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March 8, 2021

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

Attention: Dr. Susan Carlson
Re: GRAS Notice – *Glucosylated Steviol Glycosides*

Dear Dr. Carlson:

GRAS Associates, LLC, acting as the Agent for Zhucheng Haotian Pharm Co., Ltd. ("ZCHT"), is submitting for FDA review Form 3667 and the enclosed CD, free of viruses, containing a GRAS Notice for *Glucosylated Steviol Glycosides.* Along with ZCHT's determination of safety, an Expert Panel of qualified persons was assembled to assess the composite safety information of the subject substance with the intended use as a table top sweetener and as a general purpose non-nutritive sweetener for incorporation into food in general, other than infant formulas and meat and poultry products, and as a flavor modifier. The attached documentation contains the specific information that addresses the safe human food uses for the subject notified substance as discussed in the GRAS guidance document.

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

We look forward to your feedback.

Sincerely,

William J. Rowe, President
Agent for Blue California
GRAS Associates, LLC
11810 Grand Park Ave
Suite 500
North Bethesda, MD 20852
wrowe@nutrasource.ca

Enclosure: GRAS Notice for Zhucheng Haotian Pharm. Co., Ltd. – *Glucosylated Steviol Glycosides*

GRAS Notification

of

Glucosylated Steviol Glycosides

Food Usage Conditions for General Recognition of Safety

on behalf of

Zhucheng Haotian Pharm Co., Ltd.

Shandong

People's Republic of China

3/8/21

TABLE OF CONTENTS

FIGURES

TABLES

FOREWORD

Zhucheng Haotian Pharm Co., Ltd. ("ZCHT") based our Generally Recognized as Safe (GRAS) assessment of SoPure Stevia™ glucosylated steviol glycosides GSG 80 (≥80% total steviol glycosides with ≥75% glucosylated steviol glycosides and ≤20% dextrin) and GSG 95 (≥95% total steviol glycosides with ≥75% glucosylated steviol glycosides and ≤5% dextrin) preparations, primarily on the composite safety information, i.e., scientific procedures with corroboration from history of use. The safety/toxicity of steviol glycosides, history of use of steviol glycosides, and compositional details, specifications, and method of preparation of the subject ingredients were reviewed. In addition, a search of the scientific and regulatory literature was conducted through November 6, 2020 with particular attention paid to adverse reports, as well as those that supported conclusions of safety. Those references that were deemed pertinent to this review are listed in Part 7. The composite safety/toxicity studies, in concert with dietary exposure information, ultimately provide the specific scientific foundation for the GRAS conclusion.

At ZCHT's request, GRAS Associates, LLC ("GA") convened an Expert Panel to complete an independent safety evaluation of ZCHT's SoPure Stevia™ glucosylated steviol glycosides preparations. The purpose of the evaluation is to ascertain whether ZCHT's SoPure Stevia™ glucosylated steviol glycosides preparations as described in Part 3 are generally recognized as safe, i.e., GRAS, under the intended conditions of use. In addition, ZCHT has asked GA to act as Agent for the submission of this GRAS notice.

PART 1. SIGNED STATEMENTS AND CERTIFICATION

A. Claim of Exclusion from the Requirement for Premarket Approval Pursuant to 21 CFR 170 Subpart E[1]

ZCHT has concluded that our glucosylated steviol glycosides GSG 80 (≥80% total steviol glycosides with ≥75% glucosylated steviol glycosides and ≤ 20% dextrin) and GSG 95 (≥95% total steviol glycosides with ≥75% glucosylated steviol glycosides and ≤5% dextrin) preparations, referred to collectively herein as "SoPure Stevia™", and which meet the specifications described below, are GRAS in accordance with Section 201(s) of the Federal Food, Drug, and Cosmetic Act (FD&C). This determination was made in concert with an appropriately convened panel of experts who are qualified by scientific training and experience. The GRAS determination is based on scientific procedures as described in the sections that follow. The evaluation accurately reflects the intended conditions of food use for the designated glucosylated steviol glycosides preparations.

[1] See 81 FR 54960, 17 August 2016. Accessible at: https://www.gpo.gov/fdsys/pkg/FR-2016-08-17/pdf/2016-19164.pdf (Accessed 11/5/20).

Signed:

Agent for ZCHT

William J. Rowe
President
GRAS Associates, LLC
11810 Grand Park Ave.
Suite 500
North Bethesda, MD
20852

Date: March 8, 2021

B. Name and Address of Responsible Party

Zhucheng Haotian Pharm Co., Ltd.
Xinxing, Zhucheng,
Shandong Province
262218
The People's Republic of China

As the Responsible Party, ZCHT accepts responsibility for the GRAS conclusion that has been made for our SoPure Stevia™ glucosylated steviol glycosides preparations as described in the subject safety evaluation; consequently, the purified glucosylated steviol glycosides preparations having acceptable steviol glycosides compositions which meet the conditions described herein are not subject to premarket approval requirements for food ingredients.

C. Common Name and Identity of Notified Substance

The common names of the ingredients are "glucosylated steviol glycosides," "glucosyl steviol glycosides," "glucosylated steviol glycosides," "glucosylated stevia extract," "enzyme modified stevia," and "enzyme modified steviol glycosides." ZCHT also plans to market our glucosylated steviol glycosides preparations under the trade name "SoPure Stevia™."

D. Conditions of Intended Use in Food

ZCHT's SoPure Stevia™ glucosylated steviol glycosides GSG 80 (≥80% total steviol glycosides with ≥75% glucosylated steviol glycosides and ≤20% dextrin) and GSG 95 (≥95% total steviol glycosides with ≥75% glucosylated steviol glycosides and ≤5% dextrin) preparations are

intended for use as general purpose sweeteners in foods, excluding meat and poultry products and infant formulas, at levels determined by Current Good Manufacturing Practices (CGMP).

E. Basis for GRAS Conclusion

Pursuant to 21 CFR 170.30(a) and (b)[2], ZCHT's SoPure Stevia™ glucosylated steviol glycosides GSG 80 (≥80% total steviol glycosides with ≥75% glucosylated steviol glycosides and ≤20% dextrin) and GSG 95 (≥95% total steviol glycosides with ≥75% glucosylated steviol glycosides and ≤5% dextrin) preparations have been concluded to be GRAS on the basis of scientific procedures as discussed in the detailed description provided below.

ZCHT's SoPure Stevia™ glucosylated steviol glycosides preparations are not subject to premarket approval requirements of the FD&C Act based on ZCHT's conclusion that the substances are GRAS under the conditions of its intended food use.

ZCHT certifies, to the best of our knowledge, that this GRAS notice is a complete, representative, and balanced assessment that includes all relevant information, both favorable and unfavorable, available and pertinent to the evaluation of the safety and GRAS status of ZCHT's SoPure Stevia™ glucosylated steviol glycosides preparations.

F. Availability of Information

The data and information that serve as the bases for this GRAS notice will be maintained at the offices of ZCHT and will be made available during customary business hours.

ZCHT certifies that no data or information contained herein are exempt from disclosure under the Freedom of Information Act (FOIA). No non-public, safety-related data were used by the Expert Panel to reach a GRAS conclusion.

PART 2. IDENTITY, METHOD OF MANUFACTURE, SPECIFICATIONS, AND PHYSICAL OR TECHNICAL EFFECT

A. Chemical Identity of Ingredient

"Enzyme modified steviol glycosides," "glucosylated steviol glycosides," "glucosyl steviol glycosides," "glucosylated stevia extract," and "enzyme modified stevia," are the common or usual names of the non-nutritive sweetener derived from the enzymatic glycosylation of a high purity extract of *Stevia rebaudiana* Bertoni. The compositional features of ZCHT's glucosylated steviol glycosides preparations are described in more detail in this section. The preparation is also marketed as SoPure Stevia™.

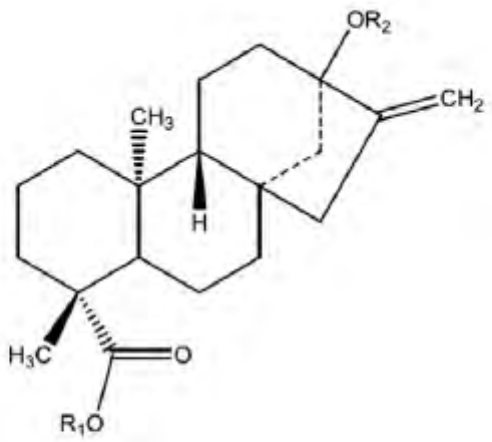
[2] https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=170.30 (Accessed 8/17/20).

The general chemistry of steviol glycosides and enzyme modified steviol glycosides has previously been reviewed in ten GRAS Notices (GRNs): GRN 337 (NOW Foods, 2010), GRN 375 (Toyo Sugar and Nippon Paper, 2011), GRN 448 (Daepyung, 2012), GRN 452 (Daepyung, 2013), GRN 607 (PureCircle, 2015), GRN 656 (GLG LIfe Tech, 2016), GRN 662 (PureCircle, 2016), GRN 821 (Haigen-BGG, 2019), GRN 858 (Qufu Shengren, 2019), and GRN 878 (Daepyung, 2019).

Representative chemical structures of some of the steviol glycosides that have been identified in *Stevia rebaudiana* Bertoni to date are presented in Figure 1.

No known toxins have been identified in stevia or stevia-derived products.

Figure 1. Chemical Structures of Various Steviol Glycosides[a]



Compound	R1	R2
Steviol	H-	H-
Stevioside	Glcß1-	Glcß(1-2)Glcß1-
Rebaudioside A	Glcß1-	Glcß(1-2)[Glcß(1-3)]Glcß1-
Rebaudioside B	H-	Glcß(1-2)[Glcß(1-3)]Glcß1-
Rebaudioside C	Glcß1-	Rhaα(1-2)[Glcβ(1-3)]Glcβ-
Rebaudioside D	Glcβ(1-2)Glcβ1-	Glcß(1-2)[Glcß(1-3)]Glcß1-
Rebaudioside E	Glcβ(1-2)Glcβ1-	Glcβ(1-2)Glcβ1-
Rebaudioside F	Glcβ1-	Xylβ(1-2)[Glcβ(1-3)]Glcβ1-
Rebaudioside M	Glcß(1-2)[Glcß(1-3)]Glcß1-	Glcß(1-2)[Glcß(1-3)]Glcß1-
Steviolbioside	H-	Glcβ(1-2)Glcβ1-
Dulcoside A	Glcβ1-	Rhaα(1-2)Glcβ1-
Rubusoside	Glcß1-	Glcß1-
Glc, Rha, and Xyl represent glucose, rhamnose, and xylose sugar moieties, respectively		

[a] From Perrier et al. (2018)

Enzyme modified steviol glycosides are produced when additional glucose moieties are bonded to the original steviol glycoside structure via α(1→4) linkages, resulting in α-glucosylated steviol glycosides. The product α-glucosylated steviol glycosides consists of a mixture of both α-D-glucosylated steviol glycosides and steviol glycosides, including rebaudioside A, rebaudioside C, dulcoside A, steviolbioside, rubusoside, and rebaudioside B. The enzyme attaches the additional glucose residues by sterio- and regio-specific 1,4-α-D-glycosidic bonds, whereas the glucose is attached by β-glycosidic bonds in naturally occurring steviol glycosides. The primary constituents of enzymatically modified stevia have been identified (Koyama et al., 2003a) and are described in Table 1. The chemical structures are shown in Figure 2.

Table 1. Components Expected to be Present in Glucosylated Steviol Glycosides[a]

Compound	Molecular Weight	Empirical Formula	Level of Enzyme Glycosylation[b]
Steviolbioside	642	$C_{32}H_{50}O_{13}$	--
Dulcoside A	788	$C_{38}H_{60}O_{17}$	--
Stevioside	804	$C_{38}H_{60}O_{18}$	--
Rebaudioside C	950	$C_{44}H_{70}O_{22}$	--
Rebaudioside A	966	$C_{44}H_{70}O_{23}$	--
Monoglucosyl rebaudioside B	966	$C_{44}H_{70}O_{23}$	+1
Monoglucosyl stevioside	966	$C_{44}H_{70}O_{23}$	+1
Monoglucosyl rebaudioside C	1112	$C_{50}H_{80}O_{27}$	+1
Monoglucosyl rebaudioside A	1128	$C_{50}H_{80}O_{28}$	+1
Diglucosyl rebaudioside B	1128	$C_{50}H_{80}O_{28}$	+2
Diglucosylstevioside	1128	$C_{50}H_{80}O_{28}$	+2
Diglucosyl rebaudioside C	1274	$C_{56}H_{90}O_{32}$	+2
Diglucosyl rebaudioside A	1290	$C_{56}H_{90}O_{33}$	+2
Triglucosyl rebaudioside B	1290	$C_{56}H_{90}O_{33}$	+3
Triglucosyl rebaudioside A	1452	$C_{62}H_{100}O_{38}$	+3

[a] Data from Koyama et al. (2003a)
[b] The level of enzymatic glycosylation indicates the number of glucose units that have been added via enzyme modification.

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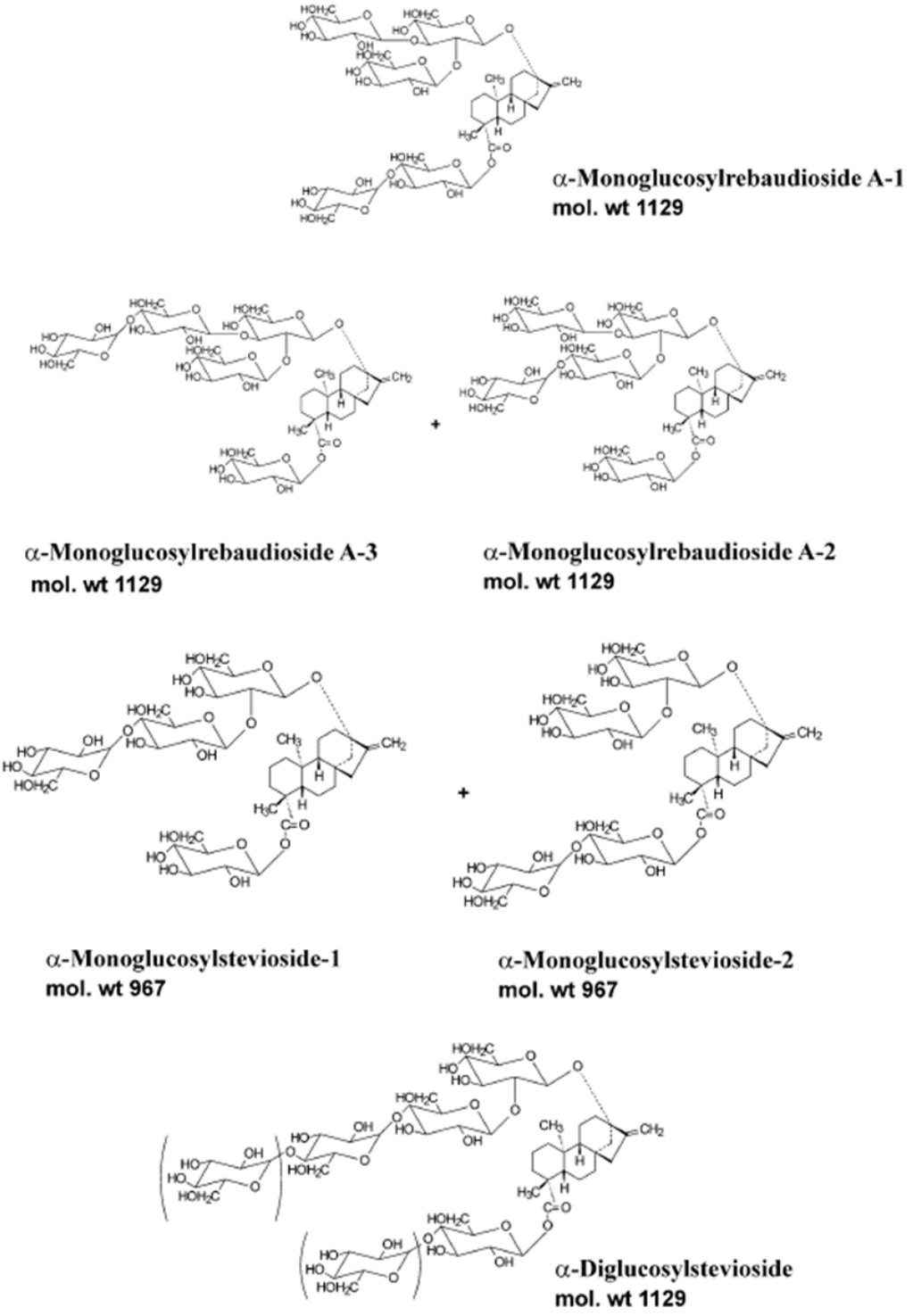
Figure 2. Chemical Structures of Various Glucosylated Steviol Glycosides[a]



[a] From Koyama et al. (2003a)

B. Manufacturing Processes

ZCHT's SoPure Stevia™ glucosylated steviol glycosides preparations are manufactured via an enzymatic reaction with *Stevia rebaudiana* Bertoni extract [>95% total steviol glycosides, which meets Joint FAO/WHO Expert Committee on Food Additives (JECFA) specifications] using cyclomaltodextrin glucanotransferase (CGTase). The resulting preparation is a blend of enzyme modified steviol glycosides and residual dextrin: SoPure Stevia™ GSG 80 (≥80% total steviol glycosides comprised of ≥75% glycosylated steviol glycosides, with ≤20% dextrin). The material is further purified to remove dextrin to obtain SoPure Stevia™ GSG 95 (≥95% total steviol glycosides comprised of ≥75% glycosylated steviol glycosides, with ≤5% dextrin).

1. Steviol Glycosides Raw Material

For the manufacturing of the starting steviol glycosides, ZCHT employs a fairly typical aqueous extraction or ethanolic extraction process that are commonly used in the industry for the production of stevia extracts. In short, dried *Stevia rebaudiana* Bertoni leaves are soaked in cold drinking water or ethanol, the extract is flocculated with calcium oxide and ferrous sulfate, and the steviol glycosides are purified through filtration, adsorption and elution, and decolorization processes. The resulting extract is concentrated using membranes and evaporation to obtain an extract with ≥95% steviol glycosides, of which ≥50% is rebaudioside A, as described in the flow chart in Figure 3. All raw materials and processing aids ZCHT uses to manufacture the raw material steviol glycosides extract are food grade.

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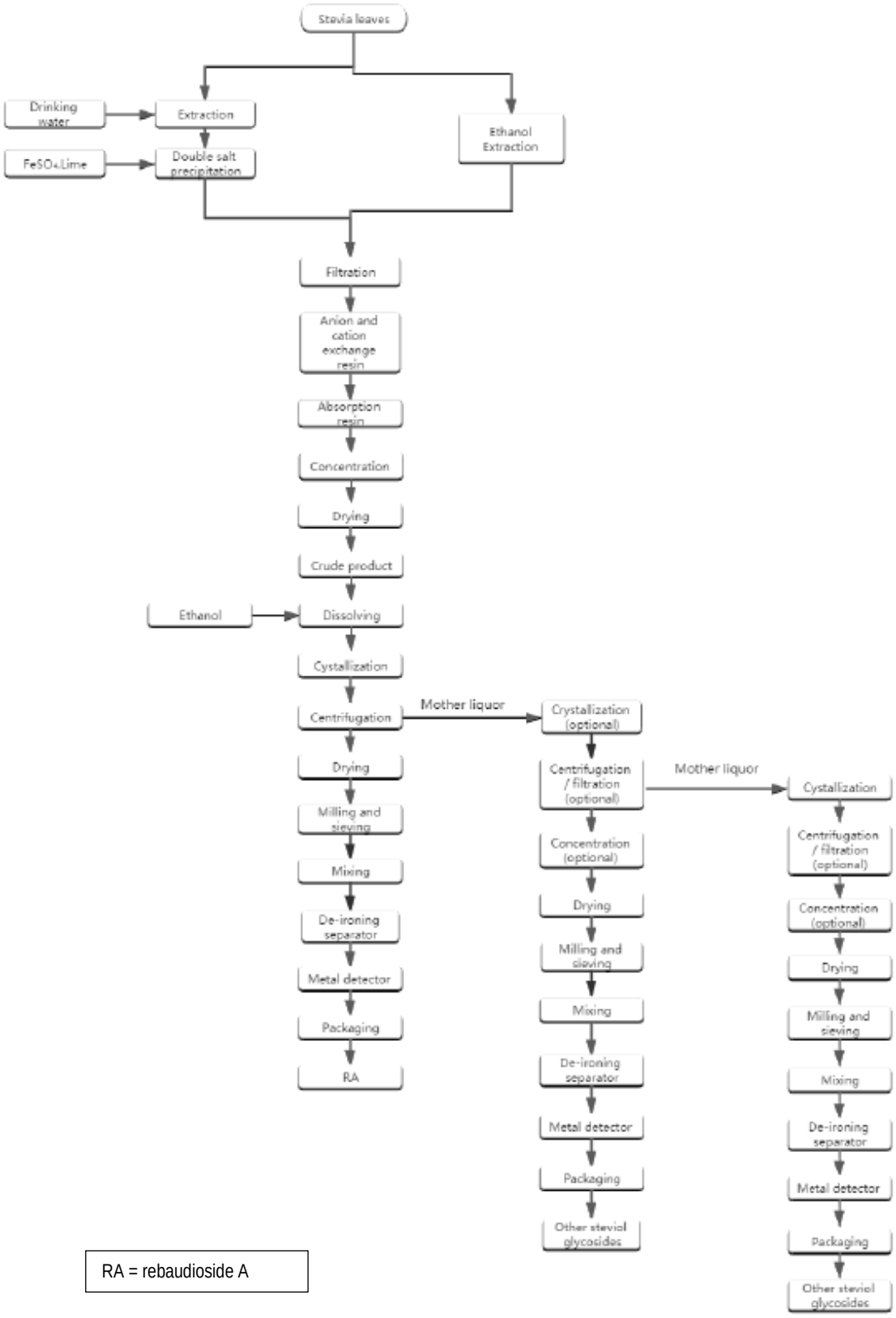
Figure 3. Flow Chart of Manufacturing Process for ZCHT's Raw Material High Purity Steviol Glycosides Preparations



RA = rebaudioside A

2. ZCHT's SoPure Stevia™ Glucosylated Steviol Glycosides

ZCHT uses the purified stevia extract product described in Part 2.B.1, dextrin, and CGTase to manufacture SoPure Stevia™ enzyme modified steviol glycosides. The purified stevia extract raw material and dextrin are solubilized in water and CGTase is added. The reaction proceeds at 70ºC for 48 hours, with adjustments to the time and temperature, as needed. The enzyme is deactivated at 100ºC for 1 hour and the denatured enzyme protein is removed through filtration. The resulting solution is evaporated and dried to produce SoPure Stevia™ GSG 80, containing a mixture of glucosylated steviol glycosides, unreacted steviol glycosides, and unreacted dextrin. Alternatively, the resulting solution can be passed through a resin column to remove unreacted dextrin to obtain SoPure Stevia™ GSG 95. The solutions are then evaporated and the resulting product is dried, milled, sieved, blended, and subjected to magnets and metal detection. The finished products are then packaged to achieve the final SoPure Stevia™ glucosylated steviol glycosides preparations.

The enzyme used to glycosylate the purified stevia extract is Toruzyme 3.0L, which is a CGTase enzyme produced by *Bacillus licheniformis*.[3] The maltodextrin (a specific type of dextrin) raw material is derived from corn. Alternative glucose donors can be either β-cyclodextrin or dextrin derived from cassava. The resin complies with 21 CFR § 173.65[4] and 21 CFR § 173.25[5] specifications. Supporting documentation for the raw materials and processing aids are provided in Appendix 1. The manufacturing process for SoPure Stevia™ glucosylated steviol glycosides preparations is summarized in the flow chart provided in Figure 4.

ZCHT's SoPure Stevia™ glucosylated steviol glycosides preparations are prepared in accordance with Current Good Manufacturing Practices (CGMP) in an FDA-registered facility (registration number 16893048990).

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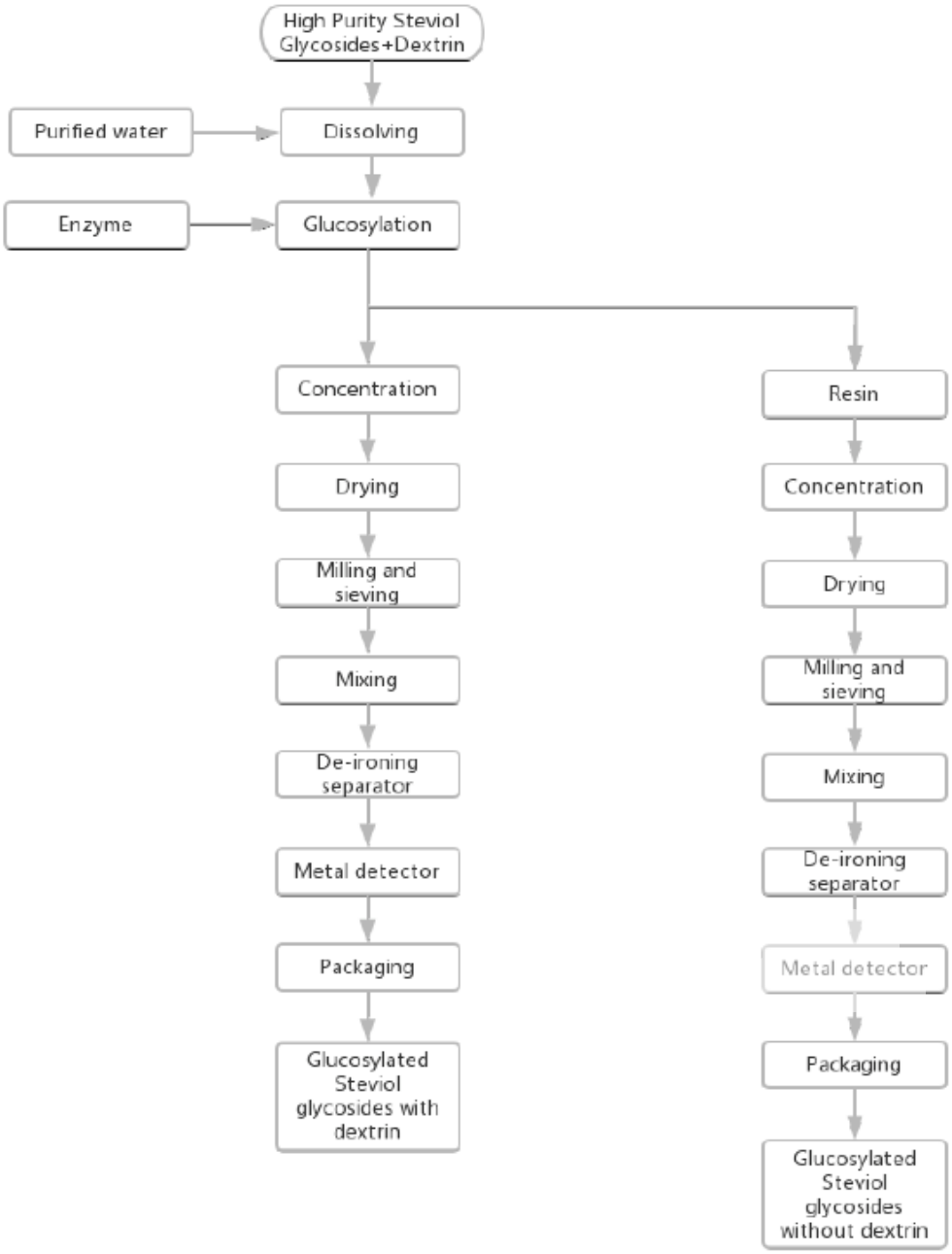
[3] Toruzyme 3.0L, manufactured by Novozymes, is a cyclomaltodextrin glucanotransferase produced by submerged fermentation of a selected strain of *Bacillus licheniformis*. It is a food grade product, complies with JECFA and FCC recommended specifications for food grade enzymes, and is GRAS as defined in 21 CFR 170.30(a).
[4] https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=173.65
[5] https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=173.25

Figure 4. Flow Chart of Manufacturing Process for ZCHT's SoPure Stevia™ Glucosylated Steviol Glycosides Preparations



C. Product Specifications

1. JECFA Specifications for Steviol Glycosides

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

In the most recent JECFA monograph, published in 2017 (FAO, 2017), steviol glycosides specifications were modified to include a minimum requirement of not less than 95% total steviol glycosides, on a dry basis, "determined as the sum of all compounds containing a steviol backbone conjugated to any number, combination or orientation of saccharides (glucose, rhamnose, fructose, deoxyglucose xylose, galactose, arabinose and xylose) occurring in the leaves of *Stevia rebaudiana* Bertoni."

JECFA's 2017 monograph describes steviol glycosides as white-to-yellow powders that are odorless or have a slight characteristic odor and exhibit a sweetness that is 200 - 300 times greater than that of sucrose. The ingredient must consist of a minimum of 95% total steviol glycosides, as defined above. The steviol glycosides are freely soluble in a 50:50 mixture of ethanol and water, and the 1 in 100 solutions have pH values between 4.5 and 7.0. The product should not have more than 1% ash, with no more than a 6% loss on drying at 105°C after 2 hours. Any residual methanol levels should not exceed 200 mg per kg and ethanol residues should not exceed 5,000 mg per kg. Arsenic and lead levels should not exceed 1 mg per kg. Microbiological criteria have also been established, with specifications of no more than 1,000 colony forming units (cfu) per g total plate count, not more than 200 cfu per g yeasts and molds, and *E. coli* and *Salmonella* negative in 1 g and 25 g, respectively.

ZCHT has adopted specifications for our purified steviol glycosides extract starting material, which are compared to the current JECFA specifications in Table 2. The typical glycosides content of production batches is provided in Table 3.

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Table 2. Specifications for Steviol Glycosides Starting Material

Physical and Chemical Parameters	JECFA[a] Specifications Steviol Glycosides	ZCHT's Specifications for Steviol Glycosides Starting Material
Appearance Form	Powder	Powder
Appearance Color	White to light yellow	White to off-white
Solubility	Freely soluble in 50:50 water: ethanol	NS
Assay	Not less than 95% total steviol glycosides[b]	≥ 95.0% (on dry basis)
Residual Ethanol	NMT 5,000 mg/kg	≤5,000 ppm
Residual Methanol	NMT 200 mg/kg	≤200 ppm
Loss on Drying	NMT 6.0%	≤6.0%
pH, 1% Solution	4.5 - 7.0	4.5 - 7.0
Total Ash	NMT 1%	NMT 1.0%
Arsenic	NMT 1 mg/kg	NMT 1 ppm
Lead	NMT 1 mg/kg	NMT 0.5 ppm
Cadmium	NS	NMT 1.0 ppm
Mercury	NS	NMT 0.1 ppm
Total Plate Count	NMT 1,000 cfu/g	NMT 1,000 cfu/g
Yeast & Mold	NMT 200 cfu/g	NMT 100 cfu/g
Salmonella	Negative in 25 g	Negative in 25 g
Escherichia coli	Negative in 1 g	Negative in 1 g

cfu – colony forming unit; g – gram; kg – kilogram; mg – milligram; NMT – not more than; NS – not specified; ppm – parts per million

[a] Prepared at 84th JECFA (2017).

[b] Total steviol glycosides as the sum of all compounds containing a steviol backbone conjugated to any number, combination, or orientation of saccharides (glucose, rhamnose, fructose, deoxyglucose xylose, galactose, arabinose, and xylose) occurring in the leaves of *Stevia rebaudiana* Bertoni

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Table 3. Typical Levels of Steviol Glycosides in Unmodified Stevia Extract and SoPure Stevia™ Glucosylated Steviol Glycosides Preparations

Component	Unmodified Stevia Extract (%)	SoPure Stevia™ GSG 80 (%)	SoPure Stevia™ GSG 95 (%)
Stevioside	36.4	5.9	7.3
Rebaudioside C	6.5		
Rebaudioside F	1.1		
Rebaudioside A	51.8		
Other steviol glycosides	1.1	0.7	0.7
n-Glucosyl Stevioside (n = 1-6)	--	19.1	22.1
n-Glucosyl Rebaudioside A (n = 1-9)	--	48.7	51.1
Other glycosylated steviol glycosides	--	19.1	16.2

ZCHT notes that individual steviol glycosides and steviol glycosides blends have varying levels of solubility in water and water:ethanol solutions. It has been previously reported that pure steviol glycosides display no or low aqueous solubility at high concentrations (Upreti et al., 2011). A study by Celaya et al. (2016) found that rebaudioside A is poorly soluble in ethanol and water and stevioside is poorly soluble in water, but that the presence of both steviol glycosides together results in higher solubilities for both. While JECFA's most recent steviol glycosides monograph[6] specifies that steviol glycosides are freely soluble in 50:50 water: ethanol solution, this is impractical for high purity preparations of a single steviol glycoside or certain steviol glycosides blends, which exhibit lower solubility in water: ethanol solutions. Based on the solubility definitions provided in Table 4, ZCHT's steviol glycosides starting material preparation ranges from freely soluble to slightly soluble.

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6 Available at: http://www.fao.org/3/BU297en/bu297en.pdf (Accessed on March 8, 2021)

Table 4. Solubility Definitions[a]

Description	Approximate Volume (mL) of Solvent Needed to Dissolve 1 g of Solute
Very soluble	Less than 1
Freely soluble	1 to 10
Soluble	10 to 30
Sparingly soluble	30 to 100
Slightly soluble	100 to 1,000
Very slightly soluble	1,000 to 10,000
Practically insoluble	Greater than 10,000

[a] Adapted from Sigma Aldrich (2019)
g – gram; mL – milliliter

2. Specifications for ZCHT's SoPure Stevia™ Glucosylated Steviol Glycosides Preparations and Supporting Methods

ZCHT has adopted product specifications for our SoPure Stevia™ glucosylated steviol glycosides preparations based upon current JECFA recommendations, while also complying with relevant Food Chemicals Codex (FCC) specifications for steviol glycosides as a consumable human food substance.[7] The compositions of five non-consecutive lots of ZCHT's SoPure Stevia™ GSG 80 (≥80% total steviol glycosides with ≥75% glucosylated steviol glycosides and ≤20% dextrin) and SoPure Stevia™ GSG 95 (≥95% total steviol glycosides with ≥75% glucosylated steviol glycosides and ≤5% dextrin) preparations are compared with the JECFA and FCC specifications in Table 5 and Table 6, respectively.

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[7] Available at: https://www.foodchemicalscodex.org/ (Accessed March 8, 2021)

Table 5. Specifications for ZCHT's SoPure Stevia™ GSG 80 Glucosylated Steviol Glycosides Preparation Compared with JECFA and FCC Specifications of Steviol Glycosides

Physical & Chemical Parameters	JECFA[a] Specifications Steviol Glycosides	FCC[b] Specifications Steviol Glycosides	ZCHT's Minimum Specifications for SoPure Stevia™ GSG 80 Glucosylated Steviol Glycosides	Results of SoPure Stevia™ GSG 80 Glucosylated Steviol Glycosides					
				20190402E3	20191001E3	20191101E3	20191104E3	20191201E3	Method of Analysis
Appearance Form	Powder	Powder, flakes, or granules	Powder	Pass	Pass	Pass	Pass	Pass	Organoleptic
Appearance Color	White to light yellow	White to light yellow	White to light yellow	Pass	Pass	Pass	Pass	Pass	Organoleptic
Solubility	Freely soluble in water: ethanol (50:50)	Freely soluble in water: ethanol (50:50)	Freely soluble in water; slightly soluble in ethanol	Pass	Pass	Pass	Pass	Pass	Organoleptic
Purity (HPLC Area)	≥ 95% Steviol Glycosides	≥ 95% Steviol Glycosides	≥ 80% Total Steviol Glycosides ≥ 75% Glucosyl Steviol Glycosides	93.8% 81.3%	94.2% 81.1%	93.7% 79.5%	93.5% 79.9%	93.3% 79.2%	NHFPC Method
Dextrin	NA	NA	≤20%	6.6%	6.1%	6.4%	6.6%	6.8%	NHFPC Method
Optical Rotation Degree	NA	NA	+65° ~ +75°	+74.8°	+73.3°	+73.8°	+71.8°	+73.1°	GB/T 14454.5
Residual Ethanol	NMT 5,000 mg/kg	NMT 0.50%	≤5,000 ppm	26 ppm	30 ppm	35 ppm	28 ppm	32 ppm	JECFA 2017
Residual Methanol	NMT 200 mg/kg	NMT 0.020%	≤200 ppm	<50 ppm	<50 ppm	<50 ppm	<50 ppm	<50 ppm	JECFA 2017
Loss on Drying	NMT 6.0%	NMT 6.0%	≤5.0%	4.3%	4.8%	4.0%	4.1%	3.7%	GB 5009.3
pH	4.5 - 7.0 (1% solution)	4.5 - 7.0 (1% solution)	4.5-7.0 (5% solution)	5.4	5.4	5.2	5.3	5.8	GB/T 9724
Relative Density	NS	NS	0.2~0.6	0.3	0.4	0.3	0.3	0.4	GB/T 11540
Total Ash	NMT 1%	NMT 1%	≤1.0%	0.05%	0.07%	0.06%	0.06%	0.07%	GB 5009.4
Arsenic	NMT 1 mg/kg	NMT 1 mg/kg	≤1.0 ppm	ND	ND	ND	ND	ND	AAS ChP2015 Part 4 (2321)
Lead	NMT 1 mg/kg	NMT 1 mg/kg	≤0.5 ppm	ND	ND	ND	ND	ND	AAS ChP2015 Part 4 (2321)
Cadmium	NS	NS	≤1.0 ppm	ND	ND	ND	ND	ND	AAS ChP2015 Part 4 (2321)

Physical & Chemical Parameters	JECFA[a] Specifications Steviol Glycosides	FCC[b] Specifications Steviol Glycosides	ZCHT's Minimum Specifications for SoPure Stevia™ GSG 80 Glucosylated Steviol Glycosides	Results of SoPure Stevia™ GSG 80 Glucosylated Steviol Glycosides					
				20190402E3	20191001E3	20191101E3	20191104E3	20191201E3	Method of Analysis
Mercury	NS	NS	≤0.1 ppm	ND	ND	ND	ND	ND	AAS ChP2015 Part 4 (2321)
Microbiological Parameters									
Total Plate Count (cfu/g)[c]	NMT 1,000	NS	$\leq 10^3$	10	10	<10	30	<10	ChP 2015 Part 4 (1105)
Yeast & Mold (cfu/g)	NMT 200	NS	$\leq 10^2$	30	20	<10	<10	<10	ChP 2015 Part 4 (1105)
E. coli	Negative in 1 g	NS	Negative/g	Negative	Negative	Negative	Negative	Negative	ChP 2015 Part 4 (1105)
Salmonella spp.	Negative in 25 g	NS	Negative/25 g	Negative	Negative	Negative	Negative	Negative	ChP 2015 Part 4 (1105)

[a] Prepared at 84th JECFA (2017)

[b] Steviol Glycosides monograph. Food Chemicals Codex (12th Ed.) (FCC, 2020)

[c] Total Plate Count and Total Aerobic Bacteria are synonyms.

AAS – atomic absorption spectrometry; cfu – colony forming units; ChP – Chinese Pharmacopeia; FCC – Food Chemicals Codex; g – gram; GB – Guobiao standard (Chinese national standard); GB/T – Guobiao standard/recommended (Chinese national standard/recommended); HPLC – high-performance liquid chromatography; JECFA – Joint FAO/WHO Expert Committee on Food Additives; kg – kilogram; mg – milligram; NA – not applicable; ND – not detected; NHFPC – National Health and Family Planning Commission (P.R. China); NMT – not more than; NS – not specified; ppm – parts per million

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Table 6. Specifications for ZCHT's SoPure Stevia™ GSG 95 Glucosylated Steviol Glycosides Preparation Compared with JECFA and FCC Specifications of Steviol Glycosides

Physical & Chemical Parameters	JECFA[a] Specifications Steviol Glycosides	FCC[b] Specifications Steviol Glycosides	ZCHT's Minimum Specifications for SoPure Stevia™ GSG 95 Glucosylated Steviol Glycosides	Results of SoPure Stevia™ GSG 95 Glucosylated Steviol Glycosides					
				G1808001	G1812001	G1907001	G1909001	G2007001	Method of Analysis
Appearance Form	Powder	Powder, flakes, or granules	Powder	Pass	Pass	Pass	Pass	Pass	Organoleptic
Appearance Color	White to light yellow	White to light yellow	White to light yellow	Pass	Pass	Pass	Pass	Pass	Organoleptic
Solubility	Freely soluble in water: ethanol (50:50)	Freely soluble in water: ethanol (50:50)	Freely soluble in water; slightly soluble in ethanol	Pass	Pass	Pass	Pass	Pass	Organoleptic
Purity (HPLC Area)	≥ 95% Steviol Glycosides	≥ 95% Steviol Glycosides	≥ 95% Total Steviol Glycosides ≥ 75% Glucosyl Steviol Glycosides	97.8% 79.6%	97.6% 79.0%	97.0% 78.8%	97.2% 79.2%	97.6% 78.8%	NHFPC Method
Dextrin	NA	NA	≤5.0%	2.2%	2.4%	3.0%	2.8%	2.4%	NHFPC Method
Optical Rotation Degree	NA	NA	+65° ~ +75°	+75.0°	+73.5°	+74.5°	+74.0°	+73.9°	GB/T 14454.5
Residual Ethanol	NMT 5,000 mg/kg	NMT 0.50%	≤5,000 ppm	527 ppm	488 ppm	537 ppm	490 ppm	570 ppm	JECFA 2017
Residual Methanol	NMT 200 mg/kg	NMT 0.020%	≤200 ppm	<50 ppm	<50 ppm	<50 ppm	<50 ppm	<50 ppm	JECFA 2017
Loss on Drying	NMT 6.0%	NMT 6.0%	≤5.0%	4.4%	4.2%	4.4%	4.3%	4.3%	GB 5009.3
pH	4.5 - 7.0 (1% solution)	4.5 - 7.0 (1% solution)	4.5-7.0 (5% solution)	5.5	5.5	5.1	5.3	5.3	GB/T 9724
Relative Density	NS	NS	0.2~0.6	0.4	0.4	0.4	0.4	0.3	GB/T 11540
Total Ash	NMT 1%	NMT 1%	≤1.0%	0.06%	0.07%	0.07%	0.07%	0.06%	GB 5009.4
Arsenic	NMT 1 mg/kg	NMT 1 mg/kg	≤1.0 ppm	ND	ND	ND	ND	ND	AAS ChP2015 Part 4 (2321)
Lead	NMT 1 mg/kg	NMT 1 mg/kg	≤0.5 ppm	ND	ND	ND	ND	ND	AAS ChP2015 Part 4 (2321)

Physical & Chemical Parameters	JECFA[a] Specifications Steviol Glycosides	FCC[b] Specifications Steviol Glycosides	ZCHT's Minimum Specifications for SoPure Stevia™ GSG 95 Glucosylated Steviol Glycosides	Results of SoPure Stevia™ GSG 95 Glucosylated Steviol Glycosides					
				G1808001	G1812001	G1907001	G1909001	G2007001	Method of Analysis
Cadmium	NS	NS	≤1.0 ppm	ND	ND	ND	ND	ND	AAS ChP2015 Part 4 (2321)
Mercury	NS	NS	≤0.1 ppm	ND	ND	ND	ND	ND	AAS ChP2015 Part 4 (2321)
Microbiological Parameters									
Total Plate Count (cfu/g)[c]	NMT 1,000	NS	$\leq 10^3$	<10	10	10	20	<10	ChP 2015 Part 4 (1105)
Yeast & Mold (cfu/g)	NMT 200	NS	$\leq 10^2$	<10	20	<10	<10	<10	ChP 2015 Part 4 (1105)
E. coli	Negative in 1 g	NS	Negative/g	Negative	Negative	Negative	Negative	Negative	ChP 2015 Part 4 (1105)
Salmonella spp.	Negative in 25 g	NS	Negative/25 g	Negative	Negative	Negative	Negative	Negative	ChP 2015 Part 4 (1105)

[a] Prepared at 84th JECFA (2017)

[b] Steviol Glycosides monograph. Food Chemicals Codex (12th Ed.) (FCC, 2020)

[c] Total Plate Count and Total Aerobic Bacteria are synonyms.

AAS – atomic absorption spectrometry; cfu – colony forming units; ChP – Chinese Pharmacopeia; FCC – Food Chemicals Codex; g – gram; GB – Guobiao standard (Chinese national standard); GB/T – Guobiao standard/recommended (Chinese national standard/recommended); HPLC – high-performance liquid chromatography; JECFA – Joint FAO/WHO Expert Committee on Food Additives; kg – kilogram; mg – milligram; NA – not applicable; ND – not detected; NHFPC – National Health and Family Planning Commission (P.R. China); NMT – not more than; NS – not specified; ppm – parts per million

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ZCHT analyzes our SoPure Stevia™ high purity steviol glycosides preparations using the National Health and Family Planning Commission (The People's Republic of China) monograph method, provided in Appendix 2. The certificates of analysis for SoPure Stevia™ GSG 80 and SoPure Stevia™ GSG 95 are presented in Appendix 3 and Appendix 4, respectively. The representative chromatograms for five representative lots of SoPure Stevia™ GSG 80 and SoPure Stevia™ GSG 95 are presented Appendix 5 and Appendix 6, respectively. Test reports for the analysis of pesticides residues in a representative lot of stevia extract raw material are provided in Appendix 7. The collection of these reports demonstrates that ZCHT's SoPure Stevia™ glucosylated steviol glycosides preparations are well characterized and meet the established purity criteria.

D. Physical or Technical Effect

ZCHT conducted sweetness equivalence evaluations for SoPure Stevia™ glucosylated steviol glycosides preparations. Taste panels determined that SoPure Stevia™ glucosylated steviol glycosides preparations are 100 - 200 times sweeter than sucrose, depending on the use level and application (Appendix 8).

In addition, ZCHT evaluated SoPure Stevia™ using the Flavor and Extract Manufacturers Association (FEMA) test method for Sensory Testing for Flavorings with Modifying properties.[8] Using the recommended level of 125 ppm for FEMA 4728, ZCHT determined that SoPure Stevia™ is significantly less sweet than 1.5% sugar, while SoPure Stevia™ enhances sweet notes when combined with sugar (Appendix 8).

E. Stability

1. Stability Data on Steviol Glycosides

The stabilities of steviol glycosides and enzyme modified steviol glycosides has previously been reviewed in ten GRAS Notices (GRNs): GRN 337 (NOW Foods, 2010), GRN 375 (Toyo Sugar and Nippon Paper, 2011), GRN 448 (Daepyung, 2012), GRN 452 (Daepyung, 2013), GRN 607 (PureCircle, 2015), GRN 656 (GLG LIfe Tech, 2016), GRN 662 (PureCircle, 2016), GRN 821 (Haigen-BGG, 2019), GRN 858 (Qufu Shengren, 2019), and GRN 878 (Daepyung, 2019).

Stevioside has been reported to be stable over the pH range 3-9 and can be heated at 100°C for 1 hour without decomposing, but, at pH greater than 9, it rapidly decomposes (Kinghorn, 2002). A series of stability studies in food applications was conducted on stevioside by Kroyer (2010). Solid stevioside was reported to be stable at up to 120°C for 1 hour. In aqueous solution, stevioside was reported to be stable at pH 2 to 10 for 2 hours at 60°C. No degradation was observed after 4 months at room temperature for 1 g per L solutions of stevioside in acetic acid (pH 3.1), citric acid (pH 2.6), and tartaric acid (pH 2.6). A 30% loss of stevioside was observed in a 1 g per L solution of phosphoric acid (pH 1.6) stored under the same conditions. In addition, degradation was

[8] Available at: https://www.femaflavor.org/sites/default/files/2019-07/FEMA%20Sensory%20Guidance%20with%20Appendix%20March%202018.pdf (Accessed on December 7, 2020)

observed in 10 g per L solutions of stevioside in acetic acid (pH 2.6, 2% loss), citric acid (pH 2.1, 22% loss), tartaric acid (pH 2.1, 33% loss), and phosphoric acid (pH 1.6, 75% loss) after 4 months.

Kroyer (2010) reported no significant changes in the concentrations of B-vitamins incubated with stevioside in aqueous solution at 80ºC for 4 hours. A decrease in the degradation rate of vitamin C was observed after 4 hours under the same conditions, indicating that stevioside provides a protective effect. No stability effects or interactions were observed between mixtures of stevioside and saccharin, cyclamate, aspartame, acesulfame, and neohesperidin stored at 80ºC for 4 hours or room temperature for 4 months. Furthermore, no stability effects or interactions were observed between stevioside and caffeine in coffee and tea beverages at 80ºC for 4 hours. These results indicate that stevioside is stable under the intended conditions of use.

Buniowska et al. (2020) studied the stability of steviol glycosides (from an aqueous *Stevia rebaudiana* Bertoni leaf extract) in a fruit juice beverage after thermal treatment ranging from 60.0 to 99.0ºC. The authors reported that decreases in rebaudioside A, rebaudioside C, and rebaudioside F concentrations were observed after thermal processing at all temperatures, independent of initial concentration. However, both temperature and concentration affected the concentration of stevioside after thermal processing, whereas stevioside was stable in solution at temperatures up to 60ºC.

In a shelf stability study conducted by Salar et al. (2020), a stevia sweetener of unknown composition that was purchased from Agriestevia S.L (Molina de Segura, Murcia, Spain) was used to sweeten a fruit juice prepared with maqui powder and lemon and other citrus juices at a concentration of 4 mg per 100 mL. The samples were pasteurized at 85ºC for 15 seconds, after which aliquots were drawn and used in studies that investigated the effects of light and temperature on the stabilities of vitamin C and phenolic compounds. The stabilities of vitamin C and phenolic compounds in the beverages prepared with sucrose and stevia were similar. Storage temperature had more of an effect on the analyzed bioactive compounds than light exposure, which was not deemed to be a “critical factor.” Compared with the sucrose-containing juice samples, there was a greater reduction in total flavonones content under light exposure at room temperature and a slightly higher loss of vitamin C during the first month was observed in juice containing the stevia sweetener. The authors noted that “stevia could be considered as an alternative sweetener by the industry,” even with these observations.

Previously submitted GRAS Notices GRN 252 (Merisant, 2008), GRN 253 (Cargill, 2008), and GRN 304 (Sunwin/WILD, 2010) reported data indicating that rebaudioside A is stable under the intended conditions of use.

Furthermore, in the 66 GRAS Notices that have been submitted to FDA and have received “no questions” letters to date, the presented stability data have supported the position that steviol glycosides are stable and well-suited for the intended uses in foods (FDA, 2020).

2. Stability Data for ZCHT's SoPure Stevia™ Glucosylated Steviol Glycosides Preparations

ZCHT conducted long-term stability studies on three lots of our SoPure Stevia™ GSG 80 glucosylated steviol glycosides preparations. Samples were stored at 25°C ± 2°C at a relative humidity of 60% ± 10%. A summary of the stability results is provided in Table 7.

Table 7. ZCHT's SoPure Stevia™ GSG 80 Glucosylated Steviol Glycosides Stability Data

Lot Number: 20170901E								
Duration	Appearance	Loss on Drying	Optical Rotation Degree	Glucosyl Steviol Glycosides	Dextrin	Total Aerobic Bacteria (cfu/g)	Mold and Yeast (cfu/g)	*E. coli* (Not detected per 1 g)
t=0	White Powder	4.3%	+72.6º	79.6%	7.7%	20	30	ND
3 months	White Powder	4.4%	+72.3º	79.2%	8.0%	<10	<10	ND
6 months	White Powder	4.3%	+72.8º	79.5%	8.4%	10	10	ND
9 months	White Powder	4.7%	+68.8º	79.8%	8.2%	20	10	ND
12 months	White Powder	4.4%	+72.5º	79.1%	6.8%	10	10	ND
18 months	White Powder	4.4%	+72.3º	79.9%	7.9%	10	10	ND
24 months	White Powder	4.3%	+72.4º	81.1%	6.3%	10	20	ND
36 months	White Powder	4.4%	+72.4º	77.6%	6.5%	10	20	ND
Lot Number: 20170902E								
Duration	Appearance	Loss on Drying	Optical Rotation Degree	Glucosyl Steviol Glycosides	Dextrin	Total Aerobic Bacteria (cfu/g)	Mold and Yeast (cfu/g)	*E. coli* (Not detected per 1 g)
t=0	White Powder	4.6%	+74.1º	80.0%	7.8%	20	<10	ND
3 months	White Powder	4.8%	+72.2º	80.9%	7.9%	<10	<10	ND
6 months	White Powder	4.7%	+74.4º	80.3%	8.0%	<10	<10	ND
9 months	White Powder	4.5%	+73.4º	80.2%	8.1%	<10	10	ND
12 months	White Powder	4.5%	+74.2º	79.5%	7.2%	<10	<10	ND
18 months	White Powder	4.6%	+72.4º	80.1%	7.8%	10	30	ND
24 months	White Powder	4.5%	+74.0º	80.4%	6.7%	20	10	ND
36 months	White Powder	4.7%	+73.6º	77.8%	6.8%	10	<10	ND

Lot Number: 20170903E								
Duration	Appearance	Loss on Drying	Optical Rotation Degree	Glucosyl Steviol Glycosides	Dextrin	Total Aerobic Bacteria (cfu/g)	Mold and Yeast (cfu/g)	*E. coli* (Not detected per 1 g)
t=0	White Powder	4.5%	+72.6º	79.2%	8.2%	30	<10	ND
3 months	White Powder	4.7%	+72.5º	79.4%	8.2%	<10	<10	ND
6 months	White Powder	4.6%	+72.9º	80.2%	8.2%	<10	<10	ND
9 months	White Powder	4.5%	+71.4º	80.0%	8.2%	<10	10	ND
12 months	White Powder	4.8%	+73.1º	79.8%	6.4%	10	<10	ND
18 months	White Powder	4.6%	+72.5º	79.2%	8.3%	10	<10	ND
24 months	White Powder	4.6%	+72.5º	80.1%	6.3%	20	<10	ND
36 months	White Powder	4.6%	+72.8º	77.0%	6.7%	20	<10	ND

cfu – colony forming unit; g – gram; ND – not detected

The stability data for steviol glycosides in the scientific literature, the JECFA report, and the extensive stability testing for structurally similar rebaudioside A as presented in GRN 252 (Merisant, 2008) and GRN 304 (Sunwin/WILD, 2010), along with ZCHT's stability testing results, support the position that ZCHT's SoPure Stevia™ GSG 80 glucosylated steviol glycosides preparations are well suited for the intended food uses.

ZCHT's SoPure Stevia™ GSG 95 glucosylated steviol glycosides preparations is a more purified finished product in which additional dextrose has been removed. As the remainder of the product is identical to SoPure Stevia™ GSG 80, it is highly unlikely that any differences in stability between the SoPure Stevia™ glucosylated steviol glycosides preparations would occur. Furthermore, the stability of steviol glycosides preparations has been well-established as discussed in Part 2.E.1. Therefore, ZCHT's SoPure Stevia™ GSG 95 glucosylated steviol glycosides preparations are well-suited for the intended food uses.

F. Calculation of Steviol Equivalents of SoPure Stevia™ Glucosylated Steviol Glycosides

For comparative purposes, the content of steviol glycosides is often expressed as steviol or steviol equivalents. Each component steviol glycoside has a steviol equivalence factor that is calculated based upon the ratio of the molecular weights (MW) of steviol and a particular steviol glycoside, as shown in Table 8.

Table 8. Steviol Equivalency Factors for Various Steviol Glycosides

Component Steviol Glycoside	Molecular Weight	Steviol Equivalency Factor[a]
Rubusoside	643	0.495
Steviolbioside	643	0.495
Dulcoside A	789	0.403
Rebaudioside B	805	0.395
Stevioside	805	0.395
Rebaudioside F	937	0.339
Rebaudioside C	951	0.334
Rebaudioside A	967	0.329
Rebaudioside E	967	0.329
Rebaudioside D	1129	0.282
Rebaudioside M	1291	0.246

[a] Calculated by dividing the molecular weight of steviol (MW=318) by the molecular weight of each glycoside.

Using these steviol equivalency factors, along with the representative percent composition of the steviol glycosides extract raw material, it is possible to determine the worst-case scenario steviol equivalency of SoPure Stevia™ glucosylated steviol glycosides, as presented in Table 9.

Table 9. Steviol Equivalency of Steviol Glycosides Extract Raw Material

Component Steviol Glycoside	Typical Composition[a] (%)	Steviol Equivalents[b] (%)
Stevioside	36.4	14.4
Rebaudioside C	6.5	2.17
Rebaudioside F	1.1	0.37
Rebaudioside A	51.8	17.0
Other steviol glycosides	1.1	1.1[c]
Total Steviol Equivalence	--	35

[a] Based on the typical levels of steviol glycosides in a representative lot of steviol glycoside extract raw material.
[b] Calculated by multiplying the % of the steviol glycoside by the steviol equivalency factor.
[c] Calculated as steviol for a worst-case-scenario equivalence.

The stevia extract starting material is enzymatically glycosylated as described in Part 2.B, in a process in which a glucosyltransferase enzyme adds glucose moieties, obtained from a dextrin

source, to the steviol glycosides present in the raw material. It is reasonable to assume that all steviol glycosides and glucosylated steviol glycosides will maintain the same level of steviol equivalence described above since no other reactions are known to occur from the known chemistry of the enzyme. Therefore, the steviol equivalency of the SoPure Stevia™ glucosylated steviol glycosides preparations is expected to be no greater than 35 g steviol per 100 g SoPure Stevia™ glucosylated steviol glycosides.

PART 3. DIETARY EXPOSURE

The subject SoPure Stevia™ glucosylated steviol glycosides preparations are intended to be used as a table top sweetener and general purpose non-nutritive sweetener in various foods, but not in infant formulas and meat and poultry, as defined in 21 CFR 170.3(o)(19).[9] ZCHT also intends for SoPure Stevia™ glucosylated steviol glycosides preparations to be used as a flavor modifier at a recommended use level of 600 mg per kg in food and 1,500 mg per kg in chewing gum, in the same manner described in GRN 607.[10]

The intended use levels will vary by food category, but the levels are self-limiting due to organoleptic factors and consumer taste considerations. However, the amounts of SoPure Stevia™ glucosylated steviol glycosides to be added to foods will not exceed the amounts reasonably required to accomplish the intended technical effect in foods as required by FDA regulation 21 CFR 182.1(b)(1).[11]

A. Estimate of Dietary Exposure to the Substance

Many scholarly estimates of potential dietary intake replacement of sweeteners, including steviol glycosides have been published (FSANZ, 2008; WHO, 2003; Renwick, 2008) or submitted to FDA (Merisant, 2008). These are summarized in Appendix 9. In GRAS Notice 301, a simplified estimate was proposed to, and accepted by, FDA based on the estimates of exposure in “sucrose equivalents” (Renwick, 2008) and the sweetness intensity of any particular sweetener (BioVittoria, 2009). As summarized in GRN 301, the intake of a sweetener that is 100 times as sweet as sucrose when used as a total sugar replacer for a 90th percentile would be a maximum of 9.9 mg per kg body weight (bw) per day for any population subgroup.

The relative sweetness intensity of SoPure Stevia™ glucosylated steviol glycosides preparations ranges between 100X and 200X times that of sucrose. A weighted sum estimate was used to determine the worst-case scenario steviol equivalency factor for SoPure Stevia™ glucosylated

[9] Non-nutritive sweeteners: Substances having less than 2 percent of the caloric value of sucrose per equivalent unit of sweetening capacity.
[10] GRN 607 available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=607&sort=GRN_No&order=DESC&startrow=1&type=basic&search=607 (Accessed March 3, 2021)
[11] Available at: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=182.1 (Accessed on March 3, 2021)

steviol glycosides, which was determined to be 35 g steviol per 100 g SoPure Stevia™ glucosylated steviol glycosides (as described in Part 2.F).

The highest 90th percentile consumption by any population subgroup of SoPure Stevia™ glucosylated steviol glycosides using the lowest relative sweetness intensity (100X) and worst-case scenario steviol equivalence (35 g steviol per 100 g SoPure Stevia™) would be approximately 9.90 mg per kg steviol glycosides bw per day. Based on a weighted sum estimate for steviol equivalents provided in Table 9, consumption would be not more than 3.47 mg per kg bw per day on a steviol equivalents basis for any population group for ZCHT's SoPure Stevia™ glucosylated steviol glycosides preparations described herein. These calculations are summarized in Table 10.

Table 10. Daily Intake of Sweeteners (in Sucrose Equivalents) & Estimated Daily Intakes of SoPure Stevia™

Population Group	Intakes of Sweeteners (mg sucrose/kg bw/day)[a]		Calculated Intake of SoPure Stevia™ (mg/kg bw/day)[b]		Calculated Intake of SoPure Stevia™ as Steviol Equivalents (mg/kg bw/day)	
	Low	High	Low	High	Low	High
Healthy Population	255	675	2.55	6.75	0.89	2.37
Diabetic Adults	280	897	2.80	8.97	0.98	3.15
Healthy Children	425	990	4.25	9.90	1.49	3.47
Diabetic Children	672	908	6.72	9.08	2.36	3.18

[a] From Renwick (2008)

[b] Calculated by dividing the sucrose intake by the minimum average relative sweetness value of 100 for SoPure Stevia™.

bw – body weight; kg – kilogram; mg – milligram

The values in Table 10 are based on the assumption that ZCHT's SoPure Stevia™ glucosylated steviol glycosides preparations constitute the entire sweetener market, which makes these estimates extremely conservative since the likelihood of that occurrence is minimal. For the general healthy adult population, the worst-case scenario estimated maximum intake of steviol glycosides is 6.75 mg per kg bw per day (2.37 mg per kg steviol equivalents) for SoPure Stevia™ glucosylated steviol glycosides preparations. For healthy children, the worst-case scenario estimated maximal intake is 9.90 mg per kg bw per day (3.47 mg per kg as steviol equivalents) for SoPure Stevia™ glucosylated steviol glycosides preparations. In all population groups, the worst-case scenario estimated daily intake of steviol glycosides, expressed as steviol equivalents, is well below the JECFA-established acceptable daily intake (ADI) of 4.0 mg per kg bw per day steviol equivalents.

As noted in GRN 607, exposure to glucosylated steviol glycosides preparations from use as a flavor modifier will be minimal compared to the EDI determined for use as a sweetener. Therefore,

the combined exposure to ZCHT's SoPure Stevia™ glucosylated steviol glycosides preparations at the recommended maximum use levels of 600 mg per kg in food and 1,500 mg per kg in chewing gum and from the proposed uses as sweeteners from other sources is not expected to exceed the JECFA-established acceptable daily intake (ADI) of 4.0 mg per kg bw per day steviol equivalents.

B. Estimated Dietary Exposure to Any Other Substance That is Expected to be Formed In or On Food

This section is not applicable to ZCHT's SoPure Stevia™ glucosylated steviol glycosides preparations, which would be chemically stable under conditions of use.

C. Dietary Exposure to Contaminants or Byproducts

While a recent publication by Kumari et al. (2016) investigated the Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and Total Antioxidant Capacity (TAC) of *S. rebaudiana* leaf --- and the observed activity has been attributed to naturally occurring phytochemicals such as phenolics, flavonoids, and pigments in the plant --- the study has minimal relevance with regard to the safety considerations of highly purified stevia extract, of which ≥ 95% consists of the most familiar steviol glycosides and their glucosylated steviol glycosides. These phytochemical contaminants, if present, are in low amounts and were likely similarly present in purified test materials that were used in the toxicology studies summarized in Appendix 10.

Furthermore, no concerns regarding dietary exposure to contaminants or byproducts have been raised by expert regulatory bodies, including the World Health Organization/Joint FAO/WHO Expert Committee on Food Additives (WHO/JECFA), European Food Safety Authority (EFSA), Food Standards Australia New Zealand (FSANZ), and FDA, since JECFA's first steviol glycosides review was performed in 2000 (WHO, 2000).

PART 4. SELF-LIMITING LEVELS OF USE

It has been well-documented in the published literature that the use of steviol glycosides is self-limiting due to organoleptic factors and consumer taste considerations (Kochikyan et al., 2006; Carakostas et al., 2008; Brandle et al., 1998; Prakash et al., 2008; Gupta et al., 2016; Gerwig et al., 2016). These organoleptic factors include bitterness and astringency, as well as a lingering metallic aftertaste (Gerwig et al., 2016).

PART 5. EXPERIENCE BASED ON COMMON USE IN FOOD BEFORE 1958

Stevia has been used as a traditional medicine and sweetener by native Guarani tribes for centuries (Esen, 2016; Gerwig et al., 2016; Brusick, 2008; Brandle et al., 1998). Hawke (2003) reported that stevia is commonly used as a treatment for type 2 diabetes in South America. However, therapeutic doses of 1 gram per person per day or more were reported to be necessary to achieve the desired effects (Gregersen et al., 2004).

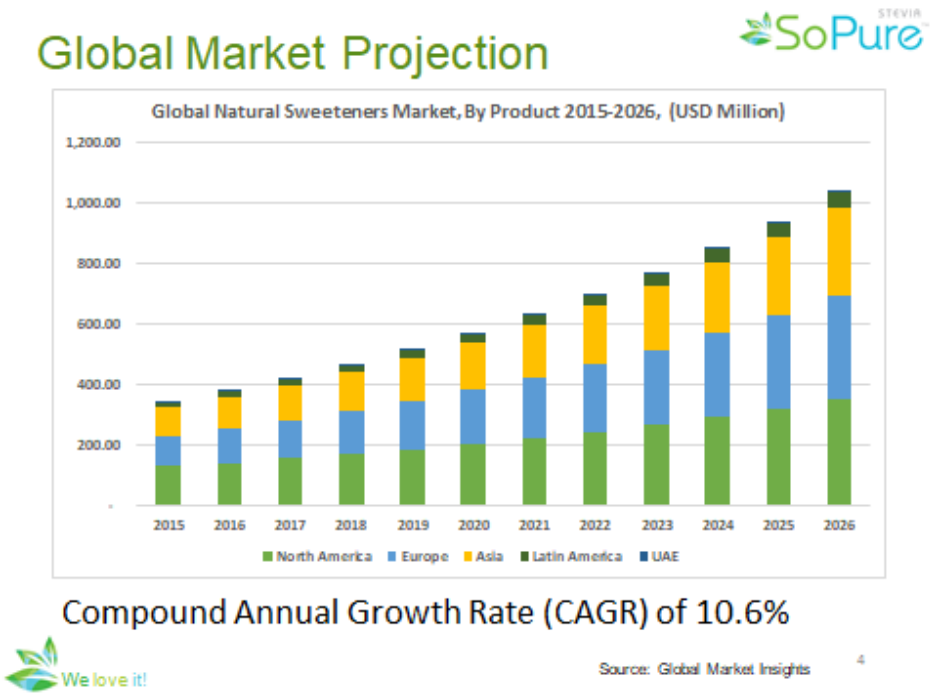
For about 30 years, consumers in Japan and Brazil, where stevia has long been approved as a food additive, have been using stevia extracts as non-caloric sweeteners (Raintree, 2012). It was previously reported that 40% of the artificial sweetener market in Japan was stevia based and that stevia is commonly used in processed foods in Japan (Lester, 1999). Use of steviol glycosides as a dietary supplement is presently permitted in the US, Canada, Australia, and New Zealand, and use as a natural health product is permitted in Canada. It has wide use in China and Japan in food and in dietary supplements. In 2005, it was estimated that sales of stevia in the US reached $45 million (Newsday, 2006).

NewHope360 reported that the global market for stevia in 2014 was $347 million and is expected to increase to $565.2 million by 2020. In addition, consumption was expected to increase from 2014 levels of 5,100.6 tons to 8,506.9 tons by 2020 (NewHope360, 2015).

Most recently, Nutritional Outlook reported that Mintel data indicated a 48% increase in stevia-containing products over the last five years (Decker and Prince, 2018).

In a global market projection prepared by Maximize Market Research Pvt. Ltd., the global stevia market was $488.8 million in 2019 and is expected to reach $980.5 million by 2027.[12] A graph depicting the compound annual growth rate of the global natural sweeteners market is shown in Figure 5.

Figure 5. Global Market Projection of Natural Sweeteners



[12] Available at: https://www.maximizemarketresearch.com/market-report/global-stevia-market/27578/ (Accessed on: November 10, 2020)

PART 6. NARRATIVE

A. Summary of Regulatory History of Enzyme Modified Steviol Glycosides

Stevia-derived sweeteners are permitted as food additives in South America and in several countries in Asia, including China, Japan, and Korea. In recent years, these sweeteners have received food usage approvals in Canada, Mexico, Peru, Uruguay, Colombia, Australia, New Zealand, Switzerland, France, Russia, Turkey, Israel, Senegal, Malaysia, Taiwan, Thailand, and Hong Kong (EFSA, 2010; Watson, 2010; Health Canada, 2012). In the United States, steviol glycosides have been used as a dietary supplement since 1995 (Geuns, 2003).

A brief overview of the most recent regulatory activity regarding steviol glycosides is presented below in Part 6.A Sections 1-5. A more detailed historical overview is provided in Appendix 11.

1. U.S. Regulatory History

Based on available information from FDA's GRAS Notice Inventory website (FDA, 2020) as of February 12, 2021, FDA has issued 66 "no questions" letters on GRAS notices on rebaudioside A, rebaudioside D, rebaudioside M, or high purity steviol glycosides, including those undergoing enzyme modification.

In addition, the Flavor and Extract Manufacturers Association (FEMA) includes 20 steviol glycosides preparations, as detailed in Appendix 11, six of which are for enzymatically modified stevia extracts, on their GRAS lists.

2. Canadian Regulatory History

On November 30, 2012, Health Canada published its final clearance for use of steviol glycosides as a sweetener in foods (Health Canada, 2012). In March 2014, Health Canada updated the List of Permitted Sweeteners (Lists of Permitted Food Additives) to include steviol glycosides in applications as a table-top sweetener and as an ingredient in a variety of foods, beverages, baked goods, meal replacement bars, condiments, and confectionary and gums (Health Canada, 2014). On January 15, 2016, Health Canada approved the use of rebaudioside M as a high-intensity sweetener under the same conditions as the previously approved steviol glycosides (Health Canada, 2016).

Health Canada's Food Directorate updated its List of Permitted Sweeteners to allow for the use of steviol glycosides as a sweetener in "unstandardized snack bars," including granola bars, cereal bars, fiber bars, and protein isolate-based bars (Health Canada, 2017b). Health Canada (2017b) also modified the List of Permitted Sweeteners to include "all the steviol glycosides in the *Stevia rebaudiana* Bertoni plant (stevia plant)."

In April 2019, Health Canada's Food Directorate modified the List of Permitted Sweeteners to allow for the use of steviol glycosides from *Stevia rebaudiana* Bertoni in canned fruit products (Health

Canada, 2019c). In May 2019, Health Canada's Food Directorate modified the List of Permitted Sweeteners to allow for the use of steviol glycosides derived from *Saccharomyces cerevisiae* strains CD15380 and CD15407 at the same maximum levels of use as steviol glycosides derived from *Stevia rebaudiana* Bertoni (Health Canada, 2019b). On June 27, 2019, Health Canada's Food Directorate modified the List of Permitted Sweeteners to allow for the use of steviol glycosides from various sources in "standardized flavoured milks" (Health Canada, 2019a).

Most recently, on September 1, 2020, Health Canada updated the List of Permitted Sweeteners to include the use of steviol glycosides produced by *Saccharomyces cerevisiae* Y63348 at the same maximum levels of use as steviol glycosides derived from *Stevia rebaudiana* Bertoni and *Saccharomyces cerevisiae* strains CD15380 and CD15407 (Health Canada, 2020).

3. European Regulatory History

An amendment to the European Union (EU) food additives regulation 231/2012, which became active on November 3, 2016, removed the previous requirement for stevia blends to contain at least 75% rebaudioside A or stevioside. In addition, the updated regulation ---(EU) 2016/1814--- now permits the following steviol glycosides in stevia blends: stevioside, rebaudiosides A, B, C, D, E, F and M, steviolbioside, rubusoside, and dulcoside (Searby, 2016).

In 2017, JECFA updated the steviol glycosides specifications to include a requirement of not less than 95% total steviol glycosides, on a dry basis, "determined as the sum of all compounds containing a steviol backbone conjugated to any number, combination or orientation of saccharides (glucose, rhamnose, fructose, deoxyglucose, xylose, galactose, arabinose and xylose) occurring in the leaves of *Stevia rebaudiana* Bertoni." Microbiological criteria were also established, with specifications of no more than 1,000 cfu per g total plate count, not more than 200 cfu per g yeasts and molds, and *E. coli* and *Salmonella* negative in 1 g and 25 g, respectively (FAO, 2017).

The European Food Safety Authority (EFSA) Panel of Food Additives and Nutrient Sources reviewed an application for glucosylated steviol glycoside preparations for use as a new food additive. The Panel concluded that the data supplied by the applicant were "insufficient to assess the safety" of the glucosylated steviol glycosides preparation. It should be noted that no safety concerns were raised in a more recent review by the EFSA Panel where their decision was based on the "limited" data provided in the dossier submitted by the applicant (EFSA, 2018).

On September 24, 2019, the EFSA Panel on Food Additives and Flavourings concluded that there is no safety concern for rebaudioside M produced via enzymatic bioconversion and recommended that the European Commission consider establishing specifications for the preparation (EFSA, 2019).

On March 24, 2020, EFSA published a scientific opinion in response to a proposed amendment of the specifications for steviol glycosides, stating that all steviol glycosides share the same metabolic fate, and therefore the safety of 60 steviol glycosides identified in the leaves of *Stevia rebaudiana*

Bertoni can be based on “read-across” from previously evaluated toxicological data. EFSA maintained that the ADI of 4 mg per kg bw applies to all 60 steviol glycosides. The EFSA Panel noted that the inclusion of more steviol glycosides, “whilst maintaining the assay value of not less than 95%, would allow less pure preparations” onto the market. The Panel stated that they “cannot conclude on the safety of the proposed amendment to the specifications of steviol glycosides (E 960) as [a] food additive if the purity assay value of not less than 95% for the total content of steviol glycosides is maintained.” Furthermore, the Panel noted that it is possible to manufacture steviol glycosides with a purity higher than 95% total steviol glycosides (EFSA, 2020).

4. Asian Regulatory History

In May 2010, Hong Kong amended its food regulations to allow the use of steviol glycosides as a permitted sweetener in foods based upon the detailed safety evaluation and favorable findings as reported by JECFA (Hong Kong Centre for Food Safety, 2010).

In July 2011, the Codex Alimentarius Commission adopted proposed maximum use levels for steviol glycosides in all major food and beverage categories which resulted in steviol glycoside approvals in Vietnam, the Philippines, Malaysia, Singapore and Thailand (Whitehead, 2013). The International Alliance of Dietary/Food Supplement Associations (IADSA) reported that the Codex Alimentarius Commission agreed to adopt the use of steviol glycosides for addition to chewable food supplements (Food Ingredients First, 2011).

On September 20, 2012, the Food Safety and Standards Authority of India (FSSAI) approved the use of steviol glycosides as a non-nutritive sweetener in a variety of foods using specifications and purity established by JECFA (FSSAI, 2012).

Since December 10, 2012, over thirty registrations have been granted by FDA Philippines to stand-alone steviol glycosides sweeteners or foods containing steviol glycosides as ingredients (Philippines, 2014).

Steviol glycosides are also listed under International Numbering System (INS) number 960 in the Food Additives Permitted Under the Singapore Food Regulations document prepared by the Agri-Food & Veterinary Authority (AVA) of Singapore (AVA, 2014).

In Japan, three stevia-derived preparations are included on the List of Existing Food Additives (Japanese Ministry of Health and Welfare, 2014): α-Glucosyltransferase Treated Stevia, described as “a substance composed mainly of α-glucosylsteviosides obtained from a ‘stevia extract’”; Powdered stevia, described as “a substance composed mainly of steviol glycosides obtained by grinding stevia leaves”; and Stevia extract, described as “a substance composed mainly of steviol glycosides obtained by extraction from stevia leaves.”

In China, stevia rebaudiana oil and steviol glycosides are permitted food additives as a natural food flavor and sweetener, respectively.[13]

"Enzymatically Modified Stevia" and "Steviol Glycoside" are listed in Korea's Food Additives Code for use as sweeteners (Korean Ministry of Food and Drug Safety Regulation, 2020).

5. Other Regulatory History

FSANZ called for submissions on permitting all minor steviol glycosides extracted from stevia leaf to be included in the definition of steviol glycosides in the Food Standards Code, noting that "[no] evidence was found to suggest that the proposed changes pose any public health and safety concerns." The submission period ended on December 19, 2016 (FSANZ, 2016). Subsequently, on February 8, 2017, FSANZ approved a draft variation of the definition of steviol glycosides to include all steviol glycosides present in the *Stevia rebaudiana* leaf (FSANZ, 2017).

FSANZ called for comments on the production of Reb M using enzymes derived from genetically modified yeast. The comment period closed on August 31, 2018 (FSANZ, 2018b). Subsequently, on October 31, 2018, FSANZ approved a draft variation to include a reference to the production method (FSANZ, 2018a).

On May 14, 2020, FSANZ published an approval report for a draft variation to amend the specification for steviol glycosides from *Stevia rebaudiana* Bertoni in section S3—35 of the Australia New Zealand Food Standards Code to include rebaudioside E produced by enzymatic conversion from stevia leaf extract. The approved draft variation allows for the use of high purity rebaudioside E (≥85% rebaudioside E; ≥95% total steviol glycosides) within the existing permissions and limits for steviol glycosides (FSANZ, 2020a). Subsequently, on July 28, 2020, Amendment No. 193 was published to include rebaudioside E produced by enzymatic conversion from stevia leaf extract (FSANZ, 2020c).

On October 21, 2020, FSANZ called for comments on permitting the use of rebaudioside M derived from *Saccharomyces cerevisiae* as a general purpose sweetening agent. FSANZ stated that a thorough safety assessment was conducted by FSANZ and "…no public health or safety concerns with this type of steviol glycoside" were found. The comment period closed on December 2, 2020 (FSANZ, 2020b).

B. Summary of the Published Literature on Enzyme Modified Steviol Glycosides

The biological, toxicological, and clinical effects of stevia and steviol glycosides have been extensively reviewed (Carakostas et al., 2008; Geuns, 2003; Huxtable, 2002). Additionally---and as noted earlier--- national and international regulatory agencies have thoroughly reviewed the safety of stevia and its glycosides. Most notably, over the years, JECFA has evaluated purified steviol

[13] A searchable permitted food additives database is available at: http://sfdachina.com/foods/Food_additive_Search.asp (Accessed on February 12, 2021).

glycosides multiple times (WHO, 2000; WHO, 2006; WHO, 2007; WHO, 2008), and their findings have been summarized in Part 5.B.3. FSANZ (2008) also evaluated steviol glycosides for use in food. The JECFA reviews, as well as the other reviews completed before 2008, primarily focused on mixtures of steviol glycosides. These studies are summarized in Appendix 12.

Since the JECFA evaluation (WHO, 2008), FDA has received and not objected to 66 GRAS notifications for steviol glycosides or enzyme modified steviol glycosides to date, many of which were discussed by Perrier et al. (2018). In each case, FDA has agreed with the conclusions that steviol glycosides are GRAS based largely on the 0-4 mg per kg bw per day ADI on a steviol equivalence basis that was established by JECFA. A publication by Roberts et al. (2016) indicates that the ADI could be higher, as discussed further in Appendix 9. Among the GRAS notifications submitted to FDA, several assessed purified preparations of rebaudioside A, and they were supported by additional toxicology and clinical studies that are summarized in Appendix 10.

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

As discussed in Appendix 12 and elsewhere, at its 51st meeting, JECFA determined that there were adequate chronic toxicity studies in rats, particularly the study by Toyoda et al. (1997), to establish a temporary ADI of 0 – 2 mg per kg bw per day with an adequate margin of safety (Toyoda et al., 1997). The Committee also critically reviewed the lack of carcinogenic response in well-conducted studies. These studies validated the Committee's conclusion that the *in vitro* mutagenic activity of steviol did not present a risk of carcinogenic effects *in vivo* and, therefore, all

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

common steviol glycosides that likely share the same basic metabolic and excretory pathway and that use high purity preparations of various steviol glycosides, are safe as sugar substitutes. Subsequently, the additional clinical data reviewed by JECFA allowed the Committee to establish a permanent ADI of 0 - 4 mg per kg bw per day (based on steviol equivalents).

More recently, JECFA published a safety evaluation of a number of food additives, including steviol glycosides (WHO, 2017). The JECFA Committee reviewed information supporting the safety of a *Yarrowia lipolytica* fermentation-produced rebaudioside A, which included a 90-day rat toxicity study and two *in vitro* genotoxicity studies, as well as *in vitro* colonic microflorae hydrolysis studies in several steviol glycosides, toxicokinetic studies of stevioside in humans and rats, and literature published since the 69th meeting.

The Committee noted that the most recent short-term toxicity studies were consistent with those reviewed at or prior to the 69th meeting, and that the new toxicokinetic study in humans did not have a large enough subject pool to provide reliable toxicokinetic estimates to derive an update ADI for steviol glycosides. The Committee confirmed the current ADI of 0 - 4 mg per kg bw steviol. In addition, the Committee prepared new “tentative” specifications for steviol glycosides, which were expanded to include “any mixture of steviol glycosides compounds derived from *S. rebaudiana* Bertoni” while retaining the requirement that the total percentage of steviol glycosides is ≥ 95% (WHO, 2017).

ZCHT critically reviewed the JECFA assessments and agrees with the calculation of the ADI for steviol glycosides.

Several published and unpublished studies (summarized in Appendix 10) on purified preparations of rebaudioside A showed an absence of toxicological effects in rats (Curry and Roberts, 2008; Nikiforov and Eapen, 2008) and dogs (Eapen, 2008) in subchronic studies, and an absence of reproductive (Curry et al., 2008; Sloter, 2008a) and developmental effects (Sloter, 2008b) in rats. Most notably, pharmacokinetic studies in rats (Roberts and Renwick, 2008) and humans (Wheeler et al., 2008) on purified rebaudioside A follow the same pathway of being degraded to steviol by intestinal bacteria with subsequent rapid glucosylation and elimination in urine and feces.

Purkayastha et al. (2014) compared the anaerobic *in vitro* metabolism of rebaudiosides A, B, D, and M with human fecal homogenates. In all cases, the rebaudiosides were hydrolyzed to steviol within 24 hours, with the majority of metabolism occurring within the first 8 hours. Metabolism of rebaudiosides took longer at higher concentrations (2.0 mg per mL vs. 0.2 mg per mL). There were no marked differences in rate or extent of hydrolysis observed between male and female fecal homogenates or individual rebaudiosides.

In a follow up study, Purkayastha et al. (2016) investigated the metabolic fates of two concentrations of steviolbioside, dulcoside A, and rebaudiosides A, B, C, D, E, F, and M in an *in vitro* study using pooled human fecal homogenates over the course of 24 to 48 hours. It was reported that the glycosidic side chains ---containing glucose, rhamnose, xylose, fructose, and

those with deoxy-glucose including combinations of α(1-2), β-1, β(1-2), β(1-3), and β(1-6) linkages ---were mostly degraded to steviol within 24 hours. This observation supports the extrapolation of safety data for specific steviol glycosides and steviol to other steviol glycosides found in *Stevia rebaudiana* leaf extract. As previously observed, the rate of metabolism was slower at higher concentrations (2.0 mg per mL vs. 0.2 mg per mL). In addition, Purkayastha et al. (2016) reported that no appreciable differences in metabolism were observed between fecal homogenates obtained from males and females or those obtained from different ethnicities.

Most recently, Purkayastha and Kwok (2020) investigated the metabolic fate of steviol glycosides in fecal homogenates collected from adults and children. Steviol glycosides obtained from stevia leaf extract (composed of more than 20 steviol glycosides, with Reb D and Reb M as the principal components), bioconversion reaction product (composed of Reb D and Reb M), minor steviol glycosides extracted from a stevia leaf extract (composed of Reb AM, Reb W2, Reb U2, Reb V, Reb N, and Reb O), enzyme modified steviol glycosides, and rebaudioside A standard were used as test samples for *in vitro* incubation in pooled human fecal homogenate samples obtained from adult and pediatric donors. Purkayastha and Kwok (2020) reported that all steviol glycosides preparations tested “shared qualitatively similar *in vitro* metabolic fates.” In addition, the authors concluded that “safety data for individual steviol glycosides can be used to support safety of all steviol glycosides produced by extraction and enzymatic conversion of stevia leaf extract.”

ZCHT concludes that these studies by Purkayastha et al. (2020; 2016; 2014) and the studies on rebaudioside A and other enzyme modified steviol glycosides preparations strengthen the argument that all steviol glycosides that follow the same metabolic pathway are safe at the JECFA established ADI.

ZCHT has also reviewed the findings from human clinical studies. ZCHT noted that the effects of steviol glycosides observed in clinical studies occur only in patients with either elevated blood glucose or blood pressure (or both). JECFA called for studies in individuals that are neither hypertensive nor diabetic (WHO, 2006). The supplemental data presented to JECFA and also published by Barriocanal et al. (2008) demonstrate the lack of pharmacological effects of steviol glycosides at 11 mg per kg bw per day in normal individuals, or approximately slightly more than 4 mg per kg bw on the basis of steviol equivalents (Barriocanal et al., 2008). Clinical studies on purified rebaudioside A showed an absence of effects on blood pressure (Maki et al., 2008a) and blood glucose levels (Maki et al., 2008b) at doses slightly higher than the exposures expected in food. ZCHT concludes that there will be no effects on blood pressure and glucose metabolism in humans at the doses of steviol glycosides expected from its use in food as a non-nutritive sweetener.

Two previously published studies summarized in Appendix 10 raised a potential concern regarding the toxicological effects of steviol glycosides. In one study, DNA damage was seen in a variety of organs as assessed by Comet assay in rats given drinking water containing 4 mg per mL steviol glycosides for up to 45 days (Nunes et al., 2007a). Several experts in the field have since

questioned the methodology used in this study (Geuns, 2007; Williams, 2007; Brusick, 2008). ZCHT has reviewed the cited publications, along with the responses made by the authors (Nunes et al., 2007b; Nunes et al., 2007c), and concurs with the challenges to the methodology utilized by Nunes et al. (2007a), thereby discounting the validity and relevance of this study.

In another study with stevioside in rats, tartrate-resistant alkaline phosphatase (TRAP) levels were measured and found to be significantly decreased at doses as low as 15 mg per kg bw (Awney et al., 2011). TRAP is an enzyme that is expressed by bone-resorbing osteoclasts, inflammatory macrophages, and dendritic cells. This enzyme was not measured in any previous toxicology studies on steviol glycosides, nor has it been adequately vetted for application in toxicological studies. Critical reviews of this study by Carakostas (2012) and Waddell (2011) revealed a poor study design that included: insufficient numbers of animals, group housing with the potential for stress related changes, unreliable access to steviol *via* drinking water resulting in suspect dosing calculations in group-housed cages, no indication of fasting prior to blood collection (which affects many chemistry and hematological values), no urine collection, and no histopathological evaluations for confirmation of findings beyond the controls. Additionally, the report did not adequately describe mean or individual organ weight data, and it lacked comparison of study findings against laboratory historical control data. ZCHT concurs with Carakostas (2012) and Waddell (2011) evaluations of the Awney et al. (2011) study and have concluded that it does not impact the safety discussion of ZCHT's SoPure Stevia™ glucosylated steviol glycosides.

Urban et al. (2013) examined the extensive genotoxicity database on steviol glycosides because some concern has been expressed in two publications (Brahmachari et al., 2011; Tandel, 2011) in which the authors concluded that additional testing is necessary to adequately address the genotoxicity profile (Urban et al., 2013). The review aimed to address this matter by evaluating the specific genotoxicity studies of concern, while evaluating the adequacy of the database that includes more recent genotoxicity data not noted in these publications. The results of this literature review showed that the current database of *in vitro* and *in vivo* studies for steviol glycosides is robust and does not indicate that either stevioside or rebaudioside A is genotoxic. This finding, combined with a paucity of evidence for neoplasm development in rat bioassays, establishes the safety of all steviol glycosides with respect to their genotoxic/carcinogenic potential. ZCHT agrees with the Urban et al. (2013) conclusions.

In addition, a paper by Shannon et al. (2016) raises a possible concern of endocrine disruption by steviol. ZCHT reviewed the publication and noted that the effects on progesterone production and on the action of progesterone (both antagonistic and agonistic) were observed *in vitro* in sperm cells. ZCHT concludes that it is difficult to translate *in vitro* concentrations to local concentrations *in vivo* at receptors and that no adverse effects were observed in well-conducted reproductive toxicology studies. Therefore, this study does not alter ZCHT's opinion that steviol glycosides preparations are generally recognized as safe. A summary of this study is provided in Appendix 10.

A commercially available steviol glycoside extract (>99%, composition and brand unknown) was used to investigate genotoxicity in human peripheral blood lymphocytes. Uçar et al. (2017) observed no significant differences in chromosomal aberration induction or micronuclei between the control and treatment groups at 24 and 48 h. These data support previous findings that steviol glycosides are not genotoxic.

Thøgersen et al. (2018) investigated the effect of rebaudioside A, stevioside, and steviol on porcine cytochrome P450 (CYP) expression and activity to assess their potential food-drug interactions in the IPEC-J2 cell line, a non-transformed cell line derived from intestinal porcine epithelial cells and in primary hepatocytes. The authors reported that there were no changes in CYP messenger ribonucleic acid (mRNA) expression following treatment of IPEC-J2 cells with rebaudioside A, stevioside, and steviol compared with control. Treatment of primary hepatocytes resulted in increases in CYP3A29 mRNA at low concentrations of rebaudioside A and steviol, and at all concentrations of stevioside. The authors reported that while treatment with the steviol glycosides tested over 24 hours resulted in minor increases in CYP3A29 mRNA expression (< 2.0-fold), "no direct effect on CYP activity" was observed. The authors concluded that rebaudioside A, stevioside, and steviol are unlikely to cause a food-drug interaction but noted that the study could not predict long term effects and effects *in vivo*.

A published study addressed the genotoxic activity of stevia (Svetia™, purity not reported[15]). Human lymphocytes were treated with 5% and 0.5% Svetia™ for 2 hours. No statistically significant difference in genetic damage was observed in the 0.5% treatment concentration compared with the negative control, while the 5% treatment concentration resulted in a statistically significant difference ($P<0.0001$) compared with the control, with a decrease in migration average. The authors described the effect as being beneficial. Human lymphocytes treated with 10% Svetia™ demonstrated significant ($P<0.0001$) genotoxic activity compared to the control; however, at treatment concentrations of 0.05%, 0.5%, and 5% Svetia™, a significant ($P<0.0001$) decrease in average migration of DNA was observed compared with the control. The authors conclude that these results demonstrate the absence of genotoxicity at concentrations up to 5% Svetia™ (Silva et al., 2018). It should be noted that these observations are consistent with the weight of the evidence supporting negative genotoxicity and at odds with data reported by Nunes et al. (2007a). As discussed above, the validity and importance of the Nunes et al. study has been discounted given the questions surrounding the methodology.

A recent study by Pasqualli et al. (2020) investigated the cytotoxicity, genotoxicity, and immunotoxicity of steviol in human cells. Lymphocytes were treated with steviol concentrations ranging from 1 to 500 μg per mL. The median lethal dose (LC_{50}) was determined to be 178.7 μg per mL. At 50 μg steviol per mL, a statistically significant decrease in lymphocytes was observed in a cell proliferation study. No effects on viability were observed at concentrations of up to 50 μg per

[15] While the purity of the material used for the study was not reported by Silva et al. (2018), a search of the manufacturer's website (www.svetia.us) indicates that the trademarked material is a blend of cane sugar and 97% pure rebaudioside A.

mL. In lymphocyte subpopulations, steviol did not display inhibitory effects in $CD3^+$ cells at any concentration, and significant decreases were observed in $CD4^+$ cells at 10 μg per mL, $CD8^+$ cells at 1 μg per mL, and double population $CD4^+CD8^+$ cells at 1 μg per mL. Results of a comet assay indicated that concentrations of 10 and 50 μg stevioside per mL led to an increase in DNA damage of approximately 62% compared with the negative control. Changes in chromosomal instability were observed at 10 and 50 μg per mL. These results are inconsistent with those reported for steviol glycosides extract by Ucar et al. (2017) and Svetia™ by Silva et al. (2018).

In a seven-week study, 13 subjects (ages 18 to 30 years) without a history of hypertension or hyperglycemia were supplemented with four commercial 1 g packets per day of steviol glycosides (equivalent to 0.1 g steviol glycosides per day, brand and composition not reported) for six weeks. Decreases in triglyceride, cholesterol, and serum tumor necrosis factor-α concentrations were observed. No adverse effects were reported (Sánchez-Delgado et al., 2019).

Wang and Wu (2019) investigated the angiotensin-converting enzyme (ACE) inhibiting activity of a 95% pure steviol glycosides extract (composition not reported) obtained from an ethanol extract of stevia leaves. Steviol glycosides were reported to have doubled the ACE inhibitory activity of an ethanolic extract of steviol leaves, were well-accepted in a sensory test in decaffeinated coffee, decaffeinated tea, and peanut protein beverages, and had a significant antihypertensive effect in spontaneously hypertensive rats. No adverse events in humans or rats were reported.

The interaction between select prescription drugs and steviol acyl glucuronide, the major metabolite of rebaudioside A, was investigated by Zhou et al. (2019). Organic anion transporter 3 (OAT3) – mediated update of steviol acyl glucuronide was examined *in vitro* using human embryonic kidney 293 (HEK293) cells. HEK293 cells were transfected with human organic anion transporter 3 (hOAT3) and rat organic anion transporter 3 (rOAT3). Both probenecid and glimepiride were potent inhibitors of hOAT3 and rOAT3 with no apparent species differences observed. Pharmacokinetic studies in male Sprague-Dawley rats revealed that both probenecid and glimepiride significantly elevated plasma steviol acyl glucuronide concentrations, particularly between 6 and 8 hours after oral administration of rebaudioside A. The inhibition of OAT3 is a potential mechanism for the interaction between steviol acyl glucuronide and probenecid and glimepiride, which could be clinically relevant. The authors concluded that “care should be given to populations with concomitant use of stevia leaf extracts and probenecid or glimepiride.”

ZCHT has reviewed the Zhou et al. (2019) publication in detail and notes that the pharmacokinetic oral dose used in the study was 15 mg per kg rebaudioside A. Plasma concentrations of steviol acyl glucuronide were observed to be dose-dependent after oral administration of rebaudioside A, with an average concentration maximum (C_{max}) of approximately 39 ng per mL at 5 mg per kg rebaudioside A and 170 ng per mL at 15 mg per kg rebaudioside A, respectively, observed at 6 hours post-dosing. Given the observed dose-dependency, it is possible that a reduced interaction would be observed between steviol acyl glucuronide and probenecid and glimiperide at lower doses of rebaudioside A. Based on a steviol equivalence factor of 0.329 for rebaudioside A as

listed in Table 8, the 15 mg per kw dose corresponds to 4.9 mg per kg steviol equivalents, which is higher than the JECFA-established ADI of 4.0 mg per kg bw per day. Given that the investigational dose is higher than the accepted ADI for steviol glycosides and the history of safe use of steviol glycosides, including the paucity of reported case studies regarding the concomitant ingestion of steviol glycosides and probenecid and glimiperide, ZCHT concludes that the use of steviol glycosides as proposed herein remains safe for the general population and agrees with Zhou et al. (2019) that care should be exercised in the small subset of the population for which probenecid and glimiperide are prescribed. However, more data is necessary to draw any specific conclusions about their findings.

Halasa et al. (2020) published a case study vignette on the investigation of the presence of steviol glycosides metabolites in plasma, cerebrospinal fluid, amniotic fluid, and cord blood in samples collected as early as 2004. The end date was not provided. Steviol glucuronide, the primary steviol metabolite, was detected in all types of samples, but was observed primarily in the plasma. Of the samples, seven of the 38 adults (18%) had detectable steviol glucuronide concentrations, while two of 13 (15%) amniotic fluid samples and one of 15 (7%) cord blood samples contained steviol glucuronide. The authors noted that steviol glucuronide was detected only in samples obtained in and after 2008, which corresponds to the dates of the first GRAS notices submitted to FDA for steviol glycosides.

It should be noted that Halasa et al. (2020) did not discuss their findings in relation to the time of consumption and intake levels of steviol glycosides. As steviol glucuronide is a known metabolite of steviol glycosides and is expected to be present in plasma following steviol glycosides ingestion, this study serves to support previous published findings.

Stamataki et al. (2020) conducted a randomized, controlled, open-label parallel arm trial on the effects of daily stevia consumption on glycemia in healthy adults. Twenty-eight subjects (ages 18 to 40 years) consumed 5 drops of SweetLeaf Stevia Sweet Drops Clear[16] (n=14, Wisdom Natural Brands, USA) twice daily for 12 weeks. The control group (n=14) maintained their usual diet. There were no significant differences in the glucose or insulin responses between groups after glucose ingestion. The authors noted that participant withdrawals were not due to study-related adverse effects.

ZCHT reviewed the publication and conclude that no safety concerns were raised by the Stamataki et al. (2020) study.

Recent studies in rats have been identified in the published literature. Assi et al. (2020) investigated the use of an ethanolic extract of dried *S. rebaudiana* leaves (chemical composition not reported) to treat diabetic rats. No adverse effects or unplanned animal deaths were reported.

[16] While no composition information was provided by Stamataki et al. (2020), the product label for SweetLeaf Stevia Sweet Drops SteviaClear® lists the following ingredients: vegetable glycerin, purified water, stevia leaf extract, water soluble dietary fiber. (Available at: https://shop.sweetleaf.com/collections/all-sweet-drops/products/sweetleaf-sweetdrops-steviaclear-1oz, Accessed on November 6, 2020).

Cho et al. (2018) investigated the impact of stevia and obesity on fertility and reproductive outcomes in Sprague Dawley rats. Rats were administered 2-3 mg per kg bw per day rebaudioside A in drinking water starting two weeks prior to mating and throughout lactation. The authors reported that obese rats supplemented with rebaudioside A displayed a lower fertility index than untreated obese rats (53.3% vs. 85.7%, respectively); however, the rate of successful pregnancies was higher in obese rats supplemented with rebaudioside A than untreated obese rats (100% vs. 60.7%). No animal deaths were reported.

A follow-up study examined the impact of maternal low-dose rebaudioside A consumption on adiposity, glucose tolerance, gut microbiota, and the mesolimbic pathway in obese dams and their offspring (Nettleton et al., 2020). Pregnant obese rats and their offspring were fed a high fat/sucrose diet plus 3 mg per kg bw per day rebaudioside A (Sigma-Aldrich) through 18 weeks postpartum. The authors noted that rebaudioside A consumption reduced the fertility of dams, as previously reported (Cho et al., 2018). The study supports findings that low-calorie sweeteners may not be metabolically inert. No animal deaths were reported.

ZCHT notes that the effect of steviol glycosides on fertility and reproductive outcomes has been the subject of a number of investigations as discussed further in Appendix 10, and that recent publications by Cho et al. (2018) and Nettleton et al. (2020) corroborate previous findings by Planas and Kuć (1968), where 5% crude stevia leaf extract was observed to reduce fertility to 21% in female rats.

The effects of non-nutritive low-calorie sweeteners on gut microbiota were reviewed by Plaza-Diaz et al. (2020). It was noted that there have been no reports of negative interactions between steviol glycosides and colonic microbiota; however, it is possible that steviol glycosides modify the gut microbiota. The authors note further studies are necessary to “clarify its specific effects.”

A recent review by Ray et al. (2020) focused on the effects of *Stevia rebaudiana* on glucose homeostasis, blood pressure, and inflammation. The authors reported that no hypersensitivities or allergies were reported since 2008, and that the few prior reports were for “improperly filtered stevia extracts.” Furthermore, Ray et al. noted that additional randomized controlled trials are needed to confirm the beneficial effects of stevia. No significant adverse effects were noted in any study included in the review.

Zhao et al. (2020) reported that stevioside improved hyperglycemia-induced cardiac dysfunction in male C57BL/6 mice. Stevioside supplementation reduced the expression levels of cardiac fibrosis producing lysyl oxidase family and weakened the collagen cross-linking lysyl oxidase-like 2 caused by hyperglycemia, as well as promoted the elimination of existing fibrosis via the regulation of matrix metalloproteinase and tissue inhibitors of metalloproteinase. No adverse events or unplanned deaths were reported.

The effect of steviol on cytotoxicity, adipogenesis, ROS concentration, and gene expression were studied in the murine 3T3-L1 cell line. Kurek et al. (2020) reported that there was no observed

effect on the proliferation of cells, lipid accumulation, or intracellular ROS generation at steviol concentrations up to 100 μM. Furthermore, it was reported that steviol reduced the expression of genes regulating the adipogenesis and lipogenesis process. Results of this study further support the safety of steviol—and by extension—steviol glycosides.

Abolhasani et al. (2020) evaluated the *in vitro* cytotoxicity of stevioside on cancerous liver (HepG2), colon (HT29), and breast (MCF7) cells, as well as normal kidney cells (Hek293), compared with cisplatin. Stevioside was reported to display higher cell growth inhibition on the HepG2 cell line and was not observed to have high toxicity on the Hek293 normal cell line. The authors concluded that stevioside "showed less cytotoxic effects compared to cisplatin" (abstract only).

ZCHT agrees with the safety conclusions of the 66 GRAS Expert Panels in the notices for steviol glycosides previously submitted to FDA that resulted in "no questions" responses from FDA, JECFA (WHO, 2006; WHO, 2008), and Renwick (2008) that a sufficient number of good quality health and safety studies exist to support the determination that purified preparations of steviol glycosides, when added to food at levels up to full replacement of sucrose on a sweetness equivalency basis, meet FDA's definition of safe.

ZCHT concludes that it is reasonable to apply the JECFA ADI of 4 mg per kg bw per day for steviol glycosides (expressed on a steviol basis) to SoPure Stevia™ glucosylated steviol glycosides. Therefore, with the steviol equivalence values shown in Table 10, ZCHT concludes that the estimated maximum daily intake of the SoPure Stevia™ preparations described herein is 9.90 mg per kg bw or 3.47 mg per kg expressed as steviol equivalents. Based upon these calculations, the intake of ZCHT's SoPure Stevia™ glucosylated steviol glycosides preparation described herein safely aligns with the 4 mg per kg bw per day ADI expressed as steviol equivalents as determined by JECFA.

The raw material steviol glycosides extract used to manufacture ZCHT's SoPure Stevia™ glucosylated steviol glycosides preparations are contain a minimum of 95% total steviol glycosides. The finished glucosylated steviol glycosides preparations are a mixture of glucosylated steviol glycosides, unreacted steviol glycosides, and unreacted dextrin, with compositions of ≥80% total steviol glycosides with ≥75% glucosylated steviol glycosides and ≤20% dextrin (SoPure Stevia™ GSG 80) and ≥95% total steviol glycosides with ≥75% glucosylated steviol glycosides and ≤5% dextrin (SoPure Stevia™ GSG 95). Given the structural similarities with rebaudioside A, stevioside, and other steviol glycosides, and considering analogous metabolic pathways for all these substances, the safety data on stevia and its other components have a direct bearing on the present safety assessment for SoPure Stevia™ glucosylated steviol glycosides. This is further supported by over a decade and a half of scientific studies on the safety of these substances, along with the fact that the major regulatory bodies view the results of toxicology studies on either stevioside or rebaudioside A as applicable to the safety assessment of all known steviol glycosides, since all are metabolized and excreted by similar pathways, with steviol being the

common metabolite for each. The foundational safety of rebaudioside A, other steviol glycosides and steviol has been summarized, with key studies summarized in Appendix 10.

Furthermore, ZCHT has reviewed this safety information and has concluded that SoPure Stevia™ glucosylated steviol glycosides preparations are generally recognized as safe for the proposed uses.

C. GRAS Criteria

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

> "…reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use."[17]

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

Furthermore, in discussing GRAS criteria, FDA notes that:

> "…General recognition of safety requires common knowledge, throughout the expert scientific community knowledgeable about the safety of substances directly or indirectly added to food, that there is reasonable certainty that the substance is not harmful under the conditions of its intended use."
>
> "'Common knowledge' can be based on either "scientific procedures" or on experience based on common use of a substance in food prior to January 1, 1958."[18]

FDA discusses in more detail what is meant by the requirement of general knowledge and acceptance of pertinent information within the scientific community, i.e., the so-called "common knowledge element," in terms of the two following component elements:[19]

- Data and information relied upon to establish safety must be generally available, and this is most commonly established by utilizing published, peer-reviewed scientific journals; and
- There must be a basis to conclude that there is consensus (but not unanimity) among qualified scientists about the safety of the substance for its intended use, and this is

[17] See 21 CFR 170.3 (e)(i) and 81 FR 54959 Available at: https://www.federalregister.gov/documents/2016/08/17/2016-19164/substances-generally-recognized-as-safe (Accessed on 6/22/20).
[18] See 81 FR 54959 Available at: https://www.federalregister.gov/documents/2016/08/17/2016-19164/substances-generally-recognized-as-safe (Accessed on 10/12/2020).
[19] See Footnote 1.

established by relying upon secondary scientific literature such as published review articles, textbooks, or compendia, or by obtaining opinions of expert panels or opinions from authoritative bodies, such as JECFA and the National Academy of Sciences.

General recognition of safety based upon scientific procedures shall require the same quantity and quality of scientific evidence as is required to obtain approval of a food additive. General recognition of safety through scientific procedures shall be based upon the application of generally available and accepted scientific data, information, or methods, which ordinarily are published, as well as the application of scientific principles, and may be corroborated by the application of unpublished scientific data, information, or methods.

The apparent imprecision of the terms “appreciable,” “at the time,” and “reasonable certainty” demonstrates that the FDA recognizes the impossibility of providing absolute safety in this or any other area (Lu, 1988; Renwick, 1990; Rulis and Levitt, 2009).

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

D. Expert Panel Findings on Safety of SoPure Stevia™ Glucosylated Steviol Glycosides

An evaluation of the safety and GRAS status of the intended use of ZCHT’s SoPure Stevia™ GSG 80 (≥80% total steviol glycosides with ≥75% glucosylated steviol glycosides and ≤20% dextrin) and GSG 95 (≥95% total steviol glycosides with ≥75% glucosylated steviol glycosides and ≤5% dextrin) preparations has been conducted by an Expert Panel convened by GRAS Associates. The Panel consisted of Robert Kapp, Ph.D., Fellow Academy of Toxicological Sciences (ATS), Fellow Royal Society of Biology (FRSB) & European Registered Toxicologist (ERT, UK); Kara Lewis, Ph.D.; and Katrina Emmel, Ph.D., as Panel Chair. The Expert Panel reviewed ZCHT’s dossier as well as other publicly available information. The individuals who served as Expert Panelists are qualified to evaluate the safety of foods and food ingredients by merit of scientific training and experience.

The GRAS Expert Panel report is provided in Appendix 13.

E. Common Knowledge Elements for GRAS Conclusions

The first common knowledge element for a GRAS conclusion requires that data and information relied upon to establish safety must be generally available; this is most commonly established by utilizing studies published in peer-reviewed scientific journals. The second common knowledge element for a GRAS conclusion requires that consensus exist within the broader scientific community.

1. Public Availability of Scientific Information

The majority of the studies reviewed on steviol glycosides and steviol have been published in the scientific literature as summarized in Appendix 10. Most of the literature relied upon by JECFA has also been published---most importantly the chronic rat studies on steviol glycosides. JECFA did make limited use of unpublished studies, and they were summarized in the two JECFA monographs. Moreover, JECFA publicly releases the results of their safety reviews, and their meeting summaries and monographs are readily available on their website.

With regard to the safety documentation, the key pharmacokinetic data establish that steviol glycosides are not absorbed through the gastrointestinal (GI) tract, *per se*; they are converted to steviol by bacteria normally present in the large intestine, and the steviol is absorbed but rapidly metabolized and excreted (Gardana et al., 2003; Koyama et al., 2003b). The action of bacteria in the large intestine is directly supported by the published study that showed that steviol glycosides can be converted to steviol in the large intestine by normal anaerobic GI flora as demonstrated by an *in vitro* study in fecal homogenates (Koyama et al., 2003b; Renwick and Tarka, 2008).

The ADI for steviol glycosides has been set largely based on a published chronic study in rats (Toyoda et al., 1997) and several published clinical studies that report no pharmacological effects in humans at doses several fold higher than the ADI (Barriocanal et al., 2006; Barriocanal et al., 2008; Wheeler et al., 2008). As mentioned above, Roberts et al. (2016) noted that the ADI could be higher using a chemical-specific adjustment factor (CSAF), as defined by the WHO in 2005, determined by comparative studies in rats and humans, which they conclude can justify an ADI value of 6-16 mg per kg bw per day for steviol glycosides.

The toxicity of the metabolite, steviol, has been well reviewed in the published literature (Geuns, 2003; WHO, 2006; Urban et al., 2013).

In addition, there is a large, publicly available, collection of GRNs regarding steviol glycosides on FDA's website.

2. Scientific Consensus

The second common knowledge element for a GRAS conclusion requires that there be a basis to conclude that consensus exists among qualified scientists about the safety of the substance for its intended use.

A number of well-respected regulatory agencies, including JECFA, EFSA, FSANZ, the Switzerland Office of Public Health, and Health Canada, as well as numerous well-respected individual scientists, have indicated that steviol glycosides are safe for human consumption at doses in the range of the JECFA ADI (FAO, 2010; EFSA, 2010; FSANZ, 2008; Switzerland Federal Office of Public Health, 2008; Health Canada, 2012; Xili et al., 1992; Toyoda et al., 1997; Geuns, 2003; Williams, 2007). Since December 2008, over sixty-five GRAS notifications have been submitted to

FDA for highly purified stevia-derived sweetener products, and FDA detailed reviews have consistently yielded “no questions” letters.

In summary, a compelling case can be made that scientific consensus exists regarding the safety of steviol glycosides when of sufficiently high purity. The central role of conversion to steviol and subsequent elimination with these naturally occurring steviol glycosides extends to the manner in which the various steviol glycosides molecules are metabolized and eliminated from the body. While the scientific conclusions are not unanimous regarding the safe human food uses of steviol glycosides, ZCHT believes that a wide consensus does exist in the scientific community to support a GRAS conclusion as evidenced by several publications (Carakostas, 2012; Geuns, 2007; Urban et al., 2013; Waddell, 2011; Williams, 2007; Brusick, 2008) that refute safety concerns expressed by a minority of scientists. Roberts et al. (2016) suggests that the ADI could be higher than has been previously accepted by the scientific community.

F. Conclusion

In consideration of the aggregate safety information available on naturally occurring steviol glycosides, ZCHT concludes that SoPure Stevia™ GSG 80 (≥80% total steviol glycosides with ≥75% glucosylated steviol glycosides and ≤20% dextrin) and GSG 95 (≥95% total steviol glycosides with ≥75% glucosylated steviol glycosides and ≤5% dextrin) defined in the subject notification are safe for use as a general purpose non-nutritive sweetener in foods other than infant formulas and meat and poultry products and as a flavor modifier at maximum recommended use levels of 600 mg per kg in foods and 1,500 mg per kg in chewing gum. The JECFA ADI for steviol glycosides of 4 mg per kg bw per day (as steviol equivalents) can be applied to ZCHT’s SoPure Stevia™ glucosylated steviol glycosides preparations. Based on published dietary exposure data for other approved sweeteners and adjusting for relative sweetness intensity, intake was estimated for healthy non-diabetic children and adults, and diabetic children and adults with the following findings.

The worst-case estimated intakes of ZCHT’s SoPure Stevia™ glucosylated steviol glycosides preparations for several population groups summarized in Part 3.A. are no greater than 3.47 mg per kg steviol equivalents per bw per day, which is well below the ADI of 4 mg per kg bw expressed as steviol equivalents as established by JECFA. The dietary levels from anticipated food consumption are not likely to exceed the ADI when ZCHT’s SoPure Stevia™ glucosylated steviol glycosides are used as a general non-nutritive sweetener and as a flavor.

Accordingly, SoPure Stevia™ GSG 80 (≥80% total steviol glycosides with ≥75% glucosylated steviol glycosides and ≤20% dextrin) and GSG 95 (≥95% total steviol glycosides with ≥75% glucosylated steviol glycosides and ≤5% dextrin) as produced by ZCHT and declared within the subject notification meet FDA’s definition of safety in that there is “reasonable certainty of no harm under the intended conditions of use” as described herein and, therefore, are generally recognized as safe (GRAS).

PART 7. LIST OF SUPPORTING DATA AND INFORMATION IN THE GRAS NOTICE.

A. References

1. List of Acronyms

AAS	Atomic Absorption Spectrometry
ACE	Angiotensin-converting enzyme
ADI	Acceptable Daily Intake
ADME	Absorption, Distribution, Metabolism and Excretion
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ATS	Academy of Toxicological Sciences
AUC	Area under the plasma-concentration time curve
AVA	Agri-food and Veterinary Authority of Singapore
BP	Blood pressure
bw	Body Weight
CFR	Code of Federal Regulations
cfu	Colony Forming Unit
CGMP	Current Good Manufacturing Practice
ChP	Chinese Pharmacopeia
C_{max}	Maximum serum concentration
CSAF	Chemical-Specific Adjustment Factor
CYP	Cytochrome P450
DBP	Diastolic blood pressure
DNA	Deoxyribonucleic Acid
EDI	Estimated daily intake
EFSA	European Food Safety Authority
EU	European Union
FCC	Food Chemicals Codex
FD&C	Federal Food Drug and Cosmetics Act
FDA	Food and Drug Administration
FEMA	Flavor and Extract Manufacturers Association
FOIA	Freedom of Information Act
FRSB	Fellow Royal Society of Biology
FSANZ	Food Standards Australia New Zealand
FSSAI	Food Safety and Standards Authority of India
g	Gram
GA	GRAS Associates
GB	Guobiao standard (Chinese national standard)
GB/T	Guobiao standard/recommended (Chinese national standard/recommended)
GEMS	Global Environment Monitoring System
GGT	Gamma-glutamyltransferase
GI	Gastrointestinal
GRAS	Generally Recognized as Safe
GRNs	GRAS Notices
HbA1c	Glycated hemoglobin
HEK293	Human embryonic kidney 293
hOAT3	Human organic anion transporter 3
HPLC	High performance liquid chromatography
IADSA	International Alliance of Dietary/Food Supplement Associations
INS	International Numbering System

JECFA	Joint FAO/WHO Expert Committee on Food Additives
kg	Kilogram
LC_{50}	Median lethal concentration
LD_{50}	Median lethal dose
mg	Milligram
mL	Milliliter
MPL	Maximum permitted level
mRNA	Messenger ribonucleic acid
MW	Molecular Weight
NA	Not applicable
ND	Not detected
ng	Nanogram
NHANES	National Health and Nutrition Examination Surveys
NHFPC	National Health and Family Planning Commission (P.R. China)
NHPs	Natural Health Products
NMT	Not more than
NOAEL	No observed adverse effect level
NS	Not specified
OAT3	Organic anion transporter 3
OECD	Organisation for Economic Co-operation and Development
ppm	Parts per million
Reb A	Rebaudioside A
Reb M	Rebaudioside M
rOAT3	Rat organic anion transporter 3
SBP	Systolic blood pressure
TAC	Total antioxidant capacity
TFC	Total flavonoid content
TPC	Total phenolic content
TRAP	Tartrate-resistant alkaline phosphatase
UDS	Unscheduled DNA synthesis
ug	Microgram
WHO	World Health Organization
WHO/JECFA	World Health Organizaiton/Joint FAO/WHO Expert Committee on Food Additives

2. References

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B. Appendices

Appendix 1 Specifications and Certificates of Analyses for Raw Materials and Production Processing Aids

Appendix 1.1 Steviol Glycosides Extract

Appendix 1.2 Dextrin

Appendix 1.3 Toruzyme 3.0L Cyclodextrin Glucosyltransferase Enzyme

Appendix 1.4 Sunresin Statement

Appendix 1.5 Adsorption Resin

Appendix 1.1 Steviol Glycosides Extract

ZHUCHENG HAOTIAN PHARM CO.,LTD.
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Http://www.zcht.cc

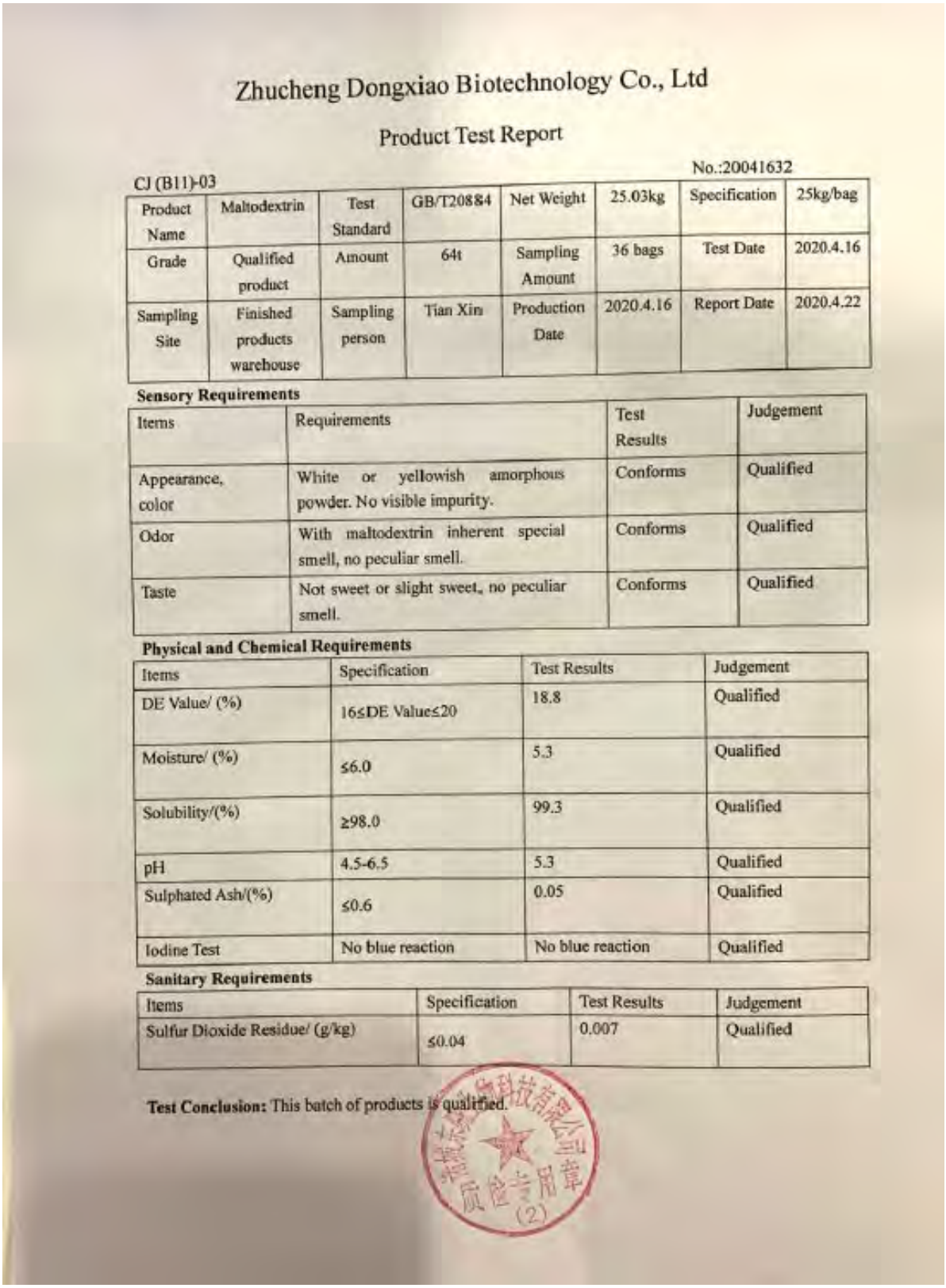
PRODUCT SPECIFICATION

Product Name: **Steviol Glycosides （Total Steviol Glycosides≥95%）**

Plant Source: **Stevia Rebaudiana (Bertoni)**

Item	Method	Specification
Appearance	Visual	White to off-white fine powder
Taste	Organoleptic	Sweet
Total Steviol Glycosides (Dry Basis, %)	JECFA 2017	Not less than 95.0
Loss On Drying (%)	JECFA 2017	Not more than 6.0
Ash (%)	JECFA 2017	Not more than 1.0
pH, 1% in Water	JECFA 2017	Not less than 4.5; Not more than 7.0
Arsenic (As)	AAS ChP2015 Part4, (2321)	Not more than 1.0 ppm
Cadmium (Cd)	AAS ChP2015 Part4, (2321)	Not more than 1.0 ppm
Lead (Pb)	AAS ChP2015 Part4, (2321)	Not more than 0.5 ppm
Mercury (Hg)	AAS ChP2015 Part4, (2321)	Not more than 0.1 ppm
Residual Solvents	JECFA 2017	Methanol, Not more than 200 ppm
		Ethanol, Not more than 5000 ppm
Total Aerobic Bacteria	ChP 2015 Part4 (1105)	Not more than 10^3 cfu/g
Mold& Yeast	ChP 2015 Part4 (1105)	Not more than 10^2 cfu/g
E.Coli	ChP 2015 Part4 (1106)	Negative/ g
Salmonella	ChP 2015 Part4 (1106)	Negative/25g
Packaging/Storage	Double food grade polyethylene bags or foil bag Store under clean dry conditions. Keep package tightly closed while not in use.	
Country of Origin	China	
Shelf Life Expectancy	3 Years	

Appendix 1.2 Dextrin



Zhucheng Dongxiao Biotechnology Co., Ltd

Product Test Report

No.:20041632

CJ (B11)-03

Product Name	Maltodextrin	Test Standard	GB/T20884	Net Weight	25.03kg	Specification	25kg/bag
Grade	Qualified product	Amount	64t	Sampling Amount	36 bags	Test Date	2020.4.16
Sampling Site	Finished products warehouse	Sampling person	Tian Xin	Production Date	2020.4.16	Report Date	2020.4.22

Sensory Requirements

Items	Requirements	Test Results	Judgement
Appearance, color	White or yellowish amorphous powder. No visible impurity.	Conforms	Qualified
Odor	With maltodextrin inherent special smell, no peculiar smell.	Conforms	Qualified
Taste	Not sweet or slight sweet, no peculiar smell.	Conforms	Qualified

Physical and Chemical Requirements

Items	Specification	Test Results	Judgement
DE Value/ (%)	16≤DE Value≤20	18.8	Qualified
Moisture/ (%)	≤6.0	5.3	Qualified
Solubility/(%)	≥98.0	99.3	Qualified
pH	4.5-6.5	5.3	Qualified
Sulphated Ash/(%)	≤0.6	0.05	Qualified
Iodine Test	No blue reaction	No blue reaction	Qualified

Sanitary Requirements

Items	Specification	Test Results	Judgement
Sulfur Dioxide Residue/ (g/kg)	≤0.04	0.007	Qualified

Test Conclusion: This batch of products is qualified.

Appendix 1.3 Toruzyme 3.0L Cyclodextrin Glucosyltransferase Enzyme

Certificate of Analysis
分析证书

novozymes
Rethink Tomorrow

Date 证书打印日期
Jan 03, 2020
Purchase order item 客户采购定单号
ENZ-20-03
Delivery 送货号
7918658
Sales order 销售定单号
5755041

Sold-to 客户名及地址

无锡市联合恒洲化工有限公司
无锡
江苏无锡市南长区清扬路123号金阳大厦509/513-520室

Ship-to 收货单位
无锡市恒慧科贸有限公司
无锡
无锡市北郊塘头通贸路中化无锡塘头仓库

Material: **Toruzyme 3.0 L**
产品名: 诺维信环糊精葡萄糖苷转移酶3.0 L

Batch: **ACN00255** Quantity: **600 KG**
批号 数量
Production Date: **Jul 31, 2019** Best before **Jul 30, 2021**
生产日期: 保质期:

Characteristic 分析项目	Unit 单位	Value 结果
环麦芽糊精葡萄糖苷基转移酶单位 KNU-CP Cyclod.Glycosyl Transf. KNU-CP	/g	3.37
pH值为25° C pH at 25° C		6.60
密度 Density	g/ml	1.033
菌落总数 Total viable count	/g	< 100
大肠菌群 Colliform bacteria	/g	< 4
大肠杆菌 E.coli		未检出/25g Not Detected, 25 g
沙门氏菌 Salmonella		未检出/25g Not Detected, 25 g

本产品同时符合食品化学品法典（FCC）和联合国粮农组织(FAO)/世界卫生组织(WHO)食品添加剂联合专家委员会（JECFA）推荐的食品级酶制剂纯度标准。
This product meet the requirements of food grade enzyme recommended by FCC and FAO/WHO JECFA.
产品符合《GB1886.174-2016 食品安全国家标准 食品添加剂 食品工业用酶制剂》关于砷、铅的指标要求。 **This product meet the requirements of Arsenic and Lead according to GB 1886.174-2016.**
砷：≤3mg/kg；铅：≤5mg/kg； **Arsenic ≤ 3mg/kg, Lead ≤ 5mg/kg**
产品抗菌活性未检出。
产品合格。

Novozymes (China)
No.160, Sun Hui Road
天津经济技术开发区

1 / 2

Certificate of Analysis
分析证书

Delivery
7918658

novozymes
Rethink Tomorrow

Ship-to 收货单位
无锡市恒懋科贸有限公司
无锡
无锡市北郊塘头通贸路中化无锡塘头仓库

The product complies with current FAO/WHO JECFA and FCC recommended purity specifications for food grade enzymes.

Quality Assurance
质量保证部

Novozymes (China)
Biotechnology Co., Ltd.
诺维信(中国)生物技术有限公司

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2 / 2

Appendix 1.4 Sunresin Statement

SUNRESIN

Sunresin-China biggest special resin manufacturer

Certificate

Product: Sunresin resins

Manufacturer: **Sunresin New Materials Co. Ltd., Xi'an**

To whom it may concern:

We hereby certificate that main components of sunresin resins are DVB、styrene.
The procedure of production was strictly made accordingly to standard of FDA 21CFR § 173.65,AP(97)1 and § 173.25.

The product is confirmed suiting for using in food processing.

Sunresin New Materials Co. Ltd., Xi'an

July 29th 2020

Appendix 1.5 Adsorption Resin

PONY 谱尼测试
Pony Testing International Group

CMA
160000343608

检 测 报 告
(Test Report)

No. GNAW6CUA1F4005334

样品名称 (Sample Description)	食品工业用吸附树脂 (Adsorption resin for food industrialuse)
委托单位 (Applicant)	西安蓝晓科技新材料股份有限公司 (Sunresin New Materials Co.Ltd.Xi'an)

PONY 谱尼测试
Pony Testing International Group
www.ponytest.com

PONY 谱尼测试 Pony Testing International Group

检 测 结 果
(Test Results)

No. GNAW6CUA1F4005334 第 1 页，共 2 页 (page 1 of 2)

样品名称 (Sample Description)	食品工业用吸附树脂 (Adsorption resin for food industrialuse)	样品规格 (Sample Specification)	——
委托单位 (Applicant)	西安蓝晓科技新材料股份有限公司 (Sunresin New Materials Co.Ltd.Xi'an)	商标 (Trade Mark)	seplite
到样日期 (Received Date)	2019-03-12	生产日期或批号 (Manufacturing Date or Lot No.)	2019-36P
检测日期 (Test Date)	2019-03-12~2019-03-20	样品等级 (Sample Grade)	食品级 (Food grade)
样品状态 (Sample Status)	固态（Solid）	检测类别 (Test Type)	委托检测 (Commissioning Test)
检测项目 (Test Items)	见下页 See next page	检测环境 (Test Environment)	符合要求 (To meet the requirements)
检测方法 (Test Methods)	见下页 See next page		
所用主要仪器 (Main Instruments)	气相色谱仪 GC 等		
备注 (Note)	1.样品来源：客户提供 Sample From: Customer supply 2.以上样品信息由委托单位提供 The information of sample was provided by the applicant. 3.该报告中检测方法由委托单位指定。 The testing methods mentioned in this report were designated by the client. 4.限值标准：GB/T 24395-2009 The limit basis of GB/T 24395-2009		

PONY专用章 (Special Stamp of PONY)	编制人 (Edited by)	
	审核人 (Checked by)	
	批准人 (Approved by)	
	签发日期 (Issued Date)	2019 年 03 月 22 日

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PONY 谱尼测试
Pony Testing International Group

检 测 结 果
(Test Results)

No. GNAW6CUA1F4005334 第 2 页，共 2 页 (page 2 of 2)

样品名称和编号 (Sample Description and Number)	检测项目 (Test Items)	单位 (Unit)	限值 (Limit)	检测结果 (Test Results)	单项结论 (Evaluation)	检测方法 (Test Methods)
A1F4005334 食品工业用吸附树脂 (Adsorption resin for food industrialuse)	重金属（以 Pb 计，干基）质量分数 Heavy metals (Pb, dry basis) mass fraction	%	≤0.0015	<0.0015	符合 (Pass)	GB/T 24396-2009 5.2
	苯 (Benzene)	mg/kg	≤2	未检出 (Not detected) (<0.6)	符合 (Pass)	GB/T 24396-2009 5.3
	1,2-二氯乙烷 (1,2-dichloroethane)	mg/kg	≤2	未检出 (Not detected) (<0.6)	符合 (Pass)	GB/T 24396-2009 5.3
	丙烯腈 (Acrylonitrile)	mg/kg	≤10	未检出 (Not detected) (<3.0)	符合 (Pass)	GB/T 24396-2009 5.3
	氯苯 (Chlorobenzene)	mg/kg	≤10	未检出 (Not detected) (<3.0)	符合 (Pass)	GB/T 24396-2009 5.3
	二乙烯苯 (Divinyl benzene)	mg/kg	≤10	未检出 (Not detected) (<3.0)	符合 (Pass)	GB/T 24396-2009 5.3
	甲苯 (Toluene)	mg/kg	≤20	未检出 (Not detected) (<6.0)	符合 (Pass)	GB/T 24396-2009 5.3
	苯乙烯 (Styrene)	mg/kg	≤20	未检出 (Not detected) (<6.0)	符合 (Pass)	GB/T 24396-2009 5.3
	二甲苯 (Xylene)	mg/kg	≤20	未检出 (Not detected) (<6.0)	符合 (Pass)	GB/T 24396-2009 5.3
	甲基丙烯酸甲酯 (Methyl methacrylate)	mg/kg	≤20	未检出 (Not detected) (<6.0)	符合 (Pass)	GB/T 24396-2009 5.3

——以下空白——
(End of Report)

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Appendix 2 Method of Analysis

Analytical Method of Glucosyl Steviol Glycosides (Translated)

Issued by: National Health and Family Planning Commission (P.R.China)
File Number: Number 8, 2016

Date Issued: June 15, 2016

English Name: Glucosyl Steviol Glycosides
Function Category: Food flavoring
(1) The dosage and the scope of use
It is formulated as a food flavoring and used in various kinds of food (except food categories listed in GB2760-2014 Table B.1) , and the dosage should be used appropriately according to production needs.
(2) Quality specification requirements

1. Scope
The specification requirements apply to the glucosyl steviol glycosides, the food additive which uses *Stevia rebaudiana* Bertoni leaves as a raw material. The stevia extract from the leaves are glycosylated by an enzyme, then concentrated by evaporation and subsequently spray-dried.

2. Technical requirements

2.1 Sensory requirements. Must meet the requirements of Table 1.

Table 1. Sensory Requirements

Items	Standard	Test Method
Color	White or light yellow color	Take appropriate sample in a clean, dry glass, observe the color and status under natural light
Appearance	Powder	

2.2 Physicochemical index： Must conform to the requirements of Table 2.

Table 2. Physical and Chemical Index

Item	**Standard**	**Test Method**
Glucosyl Steviol Glycosides（GSG）, *w*/% , ≥	75.0	Appendix A.3
Reb A+ Stevioside, *w*/% , ≤	6.0	
Reb A， *w*/% ≤	4.0	
Stevioside, *w*/% ≤	4.0	
Maltodextrin, *w*/% ≤	20.0	
Rotation	+65°~ +75°	GB/T 14454.5
Relative density	0.2~0.6	GB/T 11540
pH	4.5~7.0	GB/T 9724

Appendix A
Test method

A.1 General Provisions

The reagents and water used in the requirements of this quality specification refer to analytical reagents and Level 3 Water specified in GB/T 6682 unless other requirements are specified. The solution used in the test refers to an aqueous solution when it is not specified which solvent is used for preparation.

A.2 Identification Test

White or light yellow powder, soluble in water, slightly soluble in ethanol.

A.3 Test Method of Glucosyl Steviol Glycosides, Steviol Glycosides, and Maltodextrin

A.3.1 Principle

The total steviol glycoside content (TSG) , residual maltodextrin (RD) , unreacted steviol glycosides and glucosyl steviol glycoside ratio can be determined by adsorption chromatography and high performance liquid chromatography .

A.3.2 Scope

The scope applies to a mixture with a composition of α-1,4-glucosyl steviol glycosides（GSG）and steviol glycosides

at a content range of 60-102% solid sample on a dry basis.

A.3.3 Equipment and Reagents

A.3.3.1 High performance liquid chromatography（HPLC）equipment should be equipped with a dual pump, automatic sampler, column temperature box and Diode-Array detector (DAD), interface and data acquisition software.
A.3.3.2 HPLC amino column, 4.6mm x 250mm, 5μm particle
A.3.3.3 Accuracy of 0.0001 g analytical balance
A.3.3.4 Karl-Fischer coulomb titrimeter
A.3.3.5 Laboratory vacuum rotary evaporator
A.3.3.6 Vacuum oven
A.3.3.7 Moisture meter
A.3.3.8 Vacuum solvent filtration system, all glass
A.3.3.9 Vacuum filter system: Polypropylene material, 0.2μm， 47mm
A.3.3.10 Class A volumetric flask and a pipette
A.3.3.11 A glass column filled up with 200ml of macroporous adsorption resin (Inside diameter, 25mm)
A.3.3.12 Acetonitrile, HPLC grade
A.3.3.13 Water, HPLC grade
A.3.3.14 Ethanol, reagent grade, system device, or other equivalents
A.3.3.15 Reb-A standard sample
A.3.3.16 Stevioside standard sample
A.3.3.17 Reb-C standard sample
A.3.3.18 Reb-F standard sample
A.3.3.19 Dulcoside A standard sample
A.3.3.20 Rubusoside standard sample
A.3.3.21 Ammonium acetate, reagent grade
A.3.3.22 Glacial acetic acid, reagent grade

A.3.4 Safety Precautions

A.3.4.1 When handling materials, clean up spilled liquid and waste. Always follow the hazardous chemical materials safety measures and emergency response principles.
A.3.4.2 For the chemicals used in the above steps, all precautions and hazard precautions listed in the material safety data sheet should be followed.
A.3.4.3 Stevia glycosides, usually in the powdered form, during the process of jittering, feeding and stirring easily produces dust, which may be inhaled into the mouth and nose to produce discomfort. Therefore, it is necessary to exercise caution to avoid producing dust.

3.5 Procedure

A.3.5.1 TSG

Test solution: Weigh about 5g GSG accurately and dissolve in 250 ml water. At a rate of less than 15 ml/min, add the solution to a glass column containing 200 ml of macroporous resin, and then flush the resin with 1000 ml water. At a rate of about 15 ml/min or less, use 1000 ml of ethanol 50 % (volume) to elute the steviol glycosides adsorbed. Then evaporate the collected ethanol elution. Wash liquid separately and dry, and then place into a vacuum oven for 2 hours at 105 ℃. The dry weight of each component must be weighed and recorded, with the content (%) TSG and RD calculated using the formula below.

TSG's mass fraction w_1 is calculated by the formula（A.1）, and the mass fraction of RD's content w_2 is calculated by（A.2）:

$$w_1 = \frac{m_1}{m_2 \times (100 - w_h) \times 10^{-2}} \times 100\% \quad \text{……（A.1）}$$

Where:

m_1——the total content of ethanol components after drying in grams (g)

m_2——wet weight of the original sample in grams (g)

w_h——moisture content （%）

$$w_2 = \frac{m_3}{m_2 \times (100 - w_h) \times 10^{-2}} \times 100\% \quad \text{……（A.2）}$$

Where:

m_3——the total water components after drying in grams (g)

m_2——wet weight of the original sample in grams (g)

w_h——moisture content（ %）

Acceptance criteria:

The sample recovery rate has to be between 98.0% and 102.0%. The sample recovery rate w_3 is calculated by（A.3）:

$$w_3 = w_1 + w_2 \quad \text{……（A.3）}$$

Where:

w_1——mass fraction of TSG's total content（%）

w_2——mass fraction of RD's content（%）

If the steviol glycoside content in the washing liquid is less than 10mg/L , the washing liquid must be tested by HPLC .

A.3.5.2 The Content of Unreacted Stevia Glycosides

Weigh about 3g GSG. Add it into the buffer solution（A.3.6.1.2）to dissolve and prepare a solution of 100ml as the test solution. HPLC method to determine the content of unreacted steviol glycosides (SG) should follow （A.3.6.1）. The chromatogram of the sample should match the example chromatogram. To calculate the content of α-Glucosyl Steviol Glycosides through the total content of the following stevia glycosides（A.3.5.1）, the mass fraction of α-Glucosyl Steviol Glycoside content w_α is calculated by:

$$w_\alpha = w_1 - w_4 \quad \text{……（A.4）}$$

Where:

w_1——mass fraction of TSG（%）

w_4——mass fraction of unreacted steviol glycosides（%）

A.3.5.3 Percentage of α-Glucosyl Steviol Glycosides

Weigh about 5g of GSG and dissolve in water to make a 100ml preparation, which is used as the test solution. HPLC analysis is based on the HPLC determination procedure (A.3.6.2) of glucosyl steviol glycosides to

determine the area ratio (%) of α- glucosyl steviol glycosides .

To calculate the percentage of α-Glucosyl Steviol Glycosides, according to（A.3.5.2）， the percentage of α-Glucosyl Steviol Glycosides w_5 is calculated by the formula (A5)：

$$w_5 = w_\alpha \times A_1 \times 10^{-2} \quad \text{……（A.5）}$$

Where：

w_α——mass fraction of the content of α-Glucosyl Steviol Glycosides（%）

A_1——area ratio of α-Glucosyl Steviol Glycosides

A.3.6 HPLC Analysis

A.3.6.1 HPLC Analysis of Steviol Glycosides

A.3.6.1.1 The Moisture Balance of Standard and Samples

Stevia glycosides are hydrophilic compounds. The standard sample and test samples should have the same moisture balance before analysis. The standard sample and test sample should be put in the same room with the analytical balance, and exposed to the air for not less than 24 hrs before weighing. Stir the powder intermittently to ensure uniform moisture. At the time of weighing, use the Karl Fischer coulomb titration instrument or other moisture meter to measure the moisture value of all standard samples. The moisture value of the sample should be tested at the temperature of 105 ℃ by the loss-in-drying method.

A.3.6.1.2 Preparation of Mobile Phase Solution

Prepare appropriate mobile phase solution volume accordingly.

Aqueous buffer（0.0125％acetic acid, 0.0125％Ammonium acetate）: The buffer is prepared by dissolving 0.125g ammonium acetate (NH4OAc) in 1 L water and 125 µL glacial acetic acid (acetic acid) .

Mobile phase (Acetonitrile: buffer): Mix the acetonitrile and the buffer to prepare the mobile solution (% volume) of acetonitrile and aqueous buffer ratio to 80:20. Wait until the solution reaches room temperature for degassing of the solution.

Diluent（100％buffer solution）: Filter 1000 mL of aqueous buffer, and use it immediately.

A.3.6.1.3 Preparation of Standard Solution

Reb A standard curve: Reb A curve is composed of five concentrations points between 200mg/L-2000mg/L. Weigh Reb A (moisture balanced) samples of 5 mg, 10 mg, 25 mg, 40 mg and 50mg（±2mg）separately. Use the diluent to dissolve them individually into 25 mL volumetric flasks and dilute to the volume exactly.

Stevioside standard curve: Stevioside calibration curve is composed of 7 points: 2.5mg/L, 5mg/L, 50mg/L, 100mg/L, 500mg/L, 1000mg/L and 2000mg/L. Prepare a standard stock solution of 2000mg/L stevioside similar to the Reb-A standard reference. Dilute to the desired concentration.

Steviol glycosides: Retention time marking solution (M6), each containing the following steviol glycosides of approximately 100 mg/L (Prepared with the diluent): rubusoside, dulcoside A, stevioside, Reb C, Reb F and Reb A. Prepare the sample solution according to section A.3.5.1 and section A.3.5.2.

A.3.6.1.4 Instrument conditions are shown in Table A.1

Table A.1 Instruments Usage Conditions

Chromatographic column	NH_2 Column，250 x 4.6 mm，5μm
Temperature	30°C
Isocratic mobile phase	20％buffer solution, 80％ acetonitrile
Flow rate	1.5 mL/min
Injection volume	12 μL
Detection wavelength	UV210 nm（4 nm bw），reference：260 nm （100 nm bw ）
Run time	60 min
Automatic injector temperature	Room temperature

A.3.6.1.5 Analytical Procedure

A.3.6.1.5.1 System Startup/Applicability

Detector sensitivity tests: Inject 2.5 mg/L of the stevioside standard solution. Confirm that the stevioside peak to noise ratio is ≥3. If not, check the device and confirm that the stevioside peak signal to noise ratio is ≥3 before going on to the next step.

Tailing factor: Inject the Reb-A 2000mg/L standard sample solution and use the peak to calculate the tailing factor –T, tailing factor 0.8≤T≤2.

Signal to Noise Ratio (SNR): Calculate the SNR of the stevioside standard solution. For the standard stevioside solution with the limit of detection (LOD) of 5 mg/L, the SNR of the standard solution must be ≥10. For the standard stevioside solution with the limit of detection (LOD) of 2.5 mg/L, the SNR of the standard solution must be ≥3.

Separate the steviol glycosides. Inject the M6 sample standard solution. The stevioside and Reb C's peaks should be obviously separated. Record retention time of each steviol glycoside. （ A.3.8.1 ）.

A.3.6.1.5.2 Analytical Sequence

After the system suitability check, all remaining standard solutions are injected sequentially according to the principle of concentration from low to high, followed by sample injection. After up to 12 sample injections and after the end of the sample analysis sequence, inject 2000m/l of the stevioside standard solution and Reb-A to back up and calibrate.

A.3.6.1.5.3 Integration Parameters

Use the software tool that comes with the liquid chromatograph to complete the integration.

A.3.6.1.6 Calculation

A.3.6.1.6.1 Relative Standard Deviation of Peak Area

Relative standard deviation of peak area r_1 is calculated by（A.6）：

$$r_1 = \frac{s_1}{x} \times 100\% \qquad \text{……(A.6)}$$

Where:

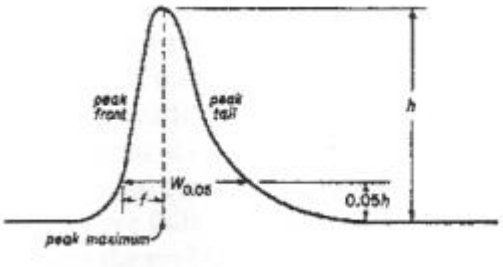
s_1——standard values of deviation = （（Σ（x-x）^{2}）/（N-1））$^{1/2}$

x——average value =（$x_1 + x_2 + x_3 + x_n$）/ N

x_n——peak area

N—— total number of samples

A.3.6.1.6.2 Tailing factor（T）



Tailing factor T is calculated by（A.7）:

$$T = \frac{W_{0.05}}{2f} \qquad \text{……(A.7)}$$

Where:

$W_{0.05}$——peak width at 5% height

f——the numerical distance from the max peak to the front peak at 5% height

A.3.6.1.6.3 The Standard Recovery

The standard recovery p is calculated by（A.8）

$$p = \frac{c_1}{c_2} \times 100\% \qquad \text{……(A.8)}$$

Where:

c_1——calculated concentration curve

c_2——theoretical concentration

A.3.6.1.6.4 Analytical Calculation

To determine the target analyte through the matching retention time of the M6 standard solution

Measure the response peak area of the target analytes in the standard solution and sample solution.

Measure the system drift of the Reb A standard sample. Measure the response peak area of Reb A under the

concentration of 2000mg/L and calculate the relative standard deviation. The relative standard deviation should meet the requirement: ≤ 2.0%.

Use the concentration (Unit: mg/L) of Reb A or stevioside as the ordinate and corresponding response area as abscissa to draw the fully fitted linear regression standard curve. Alternatively, the data acquisition software can also be used to draw a calibration curve.

From the linear regression equation of the standard curve, calculate the concentration of analyte in the sample (unit: mg/L) (Reb A uses the Reb A curve, and all other analytes use the stevioside curve) or use the data acquisition software to calculate the concentration of the analytes (using the calibration curve drawn by software). The concentration of the analyte Y is calculated according to the formula (A. 9)

$$Y = AX + B \qquad \text{……（A.9）}$$

Where：

X——peak response area

A——slope

B——y axial intercept

The concentration of each analyte in the calibration sample is as follows:

Use the concentration of each glycoside (Rubusoside, dulcosides A, Reb-C, Reb-F) and multiply by the glycosidic corrective factor, to adjust for the differences of the molecular weights from that of stevioside (see Table A.2)

The structural formula of steviol glycosides is as follows:

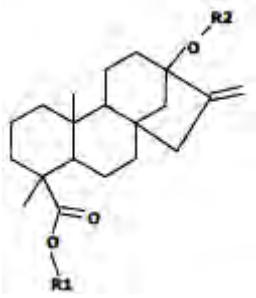


Table A.2 R1 and R2 Group of Steviol Glycosides

Name	Abbreviation	R1	R2	Molar weight（g/mol）	Correction factor
DulcosideA	Dul A	βglc-	αrha-βglc-	788.88	0.98
Reb-A	Reb A	βglc-	（βglc）2-βglc-	967.03	-
Reb-C	Reb C	βglc-	（βglc，αrha）-βglc-	951.02	1.18
Reb-F	Reb F	βglc-	（βglc，βxyl）-βglc-	936.99	1.16
Rubusoside	Rub	βglc-βglc-	βglc-βglc-	642.73	0.80

Stevioside	Stev	βglc-	βglc-βglc-	804.88	-

The weight percentage *w* of Reb A and other glycosides in the sample is calculated by（A.10）:

$$w = c_3 / c_4 \times 100 \quad \text{……（A.10）}$$

Where：

c_3——analyte concentration, mg/L

c_4——sample concentration, mg/L

The weight percentage of RebA and all other glycosides (deducting water) can be corrected by multiplying the following factor (*F*) by *W* (weight percentage) . The correction factor *F is* calculated according to the formula (A. 11) :

$$F = 100 /(100 - M) \quad \text{……（A.11）}$$

Where：

M——the sample moisture， %

The weight percentage w_{SG} of steviol glycoside (SG) in the sample is calculated by (A. 12)

$$w_{SG} = w_{Rub} + w_{DulA} + w_{RebC} + w_{RebF} + w_{Stev} + w_{RebA} \quad \text{……（A.12）}$$

Where：

w_{DulA}——DulA weight percentage in the sample，（%）

$w_{Reb\ C}$——Reb C weight percentage in the sample，（ %）

$w_{Reb\ F}$——Reb F weight percentage in the sample，（%）

w_{Stev}——Stev weight percentage in the sample，（%）

$w_{Reb\ A}$——Reb A weight percentage in the sample，（ %）

A.3.6.1.7 Acceptance Criteria

A.3.6.1.7.1 Standard Curve of the Acceptance Criteria

The standard curve of Reb A: For all different concentration levels of Reb A used in calibration curves, the standard recovery rate must be 100 ± 3%, and the acceptance criteria of the correlation coefficient of the standard curve should be ≥ 0.9900.

Stevioside standard curve: For all different concentration levels of stevioside used in the calibration curves, the standard recovery rate must be within 100.0 + /- 10%, except for the lowest concentration level (2.5mg/L) where the standard recovery rate must be within 100.0±20%. The acceptance criteria for the correlation coefficient of the standard curve is 0.9900 or higher.

A.3.6.1.7.2 Sequence Standard Sample – Standard Samples Check: The sequence standard recovery rate (see A.3.6.1.6.3) of stevioside and Reb A standard must be within 100.0± 2%。

A.3.6.1.7.3 Sample: The % relative standard deviation (RSD) of stevioside and Reb A test results of parallel samples should not exceed 2.0%. The % relative standard deviation of other glycosides should not exceed 50 % when the content is lower than 5mg/L (corresponding to 0.1 % in the sample). When the content is higher than 5mg/L, it should not exceed 20%. When the % relative standard deviation of the sample does not fall within the above range, prepare a

fresh sample until the new sample passes the quality control inspection.

A.3.6.2 The Gradient HPLC Measurement Step of Glucosyl Steviol Glycosides

A.3.6.2.1 Mobile Phase (A-acetonitrile, B-water)

Filter and de-gas the acetonitrile and water

A.3.6.2.2 Diluent (100％water）

Filter 1000mL of water and use it immediately.

A.3.6.2.3 Preparation of Standard Sample (M6）

Weigh approximately 100mg/L of each of the standard samples of Rubusoside, Dulcoside A, stevioside, Reb C, Reb F and Reb A, and prepare a mixed standard solution with the diluent.

A.3.6.2.4 Sample Preparation

According to the method described in A.3.5.3, prepare the sample solution (approximately 5%)

A.3.6.2.5 Usage Conditions of Instrument are Shown in Table A.3

Table A.3 Instruments Usage Conditions

Chromatographic column	NH_2 column， 250 x 4.6 mm， 5μm
Temperature	30°C
Gradient mobile phase	A-Acetonitrile, B-Water 0 min A：B-80：20 0～2 min A：B-80：20 2～70 min A：B-50：50
Flow rate	1.0 mL/min
Injection volume	10 μL
Detection wavelength	UV210 nm（ 4 nm bw）， reference： 260 nm （ 100 nmbw）
Run time	70 min
Automatic injector temperature	Room temperature

A.3.6.2.6 Analytical Procedure
Steviol glycoside separation: Inject the sample M6 solution. Stevioside and Reb C should have a clear separation between the two peaks. Record the retention time of each stevia glycoside (A.3.8.2)

A.3.6.2.7 Analytical Sequence
Inject samples first, then after up to 12 samples are injected or after the sample sequence test is over, the standard samples are injected for quantitative detection.

A.3.6.2.8 Integration Parameters
Use the software tool that comes with the liquid chromatograph to complete the integration. It is attached in the example chromatogram (Fig A.3) in the annex.

A.3.6.2.9 Calculation

Comparing the elution profile with the sample chromatogram (Fig A.2, Fig A.3), identify each a-Glucosyl Steviol Glycoside.

To integrate all the peak points (except unreacted glycosides), use the data acquisition software tool of the chromatograph analyzer to measure the percentage (% area) of each α-Glucosyl Steviol Glycoside.

Record percentage of each α-Glucosyl Steviol Glycoside.

A.3.7 Results Report

The concentration of unreacted stevia glycoside and TSG should be carried out based on a dry basis weight %. The percentage of a-Glucosyl Steviol Glycosides should be reported on an area % basis. The average of the repeated test results of two samples is used as the reported value.

A.3.8 Annex

A.3.8.1 M6 Sample HPLC Chromatogram

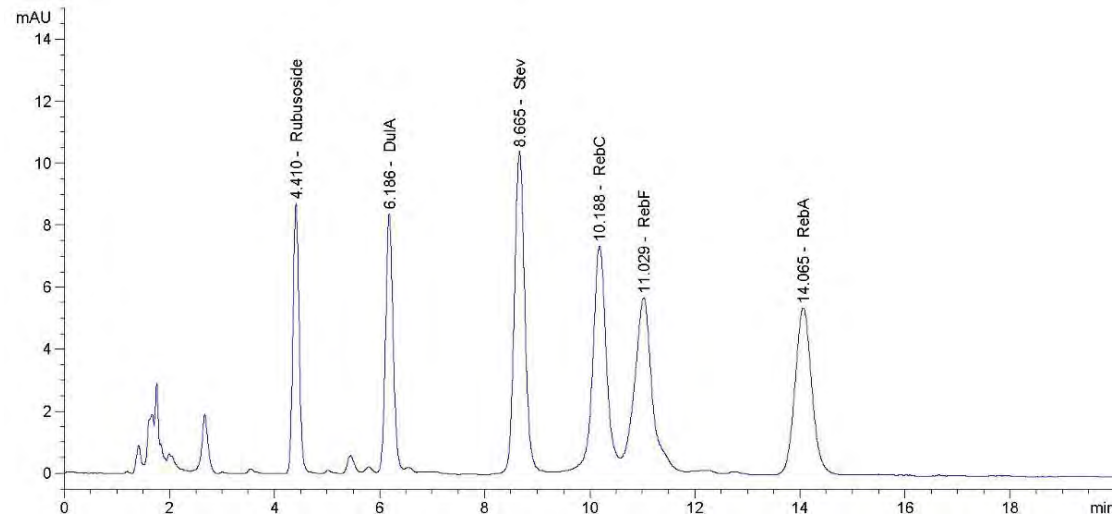


Figure A.1 M6 sample HPLC chromatogram

A.3.8.2 M6 Sample HPLC Chromatogram（Gradient）

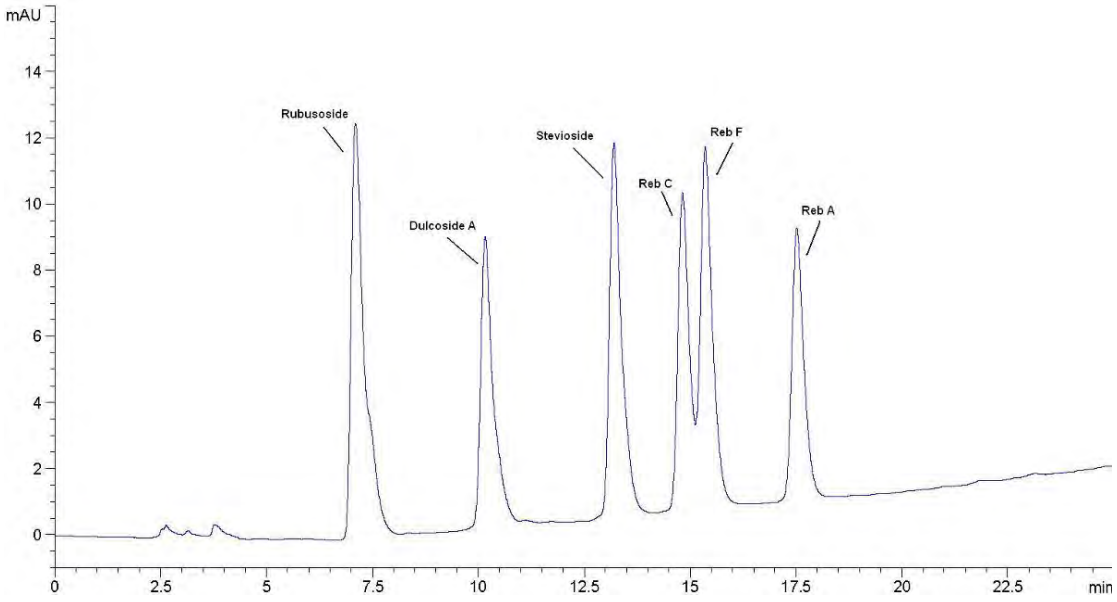


Figure A.2 M6 sample HPLC chromatogram（Gradient）

A.3.8.3 Sample Collection of Gradient Analysis Sample Chromatograms

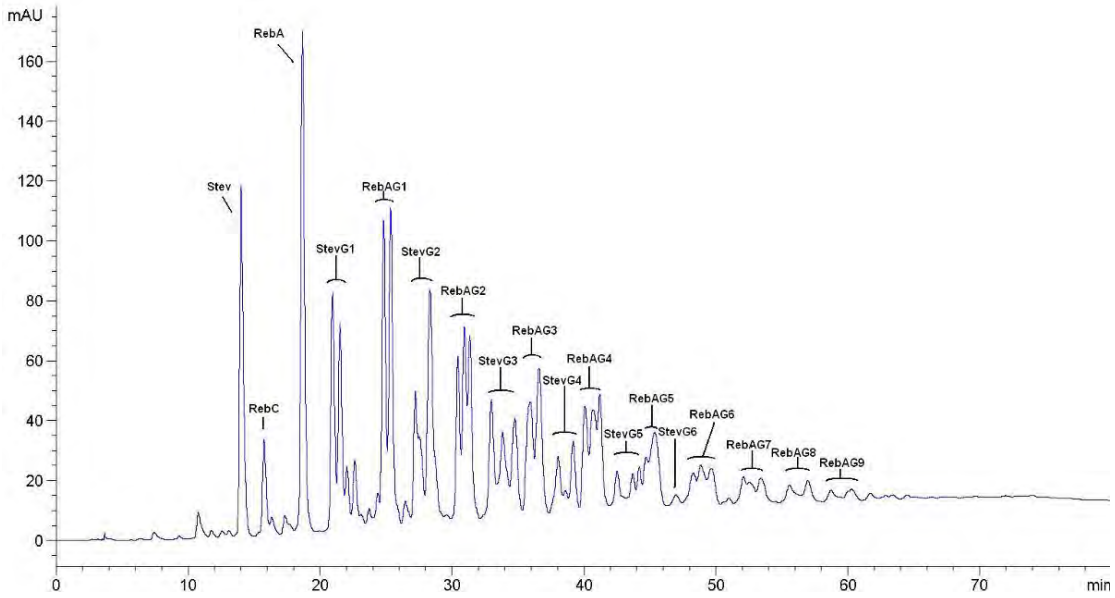


Figure A.3 Sample collection of gradient analysis sample chromatograms

Appendix 3 Certificates of Analysis for Multiple Batches of SoPure Stevia™ GSG 80 Glucosylated Steviol Glycosides

Appendix 3.1 Certificate of Analysis for SoPure Stevia™ GSG 80 Glucosylated Steviol Glycosides Batch 20190402E3

Appendix 3.2 Certificate of Analysis for SoPure Stevia™ GSG 80 Glucosylated Steviol Glycosides Batch 20191001E3

Appendix 3.3 Certificate of Analysis for SoPure Stevia™ GSG 80 Glucosylated Steviol Glycosides Batch 20191101E3

Appendix 3.4 Certificate of Analysis for SoPure Stevia™ GSG 80 Glucosylated Steviol Glycosides Batch 20191104E3

Appendix 3.5 Certificate of Analysis for SoPure Stevia™ GSG 80 Glucosylated Steviol Glycosides Batch 20191201E3

Appendix 3.1 Certificate of Analysis for SoPure Stevia™ GSG 80 Glucosylated Steviol Glycosides Batch 20190402E3

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Certificate Of Analysis

Product Name	Glucosyl Steviol Glycosides	Package size	20 kg paper carton
Batch Number	20190402E3	Quantity	1000kg
Manufacturing Date	April 18,2019	Expiry Date	April 17,2022
Report Date	May 01,2019	Packaging	Inner :laminated film pouch ,in cardboard
Specification standard	Notice of #8 in 2016 announced by National Health and Family Planning commission of P.R.C		

Items	Specifications	Result
Appearance	white or light yellow powder ,freely soluble in water, slightly soluble in ethanol	Meet spec
Total Steviol Glycosides,%	≥80.0	93.8
Glucosyl Steviol Glycosides,%	≥75.0	81.3
Dextrin,%	≤20.0	6.6
Optical Rotation Degree	+65°~+75°	+74.8°
pH (5% solution)	4.5-7.0	5.4
Relative Density	0.2~0.6	0.3
Loss on drying,%	≤5.0	4.3
Total ash,%	≤1.0	0.05
Methanol,ppm	≤200	<50
Ethanol,ppm	≤5000	26
Arsenic (As) ,ppm	≤1.0	Not Detected
Cadmium (Cd) ,ppm	≤1.0	Not Detected
Lead (Pb) ,ppm	≤0.5	Not Detected
Mercury (Hg) ,ppm	≤0.1	Not Detected
Total Aerobic Bacteria, cfu/g	$\leq 10^3$	10
Molds & Yeasts, cfu/g	$\leq 10^2$	30
Escherichia coli	Negative/g	Negative
Salmonella	Negative/25g	Negative
Conclusion: This product meets the requirement of Notice of #8 in 2016 announced by National Health and Family Planning commission of P.R.C		

Analyzed by: Checked by: Approved by:

Appendix 3.2 Certificate of Analysis for SoPure Stevia™ GSG 80 Glucosylated Steviol Glycosides Batch 20191001E3

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Certificate Of Analysis

Product Name	Glucosyl Steviol Glycosides	Packag size	20 kg paper carton
Batch Number	20191001E3	Quantity	1000kg
Manufacturing Date	Septemter 25,2019	Expiry Date	Septemter 24,2022
Report Date	October 16,2019	Packaging	Inner :laminated film pouch ,in cardboard
Specification standard	Notice of #8 in 2016 announced by National Health and Family Planning commission of P.R.C		

Items	Specifications	Result
Appearance	white or light yellow powder ,freely soluble in water, slightly soluble in ethanol	Meet spec
Total Steviol Glycosides,%	≥80.0	94.2
Glucosyl Steviol Glycosides,%	≥75.0	81.1
Dextrin,%	≤ 20.0	6.1
Optical Rotation Degree	+65°~+75°	+73.3°
pH (5% solution)	4.5-7.0	5.4
Relative Density	0.2~0.6	0.4
Loss on drying,%	≤5.0	4.8
Total ash,%	≤1.0	0.07
Methanol,ppm	≤ 200	<50
Ethanol,ppm	≤ 5000	30
Arsenic (As) ,ppm	≤1.0	Not Detected
Cadmium (Cd) ,ppm	≤1.0	Not Detected
Lead (Pb) ,ppm	≤0.5	Not Detected
Mercury (Hg) ,ppm	≤0.1	Not Detected
Total Aerobic Bacteria, cfu/g	$\leq 10^3$	10
Molds & Yeasts, cfu/g	$\leq 10^2$	20
Escherichia coli	Negative/g	Negative
Salmonella	Negative/25g	Negative
Conclusion: This product meets the requirement of Notice of #8 in 2016 announced by National Health and Family Planning commission of P.R.C		

Analyzed by: Checked by: Approved by:

Appendix 3.3 Certificate of Analysis for SoPure Stevia™ GSG 80 Glucosylated Steviol Glycosides Batch 20191101E3

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Certificate Of Analysis

Product Name	Glucosyl Steviol Glycosides	Packag size	20 kg paper carton
Batch Number	20191101E3	Quantity	1000kg
Manufacturing Date	October 24,2019	Expiry Date	October 23,2022
Report Date	November 07,2019	Packaging	Inner :laminated film pouch ,in cardboard
Specification standard	Notice of #8 in 2016 announced by National Health and Family Planning commission of P.R.C		

Items	Specifications	Result
Appearance	white or light yellow powder ,freely soluble in water, slightly soluble in ethanol	Meet spec
Total Steviol Glycosides,%	≥80.0	93.7
Glucosyl Steviol Glycosides,%	≥75.0	79.5
Dextrin,%	≤20.0	6.4
Optical Rotation Degree	+65°~+75°	+73.8°
pH (5% solution)	4.5-7.0	5.2
Relative Density	0.2~0.6	0.3
Loss on drying,%	≤5.0	4.0
Total ash,%	≤1.0	0.06
Methanol,ppm	≤200	<50
Ethanol,ppm	≤5000	35
Arsenic (As) ,ppm	≤1.0	Not Detected
Cadmium (Cd) ,ppm	≤1.0	Not Detected
Lead (Pb) ,ppm	≤0.5	Not Detected
Mercury (Hg) ,ppm	≤0.1	Not Detected
Total Aerobic Bacteria, cfu/g	$\leq 10^3$	<10
Molds & Yeasts, cfu/g	$\leq 10^2$	<10
Escherichia coli	Negative/g	Negative
Salmonella	Negative/25g	Negative

Conclusion: This product meets the requirement of Notice of #8 in 2016 announced by National Health and Family Planning commission of P.R.C

Analyzed by: Checked by: Approved by:

Appendix 3.4 Certificate of Analysis for SoPure Stevia™ GSG 80 Glucosylated Steviol Glycosides Batch 20191104E3

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Certificate Of Analysis

Product Name	Glucosyl Steviol Glycosides	Packag size	20 kg paper carton
Batch Number	20191104E3	Quantity	1000kg
Manufacturing Date	October 27,2019	Expiry Date	October 26,2022
Report Date	November 09,2019	Packaging	Inner :laminated film pouch ,in cardboard
Specification standard	Notice of #8 in 2016 announced by National Health and Family Planning commission of P.R.C		

Items	Specifications	Result
Appearance	white or light yellow powder ,freely soluble in water, slightly soluble in ethanol	Meet spec
Total Steviol Glycosides,%	≥80.0	93.5
Glucosyl Steviol Glycosides,%	≥75.0	79.9
Dextrin,%	≤20.0	6.6
Optical Rotation Degree	+65°~+75°	+71.8°
pH (5% solution)	4.5-7.0	5.3
Relative Density	0.2~0.6	0.3
Loss on drying,%	≤5.0	4.1
Total ash,%	≤1.0	0.06
Methanol,ppm	≤200	<50
Ethanol,ppm	≤5000	28
Arsenic (As) ,ppm	≤1.0	Not Detected
Cadmium (Cd) ,ppm	≤1.0	Not Detected
Lead (Pb) ,ppm	≤0.5	Not Detected
Mercury (Hg) ,ppm	≤0.1	Not Detected
Total Aerobic Bacteria, cfu/g	$\leq 10^3$	30
Molds & Yeasts, cfu/g	$\leq 10^2$	<10
Escherichia coli	Negative/g	Negative
Salmonella	Negative/25g	Negative
Conclusion: This product meets the requirement of Notice of #8 in 2016 announced by National Health and Family Planning commission of P.R.C		

Analyzed by: Checked by: Approved by:

Appendix 3.5 Certificate of Analysis for SoPure Stevia™ GSG 80 Glucosylated Steviol Glycosides Batch 20191201E3

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Certificate Of Analysis

Product Name	Glucosyl Steviol Glycosides	Package size	20 kg paper carton
Batch Number	20191201E3	Quantity	1000kg
Manufacturing Date	October 25,2019	Expiry Date	October 24,2022
Report Date	December 30,2019	Packaging	Inner laminated film pouch ,in cardboard
Specification standard	Notice of #8 in 2016 announced by National Health and Family Planning commission of P.R.C		

Items	Specifications	Result
Appearance	white or light yellow powder ,freely soluble in water, slightly soluble in ethanol	Meet spec
Total Steviol Glycosides,%	≥80.0	93.3
Glucosyl Steviol Glycosides,%	≥75.0	79.2
Dextrin,%	≤20.0	6.8
Optical Rotation Degree	+65°~+75°	+73.1°
pH (5% solution)	4.5-7.0	5.8
Relative Density	0.2~0.6	0.4
Loss on drying,%	≤5.0	3.7
Total ash,%	≤1.0	0.07
Methanol,ppm	≤200	<50
Ethanol,ppm	≤5000	32
Arsenic (As) ,ppm	≤1.0	Not Detected
Cadmium (Cd) ,ppm	≤1.0	Not Detected
Lead (Pb) ,ppm	≤0.5	Not Detected
Mercury (Hg) ,ppm	≤0.1	Not Detected
Total Aerobic Bacteria, cfu/g	$\leq 10^3$	<10
Molds & Yeasts, cfu/g	$\leq 10^2$	<10
Escherichia coli	Negative/g	Negative
Salmonella	Negative/25g	Negative
Conclusion: This product meets the requirement of Notice of #8 in 2016 announced by National Health and Family Planning commission of P.R.C		

Analyzed by: Checked by: Approved by:

Appendix 4 Certificates of Analysis for Multiple Batches of SoPure Stevia™ GSG 95 Glucosylated Steviol Glycosides

Appendix 4.1 Certificate of Analysis for SoPure Stevia™ GSG 95 Glucosylated Steviol Glycosides Batch G1808001

Appendix 4.2 Certificate of Analysis for SoPure Stevia™ GSG 95 Glucosylated Steviol Glycosides Batch G1812001

Appendix 4.3 Certificate of Analysis for SoPure Stevia™ GSG 95 Glucosylated Steviol Glycosides Batch G1907001

Appendix 4.4 Certificate of Analysis for SoPure Stevia™ GSG 95 Glucosylated Steviol Glycosides Batch G1909001

Appendix 4.5 Certificate of Analysis for SoPure Stevia™ GSG 95 Glucosylated Steviol Glycosides Batch G2007001

Appendix 4.1 Certificate of Analysis for SoPure Stevia™ GSG 95 Glucosylated Steviol Glycosides Batch G1808001

ZHUCHENG HAOTIAN PHARM CO.,LTD.
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Certificate Of Analysis

Product Name	Glucosyl Steviol Glycosides	Package size	20 kg paper carton
Batch Number	G1808001	Quantity	1000kg
Manufacturing Date	August 10,2018	Expiry Date	August 09,2021
Report Date	August 20,2018	Packaging	Inner :laminated film pouch ,in cardboard
Specification standard	Notice of #8 in 2016 announced by National Health and Family Planning commission of P.R.C		

Items	Specifications	Result
Appearance	white or light yellow powder ,freely soluble in water, slightly soluble in ethanol	Meet spec
Total Steviol Glycosides,%	≥95.0	97.8
Glucosyl Steviol Glycosides,%	≥75.0	79.6
Dextrin,%	≤5.0	2.2
Optical Rotation Degree	+65°~+75°	+75.0°
pH (5% solution)	4.5-7.0	5.5
Relative Density	0.2~0.6	0.4
Loss on drying,%	≤5.0	4.4
Total ash,%	≤1.0	0.06
Methanol,ppm	≤200	<50
Ethanol,ppm	≤5000	527
Arsenic (As) ,ppm	≤1.0	Not Detected
Cadmium (Cd) ,ppm	≤1.0	Not Detected
Lead (Pb) ,ppm	≤0.5	Not Detected
Mercury (Hg) ,ppm	≤0.1	Not Detected
Total Aerobic Bacteria, cfu/g	$\leq 10^3$	<10
Molds & Yeasts, cfu/g	$\leq 10^2$	<10
Escherichia coli	Negative/g	Negative
Salmonella	Negative/25g	Negative
Conclusion: This product meets the requirement of Notice of #8 in 2016 announced by National Health and Family Planning commission of P.R.C		

Analyzed by: Checked by: Approved by:

Appendix 4.2 Certificate of Analysis for SoPure Stevia™ GSG 95 Glucosylated Steviol Glycosides Batch G1812001

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Http://www.zcht.cc

Certificate Of Analysis

Product Name	Glucosyl Steviol Glycosides	Package size	20 kg paper carton
Batch Number	G1812001	Quantity	1000kg
Manufacturing Date	December 02,2018	Expiry Date	December 01,2021
Report Date	December 10,2018	Packaging	Inner :laminated film pouch ,in cardboard
Specification standard	Notice of #8 in 2016 announced by National Health and Family Planning commission of P.R.C		

Items	Specifications	Result
Appearance	white or light yellow powder ,freely soluble in water, slightly soluble in ethanol	Meet spec
Total Steviol Glycosides,%	≥95.0	97.6
Glucosyl Steviol Glycosides,%	≥75.0	79.0
Dextrin,%	≤5.0	2.4
Optical Rotation Degree	+65°~+75°	+73.5°
pH (5% solution)	4.5-7.0	5.5
Relative Density	0.2~0.6	0.4
Loss on drying,%	≤5.0	4.2
Total ash,%	≤1.0	0.07
Methanol,ppm	≤200	<50
Ethanol,ppm	≤5000	488
Arsenic (As) ,ppm	≤1.0	Not Detected
Cadmium (Cd) ,ppm	≤1.0	Not Detected
Lead (Pb) ,ppm	≤0.5	Not Detected
Mercury (Hg) ,ppm	≤0.1	Not Detected
Total Aerobic Bacteria, cfu/g	$\leq 10^3$	10
Molds & Yeasts, cfu/g	$\leq 10^2$	20
Escherichia coli	Negative/g	Negative
Salmonella	Negative/25g	Negative
Conclusion: This product meets the requirement of Notice of #8 in 2016 announced by National Health and Family Planning commission of P.R.C		

Analyzed by: 张全彩 Checked by: 薛雪 Approved by: 徐春荣

Appendix 4.3 Certificate of Analysis for SoPure Stevia™ GSG 95 Glucosylated Steviol Glycosides Batch G1907001

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Http://www.zcht.cc

Certificate Of Analysis

Product Name	Glucosyl Steviol Glycosides	Packag size	20 kg paper carton
Batch Number	G1907001	Quantity	1000kg
Manufacturing Date	July 06,2019	Expiry Date	July 05,2022
Report Date	July 16,2019	Packaging	Inner :laminated film pouch ,in cardboard
Specification standard	Notice of #8 in 2016 announced by National Health and Family Planning commission of P.R.C		

Items	Specifications	Result
Appearance	white or light yellow powder ,freely soluble in water, slightly soluble in ethanol	Meet spec
Total Steviol Glycosides,%	≥95.0	97.0
Glucosyl Steviol Glycosides,%	≥75.0	78.8
Dextrin,%	≤5.0	3.0
Optical Rotation Degree	+65°~+75°	+74.5°
pH (5% solution)	4.5-7.0	5.1
Relative Density	0.2~0.6	0.4
Loss on drying,%	≤5.0	4.4
Total ash,%	≤1.0	0.07
Methanol,ppm	≤200	<50
Ethanol,ppm	≤5000	537
Arsenic (As) ,ppm	≤1.0	Not Detected
Cadmium (Cd) ,ppm	≤1.0	Not Detected
Lead (Pb) ,ppm	≤0.5	Not Detected
Mercury (Hg) ,ppm	≤0.1	Not Detected
Total Aerobic Bacteria, cfu/g	$\leq 10^3$	10
Molds & Yeasts, cfu/g	$\leq 10^2$	<10
Escherichia coli	Negative/g	Negative
Salmonella	Negative/25g	Negative
Conclusion: This product meets the requirement of Notice of #8 in 2016 announced by National Health and Family Planning commission of P.R.C		

Analyzed by: Checked by: Approved by:

Appendix 4.4 Certificate of Analysis for SoPure Stevia™ GSG 95 Glucosylated Steviol Glycosides Batch G1909001

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Certificate Of Analysis

Product Name	Glucosyl Steviol Glycosides	Package size	20 kg paper carton
Batch Number	G1909001	Quantity	1000kg
Manufacturing Date	September 04,2019	Expiry Date	September 03,2022
Report Date	September 14,2019	Packaging	Inner :laminated film pouch ,In cardboard
Specification standard	Notice of #8 in 2016 announced by National Health and Family Planning commission of P.R.C		

Items	Specifications	Result
Appearance	white or light yellow powder ,freely soluble in water, slightly soluble in ethanol	Meet spec
Total Steviol Glycosides,%	≥95.0	97.2
Glucosyl Steviol Glycosides,%	≥75.0	79.2
Dextrin,%	≤5.0	2.8
Optical Rotation Degree	+65°~+75°	+74.0°
pH (5% solution)	4.5-7.0	5.3
Relative Density	0.2~0.6	0.4
Loss on drying,%	≤5.0	4.3
Total ash,%	≤1.0	0.07
Methanol,ppm	≤200	<50
Ethanol,ppm	≤5000	490
Arsenic (As) ,ppm	≤1.0	Not Detected
Cadmium (Cd) ,ppm	≤1.0	Not Detected
Lead (Pb) ,ppm	≤0.5	Not Detected
Mercury (Hg) ,ppm	≤0.1	Not Detected
Total Aerobic Bacteria, cfu/g	$\leq 10^3$	20
Molds & Yeasts, cfu/g	$\leq 10^2$	<10
Escherichia coli	Negative/g	Negative
Salmonella	Negative/25g	Negative
Conclusion: This product meets the requirement of Notice of #8 in 2016 announced by National Health and Family Planning commission of P.R.C		

Analyzed by: Checked by: Approved by:

Appendix 4.5 Certificate of Analysis for SoPure Stevia™ GSG 95 Glucosylated Steviol Glycosides Batch G2007001

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Http://www.zcht.cc

Certificate Of Analysis

Product Name	Glucosyl Steviol Glycosides	Package size	20 kg paper carton
Batch Number	G2007001	Quantity	1000kg
Manufacturing Date	July 06,2020	Expiry Date	July 05,2023
Report Date	July 15,2020	Packaging	Inner :laminated film pouch ,in cardboard
Specification standard	Notice of #8 in 2016 announced by National Health and Family Planning commission of P.R.C		

Items	Specifications	Result
Appearance	white or light yellow powder ,freely soluble in water, slightly soluble in ethanol	Meet spec
Total Steviol Glycosides,%	≥95.0	97.6
Glucosyl Steviol Glycosides,%	≥75.0	78.8
Dextrin,%	≤ 5.0	2.4
Optical Rotation Degree	+65°~+75°	+73.9°
pH (5% solution)	4.5-7.0	5.3
Relative Density	0.2~0.6	0.3
Loss on drying,%	≤ 5.0	4.3
Total ash,%	≤1.0	0.06
Methanol,ppm	≤ 200	<50
Ethanol,ppm	≤ 5000	570
Arsenic (As) ,ppm	≤1.0	Not Detected
Cadmium (Cd) ,ppm	≤1.0	Not Detected
Lead (Pb) ,ppm	≤0.5	Not Detected
Mercury (Hg) ,ppm	≤0.1	Not Detected
Total Aerobic Bacteria, cfu/g	$\leq 10^3$	<10
Molds & Yeasts, cfu/g	$\leq 10^2$	<10
Escherichia coli	Negative/g	Negative
Salmonella	Negative/25g	Negative
Conclusion: This product meets the requirement of Notice of #8 in 2016 announced by National Health and Family Planning commission of P.R.C		

Analyzed by: Checked by: Approved by:

Appendix 5 Representative Chromatograms for Multiple Batches of SoPure Stevia™ GSG 80 Glucosylated Steviol Glycosides

Appendix 5.1 Representative Chromatogram for SoPure Stevia™ GSG 80 Glucosylated Steviol Glycosides Batch 20190402E3

Appendix 5.2 Representative Chromatogram for SoPure Stevia™ GSG 80 Glucosylated Steviol Glycosides Batch 20191201E3

Appendix 5.3 Representative Chromatogram for SoPure Stevia™ GSG 80 Glucosylated Steviol Glycosides Batch 20191001E3

Appendix 5.4 Representative Chromatogram for SoPure Stevia™ GSG 80 Glucosylated Steviol Glycosides Batch 20191104E3

Appendix 5.5 Representative Chromatogram for SoPure Stevia™ GSG 80 Glucosylated Steviol Glycosides Batch 20191101E3

Appendix 5.1 Representative Chromatogram for SoPure Stevia™ GSG 80 Glucosylated Steviol Glycosides Batch 20190402E3

Empower 3

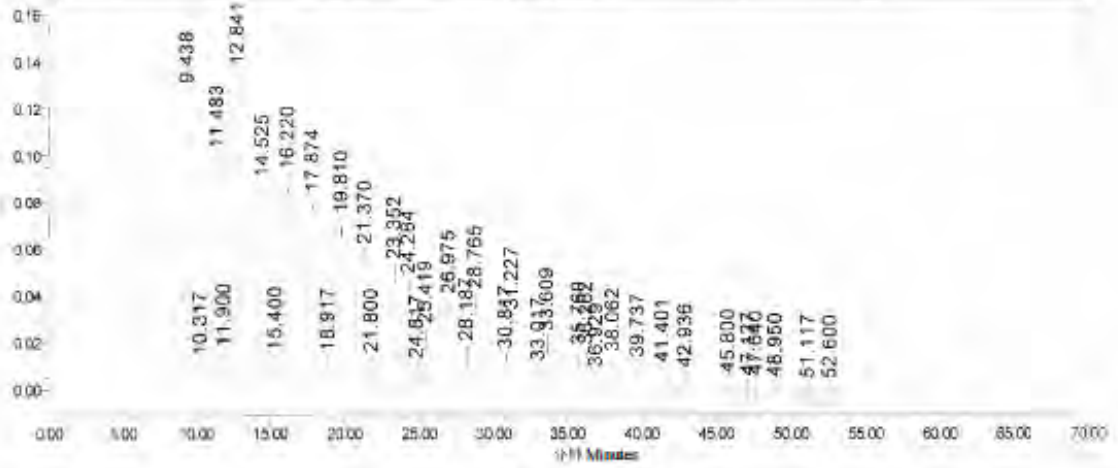
Zhucheng Haotian Pharm Co., Ltd
诸城市浩天药业有限公司

甜菊糖 Glucosyl Steviol Glycosides

样品名称 Injection Name 20190425-20190402E3
样品类型 Injection Type 未知 Unknown
样品瓶 Sample Bottles 2
进样次数 Injection Times 1
进样体积 Injection Volume 10.00 ul
运行时间 Run Time 70.0 Minutes
采集时间 Injection Date 2019/4/25 20:59:19 CST

采集者 User Name stevia2
样品组名称 Group Name 20190425葡萄糖基甜菊糖苷 GSG
采集方法组 Method Group 葡萄糖基甜菊糖糖苷梯度方法
处理方法 Processing Method 葡萄糖基甜菊糖苷梯度积分
通道名称 Channel Name W2489 ChA
处理通道说明 Wavelength W2489 ChA 210nm
处理时间 Processing Time 2019/4/26 9:43:08 CST

自动缩放色谱图



峰结果 Results

	名称	保留时间 (分钟)	面积 (微伏*秒)	高度 (微伏)	% 面积
1	STV 61	9.438	3879726	153419	9.09
2		10.317	36807	2848	0.09
3	RAG1	11.483	3566437	91422	8.36
4		11.900	76838	6691	0.18
5	STV 62	12.841	3933298	127348	9.22
6	RA G2	14.525	3749783	78606	8.79
7		15.400	69566	5580	0.16
8	STV G3	16.220	3505824	83345	8.22
9	RAG3	17.874	3387173	73671	7.94
10		18.917	63866	5666	0.15
11	STVG4	19.810	3039832	64825	7.12
12	RA G4	21.370	2446990	52019	5.73
13		21.800	73763	5533	0.17
14	STV G5	23.352	2667094	45539	6.25

	Name	Retention Time (Minutes)	Area (μV*s)	Height (μV)	Area %
	名称	保留时间 (分钟)	面积 (微伏*秒)	高度 (微伏)	% 面积
15	RAG5	24.284	1742643	39630	4.08
16		24.817	40313	3472	0.09
17	STVG6	25.419	422295	18368	0.99
18	RAG6	26.975	2761273	31480	6.47
19		28.187	217363	11256	0.51
20	RAG7	28.765	2034207	33752	4.77
21		30.817	160908	8282	0.38
22	RAG8	31.227	1498654	25032	3.51
23		33.017	62141	3497	0.15
24	RA G9	33.609	1051846	16291	2.47
25		35.760	472861	10701	1.11
26		36.262	281002	10750	0.66
27		36.929	24893	1448	0.06
28		38.062	461092	8057	1.08

报告用户: 浩顺 (stevia2) 页码: 1 (共计 2)

峰结果 Results

	名称	保留时间 (分钟)	面积 (微伏*秒)	高度 (微伏)	% 面积
29		39.737	315836	6246	0.75
30		41.401	237806	4812	0.56
31		42.938	251776	2970	0.59
32		45.800	69479	1520	0.16
33		47.127	11514	535	0.03
34		47.640	24043	862	0.06
35		48.950	20668	281	0.05
36		51.117	4577	11'	0.01
37		52.600	1976	69	0.00

2019.4.02 E3

报告用户: 张敏 (stevia2) 页码: 2 (共计 2)

Appendix 5.2 Representative Chromatogram for SoPure Stevia™ GSG 80 Glucosylated Steviol Glycosides Batch 20191201E3

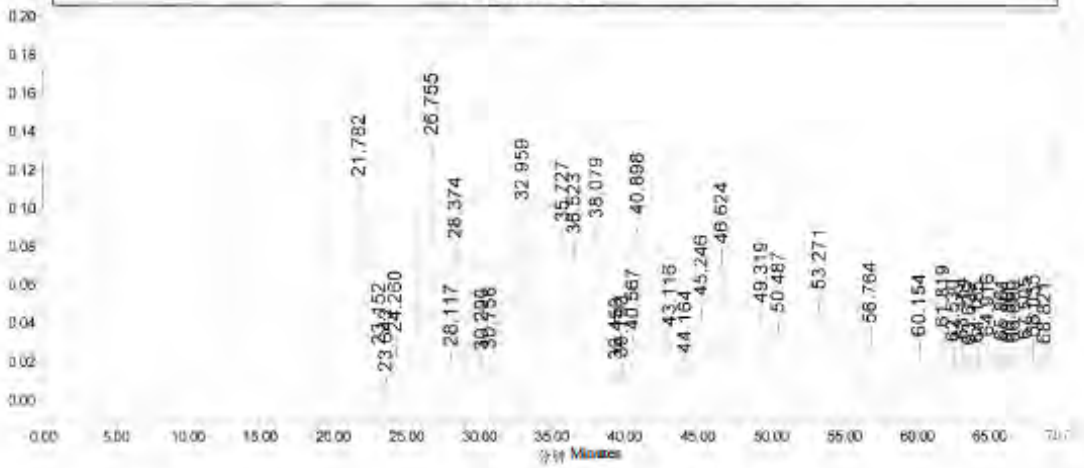
Zhucheng Haotian Pharm Co., Ltd
Empower 3
诸城市浩天药业有限公司

甜菊糖 Glucosyl Steviol Glycosides

样品名称:Injection Name 20191225-20191201E3
样品类型 Injection Type 未知 Unknown
样品瓶: Sample Bottle: 3
进样次数:Injection Times 1
进样体积:Injection Volume 0.00 ul
运行时间: Run Time 70.0 Minutes
采集时间:Injection Date 2019/12/25 18:08:19 CST

采集者: User Name stevia3
样品组名称:Group Name 20191225 葡萄糖基甜菊糖苷
采集方法组Method Group 0葡萄糖基甜菊糖苷梯度方法
处理方法Processing Method 0葡糖糖基甜菊糖苷梯度积分
通道名称:Channel Name W2489 ChA
处理通道说明Wavelength W2489 ChA 210nm
处理时间:Processing Time 2019/12/26 15:02:00 CST

自动缩放色谱图



峰结果 Results

	名称	保留时间 (分钟)	面积 (微伏*秒)	高度 (微伏)	% 面积
1	StV G1	21.782	3609589	104884	7.70
2		23.152	349831	16294	0.75
3		23.643	28949	2153	0.06
4		24.260	527319	32336	1.13
5	RA G1	26.755	4512251	124909	9.63
6		28.117	171879	13722	0.37
7	StV G2	28.374	3958462	70206	8.45
8		30.200	156189	10693	0.33
9		30.758	249003	11536	0.53
10	RA G2	32.958	4877484	88837	10.41
11	StV G3	35.727	2997829	78531	6.40
12	RA G3	36.523	2711316	69864	5.79
13	StV G4	38.079	2452030	77513	5.24
14		39.459	56703	2923	0.12

	Name	名称	Retention Time (Minutes) 保留时间 (分钟)	Area (μV*s) 面积 (微伏*秒)	Height (μV) 高度 (微伏)	Area % 面积 %
15			39.793	94421	4118	0.20
16			40.567	177499	18059	0.38
17	RA G4		40.698	5186911	78806	11.07
18			43.116	396080	19154	0.85
19			44.164	204063	5260	0.44
20	StV G5		45.246	1475183	34393	3.15
21	RA G5		46.624	2914630	61683	6.22
22	StV G6		49.319	956842	29135	2.04
23	RA G6		50.487	2242263	23720	4.79
24	RA G7		53.271	2286083	34961	4.88
25	RA G8		56.764	1626157	15997	3.47
26	RA G9		60.154	506786	8465	1.09
27			61.819	596841	12595	1.27
28			62.530	139520	4740	0.30

报告用户: 张佳英 (stevia3)

页码: 1 (共计 2)

峰结果Results

	名称	保留时间 (分钟)	面积 (微伏*秒)	高度 (微伏)	% 面积
29		63.154	160385	5525	0.34
30		63.639	61327	2962	0.13
31		64.185	79013	3646	0.17
32		64.916	400002	6958	0.85
33		65.861	152310	4133	0.33
34		66.366	50271	3006	0.11
35		66.680	71065	2950	0.15
36		67.505	115227	4217	0.25
37		68.035	251961	5468	0.54
38		68.621	43078	1571	0.09

2019.12.18

报告用户：张桂英 (stevia3) 页码: 2 (共计 2)

Appendix 5.3 Representative Chromatogram for SoPure Stevia™ GSG 80 Glucosylated Steviol Glycosides Batch 20191001E3

Zhucheng Haotian Pharm Co., Ltd

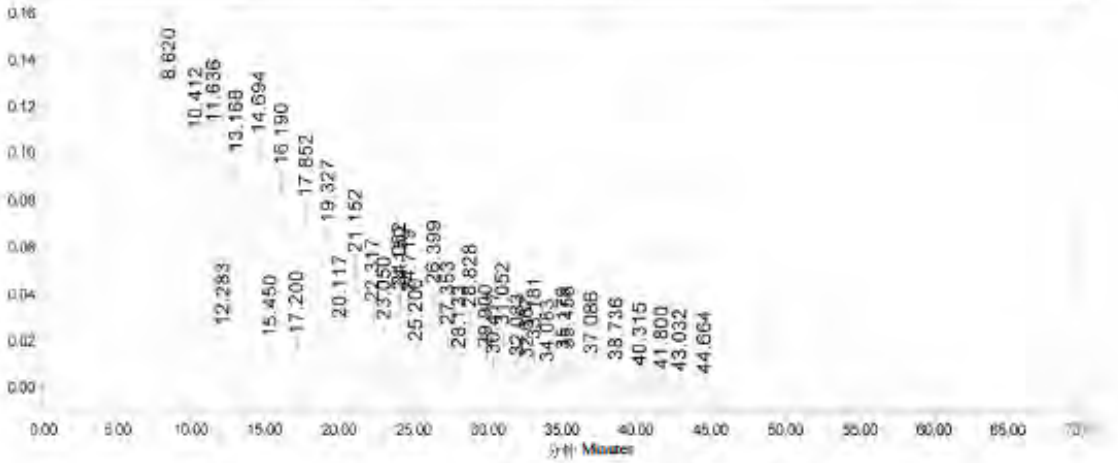
诸城市浩天药业有限公司

甜菊糖 Glucosyl Steviol Glycosides

样品名称 Injection Name 20191006-20191001E3
样品类型 Injection Type 未知 Unknown
样品瓶 Sample Bottles 53
运样次数 Injection Times 1
运样体积 Injection Volume 0.00 ul
运行时间 Run Time 70.0 Minutes
采集时间 Injection Date 2019/10/6 19:37:41 CST

采集者 User Name stevia3
样品组名称 Group Name 20191006 葡萄糖基甜菊糖苷
采集方法组 Method Group 葡萄糖基甜菊糖苷梯度方法
处理方法 Processing Method 葡萄糖基甜菊糖苷梯度积分
通道名称 Channel Name W2489 ChA
处理通道说明 Wavelength W2489 ChA 210nm
处理时间 Processing Time 2019/10/7 10:18:20 CST

自动缩放色谱图



峰结果 Results

	名称	保留时间 (分钟)	面积 (微伏*秒)	高度 (微伏)	% 面积
1	StvG1	8.620	3758690	149077	8.92
2	RAG1	10.412	3805831	93200	8.56
3	StvG2	11.636	3936722	136033	9.11
4		12.283	207350	10362	0.49
5	RAG2	13.168	3572599	84362	8.48
6	StvG3	14.694	3718027	92967	8.83
7		15.450	24823	6149	0.06
8	RAG3	16.190	3191622	79665	7.58
9		17.200	111503	8377	0.26
10	StvG4	17.852	2774712	98724	6.59
11	RAG4	19.327	2672064	56475	6.34
12		20.117	202470	18189	0.48
13	StvG5	21.152	3901011	44461	9.26
14		22.317	257956	23787	0.61

	Name 名称	Retention Time (Minutes) 保留时间 (分钟)	Area (μV*s) 面积 (微伏*秒)	Height (μV) 高度 (微伏)	Area % 面积 %
15		23.050	190279	16386	0.47
16	RA65	24.052	1519773	31251	3.61
17		24.150	349235	29221	0.83
18	StvG6	24.719	1069640	28707	2.54
19		25.200	95198	7867	0.23
20	RAG6	26.330	1866793	32754	4.43
21		27.353	397748	15743	0.94
22		28.133	54209	5827	0.13
23	RA67	28.828	1493128	23550	3.55
24		29.900	57719	6816	0.14
25		30.417	42622	4011	0.10
26	RA68	31.052	1051396	16511	2.52
27		32.033	31950	3729	0.08
28		32.657	41642	2878	0.10

报告用户：张桂英 (stevia3) 页码：1 (共计 2)

峰结果 Results

	名称	保留时间 (分钟)	面积 (微伏*秒)	高度 (微伏)	% 面积
29	RA64	33.181	690400	11272	1.64
30		34.083	12607	1947	0.03
31		35.179	279792	7569	0.66
32		35.456	246926	7967	0.58
33		37.086	351625	6184	0.83
34		38.736	212527	4227	0.50
35		40.315	120703	2493	0.29
36		41.800	63614	1374	0.15
37		43.032	6863	365	0.02
38		44.664	18905	478	0.04

20190103

Appendix 5.4 Representative Chromatogram for SoPure Stevia™ GSG 80 Glucosylated Steviol Glycosides Batch 20191104E3

Zhucheng Haotian Pharm Co., Ltd
诸城市浩天药业有限公司

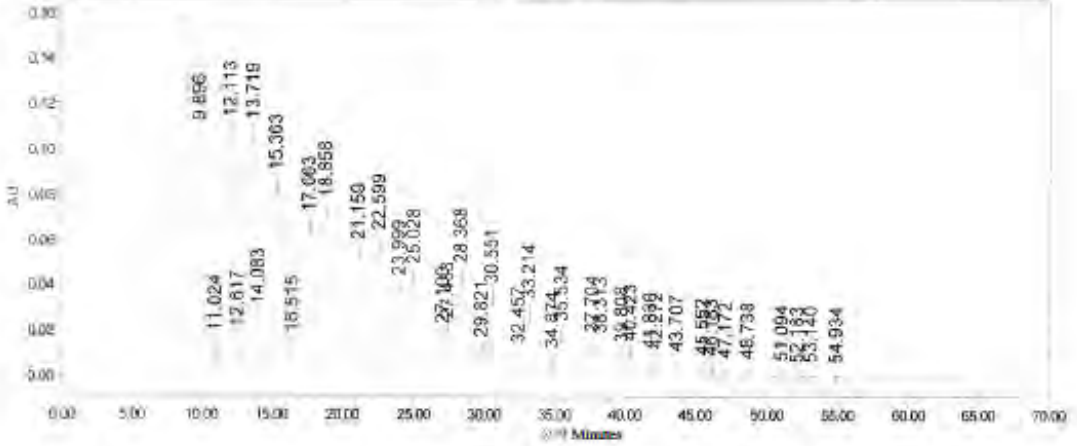
Empower 3

甜菊糖 Glucosyl Steviol Glycosides

样品名称:Injection Name 20191103-20191104E3
样品类型:Injection Type 未知 Unknown
样品瓶:Sample Bottles 5
进样次数:Injection Times
进样体积:Injection Volume 10.00 ul
运行时间:Run Time 70.0 Minutes
采集时间:Injection Date 2019/11/3 20:53:52 CST

采集者 User Name stevia5
样品组名称:Group Name 20191103 葡萄糖基甜菊糖苷
采集方法组:Method Group 葡萄糖基甜菊糖苷梯度方法
处理方法:Processing Method 葡萄糖基甜菊糖苷梯度积分
通道名称:Channel Name W2489 ChA
处理通道说明:Wavelength W2489 ChA 210nm
处理时间:Processing Time 2019/11/4 11:26:13 CST

自动缩放色谱图



峰结果 Results

	名称	保留时间 (分钟)	面积 (微伏*秒)	高度 (微伏)	% 面积
1	StvG1	9.896	3466759	133087	9.04
2		11.024	137517	6038	0.32
3	RAG1	12.113	3968319	101135	9.27
4		12.617	166423	8472	0.39
5	StvG2	13.719	3255172	100294	7.55
6		14.083	296140	18445	0.68
7	RAG2	15.363	3911663	78176	9.07
8		16.516	125349	6740	0.29
9	StvG3	17.083	3185856	60172	7.39
10	RAG3	18.858	3581319	66999	8.31
11	StvG4	21.159	2195308	48687	5.10
12	RAG4	22.599	3185350	53132	7.39
13	StvG5	23.999	2452348	32941	5.76
14	RAG5	25.028	2546327	39294	5.91

	Name	Retention Time (Minutes)	名称	保留时间 (分钟)	Area (μV*s)	面积 (微伏*秒)	Height (μV)	高度 (微伏)	Area %	% 面积
15	StvG6	27.100			377263	12408			0.87	
16		27.433			270018	14697			0.63	
17	RAG6	28.368			2698693	39928			6.26	
18		29.821			161111	7010			0.37	
19	RAG7	30.551			2109827	30384			4.89	
20		32.457			114782	4791			0.27	
21	RAG8	33.214			1581428	24060			3.67	
22		34.874			82426	2927			0.14	
23	RAG9	35.534			1061286	14889			2.46	
24		37.704			410394	10557			0.95	
25		38.313			419441	9844			0.97	
26		39.806			220913	6288			0.51	
27		40.423			351337	7313			0.81	
28		41.890			109658	3916			0.25	

报告用户: 郭凯娟 (stevia5)

页码: 1 (共计 2)

峰结果 Results

	名称	保留时间 (分钟)	面积 (微伏*秒)	高度 (微伏)	% 面积
29		42.272	193551	4068	0.45
30		43.707	211134	3175	0.49
31		45.562	62610	1879	0.15
32		46.183	56602	1785	0.13
33		47.172	41240	840	0.10
34		48.738	43184	1107	0.10
35		51.094	14620	500	0.03
36		52.183	1004	49	0.00
37		53.140	6935	298	0.02
38		54.934	7206	193	0.02

20191104E3

报告用户: 郭凤娟 (gevia5) 页码: 2 (共计 2)

Appendix 5.5 Representative Chromatogram for SoPure Stevia™ GSG 80 Glucosylated Steviol Glycosides Batch 20191101E3

Zhucheng Haotian Pharm Co., Ltd

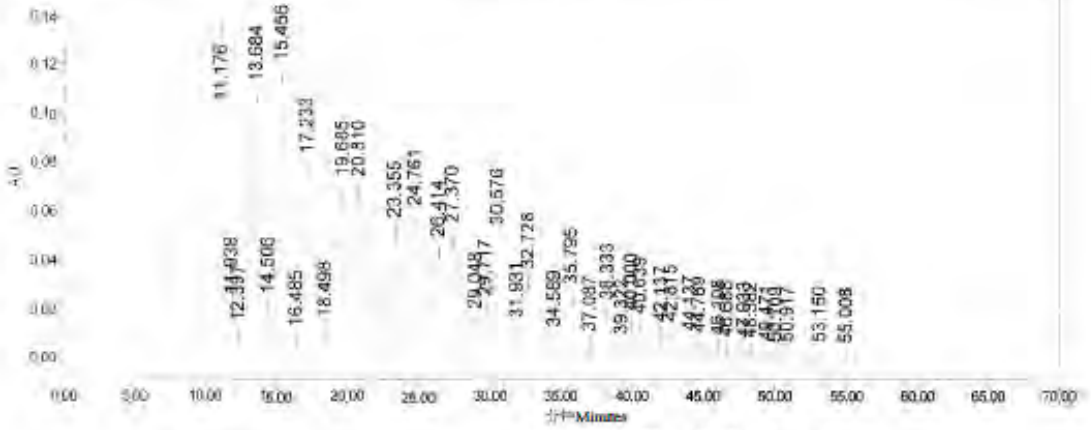
Empower 3

诸城市浩天药业有限公司

甜菊糖 Glucosyl Steviol Glycosides

样品名称:Injection Name	20191101-20191101E3	采集者:	stevia5
样品类型:Injection Type	未知 Unknown	样品组名称:	20191101 葡萄糖基甜菊糖苷
样品瓶:Sample Bottles	27	采集方法组:	α葡萄糖基甜菊糖苷梯度方法
进样次数:Injection Times	1	处理方法:	α葡萄糖基甜菊糖苷梯度积分
进样体积:Injection Volume	40.00 ul	通道名称:	W2489 ChA
运行时间:Run Time	70.0 Minutes	处理通道说明:	W2489 ChA 210nm
采集时间:Injection Date	2019/11/1 15:38:25 CST	处理时间:	2019/11/2 9:37:04 CST

自动缩放色谱图



峰结果 Results

	名称	保留时间 (分钟)	面积 (微伏*秒)	高度 (微伏)	% 面积
1	STVG1	11.176	3456835	131050	7.52
2		11.938	366192	16319	0.80
3		12.397	134557	4991	0.29
4	RAG1	13.684	3958685	102899	8.61
5		14.506	417371	16658	0.91
6	STVG2	15.486	3910566	111858	8.51
7		16.485	38287	3244	0.08
8	RAG2	17.233	3935726	73848	8.56
9		18.498	12411	7186	0.27
10	STVG3	19.685	3527159	63741	7.67
11	RAG3	20.810	3442323	64003	7.49
12	STVG4	23.355	2663804	46551	5.84
13	RAG4	24.761	3328818	53653	7.24
14	STVG5	26.414	2337809	41063	5.09

	Name	Retention Time (Minutes)	Area (μV*s)	Height (μV)	Area %
15	RAG5	27.370	2720686	46919	5.93
16	STVG6	29.048	301485	12031	0.66
17		29.717	430188	17982	0.94
18	RAG6	30.576	2848823	48307	6.19
19		31.931	196442	8642	0.43
20	RAG7	32.728	2278886	28759	4.95
21		34.589	129236	5231	0.28
22	RAG8	35.795	1688317	22525	3.67
23		37.087	90775	3584	0.20
24	RAG9	38.333	1161243	16825	2.53
25		39.322	54011	2629	0.12
26		40.000	353865	12850	0.66
27		40.639	501320	11535	1.09
28		42.137	248473	8163	0.54

报告方法: 默认值 (stevia5) 页码: 1 (共计 2)

峰结果 Results

	名称	保留时间 (分钟)	面积 (微伏*秒)	高度 (微伏)	% 面积
29		42.616	390674	8519	0.65
30		44.137	197396	4752	0.36
31		44.769	201811	4281	0.44
32		46.108	148204	3196	0.32
33		46.688	98136	2440	0.21
34		47.933	70805	2355	0.15
35		48.382	78555	2242	0.17
36		49.471	33428	1115	0.07
37		50.109	17415	569	0.04
38		50.917	26762	957	0.06
39		53.150	12543	438	0.03
40		55.008	9132	289	0.02

2019110103

报告用户: 郭凤第 (stevia5) 页码: 2 (共计 2)

Appendix 6 Representative Chromatograms for Multiple Batches of SoPure Stevia™ GSG 95 Glucosylated Steviol Glycosides

Appendix 6.1 Representative Chromatogram for SoPure Stevia™ GSG 95 Glucosylated Steviol Glycosides Batch G1808001

Appendix 6.2 Representative Chromatogram for SoPure Stevia™ GSG 95 Glucosylated Steviol Glycosides Batch G1812001

Appendix 6.3 Representative Chromatogram for SoPure Stevia™ GSG 95 Glucosylated Steviol Glycosides Batch G1907001

Appendix 6.4 Representative Chromatogram for SoPure Stevia™ GSG 95 Glucosylated Steviol Glycosides Batch G1909001

Appendix 6.5 Representative Chromatogram for SoPure Stevia™ GSG 95 Glucosylated Steviol Glycosides Batch G2007001

Appendix 6.1 Representative Chromatogram for SoPure Stevia™ GSG 95 Glucosylated Steviol Glycosides Batch G1808001

Zhucheng Haotian Pharm Co., Ltd

Empower 3

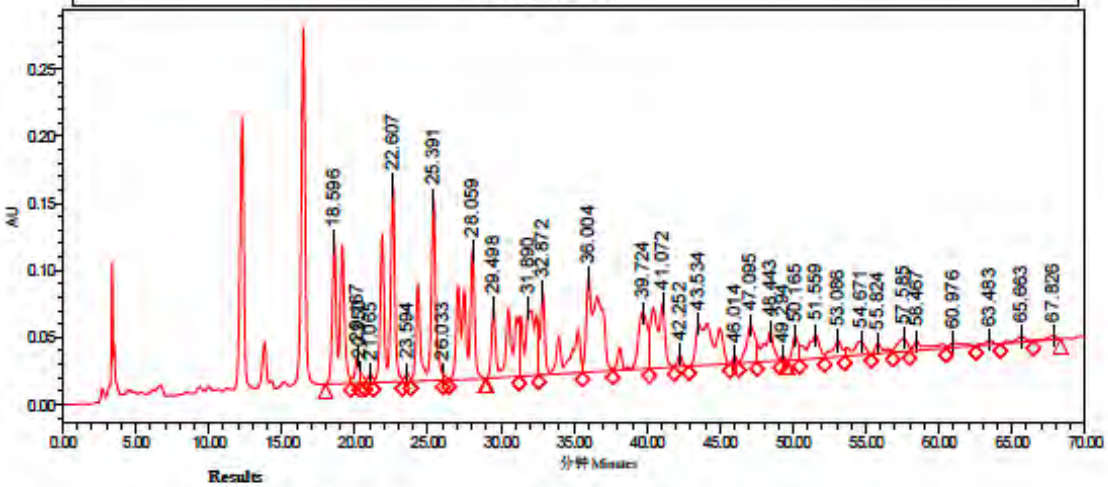
诸城市浩天药业有限公司

葡萄糖基甜菊糖苷 Glucosyl Steviol Glycosides

样品名称: Injection Name G1808001
样品类型: Injection Type 未知 Unknown
样品瓶: Sample Bottles 8
进样次数: Injection Times 1
进样体积: Injection Volume 10.00 ul
运行时间: Run Time 70.0 Minutes
采集时间: Injection Date 2018/8/12 18:45:26 CST

采集者: User Name stevia6
样品组名称: Group Name 20180812葡萄糖基甜菊糖苷 GSG
采集方法组: Method Group α葡萄糖基甜菊糖糖苷梯度方法 Gradient Method
处理方法: Processing Method α葡糖糖基甜菊糖糖苷梯度积分 Gradient Integration
通道名称: Channel Name W2489 ChA
处理通道说明: Wavelength W2489 ChA 210nm
处理时间: Processing Time 2018/8/13 11:08:56 CST

自动缩放色谱图



Results

	Name 名称	Retention Time (Minutes) 保留时间 (分钟)	Area (μV*s) 面积 (微伏*秒)	Height (μV) 高度 (微伏)	Area % 面积 %
1	$STVG_1$	18.596	4036296	106156	8.53
2		20.167	392010	20269	0.83
3		20.350	113793	8021	0.24
4		21.065	139312	6265	0.29
5	$RebAG_2$	22.607	4944132	146927	10.45
6		23.594	136134	5726	0.29
7	$STVG_3$	25.391	4444590	135105	9.39
8		26.033	62851	4452	0.13
9	$RebAG_5$	28.059	4495862	96250	9.50
10	$STVG_5$	29.498	3091701	51791	6.53
11	$RebAG_1$	31.890	3098269	50100	6.55
12	$STVG_6$	32.872	3108941	61688	6.57
13	$RebAG_6$	36.004	4380996	70189	9.26
14	$STVG_7$	39.724	2209458	41749	4.67

	Name 名称	Retention Time (Minutes) 保留时间 (分钟)	Area (μV*s) 面积 (微伏*秒)	Height (μV) 高度 (微伏)	Area % 面积 %
15	$RebAG_4$	41.072	2592703	48112	5.48
16	$STVG_8$	42.252	185740	9036	0.39
17	$RebAG_8$	43.534	2922418	31248	6.18
18		46.014	89274	5083	0.19
19		47.095	1124203	27830	2.38
20	$RebAG_7$	48.443	1096014	22495	2.32
21		49.294	50012	3229	0.11
22		50.165	487003	17788	1.03
23	$RebAG_9$	51.559	1035986	17306	2.19
24		53.086	529671	11654	1.12
25	$RebAG_{10}$	54.671	600244	9786	1.27
26		55.824	274852	7262	0.58
27		57.585	450885	9926	0.95
28		58.467	451402	6846	0.95

报告用户: 郭凤娟 (stevia5) 页码: 1 (共计 2)

Results

	Name 名称	Retention Time (Minutes) 保留时间 (分钟)	Area (μV*s) 面积 (微伏*秒)	Height (μV) 高度 (微伏)	Area % % 面积
29		60.976	231053	3604	0.49
30		63.483	141698	3193	0.30
31		65.663	243891	4014	0.52
32		67.826	157767	3088	0.33

Appendix 6.2 Representative Chromatogram for SoPure Stevia™ GSG 95 Glucosylated Steviol Glycosides Batch G1812001

Empower 3

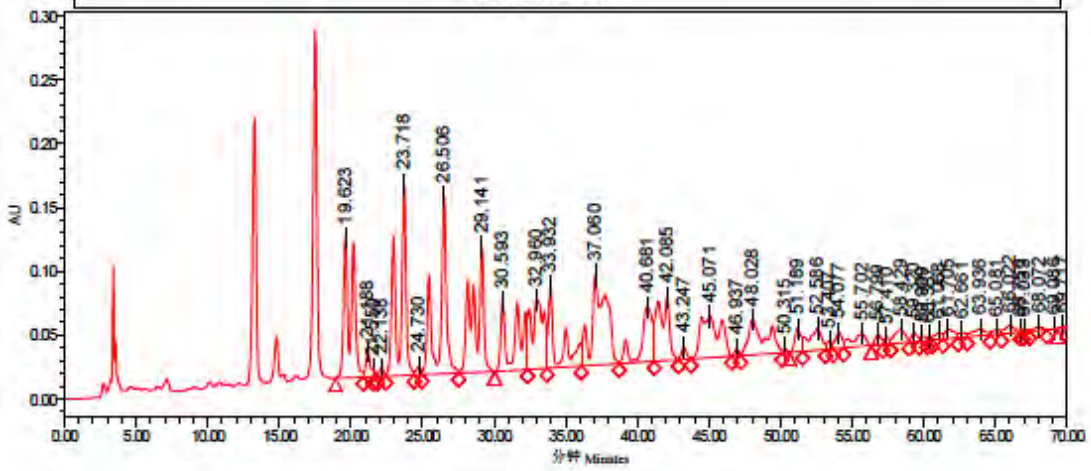
诸城市浩天药业有限公司

葡萄糖基甜菊糖苷 Glucosyl Steviol Glycosides

样品名称: Injection Name G1812001
样品类型: Injection Type 未知
样品瓶: Sample Bottles 6
进样次数: Injection Times 1
进样体积: Injection Volume 10.00 ul
运行时间: Run Time 70.0 Minutes
采集时间: Injection Date 2018/12/4 16:03:06 CST

采集者: User Name stevia8
样品组名称: Group Name 20181204葡萄糖基甜菊糖苷 GSG
采集方法组: Method Group α葡萄糖基甜菊糖糖苷梯度方法 Gradient Method
处理方法: Processing Method α葡萄糖基甜菊糖苷梯度积分 Gradient Integration
通道名称: Channel Name W2489 ChA
处理通道说明: Wavelength W2489 ChA 210nm
处理时间: Processing Time 2018/12/5 10:33:07 CST

自动增放色谱图



	Name	Retention Time (Minutes)	Area (μV*s)	Height (μV)	Area %
	名称	保留时间 (分钟)	面积 (微伏*秒)	高度 (微伏)	% 面积
1	$STVG_1$	19.623	4131119	109800	8.31
2		21.188	428947	20274	0.86
3		21.550	61290	5623	0.12
4		22.138	132011	6021	0.27
5	$RebAG_4$	23.718	4971803	149847	10.01
6		24.730	145563	6238	0.29
7	$STVG_2$	26.506	4751813	138997	9.56
8	$RebAG_2$	29.141	4589051	98108	9.24
9	$STVG_3$	30.593	3184052	54196	6.41
10	$RebAG_4$	32.960	3312764	53926	6.67
11	$STVG_4$	33.932	2456178	63549	4.94
12	$RebAG_4$	37.060	5116503	70605	10.30
13	$STVG_5$	40.681	2315729	42644	4.66
14	$RebAG_5$	42.085	2695734	49571	5.42

	Name	Retention Time (Minutes)	Area (μV*s)	Height (μV)	Area %
	名称	保留时间 (分钟)	面积 (微伏*秒)	高度 (微伏)	% 面积
15	$STVG_6$	43.247	233378	9941	0.47
16	$RebAG_6$	45.071	3124751	32324	6.29
17		46.937	108803	5617	0.22
18	$RebAG_7$	48.028	2257963	27864	4.54
19		50.315	61499	3389	0.12
20	$RebAG_8$	51.189	530099	17895	1.07
21		52.586	1044846	17022	2.10
22		53.407	158167	6114	0.32
23		54.077	367383	11947	0.74
24	$RebAG_9$	55.702	663820	9687	1.34
25		56.799	222779	8012	0.45
26		57.410	132797	4682	0.27
27		58.429	533207	10012	1.07
28		59.320	190632	6784	0.38

报告用户: 郭凤娟 (stevia5) 页码: 1 (共计 2)

	Name	Retention Time (Minutes)	Area (μV*s)	Height (μV)	Area %
	名称	保留时间 (分钟)	面积 (微伏*秒)	高度 (微伏)	% 面积
29		59.909	103662	3379	0.21
30		60.367	55852	2901	0.11
31		61.058	186610	5206	0.38
32		61.705	334797	7152	0.67
33		62.661	127380	3853	0.25
34		63.936	334003	5540	0.67
35		65.081	134956	3592	0.27
36		66.022	283187	6177	0.57
37		66.750	12964	1396	0.03
38		67.033	33385	1230	0.07
39		68.072	126026	2966	0.25
40		69.086	13327	621	0.03
41		69.717	22584	1065	0.05

Appendix 6.3 Representative Chromatogram for SoPure Stevia™ GSG 95 Glucosylated Steviol Glycosides Batch G1907001

Empower 3

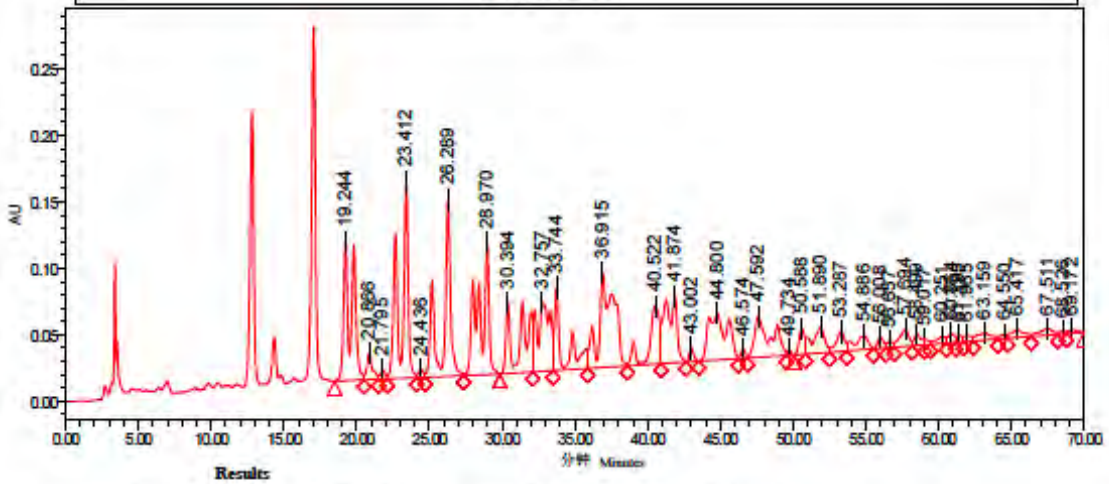
Zhucheng Haotian Pharm Co., Ltd
诸城市浩天药业有限公司

葡萄糖基甜菊糖苷 Glucosyl Steviol Glycosides

样品名称: Injection Name G1907001
样品类型: Injection Type 未知 Unknown
样品瓶: Sample Bottles 7
进样次数: Injection Times 1
进样体积: Injection Volume 10.00 ul
运行时间: Run Time 70.0 Minutes
采集时间: Injection Date 2019/7/8 17:24:18 CST

采集者: User Name stevia6
样品组名称: Group Name 20190708葡萄糖基甜菊糖苷 GSG
采集方法组: Method Group α葡萄糖基甜菊糖糖苷梯度方法 Gradient Method
处理方法: Processing Method α葡糖糖基甜菊糖苷梯度积分 Gradient Integration
通道名称: Channel Name W2489 ChA
处理通道说明: Wavelength W2489 ChA 210nm
处理时间: Processing Time 2019/7/9 10:35:32 CST

自动缩放色谱图



Results

	Name	Retention Time (Minutes) 名称 保留时间 (分钟)	Area (μV*s) 面积 (微伏*秒)	Height (μV) 高度 (微伏)	Area % 面积 % 面积
1	STVG$_3$	19.244	4106636	104612	8.22
2		20.866	509171	20171	1.02
3		21.795	153968	6610	0.31
4	RebAG$_6$	23.412	4996656	148233	10.01
5		24.436	144592	5936	0.29
6	STVG$_5$	26.289	4543134	133343	9.10
7	RebAG$_7$	28.970	4544595	99313	9.10
8	STVG$_6$	30.394	3113727	53479	6.24
9	RebAG$_8$	32.757	3291004	51758	6.59
10	STVG$_7$	33.744	2332946	62465	4.67
11	RebAG$_9$	36.915	5128198	71324	10.27
12	STVG$_8$	40.522	2158055	43362	4.32
13	RebAG$_{10}$	41.874	2794974	50132	5.60
14	STVG$_9$	43.002	220613	10080	0.44

	Name	Retention Time (Minutes) 名称 保留时间 (分钟)	Area (μV*s) 面积 (微伏*秒)	Height (μV) 高度 (微伏)	Area % 面积 % 面积
15	RebAG$_{11}$	44.800	3135758	35052	6.28
16		46.574	138874	6765	0.28
17	RebAG$_{12}$	47.592	2336280	30835	4.68
18		49.734	71477	3921	0.14
19		50.588	518105	19712	1.04
20	RebAG$_{13}$	51.890	1105249	19595	2.21
21		53.287	584933	14564	1.17
22	RebAG$_{14}$	54.886	677980	10281	1.36
23		56.008	221792	7919	0.44
24		56.657	133462	4402	0.27
25		57.694	558389	12586	1.12
26		58.499	275958	8222	0.55
27		59.017	81545	4147	0.16
28		60.251	238695	5176	0.48

报告用户: 郭凤娟 (stevia5) 页码: 1 (共计 2)

	Name 名称	Retention Time (Minutes) 保留时间 (分钟)	Area (μV*s) 面积 (微伏*秒)	Height (μV) 高度 (微伏)	Area % % 面积
29		60.864	202683	6663	0.41
30		61.395	144022	4970	0.29
31		61.965	155145	4372	0.31
32		63.159	373842	5568	0.75
33		64.550	111707	3139	0.22
34		65.417	340901	5974	0.68
35		67.511	324891	4739	0.65
36		68.526	63195	2047	0.13
37		69.172	104144	2560	0.21

Appendix 6.4 Representative Chromatogram for SoPure Stevia™ GSG 95 Glucosylated Steviol Glycosides Batch G1909001

Zhucheng Haotian Pharm Co., Ltd

Empower 3

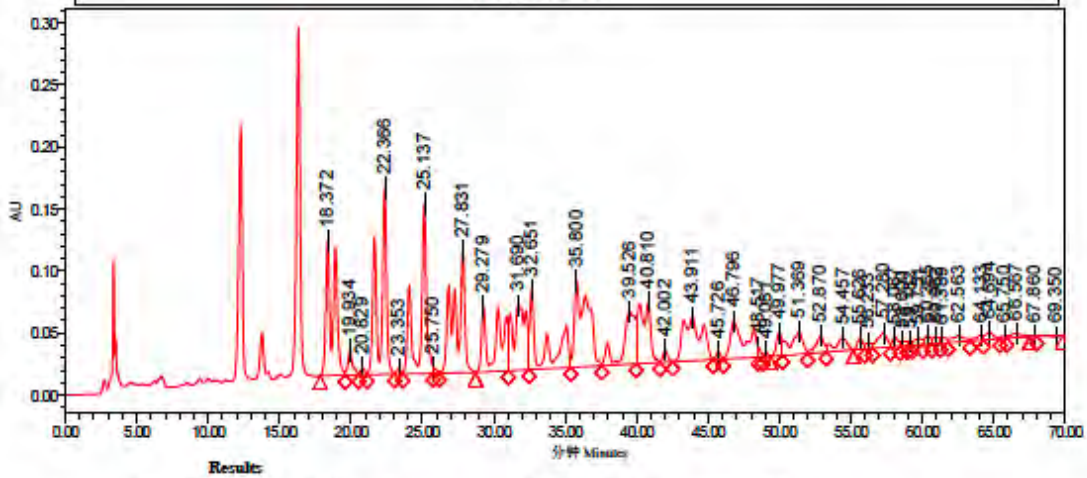
诸城市浩天药业有限公司

葡萄糖基甜菊糖苷 Glucosyl Steviol Glycosides

样品名称: Injection Name G1909001
样品类型: Injection Type 未知 Unknown
样品瓶: Sample Bottles 9
进样次数: Injection Times 1
进样体积: Injection Volume 10.00 ul
运行时间: Run Time 70.0 Minutes
采集时间: Injection Date 2019/9/6 20:06:41 CST

采集者: User Name stevia6
样品组名称: Group Name 20190906葡萄糖基甜菊糖苷GSG Gradient Method
采集方法组: Method Group d葡萄糖基甜菊糖糖苷梯度方法
处理方法: Processing Method d葡糖糖基甜菊糖苷梯度积分 Gradient Integration
通道名称: Channel Name W2489 ChA
处理通道说明: Wavelength W2489 ChA 210nm
处理时间: Processing Time 2019/9/7 11:11:26 CST

自动增放色谱图



Results

	Name	名称	Retention Time (Minutes) 保留时间 (分钟)	Area (μV*s) 面积 (微伏*秒)	Height (μV) 高度 (微伏)	Area % % 面积
1	$STVG_3$		18.372	4015116	108676	8.11
2			19.934	449070	19908	0.91
3			20.829	111452	5346	0.23
4	$RebAG_1$		22.366	4881658	150211	9.86
5			23.353	90477	4217	0.18
6	$STVG_2$		25.137	4425736	137127	8.94
7			25.750	70987	5027	0.14
8	$RebAG_2$		27.831	4558076	97885	9.21
9	$STVG_1$		29.279	3131814	52712	6.33
10	$RebAG_5$		31.690	3422089	52603	6.92
11	$STVG_4$		32.651	3122781	64195	6.31
12	$RebAG_4$		35.800	4491264	69323	9.08
13	$STVG_5$		39.526	2443685	42195	4.94
14	$RebAG_3$		40.810	2574289	49491	5.40

	Name	名称	Retention Time (Minutes) 保留时间 (分钟)	Area (μV*s) 面积 (微伏*秒)	Height (μV) 高度 (微伏)	Area % % 面积
15	$STVG_6$		42.002	223124	9893	0.45
16	$RebAG_6$		43.911	3128805	35127	6.32
17			45.726	128531	6064	0.26
18	$RebAG_7$		46.796	2231656	30249	4.51
19			48.517	85689	6245	0.17
20			49.081	67057	3559	0.14
21			49.977	470460	18527	0.95
22	$RebAG_8$		51.369	1108605	18307	2.24
23			52.870	527230	13386	1.07
24	$RebAG_9$		54.457	613210	10044	1.24
25			55.626	233003	8676	0.47
26			56.233	132965	4787	0.27
27			57.280	541434	11638	1.09
28			58.087	215947	7675	0.44

报告用户: 郭凤娟 (stevia5) 页码: 1 (共计 2)

	Name	Retention Time (Minutes)	Area (μV*s)	Height (μV)	Area %
	名称	保留时间 (分钟)	面积 (微伏*秒)	高度 (微伏)	% 面积
29		58.600	112965	3914	0.23
30		59.182	62897	3413	0.13
31		59.751	205092	5673	0.41
32		60.365	201845	7027	0.41
33		60.952	169847	5355	0.34
34		61.389	131523	4515	0.27
35		62.563	301826	4987	0.61
36		64.133	151486	3811	0.31
37		64.694	245850	5789	0.50
38		65.750	47277	1823	0.10
39		66.567	187563	3273	0.38
40		67.860	8331	475	0.02
41		69.350	64231	-973	0.13

Appendix 6.5 Representative Chromatogram for SoPure Stevia™ GSG 95 Glucosylated Steviol Glycosides Batch G2007001

Empower 3

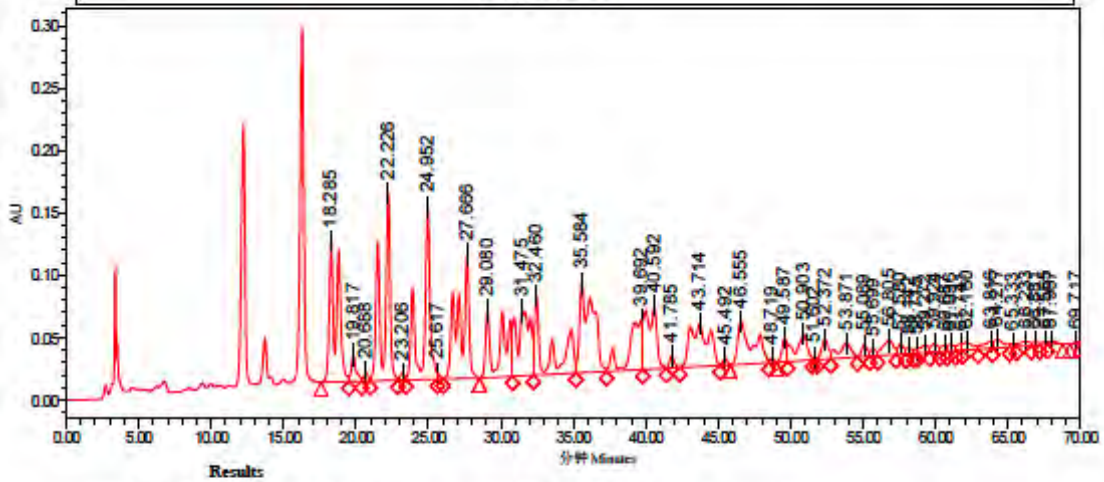
Zhucheng Haotian Pharm Co., Ltd
诸城市浩天药业有限公司

葡萄糖基甜菊糖苷 Glucosyl Steviol Glycosides

样品名称: Injection Name G2007001
样品类型: Injection Type 未知 Unknown
样品瓶: Sample Bottles 10
进样次数: Injection Times 1
进样体积: Injection Volume 10.00 ul
运行时间: Run Time 70.0 Minutes
采集时间: Injection Date 2020/7/8 21:27:53 CST

采集者: User Name stevia6
样品组名称: Group Name 20200708葡萄糖基甜菊糖苷GSG
采集方法组: Method Group α葡萄糖基甜菊糖糖苷梯度方法 (Gradient Method)
处理方法: Processing Method α葡糖糖基甜菊糖苷梯度积分 (Gradient Integration)
通道名称: Channel Name W2489 ChA
处理通道说明: Wavelength W2489 ChA 210nm
处理时间: Processing Time 2020/7/9 10:47:30 CST

自动缩放色谱图



Results

	Name	Retention Time (Minutes)	Area (μV*s)	Height (μV)	Area %
	名称	保留时间 (分钟)	面积 (微伏*秒)	高度 (微伏)	% 面积
1	$STVG_1$	18.285	4151801	112570	8.20
2		19.817	487717	21236	0.96
3		20.688	145754	6388	0.29
4	$RebAG_1$	22.226	4926138	150689	9.73
5		23.206	110605	4905	0.22
6	$STVG_2$	24.952	4515910	138587	8.92
7		25.617	59982	4683	0.12
8	$RebAG_2$	27.666	4570681	99274	9.03
9	$STVG_3$	29.080	3144347	53627	6.21
10	$RebAG_3$	31.475	3374916	52412	6.67
11	$STVG_4$	32.460	3195874	63532	6.31
12	$RebAG_4$	35.584	4545703	71965	8.98
13	$STVG_5$	39.692	2460543	39996	4.86
14	$RebAG_5$	40.592	2639098	50443	5.21

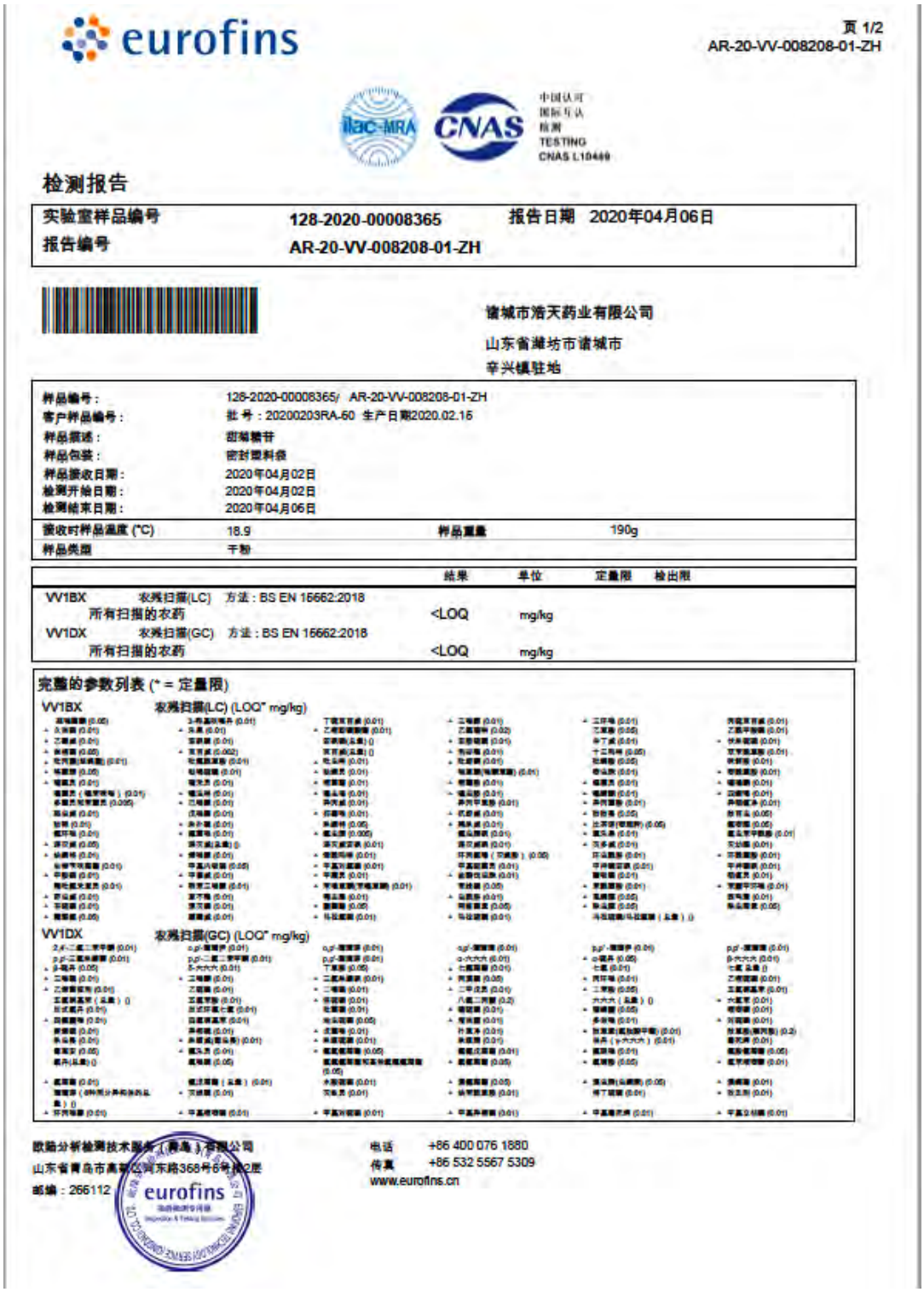
	Name	Retention Time (Minutes)	Area (μV*s)	Height (μV)	Area %
	名称	保留时间 (分钟)	面积 (微伏*秒)	高度 (微伏)	% 面积
15	$STVG_6$	41.785	225094	10042	0.45
16	$RebAG_6$	43.714	3092408	34488	6.11
17		45.492	108749	5700	0.21
18	$RebAG_7$	46.555	2365682	34444	4.67
19		48.719	65913	3617	0.13
20		49.587	443205	19150	0.88
21	$RebAG_8$	50.903	1162171	20941	2.30
22		51.602	34504	2181	0.07
23		52.372	503765	15592	1.00
24	$RebAG_9$	53.871	689599	11250	1.36
25		55.089	253239	9004	0.50
26		55.699	143627	5089	0.28
27		56.805	593710	11523	1.17
28		57.580	237314	8086	0.47

报告用户: 郭凤娟 (stevia5) 页码: 1 (共计 2)

	Name	Retention Time (Minutes)	Area (μV*s)	Height (μV)	Area %
	名称	保留时间 (分钟)	面积 (微伏*秒)	高度 (微伏)	% 面积
29		58.142	127420	4225	0.25
30		58.715	57615	3462	0.11
31		59.272	227441	5882	0.45
32		59.924	220832	6936	0.44
33		60.667	122005	4805	0.24
34		61.036	172035	5026	0.34
35		61.741	118217	4907	0.23
36		62.160	288888	6277	0.57
37		63.816	237990	5410	0.47
38		64.277	312277	6504	0.62
39		65.333	66464	2399	0.13
40		66.233	182061	3649	0.36
41		66.881	76155	2438	0.15
42		67.565	62730	2262	0.12
43		67.967	88936	2297	0.18
44		69.717	15271	704	0.03

报告用户: 郭凤娟 (stevia5) 页码: 2 (共计 2)

Appendix 7 Representative Pesticide Testing Report for Steviol Glycosides Extract Raw Material



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页 1/2
AR-20-VV-008208-01-ZH

ilac-MRA CNAS 中国认可 国际互认 检测 TESTING CNAS L10449

检测报告

实验室样品编号	128-2020-00008365	报告日期	2020年04月06日
报告编号	AR-20-VV-008208-01-ZH		

诸城市浩天药业有限公司
山东省潍坊市诸城市
辛兴镇驻地

样品编号：	128-2020-00008365/ AR-20-VV-008208-01-ZH
客户样品编号：	批 号：20200203RA-60 生产日期2020.02.16
样品描述：	甜菊糖苷
样品包装：	密封塑料袋
样品接收日期：	2020年04月02日
检测开始日期：	2020年04月02日
检测结束日期：	2020年04月06日
接收时样品温度 (°C)	18.9
样品重量	190g
样品类型	干粉

		结果	单位	定量限	检出限
VV1BX	农残扫描(LC) 方法：BS EN 15662:2018				
	所有扫描的农药	<LOQ	mg/kg		
VV1DX	农残扫描(GC) 方法：BS EN 15662:2018				
	所有扫描的农药	<LOQ	mg/kg		

完整的参数列表 (* = 定量限)

VV1BX 农残扫描(LC) (LOQ* mg/kg)

VV1DX 农残扫描(GC) (LOQ* mg/kg)

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页 2/2
AR-20-VV-008208-01-ZH

甲拌磷 (0.01)	甲拌磷(总量) ()	[illegible] (0.2)	▲ [illegible] (0.01)	[illegible] (0.01)	[illegible]（总量）()
[illegible] (0.05)	[illegible] (0.01)	▲ [illegible] (0.01)	▲ [illegible] (0.01)	▲ [illegible] (0.01)	▲ [illegible] (0.01)
艾氏剂 (0.01)	艾氏剂/狄氏剂(总量) ()	[illegible] (0.01)	[illegible] (0.01)	▲ [illegible] (0.01)	[illegible] (0.01)
[illegible] (0.01)	[illegible] (0.01)	[illegible] (0.05)	[illegible] (0.01)	▲ [illegible] (0.01)	[illegible] (0.01)
[illegible] (0.01)					

签名

Kevin Fu
授权签字人

注释

LOQ: 定量限
<LOQ: 小于定量限
N/A 表示不适用
总量结果由分量组分的定量值计算得出
▲在CNAS认可范围内
带☆的检测项目是分包给欧陆分析集团内的实验室检测
带•的检测项目是分包给欧陆分析集团外的实验室检测
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谨代表 欧陆分析检测技术服务（青岛）有限公司

报告结束

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Analytical Report

Sample Code	128-2020-00008365	Report date	06-Apr-2020
Certificate No.	AR-20-VV-008208-01-EN		

Zhucheng Haotian Pharm Co., Ltd.

Xinxing Town,Zhucheng City,Shandong Pro.CHINA

Our reference:	128-2020-00008365/ AR-20-VV-008208-01-EN
Client Sample Code:	批号：20200203RA-60 生产日期2020.02.15
Sample described as:	STEVIA EXTRACT
Sample Packaging:	Sealed plastic bag
Sample reception date:	02-Apr-2020
Analysis Starting Date:	02-Apr-2020
Analysis Ending Date:	06-Apr-2020
Arrival Temperature (°C)	18.9
Sample Weight	190g
Sample Type	Powder

		Results	Unit	LOQ	LOD
VV1BX	Pesticide Screening(LC) Method: BS EN 15662:2018				
	Screened pesticides	<LOQ	mg/kg		
VV1DX	Pesticide Screening(GC) Method: BS EN 15662:2018				
	Screened pesticides	<LOQ	mg/kg		

List of screened molecules (* = limit of quantification)

VV1BX Pesticide Screening(LC) (LOQ* mg/kg)

3-Hydroxycarbofuran (0.01)	Abamectin (0.05)	Acephate (0.01)	Acetamiprid (0.01)	Acetochlor (0.05)	* Aldicarb (0.05)
Aldicarb (sum) ()	Aldicarb-sulfone (0.01)	Aldicarb-sulfoxide (0.01)	ARAMITE (0.05)	Atrazine (0.01)	* Azinphos-methyl (0.05)
* Azoxystrobin (0.01)	Bendiocarb (0.01)	Benfuracarb (0.01)	Bioresmethrin (0.01)	* Bitertanol (0.01)	* Bracalid (0.01)
* Bupirimate (0.01)	* Buprofezin (0.01)	* Carbaryl (0.01)	Carbendazim/Benomyl (sum) (0.005)	* Carbofuran (0.002)	Carbofuran (sum) ()
Carbosulfan (0.01)	Carfentrazone-ethyl (0.01)	Chlorantraniliprole (0.01)	Chlorfluazuron (0.05)	Chloridazuron (0.01)	Chromafenozide (0.01)
* Clofentezine (0.01)	* Clothianidin (0.01)	* Cymoxanil (0.05)	Cyromazine (0.05)	Demeton-S-methyl (0.05)	Demeton-S-methyl-sulfone (0.01)
* Dichlorvos (0.05)	* Diethofencarb (0.01)	* Difenoconazole (0.01)	* Diflubenzuron (0.05)	Diflufenican (0.01)	* Dimethoate (0.01)
* Dimethomorph (0.01)	* Diniconazole (0.01)	Diphenamid (0.01)	Epoxiconazole (0.01)	Ethoxyquin (0.02)	Famoxadone (0.05)
Fenamiphos (0.05)	* Fenbuconil (0.01)	Fenbucarb (0.01)	* Fipronil (0.005)	Fipronil-sulfone (0.01)	Flazasulfop-P-butyl (0.01)
* Fludioxonil (0.05)	* Formetanate hydrochloride (0.01)	* Hexaconazole (0.01)	* Hexythiazox (0.01)	* Imazalil (0.01)	* Imidacloprid (0.01)
* Indoxacarb (sum, R+S isomers) (0.01)	Iprobenfos (0.01)	* Iprovalicarb (0.01)	Isoprocarb (0.01)	Isoprothiolane (0.01)	* Kresoxim-methyl (0.05)
* Linuron (0.01)	* Lufenuron (0.05)	* Malaoxon (0.01)	* Malathion (0.01)	Malathion/Malaoxon (sum) ()	* Metalaxyl (0.01)
* Metamitron (0.01)	* Methamidophos (0.01)	* Methidathion (0.01)	* Methomyl (0.01)	Metolachlor (0.01)	Mevinphos (0.01)
* Monocrotophos (0.01)	Neburon (0.01)	* Omethoate (0.01)	Oxydemeton-methyl (0.01)	Oxydemeton-methyl (sum) ()	
* Paraoxon-methyl (0.01)	Phorate-sulfone (0.01)	Phorate-sulfoxide (0.01)	* Phosalone (0.01)	* Pirimicarb (0.01)	* Phoxim (0.01)
* Pirimicarb (0.01)	Prochloraz (0.01)	Propamocarb (Sum of propamocarb and its salts, exp (0.01)	Propanil (0.01)	* Propargite (0.01)	* Propoxur (0.01)
* Pymetrozine (0.01)	Pyrethrins (0.05)	* Pyridaben (0.01)	Pyridaphenthion (0.01)	* Pyrifenox (0.05)	* Pyrimethanil (0.01)
* Pyriproxyfen (0.01)	* Quinoxyfen (0.01)	Quizalofop-ethyl (0.01)	Simazine (0.01)	Tebufenozide (0.01)	* Tebufenozide (0.01)
Tebufenpyrad (0.05)	Thiabendazole (0.01)	* Thiacloprid (0.01)	* Thiamethoxam (0.01)	Thiophanate-methyl (0.01)	* Triadimenol (0.01)
Trichlorfon (0.05)	* Tricyclazole (0.01)	Tridemorph (0.05)	* Triflumizole (0.01)	* Zoxamide (0.01)	

VV1DX Pesticide Screening(GC) (LOQ* mg/kg)

2-Phenylphenol (0.05)	Alachlor (0.01)	Aldrin (0.01)	Aldrin/ Dieldrin (Sum) ()	Allidochlor (0.01)	Ametryn (0.01)
* Benalaxyl (0.01)	Benoxacor (0.01)	* Bifenthrin (0.01)	* Biphenyl (0.01)	* Bromopropylate (0.01)	Butachlor (0.05)
Chlordane (total) ()	Chlordane, cis- (0.01)	Chlordane, trans- (0.01)	* Chlorfenapyr (0.05)	* Chlorfenvinphos (0.01)	Chlorpyrifos [-ethyl] (0.01)
* Chlorpyrifos-methyl (0.01)	* Chlorthal-dimethyl (0.01)	Cyanophos (0.01)	* Cyfluthrin (0.05)	Cyhalothrin, lambda-(incl. Cyhalothrin, gamma-) (0.05)	* Cypermethrin (0.05)
* Cyproconazole (0.01)	DDD, o,p- (0.01)	DDD, p,p'- (0.01)	DDE, o,p- (0.01)	DDE, p,p'- (0.01)	DDT (sum) ()
DDT, o,p'- (0.01)	DDT, p,p'- (0.01)	* Deltamethrin (0.05)	* Diazinon (0.01)	Dichlorobenzophenone (0.01)	Dichlorobenzophenone, p,p- (0.01)
* Diclofop (0.05)	Dicofol, p,p- (0.01)	* Dieldrin (0.01)	* Diphenylamine (0.05)	Endosulfan (Sum) ()	Endosulfan sulphate (0.05)
* Endosulfan, alpha- (0.05)	* Endosulfan, beta- (0.05)	EPN (0.01)	Ethion (0.01)	* Ethoprophos (0.01)	* Etofenprox (0.01)
Etrimfos (0.01)	* Fenarimol (0.01)	* Fenazaquin (0.05)	* Fenitrothion (0.01)	* Fenpropathrin (0.01)	* Fenthion (0.01)
Fenvalerate (all isomers) (0.01)	Flucythrinate (0.01)	* Flusilazole (0.01)	Fonofos (0.05)	HCH (sum) ()	HCH, alpha- (0.01)
HCH, beta- (0.01)					

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HCH, delta- (0.01)	Heptachlor (0.01)	Heptachlor (sum) ()	Heptachlor epoxide, cis- (0.01)	Heptachlor epoxide, trans- (0.01)	Heptenophos (0.01)
▲ Hexachlorobenzene (HCB) (0.01)	Isazophos (0.05)	Isocarbofos (0.01)	Isofenphos (0.01)	▲ Isofenphos-methyl (0.01)	Lindane (gamma-HCH) (0.01)
Methoxychlor (0.2)	Mirex (0.01)	▲ Myclobutanil (0.01)	Napropamide (0.2)	Nitrothal-isopropyl (0.01)	Paclobutrazol (0.01)
▲ Parathion (0.01)	▲ Parathion-methyl (0.01)	▲ Penconazole (0.01)	▲ Pendimethalin (0.01)	Pentachloroaniline (0.01)	▲ Permethrin (0.01)
Phenthoate (0.01)	Phorate (0.01)	Phosalone (sum) ()	▲ Piperonyl butoxide (0.01)	Pirimiphos-ethyl (0.01)	▲ Pirimiphos-methyl (0.01)
▲ Procymidone (0.01)	▲ Profenofos (0.05)	Prometryn (0.01)	Propachlor (0.05)	Propham (0.01)	▲ Propiconazole (0.01)
▲ Propyzamide (0.01)	Pyrazophos (0.01)	▲ Quinalphos (0.01)	Quintozene (0.01)	Quintozene (sum) ()	S 421 (0.2)
tau-Fluvalinate (0.05)	Tecnazene (0.01)	▲ Tefluthrin (0.01)	Terbufos (0.01)	Tetrachlorvinphos (0.01)	▲ Tetraconazole (0.01)
▲ Tetradifon (0.01)	▲ Tolclofos-methyl (0.01)	▲ Triadimefon (0.01)	▲ Triazophos (0.01)	▲ Trifluralin (0.01)	▲ Vinclozolin (0.01)

SIGNATURE

Kevin Fu
Authorized Signatory

EXPLANATORY NOTE

LOQ: Limit of Quantification
< LOQ: Below Limit of Quantification
N/A means Not applicable
▲ CNAS
☆ means the test is subcontracted within Eurofins group
° means the test is subcontracted outside Eurofins group

Sum compounds results are calculated from the results of each quantified compound as set by regulation
The sample description and information are provided by the Client. Eurofins is not responsible for verifying the accuracy, relevancy, adequacy and/or completeness of the information provided by the Client.
The analytical result herein is applicable for the sample(s) tested only.
This analytical report shall not be excerpted or modified without prior written approval from Eurofins. The report shall be utilized in full.
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END OF REPORT

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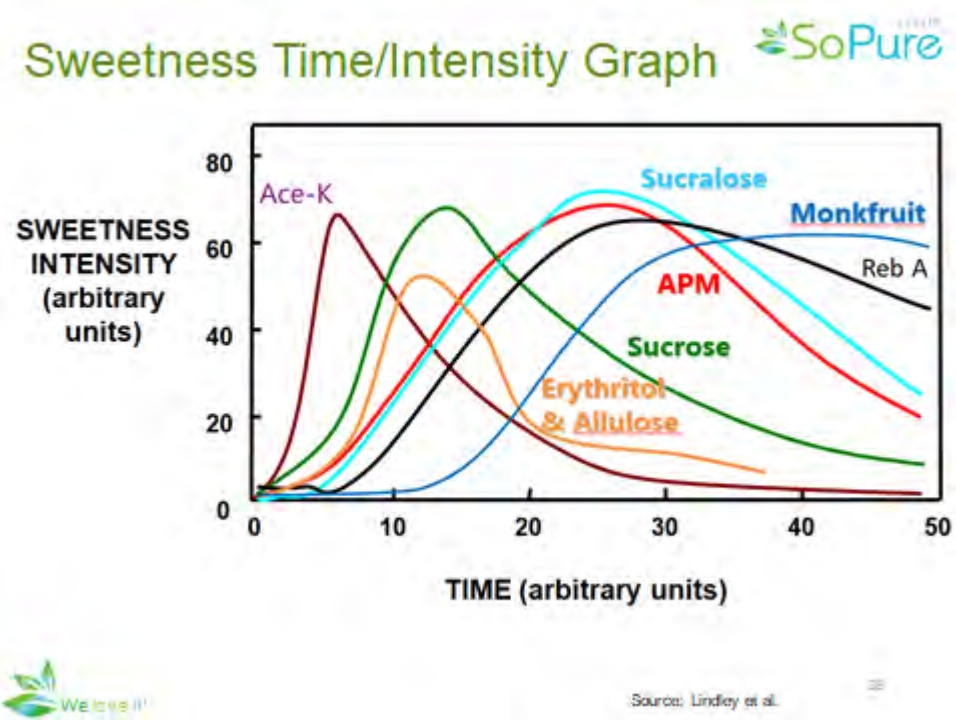
Appendix 8 Sweetness Intensity Test Reports

The sweetness of stevia is a wide range due to many factors including the composition, use level, application/ingredient interaction, personal taste preferences, adaptation, etc. Stevia has a later onset when compared to sucrose, which makes it harder to accurately compare them.



The sucrose equivalents for our products are determined through various tastings, while using literature data as a starting point. They are approximate and represent the range when used at typical use levels. The sweetness curve of stevia is unlike that of sucrose, which is a straight line. Therefore, the sweetness impact will largely depend on the use level.

Interaction of stevia with other ingredients in the formula also may have a significant impact on sweetness impact. The amount of time it takes for stevia to reach a sweetness receptor, if at all before swallowed, will impact the sweetness perception. For example, a thicker or dry baked product may need more stevia to sweeten to the same level as a thin beverage. Other ingredients like acid level and bulk sweeteners will also impact sweetness perception.

The glycoside composition of the stevia also has an effect on the sweetness. Studies performed on available GSG's showed that use level was also important in determining the enhancing sucrose equivalent. Tastings were performed in 10brix (10g/100ml) sugar in water at room temperature using an average result of 4 tasters.

FEMA 4728 ppm	sugar(g) replaced	X sugar
50	0.75	150
100	1	100
125	1.45	116
150	1.95	130
175	2.36	135
FEMA 4845 ppm	sugar(g) replaced	X sugar
50	0.7	140
100	1.55	155

In finished product, the below table includes a couple examples of the sweetness enhancement.

Category	Application	Sugar Reduction %	Stevia type	ppm	g sugar replaced	X Sugar
Desserts	Glaze	27.5%	FEMA 4728	100	2	200
Beverages	Iced Tea	27.5%	FEMA 4728	175	2.41	138

GSG products are tested according to the guidelines provided by FEMA when products are marketed to those categories. The test method is specified at: https://www.femaflavor.org/sites/default/files/2019-07/FEMA%20Sensory%20Guidance%20with%20Appendix%20March%202018.pdf. The ZCHT team tested their GSG at the recommended level of 125ppm for FEMA 4728. The results below show that it is significantly less sweet than 1.5% sugar, while enhancing sweet notes when combined with sugar.

Methodology:

Nature of Participants	Company employees
Number of Participants	30 (Female: 33%, Male: 67%)
Test Design	2-AFC, Randomized, Blind. Samples were prepared 2 hours before the test.
Environmental Condition	Standard room lightning
Sample Size	17-20ml sample liquid in a 30ml plastic drinking cup
Serving Temperature	Room temperature (~22°C)

GSG FEMA 4728

Test 1 Results:

Chose 1.5% sucrose more sweet: 27

Chose 125ppm GSG more sweet: 3

P value: 0.0001

Test 2 Results:

Chose 5% sucrose more sweet: 2

Chose 5% sucrose + 125ppm GSG more sweet: 28

P value: <0.00001

Appendix 9 Estimated Daily Intake Levels of Steviol Glycosides Preparations

Part 1. Food Uses as Addressed by JECFA, EFSA, FSANZ & Others

JECFA reviewed various estimates of possible daily intake of steviol glycosides (WHO, 2006). Merisant (2008) also listed intended use levels of rebaudioside A for various food applications in their GRAS Notice. Cargill (2008) estimated the possible daily intake of rebaudioside A assuming the use levels would be comparable to aspartame (Renwick, 2008). BioVittoria (2009) used an exposure estimate of "sucrose equivalents" and the sweetness intensity of Luo Han Guo fruit extract.

A. Estimated Daily Intake

Using different approaches, JECFA (WHO, 2006), Merisant (2008), and Cargill (2008) estimated daily intakes (EDIs) ranging from 1.3 – 4.7 mg per kg bw per day.

1. JECFA

- JECFA (WHO, 2006) evaluated information on exposure to steviol glycosides as submitted by Japan, China, and the European Commission by the Scientific Committee on Food. They used the Global Environment Monitoring System (GEMS)/Food consumption database to prepare international estimates of exposure to steviol glycosides (as steviol). JECFA assumed that steviol glycosides would replace all dietary sugars at the lowest reported relative sweetness ratio for steviol glycosides and sucrose, which is 200:1.
- The intakes ranged from 1.3 mg per kg bw per day with the African diet to 3.5 mg per kg bw per day with the European diet. Exposures to steviol glycosides assumed full replacement of all dietary sugars in the diets for Japan and the US.
- JECFA concluded that the replacement estimates were highly conservative. Furthermore, the calculated dietary exposures were overestimates and would probably be 20 – 30% of these values, or 1.0 - 1.5 mg per kg bw per day on a steviol basis or 3.0 – 4.5 mg per kg bw per day for rebaudioside A, based on the molecular weight adjustment.

2. EFSA

- On January 13, 2011, EFSA revised its dietary exposure assessment of steviol glycosides. For high consumers, revised exposure estimates to steviol glycosides remain above the established ADI of 4 mg per kg bw (steviol equivalent). For European children aged 1-14 years, revised intake estimates ranged from 1.7 to 16.3 mg per kg bw per day, and for adults, the range was reported to be from 5.6 to 6.8 mg per kg bw per day (EFSA, 2011b).

3. FSANZ

- FSANZ (2008) estimated the steviol glycoside dietary intake for adult consumers in New Zealand, assuming a full sugar replacement scenario. The estimated exposure to rebaudioside A ranged from 0.3 – 1.0 mg per kg bw per day for a consumer at the mean and 0.5 – 1.5 mg per kg bw per day for a consumer in the 90th percentile. FSANZ concluded that there were no safety concerns for either adults or children.
- In 2009, Cargill applied to FSANZ to increase the maximum usage levels of steviol glycosides in the high-volume food categories with increased usage levels by presenting market share analyses that overestimate actual intake while remaining well below the generally accepted ADI.
- FSANZ (2010) accepted the increased usage levels as requested from Cargill since no public health and safety issues were identified.

4. MERISANT

- Merisant (2008) utilized food consumption survey data from the 2003-2004 National Health and Nutrition Examination Survey (NHANES) to determine the estimated daily intake from the proposed uses of rebaudioside A.
- On a per user basis, the mean and 90th precentile daily consumption levels of rebaudioside A were estimated as 2.0 and 4.7 mg per kg bw per day, respectively.
- On a steviol equivalent basis, the Merisant estimates were calculated to be 0.7 and 1.6 mg per kg bw per day, respectively.
- On December 17, 2008, Merisant (2008) received a “no questions” letter from FDA for the use of rebaudioside A using NHANES food consumption data.

5. CARGILL

- Cargill (2008) estimated dietary intake figures for rebaudioside A by assuming that use levels of rebaudioside A would be comparable to those of aspartame in the US via post-market surveillance consumption data and published data for consumption of aspartame and other high intensity sweeteners (Renwick, 2008).
- On December 17, 2008, Cargill (2008) received a “no questions” letter from FDA for the use of rebaudioside A using comparative aspartame data.
- On May 13, 2011, FSANZ approved a Cargill application to increase the allowed maximum permitted level (MPL) of steviol glycosides (expressed as steviol equivalents) in ice cream, water based beverages, brewed soft drinks, formulated beverages and flavored soy beverages up to 200 mg per kg and in plain soy beverages up to 100 mg per kg (FSANZ, 2011).

6. BIOVITTORIA

- BioVittoria Ltd (2009) used an exposure estimate of “sucrose equivalents” and the sweetness intensity of any particular sweetener based upon data published by Renwick (2008).
- These data resulted in a maximum of 9.9 mg per kg bw per day for any population.
- BioVittoria (2010) received a “no questions” letter from FDA for the use of Luo Han Guo fruit extract using Renwick’s “sucrose equivalents.”

7. Other Publications

- Roberts et al. (2016) suggested that a higher ADI is justified based on metabolic factors to reduce the 100X safety factor. A chemical-specific adjustment factor (CSAF), as defined by the WHO in 2005, is determined by comparative studies in rats and humans.
    - A CSAF that is less than the standard 100X safety factor will result in an increase in the ADI, independent of the no observed adverse effect level (NOAEL).
    - The authors determined that using a CSAF can justify an ADI value of 6-16 mg per kg bw per day for steviol glycosides, depending on whether area under the plasma-concentration time curve (AUC) or C_{max} data are used when considering the 1,000 mg per kg bw per day NOAEL (which is equivalent to 400 mg per kg bw per day of steviol) for stevioside reported by Toyoda et al. (1997).

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Appendix 10 Studies on Steviol Glycosides Preparations

Part 1. Preparations that are Primarily Mixtures of Stevioside & Rebaudioside A

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

1. *In vivo* and *In vitro* Studies

- Studies investigating the hydrolysis of steviol glycosides by intestinal microflora have demonstrated that both stevioside and rebaudioside A are hydrolyzed to steviol following *in vitro* incubation with various cecal microflora (Wingard Jr. et al., 1980; Hutapea et al., 1997; Gardana et al., 2003; Geuns et al., 2003a).
- Various animal studies that show stevioside is not readily absorbed from the GI tract:
    - Rats - Wingard Jr. et al. (1980); Nakayama et al. (1986); Koyama et al. (2003b);
    - Hamsters - Hutapea et al. (1999);
    - Pigs - Geuns et al. (2003a); and
    - Chickens - Geuns et al. (2003b).
- *In vitro* metabolism studies show that steviol glycosides are transformed to steviol which is better absorbed in rats and humans (Geuns, 2003; Koyama et al., 2003b; Gardana et al., 2003; Wang et al., 2004).
- *In vitro* hydrolysis of rebaudioside A to steviol was found to be slower than that of stevioside (Koyama et al., 2003a).
    - The major pathway for rebaudioside A is conversion to stevioside with a minor pathway of conversion to rebaudioside B prior to being ultimately converted to steviol. Stevioside is further converted to steviolbioside, steviolmonosides, and finally steviol, with glucose being released with each subsequent hydrolysis.
- Koyama et al. (2003b) showed steviol can be converted to various glucuronides.
- Roberts and Renwick (2008) identified free steviol (82 to 86%), steviol, glucuronide (10 to 12%), and two unidentified metabolites (5-6%) in rat plasma following treatment with either stevioside or rebaudioside A eight hours post oral administration. Steviol T_{max} or plasma was noted within 30 minutes of oral administration as opposed to rebaudioside A, which has a T_{max} of 2 to 8 hours.
    - Following rebaudioside A treatment, significant amounts of unchanged rebaudioside A (29% in males and 19% in females) and stevioside (3% in males and 4% in females) were excreted in the feces.
    - Urinary excretion accounted for less than 2% of the administered dose.
    - Steviol was the predominant component found in plasma samples after oral administration of rebaudioside A, stevioside, and steviol in rats. The majority of all samples were found to be excreted rapidly---primarily in the feces---within 48 hours.
    - The predominant compound detected in the bile was steviol glucuronide, while the prominent material in the intestine was steviol.

  - The authors concluded that the overall data on toxicokinetics and metabolism indicate that rebaudioside A and stevioside are handled in an almost identical manner in the rat after oral dosing.
- Wheeler et al. (2008) assessed the comparative pharmacokinetics of steviol and steviol glucuronide following single oral doses of rebaudioside A and stevioside.
  - Following administration of rebaudioside A or stevioside, steviol glucuronide appeared in the plasma of all subjects, with median T_{max} values of 12.0 and 8.00 hours post-dose, respectively.
  - Administration of rebaudioside A resulted in a significantly (~22%) lower steviol glucuronide geometric mean C_{max} value (1,472 ng per mL) than administration of stevioside (1,886 ng per mL). The geometric mean AUC_{0-t} value for steviol glucuronide after administration of rebaudioside A (30,788 ng*h per mL) was approximately 10% lower than after administration of stevioside (34,090 ng*h per mL).
  - The authors concluded that rebaudioside A and stevioside underwent similar metabolic and elimination pathways in humans, with steviol glucuronide excreted primarily in the urine and steviol in the feces.
  - No safety concerns were noted as determined by reporting of adverse events, laboratory assessments of safety, or vital signs.
- Excretion of metabolites of stevioside after oral doses has been shown in urine and feces in rats (Sung, 2002) and hamsters (Hutapea et al., 1999).
- Oral doses in pigs led to the detection of metabolites in feces but not in urine (Geuns et al., 2003a).
- Since the individual steviol glycosides show similar pharmacokinetics in the rat and humans, the results of toxicology studies on individual steviol glycosides are applicable to the safety of steviol glycosides in general.

2. Human Studies

- Geuns et al. (2006) measured blood, urine, and fecal metabolites in 10 healthy subjects who received 3 doses of 250 mg of purified stevioside (>97%) three times per day for 3 days:
  - Free steviol was detected in feces but not in blood or urine. Steviol glucuronide was detected in blood, urine, and feces. Approximately 76% of the total steviol equivalents dosed were recovered in urine and feces.
  - The authors concluded that there was complete conversion of stevioside in the colon to steviol, which was absorbed and rapidly converted to the glucuronide.
- Renwick and Tarka (2008) reviewed studies on microbial hydrolysis of steviol glycosides and concluded that stevioside and rebaudioside A are not absorbed directly but are converted to steviol by gut microbiota in rats and in humans. This hydrolysis occurs more slowly for rebaudioside A than for stevioside.

B. Acute Toxicity Studies

A summary of the studies that investigated the acute toxicity of stevioside (96% pure) is presented in Table 10.1.

Table 10.1. Acute Toxicity of Stevioside (Purity 96%) Given Orally to Rodents

Species	Sex	LD_{50} (g/kg bw)	Reference
Mouse	Male and Female	>15	Toskulkac et al. (1997)
Mouse	Male	> 2	Medon et al. (1982)
Rat	Male and Female	>15	Toskulkac et al. (1997)
Hamster	Male and Female	>15	Toskulkac et al. (1997)

bw – body weight; Brahmachari et al. (2011)– gram; kg -- kilogram

No lethality was noted within 14 days after administration, and no clinical signs of toxicity, or morphological or histopathological changes were found, indicating that stevioside is essentially nontoxic in acute oral exposures.

C. Subchronic Toxicity Studies

- Akashi and Yokoyama (1975) dosed rats with up to 2,500 mg per kg bw per day of stevioside for 3 months and reported no adverse effects.
- Mitsuhashi (1976) added up to 7% stevioside to the diets of F344 rats for 3 months and report no adverse effects.
- Aze et al. (1990) added stevioside at 0, 0.31, 0.62, 1.25, 2.5, 5% to the diets of F344 rats for 13 weeks and reported no adverse effects. The apparent NOAEL was >5% dietary stevioside.
- The Awney et al. (2011) study revealed toxicity in rats dosed at 15 and 1,500 mg per kg stevioside, which resulted in a NOAEL of 15 mg per kg per day. This study is considered to be an outlier in critical reviews by Carakostas (2012) and Waddell (2011) for the following reasons:
    - Insufficient number of animals;
    - Animals were group housed leaving unreliable drinking water quantification;
    - No evidence of fasting before blood collection;
    - No urinalyses;
    - No histopathological confirmation of effects;
    - No organ weight data;
    - No laboratory historical control comparisons; and
    - Use of tartrate-resistant alkaline phosphatase (TRAP) enzyme, which has not been properly vetted for application on toxicological studies.

In summary, the data presented by Awney et al. (2011) are probably not representative of changes due to the subchronic dietary administration of steviol glycosides because of overall inadequate study design and reliance on the findings of the untested enzyme TRAP.

D. Chronic Toxicity Studies

- Yamada et al. (1985) added stevioside to the diets of F344 rats at 0.1, 0.3, and 1.0% with 95.2% steviol (75% stevioside/16% rebaudioside A) for 22 months in males and 24 months in females. Differences were noted in some parameters; however, the authors concluded that after 2 years of exposure, there were no significant changes that could be attributed to the administration of stevioside and reported no adverse effects. The calculated NOAEL was 550 mg per kg bw per day.
- Xili et al. (1992) added stevioside (86%) to the diets of F344 rats at 0, 0.2, 0.6, and 1.2% for 3 months and report no adverse effects. The calculated NOAEL was 794 mg per kg bw per day (high dose – 1.2%).
- Toyoda et al. (1997) added stevioside (96.5%) to the diets of F344 rats at 0, 2.5, and 5% for 104 weeks. The authors reported dose-dependent body weight gain decreases in both sexes. Kidney weights were significantly lower in 5% stevioside males; ovary, kidney and brain weights were significantly increased in 5% stevioside females; and there were decreased survival rates in males receiving 5% stevioside. However, stevioside was not carcinogenic at any level. The apparent NOAEL was the dietary level of 2.5%.
- No treatment-related increase in tumor incidence was seen in any of these studies.

E. Reproductive & Developmental Toxicity Studies

- No effects were observed in rats at doses of 96% stevioside dosed at 0, 0.15, 0.75, or 3% (equivalent to 2,000 mg per kg bw per day). The NOAEL was determined to be 2,000 mg per kg bw per day (Mori et al., 1981).
- No effect on fertility or reproductive parameters was seen in a three-generation study in hamsters at doses of 90% stevioside at 0, 500, 1,000, and 2,500 mg per kg bw per day (Yodyingyuad and Bunyawong, 1991). The NOAEL was determined to be 2,500 mg per kg bw per day.
- No teratogenic effects were observed in an additional rat study that was reviewed by Geuns (2003) in which pregnant female Wistar rats were administered stevioside (95.6%) at 0, 250, 500 or 1,000 mg per kg bw per day for 10 days (Usami et al., 1994). The NOAEL was determined to be 1,000 mg per kg bw per day.
- No effects on pregnancy or developmental parameters were observed in Swiss albino mice administered stevioside or aqueous stevia extract at doses of 500 and 800 mg per kg bw per day for 15 days to female mice (Kumar and Oommen, 2008).

F. Genotoxicity Studies

The following key genotoxicity studies have been conducted on stevia extracts and stevioside and showed negative responses:

- Bacterial mutagenicity studies negative for mutagenic response:
  - Medon et al. (1982);
  - Pezzuto et al. (1985);
  - Suttajit et al. (1993);
  - Matsui et al. (1996); and
  - Klongpanichpak et al. (1997).
- Mouse lymphoma (L5178Y/TK+/) study negative for mutagenic response:
  - Oh et al. (1999)
- Chromosome aberration studies negative for mutagenic response:
  - Human lymphocytes – Suttajit et al. (1993)
  - Chinese hamster lung fibroblasts – Nakajima (2000a); Ishidate et al. (1984)
- DNA damage (Comet assay) negative for mutagenic response:
  - Sekihashi et al. (2002); and
  - Sasaki et al. (2002)
- Mouse bone marrow/liver micronucleus studies negative for mutagenic response:
  - Oh et al. (1999)
- In two separate reviews by Carakostas et al. (2008) and Brusick (2008), research on rebaudioside A was summarized and combined with the body of knowledge on stevioside. These authors noted the following:
  - Steviol glycosides, rebaudioside A, and stevioside are not genotoxic *in vitro.*
  - Steviol glycosides, rebaudioside A, and stevioside have not been shown to be genotoxic *in vivo* in well-conducted assays.
  - The Nunes et al. (2007a) study was improperly interpreted as positive.
  - Stevioside is not a carcinogen or cancer promoter in well-conducted rodent chronic bioassays.
- Urban et al. (2013) examined the genotoxicity database on steviol glycosides concluding that the current database of *in vitro* and *in vivo* studies for steviol glycosides is robust and does not indicate that either stevioside or rebaudioside A is genotoxic.

G. Cytotoxicity

Abolhasani et al. (2020) evaluated the *in vitro* cytotoxicity of stevioside on cancerous liver (HepG2), colon (HT29), and breast (MCF7) cells, as well as normal kidney cells (Hek293), compared to cisplatin. Stevioside was reported to display higher cell growth inhibition on the HepG2 cell line and was not observed to have high toxicity on the Hek293 normal cell line. The authors concluded that stevioside “showed less cytotoxic effects compared to cisplatin.”

H. Clinical Studies & Other Reports in Humans

In South America, stevioside is used as a treatment for type 2 diabetes. These effects were key concerns for JECFA. In 2006, JECFA summarized the available clinical studies on stevioside and further studies were recommended (WHO, 2006). Subsequently, several additional studies were conducted and, in 2009, JECFA again reviewed these new studies (WHO, 2009). JECFA's summaries of the key studies are included in Table 10.2.

Table 10.2. Human Studies with Stevioside Preparations

Author/ Year	Substance Tested	Total Daily Dose	Population Characteristics	Study Design and Duration	Noted Effects Safety parameter Results
Curi et al. (1986)	Aqueous extracts *S. rebaudiana* leaves	5 g at 6 h intervals for 3 days = 20 g/day	16 healthy patients – extract/ 6 healthy patients – arabinose	3-day glucose tolerance in healthy adults	The extract of *Stevia rebaudiana* increased glucose tolerance. The extract decreased plasma glucose levels during the test and after overnight fasting in all volunteers. No adverse effects were reported.
Chan et al. (2000)	Stevioside (purity not stated)	750 mg (11 mg per kg bw/day)	60 hypertensive Chinese men and woman (aged 28-75 years) + 46 patients were given placebo.	Multicenter randomized, double-blind, placebo-controlled for 12 months	3 months: mean systolic and diastolic BP decreased and continued through the 12 months. Minor side effects occurred with 2 test group and 1 placebo group patient withdrawing. Other side effects were minor and resolved.
Hsieh et al. (2003)	Stevioside (purity not stated)	1,500 mg (21 mg/kg bw/day)	85 hypertensive Chinese men and woman (aged 20-75 years) + 89 patients were given placebo.	Multicenter randomized, double-blind, placebo-controlled for 24 months	Mean systolic and diastolic blood pressures were decreased commencing from about 1 week after the start of treatment. At 2 years test group patients had a ↓ in incidence of left ventricular hypertrophy. 3 patients withdrew. Other side effects were minor and resolved.
Anonymous (2004a)	Steviol extract: (~73% stevioside ~24% Reb A)	100 mg (3.3 mg/kg bw/day)	48 hyperlipidemic volunteers (24/24)	Randomized, double-blind, placebo-controlled for 3 months	Analyses of serum concentrations of triglycerides, liver-derived enzymes, and glucose indicated no adverse effects. 3 patients withdrew. No adverse side effects were reported.
Anonymous (2004b)	Steviol extract: (~73% stevioside ~24% Reb A)	3.25, 7.5, or 5 mg/kg bw/day	12 patients per test group	Randomized, double-blind, placebo-controlled for 30 days	No adverse responses in blood and urine biochemical parameters.

Author/ Year	Substance Tested	Total Daily Dose	Population Characteristics	Study Design and Duration	Noted Effects Safety parameter Results
Gregersen et al. (2004)	Stevioside - 91% + 9% other stevia glycosides	1 g stevioside or 1 g starch	12 patients with type 2 diabetes total	Acute paired cross-over study, single dose study	18% ↓ in glucose concentrations: Systolic and diastolic blood pressure were unchanged. No adverse effects
Temme et al. (2004)	Stevioside 97%	750 mg/kg bw/day (288 mg/kg bw steviol)	4 male and 5 female healthy patients	Short term study – 3 days	Blood chemistry, blood pressure and urinalyses were unremarkable.
Barriocanal et al. (2006)	Stevioside – 64.5% + 18.9% Reb A	750 mg/kg bw/day	Type 1 (n=8) + Type 2 (n=15) diabetics + non-diabetics (n=15) + matching controls - placebo	Double-blind, placebo-controlled trial study for 3 months	Blood chemistry, glycated hemoglobin (HbA1c), blood pressure and urinalyses were unremarkable. No adverse effects
Barriocanal et al. (2008)	Stevioside - >92%	250 mg/kg bw/day	Type 1 and Type 2 diabetics, placebo controls	Randomized, double-blind, placebo-controlled for 3 months	No changes in systolic BP, diastolic BP, glucose, or glycated hemoglobin from baseline. No adverse effects
Ferri et al. (2006)	Stevioside (purity not stated)	3.75, (7 weeks), 7.5 (11 weeks), 15 (6 weeks) + placebo (24 week) mg/kg bw/day	Patients with mild hypertension	Randomized 24 week study	No changes in systolic BP, diastolic BP. No adverse effects
Silva et al. (2006)	Stevioside: 70%	Equivalent to 1.04 mg steviol/kg bw/day + placebo	49 Mild hyperlipidemic patients: Stevioside group (n=24) placebo controls (n=25) Age: 20-70 years	Placebo-controlled double-blind trial for 90 days	No effects of treatment on ALT, AST, or GGT were found. No relevant adverse effects were noted.
Jeppesen et al. (2006)	Stevioside (purity not stated)	1,500 mg/kg bw/day or maize starch placebo	55 patients with Type 2 diabetes:	Randomized, double blinded, placebo-controlled study	No effects on the HbA1c fasting blood glucose levels, lipids, or blood pressure

ALT – alanine aminotransferase; AST – aspartate aminotransferase; BP – blood pressure; bw – body weight; Brahmachari et al. (2011)– gram; GGT – gamma-glutamyltransferase; h – hour; HbA1c – glycated hemoglobin; kg – kilogram; mg – milligram

I. Other Studies

- Thøgersen et al. (2018) investigated the effect of rebaudioside A, stevioside, and steviol on porcine cytochrome p450 (CYP) expression and activity to assess their potential food-drug interactions in the IPEC-J2 cell line.
    - There were no changes in CYP messenger ribonucleic acid (mRNA) expression following treatment of IPEC-J2 cells with rebaudioside A, stevioside, and steviol compared with control.
    - Treatment of primary hepatocytes resulted in increases in CYP329 mRNA at low concentrations of rebaudioside A and steviol, and at all concentrations of stevioside.

  - Treatment with the steviol glycosides tested over 24 hours resulted in minor increases in CYP3A29 mRNA expression (< 2.0-fold), while “no direct effect on CYP activity” was observed.
  - The authors concluded that rebaudioside A, stevioside, and steviol are unlikely to cause a food-drug interaction but noted that the study could not predict long term effects and effects *in vivo*.
- Zhao et al. (2020) studied the effect and mechanism of stevioside on preventive and therapeutic cardiac fibrosis caused by hyperglycemia in male C57BL/6 mice.
  - Stevioside supplementation reduced the expression of the cardiac fibrosis producing lysyl oxidase family (LOX) and weakened the collagen cross-linking lysyl oxidase-like 2 (LOXL2) caused by hyperglycemia.
  - Stevioside supplementation promoted the elimination of existing fibrosis via the regulation of matrix metalloproteinase (MMP 2/9) and tissue inhibitors of metalloproteinase (TIMP2/4).
  - No adverse effects were reported.

Part 2. Preparations That Are Primarily Rebaudioside A

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

Studies investigating the ADME of extracts from stevia are available on stevioside, rebaudioside A, and other steviol glycosides. Data evaluating the absorption and fate of these extracts from various animal species and humans indicate that one can extrapolate these results from rats to humans.

- Sloter (2008a) examined the potential of rebaudioside A toxicity in rats at up to 2,000 mg per kg bw per day.
  - Low levels of rebaudioside A were detected in the peripheral blood of rats post-administration of 2,000 mg per kg bw per day.
  - Estimates of absorbed dose for rebaudioside A of 0.6 µg per mL in plasma (corresponding to 0.02%) were based on amounts measured in urine collected over 24 hours in comparison to the daily administered dietary dose.
  - Mean fecal rebaudioside A and measured hydrolysis products, expressed as Total Rebaudioside A Equivalents, compared with daily administered dose results in an estimated dose recovery of approximately 84%.
- Zhou et al. (2019) investigated the interaction of organic anion transporter 3 (OAT3)-mediated update of the rebaudioside A metabolite, steviol acyl glucuronide, with selected prescription drugs.
  - The inhibitory potency of therapeutic drugs (those frequently prescribed for treating hyperglycemia, hyperlipidemia, and hyperuricemia, including probenecid and glimepiride) was examined against human renal excreter - organic anion transporter 3 (hOAT3) and rat organic anion transporter 3 (rOAT3) for uptake of steviol acyl glucuronide.

- OAT3-mediated update of steviol acyl glucuronide was examined *in vitro* using hOAT3 and rOAT3 transfected human embryonic kidney 293 (HEK203) cells. Both probenecid and glimepiride were potent inhibitors of hOAT3 and rOAT3, with no apparent species differences observed.
- Pharmacokinetic studies in male Sprague Dawley rats revealed both probenecid and glimepiride significantly elevated plasma steviol acyl glucuronide concentrations, particularly between 6 and 8 hours after oral administration of rebaudioside A.
- The inhibition of OAT3 is a potential mechanism for the interaction between steviol acyl glucuronide and probenecid or glimepiride, which can alter pharmacokinetic and safety profiles of steviol acyl glucuronide and steviol glycosides—specifically rebaudioside A.
- The authors conclude that this interaction might be clinically relevant, and that care should be given to populations with concomitant use of stevia leaf extracts and probenecid or glimepiride.

B. Subchronic Toxicity Studies

- Curry and Roberts (2008) added up to 100,000 ppm of rebaudioside A (97%) to the diets of Wistar rats for 13 weeks and reported no treatment-related adverse effects. Hence, the NOAEL was reported to be 9,938 mg per kg for males and 11,728 mg per kg for females – the highest level of treatment.
- Rebaudioside A (99.25%) was added to the diets of CRL:CD(SD) rats for 90 days at target doses of 500, 1,000, and 2,000 mg per kg bw per day with no treatment-related effects. The NOAEL was determined to be ≥2,000 mg per kg (Eapen, 2007; Nikiforov and Eapen, 2008).
- Eapen (2008) added rebaudioside A (97.5%) to the diets of Beagle dogs for 6 months at target doses of 500, 1,000, and 2,000 mg per kg bw per day and reported no adverse effects. The NOAEL was determined to be >2,000 mg per kg bw per day.
- The oral administration of fermentative rebaudioside A to Sprague Dawley rats for 91 days did not lead to any adverse effects at consumption levels up to 2,057 mg per kg bw per day for males and 2,023 mg per kg bw per day for females, which were concluded to be the NOAELs (Rumelhard et al., 2016).

C. Genotoxicity Studies

- *In vitro* and *in vivo* genotoxicity assays covering mutation, chromosome damage, and deoxyribonucleic acid (DNA) strand breakage consistently and uniformly revealed negative results for rebaudioside A.
- Evaluation of fermentation-derived rebaudioside A demonstrated a similar safety profile to plant-derived rebaudioside A (Rumelhard et al., 2016).

The following key mutagenicity studies have been conducted on rebaudioside A and are negative for mutagenic responses:

- Bacterial mutagenicity studies negative for mutagenic response:
  - Wagner and Van Dyke (2006);
  - Williams and Burdock (2009); and
  - Rumelhard et al. (2016).
- Mouse lymphoma (L5178Y/TK+/) studies negative for mutagenic response:
  - Clarke (2006); and
  - Williams and Burdock (2009).
- Human lymphocyte study negative for mutagenic response: Rumelhard et al. (2016)
- Chromosome aberration studies negative for mutagenic response:
  - Chinese hamster lung fibroblasts - Nakajima (2000a); and
  - Human lymphocytes - Williams and Burdock (2009).
- Mouse micronucleus studies negative for mutagenic response:
  - Nakajima (2000b) (BDF1 mouse bone marrow);
  - Krsmanovic and Huston (2006);
  - Williams and Burdock (2009); and
  - Unscheduled DNA synthesis (UDS) study negative for mutagenic response - Williams and Burdock (2009)
- Bacterial forward mutation study negative for mutagenic response - Pezzuto et al. (1985)

D. Reproductive & Developmental Studies on Rebaudioside A

- Curry et al. (2008) conducted a two-generation reproductive toxicity study on rebaudioside A administered in the diet at 7,500, 12,500 and 25,000 ppm in Han Wistar rats. There were no signs of toxicity or adverse effects on body weights, body weight gain, or food consumption. Rebaudioside A did not affect reproductive performance parameters including mating performance, fertility, gestation lengths, estrous cycles, or sperm motility, concentration, or morphology in either the F_0 or F_1 generations. The NOAEL for reproductive effects was 25,000 ppm, and the NOAEL for the survival, development, and general condition of the offspring also was considered to be 25,000 ppm, or 2,048 to 2,273 mg per kg bw per day (the highest dose tested).
- An unpublished study on rebaudioside A was conducted on four groups of male and female Crl:CD(SD) rats (30 per sex per group) that were fed either a basal diet or the diet containing rebaudioside A (purity 95.7%) for at least 70 consecutive days prior to mating (Sloter, 2008a). The test diet was offered to the offspring selected to become the F_1 generation following weaning (beginning on postnatal day 21). The F_0 and F_1 males continued to receive rebaudioside A throughout mating, gestation, and lactation until the day of euthanasia. Both for parental systemic and reproductive toxicity, the NOAEL was ≥2,000 mg per kg bw per day (highest dose administered).

- In another unpublished study, the embryo/fetal developmental toxicity effects of rebaudioside A when administered via gavage were studied in rats (Sloter, 2008b). The NOAEL for maternal and embryo/fetal development was determined to be >2,000 mg per kg bw per day.
- Cho et al. (2018) investigated the impact of stevia and obesity on fertility and reproductive outcomes in Sprague Dawley rats. Rats were administered 2-3 mg per kg bw per day rebaudioside A in drinking water starting two weeks prior to mating and throughout lactation. The authors reported that obese rats supplemented with rebaudioside A displayed a lower fertility index than untreated obese rats (53.3% vs. 85.7%, respectively); however, the rate of successful pregnancies was higher in obese rats supplemented with rebaudioside A than untreated obese rats (100% vs. 60.7%). No adverse effects or animal deaths were reported.
- Nettleton et al. (2020) investigated the impact of maternal low-dose rebaudioside A consumption on adiposity, glucose tolerance, gut microbiota, and the mesolimbic pathway in obese dams and their offspring. Pregnant obese rats and their offspring were fed high fat/sucrose diet plus 3 mg per kg bw per day rebaudioside A (Sigma-Aldrich) through 18 weeks postpartum. The authors noted that rebaudioside A consumption reduced the fertility of dams. The study supports findings that low-calorie sweeteners may not be metabolically inert.

E. Clinical Studies on Rebaudioside A

A summary of the clinical studies conducted on rebaudioside A is presented in Table 10.3.

Table 10.3. Human Studies with Rebaudioside A Preparations

Author/ Year	Substance Tested	Total Daily Dose	Population Characteristics	Study Design and Duration	Noted Effects Safety parameter Results
Maki et al. (2008a)	Rebaudioside A (97%)	Reb A: 1,000 mg, Placebo: 0 (n=100) Age: 18-73 years	Primarily female patients with normal and low-normal systolic blood pressure (SBP) and diastolic blood pressure (DBP)	Randomized, double-blind, placebo-controlled trial for 4 weeks	The extract of *Stevia rebaudiana* increased glucose tolerance. The extract decreased plasma glucose levels during the test and after overnight fasting in all volunteers.
Maki et al. (2008b)	Rebaudioside A (97%)	Reb A: 1,000 mg (n=60), Placebo: 0 (n=62) Age: 33-75 years	Men and women with Type 2 diabetes	Randomized, double-blind, placebo-controlled trial for 16 weeks	No treatment related changes in blood pressure, body weight, and fasting lipids were noted. Rebaudioside A was well-tolerated, and records of hypoglycemic episodes showed no excess versus placebo.

DBP – diastolic blood pressure; mg – milligram; SBP – systolic blood pressure

F. Safety of Rebaudioside A

There have been a number of studies regarding the safety and toxicity of rebaudioside A.

- GRAS Notices submitted to FDA:
    - GRN 252: Merisant (2008) conducted studies that augmented genotoxicity data in three systems recognized by FDA as good predictors of carcinogenic potential. Two of these assays were conducted in mouse systems.
    - GRN 253: Cargill (2008) conducted studies that provided significant insight into the pharmacokinetics of rebaudioside A, while demonstrating clinical safety of rebaudioside A regarding lack of effects on blood pressure and glucose metabolism that could result from doses expected from use in food.
- JECFA concluded that all naturally occurring steviol glycosides are safe as long as there is a combined purity of not less than 95% and determined the ADI of the steviol glycosides applied to rebaudioside A because the pharmacokinetics are virtually the same (FAO, 2017).
    - Carakostas et al. (2008) summarized the Cargill research program findings on rebaudioside A:
    - Steviol glycosides, rebaudioside A, and stevioside are not genotoxic *in vitro.*
    - In well-conducted *in vivo* assays, steviol glycosides, rebaudioside A, and stevioside have not been found to be genotoxic.
    - A report indicating that stevioside produces DNA breakage *in vivo* appears to be flawed (Nunes et al., 2007a) and was improperly interpreted as a positive response.
    - Steviol genotoxicity in mammalian cells is limited to *in vitro* tests that may be affected by excessive concentrations of the compound.
    - The primary evidence for steviol genotoxicity is derived from very specific bacterial tests or purified plasmid DNA that lack DNA repair capabilities.
    - Stevioside is not a carcinogen or cancer promoter in well-conducted rodent chronic bioassays.
    - While studies with rebaudioside A indicated minimal gastrointestinal (GI) absorption of the glycoside per se, the predominant metabolic pathway is comparable to that of stevioside. The use of the ADI established by JECFA, which was determined in studies employing stevioside as the main component, can be used as the ADI for rebaudioside A.
    - The dietary levels expected from consumption of rebaudioside A as a total replacement of sugar (Renwick, 2008) are lower than the ADI and, therefore, there is no safety concern for consumers.
- JECFA has evaluated the use of steviol glycosides in foods and agrees that, at the present time, the ADI for steviol glycosides of adequate purity, as defined by JECFA specifications, has been properly determined to be 4 mg per kg bw per person as steviol equivalents, which corresponds to 12 mg per kg bw per day for rebaudioside A, on a dry weight basis. Therefore, the JECFA-derived ADI was adopted as a safe exposure for rebaudioside A and

the corresponding food uses meeting the specifications within the limits determined by this esteemed international body of food safety experts can be considered to be GRAS.

- Williams and Burdock (2009) reviewed 3 *in vitro* and 2 *in vivo* genotoxicity and mutagenicity studies on rebaudioside A conducted according to Organisation for Economic Co-operation and Development (OECD) guidelines and found the studies revealed that rebaudioside A is:
    - non-mutagenic in an Ames test using *Salmonella typhimurium* and *Escherichia coli*;
    - non-mutagenic in a chromosomal aberration test using Chinese hamster V79 cells;
    - non-mutagenic in a mouse lymphoma assay using L5178Y+/- cells;
    - non-mutagenic a bone marrow micronucleus test in mice at doses up 750 mg per kg bw; and
    - non-mutagenic in an unscheduled DNA synthesis test in rats at 2,000 mg per kg bw.
    - The authors note that these studies provide additional evidence that rebaudioside A is not genotoxic at the doses tested and further support the GRAS determination of rebaudioside A.

Part 3. Studies on Other Steviol Glycosides Preparations

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

- Koyama et al. (2003b) published an *in vitro* study where α-glucosylated steviol glycosides were degraded by fecal microflora to steviol glycosides. These glycosides are subsequently hydrolyzed to the aglycone, steviol, demonstrating that the metabolic fate of α-glucosylated steviol glycosides follows that of non-modified steviol glycosides. Due to the similarities in metabolic fate, the safety of α-glucosylated steviol glycosides can be established based on studies conducted with non-modified steviol glycosides.
- Purkayastha et al. (2014) compared the anaerobic *in vitro* metabolism of rebaudiosides A, B, D, and M with human fecal homogenates.
    - The rebaudiosides were hydrolyzed to steviol within 24 hours, with the majority of metabolism occurring within the first 8 hours.
    - Metabolism of rebaudiosides took longer at higher concentrations (2.0 mg per mL vs. 0.2 mg per mL).
    - There were no marked differences in rate or extent of hydrolysis observed between male and female fecal homogenates or individual rebaudiosides.
- Purkayastha et al. (2016) investigated the metabolic fate of steviolbioside, dulcoside A, and rebaudiosides A, B, C, D, E, F, and M in an *in vitro* study using pooled human fecal homogenates over the course of 24 to 48 hours.
    - The glycosidic side chains ---containing glucose, rhamnose, xylose, fructose, and those with deoxy-glucose including combinations of α(1-2), β-1, β(1-2), β(1-3), and β(1-6) linkages ---were mostly degraded to steviol within 24 hours.
    - The rate of metabolism was slower at higher concentrations (2.0 mg per mL vs. 0.2 mg per mL).

  - No appreciable differences in metabolism were observed between fecal homogenates obtained from males and females or those obtained from different ethnicities.
- Purkayastha and Kwok (2020) investigated the *in vitro* metabolic fate of steviol glycosides in fecal homogenates collected from adults and children.
  - Steviol glycosides obtained from stevia leaf extract (composed of more than 20 steviol glycosides, with Reb D and Reb M as the principal components), bioconversion reaction product (composed of Reb D and Reb M), minor steviol glycosides extracted from a stevia leaf extract (composed of Reb AM, Reb W2, Reb U2, Reb V, Reb N, and Reb O), enzyme modified steviol glycosides, and rebaudioside A standard were used as test samples.
  - All steviol glycosides preparations tested "shared qualitatively similar *in vitro* metabolic fates."
  - The authors concluded that "safety data for individual steviol glycosides can be used to support safety of all steviol glycosides produced by extraction and enzymatic conversion of stevia leaf extract."

B. Toxicity Studies

- One study showed a toxic response and was conducted by Nunes et al. (2007a). In the Nunes study, rats were dosed with 4 mg per mL steviol glycosides in drinking water (estimated 80 to 500 mg per kg bw per day) for 45 days. Positive findings were reported in the liver, brain, and spleen, but most notably the liver. This study is considered to be an outlier in critical reviews conducted by Geuns (2007), Williams (2007), and Brusick (2008). The authors responded to these critiques (Nunes et al., 2007b; Nunes et al., 2007c). However, the consensus appears to be that Nunes et al. (2007a) used flawed methodology and improperly interpreted data as a positive response.
- Silva et al. (2018) addressed the genotoxic activity of stevia (Svetia™, purity not reported[20]).
  - Human lymphocytes were treated with 5% and 0.5% Svetia™ for 2 hours.
  - No statistically significant difference in genetic damage was observed in the 0.5% treatment concentration compared with the negative control, while the 5% treatment concentration resulted in a statistically significant difference ($P<0.0001$) compared with the control, with a decrease in migration average.
  - Human lymphocytes treated with 10% Svetia™ demonstrated significant ($P<0.0001$) genotoxic activity compared to the control; however, at treatment concentrations of 0.05%, 0.5%, and 5% Svetia™, a significant ($P<0.0001$) decrease in average migration of DNA was observed compared with the control.
  - The authors conclude that these results demonstrate the absence of genotoxicity at concentrations of up to 5% Svetia™ (Silva et al., 2018). It should be noted that these

[20] While the purity of the material used for the study was not reported by Silva et al. (2018), a search of the manufacturer's website (www.svetia.us) indicates that the trademarked material is a blend of cane sugar and 97% pure rebaudioside A.

observations are inconsistent with data reported by Nunes et al. (2007a); however, as discussed above, the validity and importance of the Nunes et al. study has been discounted given the questions surrounding the methodology.

C. Other Studies

- Sánchez-Delgado et al. (2019) studied the effects of steviol glycosides in a seven-week study on healthy young adults aged 18-30 years old. Thirty-eight patients were assigned to one of three study groups and "washed out" for one week prior to study initiation.
    - For six weeks, study participants were administered one of the following dosage regimes:
        - Sucrose (8 X 5 g packets per day)
        - Sucralose (8 X 5 g packets per day)
        - Steviol glycosides (4 X 1 g packets per day)
        - Note: the authors did not indicate if any bulking agents were present in the packets.
    - Results were as follows:
        - Subjects in the sucrose treatment group showed increased triglycerides and cholesterol.
        - Subjects in the sucralose treatment group showed increased body weight.
        - Subjects in the steviol glycosides treatment group show decreased fat mass, decreased triglycerides, and decreased tumor necrosis factor-α.
    - The authors concluded that steviol glycosides may have positive effects on metabolic parameters.
- Halasa et al. (2020) published a case study vignette on the investigation of the presence of steviol glycosides metabolites in plasma, cerebrospinal fluid, amniotic fluid, and cord blood samples from as early as 2004. The end date was not provided.
    - Steviol glucuronide was detected primarily in the plasma.
    - Seven of the 38 adults (18%) had detectable steviol glucuronide concentrations, while two of 13 (15%) amniotic fluid samples and one of 15 (7%) cord blood samples were observed to contain steviol glucuronide.

Part 4. Studies on Crude Stevia Extracts

In several studies, pharmacological and biochemical effects of crude extracts of stevia leaves and purified steviol glycosides have been investigated.

- In experimental studies in rats, crude stevia leaf extract (5%) was administered to female rats at 0 or 5% for 12 days. The female rats were subsequently mated with untreated males for the last 6 days, making a total of 18 days of exposure for the females (Planas and Kuć, 1968). Fertility was reduced to 21% of the fertility of control rats and remained reduced during the 50- to 60-day recovery period. The study report did not discuss histological examinations, weights of organs, blood analysis, urine chemistry, and necropsy.

- The use of *S. rebaudiana* as an oral contraceptive has been reported by indigenous populations in Paraguay (Planas and Kuć, 1968; Schvartaman et al., 1977).
- In rat studies, dried stevia leaves were administered at 0.67 g per mL in 2 mL doses twice per day for 60 days (Oliveira-Filho et al., 1989). The only difference due to treatment was seminal vesicle weight, which fell to 60% compared with control. No treatment-related adverse effects were noted.
- Wang and Wu (2019) studied the angiotensin-converting enzyme (ACE) inhibiting activity of an ethanolic extract of stevia leaves and purified steviol glycosides from the ethanol extract.
    - Steviol glycosides were reported to have double the ACE inhibitory activity of the ethanolic extract from stevia leaves.
    - Sensory tests in decaffeinated coffee, decaffeinated tea, and peanut protein beverages prepared with steviol glycosides demonstrated the preparations were well-accepted.
    - Steviol glycosides had a significant antihypertensive effect in spontaneously hypertensive rats. The authors suggest that the effect was dosage-dependent.
    - No adverse effects were reported.
- Assi et al. (2020) studied the efficacy of stevia extract alone and in combination with the commonly used sulfonylurea, glimepiride, in a trial to introduce a new effective therapeutic regimen for type 2 diabetes mellitus.
    - Rats with type 2 diabetes were treated orally with 300 mg per kg per day stevia extract for 21 days.
    - Results indicated that treatment with stevia extract showed good control of blood glucose levels and that a significant elevation in insulin release to glimepiride was observed.
    - The authors reported that stevia extract reduced blood glucose, triglycerides, cholesterol, ALT, AST, urea, creatinine, tumor necrosis factor, and malondialdehyde levels, while improving insulin and adiponectin levels.
    - No adverse effects were reported.
- Ray et al. (2020) studied the effects of *Stevia rebaudiana* on glucose homeostasis, blood pressure, and inflammation.
    - No hypersensitivities or allergies were reported since 2008, and that the few prior reports were for “improperly filtered stevia extracts.”
    - No significant adverse effects were noted from any study included in the review.

Part 5. Studies on Principal Metabolite: Steviol

A. Acute Toxicity Studies

- Toskulkac et al. (1997) administered single doses of steviol (90%) to rats and hamsters:
    - Rat, oral LD_{50} >15 g per kg; and
    - Hamster, oral LD_{50} 5.2 g per kg bw in males and 6.1 g per kg bw in females.

- Histopathological examination of the kidneys of hamsters revealed severe degeneration of the proximal tubular cells, and these structural alterations were correlated with increased serum blood urea nitrogen and creatinine. The authors concluded that the cause of death was acute renal failure.

B. Developmental Toxicity Studies

- Wasuntarawat et al. (1998) treated groups of pregnant golden hamsters with steviol (90%) at doses of 0 mg (n not reported), 250 mg (n=20), 500 mg (n=20), or 1,000 mg (n=12) per kg bw per day by gavage in corn oil on days 6 -10 of gestation.
  - A significant decrease in body weight gain and increased mortality (1/20, 7/20, and 5/12) were observed at the three highest doses.
  - The number of live fetuses per litter and mean fetal weight decreased in parallel.
  - No dose-dependent teratogenic effects were seen.
  - The NOEL for both maternal and developmental toxicity was 250 mg per kg bw per day.

C. Mutagenicity & Genotoxicity Studies

The following key mutagenicity studies have been conducted on steviol and are negative for mutagenic responses:

- Bacterial mutagenicity studies negative for mutagenic response:
  - Compadre et al. (1988);
  - Procinska et al. (1991); and
  - Klongpanichpak et al. (1997).
- Chromosome aberration studies negative for mutagenic response:
  - Chinese hamster lung fibroblasts – Matsui et al. (1996)
- DNA damage (Comet assay)
  - Sekihashi et al. (2002)
- Mouse bone marrow/liver micronucleus studies negative for mutagenic response:
  - Oh et al. (1999)
- Micronucleus studies negative for mutagenic response:
  - Matsui et al. (1996) (mouse);
  - Temcharoen et al. (2000) (rat);
  - Temcharoen et al. (2000) (mouse); and
  - Temcharoen et al. (2000) (hamster).

The following key mutagenicity studies have been conducted on steviol and are positive or equivocal for mutagenic responses:

- Bacterial mutagenicity studies positive for mutagenic response:

  - Pezzuto et al. (1985) – Mutagenicity was dependent on pretreatment of rats with Aroclor and NADPH addition, as unmetabolized steviol was inactive. None of the other metabolites tested was mutagenic.
  - Compadre et al. (1988) – Mass spectral analysis of steviol and analogues under conditions known to produce a mutagenic response. 15-oxo-steviol, a product of the metabolite, 15-alpha-hydroxysteviol was found to be a direct-acting mutagen.
  - Matsui et al. (1996) – Steviol was equivocal for mutagenicity. Steviol was weakly positive in Umu chromotest, either with or without metabolic activation. Steviol was negative in the reverse mutation and other bacterial assays even in presence of S9 activation.
  - Temcharoen et al. (2000) – Mutagenic effects of steviol and/or metabolites found in *S. typhimurium* TM677 by tranversions, transitions, duplications, and deletions at the guanine phosphoribosyltransferase (gpt) gene.
  - Terai et al. (2002) – Steviol was found to be mutagenic in Aroclor-induced rat liver S9 fraction.
- Chinese hamster lung fibroblast study positive for mutagenic response:
  - Matsui et al. (1996) – Gene mutations found in Chinese hamster lung fibroblasts after metabolic activation of steviol. In hamsters, several metabolites of stevioside found that have not been found in rats or humans. Therefore, experimental relevance should be questioned when hamsters are used.

Each of the positive mutagenicity studies noted above had special circumstances or slightly different procedures. The positive mutagenicity studies were collectively not believed to present sufficient toxicological concern as determined by JECFA (WHO, 2006).

D. Endocrine Disruption Studies

- Shannon et al. (2016) investigated the endocrine disrupting potential of stevioside, rebaudioside A, and steviol in a series of *in vitro* bioassays and found that steviol:
  - Antagonizes progesterone nuclear receptor transcriptional activity,
  - Increases progesterone production, and
  - Induces an agonistic response on the progesterone receptor of sperm cells (Catsper).
  - The authors conclude that steviol might not qualify as a safer alternative to sugar or synthetic sweeteners. However, one must consider the fact that it is difficult to translate *in vitro* concentrations to local concentrations *in vivo* at the receptor level and no adverse effects have been noted in any reproductive studies.

E. Other Studies

- Kurek et al. (2020) reported on the effect of steviol on cytotoxicity, adipogenesis, ROS concentration, and gene expression in the murine 3T3-L1 cell line.

- There was no observed effect on the proliferation of cells, lipid accumulation, or intracellular ROS generation at steviol concentrations up to 100 μM.
- Furthermore, it was reported that steviol reduced the expression of genes regulating the adipogenesis and lipogenesis process.

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Appendix 11 Summary of the Regulatory History of Steviol Glycosides

A. History of Traditional Medicinal and Human Food Use

- Stevia use as a sweetener and in traditional medicine by the Guarani tribes can be traced back for centuries (Esen, 2016; Gerwig et al., 2016; Brusick, 2008; Brandle et al., 1998).
- Commonly used to treat Type 2 diabetes in South America (Hawke, 2003). Doses in the range of 1 gram per person per day or more were reported to be necessary for therapeutic effects (Gregersen et al., 2004).
- Japan and Brazil approved stevia as a food additive in the 1980s (Raintree, 2012). (Lester, 1999) reported that 40% of the artificial sweetener market in Japan was stevia based.
- Use of steviol glycosides as a dietary supplement is presently permitted in the US, Canada, Australia, and New Zealand, and use as a natural health product is permitted in Canada.
- In 2005, it was estimated that sales of stevia in the US reached $45 million (Newsday, 2006).
- In 2010, Zenith International estimated stevia sales of 3,500 metric tons, which represents a 27% increase over 2009 figures. The market value is estimated to have increased to $285 million (Zenith, 2011).
- In 2013, worldwide sales of stevia were reported at 4,100 tons – representing a 6.5% increase over 2011 figures with an overall market value of $304 million (Zenith, 2013).
- In October 2014, it was reported that worldwide stevia sales increased 14% to 4,670 tons, with a market value of $336 million. It has been projected that the total market for stevia in 2017 would be 7,150 tons with an associated market value of $578 million (Zenith, 2014).
- NewHope360 reported that the global market for stevia in 2014 was $347 million, and that is expected to increase to $565.2 million by 2020. In addition, consumption is expected to increase from 2014 levels of 5,100.6 tons to 8,506.9 tons by 2020 (NewHope360, 2015).
- Nutritional Outlook reported that Mintel data indicated a 48% increase in stevia-containing products over the last five years (Decker and Prince, 2018).

B. Summary of Regulatory History of Enzyme Modified Steviol Glycosides

1. U.S. Regulatory History

As of December 17, 2020, FDA has issued 66 “no questions” letters on GRAS Notices on rebaudioside A, rebaudioside D, rebaudioside M, or steviol glycosides, including those undergoing enzyme treatment (FDA, 2020).

In addition, the Flavor and Extract Manufacturers Association (FEMA) has included several steviol glycosides preparations that are used to formulate flavors on their GRAS lists as shown in Table 11.1.

Table 11.1. FEMA GRAS Status for Steviol Glycoside Preparations

Steviol Glycosides Preparation	FEMA Number	Reference
Rebaudioside A	4601	Smith et al. (2009)
Rebaudioside C; dulcoside B	4720	Leffingwell (2011)
Glucosyl steviol glycosides; enzymatically modified stevia extract	4728	Leffingwell and Leffingwell (2014); Marnett et al. (2013)
Stevioside	4763	Leffingwell and Leffingwell (2014); Marnett et al. (2013)
Steviol glycoside extract, *Stevia rebaudiana*, Rebaudioside A 60%	4771	Marnett et al. (2013)
Steviol glycoside extract, *Stevia rebaudiana*, Rebaudioside A 80%	4772	Marnett et al. (2013)
Steviol glycoside extract, *Stevia rebaudiana*, Rebaudioside C 30%	4796	Cohen et al. (2015a); Cohen et al. (2015b)
Steviol glycoside extract, *Stevia rebaudiana,* Rebaudioside A 22%	4805	Cohen et al. (2015a); Cohen et al. (2015b)
Steviol glycoside extract, *Stevia rebaudiana* Rebaudioside C 22%	4806	Cohen et al. (2015a); Cohen et al. (2015b)
Glucosylated stevia extract Steviol glycosides 80%	4845	Cohen et al. (2017)
Enzyme modified stevia, stevioside 20%	4876	Cohen et al. (2017)
Rebaudioside M	4895	Cohen et al. (2020)
Glucosylated steviol glycosides, 70-80%	4909	Cohen et al. (2020)
Glucosylated steviol glycosides, 40%	4910	Cohen et al. (2020)
Stevia extract stevioside, 70%	4911	Cohen et al. (2020)
Rebaudioside D 95%	4921	Cohen et al. (2020)
Rebaudioside M 95%	4922	Cohen et al. (2020)
Glucosylated steviol glycosides, 90%	4931	Cohen et al. (2020)
Rebaudioside E ≥85%	4936	Cohen et al. (2020)
Rebaudioside I 95%	4937	Cohen et al. (2020)

2. Canadian Regulatory History

- On September 18, 2009, the Natural Health Products Directorate, Health Canada (Health Canada, 2009) adopted and revised the maximum limit for steviol glycosides in Natural Health products (NHPs) to be in accordance with the full ADI of 4 mg steviol per kg bw established by JECFA (WHO, 2008).

  - As a Medicinal Ingredient: The maximum daily limit without cautionary labelling and additional safety evidence was set at 4 mg per kg bw per day expressed as steviol content. This limit is equivalent to 10 mg per kg bw per day (i.e. ~ 710 mg per day for an adult) for stevioside or mixed steviol glycosides, 12 mg per kg bw per day (i.e. ~ 850 mg per day for an adult) for rebaudioside A, or 50 mg per kg bw per day (i.e. ~ 3,550 mg per day for an adult) of stevia leaf.
  - As a Non-Medicinal Ingredient: As a sweetener or flavor enhancer, the quantity used should be according to conditions of CGMP and should not exceed the amount required to accomplish the purpose for which that non-medicinal ingredient is permitted to be added. As a non-medicinal ingredient, it should not exceed 4 mg per kg bw per day expressed as steviol content.
- On November 30, 2012, Health Canada published its final clearance for use of steviol glycosides as a sweetener in foods (Health Canada, 2012).
- In March 2014, Health Canada updated the List of Permitted Sweeteners (Lists of Permitted Food Additives) to include steviol glycosides in applications as a table-top sweetener and as an ingredient in a variety of foods, beverages, baked goods, meal replacement bars, condiments, and confectionary and gums (Health Canada, 2014).
- On January 15, 2016, Health Canada approved the use of rebaudioside M for use as a high-intensity sweetener under the same conditions as the previously approved steviol glycosides (Health Canada, 2016).
- (Health Canada, 2017b) also modified the List of Permitted Sweeteners to include “all the steviol glycosides in the *Stevia rebaudiana* Bertoni plant (stevia plant).”
- On August 30, 2017, Health Canada’s Food Directorate updated its List of Permitted Sweeteners to allow for the use of steviol glycosides as a sweetener in ‘unstandardized snack bars,’ including granola bars, cereal bars, fiber bars, and protein isolate-based bars (Health Canada, 2017a).
- On August 27, 2018, Health Canada’s Food Directorate updated its List of Permitted Sweeteners to provide stakeholders with further information on the Lists of Permitted Food Additives as well as guidance on how to interpret and use these lists (Health Canada, 2018).
- On April 3, 2019, Health Canada’s Food Directorate modified the List of Permitted Sweeteners to allow for the use of steviol glycosides from *Stevia rebaudiana* Bertoni in canned fruit products (Health Canada, 2019c).
- On May 14, 2019, Health Canada’s Food Directorate modified the List of Permitted Sweeteners to allow for the use of steviol glycosides derived from *Saccharomyces cerevisiae* strains CD15380 and CD15407 at the same maximum levels of use as steviol glycosides derived from *Stevia rebaudiana* Bertoni (Health Canada, 2019b).
- On June 27, 2019, Health Canada’s Food Directorate modified the List of Permitted Sweeteners to allow for the use of steviol glycosides from various sources in “standardized flavoured milks” (Health Canada, 2019a).

- Most recently, on September 1, 2020, Health Canada updated the List of Permitted sweeteners to include the use of steviol glycosides produced by *Saccharomyces cerevisiae* Y63348 at the same maximum levels of use as steviol glycosides derived from *Stevia rebaudiana* Bertoni and *Saccharomyces cerevisiae* strains CD15380 and CD15407 (Health Canada, 2020).

3. European Regulatory History

- The Joint Expert Committee on Food Additives (JECFA) reviewed steviol glycosides at its 51st, 63rd, 68th and 73rd meetings and published its original review in 2000 (WHO, 2000).
- In 2006, JECFA established a temporary ADI (acceptable daily intake) of 0 - 2 mg per kg (on a steviol basis) at its 63rd meeting (WHO, 2006).
- In 2007, JECFA finalized food grade specifications (FAO, 2007b), although they were subsequently updated in 2008 (FAO, 2008) and 2010 (FAO, 2010).
- In 2008, Switzerland's Federal Office for Public Health approved the use of stevia as a sweetener citing the favorable actions of JECFA (Switzerland Federal Office of Public Health, 2008).
- In June 2008, the European Commission requested for EFSA to deliver a scientific opinion on the safety of steviol glycosides as a sweetener for use in the food categories specified in the dossiers from three petitioners.
    - EFSA reexamined the safety of steviol glycosides (EFSA, 2010) and the EFSA Panel established an ADI for steviol glycosides, expressed as steviol equivalents, of 4 mg per bw per day, which is similar to JECFA's determination.
    - On May 25, 2011, EFSA published the daily dietary intake for use of rebaudioside A as a flavoring substance in a variety of foods would be less than the ADI for steviol glycosides (EFSA, 2011a).
    - In 2014, EFSA evaluated extending the use of steviol glycosides as ingredients in food categories to include coffee, tea, and herbal and fruit infusions (assessed at 10 mg per L steviol glycosides) (EFSA, 2014).
    - In 2015, EFSA revised exposure estimates based on the EFSA Comprehensive European Food Consumption Database and the proposed extension of use for tea beverages and instant coffee and cappuccino products up to 29 mg per L of steviol equivalents, rather than 10 mg per L, as assessed in the previous 2014 EFSA opinion. EFSA noted that the mean exposure estimates remain below the ADI of 4 mg per kg bw per day for all population groups, with the exception of toddlers (in one country) at the upper range of the high-level exposure estimates (95th percentile: 4.3 mg per kg bw per day), which remains above the ADI. EFSA concluded that dietary exposure to steviol glycosides (E 960) is similar to the exposure estimated in 2014 and therefore does not change the outcome of the safety assessment (EFSA, 2015).
- In 2009, at the 69th meeting, the temporary status of the ADI was removed, and the ADI was raised to 0 - 4 mg per kg bw per day (on a steviol basis) as a result of the JECFA review of

more recently completed clinical studies with steviol glycosides (WHO, 2008). In 2009, JECFA published a final monograph addendum on steviol glycosides (WHO, 2009).

- In 2009, several countries and the Calorie Control Council submitted a request to the Codex Committee on Food Additives to modify the JECFA specifications for steviol glycosides to include rebaudioside D and rebaudioside F as specifically named acceptable glycosides that would be considered as part of the minimum 95% steviol glycosides composition (CCFA, 2009). The proposal was discussed at the June, 2010 JECFA Meeting (FAO/WHO, 2009), and JECFA subsequently took final action in approving the modified steviol glycosides specifications to include rebaudioside D and rebaudioside F (FAO, 2010).
- In 2009, France published its approval for the food uses of rebaudioside A with a purity of 97% (AFSSA, 2009a; AFSSA, 2009b).
- On December 2, 2011, the EU approved steviol glycosides use as food additives (EU, 2011) based upon agreement between the JECFA and EFSA that steviol glycosides are safe for all populations to consume and are a suitable sweetening option for diabetics.
- On October 13, 2016, the EU updated regulation EU 2016/1814 to permit the following steviol glycosides in stevia blends: stevioside, rebaudiosides A, B, C, D, E, F and M, steviolbioside, rubusoside, and dulcoside (Searby, 2016).
- On November 3, 2016, the EU food additives regulation 231/2012 was amended to remove the previous requirement for stevia blends to contain at least 75% rebaudioside A or stevioside.
- On January 31, 2018, the EFSA Panel of Food Additives and Nutrient Sources reviewed an application for glucosylated steviol glycoside preparations for use as a new food additive. The Panel concluded that the data supplied by the applicant were “insufficient to assess the safety” of the preparation. No safety concerns were raised by the EFSA Panel; however, their decision was based on the “limited” data provided in the dossier submitted by the applicant (EFSA, 2018).
- On September 24, 2019, the EFSA Panel on Food Additives and Flavourings concluded that there is no safety concern for rebaudioside M produced via enzymatic bioconversion and recommended that the European Commission consider establishing specifications for the preparation (EFSA, 2019).
- On March 24, 2020, EFSA published a scientific opinion in response to a proposed amendment of the specifications for steviol glycosides, stating that all steviol glycosides share the same metabolic fate, and therefore the safety of 60 steviol glycosides identified in the leaves of *Stevia rebaudiana* Bertoni can be based on “read-across” from previously evaluated toxicological data. EFSA maintained that the ADI of 4 mg per kg bw applies to all 60 steviol glycosides. The EFSA Panel noted that the inclusion of more steviol glycosides, “whilst maintaining the assay value of not less than 95%, would allow less pure preparations” onto the market. The Panel stated that they “cannot conclude on the safety of the proposed amendment to the specifications of steviol glycosides (E 960) as [a] food additive if the purity assay value of not less than 95% for the total content of steviol glycosides is maintained.” Furthermore, the Panel noted that it is possible to manufacture steviol glycosides with a purity higher than 95% total steviol glycosides (EFSA, 2020).

4. Asian Regulatory History

- In May 2010, Hong Kong amended its food regulations to allow the use of steviol glycosides as a permitted sweetener in foods based upon the detailed safety evaluation and favorable findings as reported by JECFA (Hong Kong Centre for Food Safety, 2010).
- In July 2011, the Codex Alimentarius Commission adopted proposed maximum use levels for steviol glycosides in all major food and beverage categories which resulted in steviol glycoside approvals in Vietnam, the Philippines, Malaysia, Singapore and Thailand (Whitehead, 2013).
- The International Alliance of Dietary/Food Supplement Associations (IADSA) reported that the Codex Alimentarius Commission agreed to adopt the use of steviol glycosides for addition to chewable food supplements (Food Ingredients First, 2011).
- On September 20, 2012, the Food Safety and Standards Authority of India (FSSAI) approved the use of steviol glycosides as a non-nutritive sweetener in a variety of foods using specifications and purity established by JECFA (FSSAI, 2012).
- Since December 10, 2012, over thirty registrations have been granted by FDA Philippines to stand-alone steviol glycosides sweeteners or foods containing steviol glycosides as ingredients (Philippines, 2014).
- Steviol glycosides are also listed under International Numbering System (INS) number 960 in the Food Additives Permitted Under the Singapore Food Regulations document prepared by the Agri-Food & Veterinary Authority (AVA) of Singapore (AVA, 2014).
- In China, steviol glycosides have been approved to be used as sweetener according to GB2760-2014 Standard for the Use of Food Additives issued by the National Health and Family Planning Commission of the People's Republic of China since 1984. Glucosyl Steviol Glycosides have been approved to be used as a flavoring substance since 2016.
- Steviol glycosides was approved in Korea in 1984 as a food additive by Ministry of Food and Drug Safety Regulation. Enzymatically modified stevia was approved in 2000.

5. Australia and New Zealand Regulation History

- In 2008, the Food Standards Australia New Zealand (FSANZ) completed its evaluation of an application for use of steviol glycosides in foods and recommended that the Australia and New Zealand Food Regulation Ministerial Council (Ministerial Council) amend the Australia New Zealand Food Standards Code to allow the use of steviol glycosides in food (FSANZ, 2008).
- On May 13, 2011, FSANZ approved an increase in the maximum permitted level (MPL) of steviol glycosides (expressed as steviol equivalents) in ice cream, water based beverages, brewed soft drinks, formulated beverages, and flavored soy beverages up to 200 mg per kg, and in plain soy beverages up to 100 mg per kg (FSANZ, 2011).
- In 2015, FSANZ concluded that the use of rebaudioside M does not pose any “public health and safety issues” (FSANZ, 2015).

- On January 14, 2016, rebaudioside M was approved for use "as a food additive in accordance with the current permissions for steviol glycosides" (FSANZ, 2016).
- In 2016, FSANZ called for submissions on permitting all minor steviol glycosides extracted from stevia leaf to be included in the definition of steviol glycosides in the Food Standards Code, noting that "[no] evidence was found to suggest that the proposed changes pose any public health and safety concerns" (FSANZ, 2016).
- On February 8, 2017, FSANZ approved a draft variation of the definition of steviol glycosides to include all steviol glycosides present in the *Stevia rebaudiana* leaf (FSANZ, 2017).
- In 2018, FSANZ called for comments on the production of rebaudioside M using enzymes derived from genetically modified yeast. The comment period closed on August 31, 2018 (FSANZ, 2018b). Subsequently, on October 31, 2018, FSANZ approved a draft variation to include a reference to the production method (FSANZ, 2018a).
- On May 14, 2020, FSANZ published an approval report for a draft variation to amend the specification for steviol glycosides from *Stevia rebaudiana* Bertoni in section S3—35 of the Australia New Zealand Food Standards Code to include rebaudioside E produced by enzymatic conversion from stevia leaf extract. The approved draft variation allows for the use of high purity rebaudioside E (≥85% rebaudioside E; ≥95% total steviol glycosides) within the existing permissions and limits for steviol glycosides (FSANZ, 2020a). Subsequently, on July 28, 2020, Amendment No. 193 was published to include rebaudioside E produced by enzymatic conversion from stevia leaf extract (FSANZ, 2020c).
- On October 21, 2020, FSANZ called for comments on permitting the use of rebaudioside M derived from *Saccharomyces cerevisiae* as a general purpose sweetening agent. Sandra Cuthbert, acting FSANZ Chief Executive Officer, stated that a thorough safety assessment was conducted by FSANZ and "…no public health or safety concerns with this type of steviol glycoside" were found. The comment period is scheduled to close on December 2, 2020 (FSANZ, 2020b).

6. South Africa

- On September 10, 2012, the South African Department of Health promulgated a new sweetener regulation: Regulation R733 (Regulations Relating to the Use of Sweeteners in Foodstuffs), allowed for the use of extracts of stevia rebaudiana, in composition and quantities in line with Codex standards, in food and beverages. Steviol glycosides can be used to a maximum level of 330 mg per kg (Food Stuff South Africa, 2012).

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Appendix 12 Summary of Published Safety Reviews

A. Summary of JECFA Reviews

- 51st Meeting (WHO, 2000) – Stevioside evaluation determined that there was insufficient and inconsistent information on the stevioside or steviol. No human metabolism data or mutagenicity data were available. JECFA determined that the ADI could not be determined without further data.
- 63rd Meeting (WHO, 2006) – More data were submitted; however, the data were inadequate to assess whether these pharmacological effects would also occur at lower levels of dietary exposure, which could lead to adverse effects in some individuals (e.g., those with hypotension or diabetes). The Committee allocated a temporary ADI, pending submission of further data on the pharmacological effects of steviol glycosides in humans. A temporary ADI of 0–2 mg per kg bw was established for steviol glycosides, expressed as steviol, based on a NOEL for stevioside of 970 mg per kg bw per day (or 383 mg per kg bw per day, expressed as steviol) in the two-year study in rats and a safety factor of 200.
- 68th Meeting (WHO, 2007) – Further data were submitted showing the purity at 95% and that all steviol glycosides hydrolyze to steviol upon ingestion. JECFA determined that it was unnecessary to maintain a limit for the sum of stevioside and rebaudioside content that could include product that was at least 95% stevioside or at least 95% rebaudioside A. The Chemical and Technical Assessment report, written after the 2007 meeting, explained the Committee's thinking, which resulted in flexibility in the identity specifications (FAO, 2007a; FAO, 2007b).
- 69th Meeting (WHO, 2008) – Based on additional clinical studies, JECFA finalized the evaluation of steviol glycosides and raised the ADI to 0 - 4 mg per kg bw per day and removed the "temporary" designation. A summary of the Committee's key conclusions was published in the final toxicology monograph addendum (WHO, 2009).

B. Summary of FSANZ Review of Steviol Glycosides

- In 2008, FSANZ reviewed the safety of steviol glycosides and concluded that they are well-tolerated and unlikely to have adverse effects on blood pressure, blood glucose, or other parameters in normal, hypotensive, or diabetic subjects at doses of up to 11 mg per kg bw per day. FSANZ agreed with JECFA in setting an ADI of 4 mg steviol equivalents per kg bw per day (FSANZ, 2008).
- On May 13, 2011, FSANZ approved an increase in the maximum permitted level (MPL) of steviol glycosides (expressed as steviol equivalents) in ice cream, water-based beverages, brewed soft drinks, formulated beverages and flavored soy beverages to 200 mg per kg and in plain soy beverages to 100 mg per kg (FSANZ, 2011).
- On January 16, 2016, FSANZ approved the addition of rebaudioside M as a steviol glycoside intense sweetener (FSANZ, 2016).

- On February 20, 2017, FSANZ broadened the definition and, hence, specification for steviol glycosides preparations to include any mixture of individual steviol glycosides extracted from the stevia leaf.

C. Summary of EFSA Review of Steviol Glycosides

- On March 10, 2010, EFSA adopted a scientific opinion on the safety of steviol glycosides (mixtures that comprise not less than 95% of stevioside and/or rebaudioside A) as a food additive based upon JECFA's 2008 findings and in response to the European Commission's request to re-evaluate the safety of steviol glycosides as a sweetener (EFSA, 2010).
    - EFSA agreed that the results of toxicology studies on either stevioside or rebaudioside A are applicable for the safety assessment of steviol glycosides.
    - EFSA established an ADI for steviol glycosides, expressed as steviol equivalents, of 4 mg per kg bw per day primarily based on the application of a 100-fold uncertainty factor to the NOAEL in the two-year carcinogenicity study in the rat when administering 2.5% stevioside in the diet (Toyoda et al., 1997).
- On January 11, 2011, EFSA revised the exposure assessment of steviol glycosides from its use as a food additive, for children and adults, based on the revised proposed uses presented.
    - EFSA reduced usage levels in 16 foods by a factor of 1.5 to 3, with no changes for 12 food groups.
    - The mean estimated exposure to steviol glycosides (equivalents) in European children (aged 1-14 years) ranged from 0.4 to 6.4 mg per kg bw per day and from 1.7 to 16.3 mg per kg bw per day at the 95th percentile.
    - A correction was considered to be necessary for the consumption of non-alcoholic flavored drinks (soft drinks) by children, and the corrected exposure estimate at the 95th percentile for children ranged from 1.0 to 12.7 mg per kg bw per day.
    - For adults, the mean and 97.5th percentile intakes were estimated to range from 1.9 to 2.3 and 5.6 to 6.8 mg per kg bw per day, respectively.
    - These revised exposure estimates to steviol glycosides remain above the established ADI of 4 mg per kg bw (steviol equivalent).

D. Other Published Reviews

- Stevia and steviol glycosides have been extensively investigated for their biological, toxicological, and clinical effects (Carakostas et al., 2008; Geuns, 2003; Huxtable, 2002).
- Four additional reviews have appeared on the toxicology and biological activity of stevia extracts and steviol glycosides (Yadav and Guleria, 2012; Brown and Rother, 2012; Brahmachari et al., 2011; Chatsudthipong and Muanprasat, 2009). The studies are not always closely comparable because:
    - These reviews do not clearly differentiate between studies on crude stevia extract and purified steviol glycosides.

  - Studies on biological activity used routes of administration other than oral.
  - Some studies may have used doses that are much higher than anticipated human use levels.
  - Roberts and Munro (2009) criticized the Chatsudthipong and Muanprasat (2009) review with points that are applicable – in general – to all the reviews:
    - Lack of purity of the material,
    - Route of exposure in relation to metabolism and safety assessment - *in vitro* and intravenous, intraperitoneal, or subcutaneous dosing studies are not relevant to the safety of steviol glycosides consumed orally.
    - Paucity of discussion of worldwide regulatory authorities affirming the safety of purified forms of stevioside and rebaudioside A as a food ingredient.

- In 2015, Urban et al. reviewed the potential allergenicity of steviol glycosides. The authors noted that: “hypersensitivity reactions to stevia in any form are rare” and concluded that current data do not support claims that steviol glycosides are allergenic. In addition, the authors stated that there is “little substantiated scientific evidence” to warrant consumer warning statements to consumers about allergy to highly purified stevia extracts.
- The effects of non-nutritive low-calorie sweeteners on gut microbiota were reviewed by Plaza-Diaz et al. (2020). It was noted that there have been no reports of negative interactions between steviol glycosides and colonic microbiota; however, it is possible that steviol glycosides modify the gut microbiota. The authors note that further studies are necessary to “clarify its specific effects.”
- A recent review by Ray et al. (2020) focused on the effects of *Stevia rebaudiana* on glucose homeostasis, blood pressure, and inflammation. The authors reported that no hypersensitivities or allergies were reported since 2008, and that the few prior reports were for “improperly filtered stevia extracts.” Furthermore, Ray et al. notes that additional randomized controlled trials are needed to confirm the beneficial effects of stevia. No significant adverse effects were noted from any study included in the review.

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Appendix 13 GRAS Associates Expert Panel Report

GRAS ASSOCIATES, LLC

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THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF THE PROPOSED USES OF ZHUCHENG HAOTIAN PHAM CO., LTD'S SOPURE STEVIA™ GLUCOSYLATED STEVIOL GLYCOSIDES

March 8, 2021

Foreword

An independent panel of experts ("Expert Panel") was convened by GRAS Associates, LLC on behalf of their client, Zhucheng Haotian Pharm Co., Ltd. ("ZCHT"), to evaluate the safety and Generally Recognized as Safe (GRAS) status of ZCHT's proposed uses of SoPure Stevia™ glucosylated steviol glycosides GSG 80 (≥80% total steviol glycosides with ≥75% glucosylated steviol glycosides and ≤ 20% dextrin) and GSG 95 (≥95% total steviol glycosides with ≥75% glucosylated steviol glycosides and ≤5% dextrin) preparations in conventional foods. The members of this Expert Panel[†] are qualified to serve in this capacity by qualification of scientific training and experience in the safety of food and food ingredients.

Discussion

A significant amount of safety information related to the consumption of steviol glycosides in generally available, and has been discussed in Part 6, as well as in Appendices 9-12, of ZCHT's SoPure Stevia™ glucosylated steviol glycosides GSG 80 (≥80% total steviol glycosides with ≥75% glucosylated steviol glycosides and ≤ 20% dextrin) and GSG 95 (≥95% total steviol glycosides with ≥75% glucosylated steviol glycosides and ≤5% dextrin) GRAS dossier. First, there is a history of safe consumption of steviol glycosides when used as an ingredient in food products in the U.S., Canada, South America, Europe, Asia, and Australia and New Zealand. Second, a number of experimental studies have investigated the safety of steviol glycosides, including those derived from enzymatic glycosylation processes. The composite evidence from historical safe consumption and experimental studies collectively demonstrates the safety of SoPure Stevia™ glucosylated steviol glycosides preparations for human food consumption.

The majority of the studies reviewed on steviol glycosides, steviol, and enzyme modified steviol glycosides have been discussed in detail in previous GRAS Notices (GRNs), including GRN 337, GRN 375, GRN 448, GRN 452, GRN 607, GRN 656, GRN 662, GRN 821, GRN 858, and GRN 878.

[†] Dr. Emmel, Chair of the Expert Panel, is a chemist with substantial food safety experience in addressing steviol glycosides and other food ingredients. Dr. Kapp is a toxicologist with over 35 years of experience. He is a Fellow of the Academy of Toxicological Sciences, a Fellow of the Royal Society of Biology, and a European Registered Toxicologist. Dr. Lewis is a biologist with more than 10 years of experience preparing GRAS dossiers. All three panelists have extensive technical backgrounds in the evaluation of food ingredient safety and in participating in deliberations of GRAS Expert Panels.

With regard to the safety documentation, the key pharmacokinetic data establish that steviol glycosides are not absorbed through the gastrointestinal (GI) tract, *per se*; they are converted to steviol by bacteria normally present in the large intestine, and the steviol is absorbed but rapidly metabolized to steviol glucuronide and excreted. It has been well-established experimentally from various published studies that the steviol glycoside molecules are not absorbed from the GI tract (Gardana et al., 2003; Koyama et al., 2003b). The action of bacteria in the large intestine is directly supported by the published study that showed that steviol glycosides can be converted to steviol in the large intestine by normal anaerobic GI flora as demonstrated by an *in vitro* study in fecal homogenates (Koyama et al., 2003a; Renwick and Tarka, 2008). Furthermore, Purkayastha *et al.* (2014; 2016) reported that rebaudioside B and rebaudioside A are metabolized to steviol in a similar, concentration-dependent manner in a pair of *in vitro* fecal homogenate studies. Most recently, Purkayastha and Kwok (2020) concluded that samples of stevia leaf extract, bioconverted steviol glycosides, a preparation of minor steviol glycosides, and enzyme modified steviol glycosides shared qualitatively similar *in vitro* metabolic fates as rebaudioside A in pooled human fecal homogenate samples, leading the authors to conclude that "safety data for individual steviol glycosides can be used to support safety of all steviol glycosides produced by extraction and enzymatic conversion of stevia leaf extract."

The Expert Panel reviewed other recent publications on steviol glycosides including those by Abolhasani et al. (2020), Afonso et al. (2020), Assi et al. (2020), Halasa et al. (2020), Kurek et al. (2020), Nettleton et al. (2020), Plaza-Diaz et al. (2020), Ray et al. (2020), and Zhao et al. (2020), and did not identify any that raise safety concerns with regard to the use of steviol glycosides in conventional foods.

The acceptable daily intake (ADI) for steviol glycosides has been set largely based on a published chronic study in rats (Toyoda et al., 1997) and several published studies that show there are no pharmacological effects in humans at doses several fold higher than the ADI (Barriocanal et al., 2006; Barriocanal et al., 2008; Wheeler et al., 2008). Roberts et al. (2016) noted in a persuasive argument using the World Health Organization (WHO) chemical-specific adjustment factor (CSAF)[1] that the ADI for steviol glycosides could justifiably range from 6 – 16 mg per kg bw per day. The toxicity of the metabolite, steviol, has been well reviewed in the published literature (Geuns, 2003; WHO, 2006; Urban et al., 2013). In addition, FDA has issued "no questions" letters in response to 66 GRN submissions for steviol glycosides preparations to date.

The Expert Panel notes that ZCHT's manufacturing process for their SoPure Stevia™ glucosylated steviol glycosides preparations is similar to the processes described for other GRAS enzyme modified steviol glycosides materials, as described in GRN 337, GRN 375, GRN 448, GRN 452, GRN 607, GRN 656, GRN 662, GRN 821, GRN 858, and GRN 878.

The GRAS Associates Expert Panel convened on behalf of ZCHT reviewed the proposed uses for SoPure Stevia™ glucosylated steviol glycosides GSG 80 (≥80% total steviol glycosides with ≥75%

[1] More information available at:
https://apps.who.int/iris/bitstream/handle/10665/43294/9241546786_eng.pdf;jsessionid=5605076D31FDBA3C3F13DDA0F6A9207F?sequence=1 (Accessed March 3, 2021)

glucosylated steviol glycosides and ≤20% dextrin) and GSG 95 (≥95% total steviol glycosides with ≥75% glucosylated steviol glycosides and ≤5% dextrin) preparations as a sweetener and as a flavor modifier. The highest 90th percentile consumption by any population subgroup of SoPure Stevia™ glucosylated steviol glycosides, based upon the lowest relative sweetness intensity and greatest calculated steviol equivalence, was calculated to be approximately 9.90 mg per kg body weight (bw) per day, which is equivalent to 3.47 mg per kg bw per day steviol equivalents (calculated by a weighted-sum estimate) on a worst-case scenario basis. This estimated intake is well below the JECFA ADI of 4 mg per kg bw per day expressed as steviol equivalents. The Expert Panel agrees with ZCHT's assessment that the total consumption of SoPure Stevia™ glucosylated steviol glycosides used as a sweetener and as a flavor modifier at maximum recommended use levels of 600 mg per kg in foods and 1,500 mg per kg in chewing gum is unlikely to exceed the established JECFA ADI. Therefore, SoPure Stevia™ glucosylated steviol glycosides preparations are expected to be safe within established allowable limits.

A compelling case can be made that scientific consensus exists regarding the safety of steviol glycosides when of sufficiently high purity. The central role of conversion to steviol and subsequent elimination with these naturally occurring steviol glycosides extends to the manner in which the various steviol glycosides molecules are metabolized and eliminated from the body. While the scientific conclusions are not unanimous regarding the safe human food uses of steviol glycosides, the Panel believes that a wide consensus does exist in the scientific community to support a GRAS conclusion as evidenced by several in-depth review publications (Geuns, 2007; Williams, 2007; Brusick, 2008; Waddell, 2011; Carakostas, 2012; Urban et al., 2013) that refute safety concerns expressed by a minority of scientists.

In summary, sufficient qualitative and quantitative scientific evidence in the composite is available to support the safety-in-use of ZCHT's SoPure Stevia™ glucosylated purity steviol glycosides GSG 80 (≥80% total steviol glycosides with ≥75% glucosylated steviol glycosides and ≤20% dextrin) and GSG 95 (≥95% total steviol glycosides with ≥75% glucosylated steviol glycosides and ≤5% dextrin) preparations given the following conditions:

- ZCHT's SoPure Stevia™ glucosylated steviol glycosides GSG 80 (≥80% total steviol glycosides with ≥75% glucosylated steviol glycosides and ≤20% dextrin) and GSG 95 (≥95% total steviol glycosides with ≥75% glucosylated steviol glycosides and ≤5% dextrin) continue to meet the designated specifications;
- The minimum sweetness intensity for SoPure Stevia™ glucosylated steviol glycosides preparations remains unchanged; and
- SoPure Stevia™ glucosylated steviol glycosides preparations are produced in accordance with Current Good Manufacturing Practices (CGMPs).

Conclusion

The Expert Panel critically reviewed the data provided by ZCHT for their SoPure Stevia™ glucosylated steviol glycosides GSG 80 (≥80% total steviol glycosides with ≥75% glucosylated steviol glycosides and ≤20% dextrin) and GSG 95 (≥95% total steviol glycosides with ≥75% glucosylated steviol glycosides and ≤5% dextrin) preparations, as well as publicly available

published information obtained from peer-reviewed journals and other safety assessments prepared by other Expert Panels and well-respected international regulatory bodies.

The ingestion of ZCHT's SoPure Stevia™ glucosylated steviol glycosides GSG 80 (≥80% total steviol glycosides with ≥75% glucosylated steviol glycosides and ≤20% dextrin) and GSG 95 (≥95% total steviol glycosides with ≥75% glucosylated steviol glycosides and ≤5% dextrin) from the intended uses results in intakes that are safe within the limits of established historical use and published safety studies and the widely accepted ADI of 4 mg per kg bw per day steviol equivalents.

The Expert Panel unanimously concluded that the proposed uses of ZCHT's SoPure Stevia™ glucosylated steviol glycosides preparations, manufactured as described in Part 2.b. of ZCHT's GRAS dossier, and declared within the subject notification meets the FDA definition of safety in that there is "reasonable certainty of no harm under the intended conditions of use" as described herein, and ZCHT's SoPure Stevia™ glucosylated steviol glycosides GSG 80 (≥80% total steviol glycosides with ≥75% glucosylated steviol glycosides and ≤20% dextrin) and GSG 95 (≥95% total steviol glycosides with ≥75% glucosylated steviol glycosides and ≤5% dextrin) preparations are generally recognized as safe (GRAS).

Robert W. Kapp, Jr., Ph.D.
Fellow ATS, FRSB, & ERT (UK)

Kara Lewis, Ph.D.

Katrina V. Emmel, Ph.D.
Panel Chair

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END

END

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

GENERALLY RECOGNIZED AS SAFE (GRAS) NOTICE

Form Approved: OMB No. ; Expiration Date:
(See last page for OMB Statement)

FDA USE ONLY

GRN NUMBER	DATE OF RECEIPT
ESTIMATED DAILY INTAKE	INTENDED USE FOR INTERNET
NAME FOR INTERNET	
KEYWORDS	

Transmit completed form and attachments electronically via the Electronic Submission Gateway *(see Instructions)*; OR Transmit completed form and attachments in paper format or on physical media to: Office of Food Additive Safety *(HFS-200)*, Center for Food Safety and Applied Nutrition, Food and Drug Administration, 5100 Paint Branch Pkwy., College Park, MD 20740-3835.

PART I – INTRODUCTORY INFORMATION ABOUT THE SUBMISSION

1. Type of Submission *(Check one)*

☒ New ☐ Amendment to GRN No. _____ ☐ Supplement to GRN No. _____

2. ☒ All electronic files included in this submission have been checked and found to be virus free. *(Check box to verify)*

3a. For New Submissions Only: Most recent presubmission meeting *(if any)* with FDA on the subject substance *(yyyy/mm/dd)*: N/A

3b. For Amendments or Supplements: Is your amendment or supplement submitted in response to a communication from FDA? *(Check one)* ☐ Yes ☐ No If yes, enter the date of communication *(yyyy/mm/dd)*: _____

PART II – INFORMATION ABOUT THE NOTIFIER

1a. Notifier

Name of Contact Person	Position
Hank Wang	Technical Director

Company *(if applicable)*: Nascent Health Sciences (US Division of ZCHT)

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Telephone Number	Fax Number	E-Mail Address
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1b. Agent or Attorney *(if applicable)*

Name of Contact Person	Position
William J. Rowe	President

Company *(if applicable)*: GRAS Associates

Mailing Address *(number and street)*: 11810 Grand Park Ave., Suite 500

City	State or Province	Zip Code/Postal Code	Country
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Telephone Number	Fax Number	E-Mail Address
519-341-3367	888-531-3466	wrowe@nutrasource.ca

PART III – GENERAL ADMINISTRATIVE INFORMATION

1. Name of Substance

SoPure Stevia™ Glucosylated Steviol Glycosides

2. Submission Format: *(Check appropriate box(es))*

☐ Electronic Submission Gateway

☐ Paper

☒ Electronic files on physical media with paper signature page

If applicable give number and type of physical media

_____

3. For paper submissions only:

Number of volumes: _____

Total number of pages: _____

4. Does this submission incorporate any information in FDA's files by reference? *(Check one)*

☐ Yes *(Proceed to Item 5)* ☒ No *(Proceed to Item 6)*

5. The submission incorporates by reference information from a previous submission to FDA as indicated below *(Check all that apply)*

☐ a) GRAS Notice No. GRN _____

☐ b) GRAS Affirmation Petition No. GRP _____

☐ c) Food Additive Petition No. FAP _____

☐ d) Food Master File No. FMF _____

☐ e) Other or Additional *(describe or enter information as above)* _____

6. Statutory basis for determination of GRAS status *(Check one)*

☒ Scientific Procedures *(21 CFR 170.30(b))* ☐ Experience based on common use in food *(21 CFR 170.30(c))*

7. Does the submission (including information that you are incorporating by reference) contain information that you view as trade secret or as confidential commercial or financial information?

☐ Yes *(Proceed to Item 8)*

☒ No *(Proceed to Part IV)*

8. Have you designated information in your submission that you view as trade secret or as confidential commercial or financial information *(Check all that apply)*

☐ Yes, see attached Designation of Confidential Information

☐ Yes, information is designated at the place where it occurs in the submission

☐ No

9. Have you attached a redacted copy of some or all of the submission? *(Check one)*

☐ Yes, a redacted copy of the complete submission

☐ Yes, a redacted copy of part(s) of the submission

☐ No

PART IV – INTENDED USE

1. Describe the intended use of the notified substance including the foods in which the substance will be used, the levels of use in such foods, the purpose for which the substance will be used, and any special population that will consume the substance *(e.g., when a substance would be an ingredient in infant formula, identify infants as a special population).*

Intended to be used as a table top sweetener, as a general purpose non-nutritive sweetener, and as a flavor modifier for incorporation into foods in general, other than infant formulas and meat and poultry products, 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.

2. Does the intended use of the notified substance include any use in meat, meat food product, poultry product, or egg product? *(Check one)*

☐ Yes ☒ No

PART V – IDENTITY

1. Information about the Identity of the Substance

	Name of Substance[1]	Registry Used *(CAS, EC)*	Registry No.[2]	Biological Source *(if applicable)*	Substance Category *(FOR FDA USE ONLY)*
1	Glucosyalted steviol glycosides	N/A	N/A	*Stevia rebaudiana* leaf extract	
2	Maltodextrin	CAS	9050-36-6		
3					

[1] Include chemical name or common name. Put synonyms *(whether chemical name, other scientific name, or common name)* for each respective item (1 - 3) in Item 3 of Part V *(synonyms)*

[2] Registry used e.g., CAS *(Chemical Abstracts Service)* and EC *(Refers to Enzyme Commission of the International Union of Biochemistry (IUB), now carried out by the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (IUBMB))*

2. Description

Provide additional information to identify the notified substance*(s)*, which may include chemical formula*(s)*, empirical formula*(s)*, structural formula*(s)*, quantitative composition, characteristic properties *(such as molecular weight(s))*, and general composition of the substance. For substances from biological sources, you should include scientific information sufficient to identify the source *(e.g., genus, species, variety, strain, part of a plant source (such as roots or leaves), and organ or tissue of an animal source)*, and include any known toxicants that could be in the source.

SoPure Stevia™ Glucosylated Steviol Glycosides are prepared from the enzymatic glucosylation of a purified *Stevia rebaudiana* leaf extract using a cyclodextrin glucanotransferase enzyme produced by *Bacillus licheniformis* and dextrin as the glucose source.

SoPure Stevia™ GSG 80 is composed of ≥80% total steviol glycosides with ≥75% glucosylated steviol glycosides and ≤20% dextrin.

SoPure Stevia™ GSG 95 is composed of ≥95% total steviol glycosides with ≥75% glucosylated steviol glycosides and ≤5% dextrin.

There are no known toxicants.

3. Synonyms

Provide as available or relevant:

1	Enzyme modified steviol glycosides; glucosylated steviol glycosides; SoPure Stevia™
2	
3	

PART VI – OTHER ELEMENTS IN YOUR GRAS NOTICE

(check list to help ensure your submission is complete – check all that apply)

- ☒ Any additional information about identity not covered in Part V of this form
- ☒ Method of Manufacture
- ☒ Specifications for food-grade material
- ☒ Information about dietary exposure
- ☒ Information about any self-limiting levels of use *(which may include a statement that the intended use of the notified substance is not-self-limiting)*
- ☒ Use in food before 1958 *(which may include a statement that there is no information about use of the notified substance in food prior to 1958)*
- ☒ Comprehensive discussion of the basis for the determination of GRAS status
- ☒ Bibliography

Other Information

Did you include any other information that you want FDA to consider in evaluating your GRAS notice?

☒ Yes ☐ No

Did you include this other information in the list of attachments?

☒ Yes ☐ No

PART VII – SIGNATURE

1. The undersigned is informing FDA that Zhucheng Haotian Pharm. Co., Ltd *(name of notifier)* has concluded that the intended use(s) of SoPure Stevia™ Glucosylated Steviol Glycosides *(name of notified substance)* described on this form, as discussed in the attached notice, is (are) exempt from the premarket approval requirements of section 409 of the Federal Food, Drug, and Cosmetic Act because the intended use(s) is (are) generally recognized as safe.

2. ☒ Zhucheng Haotian Pharm. Co., Ltd *(name of notifier)* agrees to make the data and information that are the basis for the determination of GRAS status available to FDA if FDA asks to see them.

Zhucheng Haotian Pharm. Co., Ltd *(name of notifier)* agrees to allow FDA to review and copy these data and information during customary business hours at the following location if FDA asks to do so.

Xinxing, Zhucheng, Shandong Province, 262218, The People's Republic of China *(address of notifier or other location)*

Zhucheng Haotian Pharm. Co., Ltd *(name of notifier)* agrees to send these data and information to FDA if FDA asks to do so.

OR

☐ The complete record that supports the determination of GRAS status is available to FDA in the submitted notice and in GRP No. ---------- *(GRAS Affirmation Petition No.)*

3. Signature of Responsible Official, Agent, or Attorney	Printed Name and Title	Date (mm/dd/yyyy)
Katrina Emmel Digitally signed by Katrina Emmel Date: 2021.03.08 10:36:05 -08'00'	Katrina Emmel on behalf of William J. Rowe, President	03/08/2021

PART VIII – LIST OF ATTACHMENTS

List your attached files or documents containing your submission, forms, amendments or supplements, and other pertinent information. Clearly identify the attachment with appropriate descriptive file names (or titles for paper documents), preferably as suggested in the guidance associated with this form. Number your attachments consecutively. When submitting paper documents, enter the inclusive page numbers of each portion of the document below.

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	Appendices 1-13 in the body of the dossier	

OMB Statement: Public reporting burden for this collection of information is estimated to average XX hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to: Department of Health and Human Services,Food and Drug Administration, Office of Chief Information Officer, 1350 Piccard Drive, Room 400, Rockville, MD 20850. (Please do NOT return the form to this address.). An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number.