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February 3, 2010

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Division of Biotechnology and GRAS Notice Review

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

Attention: Dr. Robert L. Martin

Re: GRAS Notification – High Purity Steviol Glycosides

Dear Dr. Martin:

On behalf of PureCircle USA, Inc. of Oak Brook, IL, we are submitting for FDA review a GRAS notification for High Purity Steviol Glycosides. The attached documentation contains the specific information that addresses the safe human food uses for the subject notified substance as discussed in the GRAS guidance document.

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

We look forward to your feedback.

Sincerely,

(b) (6)

Robert S. McQuate, Ph.D.
CEO & Co-Founder
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Enclosure: GRAS Notification – High Purity Steviol Glycosides (in triplicate)

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GRAS ASSOCIATES, LLC

GRAS ASSESSMENT

OF

HIGH PURITY STEVIOL GLYCOSIDES

Food Usage Conditions for General Recognition of Safety

For

PURECIRCLE USA, INC.

Oak Brook, IL

Evaluation by GRAS Expert Panel

Richard C. Kraska, Ph.D., DABT
Robert S. McQuate, Ph.D.
Wayne R. Bidlack, Ph.D.

February 3, 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]

PureCircle USA, Inc.'s High Purity Steviol Glycosides product, which is sold under the name of SG95, and which meet the specifications for High Purity Steviol Glycosides as described below, has been determined to be Generally Recognized As Safe (GRAS), in accordance with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act*. This determination was made by an appropriately convened panel of experts who are qualified by scientific training and experience; the GRAS evaluation is based on scientific procedures as described in the following sections; and the evaluation accurately reflects the conditions of the stevia-derived sweetener's intended use in foods.

Signed:

(b) (6)

2/1/2010

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

Date

B. Name & Address of Notifier

PureCircle USA, Inc.
915 Harger Road, Suite 250
Oak Brook, IL 60523

As the notifier, PureCircle USA, Inc. ("PureCircle") accepts responsibility for the GRAS determination that has been made for the high purity steviol glycosides as described in the subject notification; consequently, the high purity steviol glycosides preparation meeting the conditions described herein is exempt from premarket approval requirements for food ingredients.

[1] See 62 FR 18938 (17 April 1997) which is accessible at http://www.gpo.gov/fdsys/pkg/FR-1997-04-17/html/97-97-9706.htm.

C. Common Name & Identity of the Notified Substance

High Purity Steviol Glycosides; also see Section III.A.

D. Conditions of Intended Use in Food

The High Purity Steviol Glycosides preparation is intended to be used as a general purpose non-nutritive sweetener as a table top sweetener and for incorporation into various food categories other than 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, High Purity Steviol Glycosides have 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 PureCircle, GRAS Associates, LLC ("GA") has undertaken an independent safety evaluation of PureCircle's SG95 High Purity Steviol Glycosides preparation. The preparation is composed primarily of two steviol glycosides, namely stevioside and rebaudioside (Reb) A, with minor amounts of several other glycosides. The purpose of the evaluation is to ascertain whether the intended food uses of High Purity Steviol Glycosides as a general purpose non-nutritive sweetener are generally recognized as safe, i.e., GRAS, under the intended conditions of use.

B. Foreword

PureCircle provided GA with substantial background information needed to enable the GRAS assessment to be undertaken. In particular, the information 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 PureCircle's SG95. PureCircle 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. PureCircle 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 "doses" or use levels, is critical in extrapolating to safe exposures for High Purity Steviol Glycosides 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.

PureCircle supplied the requested safety/toxicity studies and consumption/exposure information, along with other related documentation. This was augmented with an independent search of the scientific and regulatory literature extending through December 2009. A GRAS assessment based primarily on the composite safety information, that is, based on scientific procedures, with supportive information provided by common use in food, 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 & Stevia-Derived Sweeteners

Stevia derived-sweeteners are permitted as a food additive in South America and in several countries in Asia, including China, Japan, and Korea. As discussed more fully below, over the past several months, the subject sweeteners have received approvals in Australia, New Zealand, and Switzerland, and there are reports that Mexico has authorized food uses as well. The US FDA has issued six "no objection letters" in response to the GRAS notifications filed on behalf of Reb A and steviol glycosides food uses.

In the US, steviol glycosides have been used as a dietary supplement since 1995 (Geuns, 2003). No application for dietary supplement use of purified Reb A is known to have been made. Prior to the submission of the GRAS notifications, at least two GRAS petitions seeking authorization for the addition of stevioside or steviol glycosides to foods had been submitted to FDA since 1989, yet no authorizations had been issued by FDA in response to these filings, presumably because the previously available safety data---including purity considerations---for stevia, stevioside, or steviol glycosides were viewed as being inadequate. These petitions were subsequently withdrawn.

Individual GRAS nofitications were submitted by Merisant and Cargill to FDA in May, 2008 for Reb A, both more highly purified forms of the steviol glycosides. FDA issued "no objection" letters for each of these GRAS notices on December 17, 2008. McNeil Nutritionals, LLC submitted a GRAS notification to FDA in December, 2008 for its purified steviol glycosides with rebaudioside A consisting of the principal component. FDA issued a "no objection" letter to McNeil Nutritionals on June 11, 2009. Blue California submitted its GRAS notification for highly purified Reb A in January, 2009, and FDA issued a "no objection" letter to Blue California on July 20, 2009. GRAS notifications were also submitted to FDA by Sweet Green Fields, LLC and by Wisdom Natural Brands for their stevia-derived sweetener products, and each firm received a "no objection" letter from FDA in August, 2009.[2]

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

Steviol glycosides have been under a lengthy review by the Joint FAO/WHO Expert Committee on Food Additives ("JECFA"). The original review was published in 2000 (WHO, 2000). A draft monograph was reviewed at the 51st, 63rd and 68th JECFA meetings. A temporary ADI (acceptable daily intake) of 0-2 mg/kg bw (on a steviol basis) was established at the 63rd meeting (WHO, 2006). In addition, food grade specifications were made final by JECFA (FAO, 2007a), although they were subsequently updated in 2008 (FAO, 2008). 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). JECFA has published its 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

[2] GRAS notification 252 which was submitted by Merisant, GRAS notification 253 which was submitted by Cargill, GRAS notification 275 which was submitted by McNeil Nutritionals, GRAS notification 278 which ws submitted by Blue California, GRAS notification 282 which was submitted by Sweet Green Fields, and GRAS notification 287 which was submitted by Wisdom Natural Brands are listed on FDA's website at http://www.accessdata.fda.gov/scripts/fc/fcnNavigation.cfm?rpt=grasListing, along with their respective "no objection" letters.

considered as part of the minimum 95% steviol glycoside composition (CCFA, 2009). The matter is under active consideration and is on the agenda for discussion at the JECFA Meeting in June, 2010 (FAO/WHO, 2009).

In August 2008, Switzerland's Federal Office for Public Health cited the favorable actions of JECFA in issuing its approval for the use of stevia as a sweetener (Switzerland Office of Public Health, 2008).

In September 2009, France published its approval for the food uses of Reb A with a purity of 97% (AFSSA, 2009). In January 2010, French authorities updated its approval for food and beverage uses of the high purity Reb A. Levels of Reb A permitted in selected categories of reduced calorie and no sugar added foods were increased by around 40% over the levels originally authorized (FoodNavigator, 2010).

The stevia-derived sweeteners are not presently permitted as an ingredient in conventional food in the EU, UK, Hong Kong, or Canada (Hawke, 2003). This likely reflects a lack of review of new data on the sweeteners rather than a continuing concern about safety.

Hong Kong maintains that stevia is not permitted as a sweetener, as cited on the government website (Hong Kong Government, 2002). 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.

Other international bodies have investigated the safety aspects of stevia and steviol glycosides use in foods. In 1999 in the EU, the Scientific Committee on Food (SCF) for the European Commission concluded that "there are no satisfactory data to support the safe use of these stevia plants and leaves," as reported in a five-page opinion dated June 17, 1999 (European Commission, 1999a). The Committee also reiterated "its earlier opinion that stevioside is not acceptable as a sweetener on the presently available data," in a seven-page opinion also dated June 17, 1999 (European Commission, 1999b).

The European Food Safety Authority (EFSA) is reexamining the safety of steviol glycosides in light of JECFA's 2008 findings and in response to a June 2008 request by the European Commission for EFSA to evaluate steviol glycosides (EFSA, 2008). EFSA's Food Additives & Nutrient Sources (ANS) Panel has been given a deadline of March 31, 2010 to answer this request. Furthermore, three petitions have been submitted to EFSA that seek authorization for steviol glycoside food additive use, and it is anticipated that positive opinions will be issued by EFSA in 2010 or 2011 (Nutraingredients, 2009).

On September 24, 1998 in the UK, the Advisory Committee on Novel Foods and Processes for the Ministry of Agriculture, Fisheries and Food 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."[3]

[3] See http://www.maff.gov.uk/food/novel/980924.html.

D. FDA Regulatory Framework

Stevia, also known as steviol glycosides or steviosides, has been used in dietary supplements in the US since 1995 (Geuns, 2003) and is widely available to consumers in the US through retail outlets and Internet purchases (Al-Achi and Greenwood, 2000).

In accordance with FDA regulation of foods, however, dietary supplements cannot legally be added to conventional foods. Such ingredients must undergo premarket approval by FDA as food additives or, alternatively, the 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.[4]

In 1997, FDA altered the GRAS determination process by eliminating the formal GRAS petitioning process and replacing the petitioning process with a notification procedure.[5] While outlining the necessary content to be considered in making a GRAS determination, FDA encouraged that such determinations should be provided to FDA in the form of a notification. However, notifying FDA of such determinations is strictly voluntary.

[4] See 21 CFR 170.3(i)(3).
[5] See Footnote 1.

III. CHEMISTRY & MANUFACTURE OF HIGH PURITY STEVIOL GLYCOSIDES & RELATED PURECIRCLE PRODUCT

A. Common or Usual Name

High Purity Steviol Glycosides is the common or usual name of the non-nutritive sweetener derived from *Stevia rebaudiana* Bertoni that is the subject of the GRAS evaluation, and the specific substance that is the subject of this safety evaluation is identified as SG95 as produced and sold by PureCircle. The compositional features of the subject PureCircle high purity steviol glycosides are described in more detail elsewhere in this Section.

JECFA adopted the term, steviol glycosides, for the family of chemical substances with sweetness properties that are derived from stevia. The term, stevia, is used more broadly to describe the plant or crude extracts of the plant, while stevioside is the common name for one of the specific glycosides that is commonly extracted from stevia.

B. Chemistry of Steviol Glycosides

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 & De Oliveira, 1993).

The predominant sweetener components of stevia extracts have been identified as stevioside and Reb A. The chemical identities and key chemical identifiers for the two major components are shown below.

Common Name:	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

Common Name:	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

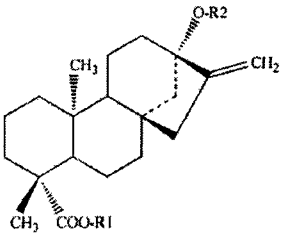
SG95 differs somewhat compared to other steviol glycosides preparations on the market in that it contains rebaudioside F and may contain rebaudioside D at levels that are slightly elevated when compared with other steviol glycosides compositions. Pertinent descriptive information on these materials appears below.

Common Name:	Rebaudioside F
Chemical Name:	13-[(2-O-β-D-xylofurananosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl) oxy] kaur-16-en-18-oic acid, β-D-glucopyranosyl ester
Chemical Formula:	$C_{43}H_{68}O_{22}$
Formula Weight:	936.42
CAS Number:	438045-89-7

Common Name:	Rebaudioside D
Chemical Name:	13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl) oxy] kaur-16-en-18-oic acid, 2-O-β-D-glucopyranosyl-β-D-glucopyranosyl ester
Chemical Formula:	$C_{50}H_{80}O_{28}$
Formula Weight:	1128.48
CAS Number:	63279-13-0

In the most recent Chemical and Technical Assessment (FAO, 2007b), JECFA identified the sweetener components. They updated the list of common glycosides and their chemical stuctures, which are slightly different from compounds depicted in older publications (Nanayakkara et al., 1987; Suttajit et al., 1993). They are shown in Figure 1.

Figure 1. Chemical Structures of Various Steviol Glycosides[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.

The structures of the components of steviol glycosides were also described in reviews by Kinghorn (2002), Kennelly (2002), and Geuns (2003). Non-sweet elements include the labdane diterpenes, triterpenes, sterols and flavonoid glycosides. Gas chromatography, infrared spectroscopy and mass spectroscopy have also revealed 31 constituents of volatile oil (Kinghorn, 2002). The structures, stereochemistry, and absolute configurations of steviol and isosteviol were established through a series of chemical reactions and analyses over a 20-year period after the seminal work of Bridel and Lavieille (1931) in France. Support for the proposed structures was achieved by the synthetic transformation of steviol into stevioside by Ogawa et al. in 1980 (cited in Brandle et al., 1998). Two of the other sweet diterpenoid glycosides, Reb A and B, were obtained from methanol extracts of stevia leaves, along with the major sweet principle constituent, stevioside, and steviolbioside, a minor constituent, which was first prepared from stevioside by alkaline hydrolysis by Wood et al. in 1955 (cited in Brandle et al., 1998). It was suggested subsequently that Reb B was an artifact formed from Reb A during isolation (Brandle et al., 1998; Kennelly, 2002). Furthermore, stevioside can be converted both chemically and

enzymatically to Reb A. Further fractionation led to the isolation and identification of three other sweet glycosides respectively named Reb C, D, and E. It was reported that Reb A and D could be converted to Reb B by alkaline hydrolysis showing that only the ester functionality differed (Brandle et al., 1998). Dulcosides A and B were also described by Kobayashi et al. (1977). Dulcoside B was subsequently shown to have the identical structure as Reb C.

C. Accepted Identity Specifications for Food Grade Steviol Glycosides

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

In 2007, JECFA recommended that food grade specifications for steviol glycosides consist of a minimum of 95% on a dried weight basis of seven specific steviol glycosides (FAO, 2007a, see Appendix A-1). The JECFA recommendation included appropriate analytical methods. Stevioside and Reb A are the component glycosides of interest for their sweetening property. The five other associated glycosides found in preparations of steviol glycosides accepted by the JECFA specification for the 95% requirement are Reb C, dulcoside A, rubusoside, steviolbioside, and Reb B. These, however, are typically found at substantially lower levels than the stevioside or Reb A. JECFA updated the specifications for steviol glycosides in 2008, and they are found in Appendix A-2 (FAO, 2008).

In early 2009, Australia submitted a request to the Codex Committee on Food Additives in which it was proposed that the JECFA specifications for Steviol Glycosides would be modified to allow inclusion of Rebaudioside D and Rebaudioside F as permitted glycosides contributing to the minimum 95% steviol glycoside composition. This proposed modification has been endorsed by the Codex Alimentarius Committee in July 2009, and the matter is under active consideration by JECFA who will address this topic at their June 2010 meeting. It should be noted that PureCircle has made application to JECFA for the comparable expanded specifications on allowable steviol glycosides (Appendix A-3).

D. Manufacturing Processes

Manufacturing processes for stevia-derived sweeteners have been described in the scientific and patent literature as noted below, and PureCircle's High Purity Steviol Glycosides manufacturing process is also discussed.

1. Scientific & Patent Literature

Typically, steviol glycosides are obtained by extracting leaves of *Stevia rebaudiana* Bertoni with hot water or alcohols (ethanol or methanol); the obtained extract is a dark particulate solution containing all the active principles plus 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, 2002). There are dozens of patents for

the extraction of steviol glycosides. Kinghorn (2002) has 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. Methods using ultrafiltration, metallic ions, supercritical fluid extraction with CO_2, and extract clarification with zeolite are found within the body of newer patents.

At the 68th JECFA meeting in 2007, steviol glycosides were defined as the products obtained from the leaves of *Stevia rebaudiana* Bertoni. As cited by JECFA, the typical manufacture starts with extracting leaves with hot water, and the aqueous extract is then 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. Processing to Provide PureCircle's High Purity Steviol Glycosides

The process used by PureCircle is fairly typical and similar to that yielding other related stevia-derived products on the market. The process uses food grade ethanol as an extraction solvent. Ethanol specifications are attached as Appendix B-1. Specifications for resins and filters are also found in Appendix B-2. The manufacturing process complies with the international GMP standard ISO 21000. A HACCP certification for the manucturing plant is included as Appendix B-3.

The raw materials used in the manufacturing process of steviol glycosides preparations are the crushed leaves of the perennial shrub *Stevia rebaudiana* (Bertoni) Bertoni of the family *Asteraceae (Compositae)*. Ethyl alcohol and ion exchange resins are used in the manufacturing. Stevia leaves are extracted with hot water at 50-60°C for 1-2 hrs with permanent agitation. The separation of filtrate is carried out by gravity flow via dross trap and collected in the holding tank. In order to remove the mechanical particles, proteins and some coloring agents the filtrate is treated with calcium hydroxide. The resulting suspension is maintained at ambient temperature for about 30 min. The separation of precipitate is realized on the plate-and-frame filter press using cotton cloth as the filtration material. Desalting of solution is carried out using ion-exchange resins of (H^+) and (OH^-) forms by usual technique. The further purification of the filtrate is developed by adsorption of sweet glycosides on specific resin packed into the columns. The resin is washed with deionized water. Desorption of sweet glycosides from columns is realized by aqueous solution of ethyl alcohol. Decoloration is carried out at room temperature by treating with activated carbon. The separation of used carbon is realized on the plate-and-frame filter press using cotton cloth as the filtration material. The removal of ethanol from glycosides purified solution is developed on usual type of evaporator without vacuum. Desalting of solution is carried out using ion-exchange resins of (H^+) and (OH^-) forms by usual technique. The refined solution is transferred to nanofiltration unit and concentrated. The drying of the concentrate is realized in the spray dryer according to the operating instructions. The final product is packaged in polyethylene bags and additionally into the carton boxes.

The manufacturing process is presented as a flow sheet in Figure 2.

Figure 2. Flow Diagram of PureCircle Manufacturing Process

Stevia leaves
↓ - Hot water
- Extraction
- Filtration

Crude Extract-1
↓ - $Ca(OH)_2$
- Flocculation
- Filtration

Crude Extract-2
↓ - Deionization
- Adsorption
- Desorption

Sollution of steviol glycosides-1
↓ - Active carbon
- Filtration

Sollution of steviol glycosides-2
↓ - Distillation

Sollution of steviol glycosides-3
↓ - Deionization
- Adsorption
- Desorption

Refined sollution of steviol glycosides
↓ - Nanofiltration

Steviol glycosides concentrated
↓ - Spray drying

Steviol glycosides powder

E. Product Specifications & Supporting Methods

1. JECFA Specifications

JECFA finalized food grade specifications at the 68th JECFA meeting, which were then published in FAO JECFA Monograph 4 (FAO, 2007a) and were subsequently revised (FAO, 2008). 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 7 specific steviol glycosides as specified in Appendix A-1 and A-2. The steviol glycosides are freely soluble in water and ethanol, and the 1 in 100 solutions exhibit pH values in the range of 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. Residual methanol levels should not exceed 200 mg/kg, and ethanol levels should not exceed 5000 mg/kg. Arsenic levels should not exceed 1 mg/kg (determined by the atomic absorption hydride technique). Lead levels should not exceed 1 mg/kg. The complete JECFA specifications including recommended analytical methods are contained in Appendix A-2.

2. Specifications for PureCircle SG95 Product

PureCircle has adopted product specifications for SG95 to meet or exceed most current JECFA recommendations. The only exception is that it is proposed that a level of < 3% rebaudioside D and <2.5% rebaudioside F are to be included among the glycosides for inclusion within the 95% total composition for steviol glycosides purity. The specifications provided by PureCircle for the final dried product are given in Table 1 and Appendix C-1 Table 1 also includes data from five production batches to show that specifcations are met. Certificates of analysis for 5 production batches are included as Appendix C-2 to demonstrate that the product routinely meets specifications. A full report of the analytical study on these batches for steviol glycoside content is included as Appendix C-3. Finally certificates of analyses are provided in Appendix C-4 to demonstrate the absence of pesticide residues and other environmental contaminants.

F. Stability Data for Stevioside & Rebaudioside A

1. Scientific Literature

Stevioside is a stable molecule over the pH range 3 - 9 and can be heated at 100°C for 1 hour, but rapidly decomposes at pH levels greater than 9 under otherwise comparable conditions (Kinghorn, 2002). It is speculated that steviobioside produced from stevioside by alkaline hydrolysis would be the major decomposition product obtained at pH 10 (Kinghorn, 2002).

Chang and Cook (1983) tested the stability of pure stevioside and Reb A in carbonated phosphoric and citric acidified beverages and reported some degradation of both sweetening components after 2 months of storage at 37°C; however, there was no significant change at room temperature or below following 5 months storage of stevioside and 3 months storage of Reb A. They also reported that exposure to 1 week of sunlight did not affect stevioside, but it did result in

approximately 20% loss of Reb A. Heating at 60°C for 6 days resulted in 0 - 6% loss of sweeteners.

As summarized below, extensive stability testing results were compiled for inclusion in both the Merisant and Cargill GRAS notifications.

Detailed stability testing was conducted by Merisant on Reb A as a powder, as a pure sweetener in solution, and on both cola-type and citrus carbonated beverages. No degradation was detected when the powder was stored at 105°C for 96 hours, and it was concluded that the powder was stable when stored for 26 weeks at 40±2°C with relative humidity of 75±5%. When considering Merisant's results of the stability investigatiothere was considerable degradation noted after 13 hours at 100°C for carbonated beverage solutions and pure sweetener solutions (Merisant, 2008).

Cargill (2008) conducted detailed stability testing on Reb A as a powder under various storage conditions and under a range of pH and temperatures. In addition, Cargill assessed Reb 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, lemon-lime, and root beer, yogurt, thermally processed beverages, and white cake. The stability testing revealed some degradation products that had not been detected in bulk Reb A. However, it was noted that these degradation products were structurally related to the steviol glycosides that are extracted from the leaves of *Stevia rebaudiana Bertoni.* The degradation products all share the same steviol aglycone backbone structure as found in stevioside and Reb A, but they differ by virtue of the glucose moities present.

Cargill (2008; published as Clos et al., 2008) also conducted photostability studies on the dry powder and mock beverages to ascertain Reb A behavior under defined conditions of fluorescent and near UV light exposure. Reb A was determined to be photostable under the defined conditions of analysis. The authors stated that the observation of better stability than in the work by Chang and Cook (1983) is due to differences in analytical methods.

From the stability testing reported, it was concluded that Reb 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, Reb A is more stable in the pH range 4 to 6 and at temperatures from 5°C to 25°C (Cargill, 2008).

2. Stability of PureCircle High Purity Steviol Glycosides SG95 Product

PureCircle is conducting stability studies on SG95 under extreme storage conditions and at different pH values from 2.0 to 8.0 to ascertain product shelf life. No significant degradation of any glycoside was observed after three weeks. Preliminary reports of results at the three week time point are included in Appendices D-1 and D-2.

Table 1. Specifications for PureCircle High Purity Steviol Glycosides SG95 Product & Results of Analyses of Five Production Batches

PARAMETER	PURECIRCLE SPECIFICATIONS SG95	LOT NO. A0912266	LOT NO. A0912267	LOT NO. A0912268	LOT NO. A0912269	LOT NO. A0912270
Appearance	Off-white to white powder	Approved	Approved	Approved	Approved	Approved
Odor	Slight, characteristic	Approved	Approved	Approved	Approved	Approved
Taste	Characteristic, sweet, slightly bitter	Approved	Approved	Approved	Approved	Approved
Sweetness (fold sweetness to sucrose)	225-275	250	250	250	250	250
Chemical Specifications						
Total Steviol Glycosides, % (anhydrous basis)	≥95.0	96.50	96.11	96.86	96.41	95.86
Stevioside, %	15.0 - 30.0	18 31	19 37	20 15	19 71	18.71
Rebaudioside A, %	50.0 - 65.0	64.56	61.25	61.58	61 45	62 73
Rebaudioside B, %	≤2.0	1.01	0.99	1.04	1.05	1 01
Rebaudioside C, %	≤15.0	9.85	11.13	10 39	10.66	10 35
Rebaudioside D, %	≤2 5	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
Rebaudioside F, %	≤3 0	1 93	2.21	2.11	2.17	2.07
Rubusoside, %	≤2.0	0.21	0.35	0.76	0.57	0.27
Steviolbioside, %	≤2.0	0.23	0.35	0 30	0 30	0 28
Dulcoside A, %	≤2.0	0.39	0 45	0.50	0.47	0.41
Loss on Drying, %	<6.0	1.42	3.56	1.93	2.92	3.25
Solubility, %	Freely Soluble	Approved	Approved	Approved	Approved	Approved
pH, 1% in water	4.5 - 7.0	6.2	6.2	6.2	6.1	6.0
Residual Ethanol, %	<0 5	0 050	0.180	0 300	0.270	0.130
Residual Methanol, %	<0.02	0.005	0 005	0.005	0.004	0.004
Ash, %	<1.0	<0 1	0 2	0.2	<0.1	<0.1
Lead (as Pb), ppm	<1.0	0.041	0.026	0.026	0.032	0.029
Arsenic (as As), ppm	<1.0	0 013	0 005	<0 005	0 005	<0.005
Cadmium (as Cd), ppm	<1.0	<0.005	<0 005	<0.005	<0 005	<0.005
Optical Rotation, $[\alpha]^{25}_{589}$	-30.0° to -38 0°	-32.80°	-34.20°	-34.20°	-34.50°	-33.70°
Microbiological Specifications						
Total Plate Count, CFU/g	<1000	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
Yeast and Mold, CFU/g	<200	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
Total Coliforms, MPN/g	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
E.coli count, MPN/g	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
Salmonella sp.	Negative/25g	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
Pesticides	None detected	None detected	None detected	None detected	None detected	None detected

Abbreviations: Stv = Stevioside; Reb A = Rebaudioside A; Reb B = Rebaudioside B; Reb C = Rebaudioside C; Reb D = Rebaudioside D; Reb F = Rebaudioside F; Dulc A = Dulcoside A; Sbio = Steviolbioside; and Rub = Rubioside.

IV. INTENDED DIETARY USES

A. Intended Uses

PureCircle intends to market its High Purity Steviol Glycosides as a table top sweetener and for incorporation into various food categories as a general-purpose sweetener, including specific food uses as reported in other countries and as depicted in Table 2.[6] High Purity Steviol Glycosides will function as a non-nutritive sweetener as defined in 21 CFR 170.3(o)(19). The use levels will vary by food category, but the levels are self-limiting due to organoleptic factors and consumer taste considerations. The amounts of High Purity Steviol Glycosides 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.[7]

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

Many estimates of possible consumption of stevia-based sweeteners have been published. Both JECFA and FSANZ reviewed estimates of possible consumption of mixed steviol glycosides as part of their safety deliberations. In addition, Merisant and Cargill estimated the consumption of Reb A in their submissions to FDA.

Estimated maximum use levels in various foods as evaluated by JECFA are summarized in Table 2.

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

FOOD USES	MAXIMUM USE LEVEL REPORTED[a] (mg STEVIOL GLYCOSIDES / kg OF FOOD)	MAXIMUM USE LEVEL CALCULATED[b] (mg STEVIOL EQUIVALENTS / kg OF FOOD)
Desserts	500	200
Cold confectionery	500	200
Pickles	1000	400
Sweetcorn	200	80
Biscuits	300	120
Beverages	500	200
Yogurt	500	200
Sauces	1000	400
Delicacies	1000	400
Bread	160	64

[a] From WHO, 2006. [b] Calculated by Expert Panel by multiplying by ratio of molecular weight of steviol to molecular weight of stevioside.

6 PureCircle does not intend to incorporate its SG95 into infant formulas or meat and poultry products.
7 See 21 CFR 182.1(b)(1).

Merisant listed expected levels of use for Reb A for various food applications in their GRAS Notification. Their consumer estimates were largely based on food consumption survey data from 2003-2004 NHANES, a resource that reflects food intake over a two-day time period. Statistically weighted values were utilized to provide reliable quantitative findings that are representative of food consumption of actual "users" within the US population. The 2-day food surveys are known to overestimate actual consumption levels when compared to longer term food surveys, such as those based on 14-day surveys. On a per user basis, the mean daily consumption of Reb A was calculated to be 2.0 mg/kg bw/day, and that for the 90th percentile consumer was found to be 4.7 mg/kg bw/day. Specific food uses and use levels are given in Table 3.

Table 3. Proposed Uses & Levels of Rebaudioside A by Merisant (2008)

FOOD USES	REB A (PPM)
Tabletop sweeteners	30,000[a]
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] Reb A content of sachet prior to dilution and not representative of "as consumed."

Cargill utilized a different approach in estimating dietary intake figures for Reb A when incorporated as a general sweetener in a broad cross-section of processed foods (Cargill, 2008). Cargill reasoned that Reb A uses and use levels would be rather comparable to aspartame uses in the US with a few minor exceptions. They performed a side-by-side consumption analysis for Reb A versus aspartame, using post-market surveillance consumption data and published data for consumption of aspartame and other high intensity sweeteners (Renwick, 2008). Their findings are considered further in Section IV.C.

C. Estimated Daily Intake

PureCircle intends to incorporate its SG95 products into a broad selection of foods as a general-purpose sweetener. The very conservative consumer intake estimates provided by JECFA (as shown above in Table 2) were utilized to gauge the potential human exposures of the subject steviol glycosides in foods as reported in the US and in other countries.

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 was 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.

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. Table 4 summarizes the exposures to steviol glycosides (as steviol) as evaluated or derived by the Committee.

Table 4. 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.

JECFA concluded that the replacement estimates were highly conservative---that is, 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.**

FSANZ (2008) similarly 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 for the mean and 90th percentile consumer, respectively. FSANZ examined consumption in other age groups and concluded there was no safety concern in children of any age.

Merisant also calculated a dietary estimate for Reb 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. On a steviol equivalent basis, the Merisant estimates would be 0.7 and 1.6 mg/kg bw/day, respectively.

In another recent consumption estimate review conducted on behalf of Cargill and included in their GRAS notification, the intake of Reb A when used as a complete sugar replacement was estimated at 1.3 - 3.4 mg/kg bw/day when calculated as Reb A or 0.4 - 1.1 mg/kg bw/day on a steviol basis (Renwick, 2008).

In concert with the JECFA intake estimates, the anticipated human exposures as projected independently and with different approaches by both Merisant and Cargill in compiling their GRAS dossiers for Reb A (Merisant, 2008 and Cargill, 2008) tended to converge to yield estimated daily intakes (EDIs) in the range 0.4 - 1.6 mg/kg bw/day on a steviol basis or 1.3 - 4.7 mg/kg bw/day on a Reb A basis

The actual daily intakes of stevioside and Reb A from products such as PureCircle's SG95 offerings and from Merisant, Cargill, McNeil Nutritional, Blue California, Sweet Green Fields, and Wisdom Natural Brands and possibly other manufacturers' and suppliers' products cannot yet be determined with accuracy. The extent that stevia-based sweeteners will penetrate the US food supply and the extent the market will select mixed steviol glycoside products versus Reb A products is uncertain. Furthermore, many competing non-caloric sweeteners are currently available to consumers which have been successful in the marketplace, most notably aspartame and sucralose.

In light of the above considerations, the intake estimates presented here are viewed as being overestimates of actual consumption. When comparing these EDI assessments for steviol glycosides, we see that total daily consumption of the steviol glycosides and Reb A for defined food uses and as a general purpose sweetener is expected to be substantially less than the acceptable daily intake values discussed more fully in Section VI.B.1.

D. Other Information on Human Exposure to Stevia

The predominant use of stevia as a food ingredient presently occurs in Brazil and Japan.[8] It is reported that 40% of the artificial sweetener market in Japan is stevia based and that stevia is commonly used in processed foods in Japan (Lester, 1999).

Stevia usage 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 are available on the daily consumption levels of steviol glycosides consumed in the US via dietary supplements in the form of capsules, softgels, tablets, etc.

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

In South America, stevia is commonly used as a treatment for type 2 diabetes (Hawke, 2003). However, elevated doses in the range of 1 gram per person per day or more were reported to be necessary to achieve this therapeutic effect (Gregersen et al., 2004).

V. SAFETY DATA FOR STEVIOL GLYCOSIDES

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

Because PureCircle's High Purity Steviol Glycosides is a mixed glycoside product composed of two major ingredients, stevioside and reb A, the data on each component are relevant to this review. The biological, toxicological and clinical data on stevia and steviol glycosides have been assessed by a number of reviewers (Carakostas et al., 2008; Geuns, 2003; Huxtable, 2002) and most notably through the on-going evaluation by JECFA (WHO, 2000, 2006, 2007, 2008) and a review by Food Standards Australia New Zealand (FSANZ, 2008) for use in food. Most of these reviews primarily focused on mixtures of steviol glycosides. The early reviews tracked the development of better toxicology studies on purer samples of steviol glycosides. These reviews followed with keen interest whether effects of concern seen in various toxicology studies, such as the decrease in fertility with crude stevia preparations and the mutagenic activity of the principle metabolite steviol, would be manifest in comprehensive studies using modern test protocols with pure test materials. In addition, JECFA encouraged the further elucidation of clinical effects on blood pressure and glucose metabolism in hypertensive and diabetic individuals, respectively, in comparison to normal human subjects. By 2006, sufficient favorable data were generated for JECFA to yield a temporary ADI which was finalized at an elevated level in 2008. More details on the JECFA reviews are discussed in Section V.A.1. The key toxicology and clinical data on steviol glycosides (primarily stevioside), more recently developed data on Reb A, and data on the principle metabolite, steviol, as reviewed by JECFA and other reviewers are summarized in Sections V.B, V.C and V.D, respectively.

1. Summary of Interim & Final JECFA Reviews

In 1999, the 51st meeting of 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*.

Additional data were requested and subsequently provided on the metabolism of steviol glycosides. These data clarified 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 Committee came to the conclusion that steviol glycosides are not mutagenic and that steviol is mutagenic only in *in vitro* studies but not *in vivo*. The Committee also became convinced that 1) purified steviol glycosides did not impair reproductive performance as did crude preparations of stevia and 2) there were sufficient chronic studies in rats with adequate no observed adverse effect levels (NOAELs) to support a reasonable temporary 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 is 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.

At the 68th meeting in 2007, JECFA apparently felt that sufficient progress had been made on the clinical studies and extended the temporary ADI until 2008 (WHO, 2007). Furthermore, sufficient data had been received 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.

At the 69th meeting in 2008, JECFA came to a final evaluation (WHO, 2008) based on their satisfaction with the completed clinical studies, and the Committee actually raised the ADI to 0 – 4 mg/kg bw/day and removed the "temporary" designation. In the final toxicology monograph addendum (WHO, 2009), the summary of the Committee's key conclusions 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/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/day (383 mg/kg bw/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/day (5–10 mg/kg bw/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/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

Food Standards Australia New Zealand (FSANZ) completed a review of the safety of steviol glycosides for use as a sweetener in foods in 2008. The risk assessments undertaken by 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. (2008).

In their early reviews, FSANZ expressed the opinion that new data in humans provide 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. FSANZ anticipated that JECFA would raise the ADI at the summer meeting in 2007. At that 2007 meeting, JECFA elected to extend its current temporary ADI for another year, apparently awaiting the completion of the clinical studies. Therefore, FSANZ established an ADI of 4 mg/kg bw/day, 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). It is important to note that JECFA and FSANZ arrived at the same ADI.

B. Safety Data on Stevioside & Stevia Extracts that are Predominantly Stevioside

This Section summarizes studies on stevioside or stevia extracts that were identified compositionally as predominantly stevioside. Related studies on Reb A are discussed in Section V.C, Safety Data on Rebaudioside A.

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

Many studies in rats (Wingard et al., 1980; Nakayama et al., 1986; Koyama et al., 2003a) and other animal models, including chickens (Geuns et al., 2003a), hamsters (Hutapea et al., 1999), and pigs (Geuns et al., 2003b) indicate that stevioside is not readily absorbed from the GI tract.

There is evidence from *in vitro* metabolism studies that bacteria in the colon of rats and humans can transform various stevia glycosides into steviol (Gardana et al., 2003). Steviol was shown to be more readily transported with *in vitro* intestinal preparations than various steviosides (Geuns, 2003, Koyama et al., 2003b). Slow absorption of steviol was indicated by detection in the plasma of rats given oral stevioside (Wang et al., 2004). However, Sung (2002) did not detect plasma steviol after oral doses of steviosides when administered to rats. In studies with human and rat liver extracts, it was demonstrated that steviol can be converted to various glucuronides (Koyama et al., 2003b).

Excretion of metabolites of stevioside after oral doses has been shown in urine and feces in rats (Sung, 2002) and hamsters (Hutapea et al., 1999). Oral doses in pigs led to the detection of metabolites in feces but not in urine (Geuns et al., 2003b).

In a study using 10 healthy human subjects, blood, urine and fecal metabolites were measured after subjects received 3 doses of 250 mg of purified stevioside (>97%) 3 times a day for 3 days. Urine was collected for 24 hours on day 3 and blood and fecal samples were also taken on day 3. Free steviol was detected in feces but not in blood or urine. Steviol glucuronide was detected in blood, urine and feces. 76% of the total steviol equivalents dosed were recovered in urine and feces. Based on these measurements, the authors concluded that there was complete conversion of stevioside in the colon to steviol, which was absorbed and rapidly converted to the glucuronide (Geuns et al., 2006).

These studies were recently reviewed by Renwick and Tarka (2008). The reviewers concluded that stevioside and Reb A are not absorebed directly and both are converted to steviol by gut microbiota in rats and in humans. This hydrolysis occurs more slowly for Reb A than for stevioside. Studies have shown that steviol-16, 17 epoxide is not a microbial metabolite. The authors concluded that there is a single hydrolysis product and that toxicological studies on stevioside are relevant to the safety assessment for Reb A.

2. Acute Toxicity Studies

Studies of the toxicity of stevioside (purity, 96%) given as single oral doses to rodents are summarized in Table 5. No lethality was seen within 14 days after administration, and no clinical

signs of toxicity or morphological or histopathological changes were found, indicating that stevioside is very non-toxic.

Table 5. Acute Toxicity of Stevioside (Purity 96%) Given Orally to Rodents

SPECIES	SEX	LD_{50} (g/kg bw)	REFERENCE
Mouse	Male and Female	>15	Toskulkao et al., (1997)
Mouse	Male	> 2	Medon et al., (1982)
Rat	Male and Female	>15	Toskulkao et al., (1997)
Hamster	Male and Female	>15	Toskulkao et al., (1997)

3. Subchronic Toxicity Studies

Three published subchronic studies with oral administration of stevioside have been conducted in rats. In addition, a reproduction study in hamsters included subchronic phases on the F_0, F_1 and F_2 generations. These are summarized in Table 6.

One of these studies was particularly important because it served as a range-finding study for two subsequent chronic studies. A 13-week toxicity study was carried out in Fischer 344 rats given doses of 0, 0.31, 0.62, 1.25, 2.5, or 5% in the diet (equivalent to 160, 310, 630, 1300, and 2500 mg/kg bw/day) to determine the appropriate doses for a two-year study for carcinogenicity. The rats were randomly allocated to six groups, each consisting of 10 males and 10 females. None of the animals died during the administration period, and there was no difference in body-weight gain between the control and treated groups during administration or in food consumption in the latter part of the study. The activity of lactic dehydrogenase and the incidence of single-cell necrosis in the liver were increased in all groups of treated males. The authors considered these effects to be nonspecific, because of the lack of a clear dose-response relationship, the relatively low severity, and their limitation to males. Other statistically significant differences in hematological and biochemical parameters were also considered to be of minor toxicological significance. The authors concluded that a concentration of 5% in the diet was a suitable maximum tolerable dose of stevioside for a two-year study in rats (Aze et al., 1991).

In earlier 3-month rat studies reviewed by Geuns (2003)---the sample purity, doses, strain of rat were not reported---a no effect level was determined to be in excess of 2500 mg/kg bw/day and 7% of the diet, apparently due to lack of effects at the highest dose tested in both studies (Akashi and Yokoyama, 1975).

Table 6. Subchronic Studies on Stevioside

STUDY	ANIMAL MODEL/ GROUP SIZE	TEST MATERIAL/ SAMPLE PURITY	DOSES / DURATION	AUTHOR ASSIGNED NOAEL (mg/kg bw/day)	RESULTS AND REMARKS
Aze et al., 1991[a]	F344 rat/ 10 females and 10 males in each of 6 groups	Stevioside/ Not reported	0, 0.31, 0.62, 1.25, 2.5, 5% in diet/13 weeks	Not reported	No effects observed on mortality, body weight or food consumption. Clinical chemistry investigation revealed increased LDH levels and histopathological investigation indicated increased incidence of single-cell liver necrosis in all male treated groups, but not in a clear dose-response relationship. Invstigators did not consider these changes to be treatment related due to the small magnitude and low severity of changes, the lack of a clear dose relationship and the limitation to males only. Organ weights, urine chemistry and gross necropsy not discussed. Authors concluded that 5% stevioside in diet is a tolerable dose for a 2 year study.
Yodyingyuad and Bunyawong, 1991[a]	Hamster/ four groups of 20 (10 male, 10 female)	Stevioside/ 90%	0, 0.5, 1.0, 2.5 g/kg bw/day/ duration unclear/ 3 months	2500	F_0, F_1 and F_2 generations in reproductive study were dosed for 90 days. Histological examination showed no effect at any dose. Weights of organs, blood analysis, urine chemistry and gross necropsy not discussed. The F_1 and F_2 hamsters continued to receive stevioside (via drinking water for one month, then at same dose as parents).
Mitsuhashi, 1976[b]	Rat (strain not reported)	Stevioside/ Not reported	Dietary concentrations up to 7%/ 3 months	Not reported	No effects noted at all doses tested. Experimental details such as body weight, organ weight, blood analysis, urine chemistry, gross necropsy and histopathology not discussed.
Akashi and Yokoyama, 1975[b]	Rat (strain not reported)	Stevioside/ Not reported	Oral doses up to 2500 mg/kg bw/3 months	2500	No effects noted at all doses tested. Experimental details such as body weight, organ weight, blood analysis, urine chemistry, gross necropsy and histopathology not discussed.

[a] Abstract only. [b] As reported by Geuns, 2003.

4. Chronic Toxicity Studies

There have been three chronic rat studies conducted on stevioside which are summarized in Table 7. No treatment-related increase in tumor incidence was seen in any of these studies. In the most recent and best-documented study (additional study details were presented to JECFA in 2006), the apparent no observed adverse effect level (NOAEL) in F344 rats was the dietary level of 2.5% (test sample purity 96%, Toyoda et al., 1997). At 5% of the diet, statistically significant decreases in body weight, percent survival and kidney weight were seen. The author attributed these effects to various factors. The decrease in body weight was attributed to an inhibition of glucose utilization. The decrease in survival seemed to have been caused by an unusual late onset of large granular lymphocyte leukemia in high dose males. The authors reported that this tumor is rather common in F344 rats and that the overall incidence in male rats was actually within the historical control range experienced in the particular laboratory. The authors attributed

the decrease in kidney weight as probably due to a decrease in chronic inflammation found in the histopathological examination relative to control animals.

Table 7. Chronic Toxicity Studies on Stevioside

STUDY	ANIMAL MODEL/ GROUP SIZE	TEST MATERIAL/ SAMPLE PURITY	DOSES / DURATION	AUTHOR ASSIGNED NOAEL (mg/kg bw/day)	RESULTS AND REMARKS
Toyoda et al., 1997	F344 rat/ 50 per sex per group	95.6% Stevioside	*Ad libitum* 0,2.5, 5% of diet/~24 months (104 weeks)	Author did not assign a NOAEL. (Mid-dose calculates to 970 in males; JECFA, 2006)	A significant decrease in survival rates in males receiving 5%. General condition, body weight, food intake, mortality, hematological, histopathological and organ weights were observed. Body weight gains dose-dependently decreased in both sexes. Kidney weights were significantly lower in 5% males and ovary, kidney and brain weights were significantly increased in 5% females. Tumors and non-neoplastic lesions found in all groups, and were not correlated to treatment. Conclusion was that stevioside is not carcinogenic under these experimental conditions.
Xili et al., 1992[a]	Wistar rat/ 45 per sex per group	85% Stevioside	0, 0.2, 0.6, 1.2 % of diet/24 months	794 (high dose)	After 6, 12 and 24 months five rats from each group were sacrificed for analysis. No effects observed on growth, food utilization, general appearance, mortality or lifespan. No changes in hematological, urinary or clinical biochemical values. Histopathological analysis showed that the neoplastic and non-neoplastic lesions were unrelated to the level of stevioside in the diet.
Yamada et al., 1985	F344 rat/ 70 per sex per group, 30 per sex per group in low-dose	95.2% Steviol glycosides (75% stevioside; 16% Reb A)	0.1, 0.3, 1% of diet/22 months for males, 24 months for females	550 (high dose)	At 6 and 12 months, 10 males and 10 females were sacrificed for analysis. General behavior, growth and mortality were same among groups throughout the experiment. At 6 months, proteinurea was significantly increased in females, and blood glucose was increased in both sexes, although urinary glucose not detected. Weights of liver, kidney, heart, prostate and testes were increased in males at 6 months, and weight of ovaries was decreased in females in dose-dependent manner. Histopathological examination showed differences in various organs at 6 months that were unrelated to stevioside dose. These differences were not found at 12 months. Authors concluded that there were no significant changes after 2 years.

[a] Only abstract available.

5. Reproductive & Developmental Toxicity Studies

S. rebaudiana has been used by Indians in Paraguay as an oral contraceptive (Planas and Kuc, 1968; Schvartzman et al., 1977). Crude stevia leaf extract has been shown to inhibit fertility in rats (Planas and Kuc, 1968). Several reproductive toxicity studies have been conducted with orally administered purified stevioside as tabulated in Table 8, No effect on fertility or reproductive parameters was seen in a three-generation study in hamsters at doses up to 2500 mg/kg/day (Yodyingyuad and Bunyawong, 1991). There was an absence of statistically significant effects at doses up to 3% (equivalent to 3000 mg/kg bw/day; sample purity 96%; Mori et al., 1981). Similar

results were observed in an additional rat study that was reviewed by Geuns (2003) where limited information is available in English (Usami et al., 1995). More recently, no effect on pregnancy or developmental parameters were observed in Swiss albino mice with stevioside or aqueous stevia extract at doses up to 800 mg/kg bw/day in female mice (Kumar and Oommen, 2008). Further details on these studies to the extent available are given in Table 8.

6. Mutagenicity & Genotoxicity Studies

Mutagenicity and genotoxicity studies on stevia and stevioside are summarized in Table 9. All studies were negative with the exception of a comet assay done in rats (Nunes et al., 2007a). The methodology used in this study and the resulting conclusions have been questioned (Geuns, 2007; Nunes et al., 2007b and 2007c; Williams, 2007; and Brusick, 2008).

7. Clinical Studies & Other Reports in Humans

Several pharmacological and biochemical effects have been reported for crude extracts of stevia leaves and purified steviol glycosides. These include effects on glucose uptake, insulin secretion, and blood pressure (Geuns, 2003a). Stevioside is used in South America as a treatment for Type II diabetes. These were key concerns for JECFA. The available clinical studies were summarized in 2006, and further studies were recommended (WHO, 2006). Several studies were subsequently conducted, and JECFA reviewed these studies in 2009 (WHO, 2009). JECFA's summaries of the key studies are included below.

a. Studies Summarized in 2006

Aqueous extracts of 5 g of *S. rebaudiana* leaves were administered to 16 volunteers at 6 hour intervals for three days, and glucose tolerance tests were performed before and after administration. Another six volunteers were given an aqueous solution of arabinose in order to eliminate possible effects of stress. The extract increased glucose tolerance and significantly decreased plasma glucose concentrations during the test and after overnight fasting in all volunteers (Curi et al., 1986).

In a multi-center randomized, double-blind, placebo-controlled trial of hypertensive Chinese men and women (aged 28–75 years), 60 patients were given capsules containing 250 mg of stevioside (purity not stated) three times per day, corresponding to a total intake of 750 mg of stevioside per day (equivalent to 11 mg/kg bw/day as calculated by FSANZ, 2008) and followed up at monthly intervals for one year. Forty-six patients were given a placebo. After 3 months, systolic and diastolic blood pressure in men and women receiving stevioside decreased significantly, and the effect persisted over the year. Blood biochemistry parameters, including lipids and glucose, showed no significant changes. Three patients receiving stevioside and one receiving the placebo withdrew from the study as a result of side effects (nausea, abdominal fullness, dizziness). In addition, four patients receiving stevioside experienced abdominal fullness, muscle tenderness, nausea and asthenia within the first week of treatment. These effects subsequently resolved, and the patients remained in the study (Chan et al., 2000).

Table 8. Reproductive Toxicity Studies on Steviol Glycosides

Study	Animal Model/ Group size	Test Sample Purity Stevioside (unless Otherwise Noted)	Doses / Duration	Author Assigned NOAEL (mg/kg bw/day)	Results and Remarks
Kumar and Oommen, 2008	Swiss albino mice/ 4 groups of 5 females	Not reported	500 and 800 mg/kg bw/15 days	800	Stevioside and stevia extract (purity and composition not reported) did not have any effect on reproductive parameters in mice when administered to female mice before or during pregnancy. No changes seen in number of implantations or uterine resorptions. No gross anatomical or histopathologic effects seen in 16-day embryos.
Usami et al., 1995[a]	Wistar Rat/4 groups of 25 or 26 pregnant rats	95.6%[b]	0, 250, 500, 1000 mg/kg bw/10 days	1000	Pregnant rats given doses of stevioside by gavage once a day on days 6-15 of gestation and were sacrificed on day 20 of gestation. Fetuses were examined for malformations in addition to maternal and fetal body weight, number of live fetuses, sex distribution, and numbers of resorptions or dead fetuses. No treatment-related effects observed. Authors concluded that orally administered stevioside is not teratogenic in rats.
Yodyingyuad and Bunyawong, 1991	Hamster/ 10 male, 10 female per group (40 total)	90%	0, 500, 1000, 2500 mg/kg bw/day/ duration unclear/ 3 months	2500	Males from each group were mated to females from respective dose group. Each female was allowed to bear 3 litters during the course of experiment. Stevioside had no effect on pregnancies of females at any dose. The F_1 and F_2 hamsters continued to receive stevioside (via drinking water for one month, then at same dose as parents); showed normal growth and fertility. Histological examination showed no effect on reproductive organs at any dose.
Oliveira-Filho et al., 1989[a]	Rat/number not reported	Not reported (Dried Stevia Leaves)	0 or 0.67 g dried leaves /ml, 2 ml twice per day/ 60 days	Not reported	Prepubertal rats (25-30 days old) tested for glycemia; serum concentrations of thyroxine; tri-iodothyroxine; available binding sites in thyroid hormone-binding proteins; binding of ^{3}H-methyltrienolone (a specific ligand of androgen receptors) to prostate cytosol; zinc content of prostate, testis, submandibular salivary gland, and pancreas; water content of testes and prostate; body-weight gain; and final weights of testes, prostate, seminal vesicle, submandibular salivary gland, and adrenal. Only difference due to treatment was seminal vesicle weight, which fell to 60% compared to control.
Mori et al., 1981	Rat/11 male, 11 female per group (44 total)	96%	0, 0.15, 0.75 or 3 % of feed/60 days	2000	Males given stevioside dose in diet for 60 days before and during mating with females who received same diet (as mated male) 14 days before mating and 7 days during gestation. No effect due to treatment on fertility or mating performance, and no effect of fetal development. Rats of each sex had slightly decreased body weight gain at highest dose with non-significant increase in number of dead and resorbed fetuses at highest dose.
Planas and Kuc, 1968[c]	Rat/14 per group (28 total)	Not reported (Crude stevia extract)	0 or 5% Crude stevia extract /18 days	Not reported	Extract given orally to adult female rats for 12 days, who were mated with untreated males during the last 6 days. Fertility reduced to 21% of fertility in control rats and remained reduced in a 50-60 day recovery. Histological examination, weights of organs, blood analysis, urine chemistry and gross necropsy not discussed.

[a] Only abstract available. [b] As reported by European Commision, 1999b.

Table 9. Mutagenicity & Genotoxicity Studies on Stevia Extracts & Stevioside

END-POINT	TEST SYSTEM	MATERIAL	PURITY (%)	CONCEN-TRATION / DOSE	RESULT	REFERENCE
Reverse mutation	*S. typhimurium* TA97, TA98, TA100, TA102, TA104, TA1535, TA1537	Stevioside	83	5 mg/plate[a] 1 mg/plate[b]	Negative	Matsui et al. (1996)
Reverse mutation	*S. typhimurium* TA98, TA100	Stevioside	99	50 mg/plate	Negative[c]	Suttajit et al. (1993)
Forward mutation	*S. typhimurium* TM677	Stevioside	83	10 mg/plate	Negative[c]	Matsui et al. (1996)
Forward mutation	*S .typhimurium* TM677	Stevioside	NS	10 mg/plate	Negative[c]	Pezzuto et al. (1985)
Forward mutation	*S. typhimurium* TM677	Stevioside	NS	Not specified	Negative[c]	Medon et al. (1982)
Gene mutation	Mouse lymphoma L5178Y cells, TK-locus	Stevioside	NS	5 mg/mL	Negative[c,d]	Oh et al. (1999)
Gene mutation (umu)	*S. typhimurium* TA1535/pSK1002	Stevioside	83	5 mg/plate	Negative[c]	Matsui et al. (1996)
Gene mutation	*B. subtilis* H17 rec+, M45 rec-	Stevioside	83	10 mg/disk	Negative[c]	Matsui et al. (1996)
Chromosomal aberration	Chinese hamster lung fibroblasts	Stevioside	83	8 mg/mL 12 mg/mL	Negative	Matsui et al. (1996)
Chromosomal aberration	Human lymphocytes	Stevioside	NS	10 mg/mL	Negative	Suttajit et al. (1993)
Chromosomal aberration	Chinese hamster lung fibroblasts	Stevioside	85	12 mg/mL	Negative[a]	Ishidate et al. (1984)
DNA damage (comet assay)	Wistar rats; liver, brain and spleen	Stevioside	88.62	4 mg/L (estimated to be 80 - 500 mg/kg bw/day) in drinking water for 45 days	Positive in all tissues examined, most notably in liver	Nunes et al. (2007a)
DNA damage (comet assay)	Male BDF1 mouse stomach, colon, liver	Stevia extract	Stevioside, 52; Reb A, 22	250 - 2000 mg/kg bw	Negative[e]	Sekihashi et al. (2002)
DNA damage (comet assay)	Male ddY mouse stomach, colon, liver, kidney, bladder, lung, brain, bone marrow	Stevia	NS	2000 mg/kg bw	Negative[e]	Sasaki et al. (2002)
Micronucleus formation	ddY mouse bone marrow and regenerating liver	Stevioside	NS	62.5 - 250 mg/kg bw	Negative	Oh et al. (1999)
Mutation	*D. melanogaster* Muller 5 strain	Stevioside	NS	2% in feed	Negative	Kerr et al. (1983)

NS = Not specified. [a] Without metabolic activation. [b] As calculated by Williams, 2007 [c] With and without metabolic activation (source not specified in original monograph). [d] Inadequate detail available. [e] Sacrificed at 3 hours and 24 hours.

A follow-up multi-center randomized, double-blind, placebo-controlled trial was conducted in hypertensive Chinese men and women (aged 20–75 years). Eighty-five patients were given capsules containing 500 mg of stevioside (purity not stated) three times per day, corresponding to a total intake of 1500 mg of stevioside per day (equivalent to 21 mg/kg bw/day, as calculated by FSANZ, 2008). Eighty-nine patients were given a placebo. Three patients in each group withdrew during the course of the study. There were no significant changes in body mass index or blood biochemistry parameters throughout the study. In the group receiving stevioside, mean

systolic and diastolic blood pressures were significantly decreased compared with the baseline, commencing from about 1 week after the start of treatment. After 2 years, 6 out of 52 patients (11.5%) in the group receiving stevioside had left ventricular hypertrophy compared with 17 of 50 patients (34%) in the group receiving the placebo ($p < 0.001$). Eight patients in each group reported minor side effects (nausea, dizziness and asthenia), which led two patients in each group to withdraw from the study. Four patients in the group receiving stevioside experienced abdominal fullness, muscle tenderness, nausea and asthenia within the first week of treatment. These effects subsequently resolved and the patients remained in the study (Hsieh et al., 2003).

In a paired cross-over study, 12 patients with Type II diabetes were given either 1 g of stevioside (stevioside, 91%; other stevia glycosides, 9%) or 1 g of maize starch (control group), which was taken with a standard carbohydrate-rich test meal. Blood samples were drawn at 30 minutes before and for 240 minutes after ingestion of the test meal. Stevioside reduced postprandial blood glucose concentrations by an average of 18% and increased the insulinogenic index by an average of 40%, indicating beneficial effects on glucose metabolism. Insulin secretion was not significantly increased. No hypoglycemic or adverse effects were reported by the patients or observed by the investigators. Systolic and diastolic blood pressure was not altered by stevioside administration (Gregersen et al., 2004).

Forty-eight hyperlipidemic volunteers were recruited to a randomized, double-blind trial designed to investigate the hypolipidemic and hepatotoxic potential of steviol glycoside extract. The extract used in this study was a product containing stevioside (73 ± 2%), rebaudioside A (24 ± 2%) and other plant polysaccharides (3%). The subjects were given two capsules, each containing 50 mg of steviol glycoside extract or placebo, twice daily (i.e., 200 mg/day, equivalent to 3.3 mg/kg bw/day assuming an average body weight of 60 kg), for 3 months. One volunteer receiving placebo and three volunteers receiving steviol glycoside failed to complete the study for personal reasons, not related to adverse reactions. At the end of the study, both groups showed decreased serum concentrations of total cholesterol and of low-density lipoproteins. Analyses of serum concentrations of triglycerides, liver-derived enzymes and glucose indicated no adverse effects. The authors questioned the subjects' compliance with the dosing regime, in view of the similarity of effect between treatment and placebo (Anonymous, 2004a). In a follow-up study, 12 patients were given steviol glycosides extract in incremental doses of 3.25, 7.5 and 15 mg/kg bw/day for 30 days per dose. Preliminary results indicated no adverse responses in blood and urine biochemical parameters (Anonymous, 2004b).

b. Studies Summarized in 2009

Four male and five female healthy volunteers (aged 21–29 years) were provided with capsules containing 250 mg stevioside (97% purity) to be taken 3 times per day for 3 days. Doses, expressed as steviol, were 288 mg/day or 4.4 mg/kg bw/day for females and 3.9 mg/kg bw/day for males. Twenty-four hour urine samples were taken before dosing on day 1 and after dosing on day 3. Fasting blood samples were taken before dosing on day 1, and six samples were taken at different time points on day 3 after dosing. Fasting blood pressure measurements were taken before the first capsule and at six different time intervals after the first dose. Urine was analysed for creatinine, sodium, potassium, calcium, and urea. Blood was analysed for plasma glucose, plasma insulin, alkaline phosphatase, ALT, GPT, creatine kinase, and lactate dehydrogenase.

The clinical analyses of blood, blood pressure, and urine showed no differences between samples taken before or after dosing. This study was approved by the local ethics committee (Temme et al., 2004).

In a study unpublished at the time of the sixty-eighth meeting, 250 mg of a product containing 91.7% total steviol glycosides, including 64.5% stevioside and 18.9% rebaudioside A, was administered to groups of type 1 (n = 8) and type 2 diabetics (n = 15) and non-diabetics (n = 15) 3 times daily for 3 months in a double-blind, placebo-controlled trial. Control groups with the same number of subjects received a placebo. After 3 months, there were no significant changes in systolic or diastolic blood pressure, glycated haemoglobin (HbA1c), blood lipids, or renal or hepatic function. No side effects were reported. This study was approved by the local ethics committee and met the requirements of the Declaration of Helsinki (Barriocanal et al., 2006, 2008). The Committee previously noted that this product did not meet the proposed specification of "not less than 95% steviol glycosides" and that the study was conducted in a small number of subjects.

A study of antihypertensive effects was conducted in previously untreated mild hypertensive patients with crude stevioside obtained from the leaves of *S. rebaudiana*. Patients with essential hypertension were subjected to a placebo phase for 4 weeks and then received either capsules containing placebo for 24 weeks or crude stevioside at consecutive doses of 3.75 mg/kg bw/day (7 weeks), 7.5 mg/kg bw/day (11 weeks) and 15 mg/kg bw/day (6 weeks). Comparison of patients receiving stevioside with those on placebo showed neither antihypertensive nor adverse effects of stevioside. This study was approved by the local ethics committee and met the requirements of the Declaration of Helsinki (Ferri et al., 2006). The product in this study also did not meet the proposed specification. According to a study available in abstract form only, a randomized placebo-controlled double-blind study was conducted in subjects with type 2 diabetes. Fifty-five subjects received 500 mg stevioside (purity unspecified) or placebo (maize starch) 3 times daily for 3 months. Compared with the placebo, stevioside did not reduce the incremental area under the glucose response curve and maintained the insulin response and HbA1c and fasting blood glucose levels. HbA1c is an indicator of mean glucose levels and is used in identifying effects on the control of diabetes. No differences in lipids or blood pressure were observed. It is not clear whether this study was approved by the local ethics committee or met the requirements of the Declaration of Helsinki (Jeppesen et al., 2006).

A placebo-controlled double-blind trial was carried out in 49 hyperlipidemic patients (aged 20–70 years, number of males and females not supplied) not undergoing treatment. The study was approved by the local ethics committee and complied with the principles of the Declaration of Helsinki. Individuals were divided into two groups, with 24 subjects receiving placebo capsules and 25 receiving capsules containing a dose of 50 mg steviol glycosides (70% stevioside, 20% Rebaudioside A), equivalent to 1.04 mg steviol/kg bw/day, using the mean body weight of the treatment group, 72.7 kg. Two capsules were taken before lunch and two before dinner each day for 90 days. Six subjects withdrew from the study, four in the placebo group and two in the test group. Self-reported adverse reactions were recorded, and fasting blood samples were taken at the end of the study and analyzed for ALT, aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low density lipoprotein (VLDL), and triglycerides. No effects of treatment

on ALT, AST, or GGT were found. Decreases in the total cholesterol and LDL were observed in both the stevioside group and the placebo group, which were not treatment related. No adverse effects were observed (Cavalcante da Silva et al., 2006). The Committee noted at its sixty-eighth meeting that the product used in this study did not meet the proposed specification.

C. Safety Data on Rebaudioside A

Only limited studies were available on Reb A during the JECFA reviews. Several toxicology studies have been recently reported on purified Reb A (published in Food and Chemical Toxicology, Supplement 1, July 2008), although it is uncertain whether or not these studies were reviewed as pre-publication manuscripts by JECFA during its 2008 deliberations. These studies include additional mutagenicity data, comparative pharmacokinetic studies with stevioside in rats and humans, several subchronic studies in rats and one in dogs, and additional reproductive and developmental toxicity studies in rats as well as additional clinical studies.

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

Three recently completed studies have shed light on the absorption and fate of Reb A in rats and humans.

The toxicokinetics and metabolism of Reb A, stevioside, and steviol were examined in rats for comparative purposes to determine whether toxicological studies conducted previously with stevioside would be applicable to the structurally-related glycoside, Reb A (Roberts and Renwick, 2008). Single, oral doses of the radiolabelled compounds were extensively and rapidly absorbed, with plasma concentration-time profiles following similar patterns for stevioside and Reb A. Elimination of radioactivity from plasma was essentially complete within 72 hours. 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. Reb 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 and the radioactivity in the feces was identified primarily as steviol. The predominant radioactive compound detected in the bile was steviol glucuronide(s), indicating de-conjugation in the lower intestine. The authors concluded that the overall data on toxicokinetics and metabolism indicate that Reb A and stevioside are handled in an almost identical manner in the rat after oral dosing.

This randomized, double-blind, cross-over study assessed the comparative pharmacokinetics of steviol and steviol glucuronide following single oral doses of Reb A and stevioside in healthy adult male subjects (Wheeler et al., 2008). Steviol glucuronide appeared in the plasma of all subjects after administration of Reb A or stevioside, with median T_{max} (time to maximum concentration) values of 12.0 and 8.00 hours post-dose, respectively. The $t_{1/2}$ values for elimination of steviol glucuronide were approximately 14 hours when either compound was given. Administration of Reb A resulted in a significantly (approximately 22%) lower steviol glucuronide geometric mean C_{max} (concentration maximum) value (1472 ng/ml) than administration of stevioside (1886

ng/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 Reb A and stevioside doses, respectively. No steviol glucuronide was detected in feces. Pharmacokinetic analysis indicated that both

Reb 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 (Reb A: 0.04%; stevioside: 0.02%). The authors concluded that Reb 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 toxicity of Reb A in rats at levels up to 2000 mg/kg bw/day (Sloter, 2008). Reb A and total steviol were detected in peripheral blood of rats during daily administration of 2000 mg/kg bw/day of Reb A at extremely low levels, with mean plasma concentrations of approximately 0.6 and 12 µg/mL, respectively. Estimates of absorbed dose for Reb 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 Reb A and measured hydrolysis products expressed as Total Reb A Equivalents compared to daily administered dose resulted in an estimate of percentage of dose recovered ≈ 84%.

2. Subchronic Toxicity Studies

Two repeated-dose studies were conducted by the oral route in Wistar rats (Curry and Roberts, 2008). In a 4-week study, Reb A (97% purity) was administered at dietary concentrations of 0, 25,000, 50,000, 75,000 and 100,000 ppm. The NOAEL, including an evaluation of testes histopathology, was determined to be 100,000 ppm. In the 13-week study, Wistar rats were administered Reb A at dietary concentrations of 0, 12,500, 25,000 and 50,000 ppm. Reductions in body weight gain attributable to initial taste aversion and lower caloric density of the diet were observed in high-dose male and female groups. Inconsistent reductions in serum bile acids and cholesterol were attributed to physiological changes in bile acid metabolism due to excretion of high levels of Reb A via the liver. All other hepatic function test results and liver histopathology were within normal limits. Significant changes in other clinical pathology results, organ weights and functional observational battery test results were not observed. Macroscopic and microscopic examinations of all organs, including testes and kidneys, 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 4,161 and 4,645 mg/kg bw/day in male and female rats, respectively.

Reb A (99.5% purity) was administered in the diet at target exposure levels of 500, 1000, and 2000 mg/kg bw/day to Sprague-Dawley rats for 90 days (Nikiforov and Eaton, 2008). There were no treatment-related effects on the general condition and behavior of the animals as determined by clinical observations, functional observational battery, and locomotor activity assessments. Evaluation of clinical pathology parameters revealed no toxicologically relevant, treatment-related

effects on hematology, serum chemistry, or urinalysis. Macroscopic and microscopic findings revealed no treatment-related effects on any organ evaluated. Lower mean body weight gains were noted in males in the 2000 mg/kg bw/day group throughout the study, which was considered by the authors to be test article related; however, given the small magnitude of the difference as compared to controls, this effect was not considered to be adverse.

A 90-day dietary toxicity study was conducted in Crl:CD(SD) rats with Reb A (99.5% purity) doses of 500, 1000 and 2000 mg/kg bw/day (Eapen, 2007). Each group consisted of 20/animals/sex. There were no treatment-related effects on clinical observations, food consumption, and functional observational or locomotor activity parameters. 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. The body weight in males was 9.1% lower than the control group at the end of the dosing period (study week 13). The investigators did not consider this result to be adverse due to the small magnitude of difference from the control group value, and they suggested that the reduction was most likely due to the large proportion of the diet represented by the test material. The assigned NOAEL was ≥ 2000 mg/kg bw/day.

A 6-month dietary toxicity study in Beagle dogs was conducted to evaluate the potential toxic effects of Reb A (97.5% purity) at dosage levels of 0, 500, 1000 or 2000 mg/kg bw/day (Eapen, 2008). All groups consisted of 4 males and 4 females. During the course of the study, there were no unscheduled deaths. No treatment-related clinical observations were noted. Home cage, open field observations and functional observations and measurements were unaffected by the administration of Reb A. There were no differences in hematology findings, serum chemistry findings, or urinalysis findings between groups. In addition, no treatment related gross necropsy observations, alterations in final body weight, alterations in organ weights, or histological changes were noted. Based on the results of this study, the authors concluded that no systemic toxicity of Reb A was observed at dosage levels up to 2000 mg/kg bw/day and the assigned NOAEL was ≥2000 mg/kg bw/day.

3. Reproductive & Developmental Toxicity Studies

Reb A (97% purity) was administered via the diet to male and female Han Wistar rats at 0, 7,500, 12,500, and 25,000 ppm for two generations (Curry et al., 2008). Reb A treatment was not associated with any signs of clinical toxicity or adverse effects on body weight, body weight gain, or food consumption. No treatment-related effects of Reb A were observed in either the F_0 or F_1 generations on reproductive performance parameters including mating performance, fertility, gestation lengths, estrous cycles, or sperm motility, concentration, or morphology. 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 Reb 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 bw/day.

In an unpublished two-generation dietary reproduction study, four groups of male and female Crl:CD(SD) rats (30/sex/group) were offered either basal diet or the test article, Reb A (purity

95.7%), continuously in the diet for at least 70 consecutive days prior to mating (Sloter, 2008). Reb A doses were 0, 500, 1000 and 2000 mg/kg bw/day for the F_0 and F_1 generations. F_0 animals were approximately 7 weeks of age at the initiation of test diet exposure. The test diet was offered to the offspring selected to become the F_1 generation following weaning beginning on postnatal day 21. The F_0 and F_1 males continued to receive Reb A throughout mating and continuing through the day of euthanasia. The F_0 and F_1 females continued to receive Reb A throughout mating, gestation and lactation, until day of euthanasia. The authors concluded that there were no effects on reproduction in males or females (estrus cycles, mating, fertility, conception or copulation indices, number of days between pairing and coitus, gestation length, and spermatogenic endpoints). A dose level ≥ 2000 mg/kg bw/day (highest dose administered) was assigned to be the NOAEL for parental systemic and reproductive toxicity.

4. Mutagenicity & Genotoxicity Studies

The mutagenicity and genotoxicity data on Reb A have greatly increased recently.

Reb A was evaluated for genotoxicity with a set of *in vitro* and *in vivo* assays covering mutation, chromosome damage and DNA strand breakage with consistent and uniformly negative results (Pezzuto et al, 1985; Nakajima, 2000a; Nakajima, 2000b; Sekihashi et al., 2002) as reviewed by Brusick (2008). An unpublished chromosome aberration assay of Reb A in cultured mammalian cells was submitted for JECFA review (Nakajima, 2000a). The JECFA review of this study indicated that no increases in chromosome aberrations were found.

More recently, in their GRAS Notification, Merisant submitted three unpublished studies on Reb 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). All three studies indicated lack of mutagenic or genotoxic activity. In addition, there is another set of published studies that indicate lack of mutagenicity *in vitro* in *Salmonella, E. coli,* and mouse lymphoma cells, lack of *in vitro* clastogenic effects in Chinese hamster V79 cells, the absence of *in vivo* effects in a mouse micronucleus assay, and a rat study for unscheduled DNA synthesis (Williams and Burdock, 2009).

Table 10 summarizes these results.

Table 10. Mutagenicity & Genotoxicity 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	5 Salmonella strains and 1 E coli strain with and without exogenous metabolic activation system	Reb A		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		Up to 5000 µg/mL	No mutagenic or clastogenic response	Williams and Burdock (2009)
Chromosome Aberration	Chinese Hamster V79 cells	reb A		Up to 5000 µg/mL		Williams and Burdock (2009)
Mouse Micronucleus	Micronucleus study consisted of 7 groups, each containing 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		Reb A		Up to 750 mg/kg bw	No increase in micronuclei formation	Williams and Burdock (2009)
Unscheduled DNA Synthesis	*In vivo* rat	Reb A		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.

5. Clinical Studies

A randomized, double-blind trial evaluated the hemodynamic effects of four weeks' consumption of 1000 mg/day Reb A (97% purity) versus placebo in 100 individuals with normal and low-normal systolic blood pressure (SBP) and diastolic blood pressure (DBP) (Maki et al., 2008a). Subjects were predominantly female (76%, Reb 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 Reb A and placebo groups, respectively. Compared with placebo, Reb A did not significantly alter resting, seated SBP, DBP, mean arterial pressure (MAP), heart rate (HR) or 24-hour ambulatory blood pressure responses. The authors concluded that these results indicated that consumption of as much as 1000 mg/day of Reb A produced no clinically important changes in blood pressure in healthy adults with normal and low-normal blood pressure.

Another randomized, double-blind, placebo-controlled clinical trial (conducted at six research sites in the US) evaluated the effects of 16 weeks of consumption of 1000 mg Reb A (97% purity, n=60) compared to placebo (n = 62) in men and women (33-75 years of age) with type 2 diabetes mellitus (Maki et al., 2008b). Mean ± standard error changes in glycosylated hemoglobin levels did not differ significantly between the Reb A (0.11 ± 0.06%) and placebo (0.09 ± 0.05%; $p = 0.355$) groups. Changes from baseline for Reb 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 C-peptide (0.13 ± 0.09 ng/mL and 0.42 ± 0.14 ng/mL) did not differ significantly ($p > 0.05$ for all). Assessments of changes in blood pressure, body weight, and fasting lipids indicated no differences by treatment. Reb A was well tolerated, and records of hypoglycemic episodes showed no excess versus placebo. The authors suggested that chronic use of 1000 mg Reb A did not alter glucose homeostasis or blood pressure in individuals with type 2 diabetes mellitus.

D. Safety of Rebaudioside D & Rebaudioside F

As mentioned above, PureCircle's specifications for SG95 include up to 2.5% reb D and up to 3% reb F. No studies were located in the literature that focused specifically on the metabolism or toxicology of Reb D or Reb F. The sweetness properties of Reb D have been described as the highest taste quality among the naturally occurring steviol glycosides (Tanaka, 1997). The safety of the steviol glycosides is based on the lack of toxicity of samples that are primarily stevioside or rebaudioside A. It is reported that the test samples in some of the subchronic and reproductive tests had low but measureable levels of Reb D and Reb F (CCFA, 2009). The lack of carcinogenicity of the glycosides is inferred by the lack of tumors found in chronic studies in rats fed samples that were largely described as stevioside. The inference of lack of carcinogenicity of the various steviol glycosides is predicated on a similar metabolic pathway. As pointed out by the review by Renwick and Tarka (2008), the metabolic data are similar from all of several laboratories that Reb A, Reb C and stevioside evidence are not digested or absorbed in the stomach or small intestine, but are converted to steviol in the large intestine by the action of indigenous microflora. According to Renwick and Tarka (2008), the lability of Reb C is particularly significant because it indicates that any glucose-rhamnose sugar linkages present in Reb C are labile to the enzyme activity due to microflora in the large intestine. The glycosidase activity in these microflora has been characterized as diverse (Hawksworth and Hill, 1971), and

this enzymatic actions can readily degrade a wide variety of xenobiotic glycosides (Tamara et al., 1980).

Therefore, there exists a strong presumption that Reb D and Reb F (including the glucose-xylose linkage in Reb F) would be metabolized similarly to other glycosides; i.e., they would not be digested or absorbed in the stomach or small intestine and would be converted to steviol in the large intestine by the action of indigenous microflora.

E. Studies on the Principal Metabolite: Steviol

A number of studies have been conducted on steviol, the principal mammalian metabolite of stevioside, and the results of these studies are provided below.

1. Acute Toxicity Studies

In male and female mice and rats given steviol (purity, 90%) orally, the LD_{50} was > 15 g/kg bw, and 1/15 animals died within 14 days of administration. The LD_{50} values in hamsters given steviol orally were 5.2 g/kg bw in males and 6.1 g/kg bw in females. Histopathological examination of the kidneys revealed severe degeneration of the proximal tubular cells, and these structural alterations were correlated with increased serum blood urea nitrogen and creatinine. The authors concluded that the cause of death was acute renal failure (Toskulkao et al., 1997).

2. Developmental Toxicity Studies

Groups of 20 pregnant golden hamsters were given steviol (purity, 90%) at doses of 0, 250, 500, 750, or 1000 mg/kg bw/day (only 12 animals at the highest dose) by gavage in corn oil on days 6 - 10 of gestation. A significant decrease in body weight gain and increased mortality (1/20, 7/20, and 5/12) were observed at the three highest doses, and the number of live fetuses per litter and mean fetal weight decreased in parallel. Histopathological examination of the maternal kidneys showed a dose-dependent increase in the severity of effects on the convoluted tubules (dilatation, hyaline droplets). However, no dose-dependent teratogenic effects were seen. The NOEL was 250 mg/kg bw/day for both maternal and developmental toxicity (Wasuntarawat et al., 1998).

3. Mutagenicity & Genotoxicity Studies

Several mutagenicity and genotoxicity studies have been conducted on steviol. The studies reviewed by JECFA are summarized in Table 11.

Table 11. Mutagenicity & Genotoxicity Studies on Steviol

STUDY	*IN VIVO/IN VITRO*	SYSTEM	TEST SAMPLE PURITY	AUTHOR CONCLUSION	RESULTS AND REMARKS
Sekihashi et al., 2002[a]	*In Vivo/In Vitro*	Comet Assay	Not reported	Negative	In *in vitro* study, steviol at 62.5, 125, 250 and 500 µg/ml did not damage DNA of TK6 and WTK1 cells in presence or absence of S9 mix. In *in vivo* study, mice sacrificed 3 or 24 hours after one-time oral administration of 250, 500, 1000 or 2000 mg/kg of steviol. Stomach, colon, kidneys, testis and liver DNA not damaged. An identical *in vivo* experiment with stevia extract performed, which also gave negative results.
Oh et al., 1999[b]	*In Vivo?*	Cell Mutation and DNA damage	Not reported	Negative	Steviol gave negative results for cell mutation and DNA damage in cultured cells.
Matsui et al., 1996[c]	*In Vivo?*	Mutagenicity and Chromosome aberration (Chinese hamster lung fibroblasts)	Not reported	Positive	Gene mutation and chromosomal aberration found in Chinese hamster lung fibroblasts after metabolic activation of steviol. In hamsters, several metabolites of stevioside found that have not been found in rats or humans. Therefore, experimental relevance should be questioned when hamsters are used.
Terai et al., 2002[a]	*In Vitro*	Bacterial Mutagenicity	Not Reported	Positive	Steviol found to be mutagenic in Aroclor induced rat liver S9 fraction. 15-oxo-steviol found to be mutagenic at 10% level of steviol. Specific mutagenicity of lactone derivative in presence of S9 mixture 10x lower than that of derivative without S9 mixture.
Temcharoen et al., 1998[c]	*In Vitro*	Bacterial Mutagenicity	Not Reported	Positive	Mutagenic effects of steviol and/or metabolites found in *S.typhimurium* TM677 by tranversions, transitions, duplications, and deletions at the guanine phosphoribosyltransferase (gpt) gene. Magnitude of increase of these mutations over the control not reported.
Klongpanich-pak et al., 1997[c]	*In Vitro*	Bacterial Mutagenicity	Not Reported	Negative	Steviol and stevioside inactive in TA strains of *S. typhimurium, e. coli WP2, uvrA/PKM101* and rec assay using *B. subtilis* even when microsomal activated fraction present. Magnitude of increase of these mutations over the control not reported.
Matsui et al., 1996[a]	*In Vitro*	Bacterial Mutagenicity	Not Reported	Negative	Testing of Southern Blot technique with probe for gpt gene DNA of *E. coli.* The chromosomal DNA of TM677 and steviol-induced TM677 mutants digested by restriction enzymes and probed. No significant differences found in fragment length between wild-type and mutant DNA.
Matsui et al., 1996[a]	*In Vitro*	Bacterial Mutagenicity	Not Reported	Both	Steviol weakly positive in umu test, either with or without metabolic activation. Steviol negative in reverse mutation and other bacterial assays even in presence of S9 activation.

STUDY	*IN VIVO/IN VITRO*	SYSTEM	TEST SAMPLE PURITY	AUTHOR CONCLUSION	RESULTS AND REMARKS
Procinska et al., 1991[c]	*In Vitro*	Bacterial Mutagenicity	Not Reported	Negative	The direct mutagenic activity of 15-oxo-steviol was refuted.
Compadre et al., 1988[a]	*In Vitro*	Bacterial Mutagenicity, Mass Spec	Not Reported	Positive	Mass spectral analysis of steviol and analogues under conditions known to produce a mutagenic response. 15-oxo-steviol, a product of the metabolite, 15-alpha-hydroxysteviol was found to be direct-acting mutagen. Magnitude of increase over control in assay not discussed.
Pezzuto et al., 1985[d]	*In Vitro*	Bacterial Mutagenicity	Not Reported	Positive	Using *S. typhimurium* TM677 strain, steviol found to be highly mutagenic in presence of 9000 x g supernatant from livers of Aroclor 1254-pretreated rats. This mutagenicty dependent on pretreatment of rats with Aroclor and NADPH addition, as unmetabolized steviol was inactive. None of other metabolites tested was mutagenic. Authors concluded that structural features of requisite importance for the expression of mutagenic activity may include a hydroxy group at position 13 and an unsaturated bond joining the carbon atoms at positions 16 and 17.
Temacha-roen et al., 2000[c]	*In Vivo*	Micronucleus (rat)	90%	Negative	Very high doses (8 g/kg bw) given to rats did not induce micronucleus in bone marrow erythrocytes in male and female animals.
Temacha-roen et al., 2000[c]	*In Vivo*	Micronucleus (mouse)	90%	Negative	Very high doses (8 g/kg bw) given to rats did not induce micronucleus in bone marrow erythrocytes in male and female animals.
Matsui et al., 1996[a]	*In Vivo*	Micronucleus (mouse)	Not Reported	Negative	Steviol did not increase number of micronuclei observed in this study.
Temacha-roen et al., 2000[c]	*In Vivo*	Micronucleus (hamster)	90%	Negative	Very high doses (4 g/kg bw) given to rats did not induce micronucleus in bone marrow erythrocytes in male and female animals.

[a] Abstract only. [b] As reported in JECFA, 2006. [c] As reviewed by Geuns, 2003. [d] Full article.

VI. DISCUSSION OF GRAS CRITERIA & PANEL SAFETY FINDINGS

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."[9]

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."[10]

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:[11]

- 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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[9] See 21 CFR 170.3(i).
[10] See 21 CFR 170.30(a).
[11] See Footnote 1.

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, this safety assessment to ascertain GRAS status for steviol glycosides for the defined food uses meets FDA criteria for reasonable certainty of no harm by considering both the technical and common knowledge elements.

2. Panel Findings on Safety Studies of Steviol Glycosides

The PureCircle SG95 product identified in the subject notification largely meets the JECFA specifications for purity in that the glycosides constitute 95% or more of the dry weight. SG95 differs slightly from other products on the market in that it contains 1-3% Reb D and Reb F in addition to the seven glycosides recognized in the JECFA specification. In addition, the products consist primarily of stevioside and Reb A. Both of these steviol glycosides have been well studied in clinical, pharmacokinetic and toxicological studies. A substantial majority of these studies have been published in peer reviewed journals. The Panel has reviewed the data on both glycosides as well as the data on steviol, the principal metabolite, and the Panel has also addressed several reviews which have expressed some concerns on the safety of steviol glycosides.

1. Safety Data on Stevioside & Stevia Extracts that are Predominantly Stevioside

Steviol glycosides are unique compounds in that they have viable uses as non-nutritive sweeteners in foods.[12] The series of reviews by JECFA over several years indicate the progression of knowledge on the toxicology and clinical effects of these compounds. The early toxicology studies were largely performed on crude extracts of stevia. Several early concerns were noted, including impairment of fertility and mutagenicity. As more studies were done on purified glycosides, the toxicology profile of steviol glycosides eventually proved to be rather unremarkable. A number of subchronic, reproductive and chronic studies have been conducted in laboratory animals. The studies were, in general, adequately designed with appropriately high doses and adequate numbers of animals to maximize the probability of detection of important adverse effects. Notably, the reproductive toxicity studies with purified steviol glycosides refuted the concern over effects on fertility that were initially reported with stevia leaves or crude extracts.

As discussed in Section V.A, JECFA reasoned that there were adequate chronic studies in rats, particularly the study by Toyoda et al. (1997), on which to base a temporary ADI with an adequate margin of safety. The Committee was satisfied that the lack of carcinogenic response

[12] 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) recently 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 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.

in these well-conducted studies justified their conclusion that the *in vitro* mutagenic activity of steviol, buttressed by the evidence of rapid biotransformation and elimination of aborbed steviol, did not present a risk of carcinogenic effects *in vivo*. In addition, they concluded that all common steviol glycosides share the same basic metabolic and excretory pathways. Therefore, JECFA has concluded that high purity preparations of various steviol glycosides are safe to use as a non-nutritive sweetener. The additional clinical data subsequently presented allowed JECFA to establish a permanent ADI of 0 - 4 mg/kg bw/day (based on steviol equivalents), which translates to 0 -10 mg/kg bw/day for stevioside. The estimated consumption levels for stevioside containing sweeteners summarized in Section IV are comfortably within the JECFA ADI.

The Panel agrees with this reasoning. It should be noted that in a recent study, DNA damage was seen in a variety of organs in a comet assay in rats maintained on drinking water containing 4 mg/mL steviol glycosides for up to 45 days (Nunes et al., 2007a). Several experts in the field have since questioned the methodology used in this study (Geuns, 2007; Williams, 2007; Brusick, 2008). The Panel has reviewed the cited publications and concurs with the challenges to the methodology utilized by Nunes et al., 2007a, thereby discounting the validity and importance of this study.

Regarding pharmacological effects in humans, JECFA called for additional studies in individuals that are neither hypertensive nor diabetic (WHO, 2006) to corroborate the observations that the effects of steviol glycosides to reduce blood glucose or blood pressure (or both) only occur in patients who already have these diseases. As reviewed by FSANZ, new data presented to JECFA demonstrated the lack of pharmacological effects of steviol glycosides at 11 mg/kg bw/day in normal individuals (Barriocanal et al., 2008). This level of intake is only slightly more than 4 mg/kg bw/day on the basis of steviol equivalents. The Panel has reviewed the clinical studies and concludes that there should be no effects on reducing blood pressure or glucose metabolism in humans at the doses of stevioside expected from use in food as a non-nutritive sweetener.

2. Safety Data on Rebaudioside A

In addition to the information on steviol glycosides discussed in the previous section, there are recent additional data specific to Reb A. Eleven papers describing the results of a comprehensive research program on Reb A have been published in July, 2008 (*Food and Chemical Toxicology*, Supplement 1, 2008). These studies formed the basis of the Cargill GRAS notification (GRN 253). Several other studies were sponsored by Merisant, and these were also then submitted with their GRAS notification (GRN 252). Previously, only a limited number of toxicology studies and clinical studies on Reb A were conducted and reported. As in the previous section on steviol glycosides, JECFA had concluded, even before these new studies were completed, that seven common steviol glycosides are safe for use as sweetener preparations when present in any combination at a combined purity of 95% or more.

The strategy of the new research on Reb A was to conduct a limited number of toxicology and clinical studies on the specific compound and to demonstrate in rats and in humans that it is pharmacokinetically similar to stevioside, which is the steviol glycoside on which most previous pharmacokinetic research was conducted. These studies were also done to help justify using the

JECFA-generated ADI (for steviol glycosides, expressed as steviol) without having to conduct a new chronic study in rats on Reb A. In addition, Merisant (see Table 10) as well as another group (Williams and Burdock, 2009) upgraded the mutagenicity and genotoxicity data available on Reb A with three assays that FDA reportedly believes are most predictive for carcinogenicity. The Cargill group also conducted two clinical studies to assure that Reb A does not have potentially adverse pharmacological effects on blood glucose and blood pressure as was previously demonstrated in some stevioside studies.

The most recent research on Reb A was summarized and combined with the body of knowledge on stevioside by Carakostas et al. (2008) and Brusick (2008). These reviews summarized the findings of the Cargill research program as follows:

- Steviol glycosides, Reb A, and stevioside are not genotoxic *in vitro*.
- Steviol glycosides, Reb A, and stevioside have not been shown to be genotoxic *in vivo* in well-conducted assays.
- A report indicating that stevioside produces DNA breakage *in vivo* appears to be flawed (Nunes et al., 2007a) 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 Reb 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 Reb A.
- The dietary levels expected from consumption of Reb A as a total replacement of sugar (Renwick, 2008) are less than the ADI and, therefore, there is no safety concern for consumers.

Regarding possible pharmacological effects of Reb A in decreasing blood pressure and blood glucose, there is a recently published clinical data study on Reb A (Maki et al., 2008a, b). The Panel has reviewed these clinical studies and concludes that there should be no effects on blood pressure and glucose metabolism in humans at the doses of Reb A expected from use in food as a non-nutritive sweetener.

3. Safety Considerations for Rebaudioside D & Rebaudioside F

The Panel belives that there is a strong presumption of safety for Reb D and Reb F because they are likely to be metabolized in the same manner as the seven glycosides recognized by JECFA as safe; i.e., Reb D and Reb F would not be digested or absorbed in the stomach or small intestine, like the related steviol glycosides, but would be converted to steviol in the large intestine by the action of indigenous microflora. The Panel agrees with the conclusions of the review by Renwick and Tarka (2008) that the glucosidase activity of the intestinal microflora is diverse and capable of completely degrading a wide variety of xenobiotic glycosides, and this position is

consistent with the view that sufficient experimental evidence exists on the degradation of stevioside, Reb A, and Reb C to conclude that all steviol glycosides in PureCircle's SG95 will be completely degraded to steviol. The Panel also supports the 2009 actions by Codex Alimentarius Commission in endorsing the actions to expand the steviol glycosides specifications to include Reb D and Reb F as will be considered in mid-2010 by JECFA.

C. Safety Concerns Expressed by Others

Over the last two decades, concerns have been voiced by FDA and selected others about the safety of sweeteners derived from stevia. The studies which showed possible adverse effects included several older toxcology studies using crude extracts of stevia or various *in vitro* mutagenicity studies employing high concentrations. Although no formal review was ever published by FDA, similar concerns were expressed by Dr. Ryan J. Huxtable, a professor of pharmacology at the University of Arizona. More recently, a review of Reb A toxicology (sponsored by the Center for Science in the Public Interest) was issued by two UCLA scientists who questioned the safety of Reb A. The Panel's evaluation of their respective concerns is summarized below.

1. Earlier FDA Concerns

FDA has previously expressed concerns about the safety of consumption of stevia and stevia extracts, based on selected publications that addressed questions about steviol glycoside metabolism, effects on glucose metabolism, effects on fertility, mutagenicity, and renal effects in rats.

As part of its broader safety evaluation of high purity steviol glycosides, the Panel has reviewed the older studies cited by FDA and has made the following general observations about the studies taken as a group. All of the cited studies were published in or before 1996, and there has been significant research reported since that time in most of the areas of concern. In addition, a number of these earlier studies are not particularly applicable to newly anticipated human intakes of high purity steviol glycosides because they used very high doses, impure extracts or because the route of exposure was not oral. Finally, many of the studies exist only as abstracts without critical details needed to determine the validity of the results.

The Panel has concluded that the concerns covered by these studies can be dismissed based on several factors: (1) the favorable results observed in a number of studies conducted on the key toxicity and clinical endpoints subsequent to the safety concerns cited by FDA; (2) the careful, ongoing review and final evaluation by JECFA and other international regulatory bodies (through 2008) that steviol glycosides can be considered to be safe at the recommended ADI of 4 mg/kg bw/day (steviol basis); (3) FDA's own independent GRAS evaluations of the more recently developed safety information as contained in the Merisant and Cargill GRAS notifications; and (4) the Panel's own conclusions regarding the toxicity and clinical endpoints previously considered.

2. Huxtable Concerns

Huxtable published a non-peer reviewed book chapter (Huxtable, 2002) in which he expressed misgivings about the safety of stevioside glycosides, most notably due to effects on glucose metabolism and the metabolic conversion to steviol. Though Huxtable acknowledged that animal and human testing to that date had not shown any problems, he discussed concern with potential problems arising from these mechanistic research studies.

The Panel notes that no adverse health findings have been demonstrated in the improved animal or human testing which has taken place since his review. Huxtable's concerns, therefore, appear to be driven by the "potential" effects of high-dose *in vitro* and *in vivo* testing, without the proper consideration given to the problems found when extrapolating these potential effects to the vastly lower dietary intakes by consumers. Consequently, the Panel concludes that Huxtable's expressed safety concerns can now be dismissed.

3. UCLA Researchers & the Center for Science in the Public Interest (CSPI) Concerns

In August 2008, two UCLA scientists published a criticism of the GRAS Assessment by Cargill (Kobylewski and Eckhert, 2008). These scientists were recruited for this task by CSPI, a public interest group which has historically commented on a number of issues related to food ingredient safety. The basic criticism of the UCLA evaluation can be summarized as follows:

- There is insufficient mutagenicity and genotoxicity data on Reb A as compared to stevioside to confirm that it is not likely to have carcinogenic properties.
- The metabolism of Reb A is too different from stevioside to rely on the rat chronic studies on stevioside to set an ADI for Reb A.
- The carcinogenic potential of both stevioside and Reb A should be examined in a second rodent species. They suggest that a mouse study is needed in accordance with FDA Redbook guidelines.

The Panel has reviewed the UCLA evaluation and the Reb A studies submitted by Merisant and Cargill. The Panel understands that it is easier to avoid making a safety decision while asking for more data. A good deal of scientific judgment is certainly needed to conclude that any data set is sufficient and that the ingredient is safe. The Panel notes that JECFA is composed of dozens of scientists who are expert in food ingredient safety and that JECFA expert panels have been establishing ADIs for food ingredients for over 40 years.

Both Merisant and Cargill took rigorous scientific approaches to demonstrate the safety of Reb A. The studies were as well conducted as those supporting stevioside safety. The safety profiles compiled by Merisant and Cargill differ somewhat, yet the results are complementary and are mutually reinforcing of Reb A safety. We also note that the UCLA evaluation did not review the Merisant studies.

The Cargill studies and evaluation provided significant insight into the pharmacokinetics of Reb A while demonstrating clinical safety of Reb 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. Merisant also added a subchronic toxicity 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 Reb A metabolism and excretion.

Therefore, the Panel disagrees with the conclusions of the UCLA/CSPI evaluation. The pharmacokinetic work shows that stevioside and Reb A are not absorbed *per se* but are converted to steviol in the GI tract. This occurs more slowly for Reb A due to the fact that it has a disaccharide side chain instead of stevioside's monosaccharide side chain. In both humans and rats, the steviol is rapidly converted to the glucuronide, and the glucuronide is not further metabolized but is efficiently excreted. In the rat, elimination occurs in the bile to the large intestine. In humans, elimination of the glucuronide occurs both in bile and urine. The UCLA evaluation indicates that this is a profound difference, which makes the rat a poor model for the extrapolation of an ADI to humans. The Panel disagrees with this concern. It is more important that the glucuronide is not expected to be toxic and is not further metabolized, but instead is efficiently eliminated. The route of elimination is different, but glucuronide elimination is a detoxification mechanism. The Panel believes that a mouse carcinogenicity study is not indicated because the rat is pharmacokinetically a model sufficiently similar to the human. Moreover, there are no data to indicate that the mouse is a better model than the rat. In addition, the Merisant mouse lymphoma and mouse micronucleus studies, as well as several mouse micronucleus studies conducted by others, do not indicate that the mouse is especially sensitive to Reb A, other steviol glycosides or steviol. These mouse studies give no indication that there are any undiscovered carcinogenic pathways that may operate in the mouse.

The Panel rejects the concerns contained in the UCLA evaluation and endorses instead the JECFA, FSANZ, Cargill and Merisant conclusion that sufficient data have been generated on steviol glycosides, partculary on test samples rich in stevioside and Reb A (as is the case with the High Purity Steviol Glycoside PureCircle products) to determine that these substances can be safely used at expected levels in food.

4. Panel's Overall Conclusions on These Concerns

Over the last several decades, there have been many *in vivo* and *in vitro* studies of a wide variety conducted on stevia extracts and steviol glycosides of varying purity. It is not surprising to find biological activity related to glucose metabolism since stevia products have been reported to be useful in the treatment of type 2 diabetes at doses higher than those projected to be used as a sweetener in foods. Moreover, there are three basic tenets of food ingredient safety methodology that need to be kept in mind. The first is that various effects observed *in vitro* can be refuted by well-conducted studies done *in vivo.* The second is that the higher concentrations used in *in vitro* studies and doses administered in *in vivo* studies (both animal and human) are very important to compare when assessing the significance of a potentially adverse *in vitro* finding. Oftentimes as noted in this evaluation, the *in vitro* doses can be orders of magnitude higher than the intakes that even high consumers of a food ingredient can be expected to ingest throughout their lifetimes. The third tenet is that primary evidence for human safety comes from studies designed to

investigate safety *per se*. As evidenced by the JECFA review and evaluation and the Panel's findings in Section VI.B., the Panel agrees that there is an ample margin of safety demonstrated by the doses used in the *in vivo* studies that meet the accepted design criteria for safety studies; furthermore, the studies of concern cited by FDA, the theoretical concerns raised by Huxtable, and the issues about Reb A described by UCLA/CSPI can be discounted based on sound use of safety and risk assessment principles.

It is the conclusion of the Panel that the safety concerns expressed above have been answered satisfactorily in the final JECFA opinion, the FSANZ review, the assessment by the Cargill and Merisant teams (as found acceptable by FDA) and in the Panel's own comprehensive, systematic review.

D. Common Knowledge Elements for a 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 studies published in peer-reviewed scientific journals. The majority of the studies reviewed in this safety assessment have been published in the scientific literature as reported in Section V. 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 reviewed only a limited number of studies conducted specifically on Reb A. The collection of supporting data on Reb A has recently been enhanced by the publication of the 2008 studies. The newest clinical studies that address JECFA's concern on unwanted pharmacological effects with steviol glycosides (Barriocanal et al., 2008) and with Reb A (Maki et al., 2008 a, b) are now published in the scientific literature.

To be sure, 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 studies have raised safety concerns, and the Panel has given careful attention to such concerns. The overriding evidence, particularly with high purity steviol glycosides, has certainly diminished the Panel's concerns based on better study designs, better study execution and new investigations that better reflect state-of-the-art toxicological and clinical 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 for its intended use. The 2008 JECFA final 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 participates in JECFA deliberations.

The JECFA conclusion has been reviewed and validated by other respected regulatory agencies, including FSANZ and the Switzerland Office of Public Health (FSANZ, 2008 and Switzerland Office of Public Health, 2008). A number of other 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 well-respected scientists that participated in the Cargill-sponsored research conducted on Reb A, most notably David Brusick, Nigel Brown, and Andrew Renwick. An assertion of "general recognition of safety" was also made by Carakostas et al. (2008). We also note that the favorable safety conclusions on McNeil Nutritionals GRAS notification on steviol glycosides with Reb A as a principal component were reported by the McNeil Expert Panel, along with FDA's concurrence with Blue California's GRAS designation for its high purity Reb A. Similar safety conclusions were noted with FDA "no objection" letters as issued to Sweet Green Fields and Wisdom Natural Brands. In summary, many diverse groups of scientists from all corners of the globe 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 objection" determinations by FDA for the GRAS notifications for Reb A as submitted by Merisant and Cargill and the more recent comparable findings by FDA with the additional GRAS notifications cited above.

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 a GRAS conclusion as outlined in this evaluation. The scientific community will likely conclude that concerns expressed by others over the years (Huxtable, 2002) are likely to be satisfied by newer data on more purified materials and the rigid specifications for purity published by JECFA for steviol glycosides, including Reb A. Most notably the concerns on effects of fertility with crude extract have been addressed with a number of reproductive effects in rats and hamsters with purer materials (Usami et al., 1995a; Yodyingyuad and Bunyawong, 1991; Mori et al.,1981). Several chronic rat studies with sufficiently high no effect levels (most notably the study by Toyoda et al. (1997) are available to set an acceptable ADI based on FDA tested review methodology. The recent clinical studies put to rest the concern that effects on blood pressure and blood glucose will be seen at the dietary levels expected (Barriocanal et al., 2008). There is also a wide consensus that the body of new research on Reb A is sufficient to establish safety, 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[13]

Part of JECFA's safety review included anticipated dietary patterns and the use concentrations expected in various foods in order to calculate the EDI (WHO, 2003, 2006). For US consumption, based on the very conservative assumption of 100% substitution of steviol glycosides for all sugars, an EDI of 5 mg/kg bw/day steviol was calculated by JECFA, however, JECFA concluded 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. In addition, Renwick (2008) concluded that if only Reb A were used as a total sugar replacement, the levels would be below the JECFA ADI. The Panel concurs that an EDI of 5 mg/kg bw/day steviol very conservatively represents a potential high user of steviol glycosides, if this non-nutritive sweetener becomes widely available in food. As part of this GRAS evaluation, the Panel agrees with the JECFA EDI for application to the PureCircle High Purity Steviol Glycosides product.

In consideration of the aggregate safety information available, the Panel concludes that JECFA has conducted an expert evaluation and agrees with JECFA's conclusion. The per person ADI for steviol glycosides of adequate purity as defined by JECFA specifications has been properly determined to be 4 mg/kg bw/day (as steviol equivalents). The Panel calculates that this is equivalent to 8 mg/kg bw/day for stevioside and 12 mg/kg bw/day for Reb A on a weight basis. The Panel agrees that adverse pharmacological effects are not likely to occur at this level and that even high consumers of steviol glycosides are not likely to exceed this level. The Panel further agrees that the presence of low levels of Reb D and Reb F does not present any safety concerns due to the likelihood that Reb D and Reb F will be metabolized *via* the same pathway as the other naturally occurring steviol glycosides. Therefore, the Panel agrees with the JECFA-derived ADI as a safe intake level of steviol glycosides and that food uses meeting the JECFA specifications, within the intake limits determined by JECFA, can be considered to be generally recognized as safe (GRAS) within the meaning of the Food, Drug, and Cosmetic Act.

[13] The detailed educational and professional credentials for the individuals serving on the Expert Panel can be found on the GRAS Associates website at www.gras-associates.com. Richard C. Kraska, Ph.D., DABT, Robert S. McQuate, Ph.D. and Wayne R. Bidlack, Ph.D. have extensive technical backgrounds in the evaluation of food ingredient safety. Drs. Kraska and McQuate worked on GRAS and food additive safety issues within FDA's GRAS Review Branch earlier in their careers and subsequently continued working within this area in the private sector. Dr. Bidlack is Professor of Food Science and former Dean of the College of Agriculture at California State Polytechnic University, Pomona. He has worked extensively in food safety matters over the years and frequently serves as a consultant to the food industry. Dr. Kraska served as Chair of the Panel.

High Purity Steviol Glycosides, with the composition as established for PureCircle's SG95, which are produced in accordance with FDA Good Manufacturing Practices requirements and which meet at a minimum the JECFA purity specifications for steviol glycosides with appropriate allowance for low levels of Reb D and Reb F as described herein are Generally Recognized As Safe when consumed as a non-nutritive sweetener within the JECFA ADI of 4 mg/kg bw/day on a steviol equivalent 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 should 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.

(b) (6)

Wayne R. Bidlack, Ph.D.

DATE: February 3, 2010

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APPENDIX A

JECFA Steviol Glycosides Specifications & Analytical Method

A-1 JECFA Steviol Glycosides Specifications & Analytical Method - 2007

A-2 JECFA Steviol Glycosides Specifications & Analytical Method – 2008

A-3 Application by PureCircle to JECFA

APPENDIX A-1

STEVIOL GLYCOSIDES

Prepared at the 68th JECFA (2007) and published in FAO JECFA Monographs 4 (2007), superseding tentative specifications prepared at the 63rd JECFA (2004), in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). A temporary ADI of 0-2 mg/kg bw (expressed as steviol) was established at the 63rd JECFA (2004).

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 methanol to release the glycosides and product is recrystallized with methanol. 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 C, dulcoside A, rubusoside, steviolbioside, and rebaudioside B 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-6-en-8-oic acid, β-D-glucopyranosyl ester

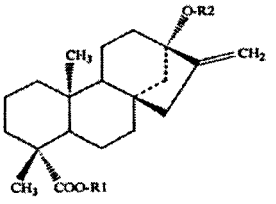
C.A.S. number

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 seven 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 C	β-Glc	β-Glc-α-Rha(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)
Rebaudioside B	H	β-Glc-β-Glc(2→1) | β-Glc(3→1)

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

Formula weight	Stevioside: 804.88 Rebaudioside A: 967.03
Assay	Not less than 95% of the total of the seven 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 and in ethanol
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 (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 high pressure liquid chromatography (Volume 4).

Standards
Stevioside, >99.0% purity and rebaudioside A, >97% purity (available from Wako pure Chemical Industries, Ltd. Japan).

Mobile phase
Mix HPLC-grade acetonitrile and water (80:20). Adjust the pH to 3.0 with phosphoric acid (85% reagent grade). Filter through 0.22 µm Millipore filter or equivalent.

Standard solutions
(a) Accurately weigh 50 mg of dried (105°, 2 h) stevioside standard into a 100-ml volumetric flask. Dissolve with mobile phase and dilute to volume with mobile phase.
(b) Repeat with previously dried rebaudioside A standard.

Sample solution
Accurately weigh 60-120 mg of dried (105°, 2 h) sample into a 100-ml volumetric flask. Dissolve with mobile phase and dilute to volume with the mobile phase.

Chromatography Conditions
Column: Supelcosil LC-NH2 or equivalent (length: 15-30 cm; inner diameter: 3.9-4.6 mm)
Mobile phase: A 80:20 mixture of acetonitrile and water (see above)
Flow rate: Adjust so that the retention time of rebaudioside A is about 21 min.
Injection volume: 5-10 µl
Detector: UV at 210 nm
Column temperature: 40°

Procedure
Equilibrate the instrument by pumping mobile phase through it until a drift-free baseline is obtained. Record the chromatograms of the sample solution and of the standard solutions.

The retention times relative to rebaudioside A (1.00) are:

0.45-0.48 for stevioside	0.12-0.16 for rubusoside
0.25-0.30 for dulcoside A	0.35-0.41 for steviolbioside
0.63-0.69 for rebaudioside C	0.73-0.79 for rebaudioside B

Measure the peak areas for the seven steviol glycosides from the sample solution (the minor components might not be detected). Measure the peak area for stevioside for the standard solution.

Calculate the percentage of each of the seven steviol glycosides, *X*, in the sample from the formula:

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

where

W_S is the amount (mg) of stevioside in the standard solution

W is the amount (mg) of sample in the sample solution

A_S is the peak area for stevioside from the standard solution

A_X is the peak area of *X* for the sample solution

f_X is the ratio of the formula weight of *X* to the formula weight of stevioside: 1.00 (stevioside), 0.98 (dulcoside A), 1.20 (rebaudioside A), 1.18 (rebaudioside C), 0.80 (rubusoside), 0.80 (steviolbioside), and 1.00 (rebaudioside B).

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

APPENDIX A-2

STEVIOL GLYCOSIDES

Prepared at the 69th JECFA (2008), published in FAO JECFA Monographs 5 (2008), superseding specifications prepared at the 68th JECFA (2007), 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 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 C, dulcoside A, rubusoside, steviolbioside, and rebaudioside B 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-6-en-8-oic acid, β-D-glucopyranosyl ester

C.A.S. number

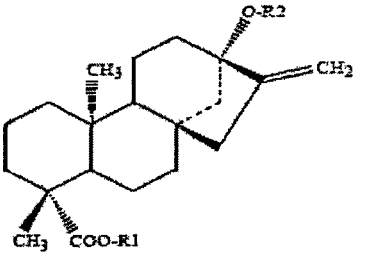
Stevioside: 57817-89-7
Rebaudioside A: 58543-16-1

Chemical formula

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

Structural Formula

The seven 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 C	β-Glc	β-Glc-α-Rha(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)
Rebaudioside B	H	β-Glc-β-Glc(2→1) | β-Glc(3→1)

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

Formula weight

Stevioside: 804.88
Rebaudioside A: 967.03

Assay

Not less than 95% of the total of the seven 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 Volume 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 Volume 4 (under "General Methods, Metallic Impurities").
METHOD OF ASSAY	Determine the percentages of the individual steviol glycosides by high pressure liquid chromatography (Volume 4).

Standards
Stevioside, >99.0% purity and rebaudioside A, >97% purity (available from Wako pure Chemical Industries, Ltd. Japan).

Mobile phase
Mix HPLC-grade acetonitrile and water (80:20). Adjust the pH to 3.0 with phosphoric acid (85% reagent grade). Filter through 0.22 µm Millipore filter or equivalent.

Standard solutions
(a) Accurately weigh 50 mg of dried (105°, 2 h) stevioside standard into a 100-ml volumetric flask. Dissolve with mobile phase and dilute to volume with mobile phase.
(b) Repeat with previously dried rebaudioside A standard.

Sample solution
Accurately weigh 60-120 mg of dried (105°, 2 h) sample into a 100-ml volumetric flask. Dissolve with mobile phase and dilute to volume with

the mobile phase.

Chromatography Conditions

Column: Supelcosil LC-NH_2 or equivalent (length: 15-30 cm; inner diameter: 3.9-4.6 mm)
Mobile phase: A 80:20 mixture of acetonitrile and water (see above)
Flow rate: Adjust so that the retention time of rebaudioside A is about 21 min.
Injection volume: 5-10 µl
Detector: UV at 210 nm
Column temperature: 40°

Procedure

Equilibrate the instrument by pumping mobile phase through it until a drift-free baseline is obtained. Record the chromatograms of the sample solution and of the standard solutions.

The retention times relative to rebaudioside A (1.00) are:

0.45-0.48 for stevioside 0.12-0.16 for rubusoside
0.25-0.30 for dulcoside A 0.35-0.41 for steviolbioside
0.63-0.69 for rebaudioside C 0.73-0.79 for rebaudioside B

Measure the peak areas for the seven steviol glycosides from the sample solution (the minor components might not be detected). Measure the peak area for stevioside for the standard solution.

Calculate the percentage of each of the seven steviol glycosides, X, in the sample from the formula:

$$\%X = [W_s/W] \times [f_x A_x/A_s] \times 100$$

where

W_s is the amount (mg) of stevioside in the standard solution
W is the amount (mg) of sample in the sample solution
A_s is the peak area for stevioside from the standard solution
A_x is the peak area of X for the sample solution
f_x is the ratio of the formula weight of X to the formula weight of stevioside: 1.00 (stevioside), 0.98 (dulcoside A), 1.20 (rebaudioside A), 1.18 (rebaudioside C), 0.80 (rubusoside), 0.80 (steviolbioside), and 1.00 (rebaudioside B).

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

Appendix A-3

Proposed Changes to JECFA Specifications for Steviol Glycosides

For consideration by the Joint FAO/WHO Expert Committee on Food Additives, 73rd Meeting, Geneva, June 2010

Submission date:

December 1, 2009

Submitted to:

Nutrition and Consumer Protection Division
Attention: Dr. Annika Wennberg
Food and Agriculture Organization of the United Nations
Via delle Terme di Caracalla
00153 Rome
Italy

Submitted by:

Calorie Control Council
1100 Johnson Ferry Road
Suite 300
Atlanta, GA 30342
404-252-3663
Contact: Lyn O'Brien Nabors

Introduction

Steviol glycosides are scheduled for re-evaluation at the 73rd meeting of JECFA in Geneva, June 2010. Steviol glycosides appears on the list of food additives scheduled for revision of specifications, in particular on the content of rebaudiosides D and F in materials of commerce and analytical methods for quantification.

These comments are submitted by the Calorie Control Council (the "Council"), an international association of companies that make low-calorie foods and beverages and the ingredients for those products. Companies that make and use steviol glycosides are among the Council's members.

Background

The specifications for steviol glycosides were most recently evaluated at the 69th meeting of the Committee and although the specifications were revised, the assay value of "not less than 95% of the total of the seven named steviol glycosides, on the dried basis" was maintained (FAO, 2008). The list of the seven named glycosides does not include rebaudiosides D and F. Rebaudioside D and F are both sweet tasting glycosides present in materials of commerce.

Data to Support Addition of Rebaudioside D and F to Existing Specifications

Analytical data demonstrate the presence of rebaudiosides D and F in commercial sweeteners produced using purification techniques previously described by JECFA (Wallin, 2004). Table 1 presents data from Pure Circle that were obtained using the JECFA analytical method (Pure Circle, 2009). The materials represented here are commercially available in Asia. The representative lots presented demonstrate that the materials will not comply with JECFA specifications without the inclusion of rebaudioside D and F in the list of the named glycosides. Historical observation of steviol glycoside sweetener composition has also demonstrated the presence of up to 1.0% of rebaudioside E. Consideration should also be given to the inclusion of rebaudioside E in the list of named glycosides.

Table 1 Analysis Results for Pure Circle Steviol Glycoside Sweetener (weight percent, dry basis)

	Lot#B0910068	Lot#B0910069	Historical Range
Rebaudioside A*	47.94	57.72	47.0-58.0**
Stevioside*	29.72	23.83	23.0-30.0
Rebaudioside C*	11.43	9.8	9.0-12.0
Dulcoside A*	1.3	0.6	0.-1.5
Rebaudioside E	ND***	ND	0-1.0
Rebaudioside B*	0.16	0.1	0-0.5
Rubusoside*	ND	ND	0-0.5
Steviolbioside*	ND	ND	0-0.5
Total SG content	90.55	92.05	

without Reb D/F			
Rebaudioside F	2.64	2.22	2.0-3.5
Rebaudioside D	1.84	1.4	0.3-2.0
Total SG content	95.03	95.67	

*Currently listed by JECFA as a "named" glycoside; **Expressed as percent; ***Not Detected.

Table 2 presents data from Cargill. These data represent 3 lots of Cargill material that is in commerce. The analytical method used to produce the data shown is slightly different from the method provided by the JECFA specification, but is an HPLC method (Cargill, 2009). The materials comply with JECFA specifications without including contribution by rebaudioside D and F because of the high rebaudioside A content. However, it is clear from these data that rebaudiosides D and F are consistently present in the sweetener.

Table 2 Analysis Results for Cargill Steviol Glycoside Sweetener (weight percent, dry basis)

	CV 09010002	ANCA 000118	CV 09010006
Rebaudioside A	97.09	97.43	98.14
Stevioside	0.28	0.09	0.25
Rebaudioside C	0.15	0.07	0.15
Dulcoside A	ND*	ND	ND
Rubusoside	0.05	ND	0.05
Steviolbioside	ND	ND	ND
Rebaudioside B	0.85	1.11	0.83
Rebaudioside F	**0.63**	**1.16**	**1.24**
Rebaudioside D	**0.16**	**0.14**	**0.13**
Total SG content	99.22	100.01	100.79

*Not detected.

Discussion

The data presented in this document demonstrate the presence of rebaudiosides D and F in materials in commerce. The addition of rebaudiosides D and F to the existing list of steviol glycosides will not preclude commercial products that do not contain these glycosides from meeting the JECFA specification. The addition of rebaudiosides D and F would expand the range of possible compositions of high purity materials that can meet the specification. Based on historical observations of rebaudioside E, consideration should be given to including this glycoside in the specification. Further, the Council notes that no synonyms are included in the current specification. The Council proposes that JECFA include the following commonly used synonyms in the specification: stevioside, rebaudioside, stevia extract, stevia leaf extract.

Thank you for your consideration.

APPENDIX B

Manufacturing Information & Specifications of Materials Used in Manufacture

B-1 Ethanol Specifications & Supplier Certification

B-2 Specifications for Resins & Filters Used in Manufacturing Process

B-3 HACCP Certification for Manufacturing Plant

Appendix B-1

广东中能酒精有限公司
Guangdongzhongneng Co., Ltd
原材料明细表
Raw Material Specification

检验货物名称：食用酒精 Name of material:Ethyl Alcohol(Ethanol)		
检验项目 Parmeter	检验标准 Acceptance criteria	检验方法 Test Method
含量 Assay,%	>94.9	FCCV
含水量 Water Content,%	<5.1	Karl Fischer Titration
酸性（乙酸） Acidity(as acetic acid),%	<0.003	FCCV
碱性 Alkalinity(as NH3),ppm	<3	FCCV
杂醇油 Fusel Oil	passes test	FCCV
酮，异丙醇 Ketones,Isopropyl Alcohol	passes test	FCCV
铅 Lead,ppm	<0.5	FCCV
甲醇 Methanol	passes test	FCCV
不挥发物 Non-volatile Residue,%	<0.003	FCCV
可溶性（在水中溶解度） Solubility in water	passes test	FCCV
硫酸实验 Substance Darkened by H2SO4	passes test	FCCV
高锰酸盐物质 Substance Reducing Permanganate	passes test	FCCV
甲苯 Toluene,ppb	<10	USPM 8260B

*Note : Food Chemicals Codex-5th ed.(2004),pp.157-158.

Prepared by:
Date:

Reviewed by:
Date:

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Guangdongzhongneng Co.,ltd
STATEMENT

Our company Guangdongzhongneng Co.,ltd statement which mentioned our Ethanol complies with FCC specifications.

Appendix B-2

SGS

Test Report No. TJTC 0861936/CHEM Date: December 1, 2009 Page 1 of 2

CANGZHOU YUANWEI CHEMICAL ENGINEERING CO., LTD
NO.18 GUOZHUANG INDUSTRIAL AREA, XIANXIAN, HEBEI 062254 P.R.CHINA

The following sample(s) was/were submitted and identified by/on behalf of the client as. MACROPOROUS ADSORPTION RESIN

SGS Ref No	: 1002788
Composition	: DIVINYL BENZENE
Model No.	: 03F
Supplier	: CANGZHOU YUANWEI CHEMICAL ENGINEERING CO., LTD

Sample Receiving Date	. November 18, 2009
Testing Period	: November 18 to December 01, 2009

Test Requested : 1. To determine the Lead content in the submitted sample.
2 *As per client's request, to determine Volatile Organic Compounds (VOC)- Benzene, Toluene, m- & p-Xylene, o-Xylene in the submitted sample.

Test method : 1. With reference to IEC 62321 Ed.1: 2008 for Lead content
Analysis was performed by ICP-AES.
2 *In-house method.
Analysis was performed by Headspace Gas Chromatograph / Mass Spectrometer (HS-GC-MS).

Test Results : Please refer to next pages

Signed for and on behalf of
SGS-CSTC Chemical Laboratory
(b) (6)

Lucid Qi
Lab Manager

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SGS

C1-6 to 9, 1/F, No.41, The 5th Avenue TEDA, Tianjin, China 300457 | (86-22) 25290999 | (86-22) 25290258 www.cn.sgs.com
中国·天津·经济技术开发区第五大街41号1层C区6-9号 邮编: 300457 | (86-22) 25290999 | (86-22) 25290262 e sgs.china@sgs.com

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SGS

Test Report **No. TJTC 0961936/CHEM** Date: December 1, 2009 Page 2 of 2

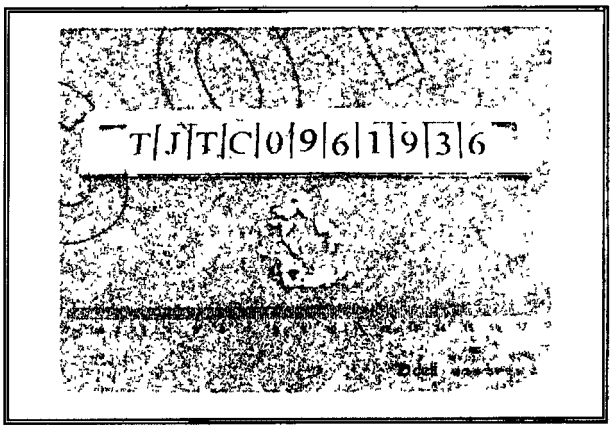
Test results by chemical method (Unit: mg/kg)

Test Item(s).	Method (refer to)	A	MDL
Lead (Pb)	(1)	n.d.	2
Benzene*	(2)	n.d.	5
Toluene*		11	5
m- & p-Xylene*		n.d.	10
o-Xylene*		n.d.	5

Test Part Description:
A. white grains

Note : (1) mg/kg = ppm
(2) n.d. = Not Detected
(3) MDL = Method Detection Limit
(4)*This test was subcontracted to SGS Shanghai chemical lab
(5)*Results reported on the submitted sample as received

Sample photo.



SGS authenticate the photo on original report only

*** End of Report ***

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SGS
TJCM 238924
C1-8 to 9.1/F,No.41,The 5th Avenue TEDA,Tianjin,China 300457 t (86-22) 25293899 f (86-22) 25295252 www.cn.sgs.com
中国·天津·经济技术开发区第五大街41号1层C区8-9号 邮编: 300457 t (86-22) 25293899 f (86-22) 25295252 e sgs.china@sgs.com
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Test Report **No. TJ505961/CHEM** Date: 11.30.2005 Page 1 of 3

ZIBO DONGDA CHEMICAL CO.,LTD.
NO. 21 XINCUN EAST ROAD,ZHANGDIAN DISTRICT,ZIBO CITY,SHANDONG PROVINCE,CHINA

The following sample(s) was/were submitted and identified on behalf of the applicant as:

Sample Name : ION EXCHANGE RESIN
SGS Ref No. : TJECO051102708
SGS Order No. : BJFDO051100358FD
Main substance : ION EXCHANGE RESIN

Sample Receiving Date : November 23, 2005
Testing Period : November 23 to November 29, 2005

Test Requested :
1) To determine the Cadmium content of the submitted sample.
2) To determine the Lead content of the submitted sample.
3) To determine Mercury content of the submitted sample.
4) To determine Hexavalent Chromium content of the submitted sample.
5) To determine the PBBs(Polybrominated biphenyls) PBBEs(PBDEs) (Polybrominated biphenyl ethers) content of the submitted sample.

Test method :
1) With reference to BS EN 1122:2001, Method B or other acid digestion. Analysis was performed by Inductively Coupled Argon Plasma – Atomic Emission Spectrometry (ICP-AES) or Atomic Absorption Spectrometry.
2) With reference to US EPA Method 3050B or other acid digestion. Analysis was performed by Inductively Coupled Argon Plasma – Atomic Emission Spectrometry (ICP-AES) or Atomic Absorption Spectrometry.
3) With reference to US EPA 3052 or other acid digestion. Analysis was performed byInductively Coupled Argon Plasma – Atomic Emission Spectrometry (ICP-AES) or US EPA7473 Analysis was performed by Hg Analyzer
4) With reference to US EPA3060A and US EPA7196A Analysis was performed by UV-VIS Spectrometric method.
5) With reference to USEPA 8081A/8270C/3540C/3550B Analysis was performed by GC/MS

Test Results : Please refer to next page

Signed for and on behalf of
SGS-CSTC Chemical Laboratory

(b) (6)
Scott Wang
Supervisor

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TJCH013669

SGS-CSTC Standards Technical Services Co. Ltd. Tianjin Branch Chemical Laboratory
01-B to 3, 1F, No 41, The 5th Avenue, TEDA, Tianjin, China

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Test Report **No. TJ505961/CHEM** Date: 11.30.2005 Page 2 of 3

Test Results

No.	Item	Unit	MDL	A
1	Cadmium (Cd)	ppm	2	N.D.
2	Lead (Pb)	ppm	2	2
3	Mercury(Hg)	ppm	2	N.D.
4	Hexavalent Chromium(Cr VI)	ppm	2	N.D.
5	PBBs(Polybrominated biphenyls)	---	---	---
	PBBs(Bromobiphenyl)	ppm	5	N.D.
	PBBs(Dibromobiphenyl)	ppm	5	N.D.
	PBBs(Tribromobiphenyl)	ppm	5	N.D.
	PBBs(Tetrabromobiphenyl)	ppm	5	N.D.
	PBBs(Pentabromobiphenyl)	ppm	5	N.D.
	PBBs(Hexabromobiphenyl)	ppm	5	N.D.
	PBBs(Heptabromobiphenyl)	ppm	5	N.D.
	PBBs(Octabromobiphenyl)	ppm	5	N.D.
	PBBs(Nonabromobiphenyl)	ppm	5	N.D.
	PBBs(Decabromobiphenyl)	ppm	5	N.D.
	PBBEs(PBDEs)(Polybrominated biphenyl ethers)	---	---	---
	PBBEs(PBDEs)(Monobromobiphenyl ethers)	ppm	5	N.D.
	PBBEs(PBDEs)(Dibromobiphenyl ethers)	ppm	5	N.D.
	PBBEs(PBDEs)(Tribromobiphenyl ethers)	ppm	5	N.D.
	PBBEs(PBDEs)(Tetrabromobiphenyl ethers)	ppm	5	N.D.
	PBBEs(PBDEs)(Pentabromobiphenyl ethers)	ppm	5	N.D.
	PBBEs(PBDEs)(Hexabromobiphenyl ethers)	ppm	5	N.D.
	PBBEs(PBDEs)(Heptabromobiphenyl ethers)	ppm	5	N.D.
	PBBEs(PBDEs)(Octabromobiphenyl ethers)	ppm	5	N.D.
	PBBEs(PBDEs)(Nonabromobiphenyl ethers)	ppm	5	N.D.
	PBBEs(PBDEs)(Decabromobiphenyl ethers)	ppm	5	N.D.

Sample Description:
A. yellow grains
Note : ppm=mg/kg
MDL= Method Detection Limit
N.D. = Not detected.(<MDL)
Photo appendix is as follow

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TJC505961

OCT-10-2009 08:01 From:SUQING 8651086338655 P.1

陈家洲号 <1>

Certificate Of Fda Registration

200908-201007

This is certified that:

At The Address Stated Below Has Completed U.S. FOOD And DRUG ADMINISTRATION Food Facility Registration And Test Through MANTONG.

Jiangsu Suqing Water Treatment Engineering Group Co., Ltd.

Add: No.89 Changqing Road, Hetang, Jiangyin, Jiangsu, 214419, CHINA

Product Description:	Strong Acid Cation Exchange Resin 001x8 Strong Acid Cation Exchange Resin 001x16 Strong Acid Cation Exchange Resin D001 Weak Acid Cation Exchange Resin D113-III Weak Base Anion Exchange Resin D301-G Anion Exchange Resin SQD-913 Anion Exchange Resin SQ-338
Investigation Requested	FDA Food Contact Article test in accordance with 21 CFR 173.25
Test Performing Date:	2009-06-26 to 2009-07-03 &2009-08-03 to 2009-08-07
Test Laboratory	The Hong Kong Standards and Testing Centre Ltd
Registration Number	16469345068

(b) (6)

MTG IDC
REGISTER CENTER

Executive Director

Date 08 20 2009

Appendix B-3

HACCP CERTIFICATE

Certificate No.: CQC08H10068R0M/3600

THIS IS TO CERTIFY THAT

Processing of Harvested Stevia Leaves for Steviol Glycosides (Steviosides)

in **Purecircle (Jiangxi) Co.,Ltd.**

Yangtang Industry Zone,Maodian Town,Ganxian,Jiangxi Province,China.

is in full compliance and continually operating with
CAC/RCP1-1969, Rev.4(2003) Guidelines for the Application of
the Hazard Analysis Critical Control Points System

Certified since: Feb. 25, 2008 Valid from: Nov. 26, 2008 Valid until: Feb. 24, 2011

After a surveillance cycle, the certificate is valid only when used together with an Acceptance Notice of Surveillance Audit issued by CQC
Please access www.cqc.com cn for checking validity of the certificate

(b) (6)

Signed by: Wang Kejiao

CHINA QUALITY CERTIFICATION CENTRE

Section 9, No.188, Nanshuan(the South Fourth Ring Road) Xilu(West Road), Beijing 100070,China
http://www.cqc.com.cn

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APPENDIX C

Specifications & Specific Analyses of Multiple Production Lots for PureCircle High Purity Steviol Glycosides Product

C-1 Specifications for PureCircle SG95

C-2 Certificates of Analysis for 5 Production Batches of SG95

C-3 Analytical Report on Determination of Steviol Glycosides Content

C-4 Certificates of Analysis for Pesticide Residues

Appendix C-1

NAME OF PRODUCT: **Steviol Glycosides 95 (SG95)**

PARAMETER	METHOD	SPECIFICATION
Appearance	Sensory Evaluation	Off-white to white powder
Odor	" "	Slight, characteristic
Taste	" "	Characteristic, sweet, slightly bitter
Sweetness, fold sweetness to sucrose	" "	>230
Total Steviol Glycosides, % (anhydrous basis)	FCC VI	≥95.0
- Stevioside, %	" "	15.0 – 30.0
- Rebaudioside A, %	" "	50.0 – 65.0
- Rebaudioside B, %	" "	≤2.0
- Rebaudioside C, %	" "	≤15.0
- Rebaudioside D, %	" "	≤2.5
- Rebaudioside F, %	" "	≤3.0
- Rubusoside, %	" "	≤2.0
- Steviolbioside, %	" "	≤2.0
- Dulcoside A, %	" "	≤2.0
Loss on Drying, %	JECFA Vol.4	<6.0
Solubility, %	" "	Freely soluble
pH, 1% in water	" "	4.5–7.0
Residual Ethanol, %	USP <467>	<0.5
Residual Methanol, %	" "	<0.02
Ash, %	JECFA Vol.4	<1.0
Lead (as Pb), ppm	ICP-MS AOAC	<1.0
Arsenic (as As), ppm	" "	<1.0
Cadmium (as Cd), ppm	" "	<1.0
Optical Rotation, $[\alpha]^{25}_{589}$	USP <781>	-30.0° to -38.0°
Total Plate Count, CFU/g	AOAC 990.12	<1000
Yeast and Mold, CFU/g	AOAC 997.02	<200
Total Coliforms, MPN/g	Chapter 4, FDA	Not Detected
E. coli count, MPN/g	" "	Not Detected
Salmonella sp.	Chapter 36, AOAC, 2001	Negative/25g

Prepared by: _____

Lai Chee Seng
Senior QA & Food Technologist
Date: 29/01/2010

Reviewed by: _____

Avetik A. Markosyan, MD, PhD
Director R&D / QA&QC
Date: 29/01/2010

Appendix C-2

NAME OF PRODUCT: **Steviol Glycosides (SG95)**
BATCH #: **A0912266**
MANUFACTURING DATE: **13th DECEMBER 2009**
EXPIRY DATE: **2 Years from Manufacturing Date**
STORAGE CONDITIONS: **Store in cool, dry and ventilated area.**

PARAMETER	METHODS	SPECIFICATION	RESULT
Appearance	Sensory Evaluation	Off-white to white powder	Approved
Odor	" "	Slight, characteristic	Approved
Taste	" "	Characteristic, sweet, slightly bitter	Approved
Sweetness, fold sweetness to sucrose	" "	>230	250
Total Steviol Glycosides, % (anhydrous basis)	FCC VI	≥95 0	96.50
- Stevioside, %	" "	15 0 - 30 0	18 31
- Rebaudioside A, %	" "	50 0 - 65 0	64.56
- Rebaudioside B, %	" "	≤2 0	1 01
- Rebaudioside C, %	" "	≤15.0	9.85
- Rebaudioside D, %	" "	≤2 5	Not Detected
- Rebaudioside F, %	" "	≤3.0	1.93
- Rubusoside, %	" "	≤2 0	0 21
- Steviolbioside, %	" "	≤2 0	0 23
- Dulcoside A, %	" "	≤2.0	0 39
Loss on Drying, %	JECFA Vol.4	<6 0	1.42
Solubility, %	" "	Freely Soluble	Approved
pH, 1% in water	" "	4.5 - 7 0	6 2
Residual Ethanol, %	USP<467>	<0 5	0.050
Residual Methanol, %	" "	<0 02	0.005
Ash, %	JECFA Vol.4	<1.0	<0 1
Lead (as Pb), ppm	ICP-MS AOAC	<1 0	0 041
Arsenic (as As), ppm	" "	<1.0	0.013
Cadmium (as Cd), ppm	" "	<1 0	<0.005
Optical Rotation, $[\alpha]^{25}_{589}$	USP<781>	-30 0° to -38 0°	-32 80°
Total Plate Count, CFU/g	AOAC 990 12	<1000	Not Detected
Yeast and Mold, CFU/g	AOAC 997.02	<200	Not Detected
Total Coliforms, MPN/g	Chapter 4, FDA	Not Detected	Not Detected
E.coli count, MPN/g	" "	Not Detected	Not Detected
Salmonella sp.	Chapter 36, AOAC, 2001	Negative/25g	Not Detected

Prepared by: _____
Choung Chia Mei
Quality Assurance Department
Date: 28th January 2010

Reviewed by: _____
Mr. Lai Chee Seng
Quality Assurance Department
Date: 28th January 2010

NAME OF PRODUCT: **Steviol Glycosides (SG95)**
BATCH #: **A0912267**
MANUFACTURING DATE: **14th DECEMBER 2009**
EXPIRY DATE: **2 Years from Manufacturing Date**
STORAGE CONDITIONS: **Store in cool, dry and ventilated area.**

PARAMETER	METHODS	SPECIFICATION	RESULT
Appearance	Sensory Evaluation	Off-white to white powder	Approved
Odor	" "	Slight, characteristic	Approved
Taste	" "	Characteristic, sweet, slightly bitter	Approved
Sweetness, fold sweetness to sucrose	" "	>230	250
Total Steviol Glycosides, % (anhydrous basis)	FCC VI	≥95 0	96 11
- Stevioside, %	" "	15 0 - 30 0	19 37
- Rebaudioside A, %	" "	50.0 - 65.0	61 25
- Rebaudioside B, %	" "	≤2 0	0 99
- Rebaudioside C, %	" "	≤15.0	11.13
- Rebaudioside D, %	" "	≤2.5	Not Detected
- Rebaudioside F, %	" "	≤3 0	2.21
- Rubusoside, %	" "	≤2 0	0 35
- Steviolbioside, %	" "	≤2.0	0 35
- Dulcoside A, %	" "	≤2 0	0.45
Loss on Drying, %	JECFA Vol 4	<6.0	3.56
Solubility, %	" "	Freely Soluble	Approved
pH, 1% in water	" "	4.5 - 7.0	6 2
Residual Ethanol, %	USP<467>	<0 5	0 180
Residual Methanol, %	" "	<0.02	0.005
Ash, %	JECFA Vol 4	<1 0	0 2
Lead (as Pb), ppm	ICP-MS AOAC	<1.0	0.026
Arsenic (as As), ppm	" "	<1.0	0 005
Cadmium (as Cd), ppm	" "	<1 0	<0.005
Optical Rotation, $[\alpha]^{25}_{589}$	USP<781>	-30 0° to -38.0°	-34 20°
Total Plate Count, CFU/g	AOAC 990.12	<1000	Not Detected
Yeast and Mold, CFU/g	AOAC 997.02	<200	Not Detected
Total Coliforms, MPN/g	Chapter 4, FDA	Not Detected	Not Detected
E.coli count, MPN/g	" "	Not Detected	Not Detected
Salmonella sp	Chapter 36, AOAC, 2001	Negative/25g	Not Detected

Prepared by: _____
Choung Chia Mei
Quality Assurance Department
Date: 28th January 2010

Reviewed by: _____
Mr. Lai Chee Seng
Quality Assurance Department
Date: 28th January 2010

NAME OF PRODUCT: **Steviol Glycosides (SG95)**
BATCH #: **A0912268**
MANUFACTURING DATE: **15th DECEMBER 2009**
EXPIRY DATE: **2 Years from Manufacturing Date**
STORAGE CONDITIONS: **Store in cool, dry and ventilated area.**

PARAMETER	METHODS	SPECIFICATION	RESULT
Appearance	Sensory Evaluation	Off-white to white powder	Approved
Odor	" "	Slight, characteristic	Approved
Taste	" "	Characteristic, sweet, slightly bitter	Approved
Sweetness, fold sweetness to sucrose	" "	>230	250
Total Steviol Glycosides, % (anhydrous basis)	FCC VI	≥95.0	96.86
- Stevioside, %	" "	15 0 - 30 0	20.15
- Rebaudioside A, %	" "	50 0 - 65 0	61.58
- Rebaudioside B, %	" "	≤2.0	1.04
- Rebaudioside C, %	" "	≤15.0	10 39
- Rebaudioside D, %	" "	≤2 5	Not Detected
- Rebaudioside F, %	" "	≤3 0	2.11
- Rubusoside, %	" "	≤2 0	0.76
- Steviolbioside, %	" "	≤2.0	0.30
- Dulcoside A, %	" "	≤2 0	0 50
Loss on Drying, %	JECFA Vol.4	<6.0	1.93
Solubility, %	" "	Freely Soluble	Approved
pH, 1% in water	" "	4 5 - 7 0	6 2
Residual Ethanol, %	USP<467>	<0 5	0.300
Residual Methanol, %	" "	<0 02	0.005
Ash, %	JECFA Vol 4	<1.0	0 2
Lead (as Pb), ppm	ICP-MS AOAC	<1.0	0.026
Arsenic (as As), ppm	" "	<1 0	<0 005
Cadmium (as Cd), ppm	" "	<1.0	<0.005
Optical Rotation, $[\alpha]^{25}_{589}$	USP<781>	-30.0° to -38.0°	-34.20°
Total Plate Count, CFU/g	AOAC 990.12	<1000	Not Detected
Yeast and Mold, CFU/g	AOAC 997.02	<200	Not Detected
Total Coliforms, MPN/g	Chapter 4, FDA	Not Detected	Not Detected
E.coli count, MPN/g	" "	Not Detected	Not Detected
Salmonella sp.	Chapter 36, AOAC, 2001	Negative/25g	Not Detected

Prepared by: _____
Choung Chia Mei
Quality Assurance Department
Date: 28th January 2010

Reviewed by: _____
Mr. Lai Chee Seng
Quality Assurance Department
Date: 28th January 2010

NAME OF PRODUCT: **Steviol Glycosides (SG95)**
BATCH #: **A0912269**
MANUFACTURING DATE: **16th DECEMBER 2009**
EXPIRY DATE: **2 Years from Manufacturing Date**
STORAGE CONDITIONS: **Store in cool, dry and ventilated area.**

PARAMETER	METHODS	SPECIFICATION	RESULT
Appearance	Sensory Evaluation	Off-white to white powder	Approved
Odor	" "	Slight, characteristic	Approved
Taste	" "	Characteristic, sweet, slightly bitter	Approved
Sweetness, fold sweetness to sucrose	" "	>230	250
Total Steviol Glycosides, % (anhydrous basis)	FCC VI	≥95.0	96 41
- Stevioside, %	" "	15 0 - 30 0	19 71
- Rebaudioside A, %	" "	50.0 - 65.0	61 45
- Rebaudioside B, %	" "	≤2 0	1 05
- Rebaudioside C, %	" "	≤15 0	10.66
- Rebaudioside D, %	" "	≤2 5	Not Detected
- Rebaudioside F, %	" "	≤3 0	2 17
- Rubusoside, %	" "	≤2.0	0 57
- Steviolbioside, %	" "	≤2.0	0.30
- Dulcoside A, %	" "	≤2 0	0.47
Loss on Drying, %	JECFA Vol 4	<6 0	2 92
Solubility, %	" "	Freely Soluble	Approved
pH, 1% in water	" "	4.5 - 7 0	6.1
Residual Ethanol, %	USP<467>	<0.5	0 270
Residual Methanol, %	" "	<0 02	0 004
Ash, %	JECFA Vol 4	<1.0	<0.1
Lead (as Pb), ppm	ICP-MS AOAC	<1.0	0 032
Arsenic (as As), ppm	" "	<1 0	0 005
Cadmium (as Cd), ppm	" "	<1 0	<0.005
Optical Rotation, $[\alpha]^{25}_{589}$	USP<781>	-30.0° to -38.0°	-34.50°
Total Plate Count, CFU/g	AOAC 990.12	<1000	Not Detected
Yeast and Mold, CFU/g	AOAC 997.02	<200	Not Detected
Total Coliforms, MPN/g	Chapter 4, FDA	Not Detected	Not Detected
E.coli count, MPN/g	" "	Not Detected	Not Detected
Salmonella sp	Chapter 36, AOAC, 2001	Negative/25g	Not Detected

Prepared by: _____
Choung Chia Mei
Quality Assurance Department
Date: 28th January 2010

Reviewed by: _____
Mr. Lai Chee Seng
Quality Assurance Department
Date: 28th January 2010

NAME OF PRODUCT: **Steviol Glycosides (SG95)**
BATCH #: **A0912270**
MANUFACTURING DATE: **17th DECEMBER 2009**
EXPIRY DATE: **2 Years from Manufacturing Date**
STORAGE CONDITIONS: **Store in cool, dry and ventilated area.**

PARAMETER	METHODS	SPECIFICATION	RESULT
Appearance	Sensory Evaluation	Off-white to white powder	Approved
Odor	" "	Slight, characteristic	Approved
Taste	" "	Characteristic, sweet, slightly bitter	Approved
Sweetness, fold sweetness to sucrose	" "	>230	250
Total Steviol Glycosides, % (anhydrous basis)	FCC VI	≥95 0	95 86
- Stevioside, %	" "	15.0 - 30.0	18 71
- Rebaudioside A, %	" "	50.0 - 65 0	62 73
- Rebaudioside B, %	" "	≤2 0	1.01
- Rebaudioside C, %	" "	≤15.0	10 35
- Rebaudioside D, %	" "	≤2 5	Not Detected
- Rebaudioside F, %	" "	≤3.0	2 07
- Rubusoside, %	" "	≤2 0	0 27
- Steviolbioside, %	" "	≤2 0	0 28
- Dulcoside A, %	" "	≤2 0	0 41
Loss on Drying, %	JECFA Vol 4	<6 0	3.25
Solubility, %	" "	Freely Soluble	Approved
pH, 1% in water	" "	4 5 - 7 0	6 0
Residual Ethanol, %	USP<467>	<0 5	0 130
Residual Methanol, %	" "	<0.02	0 004
Ash, %	JECFA Vol 4	<1 0	<0.1
Lead (as Pb), ppm	ICP-MS AOAC	<1 0	0 029
Arsenic (as As), ppm	" "	<1 0	<0.005
Cadmium (as Cd), ppm	" "	<1.0	<0 005
Optical Rotation, $[\alpha]^{25}_{589}$	USP<781>	-30.0° to -38.0°	-33 70°
Total Plate Count, CFU/g	AOAC 990.12	<1000	Not Detected
Yeast and Mold, CFU/g	AOAC 997.02	<200	Not Detected
Total Coliforms, MPN/g	Chapter 4, FDA	Not Detected	Not Detected
E.coli count, MPN/g	" "	Not Detected	Not Detected
Salmonella sp.	Chapter 36, AOAC, 2001	Negative/25g	Not Detected

Prepared by: _____
Choung Chia Mei
Quality Assurance Department
Date: 28th January 2010

Reviewed by: _____
Mr. Lai Chee Seng
Quality Assurance Department
Date: 28th January 2010

Appendix C-3

See following 111 pages

Not included in sequence with main document page numbers.

HPLC Assay of Rebaudioside A and related Steviol Glycosides in 5 commercial lots of Steviol Glycosides

Prepared by: (b) (6) 01/ 09 / 2010

Avetik A. Markosyan (Principal Scientist) — Date

Approved by: (b) (6) 01/ 09/ 2010

Varuzhan H. Abelyan (Vice President; R&D and Process Innovation) — Date

Objective

To determine rebaudioside A and related steviol glycosides content in five commercial lots of *Steviol Glycosides* produced by *PureCircle.*

Samples

Five samples representing commercial lots of *Steviol Glycosides* labeled as "A0912266", "A0912267", "A0912268", "A0912269", and "A0912270".

Standards

1. USP Rebaudioside A Standard; Lot#F0I077 (USP Rockville, MD USA);
2. USP Stevioside Standard; Lot#F0I080 (USP Rockville, MD USA);
3. Rebaudioside B Standard; Lot#18227-101 (Chromadex Inc. Irvine, CA USA);
4. Rebaudioside C Standard; Lot#00018228-3962 (Chromadex Inc. Irvine, CA USA);
5. Rebaudioside D Standard; Lot#00018229-4104 (Chromadex Inc. Irvine, CA USA);
6. Steviolbioside Standard; Lot#00019349-1821 (Chromadex Inc. Irvine, CA USA);
7. Dulcoside A Standard; Lot#04949-002 (Chromadex Inc. Irvine, CA USA);
8. Rubusoside Standard; Lot#270308 (PureCircle Sdn. Bhd., Malaysia).

Solvents and Reagents

- Acetonitrile, HPLC grade (JT Baker, USA);

- Water, HPLC grade (JT Baker, USA);
- Ammonium acetate, reagent grade (Merck, Germany);
- Acetic acid, reagent grade (Merck, Germany).

Apparatus

1. Agilent 1200 HPLC system equipped with binary pump (G1312B), auto sampler (G1367D), thermostatted column compartment (G1316B) and DAD detector (G1315C), (Agilent Technologies, USA);
2. Analytical column, Zorbax NH_2, 4.6x250mm, 5µm (PN 880952-708), (Agilent Technologies, USA);
3. Analytical balance, XS205, (Mettler Toledo, USA);
4. Karl Fischer coulometer, Mettler Toledo DL-39, (Mettler Toledo, USA);
5. Volumetric (class *A*) and Laboratory glassware.

Solution Preparation

Acetate buffer, *Mobile Phase*, and *Diluent* were prepared as per *FCC 6* monograph method (see appendix A).

Standard solutions

Rebaudioside A: approx. 1000, 2000, 3000, and 4000 mg/L USP Rebaudioside A solutions in *Diluent*.

Stevioside: approx. 2.5, 10, 100, 500, 1000, and 2000 mg/L USP Stevioside solutions in *Diluent*.

Steviol Glycosides retention time marker solution (M8): approx. 100 mg/L (each) of Rubusoside, Dulcoside A, Stevioside, Rebaudioside C, Rebaudioside A, Rebaudioside D, Steviolbioside, and Rebaudioside B solution in *Diluent.*

Sample solution

Sample solution was prepared at approx. 5000 mg/L in *Diluent.* Water content was determined by Karl Fischer method.

Chromatographic system

- Chromatograph – *Agilent 1200 series* HPLC equipped with binary pump, autosampler, thermostatted column compartment and DAD detector;
- Column – *Zorbax* NH_2 4.6 x 250 mm; 5 µm at 30°C;
- Flow rate – 2.0 mL/min;
- Injection volume – 12 µL;
- Detector – UV 210 nm.

System Suitability Requirements

System suitability was checked as per *FCC 6* monograph method.

- **Detector response:** Peak to noise ratio for 2.5 mg/L Stevioside Standard solution – NLT 3, (see appendix B);
- **Relative Standard Deviation:** Rebaudioside A peak area and retention time – NMT 2.0%, (see appendix B);
- **Column efficiency:** NLT 5000 theoretical plates count, N, using Rebaudioside B peak from Sample solution, (see appendix B);

- **Retention Time:** Rebaudioside A retention time less than 8.0 min, (Not applicable, as longer column is used);
- **Tailing Factor:** NMT 2.0 for Rebaudioside B peak from Sample solution, (see appendix B).

Analysis

Analysis and calculations were performed as per *FCC 6* monograph method for Rebaudioside A and Related Steviol Glycosides (see appendix A).

The assay results are summarized in Table 1.

Table 1

Rebaudioside A and related steviol glycosides assay

Lot#	Steviol Glycosides, % dry basis*									
	Rub	*DulA*	*Stev*	*RebC*	*RebF*	*RebA*	*RebD*	*Sbio*	*RebB*	*TSG*
A0915266	0.21	0.39	18.31	9.85	1.93	64.56	ND	0.23	1.01	96.50
A0915267	0.35	0.45	19.37	11.13	2.21	61.25	ND	0.35	0.99	96.11
A0915268	0.76	0.50	20.15	10.39	2.11	61.58	ND	0.30	1.04	96.86
A0915269	0.57	0.47	19.71	10.66	2.17	61.45	ND	0.30	1.05	96.41
A0915270	0.27	0.41	18.71	10.35	2.07	62.73	ND	0.28	1.01	95.86

* Average of 2 duplicates is reported.
Rub – Rubusoside
DulA – Dulcoside A
RebA, B, C, D, F – Rebaudioside A, B, C, D, F
Sbio – Steviolbioside
TSG – Total Steviol Glycosides
ND – Not Detected

Reference

1. Rebaudioside A monograph, *Food Chemicals Codex (FCC) 6, Second Supplement*, 2009, p. 1438-42.

APPENDIX A

Rebaudioside A and related steviol glycosides assay, FCC 6, Second Supplement, 2009, p.1438-42.

ASSAY

PROCEDURE

Acetate buffer: Dissolve 0.125 g ammonium acetate in 900 mL of water, adjust pH to 4.3 with glacial acetic acid solution and dilute to 1 L. [NOTE: It may be necessary to adjust the ratio of ammonium acetate to acetic acid. Changing the pH adjusts the retention time of rebaudioside A and related glycosides. Decreasing the pH of the buffer will decrease the retention time of rebaudioside A.]

Mobile phase: 20% (v/v) *Acetate buffer* in acetonitrile [NOTE: Equilibrate the column by flushing with 75 mL of isopropyl alcohol and then 50 mL of acetonitrile. After that, flush the column with *Mobile phase* until the retention time of rebaudioside A is less than 8 min (may take up to one day of flushing).]

Diluent: 25% (v/v) *Acetate buffer* in acetonitrile [NOTE: Allow *Diluent* to come to room temperature before use.]

Rebaudioside A standard solutions: 4500, 4750, 5250, and 5500 mg/L USP Rebaudioside A RS in *Diluent*

Stevioside standard stock solution: 250 mg/L USP Stevioside RS in *Diluent*

Stevioside standard solutions: 2.5, 5.0, 10, 25, and 50 mg/L USP Stevioside RS in *Diluent:* from *Stevioside standard stock solution*

Sample solution: 5000 mg/L in *Diluent*

Chromatographic system, Appendix IIA

Mode: High-performance liquid chromatography

Detector: UV(210nm)

Column: 15-cm x 4.6-mm, packed with a propylamino silane phase bonded to silica gel (5-µm particle diameter)[1]

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection size: 12 µL

System suitability

Samples: 5250 mg/L *Rebaudioside A standard solution,* 2.5 mg/L *Stevioside standard solution, Sample solution*

Suitability requirements

Detector response: Peak to noise ratio (peak height /baseline noise) is NLT 3 for the stevioside peak from the 2.5 mg/L *Stevioside standard solution,* where peak height is expressed in mAU, and baseline noise is the maximum deflection of the baseline (mAU) in a blank at the retention time of stevioside over the same baseline peak width in min.

Relative standard deviation: NMT 2.0% for rebaudioside A peak area and retention time from the 5250 mg/L *Rebaudioside A standard solution*

Column efficiency: NLT 5000 theoretical plates count, *N,* using the rebaudioside B peak from the *Sample solution*

Retention time: The retention time for the rebaudioside A peak from the 5250 mg/L *Rebaudioside A standard solution* is less than 8.0 min.

Tailing factor: NMT 2.0 for the rebaudioside B peak from the *Sample solution*

---

[1] *Zorbax* NH_2 (Agilent Technologies), or equivalent.

Rebaudioside A and related steviol glycosides assay, FCC 6, Second Supplement, 2009, p.1438-42.

Analysis: Separately inject equal volumes of the *Rebaudioside A standard solutions, Stevioside standard solutions,* and *Sample solution* into the chromatograph, and measure the responses for the major peaks on the resulting chromatograms. [NOTE: The approximate retention times for rebaudioside A and its related steviol glycosides are listed below in *Chromatographic Profile Table 1.* If the retention time for rebaudioside A is below 6 min, adjust the ratio of ammonium acetate to acetic acid.]

Chromatographic Profile Table 1

Compound	Approx. Retention Time (min)	Molecular Weight (g/mol)
Rubusoside	3.0	642.73
Dulcoside A	3.9	788.87
Stevioside	4.5	804.88
Rebaudioside C	5.2	951.01
Rebaudioside F	5.6	936.99
Rebaudioside A	7.1	967.01
Rebaudioside D	16	1129.15
Steviolbioside	18	642.73
Rebaudioside B	36	804.88

Prepare a standard curve for rebaudioside A by plotting rebaudioside A peak areas versus concentrations in mg/L, corrected for purity, based on the USP Reference Standard label claim. [NOTE: Peak responses for all other steviol glycosides besides rebaudioside A are used below in the *Related Steviol Glycosides* impurities test procedure.] From the standard curve, calculate the concentration *(C_U)* of rebaudioside A in the *Sample solution* in mg/L. Calculate the percentage of rebaudioside A in the portion of the sample taken by the formula:

$$C_U/C_{SMP} X 100\%$$

C_U = Concentration of rebaudioside A in the *Sample solution* determined from the standard curve (mg/L)

C_{SMP} = Concentration of the sample in the *Sample solution* (mg/L)

RELATED STEVIOL GLYCOSIDES

Acetate buffer, Mobile phase, Diluent, Rebaudioside A standard solutions, Stevioside standard stock solution, Stevioside standard solutions, Sample solu-

tion, Chromatographic system, and System suitability: Prepare as directed in the *Assay* (above).

Analysis: Proceed as directed above in the *Assay,* but with the following modifications for the standard curve and calculations.

Using the peak area responses from the *Stevioside standard solutions,* prepare a standard curve for stevioside by plotting stevioside peak areas versus concentrations, in mg/L, corrected for purity, based on the USP Reference Standard label claim. From this standard curve, determine the concentration (mg/L) of stevioside in the *Sample solution.* Calculate the percent of stevioside in the sample taken using the following formula:

$$C_U/C_{SMP} \times 100\%$$

C_U = Concentration of stevioside in the *Sample solution* determined from the standard curve (mg/L)

C_{SMP} = Concentration of the sample in the *Sample solution* (mg/L)

For the seven other steviol glycoside impurities (rubusoside, dulcoside A, rebaudioside C rebaudioside F rebaudioside D, steviolbioside, and rebaudioside B) use the stevioside standard curve prepared above to calculate the mg/L stevioside equivalents for each. Separately calculate the percents of each analyte (rubusoside, dulcoside A, rebaudioside C, rebaudioside F, rebaudioside D, steviolbioside, and rebaudioside B) in the sample taken using the following formula which takes into account the differences in molecular weights between the analytes and stevioside:

$$(C_U \times M_{r1} / M_{r2}) / C_{SMP} \times 100\%$$

C_U = concentration of stevioside equivalents in the *Sample solution* determined from the standard curve *(mg/*L)

M_{r1} = Molecular weight of the analyte (see *Chromatographic Profile Table* 1, above under *Assay*)

M_{r2} = Molecular weight of stevioside 804.88

C_{SMP} = Concentration of the sample in the *Sample solution* (mg/L)

System Suitability Check

B1 Detector response

B2 %RSD of Rebaudioside A peak area and retention time

B3 Column efficiency and tailing factor

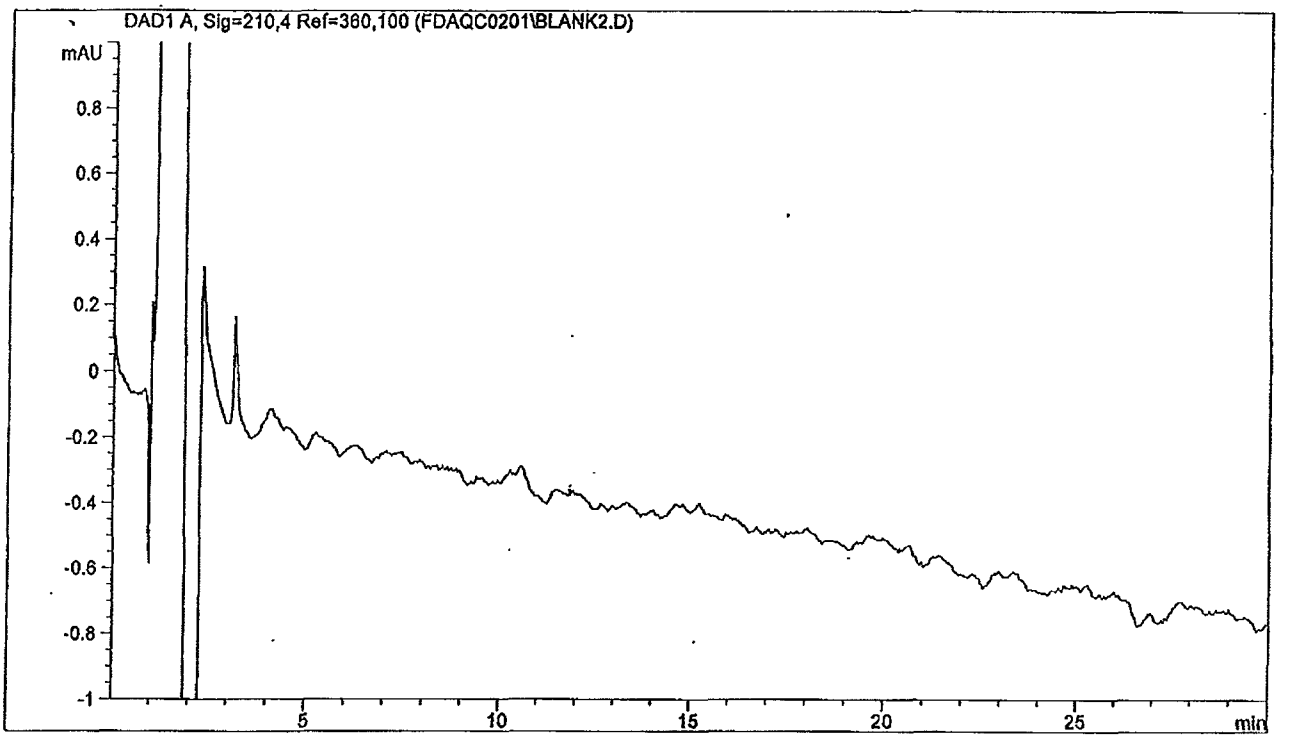
Detector response

Data File C:\CHEM32\1\DATA\FDAQC0201\BLANK2.D
Sample Name: Blank

Acq. Operator	: PureCircleQC001	Seq. Line :	1
Acq. Instrument	: Instrument 1	Location :	P1-F-01
Injection Date	: 1/2/2010 6:21:36 PM	Inj :	2
		Inj Volume :	12.0 µl

Acq. Method : C:\CHEM32\1\METHODS\NOMSD30.M
Last changed : 1/2/2010 5:50:18 PM by PureCircleQC001
(modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\NOMSDDATA.M
Last changed : 1/3/2010 1:30:37 PM
(modified after loading)
Method Info : No MSD Method

Sample Info : Blank



Area Percent Report

Sorted By : Signal
Calib. Data Modified : 1/3/2010 1:30:37 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
1	3.216		0.0000	0.00000	0.0000	Rub
2	4.517		0.0000	0.00000	0.0000	DulA
3	6.459		0.0000	0.00000	0.0000	Stev
4	7.449		0.0000	0.00000	0.0000	RebC

Pureclicle Sdn Bhd (Formerly known as Stevian Biotechnology Corporation Sdn Bhd)
576803-K
04 JAN 2010
LABORATORY

000107

Data File C:\CHEM32\1\DATA\FDAQC0201\BLANK2.D
Sample Name: Blank

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
5	7.990		0.0000	0.00000	0.0000	RebF
6	10.302		0.0000	0.00000	0.0000	RebA
7	24.136		0.0000	0.00000	0.0000	RebD
8	31.426		0.0000	0.00000	0.0000	Sbio
9	65.820		0.0000	0.00000	0.0000	RebB

Totals : 0.00000

1 Warnings or Errors :

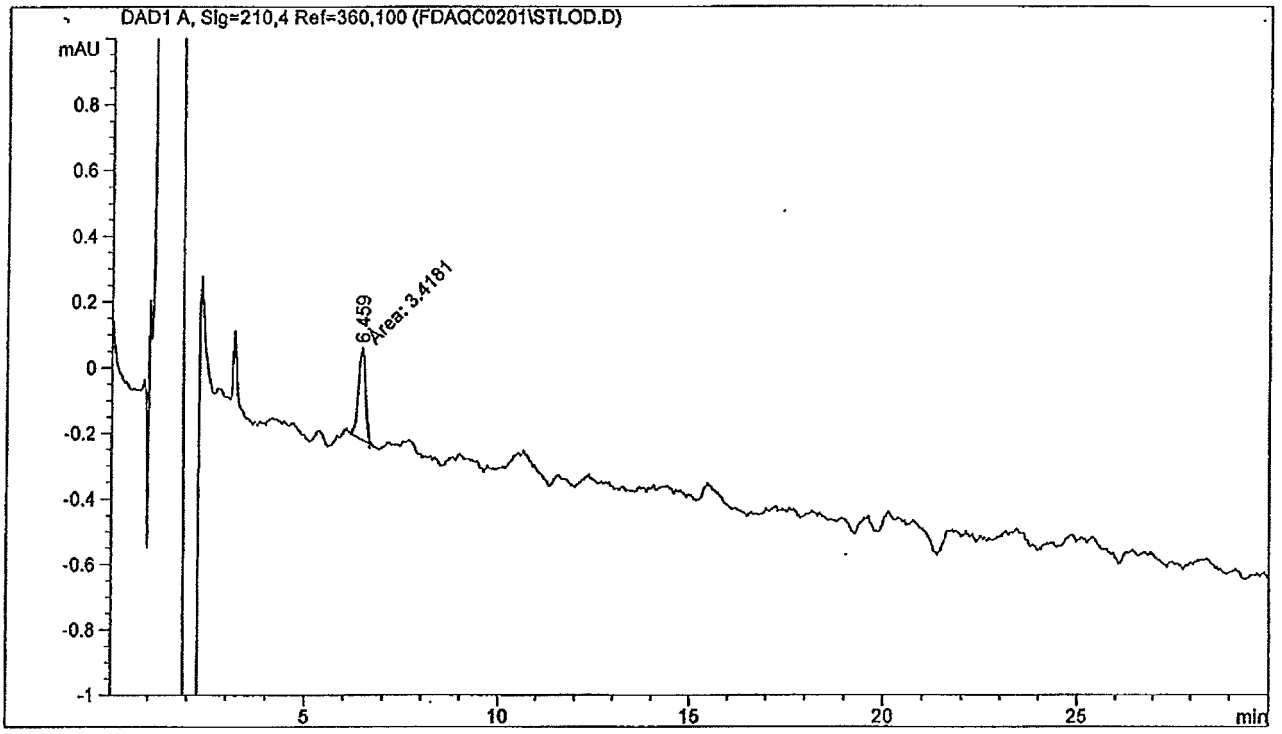
Warning : Calibrated compound(s) not found

*** End of Report ***

Purchase San Erg Kyowa Hakko as Stevian Biotechnology Corporation, San Bruno
578303-K
04 JAN 2010
LABORATORY

Data File C:\CHEM32\1\DATA\FDAQC0201\STLOD.D
Sample Name: STLOD

Acq. Operator	: PureCircleQC001	Seq. Line :	2
Acq. Instrument	: Instrument 1	Location :	P1-F-02
Injection Date	: 1/2/2010 6:52:45 PM	Inj :	1
		Inj Volume :	12.0 µl
Acq. Method	: C:\CHEM32\1\METHODS\NOMSD30.M		
Last changed	: 1/2/2010 5:50:18 PM by PureCircleQC001 (modified after loading)		
Analysis Method	: C:\CHEM32\1\METHODS\NOMSDDATA.M		
Last changed	: 1/3/2010 1:30:37 PM (modified after loading)		
Method Info	: No MSD Method		
Sample Info	: Stev 2.5ppm (LOD)		



Area Percent Report

Sorted By : Signal
Calib. Data Modified : 1/3/2010 1:30:37 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
1	3.216		0.0000	0.00000	0.0000	Rub
2	4.517		0.0000	0.00000	0.0000	DulA
3	6.459	MM	0.2039	3.41810	100.0000	Stev
4	7.449		0.0000	0.00000	0.0000	RebC

Pureclice Sdn Bhd (Formerly known as Slovian Biotechnology Corporation Sdn Bhd)
B7803-K
04 JAN 2010
LABORATORY

000109

Data File C:\CHEM32\1\DATA\FDAQC0201\STLOD.D
Sample Name: STLOD

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
5	7.990		0.0000	0.00000	0.0000	RebF
6	10.302		0.0000	0.00000	0.0000	RebA
7	24.136		0.0000	0.00000	0.0000	RebD
8	31.426		0.0000	0.00000	0.0000	Sbio
9	65.820		0.0000	0.00000	0.0000	RebB

Totals : 3.41810

1 Warnings or Errors :

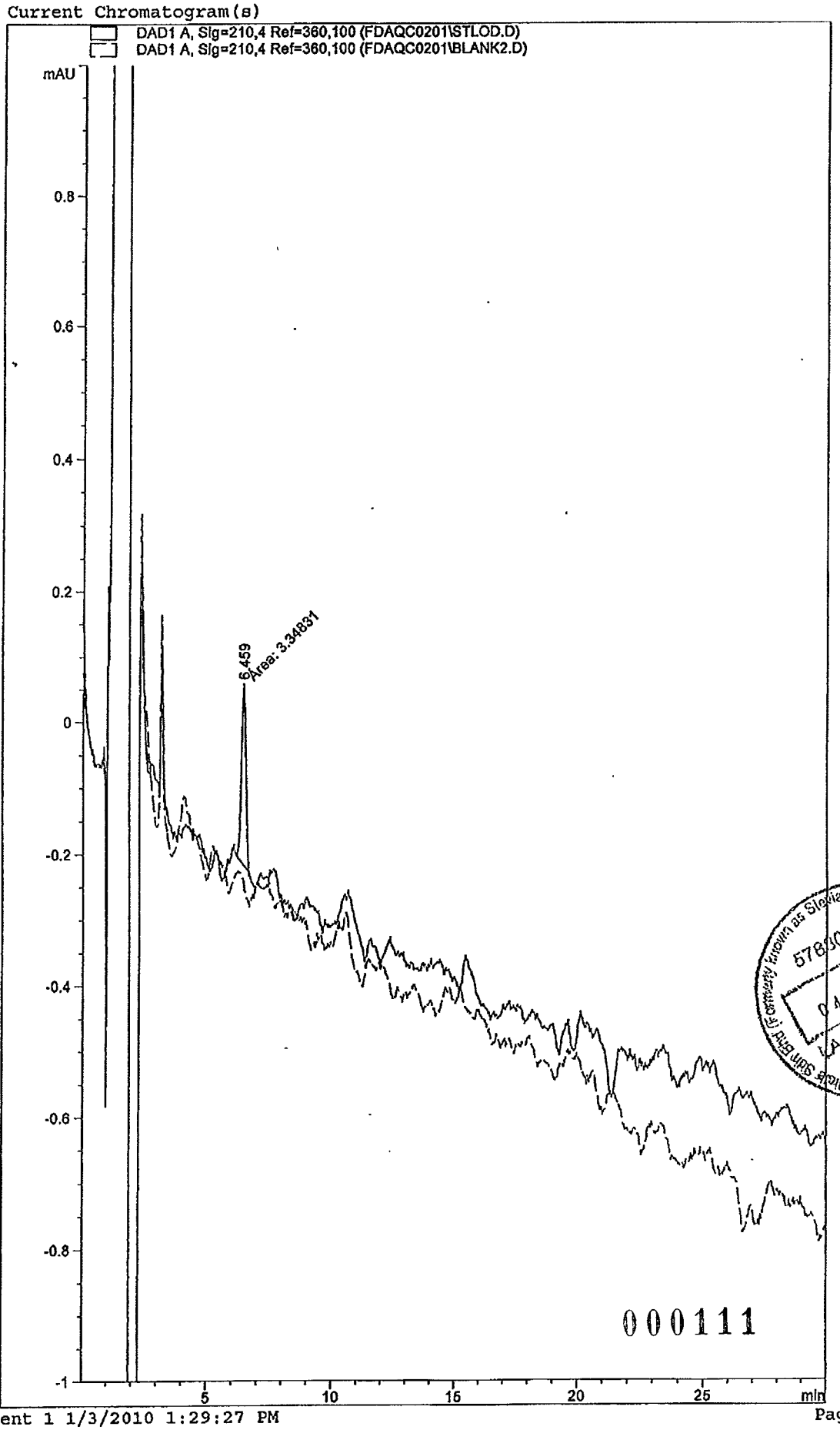
Warning : Calibrated compound(s) not found

*** End of Report ***

Stevian Biotechnology Corporation Sdn Bhd
578803-K
04 JAN 2010
LABORATORY

000110

Current Chromatogram(s)



Pwedlife Sdn Bhd (Formerly known as Slavian Biotechnology Corporation Sdn Bhd)
578303-K
04 JAN 2010
LABORATORY

000111

%RSD of Rebaudioside A peak area and retention time

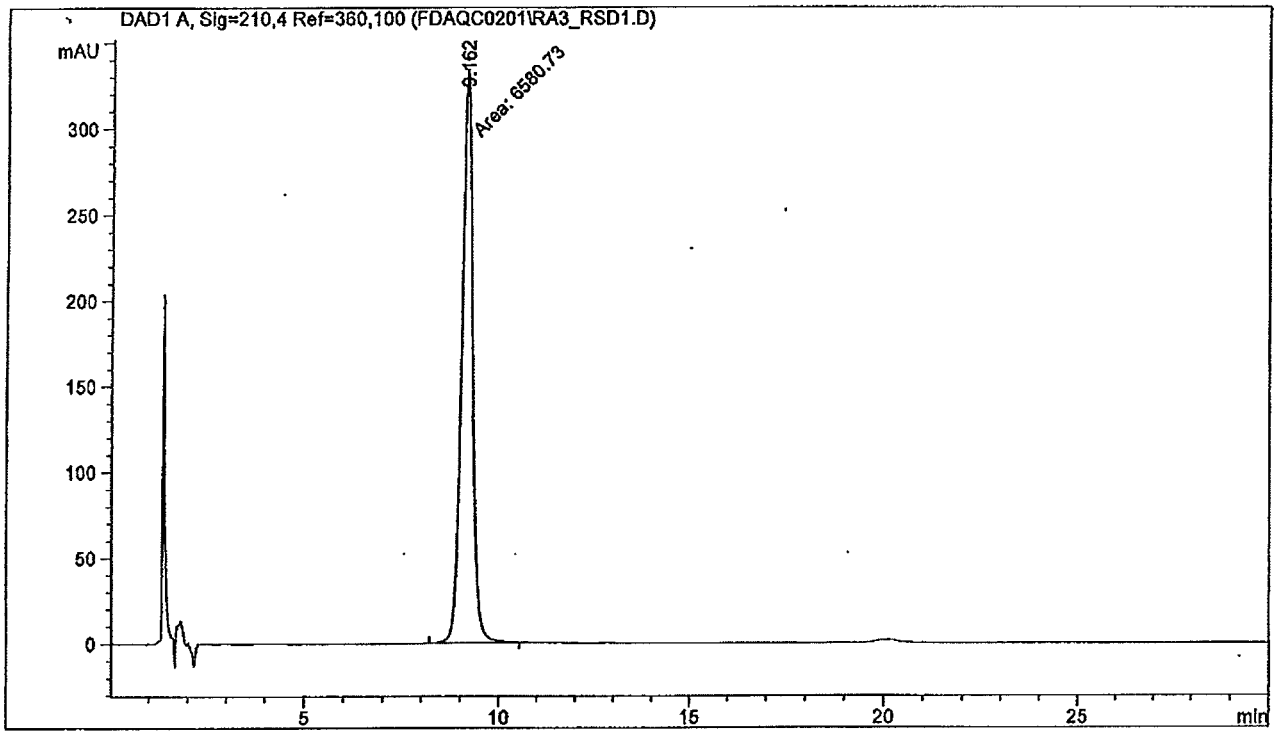
Data File C:\CHEM32\1\DATA\FDAQC0201\RA3_RSD1.D
Sample Name: RA5250

==========

Acq. Operator	: PureCircleQC001	Seq. Line	: 32
Acq. Instrument	: Instrument 1	Location	: P1-F-08
Injection Date	: 1/3/2010 5:53:07 PM	Inj	: 1
		Inj Volume	: 12.0 µl

Acq. Method : C:\CHEM32\1\METHODS\NOMSD30.M
Last changed : 1/3/2010 3:48:22 PM by PureCircleQC001
(modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\NOMSDDATA.M
Last changed : 1/4/2010 9:40:17 AM
(modified after loading)
Method Info : No MSD Method

Sample Info : RebA 5250 ppm



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Area Percent Report

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Sorted By : Signal
Calib. Data Modified : Monday, January 04, 2010 9:40:16 AM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Area % RSD 0,22
RT % RSD 0,62

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
1	3.172		0.0000	0.00000	0.0000	Rub
2	4.340		0.0000	0.00000	0.0000	DulA
3	5.900		0.0000	0.00000	0.0000	Stev
4	6.875		0.0000	0.00000	0.0000	RebC

000113

Pureciecle Sdn Bhd (Formerly known as Stevian Biotechnology Corporation Sdn Bhd)
578803-K
04 JAN 2010
LABORATORY

Data File C:\CHEM32\1\DATA\FDAQC0201\RA3_RSD1.D
Sample Name: RA5250

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
5	7.396		0.0000	0.00000	0.0000	RebF
6	9.162	MM	0.3282	6580.73047	100.0000	RebA
7	20.900		0.0000	0.00000	0.0000	RebD
8	25.739		0.0000	0.00000	0.0000	Sbio
9	44.221		0.0000	0.00000	0.0000	RebB

Totals : 6580.73047

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

*** End of Report ***

Pureclice Sdn Bhd (Formerly known as Stevian Biotechnology Corporation Sdn Bhd)
578803-K
04 JAN 2010
LABORATORY

000114

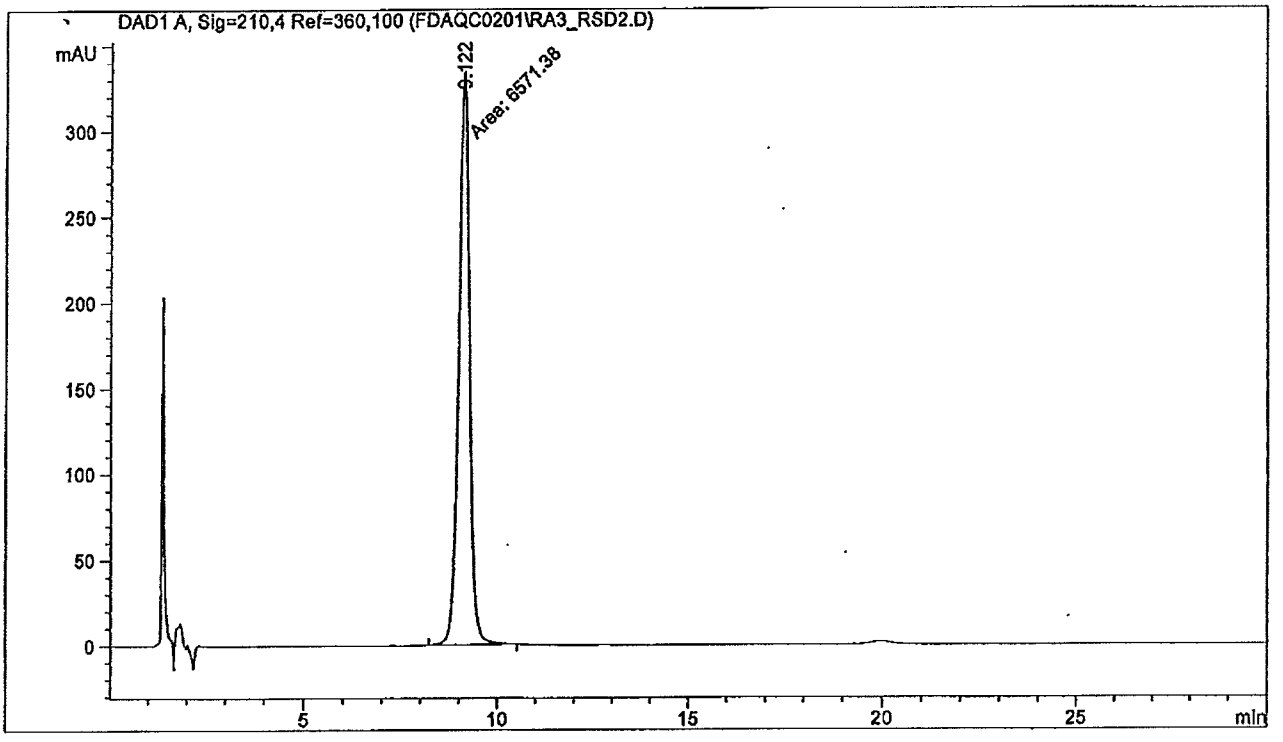
Data File C:\CHEM32\1\DATA\FDAQC0201\RA3_RSD2.D
Sample Name: RA5250

==========

Acq. Operator	: PureCircleQC001	Seq. Line :	32
Acq. Instrument	: Instrument 1	Location :	P1-F-08
Injection Date	: 1/3/2010 6:24:16 PM	Inj :	2
		Inj Volume :	12.0 µl

Acq. Method : C:\CHEM32\1\METHODS\NOMSD30.M
Last changed : 1/3/2010 3:48:22 PM by PureCircleQC001
(modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\NOMSDDATA.M
Last changed : 1/4/2010 9:40:52 AM
(modified after loading)
Method Info : No MSD Method

Sample Info : RebA 5250 ppm



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Area Percent Report

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Sorted By : Signal
Calib. Data Modified : Monday, January 04, 2010 9:40:16 AM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
1	3.172		0.0000	0.00000	0.0000	Rub
2	4.340		0.0000	0.00000	0.0000	DulA
3	5.900		0.0000	0.00000	0.0000	Stev
4	6.875		0.0000	0.00000	0.0000	RebC

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578803-K
04 JAN 2010
LABORATORY

Data File C:\CHEM32\1\DATA\FDAQC0201\RA3_RSD2.D
Sample Name: RA5250

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
5	7.396		0.0000	0.00000	0.0000	RebF
6	9.122	MM	0.3273	6571.38232	100.0000	RebA
7	20.900		0.0000	0.00000	0.0000	RebD
8	25.739		0.0000	0.00000	0.0000	Sbio
9	44.221		0.0000	0.00000	0.0000	RebB

Totals : 6571.38232

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

==========

*** End of Report ***

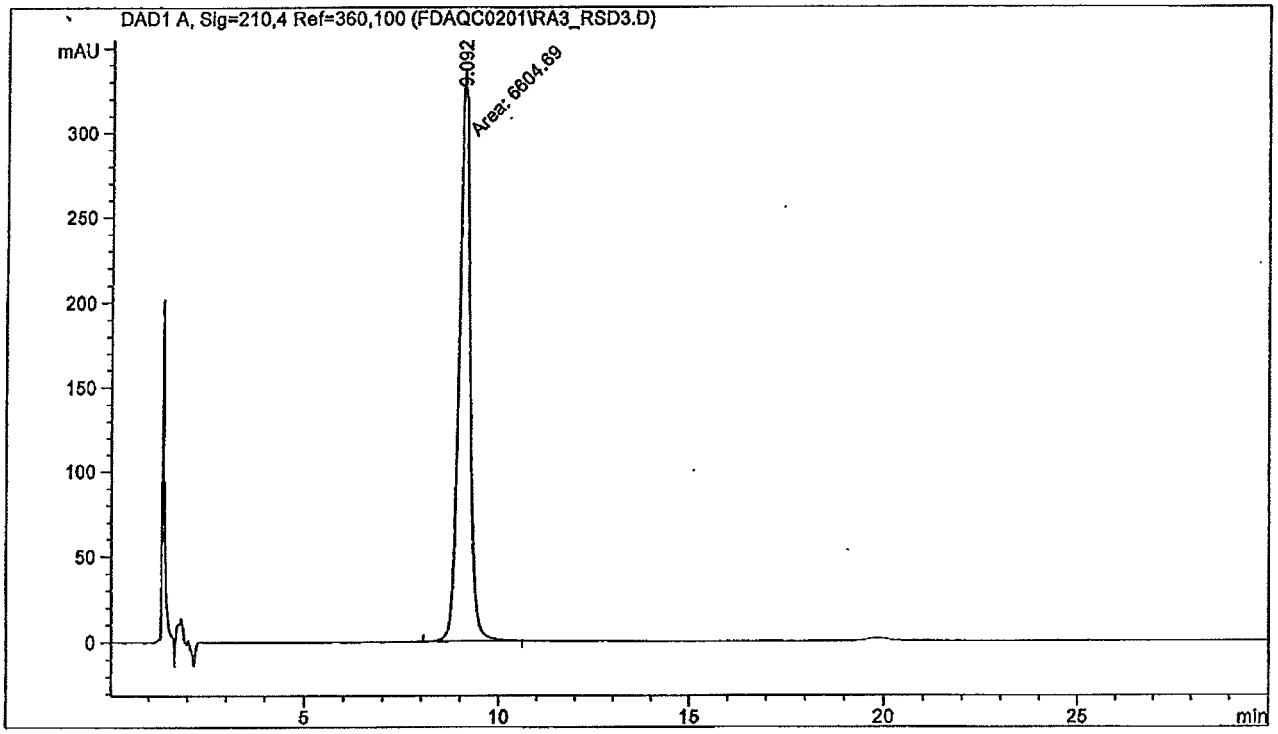
Pureclice Sdn Bhd (Formerly known as Stevian Biotechnology Corporation Sdn Bhd)
578803-K
04 JAN 2010
LABORATORY

Data File C:\CHEM32\1\DATA\FDAQC0201\RA3_RSD3.D
Sample Name: RA5250

Acq. Operator	: PureCircleQC001	Seq. Line :	32
Acq. Instrument	: Instrument 1	Location :	P1-F-08
Injection Date	: 1/3/2010 6:55:24 PM	Inj :	3
		Inj Volume :	12.0 µl

Acq. Method : C:\CHEM32\1\METHODS\NOMSD30.M
Last changed : 1/3/2010 3:48:22 PM by PureCircleQC001
(modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\NOMSDDATA.M
Last changed : 1/4/2010 9:41:35 AM
(modified after loading)
Method Info : No MSD Method

Sample Info : RebA 5250 ppm



Area Percent Report

Sorted By : Signal
Calib. Data Modified : Monday, January 04, 2010 9:40:16 AM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
1	3.172		0.0000	0.00000	0.0000	Rub
2	4.340		0.0000	0.00000	0.0000	DulA
3	5.900		0.0000	0.00000	0.0000	Stev
4	6.875		0.0000	0.00000	0.0000	RebC

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578803-K
04 JAN 2010
LABORATORY
PureCircle Sdn Bhd

000117

Data File C:\CHEM32\1\DATA\FDAQC0201\RA3_RSD3.D
Sample Name: RA5250

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
5	7.396		0.0000	0.00000	0.0000	RebF
6	9.092	MM	0.3265	6604.69385	100.0000	RebA
7	20.900		0.0000	0.00000	0.0000	RebD
8	25.739		0.0000	0.00000	0.0000	Sbio
9	44.221		0.0000	0.00000	0.0000	RebB

Totals : 6604.69385

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

==========

*** End of Report ***

Purecircle Sdn Bhd (Formerly known as Stevian Biotechnology Corporation Sdn Bhd)
578803-K
04 JAN 2010
LABORATORY

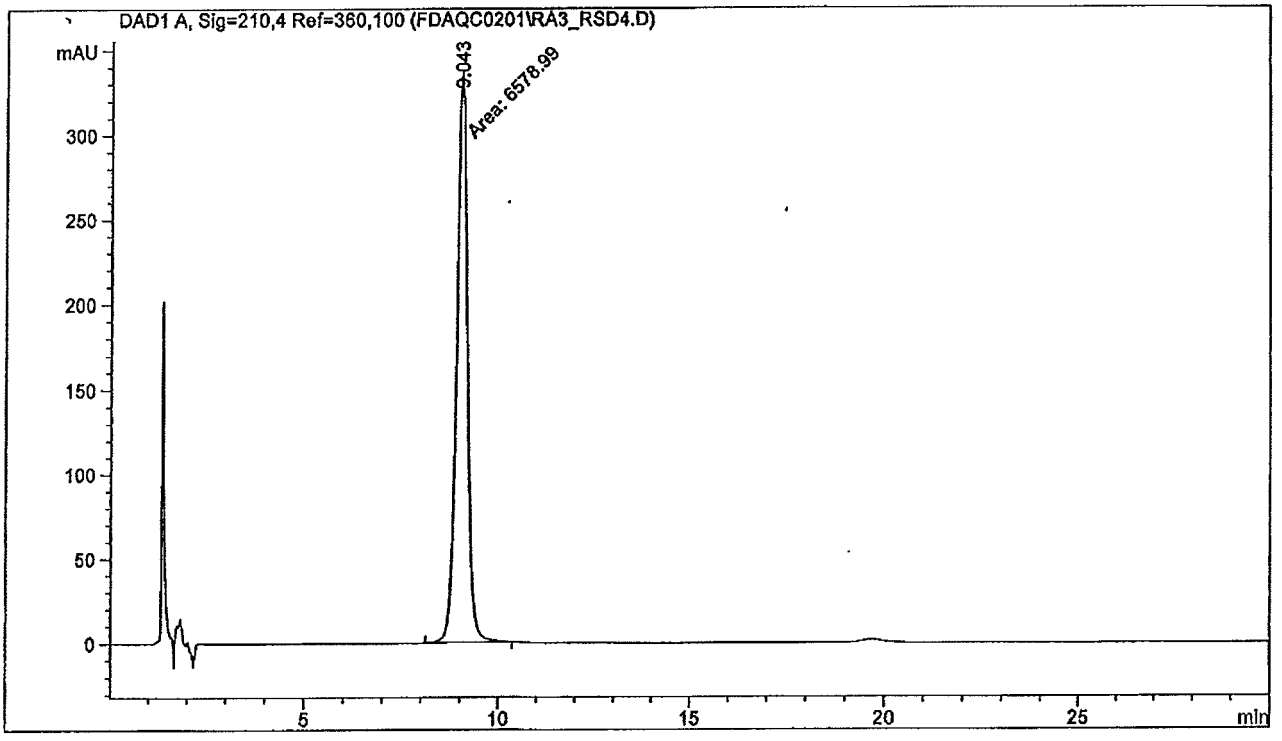
000118

Data File C:\CHEM32\1\DATA\FDAQC0201\RA3_RSD4.D
Sample Name: RA5250

Acq. Operator	: PureCircleQC001	Seq. Line	: 32
Acq. Instrument	: Instrument 1	Location	: P1-F-08
Injection Date	: 1/3/2010 7:26:33 PM	Inj	: 4
		Inj Volume	: 12.0 µl

Acq. Method : C:\CHEM32\1\METHODS\NOMSD30.M
Last changed : 1/3/2010 3:48:22 PM by PureCircleQC001
(modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\NOMSDDATA.M
Last changed : 1/4/2010 9:42:30 AM
(modified after loading)
Method Info : No MSD Method

Sample Info : RebA 5250 ppm



Area Percent Report

Sorted By : Signal
Calib. Data Modified : Monday, January 04, 2010 9:40:16 AM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
1	3.172		0.0000	0.00000	0.0000	Rub
2	4.340		0.0000	0.00000	0.0000	DulA
3	5.900		0.0000	0.00000	0.0000	Stev
4	6.875		0.0000	0.00000	0.0000	RebC

Pure Circle Sdn Bhd (formerly known as Stevian Biotechnology Corporation Sdn Bhd)
578803-K
04 JAN 2010
LABORATORY

000119

Data File C:\CHEM32\1\DATA\FDAQC0201\RA3_RSD4.D
Sample Name: RA5250

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
5	7.396		0.0000	0.00000	0.0000	RebF
6	9.043	MM	0.3246	6578.98730	100.0000	RebA
7	20.900		0.0000	0.00000	0.0000	RebD
8	25.739		0.0000	0.00000	0.0000	Sbio
9	44.221		0.0000	0.00000	0.0000	RebB

Totals : 6578.98730

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

*** End of Report ***

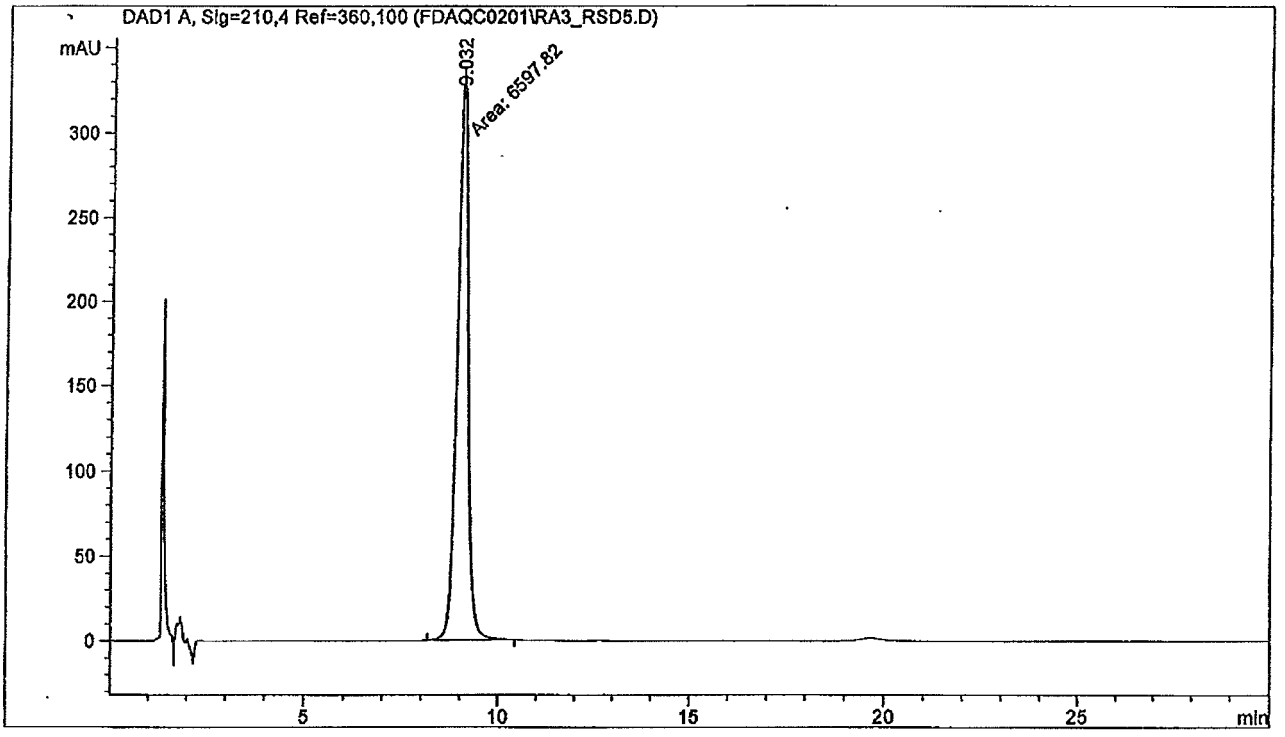
Pureclicle Sdn Bhd (formerly known as Stevian Biotechnology Corporation Sdn Bhd)
578803-K
04 JAN 2010
LABORATORY

Data File C:\CHEM32\1\DATA\FDAQC0201\RA3_RSD5.D
Sample Name: RA5250

Acq. Operator	: PureCircleQC001	Seq. Line	: 32
Acq. Instrument	: Instrument 1	Location	: P1-F-08
Injection Date	: 1/3/2010 7:57:41 PM	Inj	: 5
		Inj Volume	: 12.0 µl

Acq. Method : C:\CHEM32\1\METHODS\NOMSD30.M
Last changed : 1/3/2010 3:48:22 PM by PureCircleQC001
(modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\NOMSDDATA.M
Last changed : 1/4/2010 9:43:36 AM
(modified after loading)
Method Info : No MSD Method

Sample Info : RebA 5250 ppm



Area Percent Report

Sorted By : Signal
Calib. Data Modified : Monday, January 04, 2010 9:40:16 AM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
1	3.172		0.0000	0.00000	0.0000	Rub
2	4.340		0.0000	0.00000	0.0000	DulA
3	5.900		0.0000	0.00000	0.0000	Stev
4	6.875		0.0000	0.00000	0.0000	RebC

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578803-K
04 JAN 2010
LABORATORY
PureCircle Sdn Bhd

000121

Data File C:\CHEM32\1\DATA\FDAQC0201\RA3_RSD5.D
Sample Name: RA5250

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
5	7.396		0.0000	0.00000	0.0000	RebF
6	9.032	MM	0.3254	6597.81885	100.0000	RebA
7	20.900		0.0000	0.00000	0.0000	RebD
8	25.739		0.0000	0.00000	0.0000	Sbio
9	44.221		0.0000	0.00000	0.0000	RebB

Totals : 6597.81885

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

*** End of Report ***

000122

Purecircle Sdn Bhd (formerly known as Stevian Biotechnology Corporation Sdn Bhd)
578803-K
04 JAN 2010
LABORATORY

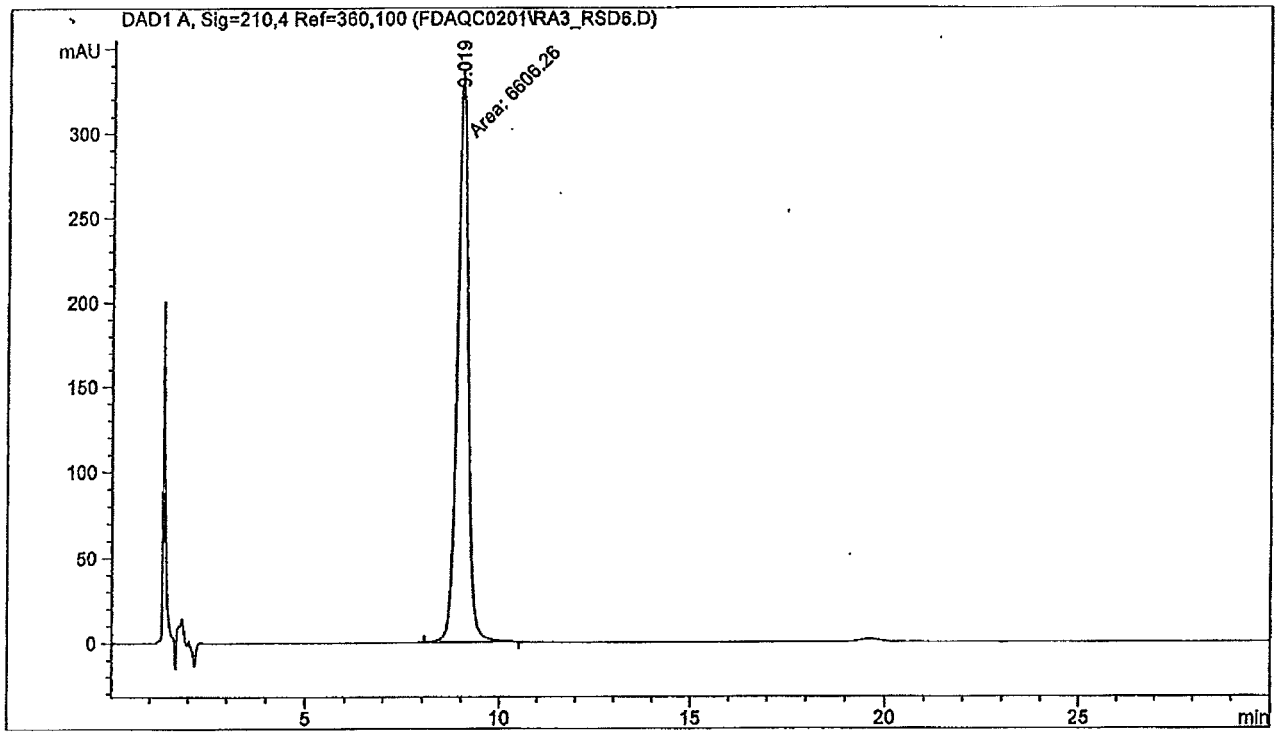
Data File C:\CHEM32\1\DATA\FDAQC0201\RA3_RSD6.D
Sample Name: RA5250

==========

Acq. Operator	: PureCircleQC001	Seq. Line	: 32
Acq. Instrument	: Instrument 1	Location	: P1-F-08
Injection Date	: 1/3/2010 8:28:50 PM	Inj	: 6
		Inj Volume	: 12.0 µl

Acq. Method : C:\CHEM32\1\METHODS\NOMSD30.M
Last changed : 1/3/2010 3:48:22 PM by PureCircleQC001
(modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\NOMSDDATA.M
Last changed : 1/4/2010 9:44:12 AM
(modified after loading)
Method Info : No MSD Method

Sample Info : RebA 5250 ppm



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Area Percent Report

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Sorted By : Signal
Calib. Data Modified : Monday, January 04, 2010 9:40:16 AM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
1	3.172		0.0000	0.00000	0.0000	Rub
2	4.340		0.0000	0.00000	0.0000	DulA
3	5.900		0.0000	0.00000	0.0000	Stev
4	6.875		0.0000	0.00000	0.0000	RebC

Purecircle Sdn Bhd (Formerly known as Stevian Biotechnology Corporation Sdn Bhd)
578803-K
04 JAN 2010
LABORATORY

000123

Data File C:\CHEM32\1\DATA\FDAQC0201\RA3_RSD6.D
Sample Name: RA5250

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
5	7.396		0.0000	0.00000	0.0000	RebF
6	9.019	MM	0.3263	6606.26416	100.0000	RebA
7	20.900		0.0000	0.00000	0.0000	RebD
8	25.739		0.0000	0.00000	0.0000	Sbio
9	44.221		0.0000	0.00000	0.0000	RebB

Totals : 6606.26416

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

==========

*** End of Report ***

Purecircle Sdn Bhd (Formerly Known as Stevian Biotechnology Corporation Sdn Bhd)
578803-K
04 JAN 2010
LABORATORY

Column efficiency and tailing factor

Data File C:\CHEM32\1\DATA\FDAQC0201\SERB.D
Sample Name: SERB

Extended Performance Report

Instrument: Instrument 1

Module	Type	Firmware rev.	Serial number
1200 Binary Pump SL	G1312B	A.06.10 [005]	DE63059499
1100/1200 Diode Array Detector SL	G1315C	B.01.06 [0001]	DE73457979
1200 Column Thermostat SL	G1316B	A.06.10 [004]	DE60559042
1200 High Performance Autosampler SL+	G1367D	A.06.12 [003]	DE82555258

Software Revision: Rev. B.04.01 SP1 [650] Copyright © Agilent Technologies

Column Description : Zorbax NH2
Product# : 880952-708 Batch#: B09019
Serial# : UST0012362
Diameter : 4.6 mm Length : 250.0 mm
Particle size : 5.0 µm Void volume : 60.0 %
Maximum Pressure : 200 bar Maximum pH : 9
Maximum Temperature: 60 °C
Comment :

Analysis method:
Path: C:\CHEM32\1\METHODS\NOMSDDATA.M
Name: NOMSDDATA.M

Sample information for Location: P1-F-04

Sample Name: SERB Multiplier: 1.00
Injection#: 1 Dilution: 1.00
Injection volume: 12.0 µl

Acquisition information:

Operator: PureCircleQC001
Date/Time: 1/2/2010 8:30:08 PM
Data file:
Path: C:\CHEM32\1\DATA\FDAQC0201\
Name: SERB.D
Method file:
Path: C:\CHEM32\1\METHODS\
Name: NOMSD.M

Flow: 2.000 ml/min
Pressure at start: 98 bar Pressure at end: 105 bar
Left Temp. at start: 30.0°C Left Temp. at end: 30.0 °C
Right Temp. at start: 30.0°C Right Temp. at end: 30.0 °C

PureCircle Sdn Bhd (Formerly known as Stevian Biotechnology Corporation Sdn Bhd)
578803-K
04 JAN 2010
LABORATORY

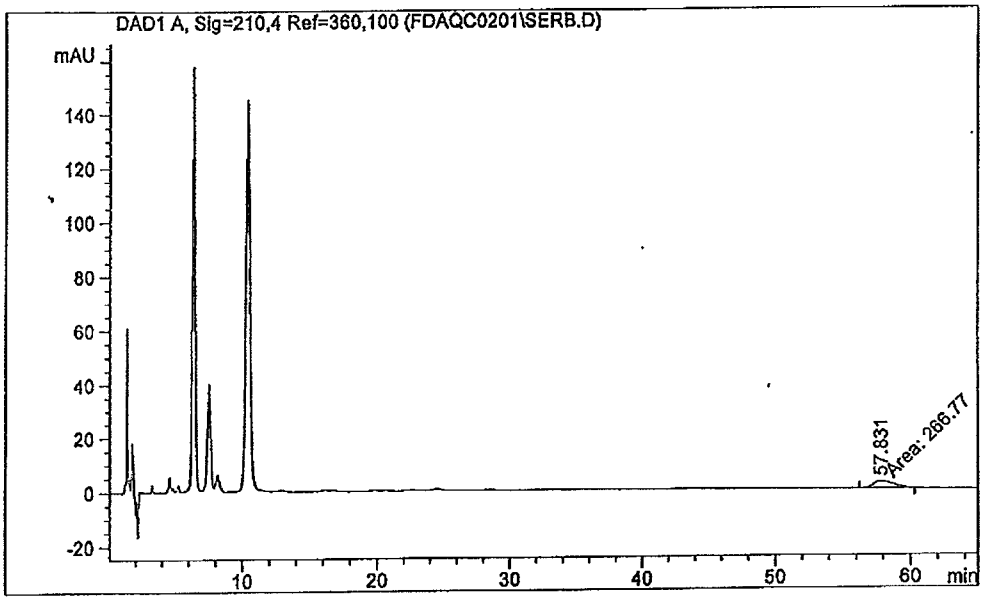
000126

Data File C:\CHEM32\1\DATA\FDAQC0201\SERB.D
Sample Name: SERB

Solvents: PMP1 , Solvent H2O -
PMP1 , Solvent ACN -

Extended Memory (DAD1)
Compact Flashcard: 1024MB

Signal description: DAD1 A, Sig=210,4 Ref=360,100



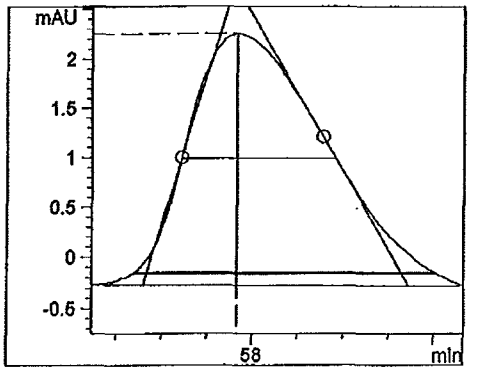
Formerly known as Slevian Biotechnology Corporation Sdn Bhd
578803-K
04 JAN 2010
LABORATORY
PureCircle Sdn Bhd

Data File C:\CHEM32\1\DATA\FDAQC0201\SERB.D
Sample Name: SERB

Compound# 9 : RebB
Amount [ ]: 180.4027

Peak description [min]:
Signal: DAD1 A, Sig=210,4 Ref=360,100

RetTime:	57.831	k':	45.397
Height:	2.53	Area:	266.8
Start:	56.265	End:	60.295
Skew:	0.380	Excess:	-0.318
Width at half height:			1.693
5 sigma:			3.353
tangent:			2.919
tailing:			3.307
Symmetry:			0.551
USP Tailing:			1.476
Integration type:			MM
Time increment [msec]:			400.0
Data points:			1322



Statistical moments (BB peak detection):

			Efficiency: Plates per .. column	meter
M0:	265.1			
M1:	58.156	Tangent method	6279	25115
M2:	0.466056	Halfwidth method	6462	25847
M3:	0.12084	5 sigma method	7435	29742
M4:	0.582445	Statistical	7257	29028

Relationship to preceeding peak:

		Selectivity:	-1.000
Resolution Tangent method:	-1.000	5 sigma method	-1.000
Halfwidth method	-1.000	Statistical method	-1.000

Puteclice Sdn Bhd (Formerly known as Stevian Biotechnology Corporation Sdn Bhd)
578803-K
04 JAN 2010
LABORATORY

000128

Data File C:\CHEM32\1\DATA\FDAQC0201\SERB.D
Sample Name: SERB

#	Ret.Time [min]	Amount []	Name	Page #
1	57.831	180.4027	RebB	3
	Total:	======== 180.4027		

*** End of Report ***

Purecircle Sdn Bhd (formerly known as Stevian Biotechnology Corporation Sdn Bhd)
578803-K
04 JAN 2010
LABORATORY

000129

Standard Chromatograms

C1 Stevioside

C2 Rebaudioside A

C3 M8 retention time marker (beginning and end of the assay)

C4 In-assay standard recovery check (Stevioside and Rebaudioside A)

APPENDIX C1

Stevioside

PureCircle Sdn Bhd	Document#: PCL-LAB-004	Issue date: 01/12/07
		Revision date: -------
HPLC reference standard preparation form		Revision No: 00

Standard Info	Stevioside (USP)
Lot#	F0I080
Purity, %wt ("as is")	97.0
Purity, %wt ("dry basis")	N/A

Stock Solution	
Weight, mg	51,13
Volumetric flask volume, mL	25
Gross concentration, mg/L	2045
Water, %wt (Karl Fischer)	—
Residual Solvent, %wt	—
Adjusted concentration, mg/L	1984
Remarks	—

Dilution Levels

Level#	Dilution	Concentration, mg/L
1	x 200	9,9
2	x 20	99,2
3	x 4	496
4	x 2	992
5	x 1	1984
Z	Z	Z
Z	Z	Z

Remarks —

Done by: (b) (6) KARTHIGAH, QC EXEC — Name, Position — 31. 12. 2009 — Date

Checked by: (b) (6) Lai Chee Seng, Senior QA — Name, Position — 31/12/2009 — Date

PureCircle Sdn Bhd (Formerly known as Stevian Biotechnology Corporation Sdn Bhd) 578803-K 31 DEC 2009 LABORATORY

000132

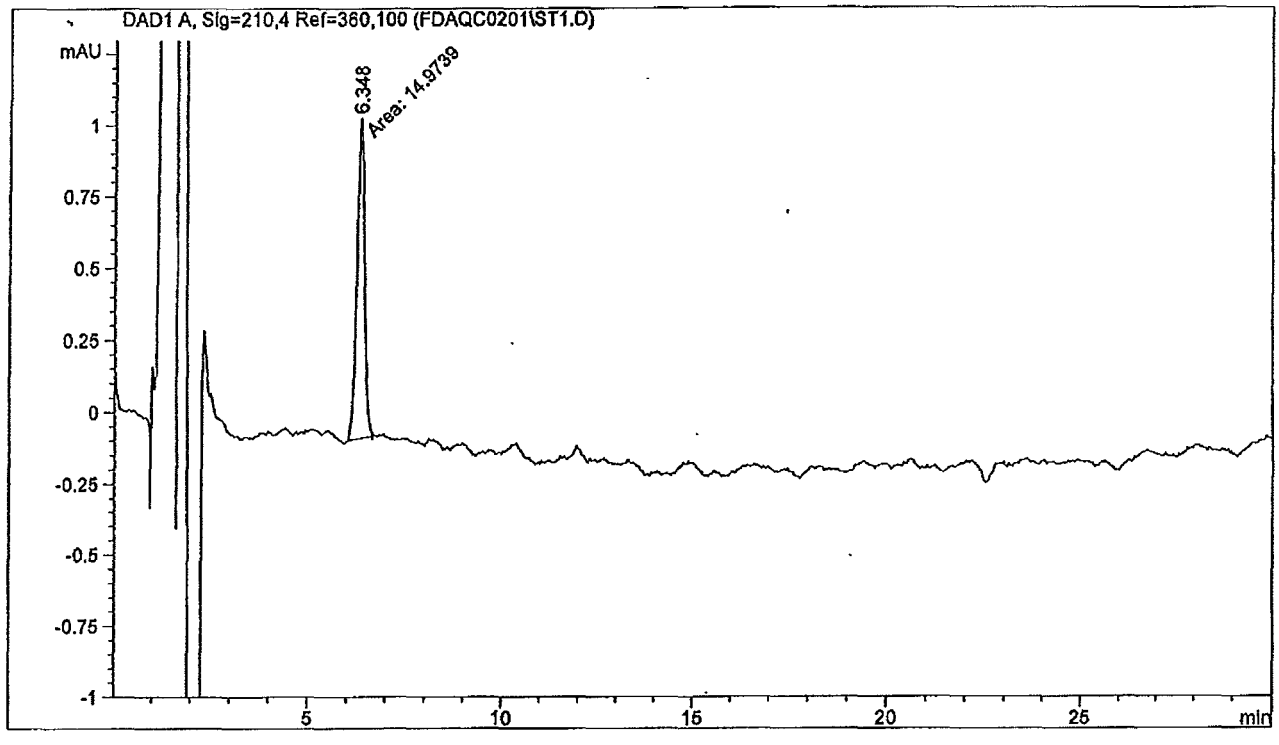
Data File C:\CHEM32\1\DATA\FDAQC0201\ST1.D
Sample Name: STL1

==========

Acq. Operator	: PureCircleQC001	Seq. Line :	5
Acq. Instrument	: Instrument 1	Location :	P1-E-02
Injection Date	: 1/2/2010 9:36:24 PM	Inj :	1
		Inj Volume :	12.0 µl

Acq. Method : C:\CHEM32\1\METHODS\NOMSD30.M
Last changed : 1/2/2010 9:36:13 PM by PureCircleQC001
(modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\NOMSDDATA.M
Last changed : 1/3/2010 2:03:49 PM
(modified after loading)
Method Info : No MSD Method

Sample Info : Stev L1 9.9ppm



==========

Area Percent Report

==========

Sorted By : Signal
Calib. Data Modified : Sunday, January 03, 2010 2:03:49 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
1	3.251		0.0000	0.00000	0.0000	Rub
2	4.585		0.0000	0.00000	0.0000	DulA
3	6.348	MM	0.2235	14.97385	100.0000	Stev
4	7.593		0.0000	0.00000	0.0000	RebC

Pure Circle Sdn Bhd (Formerly known as Stevian Biotechnology Corporation Sdn Bhd)
576803-K
04 JAN 2010
LABORATORY

000133

Data File C:\CHEM32\1\DATA\FDAQC0201\ST1.D
Sample Name: STL1

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
5	7.990		0.0000	0.00000	0.0000	RebF
6	10.094		0.0000	0.00000	0.0000	RebA
7	24.943		0.0000	0.00000	0.0000	RebD
8	33.646		0.0000	0.00000	0.0000	Sbio
9	57.831		0.0000	0.00000	0.0000	RebB

Totals : 14.97385

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

==========

*** End of Report ***

Pureclice Sdn Bhd (formerly known as Stevian Biotechnology Corporation Sdn Bhd)
578803-K
04 JAN 2010
LABORATORY

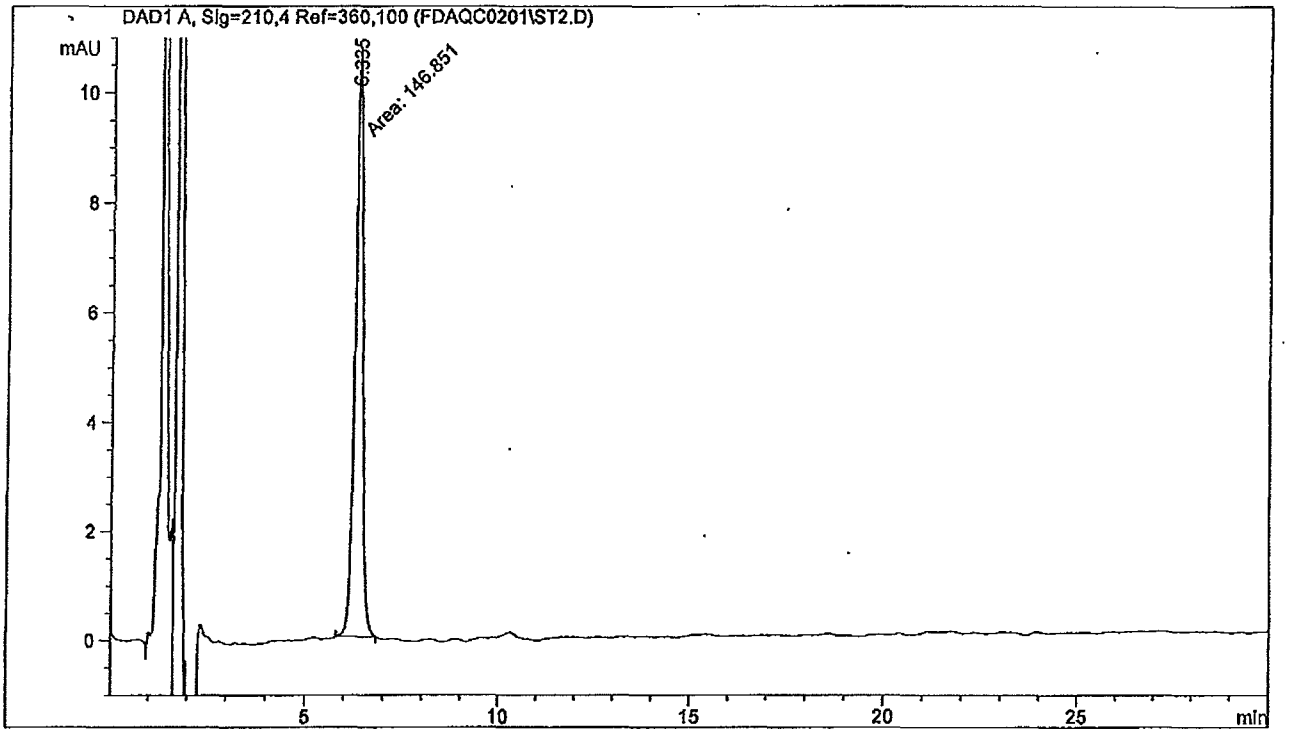
000134

Data File C:\CHEM32\1\DATA\FDAQC0201\ST2.D
Sample Name: STL2

Acq. Operator	: PureCircleQC001	Seq. Line :	6
Acq. Instrument	: Instrument 1	Location :	P1-E-03
Injection Date	: 1/2/2010 10:07:32 PM	Inj :	1
		Inj Volume :	12.0 µl

Acq. Method : C:\CHEM32\1\METHODS\NOMSD30.M
Last changed : 1/2/2010 9:36:13 PM by PureCircleQC001
(modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\NOMSDDATA.M
Last changed : 1/3/2010 2:05:14 PM
(modified after loading)
Method Info : No MSD Method

Sample Info : Stev L2 99.2ppm



Area Percent Report

Sorted By : Signal
Calib. Data Modified : 1/3/2010 2:05:02 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
1	3.251		0.0000	0.00000	0.0000	Rub
2	4.585		0.0000	0.00000	0.0000	DulA
3	6.335	MM	0.2277	146.85098	100.0000	Stev
4	7.593		0.0000	0.00000	0.0000	RebC

Formerly known as Stevian Biotechnology Corporation Sdn Bhd
578803-K
04 JAN 2010
LABORATORY
PureCircle Sdn Bhd

000135

Data File C:\CHEM32\1\DATA\FDAQC0201\ST2.D
Sample Name: STL2

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
5	7.990		0.0000	0.00000	0.0000	RebF
6	10.094		0.0000	0.00000	0.0000	RebA
7	24.943		0.0000	0.00000	0.0000	RebD
8	33.646		0.0000	0.00000	0.0000	Sbio
9	57.831		0.0000	0.00000	0.0000	RebB

Totals : 146.85098

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

*** End of Report ***

Stevian Biotechnology Corporation Sdn Bhd (Formerly known as Pueguda Sdn Bhd)
578803-K
04 JAN 2010
LABORATORY

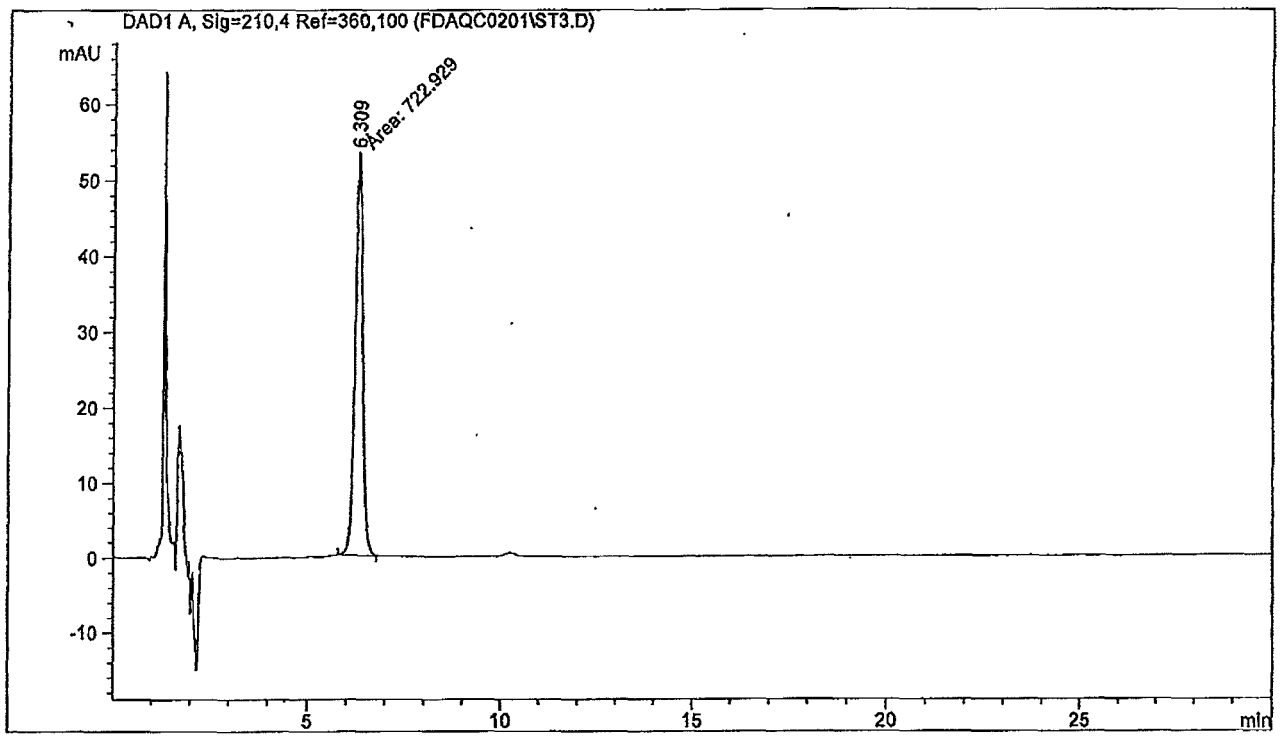
000136

Data File C:\CHEM32\1\DATA\FDAQC0201\ST3.D
Sample Name: STL3

Acq. Operator	: PureCircleQC001	Seq. Line :	7
Acq. Instrument	: Instrument 1	Location :	P1-E-04
Injection Date	: 1/2/2010 10:38:41 PM	Inj :	1
		Inj Volume :	12.0 µl

Acq. Method : C:\CHEM32\1\METHODS\NOMSD30.M
Last changed : 1/2/2010 9:36:13 PM by PureCircleQC001
(modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\NOMSDDATA.M
Last changed : 1/3/2010 2:05:58 PM
(modified after loading)
Method Info : No MSD Method

Sample Info : Stev L3 496.0ppm



Area Percent Report

Sorted By : Signal
Calib. Data Modified : 1/3/2010 2:05:50 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

000137

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
1	3.251		0.0000	0.00000	0.0000	Rub
2	4.585		0.0000	0.00000	0.0000	DulA
3	6.309	MM	0.2261	722.92896	100.0000	Stev
4	7.593		0.0000	0.00000	0.0000	RebC

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578803-K
04 JAN 2010
LABORATORY

Data File C:\CHEM32\1\DATA\FDAQC0201\ST3.D
Sample Name: STL3

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
5	7.990		0.0000	0.00000	0.0000	RebF
6	10.094		0.0000	0.00000	0.0000	RebA
7	24.943		0.0000	0.00000	0.0000	RebD
8	33.646		0.0000	0.00000	0.0000	Sbio
9	57.831		0.0000	0.00000	0.0000	RebB

Totals : 722.92896

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

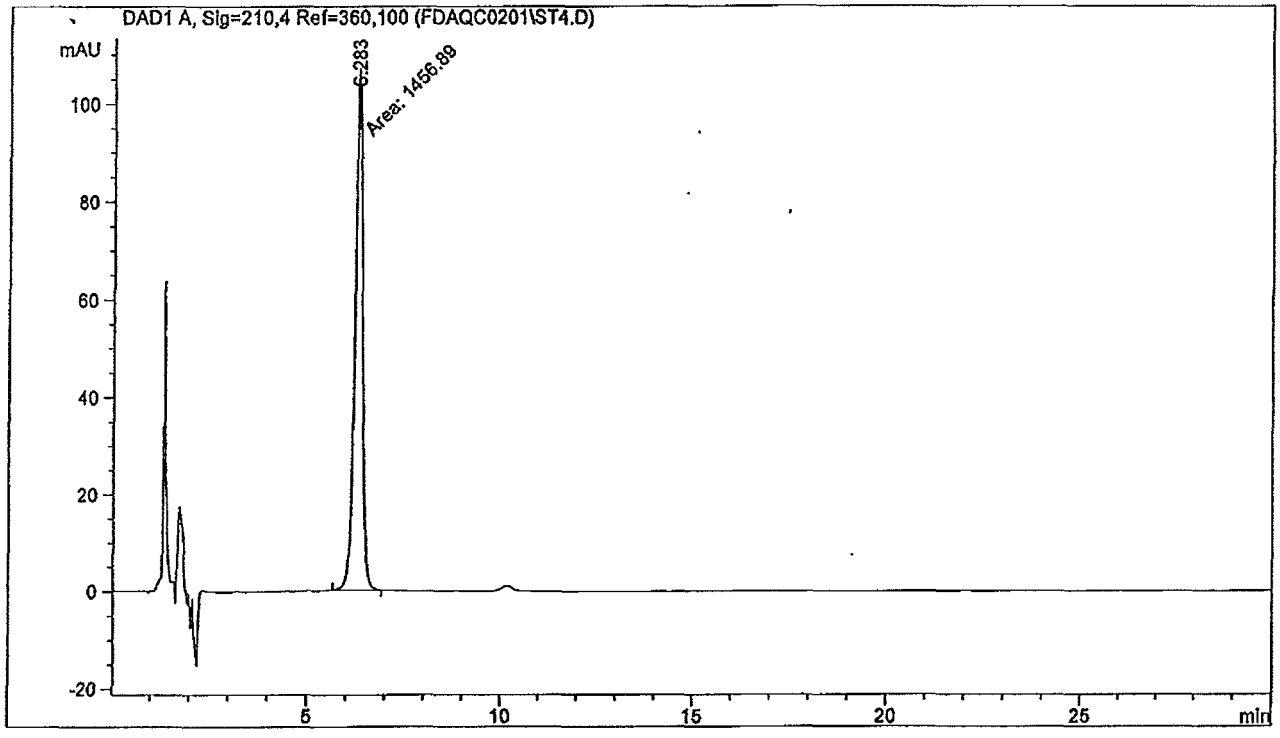
*** End of Report ***

000138

Purecircle Sdn Bhd (formerly known as Stevian Biotechnology Corporation Sdn Bhd)
573803-K
04 JAN 2010
LABORATORY

Data File C:\CHEM32\1\DATA\FDAQC0201\ST4.D
Sample Name: STL4

Acq. Operator	: PureCircleQC001	Seq. Line :	8
Acq. Instrument	: Instrument 1	Location :	P1-E-05
Injection Date	: 1/2/2010 11:09:50 PM	Inj :	1
		Inj Volume :	12.0 µl
Acq. Method	: C:\CHEM32\1\METHODS\NOMSD30.M		
Last changed	: 1/2/2010 9:36:13 PM by PureCircleQC001 (modified after loading)		
Analysis Method	: C:\CHEM32\1\METHODS\NOMSDDATA.M		
Last changed	: 1/3/2010 2:06:43 PM (modified after loading)		
Method Info	: No MSD Method		
Sample Info	: Stev L4 992.0ppm		



Area Percent Report

Sorted By : Signal
Calib. Data Modified : 1/3/2010 2:06:37 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
1	3.251		0.0000	0.00000	0.0000	Rub
2	4.585		0.0000	0.00000	0.0000	DulA
3	6.283	MM	0.2268	1456.89441	100.0000	Stev
4	7.593		0.0000	0.00000	0.0000	RebC

Pureclrcle Sdn Bhd (formerly known as Stevian Biotechnology Corporation Sdn Bhd)
578803-K
04 JAN 2010
LABORATORY

000139

Data File C:\CHEM32\1\DATA\FDAQC0201\ST4.D
Sample Name: STL4

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
5	7.990		0.0000	0.00000	0.0000	RebF
6	10.094		0.0000	0.00000	0.0000	RebA
7	24.943		0.0000	0.00000	0.0000	RebD
8	33.646		0.0000	0.00000	0.0000	Sbio
9	57.831		0.0000	0.00000	0.0000	RebB

Totals : 1456.89441

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

==========

*** End of Report ***

Purecircle Sdn Bhd (formerly known as Stevian Biotechnology Corporation Sdn Bhd)
578803-K
04 JAN 2010
LABORATORY

000140

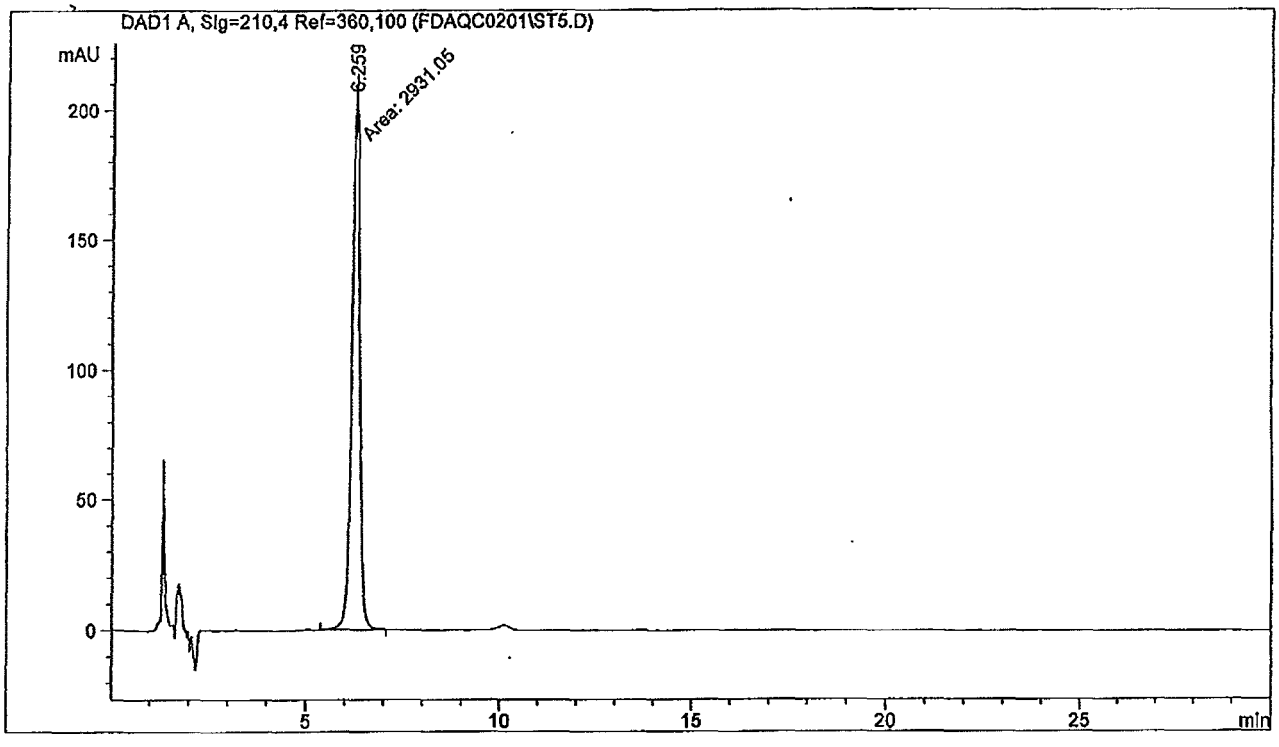
Data File C:\CHEM32\1\DATA\FDAQC0201\ST5.D
Sample Name: STL5

==========

Acq. Operator	: PureCircleQC001	Seq. Line :	9
Acq. Instrument	: Instrument 1	Location :	P1-E-06
Injection Date	: 1/2/2010 11:40:59 PM	Inj :	1
		Inj Volume :	12.0 µl

Acq. Method : C:\CHEM32\1\METHODS\NOMSD30.M
Last changed : 1/2/2010 9:36:13 PM by PureCircleQC001
(modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\NOMSDDATA.M
Last changed : 1/3/2010 2:09:06 PM
(modified after loading)
Method Info : No MSD Method

Sample Info : Stev L5 1984.0ppm



==========

Area Percent Report

==========

Sorted By : Signal
Calib. Data Modified : 1/3/2010 2:08:59 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
1	3.251		0.0000	0.00000	0.0000	Rub
2	4.585		0.0000	0.00000	0.0000	DulA
3	6.259	MM	0.2281	2931.04565	100.0000	Stev
4	7.593		0.0000	0.00000	0.0000	RebC

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578803-K
04 JAN 2010
LABORATORY

000141

Data File C:\CHEM32\1\DATA\FDAQC0201\ST5.D
Sample Name: STL5

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
5	7.990		0.0000	0.00000	0.0000	RebF
6	10.094		0.0000	0.00000	0.0000	RebA
7	24.943		0.0000	0.00000	0.0000	RebD
8	33.646		0.0000	0.00000	0.0000	Sbio
9	57.831		0.0000	0.00000	0.0000	RebB

Totals : 2931.04565

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

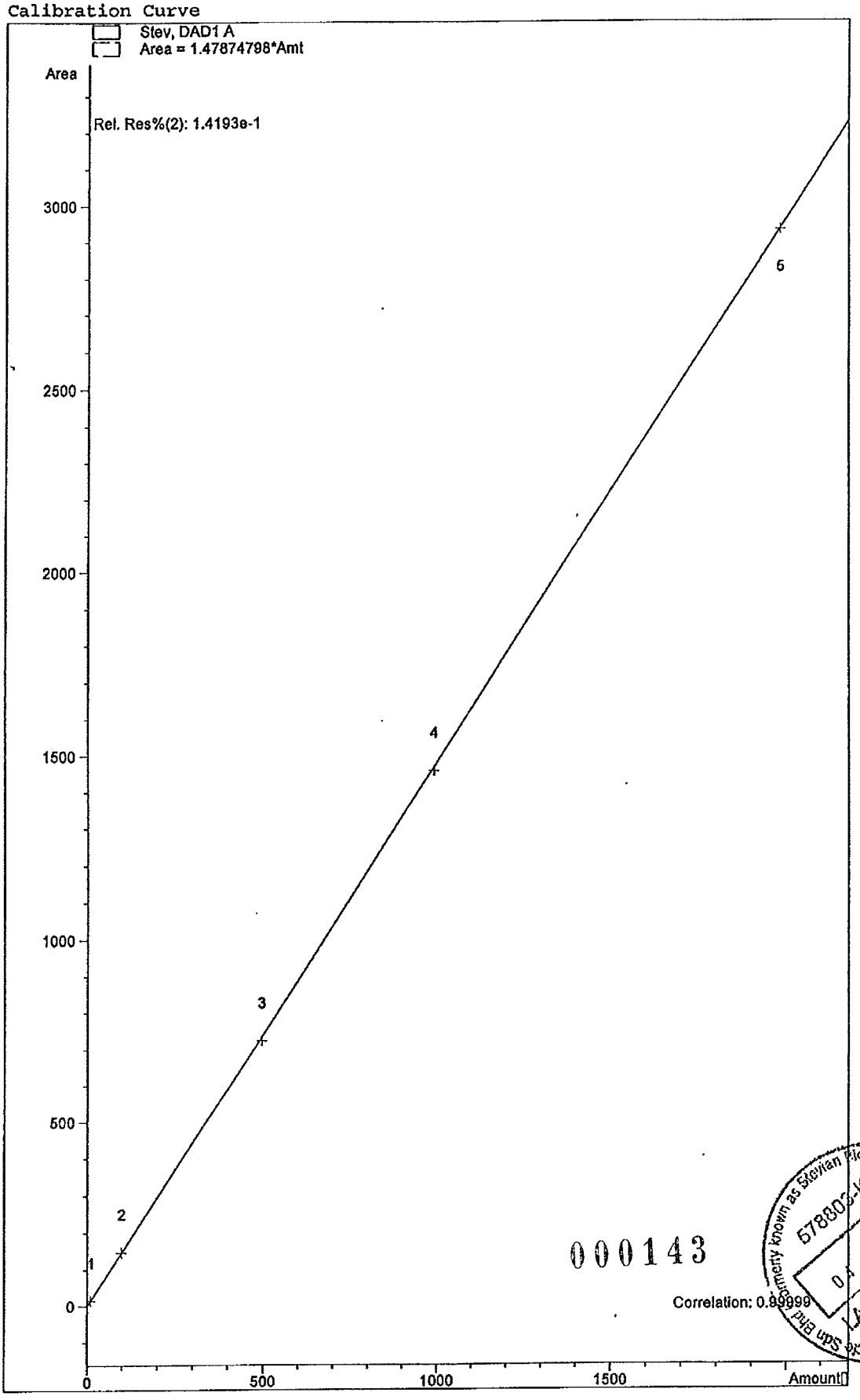
*** End of Report ***

000142

Pureclice Sdn Bhd (formerly known as Stevian Biotechnology Corporation Sdn Bhd)
578803-K
04 JAN 2010
LABORATORY

Print of window 66: Calibration Curve

Calibration Curve



000143

Formerly known as Stevian Biotechnology Corporation Sdn Bhd
578803-K
04 JAN 2010
LABORATORY
Purecircle Sdn Bhd

Rebaudioside A

PureCircle Sdn Bhd	Document#: PCL-LAB-004	Issue date: 01/12/07
		Revision date: -------
HPLC reference standard preparation form		Revision No: 00

Standard Info	Rebaudioside A (USP)
Lot#	F01077
Purity, %wt ("as is")	N/A
Purity, %wt ("dry basis")	96.9

Stock Solution	
Weight, mg	105.10
Volumetric flask volume, mL	25
Gross concentration, mg/L	4204
Water, %wt (Karl Fischer)	1.85
Residual Solvent, %wt	—
Adjusted concentration, mg/L	3994.1
Remarks	—

Dilution Levels

Level#	Dilution	Concentration, mg/L
1	x 4	998.5
2	x 2	1997.1
3	x 4/3	2995.6
4	x 1	3994.1

Remarks —

Done by: (b) (6) KARTHIGAH, QC EXEC — Name, Position — 31. 12. 2009 — Date

Checked by: (b) (6) Lai Chee Seng, Senior QA — Name, Position — 31/12/2009 — Date

Purecircle Sdn Bhd (Formerly known as Stevian Biotechnology Corporation Sdn Bhd) 578803-K 31 DEC 2009 LABORATORY

000145

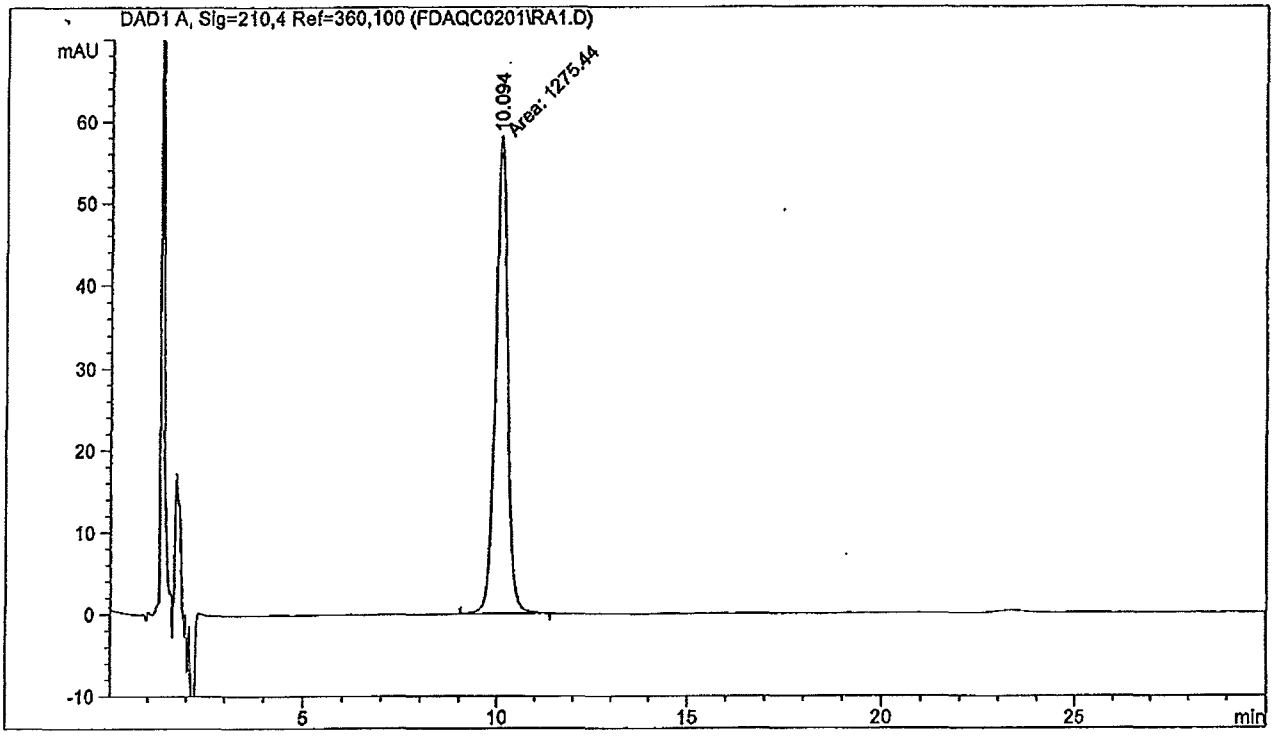
Data File C:\CHEM32\1\DATA\FDAQC0201\RA1.D
Sample Name: RAL1

==========

Acq. Operator	: PureCircleQC001	Seq. Line	: 10
Acq. Instrument	: Instrument 1	Location	: P1-E-07
Injection Date	: 1/3/2010 12:12:06 AM	Inj	: 1
		Inj Volume	: 12.0 µl

Acq. Method : C:\CHEM32\1\METHODS\NOMSD30.M
Last changed : 1/2/2010 9:36:13 PM by PureCircleQC001
(modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\NOMSDDATA.M
Last changed : 1/3/2010 1:38:52 PM
(modified after loading)
Method Info : No MSD Method

Sample Info : RebA L1 998.5ppm



==========

Area Percent Report

==========

Sorted By : Signal
Calib. Data Modified : 1/3/2010 1:38:29 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
1	3.251		0.0000	0.00000	0.0000	Rub
2	4.585		0.0000	0.00000	0.0000	DulA
3	6.427		0.0000	0.00000	0.0000	Stev
4	7.593		0.0000	0.00000	0.0000	RebC

Formerly known as Stevian Biotechnology Corporation Sdn Bhd
578803-K
04 JAN 2010
LABORATORY
PureCircle Sdn Bhd

000146

Data File C:\CHEM32\1\DATA\FDAQC0201\RA1.D
Sample Name: RAL1

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
5	7.990		0.0000	0.00000	0.0000	RebF
6	10.094	MM	0.3659	1275.43530	100.0000	RebA
7	24.943		0.0000	0.00000	0.0000	RebD
8	33.646		0.0000	0.00000	0.0000	Sbio
9	57.831		0.0000	0.00000	0.0000	RebB

Totals : 1275.43530

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

*** End of Report ***

Pureclide Sdn Bhd (formerly known as Slevian Biotechnology Corporation Sdn Bhd)
578803-K
04 JAN 2010
LABORATORY

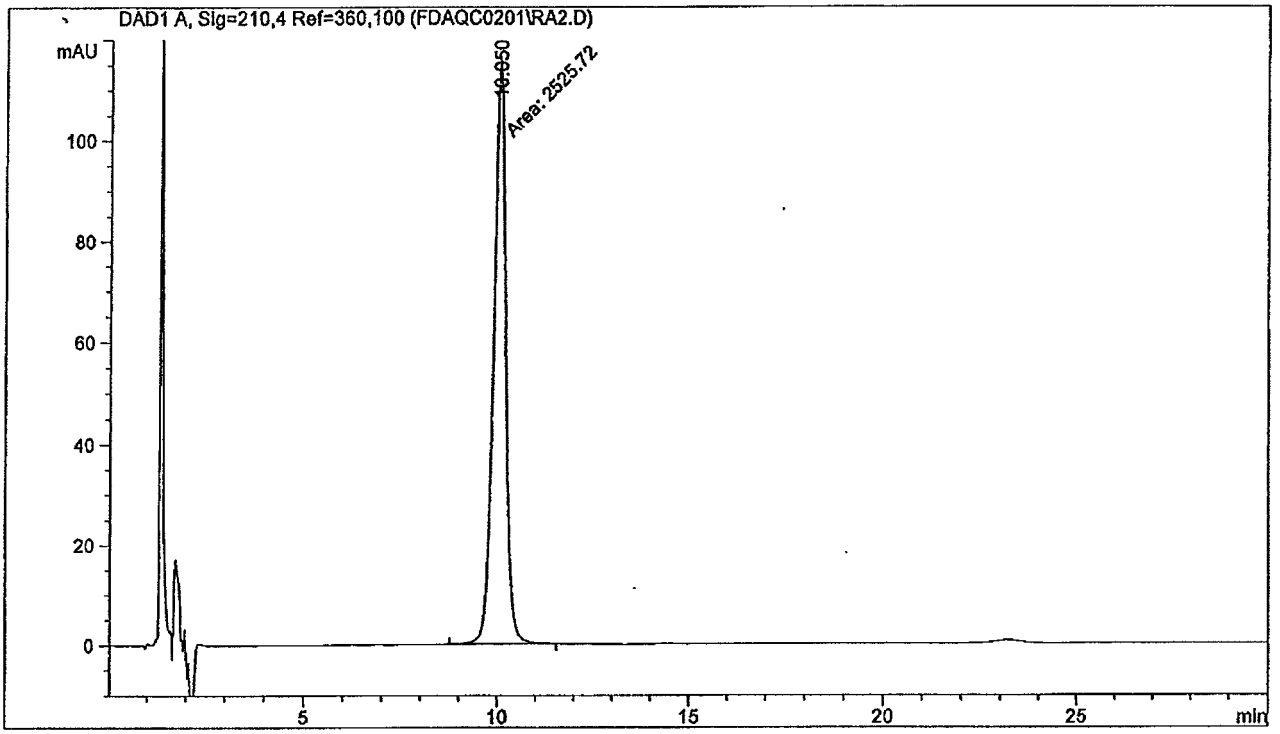
000147

Data File C:\CHEM32\1\DATA\FDAQC0201\RA2.D
Sample Name: RAL2

Acq. Operator	: PureCircleQC001	Seq. Line	: 11
Acq. Instrument	: Instrument 1	Location	: P1-E-08
Injection Date	: 1/3/2010 12:43:14 AM	Inj	: 1
		Inj Volume	: 12.0 µl

Acq. Method : C:\CHEM32\1\METHODS\NOMSD30.M
Last changed : 1/2/2010 9:36:13 PM by PureCircleQC001
(modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\NOMSDDATA.M
Last changed : 1/3/2010 1:40:11 PM
(modified after loading)
Method Info : No MSD Method

Sample Info : RebA L2 1997.1ppm



Area Percent Report

Sorted By : Signal
Calib. Data Modified : 1/3/2010 1:39:53 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
1	3.251		0.0000	0.00000	0.0000	Rub
2	4.585		0.0000	0.00000	0.0000	DulA
3	6.427		0.0000	0.00000	0.0000	Stev
4	7.593		0.0000	0.00000	0.0000	RebC

Pure Circle Sdn Bhd (formerly known as Stevian Biotechnology Corporation Sdn Bhd)
578803-K
04 JAN 2010
LABORATORY

000148

Data File C:\CHEM32\1\DATA\FDAQC0201\RA2.D
Sample Name: RAL2

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
5	7.990		0.0000	0.00000	0.0000	RebF
6	10.050	MM	0.3644	2525.72290	100.0000	RebA
7	24.943		0.0000	0.00000	0.0000	RebD
8	33.646		0.0000	0.00000	0.0000	Sbio
9	57.831		0.0000	0.00000	0.0000	RebB

Totals : 2525.72290

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

==========

*** End of Report ***

Pharmade Sdn Bhd (formerly known as Stevian Biotechnology Corporation Sdn Bhd)
578803-K
04 JAN 2010
LABORATORY

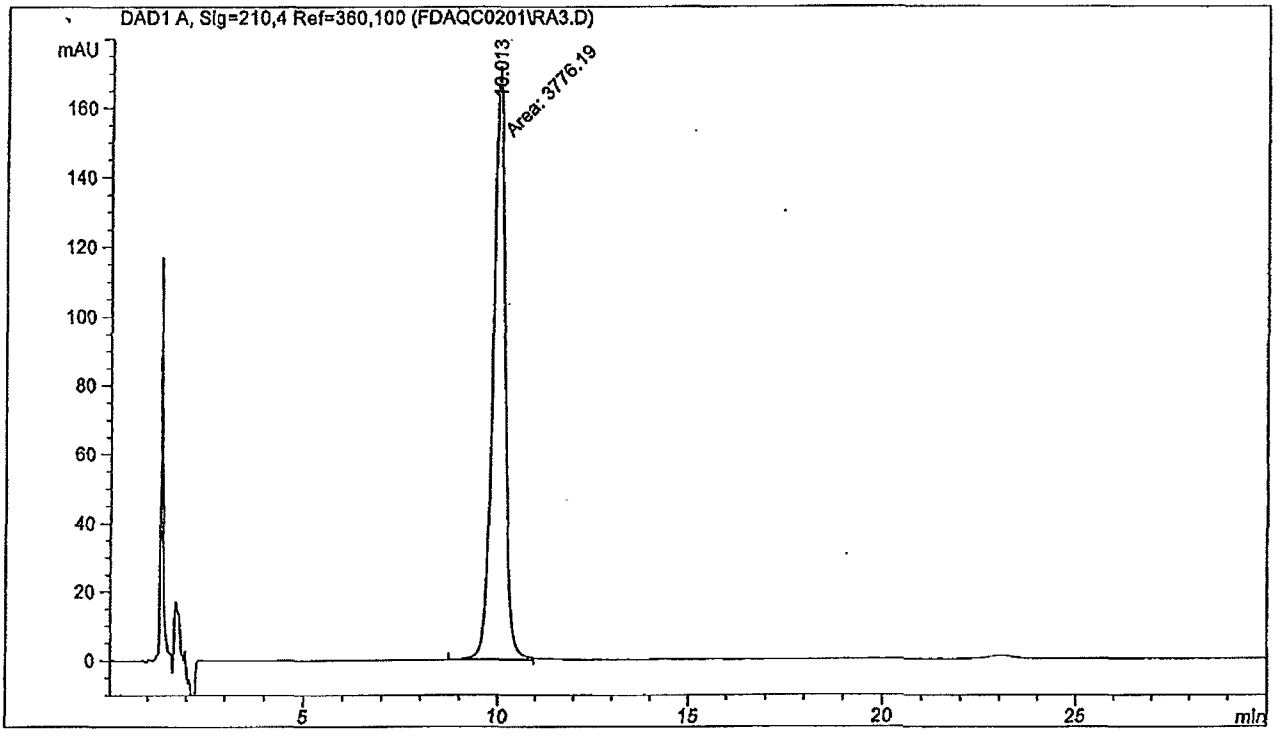
000149

Data File C:\CHEM32\1\DATA\FDAQC0201\RA3.D
Sample Name: RAL3

Acq. Operator	: PureCircleQC001	Seq. Line	: 12
Acq. Instrument	: Instrument 1	Location	: P1-E-09
Injection Date	: 1/3/2010 1:14:24 AM	Inj	: 1
		Inj Volume	: 12.0 µl

Acq. Method : C:\CHEM32\1\METHODS\NOMSD30.M
Last changed : 1/2/2010 9:36:13 PM by PureCircleQC001
(modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\NOMSDDATA.M
Last changed : 1/3/2010 1:42:38 PM
(modified after loading)
Method Info : No MSD Method

Sample Info : RebA L3 2995.6ppm



Area Percent Report

Sorted By : Signal
Calib. Data Modified : 1/3/2010 1:42:41 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
1	3.251		0.0000	0.00000	0.0000	Rub
2	4.585		0.0000	0.00000	0.0000	DulA
3	6.427		0.0000	0.00000	0.0000	Stev
4	7.593		0.0000	0.00000	0.0000	RebC

Pureciecle Sdn Bhd (Formerly known as Stevian Biotechnology Corporation)
578803-K
04 JAN 2010
LABORATORY

000150

Data File C:\CHEM32\1\DATA\FDAQC0201\RA3.D
Sample Name: RAL3

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
5	7.990		0.0000	0.00000	0.0000	RebF
6	10.013	MF	0.3665	3776.18970	100.0000	RebA
7	24.943		0.0000	0.00000	0.0000	RebD
8	33.646		0.0000	0.00000	0.0000	Sbio
9	57.831		0.0000	0.00000	0.0000	RebB

Totals : 3776.18970

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

*** End of Report ***

Pueclinte Sdn Bhd (Formerly known as Stevian Biotechnology Corporation Sdn Bhd)
573803-K
04 JAN 2010
LABORATORY

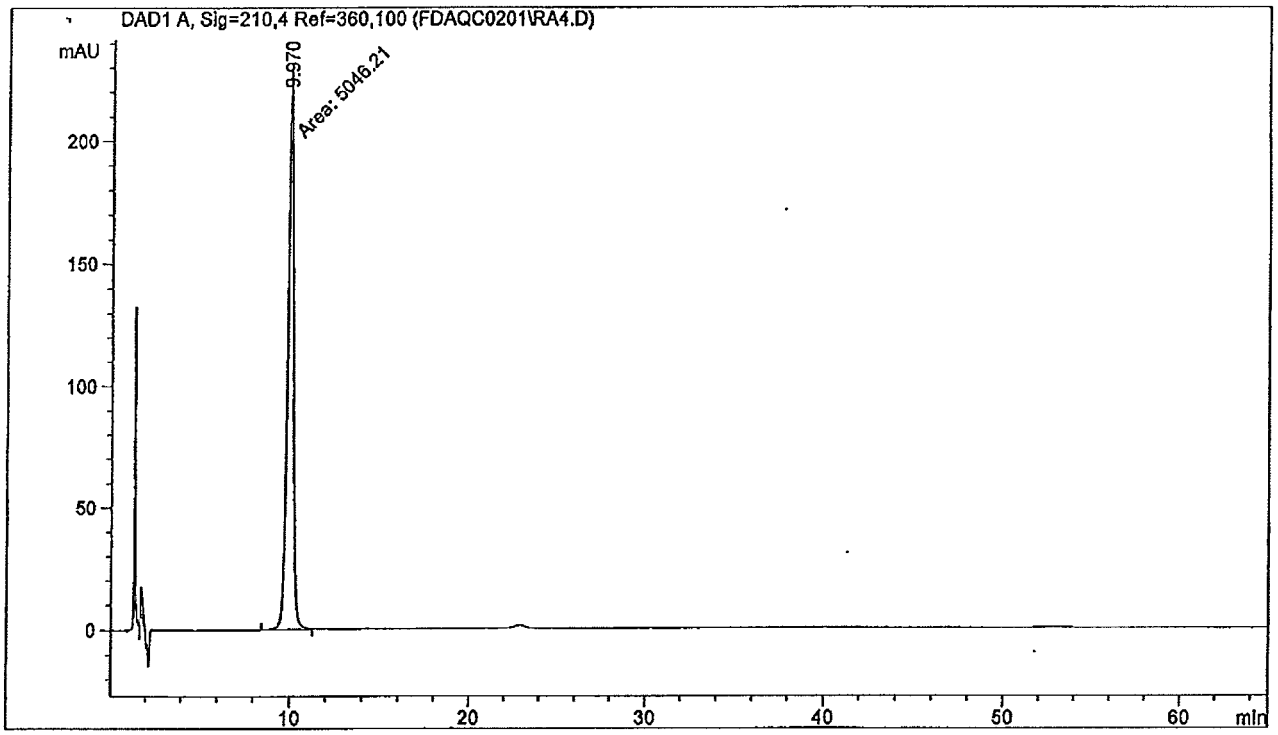
000151

Data File C:\CHEM32\1\DATA\FDAQC0201\RA4.D
Sample Name: RAL4

Acq. Operator	: PureCircleQC001	Seq. Line	: 13
Acq. Instrument	: Instrument 1	Location	: P1-F-09
Injection Date	: 1/3/2010 1:45:38 AM	Inj	: 1
		Inj Volume	: 12.0 µl

Acq. Method : C:\CHEM32\1\METHODS\NOMSD.M
Last changed : 1/3/2010 1:45:28 AM by PureCircleQC001
(modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\NOMSDDATA.M
Last changed : 1/3/2010 1:45:07 PM
(modified after loading)
Method Info : No MSD Method

Sample Info : RebA L4 3994.1ppm



Area Percent Report

Sorted By : Signal
Calib. Data Modified : 1/3/2010 1:45:43 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
1	3.251		0.0000	0.00000	0.0000	Rub
2	4.585		0.0000	0.00000	0.0000	DulA
3	6.427		0.0000	0.00000	0.0000	Stev
4	7.593		0.0000	0.00000	0.0000	RebC

Formerly known as Stevian Biotechnology Corporation Sdn Bhd (PureCircle Sdn Bhd)
578803-K
04 JAN 2010
LABORATORY

000152

Data File C:\CHEM32\1\DATA\FDAQC0201\RA4.D
Sample Name: RAL4

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
5	7.990		0.0000	0.00000	0.0000	RebF
6	9.970	MF	0.3672	5046.21143	100.0000	RebA
7	24.943		0.0000	0.00000	0.0000	RebD
8	33.646		0.0000	0.00000	0.0000	Sbio
9	57.831		0.0000	0.00000	0.0000	RebB

Totals : 5046.21143

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

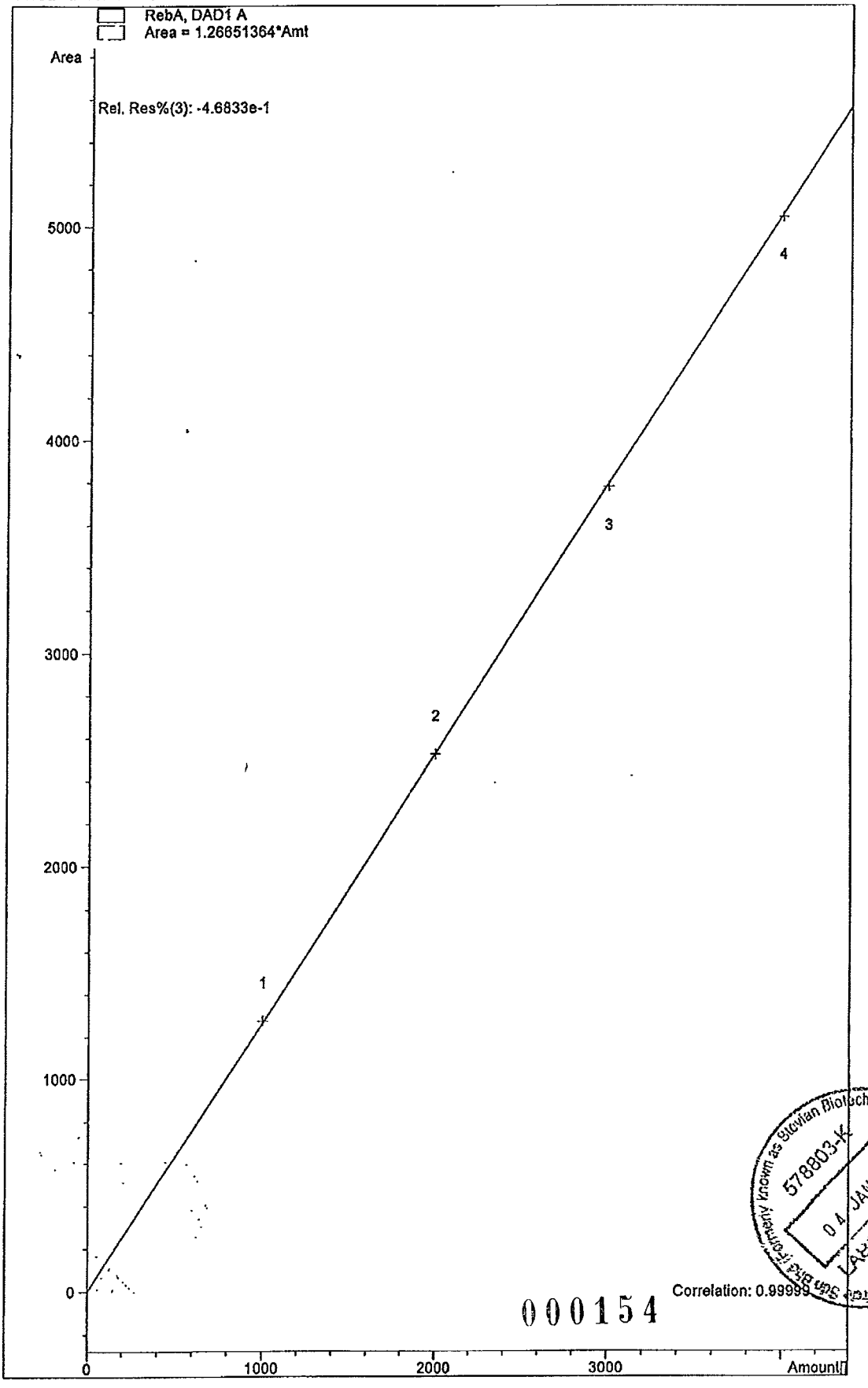
==========

*** End of Report ***

Pureclinic Sdn Bhd (Formerly known as Slevian Biotechnology Corporation Sdn Bhd)
578803-K
04 JAN 2010
LABORATORY

000153

Calibration Curve



Purechem Sdn Bhd (formerly known as Stevian Biotechnology Corporation Sdn Bhd)
578803-K
04 JAN 2010
LABORATORY

000154

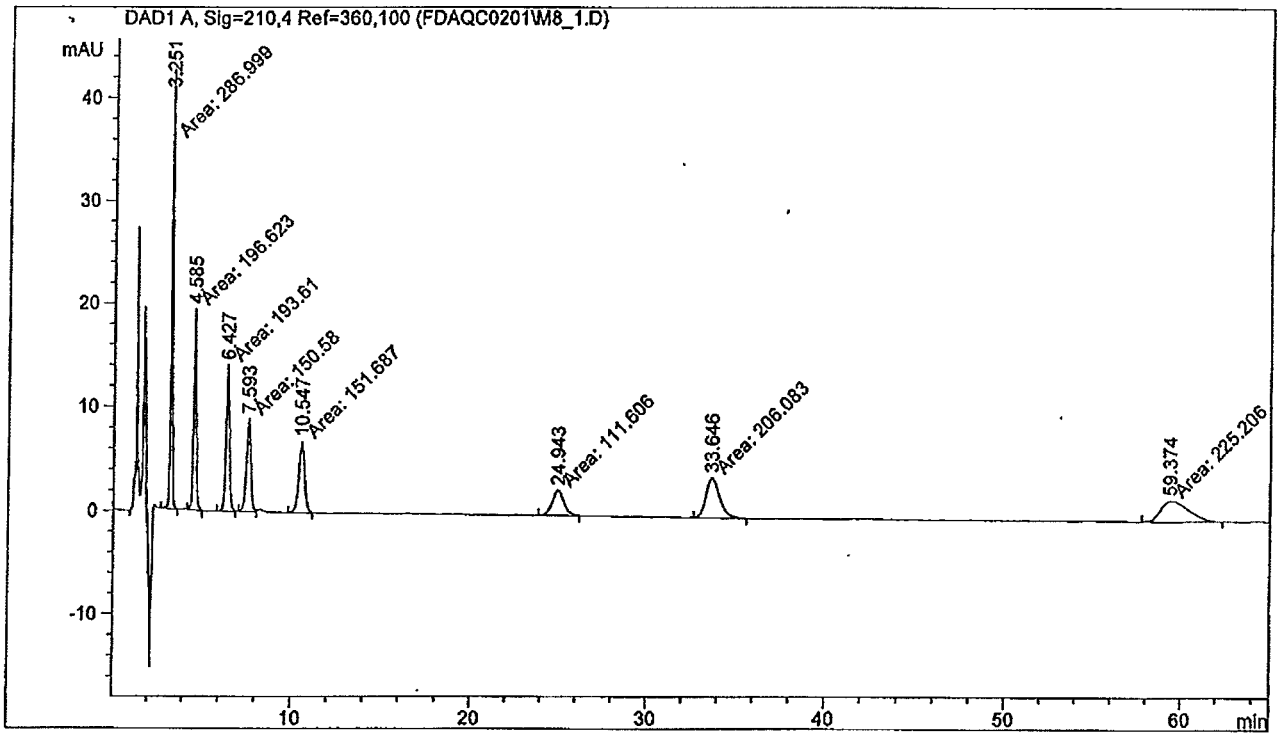
M8 retention time marker (beginning and end of the assay)

Data File C:\CHEM32\1\DATA\FDAQC0201\M8_1.D
Sample Name: M8

Acq. Operator	: PureCircleQC001	Seq. Line :	3
Acq. Instrument	: Instrument 1	Location :	P1-F-03
Injection Date	: 1/2/2010 7:24:00 PM	Inj :	1
		Inj Volume :	12.0 µl

Acq. Method : C:\CHEM32\1\METHODS\NOMSD.M
Last changed : 1/2/2010 7:23:49 PM by PureCircleQC001
(modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\NOMSDDATA.M
Last changed : 1/3/2010 1:34:25 PM
(modified after loading)
Method Info : No MSD Method

Sample Info : M8



Area Percent Report

Sorted By : Signal
Calib. Data Modified : 1/3/2010 1:34:28 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

000156

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
1	3.251	MM	0.1121	286.99930	18.8518	Rub
2	4.585	FM	0.1687	196.62277	12.9154	DulA
3	6.427	MF	0.2295	193.61029	12.7175	Stev
4	7.593	FM	0.2814	150.57967	9.8910	RebC

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578803-K
04 JAN 2010
LABORATORY

Data File C:\CHEM32\1\DATA\FDAQC0201\M8_1.D
Sample Name: M8

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
5	7.990		0.0000	0.00000	0.0000	RebF
6	10.547	MM	0.3726	151.68701	9.9637	RebA
7	24.943	MM	0.7657	111.60615	7.3310	RebD
8	33.646	MM	0.8978	206.08289	13.5368	Sbio
9	59.374	MM	1.8907	225.20590	14.7929	RebB

Totals : 1522.39397

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

*** End of Report ***

Purecircle Sdn Bhd (Formerly known as Stevian Biotechnology Corporation Sdn Bhd)
578803-K
04 JAN 2010
LABORATORY

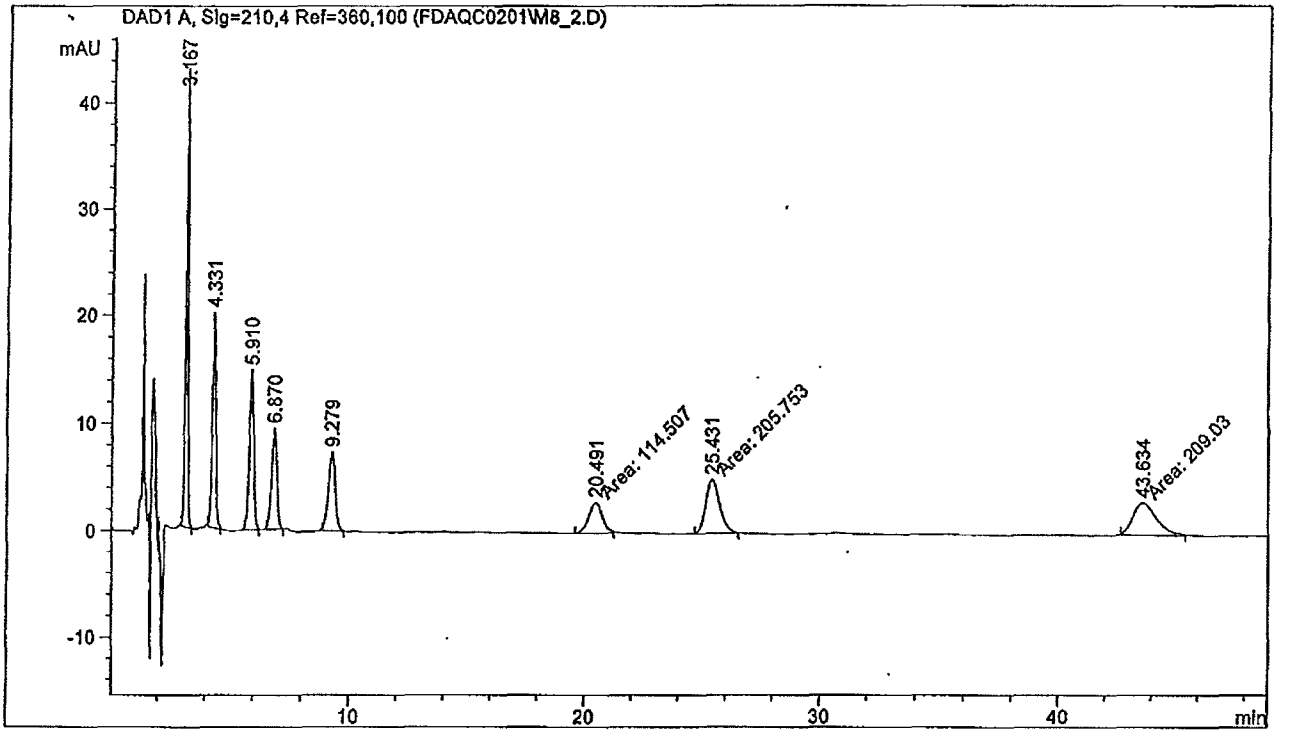
000157

Data File C:\CHEM32\1\DATA\FDAQC0201\M8_2.D
Sample Name: M8

Acq. Operator	: PureCircleQC001	Seq. Line	: 26
Acq. Instrument	: Instrument 1	Location	: P1-F-03
Injection Date	: 1/3/2010 2:10:09 PM	Inj	: 1
		Inj Volume	: 12.0 µl

Acq. Method : C:\CHEM32\1\METHODS\NOMSD.M
Last changed : 1/3/2010 2:59:37 PM by PureCircleQC001
(modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\NOMSDDATA.M
Last changed : 1/3/2010 3:02:56 PM
(modified after loading)
Method Info : No MSD Method

Sample Info : M8



Area Percent Report

Sorted By : Signal
Calib. Data Modified : 1/3/2010 3:02:56 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
1	3.167	BB	0.0979	279.61057	18.9294	Rub
2	4.331	BB	0.1427	190.14603	12.8727	DulA
3	5.910	BB	0.1951	189.15251	12.8054	Stev
4	6.870	BB	0.2304	141.88062	9.6052	RebC

Purecircle Sdn Bhd (Formerly known as Stevian Biotechnology Corporation Sdn Bhd)
578803-K
04 JAN 2010
LABORATORY

000158

Data File C:\CHEM32\1\DATA\FDAQC0201\M8_2.D
Sample Name: M8

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
5	7.447		0.0000	0.00000	0.0000	RebF
6	9.279	BB	0.3061	147.04613	9.9549	RebA
7	20.491	MM	0.6741	114.50650	7.7520	RebD
8	25.431	MM	0.6910	205.75342	13.9293	Sbio
9	43.634	MM	1.1779	209.03041	14.1512	RebB

Totals : 1477.12617

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

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*** End of Report ***

Pureclice Sdn Bhd (Formerly known as Slevian Biotechnology Corporation Sdn Bhd)
578803-K
04 JAN 2010
LABORATORY

000159

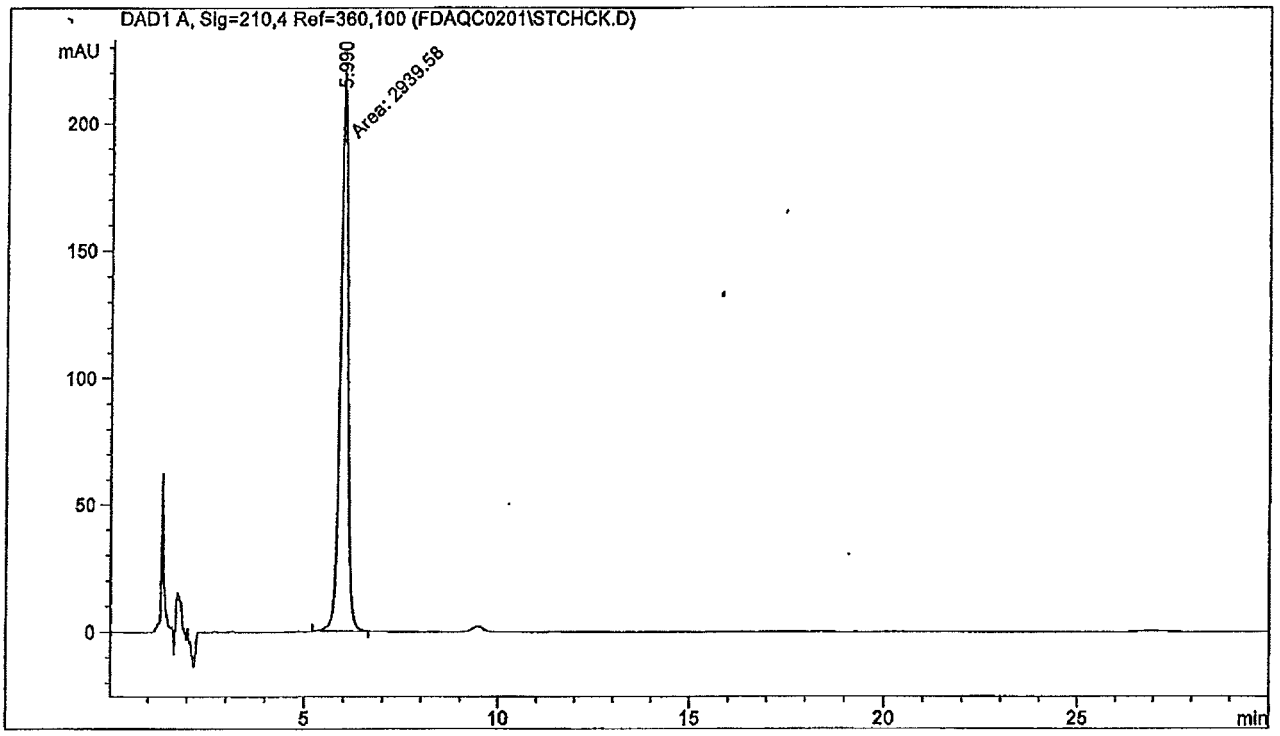
In-assay standard recovery check (Stevioside and Rebaudioside A)

Data File C:\CHEM32\1\DATA\FDAQC0201\STCHCK.D
Sample Name: STL5

Acq. Operator	: PureCircleQC001	Seq. Line	: 20
Acq. Instrument	: Instrument 1	Location	: P1-E-06
Injection Date	: 1/3/2010 9:28:47 AM	Inj	: 1
		Inj Volume	: 12.0 µl

Acq. Method : C:\CHEM32\1\METHODS\NOMSD30.M
Last changed : 1/3/2010 9:28:37 AM by PureCircleQC001
(modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\NOMSDDATA.M
Last changed : 1/3/2010 2:14:24 PM
(modified after loading)
Method Info : No MSD Method

Sample Info : Stev L5 1984.0ppm



External Standard Report

Sorted By : Signal
Calib. Data Modified : 1/3/2010 2:11:10 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount	Grp	Name
3.251		-	-	-		Rub
4.585		-	-	-		DulA
5.990	MM	2939.57690	6.76248e-1	1987.88227		Stev
7.593		-	-	-		RebC
7.990		-	-	-		RebF

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578803-K
04 JAN 2010
LABORATORY

000161

Data File C:\CHEM32\1\DATA\FDAQC0201\STCHCK.D
Sample Name: STL5

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount	Grp	Name
10.094		-	-	-		RebA
24.943		-	-	-		RebD
33.646		-	-	-		Sbio
57.831		-	-	-		RebB

Totals : 1987.88227

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

*** End of Report ***

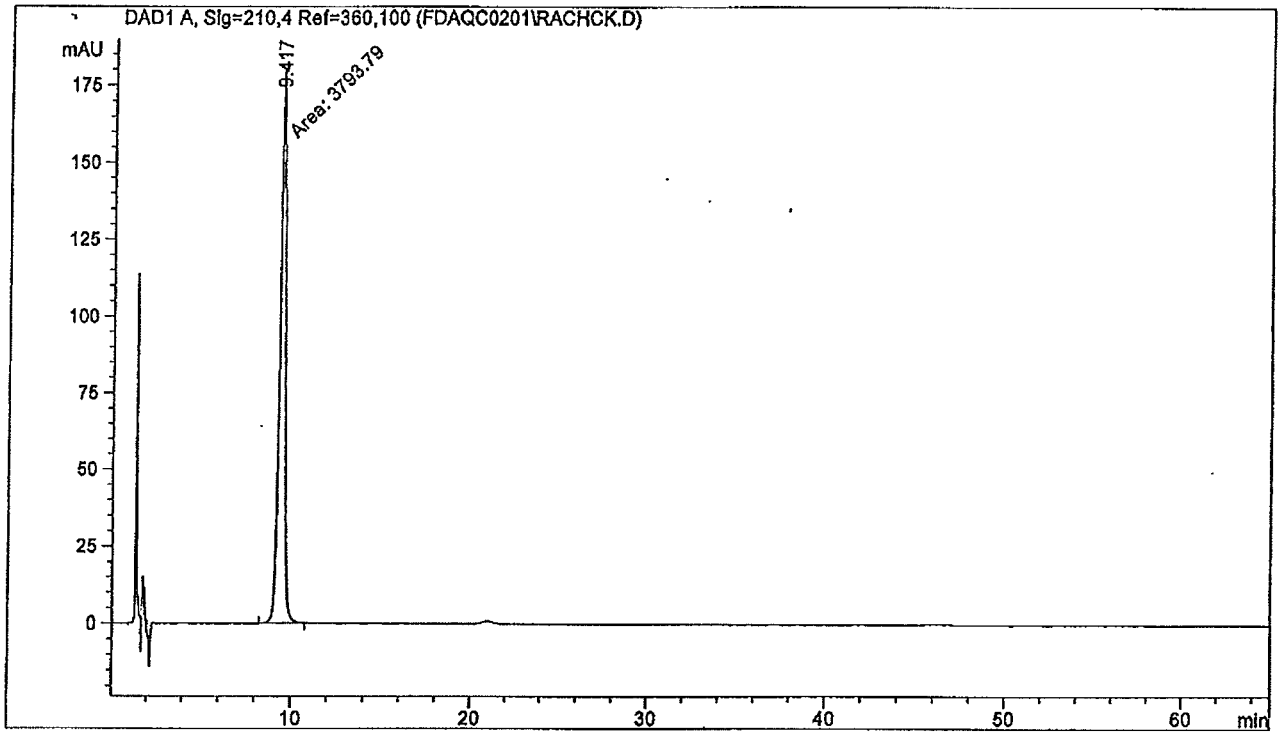
Stevian Biotechnology Corporation Sdn Bhd (formerly known as Pureforce Sdn Bhd)
578803-K
04 JAN 2010
LABORATORY

Data File C:\CHEM32\1\DATA\FDAQC0201\RACHCK.D
Sample Name: RAL3

Acq. Operator	: PureCircleQC001	Seq. Line	: 21
Acq. Instrument	: Instrument 1	Location	: P1-E-09
Injection Date	: 1/3/2010 10:00:02 AM	Inj	: 1
		Inj Volume	: 12.0 µl

Acq. Method : C:\CHEM32\1\METHODS\NOMSD.M
Last changed : 1/3/2010 9:59:52 AM by PureCircleQC001
(modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\NOMSDDATA.M
Last changed : 1/3/2010 2:15:33 PM
(modified after loading)
Method Info : No MSD Method

Sample Info : RebA L3 2995.6ppm



External Standard Report

Sorted By : Signal
Calib. Data Modified : 1/3/2010 2:15:16 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount	Grp	Name
3.251		-	-	-		Rub
4.585		-	-	-		DulA
5.990		-	-	-		Stev
7.593		-	-	-		RebC
7.990		-	-	-		RebF

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578803-K
04 JAN 2010
LABORATORY
PureCircle Sdn Bhd

000163

Data File C:\CHEM32\1\DATA\FDAQC0201\RACHCK.D
Sample Name: RAL3

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount	Grp	Name
9.417	MM	3793.78564	7.89569e-1	2995.45582		RebA
24.943		-	-	-		RebD
33.646		-	-	-		Sbio
57.831		-	-	-		RebB

Totals : 2995.45582

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

==========

*** End of Report ***

Pureclice Sdn Bhd (Formerly known as Stevian Biotechnology Corporation Sdn Bhd)
578803-K
04 JAN 2010
LABORATORY

000164

APPENDIX D

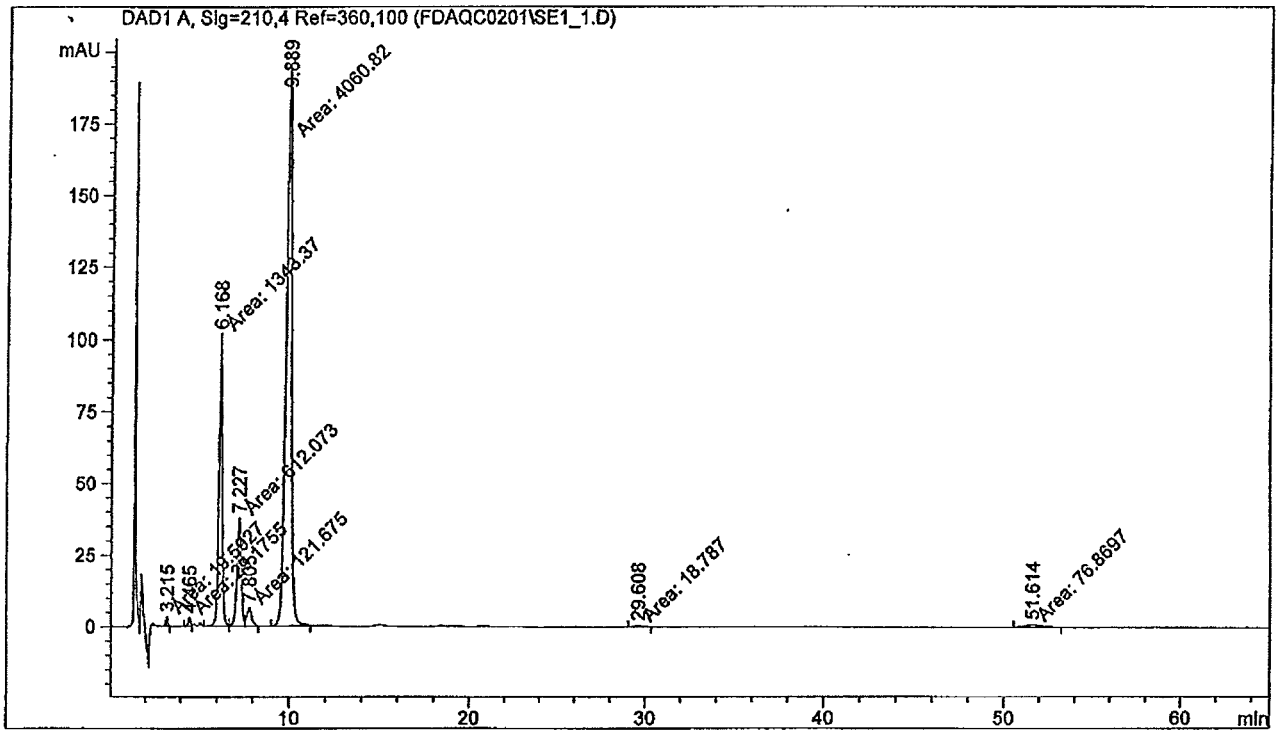
Sample Chromatograms

Data File C:\CHEM32\1\DATA\FDAQC0201\SE1_1.D
Sample Name: SE1_1

Acq. Operator	: PureCircleQC001	Seq. Line	: 14
Acq. Instrument	: Instrument 1	Location	: P1-D-01
Injection Date	: 1/3/2010 2:51:47 AM	Inj	: 1
		Inj Volume	: 12.0 µl

Acq. Method : C:\CHEM32\1\METHODS\NOMSD.M
Last changed : 1/3/2010 1:45:28 AM by PureCircleQC001
(modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\NOMSDDATA.M
Last changed : 1/3/2010 3:25:45 PM
(modified after loading)
Method Info : No MSD Method

Sample Info : SE (1_1) Lot# A0912266 5012 mg/L (KF 0.8%)



External Standard Report

Sorted By : Signal
Calib. Data Modified : Sunday, January 03, 2010 3:20:32 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount	Grp	Name
3.215	MM	19.59269	6.76248e-1	10.59961		Rub
4.465	MF	29.17554	6.76248e-1	19.33531		DulA
6.168	MF	1343.37036	6.76248e-1	908.45119		Stev
7.227	MF	612.07312	6.76248e-1	488.41760		RebC
7.805	FM	121.67471	6.76248e-1	95.44744		RebF

PureCircle Sdn Bhd (Formerly Known as Stevian Biotechnology Corporation Sdn Bhd)
578803-K
04 JAN 2010
LABORATORY

000166

Data File C:\CHEM32\1\DATA\FDAQC0201\SE1_1.D
Sample Name: SE1_1

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount	Grp	Name
9.889	MM	4060.82373	7.89569e-1	3206.30084		RebA
20.900		-	-	-		RebD
29.608	MM	18.78704	6.76248e-1	10.16376		Sbio
51.614	MM	76.86970	6.76248e-1	51.98298		RebB

Totals : 4790.69873

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

*** End of Report ***

Pureforce Sdn Bhd (formerly known as Siswan Biotechnology Corporation Sdn Bhd)
578803-K
04 JAN 2010
LABORATORY

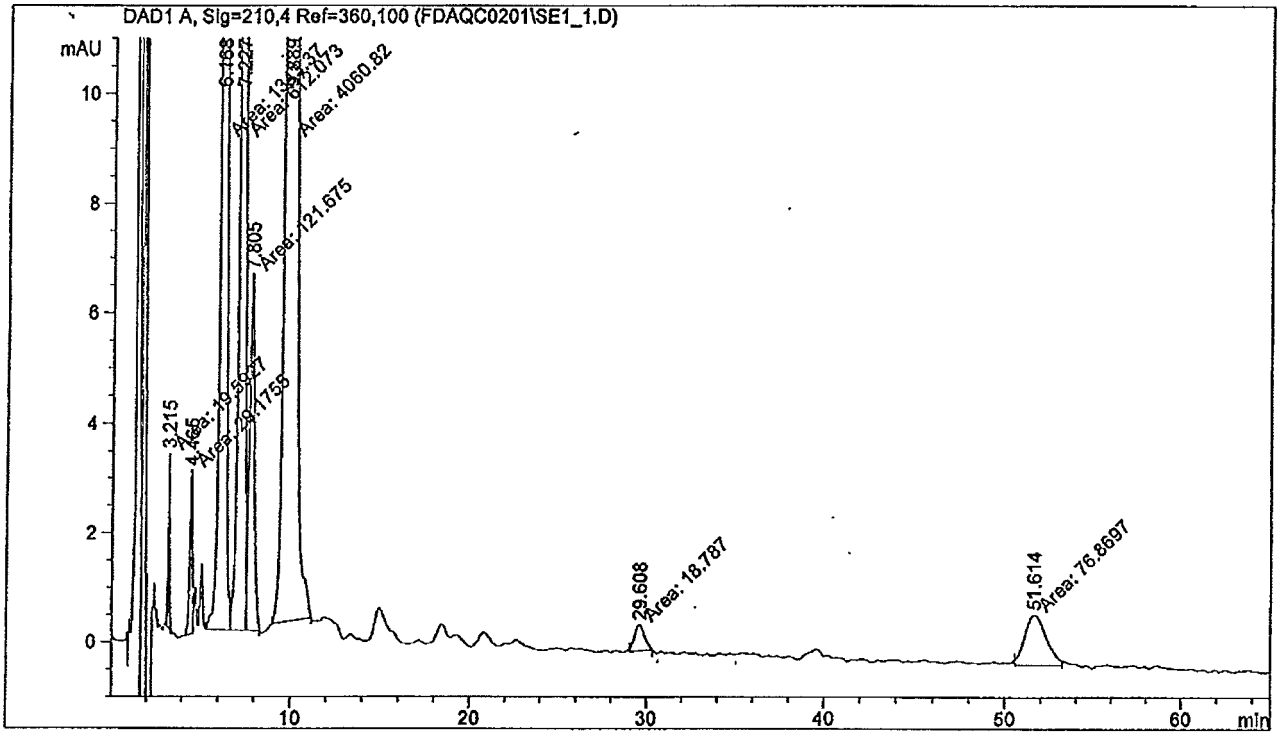
000167

Data File C:\CHEM32\1\DATA\FDAQC0201\SE1_1.D
Sample Name: SE1_1

Acq. Operator	: PureCircleQC001	Seq. Line	: 14
Acq. Instrument	: Instrument 1	Location	: P1-D-01
Injection Date	: 1/3/2010 2:51:47 AM	Inj	: 1
		Inj Volume	: 12.0 µl

Acq. Method : C:\CHEM32\1\METHODS\NOMSD.M
Last changed : 1/3/2010 1:45:28 AM by PureCircleQC001
(modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\NOMSDDATA.M
Last changed : 1/3/2010 3:20:33 PM
(modified after loading)
Method Info : No MSD Method

Sample Info : SE (1_1) Lot# A0912266 5012 mg/L (KF 0.8%)



External Standard Report

Sorted By : Signal
Calib. Data Modified : Sunday, January 03, 2010 3:20:32 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount	Grp	Name
3.215	MM	19.59269	6.76248e-1	10.59961		Rub 0,21
4.465	MF	29.17554	6.76248e-1	19.33531		DulA 0,39
6.168	MF	1343.37036	6.76248e-1	908.45119		Stev 18,27
7.227	MF	612.07312	6.76248e-1	488.41760		RebC 9,82
7.805	FM	121.67471	6.76248e-1	95.44744		RebF 1,92

000168

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578803-K
04 JAN 2010
LABORATORY
PureCircle Sdn Bhd

Data File C:\CHEM32\1\DATA\FDAQC0201\SE1_1.D
Sample Name: SE1_1

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount	Grp	Name	
9.889	MM	4060.82373	7.89569e-1	3206.30084		RebA	64,48
20.900		-	-	-		RebD	
29.608	MM	18.78704	6.76248e-1	10.16376		Sbio	0,20
51.614	MM	76.86970	6.76248e-1	51.98298		RebB	1,05
Totals :				4790.69873			96,36%

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

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

000169

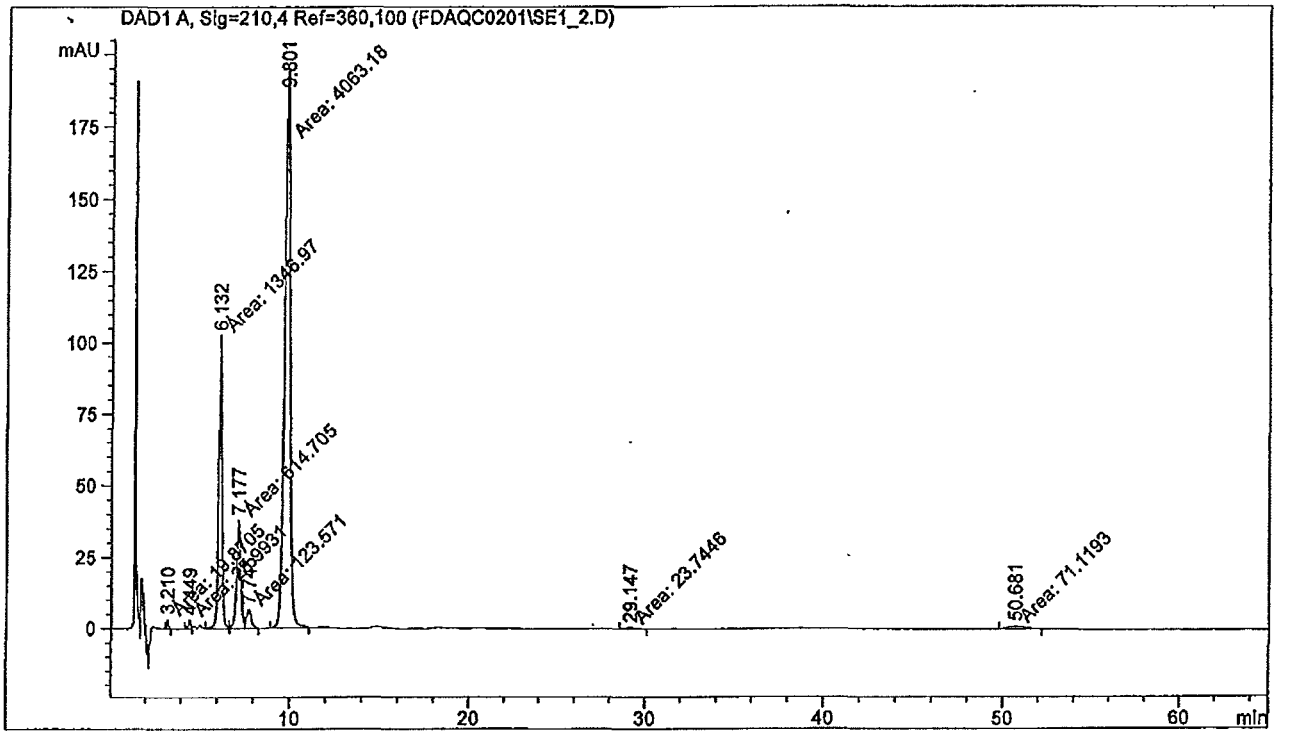
Puteclice Sdn Bhd (formerly known as Stevian Biotechnology Corporation Sdn Bhd)
573803-K
04 JAN 2010
LABORATORY

Data File C:\CHEM32\1\DATA\FDAQC0201\SE1_2.D
Sample Name: SE1_2

Acq. Operator	: PureCircleQC001	Seq. Line	: 15
Acq. Instrument	: Instrument 1	Location	: P1-D-02
Injection Date	: 1/3/2010 3:57:55 AM	Inj	: 1
		Inj Volume	: 12.0 µl

Acq. Method : C:\CHEM32\1\METHODS\NOMSD.M
Last changed : 1/3/2010 1:45:28 AM by PureCircleQC001
(modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\NOMSDDATA.M
Last changed : 1/3/2010 3:33:47 PM
(modified after loading)
Method Info : No MSD Method

Sample Info : SE (1_2) Lot# A0912266 5004 mg/L (KF 0.8%)



External Standard Report

Sorted By : Signal
Calib. Data Modified : 1/3/2010 3:26:36 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount	Grp	Name
3.210	MM	19.87049	6.76248e-1	10.74990		Rub
4.449	MF	28.99305	6.76248e-1	19.21436		DulA
6.132	MF	1346.97180	6.76248e-1	910.88665		Stev
7.177	MF	614.70490	6.76248e-1	490.51769		RebC
7.745	FM	123.57104	6.76248e-1	96.93502		RebF

Pureciale Sdn Bhd (formerly known as Stevian Biotechnology Corporation Sdn Bhd)
578803-K
04 JAN 2010
LABORATORY

000170

Data File C:\CHEM32\1\DATA\FDAQC0201\SE1_2.D
Sample Name: SE1_2

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount	Grp	Name
9.801	MM	4063.17700	7.89569e-1	3208.15891		RebA
20.900		-	-	-		RebD
29.147	MM	23.74459	6.76248e-1	12.84578		Sbio
50.681	MM	71.11926	6.76248e-1	48.09426		RebB

Totals : 4797.40258

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

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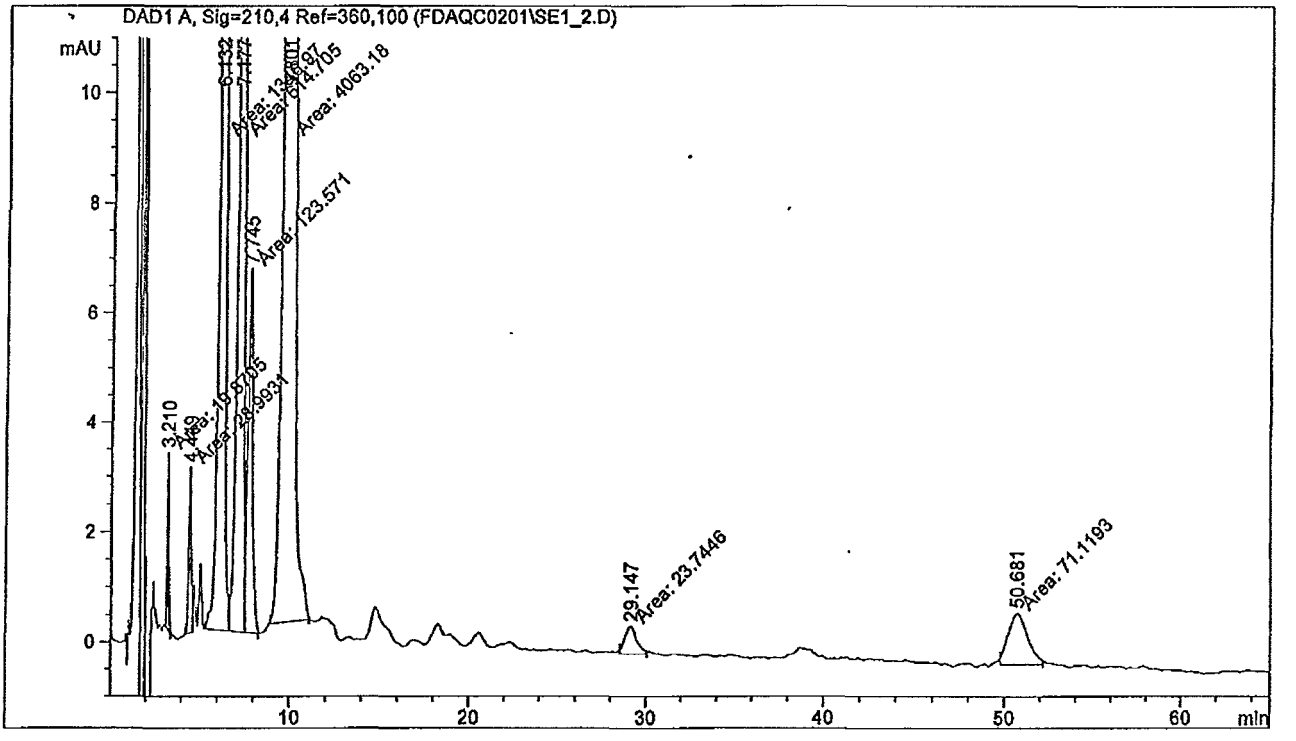
*** End of Report ***

Stevian Biotechnology Corporation Sdn Bhd
Puncak Sdn Bhd (formerly known as)
57803-K
0 4 JAN 2010
LABORATORY

Data File C:\CHEM32\1\DATA\FDAQC0201\SE1_2.D
Sample Name: SE1_2

Acq. Operator	: PureCircleQC001	Seq. Line :	15
Acq. Instrument	: Instrument 1	Location :	P1-D-02
Injection Date	: 1/3/2010 3:57:55 AM	Inj :	1
		Inj Volume :	12.0 µl
Acq. Method	: C:\CHEM32\1\METHODS\NOMSD.M		
Last changed	: 1/3/2010 1:45:28 AM by PureCircleQC001 (modified after loading)		
Analysis Method	: C:\CHEM32\1\METHODS\NOMSDDATA.M		
Last changed	: 1/3/2010 3:29:15 PM (modified after loading)		
Method Info	: No MSD Method		
Sample Info	: SE (1_2) Lot# A0912266 5004 mg/L (KF 0.8%)		



External Standard Report

Sorted By	:	Signal
Calib. Data Modified	:	1/3/2010 3:26:36 PM
Multiplier:	:	1.0000
Dilution:	:	1.0000

Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount	Grp	Name
3.210	MM	19.87049	6.76248e-1	10.74990		Rub 0,22
4.449	MF	28.99305	6.76248e-1	19.21436		DulA 0,39
6.132	MF	1346.97180	6.76248e-1	910.88665		Stev 18,35
7.177	MF	614.70490	6.76248e-1	490.51769		RebC 9,88
7.745	FM	123.57104	6.76248e-1	96.93502		RebF 1,95

000172

Purecircle Sdn Bhd (formerly known as Stevian Biotechnology Corporation Sdn Bhd)
578303-K
04 JAN 2010
LABORATORY

Data File C:\CHEM32\1\DATA\FDAQC0201\SE1_2.D
Sample Name: SE1_2

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount	Grp	Name	
9.801	MM	4063.17700	7.89569e-1	3208.15891		RebA	64,63
20.900		-	-	-		RebD	
29.147	MM	23.74459	6.76248e-1	12.84578		Sbio	0,26
50.681	MM	71.11926	6.76248e-1	48.09426		RebB	0,97
Totals :				4797.40258			96,64 %

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

==========

*** End of Report ***

000173

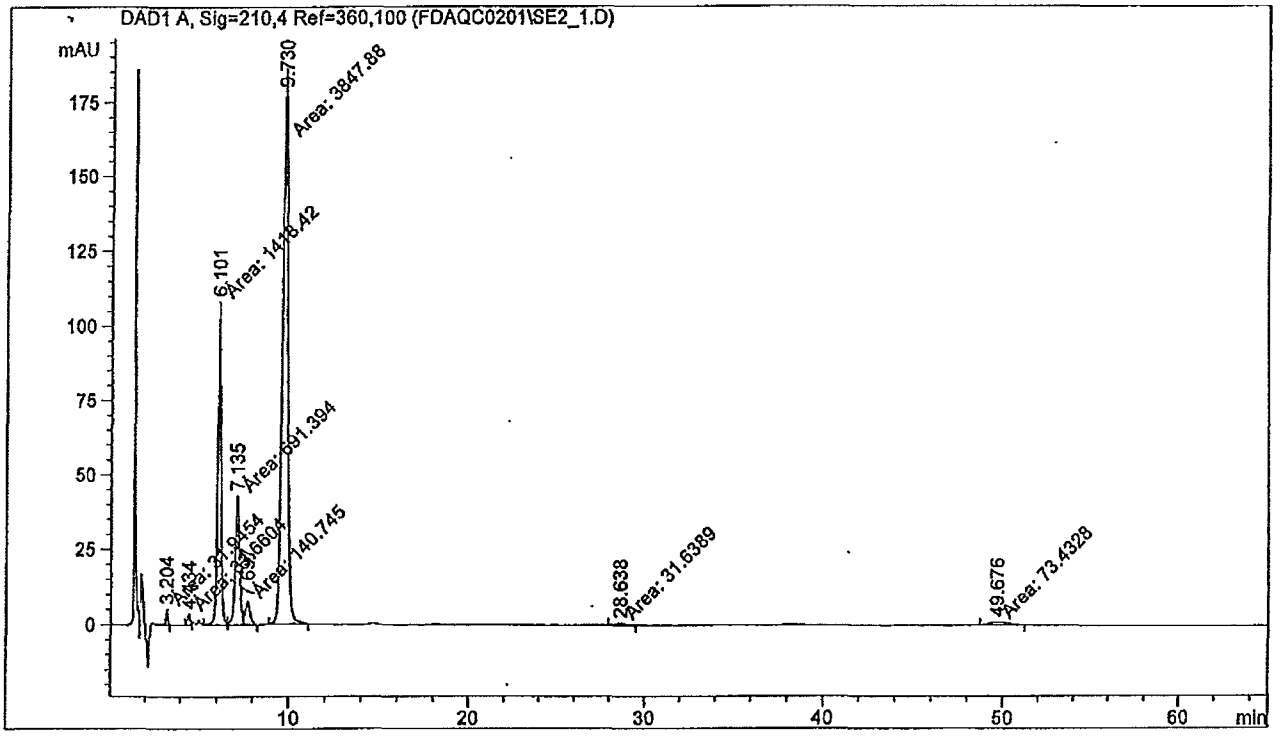
Puncture Sdn Bhd (Formerly known as Stevian Biotechnology Corporation Sdn Bhd)
573803-K
04 JAN 2010
LABORATORY

Data File C:\CHEM32\1\DATA\FDAQC0201\SE2_1.D
Sample Name: SE2_1

Acq. Operator	: PureCircleQC001	Seq. Line	: 16
Acq. Instrument	: Instrument 1	Location	: P1-D-03
Injection Date	: 1/3/2010 5:04:04 AM	Inj	: 1
		Inj Volume	: 12.0 µl

Acq. Method : C:\CHEM32\1\METHODS\NOMSD.M
Last changed : 1/3/2010 1:45:28 AM by PureCircleQC001
(modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\NOMSDDATA.M
Last changed : 1/3/2010 3:45:11 PM
(modified after loading)
Method Info : No MSD Method

Sample Info : SE (2_1) Lot# A0912267 5004 mg/L (KF 1.0%)



External Standard Report

Sorted By : Signal
Calib. Data Modified : 1/3/2010 3:37:45 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount	Grp	Name
3.204	MM	31.94542	6.76248e-1	17.28242		Rub
4.434	MF	33.66042	6.76248e-1	22.30753		DulA
6.101	MF	1418.41589	6.76248e-1	959.20056		Stev
7.135	FM	691.39392	6.76248e-1	551.71343		RebC
7.697	FM	140.74516	6.76248e-1	110.40722		RebF

Pure Circle Sdn Bhd (Formerly known as Stevian Biotechnology Corporation Sdn Bhd)
576803-K
04 JAN 2010
LABORATORY

000174

Data File C:\CHEM32\1\DATA\FDAQC0201\SE2_1.D
Sample Name: SE2_1

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount	Grp	Name
9.730	MM	3847.87500	7.89569e-1	3038.16310		RebA
20.900		-	-	-		RebD
28.638	MM	31.63887	6.76248e-1	17.11658		Sbio
49.676	MM	73.43282	6.76248e-1	49.65879		RebB

Totals : 4765.84964

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

*** End of Report ***

Stevian Biotechnology Corporation Sdn Bhd
Pusat Sun Bhd (formerly known as Stevian Biotechnology)
STB30-K
0 4 JAN 2010
LABORATORY

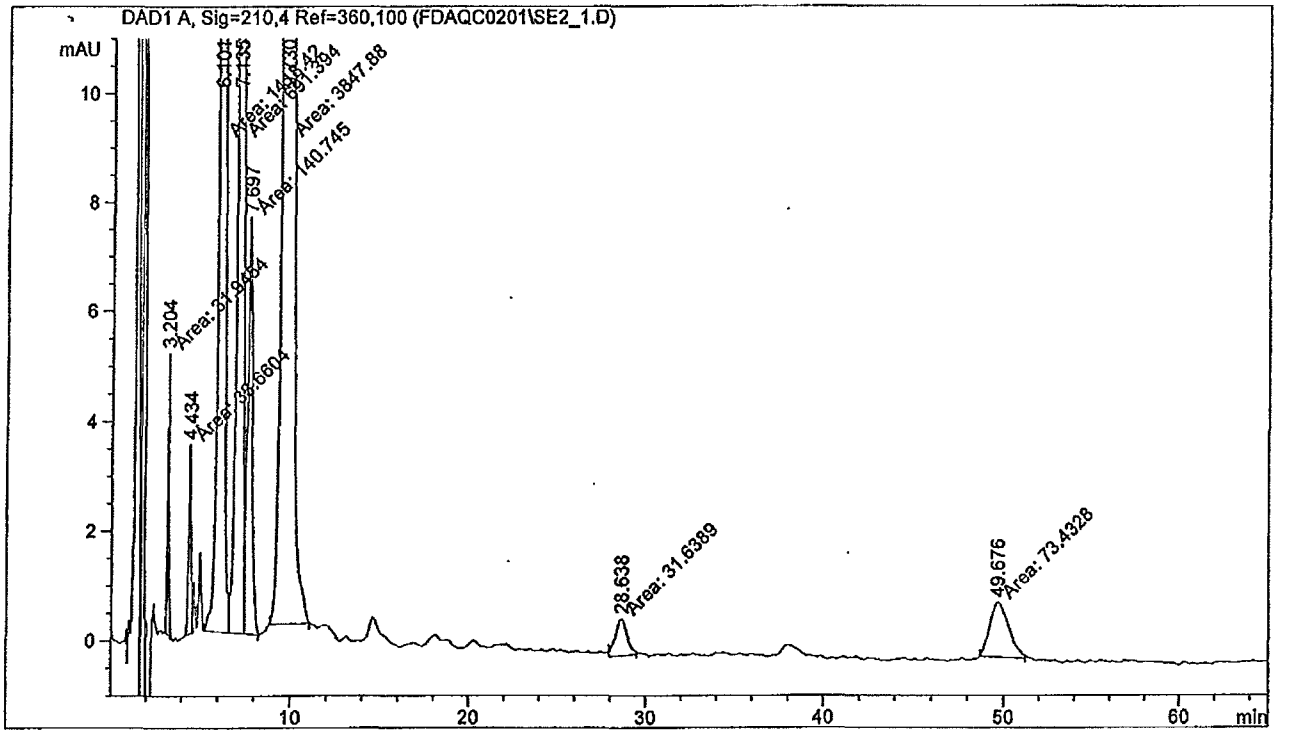
000175

Data File C:\CHEM32\1\DATA\FDAQC0201\SE2_1.D
Sample Name: SE2_1

Acq. Operator	: PureCircleQC001	Seq. Line	: 16
Acq. Instrument	: Instrument 1	Location	: P1-D-03
Injection Date	: 1/3/2010 5:04:04 AM	Inj	: 1
		Inj Volume	: 12.0 µl

Acq. Method : C:\CHEM32\1\METHODS\NOMSD.M
Last changed : 1/3/2010 1:45:28 AM by PureCircleQC001
(modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\NOMSDDATA.M
Last changed : 1/3/2010 3:41:05 PM
(modified after loading)
Method Info : No MSD Method

Sample Info : SE (2_1) Lot# A0912267 5004 mg/L (KF 1.0%)



External Standard Report

Sorted By : Signal
Calib. Data Modified : 1/3/2010 3:37:45 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

000176

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount	Grp	Name
3.204	MM	31.94542	6.76248e-1	17.28242		Rub 0,35
4.434	MF	33.66042	6.76248e-1	22.30753		DulA 0,45
6.101	MF	1418.41589	6.76248e-1	959.20056		Stev 19,36
7.135	FM	691.39392	6.76248e-1	551.71343		RebC 11,14
7.697	FM	140.74516	6.76248e-1	110.40722		RebF 2,23

Purecircle Sdn Bhd (Formerly known as Stevian Biotechnology Corporation Sdn Bhd)
578803-K
04 JAN 2010
LABORATORY

Data File C:\CHEM32\1\DATA\FDAQC0201\SE2_1.D
Sample Name: SE2_1

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount	Grp	Name
9.730	MM	3847.87500	7.89569e-1	3038.16310		RebA 61,33
20.900		-	-	-		RebD
28.638	MM	31.63887	6.76248e-1	17.11658		Sbio 0,35
49.676	MM	73.43282	6.76248e-1	49.65879		RebB 1,00
Totals :				4765.84964		96,20

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

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*** End of Report ***

000177

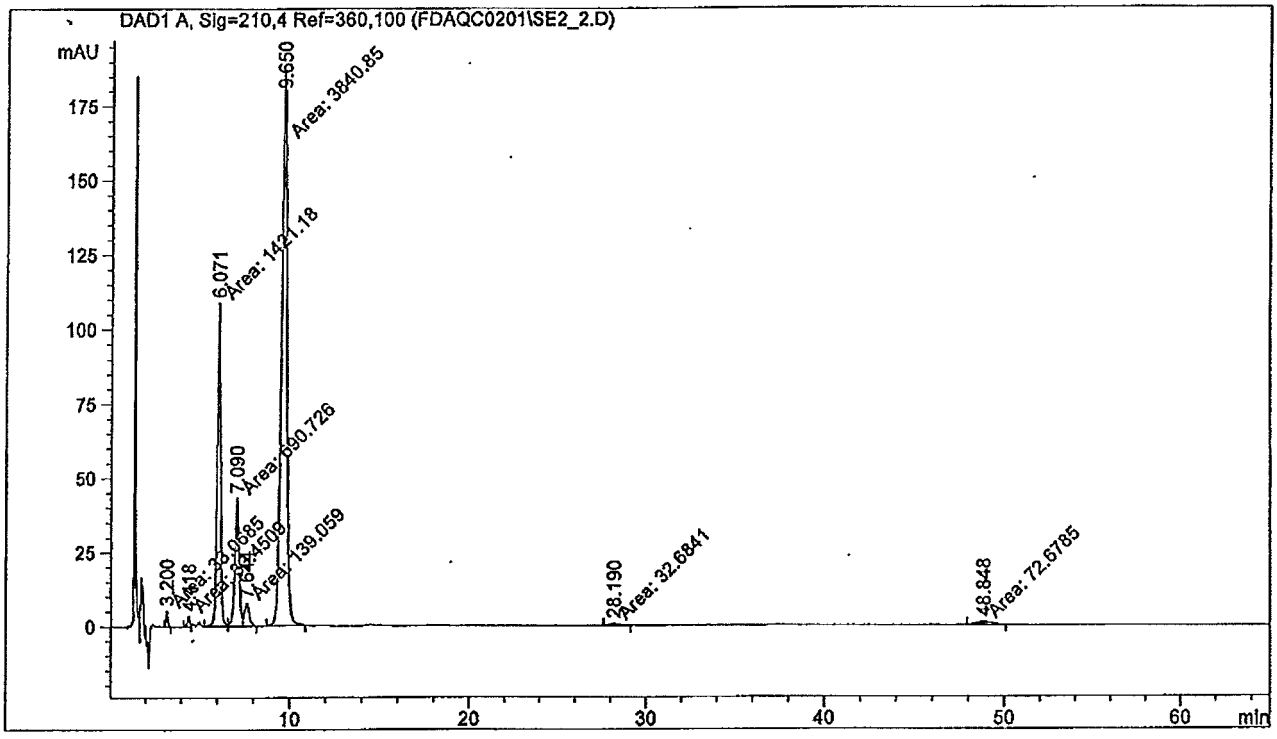
Stevian Biotechnology Corporation Sdn Bhd
572203-K
04 JAN 2010
LABORATORY
Puncak Sdn Bhd

Data File C:\CHEM32\1\DATA\FDAQC0201\SE2_2.D
Sample Name: SE2_2

Acq. Operator	: PureCircleQC001	Seq. Line	: 17
Acq. Instrument	: Instrument 1	Location	: P1-D-04
Injection Date	: 1/3/2010 6:10:13 AM	Inj	: 1
		Inj Volume	: 12.0 µl

Acq. Method : C:\CHEM32\1\METHODS\NOMSD.M
Last changed : 1/3/2010 1:45:28 AM by PureCircleQC001
(modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\NOMSDDATA.M
Last changed : 1/3/2010 3:51:41 PM
(modified after loading)
Method Info : No MSD Method

Sample Info : SE (2_2) Lot# A0912267 5008 mg/L (KF 1.0%)



External Standard Report

Sorted By : Signal
Calib. Data Modified : 1/3/2010 3:37:45 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

000178

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount	Grp	Name
3.200	MM	33.06848	6.76248e-1	17.89000		Rub
4.418	MF	33.45094	6.76248e-1	22.16871		DulA
6.071	MF	1421.17993	6.76248e-1	961.06973		Stev
7.090	MF	690.72565	6.76248e-1	551.18017		RebC
7.644	FM	139.05946	6.76248e-1	109.08487		RebF

PureCircle Sdn Bhd (Formerly known as Stevian Biotechnology Corporation Sdn Bhd)
578803-K
04 JAN 2010
LABORATORY

Data File C:\CHEM32\1\DATA\FDAQC0201\SE2_2.D
Sample Name: SE2_2

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount	Grp	Name
9.650	MM	3840.85400	7.89569e-1	3032.61954		RebA
20.900		-	-	-		RebD
28.190	MM	32.68412	6.76248e-1	17.68206		Sbio
48.848	MM	72.67847	6.76248e-1	49.14867		RebB

Totals : 4760.84374

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

*** End of Report ***

Stevian Biotechnology Corporation Sdn Bhd (formerly known as Stevian Life Sciences Sdn Bhd)
578803-K
04 JAN 2010
LABORATORY

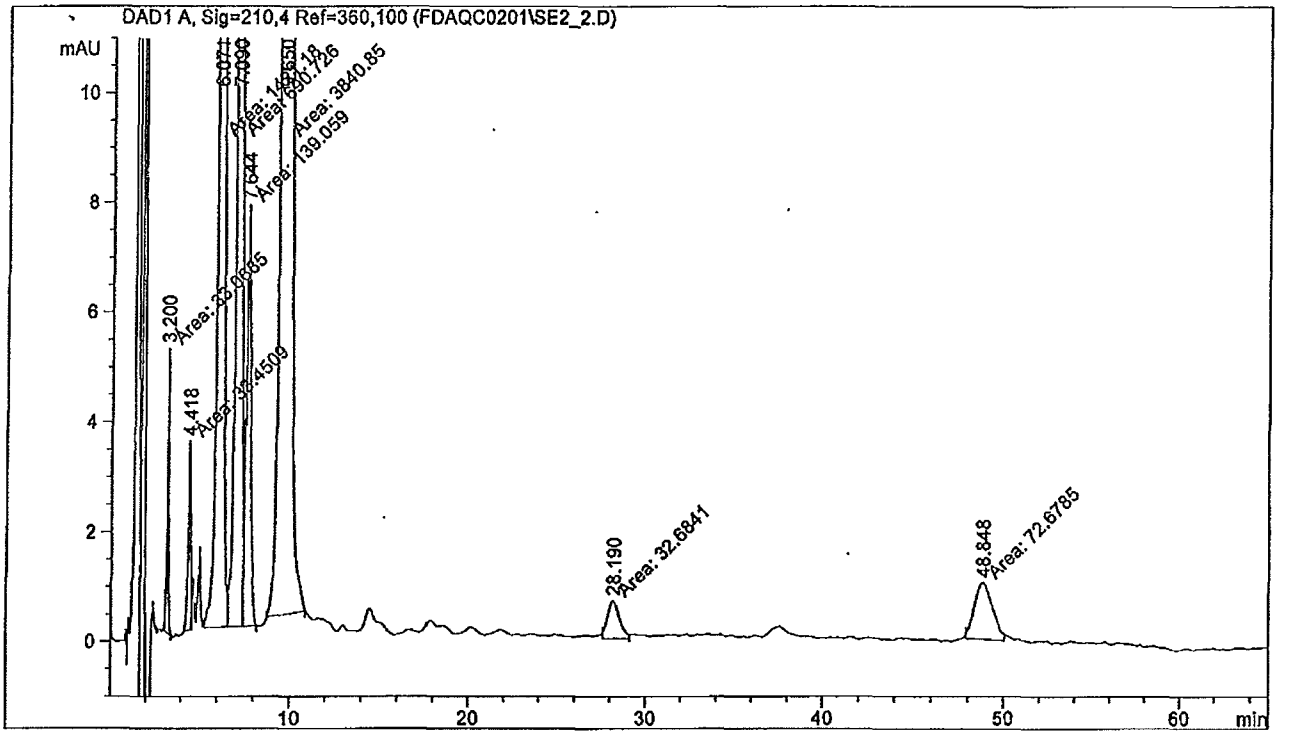
000179

Data File C:\CHEM32\1\DATA\FDAQC0201\SE2_2.D
Sample Name: SE2_2

Acq. Operator	: PureCircleQC001	Seq. Line	: 17
Acq. Instrument	: Instrument 1	Location	: P1-D-04
Injection Date	: 1/3/2010 6:10:13 AM	Inj	: 1
		Inj Volume	: 12.0 µl

Acq. Method : C:\CHEM32\1\METHODS\NOMSD.M
Last changed : 1/3/2010 1:45:28 AM by PureCircleQC001
(modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\NOMSDDATA.M
Last changed : 1/3/2010 3:46:00 PM
(modified after loading)
Method Info : No MSD Method

Sample Info : SE (2_2) Lot# A0912267 5008 mg/L (KF 1.0%)



External Standard Report

Sorted By : Signal
Calib. Data Modified : 1/3/2010 3:37:45 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

000180

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount	Grp	Name	
3.200	MM	33.06848	6.76248e-1	17.89000		Rub	0,36
4.418	MF	33.45094	6.76248e-1	22.16871		DulA	0,45
6.071	MF	1421.17993	6.76248e-1	961.06973		Stev	19,38
7.090	MF	690.72565	6.76248e-1	551.18017		RebC	11,12
7.644	FM	139.05946	6.76248e-1	109.08487		RebF	2,20

PureCircle Sdn Bhd (Formerly known as Stevian Biotechnology Corporation Sdn Bhd)
578803-K
04 JAN 2010
LABORATORY

Data File C:\CHEM32\1\DATA\FDAQC0201\SE2_2.D
Sample Name: SE2_2

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount	Grp	Name	
9.650	MM	3840.85400	7.89569e-1	3032.61954		RebA	61,17
20.900		-	-	-		RebD	
28.190	MM	32.68412	6.76248e-1	17.68206		Sbio	0,36
48.848	MM	72.67847	6.76248e-1	49.14867		RebB	0,99

Totals : 4760.84374 96,03%

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

*** End of Report ***

000181

Stevian Biotechnology Corporation Sdn Bhd (formerly known as Pureclure Sdn Bhd)
578903-K
04 JAN 2010
LABORATORY

Data File C:\CHEM32\1\DATA\FDAQC0201\SE3_1.D
Sample Name: SE3_1

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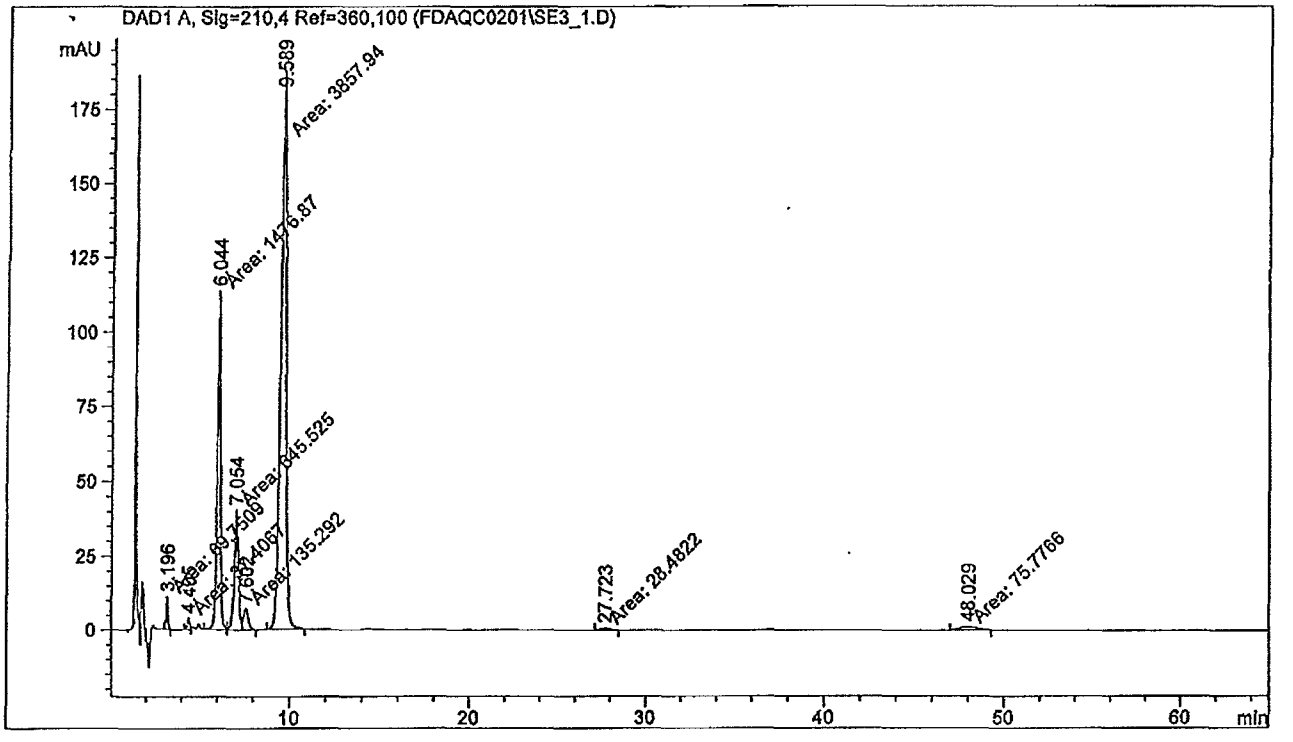
Acq. Operator	: PureCircleQC001	Seq. Line	: 18
Acq. Instrument	: Instrument 1	Location	: P1-D-05
Injection Date	: 1/3/2010 7:16:23 AM	Inj	: 1
		Inj Volume	: 12.0 µl

Acq. Method : C:\CHEM32\1\METHODS\NOMSD.M
Last changed : 1/3/2010 1:45:28 AM by PureCircleQC001
(modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\NOMSDDATA.M
Last changed : 1/3/2010 3:59:45 PM
(modified after loading)
Method Info : No MSD Method

Sample Info : SE (3_1) Lot# A0912268 5008 mg/L (KF 1.2%)



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External Standard Report

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Sorted By : Signal
Calib. Data Modified : 1/3/2010 3:55:51 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount	Grp	Name
3.196	MM	69.75095	6.76248e-1	37.73515		Rub
4.405	MF	37.40674	6.76248e-1	24.79031		DulA
6.044	MF	1476.87146	6.76248e-1	998.73100		Stev
7.054	MF	645.52466	6.76248e-1	515.11102		RebC
7.602	FM	135.29163	6.76248e-1	106.12920		RebF

000182

Pureface Sdn Bhd (Formerly known as Stevian Biotechnology Corporation Sdn Bhd)
578303-K
04 JAN 2010
LABORATORY

Data File C:\CHEM32\1\DATA\FDAQC0201\SE3_1.D
Sample Name: SE3_1

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount	Grp	Name
9.589	MM	3857.93896	7.89569e-1	3046.10930		RebA
20.900		-	-	-		RebD
27.723	MM	28.48216	6.76248e-1	15.40880		Sbio
48.029	MM	75.77657	6.76248e-1	51.24375		RebB

Totals : 4795.25854

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

*** End of Report ***

000183

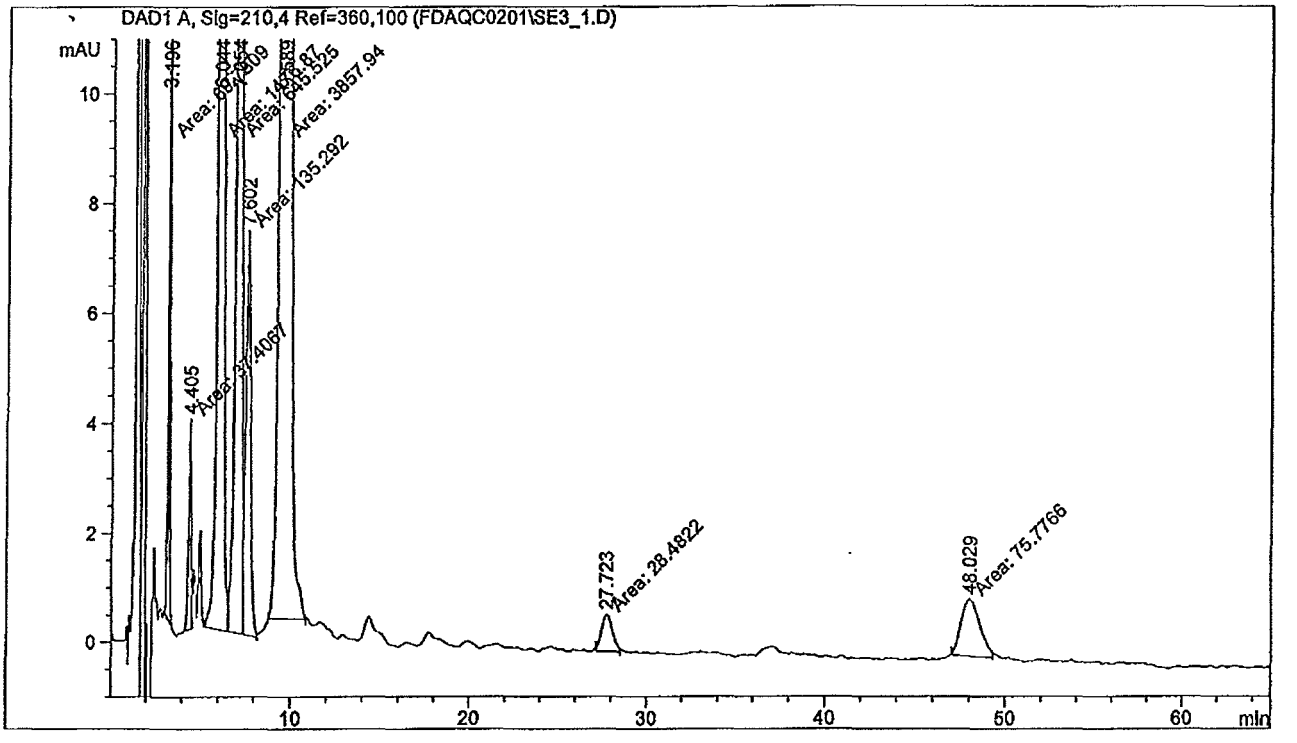
Pureclice Sdn Bhd (formerly known as Stevian Biotechnology Corporation Sdn Bhd)
575903-K
LABORATORY

Data File C:\CHEM32\1\DATA\FDAQC0201\SE3_1.D
Sample Name: SE3_1

Acq. Operator	: PureCircleQC001	Seq. Line	: 18
Acq. Instrument	: Instrument 1	Location	: P1-D-05
Injection Date	: 1/3/2010 7:16:23 AM	Inj	: 1
		Inj Volume	: 12.0 µl

Acq. Method : C:\CHEM32\1\METHODS\NOMSD.M
Last changed : 1/3/2010 1:45:28 AM by PureCircleQC001
(modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\NOMSDDATA.M
Last changed : 1/3/2010 3:55:46 PM
(modified after loading)
Method Info : No MSD Method

Sample Info : SE (3_1) Lot# A0912268 5008 mg/L (KF 1.2%)



External Standard Report

Sorted By : Signal
Calib. Data Modified : 1/3/2010 3:55:51 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount	Grp	Name
3.196	MM	69.75095	6.76248e-1	37.73515		Rub 0,76
4.405	MF	37.40674	6.76248e-1	24.79031		DulA 0,50
6.044	MF	1476.87146	6.76248e-1	998.73100		Stev 20,18
7.054	MF	645.52466	6.76248e-1	515.11102		RebC 10,41
7.602	FM	135.29163	6.76248e-1	106.12920		RebF 2,14

Purecircle Sdn Bhd (Formerly known as Slevian Biotechnology Corporation Sdn Bhd) 578803-K 04 JAN 2010 LABORATORY

000184

Data File C:\CHEM32\1\DATA\FDAQC0201\SE3_1.D
Sample Name: SE3_1

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount	Grp	Name	
9.589	MM	3857.93896	7.89569e-1	3046.10930		RebA	61.56
20.900		-	-	-		RebD	
27.723	MM	28.48216	6.76248e-1	15.40880		Sbio	0.31
48.029	MM	75.77657	6.76248e-1	51.24375		RebB	1.04

Totals : 4795.25854 96.91%

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

*** End of Report ***

Stevian Biotechnology Corporation Sdn Bhd
Purecircle Sdn Bhd (formerly known as)
578503-K
04 JAN 2010
LABORATORY

000185

Data File C:\CHEM32\1\DATA\FDAQC0201\SE3_2.D
Sample Name: SE3_2

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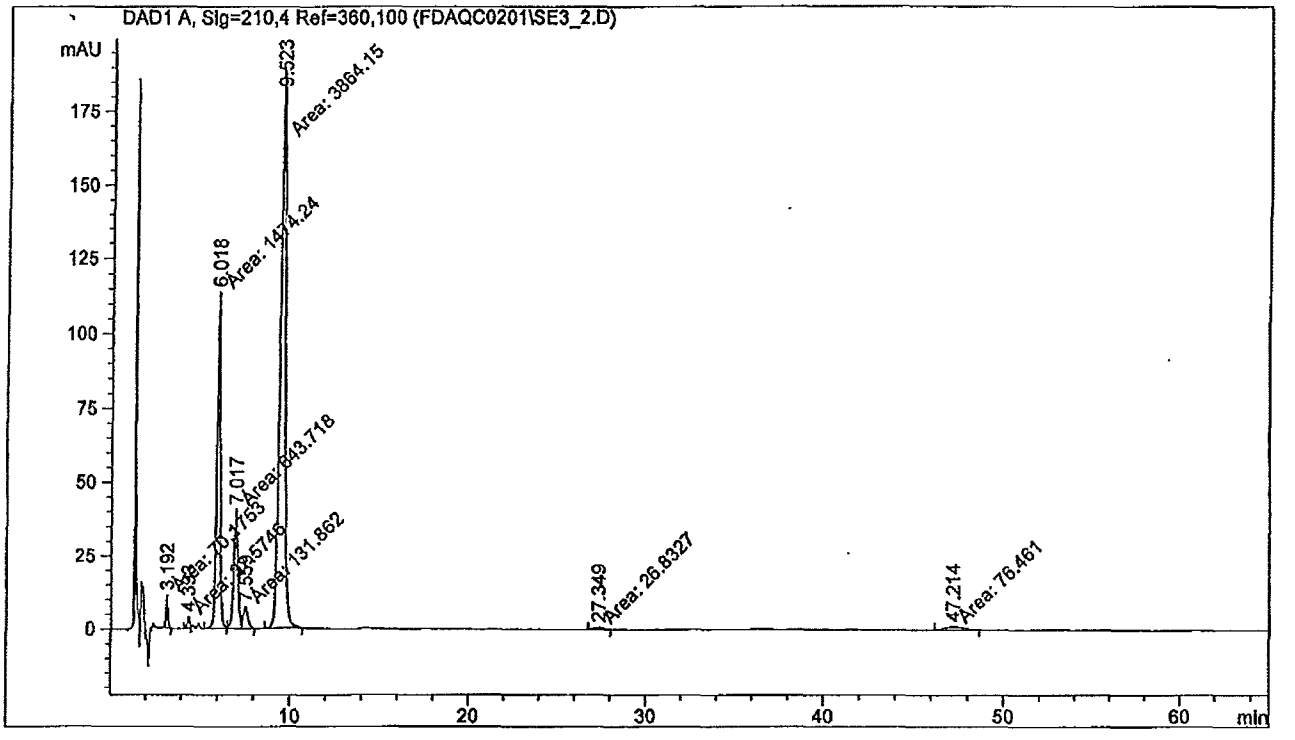
Acq. Operator	: PureCircleQC001	Seq. Line :	19
Acq. Instrument	: Instrument 1	Location :	P1-D-06
Injection Date	: 1/3/2010 8:22:31 AM	Inj :	1
		Inj Volume :	12.0 µl

Acq. Method : C:\CHEM32\1\METHODS\NOMSD.M
Last changed : 1/3/2010 1:45:28 AM by PureCircleQC001
(modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\NOMSDDATA.M
Last changed : 1/3/2010 4:05:42 PM
(modified after loading)
Method Info : No MSD Method

Sample Info : SE (3_2) Lot# A0912268 5012 mg/L (KF 1.2%)



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External Standard Report

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Sorted By : Signal
Calib. Data Modified : 1/3/2010 3:55:51 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount	Grp	Name
3.192	MM	70.17528	6.76248e-1	37.96471		Rub
4.393	MF	37.57458	6.76248e-1	24.90154		DulA
6.018	MF	1474.24377	6.76248e-1	996.95404		Stev
7.017	FM	643.71765	6.76248e-1	513.66907		RebC
7.559	FM	131.86214	6.76248e-1	103.43895		RebF

Stevian Biotechnology Corporation Sdn Bhd (Formerly Known as Pureciccle Sdn Bhd)
572803-K
04 JAN 2010
LABORATORY

000186

Data File C:\CHEM32\1\DATA\FDAQC0201\SE3_2.D
Sample Name: SE3_2

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount	Grp	Name
9.523	MM	3864.14990	7.89569e-1	3051.01326		RebA
20.900		-	-	-		RebD
27.349	MM	26.83273	6.76248e-1	14.51646		Sbio
47.214	MM	76.46098	6.76248e-1	51.70659		RebB

Totals : 4794.16463

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

*** End of Report ***

Stevian Biotechnology Corporation Sdn Bhd (formerly known as Pureclude Sdn Bhd)
572903-K
04 JAN 2010
LABORATORY

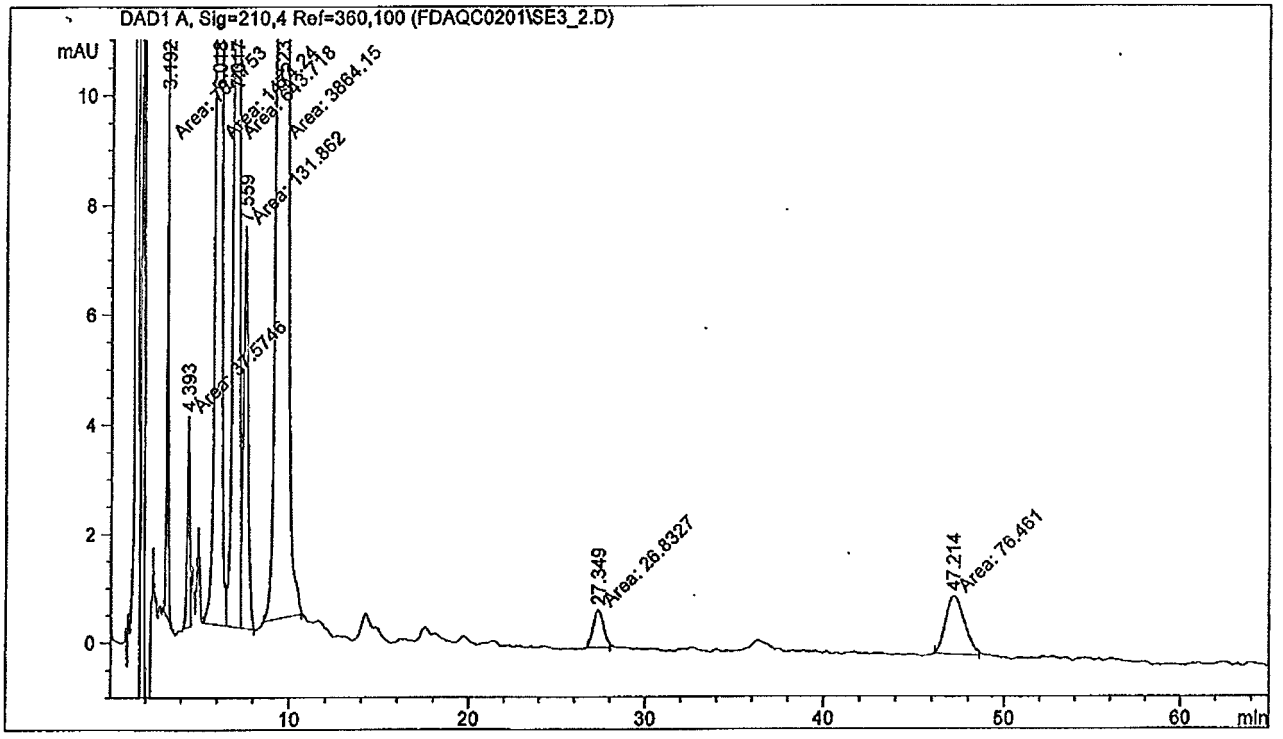
000187

Data File C:\CHEM32\1\DATA\FDAQC0201\SE3_2.D
Sample Name: SE3_2

Acq. Operator	: PureCircleQC001	Seq. Line	: 19
Acq. Instrument	: Instrument 1	Location	: P1-D-06
Injection Date	: 1/3/2010 8:22:31 AM	Inj	: 1
		Inj Volume	: 12.0 µl

Acq. Method : C:\CHEM32\1\METHODS\NOMSD.M
Last changed : 1/3/2010 1:45:28 AM by PureCircleQC001
(modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\NOMSDDATA.M
Last changed : 1/3/2010 4:04:19 PM
(modified after loading)
Method Info : No MSD Method

Sample Info : SE (3_2) Lot# A0912268 5012 mg/L (KF 1.2%)



External Standard Report

Sorted By : Signal
Calib. Data Modified : 1/3/2010 3:55:51 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount	Grp	Name
3.192	MM	70.17528	6.76248e-1	37.96471		Rub 0,7%
4.393	MF	37.57458	6.76248e-1	24.90154		DulA 0,50
6.018	MF	1474.24377	6.76248e-1	996.95404		Stev 20,13
7.017	FM	643.71765	6.76248e-1	513.66907		RebC 10,3%
7.559	FM	131.86214	6.76248e-1	103.43895		RebF 2,09

Formerly known as Stevian Biotechnology Corporation Sdn Bhd
578803-K
04 JAN 2010
LABORATORY

000188

Data File C:\CHEM32\1\DATA\FDAQC0201\SE3_2.D
Sample Name: SE3_2

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount	Grp	Name
9.523	MM	3864.14990	7.89569e-1	3051.01326		RebA 61,61
20.900		-	-	-		RebD
27.349	MM	26.83273	6.76248e-1	14.51646		Sbio 0,29
47.214	MM	76.46098	6.76248e-1	51.70659		RebB 1,04

Totals : 4794.16463 96,82%

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

==========

*** End of Report ***

000189

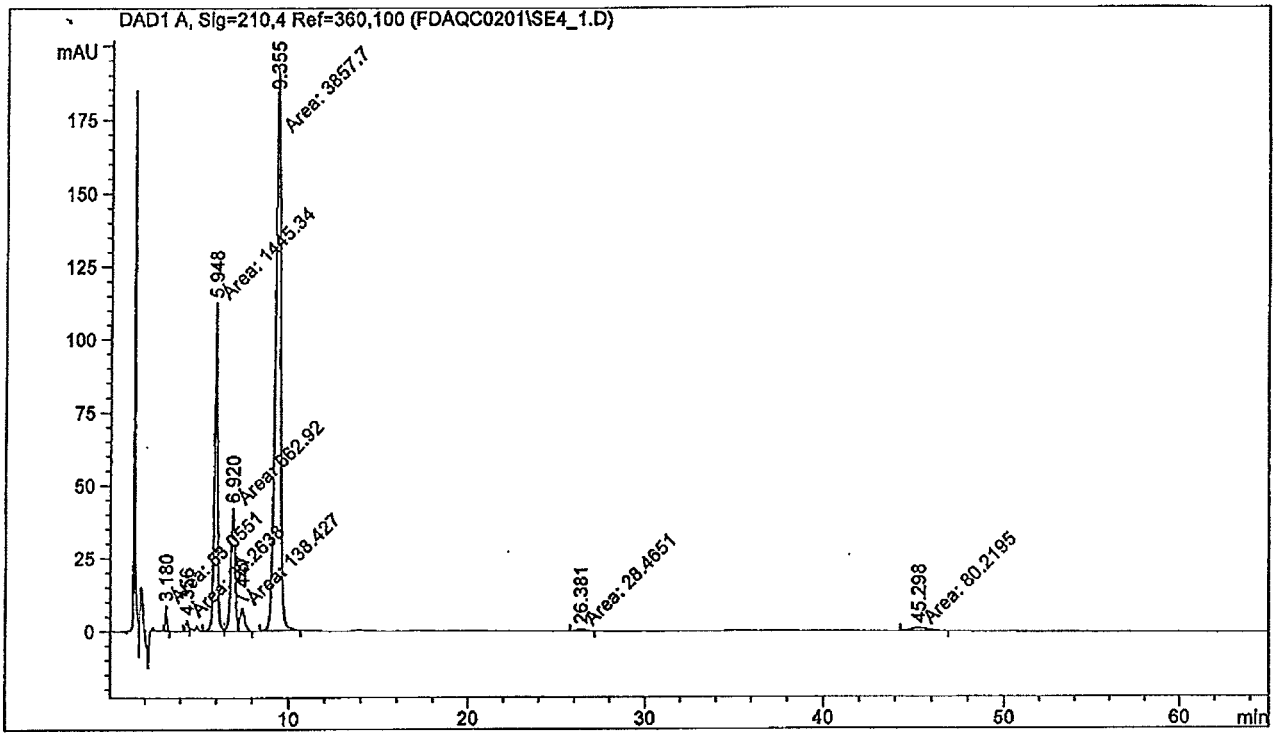
Purechide Sdn Bhd (formerly known as Stevian Biotechnology Corporation Sdn Bhd)
578803-K
04 JAN 2010
LABORATORY

Data File C:\CHEM32\1\DATA\FDAQC0201\SE4_1.D
Sample Name: SE4_1

Acq. Operator	: PureCircleQC001	Seq. Line :	22
Acq. Instrument	: Instrument 1	Location :	P1-D-07
Injection Date	: 1/3/2010 11:06:10 AM	Inj :	1
		Inj Volume :	12.0 µl

Acq. Method : C:\CHEM32\1\METHODS\NOMSD.M
Last changed : 1/3/2010 9:59:52 AM by PureCircleQC001
(modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\NOMSDDATA.M
Last changed : 1/3/2010 4:18:53 PM
(modified after loading)
Method Info : No MSD Method

Sample Info : SE (4_1) Lot# A0912269 5008 mg/L (KF 0.9%)



External Standard Report

Sorted By : Signal
Calib. Data Modified : 1/3/2010 4:13:25 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount	Grp	Name
3.180	MM	53.05507	6.76248e-1	28.70271		Rub
4.356	MF	35.26381	6.76248e-1	23.37014		DulA
5.948	MF	1445.33569	6.76248e-1	977.40501		Stev
6.920	MF	662.92047	6.76248e-1	528.99240		RebC
7.447	FM	138.42651	6.76248e-1	108.58836		RebF

Pureciecle Sdn Bhd (Formerly known as Stevian Biotechnology Corporation Sdn Bhd)
579803-K
04 JAN 2010
LABORATORY

000190

Data File C:\CHEM32\1\DATA\FDAQC0201\SE4_1.D
Sample Name: SE4_1

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount	Grp.	Name
9.355	MM	3857.70361	7.89569e-1	3045.92347		RebA
20.900		-	-	-		RebD
26.381	MM	28.46507	6.76248e-1	15.39956		Sbio
45.298	MM	80.21954	6.76248e-1	54.24830		RebB

Totals : 4782.62995

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

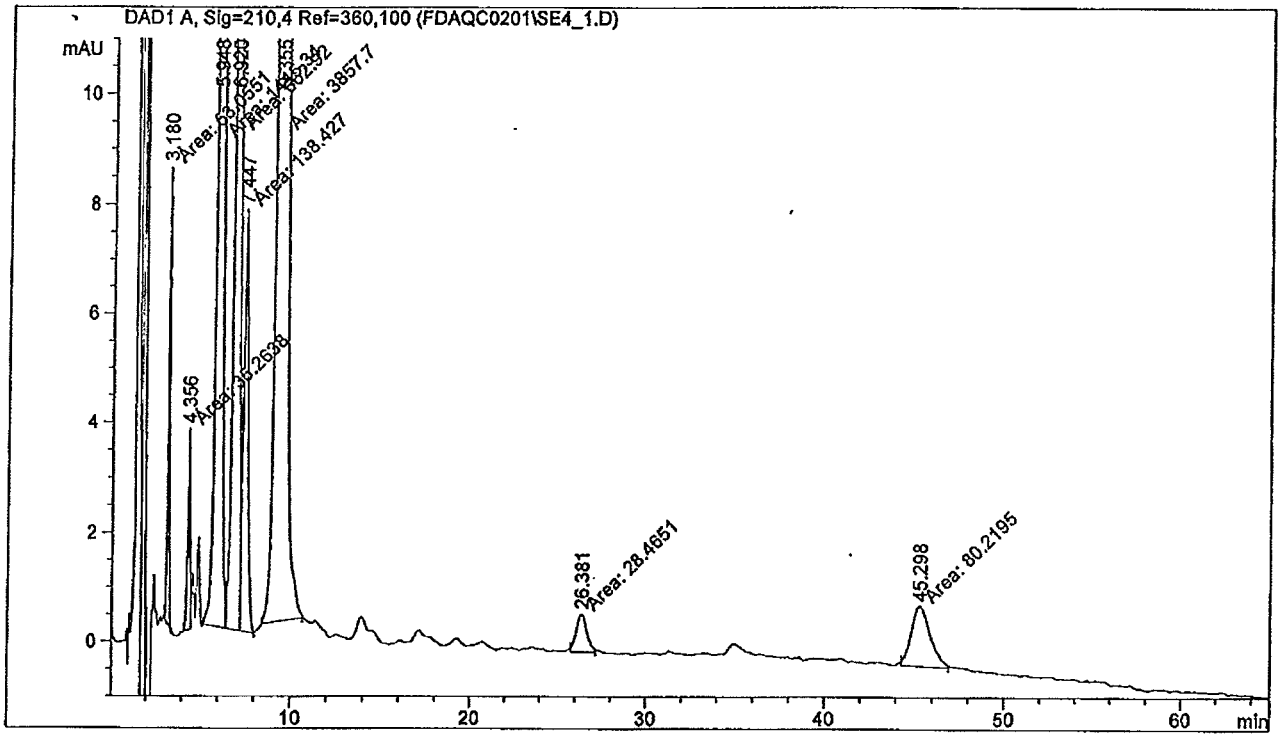
*** End of Report ***

Purecircle Sdn Bhd (formerly known as Stevian Biotechnology Corporation Sdn Bhd)
578803-K
04 JAN 2010
LABORATORY

000191

Data File C:\CHEM32\1\DATA\FDAQC0201\SE4_1.D
Sample Name: SE4_1

Acq. Operator	: PureCircleQC001	Seq. Line	: 22
Acq. Instrument	: Instrument 1	Location	: P1-D-07
Injection Date	: 1/3/2010 11:06:10 AM	Inj	: 1
		Inj Volume	: 12.0 µl
Acq. Method	: C:\CHEM32\1\METHODS\NOMSD.M		
Last changed	: 1/3/2010 9:59:52 AM by PureCircleQC001 (modified after loading)		
Analysis Method	: C:\CHEM32\1\METHODS\NOMSDDATA.M		
Last changed	: 1/3/2010 4:19:02 PM (modified after loading)		
Method Info	: No MSD Method		
Sample Info	: SE (4_1) Lot# A0912269 5008 mg/L (KF 0.9%)		



External Standard Report

Sorted By : Signal
Calib. Data Modified : 1/3/2010 4:13:25 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

000192

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount	Grp	Name
3.180	MM	53.05507	6.76248e-1	28.70271		Rub 0,58
4.356	MF	35.26381	6.76248e-1	23.37014		DulA 0,47
5.948	MF	1445.33569	6.76248e-1	977.40501		Stev 19,69
6.920	MF	662.92047	6.76248e-1	528.99240		RebC 10,66
7.447	FM	138.42651	6.76248e-1	108.58836		RebF 2,18

PureCircle Sdn Bhd (Formerly known as Stevian Biotechnology Corporation Sdn Bhd)
572803-K
04 JAN 2010
LABORATORY

Data File C:\CHEM32\1\DATA\FDAQC0201\SE4_1.D
Sample Name: SE4_1

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount	Grp	Name
9.355	MM	3857.70361	7.89569e-1	3045.92347		RebA 61,37
20.900		-	-	-		RebD
26.381	MM	28.46507	6.76248e-1	15.39956		Sbio 0,31
45.298	MM	80.21954	6.76248e-1	54.24830		RebB 1,09
Totals :				4782.62995		96,37 %

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

==========

*** End of Report ***

000193

Pureclice Sdn Bhd (Formerly known as Stevian Biotechnology Corporation Sdn Bhd)
579803-K
04 JAN 2010
LABORATORY

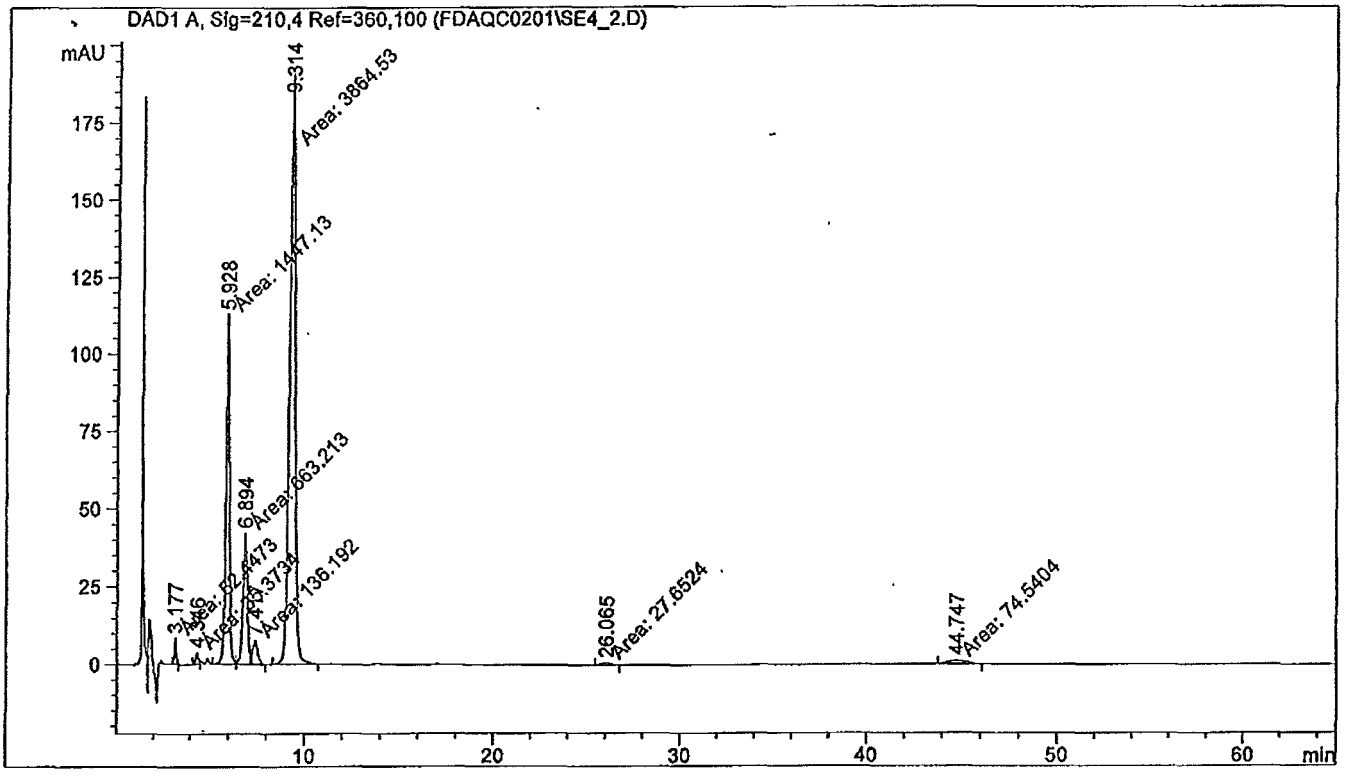
Data File C:\CHEM32\1\DATA\FDAQC0201\SE4_2.D
Sample Name: SE4_2

==========

Acq. Operator	: PureCircleQC001	Seq. Line :	23
Acq. Instrument	: Instrument 1	Location :	P1-D-08
Injection Date	: 1/3/2010 12:12:19 PM	Inj :	1
		Inj Volume :	12.0 µl

Acq. Method : C:\CHEM32\1\METHODS\NOMSD.M
Last changed : 1/3/2010 9:59:52 AM by PureCircleQC001
(modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\NOMSDDATA.M
Last changed : 1/3/2010 4:26:17 PM
(modified after loading)
Method Info : No MSD Method

Sample Info : SE (4_2) Lot# A0912269 5004 mg/L (KF 0.9%)



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External Standard Report

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Sorted By : Signal
Calib. Data Modified : 1/3/2010 4:13:25 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount	Grp	Name
3.177	MM	52.44735	6.76248e-1	28.37393		Rub
4.346	MF	35.37342	6.76248e-1	23.44278		DulA
5.928	MF	1447.12915	6.76248e-1	978.61783		Stev
6.894	MF	663.21344	6.76248e-1	529.22618		RebC

Pureclice Sdn Bhd (Formerly known as Stevian Biotechnology Corporation Sdn Bhd)
572803-K
04 JAN 2010
LABORATORY

Data File C:\CHEM32\1\DATA\FDAQC0201\SE4_2.D
Sample Name: SE4_2

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount	Grp	Name
9.314	MM	3864.52856	7.89569e-1	3051.31224		RebA
20.900		-	-	-		RebD
26.065	MM	27.65244	6.76248e-1	14.95993		Sbio
44.747	MM	74.54044	6.76248e-1	50.40783		RebB

Totals : 4783.17658

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

==========

*** End of Report ***

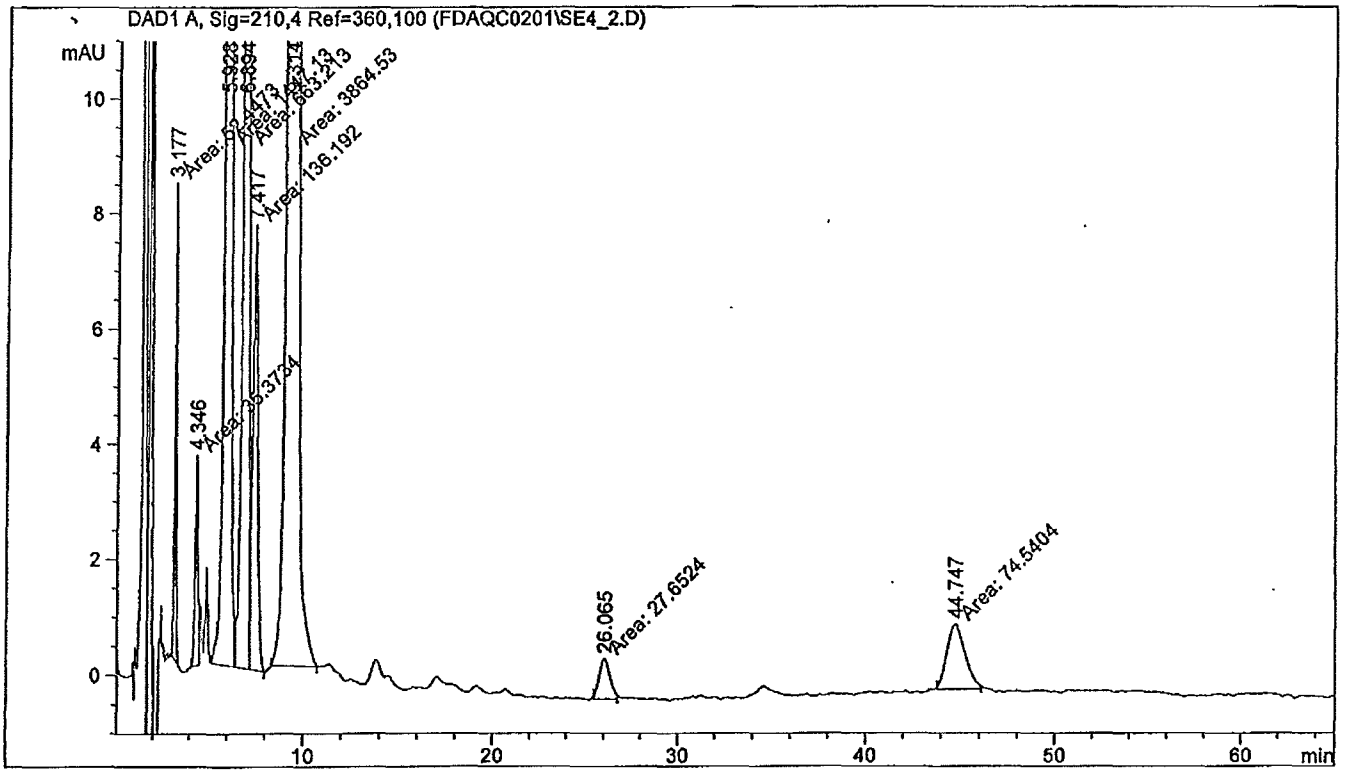
Pureclide Sdn Bhd (Formerly known as Stevian Biotechnology Corporation Sdn Bhd)
573803-K
04 JAN 2010
LABORATORY

Data File C:\CHEM32\1\DATA\FDAQC0201\SE4_2.D
Sample Name: SE4_2

Acq. Operator	: PureCircleQC001	Seq. Line	: 23
Acq. Instrument	: Instrument 1	Location	: P1-D-08
Injection Date	: 1/3/2010 12:12:19 PM	Inj	: 1
		Inj Volume	: 12.0 µl

Acq. Method : C:\CHEM32\1\METHODS\NOMSD.M
Last changed : 1/3/2010 9:59:52 AM by PureCircleQC001
(modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\NOMSDDATA.M
Last changed : 1/3/2010 4:26:26 PM
(modified after loading)
Method Info : No MSD Method

Sample Info : SE (4_2) Lot# A0912269 5004 mg/L (KF 0.9%)



External Standard Report

Sorted By : Signal
Calib. Data Modified : 1/3/2010 4:13:25 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

000196

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount	Grp	Name
3.177	MM	52.44735	6.76248e-1	28.37393		Rub 0,57
4.346	MF	35.37342	6.76248e-1	23.44278		DulA 0,47
5.928	MF	1447.12915	6.76248e-1	978.61783		Stev 19,73
6.894	MF	663.21344	6.76248e-1	529.22618		RebC 10,67

PureCircle Sdn Bhd (Formerly known as Stevian Biotechnology Corporation Sdn Bhd)
572803-K
04 JAN 2010
LABORATORY

Data File C:\CHEM32\1\DATA\FDAQC0201\SE4_2.D
Sample Name: SE4_2

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount	Grp	Name
9.314	MM	3864.52856	7.89569e-1	3051.31224		RebA 61,53
20.900		-	-	-		RebD
26.065	MM	27.65244	6.76248e-1	14.95993		Sbio 0,30
44.747	MM	74.54044	6.76248e-1	50.40783		RebB 1,02
Totals :				4783.17658		96,46%

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

==========

*** End of Report ***

000197

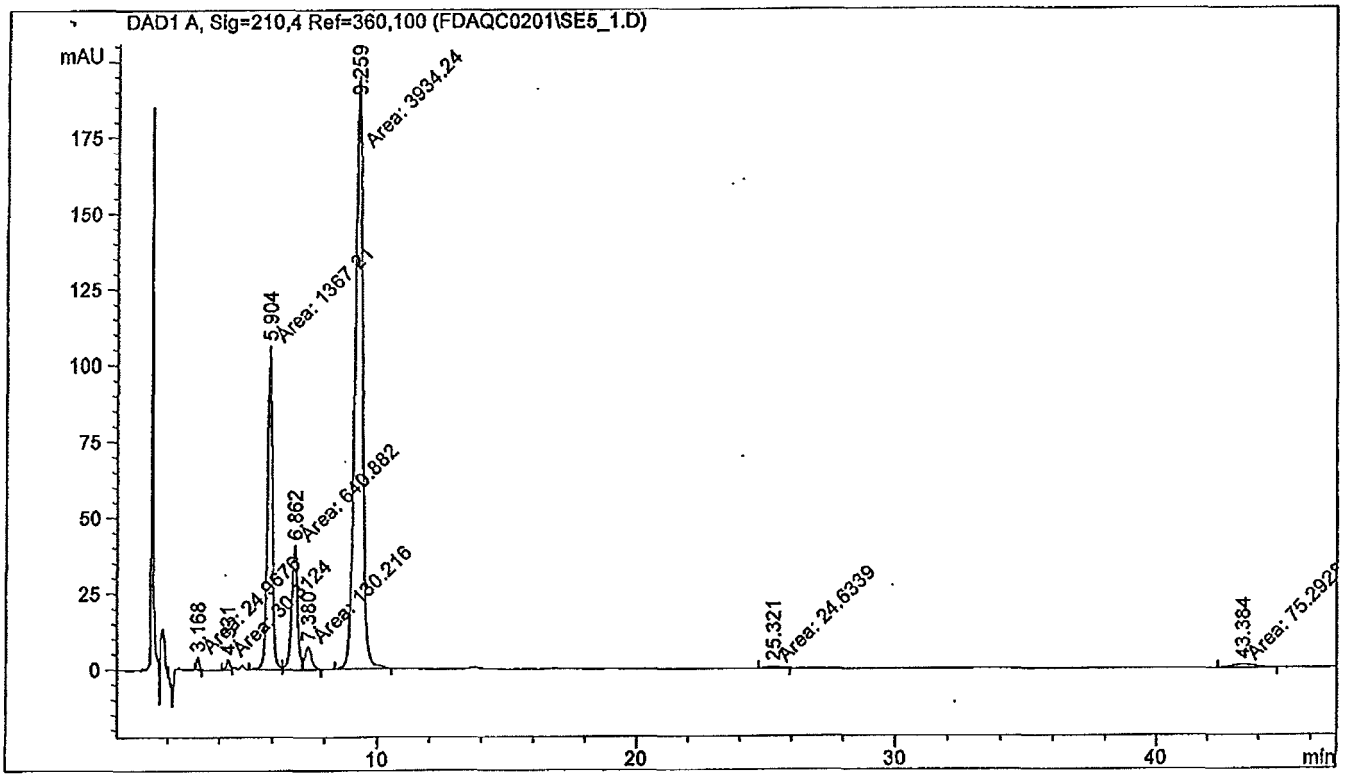
Pureciale Sdn Bhd (formerly known as Stevian Biotechnology Corporation Sdn Bhd)
578303-K
04 JAN 2010
LABORATORY

Data File C:\CHEM32\1\DATA\FDAQC0201\SE5_1.D
Sample Name: SE5_1

Acq. Operator	: PureCircleQC001	Seq. Line	: 27
Acq. Instrument	: Instrument 1	Location	: P1-D-09
Injection Date	: 1/3/2010 3:00:14 PM	Inj	: 1
		Inj Volume	: 12.0 µl

Acq. Method : C:\CHEM32\1\METHODS\NOMSD.M
Last changed : 1/3/2010 2:59:37 PM by PureCircleQC001
(modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\NOMSDDATA.M
Last changed : 1/3/2010 4:44:54 PM
(modified after loading)
Method Info : No MSD Method

Sample Info : SE (5_1) Lot# A0912270 5008 mg/L (KF 1.2%)



External Standard Report

Sorted By : Signal
Calib. Data Modified : 1/3/2010 4:35:43 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount	Grp	Name
3.168	MM	24.96765	6.76248e-1	13.50746		Rub
4.331	MF	30.31236	6.76248e-1	20.08870		DulA
5.904	MF	1367.21155	6.76248e-1	924.57374		Stev
6.862	FM	640.88165	6.76248e-1	511.40602		RebC

000198

PureCircle Sdn Bhd (Formerly Known as Cargill Biotechnology Corporation Sdn Bhd)
573803-K
04 JAN 2010
LABORATORY

Data File C:\CHEM32\1\DATA\FDAQC0201\SE5_1.D
Sample Name: SE5_1

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount	Grp	Name
9.259	MM	3934.23901	7.89569e-1	3106.35346		RebA
20.900		-	-	-		RebD
25.321	MM	24.63385	6.76248e-1	13.32688		Sbio
43.384	MM	75.29283	6.76248e-1	50.91663		RebB

Totals : 4742.32066

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

*** End of Report ***

Puncture Sdn Bhd (Formerly known as Stevian Biotechnology Corporation Sdn Bhd)
578803-K
04 JAN 2013
LABORATORY

Data File C:\CHEM32\1\DATA\FDAQC0201\SE5_1.D
Sample Name: SE5_1

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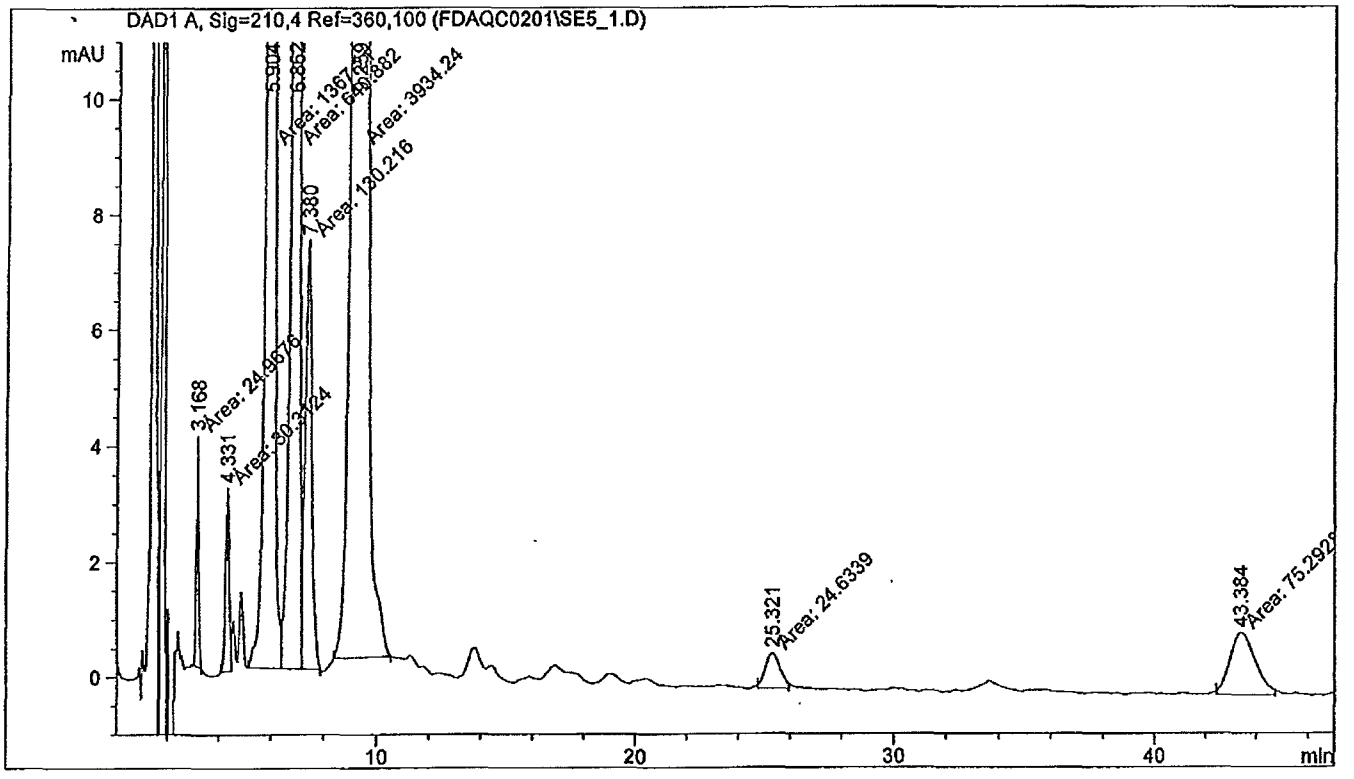
Acq. Operator	: PureCircleQC001	Seq. Line :	27
Acq. Instrument	: Instrument 1	Location :	P1-D-09
Injection Date	: 1/3/2010 3:00:14 PM	Inj :	1
		Inj Volume :	12.0 µl

Acq. Method : C:\CHEM32\1\METHODS\NOMSD.M
Last changed : 1/3/2010 2:59:37 PM by PureCircleQC001
(modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\NOMSDDATA.M
Last changed : 1/3/2010 4:43:27 PM
(modified after loading)
Method Info : No MSD Method

Sample Info : SE (5_1) Lot# A0912270 5008 mg/L (KF 1.2%)



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External Standard Report

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Sorted By : Signal
Calib. Data Modified : 1/3/2010 4:35:43 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

000200

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount	Grp	Name
3.168	MM	24.96765	6.76248e-1	13.50746		Rub 0,27
4.331	MF	30.31236	6.76248e-1	20.08870		DulA 0,41
5.904	MF	1367.21155	6.76248e-1	924.57374		Stev 18,69
6.862	FM	640.88165	6.76248e-1	511.40602		RebC 10,34

Formerly known as Stevian Biotechnology Corporation Sdn Bhd
573803-K
04 JAN 2010
LABORATORY

Data File C:\CHEM32\1\DATA\FDAQC0201\SE5_1.D
Sample Name: SE5_1

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount	Grp	Name	
9.259	MM	3934.23901	7.89569e-1	3106.35346		RebA	62,78
20.900		-	-	-		RebD	
25.321	MM	24.63385	6.76248e-1	13.32688		Sbio	0,27
43.384	MM	75.29283	6.76248e-1	50.91663		RebB	1,03

Totals : 4742.32066 95,85%

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

==========

*** End of Report ***

000201

Pureclice Sdn Bhd (formerly known as Stevian Biotechnology Corporation Sdn Bhd)
578903-K
04 JAN 2010
LABORATORY

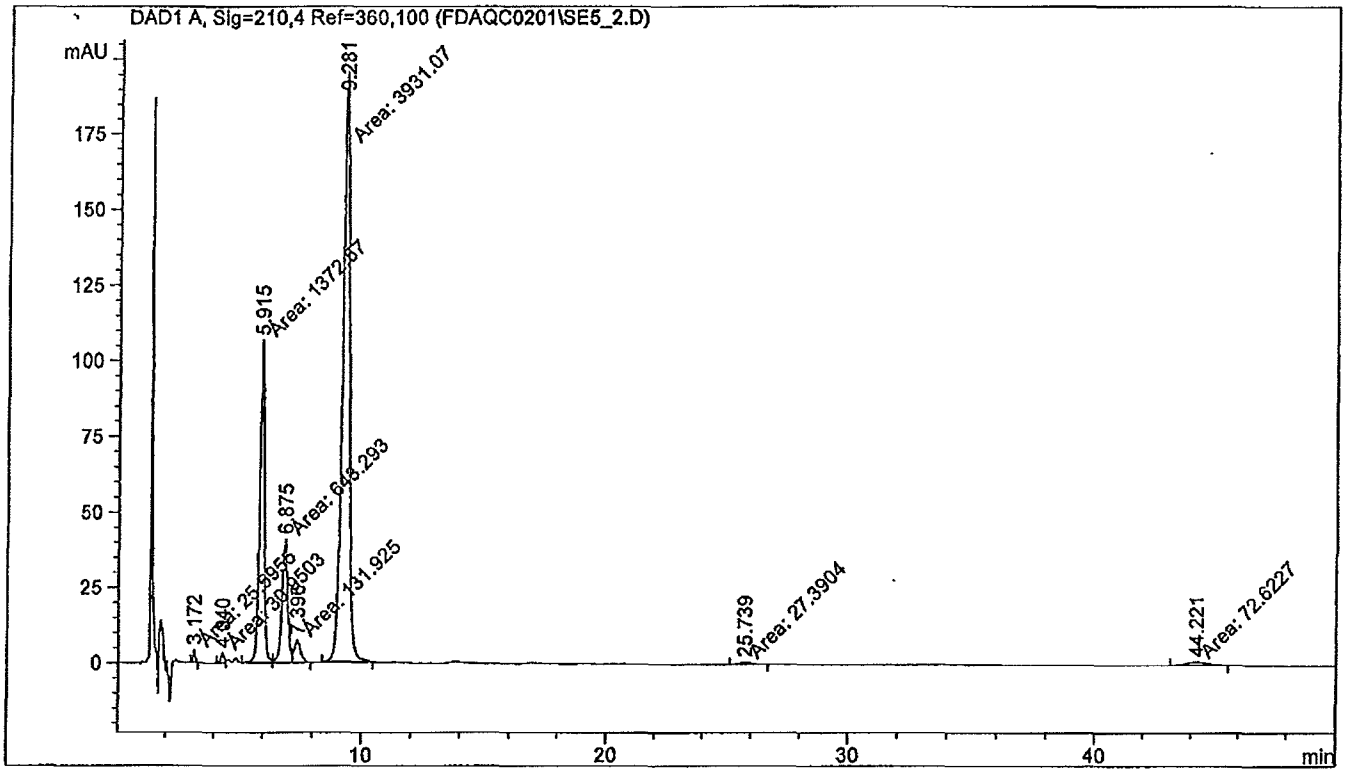
Data File C:\CHEM32\1\DATA\FDAQC0201\SE5_2.D
Sample Name: SE5_2

==========

Acq. Operator	: PureCircleQC001	Seq. Line :	25
Acq. Instrument	: Instrument 1	Location :	P1-E-01
Injection Date	: 1/3/2010 1:19:08 PM	Inj :	1
		Inj Volume :	12.0 µl

Acq. Method : C:\CHEM32\1\METHODS\NOMSD.M
Last changed : 1/3/2010 1:24:41 PM by PureCircleQC001
(modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\NOMSDDATA.M
Last changed : 1/3/2010 4:38:35 PM
(modified after loading)
Method Info : No MSD Method

Sample Info : SE (5_2) Lot# A0912270 5012 mg/L (KF 1.2%)



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External Standard Report

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Sorted By : Signal
Calib. Data Modified : 1/3/2010 4:35:43 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

000202

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount	Grp	Name
3.172	MM	25.59548	6.76248e-1	13.84711		Rub
4.340	MF	30.95033	6.76248e-1	20.51149		DulA
5.915	MF	1372.57117	6.76248e-1	928.19817		Stev
6.875	FM	643.29340	6.76248e-1	513.33053		RebC

Sdn Bhd (Formerly known as Stevian Biotechnology Corporation Sdn Bhd)
578803-K
04 JAN 2010
LABORATORY

Data File C:\CHEM32\1\DATA\FDAQC0201\SE5_2.D
Sample Name: SE5_2

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount	Grp	Name
9.281	MM	3931.06543	7.89569e-1	3103.84769		RebA
20.900		-	-	-		RebD
25.739	MM	27.39042	6.76248e-1	14.81817		Sbio
44.221	MM	72.62270	6.76248e-1	49.11095		RebB

Totals : 4747.15218

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

==========

*** End of Report ***

000203

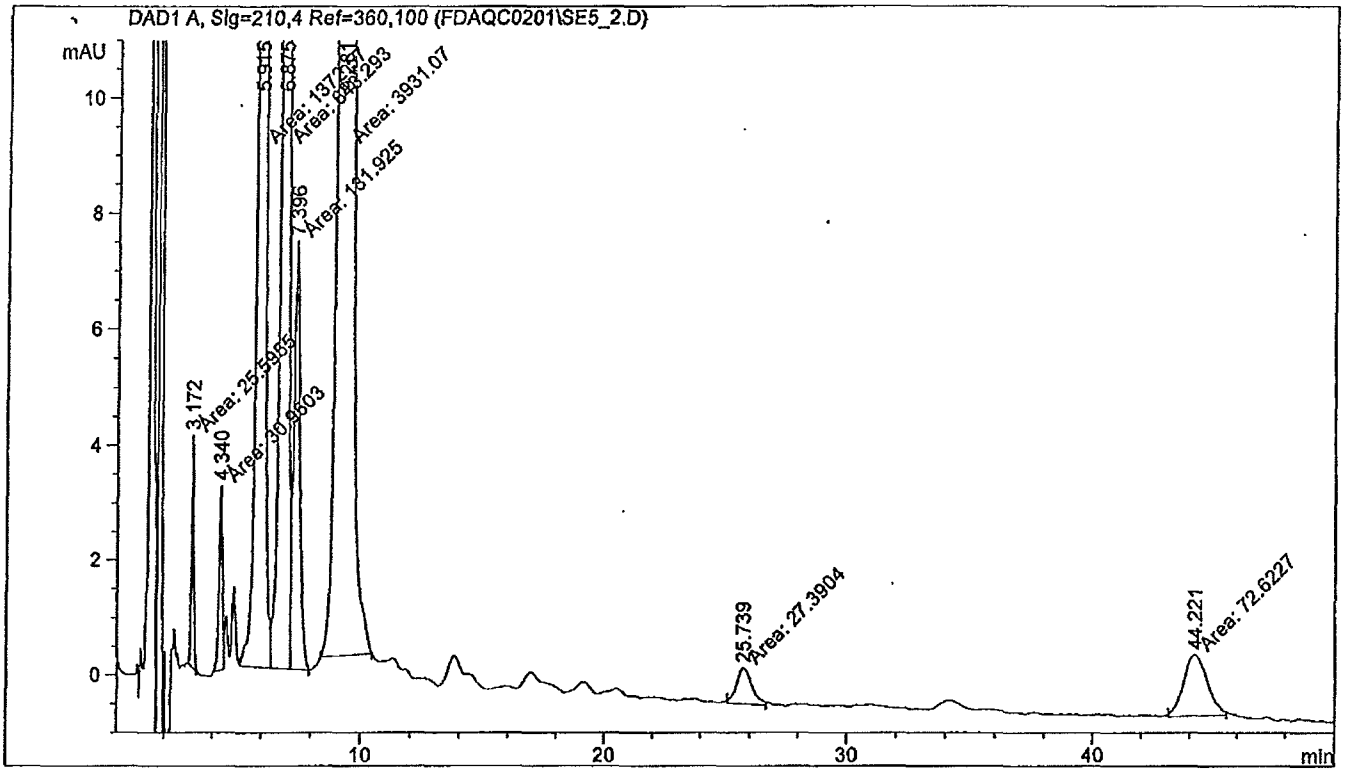
Purecircle Sdn Bhd (Formerly known as Stevian Biotechnology Corporation Sdn Bhd)
578803-K
04 JAN 2010
LABORATORY

Data File C:\CHEM32\1\DATA\FDAQC0201\SE5_2.D
Sample Name: SE5_2

Acq. Operator	: PureCircleQC001	Seq. Line	: 25
Acq. Instrument	: Instrument 1	Location	: P1-E-01
Injection Date	: 1/3/2010 1:19:08 PM	Inj	: 1
		Inj Volume	: 12.0 µl

Acq. Method : C:\CHEM32\1\METHODS\NOMSD.M
Last changed : 1/3/2010 1:24:41 PM by PureCircleQC001
(modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\NOMSDDATA.M
Last changed : 1/3/2010 4:38:10 PM
(modified after loading)
Method Info : No MSD Method

Sample Info : SE (5_2) Lot# A0912270 5012 mg/L (KF 1.2%)



External Standard Report

Sorted By : Signal
Calib. Data Modified : 1/3/2010 4:35:43 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

000204

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount	Grp	Name
3.172	MM	25.59548	6.76248e-1	13.84711		Rub 0,28
4.340	MF	30.95033	6.76248e-1	20.51149		DulA 0,41
5.915	MF	1372.57117	6.76248e-1	928.19817		Stev 18,74
6.875	FM	643.29340	6.76248e-1	513.33053		RebC 10,37

PureCircle Sdn Bhd (Formerly known as Stevian Biotechnology Corporation Sdn Bhd)
578303-K
04 JAN 2010
LABORATORY

Data File C:\CHEM32\1\DATA\FDAQC0201\SE5_2.D
Sample Name: SE5_2

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount	Grp.	Name
9.281	MM	3931.06543	7.89569e-1	3103.84769		RebA 62,68
20.900		-	-	-		RebD
25.739	MM	27.39042	6.76248e-1	14.81817		Sbio 0,30
44.221	MM	72.62270	6.76248e-1	49.11095		RebB 0,99
Totals :				4747.15218		95,87%

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

==========

*** End of Report ***

000205

Pureclice Sdn Bhd (formerly known as Stevian Biotechnology Corporation Sdn Bhd)
576303-K
04 JAN 2010
LABORATORY

PureCircle Sdn Bhd	Document#: PCL-LAB-003	Issue date: 01/12/07
		Revision date: -------
HPLC sample data record		Revision No: 00

Sample Info	Lot # A0912266	
	Duplicate I	Duplicate II
Sample ID	SE 1-1	SE 1-2
Weight, mg	125.3	125.1
Volumetric flask volume, mL	25	25
Concentration, mg/L	5012	5004
Water, %wt (Karl Fischer)	0.8	0.8
Remarks	—	

Sample Info	Lot # A0912267	
	Duplicate I	Duplicate II
Sample ID	SE 2-1	SE 2-2
Weight, mg	125.1	125.2
Volumetric flask volume, mL	25	25
Concentration, mg/L	5004	5008
Water, %wt (Karl Fischer)	1.0	1.0
Remarks	—	

Sample Info	Lot# A0912268	
	Duplicate I	Duplicate II
Sample ID	SE 3-1	SE 3-2
Weight, mg	125.2	125.3
Volumetric flask volume, mL	25	25
Concentration, mg/L	5008	5012
Water, %wt (Karl Fischer)	1.2	1.2
Remarks	—	

Done by: (b) (6) KARTHIGAH, QC EXEC — Name, Position — 31.12.2009 — Date

Checked by: (b) (6) Lai Chee Seng, Senior QA — Name, Position — 31/12/2009 — Date

000206

(Formerly known as Stevian Biotechnology Corporation Sdn Bhd) 578803-K 31 DEC 2009 LABORATORY

PureCircle Sdn Bhd	Document#: PCL-LAB-003	Issue date: 01/12/07
		Revision date: -------
HPLC sample data record		Revision No: 00

Sample Info	Lot# A0912269	
	Duplicate I	Duplicate II
Sample ID	SE 4-1	SE 4-2
Weight, mg	125,2	125,1
Volumetric flask volume, mL	25	25
Concentration, mg/L	5008	5004
Water, %wt (Karl Fischer)	0,9	0,9
Remarks	—	

Sample Info	Lot# A0912270	
	Duplicate I	Duplicate II
Sample ID	SE 5-1	SE 5-2
Weight, mg	125,2	125,3
Volumetric flask volume, mL	25	25
Concentration, mg/L	5008	5012
Water, %wt (Karl Fischer)	1,2	1,2
Remarks	—	

Sample Info		
	Duplicate I	Duplicate II
Sample ID		
Weight, mg		
Volumetric flask volume, mL		
Concentration, mg/L		
Water, %wt (Karl Fischer)		
Remarks		

Done by: (b) (6) KARTHIGAH, QC EXEC — Name, Position — 31. 12. 2009 — Date

Checked by: (b) (6) Lai Chee Seng, Senior QA — Name, Position — 31/12/2009 — Date

000207

(Formerly known as Stevian Biotechnology Corporation Sdn. Bhd.) 578803-K 31 DEC 2009 LABORATORY

Appendix C-4

See following 35 pages

Not included in sequence with main document page numbers.

Environmental Micro Analysis, Inc. ELAP Certificate #2211
460 N East Street
Woodland, CA 95776

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e-mail: emalab@emalab.com website: www.emalab.com

Analytical Report

January 11, 2010

Client: Jianning Chen
PureCircle Co., Ltd China
Yangtang Industry Zone, Ganxian
Jiangxi Province, .
Phone: (+86) 797-4601666
Fax: (+86) 797-4601663
Email: jianning.chen@purecircle.com

Project No:

PO No:

Client Sample ID: A0912266

EMA Sample No: 09122805-01

Date Received: 12/28/2009

Sample Matrix: Steviol Glycosides Powder

Analytical Method: FDA IMP-OC1

Extraction Method: FDA 302

Date Extracted: 12/29/2009

Date Completed: 1/2/2010

Analyte	Amount	RL ppm	Tolerance ppm
Acrinathrin	ND	0.010	
Aldrin	ND	0.010	
alpha-BHC	ND	0.010	
Atrazine	ND	0.010	
Benfluralin	ND	0.010	
beta Chlordane	ND	0.010	
Bifenthrin	ND	0.010	
Boscalid	ND	0.010	
Bromopropylate	ND	0.010	
Captan	ND	0.010	
Chlorodimeform	ND	0.010	
Chlorobenzilate	ND	0.010	
Chloroneb	ND	0.010	
Chlorothalonil	ND	0.010	
CIPC	ND	0.010	
cis-Chlordane	ND	0.010	
cis-Nonachlor	ND	0.010	
cis-Permethrin	ND	0.010	
Clomazone	ND	0.010	
Cyanazine	ND	0.010	
Cypermethrin	ND	0.010	
Cyproconazole	ND	0.010	
Dacthal	ND	0.010	
Decamethrin	ND	0.010	
delta-BHC	ND	0.010	
Diallate-1	ND	0.010	
Diallate-2	ND	0.010	
3,4-Dichloroanaline	ND	0.010	
Dichlobenil	ND	0.010	
Dichlofluanid	ND	0.010	
Dichloran	ND	0.010	
Dieldrin	ND	0.010	
Difenoconazole	ND	0.010	
Dimethachlor	ND	0.010	
Dimethmorph	ND	0.010	
Dinitramine	ND	0.010	
Dursban	ND	0.010	
Endosulfan Sulfate	ND	0.010	
Endosulfan-I	ND	0.010	
Endosulfan-II	ND	0.010	
Endrin	ND	0.010	
Epoxiconazole	ND	0.010	
Etaconazole-1	ND	0.010	
Etaconazole-2	ND	0.010	
Ethafluralin	ND	0.010	
Etridiazole	ND	0.010	
Fenarimol	ND	0.010	
Fenbuconazole	ND	0.010	
Fenhexamid	ND	0.010	
Fenvalerate-1	ND	0.010	
Fenvalerate-2	ND	0.010	
Fipronil	ND	0.010	
Fluchloralin	ND	0.010	
Flucythrinate	ND	0.010	
Fludioxanil	ND	0.010	
Flugquinconazole	ND	0.010	
Flutolanil	ND	0.010	
Fluvalinate	ND	0.010	
Folpet	ND	0.010	
gamma-BHC/Lindane	ND	0.010	
gamma-Chlordane	ND	0.010	
Heptachlor	ND	0.010	
Heptachlor Epoxide	ND	0.010	
Hexachlorobenzene	ND	0.010	

ND = Not Detected at or above Environmental Micro Analysis, Inc. Reporting Limit (RL)

Date: Reviewed by: Don Peterson, Laboratory Director

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Phone: (+86) 797-4601666
Fax: (+86) 797-4601663
Email: jianning.chen@purecircle.com

Project No:

PO No:

Client Sample ID: A0912266

EMA Sample No: 09122805-01

Date Received: 12/28/2009

Sample Matrix: Steviol Glycosides Powder

Analytical Method: FDA IMP-OC2

Extraction Method: FDA 302

Date Extracted: 12/29/2009

Date Completed: 1/2/2010

Analyte	Amount	RL ppm	Tolerance ppm
Hexaconazole	ND	0.010	
Imazalil	ND	0.010	
Iprodione	ND	0.010	
lambda-Cyhalothrin	ND	0.010	
Linuron	ND	0.010	
Metolachlor	ND	0.010	
Mirex	ND	0.010	
Monolinuron	ND	0.010	
Myclobutanil	ND	0.010	
Nitrofen	ND	0.010	
Nuarimol	ND	0.010	
o,p'-DDT	ND	0.010	
o,p'-Methoxychlor	ND	0.010	
Octachlor Epoxide	ND	0.010	
p,p'-DDE	ND	0.010	
p,p'-DDT	ND	0.010	
p,p'-Methoxychlor	ND	0.010	
Penconazole	ND	0.010	
Pentachloroanaline	ND	0.010	
Pentachlorobenzene	ND	0.010	
Pentachlorobenzonitrile	ND	0.010	
Pentachlorothioanisole	ND	0.010	
Prochloraz	ND	0.010	
Procymidone	ND	0.010	
Profluralin	ND	0.010	
Pronamide	ND	0.010	
Propachlor	ND	0.010	
Propanil	ND	0.010	
Propiconizole/Tilt-1	ND	0.010	
Propiconizole/Tilt-2	ND	0.010	
Pyraclostrobin	ND	0.010	
Pyridaben	ND	0.010	
Pyrifenox-1	ND	0.010	
Pyrifenox-2	ND	0.010	
Quinoxyfen	ND	0.010	
Quintozene	ND	0.010	
Simazine	ND	0.010	
Tebuconazole	ND	0.010	
Tebufenpyrad	ND	0.010	
Tecnazene	ND	0.010	
Tefluthrin	ND	0.010	
Terbuthylazine	ND	0.010	
2,3,5,6-Tetrachloroanaline	ND	0.010	
Tetradifon	ND	0.010	
Tolyfluanid	ND	0.010	
trans-Chlordane	ND	0.010	
trans-Nonachlor	ND	0.010	
trans-Permethrin	ND	0.010	
Triadimefon	ND	0.010	
Triadimenol	ND	0.010	
Triallate	ND	0.010	
Trifloxystrobin	ND	0.010	
Triflumizole	ND	0.010	
Trifluralin	ND	0.010	
Triticonazole	ND	0.010	
Vinclozolin	ND	0.010	

ND = Not Detected at or above Environmental Micro Analysis, Inc. Reporting Limit (RL)

Date: Reviewed by: Don Peterson, Laboratory Director

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Jiangxi Province.

Phone: (+86) 797-4601666
Fax: (+86) 797-4601663
Email: jianning.chen@purecircle.com

Project No:

PO No:

Client Sample ID: A0912266

EMA Sample No: 09122805-01

Date Received: 12/28/2009

Sample Matrix: Steviol Glycosides Powder

Analytical Method: FDA IMP-NSO-1

Extraction Method: FDA 302

Date Extracted: 12/29/2009

Date Completed: 1/5/2010

Analyte	Amount	RL ppm	Tolerance ppm
Allethrin	ND	0.010	
Acetamiprid	ND	0.010	
alpha-Spiroxamine	ND	0.010	
Ametryn	ND	0.010	
Aminocarb	ND	0.010	
Amitraz	ND	0.010	
Aniten/Flurecol Butyl E.	ND	0.010	
Azoxystrobin	ND	0.010	
Benalaxyl	ND	0.010	
Bendiocarb	ND	0.010	
Bendiocarb - C2H3NO	ND	0.010	
beta-Spiroxamine	ND	0.010	
Biphenyl	ND	0.010	
Biteranol-1	ND	0.010	
Biteranol-2	ND	0.010	
Bufenocarb-1	ND	0.010	
Bufenocarb-2	ND	0.010	
Bupirimate	ND	0.010	
Buprofezin	ND	0.010	
Butralin	ND	0.010	
Butylate	ND	0.010	
3-OH Carbofuran	ND	0.010	
Carbaryl	ND	0.010	
Carbaryl - C2H3NO	ND	0.010	
Carbofuran	ND	0.010	
Carbofuran - C2H3NO	ND	0.010	
Carbosulfan	ND	0.010	
Carboxin	ND	0.010	
Cycloate/Ro Neet	ND	0.010	
Cycluron	ND	0.010	
Cymiazole	ND	0.010	
Cyprodinil	ND	0.010	
Desmedipham	ND	0.010	
Desmethyl Diphenamid	ND	0.010	
Desmetryn	ND	0.010	
Diethofencarb	ND	0.010	
Diniconazole	ND	0.010	
Dioxacarb	ND	0.010	
Dioxacarb - C2H3NO	ND	0.010	
Diphenamid	ND	0.010	
d-Phenothrin #	ND	0.010	
EPTC/Eptam	ND	0.010	
Ethiofencarb	ND	0.010	
Ethiolate	ND	0.010	
Ethiolate	ND	0.010	
Ethofumesate	ND	0.010	
Ethoxyquin	ND	0.010	
Etofenprox	ND	0.010	
Famoxadone	ND	0.010	
Fenazaquin	ND	0.010	
Fenfuram	ND	0.010	
Fenobucarb	ND	0.010	
Fenoxycarb	ND	0.010	
Fenpropathrin	ND	0.010	
Fenpropimorph	ND	0.010	
Flusilazole	ND	0.010	
Fuberdiazole	ND	0.010	
Furalaxyl	ND	0.010	
Hexazinone	ND	0.010	
Isocarbamid	ND	0.010	

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Project No:

PO No:

Client Sample ID: A0912266

EMA Sample No: 09122805-01

Date Received: 12/28/2009

Sample Matrix: Steviol Glycosides Powder

Analytical Method: FDA IMP-NSO-2

Extraction Method: FDA 302

Date Extracted: 12/29/2009

Date Completed: 1/5/2010

Analyte	Amount	RL ppm	Tolerance ppm
Isoprocarb	ND	0.010	
Isopropalin	ND	0.010	
Isoprothiolane	ND	0.010	
Isoproturon (frag)	ND	0.010	
Kresoxim-Methyl	ND	0.010	
Lenacil	ND	0.010	
MBTZ	ND	0.010	
Mepronil	ND	0.010	
Metalaxyl	ND	0.010	
Metaldehyde	ND	0.010	
Methfuroxan	ND	0.010	
Methiocarb	ND	0.010	
Methoprotryne	ND	0.010	
Metolacarb	ND	0.010	
Metribuzin	ND	0.010	
Mexacarbate	ND	0.010	
MGK-264	ND	0.010	
Molinate	ND	0.010	
Naphthalene Acetamide	ND	0.010	
Naproanalid	ND	0.010	
Napropamide	ND	0.010	
Nitralin	ND	0.010	
Nitrothal Isopropyl	ND	0.010	
Norea	ND	0.010	
Octhilinone	ND	0.010	
o-Phenylphenol	ND	0.010	
Pebulate	ND	0.010	
Pendimethalin	ND	0.010	
Phenmedipham	ND	0.010	
Piperonyl Butoxide	ND	0.010	
Pirimacarb	ND	0.010	
Promecarb	ND	0.010	
Prometon	ND	0.010	
Prometryn	ND	0.010	
Propargite	ND	0.010	
Propham	ND	0.010	
Propoxur	ND	0.010	
Propoxur - C2H3NO	ND	0.010	
Pyracarbolid	ND	0.010	
Pyrethrin-1	ND	0.010	
Pyrethrin-2	ND	0.010	
Pyrimethanil	ND	0.010	
Pyriproxifen	ND	0.010	
Resmethrin	ND	0.010	
Secbumeton	ND	0.010	
Sethoxydim	ND	0.010	
Simetryn	ND	0.010	
Tebutam	ND	0.010	
Tebuthiuron	ND	0.010	
Terbumeton	ND	0.010	
Terbutryn	ND	0.010	
Tetraconazole	ND	0.010	
Tetramethrin-1	ND	0.010	
Tetramethrin-2	ND	0.010	
Thiabendazole	ND	0.010	
THPI	ND	0.010	
Tralkoxydim	ND	0.010	
Tricyclazole	ND	0.010	
2,3,5-Trimethacarb	ND	0.010	
3,4,5-Trimethacarb	ND	0.010	
Vernolate	ND	0.010	

ND = Not Detected at or above Environmental Micro Analysis, Inc. Reporting Limit (RL)

Date: Reviewed by: Don Peterson, Laboratory Director

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Analytical Report

January 11, 2010

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Project No:

PO No:

Client Sample ID: A0912266

EMA Sample No: 09122805-01

Date Received: 12/28/2009

Sample Matrix: Steviol Glycosides Powder

Analytical Method: FDA IMP-PHOS-1

Extraction Method: FDA 302

Date Extracted: 12/29/2009

Date Completed: 1/5/2010

Analyte	Amount	RL ppm	Tolerance ppm
Acephate	ND	0.010	
Azinphos-methyl	ND	0.010	
Azinphos-methyl OA	ND	0.010	
beta-chlorfenvinphos	ND	0.010	
Cadusafos	ND	0.010	
Carbofenothion	ND	0.010	
Chlorpyrifos	ND	0.010	
Chlorpyrifos OA	ND	0.010	
Chlorpyrifos-methyl	ND	0.010	
Chlorthiophos	ND	0.010	
Coumaphos	ND	0.010	
Coumaphos OA	ND	0.010	
Cyanofenphos	ND	0.010	
Cyanophos	ND	0.010	
DEF	ND	0.010	
Demeton S Sulfone	ND	0.010	
Diazinon	ND	0.010	
Diazinon OA	ND	0.010	
Dichlorvos	ND	0.010	
Dicrotophos	ND	0.010	
Dimethoate	ND	0.010	
Dioxathion	ND	0.010	
Disulfoton	ND	0.010	
Edifenphos	ND	0.010	
EPN	ND	0.010	
Ethion	ND	0.010	
Ethion OA	ND	0.010	
Ethoprop	ND	0.010	
2-Ethylhexyldiphenyl phos	ND	0.010	
Fenamiphos	ND	0.010	
Fenamiphos Sulfone	ND	0.010	
Fenitrothion	ND	0.010	
Fenthion	ND	0.010	
Fonophos	ND	0.010	
Heptenophos	ND	0.010	
Isofenphos	ND	0.010	
Leptophos	ND	0.010	
Malathion	ND	0.010	
Malathion OA	ND	0.010	

ND = Not Detected at or above Environmental Micro Analysis, Inc. Reporting Limit (RL)

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Email: jianning.chen@purecircle.com

Project No:

PO No:

Client Sample ID: A0912266

EMA Sample No: 09122805-01

Date Received: 12/28/2009

Sample Matrix: Steviol Glycosides Powder

Analytical Method: FDA IMP-PHOS-2

Extraction Method: FDA 302

Date Extracted: 12/29/2009

Date Completed: 1/5/2010

Analyte	Amount	RL ppm	Tolerance ppm
Mercarbam	ND	0.010	
Methamidophos	ND	0.010	
Methidathion	ND	0.010	
Methyl Parathion	ND	0.010	
Methyl Parathion OA	ND	0.010	
Mevinphos	ND	0.010	
Monocrotophos	ND	0.010	
Omethaote	ND	0.010	
Oxydemeton Methyl Sulfone	ND	0.010	
Parathion	ND	0.010	
Parathion OA	ND	0.010	
Phorate	ND	0.010	
Phorate OA	ND	0.010	
Phorate Sulfone	ND	0.010	
Phorate Sulfoxide	ND	0.010	
Phosalone	ND	0.010	
Phosmet	ND	0.010	
Phosmet Oxon	ND	0.010	
Pirimiphos-methy	ND	0.010	
Profenophos	ND	0.010	
Prothiophos	ND	0.010	
Prothoate	ND	0.010	
Pyrazophos	ND	0.010	
Pyridaphenthion	ND	0.010	
Quinalphos	ND	0.010	
Sulfotep	ND	0.010	
Sulprofos	ND	0.010	
Terbufos	ND	0.010	
Tetrachlorvinphos	ND	0.010	
Tolclophos Methyl	ND	0.010	
Trizaphos	ND	0.010	
Trichlorfon	ND	0.010	
Tris (Chloropropyl) Phosphate-1	ND	0.010	
Tris (Chloropropyl) Phosphate-2	ND	0.010	
Tris 2-Butoxyethyl Phosphate	ND	0.010	

ND = Not Detected at or above Environmental Micro Analysis, Inc. Reporting Limit (RL)

Date: Reviewed by: Don Peterson, Laboratory Director

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Email: jianning.chen@purecircle.com

Project No:

PO No:

Client Sample ID: A0912266

EMA Sample No: 09122805-01

Date Received: 12/28/2009

Sample Matrix: Steviol Glycosides Powder

Analytical Method: EBDC Screen

Extraction Method: CDFA

Date Extracted: 1/4/2010

Date Completed: 1/5/2010

Analyte	Amount	RL ppm	Tolerance ppm
Ferbam	ND	0.030	
Maneb (Dithane M-22)	ND	0.030	
Mancozeb	ND	0.030	
Metiram	ND	0.030	
Nabam	ND	0.030	
Thiram	ND	0.030	
Zineb	ND	0.030	
Ziram	ND	0.030	

ND = Not Detected at or above Environmental Micro Analysis, Inc. Reporting Limit (RL)

Date: Reviewed by: Don Peterson, Laboratory Director

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Email: jianning.chen@purecircle.com

Project No:

PO No:

Client Sample ID: A0912267

EMA Sample No: 09122805-02

Date Received: 12/28/2009

Sample Matrix: Steviol Glycosides Powder

Analytical Method: FDA IMP-OC1

Extraction Method: FDA 302

Date Extracted: 12/29/2009

Date Completed: 1/2/2010

Analyte	Amount	RL ppm	Tolerance ppm
Acrinathrin	ND	0.010	
Aldrin	ND	0.010	
alpha-BHC	ND	0.010	
Atrazine	ND	0.010	
Benfluralin	ND	0.010	
beta Chlordane	ND	0.010	
Bifenthrin	ND	0.010	
Boscalid	ND	0.010	
Bromopropylate	ND	0.010	
Captan	ND	0.010	
Chlorodimeform	ND	0.010	
Chlorobenzilate	ND	0.010	
Chloroneb	ND	0.010	
Chlorothalonil	ND	0.010	
CIPC	ND	0.010	
cis-Chlordane	ND	0.010	
cis-Nonachlor	ND	0.010	
cis-Permethrin	ND	0.010	
Clomazone	ND	0.010	
Cyanazine	ND	0.010	
Cypermethrin	ND	0.010	
Cyproconazole	ND	0.010	
Dacthal	ND	0.010	
Decamethrin	ND	0.010	
delta-BHC	ND	0.010	
Diallate-1	ND	0.010	
Diallate-2	ND	0.010	
3,4-Dichloroanaline	ND	0.010	
Dichlobenil	ND	0.010	
Dichlofluanid	ND	0.010	
Dichloran	ND	0.010	
Dieldrin	ND	0.010	
Difenoconazole	ND	0.010	
Dimethachlor	ND	0.010	
Dimethmorph	ND	0.010	
Dinitramine	ND	0.010	
Dursban	ND	0.010	
Endosulfan Sulfate	ND	0.010	
Endosulfan-I	ND	0.010	
Endosulfan-II	ND	0.010	
Endrin	ND	0.010	
Epoxiconazole	ND	0.010	
Etaconazole-1	ND	0.010	
Etaconazole-2	ND	0.010	
Ethafluralin	ND	0.010	
Etridiazole	ND	0.010	
Fentarimol	ND	0.010	
Fenbuconazole	ND	0.010	
Fenhexamid	ND	0.010	
Fenvalerate-1	ND	0.010	
Fenvalerate-2	ND	0.010	
Fipronil	ND	0.010	
Fluchloralin	ND	0.010	
Flucythrinate	ND	0.010	
Fludioxanil	ND	0.010	
Fluquinconazole	ND	0.010	
Flutolanil	ND	0.010	
Fluvalinate	ND	0.010	
Folpet	ND	0.010	
gamma-BHC/Lindane	ND	0.010	
gamma-Chlordane	ND	0.010	
Heptachlor	ND	0.010	
Heptachlor Epoxide	ND	0.010	
Hexachlorobenzene	ND	0.010	

ND = Not Detected at or above Environmental Micro Analysis, Inc. Reporting Limit (RL)

Date: Reviewed by: Don Peterson, Laboratory Director

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460 N East Street
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Analytical Report

January 11, 2010

Client: Jianning Chen
PureCircle Co., Ltd China
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Jiangxi Province, .
Phone: (+86) 797-4601666
Fax: (+86) 797-4601663
Email: jianning.chen@purecircle.com

Project No:

PO No:

Client Sample ID: A0912267

EMA Sample No: 09122805-02

Date Received: 12/28/2009

Sample Matrix: Steviol Glycosides Powder

Analytical Method: FDA IMP-OC2

Extraction Method: FDA 302

Date Extracted: 12/29/2009

Date Completed: 1/2/2010

Analyte	Amount	RL ppm	Tolerance ppm
Hexaconazole	ND	0.010	
Imazalil	ND	0.010	
Iprodione	ND	0.010	
lambda-Cyhalothrin	ND	0.010	
Linuron	ND	0.010	
Metolachlor	ND	0.010	
Mirex	ND	0.010	
Monolinuron	ND	0.010	
Myclobutanil	ND	0.010	
Nitrofen	ND	0.010	
Nuarimol	ND	0.010	
o,p'-DDT	ND	0.010	
o,p'-Methoxychlor	ND	0.010	
Octachlor Epoxide	ND	0.010	
p,p'-DDE	ND	0.010	
p,p'-DDT	ND	0.010	
p,p'-Methoxychlor	ND	0.010	
Penconazole	ND	0.010	
Pentachloroanaline	ND	0.010	
Pentachlorobenzene	ND	0.010	
Pentachlorobenzonitrile	ND	0.010	
Pentachlorothioanisole	ND	0.010	
Prochloraz	ND	0.010	
Procymidone	ND	0.010	
Profluralin	ND	0.010	
Pronamide	ND	0.010	
Propachlor	ND	0.010	
Propanil	ND	0.010	
Propiconizole/Tilt-1	ND	0.010	
Propiconizole/Tilt-2	ND	0.010	
Pyraclostrobin	ND	0.010	
Pyridaben	ND	0.010	
Pyrifenox-1	ND	0.010	
Pyrifenox-2	ND	0.010	
Quinoxyfen	ND	0.010	
Quintozene	ND	0.010	
Simazine	ND	0.010	
Tebuconazole	ND	0.010	
Tebufenpyrad	ND	0.010	
Tecnazene	ND	0.010	
Tefluthrin	ND	0.010	
Terbuthylazine	ND	0.010	
2,3,5,6-Tetrachloroanaline	ND	0.010	
Tetradifon	ND	0.010	
Tolylfluanid	ND	0.010	
trans-Chlordane	ND	0.010	
trans-Nonachlor	ND	0.010	
trans-Permethrin	ND	0.010	
Triadimefon	ND	0.010	
Triadimenol	ND	0.010	
Triallate	ND	0.010	
Trifloxystrobin	ND	0.010	
Triflumizole	ND	0.010	
Trifluralin	ND	0.010	
Triticonazole	ND	0.010	
Vinclozolin	ND	0.010	

ND = Not Detected at or above Environmental Micro Analysis, Inc. Reporting Limit (RL)

Date: Reviewed by: Don Peterson, Laboratory Director

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Analytical Report

January 11, 2010

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Project No:

PO No:

Client Sample ID: A0912267

EMA Sample No: 09122805-02

Date Received: 12/28/2009

Sample Matrix: Steviol Glycosides Powder

Analytical Method: FDA IMP-NSO-1

Extraction Method: FDA 302

Date Extracted: 12/29/2009

Date Completed: 1/5/2010

Analyte	Amount	RL ppm	Tolerance ppm
Allethrin	ND	0.010	
Acetamiprid	ND	0.010	
alpha-Spiroxamine	ND	0.010	
Ametryn	ND	0.010	
Aminocarb	ND	0.010	
Amitraz	ND	0.010	
Aniten/Flurecol Butyl E.	ND	0.010	
Azoxystrobin	ND	0.010	
Benalaxyl	ND	0.010	
Bendiocarb	ND	0.010	
Bendiocarb - C2H3NO	ND	0.010	
beta-Spiroxamine	ND	0.010	
Biphenyl	ND	0.010	
Biteranol-1	ND	0.010	
Biteranol-2	ND	0.010	
Bufenocarb-1	ND	0.010	
Bufenocarb-2	ND	0.010	
Bupirimate	ND	0.010	
Buprofezin	ND	0.010	
Butralin	ND	0.010	
Butylate	ND	0.010	
3-OH Carbofuran	ND	0.010	
Carbaryl	ND	0.010	
Carbaryl - C2H3NO	ND	0.010	
Carbofuran	ND	0.010	
Carbofuran - C2H3NO	ND	0.010	
Carbosulfan	ND	0.010	
Carboxin	ND	0.010	
Cycloate/Ro Neet	ND	0.010	
Cycluron	ND	0.010	
Cymiazole	ND	0.010	
Cyprodinil	ND	0.010	
Desmedipham	ND	0.010	
Desmethyl Diphenamid	ND	0.010	
Desmetryn	ND	0.010	
Diethofencarb	ND	0.010	
Diniconazole	ND	0.010	
Dioxacarb	ND	0.010	
Dioxacarb - C2H3NO	ND	0.010	
Diphenamid	ND	0.010	
d-Phenothrin #	ND	0.010	
EPTC/Eptam	ND	0.010	
Ethiofencarb	ND	0.010	
Ethiolate	ND	0.010	
Ethiolate	ND	0.010	
Ethofumesate	ND	0.010	
Ethoxyquin	ND	0.010	
Etofenprox	ND	0.010	
Famoxadone	ND	0.010	
Fenazaquin	ND	0.010	
Fenfuram	ND	0.010	
Fenobucarb	ND	0.010	
Fenoxycarb	ND	0.010	
Fenpropathrin	ND	0.010	
Fenpropimorph	ND	0.010	
Flusilazole	ND	0.010	
Fuberdiazole	ND	0.010	
Furalaxyl	ND	0.010	
Hexazinone	ND	0.010	
Isocarbamid	ND	0.010	

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Project No:

PO No:

Client Sample ID: A0912267

EMA Sample No: 09122805-02

Date Received: 12/28/2009

Sample Matrix: Steviol Glycosides Powder

Analytical Method: FDA IMP-NSO-2

Extraction Method: FDA 302

Date Extracted: 12/29/2009

Date Completed: 1/5/2010

Analyte	Amount	RL ppm	Tolerance ppm
Isoprocarb	ND	0.010	
Isopropalin	ND	0.010	
Isoprothiolane	ND	0.010	
Isoproturon (frag)	ND	0.010	
Kresoxim-Methyl	ND	0.010	
Lenacil	ND	0.010	
MBTZ	ND	0.010	
Mepronil	ND	0.010	
Metalaxyl	ND	0.010	
Metaldehyde	ND	0.010	
Methfuroxan	ND	0.010	
Methiocarb	ND	0.010	
Methoprotryne	ND	0.010	
Metolacarb	ND	0.010	
Metribuzin	ND	0.010	
Mexacarbate	ND	0.010	
MGK-264	ND	0.010	
Molinate	ND	0.010	
Naphthalene Acetamide	ND	0.010	
Naproanalid	ND	0.010	
Napropamide	ND	0.010	
Nitralin	ND	0.010	
Nitrothal Isopropyl	ND	0.010	
Norea	ND	0.010	
Octhilinone	ND	0.010	
o-Phenylphenol	ND	0.010	
Pebulate	ND	0.010	
Pendimethalin	ND	0.010	
Phenmedipham	ND	0.010	
Piperonyl Butoxide	ND	0.010	
Pirimacarb	ND	0.010	
Promecarb	ND	0.010	
Prometon	ND	0.010	
Prometryn	ND	0.010	
Propargite	ND	0.010	
Propham	ND	0.010	
Propoxur	ND	0.010	
Propoxur - C2H3NO	ND	0.010	
Pyracarbolid	ND	0.010	
Pyrethrin-1	ND	0.010	
Pyrethrin-2	ND	0.010	
Pyrimethanil	ND	0.010	
Pyriproxifen	ND	0.010	
Resmethrin	ND	0.010	
Secbumeton	ND	0.010	
Sethoxydim	ND	0.010	
Simetryn	ND	0.010	
Tebutam	ND	0.010	
Tebuthiuron	ND	0.010	
Terbumeton	ND	0.010	
Terbutryn	ND	0.010	
Tetraconazole	ND	0.010	
Tetramethrin-1	ND	0.010	
Tetramethrin-2	ND	0.010	
Thiabendazole	ND	0.010	
THPI	ND	0.010	
Tralkoxydim	ND	0.010	
Tricyclazole	ND	0.010	
2,3,5-Trimethacarb	ND	0.010	
3,4,5-Trimethacarb	ND	0.010	
Vernolate	ND	0.010	

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Project No:

PO No:

Client Sample ID: A0912267

EMA Sample No: 09122805-02

Date Received: 12/28/2009

Sample Matrix: Steviol Glycosides Powder

Analytical Method: FDA IMP-PHOS-1

Extraction Method: FDA 302

Date Extracted: 12/29/2009

Date Completed: 1/5/2010

Analyte	Amount	RL ppm	Tolerance ppm
Acephate	ND	0.010	
Azinphos-methyl	ND	0.010	
Azinphos-methyl OA	ND	0.010	
beta-chlorfenvinphos	ND	0.010	
Cadusafos	ND	0.010	
Carbofenothion	ND	0.010	
Chlorpyrifos	ND	0.010	
Chlorpyrifos OA	ND	0.010	
Chlorpyrifos-methyl	ND	0.010	
Chlorthiophos	ND	0.010	
Coumaphos	ND	0.010	
Coumaphos OA	ND	0.010	
Cyanofenphos	ND	0.010	
Cyanophos	ND	0.010	
DEF	ND	0.010	
Demeton S Sulfone	ND	0.010	
Diazinon	ND	0.010	
Diazinon OA	ND	0.010	
Dichlorvos	ND	0.010	
Dicrotophos	ND	0.010	
Dimethoate	ND	0.010	
Dioxathion	ND	0.010	
Disulfoton	ND	0.010	
Edifenphos	ND	0.010	
EPN	ND	0.010	
Ethion	ND	0.010	
Ethion OA	ND	0.010	
Ethoprop	ND	0.010	
2-Ethylhexyldiphenyl phos	ND	0.010	
Fenamiphos	ND	0.010	
Fenamiphos Sulfone	ND	0.010	
Fenitrothion	ND	0.010	
Fenthion	ND	0.010	
Fonophos	ND	0.010	
Heptenophos	ND	0.010	
Isofenphos	ND	0.010	
Leptophos	ND	0.010	
Malathion	ND	0.010	
Malathion OA	ND	0.010	

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Date. Reviewed by: Don Peterson, Laboratory Director

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Project No:

PO No:

Client Sample ID: A0912267

EMA Sample No: 09122805-02

Date Received: 12/28/2009

Sample Matrix: Steviol Glycosides Powder

Analytical Method: FDA IMP-PHOS-2

Extraction Method: FDA 302

Date Extracted: 12/29/2009

Date Completed: 1/5/2010

Analyte	Amount	RL ppm	Tolerance ppm
Mercarbam	ND	0.010	
Methamidophos	ND	0.010	
Methidathion	ND	0.010	
Methyl Parathion	ND	0.010	
Methyl Parathion OA	ND	0.010	
Mevinphos	ND	0.010	
Monocrotophos	ND	0.010	
Omethaote	ND	0.010	
Oxydemeton Methyl Sulfone	ND	0.010	
Parathion	ND	0.010	
Parathion OA	ND	0.010	
Phorate	ND	0.010	
Phorate OA	ND	0.010	
Phorate Sulfone	ND	0.010	
Phorate Sulfoxide	ND	0.010	
Phosalone	ND	0.010	
Phosmet	ND	0.010	
Phosmet Oxon	ND	0.010	
Pirimiphos-methy	ND	0.010	
Profenophos	ND	0.010	
Prothiophos	ND	0.010	
Prothoate	ND	0.010	
Pyrazophos	ND	0.010	
Pyridaphenthion	ND	0.010	
Quinalphos	ND	0.010	
Sulfotep	ND	0.010	
Sulprofos	ND	0.010	
Terbufos	ND	0.010	
Tetrachlorvinphos	ND	0.010	
Tolclophos Methyl	ND	0.010	
Trizaphos	ND	0.010	
Trichlorfon	ND	0.010	
Tris (Chloropropyl) Phosphate-1	ND	0.010	
Tris (Chloropropyl) Phosphate-2	ND	0.010	
Tris 2-Butoxyethyl Phosphate	ND	0.010	

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Project No:

PO No:

Client Sample ID: A0912267

EMA Sample No: 09122805-02

Date Received: 12/28/2009

Sample Matrix: Steviol Glycosides Powder

Analytical Method: EBDC Screen

Extraction Method: CDFA

Date Extracted: 1/4/2010

Date Completed: 1/5/2010

Analyte	Amount	RL ppm	Tolerance ppm
Ferbam	ND	0.030	
Maneb (Dithane M-22)	ND	0.030	
Mancozeb	ND	0.030	
Metiram	ND	0.030	
Nabam	ND	0.030	
Thiram	ND	0.030	
Zineb	ND	0.030	
Ziram	ND	0.030	

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Analytical Report

January 11, 2010

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Project No:

PO No:

Client Sample ID: A0912268

EMA Sample No: 09122805-03

Date Received: 12/28/2009

Sample Matrix: Steviol Glycosides Powder

Analytical Method: FDA IMP-OC1

Extraction Method: FDA 302

Date Extracted: 12/29/2009

Date Completed: 1/2/2010

Analyte	Amount	RL ppm	Tolerance ppm
Acrinathrin	ND	0.010	
Aldrin	ND	0.010	
alpha-BHC	ND	0.010	
Atrazine	ND	0.010	
Benfluralin	ND	0.010	
beta Chlordane	ND	0.010	
Bifenthrin	ND	0.010	
Boscalid	ND	0.010	
Bromopropylate	ND	0.010	
Captan	ND	0.010	
Chlorodimeform	ND	0.010	
Chlorobenzilate	ND	0.010	
Chloroneb	ND	0.010	
Chlorothalonil	ND	0.010	
CIPC	ND	0.010	
cis-Chlordane	ND	0.010	
cis-Nonachlor	ND	0.010	
cis-Permethrin	ND	0.010	
Clomazone	ND	0.010	
Cyanazine	ND	0.010	
Cypermethrin	ND	0.010	
Cyproconazole	ND	0.010	
Dacthal	ND	0.010	
Decamethrin	ND	0.010	
delta-BHC	ND	0.010	
Diallate-1	ND	0.010	
Diallate-2	ND	0.010	
3,4-Dichloroanaline	ND	0.010	
Dichlobenil	ND	0.010	
Dichlofluanid	ND	0.010	
Dichloran	ND	0.010	
Dieldrin	ND	0.010	
Difenoconazole	ND	0.010	
Dimethachlor	ND	0.010	
Dimethmorph	ND	0.010	
Dinitramine	ND	0.010	
Dursban	ND	0.010	
Endosulfan Sulfate	ND	0.010	
Endosulfan-I	ND	0.010	
Endosulfan-II	ND	0.010	
Endrin	ND	0.010	
Epoxiconazole	ND	0.010	
Etaconazole-1	ND	0.010	
Etaconazole-2	ND	0.010	
Ethafluralin	ND	0.010	
Etridiazole	ND	0.010	
Fenarimol	ND	0.010	
Fenbuconazole	ND	0.010	
Fenhexamid	ND	0.010	
Fenvalerate-1	ND	0.010	
Fenvalerate-2	ND	0.010	
Fipronil	ND	0.010	
Fluchloralin	ND	0.010	
Flucythrinate	ND	0.010	
Fludioxanil	ND	0.010	
Fluquinconazole	ND	0.010	
Flutolanil	ND	0.010	
Fluvalinate	ND	0.010	
Folpet	ND	0.010	
gamma-BHC/Lindane	ND	0.010	
gamma-Chlordane	ND	0.010	
Heptachlor	ND	0.010	
Heptachlor Epoxide	ND	0.010	
Hexachlorobenzene	ND	0.010	

ND = Not Detected at or above Environmental Micro Analysis, Inc. Reporting Limit (RL)

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January 11, 2010

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Project No:

PO No:

Client Sample ID: A0912268

EMA Sample No: 09122805-03

Date Received: 12/28/2009

Sample Matrix: Steviol Glycosides Powder

Analytical Method: FDA IMP-OC2

Extraction Method: FDA 302

Date Extracted: 12/29/2009

Date Completed: 1/2/2010

Analyte	Amount	RL ppm	Tolerance ppm
Hexaconazole	ND	0.010	
Imazalil	ND	0.010	
Iprodione	ND	0.010	
lambda-Cyhalothrin	ND	0.010	
Linuron	ND	0.010	
Metolachlor	ND	0.010	
Mirex	ND	0.010	
Monolinuron	ND	0.010	
Myclobutanil	ND	0.010	
Nitrofen	ND	0.010	
Nuarimol	ND	0.010	
o,p'-DDT	ND	0.010	
o,p'-Methoxychlor	ND	0.010	
Octachlor Epoxide	ND	0.010	
p,p'-DDE	ND	0.010	
p,p'-DDT	ND	0.010	
p,p'-Methoxychlor	ND	0.010	
Penconazole	ND	0.010	
Pentachloroanaline	ND	0.010	
Pentachlorobenzene	ND	0.010	
Pentachlorobenzonitrile	ND	0.010	
Pentachlorothioanisole	ND	0.010	
Prochloraz	ND	0.010	
Procymidone	ND	0.010	
Profluralin	ND	0.010	
Pronamide	ND	0.010	
Propachlor	ND	0.010	
Propanil	ND	0.010	
Propiconizole/Tilt-1	ND	0.010	
Propiconizole/Tilt-2	ND	0.010	
Pyraclostrobin	ND	0.010	
Pyridaben	ND	0.010	
Pyrifenox-1	ND	0.010	
Pyrifenox-2	ND	0.010	
Quinoxyfen	ND	0.010	
Quintozene	ND	0.010	
Simazine	ND	0.010	
Tebuconazole	ND	0.010	
Tebufenpyrad	ND	0.010	
Tecnazene	ND	0.010	
Tefluthrin	ND	0.010	
Terbuthylazine	ND	0.010	
2,3,5,6-Tetrachloroanaline	ND	0.010	
Tetradifon	ND	0.010	
Tolylfluanid	ND	0.010	
trans-Chlordane	ND	0.010	
trans-Nonachlor	ND	0.010	
trans-Permethrin	ND	0.010	
Triadimefon	ND	0.010	
Triadimenol	ND	0.010	
Triallate	ND	0.010	
Trifloxystrobin	ND	0.010	
Triflumizole	ND	0.010	
Trifluralin	ND	0.010	
Triticonazole	ND	0.010	
Vinclozolin	ND	0.010	

ND = Not Detected at or above Environmental Micro Analysis, Inc. Reporting Limit (RL)

Date: Reviewed by: Don Peterson Laboratory Director

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Project No:

PO No:

Client Sample ID: A0912268

EMA Sample No: 09122805-03

Date Received: 12/28/2009

Sample Matrix: Steviol Glycosides Powder

Analytical Method: FDA IMP-NSO-1

Extraction Method: FDA 302

Date Extracted: 12/29/2009

Date Completed: 1/5/2010

Analyte	Amount	RL ppm	Tolerance ppm
Allethrin	ND	0.010	
Acetamiprid	ND	0.010	
alpha-Spiroxamine	ND	0.010	
Ametryn	ND	0.010	
Aminocarb	ND	0.010	
Amitraz	ND	0.010	
Aniten/Flurecol Butyl E.	ND	0.010	
Azoxystrobin	ND	0.010	
Benalaxyl	ND	0.010	
Bendiocarb	ND	0.010	
Bendiocarb - C2H3NO	ND	0.010	
beta-Spiroxamine	ND	0.010	
Biphenyl	ND	0.010	
Biteranol-1	ND	0.010	
Biteranol-2	ND	0.010	
Bufenocarb-1	ND	0.010	
Bufenocarb-2	ND	0.010	
Bupirimate	ND	0.010	
Buprofezin	ND	0.010	
Butralin	ND	0.010	
Butylate	ND	0.010	
3-OH Carbofuran	ND	0.010	
Carbaryl	ND	0.010	
Carbaryl - C2H3NO	ND	0.010	
Carbofuran	ND	0.010	
Carbofuran - C2H3NO	ND	0.010	
Carbosulfan	ND	0.010	
Carboxin	ND	0.010	
Cycloate/Ro Neet	ND	0.010	
Cycluron	ND	0.010	
Cymiazole	ND	0.010	
Cyprodinil	ND	0.010	
Desmedipham	ND	0.010	
Desmethyl Diphenamid	ND	0.010	
Desmetryn	ND	0.010	
Diethofenocarb	ND	0.010	
Diniconazole	ND	0.010	
Dioxacarb	ND	0.010	
Dioxacarb - C2H3NO	ND	0.010	
Diphenamid	ND	0.010	
d-Phenothrin #	ND	0.010	
EPTC/Eptam	ND	0.010	
Ethiofencarb	ND	0.010	
Ethiolate	ND	0.010	
Ethiolate	ND	0.010	
Ethofumesate	ND	0.010	
Ethoxyquin	ND	0.010	
Etofenprox	ND	0.010	
Famoxadone	ND	0.010	
Fenazaquin	ND	0.010	
Fenfuram	ND	0.010	
Fenobucarb	ND	0.010	
Fenoxycarb	ND	0.010	
Fenpropathrin	ND	0.010	
Fenpropimorph	ND	0.010	
Flusilazole	ND	0.010	
Fuberdiazole	ND	0.010	
Furalaxyl	ND	0.010	
Hexazinone	ND	0.010	
Isocarbamid	ND	0.010	

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Environmental Micro Analysis, Inc. ELAP Certificate #2211
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Analytical Report

January 11, 2010

Client: Jianning Chen
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Phone: (+86) 797-4601666
Fax: (+86) 797-4601663
Email: jianning.chen@purecircle.com

Project No:

PO No:

Client Sample ID: A0912268

EMA Sample No: 09122805-03

Date Received: 12/28/2009

Sample Matrix: Steviol Glycosides Powder

Analytical Method: FDA IMP-NSO-2

Extraction Method: FDA 302

Date Extracted: 12/29/2009

Date Completed: 1/5/2010

Analyte	Amount	RL ppm	Tolerance ppm
Isoprocarb	ND	0.010	
Isopropalin	ND	0.010	
Isoprothiolane	ND	0.010	
Isoproturon (frag)	ND	0.010	
Kresoxim-Methyl	ND	0.010	
Lenacil	ND	0.010	
MBTZ	ND	0.010	
Mepronil	ND	0.010	
Metalaxyl	ND	0.010	
Metaldehyde	ND	0.010	
Methfuroxan	ND	0.010	
Methiocarb	ND	0.010	
Methoprotryne	ND	0.010	
Metolacarb	ND	0.010	
Metribuzin	ND	0.010	
Mexacarbate	ND	0.010	
MGK-264	ND	0.010	
Molinate	ND	0.010	
Naphthalene Acetamide	ND	0.010	
Naproanalid	ND	0.010	
Napropamide	ND	0.010	
Nitralin	ND	0.010	
Nitrothal Isopropyl	ND	0.010	
Norea	ND	0.010	
Octhilinone	ND	0.010	
o-Phenylphenol	ND	0.010	
Pebulate	ND	0.010	
Pendimethalin	ND	0.010	
Phenmedipham	ND	0.010	
Piperonyl Butoxide	ND	0.010	
Pirimacarb	ND	0.010	
Promecarb	ND	0.010	
Prometon	ND	0.010	
Prometryn	ND	0.010	
Propargite	ND	0.010	
Propham	ND	0.010	
Propoxur	ND	0.010	
Propoxur - C2H3NO	ND	0.010	
Pyracarbolid	ND	0.010	
Pyrethrin-1	ND	0.010	
Pyrethrin-2	ND	0.010	
Pyrimethanil	ND	0.010	
Pyriproxifen	ND	0.010	
Resmethrin	ND	0.010	
Secbumeton	ND	0.010	
Sethoxydim	ND	0.010	
Simetryn	ND	0.010	
Tebutam	ND	0.010	
Tebuthiuron	ND	0.010	
Terbumeton	ND	0.010	
Terbutryn	ND	0.010	
Tetraconazole	ND	0.010	
Tetramethrin-1	ND	0.010	
Tetramethrin-2	ND	0.010	
Thiabendazole	ND	0.010	
THPI	ND	0.010	
Tralkoxydim	ND	0.010	
Tricyclazole	ND	0.010	
2,3,5-Trimethacarb	ND	0.010	
3,4,5-Trimethacarb	ND	0.010	
Vernolate	ND	0.010	

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Date: Reviewed by: Don Peterson, Laboratory Director

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January 11, 2010

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Project No:

PO No:

Client Sample ID: A0912268

EMA Sample No: 09122805-03

Date Received: 12/28/2009

Sample Matrix: Steviol Glycosides Powder

Analytical Method: FDA IMP-PHOS-1

Extraction Method: FDA 302

Date Extracted: 12/29/2009

Date Completed: 1/5/2010

Analyte	Amount	RL ppm	Tolerance ppm
Acephate	ND	0.010	
Azinphos-methyl	ND	0.010	
Azinphos-methyl OA	ND	0.010	
beta-chlorfenvinphos	ND	0.010	
Cadusafos	ND	0.010	
Carbofenothion	ND	0.010	
Chlorpyrifos	ND	0.010	
Chlorpyrifos OA	ND	0.010	
Chlorpyrifos-methyl	ND	0.010	
Chlorthiophos	ND	0.010	
Coumaphos	ND	0.010	
Coumaphos OA	ND	0.010	
Cyanofenphos	ND	0.010	
Cyanophos	ND	0.010	
DEF	ND	0.010	
Demeton S Sulfone	ND	0.010	
Diazinon	ND	0.010	
Diazinon OA	ND	0.010	
Dichlorvos	ND	0.010	
Dicrotophos	ND	0.010	
Dimethoate	ND	0.010	
Dioxathion	ND	0.010	
Disulfoton	ND	0.010	
Edifenphos	ND	0.010	
EPN	ND	0.010	
Ethion	ND	0.010	
Ethion OA	ND	0.010	
Ethoprop	ND	0.010	
2-Ethylhexyldiphenyl phos.	ND	0.010	
Fenamiphos	ND	0.010	
Fenamiphos Sulfone	ND	0.010	
Fenitrothion	ND	0.010	
Fenthion	ND	0.010	
Fonophos	ND	0.010	
Heptenophos	ND	0.010	
Isofenphos	ND	0.010	
Leptophos	ND	0.010	
Malathion	ND	0.010	
Malathion OA	ND	0.010	

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Project No:

PO No:

Client Sample ID: A0912268

EMA Sample No: 09122805-03

Date Received: 12/28/2009

Sample Matrix: Steviol Glycosides Powder

Analytical Method: FDA IMP-PHOS-2

Extraction Method: FDA 302

Date Extracted: 12/29/2009

Date Completed: 1/5/2010

Analyte	Amount	RL ppm	Tolerance ppm
Mercarbam	ND	0.010	
Methamidophos	ND	0.010	
Methidathion	ND	0.010	
Methyl Parathion	ND	0.010	
Methyl Parathion OA	ND	0.010	
Mevinphos	ND	0.010	
Monocrotophos	ND	0.010	
Omethaote	ND	0.010	
Oxydemeton Methyl Sulfone	ND	0.010	
Parathion	ND	0.010	
Parathion OA	ND	0.010	
Phorate	ND	0.010	
Phorate OA	ND	0.010	
Phorate Sulfone	ND	0.010	
Phorate Sulfoxide	ND	0.010	
Phosalone	ND	0.010	
Phosmet	ND	0.010	
Phosmet Oxon	ND	0.010	
Pirimiphos-methy	ND	0.010	
Profenophos	ND	0.010	
Prothiophos	ND	0.010	
Prothoate	ND	0.010	
Pyrazophos	ND	0.010	
Pyridaphenthion	ND	0.010	
Quinalphos	ND	0.010	
Sulfotep	ND	0.010	
Sulprofos	ND	0.010	
Terbufos	ND	0.010	
Tetrachlorvinphos	ND	0.010	
Tolclophos Methyl	ND	0.010	
Trizaphos	ND	0.010	
Trichlorfon	ND	0.010	
Tris (Chloropropyl) Phosphate-1	ND	0.010	
Tris (Chloropropyl) Phosphate-2	ND	0.010	
Tris 2-Butoxyethyl Phosphate	ND	0.010	

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Project No:

PO No:

Client Sample ID: A0912268

EMA Sample No: 09122805-03

Date Received: 12/28/2009

Sample Matrix: Steviol Glycosides Powder

Analytical Method: EBDC Screen

Extraction Method: CDFA

Date Extracted: 1/4/2010

Date Completed: 1/5/2010

Analyte	Amount	RL ppm	Tolerance ppm
Ferbam	ND	0.030	
Maneb (Dithane M-22)	ND	0.030	
Mancozeb	ND	0.030	
Metiram	ND	0.030	
Nabam	ND	0.030	
Thiram	ND	0.030	
Zineb	ND	0.030	
Ziram	ND	0.030	

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January 11, 2010

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Project No:

PO No:

Client Sample ID: A0912269

EMA Sample No: 09122805-04

Date Received: 12/28/2009

Sample Matrix: Steviol Glycosides Powder

Analytical Method: FDA IMP-OC1

Extraction Method: FDA 302

Date Extracted: 12/29/2009

Date Completed: 1/2/2010

Analyte	Amount	RL ppm	Tolerance ppm
Acrinathrin	ND	0.010	
Aldrin	ND	0.010	
alpha-BHC	ND	0.010	
Atrazine	ND	0.010	
Benfluralin	ND	0.010	
beta Chlordene	ND	0.010	
Bifenthrin	ND	0.010	
Boscalid	ND	0.010	
Bromopropylate	ND	0.010	
Captan	ND	0.010	
Chlorodimeform	ND	0.010	
Chlorobenzilate	ND	0.010	
Chloroneb	ND	0.010	
Chlorothalonil	ND	0.010	
CIPC	ND	0.010	
cis-Chlordane	ND	0.010	
cis-Nonachlor	ND	0.010	
cis-Permethrin	ND	0.010	
Clomazone	ND	0.010	
Cyanazine	ND	0.010	
Cypermethrin	ND	0.010	
Cyproconazole	ND	0.010	
Dacthal	ND	0.010	
Decamethrin	ND	0.010	
delta-BHC	ND	0.010	
Diallate-1	ND	0.010	
Diallate-2	ND	0.010	
3,4-Dichloroanaline	ND	0.010	
Dichlobenil	ND	0.010	
Dichlofluanid	ND	0.010	
Dichloran	ND	0.010	
Dieldrin	ND	0.010	
Difenoconazole	ND	0.010	
Dimethachlor	ND	0.010	
Dimethmorph	ND	0.010	
Dinitramine	ND	0.010	
Dursban	ND	0.010	
Endosulfan Sulfate	ND	0.010	
Endosulfan-I	ND	0.010	
Endosulfan-II	ND	0.010	
Endrin	ND	0.010	
Epoxiconazole	ND	0.010	
Etaconazole-1	ND	0.010	
Etaconazole-2	ND	0.010	
Ethafluralin	ND	0.010	
Etridiazole	ND	0.010	
Fentarimol	ND	0.010	
Fenbuconazole	ND	0.010	
Fenhexamid	ND	0.010	
Fenvalerate-1	ND	0.010	
Fenvalerate-2	ND	0.010	
Fipronil	ND	0.010	
Fluchloralin	ND	0.010	
Flucythrinate	ND	0.010	
Fludioxanil	ND	0.010	
Fluquinconazole	ND	0.010	
Flutolanil	ND	0.010	
Fluvalinate	ND	0.010	
Fosetet	ND	0.010	
gamma-BHC/Lindane	ND	0.010	
gamma-Chlordene	ND	0.010	
Heptachlor	ND	0.010	
Heptachlor Epoxide	ND	0.010	
Hexachlorobenzene	ND	0.010	

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Analytical Report

January 11, 2010

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Project No:

PO No:

Client Sample ID: A0912269

EMA Sample No: 09122805-04

Date Received: 12/28/2009

Sample Matrix: Steviol Glycosides Powder

Analytical Method: FDA IMP-OC2

Extraction Method: FDA 302

Date Extracted: 12/29/2009

Date Completed: 1/2/2010

Analyte	Amount	RL ppm	Tolerance ppm
Hexaconazole	ND	0.010	
Imazalil	ND	0.010	
Iprodione	ND	0.010	
lambda-Cyhalothrin	ND	0.010	
Linuron	ND	0.010	
Metolachlor	ND	0.010	
Mirex	ND	0.010	
Monolinuron	ND	0.010	
Myclobutanil	ND	0.010	
Nitrofen	ND	0.010	
Nuarimol	ND	0.010	
o,p'-DDT	ND	0.010	
0,p'-Methoxychlor	ND	0.010	
Octachlor Epoxide	ND	0.010	
p,p'-DDE	ND	0.010	
p,p'-DDT	ND	0.010	
p,p'-Methoxychlor	ND	0.010	
Penconazole	ND	0.010	
Pentachloroanaline	ND	0.010	
Pentachlorobenzene	ND	0.010	
Pentachlorobenzonitrile	ND	0.010	
Pentachlorothioanisole	ND	0.010	
Prochloraz	ND	0.010	
Procymidone	ND	0.010	
Profluralin	ND	0.010	
Pronamide	ND	0.010	
Propachlor	ND	0.010	
Propanil	ND	0.010	
Propiconizole/Tilt-1	ND	0.010	
Propiconizole/Tilt-2	ND	0.010	
Pyraclostrobin	ND	0.010	
Pyridaben	ND	0.010	
Pyrifenox-1	ND	0.010	
Pyrifenox-2	ND	0.010	
Quinoxyfen	ND	0.010	
Quintozene	ND	0.010	
Simazine	ND	0.010	
Tebuconazole	ND	0.010	
Tebufenpyrad	ND	0.010	
Tecnazene	ND	0.010	
Tefluthrin	ND	0.010	
Terbuthylazine	ND	0.010	
2,3,5,6-Tetrachloroanaline	ND	0.010	
Tetradifon	ND	0.010	
Tolylfluanid	ND	0.010	
trans-Chlordane	ND	0.010	
trans-Nonachlor	ND	0.010	
trans-Permethrin	ND	0.010	
Triadimefon	ND	0.010	
Triadimenol	ND	0.010	
Triallate	ND	0.010	
Trifloxystrobin	ND	0.010	
Triflumizole	ND	0.010	
Trifluralin	ND	0.010	
Triticonazole	ND	0.010	
Vinclozolin	ND	0.010	

ND = Not Detected at or above Environmental Micro Analysis, Inc. Reporting Limit (RL)

Date: Reviewed by: Don Peterson, Laboratory Director

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Project No:

PO No:

Client Sample ID: A0912269

EMA Sample No: 09122805-04

Date Received: 12/28/2009

Sample Matrix: Steviol Glycosides Powder

Analytical Method: FDA IMP-NSO-1

Extraction Method: FDA 302

Date Extracted: 12/29/2009

Date Completed: 1/5/2010

Analyte	Amount	RL ppm	Tolerance ppm
Allethrin	ND	0.010	
Acetamiprid	ND	0.010	
alpha-Spiroxamine	ND	0.010	
Ametryn	ND	0.010	
Aminocarb	ND	0.010	
Amitraz	ND	0.010	
Aniten/Flurecol Butyl E.	ND	0.010	
Azoxystrobin	ND	0.010	
Benalaxyl	ND	0.010	
Bendiocarb	ND	0.010	
Bendiocarb - C2H3NO	ND	0.010	
beta-Spiroxamine	ND	0.010	
Biphenyl	ND	0.010	
Biteranol-1	ND	0.010	
Biteranol-2	ND	0.010	
Bufenocarb-1	ND	0.010	
Bufenocarb-2	ND	0.010	
Bupirimate	ND	0.010	
Buprofezin	ND	0.010	
Butralin	ND	0.010	
Butylate	ND	0.010	
3-OH Carbofuran	ND	0.010	
Carbaryl	ND	0.010	
Carbaryl - C2H3NO	ND	0.010	
Carbofuran	ND	0.010	
Carbofuran - C2H3NO	ND	0.010	
Carbosulfan	ND	0.010	
Carboxin	ND	0.010	
Cycloate/Ro Neet	ND	0.010	
Cycluron	ND	0.010	
Cymiazole	ND	0.010	
Cyprodinil	ND	0.010	
Desmedipham	ND	0.010	
Desmethyl Diphenamid	ND	0.010	
Desmetryn	ND	0.010	
Diethofenocarb	ND	0.010	
Diniconazole	ND	0.010	
Dioxacarb	ND	0.010	
Dioxacarb - C2H3NO	ND	0.010	
Diphenamid	ND	0.010	
d-Phenothrin #	ND	0.010	
EPTC/Eptam	ND	0.010	
Ethiofencarb	ND	0.010	
Ethiolate	ND	0.010	
Ethiolate	ND	0.010	
Ethofumesate	ND	0.010	
Ethoxyquin	ND	0.010	
Etofenprox	ND	0.010	
Famoxadone	ND	0.010	
Fenazaquin	ND	0.010	
Fenfuram	ND	0.010	
Fenobucarb	ND	0.010	
Fenoxycarb	ND	0.010	
Fenpropathrin	ND	0.010	
Fenpropimorph	ND	0.010	
Flusilazole	ND	0.010	
Fuberdiazole	ND	0.010	
Furalaxyl	ND	0.010	
Hexazinone	ND	0.010	
Isocarbamid	ND	0.010	

ND = Not Detected at or above Environmental Micro Analysis, Inc. Reporting Limit (RL)

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Project No:

PO No:

Client Sample ID: A0912269

EMA Sample No: 09122805-04

Date Received: 12/28/2009

Sample Matrix: Steviol Glycosides Powder

Analytical Method: FDA IMP-NSO-2

Extraction Method: FDA 302

Date Extracted: 12/29/2009

Date Completed: 1/5/2010

Analyte	Amount	RL ppm	Tolerance ppm
Isoprocarb	ND	0.010	
Isopropalin	ND	0.010	
Isoprothiolane	ND	0.010	
Isoproturon (frag)	ND	0.010	
Kresoxim-Methyl	ND	0.010	
Lenacil	ND	0.010	
MBTZ	ND	0.010	
Mepronil	ND	0.010	
Metalaxyl	ND	0.010	
Metaldehyde	ND	0.010	
Methfuroxan	ND	0.010	
Methiocarb	ND	0.010	
Methoprotryne	ND	0.010	
Metolacarb	ND	0.010	
Metribuzin	ND	0.010	
Mexacarbate	ND	0.010	
MGK-264	ND	0.010	
Molinate	ND	0.010	
Naphthalene Acetamide	ND	0.010	
Naproanalid	ND	0.010	
Napropamide	ND	0.010	
Nitralin	ND	0.010	
Nitrothal Isopropyl	ND	0.010	
Norea	ND	0.010	
Octhilinone	ND	0.010	
o-Phenylphenol	ND	0.010	
Pebulate	ND	0.010	
Pendimethalin	ND	0.010	
Phenmedipham	ND	0.010	
Piperonyl Butoxide	ND	0.010	
Pirimicarb	ND	0.010	
Promecarb	ND	0.010	
Prometon	ND	0.010	
Prometryn	ND	0.010	
Propargite	ND	0.010	
Propham	ND	0.010	
Propoxur	ND	0.010	
Propoxur - C2H3NO	ND	0.010	
Pyracarbolid	ND	0.010	
Pyrethrin-1	ND	0.010	
Pyrethrin-2	ND	0.010	
Pyrimethanil	ND	0.010	
Pyriproxifen	ND	0.010	
Resmethrin	ND	0.010	
Secbumeton	ND	0.010	
Sethoxydim	ND	0.010	
Simetryn	ND	0.010	
Tebutam	ND	0.010	
Tebuthiuron	ND	0.010	
Terbumeton	ND	0.010	
Terbutryn	ND	0.010	
Tetraconazole	ND	0.010	
Tetramethrin-1	ND	0.010	
Tetramethrin-2	ND	0.010	
Thiabendazole	ND	0.010	
THPI	ND	0.010	
Tralkoxydim	ND	0.010	
Tricyclazole	ND	0.010	
2,3,5-Trimethacarb	ND	0.010	
3,4,5-Trimethacarb	ND	0.010	
Vernolate	ND	0.010	

ND = Not Detected at or above Environmental Micro Analysis, Inc. Reporting Limit (RL)

Date: Reviewed by: Don Peterson, Laboratory Director

Environmental Micro Analysis, Inc. ELAP Certificate #2211
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Analytical Report

January 11, 2010

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Email: jianning.chen@purecircle.com

Project No:

PO No:

Client Sample ID: A0912269

EMA Sample No: 09122805-04

Date Received: 12/28/2009

Sample Matrix: Steviol Glycosides Powder

Analytical Method: FDA IMP-PHOS-1

Extraction Method: FDA 302

Date Extracted: 12/29/2009

Date Completed: 1/5/2010

Analyte	Amount	RL ppm	Tolerance ppm
Acephate	ND	0.010	
Azinphos-methyl	ND	0.010	
Azinphos-methyl OA	ND	0.010	
beta-chlorfenvinphos	ND	0.010	
Cadusafos	ND	0.010	
Carbofenothion	ND	0.010	
Chlorpyrifos	ND	0.010	
Chlorpyrifos OA	ND	0.010	
Chlorpyrifos-methyl	ND	0.010	
Chlorthiophos	ND	0.010	
Coumaphos	ND	0.010	
Coumaphos OA	ND	0.010	
Cyanofenphos	ND	0.010	
Cyanophos	ND	0.010	
DEF	ND	0.010	
Demeton S Sulfone	ND	0.010	
Diazinon	ND	0.010	
Diazinon OA	ND	0.010	
Dichlorvos	ND	0.010	
Dicrotophos	ND	0.010	
Dimethoate	ND	0.010	
Dioxathion	ND	0.010	
Disulfoton	ND	0.010	
Edifenphos	ND	0.010	
EPN	ND	0.010	
Ethion	ND	0.010	
Ethion OA	ND	0.010	
Ethoprop	ND	0.010	
2-Ethylhexyldiphenyl phos.	ND	0.010	
Fenamiphos	ND	0.010	
Fenamiphos Sulfone	ND	0.010	
Fenitrothion	ND	0.010	
Fenthion	ND	0.010	
Fonophos	ND	0.010	
Heptenophos	ND	0.010	
Isofenphos	ND	0.010	
Leptophos	ND	0.010	
Malathion	ND	0.010	
Malathion OA	ND	0.010	

ND = Not Detected at or above Environmental Micro Analysis, Inc. Reporting Limit (RL)

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Project No:

PO No:

Client Sample ID: A0912269

EMA Sample No: 09122805-04

Date Received: 12/28/2009

Sample Matrix: Steviol Glycosides Powder

Analytical Method: FDA IMP-PHOS-2

Extraction Method: FDA 302

Date Extracted: 12/29/2009

Date Completed: 1/5/2010

Analyte	Amount	RL ppm	Tolerance ppm
Mercarbam	ND	0.010	
Methamidophos	ND	0.010	
Methidathion	ND	0.010	
Methyl Parathion	ND	0.010	
Methyl Parathion OA	ND	0.010	
Mevinphos	ND	0.010	
Monocrotophos	ND	0.010	
Omethaote	ND	0.010	
Oxydemeton Methyl Sulfone	ND	0.010	
Parathion	ND	0.010	
Parathion OA	ND	0.010	
Phorate	ND	0.010	
Phorate OA	ND	0.010	
Phorate Sulfone	ND	0.010	
Phorate Sulfoxide	ND	0.010	
Phosalone	ND	0.010	
Phosmet	ND	0.010	
Phosmet Oxon	ND	0.010	
Pirimiphos-methy	ND	0.010	
Profenophos	ND	0.010	
Prothiophos	ND	0.010	
Prothoate	ND	0.010	
Pyrazophos	ND	0.010	
Pyridaphenthion	ND	0.010	
Quinalphos	ND	0.010	
Sulfotep	ND	0.010	
Sulprofos	ND	0.010	
Terbufos	ND	0.010	
Tetrachlorvinphos	ND	0.010	
Tolclophos Methyl	ND	0.010	
Trizaphos	ND	0.010	
Trichlorfon	ND	0.010	
Tris (Chloropropyl) Phosphate-1	ND	0.010	
Tris (Chloropropyl) Phosphate-2	ND	0.010	
Tris 2-Butoxyethyl Phosphate	ND	0.010	

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Project No:

PO No:

Client Sample ID: A0912269

EMA Sample No: 09122805-04

Date Received: 12/28/2009

Sample Matrix: Steviol Glycosides Powder

Analytical Method: EBDC Screen

Extraction Method: CDFA

Date Extracted: 1/4/2010

Date Completed: 1/5/2010

Analyte	Amount	RL ppm	Tolerance ppm
Ferbam	ND	0.030	
Maneb (Dithane M-22)	ND	0.030	
Mancozeb	ND	0.030	
Metiram	ND	0.030	
Nabam	ND	0.030	
Thiram	ND	0.030	
Zineb	ND	0.030	
Ziram	ND	0.030	

ND = Not Detected at or above Environmental Micro Analysis, Inc. Reporting Limit (RL)

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Project No:

PO No:

Client Sample ID: A0912270

EMA Sample No: 09122805-05

Date Received: 12/28/2009

Sample Matrix: Steviol Glycosides Powder

Analytical Method: FDA IMP-OC1

Extraction Method: FDA 302

Date Extracted: 12/29/2009

Date Completed: 1/2/2010

Analyte	Amount	RL ppm	Tolerance ppm
Acrinathrin	ND	0.010	
Aldrin	ND	0.010	
alpha-BHC	ND	0.010	
Atrazine	ND	0.010	
Benfluralin	ND	0.010	
beta Chlordane	ND	0.010	
Bifenthrin	ND	0.010	
Boscalid	ND	0.010	
Bromopropylate	ND	0.010	
Captan	ND	0.010	
Chlorodimeform	ND	0.010	
Chlorobenzilate	ND	0.010	
Chloroneb	ND	0.010	
Chlorothalonil	ND	0.010	
CIPC	ND	0.010	
cis-Chlordane	ND	0.010	
cis-Nonachlor	ND	0.010	
cis-Permethrin	ND	0.010	
Clomazone	ND	0.010	
Cyanazine	ND	0.010	
Cypermethrin	ND	0.010	
Cyproconazole	ND	0.010	
Dacthal	ND	0.010	
Decamethrin	ND	0.010	
delta-BHC	ND	0.010	
Diallate-1	ND	0.010	
Diallate-2	ND	0.010	
3,4-Dichloroanaline	ND	0.010	
Dichlobenil	ND	0.010	
Dichlofluanid	ND	0.010	
Dichloran	ND	0.010	
Dieldrin	ND	0.010	
Difenoconazole	ND	0.010	
Dimethachlor	ND	0.010	
Dimethmorph	ND	0.010	
Dinitramine	ND	0.010	
Dursban	ND	0.010	
Endosulfan Sulfate	ND	0.010	
Endosulfan-I	ND	0.010	
Endosulfan-II	ND	0.010	
Endrin	ND	0.010	
Epoxiconazole	ND	0.010	
Etaconazole-1	ND	0.010	
Etaconazole-2	ND	0.010	
Ethafluralin	ND	0.010	
Etridiazole	ND	0.010	
Fentarimol	ND	0.010	
Fenbuconazole	ND	0.010	
Fenhexamid	ND	0.010	
Fenvalerate-1	ND	0.010	
Fenvalerate-2	ND	0.010	
Fipronil	ND	0.010	
Fluchloralin	ND	0.010	
Flucythrinate	ND	0.010	
Fludioxanil	ND	0.010	
Fluquinconazole	ND	0.010	
Flutolanil	ND	0.010	
Fluvalinate	ND	0.010	
Folpet	ND	0.010	
gamma-BHC/Lindane	ND	0.010	
gamma-Chlordane	ND	0.010	
Heptachlor	ND	0.010	
Heptachlor Epoxide	ND	0.010	
Hexachlorobenzene	ND	0.010	

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January 11, 2010

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Project No:

PO No:

Client Sample ID: A0912270

EMA Sample No: 09122805-05

Date Received: 12/28/2009

Sample Matrix: Steviol Glycosides Powder

Analytical Method: FDA IMP-OC2

Extraction Method: FDA 302

Date Extracted: 12/29/2009

Date Completed: 1/2/2010

Analyte	Amount	RL ppm	Tolerance ppm
Hexaconazole	ND	0.010	
Imazalil	ND	0.010	
Iprodione	ND	0.010	
lambda-Cyhalothrin	ND	0.010	
Linuron	ND	0.010	
Metolachlor	ND	0.010	
Mirex	ND	0.010	
Monolinuron	ND	0.010	
Myclobutanil	ND	0.010	
Nitrofen	ND	0.010	
Nuarimol	ND	0.010	
o,p'-DDT	ND	0.010	
0,p'-Methoxychlor	ND	0.010	
Octachlor Epoxide	ND	0.010	
p,p'-DDE	ND	0.010	
p,p'-DDT	ND	0.010	
p,p'-Methoxychlor	ND	0.010	
Penconazole	ND	0.010	
Pentachloroanaline	ND	0.010	
Pentachlorobenzene	ND	0.010	
Pentachlorobenzonitrile	ND	0.010	
Pentachlorothioanisole	ND	0.010	
Prochloraz	ND	0.010	
Procymidone	ND	0.010	
Profluralin	ND	0.010	
Pronamide	ND	0.010	
Propachlor	ND	0.010	
Propanil	ND	0.010	
Propiconizole/Tilt-1	ND	0.010	
Propiconizole/Tilt-2	ND	0.010	
Pyraclostrobin	ND	0.010	
Pyridaben	ND	0.010	
Pyrifenox-1	ND	0.010	
Pyrifenox-2	ND	0.010	
Quinoxyfen	ND	0.010	
Quintozene	ND	0.010	
Simazine	ND	0.010	
Tebuconazole	ND	0.010	
Tebufenpyrad	ND	0.010	
Tecnazene	ND	0.010	
Tefluthrin	ND	0.010	
Terbuthylazine	ND	0.010	
2,3,5,6-Tetrachloroanaline	ND	0.010	
Tetradifon	ND	0.010	
Tolylfluanid	ND	0.010	
trans-Chlordane	ND	0.010	
trans-Nonachlor	ND	0.010	
trans-Permethrin	ND	0.010	
Triadimefon	ND	0.010	
Triadimenol	ND	0.010	
Triallate	ND	0.010	
Trifloxystrobin	ND	0.010	
Triflumizole	ND	0.010	
Trifluralin	ND	0.010	
Triticonazole	ND	0.010	
Vinclozolin	ND	0.010	

ND = Not Detected at or above Environmental Micro Analysis, Inc. Reporting Limit (RL)

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Analytical Report

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Project No:

PO No:

Client Sample ID: A0912270

EMA Sample No: 09122805-05

Date Received: 12/28/2009

Sample Matrix: Steviol Glycosides Powder

Analytical Method: FDA IMP-NSO-1

Extraction Method: FDA 302

Date Extracted: 12/29/2009

Date Completed: 1/5/2010

Analyte	Amount	RL ppm	Tolerance ppm
Allethrin	ND	0.010	
Acetamiprid	ND	0.010	
alpha-Spiroxamine	ND	0.010	
Ametryn	ND	0.010	
Aminocarb	ND	0.010	
Amitraz	ND	0.010	
Aniten/Flurecol Butyl E.	ND	0.010	
Azoxystrobin	ND	0.010	
Benalaxyl	ND	0.010	
Bendiocarb	ND	0.010	
Bendiocarb - C2H3NO	ND	0.010	
beta-Spiroxamine	ND	0.010	
Biphenyl	ND	0.010	
Biteranol-1	ND	0.010	
Biteranol-2	ND	0.010	
Bufenocarb-1	ND	0.010	
Bufenocarb-2	ND	0.010	
Bupirimate	ND	0.010	
Buprofezin	ND	0.010	
Butralin	ND	0.010	
Butylate	ND	0.010	
3-OH Carbofuran	ND	0.010	
Carbaryl	ND	0.010	
Carbaryl - C2H3NO	ND	0.010	
Carbofuran	ND	0.010	
Carbofuran - C2H3NO	ND	0.010	
Carbosulfan	ND	0.010	
Carboxin	ND	0.010	
Cycloate/Ro Neet	ND	0.010	
Cycluron	ND	0.010	
Cymiazole	ND	0.010	
Cyprodinil	ND	0.010	
Desmedipham	ND	0.010	
Desmethyl Diphenamid	ND	0.010	
Desmetryn	ND	0.010	
Diethofencarb	ND	0.010	
Diniconazole	ND	0.010	
Dioxacarb	ND	0.010	
Dioxacarb - C2H3NO	ND	0.010	
Diphenamid	ND	0.010	
d-Phenothrin #	ND	0.010	
EPTC/Eptam	ND	0.010	
Ethiofencarb	ND	0.010	
Ethiolate	ND	0.010	
Ethiolate	ND	0.010	
Ethofumesate	ND	0.010	
Ethoxyquin	ND	0.010	
Etofenprox	ND	0.010	
Famoxadone	ND	0.010	
Fenazaquin	ND	0.010	
Fenfuram	ND	0.010	
Fenobucarb	ND	0.010	
Fenoxycarb	ND	0.010	
Fenpropathrin	ND	0.010	
Fenpropimorph	ND	0.010	
Flusilazole	ND	0.010	
Fuberdiazole	ND	0.010	
Furalaxyl	ND	0.010	
Hexazinone	ND	0.010	
Isocarbamid	ND	0.010	

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Project No:

PO No:

Client Sample ID: A0912270

EMA Sample No: 09122805-05

Date Received: 12/28/2009

Sample Matrix: Steviol Glycosides Powder

Analytical Method: FDA IMP-NSO-2

Extraction Method: FDA 302

Date Extracted: 12/29/2009

Date Completed: 1/5/2010

Analyte	Amount	RL ppm	Tolerance ppm
Isoprocarb	ND	0.010	
Isopropalin	ND	0.010	
Isoprothiolane	ND	0.010	
Isoproturon (frag)	ND	0.010	
Kresoxim-Methyl	ND	0.010	
Lenacil	ND	0.010	
MBTZ	ND	0.010	
Mepronil	ND	0.010	
Metalaxyl	ND	0.010	
Metaldehyde	ND	0.010	
Methfuroxan	ND	0.010	
Methiocarb	ND	0.010	
Methoprotryne	ND	0.010	
Metolacarb	ND	0.010	
Metribuzin	ND	0.010	
Mexacarbate	ND	0.010	
MGK-264	ND	0.010	
Molinate	ND	0.010	
Naphthalene Acetamide	ND	0.010	
Naproanalid	ND	0.010	
Napropamide	ND	0.010	
Nitralin	ND	0.010	
Nitrothal Isopropyl	ND	0.010	
Norea	ND	0.010	
Octhilinone	ND	0.010	
o-Phenylphenol	ND	0.010	
Pebulate	ND	0.010	
Pendimethalin	ND	0.010	
Phenmedipham	ND	0.010	
Piperonyl Butoxide	ND	0.010	
Pirimicarb	ND	0.010	
Promecarb	ND	0.010	
Prometon	ND	0.010	
Prometryn	ND	0.010	
Propargite	ND	0.010	
Propham	ND	0.010	
Propoxur	ND	0.010	
Propoxur - C2H3NO	ND	0.010	
Pyracarbolid	ND	0.010	
Pyrethrin-1	ND	0.010	
Pyrethrin-2	ND	0.010	
Pyrimethanil	ND	0.010	
Pyriproxifen	ND	0.010	
Resmethrin	ND	0.010	
Secbumeton	ND	0.010	
Sethoxydim	ND	0.010	
Simetryn	ND	0.010	
Tebutam	ND	0.010	
Tebuthiuron	ND	0.010	
Terbumeton	ND	0.010	
Terbutryn	ND	0.010	
Tetraconazole	ND	0.010	
Tetramethrin-1	ND	0.010	
Tetramethrin-2	ND	0.010	
Thiabendazole	ND	0.010	
THPI	ND	0.010	
Tralkoxydim	ND	0.010	
Tricyclazole	ND	0.010	
2,3,5-Trimethacarb	ND	0.010	
3,4,5-Trimethacarb	ND	0.010	
Vernolate	ND	0.010	

ND = Not Detected at or above Environmental Micro Analysis, Inc. Reporting Limit (RL)

Date Reviewed by: Don Peterson, Laboratory Director

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Project No:

PO No:

Client Sample ID: A0912270

EMA Sample No: 09122805-05

Date Received: 12/28/2009

Sample Matrix: Steviol Glycosides Powder

Analytical Method: FDA IMP-PHOS-1

Extraction Method: FDA 302

Date Extracted: 12/29/2009

Date Completed: 1/5/2010

Analyte	Amount	RL ppm	Tolerance ppm
Acephate	ND	0.010	
Azinphos-methyl	ND	0.010	
Azinphos-methyl OA	ND	0.010	
beta-chlorfenvinphos	ND	0.010	
Cadusafos	ND	0.010	
Carbofenothion	ND	0.010	
Chlorpyrifos	ND	0.010	
Chlorpyrifos OA	ND	0.010	
Chlorpyrifos-methyl	ND	0.010	
Chlorthiophos	ND	0.010	
Coumaphos	ND	0.010	
Coumaphos OA	ND	0.010	
Cyanofenphos	ND	0.010	
Cyanophos	ND	0.010	
DEF	ND	0.010	
Demeton S Sulfone	ND	0.010	
Diazinon	ND	0.010	
Diazinon OA	ND	0.010	
Dichlorvos	ND	0.010	
Dicrotophos	ND	0.010	
Dimethoate	ND	0.010	
Dioxathion	ND	0.010	
Disulfoton	ND	0.010	
Edifenphos	ND	0.010	
EPN	ND	0.010	
Ethion	ND	0.010	
Ethion OA	ND	0.010	
Ethoprop	ND	0.010	
2-Ethylhexyldiphenyl phos.	ND	0.010	
Fenamiphos	ND	0.010	
Fenamiphos Sulfone	ND	0.010	
Fenitrothion	ND	0.010	
Fenthion	ND	0.010	
Fonophos	ND	0.010	
Heptenophos	ND	0.010	
Isofenphos	ND	0.010	
Leptophos	ND	0.010	
Malathion	ND	0.010	
Malathion OA	ND	0.010	

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Email: jianning.chen@purecircle.com

Project No:

PO No:

Client Sample ID: A0912270

EMA Sample No: 09122805-05

Date Received: 12/28/2009

Sample Matrix: Steviol Glycosides Powder

Analytical Method: FDA IMP-PHOS-2

Extraction Method: FDA 302

Date Extracted: 12/29/2009

Date Completed: 1/5/2010

Analyte	Amount	RL ppm	Tolerance ppm
Mercarbam	ND	0.010	
Methamidophos	ND	0.010	
Methidathion	ND	0.010	
Methyl Parathion	ND	0.010	
Methyl Parathion OA	ND	0.010	
Mevinphos	ND	0.010	
Monocrotophos	ND	0.010	
Omethaote	ND	0.010	
Oxydemeton Methyl Sulfone	ND	0.010	
Parathion	ND	0 010	
Parathion OA	ND	0.010	
Phorate	ND	0 010	
Phorate OA	ND	0 010	
Phorate Sulfone	ND	0 010	
Phorate Sulfoxide	ND	0.010	
Phosalone	ND	0.010	
Phosmet	ND	0.010	
Phosmet Oxon	ND	0.010	
Pirimiphos-methy	ND	0.010	
Profenophos	ND	0.010	
Prothiophos	ND	0 010	
Prothoate	ND	0 010	
Pyrazophos	ND	0 010	
Pyridaphenthion	ND	0 010	
Quinalphos	ND	0 010	
Sulfotep	ND	0 010	
Sulprofos	ND	0 010	
Terbufos	ND	0 010	
Tetrachlorvinphos	ND	0 010	
Tolclophos Methyl	ND	0 010	
Trizaphos	ND	0.010	
Trichlorfon	ND	0.010	
Tris (Chloropropyl) Phosphate-1	ND	0.010	
Tris (Chloropropyl) Phosphate-2	ND	0.010	
Tris 2-Butoxyethyl Phosphate	ND	0 010	

ND = Not Detected at or above Environmental Micro Analysis, Inc. Reporting Limit (RL)

Date. Reviewed by: Don Peterson, Laboratory Director

Environmental Micro Analysis, Inc. ELAP Certificate #2211
460 N East Street
Woodland, CA 95776

Phone: (530) 666-6890 Fax: (530) 666-2987
e-mail: emalab@emalab.com website: www.emalab.com

Analytical Report

January 11, 2010

Client: Jianning Chen
PureCircle So., Ltd China
Yangtang Industry Zone, Ganxian
Jiangxi Province, .

Phone: (+86) 797-4601666
Fax: (+86) 797-4601663
Email: jianning.chen@purecircle.com

Project No:

PO No:

Client Sample ID: A0912270

EMA Sample No: 09122805-05

Date Received: 12/28/2009

Sample Matrix: Steviol Glycosides Powder

Analytical Method: EBDC Screen

Extraction Method: CDFA

Date Extracted: 1/4/2010

Date Completed: 1/5/2010

Analyte	Amount	RL ppm	Tolerance ppm
Ferbam	ND	0.030	
Maneb (Dithane M-22)	ND	0.030	
Mancozeb	ND	0.030	
Metiram	ND	0.030	
Nabam	ND	0.030	
Thiram	ND	0.030	
Zineb	ND	0.030	
Ziram	ND	0.030	

ND = Not Detected at or above Environmental Micro Analysis, Inc. Reporting Limit (RL)

Date: Reviewed by: Don Peterson, Laboratory Director

APPENDIX D

PureCircle Stability Data for Steviol Glycosides

D-1 pH Stability of Steviol Glycosides

D-2 Storage Stability of Steviol Glycosides

Appendix D-1

See following 7 pages

Not included in sequence with main document page numbers.

pH Stability of Steviol Glycosides

Prepared by: _____ _____

Lai Chee Seng **Date (Senior QA&**
Food Technologist)

Approved by: _____ _____

Avetik A. Markosyan, MD, PhD **Date**
(Director R&D / QC)

Objective

To determine pH stability of *Steviol Glycosides* produced by *PureCircle*.

Samples

One sample representing commercial lot of *Steviol Glycosides* labeled as "A0912266".

Standards

1. USP Rebaudioside A Standard; Lot#F0I077 (USP Rockville, MD USA);
2. USP Stevioside Standard; Lot#F0I080 (USP Rockville, MD USA);
3. Rebaudioside B Standard; Lot#18227-101 (Chromadex Inc. Irvine, CA USA);
4. Rebaudioside C Standard; Lot#00018228-3962 (Chromadex Inc. Irvine, CA USA);
5. Rebaudioside D Standard; Lot#00018229-4104 (Chromadex Inc. Irvine, CA USA);
6. Steviolbioside Standard; Lot#00019349-1821 (Chromadex Inc. Irvine, CA USA);
7. Dulcoside A Standard; Lot#04949-002 (Chromadex Inc. Irvine, CA USA);
8. Rubusoside Standard; Lot#270308 (PureCircle Sdn. Bhd., Malaysia).

Solvents and Reagents

- Acetonitrile, HPLC grade (JT Baker, USA);
- Water, HPLC grade (JT Baker, USA);
- Ammonium acetate, reagent grade (Merck, Germany);
- Acetic acid, reagent grade (Merck, Germany).

Apparatus

1. Agilent 1200 HPLC system equipped with binary pump (G1312B), auto sampler (G1367D), thermostatted column compartment (G1316B) and DAD detector (G1315C), (Agilent Technologies, USA);
2. Analytical column, Zorbax NH_2, 4.6x250mm, 5μm (PN 880952-708), (Agilent Technologies, USA);
3. Analytical balance, XS205, (Mettler Toledo, USA);

4. Karl Fischer coulometer, Mettler Toledo DL-39, (Mettler Toledo, USA);
5. pH-meter, ionCheck 10, (Radiometer Analytical, France);
6. Volumetric (class *A*) and Laboratory glassware.

Solution Preparation

Sample solutions were prepared at approx. 5,000 ppm concentration in 0.1M phosphate buffer.

Sample Solution Storage

Sample solutions were stored at 20°C in amber glass bottles.

Determination of rebaudioside A and related steviol glycosides

Analysis and calculations were performed as per *FCC 6* monograph method for Rebaudioside A and Related Steviol Glycosides (see appendix A).

The assay results are summarized in Table 1.

Table 1

Rebaudioside A and related steviol glycosides assay

Total Steviol Glycosides, %*								
	pH 2.0	**pH 3.0**	**pH 4.0**	**pH 5.0**	**pH 6.0**	**pH 7.0**	**pH 8.0**	**Control****
t=0	95.87	96.53	95.96	96.43	96.32	96.29	96 62	96 46
2 weeks	95.50	96.05	95.63	95.94	96.07	96.01	96 09	96.04
Rebaudioside A, %								
t=0	64.12	64.77	64.36	64.64	64.61	64.54	64.80	64 57
2 weeks	64.12	64.70	64.40	64.55	64 65	64 60	64.60	64.65
Stevioside, %								
t=0	17.98	18.09	17.98	18.08	18.10	18.07	18.11	18.09
2 weeks	17.73	17.93	17.81	17.89	17.92	17.89	17.94	17.88
Rebaudioside C, %								
t=0	10.08	10.22	10.17	10 28	10.27	10.25	10.30	10.17
2 weeks	10.05	10.12	10.09	10.11	10.15	10.14	10.24	10.00
Rebaudioside F, %								
t=0	1.76	1.65	1.66	1.64	1.60	1.62	1.62	1.74
2 weeks	1.66	1.57	1.58	1.60	1.56	1.56	1.53	1 67
Rebaudioside B, %								

t=0	0.98	0.90	0.86	0.91	0.86	0.94	0.91	0.94
2 weeks	0.99	0.84	0.86	0.90	0.89	0.94	0.89	0.91
Dulcoside A, %								
t=0	0.45	0.42	0.44	0.43	0.43	0.40	0.42	0.44
2 weeks	0.42	0.42	0.42	0.41	0 41	0.41	0.42	0.42
Steviolbioside, %								
t=0	0.22	0 20	0.21	0.20	0.20	0.21	0.21	0 23
2 weeks	0.25	0.18	0.21	0.22	0.22	0.22	0 21	0.22
Rubusoside, %								
t=0	0.26	0.25	0.25	0.24	0.24	0.23	0 22	0.26
2 weeks	0.26	0.26	0 25	0.25	0.25	0.25	0 24	0.27

* Average of 2 duplicates is reported;
** Control was prepared using plain water.

ASSAY

PROCEDURE

Acetate buffer: Dissolve 0.125 g ammonium acetate in 900 mL of water, adjust pH to 4.3 with glacial acetic acid solution and dilute to 1 L. [NOTE: It may be necessary to adjust the ratio of ammonium acetate to acetic acid. Changing the pH adjusts the retention time of rebaudioside A and related glycosides. Decreasing the pH of the buffer will decrease the retention time of rebaudioside A.]

Mobile phase: 20% (v/v) *Acetate buffer* in acetonitrile [NOTE: Equilibrate the column by flushing with 75 mL of isopropyl alcohol and then 50 mL of acetonitrile. After that, flush the column with *Mobile phase* until the retention time of rebaudioside A is less than 8 min (may take up to one day of flushing).]

Diluent: 25% (v/v) *Acetate buffer* in acetonitrile [NOTE: Allow *Diluent* to come to room temperature before use.]

Rebaudioside A standard solutions: 4500, 4750, 5250, and 5500 mg/L USP Rebaudioside A RS in *Diluent*

Stevioside standard stock solution: 250 mg/L USP Stevioside RS in *Diluent*

Stevioside standard solutions: 2.5, 5.0, 10, 25, and 50 mg/L USP Stevioside RS in *Diluent:* from *Stevioside standard stock solution*

Sample solution: 5000 mg/L in *Diluent*

Chromatographic system, Appendix IIA

Mode: High-performance liquid chromatography

Detector: UV(210nm)

Column: 15-cm x 4.6-mm, packed with a propylamino silane phase bonded to silica gel *(5*-µm particle diameter)[1]

[1] *Zorbax* NH_2 (Agilent Technologies), or equivalent.

APPENDIX B

Column temperature: 30°
Flow rate: 1.5 mL/min
Injection size: 12 µL
System suitability
Samples: 5250 mg/L *Rebaudioside A standard solution,* 2.5 mg/L *Stevioside standard solution, Sample solution*
Suitability requirements
Detector response: Peak to noise ratio (peak height /baseline noise) is NLT 3 for the stevioside peak from the 2.5 mg/L *Stevioside standard solution,* where peak height is expressed in mAU, and baseline noise is the maximum deflection of the baseline (mAU) in a blank at the retention time of stevioside over the same baseline peak width in min.
Relative standard deviation: NMT 2.0% for rebaudioside A peak area and retention time from the 5250 mg/L *Rebaudioside A standard solution*
Column efficiency: NLT 5000 theoretical plates count, *N,* using the rebaudioside B peak from the *Sample solution*
Retention time: The retention time for the rebaudioside A peak from the 5250 mg/L *Rebaudioside A standard solution* is less than 8.0 min.
Tailing factor: NMT 2.0 for the rebaudioside B peak from the *Sample solution*
Analysis: Separately inject equal volumes of the *Rebaudioside A standard solutions, Stevioside standard solutions,* and *Sample solution* into the chromatograph, and measure the responses for the major peaks on the resulting chromatograms. [NOTE: The approximate retention times for rebaudioside A and its related steviol glycosides are listed below in *Chromatographic Profile Table 1.* If the retention time for rebaudioside A is below 6 min, adjust the ratio of ammonium acetate to acetic acid.]

Chromatographic Profile Table 1

Compound	Approx. Retention Time (min)	Molecular Weight (g/mol)
Rubusoside	3.0	642.73
Dulcoside A	3.9	788.87
Stevioside	4.5	804.88
Rebaudioside C	5.2	951.01
Rebaudioside F	5.6	936.99
Rebaudioside A	7.1	967.01

APPENDIX B

Rebaudioside D	16	1129.15
Steviolbioside	18	642.73
Rebaudioside B	36	804.88

Prepare a standard curve for rebaudioside A by plotting rebaudioside A peak areas versus concentrations in mg/L, corrected for purity, based on the USP Reference Standard label claim. [NOTE: Peak responses for all other steviol glycosides besides rebaudioside A are used below in the *Related Steviol Glycosides* impurities test procedure.] From the standard curve, calculate the concentration *(C_U)* of rebaudioside A in the *Sample solution* in mg/L. Calculate the percentage of rebaudioside A in the portion of the sample taken by the formula:

$$C_U/C_{SMP} X\ 100\%$$

C_U = Concentration of rebaudioside A in the *Sample solution* determined from the standard curve (mg/L)

C_{SMP} = Concentration of the sample in the *Sample solution* (mg/L)

RELATED STEVIOL GLYCOSIDES

Acetate buffer, Mobile phase, Diluent, Rebaudioside A standard solutions, Stevioside standard stock solution, Stevioside standard solutions, Sample solution, Chromatographic system, and **System suitability:** Prepare as directed in the *Assay* (above).

Analysis: Proceed as directed above in the *Assay,* but with the following modifications for the standard curve and calculations.

Using the peak area responses from the *Stevioside standard solutions,* prepare a standard curve for stevioside by plotting stevioside peak areas versus concentrations, in mg/L, corrected for purity, based on the USP Reference Standard label claim. From this standard curve, determine the concentration (mg/L) of stevioside in the *Sample solution.* Calculate the percent of stevioside in the sample taken using the following formula:

$$C_U/C_{SMP} X\ 100\%$$

C_U = Concentration of stevioside in the *Sample solution* determined from the standard curve (mg/L)

C_{SMP} = Concentration of the sample in the *Sample solution* (mg/L)

For the seven other steviol glycoside impurities (rubusoside, dulcoside A, rebaudioside C rebaudioside F rebaudioside D, steviolbioside, and rebaudioside B) use the stevioside standard curve prepared above to calculate the mg/L stevioside equivalents for each. Separately calculate the percents of each analyte (rubusoside, dulcoside A, rebaudioside C, rebaudioside F, rebaudioside D, steviolbioside, and rebaudioside B) in the sample taken using the following formula which takes into account the differences in molecular weights between the analytes and stevioside:

APPENDIX B

$$(C_U \times M_{r1} / M_{r2}) / C_{SMP} \times 100\%$$

C_U = concentration of stevioside equivalents in the *Sample solution* determined from the standard curve *(mg/L)*

M_{r1} = Molecular weight of the analyte (see *Chromatographic Profile Table* 1, above under *Assay*)

M_{r2} = Molecular weight of stevioside 804.88

C_{SMP} = Concentration of the sample in the *Sample solution* (mg/L)

Sample Chromatograms

Appendix D-2

See following 8 pages

Not included in sequence with main document page numbers.

Storage Stability of Steviol Glycosides

Prepared by: _____ _____

Lai Chee Seng **Date**
(Senior QA & Food Technologist)

Approved by: _____ _____

Avetik Markosyan MD, PhD **Date**
(Director R&D / QC)

Objective

To determine storage stability of *Steviol Glycosides* produced by *PureCircle*.

Samples

One sample representing commercial lot of *Steviol Glycosides* labeled as "A0912266".

Standards

1. USP Rebaudioside A Standard; Lot#F0I077 (USP Rockville, MD USA);
2. USP Stevioside Standard; Lot#F0I080 (USP Rockville, MD USA);
3. Rebaudioside B Standard; Lot#18227-101 (Chromadex Inc. Irvine, CA USA);
4. Rebaudioside C Standard; Lot#00018228-3962 (Chromadex Inc. Irvine, CA USA);
5. Rebaudioside D Standard; Lot#00018229-4104 (Chromadex Inc. Irvine, CA USA);
6. Steviolbioside Standard; Lot#00019349-1821 (Chromadex Inc. Irvine, CA USA);
7. Dulcoside A Standard; Lot#04949-002 (Chromadex Inc. Irvine, CA USA);
8. Rubusoside Standard; Lot#270308 (PureCircle Sdn. Bhd., Malaysia).

Solvents and Reagents

- Acetonitrile, HPLC grade (JT Baker, USA);
- Water, HPLC grade (JT Baker, USA);

- Ammonium acetate, reagent grade (Merck, Germany);
- Acetic acid, reagent grade (Merck, Germany).

Apparatus

1. Agilent 1200 HPLC system equipped with binary pump (G1312B), auto sampler (G1367D), thermostatted column compartment (G1316B) and DAD detector (G1315C), (Agilent Technologies, USA);
2. Analytical column, Zorbax NH_2, 4.6x250mm, 5µm (PN 880952-708), (Agilent Technologies, USA);
3. Analytical balance, XS205, (Mettler Toledo, USA);
4. Karl Fischer coulometer, Mettler Toledo DL-39, (Mettler Toledo, USA);
5. Constant climate chamber, KBF 115, (Binder, Germany);
6. Volumetric (class *A*) and Laboratory glassware.

Sample Storage

Sample was stored in original packaging at 40°C and 75% relative humidity.

Solution Preparation

Sample solutions were prepared at approx. 5,000 ppm concentration in *Diluent* (see appendix A).

Determination of rebaudioside A and related steviol glycosides

Analysis and calculations were performed as per *FCC 6* monograph method for Rebaudioside A and Related Steviol Glycosides (see appendix A).

The assay results are summarized in Table 1.

Table 1

Rebaudioside A and related steviol glycosides assay

Duration	Lot# A0912266, Steviol Glycosides, % dry basis*									
	Rub	*DulA*	*Stev*	*RebC*	*RebF*	*RebA*	*RebD*	*Sbio*	*RebB*	***TSG***
t=0	0.21	0.39	18.31	9.85	1.93	64.56	ND	0.23	1.01	96.50
3 weeks	0.26	0.44	18.14	10.05	1.81	64.96	ND	0.23	0.93	96.82

* Average of 2 duplicates is reported.
Rub – Rubusoside
DulA – Dulcoside A
RebA, B, C, D, F – Rebaudioside A, B, C, D, F
Sbio – Steviolbioside
TSG – Total Steviol Glycosides
ND – Not Detected

References

1 Stability testing of new drug substances and products, *ICH Q1A(R) stability testing guidelines*, The European Agency for the Evaluation of Medicinal Products, 2000.

2. Rebaudioside A monograph, *Food Chemicals Codex (FCC) 6, Second Supplement*, 2009, p. 1438-42.

APPENDIX A

***Rebaudioside A and related steviol glycosides assay*, FCC 6, Second Supplement, 2009, p.1438-42.**

ASSAY

PROCEDURE

Acetate buffer: Dissolve 0.125 g ammonium acetate in 900 mL of water, adjust pH to 4.3 with glacial acetic acid solution and dilute to 1 L. [NOTE: It may be necessary to adjust the ratio of ammonium acetate to acetic acid. Changing the pH adjusts the retention time of rebaudioside A and related glycosides. Decreasing the pH of the buffer will decrease the retention time of rebaudioside A.]

Mobile phase: 20% (v/v) *Acetate buffer* in acetonitrile [NOTE: Equilibrate the column by flushing with 75 mL of isopropyl alcohol and then 50 mL of acetonitrile. After that, flush the column with *Mobile phase* until the retention time of rebaudioside A is less than 8 min (may take up to one day of flushing).]

Diluent: 25% (v/v) *Acetate buffer* in acetonitrile [NOTE: Allow *Diluent* to come to room temperature before use.]

Rebaudioside A standard solutions: 4500, 4750, 5250, and 5500 mg/L USP Rebaudioside A RS in *Diluent*

Stevioside standard stock solution: 250 mg/L USP Stevioside RS in *Diluent*

Stevioside standard solutions: 2.5, 5.0, 10, 25, and 50 mg/L USP Stevioside RS in *Diluent:* from *Stevioside standard stock solution*

Sample solution: 5000 mg/L in *Diluent*

Chromatographic system, Appendix IIA

Mode: High-performance liquid chromatography

Detector: UV(210nm)

Column: 15-cm x 4.6-mm, packed with a propylamino silane phase bonded to silica gel *(5*-µm particle diameter)[1]

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection size: 12 µL

System suitability

Samples: 5250 mg/L *Rebaudioside A standard solution,* 2.5 mg/L *Stevioside standard solution, Sample solution*

Suitability requirements

Detector response: Peak to noise ratio (peak height /baseline noise) is NLT 3 for the stevioside peak from the 2.5 mg/L *Stevioside standard solution,* where peak height is expressed in mAU, and baseline noise is the maximum deflection of the baseline (mAU) in a blank at the retention time of stevioside over the same baseline peak width in min.

Relative standard deviation: NMT 2.0% for rebaudioside A peak area and retention time from the 5250 mg/L *Rebaudioside A standard solution*

Column efficiency: NLT 5000 theoretical plates count, *N,* using the rebaudioside B peak from the *Sample solution*

Retention time: The retention time for the rebaudioside A peak from the 5250 mg/L *Rebaudioside A standard solution* is less than 8.0 min.

Tailing factor: NMT 2.0 for the rebaudioside B peak from the *Sample solution*

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[1] *Zorbax* NH_2 (Agilent Technologies), or equivalent.

APPENDIX A

***Rebaudioside A and related steviol glycosides assay*, FCC 6, Second Supplement, 2009, p.1438-42.**

Analysis: Separately inject equal volumes of the *Rebaudioside A standard solutions, Stevioside standard solutions,* and *Sample solution* into the chromatograph, and measure the responses for the major peaks on the resulting chromatograms. [NOTE: The approximate retention times for rebaudioside A and its related steviol glycosides are listed below in *Chromatographic Profile Table 1.* If the retention time for rebaudioside A is below 6 min, adjust the ratio of ammonium acetate to acetic acid.]

Chromatographic Profile Table 1

Compound	Approx. Retention Time (min)	Molecular Weight (g/mol)
Rubusoside	3.0	642.73
Dulcoside A	3.9	788.87
Stevioside	4.5	804.88
Rebaudioside C	5.2	951.01
Rebaudioside F	5.6	936.99
Rebaudioside A	7.1	967.01
Rebaudioside D	16	1129.15
Steviolbioside	18	642.73
Rebaudioside B	36	804.88

Prepare a standard curve for rebaudioside A by plotting rebaudioside A peak areas versus concentrations in mg/L, corrected for purity, based on the USP Reference Standard label claim. [NOTE: Peak responses for all other steviol glycosides besides rebaudioside A are used below in the *Related Steviol Glycosides* impurities test procedure.] From the standard curve, calculate the concentration *(C_U)* of rebaudioside A in the *Sample solution* in mg/L. Calculate the percentage of rebaudioside A in the portion of the sample taken by the formula:

$$C_U/C_{SMP} X\ 100\%$$

C_U = Concentration of rebaudioside A in the *Sample solution* determined from the standard curve (mg/L)

C_{SMP} = Concentration of the sample in the *Sample solution* (mg/L)

RELATED STEVIOL GLYCOSIDES

Acetate buffer, Mobile phase, Diluent, Rebaudioside A standard solutions, Stevioside standard stock solution, Stevioside standard solutions, Sample solu-

tion, Chromatographic system, and **System suitability:** Prepare as directed in the *Assay* (above).

Analysis: Proceed as directed above in the *Assay,* but with the following modifications for the standard curve and calculations.

Using the peak area responses from the *Stevioside standard solutions,* prepare a standard curve for stevioside by plotting stevioside peak areas versus concentrations, in mg/L, corrected for purity, based on the USP Reference Standard label claim. From this standard curve, determine the concentration (mg/L) of stevioside in the *Sample solution.* Calculate the percent of stevioside in the sample taken using the following formula:

$$C_U / C_{SMP} \times 100\%$$

C_U = Concentration of stevioside in the *Sample solution* determined from the standard curve (mg/L)

C_{SMP} = Concentration of the sample in the *Sample solution* (mg/L)

For the seven other steviol glycoside impurities (rubusoside, dulcoside A, rebaudioside C rebaudioside F rebaudioside D, steviolbioside, and rebaudioside B) use the stevioside standard curve prepared above to calculate the mg/L stevioside equivalents for each. Separately calculate the percents of each analyte (rubusoside, dulcoside A, rebaudioside C, rebaudioside F, rebaudioside D, steviolbioside, and rebaudioside B) in the sample taken using the following formula which takes into account the differences in molecular weights between the analytes and stevioside:

$$(C_U \times M_{r1} / M_{r2}) / C_{SMP} \times 100\%$$

C_U = concentration of stevioside equivalents in the *Sample solution* determined from the standard curve *(mg/*L)

M_{r1} = Molecular weight of the analyte (see *Chromatographic Profile Table* 1, above under *Assay*)

M_{r2} = Molecular weight of stevioside 804.88

C_{SMP} = Concentration of the sample in the *Sample solution* (mg/L)

Sample Chromatograms

SUBMISSION END

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