GRAS Notice (GRN) No. 338
http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/GRASListings/default.htm

ORIGINAL SUBMISSION

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May 5, 2010

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Division of
Biotechnology and
GRAS Notice Review

Food and Drug Administration
Center for Food Safety & Applied Nutrition
Office of Food Additive Safety (HFS-200)
5100 Paint Branch Parkway
College Park, MD 20740-3835

Attention: Dr. Robert L. Martin

Re: GRAS Notification – L-Theanine (98%)

Dear Dr. Martin:

On behalf of Blue California of Rancho Santa Margarita, CA, we are submitting for FDA review a GRAS notification for L-Theanine (98%). The attached documentation contains the specific information that addresses the safe human food uses for the subject notified substance as discussed in the GRAS guidance document.

If additional information or clarification is needed as you and your colleagues proceed with the review, please feel free to contact me via telephone or email.

We look forward to your feedback.

Sincerely,

(b) (6)

Robert S. McQuate, Ph.D.
CEO & Co-Founder
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Enclosure: GRAS Notification – L-Theanine (98%) (in triplicate)

GRAS ASSOCIATES, LLC

GRAS ASSESSMENT

OF

L-THEANINE (98%)

Food Usage Conditions for General Recognition of Safety

For

BLUE CALIFORNIA
Rancho Santa Margarita, CA

Evaluation By

Richard C. Kraska, Ph.D., DABT
Robert S. McQuate, Ph.D.
Madhusudan G. Soni, Ph.D., FACN

May 5, 2010

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I. GRAS EXEMPTION CLAIM

A. Claim of Exemption from the Requirement for Premarket Approval Pursuant to Proposed 21 CFR 170.36(c)(1)[1]

L-Theanine (98%), meeting the specifications for Blue California's L-TeaActive™, as described below, has been determined to be Generally Recognized As Safe (GRAS) in accordance with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act*. This determination was made by experts qualified by scientific training and experience; it is based on scientific procedures as described in the following sections; and the evaluation accurately reflects the intended food uses of the tea leaf-derived food ingredient.

Signed:

(b) (6) [redacted] _____ May 5, 2010

Robert S. McQuate, Ph.D.
GRAS Associates, LLC
20482 Jacklight Lane
Bend, OR 97702-3074

Date

B. Names & Address of Notifier

Blue California
30111 Tomas
Rancho Santa Margarita, CA 92688

As the notifier, Blue California accepts responsibility for the GRAS determination that has been made for L-TeaActive™, its L-theanine (98%) product and as described in the subject notification; consequently, the L-theanine preparations, i.e., greater than or equal to 98% pure L-theanine, meeting the conditions described herein are exempt from pre-market approval requirements for food ingredients.

C. Common Name & Identity of the Notified Substance

L-Theanine (which is frequently shortened to theanine) is the common name for the notified substance. The specific material of interest to Blue California is the high purity L-theanine, also referred to as L-theanine (98%), is identified as L-TeaActive™. Also, see Section II.A.

[1] See 62 FR 18938 (17 April 1997) which is accessible at http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/ucm083058.htm.

D. Conditions of Intended Use in Food

L-Theanine (98%) is intended to be added as a nutrient supplement into a limited number of conventional food categories described in this document.

E. Basis for the GRAS Determination

Pursuant to 21 CFR § 170.30, L-theanine (98%) has been determined to be GRAS on the basis of scientific procedures as discussed in the detailed description provided below.

F. Availability of Information

The data and information that serve as the basis for this GRAS Notification will be sent to the US Food and Drug Administration (FDA) upon request or will be available for review and copying at reasonable times at the offices of GRAS Associates, LLC, located at 20482 Jacklight Lane, Bend, OR 97702-3074.

II. INTRODUCTION

A. Objective

At the request of Blue California, GRAS Associates, LLC ("GA") has undertaken an independent safety evaluation of L-TeaActive™, its L-theanine (98%) or high purity L-theanine, for use in foods. The purpose of the evaluation is to ascertain whether or not the intended food use of L-theanine (98%) can be considered to be generally recognized as safe (GRAS) when used as a nutrient supplement in selected food products.

B. Foreword

Blue California provided information on intended use, specifications for the manufactured material, manufacturing information, and unpublished toxicology studies for theanine. Determining how much theanine can be safely consumed, i.e., the so-called "dose" or use levels, is critical in the determination of safe exposure levels for theanine when consumed as a food ingredient. The composite safety/toxicity studies in concert with exposure information constitute the two critical information components that form the basis of the GRAS evaluation.

The safety/toxicity studies, consumption/exposure information, and other related documentation were augmented with an independent search of the scientific and regulatory literature, which included GRAS Notification 209. Based upon the composite information, a GRAS assessment based primarily on available safety information and common occurrence in food was undertaken. Those references that were deemed pertinent to the objective at hand are listed in Section VIII.

C. FDA Regulatory Framework

Ingredients for use in foods must undergo pre-market approval by FDA as food additives or, alternatively, the ingredients to be incorporated into foods must be determined to be generally recognized as safe (GRAS). The authority to make GRAS determinations is not restricted to FDA. In fact, GRAS determinations may be provided by experts who are qualified by scientific training and experience to evaluate the safety of food and food ingredients under the intended conditions of use.

In 1997, FDA altered the GRAS determination process by eliminating the formal GRAS petitioning process and replacing the petitioning process with a notification procedure. While outlining the necessary content to be considered in making a GRAS determination, FDA encouraged that such determinations be provided to FDA in the form of a notification. However, notifying FDA of such determinations is strictly voluntary.

D. Regulatory History of Theanine

GRAS Notification 209 was submitted by Taiyo International to the FDA in July, 2006 for Suntheanine®. Suntheanine® is purified L-theanine which is derived from enzymatic synthesis. The notice indicated that Suntheanine® is intended to be added to foods in a number of different food categories to provide up to 250 mg/serving of L-theanine. The food categories listed in the notice were fruit juices and drinks, non-herbal teas, sports beverages, specialty bottled waters, chocolate bars and chews, hard candies and breath mints, and chewing gum. The FDA issued a "no objection" letter for this notification on February 5, 2007.[2]

[2] FDA's response to GRAS Notification 209 which was submitted by Taiyo International is listed on the FDA's website at http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/GRASListings/ucm153810.htm.

III. INGREDIENT IDENTITY, CHEMICAL CHARACTERIZATION, MANUFACTURING PROCESS & PURITY

A. Common or Usual Name & Identity of the Notified Substance

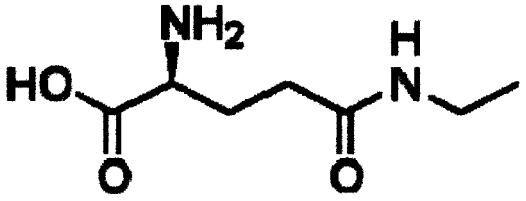
L-Theanine (which is frequently referred to as theanine) is the common name for the notified substance. The specific material of interest to Blue California is the high purity L-theanine which is also referred to as L-theanine (98%) and L-TeaActive™. The Chemical Abstract Registry (CAR) number for theanine is 3081-61-6.

B. Chemical Name

The subject ingredient, L-theanine, is an amino acid which is also known as γ-glutamylethylamide or N-ethyl-L-glutamine.

C. Chemical Identity of Theanine

Figure 1. Chemical Structure of Theanine



L-Theanine has a molecular formula of $C_7H_{14}N_2O_3$ and a molecular weight of 174.20 Daltons. Theanine is not incorporated into protein. The chemical structure of theanine is given in Figure 1. A summary of the physical and chemical properties of L-theanine may be found in Table 1.

Table 1. Physical & Chemical Properties of L-Theanine

Appearance	White crystalline powder
Appearance in solution	Transparent, colorless
Odor	None
Taste	Slightly sweet
Melting point	214-215°C
Water solubility	Soluble
Ethanol solubility	Insoluble
Ether solubility	Insoluble

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D. Manufacturing Process

L-Theanine is naturally occurring at high concentrations in the leaves of the green tea plant *Camellia sinensis*. It can also be found in the non-edible mushroom *Xerocomus badius*. L-Theanine accounts for approximately 1-2% of the dry weight of tea leaves and is water-soluble (Borzelleca et al., 2006). Steeping *C. sinensis* leaves in hot water creates tea, the most commonly consumed beverage in the world. After steeping, the majority of theanine is solubilized (Ekborg-Ott et al., 1997).

High purity L-theanine produced by Blue California is purified from green tea leaves using water and ethanol as solvents to extract the different bioactive components of the leaf. An overview of the process can be found in Figure 2. Blue California begins the purification process with cleaned green tea leaves. Theanine, which is water soluble, is extracted from the leaves by incubating the leaves with de-ionized water at 60°C for four hours. The extract is concentrated, centrifuged, and then subjected to a column separator filled with resin to concentrate the extract at pH 3. The resin is washed with ethanol to produce the L-theanine solution. Blue California separates L-theanine from tea polyphenols to 98% purity using microfiltration. During the crystallization process of caffeine, Blue California isolates L-theanine and purifies this amino acid through membrane ultrafiltration to 98% purity. A summary of the purification process is depicted in Figure 2. Blue California's L-Theanine (98%), or L-TeaActive™, is manufactured in accordance with current Good Manufacturing Practices (cGMP). The cGMP inspection certificate can be found in Appendix A.

E. Product Purity

1. Identification & Purity Analysis of L-Theanine (98%)

To insure the purified product is chemically identical to L-theanine, Blue California requested Eurofins Scientific Inc. to perform infrared spectrometry on five lots of purified L-theanine (98%). Overlap of the Fourier transform-infrared spectra of L-theanine (98%) and an L-theanine reference standard, using FTIR instrumentation, reveals that these two substances are chemically identical. The Eurofins infrared spectrometry results are attached in Appendices B1 through B6.

Blue California also requested Eurofins Scientific Inc. to perform purity analyses by high performance liquid chromatography (HPLC) of five lots of L-theanine (98%). The objective of the study was to determine the purity of the Blue California supplied production lots of L-theanine powder. Each sample of the five lots was tested in duplicate and compared to a ChromaDex L-theanine standard. Using the L-theanine standard, the limit of quantitation (LOQ) of L-theanine was projected to be 1.0%, and the limit of detection (LOD) was projected to be 0.3%, at the 1 mg/mL expected concentration of the samples. Results of the purity analysis can be found in Table 2. Overall, the study confirmed the identity of the purified product as L-theanine. The range of purity of L-theanine in the sample lots was 99.09% - 100.38%. The detailed Eurofins HPLC data are attached in Appendix C.

Table 2. Purity Analysis Testing for Blue California L-TeaActive™ from Five Production Samples

	Prep 1 Result (%w/w)	Prep 2 Result (%w/w)	Average	Mean Absolute Deviation
Sample 4968	99.6662	99.4570	99.5616	0.1046
Sample 4969	99.6200	99.0859	99.3530	0.2671
Sample 4970	99.1199	99.7460	99.4330	0.3130
Sample 4971	98.6362	98.5067	98.5715	0.0648
Sample 4972	100.3803	99.5804	99.9804	0.4000

i. Enantiomer Content of L-Theanine (98%)

Theanine in tea leaves and after solubilization is known to be a mixture of the L-theanine and D-theanine enantiomers. Ekborg-Ott et al. (1997) reported that D-theanine constitutes an average of 1.85% of the total theanine in 17 types of tea tested.

To determine the amount of the L-theanine and D-theanine enantiomers in L-theanine (98%), Blue California requested Eurofins Scientific Inc. to perform a chirality analysis by HPLC, on the same five lots from the purity analysis, and to quantify D-theanine, if any, present in the same lots.

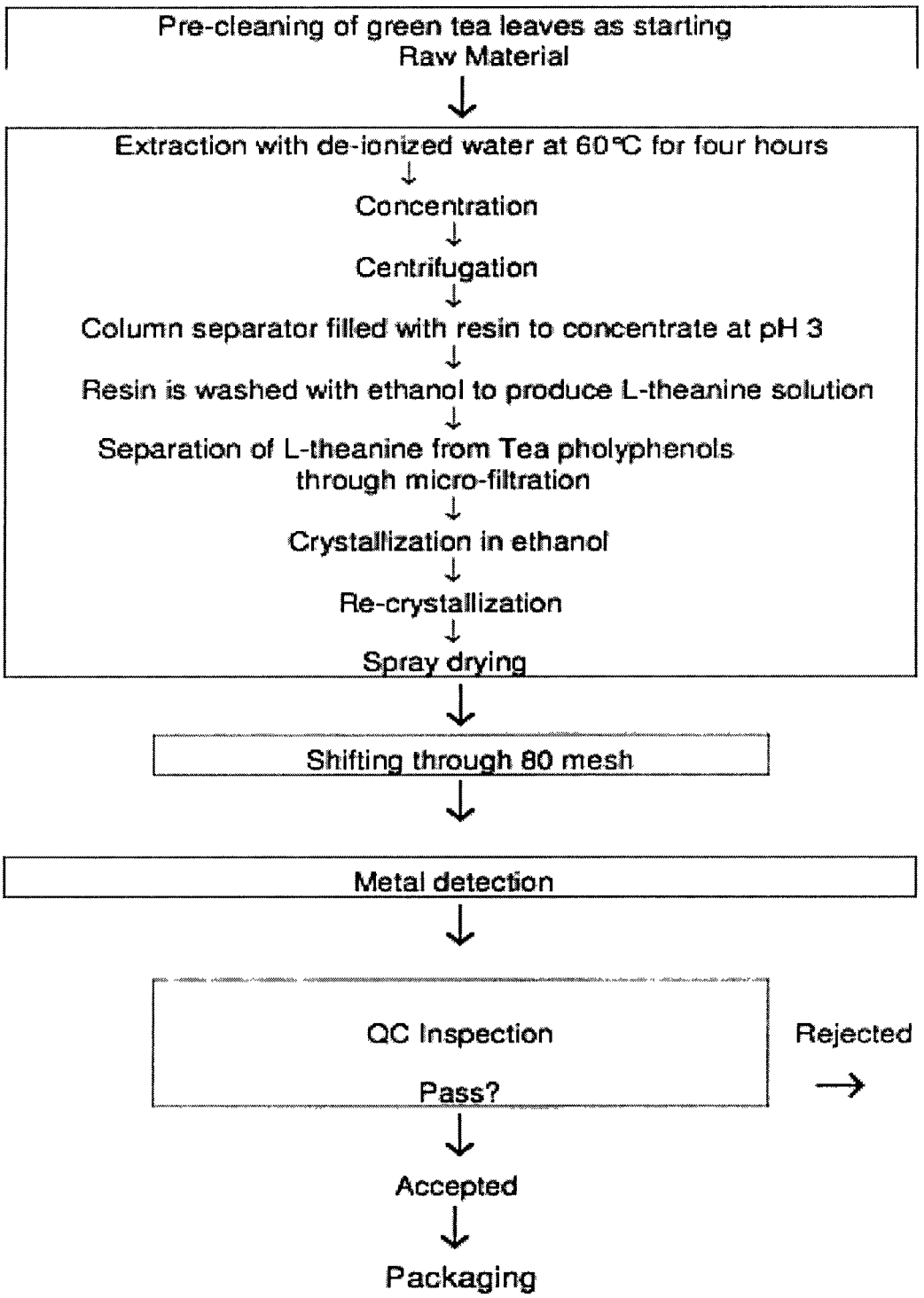
Due to the lack of availability of a commercial D-theanine standard, D-glutamine was used as the retention time marker for D-theanine. L-Theanine and L-glutamine elute with approximately the same retention time under chromatographic conditions listed in the test method. It was assumed that D-glutamine then elutes at the retention time matching to that of D-theanine due to their similarity in chemical structures. The response factor between L-theanine and L-glutamine was determined prior to LOD/LOQ determination of D-glutamine, which was then applied to calculate the LOD/LOQ of D-theanine.

D-Glutamine solution was prepared at 0.0906 mg/mL level, and 3 uL of the solution was injected in triplicate to estimate its limit of quantitation (LOQ). The signal-to-noise ratios (S/N) obtained for D-glutamine are contained in Table 3. The LOQ of D-glutamine was estimated to be 1.8% of L-theanine and the LOD is, therefore, around 0.6% of L-theanine.

Table 3. Signal-to-Noise Ratios for D-Glutamine

Injection #	D-Glutamine S/N
1	28.7
2	29.8
3	30.8
Mean	29.8
% RSD	3.5

Figure 2. Purification Summary for Blue California L-TeaActive™



Eurofins performed duplicate analyses of the same five Blue California production lots used in the purity analysis above. D-Theanine was reported to be not detected (ND) in all samples tested. Therefore, the level of D-theanine present in L-theanine (98%) was determined to be below the LOD of D-theanine. The method employed in this determination was identical to the one used for Suntheanine® described in GRN 209.

ii. Specifications of L-Theanine (98%)

Blue California has established specifications for its L-theanine (98%) which are intended to maintain the food grade status of the final product. The food-grade specifications for L-TeaActive™ are listed in Table 4.

Table 4. Food Grade Specifications for L-TeaActive™

Attribute	Specification	Methods
Appearance	White crystalline powder	VISUAL
Foreign Matter	Absent	VISUAL
Odor	Absent	OLFACTORY
Taste	Slightly Sweet	GUSTATORY
L-theanine content	≥ 98%	HPLC
Arsenic	< 2 ppm	ICP
Lead	<1 ppm	ICP
Loss on Drying	≤1%	USP 29
Residue on Ignition	≤ 0.2 %	USP 29
pH	5-7	USP 29
Chloride	≤ 0.02%	USP 29
Heavy Metals	<10 ppm	USP 29
Specific Rotation	+7.2 - 8.5 deg.	USP 29
Bulk Density	0.2 - 0.6 g/mL	USP 29
Tap Density	≥ 0.6 g/mL	USP 29
Particle Size	≥ 95% through Mesh #40 sieve	USP 29
Total Plate Count	< 3,000 cfu/gm	AOAC
Total Coliform	< 100 cfu/gm	AOAC
Yeast and Molds	< 100 cfu/gm	AOAC
E. coli	NEGATIVE	AOAC
Salmonella	NEGATIVE	AOAC
Shelf Life	2 Years	AOAC

iii. Analysis of L-Theanine (98%) Production Lots

To demonstrate conformance with the food-grade specifications in Table 4, Blue California analyzed five lots of its high purity L-theanine. The results of these analyses can be found in Table 5.

All five lots of L-theanine (98%) were within the food-grade specifications established for L-theanine (98%), indicating that the production process is capable of consistently producing food-grade product. The certificates of analysis of these lots are provided in Appendices D1 through D5.

Table 5. Results of Analysis of Blue California L-TeaActive™

Attributes	Specification	Lot Number				
		LT2008 0928	LT2008 1105	LT2009 0225	LT2009 0109	LT200 90428
Appearance	White crystalline powder	PASS	PASS	PASS	PASS	PASS
Foreign Matter	Absent	PASS	PASS	PASS	PASS	PASS
Odor	Absent	PASS	PASS	PASS	PASS	PASS
Taste	Slightly Sweet	PASS	PASS	PASS	PASS	PASS
L-theanine	≥ 98%	99.3%	99.43%	99.98%	98.57%	99.1%
Arsenic	< 2ppm	PASS	PASS	PASS	PASS	PASS
Lead	<1 ppm	PASS	PASS	PASS	PASS	PASS
Loss on Drying	≤1%	0.93%	0.95%	0.96%	0.94%	1.0%
Residue on Ignition	≤ 0.2 %	PASS	PASS	PASS	PASS	PASS
pH	5 - 7	5.80	5.7	5.7	5.7	5.7
Chloride	≤ 0.02%	PASS	PASS	PASS	PASS	PASS
Heavy Metals	<10 ppm	PASS	PASS	PASS	PASS	PASS
Specific Rotation	+7.2 - 8.5 deg	+8.1 deg	+8.1 deg	+8.2 deg	+8.1 deg	+8.0 deg
Bulk Density	0.2 - 0.6 g/mL	PASS	PASS	PASS	PASS	PASS
Tap Density	≥ 0.6 g/mL	PASS	PASS	PASS	PASS	PASS
Particle Size	≥ 95% through Mesh #40 sieve	PASS	PASS	PASS	PASS	PASS
Total Plate Count	< 3,000 cfu/gm	<1,000 cfu/gm	<1,000 cfu/gm	<1,000 cfu/gm	<1,000 cfu/gm	<1,000 cfu/gm
Total Coliform	< 100 cfu/gm	PASS	PASS	PASS	PASS	PASS
Yeast and Molds	< 100 cfu/gm	PASS	PASS	PASS	PASS	PASS
E. coli	NEGATIVE	PASS	PASS	PASS	PASS	PASS
Salmonella	NEGATIVE	PASS	PASS	PASS	PASS	PASS
Shelf Life	2 Years	PASS	PASS	PASS	PASS	PASS

F. Stability Data

A solution of L-theanine (98%) was tested at room temperature (25°C) for stability under various levels of pH over a period of 24 hours. As shown in Table 6, only minimal degradation occurred in the theanine content of the product.

Table 6. Stability of Blue California L-TeaActive™ in Various pH Solutions at 25°C

	0 hours	2 hours	6 hours	10 hours	16 hours	20 hours	24 hours
pH 2	99.8	98.2	97.4	96.9	96.1	95.8	94.9
pH 6	100.1	100.0	99.8	99.5	99.2	99.0	99.0
pH 7	99.9	99.7	99.7	99.4	99.2	99.2	98.8
pH 8	99.7	98.9	98.2	97.5	96.6	95.8	95.2
pH 10	99.8	98.2	97.3	95.9	94.6	93.2	92.9

To further analyze the stability of L-theanine (98%), a standard solution was prepared and adjusted to a range of pHs between pH 2 and pH 10. These solutions were tested for stability at 100°C over a period of 24 hours. As shown in Table 7, under these conditions the theanine content of L-theanine (98%) showed only slight degradation.

Table 7. Stability of Blue California L-TeaActive™ in Various pH Solutions at 100°C

	0 hours	2 hours	6 hours	10 hours	16 hours	20 hours	24 hours
pH 2	99.9	98.9	98.2	97.3	96.5	96.1	95.3
pH 6	100.0	99.8	99.5	99.5	99.1	98.7	98.2
pH 7	99.9	99.9	99.3	99.2	98.9	98.9	98.8
pH 8	99.7	99.1	98.6	98.2	97.3	96.7	95.0
pH 10	100.1	99.2	98.5	96.9	95.2	93.8	91.1

The stability of L-theanine (98%) under neutral conditions, but at different temperatures, was tested over a 24-hour time period. In these experiments, a solution of L-theanine (98%) was prepared and adjusted to pH 6. The samples of the theanine solution were held at 100, 110, or 120°C for the times indicated, and L-theanine content was analyzed at each time point. As shown in Table 8, the theanine content of L-theanine (98%) was essentially unchanged when kept at high temperatures for up to 24 hours.

Table 8. Stability of Blue California L-TeaActive™ in Different Temperatures at pH 6

	0 hours	2 hours	6 hours	10 hours	16 hours	20 hours	24 hours
100°C	99.9	99.9	99.5	99.5	99.3	99.2	99.0
110°C	99.7	99.4	99.3	99.2	99.0	99.0	98.8
120°C	100.2	100.1	99.8	99.8	99.7	99.6	99.2

Samples of L-theanine (98%) were packaged, tightly sealed, and stored for up to 2 years. The theanine concentration was analyzed at 6 months, 12 months, 18 months, and 24 months. As

shown in Table 9, almost no change was seen in the theanine content of the samples. This study demonstrates that the shelf life of L-theanine (98%) is at least 24 months.

Table 9. Stability of Blue California L-TeaActive™ at Room Temperature Over Time

	0 months	6 months	12 months	18 months	24 months
Content (%)	100.2	100.0	100.0	99.8	99.7
Moisture (%)	0.13	0.15	0.15	0.16	0.17

IV. INTENDED USE & DIETARY EXPOSURE

Customers of Blue California intend to use L-theanine (98%) in a limited number of human food categories where the high purity L-theanine would function as a nutrient supplement as defined in 21 CFR 170.3(o)(20). L-Theanine (98%) is intended for use in the same foods and at identical levels of addition as notified by Taiyo International, Inc., for the Suntheanine® L-theanine in GRN 209. The proposed food uses are as a food ingredient---as a dietary source of theanine---in foods such as fruit juices and drinks, non-herbal teas, sports beverages, specialty bottled waters, chocolate bars and chews, hard candies and breath mints, and chewing gum. The intended food use categories and use levels of L-theanine (98%) are presented in Table 10. The intended use of L-theanine (98%) in the same foods and at the same levels as those in GRN 209 is not expected to noticeably affect the intake of L-theanine in the overall diet of the public from introduction into the market by another supplier who will have to compete in essentially the same markets and foods. Based on a statistical analysis of potential dietary intake, in the GRN 209 notice it was estimated that the mean consumption would be 628 mg/person/day, and the 90th percentile consumption would be 1284 mg (1.28 g)/person/day.

The dietary analysis below was presented in GRN 209 and was not questioned by FDA in the response letter of February 5, 2007.

> Exponent Inc. calculated the daily intake of Suntheanine® estimated to result from its intended addition to food. These estimates were based on data collected in the United States Department of Agriculture's 1994-96 Continuing Survey of Food Intakes by Individuals (CSFII) and its Supplemental Children's Survey (CSFII 1998), as provided on CD-ROM (USDA 2000). The CSFII 1994-96 was conducted between January, 1994, and January, 1997, with noninstitutionalized individuals in the United States. Twenty-four-hour diet recall data were collected through two in-person interviews conducted between 3 and 10 days apart from a nationally representative sample of individuals of all ages. The CSFII 1998 was designed as a supplement to CSFII 1994-96 to increase the sample size for children from birth through age 9 years. This survey employed the same methodology as CSFII 1994-96. In the merged surveys (designated CSFII 1994-96, 1998), 21662 individuals provided dietary intake data on the first survey day, and 20607 provided data on the second day. The CSFII was selected, rather than the more recent NHANES 1999-2002, because the availability of 2-day data allows better estimation of the usual intake of Suntheanine® potentially resulting from its intended use.
>
> EDIs are calculated by multiplying the reported consumption of each food in categories in which Suntheanine® addition is intended by the maximum intended addition level as shown in Table 17. The total EDI is calculated by adding, at the level of the individual respondent, the intakes of Suntheanine® from each use in foods consumed by the individual. The results of these calculations are shown in Table 18.[3]
>
> Of the food categories in which Suntheanine® is intended to be used, fruit juices and drinks and chewing gum are by far the most widely consumed, by 50% or more of the United States population. Over 85% of the respondents to the CSFII reported consuming at least one of the target foods on at least one of the two survey days. On average, it is anticipated that individuals will consume 628 mg of Suntheanine® per day (11.3 mg/kg bw/day). The EDI of Suntheanine® under its intended conditions of use corresponds with the estimated 90th percentile of intake, or 1284 mg (24.2 mg/kg bw). These are roughly similar to the levels of L-theanine currently consumed by the heaviest tea drinkers in the United States, as was shown in Table 16.[4]

[3] Not reproduced in the subject GRAS Notification.
[4] The reference to Table 16 in GRN 209 is reproduced as Table 10 in the subject GRAS Notification.

Table 10. Consumption Estimate of Proposed Food Uses of L-Theanine

Food category	Serving size (RACC)	Intended Addition levels (mg/g)	% User	Daily Intake of L-Theanine			
				Per user (mg/day		Per Kg body weight (mg/kg bw/day)	
				Mean	90th percentile	Mean	90th percentile
Bottled water	240 mL	1.0	<0.1	121	242	8.1	16.2
Chocolate bars & chews	50 g	5.0	4.0	181	304	3.8	7.1
Fruit juices & drinks	240 mL	1.0	54.8	385	752	8.2	18.5
Hard candies/ mints	15 g/ 2 g	16.7/ 125	4.4	154	309	3.9	8.4
Non-herbal teas	240 mL	1.0	30.3	590	1202	8.7	17.0
Sports drinks	240 mL	1.0	2.2	360	749	6.4	13.2
Chewing gums	3 g	83.3	50.0	125	250	1.8	3.6
All categories			86.4	628	1284	11.3	24.2

Adapted from GRN 209 Table 16.

The only naturally occurring source of theanine in the American diet is tea. Behind water, tea is the most widely consumed beverage in the world. Approximately 20% of Americans consume tea each day. This percentage is higher in many other countries. In the 1999-2002 National Health and Nutrition Examination Survey (NHANES), the mean amount of tea consumed by an individual was 763 mL, but the top 10% of consumers drank 1650 mL of tea and the top 1% of consumers drank 3337 mL.

Approximately 1-2% of the dry weight of tea leaves is theanine and the majority of theanine is solubilized when tea is prepared. Recent studies by Kato et al. (2003) and Ekborg-Ott et al. (1997) found the average theanine concentration among tea varieties was approximately 1.4%. A typical cup of tea uses 3 g of tea leaves, which contains about 30 mg of theanine. Based on the typical concentration of L-theanine in tea (1-2.5%), in GRN 209 (2006) the estimated daily intake of L-theanine from tea was determined to be between 153 and 382 mg/person/day at the mean and between 330 and 825 mg/person/day at the 90th percentile. These estimates indicate that the estimated daily intake of L-theanine under the proposed intended conditions of use of L-theanine (98%) corresponds with the levels of L-theanine currently consumed by the heaviest tea drinkers in the United States.

In summary, the intended uses of L-theanine (98%) will result in an estimated 90th percentile daily intake of 1284 mg. The background 90th percentile intake of L-theanine from its presence in tea has been estimated as 825 mg/day. The estimated daily intake is roughly similar to the levels of L-theanine currently consumed by the heaviest tea drinkers in the United States.

V. REVIEW OF SAFETY DATA ON THEANINE

A. Common Knowledge of Safe Theanine Consumption

In the form of tea, theanine is a widely consumed ingredient which has been a part of the human diet for thousands of years. After water, tea is one of the most widely consumed beverages in the world (Graham 1992; Blumberg 2003). World tea production in 2001 has been reported to be 3.02 million tons. Although the United States is not considered a major tea-consuming country, over 20% of NHANES survey respondents age 13 and older and nearly 20% of respondents of all ages reported having consumed tea on the survey day. Their average intake of tea, on days they consumed tea, was nearly 800 g or about 28 ounces. The average worldwide per capita daily intake of tea has been estimated to be about 120 mL (Blumberg, 2003). Because this per capita intake includes infants and toddlers, as well as older non-tea drinkers, it is evident that consumption among older children, adolescents, and adults is much higher. Based on a 2003 world population of about 6.2 billion, per capita consumption of 120 mL of tea equates to daily consumption of nearly a million metric tons of tea. Thus, the available evidence suggests that there is a common exposure to tea and to its constituent, L-theanine. Despite this long history, no adverse effects due to ingestion of tea or L-theanine have been reported. L-Theanine has also been used as a dietary supplement in the US since the 1990s without any reports of adverse effects.

B. Absorption, Distribution, Metabolism & Excretion of Theanine

After consumption in the form of green tea, L-theanine is absorbed in the intestine and distributed throughout the body. Kitaoka et al. (1996) studied absorption of L-theanine in guinea pig ileum. Their results suggest that a sodium-coupled electrogenic transporter in the brush border of the intestine mediates absorption of L-theanine. This is the major transport system for dipolar amino acids. Kitaoka et al. (1996) found that the sodium-coupled transporter has a 7-fold lower affinity for theanine when compared to glutamine, based on the K_m value.

Following ingestion, L-theanine distributes rapidly throughout the body. Terashima et al. (1999) measured the concentration of theanine in the serum, liver, brain, and urine of rats following intragastric administration (4 g/kg bw). The concentrations of theanine in the serum, liver, and brain were significantly increased one hour after intragastric administration. Maximum levels in the serum and liver were reached after one hour, while the maximum level of theanine in the brain was reached after 5 hours. Similarly, Unno et al. (1999) found that plasma theanine levels peak at 0.5 hours after ingestion. Yokogoshi et al. (1998b) found a dose-related increase in theanine levels in rat brain at 2 hours after intragastric administration of theanine (0, 1000, 2000, 4000 mg/kg bw). At the same time, a high dose of theanine led to a significant decrease in the large neutral amino acids (phenylalanine, tryptophan, threonine, tyrosine, and branched-chain amino acids) in rat brain. The levels of other amino acids were unchanged. From these data, the authors conclude that theanine is transported across the blood-brain barrier by the same leucine-preferring transport system utilized by the large, neutral amino acids.

L-Theanine does not accumulate in the plasma but is hydrolyzed mostly in the kidney and then excreted from the body. Tsuge et al. (2003) examined 10 major tissues from Wistar rats, and detected theanine-degrading enzymatic activity in the kidney homogenate. This activity was not identified in any other tissue homogenates. Later studies indicate that some metabolism of theanine may also occur in other normal tissues, most notably the liver, mediated by glutaminase and γ-GTP (Sugiyama et al., 2004, Sadzuka et al., 2006). In an *in vitro* experiment, Tsuge et al. (2003) purified the theanine-degrading enzymatic activity from rat kidney and found it to be consistent with the previously described phosphate-independent glutaminase. This enzyme also possesses a γ-glutamyl transpeptidase activity. The phosphate-independent glutaminase degrades theanine into glutamic acid and ethylamine *via* a γ-glutamyl transpeptidase reaction. The authors state that some amount of ethylamine may recirculate in the blood, but the majority is excreted in the urine. Concentrations of glutamine and ethylamine increase in urine following theanine administration to rats in a dose-dependent manner (Unno et al., 1999). Theanine completely disappears from all tissues 24 hours after its administration (Terashima et al., 1999).

In a toxicokinetic adjunct to a subchronic study (see Section V.D.3 for additional details of this study) in rats, Borzelleca et al. (2006) found a dose-related increase in theanine plasma levels. In this study, blood samples for toxicokinetics were collected at 18:00 hour on Day 17 and at 00:00, 03:00, 06:00, 12:00, and 18:00 hours on Day 18. Blood was also collected at 18:00 hour on Day 85 and at 00:00, 03:00, 06:00, 12:00, and 18:00 hours on Day 86. The increases in C_{max} and AUC_{0-t} values were approximately proportional to the increase in the dose level during both collection intervals. For most groups, the T_{max} fell during the dark cycle, consistent with the eating pattern of rodents. Plasma concentrations, C_{max} and AUC_{0-t} were similar from week 3 to week 13, indicating theanine did not accumulate over time with repeated oral exposure.

C. Biologic Activity of Theanine

Since L-theanine crosses the blood-brain barrier, and is an analog of the major excitatory neurotransmitter glutamate, a number of studies have examined the effects of L-theanine on neural tissues. Research has shown that L-theanine is capable of binding to both glutamate receptors and glutamate transporters. Glutamate receptors are mostly found in the post-synaptic cells, and are responsible for transmitting the excitatory glutamate signal. The glutamate transporters are mostly found in neuronal and glial cells; they remove glutamate from the extracellular synaptic space and help to regulate glutamate levels and thus neuronal signaling. In brain injury and disease, an excess of glutamate causes oxidative damage, known as excitotoxicity, which can ultimately lead to neuronal cell death. Theanine appears to provide neuroprotection by reducing levels of glutamate, thus inhibiting glutamate-induced excitotoxicity. A summary of these studies is found below.

Research studies have shown that theanine binds to both the ionotropic and metabotropic glutamate receptors. The ionotropic glutamate receptors conduct neuronal signals through ligand-gated ion channels. In an early study, Shinozaki and Ishida (1978) reported that theanine functions as a glutamate antagonist at the crayfish neuromuscular junction. Later, Kakuda et al. (2000) demonstrated that theanine provided dose-dependent protection from ischemic delayed neuronal death induced by glutamic acid in field CA1 of the gerbil hippocampus. Following this study, Kakuda et al. (2002) reported that theanine binds to all three ionotropic glutamate

receptors--the NMDA receptor, the kainite receptor, and the AMPA receptor--but with an IC50 of theanine that is 80- to 30,000-fold less than that of L-glutamic acid. Their results suggest that theanine acts on the glutamate receptors as an antagonist, but the binding activity is very mild.

Other studies have examined the effects of theanine on the metabotropic glutamate receptors (mGluR). The metabotropic glutamate receptors conduct neuronal signals through a G-protein signaling cascade. Using primary cultured rat neurons, Nagasawa et al. (2004) reported that theanine inhibited the delayed death of neurons caused by a brief exposure to glutamate. This effect was abolished by group I mGluR antagonists. Nagasawa et al. (2004) also reported that theanine restored the glutamate-induced decrease in protein expression levels of PLC-β1 and –γ1. Again, this effect was abolished by group I mGluR antagonists. Nishida et al. (2008) continued these studies *in vivo* (also discussed in section V.D.2). Following oral administration of theanine (10 mmol (1.74g)/kg/day) to rats *via* gastric intubation, the authors report that protein expression of PLC-β1 and –γ1 were significantly increased in the cerebral cortex. A non-significant increase was also reported in the cerebellum, and no change was observed in the hippocampus. The authors conclude that repeated oral administration of theanine reduces oxidation levels in the brain, especially the cortex, suggesting a favorable effect on the brain *in vivo*.

Early studies indicated that theanine also binds to glutamate transporters. Sugiyama et al. (2001) reported that theanine significantly inhibited the uptake of glutamate, in a concentration-dependent manner, by M5076 ovarian sarcoma cells. RT-PCR and Western blots revealed that M5076 cells expressed the GLAST and GLT-1 glutamate transporters. The authors suggest that theanine competitively inhibits glutamate uptake by acting on these transporters. However, in a recent study, Kakuda et al. (2008) reports that the chemical structure of theanine is more similar to L-glutamine than to glutamate with regard to the amide moiety. In support of this, L-theanine is degraded by phosphate-independent glutaminase (like L-glutamine) and not by phosphate-dependent glutaminase (Tsuge et al., 2003). The authors report the saturable accumulation of [^{3}H] theanine, in a temperature – and sodium-dependent manner, in rat brain synaptosomal fractions and cultured rat cortical neurons. Based on these results, the authors suggest that theanine modulates the glutamate/glutamine cycle in neurons by inhibiting different glutamine transporters.

L-Theanine has also been linked to changes in other neurotransmitters, including serotonin, dopamine, and GABA. Yokogoshi et al. (1998a) reported an increase in brain tryptophan and a decrease in brain serotonin in rats following oral L-theanine administration. Microinjection of theanine into rat brain has been reported to increase in glycine and dopamine release in the striatum (Yokogoshi et al., 1998b; Yamada et al., 2009). The neuroprotective effect of theanine may also be mediated by GABA(A) receptors. Egashira et al. (2004) reported that theanine (1 mg/kg) reduced the size of cerebral infarcts and expression levels of NeuN, GFAP, and Iba 1 at 24 hours after a 4-hour middle cerebral artery occlusion in mice. This effect was inhibited by bicuculline (GABA(A)-receptor antagonist, 10 mg/kg) but not 3-mercaptopropionic acid (glutamate decarboxylase inhibitor).

As a neuroprotectant, theanine may be helpful to protect against Parkinson's Disease and Alzheimer's Disease. Cho et al. (2008) reported that L-theanine protects neurons against apoptosis induced by the Parkinson's Disease (PD)-related neurotoxicants rotenone and dieldrin.

Kim et al. (2009) recently reported that L-theanine (2 mg and 4 mg/kg), orally administered to mice in drinking water, attenuated Aβ(1-42)-induced neuronal cell death (a major pathological mechanism of Alzheimer's Disease) in the cortex and hippocampus of the brain.

In addition to its neuroprotectant effects, theanine may also act as a relaxant, as an immune system booster, and as an adjuvant chemotherapeutic agent. In addition, L-theanine may boost concentration and decrease blood pressure. Kobayashi et al. (1998) administered either 50 mg or 200 mg theanine in water to four high- and four low-anxiety subjects. In these subjects, an increase in the production of alpha waves (characteristic of relaxation) on electroencephalogram (EEG), but not theta waves (indicators of drowsiness) were observed beginning 30-40 minutes after administration. In a randomized placebo-controlled double-blind crossover study, Song et al. (2002) also administered 200 mg L-theanine every day for 7 days to 20 subjects classified as either high- or low-anxiety. Subjects were evaluated 1 hour after administration using the Fatigue Severity Scale and EEG. Both endpoints showed significant relaxant effects of theanine.

As summarized in GRN 209 (2006), Nozawa et al. (1995) studied the effects of theanine on brain function *in vitro* using cultured cortical neurons. The addition of theanine was found to increase the calcium in the neurons. The authors concluded that their findings suggest that theanine is a glutamate analog that is stimulatory at low concentration, but a sedative at high concentration and can suppress the excitation produced by caffeine.

L-theanine has also been implicated as an immune system booster. Kurihara et al (2007) report that co-administration of L-cystine and L-theanine to mice, prior to immunization, enhanced antigen-specific IgG production. In humans, Miyagawa et al. (2009) found that co-administration of L-cystine and L-theanine (p.o. for 14 days) to elderly patients (n=32) prior to influenza vaccination enhanced the immune response in patients with low serum total protein or hemoglobin when compared to placebo (n=33). In addition, results by Murakami et al. (2009), in a randomized, double-blind, placebo-controlled study with 15 long-distance runners, suggest that oral supplementation with L-cystine and L-theanine prevented a decrease in immune function associated with intense training.

L-Theanine may exert positive effects in other conditions of stress. Studies have shown that theanine may be effective against alcoholic liver injury (Sadzuca et al., 2005), retinopathy of prematurity (Hiramatsu et al., 2008), and may also be useful for reducing raised blood pressure (Yokogoshi et al., 1995, Rogers et al., 2008). The anti-oxidative stress activity of L-theanine may protect normal cells from some of the adverse effects of chemotherapy treatment. Available studies suggest that theanine inhibition of the glutamate transporters leads to the inhibition of efflux of chemotherapeutic agents from tumor cells, enhancing the anti-tumor effects of these agents (Sugiyama and Sadzuka 2001, Sadzuka et al., 2002a,b, Sugiyama and Sadzuka, 2003). Recently, Liu et al. (2009) found that theanine suppressed the *in vitro* and *ex vivo* growth of human non-small cell cancer A549 and leukemia K562 cell lines in dose- and time-dependent manners. In addition, theanine enhanced the anti-cancer activity of the anti-tumor agents such as trichstatin A, berbamine, and norcantharidin. As a result of these studies, L-theanine has been implicated as a potentially important adjuvant to chemotherapy.

In summary, theanine appears to reduce glutamate-induced excitotoxicity and thus protects neurons from oxidative damage. Its neuroprotective effects may be mediated by binding to

glutamate receptors, transporters, or by modulating the glutamate/glutamine cycle. In addition to its neuroprotectant effects, theanine may also act as a relaxant, as an immune system booster, and as an adjuvant chemotherapeutic agent. It may boost concentration and decrease blood pressure.

D. Toxicology Studies on Theanine

Toxicological studies of L-theanine are composed of acute, subacute, subchronic, and chronic animal toxicity studies in rats and in mice, in addition to *in vitro* assays for mutagenicity and genotoxicity.

1. Acute Studies

As reported in GRN 209, Wistar rats were orally administered 2500 or 5000 mg of Suntheanine®/kg bw dissolved in 0.1 mL saline/10 g bw. Control rats received 0.1 mL saline/10 g bw. Rats were observed for 7 days, and body weights were measured. One high-dose female rat died due to an accidental dislocation of the neck. No other effects were noted, and no statistically significant differences in body weights were observed between the control and treatment groups. These data suggest the oral LD_{50} of Suntheanine® to be >5000 mg/kg bw.

In another acute study, Sadzuka et al. (2006) administered L-theanine to male CDF mice (100 mg/kg bw/day, i.p.) for five days in the presence and absence of doxorubicin (DOX). Toxicological analysis was limited to the six cytochrome P450 enzymes (CYP1A1, CYP3A, CYP1A2, CYP2B, CYP2C9, CYP2E1, CYP3A) and glutathione S-transferase (GST) in the liver. The authors found no significant change in the cytochrome P450 content of the liver of mice treated with theanine alone. They also found that, with theanine treatment alone, there was no significant change in the level of activity of any of the CYP subtypes studied or in the level of hepatic GST activity. These results contrasted with the effects of DOX which decreased the cytochrome P450 content of the liver by 45%, had a variety of effects on the six cytochrome enzymes, and increased hepatic GST activity. The authors conclude that since theanine did not change CYP activity, it is safe as a food or supplement.

2. Subacute Studies

In one subacute theanine toxicity study, Nishida et al. (2008) orally administered L-theanine [10 mmol (1.74 g)/kg, once a day] to rats *via* gastric intubation for 2 weeks. Toxicological evaluation was limited to examining brain and body weights. Despite the administration of a high dose of theanine, equivalent to 10,000 or more cups of green tea, no change was observed in the weight of the body or the cerebral cortex (Cx), cerebellum (Cb), or hippocampus (Hip) in the brain. The authors conclude that 2-week repeated administration of theanine did not have any effect on body weight or on the 3 brain regions studied. Overall, the authors declare theanine to be a safe compound.

A second subacute study of L-theanine, performed by the Nippon Bio Research Center Co., was described in GRN 209. In this study, CRj:CD(SD) rats were gavaged with 2000 mg/kg bw/day of Suntheanine® for 28 days. The rats were monitored for changes in body weight, feeding pattern,

opthalmological examination, urine composition (color, occult blood, pH, protein, sugar, ketone bodies, urobilinogen, and bilirubin), hematology (red blood cells, hemoglobin, hematocrit, platelet, white blood cells, mean corpuscular volume, mean concentration of hemoglobin, percent white blood cells, and reticulocyte count), serum clinical chemistry (aspartate aminotransferase, alanine aminotransferase, total cholesterol, triglycerides, total protein, urea nitrogen, creatinine, total glucose, inorganic phosphorus, calcium, sodium, and potassium), and gross and histopathological examination of all of the major body organs. The authors report no adverse clinical signs, and no differences between treatment and control groups for body weight, feed consumption, opthalmological examination and urinalysis. In the hematologic and biochemical parameters measured, the only significant changes were found in treated male rats when compared to controls were an increase in fibrinogen (250.8 +/- 5.2 vs. 271 +/- 9.0 mg/dL), a decrease in platelets (118.02 +/- $6.23 \times 10^4/mm^3$ vs. 108.62 +/- $2.29 \times 10^4/mm^3$), an increased alkaline phosphatase (186.34 +/- 74.51 IU/L vs. 283.00 +/-48.10 IU/L) and an increased b-globulin in the protein fraction (11.10 +/- 0.48% vs. 11.94 +/- 0.59%). Upon examination of the organs, the authors found no gross pathological changes. They did measure a decrease in absolute brain weight (1.998+/- 0.041g vs. 1.884 +/- 0.072 g) but not relative brain weight in treated males. In addition, they observed an increase in absolute and relative liver weights. Upon histopathological examination of treated male rats, the authors report finding slight myocardial denaturation in the hearts of two rats, slight lymphocyte infilitration in the stroma of the kidney and prostate of one rat. In treated females, the authors found one rat with a slight heterotopia thyroid, one had a slight mineral deposition in the stomach, and a third rat had a vestigial postbrachial body in the thyroid. The authors concluded that these findings are spontaneous and do not constitute evidence of toxicity. The authors reported the no-observed-adverse-effect level (NOAEL) in this study to be 2000 mg/kg bw/day.

3. Subchronic Studies

In a study specifically aimed at investigating the safety of L-theanine, Borzelleca et al. (2006) performed a 90-day subchronic study measuring toxicity and toxicokinetic parameters with L-theanine in Sprague-Dawley rats. The study was conducted according to OECD and FDA guidelines for such type of study. Overall, the authors report that the oral administration of L-theanine at doses of 1500, 3000, and 4000 mg/kg bw/day resulted in a NOAEL up to the highest dose tested. The authors reported no statistically significant treatment-related adverse effects on morbidity and mortality, body weight, food consumption and efficiency, clinical chemistry, hematology, or urinalysis. Some effects observed by the authors, but not thought to be treatment-related, include a significant decrease in both body weight and food consumption at the 3000 and 4000 mg/kg dose. This decrease was considered to be related to the unpalatability of the diet and was not a toxic response. The authors also observed a decrease in locomotor activity in males when tested at week 3 (3000 mg/kg and 5000 mg/kg) and week 14 (1500 mg/kg, 3000 mg/kg and 4000 mg/kg). The biological significance of this effect in males is unknown, but the authors conclude that this is not a treatment-related adverse effect.

In their subchronic study, Borzelleca et al. (2006) also reported that no consistent treatment-related adverse effects on gross pathology, organ weights or ratios or histopathology were observed. The few effects observed were not consistent over time and/or were not dose-related. The only effects observed by the authors that were thought to be treatment-related were 1) mildly higher serum cholesterol levels in females (3000 mg/kg and 4000 mg/kg), 2) mildly lower serum

total protein in females (4000 mg/kg), and 3) mildly higher urine pH in males (3000 mg/kg and 4000 mg/kg). The authors state that these findings were mild, were not statistically significant, and are not considered adverse effects. Numerous scattered significant alterations in organ weight were observed in the groups at the two sacrifice periods. Significant changes include increased kidney-to-body weight ratios in males at all doses and females at two dose/sacrifice points. A decrease in thymus mean weights and thymus-to-body ratio, and thymus-to-brain ratio was also observed in males at all doses. The authors attribute these changes to the overall change in body weights in the treated groups. No histological changes were observed in these organs, and the authors determined that the observed changes were not biologically important.

Borzelleca et al. (2006) did observe multiple renal tubular cell adenomas and an atypical tubular hyperplasia in 2/20 (10%) of the high-dose (4000 mg/kg) and 1/20 (5%) of the mid-dose (3000 mg/kg) female rats. Except for these three females (two in the high- and one in the mid-dose group), no other females or males showed any evidence of hyperplasia or renal tumors at any of the doses. Toxic tubular changes were not seen in any other animals as would be expected in a direct toxic reaction. Hall et al. (2007) extensively investigated the mechanism behind the development of these adenomas and hyperplasia. Their investigation suggests that the increased incidence of renal tubular cell adenomas in high-dose females is due to a genetic predisposition as opposed to direct organ toxicity. The two high-dose affected females were found to be genetically related based on microsatellite DNA loci testing. Hall et al. (2007) found that the two high-dose affected females shared an uncommon polymorphism in the Birt-Hogg-Dube (*BhD*) gene. Defects in the tumor suppressor gene BhD have been previously linked to a predisposition to spontaneous renal tumors in Sprague-Dawley rats (Kouchi et al., 2006). Supporting a genetic predisposition to renal tumors in the affected rats, Hall et al. (2007) performed laser capture microdissection (LCM) of the tumor cells from the affected animals and found a loss of heterozygosity of this gene in one of the two animals. Overall, results from these investigations suggest that the tumors noted in these animals were spontaneous as a result of a shared genetic susceptibility resulting in mutation of the *Bhd* gene and development of renal tumors. The authors concluded that the development of adenomas and hyperplasia were not related to the test article (Hall et al., 2007).

Fujii and Inai (2008) reported a subchronic and a chronic study of L-theanine in mice. These studies were also summarized in GRN 209 with some additional details. For the subchronic study, B6C3F1 mice (10/sex/group) were fed a diet containing 0, 0.6, 1.25, 2.5 or 5% L-theanine. The approximate daily intake of theanine for male mice was reported as 0, 850, 1700, 3150, and 6300 mg/kg bw/day and for females the intake was 0, 550, 1450, 2550 and 5150 mg/kg bw/day, respectively. Body weight and feed consumption were recorded weekly. At the end of the 13 week test period, all animals were euthanized and autopsied. Organs and tissues, including heart, lung, submaxillary gland, esophagus, stomach, small intestine, large intestine, liver, pancreas, kidney, ovary, thyroid gland, adrenal gland, thymus, spleen, bone marrow, brain (cerebrum, cerebellum) and spinal cord were processed for histological examinations. No mortality or statistically significant differences in body weights and feed consumptions among the test groups were noted. Pathological examinations did not reveal any notable changes, including degeneration, atrophy, necrosis, inflammation or tumor in any organs or tissues. The results of this study suggest that L-theanine had no adverse effects at dose levels of up to 6300 mg/kg bw/day in male and 5150 mg/kg bw/day in female mice.

4. Chronic Studies

In a chronic toxicity study of L-theanine in mice, Fujii and Inai (2008) orally administered L-theanine at dose levels of 0 (control), 2.5 and 5% in the diet to three groups of B6C3F1 mice for 78 weeks. Some additional details of the study were also summarized in GRN 209. Based on the summary provided in the GRN 209, the doses of L-theanine were approximately 0, 2200 and 4200 mg/kg bw/day for males and 0, 2000 and 4150 mg/kg bw/day for females. Compared to control group, no significant change in the mean body weight of mice or in the survival rates in the test group were noted. Over the course of the 78 weeks, the total number of tumors decreased in a dose-dependent manner in both sexes of mice. A statistically significant decrease in the total number of tumors was observed in male mice. A non-significant decrease in tumor number was also observed in female mice. Given the lack of effect of theanine administration on body weight and organ histology, the authors conclude that the tolerated maximum dose (TMD) is greater than 5%. The investigators concluded that middle to long-term daily administration of L-theanine to mice would cause no undesirable effects.

5. Genotoxicity/Mutagenicity Studies

Ames tests of L-theanine have been reported by two different groups of investigators. Both groups found L-theanine to be non-mutagenic. Ishidate et al. (1984) performed Ames testing and chromosomal aberration tests of 242 different food additives, including L-theanine. The authors reported that an Ames test of L-theanine using *Salmonella typhimurium* and a maximum dose of 20 mg/plate of L-theanine was negative. Fourteen of the 242 food additives tested were positive. As reported in GRN 209, the Nippon Bio Research Center Co. (1999) also performed an Ames test of L-theanine using *S. typhimurium* TA100, TA98, TA1535, and TA1537 and *E coli* strains *WP2uvrA* with a maximum dose of 5000 µg/plate of Suntheanine® with and without activation (S9). Suntheanine® was not mutagenic in this assay.

Similarly, two groups have reported the results of chromosomal aberration tests. As part of the study cited above, Ishidate et al. (1984) treated the Chinese hamster fibroblast (CHL) cell line with 242 different food additives, including L-theanine at a maximum dose of 2 mg/mL. Cells were treated at 24 and 48 hours without metabolic activation. Chromosomes were isolated, and a hundred well-spread metaphases were observed under the microscope. The authors reported an incidence of 1% polyploid cells after 48 hours of L-theanine treatment, and of 4% structural chromosomal aberrations after 24 hours of L-theanine treatment. Results were considered negative if the incidence of aberrations was less than 4.9%. Untreated and solvent treated negative controls had an incidence of 3%. The results of this study suggest that L-theanine does not induce chromosomal aberrations.

In GRN 209 (2006), findings from an unpublished *in vitro* cytogenetics assay by Lloyd (2004) using duplicate human lymphocyte cultures under GLP and following OECD guideline 473 were described. The author reported that the frequency of cells with structural aberrations was similar between L-theanine-treated cultures and the negative sterile purified water controls. In contrast, the positive controls of mitomycin C (without S9) and cyclophosphamide (with S9) demonstrated a significant increase in the proportion of cells with structural aberrations. The authors concluded that L-theanine does not induce chromosome aberrations in human peripheral blood lymphocytes with or without metabolic activation.

E. Clinical Studies on Theanine

Clinical studies of theanine have been performed with theanine administered at dose ranges from 50-250 mg. The majority of human studies performed were with a small sample size and a short duration of exposure. These studies were designed to evaluate the effects of theanine on cognition, attention and stress responses. In all of the studies, limited monitoring was performed for the occurrence of any adverse effects. The results of these studies are summarized below.

A number of clinical studies have utilized ECG recordings to measure changes in alpha band activity in response to L-theanine. Alpha bands are generally thought to be a measure of relaxation. Juneja et al. (1999) found that oral administration of theanine (50 mg or 200 mg) in four high-anxiety and four low-anxiety volunteers resulted in an increase in alpha waves in the occipital and parietal regions of the brain when compared to water ingestion. Changes in alpha band activity may also be associated with selective attentional processes. Gomez-Ramirez et al. (2007) measured alpha band activity in fifteen volunteers (18-36 years) during selective attentional tasks following oral consumption of L-theanine (250 mg) or placebo. Theanine consumption resulted in an overall decrease in alpha activity while subjects were engaged in a very demanding cognitive task. However, alpha band activity was increased when subjects were under conditions requiring intersensory selective attention. The authors conclude that L-theanine may have specific effects on the brain's attention circuitry.

Since L-theanine is commonly consumed in conjunction with caffeine, clinical studies have been performed to assess the effects of L-theanine on cognition and attention in the presence and absence of caffeine. In a recent study, Owen et al. (2008) investigated the effect of caffeine (50 mg) on cognition and mood, in the presence and absence of L-theanine (100 mg). The authors found that the caffeine and theanine combination improved both speed and accuracy of performance on an attention-switching task at 60 minutes, and reduced susceptibility to distracting information in a memory task at both 60 and 90 minutes. The authors claimed that their results suggest L-theanine and caffeine together are beneficial for improving performance on cognitively demanding tasks.

In another randomized, double-blind, placebo-controlled, balanced crossover study, Haskell et al. (2008) investigated the acute cognitive and mood altering effects of L-theanine (250 mg) and caffeine (150 mg) alone and in combination. Experiments were performed with 24 volunteers, ages 18-34. The authors found that L-theanine alone had relatively few effects. Out of 38 cognitive measures performed, the authors found an effect of L-theanine on two. L-Theanine increased 'headache' ratings and decreased correct serial seven subtractions. Comparisons of the individual drinks to placebo showed that L-theanine alone significantly impaired performance on serial seven subtraction task at 90 min. The investigators suggested that beverages containing L-theanine and caffeine may have a different pharmacological profile to those containing caffeine alone.

In a third study assessing the effects of L-theanine in the presence and absence of caffeine, Kelly et al. (2008) administered 100 mg of L-theanine in the presence and absence of 50 mg of caffeine. In this study, 16 volunteers, ages 21-40 were given L-theanine and/or caffeine. Behavioral and electrophysiological analyses were performed. No effects on hit rate were observed, and no effect on tonic alpha amplitude was found with L-theanine alone. In contrast, a

decrease in tonic alpha amplitude was found with the combination. In addition, the authors stated that previous unpublished studies of L-theanine at a higher dose of 250 mg also showed a decrease of tonic alpha amplitude. Based on their data, the authors suggested that L-theanine may work to enhance the tonic apportionment of attentional resources to the visual modality when administered at high levels or in combination with caffeine.

Numerous clinical studies measuring the anti-stress effects of L-theanine have been published. Kimura et al. (2007) performed a study of the effect of L-theanine on psychological and physiological stress responses in twelve male volunteers, ages 21-25. In this study, the authors measured cardiodynamic activity utilizing ECG, and they measured volume of saliva and the concentration of s-IgA in saliva. Administration of L-theanine (200 mg, oral) to participants subjected to the stress of a mental arithmetic test showed non-significant increases in heart rate and reduced salivary IgA levels. The authors note that the small number of participants and the relatively few parameters measured limits the study. However, the investigators concluded that, with these given limitations, the administration of L-theanine reduced the stress response induced by the mental arithmetic test.

In a double-blind placebo-controlled repeated measures design, Lu et al. (2004) compared the acute effects of L-theanine with the benzodiazepine anxiolytic (anti-anxiety) alprazolam on behavioral measures of anxiety. In this study, sixteen volunteers were administered 200 mg of L-theanine, alprazolam (1 mg), or placebo. The effects of treatment were assessed under a relaxed and experimentally induced anxiety condition. The results showed some evidence for relaxing effects of L-theanine during the baseline condition on the tranquil-troubled subscale of the VAMS. Neither L-theanine nor alprazolam had any significant anxiolytic effects during the experimentally induced anxiety state. The findings from this study suggest that, while L-theanine may have some relaxing effects under resting conditions, L-theanine or alprazolam did not demonstrate any acute anxiolytic effects under conditions of increased anxiety in the anticipatory anxiety model.

In addition to the above-described studies, summaries of a series of unpublished studies, abstracts, or foreign language articles are described in GRN 209. These summaries and findings described in GRN 209 are presented below.

> In a randomized double-blind crossover study, 23 healthy male golfers received 300 mg Suntheanine® + 20 mg L-carnitine + 100 mg Dongchunghacho or a placebo for 2 days (Kim et al., 2001). Those receiving the theanine-containing beverage showed significant increases in alpha waves of the frontal and occipital regions beginning 30 minutes after the administration. Of most interest to golfers, there were significant improvements in putting success rate and in driving accuracy.
>
> In another study, "Kobayashi et al. (1998) divided 50 females age 18-22 into a high-anxiety group and a low-anxiety group based on scores on the Manifest Anxiety Scale. Four high- and four low-anxiety subjects were given either 50 mg or 200 mg theanine in water once a week. Following the administration of theanine, each subject had an electroencephalogram (EEG) for 60 minutes. Effects were noted about 30-40 minutes after the intake with an increased production of alpha waves (reported to be characteristic of relaxation), but not theta waves (reported to be indicators of drowsiness) were observed.
>
> In a randomized placebo-controlled double-blind crossover study, Song et al. (2002) investigated the effects of L-theanine in 20 subjects age 30-55 years suffering from persistent fatigue. The subjects were classified with high or low anxiety and were administered a placebo or 200 mg L-theanine every day for 7 days. Each subject's frontal and occipital EEG was measured over 1 hour immediately after the administration of L-

theanine. Results showed significant relaxant effects of theanine. In a similar follow-up randomized placebo-controlled double-blind crossover study with 20 younger males (age 18-30), Song et al. (2003) noted significant ($p<0.05$) differences in those with high anxiety, but not in those with low anxiety. The investigators concluded that the results of both studies indicate that theanine promotes brain activity related to mental relaxation and concentration.

Also summarized in GRN 209 (2006), Weiss and his colleagues "conducted a number of studies of the effects of L-theanine on the recovery of individuals from the stress of heavy exercise. In an unpublished study (Weiss et al. undated), 14 healthy male athletes exercised on a bicycle ergometer and then consumed a drink with 0, 50, or 200 mg L-theanine in a randomized double-blind crossover design. Theanine had no effect on hormones important for regeneration, but lowered prolactin (which is regulated by dopamine and serotonin). There was no significant change in brain function, but lowered β waves, reported to be an indication of relaxation, were observed. In a similar randomized double-blind crossover study, Weiss et al. (2001a) gave 14 healthy athletes drinks with 0, 50, or 200 mg theanine after exhausting exercise on a bicycle ergometer. L-Theanine had no effect on peripheral sympathetic electrodermal activity during regeneration. In another phase of this same study but reported separately (Weiss et al. 2001b), plasma levels of catecholamines, cortisole, prolactin, and serotonin were measured 0, 30, 45, 60, and 120 minutes after the drink. No effects of theanine on hormonal levels were observed. Finally, in a third phase of the study (Weiss et al. 2001c), EEG mapping was used to assess the effect of ingestion of L-theanine; the experimenters reported that L-theanine supported physiological relaxation after severe exercise.

In another unpublished study summarized in GRN 209, "Weiss and Geiss (undated) sought to determine if theanine administration had any adverse effects on cognitive function, such as poorer reaction time, concentration, alertness, or attention. In a randomized double-blind placebo-controlled crossover study, 20 male students age 19-32 ingested either placebo or 200 mg L-theanine at breakfast. No significant differences were observed in heart rate or blood pressure, blood glucose, red blood cells, hemoglobin, hematocrit, uric acid, urea, or y-glutathione. No differences were observed in reaction time or accuracy of response, concentration, or perceptual speed on a tachistoscopic test.

VI. DISCUSSION OF REVIEWED INFORMATION & GRAS CRITERIA

A. GRAS Criteria

FDA defines "safe" or "safety" as it applies to food ingredients as

> "...reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use. It is impossible in the present state of scientific knowledge to establish with complete certainty the absolute harmlessness of the use of any substance."[5]

Amplification is provided in that the determination of safety is to include probable consumption of the substance in question, the cumulative effect of the substance, and appropriate safety factors. It is FDA's operational definition of safety that serves as the framework against which this evaluation is provided.

Furthermore, in discussing GRAS criteria, FDA notes that

> "...General recognition of safety requires common knowledge about the substance throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food."
>
> "General recognition of safety through experience based on common use in food prior to January 1,1958, shall be based solely on food use of the substance prior to January 1, 1958, and shall ordinarily be based upon generally available data and information."[6]

FDA discusses in more detail what is meant by the requirement of general knowledge and acceptance of pertinent information within the scientific community, i.e., the so-called "common knowledge element," in terms of the two following elements:[7]

- Data and information relied upon to establish safety must be generally available, and this is most commonly established by utilizing published, peer-reviewed scientific journals; and
- There must be a basis to conclude that there is consensus (but not unanimity) among qualified scientists about the safety of the substance for its intended use, and this is established by relying upon secondary scientific literature such as published review articles, textbooks, or compendia, or by obtaining opinions of expert panels or opinions from authoritative bodies, such as the National Academy of Sciences.

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[5] See 21 CFR 170.3(i).
[6] See 21 CFR 170.30(a).
[7] See footnote 1.

The apparent imprecision of the terms "appreciable", "at the time" and "reasonable certainly" demonstrates that the FDA recognizes the impossibility of providing absolute safety, in this or any other area (Lu, 1988; Renwick, 1990).

B. Discussion of L-Theanine (98%) Safety

A significant amount of information related to the safety of L-theanine is available for evaluation. In addition to a long history of safe consumption of L-theanine from its natural presence in tea, the safety of L-theanine has been the subject of a number of experimental studies in both animals and humans. The evidence from historical safe consumption of L-theanine from tea and experimental studies together can be used to determine its safe use for human consumption.

As noted in Section V, tea is the only naturally occurring source of theanine in the diets of Americans and, in fact, of most people around the world. Around the world, tea is the most widely consumed beverage after water. In the US, as a beverage tea competes with coffee and a broad selection of other beverages. Available surveys suggest that approximately 20% of adults in the United States are reported to consume tea regularly. The mean amount of tea beverage consumed in the US is ~800 mL/person, while the 90th percentile consumers ingested 1650 mL of tea, and the 99th percentile consumers' intake has been estimated as 3300 mL/person. It is possible that in countries, particularly in Asia, where tea consumption is very common, the per capita tea consumption is likely to be even higher. The theanine content of a broad selection of tea leaves has been reported to range from 1 to 2.5%. Given high water solubility of theanine, nearly all of the theanine from tea leaves during tea preparation is likely to dissolve in the water and be consumed. The estimated intake of theanine by tea drinkers has been estimated to be 152-382 mg/day at the mean, between 330 and 825 mg/day at the 90th percentile, and as much as 667-1668 mg/day at the 99th percentile. Thus, the available exposure information suggests safe consumption of tea, which, in turn, supports the safety of theanine consumption from its presence in tea.

L-Theanine has been well studied in laboratory animal toxicity investigations and in clinical studies with human subjects. Available evidence from metabolism and kinetic studies suggest that, following oral ingestion, theanine is absorbed in the blood from the intestines and is distributed in many tissues, such as the liver and brain. Inside the human body, theanine is hydrolyzed to glutamine and ethylamine. Within a few hours of ingestion, both theanine and its metabolites reach peak concentrations in the plasma and in tissues followed by rapid decline, along with a concomitant increase in urinary concentrations. No evidence exists to suggest that L-theanine would bioaccumulate.

In acute toxicity studies, the oral LD_{50} of L-theanine has been reported to be >5000 mg/kg bw suggesting that L-theanine is practically non-toxic (Derelanko and Hollinger, 1995). In a short-term (28-day) oral toxicity study, the NOAEL at the only dose tested, was 2000 mg/kg bw/day. In a 13-week subchronic dietary toxicity study, rats were fed diet containing 1500, 3000, or 4000 mg theanine/kg bw/day and no toxicity was noted at any tested dose. The results of this study suggest that the NOAEL was the highest dose of 4000 mg/kg bw/day. In this study, renal tubular cell adenomas were found in two high dose and one mid dose treated female rats. Further extensive investigation revealed that these tumors were not treatment-related but were due to an unusual genetic anomaly among the population of rats used in this study. No such changes were

noted in subchronic and chronic studies in mice. Additionally the long history of safe consumption of tea and epidemiological evidence does not support any association between L-theanine and renal tubular cell adenomas. The Panel agrees that the kidney tumors noted in the rat study were an anomalous occurrence and have no bearing on the safety assessment of theanine. In a subchronic (13 weeks) and chronic (78 weeks) study of L-theanine in mice, no evidence of toxicity was noted. The results of the chronic study in mice showed no evidence of carcinogenicity of L-theanine. In genetic toxicology tests, L-theanine showed no mutagenic or clastogenic activity, further indicating that L-theanine lacks the potential to be carcinogenic. In clinical trials with human volunteers studying various possible benefits of L-theanine, no adverse effects were noted.

The no adverse effect level of 4000 mg/kg bw/day from the subchronic study in rats corresponds to a dose level of 240 g/day for an individual weighing 60 kg. Based on the intended uses of L-theanine (98%) in selected food categories identified by Blue California, the maximum daily intake has been estimated to be 1284 mg/person/day. The safety studies as corroborated by history of use information support the safety-in-use determination at the intended use levels. The Panel has concluded that the proposed average consumption of 0.63 g/person/day or 11 mg/kg bw/day and maximum consumption of approximately 1.28 g/person/day or 24 mg/kg bw/day from the use in the food categories proposed in Section IV is safe. The Panel has also determined that there is an approximate margin of safety of over 100-fold from the doses found to have no effect in long term studies in mice and rats. The estimated daily intake from the food uses proposed by Blue California, even if ingested daily over a lifetime, is considered to be safe.

Sufficient qualitative and quantitative scientific evidence exists, including human and animal data, to support the safety-in-use of L-theanine (98%). The evidence of L-theanine (98%) safety is underscored by virtue of the following:

- L-Theanine, an amino acid, is found in free form in tea and has a long safe history of human consumption.
- L-Theanine is isolated from tea leaves *(Camellia sinensis)* in accordance with current Good Manufacturing Practices.
- There is no evidence that consumption of L-theanine either in foods or as a dietary supplement has adverse effects.
- The bioavailability of the ingested L-theanine is limited as it is metabolized and rapidly excreted with no detectable levels in blood plasma 6 hours after oral administration.
- The metabolism of L-theanine has been well characterized, and neither L-theanine nor its metabolites bioaccumulate.
- A variety of animal, human and *in vitro* studies support the safety of L-theanine.

The Panel has reviewed the manufacturing procedures and specifications established by Blue California, and the Panel finds that the specifications as reported in Table 4 are adequate to define a suitable purity to be considered food grade.

C. Common Knowledge Elements

The first common knowledge element for a GRAS determination requires that data and information relied upon to establish safety must be generally available; this is most commonly established by utilizing studies published in peer-reviewed scientific journals. The majority of the studies reviewed in this safety assessment have been published in the scientific literature as reported in Section V. Specifically, studies by Borzelleca et al. (2006), Hall et al. (2007) and Fujii and Inai (2008) have investigated the subchronic and chronic toxicity of L-theanine in rats and mice. Findings from these investigations have been published in peer reviewed journals that are readily available. In addition to the many scientific studies that have been conducted and published, history of tea consumption and, in turn, intake of L-theanine since ancient times is well known around the world.

Furthermore, safety documentation for food uses of L-theanine is found in GRN 209, which also constitutes information that is generally available for review and evaluation. The composite information noted thereby fulfills the common knowledge element required for GRAS determinations.

The second common knowledge element for GRAS determinations requires that consensus exists among qualified scientists that the subject safety assessment is reasonable and appropriate. The most compelling documentation of consensus for L-theanine (98%) is the recognition that the Expert Panel undertaking the review of GRN 209 concluded that the intended food uses of comparable material were considered to be safe. Furthermore, review of this determination yielded agreement by FDA.

VII. CONCLUSIONS[8]

The Expert Panel has concluded that high purity L- theanine, i.e., L-theanine (98%), when extracted from tea leaves and produced in accordance with FDA Good Manufacturing Practices requirements and which meets appropriate food grade specifications as found with Blue California's L-TeaActive™ is Generally Recognized As Safe when consumed at levels up to 1.28 grams per person per day. In order to maintain safe levels of consumption, food uses should be maintained within the levels described in Section IV.

(b) (6)

Richard C. Kraska, Ph.D., DABT

(b) (6)

Robert S. McQuate, Ph.D.

(b) (6)

Madhusudan G. Soni, Ph.D., FACN

May 5, 2010

[8] The detailed educational and professional credentials for the individuals serving on the Expert Panel can be found on the GRAS Associates website at www.gras-associates.com. Richard C. Kraska, Ph.D., DABT, Robert S. McQuate, Ph.D. and Madhusudan G. Soni, Ph.D. have extensive technical backgrounds in the evaluation of food ingredient safety. Drs. Kraska and McQuate worked on GRAS and food additive safety issues within FDA's GRAS Review Branch earlier in their careers and subsequently continued working within this area in the private sector. Dr. Soni's curriculum vitae can be accessed at: http://www.soniassociates.net/Soni%20CV.pdf. All three individuals have previously served on multiple GRAS Expert Panels. Dr. Kraska served as Chair of the Panel.

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Weiss, M., Barthel T., Schnittger, R., Reinsberger, C., Undated. Scientific study on L-theanine containing drinks with regard on relaxation and regeneration after physical stress as measured by electroencephalography, skin conductance and stress hormones. Final Report from the Institute for Sports Medicine, University of Paderborn, Germany. Abstract summarized in GRN 209 (GRAS Notice Review), 2006. Suntheanine®. Notifier: Taiyo International Inc., Minneapolis, Minnesota. Available online: http://www.accessdata.fda.gov/scripts/fcn/gras_notices/615880A.PDF

Weiss, M., Geiss, K.R, Undated. The influence of L-theanine on reaction time and concentration. Final Report fiom the Institute for Sports Medicine, University of Paderborn, Germany. Unpublished manuscript as summarized in GRN 209 (GRAS Notice Review), 2006. Suntheanine®. Notifier: Taiyo International Inc., Minneapolis, Minnesota. Available online: http://www.accessdata.fda.gov/scripts/fcn/gras_notices/615880A.PDF

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Yokogoshi, H., Y. Kato, Sagesaka, Y.M., Takahara-Matsura, T., Kakuda, T., Takeuchi, N., 1995. Reduction effect of theanine on blood pressure and brain 5-hydroxyindoles in spontaneously hypertensive rats. Biosci Biotechnol Biochem 59(4): 615-8.

Yokogoshi, H., Kobayashi, M., 1998. Hypotensive effect of gamma-glutamylmethylamide in spontaneously hypertensive rats. Life Sci 62(12): 1065-8.

Yokogoshi, H., Mochizuki, M., Saitoh, K., 1998a. Theanine-induced reduction of brain serotonin concentration in rats. Biosci Biotechnol Biochem 62(4): 816-7.

Yokogoshi, H., Kobayashi, M., Mochizuki, M., Terashima, T., 1998b. Effect of theanine, r-glutamylethylamide, on brain monoamines and striatal dopamine release in conscious rats. Neurochem Res 23(5): 667-73.

APPENDIX A

GMP Certificate

NSF

NSF International

789 N. Dixboro Road, Ann Arbor, Michigan 48105
(800) 673-6275

INDEPENDENTLY REGISTERED NSF GMP
Manufacturing Facility

Blue California
Jiangsu Province
Jiangsu Province, China 214437

has been assessed by NSF International and found to be in compliance with:

GMP Requirements in
NSF/ANSI Standard 173, Section 8
DIETARY SUPPLEMENTS

(b) (6)

Certificate Date:	December 17, 2006
Certification Number	2WI22D
Initial Certification	April 17, 2006

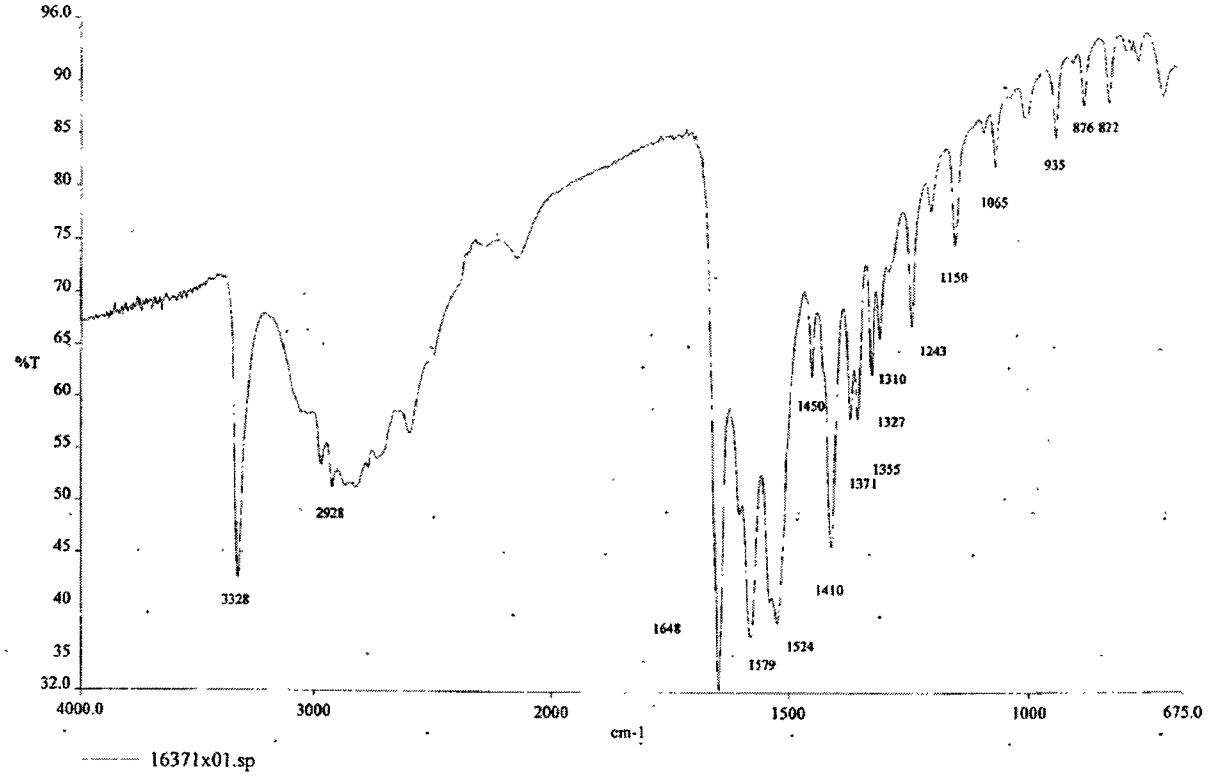
Ed Wyszumiała, General Manager
Dietary Supplements

APPENDIX B

Fourier Transform Infrared Spectra

B-1 L-Theanine Reference Standard
B-2 L-Theanine Lot #4968
B-3 L-Theanine Lot #4969
B-4 L-Theanine Lot #4970
B-5 L-Theanine Lot #4971
B-6 L-Theanine Lot #4972

B-1 L-Theanine Reference Standard



Description: L - Theanine Reference Standard

Date Created: Monday, March 08, 2010 4:39 PM Pacific Standard Time

Resolution: 4 cm-1

Detector: MCT

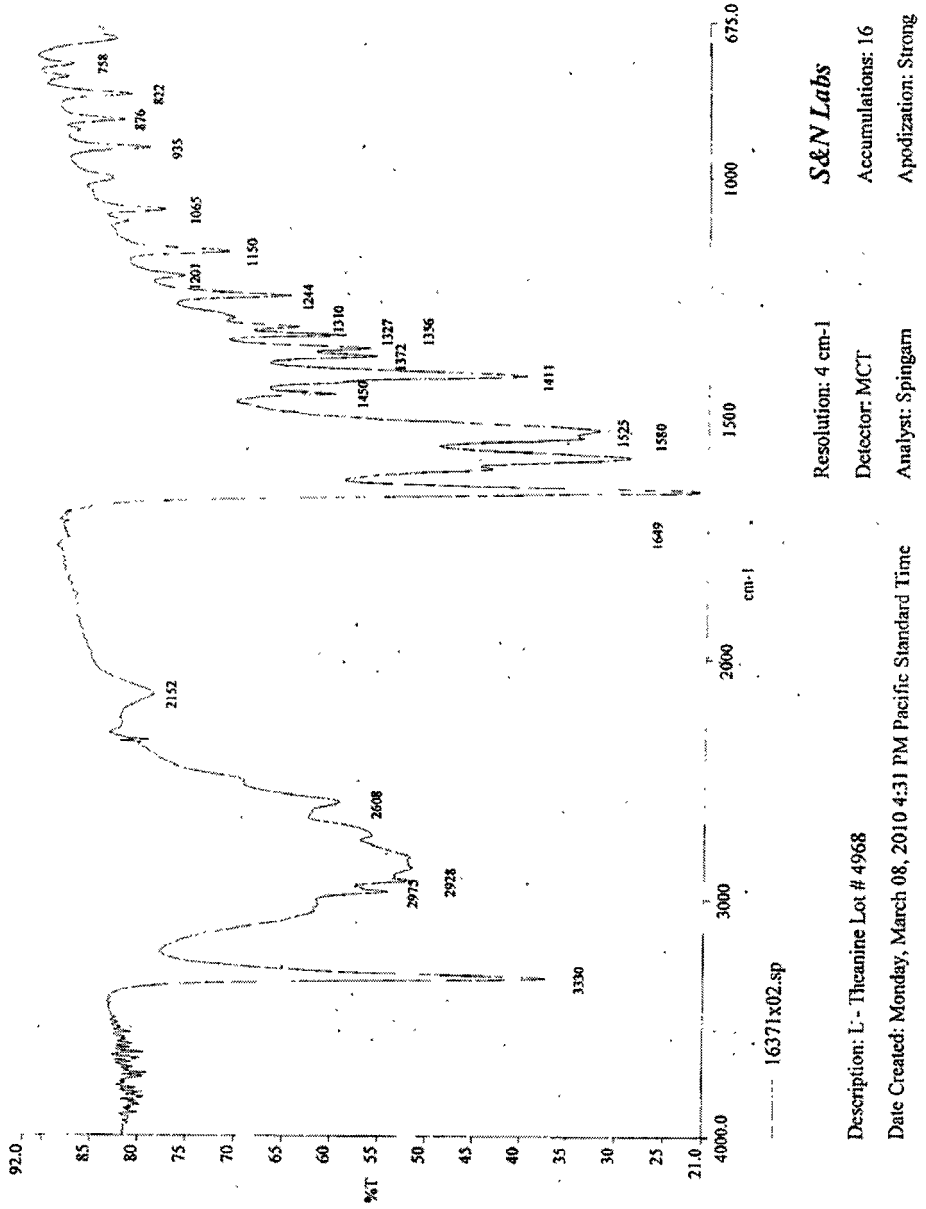
Analyst: Spingarn

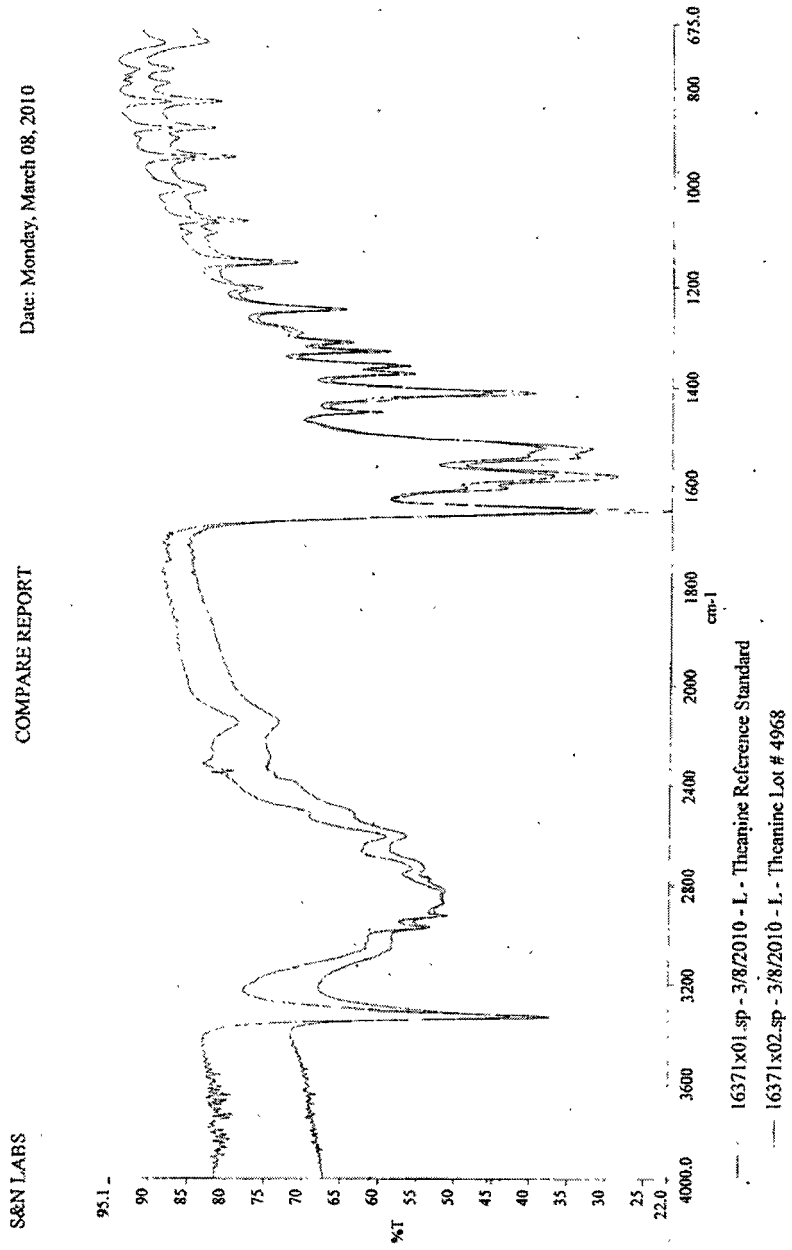
S&N Labs

Accumulations: 16

Apodization: Strong

B-2 L-Theanine Lot #4968




S&N LABS
COMPARE REPORT
Date: Monday, March 08, 2010
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55
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45
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30
25
22.0
%T
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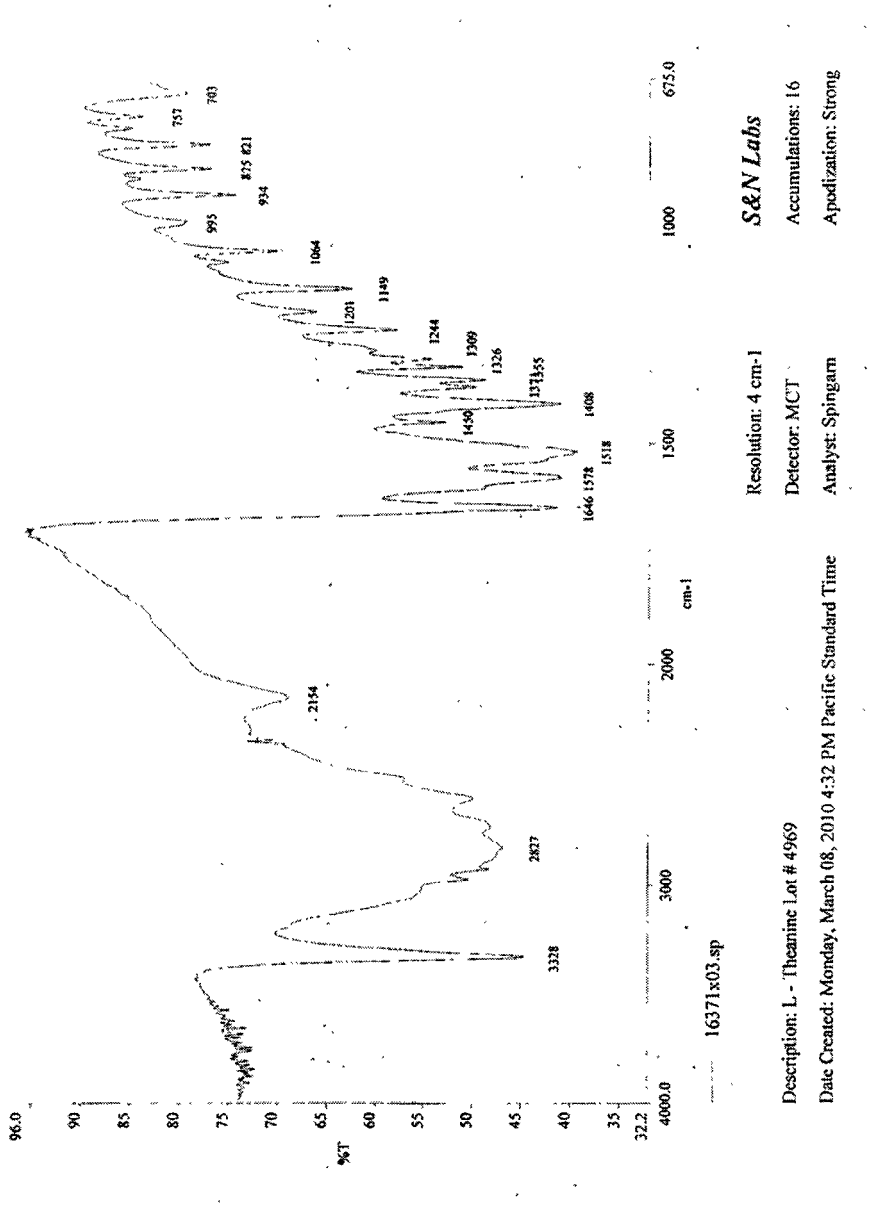
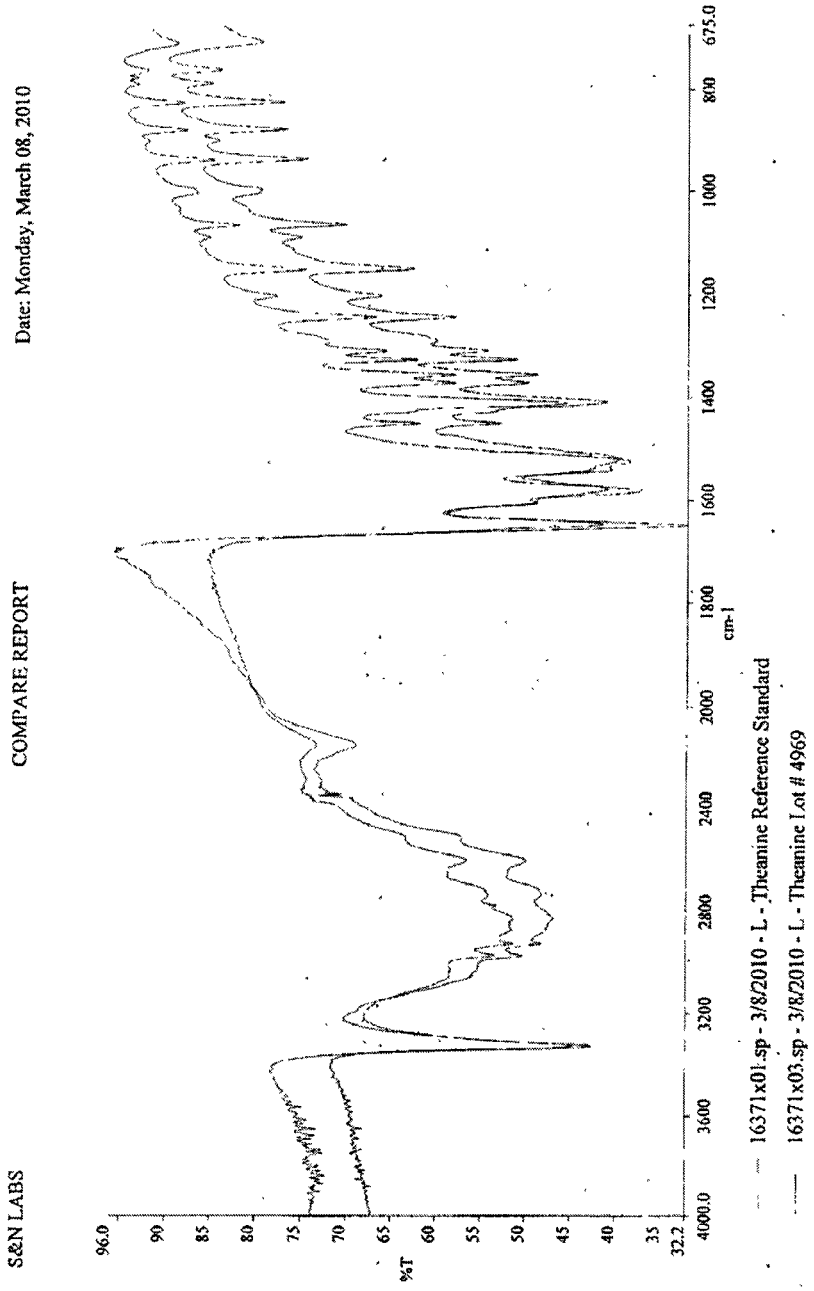
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
675.0
cm-1
16371x01.sp - 3/8/2010 - L - Theanine Reference Standard
16371x02.sp - 3/8/2010 - L - Theanine Lot # 4968

APPENDIX B-3 L-Theanine Lot #4969

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COMPARE REPORT
Date: Monday, March 08, 2010
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1800
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1400
1200
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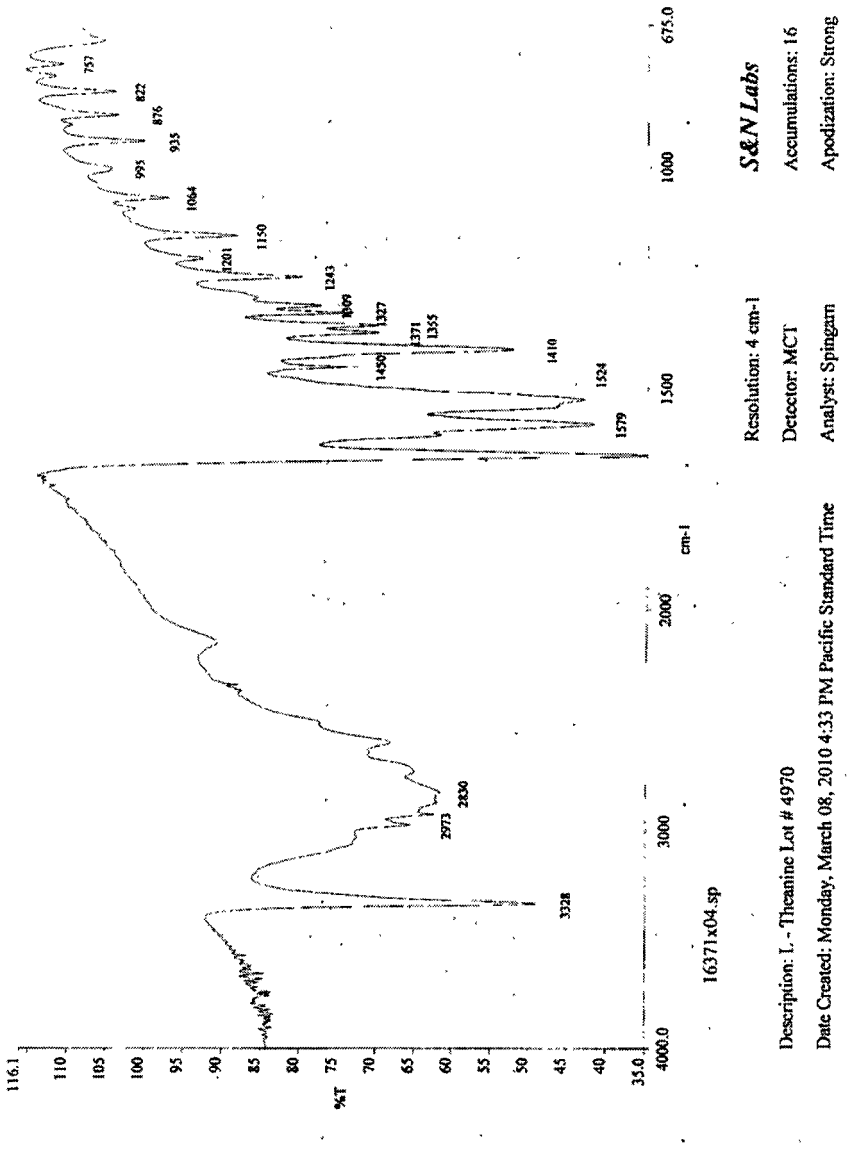
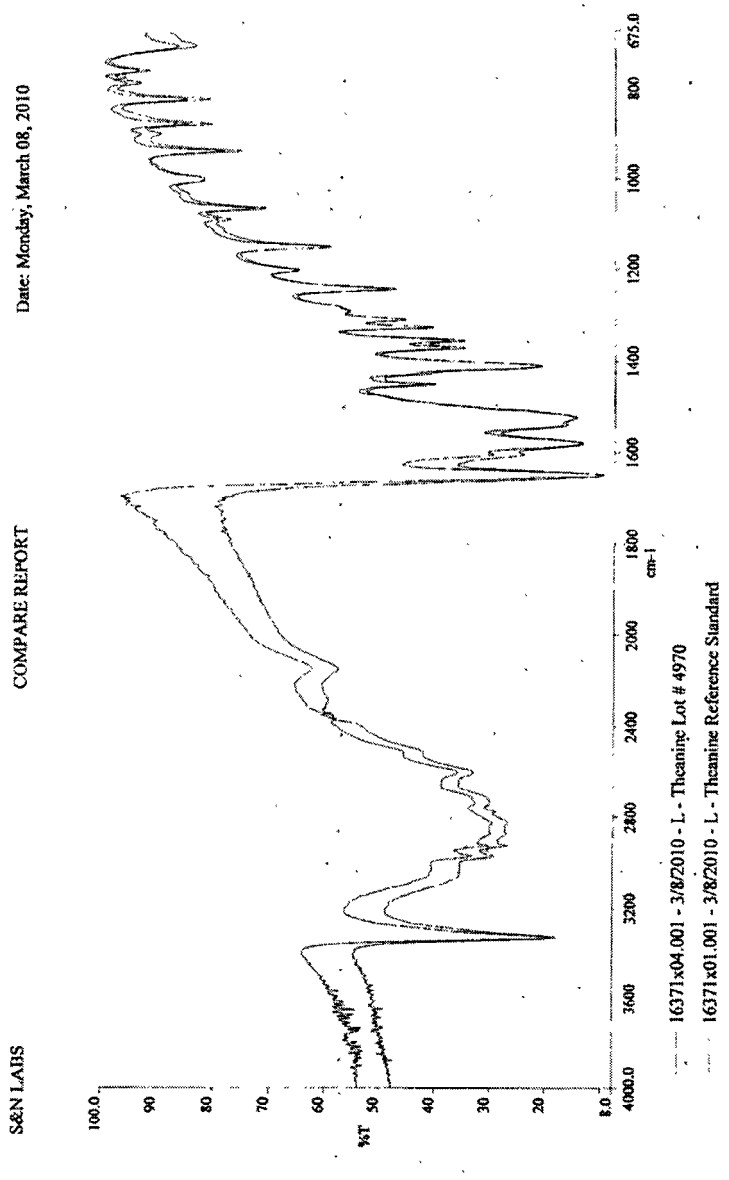
cm-1
16371x01.sp - 3/8/2010 - L - Theanine Reference Standard
16371x03.sp - 3/8/2010 - L - Theanine Lot # 4969

APPENDIX B-4 L-Theanine Lot #4970




S&N LABS
COMPARE REPORT
Date: Monday, March 08, 2010
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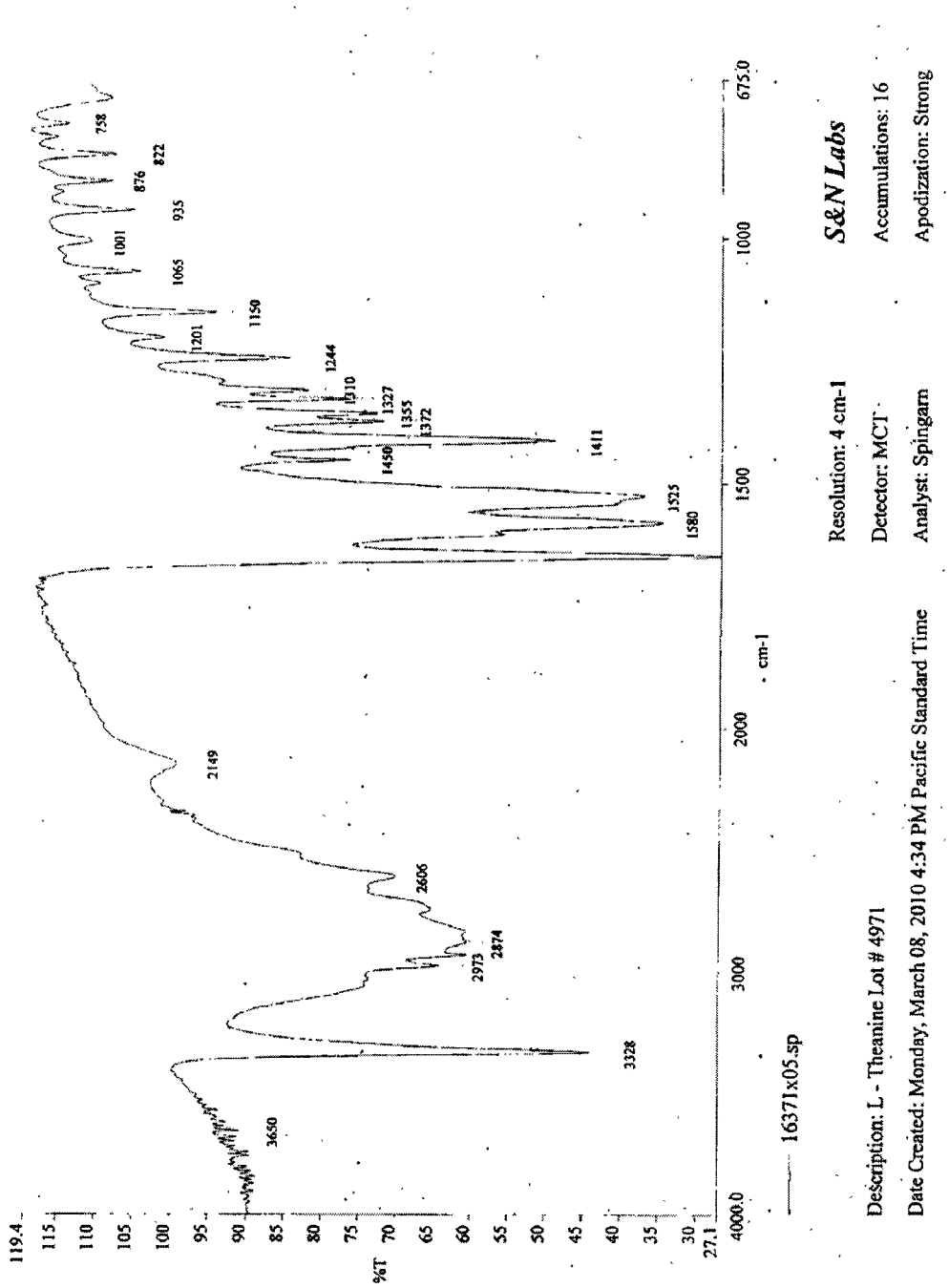
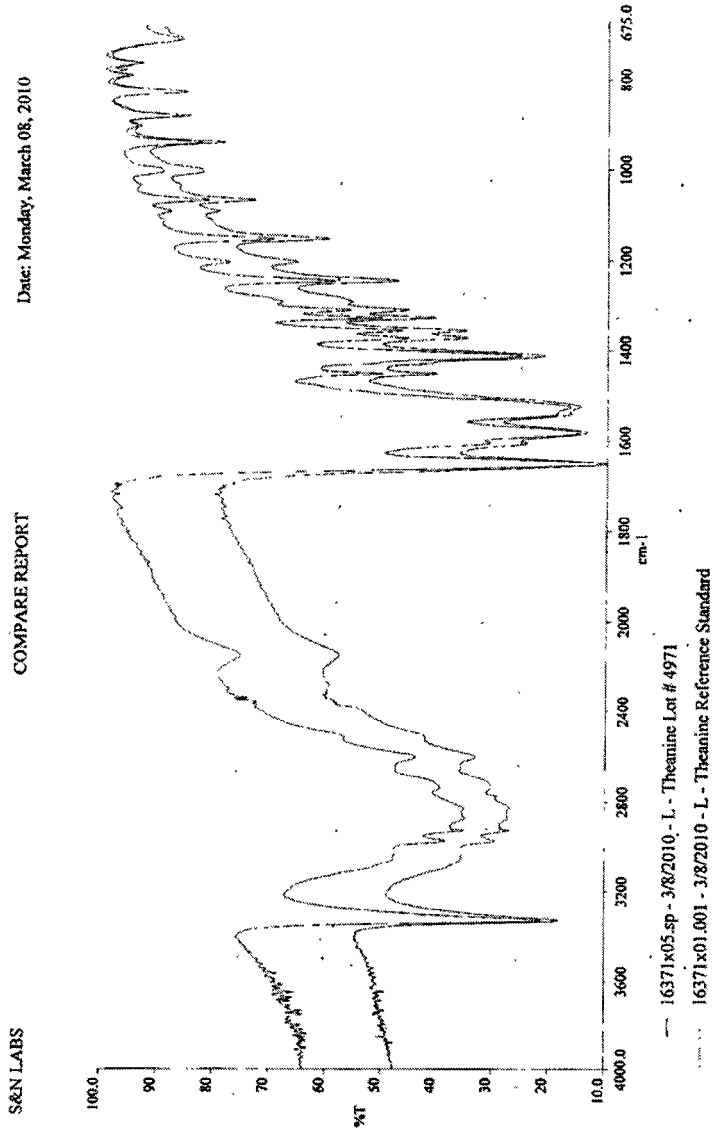
675.0
cm-1
16371x04.001 - 3/8/2010 - L - Theanine Lot # 4970
16371x01.001 - 3/8/2010 - L - Theanine Reference Standard

APPENDIX B-5 L-Theanine Lot #4971




S&N LABS
COMPARE REPORT
Date: Monday, March 08, 2010
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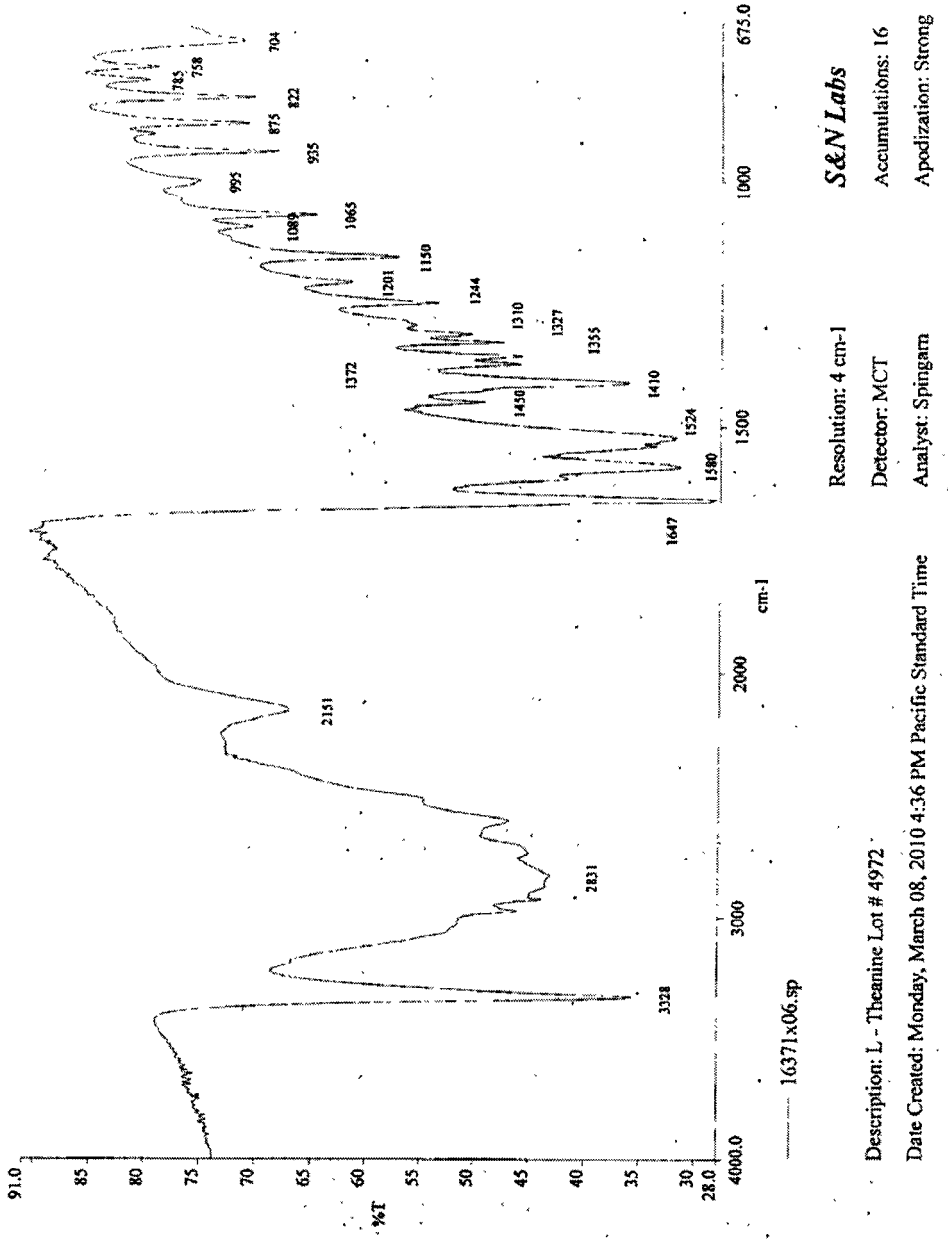
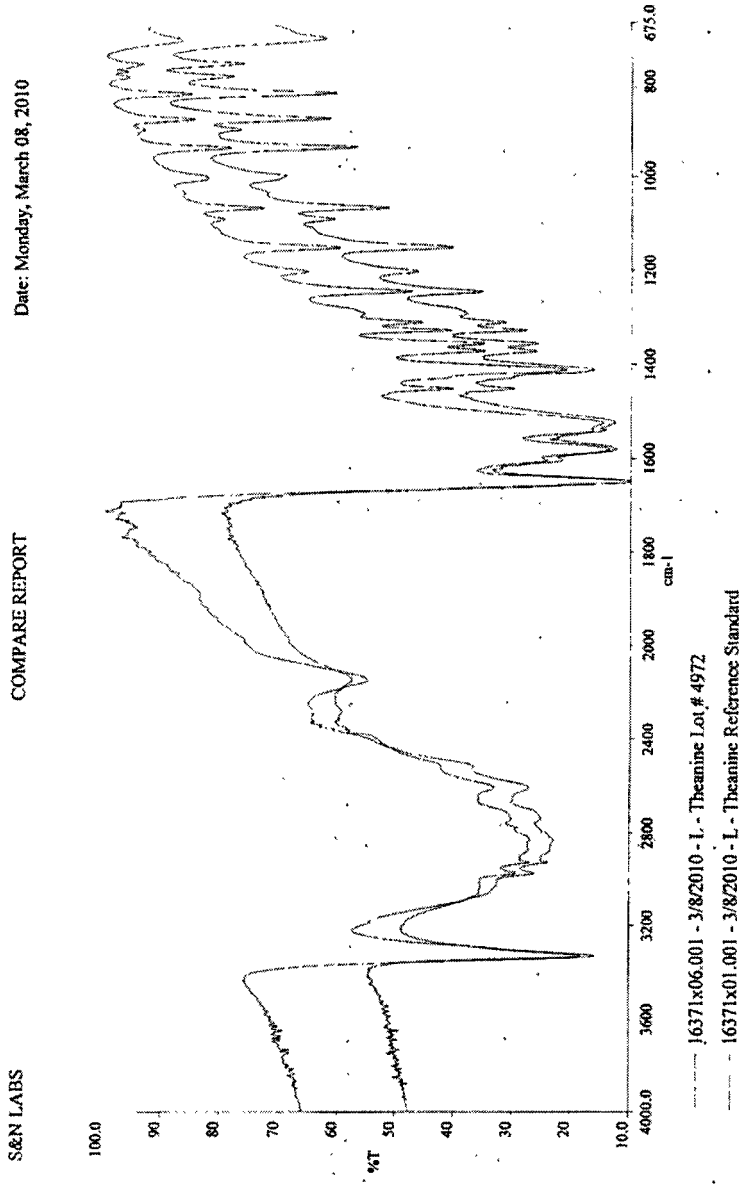
675.0
cm-1
16371x05.sp - 3/8/2010 - L - Theanine Lot # 4971
16371x01.001 - 3/8/2010 - L - Theanine Reference Standard

APPENDIX B-6 L-Theanine Lot #4972




S&N LABS
COMPARE REPORT
Date: Monday, March 08, 2010
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1000
800
675.0
cm-1
16371x06.001 - 3/8/2010 - L - Theanine Lot # 4972
16371x01.001 - 3/8/2010 - L - Theanine Reference Standard

APPENDIX C

High Performance Liquid Chromatography Data: Blue California L-Theanine (98%)

Appendix C pages (89 with 7 dividers as listed below) not included in page number count.

Summary Report
SPL Data
Resolution
LOQ
Blank
Statistics Data
Method / Seq

SUMMARY REPORT

Eurofins Scientific, Inc.
1365 Redwood Way
Petaluma, Ca 94951

Summary Report

Determination of Theanine Enantiomers by High Performance Liquid Chromatography (HPLC) and Purity Analysis of Five L-Theanine Production Lots

Prepared by (b) (6)
June Gupta, Scientist
Eurofins Scientific, Inc.

Approved by (b) (6)
Jules Skamarack, Operations Manager
Eurofins Scientific, Inc.

Approved b (b) (6)
Cecilia McCollum, Executive Vice President
Blue California Company

Date Issued: July 2009

I. Study Identification

1. Study Title:
Determination of Theanine Enantiomers by High Performance Liquid Chromatography (HPLC) and Purity Analysis of Five L-Theanine Production Lots

2. Study Objective:
The objective of this study is to determine the purity of Blue California supplied five production lots of L-Theanine powder, and quanititate D-Theanine, if any, present in the same lots.

3. Study Coordinator/Performing Laboratory:
Jules Skamarack, Eurofins Scientific, Inc.

4. Study Monitors:
Cecilia McCollum, Executive Vice President
Blue California.

5. Method References:
MJ Desai and DW Armstrong, "Analysis of Derivatized and Underivatized Theanine Enantiomers by High Performance Liquid Chromatography/Atmospheric Pressure Ionization-Mass Spectrometry", *Rapid Commun. Mass Spectrom.* **2004**; 18: 251-256.

KH Ekborg-Ott, A Taylor and DW Armstrong, "Varietal Differences in the Total and Entiomeric Composition of Theanine in Tea", *J. Agric. Food Chem.* **1997**; 45: 353-363.

II. Study Description

1. Scope:
This method is applicable to the determination of theanine enantiomers in raw materials.

2. Test Materials:
L-Theanine Powder

(1) Eurofins sample **4968**, L-Theanine Powder, Blue California Lot # LT20070608

(2) Eurofins sample **4969**, L-Theanine Powder, Blue California Lot # LT20080928

(3) Eurofins sample **4970**, L-Theanine Powder, Blue California Lot # LT20081105

(4) Eurofins sample **4971**, L-Theanine Powder, Blue California Lot # LT20090109

(5) Eurofins sample **4972**, L-Theanine Powder, Blue California Lot # LT20090225

3. Test Reagents:

(1) Methanol, HPLC Grade
Fisher P/N A452-4

(2) L-Theanine, ChromaDex, Lot # 20249-192 (98.9%)
C.A.S # 3081-61-6

(3) D-Glutamine, Sigma, Lot # 100K1780 (98%)
C.A.S # 5959-95-5

(4) L-Glutamine, Sigma, Lot # 072K0182 (99%)
C.A.S # 56-85-9

(5) AccQ-Fluor Reagent Kit, Waters, Lot #6075882061
Cat # WAT052880

(6) Ammonium Trifluoroacetate, HPLC Grade
Sigma P/N 238732

(7) Water, HPLC Grade, In-house

4. Mobile Phase Preparation:

(1) Mobile Phase A: Weigh accurately 10.0 ± 0.2 g of ammonium trifluoroacetate into a 1-L eluant bottle. Add 1000mL of HPLC grade methanol and sonicate to dissolve. Mix thoroughly. (1.0% Ammonium trifluoroacetate in Methanol)

(2) Mobile Phase B: Methanol, HPLC Grade

5. Reference Standards:

Separate Standards (L-theanine and D-glutamine)

A. Stock standards.

1. Adjust standard concentration for purity and moisture levels. Corrections are made based on suppliers C of A.

2. On a microbalance, accurately weigh 10.0 ± 1 mg of L-Theanine Chroma Dex standard and transfer to a 5-ml volumetric flask. Dissolve using about 3 mL of water and sonicate if necessary. Cool to room temperature and dilute to volume with water. Concentration is approximately 2 mg/mL of L-Theanine.

3. On a microbalance, accurately weigh 10.0 ± 1 mg of D-Glutamine Sigma standard and quantitatively transfer to a 10-mL volumetric flask. Dissolve using about 5 mL of water and sonicate if necessary. Cool to

room temperature and dilute to volume with water. Concentration is approximately 1 mg/mL of D-Glutamine. This standard is used for a retention time marker and LOD/LOQ determinations of D-Theanine.

4. On a microbalance, accurately weigh 10.0 ± 1 mg of L-Glutamine Sigma standard and transfer to a 10-ml volumetric flask.
Dissolve using about 5 mL of water and sonicate if necessary. Cool to room temperature and dilute to volume with mobile phase. Concentration is approximately 1 mg/mL of L-Glutamine. This standard is used to determine the response ratio between Glutamine and Theanine.

B. Calibration standards (mixed standard solutions was used to prepare resolution solution). The range of quantitation will roughly be between 0.2 mg and 2 mg in solution. Per ICH guidelines a 5 point curve is utilized initially for determination of linearity. A three point curve was used for routine quantitation that covers the range defined by the method and listed above. The sample test concentration will be at approximately 1 mg/mL L-Theanine, based on the expected test sample concentration. To accommodate this, dilute the stock standard volumetrically to include 1 mg/mL standard as the midpoint of calibration.

C. System suitability standards, retention time confirmation (L-Theanine ChromaDex calibration standard) and mid point calibration standards for system suitability were utilized. See results section for concentrations.

D. Derivatization Scheme
Due to the absence of commercial D-Theanine standard currently in the market, D-Glutamine was used as the retention time marker for D-Theanine. L-Theanine and L-Glutamine elute at around the same time under the chromatographic conditions listed in the test method. It is assumed that D-Glutamine then elutes at the retention time matching to that of D-Theanine due their similarity in chemical structures. Response factor between L-Theanine and L-Glutamine is to be determined prior to LOD/LOQ determination of D-Glutamine, which will then be applied to calculate LOD/LOQ of D-Theanine.

Using a qualified micropipette, 10 µL of Theanine/Glutamine solution, 70 µL of AccQ-Fluor borate buffer, and 20 µL of the AccQ-Fluor reagent solution was added to an HPLC vial. The vial was heated for 10 minutes at 55 °C on a heating block. The solution was diluted with 900 µL of water and vortexed to mix. Derivatization was performed for each of calibration standards, sample solutions and negative control.

6. Single Lab Verification Study Results:

A. Primary method: See provided method.

B. Linearity:

1. Five calibration solutions for L-theanine were prepared by serial dilution of the stock solution to create a 5-point calibration curve, with

concentrations for **L-Theanine** as follows (adjusted for standard purity and moisture):

Stock (mL)	Final volume (mL)	Concentration (mg/mL)
1	1	1.99244
1	2	0.99622
1	3	0.66415
1	6	0.33207
1	11	0.18113

Linearity Results L-Theanine:

Correlation Coefficient	**Specification**	**Result**
0.99893	**>/= 0.995**	**PASS**

Correlation coefficient between calibration levels was acceptable

C. Selectivity: For purposes of this study, selectivity is specificity

1. Perform selectivity procedures:
   a. Analyze mobile phase blanks (Both A and B).
   b. Analyze a negative control sample

2. Results:
   a. Three blanks have been provided in the study report, the mobile phase blanks and the preparation solvent blank (negative control). All chromatograms were free of interfering peaks. No additional peaks were present except for the peak due to the derivatizing agent in the negative control blank chromatogram.

   Note: chromatograms are included in hard copy.

D. System Suitability:

1. Minimum of five injections of an approximately 1.0 mg/ml standard solution were injected during each analysis sequence.

2. Acceptance criteria: The system is considered suitable if the retention times of the standard peaks do not deviate more than 1 minute during an analytical run and the RSD of the peak area is less than 3%.

Parameter	L-Theanine
Retention Time Range (minutes)	2.797 – 2.758
Retention Time % RSD	0.667
Peak Area % RSD	1.77
Number of Data Points	5

Retention Time Range meets the criteria of deviation of less than 1 minute.
Retention time % RSD = PASS
Peak Area % RSD = PASS

3. An Extended Performance report was generated using Agilent Chem Station software to include resolution between L-Theanine and D-Glutamine, tailing and theoretical plate counts of L-Theanine. Results are as follows:

USP Resolution L-Theanine/ D-Glutamine = 1.76
USP Tailing L-Theanine = 1.17 (n = 5)
USP Plate Count Tangent Method = 3175 (n = 5)

4. The retention time and identity for L-Theanine was confirmed using the ChromaDex L-Theanine standard. Chromatograms are located in the System suitability portion of the package.

D. LOD/LOQ Determination

1. D-Glutamine solution was prepared at 0.0906 mg/mL level and 3 uL of the solution was injected in triplicate to estimate its limit of quantitation (LOQ). Signal-to noise ratios (S/N) obtained for D-Glutamine are as follows:

Injection #	D-Glutamine S/N
1	28.7
2	29.8
3	30.8
Mean % RSD	29.8 3.5

LOQ of D-Glutamine is estimated to be 1.8% of L-Theanine and LOD is therefore around 0.6% of L-Theanine.

L-Theanine injected at 0.18113 mg/mL level with 3 µL injection volume yielded S/N ratio of around 145. Based on the result, LOQ and LOD of L-Theanine are projected to be 1.0% and 0.3%, respectively, of the mid-level L-Theanine standard at 1 mg/mL, which is the expected concentration of purity samples.

7. Purity Analysis of Five Production Samples:

A. Five lots of L-Theanine were analyzed for purity. Each sample was tested in duplicate for L-Theanine and D-Theanine after derivatization. The results are reported below:

Sample 4968	Prep 1	Prep 2		
Compound	Result (%w/w)	Result (%w/w)	Average	Mean Absolute Deviation
L-Theanine	99.6662	99.4570	99.5616	0.1046
D-Theanine	ND	ND	ND	N/A
Total	99.6662	99.4570	99.5616	0.1046

Sample 4969	Prep 1	Prep 2		
Compound	Result (%w/w)	Result (%w/w)	Average	Mean Absolute Deviation
L-Theanine	99.6200	99.0859	99.3530	0.2671
D-Theanine	ND	ND	ND	N/A
Total	99.6200	99.0859	99.3530	0.2671

Sample 4970	Prep 1	Prep 2		
Compound	Result (%w/w)	Result (%w/w)	Average	Mean Absolute Deviation
L-Theanine	99.1199	99.7460	99.4330	0.3130
D-Theanine	ND	ND	ND	N/A
Total	99.1199	99.7460	99.4330	0.3130

Sample 4971	Prep 1	Prep 2		
Compound	Result (%w/w)	Result (%w/w)	Average	Mean Absolute Deviation
L-Theanine	98.6362	98.5067	98.5715	0.0648
D-Theanine	ND	ND	ND	N/A
Total	98.6362	98.5067	98.5715	0.0648

Sample 4972	Prep 1	Prep 2		
Compound	Result (%w/w)	Result (%w/w)	Average	Mean Absolute Deviation
L-Theanine	100.3803	99.5804	99.9804	0.4000
D-Theanine	ND	ND	ND	N/A
Total	100.3803	99.5804	99.9804	0.4000

8. Conclusions:

The results generated meet and exceed the acceptance criteria as established in the method verification proposal. All analyses were performed on Agilent 1200 series HPLCs with Agilent Chem Station software. The primary objective of the study has been to show that the method as designed can accurately determine the concentration of L-Theanine and D-Theanine in L-Theanine raw materials supplied by Blue California. The results show that the method is precise and accurate.

Limit of detection and limit of quantitation were estimated using the low level linearity solution. The ICH visual inspection method (ICH Validation of Analytical Procedures: Methodology, section 6.1) for determining limit of detection and limit of quantitation was utilized. Limit of detection and limit of quantitation for these compounds are roughly estimated at 0.6% and 1.8% of L-Theanine, respectively. In the future additional work can be performed to statistically determine these limits if requested.

Five lots of L-Theanine powder were tested by this method. The results show that the method can accurately determine the concentration of L-Theanine in this material as well as D-Theanine enantiomer, if present.

SPL DATA

TITLE L-Theanine

Work continued from Page 86

PROJECT NO. —

BOOK NO. JG-1

Result:

[redacted]	[redacted]	D-Theanine	[redacted]
[redacted]	[redacted]	-	[redacted]
[redacted]	[redacted]	-	
[redacted]	[redacted]	-	
[redacted]	[redacted]	-	
[redacted]	[redacted]	-	
[redacted]	[redacted]	-	
[redacted]	[redacted]	-	
[redacted]	[redacted]	-	
[redacted]	[redacted]	-	
[redacted]	[redacted]	-	
[redacted]	[redacted]	-	[redacted]

D = Duplicate sample preparation.
Calibration data attached to Job # 46 96

	L-Glutamine	L-Theanine
1	1703.68	1212.54
2	1702.45	1167.37
3	1700.94	1166.996
4	—	1164.23
5	—	1165.72
$\bar{X}$	1702.36	1175.37
Conc. (mg/mL)	1.24166	0.99622

Response Ratio between Glu/Thea

$$= \frac{1702.36/1.24166}{1175.37/0.99622}$$

$= 1.16$

is between 0.80 – 1.20

∴ No correction needed.

BEST ORIGINAL COPY

LOQ of D-Glutamine/D-Theanine.

S/N = 30 @ 0.0906 mg/mL, 3 µL.

$$\text{Estimated LOQ} = \frac{(0.0906\ \text{mg/mL})\left(\frac{3\ \mu L}{5\ \mu L}\right)\left(\frac{10}{30}\right)}{1\ \text{mg/mL L-theanine}} \times 100 = 1.8\%$$

Estimated LOQ = $\frac{1.8\%}{3}$ = 0.6% L-theanine.

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Work continued to Page —

SIGNATURE (b) (6)

DATE 7/27/09

DISCLOSED TO AND UNDERSTOOD BY (b) (6)

DATE 7/27/09

WITNESS

DATE

Sample Prep: ① JG 7/23/09 Fluffy material lost during transfer.

ID	Wt (mg)	Vol. (mL)	Conc. (mg/mL)
09-4968	21.40	20.0	1.0700
09-4968D	~~24.07~~ ① ~~24.29~~ ① 20.47		① ~~1.2035~~ 1.0235
09-4969	~~30.85~~ ① 22.71		① ~~1.0425~~ 1.1355
09-4969D	26.81		1.3405
09-4970	23.99		1.1995
09-4970D	25.48		1.2740
09-4971	22.53		1.1265
09-4971D	22.71		1.1355
09-4972	20.58		1.0290
09-4972D	~~25.23~~ ① 23.81	20.0	① ~~1.2615~~ 1.1905

Resolution Soln Prep: 10 µL STD 2 + 10 µL D-Glutamine STD Soln → HPLC vial, + 70 µL borate buffer + 20 µL Reagent Soln: Heated to 55°C for 10 min, cooled to RT + 900 µL water & vortexed before analysis.

D-Glutamine LOQ Soln Prep: 1.0 mL D-Glutamine STD Soln + 10.0 mL water. 10 µL diluted soln + 20 µL Reagent Soln + 70 µL borate buffer → HPLC vial. Heated for 10 min @ 55°C, cooled to RT. + 900 µL water & vortexed before analysis. Injected X3 @ 5 µL volume. Injected X3 @ 3 µL volume.

All other STD & SPL Soln followed the same derivatization scheme: 10 µL theanine/Glutamine + 70 µL borate buffer + 20 µL Reagent Soln: Heated to 55°C for 10 min, + 900 µL water.

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Work continued to Page 87

SIGNATURE (b) (6) DATE 7/23/09

DISCLOSED TO AND UNDERSTOOD BY (b) (6) DATE 7/27/09 WITNESS DATE

BEST ORIGINAL COPY

METHOD: Theanine COLUMN ID: Antec Chirbiotic T 4.6 x 250 mm, 5 µm #108293-04
SEQUENCE: Theanine-4968 BALANCE ID: Cahn-33 / BP211D
INSTRUMENT: HPLC PIPET ID: G20, G200, P1000

Chemicals: Methanol, # 9155; milliQ Water, fresh daily
Ammonium trifluoroacetate (NH4TFA), # 9058
Waters AccQ-Fluor Reagent Kit, Lot # 6075PA2061, exp 09/2009

Mobile Phase A Prep: (exp 8/23/09)
10.0036g NH4TFA → 1 L w/ methanol

Mobile Phase B: HPLC Grade Methanol

Derivatizing Agent Prep: AccQ Reagent Powder + 1.0 mL reagent diluent. vortexed for 10 sec, heated for 10 min @ 55°C. Cooled to RT before use.

Stock STO Prep:

Analyte	wt (mg)	vol. (mL)	Conc. (mg/mL)	Info	Purity, as is
L-Theanine	10.073	5.0	1.99244	9051	98.9 %
L-Glutamine	12.542	10.0	1.24166	6399	99 %
D-Glutamine	10.170	10.0	0.99666	9179	98 %

STO Prep

ID	L-Theanine Stock STO (mL)	mL Water Added	Conc (mg/mL)
STO 1	1.0	—	1.99244
STO 2	1.0	1.0	0.99622
STO 3	1.0	2.0	0.66415
STO 4	1.0	5.0	0.33207
STO 5	1.0	10.0	0.18113

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Work continued to Page 86

SIGNATURE (b) (6) DATE 7/23/09
DISCLOSED TO (b) (6) DATE 7/27/09 WITNESS DATE

```
==================================================================
Acq. Operator    : June Gupta                    Seq. Line :  17
Acq. Instrument  : Instrument 15                  Location : P1-B-07
Injection Date   : 7/23/2009 11:06:50 PM               Inj :   1
                                               Inj Volume : 5.0 µl
Acq. Method      : C:\CHEM32\15\DATA\THEANINE-4968     2009-07-23\THEANINE.M
Last changed     : 7/23/2009 12:53:23 PM by (b) (b) (6)
Analysis Method  : C:\CHEM32\15\DATA\THEANINE-4968     2009-07-23\THEANINE.M
Last changed     : 7/24/2009 3:54:42 PM by (b) (6)
Method Info      : Chiral Analysis of Theanine

ECM Server       : http://us05sqlc/ecmwg
ECM Operator     : (b) (6)
ECM Path         : Petaluma\LC\HPLC-15\Data\THEANINE-4968     2009-07-23.SC.SSIzip
ECM Version      : 1
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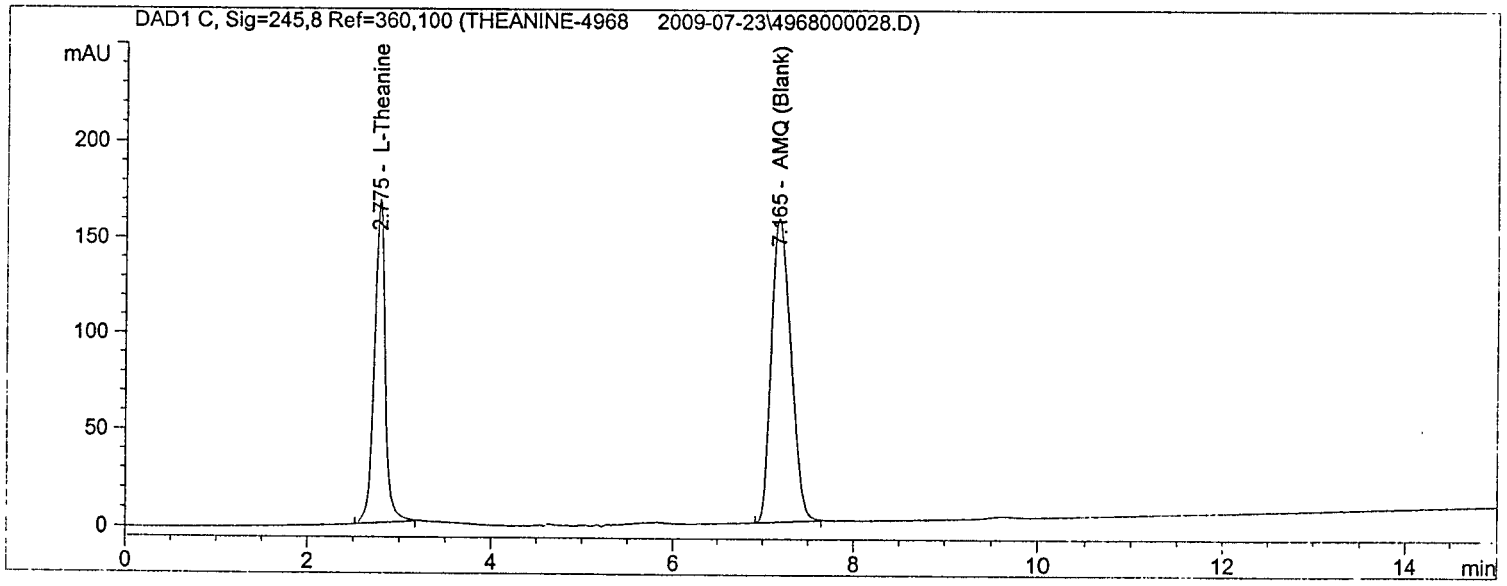



```
==================================================================
                        ESTD Percent Report
==================================================================

Sorted By                :      Signal
Calib. Data Modified     :      7/24/2009 3:38:30 PM
Multiplier:                      :      1.0000
Dilution:                        :      1.0000
Sample Amount:                   :      1.07000  [ug/mL]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=245,8 Ref=360,100

RetTime  Type     Area       Amt/Area    Amount    Grp   Name
 [min]           [mAU*s]                   %
-------|------|----------|----------|----------|--|------------------
  2.775 BB     1326.33850 8.04040e-4  99.666240     L-Theanine
  3.013            -          -          -          L-Glutamine
  3.643            -          -          -          D-Glu/Thea
  7.165 BB     2361.08496   0.00000   0.000000     AMQ (Blank)

Totals :                                99.666240
```

000071

3 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found
Warning : Invalid calibration curve, (AMQ (Blank))

==========

*** End of Report ***

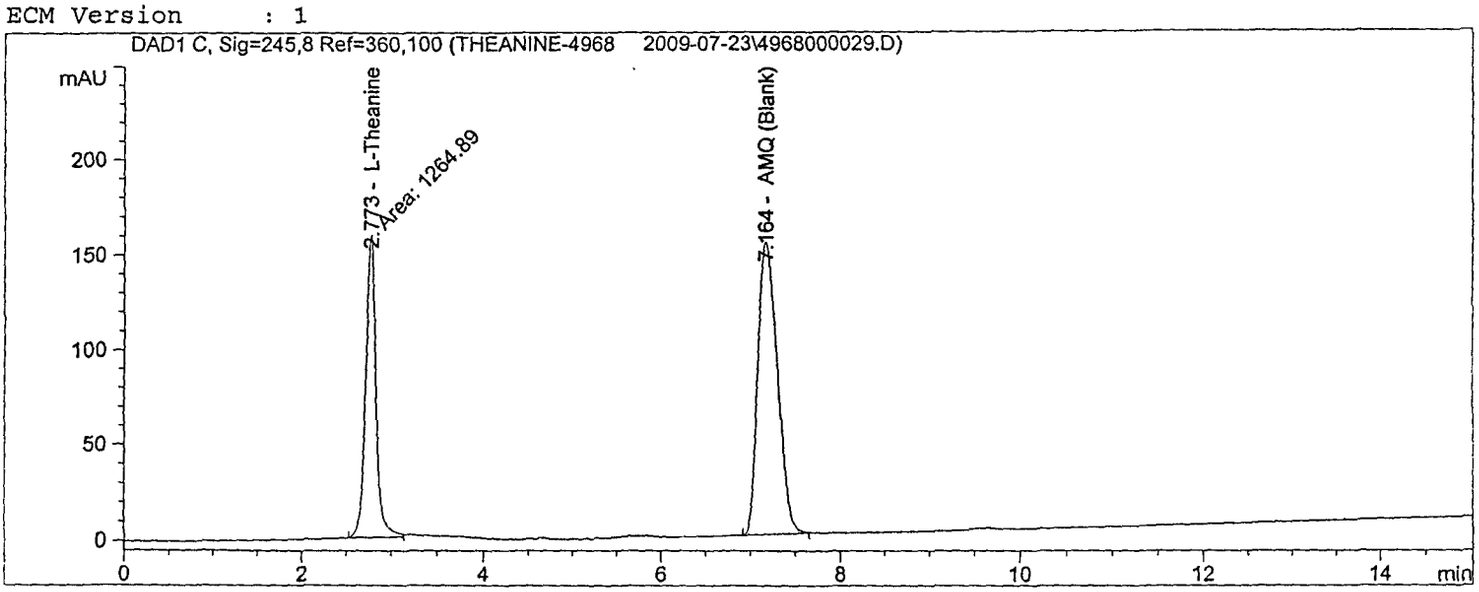
000072

==========

Acq. Operator : (b) (6) Seq. Line : 18
Acq. Instrument : Instrument 15 Location : P1-B-08
Injection Date : 7/23/2009 11:29:32 PM Inj : 1
Inj Volume : 5.0 µl

Acq. Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/23/2009 12:53:23 PM by (b) (6)
Analysis Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/24/2009 3:54:42 PM by (b) (6)
Method Info : Chiral Analysis of Theanine

ECM Server : http://us05sqlc/ecmwg
ECM Operator : (b) (6)
ECM Path : Petaluma\LC\HPLC-15\Data\THEANINE-4968 2009-07-23.SC.SSIzip
ECM Version : 1



==========

ESTD Percent Report

==========

Sorted By : Signal
Calib. Data Modified : 7/24/2009 3:38:30 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Sample Amount: : 1.02350 [ug/mL]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=245,8 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
2.773	MM	1264.89417	8.04765e-4	99.457011		L-Theanine
3.013		-	-	-		L-Glutamine
3.643		-	-	-		D-Glu/Thea
7.164	BB	2319.24927	0.00000	0.000000		AMQ (Blank)
Totals :				99.457011		

000073

3 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found
Warning : Invalid calibration curve, (AMQ (Blank))

==========

*** End of Report ***

000074

Sample Name: 4969

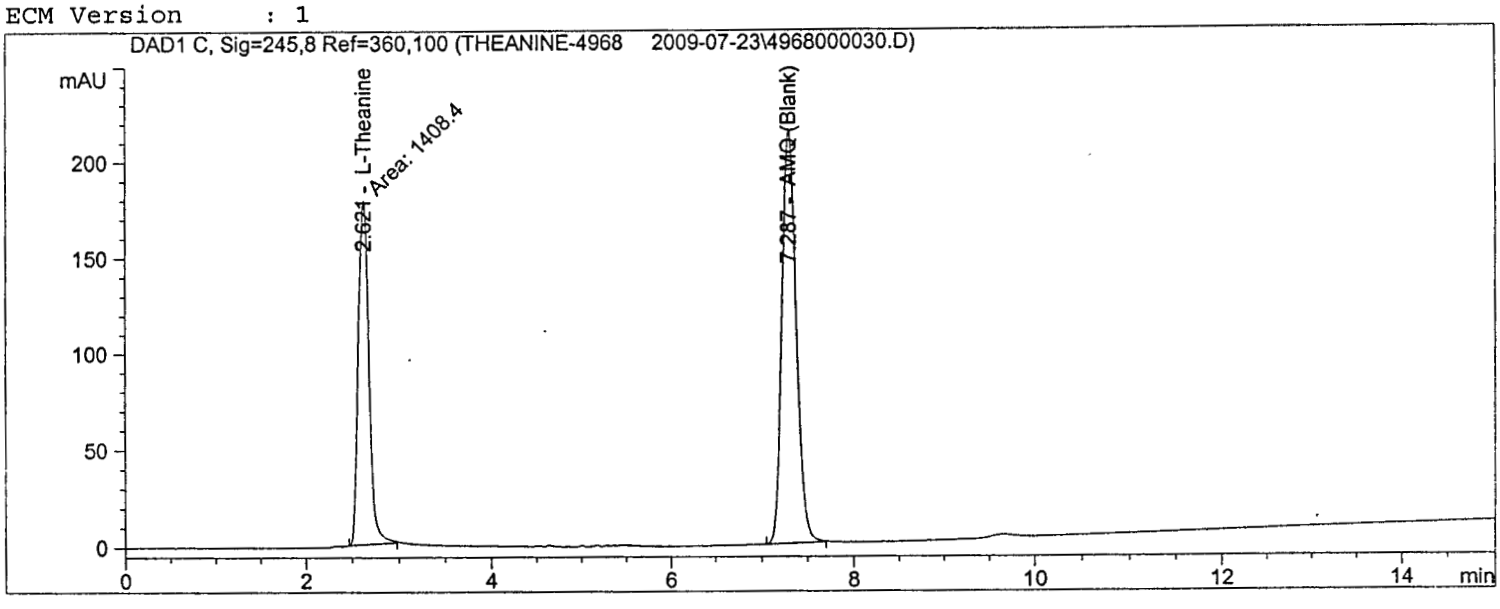
==========

Acq. Operator : (b) (6)
Acq. Instrument : Instrument 15
Injection Date : 7/24/2009 2:39:06 PM

Seq. Line : 19
Location : P1-C-01
Inj : 1
Inj Volume : 5.0 µl

Acq. Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/24/2009 10:18:08 AM by (b) (6)
Analysis Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/24/2009 3:54:42 PM by (b) (6)
Method Info : Chiral Analysis of Theanine

ECM Server : http://us05sqlc/ecmwg
ECM Operator : (b) (6)
ECM Path : Petaluma\LC\HPLC-15\Data\THEANINE-4968 2009-07-23.SC.SSIzip
ECM Version : 1



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ESTD Percent Report

==========

Sorted By : Signal
Calib. Data Modified : 7/24/2009 3:38:30 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Sample Amount: : 1.13550 [ug/mL]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=245,8 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
2.621	MM	1408.40051	8.03170e-4	99.619957		L-Theanine
3.013		-	-	-		L-Glutamine
3.643		-	-	-		D-Glu/Thea
7.287	BB	2309.71899	0.00000	0.000000		AMQ (Blank)

Totals : 99.619957

000075

3 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found
Warning : Invalid calibration curve, (AMQ (Blank))

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*** End of Report ***

000076

Sample Name: 4969D

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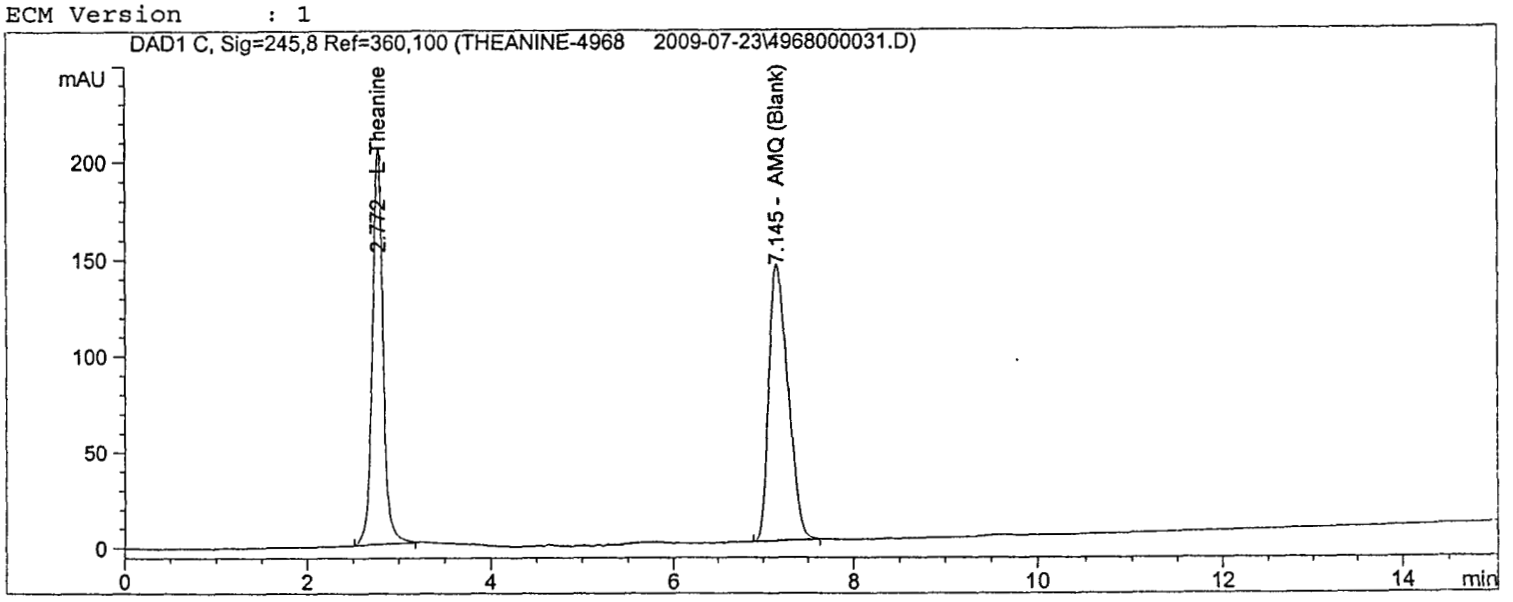
Acq. Operator : (b) (6) Seq. Line : 20
Acq. Instrument : Instrument 15 Location : P1-C-02
Injection Date : 7/24/2009 12:14:57 AM Inj : 1
Inj Volume : 5.0 µl

Acq. Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/23/2009 12:53:23 PM by (b) (6)
Analysis Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/24/2009 3:54:42 PM by (b) (6)
Method Info : Chiral Analysis of Theanine

ECM Server : http://us05sqlc/ecmwg
ECM Operator : (b) (6)
ECM Path : Petaluma\LC\HPLC-15\Data\THEANINE-4968 2009-07-23.SC.SSIzip
ECM Version : 1



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ESTD Percent Report

==========

Sorted By : Signal
Calib. Data Modified : 7/24/2009 3:38:30 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Sample Amount: : 1.34050 [ug/mL]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=245,8 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
2.772	BB	1658.12769	8.01052e-4	99.085901		L-Theanine
3.013		-	-	-		L-Glutamine
3.643		-	-	-		D-Glu/Thea
7.145	BB	2135.19775	0.00000	0.000000		AMQ (Blank)

Totals : 99.085901

000077

3 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found
Warning : Invalid calibration curve, (AMQ (Blank))

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*** End of Report ***

000078

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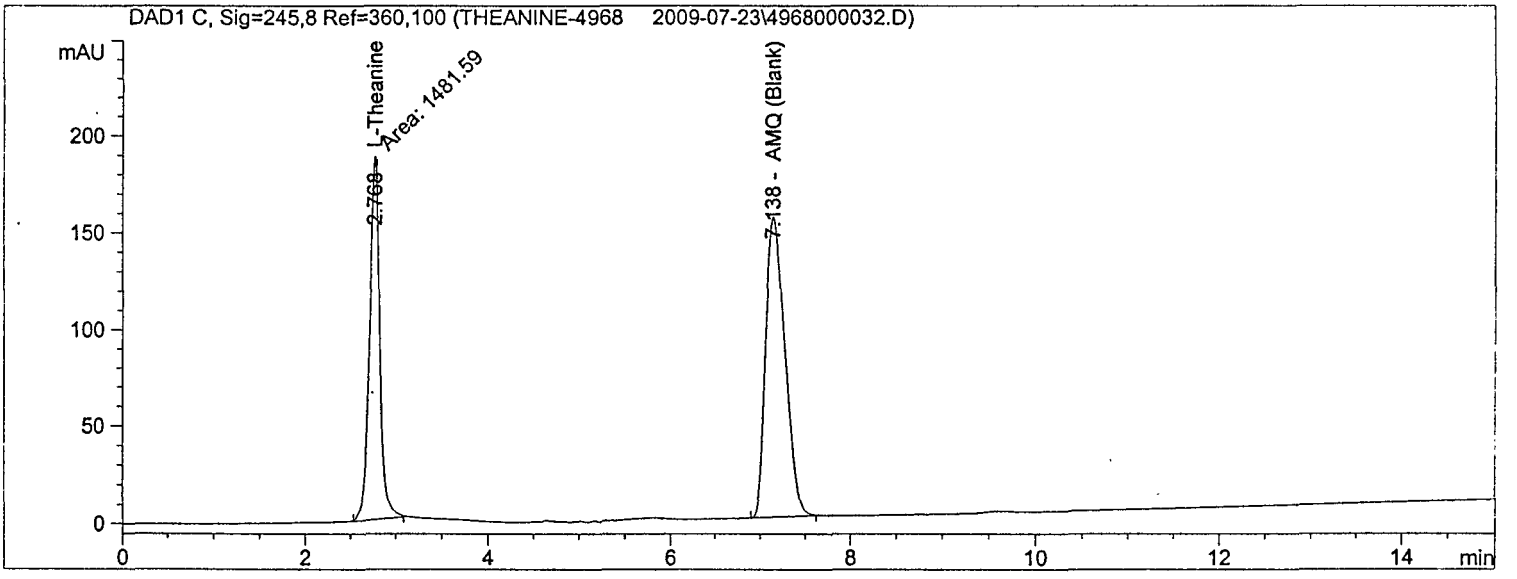
Acq. Operator : (b) (6) Seq. Line : 21
Acq. Instrument : Instrument 15 Location : P1-C-03
Injection Date : 7/24/2009 12:37:39 AM Inj : 1
Inj Volume : 5.0 µl

Acq. Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/23/2009 12:53:23 PM by (b) (6)
Analysis Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/24/2009 3:54:42 PM by (b) (6)
Method Info : Chiral Analysis of Theanine

ECM Server : http://us05sqlc/ecmwg
ECM Operator : (b) (6)
ECM Path : Petaluma\LC\HPLC-15\Data\THEANINE-4968 2009-07-23.SC.SSIzip
ECM Version : 1



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ESTD Percent Report

==========

Sorted By : Signal
Calib. Data Modified : 7/24/2009 3:38:30 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Sample Amount: : 1.19950 [ug/mL]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=245,8 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
2.768	MM	1481.59473	8.02475e-4	99.119867		L-Theanine
3.013		-	-	-		L-Glutamine
3.643		-	-	-		D-Glu/Thea
7.138	BB	2296.98291	0.00000	0.000000		AMQ (Blank)

Totals : 99.119867

000079

3 Warnings or Errors :

Sample Name: 4970

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found
Warning : Invalid calibration curve, (AMQ (Blank))

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*** End of Report ***

000080

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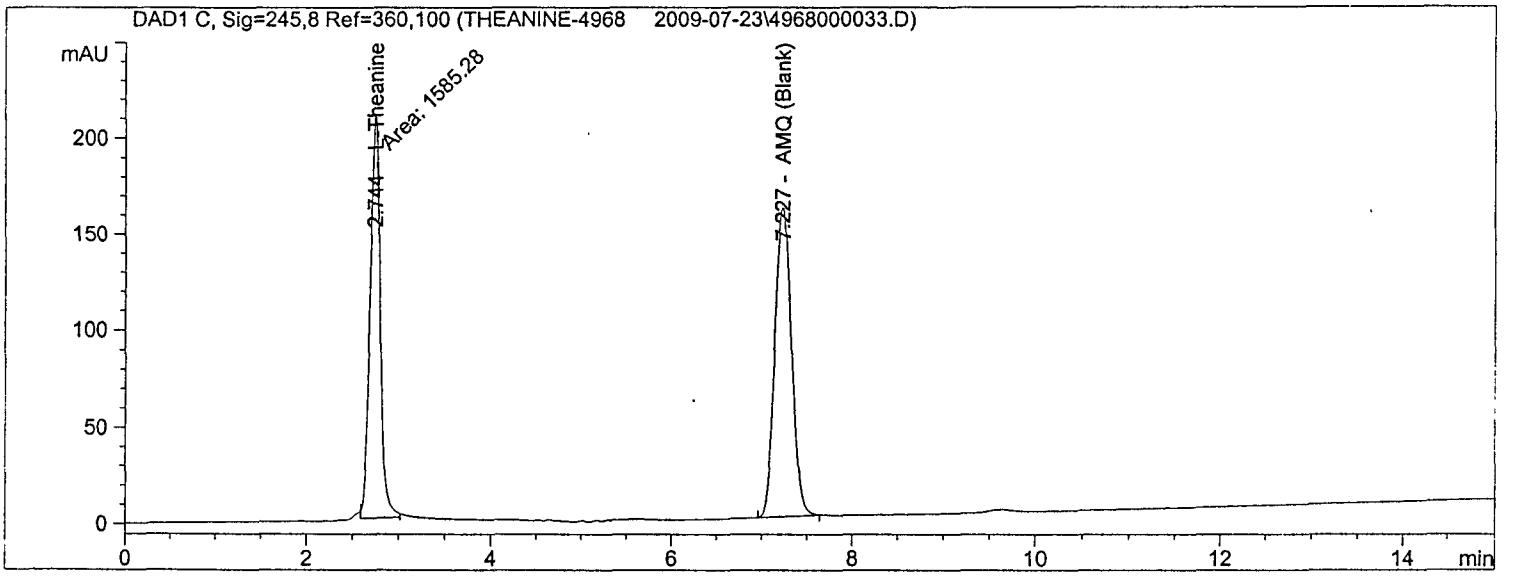
Acq. Operator : (b) (6) Seq. Line : 22
Acq. Instrument : Instrument 15 Location : P1-C-04
Injection Date : 7/24/2009 3:02:51 PM Inj : 1
Inj Volume : 5.0 µl

Acq. Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/24/2009 3:02:22 PM by (b) (6)
(modified after loading)
Analysis Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/24/2009 3:54:42 PM by (b) (6)
Method Info : Chiral Analysis of Theanine

ECM Server : http://us05sqlc/ecmwg
ECM Operator : (b) (6)
ECM Path : Petaluma\LC\HPLC-15\Data\THEANINE-4968 2009-07-23.SC.SSIzip
ECM Version : 1



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ESTD Percent Report

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Sorted By : Signal
Calib. Data Modified : 7/24/2009 3:38:30 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Sample Amount: : 1.27400 [ug/mL]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=245,8 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
2.744	MM	1585.28210	8.01601e-4	99.745951		L-Theanine
3.013		-	-	-		L-Glutamine
3.643		-	-	-		D-Glu/Thea
7.227	BB	2062.16943	0.00000	0.000000		AMQ (Blank)

Totals : 99.745951

000081

3 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found
Warning : Invalid calibration curve, (AMQ (Blank))

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*** End of Report ***

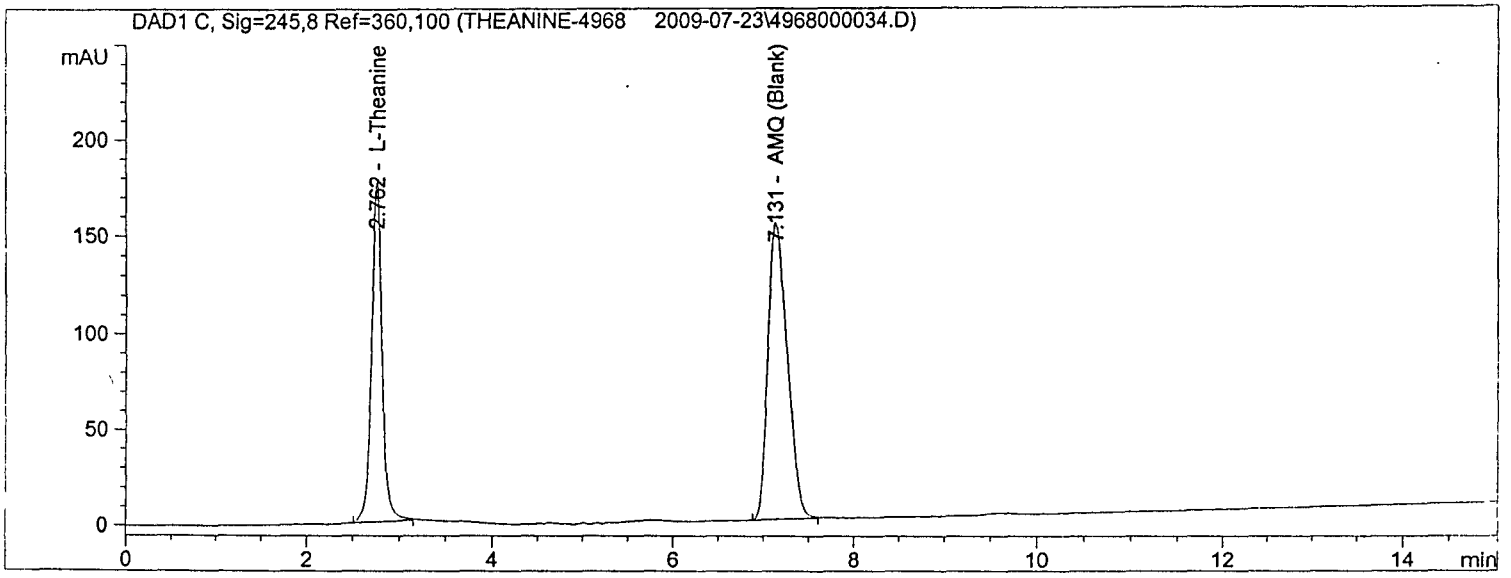
000082

==========

Acq. Operator : (b) (6) Seq. Line : 23
Acq. Instrument : Instrument 15 Location : P1-C-05
Injection Date : 7/24/2009 1:23:05 AM Inj : 1
Inj Volume : 5.0 µl

Acq. Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/23/2009 12:53:23 PM by (b) (6)
Analysis Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/24/2009 3:54:42 PM by (b) (6)
Method Info : Chiral Analysis of Theanine

ECM Server : http://us05sqlc/ecmwg
ECM Operator : (b) (6)
ECM Path : Petaluma\LC\HPLC-15\Data\THEANINE-4968 2009-07-23.SC.SSIzip
ECM Version : 1



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ESTD Percent Report

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Sorted By : Signal
Calib. Data Modified : 7/24/2009 3:38:30 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Sample Amount: : 1.12650 [ug/mL]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=245,8 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
2.762	BB	1382.99524	8.03428e-4	98.636227		L-Theanine
3.013		-	-	-		L-Glutamine
3.643		-	-	-		D-Glu/Thea
7.131	BB	2281.87866	0.00000	0.000000		AMQ (Blank)

Totals : 98.636227

000083

3 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found
Warning : Invalid calibration curve, (AMQ (Blank))

==========

*** End of Report ***

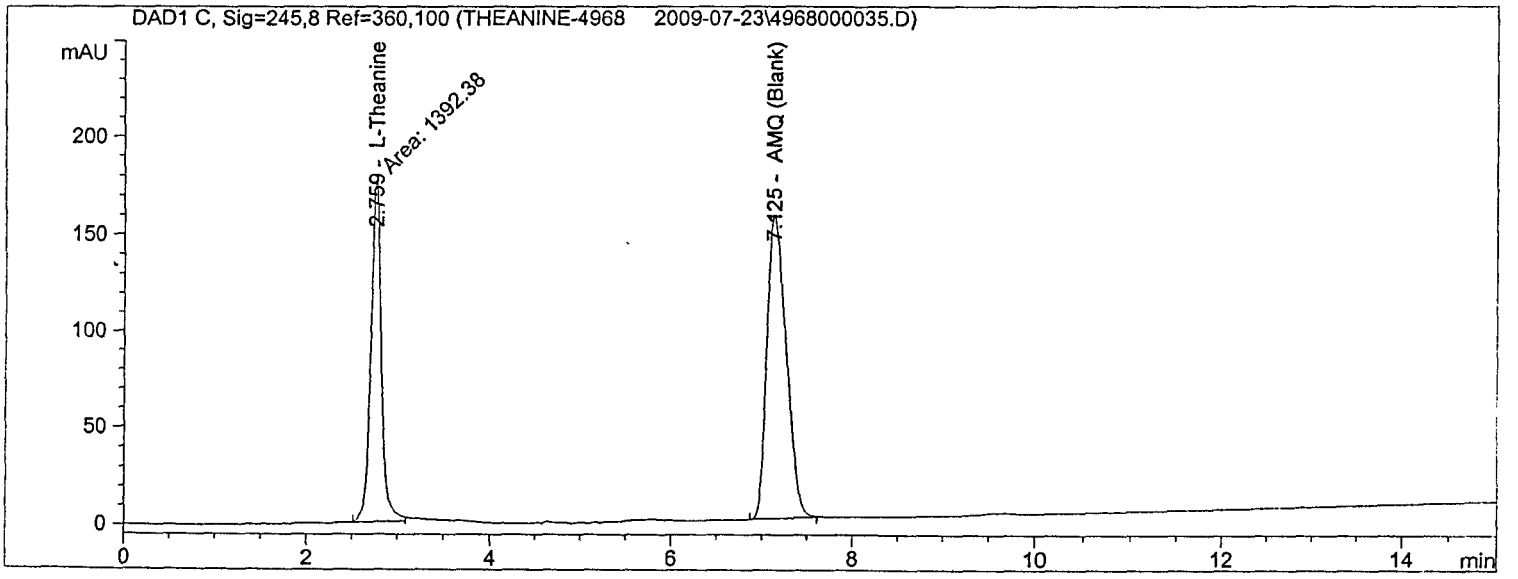
000084

==========

Acq. Operator : (b) (6) Seq. Line : 24
Acq. Instrument : Instrument 15 Location : P1-C-06
Injection Date : 7/24/2009 1:45:49 AM Inj : 1
Inj Volume : 5.0 µl

Acq. Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/23/2009 12:53:23 PM by (b) (6)
Analysis Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/24/2009 3:54:42 PM by (b) (6)
Method Info : Chiral Analysis of Theanine

ECM Server : http://us05sqlc/ecmwg
ECM Operator : (b) (6)
ECM Path : Petaluma\LC\HPLC-15\Data\THEANINE-4968 2009-07-23.SC.SSIzip
ECM Version : 1



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ESTD Percent Report

==========

Sorted By : Signal
Calib. Data Modified : 7/24/2009 3:38:30 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Sample Amount: : 1.13550 [ug/mL]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=245,8 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
2.759	MM	1392.38159	8.03331e-4	98.506732		L-Theanine
3.013		-	-	-		L-Glutamine
3.643		-	-	-		D-Glu/Thea
7.125	BB	2337.75732	0.00000	0.000000		AMQ (Blank)

Totals : 98.506732

000085

3 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found
Warning : Invalid calibration curve, (AMQ (Blank))

==========

*** End of Report ***

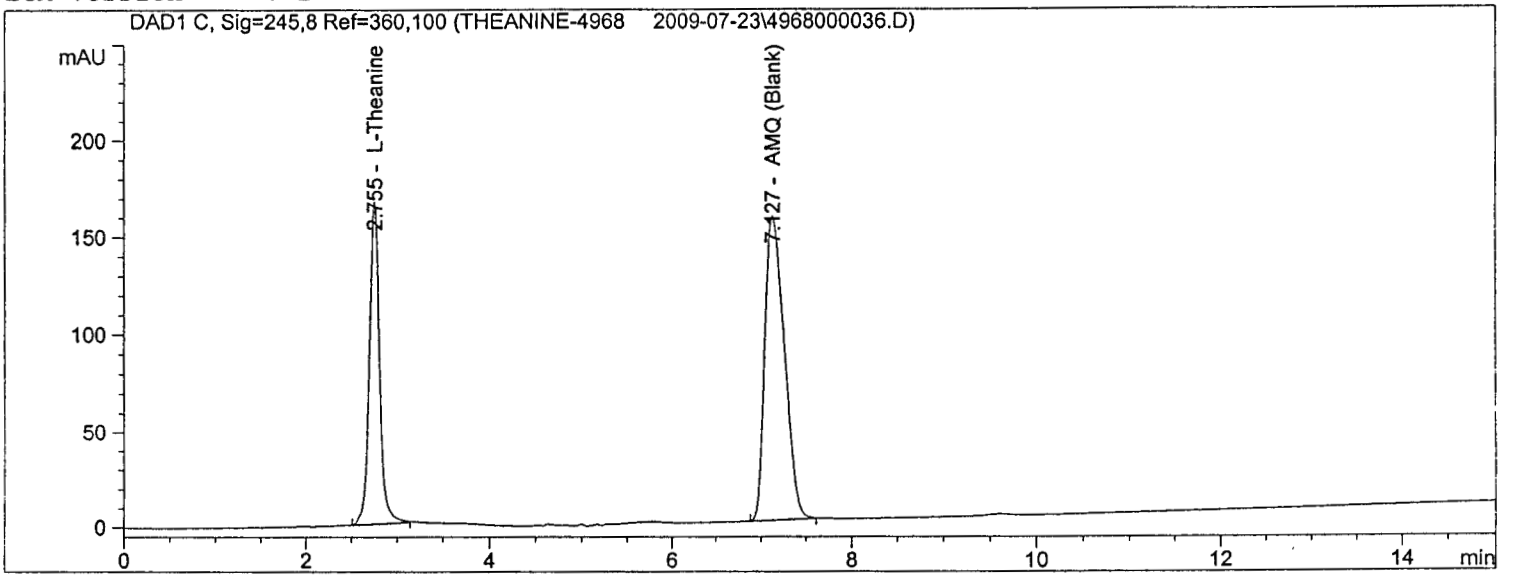
000086

==========

Acq. Operator : (b) (6) Seq. Line : 25
Acq. Instrument : Instrument 15 Location : P1-C-07
Injection Date : 7/24/2009 2:08:32 AM Inj : 1
Inj Volume : 5.0 µl

Acq. Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/23/2009 12:53:23 PM by (b) (6)
Analysis Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/24/2009 3:54:42 PM by (b) (6)
Method Info : Chiral Analysis of Theanine

ECM Server : http://us05sqlc/ecmwg
ECM Operator : (b) (6)
ECM Path : Petaluma\LC\HPLC-15\Data\THEANINE-4968 2009-07-23.SC.SSIzip
ECM Version : 1



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ESTD Percent Report

==========

Sorted By : Signal
Calib. Data Modified : 7/24/2009 3:38:30 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Sample Amount: : 1.02900 [ug/mL]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=245,8 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
2.755	BB	1283.86621	8.04534e-4	100.380322		L-Theanine
3.013		-	-	-		L-Glutamine
3.643		-	-	-		D-Glu/Thea
7.127	BB	2371.84717	0.00000	0.000000		AMQ (Blank)

Totals : 100.380322

000087

3 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found
Warning : Invalid calibration curve, (AMQ (Blank))

==========

*** End of Report ***

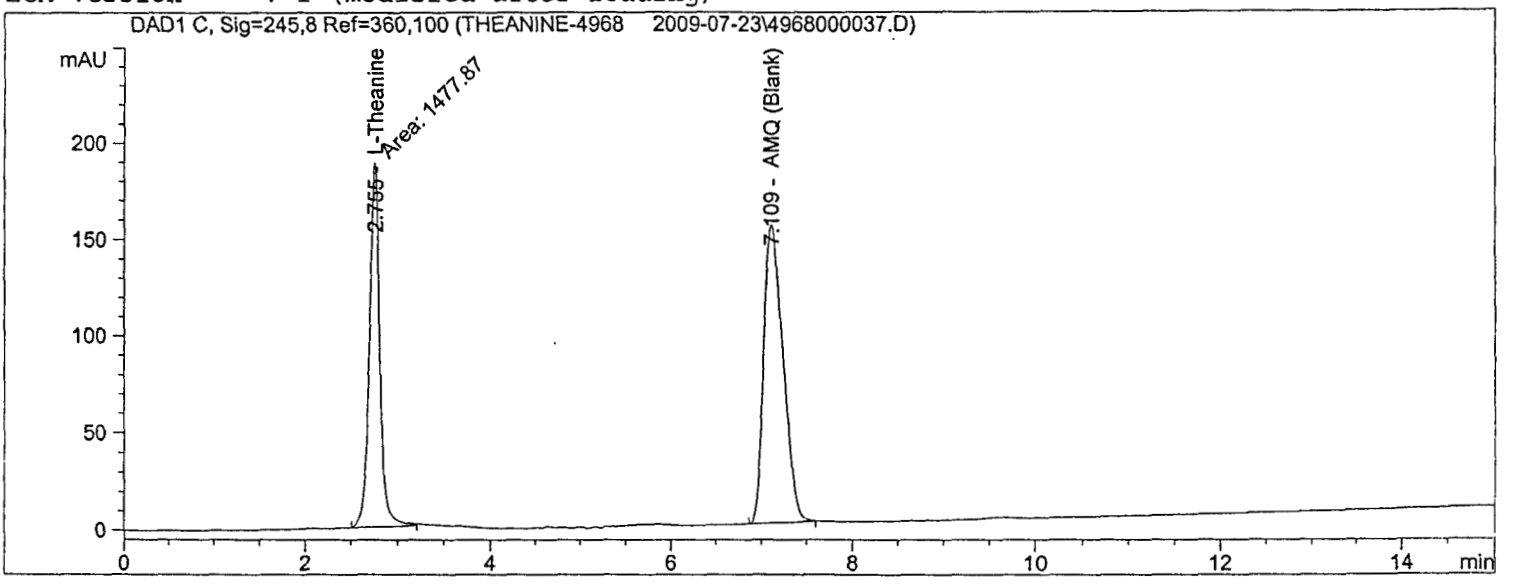
000088

==========

Acq. Operator : (b) (6) Seq. Line : 26
Acq. Instrument : Instrument 15 Location : P1-C-08
Injection Date : 7/24/2009 2:31:15 AM Inj : 1
Inj Volume : 5.0 µl

Acq. Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/23/2009 12:53:23 PM by (b) (6)
Analysis Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/27/2009 9:46:23 AM by (b) (6)
(modified after loading)
Method Info : Chiral Analysis of Theanine

ECM Server : http://us05sqlc/ecmwg
ECM Operator : (b) (6)
ECM Path : Petaluma\LC\HPLC-15\Data\THEANINE-4968 2009-07-23.SC.SSIzip
ECM Version : 1 (modified after loading)



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ESTD Percent Report

==========

Sorted By : Signal
Calib. Data Modified : 7/24/2009 5:17:06 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Sample Amount: : 1.19050 [ug/mL]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=245,8 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
2.755	MM	1477.87219	8.02170e-4	99.580395		L-Theanine
3.013		-	-	-		L-Glutamine
3.643		-	-	-		D-Glu/Thea
7.109	BB	2282.77319	0.00000	0.000000		AMQ (Blank)

Totals : 99.580395

000089

3 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found
Warning : Invalid calibration curve, (AMQ (Blank))

==========

*** End of Report ***

000090

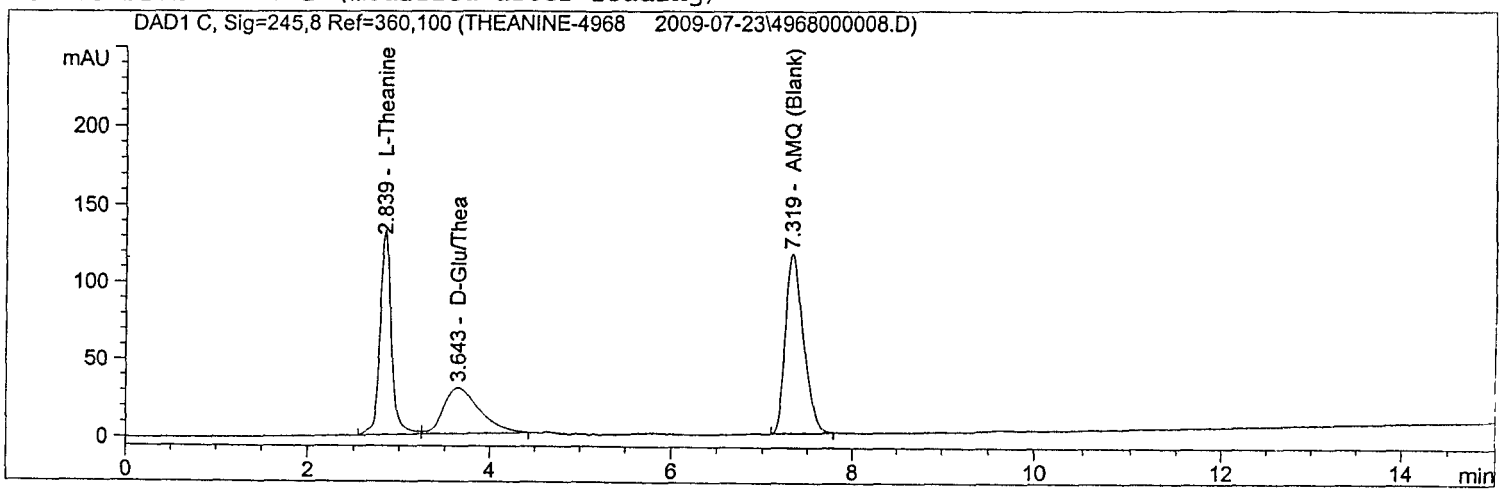
RESOLUTION

Data File C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\4968000008.D
Sample Name: Resolution Soln

==========

Acq. Operator : (b) (6) Seq. Line : 6
Acq. Instrument : Instrument 15 Location : P1-A-05
Injection Date : 7/23/2009 3:32:45 PM Inj : 1
Inj Volume : 5.0 µl
Acq. Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/23/2009 12:53:23 PM by (b) (6)
Analysis Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/24/2009 3:50:21 PM by (b) (6)
(modified after loading)
Method Info : Chiral Analysis of Theanine

ECM Server : http://us05sqlc/ecmwg
ECM Operator : (b) (6)
ECM Path : Petaluma\LC\HPLC-15\Data\THEANINE-4968 2009-07-23.SC.SSIzip
ECM Version : 1 (modified after loading)



==========

External Standard Report (Sample Amount is 0!) with Performance

==========

Calib. Data Modified : 7/24/2009 3:38:30 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=245,8 Ref=360,100

RetTime [min]	k'	Sig	Amount [ug/mL]	Symm.	Width [min]	Plates	Resolution	Name
2.839	-	1	9.15499e-1	0.80	0.1217	3015	-	L-Theanine
3.013	-	1	not f	0.00	-	-	-	L-Glutamine
3.643	-	1	0.00000	0.63	0.4167	424	1.76	D-Glu/Thea
7.319	-	1	0.00000	0.59	0.2133	6521	6.86	AMQ (Blank)

==========

*** End of Report ***

000092

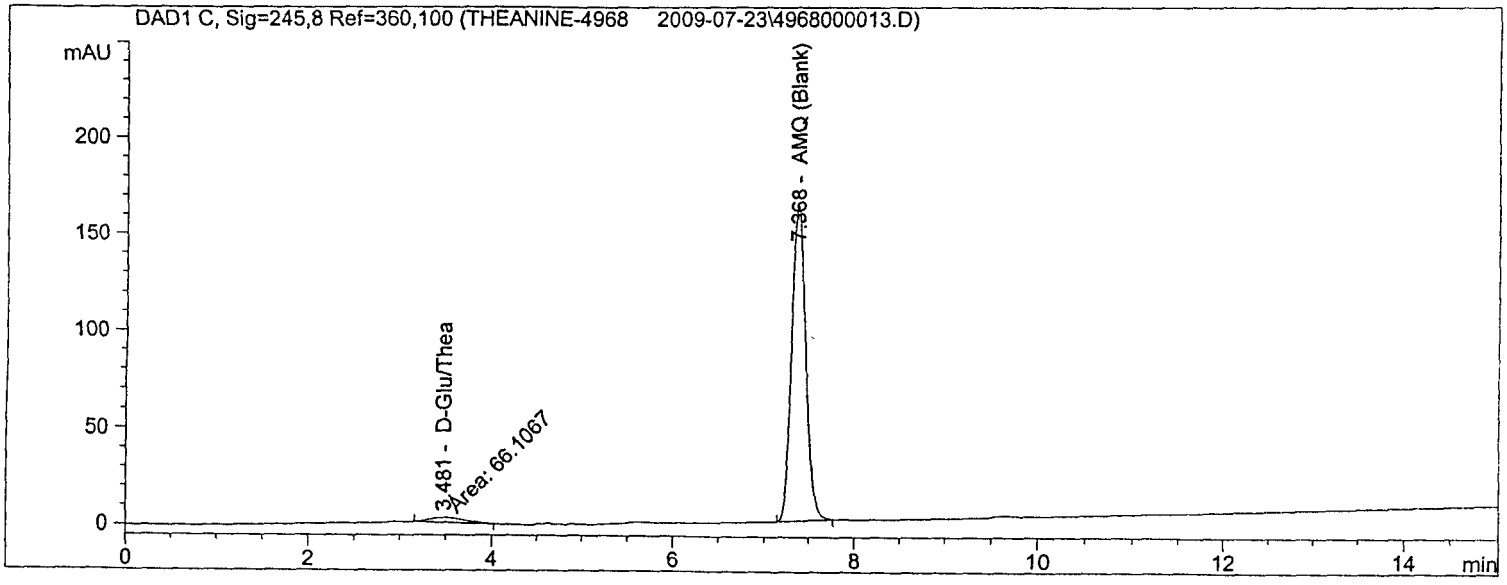
LOQ

Data File C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\4968000013.D
Sample Name: D-Glu 1:11 (LOQ)

==========

Acq. Operator : (b) (6) Seq. Line : 9
Acq. Instrument : Instrument 15 Location : P1-A-06
Injection Date : 7/23/2009 5:26:20 PM Inj : 1
Inj Volume : 5.0 µl
Different Inj Volume from Sequence ! Actual Inj Volume : 3.0 µl
Acq. Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/23/2009 12:53:23 PM by (b) (6)
Analysis Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/27/2009 10:55:03 AM by (b) (6)
Method Info : Chiral Analysis of Theanine

ECM Server : http://us05sqlc/ecmwg
ECM Operator : (b) (6)
ECM Path : Petaluma\LC\HPLC-15\Data\THEANINE-4968 2009-07-23.SC.SSIzip
ECM Version : 1



==========

External Standard Report (Sample Amount is 0!) with Performance and Noise

==========

Calib. Data Modified : Monday, July 27, 2009 10:48:08 AM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=245,8 Ref=360,100

Noise determination:

Time range from [min]	to [min]	Noise (6*SD) [mAU]	Noise (PtoP) [mAU]	Noise (ASTM) [mAU]	Wander [mAU]	Drift [mAU/h]
1.000	1.900	8.666e-2	5.361e-2	-	-	48.423

000094

Sample Name: D-Glu 1:11 (LOQ)

RetTime [min]	k'	Sig	Amount [ug/mL]	Symm.	Width [min]	Plates	Signal /Noise	Name
2.766	-	1	not f	0.00	-	-	-	L-Theanine
3.013	-	1	not f	0.00	-	-	-	L-Glutamine
3.481	-	1	0.00000	0.54	0.4100	399	28.7	D-Glu/Thea
7.368	-	1	0.00000	0.75	0.1650	11048	1878.2	AMQ (Blank)

*** End of Report ***

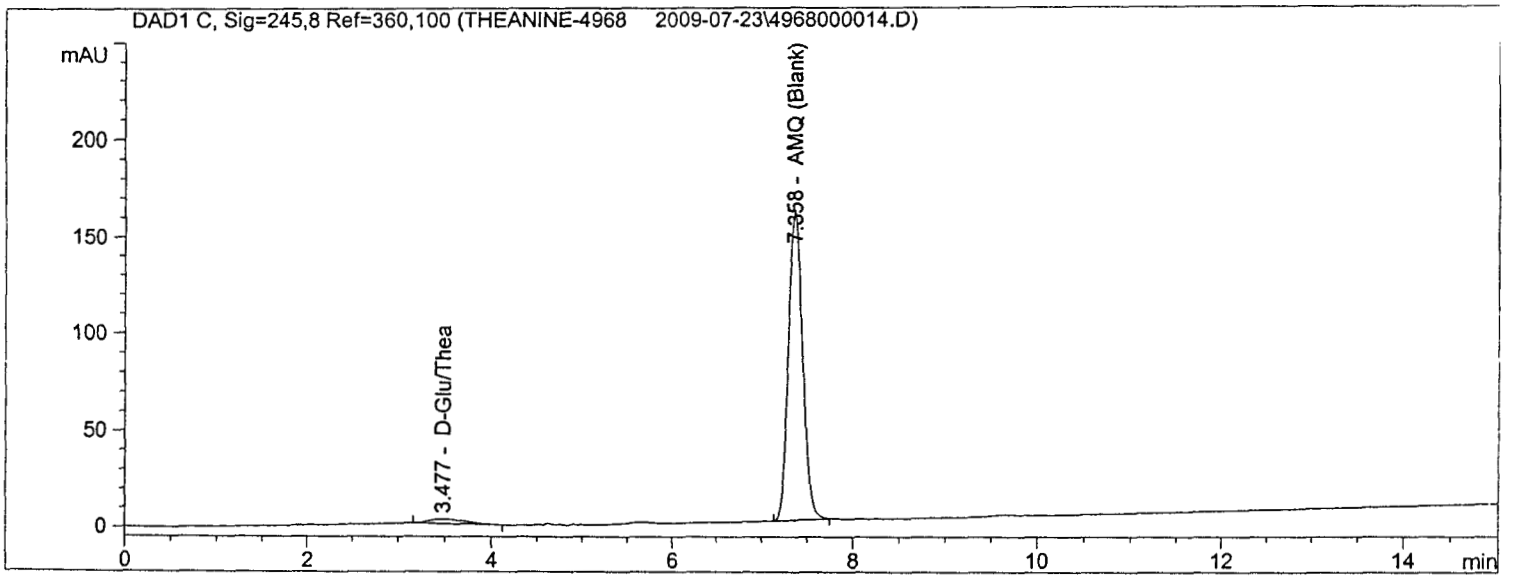
000095

Data File C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\4968000014.D
Sample Name: D-Glu 1:11 (LOQ)

==========

Acq. Operator : (b) (6) Seq. Line : 9
Acq. Instrument : Instrument 15 Location : P1-A-06
Injection Date : 7/23/2009 5:49:02 PM Inj : 2
Inj Volume : 5.0 µl
Different Inj Volume from Sequence ! Actual Inj Volume : 3.0 µl
Acq. Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/23/2009 12:53:23 PM by (b) (6)
Analysis Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/27/2009 10:55:03 AM by (b) (6)
Method Info : Chiral Analysis of Theanine

ECM Server : http://us05sqlc/ecmwg
ECM Operator : (b) (6)
ECM Path : Petaluma\LC\HPLC-15\Data\THEANINE-4968 2009-07-23.SC.SSIzip
ECM Version : 1



==========
External Standard Report (Sample Amount is 0!) with Performance and Noise
==========

Calib. Data Modified : Monday, July 27, 2009 10:48:08 AM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=245,8 Ref=360,100

Noise determination:

Time range from [min]	to [min]	Noise (6*SD) [mAU]	Noise (PtoP) [mAU]	Noise (ASTM) [mAU]	Wander [mAU]	Drift [mAU/h]
1.000	1.900	8.011e-2	5.014e-2	-	-	49.810

000096

Data File C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\4968000014.D
Sample Name: D-Glu 1:11 (LOQ)

RetTime [min]	k'	Sig	Amount [ug/mL]	Symm.	Width [min]	Plates	Signal /Noise	Name
2.766	-	1	not f	0.00	-	-	-	L-Theanine
3.013	-	1	not f	0.00	-	-	-	L-Glutamine
3.477	-	1	0.00000	0.55	0.3967	426	29.8	D-Glu/Thea
7.358	-	1	0.00000	0.75	0.1650	11017	1993.4	AMQ (Blank)

*** End of Report ***

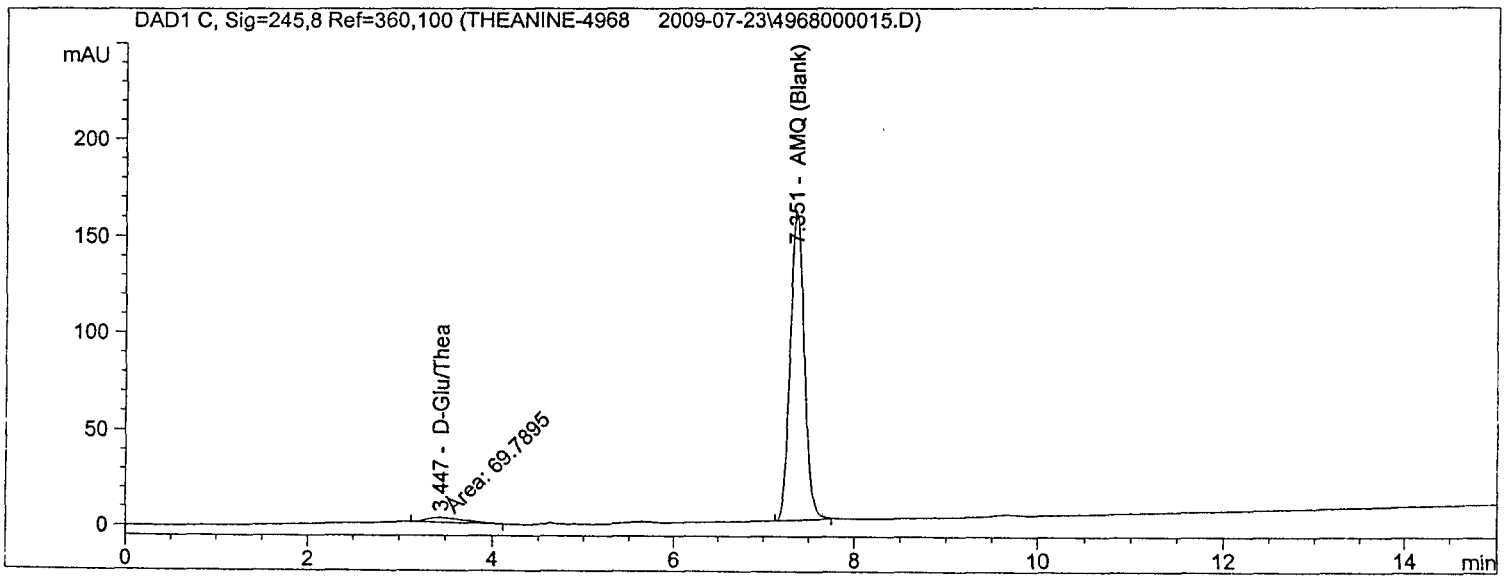
000097

Data File C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\4968000015.D
Sample Name: D-Glu 1:11 (LOQ)

==========

Acq. Operator : (b) (6) Seq. Line : 9
Acq. Instrument : Instrument 15 Location : P1-A-06
Injection Date : 7/23/2009 6:11:43 PM Inj : 3
Inj Volume : 5.0 µl
Different Inj Volume from Sequence ! Actual Inj Volume : 3.0 µl
Acq. Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/23/2009 12:53:23 PM by (b) (6)
Analysis Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/27/2009 10:55:03 AM by (b) (6)
Method Info : Chiral Analysis of Theanine

ECM Server : http://us05sqlc/ecmwg
ECM Operator : (b) (6)
ECM Path : Petaluma\LC\HPLC-15\Data\THEANINE-4968 2009-07-23.SC.SSIzip
ECM Version : 1



==========

External Standard Report (Sample Amount is 0!) with Performance and Noise

==========

Calib. Data Modified : Monday, July 27, 2009 10:48:08 AM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=245,8 Ref=360,100

Noise determination:

Time range from [min]	to [min]	Noise (6*SD) [mAU]	Noise (PtoP) [mAU]	Noise (ASTM) [mAU]	Wander [mAU]	Drift [mAU/h]
1.000	1.900	8.079e-2	4.828e-2	-	-	48.498

000098

Data File C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\4968000015.D
Sample Name: D-Glu 1:11 (LOQ)

RetTime [min]	k'	Sig	Amount [ug/mL]	Symm.	Width [min]	Plates	Signal /Noise	Name
2.766	-	1	not f	0.00	-	-	-	L-Theanine
3.013	-	1	not f	0.00	-	-	-	L-Glutamine
3.447	-	1	0.00000	0.49	0.4267	362	30.8	D-Glu/Thea
7.351	-	1	0.00000	0.75	0.1650	10997	1980.7	AMQ (Blank)

*** End of Report ***

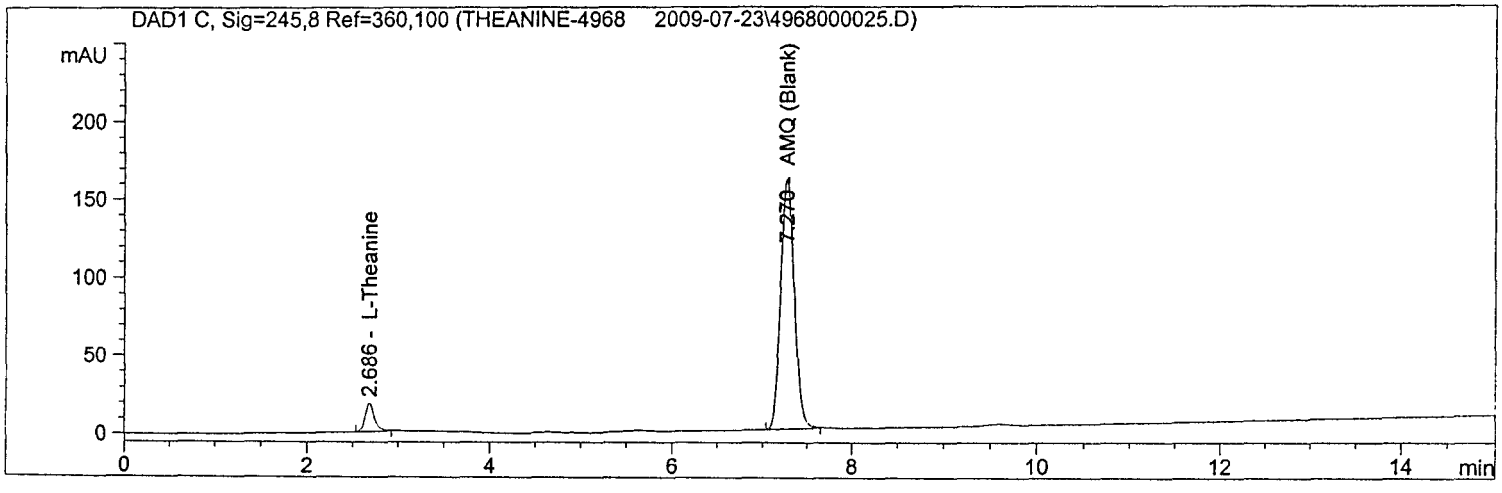
000099

Data File C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\4968000025.D
Sample Name: STD 5 (LOQ)

==========

Acq. Operator : (b) (6) Seq. Line : 16
Acq. Instrument : Instrument 15 Location : P1-B-05
Injection Date : 7/23/2009 9:58:47 PM Inj : 1
Inj Volume : 5.0 µl
Different Inj Volume from Sequence ! Actual Inj Volume : 3.0 µl
Acq. Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/23/2009 12:53:23 PM by (b) (6)
Analysis Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/24/2009 3:44:38 PM by (b) (6)
Method Info : Chiral Analysis of Theanine

ECM Server : http://us05sqlc/ecmwg
ECM Operator : (b) (6)
ECM Path : Petaluma\LC\HPLC-15\Data\THEANINE-4968 2009-07-23.SC.SSIzip
ECM Version : 1



==========
External Standard Report (Sample Amount is 0!) with Performance and Noise
==========

Calib. Data Modified : 7/24/2009 3:38:30 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=245,8 Ref=360,100

Noise determination:

Time range from [min]	to [min]	Noise (6*SD) [mAU]	Noise (PtoP) [mAU]	Noise (ASTM) [mAU]	Wander [mAU]	Drift [mAU/h]
1.000	2.000	0.1339	7.328e-2	-	-	47.296

RetTime [min]	k'	Sig	Amount [ug/mL]	Symm.	Width [min]	Plates	Signal /Noise	Name
2.686	-	1	1.23370e-1	0.72	0.1013	3894	136.6	L-Theanine
3.013	-	1	not f	0.00	-	-	-	L-Glutamine
3.643	-	1	not f	0.00	-	-	-	D-Glu/Thea

000100

Data File C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\4968000025.D
Sample Name: STD 5 (LOQ)

RetTime [min]	k'	Sig	Amount [ug/mL]	Symm.	Width [min]	Plates	Signal /Noise	Name
7.270	-	1	0.00000	0.77	0.1633	10975	1191.4	AMQ (Blank)

*** End of Report ***

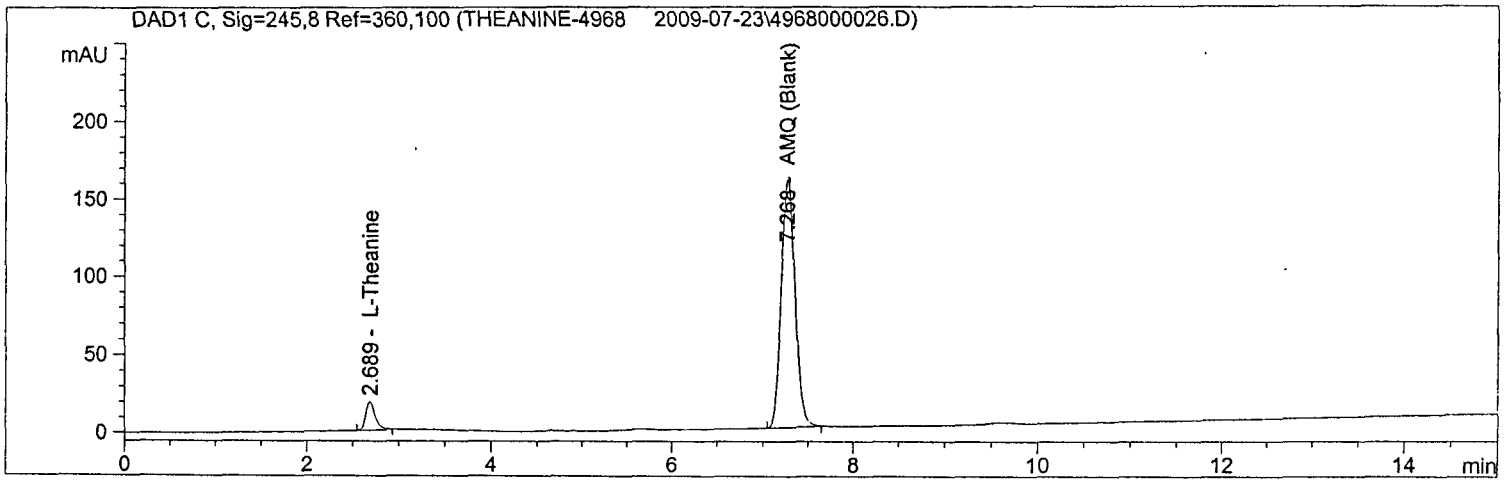
000101

Data File C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\4968000026.D
Sample Name: STD 5 (LOQ)

==========

Acq. Operator : (b) (6) Seq. Line : 16
Acq. Instrument : Instrument 15 Location : P1-B-05
Injection Date : 7/23/2009 10:21:29 PM Inj : 2
Inj Volume : 5.0 µl
Different Inj Volume from Sequence ! Actual Inj Volume : 3.0 µl
Acq. Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/23/2009 12:53:23 PM by (b) (6)
Analysis Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/24/2009 3:44:38 PM by (b) (6)
Method Info : Chiral Analysis of Theanine

ECM Server : http://us05sqlc/ecmwg
ECM Operator : (b) (6)
ECM Path : Petaluma\LC\HPLC-15\Data\THEANINE-4968 2009-07-23.SC.SSIzip
ECM Version : 1



==========
External Standard Report (Sample Amount is 0!) with Performance and Noise
==========

Calib. Data Modified : 7/24/2009 3:38:30 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=245,8 Ref=360,100

Noise determination:

Time range from [min]	to [min]	Noise (6*SD) [mAU]	Noise (PtoP) [mAU]	Noise (ASTM) [mAU]	Wander [mAU]	Drift [mAU/h]
1.000	2.000	0.1279	7.077e-2	-	-	47.319

RetTime [min]	k'	Sig	Amount [ug/mL]	Symm.	Width [min]	Plates	Signal /Noise	Name
2.689	-	1	1.23662e-1	0.71	0.1013	3902	145.2	L-Theanine
3.013	-	1	not f	0.00	-	-	-	L-Glutamine
3.643	-	1	not f	0.00	-	-	-	D-Glu/Thea

000102

Data File C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\4968000026.D
Sample Name: STD 5 (LOQ)

RetTime [min]	k'	Sig	Amount [ug/mL]	Symm.	Width [min]	Plates	Signal /Noise	Name
7.268	-	1	0.00000	0.78	0.1633	10969	1242.7	AMQ (Blank)

*** End of Report ***

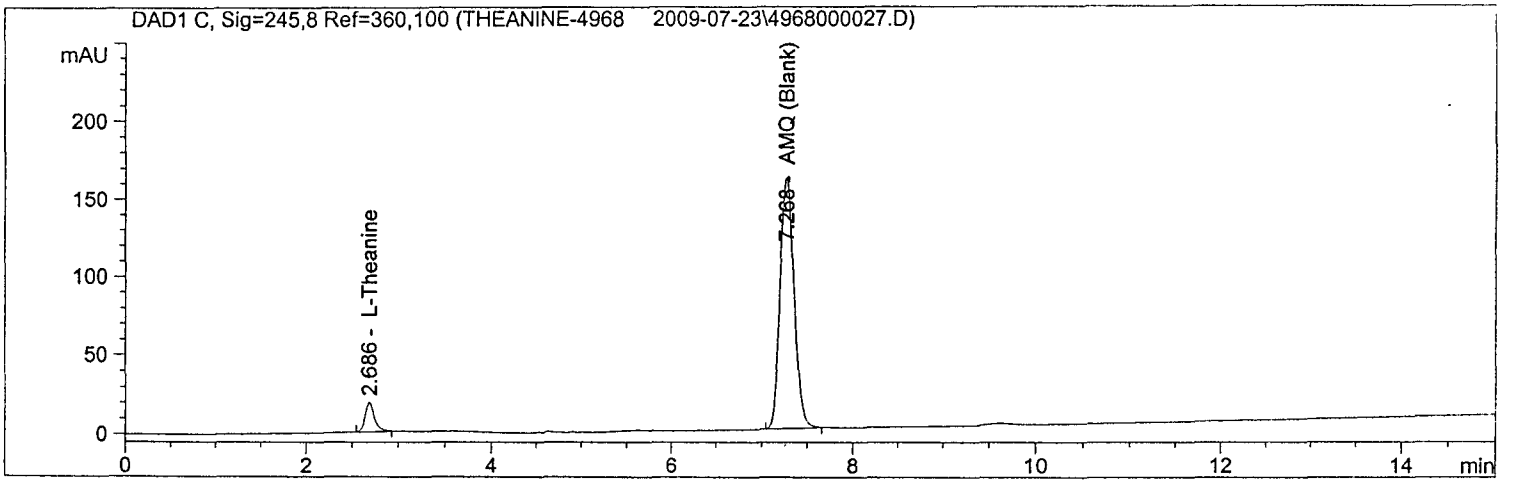
000103

Data File C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\4968000027.D
Sample Name: STD 5 (LOQ)

==========

Acq. Operator : (b) (6) Seq. Line : 16
Acq. Instrument : Instrument 15 Location : P1-B-05
Injection Date : 7/23/2009 10:44:09 PM Inj : 3
Inj Volume : 5.0 µl
Different Inj Volume from Sequence ! Actual Inj Volume : 3.0 µl
Acq. Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/23/2009 12:53:23 PM by (b) (6)
Analysis Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/24/2009 3:44:38 PM by (b) (6)
Method Info : Chiral Analysis of Theanine

ECM Server : http://us05sqlc/ecmwg
ECM Operator : (b) (6)
ECM Path : Petaluma\LC\HPLC-15\Data\THEANINE-4968 2009-07-23.SC.SSIzip
ECM Version : 1



==========
External Standard Report (Sample Amount is 0!) with Performance and Noise
==========

Calib. Data Modified : 7/24/2009 3:38:30 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=245,8 Ref=360,100

Noise determination:

Time range from [min]	to [min]	Noise (6*SD) [mAU]	Noise (PtoP) [mAU]	Noise (ASTM) [mAU]	Wander [mAU]	Drift [mAU/h]
1.000	2.000	0.1166	6.460e-2	-	-	47.776

RetTime [min]	k'	Sig	Amount [ug/mL]	Symm.	Width [min]	Plates	Signal /Noise	Name
2.686	-	1	1.24596e-1	0.72	0.1000	3998	162.3	L-Theanine
3.013	-	1	not f	0.00	-	-	-	L-Glutamine
3.643	-	1	not f	0.00	-	-	-	D-Glu/Thea

000104

Data File C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\4968000027.D
Sample Name: STD 5 (LOQ)

RetTime [min]	k'	Sig	Amount [ug/mL]	Symm.	Width [min]	Plates	Signal /Noise	Name
7.268	-	1	0.00000	0.78	0.1650	10749	1370.6	AMQ (Blank)

*** End of Report ***

000105

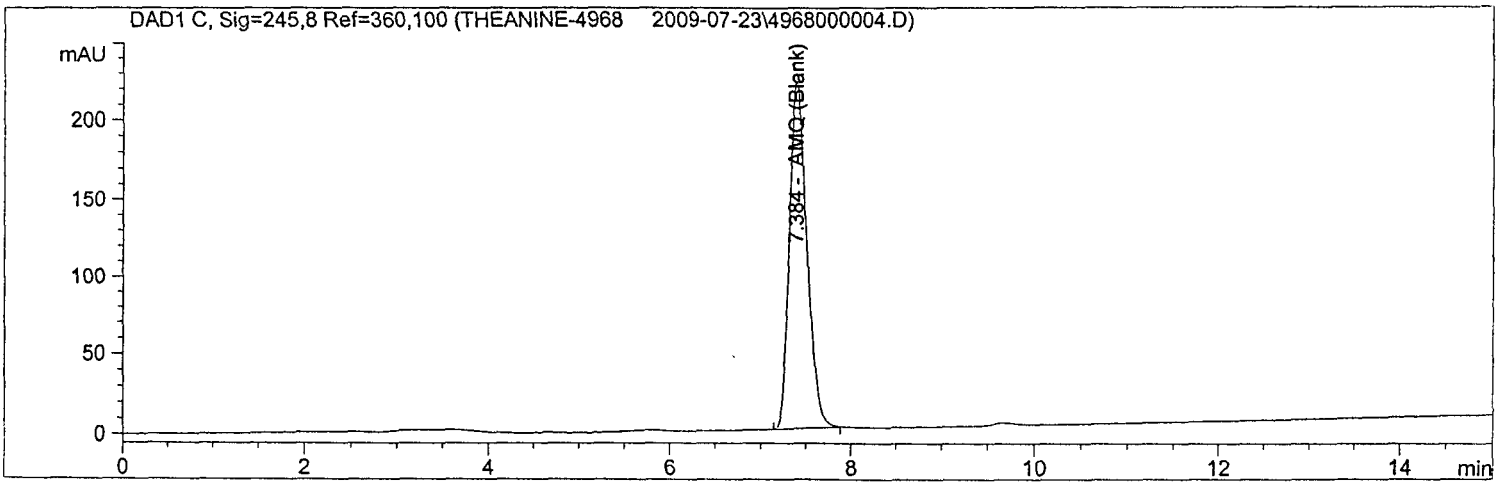
BLANK

Sample Name: Blank

==========

Acq. Operator : (b) (6) Seq. Line : 4
Acq. Instrument : Instrument 15 Location : P1-A-03
Injection Date : 7/23/2009 2:01:53 PM Inj : 1
Inj Volume : 5.0 µl
Acq. Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/23/2009 12:53:23 PM by (b) (6)
Analysis Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/24/2009 3:52:35 PM by (b) (6)
Method Info : Chiral Analysis of Theanine

ECM Server : http://us05sqlc/ecmwg
ECM Operator : (b) (6)
ECM Path : Petaluma\LC\HPLC-15\Data\THEANINE-4968 2009-07-23.SC.SSIzip
ECM Version : 1



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External Standard Report (Sample Amount is 0!)

==========

Sorted By : Signal
Calib. Data Modified : 7/24/2009 3:38:30 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=245,8 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ug/mL]	Grp	Name
2.776		-	-	-		L-Theanine
3.013		-	-	-		L-Glutamine
3.643		-	-	-		D-Glu/Thea
7.384	BB	3006.23145	0.00000	0.00000		AMQ (Blank)

Totals : 0.00000

3 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found
Warning : Invalid calibration curve, (AMQ (Blank))

000107

Sample Name: Blank

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*** End of Report ***

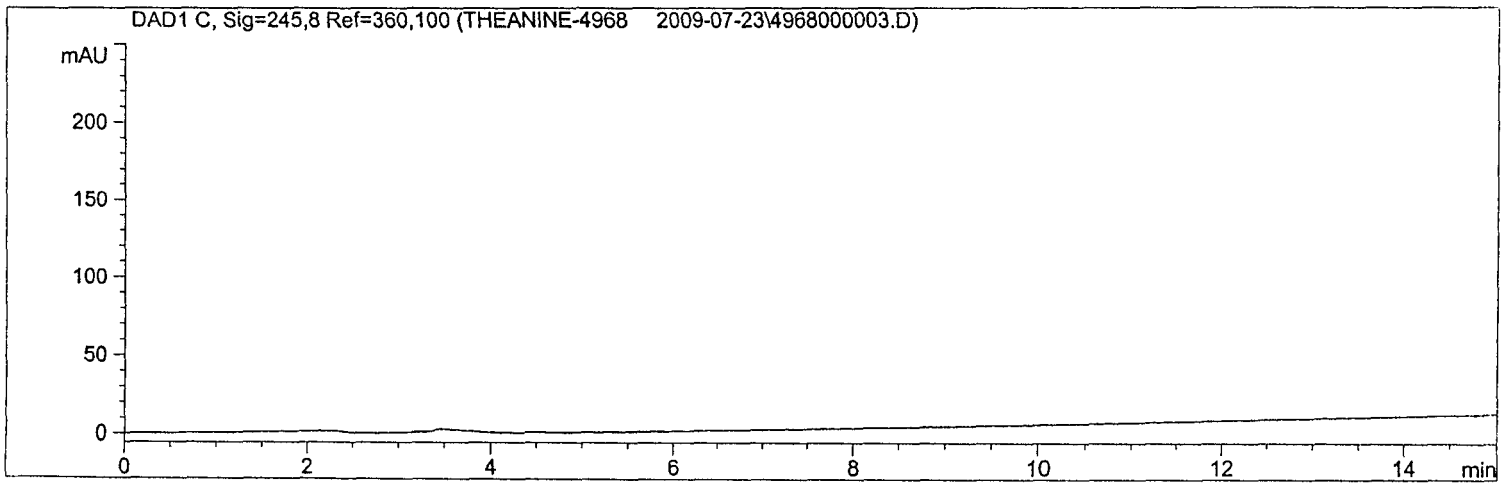
000108

Sample Name: Mobile Phase A

==========

Acq. Operator : (b) (6) Seq. Line : 3
Acq. Instrument : Instrument 15 Location : P1-A-01
Injection Date : 7/23/2009 1:39:11 PM Inj : 1
Inj Volume : 5.0 µl
Acq. Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/23/2009 12:53:23 PM by (b) (6)
Analysis Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/24/2009 3:52:35 PM by (b) (6)
Method Info : Chiral Analysis of Theanine

ECM Server : http://us05sqlc/ecmwg
ECM Operator : (b) (6)
ECM Path : Petaluma\LC\HPLC-15\Data\THEANINE-4968 2009-07-23.SC.SSIzip
ECM Version : 1



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External Standard Report (Sample Amount is 0!)

==========

Sorted By : Signal
Calib. Data Modified : 7/24/2009 3:38:30 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=245,8 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ug/mL]	Grp	Name
2.776		-	-	-		L-Theanine
3.013		-	-	-		L-Glutamine
3.643		-	-	-		D-Glu/Thea
7.197		-	-	-		AMQ (Blank)

Totals : 0.00000

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

000109

Sample Name: Mobile Phase A

==========
==========
Area Percent Report
==========

Sorted By : Signal
Calib. Data Modified : 7/24/2009 3:38:30 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=245,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
1	2.776		0.0000	0.00000	0.0000	L-Theanine
2	3.013		0.0000	0.00000	0.0000	L-Glutamine
3	3.643		0.0000	0.00000	0.0000	D-Glu/Thea
4	7.197		0.0000	0.00000	0.0000	AMQ (Blank)

Totals : 0.00000

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

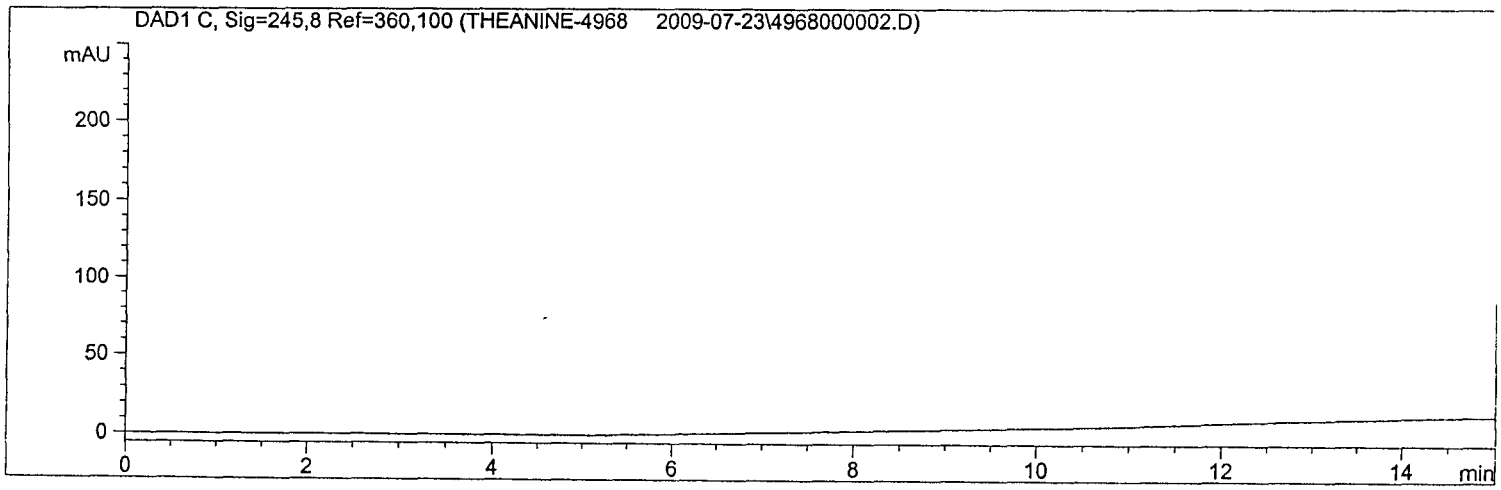
==========
*** End of Report ***

Sample Name: MP B (Methanol)

==========

Acq. Operator : (b) (6) Seq. Line : 2
Acq. Instrument : Instrument 15 Location : P1-A-02
Injection Date : 7/24/2009 10:41:12 AM Inj : 1
Inj Volume : 5.0 µl
Acq. Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/24/2009 10:18:08 AM by (b) (6)
Analysis Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/24/2009 3:52:35 PM by (b) (6)
Method Info : Chiral Analysis of Theanine

ECM Server : http://us05sqlc/ecmwg
ECM Operator : (b) (6)
ECM Path : Petaluma\LC\HPLC-15\Data\THEANINE-4968 2009-07-23.SC.SSIzip
ECM Version : 1



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External Standard Report (Sample Amount is 0!)

==========

Sorted By : Signal
Calib. Data Modified : 7/24/2009 3:38:30 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=245,8 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ug/mL]	Grp	Name
2.776		-	-	-		L-Theanine
3.013		-	-	-		L-Glutamine
3.643		-	-	-		D-Glu/Thea
7.197		-	-	-		AMQ (Blank)
Totals :				0.00000		

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

000111

Sample Name: MP B (Methanol)

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==========
Area Percent Report
==========

Sorted By : Signal
Calib. Data Modified : 7/24/2009 3:38:30 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=245,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
1	2.776		0.0000	0.00000	0.0000	L-Theanine
2	3.013		0.0000	0.00000	0.0000	L-Glutamine
3	3.643		0.0000	0.00000	0.0000	D-Glu/Thea
4	7.197		0.0000	0.00000	0.0000	AMQ (Blank)

Totals : 0.00000

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

==========
*** End of Report ***

000112

STATISTICS DATA

Statistic Report

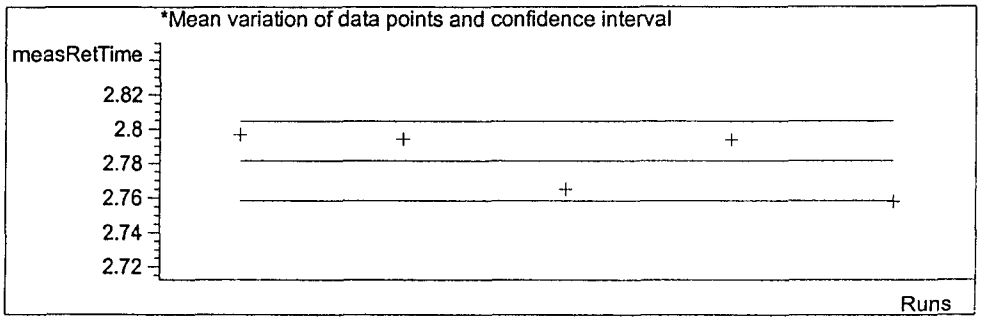
Sequence table: C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE-4968.S
Data directory path: C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\
ECM Host: http://us05sqlc
ECM Path: Petaluma\LC\HPLC-15\Data\THEANINE-4968 2009-07-23.SC.SSIzip
ECM Ver: 1
Operator: (b) (6)

Method file name: C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M

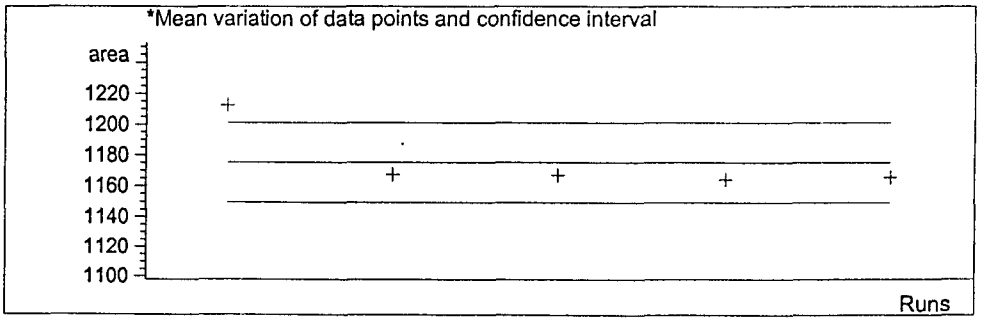
Run #	Location	Inj #	Inj. Date/Time	File Name	Sample Name
1	P1-B-02	1	7/24/2009 11:04:09 AM	4968000017.D	STD 2
2	P1-B-02	1	7/23/2009 7:19:48 PM	4968000018.D	STD 2
3	P1-B-02	2	7/23/2009 7:42:31 PM*	4968000019.D	STD 2
4	P1-B-02	3	7/23/2009 8:05:13 PM	4968000020.D	STD 2
5	P1-B-02	4	7/23/2009 8:27:56 PM	4968000021.D	STD 2

Compound: L-Theanine (Signal: DAD1 C, Sig=245,8 Ref=360,100)

Run #	RetTime [min]
1	2.79678
2	2.79422
3	2.76496
4	2.79377
5	2.75793
Mean:	2.78153
S.D.:	0.01854
RSD :	0.66660
95% CI:	0.02302



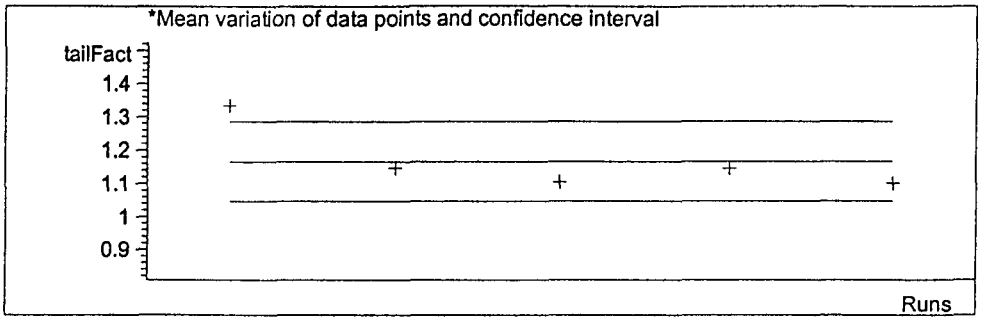
Run #	Peak area [mAU*s]
1	1212.53687
2	1167.37378
3	1166.99561
4	1164.23022
5	1165.71814
Mean:	1175.37092
S.D.:	20.81285
RSD :	1.77075
95% CI:	25.84256



000114

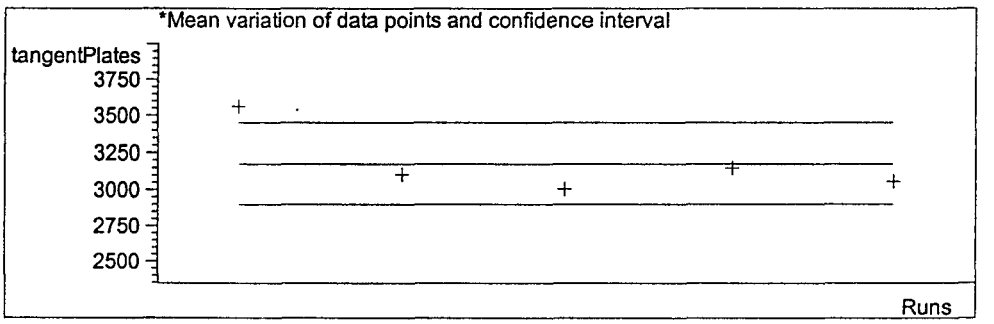
Run #	Tailing factor
1	1.33443
2	1.14780
3	1.10500
4	1.14532
5	1.10107

Mean:	1.16672
S.D.:	0.09626
RSD :	8.25020
95% CI:	0.11952



Run #	Plates (tangent method)
1	3561.46815
2	3105.01814
3	3004.21216
4	3148.05816
5	3056.79758

Mean:	3175.11084
S.D.:	222.55403
RSD :	7.00933
95% CI:	276.33729



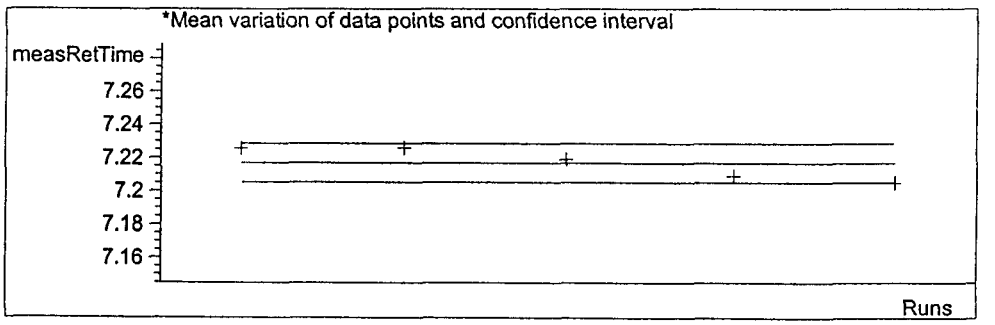
Statistic results for compound L-Glutamine not available.

Statistic results for compound D-Glu/Thea not available.

Compound: AMQ (Blank) (Signal: DAD1 C, Sig=245,8 Ref=360,100)

Run #	RetTime [min]
1	7.22562
2	7.22600
3	7.21910
4	7.20935
5	7.20521

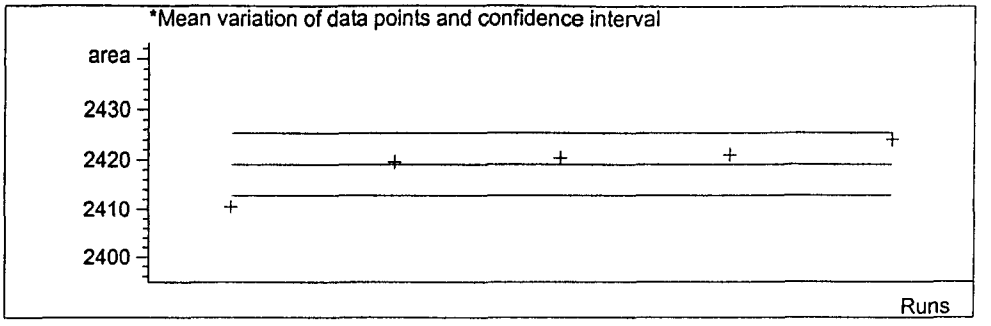
Mean:	7.21706
S.D.:	0.00945
RSD :	0.13092
95% CI:	0.01173



000115

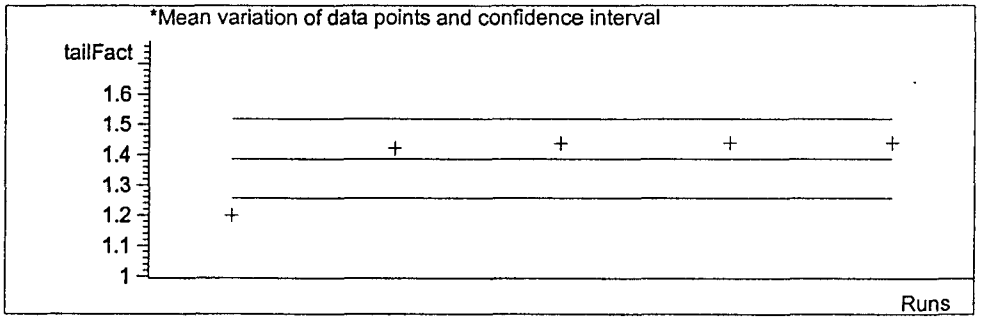
Run #	Peak area [mAU*s]
1	2410.46875
2	2419.49756
3	2420.27319
4	2420.85767
5	2423.98755

Mean:	2419.01694
S.D.:	5.07385
RSD :	0.20975
95% CI:	6.30002



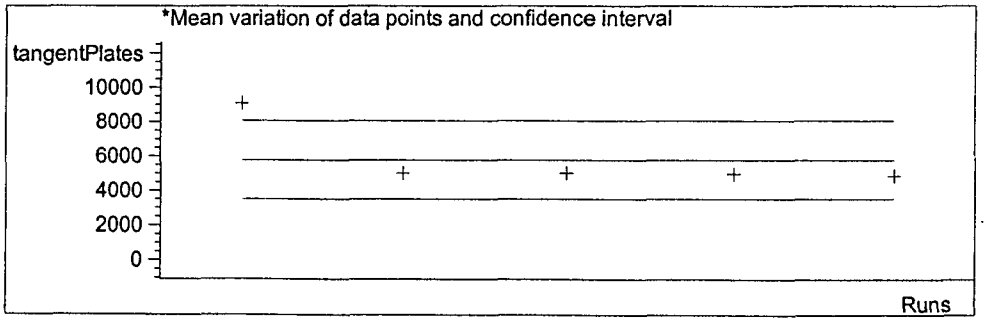
Run #	Tailing factor
1	1.19891
2	1.42072
3	1.43664
4	1.43877
5	1.43853

Mean:	1.38672
S.D.:	0.10525
RSD :	7.59010
95% CI:	0.13069



Run #	Plates (tangent method)
1	9080.96389
2	5008.38321
3	5008.04121
4	4979.82357
5	4872.50561

Mean:	5789.94350
S.D.:	1840.58479
RSD :	31.78934
95% CI:	2285.38752



000116

Calibration Table

Chiral Analysis of Theanine

Calib. Data Modified : 7/24/2009 3:38:30 PM

Rel. Reference Window : 5.000 %
Abs. Reference Window : 0.200 min
Rel. Non-ref. Window : 5.000 %
Abs. Non-ref. Window : 0.200 min
Uncalibrated Peaks : not reported
Partial Calibration : Yes, identified peaks are recalibrated
Correct All Ret. Times: No, only for identified peaks

Curve Type : Linear
Origin : Included
Weight : Equal

Recalibration Settings:
Average Response : Average all calibrations
Average Retention Time: Floating Average New 75%

Calibration Report Options :
Printout of recalibrations within a sequence:
Calibration Table after Recalibration
Normal Report after Recalibration
If the sequence is done with bracketing:
Results of first cycle (ending previous bracket)

Signal 1: DAD1 C, Sig=245,8 Ref=360,100

RetTime [min]	Lvl Sig		Amount [ug/mL]	Area	Amt/Area	Ref	Grp	Name
2.776	1	5	1.81131e-1	222.10408	8.15523e-4			L-Theanine
		4	3.32070e-1	361.60098	9.18333e-4			
		3	6.64150e-1	850.89923	7.80527e-4			
		2	9.96220e-1	1166.12925	8.54296e-4			
		1	1.99244	2528.07812	7.88124e-4			
3.013	1	1	1.00000	0.00000	0.00000			L-Glutamine
3.643	1	1	1.00000	0.00000	0.00000			D-Glu/Thea
7.197	1	1	1.00000	0.00000	0.00000			AMQ (Blank)

1 Warnings or Errors :

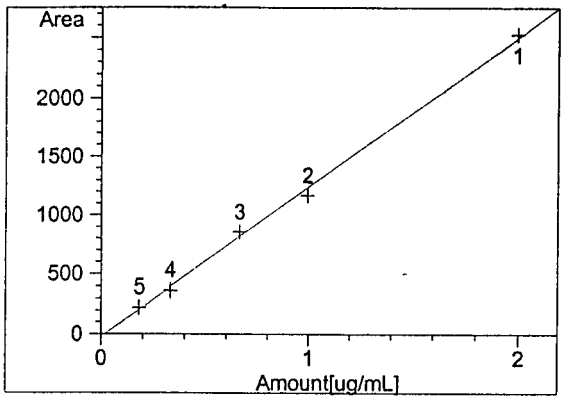
Warning : Overlapping peak time windows at 2.776 min, signal 1

Peak Sum Table

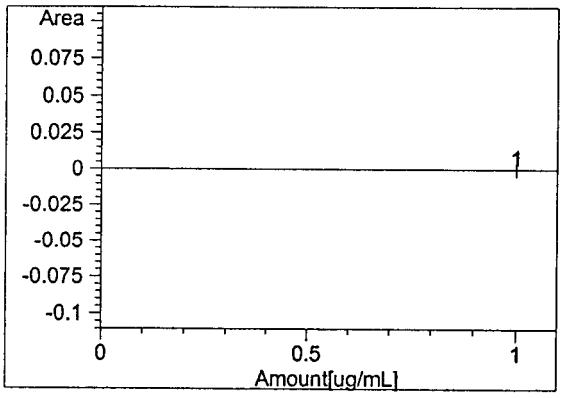
No Entries in table

000117

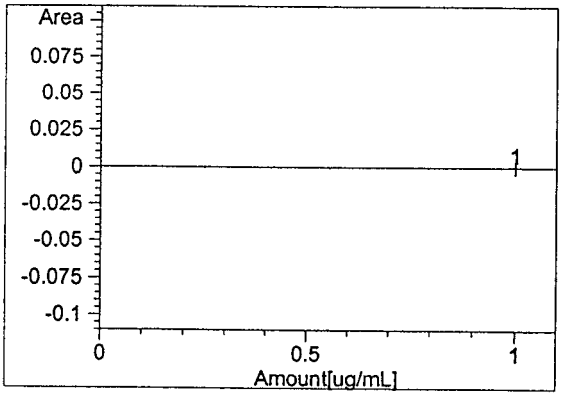
Calibration Curves



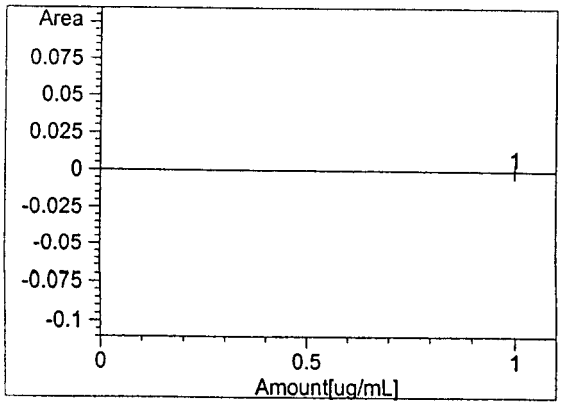
L-Theanine at exp. RT: 2.776
DAD1 C, Sig=245,8 Ref=360,100
Correlation: 0.99893
Residual Std. Dev.: 47.85007
Formula: y = mx + b
m: 1267.25252
b: -25.09605
x: Amount
y: Area



L-Glutamine at exp. RT: 3.013
DAD1 C, Sig=245,8 Ref=360,100
Correlation: 1.00000
Residual Std. Dev.: 0.00000
Formula: y = mx + b
m: 0.00000
b: 0.00000
x: Amount
y: Area



D-Glu/Thea at exp. RT: 3.643
DAD1 C, Sig=245,8 Ref=360,100
Correlation: 1.00000
Residual Std. Dev.: 0.00000
Formula: y = mx + b
m: 0.00000
b: 0.00000
x: Amount
y: Area



AMQ (Blank) at exp. RT: 7.197
DAD1 C, Sig=245,8 Ref=360,100
Correlation: 1.00000
Residual Std. Dev.: 0.00000
Formula: y = mx + b
m: 0.00000
b: 0.00000
x: Amount
y: Area

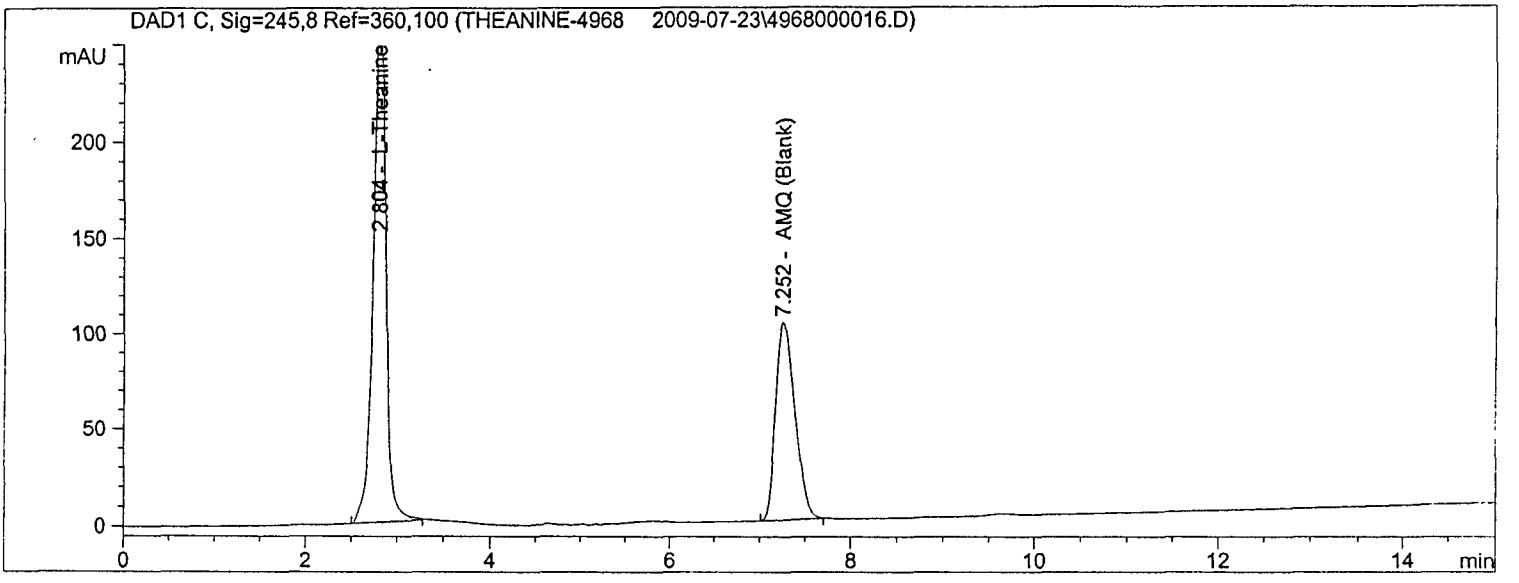
000118

Sample Name: STD 1

==========

Acq. Operator : (b) (6) Seq. Line : 10
Acq. Instrument : Instrument 15 Location : P1-B-01
Injection Date : 7/23/2009 6:34:25 PM Inj : 1
Inj Volume : 5.0 µl
Acq. Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/23/2009 12:53:23 PM by (b) (6)
Analysis Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/24/2009 3:54:42 PM by (b) (6)
Method Info : Chiral Analysis of Theanine

ECM Server : http://us05sqlc/ecmwg
ECM Operator : (b) (6)
ECM Path : Petaluma\LC\HPLC-15\Data\THEANINE-4968 2009-07-23.SC.SSIzip
ECM Version : 1



==========

External Standard Report (Sample Amount is 0!)

==========

Sorted By : Signal
Calib. Data Modified : 7/24/2009 3:38:30 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=245,8 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ug/mL]	Grp	Name
2.804	BB	2528.07812	7.96942e-4	2.01473		L-Theanine
3.013		-	-	-		L-Glutamine
3.643		-	-	-		D-Glu/Thea
7.252	BB	1504.88953	0.00000	0.00000		AMQ (Blank)

Totals : 2.01473

3 Warnings or Errors :

000119

Sample Name: STD 1

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found
Warning : Invalid calibration curve, (AMQ (Blank))

==========

*** End of Report ***

000120

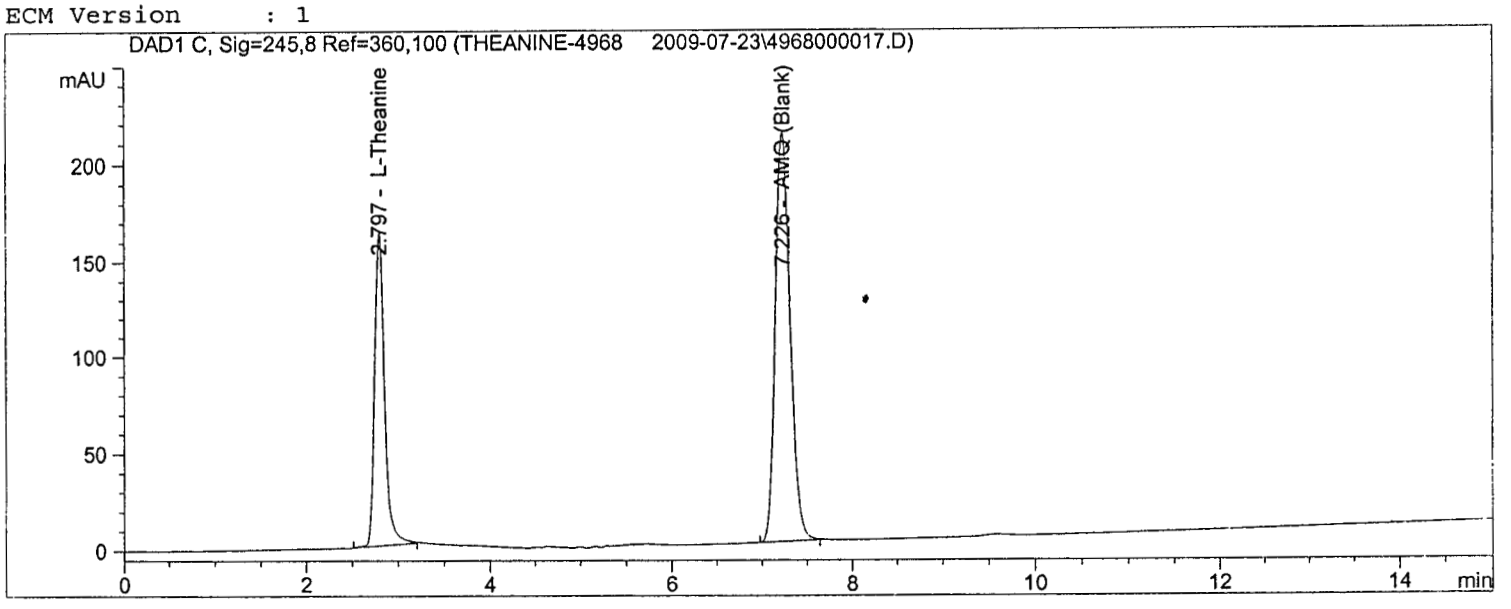
Data File C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\4968000017.D
Sample Name: STD 2

==========

Acq. Operator : (b) (6) Seq. Line : 11
Acq. Instrument : Instrument 15 Location : P1-B-02
Injection Date : 7/24/2009 11:04:09 AM Inj : 1
Inj Volume : 5.0 µl

Acq. Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/24/2009 10:18:08 AM by (b) (6)
Analysis Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/24/2009 3:54:42 PM by (b) (6)
Method Info : Chiral Analysis of Theanine

ECM Server : http://us05sqlc/ecmwg
ECM Operator : (b) (6)
ECM Path : Petaluma\LC\HPLC-15\Data\THEANINE-4968 2009-07-23.SC.SSIzip
ECM Version : 1



==========

External Standard Report (Sample Amount is 0!)

==========

Sorted By : Signal
Calib. Data Modified : 7/24/2009 3:38:30 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=245,8 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ug/mL]	Grp	Name
2.797	BB	1212.53687	8.05441e-4	9.76627e-1		L-Theanine
3.013		-	-	-		L-Glutamine
3.643		-	-	-		D-Glu/Thea
7.226	BB	2410.46875	0.00000	0.00000		AMQ (Blank)

Totals : 9.76627e-1

3 Warnings or Errors :

000121

a File C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\4968000017.D
ple Name: STD 2

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found
Warning : Invalid calibration curve, (AMQ (Blank))

==========

*** End of Report ***

000122

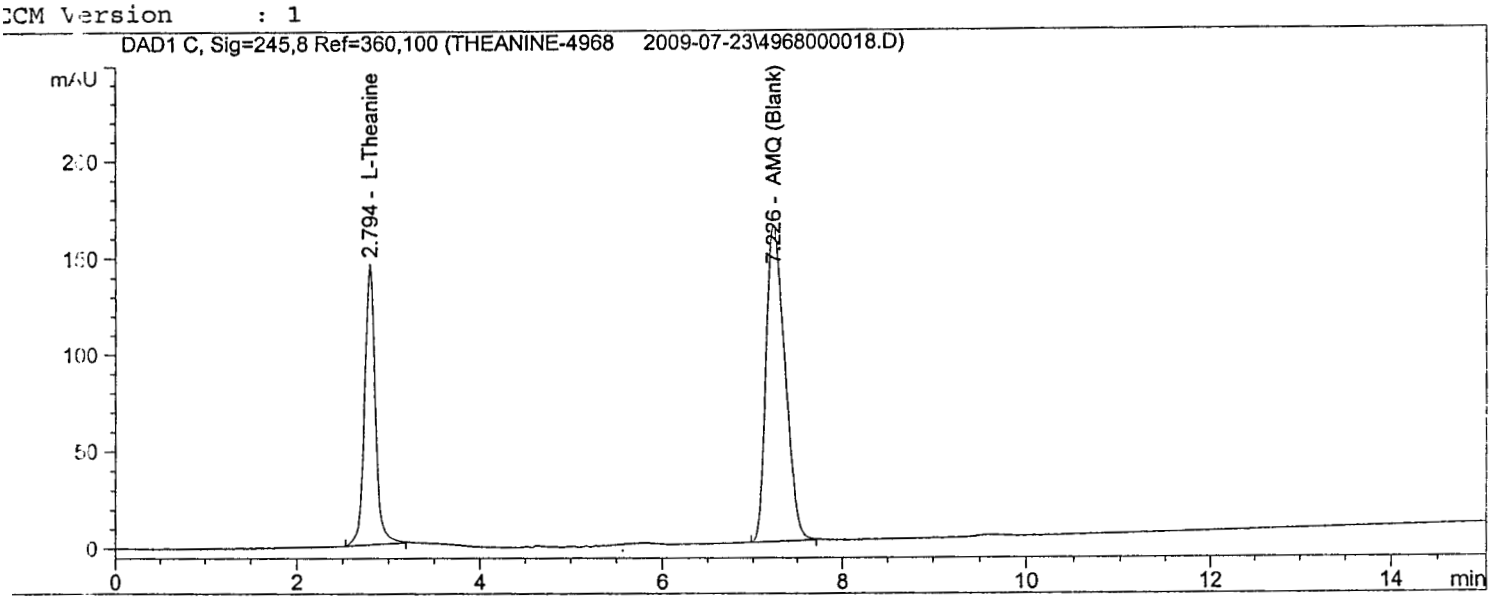
File: C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\4968000018.D
le Name: STD 2

==========

Acq. Operator : (b) (6) Seq. Line : 12
Acq. Instrument : Instrument 15 Location : P1-B-02
Injection Date : 7/23/2009 7:19:48 PM Inj : 1
Inj Volume : 5.0 µl

Acq. Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/23/2009 12:53:23 PM by (b) (6)
Analysis Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/24/2009 3:54:42 PM by (b) (6)
Method Info : Chiral Analysis of Theanine

ECM Server : http://us05sqlc/ecmwg
ECM Operator : (b) (6)
ECM Path : Petaluma\LC\HPLC-15\Data\THEANINE-4968 2009-07-23.SC.SSIzip
ECM Version : 1



==========

External Standard Report (Sample Amount is 0!)

==========

Sorted By : Signal
Calib Data Modified : 7/24/2009 3:38:30 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=245,8 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ug/mL]	Grp	Name
2.794	BB	1167.37378	8.06073e-4	9.40988e-1		L-Theanine
3.013		-	-	-		L-Glutamine
3.643		-	-	-		D-Glu/Thea
7.226	BB	2419.49756	0.00000	0.00000		AMQ (Blank)

Totals : 9.40988e-1

Warnings or Errors :

000123

Printed 15 7/24/2009 3:55:45 PM (b) (6)

le Name: STD 2

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found
Warning : Invalid calibration curve, (AMQ (Blank))

==========

*** End of Report ***

000124

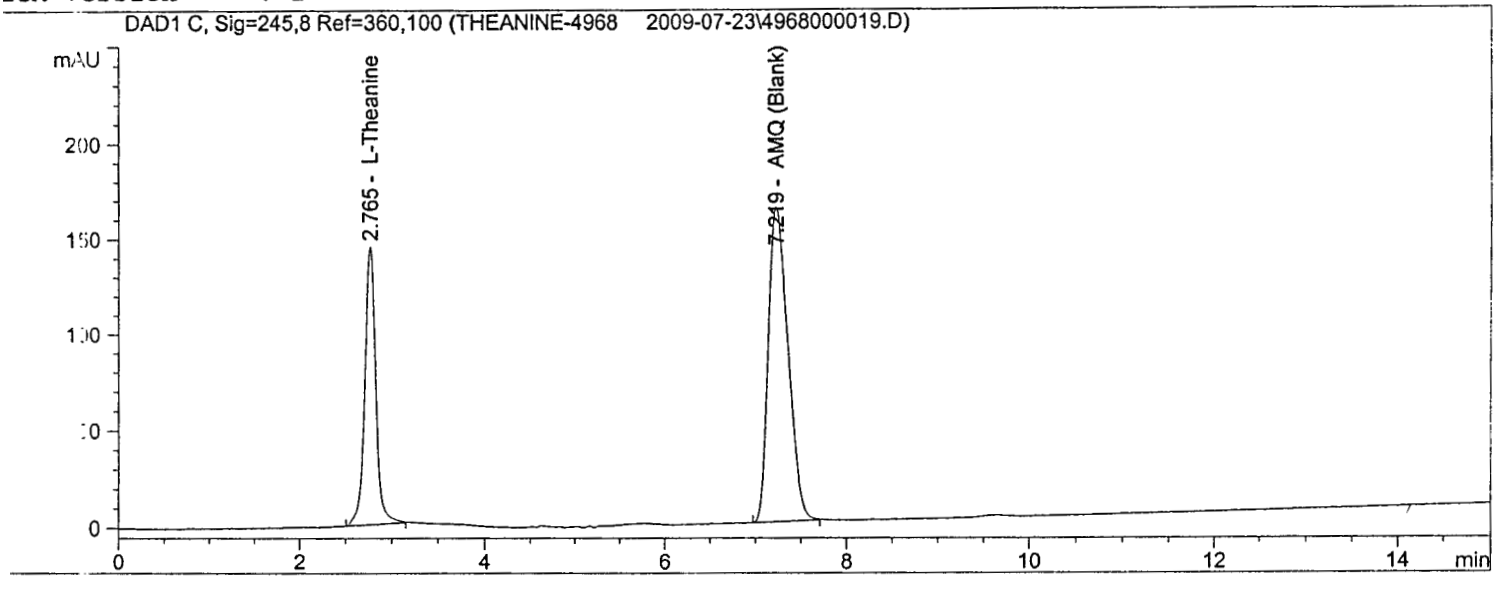
File C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\4968000019.D
le Name: STD 2

==========

Acq. Operator : (b) (6) Seq. Line : 12
Acq. Instrument : Instrument 15 Location : P1-B-02
Injection Date : 7/23/2009 7:42:31 PM Inj : 2
Inj Volume : 5.0 µl

Acq. Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/23/2009 12:53:23 PM by (b) (6)
Analysis Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/24/2009 3:54:42 PM by (b) (6)
Method Info : Chiral Analysis of Theanine

ECM Server : http://us05sqlc/ecmwg
ECM Operator : (b) (6)
ECM Path : Petaluma\LC\HPLC-15\Data\THEANINE-4968 2009-07-23.SC.SSIzip
ECM Version : 1



==========

External Standard Report (Sample Amount is 0!)

==========

orted By : Signal
alib. Data Modified : 7/24/2009 3:38:30 PM
ultiplier: : 1.0000
ilution: : 1.0000
se Multiplier & Dilution Factor with ISTDs

igna 1: DAD1 C, Sig=245,8 Ref=360,100

etTime [min	Type	Area [mAU*s]	Amt/Area	Amount [ug/mL]	Grp	Name
2.765	BB	1166.99561	8.06078e-4	9.40690e-1		L-Theanine
3.013		-	-	-		L-Glutamine
3.643		-	-	-		D-Glu/Thea
7.219	BB	2420.27319	0.00000	0.00000		AMQ (Blank)

otals : 9.40690e-1

Warnings or Errors :

000125

ument 15 7/24/2009 3:55:51 PM (b) (6)

ple Name: STD 2

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found
Warning : Invalid calibration curve, (AMQ (Blank))

==========

*** End of Report ***

000126

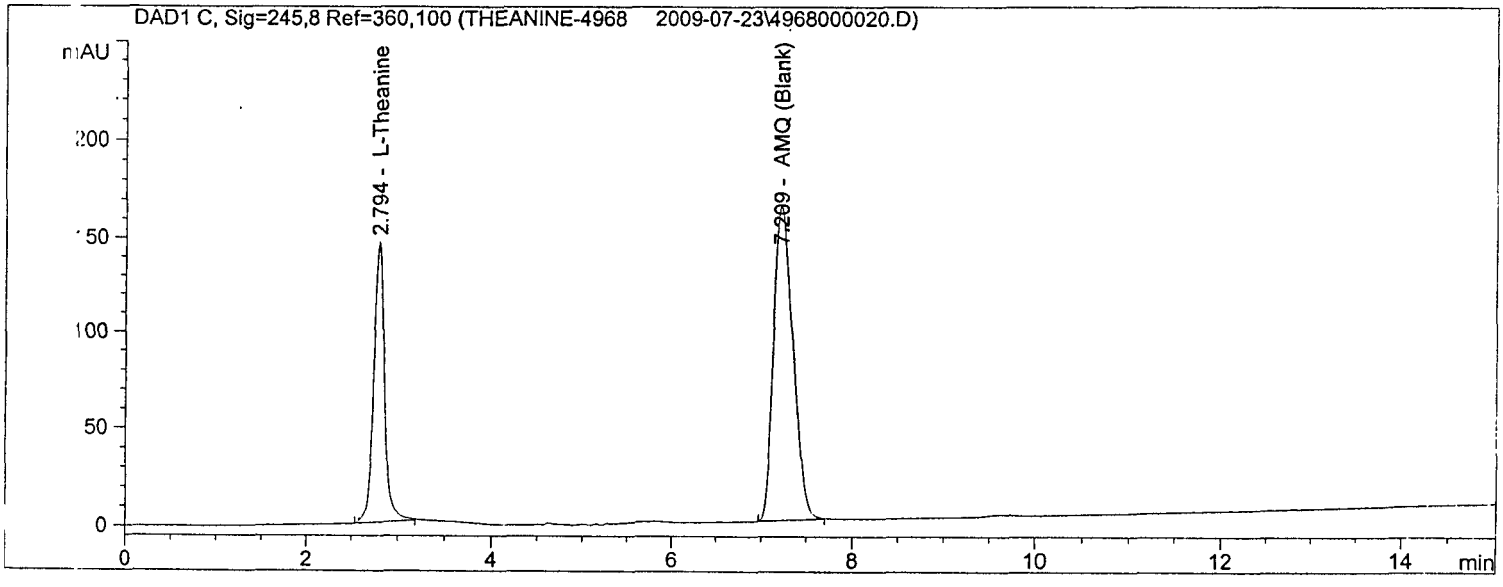
a File C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\4968000020.D
ple Name: STD 2

==========

Acq. Operator : (b) (6) Seq. Line : 12
Acq. Instrument : Instrument 15 Location : P1-B-02
Injection Date : 7/23/2009 8:05:13 PM Inj : 3
Inj Volume : 5.0 µl

Acq. Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/23/2009 12:53:23 PM by (b) (6)
Analysis Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/24/2009 3:54:42 PM by (b) (6)
Method Info : Chiral Analysis of Theanine

ECM Server : http://us05sqlc/ecmwg
ECM Operator : (b) (6)
ECM Path : Petaluma\LC\HPLC-15\Data\THEANINE-4968 2009-07-23.SC.SSIzip
ECM Version : 1



==========

External Standard Report (Sample Amount is 0!)

==========

Sorted By : Signal
Calib. Data Modified : 7/24/2009 3:38:30 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=245,8 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ug/mL]	Grp	Name
2.794	BB	1164.23022	8.06119e-4	9.38508e-1		L-Theanine
3.013		-	-	-		L-Glutamine
3.643		-	-	-		D-Glu/Thea
7.209	BB	2420.85767	0.00000	0.00000		AMQ (Blank)

Totals : 9.38508e-1

0 Warnings or Errors :

000127

le Name: STD 2

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found
Warning : Invalid calibration curve, (AMQ (Blank))

==========

*** End of Report ***

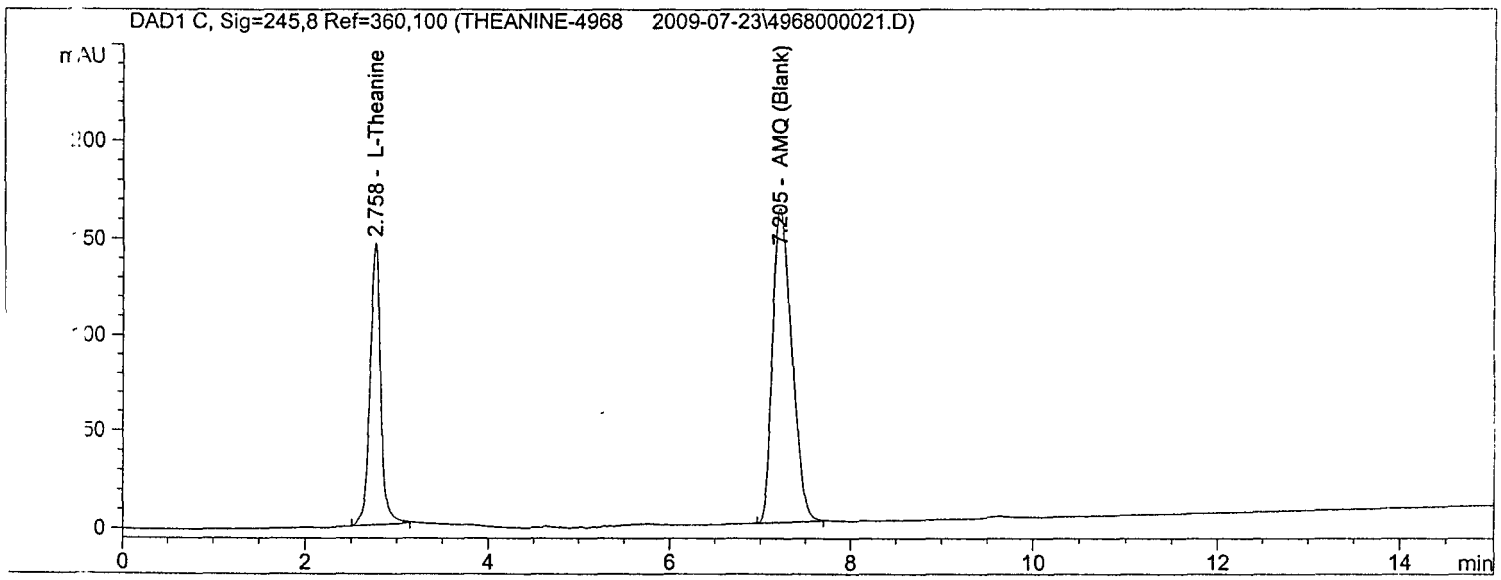
000128

C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\4968000021.D
ple Name: STD 2

==========

Acq. Operator : (b) (6) Seq. Line : 12
Acq. Instrument : Instrument 15 Location : P1-B-02
Injection Date : 7/23/2009 8:27:56 PM Inj : 4
Inj Volume : 5.0 µl
Acq. Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/23/2009 12:53:23 PM by (b) (6)
Analysis Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/24/2009 3:54:42 PM by (b) (6)
Method Info : Chiral Analysis of Theanine

ECM Server : http://us05sqlc/ecmwg
ECM Operator : (b) (6)
ECM Path : Petaluma\LC\HPLC-15\Data\THEANINE-4968 2009-07-23.SC.SSIzip
ECM Version : 1



==========

External Standard Report (Sample Amount is 0!)

==========

Sorted By : Signal
Calib. Data Modified : 7/24/2009 3:38:30 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=245,8 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ug/mL]	Grp	Name
2.758	BB	1165.71814	8.06097e-4	9.39682e-1		L-Theanine
3.013		-	-	-		L-Glutamine
3.643		-	-	-		D-Glu/Thea
7.205	BB	2423.98755	0.00000	0.00000		AMQ (Blank)

Total : 9.39682e-1

Warnings or Errors :

000129

umen: 15 7/24/2009 3:56:01 PM (b) (6)

ple Name: STD 2

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found
Warning : Invalid calibration curve, (AMQ (Blank))

==========

*** End of Report ***

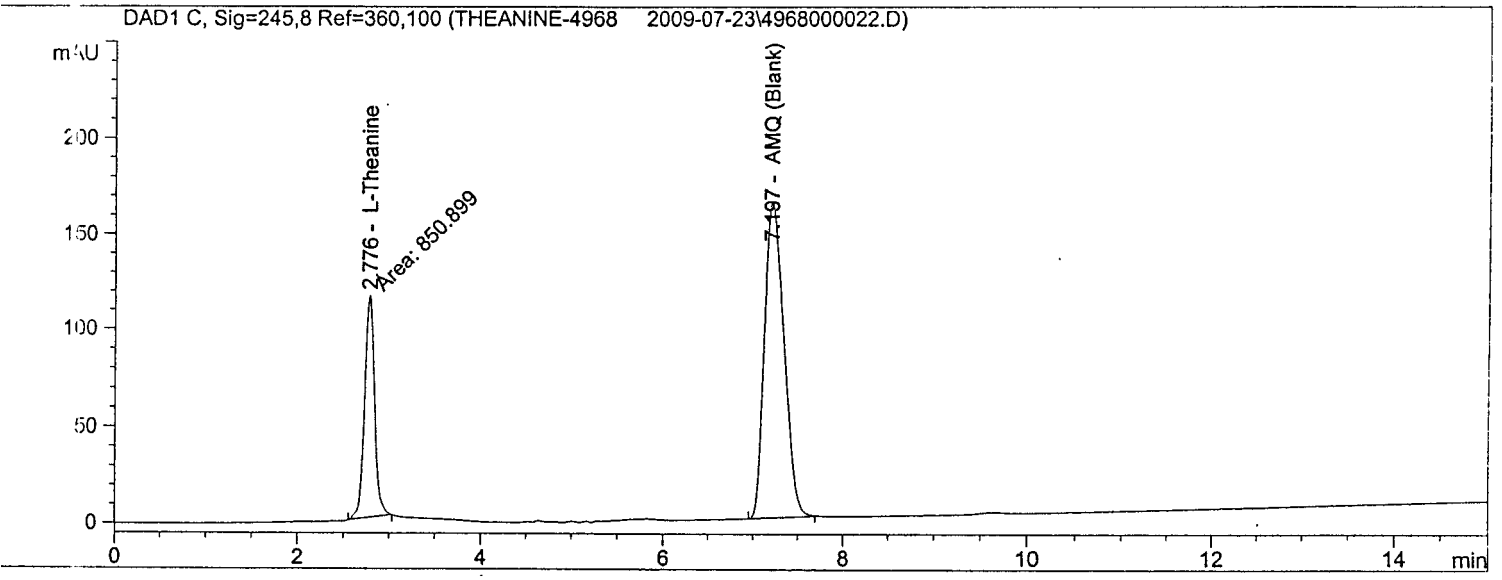
000130

le Name: STD 3

==========

Acq. Operator : (b) (6) Seq. Line : 13
Acq. Instrument : Instrument 15 Location : P1-B-03
Injection Date : 7/23/2009 8:50:38 PM Inj : 1
Inj Volume : 5.0 µl
Acq. Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/23/2009 12:53:23 PM by (b) (6)
Analysis Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/24/2009 3:54:42 PM by (b) (6)
Method Info : Chiral Analysis of Theanine

ECM Server : http://us05sqlc/ecmwg
ECM Operator : (b) (6)
ECM Path : Petaluma\LC\HPLC-15\Data\THEANINE-4968 2009-07-23.SC.SSIzip
ECM Version : 1



==========

External Standard Report (Sample Amount is 0!)

==========

Sorted By : Signal
Calib. Data Modified : 7/24/2009 3:38:30 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=245,8 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ug/mL]	Grp	Name
2.776	MM	850.89923	8.12382e-4	6.91256e-1		L-Theanine
3.013		-	-	-		L-Glutamine
3.643		-	-	-		D-Glu/Thea
7.197	BB	2465.90942	0.00000	0.00000		AMQ (Blank)
Totals :				6.91256e-1		

Warnings or Errors :

000131

ple Name: STD 3

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found
Warning : Invalid calibration curve, (AMQ (Blank))

==========

*** End of Report ***

000132

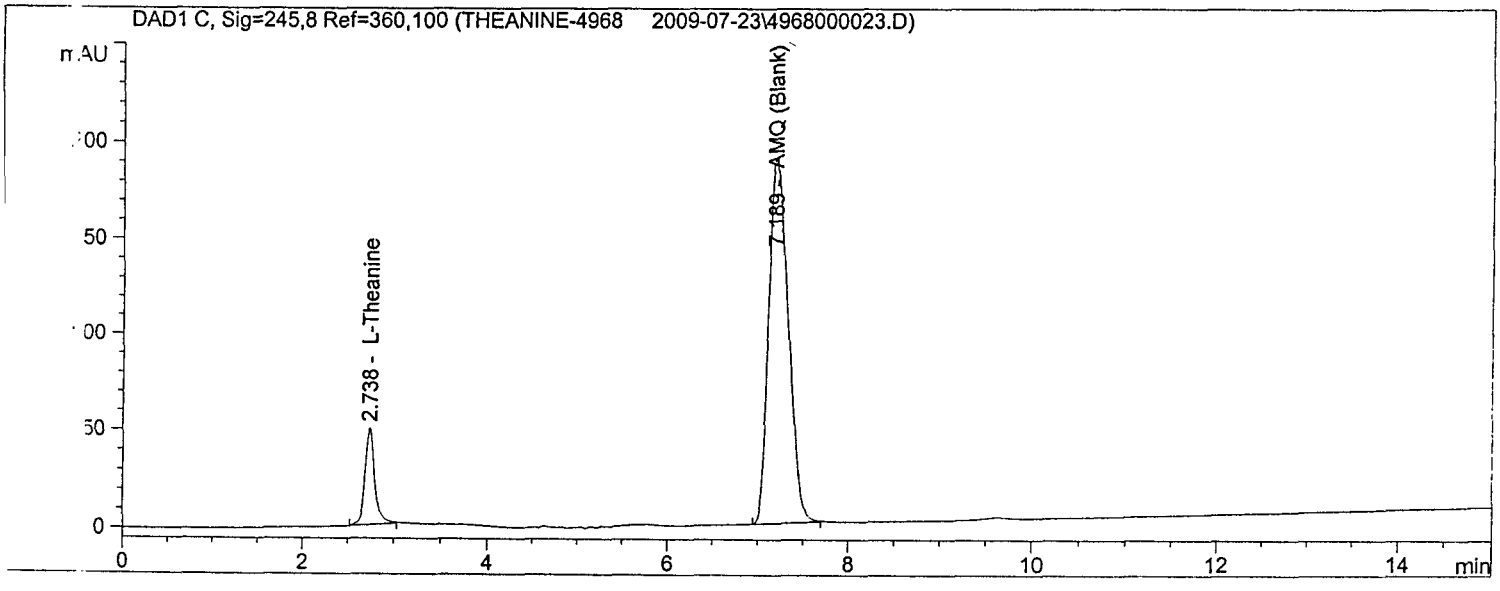
ple Name: STD 4

==========

Acq. Operator : (b) (6) Seq. Line : 14
Acq. Instrument : Instrument 15 Location : P1-B-04
Injection Date : 7/23/2009 9:13:21 PM Inj : 1
Inj Volume : 5.0 µl

Acq. Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/23/2009 12:53:23 PM by (b) (6)
Analysis Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/24/2009 3:54:42 PM by (b) (6)
Method Info : Chiral Analysis of Theanine

ECM Server : http://us05sqlc/ecmwg
ECM Operator : (b) (6)
ECM Path : Petaluma\LC\HPLC-15\Data\THEANINE-4968 2009-07-23.SC.SSIzip
ECM Version : 1



==========

External Standard Report (Sample Amount is 0!)

==========

Sorted By : Signal
Calib. Data Modified : 7/24/2009 3:38:30 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=245,8 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ug/mL]	Grp	Name
2.738	BB	361.60098	8.43875e-4	3.05146e-1		L-Theanine
3.013		-	-	-		L-Glutamine
3.613		-	-	-		D-Glu/Thea
7.189	BB	2824.62231	0.00000	0.00000		AMQ (Blank)

Total : 3.05146e-1

Warnings or Errors :

000133

ple Name: STD 4

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found
Warning : Invalid calibration curve, (AMQ (Blank))

==========

*** End of Report ***

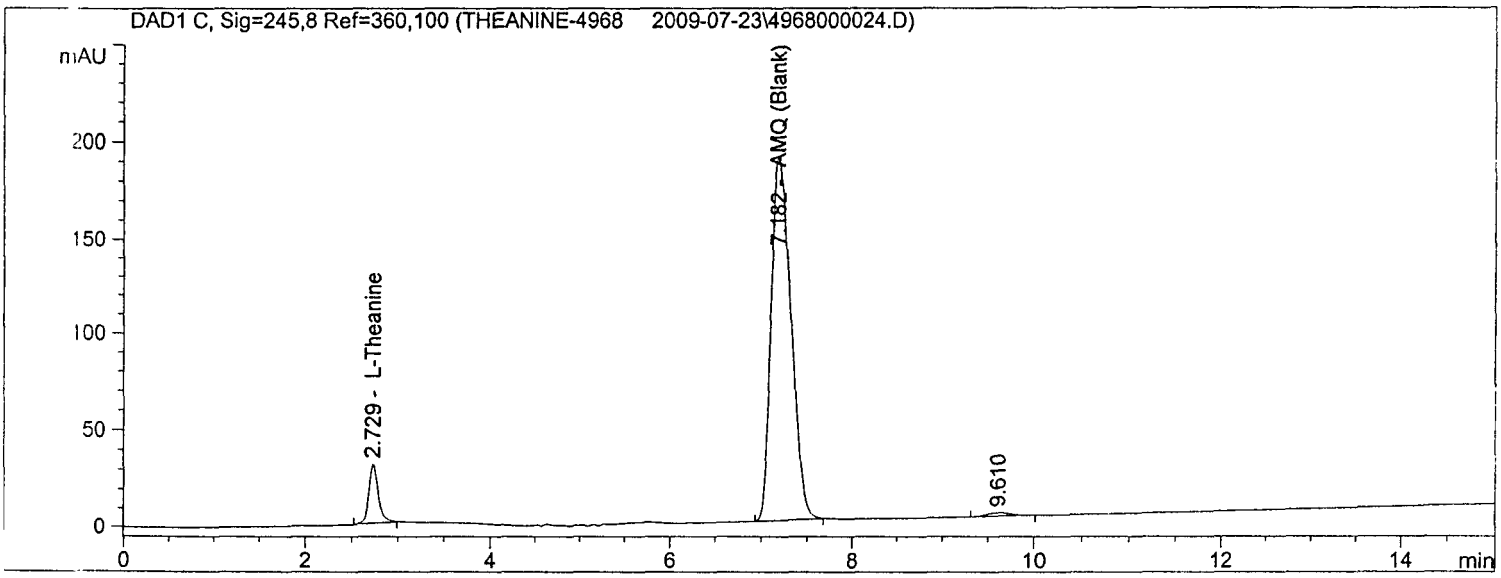
000134

File C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\4968000024.D
Sample Name: STD 5

==========

Acq. Operator : (b) (6) Seq. Line : 15
Acq. Instrument : Instrument 15 Location : P1-B-05
Injection Date : 7/23/2009 9:36:04 PM Inj : 1
Inj Volume : 5.0 µl
Acq. Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/23/2009 12:53:23 PM by (b) (6)
Analysis Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/24/2009 3:54:42 PM by (b) (6)
Method Info : Chiral Analysis of Theanine

ECM Server : http://us05sqlc/ecmwg
ECM Operator : (b) (6) a
ECM Path : Petaluma\LC\HPLC-15\Data\THEANINE-4968 2009-07-23.SC.SSIzip
ECM Version : 1



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External Standard Report (Sample Amount is 0!)

==========

Sorted By : Signal
Calib. Data Modified : 7/24/2009 3:38:30 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=245,8 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ug/mL]	Grp	Name
2.729	BB	222.10408	8.78272e-4	1.95068e-1		L-Theanine
3.013		-	-	-		L-Glutamine
3.643		-	-	-		D-Glu/Thea
7.182	BB	2840.55811	0.00000	0.00000		AMQ (Blank)

Totals : 1.95068e-1

0 Warnings or Errors :

000135

Instrument 15 7/24/2009 3:56:17 PM (b) (6)

le Name: STD 5

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found
Warning : Invalid calibration curve, (AMQ (Blank))

==========

*** End of Report ***

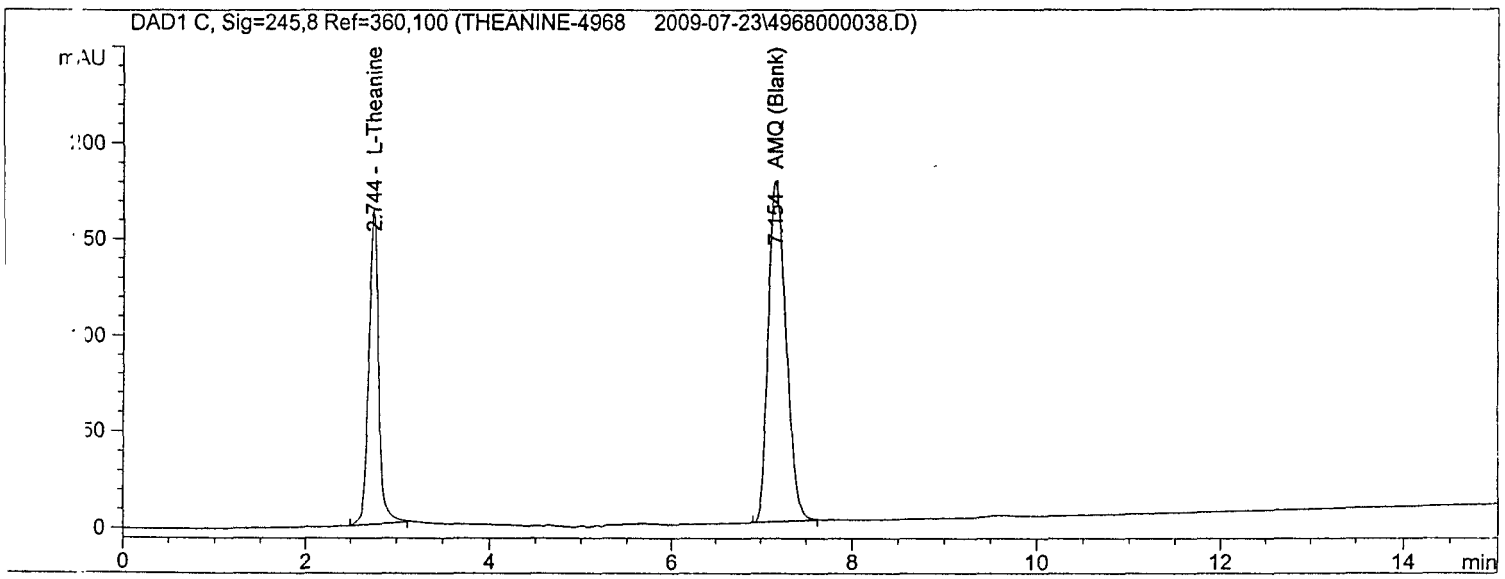
000136

ple Name: STD 2

==========

Acq. Operator : (b) (6) Seq. Line : 27
Acq. Instrument : Instrument 15 Location : P1-B-06
Injection Date : 7/24/2009 12:12:40 PM Inj : 1
Inj Volume : 5.0 µl
Acq. Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/24/2009 10:18:08 AM by (b) (6)
Analysis Method : C:\CHEM32\15\DATA\THEANINE-4968 2009-07-23\THEANINE.M
Last changed : 7/24/2009 3:54:42 PM by (b) (6)
Method Info : Chiral Analysis of Theanine

ECM Server : http://us05sqlc/ecmwg
ECM Operator : (b) (6)
ECM Path : Petaluma\LC\HPLC-15\Data\THEANINE-4968 2009-07-23.SC.SSIzip
ECM Version : 1



==========

External Standard Report (Sample Amount is 0!)

==========

Sorted By : Signal
Calib. Data Modified : 7/24/2009 3:38:30 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=245,8 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ug/mL]	Grp	Name
2.744	BB	1203.59180	8.05562e-4	9.69568e-1		L-Theanine
3.013		-	-	-		L-Glutamine
3.613		-	-	-		D-Glu/Thea
7.154	BB	2409.73413	0.00000	0.00000		AMQ (Blank)

Totals : 9.69568e-1

Warnings or Errors :

000137

ple Name: STD 2

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found
Warning : Invalid calibration curve, (AMQ (Blank))

==========

*** End of Report ***

000138

METHOD / SEQ

Sequence Table:

Sample Information Part:

Line	Location	Sample Information
1	P1-A-01	
2	P1-A-02	
3	P1-A-01	
4	P1-A-03	
5	P1-A-04	
6	P1-A-05	
7	P1-A-02	
8	P1-A-06	
9	P1-A-06	
10	P1-B-01	
11	P1-B-02	
12	P1-B-02	
13	P1-B-03	
14	P1-B-04	
15	P1-B-05	
16	P1-B-05	
17	P1-B-07	
18	P1-B-08	
19	P1-C-01	
20	P1-C-02	
21	P1-C-03	
22	P1-C-04	
23	P1-C-05	
24	P1-C-06	
25	P1-C-07	
26	P1-C-08	

000140

27 P1-B-06

28 P1-C-09

Method and Injection Info Part:

Line	Location	SampleName DataFile	Method AutoBalance	Inj LimsID	SampleType	InjVolume
1	P1-A-01	Mobile Phase A	THEANINE	1	Sample	
2	P1-A-02	MP B (Methanol)	THEANINE	1	Sample	
3	P1-A-01	Mobile Phase A	THEANINE	1	Sample	
4	P1-A-03	Blank	THEANINE	1	Sample	
5	P1-A-04	L-Glutamine	THEANINE	3	Sample	
6	P1-A-05	Resolution Soln	THEANINE	1	Sample	
7	P1-A-02	MP B	THEANINE	1	Sample	
8	P1-A-06	D-Glu 1:11 (LOQ)	THEANINE	3	Sample	
9	P1-A-06	D-Glu 1:11 (LOQ)	THEANINE	3	Sample	3
10	P1-B-01	STD 1	THEANINE	1	Calib	
11	P1-B-02	STD 2	THEANINE	1	Calib	
12	P1-B-02	STD 2	THEANINE	4	Calib	
13	P1-B-03	STD 3	THEANINE	1	Calib	
14	P1-B-04	STD 4	THEANINE	1	Calib	
15	P1-B-05	STD 5	THEANINE	1	Calib	
16	P1-B-05	STD 5 (LOQ)	THEANINE	3	Sample	3
17	P1-B-07	4968	THEANINE	1	Sample	
18	P1-B-08	4968D	THEANINE	1	Sample	
19	P1-C-01	4969	THEANINE	1	Sample	
20	P1-C-02	4969D	THEANINE	1	Sample	
21	P1-C-03	4970	THEANINE	1	Sample	
22	P1-C-04	4970D	THEANINE	1	Sample	
23	P1-C-05	4971	THEANINE	1	Sample	
24	P1-C-06	4971D	THEANINE	1	Sample	

000141

Line	Location	SampleName DataFile	Method AutoBalance	Inj LimsID	SampleType	InjVolume
25	P1-C-07	4972	THEANINE	1	Sample	
26	P1-C-08	4972D	THEANINE	1	Sample	
27	P1-B-06	STD 2	THEANINE	1	Sample	
28	P1-C-09	Glu-Theanine 1:1	THEANINE	1	Sample	

Calibration Part:

Line	Location	SampleName	Method	CalLev	Update RF	Update RT	Interval
10	P1-B-01	STD 1	THEANINE	1	Replace	Average	
11	P1-B-02	STD 2	THEANINE	2	Replace	Average	
12	P1-B-02	STD 2	THEANINE	2	Average	Average	
13	P1-B-03	STD 3	THEANINE	3	Replace	Average	
14	P1-B-04	STD 4	THEANINE	4	Replace	Average	
15	P1-B-05	STD 5	THEANINE	5	Replace	Average	

000142

Agilent 1100/1200 Binary Pump 1

Control

Column Flow	:	0.700 ml/min
Stoptime	:	15.00 min
Posttime	:	8.00 min

Solvents

Solvent A	:	0.0 % (1.0% NH4TFA in MeOH)
Solvent B	:	100.0 % (MeOH)

PressureLimits

Minimum Pressure	:	0 bar
Maximum Pressure	:	400 bar

Auxiliary

Maximal Flow Ramp	:	100.00 ml/min^2
Compressibility A	:	50*10^-6/bar
Minimal Stroke A	:	Auto
Compressibility B	:	115*10^-6/bar
Minimal Stroke B	:	Auto

Store Parameters

Store Ratio A	:	Yes
Store Ratio B	:	Yes
Store Flow	:	Yes
Store Pressure	:	Yes

Timetable

Time	Solv.B	Flow	Pressure
0.00	100.0	0.700	
15.00	65.0	0.700	

Agilent 1200 Diode Array Detector 1

Signals

Signal	Store	Signal,Bw		Reference,Bw		[nm]
A:	No	254	4	360	100	
B:	No	250	8	360	100	
C:	Yes	245	8	360	100	
D:	No	230	16	360	100	
E:	No	280	16	360	100	
F:	No	250	16	360	100	
G:	No	280	16	360	100	
H:	No	280	16	360	100	
K:	No	Board Temperature				
L:	No	Optical Unit Temperature				
M:	No	UV Lamp Anode Voltage				

Spectrum

000143

Store Spectra	:	Every 2nd spectrum
Range from	:	190 nm
Range to	:	400 nm
Range step	:	2.00 nm
Threshold	:	1.00 mAU

Time

Stoptime	:	As pump
Posttime	:	Off

Required Lamps

UV lamp required	:	Yes
Vis lamp required	:	No

Autobalance

Prerun balancing	:	Yes
Postrun balancing	:	No

Margin for negative Absorbance: 100 mAU

Peakwidth	:	> 0.1 min
Slit	:	4 nm

Analog Outputs

Zero offset ana. out. 1:	5 %
Zero offset ana. out. 2:	5 %
Attenuation ana. out. 1:	1000 mAU
Attenuation ana. out. 2:	1000 mAU

Timetable is empty

==========

Agilent 1200 High Performance Autosampler 1

==========

Injection

Injection Volume	:	5.0 µl
Injection Mode	:	Needle Wash

Time

Stoptime	:	As Pump
Posttime	:	Off

High Throughput

Autom. Delay Vol. Reduction	:	Off
Overlapped Injection	:	disabled
Minimized Carry Over	:	Off

Needle Wash

Wash Mode	:	Wash in Location

Injector Cleaning

Injection Valve Cleaning / Valve Switching

Time 1 Bypass	:	off
Time 2 Mainpass/Bypass	:	off
Time 3 Mainpass/Bypass	:	off
Time 4 Mainpass/Bypass	:	off
Valve Movements	:	1

000144

Auxiliary

Draw Speed	:	100 µl/min
Eject Speed	:	100 µl/min
Draw Position	:	0.0 mm
Equilibration Time	:	2.0 sec
Sample Flush-Out Factor	:	5.0 times Flush-Out Volume
Vial/Well Bottom Sensing	:	No

==== ==========

Agilent 1100/1200 Column Thermostat 1

==== ==========

Temperature settings

Left temperature	:	30.0°C
Right temperature	:	Same as left
Enable analysis	:	When Temp. is within setpoint +/- 1.0°C
Store left temperature	:	Yes
Store right temperature	:	No

Time

Stoptime	:	As pump
Posttime	:	Off

Column Switching Valve : Column 2

Timetable is empty

000145

APPENDIX D

Blue California Certificates of Analysis for Five Production Lots of L-Theanine (98%)

D-1 Production Lot 20080928

D-2 Production Lot 20090225

D-3 Production Lot 20080109

D-4 Production Lot 20081105

D-5 Production Lot 20090428

APPENDIX D-1

Blue California®

30111 Tomas
Rancho Santa Margarita, CA 92688
Tel: 949.635.1990
Fax: 949 635.1988

CERTIFICATE OF ANALYSIS

Product: L-Theanine 98% (*camellia sinensis, leaves*)

Lot No:	**LT20080928**	**Original Manufacturer: Blue California**	
Date of Manufacture:	**09-28-08**	**Extraction Solvent: N/A**	
Expiration/re-test date:	**09-28-11**	**Plant Extract Ratio:**	**N/A**
QC acceptance date:	**11-25-08**	**Excipients/Carriers:**	**N/A**
Food Grade		**Standard Packaging: 25 kg**	
Country of Origin: China			

ATTRIBUTES	SPECIFICATION	METHODS	RESULTS
APPEARANCE	White crystalline powder	VISUAL	PASS
FOREIGN MATTER	Absent	VISUAL	PASS
ODOR	No odor	OLFACTORY	PASS
TASTE	Slightly sweet	GUSTATORY	PASS
L-THEANINE	**> 98%**	**HPLC**	**99.3%**
ARSENIC	< 2 ppm	ICP	PASS
LEAD	< 1 ppm	ICP	PASS
LOSS ON DRYING	≤ 1%	USP 29	0.93%
RESIDUE ON IGNITION	≤ 0.2%	USP 29	PASS
PH	5 - 7	USP 29	5.80
CHLORIDE	≤ 0.02%	USP 29	PASS
HEAVY METALS	< 10 ppm	USP 29	PASS
SPECIFIC ROTATION	+7.2 - 8.5 deg.	USP 29	+8.1 deg
BULK DENSITY	0.2 - 0.6 g/ml	USP 29	PASS
TAP DENSITY	≥ 0.6 g/ml	USP 29	PASS
PARTICLE SIZE	≥ 95% through Mesh #40 Sieve	USP 29	PASS
TOTAL PLATE COUNT	< 3,000 cfu/gm	AOAC	< 1,000 cfu/gm
TOTAL COLIFORM	< 100 cfu/gm	AOAC	PASS
YEAST AND MOLDS	< 100 cfu/gm	AOAC	PASS
E. COLI:	NEGATIVE	AOAC	PASS
SALMONELLA	NEGATIVE	AOAC	PASS
SHELF LIFE	3 YEARS	AOAC	PASS

Approved by: Dr.Z.B.Yu — *12-01-2008*

QA/QC MANAGER — ISSUE DATE

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* THIS PRODUCT SHOULD BE STORED SEALED IN A COOL AND DRY PLACE.

APPENDIX D-2

Blue California®

30111 Tomas
Rancho Santa Margarita, CA 92688
Tel 949.635.1990
Fax 949.635.1988

CERTIFICATE OF ANALYSIS

Product: L-Theanine 98% *(camellia sinensis, leaves)*

Lot No:	**LT20090225**	**Original Manufacturer: Blue California**	
Date of Manufacture:	**02-25-09**	**Extraction Solvent: N/A**	
Expiration/re-test date:	**02-25-12**	**Plant Extract Ratio:**	**N/A**
QC acceptance date:	**03-21-09**	**Excipients/Carriers:**	**N/A**
Food Grade		**Standard Packaging: 25 kg**	
Country of Origin: China			

ATTRIBUTES	SPECIFICATION	METHODS	RESULTS
APPEARANCE	White crystalline powder	VISUAL	PASS
FOREIGN MATTER	Absent	VISUAL	PASS
ODOR	No odor	OLFACTORY	PASS
TASTE	Slightly sweet	GUSTATORY	PASS
L-THEANINE	**> 98%**	**HPLC**	**99.98%**
ARSENIC	< 2 ppm	ICP	PASS
LEAD	< 1 ppm	ICP	PASS
LOSS ON DRYING	≤ 1%	USP 29	0.96%
RESIDUE ON IGNITION	≤ 0.2%	USP 29	PASS
PH	5 - 7	USP 29	5.7
CHLORIDE	≤ 0.02%	USP 29	PASS
HEAVY METALS	< 10 ppm	USP 29	PASS
SPECIFIC ROTATION	+7.2 - 8.5 deg.	USP 29	+8.2 deg
BULK DENSITY	0.2 - 0.6 g/ml	USP 29	PASS
TAP DENSITY	≥ 0.6 g/ml	USP 29	PASS
PARTICLE SIZE	≥ 95% through Mesh #40 Sieve	USP 29	PASS
TOTAL PLATE COUNT	< 3,000 cfu/gm	AOAC	< 1,000 cfu/gm
TOTAL COLIFORM	< 100 cfu/gm	AOAC	PASS
YEAST AND MOLDS	< 100 cfu/gm	AOAC	PASS
E. COLI:	NEGATIVE	AOAC	PASS
SALMONELLA	NEGATIVE	AOAC	PASS
SHELF LIFE	3 YEARS	AOAC	PASS

Approved by: Dr. Z.B. Yu 03-28-2009

QA/QC MANAGER ISSUE DATE

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- THIS PRODUCT SHOULD BE STORED SEALED IN A COOL AND DRY PLACE.

APPENDIX D-3

Blue California®

30111 Tomas
Rancho Santa Margarita, CA 92688
Tel: 949.635.1990
Fax: 949.635.1988

CERTIFICATE OF ANALYSIS

Product: L-Theanine 98% (*camellia sinensis, leaves*)

Lot No:	**LT20090109**	**Original Manufacturer:**	**Blue California**
Date of Manufacture:	**01-09-09**	**Extraction Solvent: N/A**	
Expiration/re-test date:	**01-09-12**	**Plant Extract Ratio: N/A**	
QC acceptance date:	**02-15-09**	**Excipients/Carriers: N/A**	
Food Grade		**Standard Packaging: 25 kg**	
Country of Origin: China			

ATTRIBUTES	SPECIFICATION	METHODS	RESULTS
APPEARANCE	White crystalline powder	VISUAL	PASS
FOREIGN MATTER	Absent	VISUAL	PASS
ODOR	No odor	OLFACTORY	PASS
TASTE	Slightly sweet	GUSTATORY	PASS
L-THEANINE	**> 98%**	**HPLC**	**98.57%**
ARSENIC	< 2 ppm	ICP	PASS
LEAD	< 1 ppm	ICP	PASS
LOSS ON DRYING	≤ 1%	USP 29	0.94%
RESIDUE ON IGNITION	≤ 0.2%	USP 29	PASS
PH	5 - 7	USP 29	5.7
CHLORIDE	≤ 0.02%	USP 29	PASS
HEAVY METALS	< 10 ppm	USP 29	PASS
SPECIFIC ROTATION	+7.2 - 8.5 deg.	USP 29	+8.1 deg
BULK DENSITY	0.2 - 0.6 g/ml	USP 29	PASS
TAP DENSITY	≥ 0.6 g/ml	USP 29	PASS
PARTICLE SIZE	≥ 95% through Mesh #40 Sieve	USP 29	PASS
TOTAL PLATE COUNT	< 3,000 cfu/gm	AOAC	< 1,000 cfu/gm
TOTAL COLIFORM	< 100 cfu/gm	AOAC	PASS
YEAST AND MOLDS	< 100 cfu/gm	AOAC	PASS
E. COLI:	NEGATIVE	AOAC	PASS
SALMONELLA	NEGATIVE	AOAC	PASS
SHELF LIFE	3 YEARS	AOAC	PASS

Approved by: Dr. Z.B.Yu 02-19-2009

QA/QC MANAGER ISSUE DATE

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- THIS PRODUCT SHOULD BE STORED SEALED IN A COOL AND DRY PLACE.

APPENDIX D-4

Blue California®

30111 Tomas
Rancho Santa Margarita, CA 92688
Tel: 949.635.1990
Fax: 949 635 1988

CERTIFICATE OF ANALYSIS

Product: L-Theanine 98% (*camellia sinensis, leaves*)

Lot No:	**LT20081105**	**Original Manufacturer:**	**Blue California**
Date of Manufacture:	**11-05-08**	**Extraction Solvent: N/A**	
Expiration/re-test date:	**11-05-11**	**Plant Extract Ratio: N/A**	
QC acceptance date:	**01-07-09**	**Excipients/Carriers: N/A**	
Food Grade		**Standard Packaging: 25 kg**	
Country of Origin: China			

ATTRIBUTES	SPECIFICATION	METHODS	RESULTS
APPEARANCE	White crystalline powder	VISUAL	PASS
FOREIGN MATTER	Absent	VISUAL	PASS
ODOR	Absent	OLFACTORY	PASS
TASTE	Slightly Sweet	GUSTATORY	PASS
L-THEANINE	**> 98%**	**HPLC**	**99.43%**
ARSENIC	< 2 ppm	ICP	PASS
LEAD	< 1 ppm	ICP	PASS
LOSS ON DRYING	≤ 1%	USP 29	0.95%
RESIDUE ON IGNITION	≤ 0.2%	USP 29	PASS
PH	5 - 7	USP 29	5.7
CHLORIDE	≤ 0.02%	USP 29	PASS
HEAVY METALS	< 10 ppm	USP 29	PASS
SPECIFIC ROTATION	+7.2 - 8.5 deg.	USP 29	+8.1 deg
BULK DENSITY	0.2 - 0.6 g/ml	USP 29	PASS
TAP DENSITY	≥ 0.6 g/ml	USP 29	PASS
PARTICLE SIZE	≥ 95% through Mesh #40 Sieve	USP 29	PASS
TOTAL PLATE COUNT	< 3,000 cfu/gm	AOAC	< 1,000 cfu/gm
TOTAL COLIFORM	< 100 cfu/gm	AOAC	PASS
YEAST AND MOLDS	< 100 cfu/gm	AOAC	PASS
E. COLI:	NEGATIVE	AOAC	PASS
SALMONELLA	NEGATIVE	AOAC	PASS
SHELF LIFE	3 YEARS	AOAC	PASS

Approved by: Dr. Z.B.Yu 01-12-2009

QA/QC MANAGER ISSUE DATE

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APPENDIX D-5

Blue California®

30111 Tomas
Rancho Santa Margarita, CA 92688
Tel 949 635.1990
Fax: 949 635.1988

CERTIFICATE OF ANALYSIS

Product: L-Theanine 98% (*camellia sinensis, leaves*)

Lot No:	**LT20090428**	**Original Manufacturer: Blue California**	
Date of Manufacture:	**04-28-09**	**Extraction Solvent: N/A**	
Expiration/re-test date:	**04-28-12**	**Plant Extract Ratio:**	**N/A**
QC acceptance date:	**07-23-09**	**Excipients/Carriers:**	**N/A**
Food Grade		**Standard Packaging: 25 kg**	
Country of Origin: China			

ATTRIBUTES	SPECIFICATION	METHODS	RESULTS
APPEARANCE	White crystalline powder	VISUAL	PASS
FOREIGN MATTER	Absent	VISUAL	PASS
ODOR	Characteristic	OLFACTORY	PASS
TASTE	Characteristic	GUSTATORY	PASS
L-THEANINE	**> 98%**	**HPLC**	**99.1%**
ARSENIC	< 2 ppm	ICP	PASS
LEAD	< 1 ppm	ICP	PASS
LOSS ON DRYING	≤ 1%	USP 29	1.0%
RESIDUE ON IGNITION	≤ 0.2%	USP 29	PASS
PH	5 - 7	USP 29	5.7
CHLORIDE	≤ 0.02%	USP 29	PASS
HEAVY METALS	< 10 ppm	USP 29	PASS
SPECIFIC ROTATION	+7.2 - 8.5 deg.	USP 29	+ 8.0 deg
BULK DENSITY	0.2 - 0.6 g/ml	USP 29	PASS
TAP DENSITY	≥ 0.6 g/ml	USP 29	PASS
PARTICLE SIZE	≥ 95% through Mesh #40 Sieve	USP 29	PASS
TOTAL PLATE COUNT	< 3,000 cfu/gm	AOAC	< 1,000 cfu/gm
TOTAL COLIFORM	< 100 cfu/gm	AOAC	PASS
YEAST AND MOLDS	< 100 cfu/gm	AOAC	PASS
E. COLI:	NEGATIVE	AOAC	PASS
SALMONELLA	NEGATIVE	AOAC	PASS
SHELF LIFE	3 YEARS	AOAC	PASS

Approved by: Dr. Z.B.Yu — *07-27-2009*

QA/QC MANAGER — ISSUE DATE

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SUBMISSION END

AM

Fus, Andrea *

From: Bob McQuate [McQuate@Gras-Associates.com]
Sent: Wednesday, August 11, 2010 3:00 PM
To: Fus, Andrea *
Cc: Fasano, Jeremiah; Merker, Robert I; 'Richard Kraska'
Subject: RE: GRN 000338 Additional Information and Clarification from Blue California
Attachments: FDA Questions Ltr 08 10 11.doc; WATER QUALITY Analyses- JULY 2010.pdf; Ethanol Specifications .doc

Dear Dr. Fus,

In response to your earlier request for additional information regarding GRN 338, we offer the attached letter and supporting information. We apologize for the delay in getting this information to you.

We trust that this will enable you and your team to proceed with your review.

Thank you.

Bob
Robert S. McQuate, Ph.D.
CEO & Co-Founder
GRAS Associates, LLC
20482 Jacklight Lane
Bend, OR 97702-3074
541-678-5522
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August 11, 2010

Dr. Andrea F. Fus
U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Division of Biotechnology and GRAS Notice Review
5100 Paint Branch Parkway
College Park, MD 20740

RE: L-Theanine (98%) GRN 338

Dear Dr. Fus:

In your email of July 1, 2010, you requested additional information and clarification regarding Blue California's manufacturing process and product purity of L-theanine (98%) as described in Section III, Part D and Part F and Figure 2 of the above-referenced GRAS notification. We are happy to relay the following information to you.

Blue California reports that only deionized water and high purity ethanol are used in the extraction and purification of the subject L-theanine from tea leaves. Attached you will find the documentation regarding the purity/quality of these solvents. The extract is concentrated and then undergoes several filtration and purification steps using membrane technology that sorts by molecular size. The filters and membranes used meet FDA requirements listed in 21 CFR 173.20.

We trust that we have responded fully to your request for additional information.

If there are remaining questions, please contact me at your earliest convenience.

Sincerely,

Robert S. McQuate, Ph.D.
CEO & Co-Founder
GRAS Associates, LLC.

Attachments: Water Quality Specifications and Analyses
Ethanol Specifications

C: Blue California

Blue California

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PRODUCT SPECIFICATIONS

QUALITY STANDARD OF ETHANOL

Appearance	Transparent liquid
Odor	Slight characteristic odor
Taste	Slight characteristic taste
Ethanol/% (volume fraction)	95.0
Sulphuric acid test/roar	≤10
Oxidation test	≥30
Aldehyde (cetaldehyde) g/100ml	0.0003
Methanol g/100 ml	0.01
Normal propyl alcohol/mg/L	≤35
Isobutyl alcohol isoamylol/mg/L	≤2
Acid (acetic to total acid)	≤10
Ester (ethyl acetate)/mg/L	≤18
Non-volatile/mg/L	≤20
Heavy metal mg/l	≤1
Prussiate(HCN)/mg/L	≤1

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Corporate Headquarters
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Rancho Santa Margarita, CA 92688

Tel: 949-635-1991
Fax: 949-635-1988

Blue California
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WATER QUALITY PUBLICATION FROM OCT. TO DEC. IN 2009
Manufacturing Facility: Jiangsu, China

Items	Unit	Specification (GB5749-2006)	Test Result
Arsenic	mg/L	0.01	0.00025
Cadmium	mg/L	0.005	0.0001
Chromium(VI)	mg/L	0.05	0.002
Lead	mg/L	0.01	0.0025
Hg	mg/L	0.001	0.00003
Selenium	mg/L	0.01	0.00025
Cyanide	mg/L	0.05	0.001
Fluoride	mg/L	1.0	0.428
Nitrate(N total)	mg/L	10	1.810
Chloroform	mg/L	0.06	0.005
Carbon tetrachloride	mg/L	0.002	0.003
Chroma	Pt-Co	15	2.5
Odor & taste		Characteristic	none
Visible objects		none	none
PH		6.5-8.5	7.589
Aluminum	mg/L	0.2	0.004
Fe	mg/L	0.3	0.025
Manganese	mg/L	0.1	0.025
Cu	mg/L	1.0	0.003
Zinc	mg/L	1.0	0.01
Chloride	mg/L	250	25.86
Sulfate	mg/L	250	39.27
Total dissolved solid	mg/L	1000	224.2
Total hardness(as $CaCO_3$)	mg/L	450	129.3
Oxygen consumption(as O_2)	mg/L	3	1.15
Volatile Phenols (as phenol)	mg/L	0.002	0.001
Anion synthetic detergent	mg/L	0.3	0.025
Grossαradioactivity	Bq/L	0.5	0.028
Gross βradioactivity	Bq/L	1	0.082
Pipeline turbidity	NTU	1	0.298
Concentration of residual chlorine	mg/L	≥0.05	0.20
Pipeline total plate count	CFU/mL	100	0.52
Pipeline total coliform	MPN/100mL	Negative	0
Passing rate of Water Quality	%		100

Note: GB5749-2006 is Sanitary Standard for drinking water issued by Health Ministry of P.R.C.

Corporate Headquarters
30111 Tomas, Rancho Santa Margarita, CA 92688 **Tel:** 949-635-1990 **Fax:** 949-635-1984 **Website:** www.bluecal-ingredients.com