史迪威生物科技（苏州）有限公司地址：江苏省苏州市工业园区东富路 48 号
Steviana Bioscience（SuZhou）INC 电话：0512-62981591 传真：0512-62981590

June 11, 2018

#795

RECEIVED
JUN 29 2018
OFFICE OF
FOOD ADDITIVE SAFETY

Dr. Paulette Gaynor
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5001 Campus Drive
College Park, MD 20740-3835

Dear Dr. Gaynor:

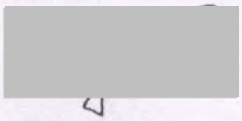
Re: GRAS Notice for High-Purity Steviol Glycoside Ingredients

In accordance with 21 CFR §170 Subpart E consisting of §§170.203 through 170.285, Steviana Bioscience (Suzhou) Inc. hereby informs the U.S. Food and Drug Administration (FDA) of the conclusion that high-purity steviol glycosides as manufactured by Steviana, are Generally Recognized as Safe (GRAS) for its intended conditions of use in food as described in the enclosed notice, and therefore is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act.

I verify that the enclosed electronic files were scanned for viruses prior to submission and are thus certified as being virus-free using Symantec Endpoint Protection Virus and Spyware Protection.

Should you have any questions or concerns regarding this GRAS Notice, please do not hesitate to contact me at any point during the review process so that we may provide a response in a timely manner.

Sincerely,



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GRAS NOTICE FOR STEVIOL GLYCOSIDES

PREPARED FOR:
Center for Food Safety and Applied Nutrition
United States Food and Drug Administration
5001 Campus Drive
College Park, MD
20740 USA

SUBMITTED BY:
Steviana Bioscience (Suzhou) Inc.
Building A
48 Dongfu Road, SIP
Suzhou, Jiangsu Province
China

DATE:
27 May 2018

GRAS Notice for Steviol Glycosides

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GRAS Notice for Steviol Glycosides

Part 1. §170.225 Signed Statements and Certification

In accordance with 21 CFR §170 Subpart E consisting of §170.203 through 170.285, Steviana Bioscience (Suzhou) Inc. (Steviana) hereby informs the United States (U.S.) Food and Drug Administration (FDA) that high-purity (≥95%) steviol glycosides rebaudioside A (Reb A 95), rebaudioside C (Reb C 95), rebaudioside D (Reb D 95), stevioside (Stevioside 95), and a mixture of rebaudioside A + rebaudioside D (90%:5%; Reb AD 95), as manufactured by Steviana, are not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on Steviana's view that the notified substances are Generally Recognized as Safe (GRAS) under the conditions of their intended use described in Section 1.3 below. In addition, as a responsible official of Steviana, Jian Liu hereby certifies that all data and information presented in this notice represents a complete, representative, and balanced submission, and which considered all unfavorable as well as favorable information known to Steviana and pertinent to the evaluation of the safety and GRAS status of the use of Steviana's steviol glycosides as general-purpose sweeteners for addition to food.

Signed,

June 14, 2018

Jian Liu, Ph.D.
CEO
Steviana Bioscience (Suzhou) Inc.
jianl@stevianabio.com

Date

1.1 Name and Address of Notifier

Steviana Bioscience (Suzhou) Inc.
Building A
48 Dongfu Road, SIP
Suzhou, Jiangsu Province
China

1.2 Common Name of Notified Substance(s)

The common name of the notified substances is steviol glycosides. Steviana's GRAS-Notified products include Rebaudioside A 95 (Reb A95), Rebaudioside C 95 (Reb C95), Rebaudioside D 95 (Reb D95), Stevioside 95, and Rebaudioside A plus D (Reb AD95). Each of these products contains at least 95% total glycosides on a w/w basis.

1.3 Conditions of Use

The steviol glycosides manufactured by Steviana are intended for use as table top sweeteners and as general purpose non-nutritive sweeteners for addition to foods in general at per serving levels reflecting good manufacturing practices and principles, in that the quantity added to foods should not exceed the amount reasonably required to accomplish its intended technical effect. Steviana's steviol glycoside products will serve as an alternative to existing GRAS sources of steviol glycosides available in the U.S. marketplace, and the introduction of the ingredient would not change the dietary exposure to steviol glycosides among U.S. consumers of foods to which steviol glycosides may be added.

Steviana's steviol glycosides are not intended for use in meat and poultry or meat and poultry-containing products or infant formulas.

1.4 Basis for GRAS

Pursuant to 21 CFR §170.30 (a) and (b) of the Code of Federal Regulations (CFR) (U.S. FDA, 2017b), steviol glycosides manufactured by Steviana (rebaudioside A [Reb A 95], rebaudioside C [Reb C 95], rebaudioside D [Reb D 95], stevioside [Stevioside 95], and a combination rebaudioside A + rebaudioside D [90%:5%; Reb AD 95]) have been concluded to have GRAS status for use as ingredients for addition to specified conventional food and beverage products as described in Section 1.3 on the basis of scientific procedures.

1.5 Availability of Information

The data and information that serve as the basis for this GRAS Notification will be made available to the FDA for review and copying upon request during business hours at the offices of:

Steviana Bioscience (Suzhou) Inc.
Building A
48 Dongfu Road, SIP
Suzhou, Jiangsu Province
China

In addition, should the FDA have any questions or additional information requests regarding this notification during or after the Agency's review of the notice, Steviana will supply these data and information.

1.6 Freedom of Information Act, 5 U.S.C. 552

It is Steviana's view that all data and information presented in Parts 2 through 7 of this Notice do not contain any trade secret, commercial, or financial information that is privileged or confidential, and therefore all data and information presented herein are not exempt from the Freedom of Information Act, 5 U.S.C. 552.

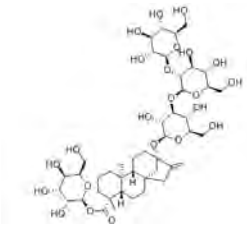
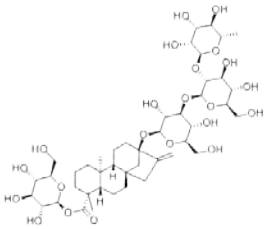
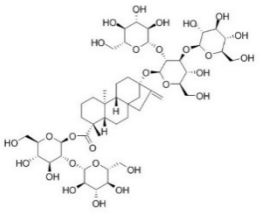
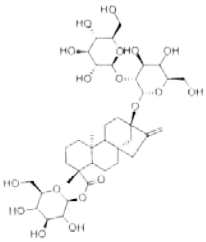
Part 2. §170.230 Identity, Method of Manufacture, Specifications, and Physical or Technical Effect

2.1 Identity

The *Stevia rebaudiana* Bertoni (*S. rebaudiana*) plant is a perennial shrub of the Compositae family, native to Northeastern Paraguay, Brazil, and other South American regions for over 1,500 years (Geuns, 2003; Ferlow, 2005). Approximately 40 steviol glycosides have been isolated from *S. rebaudiana*, which share a common steviol backbone and are conjugated with various numbers of glucose, xylose, rhamnose, fructose, and/or deoxyglucose moieties (Ihrahim *et al.*, 2016; Purkayastha *et al.*, 2016). The glycosides can be obtained by extracting stevia leaves with hot water followed by solvent purification of the water-soluble extract. The water extracts, obtained from the crushed stevia leaves, have a long history of use primarily for their sweetening properties.

A summary of the chemical-chemical characteristics of Steviana's steviol glycosides are presented below in Table 2.1-1.

Table 2.1-1 Description of Steviana's Steviol Glycosides

Common Name	Rebaudioside A (Reb A)	Rebaudioside C (Reb C)	Rebaudioside D (Reb D)	Stevioside
Trade Name	Rebaudioside A 95, Reb A 95	Rebaudioside C 95, Reb C 95	Rebaudioside D 95, Reb D 95	Stevioside
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	13-[(2-O-6–deoxy-β-L-mannopyranosyl-3-O-β–D-glucopyranosyl-β-D-glucopyranosyl)oxy] kaur-16-en-18-oic acid β-D-glucopyranosyl ester	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	13-[(2-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy] kaur-16-en-18-oic acid, β-D-glucopyranosyl ester
CAS Number	58543-16-1	63550-99-2	63279-13-0	57817-89-7
Molecular Weight (g/mol)	967	951	1,129	804.88
Physical Form	White powder	White powder	White powder	White powder
Chemical Structure	Reb A: 	Reb C: 	Reb D: 	Stevioside: 

CAS = Chemical Abstracts Service.

2.2 Manufacturing

2.2.1 Production Details and Schematics

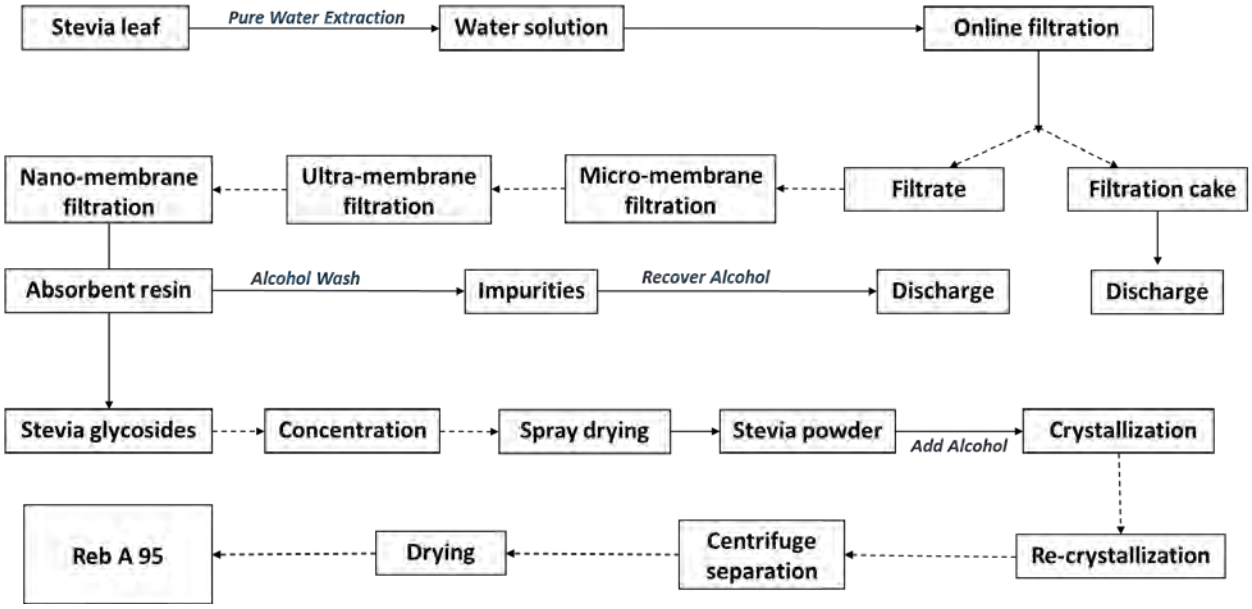
The manufacturing process is conducted in accordance with current Good Manufacturing Practices (cGMP) and is described briefly as follows. Raw stevia leaves undergo an initial hot water extraction (25 to 30°C; aqueous extract), which is subsequently filtered through micro-filters to remove suspended solids, followed by ultra-filtration for removal of pigments/proteins and other substances with higher molecular weights than steviol glycosides. A final nano-filtration step is employed to remove any small molecular weight impurities. The nano-filters are washed, and the solution is passed over an adsorbent resin to remove impurities. The material is then concentrated, and spray dried into a primary stevia extract (also referred to as mother liquor or "stevia powder") consisting of rebaudioside A, C, D, stevioside, as well as other steviol glycosides.

Steviana may also purchase primary stevia extract directly from other suppliers, which has gone through an initial rebaudioside A extraction, leaving typically >75% steviol glycosides and variable levels of rebaudioside A, from which the specific steviol glycosides are separated.

The primary stevia extract is dissolved in alcohol, re-crystallized at 60 to 70°C and then centrifuged to concentrate and extract rebaudioside A (≥95% purity). High-performance liquid chromatography (HPLC) analysis with ultraviolet (UV) detection at 210 nm is conducted to ensure the composition meets the product specification. The leftover stream from Reb A 95 production is then further refined and purified to specifically concentrate and extract high-purity stevioside (STV), as well as rebaudioside D and rebaudioside C *via* re-crystallization and column chromatographic separation. A schematic of the manufacturing process for the steviol glycoside ingredients are provided in Figures 2.2.1-1 and 2.2.1-2.

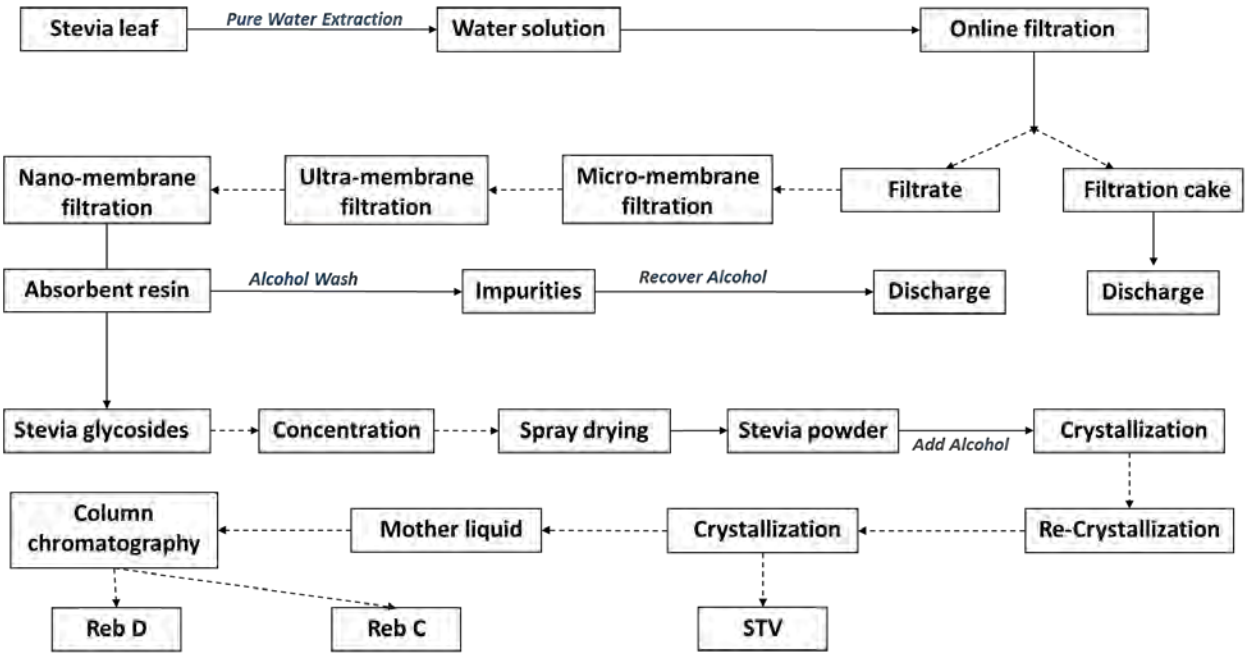
The corresponding batch analyses confirm that the manufacturing process produces a product that is consistent with the established product specifications for Steviana's steviol glycoside ingredients (see Section 2.4).

Figure 2.2.1-1 Schematic Overview of the Manufacturing Process for Steviana's Rebaudioside A



Reb A 95 = rebaudioside A 95.

Figure 2.2.1-2 Schematic Overview of the Manufacturing Process for Steviana's Rebaudioside C, D, and Stevioside



Reb D = rebaudioside D; Reb C = rebaudioside C; STV = stevioside.

2.3 Product Specifications and Batch Analysis

The product specifications established for Steviana's steviol glycosides are outlined in Table 2.3-1 and are consistent with the steviol glycosides monograph published by JECFA (2010), which are presented alongside Steviana's specifications. Analysis of 3 non-consecutive lots of each of the 5 ingredients (Reb A 95, Reb D 95, Reb C 95, Stevioside 95, and Reb AD 95) demonstrate that the manufacturing process produces a consistent product that conforms to the established specifications (see Appendix B).

Table 2.3-1 Steviana's Steviol Glycoside Product Specifications

Characteristic	Steviana Specification for All Steviol Glycoside Products	General Specification for Steviol Glycosides (JECFA)	Steviana's Test Methods
Appearance color	White	White to light yellow	Visual
Form	Powder	Powder	Visual
Odor	Slight Characteristic	NS	Organoleptic
Total steviol glycosides	≥95%	≥95% for individual or all combined steviol glycosides	Compliant with JECFA
Moisture (LOD)	≤6%	≤6%	Compliant with JECFA
Ash	≤1%	≤1%	Compliant with JECFA
Solubility	Freely soluble in water and ethanol	Freely soluble in water	Compliant with JECFA
pH (1% solution)	4.5 to 7.0	4.5 to 7.0	Compliant with JECFA
Residual solvents: Ethanol	≤5,000 ppm	≤5,000 ppm	Compliant with JECFA
Residual solvents: Methanol	≤200 ppm	≤200 ppm	Compliant with JECFA
Heavy Metal Specifications			
Lead (Pb)	≤1 ppm	≤1 ppm	Compliant with JECFA
Arsenic (As)	≤1 ppm	≤1 ppm	Compliant with JECFA
Microbiological Specifications			
Total plate count	≤1,000 CFU/g	NS	CP2010

CFU = colony-forming units; JECFA = Joint FAO/WHO Expert Committee on Food Additives; LOD = loss on drying; NS = not specified; ppm = part per million.

2.4 Stability

In acidic solutions (pH 2 to 4), steviol glycosides (approximately 90 to 94% purity) are stable for at least 180 days when stored at temperatures up to 24°C. However, when exposed to elevated temperatures of 80°C for 8 hours in water, 4 and 8% decomposition was reported in solutions of steviol glycosides at pH 4.0 and 3.0, respectively, indicating that the stability is pH and temperature dependent. When the temperature was increased to 100°C, expectedly higher rates of steviol glycoside decomposition (10 and 40% at pH 4.0 and 3.0, respectively) were reported. Based on the above findings, as well as additional publicly available stability studies, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded that "*steviol glycosides are thermally and hydrolytically stable for use in foods and acidic beverages under normal processing and storage conditions*" (JECFA, 2007).

Part 3. §170.235 Dietary Exposure

3.1 Estimated Intake of Steviol Glycosides

The intended use levels of Steviana's steviol glycosides as sweeteners in foods and beverages will be based on serving levels reflecting cGMP such that the quantity used will not exceed the amount reasonably required to accomplish the required technical sweetening effect[1].

A conservative approach for estimating steviol glycoside intake can be made based on the intake figures reported in numerous studies conducted in the U.S., Canada, Australia/New Zealand, and various countries in the European Union (EU), in which the intakes of aspartame and other high-intensity sweeteners were calculated *via* post-market surveillance data (Renwick, 2008). Renwick (2008) used this published data, adjusting for the relative sweetness intensity of steviol glycosides being 200 times that of sucrose. For the purposes of the intake assessment for steviol glycoside preparations, it was assumed that the composition of Reb A 97 is 100% rebaudioside A. The data used in these analyses were primarily from studies that used specifically designed food diaries combined with actual use levels or approved levels in different foods and beverages (Renwick, 2008). These data were pooled to provide a realistic, but conservative estimate of potential consumption of Reb A 97.

A similar approach was used to estimate intakes to Steviana's steviol glycosides, tailored to the specific relative sweetness of each extract (Table 3.1-1).

Table 3.1-1 Relative Sweetness Potency of Steviana's Steviol Glycosides

Steviol Glycoside	Sweetness Potency Compared to Sucrose
Rebaudioside A 95	350 to 380-fold
Rebaudioside D 95	450 to 500-fold
Rebaudioside C 95	40 to 60-fold
Stevioside	250 to 300-fold
Rebaudioside AD 95	400-fold

The calculated intakes of intense sweeteners (as sucrose equivalents) based on published data, and the corresponding predicted intake of Steviana's steviol glycosides, assuming complete replacement of other intense sweeteners are presented in Tables 3.1-2 to 3.1-4. The predicted intakes of Steviana's steviol glycosides are all below the current acceptable daily intake (ADI) defined by JECFA for steviol glycosides (JECFA, 2008) of 0 to 4 mg/kg body weight/day as steviol equivalents.

[1] Due to the low relative sweetness of Steviana's Reb C (only 40- to 60-fold that of sucrose), it is not used specifically for sweetness but at much lower levels for other taste properties (*e.g.*, mouth-feel) in conjunction with other steviol glycosides.

Table 3.1-2 Intakes of Intense Sweeteners and Predicted Intakes of Reb A 95

Population Group	Intakes of Intense Sweeteners (as sucrose equivalents)		Predicted Intakes of Reb A 95[a]		Predicted Intakes of Reb A 95 (as steviol equivalents)[b]	
	Mean Intake (mg/kg bw/d)	Heavy Consumer (mg/kg bw/d)	Mean Intake (mg/kg bw/d)	Heavy Consumer (mg/kg bw/d)	Mean Intake (mg/kg bw/d)	Heavy Consumer (mg/kg bw/d)
Adults	255	675	0.73	1.93	0.24	0.64
Adults with Diabetes	280	897	0.80	2.56	0.26	0.85
Children	425	990	1.21	2.83	0.40	0.93
Children with Diabetes	672	908	1.92	2.59	0.63	0.86

bw = body weight; d = day; Reb A 95 = rebaudioside A 95.

[a] Calculated by dividing the sucrose intake by the minimum relative sweetness value for Reb A 95 of 350 (notifier data).

[b] Steviol equivalents are calculated by multiplying the steviol glycosides intake estimates by a factor of 0.33 for Reb A 95 (the molar mass ratio between steviol [318.45 g/mol] and rebaudioside A [967 g/mol]).

Table 3.1-3 Intakes of Intense Sweeteners and Predicted Intakes of Reb D 95

Population Group	Intakes of Intense Sweeteners (as sucrose equivalents)		Predicted Intakes of Reb D 95[a]		Predicted Intakes of Reb D 95 (as steviol equivalents)[b]	
	Mean Intake (mg/kg bw/d)	Heavy Consumer (mg/kg bw/d)	Mean Intake (mg/kg bw/d)	Heavy Consumer (mg/kg bw/d)	Mean Intake (mg/kg bw/d)	Heavy Consumer (mg/kg bw/d)
Adults	255	675	0.57	1.50	0.16	0.42
Adults with Diabetes	280	897	0.62	1.99	0.17	0.56
Children	425	990	0.94	2.20	0.26	0.62
Children with Diabetes	672	908	1.49	2.02	0.42	0.56

bw = body weight; d = day; Reb D 95 = rebaudioside D 95.

[a] Calculated by dividing the sucrose intake by the minimum relative sweetness values of 400 for Reb D95 (notifier data).

[b] Steviol equivalents are calculated by multiplying the steviol glycosides intake estimates by a factor of 0.28 for Reb D 95 (the molar mass ratio between steviol [318.45 g/mol] and rebaudioside D [1,129 g/mol]).

Table 3.1-4 Intakes of Intense Sweeteners and Predicted Intakes of Stevioside

Population Group	Intakes of Intense Sweeteners (as sucrose equivalents)		Predicted Intakes of Stevioside[a]		Predicted Intakes of Stevioside (as steviol equivalents)[b]	
	Mean Intake (mg/kg bw/d)	Heavy Consumer (mg/kg bw/d)	Mean Intake (mg/kg bw/d)	Heavy Consumer (mg/kg bw/d)	Mean Intake (mg/kg bw/d)	Heavy Consumer (mg/kg bw/d)
Adults	255	675	1.02	2.70	0.41	1.08
Adults with Diabetes	280	897	1.12	3.59	0.45	1.44
Children	425	990	1.70	3.96	0.68	1.58
Children with Diabetes	672	908	2.69	3.63	1.08	1.45

bw = body weight; d = day.

[a] Calculated by dividing the sucrose intake by the minimum relative sweetness value of 250 for stevioside (notifier data).

[b] Steviol equivalents are calculated by multiplying the steviol glycosides intake estimates by a factor of 0.40 for stevioside (the molar mass ratio between steviol [318.45 g/mol] and stevioside [804.88 g/mol]).

Steviana's Reb C 95 has very little sweetening properties (only a 40 to 60 relative sweetness factor compared to sucrose) and is therefore typically not used for sweetness replacement in foods but is used at low levels for other organoleptic/flavor-enhancing properties, such as 'mouth-feel'. Applying the conservative intake estimates approach as presented above to Reb C 95 would exaggerate the estimated exposure to this steviol glycoside, as it would never be used at a level approaching full sucrose replacement due to cost and other factors. In practice, Reb C 95 would be used in combination with other steviol glycosides at levels of approximately 30 to 50 parts per million (ppm) in the final food product. In comparison, other steviol glycoside ingredients are typically used at levels of approximately 200 to 300 ppm.

Using this information, estimated intakes for Reb C 95 can be accounted for by increasing the estimated intakes of other steviol glycosides by a factor of 25% (200 ppm:50 ppm). As the highest estimated intakes for Steviana's steviol glycosides were calculated with stevioside (Table 3.1-4), the application of a 1.25-fold intake factor to account for a 25% use level of Reb C would result in estimated exposures ranging from 0.51 to 1.35 mg/kg bodyweight/day in mean intake users, and 1.35 to 1.98 mg/kg body weight/day in heavy users (Table 3.1-5). Even with this adjustment, the ADI is not exceeded, and the estimated intake to total steviol glycosides from the addition of 25% of Reb C 95 to any other of Steviana's extracts would be even lower. The estimated intake of Steviana's Reb AD 95 extract would be accounted for in the intake estimate for Reb A 95, as the sucrose replacement calculation assumed the sweetener consisted of 100% Reb A 95.

Table 3.1-5 Intakes of Intense Sweeteners and Predicted Intakes of Stevioside (plus 25% Reb C 95)

Population Group	Intakes of Intense Sweeteners (as sucrose equivalents)		Predicted Intakes of Stevioside[a]		Predicted Intakes of Stevioside plus 25% Reb C 95 (as steviol equivalents)[b]	
	Mean Intake (mg/kg bw/d)	Heavy Consumer (mg/kg bw/d)	Mean Intake (mg/kg bw/d)	Heavy Consumer (mg/kg bw/d)	Mean Intake (mg/kg bw/d)	Heavy Consumer (mg/kg bw/d)
Adults	255	675	1.02	2.70	0.51	1.35
Adults with Diabetes	280	897	1.12	3.59	0.56	1.8
Children	425	990	1.70	3.96	0.85	1.98
Children with Diabetes	672	908	2.69	3.63	1.35	1.81

bw = body weight; d = day; Reb C 95 = rebaudioside C 95.

[a] Calculated by dividing the sucrose intake by the minimum relative sweetness value of 250 for stevioside (client data);

[b] Steviol equivalents are calculated by multiplying the stevioside intakes Table IV.A-3 by a factor of 1.25 to account for a 25% addition of Reb C 95.

It should be noted that these intake estimates are expected to over-estimate daily intake of steviol glycosides in consumers, as it is assumed that all high-intensity sweeteners (HIS) currently on the market are replaced by steviol glycosides, and all food categories contain the maximum intended use levels of each steviol glycosides.

Additional dietary intake estimates for non-nutritive sweeteners, including steviol glycosides have been conducted by the European Food Safety Authority (EFSA) and others, as summarized below. These data support that intakes of steviol glycosides generally does not exceed the established ADI.

The EFSA conducted additional and more refined exposure analysis for estimating intakes of steviol glycosides subsequent to its original evaluation in 2010 (EFSA, 2014). These evaluations were based on maximum permitted use levels, the addition of proposed extension of uses, and the EFSA Comprehensive Food Consumption Database. Mean and 95th percentile estimated intakes were below the ADI of 4 mg/kg body weight/day for all age groups, except for 95th percentile intakes in toddlers (12 to 35 months), which

ranged from 2.0 to 4.3 mg /kg body weight/day (as steviol equivalents). The EFSA Panel concluded that the dietary exposure to steviol glycosides were considerably lower than the exposure estimated in the previous exposure assessment.

A comprehensive review of global dietary intake estimates for sweeteners, including steviol glycosides, conducted by Martyn *et al*. (2018), confirm the conclusion that estimated intakes of steviol glycosides do not exceed the ADI. In this review, the authors reviewed all available dietary intake estimates conducted since 2008 (published since Renwick, 2008) and were organized by region: Asia, Australia/New Zealand, Europe, Latin America, and estimates conducted by JECFA. Approximately 50 publications were reviewed, although not all were related to steviol glycosides.

Part 4. §170.240 Self-Limiting Levels of Use

The use of steviol glycosides in food is largely limited by the desired sweetness intended for a particular food or beverage product; therefore, the use of steviol glycosides as table top sweeteners and general-purpose sweeteners in foods is self-limiting based on their organoleptic properties.

Part 5. §170.245 Experience Based on Common Use in Food Before 1958

Not applicable.

Part 6. §170.250 Narrative and Safety Information

6.1 Introduction

Since steviol glycosides manufactured by Steviana are chemically representative of other steviol glycoside preparations that have been determined to be GRAS (*e.g*., GRAS Notice [GRN] 715, GRN 702[2]), a discussion of publicly available data and information relevant to the safety of steviol glycosides is incorporated by reference to pivotal studies discussed in the most recent GRAS Notice, GRN 715[3] (U.S. FDA, 2017a). Brief summaries of the published literature pertaining to the absorption, distribution, metabolism, and excretion of steviol glycosides are presented in Section 6.2, and an overview of published studies characterizing the toxicity in animal models and safety in humans is discussed in Sections 6.3 through 6.4. To identify new data pertinent to the safety of steviol glycosides published since the GRAS status of steviol glycosides was last evaluated in 2017 (*i.e*., GRN 715) (U.S. FDA, 2017a), a comprehensive search of the published scientific literature was conducted for the period spanning from June 2017 through April 2018 (see Appendix A for literature search report). The search was conducted using the electronic search tool, ProQuest Dialog™, with several databases, including Adis Clinical Trials Insight, AGRICOLA, AGRIS, Allied & Complementary Medicine™, BIOSIS® Toxicology, BIOSIS Previews®, CAB ABSTRACTS, Embase®, Foodline®: SCIENCE, FSTA®, MEDLINE®, NTIS: National Technical Information Service, and Toxfile®. Results of the pertinent toxicological studies from prior GRAS Notifications and newly identified studies relevant to steviol glycoside safety and

[2] U.S. FDA (2017). Agency Response Letter GRAS Notice No. GRN 000702 [Purified steviol glycosides, Hauppauge (NY): Summit Life Science, Inc. for Jiangsu Province, China: Xinghua GL Stevia Co., Ltd.]. Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=702 [Sep. 28, 2017].

[3] GRN 733 related to purified steviol glycosides (8 January 2018) received a "no question" letter; however, the GRAS notice has not been published so it could not be reviewed.

tolerance in humans are summarized in their respective sections below. Consistent with the requirements of the GRAS standard, conclusions on the GRAS status of steviol glycosides have considered all publicly available sources of information including favorable and potentially unfavorable information. Based on Steviana's updated search of the literature, Steviana is not aware of newly published studies to suggest the steviol glycosides are unsafe for use as food ingredients.

The totality of publicly available scientific literature relevant to the safe use of steviol glycosides as ingredients in food has been comprehensively evaluated, using scientific procedures, by a number of independent scientific experts, including the FDA. Approximately 40 GRAS Notices for various high-purity steviol glycosides have been reviewed by the FDA and have received a "no questions" response since 2008 (most recently GRN 715[4]; U.S. FDA, 2017a). These GRAS Notifications have consistently concluded that the addition of steviol glycosides to food is GRAS under their respective conditions of intended use.

The safety of steviol glycosides for use as general-purpose sweeteners for use in foods has also been evaluated by JECFA (JECFA, 1998, 2004, 2007, 2008, 2009), the EFSA (EFSA, 2010), Food Standards Australia New Zealand (FSANZ) (FSANZ, 2008, 2011), the Flavor and Extract Manufacturers Association of the United States (FEMA) Expert Panel[5], and Health Canada (2012). Following the safety conclusions published by JECFA in 2010, highly refined steviol glycoside extracts have been permitted for use as sweeteners in most jurisdictions across the globe (Global Stevia Institute, 2018). The safety of steviol glycosides is based on the general recognition that all glycosides are degraded to the aglycone steviol and that the safety demonstrated for one glycoside is relevant to all glycosides in general. These evaluations included a thorough examination of data on the comparative metabolism and pharmacokinetics of steviol glycosides in experimental animals and humans, acute toxicity studies, short- and long-term toxicity and carcinogenicity studies, reproductive and developmental toxicology studies, *in vitro* and *in vivo* mutagenicity/genotoxicity studies, and human studies. Based on these safety reviews, JECFA, EFSA, and FSANZ independently derived the same ADI of 0 to 4 mg/kg body weight/day, based on steviol equivalents (JECFA, 2008, 2009; FSANZ, 2008; EFSA, 2010).

Based on conclusions from previous expert panels on the GRAS status of steviol glycosides, corresponding no objection letters issued by the FDA, the widespread history of use of steviol glycosides as food ingredients globally, and conclusions from other authoritative bodies on the safety of steviol glycosides as food ingredients (*e.g.*, JECFA, FSANZ, EFSA, Health Canada), Steviana has therefore concluded that the company's steviol glycosides ingredients, as described herein, are GRAS for the specified uses in conventional food products based on scientific procedures.

6.2 Metabolic Fate

The absorption, distribution, metabolism, and excretion of steviol glycosides along with the physiological effects on the gastrointestinal tract related to steviol glycoside ingestion are well characterized and have been previously described in detail (FSANZ, 2008, 2011; JECFA, 2009; EFSA, 2010; U.S. FDA, 2017a). Generally, pharmacokinetic studies in rats and humans have confirmed that intact steviol glycosides are not absorbed from the upper gastrointestinal tract but are hydrolyzed by colonic microflora to the aglycone steviol, which is then absorbed. In humans, the aglycone steviol is primarily metabolized to steviol

[4] U.S. FDA (2018). *Agency Response Letter GRAS Notice No. GRN 000733 [Purified steviol glycosides, Qufu, Shandong Province, China: Shangdong Shengxiangyuan Biotechnology]*. Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fdcc/?set=GRASNotices&id=733 [Open pdf Letter dated Jan. 9, 2018].

[5] FEMA Numbers: 4805, 4796, 4772, 4771, 4763, 4728, 4720, 4601 (Cohen *et al.*, 2015a,b; Marnett *et al.*, 2013; Leffingwell and Leffingwell, 2014; Leffingwell, 2011; Smith *et al.*, 2009)

glucuronide, which is excreted in the urine. This finding has been further researched and reaffirmed more recently, based on *in vitro* human fecal homogenate incubation assays using rebaudiosides A, B, C, D, E, F, and M, as well as steviolbioside and dulcoside A, which showed that all steviol glycosides share the same metabolic fate (Purkayastha *et al*., 2016). The same findings have been reported in other studies for various steviol glycosides (Nikiforov *et al*., 2013; Purkayastha *et al*., 2014).

6.3 Toxicological Studies

The existing ADI for steviol glycosides is derived from a 2-year chronic study in rats, in which the no-observed-adverse-effect level (NOAEL) was concluded to be 970 mg/kg body weight/day (Toyoda *et al*., 1997; JECFA, 2009). Additional sub-chronic, chronic, reproductive, and developmental toxicity studies have been previously discussed in detail, which indicated a lack of adverse effects (JECFA, 2009). Additional 28-day feeding studies with Reb A and Reb D have been conducted in which the dietary administration of 2,000 mg/kg body weight/day (Reb A) or 500, 1,000, or 2,000 mg/kg body weight/day (Reb D) administered in the diet of male and female Sprague Dawley rats (10/sex/group) did not elicit any adverse effects in relation to clinical signs of toxicity, body weights, serum chemistry, functional battery tests, urinalysis, organ weights, or macroscopic/microscopic pathology evaluations (Nikiforov *et al*., 2013). The authors concluded that the highest doses tested with Reb A and Reb D, 2,000 mg/kg body weight/day was the NOAEL.

Subsequent to the sub-chronic studies conducted by Nikiforov *et al*. (2013), an additional 90-day feeding study was identified in which a rebaudioside A extract (meeting JECFA steviol glycoside specifications) derived from the fermentation of the genetically modified yeast, *Yarrowia lipolytica*, was evaluated in male and female Sprague Dawley rats (10/sex/group; aged 5 to 6 weeks) (Rumelhard *et al*., 2016). The target dietary intake levels were 500, 1,000, and 2,000 mg/kg body weight/day of Reb A, which corresponded to actual intakes of 516, 1,026, and 2,057 mg/kg body weight/day in males and 509, 1,016, and 2,021 mg/kg body weight/day in females. At the end of the study, high-dose male rats had a slight (5.9%) but statistically significantly decreased final body weight compared to control animals. No treatment-related adverse effects were reported in relation to clinical signs of toxicity, food intake (including high-dose males), ophthalmologic evaluations, hematology and coagulation parameters, clinical chemistries, urinalysis parameters, organ weights, or macroscopic or microscopic pathology. A slight but statistically significant increase in prothrombin time was reported in all male groups compared to controls; however, the magnitude was small (17.0 seconds in the control group *vs.* 18.0, 17.8, and 17.8 seconds in the low, mid, and high-dose groups, respectively) and there were no significant effects in other parameters, such as red blood cells, platelets, or activated partial thromboplastin time, which would indicate an adverse effect on blood clotting in these animals. Therefore, these effects were not considered toxicologically relevant by the investigators and the NOAEL was 2,000 mg/kg body weight/day, the highest dose tested (Rumelhard *et al*., 2016).

A rebaudioside A extract was administered to a group of C57BL6/J male mice (n=10; 1 month of age) in drinking water at a concentration of 0.1% for 6 months (Reynolds *et al*., 2017). Over this time period, evaluations reported included effect on circadian rhythms for wheel running activity, body weight, glucose/pyruvate/insulin tolerance, caloric and water intakes, obesity susceptibility when consuming high fat diet for 18 weeks. Results were compared to a control group of mice. Rebaudioside A intake (approximately 5.9 mg/mouse/day) did not statistically significantly alter running wheel rhythms, body weights, response to glucose, insulin, or pyruvate, caloric intake during regular diet, body weight gain, or caloric intake during high-fat diet phase compared to control mice. Rebaudioside A-consuming mice consumed statistically significantly more water compared to control mice. Although the study used only a

small number of animals, it further supports the conclusions that steviol glycosides do not alter body weight or sugar regulation in mice.

Stevioside (98% purity) was administered to male NMRI Haan strain mice (6 per group; age not reported) at a dose of 20 mg/kg body weight/day by gavage for 10 days to evaluate the effects on the oral glucose tolerance test (OGTT), adrenaline test, and alloxan-induced diabetes tests compared to saline control animals (Ilic *et al*., 2017). Glucose levels after 10 days of dosing were not statistically significantly different between stevioside and control groups; glucose administration significantly increased plasma glucose levels in the control group[6]. In the adrenaline test, no significant differences between groups were reported in relation to glycemic values before or after adrenaline administration. When alloxan was administered at the end of the 10-day dosing period, animals in the stevioside group had a statistically significantly lower rise in glycemic response 48 hours after alloxan administration compared to control animals. No significant differences in glycemic response were reported between groups when alloxan was administered prior to 10-day dosing with stevioside or saline.

The antihyperglycemic properties of minor steviol glycosides (*i.e.*, Dulcoside A, Rebaudioside B, C, D, and Steviolbioside) were evaluated in normal and streptozotocin-induced diabetic model male Wistar rats (4 to 6 per group; age not reported) at a dose of 20 mg/kg body weight and compared to control groups (Aranda-González *et al*., 2016). In the acute study, an intraperitoneal glucose tolerance test (IPGTT) was conducted in conjunction with a single intraperitoneal administration of the steviol glycoside. Blood glucose levels were measured after 0, 15, 30, 60, and 120 minutes. In a sub-chronic study, the same animal groups were subsequently administered the same dose of steviol glycosides delivered in the form of a dosed pellet on a daily basis for 28 days. The same IPGTT was conducted at the completion of the study. None of the minor steviol glycosides elicited a statistically significant effect on blood glucose levels in normal or diabetic rats following acute or chronic administration compared to control groups.

Jiang *et al*. (2017) administered Rebaudioside A in the drinking water of female weanling Sprague Dawley rats (6 per group) at concentrations of 0, 0.5, or 2.5 mM for 48 days, which was reported to be equivalent to approximately 0.21 g/kg body weight/day and 1.43 g/kg body weight/day, respectively. The animals were evaluated for food and water intake, body weight changes, as well as ovarian biological functions. At the completion of testing period, body weights and food intake were comparable between groups, while water intake was statistically significantly increased in both rebaudioside A groups in the last 3 weeks of the study, compared to the control group. Rebaudioside A intake was not associated with any significant effects on puberty onset (*i.e.*, day of vaginal opening) or number of abnormal estrous cycles, corpora lutea, or ovarian cysts compared to control group. Both rebaudioside A-dosed groups had statistically significantly decreased serum progesterone level *vs*. the control group but were not significantly different between low- and high-dose groups. Based on Western blotting analysis, the authors suggested that decreased gene expression of several steroidogenesis-related factors was related to the decreased serum progesterone levels. The biological significance of these results is unclear as no biological adverse effects were reported in relation to the decreased progesterone levels and there was a lack of dose-response. The previously summarized reproductive toxicity studies in animal models support a lack of adverse effects related to steviol glycoside administration (GRN 715; U.S. FDA, 2017a).

[6] It should be noted that glucose concentration values appeared to be subject to wide standard deviations due to the small sample sizes.

6.3.1 Genotoxicity

Steviol glycosides are not mutagenic or genotoxic based on *in vitro* and *in vivo* assays (JECFA, 2008) and this conclusion has been re-confirmed in a comprehensive review of the genotoxicity database related to steviol glycosides, conducted by Urban *et al.* (2013). An updated search of the of the published literature identified a study in which a rebaudioside A extract derived from the fermentation of the genetically modified yeast, *Yarrowia lipolytica*, was evaluated in several genotoxicity assays (Rumelhard *et al*., 2016). Reb A was not mutagenic in a bacterial reverse mutation assay *Escherichia coli* strain WP2 uvrA or *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100, in the presence and absence of metabolic activation, at Reb A concentration of up to 5,000 µg/plate. Reb A did not elicit a statistically significant increase in micronuclei *in vitro* in cultured peripheral human lymphocytes with or without metabolic activation at concentrations of up to 5,000 µg/mL (Rumelhard *et al*., 2016).

A steviol glycoside preparation ('Stevia', with 99% purity) was evaluated for its potential to induce chromosome aberrations and micronuclei in human lymphocytes in 2 separate assays (Uçar *et al*., 2018). Concentrations of 1, 2, 4, 8, and 16 µg/mL with the incorporation of negative (water) and positive controls (mitomycin C) were evaluated in both tests. Lymphocytes derived from healthy male and female donors (n=4) were incubated at 37°C for 24 or 48 hours prior to addition of the steviol glycosides or control substances. Following addition of colchicine (hour 70) and fixation, 100 well-spread metaphases per donor were analyzed for chromosome aberrations (400 metaphases per concentration). For the micronucleus test, lymphocytes were incubated for 44 or 72 hours prior to addition of steviol glycosides, followed by the addition of cytochalasin B. The incidence of micronuclei was evaluated in 4,000-binucleated cells per concentration. The authors reported that there was no significant increase in the incidence of chromosome aberrations or micronuclei compared to the negative control (Uçar *et al*., 2018).

6.3.2 Other Animal Studies

Updated searches of publicly available literature published since the GRAS status of steviol glycosides (specific to Rebaudioside D) was last evaluated in GRN 715[7] identified a limited number of new studies evaluating safety-related endpoints in various animal models. No new evidence was found that would suggest that the use of steviol glycosides as food ingredients would present unsafe or undesirable effects. The full texts and/or abstracts are provided in Appendix A for further supporting information.

6.4 Clinical Studies

Steviol glycosides have been safely consumed in human studies (*i.e.*, healthy, diabetic, and hypertensive subjects) at doses of up to 1,500 mg/day (approximately 25 mg/kg body weight/day) for up to 2 years (JECFA, 2009 and EFSA, 2010 and in recent GRAS Notifications, such as GRN 715; U.S. FDA, 2017a). Steviol glycosides did not affect glucose homeostasis in healthy subjects or individuals with diabetes and although some antihypertensive effects have been reported in long-term studies in mildly hypertensive subjects (Chan *et al*., 2000; Hsieh *et al*., 2003), these effects were noted at doses that are 6-fold higher than the established ADI and are not relevant to estimated intake levels of Steviana's steviol glycosides from intended uses. A meta-analysis evaluating the potential effects of steviol glycosides on cardiovascular risk factors has been published based on 9 clinical studies, which were all published before 2009 (Onakpoya and Heneghan, 2015). No significant effects on blood pressure or cardiovascular risk factors were reported.

[7] At the time of this dossier preparation, GRN 715 was the most recent steviol glycosides (specific to Rebaudioside D 95%) GRAS to receive a "no questions" letter from the U.S. FDA which summarized literature prior to June 2017 (U.S. FDA, 2017c).

Only 1 additional human study was identified in the literature for 2017 to 2018. The intake of 64 mg of rebaudioside A in conjunction with approximately 4 g of erythritol per day (dissolved in water) in a population of 25 pre-diabetic individuals for 2 weeks did not elicit any statistically significant effects on fructosamine levels, fasting plasma glucose, 2-hour plasma glucose, fasting insulin, fasting C-peptide levels or homeostasis model assessment (HOMA) insulin or β-cell function values compared to baseline (Shin *et al*., 2016).

The current ADI of 4 mg/kg body weight/day is calculated based on the application of a 100-fold safety factor to the chronic rodent study NOAEL of 987 mg/kg body weight/day (equivalent to 383 mg steviol equivalents/kg body weight/day) determined by Toyoda *et al.* (1997). Roberts *et al.* (2016) proposed that based on more accurate adjustment chemical-specific adjustment factors (CSAF) as defined by the World Health Organization (JECFA, 2005) used in extrapolating toxicokinetics from rats to humans, derived from rodent and human toxicokinetics investigations, a lower safety fold factor could be used. Instead of a 100-fold safety factor for inter-and intra-species differences (10-fold, each) Roberts *et al*. (2016) concluded that the CSAF for toxicokinetic differences between rats and humans can be estimated to range between 1, based on maximal plasma concentration (C_{max}) values (ratio of free plasma steviol between humans and rats), and 2.8, based on the area under the concentration-time curve (AUC) values (ratio of AUC for steviol between humans and rats). As a result, the safety factor for determining the ADI for steviol glycosides can be revised to 25 (*i.e.*, 1 x 2.5 x 10 [human variability]) or 70 (*i.e.*, 2.8 x 2.5 x 10 [human variability]), providing an ADI between 6 and 16 mg/kg body weight, as steviol equivalents. The investigations by Roberts *et al.* (2016) lend support that the margin of safety between estimated daily intakes from intake of steviol glycosides and a more precise ADI may be even greater than has been currently established. The intended uses of Steviana's steviol glycoside ingredients would result in estimated daily below the established ADI of 4 mg steviol equivalents/kg body weight/day.

6.5 Expert Panel Evaluation

Steviana has concluded that its steviol glycosides as described herein meeting appropriate food-grade specifications and manufactured consistent with cGMP, is GRAS for use as general-purpose sweeteners in conventional food and beverage products, as described in Part 1.3, on the basis of scientific procedures.

This GRAS conclusion is based on data generally available in the public domain pertaining to the safety of steviol glycosides and on a unanimous opinion among a panel of experts (the Expert Panel) who are qualified by scientific training and experience to evaluate the safety of food ingredients. The Expert Panel consisted of the following scientific experts: Prof. Emer. Joseph F. Borzelleca, Ph.D. (Virginia Commonwealth University School of Medicine), Prof. Robert Nicolosi, Ph.D. (University of Massachusetts Lowell), and Prof. John A. Thomas, Ph.D. (Indiana University School of Medicine)[8].

The Expert Panel, convened by Steviana, independently and critically evaluated all data and information presented herein, and concluded that steviol glycosides as manufactured by Steviana, were GRAS for use in food as described in Section 1.3 based on scientific procedures. A summary of data and information reviewed by the Expert Panel, and evaluation of such data as it pertains to the proposed GRAS uses of steviol glycosides is presented in Appendix D.

[8] The panelists participated in their individual capacities. Institutional affiliations are provided for identification purposes only.

6.6 Conclusions

The data and information summarized in this dossier demonstrate that steviol glycosides (Reb A 95, Reb D 95, Reb C 95, Stevioside 95, and Reb AD 95), as manufactured by Steviana, produced using cGMP and meeting appropriate food-grade specifications, are GRAS, based on scientific procedures, under the conditions of intended use in foods, as described herein.

Part 7. §170.255 List of Supporting Data and Information

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Appendix A
Literature Search Report

Identification of Pertinent Scientific Literature Regarding the Metabolism, Pre-clinical, and Clinical Safety of Steviol Glycosides

TABLE OF CONTENTS

List of Tables

Identification of Pertinent Scientific Literature Regarding the Metabolism, Pre-clinical, and Clinical Safety of Steviol Glycosides

1.0 INTRODUCTION

To identify pertinent scientific literature regarding the metabolism, pre-clinical, and clinical safety of steviol glycosides, a comprehensive search of the scientific literature was conducted using the electronic search tool ProQuest Dialog™. As the safety of steviol glycosides has been recently evaluated by the United States (U.S.) Food and Drug Administration (FDA) in 2017 (*i.e*., Generally Recognized as Safe [GRAS] Notice [GRN] 715) (U.S. FDA, 2017), a comprehensive search of the published scientific literature was conducted for the period spanning from June 2017 through April 2018

The methods used to identify the scientific literature and the results of the literature search are provided in Section 2.0 and 3.0, respectively.

2.0 IDENTIFICATION OF PERTINENT STUDIES

2.1 Literature Search Strategy

To retrieve relevant literature on the metabolism, pre-clinical, and clinical safety of steviol glycosides, 13 literature databases were searched in September of 2017 and April of 2018 using the electronic search tool ProQuest Dialog™. The databases that were searched, as well as the search terms that were used, are listed in Table 2.1-1 and Tables 2.1-2 to 2.1.4, respectively. The search was limited to articles with full texts in English language and publication dates after 01 June 2017[1]. Studies published as abstracts, commentaries, *etc*. were also excluded.

Table 2.1-1 Electronic Databases Used to Retrieve Literature

Electronic Database	Date Range	Update Frequency
Adis Clinical Trials Insight	1990 to present	Weekly
AGRICOLA	1970 to present	Monthly
AGRIS	1975 to present	Monthly
Allied & Complimentary Medicine™	1985 to present	Monthly
BIOSIS® Toxicology	1969 to present	Weekly
BIOSIS Previews®	1926 to present	Weekly
CAB ABSTRACTS	1910 to present	Weekly
EMBASE®	1947 to present	Daily
Foodline®: SCIENCE	1972 to 2016	Stopped updating April 2016; previously twice weekly
FSTA®	1969 to present	Weekly
MEDLINE®	1946 to present	Daily with annual refresh

[1] At the time of this dossier preparation, GRN 715 was the most recent steviol glycosides to receive a "no questions" letter from the U.S. FDA which summarized literature prior to June 2017.

Table 2.1-1 Electronic Databases Used to Retrieve Literature

Electronic Database	Date Range	Update Frequency
NTIS: National Technical Information Service	1964 to present	Weekly
ToxFile®	1946 to present	Daily with annual refresh

The literature search strategy to identify pre-clinical safety data related steviol glycosides is provided below in Table 2.1-2.

Table 2.1-2 Keywords Used to Retrieve Pre-clinical Safety Literature During the Updated Literature Search (01 June 2017 to 15 April 2018)

Strategy[a]	
Set 1: Substance terms	Searched for records containing the following chemical names and synonyms: • Steviol or rebaudioside or stevioside
Set 2: Animal study terms	Within the results from Set 1, searched for records containing the following terms within the publication: • animal or rat or mouse or mice or dog or rabbit or pig or hamster or monkey or rodent or pig or piglet
Set 3: Route of administration terms	Within the results from Set 2, searched for records containing the following terms within the publication: • oral* or gavage or feeding or diet or dietary or intub* or "drinking water" or intragastric
Set 4: Safety terms	Within the results from Set 3, searched for records containing the following terms within the publication: • toxic* or mortal* or lethal* or adverse* or safe* or risk* or hazard*
Set 5: Acute/repeat dose study terms	Within the results from Set 4, searched for records containing the following terms within the publication: • acute* or subacute or "sub acute" or "single dose" or "short term" or subchronic* or "sub chronic*" or chronic* or "long term" or day or week or month or year
Set 6: Safety factors terms	Within the results from Set 3, searched for records containing the following terms within the publication: • LD50 or NOAEL or LOAEL or "no observed adverse effect*" or "low* observed adverse effect*" or NOEL or LOEL or "no observed effect level" or "low* observed effect level" or "maximum tolerated dose" or safety NEAR/2 assess* or risk NEAR/2 assess*
Set 7: Carcinogenicity terms	Within the results from Set 3, searched for records containing the following terms within the publication: • carcino* or tumor* or tumour* or neoplas* or oncogen* or cancer*
Set 8: Reproductive toxicity terms	Within the results from Set 3, searched for records containing the following terms within the publication: • teratol* or teratogen* or reproduct* NEAR/5 toxic* or development* NEAR/5 toxic* or reproduct* NEAR/5 effect* or development* NEAR/5 effect* or fetus or foetus or fetal or foetal or prenatal* or postnatal* or perinatal* or litter or litters or "2 generation*" or "two generation*" or "multi generation*"
Set 9: Genotoxicity terms	Within the results from Set 1, searched for records containing the following terms within the publication: • genotox* or genetox*or mutagen* or mutat* or Ames or "dna repair" or "dna lesion*" or micronucle* or clastogen* or “DNA adduct*” or “comet assay*”

[a] Syntax for the search strategy is as follows: " " = search terms must appear directly beside each other in the exact order; * = truncation; NEAR/5 = search terms may appear within 5 words of each other with either term appearing first within the record.

The literature search strategy to identify clinical safety data related steviol glycosides is provided below in Table 2.1-3.

Table 2.1-3 Keywords Used to Retrieve Clinical Safety Literature During the Updated Literature Search (01 June 2017 to 15 April 2018)

Strategy[a]	
Set 1: Substance terms	Searched for records containing the following chemical names and synonyms within the titles and abstracts: • Steviol or rebaudioside or stevioside
Set 2: Keywords used to identify human studies	Within the results from Set 1, searched for records containing the following terms within the publication: • human or humans or subject or subjects or patient* or clinical* or volunteer* or men or women or "double blind*" or "single blind*" or "open label*" or "cross over" or crossover or cohort or randomiz* or randomis* or "placebo control*"
Set 3: Keywords used to identify route of administration	Within the results from Set 2, searched for records containing the following terms within the publication: • oral* or diet or dietary or ingest* or capsule or tablet or supplement* or consum*
Set 4: Safety terms	Within the results from Set 3, searched for records containing the following terms within the titles and abstractsb: • safe* or risk or "adverse effect*" or "adverse event*" or "adverse reaction*" or "maximum tolerated dose" or "permissible dose level" or "maximum dose level" or threshold or tolerability or tolera*)

[a] Syntax for the search strategy is as follows: " " = search terms must appear directly beside each other in the exact order;
* = truncation; NEAR/5 = search terms may appear within 5 words of each other with either term appearing first within the record.

The literature search strategy to identify nutritive safety and metabolism data related steviol glycosides is provided below in Table 2.1-4.

Table 2.1-4 Keywords Used to Retrieve Nutritive Safety and Metabolism Literature During the Updated Literature Search (01 June 2017 to 15 April 2018)

Strategy[a]	
Set 1: Keywords used for exposure	Searched for records containing the following chemical names and synonyms within the publication: • Steviol or rebaudioside or stevioside
Set 2: Animal study terms	Within the results from Set 1, searched for records containing the following terms within the publication: • animal or rat or mouse or mice or dog or rabbit or pig or hamster or monkey or rodent or pig or piglet
Set 3: Route of administration terms	Within the results from Set 2, searched for records containing the following terms within the publication[b]: • oral* or gavage or feeding or diet or dietary or intub* or "drinking water" or intragastric
Set 4: Metabolism terms	Within the results from Set 1, searched for records containing the following terms within the publication: • metabolis* or metaboliz* or "metabolic* fate*" or "metabolic* path*" or hydroly* or absorb* or absorp* or excret* or eliminat* or pharmacokinetic* or pharmacodynamic* or toxicokinetic* or toxicodynamic* or bioavailab* or biotransform* or ferment* or digest* or fecal or feacal or bowel or "short chain fatty acid*" or SCFA or mineral* or colon or caecum or cecum or fecal or faecal or bile or micro*

[a] Syntax for the search strategy is as follows: " " = search terms must appear directly beside each other in the exact order;
* = truncation.

2.2 Literature Filtration

Once the search strategy was implemented and the publication titles were retrieved, the relevance of the publications was determined at 3 stages using the titles, abstracts, and the full-text of publications. At each stage, the output was manually reviewed against the inclusion/exclusion criteria listed in Table 2.2-1 were applied to determine literature relevance. The 3 stages are outlined below in greater detail.

- Stage 1: Titles of articles were reviewed, and abstracts of titles determined to be potentially relevant were retrieved.
- Stage 2: Abstracts were reviewed, and full-length articles of abstracts determined to be potentially relevant were retrieved.
- Stage 3: Full-length articles were reviewed, and those determined not to meet all the inclusion criteria specified in Table 2.2-1 were excluded.

Table 2.2-1 Inclusion and Exclusion Criteria Used to Filter the Identified Literature

Inclusion Criteria
• The food/food constituent studied was a steviol glycoside • A full-length article published in a peer-reviewed journal • The report analyzed safety-related parameters or metabolism parameters
Exclusion Criteria
• The food/food constituent studied was not a steviol glycoside • A full-length article published in a non-peer-reviewed source (website, magazine, *etc.*) • Published in abstract form only or as a short communication (conference abstract, letter to the editor, commentary, *etc.*) • A research synthesis study (narrative review, systematic review, meta-analysis, *etc.*) • The report did not consider safety-related parameters or metabolism parameters • The study was a duplicate record in the literature search • Full publication study report not in English language

3.0 LITERATURE SEARCH RESULTS

The literature searches resulted in the identification of 73 potentially relevant titles, and abstracts were retrieved for 17 records. Following review of the 17 abstracts and recalling 11 relevant full texts. These studies are tabulated in the GRAS dossier. The results from relevant animal studies identified during the literature search did not present findings that are inconsistent with the GRAS status of steviol glycosides for use as a food ingredient. The full texts and/or abstracts for these articles are provided below in Table 3-1.

Table 3-1 Full-Text and/or Abstracts for Potentially Relevant Studies Identified in the Updated Literature Search (01 June 2017 to 15 April 2018)

Reference[2]	Electronic Copy of Publication
A research on the genotoxicity of stevia in human lymphocytes **Authors:** Uçar, Aslı; Yılmaz, Serkan; Yılmaz, Şemsigül ; Kılıç, Mustafa Sefa **Journal:** Drug and Chemical Toxicology 41.2 (Apr 3, 2018): 221-224. Stevia extracts are obtained from Stevia rebaudiana commonly used as natural sweeteners. It is ~250–300 times sweeter than sucrose. Common use of stevia prompted us to investigate its genotoxicity in human peripheral blood lymphocytes. Stevia (active ingredient steviol glycoside) was dissolved in pure water. Dose selection was done using ADI (acceptable daily intake) value. Negative control (pure water), 1, 2, 4, 8 and 16 µg/ml concentrations which were equivalent to ADI/4, ADI/2, ADI, ADI × 2 and ADI × 4 of Stevia were added to whole-blood culture. Two repetitive experiments were conducted. Our results showed that there was no significant difference in the induction of chromosomal aberrations and micronuclei between the groups treated with the concentrations of Stevia and the negative control at 24 and 48 h treatment periods. The data showed that stevia (active ingredient steviol glycosides) has no genotoxic activity in both test systems. Our results clearly supports previous findings.	Ucar et al 2018.pdf
Low-/no-calorie sweeteners: A review of global intakes **Authors:** Martyn, Danika; Darch, Maryse; Roberts, Ashley; Lee, Han Youl; Tian, Tina Yaqiong; Kaburagi, Naoko; Belmar, Pablo **Journal:** Nutrients 10.3 (Mar 15, 2018). The current review examined published data on the intake of all major low-/no-calorie sweeteners—aspartame, acesulfame-K, saccharin, sucralose, cyclamate, thaumatin and steviol glycosides—globally over the last decade. The most detailed and complex exposure assessments were conducted in Europe, following a standardized approach. Japan and Korea similarly had up-to-date and regular intake data available. The data for other Asian countries, Latin America, Australia/New Zealand and global estimates, evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), while available, were shown to be more limited in terms of design. Overall, the studies conducted since 2008 raised no concerns with respect to exceedance of individual sweetener acceptable daily intake (ADIs) among the general population globally. The data identified do not suggest a shift in exposure over time, with several studies indicating a reduction in intake. However, some data suggest there may have been an increase in the numbers of consumers of low-/no-calorie-sweetened products. Future research should consider a more standardized approach to allow the monitoring of potential changes in exposure based upon events such as sugar reduction recommendations, to ensure there is no shift in intake, particularly for high-risk individuals, including diabetics and children with specific dietary requirements, and to ensure risk management decisions are based on quality intake analyses.	Martyn et al 2018.pdf

[2] It should be noted that the reference and abstracts for the potentially relevant full texts have been copied directly from the published abstract.

Table 3-1 Full-Text and/or Abstracts for Potentially Relevant Studies Identified in the Updated Literature Search (01 June 2017 to 15 April 2018)

Reference[2]	Electronic Copy of Publication
Dietary intakes of six intense sweeteners by Irish adults **Authors:** Buffini, Maria; Goscinny, Séverin; Van Loco, Joris; Nugent, Anne P.; Walton, Janette; Flynn, Albert; Gibney, Michael J.; McNulty, Breige A **Journal:** Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment 35.3 (Mar 4, 2018): 425-438. This research investigated the intakes of six intense sweeteners: acesulfame-K (E950), aspartame (E951), cyclamate (E952), saccharin (E954), sucralose (E955), and steviol glycosides (E960) in the diets of Irish adults, using data from the National Adult Nutrition Survey. A food label survey that included products currently available on the Irish market supplemented the analysis. Sweetener intakes were investigated using three different exposure scenarios; beginning with a crude assessment which assumed that all foods permitted to contain the additives of interest always did contain them, and at their maximum permitted level (Tier 1). Refined assessments estimated intakes of the six sweeteners using food consumption data up to brand level with additive occurrence data from a survey of products currently available on the Irish market (Tier 2) and sweetener concentration data (Tier 3). Results of all exposure assessment scenarios demonstrate that intakes of each of the sweeteners of interest by the total population were below the relevant ADI level (mg kg^{-1} $bodyweight^{-1}$), even by high consumers (P99). The three sweeteners consumed in highest amounts were acesulfame-k, aspartame, and sucralose. The main sources of these sweeteners in the diet were 'cider and perry', 'energy reduced and no added sugar (ER and NAS) carbonated flavoured drinks', 'table-top sweeteners', 'dairy products', 'solid food supplements', and 'sauces'. Intakes of the six intense sweeteners are currently not a concern among Irish adults. However, exposure to these chemicals should be monitored on a regular basis due to evolving market and consumption patterns.	 Buffini et al., 2018.pdf
Chronic Intake of Commercial Sweeteners Induces Changes in Feeding Behavior and Signaling Pathways Related to the Control of Appetite in BALB/c Mic **Authors:** Barrios-Correa, Alberto A.; Estrada, José A.; Martel, Caroline; Olivier, Martin; López-Santiago, Rubén; Contreras, Irazú **Journal:** BioMed Research International 2018 Nonnutritive sweetener use is a common practice worldwide. Although considered safe for human consumption, accumulating evidence suggests these compounds may affect metabolic homeostasis; however, there is no consensus on the role of frequent sweetener intake in appetite and weight loss. We sought to determine whether frequent intake of commercial sweeteners induces changes in the JAK2/STAT3 signaling pathway in the brain of mice, as it is involved in the regulation of appetite and body composition. We supplemented adult BALB/c mice with sucrose, steviol glycosides (SG), or sucralose, daily, for 6 weeks. After supplementation, we evaluated body composition and expression of total and phosphorylated JAK2, STAT3, and Akt, as well as SOCS3 and ObRb, in brain tissue. Our results show that frequent intake of commercial SG decreases energy intake, adiposity, and weight gain in male animals, while increasing the expression of pJAK2 and pSTAT3 in the brain, whereas sucralose increases weight gain and pJAK2 expression in females. Our results suggest that chronic intake of commercial sweeteners elicits changes in signaling pathways that have been related to the control of appetite and energy balance in vivo, which may have relevant consequences for the nutritional state and long term health of the organism.	 Barrios-Correa et al., 2017.pdf

Table 3-1 Full-Text and/or Abstracts for Potentially Relevant Studies Identified in the Updated Literature Search (01 June 2017 to 15 April 2018)

Reference[2]	Electronic Copy of Publication
Predictive modelling of the exposure to steviol glycosides in Irish patients aged 1-3 years with phenylketonuria and cow's milk protein allergy **Author:** O'Sullivan, Aaron J 1 ; Pigat, Sandrine 2 ; O'Mahony, Cian 2 ; Gibney, Michael J 1 ; McKevitt, Aideen **Journals:** Food additives & contaminants. Part A, Chemistry, analysis, control, exposure & risk assessment 35.1 (Jan 2018): 40-48. Children with Phenylketonuria (PKU) and severe cow's milk protein allergy (CMPA) consume prescribed, specially formulated, foods for special medical purposes (FSMPs) as well as restricted amounts of normal foods. These patients are exposed to artificial sweeteners from the consumption of a combination of free and prescribed foods. Young patients with PKU and CMPA have a higher risk of exceeding acceptable daily intakes (ADI) for additives than age-matched healthy children. A predictive modelling approach has been adapted successfully to assess the additive exposure of young patients with PKU and CMPA to artificial sweeteners. Steviol glycosides (E960) are at various stages of regulatory approval for the various food categories in the EU but are not as yet permitted for use in products intended for young children. The aim of this study was to predict potential steviol glycoside exposure in young children with PKU and CMPA considering the potential for future provisions for the use of this sweetener. The recent introduction of steviol glycosides means that no exposure data are available for children with CMPA and PKU. Food consumption data were derived from the food consumption survey data of healthy young children in Ireland from the National Preschool and Nutrition Survey (NPNS, 2010-11). Specially formulated amino acid-based FSMPs are used to replace whole or milk protein foods and were included in the exposure model to replace restricted foods. The recommendations to ensure adequate protein intake in these patients were used to determine FSMP intake. Exposure assessment results indicated that the maximum permitted level (MPL) for FSMPs would warrant careful consideration to avoid exposures above the ADI. These data can be used to inform recommendations for the medical nutrition industry.	Full text not retrieved
Health outcomes of non-nutritive sweeteners: Analysis of the research landscape **Authors:** Lohner, Szimonetta; Toews, Ingrid; Meerpohl, Joerg J. **Journal:** Nutrition Journal 16.1 (Sep 8, 2017). Background: Food products containing non-nutritive sweeteners (NNSs) instead of sugar have become increasingly popular in the last decades. Their appeal is obviously related to their calorie-free sweet taste. However, with the dramatic increase in their consumption, it is reasonable and timely to evaluate their potential health benefits and, more importantly, potential adverse effects. The main aim of this scoping review was to map the evidence about health outcomes possibly associated with regular NNS consumption by examining the extent, range, and nature of research activity in this area. Methods: We systematically searched Ovid MEDLINE, EMBASE and the Cochrane CENTRAL databases for studies on NNSs (artificial sweeteners or natural, non-caloric sweeteners, either used individually or in combination) using text terms with appropriate truncation and relevant indexing terms. All human studies investigating any health outcomes of a NNS intervention or exposure were eligible for inclusion. No studies were excluded based on language, study design or methodological quality. Data for each health outcome were summarized in tabular form and were discussed narratively. Results: Finally, we included 372 studies in our scoping review, comprising 15 systematic reviews, 155 randomized controlled trials (RCTs), 23 non-randomized controlled trials, 57 cohort studies, 52 case-control studies, 28 cross sectional studies and 42 case series/case reports. In healthy subjects, appetite and short term food intake, risk of cancer, risk of diabetes, risk of dental caries, weight gain and risk of obesity are the most investigated health outcomes. Overall there is no conclusive evidence for beneficial and harmful effects on those outcomes. Numerous health outcomes including headaches, depression, behavioral and cognitive effects, neurological effects, risk of preterm delivery, cardiovascular effects or risk of chronic kidney disease were investigated in fewer studies and further research is needed. In subjects with diabetes and hypertension, the evidence regarding health outcomes of NNS use is also inconsistent. Conclusions: This scoping review identifies the needs for future research to address the numerous evidence	 Lohner et al 2017.pdf

Table 3-1 Full-Text and/or Abstracts for Potentially Relevant Studies Identified in the Updated Literature Search (01 June 2017 to 15 April 2018)

Reference[2]	Electronic Copy of Publication
gaps related to health effects of NNSs use.It also specifies the research questions and areas where a systematic review with meta-analyses is required for the proper evaluation of health outcomes associated to regular NNSs consumption.	
Nonnutritive sweeteners and cardiometabolic health: A systematic review and meta-analysis of randomized controlled trials and prospective cohort studies **Authors**: Azad, Meghan B.; Abou-Setta, Ahmed M.; Chauhan, Bhupendrasinh F.; Rabbani, Rasheda et al. **Journal**: CMAJ 189.28 (Jul 17, 2017): E929-E939. BACKGROUND: Nonnutritive sweeteners, such as aspartame, sucralose and stevioside, are widely consumed, yet their long-term health impact is uncertain. We synthesized evidence from prospective studies to determine whether routine consumption of nonnutritive sweeteners was associated with long-term adverse cardiometabolic effects. METHODS: We searched MEDLINE, Embase and Cochrane Library (inception to January 2016) for randomized controlled trials (RCTs) that evaluated interventions for nonnutritive sweeteners and prospective cohort studies that reported on consumption of nonnutritive sweeteners among adults and adolescents. The primary outcome was body mass index (BMI). Secondary outcomes included weight, obesity and other cardiometabolic end points. RESULTS: From 11 774 citations, we included 7 trials (1003 participants; median follow-up 6 mo) and 30 cohort studies (405 907 participants; median follow-up 10 yr). In the included RCTs, nonnutritive sweeteners had no significant effect on BMI (mean difference -0.37 kg/m2; 95% confidence interval [CI] -1.10 to 0.36; I2 9%; 242 participants). In the included cohort studies, consumption of nonnutritive sweeteners was associated with a modest increase in BMI (mean correlation 0.05, 95% CI 0.03 to 0.06; I2 0%; 21 256 participants). Data from RCTs showed no consistent effects of nonnutritive sweeteners on other measures of body composition and reported no further secondary outcomes. In the cohort studies, consumption of nonnutritive sweeteners was associated with increases in weight and waist circumference, and higher incidence of obesity, hypertension, metabolic syndrome, type 2 diabetes and cardiovascular events. Publication bias was indicated for studies with diabetes as an outcome. INTERPRETATION: Evidence from RCTs does not clearly support the intended benefits of nonnutritive sweeteners for weight management, and observational data suggest that routine intake of nonnutritive sweeteners may be associated with increased BMI and cardiometabolic risk. Further research is needed to fully characterize the long-term risks and benefits of nonnutritive sweeteners.	 Azad et al 2017.pdf
Insight into anti-diabetic effect of low dose of stevioside **Author:** Ilić, Vladimirka; Vukmirović, Saša; Stilinović, Nebojša; Čapo, Ivan; Arsenović, Milan; Milijašević, Boris **Journal:** Biomedicine and Pharmacotherapy 90 (Jun 1, 2017): 216-221. Diabetes mellitus is a chronic disease characterized by abnormal carbohydrate, lipid and protein metabolism due to a lack of insulin or reduced target cell sensitivity to insulin. Stevia rebaudiana is an important source of biochemically active substances with proven anti-diabetic effect. The aim of this study was to determine anti-diabetic effects of the low dose of stevioside in NMRI Haan mice. Aqueous stevioside solution (20 mg/kg body weight) was administered by oral route of administration. Anti-diabetic effect of stevioside was estimated by oral glucose tolerance test, adrenaline test after a 10 day stevioside treatment, and alloxan induced hyperglycaemia in mice (two experimental groups, 10 day stevioside treatment before and after alloxan administration). Aqueous stevioside solution prevented significant increase in glycaemia in oral glucose tolerance test (9.22 ± 1.13 to 9.85 ± 1.32 mmol/l, $P < 0.05$), and not in adrenaline test. Significant difference in glycaemia was detected in mice pre-treated with saline and stevioside in alloxan induced hyperglycaemia (saline 23.32 ± 2.14, stevioside 14.70 ± 4.95 mmol/l, $P < 0.05$). In mice pre-treated with stevioside, smallest β cells loss was found compared to other alloxan treated groups. Preserved normal cytoarchitectonic arrangement in islets was detected. Based on the given results we presume there exist a potential therapeutic use of low dose stevioside in diabetes.	 Ilic et al 2017.pdf

Table 3-1 Full-Text and/or Abstracts for Potentially Relevant Studies Identified in the Updated Literature Search (01 June 2017 to 15 April 2018)

Reference[2]	Electronic Copy of Publication
Effect of stevia consumption on blood pressure, stress hormone levels and anthropometrical parameters in healthy persons **Authors:** Al-Dujaili, Emad A. S; Twaij, Husni; Bataineh, Yazan A.; Arshad, Unam; Amjid, Faiza **Journal:** American Journal of Pharmacology and Toxicology 12.1 (2017): 7-17. Stevia is a natural sweetener containing steviol glycosides known to be several times sweeter than sucrose. It is thought to have several beneficial properties though some evidence state it may have detrimental effects. The aim of this study was to investigate the potential beneficial or harmful effects of stevia consumption by exploring its effects on blood pressure, stress hormone levels and anthropometrical markers in A crossover placebo-controlled study was conducted on 16 volunteers randomly assigned to consume either stevia or a placebo (sugar) for one week. The measurements were attained on three different occasions and each volunteer was allowed a 3-day initiation period before baseline and in between interventions. The systolic BP increased following stevia intake from 114.5±12.7 to 119.9±12.9mmHg ($p<0.001$) and diastolic BP from 70.8±9.4 to 75.7±9.6mmHg ($p<0.01$). Systolic BP increased slightly after the sugar placebo to 115.3±13.6 mmHg (not significant). The mean free cortisol excreted in urine has increased from 91.8±49.1 to 125.7±60.5nmole/day ($p<0.01$) after the stevia and to 109.1±42.6nmole/day after the placebo ($p = 0.210$). The ratio of urinary free cortisol/cortisone showed a statistically significant increase from 1.73±0.78 to 2.65±1.03 after stevia ($p<0.0001$). Salivary cortisol levels have also increased ($p<0.01$ at AM) after stevia. Placebo intake did not produce a significant change in salivary cortisol. The ratio of salivary cortisol/cortisone during the stevia has increased only in the morning (from 1.22±0.65 to 1.75±0.72, $p = 0.05$) and a modest increase in the daily average of salivary cortisol/cortisone. There was small insignificant reduction in weight and BMI after stevia intervention ($p = 0.246$ and $p = 0.249$ respectively). In conclusion, we have shown that short term stevia intake produced a small but significant increase in BP and effect on body weight and BMI were not significant. The rise in BP might be due to the increase in cortisol levels and cortisol/cortisone ratio indicating that stevia may possibly inhibit 11β-HSD2 enzyme by reducing the conversion of cortisol into cortisone. Therefore, caution should be taken by the public who want to consume stevia for longer period of time as a weight reducing sweetener.	 Al-Dujaili et al., 2017.pdf
Biological activity of *Stevia rebaudiana* Bertoni and their relationship to health. **Authors:** Ruiz-Ruiz, J C; Moguel-Ordoñez, Y B; Segura-Campos, M R **Journal:** Critical Reviews in Food Science and Nutrition 57.12 (2017): 2680-2690. The leaves of *Stevia rebaudiana* Bertoni has nutrients and phytochemicals, which make it an adequate source for the extraction and production of functional food ingredients. Preclinical and clinical studies suggest therapeutic and pharmacological applications for stevia and their extracts because they are not toxic and exhibit several biological activities. This review presents the biological activity of *Stevia rebaudiana* Bertoni and their relationship to antidiabetic, anticariogenic, antioxidant, hypotensive, antihypertensive, antimicrobial, anti-inflammatory and antitumor activities. Consumption and adverse effects were also reviewed.	 Ruiz-Ruiz et al 2017.pdf

Table 3-1 Full-Text and/or Abstracts for Potentially Relevant Studies Identified in the Updated Literature Search (01 June 2017 to 15 April 2018)

Reference[2]	Electronic Copy of Publication
A review on the pharmacology and toxicology of steviol glycosides extracted from Stevia rebaudiana **Authors:** Momtazi-Borojeni, Amir Abbas; Esmaeili, Seyed-Alireza; Abdollahi, Elham; Sahebkar, Amirhossein **Journal:** Current Pharmaceutical Design 23.11 (2017): 1616-1622. Stevia rebaudiana Bertoni is a sweet and nutrient-rich plant belonging to the Asteraceae family. Stevia leaves contain steviol glycosides including stevioside, rebaudioside (A to F), steviolbioside, and isosteviol, which are responsible for the plant's sweet taste, and have commercial value all over the world as a sugar substitute in foods, beverages and medicines. Among the various steviol glycosides, stevioside, rebaudioside A and rebaudioside C are the major metabolites and these compounds are on average 250-300 times sweeter than sucrose. Steviol is the final product of Stevia metabolism. The metabolized components essentially leave the body and there is no accumulation. Beyond their value as sweeteners, Stevia and its glycosdies possess therapeutic effects against several diseases such as cancer, diabetes mellitus, hypertension, inflammation, cystic fibrosis, obesity and tooth decay. Studies have shown that steviol glycosides found in Stevia are not teratogenic, mutagenic or carcinogenic and cause no acute and subacute toxicity. The present review provides a summary on the biological and pharmacological properties of steviol glycosides that might be relevant for the treatment of human diseases.	 Momtazi-Borojeni et al 2017.pdf
Safety assessment of 16 sweeteners for the Korean population using dietary intake monitoring and poundage method. **Authors:** Kim, MeeKyung; Lee, GunYoung; Lim HoSoo; SangSoon, Yun; Hwang MyungSil; Hong JinHwan; Kwon HoonJeong **Journal:** Food Additives and Contaminants A 34.9 (2017): 1500-1509. A sweetener is a food additive that imparts a sweet taste to food products. Sweeteners have been increasingly used in Korea since the approval of sodium saccharin and D-sorbitol in 1962. Unlike food contaminants, humans are exposed to food additives only through the consumption of processed food products. For exposure assessments of sweeteners, the dietary intakes of food products containing acesulfame-K, aspartame, saccharin-Na, and sucralose were determined, and the resulting calculated estimated daily intake (EDI) values were compared directly with each additive's ADI. The poundage method was used to calculate the daily intake per capita for 12 additional sweeteners, such as lactitol, for which appropriate analytical methods for food products do not exist. The risk, as evaluated by comparing the EDI with the ADI, was determined to be 2.9% for acesulfame-K, 0.8% for aspartame, 3.6% for saccharin-Na, 4.3% for steviol glycosides, and 2.1% for sucralose. No hazardous effect was predicted for the other 11 sweeteners, including lactitol.	 Kim et al 2017.pdf

Table 3-1 Full-Text and/or Abstracts for Potentially Relevant Studies Identified in the Updated Literature Search (01 June 2017 to 15 April 2018)

Reference[2]	Electronic Copy of Publication
Cytotoxicity and genotoxicity evaluation of stevioside on CCD18Co and HCT 116 cell lines. **Authors:** Sharif, R; Chan, K M; Ooi, T C; Mohammad, N F **Journal:** International Food Research Journal 24.1 (2017): 341-345. Recent findings showed that stevioside can demonstrate anti-cancer property in selected cell lines. In this study, the cytotoxicity and genotoxicity of stevioside were examined on human colon carcinoma cell, HCT 116 (targeted cell) and human colon derived CCD18Co myofibroblast cell lines (non-targeted cell) using the MTT (3-(4, 5-dimethylthiazol-2-yl)- 2,5-diphenyltettrazolium bromide) assay and alkaline comet assay, respectively. Result demonstrated that stevioside induced cell death on both HCT 116 and CCD18Co cell lines only at the highest concentration, 200 µM by causing not more than 20 and 30 percent of cell death on CCD18Co and HCT 116 cell lines, respectively ($p<0.05$). The DNA strand break measured via alkaline comet assay showed that it did not cause DNA damage at the same concentration on CCD18Co as well as in HCT 116 cell lines ($p>0.05$). In conclusion, stevioside did not exhibit cytotoxic and genotoxic effect on HCT 116 and CCD18Co cell lines respectively hence secured its uses as a non-caloric sweetener.	Full text not retrieved
Critical review on steviol glycosides: Pharmacological, toxicological and therapeutic aspects of high potency zero caloric sweetener **Authors:** Mathur, Shaifali; Bulchandani, Neha; Parihar, Suman; Shekhawat, Gyan Singh **Journal:** International Journal of Pharmacology 13.7 (2017): 916-928. Stevia rebaudiana Bertoni is a sweet tasting medicinal herb; its leaves are rich source of sweetener "steviosides", which are up to three hundred times sweeter than sucrose, more than half of which is composed by Stevioside and Rebaudioside. Due to its sweet taste it has high commercial value throughout the world as sugar substitute in medicine, foods products and beverages. The increased market share of Stevia sweeteners has established a lasting increase in the demand for constant high quality and high purity of Stevia products. Clinical examinations performed on Steviol glycosides have shown that it is non-toxic and exert hypotensive, cardiotonic, anti-diabetic, anti-carcinogenic, anti-inflammatory, anti-viral and anti-bacterial actions. Stevia leaves, steviosides and highly refined extracts of the leaves are now officially used as a low calorie natural sweetener and dietary supplement in many countries. In future, there is possibility that Stevia could become a major source of high potency low calorie sweetener for growing demand in natural food market. This manuscript focuses on the phytochemistry, medicinal applications, pharmaco kinetics and safety evaluations of Stevia products. Besides this, recent developments in agricultural breeding, biotechnological approaches through cell and tissue culture, improved extraction procedures and biotransformation for taste improvement in S. rebaudiana have also been discussed. Future prospects for realization of commercial production of Steviol glycosides are critically evaluated.	 Mathur et al 2017.pdf
Long term rebaudioside A treatment does not alter circadian activity rhythms, adiposity, or insulin action in male mice **Authors:** Reynolds, Thomas H; Soriano, Rachelle A; Obadi, Obadi A; Murkland, Stanley; Possidente, Bernard **Journal:** PloS one 12.5 (2017): e0177138. Obesity is a major public health problem that is highly associated with insulin resistance and type 2 diabetes, two conditions associated with circadian disruption. To date, dieting is one of the only interventions that result in substantial weight loss, but restricting caloric intake is difficult to maintain long-term. The use of artificial sweeteners, particularly in individuals that consume sugar sweetened beverages (energy drinks, soda), can reduce caloric intake and possibly facilitate weight loss. The purpose of the present study was to examine the effects of the artificial sweetener, rebaudioside A (Reb-A), on circadian rhythms, in vivo insulin action, and the susceptibility to diet-induced obesity. Six month old male C57BL/6 mice were assigned to a	 Reynolds et al 2017.pdf

Table 3-1 Full-Text and/or Abstracts for Potentially Relevant Studies Identified in the Updated Literature Search (01 June 2017 to 15 April 2018)

Reference[2]	Electronic Copy of Publication
control or Reb-A (0.1% Reb-A supplemented drinking water) group for six months. Circadian wheel running rhythms, body weight, caloric intake, insulin action, and susceptibility to diet-induced obesity were assessed. Time of peak physical activity under a 12:12 light-dark (LD) cycle, mean activity levels, and circadian period in constant dark were not significantly different in mice that consumed Reb-A supplemented water compared to normal drinking water, indicating that circadian rhythms and biological clock function were unaltered. Although wheel running significantly reduced body weight in both Reb-A and control mice (P = 0.0001), consuming Reb-A supplemented water did not alter the changes in body weight following wheel running (P = 0.916). In vivo insulin action, as assessed by glucose, insulin, and pyruvate tolerance tests, was not different between mice that consumed Reb-A treated water compared to normal drinking water. Finally, Reb-A does not appear to change the susceptibility to diet-induced obesity as both groups of mice gained similar amounts of body weight when placed on a high fat diet. Our results indicate that consuming Reb-A supplemented water does not promote circadian disruption, insulin resistance, or obesity.	
Toxicological evaluation of ethanolic extract from *Stevia rebaudiana* Bertoni leaves: genotoxicity and subchronic oral toxicity. **Authors:** Regulatory Toxicology and Pharmacology 86 (2017): 253-259. **Journal:** PloS one 12.5 (2017): e0177138. *Stevia rebaudiana* Bertoni leaves have a long history of use as an abundant source of sweetener. The aqueous extract of stevia leaves and the predominant constitutes steviol glycosides have been intensively investigated. However, rare studies provided toxicological evaluation of bioactive components in the polar extract regarding their safety on human health. This study aimed to evaluate the toxicity of ethanolic extract of *Stevia rebaudiana* Bertoni leaves through a battery of in vitro and *in vivo* tests. Negative results were unanimously obtained from bacterial reverse mutation assay, mouse bone marrow micronucleus assay and mouse sperm malformation assay. Oral administration at dietary levels of 1.04%, 2.08% and 3.12% for 90 days did not induce significant behavioral, hematological, clinical, or histopathological changes in rats. Significant reduction of cholesterol, total protein and albumin was observed in female animals only at high dose level. The results demonstrated that *Stevia rebaudiana* Bertoni leaves ethanolic extract, which is rich in isochlorogenic acids, does not possess adverse effects through oral administration in this study. Our data provided supportive evidence for the safety of *Stevia rebaudiana* Bertoni leaves that may potentially be used in functional foods as well as nutritional supplements beyond sweetener.	Full text not retrieved

史迪威生物科技（苏州）有限公司

STEVIANA

Steviana Bioscience(Suzhou) Inc.

产品名称（Product Name）: Rebaudiosid A(Reb A97)
批号（Lot No）:A2017072102
电话（Tel） : (+86)0512-62981591
生产地址（Place of Manufacturing）: NO.48 Dongfu RoadLoufeng,Industrial Park,Suzhou,Jiangsu,China.

分析报告

Certificate of Analysis

项目 Category	标准值 Specification	实测值 Result	检测方法 Test Mothods
状态 Appearance	白色粉末 white fine powder	白色粉末 white fine powder	JECFA
溶解性 Solubility	溶于水/乙醇 Soluble in water/ethanol	溶于水/乙醇 Soluble in water/ethanol	JECFA
重量 Weight	20kg/箱	120Kg	JECFA
含量测定 Assay	RebA≥95%	97.07%	JECFA
干燥失重 Loss on drying	<6.0%	1.46%	JECFA
灰分 Ash total	<1%	<0.1%	JECFA
溶剂残留 Residual Solvent	Ethanol <5000mg/Kg Methanol<200mg/Kg	Ethanol 1590.95ppm Methanol 17.98ppm	JECFA
重金属（铅） Heavy metal(Pb)	<1ppm	0.0037ppm	JECFA
重金属（砷） Heavy metal(As)	<1ppm	0.025pm	JECFA
菌落总数 TotalPlate Count	<1,000cfu/g	<10cfu/g	CP2010
标准（National Standard）： JECFA-2010			
结论（Conclusion）：			
检验员 （Analyst）：		核对员（Checker）：	
检测时间（Analysis Time）：			

质检报告专用章

2017.8.7

史迪威生物科技（苏州）有限公司

STEVIANA

Steviana Bioscience(Suzhou) Inc.

产品名称（Product Name）: Rebaudiosid A(Reb A97)
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分析报告

Certificate of Analysis

项目 Category	标准值 Specification	实测值 Result	检测方法 Test Mothods
状态 Appearance	白色粉末 white fine powder	白色粉末 white fine powder	JECFA
溶解性 Solubility	溶于水/乙醇 Soluble in water/ethanol	溶于水/乙醇 Soluble in water/ethanol	JECFA
重量 Weight	20kg/箱	280kg	JECFA
含量测定 Assay	RebA≥95%	97.68%	JECFA
干燥失重 Loss on drying	<6.0%	0.96%	JECFA
灰分 Ash total	<1%	<0.1%	JECFA
溶剂残留 Residual Solvent	Ethanol <5000mg/Kg Methanol<200mg/Kg	Ethanol 2683.46ppm Methanol 27.06ppm	JECFA
重金属（铅） Heavy metal(Pb)	<1ppm	0.0035ppm	JECFA
重金属（砷） Heavy metal(As)	<1ppm	0.021ppm	JECFA
菌落总数 TotalPlate Count	<1,000cfu/g	<10cfu/g	CP2010
标准（National Standard）： JECFA-2010			
结论（Conclusion）：			
检验员 （Analyst）：		核对员（Checker）：	
检测时间（Analysis Time）： 2017.8.7			

质检报告专用章

史迪威生物科技（苏州）有限公司

STEVIANA

Steviana Bioscience(Suzhou) Inc.

产品名称（Product Name）: Rebaudiosid A(Reb A99)

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分析报告

Certificate of Analysis

项目 Category	标准值 Specification	实测值 Result	检测方法 Test Mothods
状态 Appearance	白色粉末 white fine powder	白色粉末 white fine powder	JECFA
溶解性 Solubility	溶于水/乙醇 Soluble in water/ethanol	溶于水/乙醇 Soluble in water/ethanol	JECFA
重量 Weight	20kg/箱	160 kg	JECFA
含量测定 Assay	RebA≥99%	99. 05%	JECFA
干燥失重 Loss on drying	<6.0%	1.21%	JECFA
灰分 Ash total	<1%	<0.1%	JECFA
溶剂残留 Residual Solvent	Ethanol <5000mg/Kg Methanol<200mg/Kg	Ethanol 417.95 ppm Methanol 50 ppm	JECFA
重金属（铅） Heavy metal(Pb)	<1ppm	0.0025ppm	JECFA
重金属（砷） Heavy metal(As)	<1ppm	0.022ppm	JECFA
菌落总数 TotalPlate Count	<1,000cfu/g	<10cfu/g	CP2010
标准（National Standard）: JECFA-2010			
结论（Conclusion）:			
检验员 （Analyst）:		核对员（Checker）:	
检测时间（Analysis Time）:	2017.6.7		

史迪威生物科技（苏州）有限公司

STEVIANA

Steviana Bioscience(Suzhou) Inc.

产品名称（Product Name）：Viána AD 95
批号（Lot No）：20161121
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分析报告

Certificate of Analysis

项目 Category	标准值 Specification	实测值 Result	检测方法 Test Mothods
状态 Appearance	白色粉末 white fine powder	白色粉末 white fine powder	JECFA
溶解性 Solubility	溶于水/乙醇 Soluble in water/ethanol	溶于水/乙醇 Soluble in water/ethanol	JECFA
含量测定 Assay	TSG≥95%	97.25%	JECFA
1	RebA≥90%	90.93%	JECFA
2	RD≥5%	6.32%	JECFA
干燥失重 Loss on drying	<6.0%	1.7%	JECFA
灰分 Ash total	<1%	<0.1%	JECFA
溶剂残留 Residual Solvent	Ethanol <5000mg/Kg Methanol<200mg/Kg	Ethanol 2406.37ppm Methanol N D	JECFA
重金属（铅） Heavy metal(Pb)	<1ppm	0.0037ppm	JECFA
重金属（砷） Heavy metal(As)	<1ppm	0.028ppm	JECFA
菌落总数 TotalPlate Count	<1,000cfu/g	<10cfu/g	CP2010
标准（National Standard）： JECFA-2010			
结论（Conclusion）：			
检验员 （Analyst）：		核对员（Checker）：	
检测时间（Analysis Time）：2017.6.8			

史迪威生物科技（苏州）有限公司

STEVIANA

Steviana Bioscience(Suzhou) Inc.

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分析报告

Certificate of Analysis

项目 Category	标准值 Specification	实测值 Result	检测方法 Test Mothods
状态 Appearance	白色粉末 white fine powder	白色粉末 white fine powder	JECFA
溶解性 Solubility	溶于水/乙醇 Soluble in water/ethanol	溶于水/乙醇 Soluble in water/ethanol	JECFA
含量测定 Assay	TSG≥95%	98.83%	JECFA
1	RebA≥90%	93.6%	JECFA
2	RD≥5%	5.23%	JECFA
干燥失重 Loss on drying	<6.0%	4.62%	JECFA
灰分 Ash total	<1%	<0.1%	JECFA
溶剂残留 Residual Solvent	Ethanol <5000mg/Kg Methanol<200mg/Kg	Ethanol 500ppm Methanol N D	JECFA
重金属（铅） Heavy metal(Pb)	<1ppm	0.0037ppm	JECFA
重金属（砷） Heavy metal(As)	<1ppm	0.028ppm	JECFA
菌落总数 TotalPlate Count	<1,000cfu/g	<10cfu/g	CP2010
标准（National Standard）： JECFA-2010			
结论（Conclusion）：			
检验员 （Analyst）：		核对员（Checker）：	
检测时间（Analysis Time）：			

史迪威生物科技（苏州）有限公司

STEVIANA

Steviana Bioscience(Suzhou) Inc.

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分析报告

Certificate of Analysis

项目 Category	标准值 Specification	实测值 Result	检测方法 Test Mothods
状态 Appearance	白色粉末 white fine powder	白色粉末 white fine powder	JECFA
溶解性 Solubility	溶于水/乙醇 Soluble in water/ethanol	溶于水/乙醇 Soluble in water/ethanol	JECFA
重量 Weight	20kg/箱	12 箱	JECFA
含量测定 Assay	Viána AD≥95%	≥99%	JECFA
1	Reb A ≥90%	94.41%	JECFA
2	Reb D ≥5%	6.7%	JECFA
干燥失重 Loss on drying	<6.0%	1.28%	JECFA
灰分 Ash total	<1%	<0.1%	JECFA
溶剂残留 Residual Solvent	Ethanol <5000mg/Kg Methanol<200mg/Kg	Ethanol 2423.72ppm Methanol 29.23ppm	JECFA
重金属（铅） Heavy metal(Pb)	<1ppm	0.0029ppm	JECFA
重金属（砷） Heavy metal(As)	<1ppm	0.031ppm	JECFA
菌落总数 TotalPlate Count	<1,000cfu/g	<10cfu/g	CP2010
标准（National Standard）： JECFA-2010			
结论（Conclusion）：			
检验员 （Analyst）：		核对员（Checker）：	
检测时间（Analysis Time）：	2017.8.7		

史迪威生物科技（苏州）有限公司

STEVIANA

Steviana Bioscience(Suzhou) Inc.

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分析报告

Certificate of Analysis

项目 Category	标准值 Specification	实测值 Result	检测方法 Test Mothods
状态 Appearance	白色粉末 white fine powder	白色粉末 white fine powder	JECFA
溶解性 Solubility	溶于水/乙醇 Soluble in water/ethanol	溶于水/乙醇 Soluble in water/ethanol	JECFA
含量测定 Assay	RebC≥95%	95.1%	JECFA
干燥失重 Loss on drying	<6.0%	4.3%	JECFA
灰分 Ash total	<1%	<0.1%	JECFA
溶剂残留 Residual Solvent	Ethanol <5000mg/Kg Methanol<200mg/Kg	Ethanol 105.13ppm Methanol 82.58ppm	JECFA
重金属（铅） Heavy metal(Pb)	<1ppm	0.0015ppm	JECFA
重金属（砷） Heavy metal(As)	<1ppm	0.023ppm	JECFA
菌落总数 TotalPlate Count	<1,000cfu/g	<10cfu/g	CP2010
标准（National Standard）： JECFA-2010			
结论（Conclusion）：			
检验员 （Analyst）：		核对员（Checker）：	
检测时间（Analysis Time）：			

质检报告专用章

史迪威生物科技（苏州）有限公司

STEVIANA

Steviana Bioscience(Suzhou) Inc.

产品名称（Product Name）：Rebaudiosid C (Reb C95)

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电话（Tel）：（+86）0512-62981591

生产地址（Place of Manufacturing）：NO.48 Dongfu RoadLoufeng,Industrial Park,Suzhou,Jiangsu,China.

分析报告

Certificate of Analysis

项目 Category	标准值 Specification	实测值 Result	检测方法 Test Mothods
状态 Appearance	白色粉末 white fine powder	白色粉末 white fine powder	JECFA
溶解性 Solubility	溶于水/乙醇 Soluble in water/ethanol	溶于水/乙醇 Soluble in water/ethanol	JECFA
含量测定 Assay	RebC≥95%	95. 06%	JECFA
干燥失重 Loss on drying	<6.0%	4.7%	JECFA
灰分 Ash total	<1%	<0.1%	JECFA
溶剂残留 Residual Solvent	Ethanol <5000mg/Kg Methanol<200mg/Kg	Ethanol 1013. 3ppm Methanol N D	JECFA
重金属（铅） Heavy metal(Pb)	<1ppm	0.0015ppm	JECFA
重金属（砷） Heavy metal(As)	<1ppm	0.023ppm	JECFA
菌落总数 TotalPlate Count	<1,000cfu/g	<10cfu/g	CP2010
标准（National Standard）：JECFA-2010			
结论（Conclusion）：			
检验员 （Analyst）：		核对员（Checker）：	
检测时间（Analysis Time）：2017. 5. 2			

史迪威生物科技（苏州）有限公司

Steviana Bioscience(Suzhou) Inc.

产品名称（Product Name）: Rebaudioside C(Reb C95)
批号（Lot No）:20170724
电话（Tel） : (+86)0512-62981591
生产地址（Place of Manufacturing）: NO.48 Dongfu RoadLoufeng,Industrial Park,Suzhou,Jiangsu,China.

分析报告

Certificate of Analysis

项目 Category	标准值 Specification	实测值 Result	检测方法 Test Mothods
状态 Appearance	白色粉末 white fine powder	白色粉末 white fine powder	JECFA
溶解性 Solubility	溶于水/乙醇 Soluble in water/ethanol	溶于水/乙醇 Soluble in water/ethanol	JECFA
含量测定 Assay	RebC≥95%	97.08%	JECFA
干燥失重 Loss on drying	<6.0%	4.02%	JECFA
灰分 Ash total	<1%	<0.1%	JECFA
溶剂残留 Residual Solvent	Ethanol <5000mg/Kg Methanol<200mg/Kg	Ethanol 92.93ppm Methanol 142.98ppm	JECFA
重金属（铅） Heavy metal(Pb)	<1ppm	0.0031ppm	JECFA
重金属（砷） Heavy metal(As)	<1ppm	0.038ppm	JECFA
菌落总数 TotalPlate Count	<1,000cfu/g	<10cfu/g	CP2010
标准（National Standard）： JECFA-2010			
结论（Conclusion）：			
检验员 （Analyst）：		核对员（Checker）：	
检测时间（Analysis Time）： 2017.7.28			

史迪威生物科技（苏州）有限公司

STEVIANA

Steviana Bioscience(Suzhou) Inc.

产品名称（Product Name）：Rebaudiosid C (Reb C95)
批号（Lot No）： 20160722
电话（Tel） ： （+86）0512-62981591
生产地址（Place of Manufacturing）：NO.48 Dongfu RoadLoufeng,Industrial Park,Suzhou,Jiangsu,China.

分析报告

Certificate of Analysis

项目 Category	标准值 Specification	实测值 Result	检测方法 Test Mothods
状态 Appearance	白色粉末 white fine powder	白色粉末 white fine powder	JECFA
溶解性 Solubility	溶于水/乙醇 Soluble in water/ethanol	溶于水/乙醇 Soluble in water/ethanol	JECFA
含量测定 Assay	RebC≥95%	95.1%	JECFA
干燥失重 Loss on drying	≤6.0%	4.3%	JECFA
灼烧残渣 Ash total	≤1%	<0.1%	JECFA
甲醇 Methanol	≤200mg/Kg	82.58mg/Kg	JECFA
乙醇 Ethanol	≤5000mg/Kg	105.13mg/Kg	JECFA
丙酮 Acetone		Not Detected	JECFA
铅 Pb	≤1mg/Kg	Conform	JECFA
砷 As	≤1mg/Kg	Conform	JECFA

国家标准 National Standard ：	JECFA-2010		
结论 Conclusion:	合格		
检验员 Analyst :	杨东	核对员 Checker :	陈美娟
检测时间 Analysis Time:	2018.1.9		

质检报告专用章

史迪威生物科技（苏州）有限公司

STEVIANA

Steviana Bioscience(Suzhou) Inc.

产品名称（Product Name）：Rebaudiosid C (Reb C95)
批号（Lot No）： 20161010
电话（Tel）：（+86）0512-62981591
生产地址（Place of Manufacturing）：NO.48 Dongfu RoadLoufeng,Industrial Park,Suzhou,Jiangsu,China.

分析报告

Certificate of Analysis

项目 Category	标准值 Specification	实测值 Result	检测方法 Test Mothods
状态 Appearance	白色粉末 white fine powder	白色粉末 white fine powder	JECFA
溶解性 Solubility	溶于水/乙醇 Soluble in water/ethanol	溶于水/乙醇 Soluble in water/ethanol	JECFA
含量测定 Assay	RebC≥95%	95.06%	JECFA
干燥失重 Loss on drying	≤6.0%	4.7%	JECFA
灼烧残渣 Ash total	≤1%	<0.1%	JECFA
甲醇 Methanol	≤200mg/Kg	23.26mg/Kg	JECFA
乙醇 Ethanol	≤5000mg/Kg	1013.3mg/Kg	JECFA
丙酮 Acetone		Not Detected	JECFA
铅 Pb	≤1mg/Kg	Conform	JECFA
砷 As	≤1mg/Kg	Conform	JECFA

国家标准 National Standard：	JECFA-2010		
结论 Conclusion:	合格		
检验员 Analyst：	杨东	核对员 Checker：	陈美娟
检测时间 Analysis Time：	2018.1.9		

质检报告专用章

史迪威生物科技（苏州）有限公司

STEVIANA

Steviana Bioscience(Suzhou) Inc.

产品名称（Product Name）: Rebaudioside C(Reb C95)
批号（Lot No）:20170724
电话（Tel） : (+86)0512-62981591
生产地址（Place of Manufacturing）: NO.48 Dongfu RoadLoufeng,Industrial Park,Suzhou,Jiangsu,China.

分析报告

Certificate of Analysis

项目 Category	标准值 Specification	实测值 Result	检测方法 Test Mothods
状态 Appearance	白色粉末 white fine powder	白色粉末 white fine powder	JECFA
溶解性 Solubility	溶于水/乙醇 Soluble in water/ethanol	溶于水/乙醇 Soluble in water/ethanol	JECFA
含量测定 Assay	Reb C≥95%	96.8%	JECFA
干燥失重 Loss on drying	≤6.0%	3.26%	JECFA
灼烧残渣 Ash total	≤1%	<0.1%	JECFA
甲醇 Methanol	≤200mg/Kg	9.69mg/Kg	JECFA
乙醇 Ethanol	≤5000mg/Kg	54.35mg/Kg	JECFA
丙酮 Acetone		Not Detected	JECFA
铅 Pb	≤1mg/Kg	Conform	JECFA
砷 As	≤1mg/Kg	Conform	JECFA

国家标准 National Standard :	JECFA-2010		
结论 Conclusion:	合格		
检验员 Analyst :	杨东	核对员 Checker :	陈美娟
检测时间 Analysis Time:	2018.1.9		

质检报告专用章

史迪威生物科技（苏州）有限公司

STEVIANA

Steviana Bioscience(Suzhou) Inc.

产品名称（Product Name）：Rebaudioside D (Reb D)
批号（Lot No）： 20170303
电话（Tel） ： （+86）0512-62981591
生产地址（Place of Manufacturing）：NO.48 Dongfu RoadLoufeng,Industrial Park,Suzhou,Jiangsu,China.

分析报告

Certificate of Analysis

项目 Category	标准值 Specification	实测值 Result	检测方法 Test Mothods
状态 Appearance	白色粉末 white fine powder	白色粉末 white fine powder	JECFA
溶解性 Solubility	溶于水/乙醇 Soluble in water/ethanol	溶于水/乙醇 Soluble in water/ethanol	JECFA
含量测定 Assay	RD≥95%	95. 1%	JECFA
干燥失重 Loss on drying	<6.0%	2.09%	JECFA
灰分 Ash total	<1%	<0.1%	JECFA
溶剂残留 Residual Solvent	Ethanol <5000mg/Kg Methanol<200mg/Kg	Ethanol 1024ppm Methanol N D	JECFA
重金属（铅） Heavy metal(Pb)	<1ppm	0.0032ppm	JECFA
重金属（砷） Heavy metal(As)	<1ppm	0.024ppm	JECFA
菌落总数 TotalPlate Count	<1,000cfu/g	<10cfu/g	CP2010
标准（National Standard）：JECFA-2010			
结论（Conclusion）：			
检验员 （Analyst）：		核对员（Checker）：	
检测时间（Analysis Time）： 2017.5.30			

史迪威生物科技（苏州）有限公司

STEVIANA

Steviana Bioscience(Suzhou) Inc.

产品名称（Product Name）: Rebaudiosid D 95 (Reb D)
批号（Lot No）: 20170418
电话（Tel）：（+86）0512-62981591
生产地址（Place of Manufacturing）: NO.48 Dongfu RoadLoufeng,Industrial Park,Suzhou,Jiangsu,China.

分析报告

Certificate of Analysis

项目 Category	标准值 Specification	实测值 Result	检测方法 Test Mothods
状态 Appearance	白色粉末 white fine powder	白色粉末 white fine powder	JECFA
溶解性 Solubility	溶于水/乙醇 Soluble in water/ethanol	溶于水/乙醇 Soluble in water/ethanol	JECFA
含量测定 Assay	RD≥95%	95.4%	JECFA
干燥失重 Loss on drying	<6.0%	4.95%	JECFA
灰分 Ash total	<1%	<0.1%	JECFA
溶剂残留 Residual Solvent	Ethanol <5000mg/Kg Methanol<200mg/Kg	Ethanol 3201ppm Methanol N D	JECFA
重金属（铅） Heavy metal(Pb)	<1ppm	0.0033ppm	JECFA
重金属（砷） Heavy metal(As)	<1ppm	0.024ppm	JECFA
菌落总数 TotalPlate Count	<1,000cfu/g	<10cfu/g	CP2010
标准（National Standard）: JECFA-2010			
结论（Conclusion）:			
检验员 （Analyst）:		核对员（Checker）:	
检测时间（Analysis Time）: 2017.6.18			

检验报告专用章

史迪威生物科技（苏州）有限公司

STEVIANA

Steviana Bioscience(Suzhou) Inc.

产品名称（Product Name）: Rebaudioside D (Reb D95)
批号（Lot No）: 20170612
电话（Tel） : (+86)0512-62981597
生产地址（Place of Manufacturing）: NO.48 Dongfu RoadLoufeng,Industrial Park,Suzhou,Jiangsu,China.

分析报告

Certificate of Analysis

项目 Category	标准值 Specification	实测值 Result	检测方法 Test Mothods
状态 Appearance	白色粉末 white fine powder	白色粉末 white fine powder	JECFA
溶解性 Solubility	溶于水/乙醇 Soluble in water/ethanol	溶于水/乙醇 Soluble in water/ethanol	JECFA
含量测定 Assay	RebD≥95%	95.87%	JECFA
干燥失重 Loss on drying	<6.0%	3.05%	JECFA
灰分 Ash total	<1%	<0.1%	JECFA
溶剂残留 Residual Solvent	Ethanol <5000mg/Kg Methanol<200mg/Kg	Ethanol 705.85ppm /	JECFA
重金属（铅） Heavy metal(Pb)	<1ppm	0.0035ppm	JECFA
重金属（砷） Heavy metal(As)	<1ppm	0.027ppm	JECFA
菌落总数 TotalPlate Count	<1,000cfu/g	<10cfu/g	CP2010
标准（National Standard）： JECFA-2010			
结论（Conclusion）：			
检验员 （Analyst）：		核对员（Checker）：	
检测时间（Analysis Time）：2017.7.24			

史迪威生物科技（苏州）有限公司

STEVIANA

Steviana Bioscience(Suzhou) Inc.

产品名称（Product Name）: Stevioside 95 (STV 95)
批号（Lot No）： 20160805
电话（Tel） ： （+86）0512-62981591
生产地址（Place of Manufacturing）: NO.48 Dongfu RoadLoufeng,Industrial Park,Suzhou,Jiangsu,China.

分析报告

Certificate of Analysis

项目 Category	标准值 Specification	实测值 Result	检测方法 Test Mothods
状态 Appearance	白色粉末 white fine powder	白色粉末 white fine powder	JECFA
溶解性 Solubility	溶于水/乙醇 Soluble in water/ethanol	溶于水/乙醇 Soluble in water/ethanol	JECFA
含量测定 Assay	STV≥95%	95. 4%	JECFA
干燥失重 Loss on drying	<6.0%	4.3%	JECFA
灰分 Ash total	<1%	<0.1%	JECFA
溶剂残留 Residual Solvent	Ethanol <5000mg/Kg Methanol<200mg/Kg	Ethanol 3.65ppm Methanol 12. 58ppm	JECFA
重金属（铅） Heavy metal(Pb)	<1ppm	0.0028ppm	JECFA
重金属（砷） Heavy metal(As)	<1ppm	0.041ppm	JECFA
菌落总数 TotalPlate Count	<1,000cfu/g	<10cfu/g	CP2010
标准（National Standard）： JECFA-2010			
结论（Conclusion）：			
检验员 （Analyst）：		核对员（Checker）：	
检测时间（Analysis Time）： 2016.8.18			

史迪威生物科技（苏州）有限公司

STEVIANA

Steviana Bioscience(Suzhou) Inc.

产品名称（Product Name）： Stevioside 95（STV95）
批号（Lot No）: 20170525
电话（Tel） ：（+86）0512-62981591
生产地址（Place of Manufacturing）: NO.48 Dongfu RoadLoufeng,Industrial Park,Suzhou,Jiangsu,China.

分析报告

Certificate of Analysis

项目 Category	标准值 Specification	实测值 Result	检测方法 Test Mothods
状态 Appearance	白色粉末 white fine powder	白色粉末 white fine powder	JECFA
溶解性 Solubility	溶于水/乙醇 Soluble in water/ethanol	溶于水/乙醇 Soluble in water/ethanol	JECFA
含量测定 Assay	STV≥95%	95. 87%	JECFA
干燥失重 Loss on drying	<6.0%	3.91%	JECFA
灰分 Ash total	<1%	<0.1%	JECFA
溶剂残留 Residual Solvent	Ethanol <5000mg/Kg Methanol<200mg/Kg	Ethanol 112ppm Methanol 189ppm	JECFA
重金属（铅） Heavy metal(Pb)	<1ppm	0.0037ppm	JECFA
重金属（砷） Heavy metal(As)	<1ppm	0.031ppm	JECFA
菌落总数 TotalPlate Count	<1,000cfu/g	<10cfu/g	CP2010
标准（National Standard）： JECFA-2010			
结论（Conclusion）：			
检验员 （Analyst）：		核对员（Checker）：	
检测时间（Analysis Time）：2017.5.31			

史迪威生物科技（苏州）有限公司

STEVIANA

Steviana Bioscience(Suzhou) Inc.

产品名称（Product Name）：Stevioside 95 (STV 95)
批号（Lot No）： 20170602
电话（Tel） ： (+86)0512-62981597
生产地址（Place of Manufacturing）：NO.48 Dongfu RoadLoufeng,Industrial Park,Suzhou,Jiangsu,China.

分析报告

Certificate of Analysis

项目 Category	标准值 Specification	实测值 Result	检测方法 Test Mothods
状态 Appearance	白色粉末 white fine powder	白色粉末 white fine powder	JECFA
溶解性 Solubility	溶于水/乙醇 Soluble in water/ethanol	溶于水/乙醇 Soluble in water/ethanol	JECFA
含量测定 Assay	STV≥95%	95.57%	JECFA
干燥失重 Loss on drying	<6.0%	3.75%	JECFA
灰分 Ash total	<1%	<0.1%	JECFA
溶剂残留 Residual Solvent	Ethanol <5000mg/Kg Methanol<200mg/Kg	Ethanol 269.62ppm Methanol 95.01ppm	JECFA
重金属（铅） Heavy metal(Pb)	<1ppm	0.0028ppm	JECFA
重金属（砷） Heavy metal(As)	<1ppm	0.031ppm	JECFA
菌落总数 TotalPlate Count	<1,000cfu/g	<10cfu/g	CP2010
标准（National Standard）： JECFA-2010			
结论（Conclusion）：			
检验员 （Analyst）：		核对员（Checker）：	
检测时间（Analysis Time）： 2017.8.23			

SHIMADZU
LabSolutions

分析报告

<样品信息>

样品名	: 2017072723-1		
样品ID	:		
数据文件名	: RA定量_0706_lcb_201784_001.lcd		
方法文件名	: LC.lcm		
批处理文件名	: RA定量_0706_lcb.lcb		
样品瓶号	: 1-3	样品类型	: 未知
进样体积	: 5 uL		
分析日期	: 2017/8/4 11:30:49	分析者	: System Administrator
处理日期	: 2017/8/4 15:31:26	处理者	: System Administrator

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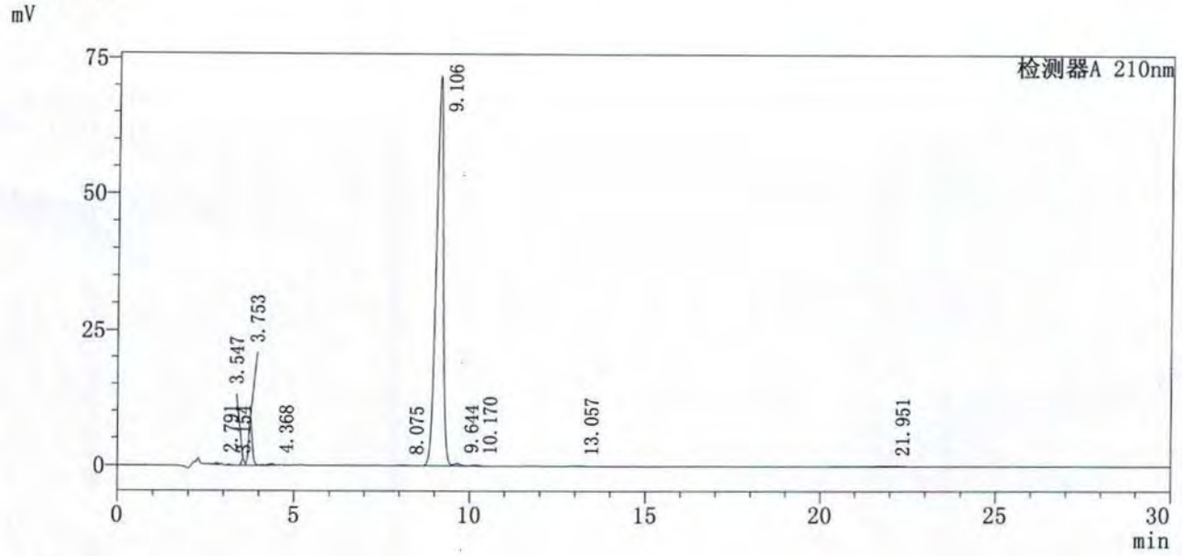


<峰表>

检测器A 210nm

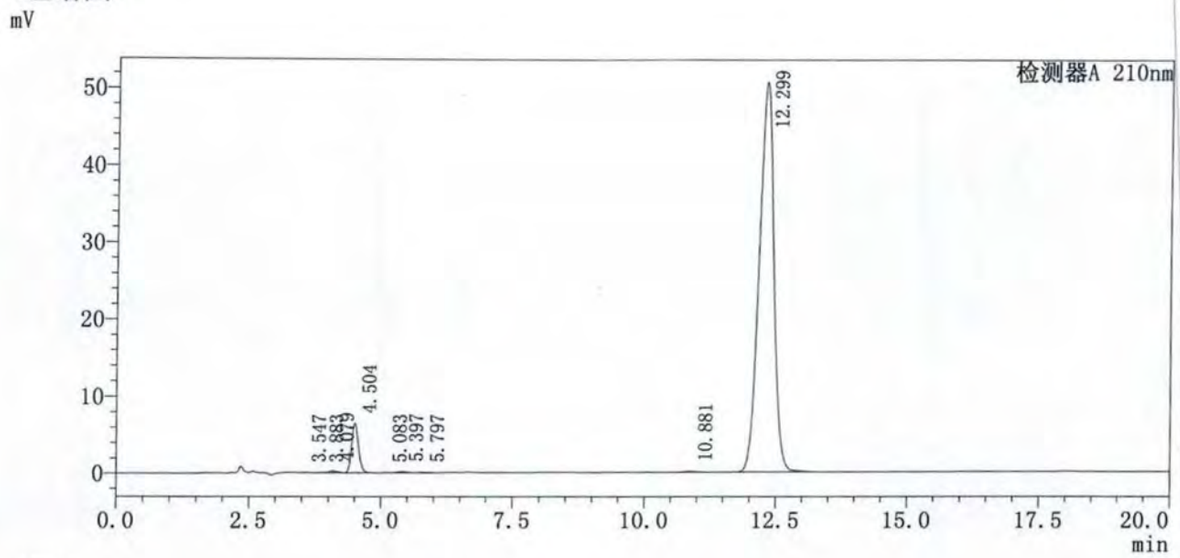
峰号	保留时间	面积	高度	浓度	浓度单位	标记	化合物名
1	2.791	2034	280	0.185			
2	3.154	1026	150	0.093			
3	3.547	6903	1103	0.629			
4	3.753	61968	8804	5.645		V M	
5	4.368	3364	265	0.306		V M	
6	8.075	1525	87	0.139			
7	9.106	1001250	71851	91.203			
8	9.644	5937	440	0.541		V	
9	10.170	2398	162	0.218		V	
10	13.057	1845	106	0.168			
11	21.951	9569	114	0.872		M	
总计		1097821	83361				

SHIMADZU
LabSolutions 分析报告

〈样品信息〉

样品名	: AD20161121		
样品ID	:		
数据文件名	: 20170612_009.lcd		
方法文件名	: LC.lcm		
批处理文件名	: STEVIA.lcb		
样品瓶号	: 1-5	样品类型	: 控制
进样体积	: 5 uL	级别号	: 1
分析日期	: 2017/6/12 19:46:08	分析者	: System Administrator
处理日期	: 2017/6/12 20:06:10	处理者	: System Administrator

〈色谱图〉



〈峰表〉

检测器A 210nm

峰号	保留时间	面积	高度	浓度	浓度单位	标记	化合物名
1	3.547	285	44	0.027		M	
2	3.883	507	59	0.048			
3	4.079	2496	239	0.238		V	
4	4.504	57914	6445	5.521		M	
5	5.083	163	16	0.016		M	
6	5.397	1842	128	0.176		V	
7	5.797	738	64	0.070		V	
8	10.881	1715	97	0.163			
9	12.299	983371	50638	93.741			
总计		1049030	57731				

SHIMADZU
LabSolutions 分析报告

〈样品信息〉

样品名	: AD20170220		
样品ID	:		
数据文件名	: 20170724_009.lcd		
方法文件名	: LC.lcm		
批处理文件名	: STEVIA.lcb		
样品瓶号	: 1-5	样品类型	: 控制
进样体积	: 5 uL	级别号	: 1
分析日期	: 2017/7/24 13:22:37	分析者	: System Administrator
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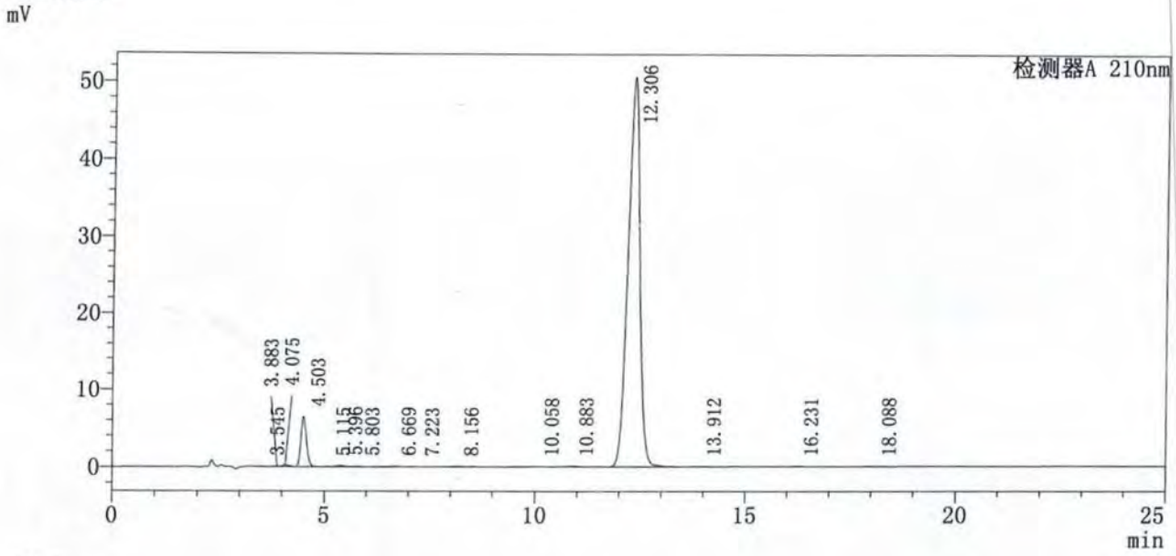
〈色谱图〉



〈峰表〉

检测器A 210nm

峰号	保留时间	面积	高度	浓度	浓度单位	标记	化合物名
1	3.545	347	52	0.033		M	
2	3.883	491	57	0.047			
3	4.075	2473	238	0.236		V	
4	4.503	57776	6408	5.508			
5	5.115	283	26	0.027		V	
6	5.396	1935	130	0.184		V	
7	5.803	747	66	0.071		V	
8	6.669	1363	77	0.130		M	
9	7.223	92	8	0.009		M	
10	8.156	1433	33	0.137		M	
11	10.058	815	24	0.078		M	
12	10.883	1648	96	0.157			
13	12.306	977716	50551	93.213			
14	13.912	259	16	0.025		M	
15	16.231	823	37	0.079		M	
16	18.088	706	28	0.067		M	
总计		1048907	57846				

SHIMADZU
LabSolutions

分析报告

<样品信息>

样品名	: A2017072102		
样品ID	:		
数据文件名	: RA定量_0706_lcb_201782_001.lcd		
方法文件名	: LC.lcm		
批处理文件名	: RA定量_0706_lcb.lcb		
样品瓶号	: 1-6	样品类型	: 标准
进样体积	: 5 uL	级别号	: 1
分析日期	: 2017/8/2 21:36:49	分析者	: System Administrator
处理日期	: 2017/8/3 15:57:24	处理者	: System Administrator

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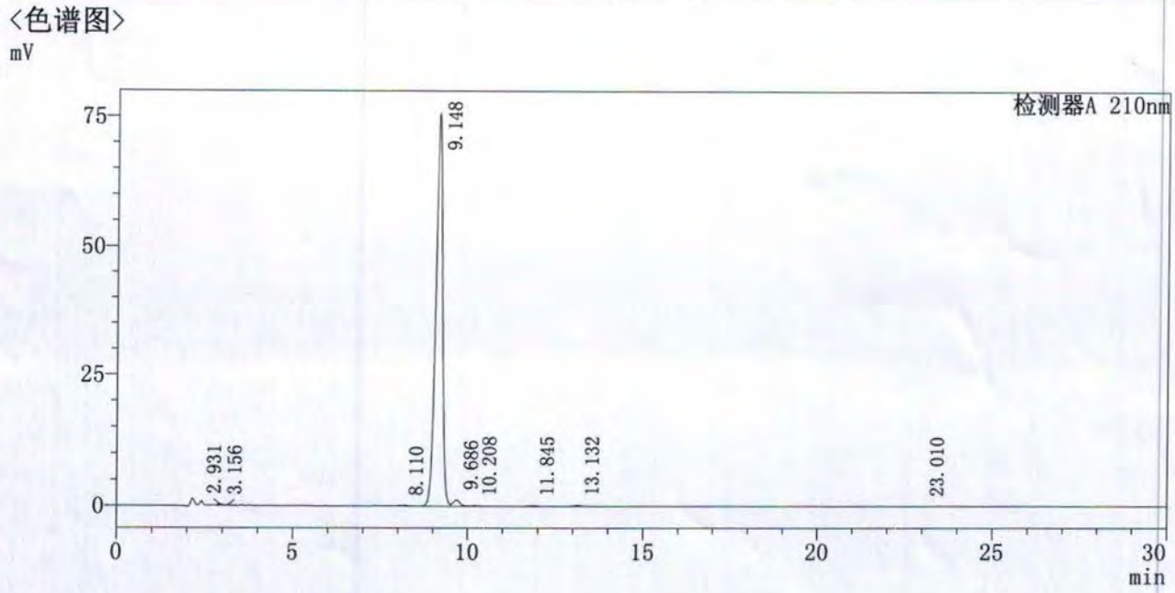


<峰表>

检测器A 210nm

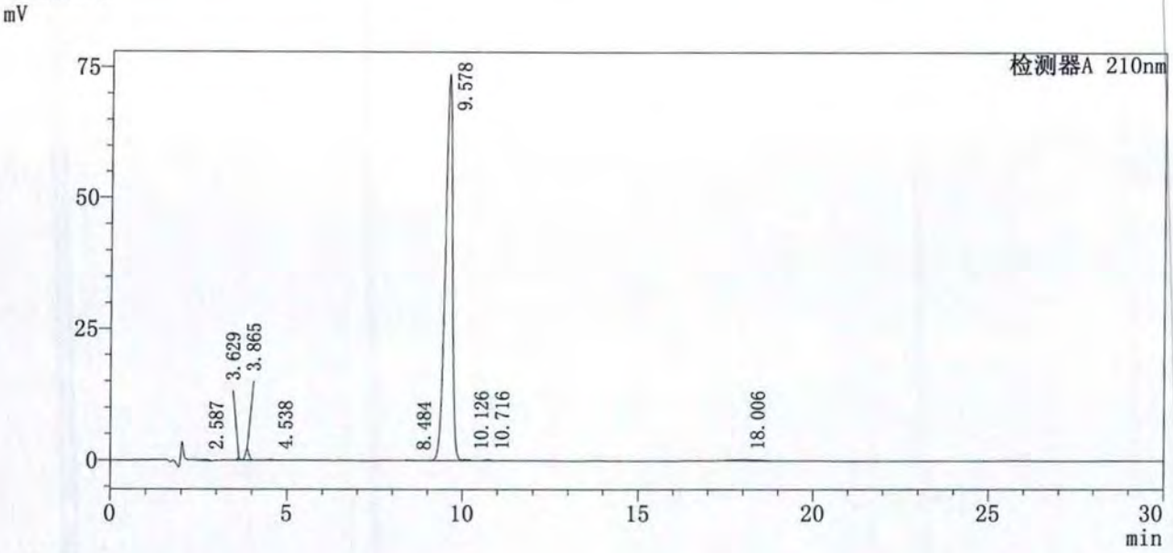
峰号	保留时间	面积	高度	浓度	浓度单位	标记	化合物名
1	2.931	2331	279	0.216		V	
2	3.156	1867	140	0.173		V	
3	8.110	1453	92	0.135			
4	9.148	1048725	75691	97.231			
5	9.686	16298	1184	1.511		V	
6	10.208	1054	76	0.098		V	
7	11.845	2022	128	0.188			
8	13.132	2761	157	0.256			
9	23.010	2077	31	0.193		M	
总计		1078589	77779				

SHIMADZU
LabSolutions 分析报告

〈样品信息〉

样品名	: 2017072803		
样品ID	:		
数据文件名	: RA定量_0706_lcb_201783_010.lcd		
方法文件名	: LC.lcm		
批处理文件名	: RA定量_0706_lcb.lcb		
样品瓶号	: 1-21	样品类型	: 标准
进样体积	: 5 uL	级别号	: 1
分析日期	: 2017/8/4 4:18:18	分析者	: System Administrator
处理日期	: 2017/8/4 9:17:52	处理者	: System Administrator

〈色谱图〉



〈峰表〉

检测器A 210nm

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1	2.587	1971	102	0.179			
2	3.629	2066	335	0.187			
3	3.865	14487	2081	1.313		V	
4	4.538	1055	123	0.096			
5	8.484	1223	78	0.111			
6	9.578	1068012	73766	96.816		M	
7	10.126	2738	206	0.248		SV M	
8	10.716	2524	170	0.229		T	
9	18.006	9055	168	0.821			
总计		1103132	77028				

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分析报告

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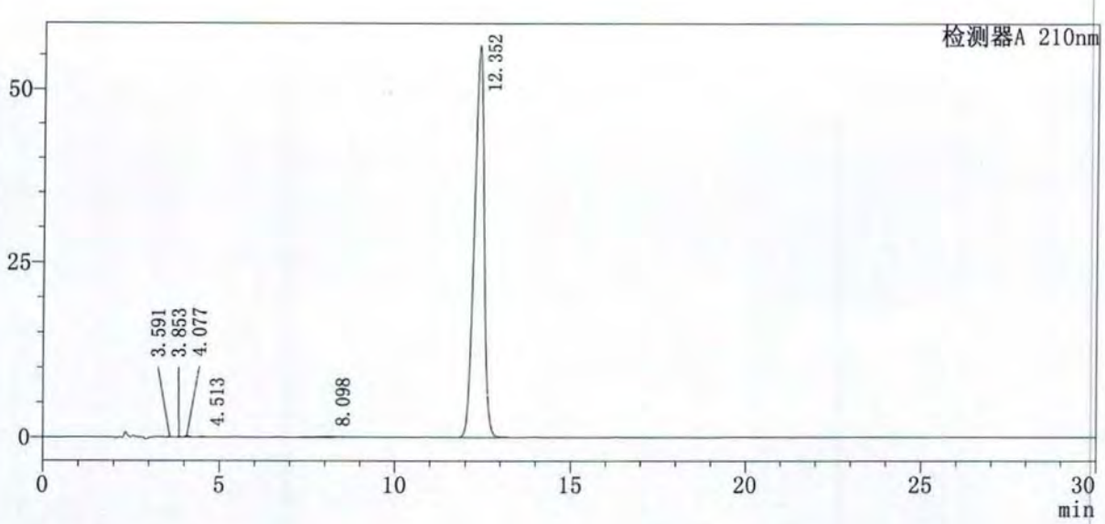

<峰表>

检测器A 210nm

峰号	保留时间	面积	高度	浓度	浓度单位	标记	化合物名
1	3.591	188	23	0.017		M	
2	3.853	298	37	0.027		V	
3	4.077	1575	178	0.144		V	
4	4.513	820	81	0.075			
5	8.098	4510	93	0.413		M	
6	12.352	1083639	56336	99.323			
总计		1091030	56748				

SHIMADZU
LabSolutions

分析报告

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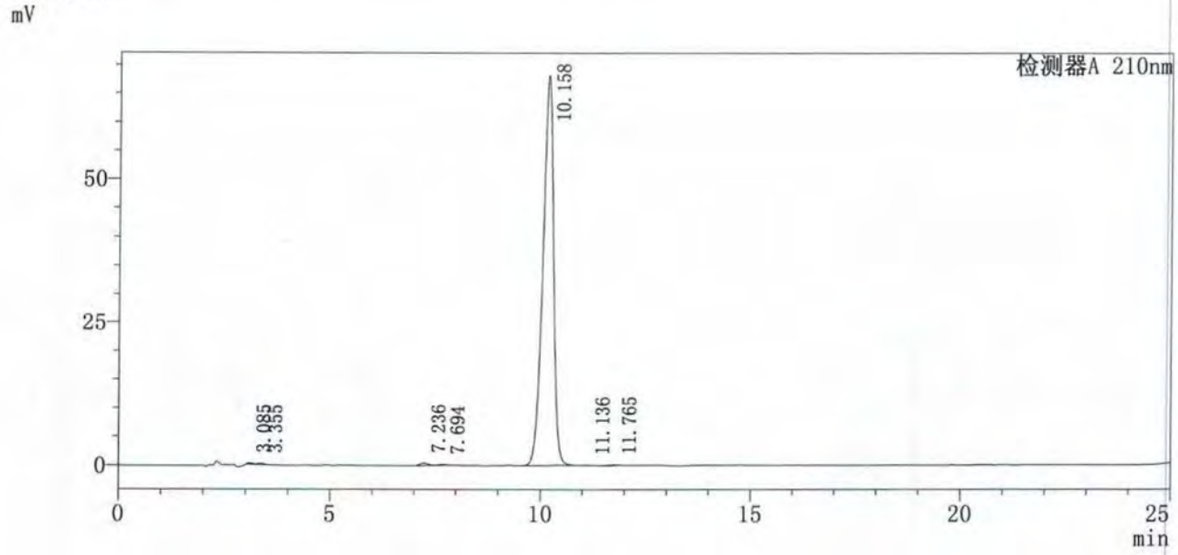

<峰表>

检测器A 210nm

峰号	保留时间	面积	高度	浓度	浓度单位	标记	化合物名
1	3.085	2156	220	0.179		M	
2	3.355	2308	221	0.191		V M	
3	7.236	5535	420	0.458			
4	7.694	1894	137	0.157		V	
5	10.158	1193678	68205	98.844		M	
6	11.136	494	37	0.041		M	
7	11.765	1575	91	0.130		M	
总计		1207639	69332				

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分析报告

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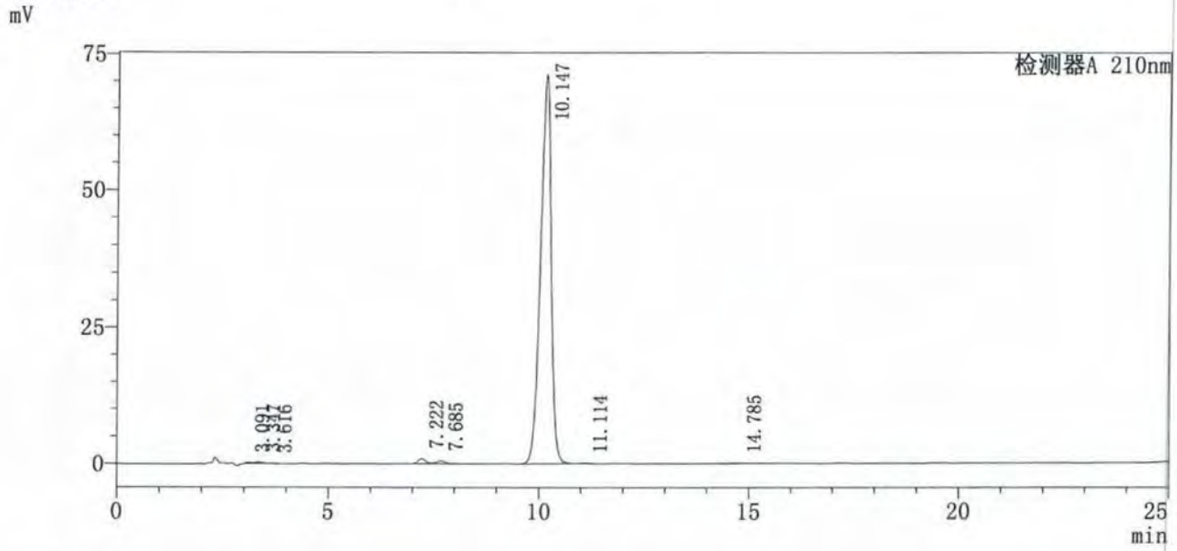

<峰表>

检测器A 210nm

峰号	保留时间	面积	高度	浓度	浓度单位	标记	化合物名
1	3.091	1416	143	0.111		M	
2	3.347	2905	238	0.229		V M	
3	3.616	653	60	0.051		V	
4	7.222	12228	933	0.963			
5	7.685	8046	539	0.633		V	
6	10.147	1241190	71202	97.713		M	
7	11.114	1413	94	0.111		M	
8	14.785	2390	78	0.188			
总计		1270241	73287				

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LabSolutions 分析报告

〈样品信息〉

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样品ID	: 024		
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方法文件名	: stevia.lcm		
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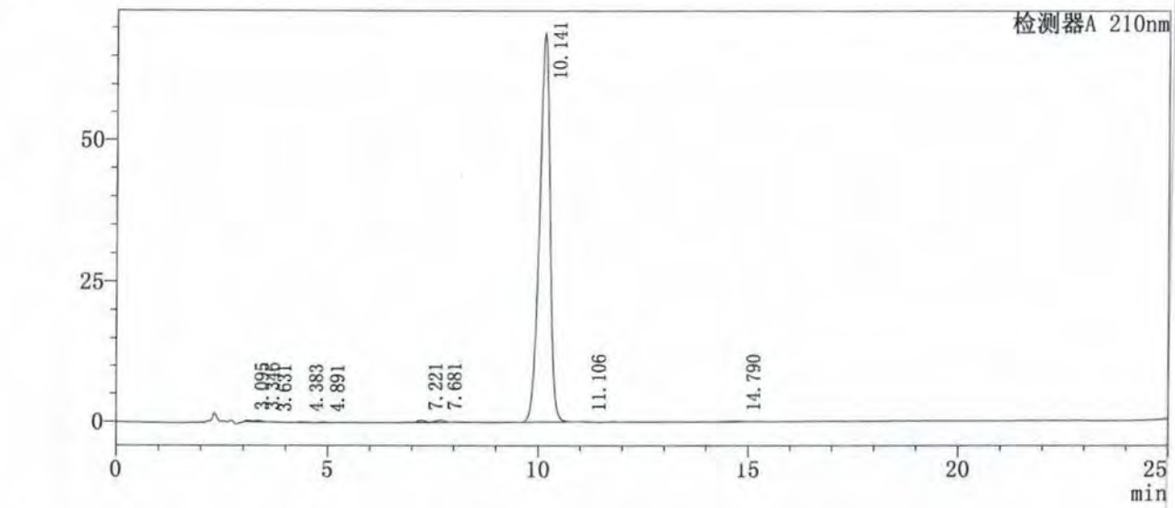

〈峰表〉

检测器A 210nm

峰号	保留时间	面积	高度	浓度	浓度单位	标记	化合物名
1	3.095	2162	227	0.178		M	
2	3.346	2407	223	0.198		V M	
3	3.631	501	33	0.041		V M	
4	4.383	1145	94	0.094			
5	4.891	827	69	0.068		V	
6	7.221	4917	373	0.404			
7	7.681	5848	385	0.480		V	
8	10.141	1196790	69175	98.299		M	
9	11.106	1054	75	0.087		M	
10	14.790	1842	64	0.151			
总计		1217493	70717				

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LabSolutions 分析报告

〈样品信息〉

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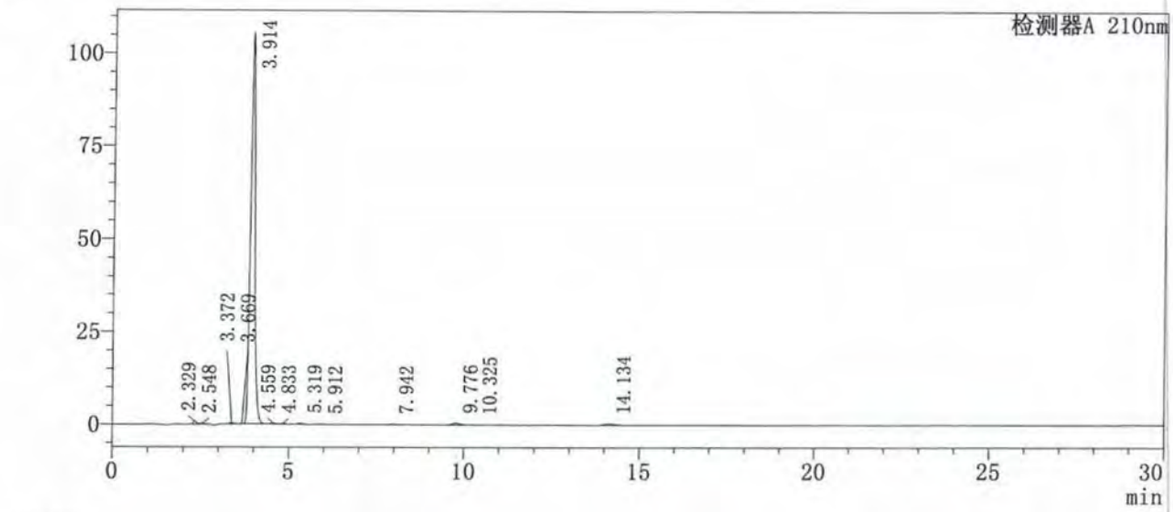

〈峰表〉

检测器A 210nm

峰号	保留时间	面积	高度	浓度	浓度单位	标记	化合物名
1	2.329	5768	890	0.600		M	
2	2.548	2298	259	0.239		V M	
3	3.372	2344	309	0.244		M	
4	3.669	183	40	0.019		M	
5	3.914	922564	105521	95.977		M	
6	4.559	2289	216	0.238		M	
7	4.833	1295	152	0.135		V	
8	5.319	3335	294	0.347			
9	5.912	886	77	0.092		V	
10	7.942	2534	155	0.264			
11	9.776	8353	477	0.869			
12	10.325	1016	59	0.106		V	
13	14.134	8364	354	0.870			
总计		961230	108803				

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分析报告

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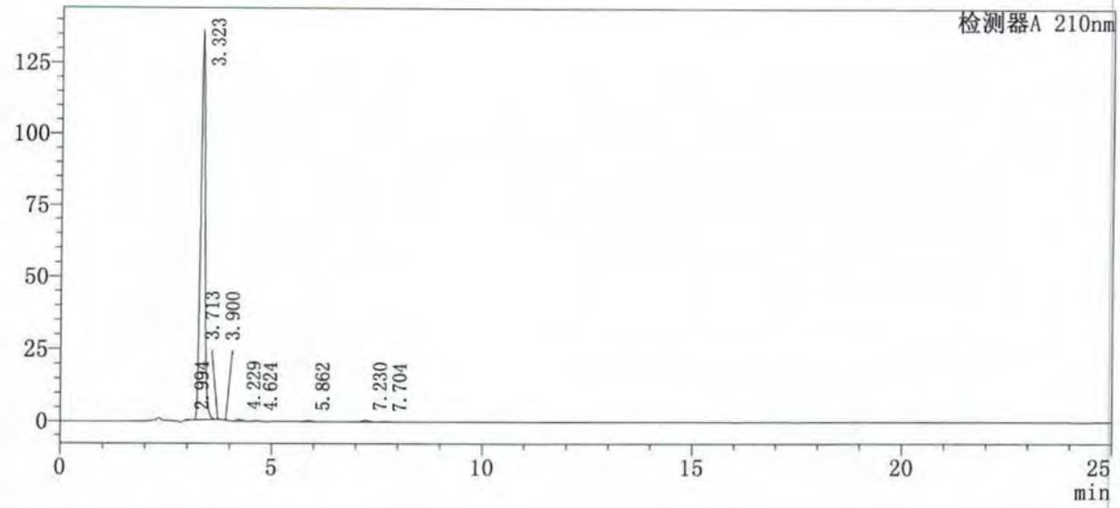

<峰表>

检测器A 210nm

峰号	保留时间	面积	高度	浓度	浓度单位	标记	化合物名
1	2.994	1492	270	0.150		M	
2	3.323	979870	135962	98.180		M	
3	3.713	198	5	0.020		M	
4	3.900	611	128	0.061		M	
5	4.229	4953	587	0.496			
6	4.624	741	131	0.074		M	
7	5.862	3652	322	0.366		V	
8	7.230	5617	447	0.563		M	
9	7.704	896	63	0.090		V M	
总计		998031	137915				

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分析报告

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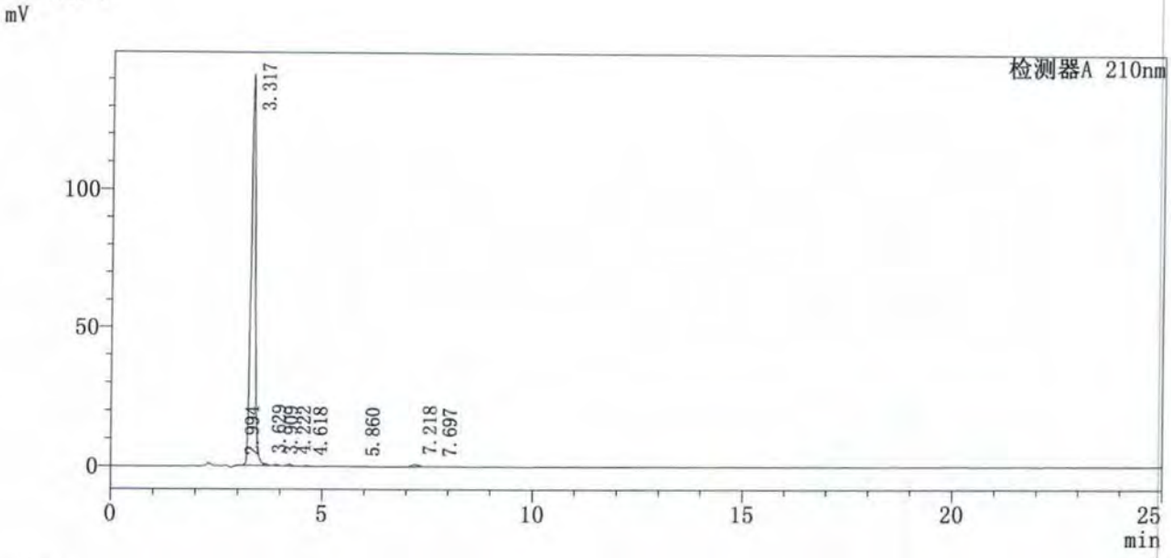

<峰表>

检测器A 210nm

峰号	保留时间	面积	高度	浓度	浓度单位	标记	化合物名
1	2.994	861	163	0.083		M	
2	3.317	1015688	141396	97.460		M	
3	3.629	3243	604	0.311		V M	
4	3.909	2680	407	0.257		M	
5	4.222	5435	633	0.521		M	
6	4.618	1811	213	0.174		M	
7	5.860	1306	108	0.125			
8	7.218	10012	774	0.961			
9	7.697	1127	66	0.108		V	
总计		1042162	144365				

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分析报告

〈样品信息〉

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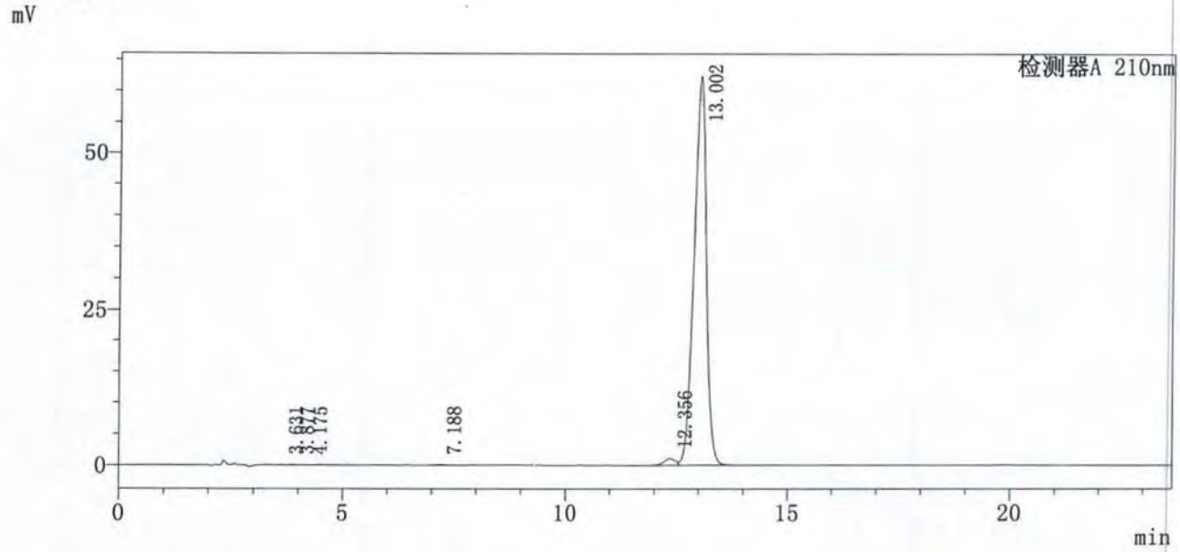

〈峰表〉

检测器A 210nm

峰号	保留时间	面积	高度	浓度	浓度单位	标记	化合物名
1	3.631	544	63	0.044		M	
2	3.877	464	57	0.037		V	
3	4.175	475	29	0.038			
4	7.188	1050	77	0.084			
5	12.356	19457	1081	1.562			
6	13.002	1223263	62447	98.234		V	
总计		1245253	63754				

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LabSolutions

分析报告

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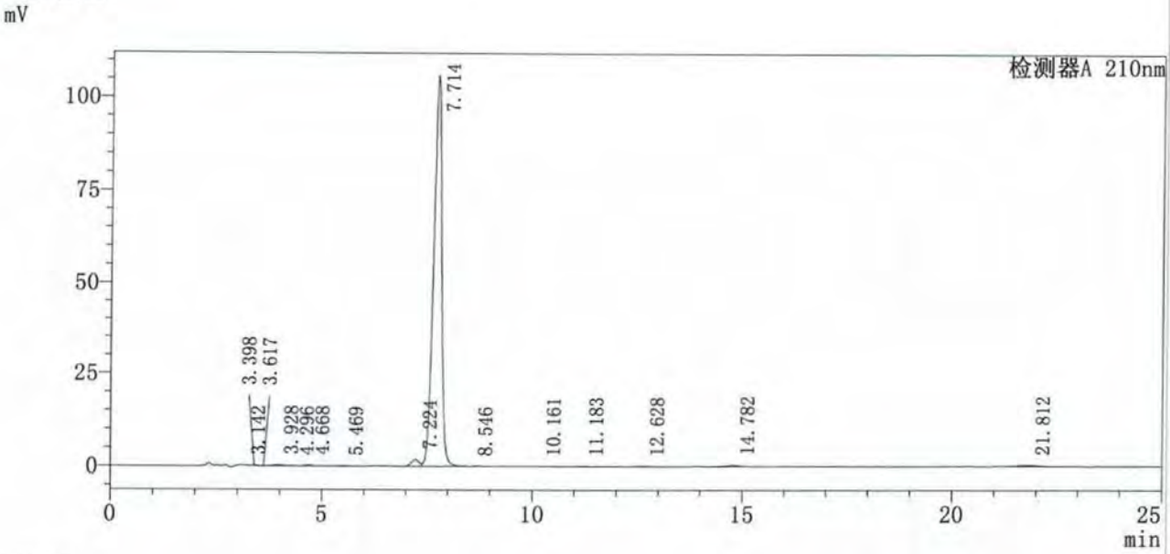

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检测器A 210nm

峰号	保留时间	面积	高度	浓度	浓度单位	标记	化合物名
1	3.142	55	26	0.004		M	
2	3.398	606	104	0.041			
3	3.617	28	8	0.002			
4	3.928	3560	275	0.242			
5	4.296	381	53	0.026		V	
6	4.668	2833	272	0.192			
7	5.469	1635	117	0.111			
8	7.224	22808	1824	1.548			
9	7.714	1419339	105922	96.338		V M	
10	8.546	0	1	0.000		S M	
11	10.161	1025	60	0.070			
12	11.183	1178	70	0.080		V	
13	12.628	1074	59	0.073			
14	14.782	7013	316	0.476			
15	21.812	11763	354	0.798			
总计		1473297	109462				

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LabSolutions

分析报告

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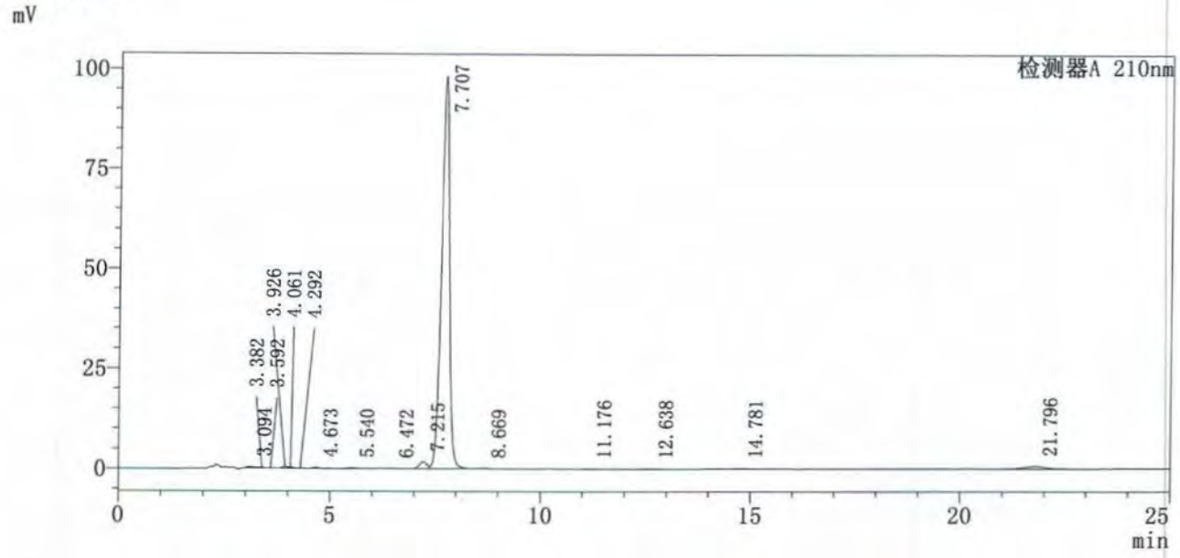

<峰表>

检测器A 210nm

峰号	保留时间	面积	高度	浓度	浓度单位	标记	化合物名
1	3.094	3386	272	0.244		M	
2	3.382	996	123	0.072		V M	
3	3.592	34	9	0.002			
4	3.926	3337	360	0.241			
5	4.061	1971	261	0.142		V	
6	4.292	568	61	0.041		V	
7	4.673	2872	249	0.207		V	
8	5.540	3093	175	0.223			
9	6.472	1184	86	0.085		V	
10	7.215	21132	1718	1.525			
11	7.707	1317930	98253	95.090		V M	
12	8.669	1180	94	0.085		M	
13	11.176	1120	71	0.081			
14	12.638	1292	73	0.093			
15	14.781	2572	131	0.186			
16	21.796	23318	674	1.682			
总计		1385985	102610				

Expert Panel Report Concerning the Generally Recognized as Safe (GRAS) Status of Steviol Glycosides for Use as General-Purpose Sweeteners in Food and Beverages

16 April 2018

INTRODUCTION

Steviana Bioscience (Suzhou) Inc. (Steviana) intends to market a range of steviol glycosides as general-purpose non-nutritive sweeteners in the United States (U.S.). An Expert Panel of independent scientists, qualified by their relevant national and international experience and scientific training to evaluate the safety of food ingredients, was convened by Steviana to conduct a critical and comprehensive evaluation of the available pertinent data and information concerning the safety and GRAS status of the proposed uses ofits steviol glycosides preparations. Steviana manufactures the following high-purity (≥95%), isolated steviol glycoside extracts: rebaudioside A ("Reb A 95"), rebaudioside C ("Reb C 95"), rebaudioside D ("Reb D 95"), stevioside ("Stevioside 95"), and a combination rebaudioside A + rebaudioside D (90%:5%; "Reb AD 95").

The Expert Panel was specifically asked to determine whether the intended uses of steviol glycosides would be Generally Recognized as Safe (GRAS), based on scientific procedures. For purposes of the Expert Panel's evaluation, "safe" or "safety" indicates that there is a reasonable certainty of no harm under the intended conditions of use of the ingredient in foods, as stated in 21 CFR §170.3(i) (U.S. FDA, 2017a).

The Expert Panel consisted of the below-signed qualified scientific experts: Joseph F. Borzelleca, Ph.D. (Virginia Commonwealth University School of Medicine); John Thomas, Ph.D. (Indiana University); and Robert Nicolosi, Ph.D. (University of Massachusetts Lowell). The Expert Panel was selected and convened in accordance with the U.S. Food and Drug Administration (FDA)'s guidance for industry on *Best Practices for Convening a GRAS Panel* (U.S., FDA 2017b). Steviana confirms that prior to convening the Expert Panel, all reasonable efforts were made to identify and select a balanced Expert Panel with expertise in appropriate scientific disciplines deemed necessary for the safety evaluation of the ingredient and efforts were placed on identifying conflicts of interest or relevant appearance issues that would potentially bias the outcome of the deliberations of the Expert Panel; no such conflicts of interest or appearance of conflicts were identified. The Expert Panel received a reasonable honorarium as compensation for the Expert Panel's time, and honoraria provided to the Expert Panel were not contingent upon the outcome of the Expert Panel deliberations.

The Expert Panel, independently and collectively, critically evaluated a supporting dossier submitted by Steviana [Documentation Supporting the Generally Recognized as Safe (GRAS) Status of Steviol Glycosides for Use as General-Purpose Sweeteners in Food and Beverages; March 2018]. This dossier is a comprehensive package of data and information, including the method of manufacture, product specifications and analytical data, stability, intended conditions of use, estimated intake of steviol glycosides based on all intended uses, and a summary of the available scientific information and data pertinent to the safety of steviol glycosides. This information was provided by Steviana together with any additional information relevant to the safety of steviol glycosides identified in a comprehensive search of the published literature through April 2018.

Following its independent and collaborative critical evaluation of the data and information, the Expert Panel convened *via* teleconference on April 16th, 2018. The Expert Panel reviewed their findings and, following discussion, unanimously concluded that the intended uses described herein of steviol glycosides, meeting appropriate food-grade specifications and manufactured consistent with current Good Manufacturing Practices (cGMP), are GRAS based on scientific procedures. A summary of the basis for the Expert Panel's conclusion is provided in the following section.

SUMMARY AND BASIS FOR GRAS

Steviol glycosides, consisting of steviol conjugated with glucose, xylose, and/or rhamnose, are natural constituents of the *Stevia rebaudiana* Bertoni (*S. rebaudiana*) plant, which is native to various South American regions and which has been used as a sweetening agent for over 1,500 years (Geuns, 2003; Ferlow, 2005). The main steviol glycosides that have been isolated and purified from the leaves of *S. rebaudiana* include stevioside, rebaudioside A, B, C, D, E, and F, dulcoside A, rubusoside, and steviolbioside, although many more have been identified (Purkayastha *et al*., 2016). The water-soluble glycosides are obtained *via* hot water extraction from the stevia leaves, followed by solvent purification. The principle constituents that give rise to the sweetening flavor of these extracts are rebaudioside A and stevioside (a closely related structural analogue). Depending on the manufacturing process, individual steviol glycosides can be isolated and purified, which provides specific sweetening profiles as needed by food manufacturers.

Steviana manufactures the following high-purity (≥95%) steviol glycoside extracts: rebaudioside A ("Reb A 95"), rebaudioside C ("Reb C 95"), rebaudioside D ("Reb D 95"), stevioside ("Stevioside 95"), and a combination rebaudioside A + rebaudioside D (90%:5%; "Reb AD 95"). These steviol glycosides are manufactured in compliance with cGMP, and all additives and processing aids used in the production of steviol glycosides are food-grade and meet Food Chemical Codex (FCC), United States Pharmacopeia (USP), or other equivalent international quality standards.

Steviana's steviol glycosides are produced either from hot water extraction of the raw stevia leaves or processing stevia extract after it has undergone an initial rebaudioside A extraction. Individual steviol glycosides are separated and concentrated *via* sequential filtration, crystallization, and column chromatographic separation techniques. The quality and purity of each steviol glycoside preparation is confirmed by high-performance liquid chromatography (HPLC). The Expert Panel reviewed analytical data obtained from 3 non-consecutive production batches of each product, which demonstrated that the manufacturing process consistently results in products that comply with the established specifications. Steviana's steviol glycosides also meet the Joint FAO/WHO Expert Committee on Food Additives (JECFA) specifications for steviol glycosides (JECFA, 2010).

Steviana intends to market steviol glycosides as general-purpose non-nutritive sweeteners for use in foods and beverages that are substitutional for uses that have previously been concluded to be GRAS (there have been approximately 41 GRAS determinations notified to the U.S. FDA for various steviol glycoside preparations between 2008 and 2018). The Expert Panel concluded that the introduction of Steviana's steviol glycoside ingredients to the U.S. marketplace would not increase dietary intakes of steviol glycosides among U.S. consumers.

The Expert Panel critically evaluated the data and information characterizing the safety of steviol glycosides.

Since 1998, the safety of steviol glycosides has been considered by several scientific bodies and regulatory agencies, including the U.S. FDA, the European Food Safety Authority (EFSA), Food Standards Australia New Zealand (FSANZ), JECFA, and Health Canada. These evaluations included a thorough examination of data on the comparative metabolism and pharmacokinetics of steviol glycosides in experimental animals and humans, acute toxicity studies, short- and long-term toxicity and carcinogenicity studies, reproductive and developmental toxicology studies, *in vitro* and *in vivo* mutagenicity/genotoxicity studies, and human studies.

It has been concluded that the safety of steviol glycosides is based on the general recognition that all glycosides are degraded to the aglycone steviol and that the safety demonstrated for one glycoside is relevant to all glycosides in general (JECFA, 2008; EFSA, 2010).

In general, pharmacokinetic studies in rats and humans have confirmed that intact steviol glycosides are not absorbed from the upper gastrointestinal tract but are hydrolyzed by colonic microflora to the aglycone steviol, which is then absorbed (JECFA, 2008). In humans, the aglycone steviol is primarily metabolized to steviol glucuronide, which is excreted in the urine. This conclusion has been further researched and reaffirmed most recently, based on *in vitro* human fecal homogenate incubation assays using rebaudiosides A, B, C, D, E, F, and M, as well as steviolbioside and dulcoside A, which showed that all steviol glycosides share the same metabolic fate (Purkayastha *et al*., 2016). The same findings have been reported in other studies for various steviol glycosides (Nikiforov *et al*., 2013; Purkayastha *et al*., 2014).

It has been concluded that steviol glycosides are not mutagenic or genotoxic based on *in vitro* and *in vivo* assays (JECFA, 2008) and this conclusion has been re-confirmed in a comprehensive review of the genotoxicity database related to steviol glycosides, conducted by Urban *et al.* (2013).

Based on several comprehensive reviews of all relevant animal and human safety data on steviol glycosides, JECFA derived an acceptable daily intake (ADI) for steviol glycosides (0 to 4 mg/kg body weight/day based on steviol equivalents) from a 2-year chronic study in rats, in which the no-observed-adverse-effect-level (NOAEL) was concluded to be 970 mg/kg body weight/day (approximately 388 mg/kg steviol equivalents/kg body weight/day) to which a 100-fold safety factor was applied (Toyoda *et al*., 1997; JECFA, 2009). JECFA also established ingredient specifications for steviol glycosides to require purity levels of greater than 95%. Based on separate reviews of the safety of steviol glycoside, FSANZ and the EFSA derived the same ADI from the same 2-year study in rats (FSANZ, 2008; EFSA, 2010).

As reviewed by JECFA (JECFA, 2009) and EFSA (EFSA, 2010) and in recent GRAS notifications (GRN 715; U.S. FDA, 2017c), steviol glycosides have been safely consumed in human studies (*i.e.*, healthy, diabetic, and hypertensive subjects) at doses of up to 1,500 mg/day (approximately 25 mg/kg body weight/day) for up to 2 years. Steviol glycosides did not affect glucose homeostasis in healthy or diabetic subjects and although some antihypertensive effects have been reported in long-term studies in mildly hypertensive subjects (Chan *et al*., 2000; Hsieh *et al*., 2003), these effects were noted at doses that are 6-fold higher than the established ADI and are not relevant to estimated intake levels of Steviana's steviol glycosides from intended uses. A meta-analysis evaluating the potential effects of steviol glycosides on cardiovascular risk factors has been published based on 9 clinical studies, which were all published before 2009 (Onakpoya and Heneghan, 2015). No significant effects on blood pressure or cardiovascular risk factors was reported.

A conservative approach for estimating steviol glycoside intake can be made based on the intake figures reported in numerous studies conducted in the U.S., Canada, Australia/New Zealand, and various countries in the European Union (EU), in which the intakes of aspartame and other high-intensity sweeteners were calculated *via* post-market surveillance data (Renwick, 2008). Based on these studies, the highest mean intake of Steviana's steviol glycosides by diabetic and non-diabetic children and adults, as steviol equivalents, was 0.51 to 1.35 mg/kg body weight/day, while intake in heavy users ranged from 1.35 to

1.98 mg/kg body weight/day. These intake estimates are expected to over-estimate potential exposure, as these estimates assume that all products consumed by an individual contain steviol glycosides and all food consumed contain steviol glycosides at the maximum intended use level. Regardless, all estimated intakes were below the ADI of 4 mg/kg body weight/day. Additional intake estimates for various populations support that intakes of steviol glycosides from intended conditions of use are generally below the ADI or not consumed at levels that would raise safety concerns (EFSA, 2014; Dewinter *et al*., 2016; Kim *et al*. 2017; Le Donne *et al*., 2017). A comprehensive review of global dietary intake estimates for sweeteners, including steviol glycosides, conducted by Martyn *et al*. (2018) also confirm these conclusions.

These estimated intake levels are comparable to the intakes of steviol glycosides that have been determined to be GRAS in previous GRAS notifications (most recently GRN 715; U.S. FDA, 2017c). It is anticipated that the intake of foods and beverages with Steviana's steviol glycosides would replace foods and beverages already supplemented with steviol glycosides and their use would thus be substitutional in nature.

A critical evaluation of the available evidence reviewed demonstrates that the intended uses as ingredients in food and beverages of Steviana's steviol glycosides, manufactured consistent with cGMP and meeting appropriate food-grade specifications, are safe and suitable and GRAS, based on scientific procedures. It is noted that Steviana's steviol glycosides are chemically identical to the steviol glycosides that have been previously determined to be GRAS for food and beverage uses and notified to the U.S. FDA without questions.

CONCLUSION

We, the members of the Expert Panel, have independently and collectively critically evaluated the data and information deemed pertinent to the safety of the intended conditions of use for Steviana's steviol glycoside preparations. We unanimously conclude that the proposed uses of steviol glycosides as general-purposes sweeteners in food and beverages, produced consistent with current Good Manufacturing Practices (cGMP) and meeting appropriate food grade specifications, are Generally Recognized as Safe (GRAS) based on scientific procedures.

It is our opinion that other qualified experts would concur with this conclusion.

[redacted]

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