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GRAS ASSOCIATES, LLC
20482 Jacklight Lane
Bend, OR 97702-3074
541-678-5522
mcquate@gras-associates.com

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Division of
Biotechnology and
GRAS Notice Review

August 24, 2010

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 – High Purity Rebaudioside A (≥ 97%)

Dear Dr. Martin:

On behalf of Guilin Layn Natural Ingredients Corp. of Guilin, China, we are submitting for FDA review a GRAS notification for High Purity Rebaudioside A (≥ 97%). 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
GRAS Associates, LLC
20482 Jacklight Lane
Bend, OR 97702-3074
541-678-5522
mcquate@gras-associates.com
www.gras-associates.com

Enclosure: GRAS Notification – High Purity Rebaudioside A (≥ 97%) (in triplicate)

GRAS ASSOCIATES, LLC

GRAS ASSESSMENT

REBAUDIOSIDE A (≥ 97%)

Food Usage Conditions for General Recognition of Safety

For

Guilin Layn Natural Ingredients Corp.

Guilin, China

Evaluation by

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

August 16, 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]

Rebaudioside A, having purity ≥ 97% and meeting the specifications for Guilin Layn Natural Ingredients Corp. 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, made by experts qualified by scientific training and experience, is based on scientific procedures as described in the following sections. The evaluation accurately reflects the conditions of the stevia-derived sweeteners' intended uses in foods.

Signed:

(b) (6)

August 24, 2010

Date

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

B. Name & Address of Notifier

Guilin Layn Natural Ingredients Corp.
#18 Xiangjiang Rd. Xing'an County, Guilin 541300, China
Xicheng Rd. Lingui County, Guilin 541100, China

As the notifier, Guilin Layn Natural Ingredients Corp. accepts responsibility for the GRAS determination that has been made for its purified rebaudioside A product[2] as described in the subject notification; consequently, these rebaudioside A preparations, i.e., having purities of no less than 97% rebaudioside A, meeting the conditions described herein are exempt from pre-market approval requirements for food ingredients.

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

2 Guilin Layn Natural Ingredients Corp refers to its high purity Rebaudioside A product from leaves of *Stevia rebaudiana* Bertoni with the tradename of Govia™.

C. Common Name & Identity of Notified Substance

Rebaudioside A, commonly shortened to reb A or Reb A, is the common name for the notified substance; also see Section III.A.

D. Conditions of Intended Uses in Food

The subject high purity rebaudioside A preparations are intended to be used as a table top sweetener and as a general purpose non-nutritive sweetener for incorporation into foods in general, other than in infant formulas and meat and poultry products, at per serving levels that reflect good manufacturing practices principles in that the quantity added to foods should not exceed the amount reasonably required to accomplish its intended technical effect.

E. Basis for the GRAS Determination

Pursuant to 21 CFR § 170.30, Guilin Layn Natural Ingredients Corp's standardized rebaudioside A preparation from the leaves of *Stevia rebaudiana* Bertoni 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 Guilin Layn Natural Ingredients Corp. ("Layn"), GRAS Associates, LLC ("GA") has undertaken an independent safety evaluation of Layn's rebaudioside A which is extracted from the leaves of *Stevia rebaudiana* Bertoni and is subsequently purified to yield rebaudioside A with a purity of ≥ 97%. The purpose of the evaluation is to ascertain whether or not the intended food uses of the subject rebaudioside A as a non-nutritive general purpose sweetener are generally recognized as safe, i.e., GRAS, when incorporated into various food categories other than infant formulas and meat and poultry products.

B. Foreword

Layn provided GA with background information needed to enable the GRAS assessment to be undertaken. In particular, the information that was provided addressed the safety/toxicity of steviol glycosides; the history of use of stevia in food; and compositional details, specifications, and method of preparation of its purified rebaudioside A. Layn was asked to provide adverse reports, as well as those that supported conclusions of safety.

Safety/toxicity studies performed with animals were noted to have value, along with available human testing. Layn was also asked to supply past and present human food use information. Knowing how much steviol glycosides has been safely consumed, i.e., the so-called "dose" or use levels, is critical in extrapolating to safe exposures for rebaudioside A when consumed as a food ingredient. The composite safety/toxicity studies, in concert with exposure information, ultimately provide the specific scientific foundation for the GRAS determination.

Layn provided the product specifications and chemical properties and some consumption/ exposure information, along with other related documentation. This was augmented with an independent search of the scientific and regulatory literature extending through August 3, 2010. A GRAS assessment based on the composite safety information that is based on scientific procedures was undertaken. Those references that were deemed pertinent to the objective at hand are listed in Section VIII.

C. Summary of Regulatory History of Stevia

Sweeteners derived from stevia are permitted as food additive in South America and in several countries in Asia, including China, Japan, and Korea. In recent years, the subject sweeteners have received food usage approvals in Mexico, Australia, New Zealand, and Switzerland. Steviol glycosides have been used as a dietary supplement in the US since 1995 (Geuns, 2003). Based on the available information, no New Dietary Ingredient Notification for dietary supplement use of purified rebaudioside A has been made to the US FDA. Since 1989 and prior to 2008, at least two GRAS petitions seeking authorization for the addition of stevioside or steviol glycosides to

foods had been submitted to FDA. However, no authorizations had been issued by FDA in response to these filings, and subsequently these petitions were withdrawn. It appears that the previously available safety data---including purity considerations---for stevia, stevioside, or steviol glycosides were inadequate.

Based on the information available from FDA's GRAS Notice Inventory[3] website, since 2008 and as of August 5, 2010, the agency has received 12 notices on rebaudioside A or steviol glycosides. Of these notices, ten have received "No Question" letters from the FDA, while two notices are under review. In May 2008, Merisant and Cargill independently submitted GRAS notifications for rebaudioside A, highly purified forms of the steviol glycosides to FDA. On December 17, 2008, FDA issued "No Question" letters for each of these GRAS notices. Since December 2008, a series of GRAS notifications were submitted to FDA for stevia-derived sweetener products by the following companies: McNeil Nutritionals, LLC; Blue California; Sweet Green Fields, LLC; Wisdom Natural Brands; Sunwin and Wild Flavors (two notifications); Pyure Brands, LLC; and PureCircle USA, Inc. Each of these firms received a "No Question" letter from FDA.[4] In addition to the above described GRAS notifications that received "No Question" letters, currently two notifications submitted to FDA by GLG Life Tech Corp. and NOW Foods, are pending with the agency.

The Food Standards Australia New Zealand (FSANZ) has completed evaluation of an application for use of steviol glycosides in foods in 2008. FSANZ recommended the Australia and New Zealand Food Regulation Ministerial Council (Ministerial Council) to amend the Australia New Zealand Food Standards Code to allow the use of steviol glycosides in food (FSANZ, 2008).

The Joint Expert Committee on Food Additives (JECFA) has reviewed steviol glycosides at its 51st, 63rd, 68th and 73rd meetings. In 2000, JECFA published the original review on steviol glycosides (WHO, 2000). JECFA established a temporary ADI (acceptable daily intake) of 0-2 mg/kg (on a steviol basis) at its 63rd meeting (WHO, 2006). Additionally, JECFA finalized food grade specifications (FAO, 2007a), although they were subsequently updated in 2008 and 2010 (FAO, 2008; FAO, 2010). At the 69th meeting, the temporary status of the ADI was removed, and the ADI was raised to 0-4 mg/kg bw/day (on a steviol basis) as a result of the JECFA review of recently completed clinical studies with steviol glycosides (WHO, 2008). In 2009, JECFA published a final monograph addendum on steviol glycosides (WHO, 2009).

In early 2009, a number of parties, including the government of Australia and the Calorie Control Council, submitted a request to the Codex Committee on Food Additives in which it was proposed that the JECFA specifications for steviol glycosides should be modified to allow inclusion of Rebaudioside D and Rebaudioside F as specifically named acceptable glycosides that would be considered as part of the minimum 95% steviol glycosides composition (CCFA, 2009). This

[3] Accessible at: http://www.accessdata.fda.gov/scripts/fcn/fcnNavigation.cfm?rpt=grasListing&displayAll=true.

[4] GRAS notification 252 was submitted by Merisant, GRAS notification 253 was submitted by Cargill, GRAS notification 275 was submitted by McNeil Nutritionals, GRAS notification 278 was submitted by Blue California, GRAS notification 282 was submitted by Sweet Green Fields, GRAS notification 287 was submitted by Wisdom Natural Brands, GRAS notifications 303 and 304 were submitted by Sunwin and Wild Flavors, GRAS notification 318 was submitted by Pyure Brands, and GRAS notification 323 was submitted by PureCircle USA; information pertaining to these notifications are listed on FDA's are listed on FDA's website at http://www.accessdata.fda.gov/scripts/fc/fcnNavigation.cfm?rpt=grasListing, along with their respective "no question" letters.

proposed modification was endorsed by the Codex Alimentarius Committee in July 2009; it was on the agenda for discussion at the JECFA Meeting in June, 2010 (WHO/FAO, 2009), and JECFA recently took final action in approving the modified steviol glycosides specifications to include Rebaudioside D and Rebaudioside F (FAO, 2010) (see Appendix A).

In 2008, Switzerland's Federal Office for Public Health (2008) approved the use of stevia as a sweetener citing the favorable actions of JECFA. Subsequently, France published its approval for the food uses of rebaudioside A with a purity of 97% (AFSSA, 2009).

Stevia-derived sweeteners are not permitted as an ingredient in conventional food in Hong Kong. This appears to be related to a lack of review of new data on the sweeteners rather than a safety concern. Although the government website cites permission to use stevia (Hong Kong Government, 2002), Hong Kong maintains that stevia is not permitted as a sweetener. The Hong Kong Government was reported to be waiting for the JECFA determination on the safety of steviol glycosides. However, no further official actions have been noted since JECFA's final resolution was reported in June 2008 or following subsequent JECFA actions in 2009 or 2010.

On September 18, 2009, based on a review of the international regulation of *Stevia rebaudiana* and the clinical evidence for safety and efficacy, the Natural Health Products Directorate, Health Canada (2009), has adopted the following guidelines for the use of *Stevia* and steviol glycosides in Natural Health Products (NHPs). The revised recommendation for the maximum limit for steviol glycosides in NHPs is in accordance with the full ADI (acceptable daily intake) of 4 mg steviol/kg body weight established by JECFA (2008).

In light of JECFA's 2008 findings and in response to a June 2008 request by the European Commission for European Food Safety Authority (EFSA) to deliver a scientific opinion on the safety of steviol glycosides as a sweetener for use in the food categories specified in the dossiers from the three petitioners, EFSA reexamined the safety of steviol glycosides (EFSA, 2010). After considering all the data on stability, degradation products, metabolism and toxicology, the EFSA Panel established an ADI for steviol glycosides, expressed as steviol equivalents, of 4 mg/kg bw/day, which is similar to JECFA's determination.[5]

D. FDA Regulatory Framework

In the US, steviol glycosides (or stevioside) have been used in dietary supplements since 1995 (Geuns, 2003). Steviol glycosides or steviosides are widely available to consumers in the US through retail outlets and Internet purchases (Al-Achi and Greenwood, 2000). As per FDA regulation of foods, dietary supplements cannot legally be added to conventional foods. Dietary supplements must undergo premarket approval by FDA as food additives or, alternatively, the

[5] From a historical perspective, it is noted that the UK's Advisory Committee on Novel Foods and Processes for the Ministry of Agriculture, Fisheries and Food on September 24, 1998 rejected an application for use of steviol glycosides as a sweetener in herbal teas because "the applicant had not provided all of the information necessary to enable an assessment to be made." (See http://www.maff.gov.uk/food/novel/980924.html.) In 1999, the Scientific Committee on Food for the European Commission concluded that "there are no satisfactory data to support the safe use of these stevia plants and leaves" (European Commission, 1999a). In another opinion also dated June 17, 1999, the Committee also reiterated "its earlier opinion that stevioside is not acceptable as a sweetener on the presently available data" (European Commission, 1999b).

ingredients to be incorporated into conventional 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.[6]

In 1997, FDA altered the GRAS determination process by eliminating the formal GRAS petitioning process. At that time, the petitioning process was replaced with a notification procedure.[7] 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.

[6] See 21 CFR 170.3(i)(3).

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

III. CHEMISTRY & MANUFACTURE OF REBAUDIOSIDE A (≥97%)

A. Common or Usual Name

Rebaudioside A, also referred to as Reb A or reb A, is one of the common steviol glycosides found in nature. The common or usual name for the products that are the subject of this notification is rebaudioside A, which is derived from the leaves of *Stevia rebaudiana* Bertoni. The rebaudioside A content of the commercial production is equal to or higher than 97%. Govia™ is the commercial name used by Layn in referring to the notified substance. In the scientific literature, steviol glycosides have been referred to as stevia, stevioside, steviol glycosides, and stevia glycoside. JECFA adopted the term, steviol glycosides, for the family of steviol derivatives with sweetness properties that are derived from the stevia plant. Presently, the term, stevia, is used more narrowly to describe the plant or crude extracts of the plant, while stevioside is the common name for another one of the specific glycosides that is extracted from stevia leaves.

B. Description

As described in Food Chemicals Codex (FCC, 2009), rebaudioside A is a white to off-white, hygroscopic fine crystal, granule, or powder having a sweet taste. It is freely soluble in ethanol:water 50/50 (v/v) and is sparingly soluble in water and in ethanol. Rebaudioside A is obtained from the leaves of the *Stevia rebaudiana* Bertoni plant in a multistep separation and purification process. Principle steps of manufacturing include extraction of steviol glycosides from the leaves using an aqueous or aqueous alcoholic (ethanol or methanol) solvent, and purification of rebaudioside A from the resulting mixture of steviol glycosides by resin absorption followed by recrystallization from an aqueous or aqueous alcoholic (ethanol or methanol) solvent. It is primarily composed of rebaudioside A, a glycoside of the *ent*-kaurenoid diterpenoid aglycone known as steviol (FCC, 2009).

C. Chemistry of Rebaudioside A

At its 51st meeting JECFA reviewed the safety related information, including chemistry on stevioside. The following description is taken from the original JECFA monograph (WHO, 2000).

> Stevioside is a glycoside of the diterpene derivative steviol (ent-13-hydroxykaur-16-en-19-oic acid). Steviol glycosides are natural constituents of the plant *Stevia rebaudiana Bertoni*, belonging to the Compositae family. The leaves of *S. rebaudiana Bertoni* contain eight different steviol glycosides, the major constituent being stevioside (triglucosylated steviol), constituting about 5-10% in dry leaves. Other main constituents are rebaudioside A (tetraglucosylated steviol), rebaudioside C, and dulcoside A. *S. rebaudiana* is native to South America and has been used to sweeten beverages and food for several centuries. The plant has also been distributed to Southeast Asia. Stevioside has a sweetening potency 250-300 times that of sucrose and is stable to heat. In a 62-year-old sample from a herbarium, the intense sweetness of *S. rebaudiana* was conserved, indicating the stability of stevioside to drying, preservation, and storage (Soejarto et al., 1982; Hanson and De Oliveira, 1993).

Of the nine different steviol glycosides, the two principal sweeteners of stevia extracts have been identified as rebaudioside A and stevioside. The chemical identities and key chemical identifiers for the two major components are shown below.

Rebaudioside A

Chemical Name:	13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl) oxy] kaur-16-en-18-oic acid, β-D-glucopyranosyl ester
Chemical Formula:	$C_{44}H_{70}O_{23}$
Formula Weight:	967.03
CAS Number:	58543-16-1

Stevioside

Chemical Name:	13-[2-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy] kaur-16-en-18-oic acid, β-D-glucopyranosyl ester
Chemical formula:	$C_{38}H_{60}O_{18}$
Formula Weight:	804.88
CAS Number:	57817-89-7

The chemical structure of rebaudioside A is presented in Figure 1. Recently, JECFA (FAO, 2007b) identified the sweetener components of stevia and updated the list of common glycosides and their chemical structures (Figure 2) that are slightly different than compounds shown in other older publications (Nanayakkara et al., 1987; Suttajit et al., 1993). The structures of the components of stevia glycosides were also described in reviews by Kinghorn and Soejarto (1985), Kennelly (2002), and Geuns (2003). Other substances that lack sweetness include the labdane diterpenes, triterpenes, sterols and flavonoid glycosides.

Figure 1. Chemical Structure of Rebaudioside A

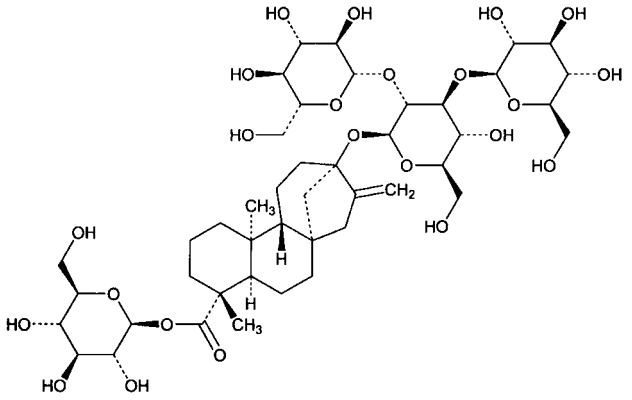
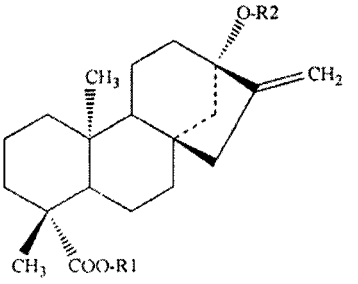


Figure 2. Chemical Structures of Various Steviol Glycosides Reproduced from FAO[a, b]



	Compound name	C.A.S. No.	R1	R2
1	Steviol	471-80-7	H	H
2	Steviolbioside	41093-60-1	H	β-Glc-β-Glc(2→1)
3	Stevioside	57817-89-7	β-Glc	β-Glc-β-Glc(2→1)
4	Rebaudioside A	58543-16-1	β-Glc	β-Glc-β-Glc(2→1) | β-Glc(3→1)
5	Rebaudioside B	58543-17-2	H	β-Glc-β-Glc(2→1) | β-Glc(3→1)
6	Rebaudioside C (dulcoside B)	63550-99-2	β-Glc	β-Glc-α-Rha(2→1) | β-Glc(3→1)
7	Rebaudioside D	63279-13-0	β-Glc-β-Glc(2→1)	β-Glc-β-Glc(2→1) | β-Glc(3→1)
8	Rebaudioside E	63279-14-1	β-Glc-β-Glc(2→1)	β-Glc-β-Glc(2→1)
9	Rebaudioside F	438045-89-7	β-Glc	β-Glc-β-Xyl(2→1) | β-Glc(3→1)
10	Rubusoside	63849-39-4	β-Glc	β-Glc
11	dulcoside A	64432-06-0	β-Glc	β-Glc-α-Rha(2→1)

[a] From FAO, 2007b.
[b] The indicated C.A.S. No. for Rubusoside as reported in the cited reference is incorrect and should be 64849-39-4.

D. Manufacturing Processes

Based on available scientific and patent literature, several manufacturing processes for steviol glycosides have been reported. These processes are summarized below, along with Layn's manufacturing process for its rebaudioside A (≥97%).

1. Scientific & Patent Literature

Steviol glycosides are obtained by extracting leaves of *Stevia rebaudiana* Bertoni with hot water or alcohols (ethanol or methanol). The extract is a dark particulate solution containing all the active principles along with leaf pigments, soluble polysaccharides, and other impurities. Some processes remove the "grease" from the leaves with solvents such as chloroform or hexane before extraction occurs (Kinghorn and Soejarto, 1985). There are several extraction patents for the isolation of steviol glycosides. Kinghorn and Soejarto (1985) have categorized the extraction patents into those based on solvent, solvent plus a decolorizing agent, adsorption and column chromatography, ion exchange resin, and selective precipitation of individual glycosides. In recent patents, methods using ultrafiltration, metallic ions, supercritical fluid extraction with CO_2 and extract clarification with zeolite are employed.

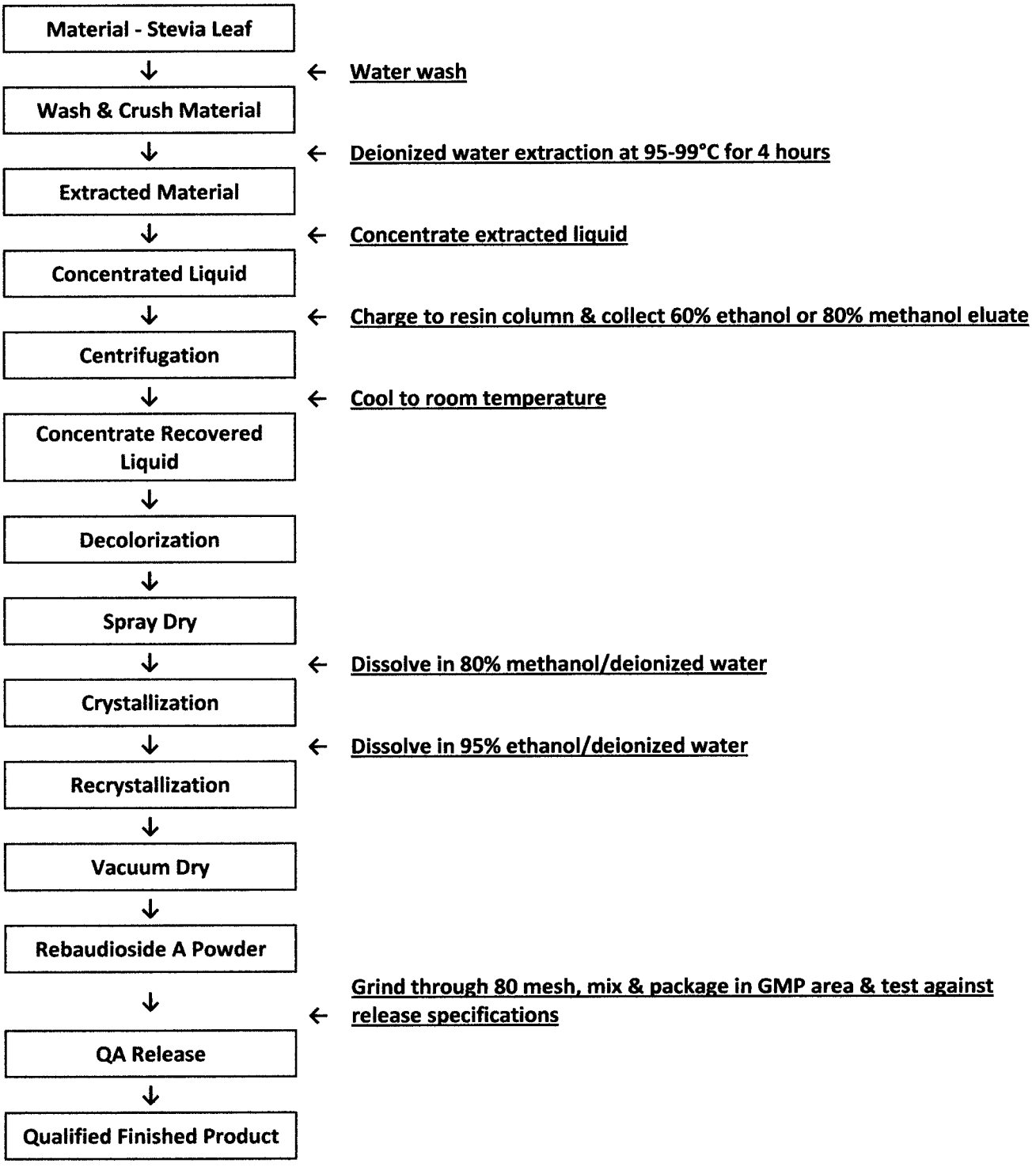
At the 68th JECFA meeting, steviol glycosides were defined as the products obtained from the leaves of *Stevia rebaudiana* Bertoni. As described by JECFA, the typical manufacture starts with extracting leaves with hot water and the aqueous extract is passed through an adsorption resin to trap and concentrate the component steviol glycosides. The resin is washed with methanol to release the glycosides and the product is recrystallized with methanol. Ion-exchange resins may be used in the purification process. The final product is commonly spray-dried.

2. Layn's Manufacturing Process for Purified Rebaudioside A

The source of Layn's rebaudioside A is the leaves of the *Stevia rebaudiana* Bertoni plant. Several factors including the temperature while cultivating, latitude where stevia is grown, and the timing of harvest are known to influence the steviol glycosides content. Layn acquires the stevia leaves as raw material that contains >6% rebaudioside A. The raw material is crushed to the size of about 20 mesh, then the leaves are washed by soaking with purified water (50% of weight of leaves, w/w) for 20 minutes. After removal of the wash water, the leaves are extracted twice with deionized water at 95-99°C. The extract liquid is concentrated first under vacuum and then by centrifugation. The extract is then applied to a D101 macroporous resin column, and the glycosides are eluted with either 60% ethanol or 80% methanol. This fraction is then charged into strong base anion resin for decolorization, and the eluate is dried under vacuum. The dried powder is dissolved in 80% methanol/deionized water solution for the first recrystallization, and the second recrystallization is done in 95% ethanol/deionized water solution. The recrystallized product is dried under vacuum, passed through an 80 mesh screen and then packaged. The product is not released for sale until QC-testing is completed.

The overview of the manufacturing processes for purified rebaudioside A (>97%) from the leaves of *Stevia rebaudiana* Bertoni is presented in Figure 3. Certificates demonstrating the suitability of the resins used for the purification and decolorization are attached as Appendices B-1 and B-2. Processing aids such as ethanol and methanol used in the manufacturing are food grade and comply with the specifications described in the 5th edition of Food Chemicals Codex with the exception of the moisture specification. Vendor specifications for the ethanol and methanol are attached as Appendices B-3 and B-4. Rebaudioside A is manufactured in accordance with current Good Manufacturing Practices (cGMP).

Figure 3. Overview of Primary Stevia Extract Production Processing for Rebaudioside A 97%



E. Product Specifications & Supporting Methods

1. JECFA Specifications for Steviol Glycosides

The composition of extracts of *Stevia rebaudiana* Bertoni depends upon the composition of the harvested leaves, which are, in turn, influenced by soil, climate, and the manufacturing process itself (FAO, 2007b).

In 2007, JECFA recommended that the method of assay should include a minimum requirement of 95% of the total of 7 specific steviol glycosides on a dried weight basis, and JECFA finalized food grade specifications at the 68th JECFA meeting with publication in the FAO JECFA Monograph 4 (FAO, 2007a). Stevioside and rebaudioside A are the major component glycosides of interest because of their sweetening property. The 5 other associated glycosides found in preparations of steviol glycosides accepted by the JECFA specifications with the 95% requirement are rebaudioside C, dulcoside A, rubusoside, steviolbioside, and rebaudioside B. These, however, are typically found at much lower levels than stevioside or rebaudioside A. JECFA updated the specifications for steviol glycosides in 2008 (FAO, 2008), and then again in 2010 when the specifications were expanded to include the original seven specific steviol glycosides plus Reb D and Reb F (FAO, 2010); also see Appendix A.

Steviol glycosides are described as a white to yellow powder, odorless to having a slight characteristic odor, and exhibiting a sweetness that is 200-300 times greater than sucrose. The ingredient must consist of a minimum of 95% of 9 specific steviol glycosides. The steviol glycosides are freely soluble in water and ethanol, and the 1 in 100 solutions exhibit pH values between 4.5 - 7.0. The product should not have more than 1% ash with no more than a 6% loss on drying at 105°C for 2 hours. Any residual methanol levels should not exceed 200 ppm, and ethanol residues should not exceed 5000 ppm. Arsenic levels should not exceed 1 ppm as determined by the atomic absorption hydride technique. Lead levels should not exceed 1 ppm.

2. Specifications for Layn's Purified Rebaudioside A (≥97%)

Layn has established product specifications for its purified rebaudioside A product that meet or exceed JECFA recommendations while also complying with Food Chemicals Codex (FCC, 2009) specifications for rebaudioside A. A comparison of the specifications provided by Layn and those from JECFA and FCC is presented in Table 1. Certificates of Analysis demonstrating that 5 production batches of the subject materials meet these specifications are provided in Appendix C. Over 140 pesticides or their residues were tested from these five production lots, and none of the pesticides or their residues were detected in any of the samples. The pesticide residue analysis test report from one of the batches is included in Appendix D. Detailed identity-related analyses for rebaudioside A and other steviol glycosides from five lots, along with representative chromatograms, are presented in Appendix E.

Table 1. Specifications for Layn's Rebaudioside A (≥ 97%) Product

PARAMETER	JECFA[a] SPECIFICATIONS STEVIOL GLYCOSIDES	FCC SPECIFICATIONS REBAUDIOSIDE A	LAYN SPECIFICATIONS REBAUDIOSIDE A (≥97%)	METHODS
Sweetness	200-300 times sweeter than sucrose	NA	300-400 times sweeter than sugar	Gustatory
Rebaudioside A	NA	NLT 95%	≥ 97%	
Other Related Steviol Glycosides (as Stev, Reb A, B, C, Dulc A, Rubu, and SB) on dry weight basis	NLT 95%	NMT 5%[b]	NS	JECFA, 2007
Moisture (loss on drying)	NMT 6%	NMT 6%	≤ 5%	USP32 <731>
Ash	NMT 1%	NMT 1%	< 1%	USP32 <561>
Optical rotation	NS	NS	-30° to -38°C	USP32 <781>
Solubility	Freely soluble in water and ethanol	Freely soluble in water:ethanol (50:50)	Soluble in water and ethanol	USP32<561>
pH (1% solution)	4.5 - 7.0	4.5 - 7.0	4.5-7.0	USP32<791>
Residual solvent levels				
Residual Methanol	NMT 200 mg/kg	NMT 0.02%	<200 ppm	USP32 <467>
Residual Ethanol	NMT 5000 mg/kg	NMT 0.5%	<2000 ppm	USP32 <467>
Heavy metals				
Heavy metals	NS	NS	< 3 mg/kg	USP32 <231>
Lead	NMT 1 mg/kg	NMT 1 mg/kg	< 0.5 mg/kg	AOAC 993.14
Arsenic	NMT 1 mg/kg	NMT 1 mg/kg	< 0.05 mg/kg	AOAC 993.14
Microbiological				
Total Plate Count	NA	NA	< 1000 cfu/g	USP32 <61>
Yeast and Mold	NA	NA	≤ 100 cfu/g	USP32 <61>
Total coliform	NA	NA	≤ 100 cfu/g	USP32 <61>
Salmonella	NA	NA	Negative	USP32 <61>
Escherichia coli	NA	NA	Negative	USP32 <61>
Staphylococcus aureus	NA	NA	Negative	USP32 <61>

[a] Prepared at 69th JECFA (2008). [b] Excludes Reb A but includes additional two glycosides Reb D and Reb F; Abbreviations: St = Stevioside; Reb A = Rebaudioside A; Reb B = Rebaudioside B; Reb C = Rebaudioside C; Dulc A = Dulcoside A; Rub = Rubusoside; SB = Steviolbioside; NS = not specified; NA = not applicable; NLT = not less than; NMT = not more than.

F. Stability Data

Kinghorn and Soejarto (1985) reported that stevioside is a stable molecule over the pH range 3-9 and can be heated at 100°C for 1 hour, but at pH levels greater than 9 under these conditions it rapidly decomposes. These investigators also speculated that at pH 10 steviolbioside would be the major decomposition product produced from stevioside by alkaline hydrolysis. In another study, Chang and Cook (1983) investigated the stability of pure stevioside and rebaudioside A in carbonated phosphoric and citric acidified beverages. Some degradation of each sweetening component after 2 months of storage at 37°C was noted. However, no significant change at room temperature or below following 5 months of storage of stevioside and 3 months of storage of rebaudioside A was noted. Exposure to 1 week of sunlight did not affect stevioside, but resulted in approximately 20% loss of rebaudioside A. Heating at 60°C for 6 days resulted in 0-6% loss of rebaudioside A.

In an extensive stability testing, Merisant (2008) conducted experiments with rebaudioside A (1) as a powder, (2) as a pure sweetener in solution, and (3) on both cola-type and citrus carbonated beverages. In these investigations no degradation was detected when the powder was stored at 105°C for 96 hours. It was concluded that the powder was stable when stored for 26 weeks at 40±2°C with relative humidity of 75±5%. Both published and unpublished testing results from Merisant revealed that rebaudioside A in carbonated citric acid beverages and phosphoric acid beverages did not significantly degrade during prolonged storage at refrigeration, normal ambient, or elevated ambient temperatures. Minimal loss of rebaudioside A was detected after storage at 60°C, with considerable degradation noted after 13 hours at 100°C for carbonated beverage solutions and pure sweetener solutions (Merisant, 2008).

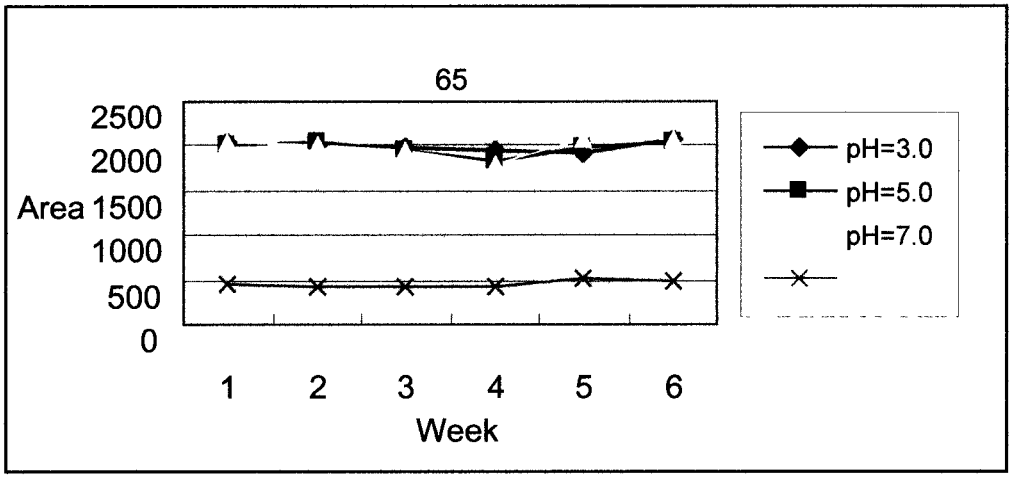
Cargill (2008) also conducted extensive stability testing on rebaudioside A as a powder under various storage conditions and under a range of pH and temperatures. Additionally, Cargill also investigated rebaudioside A stability in several representative food matrices at room temperature and elevated temperatures. Stability profiles were created for table top sweetener applications, mock beverages including cola, root beer and lemon-lime, thermally processed beverages, yogurt, and white cake. The results of stability testing revealed some degradation products that had not been detected in bulk rebaudioside A. These degradation products were structurally related to the steviol glycosides that are extracted from the leaves of *Stevia rebaudiana* Bertoni. All the degradation products were found to share the same steviol aglycone backbone structure as found in stevioside and rebaudioside A, but they differ by virtue of the glucose moities present. The results of stability testing revealed that rebaudioside A is stable in various food matrices following several days or weeks of storage. The extent and rate of degradation is dependent on pH, temperature, and time. When placed in beverages, rebaudioside A is more stable in the pH range 4 to 6 and at temperatures from 5°C to 25°C (Cargill, 2008).

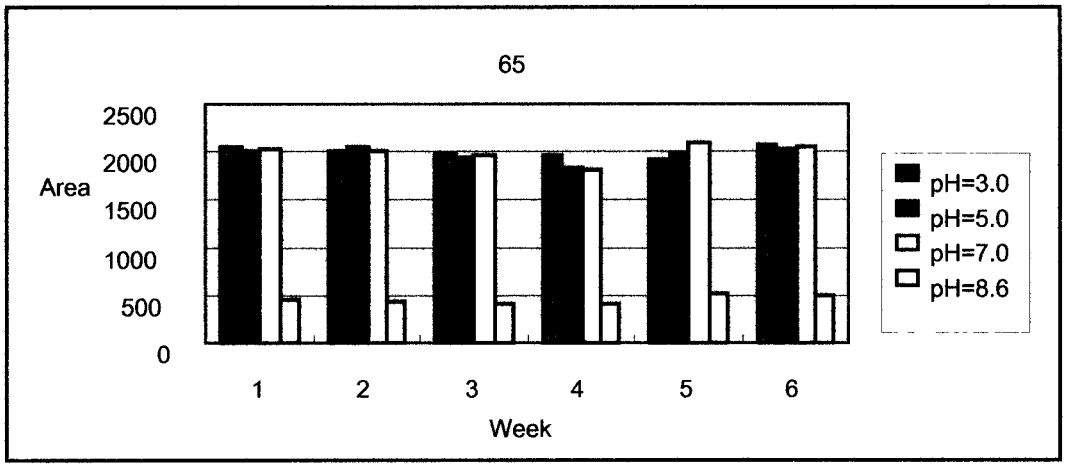
In photostability studies of the dry powder and mock beverages to ascertain rebaudioside A behavior under defined conditions of fluorescent and near UV light exposure, rebaudioside A was found to be photostable under the defined conditions of analysis (Clos et al., 2008).

Layn's purified rebaudioside A (≥97%) is comparable in composition to the Merisant materials, and the stability characteristics are expected to be equivalent to that exhibited by the materials

studied by Merisant and Cargill. This was supported by a series of experiments conducted by Layn. In these experiments, stability of purified rebaudioside A (≥97%) in different pH solutions under various storage conditions was determined from six different batches. These experiments revealed that rebaudioside A was stable as no significant changes were noted during 5 weeks of storage at 4°C, at 22°C, and at 37°C in different pH solutions except for pH=8.6 (see Figure 4). Additional experiments with rebaudioside A (≥97%) solutions (0.1 mg/mL) that were stored at 65°C for 6 weeks also confirmed the stability as depicted in Figure 4. The results also revealed that rebaudioside A exhibited greater stability in an acidic system than in an alkaline system.

Figure 4. HPLC Analyses of Rebaudioside A (97%) Solutions (0.1 mg/mL) Stored at 65°C Over a Period of Six Weeks





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IV. INTENDED DIETARY USES

A. Intended Uses

Layn intends to use purified rebaudioside A (≥97%) as a general purpose non-nutritive sweetener as a table top sweetener and in various foods other than infant formulas and meat and poultry products The intended use will be as a non-nutritive sweetener as defined under 21 CFR 170.3(o)(19)[8]. The intended use levels will vary by actual food category, but the actual levels are self-limiting due to organoleptic factors and consumer taste considerations. However, the amounts of purified rebaudioside A (≥97%) to be added to foods will not exceed the amounts reasonably required to accomplish its intended technical effect in foods as required by FDA regulation.[9] The intended use levels of purified rebaudioside A and the food categories in which it is used are identical to those described by Cargill (2008).

B. Food Uses As Addressed by JECFA, Merisant & Cargill

As part of its safety deliberations, JECFA reviewed various estimates of possible daily intake of steviol glycosides (WHO, 2006). These estimates are presented in Table 2. Merisant also listed intended use levels of rebaudioside A for various food applications in their GRAS Notification (Table 3). Merisant utilized food consumption survey data from 2003-2004 NHANES to determine the estimated daily intake from the proposed uses of rebaudioside A. On a per user basis, the mean and 90th precentile daily consumption of rebaudioside A was estimated as 2.0 and 4.7 mg/kg bw/day, respectively. In its notification, Cargill (2008) utilized a different approach in estimating dietary intake figures for rebaudioside A when incorporated as a general sweetener in a broad cross-section of processed foods. Cargill considered that with a few minor exceptions rebaudioside A uses and use levels would be comparable to those of aspartame uses in the US. Using post-market surveillance consumption data and published data for consumption of aspartame and other high intensity sweeteners (Renwick, 2008), Cargill performed a side-by-side consumption analysis for rebaudioside A versus aspartame. Findings from the above-described different sources along with FSANZ estimates are further discussed in Section IV.C, and the intake estimates are presented in Table 4.

C. Estimated Daily Intake

The very conservative consumer intake estimates provided by JECFA as shown in Table 2 were utilized to gauge the potential human exposures of steviol glycosides and rebaudioside A in foods as reported in the US and in other countries. As rebaudioside A is about twice as sweet as the mixed glycosides, these levels can be adjusted accordingly. Layn intends to use rebaudioside A in a number of food categories at levels that comply with GMP uses. The application of rebaudioside A to the same foods and at the same levels as those described in earlier FDA

[8] Non-nutritive sweeteners: Substances having less than 2 percent of the caloric value of sucrose per equivalent unit of sweetening capacity.
[9] See 21 CFR 182.1(b)(1).

notices by Merisant and Cargill is unlikely to affect the dietary intake of rebaudioside A from introduction into the market by another supplier who will have to compete in essentially the same markets and foods. This also negates the need for cumulative intake analysis.

Table 2. Food Uses of Steviol Glycosides Reported to JECFA with Calculated Steviol Equivalents

Food Type	Maximum Use Level Reported[a] (mg steviol glycosides /kg of food)	Maximum Use Level Calculated for rebaudioside A[b] mg rebaudioside A /kg of food	Maximum Use Level Calculated for rebaudioside A[b] mg Steviol Equivalents /kg of food
Desserts	500	250	83
Cold confectionery	500	250	83
Pickles	1000	500	167
Sweet corn	200	100	33
Biscuits	300	150	50
Beverages	500	250	83
Yogurt	500	250	83
Sauces	1000	500	167
Delicacies	1000	500	167
Bread	160	80	27

[a] Reproduced from WHO, 2006.
[b] Calculated by Expert Panel assuming twice the sweetness intensity for rebaudioside A and three-fold difference in molecular weight between rebaudioside A and steviol.

Table 3. Proposed Uses & Levels of Rebaudioside A by Merisant[a]

Food Group	Rebaudioside A (ppm)
Tabletop sweeteners	30,000[b]
Sweetened ready-to-drink teas	90-450
Fruit juice drinks	150-500
Diet soft drinks	150-500
Energy drinks	150
Flavored water	150
Cereals (oatmeal, cold cereal, cereal bars)	150

[a] Merisant, 2008.
[b] Reb A content of sachet prior to dilution and not representative of "as consumed."

Table 4. Summary of Estimated Daily Intake Assessments for Rebaudioside A & Calculation of Rebaudioside A Values from JECFA & FSANZ Estimates of the EDI

Scenarios	EDI		
	As Steviol[a] (mg/kg bw/day)	As Rebaudioside A[b] (mg/kg bw/day)	Total Daily Intake[c] (mg/day)
JECFA			
100% Reb A replacement of sugars	5.0	7.5	450
20-30% Reb A replacement of sugars	1.0 - 1.5	1.5 - 2.3	90 - 140
FSANZ			
100% Reb A replacement of sugars	0.3 - 1.0	0.5 - 1.5	30 - 90
MERISANT			
		2.0 - 4.7[d]	120 - 282
CARGILL			
		1.3 - 3.4[d]	78 - 204

[a] Published values for mixed steviol glycosides consumption listed in this column were used for the calculation of Reb A consumption values appearing in next two columns.
[b] Estimates for Reb A consumption were calculated from JECFA and FSANZ estimates as steviol by multiplying by 3 to correct for the molecular weight of Reb A compared to steviol and by subsequently dividing by 2 because of the increased inherent sweetness of Reb A compared to the mixed steviol glycosides.
[c] Total daily intake figures were calculated for a 60 kg adult.
[d] Published values are shown for comparison purposes.

Further consideration was given to anticipated human exposures as projected independently and with different approaches by JECFA (WHO, 2006), Merisant (2008), and Cargill (2008). As described below, the multiple approaches tended to converge to yield estimated daily intakes (EDIs) in the range of 1.3 – 4.7 mg/kg bw/day that, when compared to the acceptable daily intake (ADI), constitutes an integral component in the subject GRAS evaluation.

JECFA evaluated information on exposure to steviol glycosides as submitted by Japan and China. Additional information was available from a report on *Stevia rebaudiana* Bertoni plants and leaves that were prepared for the European Commission by the Scientific Committee on Food. JECFA used the GEMS/Food database to prepare international estimates of exposure to steviol glycosides (as steviol). JECFA assumed that steviol glycosides would replace all dietary sugars, at the lowest reported relative sweetness ratio for steviol glycosides and sucrose, which is 200:1. The intakes ranged from 1.3 mg/kg bw/day with the African diet to 3.5 mg/kg bw/day with the European diet. Additionally, JECFA also estimated the per capita exposure derived from disappearance (poundage) data supplied by Japan and China. The

Committee evaluated exposures to steviol glycosides by assuming full replacement of all dietary sugars in the diets for Japan and the US. The exposures to steviol glycosides (as steviol) as evaluated or derived by the Committee are summarized in Table 5.

Table 5. Summary of Estimates of Exposure to Steviol Glycosides (as Steviol)

ESTIMATE	EXPOSURE (mg/kg BW/DAY)
GEMS/Food (International)[a]	1.3--3.5 (for a 60 kg person)
Japan, Per Capita	0.04
Japan, Replacement Estimate[b]	3
US, Replacement Estimate[b]	5

[a] WHO Global Environment Monitoring System — Food Contamination Monitoring and Assessment Programme.
[b] These estimates were prepared in parallel to those for the international estimates; it was assumed that all dietary sugars in diets in Japan and the US would be replaced by steviol glycosides on a sweetness equivalent basis, at a ratio of 200:1.

In its assessment, JECFA concluded that the replacement estimates were highly conservative as the calculated dietary exposure overestimates likely consumption and that true dietary intakes of steviol glycosides (as steviol) would probably be 20 – 30% of these values or 1.0 - 1.5 mg/kg bw/day on a steviol basis, or 3.0 – 4.5 mg/kg bw/day for rebaudioside A based on the molecular weight adjustment. Furthermore, by adjusting for the 400-fold increased sweetness of rebaudioside A relative to sucrose compared to the mixed steviol glycosides sweetness factor of 200-fold relative to sucrose assumed by JECFA, the estimated dietary intake of rebaudioside A would likely be about 1.5 to ~ 2.3 mg/kg bw/day.

Similar to JECFA, FSANZ (2008) also estimated steviol glycoside dietary intake for adult consumers in New Zealand, assuming a full sugar replacement scenario which resulted in estimated exposures of 0.3 - 1.0 mg/kg bw/day on a steviol basis, or 0.5 – 1.5 mg/kg bw/day for rebaudioside A when making both the molecular weight and sweetness equivalency calculations. Merisant also calculated a dietary estimate for rebaudioside A of 2.0 mg/kg bw/day for the average consumer of the foods listed in Table 3 and 4.7 mg/kg bw/day for a 90th percentile consumer. In another review conducted on behalf of Cargill and included in their GRAS notification, the intake of rebaudioside A when used as a complete sugar replacement was estimated at 1.3 – 3.4 mg/kg bw/day when calculated as rebaudioside A (Renwick, 2008). The estimated daily intake assessments have been compiled in Table 4. These different assessments suggest that total daily consumption of rebaudioside A for specified food categories and as a general purpose sweetener is unlikely to exceed 5 mg/kg bw/day, for a total daily dietary exposure of up to 300 mg rebaudioside Reb for an adult weighing 60 kg.

D. Other Information on Human Exposure to Stevia: Use as Food Ingredient & Other Uses

For about 20 years, consumers in Japan and Brazil, where stevia has long been approved as a food additive, have been using stevia extracts as non-caloric sweeteners.[10] It is reported that

[10] See Raintree NutritionTropical Plant Database (www.rain-tree.com/stevia.htm).

40% of the artificial sweetener market in Japan is stevia based and that stevia is commonly used in processed foods in Japan (Lester, 1999). Although there are no reported uses of rebaudioside A as a dietary supplement, use of steviol glycoside as a dietary supplement is presently permitted in the US, Canada, Australia, and New Zealand. It has wide use in China and Japan in food and in dietary supplements. In the US, stevia is available in packets containing 60 - 90 mg steviol glycoside for home supplement uses, such as in beverages or other foods. It is estimated that sales of stevia in the US reached $45 million in 2005 (The Food Institute Report, 2006). No estimates were located on the daily consumption levels of steviol glycosides consumed in the US *via* dietary supplements.

As a result of selected firms obtaining independent GRAS determinations for the stevia-derived sweeteners during the second quarter in 2008 in the US, these ingredients have begun to be incorporated into foods. In light of FDA's review of the Merisant, Cargill, McNeil Nutritionals, Blue California, Sweet Green Fields, Wisdom Natural Brands, Sunwin/WILD (two submissions), Pyure Brands, and PureCircle USA GRAS notifications and issuance of "no questions" letters, the use of steviol glycosides sweeteners, such as rebaudioside A, is anticipated to grow substantially in the US, and international uses are also expected to increase with the favorable JECFA determination at its 2008 meeting and the more recent scientific opinion offered by EFSA on the safety of steviol glycosides as noted in Section II.C.

Hawke (2003) reported that stevia is commonly used as a treatment for Type 2 diabetes use in South America. However, for its therapeutic effects elevated doses in the range of 1 g/person/day or more were reported to be necessary (Gregersen et al., 2004).

V. SAFETY DATA FOR REBAUDIOSIDE A

A. Safety Data on Steviol Glycosides: Reviews by Expert Bodies & Other Scientists

Stevia and steviol glycosides have been extensively investigated for their biological, toxicological, and clinical effects (Carakostas et al., 2008; Geuns, 2003; Huxtable, 2002). Additionally, the national and international regulatory agencies have thoroughly reviewed the safety of stevia and its glycosides. Most notably, over the years JECFA has evaluated stevia and steviol glycoside multiple times (WHO, 2000, 2006, 2007, 2008). Recently FSANZ (2008) also evaluated steviol glycosides for use in food. The JECFA reviews, as well as the other reviews completed before 2008, primarily focused on mixtures of steviol glycosides typically and were not specific for purified rebaudioside A.

From the safety perspective, some of the earliest studies on steviol glycosides were of limited value as the actual compositions of materials investigated and their questionable purities undermined drawing firm toxicological conclusions. These early studies reported a decrease in fertility with crude stevia preparations and increased mutagenic activity of the principle metabolite, steviol. Based on these and other questions raised about safety by studies with materials of lesser purity and by studies with unusual protocols in *in vivo* and in *in vitro* systems usually employing high doses or high concentrations of test materials, FDA was reluctant to authorize the use of stevia. These concerns included renal toxicity, effects on glucose metabolism, and inhibition of mitochondrial enzymes. Over the last decade and half, the safety of steviol glycosides and rebaudioside A in particular have been extensively investigated employing comprehensive and modern toxicology protocols using scientifically accepted dosing regimens of purified and standardized test substances. The findings from these investigations are discussed below.

JECFA encouraged the further elucidation of clinical effects on blood pressure and glucose metabolism on hypertensive and diabetic individuals, respectively, in parallel with normal human subjects. By 2006, sufficient data were generated for JECFA to satisfactorily establish a temporary ADI, which was finalized in 2008. Additional details on the JECFA reviews are discussed below.

1. Summary of JECFA Reviews

Earlier at its 51st meeting, JECFA (WHO, 2000) expressed the following reservations about the safety data available at that time for steviol glycosides:

> The Committee noted several shortcomings in the information available on stevioside. In some studies, the material tested (stevioside or steviol) was poorly specified or of variable quality, and no information was available on other constituents or contaminants. Furthermore, no studies of human metabolism of stevioside and steviol were available. In addition, data on long-term toxicity and carcinogenicity were available for stevioside in only one species. The mutagenic potential of steviol has been tested sufficiently only *in vitro*.

Subsequently, additional data were generated on the metabolism of steviol glycosides and submitted to JECFA. This information suggested that the common steviol glycosides are

converted to steviol by intestinal bacteria and then rapidly converted to glucuronides that are excreted. The committee now had a molecular basis to become comfortable with studies on test materials which consisted of variable composition but were relatively high purity mixtures of the common steviol glycosides. The new information also revealed that in *in vitro* studies steviol is mutagenic, while *in vivo* condition it is not mutagenic. The committee became convinced that purified steviol glycosides did not impair reproductive performance as did crude preparations of stevia and that there was sufficient chronic studies in rats with adequate no observed effect levels (NOEL) that could support a reasonable acceptable daily intake (ADI) in the range of doses that would be encountered by the use of steviol glycosides as a sugar substitute. However, JECFA wanted more clinical data to rule out pharmacological effects at the expected doses. The following excerpt was taken from the report of the 63rd meeting (WHO, 2006):

> The Committee noted that most of the data requested at its fifty-first meeting, e.g., data on the metabolism of stevioside in humans, and on the activity of steviol in suitable studies of genotoxicity *in vivo*, had been made available. The Committee concluded that stevioside and rebaudioside A are not genotoxic *in vitro* or *in vivo* and that the genotoxicity of steviol and some of its oxidative derivatives *in vitro* is not expressed *in vivo*.
>
> The NOEL for stevioside was 970 mg/kg bw/day in a long-term study (Toyoda et al., 1997) evaluated by the Committee at its fifty-first meeting. The Committee noted that stevioside has shown some evidence of pharmacological effects in patients with hypertension or with type-2 diabetes at doses corresponding to about 12.5–25 mg/kg bw/day (equivalent to 5–10 mg/kg bw/day expressed as steviol). The evidence available at present was inadequate to assess whether these pharmacological effects would also occur at lower levels of dietary exposure, which could lead to adverse effects in some individuals (e.g., those with hypotension or diabetes).
>
> The Committee therefore decided to allocate a temporary ADI, pending submission of further data on the pharmacological effects of steviol glycosides in humans. A temporary ADI of 0–2 mg/kg bw was established for steviol glycosides, expressed as steviol, on the basis of the NOEL for stevioside of 970 mg/kg bw/day (or 383 mg/kg bw/day, expressed as steviol) in the 2-year study in rats and a safety factor of 200. This safety factor incorporates a factor of 100 for inter- and intra-species differences and an additional factor of 2 because of the need for further information. The Committee noted that this temporary ADI only applies to products complying with the specifications.
>
> The Committee required additional information, to be provided by 2007, on the pharmacological effects of steviol glycosides in humans. These studies should involve repeated exposure to dietary and therapeutic doses, in normotensive and hypotensive individuals and in insulin-dependent and insulin-independent diabetics.

In 2007, at its 68th meeting, JECFA (WHO, 2007) concluded that sufficient progress had been made on the clinical studies and extended the temporary ADI until 2008. Subsequently, sufficient data had been received by JECFA to revise and finalize food additive specifications for steviol glycosides (FAO, 2007a). The Chemical and Technical Assessment report written after the 2007 meeting, explained the Committee's thinking which resulted in flexibility in the identity specifications (FAO, 2007b).

> In response to the call for data on "stevioside" for the 63rd meeting of the Committee, submissions from several countries showed that the main components of the commercially available extracts of stevia are stevioside and rebaudioside A, in various amounts ranging from about 10-70% stevioside and 20-70% rebaudioside A. The information indicated that most commercial products contained more than 90% steviol glycosides with the two main steviol glycosides comprising about 80% of the material. The 63rd JECFA required that the summed content of stevioside and rebaudioside A was not less than 70% and established a minimum purity of 95% total steviol glycosides. Analytical data showed that most of the remaining 5% could be accounted for by saccharides other than those associated with the individual steviol glycosides.

> Noting that the additive could be produced with high purity (at least 95%) and that all the steviol glycosides hydrolyze upon ingestion to steviol, on which the temporary ADI is based, the 68th JECFA decided it was unnecessary to maintain a limit for the sum of stevioside and rebaudioside content. The Committee recognized that the newly revised specifications would cover a range of compositions that could include, on the dried basis, product that was at least 95% stevioside or at least 95% rebaudioside A.

In 2008, based on additional clinical studies, at its 69th meeting, JECFA finalized the evaluation of steviol glycosides (WHO, 2008) and raised the ADI to 0 – 4 mg/kg bw/day and removed the "temporary" designation. The summary of the Committee's key conclusions in the final toxicology monograph addendum (WHO, 2009) were stated as follows:

> From a long-term study with stevioside, which had already been discussed by the Committee at its fifty-first meeting, a NOEL of 970 mg/kg bw per day was identified. At its sixty-third meeting, the Committee set a temporary ADI of 0–2 mg/kg bw for steviol glycosides, expressed as steviol, on the basis of this NOEL for stevioside of 970 mg/kg bw per day (383 mg/kg bw per day expressed as steviol) and a safety factor of 200, pending further information. The further information was required because the Committee had noted that stevioside had shown some evidence of pharmacological effects in patients with hypertension or with type 2 diabetes at doses corresponding to about 12.5–25.0 mg/kg bw per day (5–10 mg/kg bw per day expressed as steviol).
>
> The results of the new studies presented to the Committee at its present meeting have shown no adverse effects of steviol glycosides when taken at doses of about 4 mg/kg bw per day, expressed as steviol, for up to 16 weeks by individuals with type 2 diabetes mellitus and individuals with normal or low-normal blood pressure for 4 weeks. The Committee concluded that the new data were sufficient to allow the additional safety factor of 2 and the temporary designation to be removed and established an ADI for steviol glycosides of 0–4 mg/kg bw expressed as steviol.
>
> The Committee noted that some estimates of high-percentile dietary exposure to steviol glycosides exceeded the ADI, particularly when assuming complete replacement of caloric sweeteners with steviol glycosides, but recognized that these estimates were highly conservative and that actual intakes were likely to be within the ADI range.

2. Summary of FSANZ Review of Steviol Glycosides

In 2008, FSANZ completed a review of the safety of steviol glycosides for use as a sweetener in foods. FSANZ concluded that steviol glycosides are well tolerated and unlikely to have adverse effects on blood pressure, blood glucose or other parameters in normal, hypotensive or diabetic subjects at doses up to 11 mg/kg bw/day. The FSANZ review discussed the adequacy of the existing database and several new studies, including the clinical studies reviewed by JECFA in the summer of 2007, most notably the work of Barriocanal et al., which was later published in 2008.

In their draft document, FSANZ also indicated that the new data in humans provides a basis for revising the uncertainty factors that were used by JECFA to derive the temporary ADI for steviol glycosides in 2005. In particular, the evidence surrounding the pharmacological effects of steviol glycosides on blood pressure and blood glucose has been strengthened so that the additional 2-fold safety factor for uncertainty related to effects in normotensive or diabetic individuals is no longer required. Therefore, FSANZ established an ADI of 4 mg/kg bw/day for steviol glycosides as steviol equivalents, derived by applying a 100-fold safety factor to the NOEL of 970 mg/kg bw/day (equivalent to 383 mg/kg bw/day steviol) in a 2-year rat study (FSANZ, 2008).

3. Summary of EFSA Review of Steviol Glycosides

On March 10, 2010, EFSA adopted a scientific opinion on the safety of steviol glycosides (mixtures that comprise not less than 95% of stevioside and/or rebaudioside A) as a food additive. Earlier---in 1984, 1989 and 1999---the Scientific Committee for Food (SCF) evaluated stevioside as a sweetener. At the time, the SCF concluded that the use of stevioside was "toxicologically not acceptable" due to insufficient available data to assess its safety. However, in light of JECFA's 2008 findings and in response to a June 2008 request by the European Commission, EFSA reevaluated the safety of steviol glycosides as a sweetener. As both rebaudioside A and stevioside are metabolized and excreted by similar pathways, with steviol being the common metabolite for both glycosides, the EFSA Panel agreed that the results of toxicology studies on either stevioside or rebaudioside A are applicable for the safety assessment of steviol glycosides. Considering the available safety data (*in vitro* and *in vivo* animal studies and some human tolerance studies), the EFSA Panel concluded that steviol glycosides, complying with JECFA specifications, are not carcinogenic, genotoxic, or associated with any reproductive/developmental toxicity. The EFSA Panel established an ADI for steviol glycosides, expressed as steviol equivalents, of 4 mg/kg bw/day based on the application of a 100-fold uncertainty factor to the NOAEL in the 2-year carcinogenicity study in the rat when administering 2.5% stevioside in the diet. This is equal to 967 mg stevioside/kg bw/day (corresponding to approximately 388 mg steviol equivalents/kg bw/day). Conservative estimates of steviol glycosides exposures both in adults and in children suggest that the ADI could possibly be exceeded by European consumers of certain ages and geographies at the maximum proposed use levels.

B. Safety Data on Rebaudioside A

Since 2008, several well-designed toxicology studies that followed the current regulatory and other guidelines for such studies have been reported on purified rebaudioside A. These investigations included additional subchronic studies in rats and one in dogs, mutagenicity studies, reproduction and developmental studies in rats, and comparative pharmacokinetic studies with stevioside in rats and humans, as well as additional clinical studies.

1. Subchronic Studies

Recently, Curry and Roberts (2008) reported the results of two repeat dose studies of rebaudioside A in Wistar rats. The results of these investigations suggest that administration of rebaudioside A to Han Wistar rats at dietary concentrations of up to 100,000 ppm (9938 and 11,728 mg/kg bw/day for males and females, respectively) for 4 weeks or 50,000 ppm (4161 and 4645 mg/kg bw/day for males and females, respectively) for 13 weeks did not present any evidence of systemic toxicity. In the 4-week study, rebaudioside A (97% purity) was administered at dietary concentrations of 0, 25,000, 50,000, 75,000 and 100,000 ppm to male and female rats. The NOAEL, including an evaluation of testes histopathology, was determined to be 100,000 ppm. In the 13-week study, Wistar rats were fed diets containing rebaudioside A at dietary concentrations of 0, 12,500, 25,000 and 50,000 ppm. In high-dose male and females groups, reductions in body weight gain attributable to initial taste aversion and lower caloric density of the

feed were observed. Inconsistent reductions in serum bile acids and cholesterol were attributed to physiological changes in bile acid metabolism due to excretion of high levels of rebaudioside A *via* the liver. All other hepatic function test results and liver histopathology were within normal limits. No significant changes in other clinical pathology results, organ weights and functional observational battery test results were noted. Macroscopic and microscopic examinations of all organs were unremarkable with respect to treatment-related findings. The NOAEL in the 13-week toxicity study was considered to be 50,000 ppm or approximately 4161 and 4645 mg/kg bw/day in male and female rats, respectively (Curry and Roberts, 2008).

In another 90-day dietary admix toxicity study, effects of rebaudioside A (99.5% purity) at target exposure levels of 500, 1000 and 2000 mg/kg bw/day were tested in Crl:CD(SD) rats (Nikiforov and Eapen, 2008; Eapen, 2007). Each group consisted of 20/animals/sex. No treatment related effects on clinical observations, food consumption, and functional observational or locomotor activity parameters were noted. There were no treatment related macroscopic, organ weight or microscopic findings. Significantly lower body weight gains were noted in the 2000 mg/kg bw/day group in males but not females. At the end of the dosing period, the body weight in males was 9.1% lower than the control group. Due to the small magnitude of difference from the control group value, the investigators did not consider this result to be adverse. The decrease was most likely due to the large proportion of the diet represented by the test material. The NOAEL was determined as ≥2000 mg/kg bw/day.

A 6-month dietary toxicity study in Beagle dogs (4/sex/group) was conducted to investigate the potential adverse effects of rebaudioside A (97.5% purity) at dosage levels of 0, 500, 1000 or 2000 mg/kg bw/day (Eapen, 2008). There were no unscheduled deaths during the course of the study. No treatment-related clinical observations were noted. Administration of rebaudioside A did not affect home cage, open field observations and functional observations and measurements. No differences in hematology findings, serum chemistry findings, or urinalysis findings between the groups were noted. Additionally, no treatment related gross necropsy observations, alterations in final body weight, alterations in organ weights, or histological changes were noted. The investigators concluded that no systemic toxicity of rebaudioside A was observed at dosage levels up to 2000 mg/kg bw/day and the assigned NOAEL was ≥2000 mg/kg bw/day.

2. Mutagenicity Studies

In a set of *in vitro* and *in vivo* genotoxicity assays covering mutation, chromosome damage and DNA strand breakage, rebaudioside A consistently and uniformly revealed negative results (Pezzuto et al, 1985; Nakajima, 2000a; Nakajima, 2000b; Sekihashi et al., 2002. These studies are critically reviewed by Brusick (2008). JECFA also reviewed an unpublished chromosome aberration assay of rebaudioside A in cultured mammalian cells (Nakajima, 2000a) and did not find increases in chromosome aberrations.

Additionally, FDA also reviewed three unpublished studies on rebaudioside A including a bacterial mutagenicity study (Wagner and Van Dyke, 2006), a mouse lymphoma study (Clarke, 2006) and a mouse micronucleus study (Krsmanovic and Huston, 2006) submitted by Merisant as part of the GRAS Notification. All three studies demonstrated lack of mutagenic or genotoxic activity. Additionally, Williams and Burdock (2009) also reported lack of genotoxicity in another set of

published studies that included *in vitro* mutagenicity assays with *Salmonella, E. coli,* and mouse lymphoma cells. These investigators also reported lack of *in vitro* clastogenic effects in Chinese hamster V79 cells and the absence of *in vivo* effects in a mouse micronucleus assay and a rat study for unscheduled DNA synthesis. The key mutagenicity testing results for rebaudioside A are summarized in Table 6.

3. Reproduction & Developmental Studies

In a two-generation reproductive toxicity study, rebaudioside A (97 % purity) at 0, 7,500, 12,500, and 25,000 ppm was administered in diet to male and female Han Wistar rats (Curry, et al., 2008). Administration of rebaudioside A was not associated with any signs of clinical toxicity or adverse effects on body weight, body weight gain, or food consumption. Similarly, administration of rebaudioside A did not affect reproductive performance parameters including mating performance, fertility, gestation lengths, estrous cycles, or sperm motility, concentration, or morphology in either the F_0 or F_1 generations. The survival and general condition of the F_1 and F_2 offspring, their pre-weaning reflex development, overall body weight gains, and the timing of sexual maturation, were not adversely affected by rebaudioside A treatment. The NOAEL for reproductive effects was 25,000 ppm and the NOAEL for the survival, development, and general condition of the offspring also was considered to be 25,000 ppm or 2,048 to 2,273 mg/kg body weight/day (the highest dose tested).

The results from two unpublished studies with rebaudioside A (Sloter 2008a, b) further support the above described findings from published studies. In a two-generation dietary reproduction study, four groups of male and female Crl:CD(SD) rats (30/sex/group) were fed either basal diet or the diet containing rebaudioside A (purity 95.7%) for at least 70 consecutive days prior to mating (Sloter 2008a). For the F_0 and F_1 generations rebaudioside A doses were 0, 500, 1000 and 2000 mg/kg/day. At initiation of study, F_0 animals were approximately 7 weeks of age. The test diet was offered to the offspring selected to become the F_1 generation following weaning [beginning on postnatal day (PND) 21]. The F_0 and F_1 males continued to receive rebaudioside A throughout mating, continuing through the day of euthanasia. The F_0 and F_1 females continued to receive rebaudioside A throughout mating, gestation and lactation until day of euthanasia. The authors concluded that there were no effects on reproduction in males or females as evaluated by estrus cycles, mating, fertility, conception or copulation indices, number of days between pairing and coitus, gestation length, and spermatogenic endpoints. Both for parental systemic and reproductive toxicity a dose level ≥2000 mg/kg bw/day (highest dose administered) was assigned to be the NOAEL.

In an embryo/fetal developmental toxicity study in rats (Sloter, 2008b), effects of rebaudioside A administered via gavage was tested. Rebaudioside A administration did not affect intrauterine growth and survival, and there were no test article-related fetal malformations or developmental variations at any dosage level. In the absence of maternal or developmental toxicity a dose level ≥2000 mg/kg bw/day (highest dose administered) was considered to be the NOAEL for maternal and embryo/fetal developmental toxicity.

Table 6. Mutagenicity Studies on Rebaudioside A

END-POINT	TEST SYSTEM	MATERIAL	PURITY (%)	CONCENTRATION / DOSE	RESULT	REFERENCE
Bacterial Mutagenicity	5 Salmonella strains with and without exogenous metabolic activation system	Reb A	99.5	1.5, 5.0, 15, 50, 150, 500, 1500 and 5000 µg per plate	No mutagenic response	Wagner and Van Dyke (2006)
Bacterial Mutagenicity	4 Salmonella strains and 1 *E. coli* strain with and without exogenous metabolic activation system	Reb A	95.6	Up to 5000 µg per plate	No mutagenic response	Williams and Burdock (2009)
Mouse Lymphoma	L5178Y/TK+/- mouse lymphoma mutagenesis assay in the absence and presence of exogenous metabolic activation system	Reb A	99.5	Cloning conc. of 500, 1000, 2000, 3000, 4000 and 5000 µg/mL	No mutagenic or clastogenic response	Clarke (2006)
Mouse Lymphoma	L5178Y/TK+/- mouse lymphoma mutagenesis assay in the absence and presence of exogenous metabolic activation system	Reb A	95.6	Up to 5000 µg/mL	No mutagenic or clastogenic response	Williams and Burdock (2009)
Chromosome Aberration	Human lymphocytes in absence and presence of exogenous metabolic activation system	Reb A	95.6	Up to 5000 µg/mL	No mutagenic or clastogenic response	Williams and Burdock (2009)
Mouse Micronucleus	Micronucleus study in groups of 5 male and 5 female ICR mice	Reb A	99.5	500, 1000 and 2000 mg/kg bw	No increase in micronuclei formation	Krsmanovic and Huston (2006)
Mouse Micronucleus	Micronucleus study in groups of 5 male and 5 female NMRI mice	Reb A	95.6	Up to 750 mg/kg bw	No increase in micronuclei formation	Williams and Burdock (2009)
Unscheduled DNA Synthesis	Unscheduled DNA synthesis in one group of 4 Wistar rats	Reb A	95.6	Up to 2000 mg/kg bw	No increase in unscheduled DNA synthesis	Williams and Burdock (2009)
DNA damage (comet assay)	Male BDF1 mouse stomach, colon, liver	Stevia extract	Stevio-side, 52%; Reb A, 22%	250 - 2000 mg/kg bw	Negative[a]	Sekihashi et al. (2002)
Chromosomal aberration	CHL/IU Chinese hamster lung fibroblasts	Reb A	NS	1.2 - 55 mg/mL	Negative[b]	Nakajima (2000a)
Micronucleus formation	BDF1 mouse bone marrow	Reb A	NS	500-2000 mg/kg bw per day for 2 days	Negative[c]	Nakajima (2000b)
Forward mutation	*S. typhimurium* TM677	Reb A	NS	10 mg/plate	Negative[b]	Pezzuto et al. (1985)

NS = Not specified.
[a] Sacrificed at 3 hours and 24 hours.
[b] With or without metabolic activation (source not specified in original monograph).
[c] Sacrificed at 30 hours after 2nd administration.

4. Clinical Studies on Rebaudioside A

In a four week randomized, double-blind, placebo controlled trial, hemodynamic effects of rebaudioside A at a dose of 1000 mg/day rebaudioside A (97% purity) or placebo in 100 individuals with normal and low-normal systolic blood pressure (SBP) and diastolic blood pressure (DBP) were investigated (Maki et al., 2008a). Subjects were predominantly female (76%, rebaudioside A and 82%, placebo) with a mean age of ~41 (range 18 to 73) years. At

baseline, mean resting, seated SBP/DBP was 110.0/70.3 mm Hg and 110.7/71.2 mm Hg for the rebaudioside A and placebo groups, respectively. Compared with placebo, administration of rebaudioside A did not significantly alter resting, seated SBP, DBP, mean arterial pressure (MAP), heart rate (HR) or 24-hour ambulatory blood pressure responses. The investigators concluded that consumption of 1000 mg/day of rebaudioside A produced no clinically important changes in blood pressure in healthy adults with normal and low-normal blood pressure.

In another trial, effects of 16 weeks of consumption of 1000 mg rebaudioside A (97% purity, n = 60) were compared to placebo (n = 62) in men and women (33-75 years of age) with type 2 diabetes mellitus (Maki, et al., 2008b). Changes in glycosylated hemoglobin levels did not differ significantly between the rebaudioside A (0.11 ± 0.06%, mean ± standard error) and placebo (0.09 ± 0.05%; p = 0.355) groups. Similarly, no significant (p > 0.05 for all) changes from baseline for rebaudioside A and placebo, respectively, in fasting glucose (7.5 ± 3.7 mg/dL and 11.2 ± 4.5 mg/dL), insulin (1.0 ± 0.64 μU/mL and 3.3 ± 1.5 μU/mL), and Cpeptide (0.13 ± 0.09 ng/mL and 0.42 ± 0.14 ng/mL) were noted. No treatment related changes in blood pressure, body weight, and fasting lipids were noted. Rebaudioside A was well-tolerated, and records of hypoglycemic episodes showed no excess versus placebo. Based on these results, the investigators suggested that chronic use of 1000 mg rebaudioside A does not alter glucose homeostasis or blood pressure in individuals with type 2 diabetes mellitus.

5. Absorption, Distribution, Metabolism & Excretion (ADME) Studies

In three recently completed studies, absorption and fate of rebaudioside A was systematically investigated in rats and humans.

For comparative purposes to determine whether toxicological studies conducted previously with stevioside would be applicable to the structurally-related glycoside, rebaudioside A, toxicokinetics and metabolism of rebaudioside A, stevioside, and steviol were examined in rats (Roberts and Renwick, 2008). Orally administered single doses of the radiolabelled compounds were extensively and rapidly absorbed with plasma concentration-time profiles following similar patterns for stevioside and rebaudioside A. Within 72 hours of administration, elimination of radioactivity from plasma was essentially complete. All plasma samples had similar metabolite profiles; the predominant radioactive component in all samples was steviol, with lower amounts of steviol glucuronide(s) and low levels of one or two other metabolites. Rebaudioside A, stevioside, and steviol were metabolized and excreted rapidly, with the majority of the radioactivity eliminated in the feces within 48 hours. Urinary excretion accounted for less than 2% of the administered dose for all compounds in both intact and bile duct-cannulated rats, and the majority of the absorbed dose was excreted *via* the bile. After administration of the compounds to intact and bile duct-cannulated rats, radioactivity in the feces was present primarily as steviol. The predominant radioactive compound detected in the bile of all cannulated rats was steviol glucuronide(s), indicating de-conjugation in the lower intestine. The authors concluded that the overall data on toxicokinetics and metabolism indicate that rebaudioside A and stevioside are handled in an almost identical manner in the rat after oral dosing.

In a randomized, double blind, cross-over study in healthy male subjects, Wheeler et al. (2008) assessed the comparative pharmacokinetics of steviol and steviol glucuronide following single oral doses of rebaudioside A and stevioside. Following administration of rebaudioside A or

stevioside, steviol glucuronide appeared in the plasma of all subjects, with median Tmax values of 12.00 and 8.00 hours post-dose, respectively. Steviol glucuronide was eliminated from the plasma, with similar $t_{1/2}$ values of approximately 14 hours for both compounds. Administration of rebaudioside A resulted in a significantly (approximately 22%) lower steviol glucuronide geometric mean C_{max} value (1472 ng/ml) than administration of stevioside (1886 ng/mL). The geometric mean AUC_{0-t} value for steviol glucuronide after administration of rebaudioside A (30788 ng*hr/mL) was approximately 10% lower than after administration of stevioside (34090 ng*hr/mL). Steviol glucuronide was excreted primarily in the urine of the subjects during the 72-hour collection period, accounting for 59% and 62% of the rebaudioside A and stevioside doses, respectively. No steviol glucuronide was detected in feces. Pharmacokinetic analysis indicated that both rebaudioside A and stevioside were hydrolyzed to steviol in the gastrointestinal tract prior to absorption. The majority of circulatory steviol was in the form of steviol glucuronide indicating rapid first-pass conjugation prior to urinary excretion. Only a small amount of steviol was detected in urine (rebaudioside A: 0.04%; stevioside: 0.02%). The investigators concluded that rebaudioside A and stevioside underwent similar metabolic and elimination pathways in humans with steviol glucuronide excreted primarily in the urine and steviol in the feces. No safety concerns were noted as determined by reporting of adverse events, laboratory assessments of safety or vital signs.

Another pharmacokinetic investigation was done as a toxicokinetic (TK) phase of a dietary study to determine the potential of rebaudioside A toxicity in rats at levels up to 2000 mg/kg bw/day (Sloter, 2008a). Rebaudioside A and total steviol were detected in peripheral blood of rats during daily administration of 2000 mg/kg bw/day of rebaudioside A at extremely low levels, with mean plasma concentrations of approximately 0.6 and 12 ug/mL, respectively. Estimates of absorbed dose for rebaudioside A and total steviol were approximately 0.02% and 0.06%, respectively, based on the amounts measured in urine collected over 24 hours in comparison to daily administered dietary dose to rats. Mean fecal rebaudioside A and measured hydrolysis products expressed as *Total Rebaudioside A Equivalents* compared to daily administered dose results in an estimate of percent of dose recovered ≈ 84%.

VI. DISCUSSION OF GRAS CRITERIA & REVIEWED INFORMATION

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."[11]

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."[12]

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 component elements:[13]

- 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 JECFA and the National Academy of Sciences.

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[11] See 21 CFR 170.3(i).
[12] See 21 CFR 170.30(a).
[13] See 62 FR 18938 (17 April 1997) which is accessible at http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/ucm083058.htm.

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

As noted below, the safety assessment to ascertain GRAS status for rebaudioside A with the defined food uses meets FDA criteria for reasonable certainty of no harm by considering both the technical and common knowledge elements.

B. Discussion of Expert Safety Reviews of Steviol Glycosides

Because of their sweetness characters, steviol glycosides are unique as they have viable uses as a non-nutritive sweetener in foods.[14] Periodic reviews by JECFA over the years indicate the progress of knowledge on the toxicology of steviol glycosides. Several early safety-related studies on these compounds were performed on crude extracts of stevia. These studies also included multiple investigations with *in vivo* and *in vitro* models which explored the biological activity of stevia extracts at high doses or high concentrations. These early investigations raised several concerns, including impairment of fertility, renal effects, interference with glucose metabolism, and inhibition of mitochondrial enzymes. In recent years as more and more studies were performed on purified glycosides, the toxicology profile of steviol glycosides eventually proved to be rather unremarkable. A number of subchronic, chronic and reproductive studies have been conducted in laboratory animals. These studies were well designed with appropriate dosing regimens and adequate numbers of animals to maximize the probability of detection of important effects. Notably, the initially reported concerns related to the effects of stevia leaves or crude extracts on fertility were refuted by the well-designed reproductive studies with purified steviol glycosides. All other concerns failed to manifest themselves at the doses employed in the long-term rat studies.

As discussed in Section V, at its fifty-first meeting, JECFA determined that there were adequate chronic studies in rats, particularly the study by Toyoda et al. (1997), to establish a temporary ADI of 0 - 2 mg/kg bw/day with an adequate margin of safety. The committee also critically reviewed the lack of carcinogenic response in well-conducted studies. These studies justified the Committee conclusion that the *in vitro* mutagenic activity of steviol did not present a risk of carcinogenic effects *in vivo* and, therefore, all common steviol glycosides which share the same basic metabolic and excretory pathway and that the use of high purity preparations of various steviol glycosides are safe to use as a sugar substitute. Subsequently, the additional clinical data reviewed by JECFA allowed the Committee to establish a permanent ADI of 0 - 4 mg/kg bw/day

[14] It has also been reported that steviol glycosides may have pharmacological properties, which can be used to treat certain disease conditions such as hypertension and Type 2 diabetes. Chatsudthipong and Muanprasat (2009) published a comprehensive review where they note that such therapeutic applications have not been firmly established as being due to steviol glycosides. The reviewers point out that the effects occur at higher doses than would be used for sweetening purposes. Furthermore, many effects noted in older studies may have been due to impurities in preparations that do not meet the contemporary purity specifications established by JECFA for use as a sweetener. If oral doses of steviol glycosides impart pharmacological effects, such effects would undoubtedly occur due to actions of the principle metabolite, steviol, but the pharmacological effects of steviol have not been comprehensively investigated.

(based on steviol equivalents) or 0 - 12 mg/kg bw for rebaudioside A. The GRAS Expert Panel critically reviewed the JECFA assessment and agrees with this reasoning.

The Panel also noted from a recent study that DNA damage was seen in a variety of organs as assessed by comet assay in rats given drinking water containing 4 mg/mL steviol glycosides for up to 45 days (Nunes et al., 2007). The methodology used in this study was questioned by several experts in the field (Geuns, 2007; Williams, 2007; Brusick, 2008). The Panel has reviewed the cited publications and agrees and discounts the importance of the Nunes et al. (2007) study.

The Panel has reviewed the findings from human clinical studies. The Panel noted that as regards to the clinical effects noted in humans, in order to corroborate the observations in these studies that these effects of steviol glycosides only occur in patients with either elevated blood glucose or blood pressure (or both), JECFA called for studies in individuals that are neither hypertensive nor diabetic (WHO, 2006). The new data presented to JECFA and also published by Barriocanal et al. (2008) demonstrate the lack of pharmacological effects of steviol glycosides at 11 mg/kg bw/day in normal individuals or approximately slightly more than 4 mg/kg bw on the basis of steviol equivalents. It is possible that JECFA may also have reviewed the preliminary results associated with the recently published clinical studies on rebaudioside A (Maki et al., 2008a, b). The Panel concludes that there will be no effects on blood pressure and glucose metabolism in humans at the doses of rebaudioside A expected from its use in food as a non-nutritive sweetener.

JECFA's review also included anticipated dietary patterns and the use concentrations expected in various foods in order to calculate an estimated daily intake (EDI) (WHO, 2003, 2006). Based on the assumption of 100% substitution of steviol glycosides for sugar, an EDI of 5 mg/kg bw/day of steviol was calculated for US consumption. JECFA noted that the replacement estimates were highly conservative and that this calculated intake of steviol glycosides (as steviol) would more likely be 20–30% of these values. Except for the scenario developed by JECFA with 100% replacement of sugars by steviol glycosides, and as discussed in Section IV.C and summarized in Table 4, the highest dietary estimate for use in foods for rebaudioside A is 4.7 mg/kg bw/day. The Panel agrees with the JECFA ADI of 4 mg/kg bw/day based on steviol equivalents which corresponds to 12 mg/kg bw/day for rebaudioside A and notes that the estimates as contained in Table 4 of anticipated dietary intake are below the ADI.

C. Discussion of Safety of Rebaudioside A[15]

Since July 2008, over ten papers describing the results of a comprehensive research program by different groups on rebaudioside A have been published. These and some other unpublished studies formed the basis of the two initial GRAS notifications to FDA each by Cargill (GRN 253) and Merisant (GRN 252). Prior to this, a limited number of toxicology studies specifically on

[15] Questions about the safety of rebaudioside A were previously raised by Huxtable (2002) and Kobylewski and Eckhert (2008). Their respective concerns, as well as opposing views supporting the safety of designated food uses of rebaudioside A expressed by Expert Panels have been outlined in other GRAS notifications that were submitted to FDA. A more detailed account can be found in GRAS notifications 278, 287, 303, and 304.

rebaudioside A were conducted. Even before these new studies were completed and as noted in the previous section, JECFA concluded that seven common steviol glycosides are safe for use as sweetener preparations when present in any combination as long as the combined purity of 95% or more was established.

Since a majority of the previous pharmacokinetic research was conducted with steviol glycosides, the presumed strategy adopted for the more recent research on rebaudioside A was to conduct a limited number of well-designed and executed toxicology studies on rebaudioside A itself and to demonstrate in rats and in humans that it is handled pharmacokinetically similarly to stevioside. This approach appears to have been undertaken to justify the JECFA-generated ADI without having to conduct a chronic study in rats with rebaudioside A. Additionally, the Merisant group conducted three mutagenicity assays on rebaudioside A that FDA generally considers to be most predictive for carcinogenicity potential. The Cargill group conducted two clinical studies to assure that rebaudioside A does not have potentially problematic pharmacological effects on blood glucose and blood pressure.

In a review article, Carakostas et al. (2008) summarized the most recent research on rebaudioside A. This review summarized the findings of the Cargill research program as follows:

- Steviol glycosides, rebaudioside A, and stevioside are not genotoxic *in vitro*.
- In well-conducted *in vivo* assays, steviol glycosides, rebaudioside A, and stevioside have not been found to be genotoxic.
- A report indicating that stevioside produces DNA breakage *in vivo* appears to be flawed (Nunes, et al., 2007) and was improperly interpreted as a positive response.
- Steviol genotoxicity in mammalian cells is limited to *in vitro* tests that may be affected by excessive concentrations of the compound.
- The primary evidence for steviol genotoxicity is derived from very specific bacterial tests or purified plasmid DNA that lack DNA repair capabilities.
- Stevioside is not a carcinogen or cancer promoter in well-conducted rodent chronic bioassays.
- The pharmacokinetic similarity between rebaudioside A and stevioside justifies the use of the ADI established by JECFA that was determined on studies employing stevioside as the main component as the ADI for rebaudioside A.
- The dietary levels expected from consumption of rebaudioside A as a total replacement of sugar (Renwick, 2008) are less than the ADI and, therefore, there is no safety concern for consumers.

The Panel concurs that the consumption estimates described by both JECFA and Renwick (2008) very conservatively represent a potential high user of rebaudioside A if this non-nutritive sweetener becomes widely available in food. As part of the present GRAS evaluation, the Panel adopts the JECFA EDI for application to Layn's purified rebaudioside A (≥97%).

Regarding the available aggregate safety information, the Panel has concluded that JECFA has critically and extensively evaluated the use of steviol glycosides in foods and agrees that, at the present time, the ADI for steviol glycosides of adequate purity as defined by JECFA specifications has been properly determined to be 4 mg/kg bw/person as steviol equivalents, which corresponds to 12 mg/kg bw/day for rebaudioside A on a dry weight basis. The Panel agrees that unwanted pharmacological effects are not likely to occur at this level and that high consumers of

rebaudioside A are not likely to exceed this level. Therefore, the Panel adopts the JECFA-derived ADI as a safe exposure for rebaudioside A and that food uses meeting the specifications within the limits determined by this esteemed international body of food safety experts can be considered to be generally recognized as safe (GRAS).

The Panel recognizes that JECFA is composed of dozens of scientists that are internationally known experts on food ingredient safety that have established ADIs for food ingredients over the last 40 years. Both Merisant and Cargill took rather rigorous scientific approaches to demonstrate the safety of rebaudioside A. The studies were equally well conducted. The safety profiles compiled by Merisant and Cargill differ somewhat, yet the results are complementary and are mutually reinforcing of rebaudioside A safety.

The studies conducted by Cargill provided significant insight into the pharmacokinetics of rebaudioside A while demonstrating clinical safety of rebaudioside A regarding lack of effects on blood pressure and glucose metabolism that could result from doses expected from use in food. The Merisant notification augmented genotoxicity data in three systems recognized by FDA as good predictors of carcinogenic potential. Two of these assays were conducted in mouse systems. Additional mutagenicity and genotoxicity studies have been published on rebaudioside A (Williams and Burdock, 2009). Merisant added a subchronic study in dogs and a teratology study in rats. Both Cargill and Merisant relied on the JECFA ADI for steviol glycosides as determined largely by published chronic studies in rats. Both groups justified the use of the ADI on pharmacokinetic arguments showing the similarity of stevioside and rebaudioside A metabolism and excretion.

The Panel agrees with the conclusion of JECFA and the Cargill and Merisant Expert Panels that there are a sufficient number of good quality health and safety studies to support the determination that the intended use of purified preparations of steviol glycosides, including rebaudioside A, when added to food at levels up to full replacement of sugar on a sweetness equivalency basis, meets FDA's definition of safe.

D. Common Knowledge Elements of GRAS Determination

The first common knowledge element for a GRAS determination is that data and information relied upon to establish safety must be generally available; this is most commonly established by utilizing published, peer-reviewed scientific journals. The majority of studies reviewed as part of this safety assessment have been published in the scientific literature as reported in Section V. Most of the literature relied upon by JECFA has also been published, most importantly the chronic rat studies on steviol glycosides. JECFA did make limited use of unpublished studies, and they were summarized in the two JECFA monographs. Moreover, JECFA publicly releases the results of their safety reviews, and their meeting summaries and monographs are readily available on their website. Thus, these studies become generally available to the scientific community. JECFA only reviewed a limited number of studies conducted specifically on rebaudioside A. The collection of supporting data on rebaudioside A has recently been enhanced by a series of studies published during 2008 and cited earlier. The newest clinical studies that address JECFA's concern on unwanted pharmacological effects with steviol glycosides (Barriocanal et al., 2008) and with rebaudioside A (Maki et al., 2008 a, b) are also published in the peer-reviewed scientific literature.

The Panel recognizes that the safety of steviol glycoside in human foods has been the subject of interest for many years. In addition to the reported substantial history of consumption of stevia, especially in South America and Asia, many scientific studies have been conducted and published. Some of the earlier studies have raised concerns about the safety, and the Panel has given careful attention to such concerns. The overriding evidence has diminished the Panel's concerns based on better study designs, better execution, or simply updated investigations that better reflect state-of-the art toxicological principles and findings.

The remaining common knowledge element for a GRAS determination is that there must be a basis to conclude that there is consensus among qualified scientists about the safety of the substance with its intended use. The JECFA opinion largely meets the common knowledge test on its own. The Panel is cognizant of the scientific rigor and broad base of scientific expertise that resides with the prestigious JECFA. JECFA is composed of expert scientists from various regulatory agencies around the world, as well as other scientists chosen because of their specific expertise on various classes of food ingredients. In addition, FDA participated in the JECFA deliberations.

The JECFA conclusion has been reviewed and validated by other respected regulatory agencies including FSANZ, the Switzerland Office of Public Health, and France's Agence Francais De Securite Sanitaire Des Alimenta (FSANZ, 2008; Switzerland Office of Public Health, 2008; AFSSA, 2009). Furthermore, the favorable scientific opinion on the safety of steviol glycosides use as a sweetener in foods as issued by EFSA in 2010 reinforces the safety determinations of many other qualified organizations (EFSA, 2010). In addition, a number of individual well-respected scientists have indicated that steviol glycosides are safe for human consumption at doses in the range of the JECFA ADI (Xili et al., 1992; Toyoda et al., 1997; Geuns, 2003; Williams, 2007).

The common knowledge element has been embellished by the many respected scientists that participated in the Cargill-sponsored new research conducted on rebaudioside A, most notably Brusick and Renwick. An assertion of "general recognition of safety" was made by Carakostas et al. (2008). In summary, there are many diverse groups of scientists from all corners of the globe that together provide strong fulfillment of the consensus requirement. Of particular significance from the perspective of establishing consensus for the safety of high purity steviol glycosides are the mid-December 2008 "no questions" determinations by FDA for the GRAS notifications for rebaudioside A as submitted by Merisant and Cargill and the more recent comparable findings by FDA with the additional GRAS notifications cited elsewhere.

While the scientific conclusions are not unanimous regarding the safe human food uses of steviol glycosides, the Panel believes that a wide consensus does exist in the scientific community to support the GRAS conclusion on rebaudioside A as outlined in this evaluation. The broader scientific community has concluded that past concerns expressed by others over the years (Huxtable, 2002) and earlier safety issues noted by FDA have been resolved by newer data on more purified test materials and the rigid specifications for purity published by JECFA for steviol glycosides, including rebaudioside A. Indeed, scientists from FDA are members of JECFA and have not objected to the safety decision on steviol glycosides. There is also a wider consensus that the body of new research on rebaudioside A is sufficient as opposed to the small group of scientists that argue that more studies need to be done before the sweetener is made available in the US.

VII. CONCLUSIONS[16]

Layn's purified rebaudioside A (≥97%) as expressed on a dry weight basis is Generally Recognized As Safe when consumed as a general purpose non-nutritive sweetener in foods other than infant formulas and meat and poultry products when: (1) it is produced in accordance with FDA Good Manufacturing Practices requirements; (2) it meets or exceeds the JECFA purity specifications for steviol glycosides; and (3) it is consumed within the designated JECFA ADI of 12 mg/kg bw/day on a rebaudioside A basis. In order to remain within the designated ADI, it is important to observe good manufacturing practices principles in that the quantity of a substance added to food shall not exceed the amount reasonably required to accomplish its intended technical effect.

This declaration has been made in accordance with FDA's standard for food ingredient safety, i.e., reasonable certainty of no harm under the intended conditions of use.

(b) (6)

Richard C. Kraska, Ph.D., DABT
Chair

(b) (6)

Robert S. McQuate, Ph.D.

Madhusudan G. Soni, Ph.D., FACN

August 16, 2010

[16] 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. 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 panelists have extensive technical backgrounds in the evaluation of food ingredient safety. All three individuals have previously served on multiple GRAS Expert Panels. Dr. Kraska served as Chair of the Panel.

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APPENDIX A

Updated JECFA Specifications for Steviol Glycosides - 2010

STEVIOL GLYCOSIDES

Prepared at the 73rd JECFA (2010) and published in FAO JECFA Monographs 10 (2010), superseding specifications prepared at the 69th JECFA (2008) and published in FAO JECFA Monographs 5 (2008). An ADI of 0 - 4 mg/kg bw (expressed as steviol) was established at the 69th JECFA (2008).

SYNONYMS

INS no. 960

DEFINITION

The product is obtained from the leaves of *Stevia rebaudiana* Bertoni. The leaves are extracted with hot water and the aqueous extract is passed through an adsorption resin to trap and concentrate the component steviol glycosides. The resin is washed with a solvent alcohol to release the glycosides and the product is recrystallized from methanol or aqueous ethanol. Ion exchange resins may be used in the purification process. The final product may be spray-dried.

Stevioside and rebaudioside A are the component glycosides of principal interest for their sweetening property. Associated glycosides include rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside F, dulcoside A, rubusoside and steviolbioside which are generally present in preparations of steviol glycosides at levels lower than stevioside or rebaudioside A.

Chemical name

Stevioside: 13-[(2-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy] kaur-16-en-18-oic acid, β-D-glucopyranosyl ester

Rebaudioside A: 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester

C.A.S. number

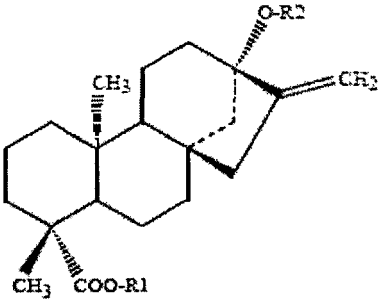
Stevioside: 57817-89-7
Rebaudioside A: 58543-16-1

Chemical formula

Stevioside: $C_{38}H_{60}O_{18}$
Rebaudioside A: $C_{44}H_{70}O_{23}$

Structural Formula

The nine named steviol glycosides:



Compound name	*R1*	*R2*
Stevioside	β-Glc	β-Glc-β-Glc(2→1)
Rebaudioside A	β-Glc	β-Glc-β-Glc(2→1) | β-Glc(3→1)
Rebaudioside B	H	β-Glc-β-Glc(2→1) | β-Glc(3→1)
Rebaudioside C	β-Glc	β-Glc-α-Rha(2→1) | β-Glc(3→1)
Rebaudioside D	β-Glc-β-Glc(2→1)	β-Glc-β-Glc(2→1) | β-Glc(3→1)
Rebaudioside F	β-Glc	β-Glc-β-Xyl(2→1) | β-Glc(3→1)
Dulcoside A	β-Glc	β-Glc-α-Rha(2→1)
Rubusoside	β-Glc	β-Glc
Steviolbioside	H	β-Glc-β-Glc(2→1)

Steviol (R1 = R2 = H) is the aglycone of the steviol glycosides. Glc, Rha and Xyl represent, respectively, glucose, rhamnose and xylose sugar moieties.

Formula weight

Stevioside: 804.88
Rebaudioside A: 967.03

Assay	Not less than 95% of the total of the nine named steviol glycosides on the dried basis.
DESCRIPTION	White to light yellow powder, odourless or having a slight characteristic odour. About 200 - 300 times sweeter than sucrose.
FUNCTIONAL USES	Sweetener
CHARACTERISTICS	
IDENTIFICATION	
Solubility (Vol. 4)	Freely soluble in water
Stevioside and rebaudioside A	The main peak in the chromatogram obtained by following the procedure in Method of Assay corresponds to either stevioside or rebaudioside A.
pH (Vol. 4)	Between 4.5 and 7.0 (1 in 100 solution)
PURITY	
Total ash (Vol. 4)	Not more than 1%
Loss on drying (Vol. 4)	Not more than 6% (105°, 2h)
Residual solvents (Vol. 4)	Not more than 200 mg/kg methanol and not more than 5000 mg/kg ethanol (Method I in Vol. 4, General Methods, Organic Components, Residual Solvents)
Arsenic (Vol. 4)	Not more than 1 mg/kg Determine by the atomic absorption hydride technique (Use Method II to prepare the test (sample) solution)
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Vol. 4 (under "General Methods, Metallic Impurities").
METHOD OF ASSAY	Determine the percentages of the individual steviol glycosides by HPLC (Vol. 4) under the following conditions. Reagents Acetonitrile: more than 95% transmittance at 210 nm. Standards Stevioside: more than 99.0% purity on the dried basis. Rebaudioside A: more than 99.0% purity on the dried basis. Mixture of nine steviol glycosides standard solution: Containing stevioside, rebaudioside A, rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside F, dulcoside A, rubusoside and

steviolbioside. This solution is diluted with water-acetonitrile (7:3) accordingly and is used for the confirmation of retention times. Standards are available from Wako Pure Chemical Industries, Ltd. Japan and ChromaDex, USA.

Standard solution
Accurately weigh 50 mg of stevioside and rebaudioside A standard into each of two 50-ml volumetric flasks. Dissolve and make up to volume with water-acetonitrile (7:3).

Sample solution
Accurately weigh 50-100 mg of sample into a 50-ml volumetric flask. Dissolve and make up to volume with water-acetonitrile (7:3).

Procedure
Inject 5 µl of sample solution under the following conditions.
Column: Capcell pak C_{18} MG II (Shiseido Co.Ltd) or Luna 5µ C18(2) 100A (Phenomenex) or equivalent (length: 250 mm; inner diameter: 4.6 mm, particle size: 5µm)
Mobile phase: 32:68 mixture of acetonitrile and 10 mmol/L sodium phosphate buffer (pH 2.6)
Flow rate: 1.0 ml/min
Detector: UV at 210 nm
Column temperature: 40°
Record the chromatogram for about 30 min.

Identification of the peaks and Calculation
Identify the peaks from the sample solution by comparing the retention time with the peaks from the mixture of nine steviol glycosides standard solution (see under figure). Measure the peak areas for the nine steviol glycosides from the sample solution. Measure the peak area for stevioside and rebaudioside A from their standard solutions.
Calculate the percentage of each of the eight steviol glycosides except rebaudioside A in the sample from the formula:

$$\%X = [W_S/W] \times [f_X A_X/A_S] \times 100$$

Calculate the percentage of rebaudioside A in the sample from the formula:

$$\%Rebaudioside\ A = [W_R/W] \times [A_X/A_R] \times 100$$

where
X is each steviol glycoside;
W_S is the amount (mg) calculated on the dried basis of stevioside in the standard solution;
W_R is the amount (mg) calculated on the dried basis of rebaudioside A in the standard solution;
W is the amount (mg) calculated on the dried basis of sample in the sample solution;
A_S is the peak area for stevioside from the standard solution;
A_R is the peak area for rebaudioside from the standard solution;

A_X is the peak area of X for the sample solution; and
f_X is the ratio of the formula weight of X to the formula weight of stevioside: 1.00 (stevioside), 1.20 (rebaudioside A), 1.00 (rebaudioside B), 1.18 (rebaudioside C), 1.40 (rebaudioside D), 1.16 (rebaudioside F), 0.98 (dulcoside A), 0.80 (rubusoside) and 0.80 (steviolbioside).

Calculate the percentage of total steviol glycosides (sum the nine percentages).

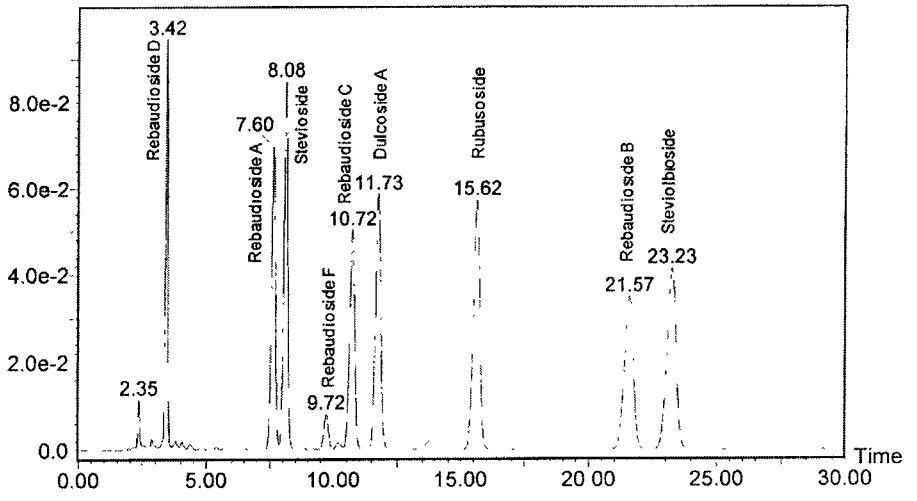


Figure. Chromatogram of mixture of nine steviol glycosides standard solution
Column: Capcell pak C_{18} MG II
Concentration: 0.5 mg/ml each except rebaudioside F (about 0.1 mg/ml)

APPENDIX B

Certificates & Analyses of Production Materials

B-1 Water Quality Certification – Absorbent Media

B-2 Water Quality Certification – Ion Exchange Resin

B-3 Analytical Test Results for Ethyl Alcohol

B-4 Analytical Test Results for Methyl Alcohol

B-1

Water Quality Association
International Headquarters & Laboratory

4151 Naperville Road
Lisle, Illinois 60532-3696 USA
Phone 630 505 0160
Fax 630 505 9637
www.wqa.org

A not-for-profit organization

GOLD SEAL CERTIFICATE

This is to certify that the drinking water system component herein has been independently tested and certified by the Water Quality Association in accordance with "Drinking Water System Components - Health Effects," NSF/ANSI-61. The material safety of the component listed has earned the Gold Seal.

Manufacturer:	SunResin Technology LTD
Address:	No 72 Keji 2nd Rd., Tianze Buldg Industrial Development Zone. Xian. Shaanxi 710075 CHINA
Model:	LXA-16
Product Type:	Adsorbent media
Size:	315-1250 microns
Water Contact Temp:	CLD
Water Contact Material:	SYN
AB 1953 Complaince:	Not Evaluated
Certificate Type:	Final
Issue Date:	Tuesday. December 08, 2009
Expiration Date:	Monday, June 30, 2014
Test Unit Number:	6316.0901C.01
Certificate Number:	CRT.081909.63160901C01.R1

(b) (6)

Joseph F. Harrison, P.E., CWS-VI

15 December 2009

Effective Date

B-2

Water Quality Association
International Headquarters & Laboratory

4151 Naperville Road
Lisle, Illinois 60532-3696 USA
Phone 630-505-0160
Fax 630-505-9637
www.wqa.org

A not-for-profit organization

GOLD SEAL CERTIFICATE

This is to certify that the drinking water system component herein has been independently tested and certified by the Water Quality Association in accordance with "Drinking Water System Components - Health Effects," NSF/ANSI-61. The material safety of the component listed has earned the Gold Seal.

Manufacturer:	SunResin Technology LTD
Address:	No 72 Keji 2nd Rd., Tianze Buldg Industrial Development Zone, Xian, Shaanxi 710075 CHINA
Model:	SC 130
Product Type:	Ion Exchange Resin
Size:	315-1250 microns
Water Contact Temp:	CLD
Water Contact Material:	SYN
Listing Notes:	This resin is certified with a minimum flow restriction of 0.10 gpm per cubic feet of media when used in a Point of Entry System.
AB 1953 Complaince:	Not Evaluated
Certificate Type:	Final
Issue Date:	Tuesday, December 08, 2009
Expiration Date:	Wednesday, June 25, 2014
Test Unit Number:	6316.0901C.02
Certificate Number:	CRT.081909.63160901C02.R1

(b) (6)

Joseph F. Harrison, P.E., CWS-VI

15 December 2009

Effective Date

B-3

中国广州分析测试中心
CHINA NATIONAL ANALYTICAL CENTER, GUANGZHOU

检 测 报 告
TEST REPORT

委托单位：桂林莱茵生物技术有限公司
Customer Guilin Layn Biotechnology Co., Ltd.
样品名称： 酒精 100526-01
Sample Name Ethyl Alcohol 100526-01
受检样品数量： 1
Quantity Received 1
样品批号： ——
Sample Lot №./Batch №: ——

报告编号： 2010003701-2 b
Report №. 2010003701-2 b
接样日期： 2010年6月12日
Sample Receiving Date 12-Jun-10
检测日期： 6月12日 至 6月25日
Testing Period 12-Jun to 25-Jun
签发日期： 2010年6月25日
Date for Reporting 25-Jun-10

分析检测结果
Test Results

分析项目 Item	检测结果 Result	计量单位 Unit	检测方法 Method
含量 Assay	>94.9	%	FCC V
杂醇油 Fusel Oil	通过试验 Pass	—	FCC V
酸度(以CH_3COOH计) Acidity(as acetic acid)	<0.003	%	FCC V
碱度(以NH_3计) Alkalinity(as NH_3)	<3	mg/kg	FCC V
铅 Lead (Pb)	<0.5	mg/kg	GFAAS(FCC V)
不挥发物 Nonvolatile Residue	<0.003	%	FCC V
水溶性 Solubility in Water	通过试验 Pass	—	FCC V
与硫酸不溶物 Substances Darkened by Sulfuric Acid	通过试验 Pass	-	FCC V
高锰酸钾消耗量 Wastage of potassium permanganate	通过试验 Pass	—	FCC V
丙酮，异丙醇 Ketones, Isopropyl Alcohol	通过试验 Pass	—	FCC V
甲醇 Methanol	通过试验 Pass	—	FCC V

备 注 Note	Gravity= 0.7902g/cm³ at 25℃
声 明 Declaration	(1)未经本中心的书面批准不得部分复制本报告（全部复制除外）。 Without the written approval of the laboratory,the report shall not be reproduced except in full. (2)送检样品，只对来样负责。 For sample submitted by customer, the results of the report is responsible for the sample only.

编审： 廖耀承 (b) (6) 批准： 车德海 (b) (6) 盖章：
Organizer and Checker Technique Controller Official Seal
职务： 工程师 职务： 研究员
Business: Engineer Business: Research Professor&quot

广州分析测试中心 检测报告专用章 (1)

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B-4

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中国广州分析测试中心
CHINA NATIONAL ANALYTICAL CENTER, GUANGZHOU

To: 黄华国

检 测 报 告
TEST REPORT

委托单位：桂林莱茵生物技术有限公司
Customer Guilin Layn Biotechnology Co., Ltd.
样品名称：甲醇 100605-01
Sample Name Methyl Alcohol 100605-01
受检样品数量：1
Quantity Received 1
样品批号：—
Sample Lot № /Batch №: ——

报告编号：2010003701-1 b
Report №. 2010003701-1 b
接样日期：2010年6月12日
Sample Receiving Date 12-Jun-10
检测日期：6月12日 至 6月25日
Testing Period 12-Jun to 25-Jun
签发日期：2010年6月25日
Date for Reporting 25-Jun-10

分析检测结果
Test Results

分析项目 Item	检测结果 Result	计量单位 Unit	检测方法 Method
含量Assay	>99.8%	g/cm	FCC V
酮和醛 Acetone and Aldehydes	<0.003	mg/kg	FCC V
酸度(以HCOOH计) Acidity(as formic acid)	<0.0015	%	FCC V
碱度(以NH_3计) Alkalinity(as NH_3)	<3	mg/kg	FCC V
铅 Lead (Pb)	<0.5	mg/kg	GFAAS(FCC V)
不挥发物 Nonvolatile Residue	<10	mg/kg	FCC V
易碳化物 Readily Carbonizable Substances	通过试验 Pass	—	FCC V
水溶性 Solubility in Water	通过试验 Pass	—	FCC V
高锰酸钾消耗量 Wastage of potassium permanganate	通过试验 Pass	—	FCC V
水分 Moisture	0.2%	%	FCC V
备注 Note	Gravity=0.7880g/cm³ at 25℃.		

声明 Declaration
(1)未经本实验室的书面批准不得部分复制本报告（全部复制除外）。
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(2)送检样品，只对来样负责。
For samples submitted by customer, the results of the report is responsible for the samples only.

编审：廖耀承 (b) (6)
Organizer and Checker
职务：工程师
Business: Engineer

批准：牟德海 (b) (6)
Technique Controller
职务：研究员
Business: Research Professor&quot

盖章 Official Seal
检测报告专用章 (1)

CNACF WI(3)-C 032.08.01 F00

第 2 页 共 3 页

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APPENDIX C

Certificates of Analyses for Layn's Purified Rebaudioside A Production Lots

C-1 Specifications of Rebaudioside A from Batch No. 100219-01

C-2 Specifications of Rebaudioside A from Batch No. 100219-02

C-3 Specifications of Rebaudioside A from Batch No. 100219-03

C-4 Specifications of Rebaudioside A from Batch No. 100219-04

C-5 Specifications of Rebaudioside A from Batch No. 100219-05

C-1 Specifications of Rebaudioside A from Batch No. 100219-01

Product Name:	Stevia rebaudiana P.E.	Manufacture Date:	2010-FEB-19
Latin Name:	Stevia rebaudiana (Bertoni) Hemsl	Testing Date:	2010-FEB-19
Batch Number:	100219-01	Expire Date:	2012-FEB-18
Quantity:		Shelf Life:	2 years

ATTRIBUTES	SPECIFICATION	METHODS	RESULTS
DESCRIPTION			
APPEARANCE	White powder	Visual	COMPLIES
FOREIGN MATTER	ABSENT	Visual	COMPLIES
ODOR	CHARACTERISTIC	OLFACTORY	COMPLIES
Taste	300-400 FOLD SWEETER THAN SUGAR	GUSTATORY	COMPLIES
SOLUBILITY	SOLUBILITY IN WATER AND ALCOHOL	USP32<561>	COMPLIES
CHEMICAL TEST			
REBAUDIOSIDE A	≥97%	JECFA, 2007	98.57%
LOSS ON DRYING	≤5%	USP32 <731>	1.12%
Total ASH	< 1%	USP32 <561>	0.16%
Optical Rotation	-30° to-38°	USP32 <781>	COMPLIES
pH(1% solution)	4.5-7.0	USP32<791 >	COMPLIES
SOLVENT RESIDUE			
ETHANOL	< 500 mg/kg	USP32 <467>	COMPLIES
METHANOL	< 100 mg/kg	USP32 <467>	COMPLIES
HEAVY METAL			
Heavy metals	< 3mg/kg	USP32<231>	COMPLIES
Arsenic	< 0.05 mg/kg	AOAC 993.14	COMPLIES
Lead	< 0.5 mg/kg	AOAC 993.14	COMPLIES
MICRO-BIOLOGICAL TEST			
TOTAL PLATE COUNT	<1000 cfu/g	USP32 <61>	COMPLIES
TOTAL COLIFORM	<100 cfu/g	USP32 <61>	COMPLIES

MOLD AND YEAST	<100 cfu/g	USP32 <61>	COMPLIES
SALMONELLA	NEGATIVE	USP32 <61>	COMPLIES
E.COLI	NEGATIVE	USP32 <61>	COMPLIES
STAPHYLOCOCCUS AUREUS	NEGATIVE	USP32 <61>	COMPLIES
PESTICIDES RESIDUE ANALYSIS			
PESTICIDES	None detected (see Appendix C)	USP32<561>	COMPLIES

C-2 Specifications of Rebaudioside A from Batch No. 100219-02

Product Name:	Stevia rebaudiana P.E.	**Manufacture Date:**	2010-FEB-19
Latin Name:	Stevia rebaudiana (Bertoni) Hemsl	**Testing Date:**	2010-FEB-19
Batch Number:	100219-02	**Expire Date:**	2012-FEB-18
Quantity:		**Shelf Life:**	2 years

ATTRIBUTES	SPECIFICATION	METHODS	RESULTS
DESCRIPTION			
APPEARANCE	White powder	Visual	COMPLIES
FOREIGN MATTER	ABSENT	Visual	COMPLIES
ODOR	CHARACTERISTIC	OLFACTORY	COMPLIES
Taste	300-400 FOLD SWEETER THAN SUGAR	GUSTATORY	COMPLIES
SOLUBILITY	SOLUBILITY IN WATER AND ALCOHOL	USP32<561>	COMPLIES
CHEMICAL TEST			
REBAUDIOSIDE A	≥97%	JECFA, 2007	98.62%
LOSS ON DRYING	≤5%	USP32 <731>	0.87%
Total ASH	< 1%	USP32 <561>	0.08%
Optical Rotation	-30° to-38°	USP32 <781>	COMPLIES
pH(1% solution)	4.5-7.0	USP32<791 >	COMPLIES
SOLVENT RESIDUE			
ETHANOL	< 500 mg/kg	USP32 <467>	COMPLIES
METHANOL	< 100 mg/kg	USP32 <467>	COMPLIES
HEAVY METAL			
Heavy metals	< 3mg/kg	USP32<231>	COMPLIES
Arsenic	< 0.05 mg/kg	AOAC 993.14	COMPLIES
Lead	< 0.5 mg/kg	AOAC 993.14	COMPLIES
MICRO-BIOLOGICAL TEST			
TOTAL PLATE COUNT	<1000 cfu/g	USP32 <61>	COMPLIES
TOTAL COLIFORM	<100 cfu/g	USP32 <61>	COMPLIES
MOLD AND YEAST	<100 cfu/g	USP32 <61>	COMPLIES

SALMONELLA	NEGATIVE	USP32 <61>	COMPLIES
E.COLI	NEGATIVE	USP32 <61>	COMPLIES
STAPHYLOCOCCUS AUREUS	NEGATIVE	USP32 <61>	COMPLIES
PESTICIDES RESIDUE ANALYSIS			
PESTICIDES	None detected	USP32<561>	COMPLIES

C-3 Specifications of Rebaudioside A from Batch No. 100219-03

Product Name:	Stevia rebaudiana P.E.	Manufacture Date:	2010-FEB-19
Latin Name:	Stevia rebaudiana (Bertoni) Hemsl	Testing Date:	2010-FEB-19
Batch Number:	100219-03	Expire Date:	2012-FEB-18
Quantity:		Shelf Life:	2 years

ATTRIBUTES	SPECIFICATION	METHODS	RESULTS
DESCRIPTION			
APPEARANCE	White powder	Visual	COMPLIES
FOREIGN MATTER	ABSENT	Visual	COMPLIES
ODOR	CHARACTERISTIC	OLFACTORY	COMPLIES
Taste	300-400 FOLD SWEETER THAN SUGAR	GUSTATORY	COMPLIES
SOLUBILITY	SOLUBILITY IN WATER AND ALCOHOL	USP32<561>	COMPLIES
CHEMICAL TEST			
REBAUDIOSIDE A	≥97%	JECFA, 2007	98.77%
LOSS ON DRYING	≤5%	USP32 <731>	1.22%
Total ASH	< 1%	USP32 <561>	0.13%
Optical Rotation	-30° to -38°	USP32 <781>	COMPLIES
pH(1% solution)	4.5-7.0	USP32<791>	COMPLIES
SOLVENT RESIDUE			
ETHANOL	< 500 mg/kg	USP32 <467>	COMPLIES
METHANOL	< 100 mg/kg	USP32 <467>	COMPLIES
HEAVY METAL			
Heavy metals	< 3mg/kg	USP32<231>	COMPLIES
Arsenic	< 0.05 mg/kg	AOAC 993.14	COMPLIES
Lead	< 0.5 mg/kg	AOAC 993.14	COMPLIES
MICRO-BIOLOGICAL TEST			
TOTAL PLATE COUNT	<1000 cfu/g	USP32 <61>	COMPLIES
TOTAL COLIFORM	<100 cfu/g	USP32 <61>	COMPLIES
MOLD AND YEAST	<100 cfu/g	USP32 <61>	COMPLIES

SALMONELLA	NEGATIVE	USP32 <61>	COMPLIES
E.COLI	NEGATIVE	USP32 <61>	COMPLIES
STAPHYLOCOCCUS AUREUS	NEGATIVE	USP32 <61>	COMPLIES
PESTICIDES RESIDUE ANALYSIS			
PESTICIDES	None detected	USP32<561>	COMPLIES

C-4 Specifications of Rebaudioside A from Batch No. 100219-04

Product Name:	Stevia rebaudiana P.E.	Manufacture Date:	2010-FEB-19
Latin Name:	Stevia rebaudiana (Bertoni) Hemsl	Testing Date:	2010-FEB-19
Batch Number:	100219-04	Expire Date:	2012-FEB-18
Quantity:		Shelf Life:	2 years

ATTRIBUTES	SPECIFICATION	METHODS	RESULTS
DESCRIPTION			
APPEARANCE	White powder	Visual	COMPLIES
FOREIGN MATTER	ABSENT	Visual	COMPLIES
ODOR	CHARACTERISTIC	OLFACTORY	COMPLIES
Taste	300-400 FOLD SWEETER THAN SUGAR	GUSTATORY	COMPLIES
SOLUBILITY	SOLUBILITY IN WATER AND ALCOHOL	USP32<561>	COMPLIES
CHEMICAL TEST			
REBAUDIOSIDE A	≥97%	JECFA, 2007	98.70%
LOSS ON DRYING	≤5%	USP32 <731>	1.01 %
Total ASH	< 1%	USP32 <561>	0.08%
Optical Rotation	-30° to -38°	USP32 <781>	COMPLIES
pH(1% solution)	4.5-7.0	USP32<791 >	COMPLIES
SOLVENT RESIDUE			
ETHANOL	< 500 mg/kg	USP32 <467>	COMPLIES
METHANOL	< 100 mg/kg	USP32 <467>	COMPLIES
HEAVY METAL			
Heavy metals	< 3mg/kg	USP32<231>	COMPLIES
Arsenic	< 0.05 mg/kg	AOAC 993.14	COMPLIES
Lead	< 0.5 mg/kg	AOAC 993.14	COMPLIES
MICRO-BIOLOGICAL TEST			
TOTAL PLATE COUNT	<1000 cfu/g	USP32 <61>	COMPLIES
TOTAL COLIFORM	<100 cfu/g	USP32 <61>	COMPLIES
MOLD AND YEAST	<100 cfu/g	USP32 <61>	COMPLIES

SALMONELLA	NEGATIVE	USP32 <61>	COMPLIES
E.COLI	NEGATIVE	USP32 <61>	COMPLIES
STAPHYLOCOCCUS AUREUS	NEGATIVE	USP32 <61>	COMPLIES
PESTICIDES RESIDUE ANALYSIS			
PESTICIDES	None detected	USP32<561>	COMPLIES

C-5 Specifications of Rebaudioside A from Batch No. 100219-05

Product Name:	Stevia rebaudiana P.E.	Manufacture Date:	2010-FEB-19
Latin Name:	Stevia rebaudiana (Bertoni) Hemsl	Testing Date:	2010-FEB-19
Batch Number:	100219-05	Expire Date:	2012-FEB-18
Quantity:		Shelf Life:	2 years

ATTRIBUTES	SPECIFICATION	METHODS	RESULTS
DESCRIPTION			
APPEARANCE	White powder	Visual	COMPLIES
FOREIGN MATTER	ABSENT	Visual	COMPLIES
ODOR	CHARACTERISTIC	OLFACTORY	COMPLIES
Taste	300-400 FOLD SWEETER THAN SUGAR	GUSTATORY	COMPLIES
SOLUBILITY	SOLUBILITY IN WATER AND ALCOHOL	USP32<561>	COMPLIES
CHEMICAL TEST			
REBAUDIOSIDE A	≥97%	JECFA, 2007	98.77%
LOSS ON DRYING	≤5%	USP32 <731>	0.84 %
Total ASH	< 1%	USP32 <561>	0.09%
Optical Rotation	-30° to -38°	USP32 <781>	COMPLIES
pH(1% solution)	4.5-7.0	USP32<791 >	COMPLIES
SOLVENT RESIDUE			
ETHANOL	< 500 mg/kg	USP32 <467>	COMPLIES
METHANOL	< 100 mg/kg	USP32 <467>	COMPLIES
HEAVY METAL			
Heavy metals	< 3mg/kg	USP32<231>	COMPLIES
Arsenic	< 0.05 mg/kg	AOAC 993.14	COMPLIES
Lead	< 0.5 mg/kg	AOAC 993.14	COMPLIES
MICRO-BIOLOGICAL TEST			
TOTAL PLATE COUNT	<1000 cfu/g	USP32 <61>	COMPLIES
TOTAL COLIFORM	<100 cfu/g	USP32 <61>	COMPLIES

MOLD AND YEAST	<100 cfu/g	USP32 <61>	COMPLIES
SALMONELLA	NEGATIVE	USP32 <61>	COMPLIES
E.COLI	NEGATIVE	USP32 <61>	COMPLIES
STAPHYLOCOCCUS AUREUS	NEGATIVE	USP32 <61>	COMPLIES
PESTICIDES RESIDUE ANALYSIS			
PESTICIDES	None detected	USP32<561>	COMPLIES

APPENDIX D

Pesticide Residue Analysis for Layn's High Purity Rebaudioside A

Pesticide Residue Tested	LOQ(mg/kg)
2-Phenylphenol	0.01
Acetochlor	0.02
Aclonifen	0.05
Aldrin	0.02
Ametryne	0.02
Aramite	0.05
Atrazine	0.02
Benfluralin	0.02
Bifenthrin	0.01
Biphenyl	0.02
Bromopropylate	0.02
Butachlor	0.01
Cadusafos	0.04
Captan	0.05
Chlorbenside	0.04
Chlordane(Sum)	
Chlordane,alpha	0.01
Chlordane,gamma	0.01
Chlorfenapyr	0.05
Chlorfenvinphos	0.02
Chlorobenzilate	0.01
Chlorothalonil	0.01
Chlorpyrifos	0.02
Chlorpyrifos-methyl	0.01
Chlorthal-dimethyl	0.01
Cyanazine	0.04
Cyanophos	0.04
Cyfluthrin	0.05
Cyhalothrin lambda	0.02
Cypermethrin	0.05
DDD, o, p'-	0.01
DDD,p, p'-	0.01
DDE ,o, p'-	0.01

DDE ,p, p′-	0.01
DDT(Sum)	
DDT, o, p′-	0.01
DDT, p, p′-	0.01
Deltamethrin	0.06
Dichlofluanid	0.02
Dichlorobenzophenone o, p′	0.02
Dichlorobenzophenone p, p′	0.02
Dichlorvos	0.05
Dicloran	0.05
Dicofol(Sum)	
Dicofol, o, p′-	0.02
Dicofol, p, p′-	0.02
Dieldrin	0.02
Dieldrin(Sum)	
Diphenylamine	0.02
Edifenphos	0.02
Endosulfan(Sum)	
Endosulfan,alpha-	0.05
Endosulfan,beta-	0.05
Endosulfan,sulfat-	0.02
Endrin	0.04
Ethion	0.04
Etridiazole	0.04
Etrimfos	0.02
Famoxadone	0.04
Fenamiphos	0.05
Fenitrothion	0.04
Fenpropathrin	0.04
Fenthion	0.04
Fenvalerate(RR-/SS)	0.04
Fenvalerate(RS-/SR)	0.04
Flucythrinate	0.05
Flucmioxazin	0.05
Fluquinconazole	0.04
Fluvalinate-tau	0.02

Folpet	0.05
Fonofos	0.04
Formothion	0.06
HCB	0.01
HCH(Sum, without Lindan)	
HCH gamma(Lindan)	0.02
HCH,alpha-	0.02
HCH,beta-	0.02
HCH,delta-	0.02
HCH,epsilon-	0.02
Heptachlor	0.01
Heptachlor(Sum)	
Heptachlor epoxide cis	0.01
Heptachlor epoxide trans	0.02
Heptenophos	0.02
Isocarbophos	0.04
Isodrin	0.04
Isofenphos	0.04
Isofenphos-methyl	0.01
Isoprothiolane	0.02
Kresoxim-methyl	0.01
Malaoxon	0.05
Malathion(Sum)	
Mecarbam	0.04
Mepronil	0.04
Methidathion	0.04
Metribuzin	0.04
Mevinphos	0.02
Nitrofen	0.02
Nitrothal-isopropyl	0.02
Octachlorodipropyl ether(S-421)	0.05
Ofurace	0.04
Oxadiazon	0.02
Oxyfluorfen	0.02
Paclobutrazol	0.04
Parathion	0.06

Parathion-methyl	0.04
Permethrin	0.04
Phrnothrin	0.04
Phenthoate	0.04
Phorate	0.04
Phosphamidon	0.04
Picoxystrobin	0.04
Pirimphos-ethyl	0.01
Procymidone	0.01
Profenofos	0.02
Prometryn	0.02
Propanil	0.02
Propazine	0.02
Pyrazphos	0.02
Pyridalyl	0.04
Pyridaphenthion	0.02
Pyrifenox	0.04
Pyrimethanil	0.01
Quinalphos	0.02
Quintozene	0.02
Quizalofop-P-ethyl	0.04
Silafluofen	0.02
Silthiofam	0.02
Tebufenpyrad	0.02
Tecnazene	0.02
Tefluthrin	0.02
Terbufos	0.02
Tetrachlorvinphos	0.02
Tetradifon	0.02
Tolylfluanid	0.04
Triallate	0.04
Triazamate	0.04
Triazophos	0.04
Trifluralin	0.02
Triticonazole	0.04

APPENDIX E

Identity Related Information for Layn's High Purity Rebaudioside A & Other Steviol Glycosides from Different Lots

HPLC determination of Rebaudioside A content in Stevia extract

Instrument and Reagents

Agilent 1100 HPLC and Chemical workstation

Steviol glycosides reference standard which purchased from CHROMADEX Company

Regents are Chromatography pure or Analytically pure

Chromatographic conditions

Column: LUNA NH_2, 250mm×4.6mm, 5μm

Mobile phase. Acetonitrile (HPLC grade) Milli-Q Water 80.20(V/V)(pH adjusted to 3.0 with 85% phosphoric acid solution)

Flow rate: 0 8ml/min

Detection:UV detector at 210nm

Injection. 5ul

Column temperature. 25℃

Standard preparation

Respectively dry steviol glycosides reference standard at 105℃ for 2 hours, cool to room temperature. Dissolve accurately weighed appropriate amount of seven dried(105℃,2h) steviol glycosides reference standard with mobile phase to obtain standard solution

Dulcoside A 0.491 mg/ml(transfer 5.06mg of Dulcoside A (purity 97.0%), accurately weighed, to a 10-ml volumetric flask, dissolve in mobile phase and dilute with mobile phase to the volume)

Steviolbioside 0 410 mg/ml(transfer 4.10mg of Steviolbioside (purity 100.0%), accurately weighed, to a 10-ml volumetric flask dissolve in mobile phase and dilute with mobile phase to the volume)

Stevioside 0.999 mg/ml(transfer 10 06mg of Stevioside (purity 99 31%), accurately weighed, to a 10-ml volumetric flask. dissolve in mobile phase and dilute with mobile phase to the volume)

Rebaudioside C 0 374 mg/ml(transfer 4 10mg of Rebaudioside C (purity 91 3%), accurately weighed, to a 10-ml volumetric flask , dissolve in mobile phase and dilute with mobile phase to the volume)

Rebaudioside D 0.418 mg/ml(transfer 4 63mg of Rebaudioside D (purity 90.3%), accurately weighed, to a 10-ml volumetric flask. dissolve in mobile phase and dilute with mobile phase to the volume)

Rebaudioside B 0.476 mg/ml(transfer 4.76mg of Rebaudioside B (purity 100%), accurately weighed, to a 10-ml volumetric flask, dissolve in mobile phase and dilute with mobile phase to the volume)

Rebaudioside A 1.702 mg/ml(transfer 17 55mg of Rebaudioside A (purity 97 0%), accurately weighed, to a 10-ml volumetric flask dissolve in mobile phase and dilute with mobile phase to the volume)

Observe and study linear relation

Separately inject 1.0 ul, 5. 0 ul, 10 0 ul,15.0 ul,20.0 ul of seven of stevioside standard solution into the chromatograph for analysis and determination. Use μg as x-coordinate, the corresponding

peak area as y-coordinate , draw the standard curve.

	Linear regression equation	correlation coefficient (r)	linearity range	Diagram No.
Dulcoside A	Y=236.77251X+1.14425	1.00000	Between 0.490782ug to 9.81564ug showed good linear relation	D0002605.D ~ D0002609.D
Steviolbioside	Y=312.82776X-14.06984	0.99995	between 0.40953ug to 8.1906ug showed good linear relation	D0002816.D ~ D0002820.D
Stevioside	Y=191.56418X+6.07303	1.00000	between 0.999 06ug to 19.98100ug showed good linear relation	D0002566.D ~ D0002570.D
Rebaudioside C	Y=212.04421X-5.15606	0.99997	between 0.374219 ug to 7.48439ug showed good linear relation	D0002578.D ~ D0002582.D
Rebaudioside D	Y=89.88093X-2.04178e-1	1.00000	between 0.4176 to 8.352ug showed good linear relation	D0002618.D ~ D0002622.D
Rebaudioside B	Y=203.78829X-7.53834	0.99996	between 0.476435 to 9.52870ug showed good linear relation	D0002821.D ~ D0002825.D
Rebaudioside A	Y=192.06293X+9.16451	0.99999	between 1.70249 to 34.04972ug showed good linear relation	D0002544.D ~ D0002549.D

Assay preparation

Accurately weighed appropriate amount of dried(105℃,2h) sample to a 10ml volumetric flask, dissolve in mobile phase and dilute with mobile phase to the volume, mix, to obtain RA assay solution. Another accurately weighed appropriate amount of dried(105℃,2h) sample to a

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25ml volumetric flask, dissolve in mobile phase and dilute with mobile phase to the volume, mix, to obtain other six steviol glycosides assay solution.

First determination of seven steviol glycosides standard solution, and draw the standard curve (peak area A- weight C(ug) curve). Determination of steviol glycosides assay solution, record the chromatograms, and measure the responses for the rebaudioside A、rebaudioside B、rebaudioside D、rebaudioside C、stevioside、dulcoside A、steviolbioside peaks, express as A_a、A_b、A_d、A_c、A_s、A_{da} and A_{bs}. The slope of the standard curve is K=A/C. calculate the content Pi(%) of above seven components taken by the formula (1).

The results of the expression

$$P_i = \frac{A_i}{k} \times \frac{V}{m} \times 100 \cdots\cdots\cdots\cdots\cdots\cdots\cdots (1)$$

in which i a、b、d、c、s、da、bs;
V sample Volume, ml
m weight of sample(mg);

total of P_a、P_b、P_d、P_c、P_s、P_{da} and P_{bs} to obtain the content of steviol glycosides

Table 1: content of rebaudioside A

Item / Batch No.	sample weight (mg)	diagram No.	RA (%) on dry basis	average RA (%) on dry basis	RSD(%)
100219-01	24.96	D002550.D	98.43	98.57	0.14
	24.32	D002551.D	98.59		
	22.35	D002552.D	98.70		
100219-02	21.96	D002553.D	98.78	98.62	0.21
	21.87	D002554.D	98.39		
	21.56	D002555.D	98.69		
100219-03	21.44	D002556.D	98.89	98.77	0.11
	21.79	D002557.D	98.74		
	21.39	D002558.D	98.68		
100219-04	21.13	D002559.D	98.49	98.70	0.19
	20.89	D002560.D	98.78		
	20.60	D002561.D	98.84		
100219-05	24.09	D002562.D	98.87	98.77	0.15
	23.40	D002563.D	98.60		
	23.52	D002564.D	98.85		

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Table 2 : total content of steviol glycosides

Item / Batch No.	sample weight (mg)	diagram No.	total other six components (%) on dry basis		average RA (%) on dry basis	total teviol glycosides (%) on dry basis
100219-01	302.13	D002572.D	0.75	Dulcoside A:ND Steviolbioside:ND Stevioside. 0.23 Rebaudioside C :0.52 Rebaudioside D :ND Rebaudioside B :ND	98 57	99.24
100219-02	310.42	D002573.D	0.72	Dulcoside A:ND Steviolbioside: ND Stevioside. 0 21 Rebaudioside C 0 51 Rebaudioside D :ND Rebaudioside B :ND	98.62	99.34
100219-03	307.60	D002574.D	0.74	Dulcoside A: ND Steviolbioside: ND Stevioside: 0.22 Rebaudioside C :0.52 Rebaudioside D :ND Rebaudioside B	98 77	99.51
100219-04	298.44	D002575.D	0.71	Dulcoside A: ND Steviolbioside: ND Stevioside:0.21 Rebaudioside C :0.50 Rebaudioside D .ND RebaudiosideB :ND	98.70	99.41
100219-05	293.05	D002576.D	0.70	Dulcoside A:ND Steviolbioside:ND Stevioside· 0.21 RebaudiosideC : 0.49 Rebaudioside D :ND Rebaudioside B :ND	98.77	99.47

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Calibration Table

Calib. Data Modified : Tuesday, March 23, 2010 7:16:29 PM

Calculate : Area Percent

Rel. Reference Window : 5.000 %
Abs Reference Window : 0.000 min
Rel. Non-ref. Window : 5.000 %
Abs Non-ref. Window : 0.000 min
Use Multiplier & Dilution Factor with ISTDs
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: VWD1 A, 210 nm

RetTime [min]	Sig	Lvl	Amount [ug]	Area	Amt/Area	Ref	Grp	Name
9.067	1	1	4.90782e-1	115.91750	4.23369e-3			Rebaudioside A
		2	2.45391	580.24573	4.21456e-3			
		3	4.90782	1160.48572	4.20375e-3			
		4	7.36173	1745.36376	4.21790e-3			
		5	9.81564	2323.26073	4.22678e-3			

Peak Sum Table

No Entries in table

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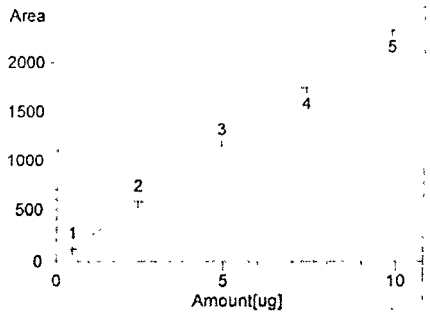
Method C:\Chem32\1\METHODS\DEF_LC.M

Calibration Curves



Dulcoside A at exp. RT: 9.607
VWD1 A, =210 nm
Correlation: 1.00000
Residual Std. Dev.: 2.82970
Formula: y = mx + b
m: 236.77251
b: 1.14425
x: Amount
y: Area

Data File D:\TJT 2010-02-25 19-56-22\D002605.D
Sample Name: b

```
==========================================================================
Acq. Operator    : WHZ                              Seq. Line :  31
Acq. Instrument  :    2                             Location : P1-D-04
Injection Date   : 2/25/2010 6:40:18 PM                   Inj :   1
                                                   Inj Volume : 5 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 1 µl
Acq. Method      : E:\Chem32\2\DATA\20100225\TJT 2010-02-25 19-56-22\TIANJUT.M
Last changed     : 2/25/2010 6:50:24 PM by WHZ
                   (modified after loading)
Analysis Method  : C:\Chem32\1\METHODS\DEF_LC.M
Last changed     : 3/23/2010 7:21:56 PM
                   (modified after loading)
```

VWD1 A, =210 nm (D:\TJT 2010-02-25 19-56-22\D002605.D)

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

Sorted By              :      Signal
Calib. Data Modified   :      3/23/2010 7:19:40 PM
Multiplier             :      1.0000
Dilution               :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A,   =210 nm

Peak RetTime  Type   Width     Area      Area    Name
  #   [min]          [min]   mAU   *s      %
----|-------|------|-------|----------|--------|--------------------
   1   9.566 BB      0.2259  115.91750 100.0000 Dulcoside A

Totals :                     115.91750

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Instrument 1 3/23/2010 7:21:58 PM

Data File D:\TJT 2010-02-25 19-56-22\D002606.D
Sample Name: b

```
==========================================================================
Acq. Operator    : WHZ                                Seq. Line :  32
Acq. Instrument  :   . 2                             Location : P1-D-04
Injection Date   : 2/25/2010 6:53:52 PM                     Inj :   1
                                                     Inj Volume : 5 µl
Acq. Method      : E:\Chem32\2\DATA\20100225\TJT 2010-02-25 19-56-22\TIANJUT.M
Last changed     : 2/25/2010 6:53:08 PM by WHZ
                   (modified after loading)
Analysis Method  : C:\Chem32\1\METHODS\DEF_LC.M
Last changed     : 3/23/2010 7:21:43 PM
                   (modified after loading)
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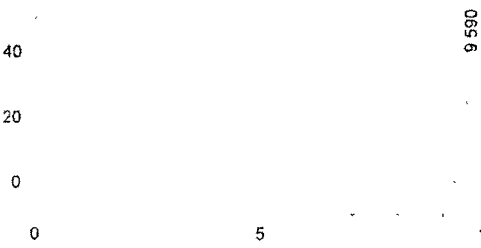
VWD1 A, =210 nm (D:\TJT 2010-02-25 19-56-22\D002606.D)

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

Sorted By              :      Signal
Calib. Data Modified   :      3/23/2010 7:19:40 PM
Multiplier             :      1.0000
Dilution               :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A,   =210 nm

Peak RetTime  Type   Width     Area      Area    Name
  #   [min]          [min]   mAU   *s       %
----|-------|------|-------|----------|--------|--------------------
   1   9.590 VB      0.2296  582.24573 100.0000 Dulcoside A

Totals :                     582.24573


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                          *** End of Report ***
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Data File D:\TJT 2010-02-25 19-56-22\D002607.D
Sample Name: b

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==================================================================
Acq. Operator    : WHZ                          Seq. Line :  33
Acq. Instrument  : [illegible] 2                Location : P1-D-04
Injection Date   : 2/25/2010 7:07:32 PM               Inj :   1
                                                Inj Volume : 5 µl
Different Inj Volume from Sequence 1     Actual Inj Volume : 10 µl
Acq. Method      : E:\Chem32\2\DATA\20100225\TJT 2010-02-25 19-56-22\TIANJUT.M
Last changed     : 2/25/2010 7:06:42 PM by WHZ
                   (modified after loading)
Analysis Method  : C:\Chem32\1\METHODS\DEF_LC.M
Last changed     : 3/23/2010 7:20:08 PM
                   (modified after loading)
```

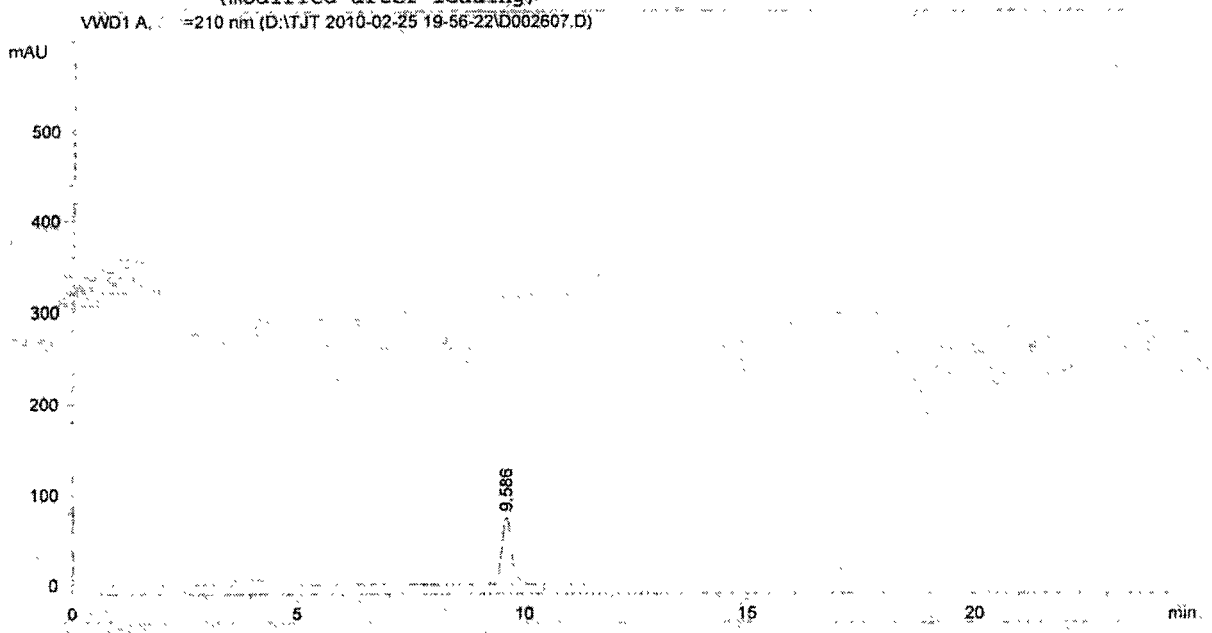


```
==================================================================
                        Area Percent Report
==================================================================

Sorted By              :      Signal
Calib. Data Modified   :      3/23/2010 7:19:40 PM
Multiplier             :      1.0000
Dilution               :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, [illegible]=210 nm

Peak RetTime Type  Width     Area      Area    Name
  #   [min]        [min]   mAU   *s      %
----|-------|------|-------|----------|--------|--------------------
   1   9.586 VB    0.2319 1167.48572 100.0000 Dulcoside A

Totals :                   1167.48572

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Instrument 1 3/23/2010 7:21:07 PM

Page 1 of 1

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Data File D:\TJT 2010-02-25 19-56-22\D002608.D
Sample Name: b

==========================================================================
Acq. Operator    : WHZ                          Seq. Line :  34
Acq. Instrument  : 2                           Location : P1-D-04
Injection Date   : 2/25/2010 7:21:17 PM                Inj :  1
                                               Inj Volume : 5 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 15 µl
Acq. Method      : E:\Chem32\2\DATA\20100225\TJT 2010-02-25 19-56-22\TIANJUT.M
Last changed     : 2/25/2010 7:20:22 PM by WHZ
                   (modified after loading)
Analysis Method  : C:\Chem32\1\METHODS\DEF_LC.M
Last changed     : 3/23/2010 7:20:08 PM
                   (modified after loading)
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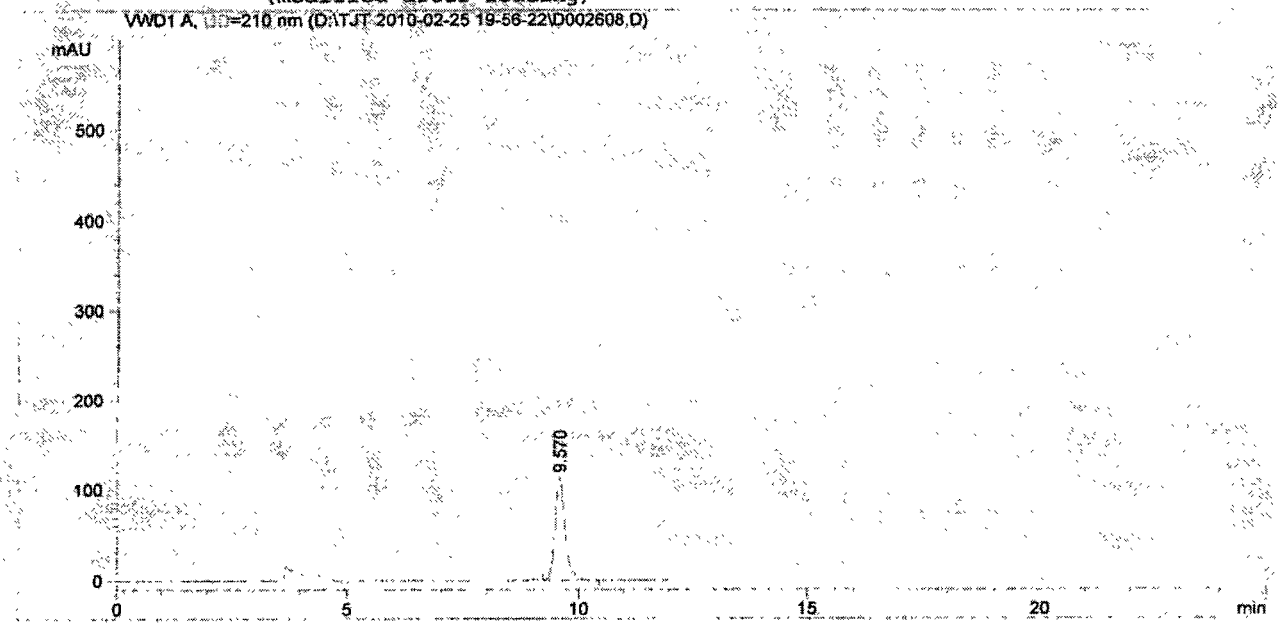


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                          Area Percent Report
==========================================================================

Sorted By              :      Signal
Calib. Data Modified   :      3/23/2010 7:19:40 PM
Multiplier             :      1.0000
Dilution               :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, =210 nm

Peak RetTime  Type    Width     Area       Area     Name
  #   [min]           [min]   mAU   *s       %
----|-------|------|-------|----------|--------|--------------------
   1   9.570 VB      0.2322 1745.35376 100.0000 Dulcoside A

Totals :                     1745.35376

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```

Data File D:\TJT 2010-02-25 19-56-22\D002609.D
Sample Name: b

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Acq. Operator : WHZ
Acq. Instrument : 2
Injection Date : 2/25/2010 7:35:10 PM

Seq. Line : 35
Location : P1-D-04
Inj : 1
Inj Volume : 5 µl

Different Inj Volume from Sequence : Actual Inj Volume : 20 µl
Acq. Method : E:\Chem32\2\DATA\20100225\TJT 2010-02-25 19-56-22\TIANJUT.M
Last changed : 2/25/2010 7:34:08 PM by WHZ
(modified after loading)
Analysis Method : C:\Chem32\1\METHODS\DEF_LC.M
Last changed : 3/23/2010 7:20:08 PM
(modified after loading)

VWD1 A, =210 nm (D:\TJT 2010-02-25 19-56-22\D002609.D)

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Area Percent Report

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Sorted By : Signal
Calib. Data Modified : 3/23/2010 7:19:40 PM
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, =210 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Area %	Name
1	9.607	VB	0.2323	2322.25073	100.0000	Dulcoside A
Totals :				2322.25073		

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Instrument 1 3/23/2010 7:20:10 PM

Page 1 of 1

Method C:\Chem32\1\METHODS\DEF_LC.M

Calibration Table

Calib. Data Modified : Tuesday, March 23, 2010 5:23:13 PM

Calculate : Area Percent

Rel. Reference Window : 5.000 %
Abs. Reference Window : 0.000 min
Rel. Non-ref. Window : 5.000 %
Abs. Non-ref. Window : 0.000 min
Use Multiplier & Dilution Factor with ISTDs
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: VWD1 A, λ=210 nm

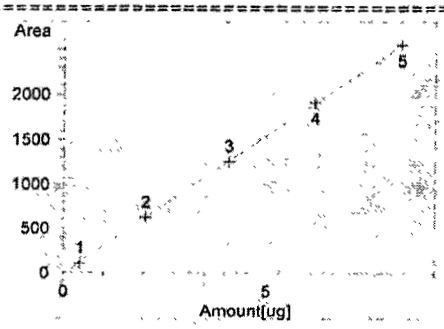
RetTime [min]	Lvl Sig		Amount [ug]	Area	Amt/Area	Ref	Grp	Name
10.820	1	1	4.09530e-1	104.00242	3.93770e-3			Steviobioside
		2	2.04765	625.81677	3.27196e-3			
		3	4.09530	1255.20642	3.26265e-3			
		4	6.14295	1915.20850	3.20746e-3			
		5	8.19060	2549.07690	3.21316e-3			

Peak Sum Table

No Entries in table

ethod C:\Chem32\1\METHODS\DEF_LC.M

Calibration Curves



Steviobioside at exp. RT: 10.820
VWD1 A, [illegible]=210 nm
Correlation: 0.99995
Residual Std. Dev.: 11.15842
Formula: y = mx + b
m: 312.82776
b: -14.06984
x: Amount
y: Area

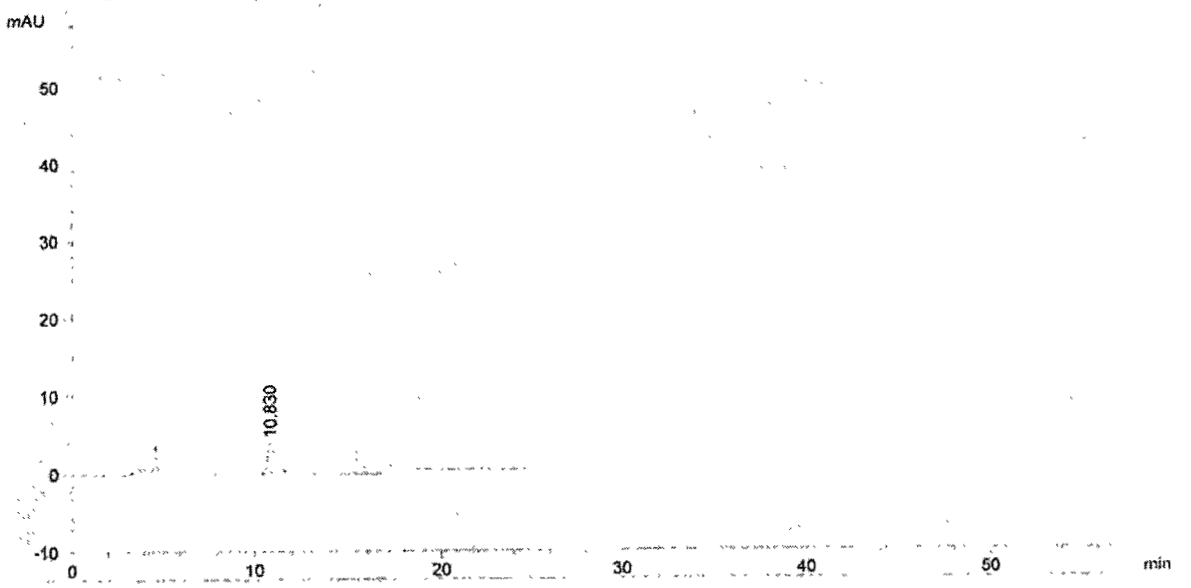
Instrument 1 3/23/2010 5:24:10 PM

Page 2 of 2

Data File D:\TYJ 2010-03-10 21-00-00\D002816.D
Sample Name: B

```
==========================================================================
Acq. Operator    : WHZ                                  Seq. Line :   4
Acq. Instrument  : [illegible] 2                        Location  : P1-B-01
Injection Date   : 3/10/2010 6:59:17 AM                       Inj :   1
                                                        Inj Volume : 5 µl
Different Inj Volume from Sequence !      Actual Inj Volume : 1 µl
Acq. Method      : E:\Chem32\2\DATA\20100310\TYJ 2010-03-10 21-00-00\TIANJUT.M
Last changed     : 3/10/2010 7:06:02 AM by WHZ
                   (modified after loading)
Analysis Method  : C:\Chem32\1\METHODS\DEF_LC.M
Last changed     : 3/23/2010 5:27:56 PM
                   (modified after loading)
```

VWD1 A, [illegible]=210 nm (D:\TYJ 2010-03-10 21-00-00\D002816.D)

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

Sorted By              :      Signal
Calib. Data Modified   :      3/23/2010 5:25:15 PM
Multiplier             :      1.0000
Dilution               :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, [illegible]=210 nm

Peak RetTime Type  Width     Area      Area    Name
  #   [min]        [min]   mAU   *s       %
----|-------|------|-------|----------|--------|--------------------
   1  10.830 BB     0.3890  104.00242 100.0000 Steviobioside

Totals :                    104.00242

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Instrument 1 3/23/2010 5:27:58 PM

Page 1 of 1

Data File D:\TYJ 2010-03-10 21-00-00\D002817.D
Sample Name: B

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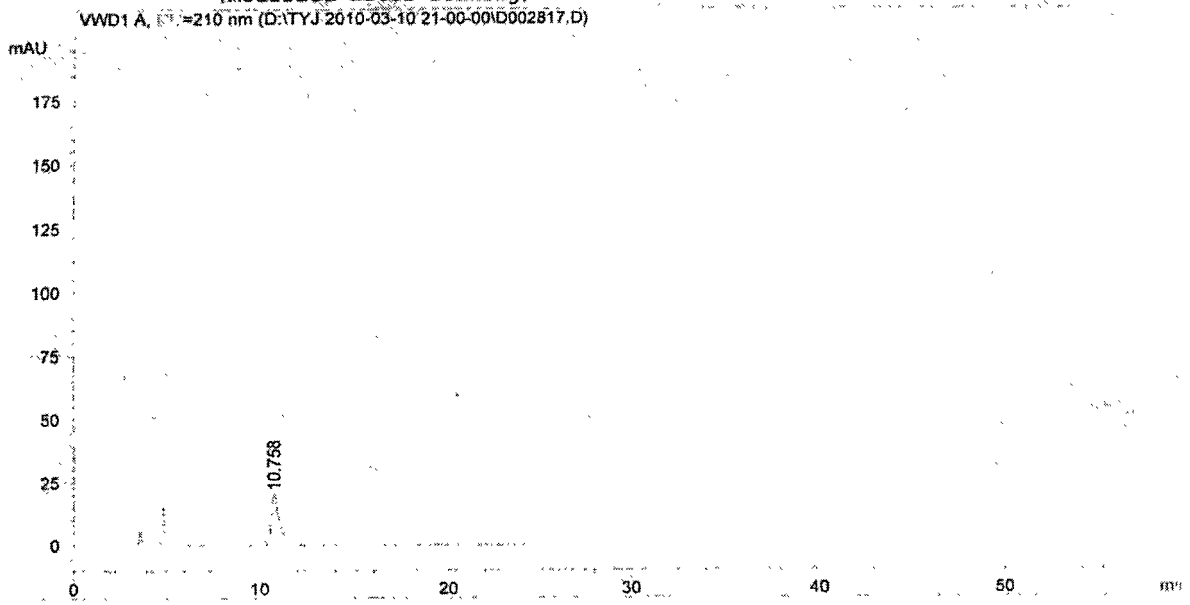
Acq. Operator : WHZ
Acq. Instrument : 2
Injection Date : 3/10/2010 7:26:52 AM

Seq. Line : 5
Location : P1-B-01
Inj : 1
Inj Volume : 5 µl

Acq. Method : E:\Chem32\2\DATA\20100310\TYJ 2010-03-10 21-00-00\TIANJUT.M
Last changed : 3/10/2010 7:26:20 AM by WHZ
(modified after loading)
Analysis Method : C:\Chem32\1\METHODS\DEF_LC.M
Last changed : 3/23/2010 5:27:35 PM
(modified after loading)



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Area Percent Report

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Sorted By : Signal
Calib. Data Modified : 3/23/2010 5:25:15 PM
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, =210 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Area %	Name
1	10.758	BB	0.4483	625.81677	100.0000	Steviobioside

Totals : 625.81677

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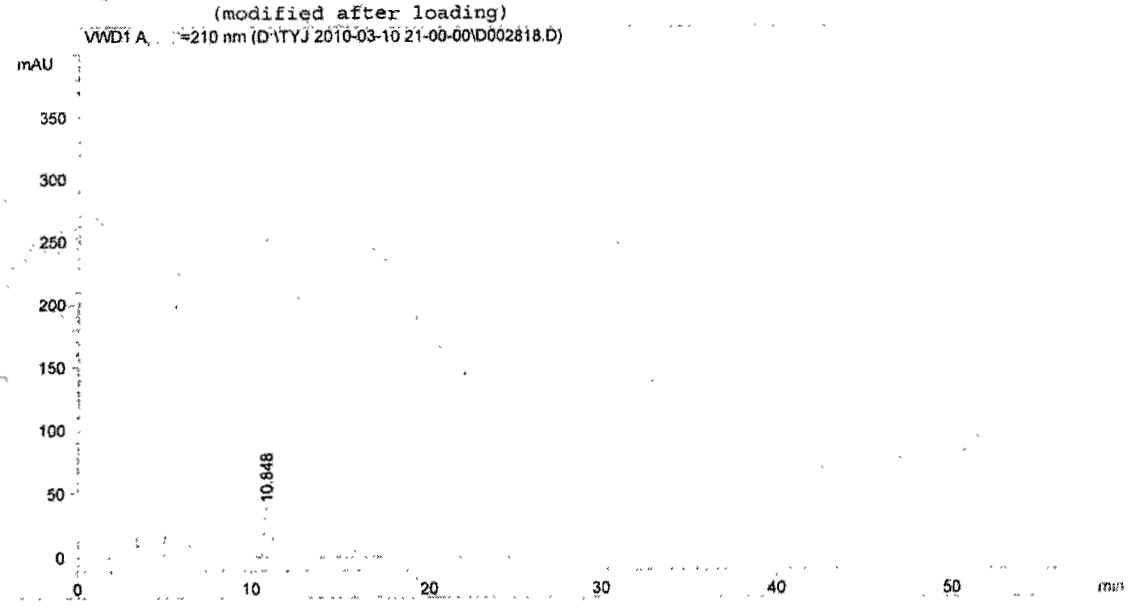
*** End of Report ***

Instrument 1 3/23/2010 5:27:37 PM

Data File D:\TYJ 2010-03-10 21-00-00\D002818.D
Sample Name: B

```
Acq. Operator    : WHZ                                Seq. Line :   6
Acq. Instrument  : [illegible] 2                      Location : P1-B-02
Injection Date   : 3/10/2010 7:54:33 AM                     Inj :   1
                                                      Inj Volume : 5 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 10 µl
Acq. Method      : E:\Chem32\2\DATA\20100310\TYJ 2010-03-10 21-00-00\TIANJUT.M
Last changed     : 3/10/2010 7:53:55 AM by WHZ
                   (modified after loading)
Analysis Method  : C:\Chem32\1\METHODS\DEF_LC.M
Last changed     : 3/23/2010 5:26:59 PM
                   (modified after loading)
```

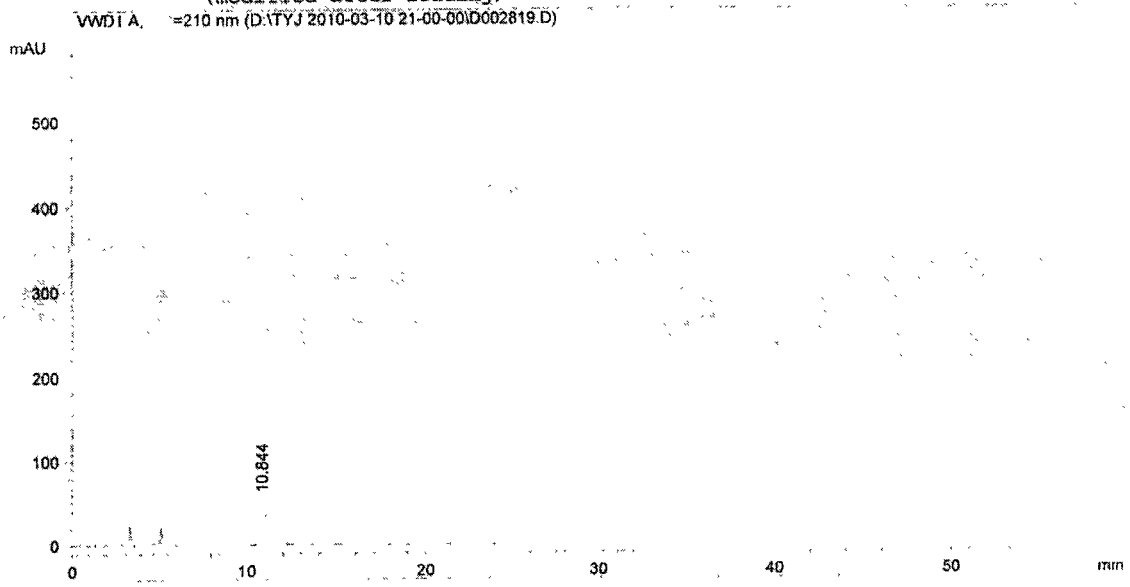



```
==================================================================
                        Area Percent Report
==================================================================

Sorted By             :      Signal
Calib. Data Modified  :      3/23/2010 5:25:15 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, [illegible]=210 nm

Peak RetTime  Type   Width     Area       Area    Name
  #   [min]          [min]   mAU   *s       %
----|-------|------|-------|----------|--------|--------------------
   1  10.848 BB      0.4541 1255.20642 100.0000 Steviobioside

Totals :                    1255.20642

==================================================================
```

Instrument 1 3/23/2010 5:27:01 PM Page 1 of 1

Data File D:\TYJ 2010-03-10 21-00-00\D002819.D
Sample Name: B

```
==========================================================================
Acq. Operator    : WHZ                                  Seq. Line :   7
Acq. Instrument  : [illegible] 2                        Location : P1-B-01
Injection Date   : 3/10/2010 8:22:20 AM                       Inj :   1
                                                        Inj Volume : 5 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 15 µl
Acq. Method      : B:\Chem32\2\DATA\20100310\TYJ 2010-03-10 21-00-00\TIANJUT.M
Last changed     : 3/10/2010 8:21:35 AM by WHZ
                   (modified after loading)
Analysis Method  : C:\Chem32\1\METHODS\DEF_LC.M
Last changed     : 3/23/2010 5:26:10 PM
                   (modified after loading)
```




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

Sorted By              :      Signal
Calib. Data Modified   :      3/23/2010 5:25:15 PM
Multiplier             :      1.0000
Dilution               :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, [illegible]=210 nm

Peak RetTime  Type   Width     Area      Area    Name
  #   [min]          [min]   mAU   *s      %
----|-------|------|-------|----------|--------|--------------------
  1  10.844 BB      0.4731 1915.20850 100.0000 Steviobioside

Totals :                   1915.20850

==========================================================================
```

Instrument 1 3/23/2010 5:26:47 PM Page 1 of 1

ata File D:\TYJ 2010-03-10 21-00-00\D002820.D
ample Name: B

Acq. Operator	: WHZ	Seq. Line :	8
Acq. Instrument	: [illegible] 2	Location :	P1-B-01
Injection Date	: 3/10/2010 8:50:12 AM	Inj :	1
		Inj Volume :	5 µl

Different Inj Volume from Sequence ! Actual Inj Volume : 20 µl
Acq. Method : E:\Chem32\2\DATA\20100310\TYJ 2010-03-10 21-00-00\TIANJUT.M
Last changed : 3/10/2010 8:49:22 AM by WHZ
(modified after loading)
Analysis Method : C:\Chem32\1\METHODS\DEF_LC.M
Last changed : 3/23/2010 5:26:10 PM
(modified after loading)

Area Percent Report

Sorted By : Signal
Calib. Data Modified : 3/23/2010 5:25:15 PM
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, [illegible]=210 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Area %	Name
1	10.820	BB	0.4781	2549.07690	100.0000	Steviobioside

Totals : 2549.07690

Instrument 1 3/23/2010 5:26:16 PM

Page 1 of 1

Calibration Table

Calib. Data Modified : 3/28/2010 12:18:17 AM

Calculate : Area Percent

Rel. Reference Window : 5.000 %
Abs. Reference Window : 0.000 min
Rel. Non-ref. Window : 5.000 %
Abs. Non-ref. Window : 0.000 min
Use Multiplier & Dilution Factor with ISTDs
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: VWD1 A, λ =210 nm

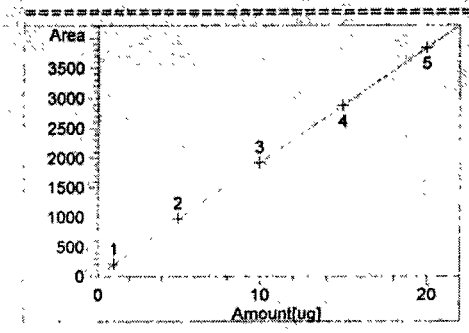
RetTime [min]	Sig	Lvl	Amount [ug]	Area	Amt/Area	Ref	Grp	Name
12.000	1	1	9.99060e-1	198.35883	5.03663e-3			Stevioside
		2	4.99500	969.80902	5.15050e-3			
		3	9.99100	1921.45984	5.19969e-3			
		4	14.98600	2874.16235	5.21404e-3			
		5	19.98100	3833.23779	5.21256e-3			

Peak Sum Table

No Entries in table

Method C:\Chem32\1\METHODS\DEF_LC.M

Calibration Curves



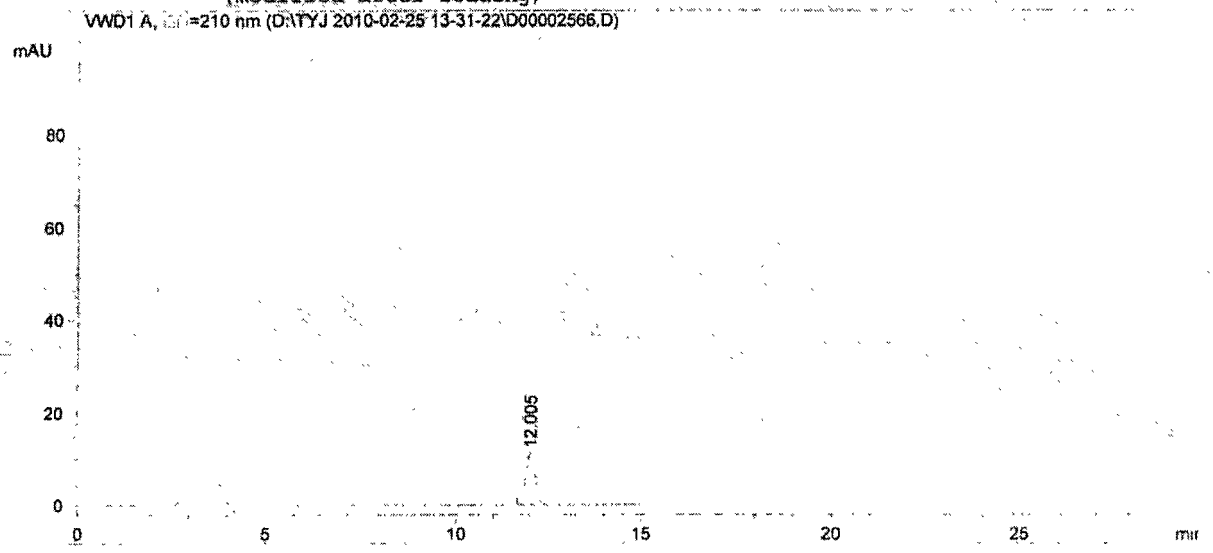
at exp. RT: 12.000
VWD1 A, [illegible]=210 nm
Correlation: 1.00000
Residual Std. Dev.: 4.86219
Formula: y = mx + b
m: 191.56418
b: 6.07303
x: Amount
y: Area

Instrument 1 3/27/2010 11:48:05 PM

Page 2 of 2

Data File D:\TYJ 2010-02-25 13-31-22\D00002566.D
Sample Name: b

```
==================================================================
Acq. Operator    : WHZ                          Seq. Line :   1
Acq. Instrument  : 2                           Location : P1-A-03
Injection Date   : 2/24/2010 9:32:01 PM                Inj :   1
                                               Inj Volume : 5 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 1 µl
Acq. Method      : E:\Chem32\2\DATA\201002250\TYJ 2010-02-25 13-31-22\TIANJUT.M
Last changed     : 2/24/2010 9:41:11 PM by WHZ
                   (modified after loading)
Analysis Method  : C:\Chem32\1\METHODS\DEF_LC.M
Last changed     : 3/27/2010 3:36:15 PM
                   (modified after loading)
```




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

Sorted By             :      Signal
Calib. Data Modified  :      3/27/2010 3:34:41 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, =210 nm

Peak RetTime  Type   Width     Area      Area    Name
  #   [min]          [min]   mAU   *s      %
----|-------|------|-------|----------|--------|--------------------
   1  12.005 BB     0.2701  198.35883 100.0000 Stevioside

Totals :                    198.35883


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

Instrument 1 3/27/2010 3:36:18 PM Page 1 of 1

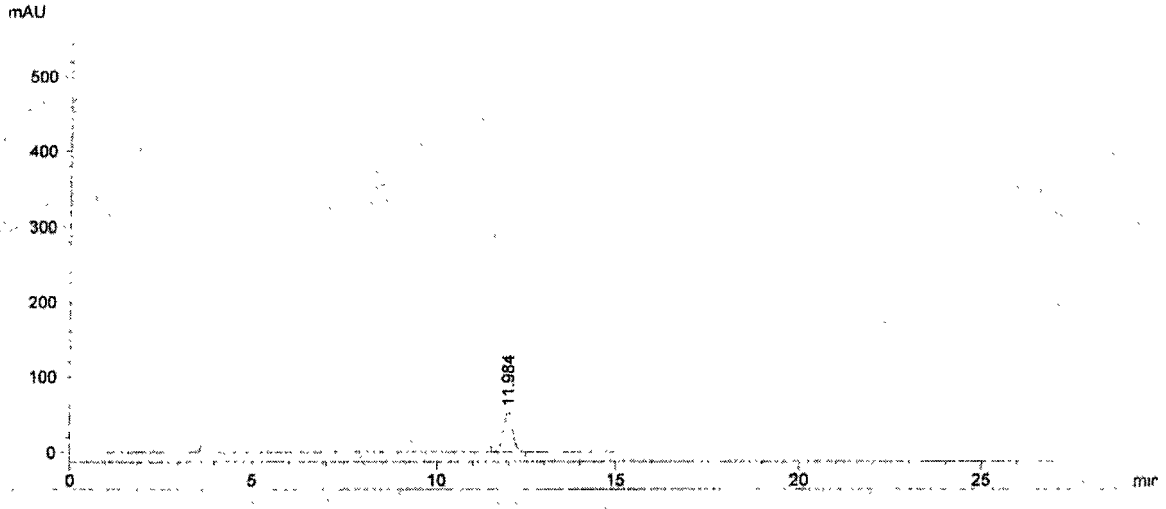
Data File D:\TYJ 2010-02-25 13-54-39\D002567.D
Sample Name: b

==========

Acq. Operator : WHZ
Acq. Instrument : LC 2
Injection Date : 2/24/2010 9:55:20 PM

Seq. Line : 1
Location : P1-A-03
Inj : 1
Inj Volume : 5 µl

Acq. Method : B:\Chem32\2\DATA\201002250\TYJ 2010-02-25 13-54-39\TIANJUT.M
Last changed : 2/24/2010 9:54:37 PM by WHZ
Analysis Method : C:\Chem32\1\METHODS\DEF_LC.M
Last changed : 3/27/2010 3:35:07 PM
(modified after loading)



==========

Area Percent Report

==========

Sorted By : Signal
Calib. Data Modified : 3/27/2010 3:34:41 PM
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, =210 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Area %	Name
1	11.984	BB	0.2771	969.80902	100.0000	Stevioside

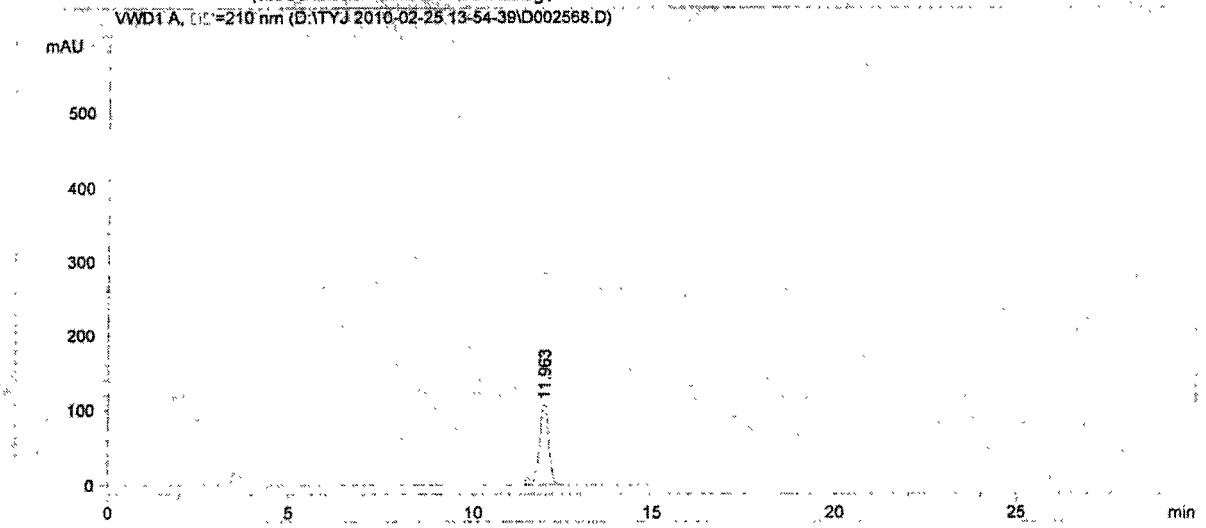
Totals : 969.80902

==========

*** End of Report ***

Data File D:\TYJ 2010-02-25 13-54-39\D002568.D
Sample Name: b

```
==================================================================
Acq. Operator    : WHZ                              Seq. Line :   2
Acq. Instrument  : [illegible] 2                    Location : P1-A-03
Injection Date   : 2/24/2010 10:13:02 PM                  Inj :   1
                                                    Inj Volume : 5 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 10 µl
Acq. Method      : E:\Chem32\2\DATA\201002250\TYJ 2010-02-25 13-54-39\TIANJUT.M
Last changed     : 2/24/2010 9:54:37 PM by WHZ
Analysis Method  : C:\Chem32\1\METHODS\DEF_LC.M
Last changed     : 3/27/2010 3:35:07 PM
                   (modified after loading)
```

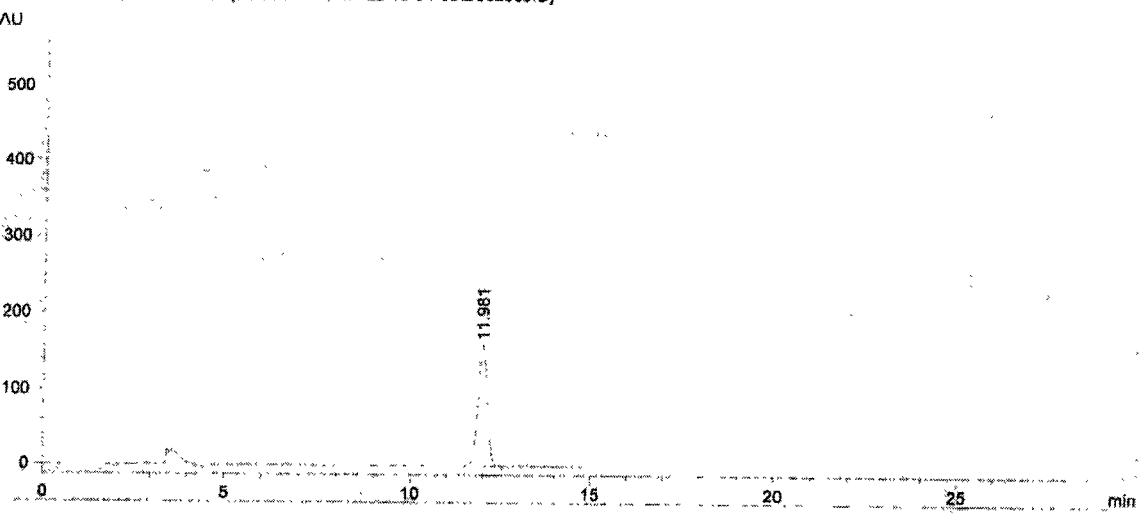



```
==================================================================
                        Area Percent Report
==================================================================

Sorted By              :      Signal
Calib. Data Modified   :      3/27/2010 3:34:41 PM
Multiplier             :      1.0000
Dilution               :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, [illegible]=210 nm

Peak RetTime  Type   Width     Area      Area    Name
  #   [min]          [min]   mAU   *s       %
----|-------|------|-------|----------|--------|--------------------
   1  11.963 BB      0.2772 1921.45984 100.0000 Stevioside

Totals :                     1921.45984


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

Sample Name: b

```
==========================================================================
Acq. Operator    : WHZ                                  Seq. Line :   3
Acq. Instrument  : LC 2                                 Location : P1-A-03
Injection Date   : 2/24/2010 10:30:50 PM                      Inj :   1
                                                        Inj Volume : 5 µl
Different Inj Volume from Sequence :      Actual Inj Volume : 15 µl
Acq. Method      : E:\Chem32\2\DATA\201002250\TYJ 2010-02-25 13-54-39\TIANJUT.M
Last changed     : 2/24/2010 9:54:37 PM by WHZ
Analysis Method  : C:\Chem32\1\METHODS\DEF_LC.M
Last changed     : 3/27/2010 3:35:07 PM
                   (modified after loading)
```

VWD1 A, =210 nm (D:\TYJ 2010-02-25 13-54-39\D002569.D)




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

Sorted By              :      Signal
Calib. Data Modified   :      3/27/2010 3:34:41 PM
Multiplier             :      1.0000
Dilution               :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, =210 nm

Peak RetTime  Type   Width     Area       Area    Name
  #   [min]          [min]   mAU   *s       %
----|-------|------|-------|----------|--------|--------------------
   1  11.981 BB     0.2770 2874.16235 100.0000 Stevioside

Totals :                    2874.16235


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


Instrument 1 3/27/2010 3:35:26 PM                          Page   1 of 1
```

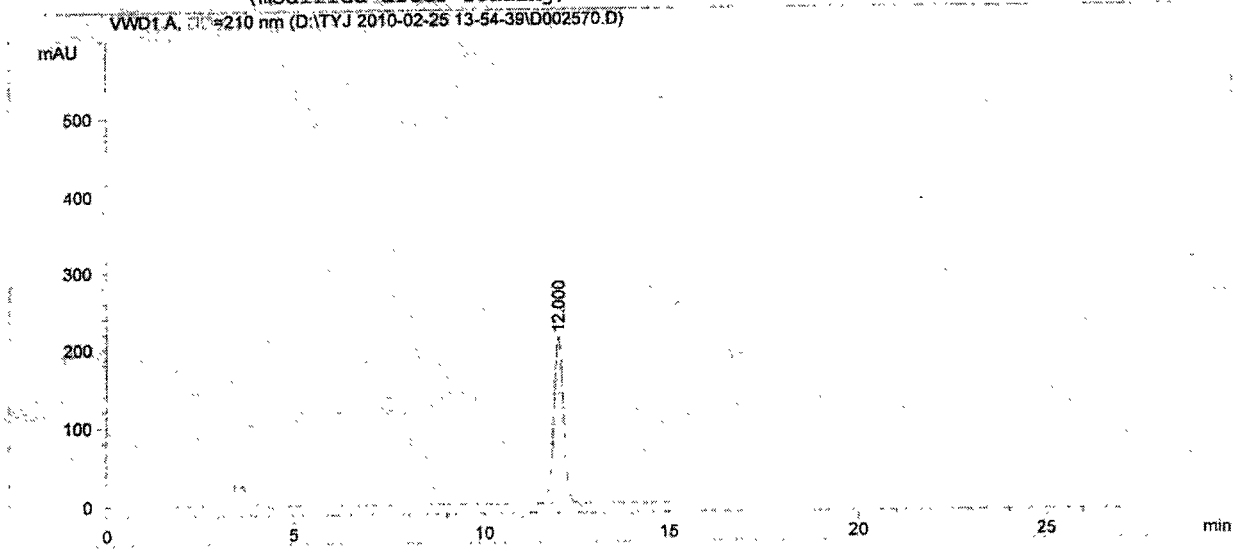
Data File D:\TYJ 2010-02-25 13-54-39\D002570.D
Sample Name: b

==========

Acq. Operator : WHZ
Acq. Instrument : [illegible] 2
Injection Date : 2/24/2010 10:48:45 PM

Seq. Line : 4
Location : P1-A-03
Inj : 1
Inj Volume : 5 µl

Different Inj Volume from Sequence ! Actual Inj Volume : 20 µl
Acq. Method : E:\Chem32\2\DATA\201002250\TYJ 2010-02-25 13-54-39\TIANJUT.M
Last changed : 2/24/2010 9:54:37 PM by WHZ
Analysis Method : C:\Chem32\1\METHODS\DEF_LC.M
Last changed : 3/27/2010 3:35:07 PM
(modified after loading)



==========

Area Percent Report

==========

Sorted By : Signal
Calib. Data Modified : 3/27/2010 3:34:41 PM
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, [illegible]=210 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Area %	Name
1	12.000	BB	0.2809	3833.23779	100.0000	Stevioside

Totals : 3833.23779

==========

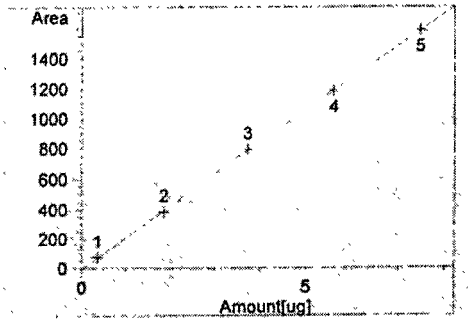
*** End of Report ***

Instrument 1 3/27/2010 3:35:12 PM

Page 1 of 1

```
==================================================================
                        Calibration Table
==================================================================

Calib. Data Modified   :     Sunday, March 28, 2010 12:27:13 AM

Calculate              :     Area Percent

Rel. Reference Window  :     5.000 %
Abs. Reference Window  :     0.000 min
Rel. Non-ref. Window   :     5.000 %
Abs. Non-ref. Window   :     0.000 min
Use Multiplier & Dilution Factor with ISTDs
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: VWD1 A, λ=210 nm

RetTime   Lvl   Amount       Area      Amt/Area  Ref Grp Name
 [min] Sig        [ug]
-------|--|--|----------|----------|----------|---|--|---------------
 15.300  1  1 3.74219e-1   74.90541 4.99589e-3        Rebaudioside C
            2    1.87110  382.66931 4.88959e-3
            3    3.74219  788.53223 4.74577e-3
            4    5.61329 1183.25110 4.74395e-3
            5    7.48439 1584.44836 4.72365e-3

1 Warnings or Errors :

Warning : Cal. table open and changed while report was generated.

==================================================================
                        Peak Sum Table
==================================================================

***No Entries in table***
==================================================================
```

Calibration Curves



Rebaudioside C at exp. RT: 15.300
VWD1 A, [illegible]=210 nm
Correlation: 0.99997
Residual Std. Dev.: 5.39014
Formula: y = mx + b
m: 212.04421
b: -5.51606
x: Amount
y: Area

nstrument 1 3/28/2010 12:34:13 AM

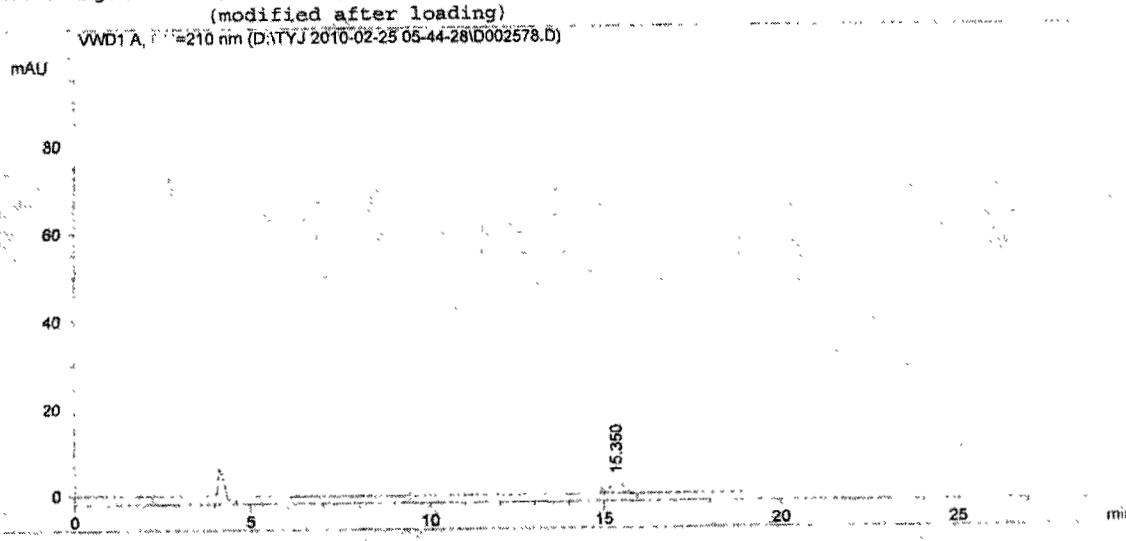
Page 2 of 2

Data File D:\TYJ 2010-02-25 05-44-28\D002578.D
Sample Name: b

Acq. Operator : WHZ
Acq. Instrument : LC 2
Injection Date : 2/24/2010 2:58:59 PM

Seq. Line : 3
Location : P1-A-04
Inj : 1
Inj Volume : 5 µl

Different Inj Volume from Sequence ! Actual Inj Volume : 1 µl
Acq. Method : E:\Chem32\2\DATA\20100225\TYJ 2010-02-25 05-44-28\TIANJUT.M
Last changed : 2/24/2010 3:16:10 PM by WHZ
(modified after loading)
Analysis Method : C:\Chem32\1\METHODS\DEF_LC.M
Last changed : 3/28/2010 12:37:02 AM
(modified after loading)



Area Percent Report

Sorted By : Signal
Calib. Data Modified : 3/28/2010 12:35:36 AM
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, =210 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Area %	Name
1	15.350	BB	0.3474	74.90541	100.0000	Rebaudioside C

Totals : 74.90541

*** End of Report ***

Instrument 1 3/28/2010 12:37:13 AM

Page 1 of 1

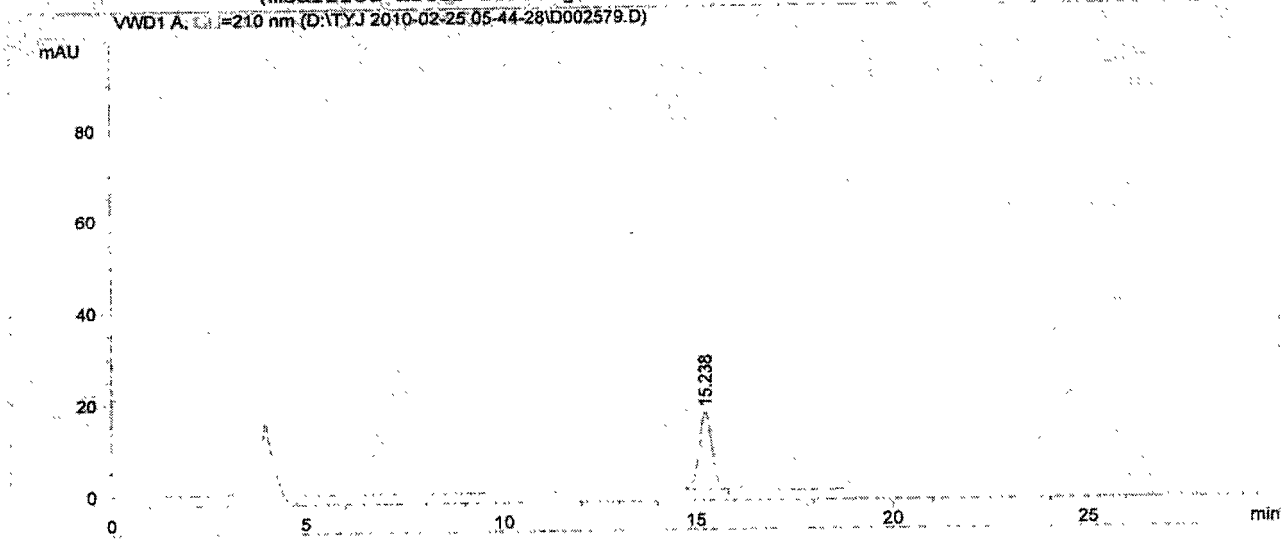
ata File D:\TYJ 2010-02-25 05-44-28\D002579.D
ample Name: b

==========

Acq. Operator : WHZ
Acq. Instrument : 1 2
Injection Date : 2/24/2010 3:18:58 PM

Seq. Line : 4
Location : P1-A-04
Inj : 1
Inj Volume : 5 µl

Acq. Method : E:\Chem32\2\DATA\20100225\TYJ 2010-02-25 05-44-28\TIANJUT.M
Last changed : 2/24/2010 3:18:23 PM by WHZ
(modified after loading)
Analysis Method : C:\Chem32\1\METHODS\DEF_LC.M
Last changed : 3/28/2010 12:37:02 AM
(modified after loading)



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Area Percent Report

==========

Sorted By : Signal
Calib. Data Modified : 3/28/2010 12:35:36 AM
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, =210 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Area %	Name
1	15.238	BB	0.3482	382.66931	100.0000	Rebaudioside C

Totals : 382.66931

==========

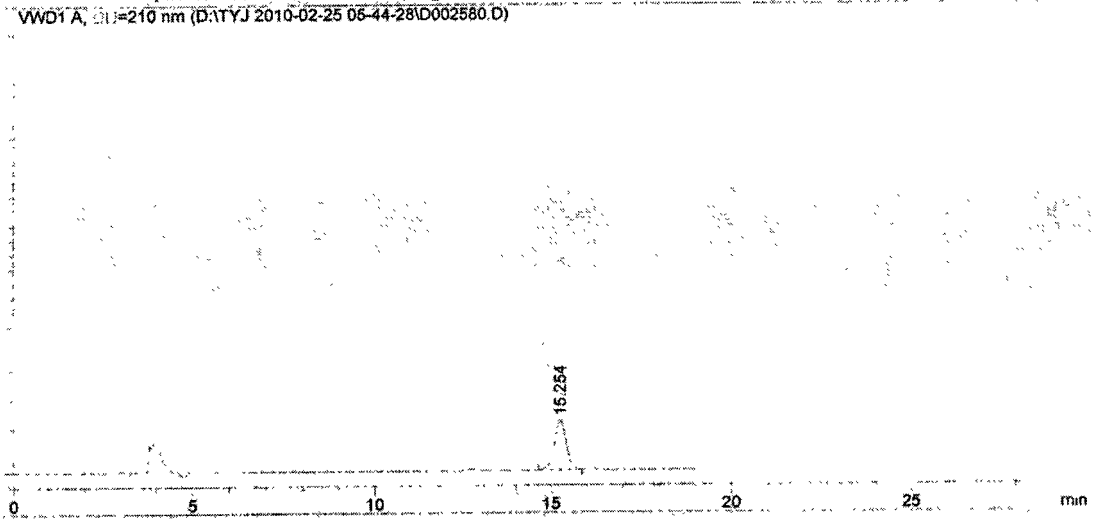
*** End of Report ***

Instrument 1 3/28/2010 12:37:05 AM

Page 1 of 1

Data File D:\TYJ 2010-02-25 05-44-28\D002580.D
Sample Name: b

```
==========================================================================
Acq. Operator    : WHZ                                  Seq. Line :   5
Acq. Instrument  : [illegible] 2                        Location  : P1-A-04
Injection Date   : 2/24/2010 3:39:00 PM                      Inj :   1
                                                        Inj Volume : 5 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 10 µl
Acq. Method      : E:\Chem32\2\DATA\20100225\TYJ 2010-02-25 05-44-28\TIANJUT.M
Last changed     : 2/24/2010 3:38:20 PM by WHZ
                   (modified after loading)
Analysis Method  : C:\Chem32\1\METHODS\DEF_LC.M
Last changed     : 3/28/2010 12:36:14 AM
                   (modified after loading)
```



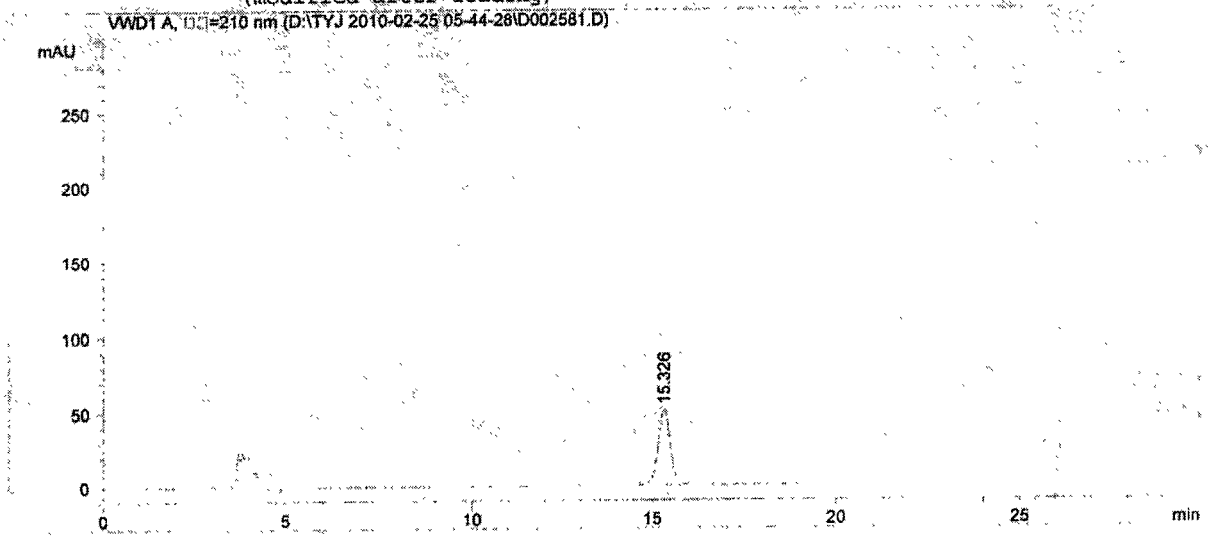

```
==========================================================================
                          Area Percent Report
==========================================================================

Sorted By              :      Signal
Calib. Data Modified   :      3/28/2010 12:35:36 AM
Multiplier             :      1.0000
Dilution               :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, [illegible]=210 nm

Peak RetTime  Type   Width     Area       Area    Name
  #   [min]          [min]   mAU   *s       %
----|-------|------|-------|----------|--------|--------------------
   1  15.254 BB      0.3564  788.53223 100.0000 Rebaudioside C

Totals :                     788.53223


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

Instrument 1 3/28/2010 12:36:44 AM

Page 1 of 1

Data File D:\TYJ 2010-02-25 05-44-28\D002581.D
Sample Name: b

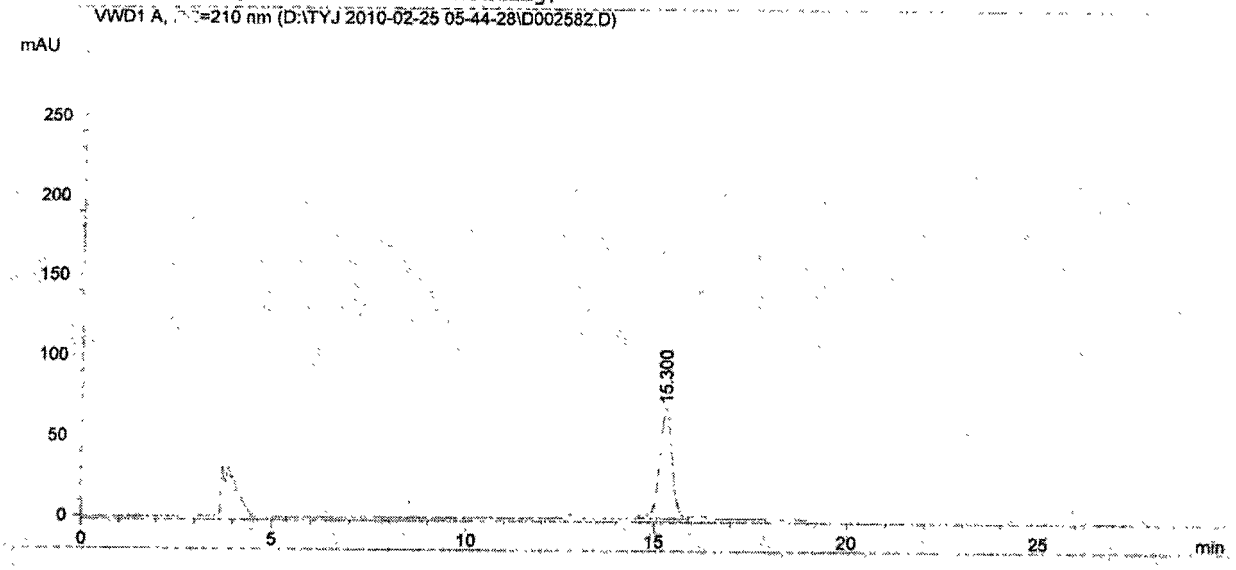
```
==========================================================================
Acq. Operator   : WHZ                                  Seq. Line :   6
Acq. Instrument : [illegible] 2                        Location : P1-A-04
Injection Date  : 2/24/2010 3:59:09 PM                       Inj :   1
                                                       Inj Volume : 5 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 15 µl
Acq. Method     : E:\Chem32\2\DATA\20100225\TYJ 2010-02-25 05-44-28\TIANJUT.M
Last changed    : 2/24/2010 3:58:23 PM by WHZ
                  (modified after loading)
Analysis Method : C:\Chem32\1\METHODS\DEF_LC.M
Last changed    : 3/28/2010 12:36:14 AM
                  (modified after loading)
```




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

Sorted By              :      Signal
Calib. Data Modified   :      3/28/2010 12:35:36 AM
Multiplier             :      1.0000
Dilution               :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, [illegible]=210 nm

Peak RetTime  Type   Width     Area       Area    Name
  #   [min]          [min]   mAU   *s       %
----|-------|------|-------|----------|--------|--------------------
   1  15.326 BB      0.3566 1183.25110 100.0000 Rebaudioside C

Totals :                     1183.25110


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

Data File D:\TYJ 2010-02-25 05-44-28\D002582.D
Sample Name: b

Acq. Operator	: WHZ	Seq. Line :	7
Acq. Instrument	: [illegible] 2	Location :	P1-A-04
Injection Date	: 2/24/2010 4:19:24 PM	Inj :	1
		Inj Volume :	5 µl
Different Inj Volume from Sequence !		Actual Inj Volume :	20 µl
Acq. Method	: E:\Chem32\2\DATA\20100225\TYJ 2010-02-25 05-44-28\TIANJUT.M		
Last changed	: 2/24/2010 4:18:33 PM by WHZ (modified after loading)		
Analysis Method	: C:\Chem32\1\METHODS\DEF_LC.M		
Last changed	: 3/28/2010 12:35:55 AM (modified after loading)		



Area Percent Report

Sorted By	:	Signal
Calib. Data Modified	:	3/28/2010 12:35:36 AM
Multiplier	:	1.0000
Dilution	:	1.0000

Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, [illegible]=210 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Area %	Name
1	15.300	BB	0.3535	1584.44836	100.0000	Rebaudioside C

Totals : 1584.44836

*** End of Report ***

Calibration Table

Calib. Data Modified : Tuesday, March 23, 2010 7:45:29 PM

Calculate : External Standard
Based on : Peak Area

Rel. Reference Window : 5.000 %
Abs. Reference Window : 0.000 min
Rel. Non-ref. Window : 5.000 %
Abs. Non-ref. Window : 0.000 min
Use Multiplier & Dilution Factor with ISTDs
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: VWD1 A, λ=210 nm

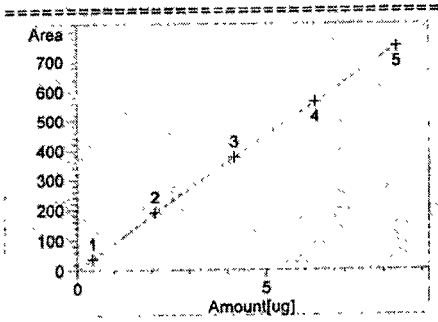
RetTime [min]	Sig	Lvl	Amount [ug]	Area	Amt/Area	Ref	Grp	Name
17.196	1	1	1.17600e-1	35.90188	1.16317e-2			Rebaudioside D
		2	2.08800	188.62643	1.10695e-2			
		3	4.17600	375.46494	1.11222e-2			
		4	6.26400	563.29016	1.11204e-2			
		5	8.35200	749.73969	1.11399e-2			

Peak Sum Table

No Entries in table

Method C:\Chem32\1\METHODS\DEF_LC.M

Calibration Curves



Rebaudioside D at exp. RT: 17.196
VWD1 A, λ=210 nm
Correlation: 1.00000
Residual Std. Dev.: 1.03832
Formula: y = mx + b
m: 89.88093
b: -2.04178e-1
x: Amount
y: Area

Instrument 1 3/23/2010 7:46:59 PM

Page 2 of 2

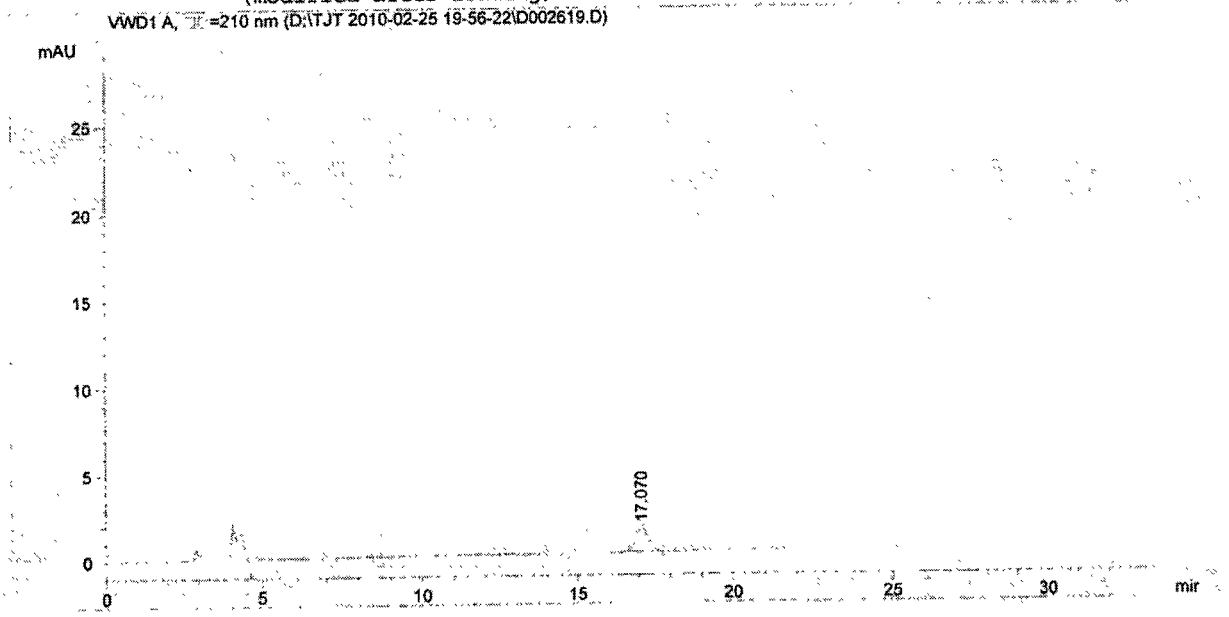
Data File D:\TJT 2010-02-25 19-56-22\D002619.D
Sample Name: B

==========

Acq. Operator : WHZ
Acq. Instrument : LC 2
Injection Date : 2/26/2010 12:45:24 AM

Seq. Line : 45
Location : P1-B-02
Inj : 1
Inj Volume : 5 µl

Different Inj Volume from Sequence ! Actual Inj Volume : 1 µl
Acq. Method : E:\Chem32\2\DATA\20100225\TJT 2010-02-25 19-56-22\TIANJUT.M
Last changed : 2/26/2010 12:44:37 AM by WHZ
(modified after loading)
Analysis Method : C:\Chem32\1\METHODS\DEF_LC.M
Last changed : 3/23/2010 7:49:00 PM
(modified after loading)



==========

External Standard Report

==========

Sorted By : Signal
Calib. Data Modified : 3/23/2010 7:47:28 PM
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, =210 nm

RetTime [min]	Type	Area mAU *s	Amt/Area	Amount [ug]	Grp	Name
17.070	BB	35.90188	1.11891e-2	4.01710e-1		Rebaudioside D

Totals : 4.01710e-1

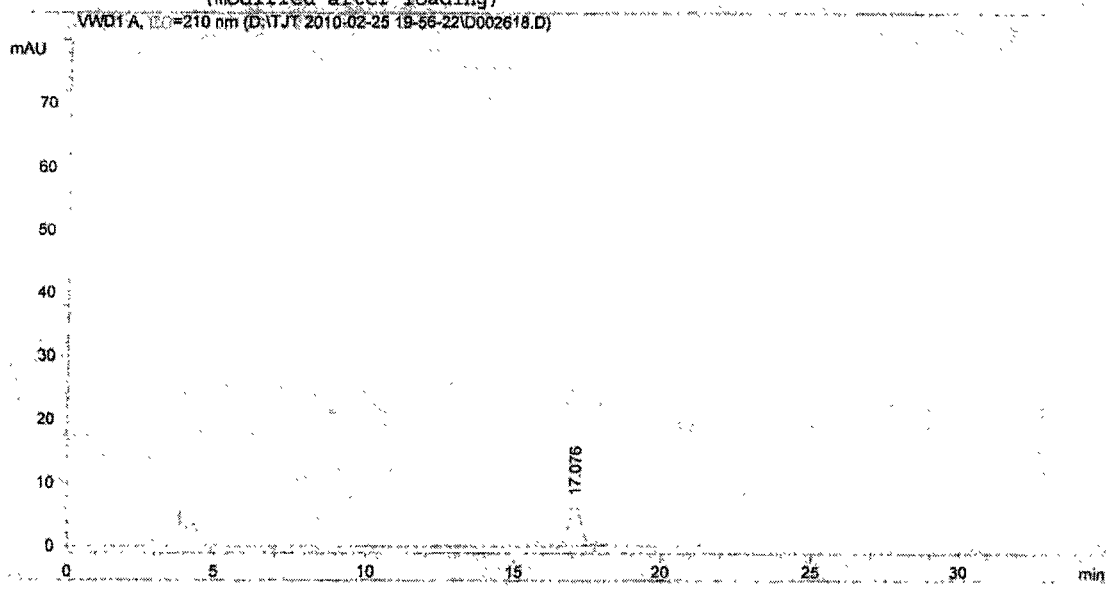
==========

*** End of Report ***

Instrument 1 3/23/2010 7:49:02 PM

Sample Name: B

```
==================================================================
Acq. Operator    : WHZ                          Seq. Line :  44
Acq. Instrument  : [illegible] 2                Location : P1-B-02
Injection Date   : 2/26/2010 12:21:48 AM              Inj :   1
                                                Inj Volume : 5 µl
Acq. Method      : E:\Chem32\2\DATA\20100225\TJT 2010-02-25 19-56-22\TIANJUT.M
Last changed     : 2/26/2010 12:20:59 AM by WHZ
                   (modified after loading)
Analysis Method  : C:\Chem32\1\METHODS\DEF_LC.M
Last changed     : 3/23/2010 7:49:18 PM
                   (modified after loading)
```

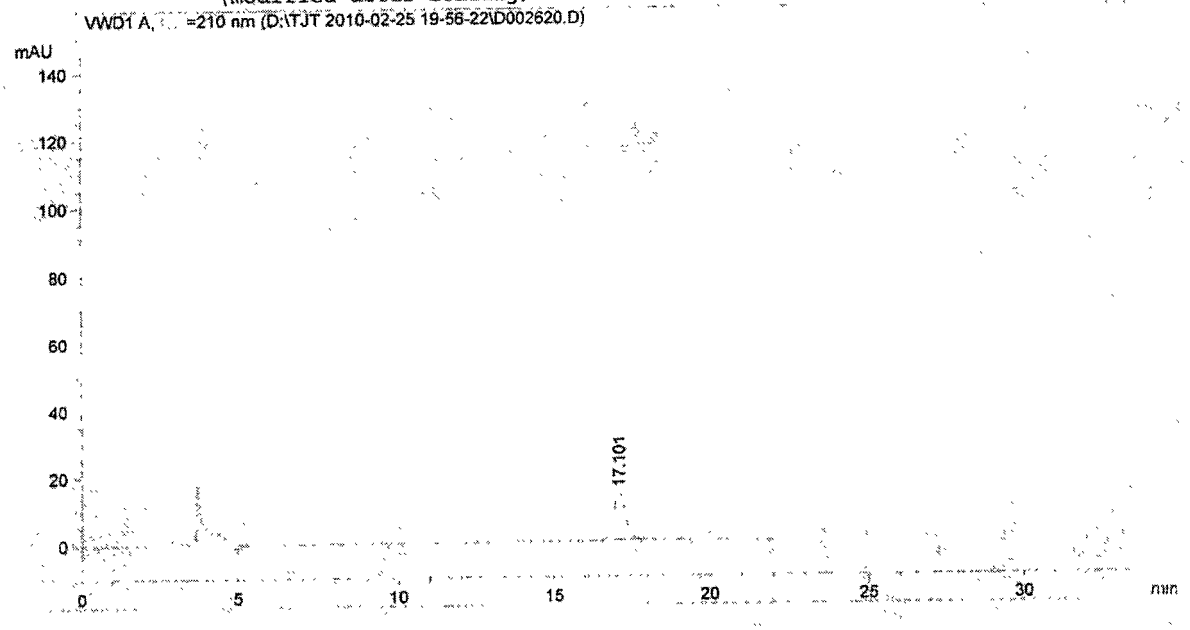



```
==================================================================
                      External Standard Report
==================================================================

Sorted By              :      Signal
Calib. Data Modified   :      3/23/2010 7:47:28 PM
Multiplier             :      1.0000
Dilution               :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, [illegible]=210 nm

RetTime  Type     Area      Amt/Area    Amount   Grp   Name
 [min]          mAU   *s                 [ug]
-------|------|----------|----------|----------|--|-----------------
 17.076 BB      188.62643 1.11379e-2    2.10090     Rebaudioside D

Totals :                                2.10090


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

Data File D:\TJT 2010-02-25 19-56-22\D002620.D
Sample Name: B

Acq. Operator	: WHZ	Seq. Line	: 46
Acq. Instrument	: [illegible] 2	Location	: P1-B-02
Injection Date	: 2/26/2010 1:09:03 AM	Inj	: 1
		Inj Volume	: 5 µl

Different Inj Volume from Sequence ! Actual Inj Volume : 10 µl
Acq. Method : E:\Chem32\2\DATA\20100225\TJT 2010-02-25 19-56-22\TIANJUT.M
Last changed : 2/26/2010 1:08:13 AM by WHZ
(modified after loading)
Analysis Method : C:\Chem32\1\METHODS\DEF_LC.M
Last changed : 3/23/2010 7:48:35 PM
(modified after loading)



External Standard Report

Sorted By : Signal
Calib. Data Modified : 3/23/2010 7:47:28 PM
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, =210 nm

RetTime [min]	Type	Area mAU *s	Amt/Area	Amount [ug]	Grp	Name
17.101	BB	375.46494	1.11319e-2	4.17963		Rebaudioside D
Totals :				4.17963		

*** End of Report ***

Instrument 1 3/23/2010 7:48:37 PM

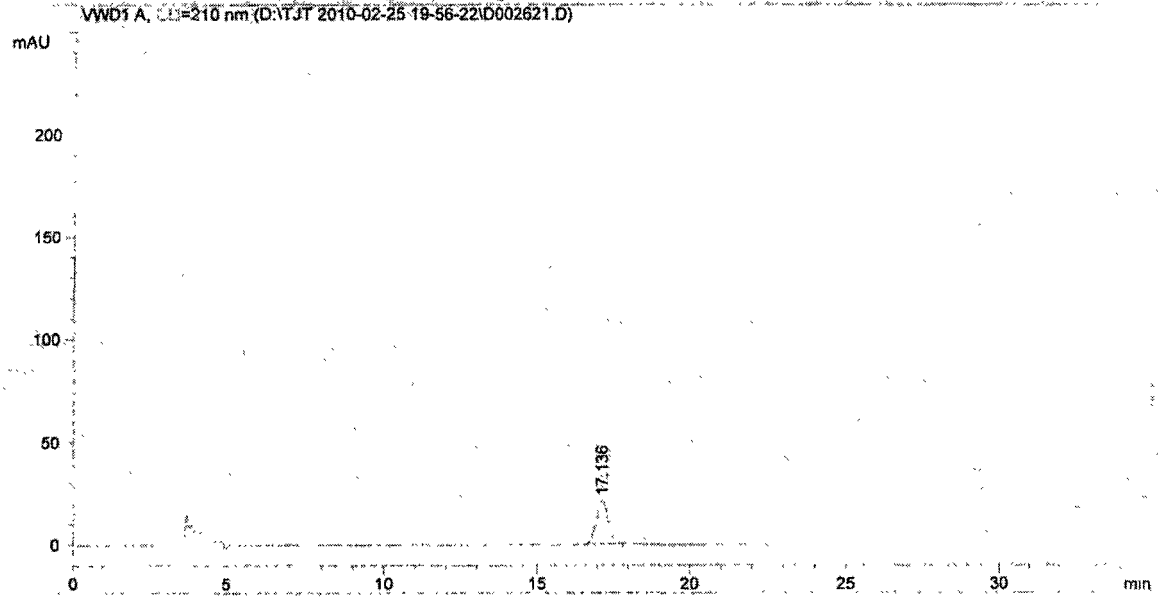
Data File D:\TJT 2010-02-25 19-56-22\D002621.D
Sample Name: B

==========

Acq. Operator : WHZ
Acq. Instrument : 2
Injection Date : 2/26/2010 1:32:50 AM

Seq. Line : 47
Location : P1-B-02
Inj : 1
Inj Volume : 5 µl

Different Inj Volume from Sequence ! Actual Inj Volume : 15 µl
Acq. Method : E:\Chem32\2\DATA\20100225\TJT 2010-02-25 19-56-22\TIANJUT.M
Last changed : 2/26/2010 1:31:52 AM by WHZ
(modified after loading)
Analysis Method : C:\Chem32\1\METHODS\DEF_LC.M
Last changed : 3/23/2010 7:48:21 PM
(modified after loading)



==========

External Standard Report

==========

Sorted By : Signal
Calib. Data Modified : 3/23/2010 7:47:28 PM
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, =210 nm

RetTime [min]	Type	Area mAU *s	Amt/Area	Amount [ug]	Grp	Name
17.136	BB	563.29016	1.11299e-2	6.26934		Rebaudioside D

Totals : 6.26934

==========

*** End of Report ***

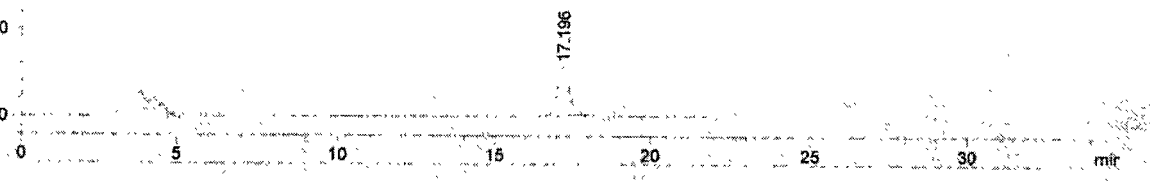
Instrument 1 3/23/2010 7:48:24 PM

Page 1 of 1

ample Name: B

```
==================================================================
Acq. Operator    : WHZ                              Seq. Line :  48
Acq. Instrument  : [illegible] 2                    Location : P1-B-02
Injection Date   : 2/26/2010 1:56:42 AM                   Inj :   1
                                                    Inj Volume : 5 µl
Different Inj Volume from Sequence !      Actual Inj Volume : 20 µl
Acq. Method      : E:\Chem32\2\DATA\20100225\TJT 2010-02-25 19-56-22\TIANJUT.M
Last changed     : 2/26/2010 1:55:39 AM by WHZ
                   (modified after loading)
Analysis Method  : C:\Chem32\1\METHODS\DEF_LC.M
Last changed     : 3/23/2010 7:47:39 PM
                   (modified after loading)
```

VWD1 A, [illegible]=210 nm (D:\TJT 2010-02-25 19-56-22\D002622.D)




```
==================================================================
                      External Standard Report
==================================================================

Sorted By              :      Signal
Calib. Data Modified   :      3/23/2010 7:47:28 PM
Multiplier             :      1.0000
Dilution               :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, [illegible]=210 nm

RetTime  Type     Area      Amt/Area    Amount   Grp   Name
 [min]          mAU   *s                [ug]
-------|------|----------|----------|----------|--|-----------------
 17.196 BB      749.73969 1.11289e-2    8.34375     Rebaudioside D

Totals :                                 8.34375


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

Method C:\Chem32\1\METHODS\DEF_LC.M

Calibration Table

Calib. Data Modified : 3/23/2010 8:01:22 PM

Calculate : External Standard
Based on : Peak Area

Rel. Reference Window : 5.000 %
Abs. Reference Window : 0.000 min
Rel. Non-ref. Window : 5.000 %
Abs. Non-ref. Window : 0.000 min
Use Multiplier & Dilution Factor with ISTDs
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: VWD1 A, λ=210 nm

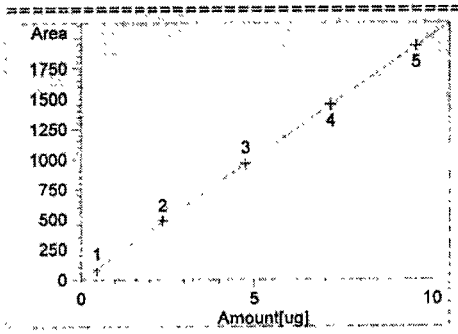
RetTime [min]	Sig	Lvl	Amount [ug]	Area	Amt/Area	Ref	Grp	Name
18.091	1	1	4.76435e-1	76.98996	6.18828e-3			Rebaudioside B
		2	2.38218	482.18515	4.94038e-3			
		3	4.76435	964.94226	4.93745e-3			
		4	7.14653	1450.51404	4.92689e-3			
		5	9.52870	1931.82617	4.93249e-3			

Peak Sum Table

No Entries in table

Method C:\Chem32\1\METHODS\DEF_LC.M

Calibration Curves



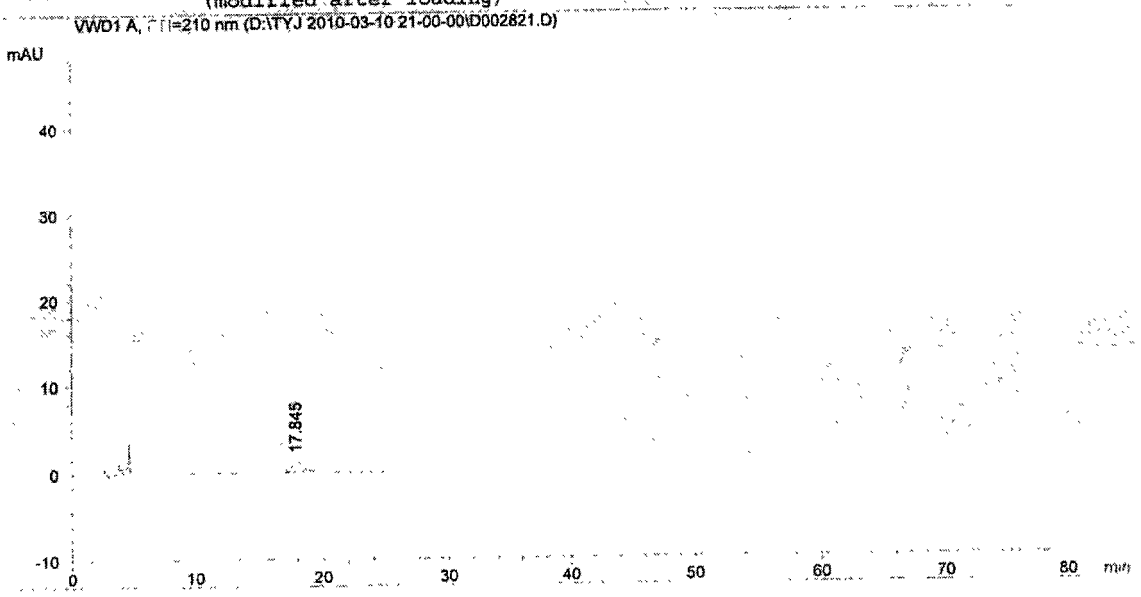
Rebaudioside B at exp. RT: 18.091
VWD1 A, λ=210 nm
Correlation: 0.99996
Residual Std. Dev.: 7.81362
Formula: y = mx + b
m: 203.78829
b: -7.53834
x: Amount
y: Area

Instrument 1 3/23/2010 8:06:24 PM

Page 2 of 2

Data File D:\TYJ 2010-03-10 21-00-00\D002821.D
Sample Name: B

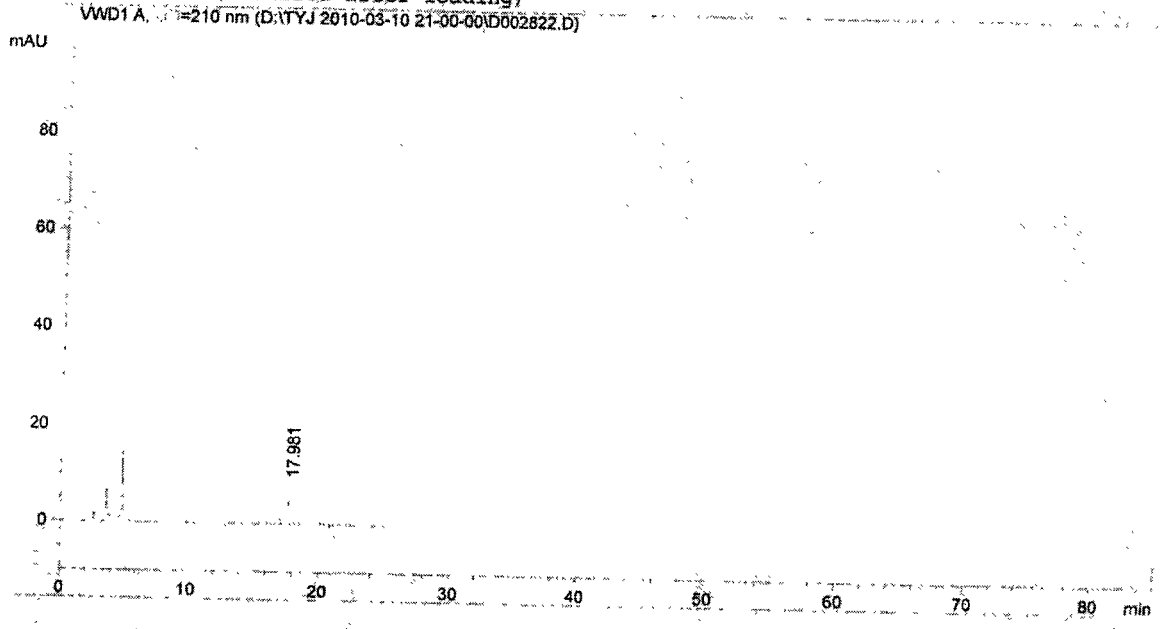
```
==================================================================
Acq. Operator    : WHZ                              Seq. Line :   9
Acq. Instrument  : 2                               Location : P1-B-02
Injection Date   : 3/10/2010 9:17:57 AM                  Inj :   1
                                                   Inj Volume : 5 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 1 µl
Acq. Method      : E:\Chem32\2\DATA\20100310\TYJ 2010-03-10 21-00-00\TIANJUT.M
Last changed     : 3/10/2010 9:17:14 AM by WHZ
                   (modified after loading)
Analysis Method  : C:\Chem32\1\METHODS\DEF_LC.M
Last changed     : 3/23/2010 8:08:43 PM
                   (modified after loading)
```




```
==================================================================
                      External Standard Report
==================================================================

Sorted By              :      Signal
Calib. Data Modified   :      3/23/2010 8:07:01 PM
Multiplier             :      1.0000
Dilution               :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, =210 nm

RetTime  Type     Area      Amt/Area    Amount   Grp   Name
 [min]          mAU   *s                [ug]
-------|------|----------|----------|----------|--|-----------------
 17.845 BB       76.98996 5.38752e-3 4.14785e-1     Rebaudioside B

Totals :                              4.14785e-1

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

Instrument 1 3/23/2010 8:08:45 PM                        Page   1 of 1
```

Sample Name: B

```
Acq. Operator    : WHZ                                  Seq. Line :  10
Acq. Instrument  : 2                                    Location : P1-B-02
Injection Date   : 3/10/2010 9:45:32 AM                        Inj :   1
                                                        Inj Volume : 5 µl
Acq. Method      : E:\Chem32\2\DATA\20100310\TYJ 2010-03-10 21-00-00\TIANJUT.M
Last changed     : 3/10/2010 9:44:59 AM by WHZ
                   (modified after loading)
Analysis Method  : C:\Chem32\1\METHODS\DEF_LC.M
Last changed     : 3/23/2010 8:08:29 PM
                   (modified after loading)
```



External Standard Report

```
Sorted By              :      Signal
Calib. Data Modified   :      3/23/2010 8:07:01 PM
Multiplier             :      1.0000
Dilution               :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, =210 nm

RetTime  Type     Area      Amt/Area     Amount    Grp   Name
 [min]          mAU   *s                  [ug]
--------|------|----------|----------|----------|--|------------------
 17.981 BB      482.18515 4.98377e-3    2.40310     Rebaudioside B

Totals :                                 2.40310
```

*** End of Report ***

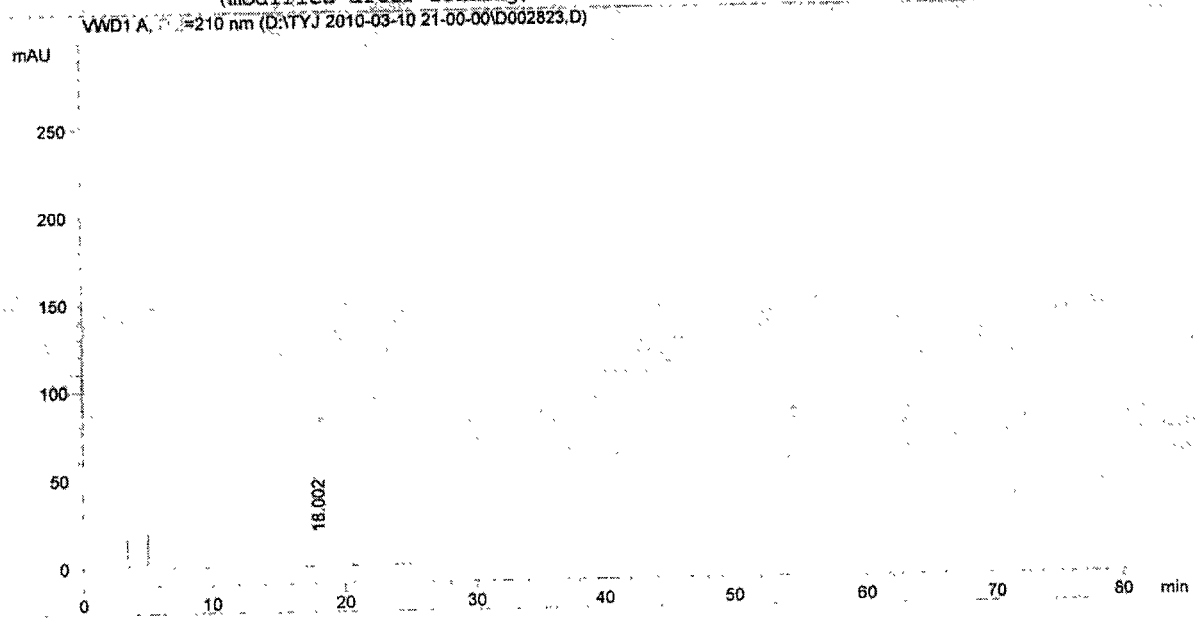
Data File D:\TYJ 2010-03-10 21-00-00\D002823.D
Sample Name: B

==========

Acq. Operator : WHZ
Acq. Instrument : 1 2
Injection Date : 3/10/2010 10:13:13 AM

Seq. Line : 11
Location : P1-B-02
Inj : 1
Inj Volume : 5 µl

Different Inj Volume from Sequence ! Actual Inj Volume : 10 µl
Acq. Method : E:\Chem32\2\DATA\20100310\TYJ 2010-03-10 21-00-00\TIANJUT.M
Last changed : 3/10/2010 10:12:34 AM by WHZ
(modified after loading)
Analysis Method : C:\Chem32\1\METHODS\DEF_LC.M
Last changed : 3/23/2010 8:07:34 PM
(modified after loading)



==========

External Standard Report

==========

Sorted By : Signal
Calib. Data Modified : 3/23/2010 8:07:01 PM
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, =210 nm

RetTime [min]	Type	Area mAU *s	Amt/Area	Amount [ug]	Grp	Name
18.002	BB	964.94226	4.94539e-3	4.77201		Rebaudioside B
Totals :				4.77201		

==========

*** End of Report ***

Instrument 1 3/23/2010 8:08:14 PM

Page 1 of 1

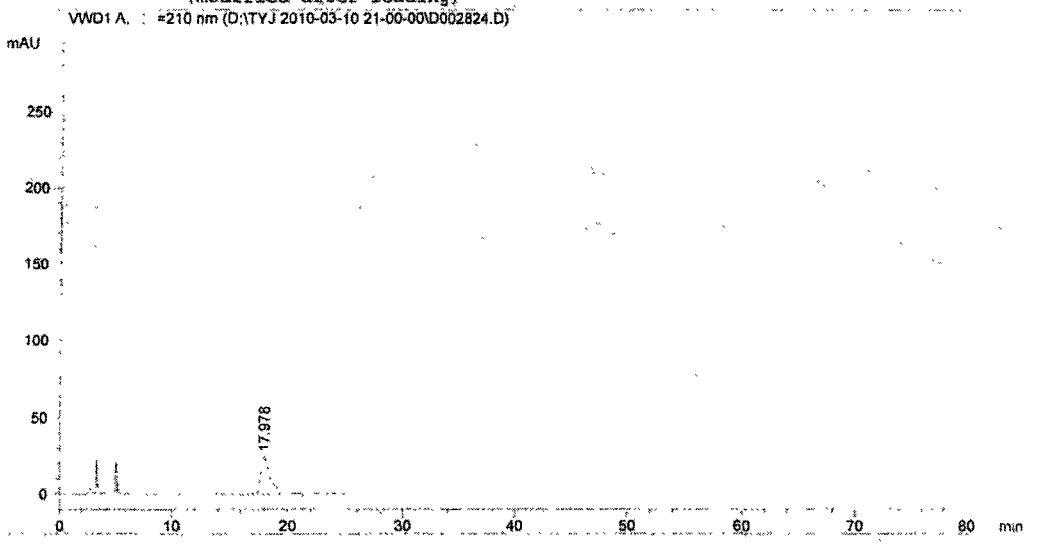
Data File D:\TYJ 2010-03-10 21-00-00\D002824.D
Sample Name: B

==========

Acq. Operator : WHZ
Acq. Instrument : 2
Injection Date : 3/10/2010 10:41:01 AM

Seq. Line : 12
Location : P1-B-02
Inj : 1
Inj Volume : 5 µl

Different Inj Volume from Sequence ! Actual Inj Volume : 15 µl
Acq. Method : E:\Chem32\2\DATA\20100310\TYJ 2010-03-10 21-00-00\TIANJUT.M
Last changed : 3/10/2010 10:40:15 AM by WHZ
(modified after loading)
Analysis Method : C:\Chem32\1\METHODS\DEF_LC.M
Last changed : 3/23/2010 8:07:34 PM
(modified after loading)



==========

External Standard Report

==========

Sorted By : Signal
Calib. Data Modified : 3/23/2010 8:07:01 PM
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, =210 nm

RetTime [min]	Type	Area mAU *s	Amt/Area	Amount [ug]	Grp	Name
17.978	BB	1450.51404	4.93256e-3	7.15474		Rebaudioside B

Totals : 7.15474

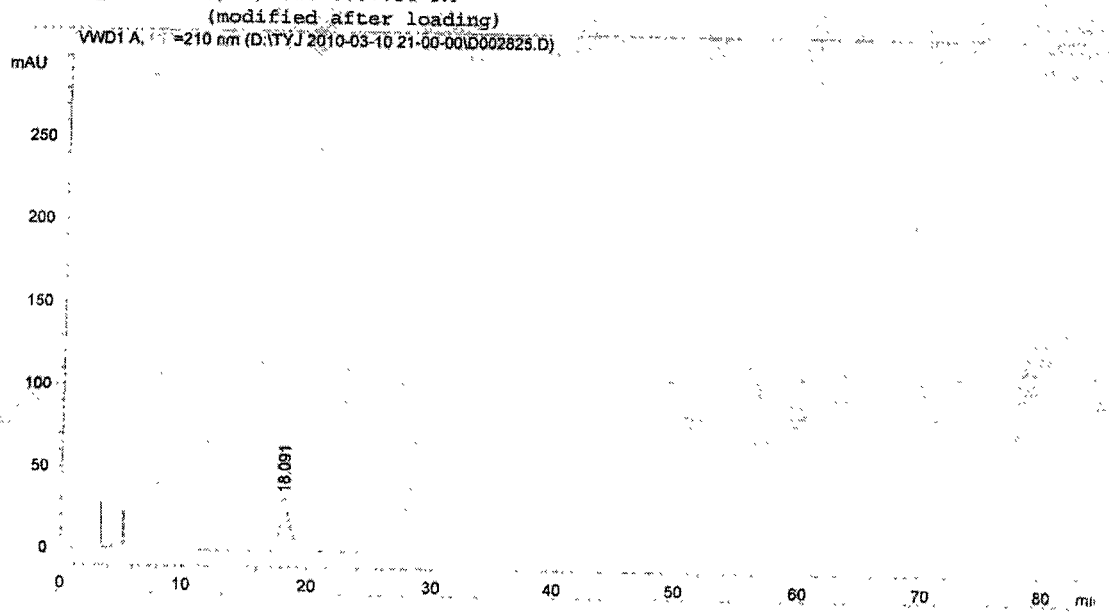
==========

*** End of Report ***

Instrument 1 3/23/2010 8:08:03 PM

Page 1 of 1

```
Acq. Operator    : WHZ                                    Seq. Line :  13
Acq. Instrument  : 2                                      Location : P1-B-02
Injection Date   : 3/10/2010 11:08:54 AM                        Inj :   1
                                                          Inj Volume : 5 µl
Different Inj Volume from Sequence !      Actual Inj Volume : 20 µl
Acq. Method      : E:\Chem32\2\DATA\20100310\TYJ 2010-03-10 21-00-00\TIANJUT.M
Last changed     : 3/10/2010 11:08:03 AM by WHZ
                   (modified after loading)
Analysis Method  : C:\Chem32\1\METHODS\DEF_LC.M
Last changed     : 3/23/2010 8:07:34 PM
                   (modified after loading)
```




```
External Standard Report

Sorted By              :      Signal
Calib. Data Modified   :      3/23/2010 8:07:01 PM
Multiplier             :      1.0000
Dilution               :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, =210 nm

RetTime  Type    Area       Amt/Area     Amount    Grp   Name
 [min]          mAU   *s                 [ug]
-------|------|----------|----------|----------|--|-----------------
 18.091 BB     1931.82617 4.92620e-3    9.51657     Rebaudioside B

Totals :                                 9.51657

*** End of Report ***

Instrument 1 3/23/2010 8:07:38 PM                                  Page  1 of 1
```

Method C:\Chem32\1\METHODS\DEF_LC.M

Calibration Table

Calib. Data Modified : Tuesday, March 23, 2010 4:29:46 PM

Calculate : Area Percent

Rel. Reference Window : 5.000 %
Abs. Reference Window : 0.000 min
Rel. Non-ref. Window : 5.000 %
Abs. Non-ref. Window : 0.000 min
Use Multiplier & Dilution Factor with ISTDs
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: VWD1 A, λ=210 nm

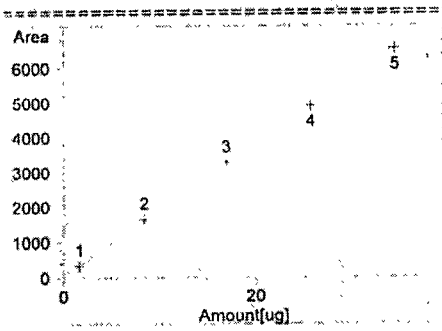
RetTime [min]	Sig	Lvl	Amount [ug]	Area	Amt/Area	Ref	Grp	Name
19.617	1	1	1.70249	329.12811	5.17272e-3			Rebaudioside A
		2	8.51786	1656.85144	5.13797e-3			
		3	17.09486	3292.04249	5.19152e-3			
		4	25.53729	4913.15576	5.19774e-3			
		5	34.04972	6540.09663	5.20630e-3			

Peak Sum Table

No Entries in table

Method C:\Chem32\1\METHODS\DEF_LC.M

Calibration Curves



Rebaudioside A at exp. RT: 18.617
VWD1 A, λ=210 nm
Correlation: 0.99999
Residual Std. Dev.: 11.63225
Formula: y = mx + b
m: 192.06293
b: 9.16451
x: Amount
y: Area

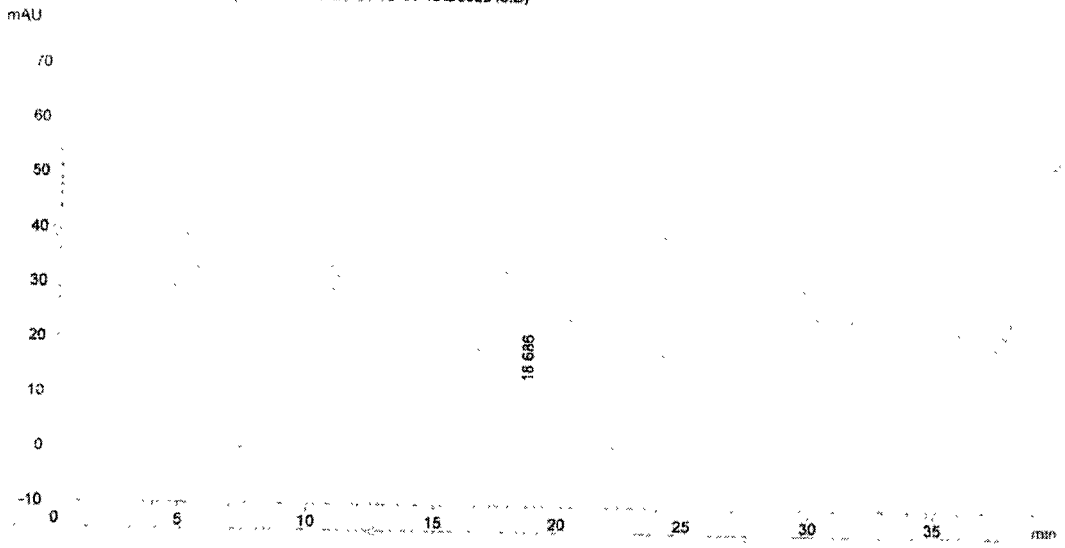
Instrument 1 3/23/2010 4:57:23 PM

Page 2 of 2

Sample Name: b

```
Acq. Operator    : wsy                                   Seq. Line :   4
Acq. Instrument  :   2                                   Location : P1-A-01
Injection Date   : 2/24/2010 4:05:08 AM                        Inj :   1
                                                         Inj Volume : 5 µl
Different Inj Volume from Sequence !      Actual Inj Volume : 1 µl
Acq. Method      : E:\Chem32\2\DATA\20100207\TJT 2010-02-24 18-41-49\TIANJUT.M
Last changed     : 2/24/2010 4:04:05 AM by wsy
                   (modified after loading)
Analysis Method  : C:\Chem32\1\METHODS\DEF_LC.M
Last changed     : 3/23/2010 4:59:45 PM
                   (modified after loading)
```

VWD1 A, =210 nm (D:\TJT 2010-02-24 18-41-49\D002546.D)



Area Percent Report

```
Sorted By              :    Signal
Calib. Data Modified   :    3/23/2010 4:59:31 PM
Multiplier             :    1.0000
Dilution               :    1.0000
Use Multiplier & Dilution Factor with ISTDs
```

Signal 1: VWD1 A, =210 nm

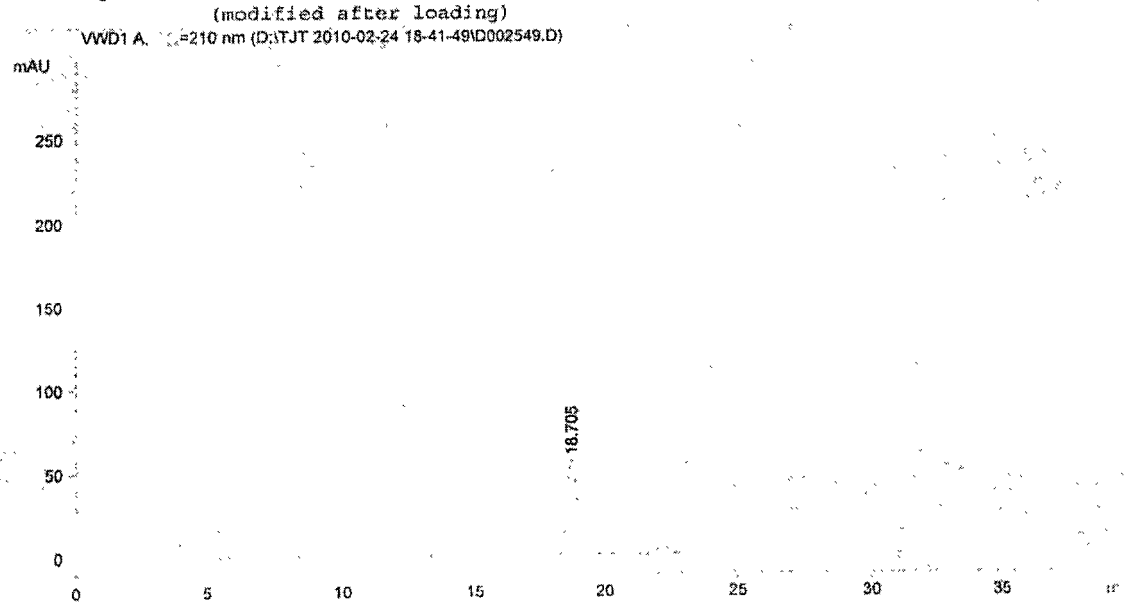
Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Area %	Name
1	16.686	BB	0.4430	329.12811	100.0000	Rebaudioside A

Totals : 329.12811

Data File D:\TJT 2010-02-24 18-41-49\D002549.D
Sample Name: b

Acq. Operator	: wsy	Seq. Line :	7
Acq. Instrument	: CE 2	Location :	P1-A-01
Injection Date	: 2/24/2010 5:19:09 AM	Inj :	1
		Inj Volume :	5 µl

Acq. Method : E:\Chem32\2\DATA\20100207\TJT 2010-02-24 18-41-49\TIANJUT.M
Last changed : 2/24/2010 5:18:24 AM by wsy
(modified after loading)
Analysis Method : C:\Chem32\1\METHODS\DEF_LC.M
Last changed : 3/22/2010 5:01:05 PM
(modified after loading)



Area Percent Report

Sorted By	:	Signal
Calib. Data Modified	:	3/23/2010 4:58:31 PM
Multiplier	:	1.0000
Dilution	:	1.0000

Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, =210 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Area %	Name
1	18.705	BB	0.4525	1656.85144	100.0000	Rebaudioside A

Totals : 1656.85144

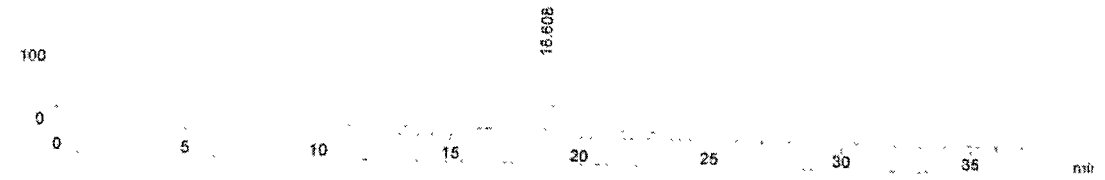
*** End of Report ***

Instrument 1 3/23/2010 5:01:07 PM

Page 1 of 1

```
Acq. Operator    : wsy                                  Seq. Line :   3
Acq. Instrument  :    2                                 Location : P1-A-01
Injection Date   : 2/24/2010 3:40:07 AM                        Inj :   1
                                                        Inj Volume : 5 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 10 µl
Acq. Method      : E:\Chem32\2\DATA\20100207\TJT 2010-02-24 18-41-49\TIANJUT.M
Last changed     : 2/24/2010 3:39:17 AM by wsy
                   (modified after loading)
Analysis Method  : C:\Chem32\1\METHODS\DEF_LC.M
Last changed     : 3/23/2010 4:58:58 PM
                   (modified after loading)
```

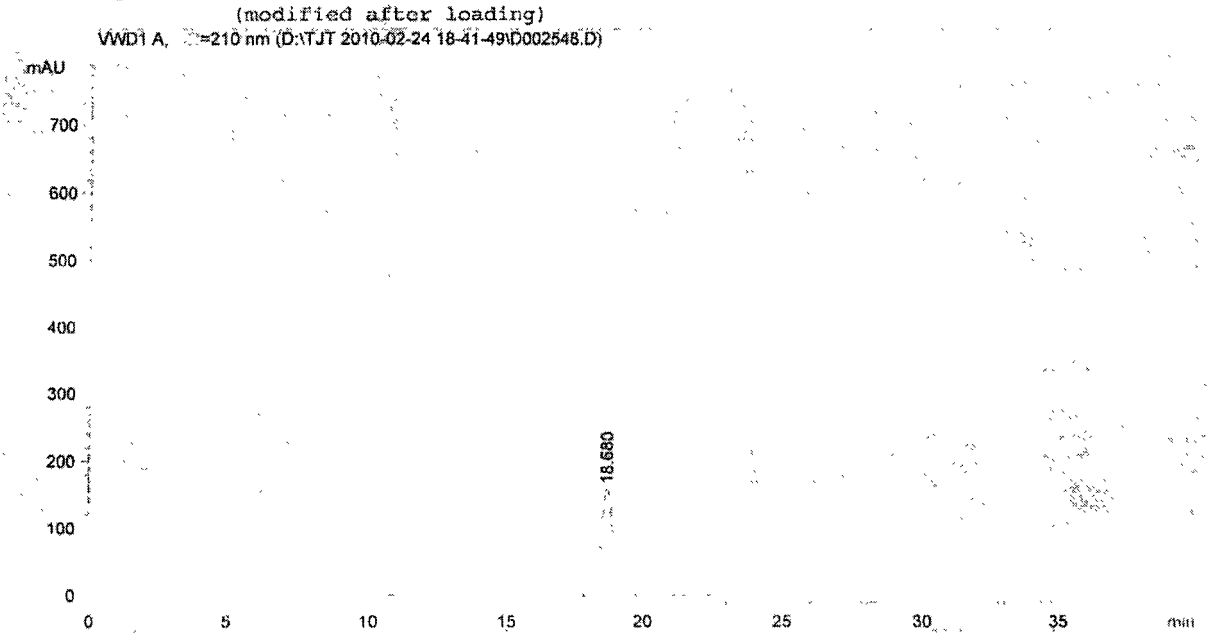
VWD1 A, λ=210 nm (D:\TJT 2010-02-24 18-41-49\0002545.D)




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

Sorted By              :      Signal
Calib. Data Modified   :      3/23/2010 4:58:31 PM
Multiplier             :      1.0000
Dilution               :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, λ=210 nm

Peak RetTime  Type   Width     Area      Area     Name
  #   [min]          [min]   mAU   *s      %
---|-------|------|-------|----------|- ----------------------------
   16.608 BB       0.4567 3292.04248 100.0000 Rebaudioside A

Totals :                  3292.04248

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

rument 1 3/23/2010 4:59:34 PM                                  Page   1 of 1
```

Data File D:\TJT 2010-02-24 18-41-49\D002548.D
Sample Name: b

```
==========================================================================
Acq. Operator    : wsy                                  Seq. Line :   6
Acq. Instrument  : CT 2                                 Location  : P1-A-01
Injection Date   : 2/24/2010 4:54:26 AM                       Inj :   1
                                                        Inj Volume : 5 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 15 µl
Acq. Method      : E:\Chem32\2\DATA\20100207\TJT 2010-02-24 18-41-49\TIANJUT.M
Last changed     : 2/24/2010 4:53:41 AM by wsy
                   (modified after loading)
Analysis Method  : C:\Chem32\1\METHODS\DEF_LC.M
Last changed     : 3/23/2010 5:00:06 PM
                   (modified after loading)
```

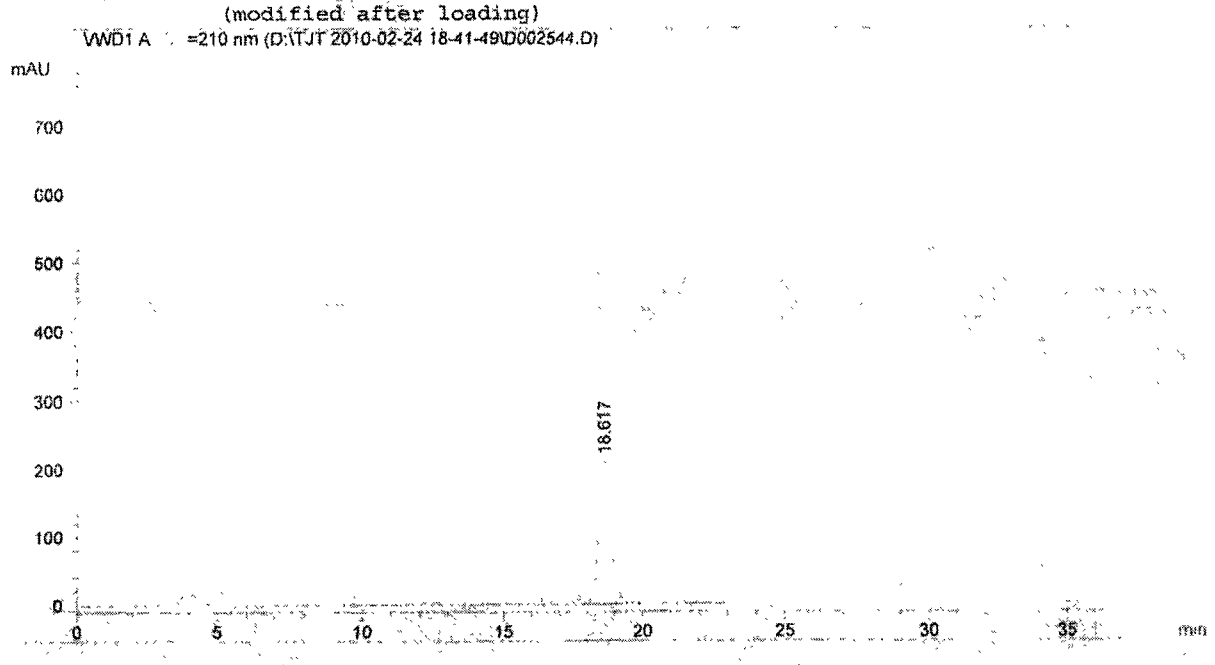



```
==========================================================================
                          Area Percent Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      3/23/2010 4:58:31 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A,   =210 nm

Peak RetTime  Type   Width     Area       Area    Name
  #   [min]          [min]   mAU   *s       %
----|-------|------|-------|----------|--------|--------------------
   1  18.680 BB      0.4704 4913.15576 100.0000 Rebaudioside A

Totals :                     4913.15576


==========================================================================
```

Instrument 1 3/23/2010 5:00:48 PM Page 1 of 1

Data File D:\TJT 2010-02-24 18-41-49\D002544.D
Sample Name: b

```
Acq. Operator    : wsy                                Seq. Line :   2
Acq. Instrument  : 2                                 Location : P1-A-C1
Injection Date   : 2/24/2010 3:15:19 AM                    Inj :   1
                                                    Inj Volume : 5 µl
Different Inj Volume from Sequence !      Actual Inj Volume : 20 µl
Acq. Method      : E:\Chem32\2\DATA\20100207\TJT 2010-02-24 18-41-49\TIANJUT.M
Last changed     : 2/24/2010 3:37:32 AM by wsy
                   (modified after loading)
Analysis Method  : C:\Chem32\1\METHODS\DEF_LC.M
Last changed     : 3/23/2010 4:58:58 PM
                   (modified after loading)
```

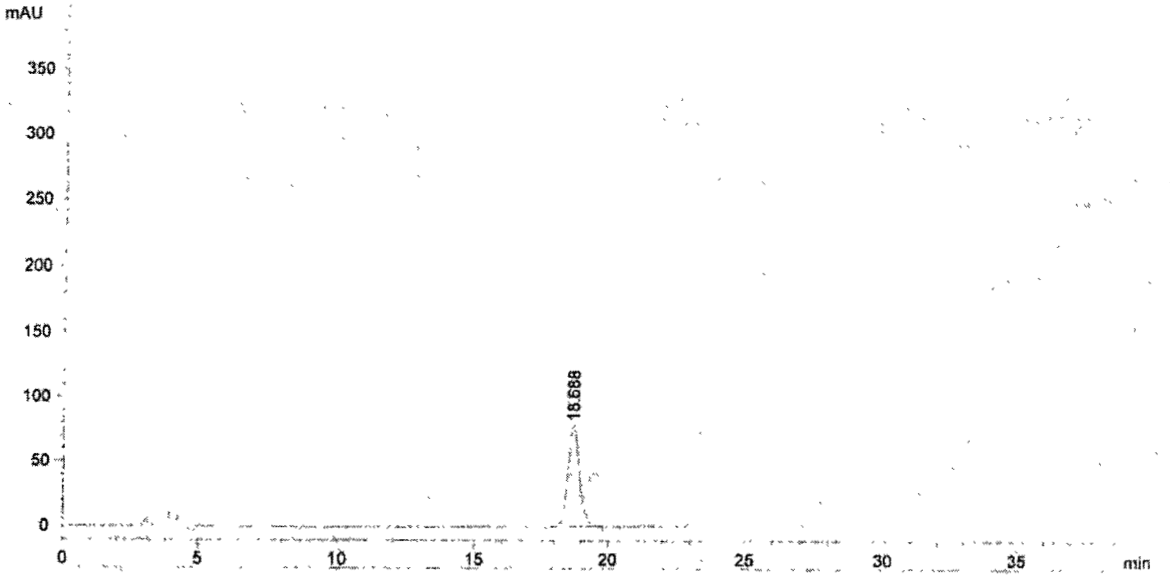



```
==================================================================
                        Area Percent Report
==================================================================

Sorted By              :      Signal
Calib. Data Modified   :      3/23/2010 4:58:31 PM
Multiplier             :      1.0000
Dilution               :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, =210 nm

Peak RetTime  Type   Width     Area      Area    Name
  #   [min]          [min]    mAU  *s      %
---|-------|------|--------|---------|--------|--------------------
   1  18.617 BB      0.4660 6540.09863 100.0000 Rebaudioside A

Totals :                    6540.09863

==================================================================
```

Sample Name: RA 1

```
Acq. Operator    : wsy                                  Seq. Line :   8
Acq. Instrument  : LC 2                                 Location : P1-A-03
Injection Date   : 2/24/2010 5:43:48 AM                       Inj :   1
                                                        Inj Volume : 5 µl
Acq. Method      : E:\Chem32\2\DATA\20100207\TJT 2010-02-24 18-41-49\TIANJUT.M
Last changed     : 2/24/2010 5:43:07 AM by wsy
                   (modified after loading)
Analysis Method  : C:\Chem32\1\METHODS\DEF_LC.M
Last changed     : 3/23/2010 8:26:41 PM
                   (modified after loading)
```

VWD1 A, λ=210 nm (D:\TJT 2010-02-24 18-41-49\D002550.D)



Area Percent Report

```
Sorted By              :      Signal
Multiplier             :      1.0000
Dilution               :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, λ=210 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]       [min]   mAU    *s   [mAU   ]      %
----|-------|----|-------|----------|----------|--------|
   1  18.688 BB    0.4605 2368.57886   78.33058 100.0000

Totals :                  2368.57886   78.33058
```

*** End of Report ***

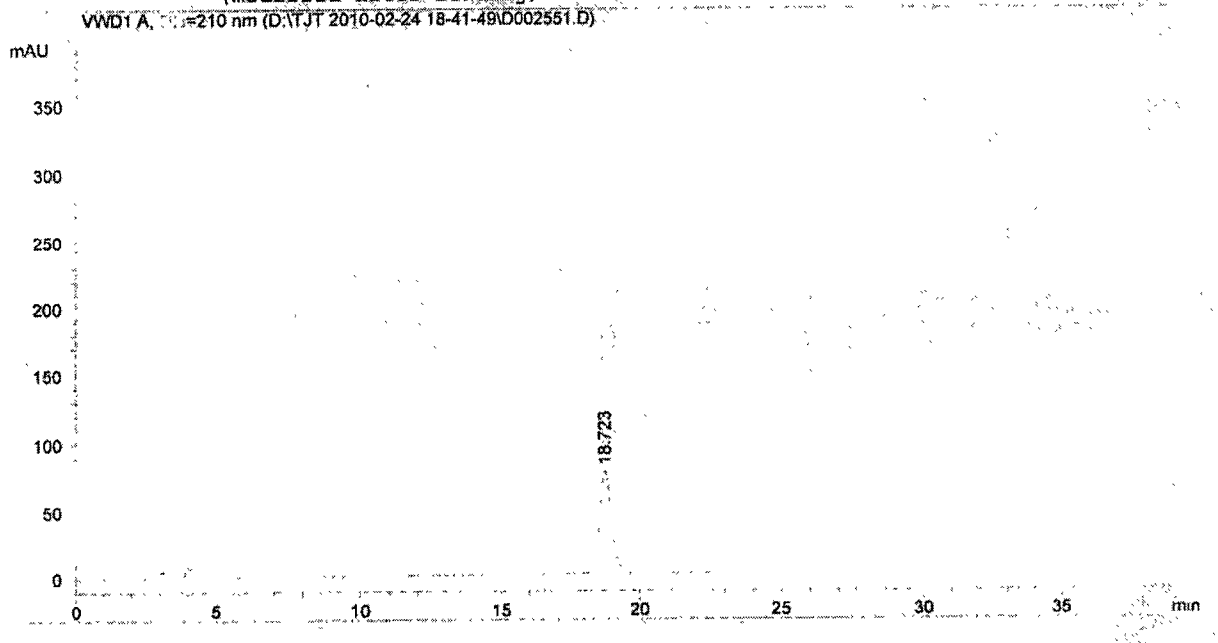
Instrument 1 3/23/2010 8:26:43 PM Page 1 of 1

Data File D:\101 2010-02-24 18-41-49\D002551.D
Sample Name: RA 2

==========

Acq. Operator	: wsy	Seq. Line :	9
Acq. Instrument	: 2	Location :	P1-A-04
Injection Date	: 2/24/2010 6:08:25 AM	Inj :	1
		Inj Volume :	5 µl

Acq. Method : E:\Chem32\2\DATA\20100207\TJT 2010-02-24 18-41-49\TIANJUT.M
Last changed : 2/24/2010 6:07:46 AM by wsy
(modified after loading)
Analysis Method : C:\Chem32\1\METHODS\DEF_LC.M
Last changed : 3/23/2010 8:26:41 PM
(modified after loading)



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Area Percent Report

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Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, =210 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	18.723	BB	0.4560	2311.81494	77.12501	100.0000
Totals :				2311.81494	77.12501	

==========

*** End of Report ***

Instrument 1 3/23/2010 8:26:50 PM

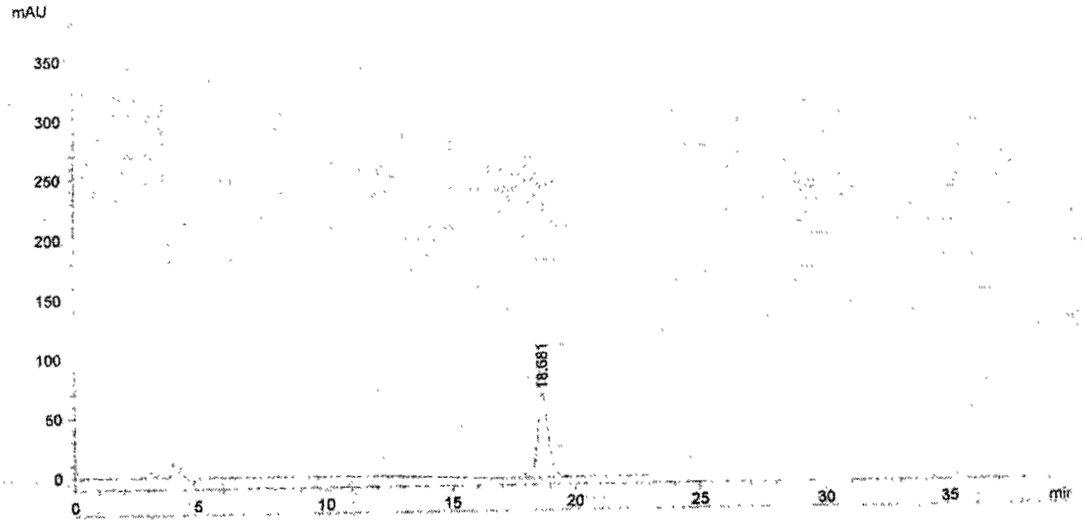
Page 1 of 1

a File D:\TJT 2010-02-24 [illegible]
ple Name: RA 3

Acq. Operator	: wsy	Seq. Line	: 10
Acq. Instrument	: [illegible] 2	Location	: P1-A-05
Injection Date	: 2/24/2010 6:33:04 AM	Inj	: 1
		Inj Volume	: 5 µl

Acq. Method : E:\Chem32\2\DATA\20100207\TJT 2010-02-24 18-41-49\TIANJUT.M
Last changed : 2/24/2010 6:32:23 AM by wsy
(modified after loading)
Analysis Method : C:\Chem32\1\METHODS\DEF_LC.M
Last changed : 3/23/2010 8:26:41 PM
(modified after loading)

VWD1 A, [illegible]=210 nm (D:\TJT 2010-02-24 18-41-49\D002552.D)



Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, [illegible]=210 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	18.681	BB	0.4594	2127.53882	70.57649	100.0000
Totals :				2127.53882	70.57649	

*** End of Report ***

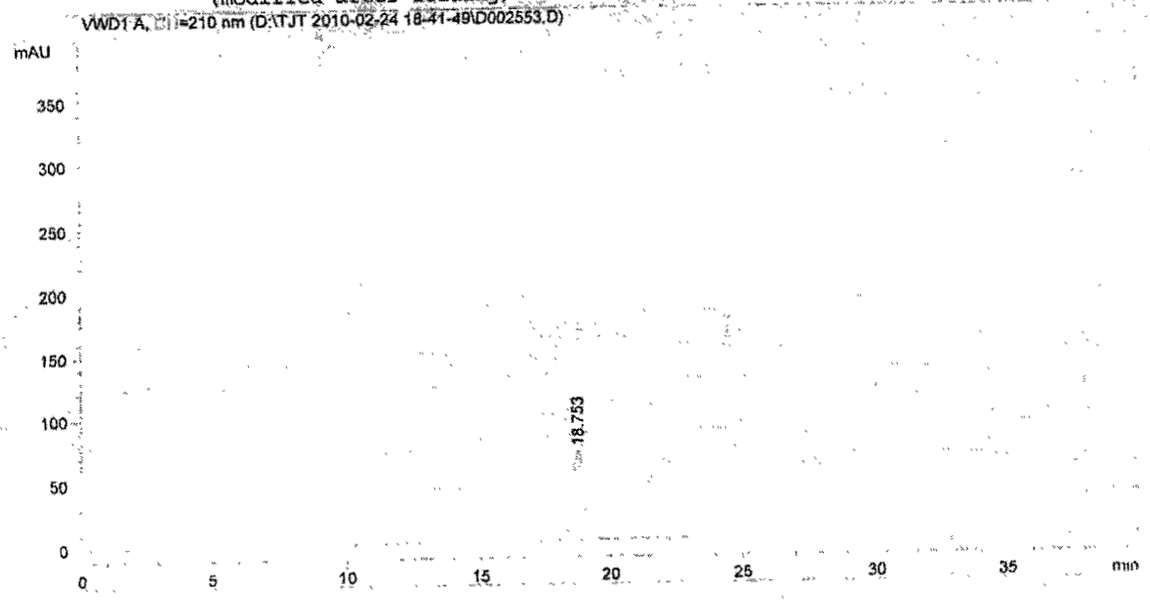
Instrument 1 3/23/2010 8:26:56 PM

Page 1 of 1

File D:\TJT 2010-02-24 18-41-49\
Sample Name: RA 4

Acq. Operator	: wsy	Seq. Line :	11
Acq. Instrument	: 2	Location :	P1-A-06
Injection Date	: 2/24/2010 6:57:43 AM	Inj :	1
		Inj Volume :	5 µl

Acq. Method : E:\Chem32\2\DATA\20100207\TJT 2010-02-24 18-41-49\TIANJUT.M
Last changed : 2/24/2010 6:57:01 AM by wsy
(modified after loading)
Analysis Method : C:\Chem32\1\METHODS\DEF_LC.M
Last changed : 3/23/2010 8:26:41 PM
(modified after loading)



Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wl=210 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	18.753	BB	0.4616	2092.35229	68.99429	100.0000
Totals :				2092.35229	68.99429	

*** End of Report ***

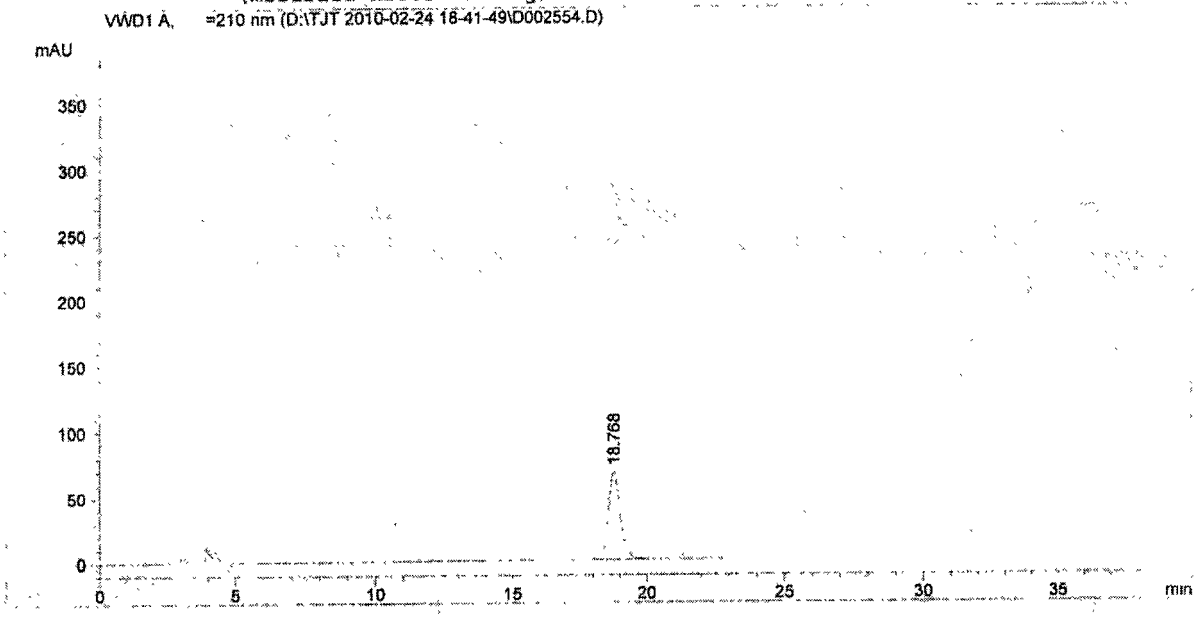
Instrument 1 3/23/2010 8:27:03 PM

Page 1 of 1

Data File D:\TJT 2010-02-24 18-41-49\D002554.D
Sample Name: RA 5

Acq. Operator	: wsy	Seq. Line	: 12
Acq. Instrument	: [illegible] 2	Location	: P1-A-07
Injection Date	: 2/24/2010 7:22:22 AM	Inj	: 1
		Inj Volume	: 5 µl

Acq. Method : E:\Chem32\2\DATA\20100207\TJT 2010-02-24 18-41-49\TIANJUT.M
Last changed : 2/24/2010 7:21:40 AM by wsy
(modified after loading)
Analysis Method : C:\Chem32\1\METHODS\DEF_LC.M
Last changed : 3/23/2010 8:26:41 PM
(modified after loading)



Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, [illegible]=210 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	18.768	BB	0.4599	2075.49951	68.48297	100.0000

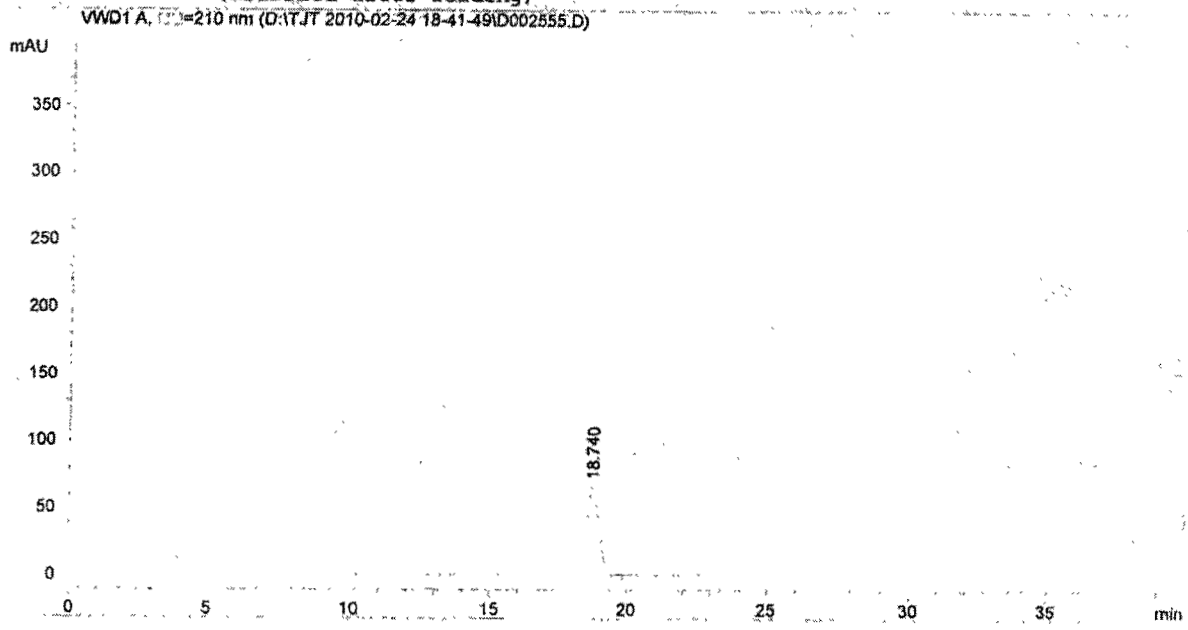
Totals : 2075.49951 68.48297

*** End of Report ***

Instrument 1 3/23/2010 8:27:10 PM

ata File D:\TJT 2010-02-24 18-41-49\D002555.D
ample Name: RA 6

Acq. Operator	: wsy	Seq. Line :	13
Acq. Instrument	: 1\ 2	Location :	P1-A-08
Injection Date	: 2/24/2010 7:47:00 AM	Inj :	1
		Inj Volume :	5 µl
Acq. Method	: E:\Chem32\2\DATA\20100207\TJT 2010-02-24 18-41-49\TIANJUT.M		
Last changed	: 2/24/2010 7:46:18 AM by wsy (modified after loading)		
Analysis Method	: C:\Chem32\1\METHODS\DEF_LC.M		
Last changed	: 3/23/2010 8:26:41 PM (modified after loading)		



Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, =210 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	18.740	BB	0.4633	2052.45117	67.34966	100.0000

Totals : 2052.45117 67.34966

*** End of Report ***

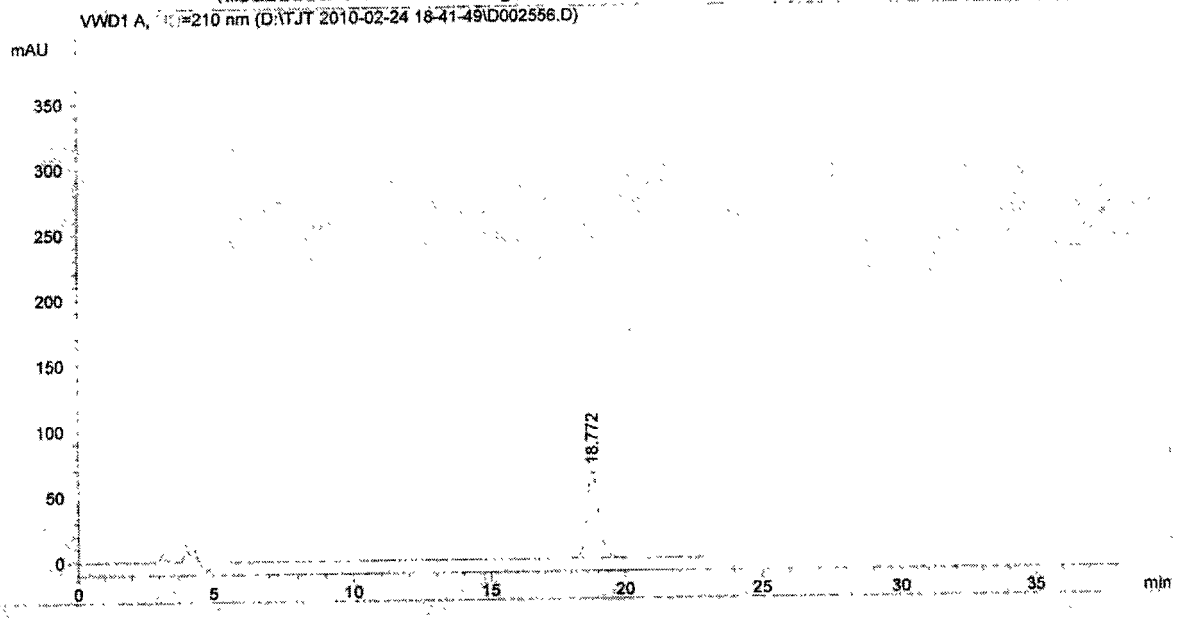
Data File D:\TJT 2010-02-24 18-41-49\D002556.D
Sample Name: RA 7

==========

Acq. Operator : wsy
Acq. Instrument : 2
Injection Date : 2/24/2010 8:11:39 AM

Seq. Line : 14
Location : P1-A-09
Inj : 1
Inj Volume : 5 µl

Acq. Method : E:\Chem32\2\DATA\20100207\TJT 2010-02-24 18-41-49\TIANJUT.M
Last changed : 2/24/2010 8:10:57 AM by wsy
(modified after loading)
Analysis Method : C:\Chem32\1\METHODS\DEF_LC.M
Last changed : 3/23/2010 8:26:41 PM
(modified after loading)



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Area Percent Report

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Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, =210 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	18.772	BB	0.4584	2045.25098	67.75658	100.0000

Totals : 2045.25098 67.75658

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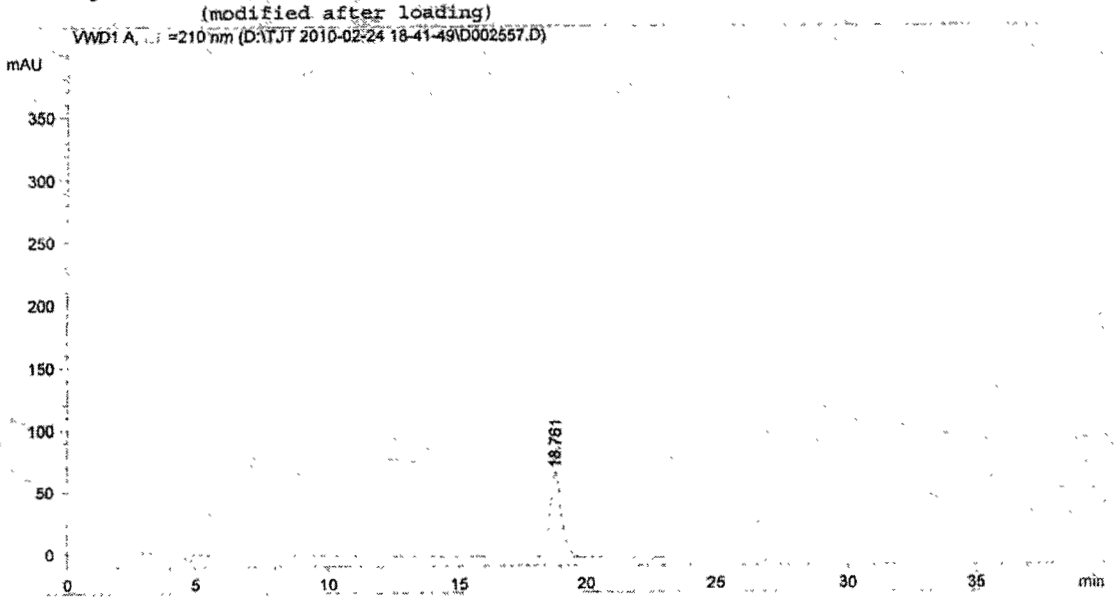
*** End of Report ***

Instrument 1 3/23/2010 8:27:25 PM

Page 1 of 1

Data File D:\TJT 2010-02-24 18-41-49\D002557.D
Sample Name: RA 8

Acq. Operator	: wsy	Seq. Line	: 15
Acq. Instrument	: [illegible] 2	Location	: P1-B-01
Injection Date	: 2/24/2010 8:36:16 AM	Inj	: 1
		Inj Volume	: 5 µl
Acq. Method	: E:\Chem32\2\DATA\20100207\TJT 2010-02-24 18-41-49\TIANJUT.M		
Last changed	: 2/24/2010 8:35:35 AM by wsy (modified after loading)		
Analysis Method	: C:\Chem32\1\METHODS\DEF_LC.M		
Last changed	: 3/23/2010 8:26:41 PM (modified after loading)		



Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, [illegible]=210 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	18.761	BB	0.4646	2075.23437	68.11649	100.0000
Totals :				2075.23437	68.11649	

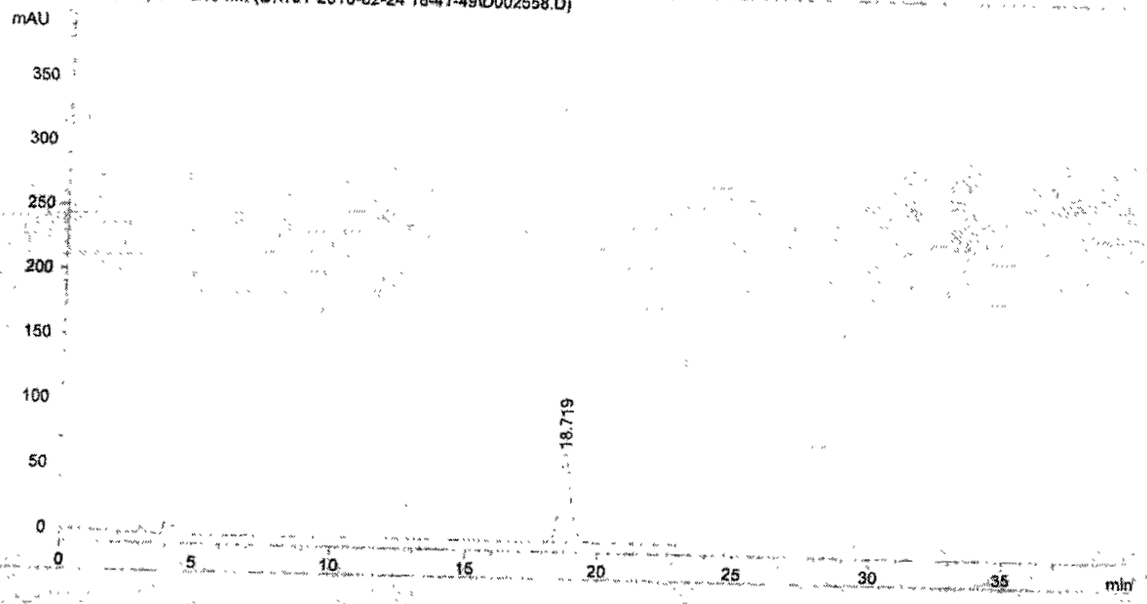
*** End of Report ***

Instrument 1 3/23/2010 8:27:32 PM

Page 1 of 1

```
Acq. Operator    : wsy                                    Seq. Line :  16
Acq. Instrument  : 2                                      Location : P1-B-02
Injection Date   : 2/24/2010 9:00:54 AM                        Inj :   1
                                                          Inj Volume : 5 µl
Acq. Method      : E:\Chem32\2\DATA\20100207\TJT 2010-02-24 18-41-49\TIANJUT.M
Last changed     : 2/24/2010 9:00:12 AM by wsy
                   (modified after loading)
Analysis Method  : C:\Chem32\1\METHODS\DEF_LC.M
Last changed     : 3/23/2010 8:26:41 PM
                   (modified after loading)
```

VWD1 A, =210 nm (D:\TJT 2010-02-24 18-41-49\0002558.D)

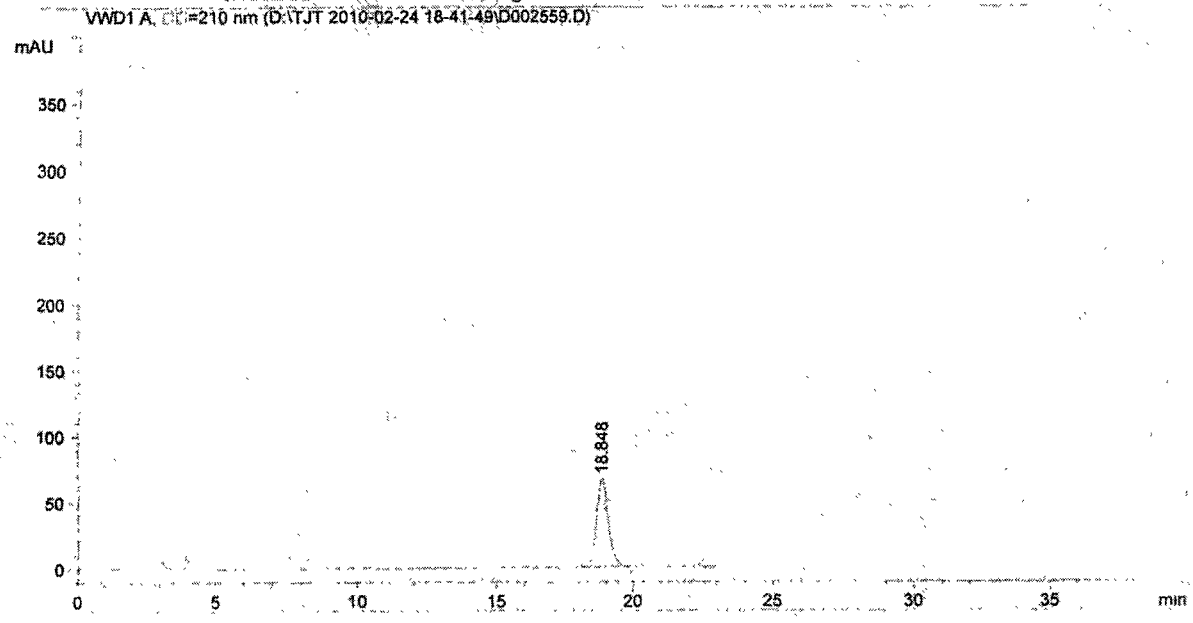



```
==========================================================================
                          Area Percent Report
==========================================================================

Sorted By              :      Signal
Multiplier             :      1.0000
Dilution               :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, =210 nm

Peak RetTime Type  Width     Area       Height      Area
  #   [min]        [min]   mAU   *s    [mAU   ]     %
----|-------|----|-------|----------|----------|--------|
   1  18.719 BB   0.4579 2036.16333   67.55595 100.0000

Totals :                  2036.16333   67.55595


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

trument 1 3/23/2010 8:27:39 PM                                  Page   1 of 1
```

Data File D:\TJT 2010-02-24 18-41-49\D002559.D
Sample Name: RA 10

Acq. Operator	: wsy	Seq. Line	: 17
Acq. Instrument	: 2	Location	: P1-B-03
Injection Date	: 2/24/2010 9:25:31 AM	Inj	: 1
		Inj Volume	: 5 µl

Acq. Method : E:\Chem32\2\DATA\20100207\TJT 2010-02-24 18-41-49\TIANJUT.M
Last changed : 2/24/2010 9:24:50 AM by wsy
(modified after loading)
Analysis Method : C:\Chem32\1\METHODS\DEF_LC.M
Last changed : 3/23/2010 8:26:41 PM
(modified after loading)



Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, =210 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	18.848	BB	0.4574	2002.06909	66.52962	100.0000
Totals :				2002.06909	66.52962	

*** End of Report ***

Instrument 1 3/23/2010 8:27:46 PM

Page 1 of 1

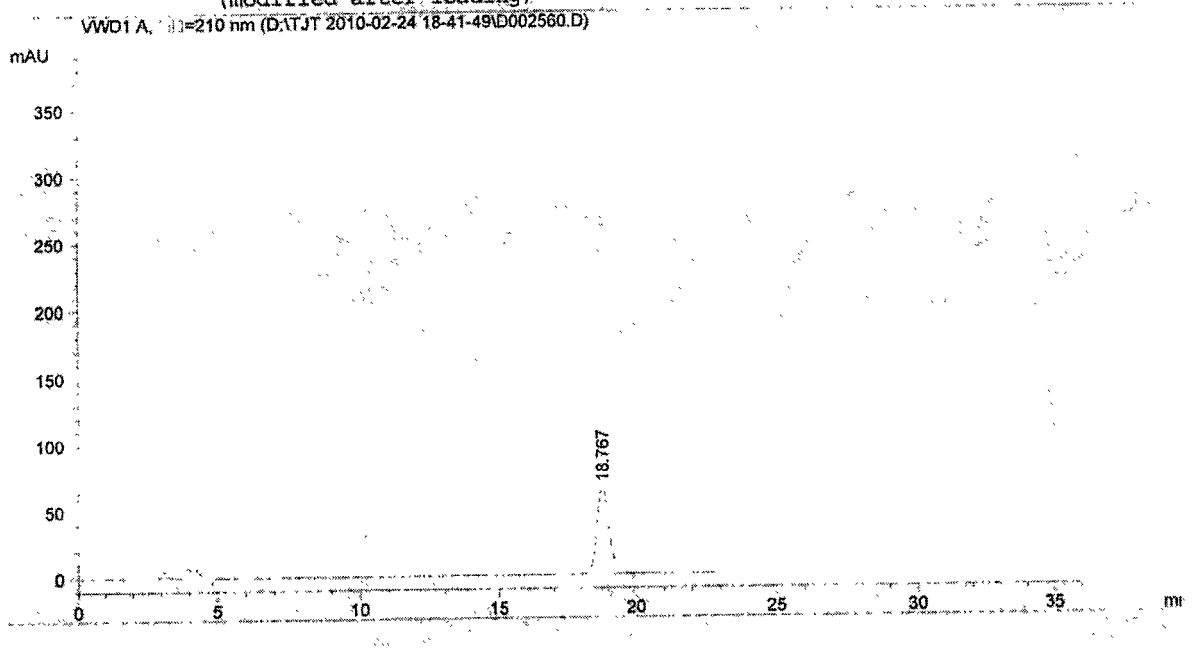
Data File D:\TJT 2010-02-24 18-41-49\D002560.D
Sample Name: RA 11

==========

Acq. Operator : wsy
Acq. Instrument : [illegible] 2
Injection Date : 2/24/2010 9:50:09 AM

Seq. Line : 18
Location : P1-B-04
Inj : 1
Inj Volume : 5 µl

Acq. Method : E:\Chem32\2\DATA\20100207\TJT 2010-02-24 18-41-49\TIANJUT.M
Last changed : 2/24/2010 9:49:28 AM by wsy
(modified after loading)
Analysis Method : C:\Chem32\1\METHODS\DEF_LC.M
Last changed : 3/23/2010 8:26:41 PM
(modified after loading)



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Area Percent Report

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Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, [illegible]=210 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	18.767	BB	0.4600	1990.80505	65.66197	100.0000
Totals :				1990.80505	65.66197	

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*** End of Report ***

Instrument 1 3/23/2010 8:27:54 PM

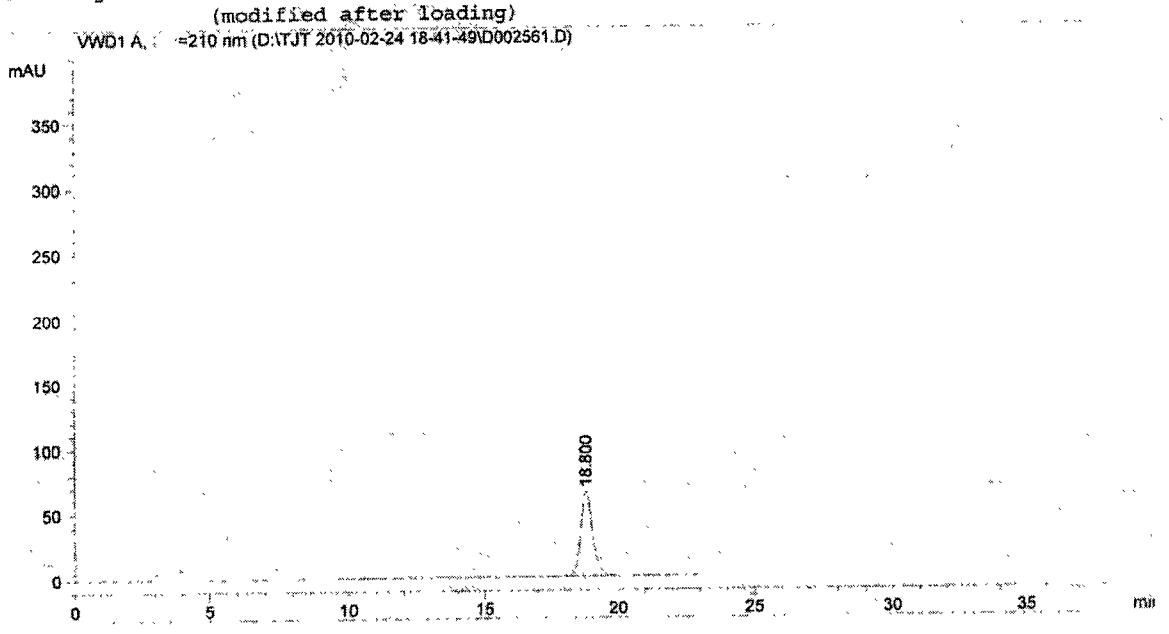
Data File D:\TJT 2010-02-24 18-41-49\D002561.D
Sample Name: RA 12

==========

Acq. Operator : wsy
Acq. Instrument : TJ 2
Injection Date : 2/24/2010 10:14:48 AM

Seq. Line : 19
Location : P1-B-05
Inj : 1
Inj Volume : 5 µl

Acq. Method : E:\Chem32\2\DATA\20100207\TJT 2010-02-24 18-41-49\TIANJUT.M
Last changed : 2/24/2010 10:14:06 AM by wsy
(modified after loading)
Analysis Method : C:\Chem32\1\METHODS\DEF_LC.M
Last changed : 3/23/2010 8:26:41 PM
(modified after loading)



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Area Percent Report

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Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, =210 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	18.800	BB	0.4626	1964.49219	64.58205	100.0000
Totals :				1964.49219	64.58205	

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*** End of Report ***

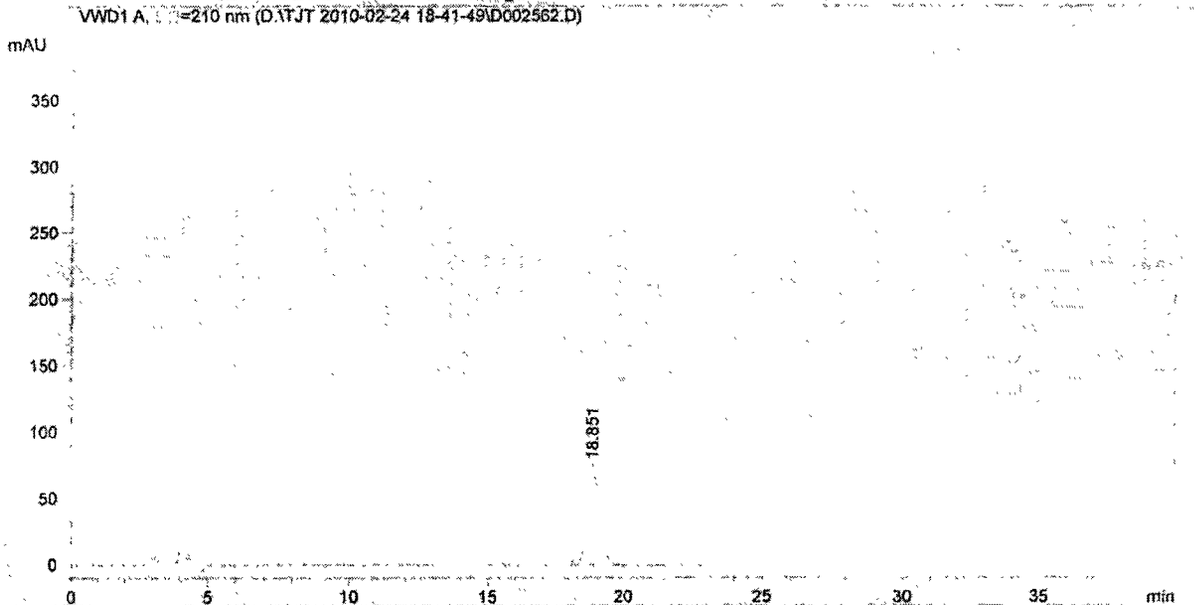
Instrument 1 3/23/2010 8:28:01 PM

Page 1 of 1

a File D:\TJT 2010-02-24 18-41-49\D002562.D
ple Name: RA 13

Acq. Operator	: wsy	Seq. Line	: 20
Acq. Instrument	: 2	Location	: P1-B-06
Injection Date	: 2/24/2010 10:39:26 AM	Inj	: 1
		Inj Volume	: 5 µl

Acq. Method : E:\Chem32\2\DATA\20100207\TJT 2010-02-24 18-41-49\TIANJUT.M
Last changed : 2/24/2010 10:38:44 AM by wsy
(modified after loading)
Analysis Method : C:\Chem32\1\METHODS\DEF_LC.M
Last changed : 3/23/2010 8:26:41 PM
(modified after loading)



Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, =210 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	18.851	BB	0.4603	2296.42383	75.99670	100.0000
Totals :				2296.42383	75.99670	

*** End of Report ***

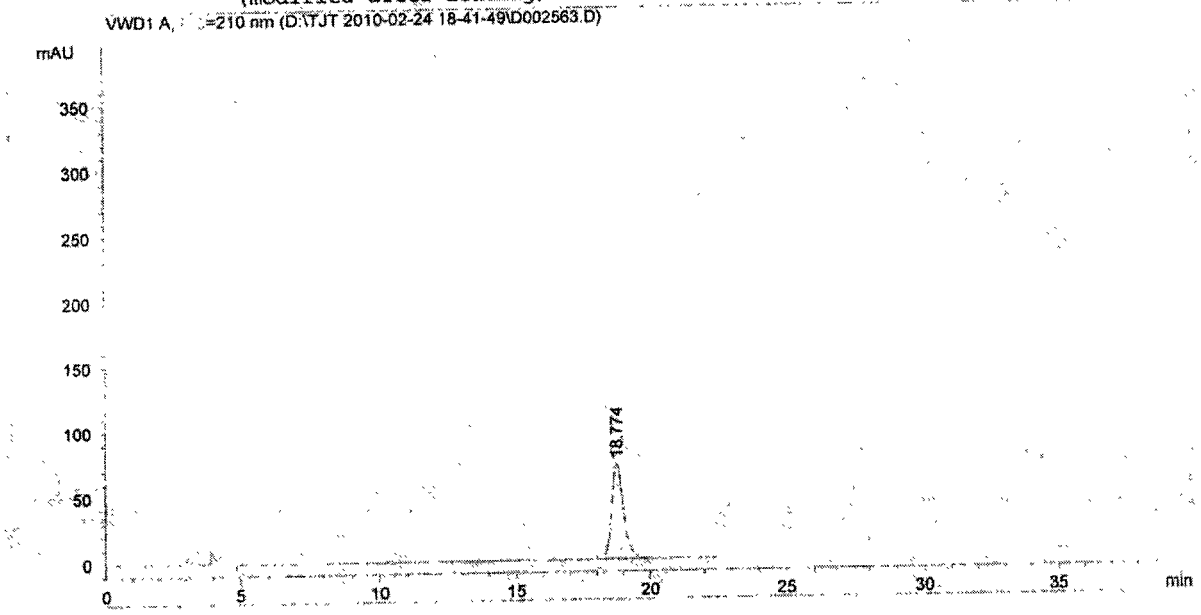
Instrument 1 3/23/2010 8:28:07 PM

Page 1 of 1

Data File D:\TJT 2010-02-24 18-41-49\D002563.D
Sample Name: RA 14

Acq. Operator	: wsy	Seq. Line :	21
Acq. Instrument	: HP 2	Location :	P1-B-07
Injection Date	: 2/24/2010 11:04:03 AM	Inj :	1
		Inj Volume :	5 µl

Acq. Method : E:\Chem32\2\DATA\20100207\TJT 2010-02-24 18-41-49\TIANJUT.M
Last changed : 2/24/2010 11:03:22 AM by wsy
(modified after loading)
Analysis Method : C:\Chem32\1\METHODS\DEF_LC.M
Last changed : 3/23/2010 8:26:41 PM
(modified after loading)



Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, =210 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	18.774	BB	0.4639	2224.76367	72.87873	100.0000
Totals :				2224.76367	72.87873	

*** End of Report ***

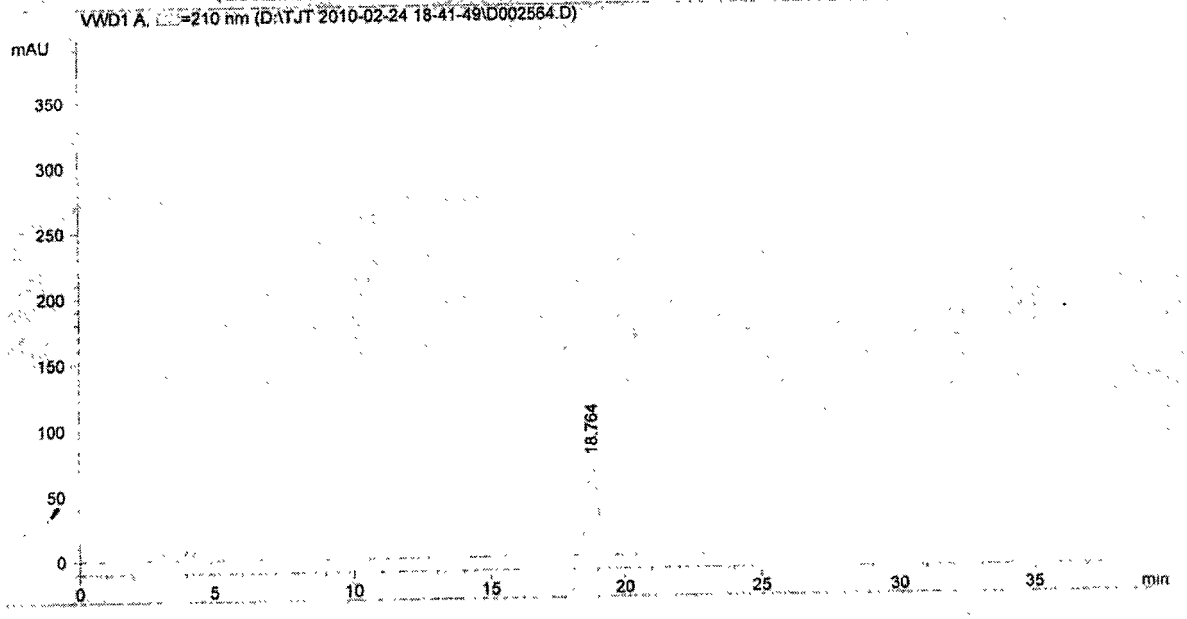
Instrument 1 3/23/2010 8:28:14 PM

Page 1 of 1

Data File D:\TJT 2010-02-24 18-41-49\D002564.D
Sample Name: RA 15

Acq. Operator	: wsy	Seq. Line :	22
Acq. Instrument	: 1 2	Location :	P1-B-08
Injection Date	: 2/24/2010 11:28:41 AM	Inj :	1
		Inj Volume :	5 µl

Acq. Method : E:\Chem32\2\DATA\20100207\TJT 2010-02-24 18-41-49\TIANJUT.M
Last changed : 2/24/2010 11:28:00 AM by wsy
(modified after loading)
Analysis Method : C:\Chem32\1\METHODS\DEF_LC.M
Last changed : 3/23/2010 8:26:41 PM
(modified after loading)



Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, =210 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	18.764	BB	0.4659	2241.81299	73.03230	100.0000
Totals :				2241.81299	73.03230	

*** End of Report ***

Instrument 1 3/23/2010 8:28:21 PM

Page 1 of 1

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==========================================================================
Acq. Operator    : WHZ                                  Seq. Line :   7
Acq. Instrument  : [illegible] 2                        Location : P1-D-01
Injection Date   : 2/25/2010 5:59:49 AM                      Inj :   1
                                                        Inj Volume : 5 µl
Acq. Method      : E:\Chem32\2\DATA\20100225\TYJ 2010-02-25 18-33-46\TIANJUT.M
Last changed     : 2/25/2010 5:59:12 AM by WHZ
                   (modified after loading)
Analysis Method  : C:\Chem32\1\METHODS\DEF_LC.M
Last changed     : 3/27/2010 12:34:57 AM
                   (modified after loading)
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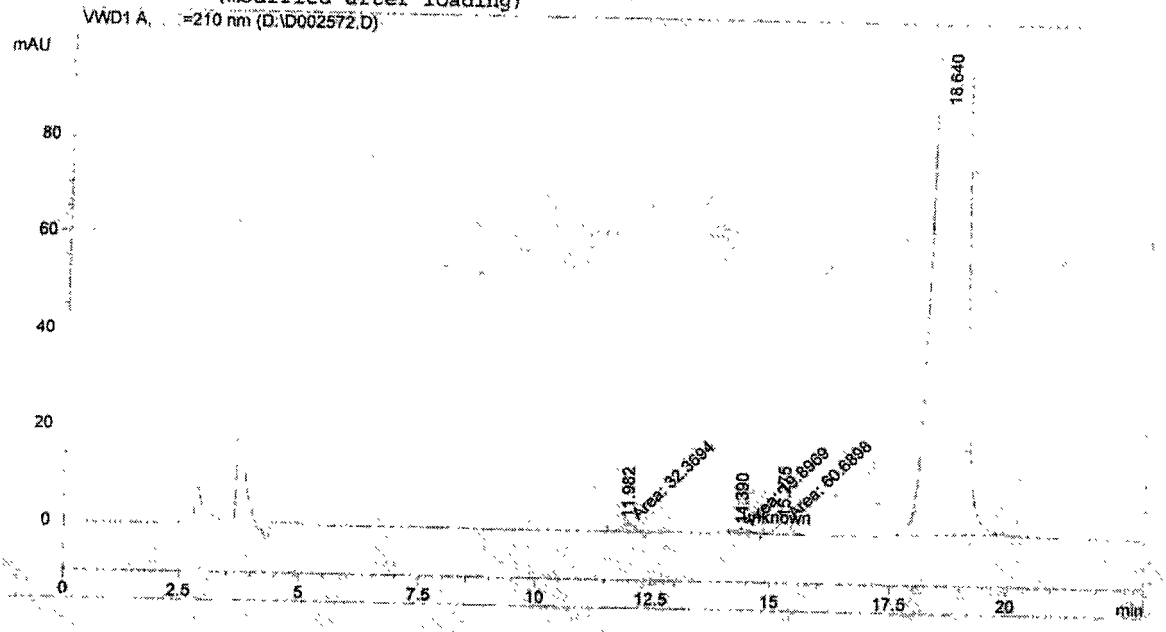


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==========================================================================
                          Area Percent Report
==========================================================================

Sorted By              :      Signal
Multiplier             :      1.0000
Dilution               :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, [illegible]=210 nm

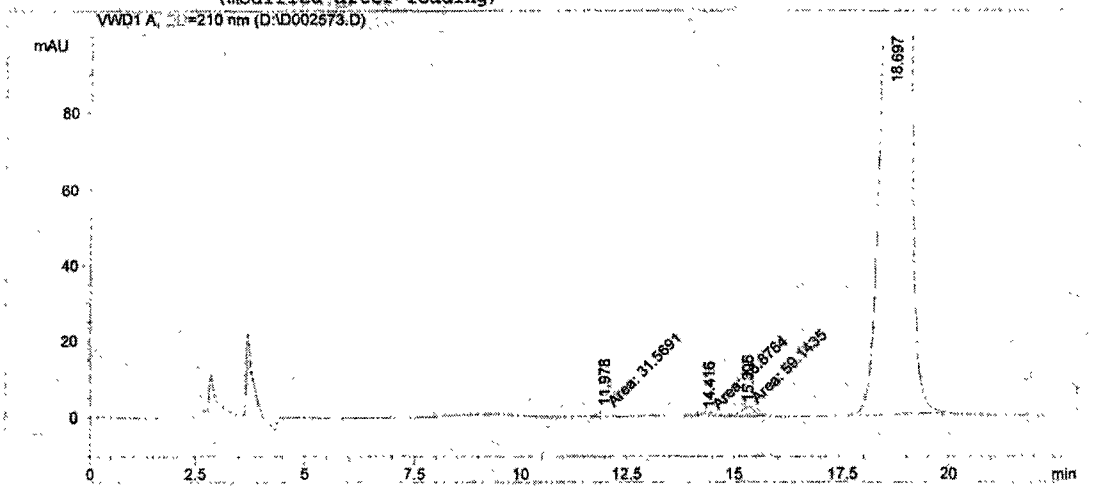
Peak RetTime Type  Width    Area      Height     Area
  #   [min]        [min]   mAU  *s    [mAU  ]     %
 ---|-------|----|-------|----------|----------|-------|
   1  11.982 MM   0.2917   32.36938    1.84922   0.2153
   2  14.390 MM   0.3550   19.89687 9.34093e-1   0.1324
   3  15.275 MM   0.3852   60.68981    2.62582   0.4037
   4  18.640 BB   0.4575 1.49188e4   506.02713  99.2486

Totals :                  1.50318e4   511.43626


[illegible]strument 1 3/27/2010 12:35:02 AM                          Page   1 of 2
```

ata File D:\D002573.D
ample Name: RA 2

```
Acq. Operator    : WHZ                              Seq. Line :   8
Acq. Instrument  : 2                               Location : P1-D-02
Injection Date   : 2/25/2010 6:27:55 AM                  Inj :   1
                                                   Inj Volume : 5 µl
Acq. Method      : E:\Chem32\2\DATA\20100225\TYJ 2010-02-25 18-33-46\TIANJUT.M
Last changed     : 2/25/2010 6:27:18 AM by WHZ
                   (modified after loading)
Analysis Method  : C:\Chem32\1\METHODS\DEF_LC.M
Last changed     : 3/27/2010 12:39:13 AM
                   (modified after loading)
```

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

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, =210 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]  mAU    *s   [mAU   ]      %
----|-------|----|-------|----------|----------|--------|
   1  11.978 MM    0.2927   31.56907    1.79780   0.2102
   2  14.416 MM    0.3463   18.87636 9.08368e-1   0.1257
   3  15.306 MM    0.3801   59.14352    2.59329   0.3938
   4  18.697 BB    0.4593 1.49090e4   503.06244  99.2703

Totals :                   1.50186e4   508.36190


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                        *** End of Report ***
```
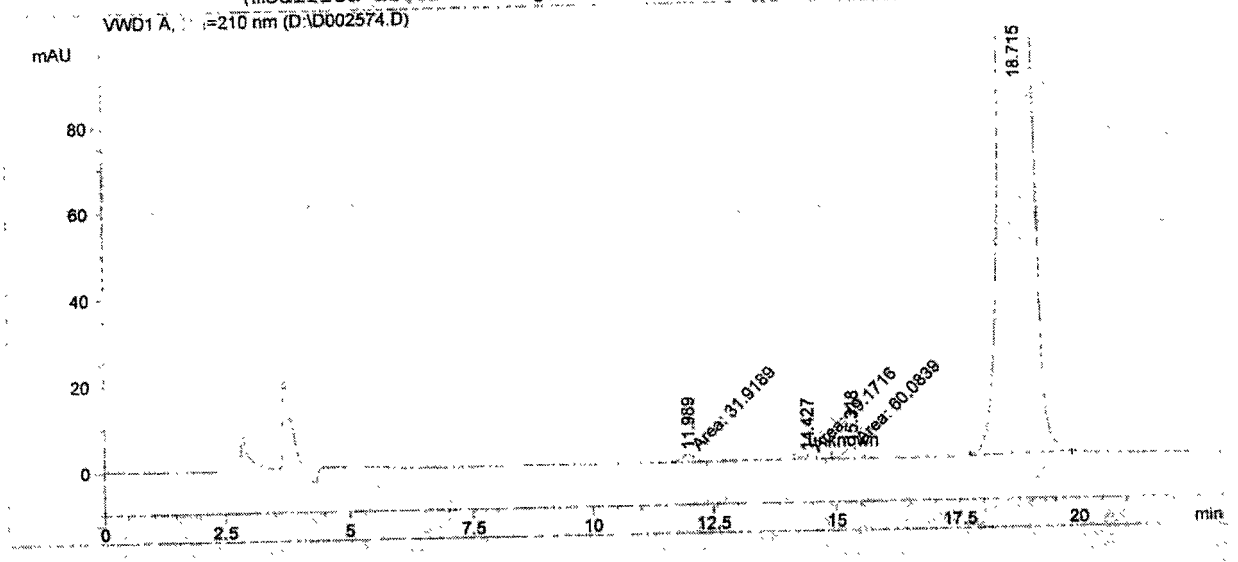

Instrument 1 3/27/2010 12:39:16 AM Page 1 of 1

Data File D:\D002574.D
Sample Name: RA 3

Acq. Operator	: WHZ	Seq. Line :	9
Acq. Instrument	: [illegible] 2	Location :	P1-D-03
Injection Date	: 2/25/2010 7:00:34 AM	Inj :	1
		Inj Volume :	5 µl

Acq. Method : E:\Chem32\2\DATA\20100225\TYJ 2010-02-25 18-33-46\TIANJUT.M
Last changed : 2/25/2010 6:59:56 AM by WHZ
(modified after loading)
Analysis Method : C:\Chem32\1\METHODS\DEF_LC.M
Last changed : 3/27/2010 12:39:13 AM
(modified after loading)



Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, [illegible]=210 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	11.989	MM	0.2967	31.91886	1.79280	0.2155
2	14.427	MM	0.3552	19.17156	8.99648e-1	0.1295
3	15.318	MM	0.3873	60.08385	2.58584	0.4057
4	18.715	BB	0.4615	1.46977e4	494.84302	99.2493
Totals :				1.48089e4	500.12131	

*** End of Report ***

Instrument 1 3/27/2010 12:41:39 AM

Page 1 of 1

Data File D:\D002575.D
Sample Name: RA 4

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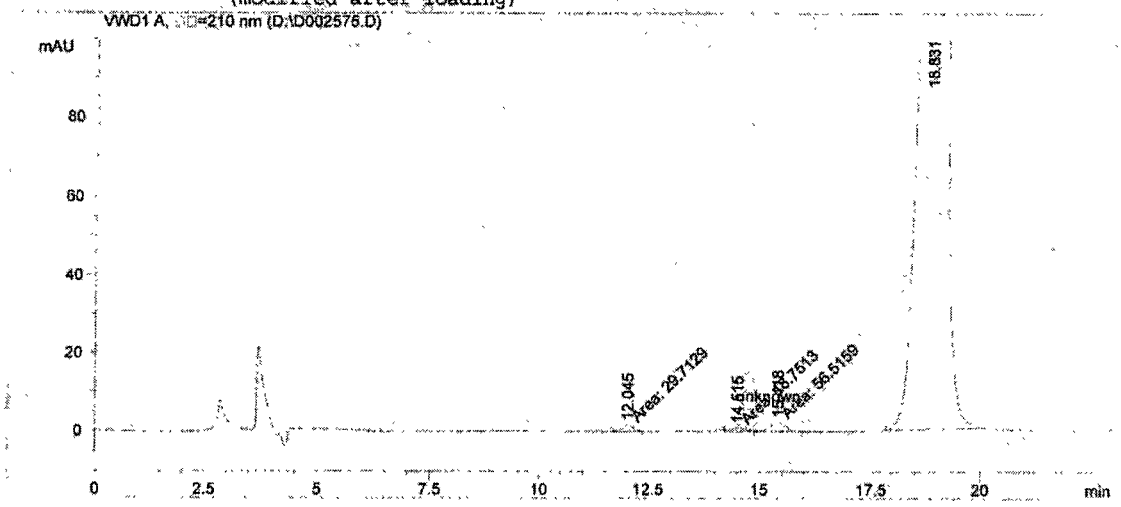
Acq. Operator	: WHZ	Seq. Line	: 10
Acq. Instrument	: [illegible] 2	Location	: P1-D-04
Injection Date	: 2/25/2010 7:33:13 AM	Inj	: 1
		Inj Volume	: 5 µl

Acq. Method : E:\Chem32\2\DATA\20100225\TYJ 2010-02-25 18-33-46\TIANJUT.M
Last changed : 2/25/2010 7:32:35 AM by WHZ
(modified after loading)
Analysis Method : C:\Chem32\1\METHODS\DEF_LC.M
Last changed : 3/27/2010 12:42:40 AM
(modified after loading)



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Area Percent Report

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Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, [illegible]=210 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	12.045	MM	0.2935	29.71290	1.68750	0.2054
2	14.515	MM	0.3641	18.75134	8.58323e-1	0.1296
3	15.418	MM	0.3864	56.51589	2.43777	0.3907
4	18.831	BB	0.4585	1.43599e4	483.64740	99.2742

Totals : 1.44649e4 488.63099

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*** End of Report ***

Instrument 1 3/27/2010 12:43:27 AM Page 1 of 1

SUBMISSION END