Generally Recognized as Safe (GRAS) Notice

for

Dried L-Valine Fermentation Product as a Source of Valine in Livestock and Poultry Feed

Prepared for:
U.S. Food and Drug Administration
Center for Veterinary Medicine
Division of Animal Feeds

Prepared by:
CheilJedang Corporation

Table of Contents

List of Tables

List of Figures

List of Appendices

PART 1 GRAS Notice

CJ CheilJedang Corporation (hereinafter referred to as 'CJ') is submitting a GRAS notice for the substance Dried L-Valine Fermentation Product as a source of L-valine in livestock and poultry diets.

1.1 Name and Address of Organization

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1.2 Name of the Notified Substance

The common or usual name of the subject substance of this notification is 'Dried L-Valine Fermentation Product' which is a source of the essential nutrient L-valine. The level of L-valine in this substance is a minimum of 72%. Dried L-Valine Fermentation Product also contains approximately 10% amino acid from biomass (dried *Corynebacterium glutamicum* cell). The trade name of the product is 'VAL Pro'.

1.3 Intended Conditions of Use

Dried L-Valine Fermentation Product is to be used as an ingredient in livestock and poultry feeds according to current good manufacturing and feeding practice as defined in 21CFR§582.1(b) ('Substances that are generally recognized as safe'). L-Valine is an essential amino acid that is typically considered to be the fifth limiting amino acid after L-tryptophan for pigs and as the fourth or fifth limiting amino acid after L-threonine for poultry. L-Valine will be incorporated into the diet at levels

commensurate with the nutritional requirement. Therefore, the required level will be decided on a case-by-case basis by animal nutritionists, based on good feeding practice for the target species.

1.4 Statutory Basis for GRAS Determination

This GRAS conclusion is based on the scientific procedures as provided in 21CFR§570.30(a) and (b).

1.5 Federal Food, Drug, and Cosmetic Act Premarket Approval Exemption

The submitter has determined that the use of Dried L-Valine Fermentation Product as produced by fermentation with *Corynebacterium glutamicum*, for use as a nutrient (L-valine) in livestock and poultry feed is Generally Recognized as Safe (GRAS) based on scientific procedure and is thus exempt from the premarket approval requirement of the Federal Food, Drug and Cosmetic Act (21 U.S.C § 301 et seq.).

1.6 Availability of Information for FDA Review

CJ agrees to make the data and information pertaining to this submission available to FDA.

CJ agrees to both of the following procedures for making the data and information available to FDA:

(A) Upon FDA's request, CJ will allow FDA to review and copy the data and information during customary business hours at the address specified for where these data and information will be available to FDA; and
(B) Upon FDA's request, CJ will provide FDA with a complete copy of the data and information either in an electronic format that is accessible for FDA evaluation or on paper.

1.7 Freedom of Information Act 5 U.S.C 552 Disclosure Exemption

CJ has placed proprietary and confidential information in three appendices: Appendix 1, 'Analytical Report; Qualitative and quantitative composition of Dried L-Valine Fermentation Product and Method validation (CONFIDENTIAL)'; Appendix 2, 'Pre-Fermentation Information (CONFIDENTIAL)'; Appendix 3, 'Manufacturing Process (CONFIDENTIAL)'; Appendix 7, 'Acute Oral Dose Toxicity Study of L-Valine (VAL Pro) in SD Rats (CONFIDENTIAL)'; Appendix 8, Bacterial Reverse Mutation

Test of L-Valine (VAL Pro) (CONFIDENTIAL); and Appendix 10, Biogenic Amine Assessment (CONFIDENTIAL).

1.8 Certification of Complete, Representative Submission

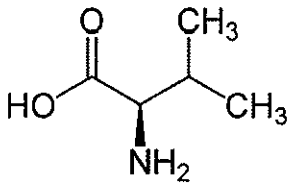
To the best of our knowledge and belief, this GRAS notice is a complete, representative and balanced submission that includes unfavorable information, as well as favorable information, known to CJ and pertinent to the evaluation of the safety and GRAS status of the use of Dried L-Valine Fermentation Product produced by fermentation with genetically engineered *Corynebacterium glutamicum* as a source of L-valine for livestock and poultry feed.

Keith D. Haydon, Ph.D.
Director of Technical Services and Marketing

PART 2 GRAS Notice: Identity, Method of Manufacture, Specifications, and Physical or Technical Effect

2.1 Scientific Data and Information that Identifies the Notified Substance

2.1.1 Name and Other Identities

Chemical name according to IUPAC nomenclature	2-amino-3-methylbutanoic acid
Synonyms	L-valine; (S)-α-Aminoisovaleric acid; L-2-Amino-3-methylbutanoic acid
CAS No.	72-18-4
EC-No.	208-220-0
Appearance	Pale or dark brown granule
Molecular mass	117.15 g/mol
Molecular formula	$C_5H_{11}NO_2$
Structural formula	

This GRAS notice covers Dried L-Valine Fermentation Product produced by fermentation with *Corynebacterium glutamicum*, with a minimum purity of 72% of L-valine. L-Valine is the active substance in the Dried L-Valine Fermentation Product. Due to its dedicated chemical properties, L-valine can only be found as free amino acid, which must not be transformed into a salt to be stable during production, storage and application.

2.1.2 Composition

The majority of Dried L-Valine Fermentation Product is L-valine (≥ 72 %). The product also contains other free amino acids (< 1 %), bound amino acids from the biomass (< 10 %), sugars (< 0.5 %), organic acids (< 0.05 %), inorganic compounds (< 10 %) and moisture (< 1 %). As shown in Table 2.1, the analysis of the five batches of Dried L-Valine Fermentation Product demonstrates that the finished product is reproducibly manufactured. The compositional analysis of Dried L-Valine Fermentation Product in Table 2.1 is without carrier. Refer to Appendix 1 for additional information regarding the analytical assessment of the product composition.

Table 2.1. Chemical composition of Dried L-Valine Fermentation Product

Test	Unit	Analysis method	Batch 01	Batch 02	Batch 03	Batch 04	Batch 05	Average
L-Valine	%	HPLC					(b) (4)	**72.38**
Hydrolyzed amino acids (in insoluble Biomass part) (Total)								**9.12**
Aspartic acid		AOAC 994.12						1.04
Threonine								0.50
Serine								0.39
Glutamic acid								1.45
Glycine								0.48
Alanine								0.98
Valine								0.60
Methionine	%	AOAC 985.28						0.25
Isoleucine		AOAC 994.12						0.39
Leucine								0.83
Tyrosine								0.24
Phenylalanine								0.46
Lysine								0.38
Histidine								0.22
Arginine								0.52
Proline								0.31
Tryptophan		AOAC 988.15						0.09
Free amino acids (Total, other than valine)		AOAC 999.13						**0.81**
Phosphoserine								0.02
Threonine								0.02
Serine								0.01
Glutamic acid								0.05
Glycine	%							0.07
Alanine								0.12
Isoleucine								0.10
Leucine								0.08
Tyrosine								0.06
Phenylalanine								0.20
Lysine								0.01
Histidine								0.07
Moisture	%	AOAC 934.01						0.82

Test	Unit	Analysis method	Batch 01	Batch 02	Batch 03	Batch 04	Batch 05	Average
Ammonium, nitrates and betaine	%						(b) (4)	**2.68**
Ammonium (as NH_3)		ASTM D 4327-97						2.48
Nitrates (as NO_3)								0.01
Betaine		Korean Feed Standard: Codex, 18 of chapter 21						0.19
Sugars (Total)	%	AOAC 995.13						**0.39**
Trehalose								0.38
Glucose								0.01
Organic acids (Succinic acid)	%	Korean Feed Standard: Codex, 1 of chapter 14						**0.02**
Inorganic anions/cations (Total)	%	ASTM D4327-03						**7.82**
Sodium								0.05
Potassium								0.46
Calcium								0.02
Magnesium								0.07
Fluoride								0.08
Chloride								0.08
Phosphate								0.30
Sulfate								6.76
Ash	%	AOAC 942.05						1.50

* Batch 01: GVAL200910, Batch 02: GVAL200911, Batch 03: GVAL200912, Batch 04: GVAL200916, Batch 05: GVAL200917
* Note that this table does not include complex carbohydrate or fats

2.1.3 Fermentation Organism

The fermentation microorganism is a genetically modified strain of *Corynebacterium glutamicum* KCCM 80240 (*C. glutamicum* KCCM 80240). The genetic modification method and characterization of the production strain can be found in Appendix 2. The safety of the production microorganism can be found in Section 6 of this dossier, Appendix 2 and Appendix 11.

2.2 Manufacturing Process

Dried L-Valine Fermentation Product is produced (b) (4)

(b) (4)

Detailed information of manufacturing process is provided in Appendix 3.

Raw materials used for producing Dried L-Valine Fermentation Product are feed grade specifications which are suitable for use in the manufacture of livestock and poultry feeds (Appendix 3). Dried L-Valine Fermentation Product is manufactured in accordance with good manufacturing practices as set forth in 21CFR§507 and meets the requirements of the US Food Safety Modernization Act (FSMA). As part of the facility's FSMA compliance, a Hazard Analysis Risk-Based Preventive Control plan has been implemented and conducted to evaluate the facility, raw materials, processes and product for potential physical, chemical and biological hazards. In order to mitigate potential risks, a hazard analysis was conducted that includes a risk assessment of the raw materials and processing steps with the implementation of appropriate preventive controls to ensure the safety of the product. These control measures are in place to effectively eliminate or reduce hazards to acceptable levels. The facility also uses prerequisite programs such as an approved supplier program to ensure the safety of the raw materials and that the raw materials are appropriate for their intended use and for the manufacture of a feed ingredient. Material suppliers are initially and periodically qualified and verification activities are performed commensurate to the risk of the material. The applicant also declares that no antimicrobial compounds (including antibiotics) were used in the production process.

2.2.1 Ingredient Stability (Shelf-life)

The stability of Dried L-Valine Fermentation Product was observed by measuring the content of L-valine and moisture under the general storage conditions (25°C, 60% RH). The content of L-valine and moisture were analyzed in real-time for 6 months. Long-term stability study on all sample tested is still on going.

As shown in Table 2.2, none of the tested samples showed a significant decrease in the level of the L-valine at the tested time points. The specified content of L-valine (minimum 72%) was maintained in all samples over the tested periods. Also, the content of moisture was maintained within the specification (maximum 5%). The full stability report is provided in Appendix 4.

Table 2.2. Shelf-life of Dried L-Valine Fermentation Product

Batch	Parameter	Storage time (month)				
		0	1	3	4	6
NGVAL191221	L-Valine (%)	(b) (4)				
	Moisture (%)					
NGVAL191222	L-Valine (%)					
	Moisture (%)					
NGVAL191223	L-Valine (%)					
	Moisture (%)					

L-valine and moisture content were stable over the 6 months of testing and it demonstrated the product stability throughout the testing period at ambient temperatures.

2.2.2 Stability Upon Addition to Animal Feed

A 3-month stability test in broiler and swine mash feed was conducted to demonstrate the stability of Dried L-Valine Fermentation Product when mixed in a complete feed. The content of L-valine was analyzed in real-time for 3 months.

As shown in Table 2.3, none of the tested samples showed a significant decrease in the level of the L-valine at the tested time points. The specified content of L-valine (minimum 72%) was maintained in all samples over the tested periods. The full study report can be found in Appendix 5.

Table 2.3. Stability of Dried L-Valine Fermentation Product in mash feed for broilers and swine

Batch	Parameter	Storage time (month)			
		0	1	2	3
		Nominal value 0.28%			
Mash feed with VAL pro GVAL200910	L-Valine (%)	(b) (4)			
Mash feed with VAL pro GVAL200911	L-Valine (%)				
Mash feed with VAL pro GVAL200912	L-Valine (%)				

This study demonstrated that Dried L-Valine Fermentation Product is a stable source of L-valine when added to complete mixed feed over a 3 month period.

2.3 Specifications

The specification of Dried L-Valine Fermentation Product is established based on the assay of 5-batch product. The analytical data supporting the specifications is reported in Table 2.1 and Appendix 1. The product specifications are provided in Table 2.4.

Table 2.4. Specification of Dried L-Valine Fermentation Product

Parameter	Specification	Analysis method
L-Valine (%)	≥ 72	HPLC (Appendix 1-Attachment 1)
Moisture (%)	≤ 5	AOAC 934.01 (105°C, 3 hr)
Ash (%)	≤ 3.5	AOAC 942.05

The hazardous substances, heavy metals, in Dried L-Valine Fermentation Product was analyzed. Certificate of analysis is provided in Appendix 6.

As shown in Table 2.5, detected level of cadmium and mercury in the Dried L-Valine Fermentation Product is below the detection. And the low concentration of other heavy metals, arsenic and lead, were detected. Hence there is no concern about the safety due to heavy metals in the animal and human, based on the NRC established tolerances (NRC, 2005).

Table 2.5. Heavy metals in Dried L-Valine Fermentation Product

Parameter	Batch No			Analysis method
	GVAL200910	GVAL200911	GVAL200912	
Lead (mg/kg)	(b) (4)			AOAC 2015.01
Arsenic (mg/kg)				
Cadmium (mg/kg)				
Mercury (μg/kg)				

2.4 Intended Use (Utility) of Dried L-Valine Fermentation Product

Dried L-Valine Fermentation Product is to be used as L-valine supplemental nutrient in livestock and poultry feeds in accordance with good manufacturing or feeding practice as defined in 21CFR§582.1(b) Substances that are generally recognized as safe. L-Valine is an essential amino acid in all animal species (EFSA, 2014). The level of supplementation varies between species and depends on the nutritional content of the diet (specifically the amino acids content). Therefore, the use of supplementation will be determined on a case-by-case basis by animal nutritionists, based on good feeding practice.

L-Valine is usually the fifth limiting amino acid after L-tryptophan for pigs and the fourth one after L-threonine for poultry. Like L-lysine, L-threonine and L-tryptophan, L-valine is an indispensable amino acid for body protein deposition, growth, and maintaining animal health. Thus a dietary deficiency in L-valine affects the utilization of previous dietary limiting amino acids and consequently animal growth and health status.

Figure 2.1. The branched chain amino acids catabolic pathway (Brosnan et al., 2006)

L-Valine is belonging to the branched-chain amino acid (BCAA) group, together with L-isoleucine and L-leucine. Due to their common metabolic pathway, some nutritional interactions/antagonisms exist between them. That is why it is very important to meet their individual dietary requirements to ensure that they are neither under- nor over supplied in animal feeds.

Dried L-Valine Fermentation Product can be added directly to the feeding stuffs/complementary feeding stuffs or via premixes. No inclusion levels are proposed as the requirements in quantitative terms depend on the species, the physiological state of the animal, the performance level and the environmental conditions, as well as the amino acid composition of the non-supplemented diet. The formulator of the feed will determine the required level of amino acid supplementation.

Dried L-Valine Fermentation Product is the subject of this application. The active substance is L-valine. Any component of Dried L-Valine Fermentation Product does not differ significantly from the constituents of the ordinary diet of target livestock and poultry species.

The biomass portion of Dried L-Valine Fermentation Product is dried, inactivated *C. glutamicum*, which is the same biomass used in the Dried L-Lysine Fermentation Product (AAFCO, 2018). According to the AAFCO Official Publication (AAFCO, 2018), Dried L-Lysine Fermentation product (AAFCO, 2018)

may be effectively used as an alternative to L-lysine monohydrochloride (L-lysine without biomass product) as a supplemental L-lysine source in swine and poultry diets. The biomass had been demonstrated to not interfere with the L-lysine availability. This has been confirmed comparing the bioavailability of L-lysine and Lysine Sulfate (Lysine Fermentation Product) in young swine (Htoo et al., 2016).

Recently, series of experiment with a spray-dried L-valine fermentation product with biomass from *C. glutamicum* are reported (Oliveira et al., 2019). The contents of L-valine in this study were 64.4%. The authors reported that the relative bioavailability by growth assay (ADG, ADFI and FCR) and blood urea nitrogen of the Dried L-Valine Fermentation Product with biomass from *C. glutamicum* was 100% as compared to commercial L-valine (98%) in weanling pigs. Therefore, there is no expectation of decreased bioavailability of L-valine by the biomass in the Dried L-Valine Fermentation Product. Additionally, a recent publication examined the bioavailability of three amino acids: L-threonine (>75%), L-valine (>70%) and L-tryptophan (>60%) fed to either broiler chicks or weanling pigs with their respective dried fermentative biomasses produced by CJ (Wensley et al., 2019). It was concluded that the respective amino acids, L-threonine, L-valine, and L-tryptophan, when formulated on an equal amino acid basis were bioequivalent to commercially available forms of the amino acids by comparison with growth parameters (ADG and FCR). Dried L-Valine Fermentation Product, the substance of this dossier, was one of the amino acids used in this study. This data clearly demonstrates that there is no expectation that the biomass will negatively impact the bioavailability of valine from the Dried L-Valine Fermentation Product.

Part of the Wensley et al. (2019) publication included a 28-day broiler utility trial conducted by Texas A&M University to specifically compare CJ's Dried L-Valine Fermentation Product to commercially available L-valine. The trial utilized 2,100 Cobb 500 male chicks averaging 39.4 grams. Chicks were blocked on weight and assigned to one of 60 pens (33 chicks/pen). Pens were randomly assigned to one of four dietary treatments. Dietary treatments were: a Positive Control (synthetic AA); a Negative Control (same as Positive Control without synthetic L-valine); a Negative Control with Dried L-Valine Fermentation Product added at 100% of Positive Control L-valine level; and a Negative Control with Dried L-Valine Fermentation Product added at 150% of Positive Control L-valine level. Pen weights and feed disappearance were recorded at day 14 (Starter Phase) and day 28 (Grower Phase). All feed was removed at day 14 and replaced with Grower Phase diets.

Table 2.6. Bioavailability results of Dried L-Valine Fermentation Product compared to positive and negative control diets as demonstrated by growth (Wensley et al., 2019)

Criteria	Positive control (PC)	Negative control (NC)	NC with Dried L-Valine Fermentation Product 100%	NC with Dried L-Valine Fermentation Product 150%	SEM	*p*-value
Body weight						
Day 0 (g)	39.4	39.4	39.5	39.3	0.03	0.764
Day 28 (kg)	1.665[a]	1.551[b]	1.684[a]	1.662[a]	0.0088	<0.001
Feed intake, g/bird/day						

Criteria	Positive control (PC)	Negative control (NC)	NC with Dried L-Valine Fermentation Product 100%	NC with Dried L-Valine Fermentation Product 150%	SEM	*p*-value
Day 0 – 28	81.4[a]	78.0[b]	82.4[a]	81.1[a]	0.39	<0.001
Average daily gain						
Day 0-28	58.1[a]	54.0[b]	58.7[a]	58.0[a]	0.34	<0.001
Gain to feed ratio						
Day 0-28	0.729[a]	0.711[b]	0.730[a]	0.728[a]	0.0031	<0.001

[a-b] Values with different superscripts differ, $p < 0.05$.

Broiler performance was negatively impacted with the reduction of L-valine level in the diet as body weight and feed intake were reduced and feed conversion ratio was increased in the NC fed broilers as compared to the PC fed broilers. Increasing the digestible L-valine level with Dried L-Valine Fermentation Product in the NC diet to equal levels of the PC diet, increased body weight and feed intake and reduced feed conversion ratio compared to the NC diet to levels similar to the PC fed broilers. Feed conversion ratio during the starter phase in the broilers fed the Dried L-Valine Fermentation Product at the equivalent level of the PC diet actually had an observed improved lower feed conversion ratio compared to the PC which may be associated with the additional nutrients contributed with the biomass. Increasing the amount of Dried L-Valine Fermentation Product to 150% the level of L-valine in the PC diet did not have any negative impacts on broiler performance. This study demonstrates the L-valine bioavailability from Dried L-Valine Fermentation Product in livestock and poultry feeds.

This broiler study was conducted to demonstrate that the limited biomass in the GRAS substance would not impact the L-valine bioavailability. The model chosen (growing poultry) has been demonstrated to be an effective model to discern the limitation of nutrient availability.

Kong and Adeola (2014) stated that bioavailability studies (which cover digestion, absorption, and utilization) are considered the absolute standard for estimating bioavailability of amino acid compared to other methods. As mentioned above, CJ completed and published a 28-day study using a broilers model (Wensley et Al., 2019). The study demonstrates that there was no impact of the biomass (28%) on L-valine bioavailability of the GRAS substance. This model suggests that the *C. glutamicum* biomass did not impact the bioavailability of L-valine in the Dried L-Valine Fermentation Product as it provided similar (P>.05) biological response (growth and feed utilization) as the 98.5% L-valine in the control diet. Swine bioavailability of L-valine of a Dried L-Valine Fermentation Product containing approximately 35% *C. glutamicum* biomass was confirmed by in a recent report of Oliveira et al. (2019). Also, Parsons (1996) review of digestible amino acids in poultry and swine reported positive correlation between cecetomized roosters and ileal-cannulated pigs. However, as Kong and Adeola (2014) noted digestibility is only one factor when assessing bioavailability. Biological responses provided are the best indicator of any biomass interference with L-valine bioavailability in the GRAS substance. As pointed out, other *C. glutamicum* amino acids sources (specifically L-lysine) has been assessed for bioavailability (AAFCO definition 36.15). There is no concern for this L-lysine source as a suitable additive for use in livestock, poultry and aquaculture. When feeding ruminants amino acids, the bacteria rich rumen

typically consumes the amino acids and building microbial proteins that are digested and absorbed later down the gastrointestinal tract.

The data presented positively demonstrates that Dried L-Valine Fermentation Product is a bioavailable source of L-valine for the intended use in livestock and poultry feeds.

Part 3 GRAS Notice: Target Animal and Human Exposures

3.1 Target Animal Exposure

L-Valine is an essential amino acid in all animal species (EFSA, 2014), including livestock and poultry (National Research Council, 1994 and 2012). The level of supplementation varies between species and is dependent on the nutritional content of the diet (specifically the amino acids content). Therefore, the use of supplementation will be determined on a case-by-case basis by animal nutritionists, based on good feeding practice.

Based on the overall level of supplementation in the most fortified diets, (for example broilers, egg layers and swine), the maximum level of use for L-valine would, in normal feeding practices, be approximately from 0.01 % to 0.30 % of the layers feed and from 0.01 to 0.40 % of the broilers feed (National Research Council, 1994). L-Valine supplementation levels in swine feeds range from 0.01 % to 0.15 % depending on production phase and feed ingredients used in the diet (National Research Council, 2012). Other species would be similar.

Therefore, the usage level of Dried L-Valine Fermentation Product in the formulated feed will be based on the L-valine naturally occurring content in the feed, a maximum usage would be considered 0.5 % of the feed.

The majority of the non-L-valine components(Table 6.1) of Dried L-Valine Fermentation Product are either essential nutrients or typical components of livestock and poultry feeds (amino acids, minerals and organic acids) and are consistent with normal components of feed, as such would not be a source of residues beyond that found in traditional livestock and poultry feeds.

3.2 Human Food Exposure

L-Valine is a required nutrient for human, since it used for muscle growth, tissue repair and energy source. It is an essential amino acid, hence it must be ingested, as a component of proteins usually obtained from soy, cheese, fish, meats and vegetables.

Dried L-Valine Fermentation Product is intended for use in livestock and poultry feeds only as a nutritional source of the essential amino acid, L-valine. Therefore, dietary intake of L-valine by animal is significantly below the amount which could cause physiological imbalances and adverse effects. The other components of the substance are nutrients and are available for uptake, metabolism and growth. Therefore, the composition of the milk, meat, and eggs from animals fed Dried L-Valine Fermentation Product, should be no different than from animals fed a nutritionally complete diet.

Also, in general, amino acids cannot be stored by the organism. Free amino acids, whether ingested in commercial synthetic form or released after the digestion of proteins by proteolytic enzymes, are absorbed through the intestinal mucosa to enter the blood stream. After absorption, alpha amino acids are directly used in protein synthesis or rapidly metabolized into intermediates in the citric cycle as evidenced by the presence of only trace amounts of alpha amino acids in the plasma.

Thus it can be concluded that there will be no additional exposure to L-valine above the natural basal content for the consumer raised by digested meat produced from animals fed with compounded feed supplemented by Dried L-Valine Fermentation Product.

Part 4 GRAS Notice: Self-Limiting Levels of Use

There is no self-limiting use information specific to this substance.

Part 5 GRAS Notice: Experience Based on Common Use in Food Before 1958

The GRAS determination is not based on common use in animal feed prior to 1958.

Part 6 GRAS Notice: Narrative

6.1 Safety of *Corynebacterium glutamicum* – Production Organism

Corynebacterium glutamicum (C. glutamicum) is a gram positive bacteria belonging to the family of *Corynebacteriaceae*. This strain is scientifically recognized as safe and provides no negative impact on human and the environment. Additionally, this strain has a long history of safe use in industrial production (Eggeling and Bott, 2005). In addition, *C. glutamicum* is a GRAS microorganism and has a 'Qualified Presumption as Safe' (QPS) status (EFSA, 2011). A description and summary of the QPS review of *C. glutamicum* is provided in Appendix 11.

C. glutamicum is an authorized source for a number of feed ingredients. It is listed as a feed ingredient in the AAFCO OP (2018). Also, it is a source organism for Condensed Extracted Glutamic Acid Fermentation Product and Dried L-lysine Fermentation Product as well as Liquid L-lysine Fermentation Product (AAFCO, 2018. 36.1; AAFCO, 2018. 36.16; AAFCO, 2018. 36.17). In 2014, the US Food and Drug Administration, Division of Animal Feeds (OS&C/FDA) had reviewed the safety assessment of this source organism for the use in animal feed. Based on that recent review, CJ extensively reviewed the recent literature since 2003 (Appendix 11). Overall, no studies were retrieved either in the electronic literature search (ELS) or follow-up selective searches that contained information indicating potential safety issues or hazards associated with *C. glutamicum*. This is consistent with the previous safety assessment completed by the US FDA, Division of Animal Feeds.

6.2 Safety Considerations due to the Nature of Modification to *Corynebacterium glutamicum*

The production microorganism used to produce Dried L-Valine Fermentation Product is a genetically modified strain of *C. glutamicum.* The details of the genetically modified strain of *C. glutamicum* are provided in Appendix 2. The assessment of the genetic engineering process demonstrates that there is no hazard imparted due to the engineering process. This data is summarized in the sections below.

Dried L-Valine Fermentation Product is intended for use as a nutrient for animal consumption. Generally, a GRAS notice addresses the potential human dietary consumption of a component of animal feed due to consumption of animal products and tissues in which the component may be present. In this case, however, there is no need to determine the estimated daily intake (EDI) of the Dried L-Valine Fermentation Product for human consumption. The Dried L-Valine Fermentation Product and any of the described biomass (see above) will be metabolized when the animal consumes and digests its feed (like all feed). Dried L-Valine Fermentation Product derived from the genetically modified *C. glutamicum* will be indistinguishable from other sources, as will be the potential non L-valine components, which are all normal components of animal feed.

1) Information on any toxic, allergenic or other harmful effects on human or animal health

The genetic modifications made, resulting in strain *C. glutamicum* KCCM 80240, exclusively correspond to the overexpression or elimination of several enzymes in its metabolism. It has been used for the manufacturing of feed additives for many years and is generally accepted as safe. The open reading frames (ORFs) of production strain were analyzed to assess the absence of shifting open reading frames which does not associated with intended genetic changes and potential of spill-over effects. Any safety concerns were not observed based on this analysis (Appendix 2).

The pathogenicity-related genes were identified using the whole genome sequence of *C. glutamicum* KCCM 80240. All protein sequences were subjected to BLAST analysis against the Virulence Factor Database. The analysis results showed no pathogenicity-related genes in the production strain *C. glutamicum* KCCM 80240 (Appendix 2, Attachment 4).

2) Potential for DNA transfer or any capacity for enhanced gene transfer

To prevent any potential transfer of genetic material to other organisms, the strategy of construction for *C. glutamicum* KCCM 80240 strain was based on procedures described below.

- Any genetic material including plasmid to be autonomously replicable was not used.

- All the genetic modifications were done on the chromosome.

3) Resistance of antibiotics of the production strain

The antibiotic minimum inhibitory concentration (MIC) for the Dried L-Valine Fermentation Product production strain was observed. The broth dilution method was used to determine the susceptibility of the production strain *C. glutamicum* KCCM 80240. In regards to antibiotic resistance, *C. glutamicum* wild-type strains has not been reported to have any antibiotic resistance. This was confirmed by the minimum inhibitory concentration (MIC) test and study report is provided in Appendix 2, Attachment 1. *C. glutamicum* KCCM 80240 showed same susceptibility to antibiotics with the wild-type *C. glutamicum* KCCM 14067. These results support that antibiotic resistance genes do not exist on the chromosome of the *C. glutamicum* KCCM 80240.

4) Absence of viable cell in final product

The absence of viable cells of the production strain in the Dried L-Valine Fermentation Product was examined in accordance with the European Food Safety Authority guidance (EFSA, 2018). According to this study, no viable cells were observed in the final product and manufacturing processes after cell inactivation of the fermentation broth (Appendix 2, Attachment 2).

6.3 Safety Considerations for L-Valine

Dried L-Valine Fermentation Product is a source of nutritional L-valine that can be safely used in the production of proteins like all other sources of L-valine.

L-valine is codified as a Generally Recognized as Safe amino acid for the use in animal feed (21CFR§582.5925). L-valine is an essential amino acid, as discussed in Section 2 and is formulated in diets based on potential natural deficiencies.

The European Food Safety Authority's (EFSA) Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) has reviewed the safety and efficacy of L-valine when used in animal diets (EFSA, 2014). According to this report, L-valine additives in animal feed are incorporated into the proteins of tissues and/or products of target animal species, and L-valine that exceeds the valine requirement of the animal is excreted as urea/uric acid and carbon dioxide. Consequently, no free L-valine occurs or accumulates in target animal tissues. L-valine is an essential amino acid for humans. There is no residue issue in free L-valine. Therefore, Dried L-Valine Fermentation Product presents no exposure risk to humans consuming tissues or products from the target animal species.

6.4 Safety Considers of Dried L-Valine Fermentation Product

As seen in Table 2.1 and Appendix 1, the majority of the substances in the product are typical components of livestock and poultry feeds.

To support the safety of Dried L-Valine Fermentation Product, the potential acute toxicity of Dried L-Valine Fermentation Product in rats was conducted in accordance with 'Acute Oral Toxicity-Fixed Dose Method (OECD, 2001)'. In this study, four fasted females were given a single oral dose of Dried L-Valine Fermentation Product as a solution in distilled water at a dose level of 2,000 mg/kg body weight following a sighting test at a dose level of 300 mg/kg and 2,000 mg/kg. No clinical signs and body weight gain related to toxicity were observed during the study. Therefore, acute oral median lethal dose (LD50) of Dried L-Valine Fermentation Product in the female Sprague-Dawley rat was estimated to be greater than 2,000 mg/kg body weight (Globally Harmonized Classification System Unclassified). Full study report is provided as Appendix 7.

Additionally, the potential mutagenicity of Dried L-Valine Fermentation Product was evaluated in accordance with 'Bacterial Reverse Mutation Assay (OECD, 1997)'. This study was conducted using *Salmonella tryphimurium* strains TA98, TA100, Ta1535 and TA1537 and *Escherichia coli* strain WP2 *uvr*A in the absence and presence of external metabolic activation. Dried L-Valine Fermentation Product showed no evidence of mutagenicity in this test. Full study report is provided as Appendix 8.

6.5 Safety Assessment of Non-Valine Components and Impurities

6.5.1 Assessment of Non-Valine Composition

The GRAS substance is 72 % L-Valine with specifications permitting up to 5 % moisture and 3.5 % ash. Section 3 of this dossier suggests the maximum level of use in the diet as 0.5 % of feed. Table 6.1 provides the compositional analysis and, by calculation, considers the contribution of this level of use to a complete diet.

Table 6.1. Contribution of non-L-valine components when Dried L-Valine Fermentation Product incorporated into a complete feed at 0.5%

Substance	Average level in Dried L-Valine Fermentation Product (%)	Contribution of the Dried L-Valine Fermentation Product incorporated in feed at 0.5% (ppm)
Ammonium (as NH_3)	2.48	124
Nitrates (as NO_3)	0.01	0.5
Betaine	0.19	9.5
Sodium	0.05	2.5
Potassium	0.46	23
Calcium	0.02	1
Magnesium	0.07	3.5
Fluoride	0.08	4
Chloride	0.08	4
Phosphate	0.3	15
Sulfate	6.76	338
Succinic Acid	0.02	1
Glucose	0.38	19
Trehalose	0.01	0.5
Phosphoserine	0.02	1
Threonine	0.02	1
Serine	0.01	0.5
Glutamic acid	0.05	2.5
Glycine	0.07	3.5
Alanine	0.12	6
Isoleucine	0.1	5
Leucine	0.08	4
Tyrosine	0.06	3
Phenylalanine	0.2	10
Lysine	0.01	0.5
Histidine	0.07	3.5

The levels of non-L-valine components are consistent with nutritional components of conventional feedstuffs, and the non-nutritional components are well below any potentials safety concern and consistent with other fermentation product components.

6.5.2 Assessment of L-Valine Derivatives

L-valine derivatives (i.e., L-α-aminobutyric acid, α-hydroxyvaline, α-thiazolealanine, and L-norvaline) which have been used for strain development were analyzed (Table 6.2). These derivatives were not found above the limit of detection except L-α-aminobutyric acid (Appendix 9). Since *C. glutamicum* has 2-ketobutyrate generation reaction which can be used as a precursor of L-α-aminobutyric acid in other amino acid synthetic pathway (Krömer et al., 2006), detected α-aminobutyric acid could be explained that it synthesized during the fermentation, not from the screening media.

Non-proteinogenic amino acids (NPAA) as well as D-isomer amino acids have been used as stabilizing agents in newer peptide therapies (Ding et al., 2020). Most NPAA are metabolized and can partially substitute for their base amino acid, as Chawla and Rudman (1974) demonstrated in nitrogen balance studies with growing rats.

The levels of non-proteinogenic derivatives of L-valine in the Dried L-Valine Fermentation Product are low. L-α-aminobutyric acid is a non-essential amino acid that is primarily derived from the catabolism of L-methionine, L-threonine, and L-serine and can be easily metabolized by livestock and poultry. L-α-aminobutyric acid levels in Dried L-Valine Fermentation Product averaged 19.07 mg/kg. Using Dried L-Valine Fermentation Products maximum inclusion level of 0.5% in a complete feed, results in only 0.0935 mg/kg concentration of L-α-aminobutyric acid; below any level of concern.

Table 6.2. L-Valine derivatives in final product

Parameter	Batch			Analysis method
	GVAL200910	GVAL200911	GVAL200912	
L-α-Aminobutyric acid (mg/kg)	(b) (4)			LC-MS/MS
α-hydroxyvaline (mg/kg)				
α-Thiazolealanine (mg/kg)				
L-Norvaline (mg/kg)				

6.5.3 Assessment of Biogenic Amines

Biogenic amines are biogenic substances with one or more amine groups. They are basic nitrogenous compounds formed mainly by decarboxylation of amino acids or by amination and transamination of aldehydes and ketones. CJ analyzed for six typical biogenic amines; tyramine, phenethylamine, putrescine, cadaverine, histamine and tryptamine in three batches of Dried L-Valine Fermentation Product.

The potential biogenic amines were also analyzed in the fermentation broths comparing against the wild-type strain (*C. glutamicum* ATCC 14067), parental strain (*C. glutamicum* CA08-0012) and production strain (*C. glutamicum* KCCM 80240). This data can be found in Appendix 2. Pre-fermentation. These data indicate that the genetic alterations did not impact the levels of biogenic amines.

Analysis of the Dried L-Valine Fermentation Product demonstrated a similar amount of each biogenic amine was detected in the three independent batches of Dried L-Valine Fermentation Product (Table 6.3). The analytical data is provided in Appendix 10. The levels of the tested biogenic amines ranged from 0.05-17.46 mg/kg in the final product.

Table 6.3. Biogenic amines in Dried L-Valine Fermentation Product

Parameter	Batch			Analysis method
	GVAL200910	GVAL200911	GVAL200912	
Cadaverine (mg/kg)	(b) (4)			LC-MS/MS
Histamine (mg/kg)				
Phenylethylamine (mg/kg)				
Putrescine (mg/kg)				
Tryptamine (mg/kg)				
Tyramine (mg/kg)				

Biogenic amines maybe present in fermented foods (whether or not fermented intentionally (spoilage)) and ingredients derived from fermentation. Studies have considered the potential concerns of biogenic amines in animal safety. Bermudez and Firman (1998) fed poultry diets supplemented with 292 ppm biogenic amines (four different biogenic amines) and found no significant response on performance, gross lesions, or histological evidence when measured after feeding for 2, 4, and 6 weeks. In a review article Feddern et al. (2019), examined US sources of animal by-product meals for a total of five biogenic amines. They reported average total of the five biogenic amines ranging from 245 ppm (meat and bone meal) - 822 ppm (poultry meal). Given the upper exposure limit outlined Section 3 of the notice (0.5% of the complete feed), the level of specific biogenic amines would range only from 0.25 ppb to 87.3 ppb. Using the highest level (worst case) of the analyzed 6 biogenic amines (from Table 6.3) the calculated addition to the complete feed is 0.244 ppm. This level is 1,200X lower than the levels which Bermudez and Firman (1998) found had no impact on poultry growth and health. Using the total of five different biogenic amines reported by Feddern et al. (2019) in US sourced poultry byproduct meals; a complete feed containing 2.5% of poultry by-product meal would have an average 20 ppm total biogenic amines in the complete feed. Hence, the amount of biogenic amines (0.244 ppm) provided by L-Valine Fermentation Product to poultry and livestock diets is numerically and biologically insignificant and would not cause a safety concern. The exposure of livestock and poultry to these insignificant levels of biogenic amines (especially in comparison to the typical dietary ingredients) and will not impact target animal safety or human food safety.

6.6 Safety Assessment for Target Animals

The notice covers the safety of the Dried L-Valine Fermentation Product from a number of perspectives. Section 6.1 covers the known safety of the host species *C. glutamicum* based on the review of literature and previous safety determinations by FDA and other authoritative bodies. The genetic modification process was exhaustively assessed to demonstrate that no unexpected changes would impact the safety of the GRAS substance (Section 6.2 and Appendix 2). L-Valine history of use and regulatory status was provided in Section 6.3 of the notice. The dossier includes both the report of the acute toxicology test and the Bacterial Reverse Mutation Assay, which further supported the safety determination (Section 6.4). Table 2.5 provides the heavy metal levels of the fermentation product which are very low, well below concern level (NRC, 2005) based on the fact that the starting materials are feed grade and have specific tight specifications. The notice includes a compositional analysis as well as the analysis on the L-valine derivatives and possible contamination through biogenic amines (Section 6.5); that demonstrates that the levels of impurities will not impact the safety of the GRAS substance. The notice provides the basis of CJ's determination that there is reasonable certainty that the Dried L-Valine Fermentation Product as a source of L-valine for livestock and poultry is not harmful and that the conclusion meets the generally recognized as safe standard.

6.7 Safety Assessment for Human Consumption

Dried L-Valine Fermentation Product is intended for use as a nutrient for animal consumption. Ordinarily, a GRAS notice will address the potential human dietary consumption of a component of animal feed due to consumption of animal products and tissues in which the component may be present. In this case, however, there is no need to determine the estimated daily intake (EDI) of the Dried L-Valine Fermentation Product for human consumption. L-valine (Dried L-Valine Fermentation Product) and any of the described non-L-valine components shown in Table 6.1 above will be metabolized when the animal consumes and digests animal feed containing Dried L-Valine Fermentation Product. Dried L-Valine Fermentation Product derived from the genetically modified *C. glutamicum* will be indistinguishable from other L-valine sources, as will be the potential non L-valine components, which are all normal components of animal feed. Non-valine components of Dried L-Valine Fermentation Product are all typical feed components, mostly nutrients and will not be a concern for residues.

This same determination was made by the FDA in their support of the AAFCO definition 36.16 Dried L-Lysine Fermentation Product 36.17 Liquid L-Lysine Fermentation product and 36.1 Condensed Extracted Glutamic Acid Fermentation Product.

In this regard, the European Food Safety Authority's (EFSA) Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) has reviewed the safety and efficacy of L-valine produced by *C. glutamicum* for use in the diets of all animal species (EFSA, 2014). According to this report, L-valine additives in animal feed will be incorporated into proteins of tissues and/or products of target

animal species. Also, doses exceeding the L-valine requirement of the animal will be excreted as urea/uric acid and carbon dioxide. Consequently, no free L-valine occurs or accumulates in target animal tissues and the only form of L-valine that humans will be exposed to from its use in animal feed is in the form of protein that will be digested, absorbed, and metabolized consistent with human nutrient needs. The absence of residual L-valine in the tissues of animals consuming L-valine in its diet will, therefore, not result in a subsequent human exposure or safety issue. As indicated by the analytical values displayed in Table 2.1, Table 6.1, and Appendix 1, residual components of Dried L-Valine Fermentation Product are at levels too low to present any risk of humans consuming the tissues of food animals fed the nutrient. All residual constituents are common metabolites or minerals and will be either excreted or metabolized. Therefore, they present no exposure risk to humans consuming tissues or products from the target animal. A review of the publicly available literature does not reveal information demonstrating that any of these residual constituents appears to present a risk of accumulation or harm to humans at the levels that would be consumed from animal tissue (Meyers et al., 2006). It should also be noted that L-valine is an essential amino acid for human nutrition is approved for direct addition to human food (21CFR§172.320).

In the Bacterial Reverse Mutation Assay of Section 6.4 in this dossier, Dried L-Valine Fermentation Product was not mutagenic in this bacterial assay system (Appendix 8). The results indicate that the test article, Dried L-Valine Fermentation Product, was not mutagenic in this bacterial assay system.

Since Dried L-Valine Fermentation Product produced by fermentation with *C. glutamicum* KCCM 80240, potential impurities occurring during the fermentation were analyzed additionally. These impurities: valine derivative (Section 6.5.2) and biogenic amines (Section 6.5.3) are all well below safety concerns and are consistent with other feedstuffs offered to livestock and poultry.

As such there is no hazard specific to these potential derivatives nor any other compounds as assessed by CJ in the full description of the GRAS substance. CJ has reasonable certainty that the substance is not harmful under the conditions of its intended use for humans consuming the products from animals provided Dried L-Valine Fermentation product.

6.8 Safety Conclusion

Based on the documentation provided in this GRAS Notification and as discussed above, CJ concludes that Dried L-Valine Fermentation Product produced by fermentation with *C. glutamicum* is generally recognized as safe via scientific procedures as a nutrient for animal consumption. CJ has reasonable certainty that the substance is not harmful under the conditions of its intended use. The notifier has reviewed the available data and information and is not aware of any data and information that is, or may appear to be, inconsistent with our conclusion of GRAS status.

Part 7 GRAS Notice: List of Supporting Data and Information

7.1 Confidential Information

The only information that is considered confidential in this GRAS Notice is the information specific to the production of the genetically modified organism, the manufacturing process, and the documentation of the assays specific for the composition of the marketed product. None of the information to support the safety narrative, Section 6 of this notice, is considered to be confidential. All this information is provided in a summary basis in the body of the submission, as required by 21 CFR§570 Subpart E. Therefore, the summary of the manufacturing process, with the full disclosure of the safety assessment, are consistent with the general recognition standards.

7.2 Supporting Data Information

All submitted data and reports were tested with samples produced on a pilot scale in CJ R&D center. The production process is the same for both the pilot scale and the commercial scale, ensuring that the identity of the final product is the same regardless of the scale.

7.3 References

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April 30, 2021

Dr. David Edwards Director
Division of Animal Feeds (HFV- 220),
Center for Veterinary Medicine,
Food and Drug Administration,
7519 Standish Pl., Rockville, MD 20855

Subject: Amendment of Filing of Animal GRAS Notification
L-Valine Fermentation Product

Notifier: CheilJedang Corporation (CJ) 330, Dongho-Ro, Jung-Gu, SEOUL,04560, KOREA

Dear Dr. Edwards:

In response to the email as provided by your staff member, Ms. Wasima Wahid, as dated April 20, 2021 which delineated a number of concerns identified in the acceptance filing procedure, we offer the following. We are grateful that we had the opportunity to respond to the raised issues.

We have numbered identified issues and responses.

Q1. The notifier does not clearly explain ①**why there are 16 more predicted open reading frames (ORF) in *C. glutamicum* KCCM 80240** when compared to the parental strain and does ②**not address whether these are, or are not, related to the genetic engineering process**. We note that firms often perform bioinformatics or "in silico" analyses to determine whether the changes in the nucleotide sequence could have created ORFs that could lead to production of polypeptides. ③**The safety of any putative polypeptide sequence that is 30 amino acids long or greater should be assessed**. The most common way this is done is ④**by conducting a FASTA amino acid sequence alignment against available databases that contain sequences for toxins and other biologically active proteins**. ⑤**Potential relationships should be assessed by comparison of the amino acid sequences, the percentage of identity, and alignment length**. If there is an alignment between a putative polypeptide and a protein of concern present in a database, then additional information and data may be required. This type of assessment should be done for each ORF locations identified during the ORF analysis. A GRAS notice should **describe the number of potential ORFs associated with the changes in the microorganism** (often a significant number of base pairs upstream and downstream of each insertion/deletion are included in this analysis), the number of putative polypeptides, the alignment of these polypeptides with proteins of concern in the databases, data supporting the acceptability of the databases for this purpose, and the notifier's conclusions about the results of the FASTA comparisons, and any additional information that is required ⑥**to demonstrate that any identified putative polypeptides do not raise a safety concerns.**

A1. In addition to the raised issues, we have noted the following error on the whole genome sequence. Due to ORF number and some minor changes, submitted WGS analysis report is revised and provided as "Revised **Appendix 2_Attachment 4_Whole genome sequence analysis"**. The number of ORFs was changed by re-identification (Table 1). **The difference in number of ORFs between parents and production strain were changed from 16 to 10**. The reason why CJ corrected the number of ORFs is explained in Q2.

Table 1. Genome features of three *C. glutamicum* strains

Feature	*C. glutamicum* strains		
	Wild-type strain ATCC 14067	Parental strain CA08-0012	Production strain KCCM 80240
Genome size (bp)	(b) (4)		
G+C content (%)			
ORFs			
tRNA			
rRNA			

(b) (4)

Table 2. 10 more predicted ORFs of *C. glutamicum* KCCM 80240 (Revised Appendix 2_Attachment 4_Whole genome sequence analysis, Page 58-59)

	Gene ID	Function	Related to Genetic Engineering
1	(b) (4)		
2			
3			
4			
5			
6			
7			
8			
9			
10			

In addition to 10 more predicted ORFs, the potential of existence of antimicrobial resistance and pathogenic genes in all ORFs of *C. glutamicum* KCCM 80240 were identified using BLAST analysis. ③ The amino acid sequences of all predicted ORFs, not limited to 30 amino acids long or greater, in ④*C. glutamicum* KCCM 80240 were searched against the two antimicrobial resistance genes databases, Resfinder (https://cge.cbs.dtu.dk/services/ResFinder/) and ARG-ANNOT databases (http://backup.mediterranee-infection.com/article.php?laref=282&titre=arg-annot), and Virulence Factor database (http://www.mgc.ac.cn/VFs/main.htm).

The ResFinder is based on a database of more than 2,000 resistance genes covering 12 types of antimicrobial resistance agents (aminoglycoside, betalactamase, fluoroquinolone, fosfomycin, fusidic acid, glycopeptide, macrolide lincosamide streptograminB, phenicol, rifampicin, sulphoamide, tetracycline, and trimethophorim). All amino acid sequences of *C. glutamicum* KCCM 80240 were

examined by BLASTP against to the genes from ResFinder database and the threshold for reporting a match was set to be at least 70% identity and 60% length of query sequence. It shows that matched gene with antimicrobial resistance gene was NOT detected. The absence of antimicrobial resistance gene in *C. glutamicum* KCCM 80240 is confirmed (Revised Appendix 2_Attachment 4_Whole genome sequence analysis, Page 23-25).

Additional analysis of antimicrobial resistance genes was conducted using another database, ARG-ANNOT (version May 2018) which contained 1,808 entries. As a result, the top 5 hits had low identity(≤50%), and there is NO hit with ≥70% identity and ≥60% length of query sequence. From the searching results, no antibiotic-resistance genes were detected in genome *C. glutamicum* KCCM 80240 (Revised Appendix 2_Attachment 4_Whole genome sequence analysis, Page 25).

The pathogen associated genes in the genome of *C. glutamicum* KCCM 80240 was analysed using VFDB (version July 2020). VFDB contains cumulative information of virulence factors for important bacteria pathogens including bacterial toxins, cell surface proteins that mediate bacterial attachment, cell surface carbohydrates and proteins that protect a bacterium, and hydrolytic enzymes that may contribute to the pathogenicity of the bacterium. To date, 32 genera of pathogens with medical importance are formally included in VFDB, and 42 additional genera are also partially included in the database.

(b) (4)

Table 3. Analysis of potential antimicrobial resistance and pathogen associated genes

	Reference Database	Threshold Setting		Results
		Identity (%)	Length of query sequence (%)	
Antimicrobial resistance genes	Resfinder (https://cge.cbs.dtu.dk/services/ResFinder/)	≥ 70	≥60	No hits
	ARG-ANNOT (http://backup.mediterranee-infection.com/article.php?laref=282&titre=arg-annot)	≥ 70	≥60	No hits
Pathogenic genes	VFDB (http://www.mgc.ac.cn/VFs/main.htm) * bacterial toxins, cell surface proteins, cell surface carbohydrates and proteins, hydrolytic enzymes (that may contribute to the pathogenicity of the bacterium)	≥ 80	≥60	1 hits (Isocitrate lyase ; a common and an essential anaplerotic enzyme for various organism)

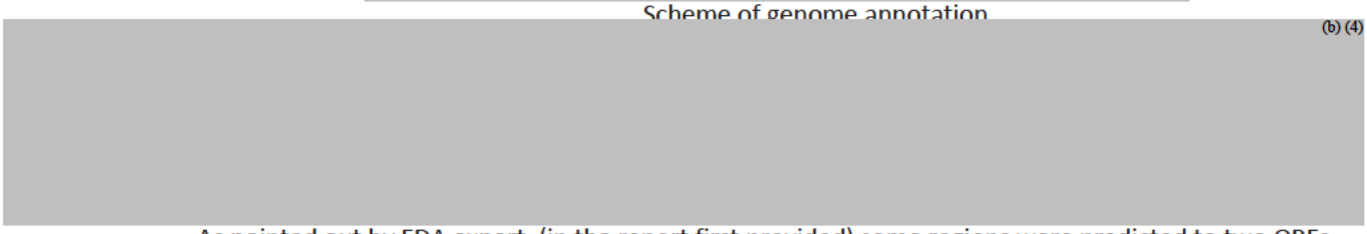
⑥**As results of analysis, there is no occurrence to express antimicrobial resistance or pathogen associated proteins and therefore the safety concern by genetic modification will not be raised.** The details of analysis result were reported in Table 10 to 12 of WGS analysis report (Revised Appendix 2_Attachment 4_Whole genome sequence analysis, Page 23-26).

Q2. The notifier states that the Glimmer program predicted two ORFs (upstream portion and downstream portion) when the genetic modification resulted in the deletion of the central portion of a gene. It does not appear that Glimmer considers that this type of deletion may produce a truncated protein or a chimeric protein. The notifier should address this issue. In addition, a GRAS notice needs to address whether any potential polypeptides expressed because of these ORF would, or would not, raise safety concerns.

A2. First, the description of ORF analysis and annotation method to predict the ORFs in *C. glutamicum* KCCM 80240 was insufficient, so detailed analytical method was added to WGS report (Revised Appendix 2_Attachment 4_Whole genome sequence analysis, Page 6). To improve the accuracy and efficiency of genome annotation, the ORF analysis and annotation of the genome were performed by running the automatic annotation pipeline Prokka which uses Glimmer algorithm for the ORF prediction and then, the automatic annotation results was corrected by manual curation.

(b) (4)

Scheme of genome annotation



As pointed out by FDA expert, (in the report first provided) some regions were predicted to two ORFs (upstream portion and downstream portion) when the genetic modification resulted in the deletion of the central portion of a gene. Some part of the genes were described as ORF which were marked with P2 although it cannot be expressed as a protein because this part was matched with a part of the gene by manual curation using TBLASTN.

In order to prevent confusion, ORFs were re-identified by exempting the sequences which cannot be translated to the proteins or peptides in the revised report. The ORFs in upstream portion would be expressed in a form of truncated, but not as an unexpected protein caused by frame shift. **No safety concern would be expected from the truncated protein since this would be translated less than 10% (42 amino acids) peptides compared to the intact protein (436 amino acids), resulting in loss of function as intended** (Revised Appendix 2_Attachment 4_Whole genome sequence analysis, Page 15 and 21-22).

Q3. In addition, the GRAS notice provides a pairwise comparison of the nucleotide sequences that were obtained from the whole genome sequencing of *C. glutamicum* ATCC 14067, *C. glutamicum* CA08-0012, and *C. gluta*micum KCCM 80240 (Revised Appendix 2_Attachment 4. Whole genome sequence analysis). This document highlights differences in the genome size and number of ORF. The notifier also states that during the development of mutagenized *C. glutamicum* CA08-0012 that there was rearrangement within the chromosome due to the insertion or deletion of transposons or integrases. The notifier does not clearly describe whether insertion/deletion of these mobile elements would lead to the production of chimeric proteins that might raise safety concerns and this should be specifically addressed.

A3. We conducted TBLASTN analysis of ORFs based on the amino acid sequence and found no chimeric protein production. All ORFs in the genome sequence were analyzed in order of automatic annotation pipeline and manual curation. As described in Table S2 and S4 (Revised Appendix 2_Attachment 4_Whole genome sequence analysis, Page 46-50 and 58-59), **unknown ORFs were NOT detected** compare to the both genome sequences of wild type (*C. glutamicum* ATCC 14067) and parental strain (*C. glutamicum* CA08-0012). Therefore, it can be concluded that there are no safety concerns by expression of unexpected chimeric protein.

Should you have any questions on the provided material, please contact the undersigned.

Sincerely,

Kristi Smedley

Digitally signed by Kristi Smedley
DN: cn=Kristi Smedley, o=Center for Regulatory Services, Inc., ou, email=smedley@cfr-services.com, c=US
Date: 2021.05.02 21:51 52 -04'00'

Kristi O. Smedley, Ph.D.
Consultant to CJ America

Cc: Min Kang, CJ America

ATTACHMENTS (each attachment should be referenced in this letter)

- Revised Appendix 2_ Pre-Fermentation Information
- Revised Appendix 2_Attachment 4_Whole genome sequence analysis
- Seemann, T. (2016). Bioinformatics (Additional reference of Revised Appendix 2)

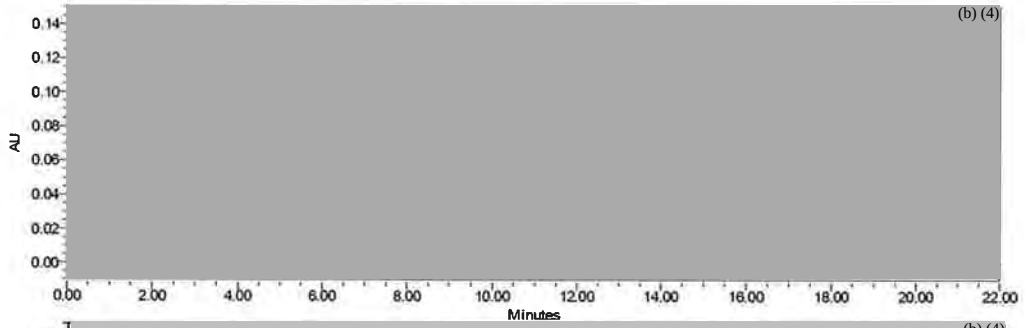

(b) (4)

1. Identity of the Notified Substance

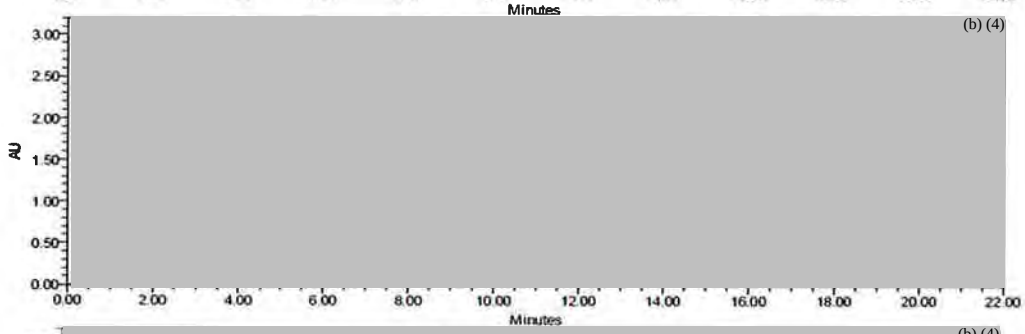
The notifier needs to provide data to demonstrate whether Dried L-Valine Fermentation Product (≥72% L-valine) contains only the L-isomer form of valine.

The chirality of valine in Dried L-Valine Fermentation Product is confirmed by experimental analysis using chiral-HPLC. Two representative batches of Dried L-Valine Fermentation Product produced at 2019 and 2020 were tested. As shown in Figure 1, Dried L-Valine Fermentation Product contains only the L-form of valine. The absence of D-valine is confirmed. Details of analysis is provided as 'Appendix A_Analytical Report; Chiral purity test of Dried L-Valine Fermentation Product'

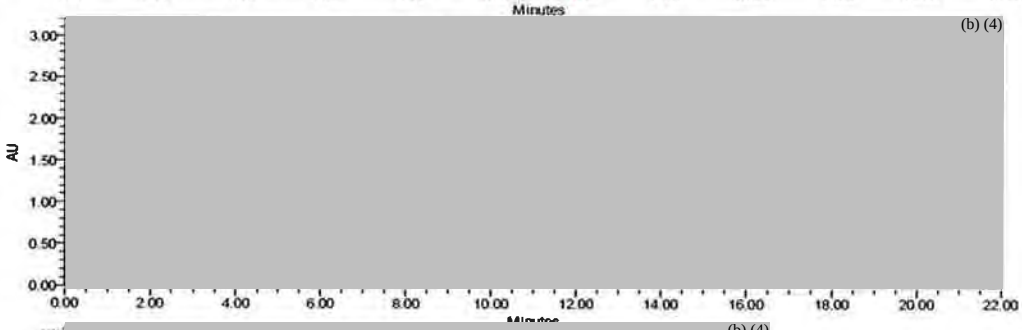
(a)



(b)



(c)



(d)

* *Lot code for the pilot scale sample was changed from NGVAL to GVAL*

(b) (4)

2. Composition of the notified substance

2.1 How the tested pilot batches can represent the expected composition of the commercial products

The process parameters for pilot scale production are the same as commercial scale (Figure 2). Also, the raw material used for fermentation and its composition is exactly the same as our planned commercial production. Therefore, the provided analytical results can representatively support the specifications (anticipated L-valine content, microbial contaminants, heavy metal contents, levels of biogenic amines and L-valine derivatives) and stability of the commercial products of the notified substance.

CJ CheilJedang has extensive experience to using different production strains for the purpose of amino acid production and all were tested in a pilot scale prior to commercialization. No differences in production yields were observed between pilot and commercial scale production, especially in fermentation process.

Figure 2. Manufacturing process

2.2 Confirmation of production strain

(b) (4)

2.3 Compositional analysis – Sample preparation, CoAs

Except for moisture analysis, test samples were analyzed after (b) (4) (b) (4) Analytical report is revised for clarification and provided as 'Revised Appendix 1_Analytical Report; Qualitative and quantitative composition of Dried L-Valine Fermentation Product and Method validation'.

Certificate of analyses regarding the compositional analysis results are attached to the end of 'Revised Appendix 1_Analytical Report; Qualitative and quantitative composition of Dried L-Valine Fermentation Product and Method validation'.

2.4 Usage of carrier

The provided analysis contained the carrier. Please refer to the CoA of the carrier used for the pilot scale samples provided as Attachment 1 of this amendment. As noted in the Table 2.1 in the previous submission (Section 2.1.2, p.9-10), we did not include complex carbohydrate and fats. The balance of the product composition is complex carbohydrates and fats mostly contributed by the carrier.

3. Heavy Metals

The Hg content for three tested batches are reported as <0.124 µg/kg, <0.117 µg/kg, and <0.096 µg/kg, respectively. The notifier needs to clarify the limit of quantification (LOQ) for Hg.

The Hg content of all samples tested were below limit of quantification, however, the levels were expressed with different dilution factors. As shown in the quantitation report provided as Appendix 6_Certificate of analysis_Heavy metals, the factors of prep dilution of each batch were different (GVAL200910-62.6364, GVAL200911-58.8295, GVAL200912-48.2866). That means the concentrations of each batch of sample for Hg analysis was not exactly same. The LOQ is automatically calculated by considering the sample dilution, therefore it makes the difference in LOQ value. The LOQ calculated from the calibration curve was 1.99 ng/kg (weight of mercury/weight of diluent).

4. Stability

A. Stability of the Dried L-Valine Fermentation Product (≥72% L-valine) (Response: Section 4.1)

The notifier needs to clarify the production scale of the three batches (NGVAL191221, 191222, and 191223) of the final product tested in the stability study. If these three batches were pilot or lab scale, the notifier needs to justify the tested batches were representative of the commercial production and the stability data collected using these batches can be used to establish the stability of the commercial product of the notified substance.

At this time, we have following questions regarding the provided 6-month stability data:

- Based on the CoAs provided in the Appendix 4, the three batches used in the stability study were manufactured on December 21, 22, and 23 of 2019, respectively. The schedule of the study provided in the Appendix 4 indicated that the duration of the reported stability study was from June 26, 2020 (initiation of the experiment) to December 11, 2020 (submission of the report). The initiation of the testing was already 6 months after the manufacturing dates. The L-valine and moisture contents of the tested batches right after the production are not provided to assess the potential change from the production to the initiation of the study.
- The analyses reported on the CoAs provided in Appendix 4 were conducted on December 11, 2020, which was supposed to be the 6-month time point. However, the analytical results reported on these CoAs are used by the notifier as the L-valine and moisture contents at the initial time point. The notifier needs to clarify this discrepancy.
- The CoAs for each batch at each time point should be provided to facilitate our evaluation.

If the notifier has collected more long-term stability data since the submission of this GRAS Notice, the notifier can provide these additional data to support the stability of the final product at recommended storage conditions.

B. Stability of Dried L-Valine Fermentation Product (≥72% L-valine) in Feed (Response: Section 4.2)

The three pilot batches (GVAL200910, 200911, and 200912) of final product were the same pilot batches 01-03 used for the compositional analysis. The notifier needs to justify the tested batches were representative of the commercial production and the stability data collected using these batches can be used to establish the stability of the commercial product of the notified substance in the complete feed for the target animal species.

4.1. Stability of the Dried L-Valine Fermentation Product (≥72% L-valine)

The three batches of the final product tested in the stability study were produced in a pilot scale.

1) CoA

As pointed out by FDA, the previously submitted CoAs were mis-attached to the study report (Appendix 4_Stability (Shelf-life)). The table of stability report is revised and the CoAs for each batch at each time point are attached. Revised report is provided as 'Revised Appendix 4_Stability (Shelf-life)'.

The pilot scale samples were manufactured in December 2019. The contents of L-valine and moisture were analyzed in February 2020. Stability study were schedule to be conducted after production, however due to COVID, test samples were stored in an ambient condition (approximately 20°C) in our storage for 6 months prior to the stability study being initiated. We apologize for missing the analysis at initial time point "0", however, based on 1-month stability data, there is no potential change from production to the initiation of the study. That is, this unintended 6-month storage prior to the stability test did not impact on the stability of the product.

Additional data for stability study is provided in the 'Revised Appendix 4_Stability (Shelf-life)'. The content of L-valine and moisture were analyzed in real-time. As requested by FDA, we have included additional data through 12-month of stability. As shown in below table, none of the test samples showed a significant change in the level of L-valine. The content of moisture tends to increase but it was within the specification. The level of L-valine is analyzed as dry matter basis.

Table 1. Stability of Dried L-Valine Fermentation Product

Lot No.	Parameter	Spec.	2020 Feb	Storage time (month)						
				0	1	3	4	6	9	12
					A:20.07.31 B:20.08.25	A:20.09.25 B:20.10.22	A:20.10.30 B:20.11.24	A:20.12.23 B:20.12.24	A:21.03.26 B:21.04.30	A:21.06.25 B:21.08.26
NGVAL191221	L-Valine (%)	≥ 72.0	72.26	(b) (4)						
	Moisture (%)	≤ 5.0	1.90							
NGVAL191222	L-Valine (%)	≥ 72.0	72.19							
	Moisture (%)	≤ 5.0	1.89							
NGVAL191223	L-Valine (%)	≥ 72.0	72.28							
	Moisture (%)	≤ 5.0	1.72							

* A: Sampling date, B: Test date (The time gap between the sampling and actual analysis date was occurred due to the analysis lab schedule.)

4.2 Stability of Dried L-Valine Fermentation Product (≥72% L-valine) in Feed

The three batches of the final product tested in the feed stability study were produced in a pilot scale. As described previously (Section 2.1 of this Amendment), the tested batches are representative of the commercial production and the stability data collected using these three batches samples demonstrate the expected stability of the commercial product of the notified substance in the complete feed for the target animal species.

5. Analytical Methods

A. Method used to detect production strain viable cells at different manufacturing steps and in the final product (Response: Section 5.1)

The Figure 1 in the Appendix 2_Attachment 2 is not properly labeled. All plates in Figure 1 needs to be labeled according to the figure legend. Page 4 of 5, GRAS Notice M-000108-Z-0004, Dried L-Valine, Livestock and poultry, September 30, 2021 teleconference

The procedure of the Control test states "2) Cell suspension was diluted up to 10^{-7} and then 500 µL of aliquots was added to 20 mL of sterile 0.9% saline (approximately 50 CFU mL^{-1})." The notifier needs to clarify whether the cell concentration of 50 CFU/mL refers to the diluted cell suspension before or after mixing with 20 mL saline. Calculations need to be provided to support the stated cell concentration.

The procedure of the Spike test calls for the addition of 1 mL of cell suspension to 1 g of final product sample. The notifier needs to clarify whether the 1 mL cell suspension used for spiking is the diluted suspension before or after mixing with 20 mL saline.

B. Method used for biogenic amines analysis (Response: Section 5.2)

The notifier needs to clarify the approach to quantify each targeted biogenic amine. If the quantification is by using external standards, the notifier needs to provide the calibration curves for each biogenic amine. The reported LOD, LOQ and method specificity for all targeted biogenic amines should be verified, corresponding chromatograms should be included.

The ratio of each tested biogenic amine between the final product and production strain fermentation broth is calculated in the table below:

Ratio of biogenic amines between the final product and the production strain fermentation broth

Biogenic amine	Final product[a] (mg/kg)	Fermentation broth[b] (mg/L)	Concentrating ratio
Cadaverine	(b) (4)		
Histamine			
Phenylethylamine			
Putrescine			
Tryptamine			
Tyramine			

[a] Average value from three batches (GVAL200910, 200911 and 200912) reported in Appendix 10.
[b] Average value from three batches (KCCM80240_200907, 200908 and 200909) reported in Appendix 10.

Based on the ratio calculated in the table above, it appears that from fermentation broth to the final product, different biogenic amines were concentrated by different factors, e.g., tyramine is only concentrated by a factor of (b) (4) while the concentrating factor is (b) (4) folds for phenylethylamine. The notifier needs to clarify this discrepancy.

C. Method used to analyze L-valine derivatives (Response: Section 5.3)

The notifier needs to provide the method procedure, including sample preparation and instrument parameters, and quantification approach. If the quantification is by using external standards, the notifier needs to provide the calibration curves for all tested L-valine derivatives. The reported LOD, LOQ and

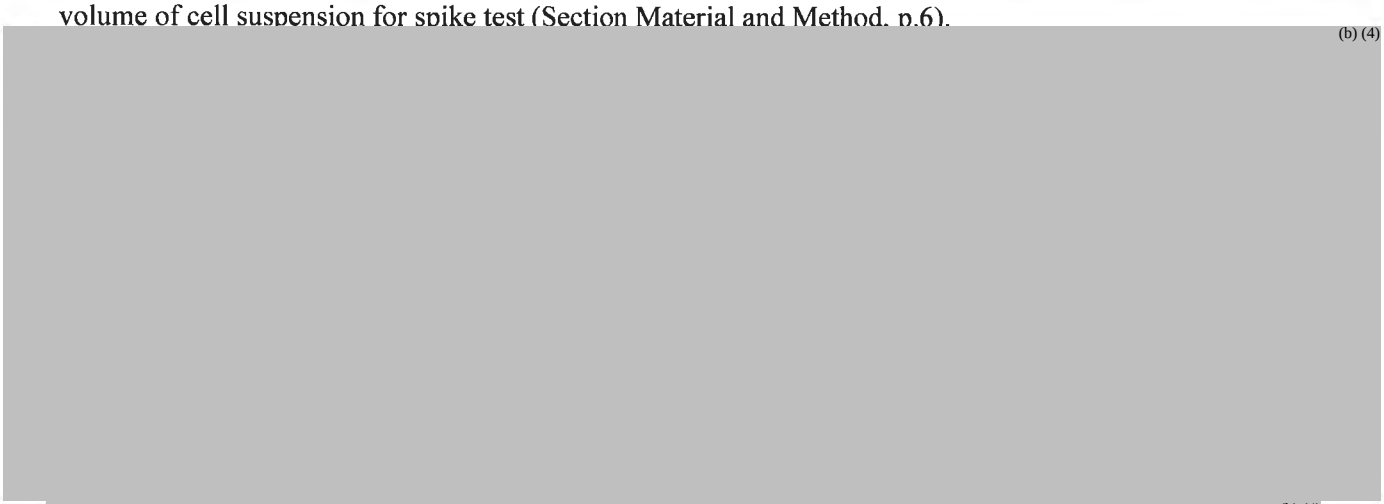
method specificity for all targeted L-valine derivatives should be verified, corresponding chromatograms should be included.

5.1 Method used to detect production strain viable cells at different manufacturing steps and in the final product

The Figure 1 in the 'Appendix 2_Attachment 2' is revised with proper label. Also, some corrections regarding the methods of Control and Spike test reflected. The study report of viable cell analysis is revised and provided as 'Revised Appendix 2_Attachment 2_Viable cell'.

Figure 5. Revised Figure 1 in the 'Appendix 2_Attachment 2'

There were clerical errors in reporting the final concentration of cell suspension for control test and used volume of cell suspension for spike test (Section Material and Method, p.6).



(b) (4)

(b) (4) In the part of sample preparation for spike test is also revised. Cell suspension for spiking test is the diluted suspension prepared by same method for control test. Entire volume of cell suspension was mixed with 1 g of test sample. Therefore, the method was revised as follows: (b) (4)

(b) (4)

5.2. Method used for biogenic amines analysis

1) Chromatogram

(b) (4)

2) Calibration curve

The concentration of biogenic amines were calculated based on the equation from the calibration curve.

(b) (4)

3) LOD/LOQ calculation

Limit of detection and limit of quantitation were determined by analyzing 7 samples of concentration near the expected limit of detection. The standard deviation of 7 samples simply multiply by the correct student's t-value. t-value for six degrees of freedom and 99% confidence level is to be 3.14. Therefore, the LOD is calculated as follows (EPA, 2016):

LOD= 3.14 × sd (standard deviation)

The limit of quantitation can also be calculated (EC, 2016):

LOQ= 10 × sd (standard deviation)

Table 2. LOD and LOQ of biogenic amines

	Biogenic amines (ng/mL)					
	Tryptamine	**Phenylethyl-amine**	**Putrescine**	**Cadaverine**	**Histamine**	**Tyramine**
1	[redacted] (b) (4)					
2						
3						
4						
5						
6						
7						
Standard deviation	0.0056	0.0063	0.0124	0.0132	0.0158	0.0131
LOD	**0.0177**	**0.0199**	**0.0391**	**0.0415**	**0.0497**	**0.0413**
LOQ	**0.0565**	**0.0634**	**0.1244**	**0.1320**	**0.1584**	**0.1315**
LOD (mg/kg)*	**0.00089**	**0.00099**	**0.00195**	**0.00207**	**0.00249**	**0.00206**
LOQ (mg/kg)*	**0.00282**	**0.00317**	**0.00622**	**0.00660**	**0.00792**	**0.00657**

* 0.1 g sample in 5 mL 0.1N hydrochloric acid

4) Correlation between fermentation broth and final product

Biogenic amine can be synthesized by decarboxylation of amino acids or by amination and transamination of aldehydes and ketones during the metabolic processes in the microorganism.

[redacted] (b) (4)

(b) (4) Consequently, the ratio of biogenic amine between the final product, Dried L-Valine Fermentation Product, and production strain fermentation broth varied.

As described in the dossier (Section 6.5.3), the level of biogenic amines in Dried L-Valine Fermentation Product is not high enough to affect growth and health of target animal when 0.5% of Dried L-Valine Fermentation Product is incorporated into the complete feed.

5.3 Method used to analyze L-valine derivatives

L-valine derivatives (L-α-aminobutyric acid, α-hydroxyvaline, 2-thiazolealanine, and L-norvaline) have been used for strain development. The residual amount of L-valine derivatives in the final product, Dried L-Valine Fermentation Product, were analyzed by using below method. Except for L-α-aminobutyric acid, other derivatives were found below the limit of detection level. The detected L-α-aminobutyric acid is expected to be derived from the fermentation, not from the screening media.

1) Analytical method

0.1 g of Dried L-Valine Fermentation Product (as-is sample) was dissolved in 5 ml distilled water.

Chromatography separation of the samples were performed on a Vanquish (Thermo Scientific, USA) system using the Eclipse XDB-C8 (4.6 mm × 150 mm, 5 um) (Agilent, USA). The column oven was operated at 45°C. Mobile phase A consisted of 10 mM ammonium formate and 0.1% formic acid in water and mobile phase B consisted of 0.1% formic acid in acetonitrile. An optimized gradient elution

with mobile phase A and mobile B (0-0.1 min, 1% B; 0.1-3 min, 50% B; 3-3.5 min, 90% B; 3.5-3.8 min, 90% B; 3.8-4.0 min, 1% B) at a flow rate of 0.5 mL min^{-1} was used.

MS analysis was carried out on a TSQ Altis triplequad mass spectra (Thermo Scientific, USA) equipped with heated electrospray ionization (H-ESI) ion source. The parameters of optimized mass spectrometry were summarized in Table 3. Selected reaction monitoring (SRM) transitions were monitored.

Table 3. Source parameters for the TSQ Altis mass spectrometer

Ion Source Parameter	**Value**
Positive Ion (V)	3500
Sheath Gas (Arb)	50
Aux Gas (Arb)	10
Sweep Gas (Arb)	1
Ion Transfer Tube Temp (°C)	325
Vaporizer Temp (°C)	350

Table 4. SRM properties for analysis of L-valine derivatives

Compound	**Precursor (m/z)**	**Product (m/z)**	**Collision Energy (V)**	**Min Dwell Time (ms)**	**RF Lens (V)**
L-2-Aminobutyric acid					(b) (4)
L-2-Aminobutyric acid					
L-2-Aminobutyric acid					
L-Norvaline					
L-Norvaline					
L-Norvaline					
2-Thiazolealanine					
2-Thiazolealanine					
2-Thiazolealanine					
α-Hydroxyvaline					
α-Hydroxyvaline					
α-Hydroxyvaline					

2) Chromatogram

(b) (4)

3) Calibration curve

The concentration of L-valine derivatives were calculated based on the equation from calibration curve.

(b) (4)

3) LOD/LOQ calculation

Limit of detection and limit of quantitation were determined by analyzing 7 samples of concentration near the expected limit of detection. The standard deviation of 7 samples simply multiply by the correct student's t-value. t-value for six degrees of freedom and 99% confidence level is to be 3.14. Therefore, the LOD is calculated as follows (EPA, 2016):

LOD= 3.14 × sd (standard deviation)

The limit of quantitation can also be calculated (EC, 2016):

LOQ= 10 × sd (standard deviation)

Table 5. LOD and LOQ of L-valine derivatives

	L-valine derivatives (ng/mL)			
	L-2-aminobutyric acid	**L-norvaline**	**2-thiazolealanine**	**α-hydroxyvaline**
1	(b) (4)			
2				
3				
4				
5				
6				
7				
Standard deviation	0.1596	0.6144	0.0676	0.0224
LOD	0.5012	1.9291	0.2124	0.0703
LOQ	1.5961	6.1437	0.6764	0.2240
LOD (mg/kg)*	**0.025**	**0.096**	**0.011**	**0.004**
LOQ (mg/kg)*	**0.080**	**0.307**	**0.034**	**0.011**

* 0.1 g sample in 5 mL water

6. Molecular Biology (MB)

- In the Revised Appendix 2_Pre-fermentation Information, there was a discrepancy between the deleted sequence in "original ilvA ORF" as underlined in Table B.5.2 on page 40 in "M-000108-T-0001_sub_001.pdf" and the sequence of primer 2 listed on page 23. After a comparison we found 3' sequence was missing a complementary base for "G" corresponding to the position (b) (4) in the *ilvA* gene. The notifier should provide a clarification in the amendment whether it is a typographical error in the primer 2 sequence. If not, please provide the information about the alignment of the primer 2 sequence and the original (undeleted) *ilvA* sequence, as well as a clear explanation on how the deletion in the *ilvA* gene in Table B.5.2 can be achieved using the current primer 2. **(Response: Section 6.1)**
- (b) (4) (b) (4) Please clarify in the amendment whether this is a typographical error. If not, the notifier should provide additional information about identity of the strain. **(Response: Section 6.2)**
- In page 36, the primer 80 used to amplify the upstream region of the *avtA* gene may contain a wrong base as shown below in red. **(Response: Section 6.3)**

  Primer 80-

(b) (4)

(b) (4)

The Primer 81 which partially overlaps the primer 80 actually confirms the correct sequence. Please clarify this issue in the amendment.

6.1 Discrepancy of sequence

There is a typographical error in the Primer 2 sequence in which 'C' residue is missed at the 5' terminus (Figure 11). The sequence of Primer 2 is corrected as follows:

(b) (4)

6.2 Origin of ilvE promoter

There is a typographical error in the strain name *C. glutamicum* VCA08- 0012. (b) (4)

(b) (4)

6.3 Sequence of Primer 80

The sequence of used Primer 80 and Primer 81 are same as described in the 'Revised Appendix 2_Pre-fermentation Information'. When developing production strain, Primer 80 and Primer 81 were designed based on the genomic DNA sequence of the (b) (4) which was used as a PCR template (Figure 12 (a)).

Although we used the Primer 80 and Primer 81, there was a discrepancy in primer sequence and genetic modification region as FDA pointed out. By WGS analysis, a point mutation was identified in the *avt*A region of (b) (4) As reported in WGS analysis report (Revised Appendix 2_Attachment 4_Whole genome sequence analysis, submitted in April 2021), 'T' residue was changed

(b) (4)

(b) (4)

(b) (4)

[Attachment 1]

CJ CHEILJEDANG CORPORATION

CERTIFICATE OF ANALYSIS

Product Name	CORN STARCH (NGMO)		
Manufactured Date	2018.02.19	Delivery Date	
Quantity	20kg		

Analysis Data

No	ITEM	SPECIFICATION	RESULT	REMARK
1	Appearance	White powder	(b) (4)	-
2	Moisture (%)	Max. 14.0		KFDA METHOD
3	pH	4.0~7.0		Starch:Water=1:2(w/w%)
4	Crude protein(%)	Max. 0.40		N×6.25
5	Ash (%)	Max. 0.15		KFDA METHOD
6	Whiteness(%)	Min. 88.0		Kett-c-1
7	SO_2 (ppm)	Max. 30.0		Quantitative analysis
8	Acidity(㎖)	Max. 3.0		KFDA METHOD
9	Starch Value (%)	Min. 98.0		DS%
10	Foreign material	Pass		-

We here certify that above figures are true and correct.

Analyzed (b) (4)

Q.C Manager :

ADD : 141. Yongdam-ro, Sangnok-gu,
Ansan-si, Gyeonggi-do, Korea
TEL. (031) 400 – 3099
FAX. (031) 438 – 1603

REPORT

Chiral purity test of Dried L-Valine Fermentation Product using HPLC

Original Final report date: Oct 12, 2021

CJ Research Institute of Biotechnology

CONTENTS

TITLE: Chiral purity test of Dried L-Valine Fermentation Product using HPLC

1. OBJECTIVE OF THE STUDY

Chiral purity test of 'Dried L-Valine Fermentation Product' using HPLC was carried out to evaluate that 'Dried L-Valine Fermentation Product' has only L-form of valine.

2. MATERIAL

(1) Standard reagent

Reagents	Supplier	Batch number
L-Valine	(b) (4)	BCBZ8642
DL-Valine		BCBR9321V

(2) Test article

1) Identity: Dried L-Valine Fermentation Product ((b) (4) , minimum of 72% of L-valine)
2) Lot No.: GVAL200910 and NGVAL191221
3) Storage conditions: Room temperature

3. METHOD

(1) Preparation of sample solution

Approximately 4.5 g of 'Dried L-Valine Fermentation Product' was weighed and put them into 50 mL of volumetric flask. It was adjusted to the volume with distilled water (64.8 g/L as L-valine), and filtered using syringe filter.

(2) Preparation of calibration standard solutions of DL-valine

Calibration standard solution was prepared by the 5, 12.5, 25.1, 50.2, and 125.5 mg/L of D-valine with distilled water as presented below.

Solution	**Dilution**	**Concentration of D-valine (mg/L)***	**Concentration of DL-valine (mg/L)**
Stock solution	-	(b) (4)	
STD 5	1/2 dilution of stock solution		

STD 4	1/5 dilution of stock solution	(b) (4)
STD 3	1/2 dilution of STD 4	
STD 2	1/10 dilution of STD 5	
STD 1	1/5 dilution of STD 3	

* It was regarded that the ratio of D-/L-valine is 50:50 in this test.

(3) Limit of detection

1) Calculation using calibration curve

Calibration curve was provided to express LOD (limit of detection). In addition, regression analysis was also carried out using this curve to figure out 'Residual standard deviation' to calculate LOD (*Anal. Chem*. 1999, 71, 2672-2677).

LOD may also be calculated based on the standard deviation of the response (σ) of the curve and the slope of the calibration curve (S) at levels approximating the LOD according to the formula: LOD = 3.3*(σ/S). The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

In this case, deviation of response would be residual standard deviation.

The residual standard deviation is a statistical term used to describe the difference in standard deviations of observed values versus predicted values as shown by points in a regression analysis. Regression analysis is a method used in statistics to show a relationship between two different variables, and to describe how well you can predict the behavior of one variable from the behavior of another.

Residual standard deviation is also referred to as the standard deviation of points around a fitted line or the standard error of estimate. The formulas for residual and residual standard deviation is :

Residual = $(Y - Y_{est})$

$$S_{res} = \sqrt{\frac{\sum(Y - Y_{est})^2}{n - 2}}$$

S_{res} = Residual standard deviation

Y= Observed value

Y_{est}= Estimated or projected value

n= Data points in population

We preformed 5 point calibration for D-valine as below and described the summary output of regression analysis.

2) Calculation using signal/noise ratio

The LOD is the concentration at which the signal level of the substance reaches at least 3 times the signal noise of the baseline. We also checked the signal/noise ratio of the peak assumed to be LOD.

(4) HPLC condition

	Analytical condition
Column	sumichiral OA 5000 (150 x 4.6mm)
Mobile phase	2 mmol/L copper(Ⅱ) sulfate in water
Flow rate	1mL/min
Analysis time	50 min
Column temperature	35 ºC
Injection volume	20 μL
Wavelength	(b) (4)

4. CHIRAL PURITY TEST OF DRIED L-VALINE FERMENTATION PRODUCT

(1) Chromatogram of standard solution

As shown in Figure 1(b), two peaks were detected in the chromatogram of DL-valine standard solution. To sort out peak for L-valine and D-valine, a standard solution of L-valine was prepared and analyzed. The L-valine was analyzed at approximately 10.2 min as shown in Figure 1(d). From this result, the retention time of L-valine and D-valine was determined as approximately (b) (4).

It was also found that when the concentration of L-valine is too high, it seemed the peak of L-valine is different from the chromatogram of standard solution due to the saturation of the L-valine peak. So, we prepared high concentration of L-valine standard solution (50 g/L) and analyzed serially diluted L-valine standard solution to clarify that saturated peak is a peak L-valine (Figure 1(d)).

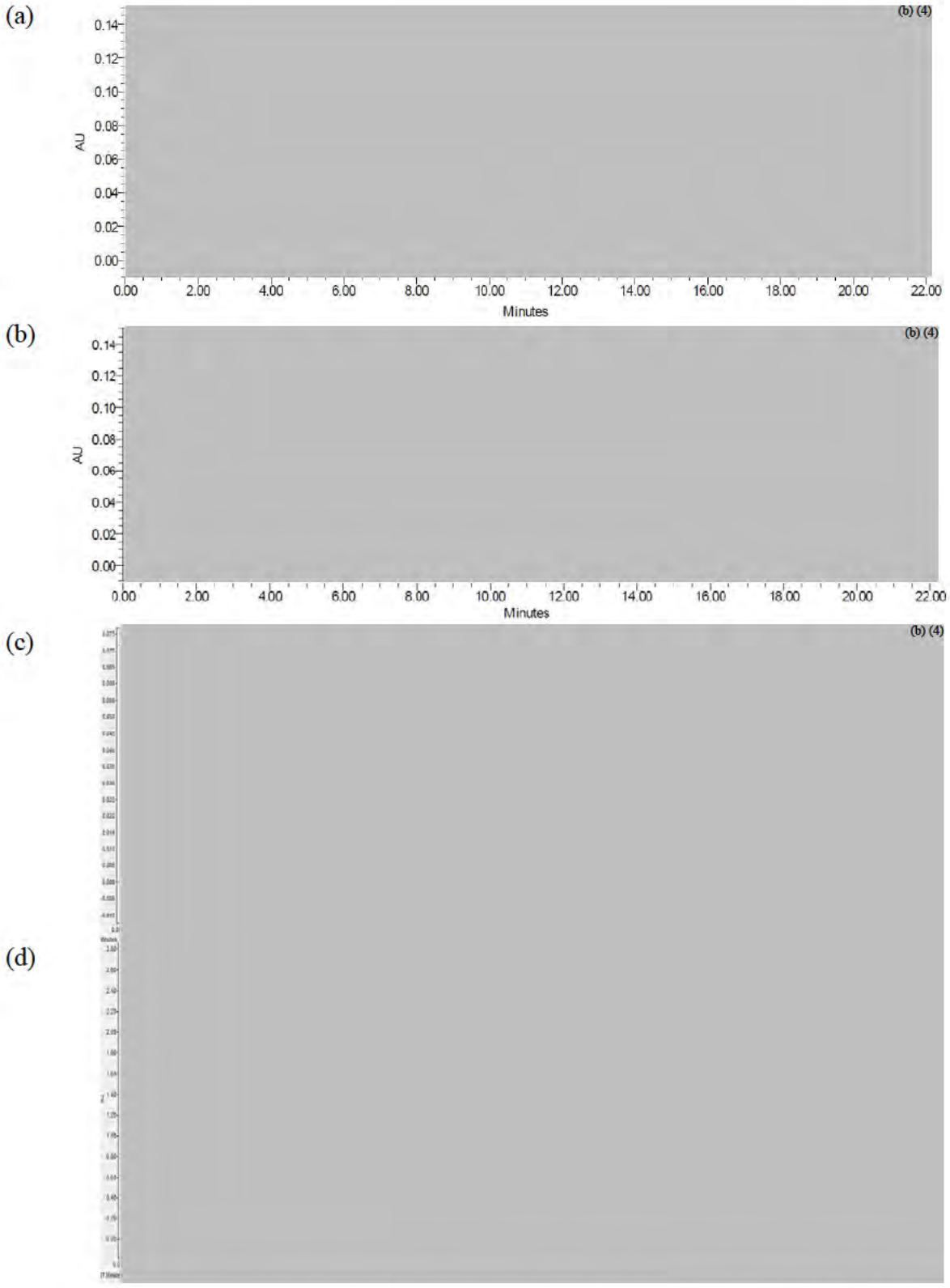


Figure 1. Chromatograms of standard solution. (a) Blank, (b) Standard solution of DL-valine, (c) Standard solution of DL-valine as diluted and (d) Identification of saturated L-valine peak with a serially diluted L-valine standard solution.

(2) Linearity

The linearity was evaluated by analyzing the calibration standard solution with a concentration of (b) (4) of D-valine. To assess the linearity of standard curve, five different concentrations of standard solution were prepared and injected to HPLC system. The regression equation was calculated in the form of Y=AX+B, where Y and X are the area of the peak and the sample amount, respectively. Correlation coefficients (r^2) obtained from regression analysis.

Table 1. Linearity of D-valine

Calibration levels (mg/L)	(b) (4)
Peak area	
Calibration curve	
Correlation coefficient (r^2)	

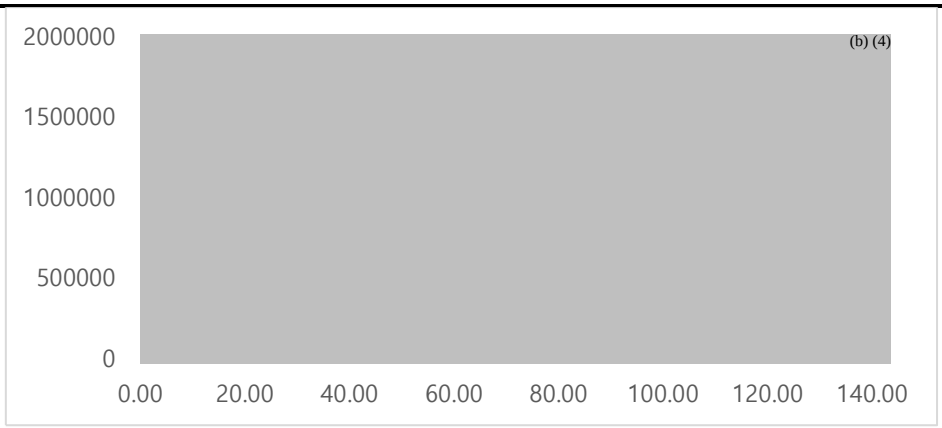


Figure 2. Linearity of D-valine

(3) Limit of detection (Calculation using regression analysis)

1) Limit of detection of D-valine

Limit of detection of D-valine were (b) (4).

2) Summary output for regression analysis study

Regression statistics	
Multiple R (Correlation coefficient)	(b) (4)
R Square (Coefficient of determination)	
Adjusted R Square	
Standard Error (Residual standard deviation)	
Observations	

3) LOD of D-valine

LOD [redacted]

(4) Limit of detection (measurement from signal/noise ration)

To clarify the calculated detection limit, we also injected 1 mg/kg of D-valine (from the calculation, LOD was [redacted]) and checked the signal/noise ratio (S/N ration) from the Empower software program. S/N ration of D-valine was 10.9 at 1 mg/kg, so injected again with half of injection volume (10 µL). Since the injection volume was half, the concentration was 0.5 mg/kg, and S/N ration of D-valine was 3.2 at 0.5 mg/kg.

So even the LOD was calculated as [redacted] from the regression analysis, the LOD of D-valine would be lower to 0.5 mg/kg.

In addition, limit of detection of D-valine in 'Dried L-Valine Fermentation Product' were approximately [redacted] due to the concentration of valine in water is about 64.8 g/L and 'Dried L-Valine Fermentation Product' contained 72% of valine.

Table 2. Limit of detection (LOD)

(a) Limit of detection (LOD) of D-valine from the chromatogram (S/N ration).

LOD	Concentration (mg/kg)
	[redacted]

(b) Limit of detection (LOD) of D-valine in 'Dried L-Valine Fermentation Product' [a].

LOD	Concentration (mg/kg)
	[redacted]

[a] The concentration of valine in water is approximately 64.8 g/L and 'Dried L-Valine Fermentation Product' contained 72% of valine (dilution factor would be approximately 11.1). Therefore, analyze D-valine in 'Dried L-Valine Fermentation Product', the LOD is different.

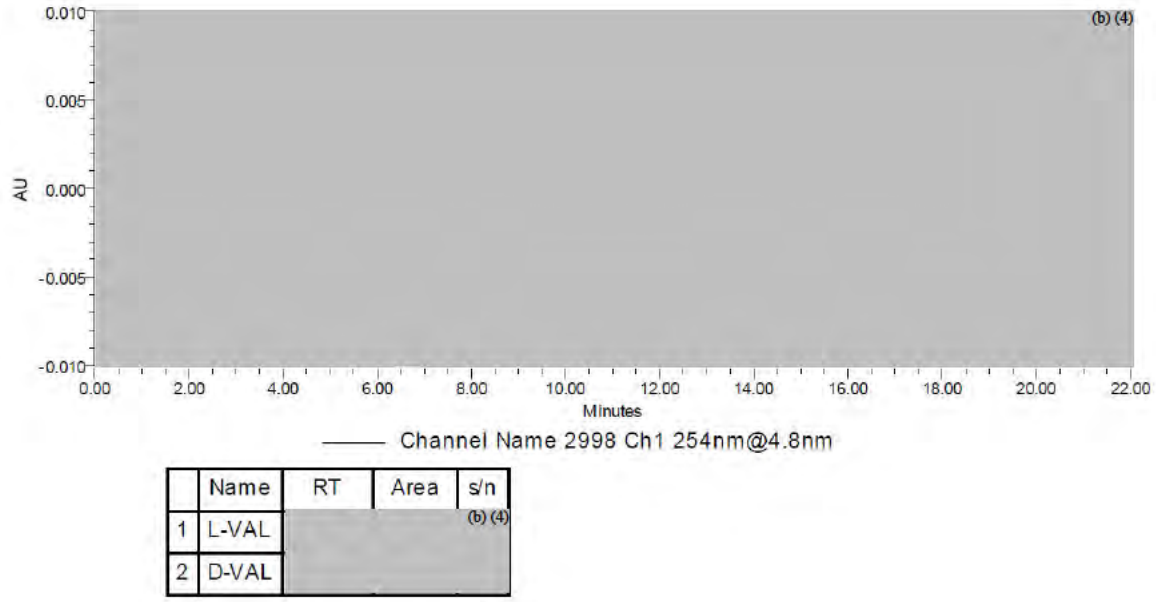


Figure 3. Chromatogram and S/N ration of 0.5 mg/kg of DL-valine

(4) Chiral purity test of 'Dried L-Valine Fermentation Product'

The developed HPLC method was applied to determine the content of D-valine in 'Dried L-Valine Fermentation Product' produced by CJ. High concentration of sample solutions were prepared (GVAL200910: 89.95 g/L, and NGVAL191221: 89.84 g/L) to prove the presence or absence of D-valine.

Compared to chromatogram of standard solution of DL-valine, no D-valine was observed in the 'Dried L-Valine Fermentation Product' produced by CJ. Chromatograms of sample solution were shown in Figure 4.

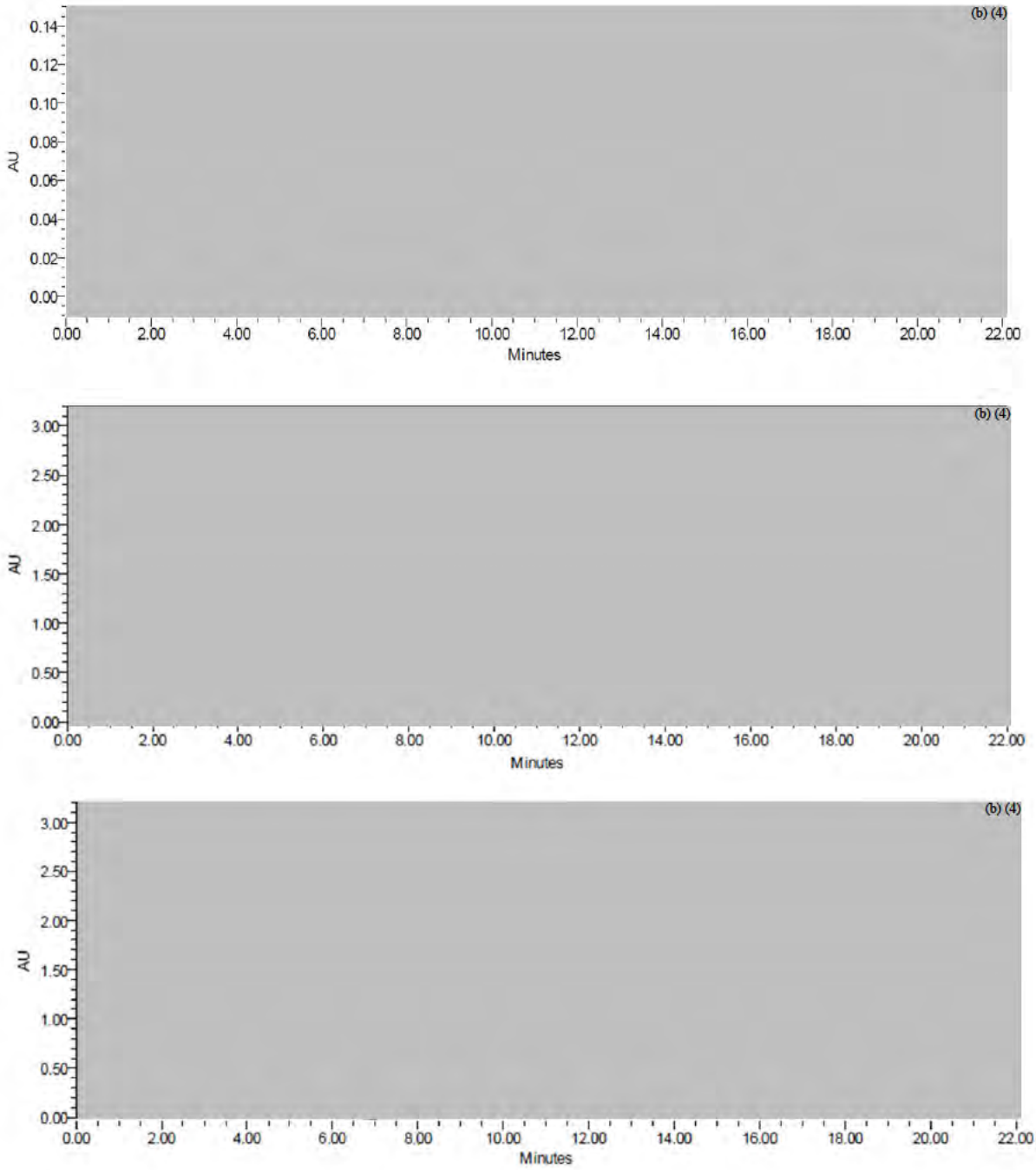


Figure 4. Chromatogram of Dried L-Valine Fermentation Product

To confirm there is no D-valine in Dried L-Valine Fermentation Product, we also compared the chromatogram with expansion version. It was also shown that D-valine is not included in Dried L-Valine Fermentation Product.

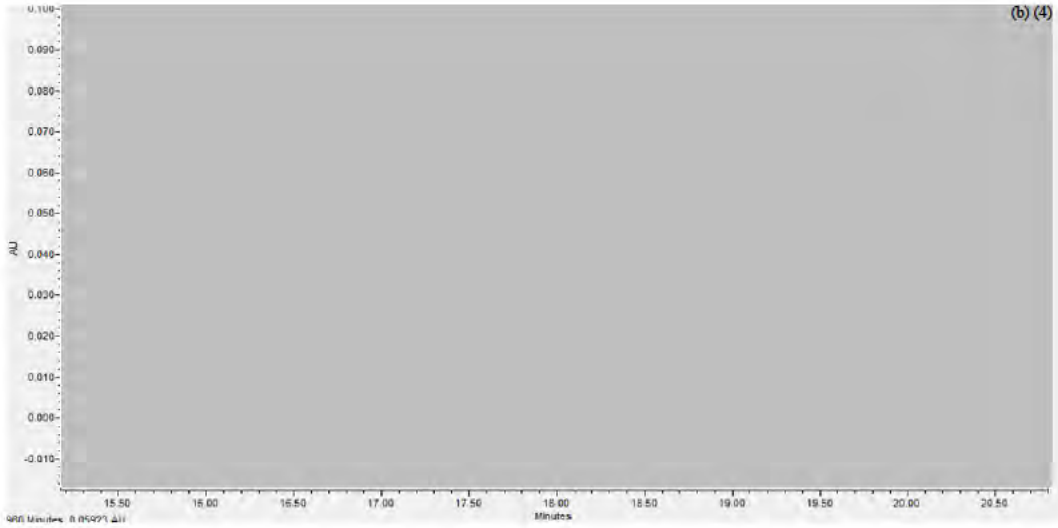


Figure 5. Chromatogram of Dried L-Valine Fermentation Product (expansion version)

Table 4. Content of D-valine in 'Dried L-Valine Fermentation Product'

Lot No	Concentration	unit
GVAL200910 NGVAL191221	(b) (4)	mg/kg

* N.D.: Not detected (LOD= (b) (4))

5. CONCLUSION

This study was conducted to evaluate the chiral purity of the Dried L-Valine Fermentation Product using HPLC. Linearity was checked in the range of 5-125.5 mg/L of D-valine. Using developed analytical method, limit of detection of test article formulation were evaluated. This method was applied to determine the content of D-valine in 'Dried L-Valine Fermentation Product' produced by CJ. Based on the results above, there was no D-valine in the 'Dried L-Valine Fermentation Product' that produced by CJ.

CONFIDENTIAL REPORT

Confirmation of production strain of Dried L-Valine Fermentation Product

Version 1.0

TITLE

Confirmation of production strain of Dried L-Valine Fermentation Product

OBJECTIVE OF THE STUDY

This study was conducted to confirm that the pilot scale batches of test sample used for the previous submission is produced by *C. glutamicum* KCCM 80240 which is used for the commercial batch of Dried L-Valine Fermentation Product.

SCHEDULE OF THE STUDY

Initiation of experiment: October 7, 2021

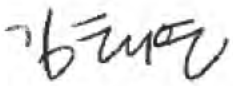
Termination of experiment: October 8, 2021

Submission of final report: October 12, 2021

TESTING FACILITY

R&BD)Regulatory Affairs, CJ CheilJedang
55, Gwanggyo-ro 42beon-gil, Yeongtong-gu, Suwon-si, Gyeonggi-do 16495, Korea

RESPONSIBLE STAFFS

Analyst and Author Taeyeon Kim 

Report approved by Yang Hee Kim 

CONTENTS

INTRODUCTION

Samples used for the previous submission of Dried L-Valine Fermentation Product were pilot scale batches of the final product. To confirm that the samples were produced by *Corynebacterium glutamicum* KCCM 80240 which will be used for the commercial production strain of Dried L-Valine Fermentation Product, PCR analysis was carried out.

Two different kinds of genetically modified region in *C. glutamicum* KCCM 80240 were selected to analyze: 1) Pcj7-*gap*N (*L.delbreuckii*) and 2) partially deleted *ilv*A gene region.

MATERIALS AND METHODS

Test sample

Eight batches of Dried L-Valine Fermentation Product were tested. The certificate of analysis of test samples are provided as Attachment 1 at the end of this test report.

- Batch No.:

  NGVAL191221, NGVAL191222, NGVAL191223 – used for stability (shelf-life) study

  GVAL200910, GVAL200911, GVAL200912, GVAL200916, GVAL200917 – used for compositional analysis, heavy metal analysis, viable cell analysis, and mash feed stability study

DNA extraction

A genomic DNA of *C. glutamicum* ATCC 14067, CA08-0012, KCCM 80240 and total DNA present in the samples were extracted by using (b) (4) as follows.

1) Prepare a sample according to the following methods:

(b) (4)

2) (b) (4)

3) (b) (4)

4) (b) (4)

5) (b) (4)

6) (b) (4)

7) (b) (4)

8) (b) (4)

9) (b) (4)

10) (b) (4)

11) (b) (4)

12) (b) (4)

13) (b) (4)

14) (b) (4)

15) (b) (4)

16) (b) (4)

PCR analysis

Primers were designed from the specific region of the production strain (Table 1).

Table 1. Primers used for this study

Target	Sequence (5'→3')	Size of PCR product (bp)	
		Before modification	After modification
*gap*N (*L. delbrueckii*)	(b) (4)		
Partially deleted *ilv*A gene			

(b) (4)

RESULTS

The amplification of target genes were observed by PCR analysis. All test sample showed specific amplification for foreign *gap*N gene (Table 2 and Figure 2) and partially deleted *ilv*A gene (Table 3 and Figure 3), which were genetically modified region of the production strain.

Based on this result, it is confirmed that the pilot batches of test samples were produced by *C. glutamicum* KCCM 80240 which is used for producing commercial batches of Dried L-Valine Fermentation Product.

Table 2. PCR analysis of Pcj7-gapN (*L. delbreuckii*) gene

		Result
Negative control	Distilled water	(b) (4)
	C. glutamicum ATCC 14067, wild-type strain	
	C. glutamicum CA08-0012, parental strain	
Positive control	*C. glutamicum* KCCM 80240	
Sample	Batch No. NGVAL191221	
	Batch No. NGVAL191222	
	Batch No. NGVAL191223	
	Batch No. GVAL200910	
	Batch No. GVAL 200911	
	Batch No. GVAL200912	
	Batch No. GVAL200916	
	Batch No. GVAL200917	

(-), no amplification; (+), specific amplification

(b) (4)

Table 3. PCR analysis of partially deleted *ilv*A gene

		Result
Negative control	Distilled water	(b) (4)
	C. glutamicum ATCC 14067, wild-type strain	
	C. glutamicum CA08-0012, parental strain	
Positive control	*C. glutamicum* KCCM 80240	
Sample	Batch No. NGVAL191221	
	Batch No. NGVAL191222	
	Batch No. NGVAL191223	
	Batch No. GVAL200910	
	Batch No. GVAL 200911	
	Batch No. GVAL200912	
	Batch No. GVAL200916	
	Batch No. GVAL200917	

(-), no amplification; (+), specific amplification

(b) (4)

[Attachment 1] Certificate of Analysis

CJ Research Institute of Biotechnology

55, Gwanggyo-ro 42beon-gil, Yeongtong-gu, Suwon-si, Gyeonggi-do, 16495, Korea

www.cj.co.kr

TEL : 031) 8099-2450 FAX : 031) 8099-2918

Certificate of analysis

Certificate No.	2020-PR-001	Receipt No.	2020-AN-001
Client	-	Date of Receipt	2020.02.12.
Client Name	-	Date of Test	2020.02.12.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (70%)
Manuf. Date	2019.12.21.
Lot. No	NGVAL191221
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 70 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (22~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

The Test Report cannot be reproduced, except in full.

(b) (4)

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Certificate of analysis

Certificate No.	2020-PR-002	Receipt No.	2020-AN-002
Client	-	Date of Receipt	2020.02.12.
Client Name	-	Date of Test	2020.02.12.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (70%)
Manuf. Date	2019.12.22.
Lot. No	NGVAL191222
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 70 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (22~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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Certificate of analysis

Certificate No.	2020-PR-003	Receipt No.	2020-AN-003
Client	-	Date of Receipt	2020.02.12.
Client Name	-	Date of Test	2020.02.12.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (70%)
Manuf. Date	2019.12.23.
Lot. No	NGVAL191223
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 70 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (22~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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Certificate of analysis

Certificate No.	2021-PR-124	Receipt No.	2021-AN-092
Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.11.22.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.10.
Lot. No	GVAL200910
Quantity (kg)	-

Test Item(s)	lot number	Test Result	Test method used
L-Valine(dry base)	Not less than 72 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01
Ash	Not more than 5 %		AOAC 942.05

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

(b) (4)

Oct, 07, 2021

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Certificate of analysis

Certificate No.	2021-PR-125	Receipt No.	2021-AN-093
Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.11.22.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.11.
Lot. No	GVAL200911
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 72 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01
Ash	Not more than 5 %		AOAC 942.05

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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Certificate of analysis

Certificate No.	2021-PR-126	Receipt No.	2021-AN-094
Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.11.22.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.12.
Lot. No	GVAL200912
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 72 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01
Ash	Not more than 5 %		AOAC 942.05

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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Certificate of analysis

Certificate No.	2021-PR-127	Receipt No.	2021-AN-095
Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.11.22.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.16.
Lot. No	GVAL200916
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 72 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01
Ash	Not more than 5 %		AOAC 942.05

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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TEL : 031) 8099-2450 FAX : 031) 8099-2918

Certificate of analysis

Certificate No.	2021-PR-128	Receipt No.	2021-AN-096
Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.11.22.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.17.
Lot. No	GVAL200917
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 72 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01
Ash	Not more than 5 %		AOAC 942.05

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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(b) (4)

Oct, 07, 2021

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REPORT

Analytical Method Validation of Dried L-Valine Fermentation Product using HPLC

(Confidential)

Original final report date: Dec 21, 2020

Study Director	Quality Assurance Manager
(b) (4)	
Dami Jeong	Seok-Hun Yun

CJ Research Institute of Biotechnology

Table of contents

1. Introduction

There are several official methods to analyze L-valine. The commonly used method of L-valine analysis is potentiometric titration with perchloric acid, however, most other amino acids could also be detected by this method. Therefore, titration method is not applicable in case of sample containing the other amino acids as an impurity.

For this reason, CJ developed the analytical method for 'Dried L-Valine Fermentation Product' and this analytical method was verified by method validation.

2. Test article

2.1. Test Article

1) Identity: Dried L-Valine Fermentation Product (VAL Pro)

2) Lot number: GVAL200910

3) Purity: > 72.0% (L-Valine, dry basis)

4) Date of receipt: November 30, 2020

5) Amount of receipt: approximately 100 g

6) Storage conditions: room temperature

7) Supplier: CJ Research Institute of Biotechnology

2.2. Reference standard

1) Identity: L-Valine

2) Product No.: V0500 (SLCD6123)

3) Purity: 100%

4) Quality release Date: October 04, 2019

5) Amount of receipt: 25 g

6) Storage conditions: room temperature

7) Supplier: (b) (4)

8) Expiry date (retest date): October, 2022

3. HPLC analytical condition

3.1. HPLC Condition

Table 1. HPLC Condition

	Condition
System	HPLC (SHIMADZU Nexera UPLC-30A)
Detector	Fluorescence detector (Excitation λ : 338nm Emission λ : 425nm)
Column	ODS C18, 150 × 4.6 mm, particle size 3 μm
Column temperature	40℃
Mobile phase	16.7 mM-KH_2PO_4 + 5 mM OSA in 12% CH_3CN, pH 2.5 (by H_3PO_4)
Flow rate of mobile phase	1.0 ml/min
Reaction reagent	201.91mM-KOH + 241.39mM-H_3BO_3 + 2.53mM-OPA + C_2H_6OS 1mL + CH_3OH 5mL + 3.5%-Brij 1.25mL
Flow rate of reaction reagent	0.5 ml/min
Sample temperature	15℃
Injection volume	5 μl
Concentration of sample and standard solution	0.1 g/L (L-valine concentration basis)

3.2. Preparation reagent for mobile phase and reaction reagent

Table 2. Preparation reagent for mobile phase and reaction reagent

Mobile phase			
	Purity	Manufacturer	Product No.
Acetonitrile(CH_3CN)	HPLC Grade	(b) (4)	(b) (4)
Potassium dihydrogen phosphate (KH_2PO_4)	≥99%	(b) (4)	(b) (4)
Phosphoric acid(H_3PO_4)	≥85%	(b) (4)	(b) (4)
1-Octanfonic acid sodium salt (OSA)	≥98%	(b) (4)	(b) (4)
Distilled water	minimum conductivity (18.2 MΩ)		

Reaction reagent			
	Purity	Manufacturer	Product No.
Potassium hydroxide	≥85%	(b) (4)	(b) (4)
Boric acid	≥99.5%	(b) (4)	(b) (4)
O-phthalaldehyde (OPA)	≥97%	(b) (4)	(b) (4)
2-Mercapto ethanol(2-ETSH)	≥99%	(b) (4)	(b) (4)
Methyl alcohol	≥98%	(b) (4)	(b) (4)
Distilled water	minimum conductivity (18.2 MΩ)		

3.3. Mobile phase solution preparation method

Table 3. Mobile phase solution preparation method

Reagent name	Concentration (mM)	Amount (g)	Total volume (mL)
Potassium dihydrogen phosphate (KH_2PO_4)	(b) (4)		1000
1-Octanfonic acid sodium salt (OSA)			
Acetonitrile (CH_3CN)			1137
Phosphoric Acid (H_3PO_4)			

(b) (4)

3.4. Reaction reagent preparation method

Table 4. Reaction reagent preparation method

Reagent name	Concentration (mM)	Amount (g)	Total volume (mL)
Potassium hydroxide	(b) (4)		
Boric acid			
O-phthalaldehyde (OPA)			1000
2-Mercaptoethanol (2-ETSH)			
Methyl alcohol			
3.5%-Brij solution			

(b) (4)

4. Standard preparation

(b) (4)

5. Sample preparation

(b) (4)

(b) (4)

6. Data processing and calculation

(b) (4)

Table 5. Data calculation

	Standard solution	Sample solution
Weight	(b) (4)	
Preparation concentration		
Area 1		
Area 2		
Area 3		
Area 4		
Average		
STDEV	(b) (4)	
%RSD*		
R.F. (Response factor		
Measurement concentration		
Result		

* If the area difference is RSD ≥ 1%, reanalyze and if the difference is still over 1%, instrument should be checked.

7. Specificity

(b) (4)

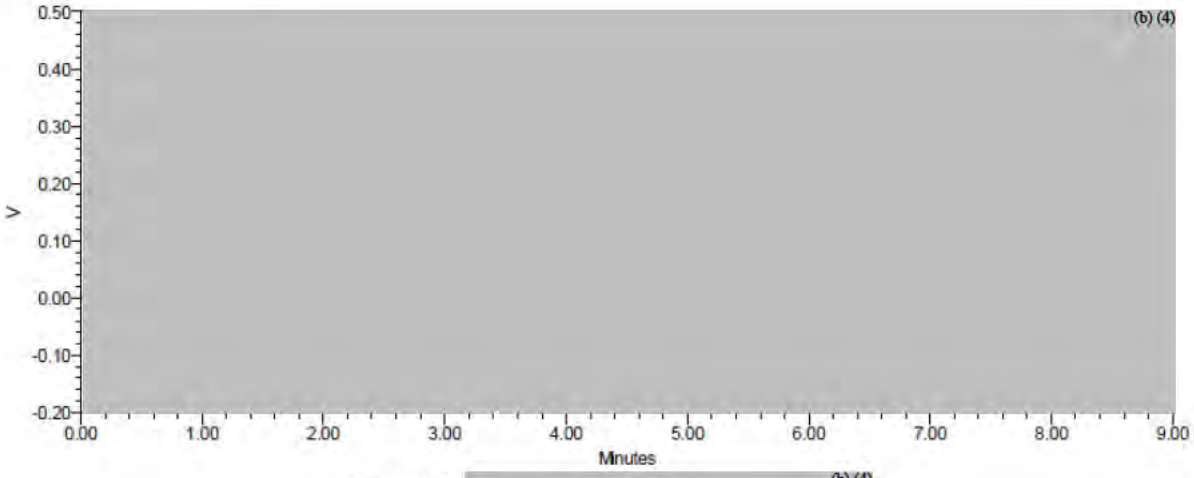


Figure 1. (b) (4)

8. System suitability

(b) (4)

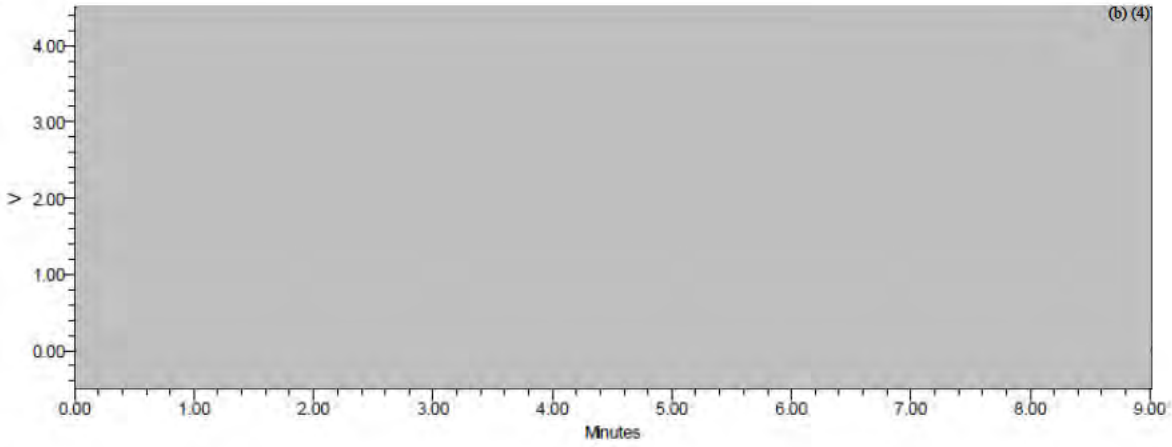
Table 6. Reference standard solution (0.025 g/L)

	Peak area (STD 1, 0.025 g/L)
1	(b) (4)
2	
3	
4	
5	
6	
7	
8	
9	
10	
%RSD	0.18%

Table 7. Reference standard solution (0.1 g/L)

	Peak area (STD 4, 0.100 g/L)
1	(b) (4)
2	
3	
4	
5	
6	
7	
8	
9	
10	
%RSD	0.16%

(a)



(b)

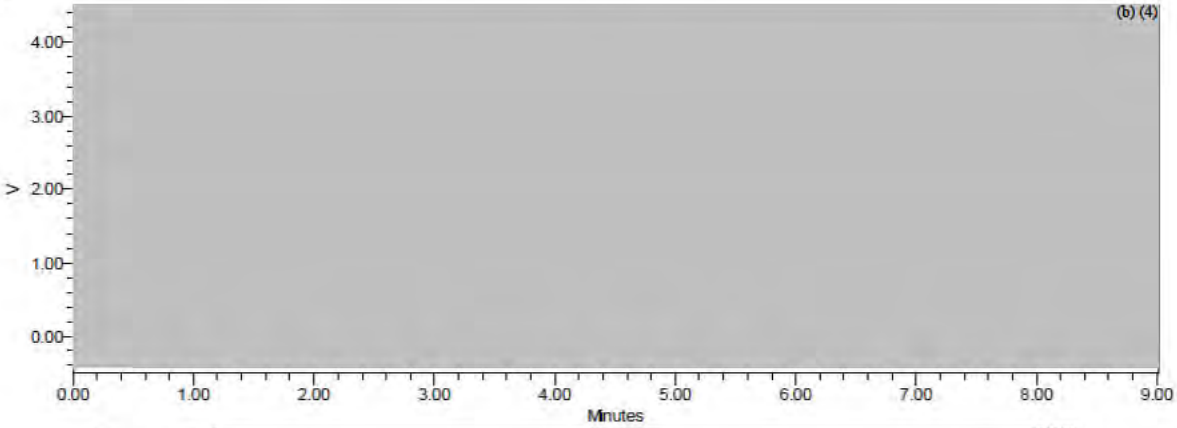


Figure 2. (b) (4)

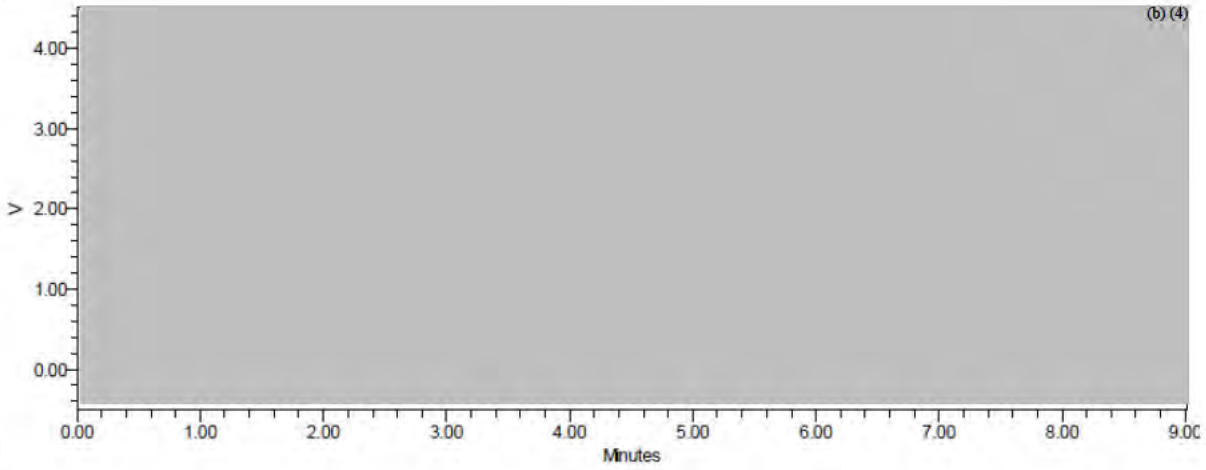
9. Homogeneity

(b) (4)

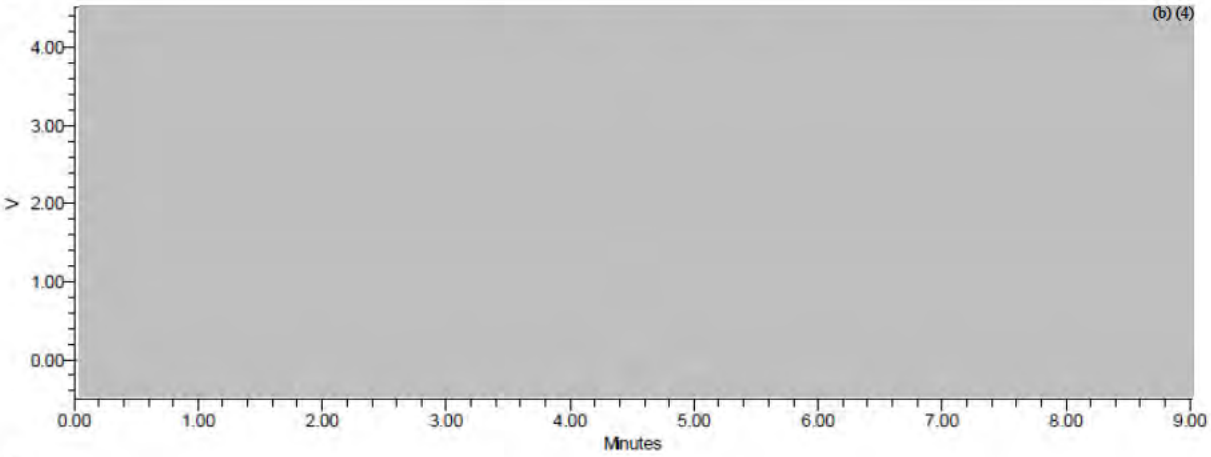
Table 8. Homogeneity of sample

Sample	Sample weight (g)	L-Valine (%)
Sampling 1	0.13775 g/1000mL	(b) (4)
Sampling 2	0.13705 g/1000mL	
Sampling 3	0.13787 g/1000mL	
Sampling 4	0.13761 g/1000mL	
Sampling 5	0.13678 g/1000mL	
Average	-	72.19
%RSD	-	0.28

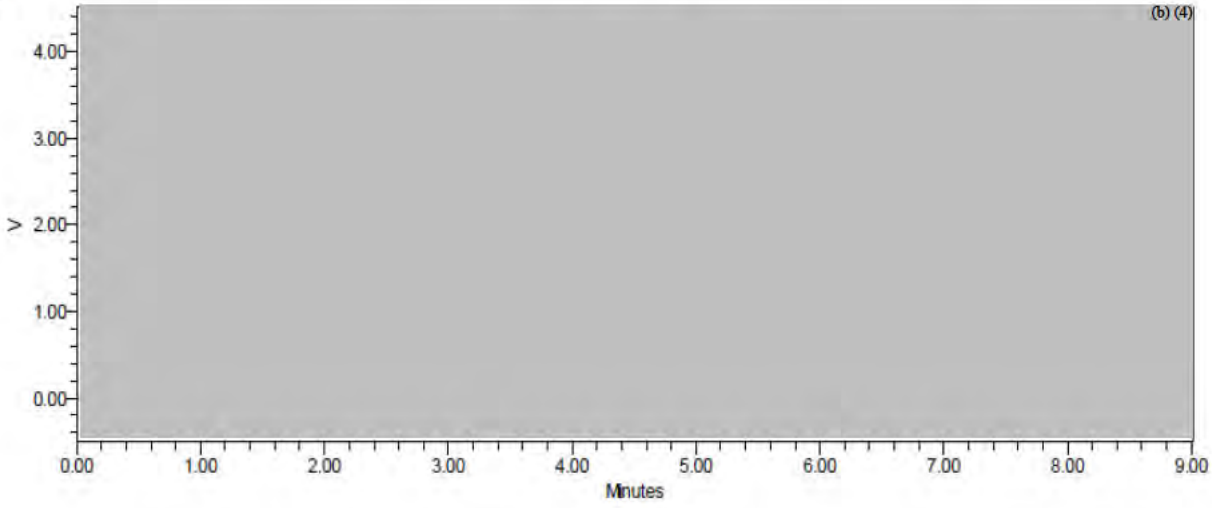
(a)


(b) (4)
4.00
3.00
V 2.00
1.00
0.00
0.00 1.00 2.00 3.00 4.00 5.00 6.00 7.00 8.00 9.00
Minutes

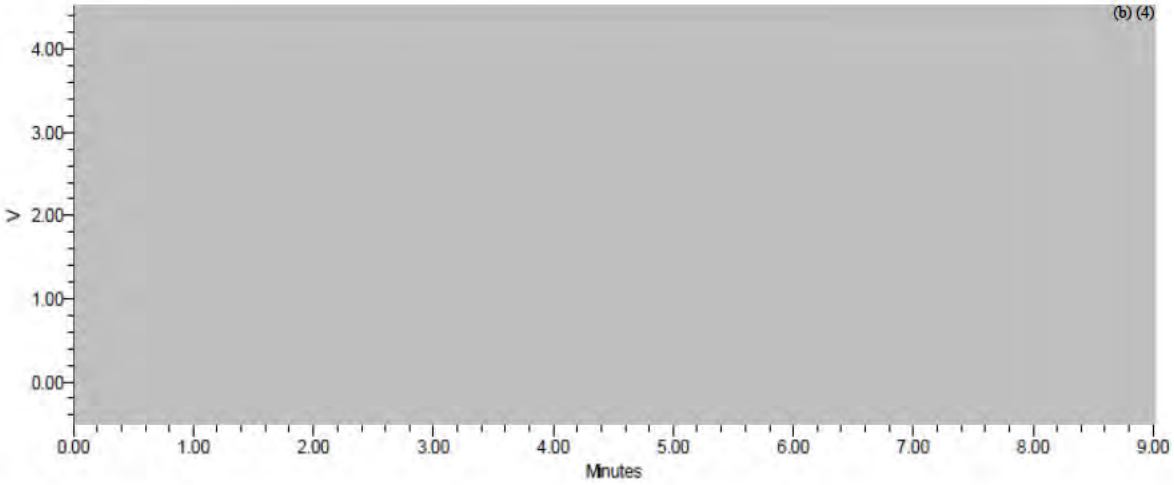
(b)


(b) (4)
4.00
3.00
V 2.00
1.00
0.00
0.00 1.00 2.00 3.00 4.00 5.00 6.00 7.00 8.00 9.00
Minutes

(c)


(b) (4)
4.00
3.00
V 2.00
1.00
0.00
0.00 1.00 2.00 3.00 4.00 5.00 6.00 7.00 8.00 9.00
Minutes

(d)



(e)

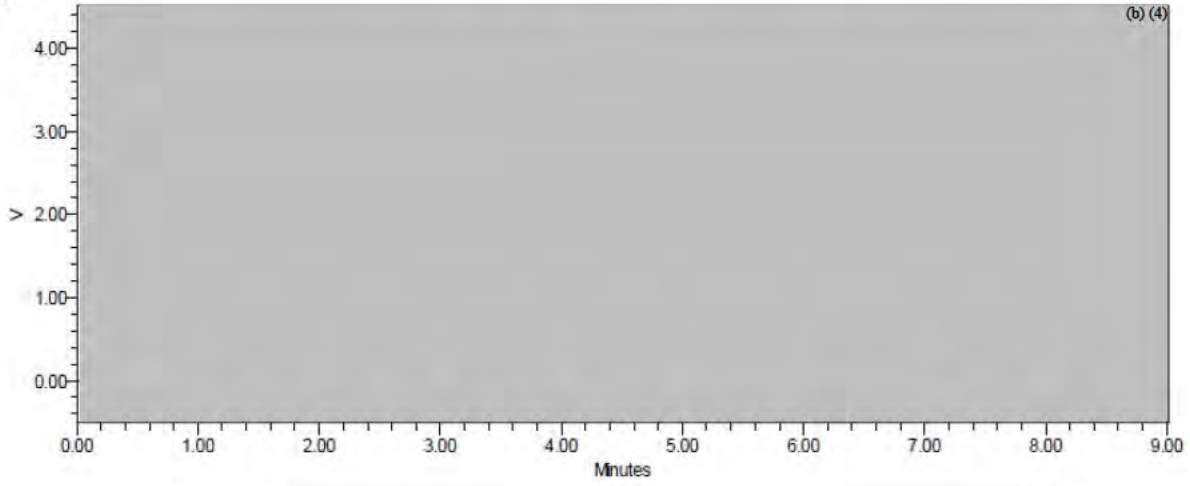


Figure 3. (b) (4)

10. Stability

(b) (4)

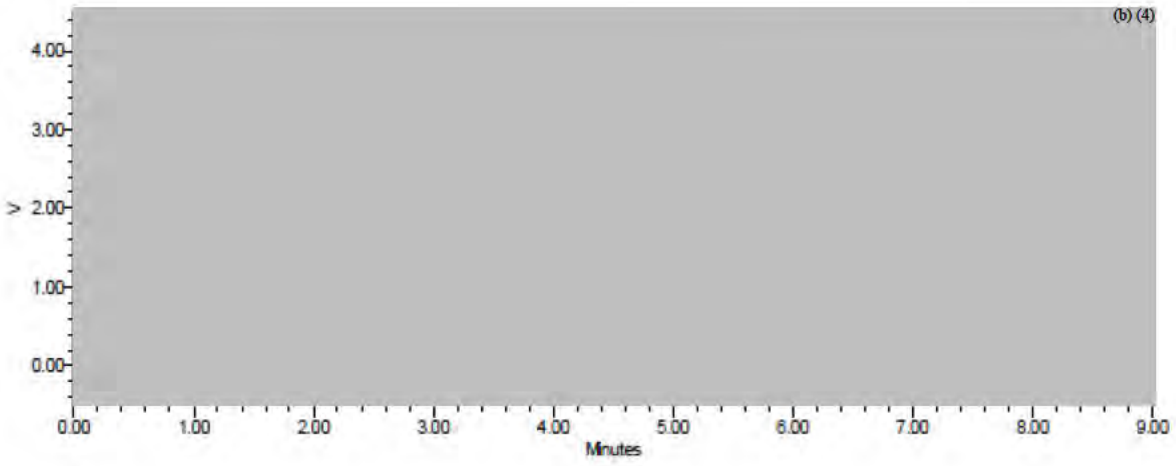
And %RSD was 0.17%.

The recovery of sample was satisfied with the acceptance criteria of 98%-102% and %RSD criteria of < 1%.

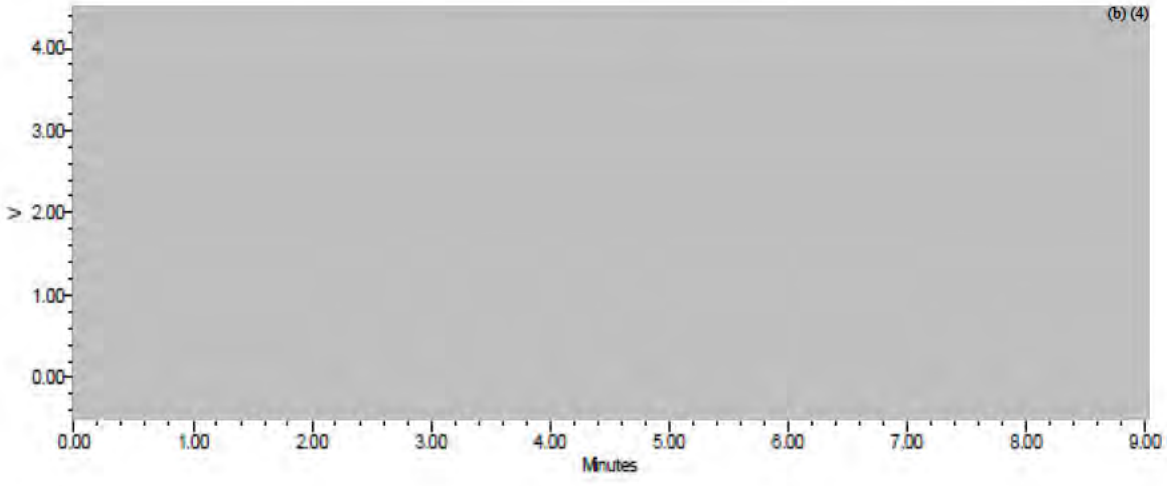
Table 9. Stability of the sample (investigation of precision of sample)

Time (day)	Time (h)	L-Valine (%)	Recovery (%)
day 1_1	0	(b) (4)	
day 1_2	5		
day 1_3	10		
day 2_1	23		
day 2_2	28		
day 2_3	32		
day 3_1	53		
day 3_2	57		
day 3_3	62		
%RSD		0.17%	-

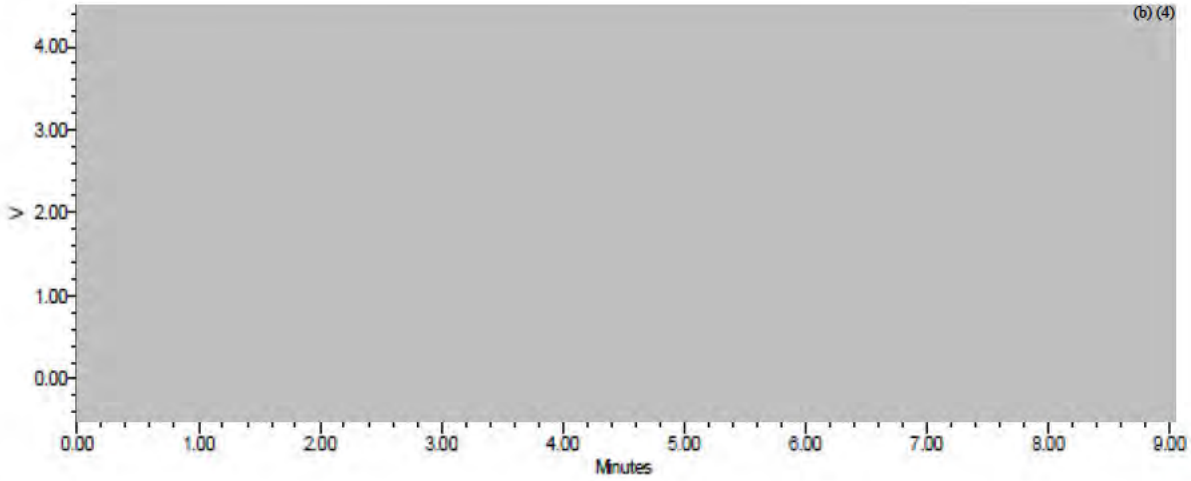
(a)



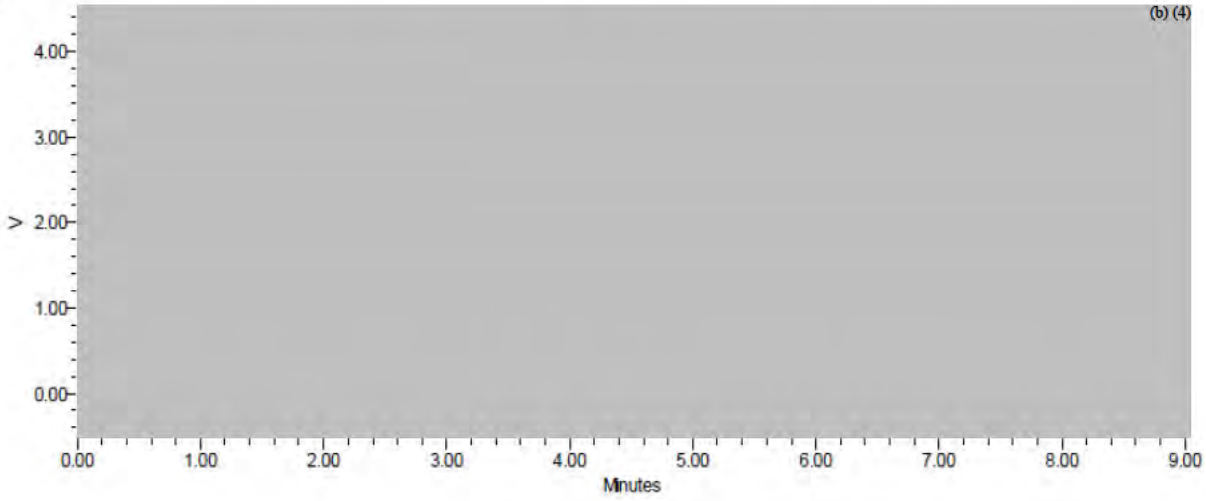
(b)



(c)


(b) (4)
4.00
3.00
V 2.00
1.00
0.00
0.00 1.00 2.00 3.00 4.00 5.00 6.00 7.00 8.00 9.00
Minutes

(d)


(b) (4)
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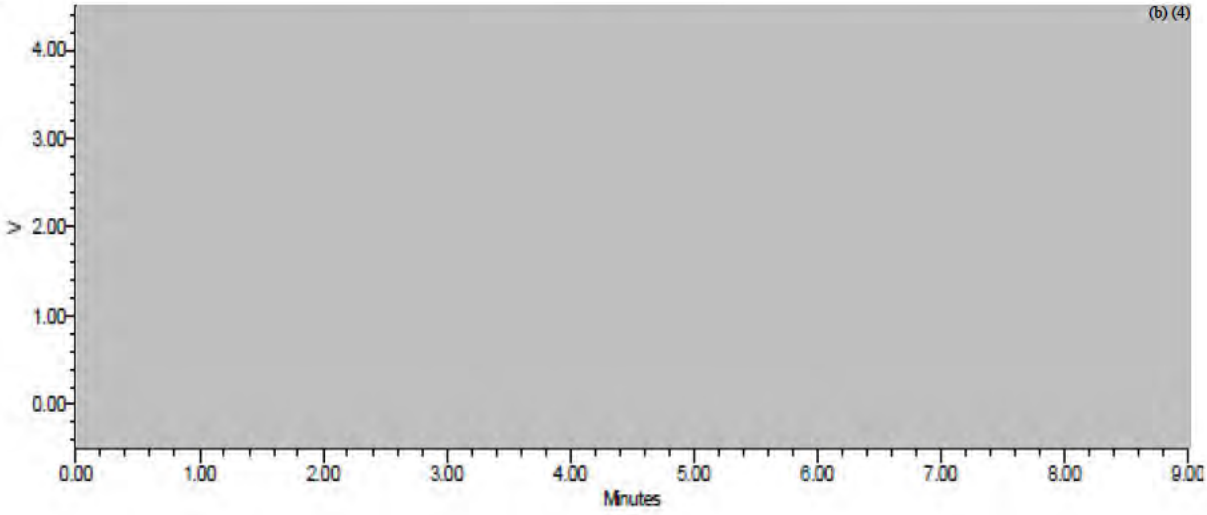
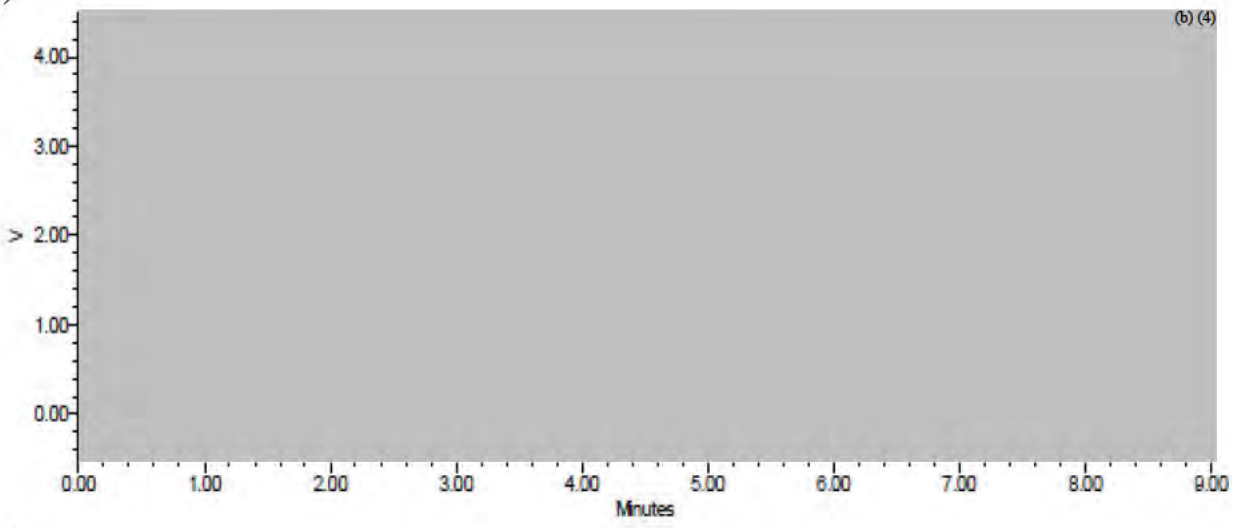
3.00
V 2.00
1.00
0.00
0.00 1.00 2.00 3.00 4.00 5.00 6.00 7.00 8.00 9.00
Minutes

(e)


(b) (4)
4.00
3.00
V 2.00
1.00
0.00
0.00 1.00 2.00 3.00 4.00 5.00 6.00 7.00 8.00 9.00
Minutes

(f)


(b) (4)
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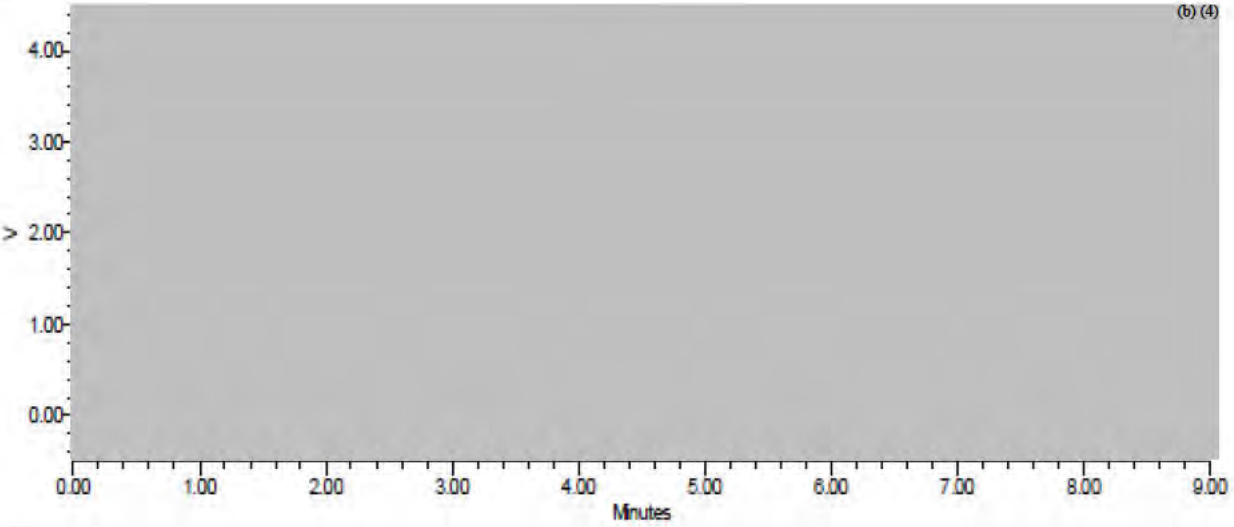
3.00
> 2.00
1.00
0.00
0.00 1.00 2.00 3.00 4.00 5.00 6.00 7.00 8.00 9.00
Minutes

(g)


(b) (4)
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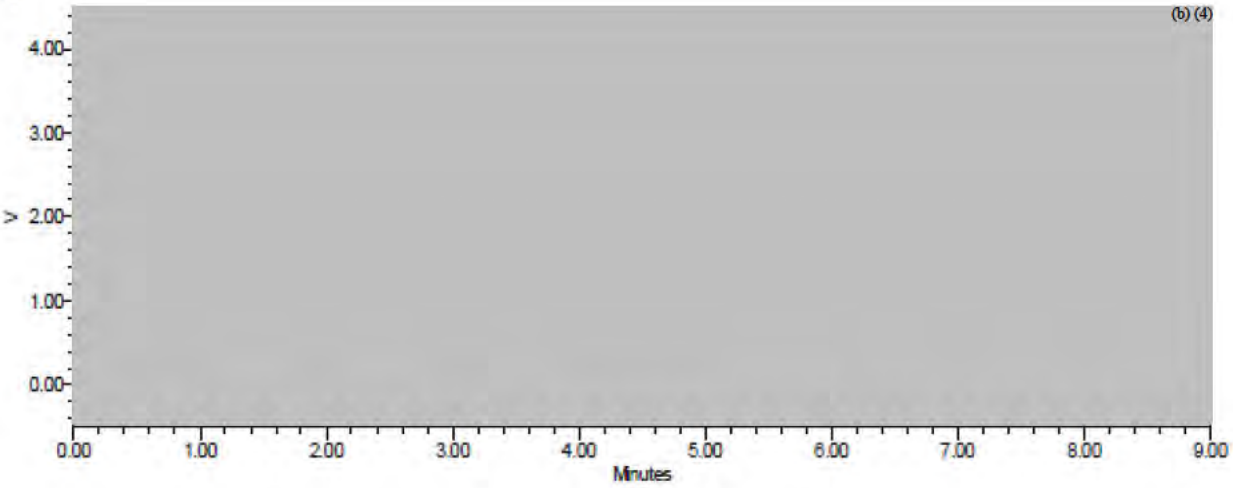
3.00
> 2.00
1.00
0.00
0.00 1.00 2.00 3.00 4.00 5.00 6.00 7.00 8.00 9.00
Minutes

(h)


(b) (4)
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3.00
> 2.00
1.00
0.00
0.00 1.00 2.00 3.00 4.00 5.00 6.00 7.00 8.00 9.00
Minutes

(i)

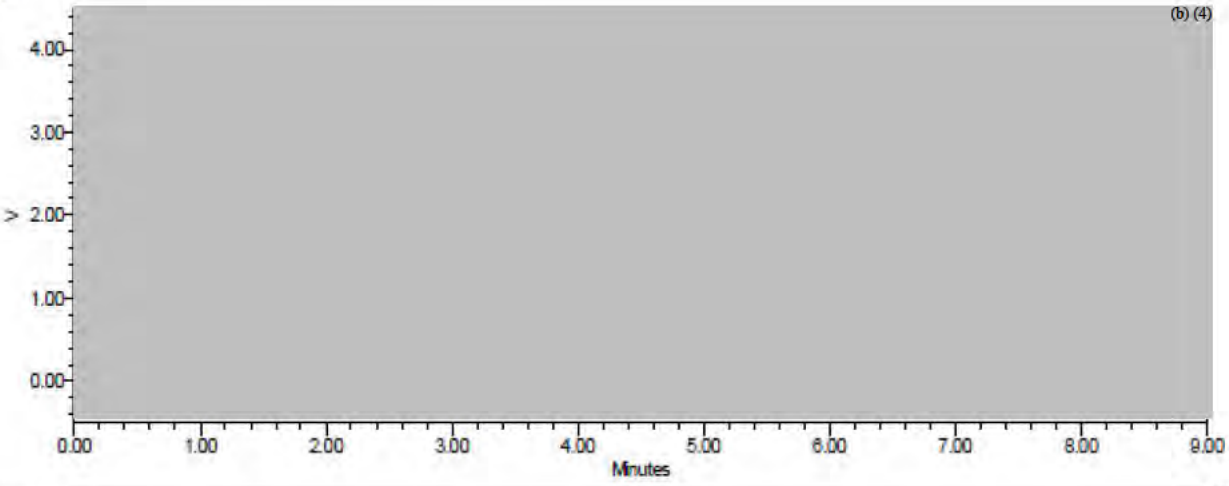


Figure 4. (b) (4)

11. Linearity

(b) (4)

Table 10. Calibration curve

	L-Valine (g/L)	Peak area*
STD 1 (25%)	(b) (4)	
STD 2 (50%)		
STD 3 (80%)		
STD 4 (100%)		
STD 5 (120%)		

* Mean area of triplet injection

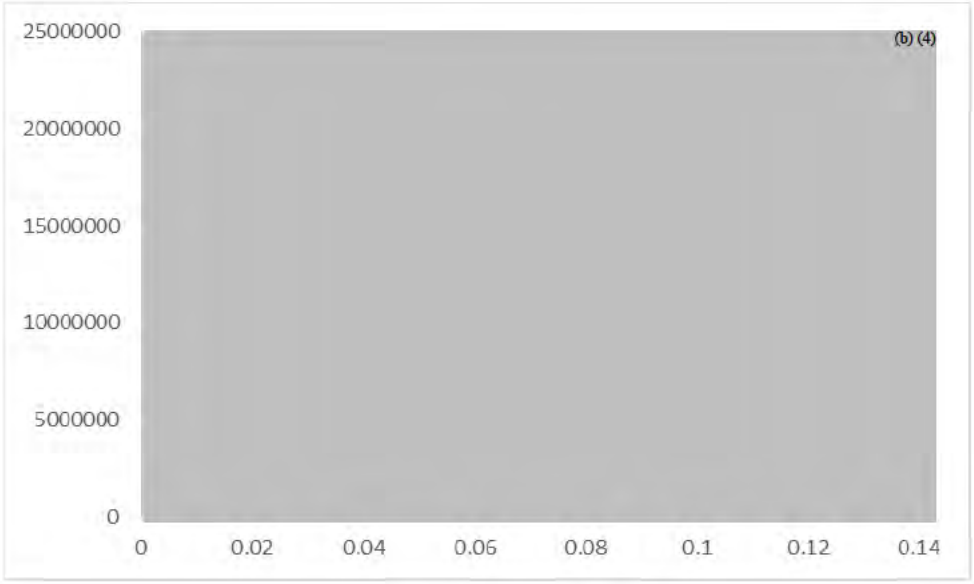
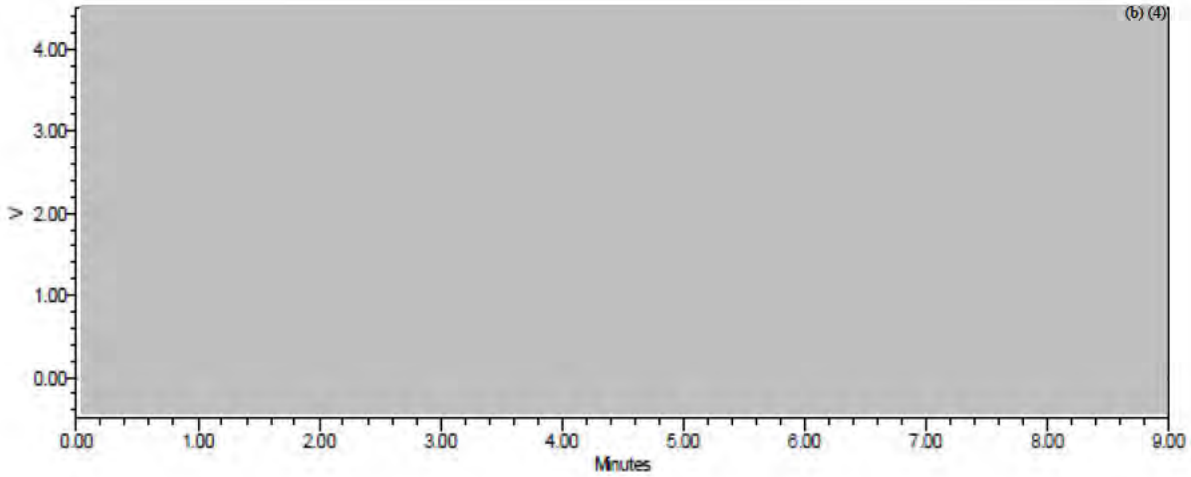
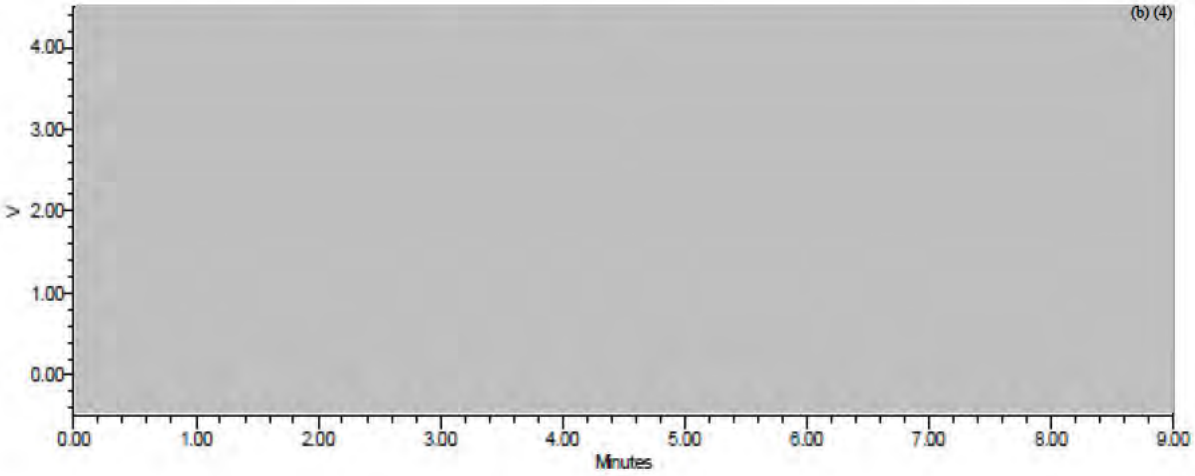


Figure 5. Calibration curve

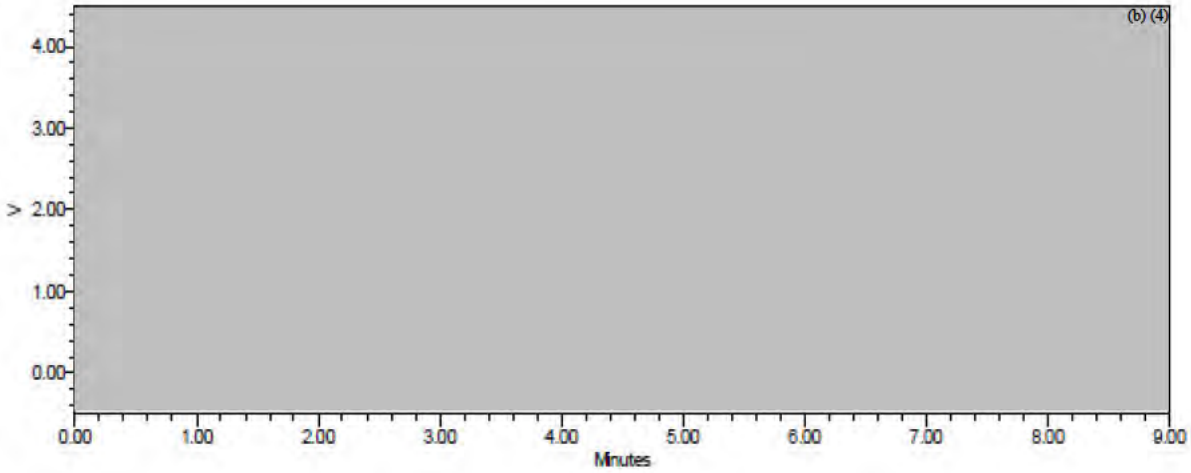
(a)



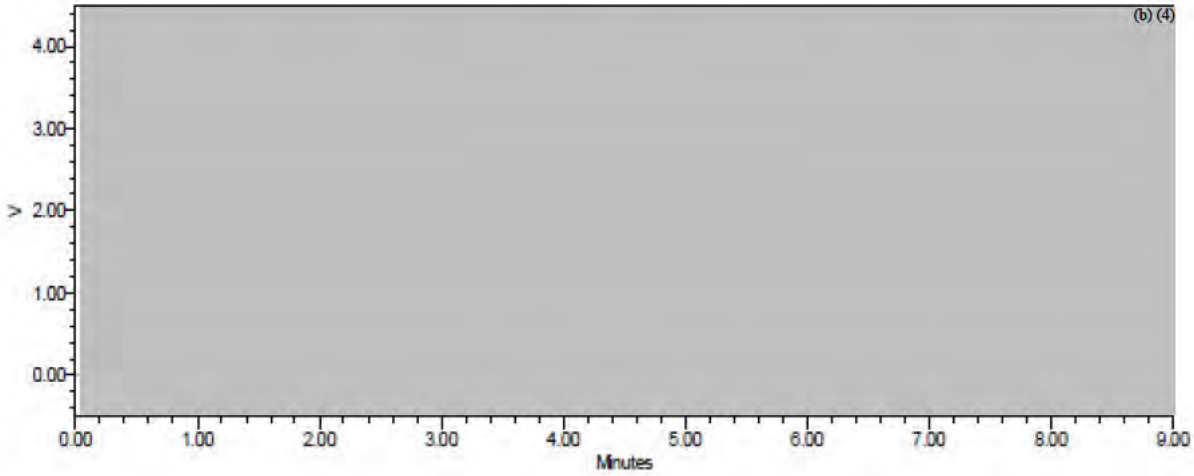
(b)



(c)


(b) (4)
4.00
3.00
> 2.00
1.00
0.00
0.00 1.00 2.00 3.00 4.00 5.00 6.00 7.00 8.00 9.00
Minutes

(d)


(b) (4)
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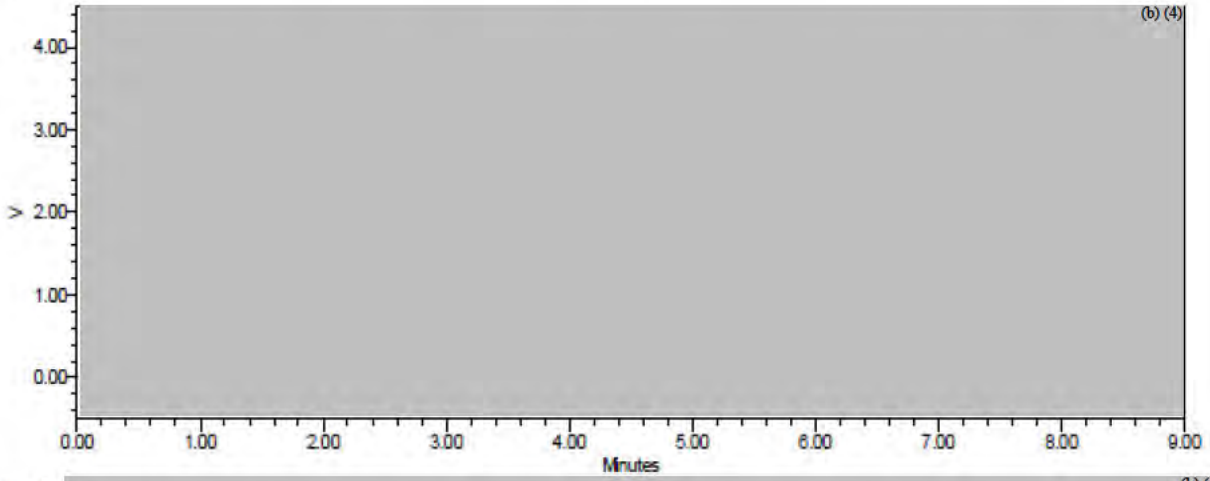
3.00
> 2.00
1.00
0.00
0.00 1.00 2.00 3.00 4.00 5.00 6.00 7.00 8.00 9.00
Minutes

(e)


(b) (4)
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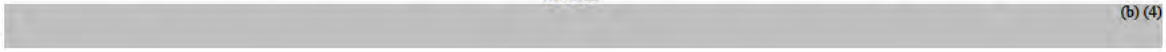
3.00
> 2.00
1.00
0.00
0.00 1.00 2.00 3.00 4.00 5.00 6.00 7.00 8.00 9.00
Minutes

Figure 6.


(b) (4)

12. Limit of detection and limit of quantification

(b) (4)

(b) (4)

(b) (4)

12.1. LOD and LOQ of L-valine

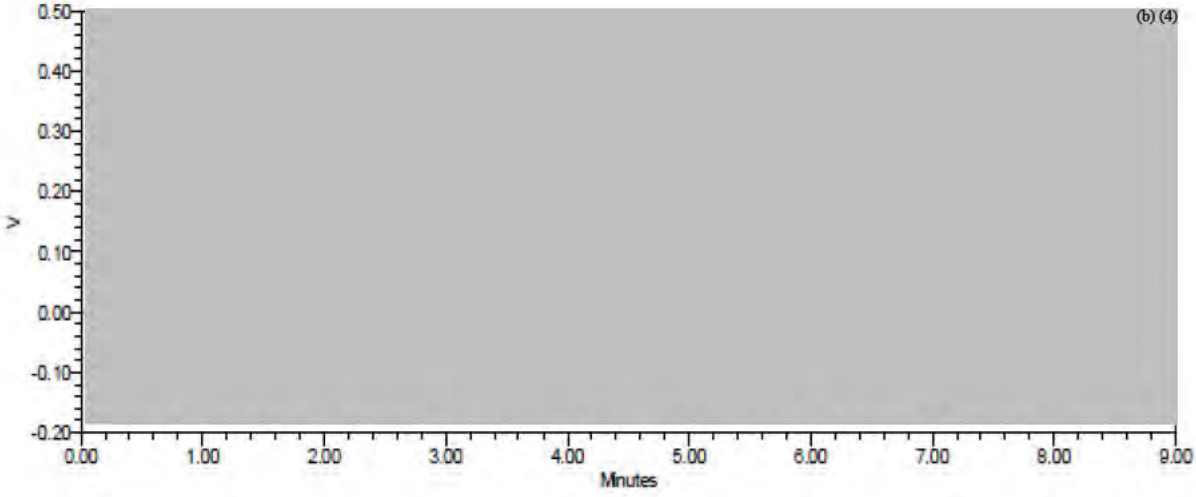
Table 11. .Summary output for regression analysis study

Regression statistics	
Multiple R (Correlation coefficient)	(b) (4)
R Square (Coefficient of determination)	(b) (4)

Adjusted R Square	(b) (4)
Standard Error (Residual standard deviation)	(b) (4)
Observations	(b) (4)

(b) (4)

(a)



(b)

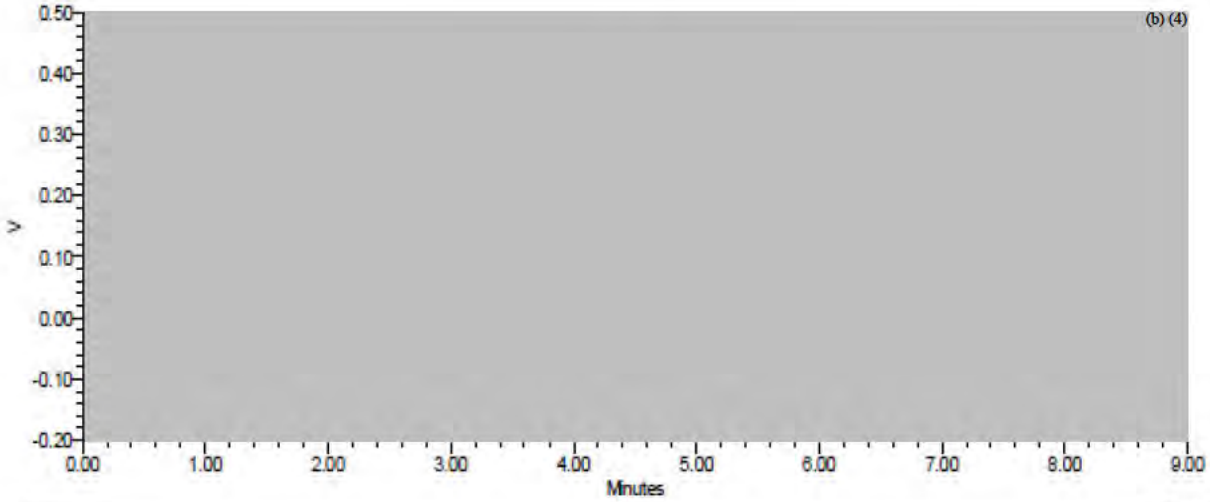


Figure 7. (b) (4)

13. Precision

(b) (4)

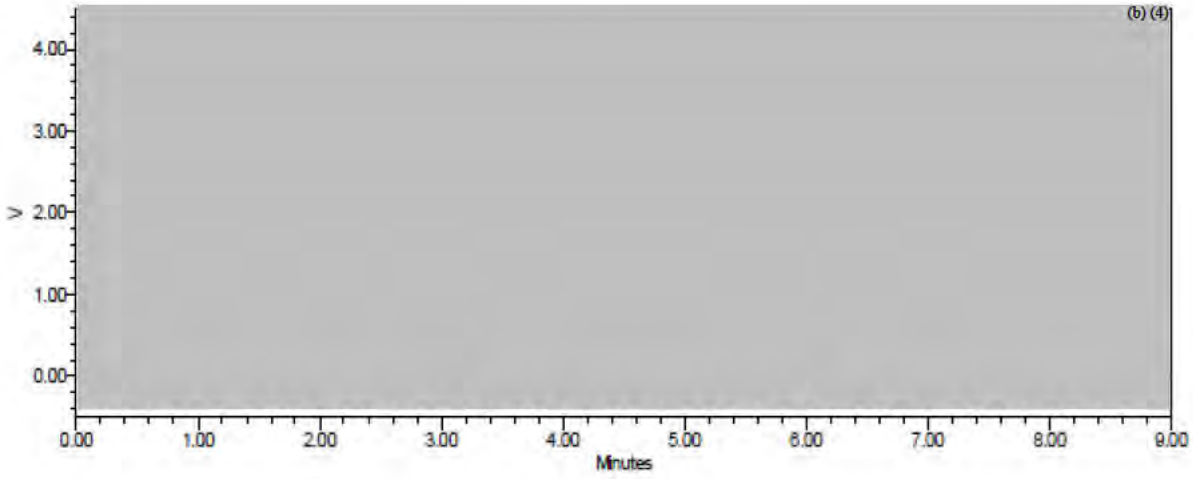
Table 12. Repeated injection of sample solution

	Sample solution
1	(b) (4)
2	
3	
4	
5	
6	
7	
8	
9	
10	
%RSD	0.17 %

Table 13. Repeated injection of CRM solution

	CRM solution
1	(b) (4)
2	
3	
4	
5	
6	
7	
8	
9	
10	
%RSD	0.12 %

(a)



(b)

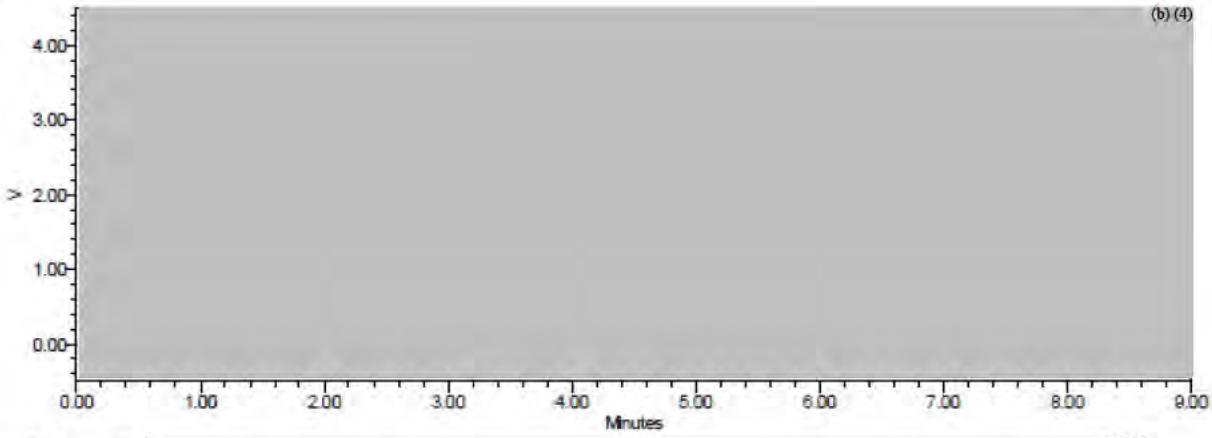


Figure 8. (b) (4)

14. Accuracy

(b) (4)

(b) (4)

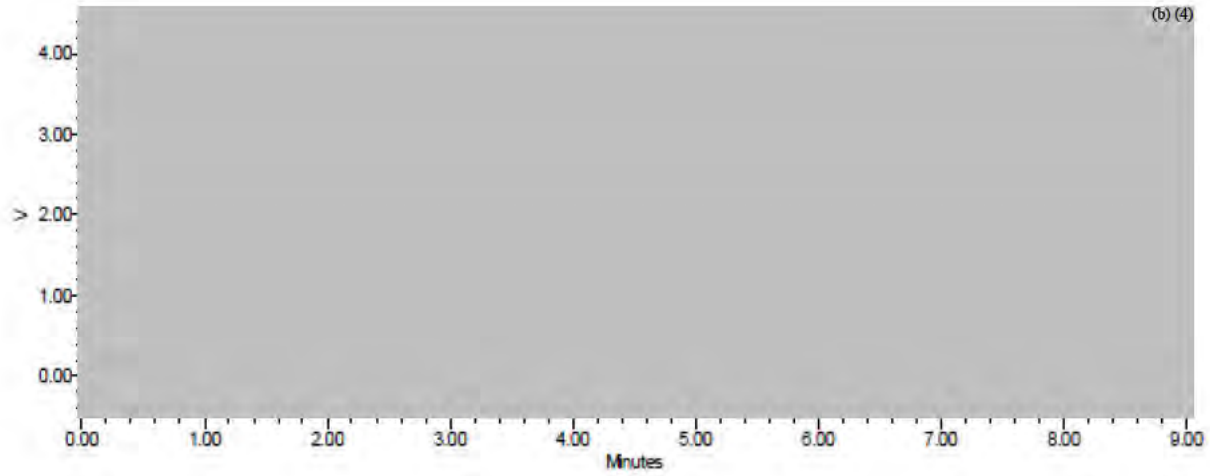
(b) (4)

(b) (4)

(b) (4)

(b) (4)

(a)


(b) (4)
4.00
3.00
> 2.00
1.00
0.00
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
9.00
Minutes

(b)

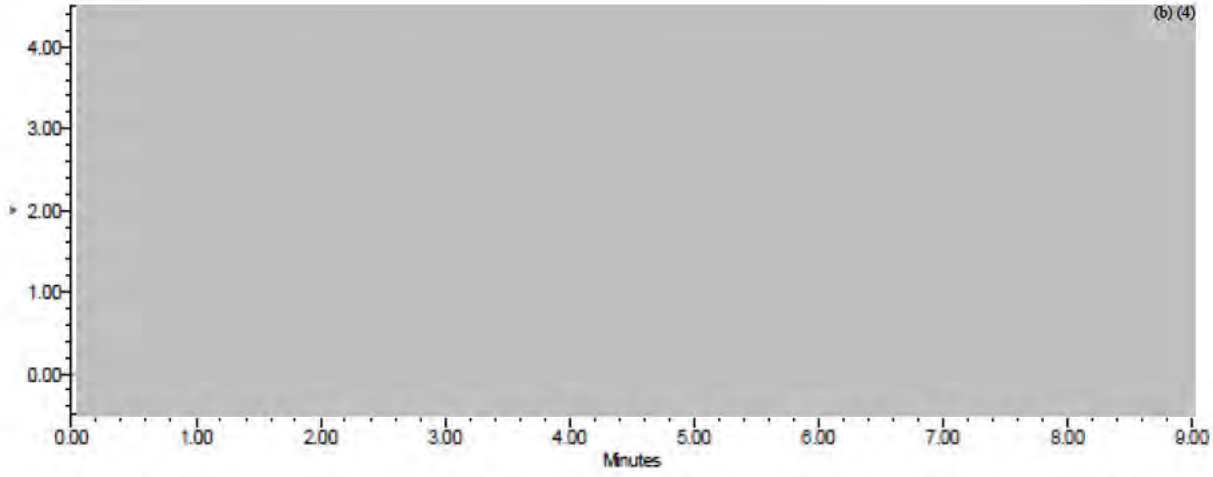


Figure 9. (b) (4)

14.1. Summary of uncertainty measurement

Mathematical model

(b) (4)

Table 14. Uncertainty measurement

Uncertainty contributor	Measurement value	Standard uncertainty	Relative standard uncertainty	Effective degree of freedom	Type	Probability distribution
14.2.1. Standard preparation	(b) (4)					
14.2.1.1. Uncertainty in weight determination						
1) Dispersion in repeated measurements						
2) Uncertainty of Balance calibration result						
14.2.1.2. Volumetric measuring						

1) Dispersion in repeated measurements	(b) (4)
2) Uncertainty of balance calibration result	
3) Uncertainty of 1000 mL volumetric flask calibration result	
14.2.2. Sample preparation	
14.2.2.1. Uncertainty in sample weight determination	
1) Dispersion in repeated measurements	
2) Uncertainty of balance calibration result	
14.2.2.2. Volumetric measuring	
1) Dispersion in repeated measurements	
2) Uncertainty of balance calibration result	
3) Uncertainty of 250 mL volumetric flask calibration result	
14.2.3. Precision of the instrument	
14.2.3.1. Uncertainty of dispersion in the standard solution repeated measurement	
14.2.3.2. Uncertainty of dispersion in the sample solution repeated measurement	

Relative combined standard uncertainty	(b) (4)
Effective degree of freedom	
Coverage factor *k*	
Expanded uncertainty	
Results	

14.2. Uncertainty measurement

14.2.1. Standard preparation

14.2.1.1. Uncertainty in weight determination

1) Dispersion in repeated measurements

Type A uncertainty

Number of sample measurements	Measurement value (g)
1	(b) (4)
2	
3	
4	
5	
Measurement value	
standard deviation	
standard uncertainty	
relative standard uncertainty	
degree of freedom	

- Standard uncertainty = (b) (4)

- Relative standard uncertainty = (b) (4)

- A Type degree of freedom = (b) (4)

2) Uncertainty of balance calibration result

Type B uncertainty

Balance	Mass (g)	Uncertainty	Standard uncertainty	Relative standard uncertainty
	0.10004	0.0005	(b) (4)	
Standard uncertainty			(b) (4)	
Relative standard uncertainty				
Degree of freedom				

(b) (4)

3) Relative combined standard uncertainty

(b) (4)

4) Effective degree of freedom

(b) (4)

14.2.1.2. Volumetric measuring

1) Dispersion in repeated measurements

Type A uncertainty

Number of sample measurements	Measurement value (g)
1	(b) (4)
2	(b) (4)

3	(b) (4)
4	(b) (4)
5	(b) (4)
Measurement value	(b) (4)
Standard deviation	(b) (4)
Standard uncertainty	(b) (4)
Relative standard uncertainty	(b) (4)
Degree of freedom	(b) (4)

(b) (4)

2) Uncertainty of balance calibration result

Type B uncertainty

Balance	Mass (g)	Uncertainty	Standard uncertainty	Relative standard uncertainty
	1246.33	0.0200	(b) (4)	(b) (4)
Standard uncertainty			(b) (4)	
Relative standard uncertainty				
Degree of freedom				

(b) (4)

3) Uncertainty of 1000 mL volumetric flask calibration result

Type B uncertainty

Volumetric flask	Volume (mL)	Uncertainty	Standard uncertainty	Relative standard uncertainty
	1000	0.220	(b) (4)	(b) (4)
Standard uncertainty			(b) (4)	
Relative standard uncertainty				
Degree of freedom				

(b) (4)

4) Relative combined standard uncertainty

(b) (4)

5) Effective degree of freedom

(b) (4)

14.2.1.4. Effective degree of freedom of standard preparation

(b) (4)

14.2.2. Sample preparation

14.2.2.1. Uncertainty in sample weight determination

1) Dispersion in repeated measurements

Type A uncertainty

Number of sample measurements	Measurement value (g)
1	(b) (4)
2	(b) (4)
3	(b) (4)
4	(b) (4)
5	(b) (4)
Measurement value	(b) (4)
Standard deviation	(b) (4)
Standard uncertainty	(b) (4)
Relative standard uncertainty	(b) (4)
Degree of freedom	(b) (4)

(b) (4)

2) Uncertainty of balance calibration result

Type B uncertainty

Balance	Mass (g)	Uncertainty	Standard uncertainty	Relative standard uncertainty
	0.10052	0.0005	(b) (4)	(b) (4)

Standard uncertainty	(b) (4)
Relative standard uncertainty	
Degree of freedom	

(b) (4)

3) Relative combined standard uncertainty

(b) (4)

4) Effective degree of freedom

(b) (4)

14.2.2.2. Volumetric measuring

1) Dispersion in repeated measurements

Type A uncertainty

Number of sample measurements	Measurement value (g)
1	(b) (4)
2	(b) (4)
3	(b) (4)
4	(b) (4)
5	(b) (4)
Measurement value	(b) (4)
Standard deviation	(b) (4)

Standard uncertainty	(b) (4)
Relative standard uncertainty	(b) (4)
Degree of freedom	(b) (

(b) (4)

2) Uncertainty of balance calibration result

Type B uncertainty

Balance	Mass (g)	Uncertainty	Standard uncertainty	Relative standard uncertainty
	1246.33	0.0200	(b) (4)	(b) (4)
Standard uncertainty			(b) (4)	
Relative standard uncertainty				
Degree of freedom				

(b) (4)

3) Uncertainty of 1000 mL volumetric flask calibration result

Type B uncertainty

Volumetric flask	Volume (mL)	Uncertainty	Standard uncertainty	Relative standard uncertainty
	1000.00	0.220	0.110	0.011
Standard uncertainty			(b) (4)	
Relative standard uncertainty			(b) (4)	

Degree of freedom	(b) (4)

(b) (4)

4) Relative combined standard uncertainty

(b) (4)

5) Effective degree of freedom

(b) (4)

14.2.2.4. Effective degree of freedom of sample preparation

(b) (4)

14.2.3. Precision of the instrument

14.2.3.1. Uncertainty of dispersion in the standard solution repeated measurement

Type A uncertainty

Number of sample measurements	Peak area

1	(b) (4)
2	(b) (4)
3	(b) (4)
4	(b) (4)
Measurement value	(b) (4)
Standard deviation	(b) (4)
Standard uncertainty	(b) (4)
Relative standard uncertainty	(b) (4)
Degree of freedom	(b) (4)

(b) (4)

14.2.3.2. Uncertainty of dispersion in the sample solution repeated measurement

Type A uncertainty

Number of sample measurements	Peak area
1	(b) (4)
2	(b) (4)
3	(b) (4)
Measurement value	(b) (4)
Standard deviation	(b) (4)
Standard uncertainty	(b) (4)
Relative standard uncertainty	(b) (4)
Degree of freedom	(b) (4)

(b) (4)

14.2.3.3. Relative combined standard uncertainty of precision of the instrument

(b) (4)

14.2.3.4. Effective degree of freedom of precision of the instrument

(b) (4)

14.2.4. Relative combined standard uncertainty of valine analysis

(b) (4)

14.2.5. Effective degree of freedom of valine analysis

(b) (4)

14.2.6. Expanded uncertainty (U)

(b) (4)

14.2.7. Result

(b) (4)

15. Robustness

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

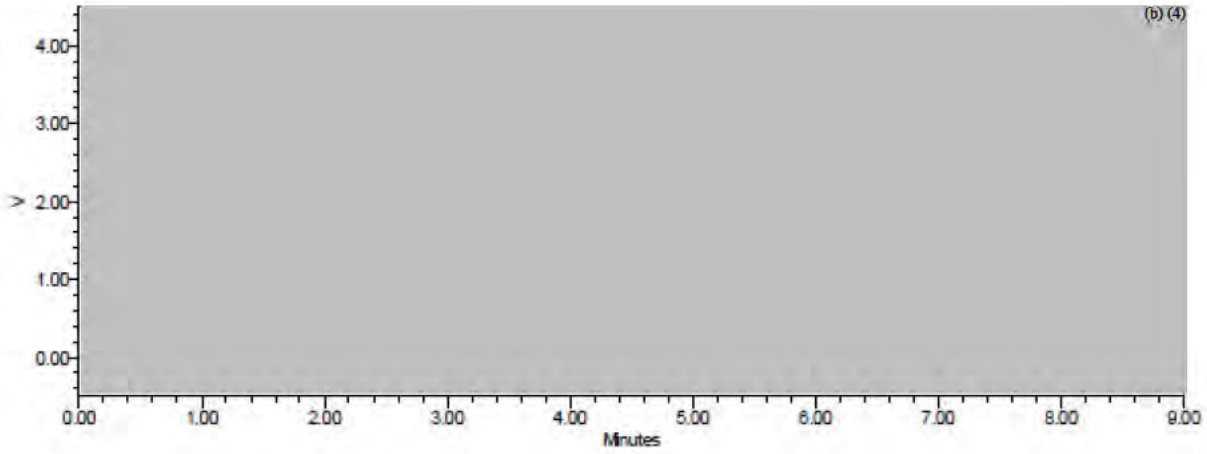
(b) (4)

(b) (4)

Table 15. Data of robustness test

Factor	L-valine (%)	Recovery (%)	Retention time of L-valine	Average peak area of standard	Average peak area of sample
Standard condition	(b) (4)			15915610	15782377
35°C				12291928	12330444
45°C				18942060	18912625
0.8 mL/min				26135362	26103728
1.2 mL/min				8381739	8388608
CH_3CN 9%				14742519	14717692
CH_3CN 15%				15232148	15206820
pH 2.3				4614004	4589815
pH 2.7				19682457	19674538

(a)



(b)

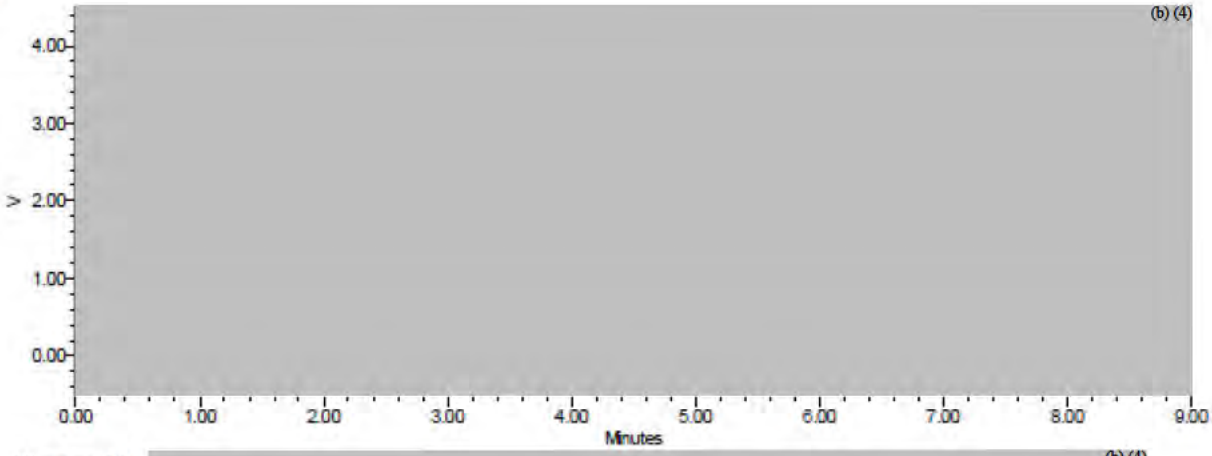
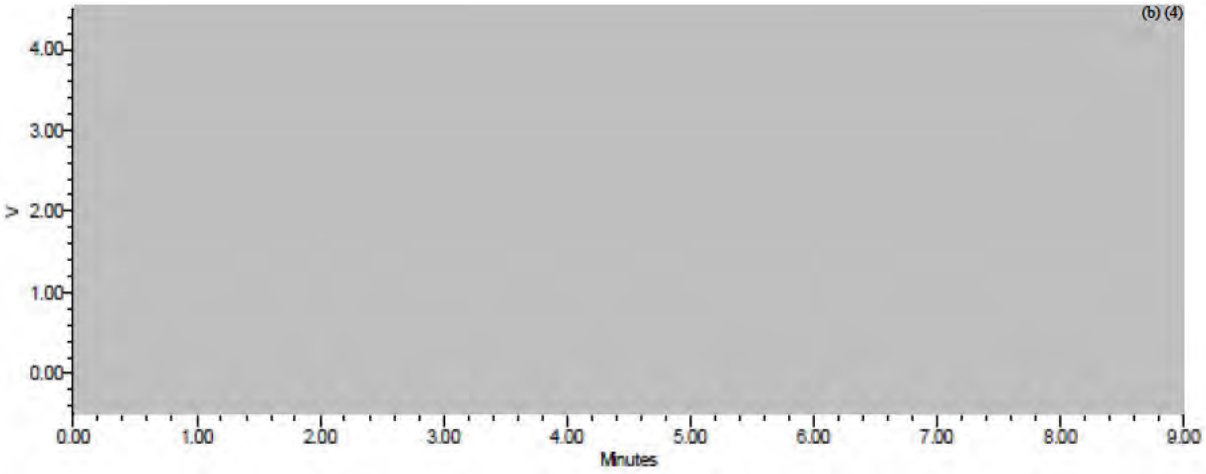


Figure 10.

(a)



(b)

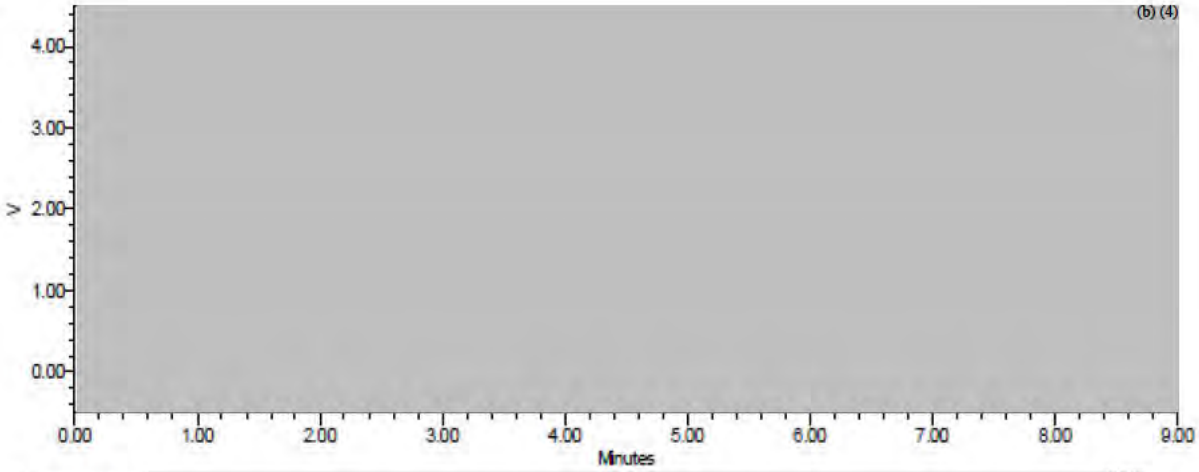
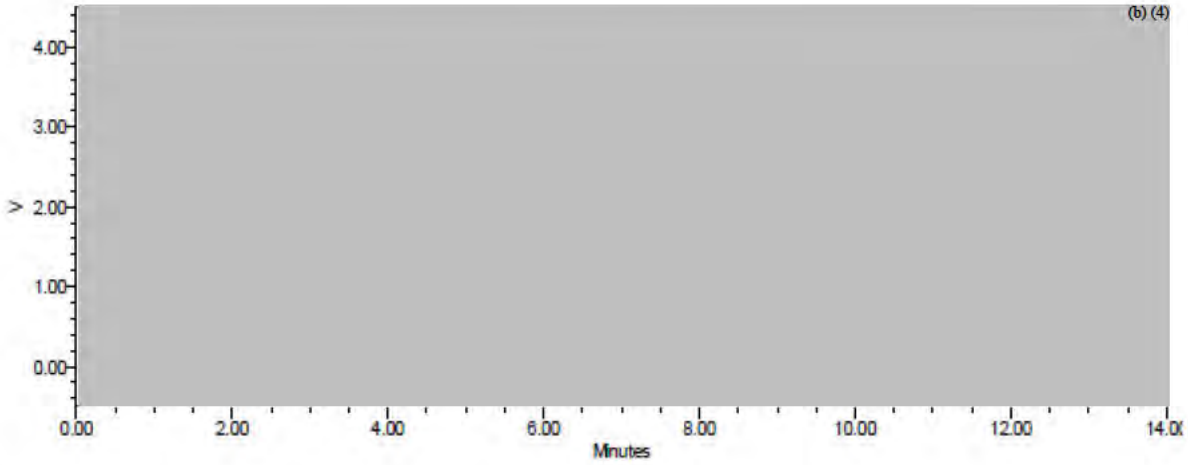


Figure 11.

(a)



(b)

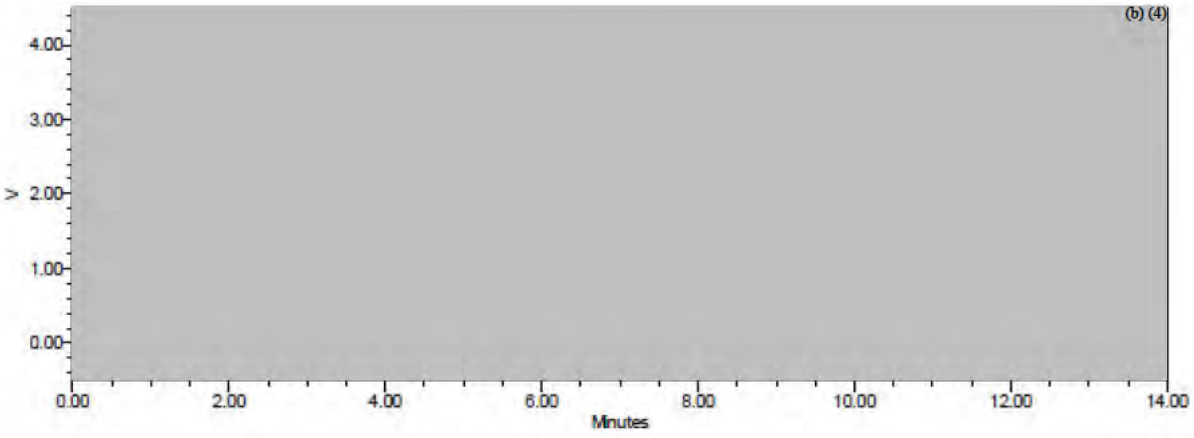
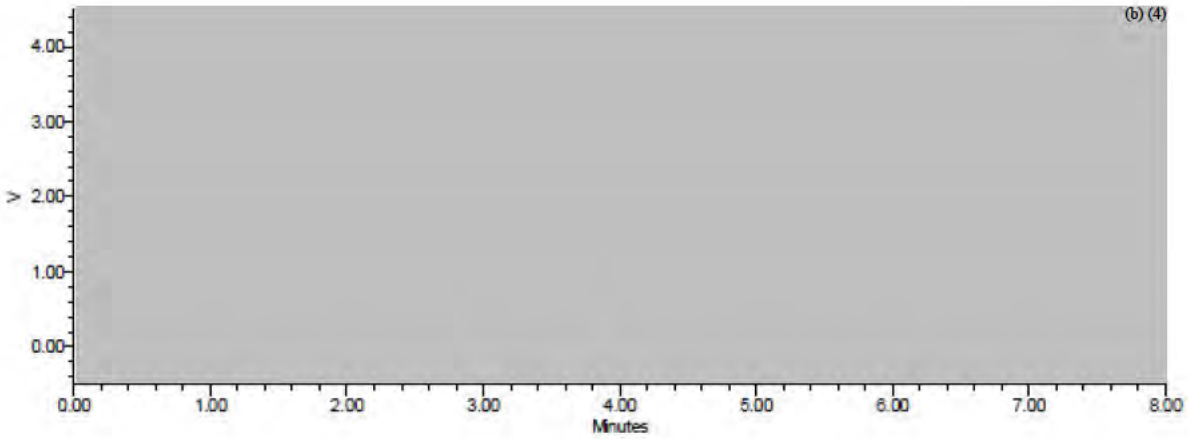


Figure 12. (b) (4)

(a)



(b)

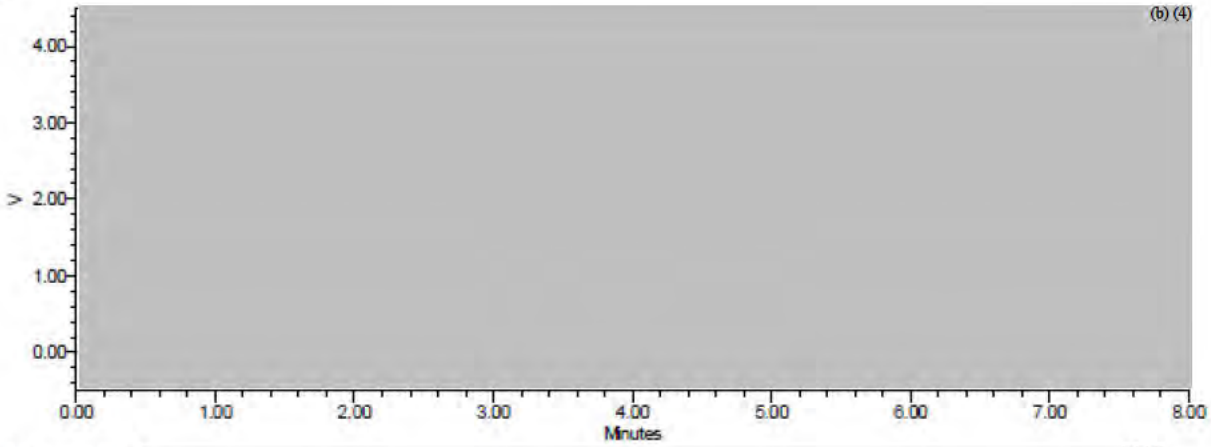
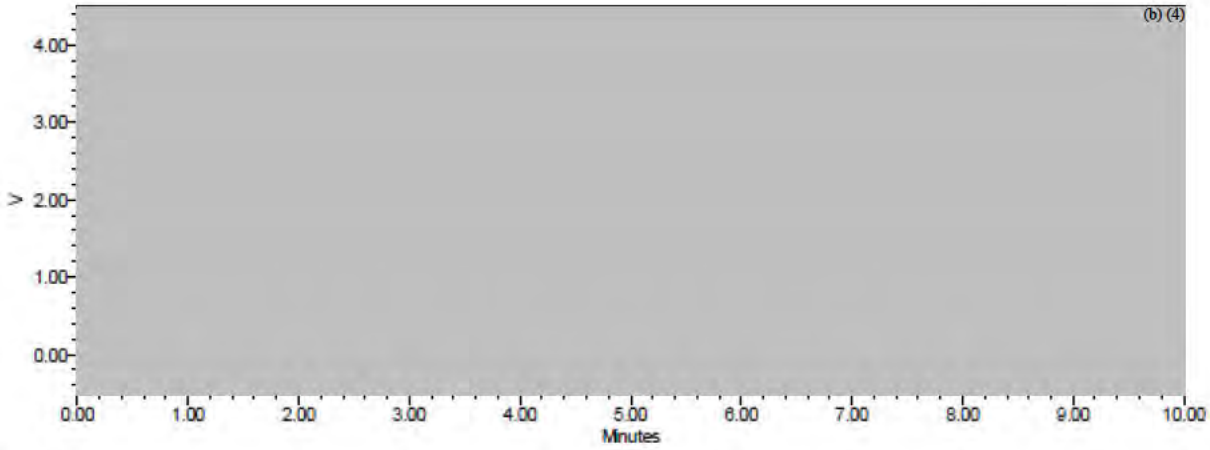


Figure 13. (b) (4)

(a)



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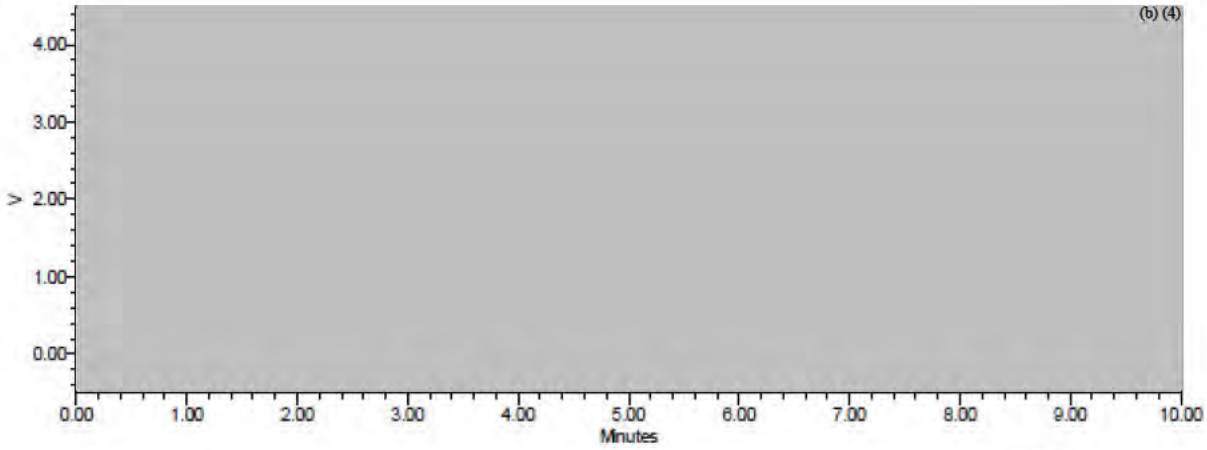
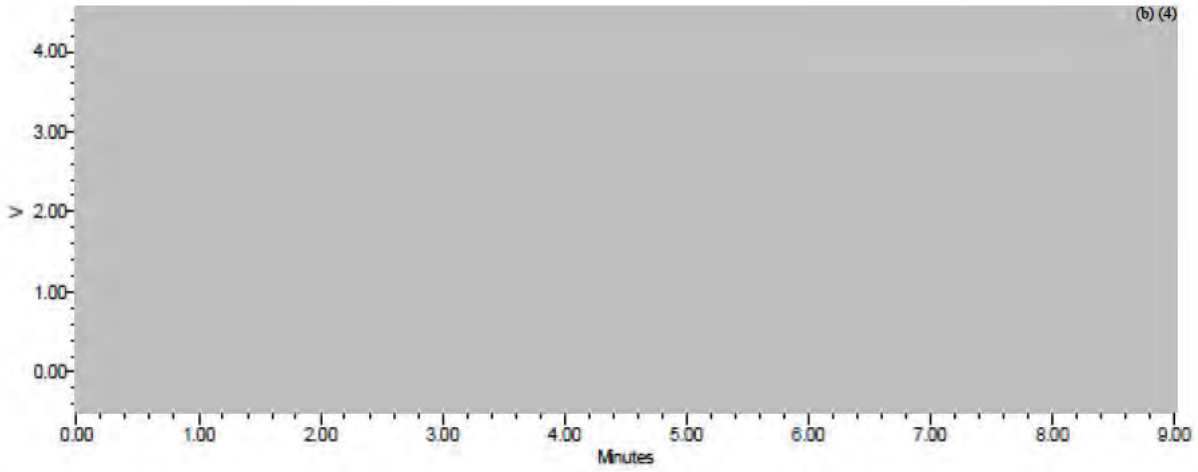


Figure 14. (b) (4)

(a)



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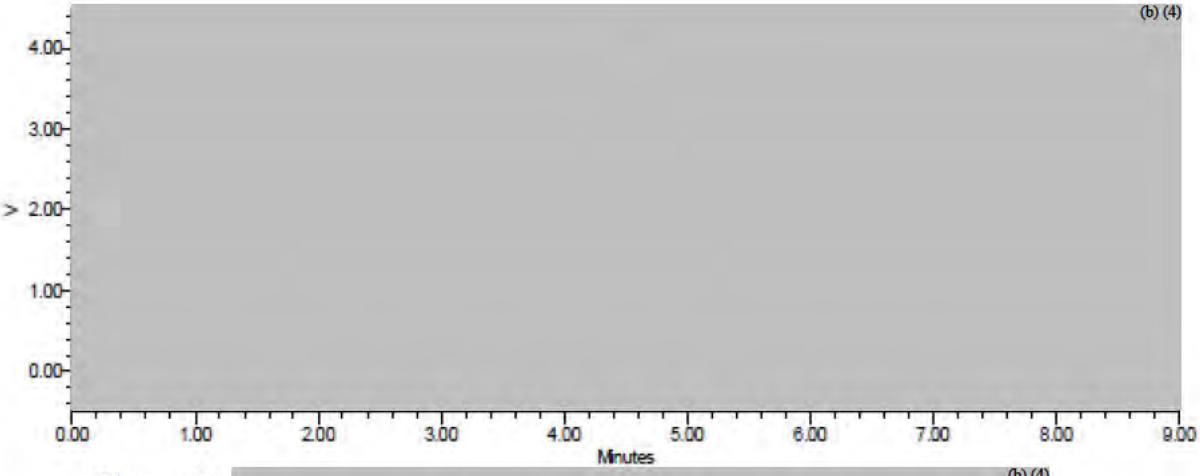
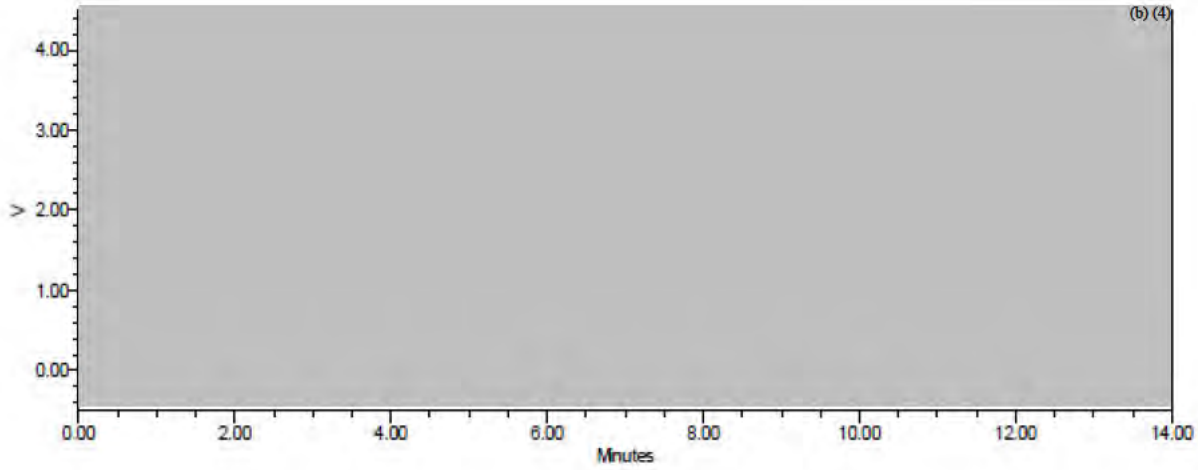


Figure 15. [redacted]

(a)



(b)

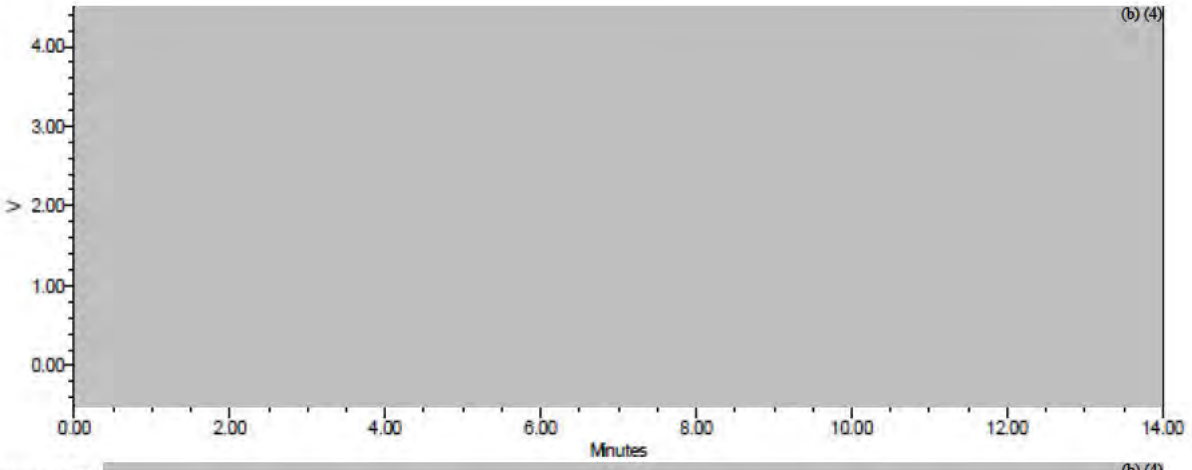
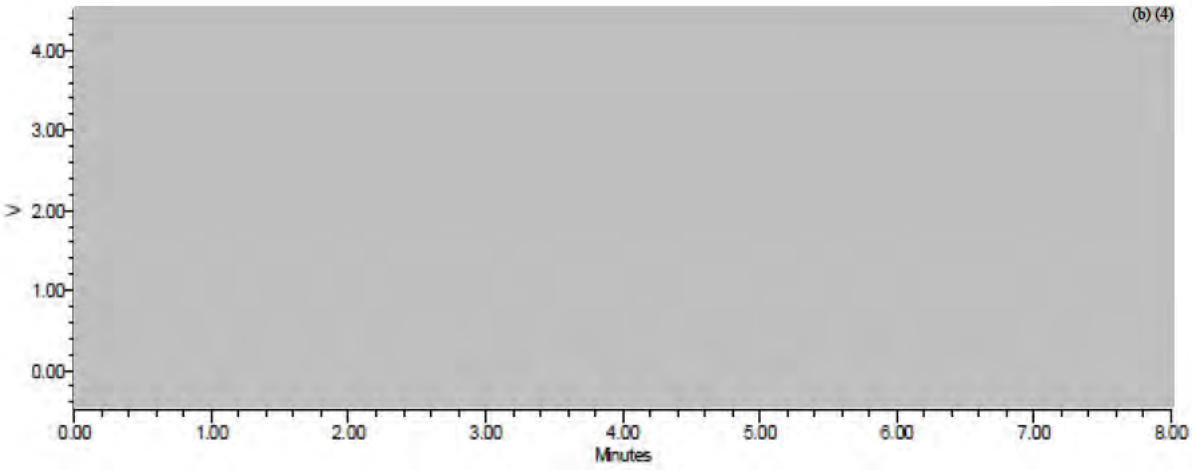


Figure 16.

(a)



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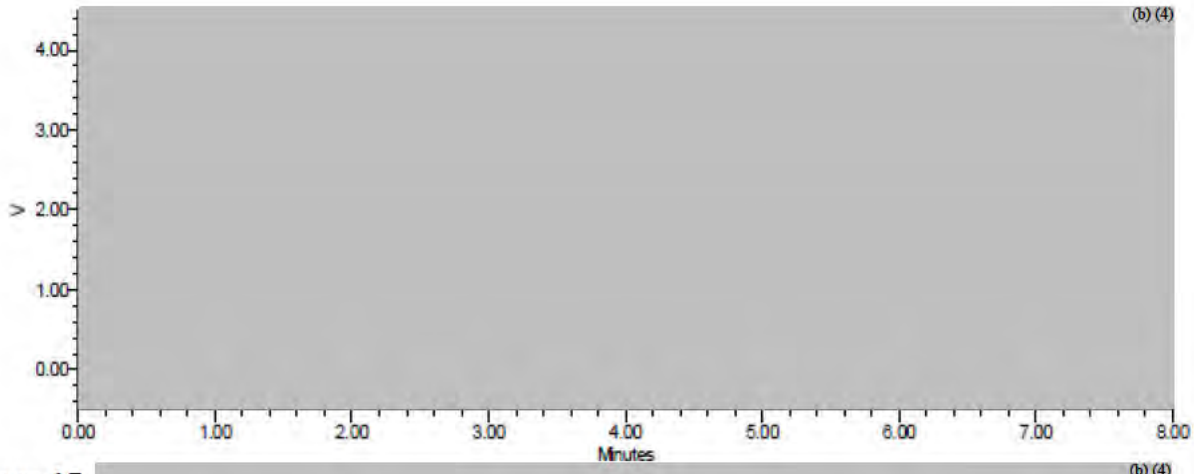


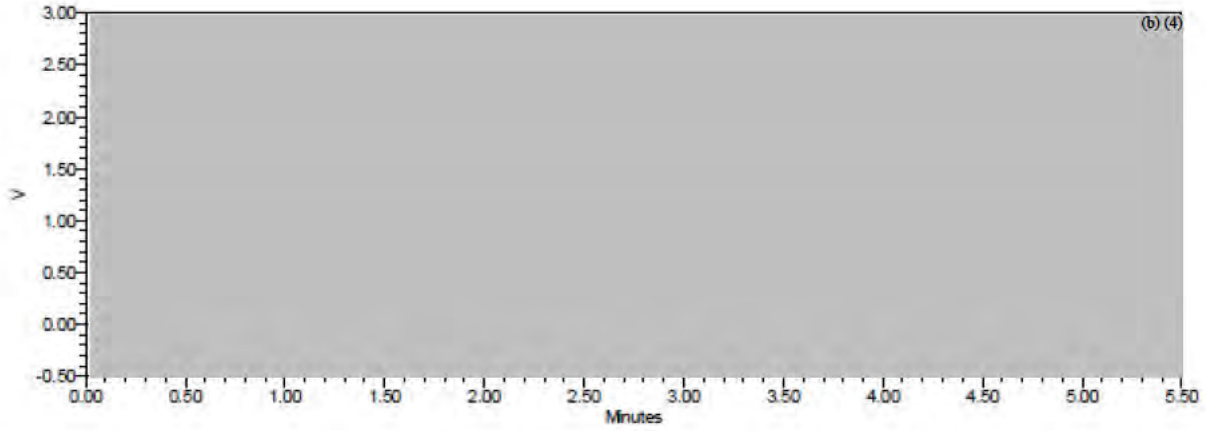
Figure 17.

16. Impurity identification

(b) (4)

(b) (4)

(a)



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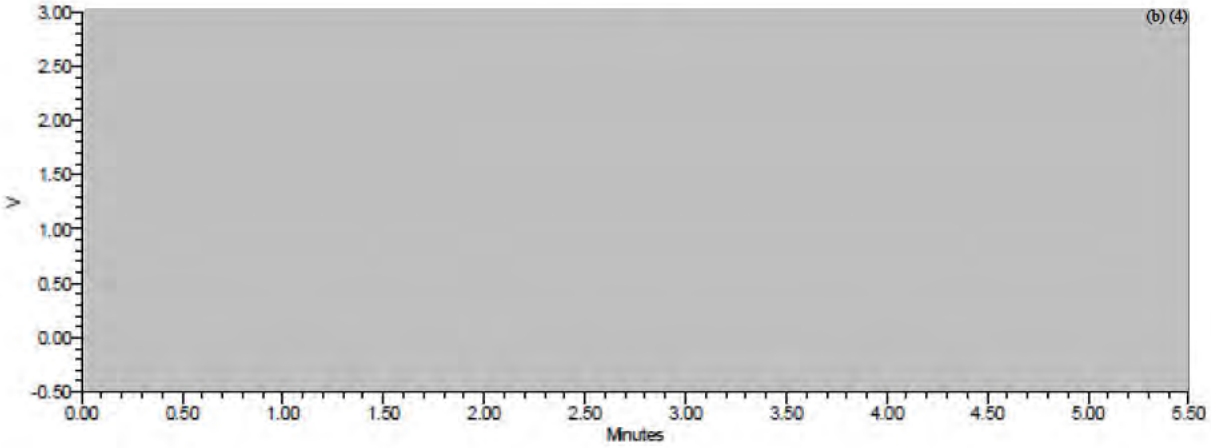
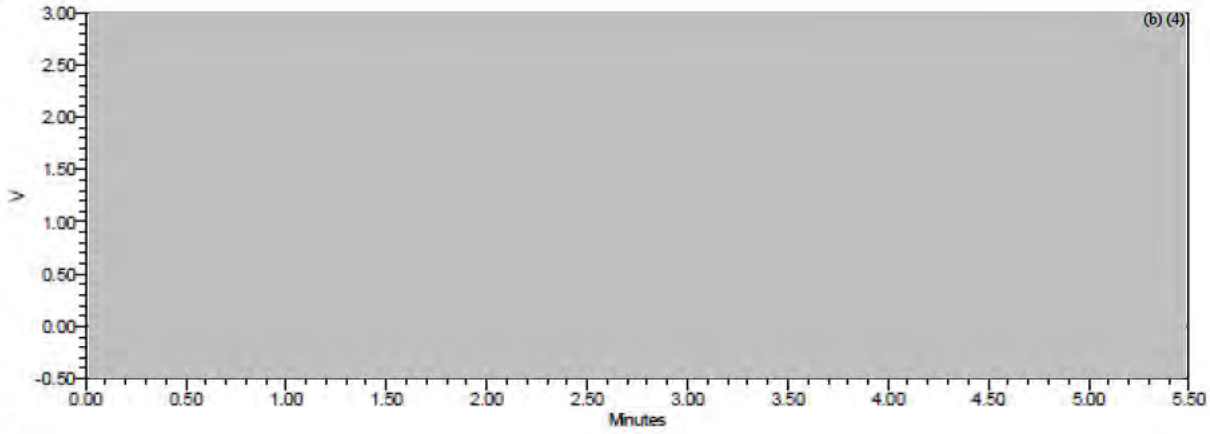
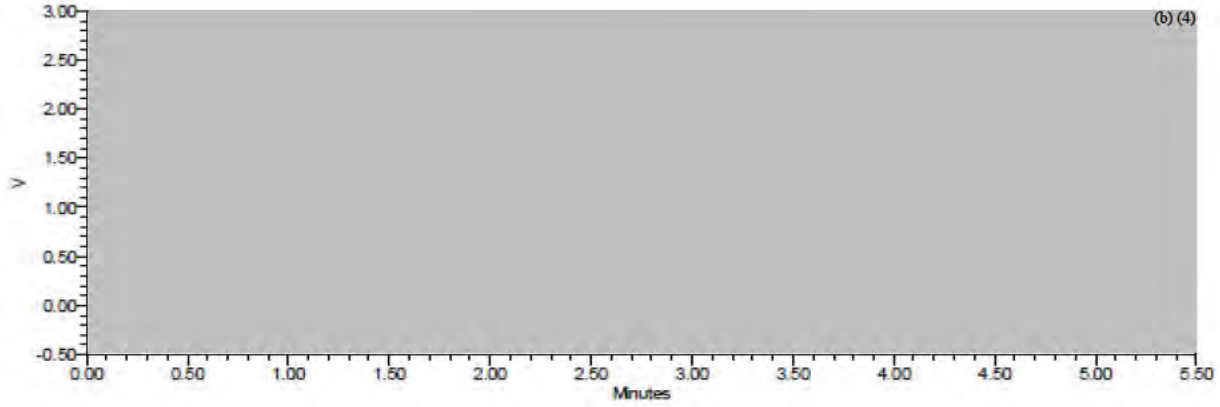


(c)



(d)



(e)

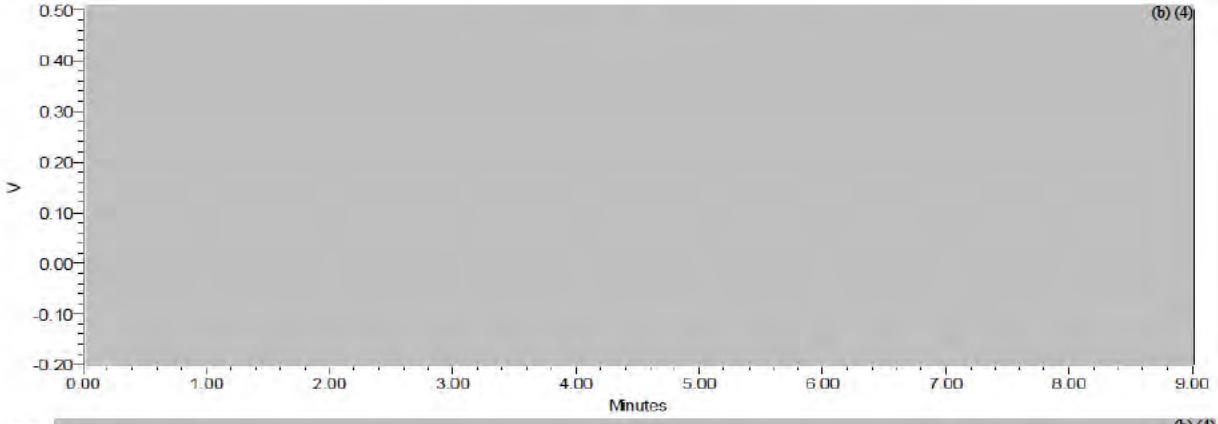
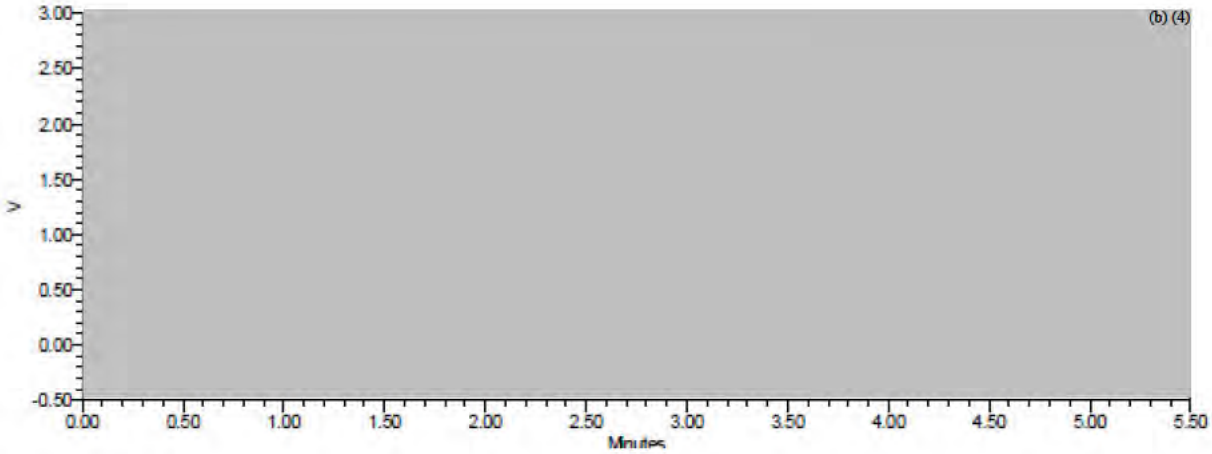
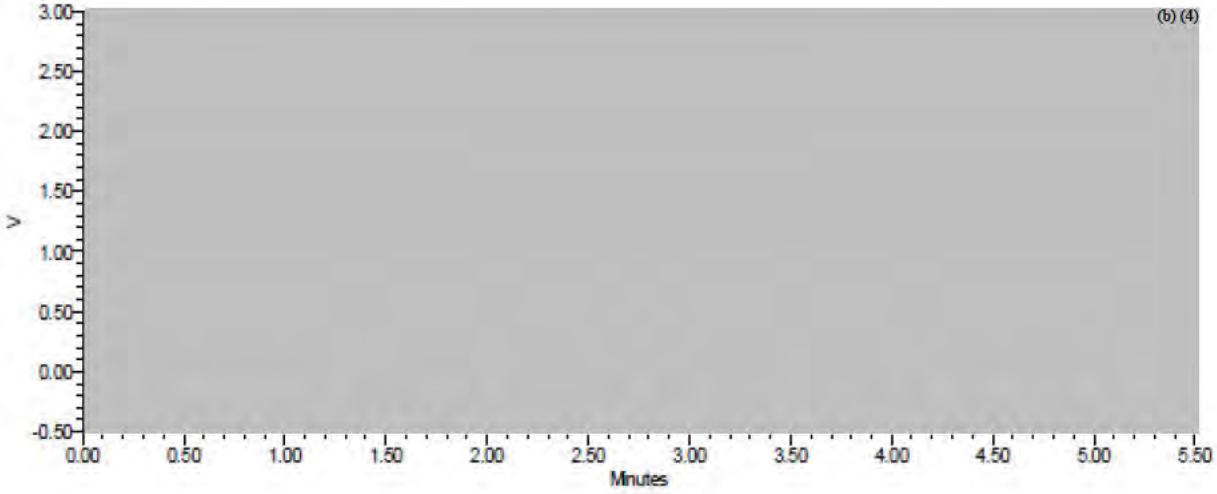


Figure 18.

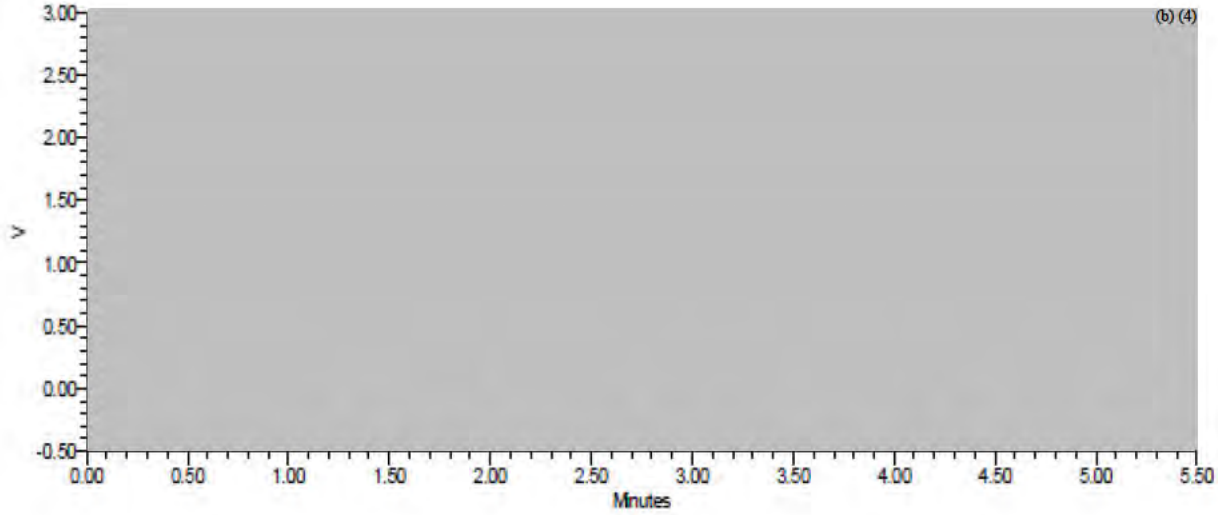
(a)


(b) (4)
V
3.00
2.50
2.00
1.50
1.00
0.50
0.00
-0.50
0.00 0.50 1.00 1.50 2.00 2.50 3.00 3.50 4.00 4.50 5.00 5.50
Minutes

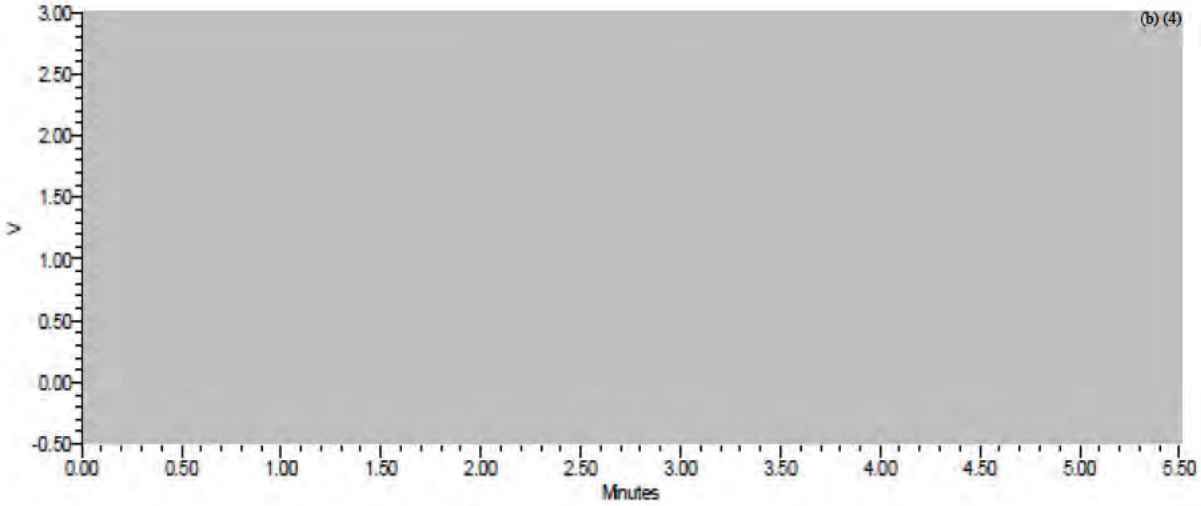
(b)


(b) (4)
V
3.00
2.50
2.00
1.50
1.00
0.50
0.00
-0.50
0.00 0.50 1.00 1.50 2.00 2.50 3.00 3.50 4.00 4.50 5.00 5.50
Minutes

(c)


(b) (4)
V
3.00
2.50
2.00
1.50
1.00
0.50
0.00
-0.50
0.00 0.50 1.00 1.50 2.00 2.50 3.00 3.50 4.00 4.50 5.00 5.50
Minutes

(d)



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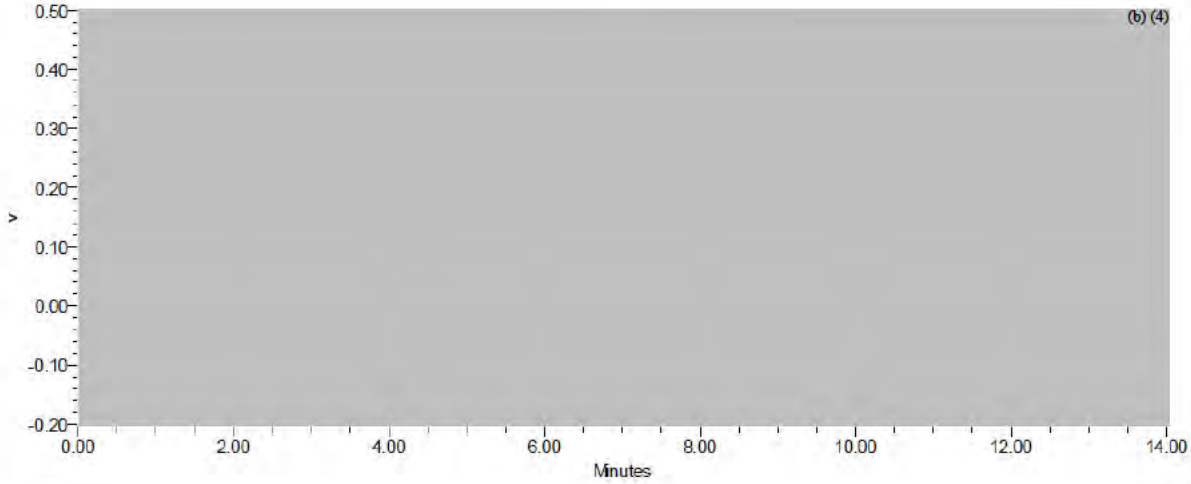


Figure 19. [redacted]

17. Conclusion

Table 16. Summary of validation test

Specificity	(b) (4)	There is no interference to peak response by diluent.
System Suitability		· %RSD < 1%
Homogeneity of sample		· %RSD < 1%
Stability of the sample		· Recovery 98% ~ 102%
		· %RSD < 1%
Linearity		· $R^2 > 0.9990$
Limit of Detection and Limit of Quantification		-
Precision		· %RSD < 1%
Accuracy		· $\|En\| \leq 1$
Robustness		· Recovery 98% ~ 102%
This validation results confirmed that all of the results were suitable for the reference value and that the analytical method could be used for rapid and accurate L-valine analysis.		

18. Raw data file

	Data file name
Specificity	(b) (4)
System Suitability	
Homogeneity of sample	
Stability of the sample	
Linearity	
Limit of Detection and Limit of Quantification	
Precision	
Accuracy	
Robustness	

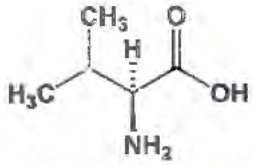
Certificate of Analysis

ISO 17034
ANAB Cert# AR-1470

ISO/IEC 17025
ANAB Cert# AT-1467

L-VALINE

CERTIFIED REFERENCE MATERIAL



CERTIFIED PURITY: 98.9%, $U_{cm} = \pm 0.07\%$ k = 2.07
(Mass Balance/as is basis)

NOMINAL PACKAGE SIZE: 1g

CATALOG #: PHR1172 **LOT #:** LRAC2856

CERTIFICATE VERSION: LRAC2856.1 **ISSUE DATE:** 22 May 2019

Note: Certificates may be updated due to Pharmacopeial Lot changes or the availability of new data. Check our website at: (b) (4) *for the most current version.*

CRM EXPIRATION: 31 May 2023 (Proper Storage and Handling Required).

RECEIPT DATE: _____
Note: this space is provided for convenience only and its use is not required.

STORAGE: Store at Room Temperature, keep container tightly closed. Attachment of a 20 mm aluminum crimp seal recommended for unused portions.

CHEMICAL FORMULA: $C_5H_{11}NO_2$ **MW:** 117.15

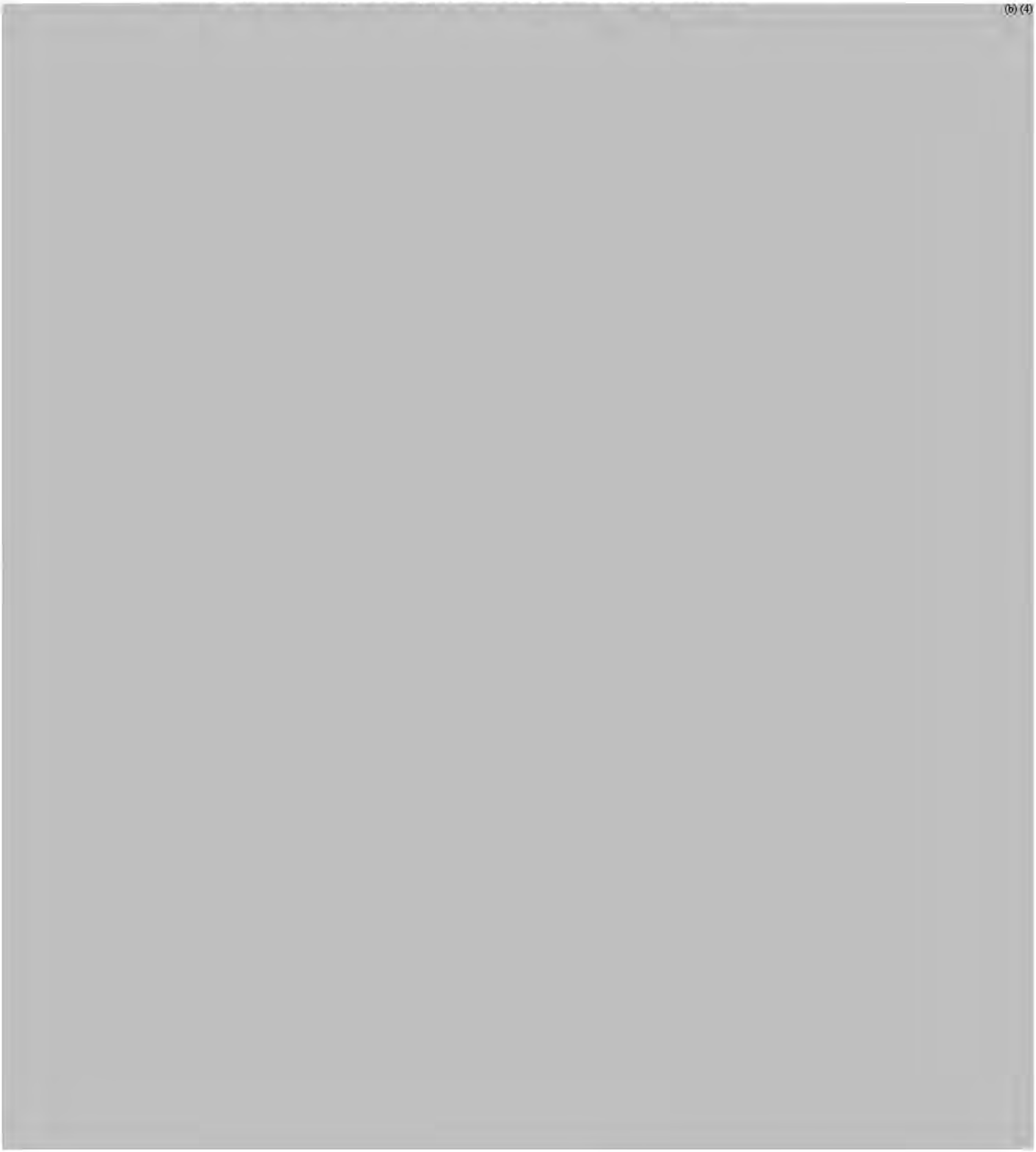
PHYSICAL DESCRIPTION: White powder in amber vial **CAS #:** 72-18-4

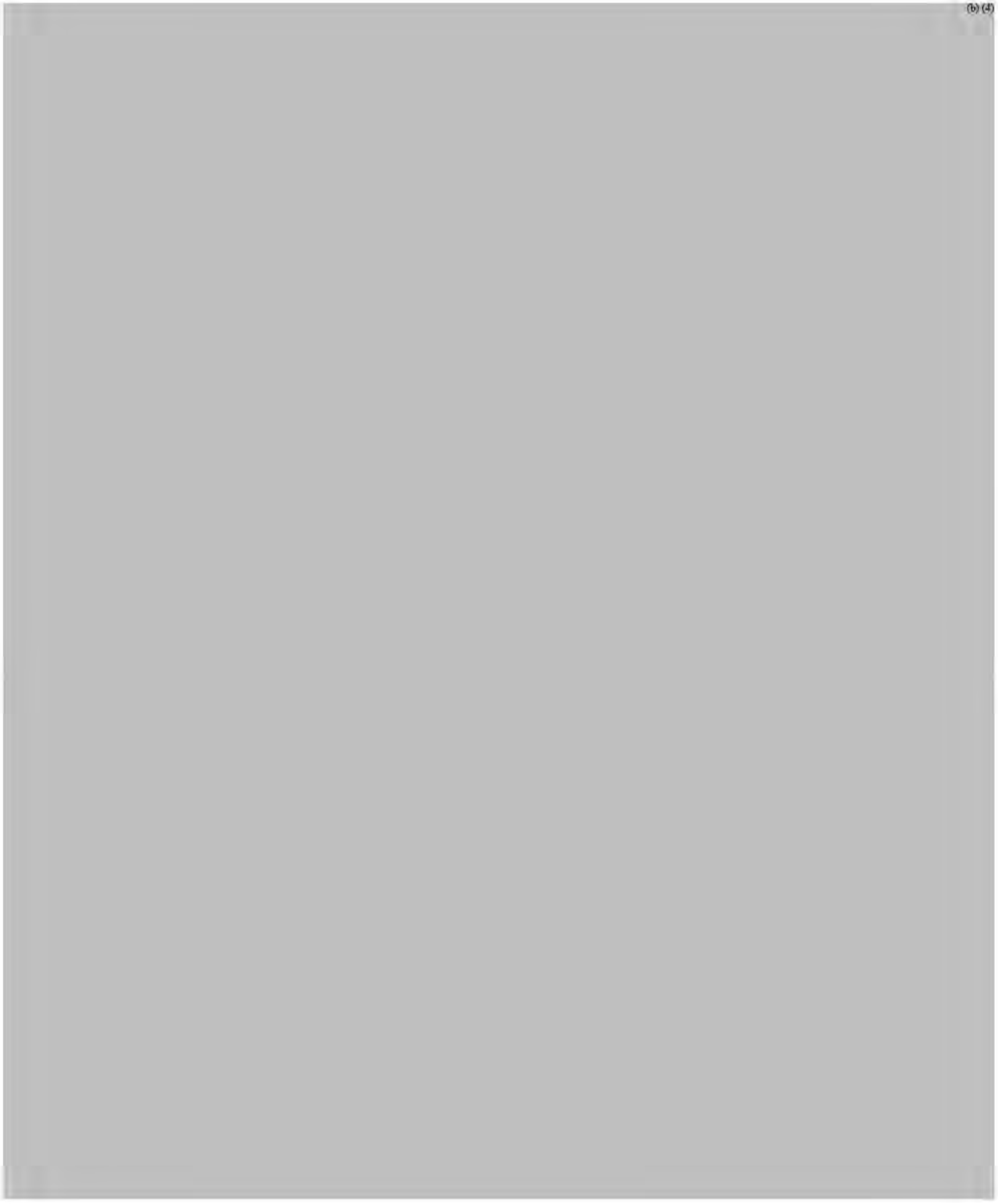
HAZARDS: Read Safety Data Sheet before using. All chemical reference materials should be considered potentially hazardous and should be used only by qualified laboratory personnel.

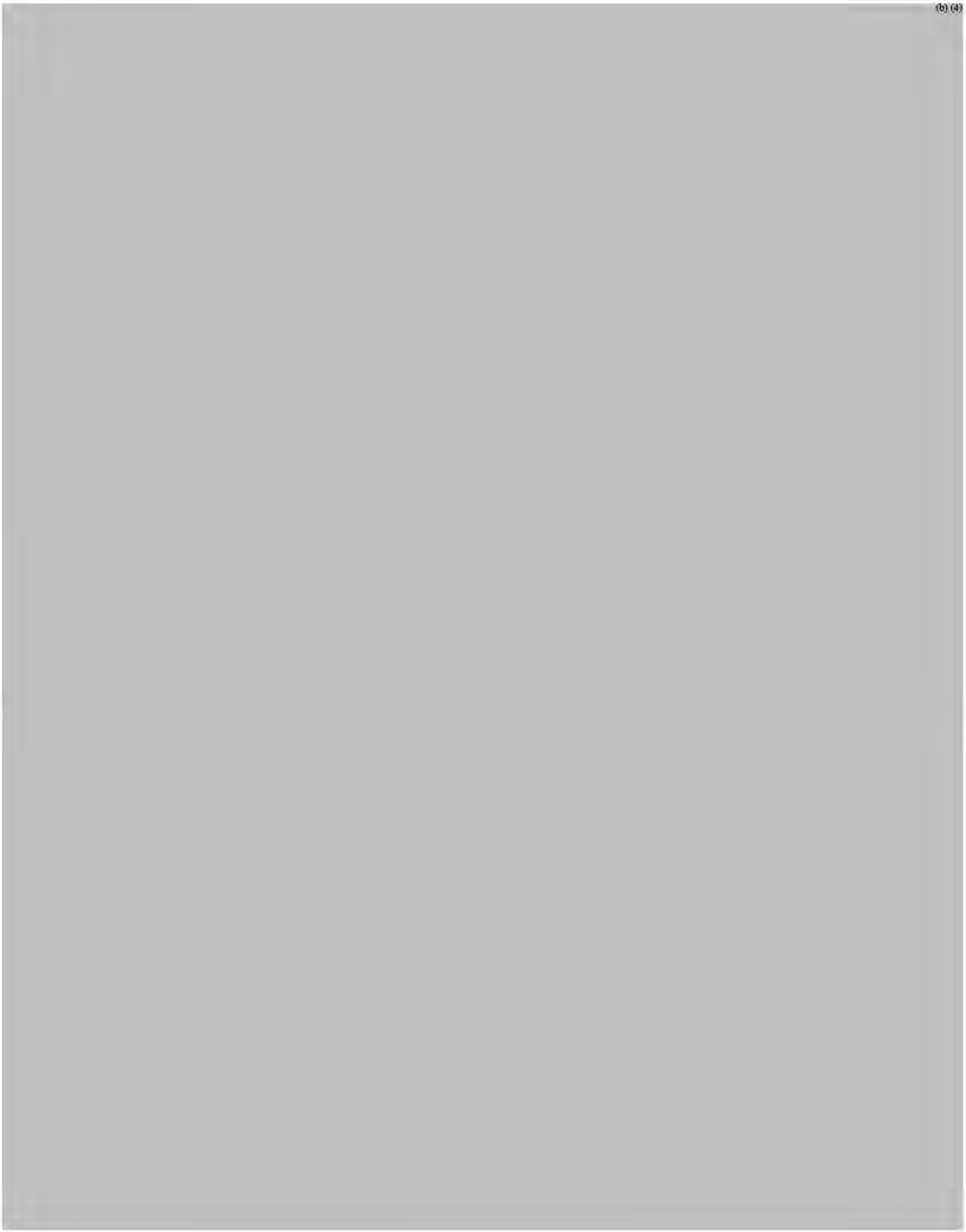
(b) (4)

INSTRUCTIONS FOR USE: Do not dry, use on the as is basis. The internal pressure of the container may be slightly different from the atmospheric pressure at the user's location. Open slowly and carefully to avoid dispersion of the material. This material is intended for Laboratory Use only. Not for drug, household or other uses.

(b) (4)

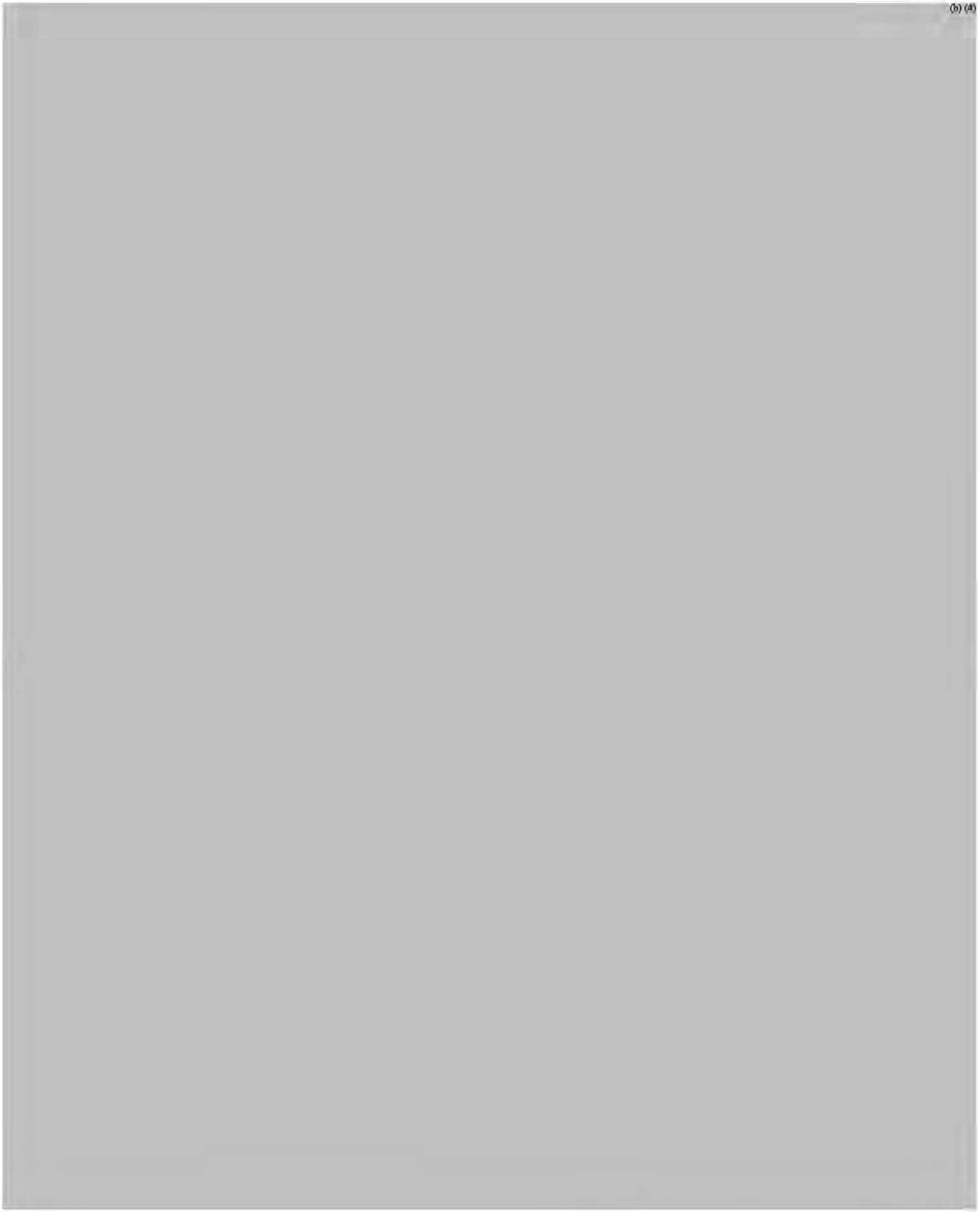



(b) (4)


(b) (4)

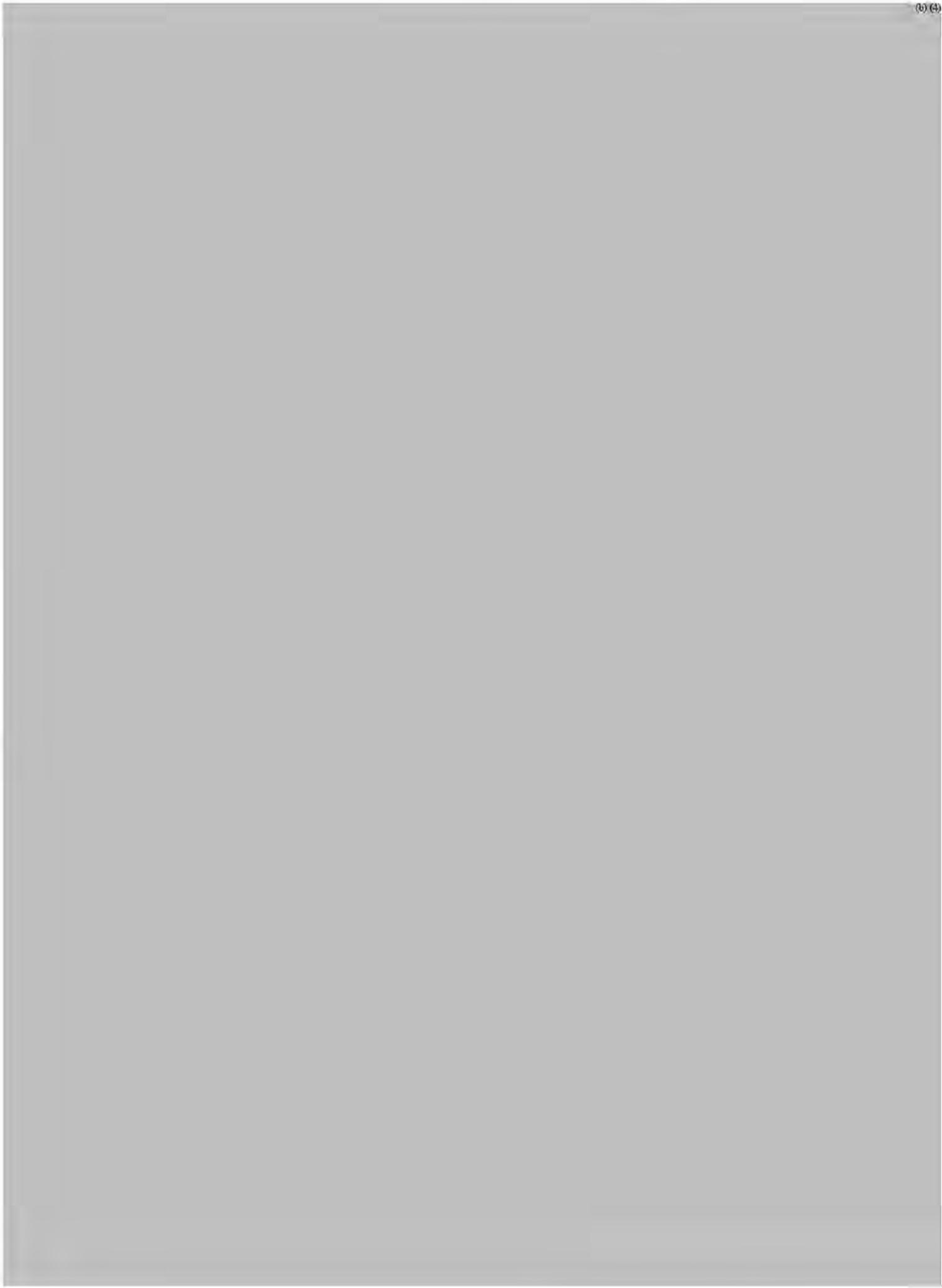

(b) (4)


(b) (4)


(b) (4)


(b) (4)


(b) (4)


(b) (4)

ANALYTICAL REPORT

Qualitative and quantitative composition of VAL Pro

(Document No.: BA20003)

CJ Research Institute of Biotechnology

CONTENTS

1. L-valine and moisture contents in 5 batches of 'VAL Pro', in g per 100 g (%) of the product as is

Component	Unit	GVAL200910	GVAL200911	GVAL200912	GVAL200916	GVAL200917
L-valine (dry basis)	%	(b) (4)				
Moisture	%					

(b) (4)

2. Nitrogen containing components in 5 batches of 'VAL Pro', in g per 100 g (%) of the product as is

Component	Unit	GVAL200910	GVAL200911	GVAL200912	GVAL200916	GVAL200917
Ammonium (as NH_3)	%	(b) (4)				
Nitrates (as NO_3)	%					
Betaine	%					
Sum of quantifiable NH_3, NO_3, betaine	**%**					

(b) (4)

3. Compositional analysis of the carbohydrates fraction in 5 batches of 'VAL Pro', in g per 100 g (%) of the product as is

Component	Unit	GVAL200910	GVAL200911	GVAL200912	GVAL200916	GVAL200917
Trehalose	%	(b) (4)				
Glucose	%					
Fructose	%	(b) (4)				
Sucrose	%					
Isomaltose	%					
Maltose	%					

Sum of quantifiable sugars	**%**	(b) (4)

(b) (4)

4. Amino acid contents in 5 batches of 'VAL Pro', in g per 100 g (%) of the product as is

Component	Unit	GVAL200910	GVAL200911	GVAL200912	GVAL200916	GVAL200917
Phosphoserine	%	(b) (4)				
Taurine	%					
Phosphoethanolamine	%					
Urea	%					
Aspartic acid	%					
Threonine	%					
Serine	%					
Glutamic acid	%					
Sarcosine	%					
α-Aminoadipic acid	%					
Glycine	%					
Alanine	%					
Citrulline	%					
α -Amino-n-butyric acid	%					
Cystine	%					
Methionine	%					
Cysthathionine	%					
Isoleucine	%					
Leucine	%					
Tyrosine	%					
Phenylalanine	%					

β-Alanine	%	(b) (4)
β-Aminoisobutyric acid	%	
γ-Amino-n-butyric acid	%	
Ethanolamine	%	
Hydroxylysine	%	
Ornithine	%	
Lysine	%	
1-Methylhistidine	%	
Histidine	%	
3-methylhistidine	%	
Asparagine	%	
Carnosine	%	
Arginine	%	
Hydroxyproline	%	
Proline	%	
Sum of amino acids other than L-valine	**%**	

(b) (4)

5. Hydrolyzed amino acids contents in insoluble part in 5 batches of 'VAL Pro', in g per 100 g (%) of the product as is

Component	Unit	GVAL200910	GVAL200911	GVAL200912	GVAL200916	GVAL200917
Aspartic acid	%					(b) (4)
Threonine	%					
Serine	%					
Glutamic acid	%					
Glycine	%					
Alanine	%					
Cystine	%					(b) (4)
Valine	%					
Methionine	%					
Isoleucine	%					
Leucine	%					
Tyrosine	%					
Phenylalanine	%					
Lysine	%					
Histidine	%					
Arginine	%					
Proline	%					
Tryptophan	%					
Sum of 'hydrolyzed amino acids' in insoluble part[1]	**%**					(b) (4)

(b) (4)

(b) (4)

6. Compositional analysis of organic acids fraction in 5 batches of 'VAL Pro', in g per 100 g (%) of the product as is

Component	Unit	GVAL200910	GVAL200911	GVAL200912	GVAL200916	GVAL200917
Citric Acid	%	(b) (4)				
Malic Acid	%					
Succinic Acid	%					
Lactic Acid	%					
Formic Acid	%					
Acetic Acid	%					
Sum of quantifiable organic acids	**%**					

(b) (4)

7. Compositional analysis of inorganic components in 5 batches of 'VAL Pro', in g per 100 g (%) of the product as is

Component	Unit	GVAL200910	GVAL200911	GVAL200912	GVAL200916	GVAL200917
Ash	%	(b) (4)				
Sodium	%					
Potassium	%					
Calcium	%					
Magnesium	%					
Fluoride	%					
Bromide	%					
Chloride	%					
Phosphate	%					
Sulfate	%					
Sum of quantifiable inorganic anions and cations	**%**					

(b) (4)

8. Overview of the quantifiable main components of 'VAL Pro', in g per 100 g (%) of the product as is

Component	Unit	GVAL200910	GVAL200911	GVAL200912	GVAL200916	GVAL200917
L-valine (as dry basis)	**%**	(b) (4)				
Hydrolyzed amino acids (in insoluble biomass part)	**%**					
Free amino acids (other than L-valine)	**%**					
Moisture	%					

Ammonium, nitrates and betaine		**%**	(b) (4)
Sugars		**%**	
Organic acids		**%**	
Inorganic anions/cations		**%**	
	Ash[1]	%	

(b) (4)

9. Results and methods of 'VAL Pro'

Component	Results[1]	Analytical method
L-valine	(b) (4)	HPLC-FLD (modified AOAC 999.13)
Hydrolyzed amino acids (in insoluble biomass part)		AOAC 994.12
		AOAC 988.15
		AOAC 985.28
Free amino acids (other than L-valine)		AOAC 999.13
Moisture		AOAC 934.01
Ammonium, nitrates and betaine		ASTM D 4327-03
		Korean Feed Standards Codex, 18 of chapter 21.
Sugars		AOAC 995.13
Organic acids		Korean Feed Standards Codex, 1 of chapter 14
Inorganic anions/cations		ASTM D 4327-03
		ASTM D 6919–03
Ash		AOAC 942.05

[1]Results are mean value of five batches

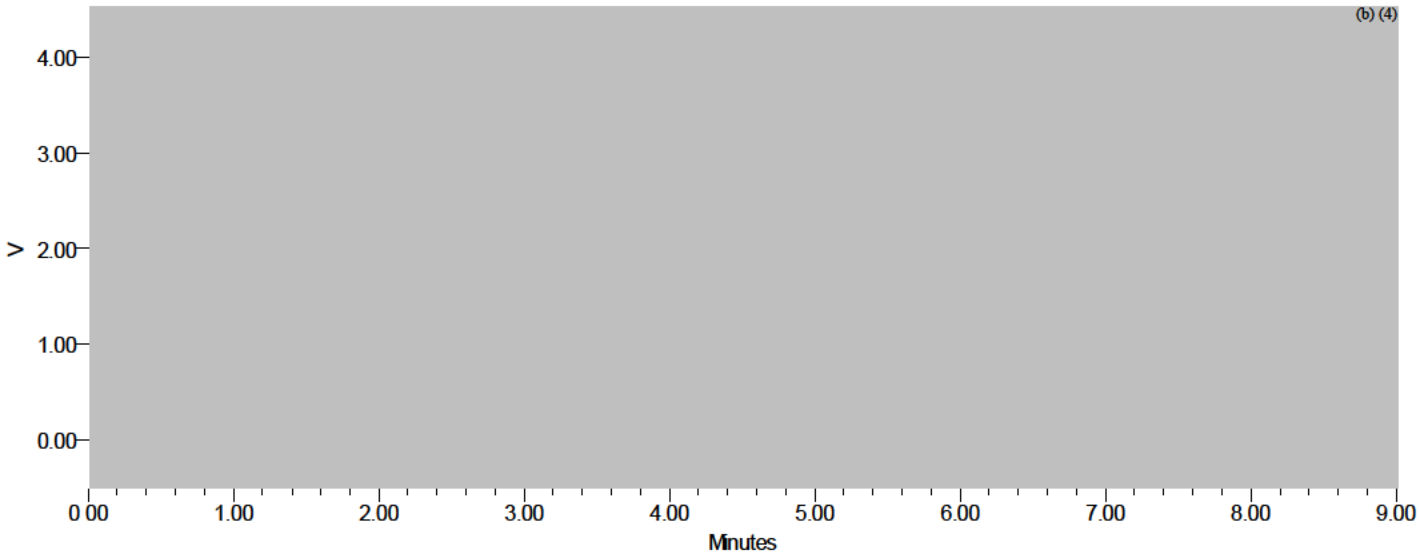
Sample Set Name:	Granule Valine_1 Day	Processed By:	System/Administrator
Sample Set Method:	Granule Valine_1 Day	Printed By:	System
System Node:	Client13	Result Set ID:	
System Name:	UC10	# of Results:	20
Acquired By:	System		
Sample Set Start Date:	12/17/2020 6:52:02 PM KST		
Sample Set Finish Date:	12/18/2020 12:24:42 PM KST		

Sample Set Table

	Sample Name	Sample Type	Vial	Inj #	Injection Volume (ul)	Acquistion Method Set	Processed Channel Descr.	(b) (4)
1	SS1_1	Unknown	68	1	5.00	VAL_ACR	Detector A	
2	SS1_2	Unknown	68	1	5.00	VAL_ACR	Detector A	
3	SS1_3	Unknown	68	1	5.00	VAL_ACR	Detector A	
4	SS1_4	Unknown	68	1	5.00	VAL_ACR	Detector A	
5	SS1_5	Unknown	68	1	5.00	VAL_ACR	Detector A	
6	SS1_6	Unknown	68	1	5.00	VAL_ACR	Detector A	
7	SS1_7	Unknown	68	1	5.00	VAL_ACR	Detector A	
8	SS1_8	Unknown	68	1	5.00	VAL_ACR	Detector A	
9	SS1_9	Unknown	68	1	5.00	VAL_ACR	Detector A	
10	SS1_10	Unknown	68	1	5.00	VAL_ACR	Detector A	
11	SS4_1	Unknown	69	1	5.00	VAL_ACR	Detector A	
12	SS4_2	Unknown	69	1	5.00	VAL_ACR	Detector A	
13	SS4_3	Unknown	69	1	5.00	VAL_ACR	Detector A	
14	SS4_4	Unknown	69	1	5.00	VAL_ACR	Detector A	
15	SS4_5	Unknown	69	1	5.00	VAL_ACR	Detector A	
16	SS4_6	Unknown	69	1	5.00	VAL_ACR	Detector A	
17	SS4_7	Unknown	69	1	5.00	VAL_ACR	Detector A	
18	SS4_8	Unknown	69	1	5.00	VAL_ACR	Detector A	
19	SS4_9	Unknown	69	1	5.00	VAL_ACR	Detector A	
20	SS4_10	Unknown	69	1	5.00	VAL_ACR	Detector A	

SAMPLE INFORMATION

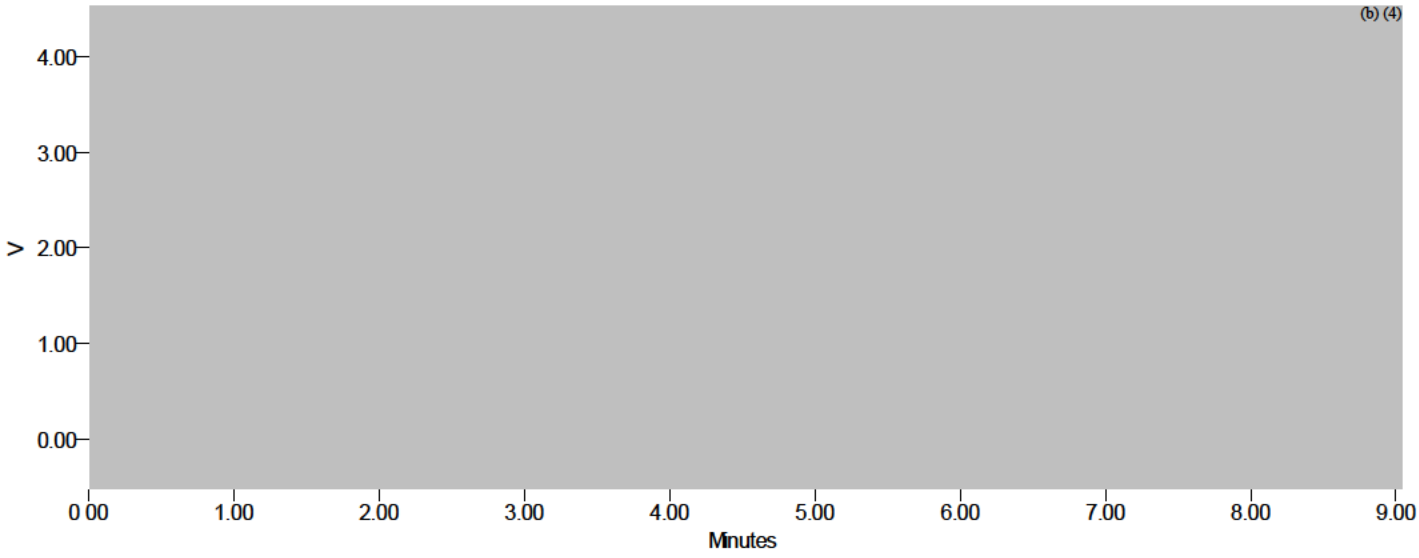
Sample Name:	SS1_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	68	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 7:54:41 AM KST		
Date Processed:	12/20/2020 5:20:03 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

Sample Name:	SS1_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	68	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 8:04:18 AM KST		
Date Processed:	12/20/2020 5:20:03 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

Sample Name:	SS1_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	68	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
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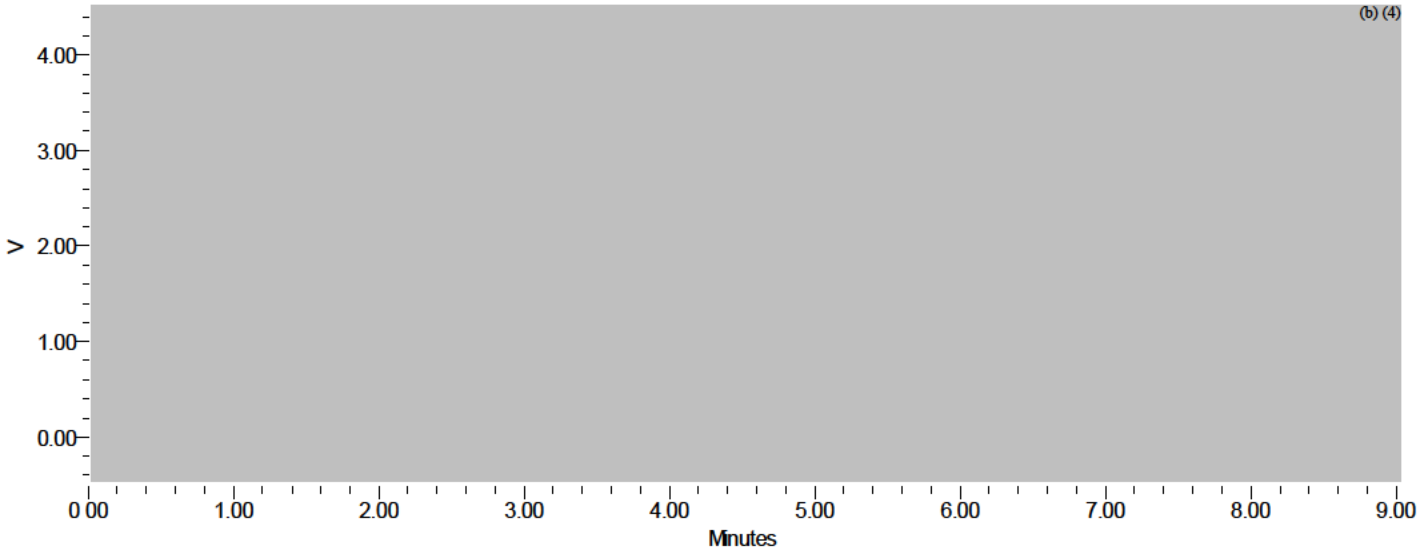
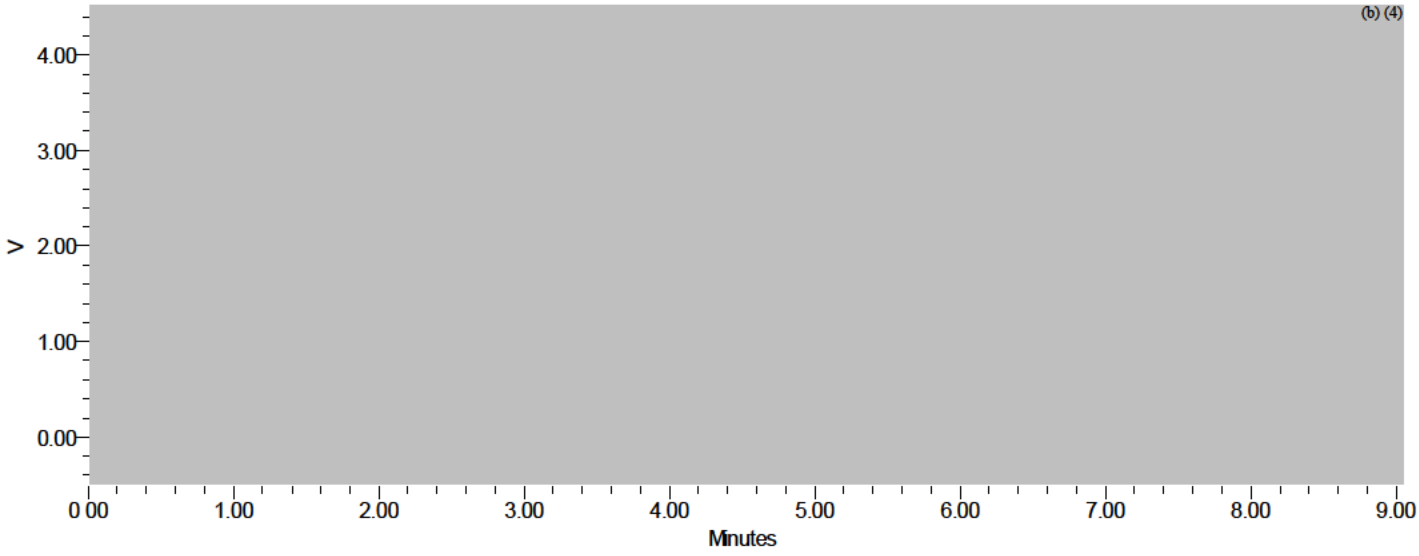
Date Processed:	12/20/2020 5:20:03 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

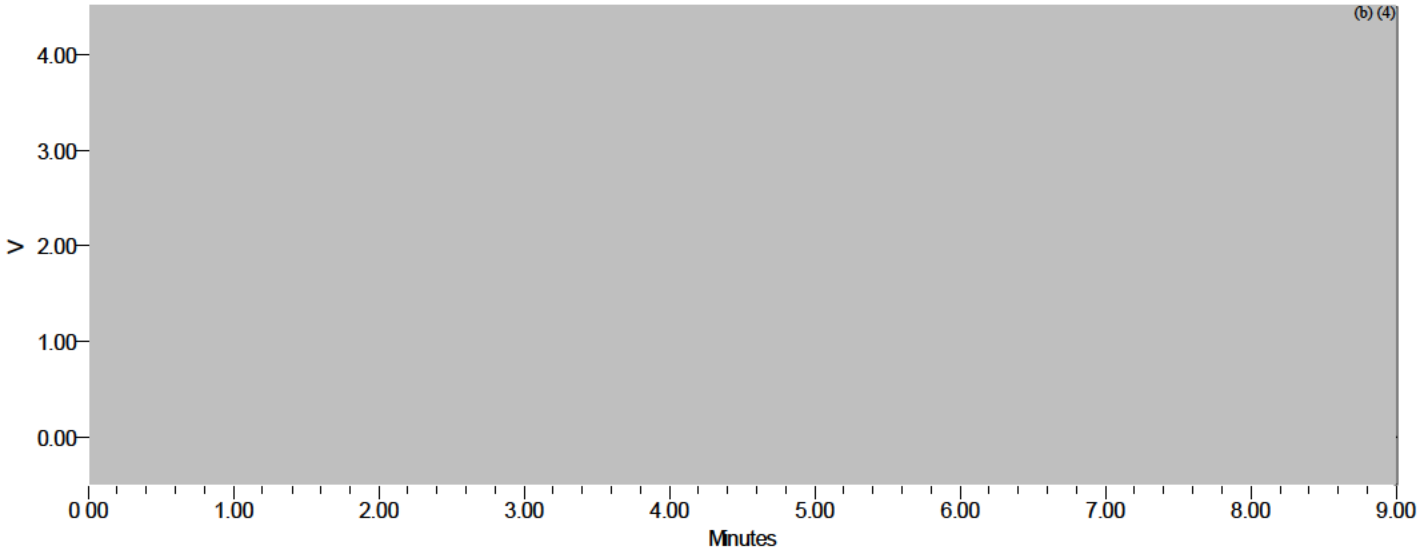
Sample Name:	SS1_4	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	68	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 8:23:38 AM KST		
Date Processed:	12/20/2020 5:20:04 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

Sample Name:	SS1_5	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	68	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 8:33:17 AM KST		
Date Processed:	12/20/2020 5:20:04 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

Sample Name:	SS1_6	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	68	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A Ex (b) (4)
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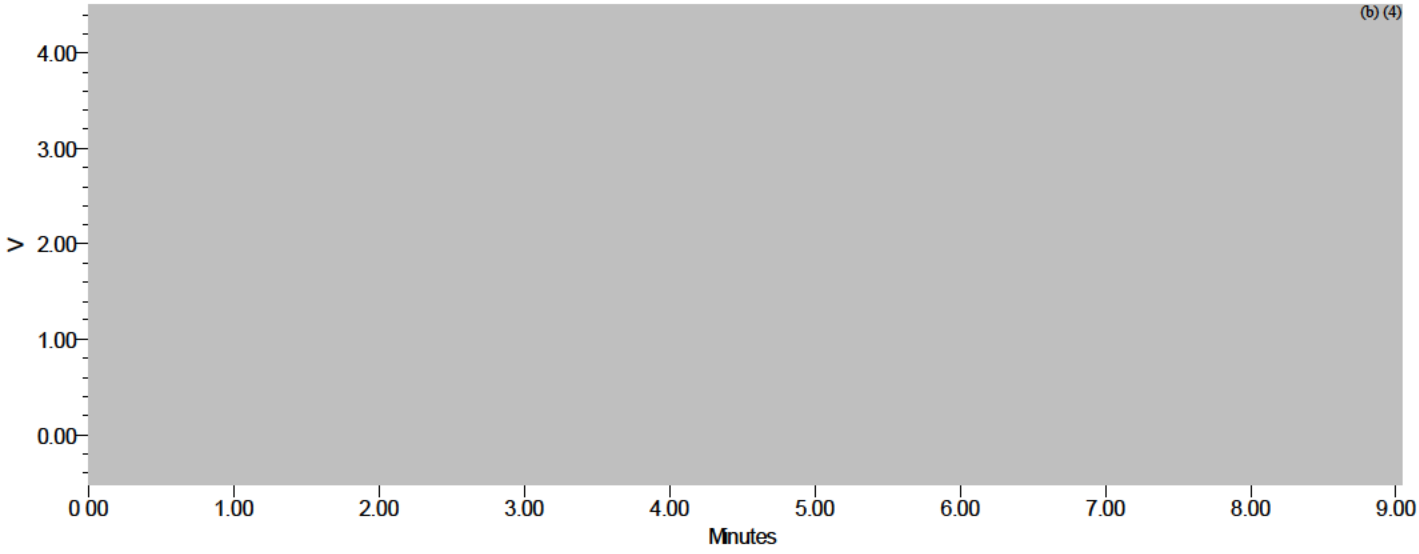
Date Processed:	12/20/2020 5:20:04 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

Sample Name:	SS1_7	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	68	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
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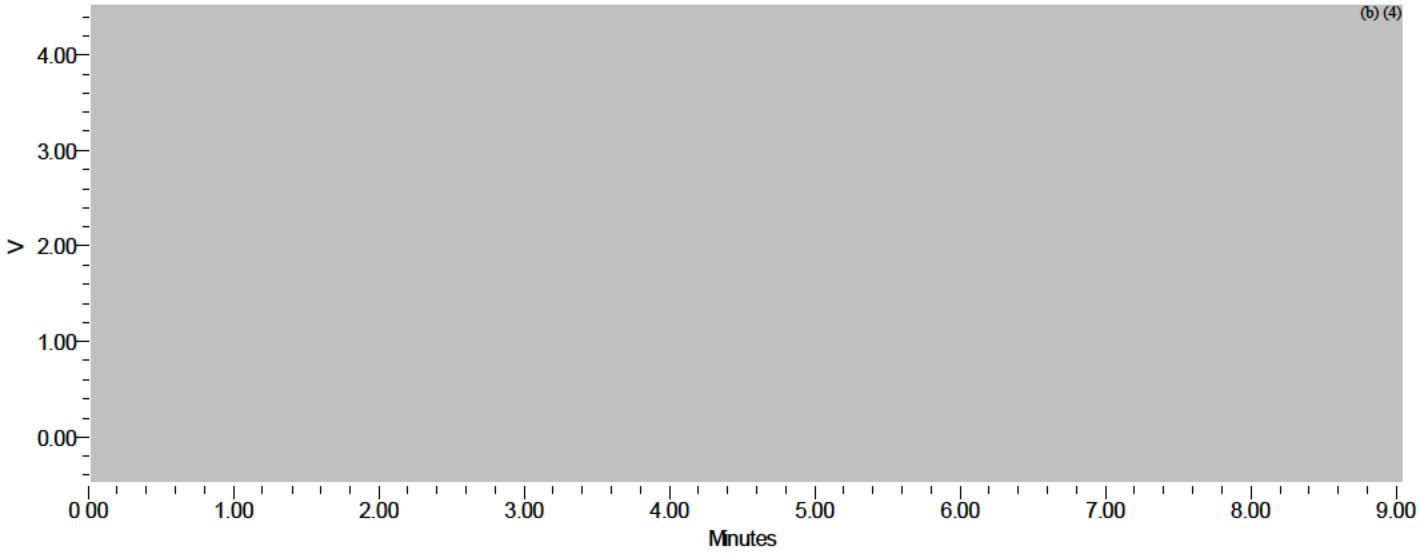
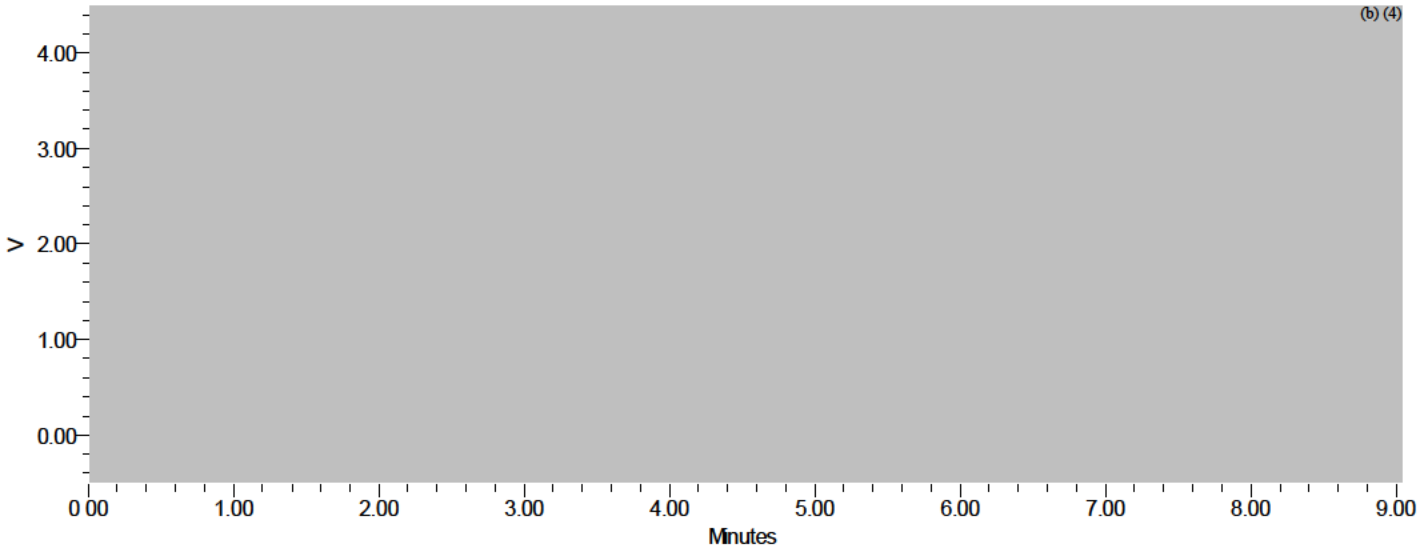
Date Processed:	12/20/2020 5:20:04 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

Sample Name:	SS1_8	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	68	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 9:02:17 AM KST		
Date Processed:	12/20/2020 5:20:04 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

Sample Name:	SS1_9	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	68	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
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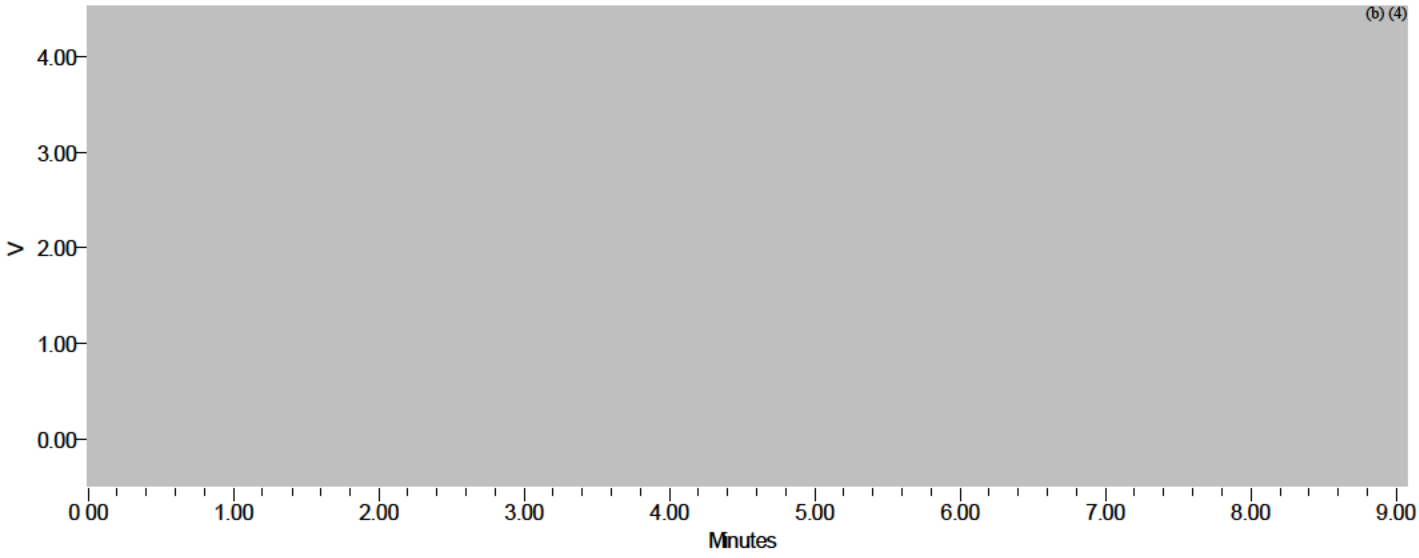
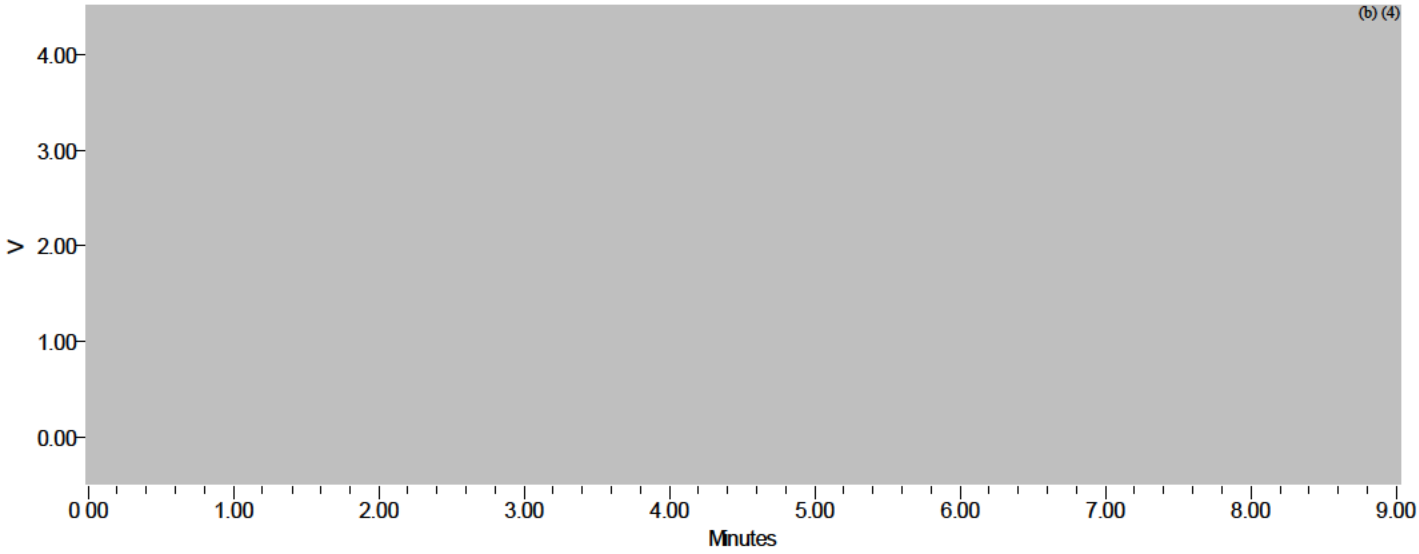
Date Processed:	12/20/2020 5:20:05 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

Sample Name:	SS1_10	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	68	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 9:21:38 AM KST		
Date Processed:	12/20/2020 5:20:05 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

Sample Name:	SS4_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	69	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 9:40:57 AM KST		
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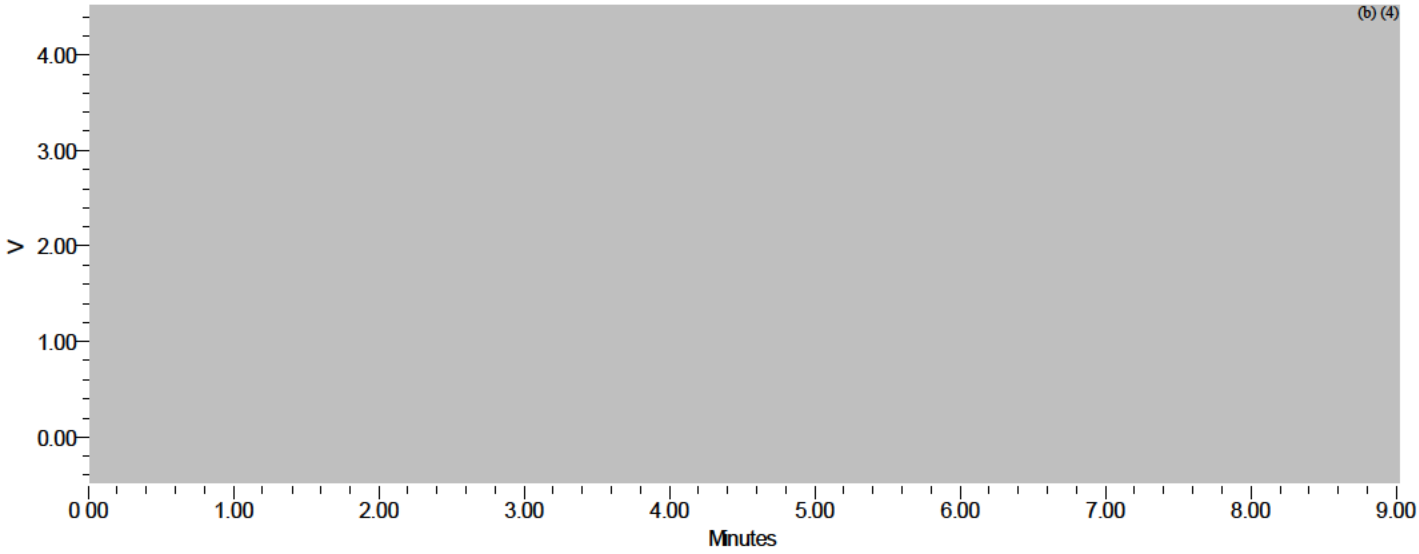

	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

Sample Name:	SS4_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	69	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
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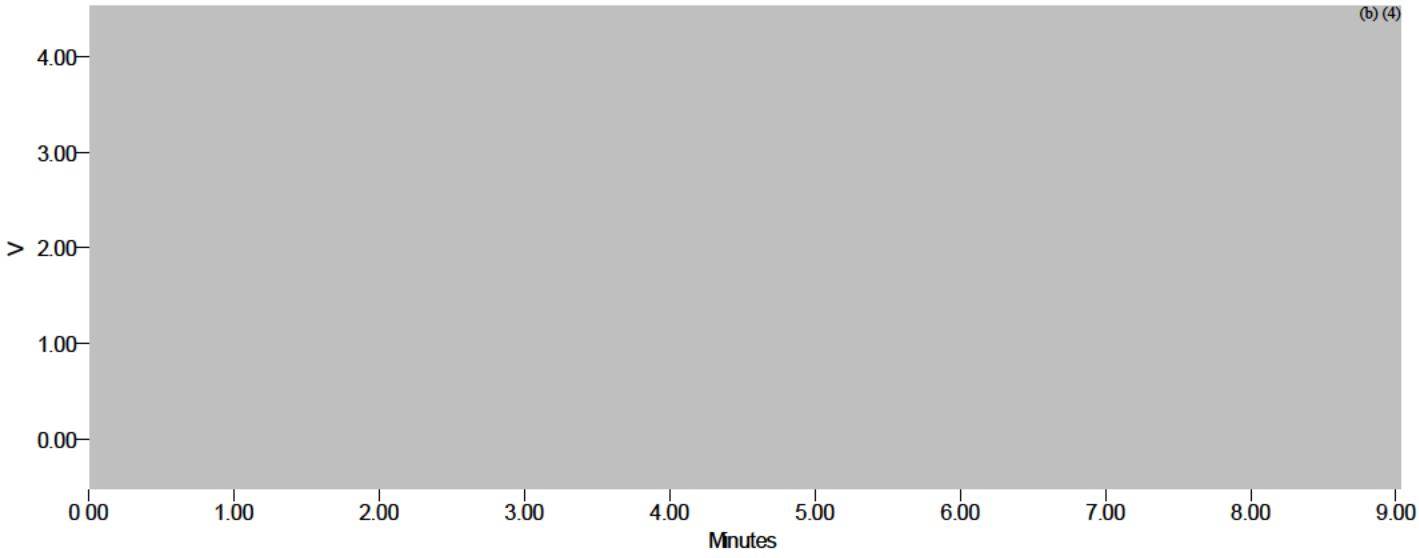
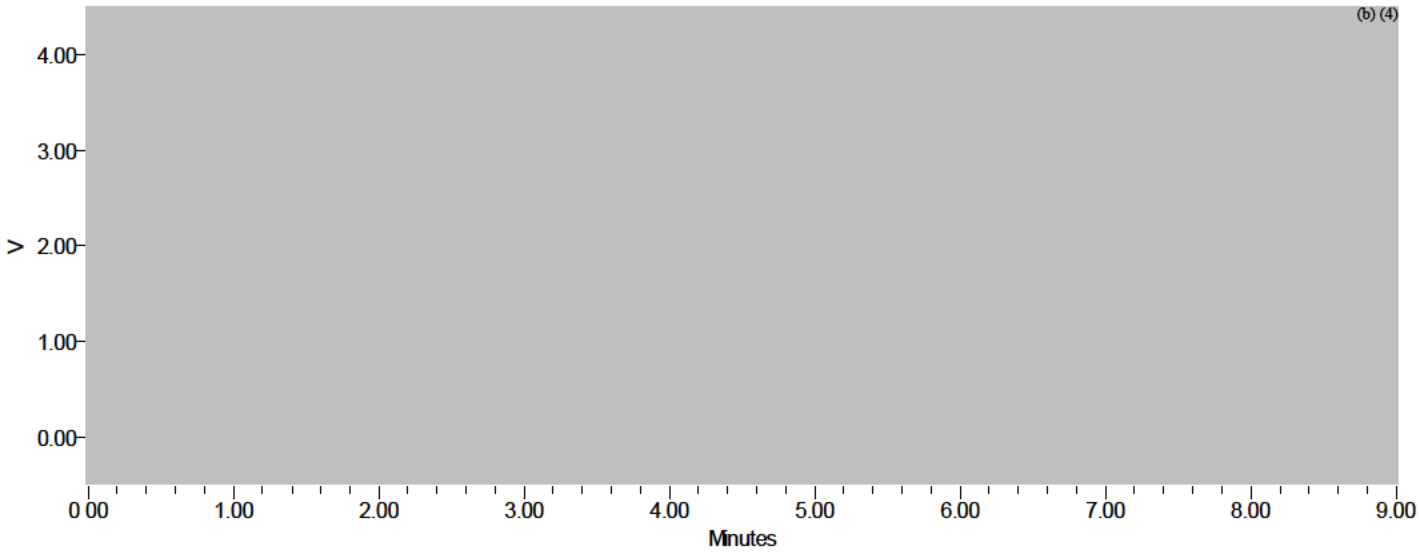
Date Processed:	12/20/2020 5:20:05 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

Sample Name:	SS4_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	69	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 10:00:17 AM KST		
Date Processed:	12/20/2020 5:20:05 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

Sample Name:	SS4_4	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	69	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 10:09:56 AM KST		
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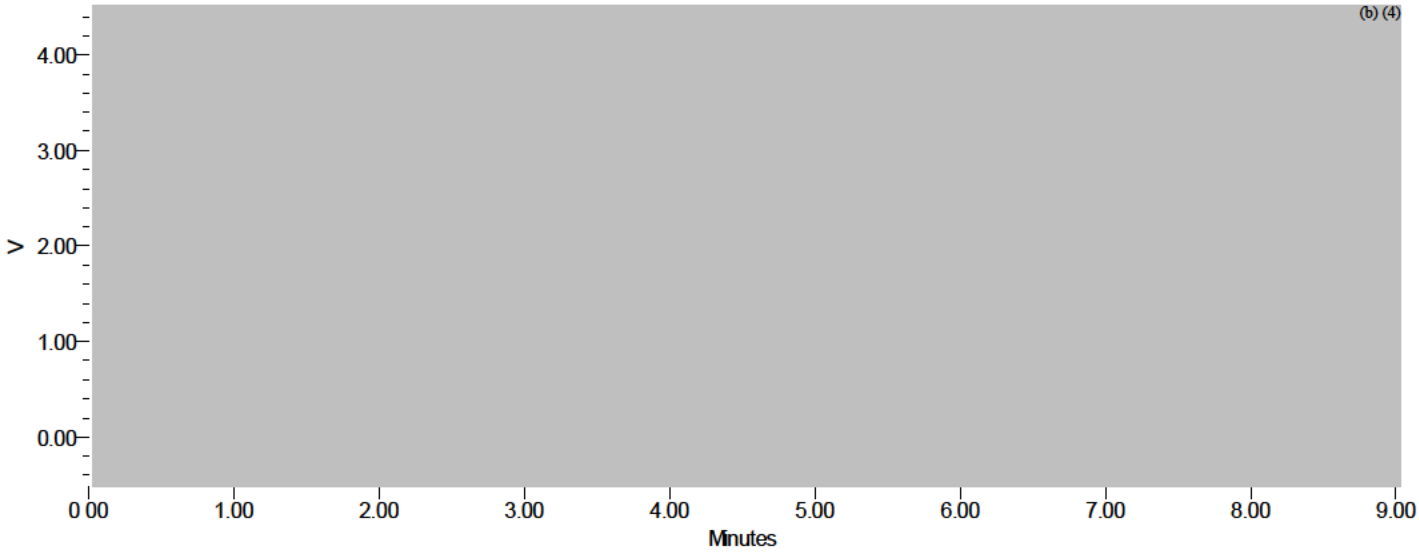

	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

Sample Name:	SS4_5	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	69	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
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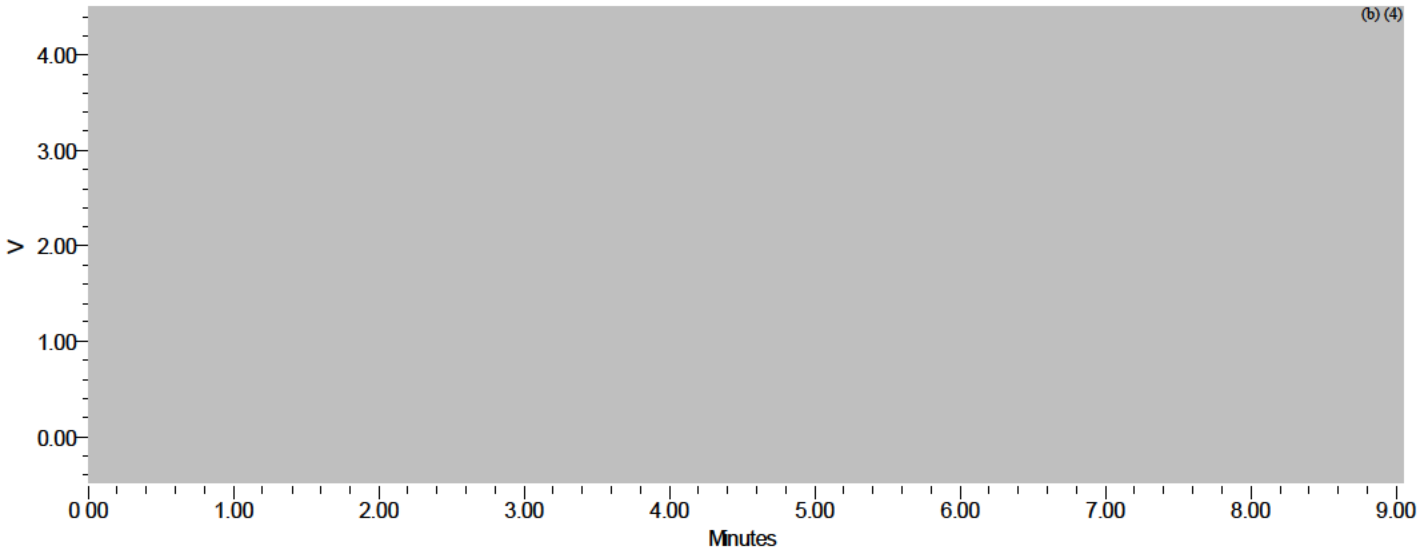

	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

Sample Name:	SS4_6	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	69	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
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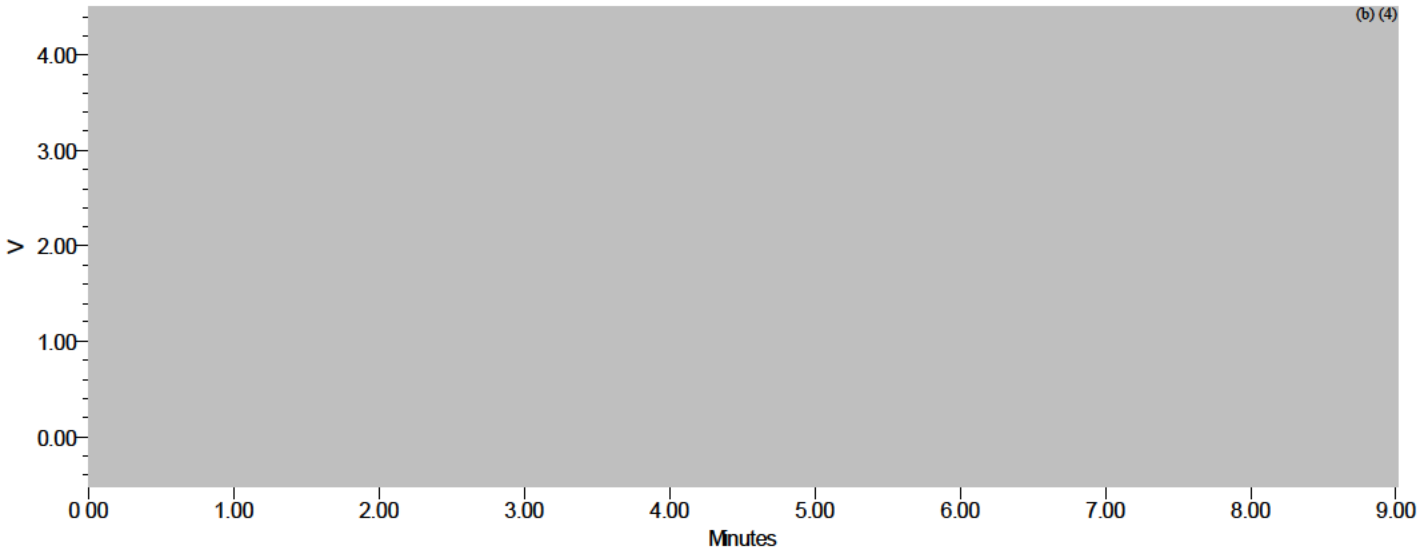
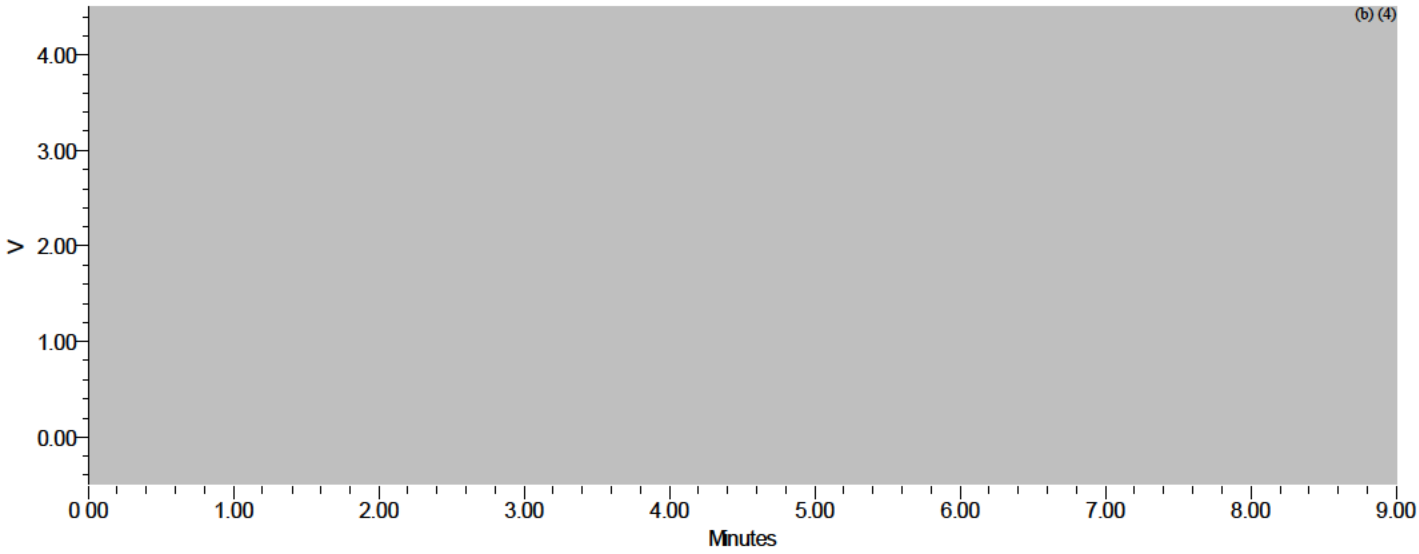


	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

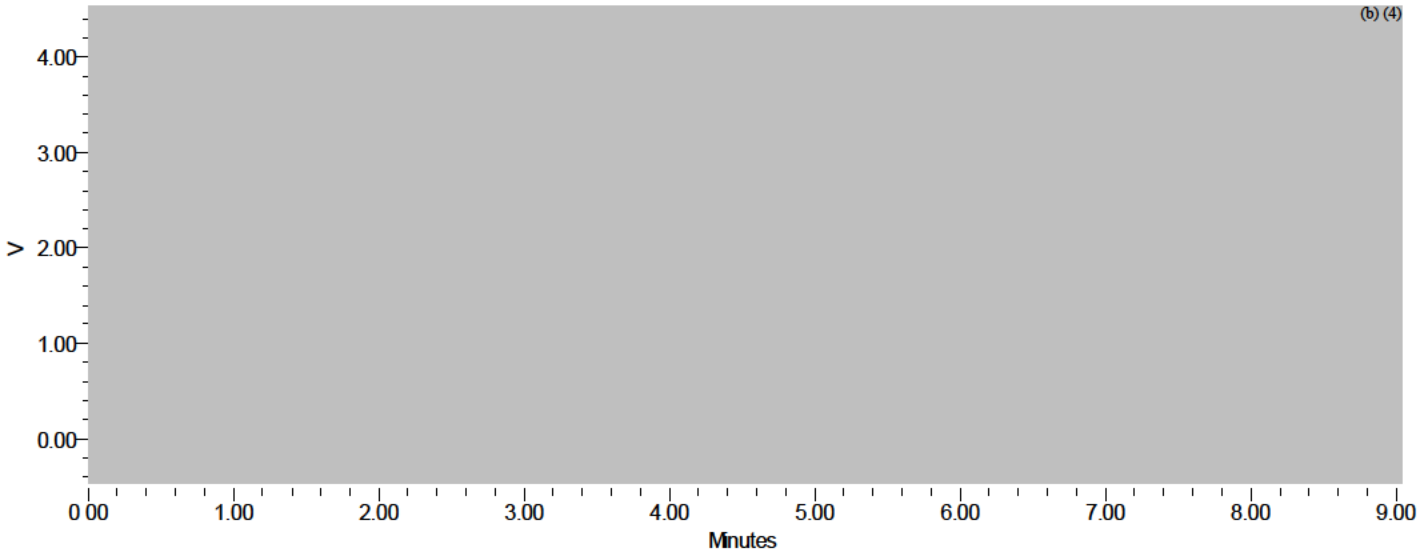
Sample Name:	SS4_7	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	69	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 10:38:58 AM KST		
Date Processed:	12/20/2020 5:20:06 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

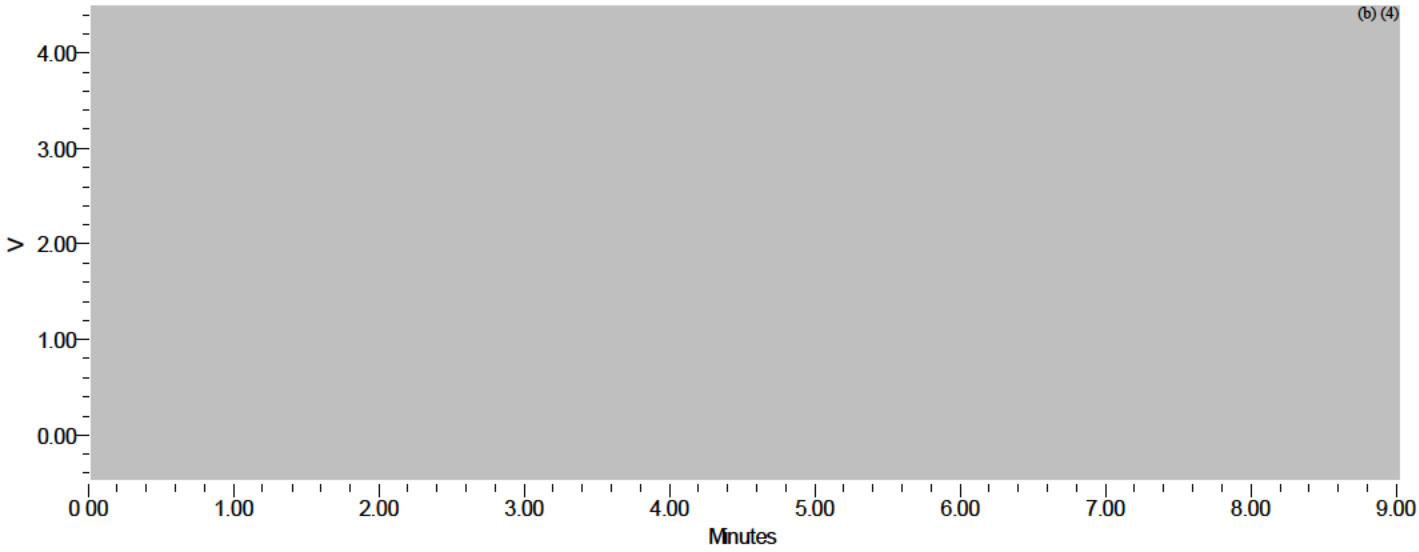
Sample Name:	SS4_8	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	69	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 10:48:37 AM KST		
Date Processed:	12/20/2020 5:20:06 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

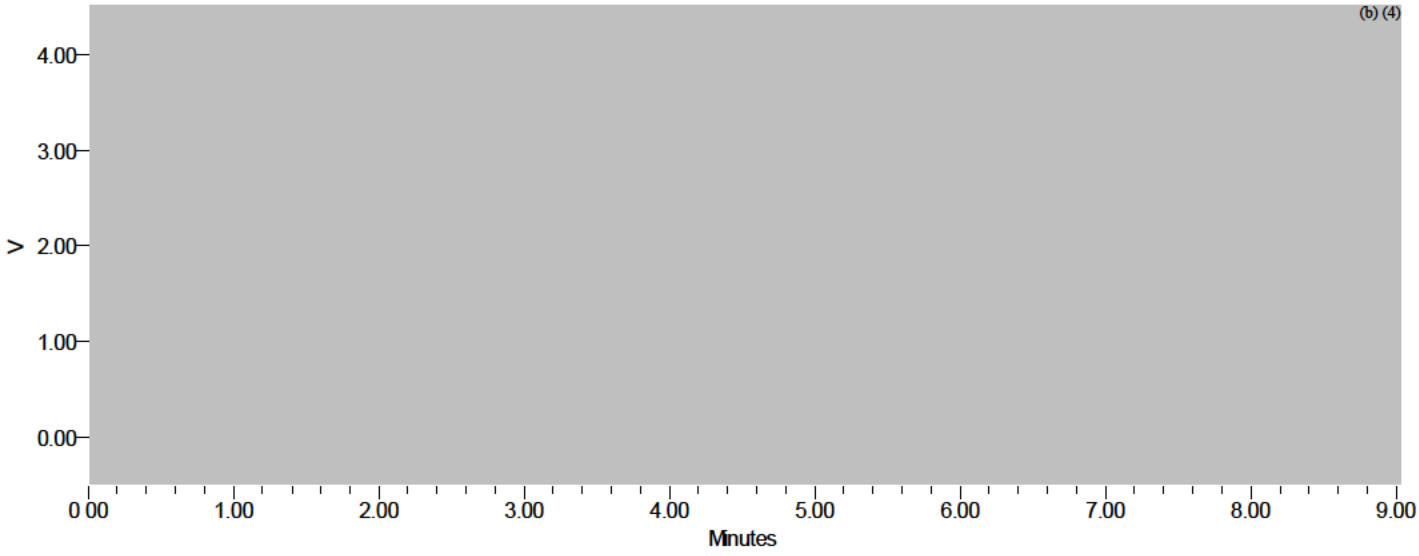
Sample Name:	SS4_9	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	69	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 10:58:16 AM KST		
Date Processed:	12/20/2020 5:20:07 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

Sample Name:	SS4_10	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	69	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 11:07:51 AM KST		
Date Processed:	12/20/2020 5:20:07 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

Software: Empower 3 Software Build 3471

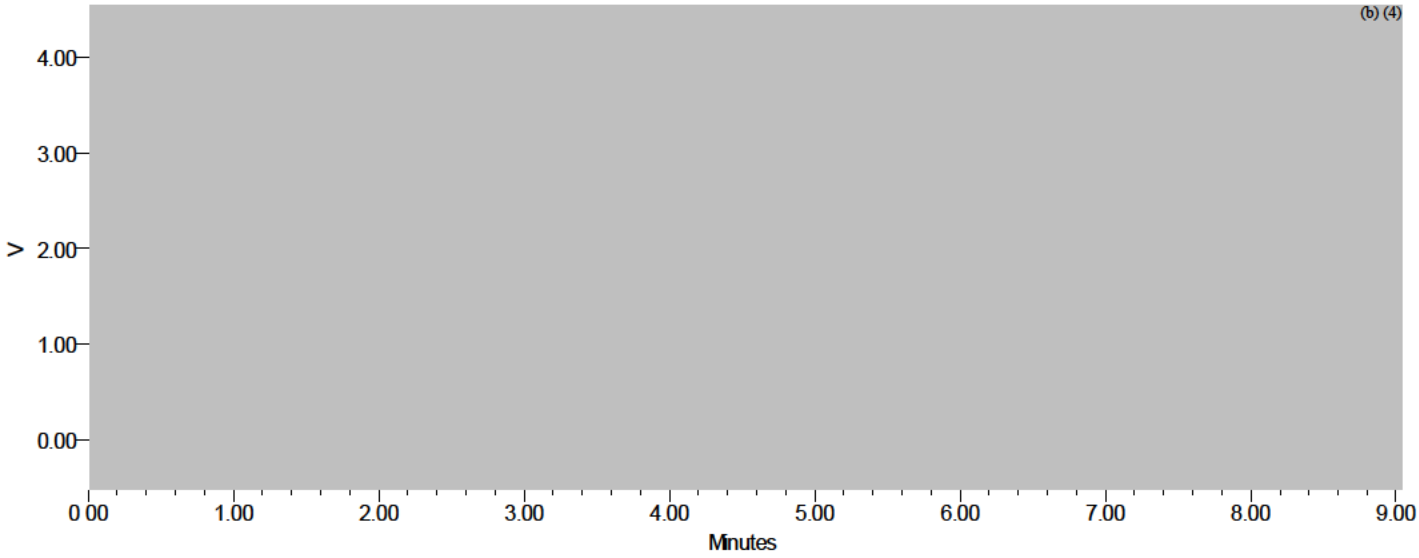
Sample Set Name:	Granule Valine_2 Day	Processed By:	System/Administrator
Sample Set Method:	Granule Valine_2 Day	Printed By:	System
System Node:	Client13	Result Set ID:	
System Name:	UC10	# of Results:	19
Acquired By:	System		
Sample Set Start Date:	12/18/2020 12:24:43 PM KST		
Sample Set Finish Date:	12/19/2020 6:05:57 AM KST		

Sample Set Table

	Sample Name	Sample Type	Vial	Inj #	Injection Volume (ul)	Acquistion Method Set	Processed Channel Descr.	(b) (4)
1	STD_1	Unknown	77	1	5.00	VAL_ACR	Detector A	
2	H1_1	Unknown	78	1	5.00	VAL_ACR	Detector A	
3	H2_1	Unknown	79	1	5.00	VAL_ACR	Detector A	
4	H3_1	Unknown	80	1	5.00	VAL_ACR	Detector A	
5	H4_1	Unknown	81	1	5.00	VAL_ACR	Detector A	
6	H5_1	Unknown	82	1	5.00	VAL_ACR	Detector A	
7	STD_2	Unknown	83	1	5.00	VAL_ACR	Detector A	
8	H1_2	Unknown	84	1	5.00	VAL_ACR	Detector A	
9	H2_2	Unknown	85	1	5.00	VAL_ACR	Detector A	
10	H3_2	Unknown	86	1	5.00	VAL_ACR	Detector A	
11	H4_2	Unknown	87	1	5.00	VAL_ACR	Detector A	
12	H5_2	Unknown	88	1	5.00	VAL_ACR	Detector A	
13	STD_3	Unknown	89	1	5.00	VAL_ACR	Detector A	
14	H1_3	Unknown	90	1	5.00	VAL_ACR	Detector A	
15	H2_3	Unknown	91	1	5.00	VAL_ACR	Detector A	
16	H3_3	Unknown	92	1	5.00	VAL_ACR	Detector A	
17	H4_3	Unknown	93	1	5.00	VAL_ACR	Detector A	
18	H5_3	Unknown	94	1	5.00	VAL_ACR	Detector A	
19	STD_4	Unknown	95	1	5.00	VAL_ACR	Detector A	

SAMPLE INFORMATION

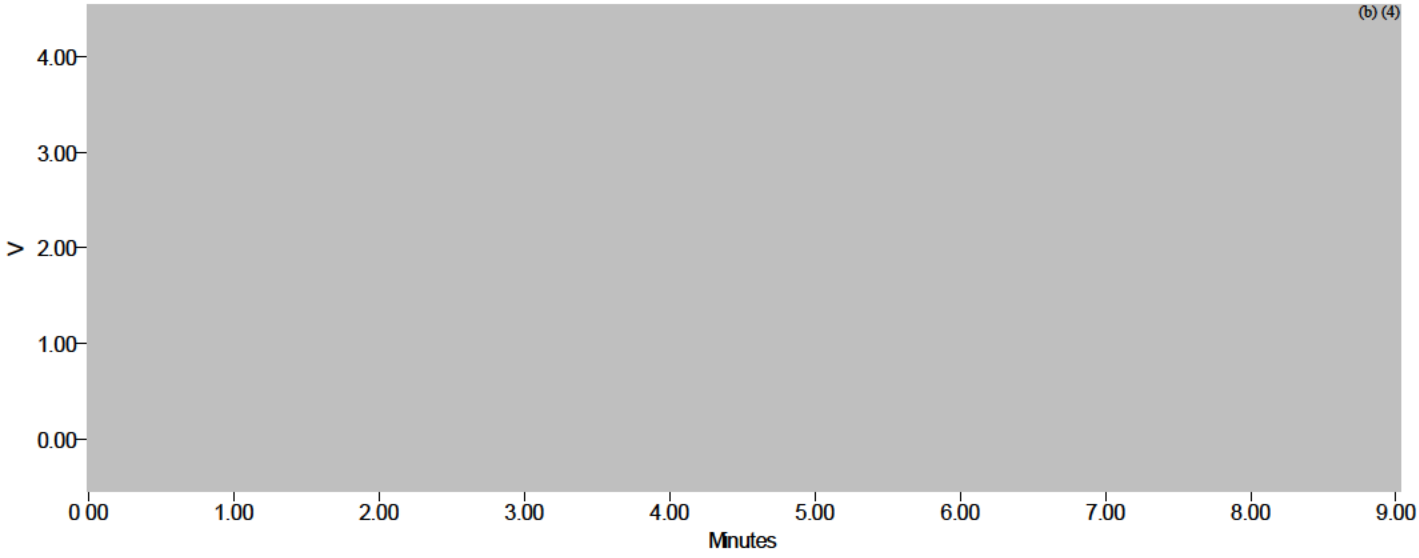
Sample Name:	STD_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_2 Day
Vial:	77	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 1:42:27 PM KST		
Date Processed:	12/20/2020 5:04:39 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

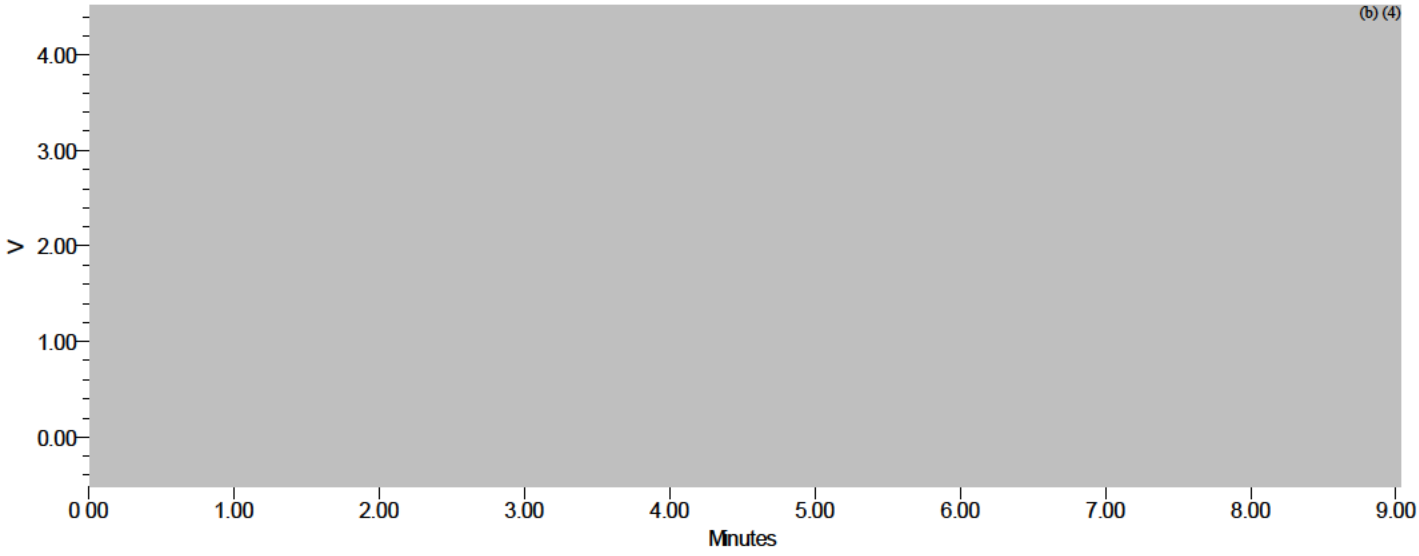
Sample Name:	H1_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_2 Day
Vial:	78	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 1:52:09 PM KST		
Date Processed:	12/20/2020 5:04:40 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

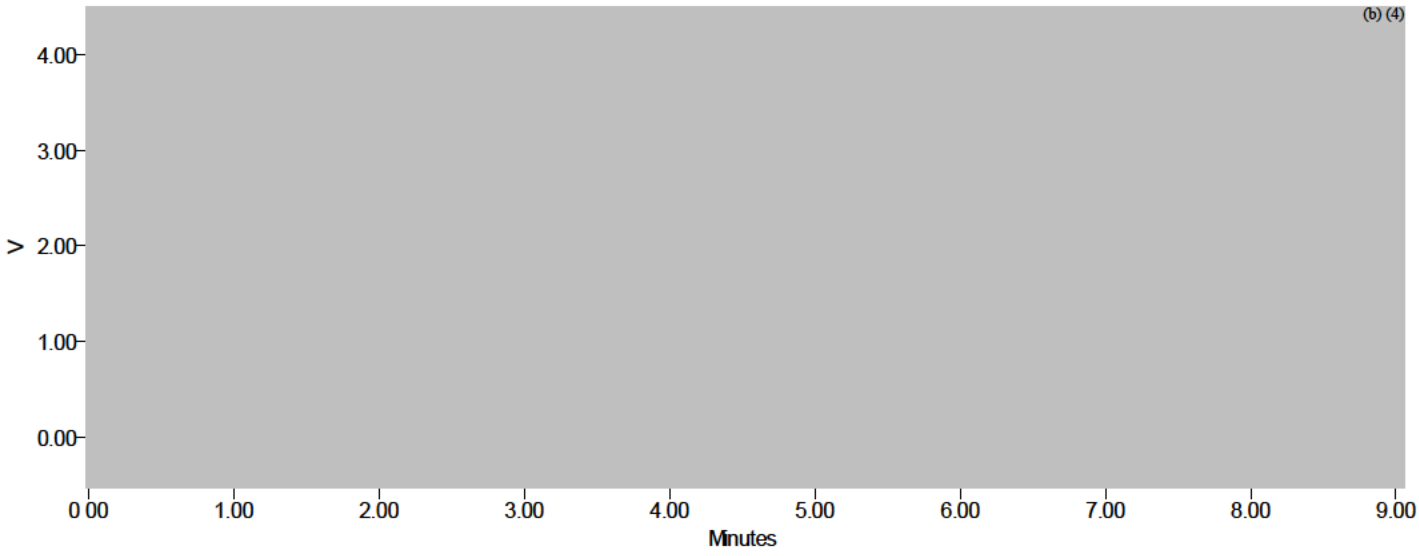
Sample Name:	H2_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_2 Day
Vial:	79	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 2:01:47 PM KST		
Date Processed:	12/20/2020 5:04:40 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

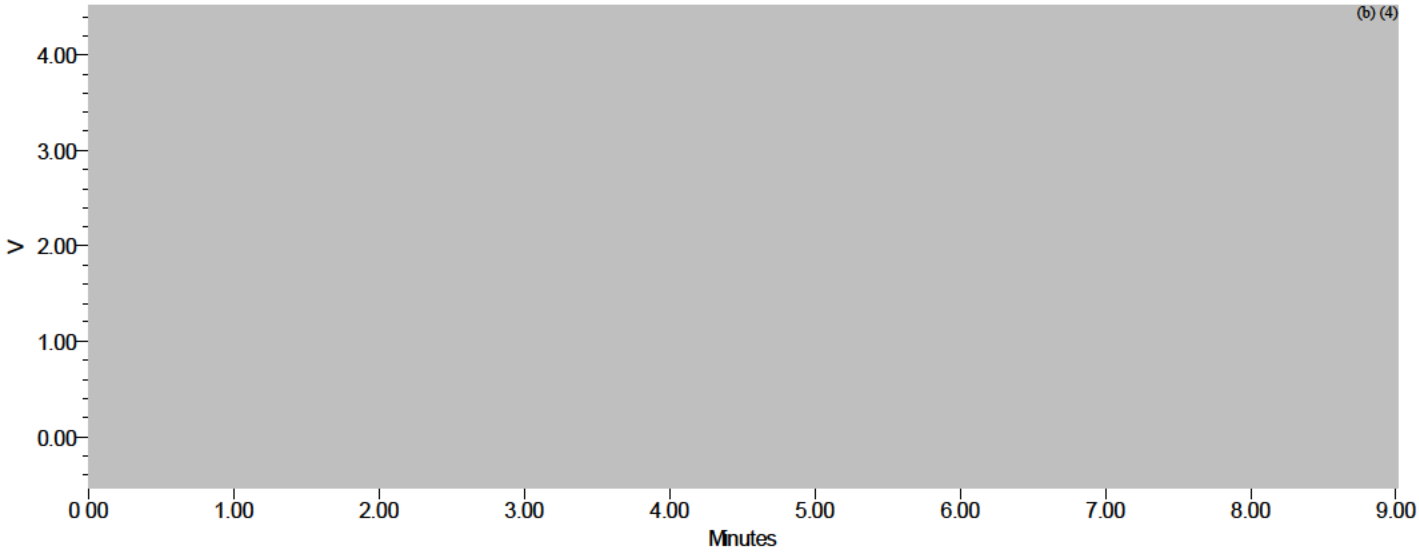
Sample Name:	H3_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_2 Day
Vial:	80	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 2:11:26 PM KST		
Date Processed:	12/20/2020 5:04:40 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

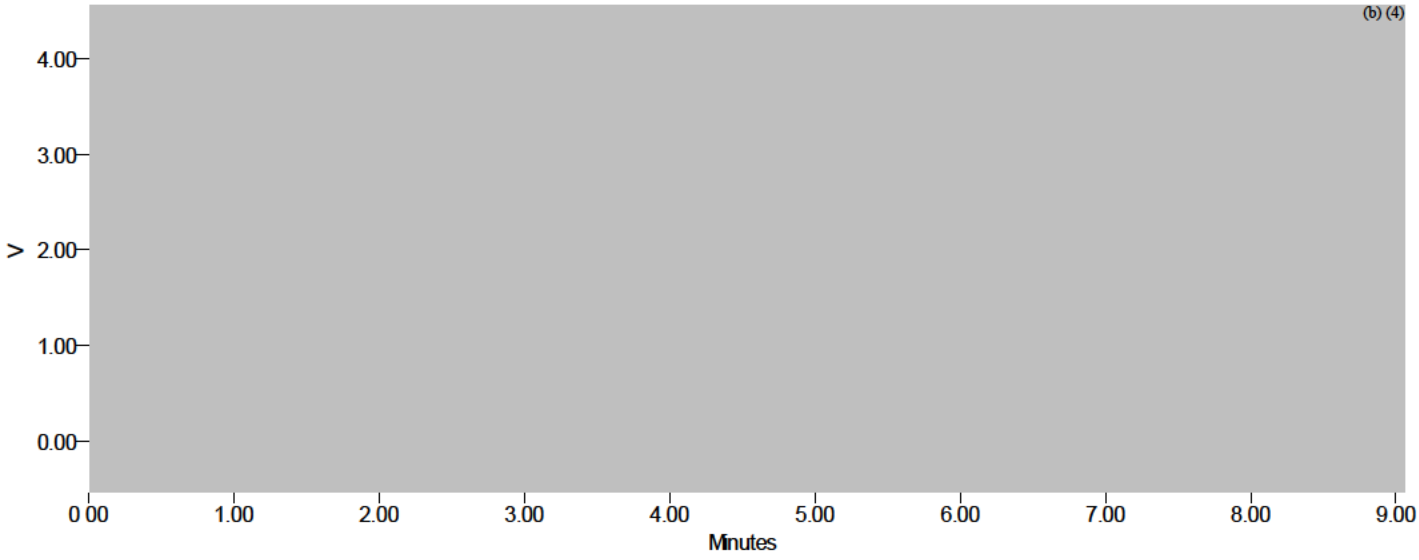
Sample Name:	H4_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_2 Day
Vial:	81	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 2:21:00 PM KST		
Date Processed:	12/20/2020 5:04:40 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

Sample Name:	H5_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_2 Day
Vial:	82	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 2:30:40 PM KST		
Date Processed:	12/20/2020 5:04:40 PM KST		

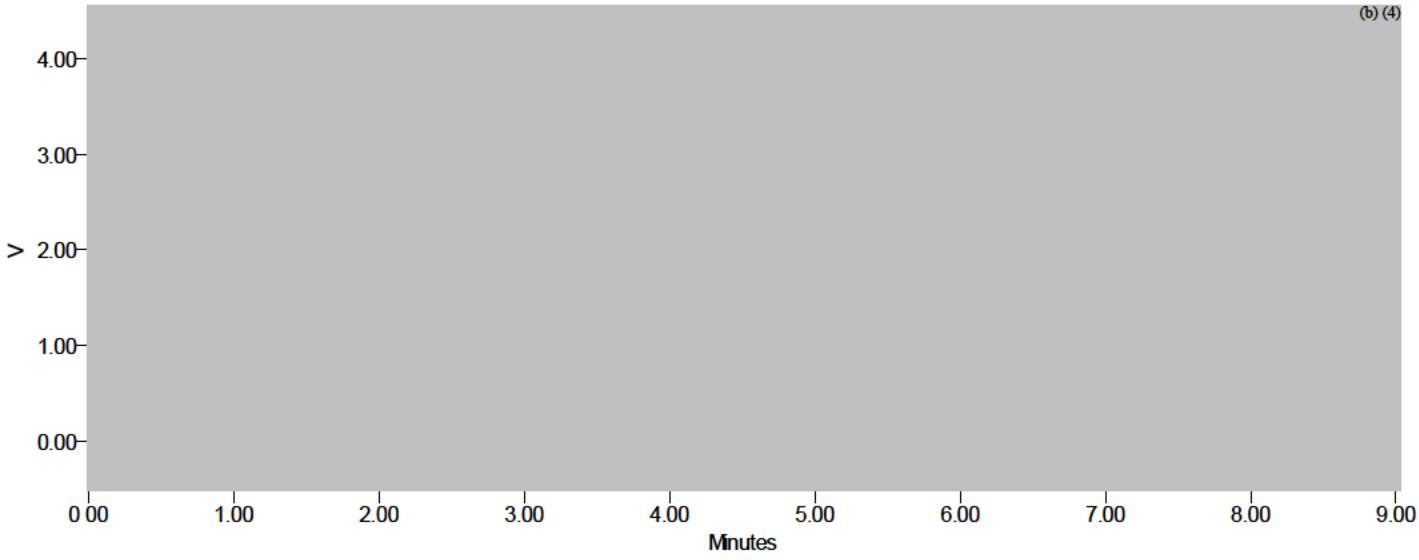


(b) (4)

1	Valine	7.064	15753265	13357

SAMPLE INFORMATION

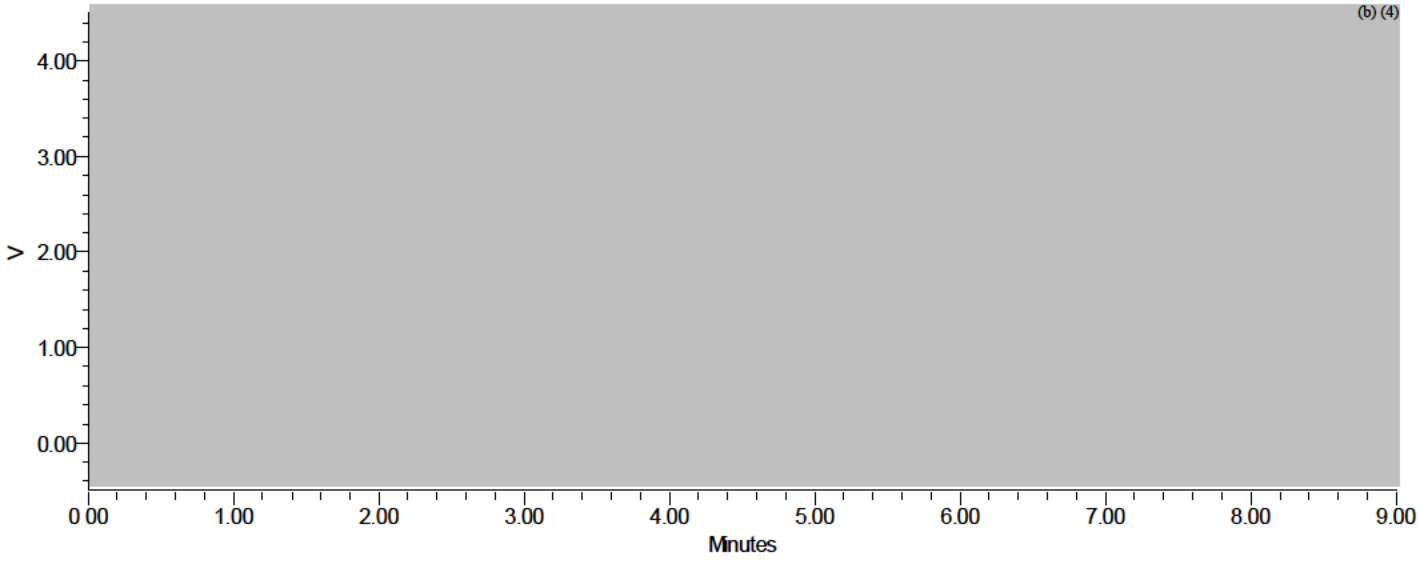
Sample Name:	STD_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_2 Day
Vial:	83	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 2:40:20 PM KST		
Date Processed:	12/20/2020 5:04:41 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

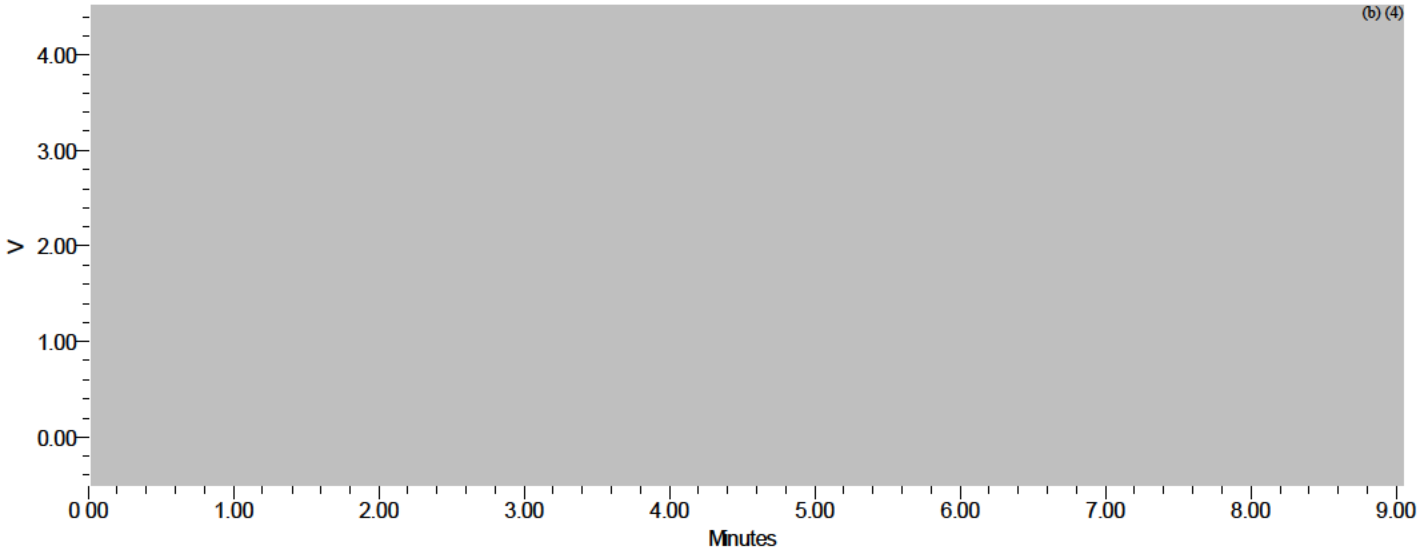
Sample Name:	H1_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_2 Day
Vial:	84	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector (b) (4)
Date Acquired:	12/18/2020 2:49:57 PM KST		
Date Processed:	12/20/2020 5:04:41 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

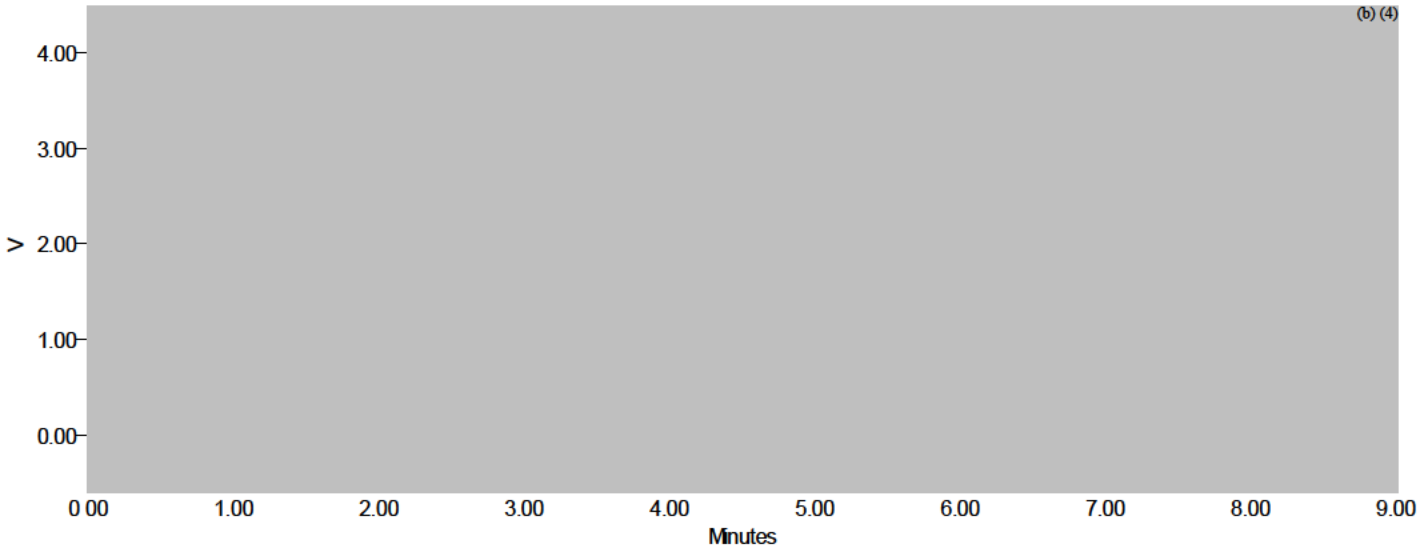
Sample Name:	H2_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_2 Day
Vial:	85	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 2:59:37 PM KST		
Date Processed:	12/20/2020 5:04:41 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

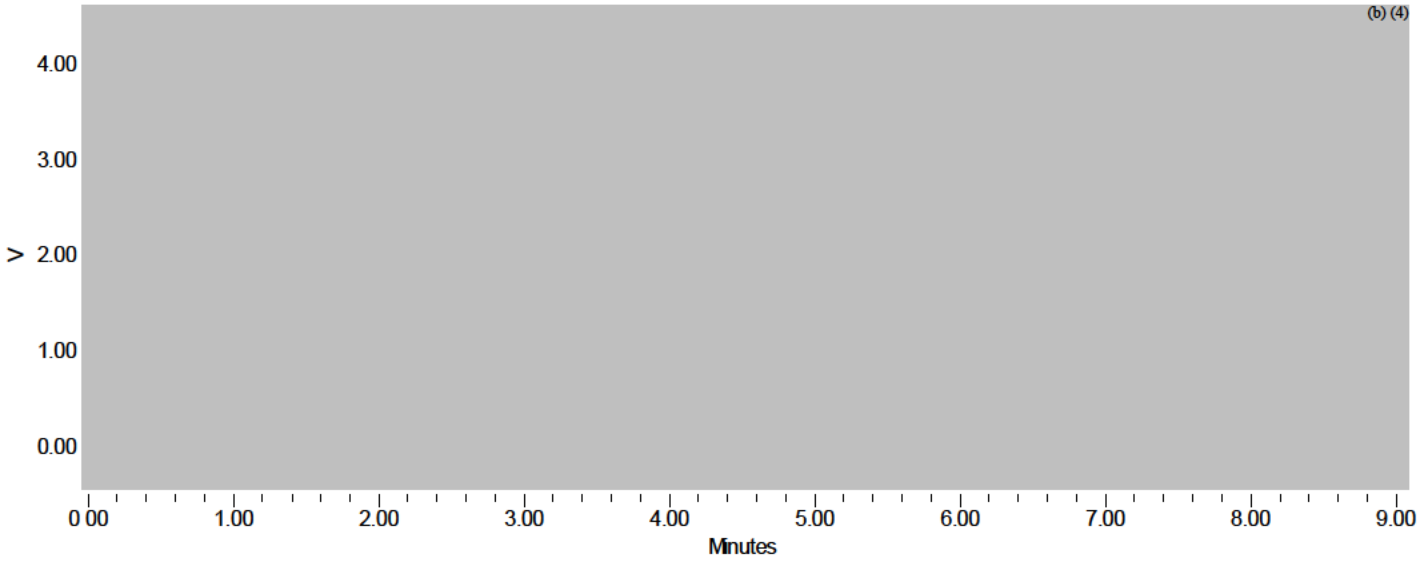
Sample Name:	H3_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_2 Day
Vial:	86	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 3:09:16 PM KST		
Date Processed:	12/20/2020 5:04:41 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

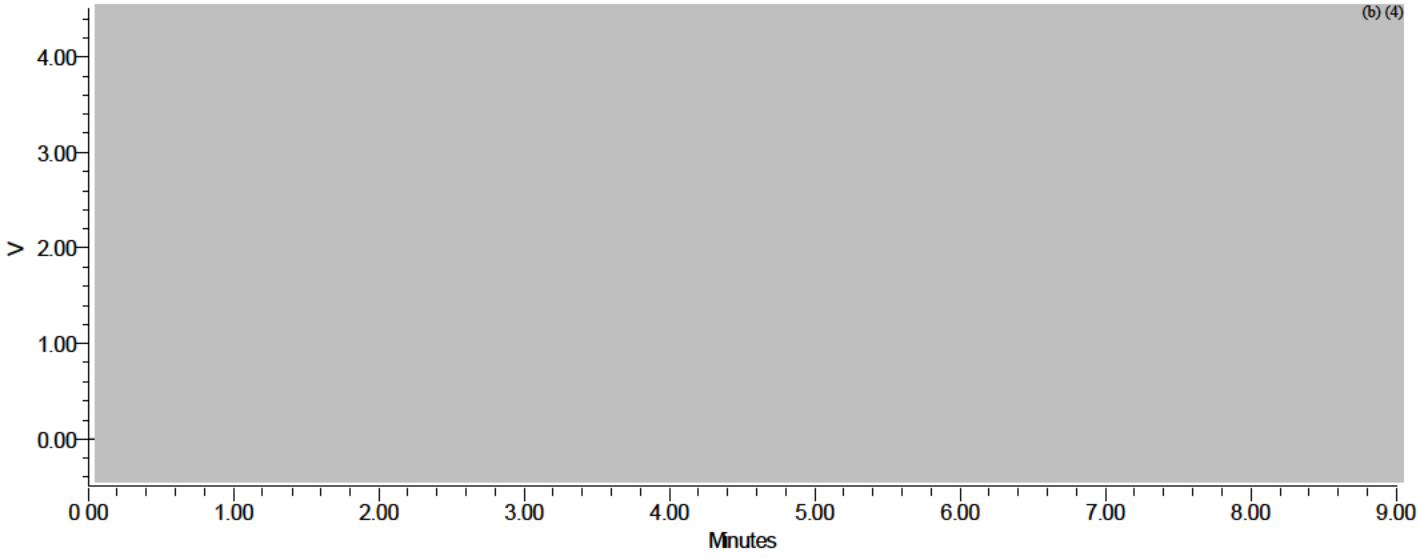
Sample Name:	H4_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_2 Day
Vial:	87	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 3:18:59 PM KST		
Date Processed:	12/20/2020 5:04:41 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

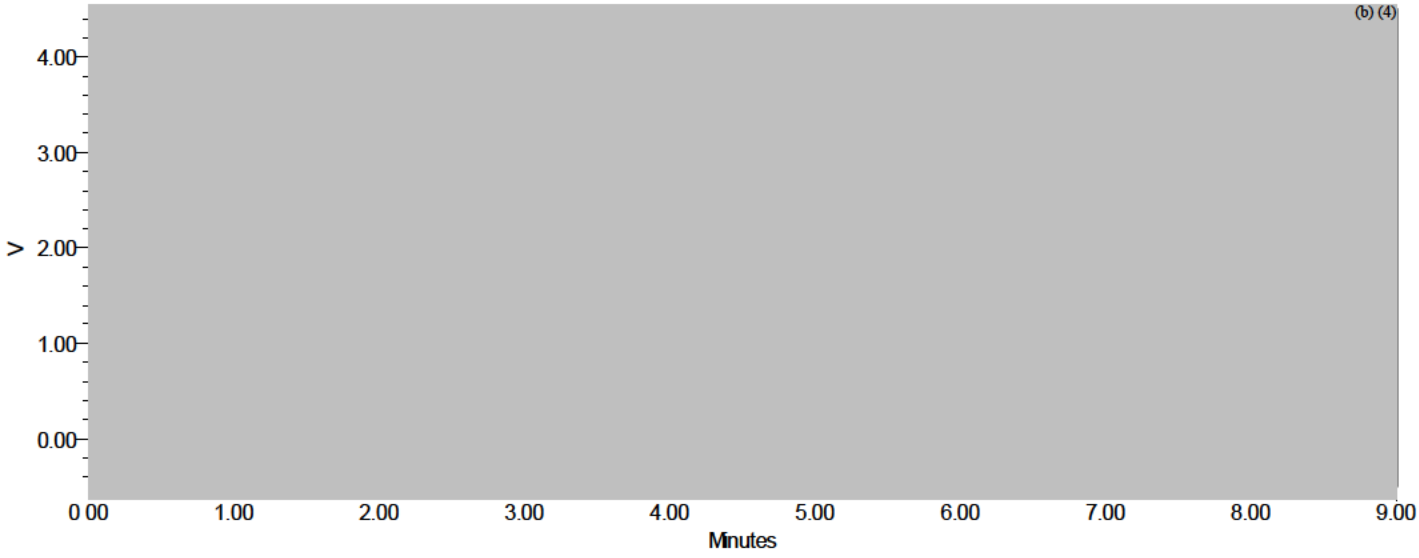
Sample Name:	H5_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_2 Day
Vial:	88	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 3:28:40 PM KST		
Date Processed:	12/20/2020 5:04:41 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

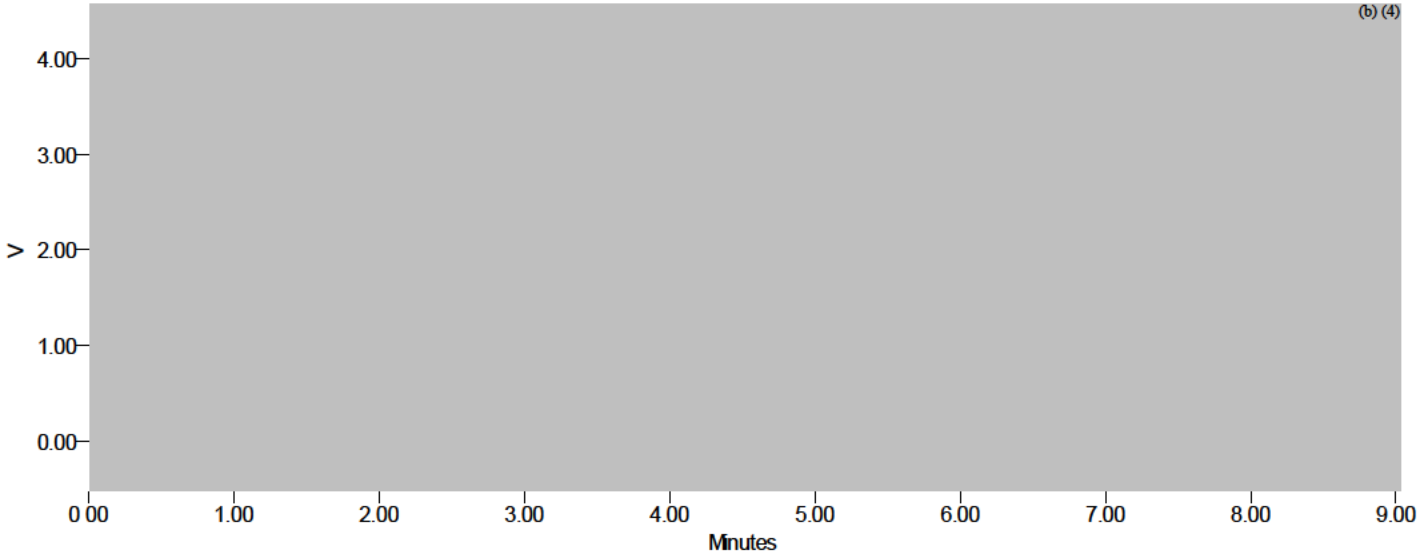
Sample Name:	STD_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_2 Day
Vial:	89	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 3:38:19 PM KST		
Date Processed:	12/20/2020 5:04:42 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

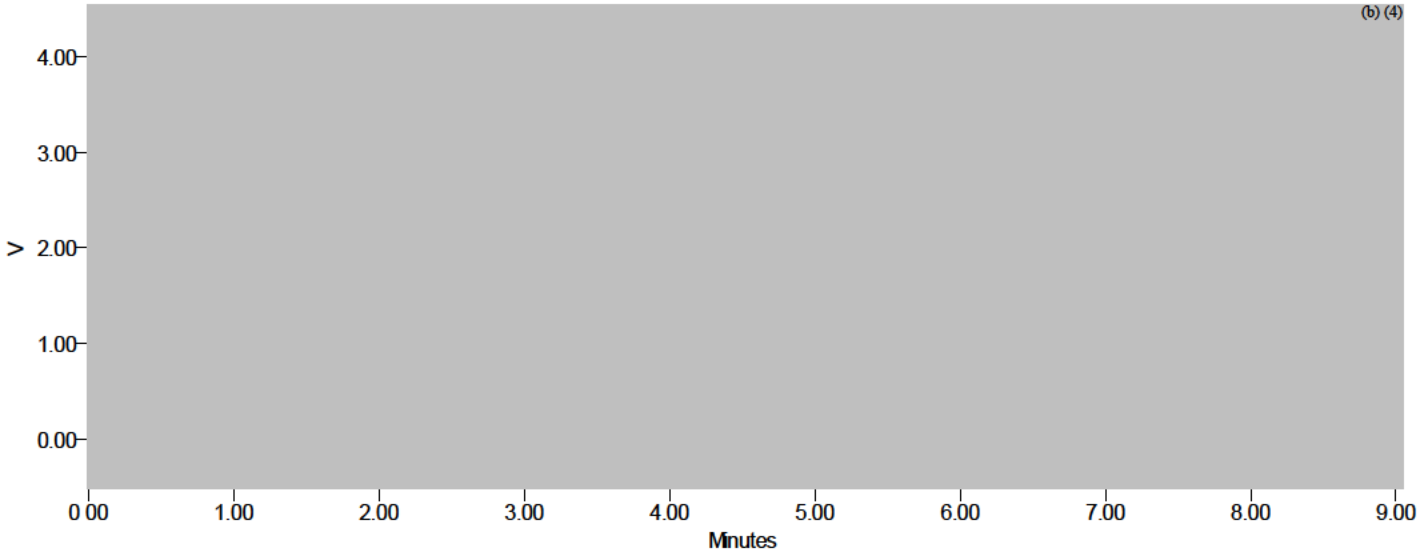
Sample Name:	H1_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_2 Day
Vial:	90	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 3:47:54 PM KST		
Date Processed:	12/20/2020 5:04:42 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

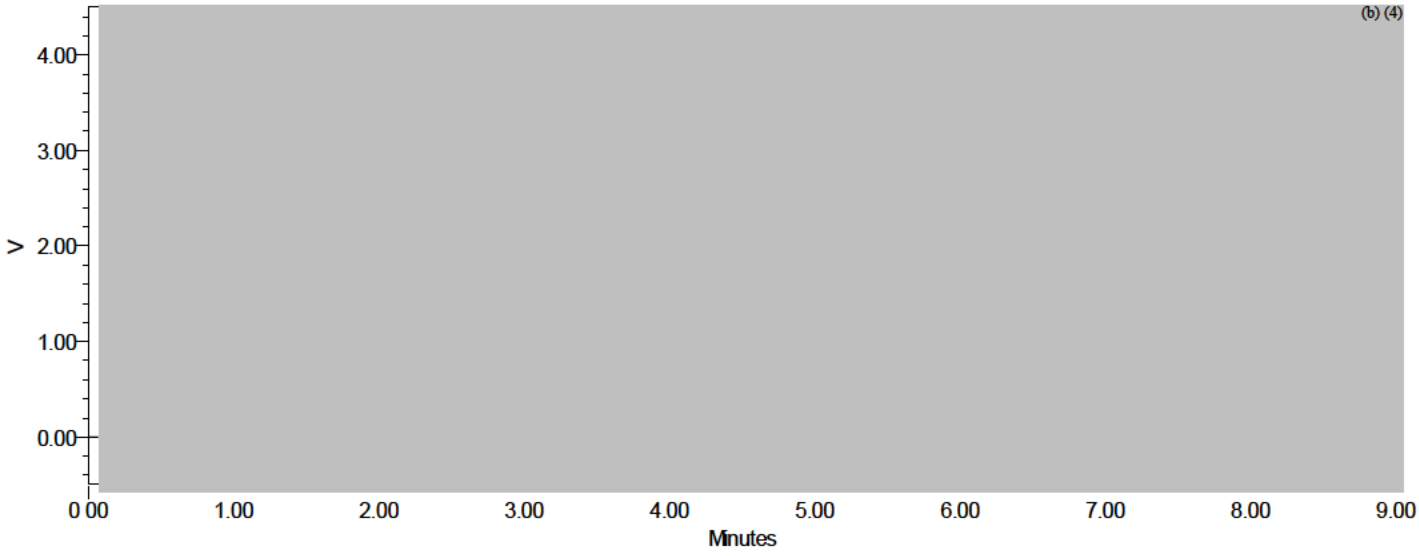
Sample Name:	H2_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_2 Day
Vial:	91	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 3:57:35 PM KST		
Date Processed:	12/20/2020 5:04:42 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

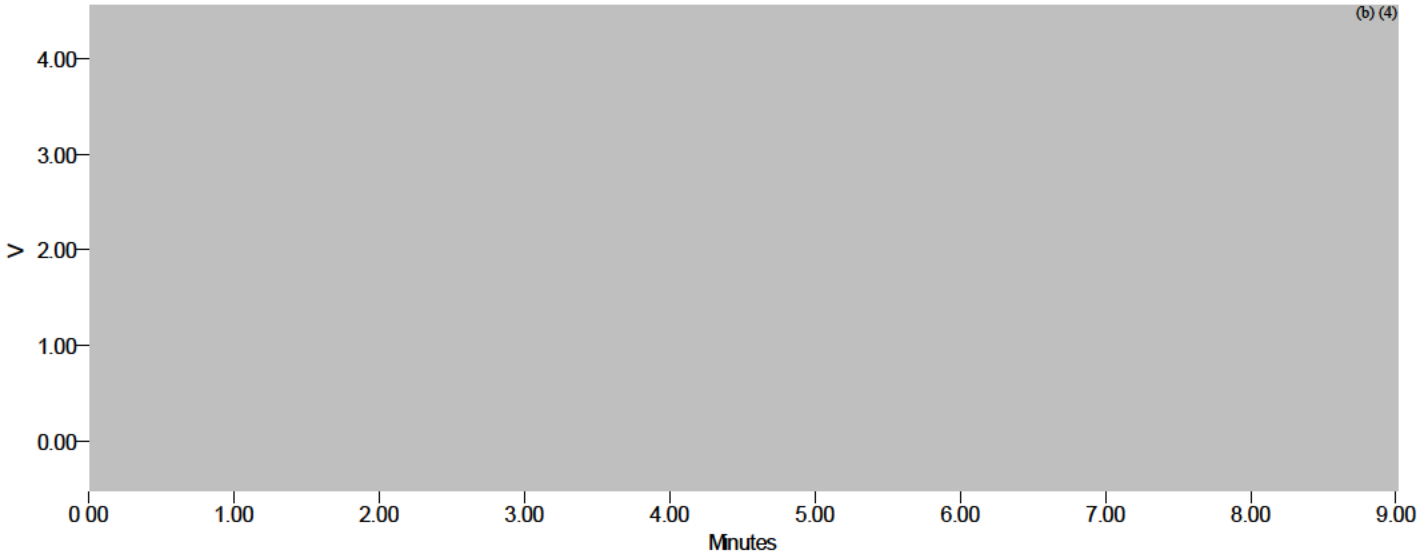
Sample Name:	H3_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_2 Day
Vial:	92	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 4:07:14 PM KST		
Date Processed:	12/20/2020 5:04:42 PM KST		



	Peak Name	RT	Area	USP Plate Count (b) (4)
1	Valine			

SAMPLE INFORMATION

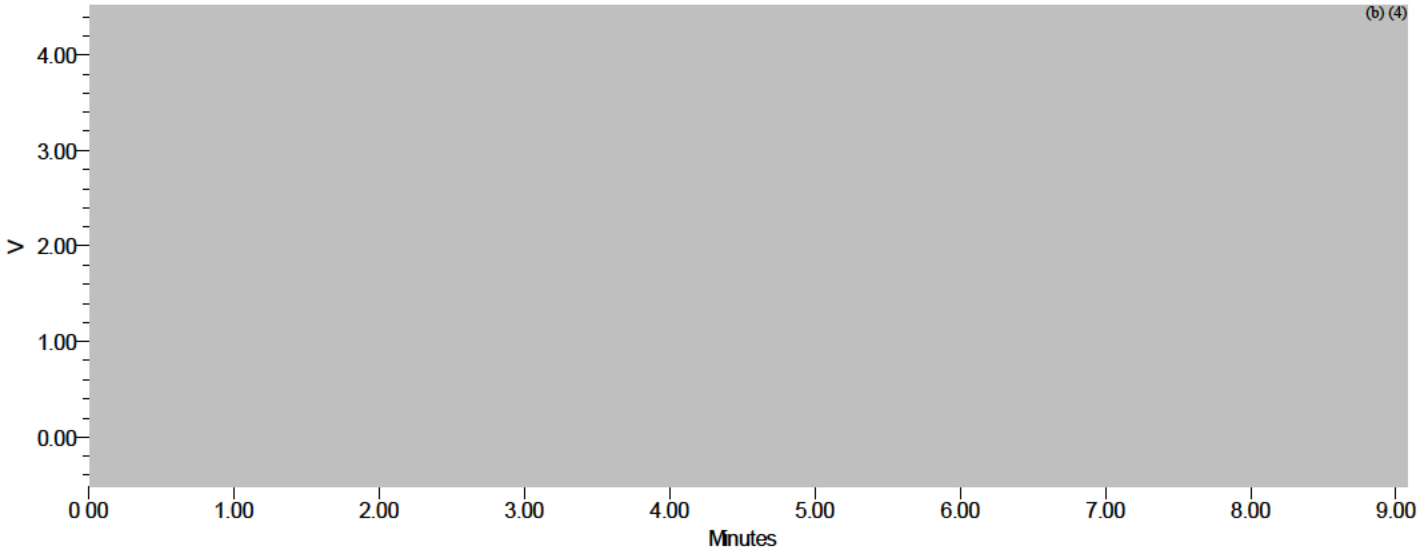
Sample Name:	H4_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_2 Day
Vial:	93	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 4:16:55 PM KST		
Date Processed:	12/20/2020 5:04:42 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

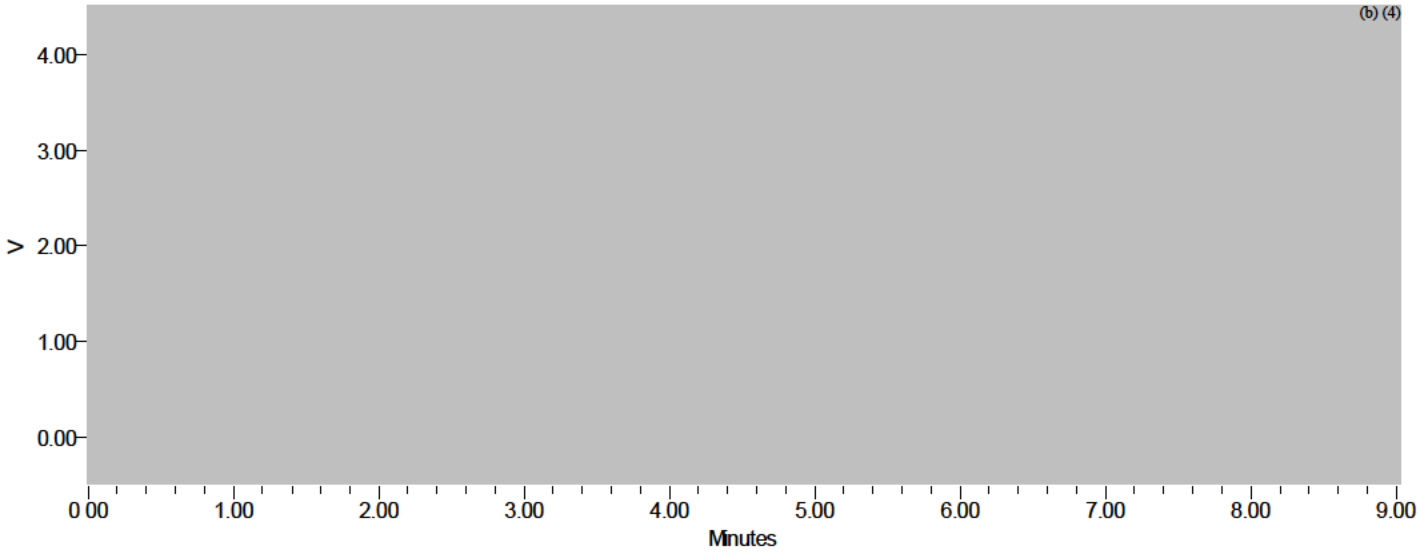
Sample Name:	H5_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_2 Day
Vial:	94	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 4:26:37 PM KST		
Date Processed:	12/20/2020 5:04:43 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

Sample Name:	STD_4	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_2 Day
Vial:	95	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 4:36:18 PM KST		
Date Processed:	12/20/2020 5:04:43 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

Software: Empower 3 Software Build 3471

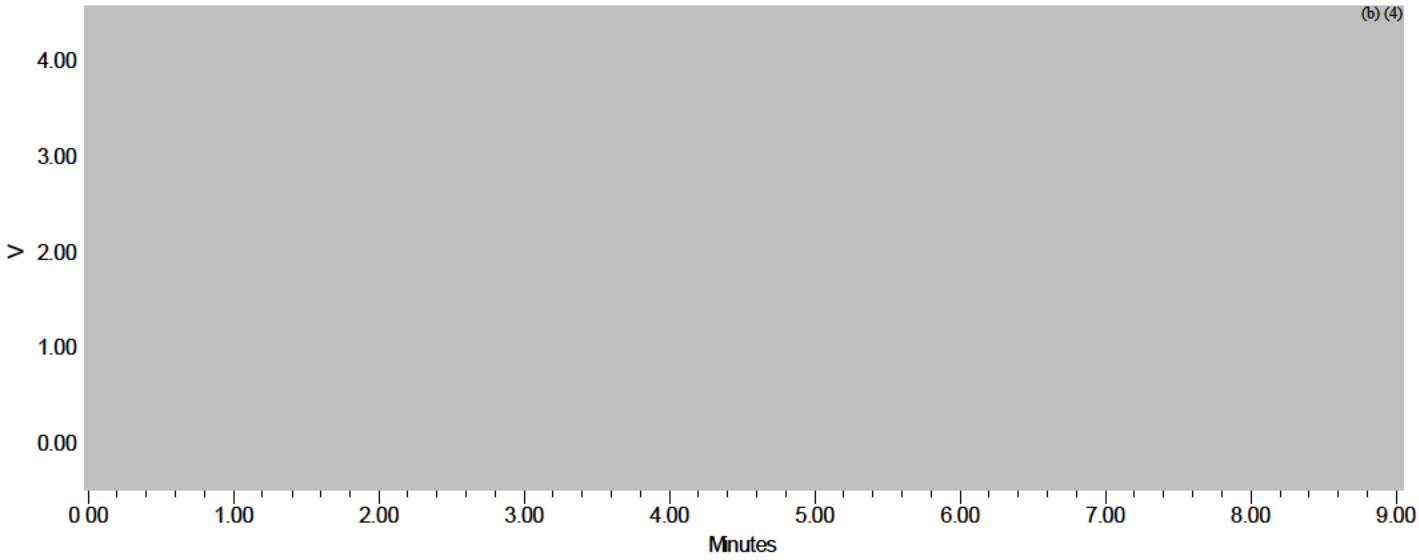
Sample Set Name:	Granule Valine_1 Day	Processed By:	System/Administrator
Sample Set Method:	Granule Valine_1 Day	Printed By:	System
System Node:	Client13	Result Set ID:	
System Name:	UC10	# of Results:	21
Acquired By:	System		
Sample Set Start Date:	12/17/2020 6:52:02 PM KST		
Sample Set Finish Date:	12/18/2020 12:24:42 PM KST		

Sample Set Table

	Sample Name	Sample Type	Vial	Inj #	Injection Volume (ul)	Acquistion Method Set	Processed Channel Descr.	(b) (4)
1	SSTD11_1	Unknown	1	1	5.00	VAL_ACR	Detector A	
2	SSPL11_1	Unknown	2	1	5.00	VAL_ACR	Detector A	
3	SSTD11_2	Unknown	3	1	5.00	VAL_ACR	Detector A	
4	SSPL11_2	Unknown	4	1	5.00	VAL_ACR	Detector A	
5	SSTD11_3	Unknown	5	1	5.00	VAL_ACR	Detector A	
6	SSPL11_3	Unknown	6	1	5.00	VAL_ACR	Detector A	
7	SSTD11_4	Unknown	7	1	5.00	VAL_ACR	Detector A	
8	SSTD12_1	Unknown	8	1	5.00	VAL_ACR	Detector A	
9	SSPL12_1	Unknown	9	1	5.00	VAL_ACR	Detector A	
10	SSTD12_2	Unknown	10	1	5.00	VAL_ACR	Detector A	
11	SSPL12_2	Unknown	11	1	5.00	VAL_ACR	Detector A	
12	SSTD12_3	Unknown	12	1	5.00	VAL_ACR	Detector A	
13	SSPL12_3	Unknown	13	1	5.00	VAL_ACR	Detector A	
14	SSTD12_4	Unknown	14	1	5.00	VAL_ACR	Detector A	
15	SSTD13_1	Unknown	15	1	5.00	VAL_ACR	Detector A	
16	SSPL13_1	Unknown	16	1	5.00	VAL_ACR	Detector A	
17	SSTD13_2	Unknown	17	1	5.00	VAL_ACR	Detector A	
18	SSPL13_2	Unknown	18	1	5.00	VAL_ACR	Detector A	
19	SSTD13_3	Unknown	19	1	5.00	VAL_ACR	Detector A	
20	SSPL13_3	Unknown	20	1	5.00	VAL_ACR	Detector A	
21	SSTD13_4	Unknown	21	1	5.00	VAL_ACR	Detector A	

SAMPLE INFORMATION

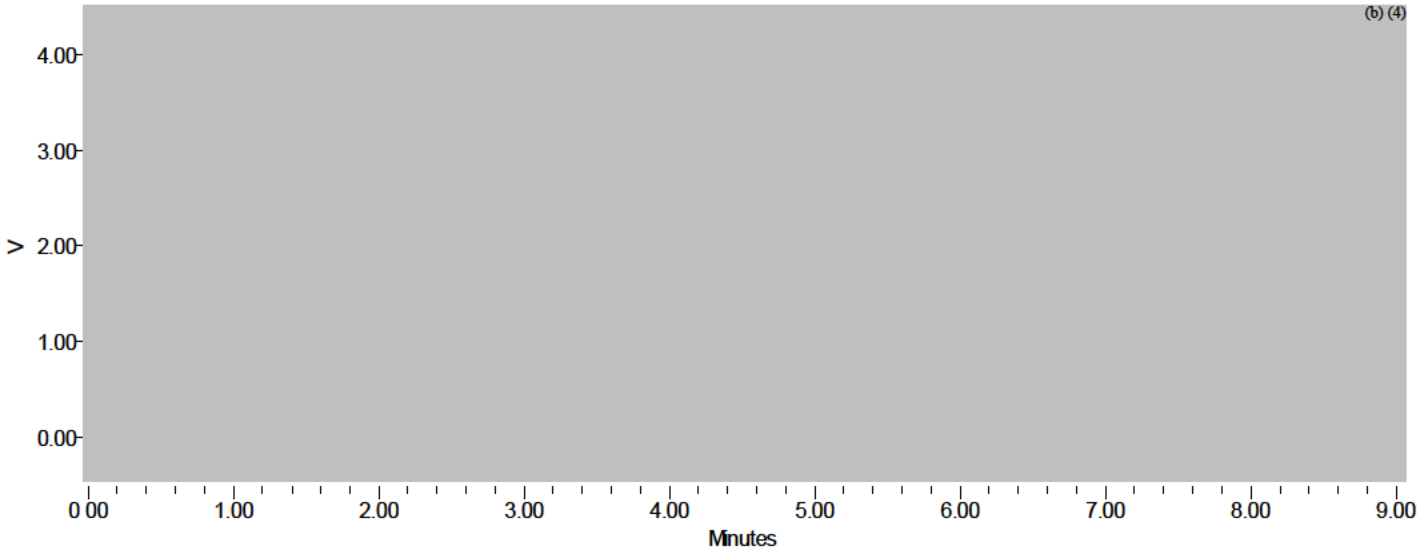
Sample Name:	SSTD11_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	1	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/17/2020 7:50:31 PM KST		
Date Processed:	12/20/2020 4:56:56 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

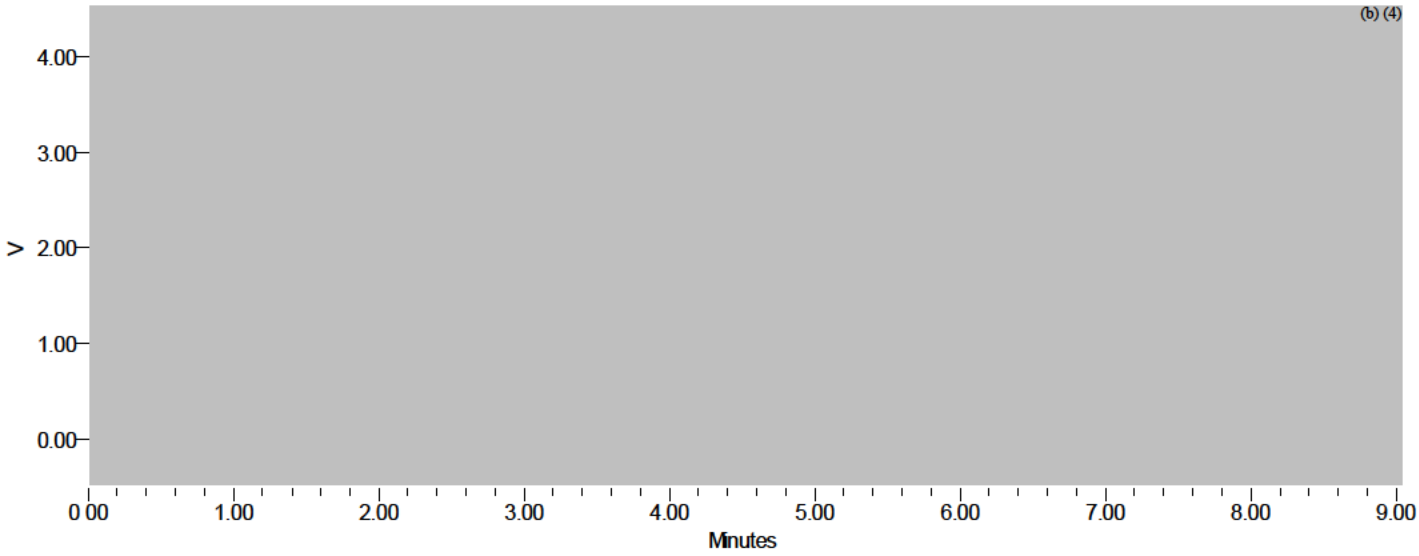
Sample Name:	SSPL11_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	2	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/17/2020 8:00:14 PM KST		
Date Processed:	12/20/2020 4:56:56 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

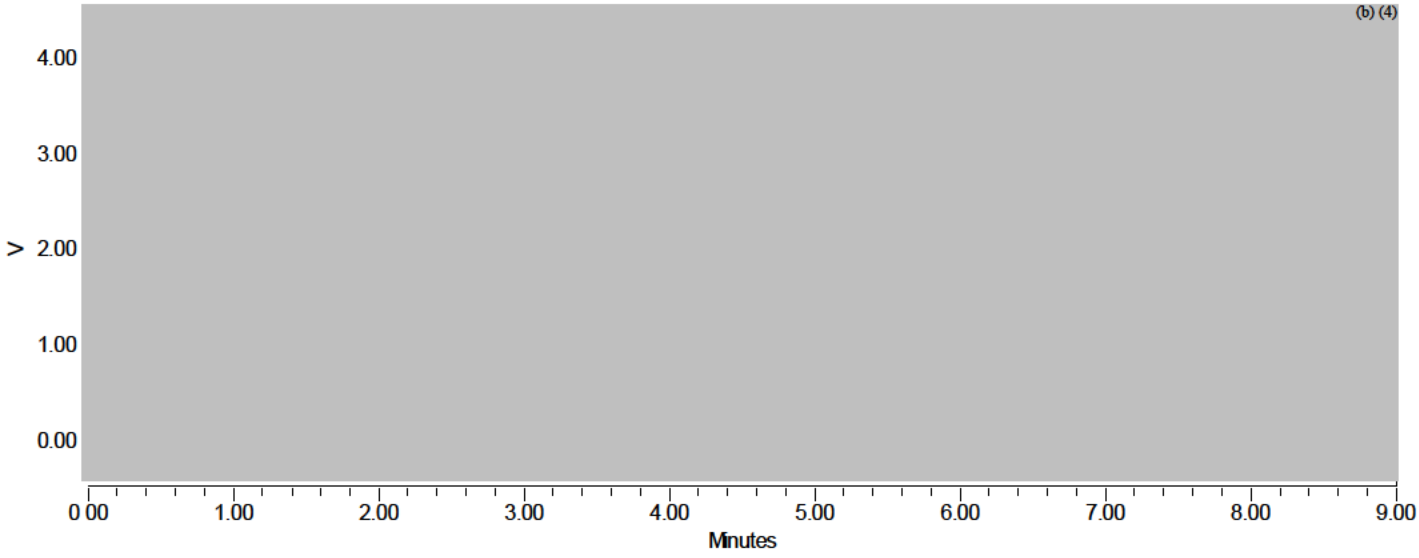
Sample Name:	SSTD11_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	3	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/17/2020 8:09:55 PM KST		
Date Processed:	12/20/2020 4:56:56 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

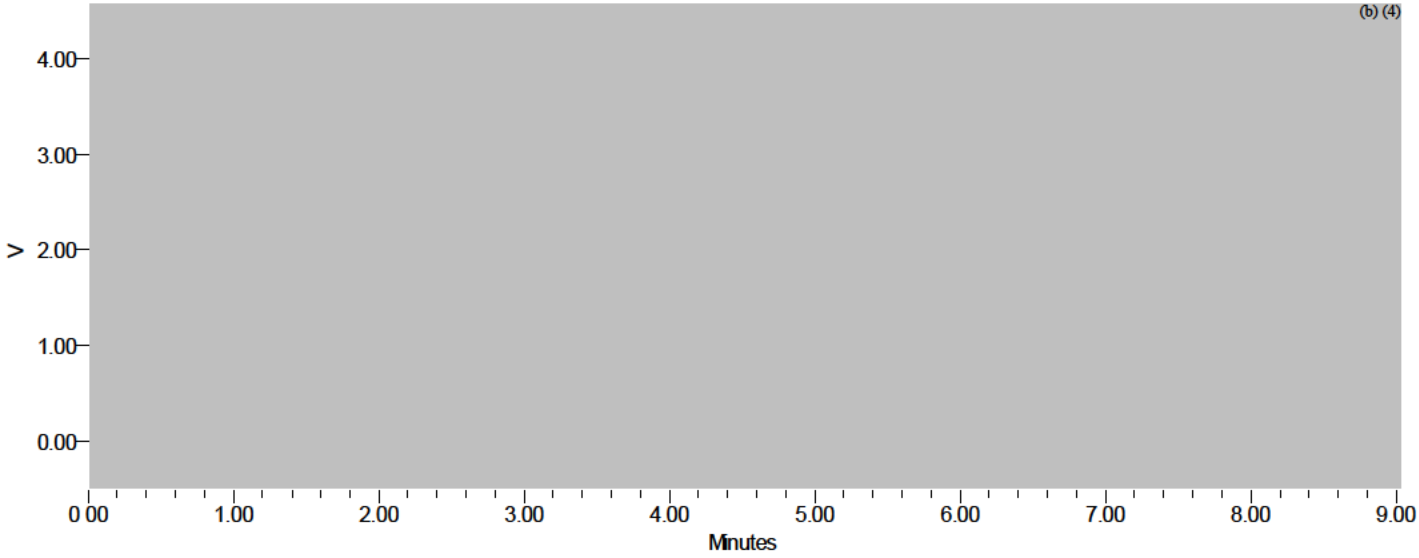
Sample Name:	SSPL11_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	4	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/17/2020 8:19:29 PM KST		
Date Processed:	12/20/2020 4:56:56 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

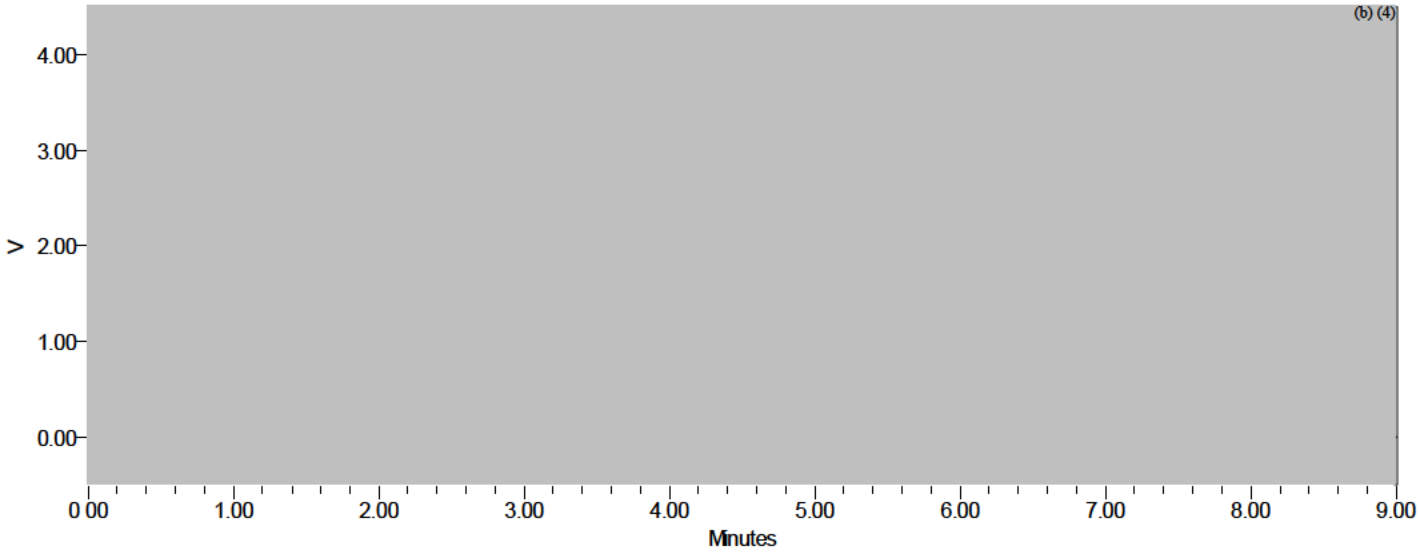
Sample Name:	SSTD11_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	5	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/17/2020 8:29:11 PM KST		
Date Processed:	12/20/2020 4:56:57 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

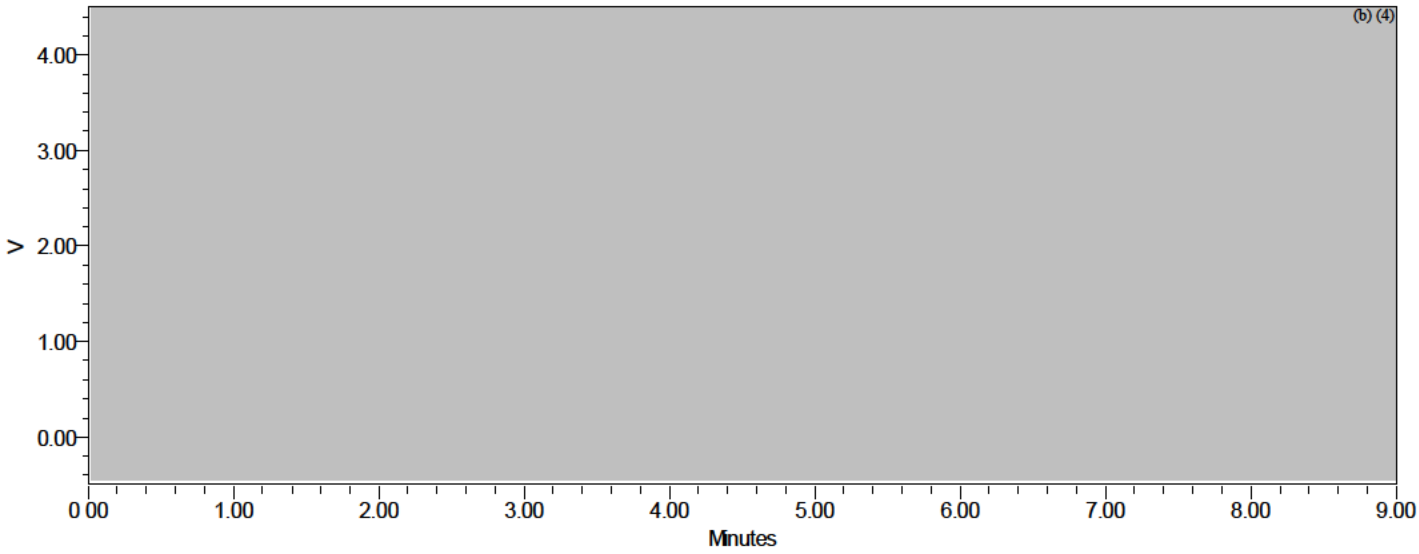
Sample Name:	SSPL11_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	6	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/17/2020 8:38:51 PM KST		
Date Processed:	12/20/2020 4:56:57 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

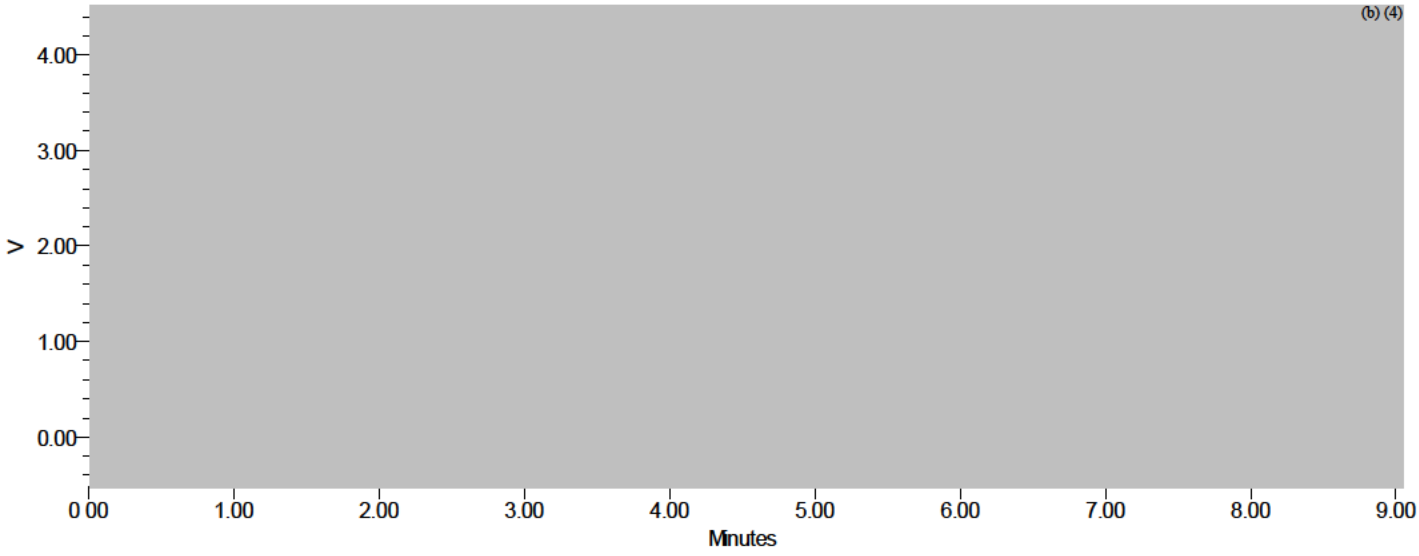
Sample Name:	SSTD11_4	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	7	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/17/2020 8:48:29 PM KST		
Date Processed:	12/20/2020 4:56:57 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

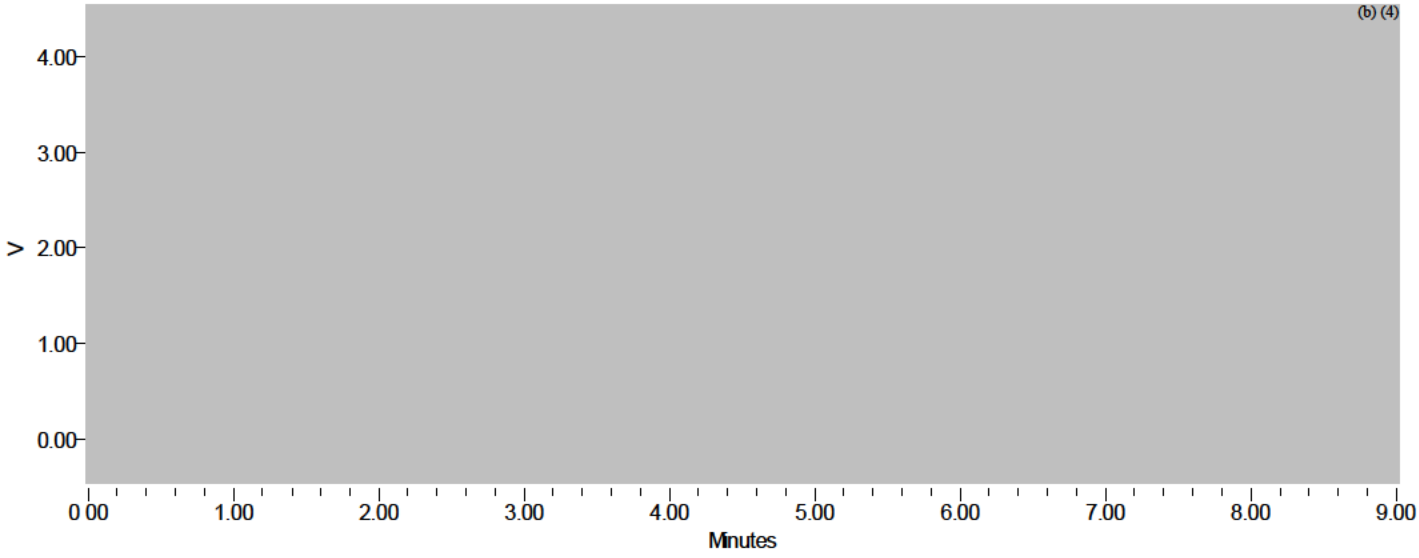
Sample Name:	SSTD12_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	8	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 12:59:39 AM KST		
Date Processed:	12/20/2020 4:57:21 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

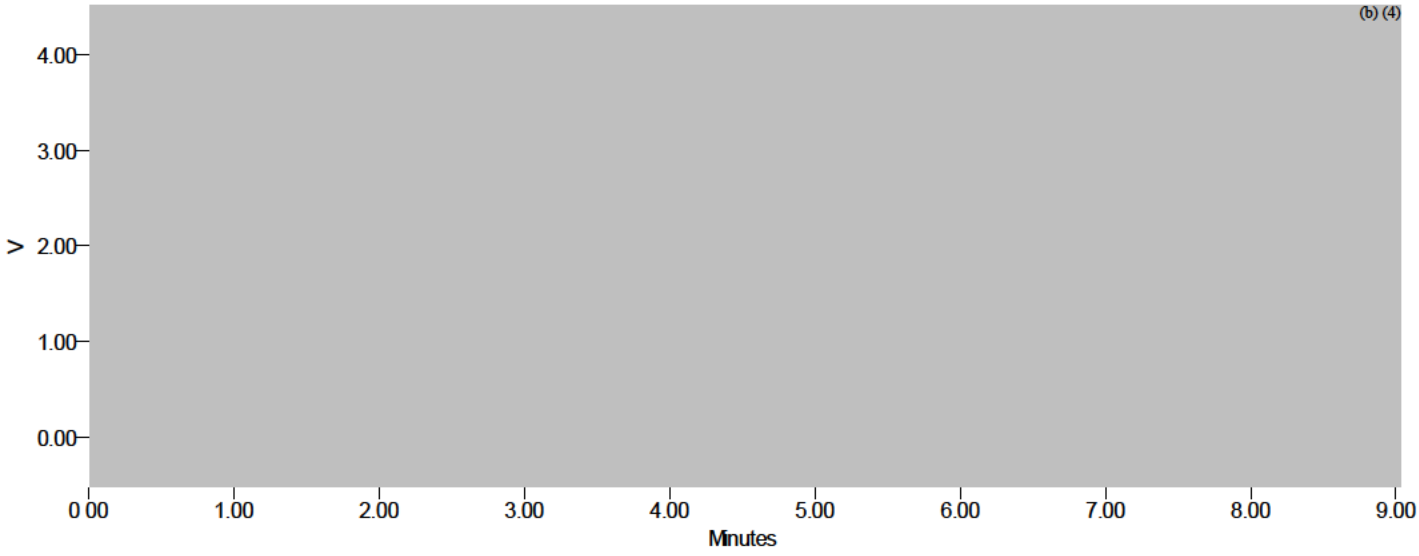
Sample Name:	SSPL12_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	9	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 1:09:21 AM KST		
Date Processed:	12/20/2020 4:57:21 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

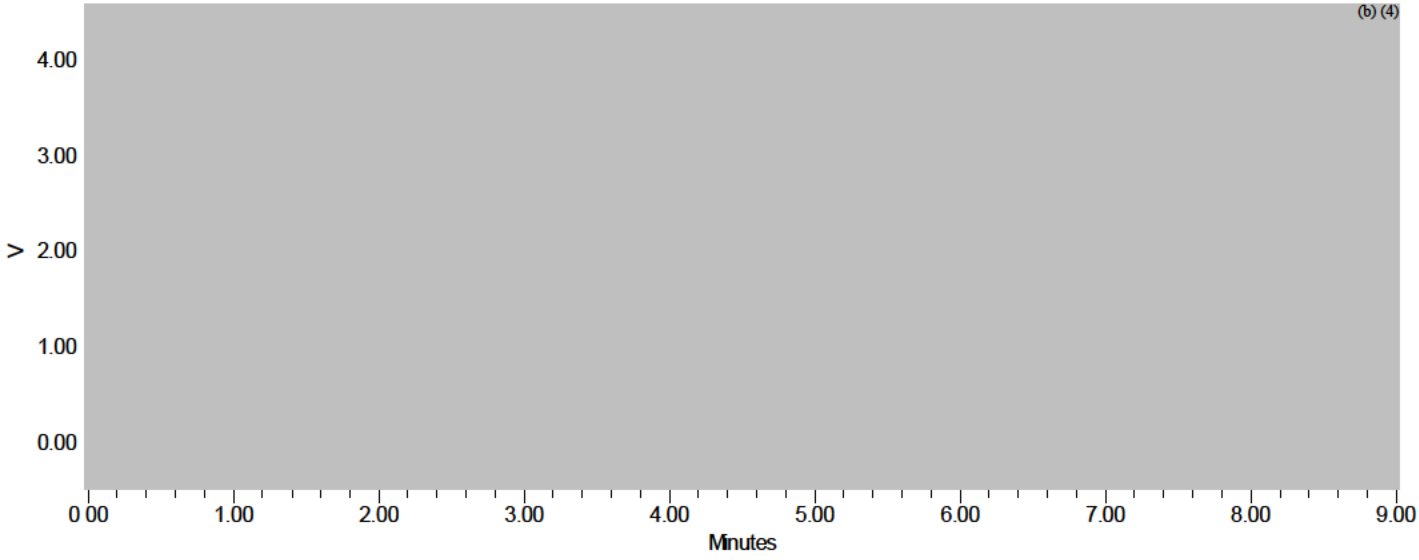
Sample Name:	SSTD12_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	10	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 1:19:01 AM KST		
Date Processed:	12/20/2020 4:57:21 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

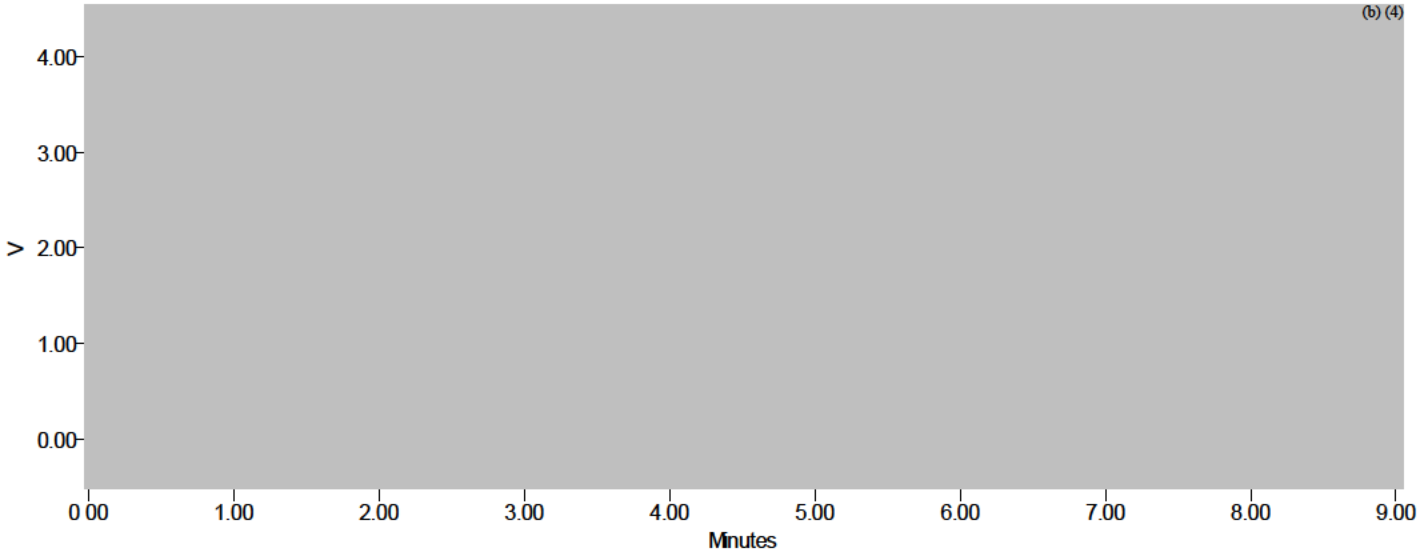
Sample Name:	SSPL12_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	11	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 1:28:43 AM KST		
Date Processed:	12/20/2020 4:57:21 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

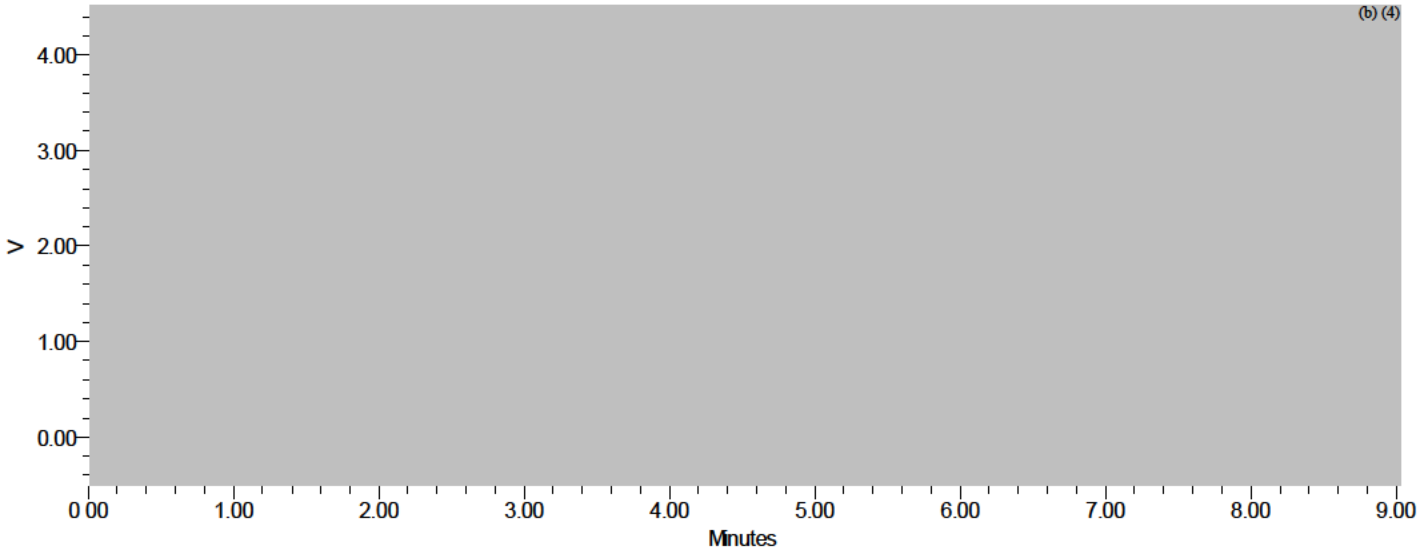
Sample Name:	SSTD12_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	12	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 1:38:25 AM KST		
Date Processed:	12/20/2020 4:57:22 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

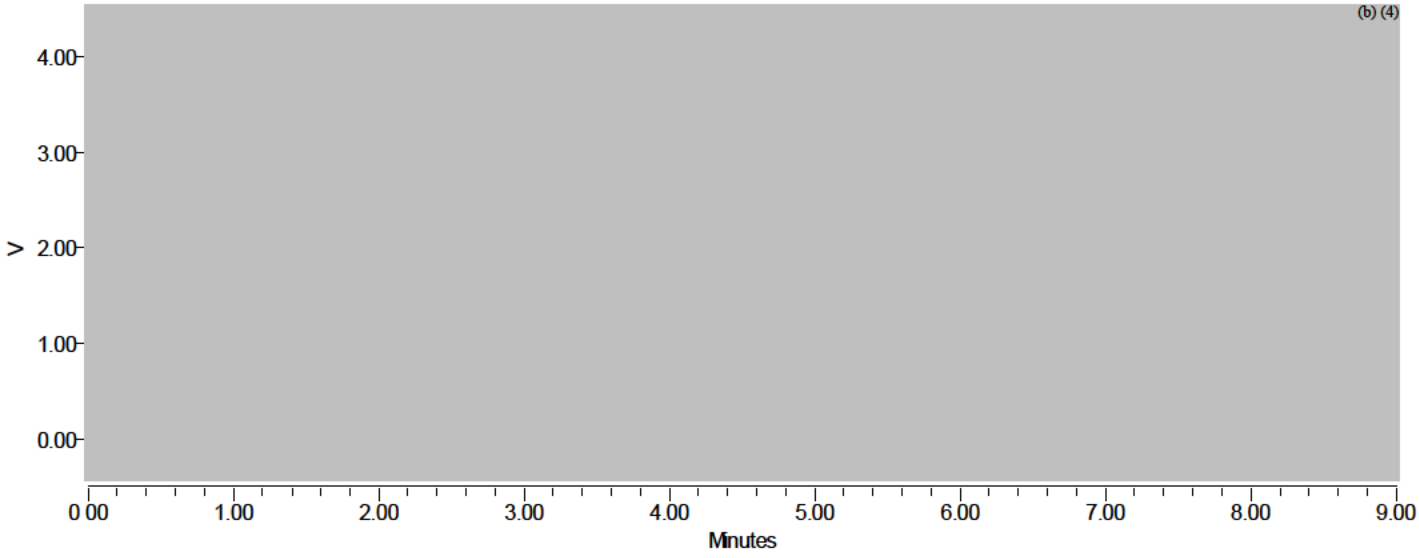
Sample Name:	SSPL12_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	13	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 1:48:06 AM KST		
Date Processed:	12/20/2020 4:57:22 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

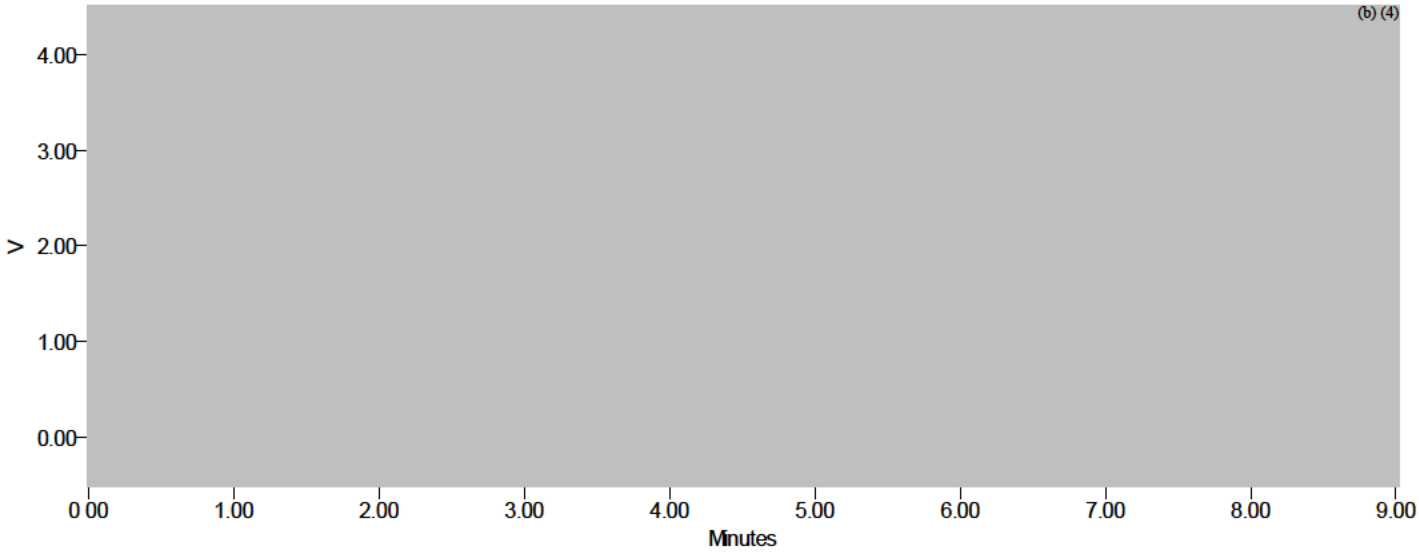
Sample Name:	SSTD12_4	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	14	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 1:57:44 AM KST		
Date Processed:	12/20/2020 4:57:22 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

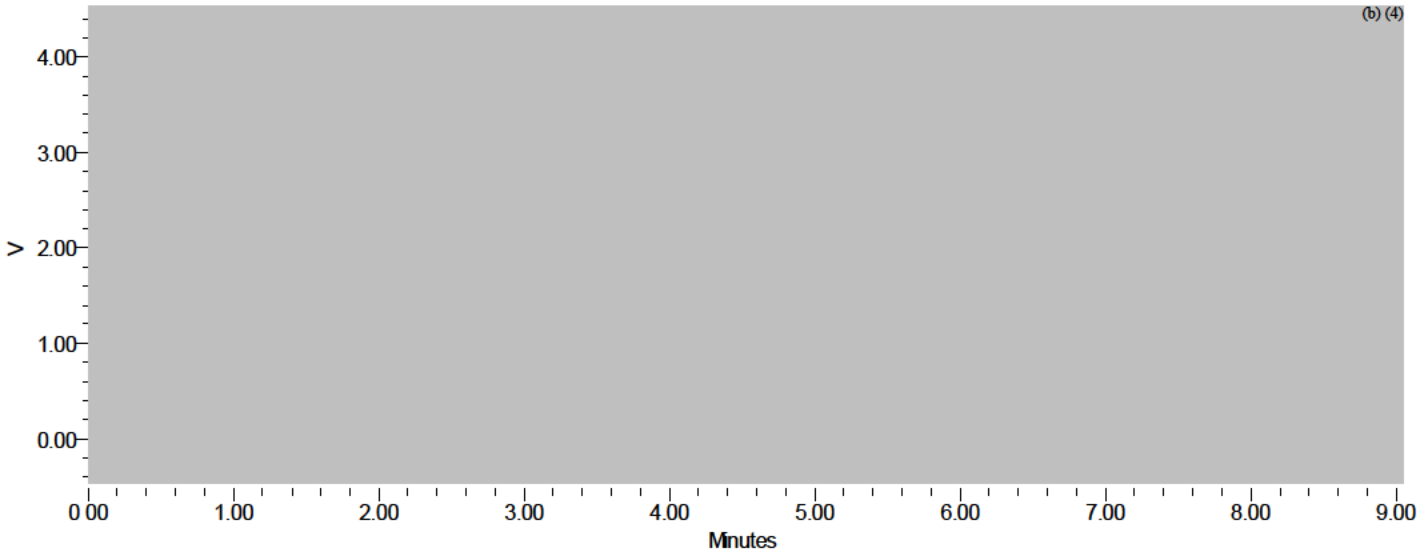
Sample Name:	SSTD13_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	15	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 5:49:14 AM KST		
Date Processed:	12/20/2020 4:57:25 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

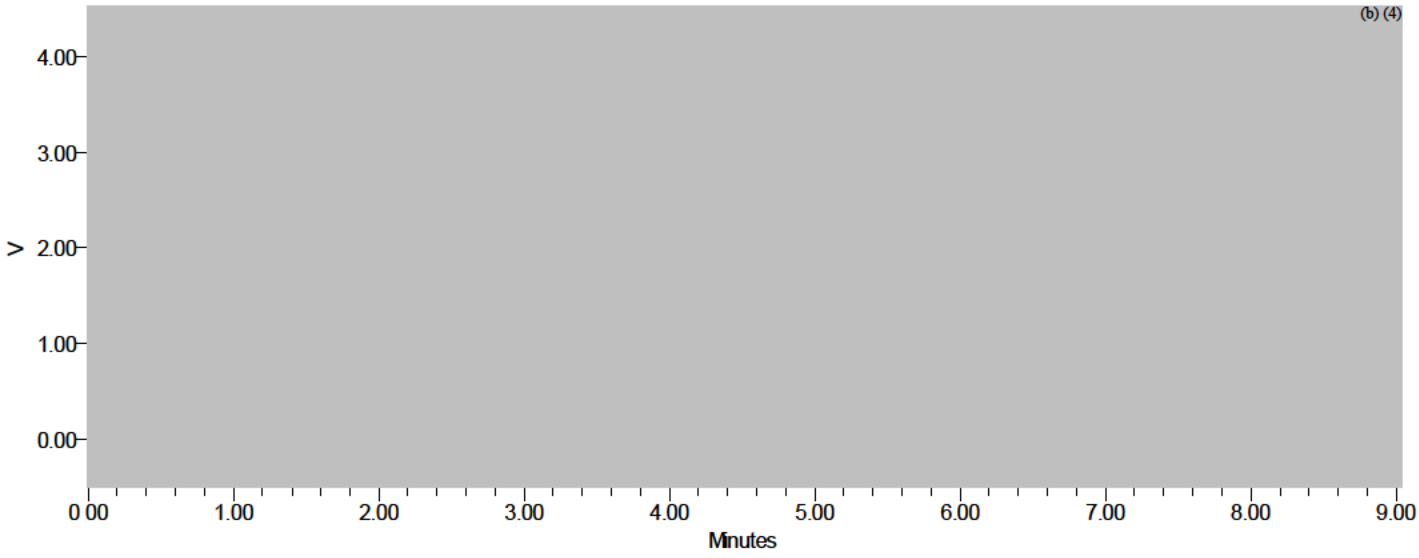
Sample Name:	SSPL13_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	16	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 5:58:57 AM KST		
Date Processed:	12/20/2020 4:57:25 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

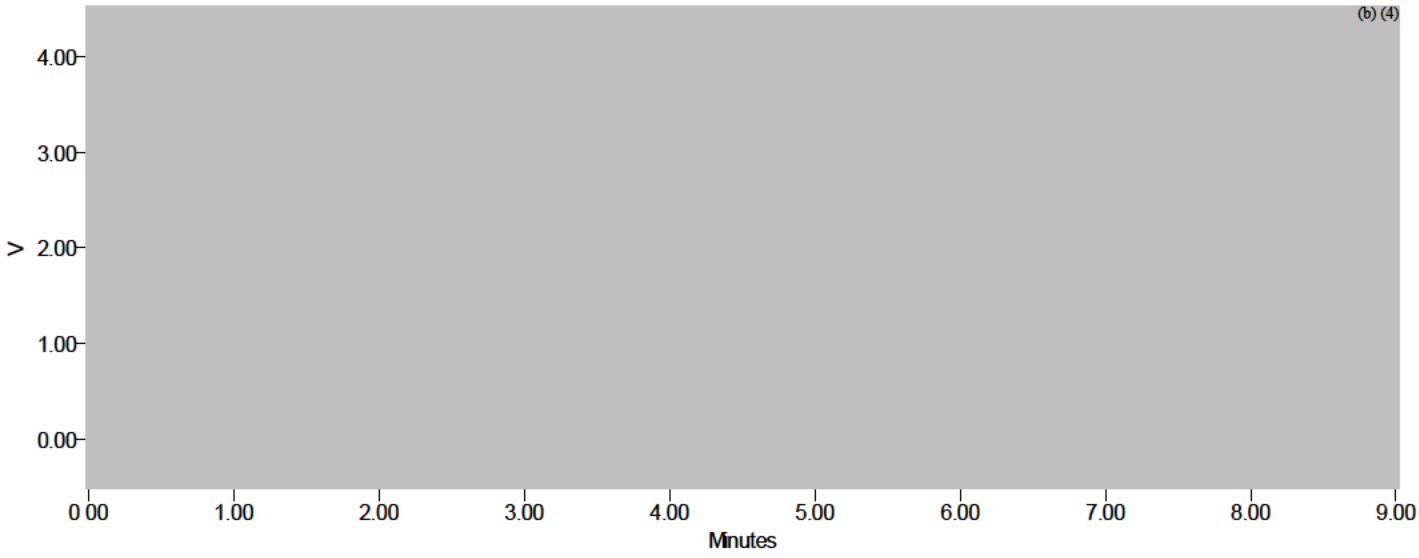
Sample Name:	SSTD13_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	17	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 6:08:39 AM KST		
Date Processed:	12/20/2020 4:57:25 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

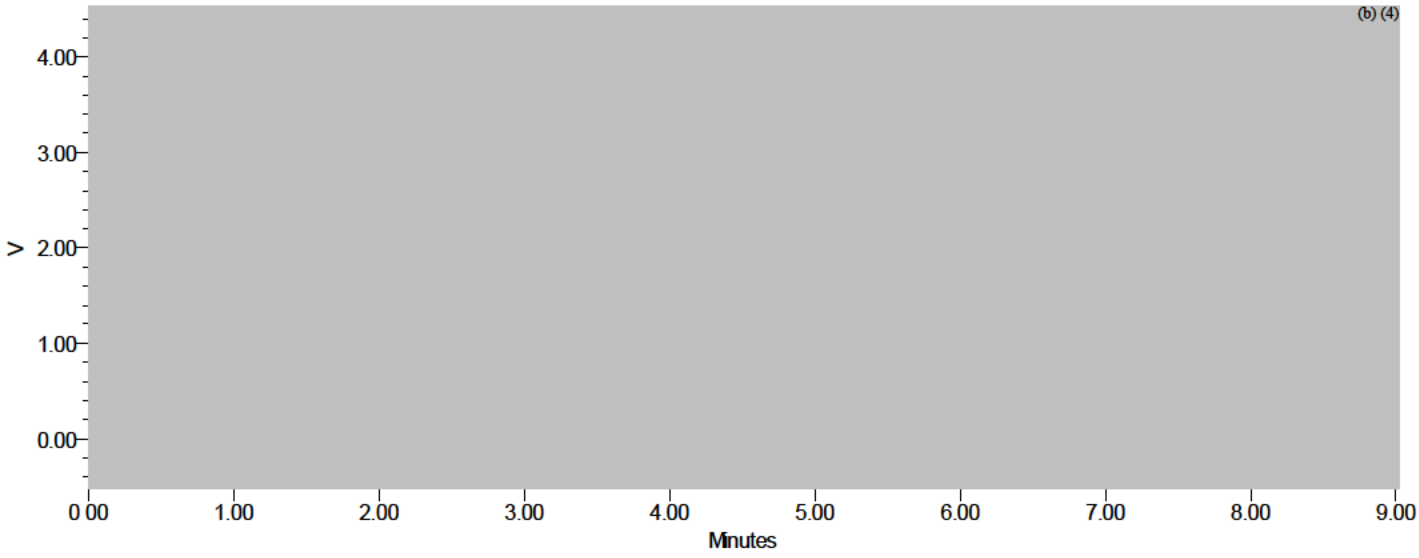
Sample Name:	SSPL13_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	18	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 6:18:19 AM KST		
Date Processed:	12/20/2020 4:57:26 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

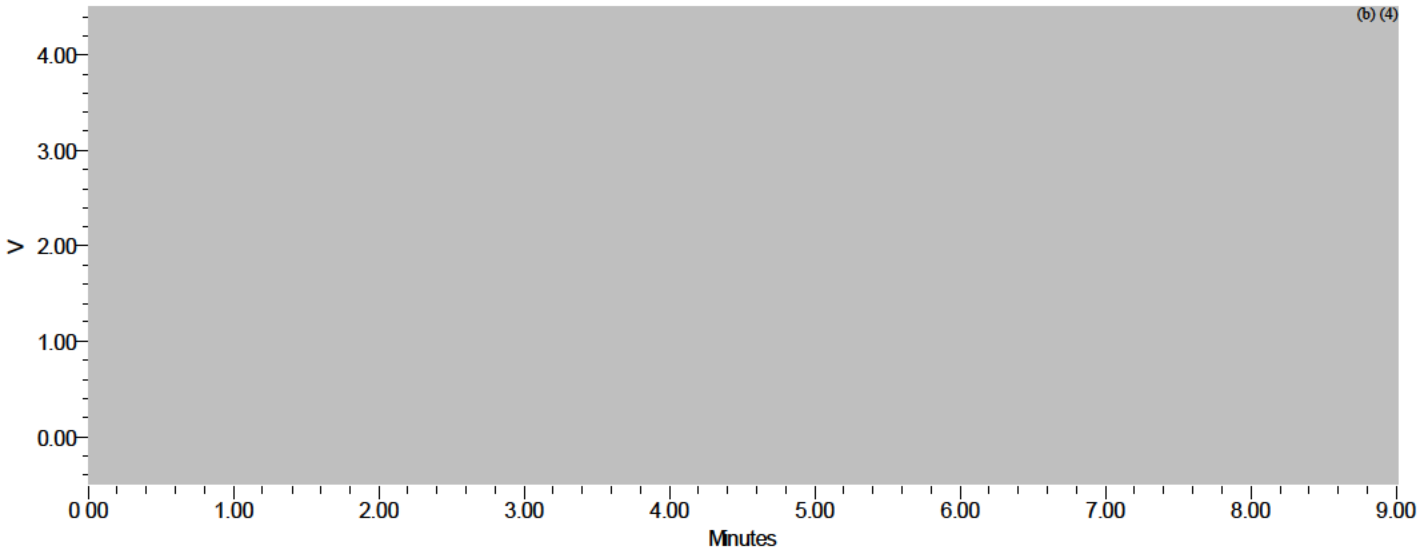
Sample Name:	SSTD13_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	19	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 6:27:56 AM KST		
Date Processed:	12/20/2020 4:57:26 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

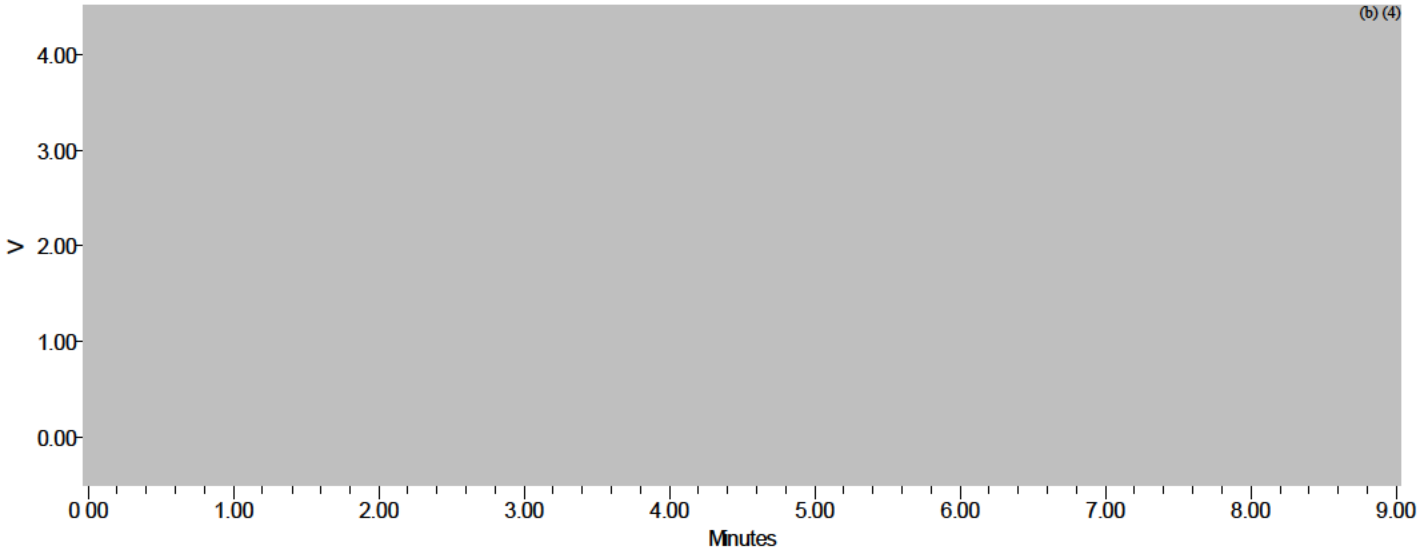
Sample Name:	SSPL13_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	20	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 6:37:32 AM KST		
Date Processed:	12/20/2020 4:57:26 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

Sample Name:	SSTD13_4	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	21	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 6:47:12 AM KST		
Date Processed:	12/20/2020 4:57:26 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

Software: Empower 3 Software Build 3471

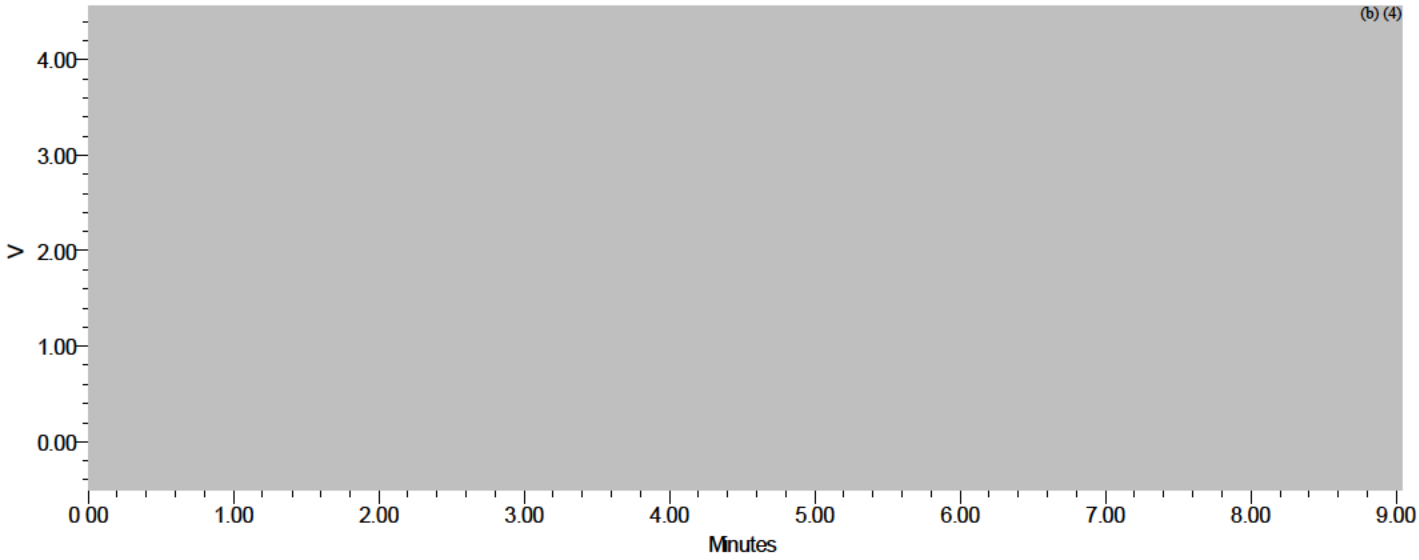
Sample Set Name:	Granule Valine_2 Day	Processed By:	System/Administrator
Sample Set Method:	Granule Valine_2 Day	Printed By:	System
System Node:	Client13	Result Set ID:	
System Name:	UC10	# of Results:	21
Acquired By:	System		
Sample Set Start Date:	12/18/2020 12:24:43 PM KST		
Sample Set Finish Date:	12/19/2020 6:05:57 AM KST		

Sample Set Table

	Sample Name	Sample Type	Vial	Inj #	Injection Volume (ul)	Acquistion Method Set	Processed Channel Descr.	(b) (4)
1	SSTD21_1	Unknown	1	1	5.00	VAL_ACR	Detector A	
2	SSPL21_1	Unknown	2	1	5.00	VAL_ACR	Detector A	
3	SSTD21_2	Unknown	3	1	5.00	VAL_ACR	Detector A	
4	SSPL21_2	Unknown	4	1	5.00	VAL_ACR	Detector A	
5	SSTD21_3	Unknown	5	1	5.00	VAL_ACR	Detector A	
6	SSPL21_3	Unknown	6	1	5.00	VAL_ACR	Detector A	
7	SSTD21_4	Unknown	7	1	5.00	VAL_ACR	Detector A	
8	SSTD22_1	Unknown	8	1	5.00	VAL_ACR	Detector A	
9	SSPL22_1	Unknown	9	1	5.00	VAL_ACR	Detector A	
10	SSTD22_2	Unknown	10	1	5.00	VAL_ACR	Detector A	
11	SSPL22_2	Unknown	11	1	5.00	VAL_ACR	Detector A	
12	SSTD22_3	Unknown	12	1	5.00	VAL_ACR	Detector A	
13	SSPL22_3	Unknown	13	1	5.00	VAL_ACR	Detector A	
14	SSTD22_4	Unknown	14	1	5.00	VAL_ACR	Detector A	
15	SSTD23_1	Unknown	15	1	5.00	VAL_ACR	Detector A	
16	SSPL23_1	Unknown	16	1	5.00	VAL_ACR	Detector A	
17	SSTD23_2	Unknown	17	1	5.00	VAL_ACR	Detector A	
18	SSPL23_2	Unknown	18	1	5.00	VAL_ACR	Detector A	
19	SSTD23_3	Unknown	19	1	5.00	VAL_ACR	Detector A	
20	SSPL23_3	Unknown	20	1	5.00	VAL_ACR	Detector A	
21	SSTD23_4	Unknown	21	1	5.00	VAL_ACR	Detector A	

SAMPLE INFORMATION

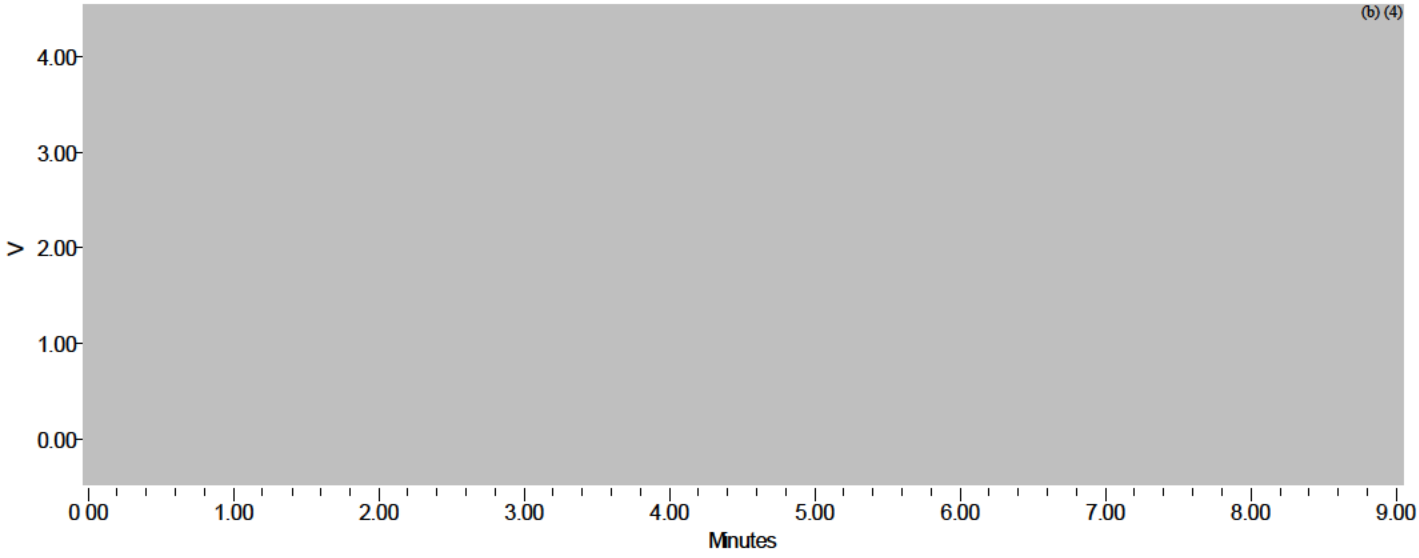
Sample Name:	SSTD21_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_2 Day
Vial:	1	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 6:54:59 PM KST		
Date Processed:	12/21/2020 11:51:12 AM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

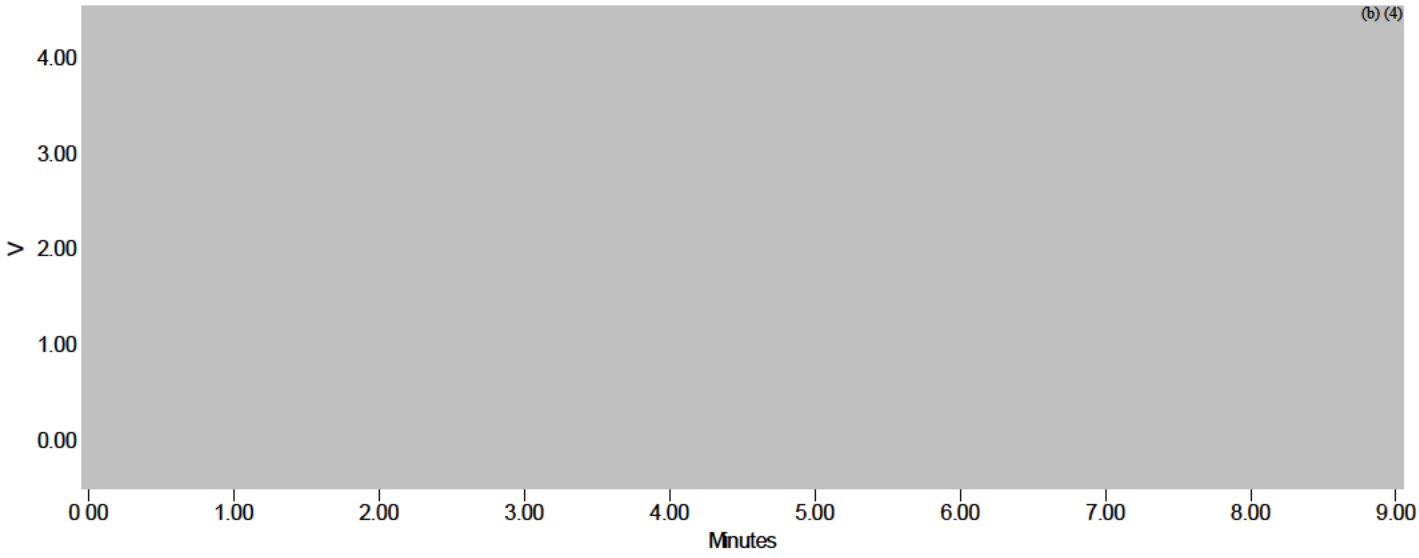
Sample Name:	SSPL21_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_2 Day
Vial:	2	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 7:23:52 PM KST		
Date Processed:	12/21/2020 11:51:13 AM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

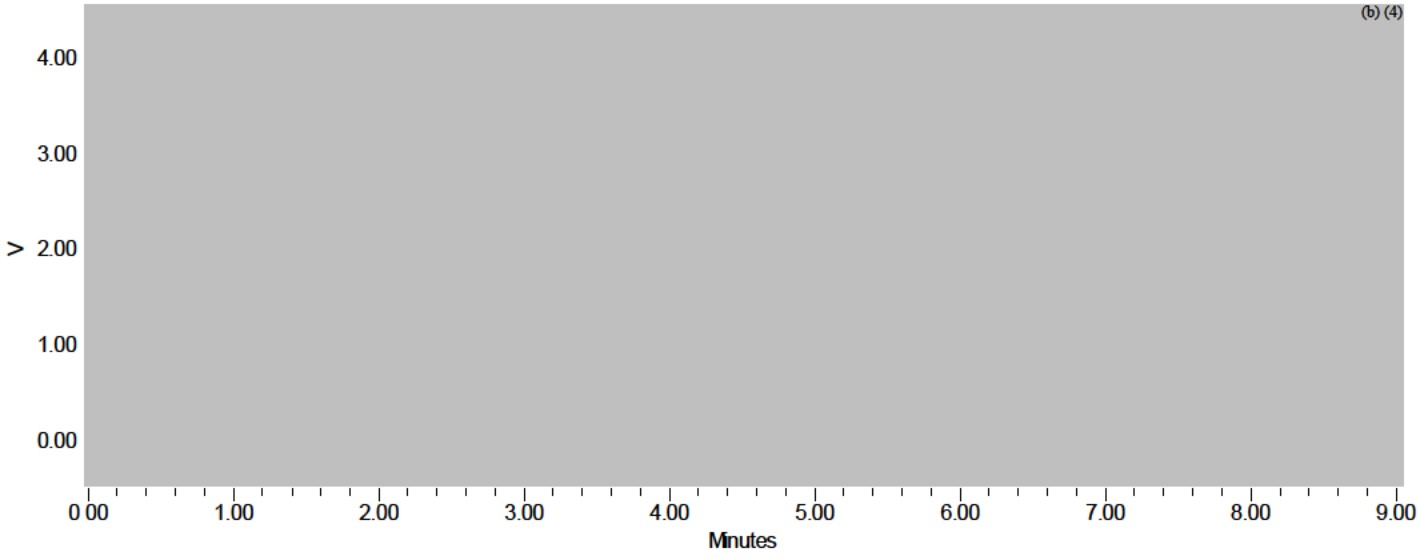
Sample Name:	SSTD21_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_2 Day
Vial:	3	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 7:33:32 PM KST		
Date Processed:	12/21/2020 11:51:13 AM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

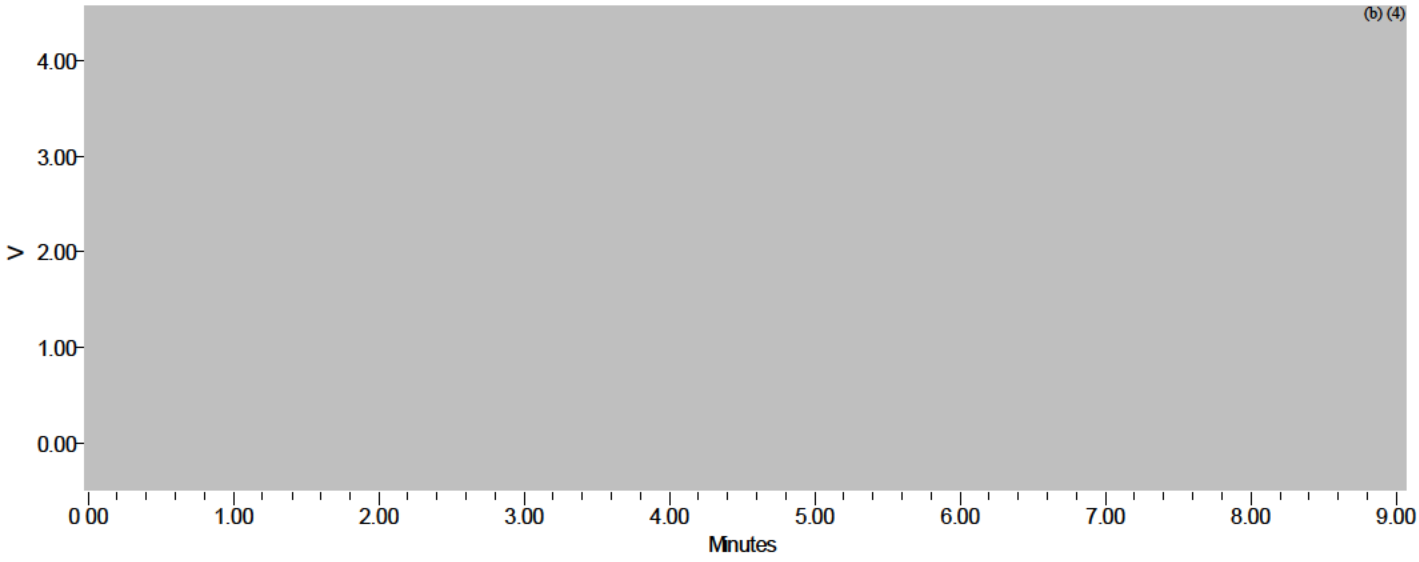
Sample Name:	SSPL21_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_2 Day
Vial:	4	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 7:43:10 PM KST		
Date Processed:	12/21/2020 11:51:13 AM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

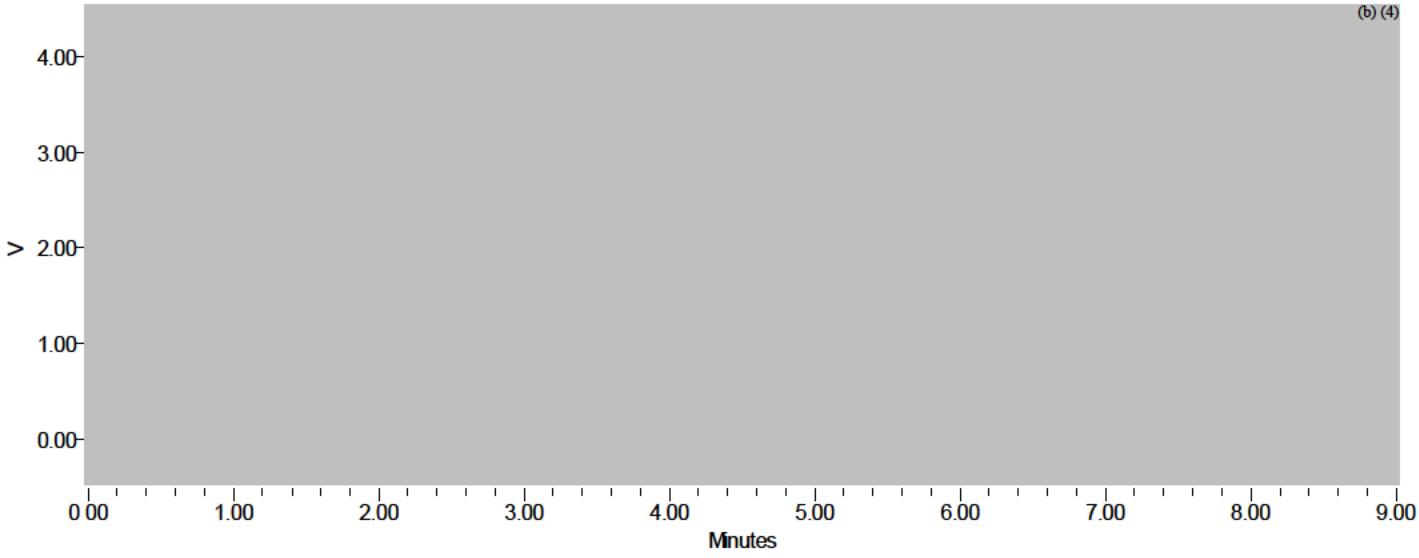
Sample Name:	SSTD21_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_2 Day
Vial:	5	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 7:52:50 PM KST		
Date Processed:	12/21/2020 11:51:13 AM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

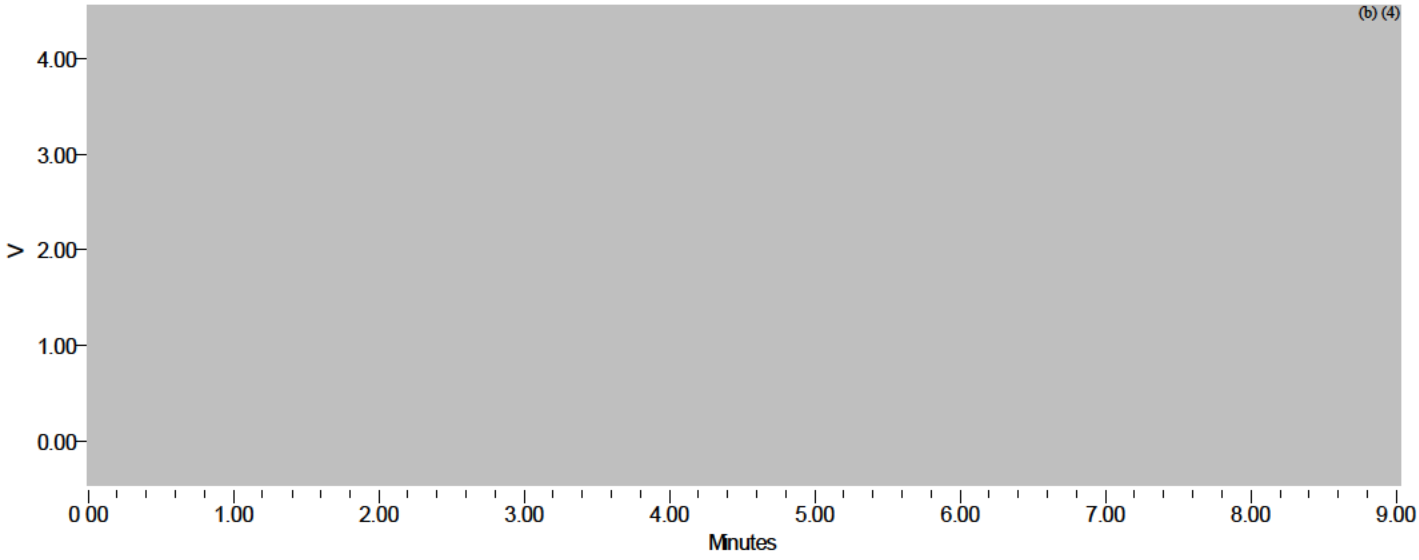
Sample Name:	SSPL21_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_2 Day
Vial:	6	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 8:02:31 PM KST		
Date Processed:	12/21/2020 11:51:13 AM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

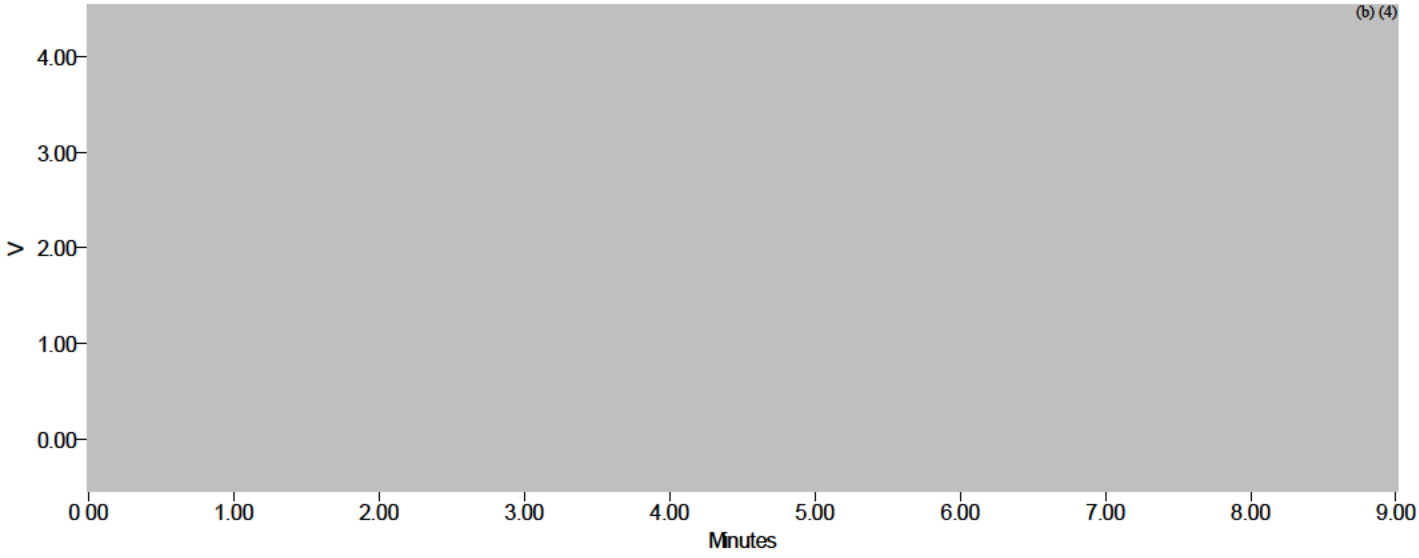
Sample Name:	SSTD21_4	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_2 Day
Vial:	7	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 8:12:10 PM KST		
Date Processed:	12/21/2020 11:51:14 AM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

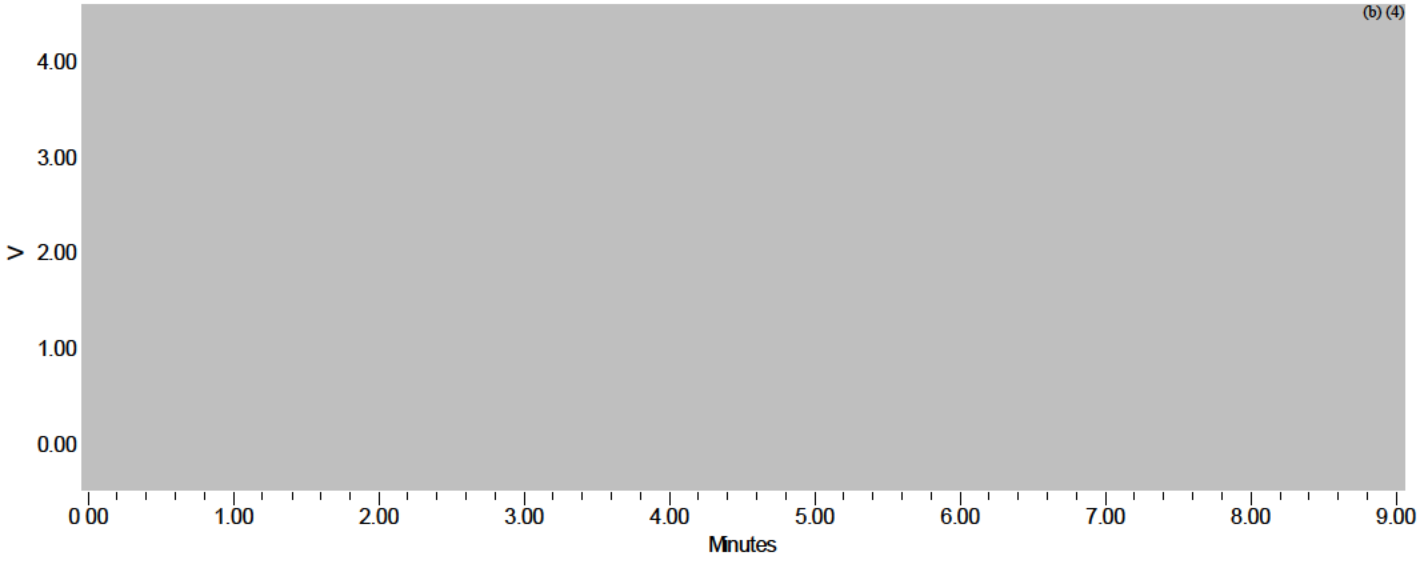
Sample Name:	SSTD22_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_2 Day
Vial:	8	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 11:21:10 PM KST		
Date Processed:	12/21/2020 11:51:17 AM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

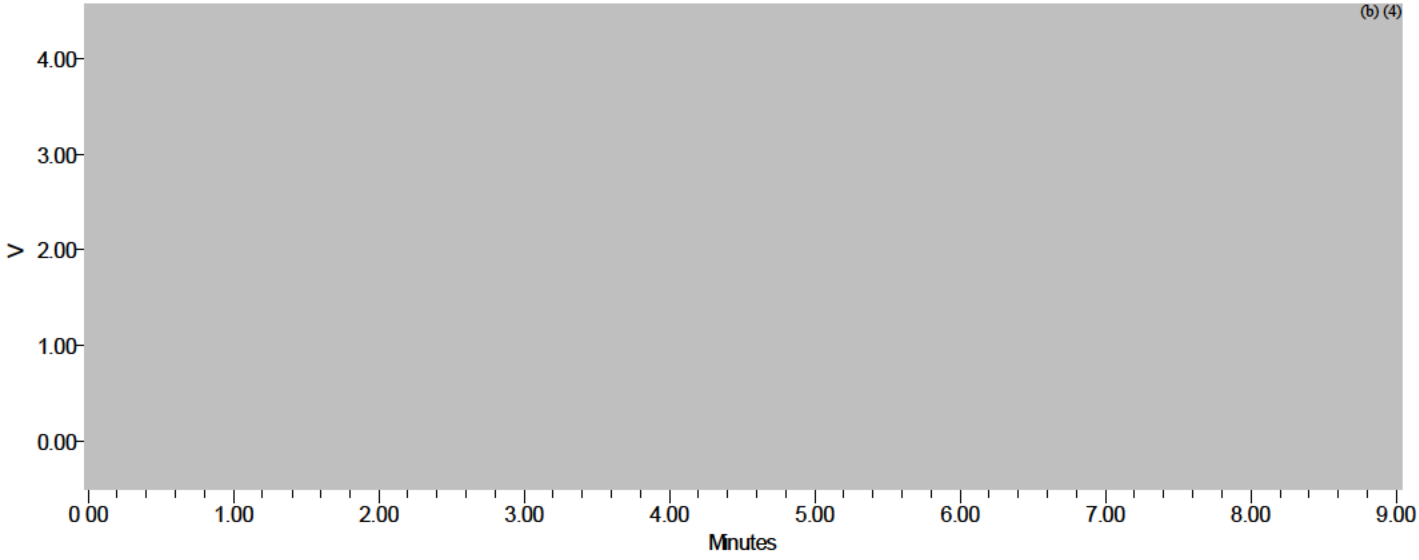
Sample Name:	SSPL22_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_2 Day
Vial:	9	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 11:30:52 PM KST		
Date Processed:	12/21/2020 11:51:17 AM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

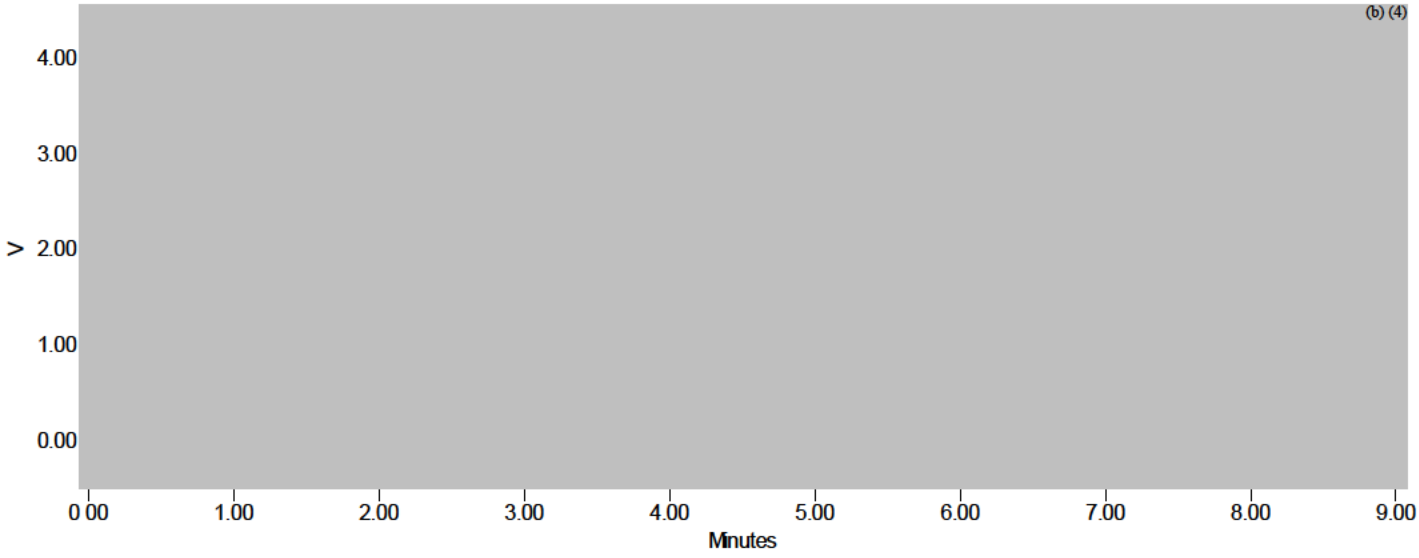
Sample Name:	SSTD22_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_2 Day
Vial:	10	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 11:40:33 PM KST		
Date Processed:	12/21/2020 11:51:18 AM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

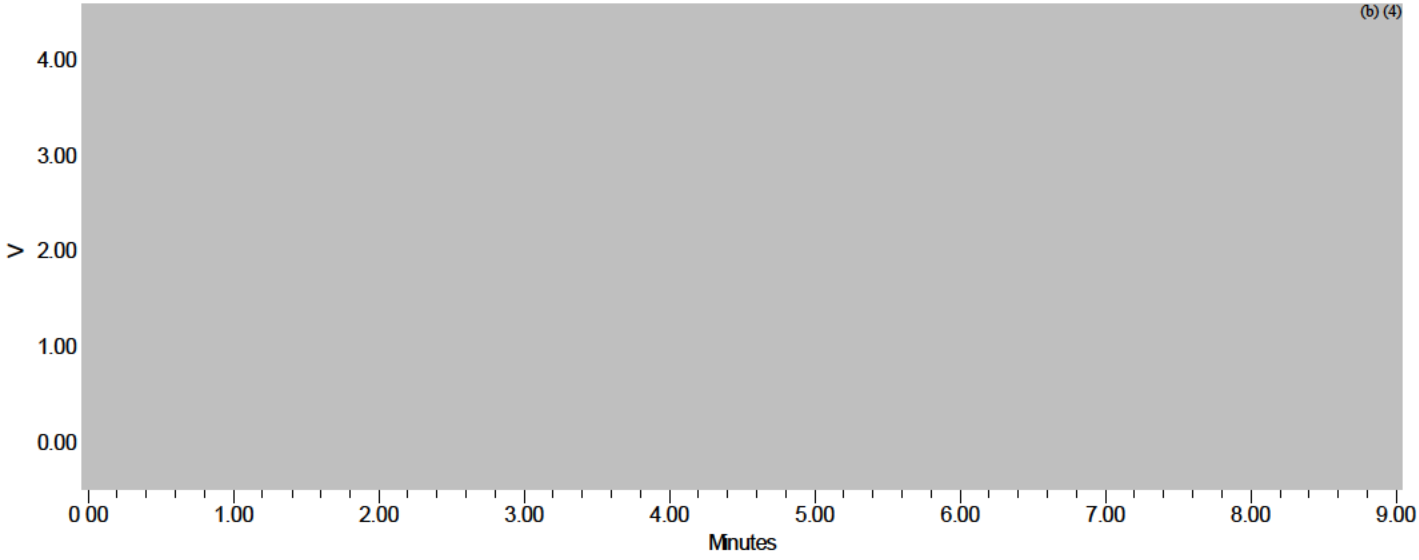
Sample Name:	SSPL22_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_2 Day
Vial:	11	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 11:50:15 PM KST		
Date Processed:	12/21/2020 11:51:18 AM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

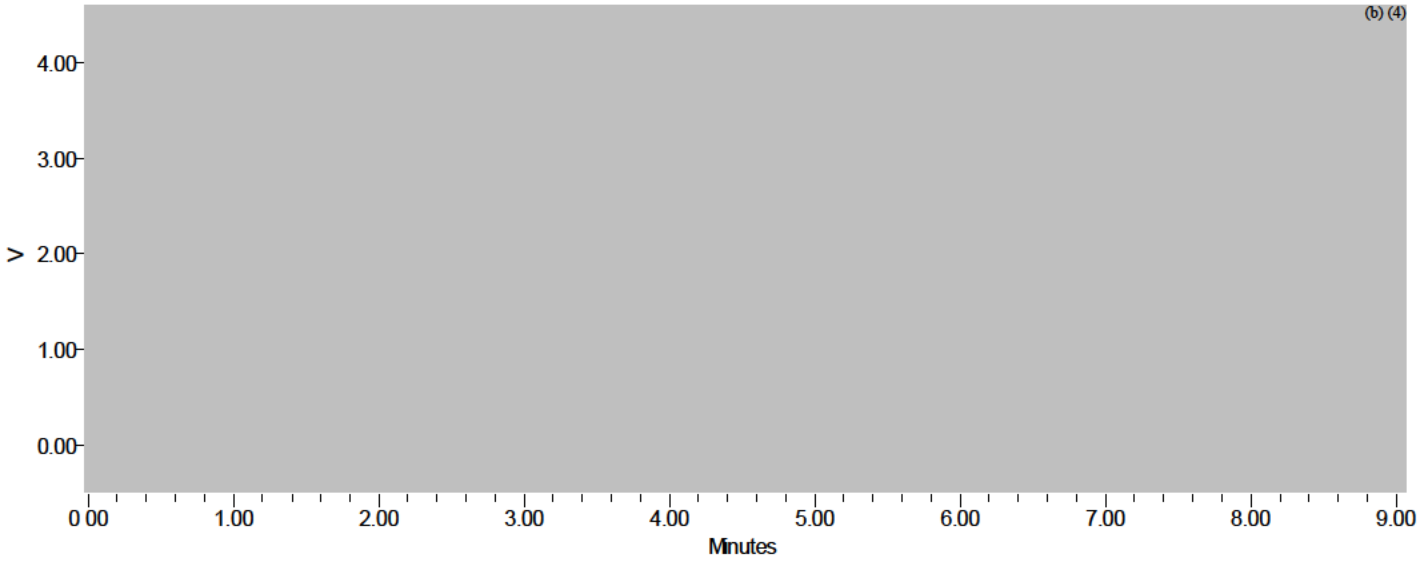
Sample Name:	SSTD22_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_2 Day
Vial:	12	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 11:59:55 PM KST		
Date Processed:	12/21/2020 11:51:18 AM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

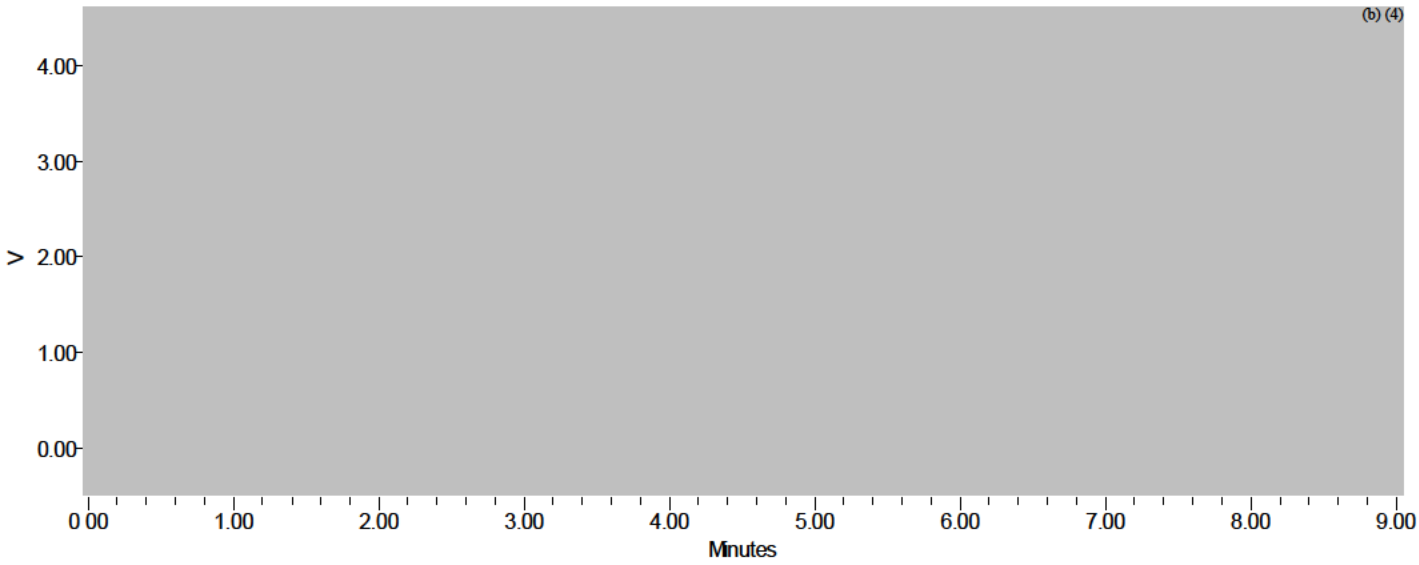
Sample Name:	SSPL22_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_2 Day
Vial:	13	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/19/2020 12:09:31 AM KST		
Date Processed:	12/21/2020 11:51:18 AM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

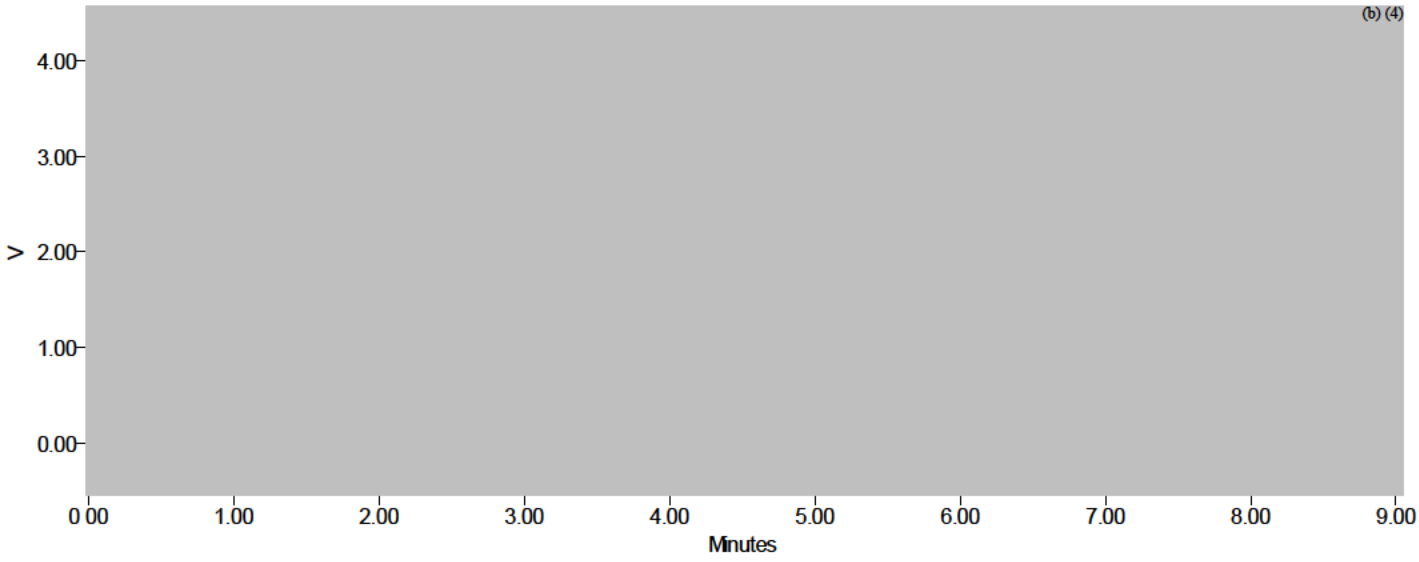
Sample Name:	SSTD22_4	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_2 Day
Vial:	14	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/19/2020 12:19:12 AM KST		
Date Processed:	12/21/2020 11:51:19 AM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

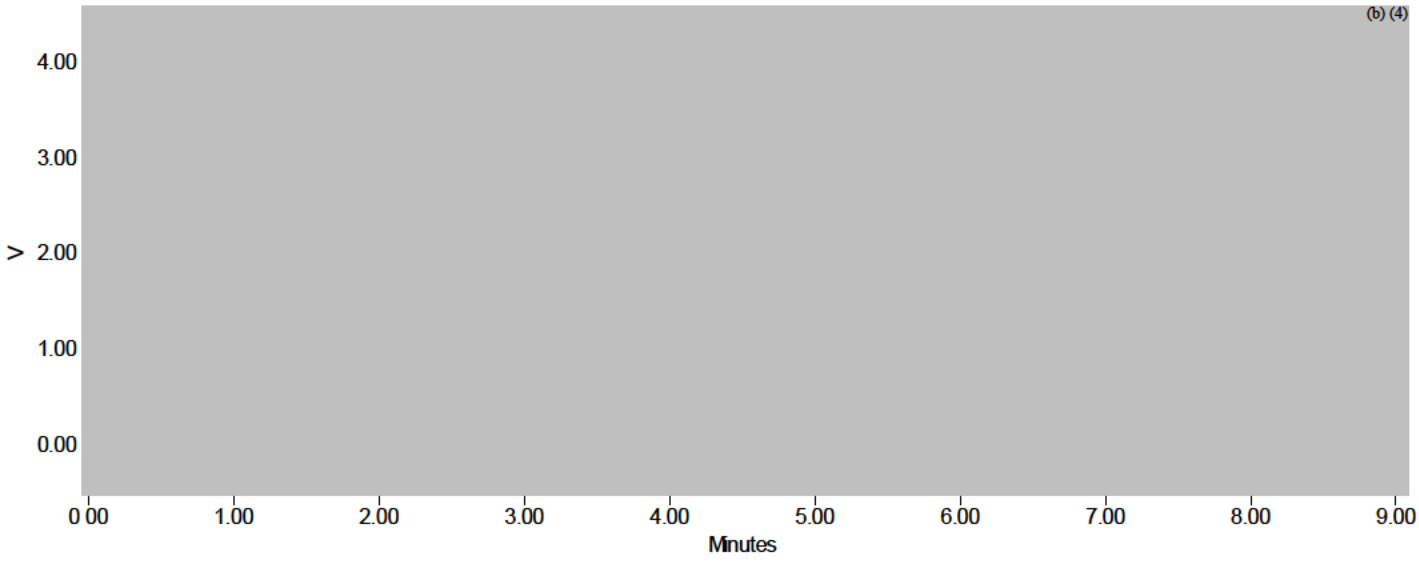
Sample Name:	SSTD23_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_2 Day
Vial:	15	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/19/2020 3:27:58 AM KST		
Date Processed:	12/21/2020 11:51:24 AM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

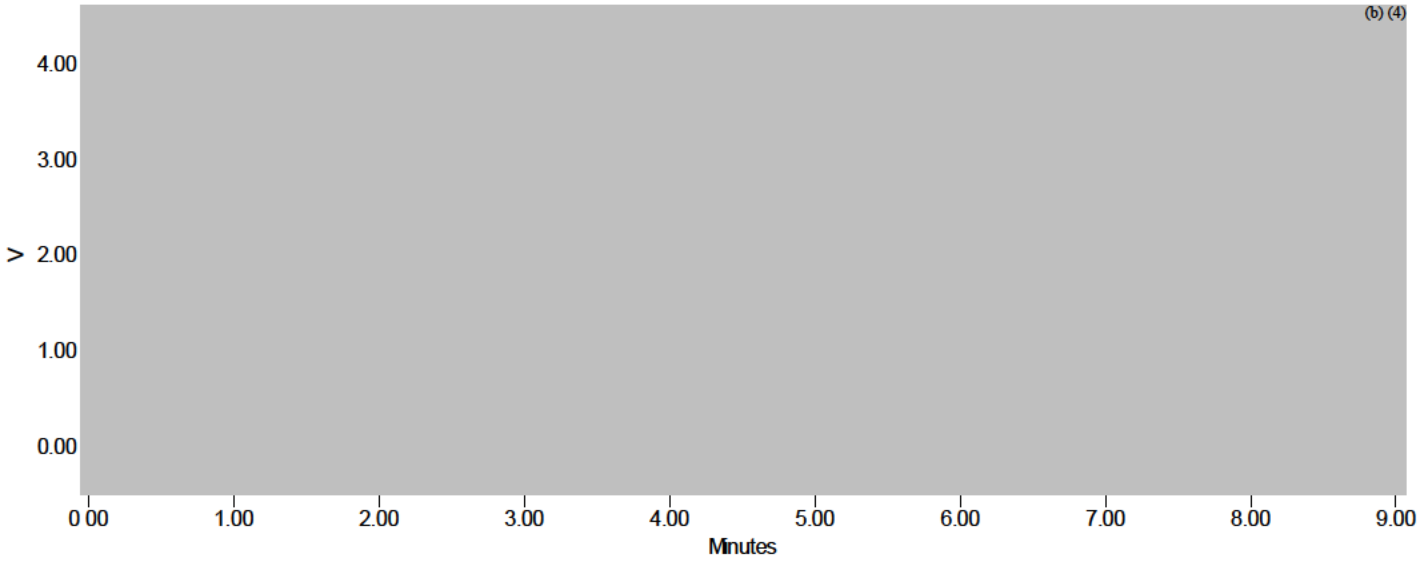
Sample Name:	SSPL23_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_2 Day
Vial:	16	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/19/2020 3:37:38 AM KST		
Date Processed:	12/21/2020 11:51:24 AM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

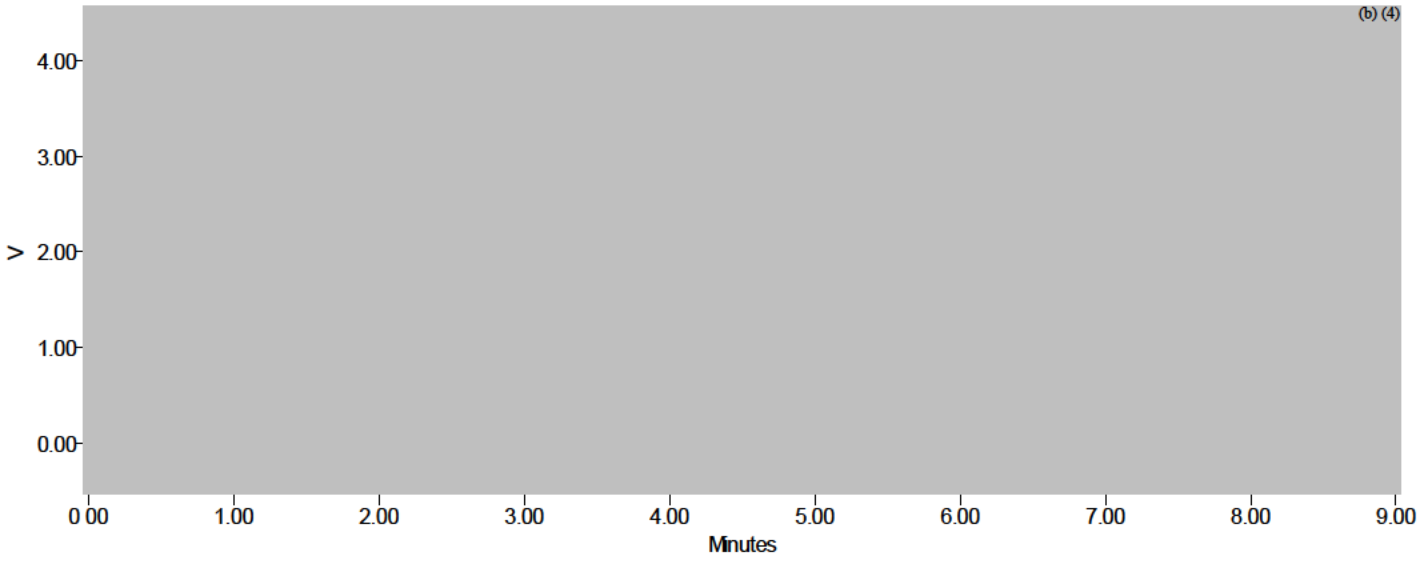
Sample Name:	SSTD23_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_2 Day
Vial:	17	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/19/2020 3:47:20 AM KST		
Date Processed:	12/21/2020 11:51:24 AM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

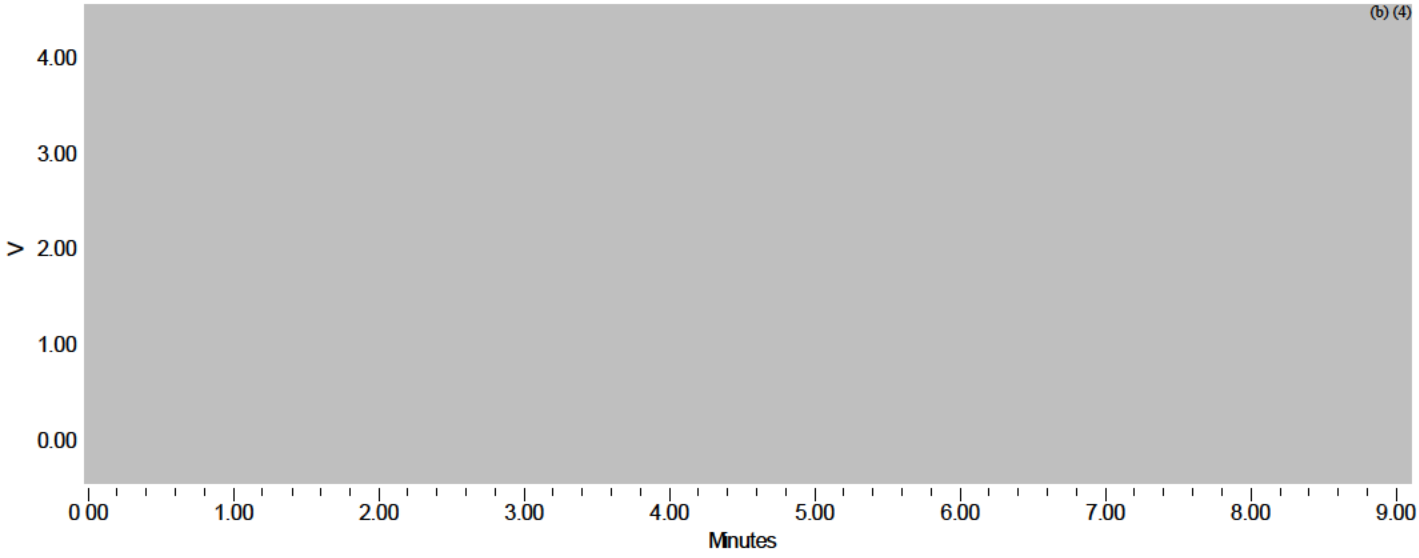
Sample Name:	SSPL23_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_2 Day
Vial:	18	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/19/2020 3:57:01 AM KST		
Date Processed:	12/21/2020 11:51:25 AM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

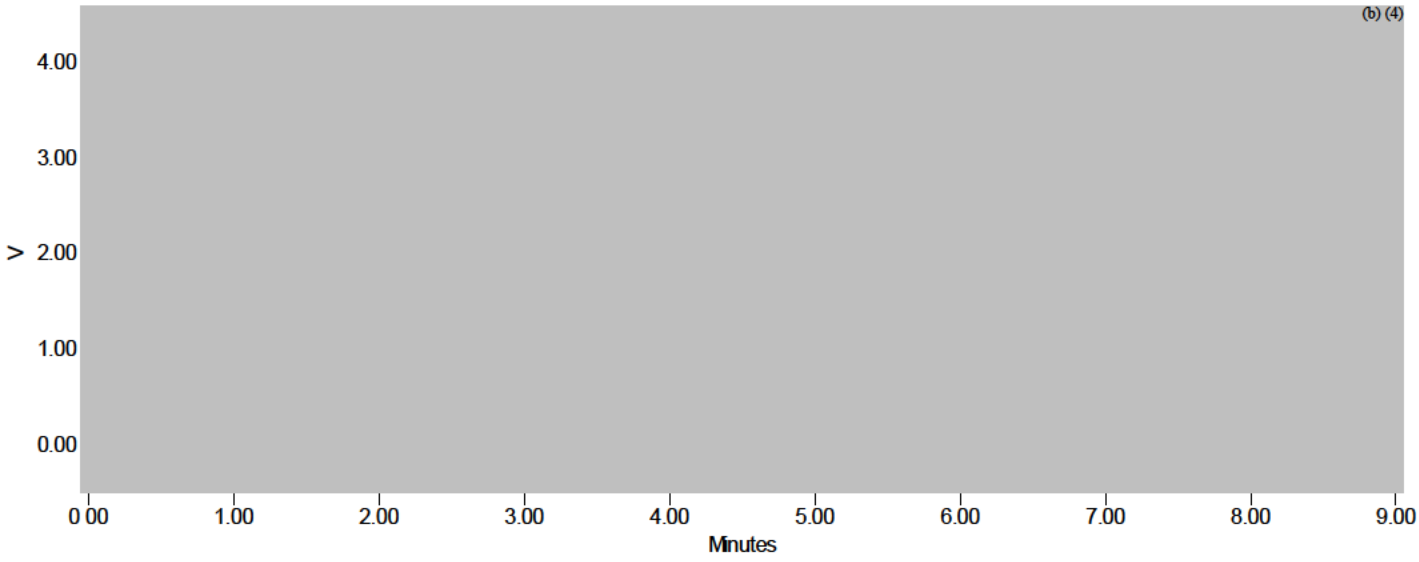
Sample Name:	SSTD23_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_2 Day
Vial:	19	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/19/2020 4:06:45 AM KST		
Date Processed:	12/21/2020 11:51:25 AM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

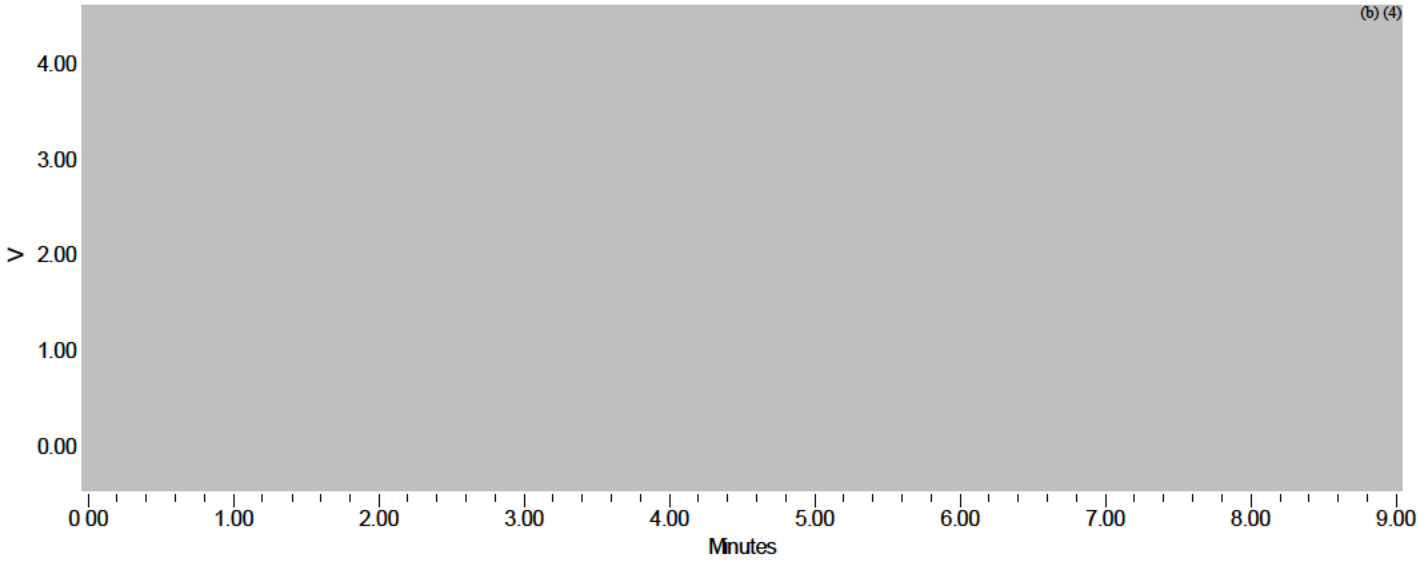
Sample Name:	SSPL23_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_2 Day
Vial:	20	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/19/2020 4:16:20 AM KST		
Date Processed:	12/21/2020 11:51:25 AM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

Sample Name:	SSTD23_4	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_2 Day
Vial:	21	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/19/2020 4:26:01 AM KST		
Date Processed:	12/21/2020 11:51:25 AM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

Software: Empower 3 Software Build 3471

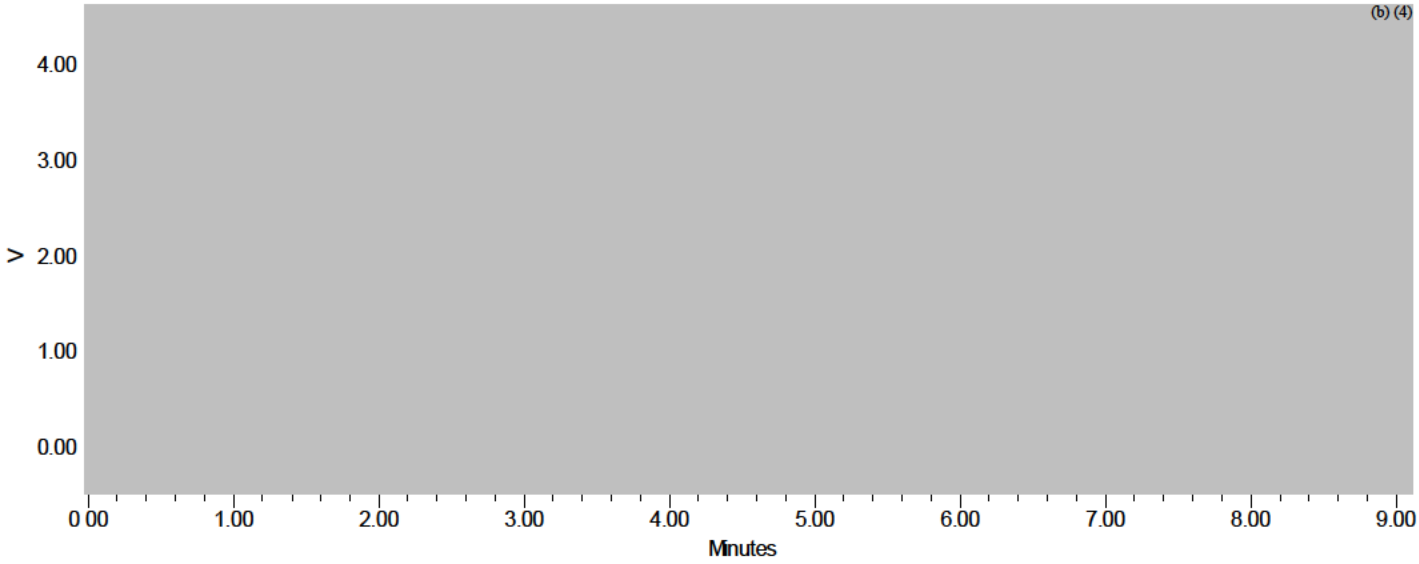
Sample Set Name:	Granule Valine_3 Day_2nd	Processed By:	System/Administrator
Sample Set Method:	Granule Valine_3 Day_2nd	Printed By:	System
System Node:	Client13	Result Set ID:	
System Name:	UC10	# of Results:	21
Acquired By:	System		
Sample Set Start Date:	12/19/2020 9:59:47 PM KST		
Sample Set Finish Date:	12/20/2020 3:30:48 PM KST		

Sample Set Table

	Sample Name	Sample Type	Vial	Inj #	Injection Volume (ul)	Acquistion Method Set	Processed Channel Descr.	(b) (4)
1	SSTD31_1	Unknown	1	1	5.00	VAL_ACR	Detector A	
2	SSPL31_1	Unknown	2	1	5.00	VAL_ACR	Detector A	
3	SSTD31_2	Unknown	3	1	5.00	VAL_ACR	Detector A	
4	SSPL31_2	Unknown	4	1	5.00	VAL_ACR	Detector A	
5	SSTD31_3	Unknown	5	1	5.00	VAL_ACR	Detector A	
6	SSPL31_3	Unknown	6	1	5.00	VAL_ACR	Detector A	
7	SSTD31_4	Unknown	7	1	5.00	VAL_ACR	Detector A	
8	SSTD32_1	Unknown	8	1	5.00	VAL_ACR	Detector A	
9	SSPL32_1	Unknown	9	1	5.00	VAL_ACR	Detector A	
10	SSTD32_2	Unknown	10	1	5.00	VAL_ACR	Detector A	
11	SSPL32_2	Unknown	11	1	5.00	VAL_ACR	Detector A	
12	SSTD32_3	Unknown	12	1	5.00	VAL_ACR	Detector A	
13	SSPL32_3	Unknown	13	1	5.00	VAL_ACR	Detector A	
14	SSTD32_4	Unknown	14	1	5.00	VAL_ACR	Detector A	
15	SSTD33_1	Unknown	15	1	5.00	VAL_ACR	Detector A	
16	SSPL33_1	Unknown	16	1	5.00	VAL_ACR	Detector A	
17	SSTD33_2	Unknown	17	1	5.00	VAL_ACR	Detector A	
18	SSPL33_2	Unknown	18	1	5.00	VAL_ACR	Detector A	
19	SSTD33_3	Unknown	19	1	5.00	VAL_ACR	Detector A	
20	SSPL33_3	Unknown	20	1	5.00	VAL_ACR	Detector A	
21	SSTD33_4	Unknown	21	1	5.00	VAL_ACR	Detector A	

SAMPLE INFORMATION

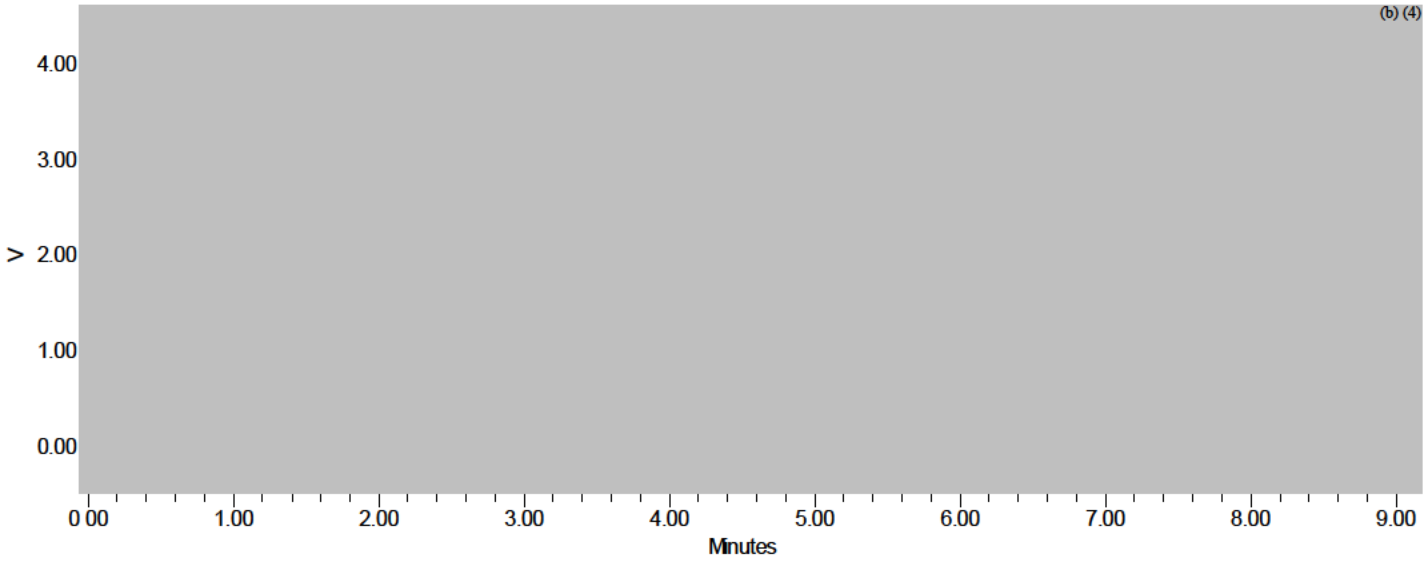
Sample Name:	SSTD31_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day_2nd
Vial:	1	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 1:02:19 AM KST		
Date Processed:	12/20/2020 5:02:46 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

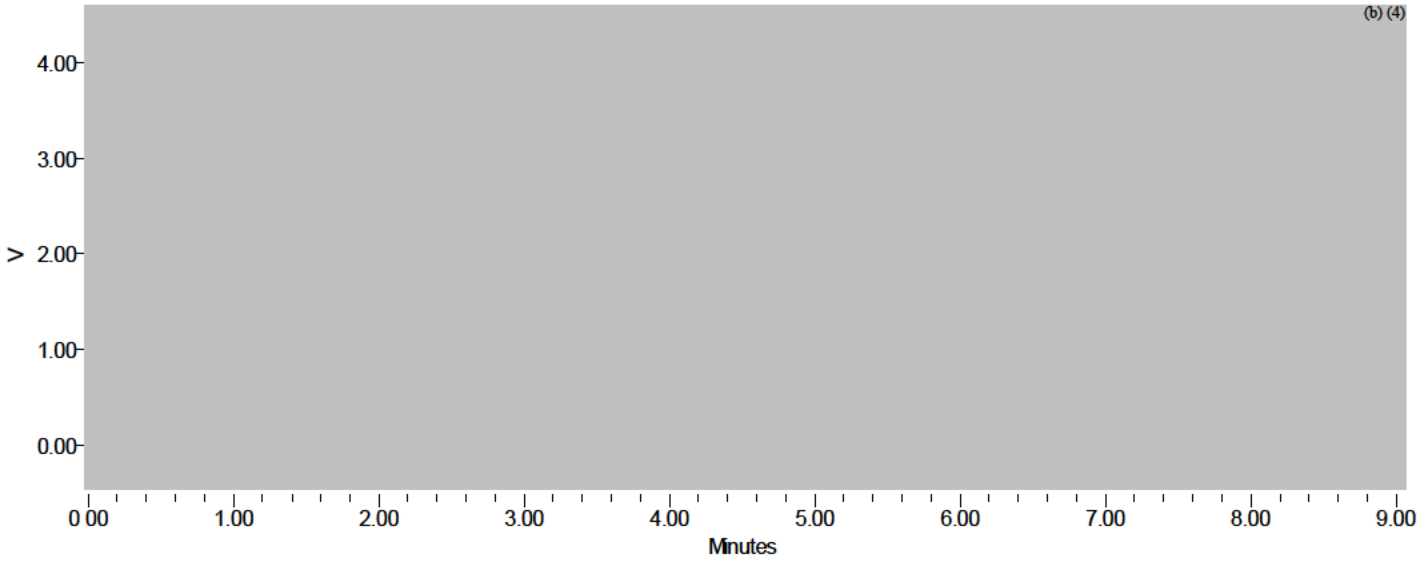
Sample Name:	SSPL31_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day_2nd
Vial:	2	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 1:12:00 AM KST		
Date Processed:	12/20/2020 5:02:46 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

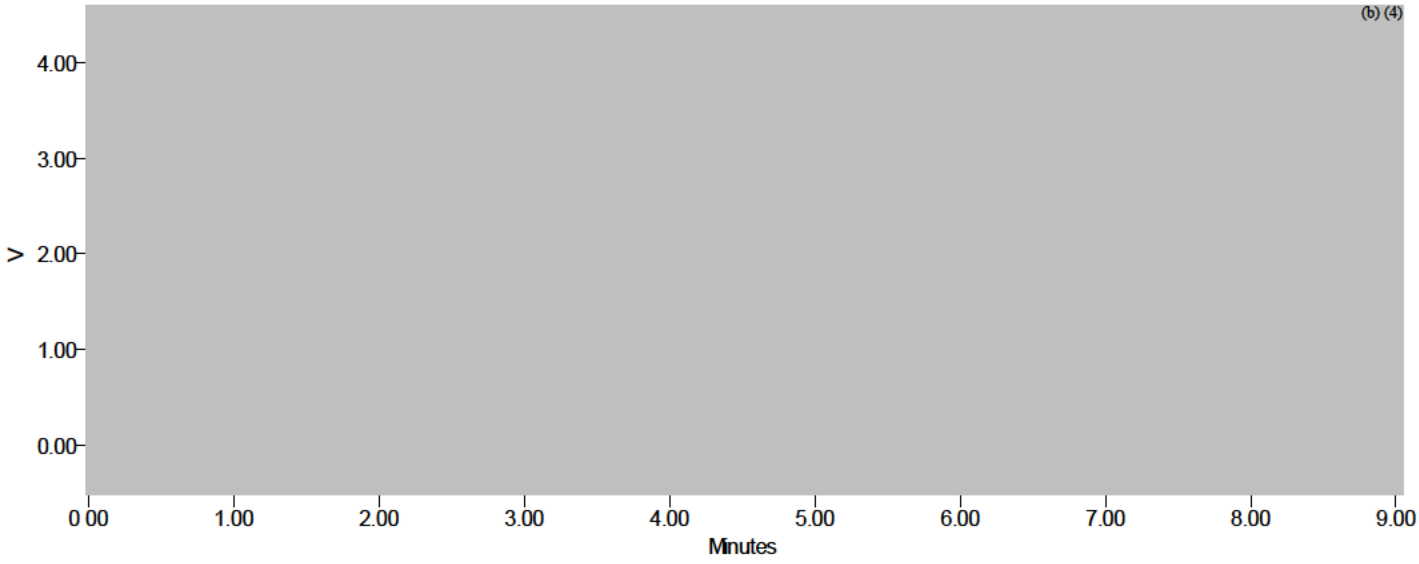
Sample Name:	SSTD31_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day_2nd
Vial:	3	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 1:21:34 AM KST		
Date Processed:	12/20/2020 5:02:47 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

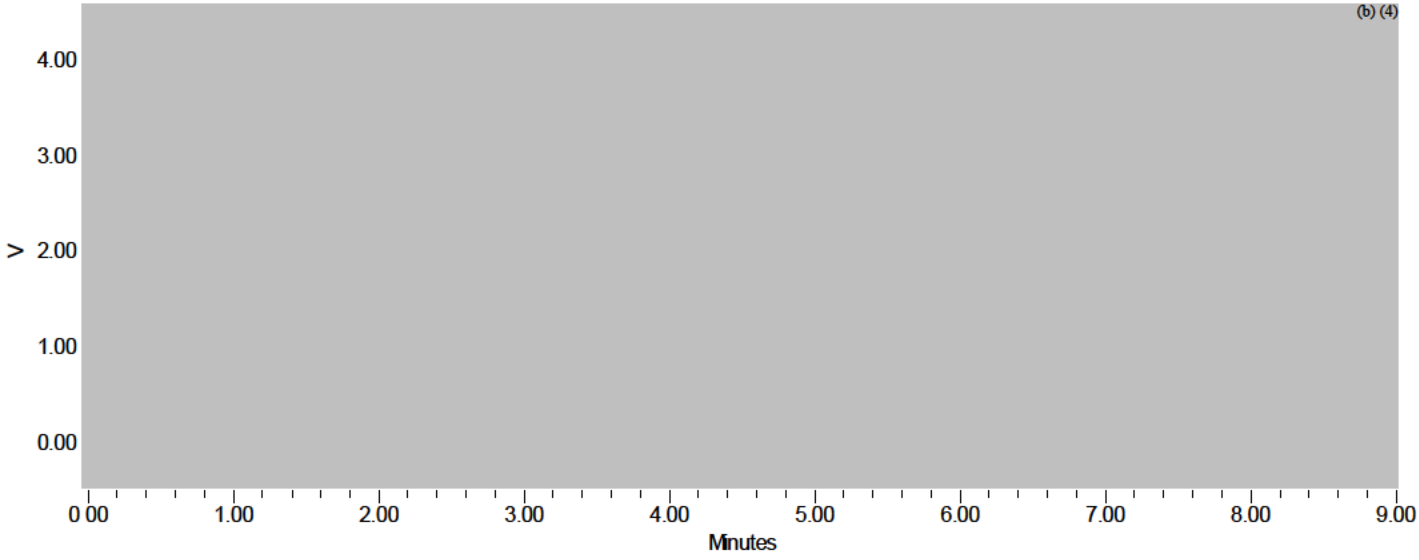
Sample Name:	SSPL31_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day_2nd
Vial:	4	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 1:31:16 AM KST		
Date Processed:	12/20/2020 5:02:47 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

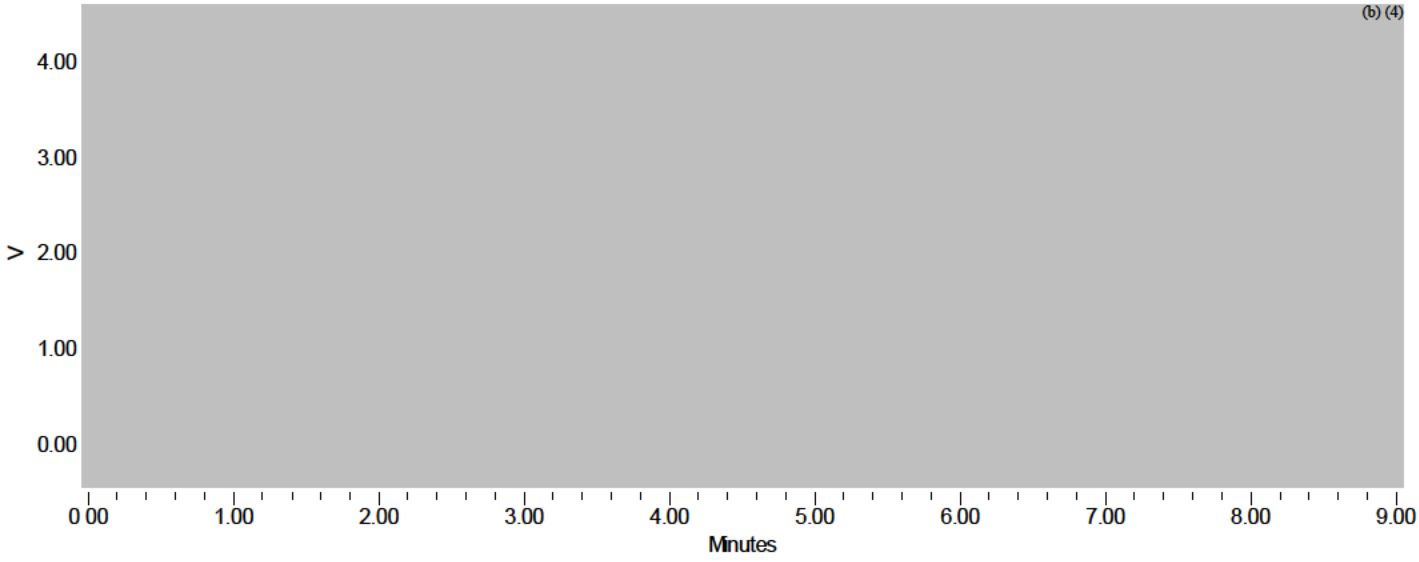
Sample Name:	SSTD31_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day_2nd
Vial:	5	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 1:40:52 AM KST		
Date Processed:	12/20/2020 5:02:47 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

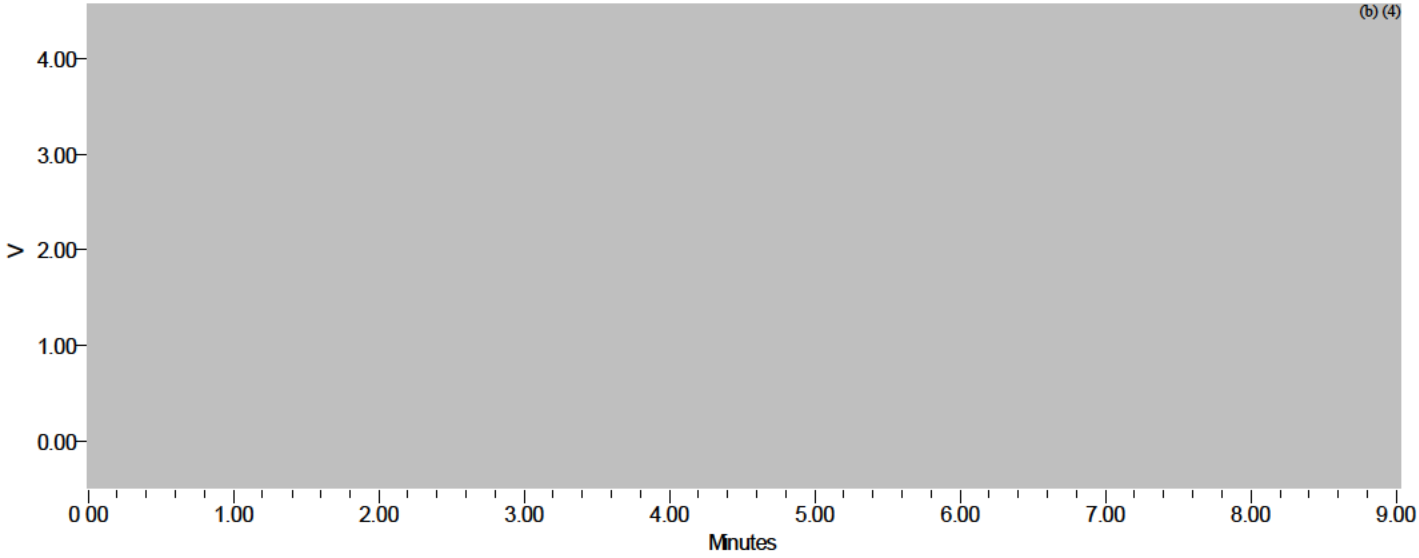
Sample Name:	SSPL31_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day_2nd
Vial:	6	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 1:50:33 AM KST		
Date Processed:	12/20/2020 5:02:47 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

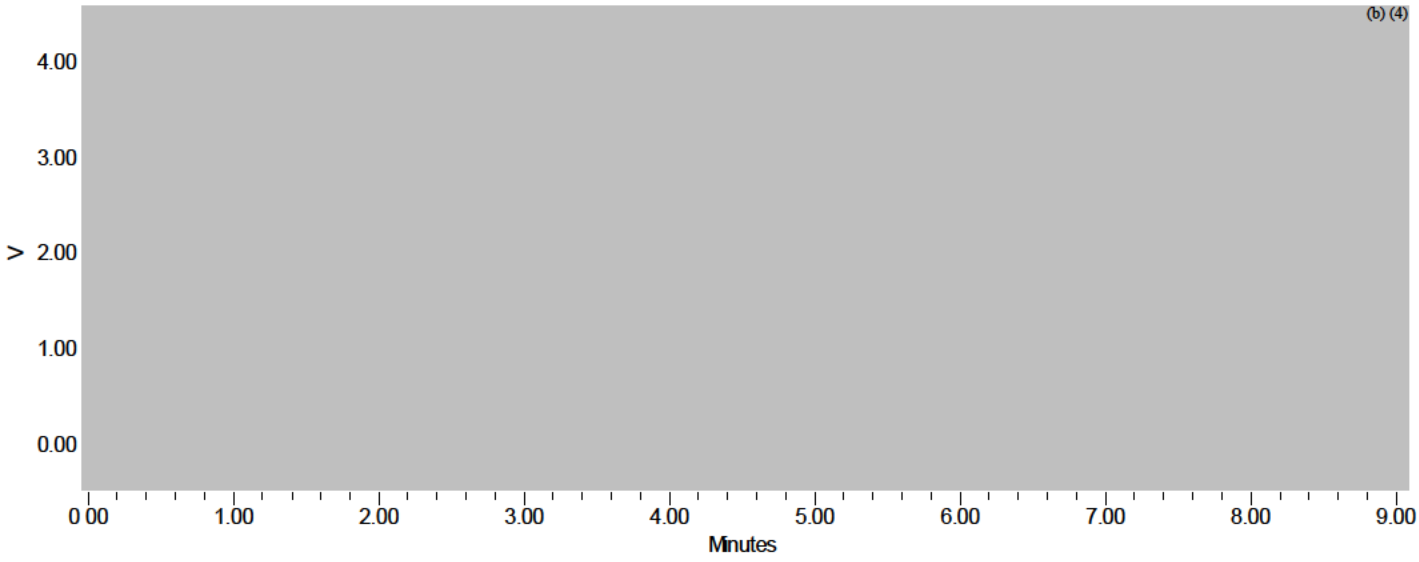
Sample Name:	SSTD31_4	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day_2nd
Vial:	7	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 2:00:12 AM KST		
Date Processed:	12/20/2020 5:02:47 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

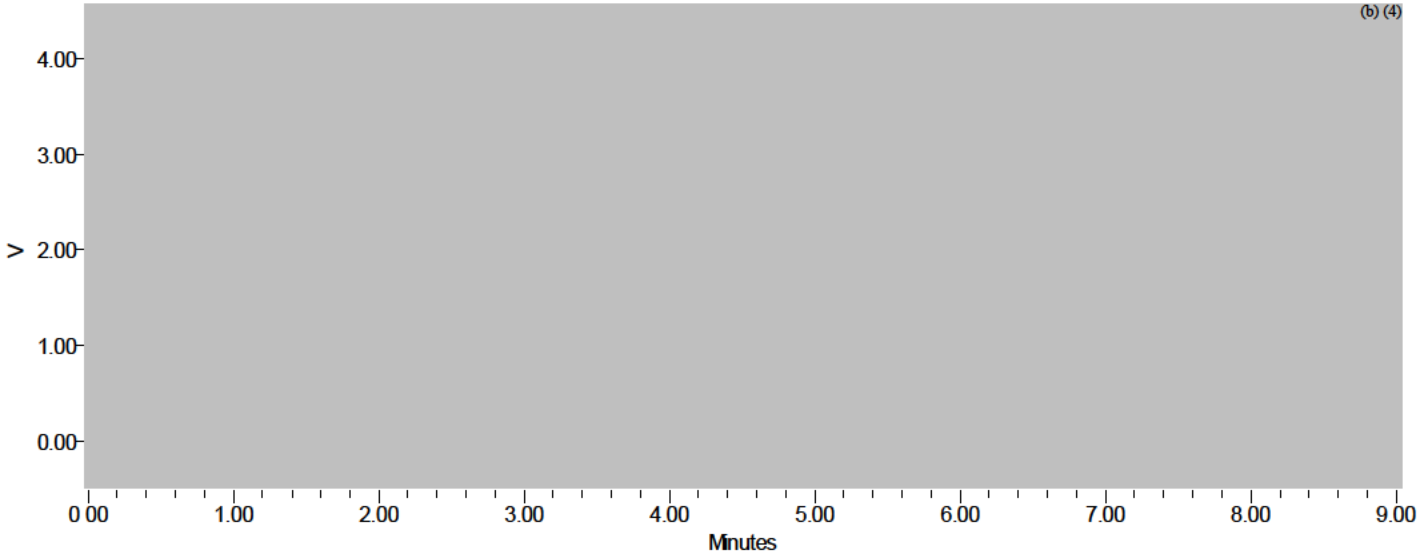
Sample Name:	SSTD32_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day_2nd
Vial:	8	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 5:19:12 AM KST		
Date Processed:	12/20/2020 5:02:59 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

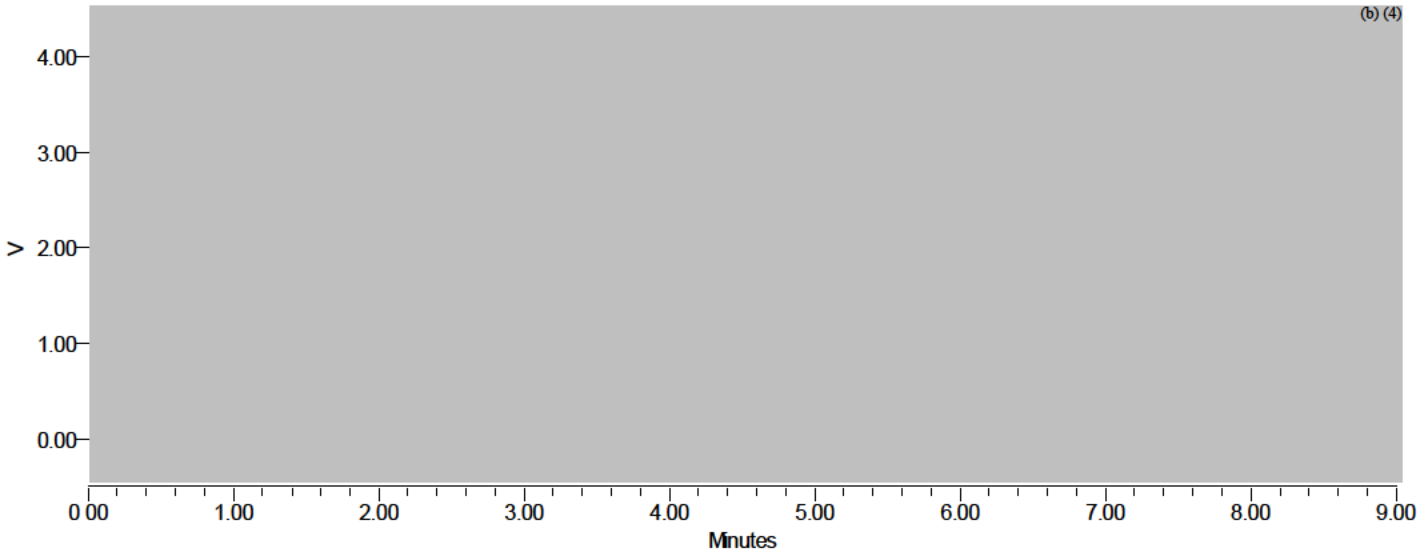
Sample Name:	SSPL32_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day_2nd
Vial:	9	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 5:28:53 AM KST		
Date Processed:	12/20/2020 5:02:59 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

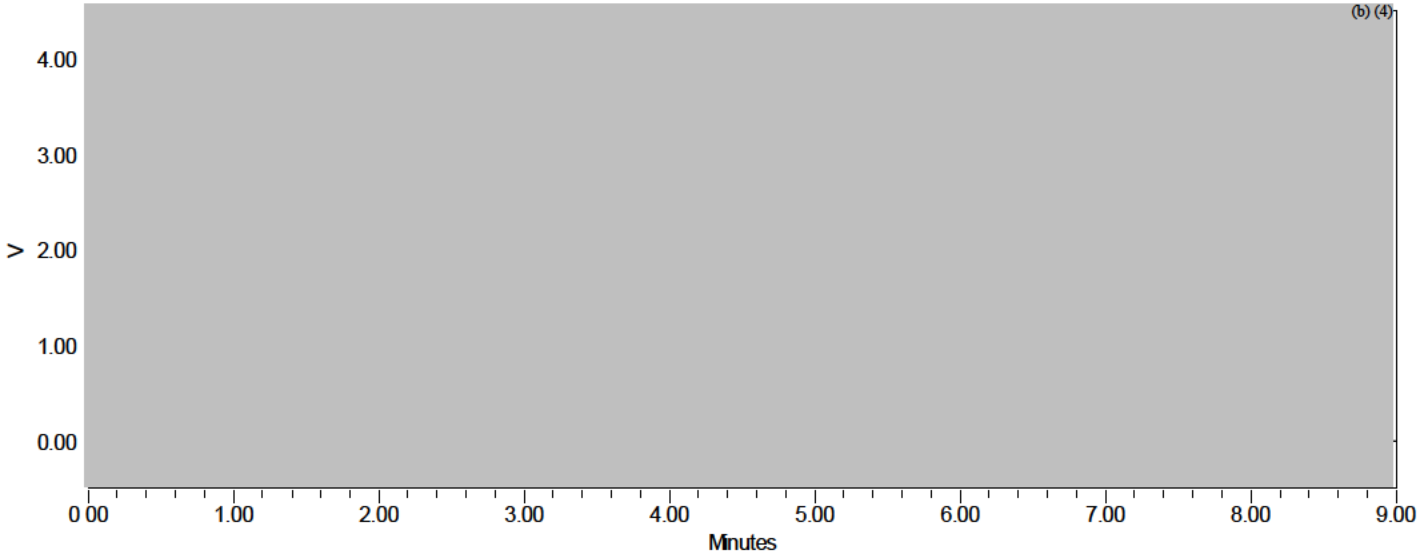
Sample Name:	SSTD32_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day_2nd
Vial:	10	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 5:38:36 AM KST		
Date Processed:	12/20/2020 5:02:59 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

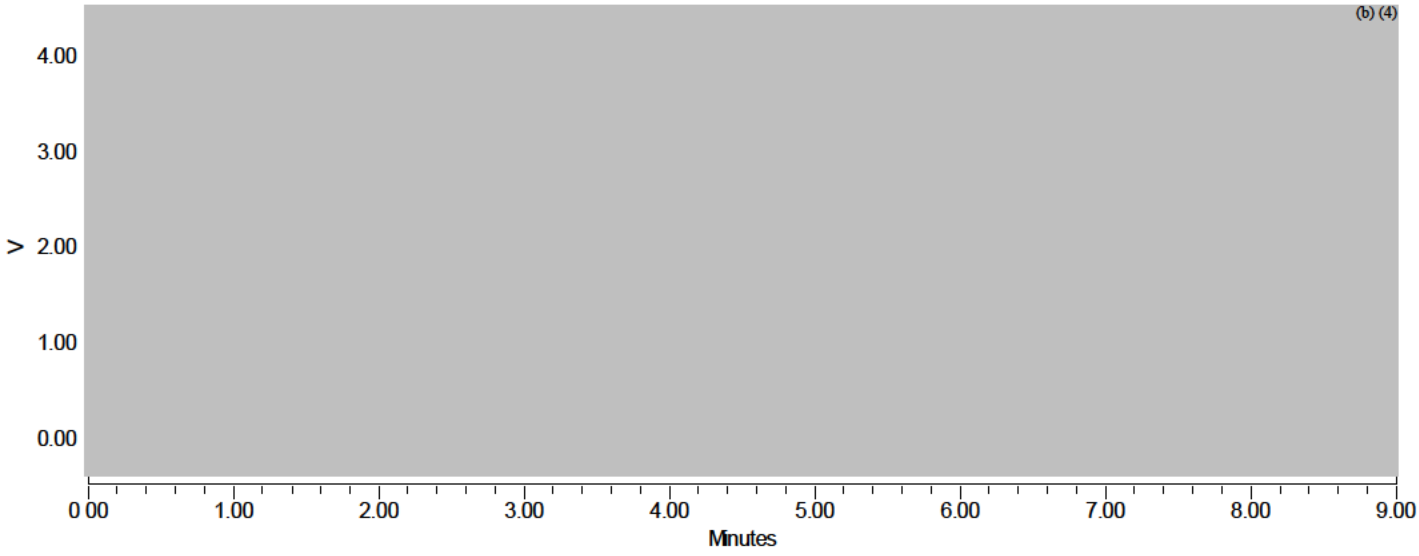
Sample Name:	SSPL32_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day_2nd
Vial:	11	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 5:48:19 AM KST		
Date Processed:	12/20/2020 5:03:00 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

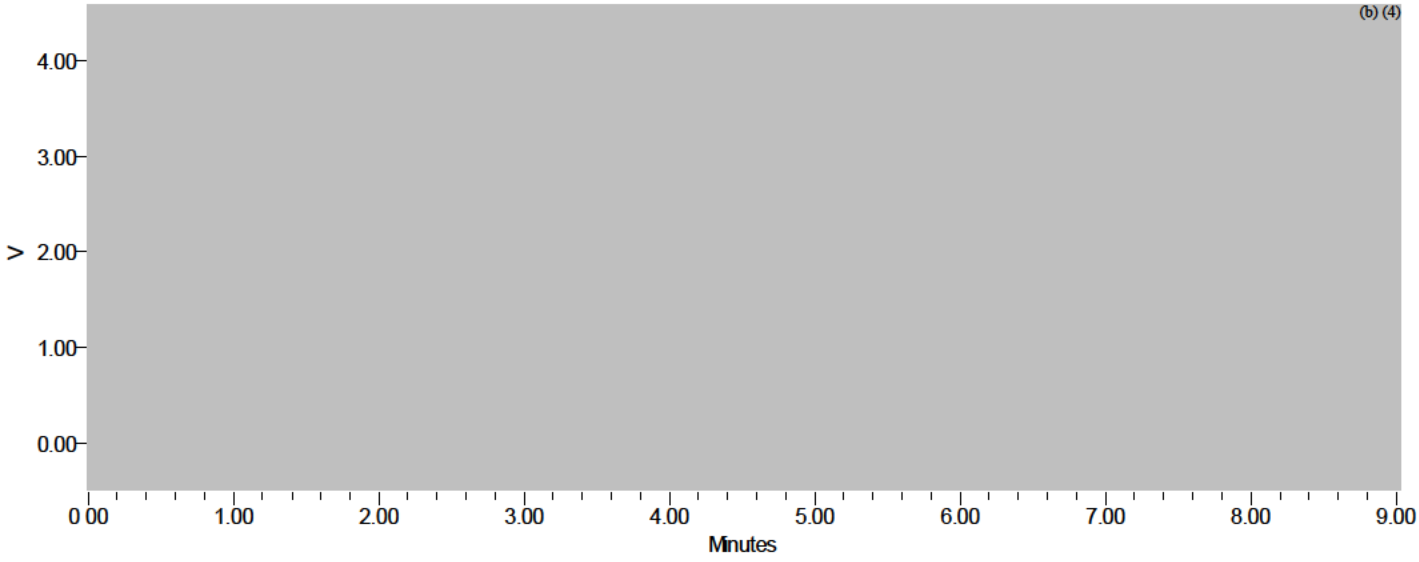
Sample Name:	SSTD32_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day_2nd
Vial:	12	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 5:57:54 AM KST		
Date Processed:	12/20/2020 5:03:00 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

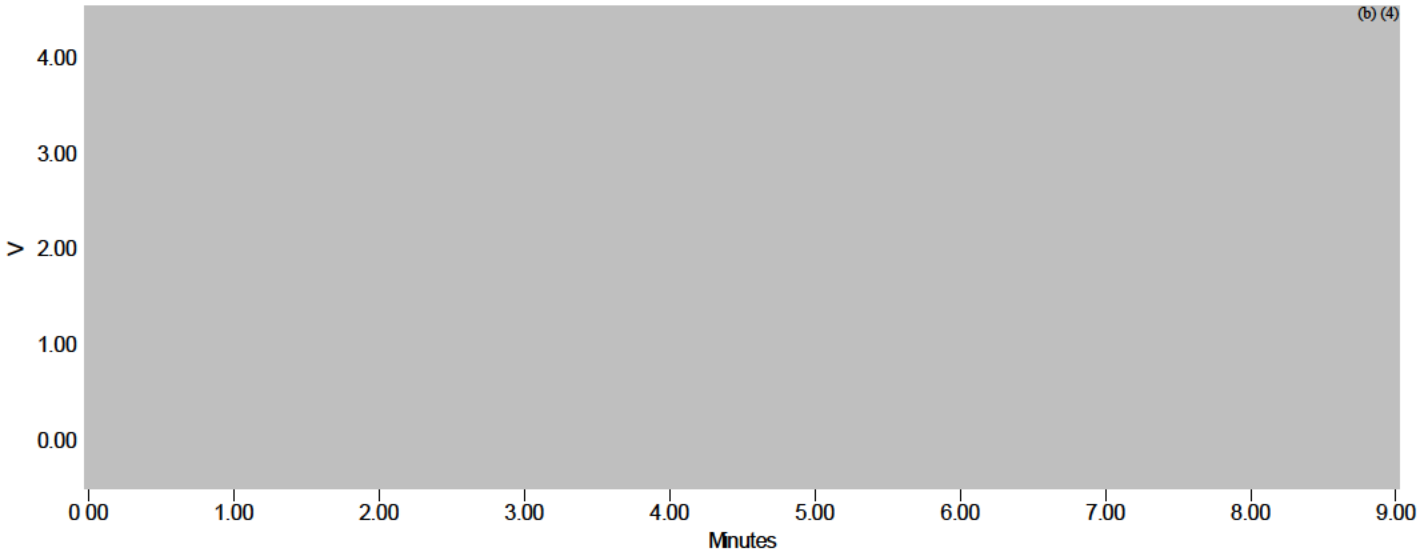
Sample Name:	SSPL32_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day_2nd
Vial:	13	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 6:07:36 AM KST		
Date Processed:	12/20/2020 5:03:00 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

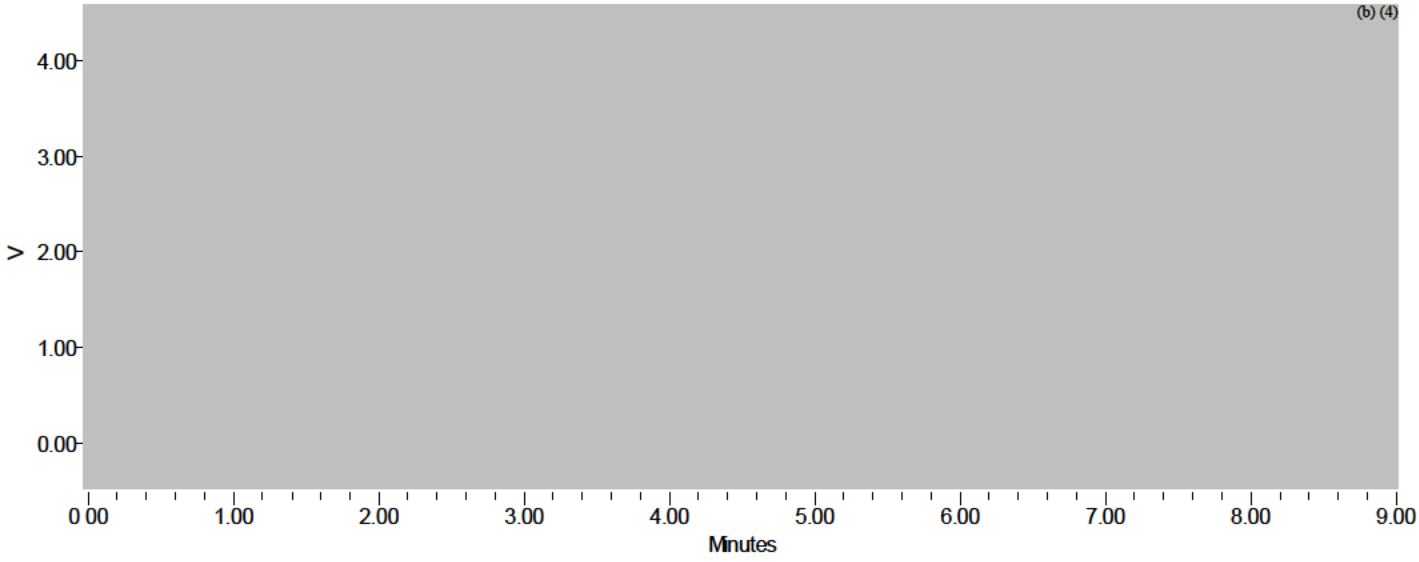
Sample Name:	SSTD32_4	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day_2nd
Vial:	14	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 6:17:17 AM KST		
Date Processed:	12/20/2020 5:03:00 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

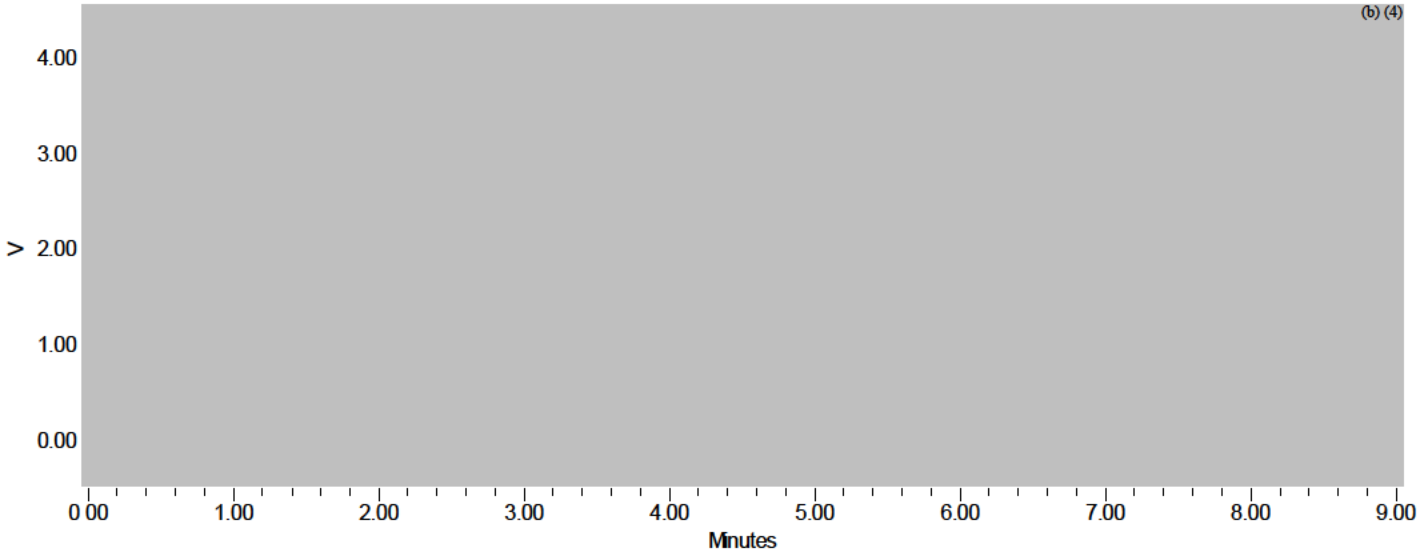
Sample Name:	SSTD33_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day_2nd
Vial:	15	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 9:36:23 AM KST		
Date Processed:	12/20/2020 5:03:09 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

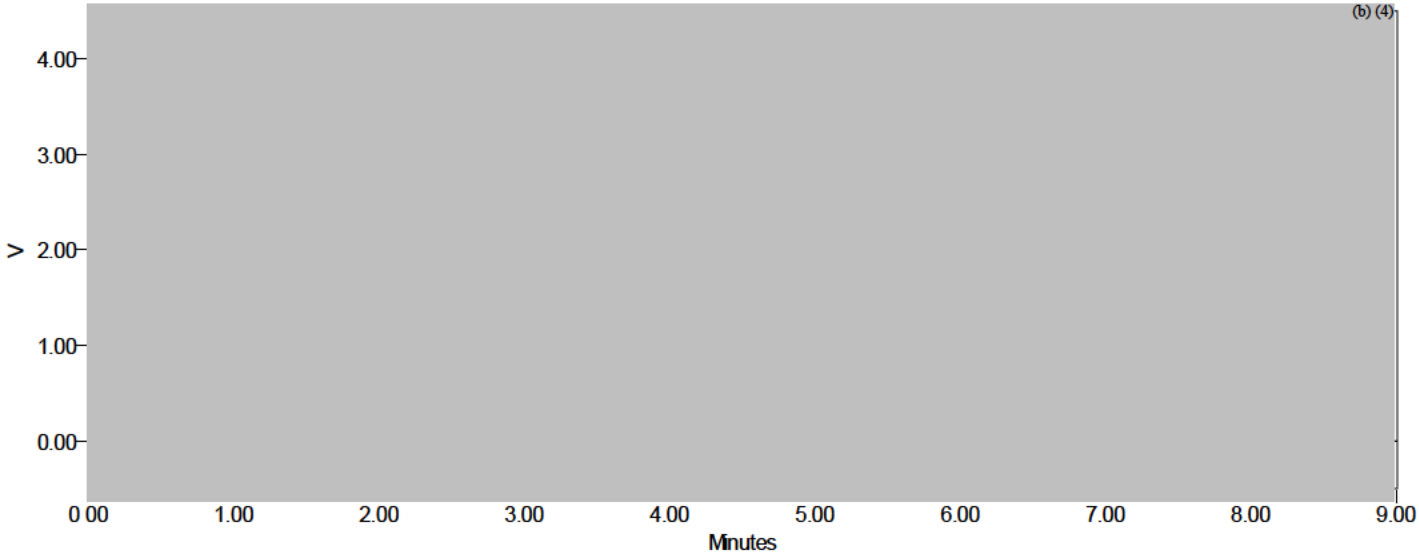
Sample Name:	SSPL33_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day_2nd
Vial:	16	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 9:46:03 AM KST		
Date Processed:	12/20/2020 5:03:10 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

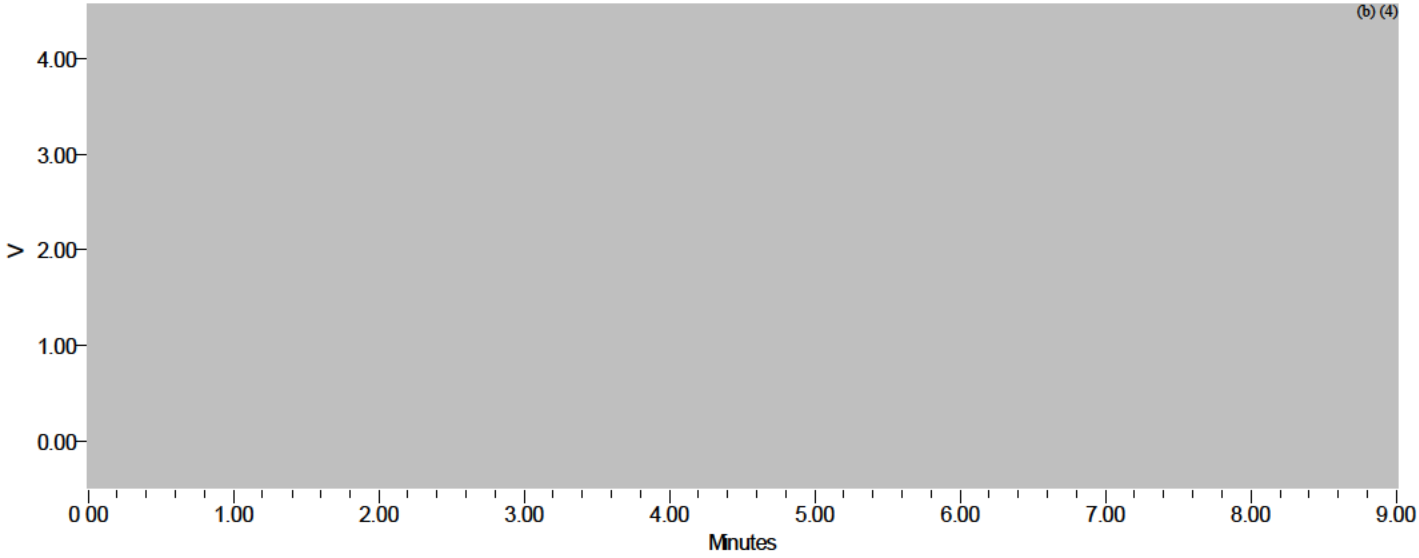
Sample Name:	SSTD33_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day_2nd
Vial:	17	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 9:55:45 AM KST		
Date Processed:	12/20/2020 5:03:10 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

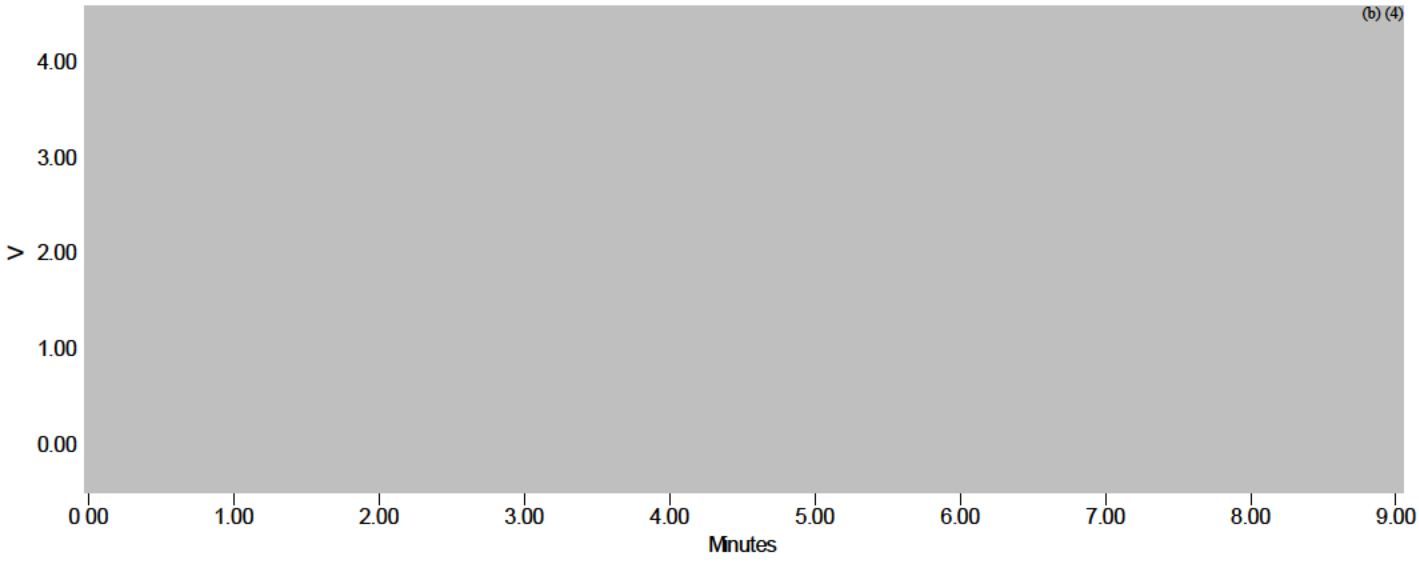
Sample Name:	SSPL33_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day_2nd
Vial:	18	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 10:05:25 AM KST		
Date Processed:	12/20/2020 5:03:10 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

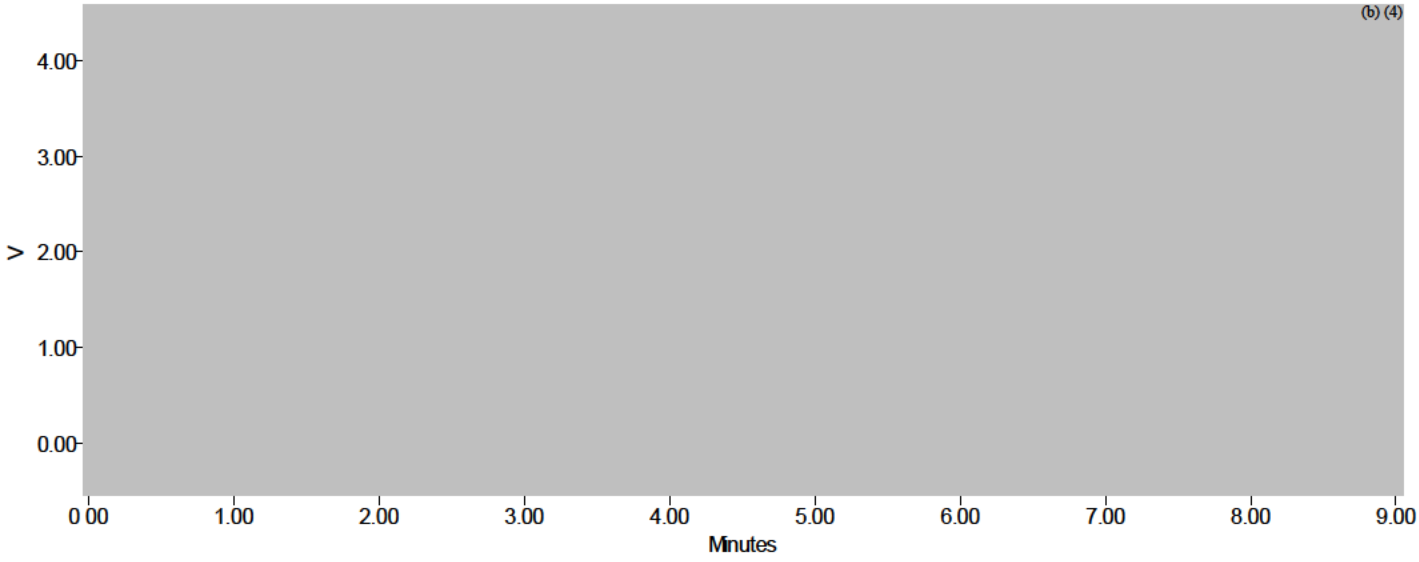
Sample Name:	SSTD33_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day_2nd
Vial:	19	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 10:15:04 AM KST		
Date Processed:	12/20/2020 5:03:10 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

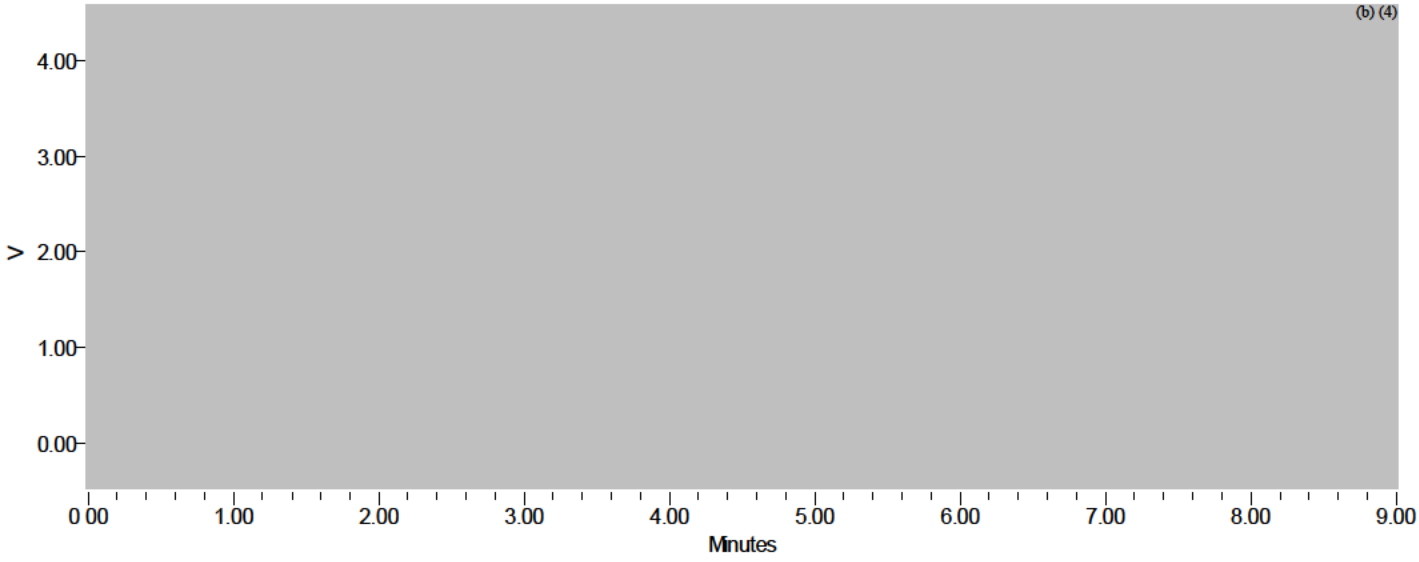
Sample Name:	SSPL33_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day_2nd
Vial:	20	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 10:24:44 AM KST		
Date Processed:	12/20/2020 5:03:10 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

Sample Name:	SSTD33_4	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day_2nd
Vial:	21	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 10:34:25 AM KST		
Date Processed:	12/20/2020 5:03:11 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

Software: Empower 3 Software Build 3471

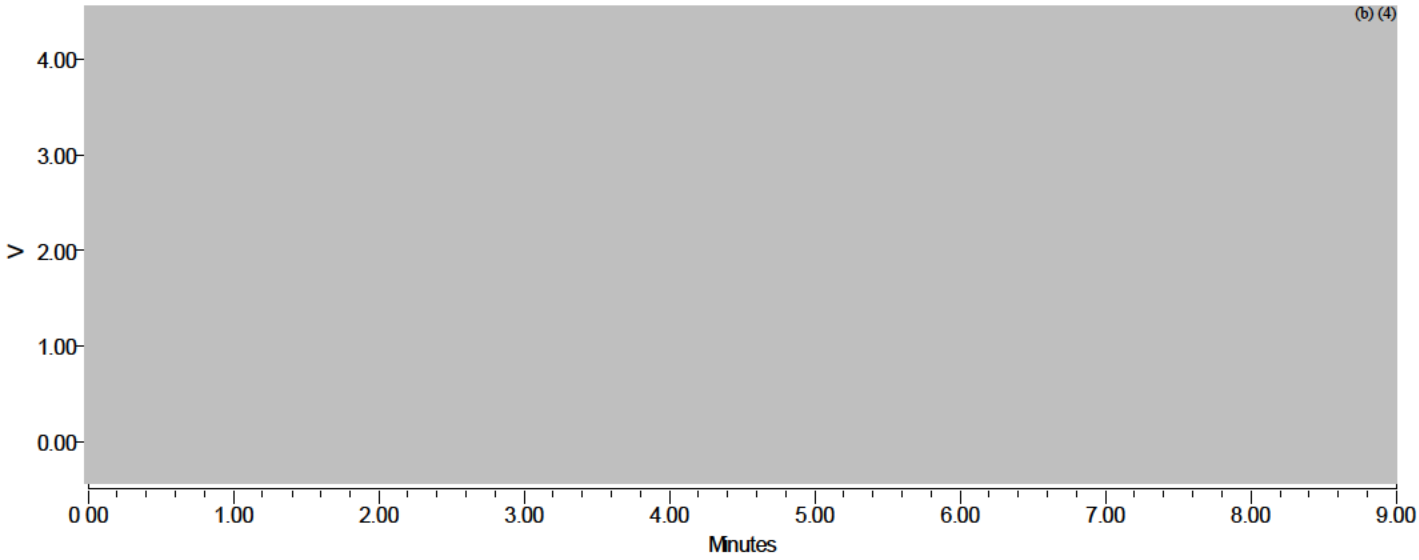
Sample Set Name:	Granule Valine_1 Day	Processed By:	System/Administrator
Sample Set Method:	Granule Valine_1 Day	Printed By:	System
System Node:	Client13	Result Set ID:	
System Name:	UC10	# of Results:	15
Acquired By:	System		
Sample Set Start Date:	12/17/2020 6:52:02 PM KST		
Sample Set Finish Date:	12/18/2020 12:24:42 PM KST		

Sample Set Table

	Sample Name	Sample Type	Vial	Inj #	Injection Volume (ul)	Acquistion Method Set	Processed Channel Descr.	(b) (4)
1	L1_1	Unknown	29	1	5.00	VAL_ACR	Detector A	
2	L1_2	Unknown	30	1	5.00	VAL_ACR	Detector A	
3	L1_3	Unknown	31	1	5.00	VAL_ACR	Detector A	
4	L2_1	Unknown	32	1	5.00	VAL_ACR	Detector A	
5	L2_2	Unknown	33	1	5.00	VAL_ACR	Detector A	
6	L2_3	Unknown	34	1	5.00	VAL_ACR	Detector A	
7	L3_1	Unknown	35	1	5.00	VAL_ACR	Detector A	
8	L3_2	Unknown	36	1	5.00	VAL_ACR	Detector A	
9	L3_3	Unknown	37	1	5.00	VAL_ACR	Detector A	
10	L4_1	Unknown	38	1	5.00	VAL_ACR	Detector A	
11	L4_2	Unknown	39	1	5.00	VAL_ACR	Detector A	
12	L4_3	Unknown	40	1	5.00	VAL_ACR	Detector A	
13	L5_1	Unknown	41	1	5.00	VAL_ACR	Detector A	
14	L5_2	Unknown	42	1	5.00	VAL_ACR	Detector A	
15	L5_3	Unknown	43	1	5.00	VAL_ACR	Detector A	

SAMPLE INFORMATION

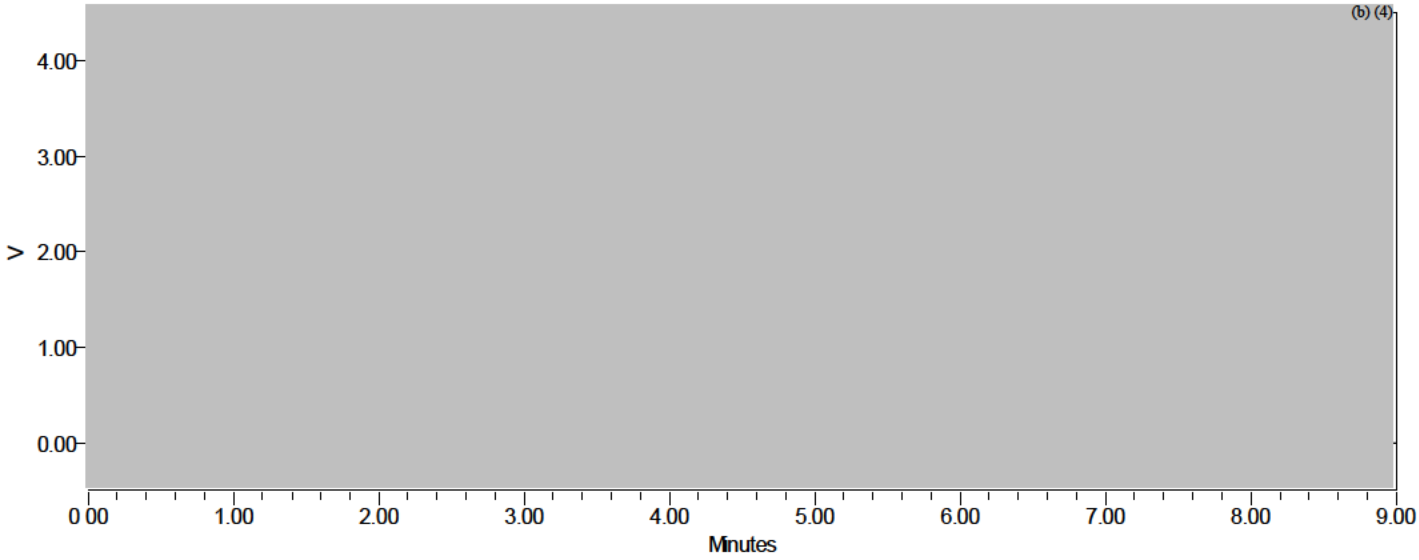
Sample Name:	L1_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	29	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/17/2020 10:25:15 PM KST		
Date Processed:	12/20/2020 5:13:50 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

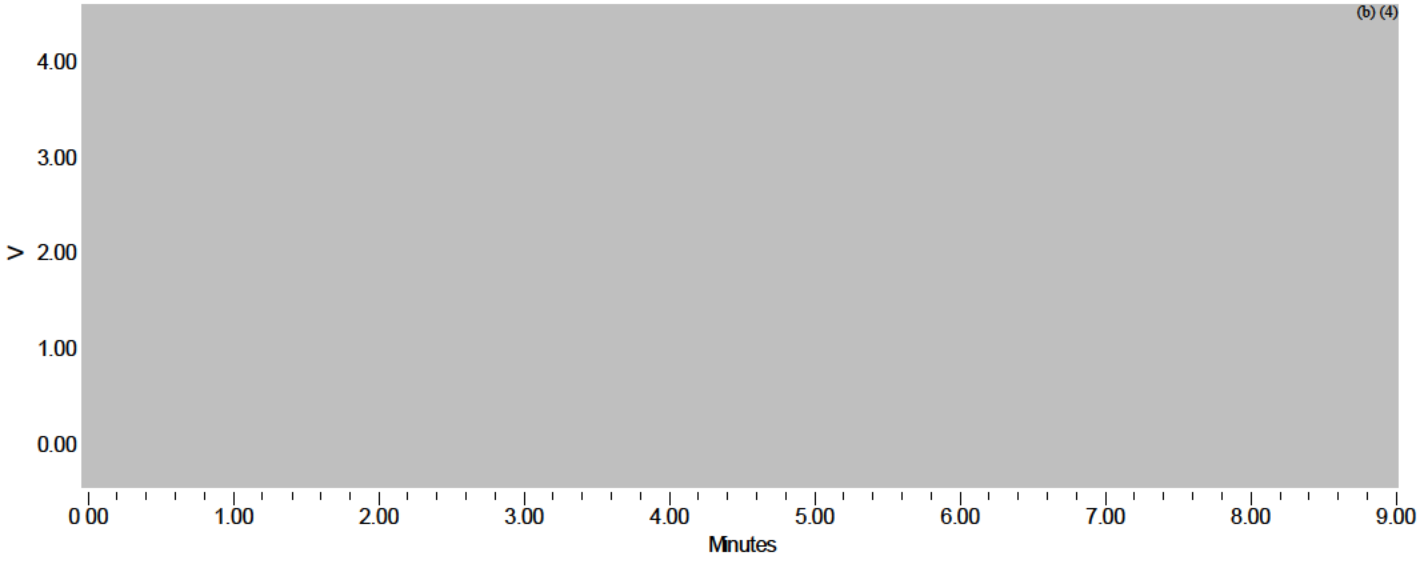
Sample Name:	L1_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	30	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/17/2020 10:34:57 PM KST		
Date Processed:	12/20/2020 5:13:50 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

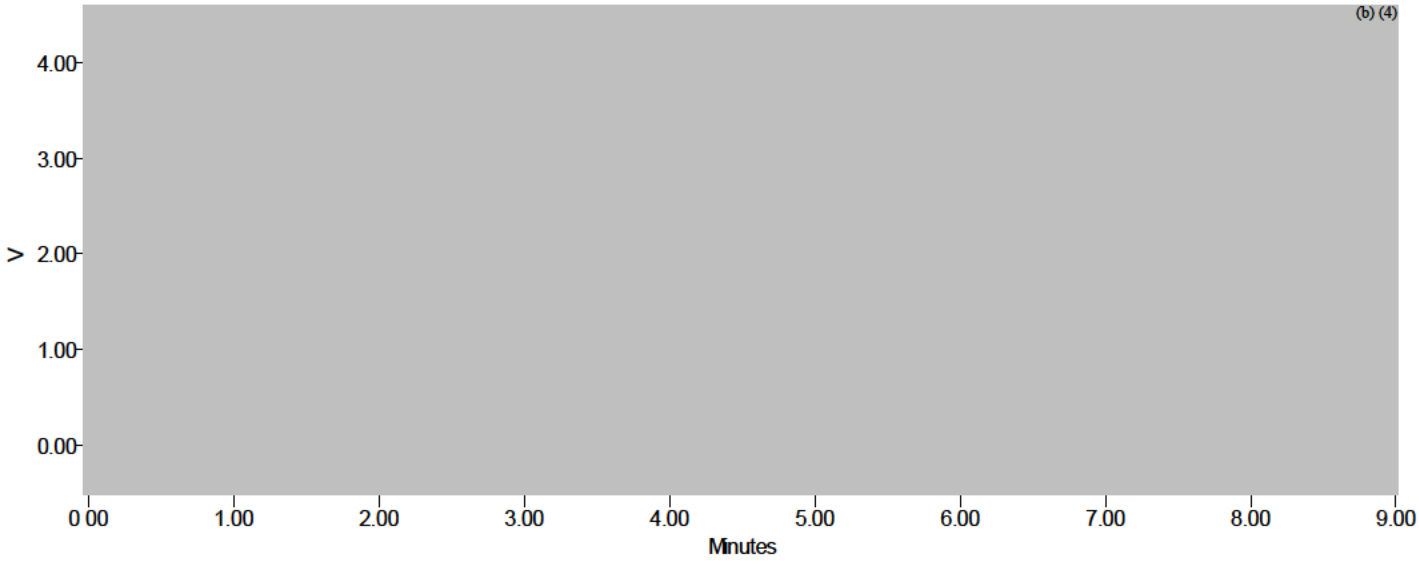
Sample Name:	L1_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	31	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/17/2020 10:44:39 PM KST		
Date Processed:	12/20/2020 5:13:50 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

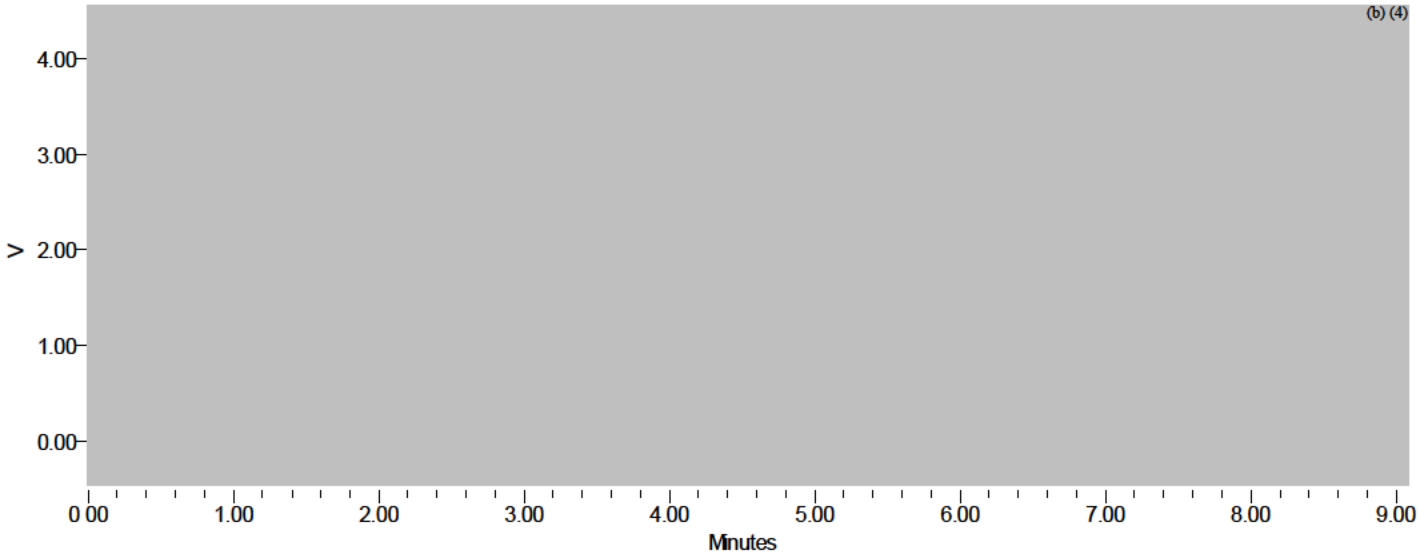
Sample Name:	L2_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	32	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/17/2020 10:54:20 PM KST		
Date Processed:	12/20/2020 5:13:50 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

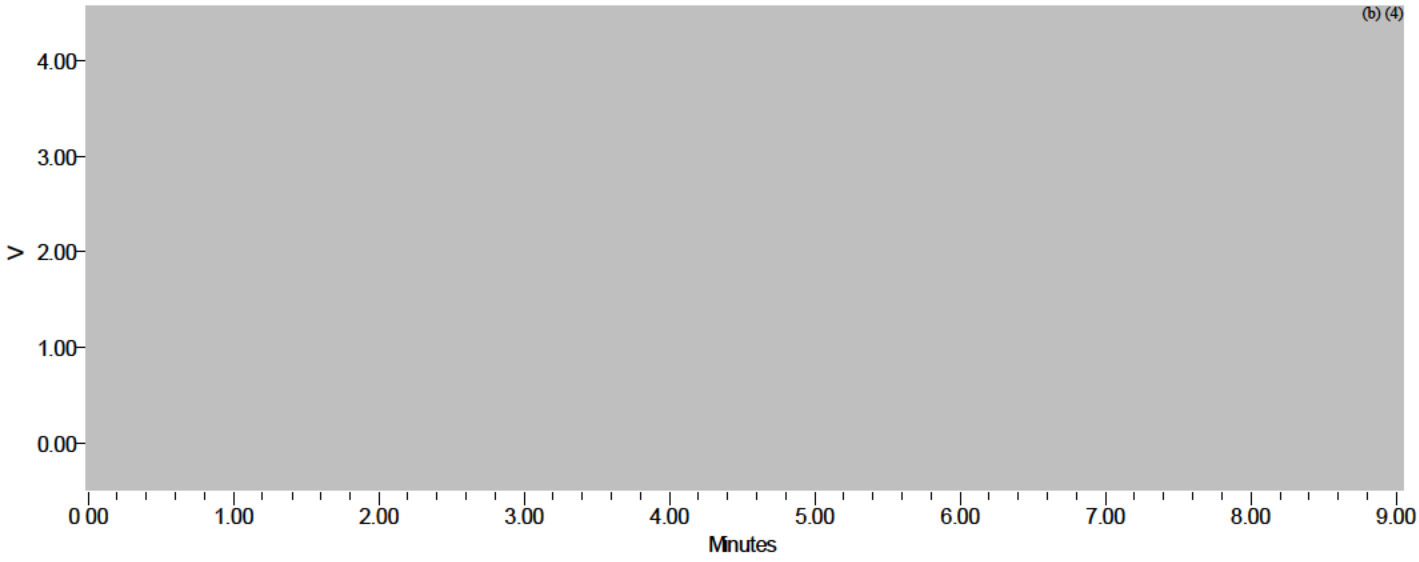
Sample Name:	L2_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	33	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/17/2020 11:04:00 PM KST		
Date Processed:	12/20/2020 5:13:50 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

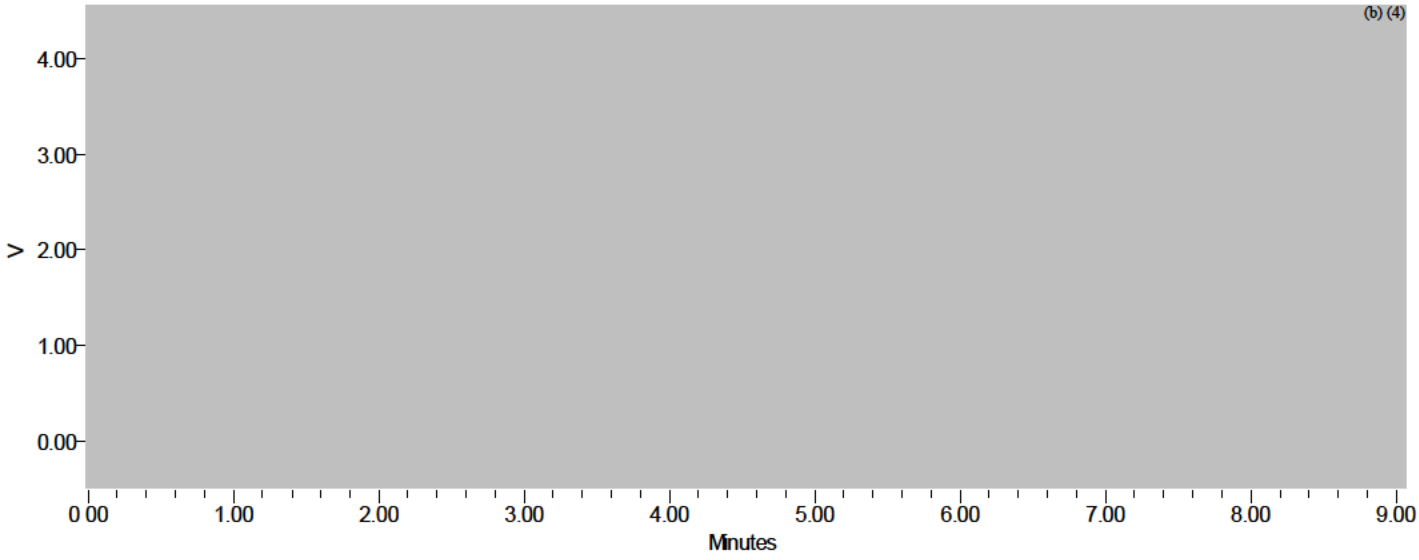
Sample Name:	L2_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	34	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/17/2020 11:13:39 PM KST		
Date Processed:	12/20/2020 5:13:51 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

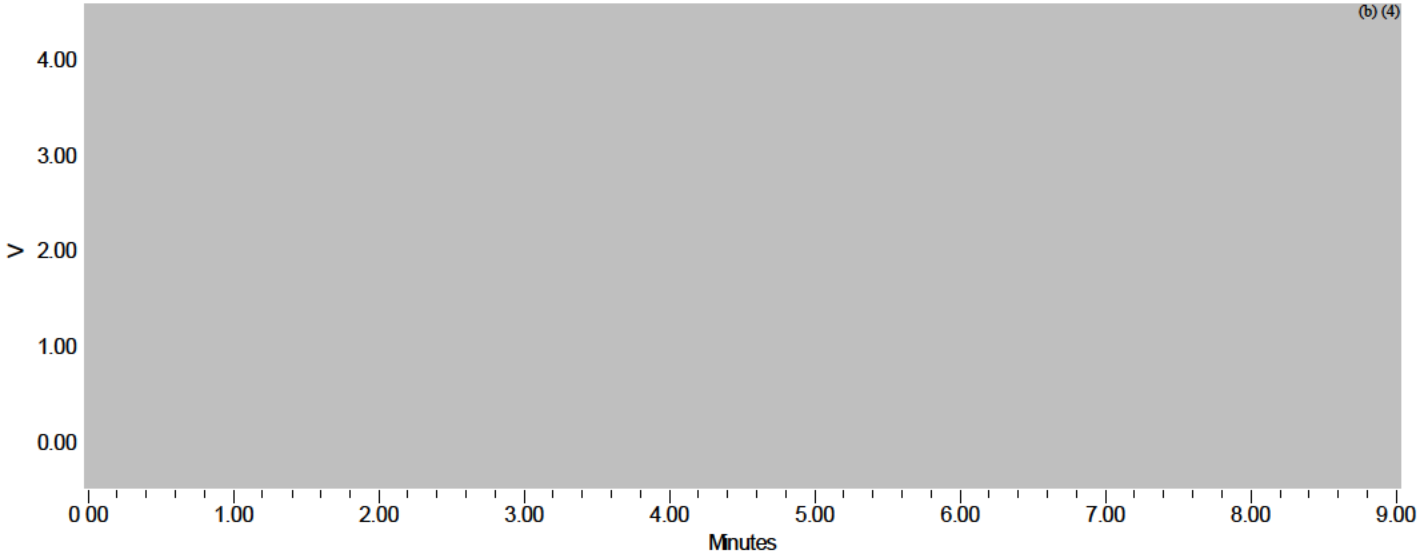
Sample Name:	L3_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	35	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/17/2020 11:23:19 PM KST		
Date Processed:	12/20/2020 5:13:51 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

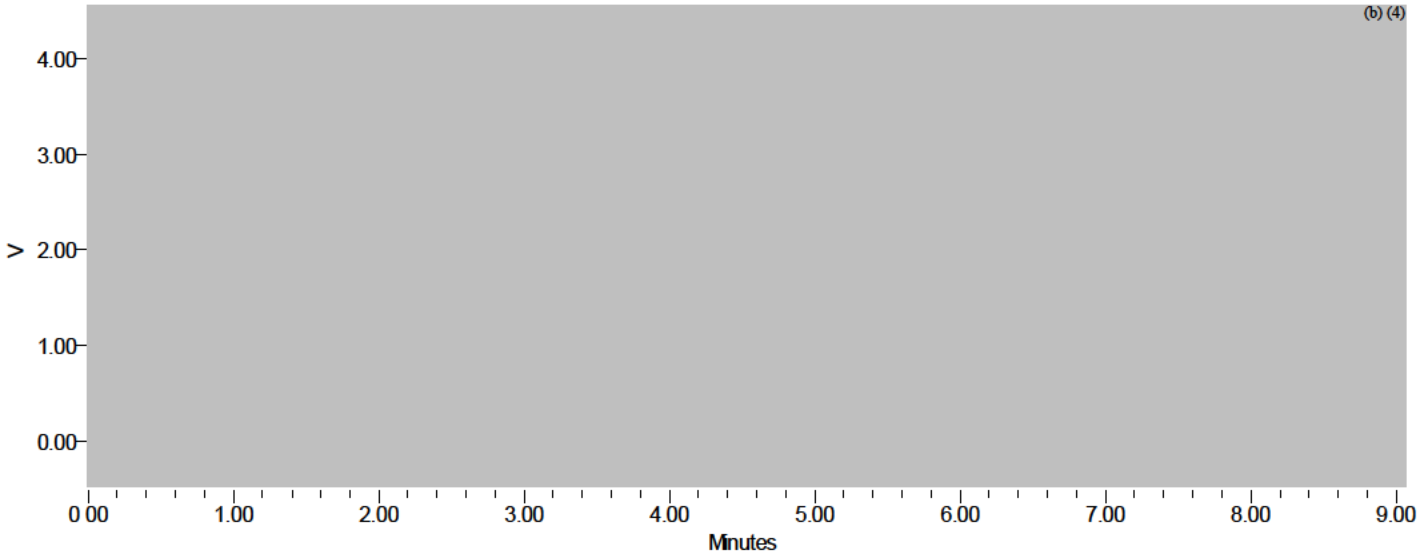
Sample Name:	L3_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	36	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/17/2020 11:32:58 PM KST		
Date Processed:	12/20/2020 5:13:51 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

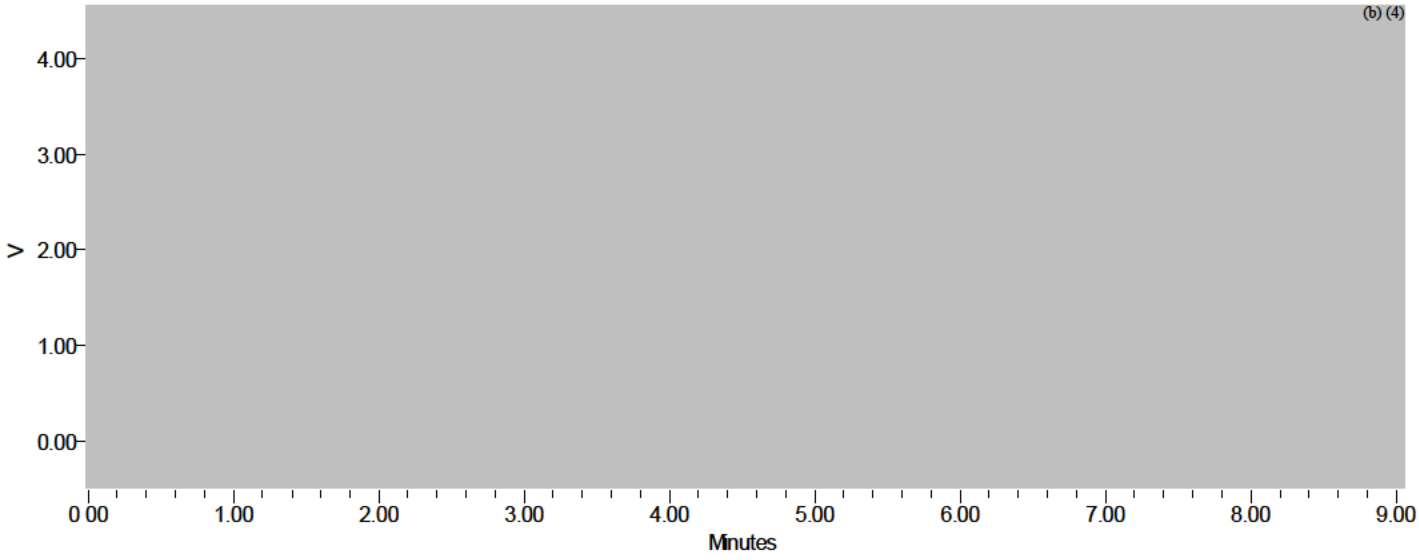
Sample Name:	L3_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	37	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/17/2020 11:42:39 PM KST		
Date Processed:	12/20/2020 5:13:51 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

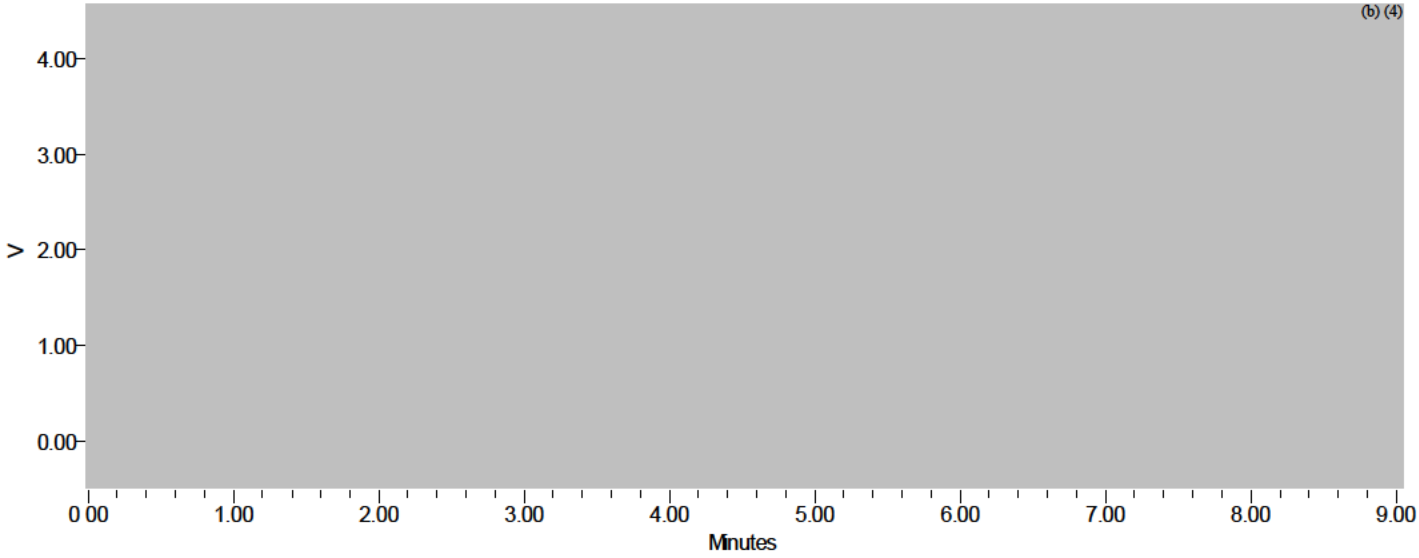
Sample Name:	L4_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	38	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/17/2020 11:52:17 PM KST		
Date Processed:	12/20/2020 5:13:51 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

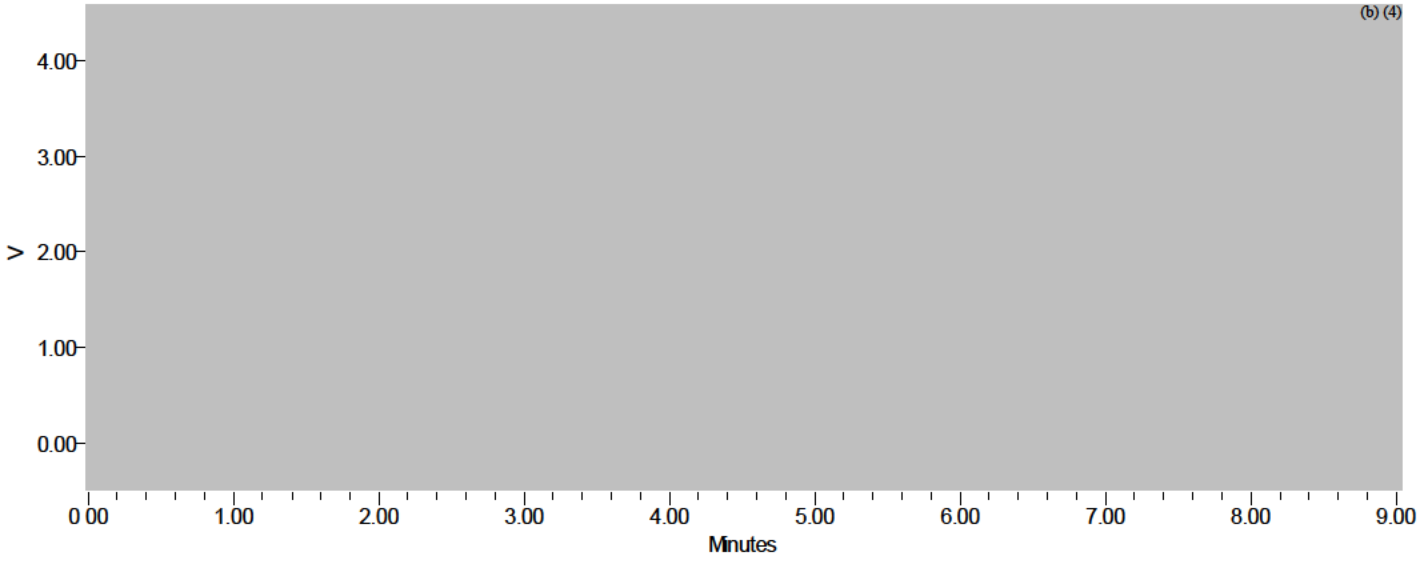
Sample Name:	L4_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	39	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 12:01:58 AM KST		
Date Processed:	12/20/2020 5:13:52 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

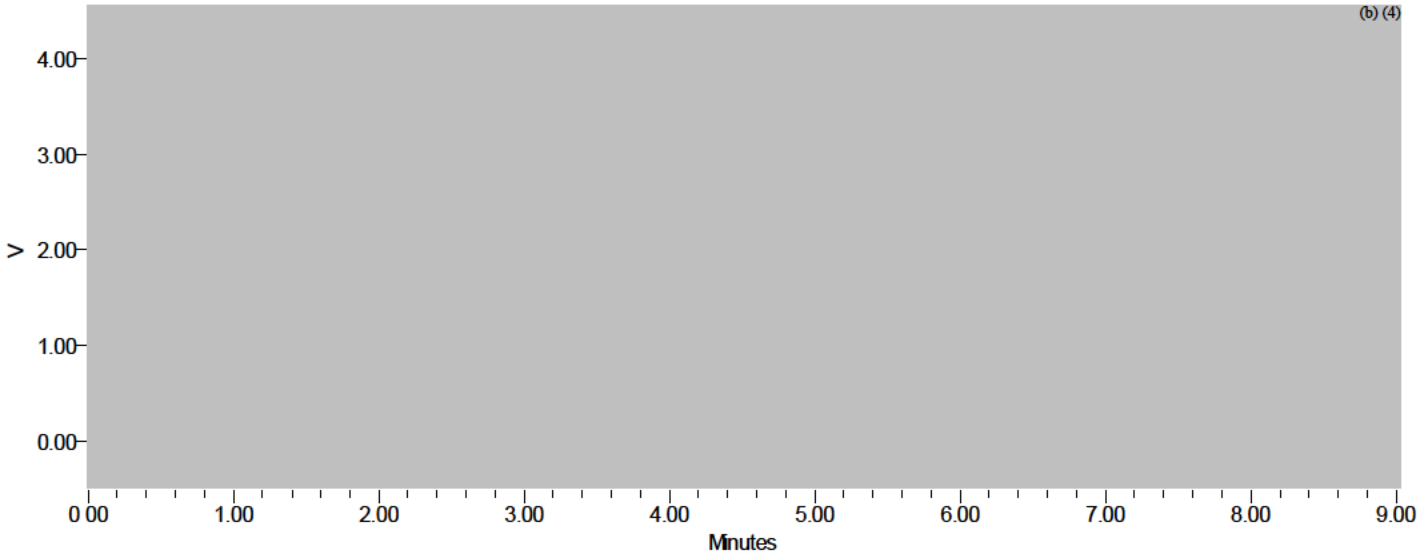
Sample Name:	L4_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	40	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 12:11:34 AM KST		
Date Processed:	12/20/2020 5:13:52 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

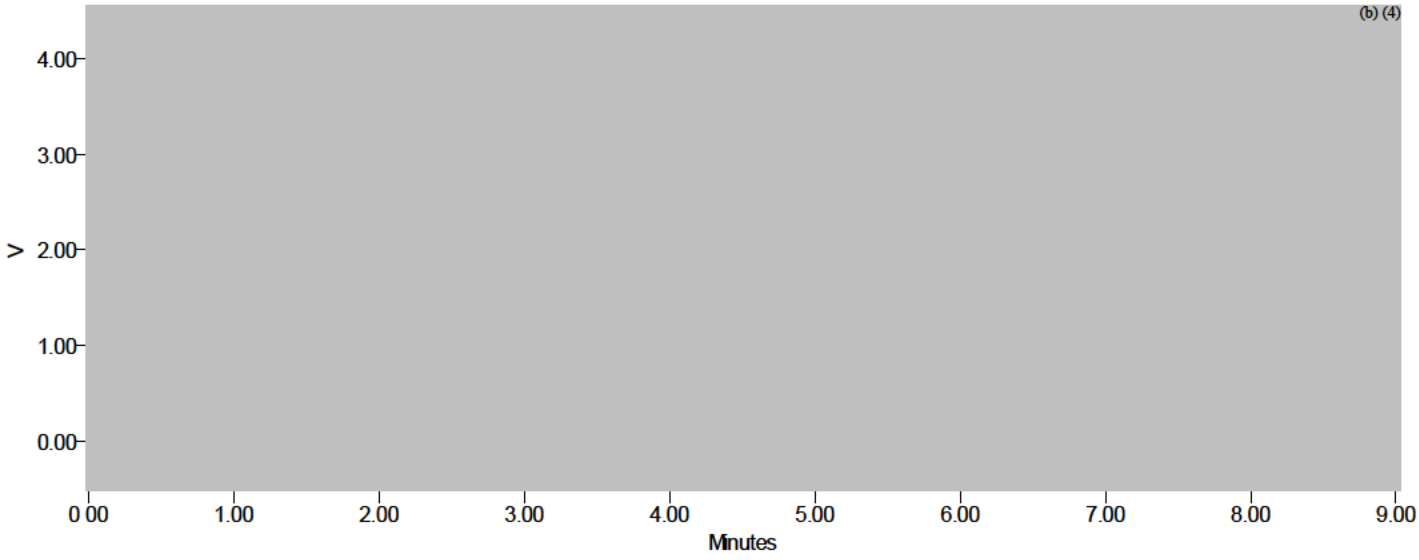
Sample Name:	L5_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	41	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 12:21:11 AM KST		
Date Processed:	12/20/2020 5:13:52 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

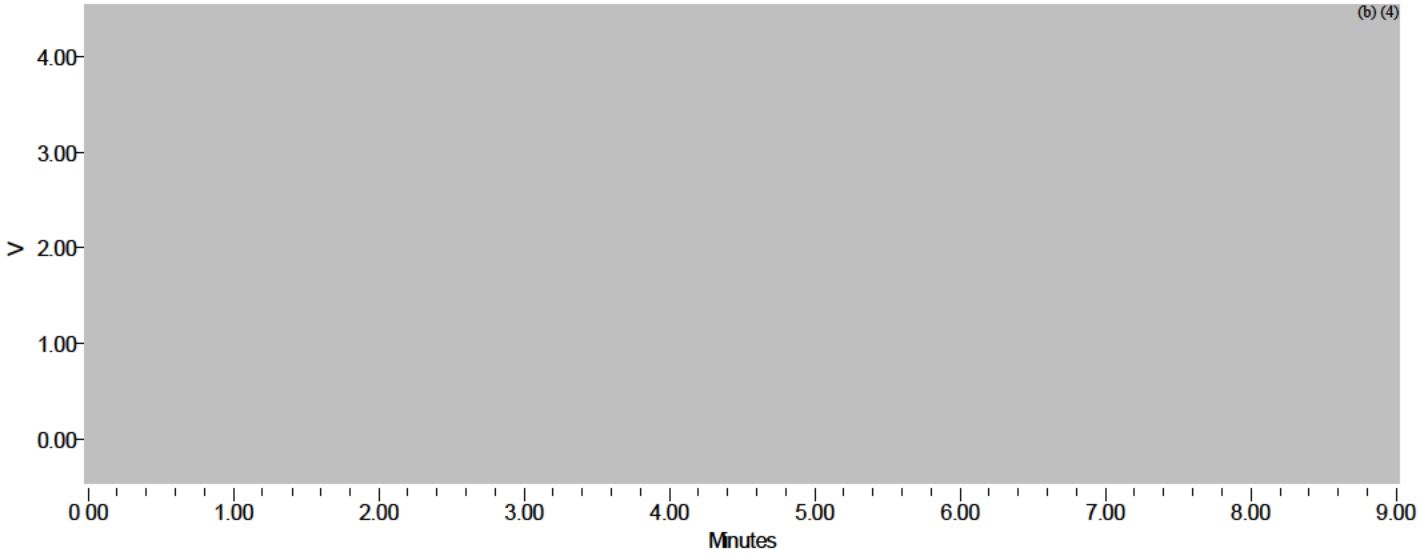
Sample Name:	L5_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	42	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 12:30:45 AM KST		
Date Processed:	12/20/2020 5:13:52 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

Sample Name:	L5_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	43	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 12:40:28 AM KST		
Date Processed:	12/20/2020 5:13:52 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

Software: Empower 3 Software Build 3471

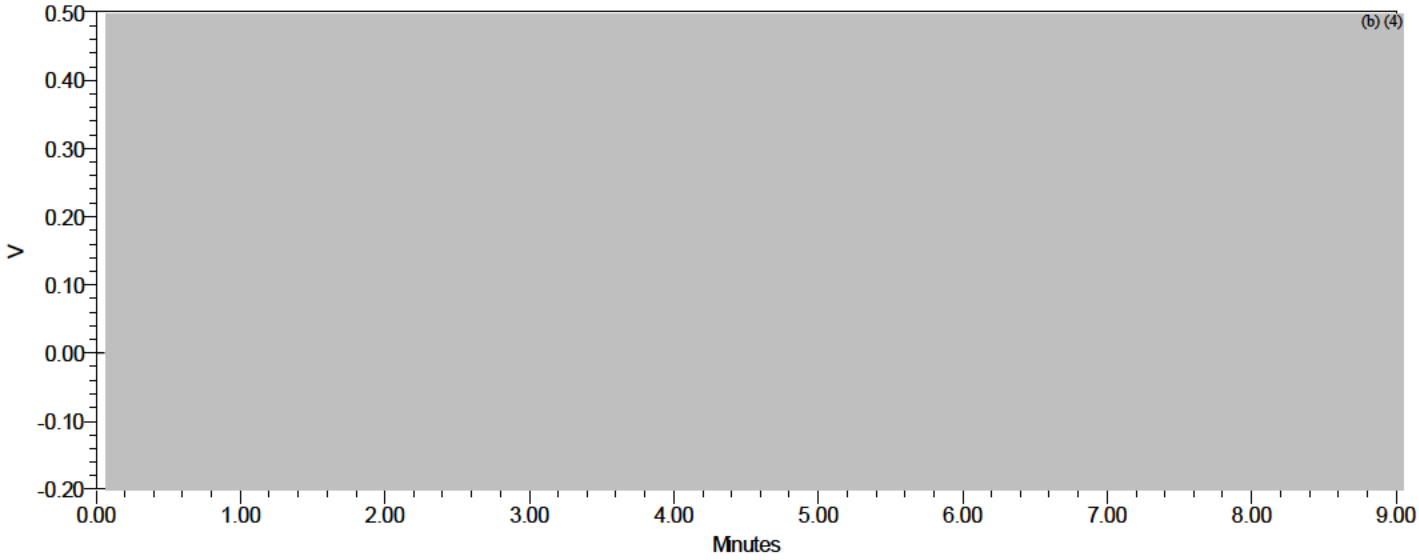
Sample Set Name:	Granule Valine_1 Day	Processed By:	System/Administrator
Sample Set Method:	Granule Valine_1 Day	Printed By:	System
System Node:	Client13	Result Set ID:	
System Name:	UC10	# of Results:	3
Acquired By:	System		
Sample Set Start Date:	12/17/2020 6:52:02 PM KST		
Sample Set Finish Date:	12/18/2020 12:24:42 PM KST		

Sample Set Table

	Sample Name	Sample Type	Vial	Inj #	Injection Volume (ul)	Acquistion Method Set	Processed Channel Descr.	(b) (4)
1	BLANK	Unknown	105	1	5.00	VAL_ACR	Detector A	
2	LOD_1ppm	Unknown	44	1	5.00	VAL_ACR	Detector A	
3	LOQ_3ppm	Unknown	46	1	5.00	VAL_ACR	Detector A	

SAMPLE INFORMATION

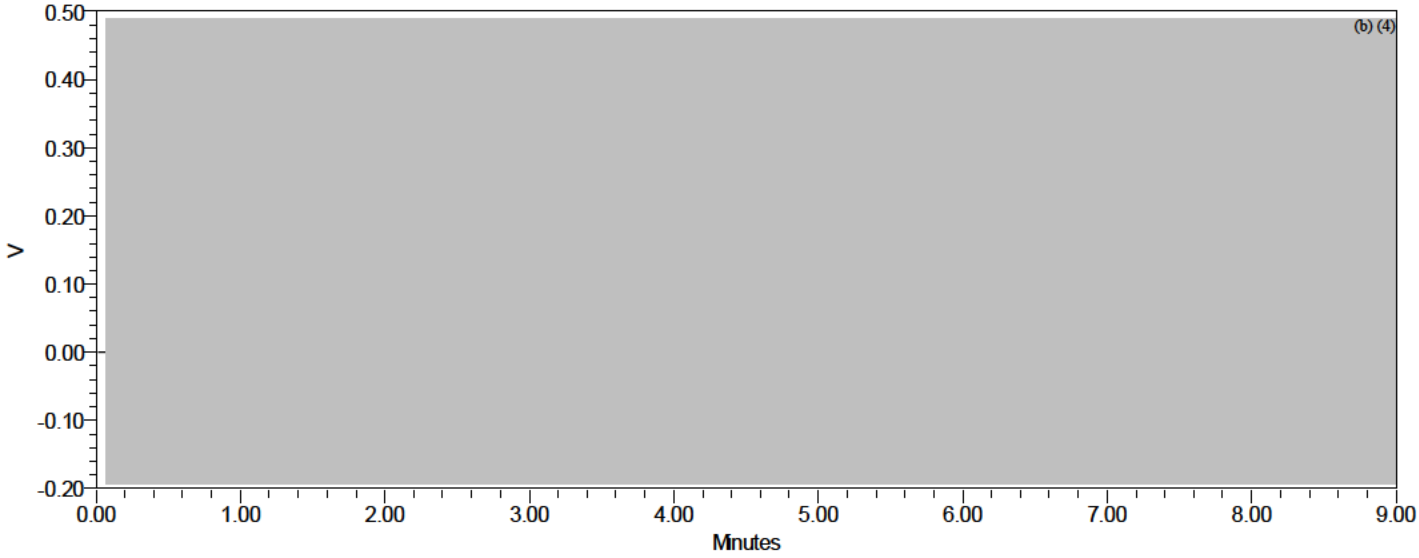
Sample Name:	BLANK	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	105	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 6:56:51 AM KST		
Date Processed:	12/20/2020 5:17:09 PM KST		



	Peak Name	RT
1	Valine	(b) (4)

SAMPLE INFORMATION

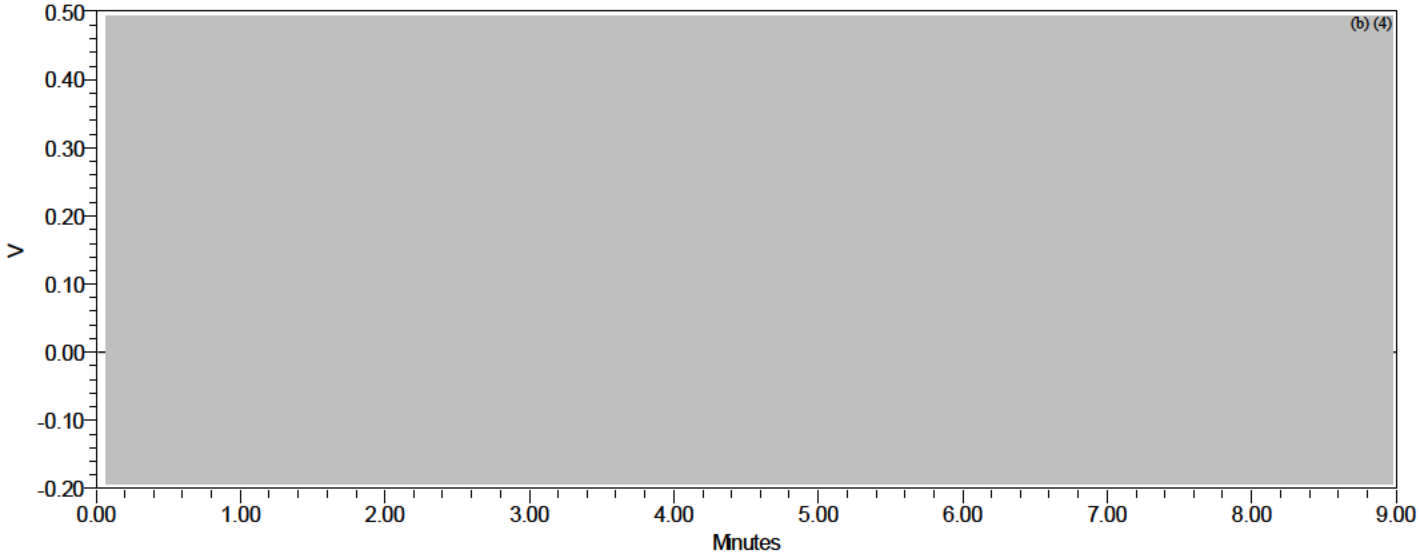
Sample Name:	LOD_1ppm	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	44	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 7:06:33 AM KST		
Date Processed:	12/20/2020 5:17:44 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

Sample Name:	LOQ_3ppm	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	46	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 7:25:48 AM KST		
Date Processed:	12/20/2020 5:17:45 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

Software: Empower 3 Software Build 3471

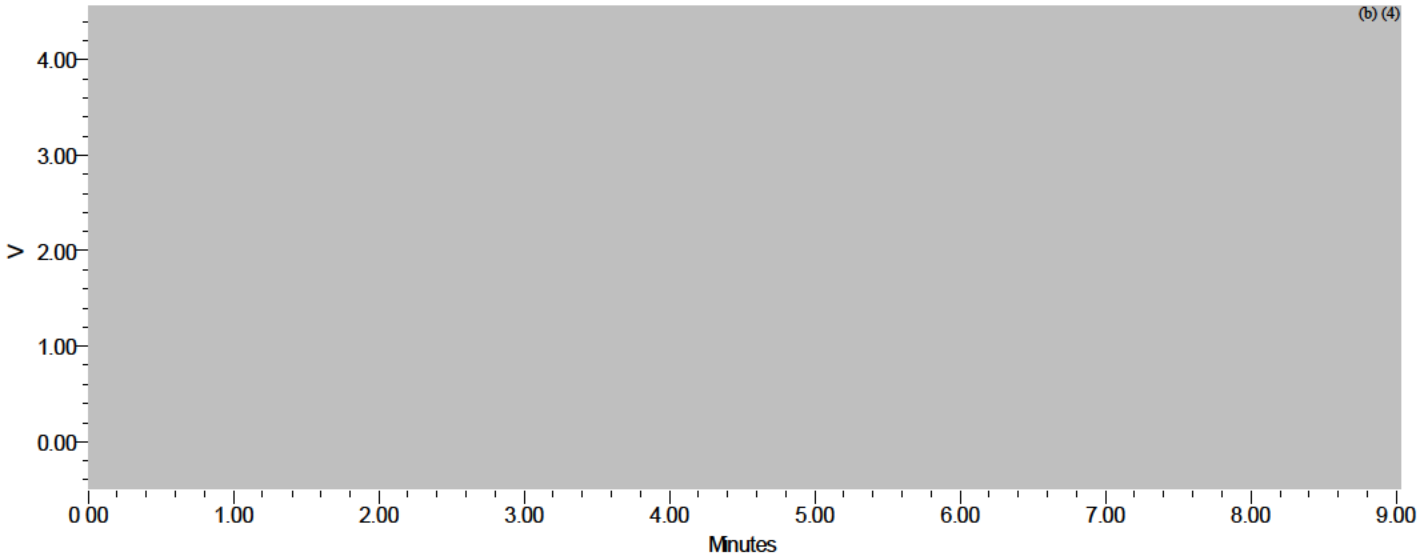
Sample Set Name:	Granule Valine_1 Day	Processed By:	System/Administrator
Sample Set Method:	Granule Valine_1 Day	Printed By:	System
System Node:	Client13	Result Set ID:	
System Name:	UC10	# of Results:	20
Acquired By:	System		
Sample Set Start Date:	12/17/2020 6:52:02 PM KST		
Sample Set Finish Date:	12/18/2020 12:24:42 PM KST		

Sample Set Table

	Sample Name	Sample Type	Vial	Inj #	Injection Volume (ul)	Acquistion Method Set	Processed Channel Descr.	(b) (4)
1	ZSTD_1	Unknown	48	1	5.00	VAL_ACR	Detector A	
2	ZSTD_2	Unknown	49	1	5.00	VAL_ACR	Detector A	
3	ZSTD_3	Unknown	50	1	5.00	VAL_ACR	Detector A	
4	ZSTD_4	Unknown	51	1	5.00	VAL_ACR	Detector A	
5	ZSTD_5	Unknown	52	1	5.00	VAL_ACR	Detector A	
6	ZSTD_6	Unknown	53	1	5.00	VAL_ACR	Detector A	
7	ZSTD_7	Unknown	54	1	5.00	VAL_ACR	Detector A	
8	ZSTD_8	Unknown	55	1	5.00	VAL_ACR	Detector A	
9	ZSTD_9	Unknown	56	1	5.00	VAL_ACR	Detector A	
10	ZSTD_10	Unknown	57	1	5.00	VAL_ACR	Detector A	
11	ZSPL_1	Unknown	58	1	5.00	VAL_ACR	Detector A	
12	ZSPL_2	Unknown	59	1	5.00	VAL_ACR	Detector A	
13	ZSPL_3	Unknown	60	1	5.00	VAL_ACR	Detector A	
14	ZSPL_4	Unknown	61	1	5.00	VAL_ACR	Detector A	
15	ZSPL_5	Unknown	62	1	5.00	VAL_ACR	Detector A	
16	ZSPL_6	Unknown	63	1	5.00	VAL_ACR	Detector A	
17	ZSPL_7	Unknown	64	1	5.00	VAL_ACR	Detector A	
18	ZSPL_8	Unknown	65	1	5.00	VAL_ACR	Detector A	
19	ZSPL_9	Unknown	66	1	5.00	VAL_ACR	Detector A	
20	ZSPL_10	Unknown	67	1	5.00	VAL_ACR	Detector A	

SAMPLE INFORMATION

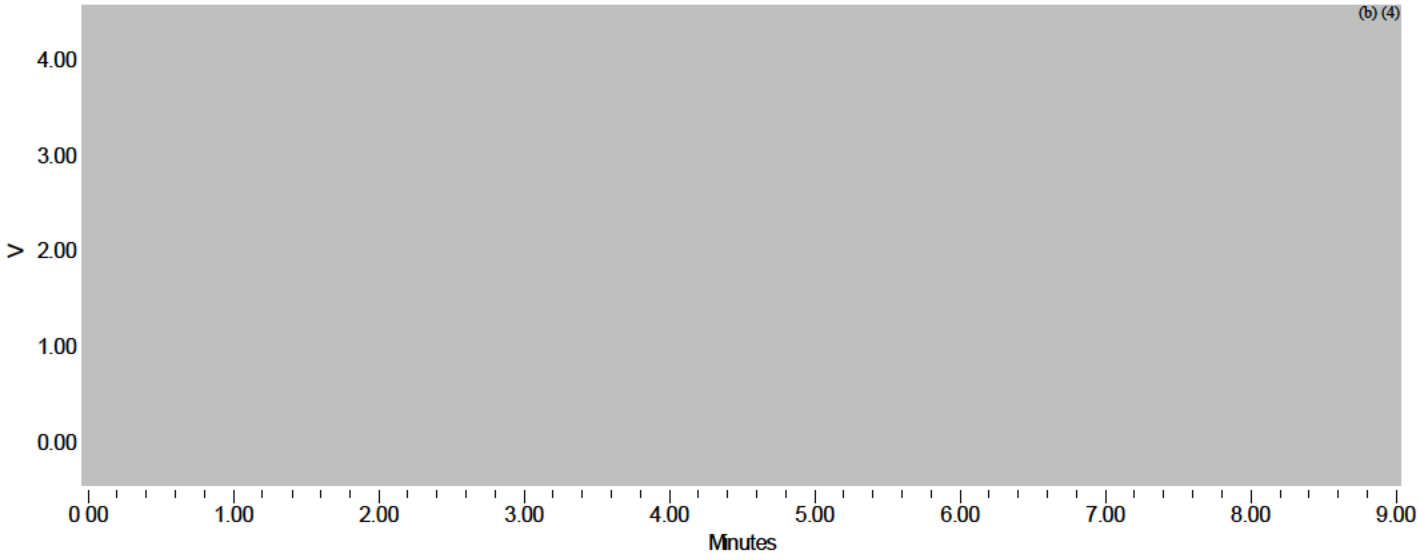
Sample Name:	ZSTD_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	48	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 2:17:05 AM KST		
Date Processed:	12/20/2020 5:15:22 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

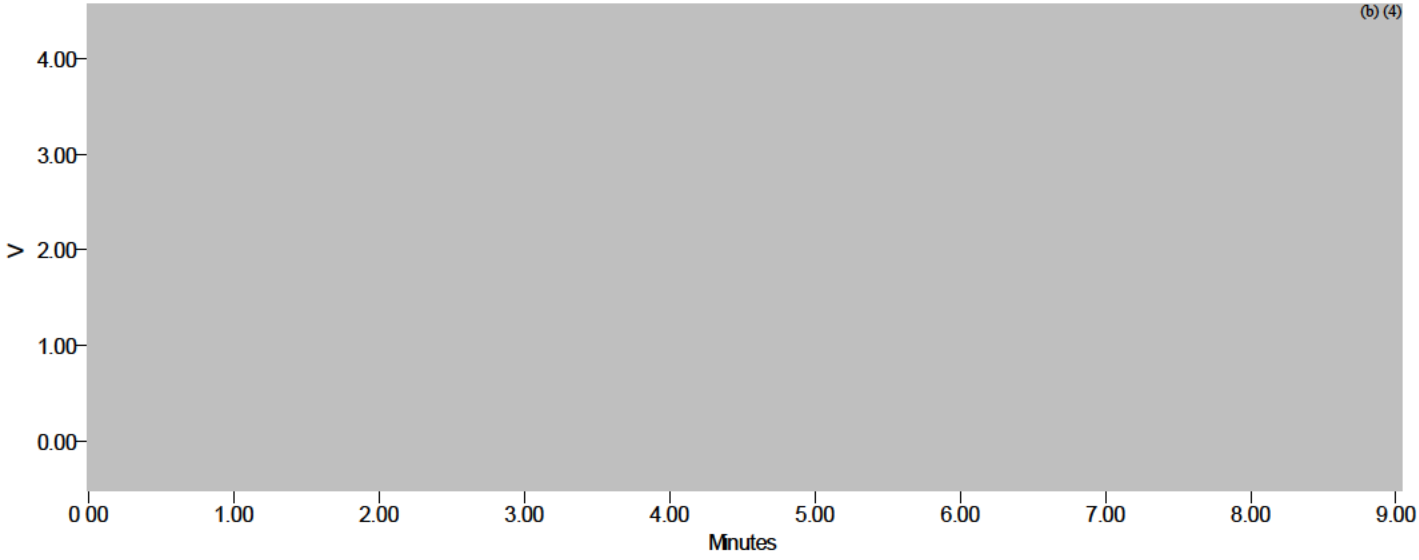
Sample Name:	ZSTD_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	49	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 2:26:41 AM KST		
Date Processed:	12/20/2020 5:15:22 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

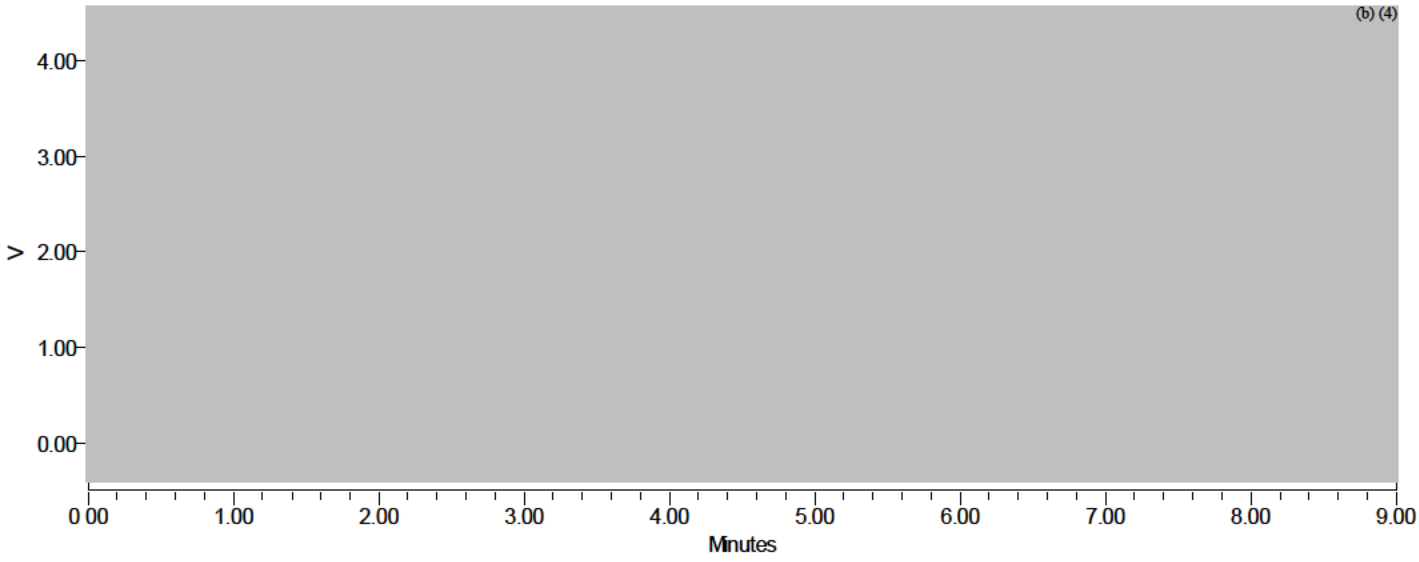
Sample Name:	ZSTD_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	50	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 2:36:21 AM KST		
Date Processed:	12/20/2020 5:15:22 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

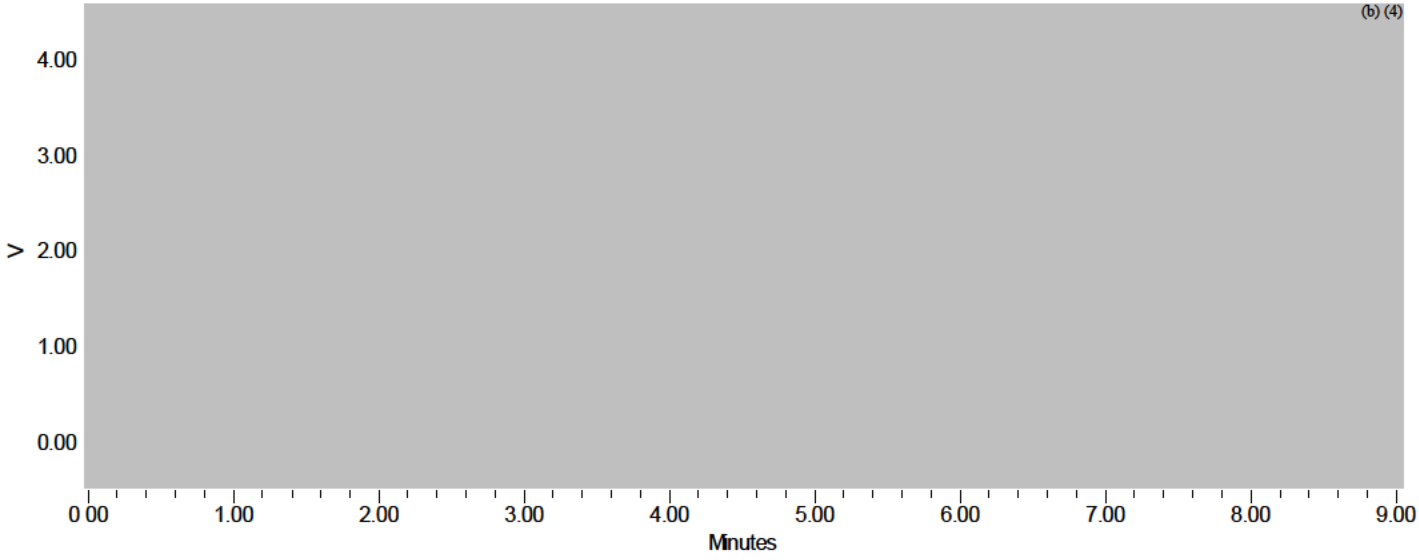
Sample Name:	ZSTD_4	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	51	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 2:46:01 AM KST		
Date Processed:	12/20/2020 5:15:23 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

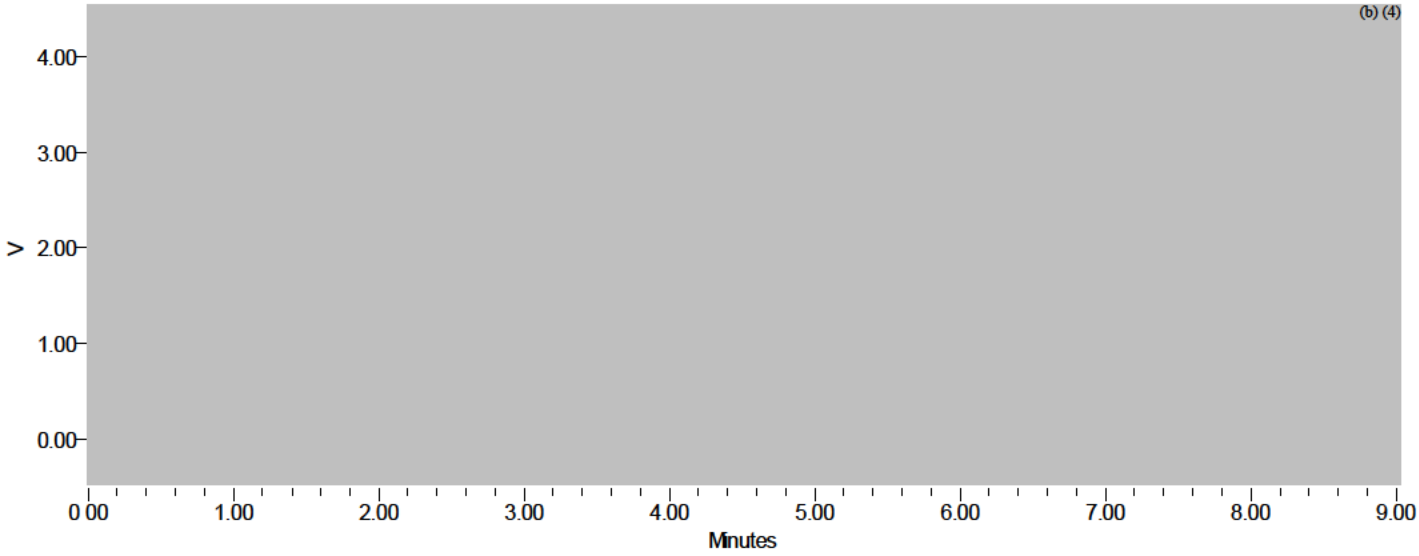
Sample Name:	ZSTD_5	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	52	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 2:55:35 AM KST		
Date Processed:	12/20/2020 5:15:23 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

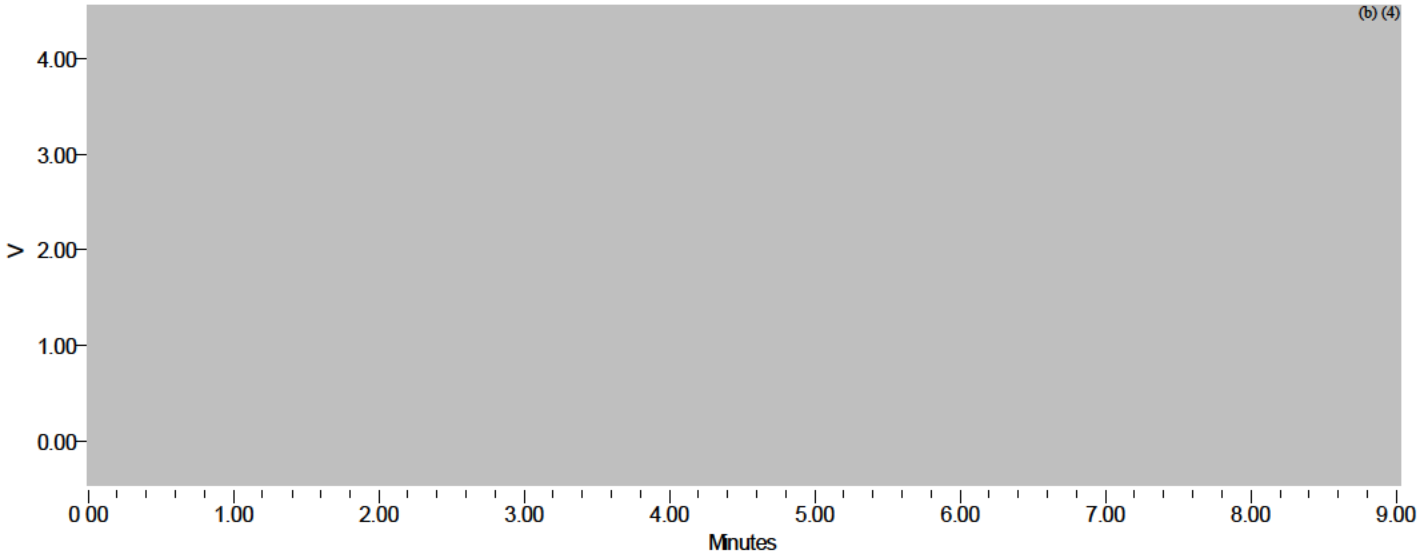
Sample Name:	ZSTD_6	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	53	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 3:05:16 AM KST		
Date Processed:	12/20/2020 5:15:23 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

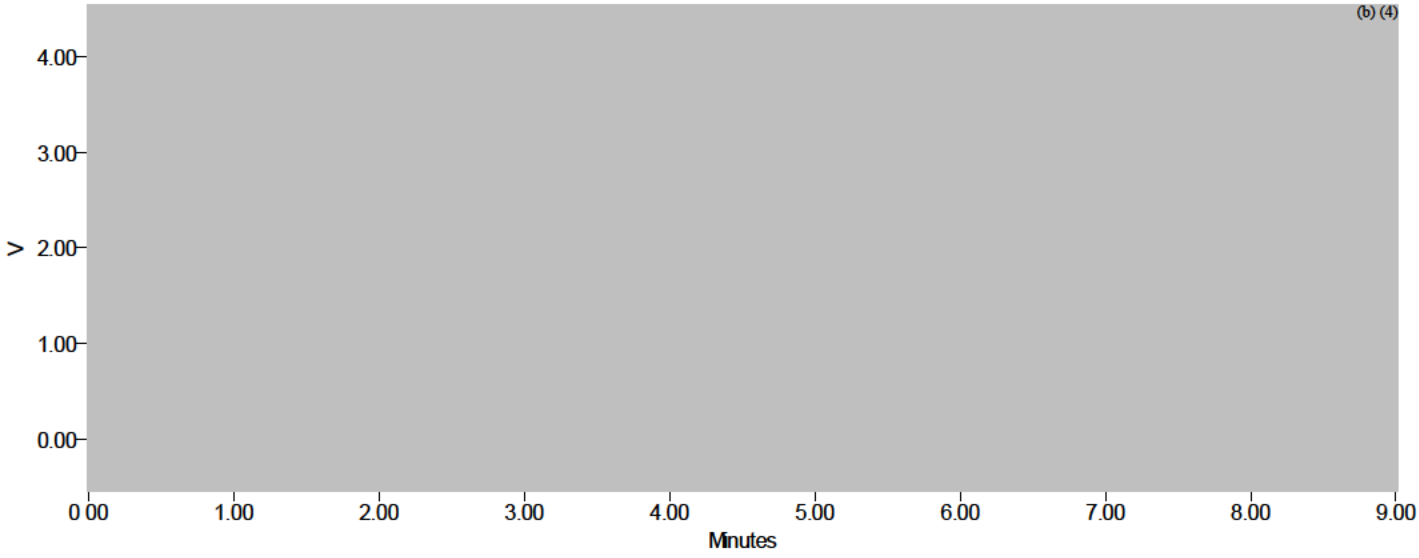
Sample Name:	ZSTD_7	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	54	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 3:14:50 AM KST		
Date Processed:	12/20/2020 5:15:23 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

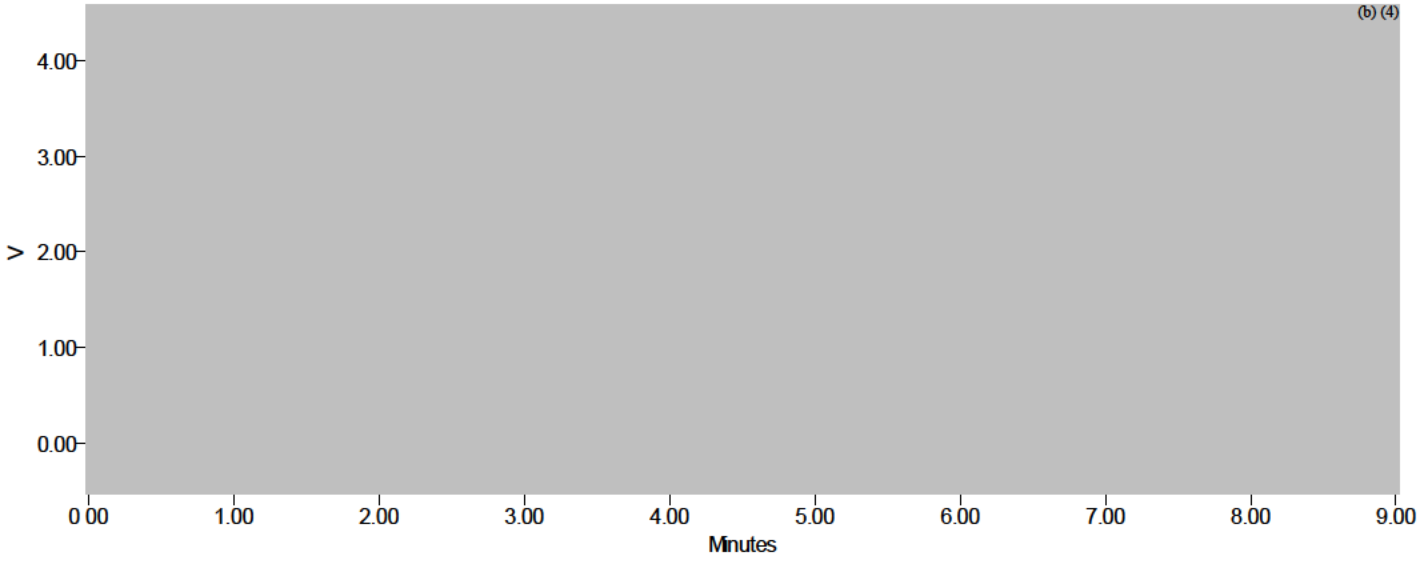
Sample Name:	ZSTD_8	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	55	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 3:24:31 AM KST		
Date Processed:	12/20/2020 5:15:23 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

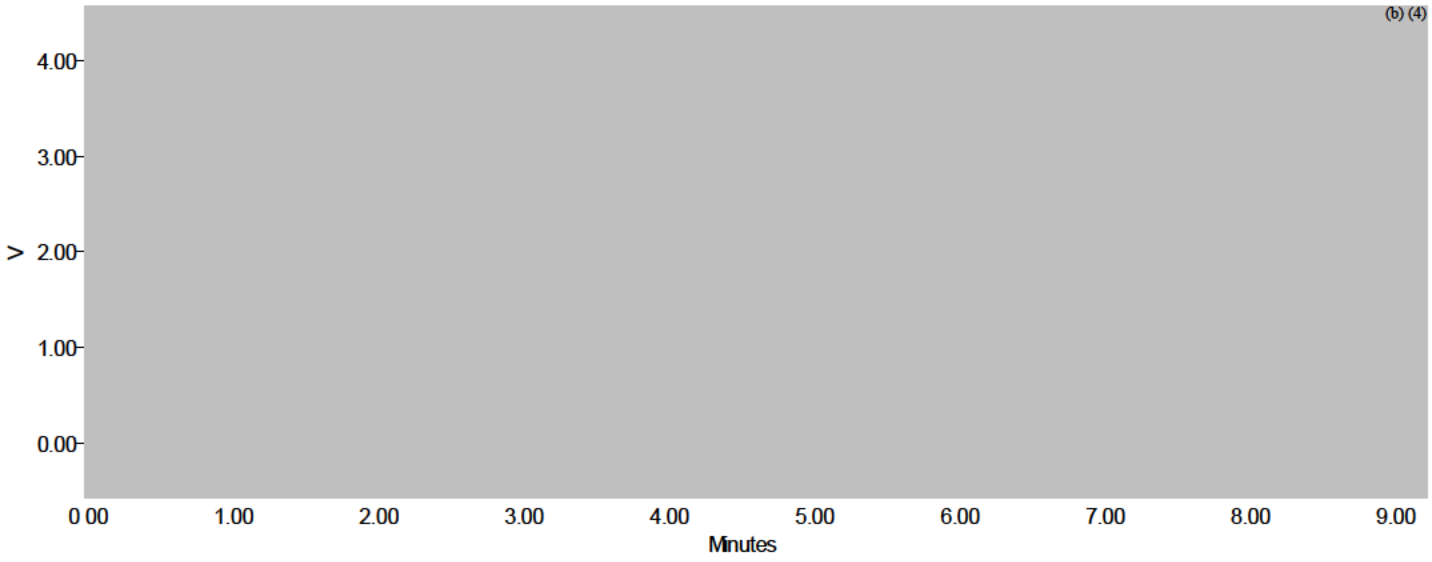
Sample Name:	ZSTD_9	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	56	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 3:34:06 AM KST		
Date Processed:	12/20/2020 5:15:24 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

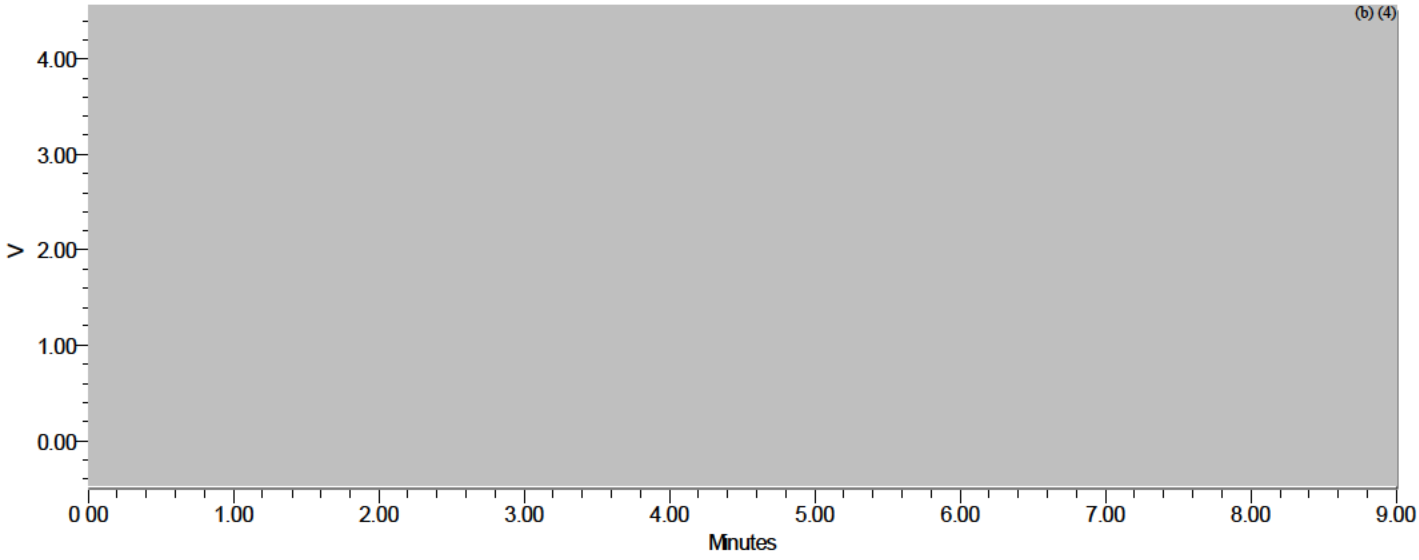
Sample Name:	ZSTD_10	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	57	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 3:43:46 AM KST		
Date Processed:	12/20/2020 5:15:24 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

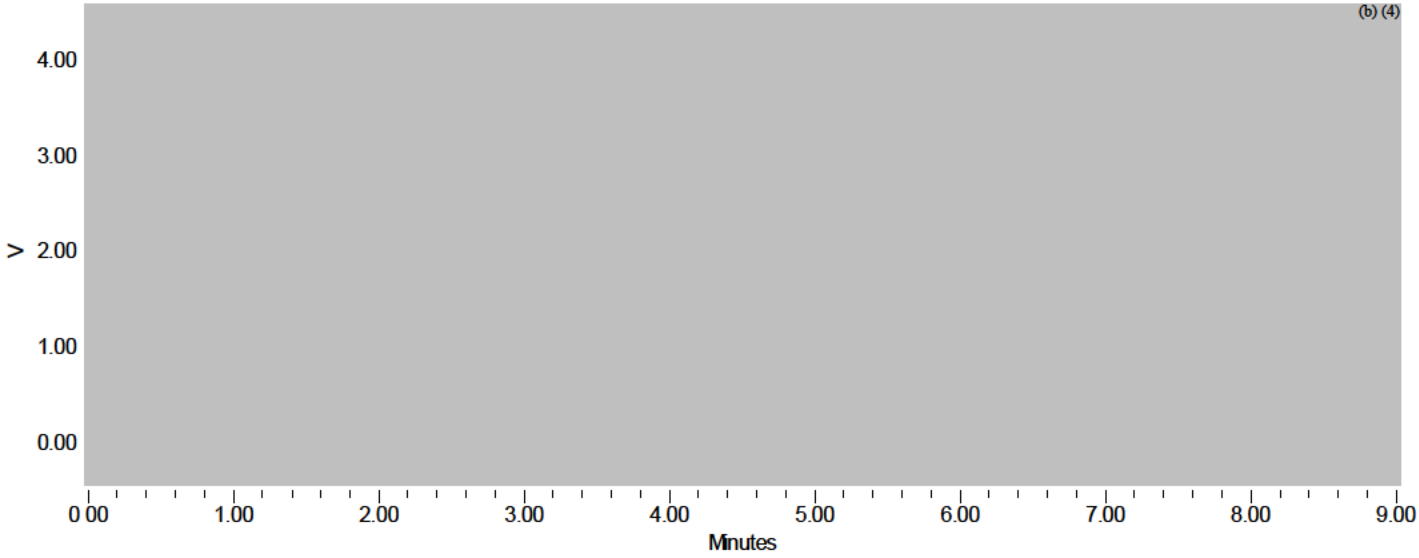
Sample Name:	ZSPL_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	58	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 4:03:03 AM KST		
Date Processed:	12/20/2020 5:15:26 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

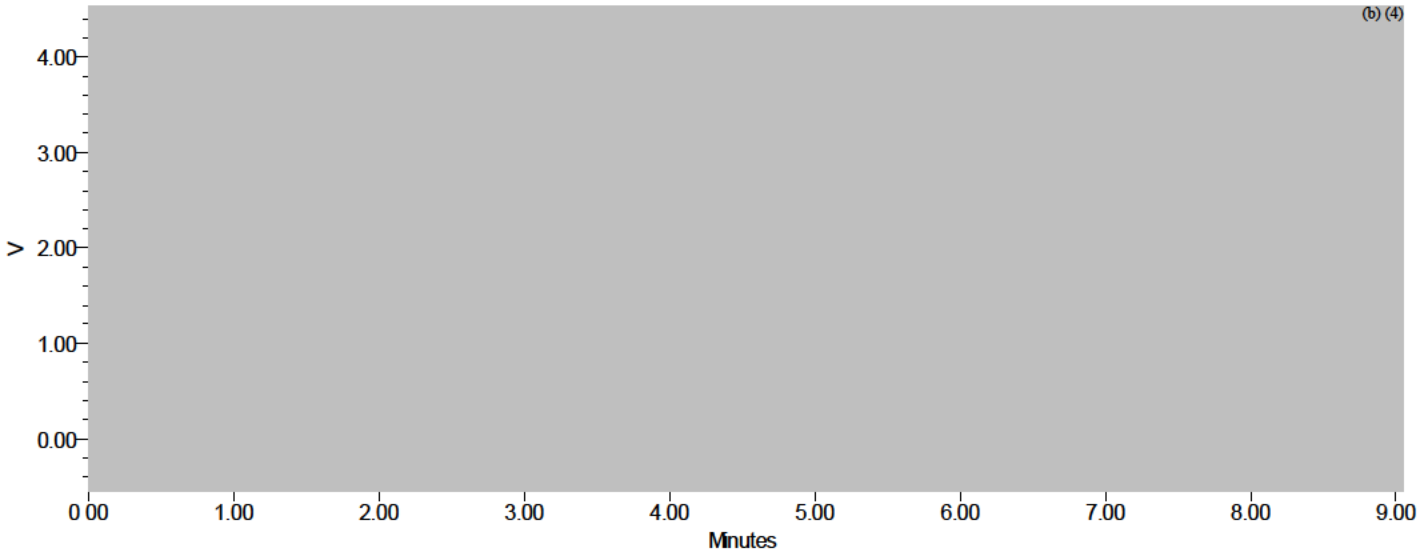
Sample Name:	ZSPL_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	59	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 4:12:44 AM KST		
Date Processed:	12/20/2020 5:15:27 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

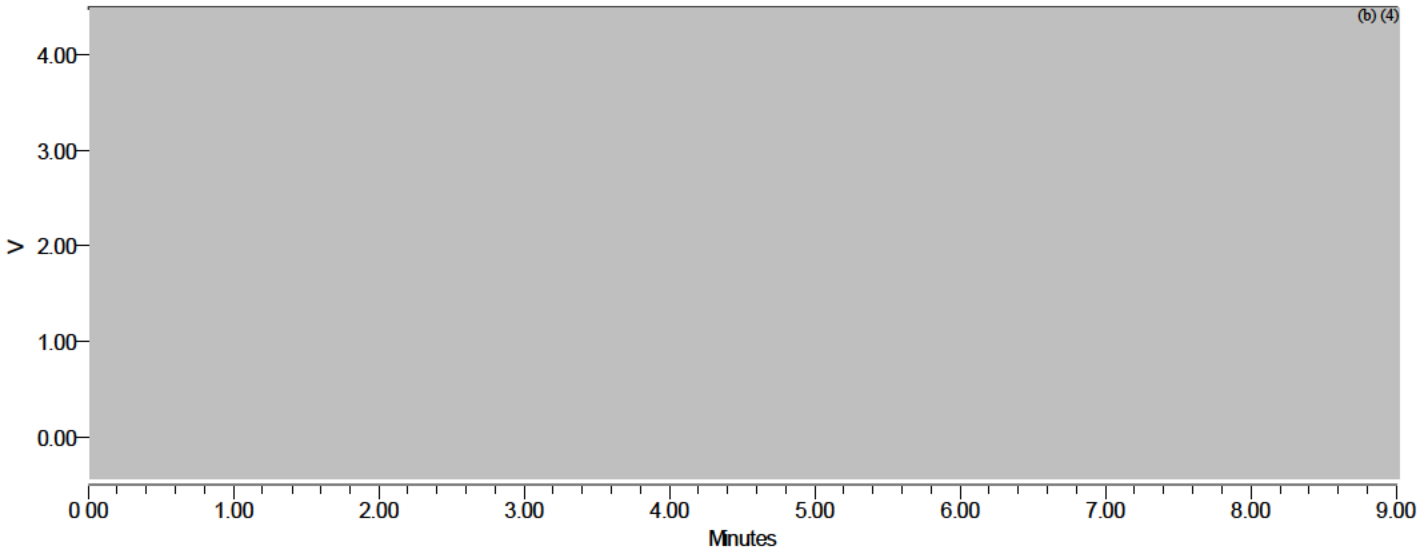
Sample Name:	ZSPL_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	60	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 4:22:24 AM KST		
Date Processed:	12/20/2020 5:15:27 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

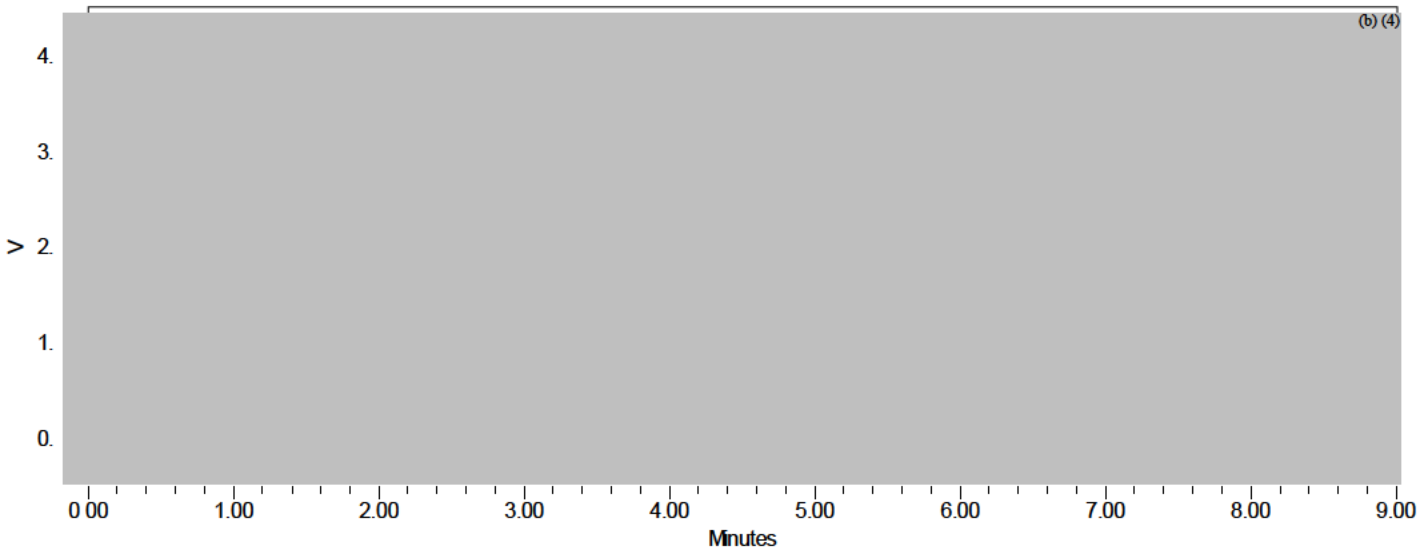
Sample Name:	ZSPL_4	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	61	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 4:32:00 AM KST		
Date Processed:	12/20/2020 5:15:27 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

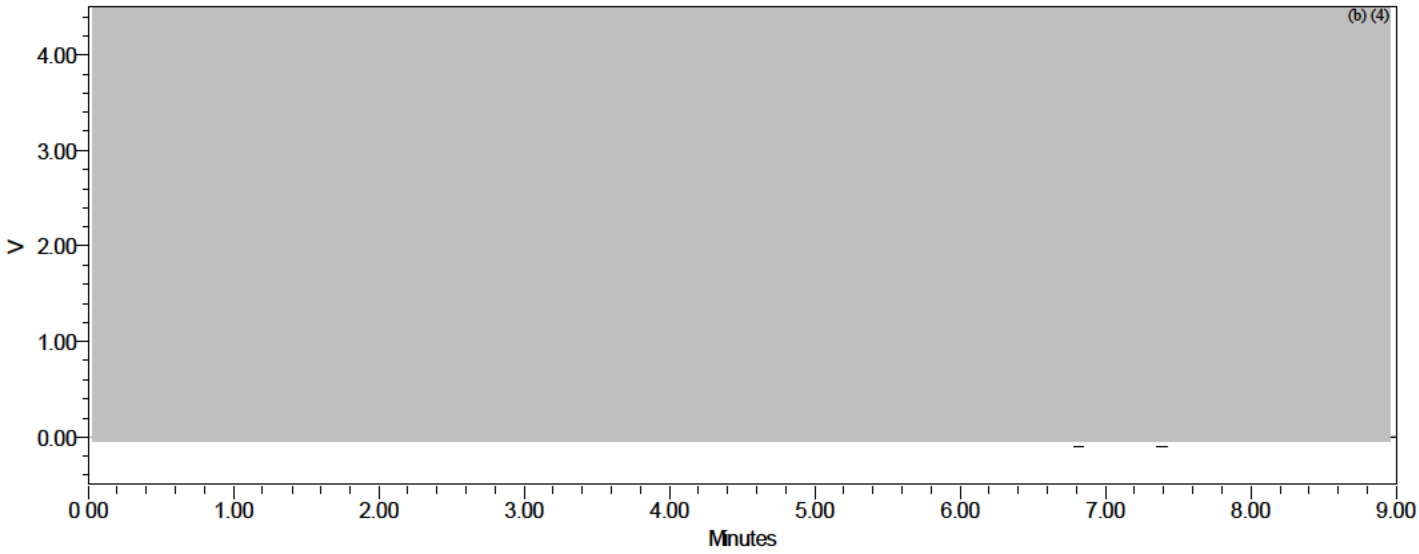
Sample Name:	ZSPL_5	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	62	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 4:41:41 AM KST		
Date Processed:	12/20/2020 5:15:27 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

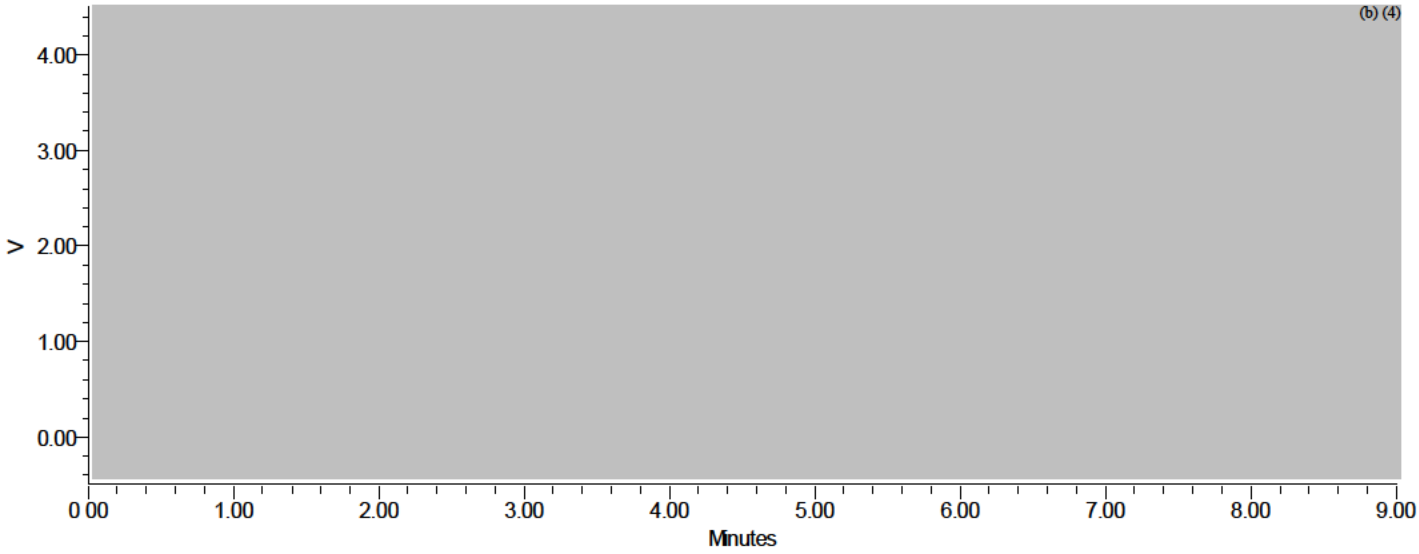
Sample Name:	ZSPL_6	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	63	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 4:51:19 AM KST		
Date Processed:	12/20/2020 5:15:27 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

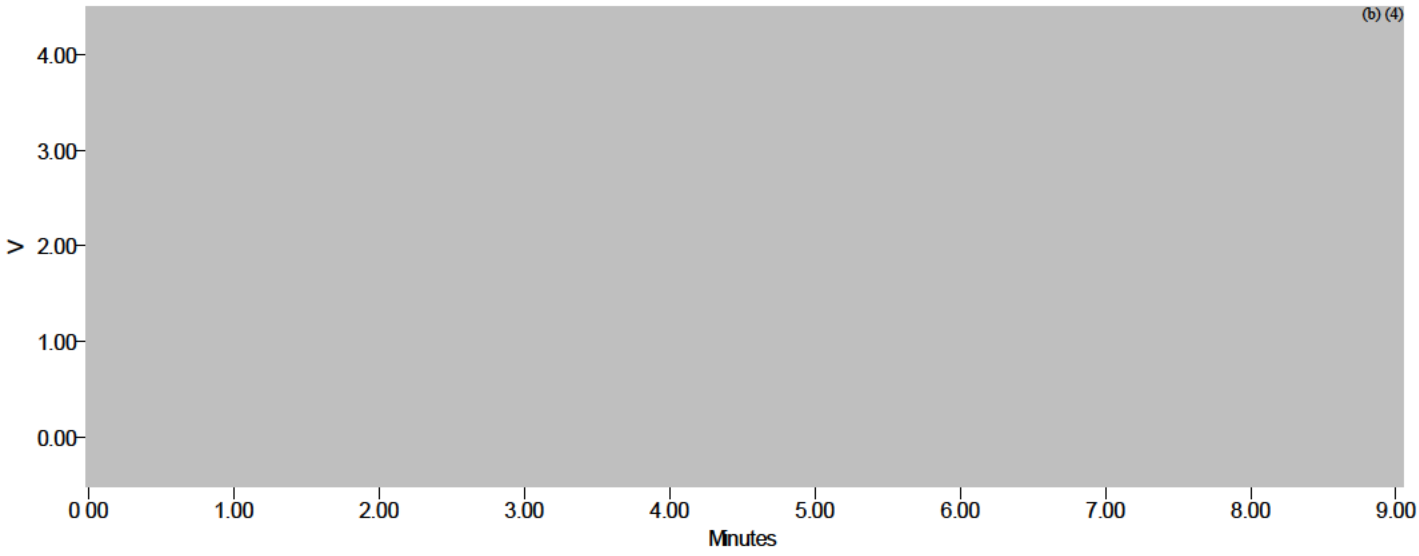
Sample Name:	ZSPL_7	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	64	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 5:00:58 AM KST		
Date Processed:	12/20/2020 5:15:28 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

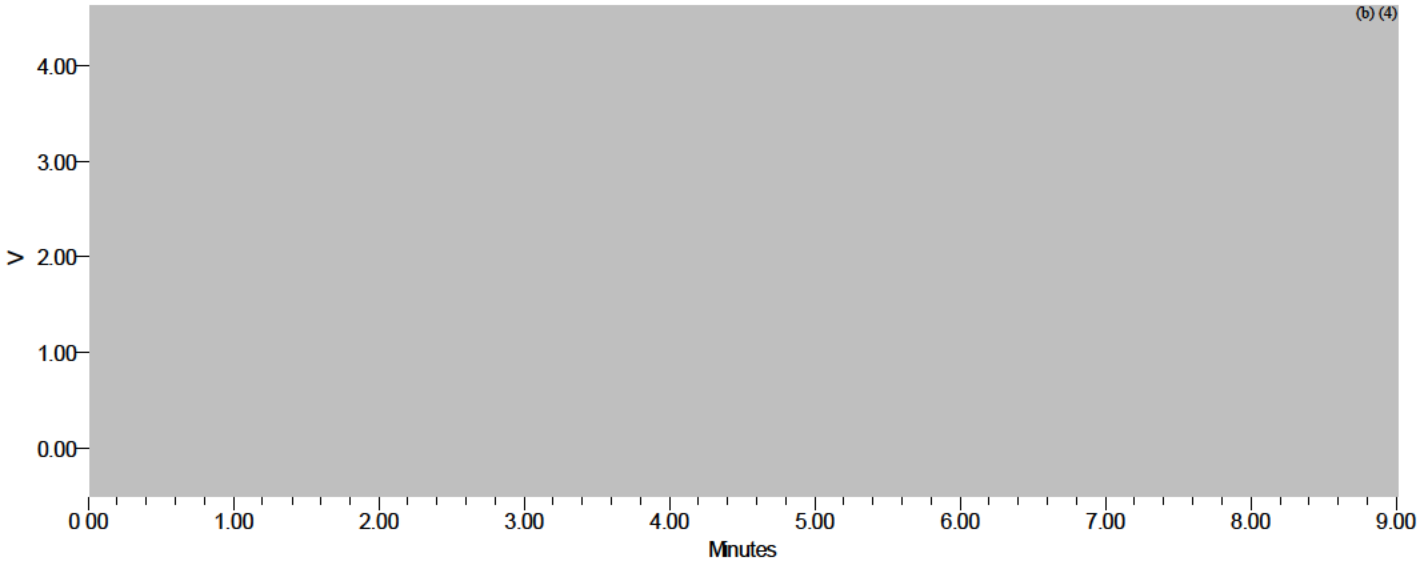
Sample Name:	ZSPL_8	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	65	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 5:10:38 AM KST		
Date Processed:	12/20/2020 5:15:28 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

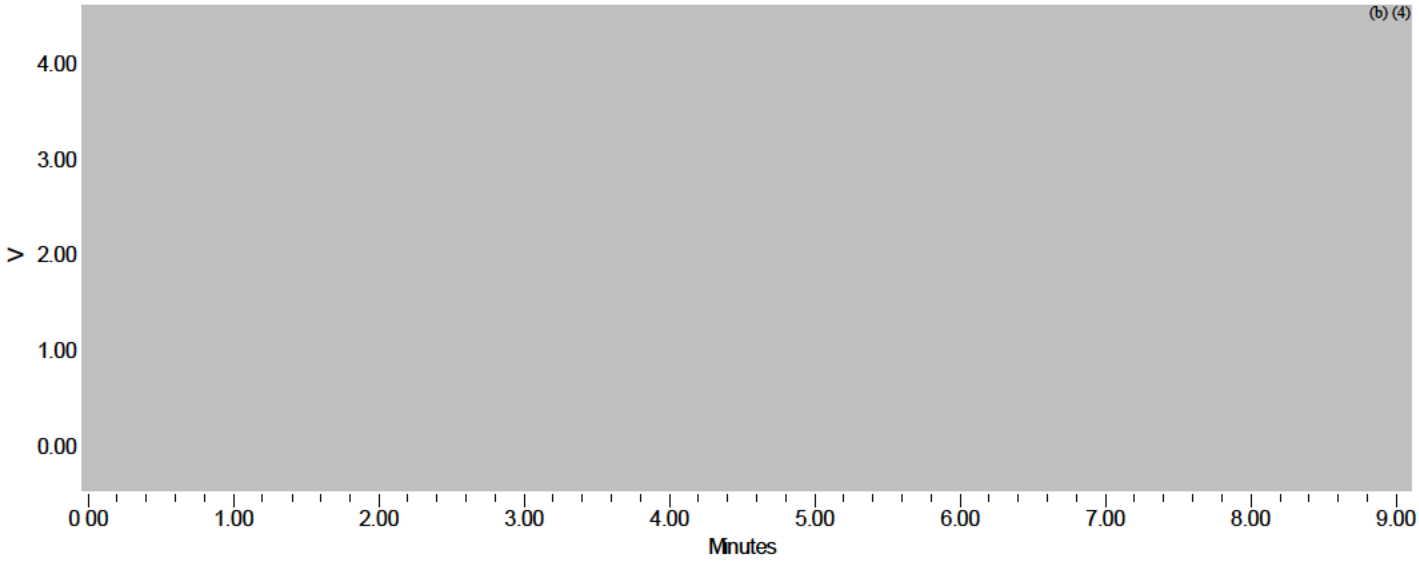
Sample Name:	ZSPL_9	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	66	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 5:20:15 AM KST		
Date Processed:	12/20/2020 5:15:28 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

Sample Name:	ZSPL_10	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	67	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 5:29:55 AM KST		
Date Processed:	12/20/2020 5:15:28 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

Software: Empower 3 Software Build 3471

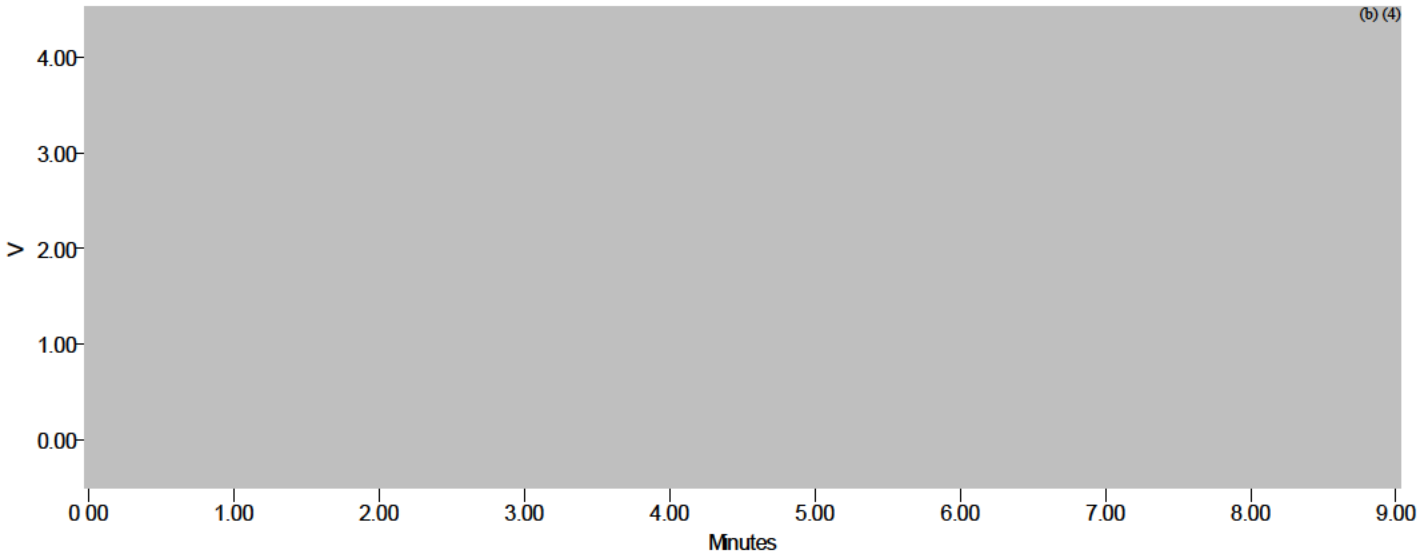
Sample Set Name:	Granule Valine_1 Day	Processed By:	System/Administrator
Sample Set Method:	Granule Valine_1 Day	Printed By:	System
System Node:	Client13	Result Set ID:	
System Name:	UC10	# of Results:	7
Acquired By:	System		
Sample Set Start Date:	12/17/2020 6:52:02 PM KST		
Sample Set Finish Date:	12/18/2020 12:24:42 PM KST		

Sample Set Table

	Sample Name	Sample Type	Vial	Inj #	Injection Volume (ul)	Acquistion Method Set	Processed Channel Descr.	(b) (4)
1	ASTD_1	Unknown	22	1	5.00	VAL_ACR	Detector A	
2	ASPL_1	Unknown	23	1	5.00	VAL_ACR	Detector A	
3	ASTD_2	Unknown	24	1	5.00	VAL_ACR	Detector A	
4	ASPL_2	Unknown	25	1	5.00	VAL_ACR	Detector A	
5	ASTD_3	Unknown	26	1	5.00	VAL_ACR	Detector A	
6	ASPL_3	Unknown	27	1	5.00	VAL_ACR	Detector A	
7	ASTD_4	Unknown	28	1	5.00	VAL_ACR	Detector A	

SAMPLE INFORMATION

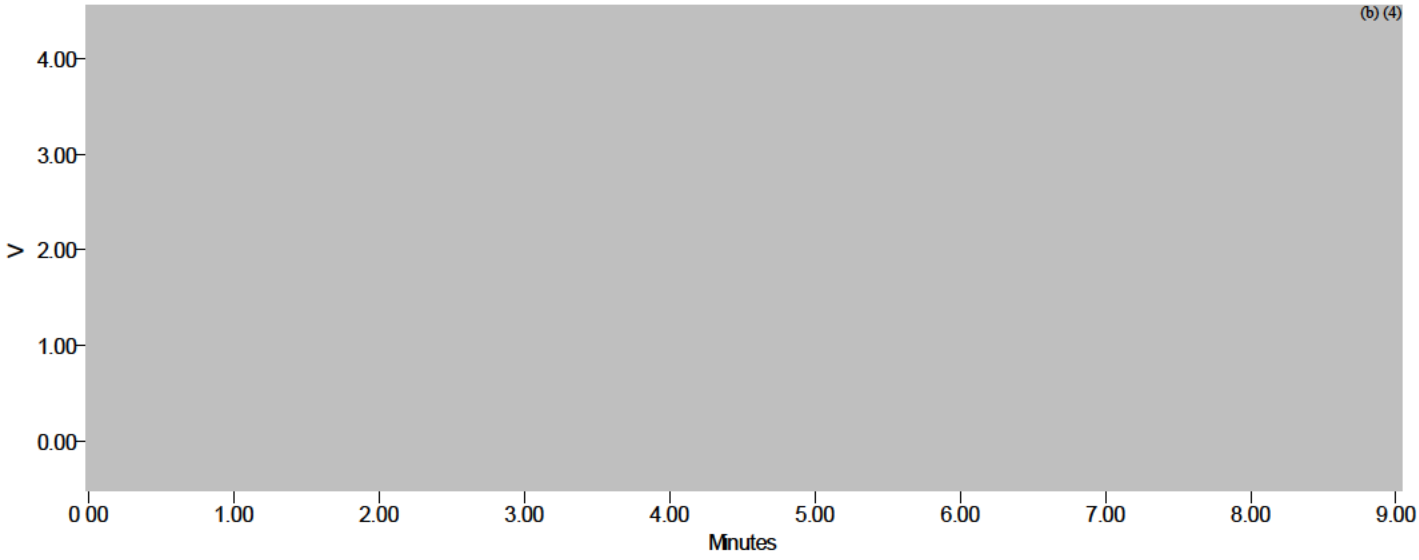
Sample Name:	ASTD_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	22	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 11:17:30 AM KST		
Date Processed:	12/20/2020 5:12:21 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

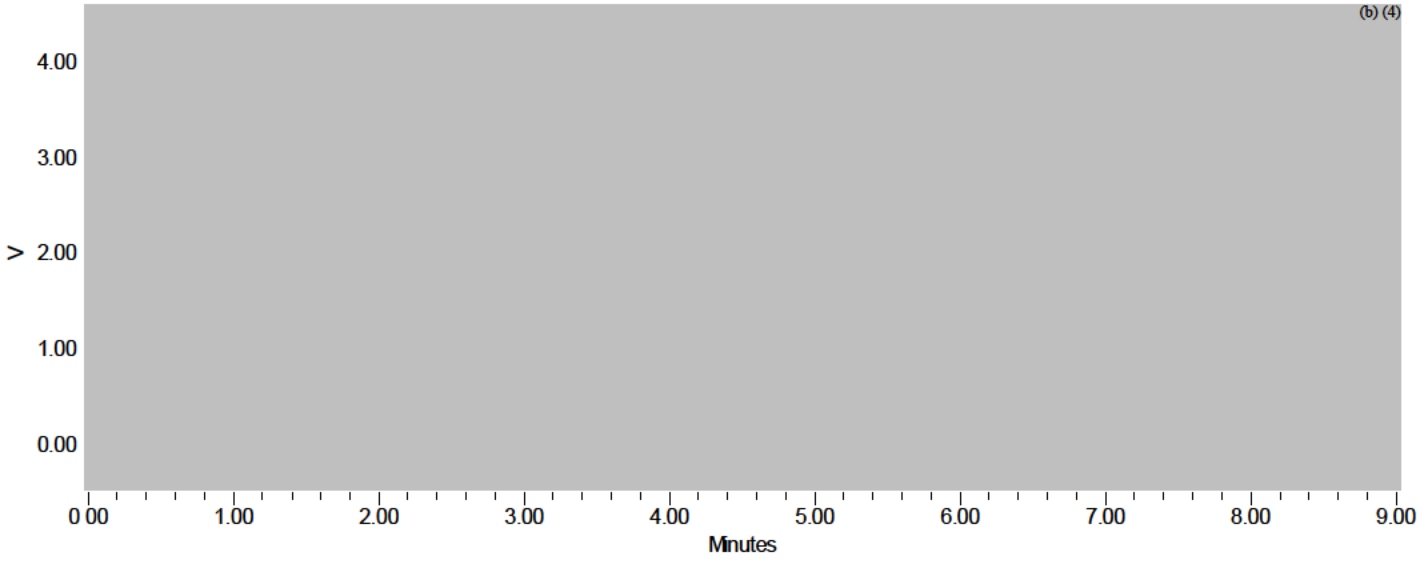
Sample Name:	ASPL_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	23	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 11:27:12 AM KST		
Date Processed:	12/20/2020 5:12:21 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

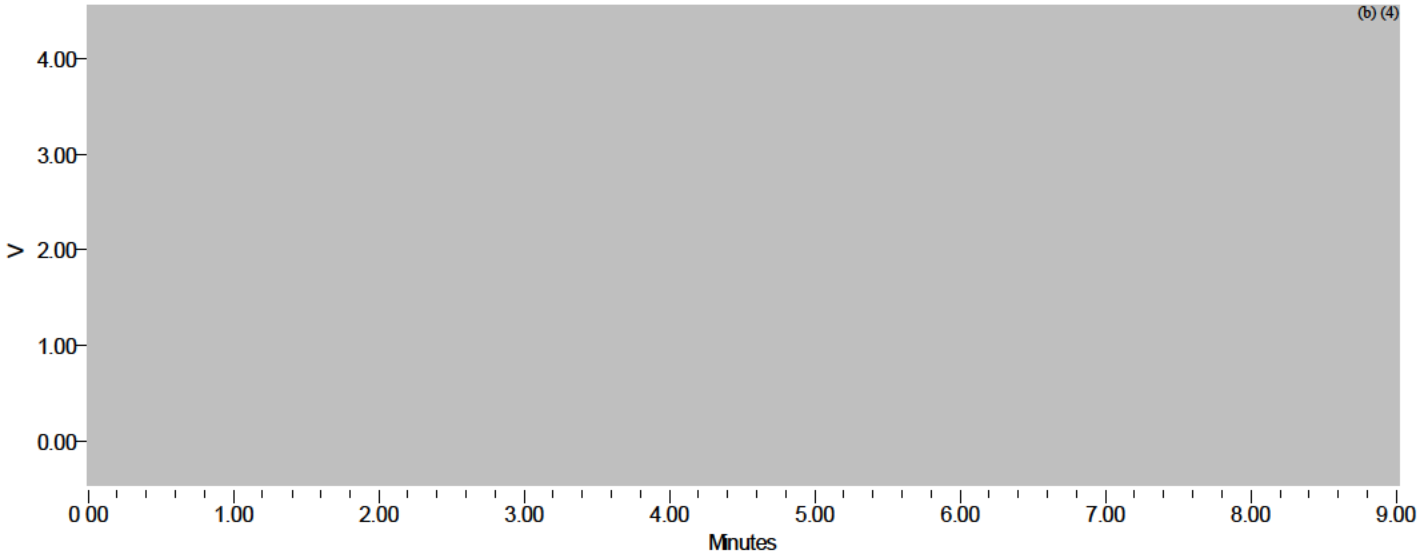
Sample Name:	ASTD_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	24	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 11:36:51 AM KST		
Date Processed:	12/20/2020 5:12:21 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

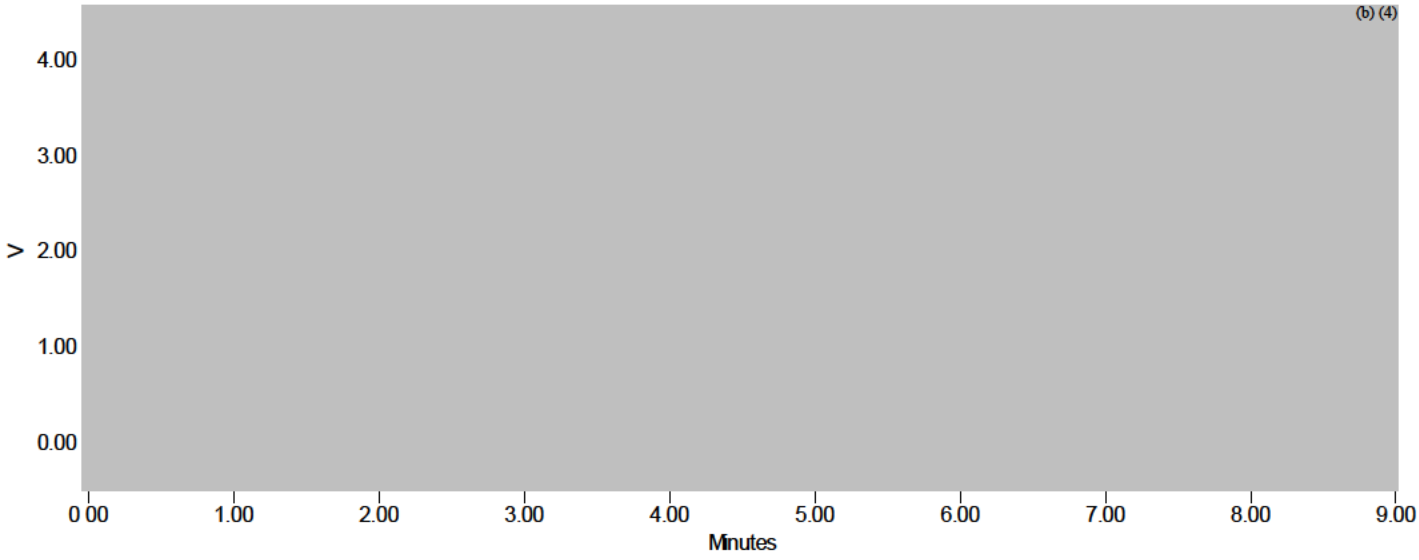
Sample Name:	ASPL_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	25	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 11:46:32 AM KST		
Date Processed:	12/20/2020 5:12:22 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

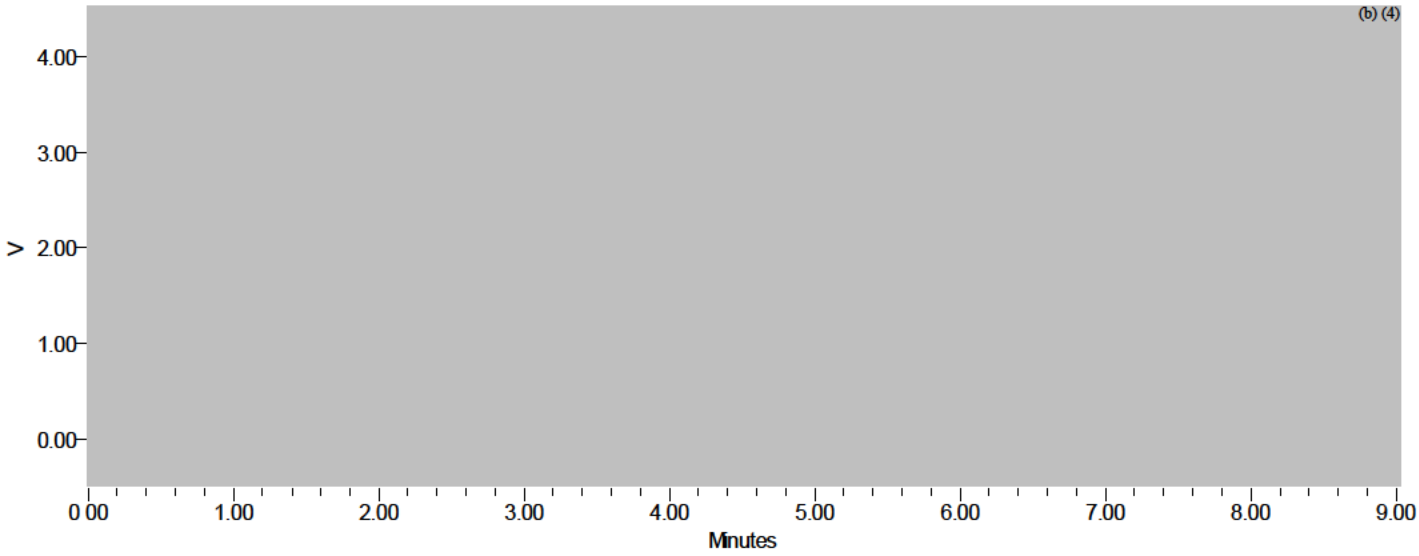
Sample Name:	ASTD_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	26	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 11:56:14 AM KST		
Date Processed:	12/20/2020 5:12:22 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

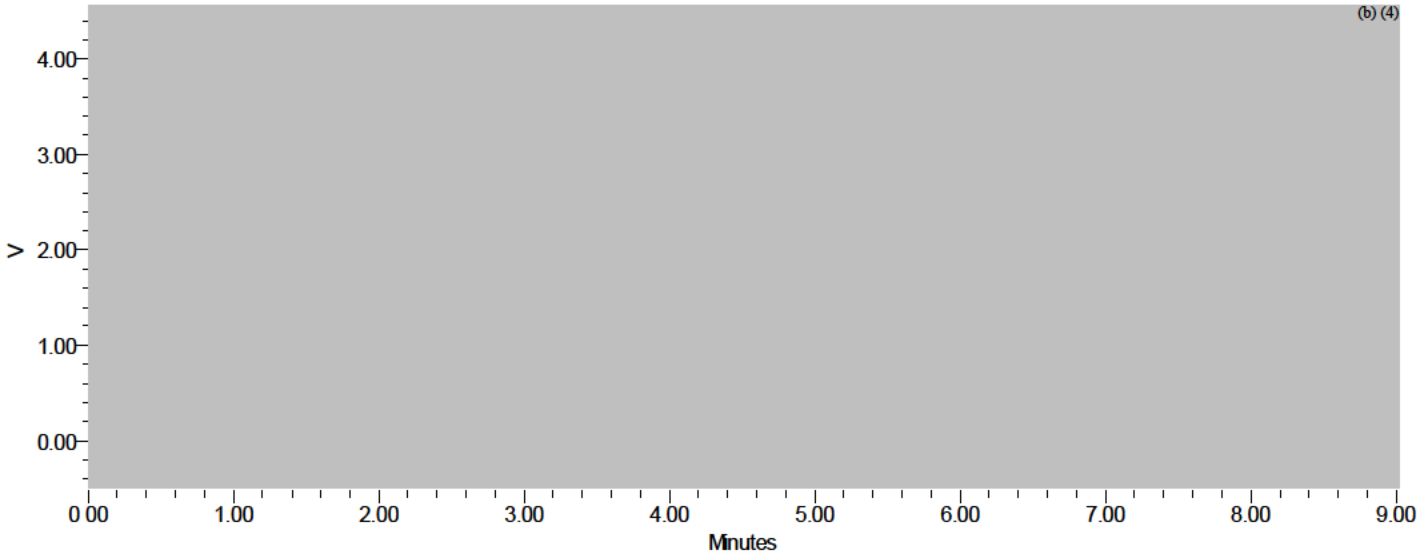
Sample Name:	ASPL_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	27	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 12:05:55 PM KST		
Date Processed:	12/20/2020 5:12:22 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

Sample Name:	ASTD_4	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_1 Day
Vial:	28	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_1
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/18/2020 12:15:37 PM KST		
Date Processed:	12/20/2020 5:12:22 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

Software: Empower 3 Software Build 3471

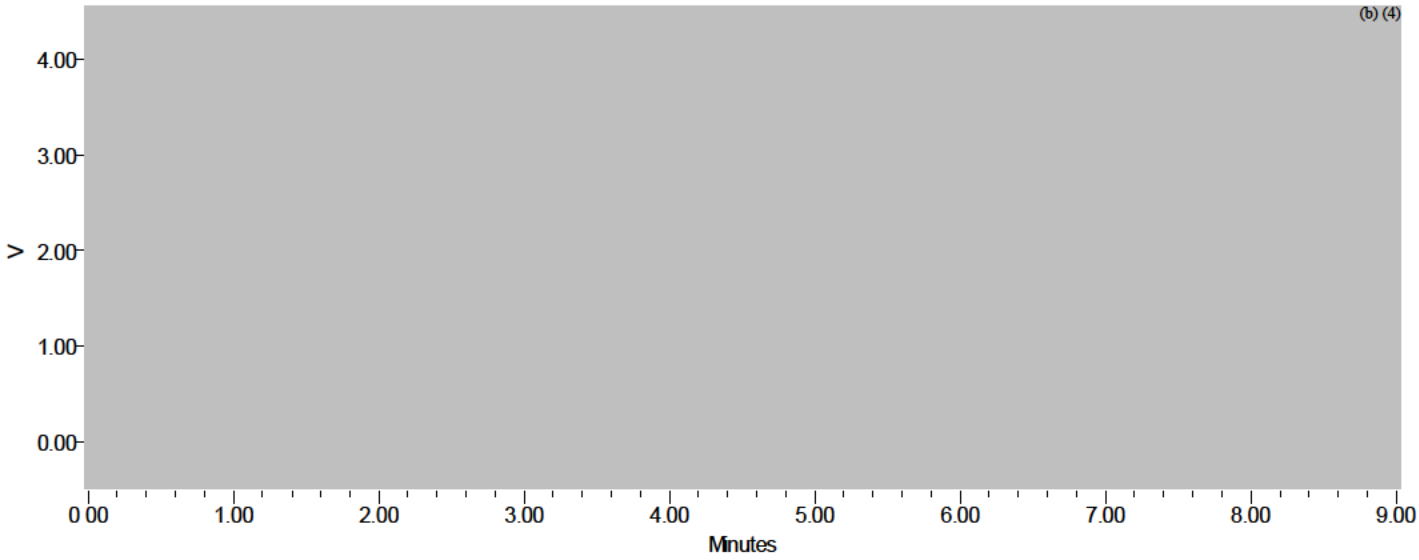
Sample Set Name:	Granule Valine_4 Day	Processed By:	System/Administrator
Sample Set Method:	Granule Valine_4 Day	Printed By:	System
System Node:	Client13	Result Set ID:	
System Name:	UC10	# of Results:	14
Acquired By:	System		
Sample Set Start Date:	12/20/2020 3:41:57 PM KST		
Sample Set Finish Date:	12/20/2020 6:26:58 PM KST		

Sample Set Table

	Sample Name	Sample Type	Vial	Inj #	Injection Volume (ul)	Acquistion Method Set	Processed Channel Descr.	(b) (4)
1	35STD_1	Unknown	36	1	5.00	VAL_ACR_Oven Temp_35	Detector A	
2	35SPL_1	Unknown	37	1	5.00	VAL_ACR_Oven Temp_35	Detector A	
3	35STD_2	Unknown	38	1	5.00	VAL_ACR_Oven Temp_35	Detector A	
4	35SPL_2	Unknown	39	1	5.00	VAL_ACR_Oven Temp_35	Detector A	
5	35STD_3	Unknown	40	1	5.00	VAL_ACR_Oven Temp_35	Detector A	
6	35SPL_3	Unknown	41	1	5.00	VAL_ACR_Oven Temp_35	Detector A	
7	35STD_4	Unknown	42	1	5.00	VAL_ACR_Oven Temp_35	Detector A	
8	45STD_1	Unknown	43	1	5.00	VAL_ACR_Oven Temp_45	Detector A	
9	45SPL_1	Unknown	44	1	5.00	VAL_ACR_Oven Temp_45	Detector A	
10	45STD_2	Unknown	45	1	5.00	VAL_ACR_Oven Temp_45	Detector A	
11	45SPL_2	Unknown	46	1	5.00	VAL_ACR_Oven Temp_45	Detector A	
12	45STD_3	Unknown	47	1	5.00	VAL_ACR_Oven Temp_45	Detector A	
13	45SPL_3	Unknown	48	1	5.00	VAL_ACR_Oven Temp_45	Detector A	
14	45STD_4	Unknown	49	1	5.00	VAL_ACR_Oven Temp_45	Detector A	

SAMPLE INFORMATION

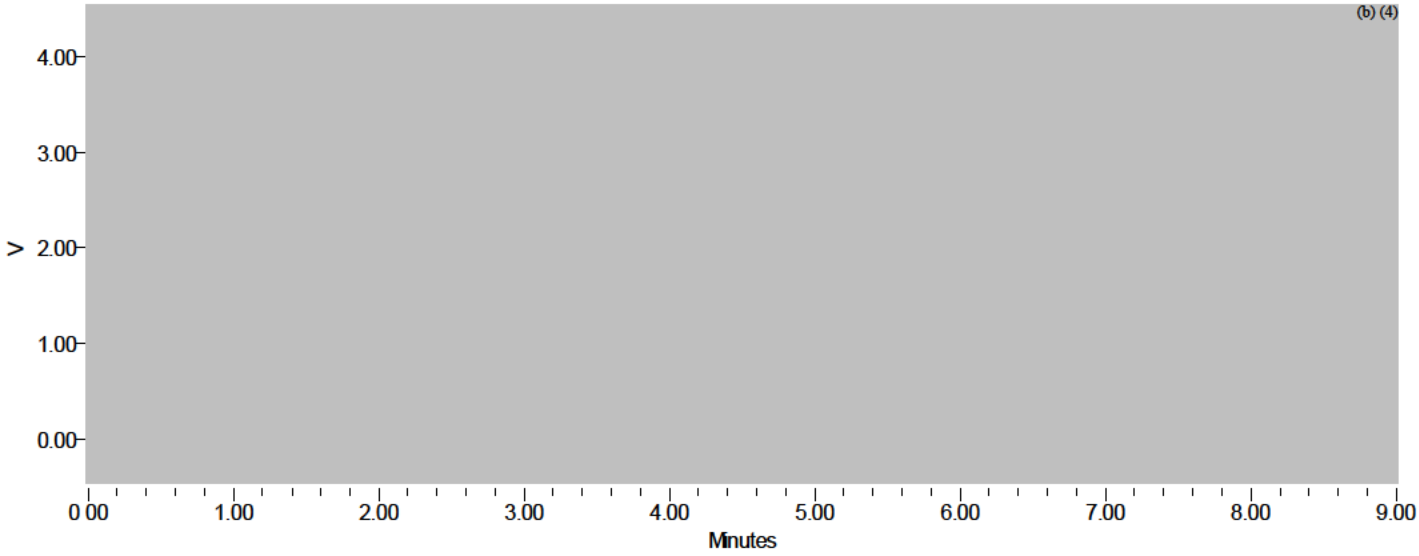
Sample Name:	35STD_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_4 Day
Vial:	36	Acq. Method Set:	VAL_ACR_Oven Temp_35
Injection #:	1	Processing Method:	Valine_Temp 35
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 3:42:32 PM KST		
Date Processed:	12/20/2020 5:41:20 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

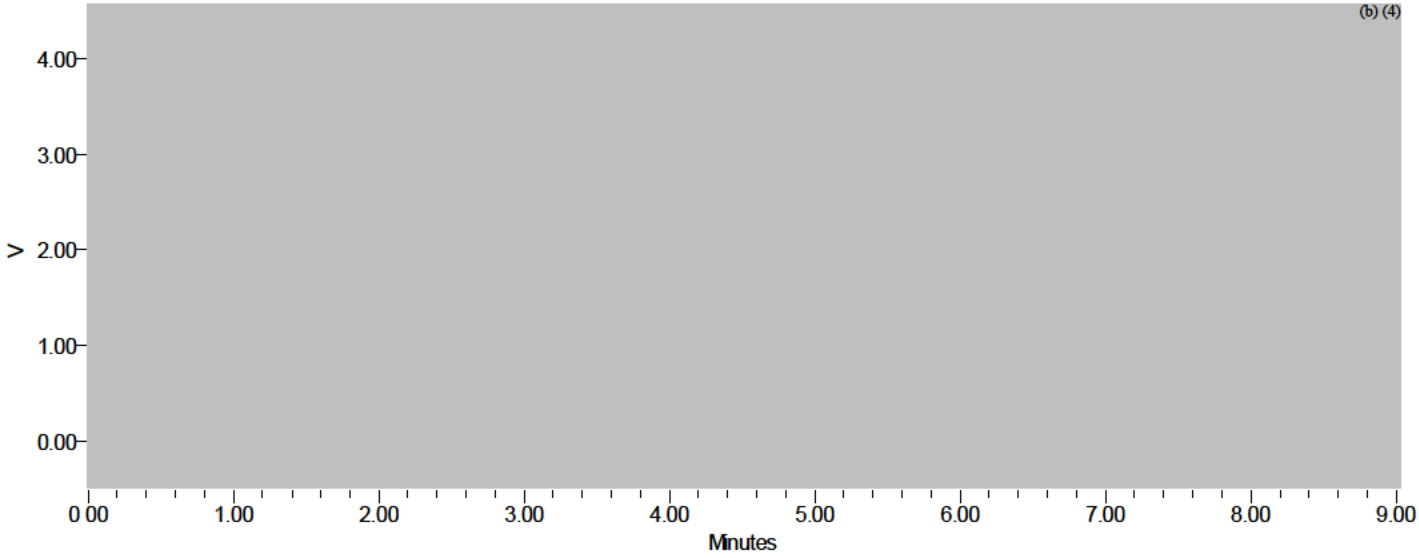
Sample Name:	35SPL_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_4 Day
Vial:	37	Acq. Method Set:	VAL_ACR_Oven Temp_35
Injection #:	1	Processing Method:	Valine_Temp 35
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 3:52:10 PM KST		
Date Processed:	12/20/2020 5:41:30 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

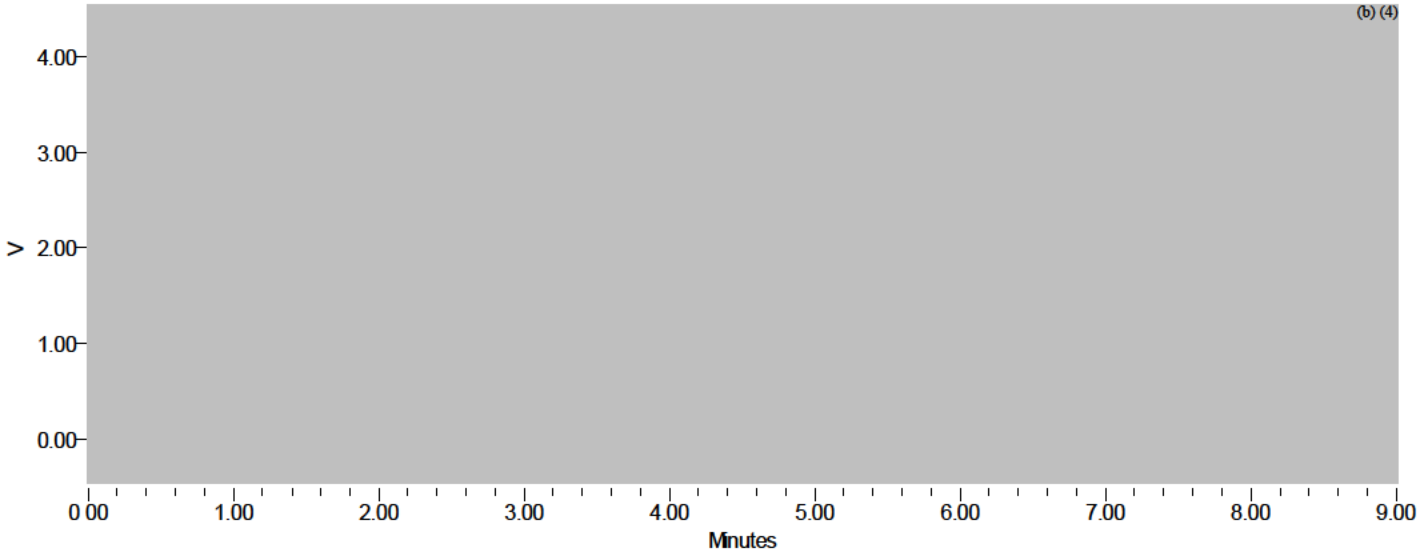
Sample Name:	35STD_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_4 Day
Vial:	38	Acq. Method Set:	VAL_ACR_Oven Temp_35
Injection #:	1	Processing Method:	Valine_Temp 35
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 4:01:51 PM KST		
Date Processed:	12/20/2020 5:41:31 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

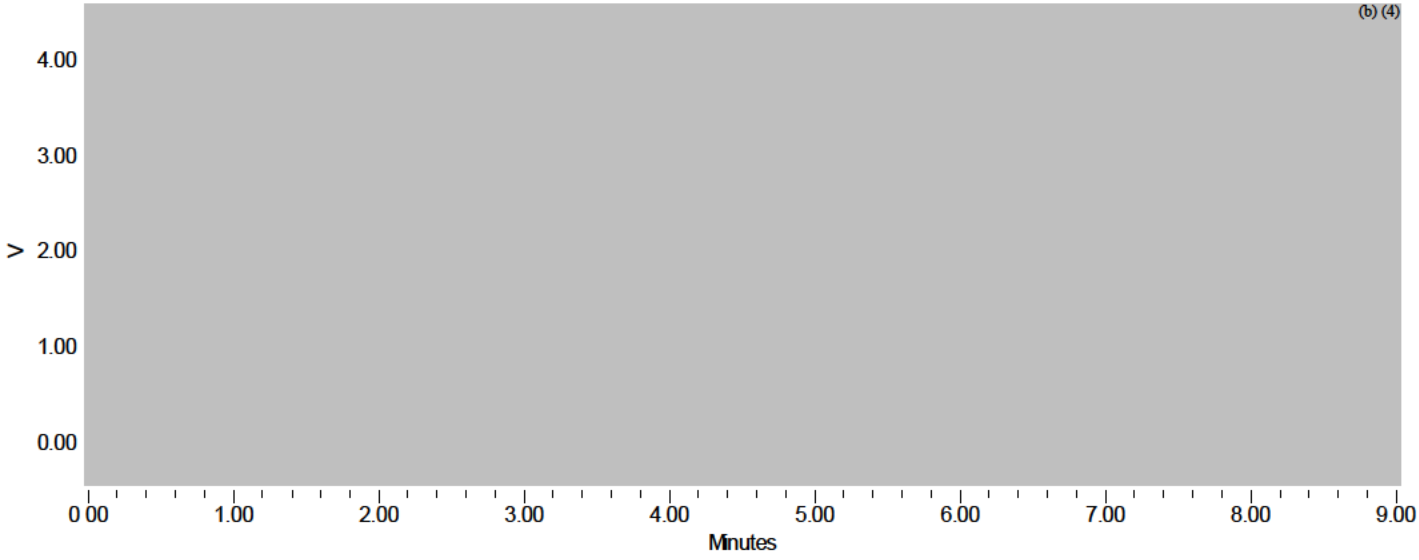
Sample Name:	35SPL_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_4 Day
Vial:	39	Acq. Method Set:	VAL_ACR_Oven Temp_35
Injection #:	1	Processing Method:	Valine_Temp 35
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 4:11:35 PM KST		
Date Processed:	12/20/2020 5:41:31 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

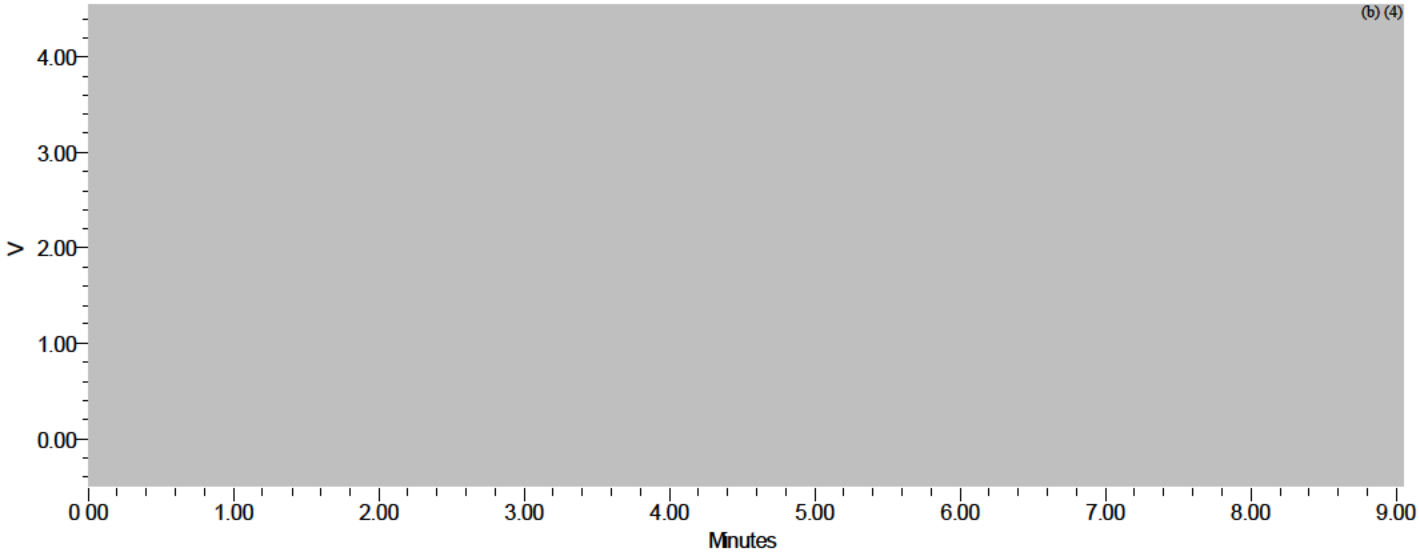
Sample Name:	35STD_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_4 Day
Vial:	40	Acq. Method Set:	VAL_ACR_Oven Temp_35
Injection #:	1	Processing Method:	Valine_Temp 35
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 4:21:16 PM KST		
Date Processed:	12/20/2020 5:41:31 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

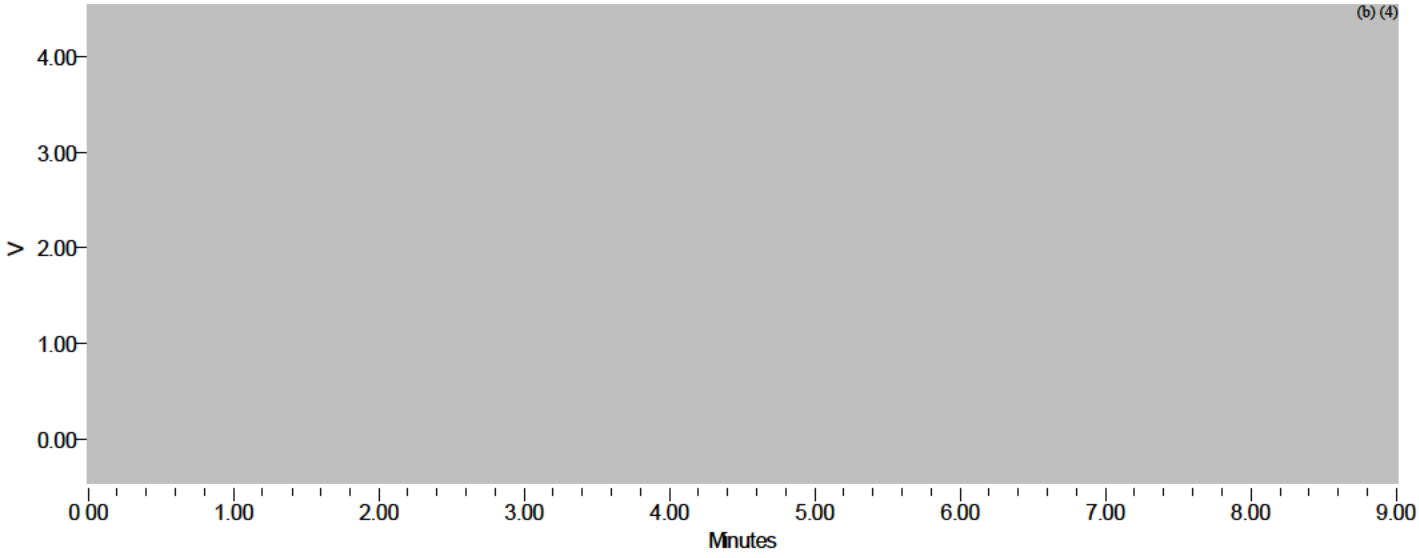
Sample Name:	35SPL_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_4 Day
Vial:	41	Acq. Method Set:	VAL_ACR_Oven Temp_35
Injection #:	1	Processing Method:	Valine_Temp 35
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 4:30:57 PM KST		
Date Processed:	12/20/2020 5:41:31 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

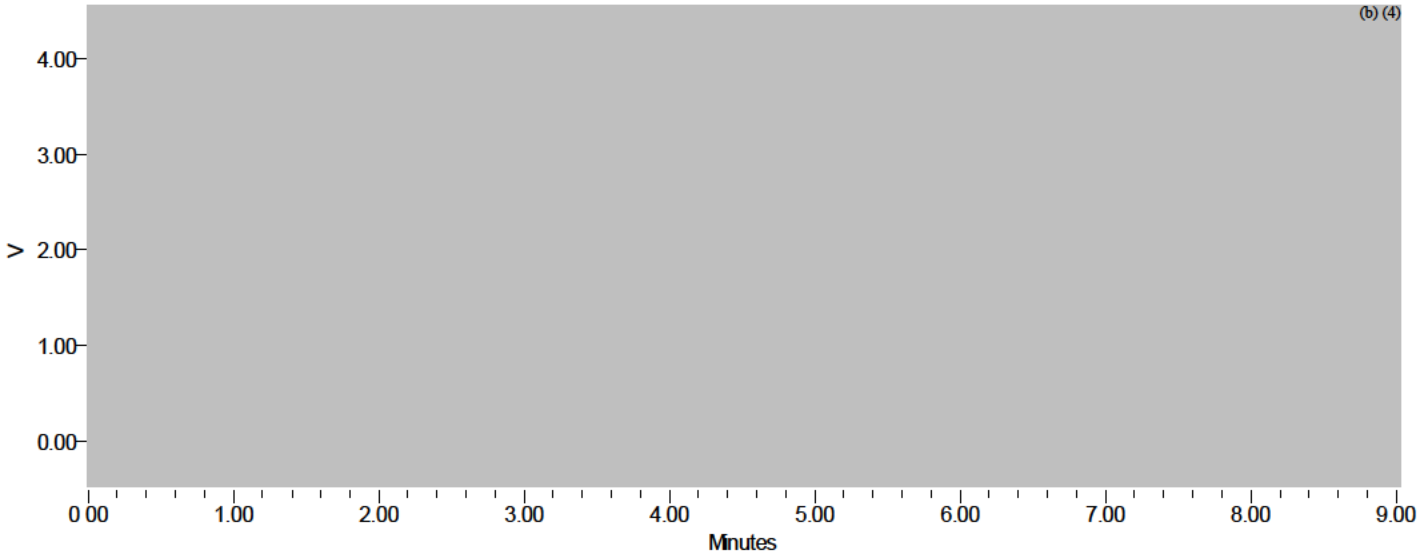
Sample Name:	35STD_4	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_4 Day
Vial:	42	Acq. Method Set:	VAL_ACR_Oven Temp_35
Injection #:	1	Processing Method:	Valine_Temp 35
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 4:40:37 PM KST		
Date Processed:	12/20/2020 5:41:32 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

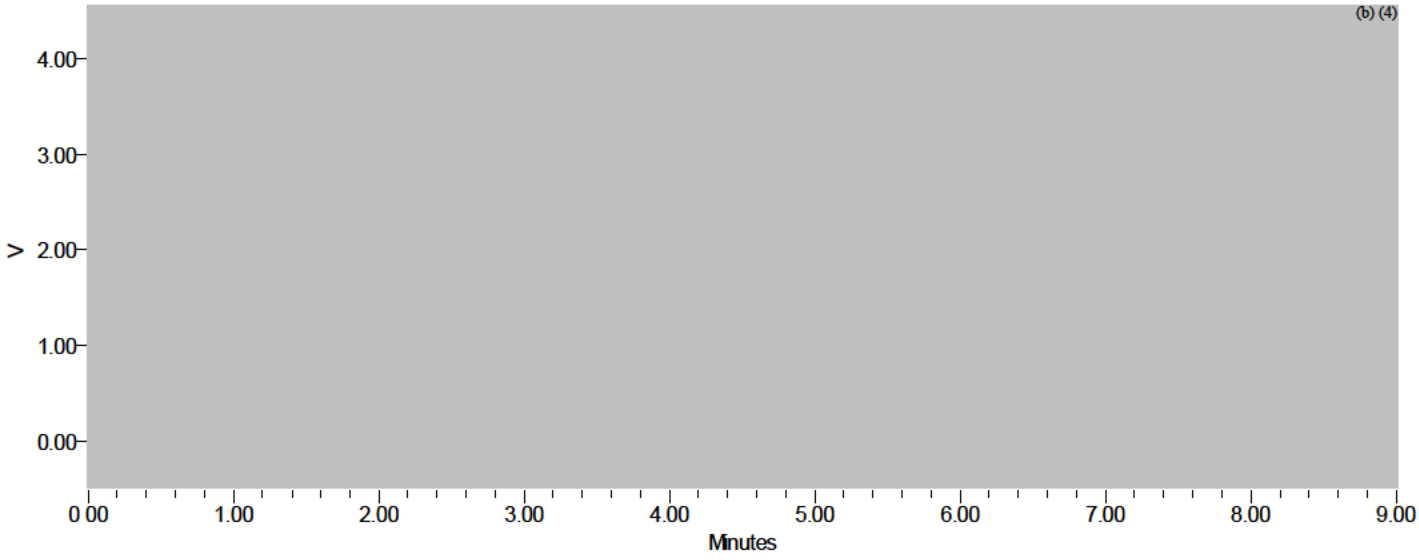
Sample Name:	45STD_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_4 Day
Vial:	43	Acq. Method Set:	VAL_ACR_Oven Temp_45
Injection #:	1	Processing Method:	Valine_Temp 45
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 5:19:58 PM KST		
Date Processed:	12/20/2020 5:42:33 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

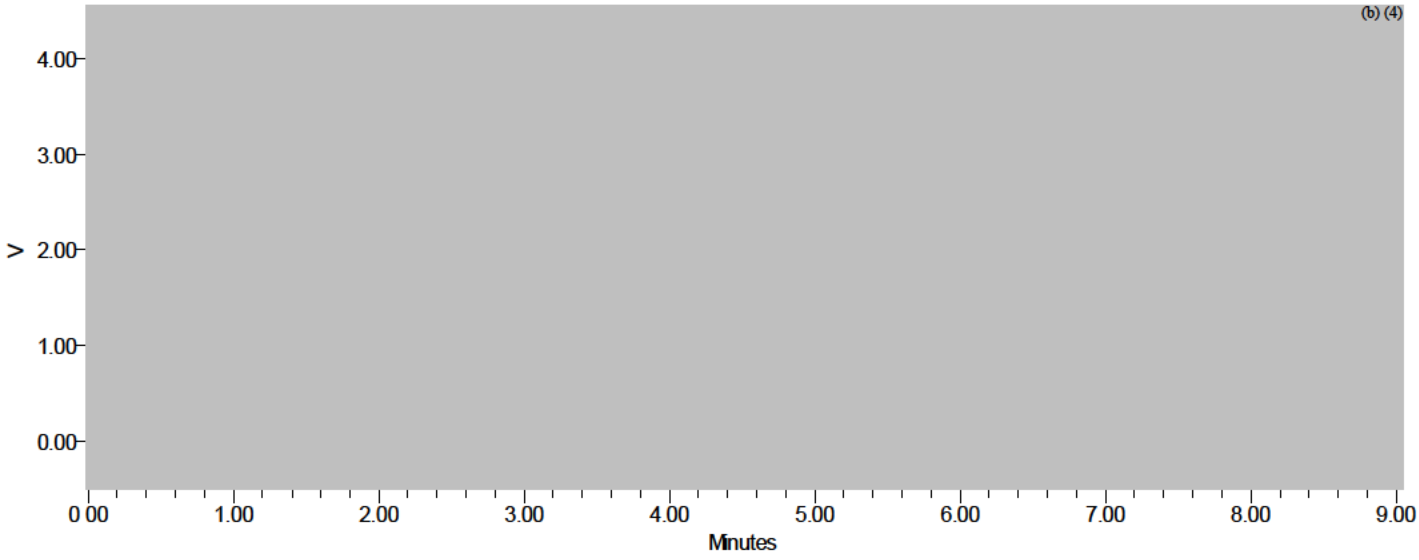
Sample Name:	45SPL_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_4 Day
Vial:	44	Acq. Method Set:	VAL_ACR_Oven Temp_45
Injection #:	1	Processing Method:	Valine_Temp 45
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 5:29:40 PM KST		
Date Processed:	12/20/2020 5:42:52 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

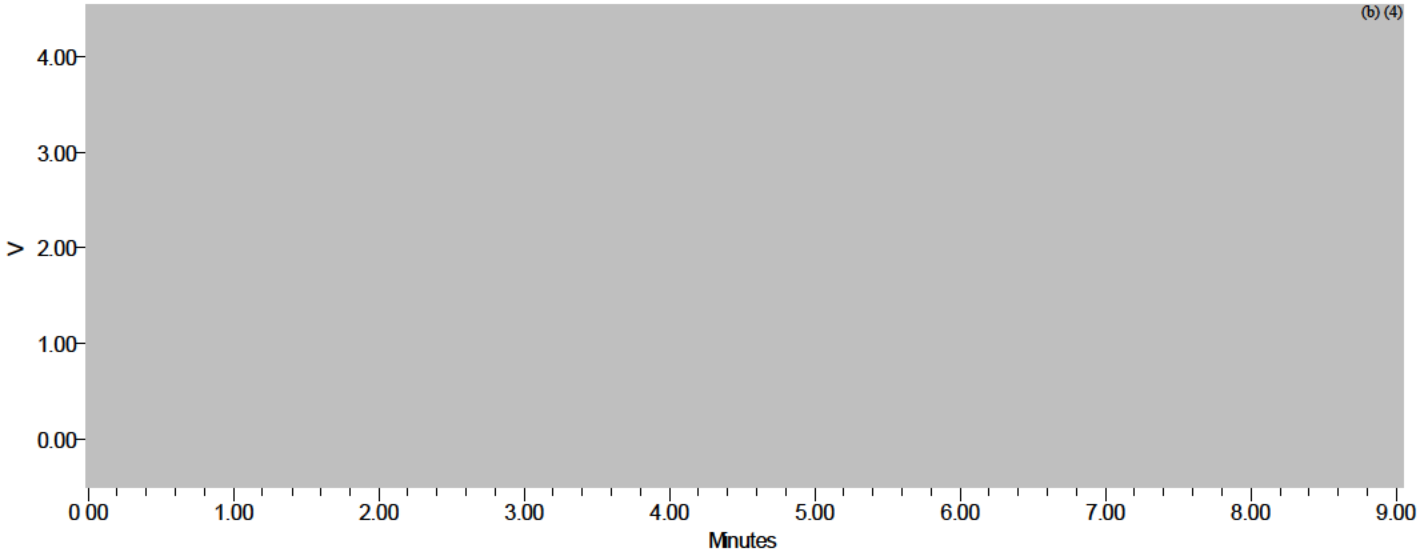
Sample Name:	45STD_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_4 Day
Vial:	45	Acq. Method Set:	VAL_ACR_Oven Temp_45
Injection #:	1	Processing Method:	Valine_Temp 45
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 5:39:17 PM KST		
Date Processed:	12/20/2020 6:25:21 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

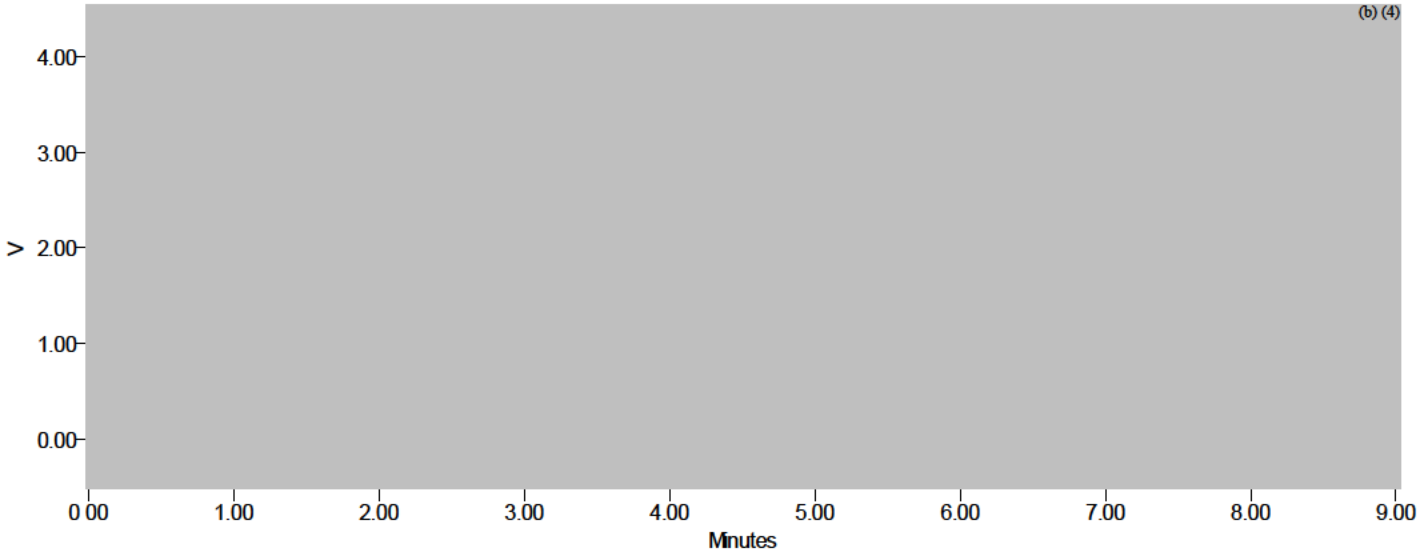
Sample Name:	45SPL_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_4 Day
Vial:	46	Acq. Method Set:	VAL_ACR_Oven Temp_45
Injection #:	1	Processing Method:	Valine_Temp 45
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 5:48:56 PM KST		
Date Processed:	12/20/2020 6:25:22 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

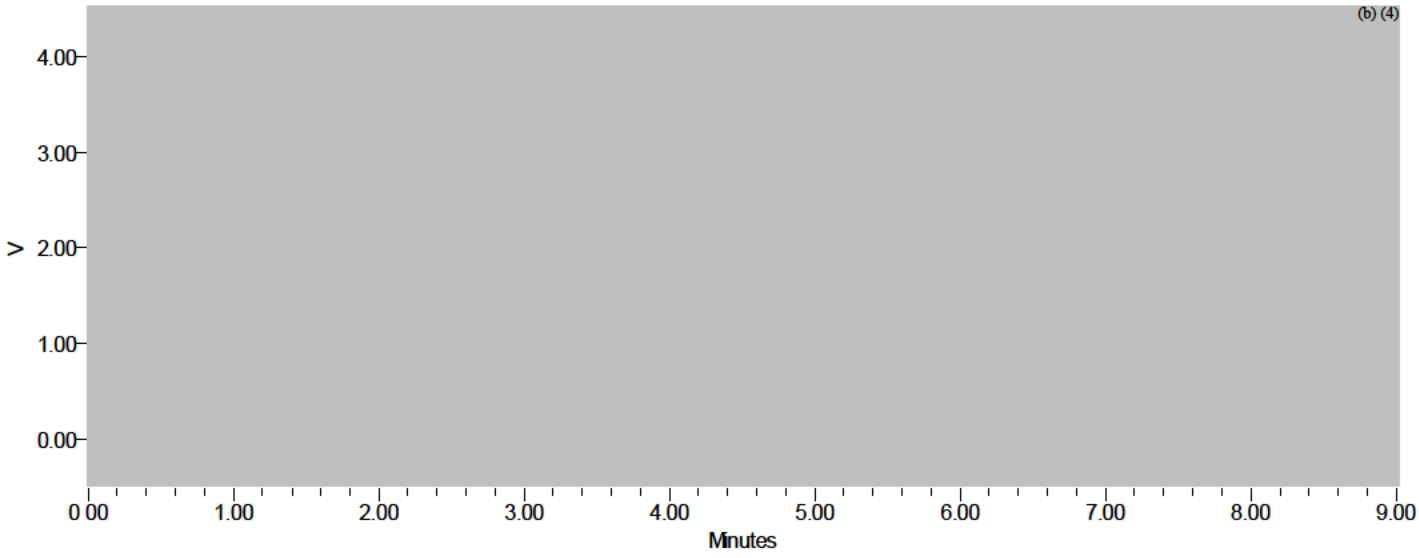
Sample Name:	45STD_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_4 Day
Vial:	47	Acq. Method Set:	VAL_ACR_Oven Temp_45
Injection #:	1	Processing Method:	Valine_Temp 45
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 5:58:37 PM KST		
Date Processed:	12/20/2020 6:25:22 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

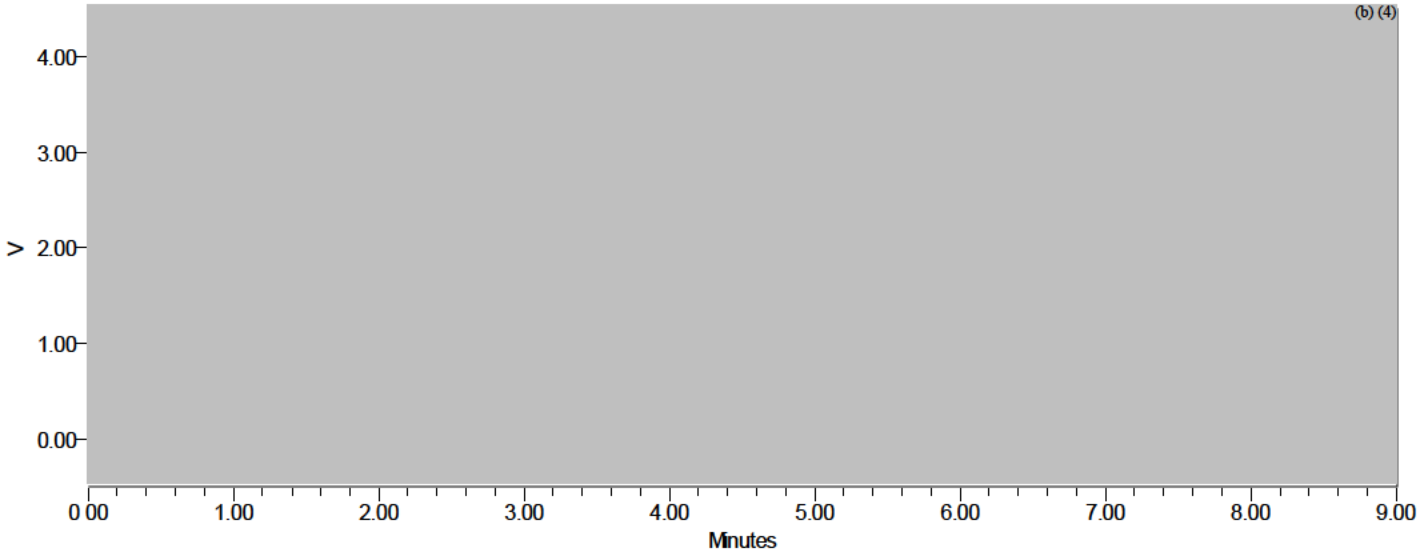
Sample Name:	45SPL_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_4 Day
Vial:	48	Acq. Method Set:	VAL_ACR_Oven Temp_45
Injection #:	1	Processing Method:	Valine_Temp 45
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 6:08:17 PM KST		
Date Processed:	12/20/2020 6:25:22 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

Sample Name:	45STD_4	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_4 Day
Vial:	49	Acq. Method Set:	VAL_ACR_Oven Temp_45
Injection #:	1	Processing Method:	Valine_Temp 45
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 6:17:55 PM KST		
Date Processed:	12/20/2020 6:27:11 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

Software: Empower 3 Software Build 3471

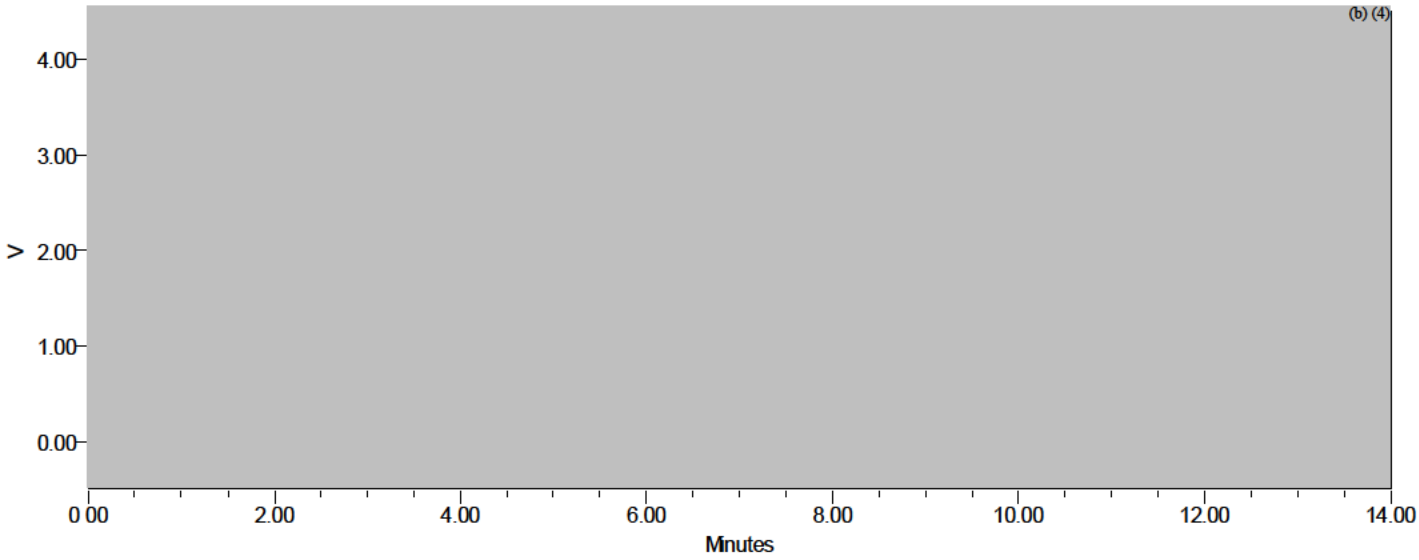
Sample Set Name:	Granule Valine_3 Day_2nd	Processed By:	System/Administrator
Sample Set Method:	Granule Valine_3 Day_2nd	Printed By:	System
System Node:	Client13	Result Set ID:	
System Name:	UC10	# of Results:	14
Acquired By:	System		
Sample Set Start Date:	12/19/2020 9:59:47 PM KST		
Sample Set Finish Date:	12/20/2020 3:30:48 PM KST		

Sample Set Table

	Sample Name	Sample Type	Vial	Inj #	Injection Volume (ul)	Acquistion Method Set	Processed Channel Descr.	(b) (4)
1	FSTD1_1	Unknown	22	1	5.00	VAL_ACR_Flow Rate 08	Detector A	
2	FSPL1_1	Unknown	23	1	5.00	VAL_ACR_Flow Rate 08	Detector A	
3	FSTD1_2	Unknown	24	1	5.00	VAL_ACR_Flow Rate 08	Detector A	
4	FSPL1_2	Unknown	25	1	5.00	VAL_ACR_Flow Rate 08	Detector A	
5	FSTD1_3	Unknown	26	1	5.00	VAL_ACR_Flow Rate 08	Detector A	
6	FSPL1_3	Unknown	27	1	5.00	VAL_ACR_Flow Rate 08	Detector A	
7	FSTD1_4	Unknown	28	1	5.00	VAL_ACR_Flow Rate 08	Detector A	
8	FSTD2_4	Unknown	35	1	5.00	VAL_ACR_Flow Rate 12	Detector A	
9	FSPL2_3	Unknown	34	1	5.00	VAL_ACR_Flow Rate 12	Detector A	
10	FSPL2_2	Unknown	32	1	5.00	VAL_ACR_Flow Rate 12	Detector A	
11	FSTD2_2	Unknown	31	1	5.00	VAL_ACR_Flow Rate 12	Detector A	
12	FSTD2_3	Unknown	33	1	5.00	VAL_ACR_Flow Rate 12	Detector A	
13	FSPL2_1	Unknown	30	1	5.00	VAL_ACR_Flow Rate 12	Detector A	
14	FSTD2_1	Unknown	29	1	5.00	VAL_ACR_Flow Rate 12	Detector A	

SAMPLE INFORMATION

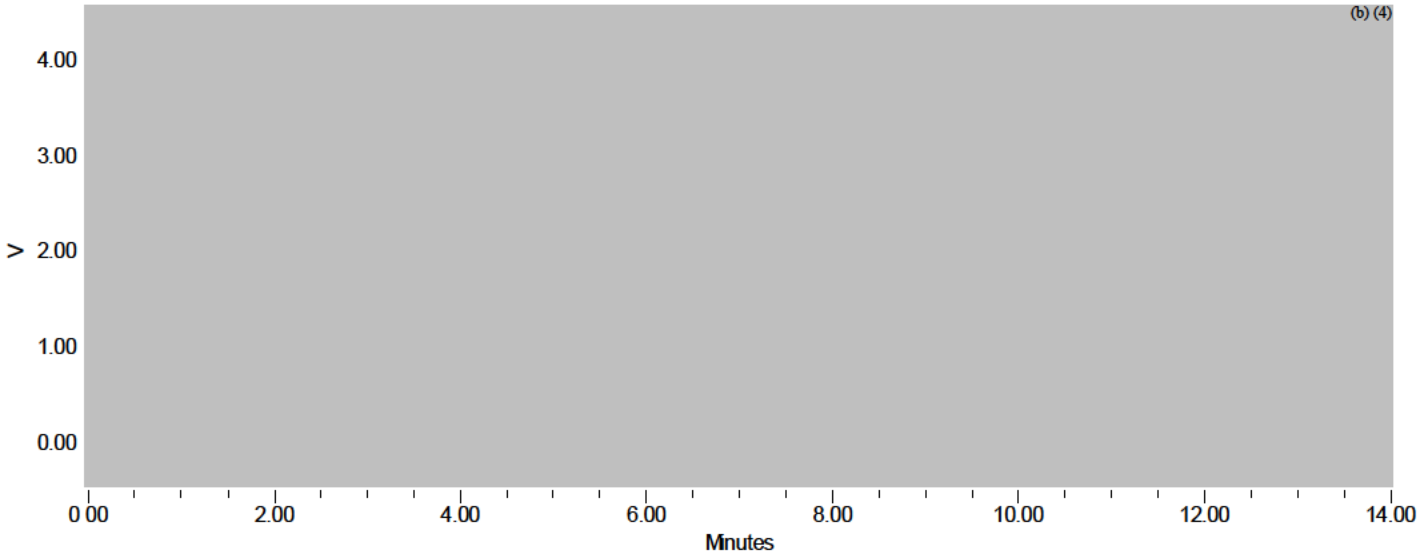
Sample Name:	FSTD1_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day_2nd
Vial:	22	Acq. Method Set:	VAL_ACR_FlowRate 08
Injection #:	1	Processing Method:	Valine_FR08
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	14.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 11:52:28 AM KST		
Date Processed:	12/21/2020 12:04:21 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

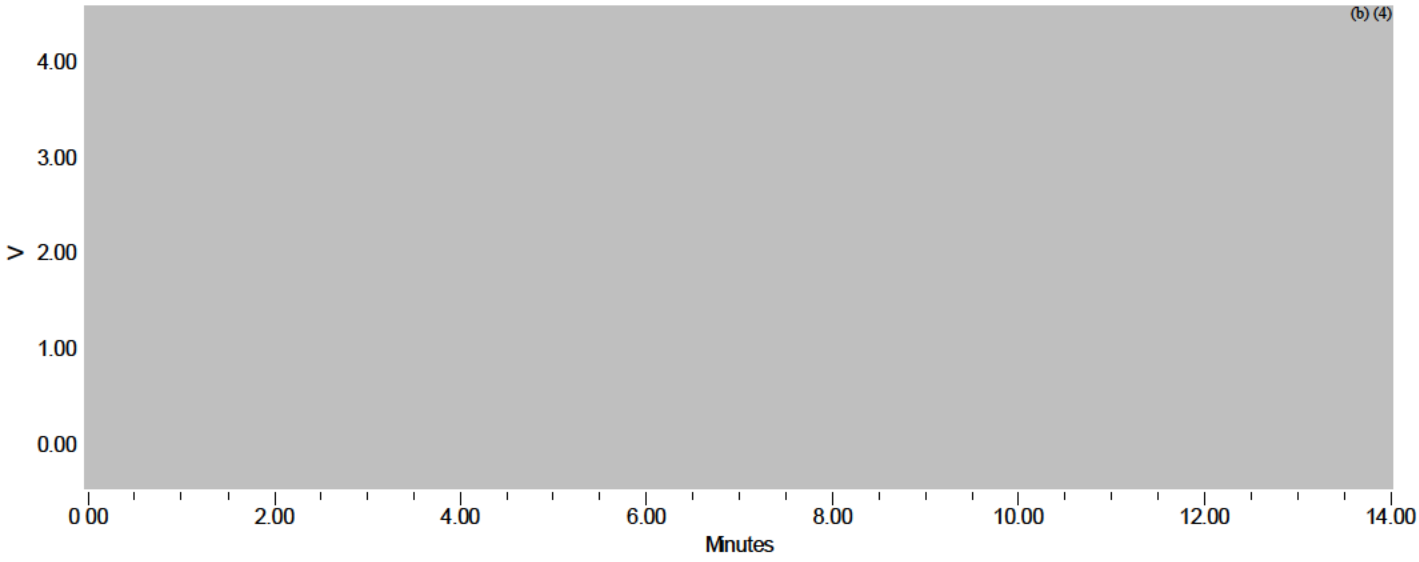
Sample Name:	FSPL1_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day_2nd
Vial:	23	Acq. Method Set:	VAL_ACR_FlowRate 08
Injection #:	1	Processing Method:	Valine_FR08
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	14.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 12:07:10 PM KST		
Date Processed:	12/21/2020 12:04:21 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

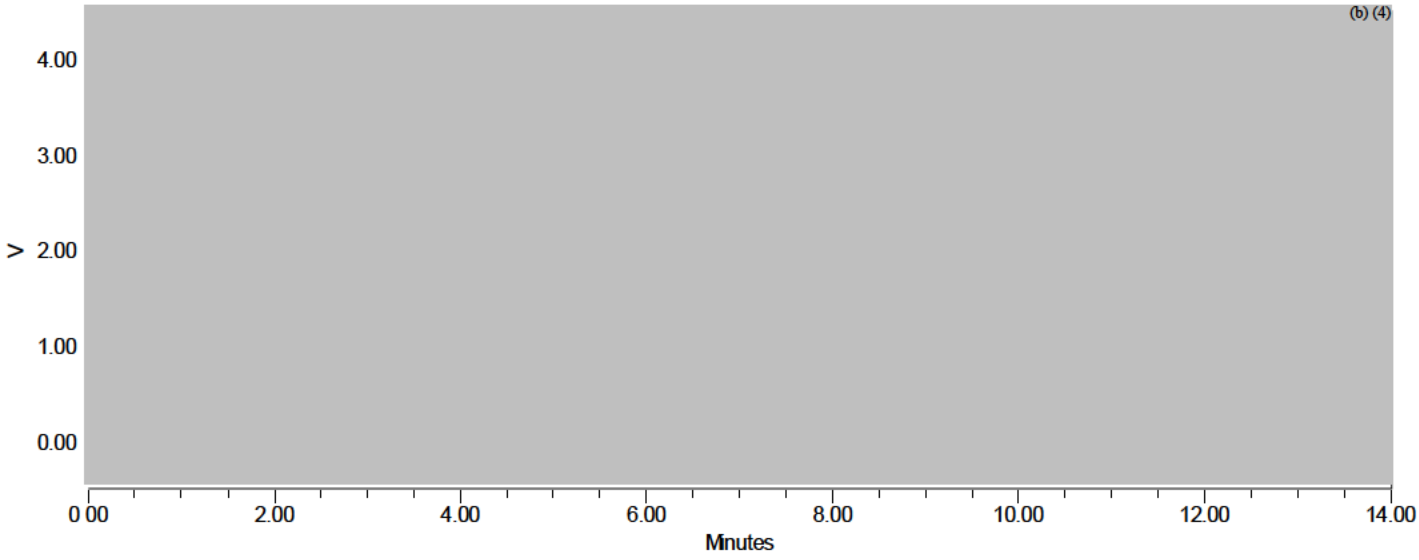
Sample Name:	FSTD1_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day_2nd
Vial:	24	Acq. Method Set:	VAL_ACR_FlowRate 08
Injection #:	1	Processing Method:	Valine_FR08
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	14.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 12:21:50 PM KST		
Date Processed:	12/21/2020 12:04:21 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

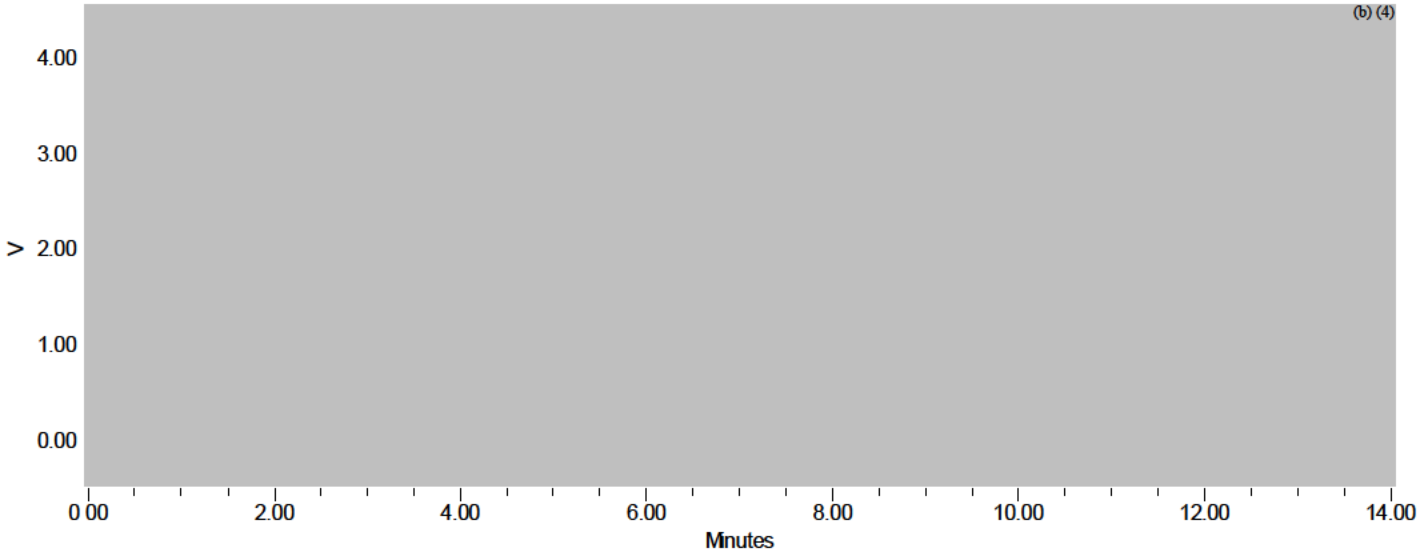
Sample Name:	FSPL1_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day_2nd
Vial:	25	Acq. Method Set:	VAL_ACR_FlowRate 08
Injection #:	1	Processing Method:	Valine_FR08
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	14.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 12:36:34 PM KST		
Date Processed:	12/21/2020 12:04:22 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

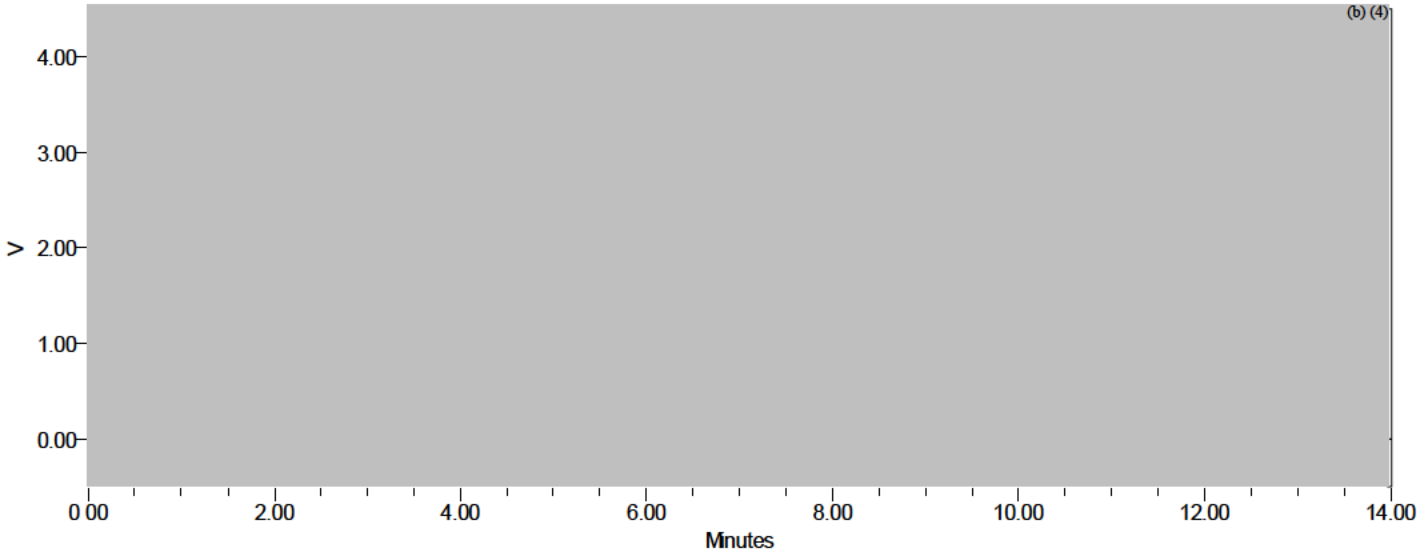
Sample Name:	FSTD1_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day_2nd
Vial:	26	Acq. Method Set:	VAL_ACR_FlowRate 08
Injection #:	1	Processing Method:	Valine_FR08
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	14.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 12:51:13 PM KST		
Date Processed:	12/21/2020 12:04:22 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

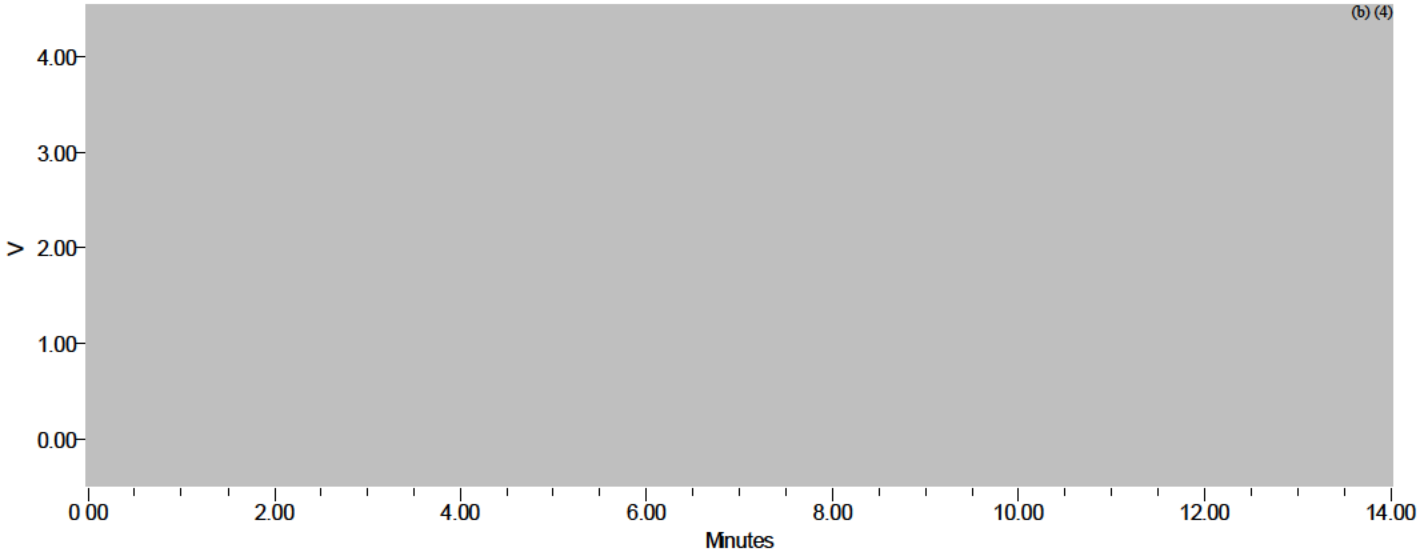
Sample Name:	FSPL1_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day_2nd
Vial:	27	Acq. Method Set:	VAL_ACR_FlowRate 08
Injection #:	1	Processing Method:	Valine_FR08
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	14.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 1:05:47 PM KST		
Date Processed:	12/21/2020 12:04:22 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

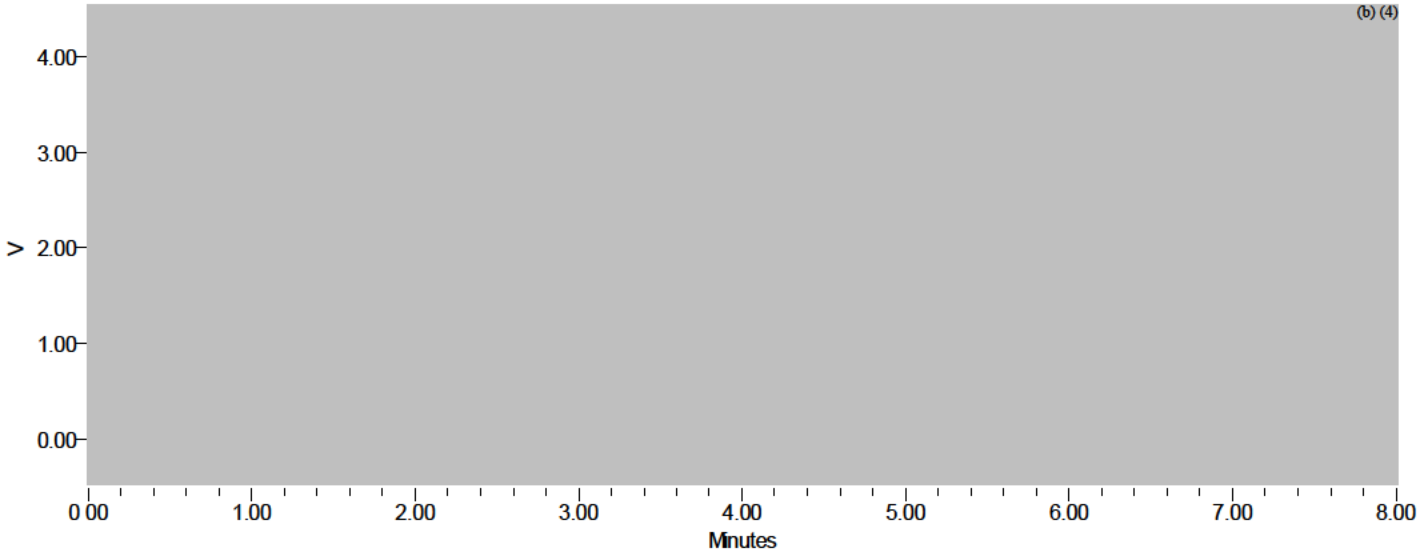
Sample Name:	FSTD1_4	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day_2nd
Vial:	28	Acq. Method Set:	VAL_ACR_FlowRate 08
Injection #:	1	Processing Method:	Valine_FR08
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	14.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 1:20:30 PM KST		
Date Processed:	12/21/2020 12:04:22 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

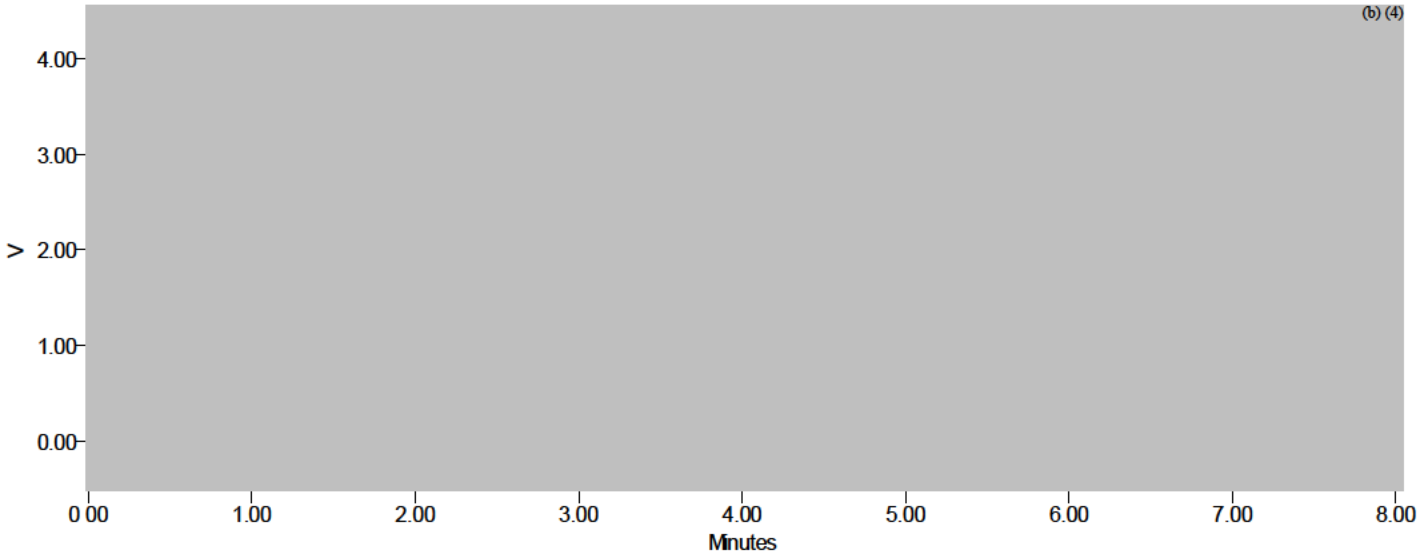
Sample Name:	FSTD2_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day_2nd
Vial:	29	Acq. Method Set:	VAL_ACR_FlowRate 12
Injection #:	1	Processing Method:	Valine_FR12
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	8.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 2:07:58 PM KST		
Date Processed:	12/21/2020 12:15:37 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

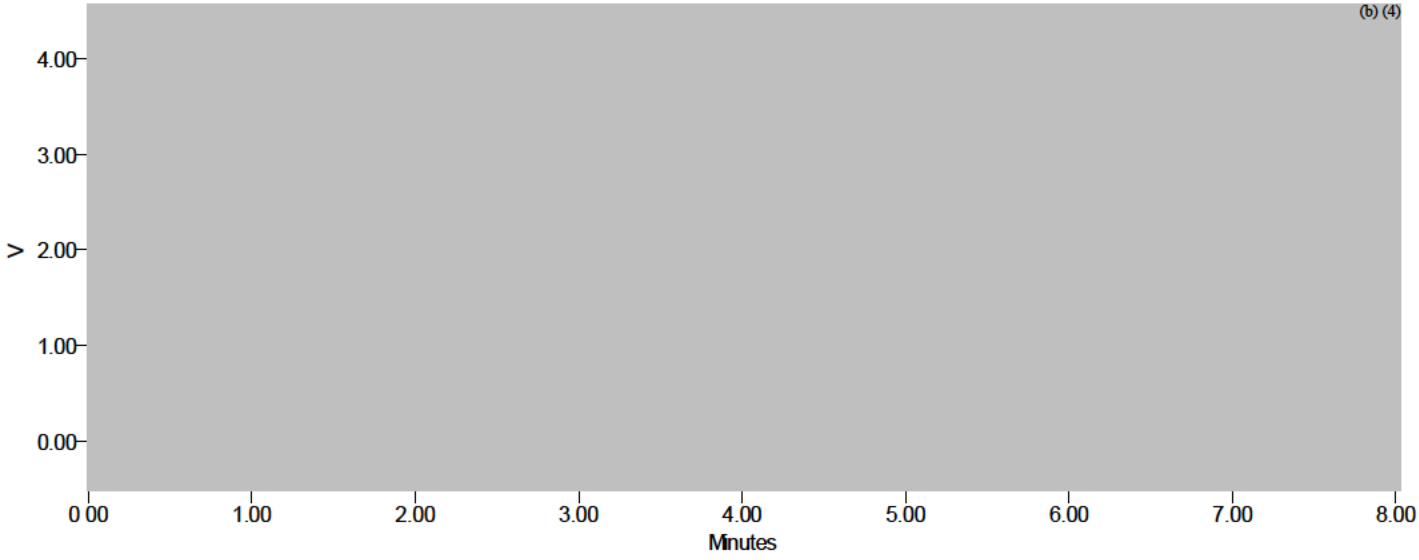
Sample Name:	FSPL2_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day_2nd
Vial:	30	Acq. Method Set:	VAL_ACR_FlowRate 12
Injection #:	1	Processing Method:	Valine_FR12
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	8.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 2:16:39 PM KST		
Date Processed:	12/21/2020 12:15:38 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

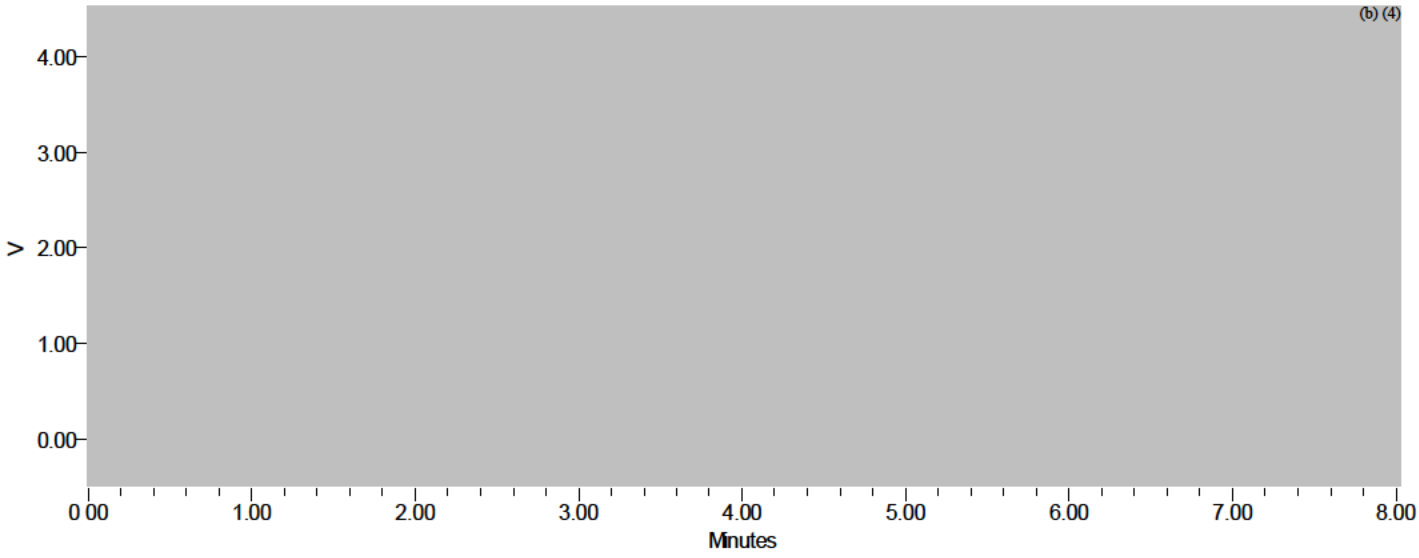
Sample Name:	FSTD2_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day_2nd
Vial:	31	Acq. Method Set:	VAL_ACR_FlowRate 12
Injection #:	1	Processing Method:	Valine_FR12
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	8.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 2:25:22 PM KST		
Date Processed:	12/21/2020 12:15:41 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

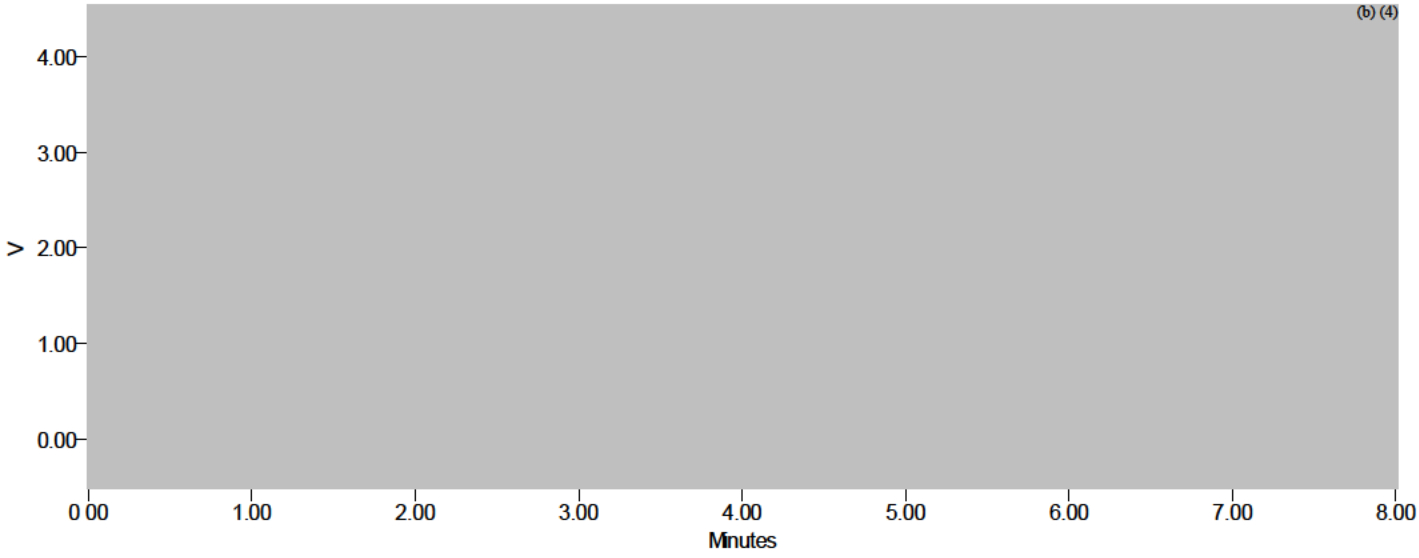
Sample Name:	FSPL2_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day_2nd
Vial:	32	Acq. Method Set:	VAL_ACR_FlowRate 12
Injection #:	1	Processing Method:	Valine_FR12
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	8.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 2:34:02 PM KST		
Date Processed:	12/21/2020 12:15:42 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

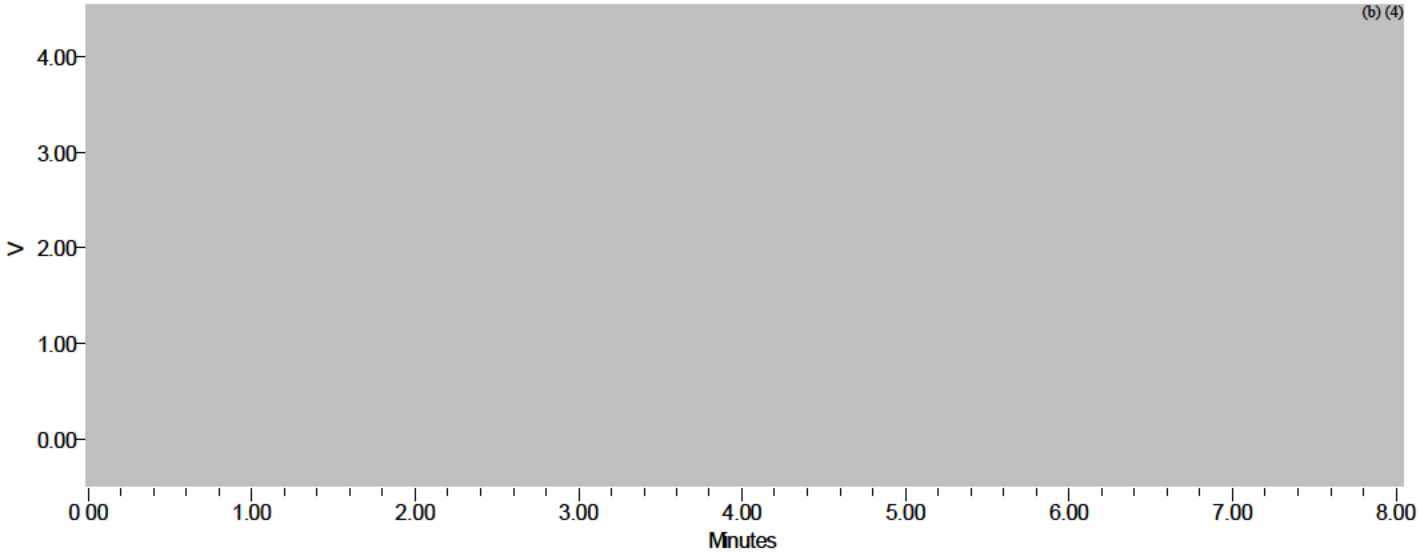
Sample Name:	FSTD2_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day_2nd
Vial:	33	Acq. Method Set:	VAL_ACR_FlowRate 12
Injection #:	1	Processing Method:	Valine_FR12
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	8.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 2:42:40 PM KST		
Date Processed:	12/21/2020 12:15:39 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

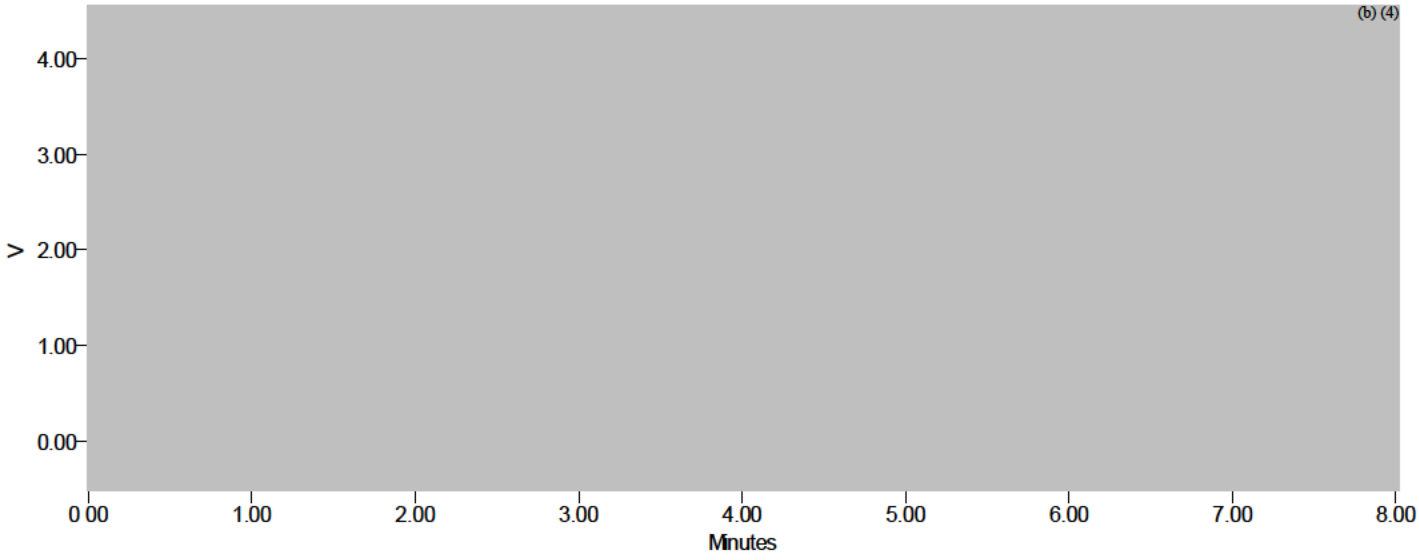
Sample Name:	FSPL2_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day_2nd
Vial:	34	Acq. Method Set:	VAL_ACR_FlowRate 12
Injection #:	1	Processing Method:	Valine_FR12
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	8.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 2:51:22 PM KST		
Date Processed:	12/21/2020 12:15:43 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

Sample Name:	FSTD2_4	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day_2nd
Vial:	35	Acq. Method Set:	VAL_ACR_FlowRate 12
Injection #:	1	Processing Method:	Valine_FR12
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	8.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 2:59:56 PM KST		
Date Processed:	12/21/2020 12:15:44 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

Software: Empower 3 Software Build 3471

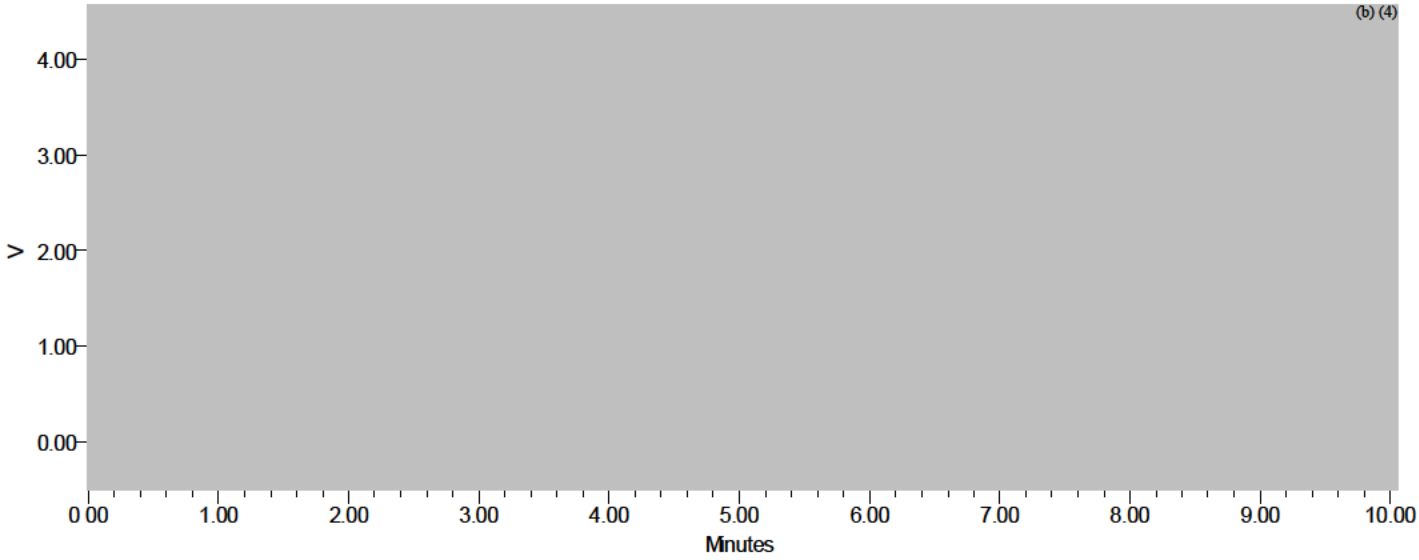
Sample Set Name:	Granule Valine_4 Day_2nd	Processed By:	System/Administrator
Sample Set Method:	Granule Valine_4 Day_2nd	Printed By:	System
System Node:	Client13	Result Set ID:	
System Name:	UC10	# of Results:	14
Acquired By:	System		
Sample Set Start Date:	12/20/2020 6:51:02 PM KST		
Sample Set Finish Date:	12/20/2020 10:57:43 PM KST		

Sample Set Table

	Sample Name	Sample Type	Vial	Inj #	Injection Volume (ul)	Acquistion Method Set	Processed Channel Descr.	(b) (4)
1	PSTD1_1	Unknown	50	1	5.00	VAL_ACR	Detector A	
2	PSPL1_1	Unknown	51	1	5.00	VAL_ACR	Detector A	
3	PSTD1_2	Unknown	52	1	5.00	VAL_ACR	Detector A	
4	PSPL1_2	Unknown	53	1	5.00	VAL_ACR	Detector A	
5	PSTD1_3	Unknown	54	1	5.00	VAL_ACR	Detector A	
6	PSPL1_3	Unknown	55	1	5.00	VAL_ACR	Detector A	
7	PSTD1_4	Unknown	56	1	5.00	VAL_ACR	Detector A	
8	PSTD2_1	Unknown	57	1	5.00	VAL_ACR	Detector A	
9	PSPL2_1	Unknown	58	1	5.00	VAL_ACR	Detector A	
10	PSTD2_2	Unknown	59	1	5.00	VAL_ACR	Detector A	
11	PSPL2_2	Unknown	60	1	5.00	VAL_ACR	Detector A	
12	PSTD2_3	Unknown	61	1	5.00	VAL_ACR	Detector A	
13	PSPL2_3	Unknown	62	1	5.00	VAL_ACR	Detector A	
14	PSTD2_4	Unknown	63	1	5.00	VAL_ACR	Detector A	

SAMPLE INFORMATION

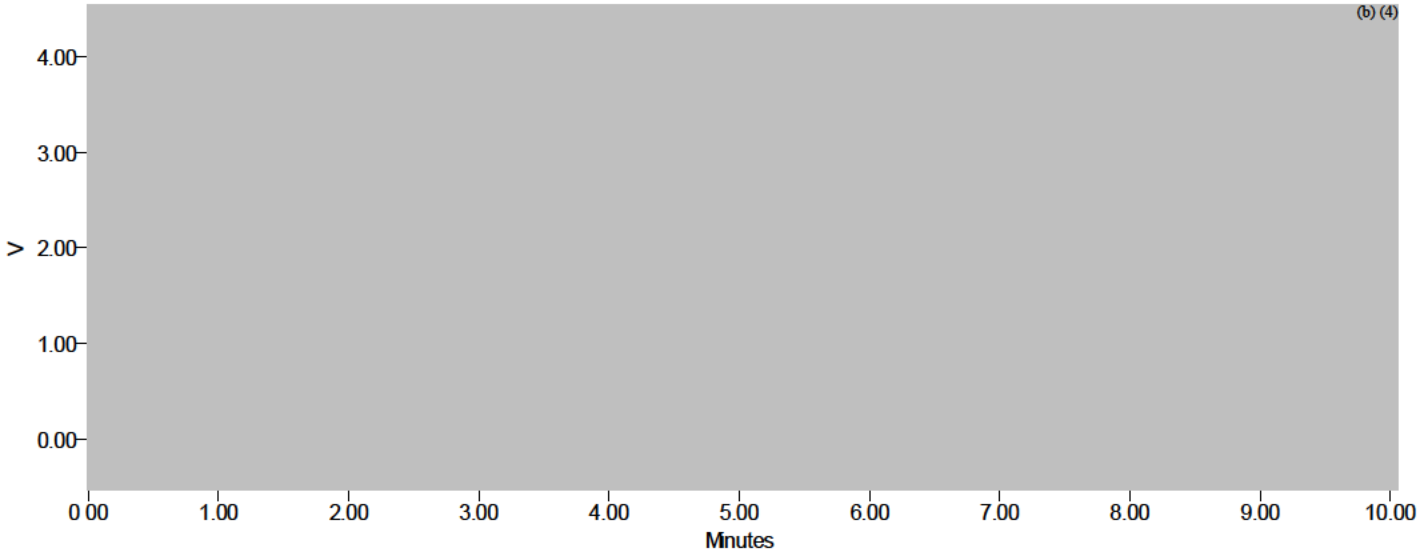
Sample Name:	PSTD1_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_4 Day_2nd
Vial:	50	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_pH2 3
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	10.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 7:45:56 PM KST		
Date Processed:	12/20/2020 8:59:13 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

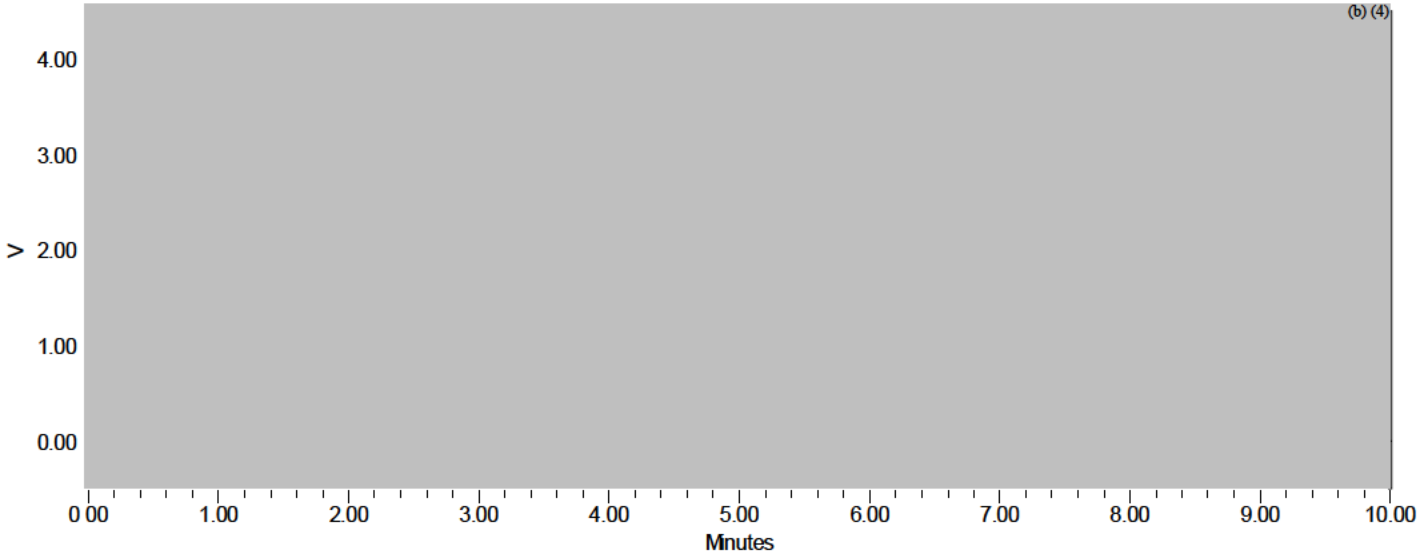
Sample Name:	PSPL1_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_4 Day_2nd
Vial:	51	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_pH2 3
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	10.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 7:56:32 PM KST		
Date Processed:	12/20/2020 8:59:40 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

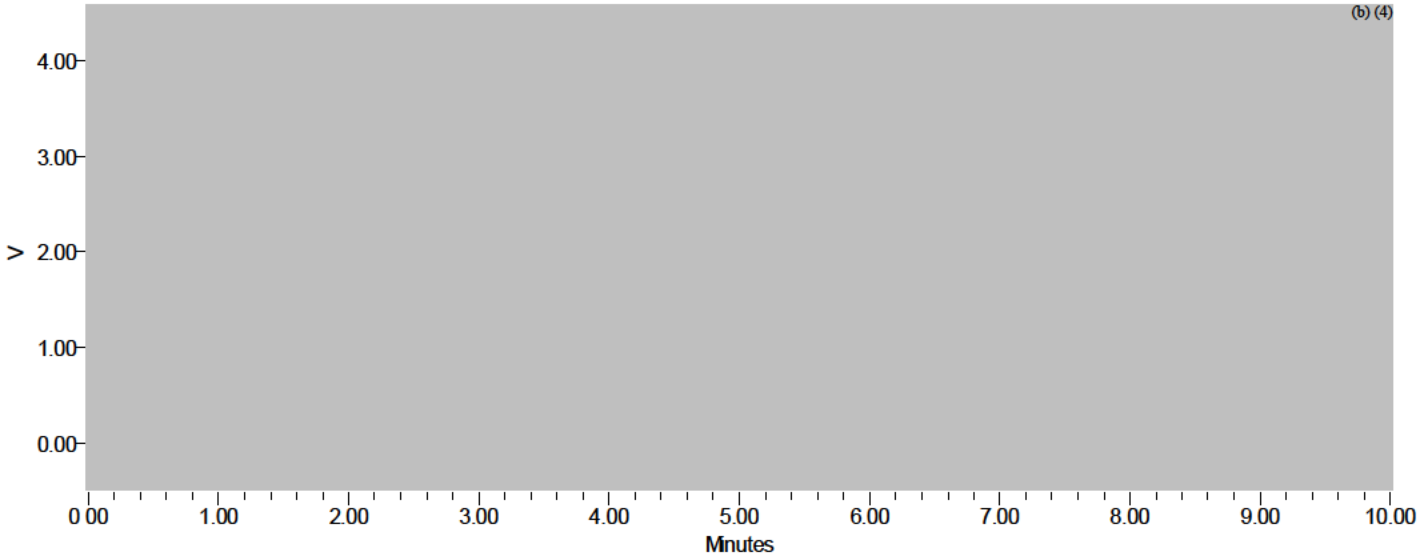
Sample Name:	PSTD1_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_4 Day_2nd
Vial:	52	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_pH2 3
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	10.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 8:07:13 PM KST		
Date Processed:	12/20/2020 8:59:41 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

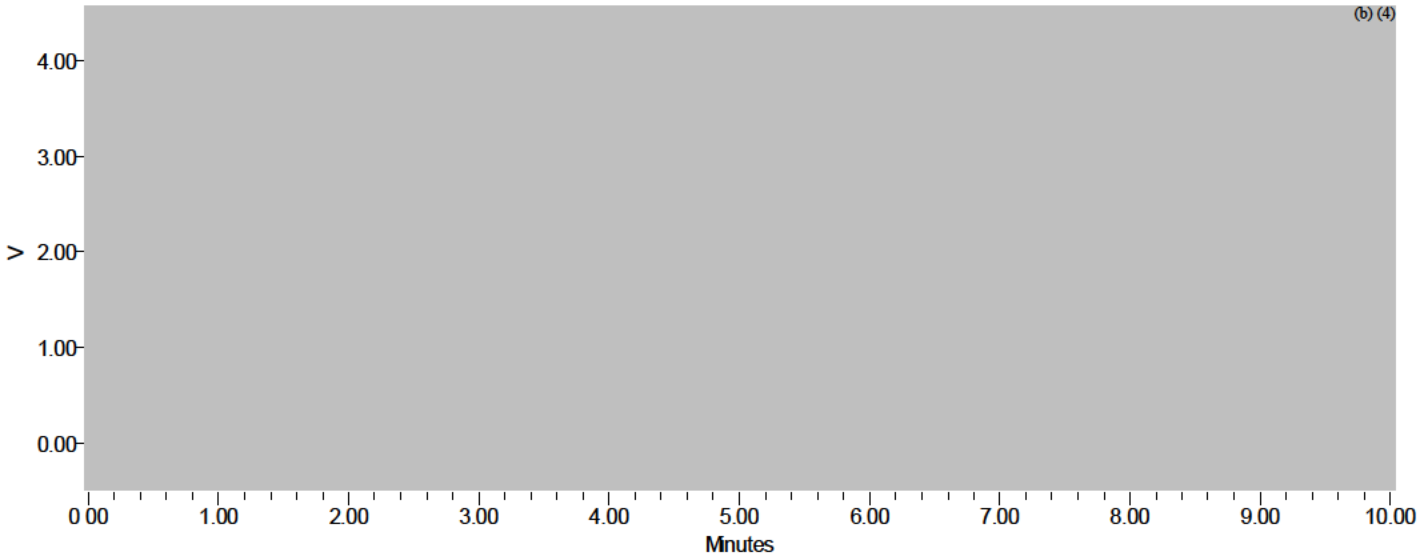
Sample Name:	PSPL1_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_4 Day_2nd
Vial:	53	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_pH2 3
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	10.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 8:17:53 PM KST		
Date Processed:	12/20/2020 8:59:41 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

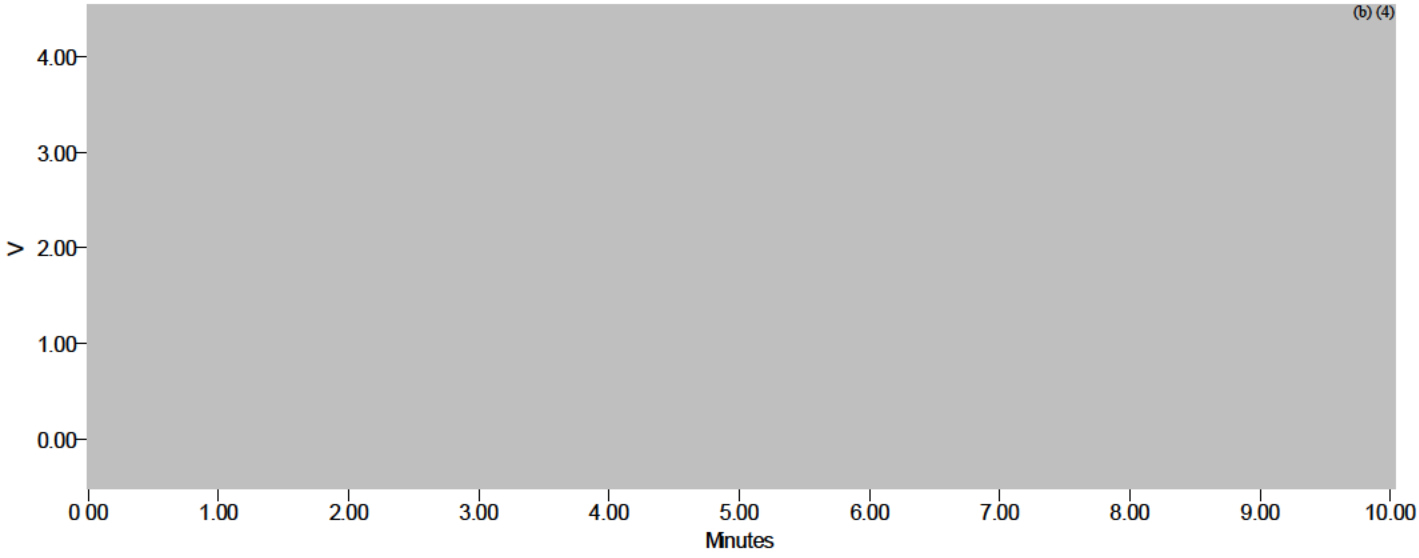
Sample Name:	PSTD1_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_4 Day_2nd
Vial:	54	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_pH2 3
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	10.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 8:28:36 PM KST		
Date Processed:	12/20/2020 8:59:41 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

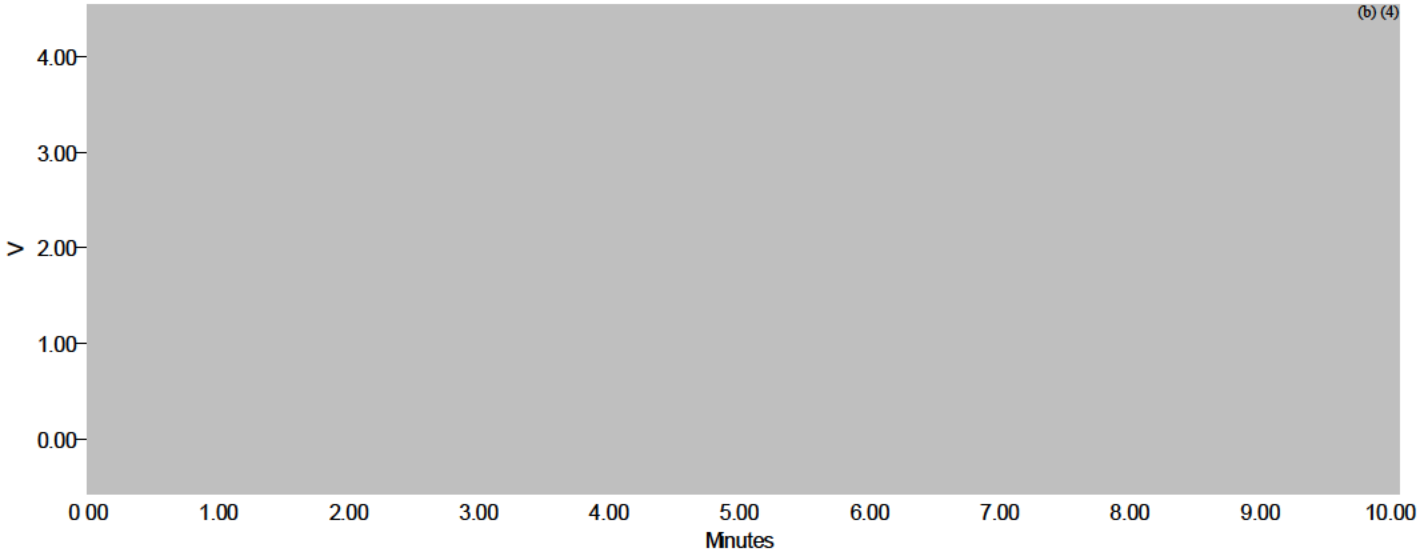
Sample Name:	PSPL1_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_4 Day_2nd
Vial:	55	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_pH2 3
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	10.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 8:39:12 PM KST		
Date Processed:	12/20/2020 8:59:41 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

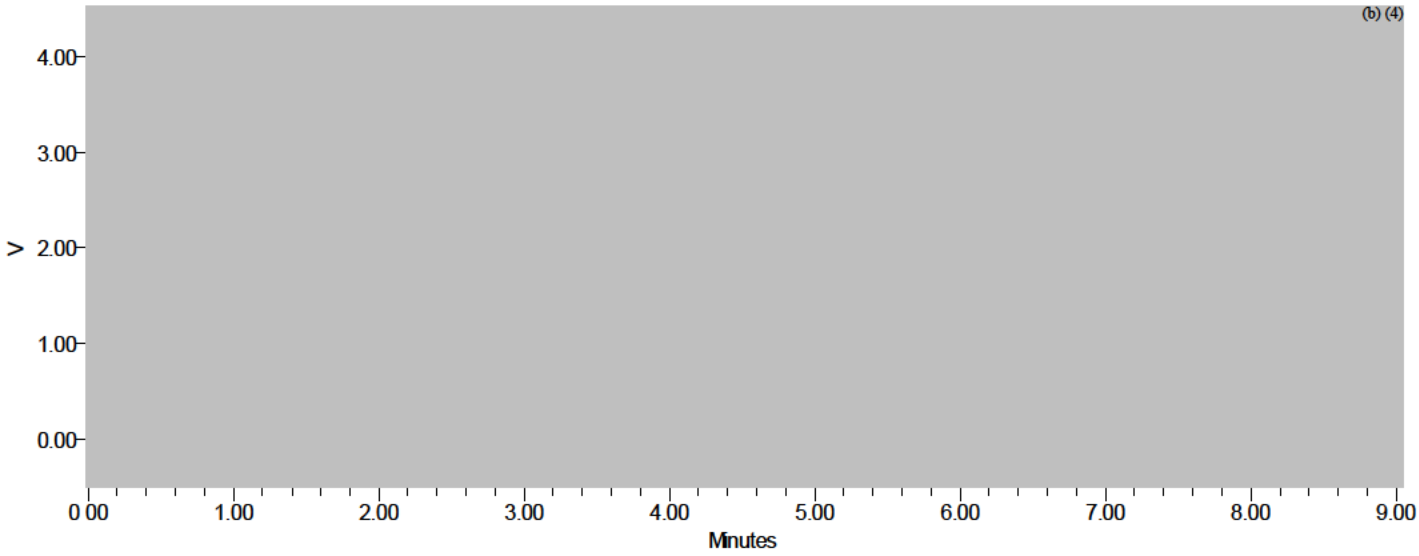
Sample Name:	PSTD1_4	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_4 Day_2nd
Vial:	56	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_pH2 3
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	10.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 8:49:53 PM KST		
Date Processed:	12/20/2020 9:00:17 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

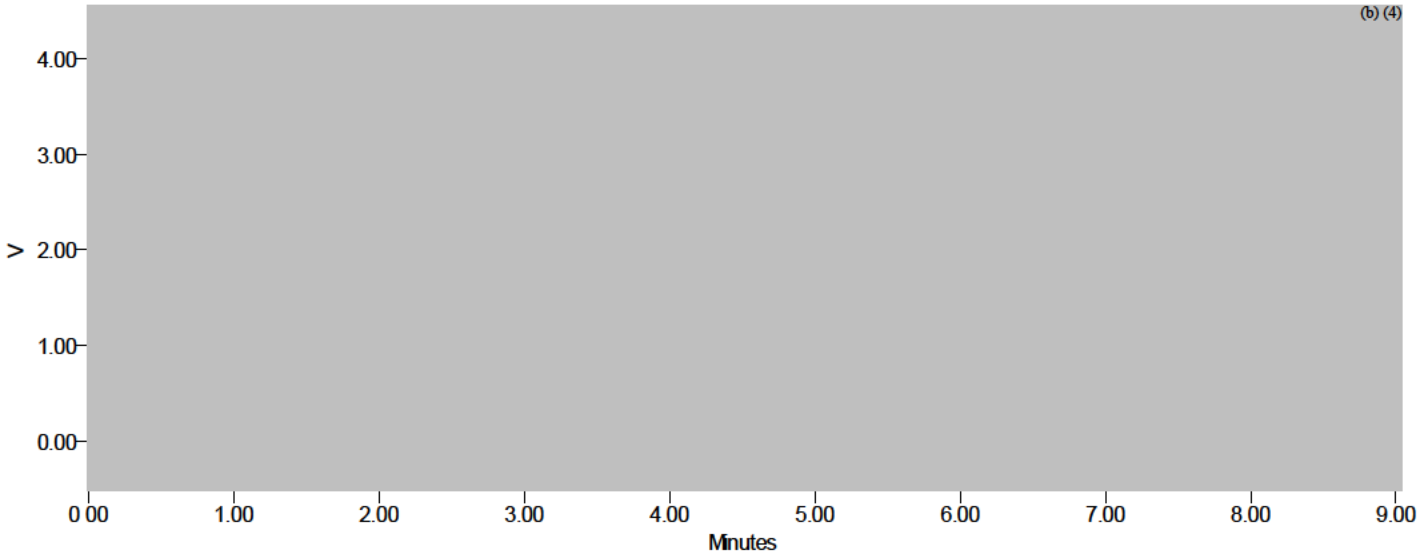
Sample Name:	PSTD2_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_4 Day_2nd
Vial:	57	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_pH 2 7
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 9:50:36 PM KST		
Date Processed:	12/20/2020 10:13:30 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

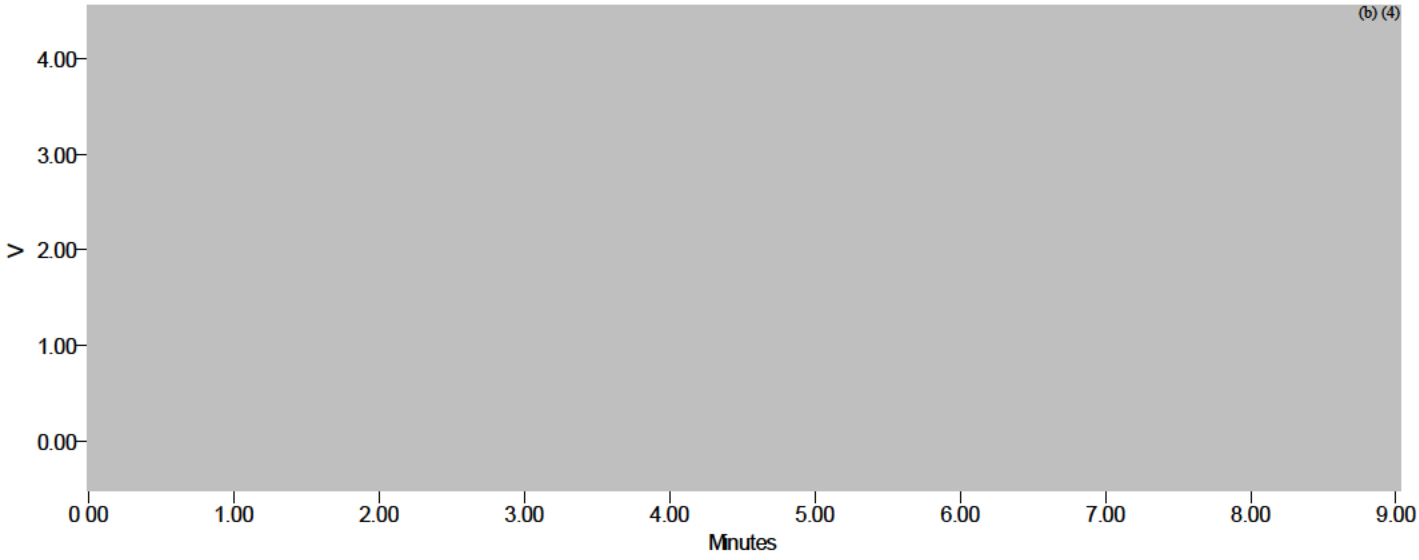
Sample Name:	PSPL2_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_4 Day_2nd
Vial:	58	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_pH 2 7
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 10:00:16 PM KST		
Date Processed:	12/20/2020 10:13:50 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

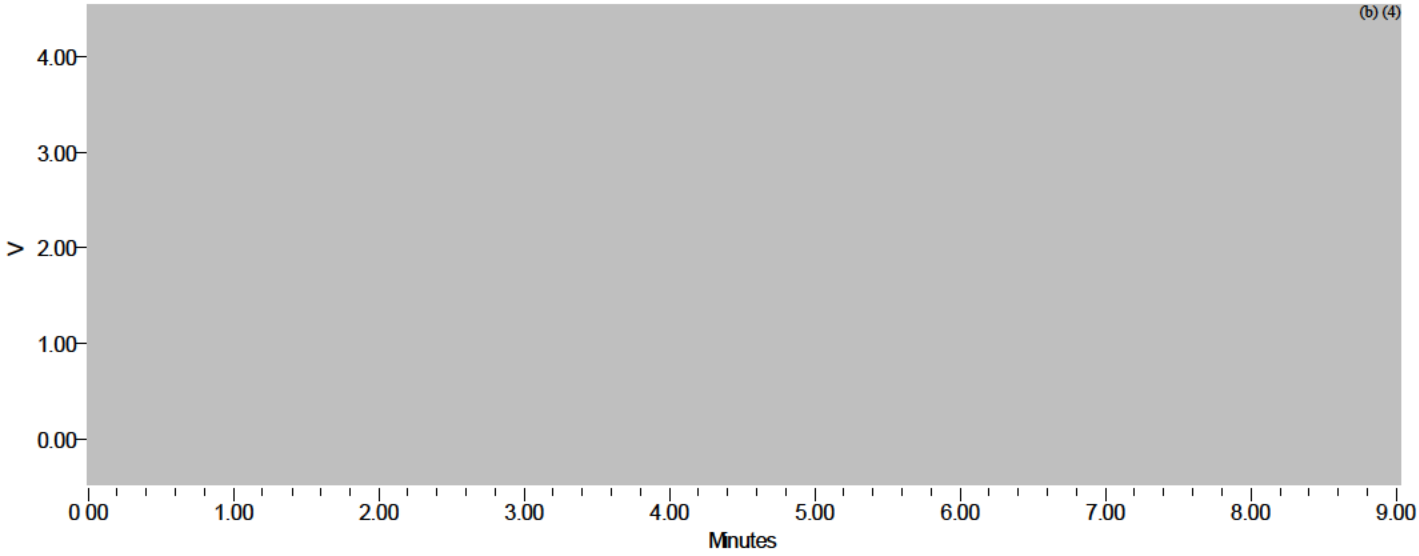
Sample Name:	PSTD2_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_4 Day_2nd
Vial:	59	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_pH 2 7
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 10:09:56 PM KST		
Date Processed:	12/20/2020 10:22:12 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

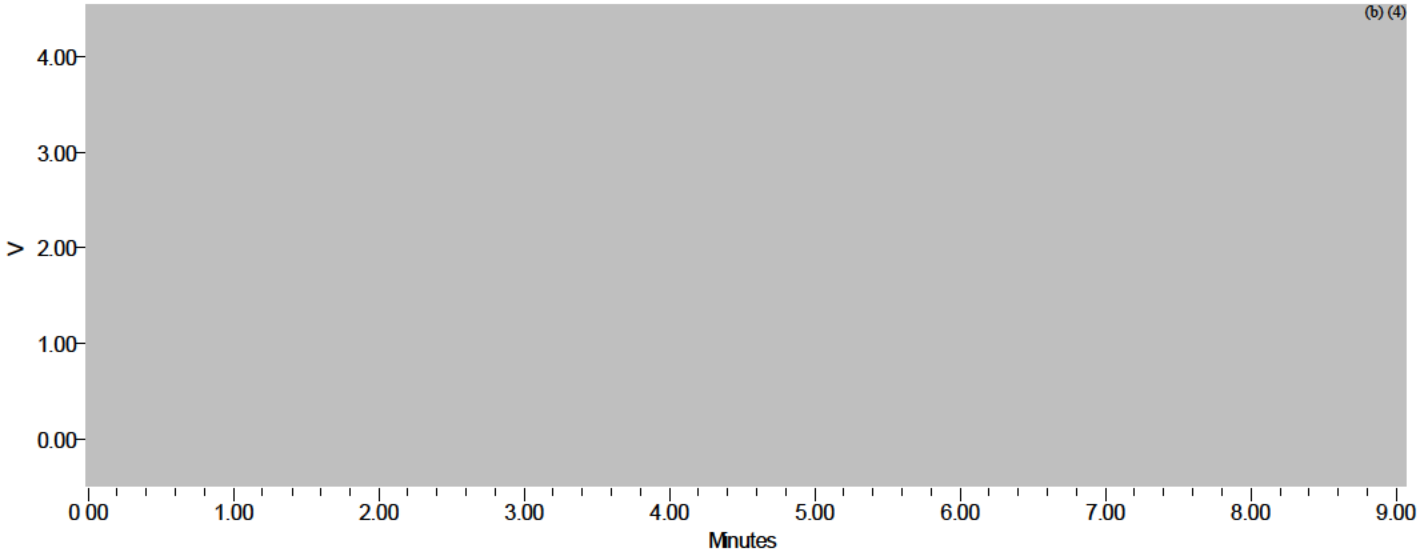
Sample Name:	PSPL2_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_4 Day_2nd
Vial:	60	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_pH 2 7
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 10:19:39 PM KST		
Date Processed:	12/20/2020 10:33:24 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

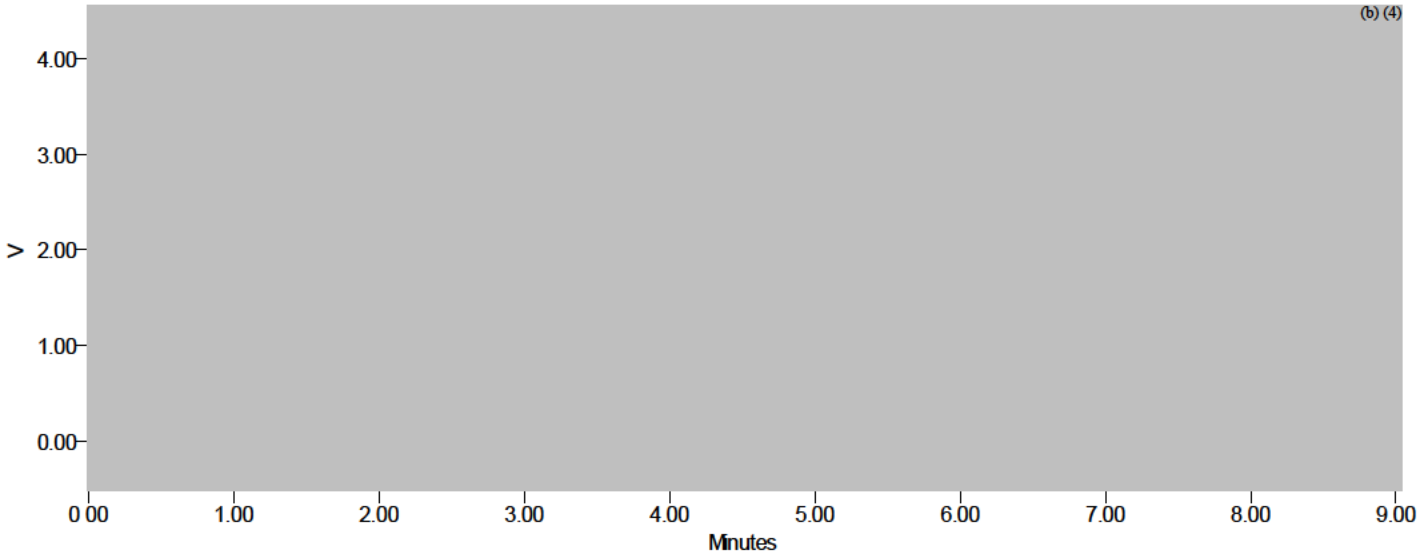
Sample Name:	PSTD2_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_4 Day_2nd
Vial:	61	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_pH 2 7
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 10:29:20 PM KST		
Date Processed:	12/20/2020 10:42:53 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

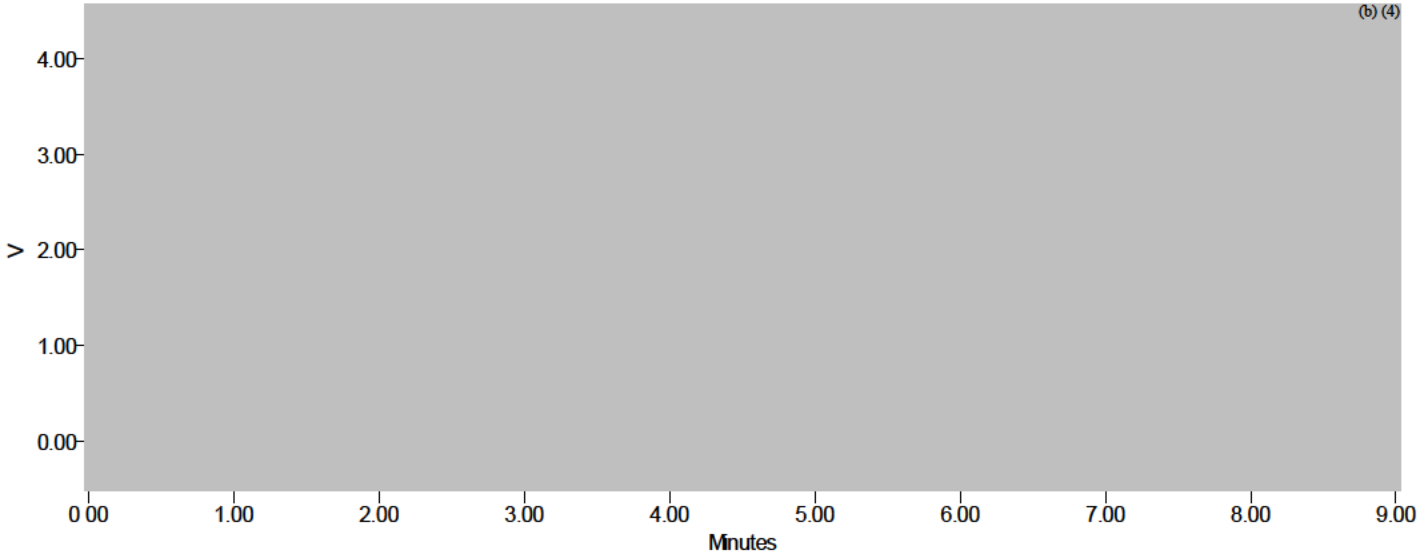
Sample Name:	PSPL2_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_4 Day_2nd
Vial:	62	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_pH 2 7
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 10:39:02 PM KST		
Date Processed:	12/20/2020 10:54:06 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

Sample Name:	PSTD2_4	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_4 Day_2nd
Vial:	63	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_pH 2 7
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/20/2020 10:48:38 PM KST		
Date Processed:	12/20/2020 11:12:11 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

Software: Empower 3 Software Build 3471

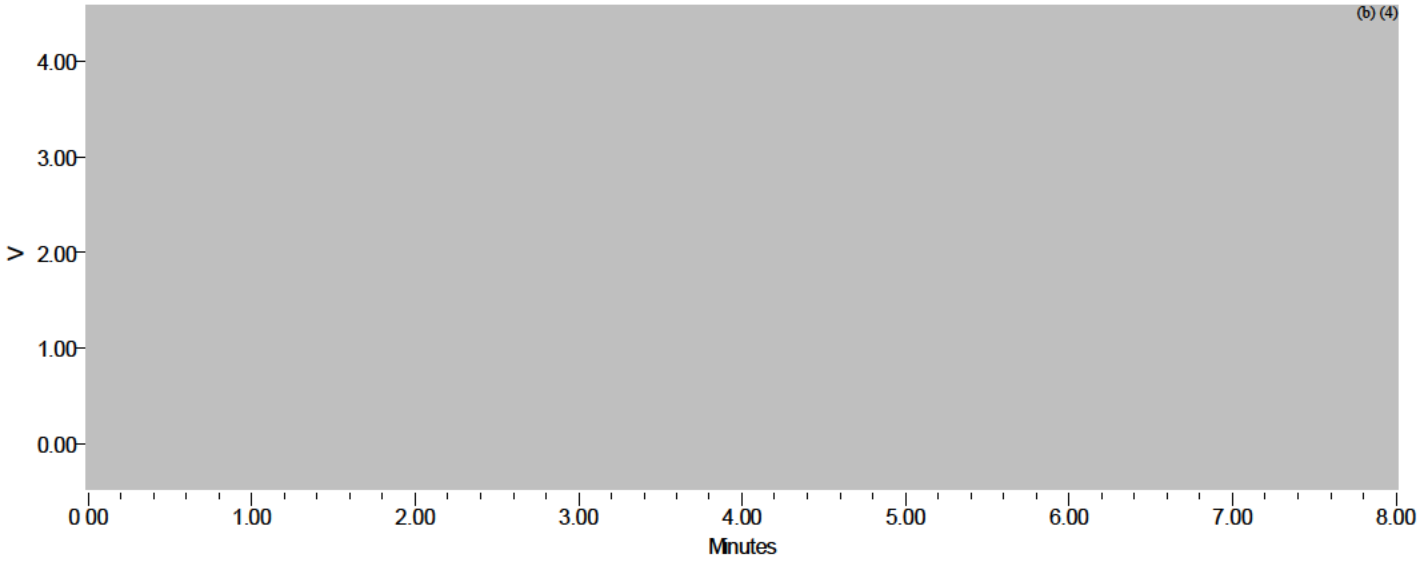
Sample Set Name:	Granule Valine_3 Day	Processed By:	System/Administrator
Sample Set Method:	Granule Valine_3 Day	Printed By:	System
System Node:	Client13	Result Set ID:	
System Name:	UC10	# of Results:	14
Acquired By:	System		
Sample Set Start Date:	12/19/2020 1:16:11 PM KST		
Sample Set Finish Date:	12/19/2020 9:44:54 PM KST		

Sample Set Table

	Sample Name	Sample Type	Vial	Inj #	Injection Volume (ul)	Acquistion Method Set	Processed Channel Descr.	(b) (4)
1	OSTD2_1	Unknown	37	1	5.00	VAL_ACR	Detector A	
2	OSPL2_1	Unknown	38	1	5.00	VAL_ACR	Detector A	
3	OSTD2_2	Unknown	39	1	5.00	VAL_ACR	Detector A	
4	OSPL2_2	Unknown	40	1	5.00	VAL_ACR	Detector A	
5	OSTD2_3	Unknown	41	1	5.00	VAL_ACR	Detector A	
6	OSPL2_3	Unknown	42	1	5.00	VAL_ACR	Detector A	
7	OSTD2_4	Unknown	43	1	5.00	VAL_ACR	Detector A	
8	OSTD1_1	Unknown	44	1	5.00	VAL_ACR	Detector A	
9	OSPL1_1	Unknown	45	1	5.00	VAL_ACR	Detector A	
10	OSTD1_2	Unknown	46	1	5.00	VAL_ACR	Detector A	
11	OSPL1_2	Unknown	47	1	5.00	VAL_ACR	Detector A	
12	OSTD1_3	Unknown	48	1	5.00	VAL_ACR	Detector A	
13	OSPL1_3	Unknown	49	1	5.00	VAL_ACR	Detector A	
14	OSTD1_4	Unknown	50	1	5.00	VAL_ACR	Detector A	

SAMPLE INFORMATION

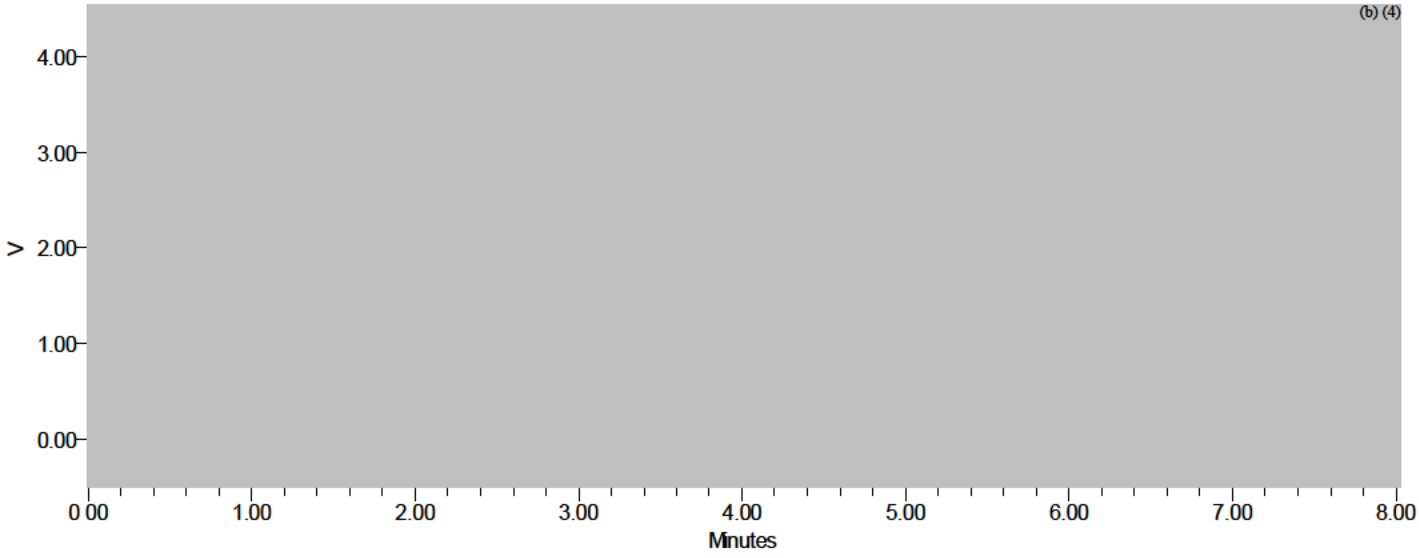
Sample Name:	OSTD2_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day
Vial:	37	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_ACN15%
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	8.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/19/2020 3:57:18 PM KST		
Date Processed:	12/20/2020 5:24:36 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

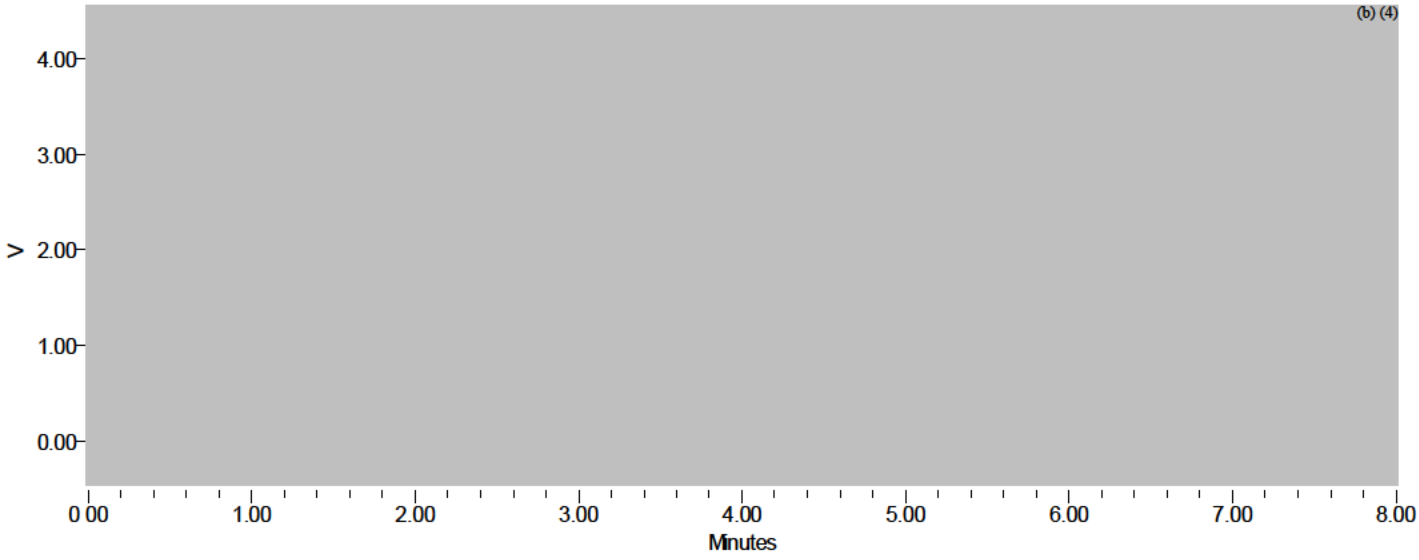
Sample Name:	OSPL2_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day
Vial:	38	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_ACN15%
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	8.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/19/2020 4:05:57 PM KST		
Date Processed:	12/20/2020 5:24:46 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

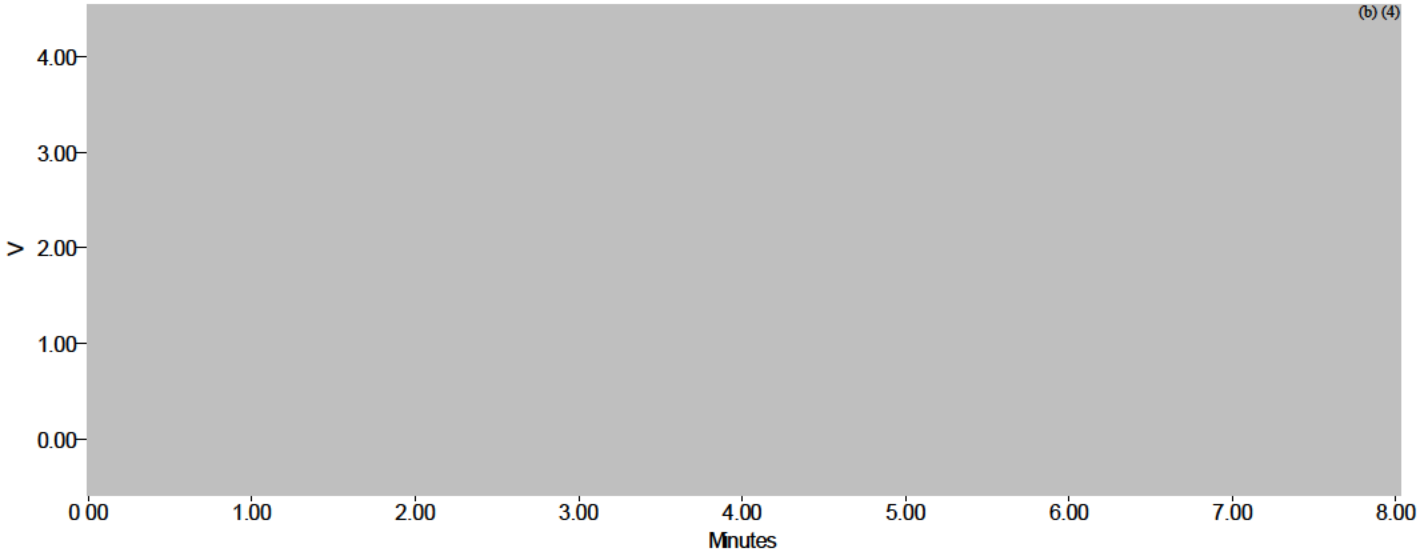
Sample Name:	OSTD2_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day
Vial:	39	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_ACN15%
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	8.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/19/2020 4:14:36 PM KST		
Date Processed:	12/20/2020 5:24:46 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

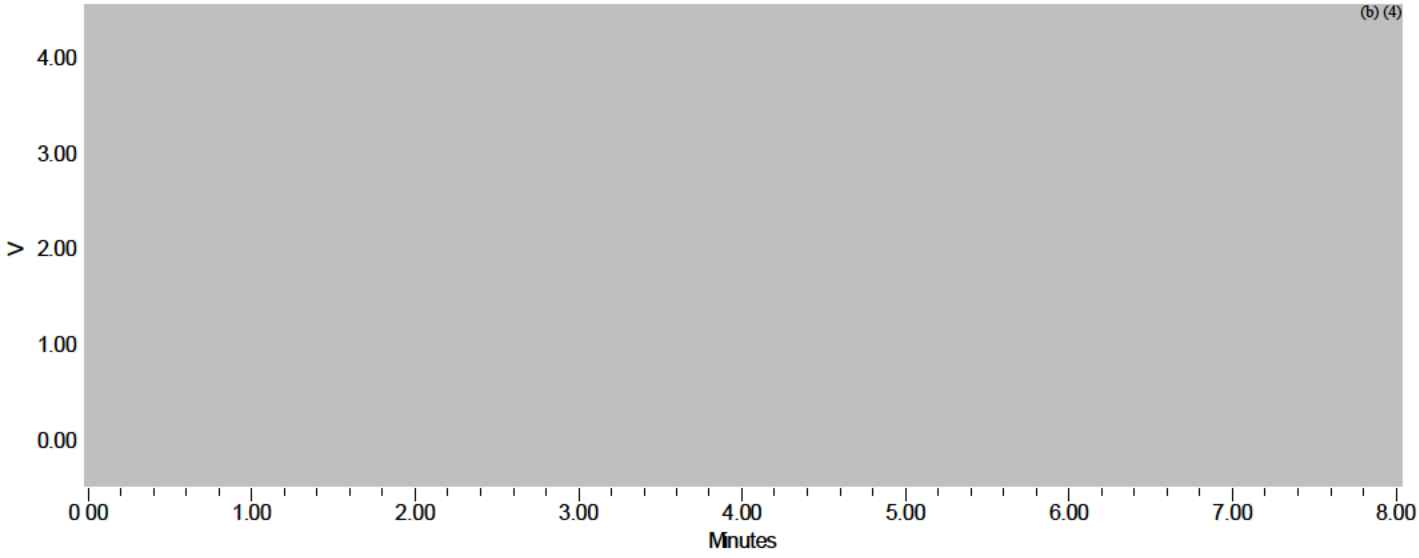
Sample Name:	OSPL2_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day
Vial:	40	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_ACN15%
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	8.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/19/2020 4:23:14 PM KST		
Date Processed:	12/20/2020 5:24:46 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

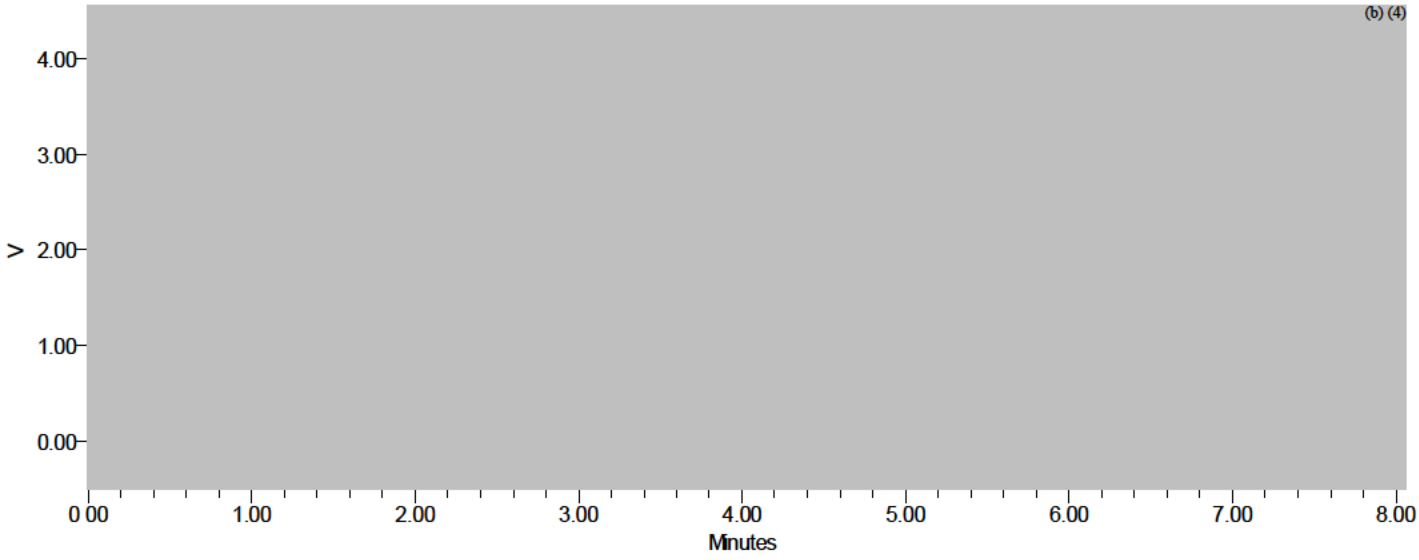
Sample Name:	OSTD2_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day
Vial:	41	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_ACN15%
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	8.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/19/2020 4:31:57 PM KST		
Date Processed:	12/20/2020 5:24:46 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

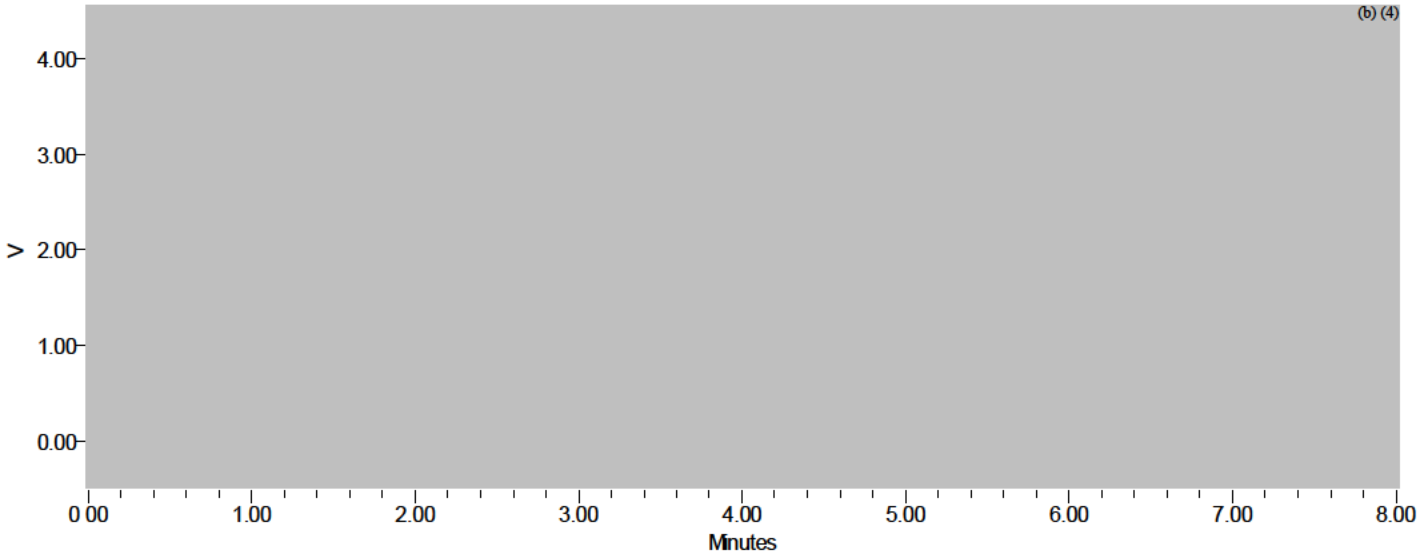
Sample Name:	OSPL2_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day
Vial:	42	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_ACN15%
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	8.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/19/2020 4:40:38 PM KST		
Date Processed:	12/20/2020 5:24:47 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

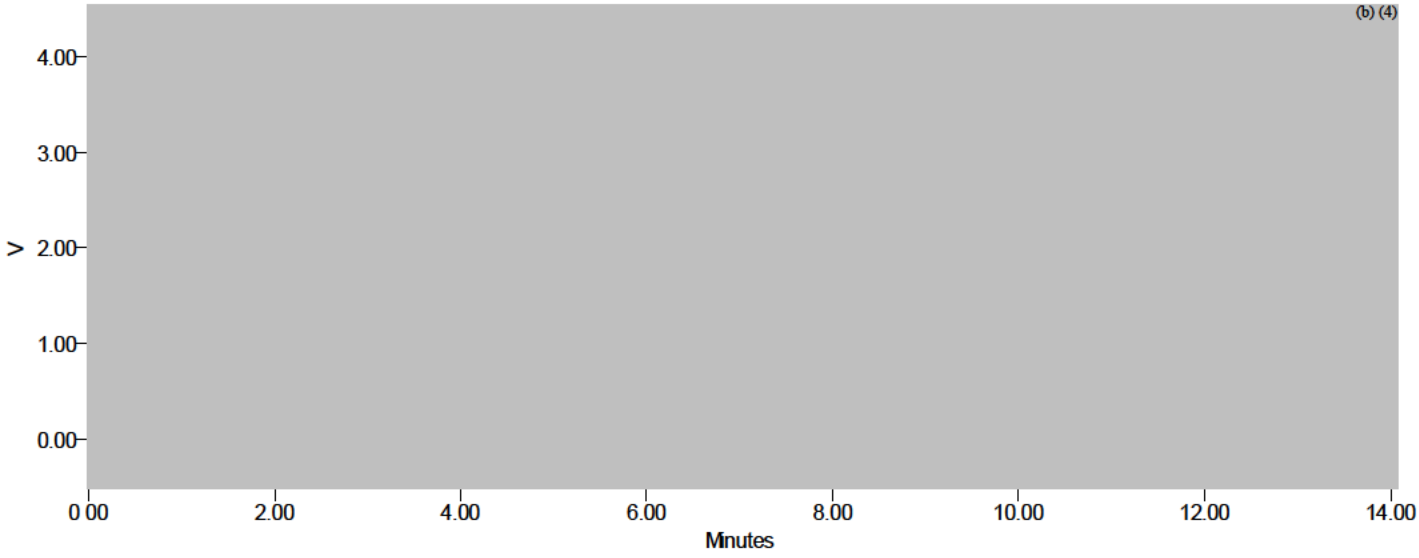
Sample Name:	OSTD2_4	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day
Vial:	43	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_ACN15%
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	8.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/19/2020 4:49:15 PM KST		
Date Processed:	12/20/2020 5:24:47 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

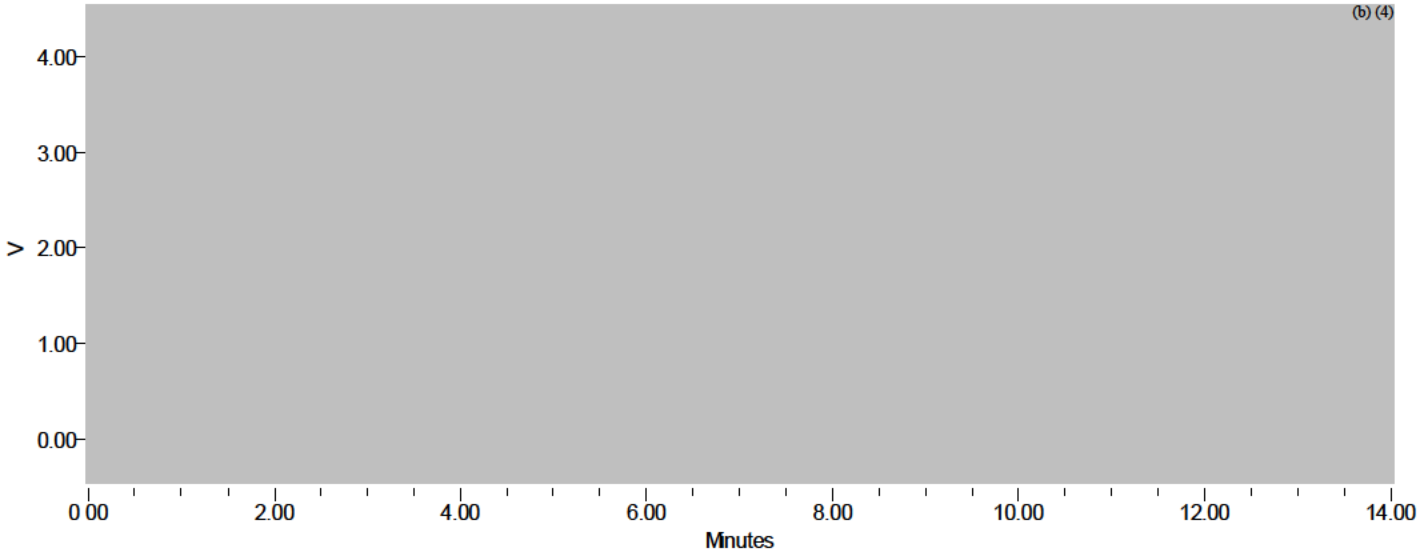
Sample Name:	OSTD1_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day
Vial:	44	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_ACN9%
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	14.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/19/2020 8:02:51 PM KST		
Date Processed:	12/20/2020 5:27:00 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

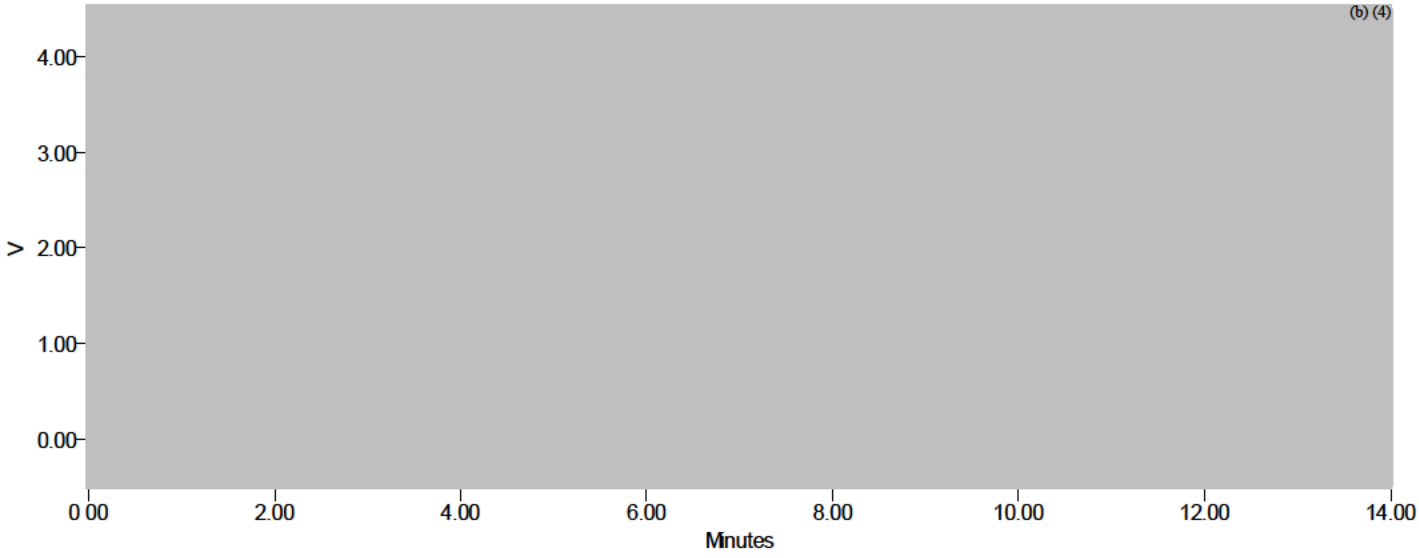
Sample Name:	OSPL1_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day
Vial:	45	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_ACN9%
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	14.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/19/2020 8:17:31 PM KST		
Date Processed:	12/20/2020 5:27:10 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

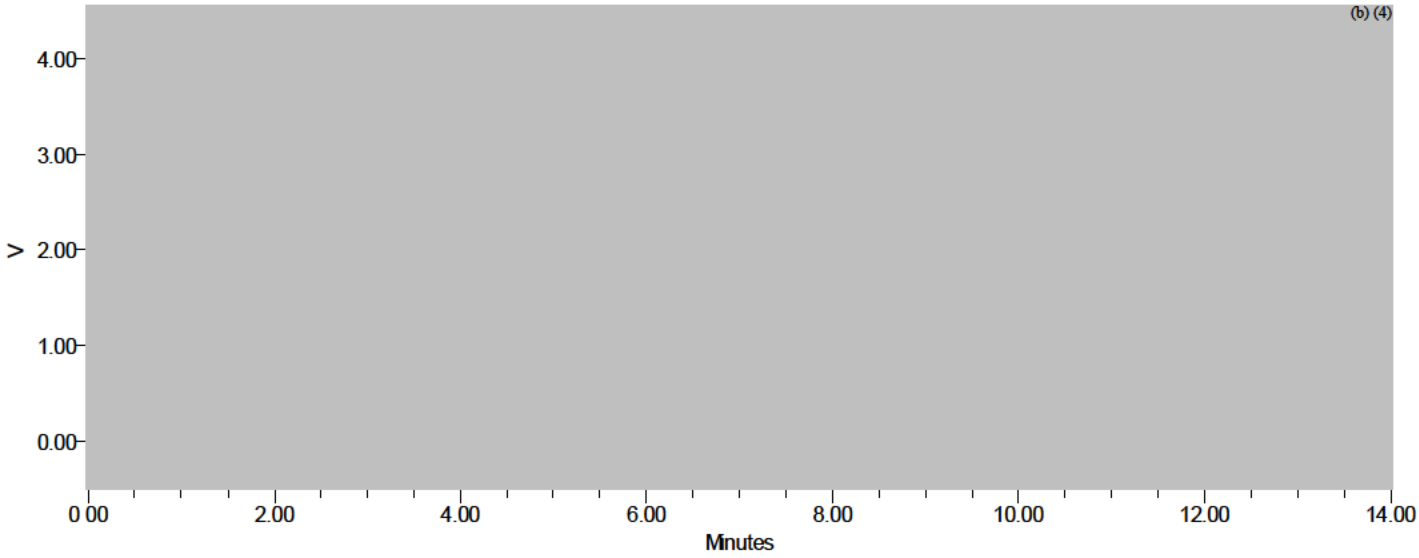
Sample Name:	OSTD1_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day
Vial:	46	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_ACN9%
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	14.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/19/2020 8:32:11 PM KST		
Date Processed:	12/20/2020 5:27:11 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

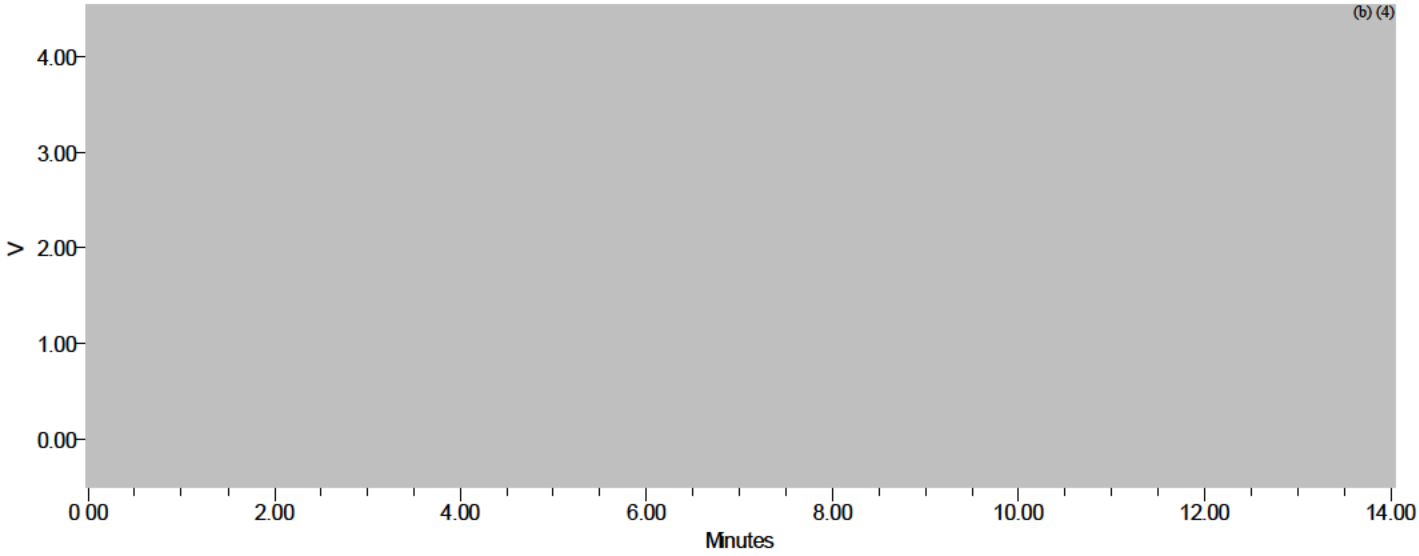
Sample Name:	OSPL1_2	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day
Vial:	47	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_ACN9%
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	14.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/19/2020 8:46:51 PM KST		
Date Processed:	12/20/2020 5:27:11 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

Sample Name:	OSTD1_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day
Vial:	48	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_ACN9%
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	14.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/19/2020 9:01:32 PM KST		
Date Processed:	12/20/2020 5:27:11 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

Sample Name:	OSPL1_3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day
Vial:	49	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_ACN9%
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	14.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
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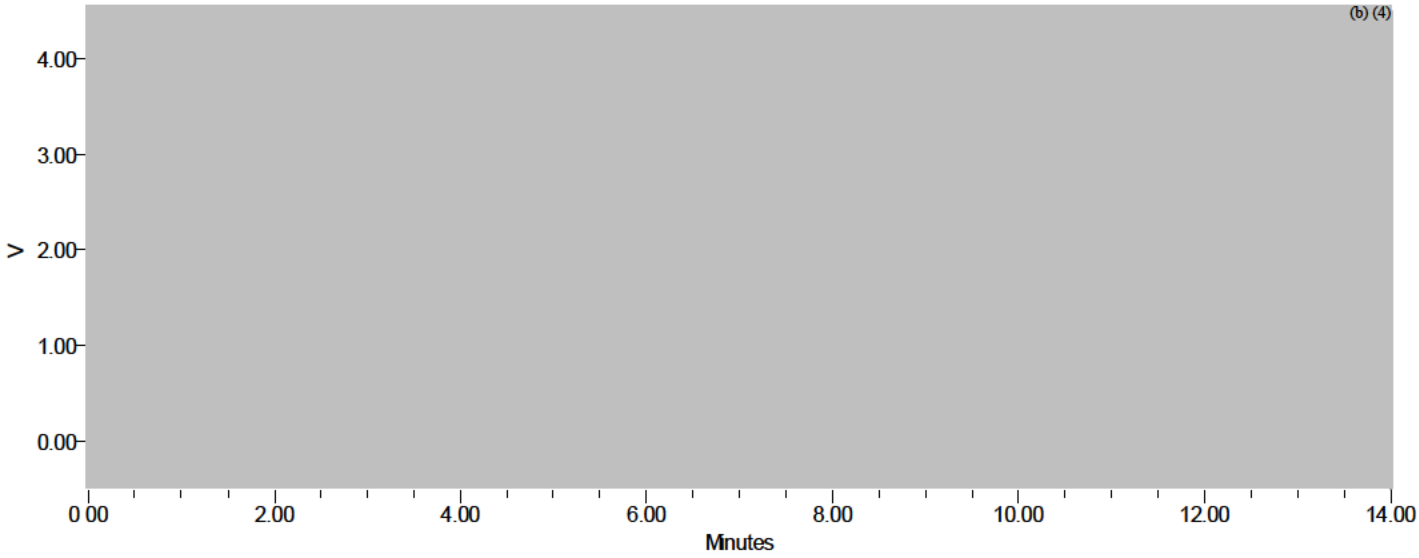
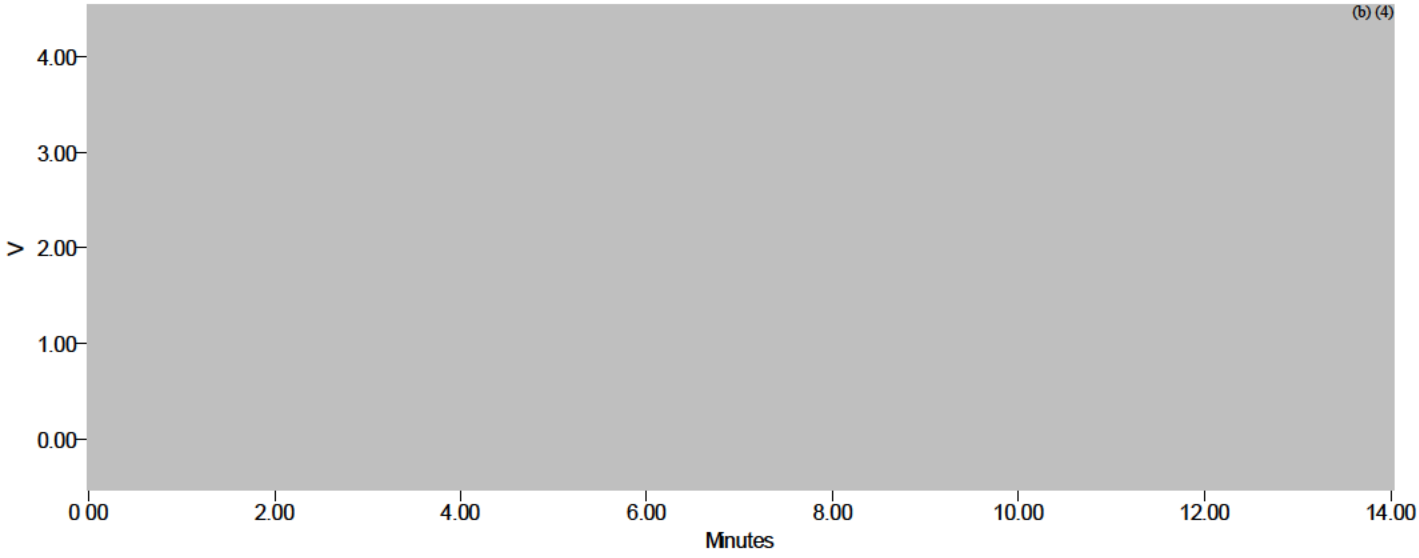


	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

SAMPLE INFORMATION

Sample Name:	OSTD1_4	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day
Vial:	50	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Valine_ACN9%
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	14.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
Date Acquired:	12/19/2020 9:30:49 PM KST		
Date Processed:	12/20/2020 5:27:12 PM KST		



	Peak Name	RT	Area	USP Plate Count
1	Valine	(b) (4)		

Software: Empower 3 Software Build 3471

Sample Set Name:	Granule Valine_2 Day	Processed By:	System/Administrator
Sample Set Method:	Granule Valine_2 Day	Printed By:	System
System Node:	Client13	Result Set ID:	
System Name:	UC10	# of Results:	4
Acquired By:	System		
Sample Set Start Date:	12/18/2020 12:24:43 PM KST		
Sample Set Finish Date:	12/19/2020 6:05:57 AM KST		

Sample Set Table

	Sample Name	Sample Type	Vial	Inj #	Injection Volume (ul)	Acquistion Method Set	Processed Channel Descr.	
1	NH4OH	Unknown	96	1	5.00	VAL_ACR	Detector A	(b) (4)
2	Glutamic acid	Unknown	73	1	5.00	VAL_ACR	Detector A	
3	Alanine	Unknown	74	1	5.00	VAL_ACR	Detector A	
4	Glycine	Unknown	76	1	5.00	VAL_ACR	Detector A	

SAMPLE INFORMATION

Sample Name:	Glutamic acid	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_2 Day
Vial:	73	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Glutamic acid
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
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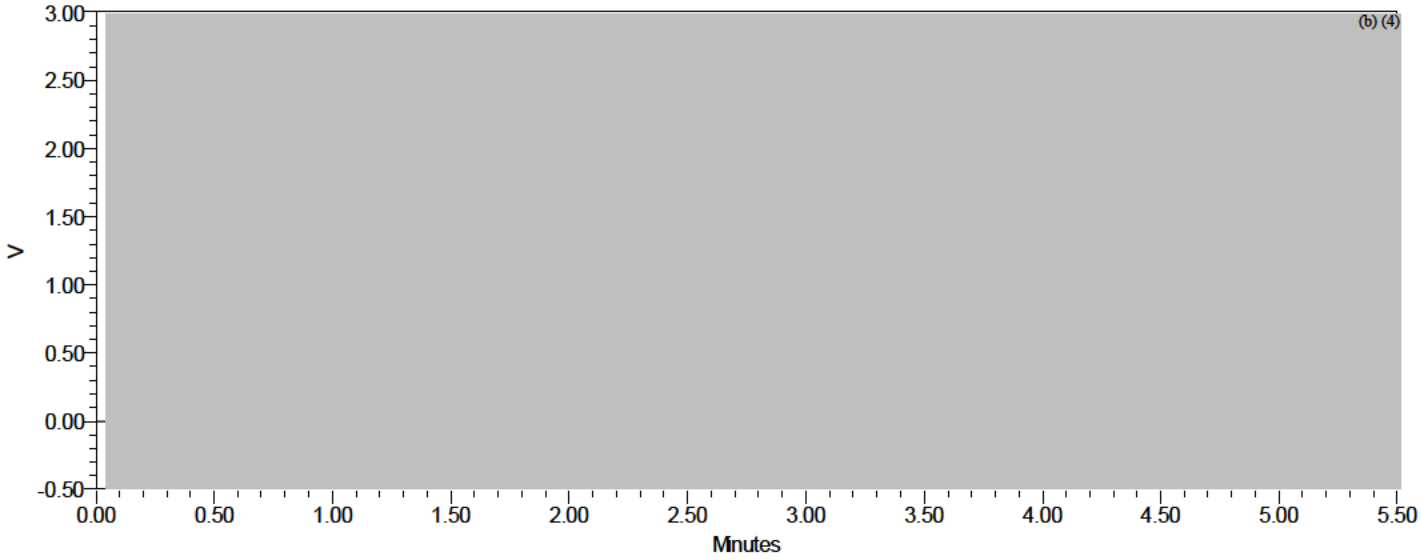

	Peak Name	RT	Area	USP Plate Count
1	Glutamic acid	(b) (4)		

SAMPLE INFORMATION

Sample Name:	Alanine	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_2 Day
Vial:	74	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Alanine
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
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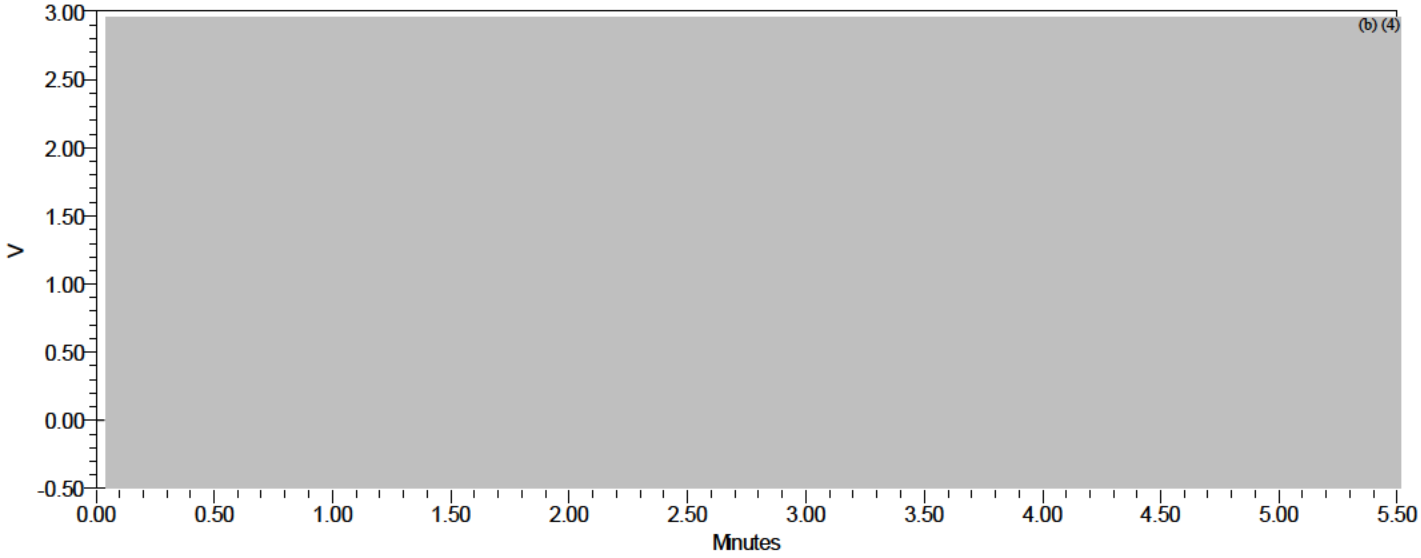

	Peak Name	RT	Area	USP Plate Count
1	Alanine	(b) (4)		

SAMPLE INFORMATION

Sample Name:	Glycine	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_2 Day
Vial:	76	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Glycine
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
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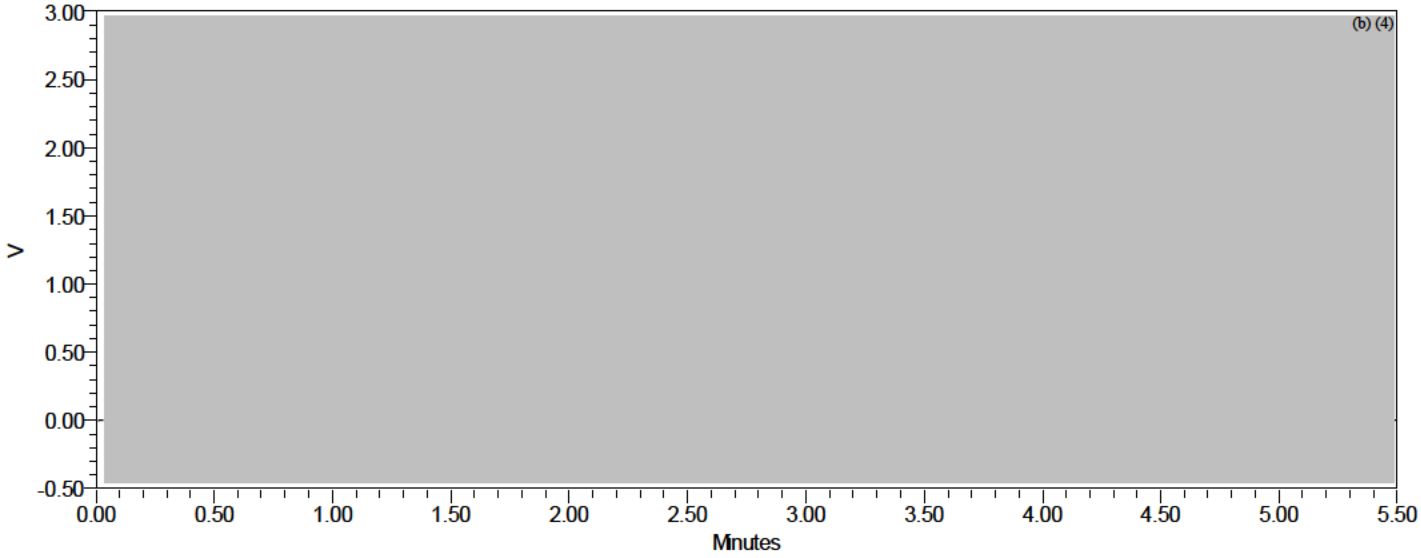

	Peak Name	RT	Area	USP Plate Count
1	Glycine	(b) (4)		

SAMPLE INFORMATION

Sample Name:	NH4OH	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_2 Day
Vial:	96	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Ammonia
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
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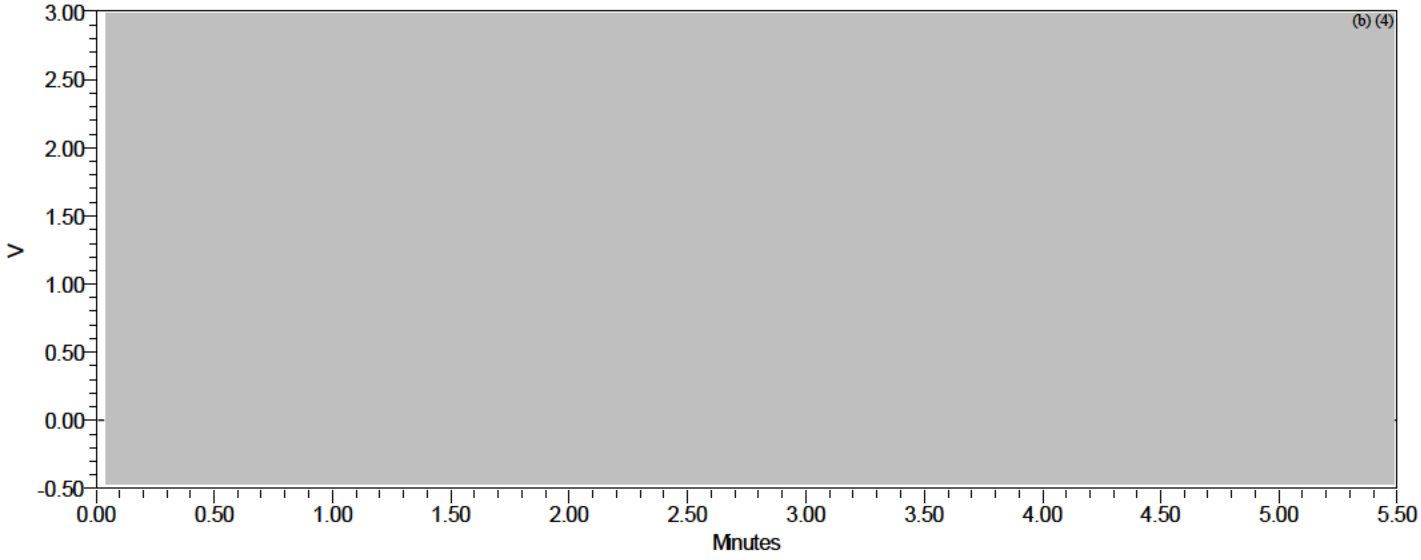

	Peak Name	RT	Area	USP Plate Count
1	Ammonia	(b) (4)		

Software: Empower 3 Software Build 3471

Sample Set Name:	Granule Valine_3 Day	Processed By:	System/Administrator
Sample Set Method:	Granule Valine_3 Day	Printed By:	System
System Node:	Client13	Result Set ID:	
System Name:	UC10	# of Results:	4
Acquired By:	System		
Sample Set Start Date:	12/19/2020 1:16:11 PM KST		
Sample Set Finish Date:	12/19/2020 9:44:54 PM KST		

Sample Set Table

	Sample Name	Sample Type	Vial	Inj #	Injection Volume (ul)	Acquistion Method Set	Processed Channel Descr.	
1	Glutamic acid	Unknown	32	1	5.00	VAL_ACR	Detector A	(b) (4)
2	Alanine	Unknown	33	1	5.00	VAL_ACR	Detector A	
3	Glycine	Unknown	34	1	5.00	VAL_ACR	Detector A	
4	NH4OH	Unknown	35	1	5.00	VAL_ACR	Detector A	

SAMPLE INFORMATION

Sample Name:	Glutamic acid	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day
Vial:	32	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	GA_ACN
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	14.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
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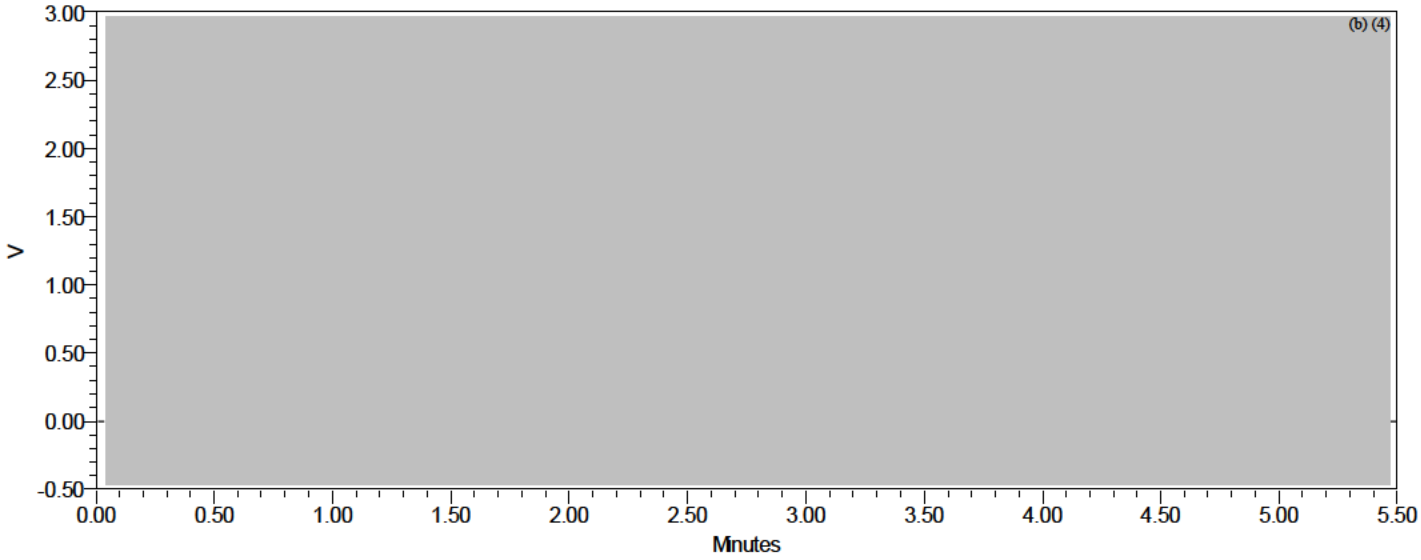

	Peak Name	RT	Area	USP Plate Count
1	Glutamic acid	(b) (4)		

SAMPLE INFORMATION

Sample Name:	Alanine	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day
Vial:	33	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Alanin_ACN
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	8.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
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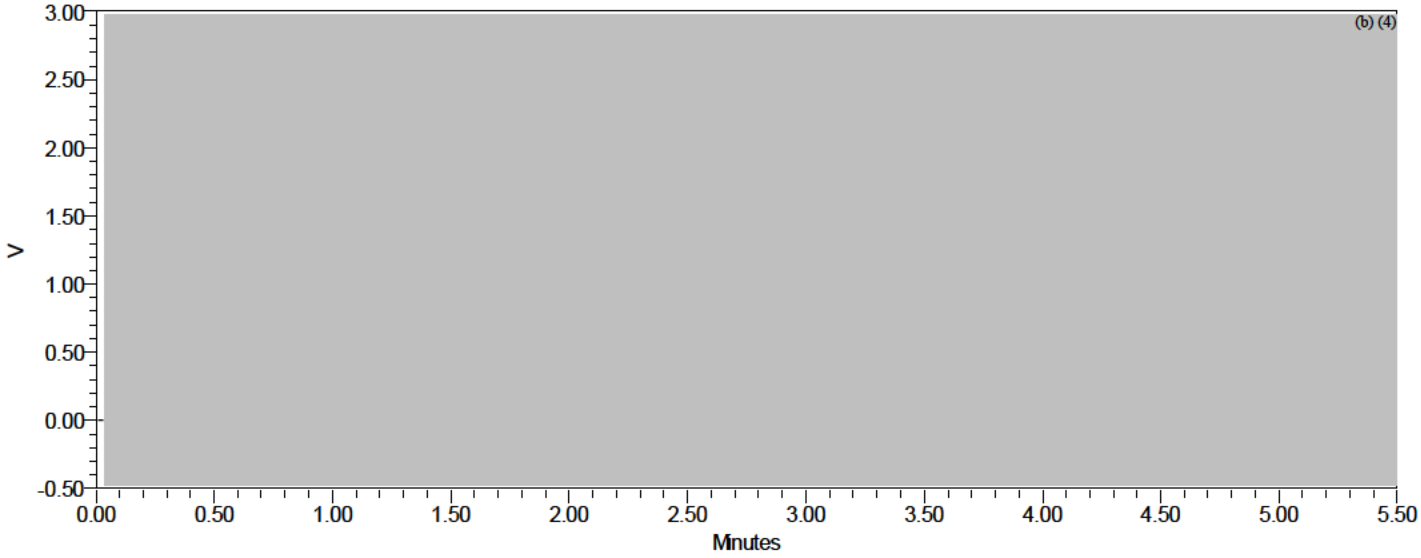

	Peak Name	RT	Area	USP Plate Count
1	Alanine	(b) (4)		

SAMPLE INFORMATION

Sample Name:	Glycine	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day
Vial:	34	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Glycine_ACN
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	8.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
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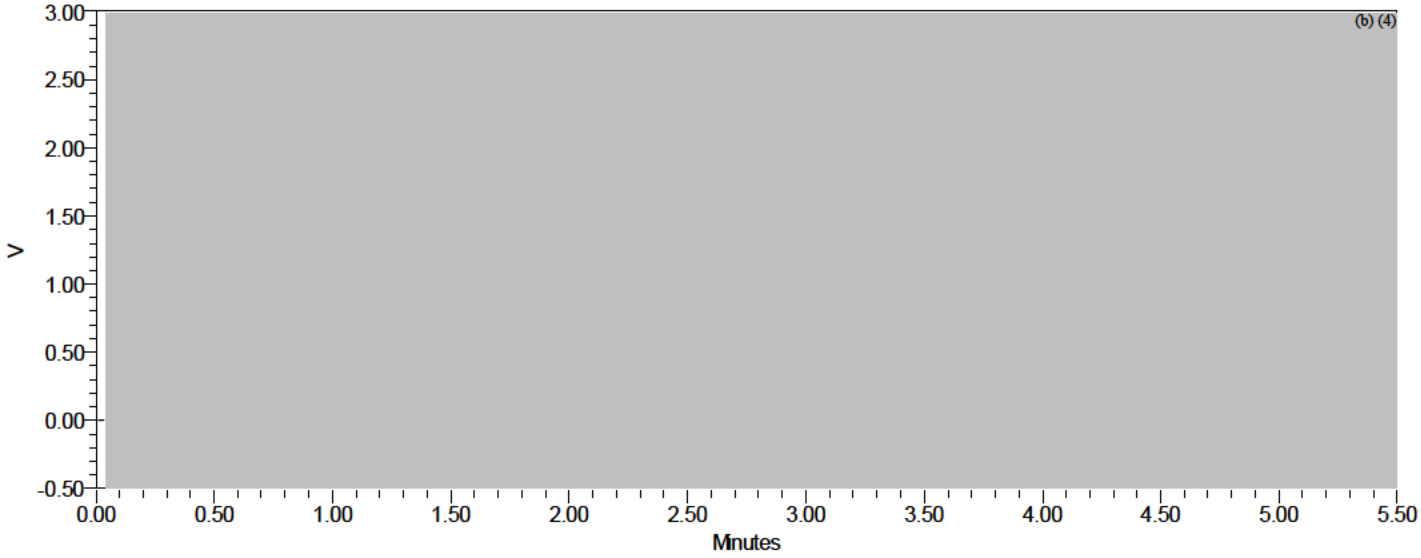

	Peak Name	RT	Area	USP Plate Count
1	Glycine	(b) (4)		

SAMPLE INFORMATION

Sample Name:	NH4OH	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day
Vial:	35	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Ammonia_ACN
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	8.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
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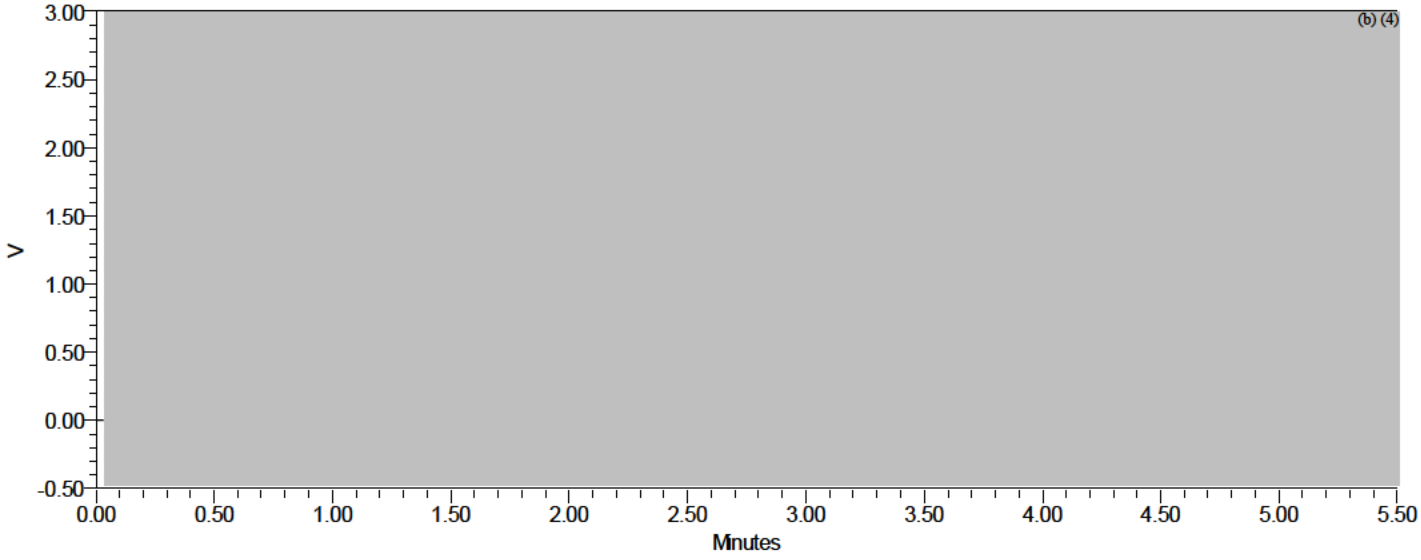

	Peak Name	RT	Area	USP Plate Count
1	Ammonia	(b) (4)		

Software: Empower 3 Software Build 3471

Sample Set Name:	Granule Valine_2 Day, Granule	Processed By:	System/Administrator
Sample Set Method:	Granule Valine_2 Day, Granule	Printed By:	System
System Node:	Client13	Result Set ID:	
System Name:	UC10	# of Results:	2
Acquired By:	System		
Sample Set Start Date:	12/18/2020 12:24:43 PM KST, 12/19/2020 1:16:11 PM KST		
Sample Set Finish Date:	12/19/2020 6:05:57 AM KST, 12/19/2020 9:44:54 PM KST		

Sample Set Table

	Sample Name	Sample Type	Vial	Inj #	Injection Volume (ul)	Acquistion Method Set	Processed Channel Descr.	
1	H1_1	Unknown	78	1	5.00	VAL_ACR	Detector A	(b) (4)
2	OSPL1_1	Unknown	45	1	5.00	VAL_ACR	Detector A	

SAMPLE INFORMATION

Sample Name:	H1_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_2 Day
Vial:	78	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Unknown ID_normal
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	9.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
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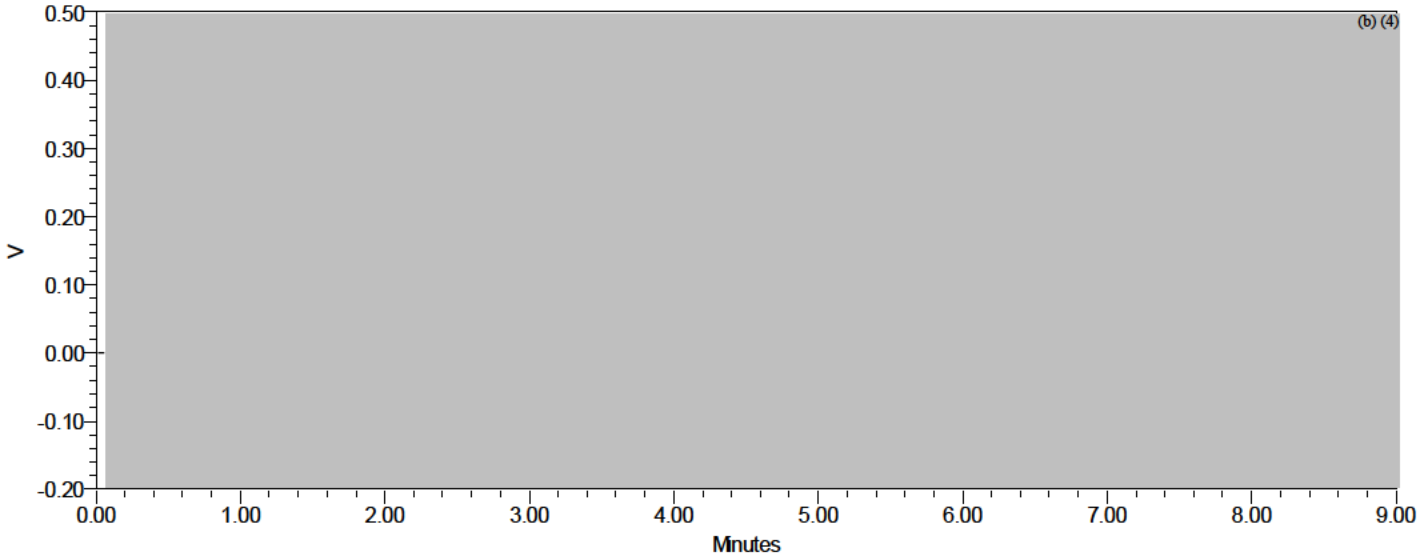

	Peak Name	RT	Area	USP Plate Count
1	Glycine+Glutamic acid	(b) (4)		
2	Alanine			
3	Ammonia			
4	Valine			

SAMPLE INFORMATION

Sample Name:	OSPL1_1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Granule Valine_3 Day
Vial:	45	Acq. Method Set:	VAL_ACR
Injection #:	1	Processing Method:	Unknown ID_ACN
Injection Volume:	5.00 ul	Channel Name:	Detector A
Run Time:	14.0 Minutes	Proc. Chnl. Descr.:	Detector A (b) (4)
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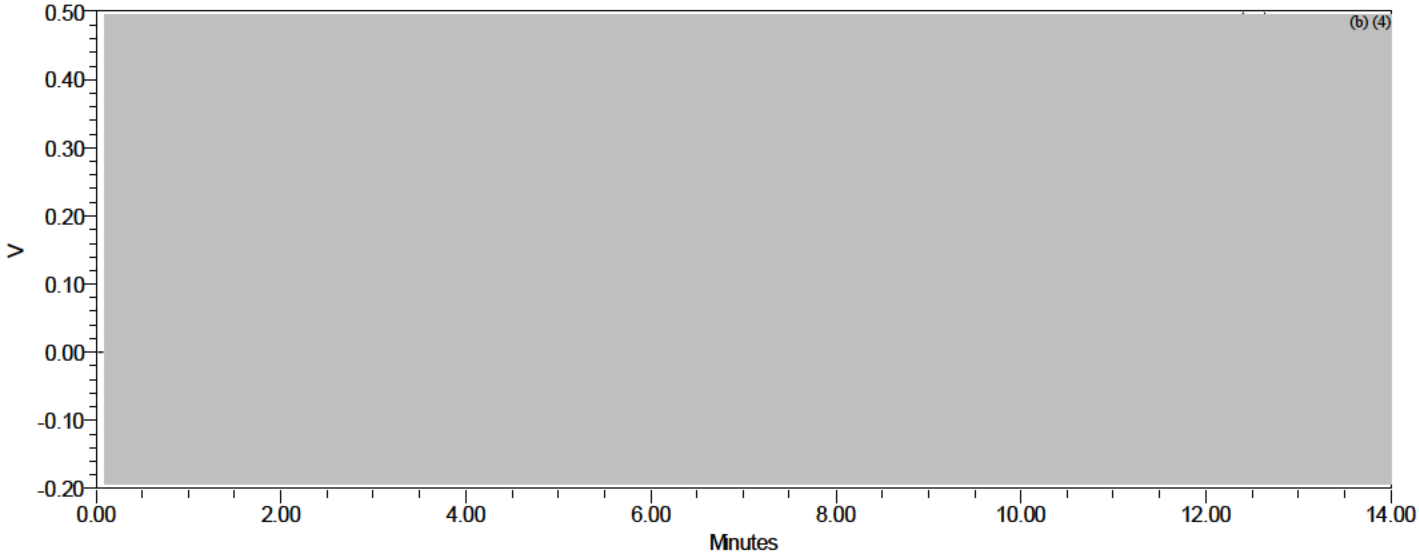


	Peak Name	RT	Area	USP Plate Count
1	Glycine	(b) (4)		
2	Glutamic acid			
3	Alanine			
4	Ammonia			
5	Valine			

Software: Empower 3 Software Build 3471

CONFIDENTIAL REPORT

Determination of antibiotic minimum inhibitory concentration (MIC) of *C. glutamicum* KCCM 80240

Version 1.0

TITLE

Determination of antibiotic minimal inhibitory concentration (MIC) of *Corynebacterium glutamicum* KCCM 80240

OBJECTIVE OF THE STUDY

This study was conducted to determine MIC of Val Pro producing strain *C. glutamicum* KCCM 80240.

SCHEDULE OF THE STUDY

Initiation of experiment: September 8, 2020

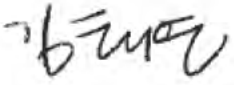
Termination of experiment: September 18, 2020

Submission of final report: December 31, 2020

TESTING FACILITY

Institute of Biotechnology) Scientific and Regulatory Affairs, CJ CheilJedang
55, Gwanggyo-ro 42beon-gil, Yeongtong-gu, Suwon-si, Gyeonggi-do 16495, Korea

RESPONSIBLE STAFFS

Analyst and Author	Taeyeon Kim	
Report approved by	Yang Hee Kim	

CONTENTS

INTRODUCTION

Corynebacterium glutamicum KCCM 80240 used as a production microorganism of L-valine. In order to observe antimicrobial susceptibility, minimum inhibitory concentration (MIC) test of *C. glutamicum* KCCM 80240 was conducted by comparing with wild-type strain *C. glutamicum* ATCC 14067 and parent strain, the NTG-treated strain, *C. glutamicum* CA08-0012. The two fold serial dilutions procedure in broth with antibiotics were used and the MIC is determined as the lowest concentration of the antibiotics that inhibits bacterial growth.

MATERIALS AND METHODS

The MIC of production strain was evaluated in accordance with the broth microdilution method given in the Clinical and Laboratory Standards Institute (CLSI) guideline [1].

Preparation of antibiotics solution

(b) (4)

(b) (4)

Preparation of inoculum

(b) (4)

Broth microdilution method

(b) (4)

RESULTS

(b) (4)

Table 1. Antimicrobial susceptibility of ATCC 14067, CA08-0012 and KCCM 80240

Antibiotics	MIC ($mg \cdot L^{-1}$)			Interpretation[a]		
	ATCC 14067	CA08-0012	KCCM 80240	ATCC 14067	CA08-0012	KCCM 80240
Ampicillin	(b) (4)					
Vancomycin						
Gentamycin						
Kanamycin						
Streptomycin						
Erythromycin						
Clindamycin						
Tetracycline						
Chloramphenicol						

(b) (4)

As shown in Table 1, the cell growth inhibition of production strain was observed in susceptible range. Therefore, it can be conclude that antimicrobial susceptibility by genetic modification is not occurred.

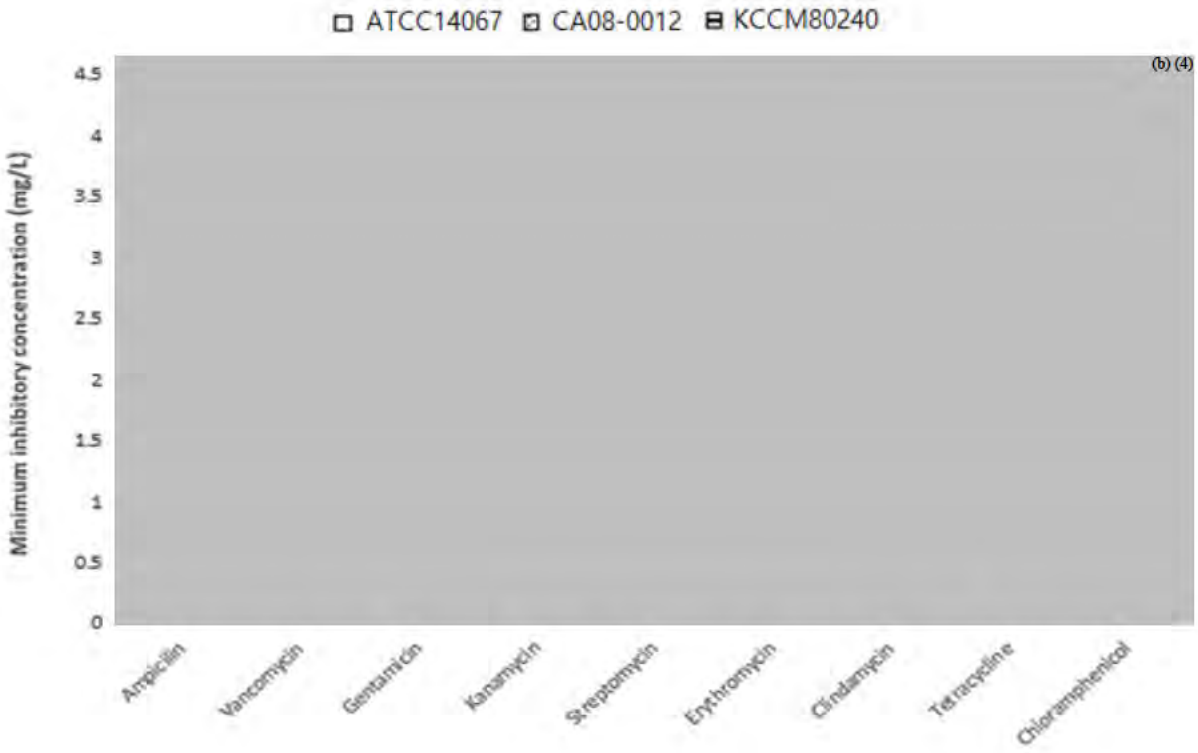


Figure 1. Graph representing the minimum inhibitory concentration (MIC) of *C. glutamicum* ATCC 14067 (wild-type strain), *C. glutamicum* CA08-0012 (parent strain) and *C. glutamicum* KCCM 80240 (L-valine production strain) measured against different antibiotics.

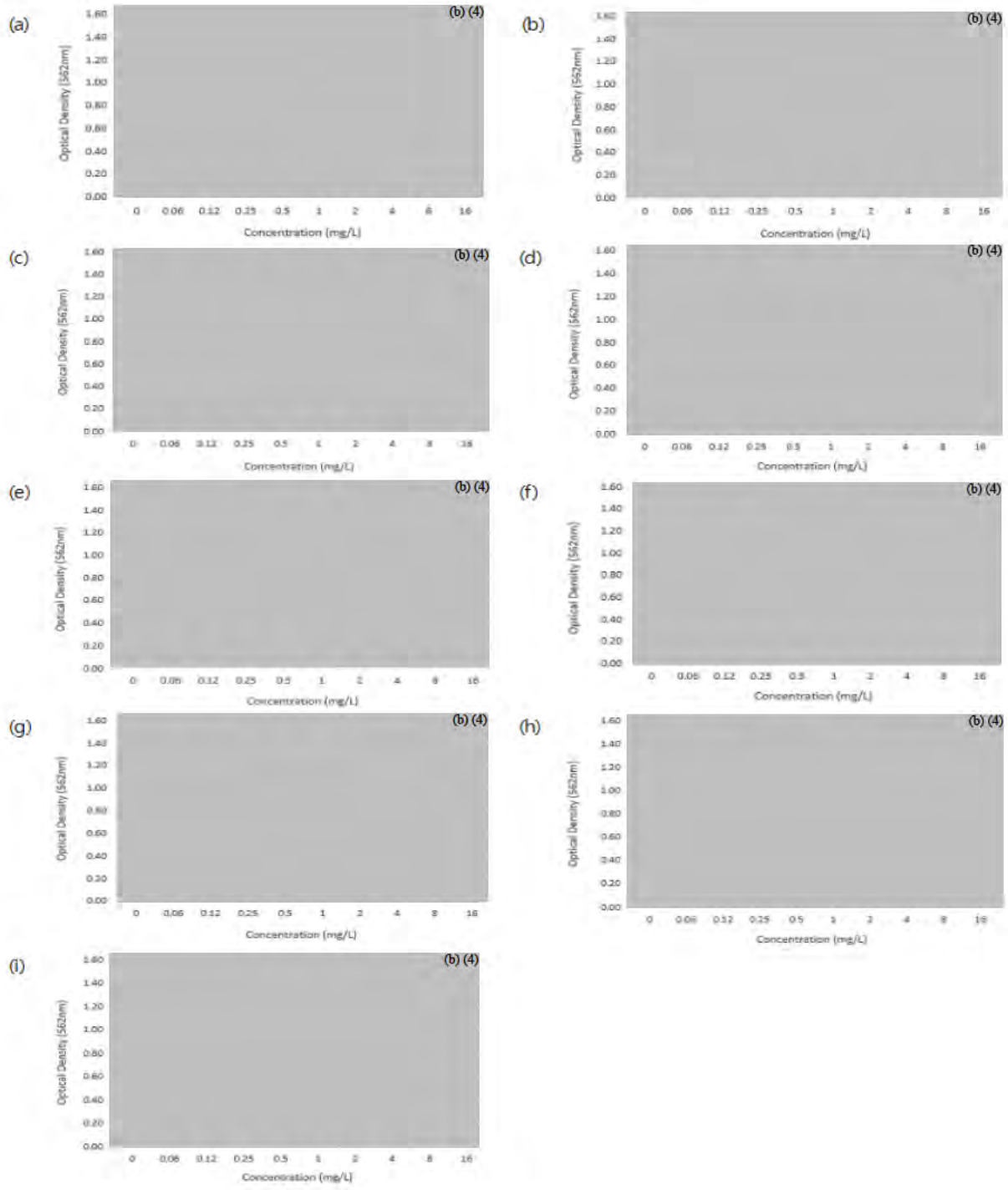


Figure 2. Effect of (a) ampicillin, (b) vancomycin, (c) gentamicin (d) kanamycin, (e) streptomycin, (f) erythromycin, (g) clindamycin, (h) tetracycline and (i) chloramphenicol on production strain (●: ATCC 14067, ■: CA08-0012, ○: KCCM 80240) growth.

REFERENCES

[1] Clinical and Laboratory Standards Institute. 2012. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard—9th ed. CLSI document M07-A9. Clinical and Laboratory Standards Institute, Wayne, PA

[2] EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2018. Guidance on the characterisation of microorganisms used as feed additives or as production. EFSA Journal, 16(3), 5206. DOI: 10.2903/j.efsa.2018.5206. Available online: https://efsa.onlinelibrary.wiley.com/doi/full/10.2903/j.efsa.2018.5206.

CONFIDENTIAL REPORT

Determination of viable cells of the production strain in Dried L-Valine Fermentation Product

Version 1.0

TITLE

Determination of viable cells of the production strain in Dried L-Valine Fermentation Product

OBJECTIVE OF THE STUDY

This study was conducted to determine the viable cells of the production strain *Corynebacterium glutamicum* KCCM 80240 in the final product and manufacturing process.

SCHEDULE OF THE STUDY

Initiation of experiment: October 23, 2020

Termination of experiment: November 5, 2020

Submission of final report: December 31, 2020

TESTING FACILITY

Institute of Biotechnology) Scientific and Regulatory Affairs, CJ CheilJedang
55, Gwanggyo-ro 42beon-gil, Yeongtong-gu, Suwon-si, Gyeonggi-do 16495, Korea

RESPONSIBLE STAFFS

Analyst and Author	Taeyeon Kim	
Report approved by	Yang Hee Kim	

CONTENTS

INTRODUCTION

Corynebacterium glutamicum KCCM 80240 is a production microorganism to produce L-valine as a fermentation product. In accordance with EFSA guidance on microorganism used as feed additives or as production organisms, the absence of the production strain in the final product should be investigated for safety aspects [1]. In order to confirm the absence of viable cells in the final products, the membrane filtration method was used.

MATERIALS AND METHODS

Test sample

(A) Detection of viable cells in the final product

Three independent batches of Dried L-Valine Fermentation Product were tested to analyse the existence of viable cell. The certificate of analysis of test samples are attached as Appendix 1.

- Batch No. : GVAL200910, GVAL200911, GVAL200912

(B) Detection of viable cells in the manufacturing process

Samples were taken from the representative step of manufacturing process to determine the existence of viable cells. The sampling point from the manufacturing process is divided into five steps: fermentation, pH adjustment, biomass inactivation, concentration and final product. Details of sampling point is marked in Appendix 2 of this report.

Limit of detection test

Sample analysis

(b) (4)

Control test

(b) (4)

Spike test

(b) (4)

(b) (4)

RESULTS

Determination of limit of detection of analysis

(b) (4)

Table 1. LOD of viable cell test (Number of viable cells in culture broth=1.0×10^9 CFU mL^{-1})

Strain	Dilution fold	Number of viable cells(CFU mL^{-1})
C. glutamicum KCCM 80240	(b) (4)	

(b) (4)

Viable cell test

(b) (4)

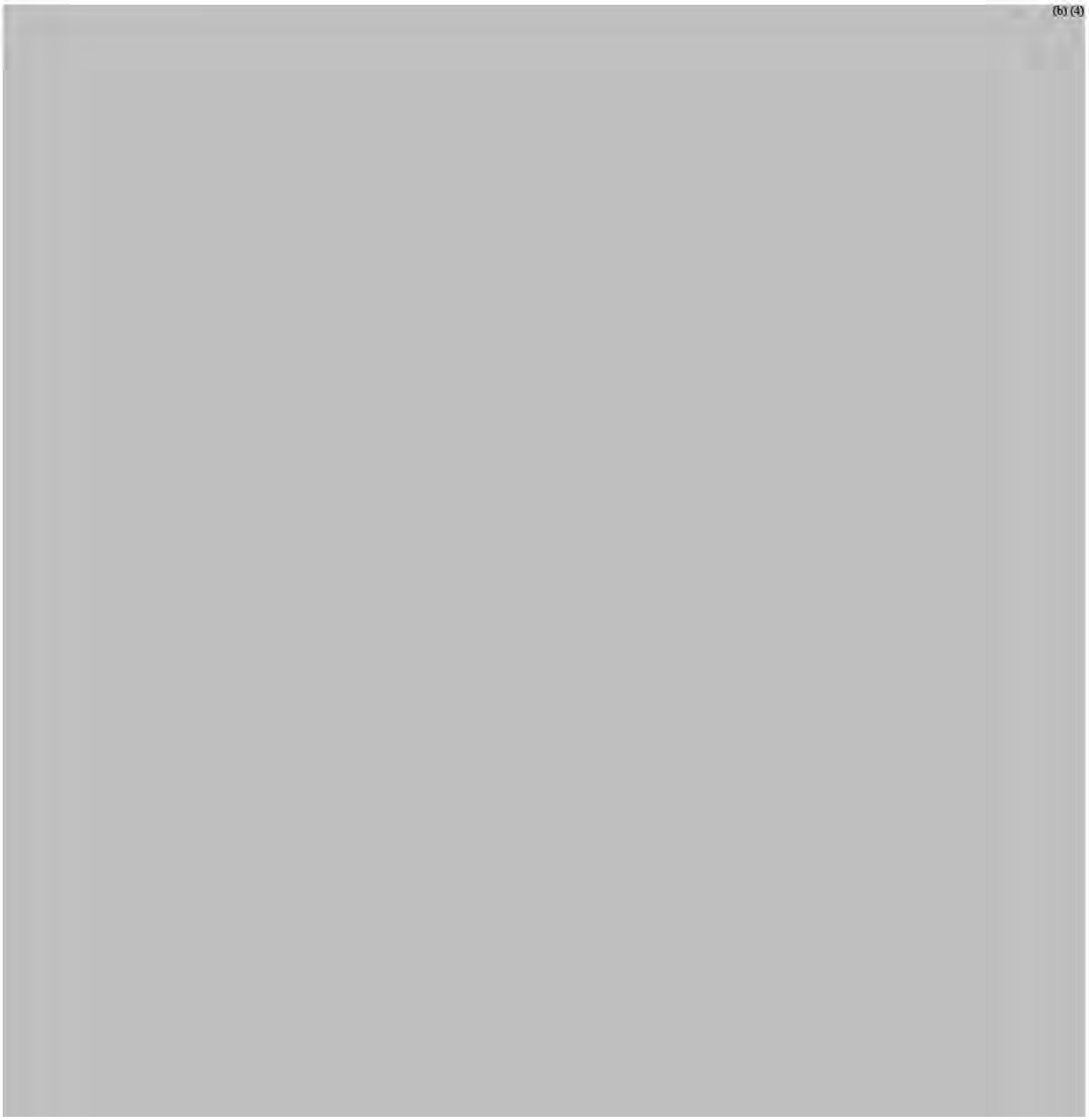
(b) (4)

(A) Detection of viable cells in the final product

(b) (4)

Table 2. Number of viable cells in Dried L-Valine Fermentation Product

Product	Batch number	Number of viable cell (CFU g^{-1})		
		1st analysis	2nd analysis	3rd analysis
Dried L-Valine Fermentation Product	GVAL200910	(b) (4)		
	GVAL200911			
	GVAL200912			

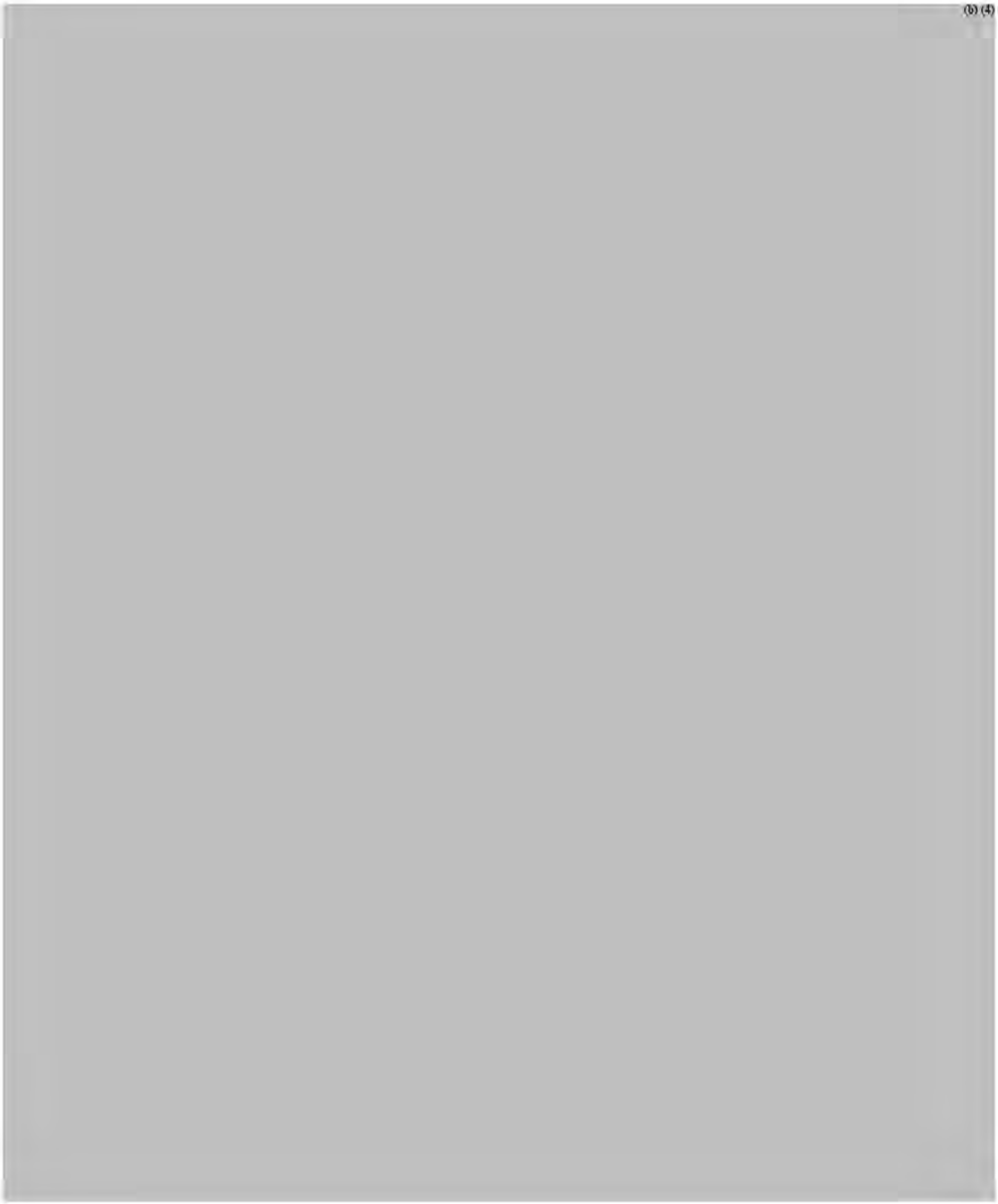

(b) (4)

(B) Detection of viable cells in the manufacturing process

(b) (4)

Table 3. Number of viable cells in Dried L-Valine Fermentation Product manufacturing process

	Number of viable cell (CFU mL^{-1})		
	1st analysis	2nd analysis	3rd analysis
Fermentation	(b) (4)		
pH adjustment			
Cell inactivation			
Concentration			
Product (CFU g^{-1})			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[APPENDIX 1] Certificate of Analysis

GVAL200910

CJ Research Institute of Biotechnology 55, Gwanggyo-ro 42beon-gil, Yeongtong-gu, Suwon-si, Gyeonggi-do, 16495, Korea www.cj.co.kr TEL : 031) 8099-2450 FAX : 031) 8099-2918	CJ CHEILJEDANG BIO

Certificate of analysis

Certificate No.	2020-PR-132	Receipt No.	2020-AN-106
Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.11.23.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.10.
Lot. No	GVAL200910
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 72 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

The Test Report cannot be reproduced, except in full.

Tested by (b) (4)

Approved by Technical Manager

Dec, 23, 2020

CJ Research Institute of Biotechnology

CJ BIO-AD form 100-01 REV.01

GVAL200911

CJ Research Institute of Biotechnology

55, Gwanggyo-ro 42beon-gil, Yeongtong-gu, Suwon-si, Gyeonggi-do, 16495, Korea

www.cj.co.kr

TEL : 031) 8099-2450 FAX : 031) 8099-2918

Certificate of analysis

Certificate No.	2020-PR-133	Receipt No.	2020-AN-107
Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.11.23.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.11.
Lot. No	GVAL200911
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 72 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.
The Test Report cannot be reproduced, except in full.

Tested by (b) (4)

Approved by Technical Manager (b) (4)

Dec, 23, 2020

CJ Research Institute of Biotechnology

CJ BIO-AD form 100-01 REV.01

GVAL200912

CJ Research Institute of Biotechnology

55, Gwanggyo-ro 42beon-gil, Yeongtong-gu, Suwon-si, Gyeonggi-do, 16495, Korea

www.cj.co.kr

TEL : 031) 8099-2450 FAX : 031) 8099-2918

Certificate of analysis

Certificate No.	2020-PR-134	Receipt No.	2020-AN-108
Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.11.23.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.12.
Lot. No	GVAL200912
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 72 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

The Test Report cannot be reproduced, except in full.

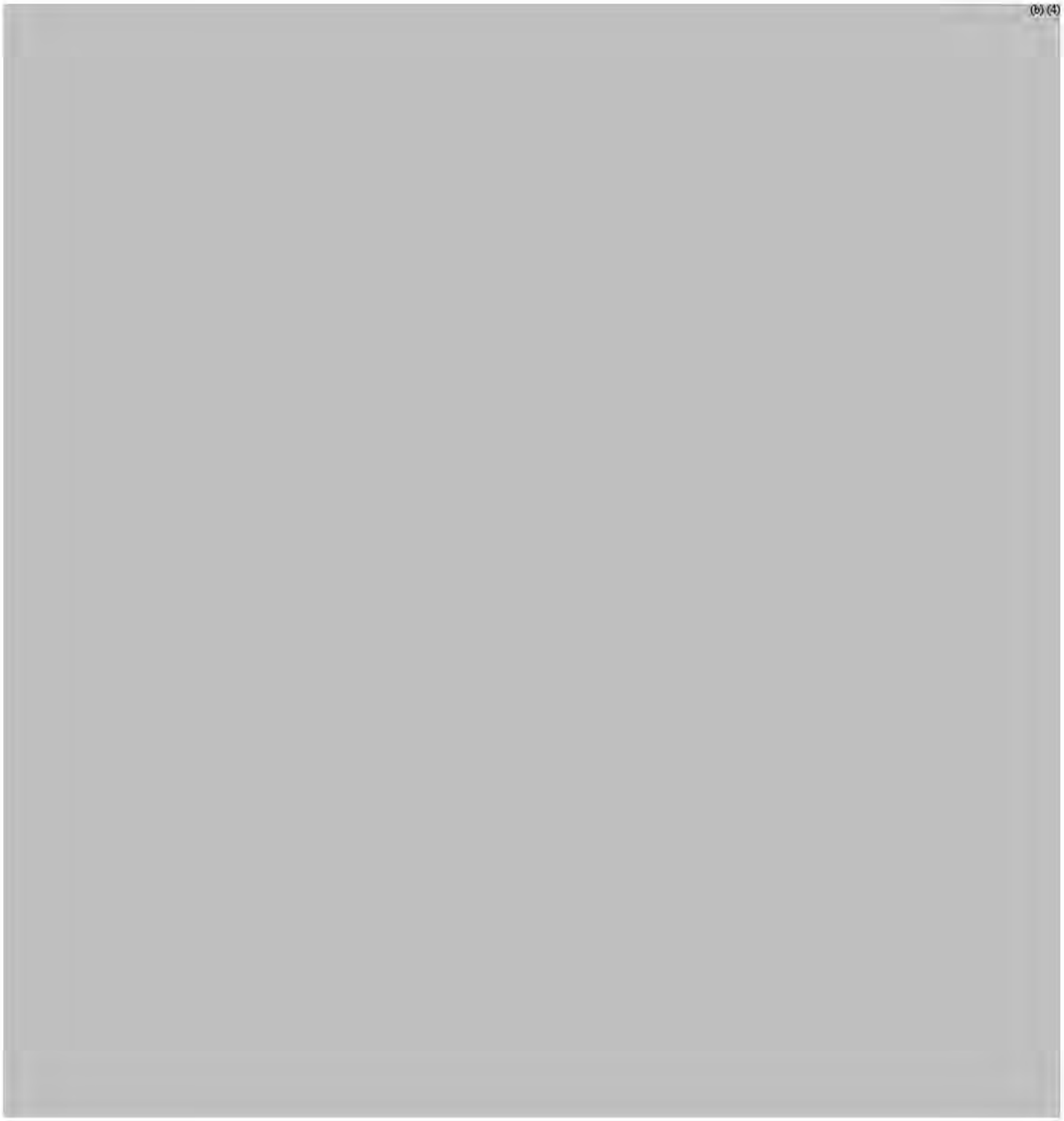
Tested by (b) (4)

Approved by Technical Manager (b) (4)

Dec, 23, 2020

CJ Research Institute of Biotechnology

CJ BIO-AD form 100-01 REV.01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CONFIDENTIAL REPORT

Genetic Stability of *C. glutamicum* KCCM 80240

Version 1.0

TITLE

Genetic stability of *C. glutamicum* KCCM 80240

OBJECTIVE OF THE STUDY

This study was conducted to examine the genetic stability of L-valine production strain, *C. glutamicum* KCCM 80240.

SCHEDULE OF THE STUDY

Initiation of experiment: November 5, 2020

Termination of experiment: November 25, 2020

Submission of final report: December 24, 2020

TESTING FACILITY

Institute of Biotechnology) Scientific and Regulatory Affairs, CJ CheilJedang
55, Gwanggyo-ro 42beon-gil, Yeongtong-gu, Suwon-si, Gyeonggi-do 16495, Korea

RESPONSIBLE STAFFS

Analyst and Author Taeyeon Kim

Report approved by Yang Hee Kim

CONTENTS

INTRODUCTION

Corynebacterium glutamicum KCCM 80240 is a genetically modified strain for L-valine production. Among the genetically modified region, Pcj7-gapN (*L. delbreuckii*) region was constructed to supplement NADPH toward L-valine production. Through this genetic modification, L-valine concentration was increased because of sufficient co-factor supply.

In this study, the genetic stability of *C. glutamicum* KCCM80240 was confirmed by detecting maintenance of specifically modified gene with PCR analysis. To verify the genetic stability of production strain, the maintenance of Pcj7-gapN (*L. delbreuckii*) region was observed during the fermentation.

MATERIALS AND METHODS

Test sample

(b) (4)

(b) (4)

(b) (4)

PCR analysis

(b) (4)


(b) (4)


(b) (4)

RESULTS

Confirmation of genetic stability

[redacted] (b) (4)

Table 2. PCR analysis of Pcj7-gapN (*L. delbreuckii*) gene

Sample		Results		
		1st analysis	2nd analysis	3rd analysis
C. glutamicum ATCC 14067	Negative control	[redacted] (b) (4)		
C. glutamicum KCCM 80240	Positive control			
	Pre-seed culture			
	Seed culture			
	Main culture			
	Final culture			

(-), no amplification; (+), specific amplification

[redacted] (b) (4)

CONFIDENTIAL REPORT

Whole genome sequence analysis of *Corynebacterium glutamicum* KCCM 80240

Version 1.0

TITLE

Whole genome sequence analysis of *Corynebacterium glutamicum* KCCM80240

OBJECTIVE OF THE STUDY

This study was conducted to analyse the genomic features of production strain, *Corynebacterium glutamicum* KCCM 80240.

SCHEDULE OF THE STUDY

Initiation of experiment: 7 September 2020

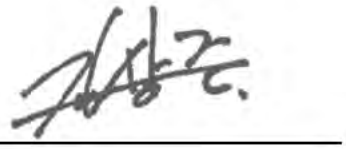
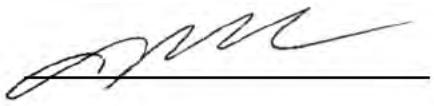
Termination of experiment: 10 December 2020

Submission of final report: 15 December 2020

TESTING FACILITY

Institute of Biotechnology) Data Science Team, CJ CheilJedang
55, Gwanggyo-ro 42beon-gil, Yeongtong-gu, Suwon-si, Gyeonggi-do 16495, Korea

RESPONSIBLE STAFFS

Analyst	Sang Jun Kim	
Author	Su Jin Kim	
Report approved by	Sung Gun Lee	

CONTENTS

INTRODUCTION

L-Valine is produced by fermentation with *Corynebacterium glutamicum* KCCM80240. The genome sequence analysis of the production strains should be performed for safety aspects in accordance with EFSA guidance on the characterisation of microorganisms used as feed additives or as production organisms [1]. This study provide the information about the analysis method and WGS-based charaterisation of the production strain *C. glutamicum* KCCM80240.

MATERIALS AND METHODS

1. Whole genome sequencing

(b) (4)

2. Bioinformatics analysis

2-1. Genome annotation

(b) (4)

2-2. Bacterial identification

(b) (4)

2-3. Identification of antimicrobial resistance (AMR) genes

(b) (4)

2-4. Identification of toxicity and pathogenicity-related genes

(b) (4)

RESULTS

1. Overview on construction of production strain

Table 1. Genome features of three *C. glutamicum* strains

Feature	*C. glutamicum strains*		
	Wild-type strain ATCC 14067	**Parental strain CA08-0012**	**Production strain KCCM 80240**
Genome size (bp)	(b) (4)		
G+C content (%)			
ORFs*			
tRNA			
rRNA			

* The number of ORFs was counted except the pseudogene.

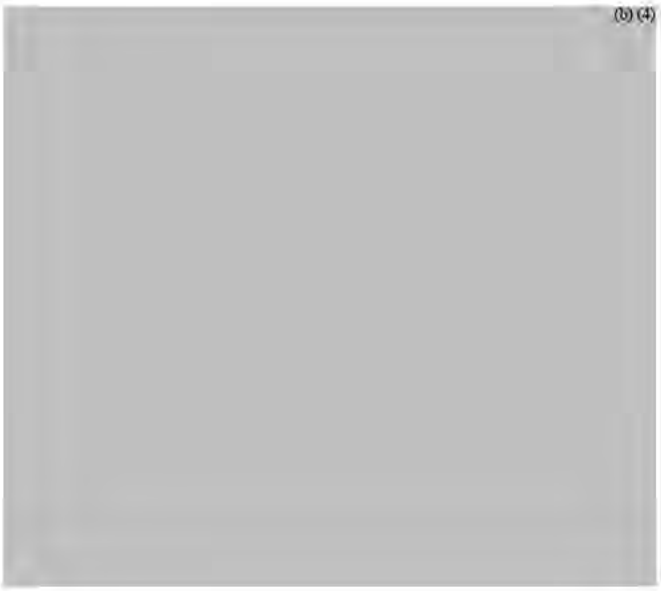
2. WGS analysis of the parental strain *C. glutamicum* CA08-0012

(b) (4)

Table2. General features of the wild-type ATCC 14067 and the NTG mutant CA08-0012 genome

Items	*C. glutamicum* ATCC 14067	*C. glutamicum* CA08-0012
Genome length (bp)	(b) (4)	
G+C contents (%)		
Predicted ORFs		
Predicted tRNAs		
Predicted rRNAs		

(b) (4)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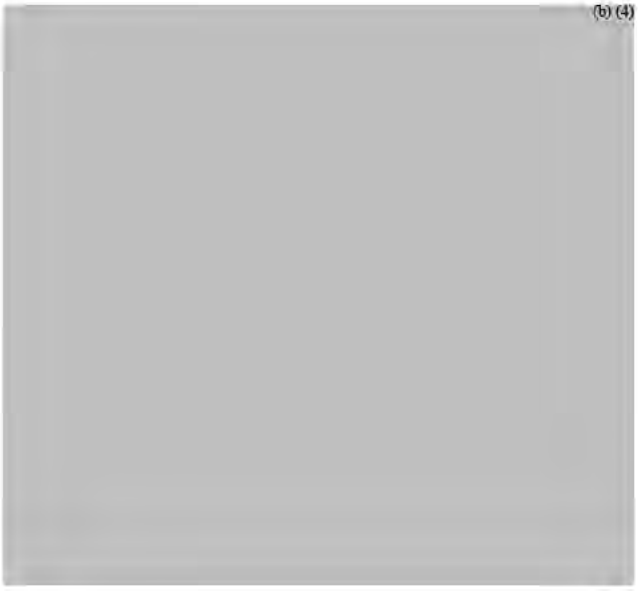
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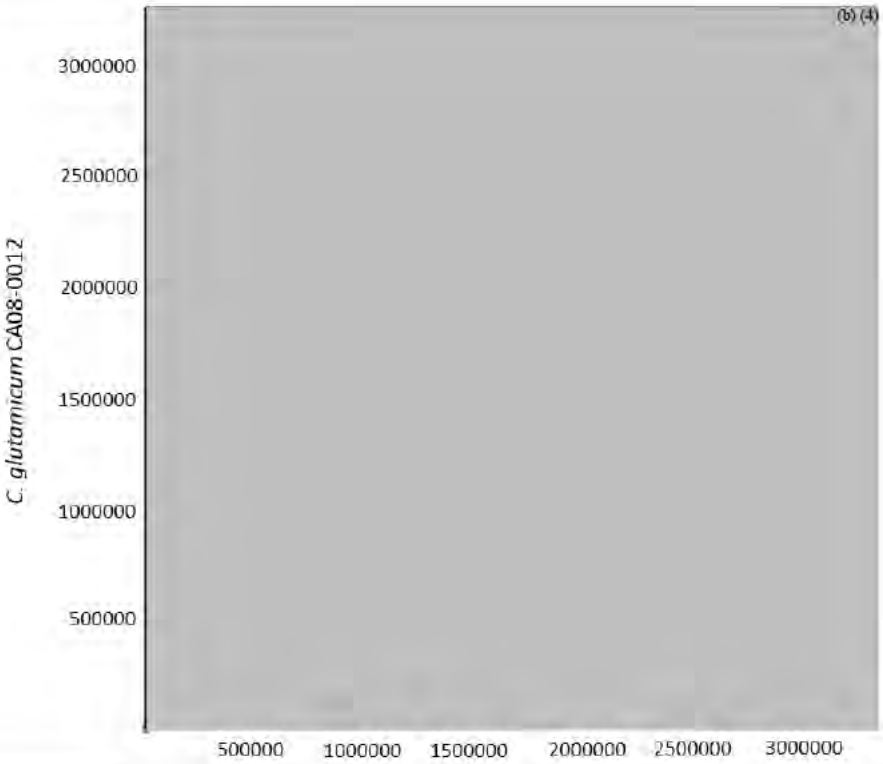
(b) (4)


(b) (4)
3000000
2500000
2000000
1500000
1000000
500000
C. glutamicum CA08-0012
500000
1000000
1500000
2000000
2500000
3000000

(b) (4)

(b) (4)

3. WGS analysis of the production strain *C. glutamicum* KCCM 80240

(b) (4)

(b) (4)

(b) (4)

(b) (4)

Table 6. General features of the *C. glutamicum* KCCM 80240 genomes

Items	Parental strain *C. glutamicum* CN08-0012	Production strain *C. glutamicum* KCCM 80240
(b) (4)	(b) (4)	

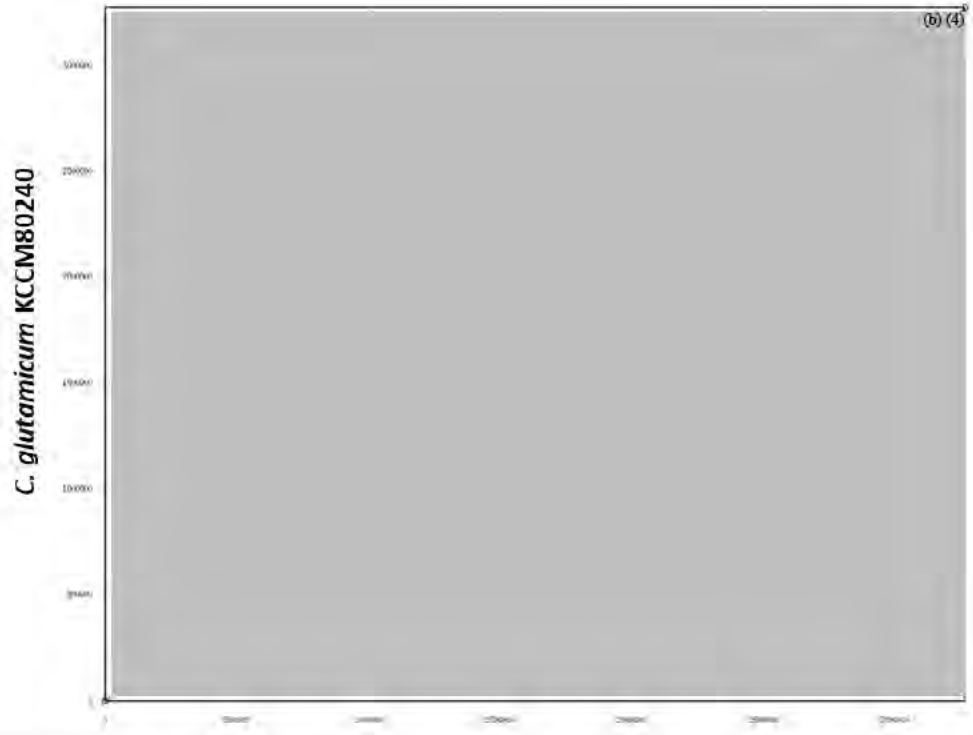
(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)



(b) (4)

Table 7. Rearranged chromosome region of *C. glutamicum* KCCM 80240

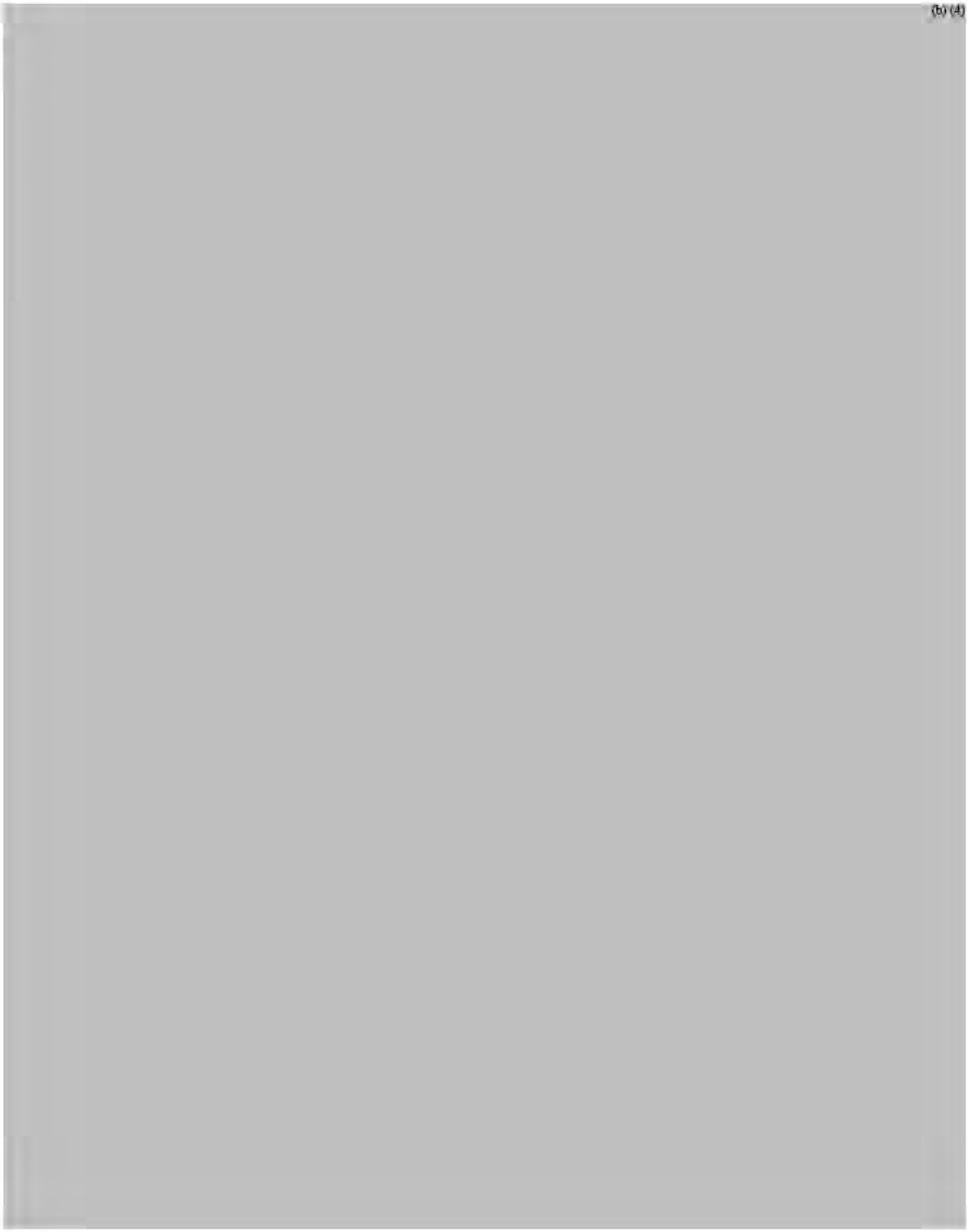
No.	Parental stain *C. glutamicum* CA08-0012 Position	Production strain *C. glutamicum* KCCM 80240 Modification type	Involved genes*
1	(b) (4)		
2			
3			
4			
5			
6			
7			
8			

* The genetic modified site is marked in red.

(b) (4)

(b) (4)

(a)



(b)

(c)

(d)

(e)

(f)

(g)

(h)

(i)

(j)

Table 8. Modified structural elements of the *C. glutamicum* KCCM 80240 chromosome

No	Genetic modification	Name	Types of structural element	Type of genetic modification	Location	Purpose and function
1	[redacted]					(b) (4)
2						
3						
4						
5						
6						


(b) (4)
7
8
9
10

4. Identification of microorganism

Table 9. ANI for *C. glutamicum* KCCM 80240 with the wild-type Corynebacterium species

Rank	Species	GenBank accession no.	ANI value
1	(b) (4)		
2			
3			
4			
5			

5. Identification of antimicrobial resistance gene

Table 10. Screening for antimicrobial resistance genes using ResFinder data base

(b) (4)

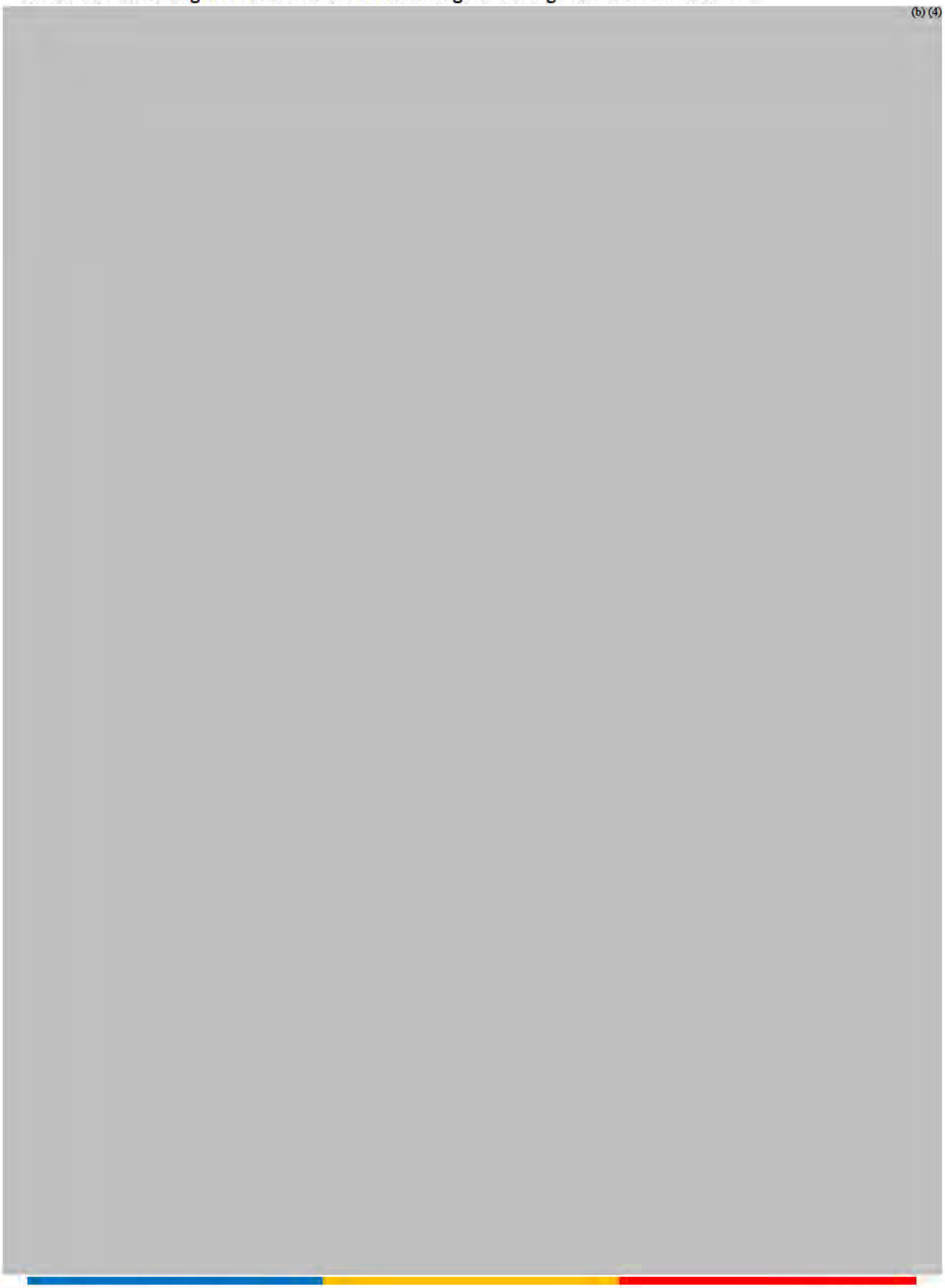


Table 11. Screening for antimicrobial resistance genes using ARG-ANNOT data base

ARG-ANNOT-V4

Gene ID of *C. glutamicum* KCCM 80240		Gene ID in ARG-ANNOT DB		Identity		Coverage
Name	Length	Name	Length	(/)	(%)	(%)

6. Identification of toxigenic and pathogenic genes

(b) (4)

(b) (4)

Table 12. Screening of toxigenic and pathogenic-related genes using VFDB data base

Gene ID in VFDB	*C. glutamicum* ATCC 14067				*C. glutamicum* KCCM 80240			
	Gene ID	Identity (/)	Identity (%)	Coverage (%)	Gene ID	Identity (/)	Identity (%)	Coverage (%)

(b) (4)

REFERENCES

[1] EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2018. Guidance on the characterisation of microorganisms used as feed additives or as production. EFSA Journal, 16(3), 5206. DOI: 10.2903/j.efsa.2018.5206. Available online: https://efsa.onlinelibrary.wiley.com/doi/full/10.2903/j.efsa.2018.5206.

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SUPPLEMENTARY DATA

Table S1. Nucleotide sequence variation of *C. glutamicum* CA08-0012 strain

No	*C. glutamicum* ATCC 14067			*C. glutamicum* CA08-0012		
	type	Ref. Position	Ref. Seq. Nuc.	Var. Nuc	Var. Position	Var. ORF Name
1	[redacted]					
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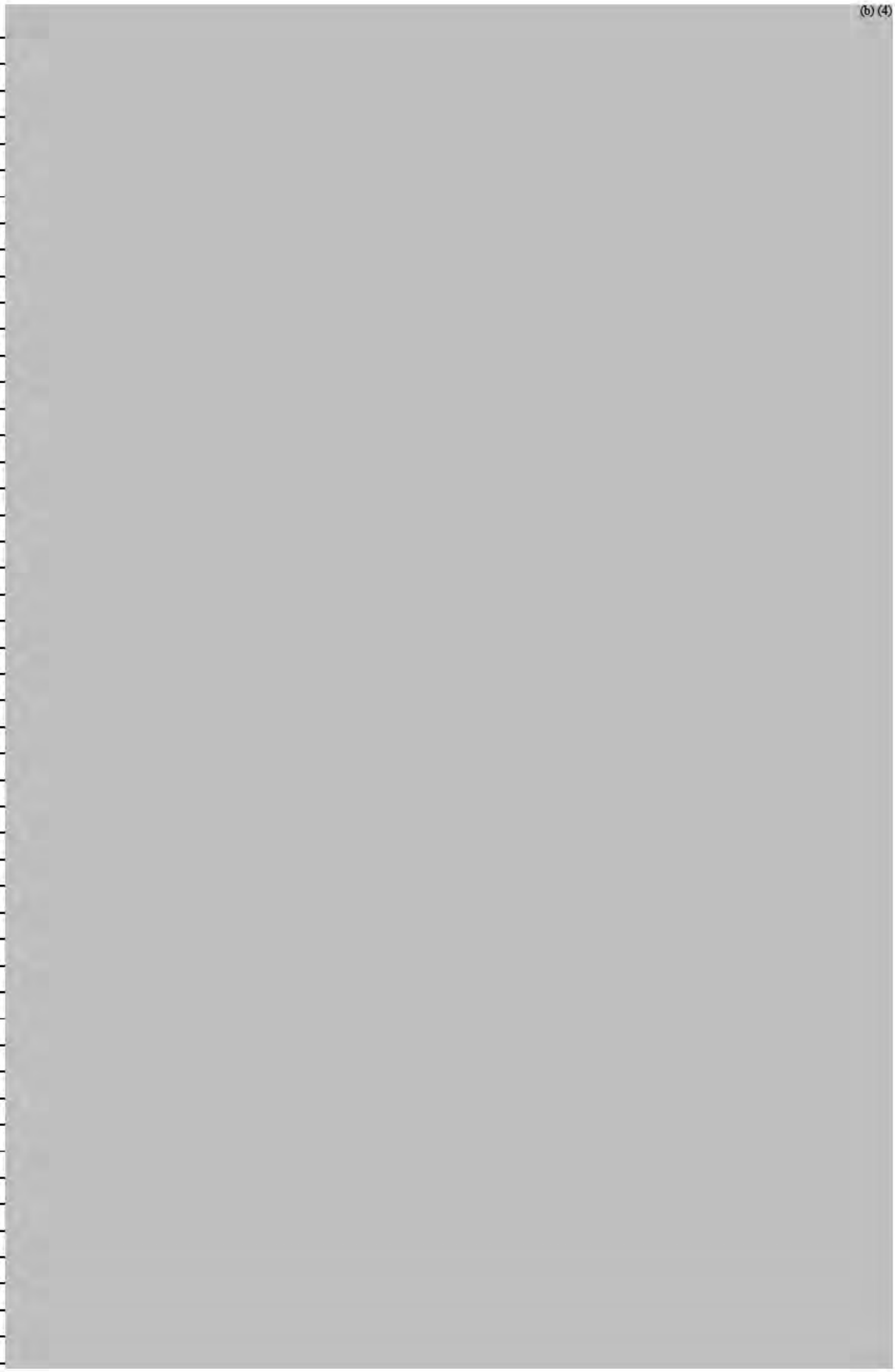

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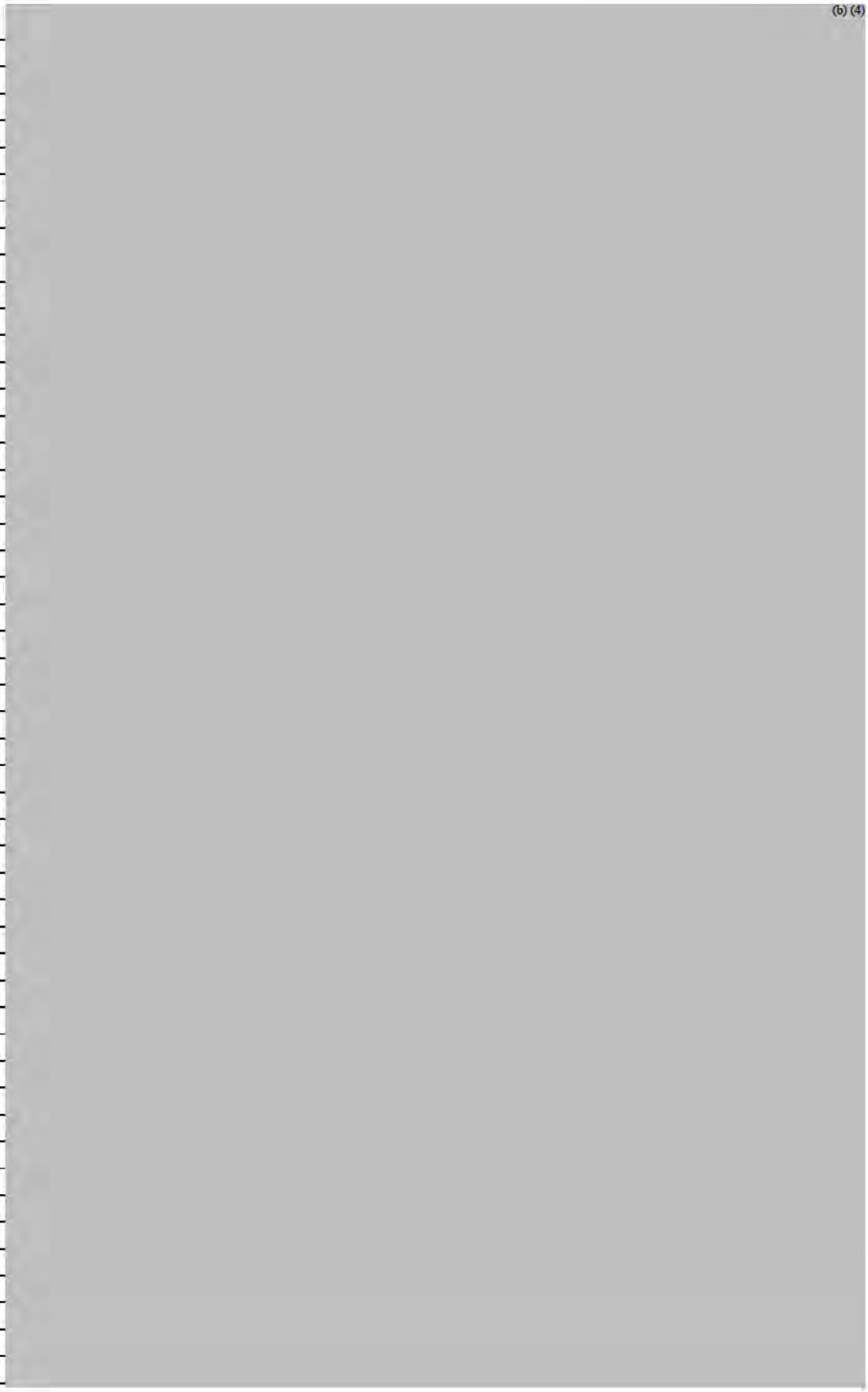

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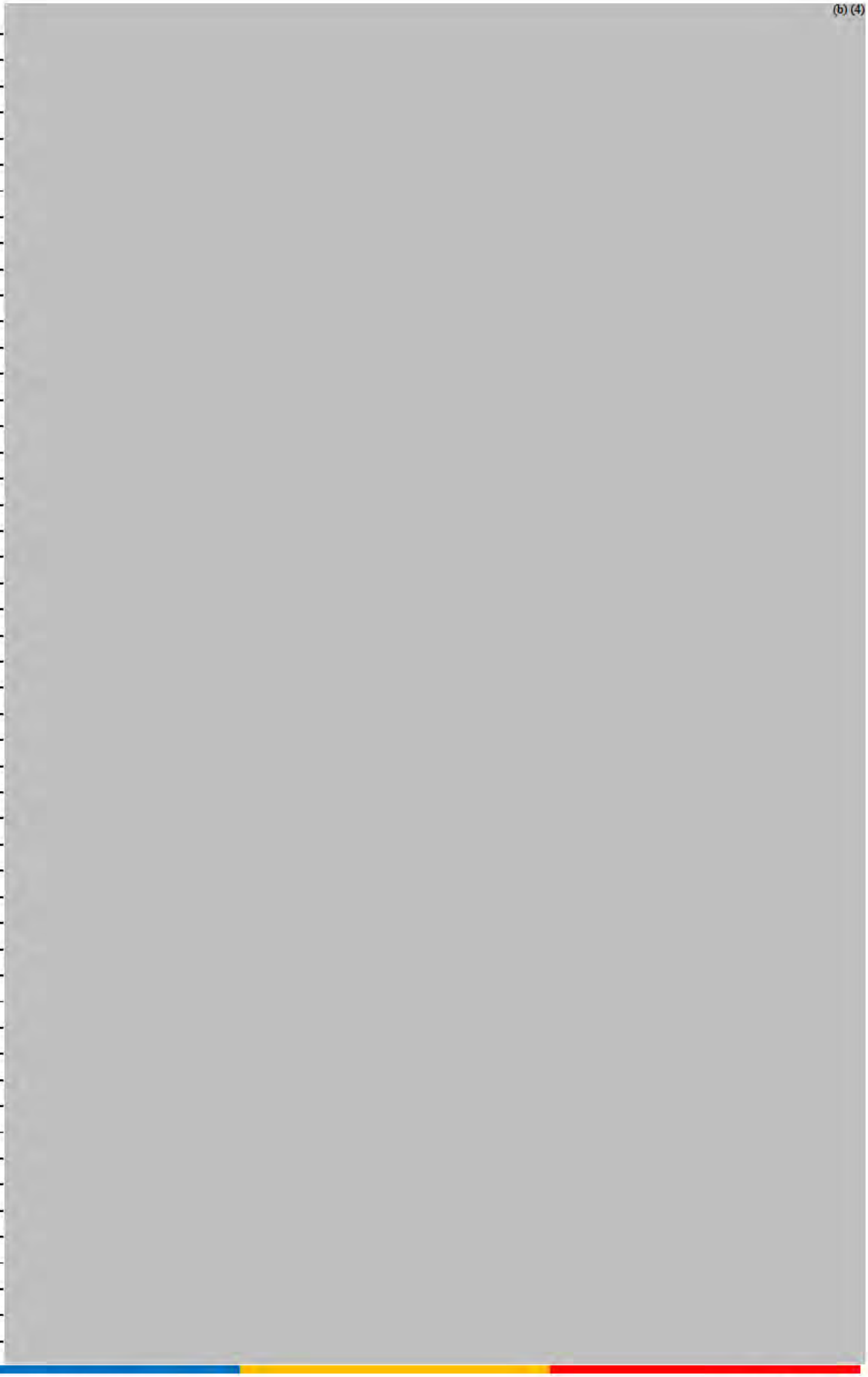

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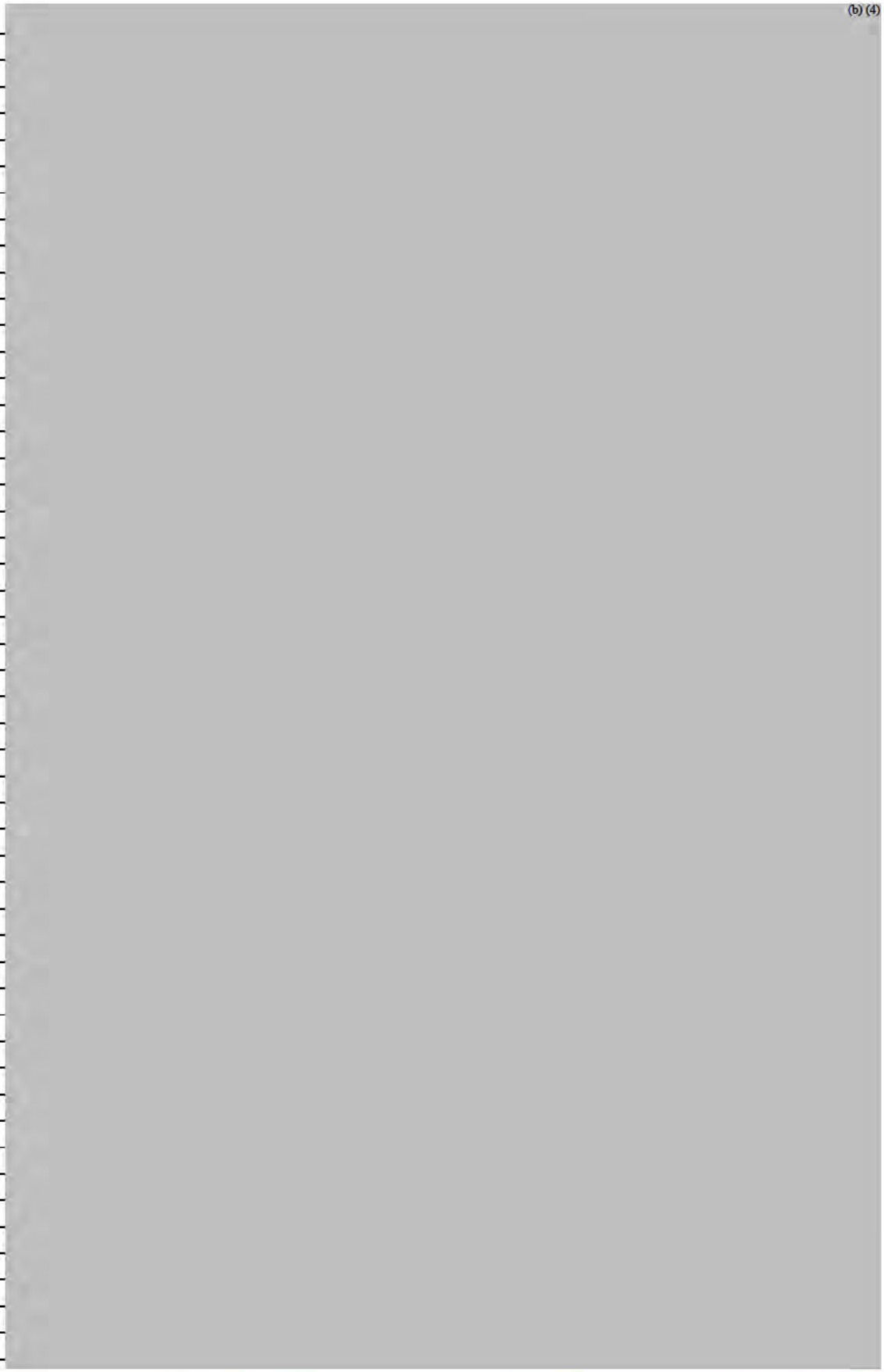
(b) (4)



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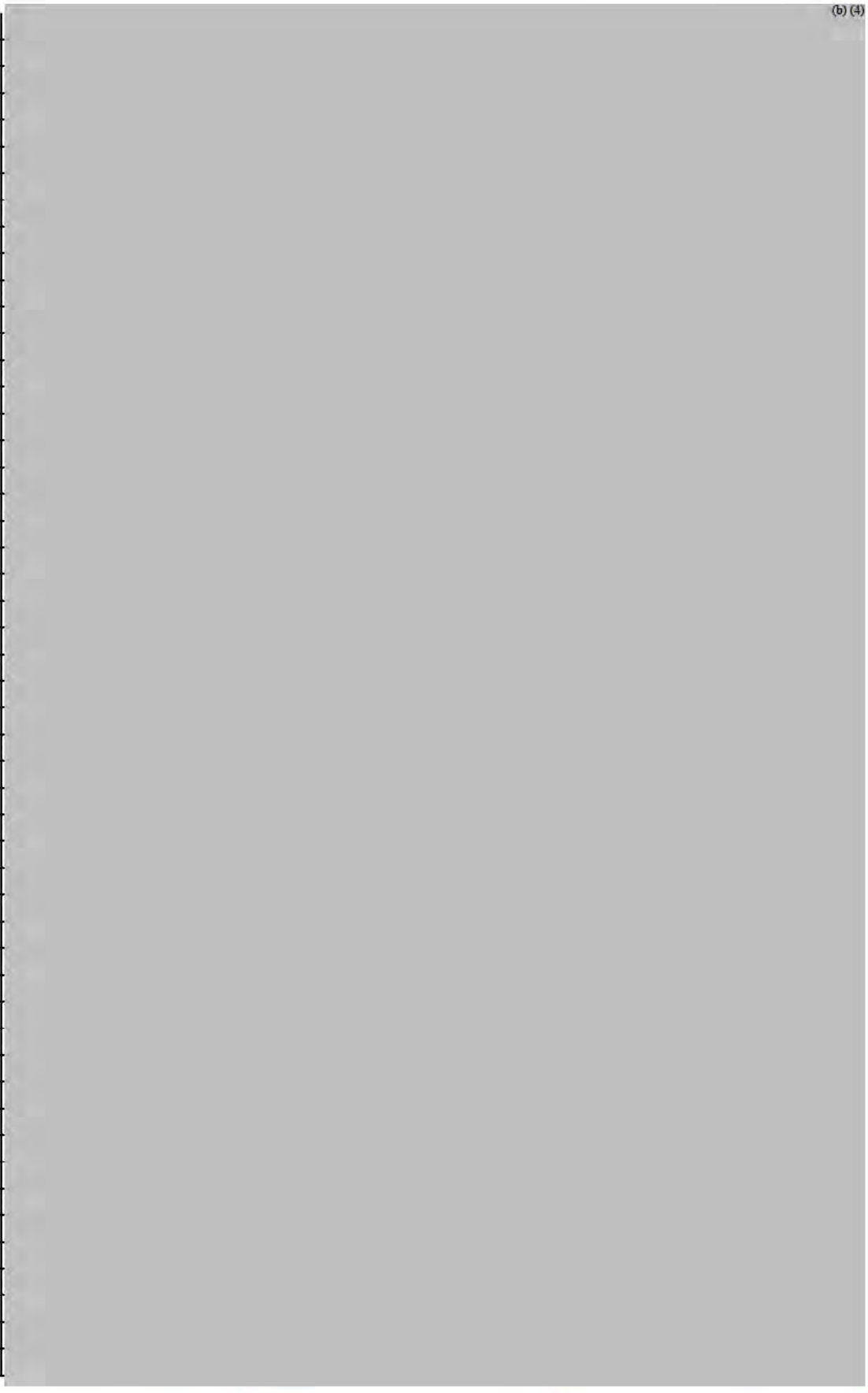

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Table S2. Gene modified regions of *C. glutamicum* CA08-0012

* MGEs related gene is highlighted in yellow.

No	*C. glutamicum* ATCC 14067						*C. glutamicum* CA08-0012		
	Gene ID	Type	Start	Gene ID	Type	Function	Gene ID	Type	Function
1	CEY17_00470	(b) (4)							(b) (4)
	CEY17_00475								
2	CEY17_01065								
	CEY17_01070								
	CEY17_01075								
	CEY17_01080								
3	CEY17_02090								
	CEY17_02095								
	CEY17_02100								
	CEY17_02105								
4	CEY17_04320								
	CEY17_04330								
	CEY17_04335								
	CEY17_04340								
	CEY17_04345								
	CEY17_04350								
	CEY17_04355								
	CEY17_04360								
	CEY17_04365								
	CEY17_04370								
	CEY17_04375								
	CEY17_04380								
5	CEY17_08925								

		(b) (4)
	CEY17_08930	
6	CEY17_06260	
	CEY17_06270	
	CEY17_06275	
7	CEY17_10440	
	CEY17_10450	
	CEY17_10455	
	CEY17_10460	
	CEY17_10465	
8	CEY17_12865	
	CEY17_12870	
9	CEY17_13185	
	CEY17_13190	
10	CEY17_15070	
	CEY17_15085	
	CEY17_15090	
	CEY17_15095	
	CEY17_15105	
	CEY17_15110	
	CEY17_15115	
	CEY17_15120	
	CEY17_15125	
	CEY17_15130	
	CEY17_15135	
	CEY17_15140	
	CEY17_15145	
	CEY17_15150	
	CEY17_15155	

CEY17_15170	(b) (4)	(b) (4)
CEY17_15175		
CEY17_15180		
CEY17_15185		
CEY17_15190		
CEY17_15195		
CEY17_15215		
CEY17_15220		
CEY17_15225		
CEY17_15230		
CEY17_15240		
CEY17_15245		
CEY17_15250		
CEY17_15255		
CEY17_15260		
CEY17_15265		
CEY17_15270		
CEY17_15280		
CEY17_15285		
CEY17_15290		
CEY17_15295		
CEY17_15300		
CEY17_15305		
CEY17_15310		
CEY17_15315		
CEY17_15320		
CEY17_15325		
CEY17_15335		
CEY17_15340		
CEY17_15345		
CEY17_15350		

	(b) (4)	(b) (4)
CEY17_15360		
CEY17_15365		
CEY17_15370		
CEY17_15380		
CEY17_15385		
CEY17_15390		
CEY17_15395		
CEY17_15400		
CEY17_15405		
CEY17_15410		
CEY17_15420		

Table S3. Nucleotide sequence variation of *C. glutamicum* KCCM 80240 strain

* The genetic modified site is marked in red.

No.	*C. glutamicm* CA08-0012		*C. glutamicm* KCCM80240			
	Ref. Position	Ref. Seq. Nuc.	Var. Nuc	Var. Position	Type	Var. ORF Name
1	(b) (4)					
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98	(b) (4)
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(b) (4)

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Table S2. Gene modified regions of *C. glutamicum* KCCM 80240

* MGEs related gene is highlighted in yellow. The genetic modified site is marked in red.

No	*C. glutamicum* CA08-0012					*C. glutamicum* KCCM 80240		
	Gene ID	Type	Position	Strd	Function	Modified type	Gene ID	Function
1								(b) (4)
2								
3								
4								
5								


(b) (4)
6
7
8

Figure S1. Phylogenetic tree of *C. glutamicum* KCCM 80240 based on 16s rDNA sequence analysis

CONFIDENTIAL REPORT

Metabolic flux analysis of *C. glutamicum* KCCM 80240

Version 1.0

TITLE

Metabolic flux analysis of *C. glutamicum* KCCM 80240

OBJECTIVE OF THE STUDY

Metabolic flux analysis was conducted to determine intracellular fluxes of *C. glutamicum* KCCM 80240.

SCHEDULE OF THE STUDY

Initiation of experiment: September 8, 2020

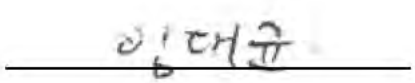
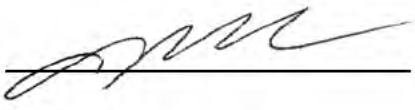
Termination of experiment: December 3, 2020

Submission of final report: December 28, 2020

TESTING FACILITY

Institute of Biotechnology) Data Science Team, CJ CheilJedang
55, Gwanggyo-ro 42beon-gil, Yeongtong-gu, Suwon-si, Gyeonggi-do 16495, Korea

RESPONSIBLE STAFFS

Analyst and Author	Daekyun Im	
Report approved by	Sung gun Lee	

CONTENTS

INTRODUCTION

Metabolic flux analysis (MFA) has been extensively used to determine intracellular fluxes of many organisms to investigate certain metabolic state through observable phenotypes, such as amount of biomass, extracellular concentrations of substrates, products and by-products [1-3].

MFA could provide valuable insights into cell physiology because it is based on a stoichiometric model which represents major metabolic pathways of organisms.

Here, we report re-directed metabolic fluxes of production strain, *C. glutamicum* KCCM 80240, compared to those of the wild type strain, *C. glutamicum* ATCC 14067, through MFA.

METHODS

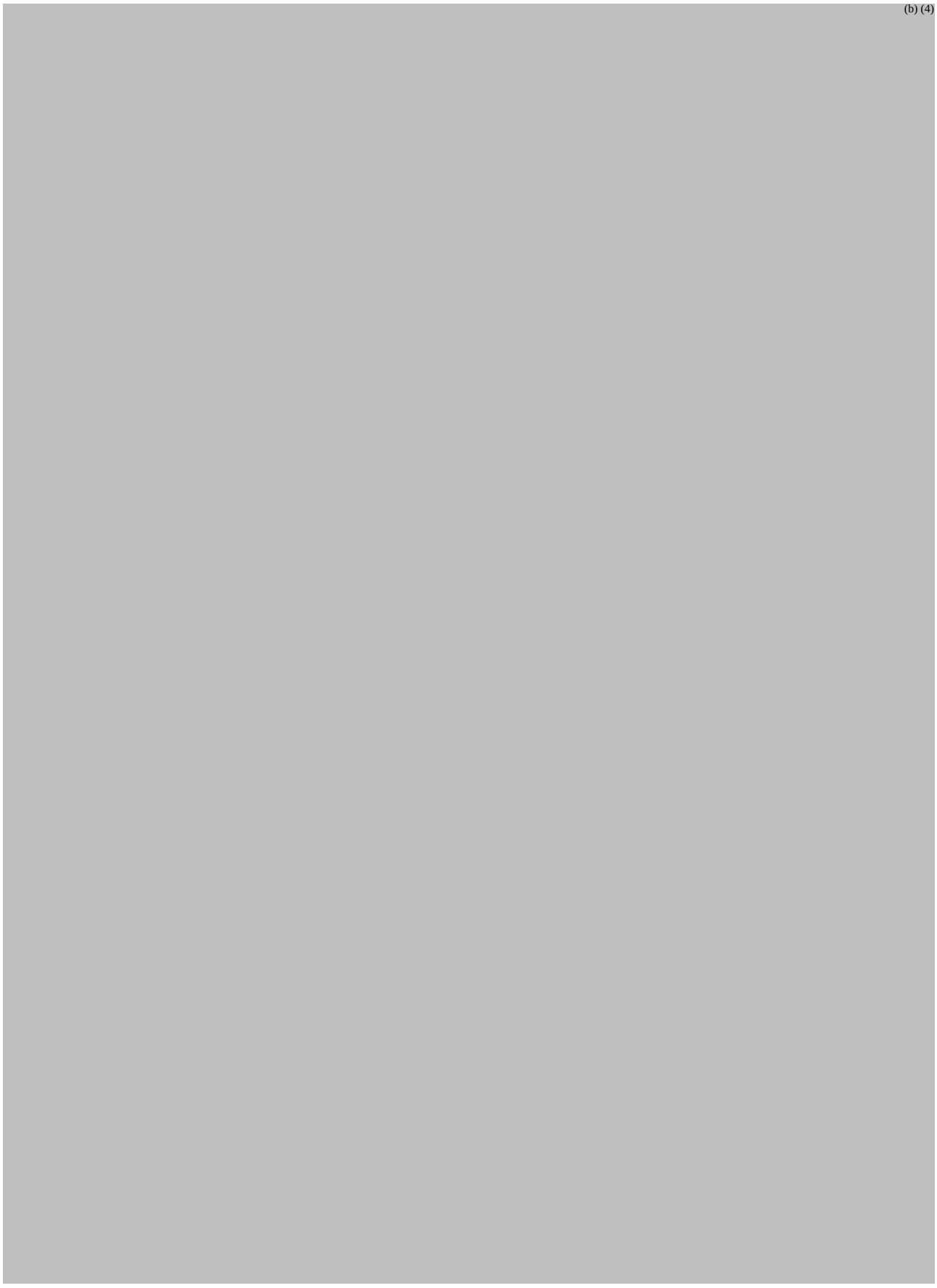
Translation level measurement

Extracellular metabolite measurement

Off-gas analysis

Metabolic network

Table 1. Metabolic network for MFA



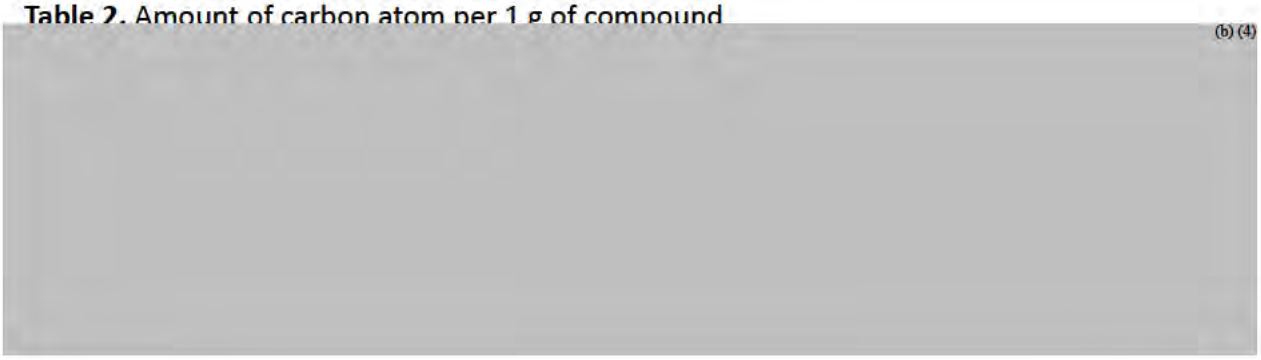
(b) (4)

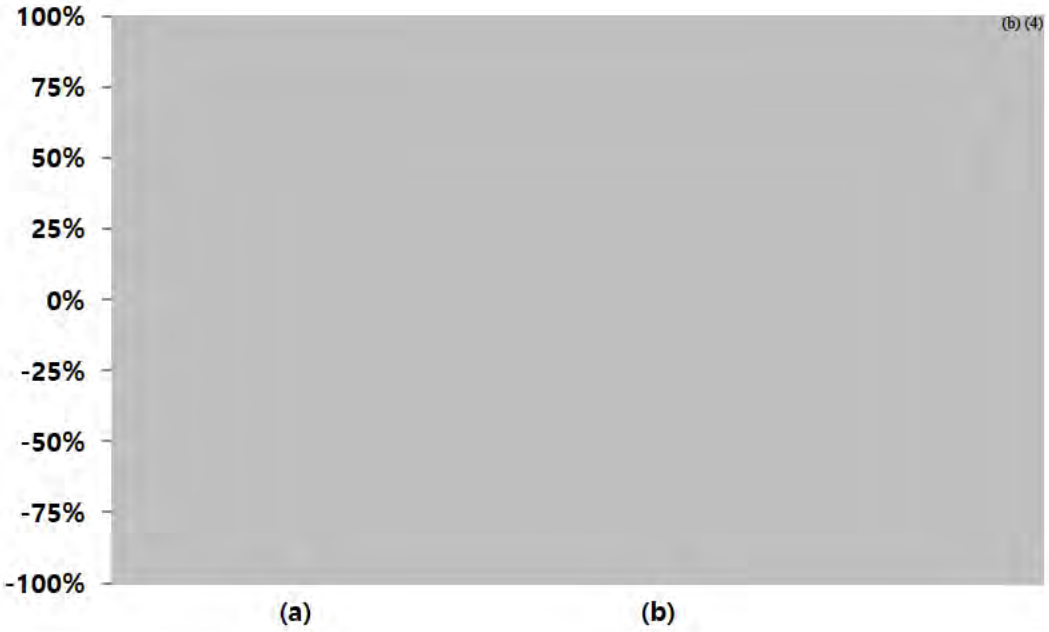
MFA computation

RESULTS

Carbon balance analysis

Table 2. Amount of carbon atom per 1 g of compound





Translation level measurement

(b) (4)

Table 1. *gapA* and *gapN* translation levels of *C. glutamicum* KCCM 80240

(b) (4)

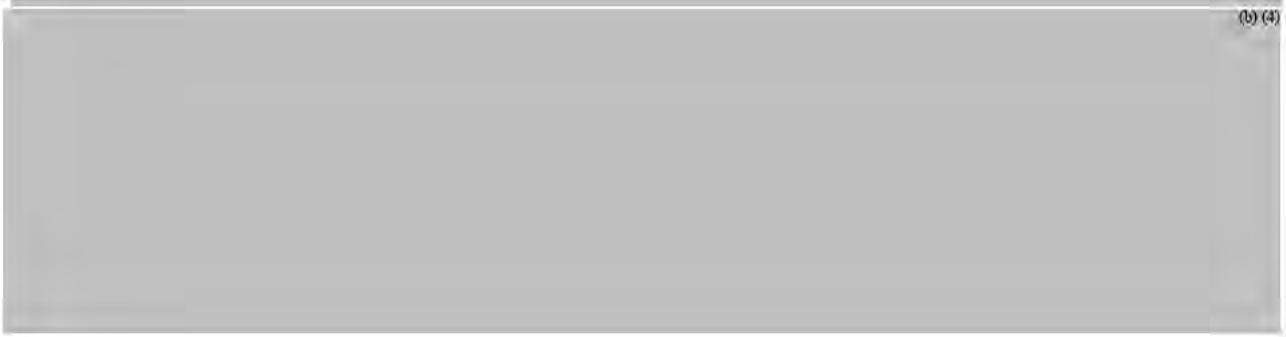
Metabolic flux analysis

(b) (4)

Table 2. Measured extracellular flux rates. Carbon consumption rates are represented by a negative signal. Unit of each measurement is mmol/gdcw/h, except biomass (/h) and each analysis was carried out in duplicate.

(b) (4)

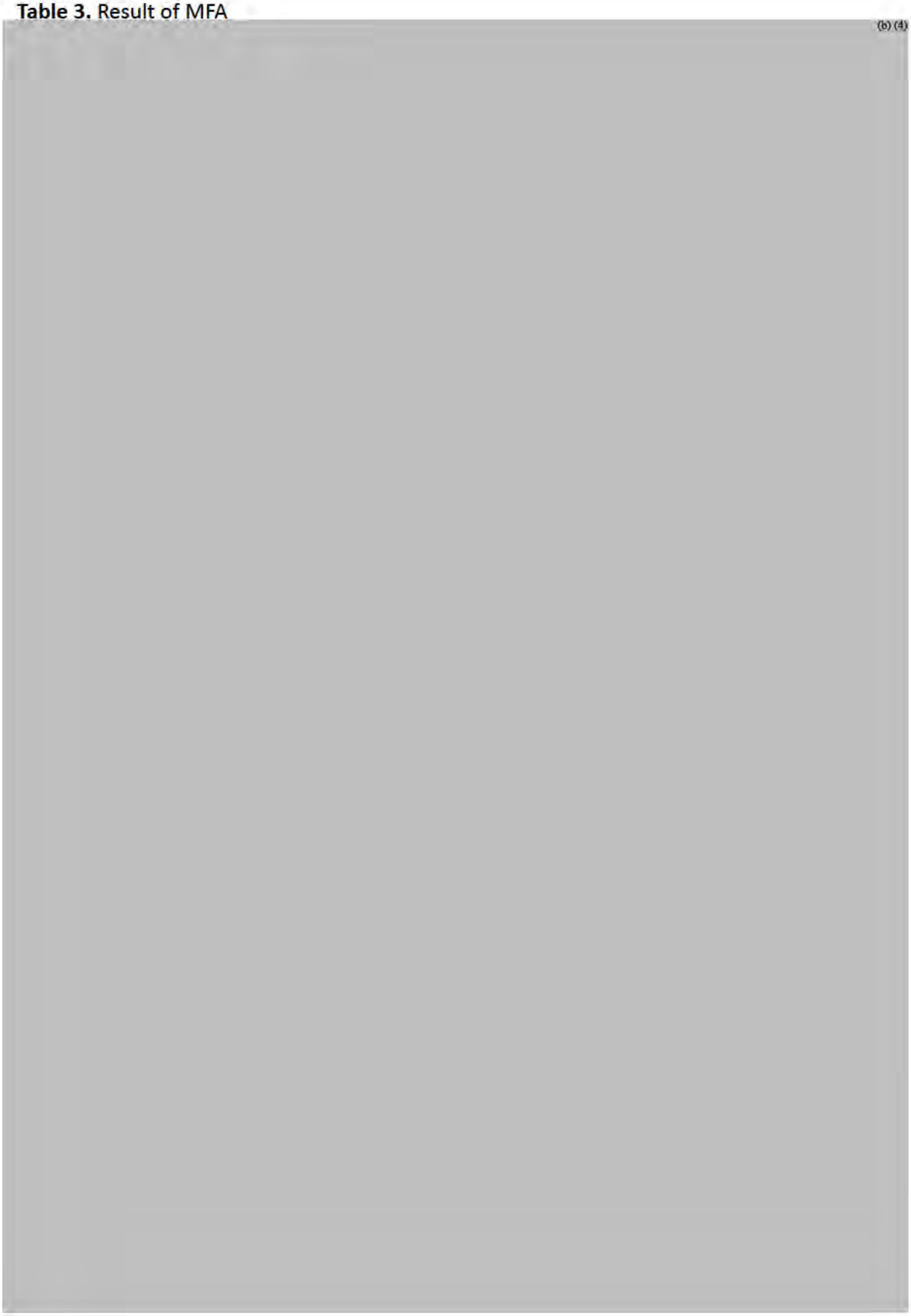

(b) (4)

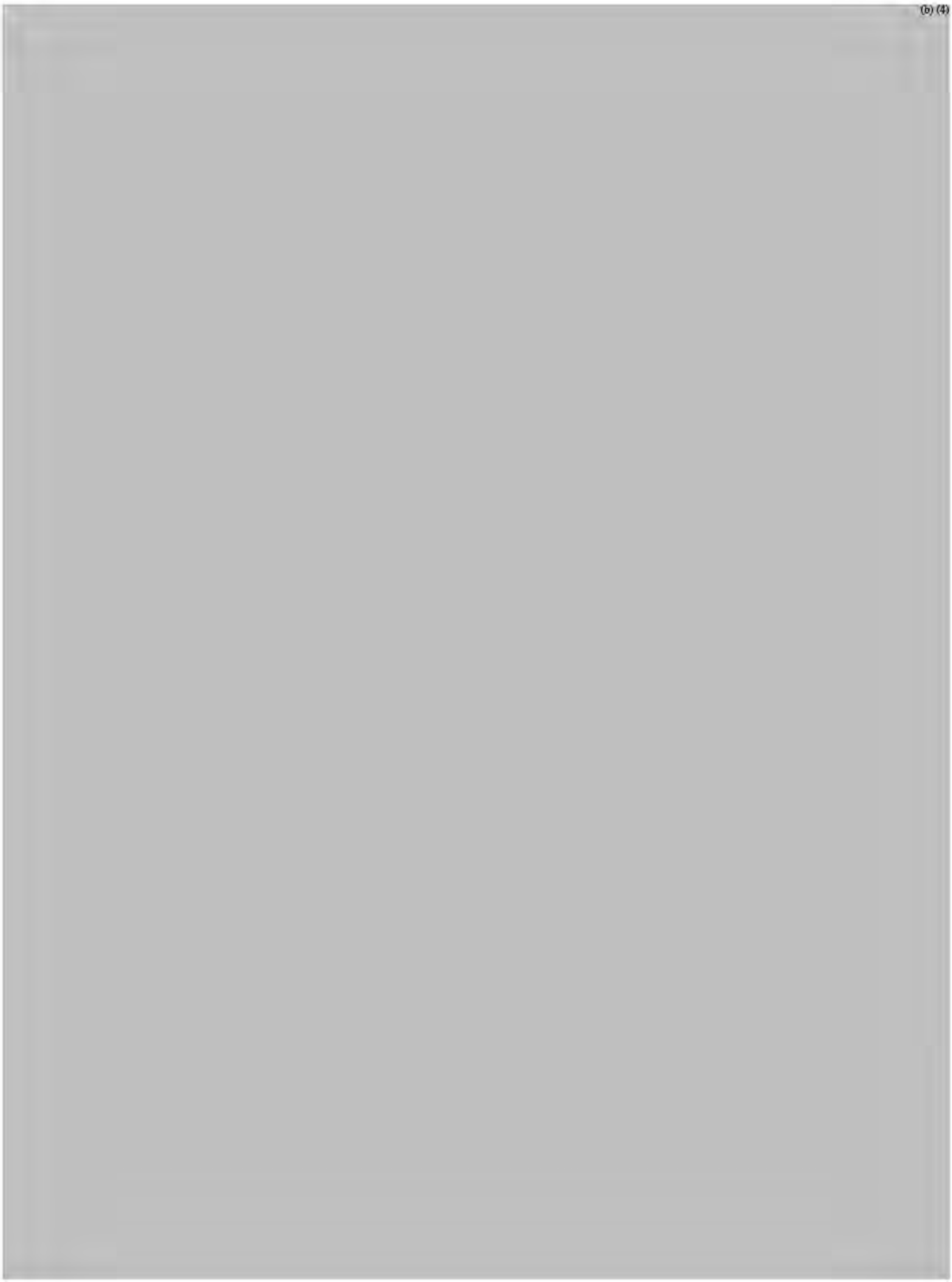

(b) (4)


(b) (4)

Table 3. Result of MFA

(b) (4)




(b) (4)


(b) (4)

REFERENCES

(b) (4)

APPENDIX 2. PRE-FERMENTATION INFORMATION (CONFIDENTIAL)

Table of Contents

List of Tables

List of Figures

A. CHARACTERIZATION OF THE PRODUCTION MICROORGANISM

(b) (4)

(b) (4)

A.1 Scientific Name and Taxonomy of *C. glutamicum* KCCM 80240

(b) (4)

(b) (4)

A.2 Nature Habitat of *C. glutamicum* and Its Ecological Role

It was reported that Corynebacteriaceae are rod-shaped, fast growing, non-sporulating gram-positive bacteria that are found widespread in nature. A large number of corynebacterial species were isolated from human clinical samples or animals, but several others were isolated from soils, cheese, dairy products, vegetables and fruits. Some of these species were also found in marine samples. It seems that these bacteria are widely spread throughout nature which induces high diversity in the Corynebacterium genus. The natural habitat of *C. glutamicum* strains have been reported in soil, soils contaminated with bird feces, sewage, manure, and vegetables and fruits (Eggeling and Bott, 2005).

A.3 Phenotypic Characteristics of *C. glutamicum* KCCM 80240

(b) (4)

[redacted] (b) (4)

Table A.3.1. Phenotypic characteristics of *C. glutamicum* ATCC14067, *C. glutamicum* CA08-0012 and *C. glutamicum* KCCM 80240

	***C. glutamicum* ATCC 14067 (Wild-type strain)**	***C. glutamicum* CA08-0012 (Parental strain)**	***C. glutamicum* KCCM 80240 (Production strain)**
Colony shape	[redacted] (b) (4)		(b) (4)
Colony color			
Cell arrangement			
Cell shape			
16s rDNA homology			
Optimal temperature range			
Optimal pH range			

A.4 Genetic Comparison of Host to Published Data of the Species

[redacted] (b) (4)

Certification of The Safety Deposit

Name and Address of Depositor To : CJ CheilJedang 330, Dongho-ro, Jung-gu, Seoul, 04560, Korea	
1. IDENTIFICATION OF THE MICROORGANISMS	
Identification reference given by the Depositor: *Corynebacterium glutamicum* CE02-CA08-1331	Accession number given by the Safety Depositary Authority: KCCM 80240
2. RECEIPT AND ACCEPTANCE	
This Safety Depositary Authority accepts the microorganism identified under 1 above, which was received it on September. 25. 2020.	
3. SAFETY DEPOSITARY AUTHORITY	
Name: Korean Culture Collection of Microorganisms Address: Yurim Bild. 45, Hongjenae 2ga-gil, Seodaemun-gu, Seoul, 03641, Republic of Korea	Signature(s) of person(s) having the power to represent the Safety Depositary Authority or of authorized official(s): Date: September. 25. 2020.

한국미생물보존센터 KOREAN CULTURE CENTER OF MICROORGANISMS

TSD-Form(5)

Figure A.1.1. Certificate of deposition (*C. glutamicum* KCCM 80240)

KCCM
KFCC 사단법인 한국종균협회 03641 서울시 서대문구 홍제내2가길 45 유원빌딩
TEL : (02)391-0950
FAX : (02)392-2859
Home Page : http://www.kccm.or.kr
한국미생물보존센터

KOREAN CULTURE CENTER OF MICROORGANISMS
FORMERLY FEDERATION OF CULTURE COLLECTIONS
45, Hongjenae 2ga-gil, Seodaemun-gu, Seoul 03641, Korea
TEL : 82-2-391-0950
FAX : 82-2-392-2859
Home Page : http://www.kccm.or.kr

No.20-83

2020-09-25

Certification of Analysis

Dear CJ CheilJedang
330, Dongho-ro, Jung-gu,
Seoul, Korea
04560

We have performed the 16S rDNA sequence analysis of your strain **KCCM80240**. The result is as follows:

KCCM80240 : *Corynebacterium glutamicum*
(GenBank Data homology search result : 99%)

Please refer to sequence and phylogeny tree.

Sincerely yours

(b) (4)

Korean Culture Collection of Microorganisms (KCCM)
45, Hongjenae 2ga-gil, Seodaemun-gu, Seoul, Korea. 03641
Tel : 82-2-391-0950
FAX: 82-2-392-2859

(b) (4)

>KCCM80240

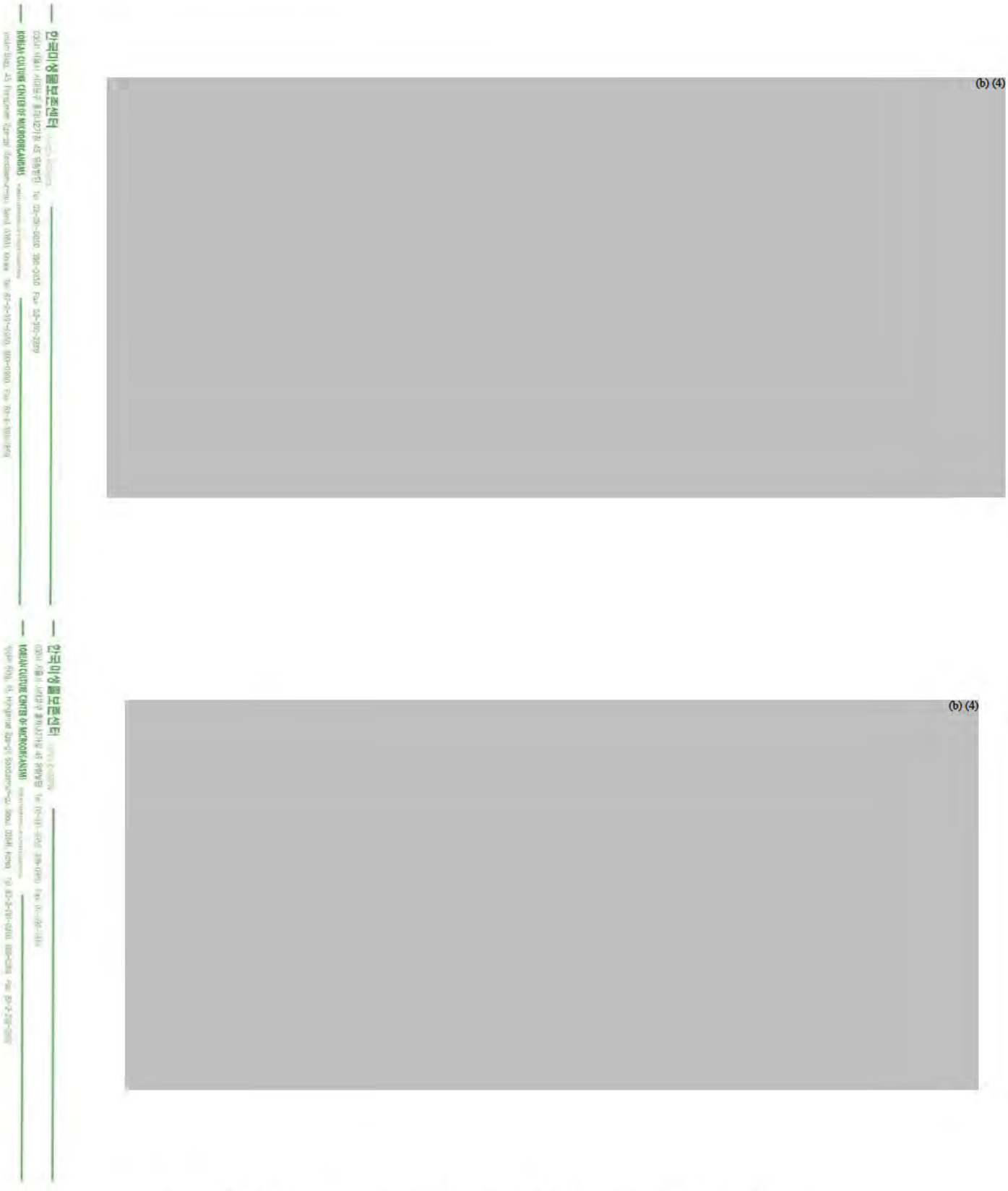


Figure A.3.1. 16s rDNA sequence analysis of *C. glutamicum* KCCM 80240

B. INFORMATION OF DRIED L-VALINE FERMENTATION PRODUCT PRODUCING STRAIN, *CORYNEBACTERIUM GLUTAMICUM* KCCM 80240

[redacted] (b) (4)

B.1 Information of Genetic Modification in *C. glutamicum* KCCM 80240

[redacted] (b) (4)

B.1.1 Random Mutagenesis

[redacted] (b) (4)

B.1.2 Site-directed Mutagenesis

[redacted] (b) (4)

(b) (4)

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B.1.3 Overexpression of Biosynthetic Genes, Especially Deregulated Genes Encoding Key Enzymes, for Producing C. glutamicum KCCM 80240

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The production strain of Dried L-Valine Fermentation Product was deposited as *C. glutamicum* KCCM 80240 at KCCM (Korea Culture Center of Microorganisms) located in the South Korea.

Table B.1.1. Summary of genetic modification in *C. glutamicum* KCCM 80240

Modified gene	Modified locus	Modification method	Copy number of integration gene	Characteristics	
				Parental organism	Donor organism
(b) (4)					

B.2 Donor Organism

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(b) (4)

B.3 Descriptions of Genetic Modification

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B.3.1 Vector Used for Genetic Modification

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(b) (4)

Figure B.3.1. Marker free insertion method

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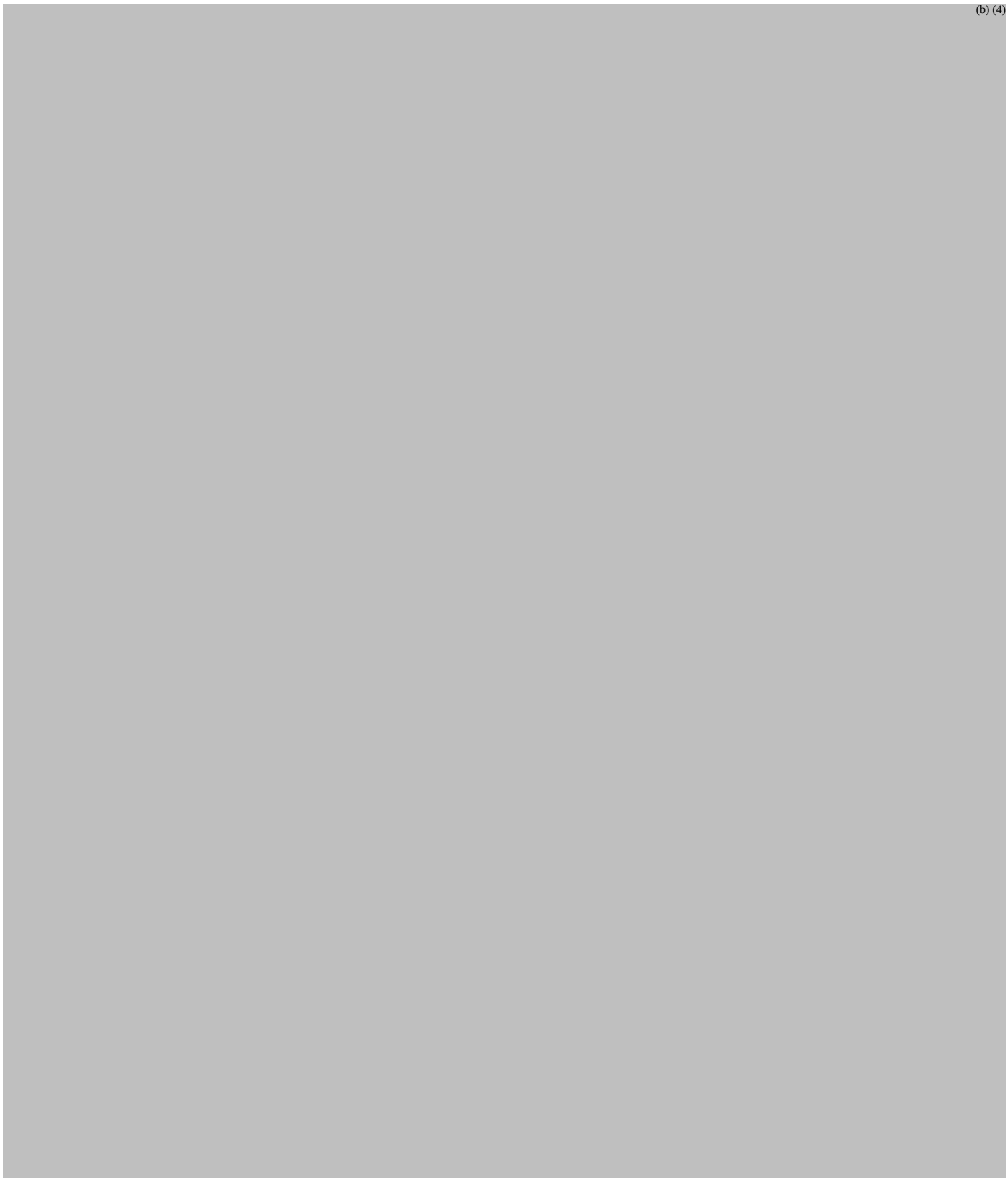

B.3.2 Partial Deletion of ilvA Gene

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(b) (4)

Table B.3.1. Primer sequence used to construct *C. glutamicum* KCCM 80240

No	SEQ ID	Sequence (5' → 3')
1	SEQ ID No 01	(b) (4)
2	SEQ ID No 02	
3	SEQ ID No 03	
4	SEQ ID No 04	
5	SEQ ID No 05	
6	SEQ ID No 06	
7	SEQ ID No 07	
8	SEQ ID No 08	
9	SEQ ID No 09	
10	SEQ ID No 10	
11	SEQ ID No 11	
12	SEQ ID No 12	
13	SEQ ID No 13	
14	SEQ ID No 14	
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16	SEQ ID No 16	
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18	SEQ ID No 18	
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34	SEQ ID No 34	
35	SEQ ID No 35	
36	SEQ ID No 36	
37	SEQ ID No 37	
38	SEQ ID No 38	
39	SEQ ID No 39	
40	SEQ ID No 40	
41	SEQ ID No 41	
42	SEQ ID No 42	
43	SEQ ID No 43	
44	SEQ ID No 44	
45	SEQ ID No 45	
46	SEQ ID No 46	

No	SEQ ID	Sequence (5' → 3')
47	SEQ ID No 47	(b) (4)
48	SEQ ID No 48	
49	SEQ ID No 49	
50	SEQ ID No 50	
51	SEQ ID No 51	
52	SEQ ID No 52	
53	SEQ ID No 53	
54	SEQ ID No 54	
55	SEQ ID No 55	
56	SEQ ID No 56	
57	SEQ ID No 57	
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76	SEQ ID No 76	
77	SEQ ID No 77	
78	SEQ ID No 78	
79	SEQ ID No 79	
80	SEQ ID No 80	
81	SEQ ID No 81	
82	SEQ ID No 82	
83	SEQ ID No 83	
84	SEQ ID No 84	
85	SEQ ID No 85	
86	SEQ ID No 86	

B.4 Identification and Detection Techniques

(b) (4)

Table B.4.1. Comparison of PCR products sizes between *C. glutamicum* ATCC 14067 and *C. glutamicum* KCCM 80240

Gene	Seq No	Primer sequence (5' → 3')	Integrated locus	PCR size (bp)	
				ATCC 14067	KCCM 80240

(b) (4)

B.5 Description of Gene Deletion Region(s)

(b) (4)

(b) (4)

Table B.5.1. Size and function of deleted gene

Deleted gene	Function	Size (bp)	
		Whole gene	Deleted gene
(b) (4)			

(b) (4)

(b) (4)

Name	Sequence (5' → 3')	Size (bp)
(b) (4)		

Name	Sequence (5' → 3')	Size (bp)
(b) (4)		

B.6 Promoter Information

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

Promoter Name	Origin	Sequence (5' → 3')	Length (bp)	Applied ORF
(b) (4)				

B.7 Description of Gene Integration

(b) (4)

(b) (4)

(b) (4)

Table B.7.1. Location of integrated genes in genome

Genes	Integrated locus	Location in genome
(b) (4)		

(b) (4)

Name	Sequence (5' → 3')	Size (bp)	Origin
(b) (4)			

(b) (4)

Name	Sequence (5' → 3')	Size (bp)	Origin
(b) (4)			
(b) (4)			
(b) (4)			
(b) (4)			

Name	Sequence (5' → 3')	Size (bp)	Origin
(b) (4)			

(b) (4)

Name	Sequence (5' → 3')	Size (bp)	Origin
(b) (4)			
(b) (4)			
(b) (4)			

Name	Sequence (5' → 3')	Size (bp)	Origin
(b) (4)			
(b) (4)			

(b) (4)

Name	Sequence (5' → 3')	Size (bp)	Origin
(b) (4)			

Name	Sequence (5' → 3')	Size (bp)	Origin

(b) (4)

Name	Sequence (5' → 3')	Size (bp)	Origin
(b) (4)			

T (b) (4)

Name	Sequence (5' → 3')	Size (bp)	Origin
(b) (4)			

Name	Sequence (5' → 3')	Size (bp)	Origin
			(b) (4)
			(b) (4)

Name	Sequence (5' → 3')	Size (bp)	Origin
(b) (4)			

(b) (4)

Name	Sequence (5' → 3')	Size (bp)	Origin
(b) (4)			

Name	Sequence (5' → 3')	Size (bp)	Origin
			(b) (4)

Name	Sequence (5' → 3')	Size (bp)	Origin
(b) (4)			
(b) (4)			

(b) (4)

Name	Sequence (5' → 3')	Size (bp)	Origin
(b) (4)			

Name	Sequence (5' → 3')	Size (bp)	Origin
			(b) (4)

Name	Sequence (5' → 3')	Size (bp)	Origin
(b) (4)			

(b) (4)

Name	Sequence (5' → 3')	Size (bp)	Origin
(b) (4)			

(b) (4)

Name	Sequence (5' → 3')	Size (bp)	Origin
			(b) (4)

(b) (4)

Name	Sequence (5' → 3')	Size (bp)	Origin
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			(b) (4)

Name	Sequence (5' → 3')	Size (bp)	Origin
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(b) (4)			

*Mutations are underlined.

Name	Sequence (5' → 3')	Size (bp)	Origin
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(b) (4)			

Name	Sequence (5' → 3')	Size (bp)	Origin
			(b) (4)
			(b) (4)

Name	Sequence (5' → 3')	Size (bp)	Origin
(b) (4)			
(b) (4)			
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B.8 Safety of DNA Modification

(b) (4)

(b) (4)

B.9 Genetic Stability of *C. glutamicum* KCCM 80240

(b) (4)

B.10 Open Reading Frame (ORF) Analysis of Genetically Modified Region

(b) (4)

(b) (4)

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(b) (4)

Table B.10.1. Location of modified gene in genome

Genes	Modification type	Locus	Location in genome
(b) (4)			

(b) (4)

(b) (4)

B.11 Open Reading Frame Analysis of Full Genome Sequence of *C. glutamicum* KCCM 80240

(b) (4)

Table B.11.1. Comparison of ORF between the *C. glutamicum* ATCC14067, *C. glutamicum* CA08-0012 and *C. glutamicum* KCCM80240

Feature	Wild-type strain ATCC 14067	Parental strain CA08-0012	Production strain KCCM 80240
(b) (4)			

(b) (4)

C. SPILL-OVER ANALYSIS

(b) (4)

(b) (4)

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(b) (4)

C.1 Comparison Metabolic Flux of *C. glutamicum* ATCC 14067 and *C. glutamicum* KCCM 80240

(b) (4)

(b) (4)

(b) (4)

Table C.1.1 MFA of *C. glutamicum* ATCC 14067 and *C. glutamicum* KCCM 80240

Pathway	ATCC 14067	KCCM 80240
		(b) (4)

Pathway	ATCC 14067	KCCM 80240 (b) (4)

Pathway	ATCC 14067	KCCM 80240
(b) (4)		

C.2 Comparison of Metabolite in *C. glutamicum* ATCC 14067 and *C. glutamicum* KCCM 80240

(b) (4)

(b) (4)

(b) (4)

(b) (4)

Table C.2.1. Amino acid of *C. glutamicum* ATCC 14067, *C. glutamicum* CA08-0012 and *C. glutamicum* KCCM 80240 fermentation broth (3KL Pilot scale, the end of fermentation)

	ATCC 14067 (g/L)	CA08-0012 (g/L)	KCCM 80240 (g/L)			
	Batch1	Batch1	Batch1	Batch2	Batch3	Ave
OD_{562}	(b) (4)					57.6
Asp						0.00
Thr						0.07
Ser						0.01
Glu						0.20
Gly						0.14
Ala						0.17
Cys						0.00
Val						92.51
Met						0.00
Ile						0.12
Leu						0.14
Tyr						0.11
Phe						0.32
Lys						0.02
His						0.31
Arg						0.00

** Asp: aspartate, Thr: threonine, Ser: serine, Glu: glutamate, Gly: glycine, Ala: alanine, Cys: cysteine, Val: valine, Met: methionine, Ile: isoleucine, Leu: leucine, Tyr: tyrosine, Phe: phenylalanine, Lys: lysine, His: histidine, Arg: arginine*

*** Analytical method: L-Valine-HPLC, Free amino acids (except L-valine)-AOAC 999.13*

(b) (4)

(b) (4)

Table C.2.2. Organic acid of *C. glutamicum* ATCC 14067, *C. glutamicum* CA08-0012 and *C. glutamicum* KCCM 80240 fermentation broth (3KL Pilot scale, the end of fermentation)

	ATCC 14067 (g/L)	CA08-0012 (g/L)	KCCM 80240 (g/L)			
	Batch1	Batch1	Batch1	Batch2	Batch3	Ave.
Citric acid	(b) (4)					0.00
Malic acid						0.04
Succinic acid						0.00
Lactic acid						0.00
Formic acid						0.00
Acetic acid						0.01

** Analytical method: Korean Feed Standards Codex, 1 of chapter 14*

(b) (4)

C.3 Biogenic Amines

(b) (4)

(b) (4)

(b) (4)

Table C.2.6. Biogenic amines of *C. glutamicum* ATCC 14067, *C. glutamicum* CA08-0012 and *C. glutamicum* KCCM 80240 fermentation broth

	ATCC 14067 (mg/L)				CA08-0012 (mg/L)				KCCM 80240 (mg/L)			
	Batch1	Batch2	Batch3	Ave.	Batch1	Batch2	Batch3	Ave.	Batch1	Batch2	Batch3	Ave.
Cadaverine	(b) (4)			0.110	(b) (4)	(b) (4)	(b) (4)	0.128	(b) (4)	(b) (4)	(b) (4)	0.020
Histamine				0.104	(b) (4)	(b) (4)	(b) (4)	0.185	(b) (4)	(b) (4)	(b) (4)	0.153
Phenylethyl-amine				0.067	(b) (4)	(b) (4)	(b) (4)	0.079	(b) (4)	(b) (4)	(b) (4)	0.077
Putrescine				1.015	(b) (4)	(b) (4)	(b) (4)	1.352	(b) (4)	(b) (4)	(b) (4)	0.248
Tryptamine				0.010	(b) (4)	(b) (4)	(b) (4)	0.010	(b) (4)	(b) (4)	(b) (4)	0.008
Tyramine				2.851	(b) (4)	(b) (4)	(b) (4)	3.351	(b) (4)	(b) (4)	(b) (4)	3.654

(b) (4)

D. LIST OF ATTACHMENTS

Attachment 1	Determination of Antibiotic Minimal Inhibitory Concentration (MIC) of *Corynebacterium glutamicum* KCCM 80240, 9 pages
Attachment 2	Determination of viable cells of the production strain in Dried L-Valine Fermentation Product, 16 pages
Attachment 3	Genetic stability of *Corynebacterium glutamicum* KCCM 80240, 7 pages
Attachment 4	Whole genome sequence analysis of *Corynebacterium glutamicum* KCCM 80240, 59 pages
Attachment 5	Metabolic flux analysis of *Corynebacterium glutamicum* KCCM 80240, 15 pages

E. LIST OF REFERENCES

(b) (4)

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APPENDIX 3 - Manufacturing Process (CONFIDENTIAL)

(b) (4)

(b) (4)

1. Raw materials

(b) (4)

Table 1 provides the list of each raw materials to produce Dried L-Valine Fermentation Product.

Table 1. Raw materials for Dried L-Valine Fermentation Product with regulatory status

Item	Regulatory Citation	
Magnesium Sulfate	FDA 21 CFR 582.5443 AAFCO 57.88	IFN 6-26-134
Potassium Phosphate	FDA 21 CFR 582.6285	IFN 6-18-673
Ammonium Sulfate	FDA 21 CFR 582.1143 AAFCO 57.27	IFN 6-09-339
Sulfuric Acid	FDA 21 CFR 582.1095	IFN 6-29-778
Manganese Sulfate	FDA 21 CFR 582.80 AAFCO 57.96	IFN 6-26-136
Iron Sulfate	FDA 21 CFR 582.80	IFN 6-20-734
Antifoam: Polyoxyethylene Polyoxypropylene Block Copolymer (CAS No. 9003-11-6)	FDA 21 CFR 173.340 FDA 21 CFR 172.808(b)(3)	FDA-ETA Letter, 2003
Beet Molasses	AAFCO 63.1	IFN 4-30-289
Biotin	FDA 21 CFR 582.5159	IFN 7-00-723
Copper Sulfate	AAFCO 57.69	IFN 6-01-717
Phosphoric Acid	FDA 21 CFR 582.1073	IFN 6-03-707
Anhydrous ammonia	AAFCO 87.11	IFN 5-14-511
Nicotinamide (Niacinamide)	FDA 21 CFR 582.5535	IFN 7-03-215
Zinc Sulfate	AAFCO 57.118	IFN 6-05-555
Corn Steep Liquor	AAFCO 48.24	
Calcium Pantothenate	FDA 21 CFR 582.5212	IFN 7-07-079
Potassium Hydroxide	AAFCO 57.124	IFN 6-20-870
Thiamine Hydrochloride	FDA 21 CFR 582.5875	IFN 7-04-828

Table 2 provides a summary of the purchasing specifications. All starting materials have been determined to be suitable for animal feed.

Table 2. Specifications of raw materials of Dried L-Valine Fermentation Product

Item	Specifications
Magnesium Sulfate	(b) (4)
Potassium Phosphate	
Ammonium Sulfate	
Sulfuric Acid	
Manganese Sulfate	
Iron Sulfate	
Antifoam: Polyoxyethylene Polyoxypropylene Block Copolymer(CAS No. 9003-11-6)	(b) (4)
Beet Molasses	
Biotin	
Copper Sulfate	
Phosphoric Acid	
Anhydrous ammonia	
Nicotinamide (Niacinamide)	
Zinc Sulfate	
Corn Steep Liquor	
Calcium Pantothenate	
Potassium Hydroxide	
Thiamine Hydrochloride	

2. Fermentation

(b) (4)

(b) (4)

3. Cell inactivation

(b) (4)

4. Concentration

(b) (4)

5. Granulation and drying

(b) (4)

6. Mesh separation and packaging

(b) (4)

CONFIDENTIAL REPORT

Interim report of stability test

: Dried L-Valine Fermentation Product (VAL Pro)

Version 1.0

TITLE

Interim report of stability test: Dried L-Valine Fermentation Product (VAL pro)

OBJECTIVE OF THE STUDY

This study was conducted to establish a shelf life for the Dried L-Valine Fermentation Product (VAL pro) under recommended storage conditions.

SCHEDULE OF THE STUDY

Initiation of experiment: June 26, 2020

Termination of experiment:

Submission of report: December 31, 2020

TESTING FACILITY

Institute of Biotechnology) Scientific and Regulatory Affairs, CJ CheilJedang
55, Gwanggyo-ro 42beon-gil, Yeongtong-gu, Suwon-si, Gyeonggi-do 16495, Korea

RESPONSIBLE STAFFS

Analyst and Author	Ran Young Yoon	
Report approved by	Yang Hee Kim	

CONTENTS

MATERIALS AND METHODS

The stability test of Dried L-Valine Fermentation Product (VAL pro) was conducted in accordance with the ICH HARMONISED TRIPARTITE GUIDELINE [1].

Information of test sample

1) Sample: Dried L-Valine Fermentation Product (VAL pro)

- L-Valine (dry base): not less than 72%
- Moisture: not more than 5.0%

2) Batch number: NGVAL191221, NGVAL191222, NGVAL191223

Storage condition

1) Packaging: Polypropylene woven bag and 1 ply polyethylene inner

2) Weight of storage sample: 50 g / bag

3) Temperature and humidity of storage

- General condition: 25°C ± 2°C and 60% RH ± 5% RH
- Accelerated condition: 40°C ± 2°C and 75% RH ± 5% RH

4) Testing frequency: Initial, 1, 3, 4, 6 months

Analysis method

1) Content of L-valine: HPLC-FLD

Parameter	Condition
System	HPLC
Detector	Fluorescence detector (Excitation λ : 338 nm Emission λ : 425 nm)
Column	ODS C18, 150 x 4.6 mm, particle size 3 µm
Column temperature	40 °C
Mobile phase	16.7 mM-KH_2PO_4 + 5 mM OSA in 12% CH_3CN, pH 2.5 (by H_3PO_4)
Flow rate of mobile phage	1.0 ml/min
Reaction reagent	201.91 mM-KOH + 241.39 mM-H_3BO_3 + 2.53 mM-OPA + C_2H_6OS 1 mL + CH_3OH 5 mL + 3.5 %-Brij 1.25 mL
Flow rate of reaction reagent	0.5 ml/min
Sample temperature	15 °C
Injection volume	5 µl

2) Moisture: Loss on drying (AOAC 934.01)

RESULTS

Table 1. General condition (25°C/60% RH)

Test items	Specification	Batch No.	Initial	1 month	3 month	4 month	6 month
L-Valine (% dry base)	≥ 72.0	NGVAL191221	(b) (4)				
		NGVAL191222					
		NGVAL191223					
Moisture (%)	≤ 5.0	NGVAL191221					
		NGVAL191222					
		NGVAL191223					

Table 2. Accelerated condition (40°C/75% RH)

Test items	Specification	Batch No.	Initial	1 month	3 month	4 month	6 month
L-Valine (% dry base)	≥ 72.0	NGVAL191221	(b) (4)				
		NGVAL191222					
		NGVAL191223					
Moisture (%)	≤ 5.0	NGVAL191221					
		NGVAL191222					
		NGVAL191223					

REFERENCES

[1] ICH Harmonised Tripartite Guideline. Q1A(R2) Stability Testing of New Drug Substances and Products. 6 February 2003.

[APPENDIX 1] Certificate of Analysis

A. CJ L-Valine Fermentation Product (Lot number: NGVAL191221)

CJ Research Institute of Biotechnology

55, Gwanggyo-ro 42beon-gil, Yeongtong-gu, Suwon-si, Gyeonggi-do, 16495, Korea

www.cj.co.kr

TEL : 031) 8099-2450 FAX : 031) 8099-2918

Certificate of analysis

Certificate No.	2020-PR-129	Receipt No.	2020-AN-103
Client	-	Date of Receipt	2020.11.25.
Client Name	-	Date of Test	2020.12.11
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine(Val pro)
Manuf. Date	2019.12.21
Lot. No	NGVAL191221
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 72 %	(b) (4)	HPLC
Moisture(Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

The Test Report cannot be reproduced, except in full.

Tested by (b) (4)

Approved by Technical Manager (b) (4)

Dec, 22, 2020

CJ Research Institute of Biotechnology

CJ BIO-AD form 100-01 REV.01

B. CJ L-Valine Fermentation Product (Lot number: NGVAL191222)

CJ Research Institute of Biotechnology

55, Gwanggyo-ro 42beon-gil, Yeongtong-gu, Suwon-si, Gyeonggi-do, 16495, Korea

www.cj.co.kr

TEL : 031) 8099-2450 FAX : 031) 8099-2918

Certificate of analysis

Certificate No.	2020-PR-130	Receipt No.	2020-AN-104
Client	-	Date of Receipt	2020.11.25.
Client Name	-	Date of Test	2020.12.11
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine(Val pro)
Manuf. Date	2019.12.22
Lot. No	NGVAL191222
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 72 %	(b) (4)	HPLC
Moisture(Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

The Test Report cannot be reproduced, except in full.

Tested by (b) (4)

Approved by Technical Manager (b) (4)

Dec, 22, 2020

CJ Research Institute of Biotechnology

CJ BIO-AD form 100-01 REV.01

C. CJ L-Valine Fermentation Product (Lot number: NGVAL191223)

CJ Research Institute of Biotechnology

55, Gwanggyo-ro 42beon-gil, Yeongtong-gu, Suwon-si, Gyeonggi-do, 16495, Korea

www.cj.co.kr

TEL : 031) 8099-2450 FAX : 031) 8099-2918

Certificate of analysis

Certificate No.	2020-PR-131	Receipt No.	2020-AN-105
Client	-	Date of Receipt	2020.11.25.
Client Name	-	Date of Test	2020.12.11
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine(Val pro)
Manuf. Date	2019.12.23
Lot. No	NGVAL191223
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 72 %	(b) (4)	HPLC
Moisture(Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.
The Test Report cannot be reproduced, except in full.

Tested by (b) (4)

Approved by Technical Manager (b) (4)

Dec, 22, 2020

CJ Research Institute of Biotechnology

CJ BIO-AD form 100-01 REV.01

CONFIDENTIAL REPORT

Stability of Dried L-Valine Fermentation Product (VAL Pro) in mash feed

Version 1.0

TITLE

Stability of Dried L-Valine Fermentation Product (VAL Pro) in mash feed

OBJECTIVE OF THE STUDY

This study was conducted to examine the stability of Dried L-Valine Fermentation Product (VAL Pro) in mash feed.

SCHEDULE OF THE STUDY

Initiation of experiment: November 24, 2020

Termination of experiment: February 26, 2021

Submission of report: February 26, 2021

TESTING FACILITY

Institute of Biotechnology) Scientific and Regulatory Affairs, CJ CheilJedang
55, Gwanggyo-ro 42beon-gil, Yeongtong-gu, Suwon-si, Gyeonggi-do 16495, Korea

RESPONSIBLE STAFFS

Analyst and Author	Ran Young Yoon	
Report approved by	Yang Hee Kim	

CONTENTS

MATERIALS AND METHODS

Information of product

1) Name: Dried L-Valine Fermentation Product (VAL pro)

2) L-Valine (dry basis): not less than 72%

3) Batch number: GVAL200910, GVAL200911, GVAL200912

Information of mash feed

1) Target animals: broiler, swine

2) Information of ingredients

Ingredient	Product name	Supplier
Corn	PNW corn	(b) (4)
Soybean meal (44% CP)	Soybean meal	CJ cheiljedang

2) Formulation of the mash feed

Composition	Percentage (%)
Corn	78.8
Soybean meal (44% CP)	19.8
Soybean oil	1.0
Analytical components	**Percentage (%)**
Crude protein	14.56
Crude fat	2.88
Crude fiber	2.39
Crude ash	2.04
Calcium	0.02
Phosphorous	0.20
Metabolisable energy	3.54 Mkcal/kg

Sample preparation

1) Added amount of Dried L-Valine Fermentation Product (VAL Pro): 0.4%

2) Preparation of mash feed with VAL pro

	Amount (g, batch)
Mash feed	48,000
Dried L-Valine Fermentation Product (VAL Pro)	200
(Total) Mash feed with VAL pro	50,000

Homogeneity test

Before the storage, homogeneity of Dried L-Valine Fermentation Product in mash feed was confirmed by analyzing L-valine in the randomly collected samples (Table 1).

Storage condition

1) Packaging: Polypropylene woven bag and 1 ply polyethylene inner

2) Weight of storage samples: 100 g/bag

3) Temperature and humidity of storage: 25°C ± 2°C and 60% RH ± 5% RH

4) Storage period: 3 months

Analysis of sample

1) Pretreatment of sample
: 2.5 g of the sample was weighed, put into 100 mL of volumetric flask and add approximately 70 ml ultra-purified water. In addition, sonicate for 30 min, reduce the temperature of solution and then adjusted the final volume to 100 mL with ultra-purified water. Filter a suitable amount of the test solution through membrane filter unit, into autosampler vials and inject into analyzer.

2) Contents of L-valine: HPLC-FLD

Parameter	Condition
System	HPLC
Detector	Fluorescence detector (Excitation λ : 338 nm Emission λ : 425 nm)
Column	ODS C18, 150 x 4.6 mm, particle size 3 µm
Column temperature	40 °C
Mobile phase	16.7 mM-KH_2PO_4 + 5 mM OSA in 12% CH_3CN, pH 2.5 (by H_3PO_4)
Flow rate of mobile phage	1.0 ml/min
Reaction reagent	201.91 mM-KOH + 241.39 mM-H_3BO_3 + 2.53 mM-OPA + C_2H_6OS 1 mL + CH_3OH 5 mL + 3.5 %-Brij 1.25 mL
Flow rate of reaction reagent	0.5 ml/min
Sample temperature	15 °C
Injection volume	5 µl

RESULTS

Dried L-Valine Fermentation Product (VAL Pro) was mixed into the mash feed with an addition rate of 0.40 %. Taking into account the L-valine content of 72.17–72.22%, the nominal content in the mash feed is approximately 0.28 %.

The homogeneity of Dried L-Valine Fermentation Product in mash feed was observed first. As shown in Table 1, analyzed sample showed good homogeneity. The result indicated that Dried L-Valine Fermentation Product is mixed well in mash feed with good homogeneity.

Table 1. Homogeneity of Dried L-Valine Fermentation Product in mash feed

Sample	L-Valine (%, dry basis) * Added amount 0.40 %, resulting nominal value approx. 0.28 %
Blank value of mash feed	(b) (4)
mash feed with VAL pro GVAL200910 - 1	
mash feed with VAL pro GVAL200910 - 2	
mash feed with VAL pro GVAL200910 - 3	
mash feed with VAL pro GVAL200911- 1	
mash feed with VAL pro GVAL200911- 2	
mash feed with VAL pro GVAL200911- 3	
mash feed with VAL pro GVAL200912 - 1	
mash feed with VAL pro GVAL200912 - 2	
mash feed with VAL pro GVAL200912 - 3	
Mean value and standard deviation	0.30 ± 0.010

The stability data of Dried L-Valine Fermentation Product in mash feed are summarized in Table 2. The contents of L-valine in mash feed was not changed for 3 months.

Table 2. Stability of Dried L-Valine Fermentation Product in mash feed

Sample	Unit	Initial	1 month	2 month	3 month
Mash feed with VAL pro GVAL200910	%	(b) (4)			
Mash feed with VAL pro GVAL200911					
Mash feed with VAL pro GVAL200912					

[APPENDIX 1] Certificate of Analysis – Test Sample

A. CJ Dried L-Valine Fermentation Product (Lot number: GVAL200910)

CJ Research Institute of Biotechnology	
55, Gwanggyo-ro 42beon-gil, Yeongtong-gu, Suwon-si, Gyeonggi-do, 16495, Korea www.cj.co.kr TEL : 031) 8099-2450 FAX : 031) 8099-2918	CJ CHEILJEDANG BIO

Certificate of analysis

Certificate No.	2020-PR-132	Receipt No.	2020-AN-106
Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.11.23.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.10.
Lot. No	GVAL200910
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 72 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.
The Test Report cannot be reproduced, except in full.

Tested by (b) (4)

Approved by Technical Manager (b) (4)

Dec, 23, 2020

CJ Research Institute of Biotechnology

CJ BIO-AD form 100-01 REV.01

B. CJ Dried L-Valine Fermentation Product (Lot number: GVAL200911)

CJ Research Institute of Biotechnology

55, Gwanggyo-ro 42beon-gil, Yeongtong-gu, Suwon-si, Gyeonggi-do, 16495, Korea

www.cj.co.kr

TEL : 031) 8099-2450 FAX : 031) 8099-2918

Certificate of analysis

Certificate No.	2020-PR-133	Receipt No.	2020-AN-107
Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.11.23.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.11.
Lot. No	GVAL200911
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 72 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

The Test Report cannot be reproduced, except in full.

Tested by (b) (4)

Approved by Technical Manager (b) (4)

Dec, 23, 2020

CJ Research Institute of Biotechnology

CJ BIO-AD form 100-01 REV.01

C. CJ Dried L-Valine Fermentation Product (Lot number: GVAL200912)

CJ Research Institute of Biotechnology

55, Gwanggyo-ro 42beon-gil, Yeongtong-gu, Suwon-si, Gyeonggi-do, 16495, Korea

www.cj.co.kr

TEL : 031) 8099-2450 FAX : 031) 8099-2918

Certificate of analysis

Certificate No.	2020-PR-134	Receipt No.	2020-AN-108
Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.11.23.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.12.
Lot. No	GVAL200912
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 72 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

The Test Report cannot be reproduced, except in full.

Tested by (b) (4)

Approved by Technical Manager (b) (4)

Dec, 23, 2020

CJ Research Institute of Biotechnology

CJ BIO-AD form 100-01 REV.01

[APPENDIX 2] Certificate of Analysis – Homogeneity and Stability

. Homogeneity_mash feed with VAL pro GVAL200910-1

CJ Research Institute of Biotechnology

55, Gwanggyo-ro 42beon-gil, Yeongtong-gu, Suwon-si, Gyeonggi-do, 16495, Korea

www.cj.co.kr

TEL : 031) 8099-2450 FAX : 031) 8099-2918

Certificate of analysis

Certificate No.	2021-PR-037	Receipt No.	2020-AN-126
Client	-	Date of Receipt	2020.11.24.
Client Name	-	Date of Test	2020.12.05.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	mash feed with VAL pro GVAL200910-1
Manuf. Date	2020.11.23.
Quantity (kg)	-

Test Item(s)	Test Result	Test method used
L-valine	(b) (4)	HPLC

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

The Test Report cannot be reproduced except in full.

Tested by (b) (4)

Approved by Technical Manager (b) (4)

Feb, 26, 2021

CJ Research Institute of Biotechnology

CJ BIO-AD form 100-01 REV.01

. Homogeneity_mash feed with VAL pro GVAL200910-2

CJ Research Institute of Biotechnology

55, Gwanggyo-ro 42beon-gil, Yeongtong-gu, Suwon-si, Gyeonggi-do, 16495, Korea

www.cj.co.kr

TEL : 031) 8099-2450 FAX : 031) 8099-2918

Certificate of analysis

Certificate No.	2021-PR-038	Receipt No.	2020-AN-127
Client	-	Date of Receipt	2020.11.24.
Client Name	-	Date of Test	2020.12.05.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	mash feed with VAL pro GVAL200910-2
Manuf. Date	2020.11.23.
Quantity (kg)	-

Test Item(s)	Test Result	Test method used
L-valine	(b) (4)	HPLC

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

The Test Report cannot be reproduced, except in full.

Tested by (b) (4)

Approved by Technical Manager (b) (4)

Feb, 26, 2021

CJ Research Institute of Biotechnology

CJ BIO-AD form 100-01 REV.01

. Homogeneity_mash feed with VAL pro GVAL200910-3

CJ Research Institute of Biotechnology

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TEL : 031) 8099-2450 FAX : 031) 8099-2918

Certificate of analysis

Certificate No.	2021-PR-039	Receipt No.	2020-AN-128
Client	-	Date of Receipt	2020.11.24.
Client Name	-	Date of Test	2020.12.05.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	mash feed with VAL pro GVAL200910-3
Manuf. Date	2020.11.23.
Quantity (kg)	-

Test Item(s)	Test Result	Test method used
L-valine	(b) (4)	HPLC

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

The Test Report cannot be reproduced, except in full.

Tested by (b) (4)

Approved by Technical Manager (b) (4)

Feb, 26, 2021

CJ Research Institute of Biotechnology

CJ BIO-AD form 100-01 REV.01

. Homogeneity_mash feed with VAL pro GVAL200911-1

CJ Research Institute of Biotechnology

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www.cj.co.kr

TEL : 031) 8099-2450 FAX : 031) 8099-2918

Certificate of analysis

Certificate No.	2021-PR-040	Receipt No.	2020-AN-129
Client	-	Date of Receipt	2020.11.24.
Client Name	-	Date of Test	2020.12.05.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	mash feed with VAL pro GVAL200911-1
Manuf. Date	2020.11.23.
Quantity (kg)	-

Test Item(s)	Test Result	Test method used
L-valine	(b) (4)	HPLC

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.
The Test Report cannot be reproduced, except in full.

Tested by (b) (4)

Approved by Technical Manager (b) (4)

Feb, 26, 2021

CJ Research Institute of Biotechnology

CJ BIO-AD form 100-01 REV.01

. Homogeneity_mash feed with VAL pro GVAL200911-2

CJ Research Institute of Biotechnology

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TEL : 031) 8099-2450 FAX : 031) 8099-2918

Certificate of analysis

Certificate No.	2021-PR-041	Receipt No.	2020-AN-130
Client	-	Date of Receipt	2020.11.24.
Client Name	-	Date of Test	2020.12.05.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	mash feed with VAL pro GVAL200911-2
Manuf. Date	2020.11.23.
Quantity (kg)	-

Test Item(s)	Test Result	Test method used
L-valine	(b) (4)	HPLC

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.
The Test Report cannot be reproduced, except in full.

Tested by (b) (4)

Approved by Technical Manager (b) (4)

Feb, 26, 2021

CJ Research Institute of Biotechnology

CJ BIO-AD form 100-01 REV.01

. Homogeneity_mash feed with VAL pro GVAL200911-3

CJ Research Institute of Biotechnology

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www.cj.co.kr

TEL : 031) 8099-2450 FAX : 031) 8099-2918

Certificate of analysis

Certificate No.	2021-PR-042	Receipt No.	2020-AN-131
Client	-	Date of Receipt	2020.11.24.
Client Name	-	Date of Test	2020.12.05.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	mash feed with VAL pro GVAL200911-3
Manuf. Date	2020.11.23.
Quantity (kg)	-

Test Item(s)	Test Result	Test method used
L-valine	(b) (4)	HPLC

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

The Test Report cannot be reproduced, except in full.

Tested by (b) (4)

Approved by Technical Manager (b) (4)

Feb, 26, 2021

CJ Research Institute of Biotechnology

CJ BIO-AD form 100-01 REV.01

. Homogeneity_mash feed with VAL pro GVAL200912-1

CJ Research Institute of Biotechnology

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www.cj.co.kr

TEL : 031) 8099-2450 FAX : 031) 8099-2918

Certificate of analysis

Certificate No.	2021-PR-043	Receipt No.	2020-AN-132
Client	-	Date of Receipt	2020.11.24.
Client Name	-	Date of Test	2020.12.05.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	mash feed with VAL pro GVAL200912-1
Manuf. Date	2020.11.23.
Quantity (kg)	-

Test Item(s)	Test Result	Test method used
L-valine	(b) (4)	HPLC

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.
The Test Report cannot be reproduced, except in full.

Tested by (b) (4)

Approved by Technical Manager (b) (4)

Feb, 26, 2021

CJ Research Institute of Biotechnology

CJ BIO-AD form 100-01 REV.01

. Homogeneity_mash feed with VAL pro GVAL200912-2

CJ Research Institute of Biotechnology

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TEL : 031) 8099-2450 FAX : 031) 8099-2918

Certificate of analysis

Certificate No.	2021-PR-044	Receipt No.	2020-AN-133
Client	-	Date of Receipt	2020.11.24.
Client Name	-	Date of Test	2020.12.05.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	mash feed with VAL pro GVAL200912-2
Manuf. Date	2020.11.23.
Quantity (kg)	-

Test Item(s)	Test Result	Test method used
L-valine	(b) (4)	HPLC

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

The Test Report cannot be reproduced, except in full.

Tested by (b) (4)

Approved by Technical Manager (b) (4)

Feb, 26, 2021

CJ Research Institute of Biotechnology

CJ BIO-AD form 100-01 REV.01

. Homogeneity_mash feed with VAL pro GVAL200912-3

CJ Research Institute of Biotechnology

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TEL : 031) 8099-2450 FAX : 031) 8099-2918

Certificate of analysis

Certificate No.	2021-PR-045	Receipt No.	2020-AN-134
Client	-	Date of Receipt	2020.11.24.
Client Name	-	Date of Test	2020.12.05.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	mash feed with VAL pro GVAL200912-3
Manuf. Date	2020.11.23.
Quantity (kg)	-

Test Item(s)	Test Result	Test method used
L-valine	(b) (4)	HPLC

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.
The Test Report cannot be reproduced, except in full.

Tested by (b) (4)

Approved by Technical Manager (b) (4)

Feb, 26, 2021

CJ Research Institute of Biotechnology

CJ BIO-AD form 100-01 REV.01

. Stability initial_mash feed with VAL pro GVAL200910

CJ Research Institute of Biotechnology

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TEL : 031) 8099-2450 FAX : 031) 8099-2918

Certificate of analysis

Certificate No.	2021-PR-046	Receipt No.	2020-AN-135
Client	-	Date of Receipt	2020.11.24.
Client Name	-	Date of Test	2020.12.05.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	mash feed with VAL pro GVAL200910
Manuf. Date	2020.11.23.
Quantity (kg)	-

Test Item(s)	Test Result	Test method used
L-valine	(b) (4)	HPLC

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.
The Test Report cannot be reproduced, except in full.

(b) (4)

Approved by Technical Manager

Feb, 26, 2021

CJ Research Institute of Biotechnology

CJ BIO-AD form 100-01 REV.01

. Stability initial_mash feed with VAL pro GVAL200911

CJ Research Institute of Biotechnology

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TEL : 031) 8099-2450 FAX : 031) 8099-2918

Certificate of analysis

Certificate No.	2021-PR-047	Receipt No.	2020-AN-136
Client	-	Date of Receipt	2020.11.24.
Client Name	-	Date of Test	2020.12.05.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	mash feed with VAL pro GVAL200911
Manuf. Date	2020.11.23.
Quantity (kg)	-

Test Item(s)	Test Result	Test method used
L-valine	(b) (4)	HPLC

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.
The Test Report cannot be reproduced, except in full.

Tested by (b) (4)

Approved by Technical Manager (b) (4)

Feb, 26, 2021

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CJ BIO-AD form 100-01 REV.01

. Stability initial_mash feed with VAL pro GVAL200912

CJ Research Institute of Biotechnology

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Certificate of analysis

Certificate No.	2021-PR-048	Receipt No.	2020-AN-137
Client	-	Date of Receipt	2020.11.24.
Client Name	-	Date of Test	2020.12.05.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	mash feed with VAL pro GVAL200912
Manuf. Date	2020.11.23.
Quantity (kg)	-

Test Item(s)	Test Result	Test method used
L-valine	(b) (4)	HPLC

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

The Test Report cannot be reproduced, except in full.

Tested by (b) (4)

Approved by Technical Manager

Feb, 26, 2021

CJ Research Institute of Biotechnology

CJ BIO-AD form 100-01 REV.01

. Stability 1 month_mash feed with VAL pro GVAL200910

CJ Research Institute of Biotechnology

55, Gwanggyo-ro 42beon-gil, Yeongtong-gu, Suwon-si, Gyeonggi-do, 16495, Korea

www.cj.co.kr

TEL : 031) 8099-2450 FAX : 031) 8099-2918

Certificate of analysis

Certificate No.	2021-PR-049	Receipt No.	2020-AN-138
Client	-	Date of Receipt	2020.12.22.
Client Name	-	Date of Test	2020.12.22.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	mash feed with VAL pro GVAL200910
Manuf. Date	2020.11.23
Quantity (kg)	-

Test Item(s)	Test Result	Test method used
L-valine	(b) (4)	HPLC

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

The Test Report cannot be reproduced, except in full.

Tested by (b) (4)

Approved by Technical Manager (b) (4)

Feb, 26, 2021

CJ Research Institute of Biotechnology

CJ BIO-AD form 100-01 REV.01

. Stability 1 month_mash feed with VAL pro GVAL200911

CJ Research Institute of Biotechnology

55, Gwanggyo-ro 42beon-gil, Yeongtong-gu, Suwon-si, Gyeonggi-do, 16495, Korea

www.cj.co.kr

TEL : 031) 8099-2450 FAX : 031) 8099-2918

Certificate of analysis

Certificate No.	2021-PR-050	Receipt No.	2020-AN-139
Client	-	Date of Receipt	2020.12.22.
Client Name	-	Date of Test	2020.12.22.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	mash feed with VAL pro GVAL200911
Manuf. Date	2020.11.23
Quantity (kg)	-

Test Item(s)	Test Result	Test method used
L-valine	(b) (4)	HPLC

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

The Test Report cannot be reproduced, except in full.

Tested by (b) (4)

Approved by Technical Manager

Feb, 26, 2021

CJ Research Institute of Biotechnology

CJ BIO-AD form 100-01 REV.01

. Stability 1 month_mash feed with VAL pro GVAL200912

CJ Research Institute of Biotechnology

55, Gwanggyo-ro 42beon-gil, Yeongtong-gu, Suwon-si, Gyeonggi-do, 16495, Korea

www.cj.co.kr

TEL : 031) 8099-2450 FAX : 031) 8099-2918

Certificate of analysis

Certificate No.	2021-PR-051	Receipt No.	2020-AN-140
Client	-	Date of Receipt	2020.12.22.
Client Name	-	Date of Test	2020.12.22.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	mash feed with VAL pro GVAL200912
Manuf. Date	2020.11.23
Quantity (kg)	-

Test Item(s)	Test Result	Test method used
L-valine	(b) (4)	HPLC

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.
The Test Report cannot be reproduced, except in full.

Tested by (b) (4)

Approved by Technical Manager

Feb, 26, 2021

CJ Research Institute of Biotechnology

CJ BIO-AD form 100-01 REV.01

. Stability 2 month_mash feed with VAL pro GVAL200910

CJ Research Institute of Biotechnology

55, Gwanggyo-ro 42beon-gil, Yeongtong-gu, Suwon-si, Gyeonggi-do, 16495, Korea

www.cj.co.kr

TEL : 031) 8099-2450 FAX : 031) 8099-2918

Certificate of analysis

Certificate No.	2021-PR-052	Receipt No.	2021-AN-025
Client	-	Date of Receipt	2021.01.23.
Client Name	-	Date of Test	2021.01.28.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	mash feed with VAL pro GVAL200910
Manuf. Date	2020.11.23
Quantity (kg)	-

Test Item(s)	Test Result	Test method used
L-valine	(b) (4)	HPLC

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

The Test Report cannot be reproduced except in full.

Tested by (b) (4)

Approved by Technical Manager (b) (4)

Feb, 26, 2021

CJ Research Institute of Biotechnology

CJ BIO-AD form 100-01 REV.01

. Stability 2 month_mash feed with VAL pro GVAL200911

CJ Research Institute of Biotechnology

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www.cj.co.kr

TEL : 031) 8099-2450 FAX : 031) 8099-2918

Certificate of analysis

Certificate No.	2021-PR-053	Receipt No.	2021-AN-026
Client	-	Date of Receipt	2021.01.23.
Client Name	-	Date of Test	2021.01.28.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	mash feed with VAL pro GVAL200911
Manuf. Date	2020.11.23
Quantity (kg)	-

Test Item(s)	Test Result	Test method used
L-valine	(b) (4)	HPLC

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

The Test Report cannot be reproduced, except in full.

Tested by (b) (4)

Approved by Technical Manager (b) (4)

Feb, 26, 2021

CJ Research Institute of Biotechnology

CJ BIO-AD form 100-01 REV.01

. Stability 2 month_mash feed with VAL pro GVAL200912

CJ Research Institute of Biotechnology

55, Gwanggyo-ro 42beon-gil, Yeongtong-gu, Suwon-si, Gyeonggi-do, 16495, Korea

www.cj.co.kr

TEL : 031) 8099-2450 FAX : 031) 8099-2918

Certificate of analysis

Certificate No.	2021-PR-054	Receipt No.	2021-AN-027
Client	-	Date of Receipt	2021.01.23.
Client Name	-	Date of Test	2021.01.28.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	mash feed with VAL pro GVAL200912
Manuf. Date	2020.11.23
Quantity (kg)	-

Test Item(s)	Test Result	Test method used
L-valine	(b) (4)	HPLC

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.
The Test Report cannot be reproduced, except in full.

Tested by (b) (4)

Approved by Technical Manager (b) (4)

Feb, 26, 2021

CJ Research Institute of Biotechnology

CJ BIO-AD form 100-01 REV.01

. Stability 3 month_mash feed with VAL pro GVAL200910

CJ Research Institute of Biotechnology

55, Gwanggyo-ro 42beon-gil, Yeongtong-gu, Suwon-si, Gyeonggi-do, 16495, Korea

www.cj.co.kr

TEL : 031) 8099-2450 FAX : 031) 8099-2918

Certificate of analysis

Certificate No.	2021-PR-055	Receipt No.	2021-AN-028
Client	-	Date of Receipt	2021.02.23.
Client Name	-	Date of Test	2021.02.24.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	mash feed with VAL pro GVAL200910
Manuf. Date	2020.11.23
Quantity (kg)	-

Test Item(s)	Test Result	Test method used
L-valine	(b) (4)	HPLC

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

The Test Report cannot be reproduced, except in full.

Tested by (b) (4)

Approved by Technical Manager (b) (4)

Feb, 26, 2021

CJ Research Institute of Biotechnology

CJ BIO-AD form 100-01 REV.01

. Stability 3 month_mash feed with VAL pro GVAL200911

CJ Research Institute of Biotechnology

55, Gwanggyo-ro 42beon-gil, Yeongtong-gu, Suwon-si, Gyeonggi-do, 16495, Korea

www.cj.co.kr

TEL : 031) 8099-2450 FAX : 031) 8099-2918

Certificate of analysis

Certificate No.	2021-PR-056	Receipt No.	2021-AN-029
Client	-	Date of Receipt	2021.02.23.
Client Name	-	Date of Test	2021.02.24.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	mash feed with VAL pro GVAL200911
Manuf. Date	2020.11.23
Quantity (kg)	-

Test Item(s)	Test Result	Test method used
L-valine	(b) (4)	HPLC

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

The Test Report cannot be reproduced, except in full.

Tested by (b) (4)

Approved by Technical Manager (b) (4)

Feb, 26, 2021

CJ Research Institute of Biotechnology

CJ BIO-AD form 100-01 REV.01

. Stability 3 month_mash feed with VAL pro GVAL200912

CJ Research Institute of Biotechnology

55, Gwanggyo-ro 42beon-gil, Yeongtong-gu, Suwon-si, Gyeonggi-do, 16495, Korea

www.cj.co.kr

TEL : 031) 8099-2450 FAX : 031) 8099-2918

Certificate of analysis

Certificate No.	2021-PR-057	Receipt No.	2021-AN-030
Client	-	Date of Receipt	2021.02.23.
Client Name	-	Date of Test	2021.02.24.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	mash feed with VAL pro GVAL200912
Manuf. Date	2020.11.23
Quantity (kg)	-

Test Item(s)	Test Result	Test method used
L-valine	(b) (4)	HPLC

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

The Test Report cannot be reproduced, except in full.

Tested by (b) (4)

Approved by Technical Manager (b) (4)

Feb, 26, 2021

CJ Research Institute of Biotechnology

CJ BIO-AD form 100-01 REV.01

AOAC Official Method 2015.01
Heavy Metals in Food

Inductively Coupled Plasma–Mass Spectrometry
First Action 2015

Note: The following is not intended to be used as a comprehensive training manual. Analytical procedures are written based on the assumption that they will be performed by technicians who are formally trained in at least the basic principles of chemical analysis and in the use of the subject technology.

{Applicable for the determination of heavy metals [arsenic (As), CAS No. 7440-38-2; cadmium (Cd), CAS No. 7440-43-9; lead (Pb), CAS No. 7439-92-1; and mercury (Hg), CAS No. 7439-97-6] at trace levels in food and beverage samples, including solid chocolate, fruit juice, fish, infant formula, and rice, using microwave digestion and inductively coupled plasma–mass spectrometry (ICP-MS).}

Caution: Nitric acid and hydrochloric acid are corrosive. When working with these acids, wear adequate protective gear, including eye protection, gloves with the appropriate resistance, and a laboratory coat. Use an adequate fume hood for all acids.

Hydrogen peroxide is a strong oxidizer and can react violently with organic material to give off oxygen gas and heat. Adequate protective gear should be worn.

Many of the chemicals have toxicities that are not well established and must be handled with care. For all known chemicals used, consult the Material Safety Data Sheet (MSDS) in advance.

The inductively coupled plasma–mass spectrometer emits UV light when the plasma is on. UV resistant goggles should be worn if working near the plasma.

The instrument generates high levels of radio frequency (RF) energy and is very hot when the plasma is on. In the case of an instrument failure, be aware of these potential dangers.

Safely store interference reduction technology (IRT) gases, such as oxygen, in a closed, ventilated cabinet. Use adequate caution with pressurized gases. Prior training or experience is necessary to change any gas cylinders. Oxygen gas can cause many materials to ignite easily.

Following microwave digestion, samples are hot to the touch. Allow the samples to cool to room temperature before opening the digestion vessels to avoid unexpected depressurization and potential release of toxic fumes.

A. Principle

Food samples are thoroughly homogenized and then prepared by microwave digestion and the addition of dilute solutions of gold (Au) and lutetium (Lu). The Au is used to stabilize the Hg in the preparation, and the Lu is used to assess the potential loss of analyte during the microwave digestion process.

A prepared, diluted, aqueous sample digestate is pumped through a nebulizer, where the liquid forms an aerosol as it enters a spray chamber. The aerosol separates into a fine aerosol mist and larger aerosol droplets. The larger droplets exit the spray chamber while the fine mist is transported into the ICP torch.

Inside the ICP torch, the aerosol mist is transported into a high-temperature plasma, where it becomes atomized and ionized as it passes through an RF load coil. The ion stream is then focused by a single ion

lens through a cylinder with a carefully controlled electrical field. For instruments equipped with dynamic reaction cell (DRC) or collision cell IRT, the focused ion stream is directed into the reaction/collision cell where, when operating with a pressurized cell, the ion beam will undergo chemical modifications and/or collisions to reduce elemental interferences. When not operating with a pressurized cell, the ion stream will remain focused as it passes through the cell with no chemical modification taking place.

The ion stream is then transported to the quadrupole mass filter, where only ions having a desired mass-to-charge ratio (*m/z*) are passed through at any moment in time. The ions exiting the mass filter are detected by a solid-state detector and the signal is processed by the data handling system.

B. Equipment

Perform routine preventative maintenance for the equipment used in this procedure.

An ultra-clean laboratory environment is critical for the successful production of quality data at ultra-low levels. All sample preparation must take place in a clean hood (Class 100). Metallic materials should be kept to a minimum in the laboratory and coated with an acrylic polymer gel where possible. Adhesive floor mats should be used at entrances to the laboratory and changed regularly to prevent the introduction of dust and dirt from the outside environment. Wear clean-room gloves and change whenever contact is made with anything non-ultra-clean. The laboratory floor should be wiped regularly to remove any particles without stirring up dust. *Note:* "Ultra-clean" (tested to be low in the analytes of interest) reagents, laboratory supplies, facilities, and sample handling techniques are required to minimize contamination in order to achieve the trace-level detection limits described herein.

(**a**) *Instrumentation*.--ICP-MS instrument, equipped with IRT with a free-running 40 MHz RF generator; and controllers for nebulizer, plasma, auxiliary, and reaction/collision flow control. The quadrupole mass spectrometer has a mass range of 5 to 270 atomic mass units (amu). The turbo molecular vacuum system achieves 10^{-6} torr or better. Recommended ICP-MS components include an RF coil, platinum skimmer and sampler cones, Peltier-cooled quartz cyclonic spray chamber, quartz or sapphire injector, micronebulizer, variable speed peristaltic pump, and various types of tubing (for gases, waste, and peristaltic pump). *Note*: The procedure is written specifically for use with a PerkinElmer ELAN DRC II ICP-MS (www.perkinelmer.com). Equivalent procedures may be performed on any type of ICP-MS instrument with equivalent IRT if the analyst is fully trained in the interpretation of spectral and matrix interferences and procedures for their correction, including the optimization of IRT. For example, collision cell IRT can be used for arsenic determination using helium gas.

(**b**) *Gases*.--High-purity grade liquid argon (>99.996%). Additional gases are required for IRT (such as ultra-x grade, 99.9999% minimum purity oxygen, used for determination of As in DRC mode with some PerkinElmer ICP-MS instruments).

(**c**) *Analytical balance*.--Standard laboratory balance suitable for sample preparation and capable of measuring to 0.1 mg.

(**d**) *Clean-room gloves*.--Tested and certified to be low in the metals of interest.

(**e**) *Microwave digestion system*.--Laboratory microwave digestion system with temperature control and an adequate supply of chemically inert digestion vessels. The microwave should be appropriately vented and corrosion resistant.

(*1*) The microwave digestion system must sense the temperature to within ±2.5°C and automatically adjust the microwave field output power within 2 s of sensing. Temperature sensors should be accurate

to ±2°C (including the final reaction temperature of 190°C). Temperature feedback control provides the primary control performance mechanism for the method.

(*2*) The use of microwave equipment with temperature feedback control is required to control the unfamiliar reactions of unique or untested food or beverage samples. These tests may require additional vessel requirements, such as increased pressure capabilities.

(**f**) *Autosampler cups.*--15 and 50 mL; vials are precleaned by soaking in 2-5% (v/v) HNO_3 overnight, rinsed three times with reagent water/deionized water (DIW), and dried in a laminar flow clean hood. For the 50 mL vials, as these are used to prepare standards and bring sample preparations to final volume, the bias and precision of the vials must be assessed and documented prior to use. The recommended procedure for this is as follows:

(1) For every case of vials from the same lot, remove 10 vials.

(2) Tare each vial on an analytical balance, and then add reagent water up to the 20 mL mark. Repeat procedure by adding reagent water up to the 50 mL mark.

(3) Measure and record the mass of reagent water added, and then calculate the mean and RSD of the 10 replicates at each volume.

(4) To evaluate bias, the mean of the measurements must be with ±3% of the nominal volume. To evaluate precision, the RSD of the measurements must be ≤3% using the stated value (20 or 50 mL) in place of the mean.

(**g**) *Spatulas*.--To weigh out samples; should be acid-cleaned plastic (ideally Teflon) and cleaned by soaking in 2% (v/v) HNO_3 prior to use.

C. Reagents and Standards

Reagents may contain elemental impurities that could negatively affect data quality. High-purity reagents should always be used. Each reagent lot should be tested and certified to be low in the elements of interest before use.

(**a**) *DIW*.--ASTM Type I; demonstrated to be free from the metals of interest and potentially interfering substances.

(**b**) *Nitric acid (HNO_3).*—Concentrated; tested and certified to be low in the metals of interest.

(c) *Hydrogen peroxide (H_2O_2).*–Optima grade or equivalent, 30-32% assay.

(**d**) *Stock standard solutions.*--Obtained from a reputable and professional commercial source.

(*1*) *Single-element standards*.--Obtained for each determined metal, as well as for any metals used as internal standards and interference checks.

(*2*) *Second source standard*.--Independent from the single-element standard; obtained for each determined metal.

(*3*) *Multi-element stock standard solution*.--Elements must be compatible and stable in solutions together. Stability is determined by the vendor; concentrations are then verified before use of the standard.

(**e**) *Internal standard solution*.--For analysis of As, Cd, Pb, and Hg in food matrices, an internal standard solution of 40 µg/L rhodium (Rh), indium (In), and thulium (Tm) is recommended. Rh is analyzed in DRC

mode for correction of the As signal. In addition, the presence of high levels of elements, such as carbon and chlorine, in samples can increase the effective ionization of the plasma and cause a higher response factor for arsenic in specific samples. This potential interference is addressed by the on-line addition of acetic acid (or another carbon source, such as methanol), which greatly increases the effective ionization of incompletely ionized analytes, and decreases the potential increase caused by sample characteristics. The internal standard solution should be prepared in 20% acetic acid.

(**f**) *Calibration standards.*--Fresh calibration standards should be prepared every day, or as needed.

(*1*) Dilute the multi-element stock standard solutions into 50 mL precleaned autosampler vials with 5% HNO_3 in such a manner as to create a calibration curve. The lowest calibration standard (STD 1) should be equal to or less than the limit of quantitation (LOQ) when recalculated in units specific to the reported sample results.

(*2*) *See* Table **2015.01A** for recommended concentrations for the calibration curve.

Table 2015.01A. Recommended concentrations for the calibration curve

Standard	As, µg/L	Cd, µg/L	Pb, µg/L	Hg, µg/L
0	0.00	0.00	0.000	0.00
1	0.01	0.01	0.005	0.01
2	0.02	0.02	0.010	0.05
3	0.10	0.10	0.050	0.10
4	0.50	0.50	0.250	0.50
5	5.00	5.00	2.500	2.00
6	20.00	20.00	10.000	5.00

(**g**) *Initial calibration verification (ICV) solution.*--Made up from second source standards in order to verify the validity of the calibration curve.

(**h**) *Calibration solutions*.--Daily optimization, tuning, and dual detector calibration solutions, as needed, should be prepared and analyzed per the instrument manufacturer's suggestions.

(**i**) *Certified Reference Materials (CRMs)*.--CRMs should preferably match the food matrix type being analyzed and contain the elements of interest at certified concentrations above the LOQ. Recommended reference materials include NIST SRM 1568a (Rice Flour), NIST SRM 1548a (Typical Diet), NRCC CRM DORM-3 (Dogfish Muscle), and NIST SRM 2976 (Mussel Tissue).

(**j**) *Spiking solution*.--50 mg/L Au and Lu in 5% (v/v) HNO_3. Prepared from single-element standards.

D. Contamination and Interferences

(**a**) Well-homogenized samples and small reproducible aliquots help minimize interferences.

(**b**) *Contamination.*—(*1*) Contamination of the samples during sample handling is a great risk. Extreme care should be taken to avoid this. Potential sources of contamination during sample handling include using metallic or metal-containing homogenization equipment, laboratory ware, containers, and sampling equipment.

(*2*) Contamination of samples by airborne particulate matter is a concern. Sample containers must remain closed as much as possible. Container lids should only be removed briefly and in a clean environment during sample preservation and processing, so that exposure to an uncontrolled environment is minimized.

(**c**) *Laboratory.*--(*1*) All laboratory ware (including pipet tips, ICP-MS autosampler vials, sample containers, extraction apparatus, and reagent bottles) should be tested for the presence of the metals of interest. If necessary, the laboratory ware should be acid-cleaned, rinsed with DIW, and dried in a Class 100 laminar flow clean hood.

(*2*) All autosampler vials should be cleaned by storing them in 2% (v/v) HNO_3 overnight and then rinsed three times with DIW. Then dry vials in a clean hood before use. Glass volumetric flasks should be soaked in about 5% HNO_3 overnight prior to use.

(*3*) All reagents used for analysis and sample preparation should be tested for the presence of the metals of interest prior to use in the laboratory. Due to the ultra-low detection limits of the method, it is imperative that all the reagents and gases be as low as possible in the metals of interest. It is often required to test several different sources of reagents until an acceptable source has been found. Metals contamination can vary greatly from lot to lot, even when ordering from the same manufacturer.

(*4*) Keep the facility free from all sources of contamination for the metals of interest. Replace laminar flow clean hood HEPA filters with new filters on a regular basis, typically once a year, to reduce airborne contaminants. Metal corrosion of any part of the facility should be addressed and replaced. Every piece of apparatus that is directly or indirectly used in the processing of samples should be free from contamination for the metals of interest.

(**d**) *Elemental interferences*.--Interference sources that may inhibit the accurate collection of ICP-MS data for trace elements are addressed below.

(*1*) *Isobaric elemental interferences*.--Isotopes of different elements that form singly or doubly charged ions of the same *m/z* and cannot be resolved by the mass spectrometer. Data obtained with isobaric overlap must be corrected for that interference.

(*2*) *Abundance sensitivity*.--Occurs when part of an elemental peak overlaps an adjacent peak. This often occurs when measuring a small *m/z* peak next to a large *m/z* peak. The abundance sensitivity is affected by ion energy and quadrupole operating pressure. Proper optimization of the resolution during tuning will minimize the potential for abundance sensitivity interferences.

(*3*) *Isobaric polyatomic interferences.*--Caused by ions, composed of multiple atoms, which have the same *m/z* as the isotope of interest, and which cannot be resolved by the mass spectrometer. These ions are commonly formed in the plasma or the interface system from the support gases or sample components. The objective of IRT is to remove these interferences, making the use of correction factors unnecessary when analyzing an element in DRC mode. Elements not determined in DRC mode can be corrected by using correction equations in the ICP-MS software.

(**e**) *Physical interferences.*--(*1*) Physical interferences occur when there are differences in the response of the instrument from the calibration standards and the samples. Physical interferences are associated with the physical processes that govern the transport of sample into the plasma, sample conversion processes in the plasma, and the transmission of ions through the plasma-mass spectrometer interface.

(*2*) Physical interferences can be associated with the transfer of solution to the nebulizer at the point of nebulization, transport of aerosol to the plasma, or during excitation and ionization processes in the plasma. High levels of dissolved solids in a sample can result in physical interferences. Proper internal

standardization (choosing internal standards that have analytical behavior similar to the associating elements) can compensate for many physical interferences.

(**f**) *Resolution of interferences.*—(*1*) For elements that are subject to isobaric or polyatomic interferences (such as As), it is advantageous to use the DRC mode of the instrument. This section specifically describes a method of using IRT for interference removal for As using a PerkinElmer DRC II and oxygen as the reaction gas. Other forms of IRT may also be appropriate.

(*a*) Arsenic, which is monoisotopic, has an *m/z* of 75 and is prone to interferences from many sources, most notably from chloride (Cl), which is common in many foods (e.g., salt). Argon (Ar), used in the ICP-MS plasma, forms a polyatomic interference with Cl at *m/z* 75 [$^{35}Cl + {}^{40}Ar = {}^{75}(ArCl)$].

(*b*) When arsenic reacts with the oxygen in the DRC cell, $^{75}As^{16}O$ is formed and measured at *m/z* 91, which is free of most interferences. The potential ^{91}Zr interference is monitored for in the following ways: ^{90}Zr and ^{94}Zr are monitored for in each analytical run, and if a significant Zr presence is detected, then $^{75}As^{16}O$ measured at *m/z* 91 is evaluated against the ^{75}As result. If a significant discrepancy is present, then samples may require analysis using alternative IRT, such as collision cell technology (helium mode).

(*c*) Instrument settings used (for PerkinElmer DRC II): DRC settings for $^{91}(AsO)$ and ^{103}Rh include an RPq value of 0.7 and a cell gas flow rate of 0.6 L/min. Cell conditions, especially cell gas flow rates, may be optimized for specific analyte/matrix combinations, as needed. In such cases, the optimized methods will often have slightly different RPq and cell gas flow values.

(*2*) For multi-isotopic elements, more than one isotope should be measured to monitor for potential interferences. For reporting purposes, the most appropriate isotope should be selected based on review of data for matrix interferences and based on the sensitivity (or relative abundance) of each isotope. The table below lists the recommended isotopes to measure. Low abundance isotopes are not recommended for this method as it is specifically applicable for ultra-low level concentrations (8-10 ppb LOQs). *See* Table **2015.01B**.

Table 2015.01B. Recommended isotopes for analysis

Element	Isotope, amu	Isotopic abundance, %	Potential interferences
Cd	111	13	MoO^+
	114	29	MoO^+, Sn^+
Hg	200	23	WO^+
	202	30	WO^+
Pb[a]	Sum of 206, 207, and 208	99	OsO^+

[a] Allowance for isotopic variability of lead isotopes.

(**g**) *Memory effects*.—Minimize carryover of elements in a previous sample in the sample tubing, cones, torch, spray chamber, connections, and autosampler probe by rinsing the instrument with a reagent blank after samples high in metals concentrations are analyzed. Memory effects for Hg can be minimized through the addition of Au to all standard, samples, and quality control (QC) samples.

E. Sample Handling and Storage

(**a**) Food and beverage samples should be stored in their typical commercial storage conditions (either frozen, refrigerated, or at room temperature) until analysis. Samples should be analyzed within 6 months of preparation.

(**b**) If food or beverage samples are subsampled from their original storage containers, ensure that containers are free from contamination for the elements of concern.

F. Sample Preparation

(**a**) Weigh out sample aliquots (typically 0.25 g of as-received or wet sample) into microwave digestion vessels.

(**b**) Add 4 mL of concentrated HNO_3 and 1 mL of 30% hydrogen peroxide (H_2O_2) to each digestion vessel.

(**c**) Add 0.1 mL of the 50 mg/L Au + Lu solution to each digestion vessel.

(**d**) Cap the vessels securely (and insert into pressure jackets, if applicable). Place the vessels into the microwave system according to the manufacturer's instructions, and connect the appropriate temperature and/or pressure sensors.

(**e**) Samples are digested at a minimum temperature of 190°C for a minimum time of 10 min. Appropriate ramp times and cool down times should be included in the microwave program, depending on the sample type and model of microwave digestion system. Microwave digestion is achieved using temperature feedback control. Microwave digestion programs will vary depending on the type of microwave digestion system used. When using this mechanism for achieving performance-based digestion targets, the number of samples that may be simultaneously digested may vary. The number will depend on the power of the unit, the number of vessels, and the heat loss characteristics of the vessels. It is essential to ensure that all vessels reach at least 190°C and be held at this temperature for at least 10 min. The monitoring of one vessel as a control for the batch/carousel may not accurately reflect the temperature in the other vessels, especially if the samples vary in composition and/or sample mass. Temperature measurement and control will depend on the particular microwave digestion system.

(1) Note: a predigestion scheme for samples that react vigorously to the addition of the acid may be required.

(2) The method performance data presented in this method was produced using a Berghof Speedwave 4 microwave digestion system, with the program listed in Table **2015.01C** (steps 1 and 2 are a predigestion step).

Table 2015.01C. Digestion program for Berghof Speedwave 4 microwave

Step	Temp., °C	Ramp, min	Hold, min
1	145	1	1
2	50	1	1
3	145	1	1
4	170	1	10
5	190	1	10

(3) Equivalent results were achieved using the program listed in Table **2015.01D** on a CEM MARS 6 microwave digestion system using the 40-position carousel and 55 mL Xpress digestion vessels.

Table 2015.01D. Digestion program for CEM MARS 6 microwave

Step	Temp., °C	Ramp, min	Hold, min
1	190	20	10
2	Cool down	NA	10

(4) For infant formula samples, the program described in Table **2015.01E** has been shown to work effectively.

Table 2015.01E. Digestion program for infant formula

Step	Temp., °C	Ramp, min	Hold, min
1	180	20	20
2	Cool down	NA	20
3	200	20	20
4	Cool down	NA	20

(**f**) Allow vessels to cool to room temperature and slowly open. Open the vessels carefully, as residual pressure may remain and digestate spray is possible. Pour the contents of each vessel into an acid-cleaned 50 mL HDPE centrifuge tube and dilute with DIW to a final volume of 20 mL.

(**g**) Digestates are diluted at least 4x prior to analysis with the 1% (v/v) HNO_3 diluent. When the metals concentration of a sample is unknown, the samples may be further diluted or analyzed using a total quantification method prior to being analyzed with a comprehensive quantitative method. This protects the instrument and the sample introduction system from potential contamination and damage.

(**h**) Food samples high in calcium carbonate ($CaCO_3$) will not fully digest. In such cases, the CRM can be used as a gauge for an appropriate digestion time.

(**i**) QC samples to be prepared with the batch (a group of samples and QC samples that are prepared together) include a minimum of three method blanks, duplicate for every 10 samples, matrix spike/matrix spike duplicate (MS/MSD) for every 10 samples, blank spike, and any matrix-relevant CRMs that are available.

G. Procedure

(**a**) *Instrument startup*.--(*1*) Instrument startup routine and initial checks should be performed per manufacturer recommendations.

(*2*) Ignite the plasma and start the peristaltic pump. Allow plasma and system to stabilize for at least 30 min.

(**b**) *Optimizations.*--(*1*) Perform an optimization of the sample introduction system (e.g., *X-Y* and *Z* optimizations) to ensure maximum sensitivity.

(*2*) Perform an instrument tuning or mass calibration routine whenever there is a need to modify the resolution for elements, or monthly (at a minimum), to ensure the instrument's quadrupole mass filtering performance is adequate. Measured masses should be ±0.1 amu of the actual mass value, and the resolution (measured peak width) should conform to manufacturer specifications.

(*3*) Optimize the nebulizer gas flow for best sensitivity while maintaining acceptable oxide and double-charged element formation ratios.

(*4*) Perform a daily check for instrument sensitivity, oxide formation ratios, double-charged element formation ratios, and background. If the performance check is not satisfactory, additional optimizations (a "full optimization") may be necessary.

(**c**) *Internal standardization and calibration*.--(*1*) Following precalibration optimizations, prepare and analyze the calibration standards prepared as described in **C**(**e**).

(*2*) Use internal standardization in all analyses to correct for instrument drift and physical interferences. Refer to **D**(**e**)(*2*). Internal standards must be present in all samples, standards, and blanks at identical concentrations. Internal standards can be added using a second channel of the peristaltic pump to produce a responses that is clear of the pulse-to-analog detector interface.

(*3*) Multiple isotopes for some analytes may be measured, with only the most appropriate isotope (as determined by the analyst) being reported.

(*4*) Use IRT for the quantification of As using the Rh internal standard.

(**d**) *Sample analysis*.--(*1*) Create a method file for the ICP-MS.

(*2*) Enter sample and calibration curve information into the ICP-MS software.

(*3*) Calibrate the instrument and ensure the resulting standard recoveries and correlation coefficients meet specifications (**H**).

(*4*) Start the analysis of the samples.

(*5*) Immediately following the calibration, an initial calibration blank (ICB) should be analyzed. This demonstrates that there is no carryover of the analytes of interest and that the analytical system is free from contamination.

(*6*) Immediately following the ICB, an ICV should be analyzed. This standard must be prepared from a different source than the calibration standards.

(*7*) A minimum of three reagent/instrument blanks should be analyzed following the ICV. These instrument blanks can be used to assess the background and variability of the system.

(*8*) A continuing calibration verification (CCV) standard should be analyzed after every 10 injections and at the end of the run. The CCV standard should be a mid-range calibration standard.

(*9*) An instrument blank should be analyzed after each CCV (called a continuing calibration blank, or CCB) to demonstrate that there is no carryover and that the analytical system is free from contamination.

(*10*) Method of Standard Additions (MSA) calibration curves may be used any time matrix interferences are suspected.

(*11*) Post-preparation spikes (PS) should be prepared and analyzed whenever there is an issue with the MS recoveries.

(**e**) Export and process instrument data.

H. Quality Control

(**a**) The correlation coefficients of the weighted-linear calibration curves for each element must be ≥0.995 to proceed with sample analysis.

(**b**) The percent recovery of the ICV standard should be 90-110% for each element being determined.

(**c**) Perform instrument rinses after any samples suspected to be high in metals, and before any method blanks, to ensure baseline sensitivity has been achieved. Run these rinses between all samples in the batch to ensure a consistent sampling method.

(**d**) Each analytical or digestion batch must have at least three preparation (or method) blanks associated with it if method blank correction is to be performed. The blanks are treated the same as the samples and must go through all of the preparative steps. If method blank correction is being used, all of the samples in the batch should be corrected using the mean concentration of these blanks. The estimated method detection limit (EMDL) for the batch is equal to 3 times the standard deviation (SD) of these blanks.

(**e**) For every 10 samples (not including quality control samples), a matrix duplicate (MD) sample should be analyzed. This is a duplicate of a sample that is subject to all of the same preparation and analysis steps as the original sample. Generally, the relative percent difference (RPD) for the replicate should be ≤30% for all food samples if the sample concentrations are greater than 5 times the LOQ. RPD is calculated as shown below. An MSD may be substituted for the MD, with the same control limits.

$$RPD = 200\ x\ \frac{|S1 - S2|}{S1 + S2}$$

where S1 = concentration in the first sample and S2 = concentration in the duplicate.

(**f**) For every 10 samples (not including quality control samples), an MS and MSD should be performed. The percent recovery of the spikes should be 70-130% with an RPD ≤30% for all food samples.

(*1*) If the spike recovery is outside of the control limits, an MSA curve that has been prepared and analyzed may be used to correct for the matrix effect. Samples may be corrected by the slope of the MSA curve if the correlation coefficient of the MSA curve is ≥0.995.

(*a*) The MSA technique involves adding known amounts of standard to one or more aliquots of the processed sample solution. This technique attempts to compensate for a sample constituent that enhances or depresses the analyte signal, thus producing a different slope from that of the calibration standards. It will not correct for additive interferences which cause a baseline shift.

(*b*) The best MSA results can be obtained by using a series of standard additions. To equal volumes of the sample are added a series of standard solutions containing different known quantities of the analyte(s), and all solutions are diluted to the same final volume. For example, addition 1 should be prepared so that the resulting concentration is approximately 50% of the expected concentration of the native sample. Additions 2 and 3 should be prepared so that the concentrations are approximately 100% and 150%, respectively, of the expected native sample concentration. Determine the concentration of each solution and then plot on the vertical axis of a graph, with the concentrations of the known standards plotted on the horizontal axis. When the resulting line is extrapolated to zero absorbance, the point of interception of the abscissa is calculated MSA-corrected concentration of the analyte in the sample. A linear regression program may be used to obtain the intercept concentration.

(*c*) For results of the MSA technique to be valid, take into consideration the following limitations:

(*i*) The apparent concentrations from the calibration curve must be linear (0.995 or greater) over the concentration range of concern.

(*ii*) The effect of the interference should not vary as the ratio of analyte concentration to sample matrix changes, and the MSA curve should respond in a similar manner as the analyte.

(*2*) If the sample concentration levels are sufficiently high, the sample may be diluted to reduce the matrix effect. Samples should be diluted with the 1% (v/v) HNO_3 diluent. For example, to dilute a sample by a 10x dilution factor, pipette 1 mL of the digested sample into an autosampler vial, and add 9 mL of the 1% (v/v) HNO_3 diluent. MS/MSD sets should be performed at the same dilution factor as the native sample.

(*3*) Spike at 1-10 times the level of a historical sample of the same matrix type, or, if unknown, spike at 1-5 times a typical value for the matrix. Spiking levels should be no lower than 10 times the LOQ.

(**g**) Percent recoveries of the CRMs should be 75-125% of their certified value.

(**h**) Percent recoveries of the CCV standards should be within 85-115%. Sample results may be CCV-corrected using the mean recovery of the bracketing CCVs. This should only be done after careful evaluation of the data. The instrument should show a trending drift of CCV recoveries and not just a few anomalous outliers.

(**i**) CCBs should be monitored for the effects of carryover and for possible system contamination. If carryover of the analyte at levels greater than 10 times the MDL is observed, the sample results may not be reportable.

(**j**) Absolute response of any one internal standard should not vary from the original response in the calibration blank by more than 60-125%. Some analytical samples, such as those containing concentrations of the internal standard and tissue digestates, can have a serious effect on the internal standard intensities, but this does not necessarily mean that the analytical system is out of control. In some situations, it is appropriate to reprocess the samples using a different internal standard monitored in the analysis. The data should be carefully evaluated before doing this.

(**k**) The recovery of the Lu that was spiked into the sample preparation prior to digestion should be evaluated to assess any potential loss of analyte during the process. The concentration of Lu in the sample preparation is 0.25 mg/L, and for samples diluted 4x at the instrument, this is equivalent to 62.5 µg/L at the instrument (if samples are diluted more than 4x, this must be taken into account). The Lu recovery should be no less than 75% of the original spiked concentration.

(**l**) Refer to Table **2015.01F** for a summary of all recommended quality control samples, minimum frequency at which they are to be analyzed, acceptance criteria for each, and appropriate corrective action if the acceptance criteria are not met.

Table 2015.01F. Summary of quality control samples

QC sample	Measure	Minimum frequency	Acceptance criteria	Corrective action
Calibration standards	Linearity of the calibration curve	Analyzed once per analytical day	Correlation coefficient ≥0.995, 1st standard ≤MRL, low standard recovery = 75-125%, all other standard recoveries = 80-120%	Reanalyze suspect calibration standard. If criteria still not met, then re-prepare standards and recalibrate the instrument.
Internal standards	Variation in sample properties between samples and standards	Each standard, blank, and sample is spiked with internal standard	60-125% recovery compared to calibration blank	If the responses of the internal standards in the following CCB are within the limit, rerun the sample at an additional 2x dilution. If not, then samples must be reanalyzed with a new calibration.
Lu digestion check spike	Assessment of potential loss during digestion	Added to every digested samples	Recovery ≥75%	Re-prepare the sample
Initial calibration verification (ICV)	Independent check of system performance	One following instrument calibration	Recovery = 90-110%	Correct problem prior to continuing analysis. Recalibrate if necessary.
Continuing calibration verification (CCV)	Accuracy	At beginning and end of analysis and one per 10 injections	Recovery = 85-115%	Halt analysis, correct problem, recalibrate, and reanalyze affected samples
Method blanks (MB)	Contamination from reagents, lab ware, etc.	Minimum of three per batch	Mean ≤ MRL; SD ≤ MDL or MBs <1/10th sample result	Determine and eliminate cause of contamination. Affected samples must be re-prepared and reanalyzed.
Method duplicates (MD)	Method precision within a given matrix	Minimum of one per 10 samples	RPD ≤ 30% or ±2x LOQ if results ≤5x LOQ	If RPD criteria not met, then sample may be re-prepared and reanalyzed, but this is not required. Sample matrix may be inhomogeneous. A post-digestion duplicate (PDD) can be analyzed to evaluate instrument precision.

Matrix spikes/matrix spike duplicates (MS/MSD)	Method accuracy and precision within a given matrix	Minimum of one per 10 samples	Recovery = 70-130% and RPD ≤ 30%	If RPD > 30%, results must be qualified
Post-preparation spike (PS)	Check for matrix interference	When required (samples spiked too low/high, dilution test fails, etc.)	Recovery = 75-125%	Analyze samples using MSA or results flagged accordingly
Laboratory fortified blank (LFB) or blank spike (BS)	Method accuracy	Minimum of one per batch	Recovery = 75-125%	If LFB recovery is outside of the control limit, then batch must be re-prepared and reanalyzed
Certified Reference Material (CRM)	Method accuracy	Must be matrix-matched to samples; minimum of one per batch	Recovery = 75-125% unless limits set by CRM manufacturer are greater or element/CRM specific limits have been established	If CRM true value is ≥5x the LOQ and recovery is outside of the control limit, then batch must be re-prepared and reanalyzed

I. Method Performance

(**a**) Limit of detection (LOD) and LOQ were determined through the analysis of 23 method blanks (*see* Table **2015.01G**). LOD was calculated as 3 times the SD of the results of the blanks, and LOQ was calculated as 2 times the value of the LOD, except where the resulting LOQ would be less than the lowest calibration point, in which case LOQ was elevated and set at the lowest calibration point and LOD was calculated as 1/3 of the LOQ. All LOQs achieved are ≤10 µg/kg for all food matrices and ≤8 µg/kg for liquid matrices, such as infant formula.

Table 2015.01G. Method blank results and LOD/LOQ, µg/kg

Method blanks	^{91}(AsO)	^{111}Cd	^{114}Cd	Pb	^{200}Hg	^{202}Hg
MB-01	2.83	0.229	0.270	1.90	1.61	0.95
MB-02	1.48	-0.088	0.270	0.14	1.48	1.13
MB-03	1.80	0.007	0.115	0.13	0.76	0.25
MB-04	1.03	0.154	0.288	0.12	1.46	0.33
MB-05	1.43	0.010	0.259	1.84	1.28	0.27
MB-06	1.07	0.105	0.096	3.02	0.87	0.76
MB-07	2.31	-0.002	0.297	2.67	0.89	0.44
MB-08	1.20	0.285	0.200	4.24	0.55	0.28
MB-09	1.05	0.002	0.182	0.09	0.96	0.25
MB-10	2.12	0.047	0.150	0.19	0.71	0.02
MB-11	2.09	-0.145	0.226	0.12	0.64	0.57
MB-12	1.44	0.037	0.165	0.18	0.45	0.50
MB-13	0.70	-0.122	0.160	0.17	0.81	0.19
MB-14	1.12	-0.001	0.074	0.14	0.85	0.21
MB-15	2.33	0.097	0.207	0.11	0.18	0.17
MB-16	1.53	-0.117	0.146	0.16	1.33	1.09
MB-17	1.79	-0.070	0.180	0.03	3.46	2.19
MB-18	1.90	0.049	0.115	0.06	3.30	2.36
MB-19	1.18	0.043	0.224	0.39	4.01	2.78
MB-20	1.24	-0.060	0.199	0.07	0.99	0.56
MB-21	0.92	0.165	0.120	0.03	0.73	0.33
MB-22	1.69	0.005	0.186	0.09	0.60	0.25
MB-23	2.13	0.171	0.152	0.08	0.41	-0.23
SD	0.54	0.113	0.063	1.18	1.01	0.77
LOD	1.6	0.50[a]	0.50[a]	3.5	3.0	2.3
LOQ	3.3	1.60[a]	1.60[a]	7.1	6.0	4.6

[a] Adjusted to conform to lowest calibration point.

(**b**) Sample-specific LOQs for several matrices, based on LOQs determined by the default method, and adjusted for changes in sample mass for particular samples, are shown in Table **2015.01H**. Values have been rounded up to the nearest part-per-billion.

Table 2015.01H. Sample-specific LOQs

Sample	LOQ, µg/kg (as received)			
	As	Cd	Pb	Hg
Infant formula	2	1	4	3
Chocolate	4	2	8	6
Rice flour	4	2	8	6
Fruit juice	1	1	2	2

(**c**) Numerous relevant CRMs were analyzed to establish method accuracy. Example percent recoveries are provided in Table **2015.01I** (recoveries have been omitted for CRMs that do not provide a certified value or if the certified value is less than the LOQ).

Table 2015.01I. Recoveries for numerous relevant CRMs

Certified Reference Material	As, %	Cd, %	Pb, %	Hg, %
DOLT-4 Dogfish Liver	104	97	87	114
DORM-3 Fish Protein	105	109	94	114
DORM-4 Fish Protein	105	91	91	81
NIST 1548a Typical Diet	103	95	113	NA
NIST 1568a Rice Flour	98	99	NA	NA
NIST 1946 Lake Superior Fish Tissue	119	NA	NA	101
TORT-2 Lobster Hepatopancreas	109	104	95	116
TORT-3 Lobster Hepatopancreas	113	89	86	86

(**d**) *Standard Method Performance Requirements*[SM] (AOAC SMPR 2012.007) for repeatability, reproducibility, and recovery for the method are shown in the Table **2015.01J**. *See* Appendix A (*J. AOAC Int*., future issue) for detailed method performance information supporting acceptance of the method.

Table 2015.01J. AOAC SMPR 2012.007

Concentration range, µg/kg	Repeatability, %	Reproducibility, %	Recovery, %
LOQ–100	15	32	60-115
100–1000	11	16	80-115
>1000	7.3	8	80-115

(**e**) Detailed method performance information supporting acceptance of the method is on file with AOAC and the method author and is available upon request. Method validation samples were prepared and analyzed for all applicable matrices. In general, all SMPR criteria were met for As, Cd, Hg, and Pb in the matrices apple juice, infant formula, cocoa powder, and rice flour.

J. AOAC Int. (future issue)

AOAC SMPR 2012.007
J. AOAC Int. **96**, 704(2013)
DOI: 10.5740/jaoac.int.2012.007

Posted: May 28, 2015

(b) (4)

FINAL REPORT

Acute Oral Dose Toxicity Study of L-Valine (VAL Pro) in Sprague-Dawley Rats (Fixed Dose Procedure)

Study No.: B20742

(b) (4)

(b) (4)

GLP COMPLIANCE STATEMENT

Study Title : Acute Oral Dose Toxicity Study of L-Valine (VAL Pro) in Sprague-Dawley Rats (Fixed Dose Procedure)

Study No. : B20742

This study was conducted in accordance with the following Good Laboratory Practice Regulations:

- "Good Laboratory Practice Regulation for Nonclinical Laboratory Studies" Notification No. 2018-93, Ministry of Food and Drug Safety, Republic of Korea (Nov. 21, 2018)
- "OECD Principles of Good Laboratory Practice" Organisation for Economic Co-operation and Development, ENV/MC/CHEM(98)17 (as revised in 1997)

(b) (4) (b) (4)

Study Director

Nov. 17, 2020
Date

(b) (4) (b) (4)

Test Facility Management

Nov. 17, 2020
Date

QUALITY ASSURANCE STATEMENT

Study Title : Acute Oral Dose Toxicity Study of L-Valine (VAL Pro) in Sprague-Dawley Rats (Fixed Dose Procedure)

Study No. : B20742

This study was audited by the Quality Assurance Unit of (b) (4) as indicated below. Audits were based on the GLPs, protocol and SOPs (b) (4) The results of all quality assurance inspections and audits were reported to the study director and test facility management.

Audit phases and dates as described below were reported to the study director and test facility management.

Audit Phase	Audit Date	To Study Director and Test Facility Management
Protocol	Sep. 21, 2020	Sep. 21, 2020
Animal receipt*	Aug. 25, 2020	Aug. 25, 2020
Storage of the test substance	Oct. 6, 2020	Oct. 6, 2020
Preparation of the dosing formulations	Oct. 6, 2020	Oct. 6, 2020
Administration	Oct. 6, 2020	Oct. 6, 2020
Clinical signs	Oct. 6, 2020	Oct. 6, 2020
Preparation of the dosing formulations	Oct. 8, 2020	Oct. 8, 2020
Necropsy	Oct. 20, 2020	Oct. 20, 2020
Raw data	Nov. 13, 2020	Nov. 13, 2020
Draft Report	Nov. 13, 2020	Nov. 13, 2020
Final Report	Nov. 17, 2020	Nov. 17, 2020

*Process-based inspections: The performance of process-based inspections covering phases which occur with a very high frequency may result in some studies not being inspected on an individual basis during their experimental phase.

This statement confirms that described methods were established, and results reflected raw data accurately in the final report.

(b) (4)
Quality Assurance Management

(b) (4)

Nov. 17, 2020
Date

CONTENTS

SUMMARY

This study was conducted to assess the potential toxicity and to classify the test substance, L-Valine (VAL Pro), under the category of GHS classification following a single oral administration to 8 − 9-week-old female Sprague-Dawley rats.

Two dose groups with one female each for the sighting study and one dose group with four females for the main study were utilized as follows:

Group 1 (Step 1): 300 mg/kg of the test substance

Groups 2 and 3 (Steps 2 and 3): 2,000 mg/kg of the test substance

Sighting study (Steps 1 and 2): A dose of 300 mg/kg was administered and no mortality was observed (Step 1). A second dose of 2,000 mg/kg was administered and no mortality was observed (Step 2).

Main study (Step 3): A dose of 2,000 mg/kg was administered and no mortality was observed (Step 3). The study was finished at that point.

All animals were monitored for clinical signs and body weight changes during the 14-day observation period after administration. They were subjected to a gross necropsy at the end of the observation period.

There were no deaths of animals at 300 and 2,000 mg/kg. No test substance-related effects were observed in clinical signs, body weight data or necropsy findings in the animals at 300 and 2,000 mg/kg.

Based on the result of the acute oral toxicity study in Sprague-Dawley rats, the test substance, L-Valine (VAL Pro), was classified to be 'Category 5 or Unclassified' according to the GHS classification.

1. EXPERIMENTAL OUTLINE

1.1 Purpose

The purpose of this study was to assess the potential toxicity and to classify the test substance, L-Valine (VAL Pro), under the category of GHS classification following a single oral administration to female Sprague-Dawley rats.

1.2 Good Laboratory Practice Regulations

This study was conducted in accordance with the following Good Laboratory Practice Regulations:

- "Good Laboratory Practice Regulation for Nonclinical Laboratory Studies"
  Notification No. 2018-93, Ministry of Food and Drug Safety, Republic of Korea (Nov. 21, 2018)
- "OECD Principles of Good Laboratory Practice"
  Organisation for Economic Co-operation and Development, ENV/MC/CHEM(98)17 (as revised in 1997)

1.3 Regulatory Guidelines

This study was conducted in accordance with the following test guideline:

- "OECD Guidelines for the Testing of Chemicals, 420, Acute Oral Toxicity-Fixed Dose Procedure"
  Organisation for Economic Co-operation and Development (Adopted: 17th December 2001)

1.4 Animal Ethics

(b) (4) received full accreditation from the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International) in 2010. This study was reviewed and approved by the (b) (4) based on Animal Protection Act of Republic of Korea (Enactment May 31, 1991, No. 4379, Revision Aug. 27, 2019, No. 16544) (Approval No.: 200492).

1.5 Veterinary Care

All procedures in this study were in compliance with the Animal Protection Act of Republic of Korea, the Guide for the Care and Use of Laboratory Animals.

1.6 Sponsor

Name	CJ CheilJedang
Address	55, Gwanggyo-ro 42beon-gil, Yeongtong-gu, Suwon, Gyeonggi, Republic of Korea
TEL	+ 82-31-8099-1515

1.7 Test Facility

Name	(b) (4)
Address	(b) (4)
TEL	(b) (4) FAX (b) (4)

1.8 Study Director

Name	(b) (4)

1.9 Study Schedule

Study initiation	Sep. 17, 2020
Experimental start	Sep. 29, 2020
Animal receipt	Sep. 29, 2020
Group assignment	Oct. 5, 2020
Administration	Oct. 6, 8 and 13, 2020
Necropsy	Oct. 20, 22 and 27, 2020
Experimental completion	Nov. 3, 2020
Study completion	Nov. 17, 2020

1.10 Key Personnel

Evaluation of animal's health condition	(b) (4)
Test substance storage and handling	(b) (4)
Pathology	(b) (4)

1.11 Retention of Raw Data

1.11.1 Duration Three years from the approval date
(Notification No. 2018-93, Ministry of Food and Drug Safety, Republic of Korea)

1.11.2 Storage facility

Name (b) (4)

Location (b) (4)

(Raw data will be retained for the first five years after completion of the study in the archives of (b) (4) Further storage will be determined in agreement with the Sponsor.)

1.11.3 Type of records and data

Protocol, final report, all raw data, documents related to the study and communications

2. MATERIALS AND METHODS

2.1 Test Substance

2.1.1	Name	L-Valine (VAL Pro)
2.1.2	Lot No./Batch No.	GVAL191121
2.1.3	Appearance	Light brown granules
2.1.4	Component · Content	Valine 72.57%
2.1.5	Date of manufacture	Nov. 21, 2019
2.1.6	Expiration date	Dec. 23, 2021
2.1.7	Storage condition	Room temperature (15 – 25°C)
2.1.8	Handling instructions	Not specific
2.1.9	Supplier	
	Name	CJ CheilJedang
	Address	55, Gwanggyo-ro 42beon-gil, Yeongtong-gu, Suwon, Gyeonggi, Republic of Korea
2.1.10	Disposition of test substance	Any remaining test substance is returned to the Sponsor.

2.2 Preparation and Analysis of the Dosing Formulations

2.2.1 Vehicle

2.2.1.1	Name	Water for injection
2.2.1.2	Lot No.	(b) (4)
2.2.1.3	Storage condition	Room temperature (1 − 30°C)
2.2.1.4	Manufacturer	(b) (4) Republic of Korea

2.2.2 Preparation of the dosing formulations

The required amount of test substance was weighed and placed in a mortar. The vehicle was added and suspended using a pestle, and the vehicle was gradually added to yield the desired concentration. Dosing formulations were prepared just prior to use on the day of administration.

2.2.3 Analysis of the dosing formulations

Analysis for stability, homogeneity and concentration of dosing formulations was not performed.

2.3 Test System

2.3.1 Species, strain Rat, Sprague-Dawley (Crl:CD(SD)), SPF

2.3.2 Producer & supplier (b) (4) Republic of Korea

2.3.3 Justification for species selection

Sprague-Dawley rats are extensively used in toxicity studies and are selected because of the abundance of historical control data to be compared.

2.3.4 Sex, number, age and body weight range of animals at receipt

Female, 11 rats, 7 weeks old, 167.9 – 185.3 g

2.3.5 Sex, number, age and body weight range of animals at administration

Female, 6 rats, 8 – 9 weeks old, 187.5 – 210.6 g

2.3.6 Quarantine and acclimation

Upon receipt, all animals were subjected to clinical examination and recorded for body weight. Clinical signs were observed once daily during the quarantine-acclimation period. However, animals were quarantined for 3 days in a quarantine room and then, moved to an animal room.

On the last day of the quarantine-acclimation period, the body weight was recorded, and then general health examination based on clinical signs and body weight changes was conducted by responsible personnel of quarantine.

2.3.7 Animal and cage identification

During the acclimation period, a temporary identification number was marked on the tail of each animal and a temporary identification card (quarantine-acclimation period) was attached to each cage.

Following group assignment, an individual identification number was marked uniquely on the tail of each animal and a color-coded cage card was attached to each cage describing the group and dose level.

2.3.8 Group assignment

On the last day of acclimation (group assignment day), ten healthy animals with body weights close to the mean body weight were allocated to the groups using the random method. The study groups were consisted of four groups, and one animal each was assigned to two groups for the sighting study and four animals each were assigned to two groups for the main study. Animals of each group were given an animal ID number (G1: 2101, G2: 2201, G3: 2301 − 2304 and G4: 2401 − 2404).

2.3.9 Disposition of remaining animals

The remaining animal not selected for the study was discarded following group assignment. Disused animals in Step 4 were excluded from the test system after dosing in Step 3.

2.4 Animal Husbandry

2.4.1	Quarantine room No.	A315
2.4.2	Animal room No.	A322
2.4.3	Type & size of cage	Stainless wire mesh cage, 260W×350D×210H (mm)
2.4.4	Number of animals per cage	One animal/cage (during the quarantine-acclimation and observation periods)
2.4.5	Temperature	Measurement value: 20.2 – 23.7°C, permissible range: 19.0 − 25.0°C
2.4.6	Relative humidity	Measurement value: 50.0 – 60.2%, permissible range: 30.0 − 70.0%
2.4.7	Air changes	10 − 15 clean, fresh, filtered air changes per hour
2.4.8	Lighting	12 hour light/dark cycle (7 AM − 7 PM via automated timer)
2.4.9	Intensity of illumination	150 − 300 Lux

2.4.10 Replacement and washing of breeding materials

Cages and feeders were replaced once every two weeks.

Breeding materials were washed using an automatic washing machine and sterilized by an autoclave.

2.5 Feed

2.5.1 Type

Pelleted rodent chow

(b) (4)

2.5.2 Lot No. (b) (4)

2.5.3 Manufacturer (b) (4)

2.5.4 Method of feeding

The feed was placed in feeders and provided *ad libitum*.

2.5.5 Analysis and confirmation of feed

The certificate of feed analysis was provided by the manufacturer, (b) (4) The results of feed analysis met the allowable standard of this facility.

2.6 Drinking Water

2.6.1 Type and method of water supply

Public tap water in (b) (4) was filtered and irradiated by ultraviolet light and provided *ad libitum*.

2.6.2 Analysis of drinking water

Samples of drinking water are analyzed for specified microorganisms once a month and all environmental contaminants once a year by the (b) (4) according to the Regulation of Quality Criteria for Potable Water and Test (Ministry of Environment Ordinance No. 833, Revision Dec. 20, 2019). The results of water analysis met the allowable standard of this facility.

2.7 Dosing

2.7.1 Route

Oral via gastric intubation

2.7.2 Justification for the route of administration

The oral route was chosen because it is the intended route of administration in animals.

2.7.3 Method of administration

Individual doses were calculated based on the animals' body weights recorded just prior to dosing at a dose volume of 10 mL/kg body weight. Animals were dosed via gastric intubation with a disposable syringe fitted with an intubation tube. Animals were fasted overnight, approximately 16 hours prior to dosing. Drinking water was provided *ad libitum*. Feed was provided approximately 4 hours after dosing.

2.8 Group Designation and Dose Levels

2.8.1 Sighting study

The starting dose level for this study is selected at 300 mg/kg because there is no available toxicity information on the test substance. Therefore, a starting dose of 300 mg/kg body weight of the test substance was administered to one animal as the dose level for the sighting study (Step 1).

The following steps were based on the results of mortality and clinical signs of animals obtained from the observations for 2 days after the administration at the previous dose level in accordance with '<Attachment 1> ANNEX 2: FLOW CHART FOR THE SIGHTING STUDY'.

2.8.2 Main study

The progress of main study was based on the mortality and clinical observations for 5 days after the administration in the sighting study. In the main study, the test substance was dosed to four female rats. The main study was performed in accordance with '<Attachment 2> ANNEX 3: FLOW CHART FOR THE MAIN STUDY'

SAFETY?

The group designation is shown as follows:

Group		Step	Dose (mg/kg)	Dose volume (mL/kg)	No. of animals (Animal ID No.)	Deaths
Sighting study	G1	Step 1	300	10	1 (2101)	None
Sighting study	G2	Step 2	2,000	10	1 (2201)	None
Main study	G3	Step 3	2,000	10	4 (2301 – 2304)	None

2.9 Parameters Evaluated

2.9.1 Clinical signs

All animals were observed for clinical signs (type, severity, time of onset and recovery, etc.) and mortality at 30 minutes and 1, 2, 4 and 6 hours after dosing on the day of dosing (Day 1), and once daily thereafter for 14 days (Day 2 to Day 15).

2.9.2 Body weights

The body weight was recorded once on the day of dosing (prior to dosing), and on Days 2, 4, 8 and 15 (the day of necropsy).

2.9.3 Necropsy

On the day of necropsy, all animals were anesthetized with CO_2 gas inhalation and exsanguinated from the abdominal aorta. Complete gross postmortem examinations were performed on all animals in the study.

2.9.4 Histopathology

Since no gross findings were observed at necropsy, histopathological examination was not performed.

2.10 Statistical Analysis

Statistical analysis was not performed. Mean scores and values were determined.

2.11 Classification of GHS Category

The classification of Globally Harmonized Classification System for Chemical Substances and Mixtures (GHS) Category was estimated based on the mortality in each step (Attachment 2).

3. RESULTS AND DISCUSSION

3.1 Mortality

(Table 1)

There were no deaths of animals at 300 and 2,000 mg/kg throughout the study.

3.2 Clinical Signs

(Table 2)

No abnormalities of clinical signs were observed in any animal at 300 and 2,000 mg/kg throughout the study.

3.3 Body Weights

(Figure 1, Figure 2, Figure 3, Table 3)

Normal body weight gain was observed in all animals at 300 and 2,000 mg/kg throughout the study.

3.4 Necropsy and Histopathological Findings

(Appendix IV)

No abnormal gross findings were observed in any animal at 300 and 2,000 mg/kg.

4. CONCLUSION

Based on the result of the acute oral toxicity study in Sprague-Dawley rats, the test substance, L-Valine (VAL Pro), was classified to be 'Category 5 or Unclassified' according to the GHS classification.

FIGURES

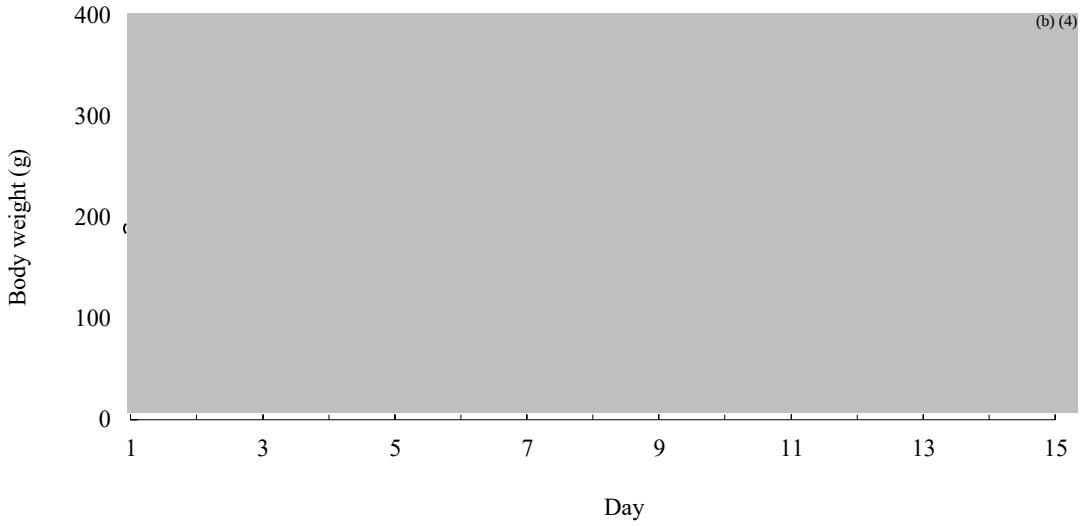


Figure 1. Body Weights (Step 1: 300 mg/kg (Sighting study))

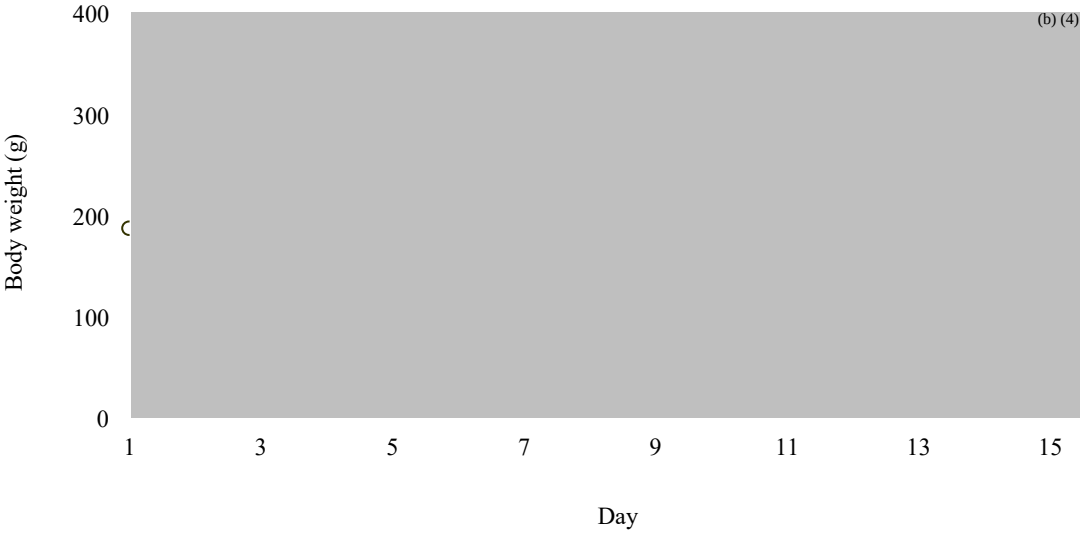


Figure 2. Body Weights (Step 2: 2,000 mg/kg (Sighting study))

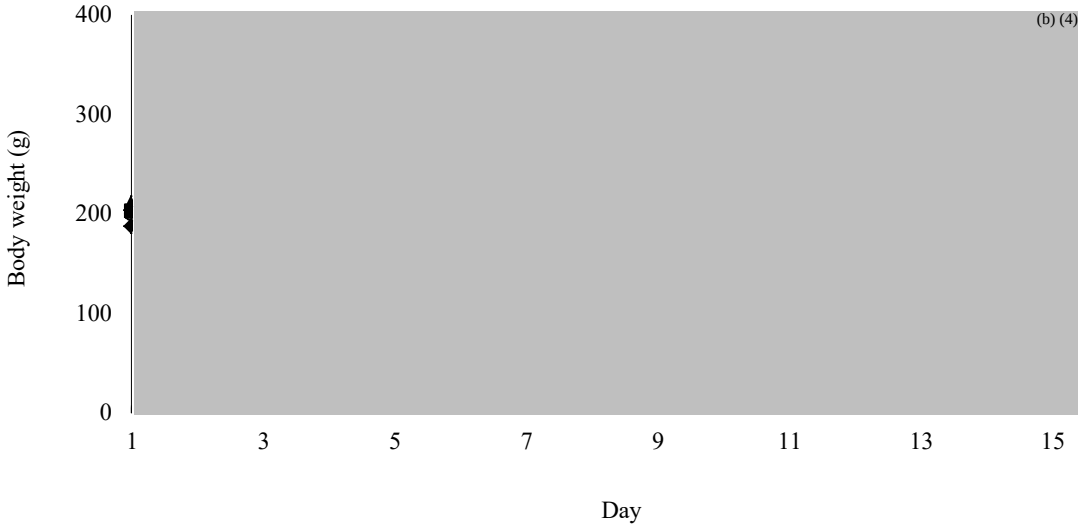


Figure 3. Body Weights (Step 3: 2,000 mg/kg (Main study))

SUMMARY TABLE

Table 1. Summary of Mortality

Step / Dose (mg/kg)	No. of animals	Day															Mortality
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
Step 1 300	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/1
Step 2 2,000	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/1
Step 3 2,000	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/4

Steps 1 & 2: Sighting study Step 3: Main study

INDIVIDUAL ANIMAL DATA

Table 2. Individual Clinical Signs

Step / Dose (mg/kg)	Animal ID	Clinical sign	Hours (Day 1) after dosing 0.5	1	2	4	6
Step 1 300	2101		(b) (4)				
Step 2 2,000	2201						
Step 3 2,000	2301						
	2302						
	2303						
	2304						

Step / Dose (mg/kg)	Animal ID	Clinical sign	Day 2	3	4	5	6	7	8	9	10	11	12	13	14	15
Step 1 300	2101		(b) (4)													
Step 2 2,000	2201															
Step 3 2,000	2301															
	2302															
	2303															
	2304															

Steps 1 & 2: Sighting study Step 3: Main study

-: No observable abnormality

Table 3. Individual Body Weights

(g)

Step / Dose (mg/kg)	Animal ID	Day 1	2	4	8	15	Gain 1～15
		(b) (4)					
Step 1 300	2101						
Step 2 2,000	2201						
Step 3 2,000	2301						
	2302						
	2303						
	2304						
	Mean	**200.4**	**216.9**	**221.3**	**230.0**	**243.1**	**42.7**
	S.D.	**9.6**	**8.2**	**7.4**	**4.7**	**6.7**	**9.6**
	N	**4**	**4**	**4**	**4**	**4**	**4**

Steps 1 & 2: Sighting study Step 3: Main study

APPENDICES

Appendix I. Protocol

(b) (4)

PROTOCOL

Acute Oral Dose Toxicity Study
of L-Valine (VAL Pro) in Sprague-Dawley Rats
(Fixed Dose Procedure)

Study No.: B20742

(b) (4)

PROTOCOL REVIEWED AND ACCEPTED BY

Study Title: Acute Oral Dose Toxicity Study of L-Valine (VAL Pro) in Sprague-Dawley Rats (Fixed Dose Procedure)

Study No.: B20742

Test Facility (b) (4)

Study Director (b) (4)

Sep. 17, 2020
Date

Test Facility Management (b) (4)

Sep. 17, 2020
Date

Sponsor CJ CheilJedang

Monitor (b) (4)

Sep. 17, 2020
Date

CONTENTS

1. EXPERIMENTAL OUTLINE

1.1 Purpose

The purpose of this study is to assess the potential toxicity and to classify the test substance, L-Valine (VAL Pro), under the category of GHS classification following a single oral administration to female Sprague-Dawley rats.

1.2 Good Laboratory Practice Regulations

This study will be conducted in accordance with the following Good Laboratory Practice Regulations:

- "Good Laboratory Practice Regulation for Nonclinical Laboratory Studies"
  Notification No. 2018-93, Ministry of Food and Drug Safety, Republic of Korea (Nov. 21, 2018)
- "OECD Principles of Good Laboratory Practice"
  Organisation for Economic Co-operation and Development, ENV/MC/CHEM(98)17 (as revised in 1997)

1.3 Regulatory Guidelines

This study will be conducted in accordance with the following test guideline:

- "OECD Guidelines for the Testing of Chemicals, 420, Acute Oral Toxicity-Fixed Dose Procedure"
  Organisation for Economic Co-operation and Development (Adopted: 17th December 2001)

1.4 Animal Ethics (SOP/GER/020)

(b) (4) received full accreditation from the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International) in 2010. This study was reviewed and approved by the (b) (4) (b) (4) based on Animal Protection Act of Republic of Korea (Enactment May 31, 1991, No. 4379, Revision Aug. 27, 2019, No. 16544) (Approval No.: 200492).

1.5 Veterinary Care (SOP/GER/020)

In accordance with the Animal Protection Act of Republic of Korea, the Guide for the Care and Use of Laboratory Animals, medical treatment necessary to prevent unacceptable pain and suffering, including euthanasia, is the sole responsibility of the attending laboratory animal veterinarian. Veterinary treatment may be conducted based upon consensus agreement with the study director, attending laboratory animal veterinarian and the Sponsor.

1.6 Sponsor

Name	CJ CheilJedang
Address	55, Gwanggyo-ro 42beon-gil, Yeongtong-gu, Suwon, Gyeonggi, Republic of Korea
TEL	+ 82-31-8099-1515

1.7 Test Facility

Name	(b) (4)
Address	
TEL	(b) (4) FAX (b) (4)

1.8 Study Director

Name	(b) (4)

1.9 Study Schedule

Study initiation	Sep. 17, 2020
Experimental start	Sep. 29, 2020
Animal receipt	Sep. 29, 2020
Group assignment	Oct. 5, 2020
Administration	Oct. 6, 8, 13 and 16, 2020
Necropsy	Oct. 20, 22, 27 and 30, 2020
Experimental completion	Nov. 6, 2020
Draft report issue	Nov. 20, 2020

1.10 Protocol Amendments

After the approval of the protocol, protocol amendments including the reason for the changes, the contents of change and date of amendment will be documented and signed by the study director. And then, they will be submitted to the Sponsor.

1.11 Final Report

The final report will be written including figures, tables, and appendices. The original document will be retained in the archives of (b) (4)

1.12 Retention of Raw Data

1.12.1 Duration Three years from the approval date
(Notification No. 2018-93, Ministry of Food and Drug Safety, Republic of Korea)

1.12.2 Storage facility

Name Archives of (b) (4)

Location (b) (4)

(Raw data will be retained for the first five years after completion of the study in the archives of (b) (4) Further storage will be determined in agreement with the Sponsor.)

1.12.3 Type of records and data

Protocol, final report, all raw data, documents related to the study, specimens (as long as quality permits evaluation), communications, etc.

2. MATERIALS AND METHODS

2.1 Test Substance

2.1.1	Name	L-Valine (VAL Pro)
2.1.2	Lot No./Batch No.	GVAL191121
2.1.3	Appearance	Light brown granules
2.1.4	Component・Content	Valine 72.57%
2.1.5	Date of manufacture	Nov. 21, 2019
2.1.6	Expiration date	Dec. 23, 2021
2.1.7	Storage condition	Room temperature (15 – 25°C)
2.1.8	Handling instructions	Not specific
2.1.9	Supplier	
	Name	CJ CheilJedang
	Address	55, Gwanggyo-ro 42beon-gil, Yeongtong-gu, Suwon, Gyeonggi, Republic of Korea
2.1.10	Disposition of test substance	Any remaining test substance is returned to the Sponsor.

2.2 Preparation and Analysis of the Dosing Formulations

2.2.1 Vehicle

2.2.1.1	Name	Water for injection
2.2.1.2	Storage condition	Room temperature (1 − 30°C)
2.2.1.3	Manufacturer	(b) (4) Republic of Korea

2.2.2 Preparation of the dosing formulations (SOP/FOT/140)

The required amount of test substance will be weighed and placed in a mortar. The vehicle will be added and suspended using a pestle, and the vehicle will be gradually added to yield the desired concentration. Dosing formulations will be prepared just prior to use on the day of administration.

2.2.3 Analysis of the dosing formulations

Analysis for stability, homogeneity and concentration of dosing formulations will not be performed.

2.3 Test System

2.3.1 Species, strain Rat, Sprague-Dawley (b) (4)

2.3.2 Producer & supplier (b) (4) Republic of Korea

2.3.3 Justification for species selection

Sprague-Dawley rats are extensively used in toxicity studies and are selected because of the abundance of historical control data to be compared.

2.3.4 Sex, number and age of animals at receipt

Female, 11 rats, 7 weeks old

2.3.5 Quarantine and acclimation (SOP/GER/011)

Upon receipt, all animals will be subjected to clinical examination and recorded for body weight. Clinical signs will be observed once daily during the quarantine-acclimation period. However, animals will be quarantined for 3 days in a quarantine room, then and moved to an animal room.

On the last day of the quarantine-acclimation period, body weight will be recorded, and then general health examination based on clinical signs and body weight changes will be conducted by responsible personnel of quarantine. Abnormal animals will be euthanized by CO_2 gas inhalation.

2.3.6 Animal and cage identification (SOP/SGE/140, 142)

During the acclimation period, a temporary identification number will be marked on the tail of each animal and a temporary identification card (quarantine-acclimation period) will be attached to each cage.

Following group assignment, an individual identification number will be marked uniquely on the tail of each animal and a color-coded cage card will be attached to each cage describing the group and dose level.

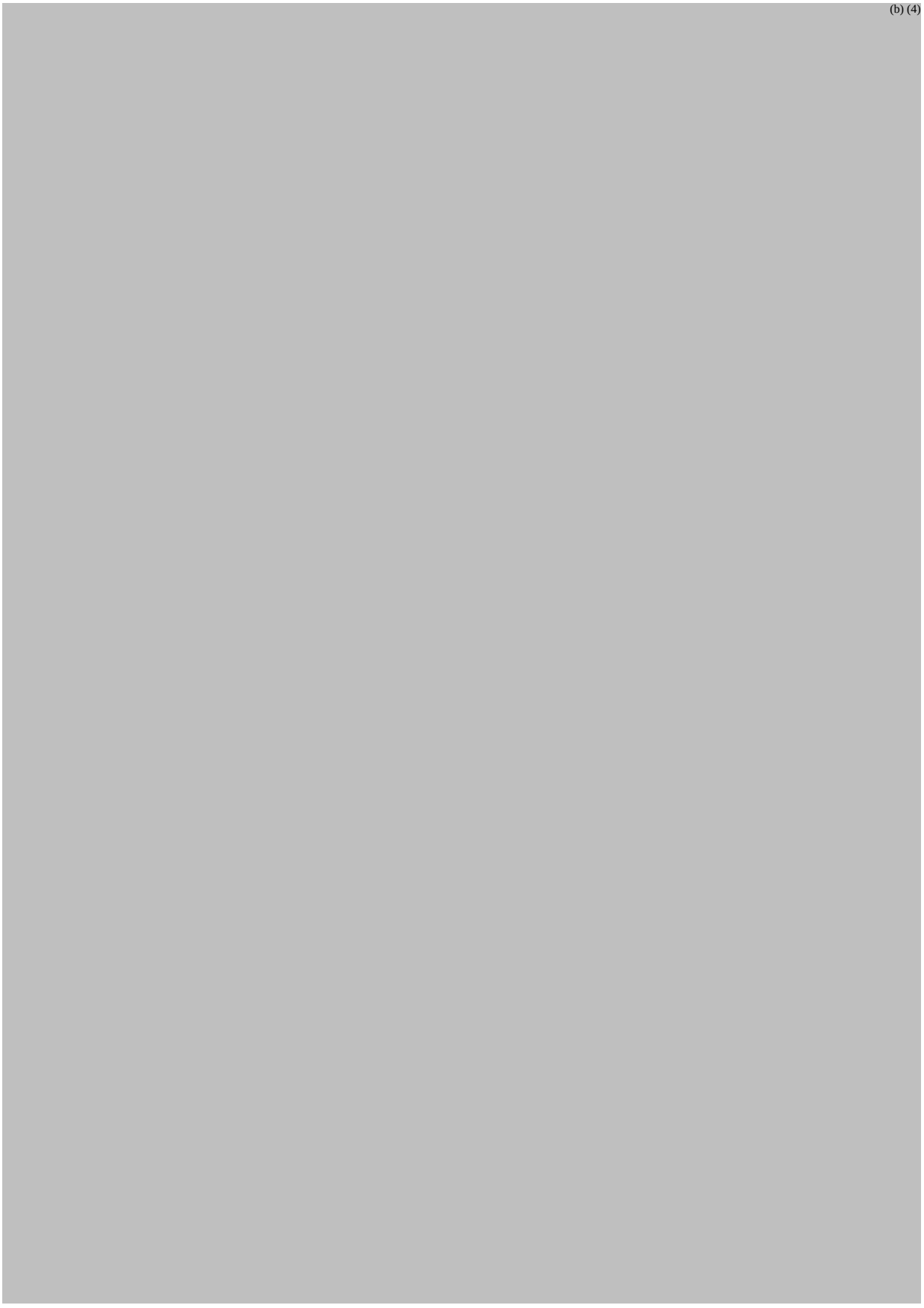
2.3.7 Group assignment (SOP/SGE/130)

On the last day of acclimation (group assignment day), ten healthy animals with body weights close to the mean body weight will be allocated to the groups using the random method. The study groups will consist of four groups, and one animal each will be assigned to two groups for the sighting study and four animals each will be assigned to two groups for the main study. Animals of each group will be given an animal ID number (G1: 2101, G2: 2201, G3: 2301 − 2304 and G4: 2401 − 2404).

2.3.8 Disposition of remaining animals (SOP/SGE/340)

The remaining animal not selected for the study will be discarded following group assignment. All animals including unused animals will be discarded after the completion of the final dosing.

(b) (4)



2.6 Drinking Water (SOP/GER/011)

2.6.1 Type and method of water supply

Public tap water in (b) (4) will be filtered and irradiated by ultraviolet light and provided *ad libitum*.

2.6.2 Analysis of drinking water

Samples of drinking water are analyzed for specified microorganisms once a month and all environmental contaminants once a year by the (b) (4) (b) (4) (b) (4) according to the Regulation of Quality Criteria for Potable Water and Test (Ministry of Environment Ordinance No. 833, Revision Dec. 20, 2019). The results of water analysis will be confirmed to meet the allowable standard of this facility.

2.7 Dosing (SOP/SGE/260)

2.7.1 Route

Oral via gastric intubation

2.7.2 Justification for the route of administration

The oral route is chosen because it is the intended route of administration in animals.

2.7.3 Method of administration

Individual doses will be calculated based on the animals' body weights recorded just prior to dosing at a dose volume of 10 mL/kg body weight. Animals will be dosed via gastric intubation with a disposable syringe fitted with an intubation tube. Animals will be fasted overnight, approximately 16 hours prior to dosing. Drinking water will be provided *ad libitum*. Feed will be provided approximately 4 hours after dosing.

2.8 Group Designation and Dose Levels

2.8.1 Sighting study

The starting dose level for this study is selected at 300 mg/kg because there is no available toxicity information on the test substance. Therefore, a starting dose of 300 mg/kg body weight of the test substance will be administered to one animal as the dose level for the sighting study (Step 1).

The following steps will be based on the results of mortality and clinical signs of animals obtained from the observations for more than 24 hours after the administration at the previous dose level in accordance with '<Attachment 1> ANNEX 2: FLOW CHART FOR THE SIGHTING STUDY'.

2.8.2 Main study

The progress of main study will be based on the mortality and clinical observations for more than 24 hours after the administration in the sighting study. In the main study, the test substance will be dosed to four female rats. The main study will be performed in accordance with '<Attachment 2> ANNEX 3: FLOW CHART FOR THE MAIN STUDY'

2.9 Parameters Evaluated

2.9.1 Clinical signs (SOP/SGE/160)

All animals will be observed for clinical signs (type, severity, time of onset and recovery, etc.) and mortality at least once for 30 minutes after dosing and at 1, 2, 4 and 6 hours after dosing on the day of dosing (Day 1), and once daily thereafter for 14 days (Day 2 to Day 15).

2.9.2 Disposition of dead animals (SOP/SGE/240)

A necropsy will be conducted as soon as possible after body weight measurement of the animals found dead during the observation period. If necropsy is not feasible immediately, the dead animals will be necropsied within 24 hours after storage under refrigeration.

2.9.3 Body weights (SOP/SGE/300)

The body weight will be recorded once on the day of dosing (prior to dosing), and on Days 2, 4, 8 and 15 (the day of necropsy).

2.9.4 Necropsy (SOP/PAT/115, 130)

On the day of necropsy, all surviving animals will be anesthetized by CO_2 gas inhalation and exsanguinated from the abdominal aorta. Complete gross postmortem examinations will be performed on all animals in the study.

2.9.5 Histopathology (SOP/PAT/190, 230, 250, 280, 300, 320)

The histopathological examination will be performed on organs and/or tissues showing morphological abnormalities or any gross lesions at necropsy, if the detailed examination is deemed necessary.

2.10 Statistical Analysis

Statistical analysis will not be performed. Mean scores and values will be determined.

2.11 Classification of GHS Category

The classification of Globally Harmonized Classification System for Chemical Substances and Mixtures (GHS) Category will be estimated based on the mortality in each step (Attachment 2).

<Attachment 1>

ANNEX 2: FLOW CHART FOR THE SIGHTING STUDY

'

(b) (4)

<Attachment 2>

ANNEX 3: FLOW CHART FOR THE MAIN STUDY

Appendix II. Protocol Amendments

Procedure	Protocol Amendments
No amendments were noted on the study.	

Appendix III. Protocol Deviations

Procedure	Protocol Deviations
No deviations were noted on the study.	

Appendix IV. Pathology Report

(b) (4)

PATHOLOGY REPORT

Study Title : Acute Oral Dose Toxicity Study of L-Valine (VAL Pro) in Sprague-Dawley Rats (Fixed Dose Procedure)

Study No. : B20742

Test Facility : (b) (4)

Study Pathologist: (b) (4)

Nov, 3, 2020
Date

1. Materials and Methods

1.1 Dose Levels

1.1.1 Sighting study
-Step 1: 300 mg/kg
-Step 2: 2,000 mg/kg

1.1.2 Main study
- Step 3: 2,000 mg/kg

1.2 Necropsy

On Day 15, all animals were anesthetized with CO_2 gas inhalation and exsanguinated from the abdominal aorta. Complete gross postmortem examination was performed on all animals in the study.

2. Results and Discussion

2.1 Necropsy

At necropsy, no remarkable findings were noted in all animals.

APPENDICES

Individual Gross Findings

Study No. : B20742
Sex : Female
Step 1 : (b) (4)
Step 2 :
Step 3 :

Individual Gross Findings

STUDY NO. : B20742
DOSE STEP : 1

*ANIMAL ID : 2101 SEX : Female

STUDY DAY : 15 STATUS AT NECROPSY : Scheduled

NECROPSY FINDINGS
No Finding Noted

Individual Gross Findings

STUDY NO. : B20742
DOSE STEP : 2

*ANIMAL ID : 2201 SEX : Female

STUDY DAY : 15 STATUS AT NECROPSY : Scheduled

NECROPSY FINDINGS
No Finding Noted

Individual Gross Findings

STUDY NO. : B20742
DOSE STEP : 3

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*ANIMAL ID : 2301 SEX : Female

STUDY DAY : 15 STATUS AT NECROPSY : Scheduled

NECROPSY FINDINGS
No Finding Noted

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*ANIMAL ID : 2302 SEX : Female

STUDY DAY : 15 STATUS AT NECROPSY : Scheduled

NECROPSY FINDINGS
No Finding Noted

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*ANIMAL ID : 2303 SEX : Female

STUDY DAY : 15 STATUS AT NECROPSY : Scheduled

NECROPSY FINDINGS
No Finding Noted

---

*ANIMAL ID : 2304 SEX : Female

STUDY DAY : 15 STATUS AT NECROPSY : Scheduled

NECROPSY FINDINGS
No Finding Noted

Appendix V. Certificate of Analysis

CJ Research Institute of Biotechnology

55, Gwanggyo-ro 42beon-gil, Yeongtong-gu, Suwon-si, Gyeonggi-do, 16495, Korea

www.cj.co.kr

TEL : 031) 8099-2450 FAX : 031) 8099-2918

Certificate of analysis

Certificate No.	2019-PR-205	Receipt No.	2019-AN-130
Client	-	Date of Receipt	2019.11.25
Client Name	-	Date of Test	2019.11.26
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine Feed Grade
Manuf. Date	2019.11.21.
Lot. No	GVAL191121
Quantity (kg)	

Test Item(s)	Test Result	Test method used
Valine	(b) (4)	HPLC
Loss on drying		AOAC 934.01

* Information

* Temperature : (22~28) ℃, Relative Humidity : (30~60) %

* N.D : not detected (not quantifiable)

* The results shown in this test report refer only to the sample tested unless otherwise stated.

The Test Report cannot be reproduced, except in full.

Tested by (b) (4) (b) (4)

Approved by Technical Manager

Nov, 29, 2019

CJ Research Institute of Biotechnology

CJ BIO-AD form 100-01 REV.01

<Attachment 1>

ANNEX 2: FLOW CHART FOR THE SIGHTING STUDY

<Attachment 2>

ANNEX 3: FLOW CHART FOR THE MAIN STUDY

(b) (4)

FINAL REPORT

Bacterial Reverse Mutation Test of L-Valine (VAL Pro)

Study No.: B20743

(b) (4)

GLP COMPLIANCE STATEMENT

Study Title: Bacterial Reverse Mutation Test of L-Valine (VAL Pro)

Study No.: B20743

This study was conducted in accordance with the following Good Laboratory Practice Regulations:

- "Good Laboratory Practice Regulation for Nonclinical Laboratory Studies"

  Notification No. 2018-93, Ministry of Food and Drug Safety, Republic of Korea (Nov. 21, 2018)
- "OECD Principles of Good Laboratory Practice"

  Organisation for Economic Co-operation and Development, ENV/MC/CHEM(98) 17 (as revised in 1997)

(b) (4)

Study Director

Dec. 17. 2020

Date

(b) (4)

Test Facility Management

Dec. 17, 2020

Date

QUALITY ASSURANCE STATEMENT

Study Title : Bacterial Reverse Mutation Test of L-Valine (VAL Pro)

Study No. : B20743

This study was audited by the Quality Assurance Unit of (b) (4) as indicated below. Audits were based on the GLPs, protocol and SOPs (b) (4) The results of all quality assurance inspections and audits were reported to the study director and test facility management.

Audit phases and dates as described below were reported to the study director and test facility management.

Audit Phase	Audit Date	To Study Director and Test Facility Management
Protocol	Oct. 12, 2020	Oct. 12, 2020
Storage of the test substance	Oct. 14, 2020	Oct. 14, 2020
[Dose range finding study]		
Preparation of the dosing formulations	Oct. 14, 2020	Oct. 14, 2020
Treatment with dosing formulations	Oct. 14, 2020	Oct. 14, 2020
[Main study]		
Inoculation of strains*	Oct. 5, 2020	Oct. 6, 2020
Preparation of the dosing formulations	Oct. 27, 2020	Oct. 27, 2020
Treatment with dosing formulations	Oct. 27, 2020	Oct. 27, 2020
Colony counting*	Oct. 8, 2020	Oct. 8, 2020
Raw data	Nov. 23, 2020	Nov. 23, 2020
Draft Report	Nov. 23, 2020	Nov. 23, 2020
Final Report	Dec. 17, 2020	Dec. 17, 2020

*Process-based inspections: The performance of process-based inspections covering phases which occur with a very high frequency may result in some studies not being inspected on an individual basis during their experimental phase.

This statement confirms that described methods were established, and results reflected raw data accurately in the final report.

(b) (4)
Quality Assurance Management

(b) (4)

Dec. 17, 2020
Date

CONTENTS

SUMMARY

This study was designed to evaluate the mutagenic potential of the test substance, L-Valine (VAL Pro), using histidine requiring *Salmonella typhimurium* (TA98, TA100, TA1535 and TA1537) strains and tryptophan requiring *Escherichia coli* (WP2*uvrA*(pKM101)) strain in the absence and presence of metabolic activation.

In order to determine the high dose level of the main study, a dose range finding study was conducted. The high dose was set at 5,000 μg/plate and it was sequentially diluted by applying a geometric ratio of 4 to produce 5 lower dose levels (1,250, 313, 78.1, 19.5 and 4.88 μg/plate). As a result, the growth inhibition by the test substance and precipitation of the test substance were not evident at any dose level of the test substance in all strains in the absence and presence of metabolic activation.

Therefore, the dose levels of the main study were selected as follows. In addition, the negative and positive control groups were set.

Strain	S9 mix	Dose levels of the main study (μg/plate)
TA98, TA100, TA1535, TA1537, WP2*uvrA*(pKM101)	-/+	5,000, 2,500, 1,250, 625, 313

Based on the result of the main study, the mean number of revertant colonies was less than twice when compared to the negative control group at all dose levels of the test substance in the absence and presence of metabolic activation.

In the positive control group, the mean number of revertant colonies for each strain was markedly increased more than twice when compared to the negative control group.

Based on the results of this study, the test substance, L-Valine (VAL Pro), did not show any indication of mutagenic potential under the conditions of this study.

1. EXPERIMENTAL OUTLINE

1.1 Purpose

The purpose of this study was to evaluate the mutagenic potential of the test substance, L-Valine (VAL Pro), using histidine requiring *Salmonella typhimurium* strains and tryptophan requiring *Escherichia coli* strain.

1.2 Good Laboratory Practice Regulations

This study was conducted in accordance with the following Good Laboratory Practice Regulations:

- "Good Laboratory Practice Regulation for Nonclinical Laboratory Studies"

  Notification No. 2018-93, Ministry of Food and Drug Safety, Republic of Korea (Nov. 21, 2018)

- "OECD Principles of Good Laboratory Practice"

  Organisation for Economic Co-operation and Development, ENV/MC/CHEM(98) 17 (as revised in 1997)

1.3 Regulatory Guidelines

This study was conducted in accordance with the following guideline:

- "OECD Guidelines for the Testing of Chemicals, 471, Bacterial Reverse Mutation Test"
  Organisation for Economic Co-operation and Development (Adopted: 26 June 2020)

1.4 Sponsor

Name	CJ CheilJedang
Address	55, Gwanggyo-ro 42beon-gil, Yeongtong-gu, Suwon-si, Gyeonggi-do, 16495, Republic of Korea
TEL	+ 82-31-8099-1515

1.5 Test Facility

Name (b) (4)

Address (b) (4)

TEL (b) (4) FAX (b) (4)

1.6 Study Director

Name (b) (4)

1.7 Study Schedule

Study initiation	Sep. 28, 2020
Experimental start	Oct. 13, 2020
<Dose range finding study>	
- Inoculation of strains	Oct. 13, 2020
- Treatment of dosing formulation	Oct. 14, 2020
- Incubation of strains	Oct. 14–16, 2020
- Colony counting	Oct. 16, 2020
<Main study>	
- Inoculation of strains	Oct. 26 and 27, 2020
- Treatment of dosing formulation	Oct. 27 and 28, 2020
- Incubation of strains	Oct. 27–29, Oct. 28–30, 2020
- Colony counting	Oct. 29 and 30, 2020
Experimental completion	Oct. 30, 2020
Study completion	Dec. 17, 2020

1.8 Key Personnel

Test substance storage and handling (b) (4)

1.9 Retention of Raw Data

1.9.1 Duration Three years from the approval date

(Notification No. 2018-93, Ministry of Food and Drug Safety, Republic of Korea)

1.9.2 Storage facility

Name (b) (4)

Address (b) (4)

(Raw data will be retained for the first five years after the completion of the study in the archives of (b) (4) Further storage will be determined in agreement with the sponsor.)

1.9.3 Type of records and data

Protocol, final report, all raw data, documents related to the study, communications

2. MATERIALS AND METHODS

2.1 Test Substance

2.1.1	Name	L-Valine (VAL Pro)
2.1.2	Lot No./Batch No.	GVAL191121
2.1.3	Appearance	Light brown granules
2.1.4	Component · Content	Valine 72.57%
2.1.5	Date of manufacture	Nov. 21, 2019
2.1.6	Date of expiration	Dec. 23, 2021
2.1.7	Storage condition	Room temperature (15 ~ 25°C)
2.1.8	Handling instructions	Not specific
2.1.9	Supplier	
	Name	CJ CheilJedang
	Address	55, Gwanggyo-ro 42beon-gil, Yeongtong-gu, Suwon-si, Gyeonggi-do, 16495, Republic of Korea
2.1.10	Disposition of test substance	Any remaining test substance is returned to the Sponsor.

2.2 Negative Control

2.2.1	Name	Water for injection
2.2.2	Lot No.	(b) (4)
2.2.3	Storage condition	Room temperature (1 ~ 30°C)
2.2.4	Manufacturer	(b) (4) (b) (4)
2.2.5	Justification for selection	Water for injection, the vehicle of the test substance, was used as the negative control.

2.3 Preparation and Analysis of the Dosing Formulations

2.3.1 Vehicle

2.3.1.1 Name Water for injection

2.3.1.2 Lot No. (b) (4)

2.3.1.3 Justification for selection

In order to produce a dose of 5,000 μg/plate, which is the high dose level of the dose range finding study, a preliminary solubility test was conducted. As a result, the test substance was suspended uniformly in water for injection. Therefore, water for injection was selected as the vehicle for this study.

2.3.2 Preparation method

All preparations were conducted on the day of treatment of dosing formulations.

The required amount of the test substance was weighed and placed in a mortar. A small amount of vehicle, water for injection, was added and the both materials were mixed using a pestle until suspended uniformly. Then, the mixture was transferred in a measuring tube and vehicle was added to yield the desired dose level. The high dose formulation was serially diluted to produce lower dose levels.

2.3.3 Analysis of the dosing formulations

Analysis for stability, homogeneity and concentration of the dosing formulations was not performed.

2.4 Positive Controls

2.4.1 Name

Name	Lot No. (#: Batch No.)	Storage condition	Manufacturer
Sodium azide (SA)	#MKBX7529V	Room temperature	(b) (4)
2-Nitrofluorene (2-NF)	#S43858V	Room temperature	
2-Aminoanthracene (2-AA)	#STBD3302V	Room temperature	
9-Aminoacridine (9-AA)	BCCB4167	Room temperature	
4-Nitroquinoline N-oxide (4-NQO)	#WXBC3635V	Room temperature	

2.4.2 Vehicle of the positive controls

Name	Lot No.	Storage condition	Manufacturer
Water for injection (SA)	19017	Room temperature	(b) (4)
Dimethyl sulfoxide (2-NF, 2-AA, 9-AA, 4-NQO)	K51447331, K51637131	Room temperature	

2.4.3 Preparation of the positive controls

The required amount of the positive controls was weighed. The positive controls were prepared in vehicle. The prepared positive controls were stored in a deep freezer (-80 ~ -60℃, (b) (4)) and thawed just prior to use.

<The type and dose of the positive controls for the respective strains>

S9 mix	Strain	Name	Dose (μg/plate)
-	TA98	2-NF	5.0
	TA100	SA	1.5
	TA1535	SA	1.5
	TA1537	9-AA	80.0
	WP2*uvrA*(pKM101)	4-NQO	0.1
+	TA98	2-AA	1.0
	TA100	2-AA	2.0
	TA1535	2-AA	3.0
	TA1537	2-AA	3.0
	WP2*uvrA*(pKM101)	2-AA	2.0

2.5 Medium

2.5.1 Nutrient broth medium

Nutrient broth (b) (4) was weighed and mixed with a small amount of ultra pure water using a stirrer until dissolved. Ultra pure water was added to yield a concentration of 0.8% and then autoclaved.

2.5.2 Minimal glucose agar plate

2.5.2.1 Storage condition Room temperature

2.5.2.2 Producer and supplier (b) (4)

<Composition of the minimal glucose agar plate>

Component	Amount of each component
Bacto agar	15 g
10-fold VB salts	100 mL
20% Glucose	100 mL
Ultra pure water	800 mL
Total volume	1 L

<Composition of the 10-fold VB salts>

Component	Used amount	Supplier
$MgSO_4 \cdot 7H_2O$	0.2 g	(b) (4)
Citric acid	1.829 g	(b) (4)
K_2HPO_4	10 g	(b) (4)
$NaNH_4HPO_4 \cdot 4H_2O$	3.58 g	(b) (4) (b) (4)
Ultra pure water	100 mL	-

2.5.3 Top agar

NaCl and bacto agar (b) (4) were weighed and ultra pure water was added to yield the concentrations of 0.5% and 0.6%, respectively, and then autoclaved. These mixtures were mixed with the 0.5 mM L-Histidine/D-Biotin ((b) (4) (b) (4)) solution at a ratio of 10 to 1 for *Salmonella typhimurium* and with the 0.5 mM L-Tryptophan (b) (4) solution at a ratio of 10 to 1 for *Escherichia coli*.

2.6 Preparation of S9 Mix

2.6.1 Name S9 and Cofactor A

2.6.2 Storage condition Deep freezer (-80 ~ -60°C)

2.6.3 Producer (b) (4)

2.6.4 Supplier (b) (4)

2.6.5 Characteristics of S9

Species and strain	Sprague-Dawley rat [Crl:CD(SD)]
Sex and age	Male, 7 weeks old
Organ	Liver
Inducing agent	Phenobarbital (PB) and 5,6-benzoflavone (BF)
Dose and frequency	PB: 30 mg/kg, once (Day 1) 60 mg/kg, once daily for 3 consecutive days (Days 2–4) BF: 80 mg/kg, once (Day 3)
Route of administration	Intraperitoneal injection

2.6.6 Composition of S9 mix

Component		Amount of each component
S9		0.1 mL
Cofactor A	0.4 mol/L $MgCl_2$	0.02 mL (8 µmol)
	1.65 mol/L KCl	0.02 mL (33 µmol)
	1.0 mol/L Glucose-6-phosphate	0.005 mL (5 µmol)
	0.1 mol/L NADPH	0.04 mL (4 µmol)
	0.1mol/L NADH	0.04 mL (4 µmol)
	0.2 mol/L Sodium phosphate buffer, pH 7.4	0.5 mL (100 µmol)
	Purified water	0.275 mL
Total volume		1 mL

2.6.7 Preparation method of S9 mix

The preparation of S9 mix was conducted immediately prior to use. The frozen S9 (Lot No.: 20080705) and Cofactor A (Lot No.: A20080405) were thawed and mixed at a ratio of 1 to 9.

2.7 Bacterial Strains

2.7.1 Species and strains

Salmonella typhimurium TA98

Salmonella typhimurium TA100

Salmonella typhimurium TA1535

Salmonella typhimurium TA1537

Escherichia coli WP2*uvrA*(pKM101)

2.7.2 Storage condition Deep freezer (-80 ~ -60℃)

2.7.3 Producer (b) (4) (b) (4)

2.7.4 Supplier (b) (4)

2.7.5 Justification for strain selection

These strains are highly sensitive to mutagens, commonly used in mutagenicity studies and recommended in the test guidelines.

2.7.6 Genotypes of each strain

Species	Strain	Genotype
Salmonella typhimurium	TA98 TA100 TA1535 TA1537	(b) (4)
Escherichia coli	WP2*uvrA*(pKM101)	

2.7.7 Pre-incubation

After confirming strain characteristics, each frozen bacterial suspension was thawed. And then, it was inoculated into the nutrient broth medium and incubated in a shaking water bath (37°C, 130 rpm, (b) (4)). Following pre-incubation, the turbidity of the cultures was measured with a UV/VIS spectrophotometer (b) (4)). Cultures with a density greater than 1×10^9 cells/mL were used in this study.

2.8 Dose Range Finding Study

A dose range finding study was conducted to determine the high dose for the main study.

2.8.1 Dose levels

The high dose of the test substance was set at 5,000 μg/plate, which is required in the test guidelines. The high dose was sequentially diluted by applying a geometric ratio of 4 to produce 5 lower dose levels (1,250, 313, 78.1, 19.5 and 4.88 μg/plate). In addition, the negative and positive control groups were set.

2.8.2 Study method

The dose range finding study was conducted using the same method and conditions as the main study. Two plates per dose were used in the dose range finding study.

2.8.3 Justification for selection of the dose levels in the main study

As a result of the dose range finding study, the growth inhibition by the test substance and precipitation of the test substance were not evident at any dose level of the test substance in all strains in the absence and presence of metabolic activation.

Therefore, the high dose in the main study was selected at 5,000 μg/plate in all strains in the absence and presence of metabolic activation and it was sequentially diluted by applying a geometric ratio of 2 to produce 4 lower dose levels (2,500, 1,250, 625 and 313 μg/plate). In addition, the negative and positive control groups were set.

Strain	S9 mix	Dose levels of the main study (μg/plate)
TA98, TA100, TA1535, TA1537, WP2*uvrA*(pKM101)	-/+	5,000, 2,500, 1,250, 625, 313

2.9 Main Study

2.9.1 Study method

The main study was conducted according to the pre-incubation method. All treatments were divided into absence and presence of metabolic activation.

Three plates per dose were used in the main study and the treatment was conducted in duplicate.

Each plate was labeled with an identification number which indicates the bacterial strain, dose, the negative and positive controls and the absence or presence of S9 mix.

2.9.2 Treatment method

(b) (4)

(b) (4)

2.9.3 Incubation method and period

After the top agar was solidified, the plates were inverted and cultured in an incubator ((b) (4)) at 37℃ for 48 hours.

2.9.4 Observation of precipitation

The precipitation of the test substance was observed with the naked eye and recorded at the time of treatment of the test substance and colony counting.

2.9.5 Revertant colony counting

Following cultivation, the number of revertant colonies was automatically counted by a colony counter (b) (4)) or by visual counting. When automatic counting was considered to be inaccurate, the number of revertant colonies was counted by visual counting.

2.9.6 Observation of background lawn

To confirm the absence or presence of growth inhibition by the test substance, the background lawn was observed using a stereoscopic microscope (45-fold magnification, (b) (4)). Growth inhibition was detected by reduction in the number of revertant colonies, or by diminution or clearing of background lawn compared to the negative control group.

2.10 Acceptance Criteria

Evaluation of the validity of the study results was conducted based on the following criteria:

・	The results of gene mutagenic potential in the main studies are reproducible.
・	There are more than 4 dose levels at which growth inhibition is not observed.
・	The mean number of revertant colonies for the negative and positive control groups is within the range of the historical control data or the mean number of revertant colonies in the positive control group is increased at least twice as compared to the negative control group.
・	No plate shows any evidence of contamination.

2.11 Evaluation Criteria

The results of the study were judged to be positive when the following conditions were met (others were considered as negative). A confirmatory study was not conducted.

- The number of revertant colonies in any strain at one or more doses is increased at least two times when compared to the negative control group. There should be dose dependency or reproducibility as dose increases.

2.12 Statistical Analysis

Individual plate was counted for revertant colonies. The average and standard deviation of the number of revertant colonies were calculated. Statistical analysis was not performed.

3. RESULTS AND DISCUSSION

3.1 Dose Range Finding Study

(Figure 1, Figure 2, Table 1, Table 2)

As a result of the dose range finding study according to the 2.8 method, the dose levels of the main study were selected as follows. In addition, the negative and positive control groups were set.

Strain	S9 mix	Dose levels of the main study (μg/plate)
TA98, TA100, TA1535, TA1537, WP2*uvrA*(pKM101)	-/+	5,000, 2,500, 1,250, 625, 313

3.2 Main Study

(Figure 3, Figure 4, Figure 5, Figure 6, Table 3, Table 4)

3.2.1 Revertant colony counting

As a result of the main study, the mean number of revertant colonies was less than twice when compared to the negative control group at all dose levels of the test substance in all strains in the absence and presence of metabolic activation.

In the positive control group, the mean number of revertant colonies for each strain was markedly increased more than twice when compared to the negative control group.

3.2.2 Growth inhibition and precipitation of the test substance

Growth inhibition by the test substance and precipitation of the test substance were not evident at any dose level of the test substance in all strains in the absence and presence of metabolic activation.

3.3 Acceptance of Study

There were more than 4 dose levels at which growth inhibition was not observed. The results of gene mutagenic potential in the main studies were reproducible. The mean number of revertant colonies in the negative and positive group was within the range of the historical control data (Table 5) and the number of revertant colonies in each strain in the positive control groups was markedly increased at least twice when compared to the negative control group. In addition, there was no contamination. Therefore, these results indicated that this study was conducted under the suitable conditions.

4. CONCLUSION

Based on the results of this study, the test substance, L-Valine (VAL Pro), did not show any indication of mutagenic potential under the conditions of this study.

FIGURES

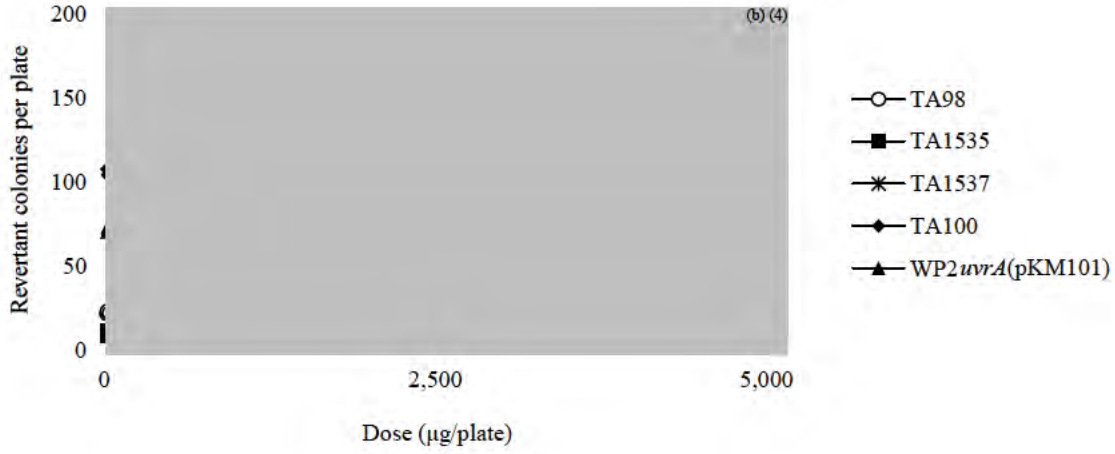


Figure 1. Dose-response Curve in the Absence of Metabolic Activation
(TA98, TA100, TA1535, TA1537 and WP2*uvrA*(pKM101), Dose Range Finding Study)

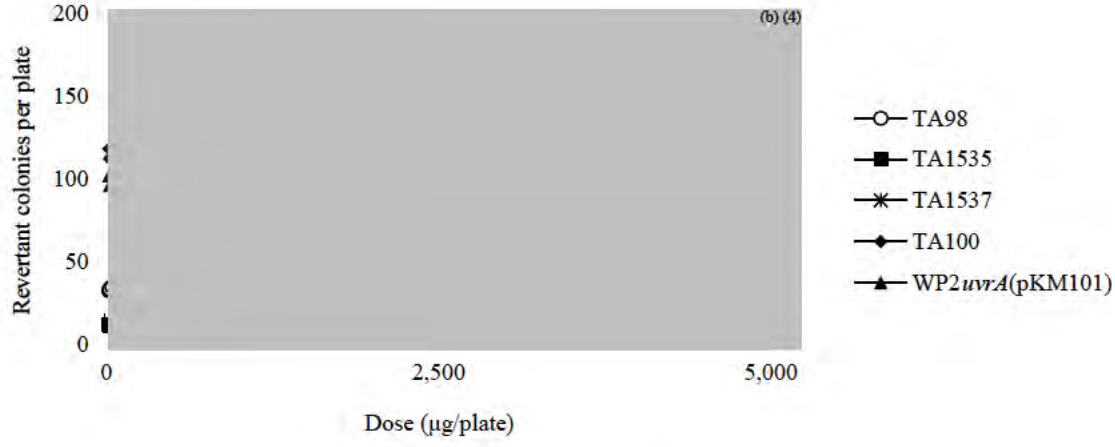


Figure 2. Dose-response Curve in the Presence of Metabolic Activation
(TA98, TA100, TA1535, TA1537 and WP2*uvrA*(pKM101), Dose Range Finding Study)

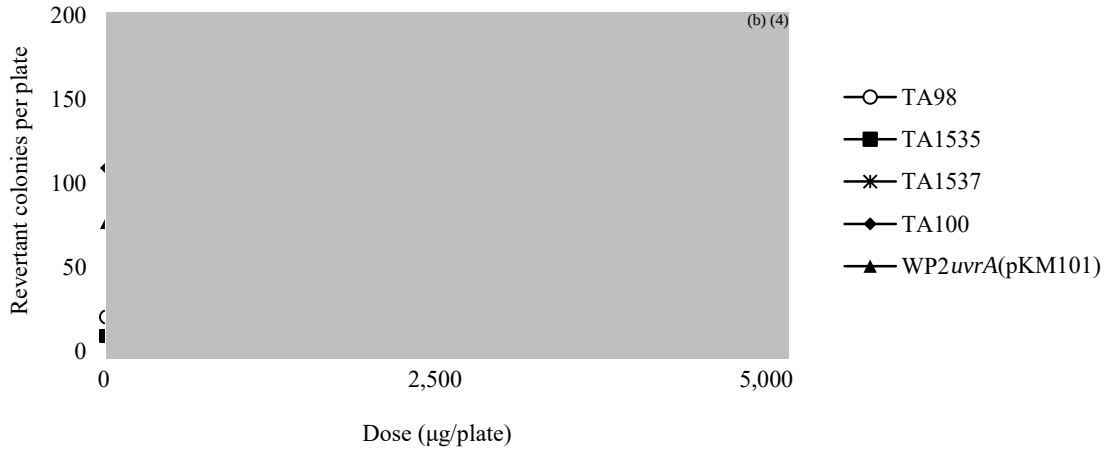


Figure 3. Dose-response Curve in the Absence of Metabolic Activation
(TA98, TA100, TA1535, TA1537 and WP2*uvrA*(pKM101), 1st Main Study)

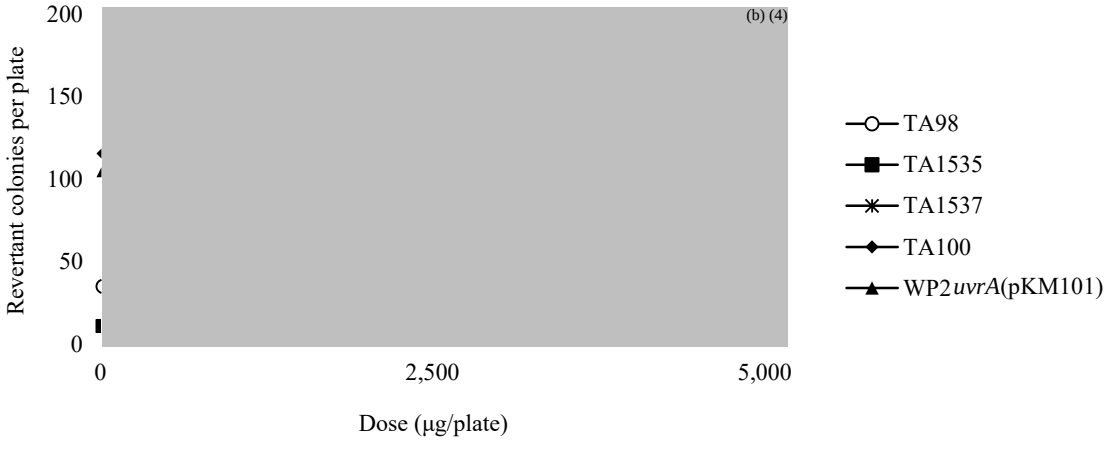


Figure 4. Dose-response Curve in the Presence of Metabolic Activation
(TA98, TA100, TA1535, TA1537 and WP2*uvrA*(pKM101), 1st Main Study)

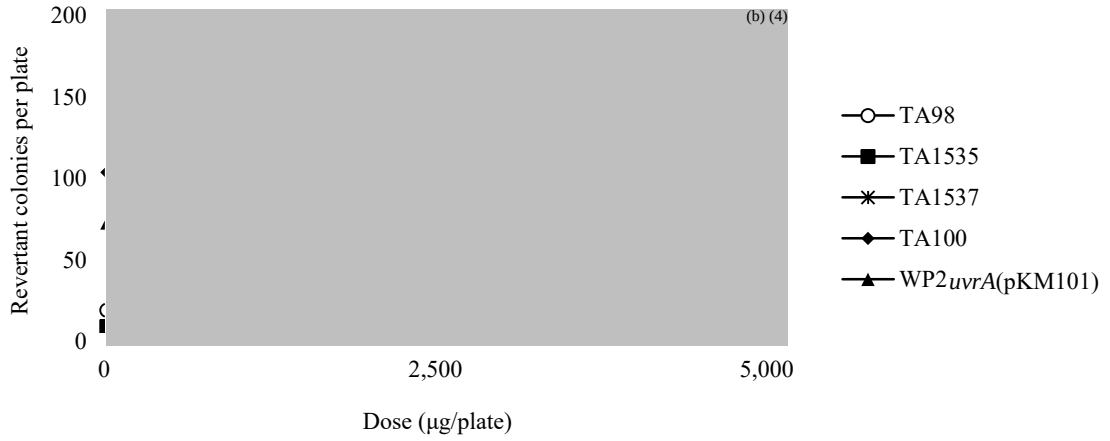


Figure 5. Dose-response Curve in the Absence of Metabolic Activation
(TA98, TA100, TA1535, TA1537 and WP2*uvrA*(pKM101), 2nd Main Study)

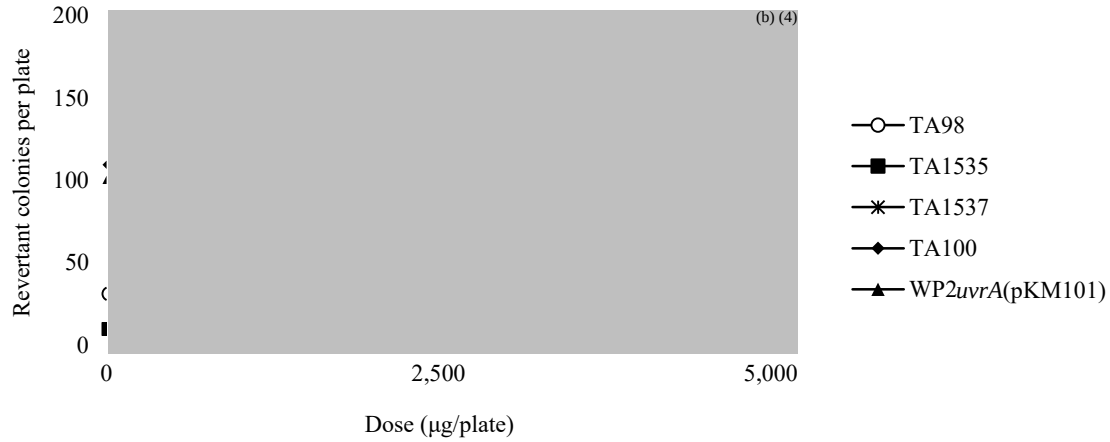


Figure 6. Dose-response Curve in the Presence of Metabolic Activation
(TA98, TA100, TA1535, TA1537 and WP2*uvrA*(pKM101), 2nd Main Study)

TABLES

Table 1. The Number of Revertant Colonies per Plate in the Absence of Metabolic Activation (Dose Range Finding Study)

Strain	Test substance	Dose (μg/plate)	Individual revertant	Mean
TA98	Water for injection	0	(b) (4)	22
	L-Valine (VAL Pro)	4.88		24
		19.5		23
		78.1		20
		313		21
		1,250		20
		5,000		24
	2-Nitrofluorene (2-NF)	5.0		708
TA100	Water for injection	0	(b) (4)	108
	L-Valine (VAL Pro)	4.88		105
		19.5		109
		78.1		99
		313		104
		1,250		103
		5,000		112
	Sodium azide (SA)	1.5		749
TA1535	Water for injection	0	(b) (4)	12
	L-Valine (VAL Pro)	4.88		9
		19.5		10
		78.1		11
		313		9
		1,250		11
		5,000		10
	Sodium azide (SA)	1.5		597
TA1537	Water for injection	0	(b) (4)	9
	L-Valine (VAL Pro)	4.88		8
		19.5		7
		78.1		8
		313		9
		1,250		8
		5,000		10
	9-Aminoacridine (9-AA)	80.0		644
WP2*uvrA* (pKM101)	Water for injection	0	(b) (4)	71
	L-Valine (VAL Pro)	4.88		74
		19.5		70
		78.1		74
		313		67
		1,250		65
		5,000		62
	4-Nitroquinoline N-oxide (4-NQO)	0.1		382

Table 2. The Number of Revertant Colonies per Plate in the Presence of Metabolic Activation (Dose Range Finding Study)

Strain	Test substance	Dose (μg/plate)	Individual revertant	Mean
TA98	Water for injection	0	(b) (4)	32
	L-Valine (VAL Pro)	4.88		33
		19.5		35
		78.1		32
		313		29
		1,250		45
		5,000		54
	2-Aminoanthracene (2-AA)	1.0		443
TA100	Water for injection	0	(b) (4)	118
	L-Valine (VAL Pro)	4.88		112
		19.5		113
		78.1		111
		313		120
		1,250		116
		5,000		121
	2-Aminoanthracene (2-AA)	2.0		949
TA1535	Water for injection	0	(b) (4)	12
	L-Valine (VAL Pro)	4.88		11
		19.5		14
		78.1		13
		313		11
		1,250		12
		5,000		14
	2-Aminoanthracene (2-AA)	3.0		165
TA1537	Water for injection	0	(b) (4)	21
	L-Valine (VAL Pro)	4.88		22
		19.5		18
		78.1		19
		313		18
		1,250		22
		5,000		24
	2-Aminoanthracene (2-AA)	3.0		249
WP2*uvrA* (pKM101)	Water for injection	0	(b) (4)	102
	L-Valine (VAL Pro)	4.88		96
		19.5		100
		78.1		94
		313		100
		1,250		99
		5,000		106
	2-Aminoanthracene (2-AA)	2.0		350

Table 3. The Number of Revertant Colonies per Plate in the Absence of Metabolic Activation (1st and 2nd Main Studies)

Strain	Test substance	Dose (μg/plate)	1st Main study			2nd Main study		
			Individual revertant colony counts	Mean	S D	Individual revertant colony counts	Mean	S D
TA98	Water for injection	0	(b) (4)	20	2	(b) (4)	19	2
	L-Valine (VAL Pro)	313		23	2		20	2
		625		24	3		21	2
		1,250		24	2		21	2
		2,500		22	2		22	2
		5,000		24	3		20	2
	2-Nitrofluorene (2-NF)	5 0		701	31		683	24
TA100	Water for injection	0	(b) (4)	109	5	(b) (4)	104	4
	L-Valine (VAL Pro)	313		111	3		107	4
		625		115	4		111	3
		1,250		122	4		117	4
		2,500		117	3		109	5
		5,000		120	4		114	3
	Sodium azide (SA)	1 5		715	31		713	23
TA1535	Water for injection	0	(b) (4)	9	1	(b) (4)	9	1
	L-Valine (VAL Pro)	313		10	1		9	1
		625		9	1		10	1
		1,250		10	2		11	1
		2,500		9	2		9	2
		5,000		11	1		10	2
	Sodium azide (SA)	1 5		593	7		579	29
TA1537	Water for injection	0	(b) (4)	9	1	(b) (4)	10	1
	L-Valine (VAL Pro)	313		9	1		9	1
		625		8	2		9	1
		1,250		8	1		8	1
		2,500		7	1		8	1
		5,000		10	1		11	1
	9-Aminoacridine (9-AA)	80 0		623	11		556	27
WP2*uvrA* (pKM101)	Water for injection	0	(b) (4)	77	4	(b) (4)	73	4
	L-Valine (VAL Pro)	313		82	4		77	3
		625		88	4		81	3
		1,250		90	5		87	4
		2,500		82	3		79	4
		5,000		86	4		82	3
	4-Nitroquinoline N-oxide (4-NQO)	0 1		401	16		405	27

S D : Standard Deviation

Table 4. The Number of Revertant Colonies per Plate in the Presence of Metabolic Activation (1st and 2nd Main Studies)

Strain	Test substance	Dose (μg/plate)	1st Main study			2nd Main study		
			Individual revertant colony counts	Mean	S D	Individual revertant colony counts	Mean	S D
TA98	Water for injection	0	(b) (4)	35	2	(b) (4)	31	1
	L-Valine (VAL Pro)	313		34	3		30	2
		625		42	3		41	2
		1,250		50	3		48	2
		2,500		53	3		49	2
		5,000		57	3		54	2
	2-Aminoanthracene (2-AA)	1 0		400	9		451	22
TA100	Water for injection	0	(b) (4)	115	4	(b) (4)	109	4
	L-Valine (VAL Pro)	313		122	4		116	4
		625		121	4		114	4
		1,250		118	3		108	3
		2,500		119	5		113	4
		5,000		123	4		118	4
	2-Aminoanthracene (2-AA)	2 0		982	23		961	21
TA1535	Water for injection	0	(b) (4)	11	1	(b) (4)	10	2
	L-Valine (VAL Pro)	313		11	1		11	1
		625		10	2		11	1
		1,250		12	1		12	1
		2,500		10	1		11	1
		5,000		13	2		12	1
	2-Aminoanthracene (2-AA)	3 0		174	11		161	25
TA1537	Water for injection	0	(b) (4)	22	2	(b) (4)	21	2
	L-Valine (VAL Pro)	313		22	2		19	3
		625		21	3		20	3
		1,250		23	2		23	2
		2,500		23	3		22	2
		5,000		25	2		24	2
	2-Aminoanthracene (2-AA)	3 0		186	6		203	16
WP2*uvrA* (pKM101)	Water for injection	0	(b) (4)	105	4	(b) (4)	102	4
	L-Valine (VAL Pro)	313		111	3		108	4
		625		103	5		101	4
		1,250		96	4		95	5
		2,500		95	4		93	5
		5,000		101	4		99	5
	2-Aminoanthracene (2-AA)	2 0		338	20		362	15

S D : Standard Deviation

Table 5. Historical Control Data

Historical negative control values of revertant colonies

Strain	S9 mix	N	Mean ± S.D.	Range	
				Lower	Upper
TA100	-	70	85.7 ± 9.5	(b) (4)	
	+	70	95.9 ± 10.3		
TA1535	-	69	11.6 ± 2.1		
	+	69	11.0 ± 1.6		
WP2*uvrA* (pKM101)	-	69	113.3 ± 20.4		
	+	69	138.9 ± 18.6		
TA98	-	70	18.8 ± 2.9		
	+	70	30.2 ± 4.9		
TA1537	-	69	8.4 ± 1.0		
	+	69	16.1 ± 2.8		

Historical positive control values of revertant colonies

Strain	S9 mix	Positive control	Dose (μg/plate)	N	Mean ± S.D.	Range	
						Lower	Upper
TA100	-	SA	1.5	70	662.1 ± 59.9	(b) (4)	
	+	2-AA	2.0	67	808.1 ± 127.6		
TA1535	-	SA	1.5	70	522.1 ± 52.8		
	+	2-AA	3.0	69	153.2 ± 21.8		
WP2*uvrA* (pKM101)	-	4-NQO	0.1	66	606.9 ± 124.8		
	+	2-AA	2.0	69	482.7 ± 76.1		
TA98	-	2-NF	5.0	70	622.9 ± 92.6		
	+	2-AA	1.0	67	392.0 ± 52.4		
TA1537	-	9-AA	80.0	70	519.1 ± 92.4		
	+	2-AA	3.0	66	204.1 ± 31.1		

Negative control: Water for injection, Dimethyl sulfoxide, Acetone, Tetrahydrofuran, *etc*.
SA: Sodium azide
2-AA: 2-Aminoanthracene
4-NQO: 4-Nitroquinoline N-oxide
2-NF: 2-Nitrofluorene
9-AA: 9-Aminoacridine
N: The total number of bacterial reverse mutation test
S.D.: Standard Deviation
The above historical control values were obtained from the data pooled from Sep. 29, 2016 to Nov. 14, 2019.
The range was calculated by the control limit of X derived from $\bar{X}$-$\bar{R}$-$\bar{R}$s value.

APPENDICES

Appendix I. Protocol

(b) (4)

PROTOCOL

Bacterial Reverse Mutation Test of L-Valine (VAL Pro)

Study No.: B20743

(b) (4)

PROTOCOL REVIEWED AND ACCEPTED BY

Study Title: Bacterial Reverse Mutation Test of L-Valine (VAL Pro)

Study No.: B20743

Test Facility (b) (4)

Study Director

(b) (4)

Sep. 28, 2020
Date

Test Facility Management

(b) (4)

Sep. 28, 2020
Date

Sponsor CJ CheilJedang

Monitor

(b) (4)

Sep 28, 2020
Date

CONTENTS

1. EXPERIMENTAL OUTLINE

1.1 Purpose

The purpose of this study is to evaluate the mutagenic potential of the test substance, L-Valine (VAL Pro), using histidine requiring *Salmonella typhimurium* strains and tryptophan requiring *Escherichia coli* strain.

1.2 Good Laboratory Practice Regulations

This study will be conducted in accordance with the following Good Laboratory Practice Regulations:

- "Good Laboratory Practice Regulation for Nonclinical Laboratory Studies"

  Notification No. 2018-93, Ministry of Food and Drug Safety, Republic of Korea (Nov. 21, 2018)

- "OECD Principles of Good Laboratory Practice"

  Organisation for Economic Co-operation and Development, ENV/MC/CHEM(98) 17 (as revised in 1997)

1.3 Regulatory Guidelines

This study will be conducted in accordance with the following guideline:

- "OECD Guidelines for the Testing of Chemicals, 471, Bacterial Reverse Mutation Test"
  Organisation for Economic Co-operation and Development (Adopted: 26 June 2020)

1.4 Sponsor

Name	CJ CheilJedang
Address	55, Gwanggyo-ro 42beon-gil, Yeongtong-gu, Suwon-si, Gyeonggi-do, 16495, Republic of Korea
TEL	+ 82-31-8099-1515

1.5 Test Facility

Name	(b) (4)
Address	(b) (4)
(b) (4)	

1.6 Study Director

Name	(b) (4)

1.7 Study Schedule

Study initiation	Sep. 28, 2020
Experimental start	Oct. 13, 2020
<Dose range finding study>	
- Inoculation of strains	Oct. 13, 2020
- Treatment of dosing formulation	Oct. 14, 2020
- Incubation of strains	Oct. 14–16, 2020
- Colony counting	Oct. 16, 2020
<Main study>	
- Inoculation of strains	Oct. 26 and 27, 2020
- Treatment of dosing formulation	Oct. 27 and 28, 2020
- Incubation of strains	Oct. 27–29, Oct. 28–30, 2020
- Colony counting	Oct. 29 and 30, 2020
Experimental completion	Oct. 30, 2020
Draft report issue	Nov. 27, 2020

1.8 Protocol Amendments

After the approval of the protocol, protocol amendments including reason for the changes, the contents of change and date of amendment will be documented and signed by the study director.

(b) (4) *Study No.: B20743*
Protocol

1.9 Final Report

The final report will be written including figures, tables and appendices. The original document will be retained in the archives of Biotoxtech Co., Ltd.

1.10 Retention of Raw Data

1.10.1 Duration Three years from the approval date

(Notification No. 2018-93, Ministry of Food and Drug Safety, Republic of Korea)

1.10.2 Storage facility

Name (b) (4)

Address (b) (4)

(b) (4)

(Raw data will be retained for the first five years after the completion of the study in the archives of (b) (4) Further storage will be determined in agreement with the Sponsor.)

1.10.3 Type of records and data

Protocol, final report, all raw data, documents related to the study, communications, etc.

2. MATERIALS AND METHODS

2.1 Test Substance

2.1.1	Name	L-Valine (VAL Pro)
2.1.2	Lot No./Batch No.	GVAL191121
2.1.3	Appearance	Light brown granules
2.1.4	Component · Content	Valine 72.57%
2.1.5	Date of manufacture	Nov. 21, 2019
2.1.6	Date of expiration	Dec. 23, 2021
2.1.7	Storage condition	Room temperature (15–25°C)
2.1.8	Handling instructions	Not specific
2.1.9	Supplier	
	Name	CJ CheilJedang
	Address	55, Gwanggyo-ro 42beon-gil, Yeongtong-gu, Suwon-si, Gyeonggi-do, 16495, Republic of Korea
2.1.10	Disposition of test substance	Any remaining test substance is returned to the Sponsor.

2.2 Negative Control

2.2.1	Name	Water for injection
2.2.2	Storage condition	Room temperature (1–30°C)
2.2.3	Manufacturer	(b) (4)
2.2.4	Justification for selection	Water for injection, the vehicle of the test substance, will be used as the negative control.

2.3 Preparation and Analysis of the Dosing Formulations

2.3.1 Vehicle

2.3.1.1 Name Water for injection

2.3.1.2 Justification for selection

In order to produce a dose of 5,000 μg/plate, which is the high dose level of the dose range finding study, a preliminary solubility test was conducted. As a result, the test substance was suspended uniformly in water for injection. Therefore, water for injection will be selected as the vehicle for this study.

2.3.2 Preparation method (SOP/FOT/140)

All preparations will be conducted on the day of treatment of dosing formulations.

The required amount of the test substance will be weighed and placed in a mortar. A small amount of vehicle, water for injection, will be added and the both materials will be mixed using a pestle until suspended uniformly. Then, the mixture will be transferred in a measuring tube and vehicle will be added to yield the desired dose level. The high dose formulation will be serially diluted to produce lower dose levels.

2.3.3 Analysis of the dosing formulations (SOP/ANA/002)

Analysis for stability, homogeneity and concentration of the dosing formulations will not be performed.

2.4 Positive Controls (SOP/GNT/004, SOP/FOT/140)

2.4.1 Name

Name	Storage condition	Manufacturer
Sodium azide (SA)	Room temperature	(b) (4)
2-Nitrofluorene (2-NF)	Room temperature	
2-Aminoanthracene (2-AA)	Room temperature	
9-Aminoacridine (9-AA)	Room temperature	
4-Nitroquinoline N-oxide (4-NQO)	Room temperature	

2.4.2 Vehicle of the positive controls

Name	Storage condition	Manufacturer
Water for injection (SA)	Room temperature	(b) (4)
Dimethyl sulfoxide (2-NF, 2-AA, 9-AA, 4-NQO)	Room temperature	

2.4.3 Preparation of the positive controls

The required amount of the positive controls will be weighed. The positive controls are prepared in vehicle. The prepared positive controls are stored in a deep freezer (-80—60°C, (b) (4) and thawed just prior to use.

<The type and dose of the positive controls for the respective strains>

S9 mix	Strain	Name	Dose (μg/plate)
-	TA98	2-NF	5.0
	TA100	SA	1.5
	TA1535	SA	1.5
	TA1537	9-AA	80.0
	WP2*uvrA*(pKM101)	4-NQO	0.1
+	TA98	2-AA	1.0
	TA100	2-AA	2.0
	TA1535	2-AA	3.0
	TA1537	2-AA	3.0
	WP2*uvrA*(pKM101)	2-AA	2.0

2.5 Medium (SOP/GNT/003)

2.5.1 Nutrient broth medium

Nutrient broth (b) (4) will be weighed and mixed with a small amount of ultra pure water using a stirrer until dissolved. Ultra pure water will be added to yield a concentration of 0.8% and then autoclaved.

2.5.2 Minimal glucose agar plate

2.5.2.1 Storage condition Room temperature

2.5.2.2 Producer and supplier (b) (4)

<Composition of the minimal glucose agar plate>

Component	Amount of each component
Bacto agar	15 g
10-fold VB salts	100 mL
20% Glucose	100 mL
Ultra pure water	800 mL
Total volume	1 L

<Composition of the 10-fold VB salts >

Component	Used amount	Supplier
$MgSO_4 \cdot 7H_2O$	0.2 g	(b) (4)
Citric acid	1.829 g	(b) (4)
K_2HPO_4	10 g	(b) (4)
$NaNH_4HPO_4 \cdot 4H_2O$	3.58 g	(b) (4)
Ultra pure water	100 mL	-

2.5.3 Top agar

NaCl and bacto agar (b) (4) will be weighed and ultra pure water will be added to yield the concentrations of 0.5% and 0.6%, respectively, and then autoclaved. These mixtures will be mixed with the 0.5 mM L-Histidine/D-Biotin (b) (4) solution at a ratio of 10 to 1 for *Salmonella typhimurium* and with the 0.5 mM L-Tryptophan (b) (4) solution at a ratio of 10 to 1 for *Escherichia coli*.

2.6 Preparation of S9 Mix (SOP/GNT/006)

2.6.1 Name S9 and Cofactor A

2.6.2 Storage condition Deep freezer (-80--60°C)

2.6.3 Producer (b) (4)

2.6.4 Supplier (b) (4)

2.6.5 Characteristics of S9

Species and strain	Sprague-Dawley rat (b) (4)
Sex and age	Male, 7 weeks old
Organ	Liver
Inducing agent	Phenobarbital (PB) and 5,6-benzoflavone (BF)
Dose and frequency	PB: 30 mg/kg, once (Day 1) 60 mg/kg, once daily for 3 consecutive days (Days 2–4) BF: 80 mg/kg, once (Day 3)
Route of administration	Intraperitoneal injection

2.6.6 Composition of S9 mix

Component		Amount of each component
S9		0.1 mL
Cofactor A	0.4 mol/L $MgCl_2$	0.02 mL (8 µmol)
	1.65 mol/L KCl	0.02 mL (33 µmol)
	1.0 mol/L Glucose-6-phosphate	0.005 mL (5 µmol)
	0.1 mol/L NADPH	0.04 mL (4 µmol)
	0.1 mol/L NADH	0.04 mL (4 µmol)
	0.2 mol/L Sodium phosphate buffer, pH 7.4	0.5 mL (100 µmol)
	Purified water	0.275 mL
Total volume		1 mL

2.6.7 Preparation method of S9 mix

The preparation of S9 mix will be conducted immediately prior to use. The frozen S9 and Cofactor A will be thawed and mixed at a ratio of 1 to 9.

2.7 Bacterial Strains (SOP/GER/030, SOP/GNT/007)

2.7.1 Species and strains

Salmonella typhimurium TA98

Salmonella typhimurium TA100

Salmonella typhimurium TA1535

Salmonella typhimurium TA1537

Escherichia coli WP2*uvrA*(pKM101)

2.7.2 Storage condition Deep freezer (-80—60°C)

2.7.3 Producer (b) (4)

2.7.4 Supplier (b) (4)

2.7.5 Justification for strain selection

These strains are highly sensitive to mutagens, commonly used in mutagenicity studies and recommended in the test guidelines.

2.7.6 Genotypes of each strain

Species	Strain	Genotype
Salmonella typhimurium	TA98 TA100 TA1535 TA1537	(b) (4)
Escherichia coli	WP2*uvrA*(pKM101)	

2.7.7 Pre-incubation

After confirming strain characteristics, each frozen bacterial suspension will be thawed. And then, it will be inoculated into the nutrient broth medium and incubated in a shaking water bath (37°C, 130 rpm, (b) (4) Following pre-incubation, the turbidity of the cultures will be measured with a UV/VIS spectrophotometer (660 nm, (b) (4) Cultures with a density greater than 1×10^9 cells/mL will be used in this study.

2.8 Dose Range Finding Study (SOP/GNT/012)

A dose range finding study will be conducted to determine the high dose for the main study.

2.8.1 Dose levels

The high dose of the test substance will be set at 5,000 μg/plate, which is required in the test guidelines. The high dose will be sequentially diluted by applying a geometric ratio of 4 to produce 5 lower dose levels (1,250, 313, 78.1, 19.5 and 4.88 μg/plate). In addition, the negative and positive control groups will be set.

2.8.2 Study method

The dose range finding study will be conducted using the same method and conditions as the main study. Two plates per dose will be used in the dose range finding study.

2.8.3 Justification for selection of the dose levels in the main study

If growth inhibition and precipitation of test substance are not evident at any dose level as a result of the dose range finding study, the high dose in the main study will be selected at 5,000 μg/plate and it will be sequentially diluted by applying a geometric ratio of 2 to produce 4 lower dose levels (2,500, 1,250, 625 and 313 μg/plate). In addition, the negative and positive control groups will be set.

If growth inhibition by the test substance is evident, the high dose in the main study will be selected at the lowest dose level at which growth inhibition is confirmed and it will be sequentially diluted by applying a geometric ratio of 2 to produce at least 5 lower dose levels in order to secure at least 4 dose levels that do not exhibit growth inhibition. In addition, the negative and positive control groups will be set.

If growth inhibition by the test substance is not evident and precipitation of the test substance is evident, the high dose in the main study will be selected at the high dose at which precipitation is observed but it does not interfere with the colony counting and it will be sequentially diluted by applying a geometric ratio of 2 to produce 4 lower dose levels. In addition, the negative and positive control groups will be set.

2.9 Main Study (SOP/GNT/012)

2.9.1 Study method

The main study will be conducted according to the pre-incubation method. All treatments will be divided into absence and presence of metabolic activation.

Three plates per dose will be used in the main study and the treatment will be conducted in duplicate.

Each plate will be labeled with an identification number which indicates the bacterial strain, dose, the negative and positive controls and the absence or presence of S9 mix.

2.9.2 Treatment method

(b) (4)

2.9.3 Incubation method and period

After the top agar is solidified, the plates will be inverted and cultured in an incubator at approximately 37°C for 48 hours.

2.9.4 Observation of precipitation

The precipitation of the test substance will be observed with the naked eye and recorded at the time of treatment of the test substance and colony counting.

2.9.5 Revertant colony counting

Following cultivation, the number of revertant colonies will be automatically counted by a colony counter (b) (4) (b) (4) or by visual counting. If automatic counting is considered to be inaccurate, the number of revertant colonies will be counted by visual counting.

2.9.6 Observation of background lawn

To confirm the absence or presence of growth inhibition by the test substance, the background lawn will be observed using a stereoscopic microscope (45-fold magnification, (b) (4)). Growth inhibition will be detected by reduction in the number of revertant colonies, or by diminution or clearing of background lawn compared to the negative control group.

(b) (4) *Study No.: B20743*
Protocol

2.10 Acceptance Criteria (SOP/GNT/012)

Evaluation of the validity of the study results will be conducted based on the following criteria:

- The results of gene mutagenic potential in the main studies are reproducible.
- There are more than 4 dose levels at which growth inhibition is not observed.
- The mean number of revertant colonies for the negative and positive control groups is within the range of the historical control data or the mean number of revertant colonies in the positive control group is increased at least twice as compared to the negative control group.
- No plate shows any evidence of contamination.

2.11 Evaluation Criteria (SOP/GNT/012)

The results of the study will be judged to be positive if the following conditions are met (others are considered as negative). A confirmatory study will be conducted if the result is not clearly positive.

- The number of revertant colonies in any strain at one or more doses is increased at least by two times when compared to the negative control group. There should be dose dependency or reproducibility as dose increases.

2.12 Statistical Analysis

Individual plates will be counted for revertant colonies. The average and standard deviation of the number of revertant colonies will be calculated. Statistical analysis will not be performed.

Appendix II. Protocol Amendments

Procedure	Protocol Amendments
No amendments were noted on the study.	

Appendix III. Protocol Deviations

Procedure	Protocol Deviations
No deviations were noted on the study.	

Appendix IV. Certificate of Analysis of Test Substance

CJ Research Institute of Biotechnology

55, Gwanggyo-ro 42beon-gil, Yeongtong-gu, Suwon-si, Gyeonggi-do, 16495, Korea

www.cj.co.kr

TEL : 031) 8099-2450 FAX : 031) 8099-2918

Certificate of analysis

Certificate No.	2019-PR-205	Receipt No.	2019-AN-130
Client	-	Date of Receipt	2019.11.25
Client Name	-	Date of Test	2019.11.26
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine Feed Grade
Manuf. Date	2019.11.21.
Lot. No	GVAL191121
Quantity (kg)	

Test Item(s)	Test Result	Test method used
Valine	(b) (4)	HPLC
Loss on drying		AOAC 934.01

* Information

* Temperature : (22~28) °C, Relative Humidity : (30~60) %

* N.D : not detected (not quantifiable)

* The results shown in this test report refer only to the sample tested unless otherwise stated.

The Test Report cannot be reproduced, except in full.

Tested by (b) (4) (b) (4)

Approved by Technical Manager (b) (4) (b) (4)

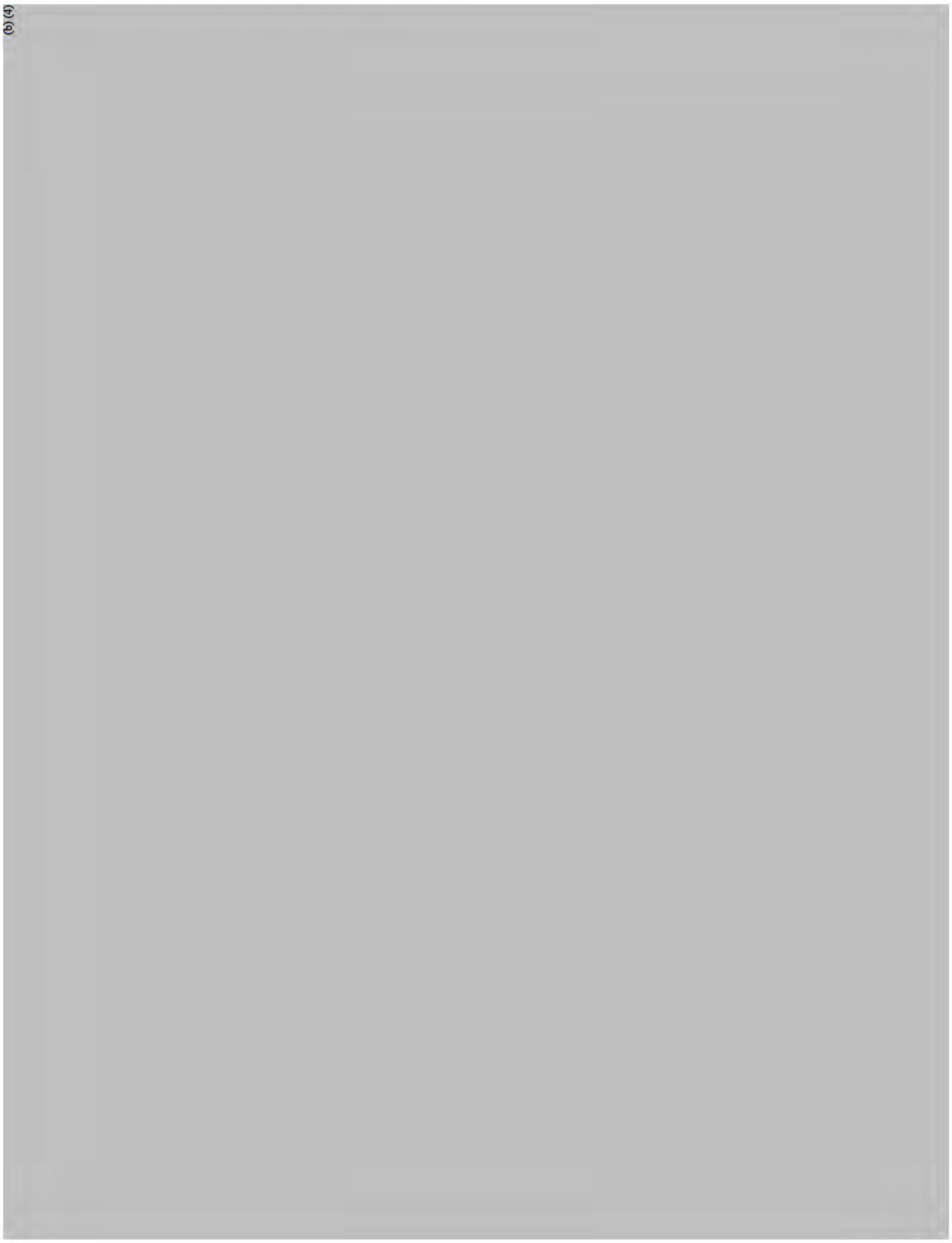
Nov. 29. 2019

CJ Research Institute of Biotechnology

CJ BIO AD form 100-01 REV.01

Appendix V. Certificate of Analysis of S9 mix

(b) (4)



Test Report

No: [redacted]

1. Client

① Name	[redacted]	② Company	CJ CheilJedang
③ Address	55, 42 beongil, gyanggyo ro, Yeongtong gu, Suwon si, Gyeonggi do Republic of Korea	④ Telephone	+82-31-8099-1539

2. Sample description

① Receipt No.	[redacted]	② Receipt Date	December 30, 2020
③ Sample Type	Powder	④ Quantity	1
⑤ Sample name	GVAL200910	⑥ Analysis Date	December 30, 2020

Test Results

Compound	Method	Unit	Test Result	LOD	LOQ
L-α-Aminobutyric acid	LC-MS/MS	mg/kg	[redacted]	0.025	0.080
α-Thiazolealanine	LC-MS/MS	mg/kg		0.011	0.034
α-Hydroxyvaline	LC-MS/MS	mg/kg		0.004	0.011
L-Norvaline	LC-MS/MS	mg/kg		0.096	0.307

This test report shall be used within the purpose of its defined usage.
The results have been made for the sample supplied by the client,
and it is the decision of the client naming the presented sample.

December 31, 2020

This is to inform the results of laboratory analysis according to the regulations for the testing and analysis at [redacted]

[redacted]

[redacted]

Test Report

No: [redacted]

1. Client

① Name	[redacted]	② Company	CJ CheilJedang
③ Address	55, 42 beongil, gyanggyo ro, Yeongtong gu, Suwon si, Gyeonggi do Republic of Korea	④ Telephone	+82-31-8099-1539

2. Sample description

① Receipt No.	[redacted]	② Receipt Date	December 30, 2020
③ Sample Type	Powder	④ Quantity	1
⑤ Sample name	GVAL200911	⑥ Analysis Date	December 30, 2020

Test Results

Compound	Method	Unit	Test Result	LOD	LOQ
L-α-Aminobutyric acid	LC-MS/MS	mg/kg	[redacted]	0.025	0.080
α-Thiazolealanine	LC-MS/MS	mg/kg		0.011	0.034
α-Hydroxyvaline	LC-MS/MS	mg/kg		0.004	0.011
L-Norvaline	LC-MS/MS	mg/kg		0.096	0.307

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and it is the decision of the client naming the presented sample.

December 31, 2020

This is to inform the results of laboratory analysis according to the regulations for the testing and analysis at [redacted]

[redacted]

[redacted]

Test Report

No: [redacted]

1. Client

① Name	[redacted]	② Company	CJ CheilJedang
③ Address	55, 42 beongil, gyanggyo ro, Yeongtong gu, Suwon si, Gyeonggi do Republic of Korea	④ Telephone	+82-31-8099-1539

2. Sample description

① Receipt No.	[redacted]	② Receipt Date	December 30, 2020
③ Sample Type	Powder	④ Quantity	1
⑤ Sample name	GVAL200912	⑥ Analysis Date	December 30, 2020

Test Results

Compound	Method	Unit	Test Result	LOD	LOQ
L-α-Aminobutyric acid	LC-MS/MS	mg/kg	[redacted]	0.025	0.080
α-Thiazolealanine	LC-MS/MS	mg/kg		0.011	0.034
α-Hydroxyvaline	LC-MS/MS	mg/kg		0.004	0.011
L-Norvaline	LC-MS/MS	mg/kg		0.096	0.307

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The results have been made for the sample supplied by the client,
and it is the decision of the client naming the presented sample.

December 31, 2020

This is to inform the results of laboratory analysis according to the regulations for the testing and analysis at [redacted]

[redacted]

[redacted]

Test Report

No: [redacted]

1. Client

① Name	[redacted]	② Company	CJ CheilJedang
③ Address	55, 42 beongil, gyanggyo ro, Yeongtong gu, Suwon si, Gyeonggi do Republic of Korea	④ Telephone	+82-31-8099-1539

2. Sample description

① Receipt No.	[redacted]	② Receipt Date	December 30, 2020
③ Sample Type	Powder	④ Quantity	1
⑤ Sample name	GVAL200910 + 5ppm standard	⑥ Analysis Date	December 30, 2020

Test Results

Compound	Method	Unit	Test Result	LOD	LOQ
L-α-Aminobutyric acid	LC-MS/MS	mg/kg	[redacted]	0.025	0.080
α-Thiazolealanine	LC-MS/MS	mg/kg		0.011	0.034
α-Hydroxyvaline	LC-MS/MS	mg/kg		0.004	0.011
L-Norvaline	LC-MS/MS	mg/kg		0.096	0.307

This test report shall be used within the purpose of its defined usage.
The results have been made for the sample supplied by the client,
and it is the decision of the client naming the presented sample.

December 31, 2020

This is to inform the results of laboratory analysis according to the regulations for the testing and analysis at [redacted]

[redacted]

[redacted]

Test Report

No: [redacted]

1. Client

① Name	[redacted]	② Company	CJ CheilJedang
③ Address	55, 42 beongil, gyanggyo ro, Yeongtong gu, Suwon si, Gyeonggi do Republic of Korea	④ Telephone	+82-31-8099-1539

2. Sample description

① Receipt No.	[redacted]	② Receipt Date	December 30, 2020
③ Sample Type	Powder	④ Quantity	1
⑤ Sample name	GVAL200911 + 5ppm standard	⑥ Analysis Date	December 30, 2020

Test Results

Compound	Method	Unit	Test Result	LOD	LOQ
L-α-Aminobutyric acid	LC-MS/MS	mg/kg	[redacted]	0.025	0.080
α-Thiazolealanine	LC-MS/MS	mg/kg		0.011	0.034
α-Hydroxyvaline	LC-MS/MS	mg/kg		0.004	0.011
L-Norvaline	LC-MS/MS	mg/kg		0.096	0.307

This test report shall be used within the purpose of its defined usage.
The results have been made for the sample supplied by the client,
and it is the decision of the client naming the presented sample.

December 31, 2020

This is to inform the results of laboratory analysis according to the regulations for the testing and analysis at [redacted]

[redacted]

[redacted]

Test Report

No: [redacted]

1. Client

① Name	[redacted]	② Company	CJ CheilJedang
③ Address	55, 42 beongil, gyanggyo ro, Yeongtong gu, Suwon si, Gyeonggi do Republic of Korea	④ Telephone	+82-31-8099-1539

2. Sample description

① Receipt No.	[redacted]	② Receipt Date	December 30, 2020
③ Sample Type	Powder	④ Quantity	1
⑤ Sample name	GVAL200912 + 5ppm standard	⑥ Analysis Date	December 30, 2020

Test Results

Compound	Method	Unit	Test Result	LOD	LOQ
L-α-Aminobutyric acid	LC-MS/MS	mg/kg	[redacted]	0.025	0.080
α-Thiazolealanine	LC-MS/MS	mg/kg		0.011	0.034
α-Hydroxyvaline	LC-MS/MS	mg/kg		0.004	0.011
L-Norvaline	LC-MS/MS	mg/kg		0.096	0.307

This test report shall be used within the purpose of its defined usage.
The results have been made for the sample supplied by the client,
and it is the decision of the client naming the presented sample.

December 31, 2020

This is to inform the results of laboratory analysis according to the regulations for the testing and analysis at [redacted]

[redacted]

[redacted]

Biogenic amine assessment

CJ CheilJedang analyzed the biogenic amines in test samples to 3rd party laboratory, [redacted] The type of samples are liquid and solid (powder) collected from the fermentation broth and final product, respectively (Table 1). In order to observe whether the matrix effect occurred during the analysis, additional analysis of each sample spiked with biogenic amine standard were conducted. Taking the analyzed concentration of biogenic amines in each sample into account, 100 ppb and 5 ppm biogenic amine standard were added into the liquid and solid (powder) sample, respectively. As shown in the Table 2 and 3, we concluded that no interference was observed by the fermentation broth components and biomass. Analytical method and test reports of each sample are attached to this document.

Table 1. Sample information

Sample name	Type	Description	Reference
ATCC14067_200907	Liquid	Fermentation broth of ATCC 14067 (wild-type strain)	Appendix 2. (C. Spill-over analysis, Table C.2.6)
ATCC14067_200908	Liquid	Fermentation broth of ATCC 14067 (wild-type strain)	
ATCC14067_200909	Liquid	Fermentation broth of ATCC 14067 (wild-type strain)	
CA08_0012_200907	Liquid	Fermentation broth of CA08_0012 (parent strain)	
CA08_0012_200908	Liquid	Fermentation broth of CA08_0012 (parent strain)	
CA08_0012_200909	Liquid	Fermentation broth of CA08_0012 (parent strain)	
KCCM80240_200907	Liquid	Fermentation broth of KCCM 80240 (production strain)	
KCCM80240_200908	Liquid	Fermentation broth of KCCM 80240 (production strain)	
KCCM80240_200909	Liquid	Fermentation broth of KCCM 80240 (production strain)	
GVAL200910	Solid (powder)	Dried L-Valine Fermentation Product	GRAS Notice (6.6 Safety Assessment for Human Consumption, Table 6.3)
GVAL200911	Solid (powder)	Dried L-Valine Fermentation Product	
GVAL200912	Solid (powder)	Dried L-Valine Fermentation Product	
ATCC14067_200907 +100ppb standard	Liquid	Fermentation broth of ATCC 14067 (wild-type strain) with 100 ppb biogenic amine standard (spike test)	Appendix 10 (Biogenic Amine Assessment, Table 2)
ATCC14067_200908+100ppb standard	Liquid	Fermentation broth of ATCC 14067 (wild-type strain) with 100 ppb biogenic amine standard (spike test)	
ATCC14067_200909+1	Liquid	Fermentation broth of ATCC 14067 (wild-	

00ppb standard		type strain) with 100 ppb biogenic amine standard (spike test)	
CA08_0012_200907+100ppb standard	Liquid	Fermentation broth of CA08_0012 (parent strain) with 100 ppb biogenic amine standard (spike test)	
CA08_0012_200908+100ppb standard	Liquid	Fermentation broth of CA08_0012 (parent strain) with 100 ppb biogenic amine standard (spike test)	
CA08_0012_200909+100ppb standard	Liquid	Fermentation broth of CA08_0012 (parent strain) with 100 ppb biogenic amine standard (spike test)	
KCCM80240_200907+100ppb standard	Liquid	Fermentation broth of KCCM 80240 (production strain) with 100 ppb biogenic amine standard (spike test)	
KCCM80240_200908+100ppb standard	Liquid	Fermentation broth of KCCM 80240 (production strain) with 100 ppb biogenic amine standard (spike test)	
KCCM80240_200909+100ppb standard	Liquid	Fermentation broth of KCCM 80240 (production strain) with 100 ppb biogenic amine standard (spike test)	
GVAL200910+5ppm standard	Solid (powder)	Dried L-Valine Fermentation Product with 5 ppm biogenic amine standard (spike test)	Appendix 10 (Biogenic Amine Assessment, Table 3)
GVAL200911+5ppm standard	Solid (powder)	Dried L-Valine Fermentation Product with 5 ppm biogenic amine standard (spike test)	
GVAL200912+5ppm standard	Solid (powder)	Dried L-Valine Fermentation Product with 5 ppm biogenic amine standard (spike test)	

Table 2. Spike test (Liquid)

Sample name	Cadaverine (μg/kg)	Histamine (μg/kg)	Phenylethyl-amine (μg/kg)	Putrescine (μg/kg)	Tyrptamine (μg/kg)	Tyramine (μg/kg)
ATCC14067_200907						(b) (4)
ATCC14067_200907 +100ppb standard						
Recovery (%)						
ATCC14067_200908						
ATCC14067_200908+100 ppb standard						
Recovery (%)						
ATCC14067_200909						
ATCC14067_200909+100 ppb standard						
Recovery (%)						
CA08_0012_200907						
CA08_0012_200907+100p pb standard						
Recovery (%)						
CA08_0012_200908						
CA08_0012_200908+100p pb standard						
Recovery (%)						
CA08_0012_200909						
CA08_0012_200909+100p pb standard						
Recovery (%)						
KCCM80240_200907						
KCCM80240_200907+100 ppb standard						
Recovery (%)						
KCCM80240_200908						
KCCM80240_200908+100 ppb standard						
Recovery (%)						
KCCM80240_200909						
KCCM80240_200909+100 ppb standard						
Recovery (%)						(b) (4)
Recovery (%) in average	**91.67±7.06**	**109.25±7.30**	**94.19±7.54**	**101.13±10.26**	**101.61±3.75**	**107.54±7.53**

Table 3. Spike test (Powder)

Sample name	Cadaverine (mg/kg)	Histamine (mg/kg)	Phenylethyl-amine (mg/kg)	Putrescine (mg/kg)	Tyrptamine (mg/kg)	Tyramine (mg/kg)
GVAL200910						(b) (4)
GVAL200910+5ppm standard						
Recovery (%)						
GVAL200911						
GVAL200911+5ppm standard						
Recovery (%)						
GVAL200912						
GVAL200912+5ppm standard						
Recovery (%)						
Recovery (%) in average	**107.53±7.91**	**109.87±10.32**	**99.60±9.18**	**104.07±10.10**	**104.13±0.58**	**107.07±3.97**

Summary of the analytical method

A. Sample preparation (Powder)

(b) (4)

B. Sample preparation (Liquid)

(b) (4)

C. Analytical condition

(a) LC condition

System	HPLC
Column	(b) (4), 2.1x150, 1.6um, particle size 1.6 µm
Mobile phase	A: 0.1% Formic acid in water, B: 0.1% Formic acid in acetonitrile
Gradient mode	

Time	Flow rate (ml/min)	%A	%B
0	0.3	(b) (4)	
1	0.3		
8	0.3		
10	0.3		
11	0.3		
15	0.3		

Column temperature	45°C
Sample temperature	10°C
Injection volume	3 µl

(b) MS/MS condition

System	Triple quadruple
Ion Source Type	H-ESI
Positive Ion (V)	3500
Ion Transfer Tube Temp	325
Vaporizer Temp (°C)	350
Polarity	Positive

MRM condition

Compound	Retention Time (min)	Precursor (m/z)	Product (m/z)	Collision Energy (V)
Tryptamine				
Tryptamine				
Tryptamine				
Phenylethylamine				
Phenylethylamine				
Phenylethylamine				
Putrescine				
Putrescine				
Putrescine				
Cadaverine				
Cadaverine				
Cadaverine				
Histamine				
Histamine				
Histamine				
Tyramine				
Tyramine				
Tyramine				

(b) (4)

Test Report

No: [redacted]

1. Client

① Name	[redacted]	② Company	CJ CheilJedang
③ Address	55, 42beon-gil, Gwanggyo-ro, Yeongtong-gu, Suwon-si, Gyeonggi-do, Republic of Korea	④ Telephone	+82-31-8099-1539

2. Sample description

① Receipt No.	[redacted]	② Receipt Date	January 26, 2021
③ Sample Type	Liquid	④ Quantity	1
⑤ Sample name	ATCC14067_200907	⑥ Analysis Date	January 26, 2021

Test Results

Compound	Method	Unit	Test Result	LOD	LOQ
Cadaverine	LC-MS/MS	ng/mL	[redacted]	0.0415	0.1320
Histamine	LC-MS/MS	ng/mL		0.0497	0.1584
Phenylethylamine	LC-MS/MS	ng/mL		0.0199	0.0634
Putrescine	LC-MS/MS	ng/mL		0.0391	0.1244
Tryptamine	LC-MS/MS	ng/mL		0.0177	0.0565
Tyramine	LC-MS/MS	ng/mL		0.0413	0.1315

This test report shall be used within the purpose of its defined usage.
The results have been made for the sample supplied by the client,
and it is the decision of the client naming the presented sample.

January 28, 2021

This is to inform the results of laboratory analysis according to the regulations for the testing and analysis at [redacted]

[redacted]

[redacted]

Test Report

No: [redacted]

1. Client

① Name	[redacted]	② Company	CJ CheilJedang
③ Address	55, 42beon-gil, Gwanggyo-ro, Yeongtong-gu, Suwon-si, Gyeonggi-do, Republic of Korea	④ Telephone	+82-31-8099-1539

2. Sample description

① Receipt No.	[redacted]	② Receipt Date	January 26, 2021
③ Sample Type	Liquid	④ Quantity	1
⑤ Sample name	ATCC14067_200908	⑥ Analysis Date	January 26, 2021

Test Results

Compound	Method	Unit	Test Result	LOD	LOQ
Cadaverine	LC-MS/MS	ng/mL	[redacted]	0.0415	0.1320
Histamine	LC-MS/MS	ng/mL		0.0497	0.1584
Phenylethylamine	LC-MS/MS	ng/mL		0.0199	0.0634
Putrescine	LC-MS/MS	ng/mL		0.0391	0.1244
Tryptamine	LC-MS/MS	ng/mL		0.0177	0.0565
Tyramine	LC-MS/MS	ng/mL		0.0413	0.1315

This test report shall be used within the purpose of its defined usage.
The results have been made for the sample supplied by the client,
and it is the decision of the client naming the presented sample.

January 28, 2021

This is to inform the results of laboratory analysis according to the regulations for the testing and analysis at [redacted]

[redacted]

[redacted]

Test Report

No: [redacted] (b) (4)

1. Client

① Name	[redacted]	② Company	CJ CheilJedang
③ Address	55, 42beon-gil, Gwanggyo-ro, Yeongtong-gu, Suwon-si, Gyeonggi-do, Republic of Korea	④ Telephone	+82-31-8099-1539

2. Sample description

① Receipt No.	[redacted] (b) (4)	② Receipt Date	January 26, 2021
③ Sample Type	Liquid	④ Quantity	1
⑤ Sample name	ATCC14067_200909	⑥ Analysis Date	January 26, 2021

Test Results

Compound	Method	Unit	Test Result	LOD	LOQ
Cadaverine	LC-MS/MS	ng/mL	[redacted] (b) (4)	0.0415	0.1320
Histamine	LC-MS/MS	ng/mL		0.0497	0.1584
Phenylethylamine	LC-MS/MS	ng/mL		0.0199	0.0634
Putrescine	LC-MS/MS	ng/mL		0.0391	0.1244
Tryptamine	LC-MS/MS	ng/mL		0.0177	0.0565
Tyramine	LC-MS/MS	ng/mL		0.0413	0.1315

This test report shall be used within the purpose of its defined usage.
The results have been made for the sample supplied by the client,
and it is the decision of the client naming the presented sample.

January 28, 2021

This is to inform the results of laboratory analysis according to the regulations for the testing and analysis at [redacted] (b) (4)

[redacted] (b) (4)

[redacted] (b) (4) [redacted]

Test Report

No: [redacted]

1. Client

① Name	[redacted]	② Company	CJ CheilJedang
③ Address	55, 42beon-gil, Gwanggyo-ro, Yeongtong-gu, Suwon-si, Gyeonggi-do, Republic of Korea	④ Telephone	+82-31-8099-1539

2. Sample description

① Receipt No.	[redacted]	② Receipt Date	January 26, 2021
③ Sample Type	Liquid	④ Quantity	1
⑤ Sample name	CA08_0012_200907	⑥ Analysis Date	January 26, 2021

Test Results

Compound	Method	Unit	Test Result	LOD	LOQ
Cadaverine	LC-MS/MS	ng/mL	[redacted]	0.0415	0.1320
Histamine	LC-MS/MS	ng/mL		0.0497	0.1584
Phenylethylamine	LC-MS/MS	ng/mL		0.0199	0.0634
Putrescine	LC-MS/MS	ng/mL		0.0391	0.1244
Tryptamine	LC-MS/MS	ng/mL		0.0177	0.0565
Tyramine	LC-MS/MS	ng/mL		0.0413	0.1315

This test report shall be used within the purpose of its defined usage.
The results have been made for the sample supplied by the client,
and it is the decision of the client naming the presented sample.

January 28, 2021

This is to inform the results of laboratory analysis according to the regulations for the testing and analysis at [redacted]

[redacted]

[redacted]

Test Report

No: [redacted] (b) (4)

1. Client

① Name	[redacted]	② Company	CJ CheilJedang
③ Address	55, 42beon-gil, Gwanggyo-ro, Yeongtong-gu, Suwon-si, Gyeonggi-do, Republic of Korea	④ Telephone	+82-31-8099-1539

2. Sample description

① Receipt No.	[redacted] (b) (4)	② Receipt Date	January 26, 2021
③ Sample Type	Liquid	④ Quantity	1
⑤ Sample name	CA08_0012_200908	⑥ Analysis Date	January 26, 2021

Test Results

Compound	Method	Unit	Test Result	LOD	LOQ
Cadaverine	LC-MS/MS	ng/mL	[redacted] (b) (4)	0.0415	0.1320
Histamine	LC-MS/MS	ng/mL		0.0497	0.1584
Phenylethylamine	LC-MS/MS	ng/mL		0.0199	0.0634
Putrescine	LC-MS/MS	ng/mL		0.0391	0.1244
Tryptamine	LC-MS/MS	ng/mL		0.0177	0.0565
Tyramine	LC-MS/MS	ng/mL		0.0413	0.1315

This test report shall be used within the purpose of its defined usage.
The results have been made for the sample supplied by the client,
and it is the decision of the client naming the presented sample.

January 28, 2021

This is to inform the results of laboratory analysis according to the regulations for the testing and analysis at [redacted] (b) (4)

[redacted] (b) (4)

[redacted] (b) (4)

Test Report

No: [redacted]

1. Client

① Name	[redacted]	② Company	CJ CheilJedang
③ Address	55, 42beon-gil, Gwanggyo-ro, Yeongtong-gu, Suwon-si, Gyeonggi-do, Republic of Korea	④ Telephone	+82-31-8099-1539

2. Sample description

① Receipt No.	[redacted]	② Receipt Date	January 26, 2021
③ Sample Type	Liquid	④ Quantity	1
⑤ Sample name	CA08_0012_200909	⑥ Analysis Date	January 26, 2021

Test Results

Compound	Method	Unit	Test Result	LOD	LOQ
Cadaverine	LC-MS/MS	ng/mL	[redacted]	0.0415	0.1320
Histamine	LC-MS/MS	ng/mL		0.0497	0.1584
Phenylethylamine	LC-MS/MS	ng/mL		0.0199	0.0634
Putrescine	LC-MS/MS	ng/mL		0.0391	0.1244
Tryptamine	LC-MS/MS	ng/mL		0.0177	0.0565
Tyramine	LC-MS/MS	ng/mL		0.0413	0.1315

This test report shall be used within the purpose of its defined usage.
The results have been made for the sample supplied by the client,
and it is the decision of the client naming the presented sample.

January 28, 2021

This is to inform the results of laboratory analysis according to the regulations for the testing and analysis at [redacted]

[redacted]

[redacted]

Test Report

No: [redacted]

1. Client

① Name	[redacted]	② Company	CJ CheilJedang
③ Address	55, 42beon-gil, Gwanggyo-ro, Yeongtong-gu, Suwon-si, Gyeonggi-do, Republic of Korea	④ Telephone	+82-31-8099-1539

2. Sample description

① Receipt No.	[redacted]	② Receipt Date	January 26, 2021
③ Sample Type	Liquid	④ Quantity	1
⑤ Sample name	KCCM80240_200907	⑥ Analysis Date	January 26, 2021

Test Results

Compound	Method	Unit	Test Result	LOD	LOQ
Cadaverine	LC-MS/MS	ng/mL	[redacted]	0.0415	0.1320
Histamine	LC-MS/MS	ng/mL		0.0497	0.1584
Phenylethylamine	LC-MS/MS	ng/mL		0.0199	0.0634
Putrescine	LC-MS/MS	ng/mL		0.0391	0.1244
Tryptamine	LC-MS/MS	ng/mL		0.0177	0.0565
Tyramine	LC-MS/MS	ng/mL		0.0413	0.1315

This test report shall be used within the purpose of its defined usage.
The results have been made for the sample supplied by the client,
and it is the decision of the client naming the presented sample.

January 28, 2021

This is to inform the results of laboratory analysis according to the regulations for the testing and analysis at [redacted]

[redacted]

[redacted]

Test Report

No: [redacted]

1. Client

① Name	[redacted]	② Company	CJ CheilJedang
③ Address	55, 42beon-gil, Gwanggyo-ro, Yeongtong-gu, Suwon-si, Gyeonggi-do, Republic of Korea	④ Telephone	+82-31-8099-1539

2. Sample description

① Receipt No.	[redacted]	② Receipt Date	January 26, 2021
③ Sample Type	Liquid	④ Quantity	1
⑤ Sample name	KCCM80240_200908	⑥ Analysis Date	January 26, 2021

Test Results

Compound	Method	Unit	Test Result	LOD	LOQ
Cadaverine	LC-MS/MS	ng/mL	[redacted]	0.0415	0.1320
Histamine	LC-MS/MS	ng/mL		0.0497	0.1584
Phenylethylamine	LC-MS/MS	ng/mL		0.0199	0.0634
Putrescine	LC-MS/MS	ng/mL		0.0391	0.1244
Tryptamine	LC-MS/MS	ng/mL		0.0177	0.0565
Tyramine	LC-MS/MS	ng/mL		0.0413	0.1315

This test report shall be used within the purpose of its defined usage.
The results have been made for the sample supplied by the client,
and it is the decision of the client naming the presented sample.

January 28, 2021

This is to inform the results of laboratory analysis according to the regulations for the testing and analysis at [redacted]

[redacted]

[redacted]

Test Report

No: [redacted]

1. Client

① Name	[redacted]	② Company	CJ CheilJedang
③ Address	55, 42beon-gil, Gwanggyo-ro, Yeongtong-gu, Suwon-si, Gyeonggi-do, Republic of Korea	④ Telephone	+82-31-8099-1539

2. Sample description

① Receipt No.	[redacted]	② Receipt Date	January 26, 2021
③ Sample Type	Liquid	④ Quantity	1
⑤ Sample name	KCCM80240_200909	⑥ Analysis Date	January 26, 2021

Test Results

Compound	Method	Unit	Test Result	LOD	LOQ
Cadaverine	LC-MS/MS	ng/mL	[redacted]	0.0415	0.1320
Histamine	LC-MS/MS	ng/mL		0.0497	0.1584
Phenylethylamine	LC-MS/MS	ng/mL		0.0199	0.0634
Putrescine	LC-MS/MS	ng/mL		0.0391	0.1244
Tryptamine	LC-MS/MS	ng/mL		0.0177	0.0565
Tyramine	LC-MS/MS	ng/mL		0.0413	0.1315

This test report shall be used within the purpose of its defined usage.
The results have been made for the sample supplied by the client,
and it is the decision of the client naming the presented sample.

January 28, 2021

This is to inform the results of laboratory analysis according to the regulations for the testing and analysis at [redacted]

[redacted]

[redacted]

Test Report

No: [redacted]

1. Client

① Name	[redacted]	② Company	CJ CheilJedang
③ Address	55, 42beon-gil, Gwanggyo-ro, Yeongtong-gu, Suwon-si, Gyeonggi-do, Republic of Korea	④ Telephone	+82-31-8099-1539

2. Sample description

① Receipt No.	[redacted]	② Receipt Date	January 26, 2021
③ Sample Type	Powder	④ Quantity	1
⑤ Sample name	GVAL200910	⑥ Analysis Date	January 26, 2021

Test Results

Compound	Method	Unit	Test Result	LOD	LOQ
Cadaverine	LC-MS/MS	mg/kg	[redacted]	0.00207	0.00660
Histamine	LC-MS/MS	mg/kg		0.00249	0.00792
Phenylethylamine	LC-MS/MS	mg/kg		0.00099	0.00317
Putrescine	LC-MS/MS	mg/kg		0.00195	0.00622
Tryptamine	LC-MS/MS	mg/kg		0.00089	0.00282
Tyramine	LC-MS/MS	mg/kg		0.00206	0.00657

This test report shall be used within the purpose of its defined usage.
The results have been made for the sample supplied by the client,
and it is the decision of the client naming the presented sample.

January 28, 2021

This is to inform the results of laboratory analysis according to the regulations for the testing and analysis at [redacted]

[redacted]

[redacted]

Test Report

No: [redacted]

1. Client

① Name	[redacted]	② Company	CJ CheilJedang
③ Address	55, 42beon-gil, Gwanggyo-ro, Yeongtong-gu, Suwon-si, Gyeonggi-do, Republic of Korea	④ Telephone	+82-31-8099-1539

2. Sample description

① Receipt No.	[redacted]	② Receipt Date	January 26, 2021
③ Sample Type	Powder	④ Quantity	1
⑤ Sample name	GVAL200911	⑥ Analysis Date	January 26, 2021

Test Results

Compound	Method	Unit	Test Result	LOD	LOQ
Cadaverine	LC-MS/MS	mg/kg	[redacted]	0.00207	0.00660
Histamine	LC-MS/MS	mg/kg		0.00249	0.00792
Phenylethylamine	LC-MS/MS	mg/kg		0.00099	0.00317
Putrescine	LC-MS/MS	mg/kg		0.00195	0.00622
Tryptamine	LC-MS/MS	mg/kg		0.00089	0.00282
Tyramine	LC-MS/MS	mg/kg		0.00206	0.00657

This test report shall be used within the purpose of its defined usage.
The results have been made for the sample supplied by the client,
and it is the decision of the client naming the presented sample.

January 28, 2021

This is to inform the results of laboratory analysis according to the regulations for the testing and analysis at [redacted]

[redacted]

[redacted]

Test Report

No: [redacted]

1. Client

① Name	[redacted]	② Company	CJ CheilJedang
③ Address	55, 42beon-gil, Gwanggyo-ro, Yeongtong-gu, Suwon-si, Gyeonggi-do, Republic of Korea	④ Telephone	+82-31-8099-1539

2. Sample description

① Receipt No.	[redacted]	② Receipt Date	January 26, 2021
③ Sample Type	Powder	④ Quantity	1
⑤ Sample name	GVAL200912	⑥ Analysis Date	January 26, 2021

Test Results

Compound	Method	Unit	Test Result	LOD	LOQ
Cadaverine	LC-MS/MS	mg/kg	[redacted]	0.00207	0.00660
Histamine	LC-MS/MS	mg/kg		0.00249	0.00792
Phenylethylamine	LC-MS/MS	mg/kg		0.00099	0.00317
Putrescine	LC-MS/MS	mg/kg		0.00195	0.00622
Tryptamine	LC-MS/MS	mg/kg		0.00089	0.00282
Tyramine	LC-MS/MS	mg/kg		0.00206	0.00657

This test report shall be used within the purpose of its defined usage.
The results have been made for the sample supplied by the client,
and it is the decision of the client naming the presented sample.

January 28, 2021

This is to inform the results of laboratory analysis according to the regulations for the testing and analysis at [redacted]

[redacted]

[redacted]

Test Report

No: [redacted]

1. Client

① Name	[redacted]	② Company	CJ CheilJedang
③ Address	55, 42beon-gil, Gwanggyo-ro, Yeongtong-gu, Suwon-si, Gyeonggi-do, Republic of Korea	④ Telephone	+82-31-8099-1539

2. Sample description

① Receipt No.	[redacted]	② Receipt Date	January 26, 2021
③ Sample Type	Liquid	④ Quantity	1
⑤ Sample name	ATCC14067_200907 + 100ppb standard	⑥ Analysis Date	January 26, 2021

Test Results

Compound	Method	Unit	Test Result	LOD	LOQ
Cadaverine	LC-MS/MS	ng/mL	[redacted]	0.0415	0.1320
Histamine	LC-MS/MS	ng/mL		0.0497	0.1584
Phenylethylamine	LC-MS/MS	ng/mL		0.0199	0.0634
Putrescine	LC-MS/MS	ng/mL		0.0391	0.1244
Tryptamine	LC-MS/MS	ng/mL		0.0177	0.0565
Tyramine	LC-MS/MS	ng/mL		0.0413	0.1315

This test report shall be used within the purpose of its defined usage.
The results have been made for the sample supplied by the client,
and it is the decision of the client naming the presented sample.

January 28, 2021

This is to inform the results of laboratory analysis according to the regulations for the testing and analysis at [redacted]

[redacted]

[redacted]

Test Report

No: [redacted]

1. Client

① Name	[redacted]	② Company	CJ CheilJedang
③ Address	55, 42beon-gil, Gwanggyo-ro, Yeongtong-gu, Suwon-si, Gyeonggi-do, Republic of Korea	④ Telephone	+82-31-8099-1539

2. Sample description

① Receipt No.	[redacted]	② Receipt Date	January 26, 2021
③ Sample Type	Liquid	④ Quantity	1
⑤ Sample name	ATCC14067_200908 + 100ppb standard	⑥ Analysis Date	January 26, 2021

Test Results

Compound	Method	Unit	Test Result	LOD	LOQ
Cadaverine	LC-MS/MS	ng/mL	[redacted]	0.0415	0.1320
Histamine	LC-MS/MS	ng/mL		0.0497	0.1584
Phenylethylamine	LC-MS/MS	ng/mL		0.0199	0.0634
Putrescine	LC-MS/MS	ng/mL		0.0391	0.1244
Tryptamine	LC-MS/MS	ng/mL		0.0177	0.0565
Tyramine	LC-MS/MS	ng/mL		0.0413	0.1315

This test report shall be used within the purpose of its defined usage.
The results have been made for the sample supplied by the client,
and it is the decision of the client naming the presented sample.

January 28, 2021

This is to inform the results of laboratory analysis according to the regulations for the testing and analysis at [redacted]

[redacted]

[redacted]

Test Report

No: [redacted]

1. Client

① Name	[redacted]	② Company	CJ CheilJedang
③ Address	55, 42beon-gil, Gwanggyo-ro, Yeongtong-gu, Suwon-si, Gyeonggi-do, Republic of Korea	④ Telephone	+82-31-8099-1539

2. Sample description

① Receipt No.	[redacted]	② Receipt Date	January 26, 2021
③ Sample Type	Liquid	④ Quantity	1
⑤ Sample name	ATCC14067_200909 + 100ppb standard	⑥ Analysis Date	January 26, 2021

Test Results

Compound	Method	Unit	Test Result	LOD	LOQ
Cadaverine	LC-MS/MS	ng/mL	[redacted]	0.0415	0.1320
Histamine	LC-MS/MS	ng/mL		0.0497	0.1584
Phenylethylamine	LC-MS/MS	ng/mL		0.0199	0.0634
Putrescine	LC-MS/MS	ng/mL		0.0391	0.1244
Tryptamine	LC-MS/MS	ng/mL		0.0177	0.0565
Tyramine	LC-MS/MS	ng/mL		0.0413	0.1315

This test report shall be used within the purpose of its defined usage.
The results have been made for the sample supplied by the client,
and it is the decision of the client naming the presented sample.

January 28, 2021

This is to inform the results of laboratory analysis according to the regulations for the testing and analysis at [redacted]

[redacted]

[redacted]

Test Report

No: [redacted]

1. Client

① Name	[redacted]	② Company	CJ CheilJedang
③ Address	55, 42beon-gil, Gwanggyo-ro, Yeongtong-gu, Suwon-si, Gyeonggi-do, Republic of Korea	④ Telephone	+82-31-8099-1539

2. Sample description

① Receipt No.	[redacted]	② Receipt Date	January 26, 2021
③ Sample Type	Liquid	④ Quantity	1
⑤ Sample name	CA08_0012_200907 + 100ppb standard	⑥ Analysis Date	January 26, 2021

Test Results

Compound	Method	Unit	Test Result	LOD	LOQ
Cadaverine	LC-MS/MS	ng/mL	[redacted]	0.0415	0.1320
Histamine	LC-MS/MS	ng/mL		0.0497	0.1584
Phenylethylamine	LC-MS/MS	ng/mL		0.0199	0.0634
Putrescine	LC-MS/MS	ng/mL		0.0391	0.1244
Tryptamine	LC-MS/MS	ng/mL		0.0177	0.0565
Tyramine	LC-MS/MS	ng/mL		0.0413	0.1315

This test report shall be used within the purpose of its defined usage.
The results have been made for the sample supplied by the client,
and it is the decision of the client naming the presented sample.

January 28, 2021

This is to inform the results of laboratory analysis according to the regulations for the testing and analysis at [redacted]

[redacted]

[redacted]

Test Report

No: [redacted]

1. Client

① Name	[redacted]	② Company	CJ CheilJedang
③ Address	55, 42beon-gil, Gwanggyo-ro, Yeongtong-gu, Suwon-si, Gyeonggi-do, Republic of Korea	④ Telephone	+82-31-8099-1539

2. Sample description

① Receipt No.	[redacted]	② Receipt Date	January 26, 2021
③ Sample Type	Liquid	④ Quantity	1
⑤ Sample name	CA08_0012_200908 + 100ppb standard	⑥ Analysis Date	January 26, 2021

Test Results

Compound	Method	Unit	Test Result	LOD	LOQ
Cadaverine	LC-MS/MS	ng/mL	[redacted]	0.0415	0.1320
Histamine	LC-MS/MS	ng/mL		0.0497	0.1584
Phenylethylamine	LC-MS/MS	ng/mL		0.0199	0.0634
Putrescine	LC-MS/MS	ng/mL		0.0391	0.1244
Tryptamine	LC-MS/MS	ng/mL		0.0177	0.0565
Tyramine	LC-MS/MS	ng/mL		0.0413	0.1315

This test report shall be used within the purpose of its defined usage.
The results have been made for the sample supplied by the client,
and it is the decision of the client naming the presented sample.

January 28, 2021

This is to inform the results of laboratory analysis according to the regulations for the testing and analysis at [redacted]

[redacted]

[redacted]

Test Report

No: [redacted]

1. Client

① Name	[redacted]	② Company	CJ CheilJedang
③ Address	55, 42beon-gil, Gwanggyo-ro, Yeongtong-gu, Suwon-si, Gyeonggi-do, Republic of Korea	④ Telephone	+82-31-8099-1539

2. Sample description

① Receipt No.	[redacted]	② Receipt Date	January 26, 2021
③ Sample Type	Liquid	④ Quantity	1
⑤ Sample name	CA08_0012_200909 + 100ppb standard	⑥ Analysis Date	January 26, 2021

Test Results

Compound	Method	Unit	Test Result	LOD	LOQ
Cadaverine	LC-MS/MS	ng/mL	[redacted]	0.0415	0.1320
Histamine	LC-MS/MS	ng/mL		0.0497	0.1584
Phenylethylamine	LC-MS/MS	ng/mL		0.0199	0.0634
Putrescine	LC-MS/MS	ng/mL		0.0391	0.1244
Tryptamine	LC-MS/MS	ng/mL		0.0177	0.0565
Tyramine	LC-MS/MS	ng/mL		0.0413	0.1315

This test report shall be used within the purpose of its defined usage.
The results have been made for the sample supplied by the client,
and it is the decision of the client naming the presented sample.

January 28, 2021

This is to inform the results of laboratory analysis according to the regulations for the testing and analysis at [redacted]

[redacted]

[redacted]

Test Report

No: [redacted]

1. Client

① Name	[redacted]	② Company	CJ CheilJedang
③ Address	55, 42beon-gil, Gwanggyo-ro, Yeongtong-gu, Suwon-si, Gyeonggi-do, Republic of Korea	④ Telephone	+82-31-8099-1539

2. Sample description

① Receipt No.	[redacted]	② Receipt Date	January 26, 2021
③ Sample Type	Liquid	④ Quantity	1
⑤ Sample name	KCCM80240_200907 + 100ppb standard	⑥ Analysis Date	January 26, 2021

Test Results

Compound	Method	Unit	Test Result	LOD	LOQ
Cadaverine	LC-MS/MS	ng/mL	[redacted]	0.0415	0.1320
Histamine	LC-MS/MS	ng/mL		0.0497	0.1584
Phenylethylamine	LC-MS/MS	ng/mL		0.0199	0.0634
Putrescine	LC-MS/MS	ng/mL		0.0391	0.1244
Tryptamine	LC-MS/MS	ng/mL		0.0177	0.0565
Tyramine	LC-MS/MS	ng/mL		0.0413	0.1315

This test report shall be used within the purpose of its defined usage.
The results have been made for the sample supplied by the client,
and it is the decision of the client naming the presented sample.

January 28, 2021

This is to inform the results of laboratory analysis according to the regulations for the testing and analysis at [redacted]

[redacted]

[redacted]

Test Report

No: [redacted]

1. Client

① Name	[redacted]	② Company	CJ CheilJedang
③ Address	55, 42beon-gil, Gwanggyo-ro, Yeongtong-gu, Suwon-si, Gyeonggi-do, Republic of Korea	④ Telephone	+82-31-8099-1539

2. Sample description

① Receipt No.	[redacted]	② Receipt Date	January 26, 2021
③ Sample Type	Liquid	④ Quantity	1
⑤ Sample name	KCCM80240_200908 + 100ppb standard	⑥ Analysis Date	January 26, 2021

Test Results

Compound	Method	Unit	Test Result	LOD	LOQ
Cadaverine	LC-MS/MS	ng/mL	[redacted]	0.0415	0.1320
Histamine	LC-MS/MS	ng/mL		0.0497	0.1584
Phenylethylamine	LC-MS/MS	ng/mL		0.0199	0.0634
Putrescine	LC-MS/MS	ng/mL		0.0391	0.1244
Tryptamine	LC-MS/MS	ng/mL		0.0177	0.0565
Tyramine	LC-MS/MS	ng/mL		0.0413	0.1315

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and it is the decision of the client naming the presented sample.

January 28, 2021

This is to inform the results of laboratory analysis according to the regulations for the testing and analysis at [redacted]

[redacted]

[redacted]

Test Report

No: [redacted]

1. Client

① Name	[redacted]	② Company	CJ CheilJedang
③ Address	55, 42beon-gil, Gwanggyo-ro, Yeongtong-gu, Suwon-si, Gyeonggi-do, Republic of Korea	④ Telephone	+82-31-8099-1539

2. Sample description

① Receipt No.	[redacted]	② Receipt Date	January 26, 2021
③ Sample Type	Liquid	④ Quantity	1
⑤ Sample name	KCCM80240_200909 + 100ppb standard	⑥ Analysis Date	January 26, 2021

Test Results

Compound	Method	Unit	Test Result	LOD	LOQ
Cadaverine	LC-MS/MS	ng/mL	[redacted]	0.0415	0.1320
Histamine	LC-MS/MS	ng/mL		0.0497	0.1584
Phenylethylamine	LC-MS/MS	ng/mL		0.0199	0.0634
Putrescine	LC-MS/MS	ng/mL		0.0391	0.1244
Tryptamine	LC-MS/MS	ng/mL		0.0177	0.0565
Tyramine	LC-MS/MS	ng/mL		0.0413	0.1315

This test report shall be used within the purpose of its defined usage.
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and it is the decision of the client naming the presented sample.

January 28, 2021

This is to inform the results of laboratory analysis according to the regulations for the testing and analysis at [redacted]

[redacted]

[redacted]

Test Report

No: [redacted]

1. Client

① Name	[redacted]	② Company	CJ CheilJedang
③ Address	55, 42beon-gil, Gwanggyo-ro, Yeongtong-gu, Suwon-si, Gyeonggi-do, Republic of Korea	④ Telephone	+82-31-8099-1539

2. Sample description

① Receipt No.	[redacted]	② Receipt Date	January 26, 2021
③ Sample Type	Powder	④ Quantity	1
⑤ Sample name	GVAL200910 + 5ppm standard	⑥ Analysis Date	January 26, 2021

Test Results

Compound	Method	Unit	Test Result	LOD	LOQ
Cadaverine	LC-MS/MS	mg/kg	[redacted]	0.00207	0.00660
Histamine	LC-MS/MS	mg/kg		0.00249	0.00792
Phenylethylamine	LC-MS/MS	mg/kg		0.00099	0.00317
Putrescine	LC-MS/MS	mg/kg		0.00195	0.00622
Tryptamine	LC-MS/MS	mg/kg		0.00089	0.00282
Tyramine	LC-MS/MS	mg/kg		0.00206	0.00657

This test report shall be used within the purpose of its defined usage.
The results have been made for the sample supplied by the client,
and it is the decision of the client naming the presented sample.

January 28, 2021

This is to inform the results of laboratory analysis according to the regulations for the testing and analysis at [redacted]

[redacted]

[redacted]

Test Report

No: [redacted]

1. Client

① Name	[redacted]	② Company	CJ CheilJedang
③ Address	55, 42beon-gil, Gwanggyo-ro, Yeongtong-gu, Suwon-si, Gyeonggi-do, Republic of Korea	④ Telephone	+82-31-8099-1539

2. Sample description

① Receipt No.	[redacted]	② Receipt Date	January 26, 2021
③ Sample Type	Powder	④ Quantity	1
⑤ Sample name	GVAL200911 + 5ppm standard	⑥ Analysis Date	January 26, 2021

Test Results

Compound	Method	Unit	Test Result	LOD	LOQ
Cadaverine	LC-MS/MS	mg/kg	[redacted]	0.00207	0.00660
Histamine	LC-MS/MS	mg/kg		0.00249	0.00792
Phenylethylamine	LC-MS/MS	mg/kg		0.00099	0.00317
Putrescine	LC-MS/MS	mg/kg		0.00195	0.00622
Tryptamine	LC-MS/MS	mg/kg		0.00089	0.00282
Tyramine	LC-MS/MS	mg/kg		0.00206	0.00657

This test report shall be used within the purpose of its defined usage.
The results have been made for the sample supplied by the client,
and it is the decision of the client naming the presented sample.

January 28, 2021

This is to inform the results of laboratory analysis according to the regulations for the testing and analysis at [redacted]

[redacted]

[redacted]

Test Report

No: [redacted]

1. Client

① Name	[redacted]	② Company	CJ CheilJedang
③ Address	55, 42beon-gil, Gwanggyo-ro, Yeongtong-gu, Suwon-si, Gyeonggi-do, Republic of Korea	④ Telephone	+82-31-8099-1539

2. Sample description

① Receipt No.	[redacted]	② Receipt Date	January 26, 2021
③ Sample Type	Powder	④ Quantity	1
⑤ Sample name	GVAL200912 + 5ppm standard	⑥ Analysis Date	January 26, 2021

Test Results

Compound	Method	Unit	Test Result	LOD	LOQ
Cadaverine	LC-MS/MS	mg/kg	[redacted]	0.00207	0.00660
Histamine	LC-MS/MS	mg/kg		0.00249	0.00792
Phenylethylamine	LC-MS/MS	mg/kg		0.00099	0.00317
Putrescine	LC-MS/MS	mg/kg		0.00195	0.00622
Tryptamine	LC-MS/MS	mg/kg		0.00089	0.00282
Tyramine	LC-MS/MS	mg/kg		0.00206	0.00657

This test report shall be used within the purpose of its defined usage.
The results have been made for the sample supplied by the client,
and it is the decision of the client naming the presented sample.

January 28, 2021

This is to inform the results of laboratory analysis according to the regulations for the testing and analysis at [redacted]

[redacted]

[redacted]

Appendix 11. Literature Review *Corynebacterium glutamicum* – with references

Review of the safety of *Corynebacterium glutamicum*

TABLE OF CONTENTS

1. INTRODUCTION

This document addresses the safety of the microorganism *Corynebacterium glutamicum*. It presents scientific data and information gathered from in-depth literature reviews which demonstrate that *C. glutamicum* can be used as a microorganism for the industrial production of amino acids and other substances which in turn can be safely added to feed for food-producing animals and poses no risk or health hazards to humans consuming products from food-producing animals consuming the substance. This review, as prescribed by the Division of Animal Feed staff, is intended to refresh the detailed safety review assessment completed in 2003 by the Division with the addition of *Corynebacterium glutamicum* and *Corynebacterium glutamicum* derived ingredients as an authorized feed ingredient.

2. EVALUATION BY EFSA

2.1 Qualified presumption of safety (QPS)

A wide variety of microorganisms are intentionally added at different stages into the food chain, either directly or as a source of food and feed additives, enzymes or plant protection products. The qualified presumption of safety (QPS) approach was developed by the EFSA Scientific Committee to provide a generic concept to prioritize and to harmonize risk assessment within EFSA of microorganisms intentionally introduced into the food chain (EFSA, 2005, 2007).

The list of QPS microorganisms has been continuously revised and updated since it was established in 2007. The publication of the overall assessment of the taxonomic units (TU) previously recommended for the QPS list is carried out every three years (EFSA, 2007, 2012). The recommendations provided concerning that list of microorganisms are maintained and re-evaluated based on extensive literature reviews and expert knowledge. (EFSA, 2007, 2018).

2.2 Re-evaluation using literature review

The bi-annual re-evaluation of microorganisms begins with a literature review for each TU that is notified to EFSA. QPS recommended TU and those which represent new TU notifications are annually reviewed (EFSA, 2007). The literature review for a new TU is broader to cover the history of use, the potential safety concerns and the ecology. Relevant databases such as Web of Science Core Collection, CAB Abstracts, BIOSIS Citation Index, MEDLINE and Food Science Technology Abstracts are searched using the TU in combination with common keywords (e.g. toxin, disease, antibiotic/antimycotic resistance, safety, syndrome) and respective animal categories. The search terms are broad and cover synonyms or former names of taxonomic units (EFSA, 2012, 2013, 2017). Findings from the literature review are then evaluated, taking into consideration recommendations given in the previous QPS Opinion. A detailed description of the methodology used in carrying out the literature review can be found in EFSA (2013, 2017). A summary of the literature search strategy for the most recent QPS update for *C. glutamicum* is given in Table 1.

Table 1.	*Corynebacterium glutamicum*
String for species	
"*Corynebacterium glutamicum*" OR "*C*	

glutamicum" OR "*Brevibacterium lactofermentum*" OR "*B lactofermentum*"	
Outcome	String
1) Antimicrobial/Antibiotic/Antimycotic	"antimicrobial resistan*" OR "antibiotic resistan*" OR "antimicrobial susceptibil*"
2) Infection/Bacteremia/Fungemia/Sepsis	infection* OR abscess* OR sepsis* or septic* OR bacteremia OR bacteraemia OR toxin* OR "pathogen*"
3) Type of disease	Not applied
4) Mortality/Morbidity	clinical* OR death* OR morbidit* OR mortalit* OR disease* OR illness*
5) Disease Risk	opportunistic OR virulen*
Flow records by search strategy resulted in 78 papers being identified using title screening, of which 8 papers were identified using title/abstract screening, of which 1 was identified using article appraisal and was considered relevant for QPS. Following the review of that paper (Yang and Yang, 2017), it was concluded that there were no safety concerns identified in the only article considered relevant for QPS exercise (EFSA, 2019).	
A literature review did not reveal new information about adverse health effects or on safety concerns since the last update (EFSA, 2013). The QPS recommendation has been confirmed.	
Source: EFSA (2018).	

2.3 QPS Classification of *Corynebacterium glutamicum*

The QPS approach is currently used for microorganisms in the three broad categories within which most of the species notified to EFSA fall: bacteria, yeasts and viruses (EFSA, 2005, 2007). Here only information as it relates to the QPS assessment of the bacterium *C. glutamicum* is presented.

As noted, each updated QPS Opinion is based on a review of newly available scientific literature and recommendations given in the previous years' opinions. Scientific opinions on the update of the list of QPS-recommended biological agents intentionally added to food or feed that include *C. glutamicum* are reported for the years 2007, 2008, 2010, 2011, 2012, 2013, 2016, 2017 and 2019. The recommendations given in each QPS Opinion for these respective years are summarized in Appendix 1. The recommendations unanimously confirm that *C. glutamicum* meets the QPS criteria for humans and animals and there are no adverse health effects or on safety concerns.

3. LITERATURE SEARCH (2003-2019)

3.1 Method Used

An electronic literature search (ELS) was conducted by saqual GmbH to collect scientific studies, articles, reports and other documents deemed to be relevant for a review of the safety/risk assessment of *C. glutamicum.* The ELS was carried out in October 30th, 2019 using the Google Scholar database and included information published from 2003 onwards. A detailed description of the ELS strategy employed and a listing of the search "strings" used and "hits" obtained is detailed in Appendix 2. The ELS was based on the search terms or

"strings" used by EFSA in the 2017 QPS re-evaluations for *C. glutamicum* (Section 2.2, Table 1), but adapted to the Google Scholar and its specific structure. The information collected from the ELS was reviewed and follow-up selective searches were made using the Web of Science Core Collection, CAB Abstracts and Global Health, BIOSIS Citation Index and Current Contents.

3.2 Relevant Records Retrieved

The "hits" or records retrieved in the ELS search were compiled and each publication was reviewed and judged whether it contained information relevant to the safety of C. glutamicum (Appendix 2, Table 2). Some examples of the topics addressing *C. glutamicum* in the records retrieved include the role of pathogenic and non-pathogenic *Corynebacterium spp*., particularly in human clinical trials (Camello et al., 2003; Roux et al., 2004; Bernard, 2005; Eguchi et al., 2008; Olender, 2012; Oliveira et al., 2017), genetic and biochemical characterization of *C. glutamicum* and site directed mutagenesis (Zhang et al., 2012), gene identification and sequencing (Ikeda and Nagakawa, 2003; Khamis et al., 2004; Ordonez et al., 2005; Yukawa et al., 2007), gene deletion and the effect on cell morphology and antibiotic resistance (Möker et al., 2004; Oritz-Pérez et al., 2010; Bernard, 2012) and carcass degradation (Kim et al., 2017).

Overall, no studies were retrieved either in the ELS or follow-up selective searches that contained information indicating potential safety issues or hazards associated with *C. glutamicum*. Those records retrieved from the searches that support the accepted safe use of different strains of C. glutamicum for amino acid production are reviewed in the following narrative.

4. NARRATIVE - CORYNEBACTERIUM GLUTAMICUM

The scientific data and information presented in the following sections demonstrate that *C. glutamicum* can be safely used as a microorganism for the industrial production of amino acids under the conditions of intended use for the target animals and humans consuming food derived from food-producing animals consuming the substance.

4.1 Taxonomy and Characteristics

The genus *Corynebacterium* belongs to the taxonomic class *Actinobacteria* that represents gram-positive bacteria with a high guanine and cytosine content in their DNA (Stackebrandt et al., 1997; Ventura et al., 2007). The genus Corynebacterium which currently has 110 validated species, is highly diversified and includes species that are of medical, veterinary, or biotechnological relevance (Pascual et al., 1995; Khamis et al., 2004; Bernard, 2012; Soares et al., 2013; Oliveira et al., 2017; Dalen et al., 2018).

One of the most prominent members among the genus *Corynebacterium* is *C. glutamicum*, a bacterium isolated in 1956 from an avian-feces-contaminated soil sample collected from Ueno Zoo in Tokyo (Japan) with a natural capacity to accumulate L-glutamate extracellularly in a biotin-limited medium (Kinoshita et al., 1957; Udaka, 1960; Shiio et al., 1962). *C. glutamicum* belongs to a broad, diverse group of mycolic acid-containing bacteria that share the property of having an unusual cell envelope composition and architecture, differing from those of other gram-positive bacteria (Peuch et al., 2001).

C. glutamicum is a nonmotile, facultative anaerobic, Gram-positive biotin-auxotrophic soil bacterium, which forms rod-shaped, straight, or slightly curved cells (Becker and Whittman, 2017). The chromosome of the wild-type strain *C. glutamicum* ATCC 14067 is 3,273,044 bp in length, with an average GC content of 54.13% (Yangyong Lv et al., 2012). *C. glutamicum* can use a variety of carbon sources as growth and energy substrates, including sugars, sugar alcohols, organic acids and aromatic compounds (Becker et al., 2016). For information on taxonomical studies see Abe et al (1967) and Liebl (2005).

Although some *Corynebacterium spp*. have been detected as components of the bacterial community of cheese surface (Monnet et al., 2006), only *C. glutamicum* is considered of relevance for industry feed and food production sectors.

4.2 Amino Acid Production

The global amino acid market is more than $US 7 billion and is forecast to reach $US 11.6 billion by the year 2015 and $US 35 billion by 2022 (Radiant Insights, Inc., 2015). Global volume consumption of feed grade amino acids, estimated at 4.5 million metric tons in 2017, is projected to reach 6.2 million metric tons by 2022. Poultry feed constitutes the largest consumer of feed amino acids globally with 2017 market share of 43.4% (Business Wire, 2017).

C. glutamicum has many fundamental physiological properties that make it an important industrial workhorse. These properties are listed by Lee et al (2016) as follows: (i) not pathogenic and generally recognized as a safe strain (GRAS); (ii) fast growth to high cell densities; (iii) genetically stable owing to the lack of a recombination repair system; (iv) limited restriction-modification system; (v) no autolysis and maintenance of metabolic activity under growth arrested conditions; (vi) low protease activity favoring recombinant protein production; (vii) plasticity of metabolism and strong secondary metabolism properties; and (viii) broad spectrum of carbon utilization (pentoses, hexoses, and alternative carbon sources); stress tolerance to carbon sources.

C. glutamicum's inability to form spores, relatively few growth requirements and natural capability to produce and secrete glutamate in high amounts makes it one of the most important platform microorganisms used for industrial production of amino acids. The practice of developing amino acid overproducing strains by mutagenesis and selection is a very well-established technique (Rowlands, 1984). Different strains have been utilized for decades by the industry to produce glutamate, lysine, tryptophan, threonine, isoleucine, valine and leucine as described in the "Handbook of *Corynebacterium glutamicum*" (Eggeling and Bott, 2005).

Amino acids have a wide variety of characteristics in terms of nutritional value, taste, medicinal action, and chemical properties, and thus have many potential uses, e.g., in food additives, feed supplements, pharmaceuticals, cosmetics, polymer materials, and agricultural chemicals (Ikeda and Takeno, 2013). Industrial amino acids produced by microorganisms are identical to those naturally found in vegetables and animals (Bercovici and Fuller, 1995).

Over the past decades, global competition among leading companies in the field steadily demanded innovation to improve key performance indicators: yield, titer, and productivity (Becker et al., 2016). For this reason, *C. glutamicum* has become one of the best characterized microorganisms worldwide with regard to substrate spectrum and nutrient requirement (Buschke et al., 2013), catabolic and anabolic pathways and their regulation (Kalinowski et al., 2003; Schroder and Tauch, 2010) underlying biochemistry (Blombach and Seibold, 2010) and response to environmental conditions (Ehira et al., 2009).

4.2.1 Production methods

The two microbiological (biotechnology) methods for the industrial production of amino acids are the use of microbial enzymes or immobilized cells (enzymatic method) and fermentation (semi or direct) (Ivanov et al., 2013). The fermentation process is briefly addressed here to illustrate that the purification step within the fermentation process ensures a safe product.

Fermentation processes typically comprise three steps: fermentation, crude isolation and purification (Kusumoto, 2011; Ikeda and Takeno, 2013; Ivanov et al., 2013). In the fermentation process, the desired amino acid is specifically produced by the fermentation microorganism (e.g. *C. glutamicum* in the production of L-glutamine, L-lysine, L-valine). During the crude isolation process, most impurities contained in the fermentation broth are removed by combining various technologies. Final purification is performed to ensure the required quality for the intended use. The final product is obtained as a crystalline powder. The product is released only after quality tests have verified that the product meets specific requirements, and the normal functioning of each process step has been verified. All manufacturing processes to produce amino acids must comply with current good manufacturing practice requirements.

4.3 Other Uses

C. glutamicum is also employed in the production of L-phenylalanine (Shu and Liao, 2002), L-serine (Stolz et al. 2007) and for secreted protein production (Kikuchi et al., 2003; Umakoshi et al., 2011). The bacterium can be engineered for production of isobutanol (Blombach et al., 2011) and succinate (Litsanov et al., 2013).

Products for health and nutrition have the longest history in industrial biotechnology, with *C. glutamicum* being one of the major producers Meanwhile, processes for other products including non-proteinogenic amino acids, vitamins, flavors and fragrances and other nutrients and health care products are also on the rise (Burnett et al., 2013; Becker et al., 2016).

4.4 Genetic engineering

The past quarter century has seen rapid developments in strain development technology. Metabolic engineering has repeatedly led to successful yield improvements, especially in the field of amino acid production by *C. glutamicum* (Kirchner and Tauch, 2003; Eggeling and Bott, 2005; Wendisch, 2006; Becker and Whittmann, 2012; Zahoor et al., 2012; Burkovski, 2013; Buschke et al, 2013; Heider and Wendisch, 2015).

4.5 Safety Concerns

The species, *C. glutamicum*, which serves as recipient and donor strain is generally considered to be non-pathogenic and no safety concerns are reported for this bacterial species for humans and animals. It is not known to produce toxins or present any other hazards (Nelson et al., 2000; Kalinowski et al., 2003; Bernard, 2005; Olender, 2012; Oliviera et al., 2017).

As discussed in Section 2, *C. glutamicum* meets the EFSA premarket qualified presumption of safety (QPS) assessment criteria when used for fermentation of amino acids.

C. glutamicum is listed as a fermentation organism in several AAFCO feed ingredient definitions (e.g. 36.1, 36.16 and 36.17 (AAFCO 2016). Moreover, amino acids produced by an aerobic fermentation process using *C. glutamicum* are generally recognized as a safe (GRAS) for humans and food producing animals.

Due to its importance as an amino acid producer, *C. glutamicum* is one of the most-investigated and documented microorganisms (Jetten and Sinskey, 1995; Sahm et al., 1995, 2000; Krömer et al., 2004; Leuchtenberger et al. 2005; Dong et al., 2011; Schneider et al., 2011; Ikeda and Takeno, 2013; Lv et al., 2015; Hirasawa and Shimizu, 2016; Wendisch et al., 2016). Lee et al (2016) reviewed the literature and found that as of 2015 over 2,700 papers and 1,700 patents have been reported relating to *C. glutamicum.* The breadth and depth of research carried out on *C. glutamicum* substantiates the accepted safety of using this bacterium by the industry.

In addition to being used for the industrial production of amino acids, *Corynebacterium spp*. have a long history of safe use in food production, including preparation of fermented maize, sorghum, millet, African oil bean seed, rice, soybean and cassava (Caplice and Fitzgerald, 1999; Tateno et al., 2007; Osungbaro, 2009).

4.5.1 Nonpathogenicity

Many of the genes present in the completely sequenced genome of *C. glutamicum* are highly conserved in sequence and gene order within the other members of the *genus Corynebacterium* (Ikeda and Nakagawa, 2003; Kalinowski *et al* 2003). As a non-pathogenic member of the genus, *C. glutamicum* is of increasing interest as a model organism for other members of the suborder including important pathogens such as *C. diphtheriae, Mycobacterium tuberculosis* and *M. leprae* (Camello et al., 2003; Gibson et al., 2003; Moeker et al., 2004; Olender et al., 2012; Tauch and Burkovski, 2015; Cashmore et al., 2017).

5. SUMMARY AND CONCLUSIONS

The data and scientific information presented in this document demonstrate that there are no known safety issues regarding the use of *C. glutamicum* in the production of compounds for use in food for humans and for food-producing animals. *C. glutamicum* is generally considered to be non-pathogenic and no safety concerns are envisaged. The ELS and follow-up selected literature reviews carried out did not reveal any hazards associated with C. glutamicum when added to food or feed. These findings agree with the EFSA QPS Opinions issued from 2005 onwards.

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7. APPENDIX 1

Scientific Opinion on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA

Scientific opinions for *C. glutamicum* for each year are extracted from the respective reference cited.

Year 2007

EFSA. 2007. Opinion of the Scientific Committee on a request from EFSA on the introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA. EFSA Journal 2007, 587:1-16.

Corynebacterium glutamicum

C. glutamicum is a soil bacterium widely used for the biotechnological production of amino acids. Amino acid producing strains have been selected and improved by mutagenesis as well as by using recombinant DNA technology. *C. glutamicum* belongs to a genus which also includes significant human pathogenic bacteria. Although some *Corynebacterium* species have been detected as components of the bacterial community of cheese surface, only *C. glutamicum* is considered of relevance for feed and food sectors. Only this species has been considered for the QPS assessment because of its significant role in the industrial production of amino acids.

Taxonomic unit defined

The genus *Corynebacterium* belongs to a branch of the *Actinomycetales* that also includes the genera *Mycobacterium*, *Nocardia* and *Rhodococcus.* Bacterial species belonging to this branch of the Gram-positive bacteria share particular characteristics, such as high G+C content (47– 74%) and a specific cell envelope organization, mainly characterized by the presence of peptidoglycan, arabinogalactan and mycolic acids. The genus currently contains 63 species, which colonize different environments.

Is the body of knowledge sufficient?

The characteristics, the physiology and the genetics of *C. glutamicum* are well known. The genome sequence of this industrial bacterium has been determined (Kalinowski et al., 2003), reflecting the considerable biotechnological importance of these organisms.

Are there safety concerns?

C. glutamicum plays an important role in the amino acid fermentation industry. No safety concerns are reported for this bacterial species for humans and animals, and no information on the presence of acquired antibiotic resistances in this bacterial species is available. However, it should be kept in mind that the direct exposure of consumers to this bacterial species is expected to be very low.

Can the safety concerns be excluded?

C. glutamicum has generally been considered to be non-pathogenic and no safety concerns are envisaged. However, its history of use is as a source of amino acids and has not, to date, involved the direct and deliberate exposure of humans or livestock.

Units proposed for QPS status

There is a long history of safe use of *C. glutamicum* as an amino acid producer; consequently, *C. glutamicum* is proposed for QPS status with the qualification that this status applies only when the species is used for production purposes only.

Year 2008

EFSA. 2008. Scientific Opinion of the Panel on Biological Hazards on a request from EFSA on the maintenance of the QPS list of microorganisms intentionally added to food or feed. EFSA Journal 2008, 923, 1-48.

Corynebacterium glutamicum

QPS status applies only when the species is used for production purposes.Year 2010

EFSA. 2010. EFSA Panel on Biological Hazards (BIOHAZ); Scientific Opinion on the maintenance of the list of QPS biological agents intentionally added to food and feed (2010 update). EFSA Journal 2010;8(12):1944. 56 pp.

Corynebacterium glutamicum

QPS recommendation only when the species is used for amino acid production.

Year 2011

EFSA. 2011. EFSA Panel on Biological Hazards (BIOHAZ); Scientific Opinion on the maintenance of the list of QPS biological agents intentionally added to food and feed (2011 update). EFSA Journal 2011;9(12):2497. 82 pp.

Corynebacteria

A literature review did not reveal new information about adverse health effects or on safety concerns since the last update (EFSA, 2010). The QPS recommendation has been confirmed.

Antimicrobial resistance aspects regarding the qualification

While no actual antibiotic MIC determinations for *C. glutamicum* appear to have been done, the antibiotic sensitivity of a strain used for amino acid production, has been tested using a disc method (Costa-Riu et al., 2003). The strain was sensitive to ampicillin, kanamycin, streptomycin, tetracycline, susceptible to gentamicin and resistant to norfloxacin, and chloramphenicol. However, the susceptibility test was not performed according to the methodology recommended by the CLSI guideline (Anonymous, 2007). There is no new information that would require a modification in the qualification of the antimicrobial resistance.

Year 2012

EFSA. 2012. Scientific Opinion on the maintenance of the list of QPS biological agents intentionally added to food and feed (2012 update). EFSA Panel on Biological Hazards. EFSA Journal 2012, 10(12):3020. 84 pp.

Corynebacteria

A literature review did not reveal new information about adverse health effects or safety concerns with regards to the last update (EFSA, 2011). The QPS recommendation has been confirmed.

Antimicrobial resistance aspects regarding the qualification

While no actual antibiotic MIC determinations for *C. glutamicum* appear to have been done, the antibiotic sensitivity of a strain used for amino acid production, has been tested using a disc method (Costa-Riu et al., 2003). The strain was sensitive to ampicillin, kanamycin, streptomycin, tetracycline, gentamicin and resistant to norfloxacin, and chloramphenicol. The susceptibility test was not performed according to the methodology recommended by the CLSI guideline (CLSI, 2007). There is no new information that would require a modification in the qualification of the antimicrobial resistance.

Year 2013

EFSA. 2013. Scientific Opinion on the maintenance of the list of QPS biological agents intentionally added to food and feed (2013 update). EFSA Panel on Biological Hazards. EFSA Journal 2013;11(11):3449, 107 pp.

Corynebacterium glutamicum

A literature review did not reveal new information about adverse health effects or safety concerns with regards to the last update (EFSA, 2012). The QPS recommendation has been confirmed.

Antimicrobial resistance aspects regarding the qualification

No new relevant information in the last year was published on the antimicrobial susceptibility or resistance of *C. glutamicum,* therefore no modifications in the qualification of the antimicrobial resistance are proposed.

Year 2017

EFSA. 2017. Scientific Opinion on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA. EFSA Journal 2017, 15(3):4664, 178 pp.

Corynebacterium glutamicum

Taxonomy

Since the last update on the QPS status (EFSA, 2013), no new information on the taxonomy of the *C. glutamicum* has been published.

Update of the body of knowledge on safety concerns

The total number of references found through the ELS was 188; after screening at title/abstract level, 33 passed to the full text phase; of those, two were considered relevant for the QPS assessment. A literature review did not reveal any new information about adverse health effects or safety concerns since the last update (EFSA, 2013).

Revision of antimicrobial resistance aspects

The involvement of class 1 integrons in the AMR towards streptomycin/spectinomycin and tetracycline in *C. glutamicum* isolates has been confirmed and reviewed by Deng et al. (2015). No additional relevant information was published in the last year on the antimicrobial susceptibility or resistance of *C. glutamicum*.

Update on other qualifications

This TU has the following qualification 'QPS only applies when the species is used for amino acid production'. Due to a lack of knowledge in relation to history of use of the viable organisms and because other members of the same genus are pathogenic, the qualification is confirmed.

Other relevant information

No new relevant information was identified.

Conclusion regarding a QPS recommendation

The QPS recommendation is confirmed for *C. glutamicum* as well as the qualification.

Year 2018

EFSA. 2018. Update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 7: suitability of taxonomic units notified to EFSA until September 2017. EFSA Journal 2018, 16(1):5131, 43 pp.

Corynebacterium glutamicum

No safety concerns identified in the only article considered relevant for QPS exercise.

Year 2019

EFSA. 2019. Update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 10: suitability of taxonomic units notified to EFSA until March 2019. EFSA Journal 2019, 17(7):5753, 79 pp.

Corynebacterium glutamicum

A search for papers potentially relevant for the QPS consideration of Corynebacterium glutamicum provided 45 references. No paper reached the final selection phase, therefore no new safety concerns were identified.

8. APPENDIX 2

Electronic Literature Search for safety / risk assessment of *Corynebacterium glutamicum*

Project: Electronic Literature Search for safety / risk assessment of *Corynebacterium glutamicum*

An electronic literature search (ELS) on *Corynebacterium glutamicum* was conducted to collect studies, articles, reports and reviews that are deemed likely to be relevant for further safety / risk assessment of *Corynebacterium glutamicum*.

The search was conducted with the following information:

1. Name of the database searched: Google Scholar (https://scholar.google.co.in).
2. Dates on which the database searched: October 30-31, 2019.
3. Time period between which the database searched: Publications between 2003 and till date.
4. Other restrictions applied: Search terms present in 'allintitle' and 'anywhere' excluding patents and citations.
5. Languages searched: For pages written in any language.
6. Publications searched: Articles published in any peer reviewed journal; book or book chapters; theses; published reviews; etc.
7. Search strategy applied, and records retrieved: Recorded in Table 1.

Selection of articles: A stepwise exercise was performed to select articles that are deemed likely to be relevant for further safety / risk assessment of *Corynebacterium glutamicum* and the shortlisted articles were made available for the 'full review' at the end of ELS.

1. Step 1: Check if the word "Corynebacterium" is mentioned in title, keywords and/or abstract
2. Step 2: Check if the term "Corynebacterium glutamicum" is described in abstract
3. Step 3: Read the abstract
4. Step 4: Select articles for the 'full review' if abstract describes "Corynebacterium glutamicum" or "Corynebacterium spp" and at least some indicative information that the article covers either safety aspects; hazards / disease events in plant, animals and humans; toxin production; or carry genes for antimicrobial resistance. Further detailed evaluation on deemed likely to be included or excluded for the 'full review' was recorded in Table 2.

Table 1: Electronic Literature Search (ELS) Strategy and Retrieved Hits:

Strategy number	Terms	Hits	Notes
#1	allintitle: "Corynebacterium glutamicum"	2780	First 50 hits were checked following 'selection of articles' as mentioned above and recorded in table 2.
#2	allintitle: "Corynebacterium"	4550	First 50 hits were checked following 'selection of articles' as mentioned above and recorded in table 2.
#3	#2 resistance	53	All hits were checked following 'selection of articles' as mentioned above and recorded in table 2.
#4	#2 resistant	52	All hits were checked following 'selection of articles' as mentioned above and recorded in table 2.
#5	#2 antibiotic resistance	4	Both hits were checked following 'selection of articles' as mentioned above and recorded in table 2.
#6	#2 antibiotic resistant	4	All hits were checked following 'selection of articles' as mentioned above and recorded in table 2.
#7	#2 antimicrobial susceptibility OR susceptibilities	10	All hits were checked following 'selection of articles' as mentioned above and recorded in table 2.
#8	#2 infection OR infections	252	All hits were checked following 'selection of articles' as mentioned above and recorded in table 2.
#9	#2 abscess OR abscesses	36	All hits were checked following 'selection of articles' as mentioned above and recorded in table 2.
#10	#2 sepsis OR septic	22	All hits were checked following 'selection of articles' as mentioned above and recorded in table 2.
#11	#2 bacteremia OR bacteraemia	27	All hits were checked following 'selection of articles' as mentioned above and recorded in table 2.
#12	#2 toxic OR toxin OR toxins	42	First 20 hits were checked following 'selection of articles' as mentioned above and recorded in table 2.

Strategy number	Terms	Hits	Notes
#13	#2 pathogen OR pathogenic OR pathogenicity	91	First 20 hits were checked following 'selection of articles' as mentioned above and recorded in table 2.
#14	#2 opportunistic OR virulence OR virulent	50	First 20 hits were checked following 'selection of articles' as mentioned above and recorded in table 2.
#15	#2 safety OR risk	28	All hits were checked following 'selection of articles' as mentioned above and recorded in table 2.
#16	#2 mutagenic OR mutagenicity	00	All hits were checked following 'selection of articles' as mentioned above and recorded in table 2.
#17	#2 toxicity OR toxicology	5	All hits were checked following 'selection of articles' as mentioned above and recorded in table 2.
#18	#2 clinical OR clinically	96	First 20 hits were checked following 'selection of articles' as mentioned above and recorded in table 2.
#19	#2 death OR deaths	2	All hits were checked following 'selection of articles' as mentioned above and recorded in table 2.
#20	#2 morbidity OR morbidities	00	
#21	#2 mortality OR mortalities	2	All hits were checked following 'selection of articles' as mentioned above and recorded in table 2.
#22	#2 disease OR diseases	24	First 20 hits were checked following 'selection of articles' as mentioned above and recorded in table 2.
#23	#2 illness OR illnesses	5	All hits were checked following 'selection of articles' as mentioned above and recorded in table 2.
#24	anywhere: "Corynebacterium glutamicum"	611	First 20 hits were checked following 'selection of articles' as mentioned above and recorded in table 2.

Strategy number	Terms	Hits	Notes
#25	#24 resistance	453	First 20 hits were checked following 'selection of articles' as mentioned above and recorded in table 2.
#26	#24 resistant	494	First 20 hits were checked following 'selection of articles' as mentioned above and recorded in table 2.
#27	#24 antibiotic resistance	436	First 20 hits were checked following 'selection of articles' as mentioned above and recorded in table 2.
#28	#24 antibiotic resistant	353	First 20 hits were checked following 'selection of articles' as mentioned above and recorded in table 2.
#29	#24 antimicrobial susceptibility OR susceptibilities	269	First 20 hits were checked following 'selection of articles' as mentioned above and recorded in table 2.
#30	#24 infection OR infections	271	First 20 hits were checked following 'selection of articles' as mentioned above and recorded in table 2.
#31	#24 abscess OR abscesses	15	All hits were checked following 'selection of articles' as mentioned above and recorded in table 2.
#32	#24 sepsis OR septic	32	All hits were checked following 'selection of articles' as mentioned above and recorded in table 2.
#33	#24 bacteremia OR bacteraemia	18	All hits were checked following 'selection of articles' as mentioned above and recorded in table 2.
#34	#24 toxic OR toxin OR toxins	300	First 20 hits were checked following 'selection of articles' as mentioned above and recorded in table 2.
#35	#24 pathogen OR pathogenic OR pathogenicity	296	First 20 hits were checked following 'selection of articles' as mentioned above and recorded in table 2.
#36	#24 opportunistic OR virulence OR virulent	217	First 20 hits were checked following 'selection of articles' as mentioned above and recorded in table 2.

Strategy number	Terms	Hits	Notes
#37	#24 safety OR risk	223	First 20 hits were checked following 'selection of articles' as mentioned above and recorded in table 2.
#38	#24 mutagenic OR mutagenicity	39	First 10 hits were checked following 'selection of articles' as mentioned above and recorded in table 2.
#39	#24 toxicity OR toxicology	205	First 20 hits were checked following 'selection of articles' as mentioned above and recorded in table 2.
#40	#24 clinical OR clinically	252	First 20 hits were checked following 'selection of articles' as mentioned above and recorded in table 2.
#41	#24 death OR deaths	219	First 20 hits were checked following 'selection of articles' as mentioned above and recorded in table 2.
#42	#24 morbidity OR morbidities	28	First 10 hits were checked following 'selection of articles' as mentioned above and recorded in table 2.
#43	#24 mortality OR mortalities	235	First 20 hits were checked following 'selection of articles' as mentioned above and recorded in table 2.
#44	#24 disease OR diseases	355	First 20 hits were checked following 'selection of articles' as mentioned above and recorded in table 2.
#45	#24 illness OR illnesses	43	First 10 hits were checked following 'selection of articles' as mentioned above and recorded in table 2.

Table 2: Relevant References / Articles:

Search Strategy No. / hits	Search Strategy	Selected Publications	Include / Exclude Justification
#1 / 2780	allintitle: "Corynebacterium glutamicum"	Handbook of Corynebacterium glutamicum Eggeling L, Bott M. CRC Press, 2005. ISBN: 9781420039696	Review / Exclude Not relevant to safety of C. glutamicum
		The Corynebacterium glutamicum genome: features and impacts on biotechnological processes agawa S. Applied Microbiology and Biotechnology, 2003. Vol. 62(2 – 3), pp 99 – 109.	Review / Exclude Not relevant to safety of C. glutamicum
		Comparative analysis of the Corynebacterium glutamicum group and complete genome sequence of strain R Yukawa H, et al. Microbiology, 2007. Vol. 153, pp. 1042 – 1058. doi: 10.1099/mic.0.2006/003657-0	Review / Exclude Not relevant to safety of C. glutamicum
		Deletion of the genes encoding the MtrA–MtrB two-component system of Corynebacterium glutamicum has a strong influence on cell morphology, antibiotics susceptibility and expression of genes involved in osmoprotection Möker N, et al. Molecular Microbiology, 2004. Vol. 54 (2), pp. 420 – 438.	Review / Exclude Not relevant to safety of C. glutamicum
#2 / 4550	allintitle: "Corynebacterium"	The Corynebacterium glutamicum genome: features and impacts on biotechnological processes M.Ikeda et al. Applied Microbiology and Biotechnology., 2003. Vol.62 (2-3), pp. 99 – 109.	Review / Exclude Not relevant to safety of C. glutamicum
		Several results repeated	
#3 / 53	allintitle: Corynebacterium resistance	Analysis of Genes Involved in Arsenic Resistance in Corynebacterium glutamicum	Review / Exclude Not relevant to safety

Search Strategy No. / hits	Search Strategy	Selected Publications	Include / Exclude Justification
		ATCC 13032Efrén Ordóñez et al. Applied of Genes Involved in Arsenic Resistance in Corynebacterium glutamicum ATCC13032, 2005. Vol. 71(10), pp. 6206 – 6215.	of C. glutamicum
		A Corynebacterium glutamicum gene conferring multidrug resistance in the heterologous host Escherichia coli.W Jäger, et al. Journal of Biotechnology 1997. Vol. 179(7), pp. 2449 – 2451.	Review / Exclude Not relevant to C. glutamicum
		The alanine racemase gene alr is an alternative to antibiotic resistance genes in cloning systems for industrial Corynebacterium glutamicum strainsAndreas Tauch, et al Journal of Biotechnology, 2002. Vol. 99(1), pp. 79 – 91.	Review / Exclude Not relevant to safety of C. glutamicum
		Mechanisms of Antibiotic Resistance in Corynebacterium spp. Causing Infections in People Olender A. 2012 https://www.intechopen.com/ https://cdn.intechopen.com/pdfs-wm/34699.pdf	Review / Exclude Not relevant to safety of C. glutamicum
		The identification and resistance analysis to 66 strains of corynebacterium clinical isolates Zhang LWZ. Chinese Journal of Laboratory Diagnosis, 2007. Vol. 7. http://en.cnki.com.cn/Article_en/CJFDTOTAL-ZSZD200707029.htm	Exclude (based on abstract; no translation of full paper)) Not relevant to safety of C. glutamicum
		Antimicrobial Resistance in Corynebacterium spp., Arcanobacterium spp., and Trueperella pyogenes. Feßler AT, Schwarz S. Microbiology Spectrum, 2017. Vol. 5(6). DOI: 10.1128/microbiolspec.ARBA-	Review / Exclude Not relevant to safety of C. glutamicum

Search Strategy No. / hits	Search Strategy	Selected Publications	Include / Exclude Justification
		0021-2017	
		Extracytoplasmic function **sigma factor σD confers** resistance to environmental stress by enhancing mycolate synthesis and modifying peptidoglycan structures in Corynebacterium glutamicum Koichi Toyoda, Toyoda K, Masayuki I. Molecular Microbiology, 2018. Vol. 107 (3), pp. 312 – 329.	Review / Exclude Not relevant to safety of C. glutamicum
		Phenotypic and genotypic characterization of high-level macrolide and lincosamide resistance in Corynebacterium species in Canada and the distribution of the ermX resistance determinant among Corynebacterium species Singh, Cathleen. Theses, 2010.	Review / Exclude Not relevant to safety of C. glutamicum
		A National Survey of Multi-Drug Resistance in Ophthalmic Clinical Isolates of Corynebacterium in Japan Eguchi H, et al., Investigative Ophthalmology and Visual Science, 2008. Vol.49, pp. 5530	Review / Exclude Not relevant to safety of C. glutamicum
		Several results repeated	
#4 / 52	allintitle: Corynebacterium resistant	Feedback-resistant acetohydroxy acid synthase increases valine production in Corynebacterium glutamicumVeronika Elišáková, et al. Genetics and Molecular Biology, 2005.,pp 207 – 213.	Review / Exclude Not relevant to safety of C. glutamicum
		Co-expression of feedback-resistant threonine dehydratase and acetohydroxy acid synthase increase l-isoleucine production in Corynebacterium glutamicumAuthor links open overlay panelLianghongYin. et al.	Review / Exclude Not relevant to safety of C. glutamicum

Search Strategy No. / hits	Search Strategy	Selected Publications	Include / Exclude Justification
		Metabolic Engineering, 2012. Vol. 14 (5), pp.542 – 550.	
		Corynebacterium resistens sp. nov., a New Multidrug-Resistant Coryneform Bacterium Isolated from Human InfectionsYoshihito Otsuka, et al. Journal of Clinical Microbiology, 2005. Vol. 43 (8), pp 3713 – 3717.	Review / Exclude Not relevant to C. glutamicum
		Adaptive evolution of Corynebacterium glutamicum resistant to oxidative stress and its global gene expression profilingJY Lee, et al. Biotechnology Letters, 2013. Vol. 35 (5), pp 709 – 717.	Review / Exclude Not relevant to safety of C. glutamicum
		Genetic and biochemical characterization of Corynebacterium glutamicum ATP phosphoribosyltransferase and its three mutants resistant to feedback inhibition by histidineYun Zhang, et al. Japanese Journal of Infectious, 2012. Vol. 94(3). Pp 829-838	Review / Exclude Not relevant to safety of C. glutamicum
		Characteristics of Multidrug-resistant Corynebacterium spp. Isolated from Blood Cultures from Hospitalized Patients in JapanLiang Qin, et al. Japanese Journal of Infectious Diseases, 2017. Vol.70(2), pp.152-157	Review / Exclude Not relevant to safety of C. glutamicum
		Generation of branched-chain amino acids resistant Corynebacterium glutamicum acetohydroxy acid synthase by site-directed mutagenesisGuo Y, et al. Biotechnology and Bioprocess Engineering, 2014. Vol. 19(3), pp. 456 – 467.	Review / Include Article discusses antibiotic resistance.
		Few results repeated	

Search Strategy No. / hits	Search Strategy	Selected Publications	Include / Exclude Justification
#5 / 4	allintitle: Corynebacterium antibiotic resistance	Results repeated	
#6 / 4	allintitle: Corynebacterium antibiotic resistant	none	
#7 / 10	allintitle: Corynebacterium antimicrobial susceptibility OR susceptibilities	Antimicrobial Susceptibility and Species Identification of Corynebacterium spp. Strains Collected in Europe and USA Medical Centers (2006-2010) Sader HS, et al. Sentry Antimicrobial Surveillance, 2012. P1092 ECCMID 2012 JMI Laboratories North Liberty, IA, USA	Review / Exclude Not relevant to safety of C. glutamicum
		Few results repeated	
#8 / 252	allintitle: Corynebacterium infection OR infections	Idiopathic Granulomatous Mastitis Associated with Corynebacterium Sp. Infection Creed Michael Stary, et al. Hawai'I Medical Journal, 2011. Vol.70 (5), pp. 99 –101.	Review / Exclude Not relevant to safety of C. glutamicum
		Corynebacterium-associated skin infections Blaise G, et al. International Journal of Dermatology, 2008. Vol. 47 (9), pp. 884 – 890.	Review / Exclude Not relevant to safety of C. glutamicum
		Corynebacterium Species Isolated from Bone and Joint Infections Identified by 16S rRNA Gene Sequence Analysis Raoult D, et al. J. Clin. Microbiol., 2004. Vol. 42 (5), pp. 2231 – 2233.	Review / Exclude Not relevant to safety of C. glutamicum
		Case of erythema nodosum associated with granulomatous mastitis probably due to Corynebacterium infection Kubo Y, et al. The Journal of Dermatology, 2014. Vol. 41(9), pp. 821 – 823.	Review / Exclude Not relevant to safety of C. glutamicum
		[Wound infections due to	Review / Exclude

Search Strategy No. / hits	Search Strategy	Selected Publications	Include / Exclude Justification
		opportunistic corynebacterium species] Olender A, Łetowska I. Medycyna Doswiadczalna i Mikrobiologia, 2010. Vol. 62 (2), pp. 135 – 140.	(based on abstract; no translation of full paper)) Not relevant to safety of C. glutamicum
		Identification of Corynebacterium spp. isolated from bovine intramammary infections by matrix-assisted laser desorption ionization-time of flight mass spectrometry dos Santos MV, et al. Veterinary Microbiology, 2014. Vol. 173 (1 – 2), pp. 147 – 151.	Review / Exclude Not relevant to safety of C. glutamicum
		Ocular Infections Caused by Corynebacterium Species Eguchi H. Infection Control, 2013. Dr. Silpi Basak (Ed.), In Tech, DOI: 10.5772/56214.	Review / Exclude Not relevant to safety of C. glutamicum
		Hardware Infection with Corynebacterium spp.: a Case Report and Review of the Literature Clarridge III JE, et al. Clinical Microbiology Newsletter, 2014. Vol. 36(2), pp. 9 – 13.	Review / Exclude Not relevant to safety of C. glutamicum
		Cerebrospinal fluid shunt infection caused by Corynebacterium sp: Case report and review Randi BA, et al. Brain Injury, 2014. Vol. 28(9), pp. 1223 – 1225.	Review / Exclude Not relevant to safety of C. glutamicum
		Transmission dynamics of intramammary infections caused by Corynebacterium species Delen G, et al. Journal of Dairy Science, 2018. Vol. 101 (1), pp. 472 – 479.	Review / Exclude Not relevant to safety of C. glutamicum
		Modelling and dynamics of intramammary infections caused by Corynebacterium species	Review / Exclude Not relevant to safety of C. glutamicum

Search Strategy No. / hits	Search Strategy	Selected Publications	Include / Exclude Justification
		Rachah A, et al. 7th International Conference on Modeling, Simulation, and Applied Optimization (ICMSAO), 2017. Conference proceedings.	
		Few results repeated	
#9 / 36	allintitle: Corynebacterium abscess OR abscesses	none	
#10 / 22	allintitle: Corynebacterium sepsis OR septic	none	
#11 / 27	allintitle: Corynebacterium bacteremia OR bacteraemia	none	
#12 / 42	allintitle: Corynebacterium toxic OR toxin OR toxins	none	
#13 / 91	allintitle: Corynebacterium pathogen OR pathogenic OR pathogenicity	Corynebacterium occurance and pathogenicity for humans and animals Banaszkiewicz T, Krukowski H. Medycyna Weterynaryjna, 2011. Vol.67 No.4 pp.229-232	Exclude (based on abstract; no translation of full paper)) Not relevant to safety of C. glutamicum
		Insight of Genus Corynebacterium: Ascertaining the Role of Pathogenic and Non-pathogenic Species Oliveira A, et al. Front. Microbiol., 2017. https://doi.org/10.3389/fmicb.2017.01937	Review / Exclude Not relevant to safety of C. glutamicum
		Few results repeated	
#14 / 50	allintitle: Corynebacterium opportunistic OR virulence OR virulent	Molecular armory or niche factors: virulence determinants of Corynebacterium species Olender A, Łetowska I Microbiology Letters, 2010. Vol. 62(2), pp.135-140	Review / Exclude Not relevant to safety of C. glutamicum

Search Strategy No. / hits	Search Strategy	Selected Publications	Include / Exclude Justification
		Few results repeated	
#15 / 28	allintitle: Corynebacterium safety OR risk	Safety and efficacy of L arginine produced by Corynebacterium glutamicum KCTC 10423BP for all animal species EFSA. EFSA Journal, 2016. DOI: 10.2903/j.efsa.2016.4345	Review / Include Assessment reviews safety, efficacy and toxicity
		Scientific Opinion on the safety and efficacy of L-valine produced by Corynebacterium glutamicum (KCCM 80058) for all animal species, based on a dossier submitted by CJ Europe GmbH EFSA. EFSA Journal, 2013. DOI: 10.2903/j.efsa.2013.3429	Review / Include Assessment reviews safety, efficacy and toxicity
		Safety and efficacy of l-arginine produced by Corynebacterium glutamicum KCCM 80099 for all animal species EFSA. EFSA Journal, 2017. DOI: 10.2903/j.efsa.2017.4858	Review / Include Assessment reviews safety, efficacy and toxicity
		Opinion of the Panel on additives and products or substances used in animal feed (FEEDAP) on the safety and efficacy of the product containing L-arginine produced by fermentation from Corynebacterium glutamicum (ATCC-13870) for all animal species EFSA. EFSA Journal, 2007. DOI: 10.2903/j.efsa.2007.473	Review / Include Assessment reviews safety, efficacy and toxicity
		Scientific Opinion on the safety and efficacy of L-valine (ValAMINO®) produced by Corynebacterium glutamicum (DSM 25202) for all animal species, based on a dossier submitted by Evonik Industries AG EFSA. EFSA Journal, 2014. DOI:	Review / Include Assessment reviews safety, efficacy and toxicity

Search Strategy No. / hits	Search Strategy	Selected Publications	Include / Exclude Justification
		10.2903/j.efsa.2014.3795	
		Scientific Opinion on the safety and efficacy of L-lysine monohydrochloride, technically pure, produced with Escherichia coli CGMCC 3705 and L-lysine sulphate produced with Corynebacterium glutamicum CGMCC 3704 for all animal species, based on a dossier submitted by HELM AG EFSA. EFSA Journal, 2015. DOI: 10.2903/j.efsa.2015.4156	Review / Include Assessment reviews safety, efficacy and toxicity
		Safety of concentrated l-lysine (base), l-lysine monohydrochloride and l-lysine sulfate produced using different strains of Corynebacterium glutamicum for all animal species based on a dossier submitted by FEFANA asbl EFSA. EFSA Journal, 2019. DOI: 10.2903/j.efsa.2019.5532	Review / Include Assessment reviews safety, efficacy and toxicity
		Safety and efficacy of l-lysine monohydrochloride and concentrated liquid l-lysine (base) produced by fermentation using Corynebacterium glutamicum strain NRRL B-50775 for all animal species based on a dossier submitted by ADM EFSA. EFSA Journal, 2019. DOI: 10.2903/j.efsa.2019.5537	Review / Include Assessment reviews safety, efficacy and toxicity
		Safety and efficacy of l-arginine produced by fermentation using Corynebacterium glutamicum KCCM 10741P for all animal species EFSA. EFSA Journal, 2018. DOI: 10.2903/j.efsa.2018.5277	Review / Include Assessment reviews safety, efficacy and toxicity

Search Strategy No. / hits	Search Strategy	Selected Publications	Include / Exclude Justification
		Safety and efficacy of l-arginine produced by fermentation with Corynebacterium glutamicum KCCM 80182 for all animal species EFSA. EFSA Journal, 2019. DOI: 10.2903/j.efsa.2019.5696	Review / Include Assessment reviews safety, efficacy and toxicity
		Safety and efficacy of l-histidine monohydrochloride monohydrate produced using Corynebacterium glutamicum KCCM 80172 for all animal species EFSA. EFSA Journal, 2019. DOI: 10.2903/j.efsa.2019.5783	Review / Include Assessment reviews safety, efficacy and toxicity
		Few results repeated	
#16/ 0	allintitle: Corynebacterium mutagenic OR mutagenicity		
#17 / 5	allintitle: Corynebacterium toxicity OR toxicology	Transcriptomic analysis of Corynebacterium glutamicum in the response to the toxicity of furfural present in lignocellulosic hydrolysates Park HS, et al. Process Biochemistry, 2015. Vol. 50(3), pp. 347 – 356.	Review / Exclude Not relevant to safety of C. glutamicum
#18 / 96	allintitle: Corynebacterium clinical OR clinically	The clinical course of peritoneal dialysis-related peritonitis caused by Corynebacterium species Szeto CC, et al. Nephrology Dialysis Transplantation, 2005. Vol. 20 (12), pp. 2793 – 2796. https://doi.org/10.1093/ndt/gfi123	Review / Exclude Not relevant to safety of C. glutamicum
		Nondiphtherial Corynebacterium species isolated from clinical specimens of patients in a university hospital, Rio de Janeiro, Brazil Camello TCF, et al. Braz. J.	Review / Exclude Not relevant to safety of C. glutamicum

Search Strategy No. / hits	Search Strategy	Selected Publications	Include / Exclude Justification
		Microbiol., 2003. Vol. 34 (1).	
		Antibiotic susceptibility of Corynebacterium isolated from clinical specimens Chen D, et al. Chinese Journal of Clinical Laboratory Science, 2011. Vol. 3	Review / Exclude Not relevant to safety of C. glutamicum
		Relationship Between Susceptibility to Quinolones in Corynebacterium Ophthalmic Clinical Isolates and the GyrA Gene Mutations Katome T, et al. Investigative Ophthalmology & Visual Science, 2008. Vol. 49 (13).	Review / Exclude Not relevant to safety of C. glutamicum
		Relationship Between Mutations in the gyrA Gene and Quinolone Resistance in Ophthalmic Clinical Isolates of Corynebacterium Species Eguchi H, et al., Investigative Ophthalmology & Visual Science, 2006. Vol. 47 (13), pp. 3566.	Review / Exclude Not relevant to safety of C. glutamicum
		Endophthalmitis Caused by Corynebacterium Species: Clinical Features, Antibiotic Susceptibility, and Treatment Outcomes Kuriyan AE, et al. Ophthalmology retina, 2017. Vol. 1 (3), pp. 200 – 205.	Review / Exclude Not relevant to safety of C. glutamicum
#19 /2	allintitle: Corynebacterium death OR deaths	none	
#20/ 0	allintitle: Corynebacterium morbidity OR morbidities	none	
#21 / 2	allintitle: Corynebacterium mortality OR mortalities	Biodegradation of Contaminated Environments Using Corynebacterium glutamicum and Its Application to Livestock Mortalities Burials [rest of the details are in Chinese]	Exclude (based on abstract; no translation of full paper)) Not relevant to safety of C. glutamicum

Search Strategy No. / hits	Search Strategy	Selected Publications	Include / Exclude Justification
#22 / 24	allintitle: Corynebacterium disease OR diseases	Corynebacterium species and coryneforms: An update on taxonomy and diseases attributed to these taxa Bernard K. Clinical Microbiology Newsletter, 2005. Vol. 27(2), pp 9 – 18. DOI: https://doi.org/10.1016/j.clinmicnews.2005.01.002.	Exclude Not relevant to safety of C. glutamicum
#23 / 5	allintitle: Corynebacterium illness OR illnesses	none	
#24 / 611	anywhere: "Corynebacterium glutamicum"	Few results repeated	
#25 / 453	anywhere: "Corynebacterium glutamicum" resistance	none	
#26 / 494	anywhere: "Corynebacterium glutamicum" resistant	none	
#27 / 436	anywhere: "Corynebacterium glutamicum" antibiotic resistance	none	
#28 / 353	anywhere: "Corynebacterium glutamicum" antibiotic resistant	Drivers of bacterial genomes plasticity and roles they play in pathogen virulence, persistence and drug resistance Patel S. Infection, Genetics and Evolution, 2016. Vol. 45, pp. 151 – 164.	Exclude Not relevant to safety of C. glutamicum
#29 / 269	anywhere: "Corynebacterium glutamicum" antimicrobial susceptibility OR susceptibilities	none	
#30 / 271	anywhere: "Corynebacteriu	none	

Search Strategy No. / hits	Search Strategy	Selected Publications	Include / Exclude Justification
	m glutamicum" infection OR infections		
#31 / 15	anywhere: "Corynebacterium glutamicum" abscess OR abscesses	Corynebacterium ulcerans, an emerging human pathogen Hacker E, et al. Future Microbiology, 2016. Vol. 11 (9). https://doi.org/10.2217/fmb-2016-0085	Exclude Not relevant to C. glutamicum
#32 / 32	anywhere: "Corynebacterium glutamicum" sepsis OR septic	none	
#33 / 18	anywhere: "Corynebacterium glutamicum" bacteremia OR bacteraemia	none	
#34 / 300	anywhere: "Corynebacterium glutamicum" toxic OR toxin OR toxins	none	
#35 / 296	anywhere: "Corynebacterium glutamicum" pathogen OR pathogenic OR pathogenicity	none	
#36 / 217	anywhere: "Corynebacterium glutamicum" opportunistic OR virulence OR virulent	none	
#37 / 223	anywhere: "Corynebacterium glutamicum" safety OR risk	none	
#38 / 39	anywhere: "Corynebacterium glutamicum" mutagenic OR mutagenicity	none	
#39 /	anywhere:	none	

Search Strategy No. / hits	Search Strategy	Selected Publications	Include / Exclude Justification
205	"Corynebacterium glutamicum" toxicity OR toxicology		
#40 / 252	anywhere: "Corynebacterium glutamicum" clinical OR clinically	none	
#41 / 219	anywhere: "Corynebacterium glutamicum" death OR deaths	none	
#42 / 28	anywhere: "Corynebacterium glutamicum" morbidity OR morbidities	none	
#43 / 235	anywhere: "Corynebacterium glutamicum" mortality OR mortalities	none	
#44 /355	anywhere: "Corynebacterium glutamicum" disease OR diseases	none	
#45 / 43	anywhere: "Corynebacterium glutamicum"	none	

REPORT

Analytical Method Validation of Dried L-Valine Fermentation Product using HPLC

(Confidential)

Original final report date: Dec 21, 2020

Study Director	Quality Assurance Manager
(b) (4)	
Dami Jeong	Seok-Hun Yun

CJ Research Institute of Biotechnology

Table of contents

1. Introduction

There are several official methods to analyze L-valine. The commonly used method of L-valine analysis is potentiometric titration with perchloric acid, however, most other amino acids could also be detected by this method. Therefore, titration method is not applicable in case of sample containing the other amino acids as an impurity.

For this reason, CJ developed the analytical method for 'Dried L-Valine Fermentation Product' and this analytical method was verified by method validation.

2. Test article

2.1. Test Article

1) Identity: Dried L-Valine Fermentation Product (VAL Pro)

2) Lot number: GVAL200910

3) Purity: > 72.0% (L-Valine, dry basis)

4) Date of receipt: November 30, 2020

5) Amount of receipt: approximately 100 g

6) Storage conditions: room temperature

7) Supplier: CJ Research Institute of Biotechnology

2.2. Reference standard

1) Identity: L-Valine

2) Product No.: V0500 (SLCD6123)

3) Purity: 100%

4) Quality release Date: October 04, 2019

5) Amount of receipt: 25 g

6) Storage conditions: room temperature

7) Supplier: (b) (4)

8) Expiry date (retest date): October, 2022

3. HPLC analytical condition

3.1. HPLC Condition

Table 1. HPLC Condition

	Condition
System	HPLC (SHIMADZU Nexera UPLC-30A)
Detector	Fluorescence detector (Excitation λ : 338nm Emission λ : 425nm)
Column	ODS C18, 150 × 4.6 mm, particle size 3 μm
Column temperature	40℃
Mobile phase	16.7 mM-KH_2PO_4 + 5 mM OSA in 12% CH_3CN, pH 2.5 (by H_3PO_4)
Flow rate of mobile phase	1.0 ml/min
Reaction reagent	201.91mM-KOH + 241.39mM-H_3BO_3 + 2.53mM-OPA + C_2H_6OS 1mL + CH_3OH 5mL + 3.5%-Brij 1.25mL
Flow rate of reaction reagent	0.5 ml/min
Sample temperature	15℃
Injection volume	5 μl
Concentration of sample and standard solution	0.1 g/L (L-valine concentration basis)

3.2. Preparation reagent for mobile phase and reaction reagent

Table 2. Preparation reagent for mobile phase and reaction reagent

Mobile phase			
	Purity	Manufacturer	Product No.
Acetonitrile(CH_3CN)	HPLC Grade	(b) (4)	(b) (4)
Potassium dihydrogen phosphate (KH_2PO_4)	≥99%	(b) (4)	(b) (4)
Phosphoric acid(H_3PO_4)	≥85%	(b) (4)	(b) (4)
1-Octanfonic acid sodium salt (OSA)	≥98%	(b) (4)	(b) (4)
Distilled water	minimum conductivity (18.2 MΩ)		

Reaction reagent			
	Purity	Manufacturer	Product No.
Potassium hydroxide	≥85%	(b) (4)	(b) (4)
Boric acid	≥99.5%	(b) (4)	(b) (4)
O-phthalaldehyde (OPA)	≥97%	(b) (4)	(b) (4)
2-Mercapto ethanol(2-ETSH)	≥99%	(b) (4)	(b) (4)
Methyl alcohol	≥98%	(b) (4)	(b) (4)
Distilled water	minimum conductivity (18.2 MΩ)		

3.3. Mobile phase solution preparation method

Table 3. Mobile phase solution preparation method

Reagent name	Concentration (mM)	Amount (g)	Total volume (mL)
Potassium dihydrogen phosphate (KH_2PO_4)	(b) (4)		1000
1-Octanfonic acid sodium salt (OSA)			
Acetonitrile (CH_3CN)			1137
Phosphoric Acid (H_3PO_4)			

(b) (4)

3.4. Reaction reagent preparation method

Table 4. Reaction reagent preparation method

Reagent name	Concentration (mM)	Amount (g)	Total volume (mL)
Potassium hydroxide	(b) (4)		
Boric acid			
O-phthalaldehyde (OPA)			1000
2-Mercaptoethanol (2-ETSH)			
Methyl alcohol			
3.5%-Brij solution			

(b) (4)

4. Standard preparation

(b) (4)

5. Sample preparation

(b) (4)

(b) (4)

6. Data processing and calculation

(b) (4)

Table 5. Data calculation

	Standard solution	Sample solution
Weight	(b) (4)	
Preparation concentration		
Area 1		
Area 2		
Area 3		
Area 4		
Average		
STDEV	(b) (4)	
%RSD*		
R.F. (Response factor		
Measurement concentration		
Result		

* If the area difference is RSD ≥ 1%, reanalyze and if the difference is still over 1%, instrument should be checked.

7. Specificity

(b) (4)

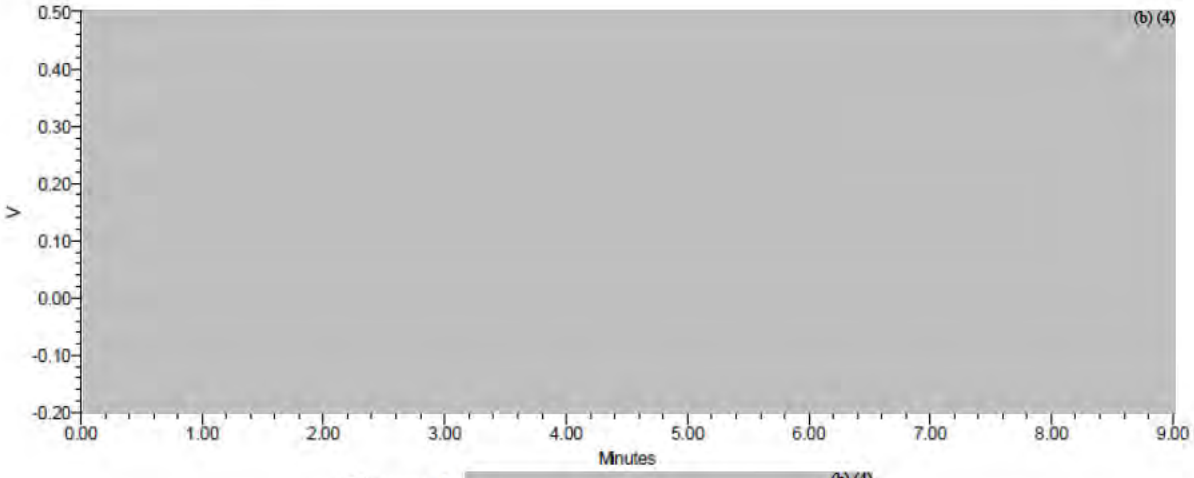


Figure 1. (b) (4)

8. System suitability

(b) (4)

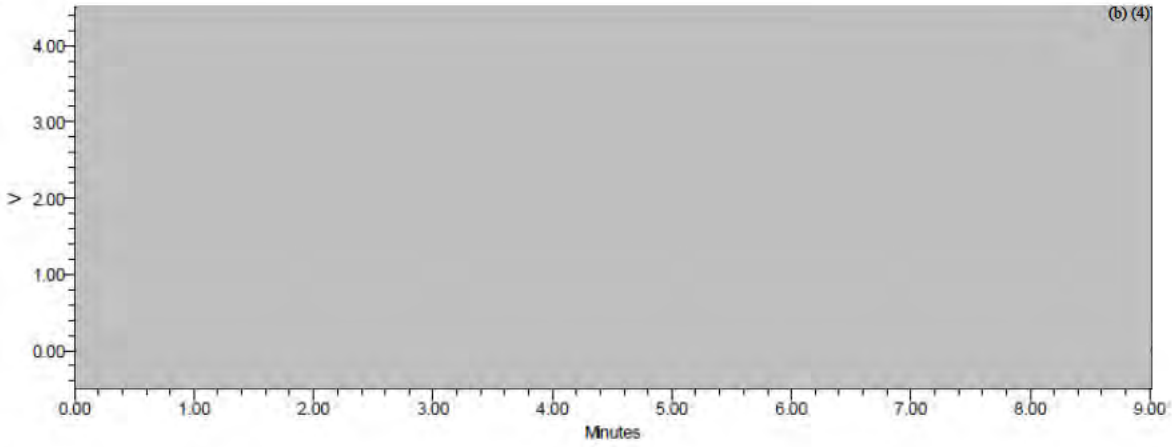
Table 6. Reference standard solution (0.025 g/L)

	Peak area (STD 1, 0.025 g/L)
1	(b) (4)
2	
3	
4	
5	
6	
7	
8	
9	
10	
%RSD	0.18%

Table 7. Reference standard solution (0.1 g/L)

	Peak area (STD 4, 0.100 g/L)
1	(b) (4)
2	
3	
4	
5	
6	
7	
8	
9	
10	
%RSD	0.16%

(a)



(b)

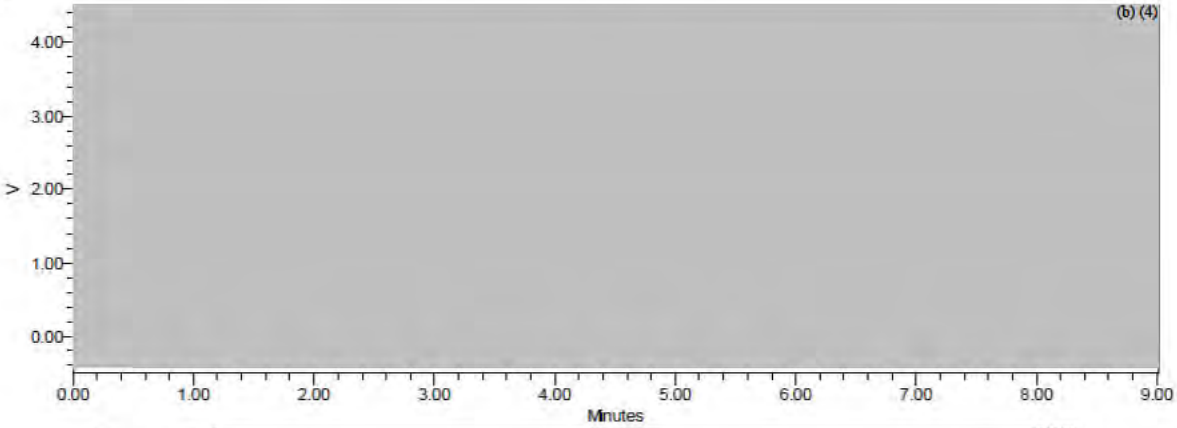


Figure 2. (b) (4)

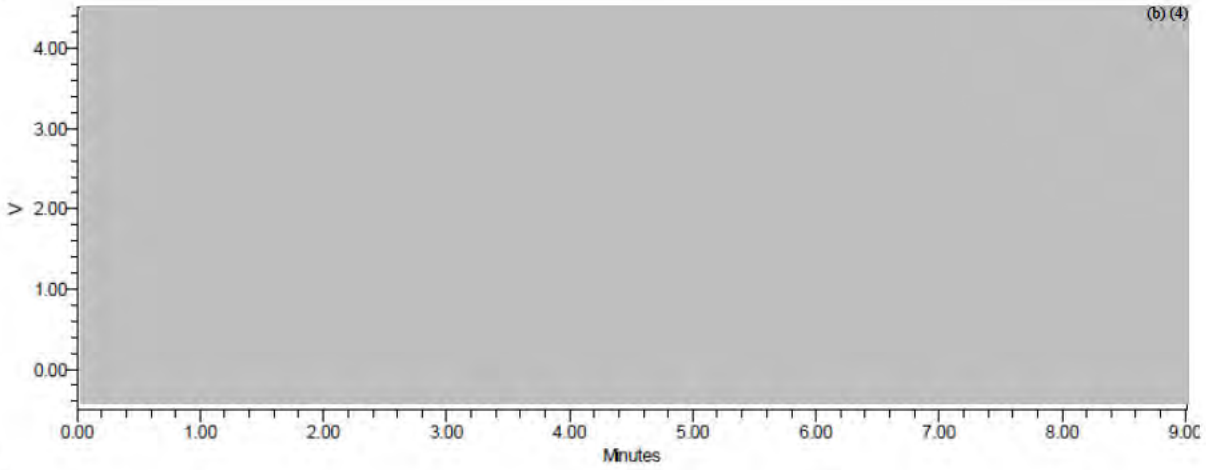
9. Homogeneity

(b) (4)

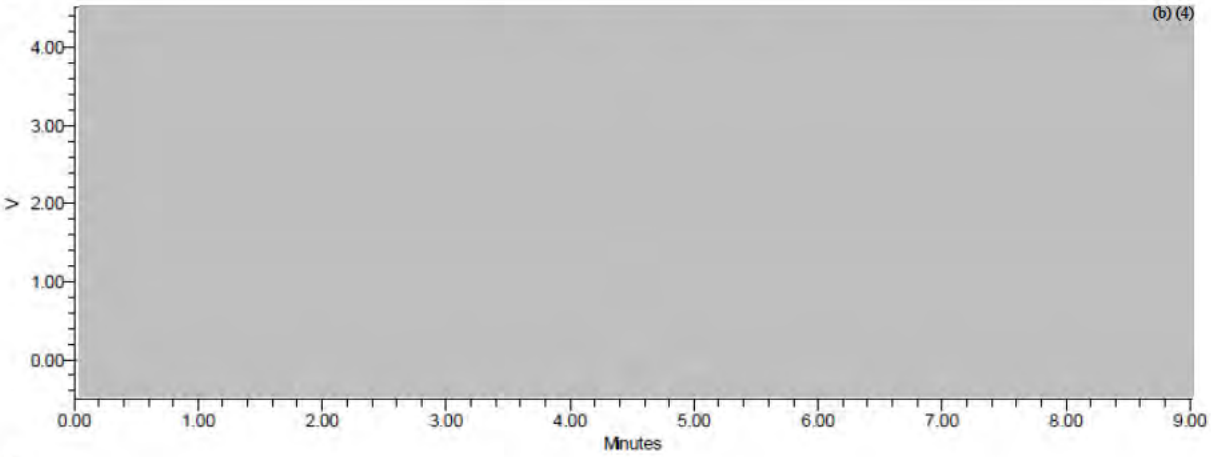
Table 8. Homogeneity of sample

Sample	Sample weight (g)	L-Valine (%)
Sampling 1	0.13775 g/1000mL	(b) (4)
Sampling 2	0.13705 g/1000mL	
Sampling 3	0.13787 g/1000mL	
Sampling 4	0.13761 g/1000mL	
Sampling 5	0.13678 g/1000mL	
Average	-	72.19
%RSD	-	0.28

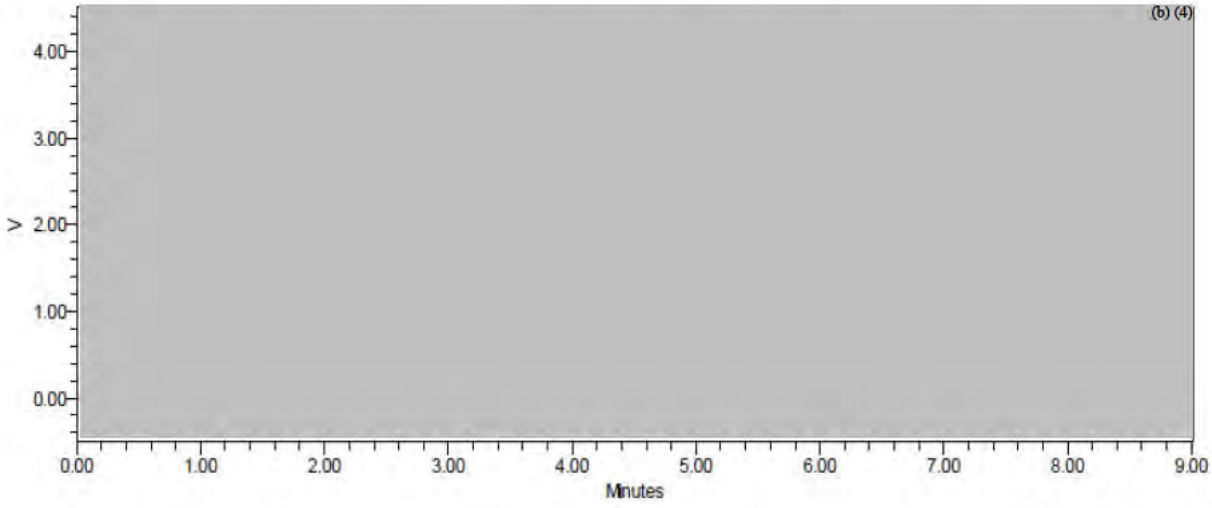
(a)


(b) (4)
4.00
3.00
V 2.00
1.00
0.00
0.00 1.00 2.00 3.00 4.00 5.00 6.00 7.00 8.00 9.00
Minutes

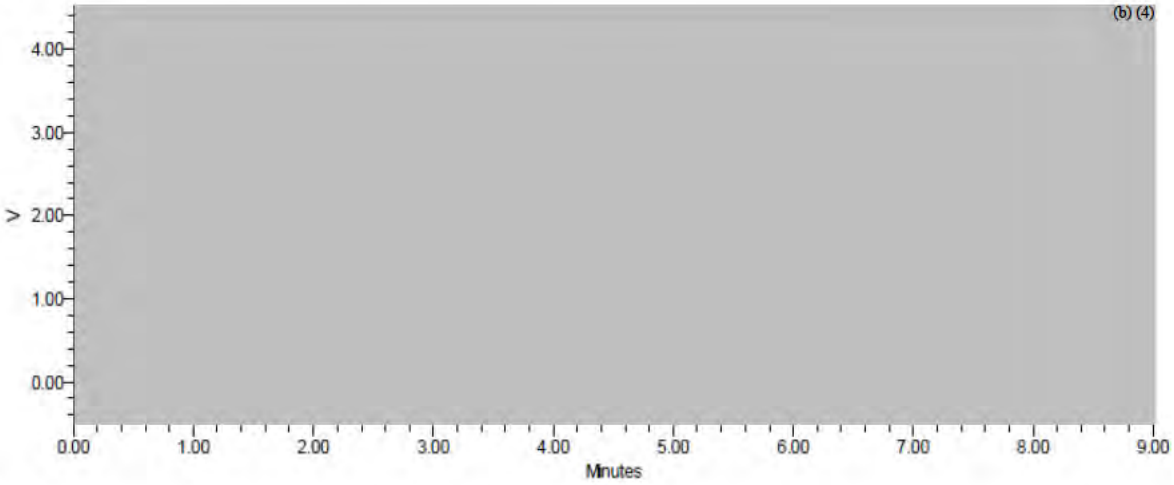
(b)


(b) (4)
4.00
3.00
V 2.00
1.00
0.00
0.00 1.00 2.00 3.00 4.00 5.00 6.00 7.00 8.00 9.00
Minutes

(c)


(b) (4)
4.00
3.00
V 2.00
1.00
0.00
0.00 1.00 2.00 3.00 4.00 5.00 6.00 7.00 8.00 9.00
Minutes

(d)



(e)

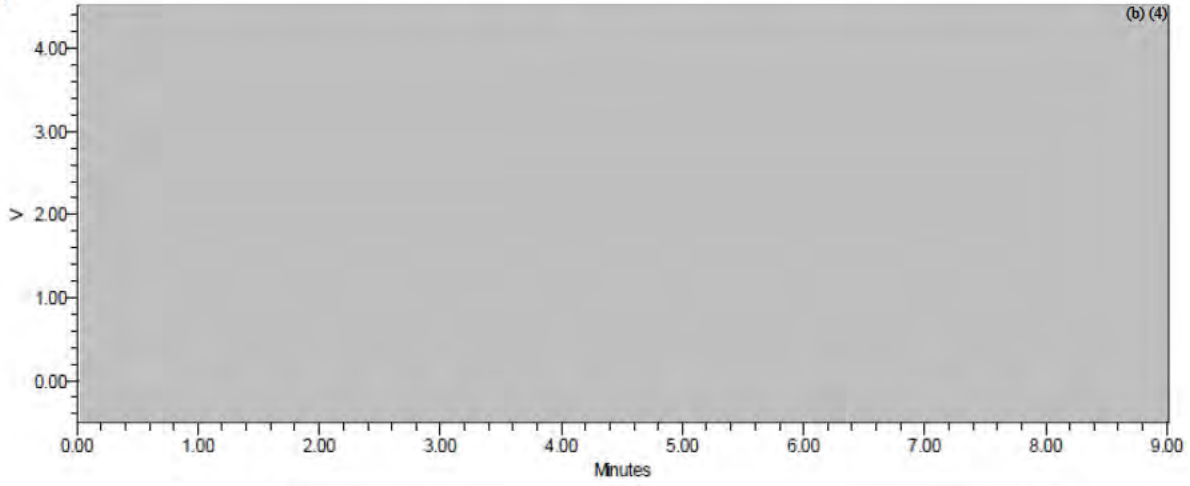


Figure 3. (b) (4)

10. Stability

(b) (4)

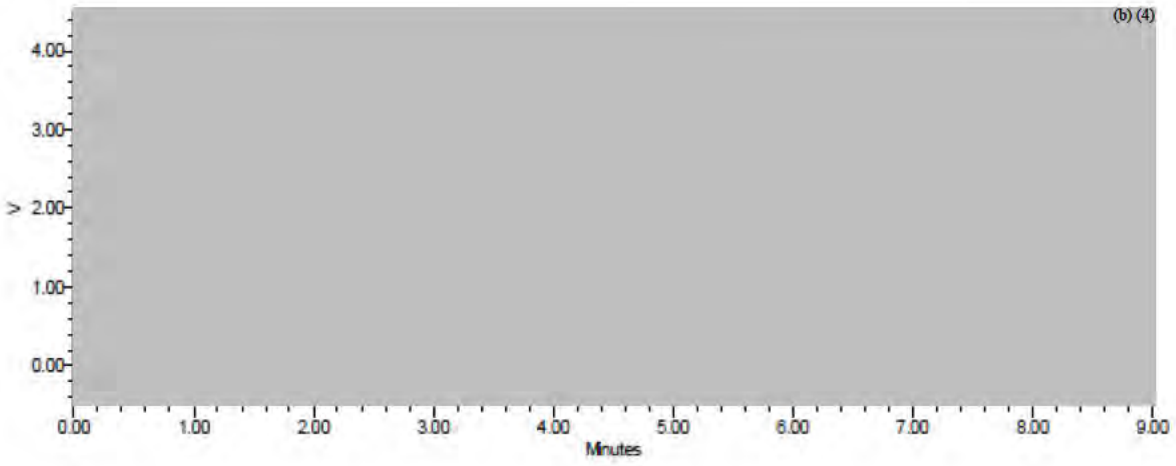
And %RSD was 0.17%.

The recovery of sample was satisfied with the acceptance criteria of 98%-102% and %RSD criteria of < 1%.

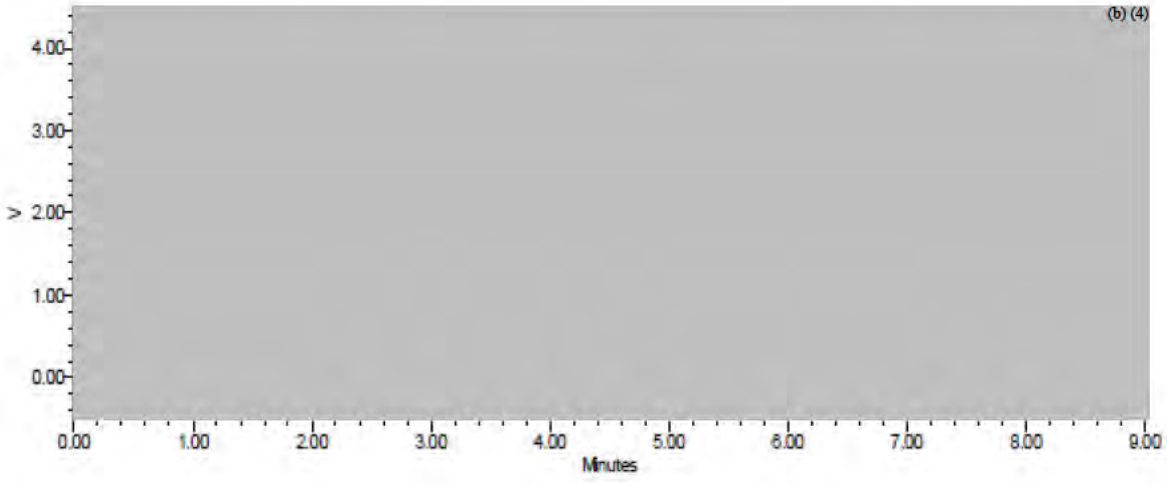
Table 9. Stability of the sample (investigation of precision of sample)

Time (day)	Time (h)	L-Valine (%)	Recovery (%)
day 1_1	0	(b) (4)	
day 1_2	5		
day 1_3	10		
day 2_1	23		
day 2_2	28		
day 2_3	32		
day 3_1	53		
day 3_2	57		
day 3_3	62		
%RSD		0.17%	-

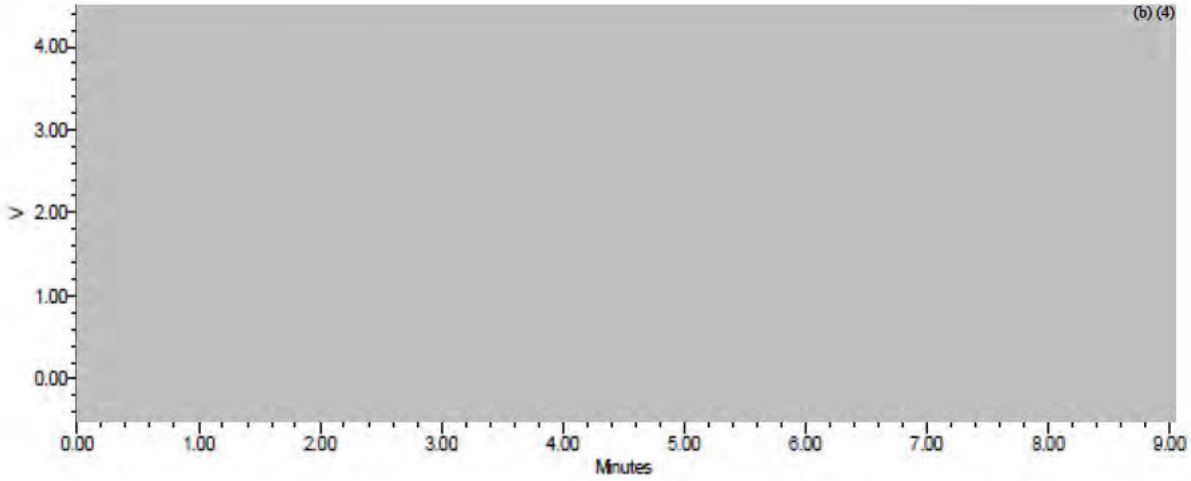
(a)



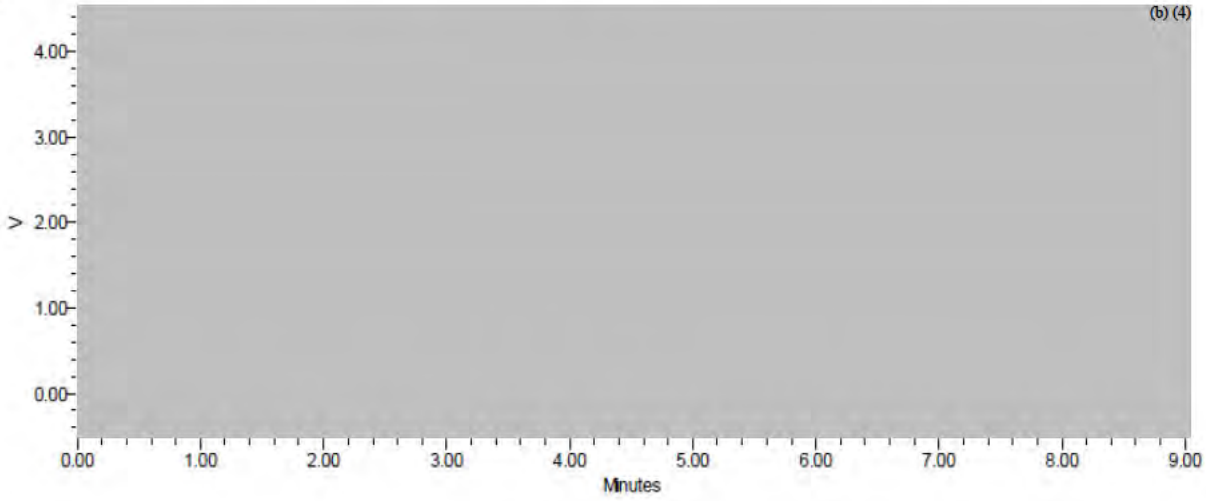
(b)



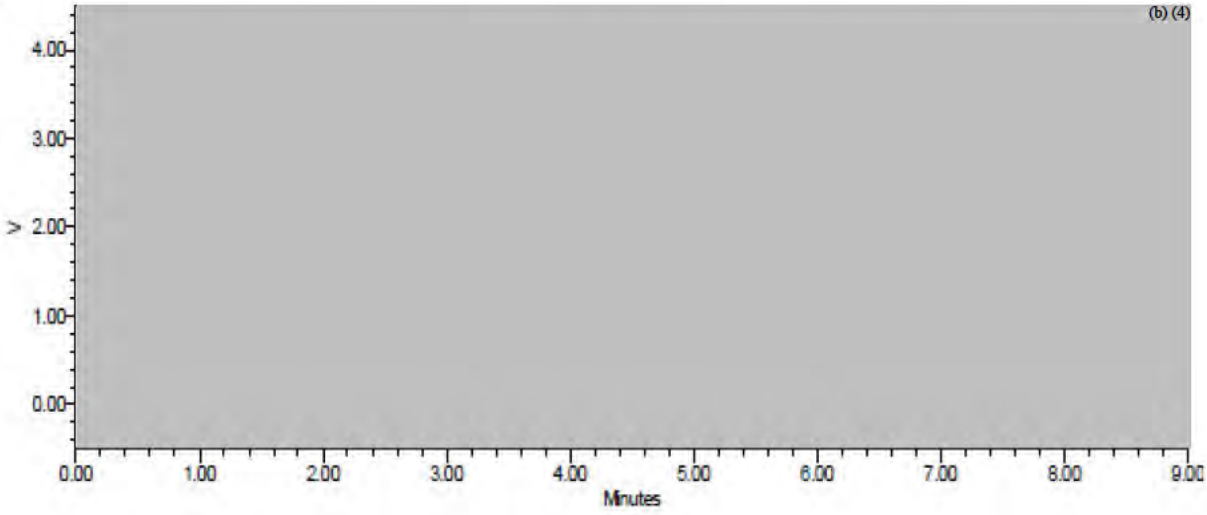
(c)


(b) (4)
4.00
3.00
V 2.00
1.00
0.00
0.00 1.00 2.00 3.00 4.00 5.00 6.00 7.00 8.00 9.00
Minutes

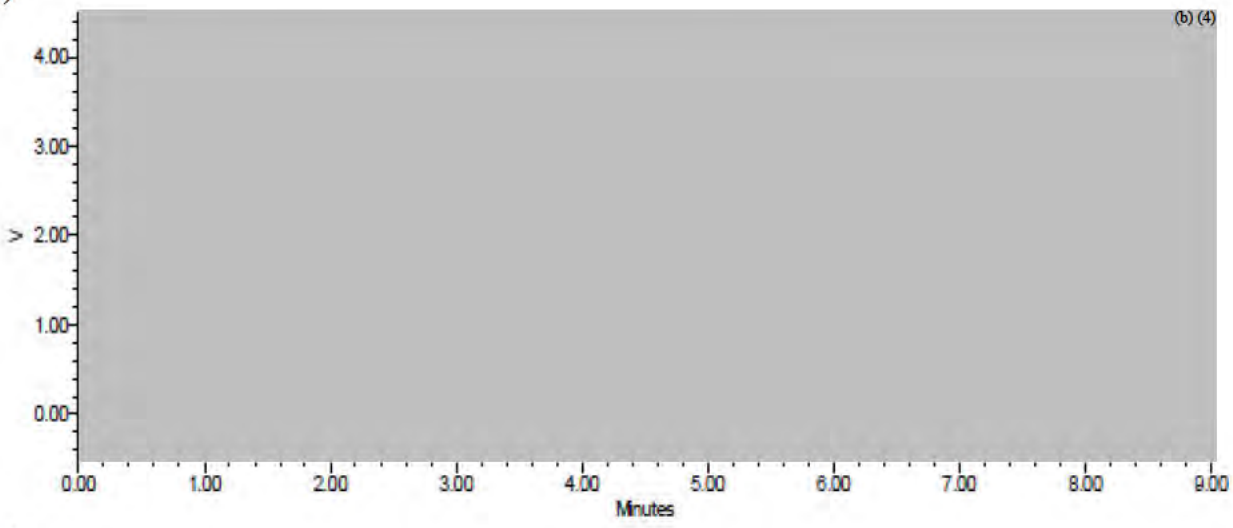
(d)


(b) (4)
4.00
3.00
V 2.00
1.00
0.00
0.00 1.00 2.00 3.00 4.00 5.00 6.00 7.00 8.00 9.00
Minutes

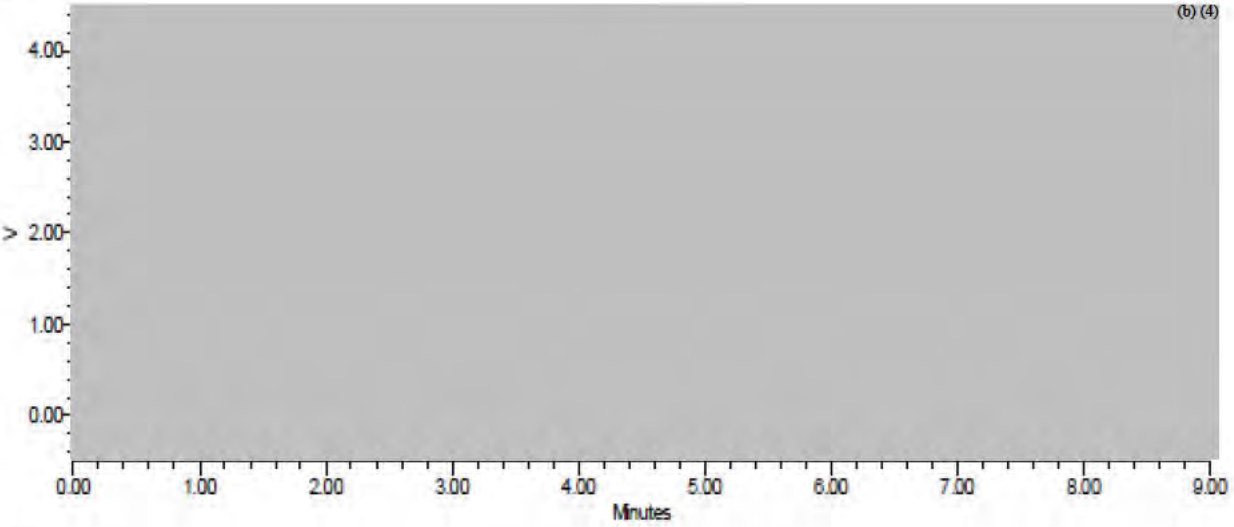
(e)


(b) (4)
4.00
3.00
V 2.00
1.00
0.00
0.00 1.00 2.00 3.00 4.00 5.00 6.00 7.00 8.00 9.00
Minutes

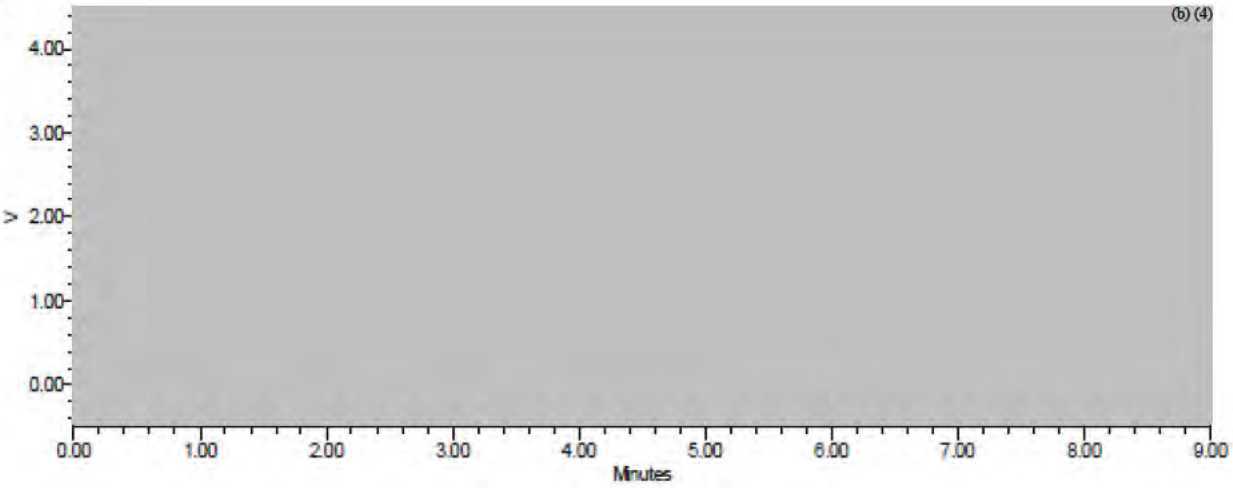
(f)


(b) (4)
4.00
3.00
> 2.00
1.00
0.00
0.00 1.00 2.00 3.00 4.00 5.00 6.00 7.00 8.00 9.00
Minutes

(g)


(b) (4)
4.00
3.00
> 2.00
1.00
0.00
0.00 1.00 2.00 3.00 4.00 5.00 6.00 7.00 8.00 9.00
Minutes

(h)


(b) (4)
4.00
3.00
> 2.00
1.00
0.00
0.00 1.00 2.00 3.00 4.00 5.00 6.00 7.00 8.00 9.00
Minutes

(i)

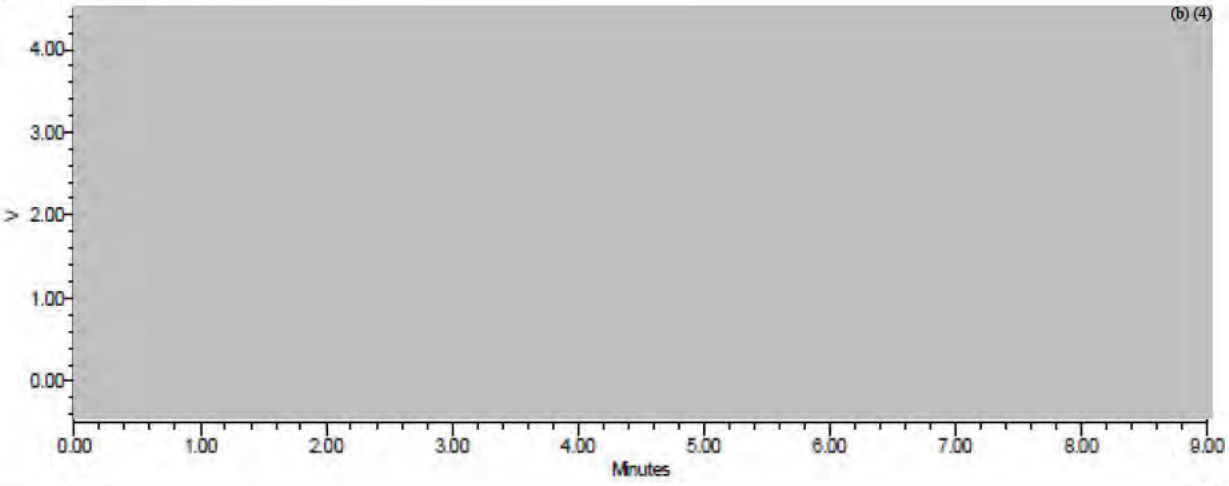


Figure 4. (b) (4)

11. Linearity

(b) (4)

Table 10. Calibration curve

	L-Valine (g/L)	Peak area*
STD 1 (25%)	(b) (4)	
STD 2 (50%)		
STD 3 (80%)		
STD 4 (100%)		
STD 5 (120%)		

* Mean area of triplet injection

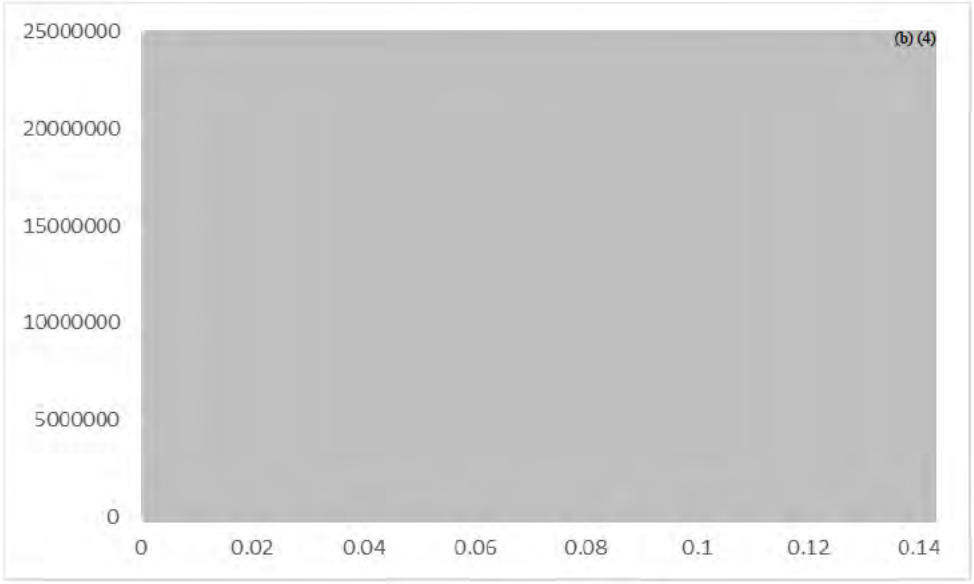
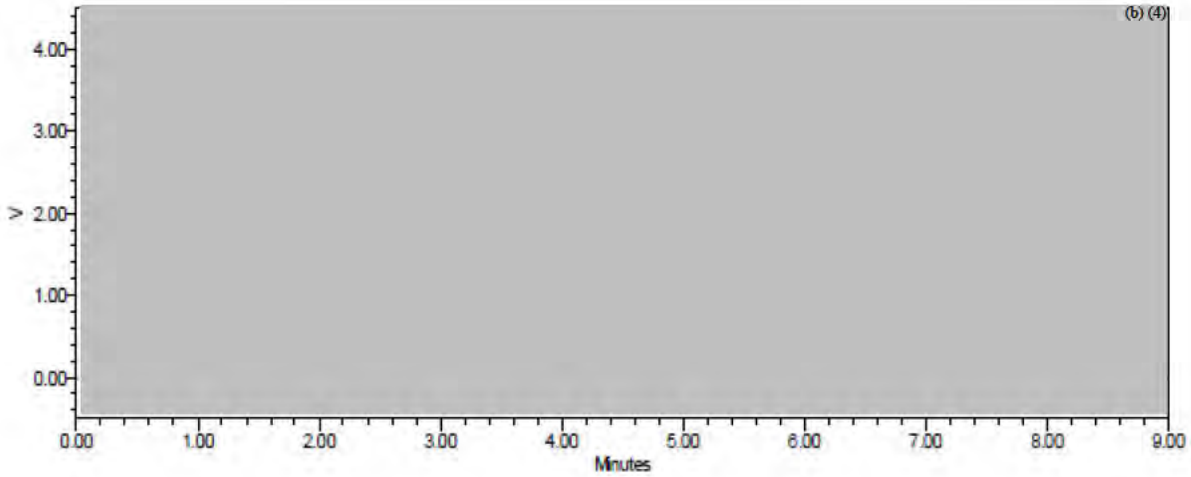
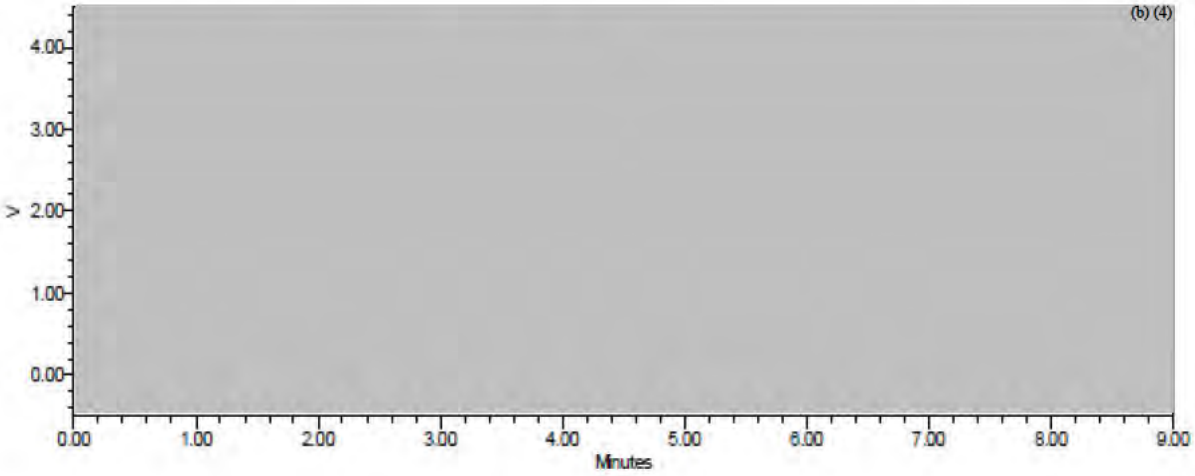


Figure 5. Calibration curve

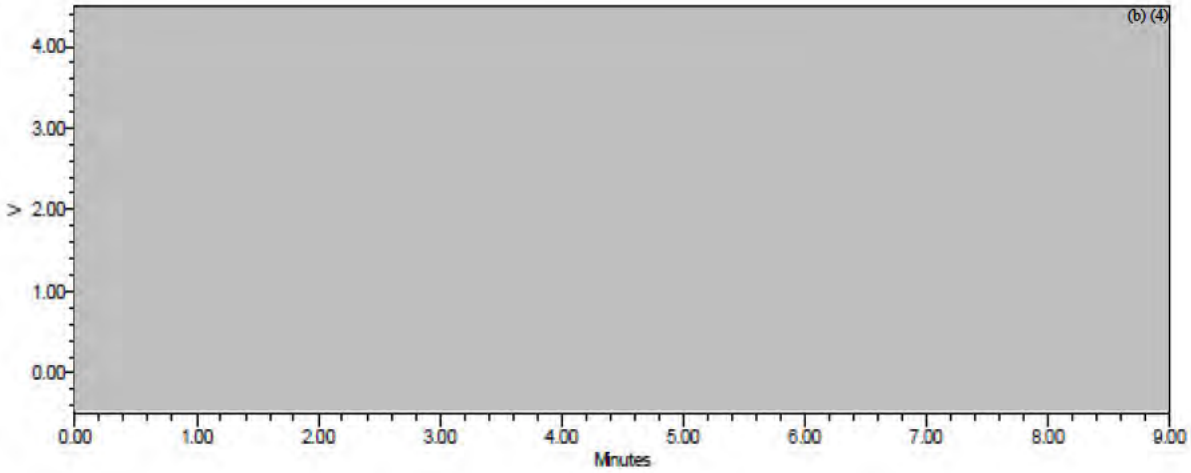
(a)



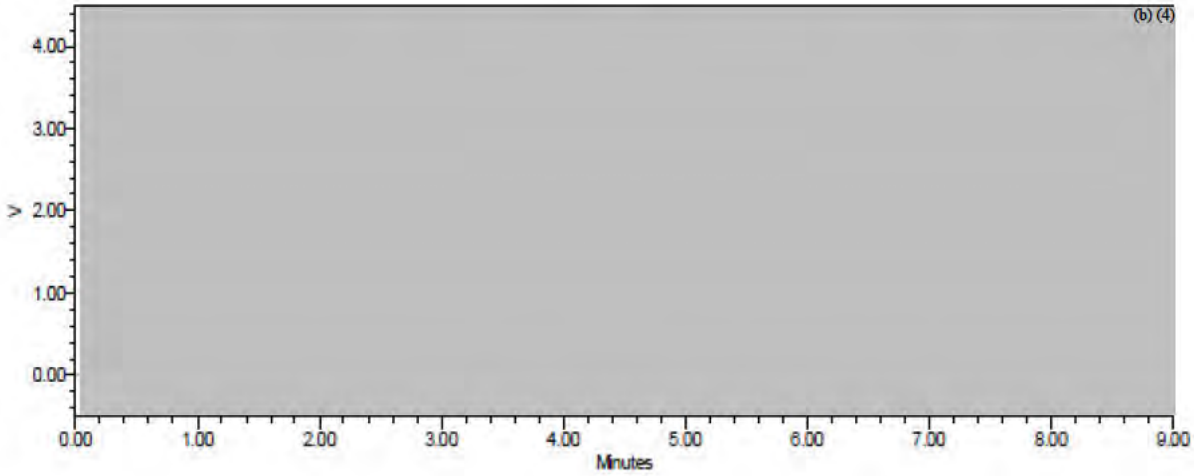
(b)



(c)


(b) (4)
4.00
3.00
> 2.00
1.00
0.00
0.00 1.00 2.00 3.00 4.00 5.00 6.00 7.00 8.00 9.00
Minutes

(d)


(b) (4)
4.00
3.00
> 2.00
1.00
0.00
0.00 1.00 2.00 3.00 4.00 5.00 6.00 7.00 8.00 9.00
Minutes

(e)

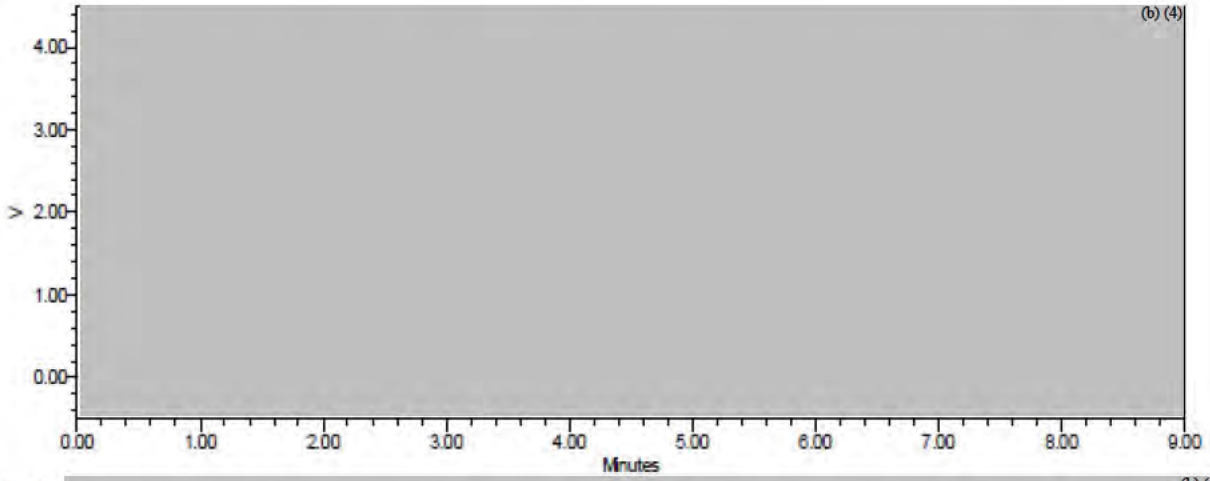
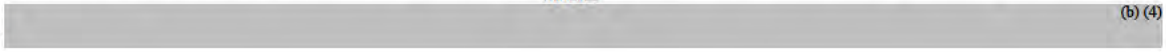

(b) (4)
4.00
3.00
> 2.00
1.00
0.00
0.00 1.00 2.00 3.00 4.00 5.00 6.00 7.00 8.00 9.00
Minutes

Figure 6.


(b) (4)

12. Limit of detection and limit of quantification

(b) (4)

(b) (4)

(b) (4)

12.1. LOD and LOQ of L-valine

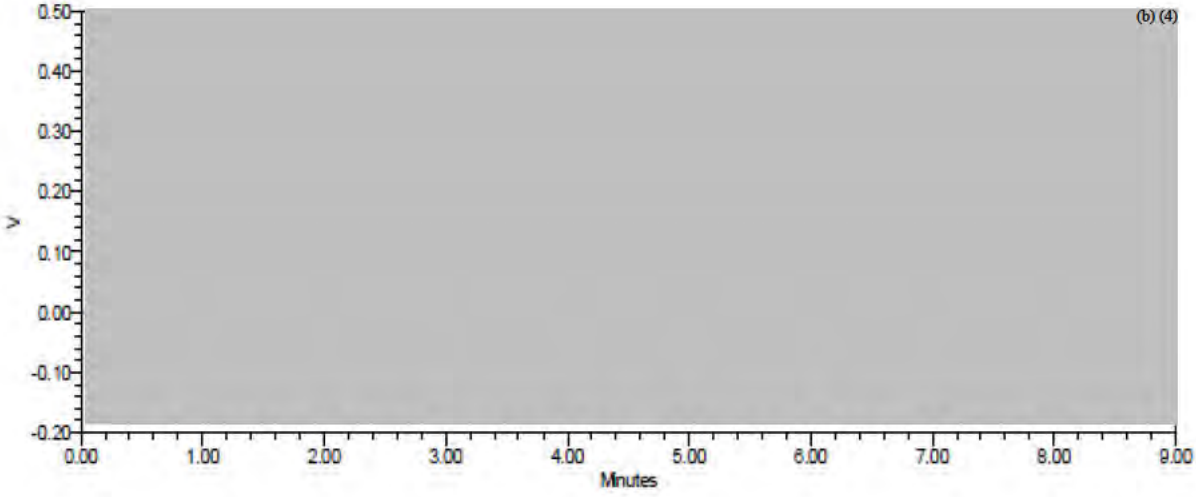
Table 11. .Summary output for regression analysis study

Regression statistics	
Multiple R (Correlation coefficient)	(b) (4)
R Square (Coefficient of determination)	(b) (4)

Adjusted R Square	(b) (4)
Standard Error (Residual standard deviation)	(b) (4)
Observations	(b) (4)

(b) (4)

(a)



(b)

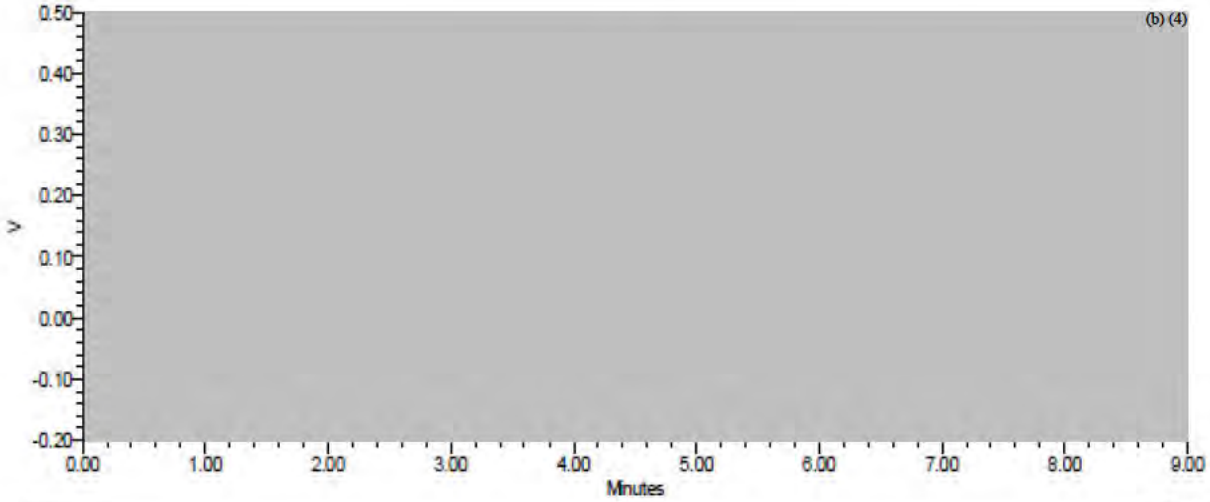


Figure 7. (b) (4)

13. Precision

(b) (4)

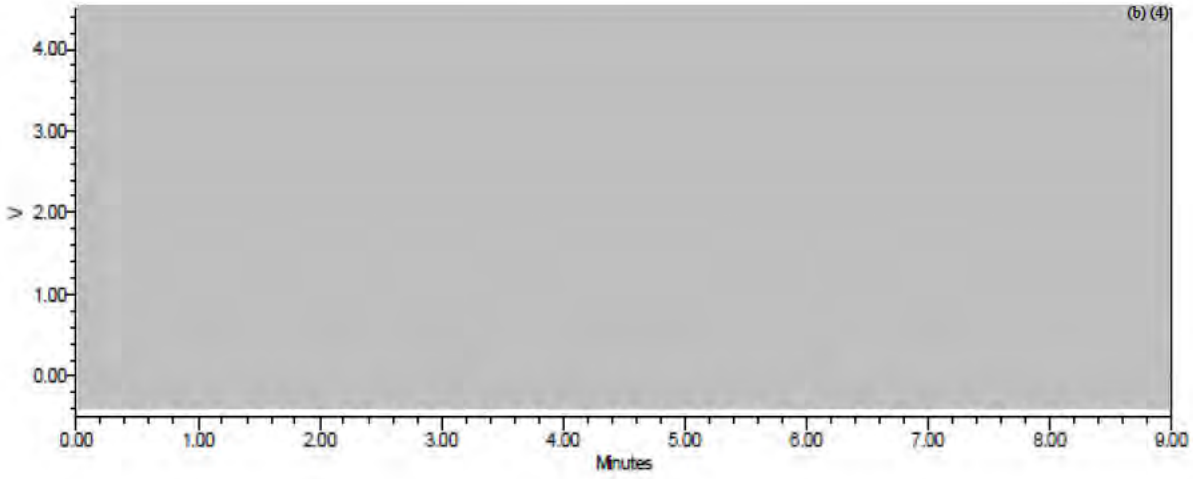
Table 12. Repeated injection of sample solution

	Sample solution
1	(b) (4)
2	
3	
4	
5	
6	
7	
8	
9	
10	
%RSD	0.17 %

Table 13. Repeated injection of CRM solution

	CRM solution
1	(b) (4)
2	
3	
4	
5	
6	
7	
8	
9	
10	
%RSD	0.12 %

(a)



(b)

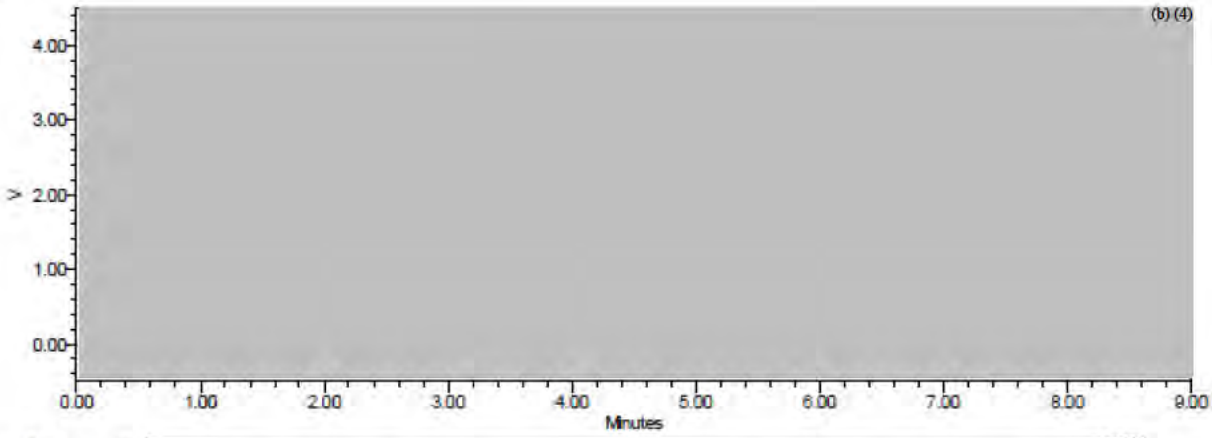


Figure 8. (b) (4)

14. Accuracy

(b) (4)

(b) (4)

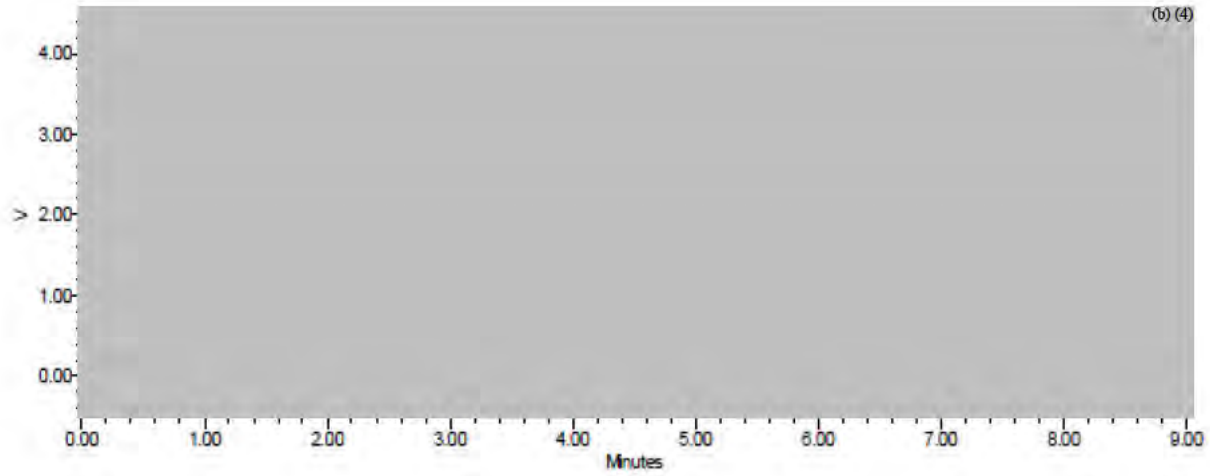
(b) (4)

(b) (4)

(b) (4)

(b) (4)

(a)


(b) (4)
4.00
3.00
> 2.00
1.00
0.00
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
9.00
Minutes

(b)

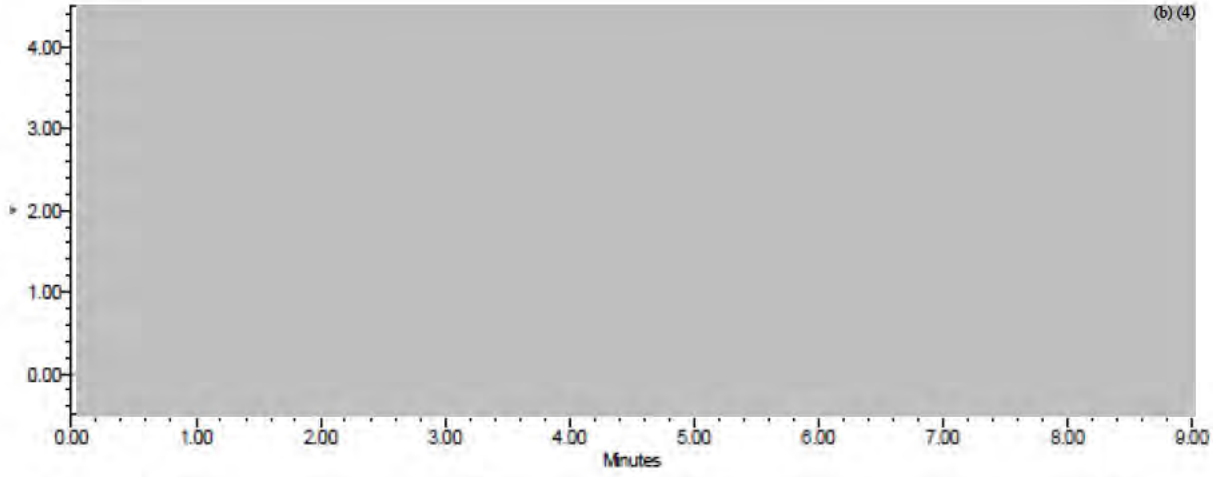


Figure 9. (b) (4)

14.1. Summary of uncertainty measurement

Mathematical model

(b) (4)

Table 14. Uncertainty measurement

Uncertainty contributor	Measurement value	Standard uncertainty	Relative standard uncertainty	Effective degree of freedom	Type	Probability distribution
14.2.1. Standard preparation	(b) (4)					
14.2.1.1. Uncertainty in weight determination						
1) Dispersion in repeated measurements						
2) Uncertainty of Balance calibration result						
14.2.1.2. Volumetric measuring						

1) Dispersion in repeated measurements	(b) (4)
2) Uncertainty of balance calibration result	
3) Uncertainty of 1000 mL volumetric flask calibration result	
14.2.2. Sample preparation	
14.2.2.1. Uncertainty in sample weight determination	
1) Dispersion in repeated measurements	
2) Uncertainty of balance calibration result	
14.2.2.2. Volumetric measuring	
1) Dispersion in repeated measurements	
2) Uncertainty of balance calibration result	
3) Uncertainty of 250 mL volumetric flask calibration result	
14.2.3. Precision of the instrument	
14.2.3.1. Uncertainty of dispersion in the standard solution repeated measurement	
14.2.3.2. Uncertainty of dispersion in the sample solution repeated measurement	

Relative combined standard uncertainty	(b) (4)
Effective degree of freedom	
Coverage factor *k*	
Expanded uncertainty	
Results	

14.2. Uncertainty measurement

14.2.1. Standard preparation

14.2.1.1. Uncertainty in weight determination

1) Dispersion in repeated measurements

Type A uncertainty

Number of sample measurements	Measurement value (g)
1	(b) (4)
2	
3	
4	
5	
Measurement value	
standard deviation	
standard uncertainty	
relative standard uncertainty	
degree of freedom	

- Standard uncertainty = (b) (4)

- Relative standard uncertainty = (b) (4)

- A Type degree of freedom = (b) (4)

2) Uncertainty of balance calibration result

Type B uncertainty

Balance	Mass (g)	Uncertainty	Standard uncertainty	Relative standard uncertainty
	0.10004	0.0005	(b) (4)	
Standard uncertainty			(b) (4)	
Relative standard uncertainty				
Degree of freedom				

(b) (4)

3) Relative combined standard uncertainty

(b) (4)

4) Effective degree of freedom

(b) (4)

14.2.1.2. Volumetric measuring

1) Dispersion in repeated measurements

Type A uncertainty

Number of sample measurements	Measurement value (g)
1	(b) (4)
2	(b) (4)

3	(b) (4)
4	(b) (4)
5	(b) (4)
Measurement value	(b) (4)
Standard deviation	(b) (4)
Standard uncertainty	(b) (4)
Relative standard uncertainty	(b) (4)
Degree of freedom	(b) (4)

(b) (4)

2) Uncertainty of balance calibration result

Type B uncertainty

Balance	Mass (g)	Uncertainty	Standard uncertainty	Relative standard uncertainty
	1246.33	0.0200	(b) (4)	(b) (4)
Standard uncertainty			(b) (4)	
Relative standard uncertainty				
Degree of freedom				

(b) (4)

3) Uncertainty of 1000 mL volumetric flask calibration result

Type B uncertainty

Volumetric flask	Volume (mL)	Uncertainty	Standard uncertainty	Relative standard uncertainty
	1000	0.220	(b) (4)	(b) (4)
Standard uncertainty			(b) (4)	
Relative standard uncertainty				
Degree of freedom				

(b) (4)

4) Relative combined standard uncertainty

(b) (4)

5) Effective degree of freedom

(b) (4)

14.2.1.4. Effective degree of freedom of standard preparation

(b) (4)

14.2.2. Sample preparation

14.2.2.1. Uncertainty in sample weight determination

1) Dispersion in repeated measurements

Type A uncertainty

Number of sample measurements	Measurement value (g)
1	(b) (4)
2	(b) (4)
3	(b) (4)
4	(b) (4)
5	(b) (4)
Measurement value	(b) (4)
Standard deviation	(b) (4)
Standard uncertainty	(b) (4)
Relative standard uncertainty	(b) (4)
Degree of freedom	(b) (4)

(b) (4)

2) Uncertainty of balance calibration result

Type B uncertainty

Balance	Mass (g)	Uncertainty	Standard uncertainty	Relative standard uncertainty
	0.10052	0.0005	(b) (4)	(b) (4)

Standard uncertainty	(b) (4)
Relative standard uncertainty	
Degree of freedom	

(b) (4)

3) Relative combined standard uncertainty

(b) (4)

4) Effective degree of freedom

(b) (4)

14.2.2.2. Volumetric measuring

1) Dispersion in repeated measurements

Type A uncertainty

Number of sample measurements	Measurement value (g)
1	(b) (4)
2	(b) (4)
3	(b) (4)
4	(b) (4)
5	(b) (4)
Measurement value	(b) (4)
Standard deviation	(b) (4)

Standard uncertainty	(b) (4)
Relative standard uncertainty	(b) (4)
Degree of freedom	(b) (

(b) (4)

2) Uncertainty of balance calibration result

Type B uncertainty

Balance	Mass (g)	Uncertainty	Standard uncertainty	Relative standard uncertainty
	1246.33	0.0200	(b) (4)	(b) (4)
Standard uncertainty			(b) (4)	
Relative standard uncertainty				
Degree of freedom				

(b) (4)

3) Uncertainty of 1000 mL volumetric flask calibration result

Type B uncertainty

Volumetric flask	Volume (mL)	Uncertainty	Standard uncertainty	Relative standard uncertainty
	1000.00	0.220	0.110	0.011
Standard uncertainty			(b) (4)	
Relative standard uncertainty			(b) (4)	

Degree of freedom	(b) (4)

(b) (4)

4) Relative combined standard uncertainty

(b) (4)

5) Effective degree of freedom

(b) (4)

14.2.2.4. Effective degree of freedom of sample preparation

(b) (4)

14.2.3. Precision of the instrument

14.2.3.1. Uncertainty of dispersion in the standard solution repeated measurement

Type A uncertainty

Number of sample measurements	Peak area

1	(b) (4)
2	(b) (4)
3	(b) (4)
4	(b) (4)
Measurement value	(b) (4)
Standard deviation	(b) (4)
Standard uncertainty	(b) (4)
Relative standard uncertainty	(b) (4)
Degree of freedom	(b) (4)

(b) (4)

14.2.3.2. Uncertainty of dispersion in the sample solution repeated measurement

Type A uncertainty

Number of sample measurements	Peak area
1	(b) (4)
2	(b) (4)
3	(b) (4)
Measurement value	(b) (4)
Standard deviation	(b) (4)
Standard uncertainty	(b) (4)
Relative standard uncertainty	(b) (4)
Degree of freedom	(b) (4)

(b) (4)

14.2.3.3. Relative combined standard uncertainty of precision of the instrument

(b) (4)

14.2.3.4. Effective degree of freedom of precision of the instrument

(b) (4)

14.2.4. Relative combined standard uncertainty of valine analysis

(b) (4)

14.2.5. Effective degree of freedom of valine analysis

(b) (4)

14.2.6. Expanded uncertainty (U)

(b) (4)

14.2.7. Result

(b) (4)

15. Robustness

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

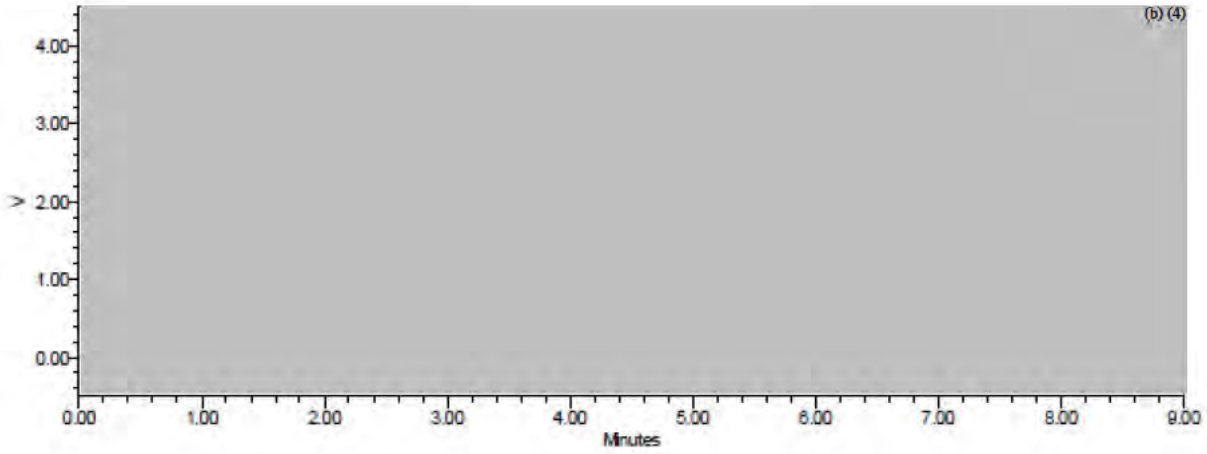
(b) (4)

(b) (4)

Table 15. Data of robustness test

Factor	L-valine (%)	Recovery (%)	Retention time of L-valine	Average peak area of standard	Average peak area of sample
Standard condition	(b) (4)			15915610	15782377
35°C				12291928	12330444
45°C				18942060	18912625
0.8 mL/min				26135362	26103728
1.2 mL/min				8381739	8388608
CH_3CN 9%				14742519	14717692
CH_3CN 15%				15232148	15206820
pH 2.3				4614004	4589815
pH 2.7				19682457	19674538

(a)



(b)

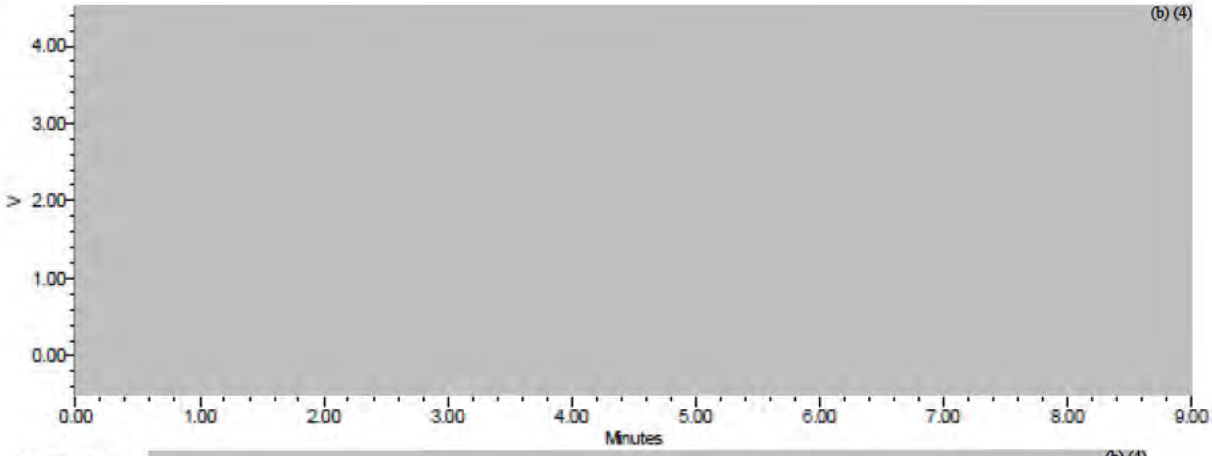
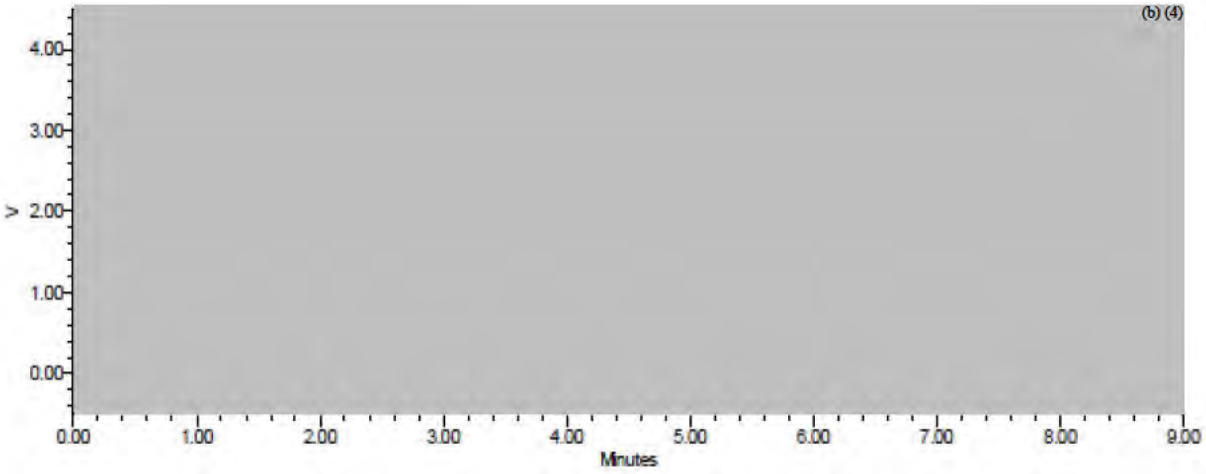


Figure 10.

(a)



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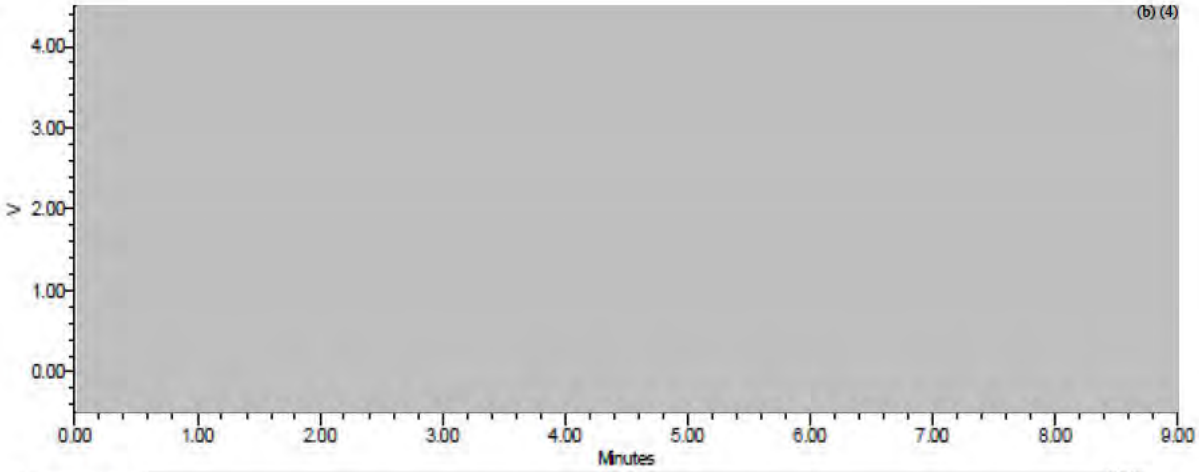
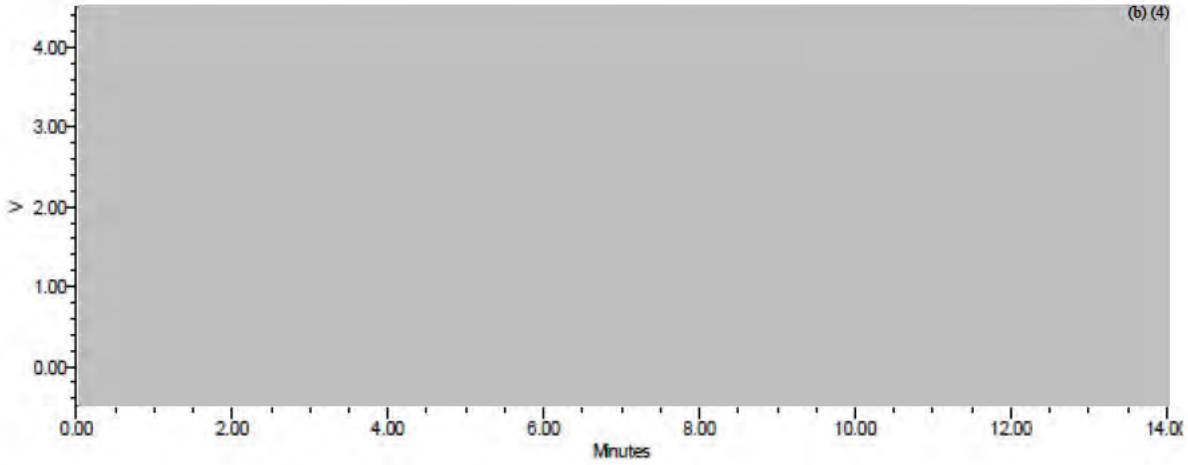


Figure 11.

(a)



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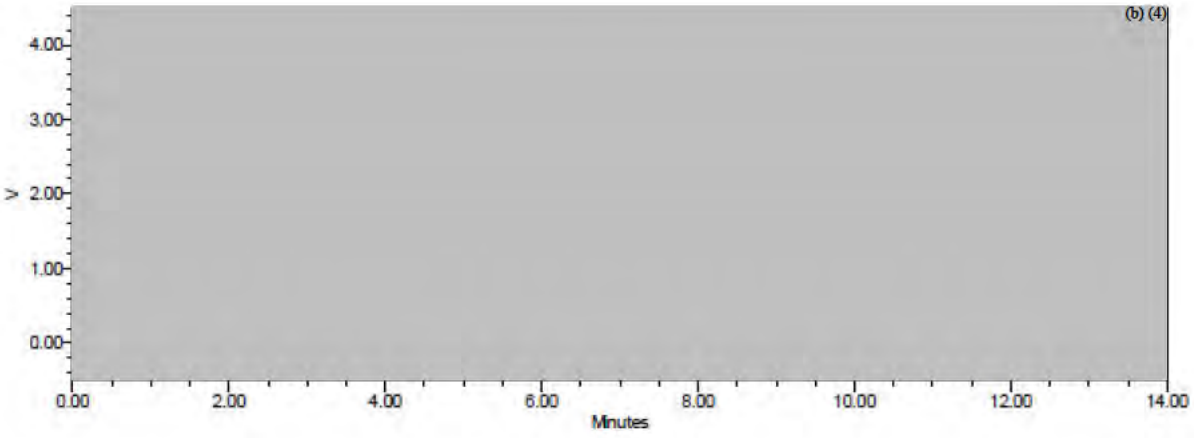
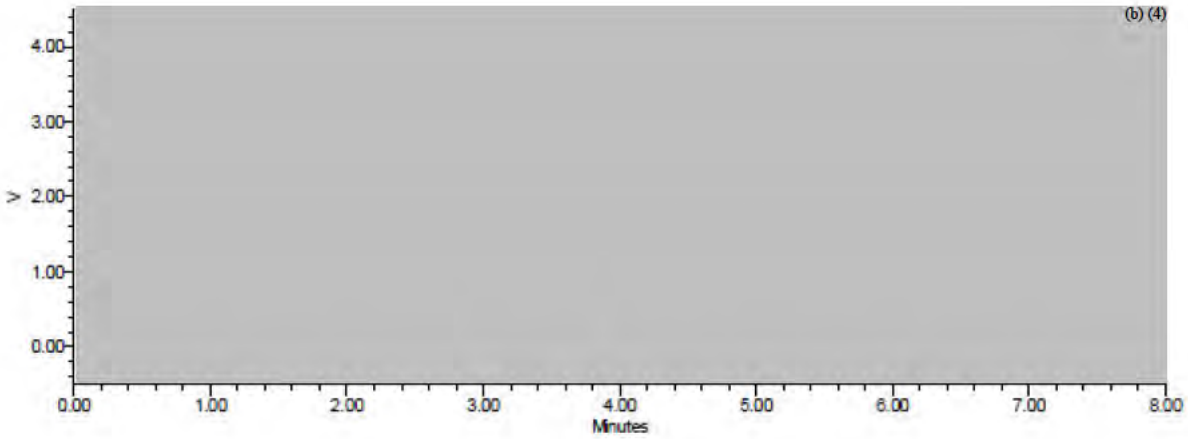


Figure 12. (b) (4)

(a)



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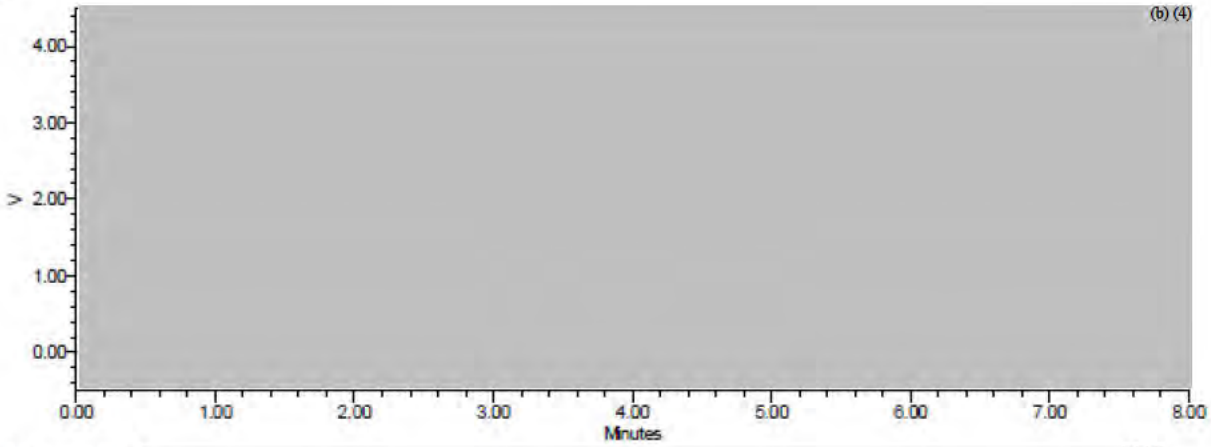
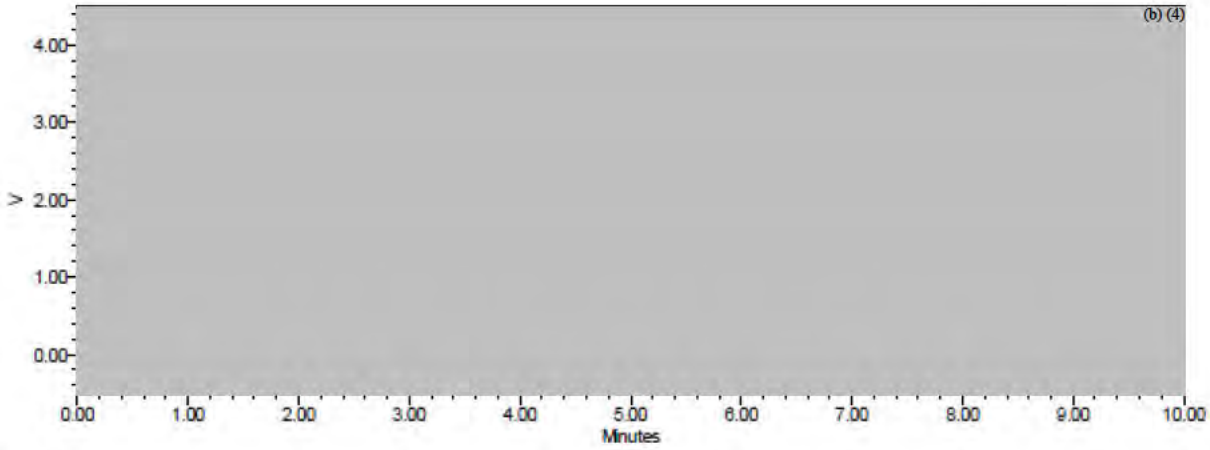


Figure 13. (b) (4)

(a)



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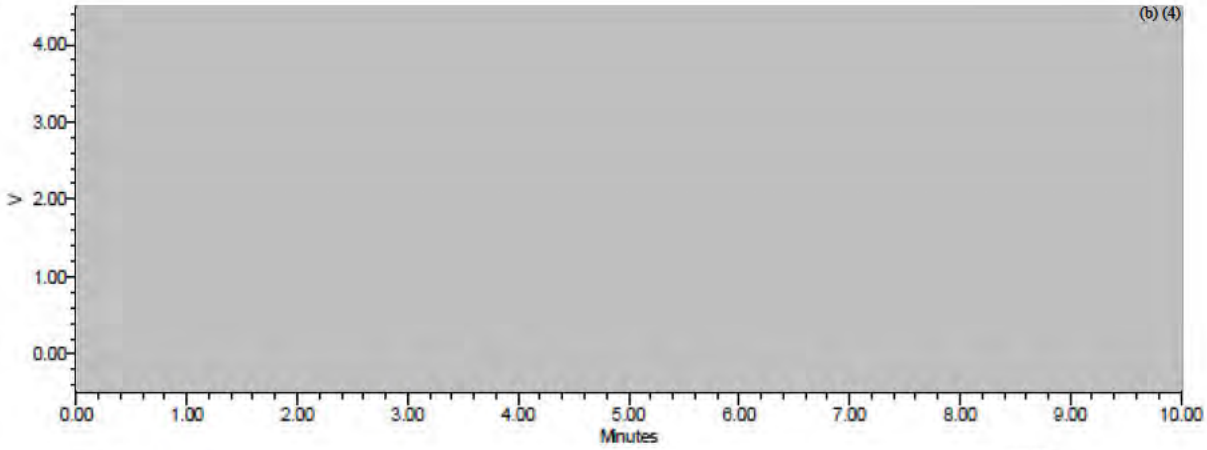
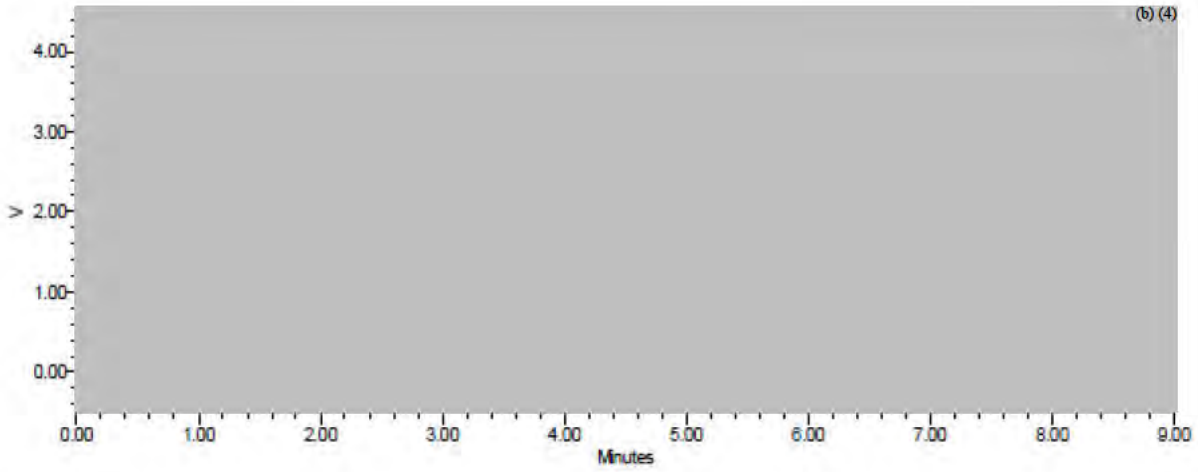


Figure 14. (b) (4)

(a)



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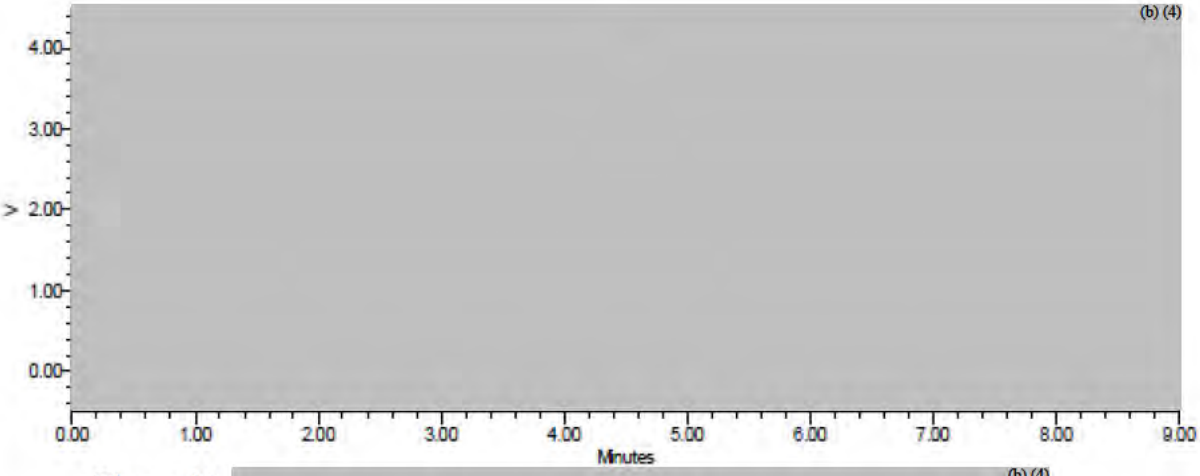
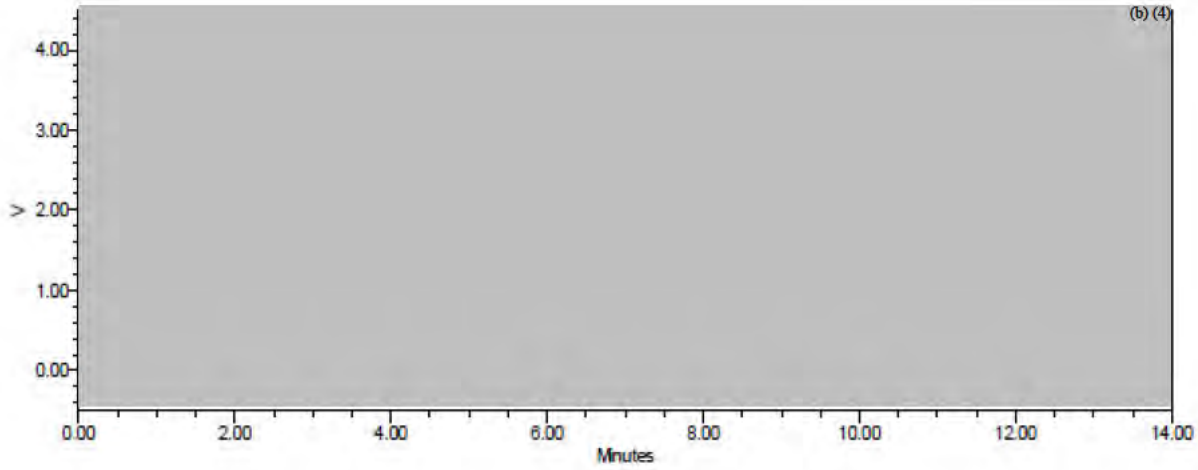


Figure 15. [redacted]

(a)



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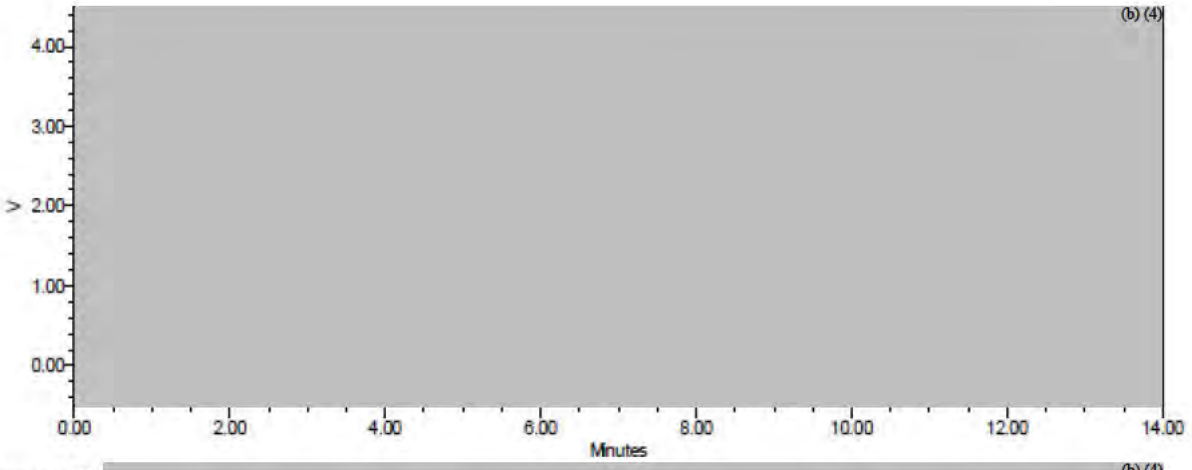
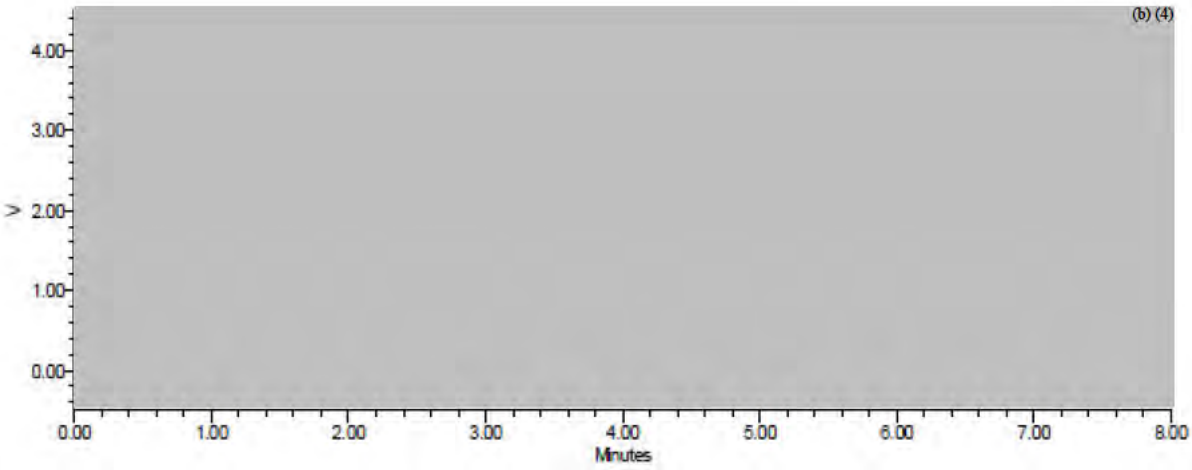


Figure 16.

(a)



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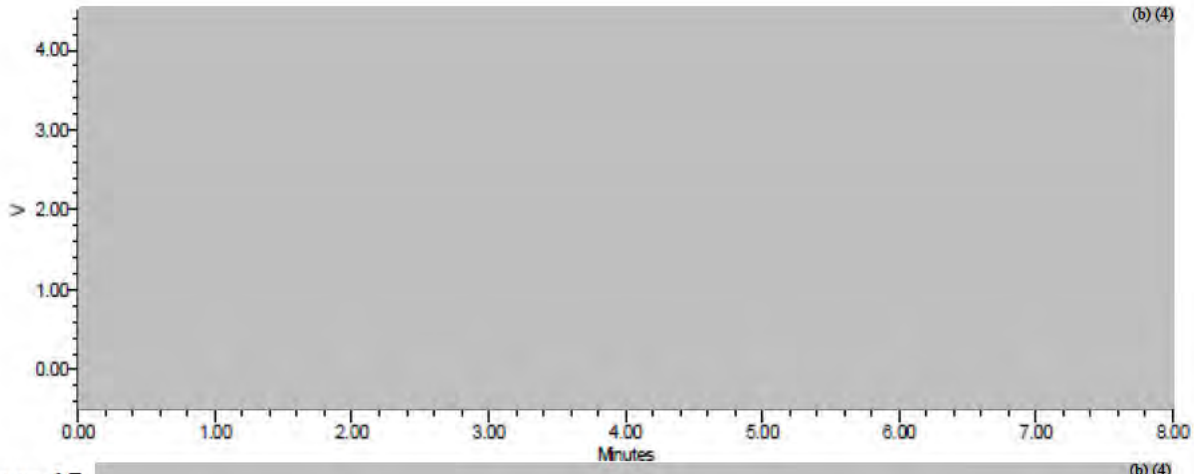


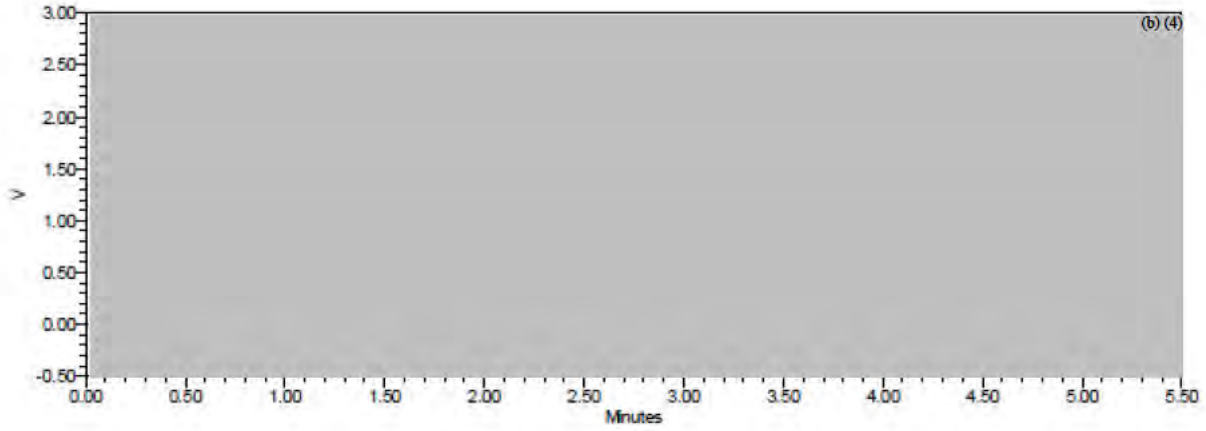
Figure 17.

16. Impurity identification

(b) (4)

(b) (4)

(a)



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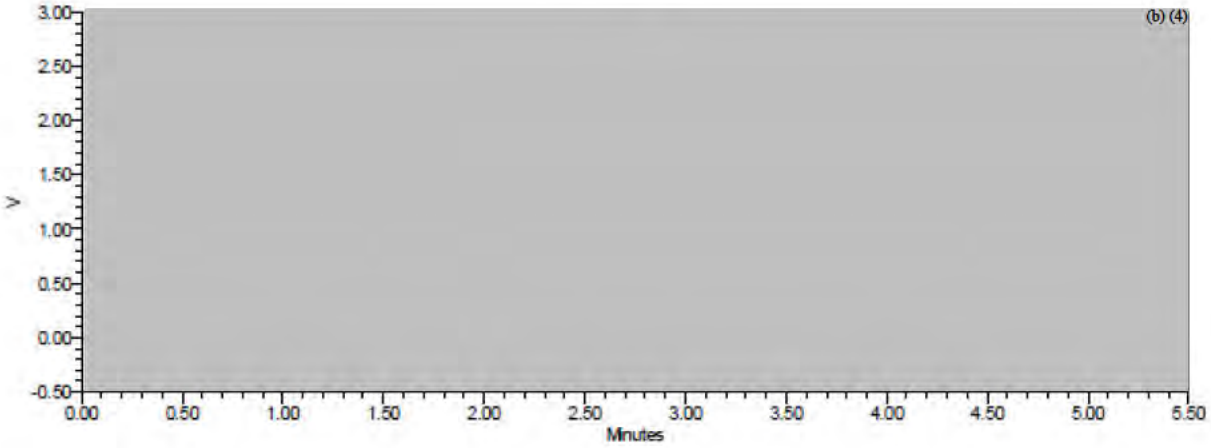

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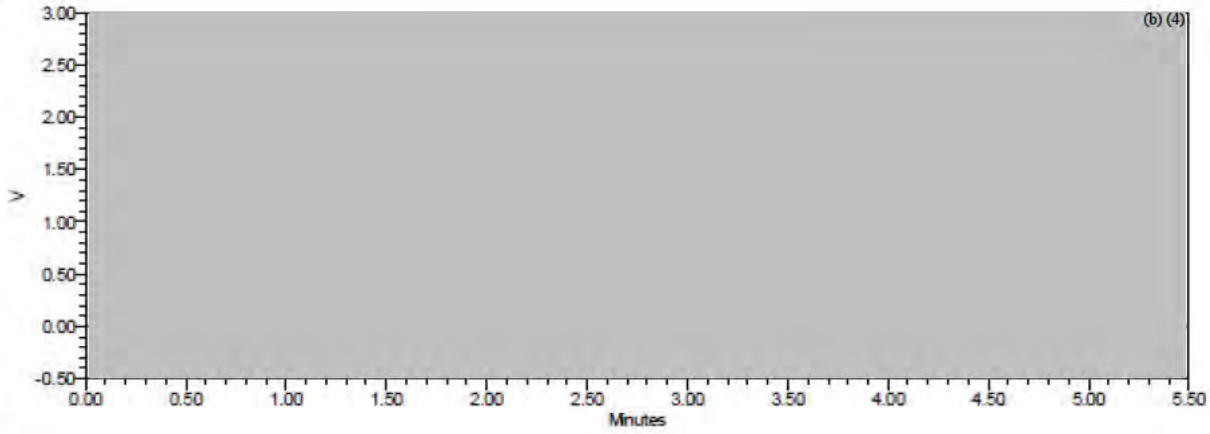
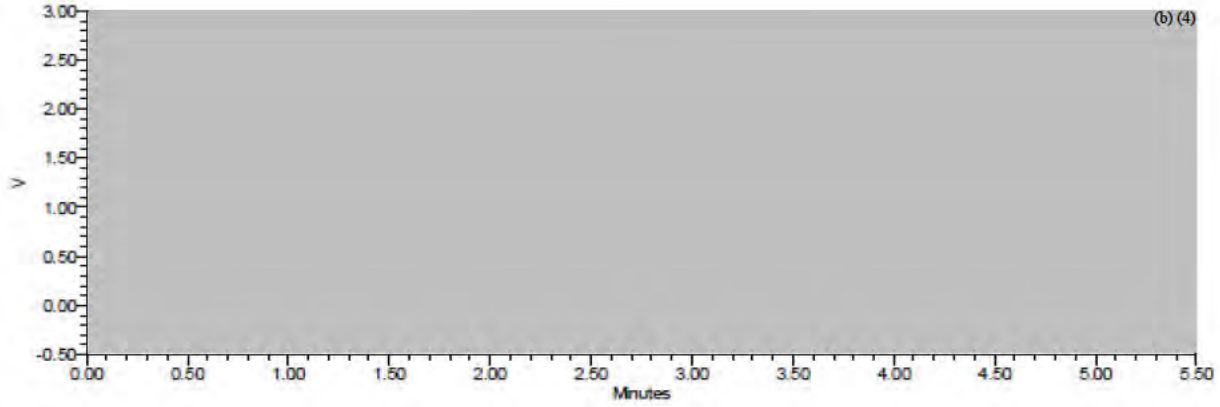


(d)



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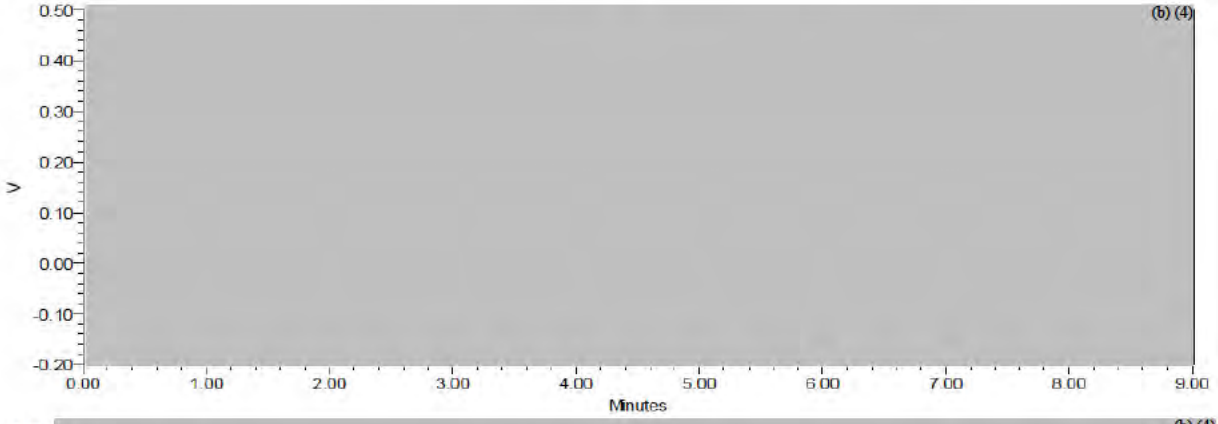
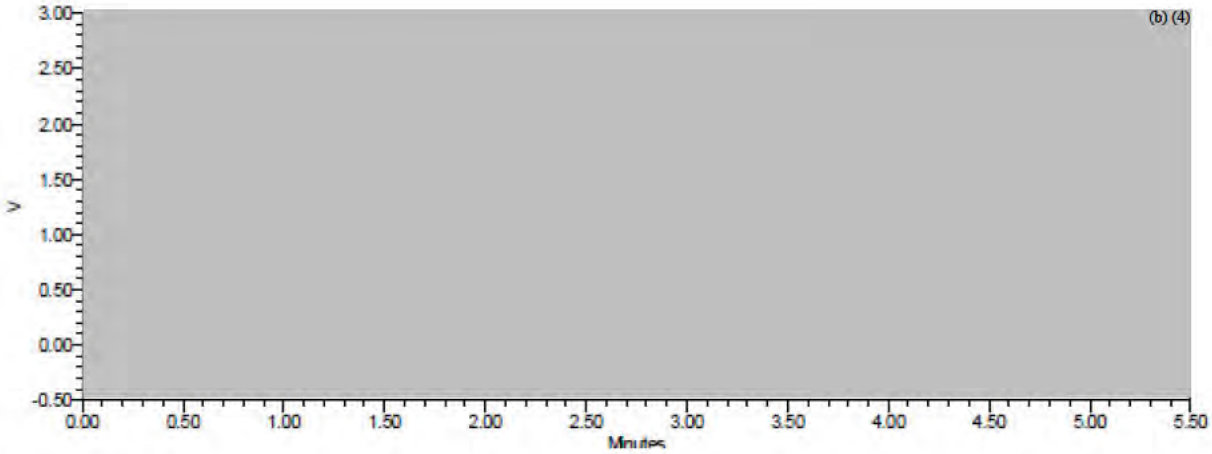
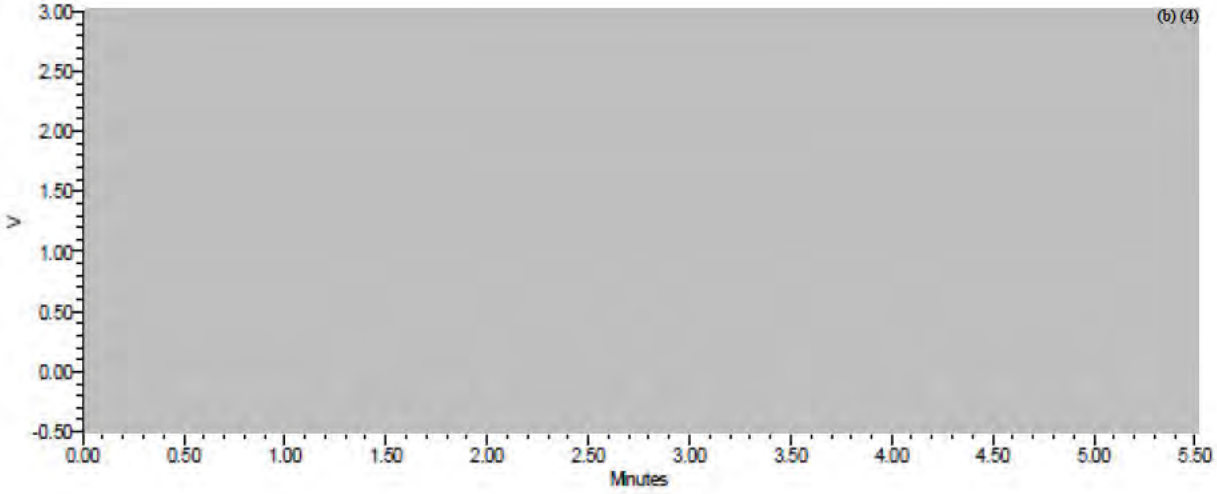


Figure 18.

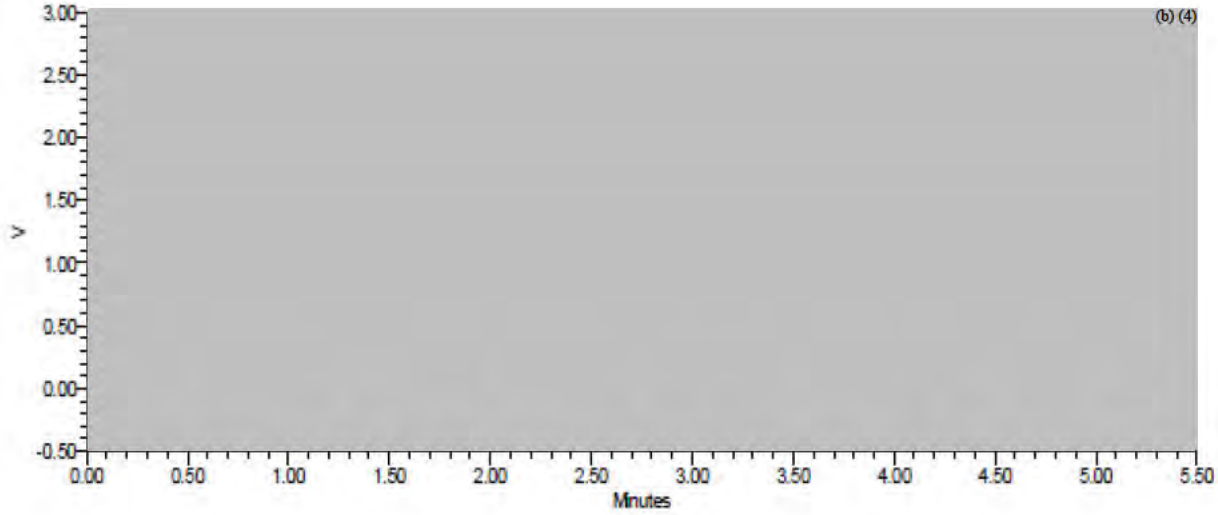
(a)


(b) (4)
V
3.00
2.50
2.00
1.50
1.00
0.50
0.00
-0.50
0.00 0.50 1.00 1.50 2.00 2.50 3.00 3.50 4.00 4.50 5.00 5.50
Minutes

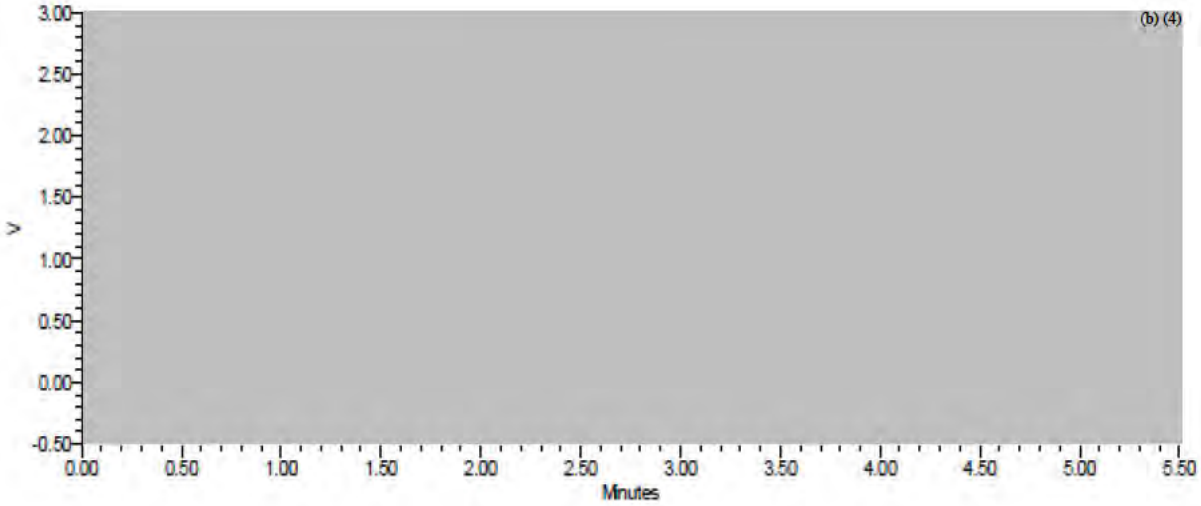
(b)


(b) (4)
V
3.00
2.50
2.00
1.50
1.00
0.50
0.00
-0.50
0.00 0.50 1.00 1.50 2.00 2.50 3.00 3.50 4.00 4.50 5.00 5.50
Minutes

(c)


(b) (4)
V
3.00
2.50
2.00
1.50
1.00
0.50
0.00
-0.50
0.00 0.50 1.00 1.50 2.00 2.50 3.00 3.50 4.00 4.50 5.00 5.50
Minutes

(d)



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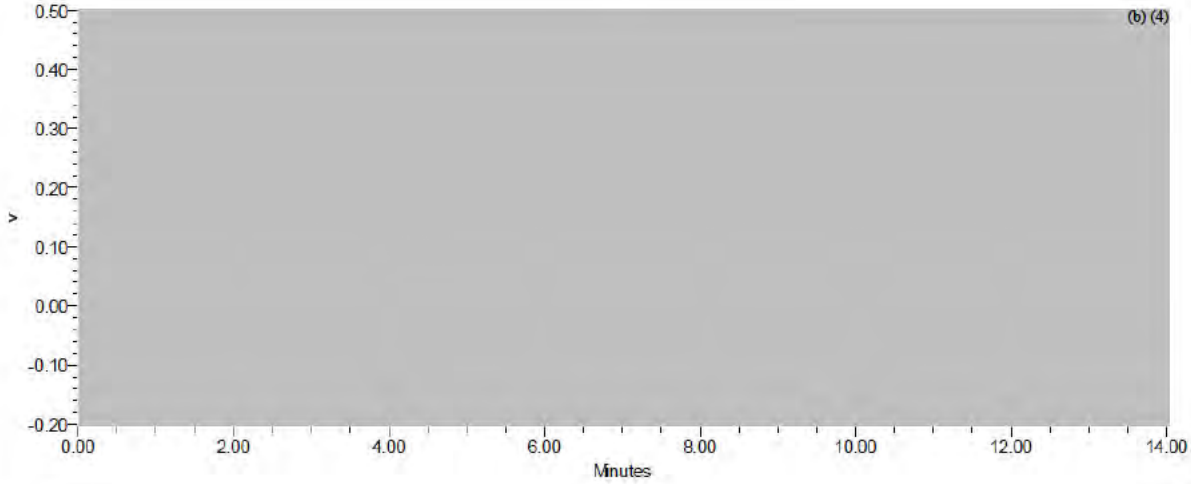


Figure 19. [redacted]

17. Conclusion

Table 16. Summary of validation test

Specificity	(b) (4)	There is no interference to peak response by diluent.
System Suitability		· %RSD < 1%
Homogeneity of sample		· %RSD < 1%
Stability of the sample		· Recovery 98% ~ 102%
		· %RSD < 1%
Linearity		· $R^2 > 0.9990$
Limit of Detection and Limit of Quantification		-
Precision		· %RSD < 1%
Accuracy		· $\|En\| \leq 1$
Robustness		· Recovery 98% ~ 102%
This validation results confirmed that all of the results were suitable for the reference value and that the analytical method could be used for rapid and accurate L-valine analysis.		

18. Raw data file

	Data file name
Specificity	(b) (4)
System Suitability	
Homogeneity of sample	
Stability of the sample	
Linearity	
Limit of Detection and Limit of Quantification	
Precision	
Accuracy	
Robustness	

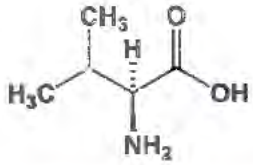
Certificate of Analysis

ISO 17034
ANAB Cert# AR-1470

ISO/IEC 17025
ANAB Cert# AT-1467

L-VALINE

CERTIFIED REFERENCE MATERIAL



CERTIFIED PURITY: 98.9%, $U_{cm} = \pm 0.07\%$ k = 2.07
(Mass Balance/as is basis)

NOMINAL PACKAGE SIZE: 1g

CATALOG #: PHR1172 **LOT #:** LRAC2856

CERTIFICATE VERSION: LRAC2856.1 **ISSUE DATE:** 22 May 2019

Note: Certificates may be updated due to Pharmacopeial Lot changes or the availability of new data. Check our website at: (b) (4) *for the most current version.*

CRM EXPIRATION: 31 May 2023 (Proper Storage and Handling Required).

RECEIPT DATE: _____
Note: this space is provided for convenience only and its use is not required.

STORAGE: Store at Room Temperature, keep container tightly closed. Attachment of a 20 mm aluminum crimp seal recommended for unused portions.

CHEMICAL FORMULA: $C_5H_{11}NO_2$ **MW:** 117.15

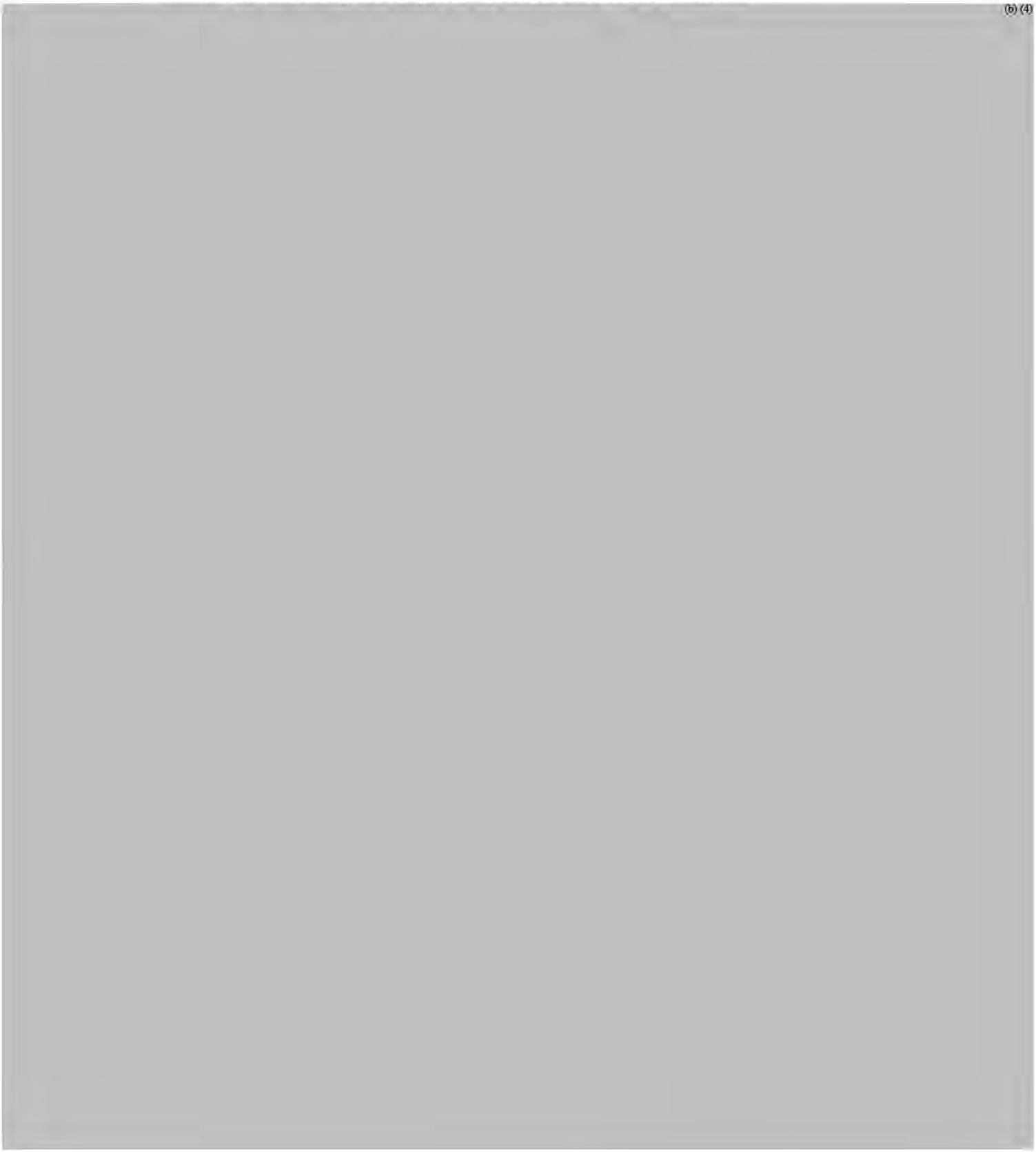
PHYSICAL DESCRIPTION: White powder in amber vial **CAS #:** 72-18-4

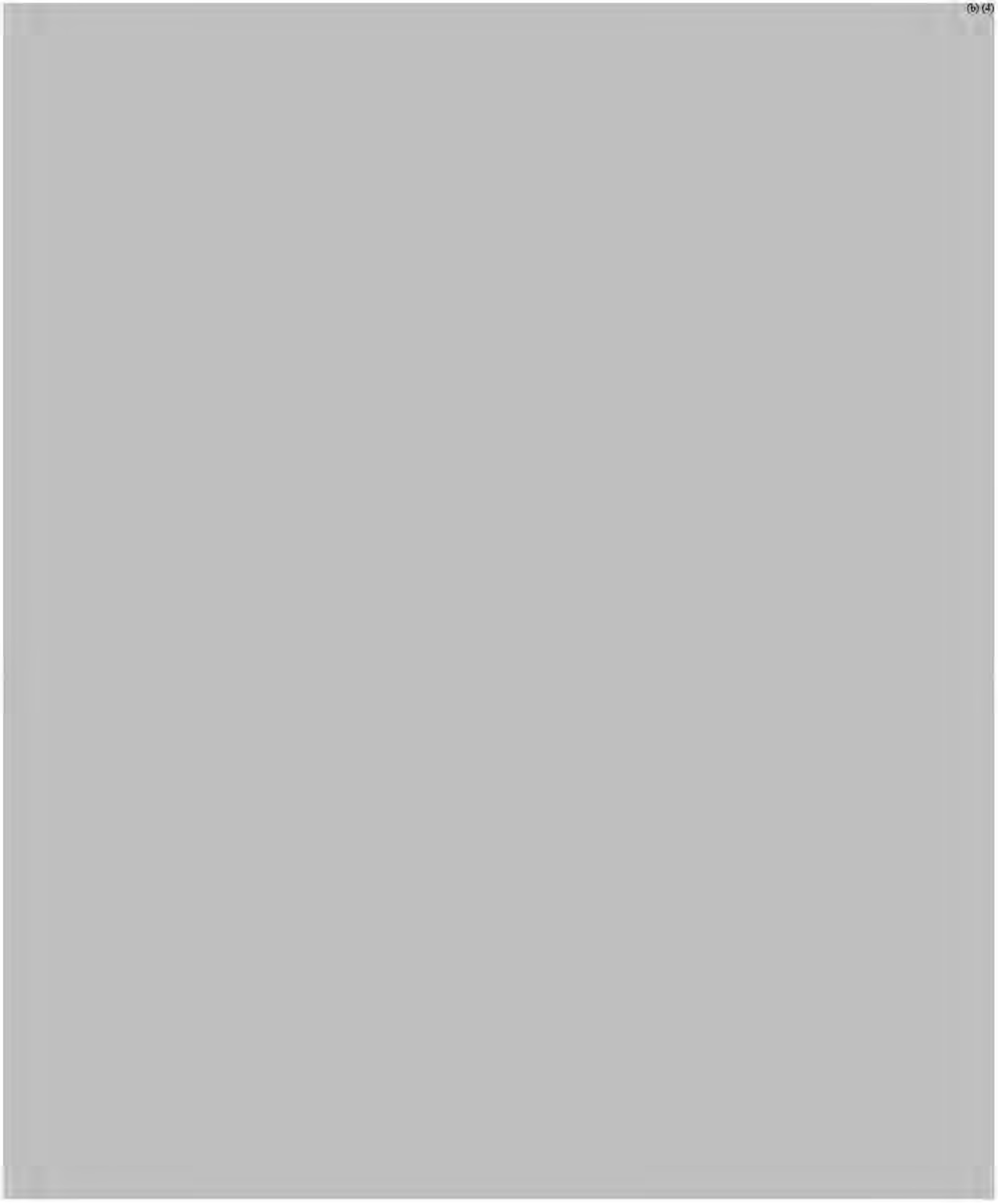
HAZARDS: Read Safety Data Sheet before using. All chemical reference materials should be considered potentially hazardous and should be used only by qualified laboratory personnel.

(b) (4)

INSTRUCTIONS FOR USE: Do not dry, use on the as is basis. The internal pressure of the container may be slightly different from the atmospheric pressure at the user's location. Open slowly and carefully to avoid dispersion of the material. This material is intended for Laboratory Use only. Not for drug, household or other uses.

(b) (4)

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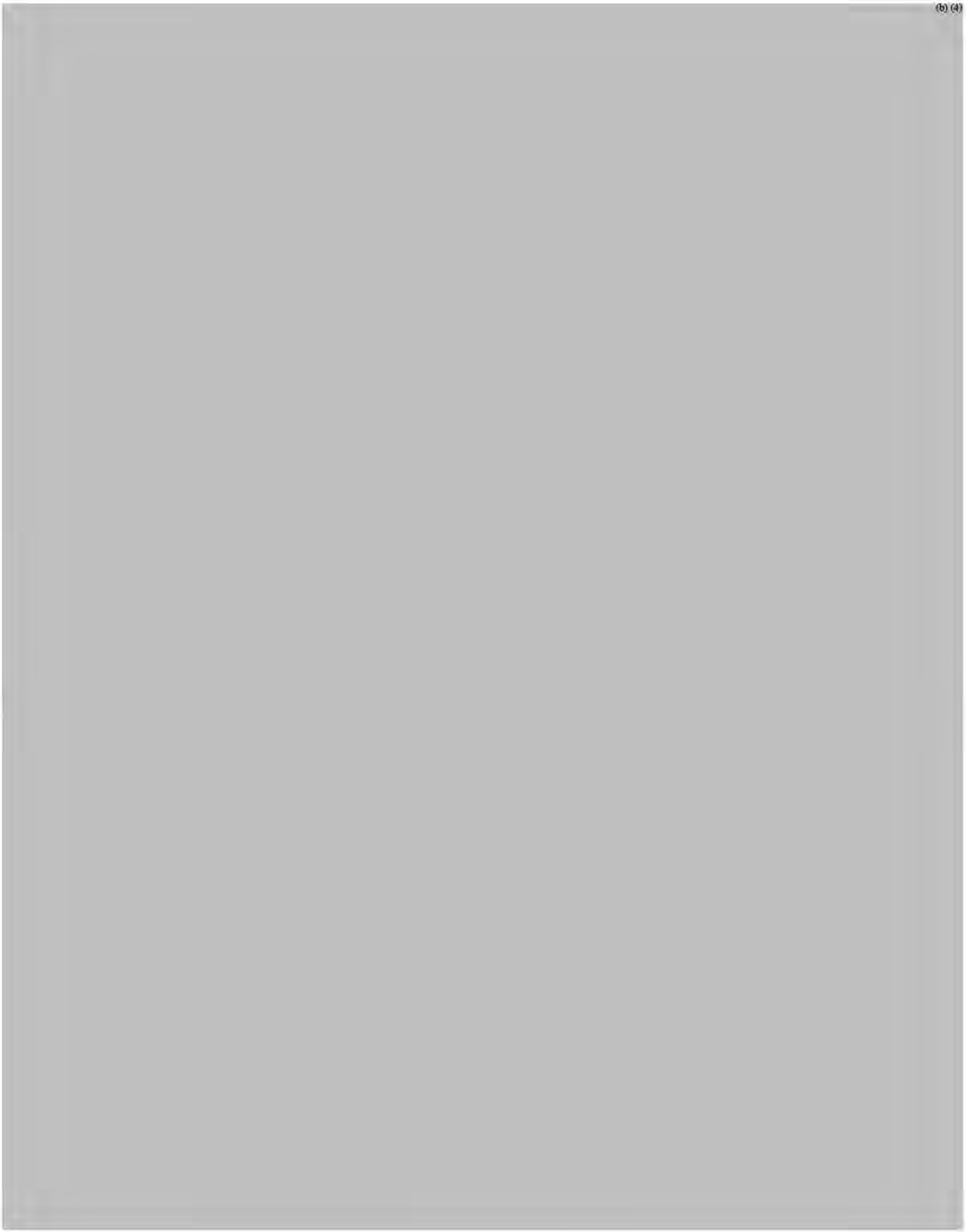
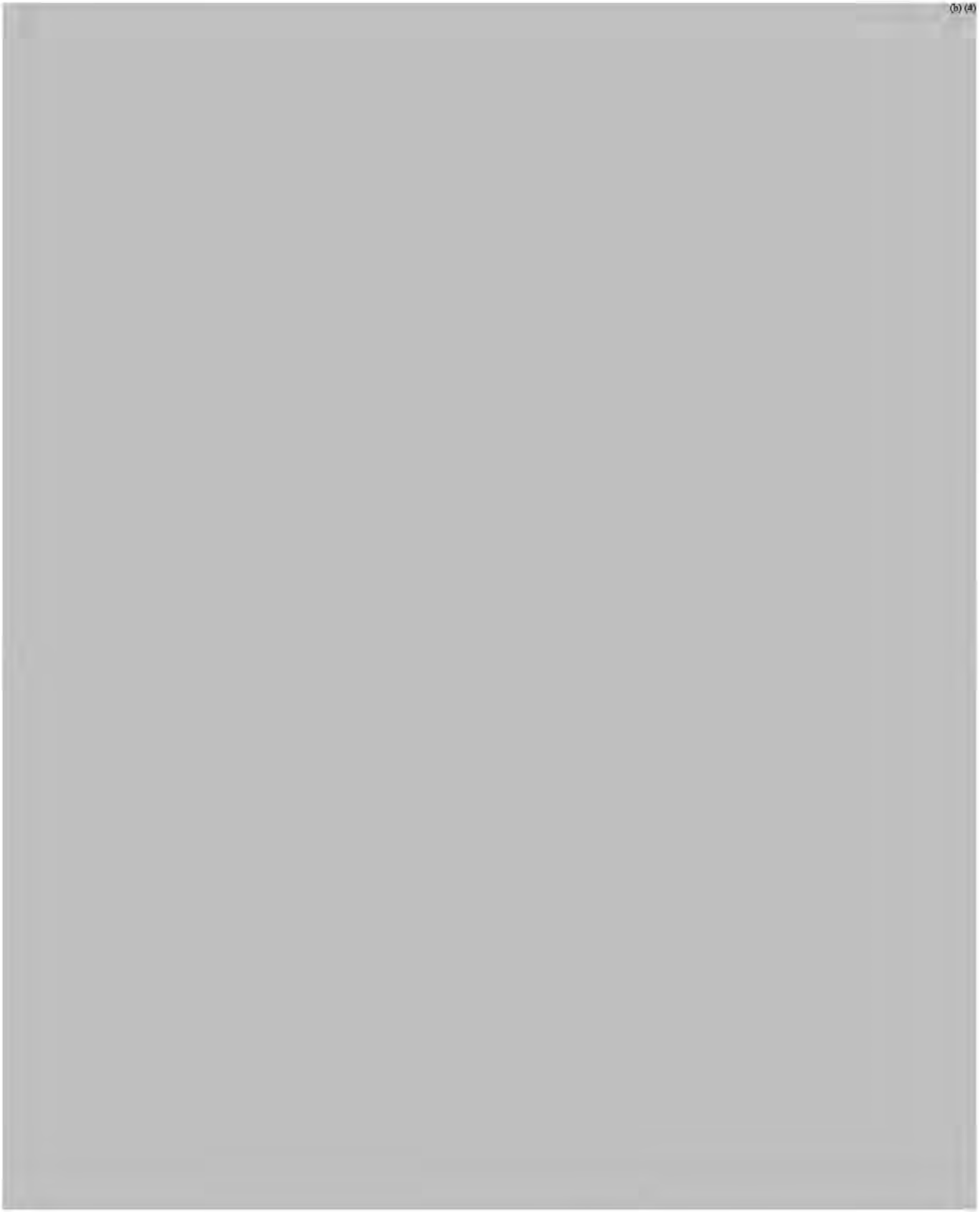

(b) (4)

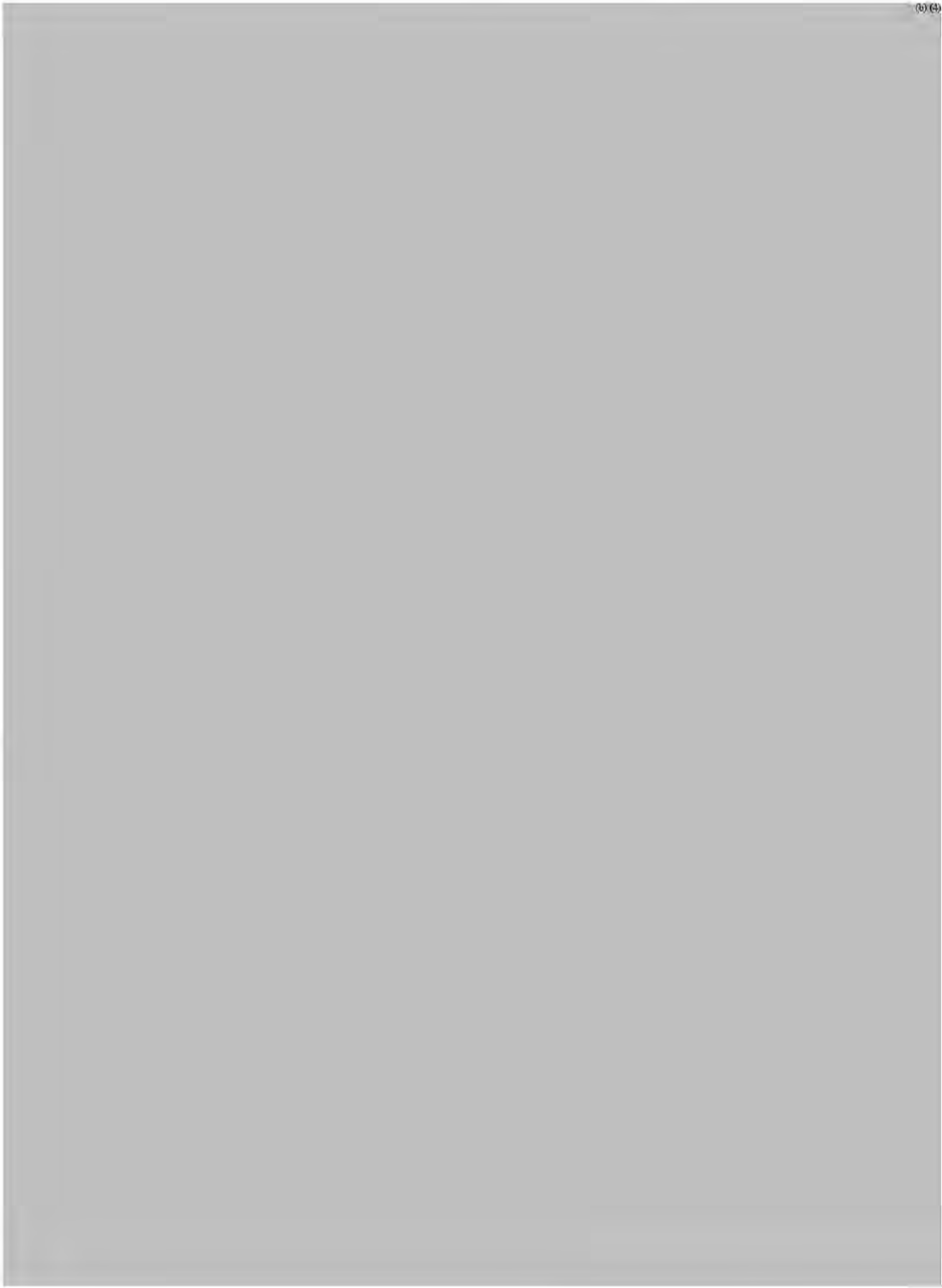

(b) (4)


(b) (4)


(b) (4)


(b) (4)


(b) (4)


(b) (4)

ANALYTICAL REPORT

Qualitative and quantitative composition of VAL Pro

(Document No.: BA20003)

CJ Research Institute of Biotechnology

CONTENTS

Prior to the compositional analysis, samples were dried at 105 °C for 3 hours. Therefore, analysed data of components in this report were provided as 'dry matter basis' (except 'Moisture').

1. L-Valine and moisture contents in 5 batches of 'VAL Pro', in g per 100 g (%) of the product

Component	Unit	GVAL200910	GVAL200911	GVAL200912	GVAL200916	GVAL200917
L-Valine	%	(b) (4)				
Moisture	%					

(b) (4)

2. Nitrogen containing components in 5 batches of 'VAL Pro', in g per 100 g (%) of the product

Component	Unit	GVAL200910	GVAL200911	GVAL200912	GVAL200916	GVAL200917
Ammonium (as NH_4)	%	(b) (4)				
Nitrates (as NO_3)	%					
Betaine	%					
Sum of quantifiable NH_3, NO_3, betaine	**%**					

(b) (4)

3. Compositional analysis of the carbohydrates fraction in 5 batches of 'VAL Pro', in g per 100 g (%) of the product

Component	Unit	GVAL200910	GVAL200911	GVAL200912	GVAL200916	GVAL200917
Trehalose	%	(b) (4)				
Glucose	%					
Fructose	%					
Sucrose	%					
Isomaltose	%					
Maltose	%					
Sum of quantifiable sugars	**%**					

(b) (4)

4. Amino acid contents in 5 batches of 'VAL Pro', in g per 100 g (%) of the product

Component	Unit	GVAL200910	GVAL200911	GVAL200912	GVAL200916	GVAL200917
Phosphoserine	%	(b) (4)				
Taurine	%					
Phosphoethanolamine	%					
Urea	%					
Aspartic acid	%					
Threonine	%					
Serine	%					
Glutamic acid	%					
Sarcosine	%					
α-Aminoadipic acid	%					
Glycine	%					
Alanine	%					

(b) (4)

Citrulline	%
α -Amino-n-butyric acid	%
Cystine	%
Methionine	%
Cysthathionine	%
Isoleucine	%
Leucine	%
Tyrosine	%
Phenylalanine	%
β-Alanine	%
β-Aminoisobutyric acid	%
γ-Amino-n-butyric acid	%
Ethanolamine	%
Hydroxylysine	%
Ornithine	%
Lysine	%
1-Methylhistidine	%
Histidine	%
3-methylhistidine	%
Asparagine	%
Carnosine	%
Arginine	%
Hydroxyproline	%
Proline	%
Sum of amino acids other than L-valine	**%**

(b) (4)

(b) (4)

5. Hydrolyzed amino acids contents in insoluble part in 5 batches of 'VAL Pro', in g per 100 g (%) of the product

Component	Unit	GVAL200910	GVAL200911	GVAL200912	GVAL200916	GVAL200917
Aspartic acid	%					
Threonine	%					
Serine	%					
Glutamic acid	%					
Glycine	%					
Alanine	%					
Cystine	%					
Valine	%					
Methionine	%					
Isoleucine	%					
Leucine	%					
Tyrosine	%					
Phenylalanine	%					
Lysine	%					
Histidine	%					
Arginine	%					
Proline	%					
Tryptophan	%					
Sum of 'hydrolyzed amino acids' in insoluble part[1]	**%**					

(b) (4)

(b) (4)

[redacted]

6. Compositional analysis of organic acids fraction in 5 batches of 'VAL Pro', in g per 100 g (%) of the product

Component	Unit	GVAL200910	GVAL200911	GVAL200912	GVAL200916	GVAL200917
Citric Acid	%					
Malic Acid	%					
Succinic Acid	%					
Lactic Acid	%					
Formic Acid	%					
Acetic Acid	%					
Sum of quantifiable organic acids	**%**					

(b) (4)

[redacted]

- Organic acids

: Samples are extracted with water. The resulting extract is analyzed using cation exchange chromatography on a HPLC with conductivity. (Korean Feed Standards Codex, 1 of chapter 14.)

7. Compositional analysis of inorganic components in 5 batches of 'VAL Pro', in g per 100 g (%) of the product

Component	Unit	GVAL200910	GVAL200911	GVAL200912	GVAL200916	GVAL200917
Ash	%	(b) (4)				
Sodium	%					
Potassium	%					
Calcium	%					
Magnesium	%					
Fluoride	%					
Bromide	%					
Chloride	%					
Phosphate	%					
Sulfate	%					
Sum of quantifiable inorganic anions and cations	**%**					

(b) (4)

8. Overview of the quantifiable main components of 'VAL Pro', in g per 100 g (%) of the product

Component	Unit	GVAL200910	GVAL200911	GVAL200912	GVAL200916	GVAL200917
L-Valine	%	(b) (4)				

Hydrolyzed amino acids (in insoluble biomass part)		%
Free amino acids (other than L-valine)		%
Moisture		%
Ammonium, nitrates and betaine		%
Sugars		%
Organic acids		%
Inorganic anions/cations		%
	Ash[1]	%

(b) (4)

(b) (4)

9. Results and methods of 'VAL Pro'

Component	Results[1]	Analytical method
L-Valine	72.38 %	HPLC-FLD (modified AOAC 999.13)
Hydrolyzed amino acids (in insoluble biomass part)	9.12 %	AOAC 994.12
		AOAC 988.15
		AOAC 985.28
Free amino acids (other than L-valine)	0.81 %	AOAC 999.13
Moisture	0.82 %	AOAC 934.01
Ammonium, nitrates and betaine	2.68 %	ASTM D 4327-03
		ASTM D 6919–03
		Korean Feed Standards Codex, 18 of chapter 21.
Sugars	0.39 %	AOAC 995.13
Organic acids	0.02 %	Korean Feed Standards Codex, 1 of chapter 14
Inorganic anions/cations	7.82 %	ASTM D 4327-03
		ASTM D 6919–03
Ash	1.50 %	AOAC 942.05

[1]Results are mean value of five batches

[Appendix] Certificate of analysis

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Certificate of analysis

Certificate No.	2021-PR-124	Receipt No.	2021-AN-092
Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.11.22.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.10.
Lot. No	GVAL200910
Quantity (kg)	-

Test Item(s)	lot number	Test Result	Test method used
L-Valine(dry base)	Not less than 72 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01
Ash	Not more than 5 %		AOAC 942.05

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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Certificate of analysis

Certificate No.	2021-PR-125	Receipt No.	2021-AN-093
Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.11.22.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.11.
Lot. No	GVAL200911
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 72 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01
Ash	Not more than 5 %		AOAC 942.05

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

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Certificate No.	2021-PR-126	Receipt No.	2021-AN-094
Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.11.22.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.12.
Lot. No	GVAL200912
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 72 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01
Ash	Not more than 5 %		AOAC 942.05

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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Certificate of analysis

Certificate No.	2021-PR-127	Receipt No.	2021-AN-095
Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.11.22.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.16.
Lot. No	GVAL200916
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 72 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01
Ash	Not more than 5 %		AOAC 942.05

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.11.22.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.17.
Lot. No	GVAL200917
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 72 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01
Ash	Not more than 5 %		AOAC 942.05

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

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Certificate No.	2021-PR-129	Receipt No.	2021-AN-097
Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.11.26.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.10.
Lot. No	GVAL200910
Quantity (kg)	-

Test Item(s)	Specification	Test Result (%)	Test method used
Citric Acid	-	(b) (4)	Korean Feed Standards Codex, 1 of chapter 14
Malic Acid	-		
Succinic Acid	-		
Lactic Acid	-		
Formic Acid	-		
Acetic Acid	-		
Trehalose	-		AOAC 995.13
Glucose	-		
Fructose	-		
Sucrose	-		
Isomaltose	-		
Maltose	-		

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* N.D. : not detected (not quantifiable)

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.11.26.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.11.
Lot. No	GVAL200911
Quantity (kg)	-

Test Item(s)	Specification	Test Result (%)	Test method used
Citric Acid	-	(b) (4)	Korean Feed Standards Codex, 1 of chapter 14
Malic Acid	-		
Succinic Acid	-		
Lactic Acid	-		
Formic Acid	-		
Acetic Acid	-		
Trehalose	-		AOAC 995.13
Glucose	-		
Fructose	-		
Sucrose	-		
Isomaltose	-		
Maltose	-		

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

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Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.11.26.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.12.
Lot. No	GVAL200912
Quantity (kg)	-

Test Item(s)	Specification	Test Result (%)	Test method used
Citric Acid	-	(b) (4)	Korean Feed Standards Codex, 1 of chapter 14
Malic Acid	-		
Succinic Acid	-		
Lactic Acid	-		
Formic Acid	-		
Acetic Acid	-		
Trehalose	-		AOAC 995.13
Glucose	-		
Fructose	-		
Sucrose	-		
Isomaltose	-		
Maltose	-		

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* N.D. : not detected (not quantifiable)

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Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.11.26.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.16.
Lot. No	GVAL200916
Quantity (kg)	-

Test Item(s)	Specification	Test Result (%)	Test method used
Citric Acid	-	(b) (4)	Korean Feed Standards Codex, 1 of chapter 14
Malic Acid	-		
Succinic Acid	-		
Lactic Acid	-		
Formic Acid	-		
Acetic Acid	-		
Trehalose	-		AOAC 995.13
Glucose	-		
Fructose	-		
Sucrose	-		
Isomaltose	-		
Maltose	-		

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* N.D. : not detected (not quantifiable)

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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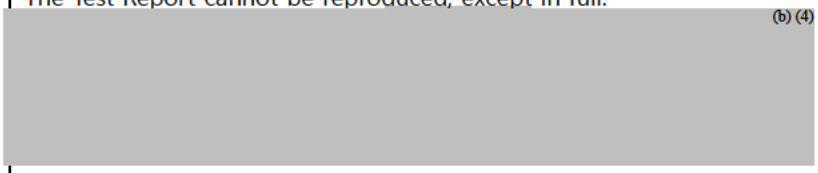

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Certificate No.	2021-PR-133	Receipt No.	2021-AN-101
Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.11.26.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.17.
Lot. No	GVAL200917
Quantity (kg)	-

Test Item(s)	Specification	Test Result (%)	Test method used
Citric Acid	-	(b) (4)	Korean Feed Standards Codex, 1 of chapter 14
Malic Acid	-		
Succinic Acid	-		
Lactic Acid	-		
Formic Acid	-		
Acetic Acid	-		
Trehalose	-		AOAC 995.13
Glucose	-		
Fructose	-		
Sucrose	-		
Isomaltose	-		
Maltose	-		

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* N.D. : not detected (not quantifiable)

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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Certificate of analysis

Certificate No.	2021-PR-134	Receipt No.	2021-AN-102
Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.11.27.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.10.
Lot. No	GVAL200910
Quantity (kg)	-

Test Item(s)	Specification	Test Result (%)	Test method used
Potassium	-	(b) (4)	ASTM D 6919–03
Calcium	-		
Magnesium	-		
Nitrate (as NO_3)	-		ASTM D 4327-03
Fluoride	-		
Bromide	-		
Chloride	-		
Phosphate	-		
Phosphoserine	-		AOAC 999.13
Taurine	-		
Phospho ethanol amine	-		
Urea	-		
Aspartic acid	-		
Threonine	-		
Serine	-		
Glutamic acid	-		
Sarcosine	-		

a-Amino adipic acid	-	(b) (4)
Glycine	-	
Alanine	-	
Citrulline	-	
a-Amino-n-butyric acid	-	
Cystine	-	
Methionine	-	
Cysthathionine	-	
Isoleucine	-	
Leucine	-	
Tyrosine	-	
Phenylalanine	-	
β-Alanine	-	
β-Amino isobutyric acid	-	
γ-Amino-n-butyric acid	-	
Ethanol amine	-	
Hydroxy lysine	-	
Ornithine	-	
Lysine	-	
1-Methylhistidine	-	
Histidine	-	
3-methylhistidine	-	
Asparagine	-	
Carnosine	-	
Arginine	-	
Hydroxy proline	-	
Proline	-	

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* N.D. : not detected (not quantifiable)

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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Certificate of analysis

Certificate No.	2021-PR-135	Receipt No.	2021-AN-103
Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.11.27.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.11.
Lot. No	GVAL200911
Quantity (kg)	-

Test Item(s)	Specification	Test Result (%)	Test method used
Potassium	-	(b) (4)	ASTM D 6919–03
Calcium	-		
Magnesium	-		
Nitrate (as NO_3)	-		ASTM D 4327-03
Fluoride	-		
Bromide	-		
Chloride	-		
Phosphate	-		
Phosphoserine	-		AOAC 999.13
Taurine	-		
Phospho ethanol amine	-		
Urea	-		
Aspartic acid	-		
Threonine	-		
Serine	-		
Glutamic acid	-		
Sarcosine	-		

a-Amino adipic acid	-	(b) (4)
Glycine	-	
Alanine	-	
Citrulline	-	
a-Amino-n-butyric acid	-	
Cystine	-	
Methionine	-	
Cysthathionine	-	
Isoleucine	-	
Leucine	-	
Tyrosine	-	
Phenylalanine	-	
β-Alanine	-	
β-Amino isobutyric acid	-	
γ-Amino-n-butyric acid	-	
Ethanol amine	-	
Hydroxy lysine	-	
Ornithine	-	
Lysine	-	
1-Methylhistidine	-	
Histidine	-	
3-methylhistidine	-	
Asparagine	-	
Carnosine	-	
Arginine	-	
Hydroxy proline	-	
Proline	-	

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* N.D. : not detected (not quantifiable)

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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Certificate of analysis

Certificate No.	2021-PR-136	Receipt No.	2021-AN-104
Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.11.27.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.12.
Lot. No	GVAL200912
Quantity (kg)	-

Test Item(s)	Specification	Test Result (%)	Test method used
Potassium	-	(b) (4)	ASTM D 6919–03
Calcium	-		
Magnesium	-		
Nitrate (as NO_3)	-		ASTM D 4327-03
Fluoride	-		
Bromide	-		
Chloride	-		
Phosphate	-		
Phosphoserine	-		AOAC 999.13
Taurine	-		
Phospho ethanol amine	-		
Urea	-		
Aspartic acid	-		
Threonine	-		
Serine	-		
Glutamic acid	-		
Sarcosine	-		

a-Amino adipic acid	-	(b) (4)
Glycine	-	
Alanine	-	
Citrulline	-	
a-Amino-n-butyric acid	-	
Cystine	-	
Methionine	-	
Cysthathionine	-	
Isoleucine	-	
Leucine	-	
Tyrosine	-	
Phenylalanine	-	
β-Alanine	-	
β-Amino isobutyric acid	-	
γ-Amino-n-butyric acid	-	
Ethanol amine	-	
Hydroxy lysine	-	
Ornithine	-	
Lysine	-	
1-Methylhistidine	-	
Histidine	-	
3-methylhistidine	-	
Asparagine	-	
Carnosine	-	
Arginine	-	
Hydroxy proline	-	
Proline	-	

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* N.D. : not detected (not quantifiable)

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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(b) (4)

(b) (4)

Approved by Technical Manager

Oct, 07, 2021

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Certificate of analysis

Certificate No.	2021-PR-137	Receipt No.	2021-AN-105
Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.11.27.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.16.
Lot. No	GVAL200916
Quantity (kg)	-

Test Item(s)	Specification	Test Result (%)	Test method used
Potassium	-	(b) (4)	ASTM D 6919–03
Calcium	-		
Magnesium	-		
Nitrate (as NO_3)	-		ASTM D 4327-03
Fluoride	-		
Bromide	-		
Chloride	-		
Phosphate	-		
Phosphoserine	-		AOAC 999.13
Taurine	-		
Phospho ethanol amine	-		
Urea	-		
Aspartic acid	-		
Threonine	-		
Serine	-		
Glutamic acid	-		
Sarcosine	-		

a-Amino adipic acid	-	(b) (4)
Glycine	-	
Alanine	-	
Citrulline	-	
a-Amino-n-butyric acid	-	
Cystine	-	
Methionine	-	
Cysthathionine	-	
Isoleucine	-	
Leucine	-	
Tyrosine	-	
Phenylalanine	-	
β-Alanine	-	
β-Amino isobutyric acid	-	
γ-Amino-n-butyric acid	-	
Ethanol amine	-	
Hydroxy lysine	-	
Ornithine	-	
Lysine	-	
1-Methylhistidine	-	
Histidine	-	
3-methylhistidine	-	
Asparagine	-	
Carnosine	-	
Arginine	-	
Hydroxy proline	-	
Proline	-	

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* N.D. : not detected (not quantifiable)

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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(b) (4)

(b) (4)

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Oct, 07, 2021

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Certificate of analysis

Certificate No.	2021-PR-138	Receipt No.	2021-AN-106
Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.11.27.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.17.
Lot. No	GVAL200917
Quantity (kg)	-

Test Item(s)	Specification	Test Result (%)	Test method used
Potassium	-	(b) (4)	ASTM D 6919–03
Calcium	-		
Magnesium	-		
Nitrate (as NO_3)	-		ASTM D 4327-03
Fluoride	-		
Bromide	-		
Chloride	-		
Phosphate	-		
Phosphoserine	-		AOAC 999.13
Taurine	-		
Phospho ethanol amine	-		
Urea	-		
Aspartic acid	-		
Threonine	-		
Serine	-		
Glutamic acid	-		
Sarcosine	-		

		(b) (4)
a-Amino adipic acid	-	
Glycine	-	
Alanine	-	
Citrulline	-	
a-Amino-n-butyric acid	-	
Cystine	-	
Methionine	-	
Cysthathionine	-	
Isoleucine	-	
Leucine	-	
Tyrosine	-	
Phenylalanine	-	
β-Alanine	-	
β-Amino isobutyric acid	-	
γ-Amino-n-butyric acid	-	
Ethanol amine	-	
Hydroxy lysine	-	
Ornithine	-	
Lysine	-	
1-Methylhistidine	-	
Histidine	-	
3-methylhistidine	-	
Asparagine	-	
Carnosine	-	
Arginine	-	
Hydroxy proline	-	
Proline	-	

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* N.D. : not detected (not quantifiable)

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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(b) (4)

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Certificate of analysis

Certificate No.	2021-PR-139	Receipt No.	2021-AN-107
Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.12.01.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.10.
Lot. No	GVAL200910
Quantity (kg)	-

Test Item(s)	Specification	Test Result (%)	Test method used
Ammonium (as NH_4)	-	(b) (4)	ASTM D 6919–03
Sodium	-		
Sulfate	-		ASTM D 4327-03

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* N.D. : not detected (not quantifiable)

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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Certificate of analysis

Certificate No.	2021-PR-140	Receipt No.	2021-AN-108
Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.12.01.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.11.
Lot. No	GVAL200911
Quantity (kg)	-

Test Item(s)	Specification	Test Result (%)	Test method used
Ammonium (as NH_4)	-	(b) (4)	ASTM D 6919–03
Sodium	-		
Sulfate	-		ASTM D 4327-03

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* N.D. : not detected (not quantifiable)

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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Certificate No.	2021-PR-141	Receipt No.	2021-AN-109
Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.12.01.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.12.
Lot. No	GVAL200912
Quantity (kg)	-

Test Item(s)	Specification	Test Result (%)	Test method used
Ammonium (as NH_4)	-	(b) (4)	ASTM D 6919–03
Sodium	-		
Sulfate	-		ASTM D 4327-03

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* N.D. : not detected (not quantifiable)

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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Certificate of analysis

Certificate No.	2021-PR-142	Receipt No.	2021-AN-110
Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.12.01.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.16.
Lot. No	GVAL200916
Quantity (kg)	-

Test Item(s)	Specification	Test Result (%)	Test method used
Ammonium (as NH_4)	-	(b) (4)	ASTM D 6919–03
Sodium	-		
Sulfate	-		ASTM D 4327-03

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* N.D. : not detected (not quantifiable)

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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Certificate of analysis

Certificate No.	2021-PR-143	Receipt No.	2021-AN-111
Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.12.01.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.17.
Lot. No	GVAL200917
Quantity (kg)	-

Test Item(s)	Specification	Test Result (%)	Test method used
Ammonium (as NH_4)	-	(b) (4)	ASTM D 6919–03
Sodium	-		
Sulfate	-		ASTM D 4327-03

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* N.D. : not detected (not quantifiable)

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Certificate of analysis

Certificate No.	2021-PR-144	Receipt No.	2021-AN-112
Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.12.07.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.10.
Lot. No	GVAL200910
Quantity (kg)	-

Test Item(s)	Specification	Test Result (%)	Test method used
(hydrolyzed) Aspartic acid	-	(b) (4)	AOAC 994.12
(hydrolyzed) Threonine	-		AOAC 994.12
(hydrolyzed) Serine	-		AOAC 994.12
(hydrolyzed) Glutamic acid	-		AOAC 994.12
(hydrolyzed) Glycine	-		AOAC 994.12
(hydrolyzed) Alanine	-		AOAC 994.12
(hydrolyzed) Cystine	-		AOAC 985.28
(hydrolyzed) Valine	-		AOAC 994.12
(hydrolyzed) Methionine	-		AOAC 985.28
(hydrolyzed) Isoleucine	-		AOAC 994.12
(hydrolyzed) Leucine	-		AOAC 994.12
(hydrolyzed) Tyrosine	-		AOAC 994.12
(hydrolyzed) Phenylalanine	-		AOAC 994.12
(hydrolyzed) Lysine	-		AOAC 994.12
(hydrolyzed) Histidine	-		AOAC 994.12
(hydrolyzed) Argnine	-		AOAC 994.12
(hydrolyzed) Proline	-		AOAC 994.12

* Information
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Certificate of analysis

Certificate No.	2021-PR-145	Receipt No.	2021-AN-113
Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.12.07.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.11.
Lot. No	GVAL200911
Quantity (kg)	-

Test Item(s)	Specification	Test Result (%)	Test method used
(hydrolyzed) Aspartic acid	-	(b) (4)	AOAC 994.12
(hydrolyzed) Threonine	-		AOAC 994.12
(hydrolyzed) Serine	-		AOAC 994.12
(hydrolyzed) Glutamic acid	-		AOAC 994.12
(hydrolyzed) Glycine	-		AOAC 994.12
(hydrolyzed) Alanine	-		AOAC 994.12
(hydrolyzed) Cystine	-		AOAC 985.28
(hydrolyzed) Valine	-		AOAC 994.12
(hydrolyzed) Methionine	-		AOAC 985.28
(hydrolyzed) Isoleucine	-		AOAC 994.12
(hydrolyzed) Leucine	-		AOAC 994.12
(hydrolyzed) Tyrosine	-		AOAC 994.12
(hydrolyzed) Phenylalanine	-		AOAC 994.12
(hydrolyzed) Lysine	-		AOAC 994.12
(hydrolyzed) Histidine	-		AOAC 994.12
(hydrolyzed) Argnine	-		AOAC 994.12
(hydrolyzed) Proline	-		AOAC 994.12

* Information
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Certificate of analysis

Certificate No.	2021-PR-146	Receipt No.	2021-AN-114
Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.12.07.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.12.
Lot. No	GVAL200912
Quantity (kg)	-

Test Item(s)	Specification	Test Result (%)	Test method used
(hydrolyzed) Aspartic acid	-	(b) (4)	AOAC 994.12
(hydrolyzed) Threonine	-		AOAC 994.12
(hydrolyzed) Serine	-		AOAC 994.12
(hydrolyzed) Glutamic acid	-		AOAC 994.12
(hydrolyzed) Glycine	-		AOAC 994.12
(hydrolyzed) Alanine	-		AOAC 994.12
(hydrolyzed) Cystine	-		AOAC 985.28
(hydrolyzed) Valine	-		AOAC 994.12
(hydrolyzed) Methionine	-		AOAC 985.28
(hydrolyzed) Isoleucine	-		AOAC 994.12
(hydrolyzed) Leucine	-		AOAC 994.12
(hydrolyzed) Tyrosine	-		AOAC 994.12
(hydrolyzed) Phenylalanine	-		AOAC 994.12
(hydrolyzed) Lysine	-		AOAC 994.12
(hydrolyzed) Histidine	-		AOAC 994.12
(hydrolyzed) Argnine	-		AOAC 994.12
(hydrolyzed) Proline	-		AOAC 994.12

* Information
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Certificate of analysis

Certificate No.	2021-PR-147	Receipt No.	2021-AN-115
Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.12.07.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.16.
Lot. No	GVAL200916
Quantity (kg)	-

Test Item(s)	Specification	Test Result (%)	Test method used
(hydrolyzed) Aspartic acid	-	(b) (4)	AOAC 994.12
(hydrolyzed) Threonine	-		AOAC 994.12
(hydrolyzed) Serine	-		AOAC 994.12
(hydrolyzed) Glutamic acid	-		AOAC 994.12
(hydrolyzed) Glycine	-		AOAC 994.12
(hydrolyzed) Alanine	-		AOAC 994.12
(hydrolyzed) Cystine	-		AOAC 985.28
(hydrolyzed) Valine	-		AOAC 994.12
(hydrolyzed) Methionine	-		AOAC 985.28
(hydrolyzed) Isoleucine	-		AOAC 994.12
(hydrolyzed) Leucine	-		AOAC 994.12
(hydrolyzed) Tyrosine	-		AOAC 994.12
(hydrolyzed) Phenylalanine	-		AOAC 994.12
(hydrolyzed) Lysine	-		AOAC 994.12
(hydrolyzed) Histidine	-		AOAC 994.12
(hydrolyzed) Argnine	-		AOAC 994.12
(hydrolyzed) Proline	-		AOAC 994.12

* Information
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Certificate of analysis

Certificate No.	2021-PR-148	Receipt No.	2021-AN-116
Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.12.07.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.17.
Lot. No	GVAL200917
Quantity (kg)	-

Test Item(s)	Specification	Test Result (%)	Test method used
(hydrolyzed) Aspartic acid	-	(b) (4)	AOAC 994.12
(hydrolyzed) Threonine	-		AOAC 994.12
(hydrolyzed) Serine	-		AOAC 994.12
(hydrolyzed) Glutamic acid	-		AOAC 994.12
(hydrolyzed) Glycine	-		AOAC 994.12
(hydrolyzed) Alanine	-		AOAC 994.12
(hydrolyzed) Cystine	-		AOAC 985.28
(hydrolyzed) Valine	-		AOAC 994.12
(hydrolyzed) Methionine	-		AOAC 985.28
(hydrolyzed) Isoleucine	-		AOAC 994.12
(hydrolyzed) Leucine	-		AOAC 994.12
(hydrolyzed) Tyrosine	-		AOAC 994.12
(hydrolyzed) Phenylalanine	-		AOAC 994.12
(hydrolyzed) Lysine	-		AOAC 994.12
(hydrolyzed) Histidine	-		AOAC 994.12
(hydrolyzed) Argnine	-		AOAC 994.12
(hydrolyzed) Proline	-		AOAC 994.12

* Information
* Temperature : (20~28) ℃, Relative Humidity : (30~60) % * N.D. : not detected (not quantifiable) * The results shown in this test report refer only to the sample tested unless otherwise stated. The Test Report cannot be reproduced, except in full. (b) (4) (b) (4) Approved by Technical Manager Oct, 07, 2021 **CJ Research Institute of Biotechnology**

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Certificate of analysis

Certificate No.	2021-PR-149	Receipt No.	2021-AN-117
Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.12.03.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.10.
Lot. No	GVAL200910
Quantity (kg)	-

Test Item(s)	Specification	Test Result (%)	Test method used
(hydrolyzed) Tryptophan	-	(b) (4)	AOAC 988.15

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* N.D. : not detected (not quantifiable)

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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Certificate of analysis

Certificate No.	2021-PR-150	Receipt No.	2021-AN-118
Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.12.03.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.11.
Lot. No	GVAL200911
Quantity (kg)	-

Test Item(s)	Specification	Test Result (%)	Test method used
(hydrolyzed) Tryptophan	-	(b) (4)	AOAC 988.15

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* N.D. : not detected (not quantifiable)

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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Certificate of analysis

Certificate No.	2021-PR-151	Receipt No.	2021-AN-119
Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.12.03.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.12.
Lot. No	GVAL200912
Quantity (kg)	-

Test Item(s)	Specification	Test Result (%)	Test method used
(hydrolyzed) Tryptophan	-	(b) (4)	AOAC 988.15

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* N.D. : not detected (not quantifiable)

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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(b) (4)

(b) (4)

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Certificate of analysis

Certificate No.	2021-PR-152	Receipt No.	2021-AN-120
Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.12.03.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.16.
Lot. No	GVAL200916
Quantity (kg)	-

Test Item(s)	Specification	Test Result (%)	Test method used
(hydrolyzed) Tryptophan	-	(b) (4)	AOAC 988.15

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* N.D. : not detected (not quantifiable)

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(b) (4)

(b) (4)

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Certificate of analysis

Certificate No.	2021-PR-153	Receipt No.	2021-AN-121
Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.12.03.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.17.
Lot. No	GVAL200917
Quantity (kg)	-

Test Item(s)	Specification	Test Result (%)	Test method used
(hydrolyzed) Tryptophan	-	(b) (4)	AOAC 988.15

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* N.D. : not detected (not quantifiable)

* The results shown in this test report refer only to the sample tested unless otherwise stated.

The Test Report cannot be reproduced, except in full.

(b) (4)

(b) (4)

Approved by Technical Manager

Oct, 07, 2021

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TEL : 031) 8099-2450 FAX : 031) 8099-2918

Certificate of analysis

Certificate No.	2021-PR-154	Receipt No.	2021-AN-122
Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.12.08.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.10.
Lot. No	GVAL200910
Quantity (kg)	-

Test Item(s)	Specification	Test Result (%)	Test method used
Betaine	-	(b) (4)	Korean Feed Standards Codex, 18 of chapter 21.

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* N.D. : not detected (not quantifiable)

* The results shown in this test report refer only to the sample tested unless otherwise stated. The Test Report cannot be reproduced, except in full.

(b) (4)

(b) (4)

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TEL : 031) 8099-2450 FAX : 031) 8099-2918

Certificate of analysis

Certificate No.	2021-PR-155	Receipt No.	2021-AN-123
Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.12.08.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.11.
Lot. No	GVAL200911
Quantity (kg)	-

Test Item(s)	Specification	Test Result (%)	Test method used
Betaine	-	(b) (4)	Korean Feed Standards Codex, 18 of chapter 21.

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* N.D. : not detected (not quantifiable)

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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(b) (4)

(b) (4)

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Oct, 07, 2021

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TEL : 031) 8099-2450 FAX : 031) 8099-2918

Certificate of analysis

Certificate No.	2021-PR-156	Receipt No.	2021-AN-124
Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.12.08.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.12.
Lot. No	GVAL200912
Quantity (kg)	-

Test Item(s)	Specification	Test Result (%)	Test method used
Betaine	-	(b) (4)	Korean Feed Standards Codex, 18 of chapter 21.

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* N.D. : not detected (not quantifiable)

* The results shown in this test report refer only to the sample tested unless otherwise stated.

The Test Report cannot be reproduced, except in full.

(b) (4)

(b) (4)

Approved by Technical Manager

Oct, 07, 2021

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Certificate of analysis

Certificate No.	2021-PR-157	Receipt No.	2021-AN-125
Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.12.08.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.16.
Lot. No	GVAL200916
Quantity (kg)	-

Test Item(s)	Specification	Test Result (%)	Test method used
Betaine	-	0.21 %	Korean Feed Standards Codex, 18 of chapter 21.

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* N.D. : not detected (not quantifiable)

* The results shown in this test report refer only to the sample tested unless otherwise stated.

The Test Report cannot be reproduced, except in full.

(b) (4)

(b) (4)

Approved by Technical Manager

Oct, 07, 2021

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TEL : 031) 8099-2450 FAX : 031) 8099-2918

Certificate of analysis

Certificate No.	2021-PR-158	Receipt No.	2021-AN-126
Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.12.08.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.17.
Lot. No	GVAL200917
Quantity (kg)	-

Test Item(s)	Specification	Test Result (%)	Test method used
Betaine	-	(b) (4)	Korean Feed Standards Codex, 18 of chapter 21.

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* N.D. : not detected (not quantifiable)

* The results shown in this test report refer only to the sample tested unless otherwise stated.

The Test Report cannot be reproduced, except in full.

(b) (4)

(b) (4)

Approved by Technical Manager

Oct, 07, 2021

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CONFIDENTIAL REPORT

Determination of viable cells of the production strain in Dried L-Valine Fermentation Product

Version 1.1

TITLE

Determination of viable cells of the production strain in Dried L-Valine Fermentation Product

OBJECTIVE OF THE STUDY

This study was conducted to determine the viable cells of the production strain *Corynebacterium glutamicum* KCCM 80240 in the final product and manufacturing process.

SCHEDULE OF THE STUDY

Initiation of experiment: October 23, 2020

Termination of experiment: November 5, 2020

Submission of final report: December 31, 2020

Submission of revised report: October 7, 2021

TESTING FACILITY

R&BD)Regulatory Affairs, CJ CheilJedang
55, Gwanggyo-ro 42beon-gil, Yeongtong-gu, Suwon-si, Gyeonggi-do 16495, Korea

RESPONSIBLE STAFFS

Analyst and Author	Taeyeon Kim	
Report approved by	Yang Hee Kim	

CONTENTS

INTRODUCTION

Corynebacterium glutamicum KCCM 80240 is a production microorganism to produce L-valine as a fermentation product. In accordance with EFSA guidance on microorganism used as feed additives or as production organisms, the absence of the production strain in the final product should be investigated for safety aspects [1]. In order to confirm the absence of viable cells in the final products, the membrane filtration method was used.

MATERIALS AND METHODS

Test sample

(A) Detection of viable cells in the final product

Three independent batches of Dried L-Valine Fermentation Product were tested to analyse the existence of viable cell. The certificate of analysis of test samples are attached as Appendix 1.

- Batch No. : GVAL200910, GVAL200911, GVAL200912

(B) Detection of viable cells in the manufacturing process

Samples were taken from the representative step of manufacturing process to determine the existence of viable cells. The sampling point from the manufacturing process is divided into five steps: fermentation, pH adjustment, biomass inactivation, concentration and final product. Details of sampling point is marked in Appendix 2 of this report.

Limit of detection test

(b) (4)

Sample analysis

(b) (4)

(b) (4)

Control test

(b) (4)

Spike test

(b) (4)

RESULTS

Determination of limit of detection of analysis

(b) (4)

Table 1. LOD of viable cell test (Number of viable cells in culture broth=1.0×10^9 CFU mL^{-1})

Strain	Dilution fold	Number of viable cells(CFU mL^{-1})
C. glutamicum KCCM 80240	(b) (4)	

(b) (4)

Viable cell test

(b) (4)

(b) (4)

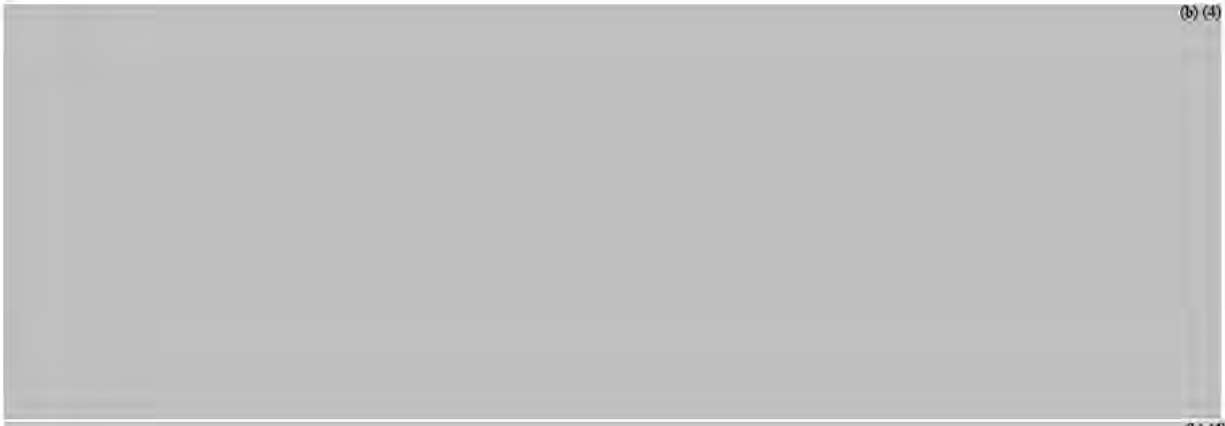
(A) Detection of viable cells in the final product

(b) (4)

Table 2. Number of viable cells in Dried L-Valine Fermentation Product

Product	Batch number	Number of viable cell (CFU g^{-1})		
		1st analysis	2nd analysis	3rd analysis
Dried L-Valine Fermentation Product	GVAL200910	(b) (4)		
	GVAL200911			
	GVAL200912			


(b) (4)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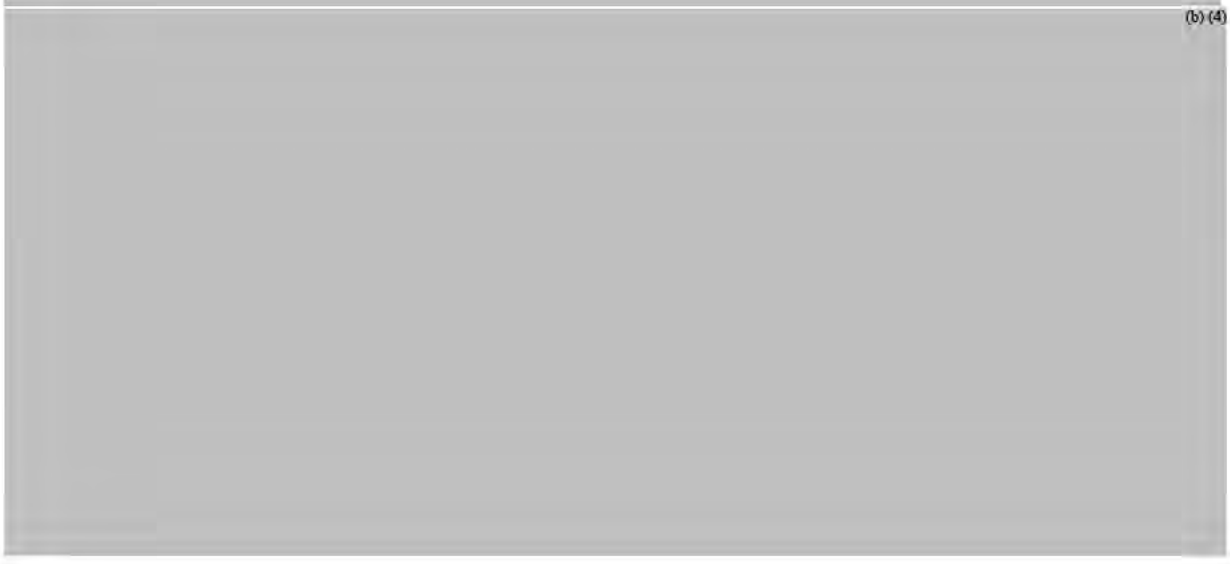
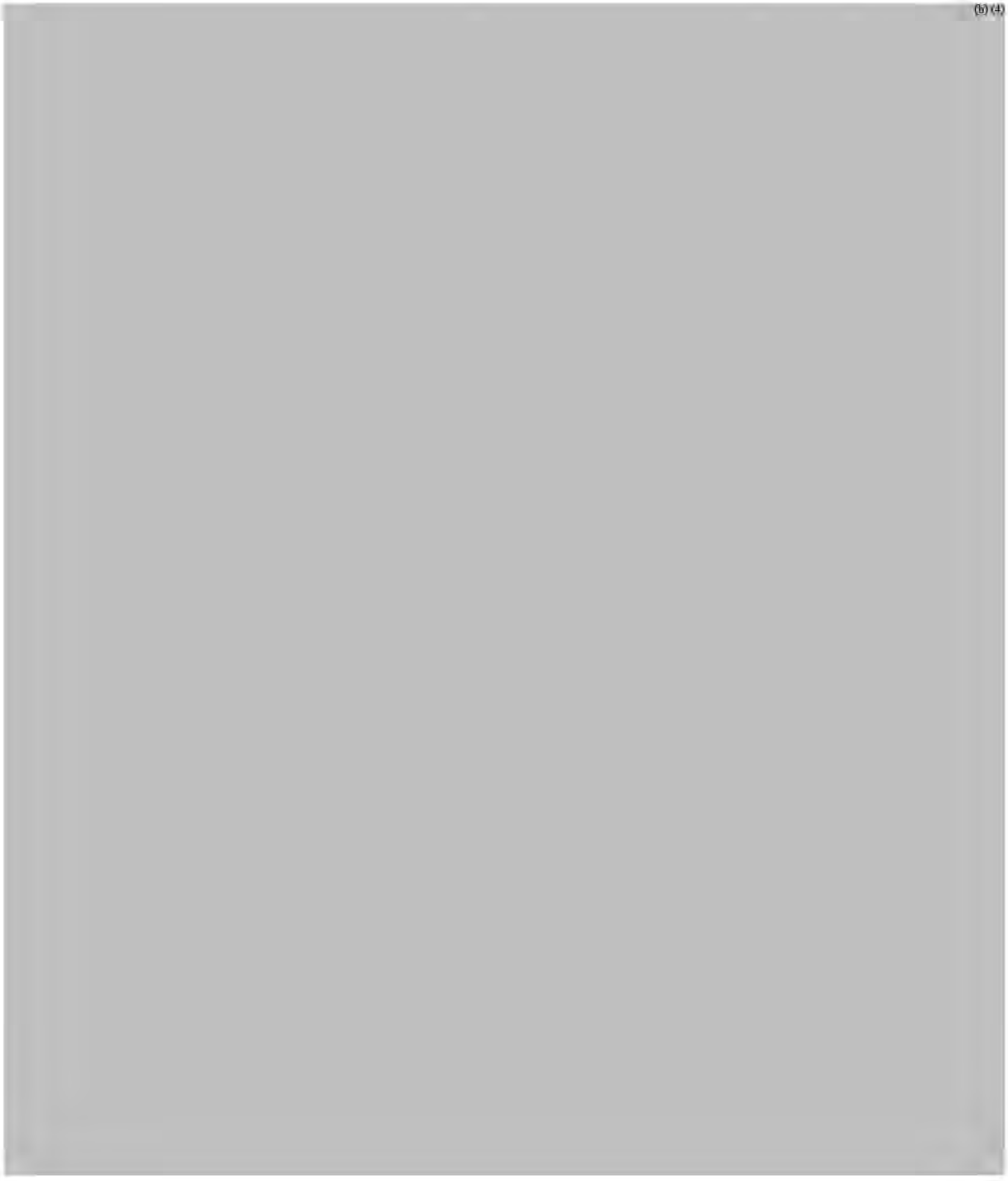

(b) (4)

(B) Detection of viable cells in the manufacturing process

(b) (4)

Table 3. Number of viable cells in Dried L-Valine Fermentation Product manufacturing process

	Number of viable cell (CFU mL^{-1})		
	1st analysis	2nd analysis	3rd analysis
Fermentation	(b) (4)		
pH adjustment			
Cell inactivation			
Concentration			
Product (CFU g^{-1})			


(b) (4)

REFERENCES

[1] EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2018. Guidance on the characterisation of microorganisms used as feed additives or as production. EFSA Journal, 16(3), 5206. DOI: 10.2903/j.efsa.2018.5206. Available online: https://efsa.onlinelibrary.wiley.com/doi/full/10.2903/j.efsa.2018.5206.

[APPENDIX 1] Certificate of Analysis

GVAL200910

CJ Research Institute of Biotechnology 55, Gwanggyo-ro 42beon-gil, Yeongtong-gu, Suwon-si, Gyeonggi-do, 16495, Korea www.cj.co.kr TEL : 031) 8099-2450 FAX : 031) 8099-2918	CJ CHEILJEDANG BIO

Certificate of analysis

Certificate No.	2020-PR-132	Receipt No.	2020-AN-106
Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.11.23.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.10.
Lot. No	GVAL200910
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 72 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.
The Test Report cannot be reproduced, except in full.

Tested by (b) (4)

Approved by Technical Manager (b) (4)

Dec, 23, 2020

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GVAL200911

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Certificate of analysis

Certificate No.	2020-PR-133	Receipt No.	2020-AN-107
Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.11.23.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.11.
Lot. No	GVAL200911
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 72 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.
The Test Report cannot be reproduced, except in full.

Tested by (b) (4)

Approved by Technical Manager (b) (4)

Dec, 23, 2020

CJ Research Institute of Biotechnology

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GVAL200912

CJ Research Institute of Biotechnology

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TEL : 031) 8099-2450 FAX : 031) 8099-2918

Certificate of analysis

Certificate No.	2020-PR-134	Receipt No.	2020-AN-108
Client	-	Date of Receipt	2020.11.19.
Client Name	-	Date of Test	2020.11.23.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (Val pro)
Manuf. Date	2020.09.12.
Lot. No	GVAL200912
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 72 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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Tested by (b) (4)

Approved by Technical Manager (b) (4)

Dec, 23, 2020

CJ Research Institute of Biotechnology

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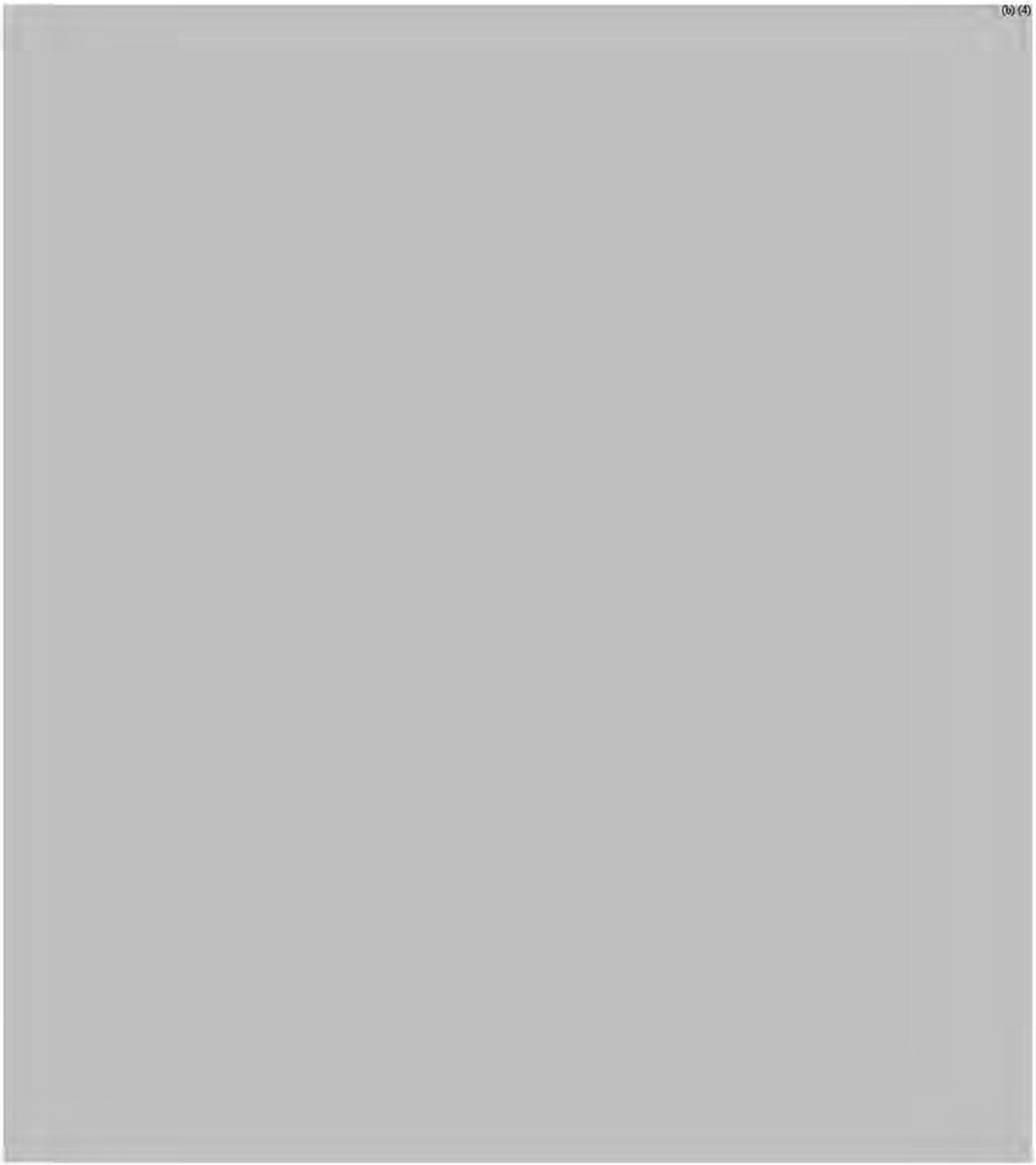
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REVISED APPENDIX 2_ATTACHMENT 4_WHOLE GENOME SEQUENCE ANALYSIS (CONFIDENTIAL)

CONFIDENTIAL REPORT

Whole genome sequence analysis of *Corynebacterium glutamicum* KCCM 80240

Version 2.0

TITLE

Whole genome sequence analysis of *Corynebacterium glutamicum* KCCM 80240

OBJECTIVE OF THE STUDY

This study was conducted to analyse the genomic features of production strain, *Corynebacterium glutamicum* KCCM 80240.

SCHEDULE OF THE STUDY

Initiation of experiment: 7 September 2020

Termination of experiment: 10 December 2020

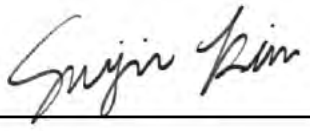
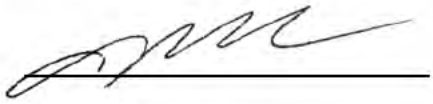
Submission of final report (Version 1.0): 15 December 2020

Submission of final report (Version 2.0): 29 April 2021

TESTING FACILITY

Institute of Biotechnology) Data Science Team, CJ CheilJedang
55, Gwanggyo-ro 42beon-gil, Yeongtong-gu, Suwon-si, Gyeonggi-do 16495, Korea

RESPONSIBLE STAFFS

Analyst	Sang Jun Kim	
Author	Su Jin Kim	
Report approved by	Sung Gun Lee	

CONTENTS

INTRODUCTION

L-Valine is produced by fermentation with *Corynebacterium glutamicum* KCCM 80240. The genome sequence analysis of the production strains should be performed for safety aspects in accordance with EFSA guidance on the characterisation of microorganisms used as feed additives or as production organisms [1]. This study provide the information about the analysis method and WGS-based charaterisation of the production strain *C. glutamicum* KCCM 80240.

MATERIALS AND METHODS

1. Whole genome sequencing

2. Bioinformatics analysis

2-1. Genome annotation

2-2. Bacterial identification

2-3. Identification of antimicrobial resistance (AMR) genes

2-4. Identification of pathogen associated genes

RESULTS

1. Overview on construction of production strain

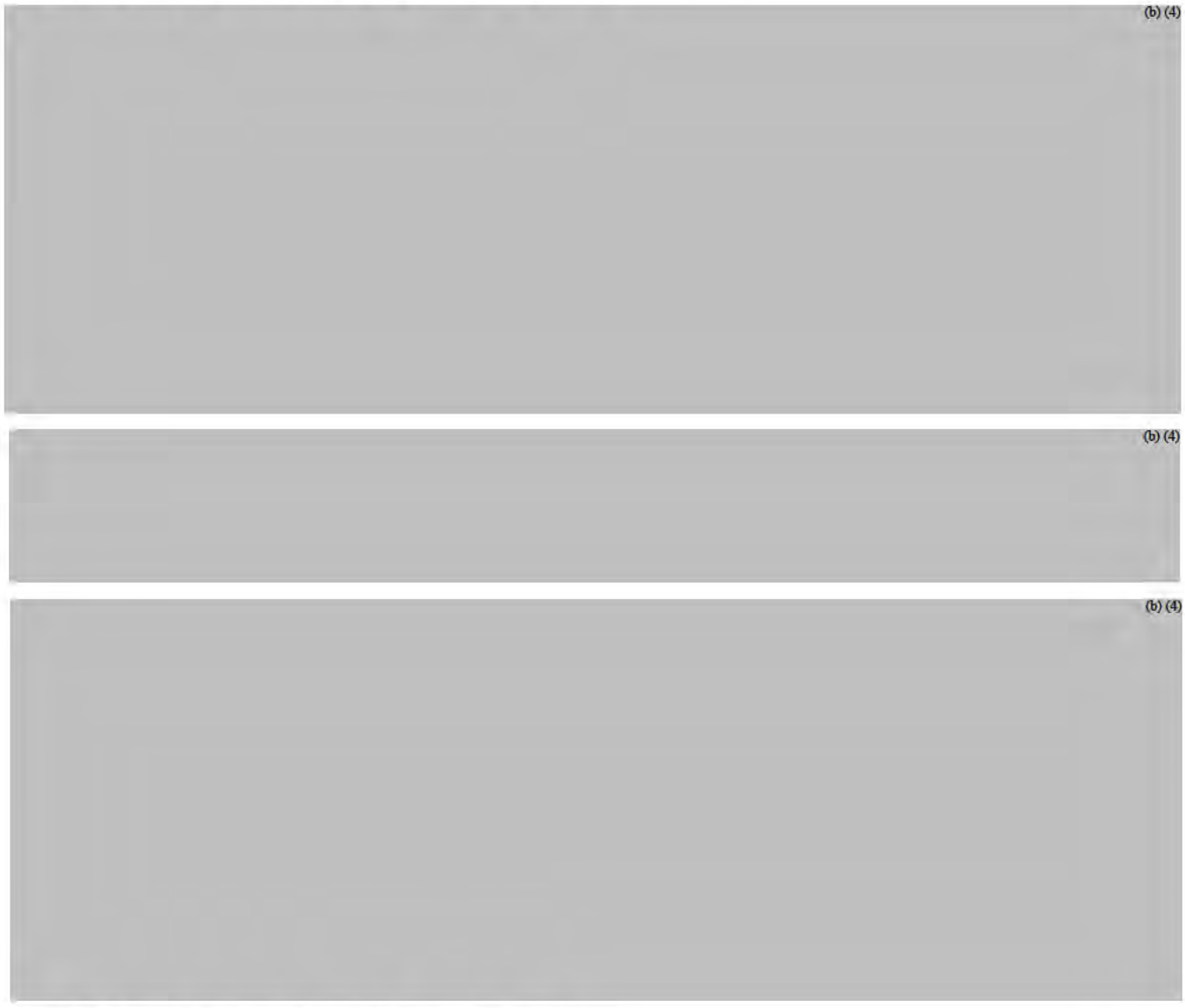


Table 1. Genome features of three *C. glutamicum* strains

Feature	*C. glutamicum strains*		
	Wild-type strain ATCC 14067	**Parental strain CA08-0012**	**Production strain KCCM 80240**
Genome size (bp)	(b) (4)		
G+C content (%)			
ORFs*			
tRNA			
rRNA			

* The number of ORFs was counted except the pseudogene.

2. WGS analysis of parental strain *C. glutamicum* CA08-0012

(b) (4)

Table2. General features of *C. glutamicum* ATCC 14067 and *C. glutamicum* CA08-0012 genome

Feature	*C. glutamicum* ATCC 14067	*C. glutamicum* CA08-0012
Genome length (bp)	(b) (4)	
G+C contents (%)		
Predicted ORFs		
Predicted tRNAs		
Predicted rRNAs		

(b) (4)

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(b) (4)


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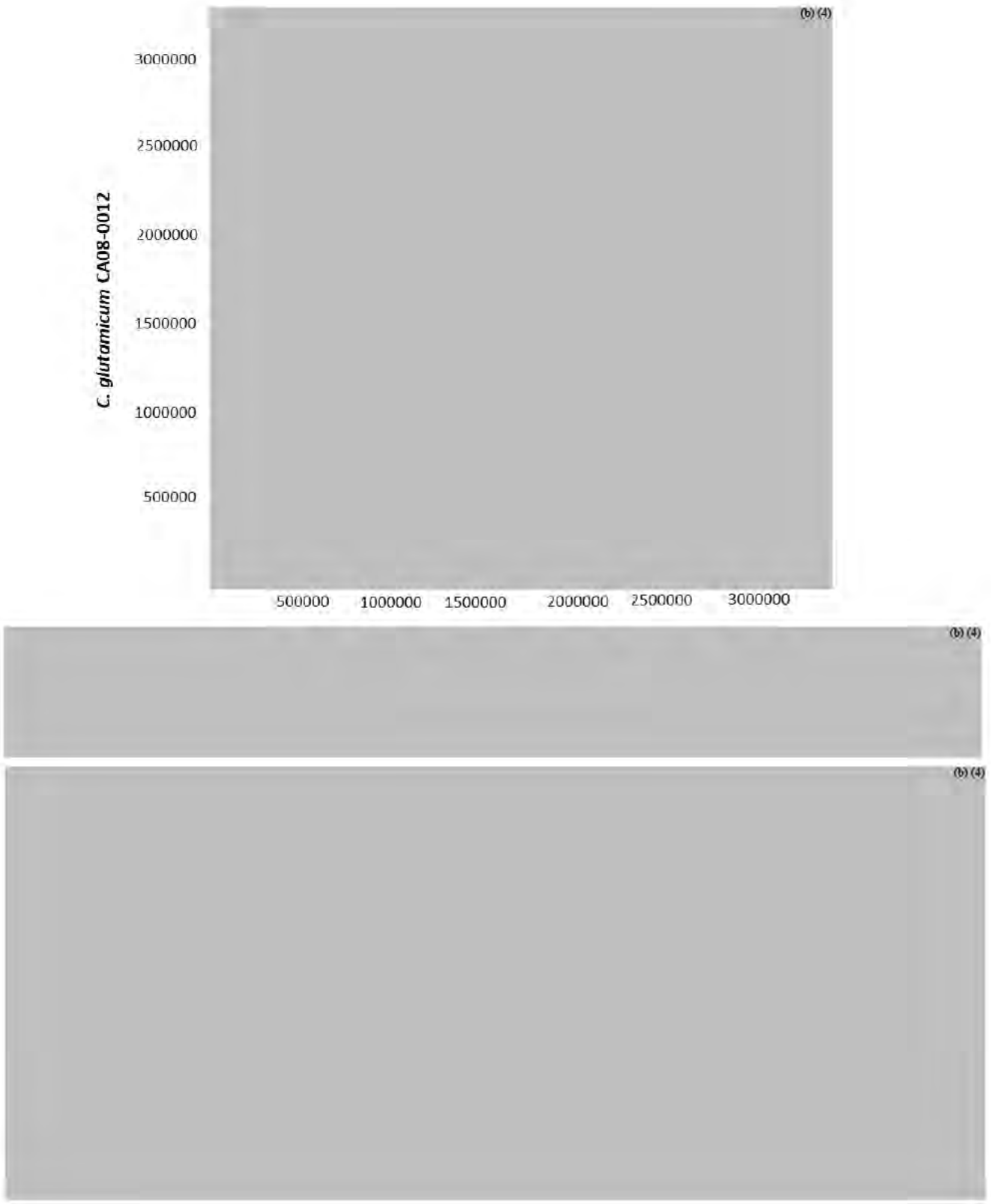
(b) (4)


(b) (4)
3000000
2500000
2000000
1500000
1000000
500000
C. glutamicum CA08-0012
500000
1000000
1500000
2000000
2500000
3000000
(b) (4)
(b) (4)

3. WGS analysis of the production strain *C. glutamicum* KCCM 80240

(b) (4)

Table 6. General features of *C. glutamicum* CN08-0012 and *C. glutamicum* KCCM 80240 genomes

Items	Parental strain *C. glutamicum* CN08-0012	Production strain *C. glutamicum* KCCM 80240
Genome length (bp)	(b) (4)	
G+C contents (%)		
Predicted ORFs		
Predicted tRNAs		
Predicted rRNAs		

(b) (4)

(b) (4)

(b) (4)

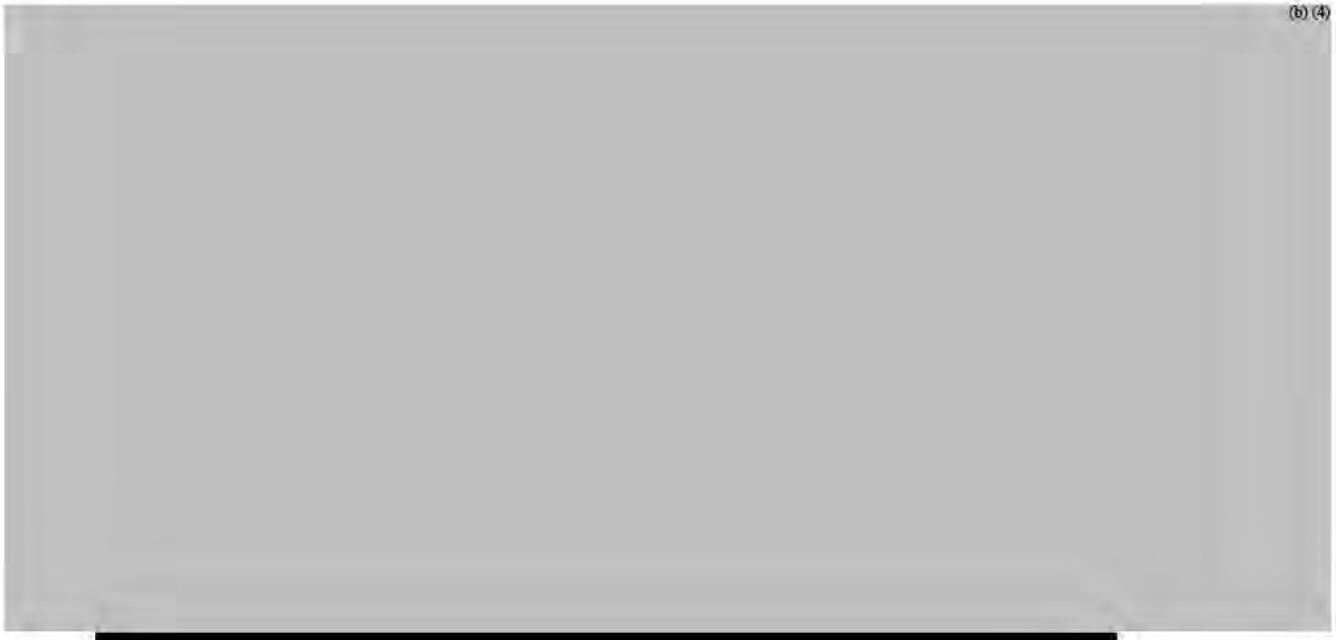
Table 7. Rearranged chromosome region of *C. glutamicum* KCCM 80240

No.	Parental stain *C. glutamicum* CA08-0012 Position	Production strain *C. glutamicum* KCCM 80240 Modification type	Involved genes*
1	(b) (4)		
2			

3 (b) (4)
4
5
6
7
8

(b) (4)

(b) (4)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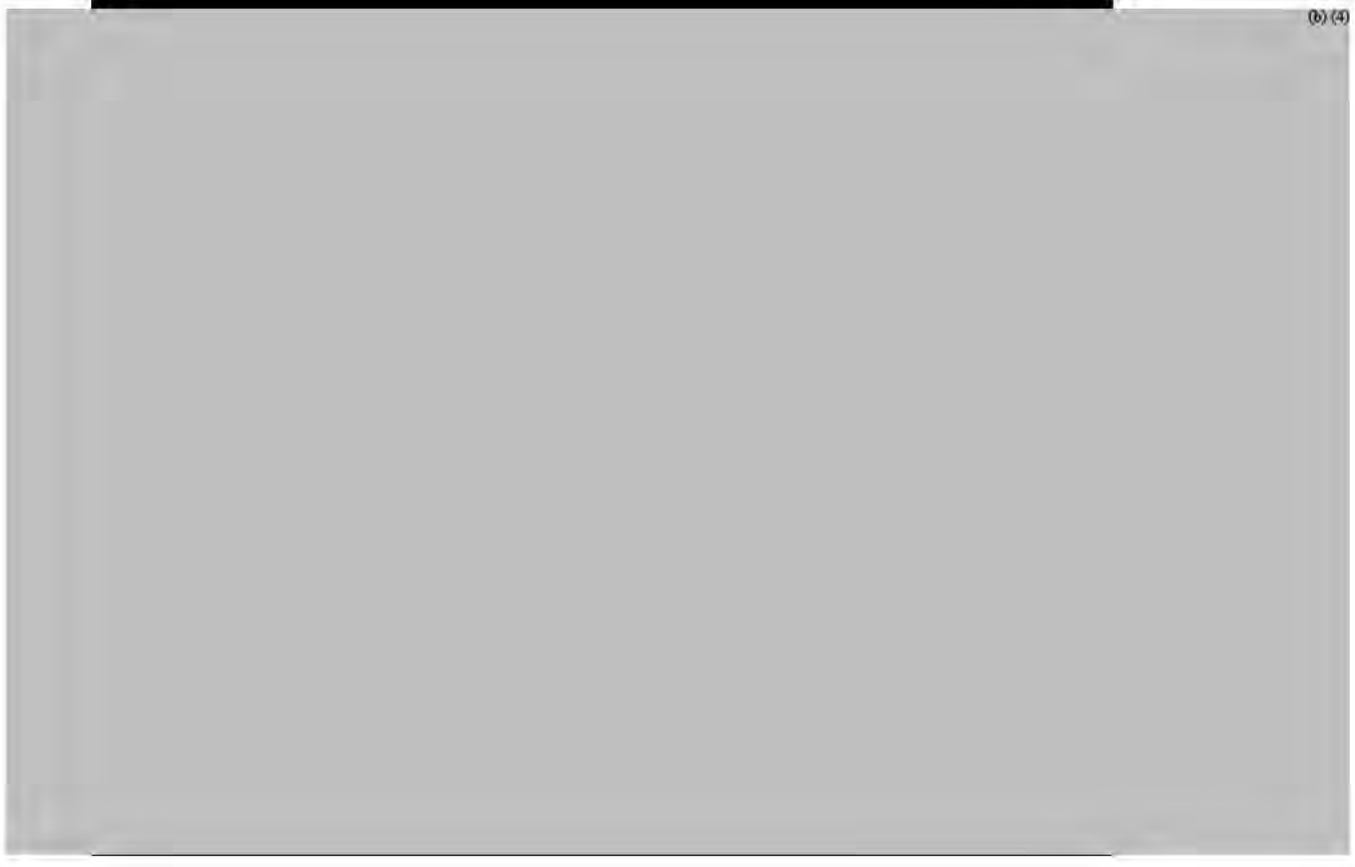
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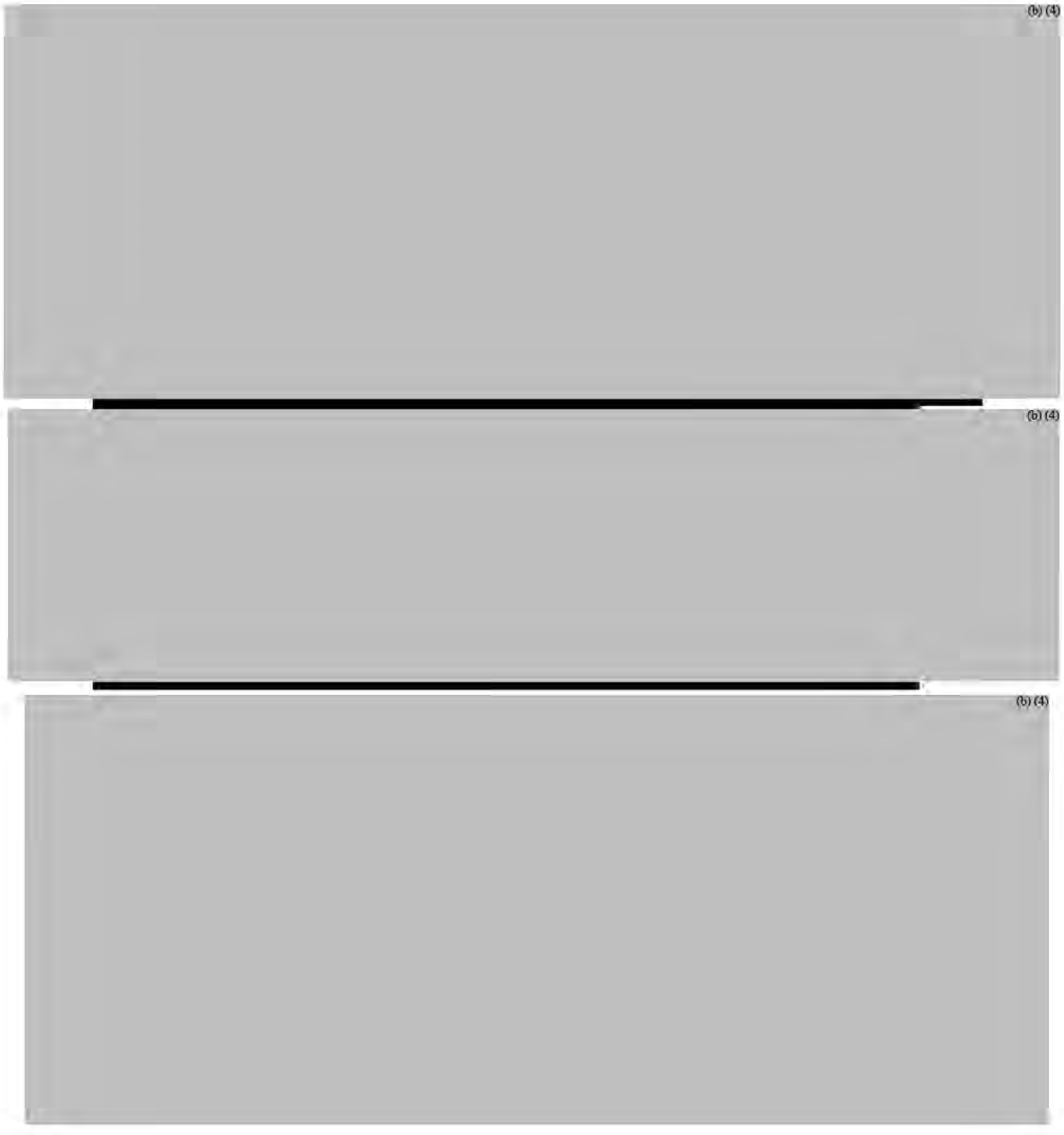
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(b) (4)
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(b) (4)


(b) (4)


(b) (4)


(b) (4)
(b) (4)

Table 8. Modified structural elements of *C. glutamicum* KCCM 80240 chromosome

No	Genetic modification	Name	Types of structural element	Type of genetic modification	Location	Purpose and function
1						(b) (4)
2						
3						
4						
5						
6						(b) (4)


(b) (4)
7
8
9
10

4. Identification of microorganism

(b) (4)

Table 9. ANI for *C. glutamicum* KCCM 80240 with wild-type Corynebacterium species

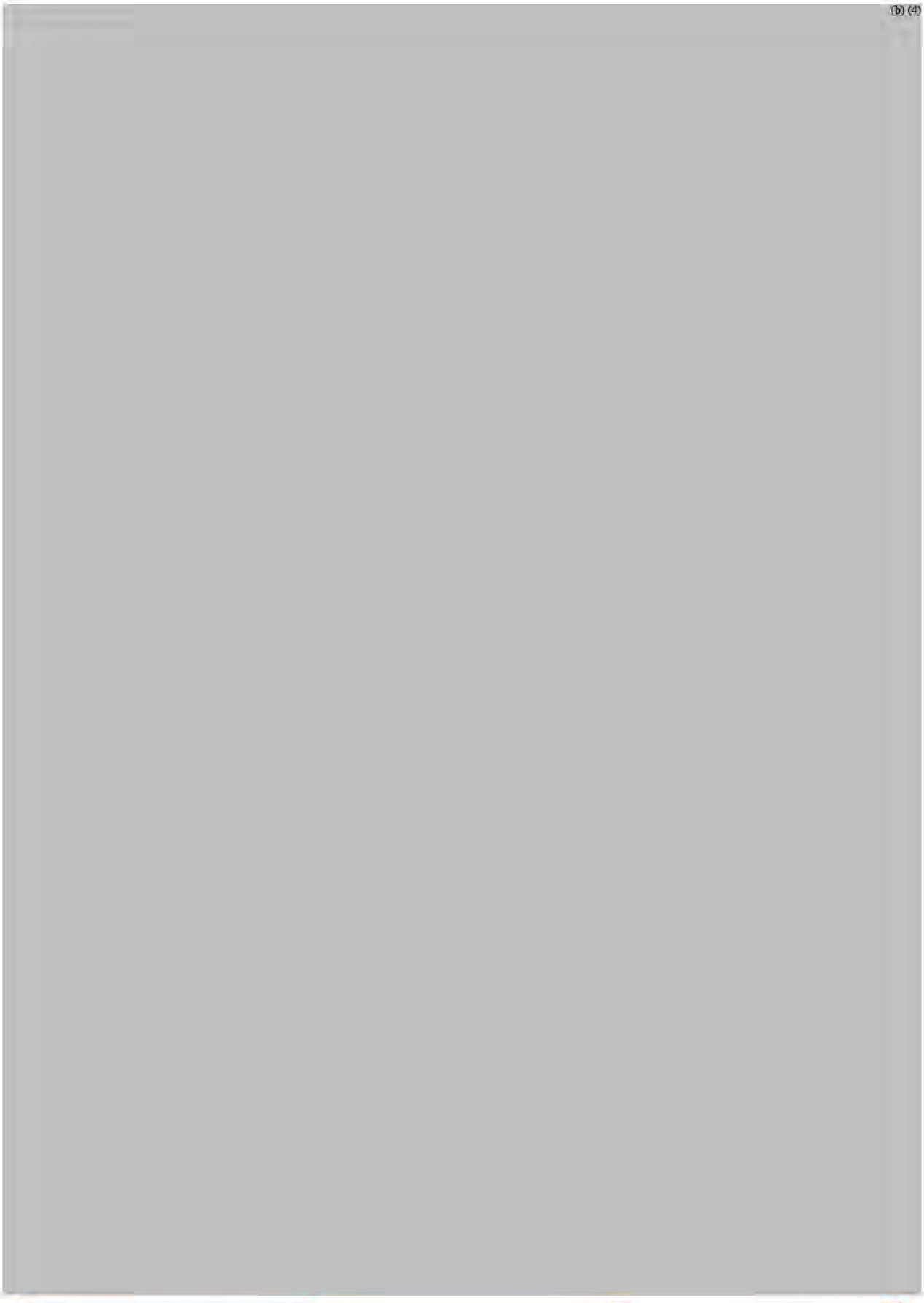
Rank	Species	GenBank accession no.	ANI value (%)
1	(b) (4)	GCA_002243555.1	99.9
2		GCA_000550785.1	83.0
3		GCA_001941425.1	81.2
4		GCA_000011325.1.	79.5
5		GCA_006539465.1.	77.7

5. Identification of antimicrobial resistance gene

(b) (4)

Table 10. Screening for antimicrobial resistance genes using ResFinder data base

(b) (4)


(b) (4)

(b) (4)

(b) (4)

Table 11. Screening for antimicrobial resistance genes using ARG-ANNOT data base

ARG-ANNOT-V4						
Gene ID of *C. glutamicum* KCCM 80240		Gene ID in ARG-ANNOT DB		Identity		Coverage
Name	Length	Name	Length	(/)	(%)	(%)

(b) (4)

6. Identification of pathogen associated genes

(b) (4)

Table 12. Screening of pathogen associated genes using VFDB data base

Gene ID in VFDB	*C. glutamicum* ATCC 14067				*C. glutamicum* KCCM 80240			
	Gene ID	Identity (/)	Identity (%)	Coverage (%)	Gene ID	Identity (/)	Identity (%)	Coverage (%)
								(b) (4)

REFERENCES

(b) (4)


(b) (4)

SUPPLEMENTARY DATA

Table S1. Nucleotide sequence variation of *C. glutamicum* CA08-0012 strain

No	*C. glutamicum* ATCC 14067			*C. glutamicum* CA08-0012		
	type	Ref. Position	Ref. Seq. Nuc.	Var. Nuc	Var. Position	Var. ORF Name
1						
2						
3						
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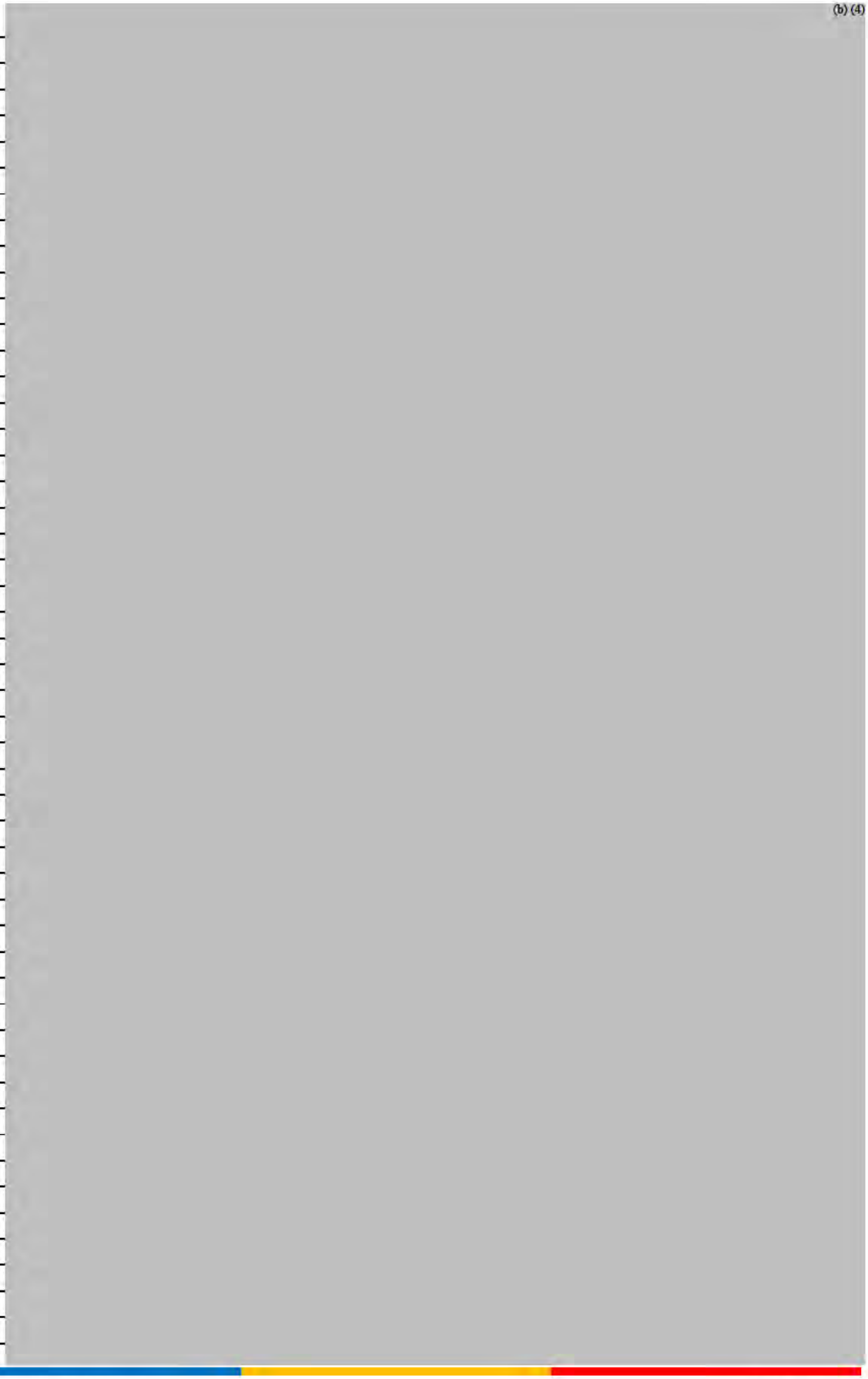

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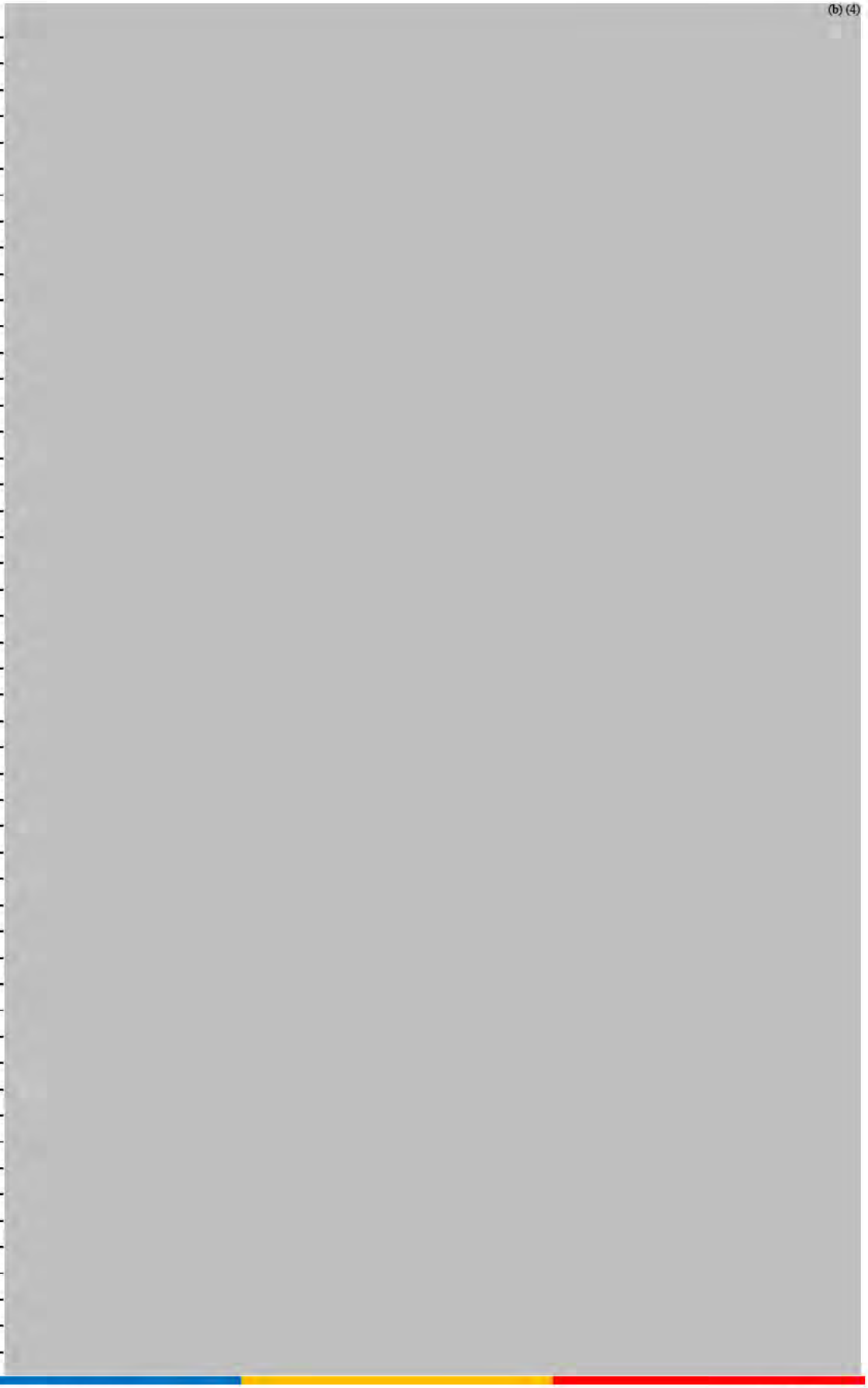

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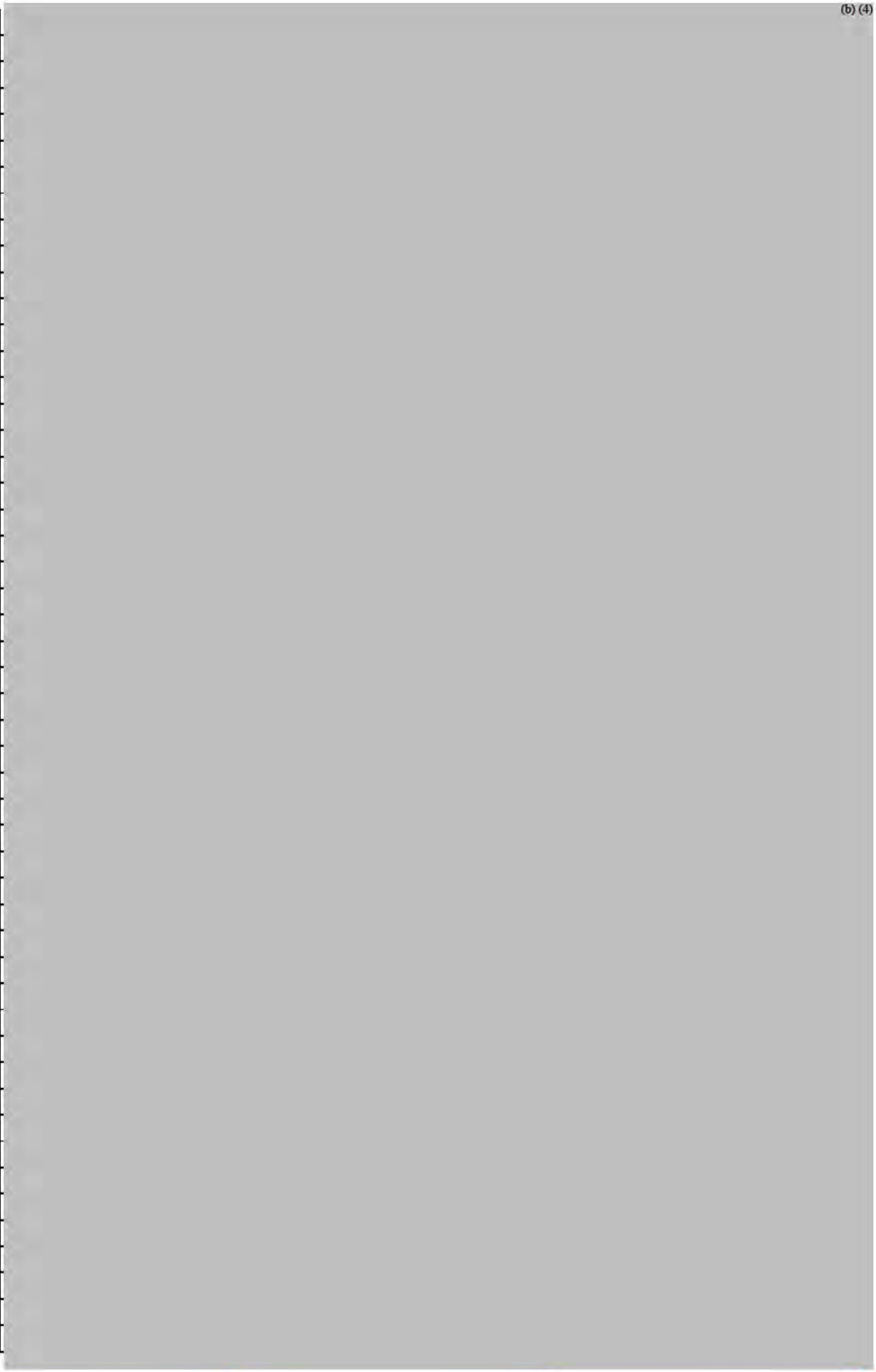

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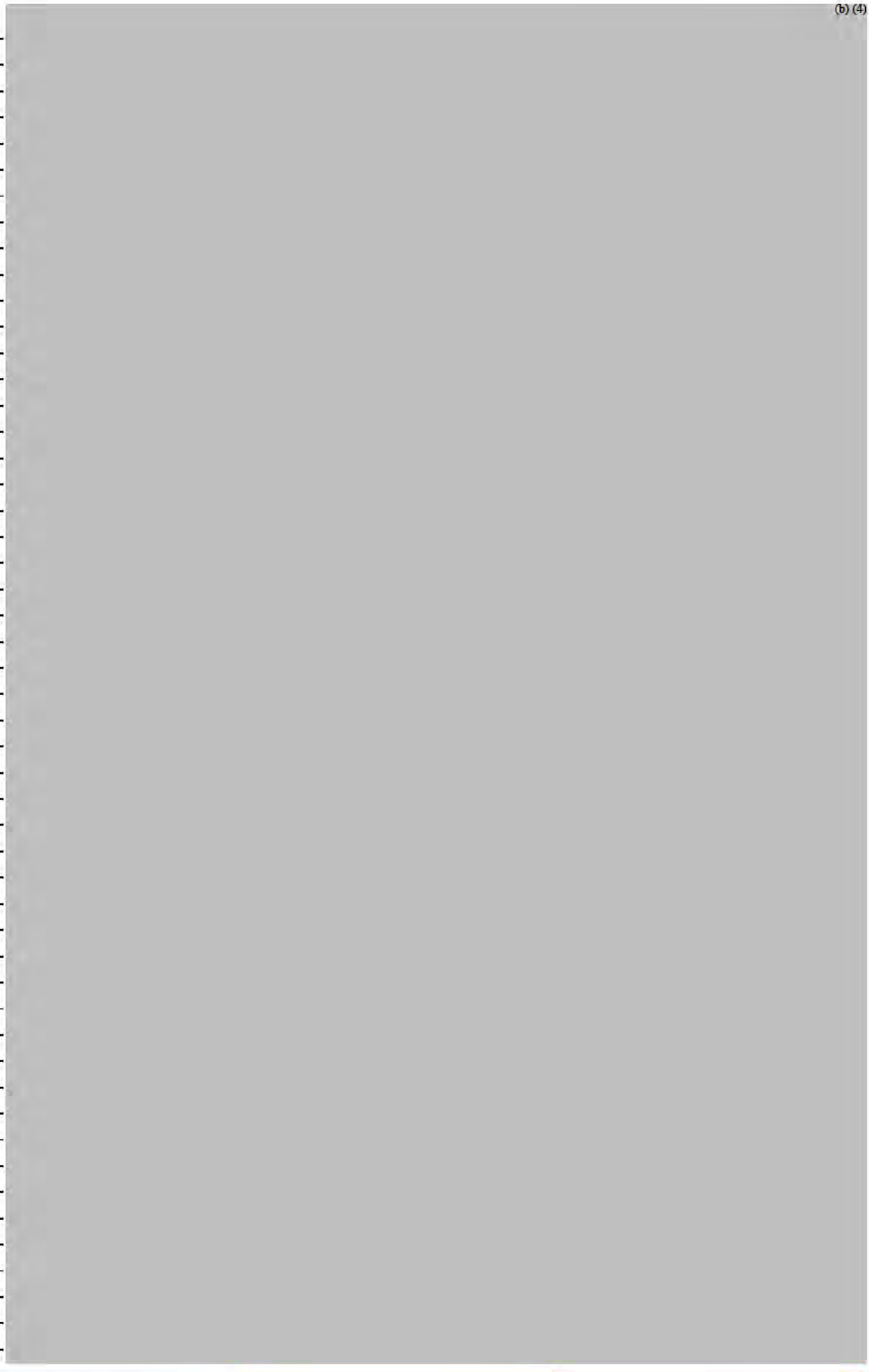

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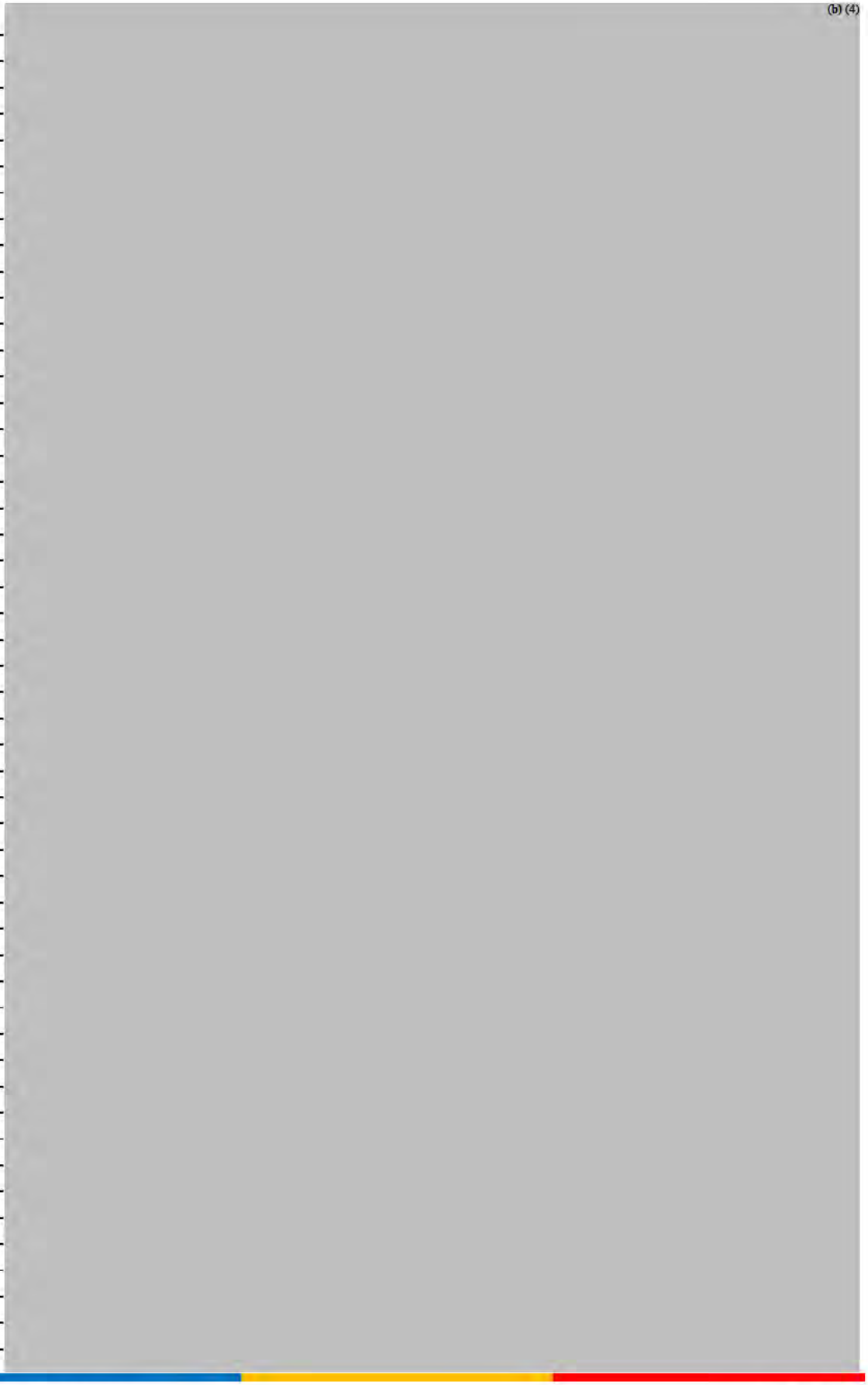

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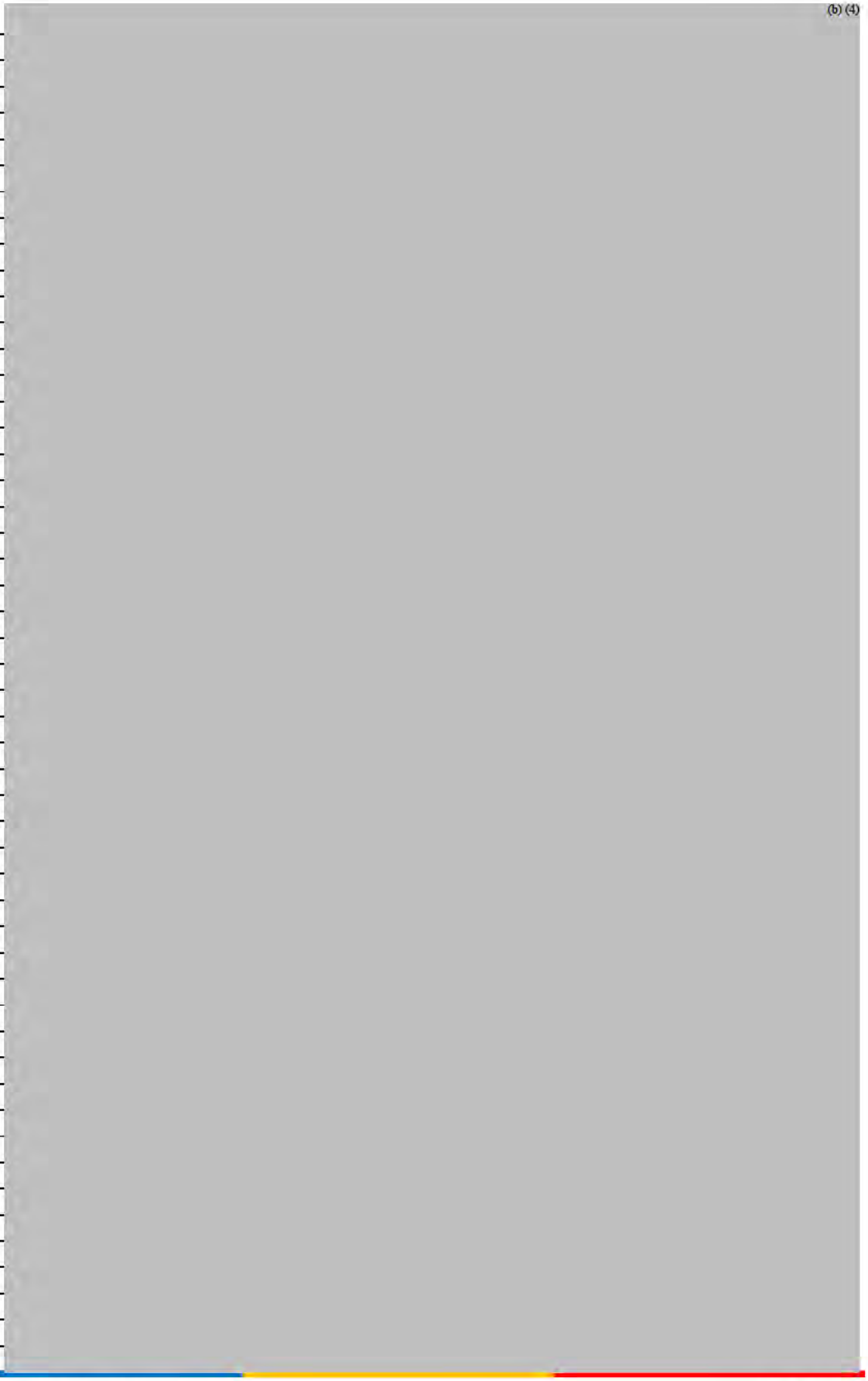

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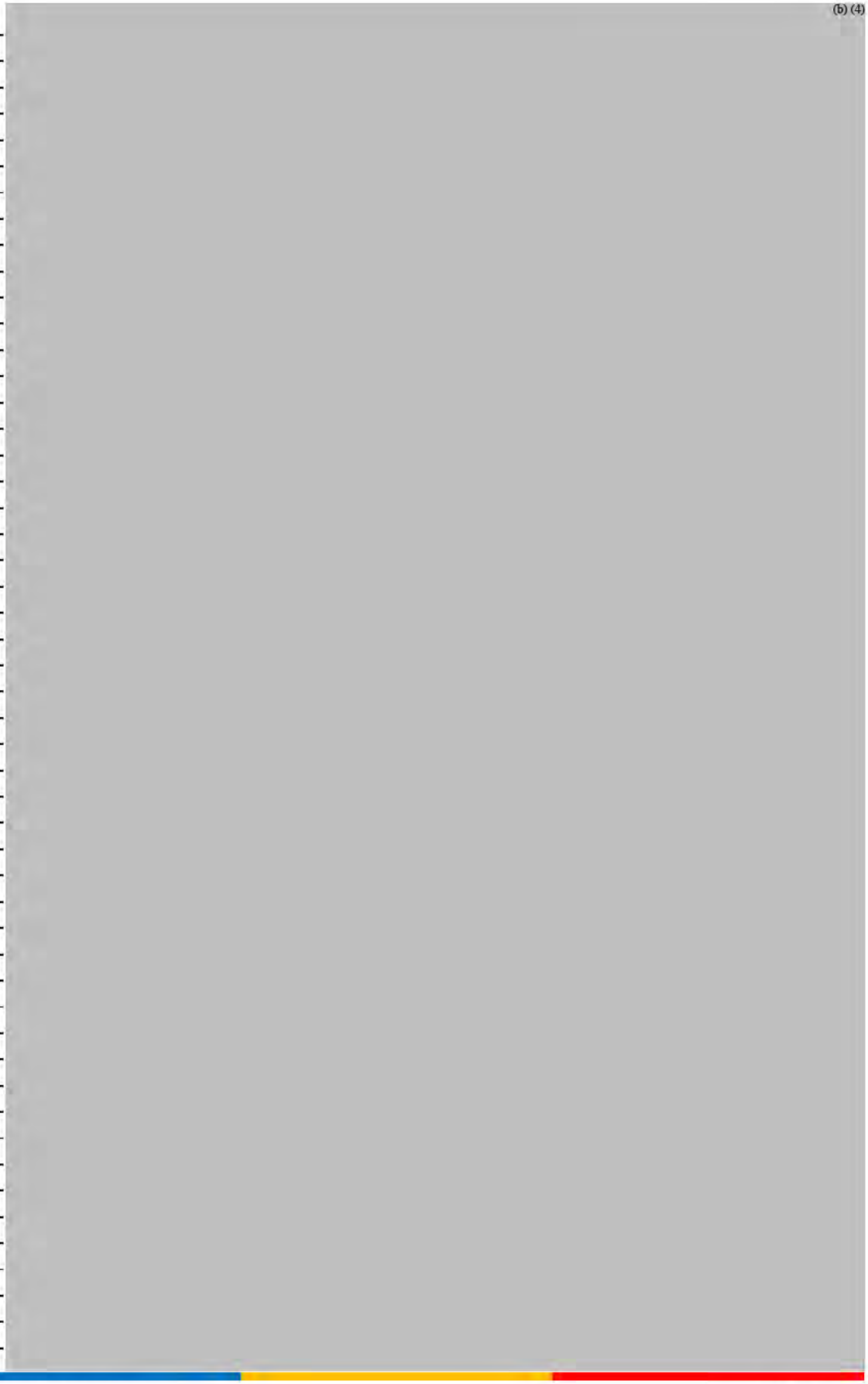

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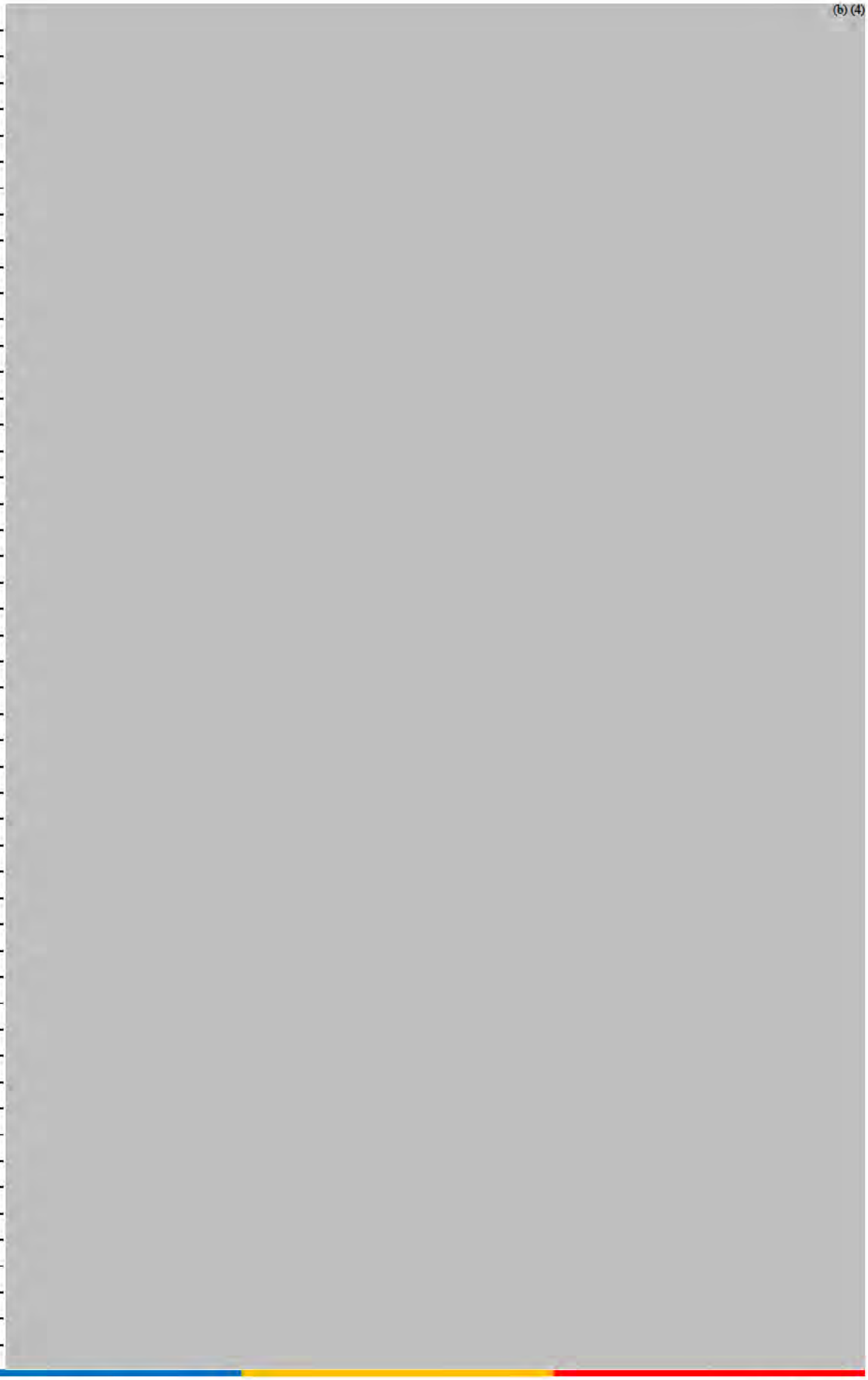

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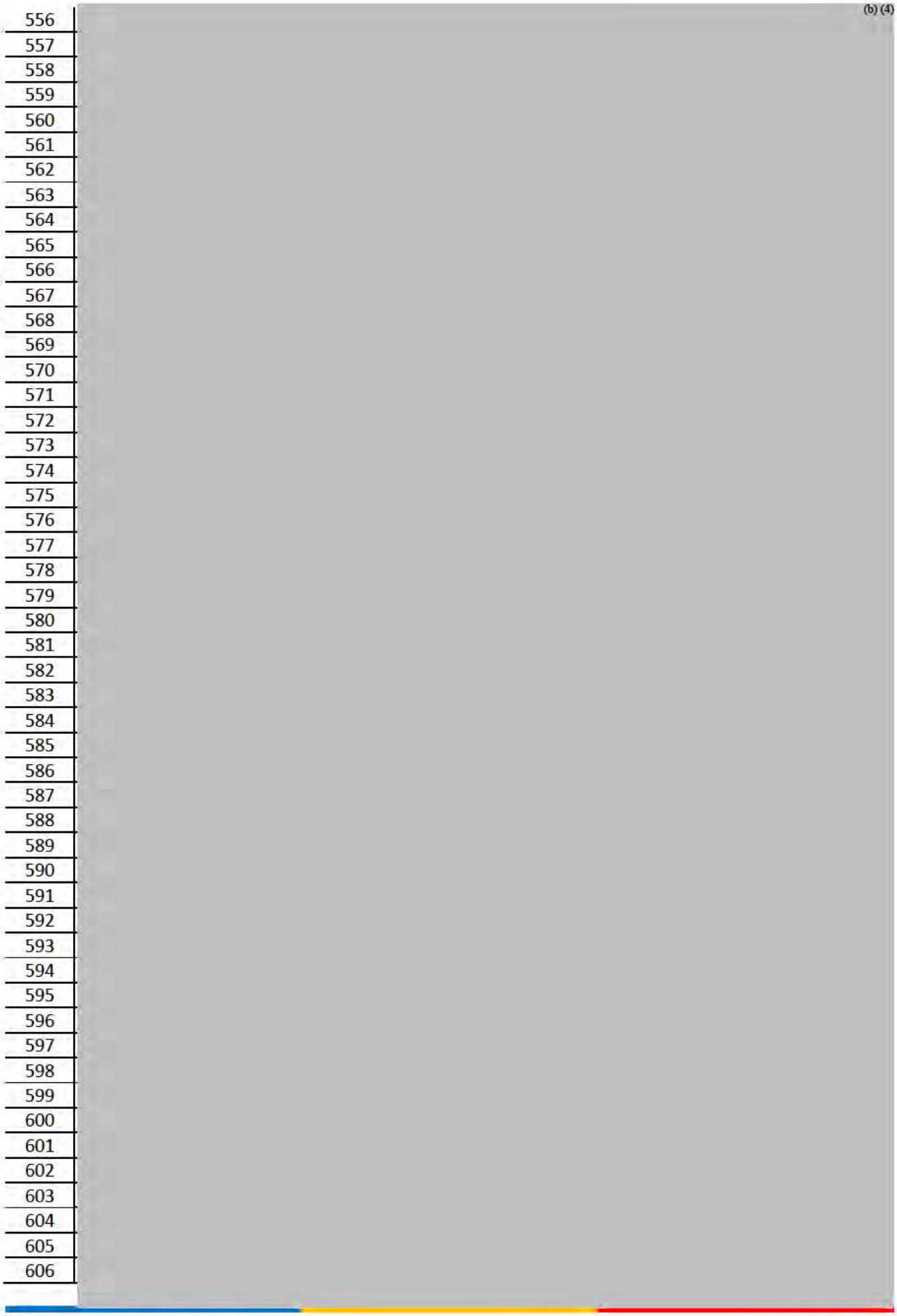

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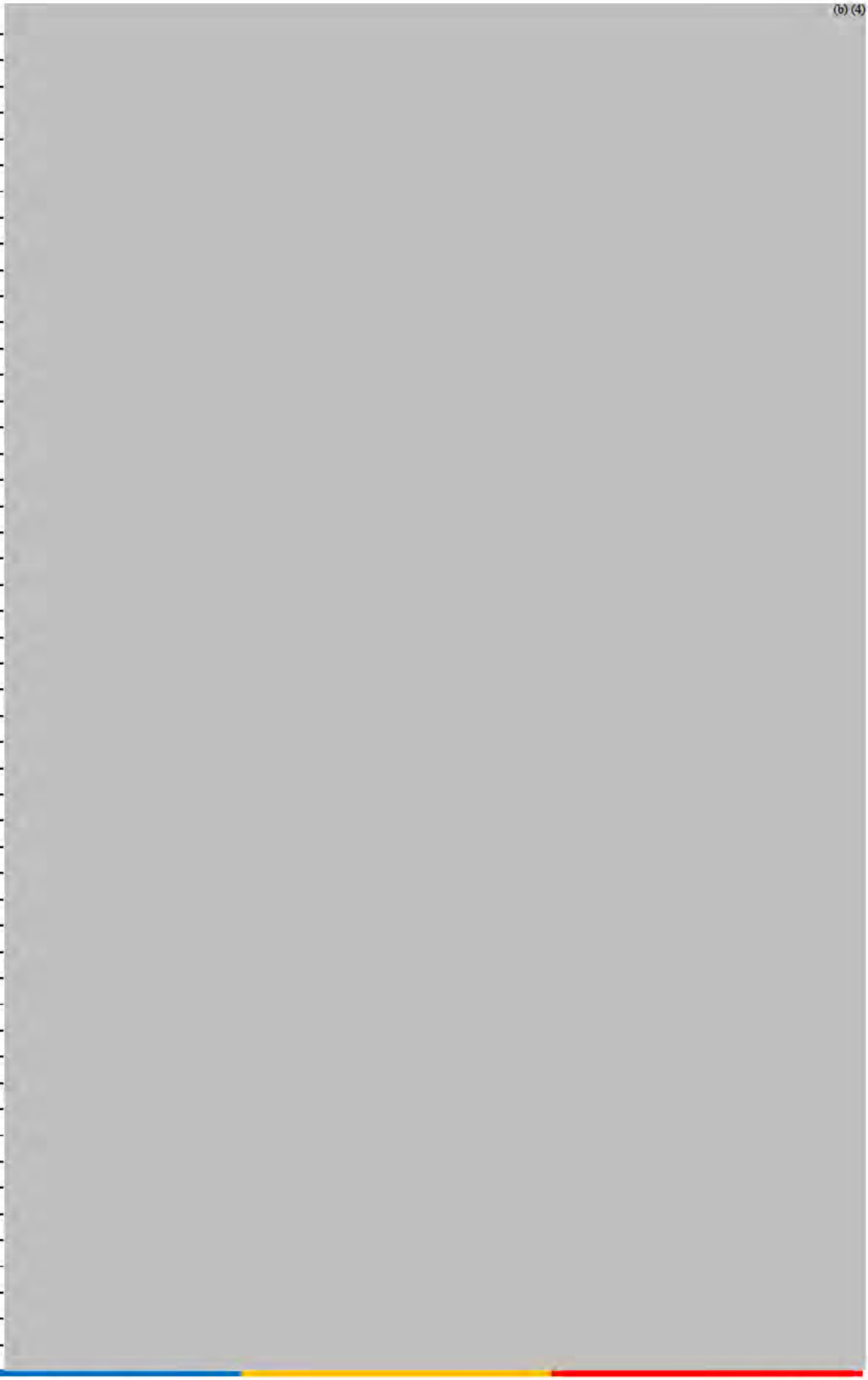

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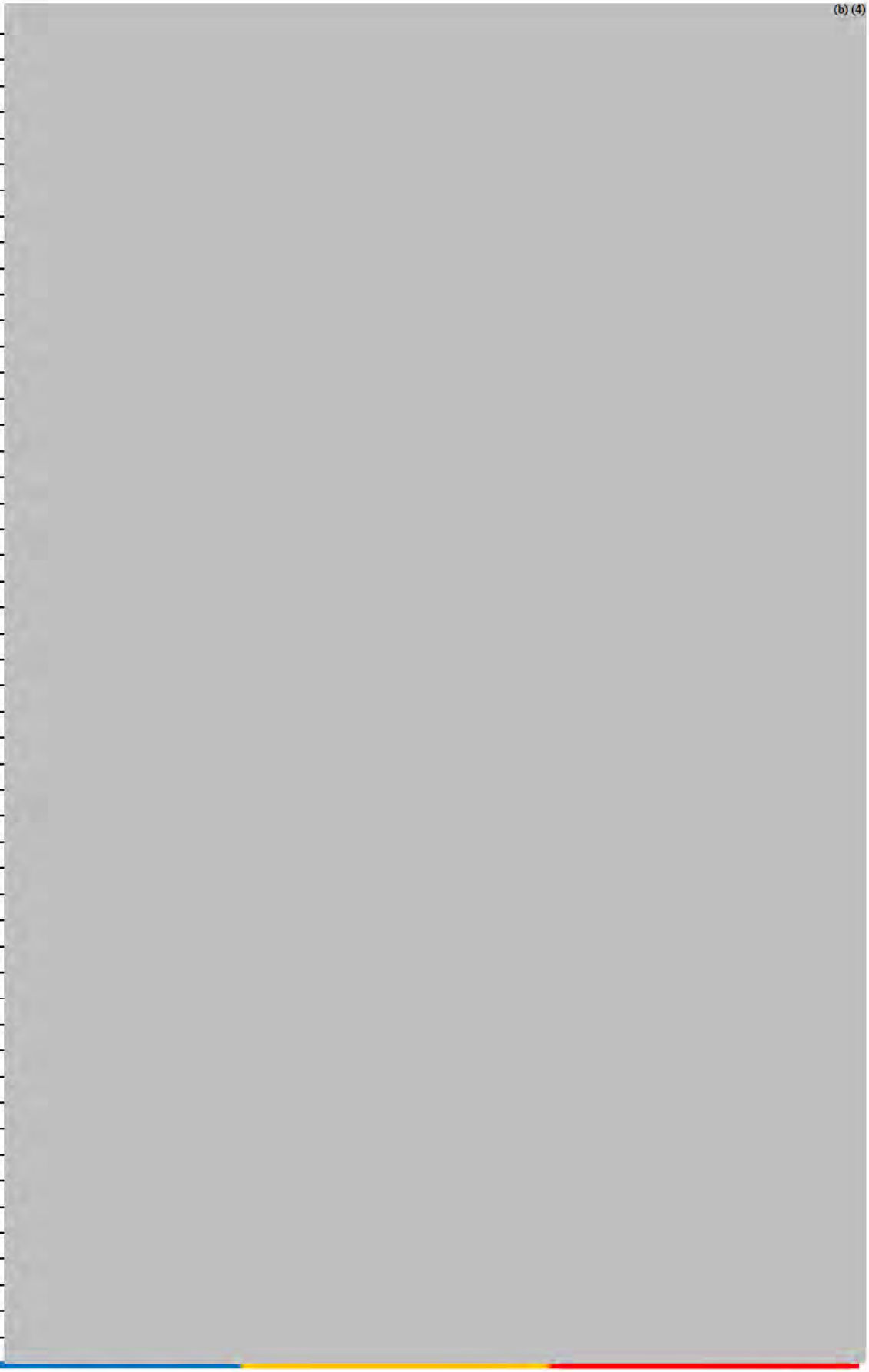

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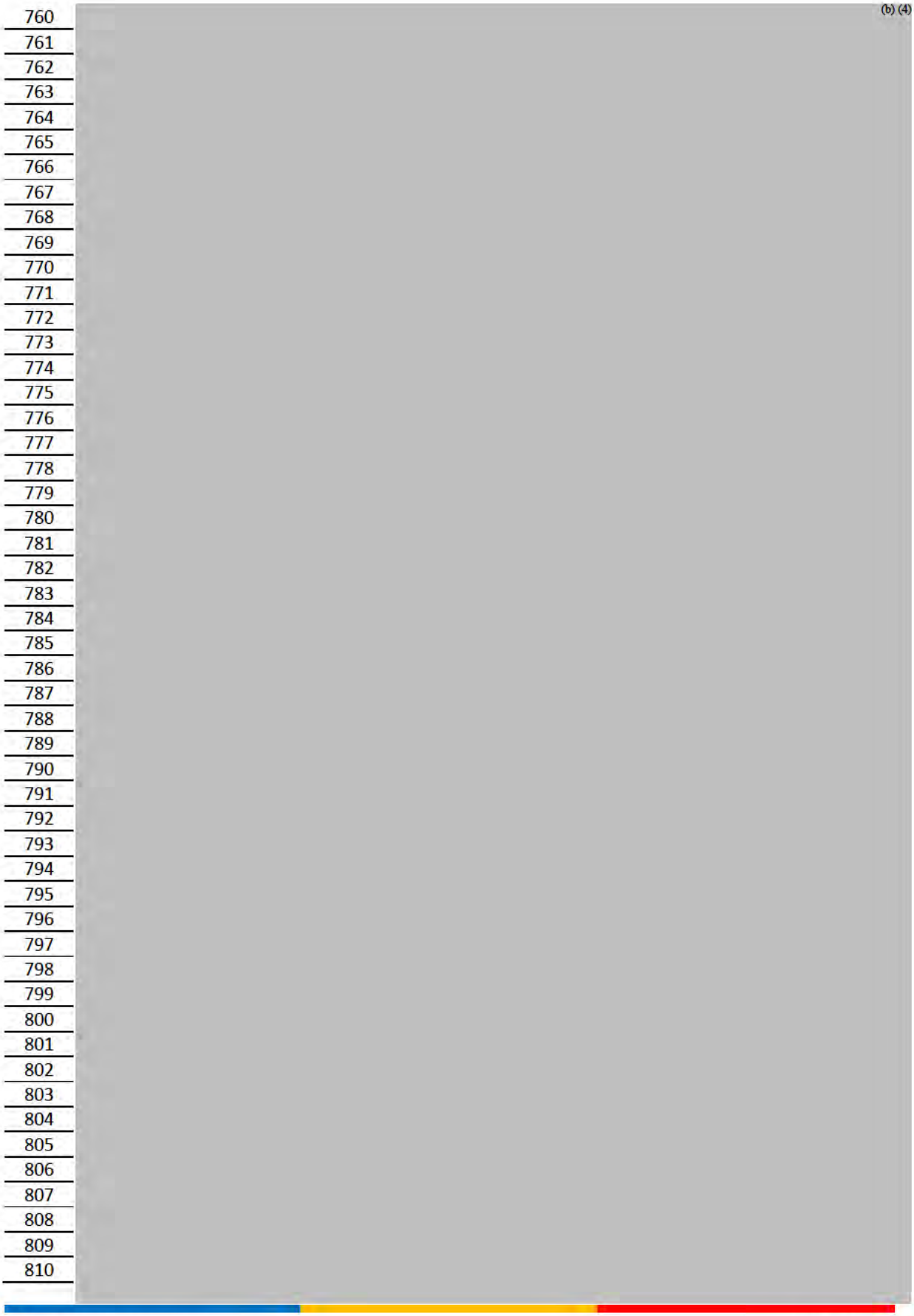

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Table S2. Gene modified regions of *C. glutamicum* CA08-0012

* MGEs related gene is highlighted in yellow.

No *	*C. glutamicum* ATCC 14067					*C. glutamicum* CA08-0012		
	Gene ID	Type	Position	Strd	Function	Modified Type	Gene ID	Function
1	CEY17_00470							
	CEY17_00475							
2	CEY17_01065							
	CEY17_01070							
	CEY17_01075							
	CEY17_01080							
3	CEY17_02095							
	CEY17_02100							
	CEY17_02105							
A	CEY17_03690							
	CEY17_03700							
4	CEY17_04320							
	CEY17_04330							
	CEY17_04335							
	CEY17_04340							
	CEY17_04345							
	CEY17_04350							
	CEY17_04355							
	CEY17_04360							
	CEY17_04365							

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	CEY17_04370
	CEY17_04375
	CEY17_04380
B	CEY17_05475
	CEY17_05485
5	CEY17_06260
	CEY17_06270
6	CEY17_08925
	CEY17_08970
C	CEY17_09245
	CEY17_09250
7	CEY17_10440
	CEY17_10450
	CEY17_10455
	CEY17_10460
8	CEY17_12865
	CEY17_12870
9	CEY17_13180
	CEY17_13185
10	CEY17_15070

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CEY17_15085	(b) (4)
CEY17_15090	
CEY17_15095	
CEY17_15105	
CEY17_15110	
CEY17_15115	
CEY17_15120	
CEY17_15125	
CEY17_15130	
CEY17_15135	
CEY17_15140	
CEY17_15145	
CEY17_15150	
CEY17_15155	
CEY17_15170	
CEY17_15175	
CEY17_15180	
CEY17_15185	
CEY17_15190	
CEY17_15195	
CEY17_15215	
CEY17_15220	
CEY17_15225	
CEY17_15230	
CEY17_15240	

CEY17_15245	
CEY17_15250	
CEY17_15255	
CEY17_15260	
CEY17_15265	
CEY17_15270	
CEY17_15280	
CEY17_15285	
CEY17_15290	
CEY17_15295	
CEY17_15300	
CEY17_15305	
CEY17_15310	
CEY17_15315	
CEY17_15320	
CEY17_15325	
CEY17_15335	
CEY17_15340	
CEY17_15345	
CEY17_15350	
CEY17_15360	
CEY17_15365	
CEY17_15370	
CEY17_15380	
CEY17_15385	

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CEY17_15390
CEY17_15395
CEY17_15400
CEY17_15405
CEY17_15410
CEY17_15420
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Table S3. Nucleotide sequence variation of *C. glutamicum* KCCM 80240 strain

No.	*C. glutamicm* CA08-0012		*C. glutamicm* KCCM80240			
	Ref. Position	Ref. Seq. Nuc.	Var. Nuc	Var. Position	Type	Var. ORF Name
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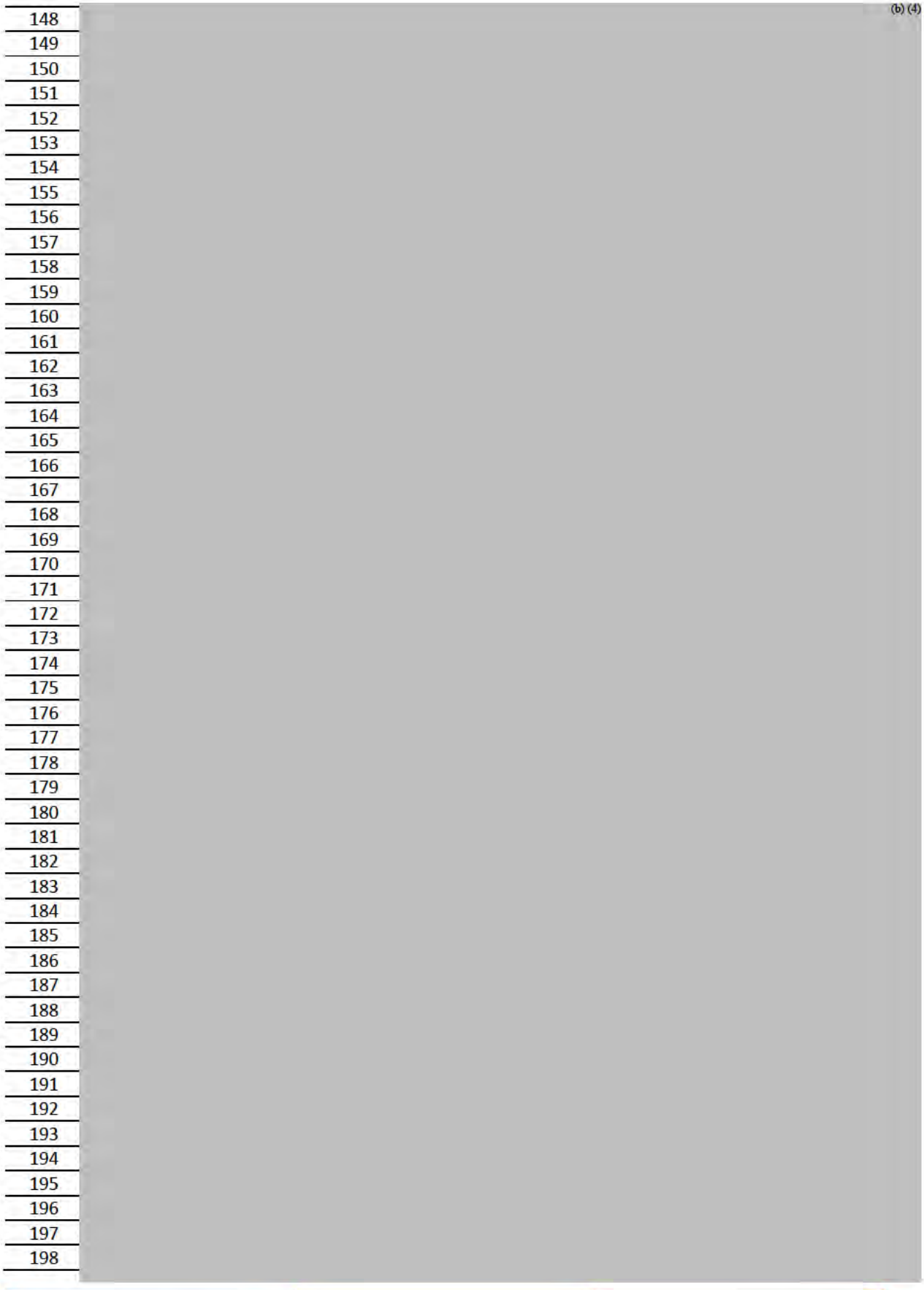

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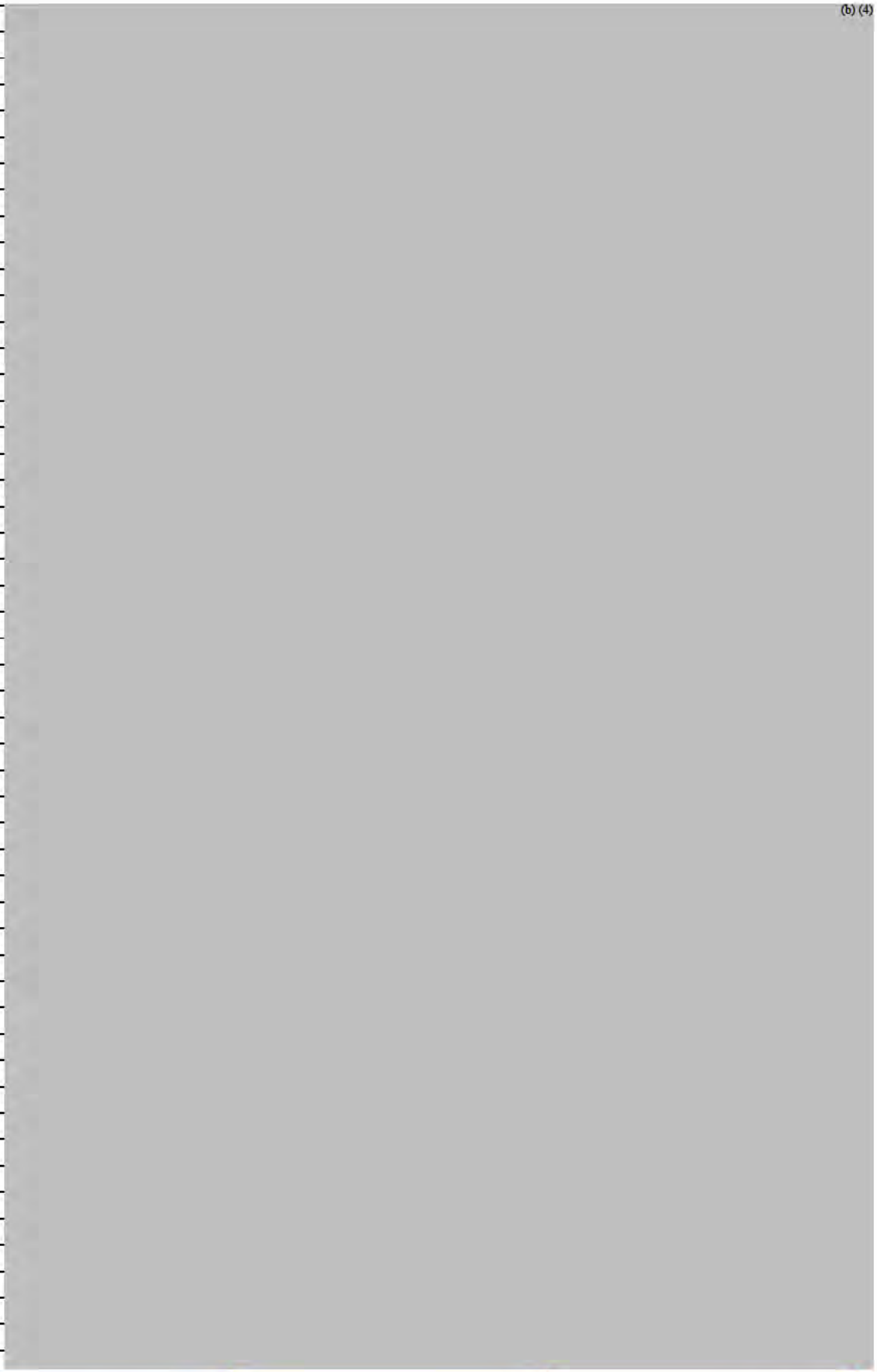

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Table S4. Gene modified regions of *C. glutamicum* KCCM 80240

* MGEs related gene is highlighted in yellow. The genetic modified site is marked in red.

No	*C. glutamicum* CA08-0012					*C. glutamicum* KCCM 80240		
	Gene ID	Type	Position	Strd	Function	Modified type	Gene ID	Function
1								
2								
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Figure S1. Phylogenetic tree of *C. glutamicum* KCCM 80240 based on 16s rDNA sequence analysis

REVISED APPENDIX 2. PRE-FERMENTATION INFORMATION (CONFIDENTIAL)

Table of Contents

List of Tables

List of Figures

A. CHARACTERIZATION OF THE PRODUCTION MICROORGANISM

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A.1 Scientific Name and Taxonomy of *C. glutamicum* KCCM 80240

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(b) (4)

A.2 Nature Habitat of *C. glutamicum* and Its Ecological Role

It was reported that Corynebacteriaceae are rod-shaped, fast growing, non-sporulating gram-positive bacteria that are found widespread in nature. A large number of corynebacterial species were isolated from human clinical samples or animals, but several others were isolated from soils, cheese, dairy products, vegetables and fruits. Some of these species were also found in marine samples. It seems that these bacteria are widely spread throughout nature which induces high diversity in the Corynebacterium genus. The natural habitat of *C. glutamicum* strains have been reported in soil, soils contaminated with bird feces, sewage, manure, and vegetables and fruits (Eggeling and Bott, 2005).

A.3 Phenotypic Characteristics of *C. glutamicum* KCCM 80240

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Table A.3.1. Phenotypic characteristics of *C. glutamicum* ATCC14067, *C. glutamicum* CA08-0012 and *C. glutamicum* KCCM 80240

	***C. glutamicum* ATCC 14067 (Wild-type strain)**	***C. glutamicum* CA08-0012 (Parental strain)**	***C. glutamicum* KCCM 80240 (Production strain)**
Colony shape	(b) (4)		(b) (4)
Colony color			
Cell arrangement			
Cell shape			
16s rDNA homology			
Optimal temperature range			
Optimal pH range			

A.4 Genetic Comparison of Host to Published Data of the Species

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Certification of The Safety Deposit

Name and Address of Depositor
To : CJ CheilJedang
330, Dongho-ro, Jung-gu, Seoul, 04560,
Korea

1. IDENTIFICATION OF THE MICROORGANISMS

Identification reference given by the Depositor:	Accession number given by the Safety Depositary Authority:
Corynebacterium glutamicum CE02-CA08-1331	KCCM 80240

2. RECEIPT AND ACCEPTANCE

This Safety Depositary Authority accepts the microorganism identified under 1 above, which was received it on September. 25. 2020.

3. SAFETY DEPOSITARY AUTHORITY

Name: Korean Culture Collection of Microorganisms Address: Yurim Bild. 45, Hongjenae 2ga-gil, Seodaemun-gu, Seoul, 03641, Republic of Korea	Signature(s) of person(s) having the power to represent the Safety Depositary Authority or of authorized official(s): Date: September. 25. 2020.

한국미생물보존센터
KOREAN CULTURE CENTER OF MICROORGANISMS

Figure A.1.1. Certificate of deposition (*C. glutamicum* KCCM 80240)

KCCM KFCC 사단법인 한국종균협회 한국미생물보존센터
03641 서울시 서대문구 홍제2가길 45 유림빌딩
TEL : (02)391-0950
FAX : (02)392-2859
Home Page : http://www.kccm.or.kr

KOREAN CULTURE CENTER OF MICROORGANISMS
FORMERLY FEDERATION OF CULTURE COLLECTIONS
45, Hongjenae 2ga-gil, Seodaemun-gu, Seoul 03641, Korea
TEL : 82-2-391-0950
FAX : 82-2-392-2859
Home Page : http://www.kccm.or.kr

No.20-83

2020-09-25

Certification of Analysis

Dear CJ CheilJedang
330, Dongho-ro, Jung-gu,
Seoul, Korea
04560

We have performed the 16S rDNA sequence analysis of your strain **KCCM80240**. The result is as follows:

KCCM80240 : *Corynebacterium glutamicum*
(GenBank Data homology search result : 99%)

Please refer to sequence and phylogeny tree.

Sincerely yours

(b) (4)

Korean Culture Collection of Microorganisms (KCCM)
45, Hongjenae 2ga-gil, Seodaemun-gu, Seoul, Korea. 03641
Tel : 82-2-391-0950
FAX: 82-2-392-2859

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>KCCM80240

Figure A.3.1. 16s rDNA sequence analysis of *C. glutamicum* KCCM 80240

B. INFORMATION OF DRIED L-VALINE FERMENTATION PRODUCT PRODUCING STRAIN, *CORYNEBACTERIUM GLUTAMICUM* KCCM 80240

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B.1 Information of Genetic Modification in *C. glutamicum* KCCM 80240

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B.1.1 Random Mutagenesis

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B.1.2 Site-directed Mutagenesis

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B.1.3 Overexpression of Biosynthetic Genes, Especially Deregulated Genes Encoding Key Enzymes, for Producing C. glutamicum KCCM 80240

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The production strain of Dried L-Valine Fermentation Product was deposited as *C. glutamicum* KCCM 80240 at KCCM (Korea Culture Center of Microorganisms) located in the South Korea.

Table B.1.1. Summary of genetic modification in *C. glutamicum* KCCM 80240

Modified gene	Modified locus	Modification method	Copy number of integration gene	Characteristics	
				Parental organism	Donor organism
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B.2 Donor Organism

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B.3 Descriptions of Genetic Modification

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B.3.1 Vector Used for Genetic Modification

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Figure B.3.1. Marker free insertion method

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Table B.3.1. Sequence of pDZ-MCS

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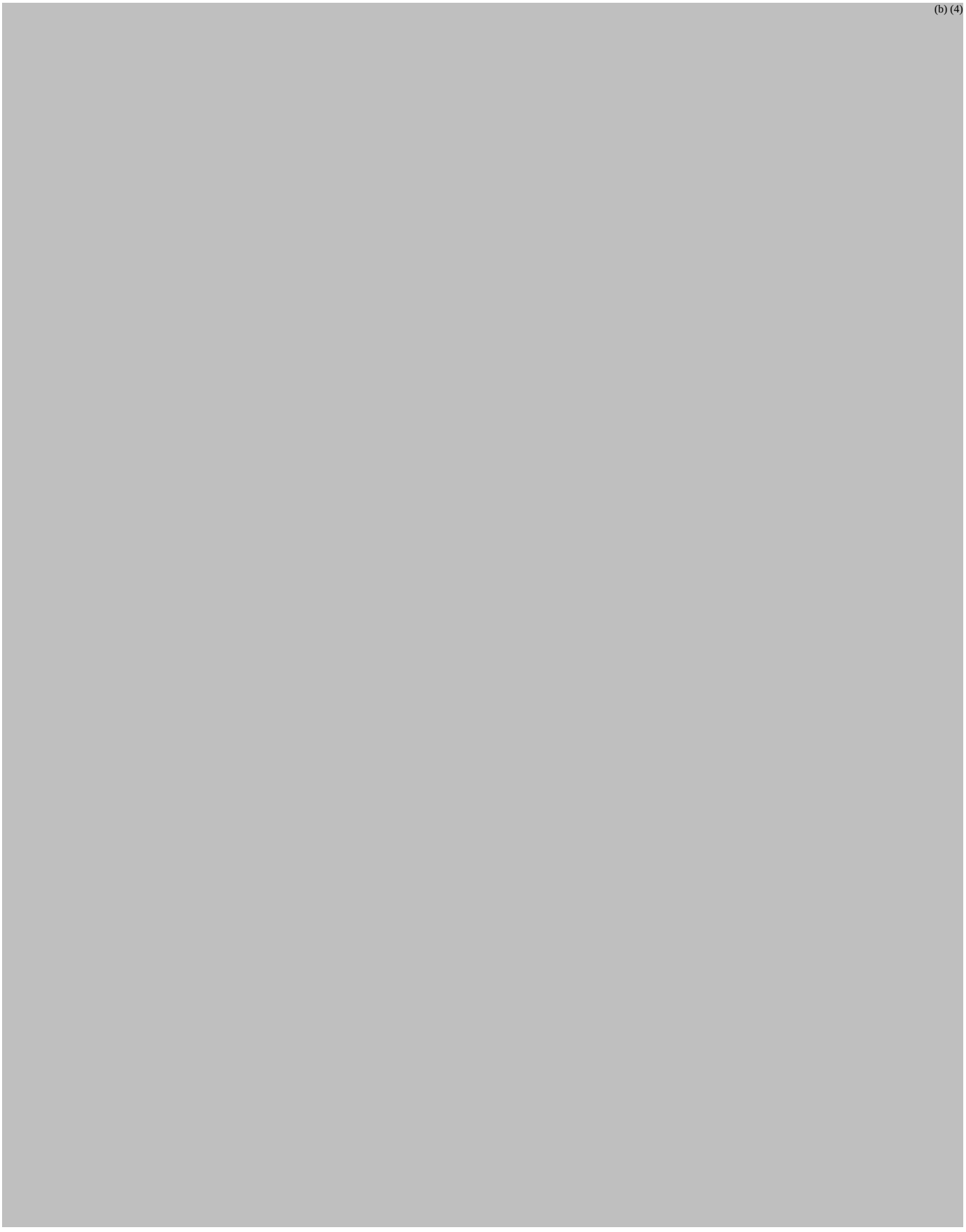

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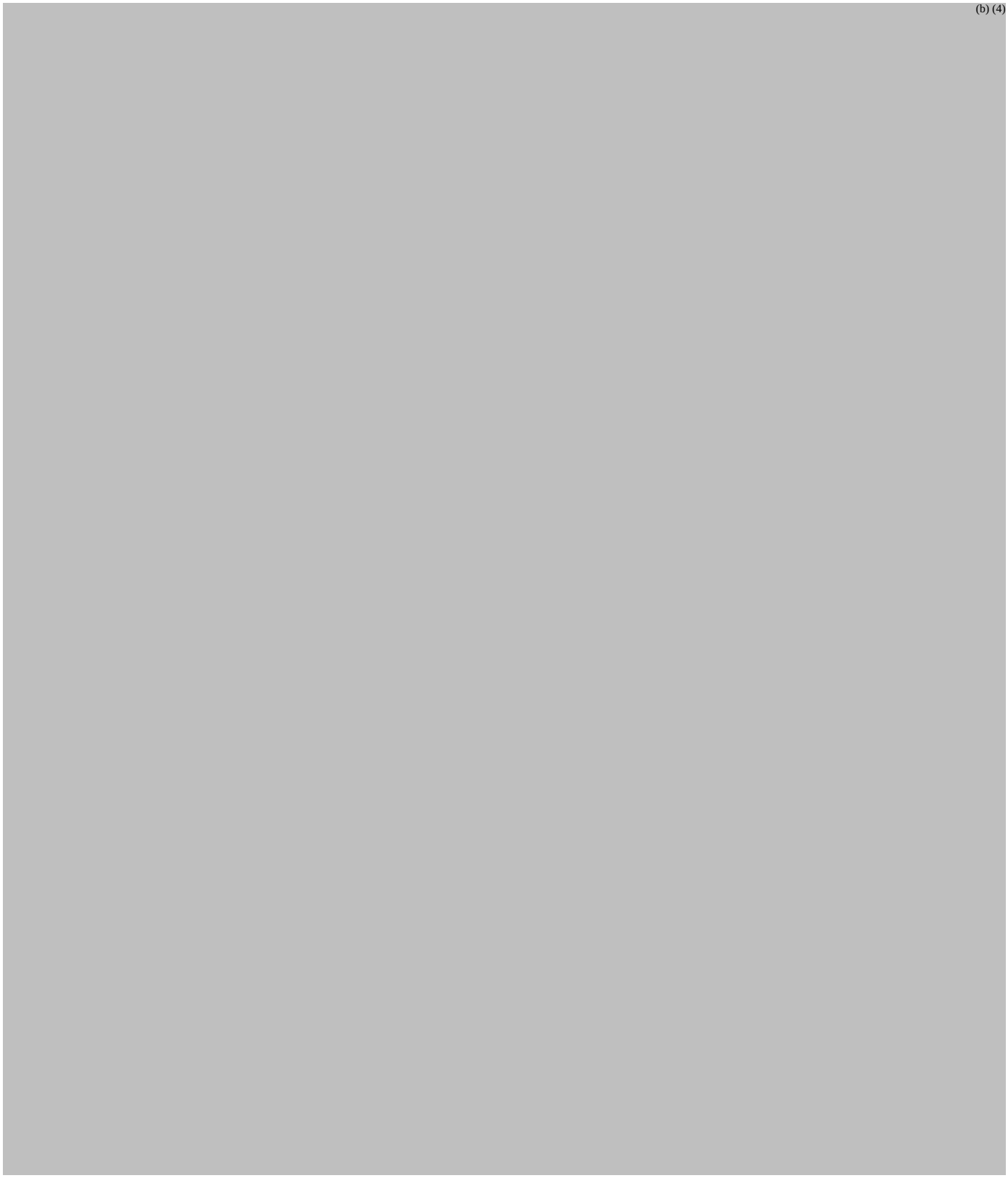

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B.3.3 Replacement of Original ilvC Gene with Duplicated ilvC Gene

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Table B.3.1. Primer sequence used to construct *C. glutamicum* KCCM 80240

No	SEQ ID	Sequence (5' → 3')
1	SEQ ID No 01	(b) (4)
2	SEQ ID No 02	
3	SEQ ID No 03	
4	SEQ ID No 04	
5	SEQ ID No 05	
6	SEQ ID No 06	
7	SEQ ID No 07	
8	SEQ ID No 08	
9	SEQ ID No 09	
10	SEQ ID No 10	
11	SEQ ID No 11	
12	SEQ ID No 12	
13	SEQ ID No 13	
14	SEQ ID No 14	
15	SEQ ID No 15	
16	SEQ ID No 16	
17	SEQ ID No 17	
18	SEQ ID No 18	
19	SEQ ID No 19	
20	SEQ ID No 20	
21	SEQ ID No 21	
22	SEQ ID No 22	
23	SEQ ID No 23	
24	SEQ ID No 24	
25	SEQ ID No 25	
26	SEQ ID No 26	
27	SEQ ID No 27	
28	SEQ ID No 28	
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30	SEQ ID No 30	
31	SEQ ID No 31	
32	SEQ ID No 32	
33	SEQ ID No 33	
34	SEQ ID No 34	
35	SEQ ID No 35	
36	SEQ ID No 36	
37	SEQ ID No 37	
38	SEQ ID No 38	
39	SEQ ID No 39	
40	SEQ ID No 40	
41	SEQ ID No 41	
42	SEQ ID No 42	
43	SEQ ID No 43	
44	SEQ ID No 44	
45	SEQ ID No 45	
46	SEQ ID No 46	

No	SEQ ID	Sequence (5' → 3')
47	SEQ ID No 47	(b) (4)
48	SEQ ID No 48	
49	SEQ ID No 49	
50	SEQ ID No 50	
51	SEQ ID No 51	
52	SEQ ID No 52	
53	SEQ ID No 53	
54	SEQ ID No 54	
55	SEQ ID No 55	
56	SEQ ID No 56	
57	SEQ ID No 57	
58	SEQ ID No 58	
59	SEQ ID No 59	
60	SEQ ID No 60	
61	SEQ ID No 61	
62	SEQ ID No 62	
63	SEQ ID No 63	
64	SEQ ID No 64	
65	SEQ ID No 65	
66	SEQ ID No 66	
67	SEQ ID No 67	
68	SEQ ID No 68	
69	SEQ ID No 69	
70	SEQ ID No 70	
71	SEQ ID No 71	
72	SEQ ID No 72	
73	SEQ ID No 73	
74	SEQ ID No 74	
75	SEQ ID No 75	
76	SEQ ID No 76	
77	SEQ ID No 77	
78	SEQ ID No 78	
79	SEQ ID No 79	
80	SEQ ID No 80	
81	SEQ ID No 81	
82	SEQ ID No 82	
83	SEQ ID No 83	
84	SEQ ID No 84	
85	SEQ ID No 85	
86	SEQ ID No 86	

B.4 Identification and Detection Techniques

(b) (4)

Table B.4.1. Comparison of PCR products sizes between *C. glutamicum* ATCC 14067 and *C. glutamicum* KCCM 80240

Gene	Seq No	Primer sequence (5' → 3')	Integrated locus	PCR size (bp)	
				ATCC 14067	KCCM 80240

(b) (4)

B.5 Description of Gene Deletion Region(s)

(b) (4)

(b) (4)

Table B.5.1. Size and function of deleted gene

Deleted gene	Function	Size (bp)	
		Whole gene	Deleted gene
(b) (4)			

(b) (4)

Name	Sequence (5' → 3')	Size (bp)
(b) (4)		

Name	Sequence (5' → 3')	Size (bp)
	(b) (4)	

B.6 Promoter Information

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

Promoter Name	Origin	Sequence (5' → 3')	Length (bp)	Applied ORF
(b) (4)				

B.7 Description of Gene Integration

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

	rated locus	Location in genome
(b) (4)		

(b) (4)

		Size (bp)	Origin
(b) (4)			
(b) (4)			
(b) (4)			

Name	Sequence (5' → 3')	Size (bp)	Origin
			(b) (4)
			(b) (4)
			(b) (4)
			(b) (4)

Name	Sequence (5' → 3')	Size (bp)	Origin
[redacted]	[redacted]	[redacted]	[redacted]

(b) (4)

Table B.7.3. Sequence of introduced Pcj7-*ilv*E

Name	Sequence (5' → 3')	Size (bp)	Origin
[redacted]	[redacted]	[redacted]	[redacted]

(b) (4)

(b) (4)

Name	Sequence (5' → 3')	Size (bp)	Origin
(b) (4)			
(b) (4)			

(b) (4)

Name	Sequence (5' → 3')	Size (bp)	Origin
(b) (4)			

Name	Sequence (5' → 3')	Size (bp)	Origin
			(b) (4)

Name	Sequence (5' → 3')	Size (bp)	Origin
(b) (4)			

(b) (4)

*Mutations are underlined.

(b) (4)

Name	Sequence (5' → 3')	Size (bp)	Origin
(b) (4)			

Name	Sequence (5' → 3')	Size (bp)	Origin
			(b) (4)
			(b) (4)

Name	Sequence (5' → 3')	Size (bp)	Origin
			(b) (4)

(b) (4)

Name	Sequence (5' → 3')	Size (bp)	Origin
			(b) (4)

Name	Sequence (5' → 3')	Size (bp)	Origin

(b) (4)

Name	Sequence (5' → 3')	Size (bp)	Origin
(b) (4)			
(b) (4)			

(b) (4)

Name	Sequence (5' → 3')	Size (bp)	Origin
(b) (4)			

Name	Sequence (5' → 3')	Size (bp)	Origin
			(b) (4)
			(b) (4)

Name	Sequence (5' → 3')	Size (bp)	Origin
			(b) (4)

(b) (4)

Name	Sequence (5' → 3')	Size (bp)	Origin
			(b) (4)

Name	Sequence (5' → 3')	Size (bp)	Origin
(b) (4)			

(b) (4)

Name	Sequence (5' → 3')	Size (bp)	Origin
			(b) (4)
			(b) (4)

Name	Sequence (5' → 3')	Size (bp)	Origin
			(b) (4)
			(b) (4)

*Mutations are underlined.

(b) (4)

Name	Sequence (5' → 3')	Size (bp)	Origin
			(b) (4)
			(b) (4)

Name	Sequence (5' → 3')	Size (bp)	Origin
			(b) (4)
			(b) (4)

Name	Sequence (5' → 3')	Size (bp)	Origin
(b) (4)			
(b) (4)			

B.8 Safety of DNA Modification

(b) (4)

(b) (4)

B.9 Genetic Stability of *C. glutamicum* KCCM 80240

(b) (4)

B.10 Open Reading Frame (ORF) Analysis of Genetically Modified Region

(b) (4)

(b) (4)

(b) (4)

(b) (4)

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(b) (4)

(b) (4)

Table B.10.1. Location of modified gene in genome

Genes	Modification type	Locus	Location in genome
(b) (4)			

(b) (4)

(b) (4)

B.11 Open Reading Frame Analysis of Full Genome Sequence of *C. glutamicum* KCCM 80240

(b) (4)

Table B.11.1. Comparison of ORF between the *C. glutamicum* ATCC14067, *C. glutamicum* CA08-0012 and *C. glutamicum* KCCM80240

Feature	Wild-type strain ATCC 14067	Parental strain CA08-0012	Production strain KCCM 80240
(b) (4)			

(b) (4)

C. SPILL-OVER ANALYSIS

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

C.1 Comparison Metabolic Flux of *C. glutamicum* ATCC 14067 and *C. glutamicum* KCCM 80240

(b) (4)

(b) (4)

(b) (4)

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(b) (4)

Table C.1.1 MFA of *C. glutamicum* ATCC 14067 and *C. glutamicum* KCCM 80240

Pathway	ATCC 14067	KCCM 80240
Glycolysis		

(b) (4)

Pathway	ATCC 14067	KCCM 80240
		(b) (4)

Pathway	ATCC 14067	KCCM 80240
(b) (4)		

C.2 Comparison of Metabolite in *C. glutamicum* ATCC 14067 and *C. glutamicum* KCCM 80240

(b) (4)

(b) (4)

(b) (4)

(b) (4)

Table C.2.1. Amino acid of *C. glutamicum* ATCC 14067, *C. glutamicum* CA08-0012 and *C. glutamicum* KCCM 80240 fermentation broth (3KL Pilot scale, the end of fermentation)

	ATCC 14067 (g/L)	CA08-0012 (g/L)	KCCM 80240 (g/L)			
	Batch1	Batch1	Batch1	Batch2	Batch3	Ave
OD_{562}					(b) (4)	57.6
Asp						0.00
Thr						0.07
Ser						0.01
Glu						0.20
Gly						0.14
Ala						0.17
Cys						0.00
Val						92.51
Met						0.00
Ile						0.12
Leu						0.14
Tyr						0.11
Phe						0.32

Lys	(b) (4)	0.02
His		0.31
Arg		0.00

** Asp: aspartate, Thr: threonine, Ser: serine, Glu: glutamate, Gly: glycine, Ala: alanine, Cys: cysteine, Val: valine, Met: methionine, Ile: isoleucine, Leu: leucine, Tyr: tyrosine, Phe: phenylalanine, Lys: lysine, His: histidine, Arg: arginine*

*** Analytical method: L-Valine-HPLC, Free amino acids (except L-valine)-AOAC 999.13*

(b) (4)

(b) (4)

Table C.2.2. Organic acid of *C. glutamicum* ATCC 14067, *C. glutamicum* CA08-0012 and *C. glutamicum* KCCM 80240 fermentation broth (3KL Pilot scale, the end of fermentation)

	ATCC 14067 (g/L)	CA08-0012 (g/L)	KCCM 80240 (g/L)			
	Batch1	Batch1	Batch1	Batch2	Batch3	Ave.
Citric acid	(b) (4)					0.00
Malic acid						0.04
Succinic acid						0.00
Lactic acid						0.00
Formic acid						0.00
Acetic acid						0.01

** Analytical method: Korean Feed Standards Codex, 1 of chapter 14*

(b) (4)

C.3 Biogenic Amines

(b) (4)

(b) (4)

(b) (4)

Table C.2.6. Biogenic amines of *C. glutamicum* ATCC 14067, *C. glutamicum* CA08-0012 and *C. glutamicum* KCCM 80240 fermentation broth

	ATCC 14067 (mg/L)				CA08-0012 (mg/L)				KCCM 80240 (mg/L)			
	Batch1	Batch2	Batch3	Ave.	Batch1	Batch2	Batch3	Ave.	Batch1	Batch2	Batch3	Ave.
Cadaverine	(b) (4)			0.110	(b) (4)			0.128	(b) (4)			0.020
Histamine				0.104				0.185				0.153
Phenylethyl-amine				0.067				0.079				0.077
Putrescine				1.015				1.352				0.248
Tryptamine				0.010				0.010				0.008
Tyramine				2.851				3.351				3.654

(b) (4)

D. LIST OF ATTACHMENTS

Attachment 1	Determination of Antibiotic Minimal Inhibitory Concentration (MIC) of *Corynebacterium glutamicum* KCCM 80240, 9 pages
Attachment 2	Determination of viable cells of the production strain in Dried L-Valine Fermentation Product, 16 pages
Attachment 3	Genetic stability of *Corynebacterium glutamicum* KCCM 80240, 7 pages
Attachment 4	Whole genome sequence analysis of *Corynebacterium glutamicum* KCCM 80240, 59 pages
Attachment 5	Metabolic flux analysis of *Corynebacterium glutamicum* KCCM 80240, 15 pages

E. LIST OF REFERENCES

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CONFIDENTIAL REPORT

Stability test

: Dried L-Valine Fermentation Product (VAL Pro)

Version 2.0

TITLE

Stability test: Dried L-Valine Fermentation Product (VAL pro)

OBJECTIVE OF THE STUDY

This study was conducted to establish a shelf life for the Dried L-Valine Fermentation Product (VAL Pro) under the recommended storage conditions.

SCHEDULE OF THE STUDY

Initiation of experiment: June 26, 2020

Termination of experiment: August 26, 2021

Submission of interim report: December 31, 2020

Submission of final report: October 7, 2021

TESTING FACILITY

Institute of Biotechnology) Scientific and Regulatory Affairs, CJ CheilJedang
55, Gwanggyo-ro 42beon-gil, Yeongtong-gu, Suwon-si, Gyeonggi-do 16495, Korea

RESPONSIBLE STAFFS

Analyst and Author	Ran Young Yoon	
Report approved by	Yang Hee Kim	

CONTENTS

MATERIALS AND METHODS

The stability test of Dried L-Valine Fermentation Product (VAL Pro) was conducted in accordance with the ICH HARMONISED TRIPARTITE GUIDELINE [1].

Information of test sample

1) Sample: Dried L-Valine Fermentation Product (VAL Pro)

- L-Valine (dry base): not less than 72%
- Moisture: not more than 5.0%

2) Batch number: NGVAL191221, NGVAL191222, NGVAL191223

Storage condition

1) Packaging: Polypropylene woven bag and 1 ply polyethylene inner

2) Weight of storage sample: 50 g / bag

3) Temperature and humidity of storage

- General condition: 25°C ± 2°C and 60% RH ± 5% RH
- Accelerated condition: 40°C ± 2°C and 75% RH ± 5% RH

4) Testing frequency: Initial, 1, 3, 4, 6 months

Analysis method

1) Content of L-valine: HPLC

Parameter	Condition
System	HPLC (SHIMADZU Nexera UPLC-30A)
Detector	Fluorescence detector (Excitation λ : 338 nm Emission λ : 425 nm)
Column	ODS C18, 150 x 4.6 mm, particle size 3 µm
Column temperature	40 °C
Mobile phase	16.7 mM-KH_2PO_4 + 5 mM OSA in 12% CH_3CN, pH 2.5 (by H_3PO_4)
Flow rate of mobile phage	1.0 ml/min
Reaction reagent	201.91 mM-KOH + 241.39 mM-H_3BO_3 + 2.53 mM-OPA + C_2H_6OS 1 mL + CH_3OH 5 mL + 3.5 %-Brij 1.25 mL
Flow rate of reaction reagent	0.5 ml/min
Sample temperature	15 °C
Injection volume	5 µl

2) Moisture: Loss on drying (AOAC 934.01)

RESULTS

Table 1. General condition (25°C/60% RH)

Test items	Specification	Batch No.		Initial	1 month	3 month
L-Valine (% dry base)	≥ 72.0	NGVAL191221	(b) (4)			
		NGVAL191222				
		NGVAL191223				
Moisture (%)	≤ 5.0	NGVAL191221				
		NGVAL191222				
		NGVAL191223				
Test items	**Specification**	**Batch No.**	**4 month**	**6 month**	**9 month**	**12 month**
L-Valine (% dry base)	≥ 72.0	NGVAL191221	(b) (4)			
		NGVAL191222				
		NGVAL191223				
Moisture (%)	≤ 5.0	NGVAL191221				
		NGVAL191222				
		NGVAL191223				

Table 2. Accelerated condition (40°C/75% RH)

Test items	Specification	Batch No.		1 month	3 month	4 month	6 month
L-Valine (% dry base)	≥ 72.0	NGVAL191221	(b) (4)				
		NGVAL191222					
		NGVAL191223					
Moisture (%)	≤ 5.0	NGVAL191221					
		NGVAL191222					
		NGVAL191223					

REFERENCES

[1] ICH Harmonised Tripartite Guideline. Q1A(R2) Stability Testing of New Drug Substances and Products. 6 February 2003.

[APPENDIX 1] Certificate of Analysis

CJ Research Institute of Biotechnology

55, Gwanggyo-ro 42beon-gil, Yeongtong-gu, Suwon-si, Gyeonggi-do, 16495, Korea

www.cj.co.kr

TEL : 031) 8099-2450 FAX : 031) 8099-2918

Certificate of analysis

Certificate No.	2020-PR-001	Receipt No.	2020-AN-001
Client	-	Date of Receipt	2020.02.12.
Client Name	-	Date of Test	2020.02.12.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (70%)
Manuf. Date	2019.12.21.
Lot. No	NGVAL191221
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 70 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (22~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

The Test Report cannot be reproduced, except in full.

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Certificate No.	2020-PR-002	Receipt No.	2020-AN-002
Client	-	Date of Receipt	2020.02.12.
Client Name	-	Date of Test	2020.02.12.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (70%)
Manuf. Date	2019.12.22.
Lot. No	NGVAL191222
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 70 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (22~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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Certificate of analysis

Certificate No.	2020-PR-003	Receipt No.	2020-AN-003
Client	-	Date of Receipt	2020.02.12.
Client Name	-	Date of Test	2020.02.12.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (70%)
Manuf. Date	2019.12.23.
Lot. No	NGVAL191223
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 70 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (22~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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Certificate No.	2021-PR-095	Receipt No.	2021-AN-062
Client	-	Date of Receipt	2020.07.31.
Client Name	-	Date of Test	2020.08.25.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (ValPro)-25
Manuf. Date	2019.12.21.
Lot. No	NGVAL191221
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 70 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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Certificate No.	2021-PR-096	Receipt No.	2021-AN-063
Client	-	Date of Receipt	2020.07.31.
Client Name	-	Date of Test	2020.08.25.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (ValPro)-40
Manuf. Date	2019.12.21.
Lot. No	NGVAL191221
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 70 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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(b) (4)

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Certificate No.	2021-PR-097	Receipt No.	2021-AN-064
Client	-	Date of Receipt	2020.07.31.
Client Name	-	Date of Test	2020.08.25.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (ValPro)-25
Manuf. Date	2019.12.22.
Lot. No	NGVAL191222
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 70 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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(b) (4)

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Certificate No.	2021-PR-098	Receipt No.	2021-AN-065
Client	-	Date of Receipt	2020.07.31.
Client Name	-	Date of Test	2020.08.25.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (ValPro)-40
Manuf. Date	2019.12.22.
Lot. No	NGVAL191222
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 70 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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Certificate of analysis

Certificate No.	2021-PR-099	Receipt No.	2021-AN-066
Client	-	Date of Receipt	2020.07.31.
Client Name	-	Date of Test	2020.08.25.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (ValPro)-25
Manuf. Date	2019.12.23.
Lot. No	NGVAL191223
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 70 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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Oct, 06, 2021

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Certificate of analysis

Certificate No.	2021-PR-100	Receipt No.	2021-AN-067
Client	-	Date of Receipt	2020.07.31.
Client Name	-	Date of Test	2020.08.25.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (ValPro)-40
Manuf. Date	2019.12.23.
Lot. No	NGVAL191223
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 70 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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Certificate of analysis

Certificate No.	2021-PR-101	Receipt No.	2021-AN-068
Client	-	Date of Receipt	2020.09.25.
Client Name	-	Date of Test	2020.10.22.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (ValPro)-25
Manuf. Date	2019.12.21.
Lot. No	NGVAL191221
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 70 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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Certificate of analysis

Certificate No.	2021-PR-102	Receipt No.	2021-AN-069
Client	-	Date of Receipt	2020.09.25.
Client Name	-	Date of Test	2020.10.22.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (ValPro)-40
Manuf. Date	2019.12.21.
Lot. No	NGVAL191221
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 70 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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Certificate of analysis

Certificate No.	2021-PR-103	Receipt No.	2021-AN-070
Client	-	Date of Receipt	2020.09.25.
Client Name	-	Date of Test	2020.10.22.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (ValPro)-25
Manuf. Date	2019.12.22.
Lot. No	NGVAL191222
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 70 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

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Certificate of analysis

Certificate No.	2021-PR-104	Receipt No.	2021-AN-071
Client	-	Date of Receipt	2020.09.25.
Client Name	-	Date of Test	2020.10.22.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (ValPro)-40
Manuf. Date	2019.12.22.
Lot. No	NGVAL191222
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 70 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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Certificate of analysis

Certificate No.	2021-PR-105	Receipt No.	2021-AN-072
Client	-	Date of Receipt	2020.09.25.
Client Name	-	Date of Test	2020.10.22.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (ValPro)-25
Manuf. Date	2019.12.23.
Lot. No	NGVAL191223
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 70 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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Certificate of analysis

Certificate No.	2021-PR-106	Receipt No.	2021-AN-073
Client	-	Date of Receipt	2020.09.25.
Client Name	-	Date of Test	2020.10.22.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (ValPro)-40
Manuf. Date	2019.12.23.
Lot. No	NGVAL191223
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 70 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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Certificate No.	2021-PR-107	Receipt No.	2021-AN-074
Client	-	Date of Receipt	2020.10.30.
Client Name	-	Date of Test	2020.11.24.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (ValPro)-25
Manuf. Date	2019.12.21.
Lot. No	NGVAL191221
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 70 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

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Certificate of analysis

Certificate No.	2021-PR-108	Receipt No.	2021-AN-075
Client	-	Date of Receipt	2020.10.30.
Client Name	-	Date of Test	2020.11.24.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (ValPro)-40
Manuf. Date	2019.12.21.
Lot. No	NGVAL191221
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 70 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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Certificate No.	2021-PR-109	Receipt No.	2021-AN-076
Client	-	Date of Receipt	2020.10.30.
Client Name	-	Date of Test	2020.11.24.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (ValPro)-25
Manuf. Date	2019.12.22
Lot. No	NGVAL191222
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 70 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

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Certificate No.	2021-PR-110	Receipt No.	2021-AN-077
Client	-	Date of Receipt	2020.10.30.
Client Name	-	Date of Test	2020.11.24.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (ValPro)-40
Manuf. Date	2019.12.22
Lot. No	NGVAL191222
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 70 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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Certificate of analysis

Certificate No.	2021-PR-111	Receipt No.	2021-AN-078
Client	-	Date of Receipt	2020.10.30.
Client Name	-	Date of Test	2020.11.24.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (ValPro)-25
Manuf. Date	2019.12.23.
Lot. No	NGVAL191223
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 70 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

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Certificate of analysis

Certificate No.	2021-PR-112	Receipt No.	2021-AN-079
Client	-	Date of Receipt	2020.10.30.
Client Name	-	Date of Test	2020.11.24.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (ValPro)-40
Manuf. Date	2019.12.23.
Lot. No	NGVAL191223
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 70 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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Certificate of analysis

Certificate No.	2021-PR-113	Receipt No.	2021-AN-080
Client	-	Date of Receipt	2020.12.23.
Client Name	-	Date of Test	2020.12.24.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (ValPro)-25
Manuf. Date	2019.12.21.
Lot. No	NGVAL191221
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 70 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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Certificate of analysis

Certificate No.	2021-PR-114	Receipt No.	2021-AN-081
Client	-	Date of Receipt	2020.12.23.
Client Name	-	Date of Test	2020.12.24.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (ValPro)-40
Manuf. Date	2019.12.21.
Lot. No	NGVAL191221
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 70 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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Certificate of analysis

Certificate No.	2021-PR-115	Receipt No.	2021-AN-082
Client	-	Date of Receipt	2020.12.23.
Client Name	-	Date of Test	2020.12.24.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (ValPro)-25
Manuf. Date	2019.12.22.
Lot. No	NGVAL191222
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 70 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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Certificate of analysis

Certificate No.	2021-PR-116	Receipt No.	2021-AN-083
Client	-	Date of Receipt	2020.12.23.
Client Name	-	Date of Test	2020.12.24.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (ValPro)-40
Manuf. Date	2019.12.22.
Lot. No	NGVAL191222
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 70 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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Certificate of analysis

Certificate No.	2021-PR-117	Receipt No.	2021-AN-084
Client	-	Date of Receipt	2020.12.23.
Client Name	-	Date of Test	2020.12.24.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (ValPro)-25
Manuf. Date	2019.12.23.
Lot. No	NGVAL191223
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 70 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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Certificate of analysis

Certificate No.	2021-PR-118	Receipt No.	2021-AN-085
Client	-	Date of Receipt	2020.12.23.
Client Name	-	Date of Test	2020.12.24.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (ValPro)-40
Manuf. Date	2019.12.23.
Lot. No	NGVAL191223
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 70 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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Certificate of analysis

Certificate No.	2021-PR-119	Receipt No.	2021-AN-086
Client	-	Date of Receipt	2021.03.26.
Client Name	-	Date of Test	2021.04.30.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (ValPro)-25
Manuf. Date	2019.12.21.
Lot. No	NGVAL191221
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 70 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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Certificate of analysis

Certificate No.	2021-PR-120	Receipt No.	2021-AN-087
Client	-	Date of Receipt	2021.03.26.
Client Name	-	Date of Test	2021.04.30.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (ValPro)-25
Manuf. Date	2019.12.22.
Lot. No	NGVAL191222
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 70 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

The Test Report cannot be reproduced, except in full.

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Approved by Technical Manager

Oct, 06, 2021

CJ Research Institute of Biotechnology

CJ BIO-AD form 100-01 REV.01

CJ Research Institute of Biotechnology

55, Gwanggyo-ro 42beon-gil, Yeongtong-gu, Suwon-si, Gyeonggi-do, 16495, Korea

www.cj.co.kr

TEL : 031) 8099-2450 FAX : 031) 8099-2918

Certificate of analysis

Certificate No.	2021-PR-121	Receipt No.	2021-AN-088
Client	-	Date of Receipt	2021.03.26.
Client Name	-	Date of Test	2021.04.30.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (ValPro)-25
Manuf. Date	2019.12.23.
Lot. No	NGVAL191223
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 70 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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Certificate of analysis

Certificate No.	2021-PR-122	Receipt No.	2021-AN-089
Client	-	Date of Receipt	2021.06.25.
Client Name	-	Date of Test	2021.08.26.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (ValPro)-25
Manuf. Date	2019.12.21.
Lot. No	NGVAL191221
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 70 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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Certificate of analysis

Certificate No.	2021-PR-123	Receipt No.	2021-AN-090
Client	-	Date of Receipt	2021.06.25.
Client Name	-	Date of Test	2021.08.26.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (ValPro)-25
Manuf. Date	2019.12.22.
Lot. No	NGVAL191222
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 70 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

The Test Report cannot be reproduced, except in full.

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TEL : 031) 8099-2450 FAX : 031) 8099-2918

Certificate of analysis

Certificate No.	2021-PR-124	Receipt No.	2021-AN-091
Client	-	Date of Receipt	2021.06.25.
Client Name	-	Date of Test	2021.08.26.
Client Tel	-	Use of Report	Reference test
Client Address	-		

Test Sample	L-Valine (ValPro)-25
Manuf. Date	2019.12.23.
Lot. No	NGVAL191223
Quantity (kg)	-

Test Item(s)	Specification	Test Result	Test method used
L-Valine(dry base)	Not less than 70 %	(b) (4)	HPLC
Moisture (Loss on drying)	Not more than 5 %		AOAC 934.01

* Information

* Temperature : (20~28) ℃, Relative Humidity : (30~60) %

* The results shown in this test report refer only to the sample tested unless otherwise stated.

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