DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

GENERALLY RECOGNIZED AS SAFE (GRAS) NOTICE (Subpart E of Part 170)

Form Approved: OMB No. 0910-0342; Expiration Date: 09/30/2019
(See last page for OMB Statement)

FDA USE ONLY

GRN NUMBER	DATE OF RECEIPT
000878	7/30/2019
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,5001 Campus Drive, College Park, MD 20740-3835.

SECTION A – 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)*

3 Most recent presubmission meeting *(if any)* with FDA on the subject substance *(yyyy/mm/dd)*: N/A

☐
☐ _____

SECTION B – INFORMATION ABOUT THE NOTIFIER

1a. Notifier	Name of Contact Person David Kim		Position or Title Managing Director	
	Organization *(if applicable)* Daepyung Co., Ltd.			
	Mailing Address *(number and street)* Leaders B/D 604, #274-4 Seohyeon-Dong, Bundang-Gu			
City Seongnam-Si	State or Province Gyeonggi-Do	Zip Code/Postal Code 463-824	Country Republic of Korea	
Telephone Number +82-31-709-7755	Fax Number +82-31-709-7756	E-Mail Address djkim@daepyung.co.kr		
1b. Agent or Attorney *(if applicable)*	Name of Contact Person William J. Rowe		Position or Title President and CEO	
	Organization *(if applicable)* GRAS Associates			
	Mailing Address *(number and street)* 27499 Riverview Center Blvd., Suite 212			
City Bonita Springs	State or Province Florida	Zip Code/Postal Code 34134	Country United States of America	
Telephone Number 239-444-1724	Fax Number 239-444-1723	E-Mail Address wrowe@nutrasource.ca		

SECTION C – GENERAL ADMINISTRATIVE INFORMATION

1. Name of notified substance, using an appropriately descriptive term

High Purity Glucosylated Steviol Glycosides; Enzyme Treated Stevia; Enzyme Modified Stevia; STEVITEN FRESH; STEVITEN RICH

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

☐ Electronic Submission Gateway

☐ Paper

☒ Electronic files on physical media

If applicable give number and type of physical media

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

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

☐

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

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

7. Does the submission (including information that you are incorporating) contain information that you view as trade secret or as confidential commercial or financial information? (see 21 CFR 170.225(c)(8))

☐ Yes *(Proceed to Item 8*

☒ No *(Proceed to Section D)*

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SECTION D – INTENDED USE

1. Describe the intended conditions of use of the notified substance, including the foods in which the substance will be used, the levels of use in such foods, and the purposes for which the substance will be used, including, when appropriate, a description of a subpopulation expected to consume the notified substance.

Intended to be used as a table top sweetener and as a general purpose non-nutritive sweetener for incorporation into foods in general, other than 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 product(s) subject to regulation by the Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture?

(Check one)

☐ Yes ☒ No

3. If your submission contains trade secrets, do you authorize FDA to provide this information to the Food Safety and Inspection Service of the U.S. Department of Agriculture?

(Check one)

☒ Yes ☐ No , you ask us to exclude trade secrets from the information FDA will send to FSIS.

SECTION E - PARTS 2 -7 OF YOUR GRAS NOTICE

☒ PART 2 of a GRAS notice: Identity, method of manufacture, specifications, and physical or technical effect (170.230).

☒ PART 3 of a GRAS notice: Dietary exposure (170.235).

☒ PART 4 of a GRAS notice: Self-limiting levels of use (170.240).

☒ PART 5 of a GRAS notice: Experience based on common use in foods before 1958 (170.245).

☒ PART 6 of a GRAS notice: Narrative (170.250).

☒ PART 7 of a GRAS notice: List of supporting data and information in your GRAS notice (170.255)

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

SECTION F - SIGNATURE AND CERTIFICATION STATEMENTS

1. The undersigned is informing FDA that Daepyung Co., Ltd.
(name of notifier)

has concluded that the intended use(s) of High Purity Glucosylated Steviol Glycosides; Enzyme Treated Stevia; Enzyme Modified Stevia
(name of notified substance)

described on this form, as discussed in the attached notice, is (are) not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on your conclusion that the substance is generally recognized as safe recognized as safe under the conditions of its intended use in accordance with § 170.30.

2. Daepyung Co., Ltd. agrees to make the data and information that are the basis for the conclusion of GRAS status available to FDA if FDA asks to see them;
(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; agrees to send these data and information to FDA if FDA asks to do so.

Daepyung, Leaders B/D 604, #274-4 Seohyeon-Dong, Bundang-Gu, Seongnam-Si, Gyeonggi-Do, Republic of South Korea
(address of notifier or other location)

The notifying party certifies that this GRAS notice is a complete, representative, and balanced submission that includes unfavorable, as well as favorable information, pertinent to the evaluation of the safety and GRAS status of the use of the substance.The notifying party certifies that the information provided herein is accurate and complete to the best or his/her knowledge. Any knowing and willful misinterpretation is subject to criminal penalty pursuant to 18 U.S.C. 1001.

3. Signature of Responsible Official, Agent, or Attorney	Printed Name and Title	Date (mm/dd/yyyy)
[redacted]	Katrina Emmel on behalf of William J. Rowe, President	07/25/2019

SECTION G – 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)
	Multiple Appendices -- Appendices 1 through 12	

GRAS ASSOCIATES, LLC

GRAS Associates, LLC
27499 Riverview Center Blvd.
Bonita Springs, FL 34134
T: 239.444.1724 | F: 239.444.1723
www.gras-associates.com

GRN 000878

July 25, 2019

Food and Drug Administration
Center for Food Safety & Applied Nutrition
Office of Food Additive Safety (HFS-255)
5001 Campus Drive
College Park, MD 20740-3835

Attention: Dr. Paulette Gaynor
Re: GRAS Notification – High Purity Glucosylated Steviol Glycosides

Dear Dr. Gaynor:

GRAS Associates, LLC, acting as the Agent for Daepyung Co., Ltd. ("Daepyung", Republic of South Korea), is submitting for FDA review Form 3667 and the enclosed CD, free of viruses, containing a GRAS Notification for *High Purity Glucosylated Steviol Glycosides*. Along with Daepyung'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. 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
27499 Riverview Center Blvd., Suite 212
Bonita Springs, FL 34134
wrowe@nutrasource.ca

Enclosure: GRAS Notification for Daepyung –*High Purity Glucosylated Steviol Glycosides*

GRAS Notification

of

High Purity Glucosylated Steviol Glycosides

Food Usage Conditions for General Recognition of Safety

on behalf of

Daepyung Co., Ltd.
Bundang-Gu, Seongnam-Si, Gyeongii-Do
Republic of South Korea (463-864)

7/23/19

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FOREWORD

Daepyung Co., Ltd. ("Daepyung") based our Generally Recognized as Safe (GRAS) assessment of high purity glucosylated steviol glycosides 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 ingredient were reviewed. In addition, a search of the scientific and regulatory literature was conducted through May 26, 2019, 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 Daepyung's request, GRAS Associates, LLC ("GA") convened an Expert Panel to complete an independent safety evaluation of Daepyung's high purity enzyme glucosylated steviol glycosides preparations. The purpose of the evaluation is to ascertain whether Daepyung's conclusion that the intended food uses of high purity glucosylated steviol glycosides as described in Part 3 are generally recognized as safe, i.e., GRAS, under the intended conditions of use. In addition, Daepyung has asked GA to act as Agent for the submission of this GRAS notification.

PART 1. SIGNED STATEMENTS AND CERTIFICATION

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

Daepyung has concluded that our high purity glucosylated steviol glycosides preparations that are a blend of glucosylated steviol glycosides, unreacted steviol glycosides, and maltodextrin, referred to as "STEVITEN FRESH" (80 - 90% total glucosylated steviol glycosides and unreacted steviol glycosides) and "STEVITEN RICH" (85 - 95% total glucosylated steviol glycosides and unreacted steviol glycosides), and which meet the specifications described below, are GRAS in accordance with Section 201(s) of the Federal Food, Drug, and Cosmetic (FD&C Act) Act. 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 following sections. The evaluation accurately reflects the intended conditions of food use for the designated high purity 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 5/23/19).

Signed:

Agent for Daepyung

William J. Rowe
President
GRAS Associates, LLC
27499 Riverview Center Blvd.
Suite 212
Bonita Springs, FL 34134

Date: 7/23/19

B. Name and Address of Responsible Parties

Daepyung Co., Ltd.
Leaders B/D 604, #274-4 Seohyeon-Dong
Bundang-Gu, Seongnam-Si, Gyeonggi-Do
Republic of South Korea (463-824)

As the Responsible Party, Daepyung accepts responsibility for the GRAS conclusion that has been made for our high purity glucosylated steviol glycosides preparations as described in the subject safety evaluation; consequently, the purified 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 name of the ingredient to be used on food labels is “Enzyme Treated Stevia” or “Enzyme Modified Stevia.” Daepyung also plans to market our high purity glucosylated steviol glycosides preparations under the trade names “STEVITEN FRESH” and “STEVITEN RICH.”

D. Conditions of Intended Use in Food

Daepyung’s STEVITEN FRESH (80 - 90% total glucosylated steviol glycosides and unreacted steviol glycosides) and STEVITEN RICH (85 - 95% total glucosylated steviol glycosides and unreacted steviol glycosides) high purity glycosylated steviol glycosides 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], Daepyung's STEVITEN FRESH (80 - 90% total glucosylated steviolglycosides and unreacted steviol glycosides) and STEVITEN RICH (85 - 95% total glucosylated steviol glycosides and unreacted steviol glycosides) high purity glucosylated steviol glycosides preparations have been concluded to be GRAS on the basis of scientific procedures as discussed in the detailed description provided below.

Purified steviol glycosides are not subject to premarket approval requirements of the FD&C Act based on Daepyung's conclusion that the substance is GRAS under the conditions of its intended food use.

Daepyung 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 high purity glucosylated steviol glycosides.

F. Availability of Information

The data and information that serve as the bases for this GRAS Notice will be maintained at the offices of Daepyung Co, Ltd. (Republic of South Korea), and will be made available during customary business hours.

Daepyung 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

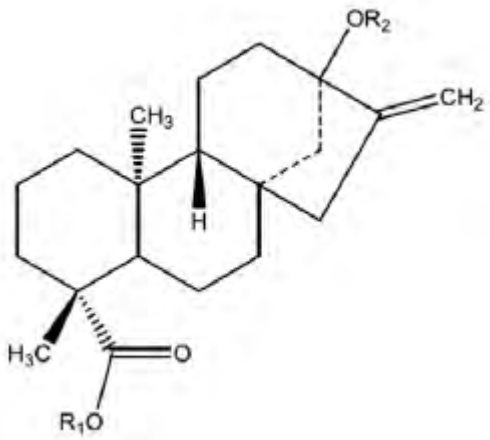
"Enzyme modified steviol glycosides" or "glucosylated steviol glycosides" are the common or usual names of the non-nutritive sweetener derived from the enzymatic glycosylation of a high purity extract of *Stevia rebaudiana* Bertoni. The compositional features of Daepyung's high purity glucosylated steviol glycosides preparations are described in more detail in this section. The preparations are also marketed as STEVITEN FRESH and STEVITEN RICH.

The general chemistry of steviol glycosides and enzyme modified steviol glycosides has previously been reviewed in a number of GRAS Notifications (GRN), including GRN 337 (NOW Foods, 2010), GRN 667 (Blue California, 2016), and GRN 715 (Blue California, 2017). Representative chemical structures of steviol glycosides that have been identified to date are presented in Figure 1.

[2] https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=170.30 (Accessed 5/22/19).

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)

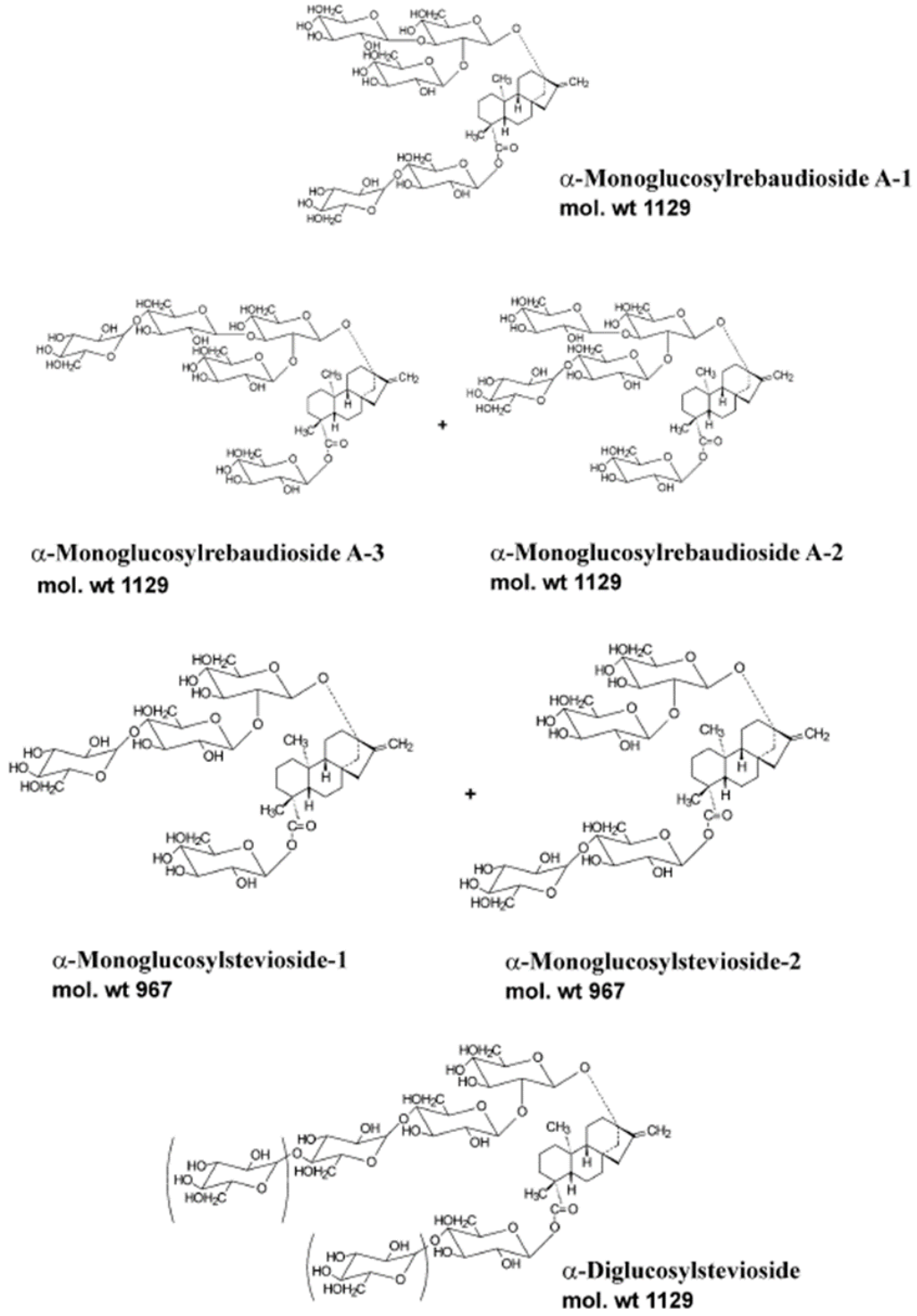
Enzyme modified steviol glycosides are produced when additional glucose moieties are bonded to the original steviol glycoside structure via α(1→4) linkages, resulting in α-glucosylated steviol glycosides. The product α-glucosylated steviol glycosides consists of a mixture of both α-D-glucosylated steviol 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.

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



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

B. Manufacturing Processes

Daepyung's STEVITEN FRESH and STEVITEN RICH 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 preparations are high purity enzyme modified steviol glycosides products: STEVITEN FRESH (80 - 90% total glucosylated steviolglycosides and unreacted steviol glycosides) and STEVITEN RICH (85 - 95% total glucosylated steviolglycosides and unreacted steviol glycosides). The remaining 5 - 20% of the finished product is unreacted maltodextrin.

1. Steviol Glycosides Raw Material

For the manufacturing of the starting steviol glycosides material, Daepyung employs a fairly typical process that is used in the industry for the production of stevia extracts. In order to extract rebaudioside A from the leaves of *Stevia rebaudiana* Bertoni, Daepyung has developed a state-of-the-art process in which a 30 - 60% rebaudioside A extract powder is prepared and further purified to obtain an extract with 95% steviol glycosides, as described in the flow chart in Figure 3.

2. STEVITEN FRESH and STEVITEN RICH Enzyme Modified Steviol Glycosides

Daepyung uses the purified stevia extract product, maltodextrin, and cyclomaltodextrin glucanotransferase enzyme to manufacture high purity enzyme modified steviol glycosides. After being heated to 78±1°C at pH 5.6 for 25 to 48 hours, the mixed starting material is deactivated at 95°C for 1 hour at pH 4.0, absorbed with absorption resin, and eluted with food grade ethanol. The eluted solution is then concentrated, filtered through diatomaceous earth (celite), sterilized by ultra-high temperature (UHT), and spray dried to obtain either the STEVITEN FRESH or STEVITEN RICH preparation, which is a mixture of glucosylated steviol glycosides and unmodified steviol glycosides, with 5 - 20% unreacted maltodextrin.

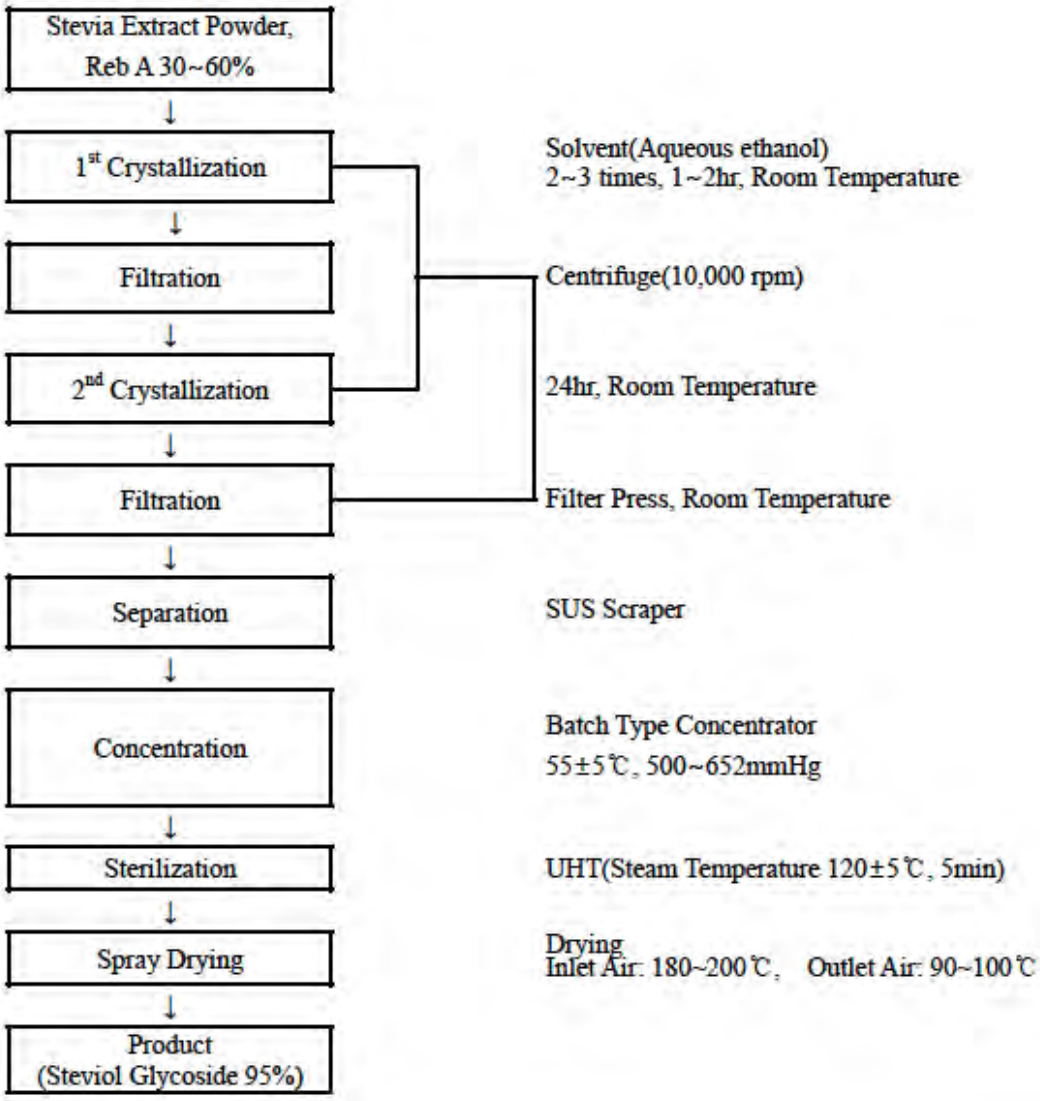
The enzyme used to glucosylate the purified stevia extract is Toruzyme 3.0L, which is a CGTase enzyme produced by a genetically modified strain of *Bacillus licheniformis*.[3] The glucose source is maltodextrin derived from tapioca. The ethanol, resin, and celite used in the purification process comply with applicable Food Chemicals Codex (FCC) or 21 CFR specifications. Supporting documentation for the raw materials and processing aids are provided in Appendix 1.

Daepyung's high purity glucosylated steviol glycosides preparations are prepared in accordance with CGMP regulations in a food facility registered with the Department of Health and Human Services/Food and Drug Administration (DHHS/FDA).

The manufacturing process is summarized in the flow charts provided in Figure 4 and Figure 5.

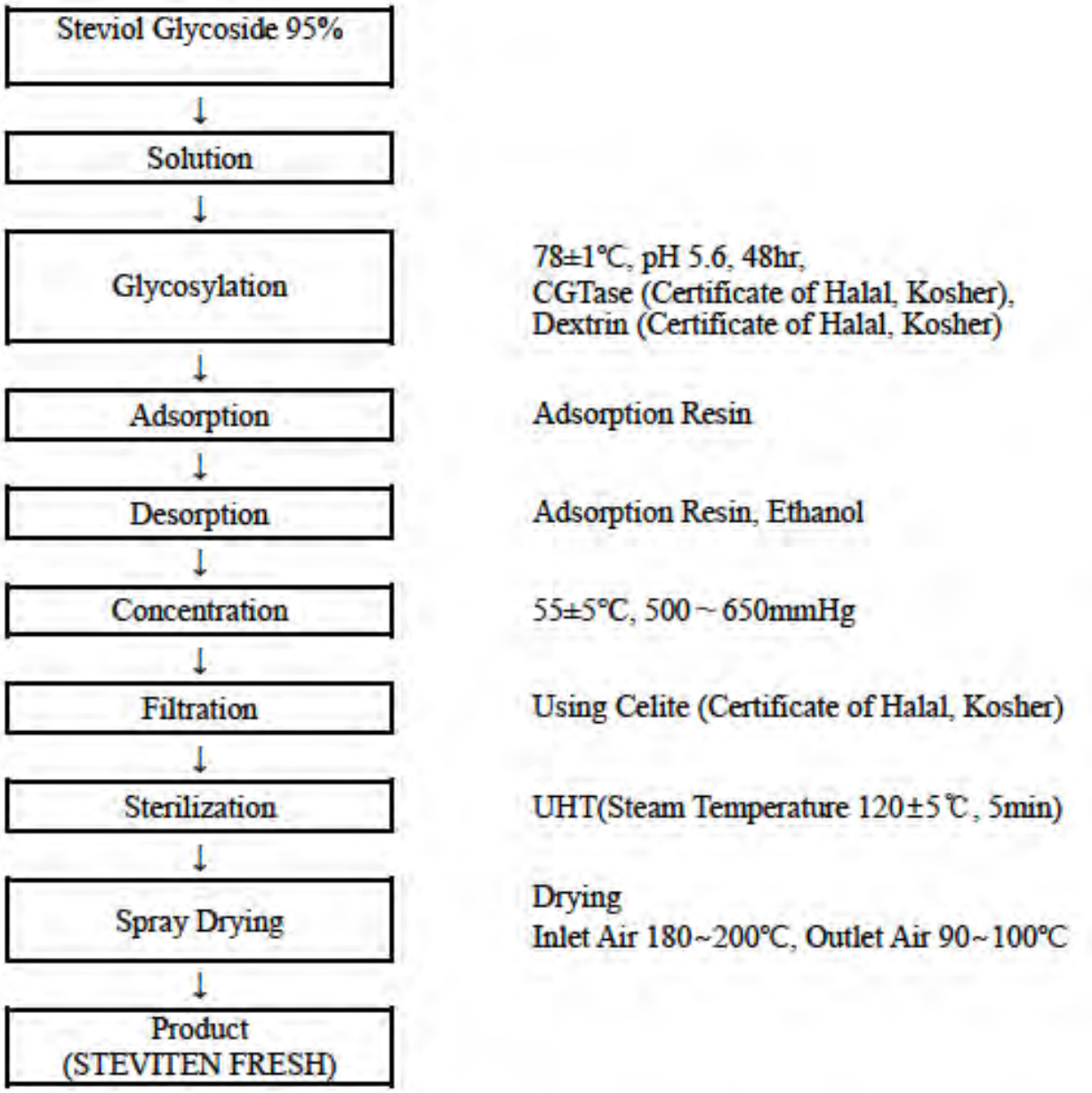
[3] Toruzyme 3.0L, manufactured by Novozymes, is a cyclomaltodextrin glucanotransferase produced by submerged fermentation of a selected strain of *Bacillus licheniformis*. It is a food grade product and complies with JECFA and FCC recommended specifications for food grade enzymes, and is GRAS as defined in 21 CFR 170.30(a).

Figure 3. Flow Chart of Manufacturing Process for Daepyung's Steviol Glycosides Raw Material (>95% Steviol Glycosides)[4]



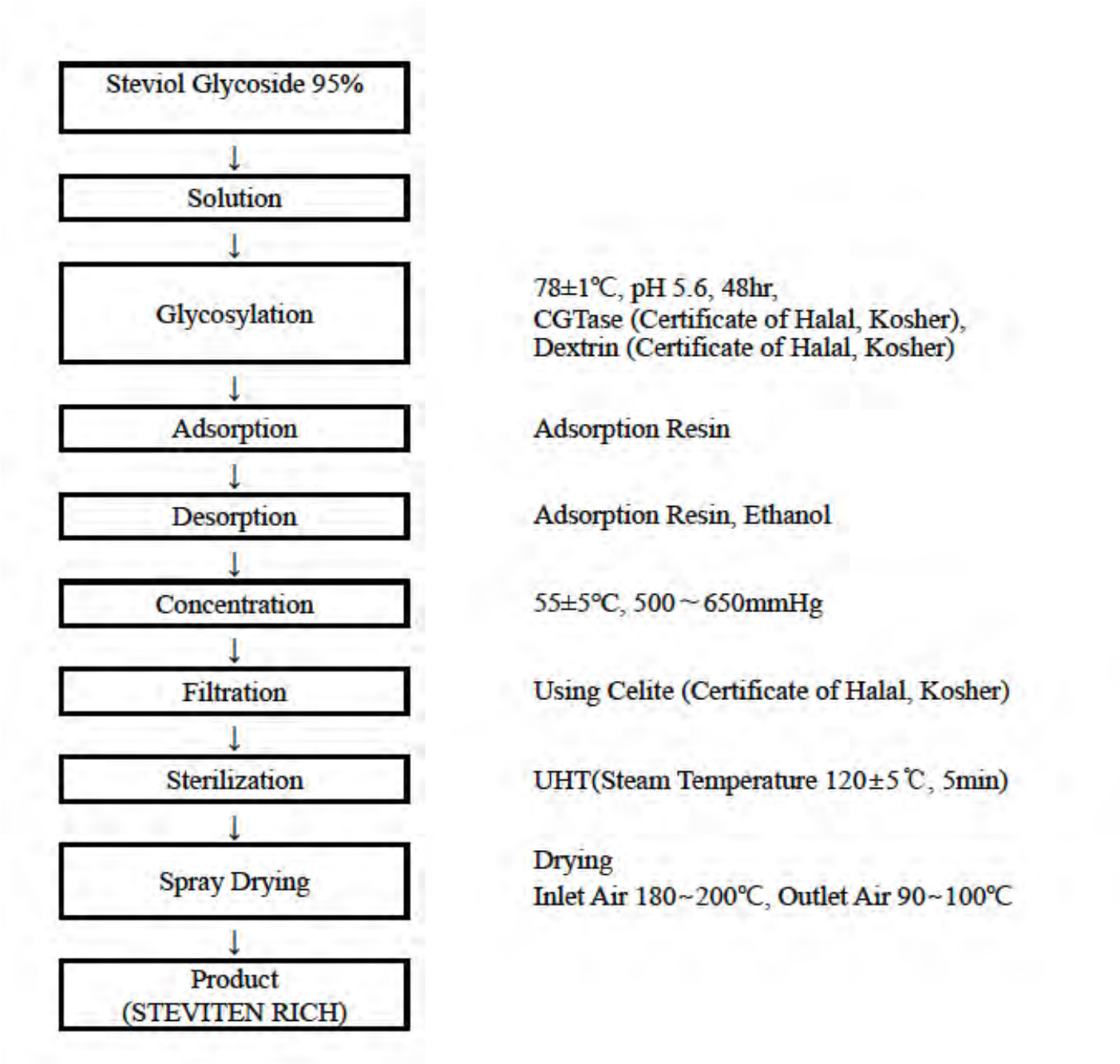
[4] UHT- Ultra-high temperature; SUS – steel use stainless, a Japanese Industrial Standards acronym for stainless steel

Figure 4. Flow Chart of Manufacturing Process for Daepyung's STEVITEN FRESH High Purity Glucosylated Steviol Glycosides Preparations[5]



[5] Maltodextrin is a short-chain dextrin commonly used in food applications. In this flowchart, the term "Dextrin" refers to the tapioca-derived maltodextrin that serves as the enzyme's glucose sources; UHT – Ultra-high temperature

Figure 5. Flow Chart of Manufacturing Process for Daepyung's STEVITEN RICH High Purity Glucosylated Steviol Glycosides Preparations[6]



[6] Maltodextrin is a short-chain dextrin commonly used in food applications. In this flowchart, the term "Dextrin" refers to the tapioca-derived maltodextrin that serves as the enzyme's glucose sources; UHT – Ultra-high temperature

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 itself (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 exhibit 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.

Daepyung has adopted specifications for our purified steviol glycosides extract starting material that meet or exceed current JECFA specifications, as demonstrated in Table 2. The typical glycosides content of production batches is provided in Table 3.

Table 2. Specifications for Steviol Glycosides Starting Material

Physical & Chemical Parameters	JECFA[a] Specifications Steviol Glycosides	Daepyung'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	Freely soluble in water and ethanol
Assay	Not less than 95% total steviol glycosides[b]	≥ 95% total steviol glycosides
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%
pH, 1% Solution	4.5 - 7.0	4.5 - 7.0
Total Ash	NMT 1%	NMT 1%
Arsenic	NMT 1 mg/kg	NMT 1 mg/kg
Lead	NMT 1 mg/kg	NMT 1 mg/kg
Total Plate Count	NMT 1,000 cfu/g	< 1,000 cfu/g
Yeast & Mold	NMT 200 cfu/g	< 100 cfu/g
Salmonella	Negative in 25 g	Negative
Escherichia coli	Negative in 1 g	≤ 10 mpn/g

NS = not specified; NMT = not more than; mpn = most probable number

[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.

Table 3. Typical Levels of Steviol Glycosides in Unmodified Stevia Extract & STEVITEN FRESH and STEVITEN RICH High Purity Glucosylated Steviol Glycosides Preparations

COMPONENT	UNMODIFIED STEVIA EXTRACT (%)	STEVITEN FRESH HIGH PURITY GLUCOSYLATED STEVIOL GLYCOSIDES (%)	STEVITEN RICH HIGH PURITY GLUCOSYLATED STEVIOL GLYCOSIDES (%)
Rubusoside	2.0 - 3.5	0.0 - 0.6	0.0 - 0.6
Steviolbioside	0.0 - 1.0	0.5 - 1.5	0.5 - 1.5
Dulcoside A	0.0 - 1.0	0.5 - 1.5	0.5 - 1.5
Rebaudioside B	0.0 - 1.0	0.0 - 1.0	0.0 - 1.0
Stevioside	46.0 - 50.0	8.0 - 10.0	6.0 - 7.0
Rebaudioside C	5.5 - 6.5	2.0 - 3.0	1.0 - 2.0
Rebaudioside F	0.5 - 1.0	0.0 - 0.5	0.0 - 0.5
Rebaudioside A	35.0 - 40.0	7.0 - 9.0	5.0 - 6.0
Rebaudioside D	0.0 - 0.5	ND	ND
Monoglucosyl stevioside m/z 966	ND	9.0 - 11.0	8.0 - 10.0
Monoglucosyl rebaudioside C m/z 1112	ND	2.0 - 2.6	1.3 - 1.6
Monoglucosyl rebaudioside A m/z 1128	ND	7.0 - 8.0	7.0 - 9.0
Diglucosyl stevioside m/z 1128	ND	9.0 - 11.0	9.0 - 12.0
Diglucosyl rebaudioside C m/z 1274	ND	1.5 - 2.5	1.5 - 2.5
Diglucosyl rebaudioside A m/z 1290	ND	8.0 - 11.0	11.0 - 13.0
Triglucosyl stevioside m/z 1290	ND	8.0 - 13.0	9.0 - 14.0
Triglucosyl rebaudioside A m/z 1452	ND	3.5 - 5.0	4.5 - 6.5
Tetraglucosyl stevioside m/z 1452	ND	7.0 - 8.5	5.0 - 8.0
Tetraglucosyl rebaudioside A m/z 1614	ND	2.5 - 3.5	4.0 - 6.0
Unidentified glucosylated steviol glycosides m/z > 1614	ND	5.0 - 6.0	4.0 - 7.0

ND = not detected

2. Specifications for Daepyung's STEVITEN FRESH and STEVITEN RICH High Purity Glucosylated Steviol Glycosides Preparations and Supporting Methods

Daepyung has adopted product specifications for our STEVITEN FRESH and STEVITEN RICH high purity glucosylated steviol glycosides preparations that meet or exceed current JECFA recommendations, while also complying with Food Chemicals Codex (FCC, 2010) specifications for rebaudioside A as a consumable human food substance. The compositions of five non-consecutive lots of Daepyung's STEVITEN FRESH (80 - 90% total glucosylated steviol glycosides and unreacted steviol glycosides) and STEVITEN RICH (85 - 95% total glucosylated steviol glycosides and unreacted steviol glycosides) preparations are compared to the JECFA and FCC specifications in Table 4 and Table 5, respectively.

Table 4. Specifications for Daepyung's STEVITEN FRESH Enzyme Modified Stevia Preparation

Physical & Chemical Parameters	JECFA[a] Specifications Steviol Glycosides	FCC[b] Specifications Rebaudioside A	Daepyung's Minimum Specifications for STEVITEN FRESH Enzyme Modified Stevia	Results of STEVITEN FRESH Enzyme Modified Stevia Preparations				
				Lot Number 190110	Lot Number 190116	Lot Number 190214	Lot Number 190308	Lot Number 190314
Appearance Form	Powder	Crystal, granule or powder	Powder	Pass	Pass	Pass	Pass	Pass
Appearance Color	White to light yellow	White to off-white	White	Pass	Pass	Pass	Pass	Pass
Solubility	Freely soluble in water: ethanol (50:50)	Freely soluble in water: ethanol (50:50)	Freely soluble in water and ethanol	Pass	Pass	Pass	Pass	Pass
Purity (HPLC Area)	≥ 95% Steviol Glycosides	≥ 95%	80.0 - 90.0% glucosylated steviol glycosides and unreacted steviol glycosides (dry weight) NLT 65.0% glucosylated steviol glycosides (dry weight)	83.4% 75.9%	83.4% 76.1%	83.6% 76.3%	83.5% 76.1%	83.4% 75.9%
Residual Ethanol	NMT 5,000 mg/kg	NMT 0.5%	NMT 5,000 mg/kg	78 mg/kg	75 mg/kg	80 mg/kg	71 mg/kg	78 mg/kg
Residual Methanol	NMT 200 mg/kg	NMT 0.02%	NMT 200 mg/kg	41 mg/kg	45 mg/kg	39 mg/kg	43 mg/kg	51 mg/kg
Loss on Drying	NMT 6.0%	NMT 6.0%	NMT 6.0%	4.1%	4.0%	4.2%	3.8%	3.5%
pH, 1% Solution	4.5 - 7.0	4.5 - 7.0	4.5 - 7.0	5.8	5.9	5.5	5.6	5.5
Total Ash	NMT 1%	NMT 1%	NMT 1.0 %	0.2%	0.2%	0.2%	0.2%	0.2%
Arsenic	NMT 1 mg/kg	NMT 1 mg/kg	NMT 1 mg/kg	ND	ND	ND	ND	ND
Lead	NMT 1 mg/kg	NMT 1 mg/kg	NMT 1 mg/kg	ND	ND	ND	ND	ND
TotalPlate Count (cfu/g, max)	NMT 1,000	NA	NMT 1,000	ND	ND	ND	ND	ND
Yeast & Mold (cfu/g, max)	NMT 200	NA	NMT 100	ND	ND	ND	ND	ND
***E. coli* (mpn/g)**	Negative in 1 g	NA	Negative in 1 g	Negative	Negative	Negative	Negative	Negative
Salmonella spp.	Negative in 25 g	NA	NMT 10 mpn per g	ND	ND	ND	ND	ND

[a] Prepared at 84th JECFA (2017)
[b] Rebaudioside A monograph. Food Chemicals Codex (7th Ed.) (FCC, 2010)
NS = not specified; NA = not applicable; NMT = not more than; NLT = not less than; mpn = most probable number; ND = not detected

Table 5. Specifications for Daepyung's STEVITEN RICH Enzyme Modified Stevia Preparation

Physical & Chemical Parameters	JECFA[a] Specifications Steviol Glycosides	FCC[b] Specifications Rebaudioside A	Daepyung's Minimum Specifications for STEVITEN RICH Enzyme Modified Stevia	Results of STEVITEN RICH Enzyme Modified Stevia Preparations				
				Lot Number 190108	Lot Number 190129	Lot Number 190221	Lot Number 190321	Lot Number 190325
Appearance Form	Powder	Crystal, granule or powder	Powder	Pass	Pass	Pass	Pass	Pass
Appearance Color	White to light yellow	White to off-white	White	Pass	Pass	Pass	Pass	Pass
Solubility	Freely soluble in water: ethanol (50:50)	Freely soluble in water: ethanol (50:50)	Freely soluble in water and ethanol	Pass	Pass	Pass	Pass	Pass
Purity (HPLC Area)	≥ 95% Steviol Glycosides	≥ 95%	85.0 - 95.0% glucosylated steviol glycosides and unreacted steviol glycosides (dry weight) NLT 70.0% glucosylated steviol glycosides (dry weight)	92.4% 86.6%	92.3% 86.4%	92.5% 86.9%	92.1% 86.5%	92.4% 86.9%
Residual Ethanol	NMT 5,000 mg/kg	NMT 0.5%	NMT 5,000 mg/kg	69 mg/kg	70 mg/kg	64 mg/kg	62 mg/kg	62 mg/kg
Residual Methanol	NMT 200 mg/kg	NMT 0.02%	NMT 200 mg/kg	38 mg/kg	35 mg/kg	31 mg/kg	35 mg/kg	41 mg/kg
Loss on Drying	NMT 6.0%	NMT 6.0%	NMT 6.0%	3.8%	4.0%	4.1%	4.0%	3.9%
pH, 1% Solution	4.5 - 7.0	4.5 - 7.0	4.5 - 7.0	5.6	5.8	5.8	5.5	5.6
Total Ash	NMT 1%	NMT 1%	NMT 1.0 %	0.2%	0.2%	0.2%	0.2%	0.2%
Arsenic	NMT 1 mg/kg	NMT 1 mg/kg	NMT 1 mg/kg	ND	ND	ND	ND	ND
Lead	NMT 1 mg/kg	NMT 1 mg/kg	NMT 1 mg/kg	ND	ND	ND	ND	ND
Total Plate Count (cfu/g, max)	NMT 1,000	NA	NMT 1,000	ND	ND	ND	ND	ND
Yeast & Mold (cfu/g, max)	NMT 200	NA	NMT 100	ND	ND	ND	ND	ND
E. coli (mpn/g)	Negative in 1 g	NA	Negative in 1 g	Negative	Negative	Negative	Negative	Negative
Salmonella spp.	Negative in 25 g	NA	NMT 10 mpn per g	ND	ND	ND	ND	ND

[a] Prepared at 84th JECFA (2017)
[b] Rebaudioside A monograph. Food Chemicals Codex (7th Ed.) (FCC, 2010)
NS = not specified; NA = not applicable; NMT = not more than; mpn = most probable number; ND = not detected

Details of the analytical methodology employed to characterize and quantitate the steviol glycosides are provided in Appendix 2. The certificates of analysis and representative chromatograms for five representative lots of material are presented in Appendix 3 and Appendix 4. A test report for the analysis of pesticides residues in the raw material stevia extract used to manufacture the high purity enzyme modified steviol glycosides is provided in Appendix 5. The collection of these reports demonstrates that Daepyung's high purity enzyme modified steviol glycosides products are well characterized and meet the established purity criteria.

D. Physical or Technical Effect

Daepyung conducted sweetness equivalence evaluations for STEVITEN FRESH and STEVITEN RICH high purity enzyme modified steviol glycosides preparations. A taste panel compared solutions of STEVITEN FRESH with 5% sucrose solution and determined that STEVITEN FRESH is 100 times sweeter than sucrose. Following the same methodology, a taste panel determined that STEVITEN RICH is 110 times sweeter than sugar.

E. Stability

1. Stability Data on Steviol Glycosides

The stability of steviol glycosides and enzyme modified steviol glycosides has previously been reviewed in a number of GRAS Notifications, including GRN 337 (NOW Foods, 2010), GRN 667 (Blue California, 2016), and GRN 715 (Blue California, 2017).

Steviol glycosides have been reported to be stable over the pH range 3-9 and can be heated at 100 ^{o}C for 1 hour, but, at pH levels greater than 9, they rapidly decompose (Kinghorn, 2002). Previously submitted GRAS Notifications, GRN 252 (Merisant, 2008), GRN 253 (Cargill, 2008), and GRN 304 (Sunwin/WILD, 2010), reported stability data indicating that Rebaudioside A is stable under the intended conditions of use.

Furthermore, in the over 57 GRAS Notifications that have been submitted to FDA to date for steviol glycosides, the presented stability data have supported the position that steviol glycosides are stable and well-suited for the intended uses in foods.

2. Stability Data for Daepyung's STEVITEN FRESH Enzyme Modified Steviol Glycosides Preparations

Daepyung conducted a battery of stability studies on our STEVITEN FRESH high purity enzyme modified steviol glycosides preparations, including powder shelf and solution stability under a variety of conditions.

Table 6. Daepyung's STEVITEN FRESH Powder Storage Stability Data

Duration	Total enzyme modified steviol glycosides and unreacted steviol glycosides (%) (25 °C)	Total enzyme modified steviol glycosides and unreacted steviol glycosides (%) (40 °C)
t = 0	83.4	83.3
6 months	83.5	83.4
12 months	83.5	83.4
18 months	83.3	83.3
24 months	83.4	83.3
30 months	83.4	83.4
36 months	83.4	83.5

Tabulated results are an average of data obtained for three lots of material: Lot 151127; Lot 151209; and Lot 151225. Individual results are provided in Appendix 6.

Table 7. Daepyung's STEVITEN FRESH Powder Microbiological Stability

Duration	Total Plate Count (cfu/g)	Yeast & Mold (cfu/g)	Salmonella	E. coli (MPN/g)
t = 0	0	0	Negative	0
6 months	0	0	Negative	0
12 months	0	0	Negative	0
18 months	0	0	Negative	0
24 months	0	0	Negative	0
30 months	0	0	Negative	0
36 months	0	0	Negative	0

Tabulated results are an average of data obtained for three lots of material: Lot 151127; Lot 151209; and Lot 151225. Individual results are provided in Appendix 6.

Table 8. Daepyung's STEVITEN FRESH Solution Stability Data (10% Water Solution, pH 5)

Duration	Total enzyme modified steviol glycosides and unreacted steviol glycosides (%)		
	5 °C	25 °C	50 °C
t = 0	83.4	83.4	83.4
20 hours	83.3	83.4	83.3
40 hours	83.4	83.4	83.4
60 hours	83.4	83.4	83.5

Tabulated results are an average of data obtained for three lots of material: Lot 151127; Lot 151209; and Lot 151225. Individual results are provided in Appendix 6.

Table 9. Daepyung's STEVITEN FRESH Solution Stability Data (1% Water Solution)

Duration	Total enzyme modified steviol glycosides and unreacted steviol glycosides (%)		
	pH 2	pH 5	pH 8
t = 0	83.5	83.4	83.4
Day 2	83.4	83.5	83.5
Day 4	83.3	83.4	83.5
Day 6	83.5	83.4	83.4

Tabulated results are an average of data obtained for three lots of material: Lot 151127; Lot 151209; and Lot 151225. Individual results are provided in Appendix 6.

A stability study report for STEVITEN FRESH is provided in Appendix 6.

These short-term solution stability studies on STEVITEN FRESH corroborate the findings of previously published stability studies on steviol glycosides solutions (Kinghorn, 2002; Merisant, 2008; Cargill, 2008; Sunwin/WILD, 2010) as described in more detail in Part 2.E.1.

From the data presented in Table 6 through Table 9, Daepyung concludes that STEVITEN FRESH preparations are shelf stable for up to 36 months, and the solutions are stable under the conditions specified.

3. Stability Data for Daepyung's STEVITEN RICH Enzyme Modified Steviol Glycosides Preparations

Daepyung conducted a battery of stability studies on our STEVITEN RICH high purity enzyme modified steviol glycosides preparations, including powder shelf and solution stability under a variety of conditions.

Table 10. Daepyung's STEVITEN RICH Powder Storage Stability Data

Duration	Total enzyme modified steviol glycosides and unreacted steviol glycosides (%) (25 °C)	Total enzyme modified steviol glycosides and unreacted steviol glycosides (%) (40 °C)
t = 0	92.5	92.4
6 months	92.4	92.5
12 months	92.5	92.4
18 months	92.4	92.5
24 months	92.4	92.4
30 months	92.4	92.4
36 months	92.4	92.4

Tabulated results are an average of data obtained for three lots of material: Lot 151130; Lot 151218; and Lot 151223. Individual results are provided in Appendix 7.

Table 11. Daepyung's STEVITEN RICH Powder Microbiological Stability

DURATION	TOTAL PLATE COUNT (CFU/G)	YEAST & MOLD (CFU/G)	*SALMONELLA*	*E. COLI* (MPN/G)
t = 0	0	0	Negative	0
6 months	0	0	Negative	0
12 months	0	0	Negative	0
18 months	0	0	Negative	0
24 months	0	0	Negative	0
30 months	0	0	Negative	0
36 months	0	0	Negative	0

Tabulated results are an average of data obtained for three lots of material: Lot 151130; Lot 151218; and Lot 151223. Individual results are provided in Appendix 7.

Table 12. Daepyung's STEVITEN RICH Solution Stability Data (10% Water Solution, pH 5)

DURATION	TOTAL ENZYME MODIFIED STEVIOL GLYCOSIDES AND UNREACTED STEVIOL GLYCOSIDES (%)		
	5 °C	25 °C	50 °C
t = 0	92.4	92.4	92.4
20 hours	92.4	92.4	92.5
40 hours	92.4	92.4	92.5
60 hours	92.4	92.3	92.5

Tabulated results are an average of data obtained for three lots of material: Lot 151130; Lot 151218; and Lot 151223. Individual results are provided in Appendix 7.

Table 13. Daepyung's STEVITEN RICH Solution Stability Data (1% Water Solution)

DURATION	TOTAL ENZYME MODIFIED STEVIOL GLYCOSIDES AND UNREACTED STEVIOL GLYCOSIDES (%)		
	PH 2	PH 5	PH 8
t = 0	92.4	92.5	92.4
Day 2	92.4	92.5	92.5
Day 4	92.4	92.4	92.5
Day 6	92.4	92.5	92.4

Tabulated results are an average of data obtained for three lots of material: Lot 151130; Lot 151218; and Lot 151223. Individual results are provided in Appendix 7.

A stability study report for STEVITEN RICH is provided in Appendix 7.

These short-term solution stability studies on STEVITEN RICH corroborate the findings of previously published stability studies on steviol glycosides solutions (Kinghorn, 2002; Merisant, 2008; Cargill, 2008; Sunwin/WILD, 2010) as described in more detail in Part 2.E.1.

From the data presented in Table 10 through Table 13, Daepyung concludes that STEVITEN FRESH preparations are shelf stable for up to 36 months, and the solutions are stable under the conditions specified.

F. Calculation of Steviol Equivalents of STEVITEN FRESH and STEVITEN RICH Enzyme Modified 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 14.

Table 14. 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 C	951	0.334
Rebaudioside F	937	0.339
Rebaudioside A	967	0.329
Rebaudioside D	1129	0.282

[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 percent composition of the stevia extract starting material, it is possible to determine the steviol equivalency of the raw material steviol glycosides extract, as presented in Table 15.

Table 15. Steviol Equivalency of Steviol Glycosides Extract Raw Material

Component Steviol Glycoside	Upper Range[a] (%)	Steviol Equivalents[b] (%)
Rubusoside	3.5	1.7
Steviolbioside	1.0	0.50
Dulcoside A	1.0	0.40
Rebaudioside B	1.0	0.40

COMPONENT STEVIOL GLYCOSIDE	UPPER RANGE[a] (%)	STEVIOL EQUIVALENTS[b] (%)
Stevioside	50.0	19.8
Rebaudioside C	6.5	2.2
Rebaudioside F	1.0	0.33
Rebaudioside A	40.0	13.2
Rebaudioside D	0.5	0.1
Total Steviol Equivalence[c]		38.6

[a] Based on the typical levels of steviol glycosides in the raw material steviol glycosides extract, as reported in Table 3.

[b] Calculated by multiplying the % of the steviol glycoside by the steviol equivalency factor.

[c] Steviol equivalent calculated on worst-case basis, using the highest typical values for all steviol glycosides.

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 maltodextrin 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 STEVITEN FRESH and STEVITEN RICH preparations is expected to be no greater than 38.6 g steviol per 100 g STEVITEN FRESH or STEVITEN RICH.

PART 3. DIETARY EXPOSURE

The subject STEVITEN FRESH and STEVITEN RICH preparations are intended to be used as table top sweeteners and general purpose non-nutritive sweeteners in various foods other than infant formulas and meat and poultry, as defined in 21 CFR 170.3(o)(19).[7] The intended use levels will vary by actual food category, but the actual levels are self-limiting due to organoleptic factors and consumer taste considerations. However, the amounts of STEVITEN FRESH and STEVITEN RICH 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.[8]

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 8. In GRAS notification 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,

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

2009). As summarized in GRN 301, the 90th percentile consumer of a sweetener that is 100 times as sweet as sucrose when used as a total sugar replacement would be a maximum of 9.9 mg per kg body weight (bw) per day for any population subgroup.

The estimated sweetness intensity for STEVITEN FRESH is approximately 100 times that of sucrose, while the estimated sweetness intensity for STEVITEN RICH is approximately 110 times that of sucrose. A weighted sum estimate was used to determine the steviol equivalency factor for the STEVITEN FRESH and STEVITEN RICH preparations on a worst-case scenario basis, and was determined to be 38.6 g steviol per 100 g STEVITEN FRESH or STEVITEN RICH (as described in Part 2.F).

The highest 90th percentile consumption by any population subgroup of either STEVITEN preparation (corresponding to STEVITEN FRESH) would consume approximately 9.90 mg per kg steviol glycosides bw per day. Based on a weighted sum estimate for steviol equivalents provided in Table 15, the consumption would be less than 3.82 mg per kg bw per day on a steviol equivalents basis for any population group, on a worst-case scenario basis, for any of Daepyung's STEVITEN preparations described herein. These calculations are summarized in Table 16 and Table 17 for STEVITEN FRESH and STEVITEN RICH, respectively.

Table 16. Daily Intake of Sweeteners (in Sucrose Equivalents) & Estimated Daily Intakes of STEVITEN FRESH

Population Group	Intakes of Sweeteners (mg sucrose/kg bw/day)[a]		Calculated Intake of STEVITEN FRESH (mg/kg bw/day)[b]		Calculated Intake of STEVITEN FRESH as Steviol Equivalents (mg/kg bw/day)	
	Low	High	Low	High	Low	High
Healthy Population	255	675	2.55	6.75	0.98	2.60
Diabetic Adults	280	897	2.80	8.97	1.08	3.46
Healthy Children	425	990	4.25	9.90	1.64	3.82
Diabetic Children	672	908	6.72	9.08	2.59	3.50

[a] From Renwick (2008)

[b] Calculated by dividing the sucrose intake by the minimum average relative sweetness value of 100 for STEVITEN FRESH.

Table 17. Daily Intake of Sweeteners (in Sucrose Equivalents) & Estimated Daily Intakes of STEVITEN RICH

Population Group	Intakes of Sweeteners (mg sucrose/kg bw/day)a		Calculated Intake of STEVITEN RICH (mg/kg bw/day)b		Calculated Intake of STEVITEN RICH as Steviol Equivalents (mg/kg bw/day)	
	Low	High	Low	High	Low	High
Healthy Population	255	675	2.32	6.14	0.89	2.37
Diabetic Adults	280	897	2.55	8.15	0.98	3.15
Healthy Children	425	990	3.86	9.00	1.49	3.47
Diabetic Children	672	908	6.11	8.25	2.36	3.18

[a] From Renwick (2008)

[b] Calculated by dividing the sucrose intake by the minimum average relative sweetness value of 110 for STEVITEN RICH.

The values in Table 16 and Table 17 assume that Daepyung's STEVITEN FRESH and STEVITEN RICH 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 estimated maximum intake of purified steviol glycosides is 6.75 mg per kg bw per day (2.60 mg per kg steviol equivalents) for STEVITEN FRESH and 6.14 mg per kg bw per day (2.37 mg per kg steviol equivalents) for STEVITEN RICH. For healthy children, the estimated maximal intake is 9.90 mg per kg bw per day (3.82 mg per kg as steviol equivalents) for STEVITEN FRESH and 9.00 mg per kg bw per day (3.47 mg per kg steviol equivalents) for STEVITEN RICH. In all population groups, the estimated daily intake of purified steviol glycosides, expressed as steviol equivalents, is below 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 Daepyung's STEVITEN FRESH and STEVITEN RICH products, 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 9.

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

A. Other Information on Dietary Exposure

1. History of Traditional Medicinal and Human Food Use

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 had been 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 is 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).

B. 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 Mexico, Australia, New Zealand, Switzerland, France, Peru, Uruguay, Colombia, Senegal, Russia, Malaysia, Turkey, Taiwan, Thailand, Israel, Canada, 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 5.B Sections 1-5; a more detailed historical overview is provided in Appendix 10.

1. U.S. Regulatory History

Based on available information from FDA's GRAS Notice Inventory website (FDA, 2019) as of May 23, 2019, FDA has issued 57 "no questions" letters on GRAS notices on rebaudioside A, rebaudioside D, rebaudioside M, or steviol glycosides, including those undergoing enzyme modification.

In addition, the Flavor and Extract Manufacturers Association (FEMA) includes 11 steviol glycosides preparations, three 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 (2017a) 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, 2019b). Most recently, 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, 2019a).

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% Reb 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 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." 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).

Most recently, 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 by the EFSA Panel, and that their decision was based on the "limited" data provided in the dossier submitted by the applicant (EFSA, 2018).

4. Asian Regulatory History

No regulatory updates have been identified in recent years. The Asian regulatory history for steviol glycosides through 2014 is presented in Appendix 10.

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, 2016b). 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).

Most recently, 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).

PART 6. NARRATIVE

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---the 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 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 11.

Since the JECFA evaluation (WHO, 2008), FDA has received and not objected to over fifty-five GRAS notifications for steviol glycosides or enzyme modified steviol glycosides, 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 recent publication by Roberts et al. (2016) indicates that the ADI could be higher, as discussed further in Appendix 8. 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 9.

Because of their sweetness characteristics, steviol glycosides have viable uses as a non-nutritive sweetener in foods.[9] Periodic reviews by JECFA over the years indicate the progression of knowledge on the toxicology of steviol glycosides. Several early safety-related studies on these compounds were performed on crude extracts of stevia. These studies also included multiple investigations with *in vivo* and *in vitro* models, which explored the biological activity of stevia extracts at high doses or high concentrations. These early investigations raised several concerns, including impairment of fertility, renal effects, interference with glucose metabolism, and inhibition of mitochondrial enzymes. In recent years, as more and more studies were performed on purified glycosides, the toxicology profile of steviol glycosides eventually proved to be rather unremarkable. A number of subchronic, chronic, and reproductive studies have been conducted in laboratory animals. These studies were well designed with appropriate dosing regimens and adequate

[9] 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.

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 11 and elsewhere, at its 51st meeting, JECFA determined that there were adequate chronic studies in rats, particularly the study by Toyoda et al. (1997), to establish a temporary ADI of 0 - 2 mg 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 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).

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).

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

Several published and unpublished studies (summarized in Appendix 9) 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.

Daepyung concluded that these studies on rebaudioside A strengthen the argument that all steviol glycosides that follow the same metabolic pathway are safe at the JECFA established ADI.

Daepyung has also reviewed the findings from human clinical studies. Daepyung noted that, with regard to the clinical effects reported in humans, in order to corroborate the observations in these studies, these effects of steviol glycosides only occur in patients with either elevated blood glucose or blood pressure (or both). JECFA called for studies in individuals that are neither hypertensive nor diabetic (WHO, 2006). The 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. Daepyung 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 9 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). Daepyung 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.

Urban et al. (2013) examined the extensive genotoxicity database on steviol glycosides because some concern has been expressed in two relatively recent 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.

In addition, a paper by Shannon et al. (2016) raises a possible concern of endocrine disruption by steviol. Daepyung 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. Daepyung 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 Daepyung's opinion that steviol glycosides preparations are generally recognized as safe. A summary of this study is provided in Appendix 9.

Philippaert et al. (2017) demonstrated that stevioside, rebaudioside A, and steviol potentiate the activity of transient receptor potential cation channel subfamily melastatin member 5 (TRPM5), a Ca^{2+}-activated cation channel that is expressed in type II taste receptor cells and pancreatic β-cells. The authors found that the steviol glycosides increased the perception of bitter, sweet, and umami tastes and also enhanced glucose-induced insulin secretion in a TRPM5-dependent manner. Furthermore, *in vivo* studies indicated that daily consumption of stevioside prevents high-fat-induced diabetic hyperglycemia development in wild-type mice. No adverse events or animal deaths were discussed.

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.

Panagiotou et al. (2018) observed that steviol and steviol glycosides exert glucocorticoid receptor-mediated effects in human leukemic T-cells (Jurkat cells) but not in normal human peripheral blood mononuclear cells, which they concluded was due to a cell-type specific manner of glucocorticoid receptor-modulation.

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, which is 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 CYP329 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 recently published study addressed the genotoxic activity of stevia (Svetia™, purity not reported[10]). 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 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.

Daepyung agrees with the safety conclusions of the 57 GRAS Expert Panels in the notifications 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.

Daepyung 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 STEVITEN FRESH and STEVITEN RICH. Therefore, with the steviol equivalence values shown in Table 16, Daepyung concludes that, for the general population, the estimated maximum daily intake of any STEVITEN preparation described herein is 9.90 mg per kg bw or 3.82 mg per kg expressed as steviol equivalents. Based upon these calculations, the intake of all of Daepyung's STEVITEN preparations 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 Daepyung's STEVITEN preparations contain not less than 95% total steviol glycoside. The finished high purity glucosylated

[10] 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 Reb A.

steviol glycosides preparations are a mixture of glucosylated steviol glycosides, unreacted steviol glycosides, and unreacted maltodextrin, where STEVITEN FRESH is 80-90% total glucosylated steviol glycosides and unreacted steviol glycosides, and STEVITEN RICH is 85-95% total glucosylated steviol glycosides and unreacted steviol glycosides. 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 STEVITEN FRESH and STEVITEN RICH. 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 Reb A, other steviol glycosides and steviol has been summarized, with key studies summarized in Appendix 9.

Furthermore, Daepyung has reviewed this safety information and have concluded that STEVITEN FRESH and STEVITEN RICH preparations are generally recognized as safe for the proposed uses.

A. GRAS Criteria

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

> “…reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use.”[11]

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.” [12]

[11] 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 9/8/18).
[12] See 81 FR 54959 Available at: https://www.federalregister.gov/documents/2016/08/17/2016-19164/substances-generally-recognized-as-safe (Accessed on 9/8/18).

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:[13]

- Data and information relied upon to establish safety must be generally available, and this is most commonly established by utilizing published, peer-reviewed scientific journals; and

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

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.

B. Expert Panel Findings on Safety of STEVITEN FRESH and STEVITEN RICH Preparations

An evaluation of the safety and GRAS status of the intended use of Daepyung's STEVITEN FRESH (80-90% total unreacted steviol glycosides and glucosylated steviol glycosides) and STEVITEN RICH (85-95% total unreacted steviol glycosides and glucosylated steviol glycosides) 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 Daepyung's dossier as well as other publicly available information available to them. 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 12.

[13] See Footnote 1.

C. 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 exists 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 9. 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 fifty-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, Daepyung 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.

D. Conclusion

In consideration of the aggregate safety information available on naturally occurring steviol glycosides, Daepyung concludes that STEVITEN FRESH (80-90% total unreacted steviol glycosides and glucosylated steviol glycosides) and STEVITEN RICH (85-95% total unreacted steviol glycosides and glucosylated steviol glycosides) preparations 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. The JECFA ADI for steviol glycosides of 4 mg per kg bw per day (as steviol equivalents) can be applied to Daepyung’s STEVITEN FRESH and STEVITEN RICH 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 Daepyung’s STEVITEN FRESH and STEVITEN RICH preparations for several population groups summarized in Part 3.A. are no greater than 3.82 mg per kg steviol equivalents per bw per day, which is 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 high purity glucosylated steviol glycosides composed of at least 80% total unreacted steviol glycosides and glucosylated steviol glycosides mixed with unreacted maltodextrin are used as a general non-nutritive sweetener.

Accordingly, STEVITEN FRESH (80-90% total unreacted steviol glycosides and glucosylated steviol glycosides) and STEVITEN RICH (85-95% total unreacted steviol glycosides and

glucosylated steviol glycosides) as produced by Daepyung 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

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
C	Celsius
CFR	Code of Federal Regulations
CFU	Colony Forming Unit
CGMP	Current Good Manufacturing Practice
CGTase	Cyclomaltodextrin glucanotransferase
C_{max}	Maximum (peak) serum concentration of substance is observed
CSAF	Chemical-specific adjustment factor
CYP	Cytochrome P450
DBP	Diastolic blood pressure
DHHS/FDA	Department of Health and Human Services/Food and Drug Administration
DNA	Deoxyribonucleic acid
EDI	Estimated daily intake
EFSA	European Food Safety Authority
ERT	European Registered Toxicologist
EU	European Union
FAO/WHO	Food and Agriculture Organization of the United Nations/World Health Organization
FCC	Food Chemicals Codex
FD&C Act	Federal Food Drug and Cosmetics Act
FDA	Food and Drug Administration
FEMA	Flavor 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
GEMS	Global Environment Monitoring System
GGT	Gamma-glutamyltransferase
GI	Gastrointestinal
GRAS	Generally Recognized as Safe
GRN	GRAS Notification
h or hr	Hour
HbA1c	Glycated hemoglobin
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	Killogram
LD_{50}	Median (50%) lethal dose
mg	Milligram
ml	Milliliter
MPL	Maximum permitted level
MPN	Most probable number
mRNA	Messenger ribonucleic acid
MW	Molecular weight
NA	Not applicable
ND	Not detected
ng	Nanogram
NHANES	National Health and Nutrition Examination Surveys
NHPs	Natural Health Products
NMT	Not more than
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NS	Not specified
OECD	Organisation for Economic Co-operation and Development
PPM	Parts per million
Reb A	Rebaudioside A
Reb M	Rebaudioside M
SBP	Systolic blood pressure
SUS	Steel use stainless
TAC	Total Antioxidant Capacity
TFC	Total Flavonoid Content
T_{max}	Time at which maximum (peak) plasma concentration (C_{max}) of substance is observed
TPC	Total Phenolic Content
TRAP	Tartrate-resistant alkaline phosphatase
TRPM5	Transient receptor potential cation channel subfamily melastatin member 5
UDS	Unscheduled DNA synthesis
ug	Microgram
UHT	Ultra-high temperature
WHO	World Health Organization
WHO/JECFA	World Health Organizaiton/Joint FAO/WHO Expert Committee on Food Additives

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B. Appendices

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

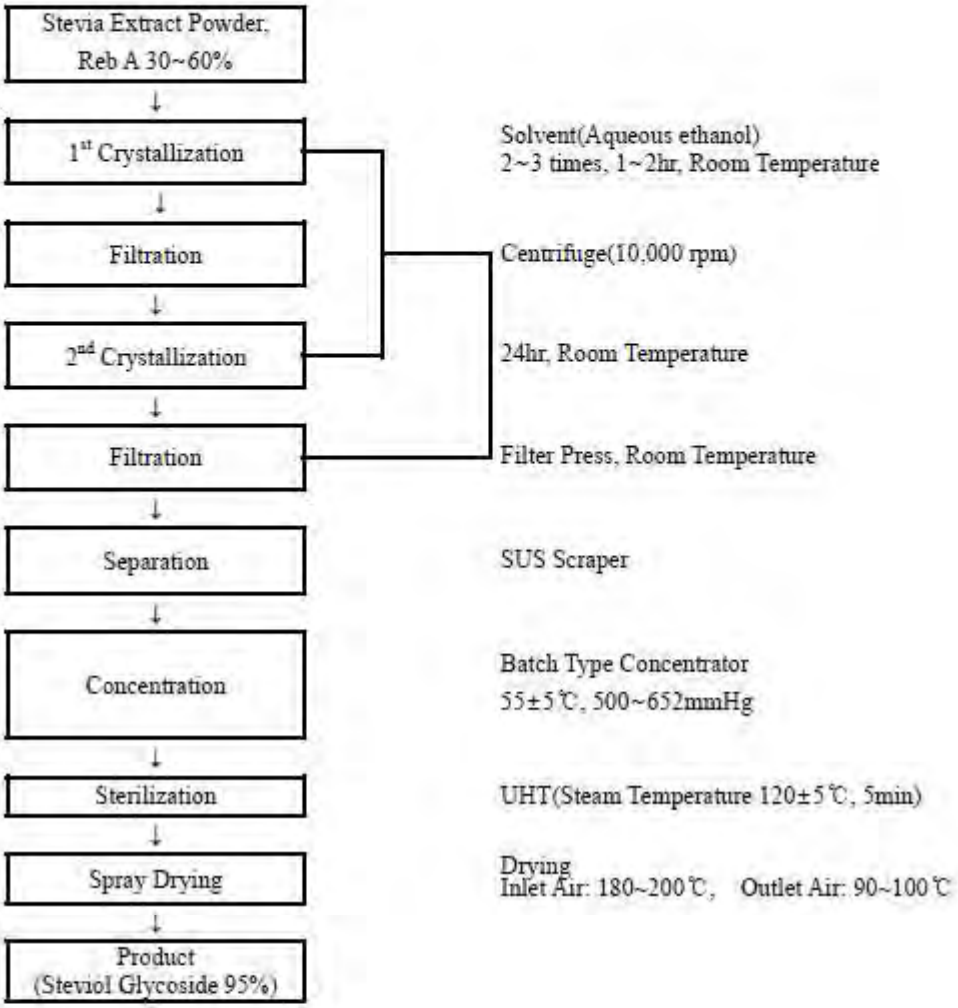
Appendix 1.1 Steviol Glycosides Extract

Specification for DAEPYUNG's STEVITEN FRESH Starting Material Product(Steviol glycosides 95%)

Comparison to JECFA Specification

Parameter	JECFA Specifications Steviol Glycosides	FCC Specifications Rebaudioside A	DAEPYUNG Specifications Steviol Glycosides	Methods
Appearance	White to light yellow powder	White to off-white hygroscopic fine crystal, granule or powder	White to off-white powder	Visual
Sweetness	200~300 times sweeter than sucrose	NA	250~300	Gustatory
Rebaudioside A	NA	NLT 95%	Minimum of 85%	JECFA HPLC
Total Steviol glycosies	NLT 95%	NA	≥95%	JECFA HPLC
Other Related Steviol Glycosides(such as Stev, Reb A, B, C, Dul c A, Rub & SB) on dry weight basis	NLT 95%	NMT 5%	NS	JECFA 2007
Residue in Ignition	NS	NS	NS	USP
Loss on drying	NMT 6%	NMT 6%	NMT 6%	USP
Ash	NMT 1%	NMT 1%	NMT 1%	JECFA Vol. 4
Optical rotation	NS	NS	NS	USP
Solubility	Freely soluble in water & ethanol	Freely soluble in water:ethanol(50:50)	Freely soluble in water & ethanol	USP
pH(1% solution)	4.5 ~ 7.0	4.5 ~ 7.0	4.5 ~ 7.0	USP
Residual Solvent Levels				
Residual Methanol	NMT 200 mg/kg	NMT 200 mg/kg	NMT 200 mg/kg	USP
Residual ethanol	NMT 5000 mg /kg	NMT 5000 mg /kg	NMT 5000 mg /kg	USP
Heavy metals				
Lead	NMT 1 mg/kg	NMT 1 mg/kg	NMT 1 mg/kg	ICP-MS AOAC
Arsenic	NMT 1 mg/kg	NMT 1 mg/kg	NMT 1 mg/kg	ICP-MS AOAC
Micribiological				
Total Plate Count	NA	NA	≤ 1000 CFU/g	AOAC 977.27
Yeast and Mold	NA	NA	≤ 100 CFU/g	AOAC 997.02
Total coliform	NA	NA	NS	AOAC 991.14
Salmonella	NA	NA	Negative	AOAC 2014.01
Escherichia coli	NA	NA	≤ 10 npm/g	AOAC 991.14
Staphylococcus aureus	NA	NA	NS	AOAC 2001.05

MANUFACTURING FLOW CHART OF Steviol Glycoside 95%



Appendix 1.2 Maltodextrin

Neotech Food Co.,Ltd.	SPECIFICATION ของผลิตภัณฑ์	หมายเลข : SD-QC02-02 หน้าที่ : 1 แก้ไขครั้งที่ 000 วันที่บังคับใช้ : 30 กันยายน 2556

SPECIFICATION OF MALTODEXTRIN

Neo-Maldex NM - 10

Item	Limit	Analysis Method
Moisture content (%)	6.00(max)	AOAC (2000),930.46 B
Ash (%)	0.20(max)	AOAC (2000),942.05
pH(20% solution)	4.0 - 4.7	AOAC (2000),981.12
Dextrose Equivalent (DE)	9.0 - 13.0	Lane and Eynon's Volumetric Method
Total Plate Count (CFU/g)	1000 (max)	BAM (1998) Revision A,Chapter 3
Yeast (CFU/g)	50 (max)	BAM (1998) Revision A,Chapter 18
Mold (CFU/g)	50 (max)	BAM (1998) Revision A,Chapter 18
E.coli in 25 g	Not detect	BAM (1998) Revision A,Chapter 4
Salmonella in 25g	Not detect	BAM (1998) Revision A,Chapter 5

Appendix 1.3 Cyclomaltodextrin Glucanotransferase Enzyme

Certificate of Analysis

novozymes
Rethink Tomorrow

Date
Jul 18, 2018
Purchase order item/date

Delivery
7770961
Sales order
5671315

Sold to
Daejong Co.
Fl.2 GeunHa Building 911-1 ShinJung
156-070 Seoul

Ship to
Daepyung
kyungbuk sangju-si hamchang-ub odon
sangju

Material: **Toruzyme 3.0 L**

Batch: **ACN00246**
Production date: **Nov 10, 2017**

Quantity: **500 KG**
Best before: **Nov 10, 2019**

Characteristic	Unit	Value
Cyclod Glycosyl Transf KNU-CP	/G	3,38
pH at 25°C		6,40
Density	G/ML	1,034
Total viable count	/G	< 100
Coliform bacteria	/G	< 4
E.coli		Not Detected, 25 g
Salmonella		Not Detected, 25 g

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

This is a computer generated document and no signature is required

Toruzyme 3.0 L Certificate of Analysis

novozymes®
Rethink Tomorrow

May 10, 2012

Re: Regulatory Status of Novozymes' Toruzyme 3.0 L in the US

To Whom It May Concern:

Toruzyme 3.0 L is a cyclomaltodextrin glucanotransferase enzyme produced by a genetically modified strain of *Bacillus licheniformis*. Toruzyme is a GRAS substance as defined in 21 CFR 170.30 (a) for use as a processing aid in the manufacture of beta cyclodextrins.

Novozymes' experts qualified by scientific training and experience have determined the Toruzyme 3.0L is a GRAS substance for use as a processing aid in the starch industry to manufacture beta cyclodextrins. This determination is based on the compilation and consideration of documentation regarding the enzyme identity, production strain construction and characterization, manufacturing process, toxicology studies, as well as general knowledge on the intended use and dietary exposure. The review and evaluation of this information was done on the basis of well established principles for determining the safety of enzyme preparations used in food processing (Panza and Foster, 1983, IFBC, 1990 and others). In the US, there is no premarket approval by FDA required for substances that have been determined to be GRAS.

Novozymes has filed a Food Master File number 534 with the US Food and Drug Administration on February 17, 1993 which contains detailed information about Toruzyme 3.0L.

Attached is a page from a February 23, 1998 edition of *Food Chemical News*, stating that an expert panel has determined that Beta-cyclodextrin produced by an enzyme from a genetically modified *Bacillus licheniformis* is GRAS.

Regulatory Affairs
Novozymes

Novozymes North America, Inc.
Regulatory Affairs
77 Perry Chapel Church Road, P.O. Box 576
Franklinton, North Carolina 27525

Levnedsmiddelstyrelsen

Sundhedsministeriet
National Food Agency of Denmark/Ministry of Health

4. kontor IM/JLJ 18 NOV. 1996 J.nr. 571.1201-0024

Novo Nordisk A/S
Novo Allé
2880 Bagsværd

Att.: Kim Rygaard Nielsen

Copy: PS (Bio Nordén) HL:1 (QA) SØJ (PM) BFJ (ERA)

Novo Nordisk A/S
Enzyme Regulatory Affairs

18. NOV 1996
KRyN

Toruzyme / Accept

Afgørelse

Enzymproduktet Toruzyme 3.0 L accepteres anvendt til stivelse til anvendelse i sukkerindustrien i en mængde på højst 17 g/kg. Vilkår for accepten fremgår af det følgende.

Baggrund

Virksomheden har med brev af 2. juli 1996 anmeldt enzymproduktet Toruzyme 3.0 L til anvendelse ved fremstilling af sukkerprodukter. Anmeldelsen er sket i henhold til § 20, stk. 1, nr. 3, i tilsætningsstofbekendtgørelsen.

Levnedsmiddelstyrelsen har med brev af 26. august 1996 anmodet om supplerende oplysninger, og i brev af 5. september 1996 er virksomheden fremkommet med disse. Herefter anser styrelsen anmeldelsen for fyldestgørende.

Enzymproduktet er fremstillet af Novo Nordisk A/S ved hjælp af gensplejsningsteknik.

Vilkår

Ved Levnedsmiddelstyrelsens accept forudsættes det, at enzymproduktet til stadighed er fremstillet i overensstemmelse med de oplysninger, der er givet til styrelsen, herunder at enzymproduktet overholder de krav til renhed, der er anført i tilsætningsstofbekendtgørelsens bilag 5 .

Postadresse/Postal address: Levnedsmiddelstyrelsen, Mørkhøj Bygade 19, DK-2860 Søborg, Denmark
Telefon/Phone: 39 69 66 00, +45 39 69 66 00
Telex: 16 298 foodin dk
Telefax: +45 39 66 01 00

Det er ligeledes en forudsætning, at de eventuelle tilsætningsstoffer, der er anvendt ved fremstilling af eller i enzymproduktet, opfylder Positivlistens krav til identitet og renhed, hvis stofferne er optaget i denne.

Herudover forudsættes det, at enzymproduktet anvendes i overensstemmelse såvel med de oplysninger, der er givet i anmeldelsen, d.v.s. alene til de deri nævnte produkter, som med god tilvirkningsmæssig praksis.

Levnedsmiddelstyrelsen vil desuden gøre opmærksom på, at den fortsatte anvendelse af enzymproduktet på et senere tidspunkt kan gøres betinget af, at der bliver fremlagt nye data, ligesom styrelsen generelt forbeholder sig ret til på et senere tidspunkt at indhente nye oplysninger om produktet. Og endelig bemærkes, at accepten kan tilbagekaldes, hvis styrelsen finder, at forholdene har udviklet sig i en sådan retning, at dette er ønskeligt eller påkrævet.

Hvis der sker ændringer i forbindelse med produktets fremstilling, eller hvis produktionen af produktet ophører, skal dette meddeles Levnedsmiddelstyrelsen. Da virksomheden er producent af det pågældende produkt, pålægges det desuden virksomheden ved videresalg af produktet til danske grossister eller levnedsmiddelproducenter at gøre opmærksom på, at produktet kun må anvendes på de i denne accept fastsatte vilkår, herunder de anførte anvendelsesbegrænsninger, og at brugeren skal indgive anmeldelse til styrelsen, hvis man ønsker at anvende produktet på andre vilkår end de i denne accept anførte.

Mærkning
Produktet skal mærkes i overensstemmelse med bestemmelserne i tilsætningsstofbekendtgørelsen.

Med venlig hilsen

[redacted]

Inge Munch

2

Positivlisten: Fortegnelse over tilsætningsstoffer til levnedsmidler, december 1995.

Tilsætningsstofbekendtgørelsen: Bekendtgørelse nr. 1055 af 18. december 1995 om tilsætningsstoffer til levnedsmidler.

Kopi til orientering til: Levnedsmiddelkontrollen I/S, Dyregårdsvej 1, 2740 Skovlunde.

3

Appendix 1.4 Resin

CANGZHOU YUANWEI CHEMICAL ENGINEERING CO.,LTD

Absorption Resin Specification

Sample Name	AB-8	Batch Number	091205
Sample Quantity	300g		
Sampling date	2009-12-18	Test Finish Date	2009-12-19
Test Basis	Q/CBN01-2006		
Main Test Equipment	Confidentiality		
Index	Technical Require		Test Result
Moisture	65-75%		72.17
Absorb hydroxybenzene quantity	≥50		74.98
Visual Density in wet state(g/ml)	0.68-0.75		0.70
Granule Diameter(0.3-1.2mm)	≥90%		93.68
Result	Eligibility		
Remark	This Test result just with the responsibility for current sample, if have the objection please require the check in 20 days, expire date will not take the transaction.		

Appendix 1.5 Ethanol

Fermentation Alcohol Specification & Analysis Report

Manufacturing Company	MH Ethanol Co,, Ltd

Analytical Item	Specification	Test Result
Odor	Foreign Odorless	Foreign Odorless
Ethanol Content	95.0±0.5 V/V%	95.0
Evaporating Residue	2.5㎎/100g Max.	Trace
Free Acid	As Acetic Acid 0.002 W/V% Max.	0.0011
Aldehydes	As Acetaldehyde 1 ㎎/100㎖ Max.	None
Methanol	0.15 ㎎/㎖ Max.	0.0096
Fusel oil	0.01 V/V% Max.	0.0003
Heavy Metals	None	None
Permanganate Time Test (Cameleon Reaction)	5 Minutes Min.	7' 20"
Substances Darkened by H_2SO_4	None	None
Chlorides	〃	〃

2019. 2. 14.

KOREA ALCOHOL SUPPLIES CO., LTD.

Fermentation Alcohol Specification & Analysis Report

Company	Il San Trading Co., Ltd (Chilseo Ethanol Factory)	Date	2019.01.12.	Quantity	A

Analytical Item	Specification	Test Result
Odor	Foreign Odorless	Pass
Ethanol Content	Not less than 94.9%(v/v)	95.2
Specific Gravity	0.8096 at 25°/25° Max	0.8093
Solubility in Water	No haze or turbidity develops	Pass
Substances Reducing Permanganate	The pink color does not entirely disappear	Pass
Substances Darkened by Sulfuric Acid	The mixture is colorless	Pass
Methanol	No violet color appears	Pass
Ketones, Isopropyl Alcohol	No precipitate within 3 min	Pass
Fusel Oil	None	Pass
Lead	0.5 mg/kg Max	0.13
Nonvolatile Residue	0.003% Max.	0.001
Alkalinity(As NH_3)	3 mg/kg Max	1
Acidity	As Acetic Acid 0.003 % Max	0.000

KOREA ALCOHOL SUPPLIES CO., LTD.

Appendix 1.6 Celite

Linjiang Imerys Diatomite Co., Ltd.
Lincheng Industrial Park, Linjiang City, Jilin Province, China
Tel:(+86) 21-22230080 Fax:(+86) 21-22230199

Certificate of Analysis

Customer Name: Korea IMKL **Product Type:** 503
Order Number: 18368 **Lot#.Production** 126-180909
Customer PO No.:
Date of issue: 16-Sep-18

Supplier Test Methods

	Min	Max	Method	Result 180909	Units
Permeability	1.0	2.5	CC-101-002	1.72	Darcy
150-mesh sieve retains	0	15	CC-101-005	4.2	%
Centrifuged wet density	--	0.42	CC-101-004	0.38	g/ml

Food Grade: Meet requirements of KFDA

Regards,

Ping Wang
Quality Control Manager

The Quality System of this facility is ISO 9001:2015 Registered by CQC

Seller's standard product inspection and testing procedures in effect at the time of testing were used to provide the information herein. Tests fixed by Seller at time of shipment shall be conclusive and binding upon Buyer as to all product sold and/or shipped. Seller represents and warrants to Buyer that all product conforms, as of shipment from Seller's plant, in all material respects to Seller's product specifications in effect at the time of shipment. Seller makes no other written, oral, express, or implied warranties. Seller disclaims all warranties of merchantability and fitness for a particular purpose.

For additional information including MSDS see http://www.imerys-filtration.com/msds.htm Page 1 of 1

Appendix 1.7 Statement of Compliance

DAE PYUNG CO., LTD.
http://www.daepyung.co.kr daepyung@daepyung.co.kr

Head Office : #19-8, Yeongdong-gil, Hamchang-eup, Sangju-si, Gyeongbuk, Korea
Tel: 82-54-541-9001 Fax: 82-54-541-9004
Bundang Office: 604Ho, Leaders B/D, #14, Hwangsaeul-ro 311beon-gil, Bundang-gu, Seongnam-si, Gyeonggi, Korea Tel: 82-31-709-7755 Fax: 82-31-709-7756

STATEMENT OF COMPLIANCE

May 3, 2019

We, DAEPYUNG CO., LTD., hereby declare that below raw materials and processing aids used to manufacture STEVITEN FRESH and STEVITEN RICH

Dextrin

Resin

Celite

Ethanol

comply with 21 CFR and FCC standards.

Su Jeong Kim
QA Manager
DAEPYUNG CO., LTD.

Appendix 2 Analytical Method

Please refer to the Appendix 2 reports, provided as separate files:

Appendix 2.a Analysis Methods & Data (STEVITEN FRESH)

Appendix 2.b Analysis Methods & Data (STEVITEN RICH).pdf

Appendix 3 Certificates of Analysis and Representative Chromatograms for Multiple Batches of STEVITEN FRESH

Appendix 3.1 STEVITEN FRESH Batch 190110

DAE PYUNG CO., LTD.
http://www.daepyung.co.kr daepyung@daepyung.co.kr

Head Office : #19-8, Yeongdong-gil, Hamchang-eup, Sangju-si, Gyeongbuk, Korea
Tel: 82-54-541-9001 Fax: 82-54-541-9004
Bundang Office: 604Ho, Leaders B/D, #14, Hwangsaeul-ro 311beon-gil, Bundang-gu, Seongnam-si, Gyeonggi, Korea Tel: 82-31-709-7755 Fax: 82-31-709-7756

CERTIFICATE OF ANALYSIS

Product Name: STEVITEN FRESH
Lot Number: 190110
Manufactured date: January 10, 2019
Expiry date: January 9, 2022
Storage: Dry/shade place and at room temperature

Analytical test	Methods	Specification	Results
Appearance	Visual	White powder	Passed
Odour	Sensory test	Slight characteristic	Passed
Teste	Sensory test	Sweet	Passed
Solubility	JECFA Vol.4	Freely soluble in water and ethanol	Passed
Total content of α-glucosyl Steviol glycosies and unreacted steviol glycoside(dry weight basis)	HPLC+UV	80.0 ~ 90.0 %	83.4
Content of α-glucosyl Steviol glycosides(dry weight basis)	UV	Not less than 65.0%	75.9
Loss on drying	JECFA Vol4	Not more than 6.0%	4.1
pH	JECFA Vol4	4.5~7.0	5.8
Ash	JECFA Vol4	Not more than 1.0%	0.2
Residual methanol	USP 467	Not more than 200 mg/kg	41
Residual ethanol	USP 467	Not more than 5000 mg/kg	78
Lead	JECFA Vol4	Not more than 1 mg/kg	Not detected
Arsenic	JECFA Vol4	Not more than 1 mg/kg	Not detected
Total plate count	AOAC 977.27	Less than 10^3cfu/g	Not detected
Yeast and mold	AOAC 997.02	Less than 10^2cfu/g	Not detected
E. coli	AOAC 983.25	Negative CFU/1g	Negative
Salmonella	AOAC 967.25	Not more than 10 npm/g	Not detected

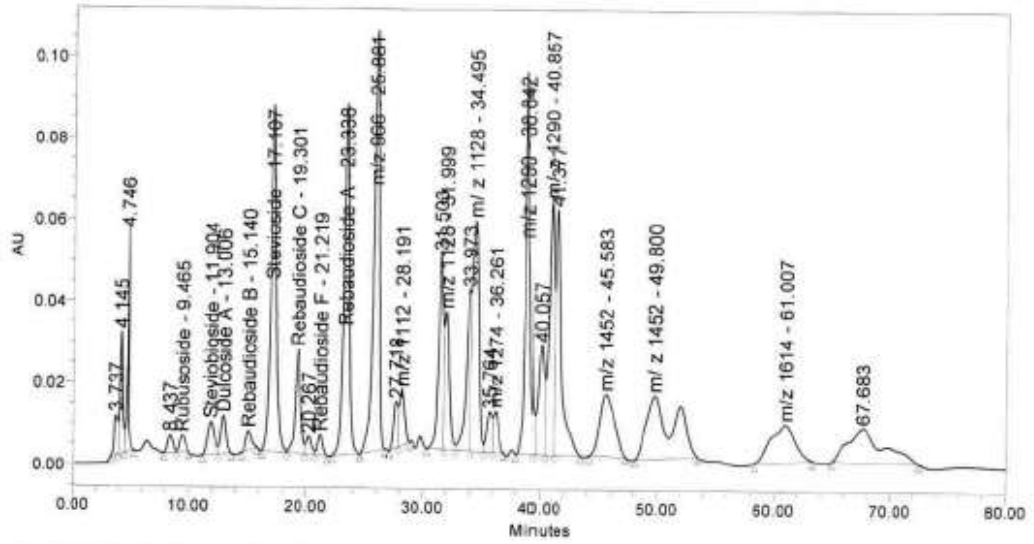
Issued by QA Department on May 14, 2019

Empower 2 SOFTWARE

GRAS

SAMPLE INFORMATION

Sample Name:	STEVITEN FRESH	Acquired By:	System
Lot:	190110	Sample Set Name:	GRAS
Vial:	5	Acq. Method Set:	G—AB—MS
Injection #:	1	Processing Methoc	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	80.0 Minutes	Proc. Chnl. Descr.:	
Date Acquired:	2019-02-25 AM 11:04:26 KST		
Date Processed:	2019-04-29 PM 3:38:35 KST		



	Peak Name	RT	Area	% Area	Height
1		3.737	201085	0.60	9590
2		4.145	484786	1.46	29764
3		4.746	448999	1.35	54011
4		8.437	127885	0.38	4271
5	Rubusoside	9.465	126038	0.38	3930
6	Steviobioside	11.904	316749	0.95	7866
7	Dulcoside A	13.006	283812	0.85	9179
8	Rebaudioside B	15.140	149783	0.45	4684
9	Stevioside	17.107	2855012	8.58	84867
10	Rebaudioside C	19.301	840705	2.53	25481
11		20.267	136248	0.41	4326
12	Rebaudioside F	21.219	139835	0.42	4978
13	Rebaudioside A	23.338	2644392	7.95	85948

	Peak Name	RT	Area	% Area	Height
14	m/z 966	25.881	3214081	9.66	102962
15		27.718	285981	0.86	11338
16	m/z 1112	28.191	417519	1.25	13445
17		31.503	1441647	4.33	47783
18	m/z 1128	31.999	996192	2.99	33710
19		33.973	1043650	3.14	39369
20	m/ z 1128	34.495	2286479	6.87	56274
21		35.764	312957	0.94	9722
22	m/z 1274	36.261	318953	0.96	9985
23	m/z 1290	38.842	2400436	7.21	93815
24		40.057	974950	2.93	26717
25	m/ z 1290	40.857	1859515	5.59	61834
26		41.377	2215235	6.66	59884

	Peak Name	RT	Area	% Area	Height
27	m/z 1452	45.583	1313661	3.95	15126
28	m/ z 1452	49.800	2406128	7.23	15384
29	m/z 1614	61.007	1295704	3.89	9087
30		67.683	1738858	5.23	8400

Appendix 3.2 STEVITEN FRESH Batch 190116

DAE PYUNG CO., LTD.
http://www.daepyung.co.kr daepyung@daepyung.co.kr

Head Office : #19-8, Yeongdong-gil, Hamchang-eup, Sangju-si, Gyeongbuk, Korea
Tel: 82-54-541-9001 Fax: 82-54-541-9004
Bundang Office: 604Ho, Leaders B/D, #14, Hwangsaeul-ro 311beon-gil, Bundang-gu, Seongnam-si, Gyeonggi, Korea Tel: 82-31-709-7755 Fax: 82-31-709-7756

CERTIFICATE OF ANALYSIS

Product Name: STEVITEN FRESH
Lot Number: 190116
Manufactured date: January 16, 2019
Expiry date: January 15, 2022
Storage: Dry/shade place and at room temperature

Analytical test	Methods	Specification	Results
Appearance	Visual	White powder	Passed
Odour	Sensory test	Slight characteristic	Passed
Teste	Sensory test	Sweet	Passed
Solubility	JECFA Vol.4	Freely soluble in water and ethanol	Passed
Total content of α-glucosyl Steviol glycosies and unreacted steviol glycoside(dry weight basis)	HPLC+UV	80.0 ~ 90.0 %	83.4
Content of α-glucosyl Steviol glycosides(dry weight basis)	UV	Not less than 65.0%	76.1
Loss on drying	JECFA Vol4	Not more than 6.0%	4.0
pH	JECFA Vol4	4.5~7.0	5.9
Ash	JECFA Vol4	Not more than 1.0%	0.2
Residual methanol	USP 467	Not more than 200 mg/kg	45
Residual ethanol	USP 467	Not more than 5000 mg/kg	75
Lead	JECFA Vol4	Not more than 1 mg/kg	Not detected
Arsenic	JECFA Vol4	Not more than 1 mg/kg	Not detected
Total plate count	AOAC 977.27	Less than 10^3cfu/g	Not detected
Yeast and mold	AOAC 997.02	Less than 10^2cfu/g	Not detected
E. coli	AOAC 983.25	Negative CFU/1g	Negative
Salmonella	AOAC 967.25	Not more than 10 npm/g	Not detected

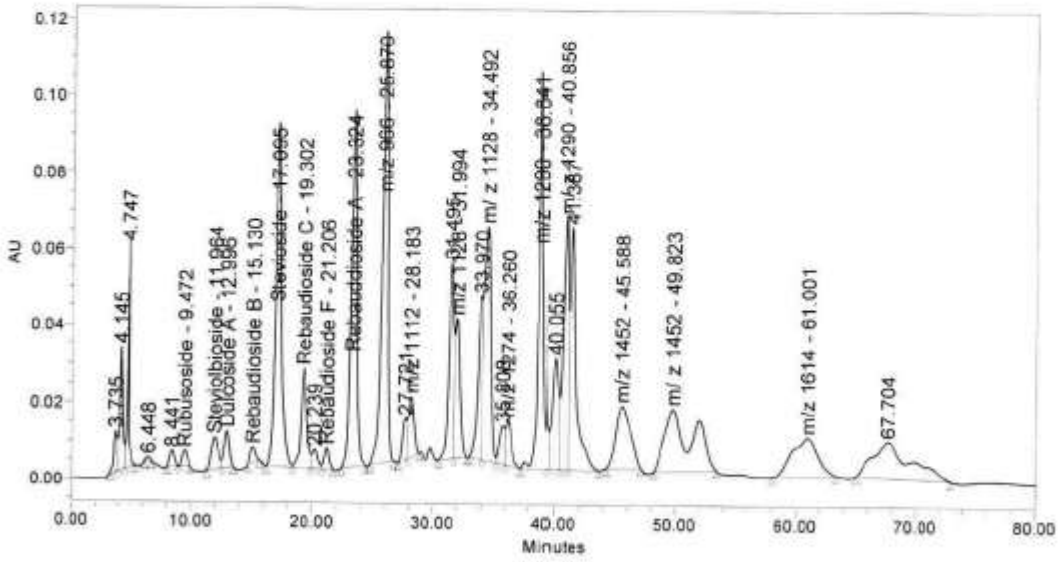
Issued by QA Department on May 14, 2019

Empower 2 software

GRAS

SAMPLE INFORMATION

Sample Name:	STEVITEN FRESH	Acquired By:	System
Lot:	190116	Sample Set Name:	GRAS
Vial:	6	Acq. Method Set:	G-AB-MS
Injection #:	1	Processing Methoc	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	80.0 Minutes	Proc. Chnl. Descr.:	
Date Acquired:	2019-02-25 PM 12:34:01 KST		
Date Processed:	2019-04-29 PM 3:44:25 KST		



	Peak Name	RT	Area	% Area	Height
1		3.735	195572	0.55	10362
2		4.145	544849	1.52	31813
3		4.747	493618	1.38	58001
4		6.448	25569	0.07	1156
5		8.441	113110	0.32	4142
6	Rubusoside	9.472	115474	0.32	3973
7	Steviolbioside	11.964	323574	0.90	8318
8	Dulcoside A	12.996	280447	0.78	9532
9	Rebaudioside B	15.130	118623	0.33	4211
10	Stevioside	17.095	3085510	8.60	89554
11	Rebaudioside C	19.302	921120	2.57	25842
12		20.239	152621	0.43	4914
13	Rebaudioside F	21.206	150061	0.42	5472

	Peak Name	RT	Area	% Area	Height
14	Rebauddioside A	23.324	2817796	7.86	92810
15	m/z 966	25.870	3468823	9.67	112611
16		27.721	251175	0.70	10805
17	m/z 1112	28.183	512918	1.43	15731
18		31.495	1459226	4.07	51199
19	m/z 1128	31.994	1116778	3.11	36233
20		33.970	1058726	2.95	42529
21	m/ z 1128	34.492	2458425	6.85	61105
22		35.808	299910	0.84	10043
23	m/z 1274	36.260	404952	1.13	11695
24	m/z 1290	38.841	2716337	7.57	103933
25		40.055	1058157	2.95	28816
26	m/ z 1290	40.856	1937413	5.40	65832
27		41.387	2375257	6.62	63300
28	m/z 1452	45.588	1370353	3.82	16247
29	m/ z 1452	49.823	2498142	6.96	16255
30	m/z 1614	61.001	1467160	4.09	10086
31		67.704	2080655	5.80	9604

Appendix 3.3 STEVITEN FRESH Batch 190214

DAE PYUNG CO., LTD.
http://www.daepyung.co.kr daepyung@daepyung.co.kr

Head Office : #19-8, Yeongdong-gil, Hamchang-eup, Sangju-si, Gyeongbuk, Korea
Tel: 82-54-541-9001 Fax: 82-54-541-9004
Bundang Office: 604Ho, Leaders B/D, #14, Hwangsaeul-ro 311beon-gil, Bundang-gu, Seongnam-si, Gyeonggi, Korea Tel: 82-31-709-7755 Fax: 82-31-709-7756

CERTIFICATE OF ANALYSIS

Product Name: STEVITEN FRESH
Lot Number: 190214
Manufactured date: February 14, 2019
Expiry date: February 13, 2022
Storage: Dry/shade place and at room temperature

Analytical test	Methods	Specification	Results
Appearance	Visual	White powder	Passed
Odour	Sensory test	Slight characteristic	Passed
Teste	Sensory test	Sweet	Passed
Solubility	JECFA Vol.4	Freely soluble in water and ethanol	Passed
Total content of α-glucosyl Steviol glycosies and unreacted steviol glycoside(dry weight basis)	HPLC+UV	80.0 ~ 90.0 %	83.6
Content of α-glucosyl Steviol glycosides(dry weight basis)	UV	Not less than 65.0%	76.3
Loss on drying	JECFA Vol4	Not more than 6.0%	4.2
pH	JECFA Vol4	4.5-7.0	5.5
Ash	JECFA Vol4	Not more than 1.0%	0.2
Residual methanol	USP 467	Not more than 200 mg/kg	39
Residual ethanol	USP 467	Not more than 5000 mg/kg	80
Lead	JECFA Vol4	Not more than 1 mg/kg	Not detected
Arsenic	JECFA Vol4	Not more than 1 mg/kg	Not detected
Total plate count	AOAC 977.27	Less than 10^3cfu/g	Not detected
Yeast and mold	AOAC 997.02	Less than 10^2cfu/g	Not detected
E. coli	AOAC 983.25	Negative CFU/1g	Negative
Salmonella	AOAC 967.25	Not more than 10 npm/g	Not detected

Issued by QA Department on May 14, 2019

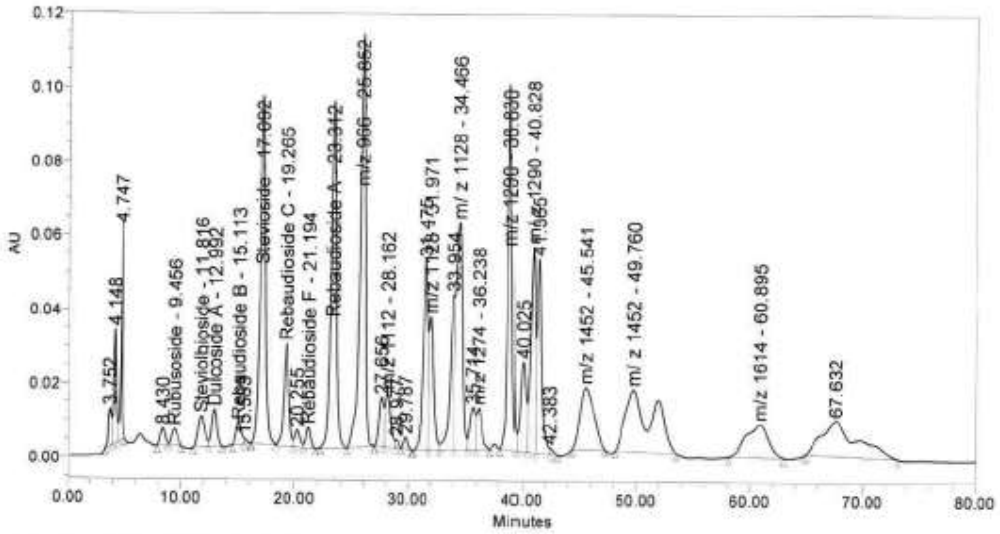
[redacted]

Empower2 software

GRAS

SAMPLE INFORMATION

Sample Name:	STEVITEN FRESH	Acquired By:	System
Lot:	190214	Sample Set Name:	GRAS
Vial:	7	Acq. Method Set:	G-AB-MS
Injection #:	1	Processing Methoc	Default1
Injection Volume	20.00 ul	Channel Name:	2487Channel 1
Run Time:	80.0 Minutes	Proc. Chnl. Descr.:	
Date Acquired:	2019-02-25 PM 1:59:24 KST		
Date Processed:	2019-04-29 PM 3:51:05 KST		



	Peak Name	RT	Area	% Area	Height
1		3.752	199051	0.57	10060
2		4.148	487966	1.41	31043
3		4.747	441009	1.27	57411
4		8.430	138333	0.40	4563
5	Rubusoside	9.456	152980	0.44	4406
6	Steviolbioside	11.816	345373	1.00	8303
7	Dulcoside A	12.992	303573	0.87	9838
8	Rebaudioside B	15.113	184103	0.53	5753
9		15.583	23118	0.07	1472
10	Stevioside	17.092	3099382	8.93	94286
11	Rebaudioside C	19.265	896084	2.58	27698
12		20.255	142038	0.41	4519
13	Rebaudioside F	21.194	152021	0.44	5409

	Peak Name	RT	Area	% Area	Height
14	Rebaudioside A	23.312	2859828	8.24	93794
15	m/z 966	25.852	3519687	10.14	111307
16		27.656	368523	1.06	13266
17	m/z 1112	28.162	486023	1.40	14555
18		28.977	43019	0.12	2054
19		29.787	75376	0.22	3115
20		31.475	1530869	4.41	51407
21	m/z 1128	31.971	1065018	3.07	36121
22		33.954	1073249	3.09	41944
23	m/ z 1128	34.466	2501212	7.21	61339
24		35.714	393420	1.13	11340
25	m/z 1274	36.238	373770	1.08	11063
26	m/z 1290	38.830	2338724	6.74	98667

	Peak Name	RT	Area	% Area	Height
27		40.025	815781	2.35	24257
28	m/ z 1290	40.828	1565662	4.51	55384
29		41.365	1596513	4.60	52328
30		42.383	33446	0.10	1726
31	m/z 1452	45.541	1439935	4.15	16818
32	m/ z 1452	49.760	2698084	7.77	16716
33	m/z 1614	60.895	1240968	3.58	9011
34		67.632	2125739	6.12	9604

Appendix 3.4 STEVITEN FRESH Batch 190308

DAE PYUNG CO., LTD.
http://www.daepyung.co.kr daepyung@daepyung.co.kr

Head Office : #19-8, Yeongdong-gil, Hamchang-eup, Sangju-si, Gyeongbuk, Korea
Tel: 82-54-541-9001 Fax: 82-54-541-9004
Bundang Office: 604Ho, Leaders B/D, #14, Hwangsaeul-ro 311beon-gil, Bundang-gu, Seongnam-si, Gyeonggi, Korea Tel: 82-31-709-7755 Fax: 82-31-709-7756

CERTIFICATE OF ANALYSIS

Product Name: STEVITEN FRESH
Lot Number: 190308
Manufactured date: March 8, 2019
Expiry date: March 7, 2022
Storage: Dry/shade place and at room temperature

Analytical test	Methods	Specification	Results
Appearance	Visual	White powder	Passed
Odour	Sensory test	Slight characteristic	Passed
Teste	Sensory test	Sweet	Passed
Solubility	JECFA Vol.4	Freely soluble in water and ethanol	Passed
Total content of α-glucosyl Steviol glycosies and unreacted steviol glycoside(dry weight basis)	HPLC+UV	80.0 - 90.0 %	83.5
Content of α-glucosyl Steviol glycosides(dry weight basis)	UV	Not less than 65.0%	76.1
Loss on drying	JECFA Vol4	Not more than 6.0%	3.8
pH	JECFA Vol4	4.5~7.0	5.6
Ash	JECFA Vol4	Not more than 1.0%	0.2
Residual methanol	USP 467	Not more than 200 mg/kg	43
Residual ethanol	USP 467	Not more than 5000 mg/kg	71
Lead	JECFA Vol4	Not more than 1 mg/kg	Not detected
Arsenic	JECFA Vol4	Not more than 1 mg/kg	Not detected
Total plate count	AOAC 977.27	Less than 10^3cfu/g	Not detected
Yeast and mold	AOAC 997.02	Less than 10^2cfu/g	Not detected
E. coli	AOAC 983.25	Negative CFU/1g	Negative
Salmonella	AOAC 967.25	Not more than 10 npm/g	Not detected

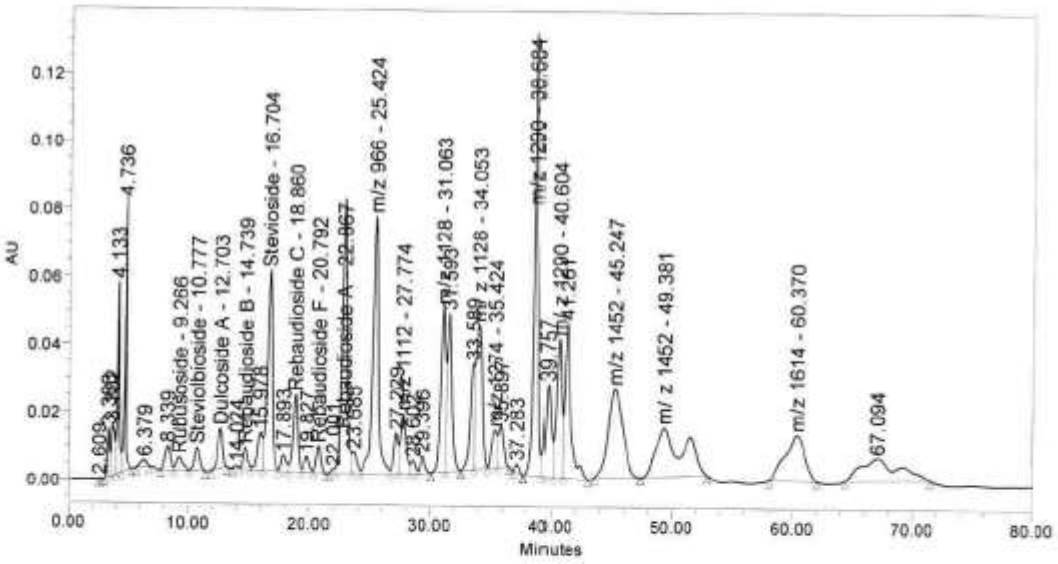
Issued by QA Department on May 14, 2019

Empower 2 software

GRAS

SAMPLE INFORMATION

Sample Name:	STEVITEN FRESH	Acquired By:	System
Lot:	190308	Sample Set Name:	GRAS
Vial:	2	Acq. Method Set:	G—AB—MS
Injection #:	1	Processing Methoc	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	80.0 Minutes	Proc. Chnl. Descr.:	
Date Acquired:	2019-04-26 PM 7:17:48 KST		
Date Processed:	2019-04-29 AM 11:53:00 KST		



	Peak Name	RT	Area	% Area	Height
1		2.609	3033	0.01	316
2		3.386	171185	0.54	14202
3		3.702	299282	0.94	14979
4		4.133	639793	2.01	56395
5		4.736	574650	1.81	78541
6		6.379	69627	0.22	2031
7		8.339	220452	0.69	7140
8	Rubusoside	9.266	146548	0.46	3927
9	Steviolbioside	10.777	209206	0.66	6951
10	Dulcoside A	12.703	330566	1.04	11708
11		14.024	21914	0.07	879
12	Rebaudioside B	14.739	179723	0.56	6558
13		15.978	319967	1.01	10997

	Peak Name	RT	Area	% Area	Height
14	Stevioside	16.704	1667304	5.24	59004
15		17.893	133712	0.42	4755
16	Rebaudioside C	18.860	614205	1.93	22704
17		19.827	139218	0.44	4685
18	Rebaudioside F	20.792	210887	0.66	8048
19		22.001	24406	0.08	1284
20	Rebaudioside A	22.867	2274030	7.15	80874
21		23.685	176811	0.56	6252
22	m/z 966	25.424	2402214	7.55	75692
23		27.229	303798	0.95	11806
24	m/z 1112	27.774	547876	1.72	17417
25		28.602	98942	0.31	3741
26		29.396	136200	0.43	5102

	Peak Name	RT	Area	% Area	Height
27	m/z 1128	31.063	1318626	4.14	48438
28		31.593	1405738	4.42	46681
29		33.589	698993	2.20	31133
30	m/ z 1128	34.053	1736790	5.46	42410
31	m/z 1274	35.424	340405	1.07	10998
32		35.897	354758	1.12	12048
33		37.283	60013	0.19	2558
34	m/z 1290	38.684	2892284	9.09	130431
35		39.757	840647	2.64	27135
36	m/ z 1290	40.604	1223548	3.85	40790
37		41.261	1640708	5.16	45668
38	m/z 1452	45.247	2172449	6.83	26084
39	m/ z 1452	49.381	2234179	7.02	14289
40	m/z 1614	60.370	1517468	4.77	13483
41		67.094	1463596	4.60	6633

Appendix 3.5 STEVITEN FRESH Batch 190314

DAE PYUNG CO., LTD.
http://www.daepyung.co.kr daepyung@daepyung.co.kr

Head Office : #19-8, Yeongdong-gil, Hamchang-eup, Sangju-si, Gyeongbuk, Korea
Tel: 82-54-541-9001 Fax: 82-54-541-9004
Bundang Office: 604Ho, Leaders B/D, #14, Hwangsaeul-ro 311beon-gil, Bundang-gu, Seongnam-si, Gyeonggi, Korea Tel: 82-31-709-7755 Fax: 82-31-709-7756

CERTIFICATE OF ANALYSIS

Product Name: STEVITEN FRESH
Lot Number: 190314
Manufactured date: March 14, 2019
Expiry date: March 13, 2022
Storage: Dry/shade place and at room temperature

Analytical test	Methods	Specification	Results
Appearance	Visual	White powder	Passed
Odour	Sensory test	Slight characteristic	Passed
Teste	Sensory test	Sweet	Passed
Solubility	JECFA Vol.4	Freely soluble in water and ethanol	Passed
Total content of α-glucosyl Steviol glycosies and unreacted steviol glycoside(dry weight basis)	HPLC+UV	80.0 - 90.0 %	83.4
Content of α-glucosyl Steviol glycosides(dry weight basis)	UV	Not less than 65.0%	75.9
Loss on drying	JECFA Vol4	Not more than 6.0%	3.5
pH	JECFA Vol4	4.5-7.0	5.5
Ash	JECFA Vol4	Not more than 1.0%	0.2
Residual methanol	USP 467	Not more than 200 mg/kg	51
Residual ethanol	USP 467	Not more than 5000 mg/kg	78
Lead	JECFA Vol4	Not more than 1 mg/kg	Not detected
Arsenic	JECFA Vol4	Not more than 1 mg/kg	Not detected
Total plate count	AOAC 977.27	Less than 10^3cfu/g	Not detected
Yeast and mold	AOAC 997.02	Less than 10^2cfu/g	Not detected
E. coli	AOAC 983.25	Negative CFU/1g	Negative
Salmonella	AOAC 967.25	Not more than 10 npm/g	Not detected

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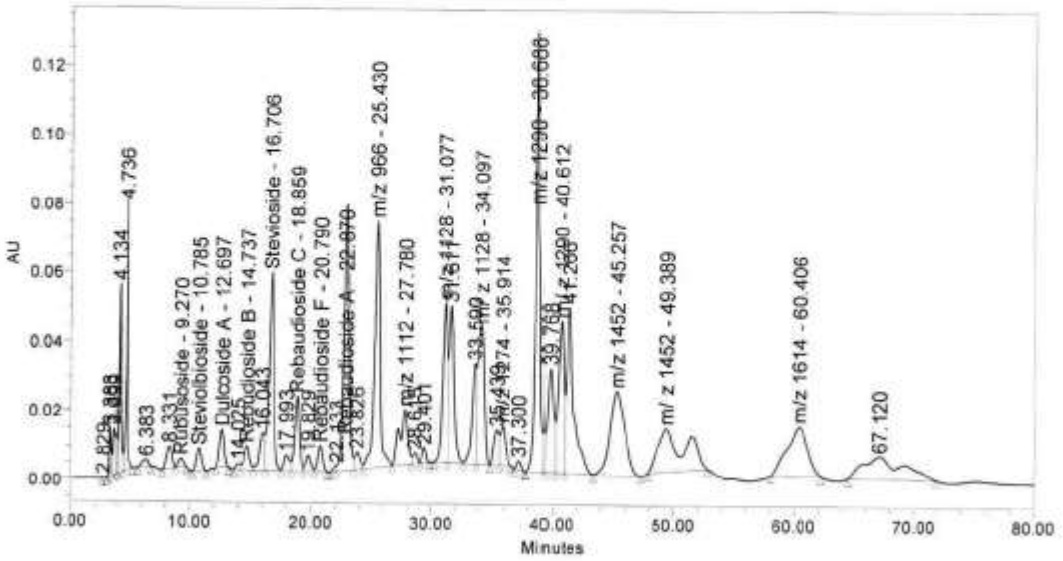
Empower 2 software

GRAS

SAMPLE INFORMATION

Sample Name:	STEVITEN FRESH	Acquired By:	System
Lot:	190314	Sample Set Name:	GRAS
Vial:	3	Acq. Method Set:	G—AB—MS
Injection #:	1	Processing Methoc	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	80.0 Minutes	Proc. Chnl. Descr.:	
Date Acquired:	2019-04-26 PM 8:38:53 KST		
Date Processed:	2019-04-29 AM 11:48:41 KST		



	Peak Name	RT	Area	% Area	Height
1		2.829	2129	0.01	205
2		3.383	160096	0.51	13818
3		3.699	249381	0.80	13386
4		4.134	604141	1.94	54515
5		4.736	559645	1.79	76337
6		6.383	71066	0.23	2105
7		8.331	189564	0.61	6470
8	Rubusoside	9.270	65609	0.21	2372
9	Steviolbioside	10.785	193993	0.62	6656
10	Dulcoside A	12.697	290213	0.93	10843
11		14.025	42507	0.14	1746
12	Rebudioside B	14.737	218979	0.70	7017
13		16.043	302391	0.97	10840

	Peak Name	RT	Area	% Area	Height
14	Stevioside	16.706	1630273	5.22	57248
15		17.993	138257	0.44	4465
16	Rebaudioside C	18.859	582843	1.87	21706
17		19.829	130980	0.42	4459
18	Rebaudioside F	20.790	199203	0.64	7564
19		22.133	23788	0.08	1537
20	Rebaudioside A	22.870	2161066	6.92	77122
21		23.826	147599	0.47	4906
22	m/z 966	25.430	2192297	7.02	71431
23	m/z 1112	27.780	751682	2.41	15982
24		28.615	75206	0.24	2998
25		29.401	95900	0.31	4303
26	m/z 1128	31.077	1245510	3.99	46032

	Peak Name	RT	Area	% Area	Height
27		31.611	1385464	4.44	45241
28		33.590	703555	2.25	29116
29	m/ z 1128	34.097	1587146	5.09	39703
30		35.439	303395	0.97	10043
31	m/z 1274	35.914	329437	1.06	11176
32		37.300	62715	0.20	2587
33	m/z 1290	38.688	2999999	9.61	127518
34		39.768	1028278	3.29	30193
35	m/ z 1290	40.612	1369398	4.39	44026
36		41.266	2096409	6.72	48049
37	m/z 1452	45.257	2037527	6.53	24292
38	m/ z 1452	49.389	1865071	5.98	12545
39	m/z 1614	60.406	1652691	5.30	14228
40		67.120	1466537	4.70	6454

Appendix 4 Certificates of Analysis and Representative Chromatograms for Multiple Batches of STEVITEN RICH

Appendix 4.1 STEVITEN RICH Batch 190108

DAE PYUNG CO., LTD.
http://www.daepyung.co.kr daepyung@daepyung.co.kr

Head Office : #19-8, Yeongdong-gil, Hamchang-eup, Sangju-si, Gyeongbuk, Korea
Tel: 82-54-541-9001 Fax: 82-54-541-9004
Bundang Office: 604Ho, Leaders B/D, #14, Hwangsaeul-ro 311beon-gil, Bundang-gu, Seongnam-si, Gyeonggi, Korea Tel: 82-31-709-7755 Fax: 82-31-709-7756

CERTIFICATE OF ANALYSIS

Product Name: STEVITEN RICH
Lot Number: 190108
Manufactured date: January 8, 2019
Expiry date: January 7, 2022
Storage: Dry/shade place and at room temperature

Analytical test	Methods	Specification	Results
Appearance	Visual	White powder	Passed
Odour	Sensory test	Slight characteristic	Passed
Teste	Sensory test	Sweet	Passed
Solubility	JECFA Vol.4	Freely soluble in water and ethanol	Passed
Total content of α-glucosyl Steviol glycosies and unreacted steviol glycoside(dry weight basis)	HPLC+UV	85.0 - 95.0 %	92.4
Content of α-glucosyl Steviol glycosides(dry weight basis)	UV	Not less than 70.0%	86.6
Loss on drying	JECFA Vol4	Not more than 6.0%	3.8
pH	JECFA Vol4	4.5~7.0	5.6
Ash	JECFA Vol4	Not more than 1.0%	0.2
Residual methanol	USP 467	Not more than 200 mg/kg	38
Residual ethanol	USP 467	Not more than 5000 mg/kg	69
Lead	JECFA Vol4	Not more than 1 mg/kg	Not detected
Arsenic	JECFA Vol4	Not more than 1 mg/kg	Not detected
Total plate count	AOAC 977.27	Less than 10^3cfu/g	Not detected
Yeast and mold	AOAC 997.02	Less than 10^2cfu/g	Not detected
E. coli	AOAC 983.25	Negative CFU/1g	Negative
Salmonella	AOAC 967.25	Not more than 10 npm/g	Not detected

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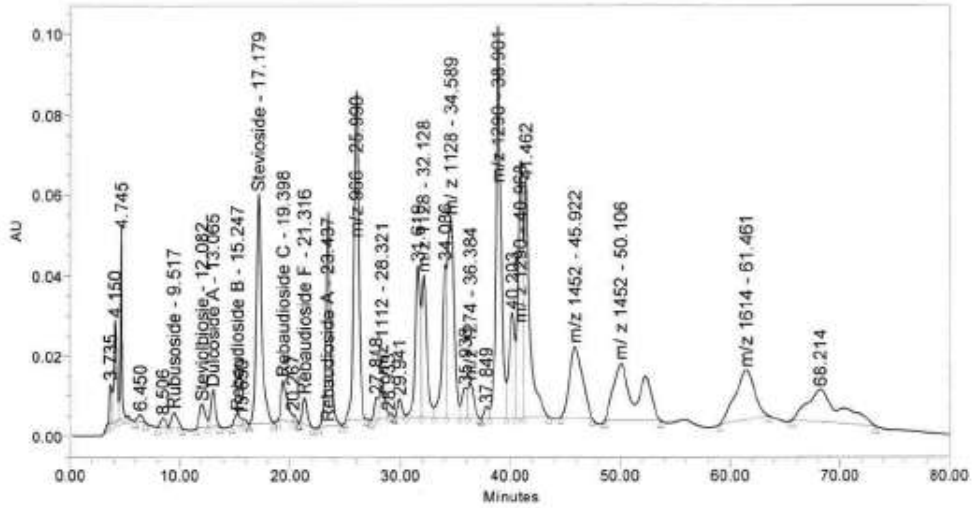
GRAS

SAMPLE INFORMATION

Sample Name:	STEVITEN RICH	Acquired By:	System
Lot:	190108	Sample Set Name:	GRAS
Vial:	8	Acq. Method Set:	G—AB—MS
Injection #:	1	Processing Methoc	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	80.0 Minutes	Proc. Chnl. Descr.:	
Date Acquired:	2019-02-25 PM 3:26:59 KST		
Date Processed:	2019-04-29 PM 4:03:44 KST		



	Peak Name	RT	Area	% Area	Height
1		3.735	158751	0.53	9869
2		4.150	450482	1.51	25164
3		4.745	377249	1.26	46912
4		6.450	54923	0.18	1870
5		8.506	68898	0.23	2203
6	Rubusoside	9.517	142674	0.48	3829
7	Steviolbiosie	12.082	207976	0.70	5710
8	Dulcoside A	13.065	270822	0.91	9166
9	Rebaudioside B	15.247	92866	0.31	2776
10		15.650	29124	0.10	1357
11	Stevioside	17.179	1910437	6.40	56955
12	Rebaudioside C	19.398	394838	1.32	10047
13		20.267	30510	0.10	1800

	Peak Name	RT	Area	% Area	Height
14	Rebaudioside F	21.316	184791	0.62	6696
15	Rebaudioside A	23.437	1632651	5.47	52969
16	m/z 966	25.990	2505548	8.39	81815
17		27.818	124285	0.42	5551
18	m/z 1112	28.321	337332	1.13	9947
19		28.933	13625	0.05	705
20		29.941	121849	0.41	4567
21		31.619	1132186	3.79	37799
22	m/z 1128	32.128	1166768	3.91	35275
23		34.086	1089046	3.65	38137
24	m/ z 1128	34.589	2048108	6.86	49373
25		35.938	239018	0.80	7425
26	m/z 1274	36.384	301981	1.01	8483

	Peak Name	RT	Area	% Area	Height
27		37.849	76437	0.26	2796
28	m/z 1290	38.901	2818857	9.44	97353
29		40.203	1003947	3.36	26230
30	m/ z 1290	40.968	1861396	6.23	63853
31		41.462	2217480	7.43	58360
32	m/z 1452	45.922	1476295	4.94	17837
33	m/ z 1452	50.106	2144896	7.18	14337
34	m/z 1614	61.461	1493473	5.00	12323
35		68.214	1680881	5.63	8149

Appendix 4.2 STEVITEN RICH Batch 190129

DAE PYUNG CO., LTD.
http://www.daepyung.co.kr daepyung@daepyung.co.kr

Head Office : #19-8, Yeongdong-gil, Hamchang-eup, Sangju-si, Gyeongbuk, Korea
Tel: 82-54-541-9001 Fax: 82-54-541-9004
Bundang Office: 604Ho, Leaders B/D, #14, Hwangsaeul-ro 311beon-gil, Bundang-gu, Seongnam-si, Gyeonggi, Korea Tel: 82-31-709-7755 Fax: 82-31-709-7756

CERTIFICATE OF ANALYSIS

Product Name: STEVITEN RICH
Lot Number: 190129
Manufactured date: January 29, 2019
Expiry date: January 285, 2022
Storage: Dry/shade place and at room temperature

Analytical test	Methods	Specification	Results
Appearance	Visual	White powder	Passed
Odour	Sensory test	Slight characteristic	Passed
Teste	Sensory test	Sweet	Passed
Solubility	JECFA Vol.4	Freely soluble in water and ethanol	Passed
Total content of α-glucosyl Steviol glycosies and unreacted steviol glycoside(dry weight basis)	HPLC+UV	85.0 ~ 95.0 %	92.3
Content of α-glucosyl Steviol glycosides(dry weight basis)	UV	Not less than 70.0%	86.4
Loss on drying	JECFA Vol4	Not more than 6.0%	4.0
pH	JECFA Vol4	4.5-7.0	5.8
Ash	JECFA Vol4	Not more than 1.0%	0.2
Residual methanol	USP 467	Not more than 200 mg/kg	35
Residual ethanol	USP 467	Not more than 5000 mg/kg	70
Lead	JECFA Vol4	Not more than 1 mg/kg	Not detected
Arsenic	JECFA Vol4	Not more than 1 mg/kg	Not detected
Total plate count	AOAC 977.27	Less than 10^3cfu/g	Not detected
Yeast and mold	AOAC 997.02	Less than 10^2cfu/g	Not detected
E. coli	AOAC 983.25	Negative CFU/1g	Negative
Salmonella	AOAC 967.25	Not more than 10 npm/g	Not detected

Issued by QA Department on May 14, 2019

[redacted]
Ra-Jeong Kim/QA Manager

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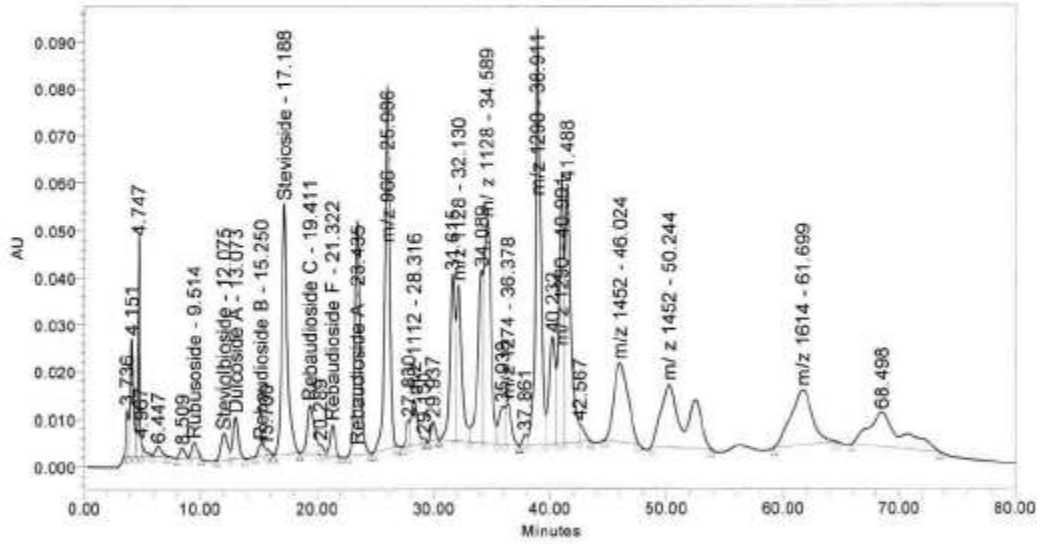
GRAS

SAMPLE INFORMATION

Sample Name:	STEVITEN RICH	Acquired By:	System
Lot:	190129	Sample Set Name:	GRAS
Vial:	9	Acq. Method Set:	G-AB-MS
Injection #:	1	Processing Methoc	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	80.0 Minutes	Proc. Chnl. Descr.:	
Date Acquired:	2019-02-25 PM 4:52:23 KST		
Date Processed:	2019-04-29 PM 4:08:33 KST		



	Peak Name	RT	Area	% Area	Height
1		3.736	170023	0.61	9992
2		4.151	456192	1.63	24843
3		4.747	400098	1.43	46149
4		4.967	61605	0.22	3077
5		6.447	75498	0.27	2000
6		8.509	58584	0.21	1949
7	Rubusoside	9.514	110988	0.40	3246
8	Steviolbioside	12.075	202870	0.73	5457
9	Dulcoside A	13.073	270569	0.97	8750
10	Rebaudioside B	15.250	87964	0.31	2601
11		15.700	21590	0.08	1162
12	Stevioside	17.188	1772205	6.34	52876
13	Rebaudioside C	19.411	429288	1.54	10319

	Peak Name	RT	Area	% Area	Height
14		20.289	58771	0.21	2267
15	Rebaudioside F	21.322	190507	0.68	6592
16	Rebaudioside A	23.435	1507167	5.39	49499
17	m/z 966	25.986	2365337	8.47	76866
18		27.830	114906	0.41	5314
19	m/z 1112	28.316	332314	1.19	9471
20		29.150	20561	0.07	961
21		29.937	95241	0.34	3924
22		31.615	1045416	3.74	35183
23	m/z 1128	32.130	1121988	4.02	33075
24		34.089	1065848	3.82	36405
25	m/ z 1128	34.589	1997759	7.15	46953
26		35.938	274313	0.98	7965

	Peak Name	RT	Area	% Area	Height
27	m/z 1274	36.378	313236	1.12	8683
28		37.861	64287	0.23	2404
29	m/z 1290	38.911	2498358	8.94	88196
30		40.232	841136	3.01	22638
31	m/ z 1290	40.991	1613605	5.78	57185
32		41.488	1893085	6.78	54545
33		42.567	99813	0.36	3722
34	m/z 1452	46.024	1386019	4.96	16432
35	m/ z 1452	50.244	2000198	7.16	13248
36	m/z 1614	61.699	1476198	5.28	11591
37		68.498	1444059	5.17	7222

Appendix 4.3 STEVITEN RICH Batch 190221

DAE PYUNG CO., LTD.
http://www.daepyung.co.kr daepyung@daepyung.co.kr

Head Office : #19-8, Yeongdong-gil, Hamchang-eup, Sangju-si, Gyeongbuk, Korea
Tel: 82-54-541-9001 Fax: 82-54-541-9004
Bundang Office: 604Ho, Leaders B/D, #14, Hwangsaeul-ro 311beon-gil, Bundang-gu, Seongnam-si, Gyeonggi, Korea Tel: 82-31-709-7755 Fax: 82-31-709-7756

CERTIFICATE OF ANALYSIS

Product Name: STEVITEN RICH
Lot Number: 190221
Manufactured date: February 21, 2019
Expiry date: February 20, 2022
Storage: Dry/shade place and at room temperature

Analytical test	Methods	Specification	Results
Appearance	Visual	White powder	Passed
Odour	Sensory test	Slight characteristic	Passed
Teste	Sensory test	Sweet	Passed
Solubility	JECFA Vol.4	Freely soluble in water and ethanol	Passed
Total content of α-glucosyl Steviol glycosies and unreacted steviol glycoside(dry weight basis)	HPLC+UV	85.0 – 95.0 %	92.5
Content of α-glucosyl Steviol glycosides(dry weight basis)	UV	Not less than 70.0%	86.9
Loss on drying	JECFA Vol4	Not more than 6.0%	4.1
pH	JECFA Vol4	4.5~7.0	5.8
Ash	JECFA Vol4	Not more than 1.0%	0.2
Residual methanol	USP 467	Not more than 200 mg/kg	31
Residual ethanol	USP 467	Not more than 5000 mg/kg	64
Lead	JECFA Vol4	Not more than 1 mg/kg	Not detected
Arsenic	JECFA Vol4	Not more than 1 mg/kg	Not detected
Total plate count	AOAC 977.27	Less than 10^3cfu/g	Not detected
Yeast and mold	AOAC 997.02	Less than 10^2cfu/g	Not detected
E. coli	AOAC 983.25	Negative CFU/1g	Negative
Salmonella	AOAC 967.25	Not more than 10 npm/g	Not detected

Issued by QA Department on May 14, 2019

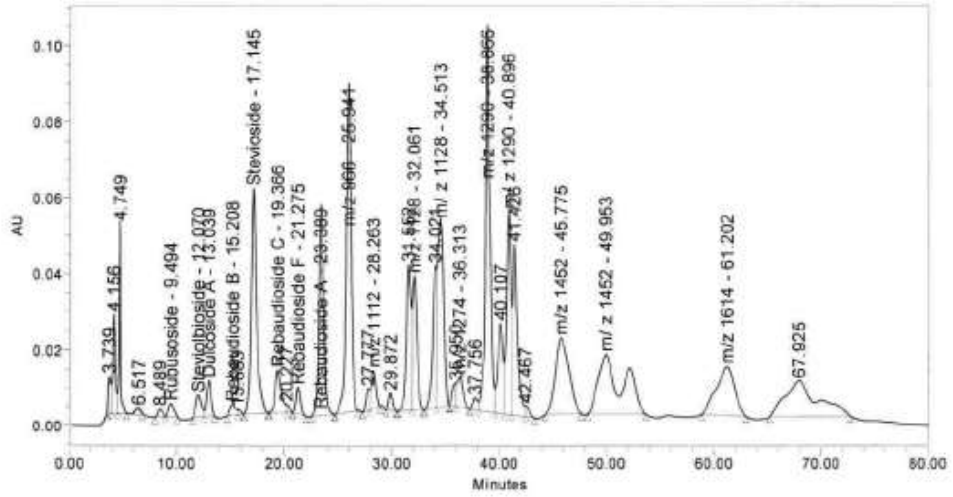
[redacted]
Su-Jeong Kim/QA Manager

Empower 2 software

GRAS

SAMPLE INFORMATION

Sample Name:	STEVITEN RICH	Acquired By:	System
Lot:	190221	Sample Set Name:	GRAS
Vial:	10	Acq. Method Set:	G—AB—MS
Injection #:	1	Processing Methoc	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	80.0 Minutes	Proc. Chnl. Descr.:	
Date Acquired:	2019-02-25 PM 6:15:38 KST		
Date Processed:	2019-04-29 PM 4:12:59 KST		



	Peak Name	RT	Area	% Area	Height
1		3.739	157371	0.54	9646
2		4.156	468416	1.60	25923
3		4.749	434924	1.48	49516
4		6.517	10036	0.03	565
5		8.489	66830	0.23	2200
6	Rubusoside	9.494	124503	0.42	3634
7	Steviolbioside	12.070	209884	0.71	5782
8	Dulcoside A	13.039	275196	0.94	9434
9	Rebaudioside B	15.208	102118	0.35	2886
10		15.683	25522	0.09	1424
11	Stevioside	17.145	1991464	6.78	59235
12	Rebaudioside C	19.366	455528	1.55	11250
13		20.227	38297	0.13	2025

	Peak Name	RT	Area	% Area	Height
14	Rebaudioside F	21.275	188174	0.64	6918
15	Rebaudioside A	23.389	1710898	5.83	55665
16	m/z 966	25.941	2672718	9.10	86444
17		27.777	127470	0.43	5739
18	m/z 1112	28.263	329487	1.12	10146
19		29.872	113670	0.39	4608
20		31.552	1112082	3.79	38153
21	m/z 1128	32.061	1048513	3.57	35179
22		34.021	911322	3.10	37299
23	m/ z 1128	34.513	1952407	6.65	48840
24		35.950	129004	0.44	6136
25	m/z 1274	36.313	314405	1.07	7951
26		37.756	93015	0.32	3196

	Peak Name	RT	Area	% Area	Height
27	m/z 1290	38.866	2781322	9.47	101510
28		40.107	850373	2.90	23649
29	m/ z 1290	40.896	1484075	5.06	53443
30		41.426	1453730	4.95	44770
31		42.467	72126	0.25	2807
32	m/z 1452	45.775	1773383	6.04	20163
33	m/ z 1452	49.953	2425995	8.26	15719
34	m/z 1614	61.202	1497705	5.10	12848
35		67.925	1954193	6.66	9396

Appendix 4.4 STEVITEN RICH Batch 190321

DAE PYUNG CO., LTD.
http://www.daepyung.co.kr daepyung@daepyung.co.kr

Head Office : #19-8, Yeongdong-gil, Hamchang-eup, Sangju-si, Gyeongbuk, Korea
Tel: 82-54-541-9001 Fax: 82-54-541-9004
Bundang Office: 604Ho, Leaders B/D, #14, Hwangsaeul-ro 311beon-gil, Bundang-gu, Seongnam-si, Gyeonggi, Korea Tel: 82-31-709-7755 Fax: 82-31-709-7756

CERTIFICATE OF ANALYSIS

Product Name: STEVITEN RICH
Lot Number: 190321
Manufactured date: March 21, 2019
Expiry date: March 20, 2022
Storage: Dry/shade place and at room temperature

Analytical test	Methods	Specification	Results
Appearance	Visual	White powder	Passed
Odour	Sensory test	Slight characteristic	Passed
Teste	Sensory test	Sweet	Passed
Solubility	JECFA Vol.4	Freely soluble in water and ethanol	Passed
Total content of α-glucosyl Steviol glycosies and unreacted steviol glycoside(dry weight basis)	HPLC+UV	85.0 ~ 95.0 %	92.1
Content of α-glucosyl Steviol glycosides(dry weight basis)	UV	Not less than 70.0%	86.5
Loss on drying	JECFA Vol4	Not more than 6.0%	4.0
pH	JECFA Vol4	4.5~7.0	5.5
Ash	JECFA Vol4	Not more than 1.0%	0.2
Residual methanol	USP 467	Not more than 200 mg/kg	35
Residual ethanol	USP 467	Not more than 5000 mg/kg	62
Lead	JECFA Vol4	Not more than 1 mg/kg	Not detected
Arsenic	JECFA Vol4	Not more than 1 mg/kg	Not detected
Total plate count	AOAC 977.27	Less than 10^3cfu/g	Not detected
Yeast and mold	AOAC 997.02	Less than 10^2cfu/g	Not detected
E. coli	AOAC 983.25	Negative CFU/1g	Negative
Salmonella	AOAC 967.25	Not more than 10 npm/g	Not detected

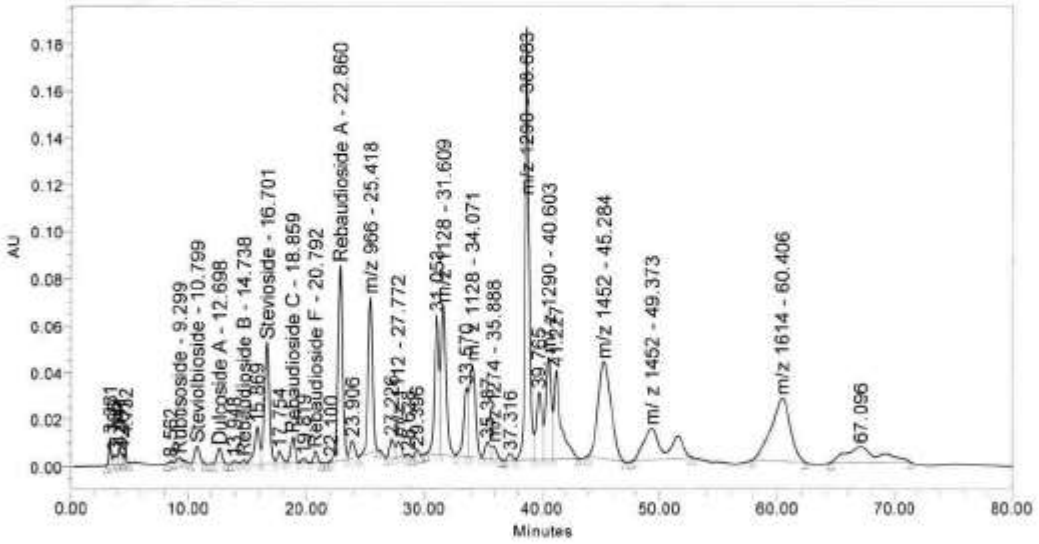
Issued by QA Department on May 14, 2019

Empower 2 software

GRAS

SAMPLE INFORMATION

Sample Name:	STEVITEN RICH	Acquired By:	System
Lot:	190321	Sample Set Name:	GRAS
Vial:	4	Acq. Method Set:	G—AB—MS
Injection #:	1	Processing Methoc	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	80.0 Minutes	Proc. Chnl. Descr.:	
Date Acquired:	2019-04-26 PM 9:59:58 KST		
Date Processed:	2019-04-29 AM 11:57:41 KST		



	Peak Name	RT	Area	% Area	Height
1		3.381	128079	0.41	11555
2		3.785	80219	0.25	4180
3		4.141	93651	0.30	6017
4		4.461	51145	0.16	4081
5		4.732	59844	0.19	9354
6		8.562	13580	0.04	654
7	Rubusoside	9.299	34433	0.11	1412
8	Steviolbioside	10.799	211845	0.67	7271
9	Dulcoside A	12.698	160049	0.51	6173
10		13.948	27150	0.09	1057
11	Rebaudioside B	14.738	34986	0.11	1451
12		15.869	449424	1.42	15412
13	Stevioside	16.701	1439685	4.56	51521

	Peak Name	RT	Area	% Area	Height
14		17.754	145507	0.46	4923
15	Rebaudioside C	18.859	285493	0.90	10823
16		19.819	49951	0.16	1796
17	Rebaudioside F	20.792	120788	0.38	4807
18		22.100	9828	0.03	869
19	Rebaudioside A	22.860	2196102	6.95	83092
20		23.906	202033	0.64	7218
21	m/z 966	25.418	1765205	5.59	66349
22		27.226	195597	0.62	7780
23	m/z 1112	27.772	206597	0.65	6790
24		28.628	25004	0.08	1086
25		29.396	55502	0.18	2665
26		31.053	1597346	5.06	59719

	Peak Name	RT	Area	% Area	Height
27	m/z 1128	31.609	1921985	6.08	63686
28		33.570	699020	2.21	29461
29	m/ z 1128	34.071	1578637	5.00	39414
30		35.387	233369	0.74	6964
31	m/z 1274	35.888	149776	0.47	5423
32		37.316	74677	0.24	2551
33	m/z 1290	38.683	4190551	13.27	183722
34		39.765	964730	3.05	28926
35	m/ z 1290	40.603	1381272	4.37	43779
36		41.227	1597099	5.06	37954
37	m/z 1452	45.284	3174728	10.05	41323
38	m/ z 1452	49.373	1755644	5.56	13239
39	m/z 1614	60.406	2970600	9.40	26796
40		67.096	1256863	3.98	6508

Appendix 4.5 STEVITEN RICH Batch 190325

DAE PYUNG CO., LTD.
http://www.daepyung.co.kr daepyung@daepyung.co.kr

Head Office : #19-8, Yeongdong-gil, Hamchang-eup, Sangju-si, Gyeongbuk, Korea
Tel: 82-54-541-9001 Fax: 82-54-541-9004
Bundang Office: 604Ho, Leaders B/D, #14, Hwangsaeul-ro 311beon-gil, Bundang-gu, Seongnam-si, Gyeonggi, Korea Tel: 82-31-709-7755 Fax: 82-31-709-7756

CERTIFICATE OF ANALYSIS

Product Name: STEVITEN RICH
Lot Number: 190325
Manufactured date: March 25, 2019
Expiry date: March 24, 2022
Storage: Dry/shade place and at room temperature

Analytical test	Methods	Specification	Results
Appearance	Visual	White powder	Passed
Odour	Sensory test	Slight characteristic	Passed
Teste	Sensory test	Sweet	Passed
Solubility	JECFA Vol.4	Freely soluble in water and ethanol	Passed
Total content of α-glucosyl Steviol glycosies and unreacted steviol glycoside(dry weight basis)	HPLC+UV	85.0 – 95.0 %	92.4
Content of α-glucosyl Steviol glycosides(dry weight basis)	UV	Not less than 70.0%	86.9
Loss on drying	JECFA Vol4	Not more than 6.0%	3.9
pH	JECFA Vol4	4.5~7.0	5.6
Ash	JECFA Vol4	Not more than 1.0%	0.2
Residual methanol	USP 467	Not more than 200 mg/kg	41
Residual ethanol	USP 467	Not more than 5000 mg/kg	62
Lead	JECFA Vol4	Not more than 1 mg/kg	Not detected
Arsenic	JECFA Vol4	Not more than 1 mg/kg	Not detected
Total plate count	AOAC 977.27	Less than 10^3cfu/g	Not detected
Yeast and mold	AOAC 997.02	Less than 10^2cfu/g	Not detected
E. coli	AOAC 983.25	Negative CFU/1g	Negative
Salmonella	AOAC 967.25	Not more than 10 npm/g	Not detected

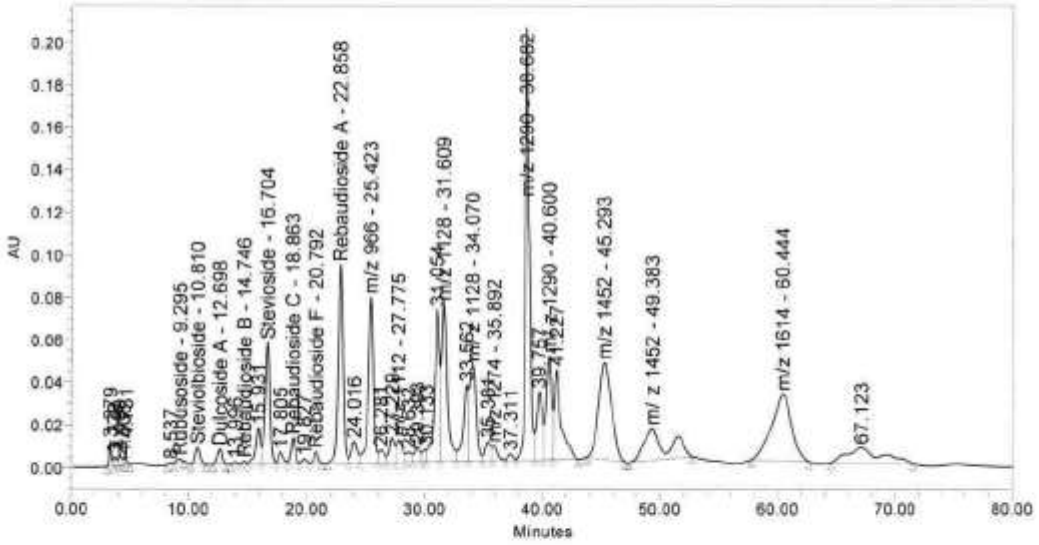
Issued by QA Department on May 14, 2019

Empower2 software

GRAS

SAMPLE INFORMATION

Sample Name:	STEVITEN RICH	Acquired By:	System
Lot:	190325	Sample Set Name:	GRAS
Vial:	5	Acq. Method Set:	G—AB—MS
Injection #:	1	Processing Method:	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	80.0 Minutes	Proc. Chnl. Descr.:	
Date Acquired:	2019-04-26 PM 11:21:03 KST		
Date Processed:	2019-04-29 PM 12:01:13 KST		



	Peak Name	RT	Area	% Area	Height
1		3.379	133644	0.35	11857
2		3.778	80137	0.21	4266
3		4.139	83044	0.22	5613
4		4.508	45980	0.12	3349
5		4.731	71251	0.19	10704
6		8.537	11095	0.03	619
7	Rubusoside	9.295	38771	0.10	1513
8	Steviolbioside	10.810	232489	0.61	7942
9	Dulcoside A	12.698	180489	0.47	6957
10		13.996	32052	0.08	1222
11	Rebaudioside B	14.746	38688	0.10	1573
12		15.931	498517	1.31	16883
13	Stevioside	16.704	1587273	4.16	56947

	Peak Name	RT	Area	% Area	Height
14		17.805	154304	0.40	5412
15	Rebaudioside C	18.863	309944	0.81	12001
16		19.827	52568	0.14	1972
17	Rebaudioside F	20.792	130689	0.34	5301
18	Rebaudioside A	22.858	2547693	6.68	93440
19		24.016	348180	0.91	10003
20	m/z 966	25.423	2338199	6.13	77649
21		26.281	201921	0.53	5702
22		27.229	341108	0.89	11361
23	m/z 1112	27.775	386820	1.01	10351
24		28.632	149757	0.39	4552
25		29.393	250600	0.66	6948
26		30.133	111345	0.29	5668

	Peak Name	RT	Area	% Area	Height
27		31.054	2199268	5.77	71460
28	m/z 1126	31.609	2503877	6.57	73896
29		33.562	987593	2.59	35399
30	m/ z 1128	34.070	1883592	4.94	44558
31		35.381	323411	0.85	8903
32	m/z 1274	35.892	212964	0.56	6894
33		37.311	87512	0.23	2967
34	m/z 1290	38.662	4666526	12.24	202904
35		39.757	1059739	2.78	31723
36	m/ z 1290	40.600	1569058	4.11	48417
37		41.227	1757023	4.61	41974
38	m/z 1452	45.293	3486809	9.14	45101
39	m/ z 1452	49.383	1949808	5.11	14668
40	m/z 1614	60.444	3612583	9.47	31008
41		67.123	1480633	3.88	7329

Appendix 5 Pesticide Testing Reports

SGS

Test Report No. F690101/LF-CTSAYFN18-36366E Issued Date : 2018. 12. 05 Page 1 of 2

DAEPYUNG CO., LTD
19-8, Yeongdong-gil,Hamchang-eup
Sangju-si,Gyeongsangbuk-do
Rep of KOREA

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

SGS File No.	: AYFN18-36366E
Product Name	: STEVIA EXTRACT
Item No./Lot No.	: Date of Receipt : 2018.11.15
Testing Period	: 2018. 11. 15 - 2018. 12. 05
Purpose of Test Report	: Data for reference
Test Items	: Pesticide 245. For the items, please refer to following page(s)
Test Method	: Analysis of hazardous substances in agricultural, GC and LC
Test Results	: 245 Not Detected

Notes 1) Not detected = ≤ LOQ (0.01mg/kg)
2) LOQ ; Limit of quantitation

Hyungsoo Jeon
Technical Manager / SGS KOREA

SGS

Test Report No. F690101/LF-CTSAYFN18-36366E Issued Date : 2018. 12. 05 Page 2 of 2

SGS File No. : AYFN18-36366E

Product Name : STEVIA EXTRACT

Pesticide 245 items

Acetamiprid, Acrinathrin, Alachlor, Aldicarb, Aldrin, Amisulbrom, Anilofos, Azinphos-methyl, Azoxystrobin, Bendiocarb, Benthiavalicarb-isopropyl, Benzoximate, BHC, Bifenox, Bifenthrin, Biteranol, Boscalid, Bromobutide, Bromopropylate, Buprofezin, Butachlor, Cadusafos, Captan, Carbaryl(NAC), Carbendazim, Carbofuran, Carbophenothion, Chinomethionat(Oxythioquinox), Chlorantraniliprole, Chlordane, Chlorfenapyr, Chlorfenvinphos, Chlorfluazuron, Chlorobenzilate, Chlorothalonil, Chlorpropham, Chlorpyrifos, Chlorpyrifos-methyl, Chromafenozide, Clofentezine, Clothianidin, Cyazofamid, Cyflufenamid, Cyfluthrin (beta), Cyhalofop-butyl, Cyhalothrin-lambda, Cymoxanil, Cypermethrin, Cyproconazole, Cyprodinil, DDT, Deltamethrin, Diazinon, Dichlofluanid, Dichlorvos/DDVP, Diclofop-methyl, Dicloran, Dicofol, Diethofencarb, Difenoconazole, Diflubenzuron, Dimepiperate, Dimethenamid, Dimethoate, Dimethomorph(E,Z), Dimethylvinphos(Z), Diniconazole, Diphenamid, Diphenylamine, Disulfoton, Dithiopyr, Diuron, Edifenphos, Endosulfan (alpha, beta,sulfate), Endrin(dieldrin), EPN, Esprocarb, Ethaboxam, Ethalfluralin, Ethiofencarb, Ethion, Ethoprophos, Ethoxazole, Etofenprox, Etridiazole, Etrimfos, Fenamidone, Fenamiphos, Fenarimol, Fenazaquin, Fenbuconazole, Fenitrothion : MEP, Fenobucarb, Fenothiocarb, Fenoxanil, Fenpropathrin, Fenpyroximate, Fenthion : MPP, Fenvalerate, Ferimzone, Fipronil, Fluacrypyrim, Flubendiamide, Flucythrinate, Fludioxonil, Flufenoxuron, Flumioxazine, Fluopicolide, Fluquinconazole, Flusilazole, Flutolanil, Folpet, Forchlorfenuron, Fosthiazate, Fthalide, Furathiocarb, Halfenprox, Heptachlor, Heptachlor epoxide, Hexaconazole, Hexaflumuron, Hexythiazox, Imazalil, Imibenconazole, Imidacloprid, Indanofan, Indoxacarb, Iprobenfos/IBP, Iprodione, Iprovalicarb, Isofenphos, Isoprocarb : MIPC, Isoprothiolane, Kresoxim-methyl, Lufenuron, Malathion, Mandipropamid, Mecarbam, Mefenacet, Mepanipyrim, Mepronil, Metalaxyl, Metamifop, Metconazole, Methabenzthiazuron, Methidathion, Methiocarb, Methomyl, Methoxychlor, Methoxyfenozide, Metobromuron, Metolachlor, Metolcarb, Metribuzin, Mevinphos, Molinate, Myclobutanil, Napropamide, Novaluron, Nuarimol, Ofurace, Oxadiazon, Oxamyl, Oxaziclomefon, Oxyfluorfen, Paclobutrazole, Parathion, Parathion-methyl, Penconazole, Pencycuron, Pendimethalin, Pentoxazone, Permethrin, Phenthoate:PAP, Phorate, Phosalone, Phosphamidone, Piperophos, Pirimicarb, Pirimiphos-ethyl, Pirimiphos-methyl, Probenazole, Prochloraz, Procymidone, Profenofos, Prometryn, Propanil, Propiconazole, Propoxur, Prothiofos, Pyraclofos, Pyraclostrobin, Pyrazophos, Pyribenzoxim, Pyributicarb, Pyridaben, Pyridalyl, Pyridaphenthion, Pyrimethanil, Pyrimidifen, Pyriminobac-methyl(E,Z), Pyriproxyfen, Pyroquilon, Quinoclamine, Quintozene(pentachloroaniline), Methyl pentachlorophenyl sulfide), Silafluofen, Simazine, Simeconazole, Simetryn, Spirodiclofen, Spiromesifen, Tebuconazole, Tebufenozide, Tebufenpyrad, Tebupirimfos, Teflubenzuron, Tefluthrin, Terbufos, Terbuthylazine, Terbutryn, Tetraconazole, Tetradifon, Thiabendazole, Thiacloprid, Thiamethoxam, Thiazopyr, Thifluzamide, Thiobencarb, Thiodicarb, Thiophanate-methyl, Tiadinil, Tolclofos-methyl, Tolylfluanid, Tralomethrin, Triadimefon, Triadimenol, Triazophos, Tricyclazole, Trifloxystrobin, Triflumizole, Triflumuron, Trifluralin, Uniconazole, Vinclozolin, Zoxamide

*** End of Report ***

Appendix 6 STEVITEN FRESH Stability Report

Report of Stability of STEVITEN FRESH

(Glucosylated Steviol Glycosides)

File No. : DP-R-ST-STEVITEN-FRESH (0)
Date : 11. Feb. 2019

Prepared by : R.K. Kim /R&D
Prepared by : S.J. Kim /QA
Approved by : K.J. Kim /President

DAEPYUNG CO., LTD.

Table 1. Summary of STEVITEN FRESH Stability Tests Completed or in Progress

Test Type	Measurement	Temperature(℃)	pH	Cycle Analysis	Planned Duration
Solid[1]	HPLC	25, 40	5	6 month	36 month
Solution[2]	HPLC	5, 25, 50	5	20hr	60hr
Water Solution[3]	HPLC	25	2, 5, 8	2day	6day

1. STEVITEN FRESH Product
2. 10% Water Solution
3. 1% Water Solution

Table 2. Summary of STEVITEN FRESH Stability Tests Completed or in Progress

Test Type	Parameter	Specification	Cycle Analysis	Planned Duration
Microbiology	Total Plate Count	≤ 1000 cfu/g	6 month	36 month
	Yeast and Mold	≤ 100 cfu/g		
	Salmonella	Negative		
	Total E. coli	≤ 10 mpn/g		

Table 3. Summary of STEVITEN FRESH Stability Result

* Assay : ≤95% Steviol glycosides and Glucosylstevioside
** Assay : Total content of ∝-Glucosyl steviol glycosides and unreacted steviol glycosides

1-1. Stability in Solid (25℃)

Initial(Month 0) - 25℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151127	95.3	4.7	83.6
151209	95.5	4.5	83.3
151225	95.5	4.5	83.4

Month 6 - 25℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151127	95.3	4.7	83.6
151209	95.4	4.6	83.4
151225	95.5	4.5	83.4

Month 12 - 25℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151127	95.4	4.6	83.5
151209	95.3	4.7	83.5
151225	95.3	4.7	83.4

Month 18 - 25℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151127	95.4	4.6	83.4
151209	95.4	4.6	83.2
151225	95.3	4.7	83.4

Month 24 - 25℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151127	95.5	4.5	83.3
151209	95.4	4.6	83.4
151225	95.4	4.6	83.4

Month 30 - 25℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151127	95.6	4.4	83.4
151209	95.5	4.5	83.3
151225	95.5	4.5	83.4

Month 36 - 25℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151127	95.4	4.6	83.4
151209	95.3	4.7	83.4
151225	95.4	4.6	83.3

1-2. Stability in Solid (40℃)

Initial(Month 0) - 40℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151127	95.4	4.6	83.4
151209	95.5	4.5	83.3
151225	95.3	4.7	83.3

Month 6 - 40℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151127	95.5	4.5	83.4
151209	95.5	4.5	83.5
151225	95.3	4.7	83.3

Month 12 - 40℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151127	95.4	4.6	83.4
151209	95.7	4.3	83.5
151225	95.4	4.6	83.3

Month 18 - 40℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151127	95.5	4.5	83.2
151209	95.4	4.6	83.5
151225	95.2	4.8	83.3

Month 24 - 40℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151127	95.5	4.5	83.2
151209	95.4	4.6	83.4
151225	95.4	4.6	83.4

Month 30 - 40℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151127	95.8	4.2	83.5
151209	95.4	4.6	83.2
151225	95.5	4.5	83.4

Month 36 - 40℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151127	95.5	4.5	83.4
151209	95.4	4.6	83.5
151225	95.4	4.6	83.5

2-1. Stability in Solution (10% Water Solution, 5 ℃)

Initial(o hr) - 5℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151127	95.5	4.5	83.2
151209	95.4	4.6	83.5
151225	95.3	4.7	83.5

20 hr - 5℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151127	95.5	4.5	83.2
151209	95.5	4.5	83.4
151225	95.5	4.5	83.4

40 hr - 5℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151127	95.5	4.5	83.4
151209	95.4	4.6	83.3
151225	95.6	4.4	83.5

60 hr - 5℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151127	95.5	4.5	83.4
151209	95.4	4.6	83.3
151225	95.6	4.4	83.5

2-2. Stability in Solution (10% Water Solution, 25 ℃)

Initial(o hr) - 25℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151127	95.8	4.2	83.5
151209	95.4	4.6	83.2
151225	95.5	4.5	83.4

20 hr - 25℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151127	95.4	4.6	83.4
151209	95.3	4.7	83.4
151225	95.4	4.6	83.3

40 hr - 25℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151127	95.5	4.5	83.4
151209	95.5	4.5	83.4
151225	95.4	4.6	83.3

60 hr - 25℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151127	95.4	4.6	83.4
151209	95.3	4.7	83.4
151225	95.4	4.6	83.3

2-3. Stability in Solution (10% Water Solution, 50 ℃)

Initial(o hr) - 50℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151127	95.6	4.4	83.5
151209	95.4	4.6	83.5
151225	95.4	4.6	83.3

20 hr - 50℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151127	95.4	4.6	83.4
151209	95.6	4.4	83.3
151225	95.4	4.6	83.3

40 hr - 50℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151127	95.3	4.7	83.4
151209	95.6	4.4	83.5
151225	95.4	4.6	83.3

60 hr - 50℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151127	95.4	4.6	83.4
151209	95.5	4.5	83.5
151225	95.4	4.6	83.5

3-1. Stability in pH(Water Solution, pH 2)

Initial(Day 0) – pH 2			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151127	95.4	4.6	83.4
151209	95.6	4.4	83.6
151225	95.5	4.5	83.5

Day 2 – pH 2			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151127	95.4	4.6	83.5
151209	95.4	4.6	83.4
151225	95.4	4.6	83.3

Day 4 – pH 2			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151127	95.3	4.7	83.3
151209	95.3	4.7	83.4
151225	95.4	4.6	83.3

Day 6 – pH 2			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151127	95.6	4.4	83.5
151209	95.6	4.4	83.4
151225	95.4	4.6	83.5

3-2. Stability in pH(Water Solution, pH 5)

Initial(Day 0) – pH 5			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151127	95.4	4.6	83.4
151209	95.5	4.5	83.5
151225	95.4	4.6	83.3

Day 2 – pH 5			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151127	95.6	4.4	83.4
151209	95.5	4.5	83.5
151225	95.4	4.6	83.5

Day 4 – pH 5			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151127	95.6	4.4	83.4
151209	95.6	4.4	83.4
151225	95.4	4.6	83.5

Day 6 – pH 5			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151127	95.6	4.4	83.4
151209	95.6	4.4	83.3
151225	95.5	4.5	83.5

3-3. Stability in pH(Water Solution , pH 8)

Initial(Day 0) – pH 8			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151127	95.6	4.4	83.4
151209	95.5	4.5	83.4
151225	95.5	4.5	83.5

Day 2 – pH 8			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151127	95.6	4.4	83.5
151209	95.5	4.5	83.4
151225	95.4	4.6	83.5

Day 4 – pH 8			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151127	95.6	4.4	83.4
151209	95.6	4.4	83.5
151225	95.5	4.5	83.5

Day 6 – pH 8			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151127	95.3	4.7	83.3
151209	95.5	4.5	83.4
151225	95.5	4.5	83.5

Table 4. Summary of STEVITEN FRESH Stability Result (Microbiology)

1.1 Total Plate Count

Initial(Month 0)		
Lot	Result(cfu/g)	Remarks
151127	0	
151209	0	
151225	0	

Initial(Month 6)		
Lot	Result(cfu/g)	Remarks
151127	0	
151209	0	
151225	0	

Initial(Month 12)		
Lot	Result(cfu/g)	Remarks
151127	0	
151209	0	
151225	0	

Initial(Month 18)		
Lot	Result(cfu/g)	Remarks
151127	0	
151209	0	
151225	0	

Initial(Month 24)		
Lot	Result(cfu/g)	Remarks
151127	0	
151209	0	
151225	0	

Initial(Month 30)		
Lot	Result(cfu/g)	Remarks
151127	0	
151209	0	
151225	0	

Initial(Month 36)		
Lot	Result(cfu/g)	Remarks
151127	0	
151209	0	
151225	0	

1.2 Yeast and Mold

Initial(Month 0)		
Lot	Result(cfu/g)	Remarks
151127	0	
151209	0	
151225	0	

Initial(Month 6)		
Lot	Result(cfu/g)	Remarks
151127	0	
151209	0	
151225	0	

Initial(Month 12)		
Lot	Result(cfu/g)	Remarks
151127	0	
151209	0	
151225	0	

Initial(Month 18)		
Lot	Result(cfu/g)	Remarks
151127	0	
151209	0	
151225	0	

Initial(Month 24)		
Lot	Result(cfu/g)	Remarks
151127	0	
151209	0	
151225	0	

Initial(Month 30)		
Lot	Result(cfu/g)	Remarks
151127	0	
151209	0	
151225	0	

Initial(Month 36)		
Lot	Result(cfu/g)	Remarks
151127	0	
151209	0	
151225	0	

1.3 Salmonella

Initial(Month 0)		
Lot	Result	Remarks
151127	Negative	
151209	Negative	
151225	Negative	

Initial(Month 6)		
Lot	Result	Remarks
151127	Negative	
151209	Negative	
151225	Negative	

Initial(Month 12)		
Lot	Result	Remarks
151127	Negative	
151209	Negative	
151225	Negative	

Initial(Month 18)		
Lot	Result	Remarks
151127	Negative	
151209	Negative	
151225	Negative	

Initial(Month 24)		
Lot	Result	Remarks
151127	Negative	
151209	Negative	
151225	Negative	

Initial(Month 30)		
Lot	Result	Remarks
151127	Negative	
151209	Negative	
151225	Negative	

Initial(Month 36)		
Lot	Result	Remarks
151127	Negative	
151209	Negative	
151225	Negative	

1.4 Total E. coli

Initial(Month 0)		
Lot	Result(mpn/g)	Remarks
151127	0	
151209	0	
151225	0	

Initial(Month 6)		
Lot	Result(mpn/g)	Remarks
151127	0	
151209	0	
151225	0	

Initial(Month 12)		
Lot	Result(mpn/g)	Remarks
151127	0	
151209	0	
151225	0	

Initial(Month 18)		
Lot	Result(mpn/g)	Remarks
151127	0	
151209	0	
151225	0	

Initial(Month 24)		
Lot	Result(mpn/g)	Remarks
151127	0	
151209	0	
151225	0	

Initial(Month 30)		
Lot	Result(mpn/g)	Remarks
151127	0	
151209	0	
151225	0	

Initial(Month 36)		
Lot	Result(mpn/g)	Remarks
151127	0	
151209	0	
151225	0	

Conclusion:

samples as i) STEVITEN FRESH, ii) 10% water solution iii) 1% water solution are tested under the condition described above.

i) STEVITEN FRESH : it shows stable. We can see a little change under 25℃ and 40℃ at PH 5 but it is not significant

ii) 10% water solution : it shows stable. We can see a little change under 50℃ comparing with 5℃ and 25℃ but it is not significant.

iii) 1% water solution: it shows stable. We can see a little change under PH 2, 5 and 8 at 25℃ but it is not significant

iv) Microbiology test: There are no contaminations. All test results are shown as zero and negative.

Appendix 7 STEVITEN RICH Stability Report

Report of Stability of STEVITEN RICH

(Glucosylated Steviol Glycosides)

File No. : DP-R-ST-STEVITEN-RICH (0)
Date : 11. Feb. 2019

Prepared by : R.K. Kim /R&D
Prepared by : S.J. Kim /QA
Approved by : K.J. Kim /President

DAEPYUNG CO., LTD.

Table 1. Summary of STEVITEN RICH Stability Tests Completed or in Progress

Test Type	Measurement	Temperature(℃)	pH	Cycle Analysis	Planned Duration
Solid[1]	HPLC	25, 40	5	6 month	36 month
Solution[2]	HPLC	5, 25, 50	5	20hr	60hr
Water Solution[3]	HPLC	25	2, 5, 8	2day	6day

1. STEVITEN RICHProduct
2. 10% Water Solution
3. 1% Water Solution

Table 2. Summary of STEVITEN RICH Stability Tests Completed or in Progress

Test Type	Parameter	Specification	Cycle Analysis	Planned Duration
Microbiology	Total Plate Count	≤ 1000 cfu/g	6 month	36 month
	Yeast and Mold	≤ 100 cfu/g		
	Salmonella	Negative		
	Total E. coli	≤ 10 mpn/g		

Table 3. Summary of STEVITEN RICH Stability Result

* Assay : ≤95% Steviol glycosides and Glucosylstevioside
** Assay : Total content of ∝-Glucosyl steviol glycosides and unreacted steviol glycosides

1-1. Stability in Solid (25℃)

Initial(Month 0) - 25℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151130	95.6	4.4	92.5
151218	95.4	4.6	92.4
151223	95.4	4.6	92.6

Month 6 - 25℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151130	95.6	4.4	92.5
151218	95.4	4.6	92.4
151223	95.4	4.6	92.4

Month 12 - 25℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151130	95.6	4.4	92.5
151218	95.5	4.5	92.5
151223	95.4	4.6	92.6

Month 18 - 25℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151130	95.5	4.5	92.4
151218	95.5	4.5	92.4
151223	95.5	4.5	92.4

Month 24 - 25℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151130	95.5	4.5	92.5
151218	95.6	4.4	92.4
151223	95.5	4.5	92.4

Month 30 - 25℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151130	95.4	4.6	92.3
151218	95.5	4.5	92.4
151223	95.5	4.5	92.4

Month 36 - 25℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151130	95.4	4.6	92.3
151218	95.5	4.5	92.4
151223	95.4	4.6	92.5

1-2. Stability in Solid (40℃)

Initial(Month 0) - 40℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151130	95.4	4.6	92.4
151218	95.5	4.5	92.4
151223	95.5	4.5	92.5

Month 6 - 40℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151130	95.4	4.6	92.4
151218	95.6	4.4	92.5
151223	95.5	4.5	92.5

Month 12 - 40℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151130	95.4	4.6	92.4
151218	95.5	4.5	92.4
151223	95.5	4.5	92.5

Month 18 - 40℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151130	95.5	4.5	92.5
151218	95.5	4.5	92.4
151223	95.5	4.5	92.5

Month 24 - 40℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151130	95.5	4.5	92.5
151218	95.4	4.6	92.4
151223	95.5	4.5	92.4

Month 30 - 40℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151130	95.6	4.4	92.5
151218	95.6	4.4	92.4
151223	95.5	4.5	92.4

Month 36 - 40℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151130	95.6	4.4	92.5
151218	95.5	4.5	92.3
151223	95.3	4.7	92.4

2-1. Stability in Solution (10% Water Solution, 5 ℃)

Initial(o hr) - 5℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151130	95.5	4.5	92.4
151218	95.5	4.5	92.3
151223	95.4	4.6	92.4

20 hr - 5℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151130	95.4	4.6	92.5
151218	95.5	4.5	92.3
151223	95.4	4.6	92.4

40 hr - 5℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151130	95.4	4.6	92.4
151218	95.5	4.5	92.4
151223	95.5	4.5	92.5

60 hr - 5℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151130	95.4	4.6	92.4
151218	95.6	4.4	92.3
151223	95.5	4.5	92.5

2-2. Stability in Solution (10% Water Solution, 25 ℃)

Initial(o hr) - 25℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151130	95.4	4.6	92.4
151218	95.5	4.5	92.4
151223	95.4	4.6	92.4

20 hr - 25℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151130	95.4	4.6	92.4
151218	95.5	4.5	92.3
151223	95.4	4.6	92.4

40 hr - 25℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151130	95.4	4.6	92.3
151218	95.5	4.5	92.4
151223	95.4	4.6	92.5

60 hr - 25℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151130	95.3	4.7	92.1
151218	95.5	4.5	92.4
151223	95.4	4.6	92.5

2-3. Stability in Solution (10% Water Solution, 50 ℃)

Initial(o hr) - 50℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151130	95.4	4.6	92.3
151218	95.6	4.4	92.5
151223	95.4	4.6	92.5

20 hr - 50℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151130	95.4	4.6	92.5
151218	95.4	4.6	92.4
151223	95.4	4.6	92.5

40 hr - 50℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151130	95.4	4.6	92.6
151218	95.5	4.5	92.4
151223	95.3	4.7	92.5

60 hr - 50℃			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151130	95.4	4.6	92.5
151218	95.5	4.5	92.4
151223	95.4	4.6	92.5

3-1. Stability in pH(Water Solution, pH 2)

Initial(Day 0) – pH 2			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151130	95.4	4.6	92.4
151218	95.5	4.5	92.4
151223	95.5	4.5	92.5

Day 2 – pH 2			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151130	95.5	4.5	92.3
151218	95.5	4.5	92.4
151223	95.5	4.5	92.5

Day 4 – pH 2			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151130	95.4	4.6	92.3
151218	95.3	4.7	92.5
151223	95.4	4.6	92.5

Day 6 – pH 2			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151130	95.4	4.6	92.3
151218	95.6	4.4	92.4
151223	95.4	4.6	92.5

3-2. Stability in pH(Water Solution, pH 5)

Initial(Day 0) – pH 5			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151130	95.6	4.4	92.6
151218	95.5	4.5	92.4
151223	95.3	4.7	92.5

Day 2 – pH 5			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151130	95.6	4.4	92.5
151218	95.5	4.5	92.4
151223	95.4	4.6	92.5

Day 4 – pH 5			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151130	95.4	4.6	92.3
151218	95.5	4.5	92.5
151223	95.3	4.7	92.5

Day 6 – pH 5			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151130	95.4	4.6	92.4
151218	95.5	4.5	92.5
151223	95.6	4.4	92.5

3-3. Stability in pH(Water Solution , pH 8)

Initial(Day 0) – pH 8			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151130	95.5	4.5	92.4
151218	95.5	4.5	92.3
151223	95.6	4.4	92.5

Day 2 – pH 8			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151130	95.4	4.6	92.6
151218	95.5	4.5	92.5
151223	95.6	4.4	92.5

Day 4 – pH 8			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151130	95.5	4.5	92.6
151218	95.5	4.5	92.5
151223	95.6	4.4	92.5

Day 6 – pH 8			
Measurement	HPLC		UV / HPLC
Lot	Assay*		Assay**
	Steviol glycosides and Glucosylstevioside(%)	Non-Steviosides(%)	
151130	95.4	4.6	92.4
151218	95.4	4.6	92.4
151223	95.6	4.4	92.5

Table 4. Summary of STEVITEN RICHStability Result (Microbiology)

1.1 Total Plate Count

Initial(Month 0)		
Lot	Result(cfu/g)	Remarks
151130	0	
151218	0	
151223	0	

Initial(Month 6)		
Lot	Result(cfu/g)	Remarks
151130	0	
151218	0	
151223	0	

Initial(Month 12)		
Lot	Result(cfu/g)	Remarks
151130	0	
151218	0	
151223	0	

Initial(Month 18)		
Lot	Result(cfu/g)	Remarks
151130	0	
151218	0	
151223	0	

Initial(Month 24)		
Lot	Result(cfu/g)	Remarks
151130	0	
151218	0	
151223	0	

Initial(Month 30)		
Lot	Result(cfu/g)	Remarks
151130	0	
151218	0	
151223	0	

Initial(Month 36)		
Lot	Result(cfu/g)	Remarks
151130	0	
151218	0	
151223	0	

1.2 Yeast and Mold

Initial(Month 0)		
Lot	Result(cfu/g)	Remarks
151130	0	
151218	0	
151223	0	

Initial(Month 6)		
Lot	Result(cfu/g)	Remarks
151130	0	
151218	0	
151223	0	

Initial(Month 12)		
Lot	Result(cfu/g)	Remarks
151130	0	
151218	0	
151223	0	

Initial(Month 18)		
Lot	Result(cfu/g)	Remarks
151130	0	
151218	0	
151223	0	

Initial(Month 24)		
Lot	Result(cfu/g)	Remarks
151130	0	
151218	0	
151223	0	

Initial(Month 30)		
Lot	Result(cfu/g)	Remarks
151130	0	
151218	0	
151223	0	

Initial(Month 36)		
Lot	Result(cfu/g)	Remarks
151130	0	
151218	0	
151223	0	

1.3 Salmonella

Initial(Month 0)		
Lot	Result	Remarks
151130	Negative	
151218	Negative	
151223	Negative	

Initial(Month 6)		
Lot	Result	Remarks
151130	Negative	
151218	Negative	
151223	Negative	

Initial(Month 12)		
Lot	Result	Remarks
151130	Negative	
151218	Negative	
151223	Negative	

Initial(Month 18)		
Lot	Result	Remarks
151130	Negative	
151218	Negative	
151223	Negative	

Initial(Month 24)		
Lot	Result	Remarks
151130	Negative	
151218	Negative	
151223	Negative	

Initial(Month 30)		
Lot	Result	Remarks
151130	Negative	
151218	Negative	
151223	Negative	

Initial(Month 36)		
Lot	Result	Remarks
151130	Negative	
151218	Negative	
151223	Negative	

1.4 Total E. coli

Initial(Month 0)		
Lot	Result(mpn/g)	Remarks
151130	0	
151218	0	
151223	0	

Initial(Month 6)		
Lot	Result(mpn/g)	Remarks
151130	0	
151218	0	
151223	0	

Initial(Month 12)		
Lot	Result(mpn/g)	Remarks
151130	0	
151218	0	
151223	0	

Initial(Month 18)		
Lot	Result(mpn/g)	Remarks
151130	0	
151218	0	
151223	0	

Initial(Month 24)		
Lot	Result(mpn/g)	Remarks
151130	0	
151218	0	
151223	0	

Initial(Month 30)		
Lot	Result(mpn/g)	Remarks
151130	0	
151218	0	
151223	0	

Initial(Month 36)		
Lot	Result(mpn/g)	Remarks
151130	0	
151218	0	
151223	0	

Conclusion:

samples as i) STEVITEN FRESH, ii) 10% water solution iii) 1% water solution are tested under the condition described above.

i) STEVITEN RICH: it shows stable. We can see a little change under 25℃ and 40℃ at PH 5 but it is not significant

ii) 10% water solution : it shows stable. We can see a little change under 50℃ comparing with 5℃ and 25℃ but it is not significant.

iii) 1% water solution: it shows stable. We can see a little change under PH 2, 5 and 8 at 25℃ but it is not significant

iv) Microbiology test: There are no contaminations. All test results are shown as zero and negative.

Appendix 8 Estimated Daily Intake Levels of Steviol Glycosides Preparations

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 Notification. Cargill (2008) estimated the possible daily intake of rebaudioside A assuming the use levels would be comparable to aspartame and (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.

B. 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 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.

C. 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).

D. 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.

E. 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.

F. 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).

G. 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.”

H. 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).

Appendix 9 Studies on Steviol Glycosides Preparations

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

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

1. Animal Studies

- 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);
  - Chickens – Geuns et al. (2003b);
  - Hamsters – Hutapea et al. (1999);
  - Pigs – Geuns et al. (2003a)
- *In vitro* metabolism studies show stevia 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).
- Koyama et al. (2003b) showed steviol can be converted to various glucuronides.
- 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).
- 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.
- 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 a 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 acute toxicity of stevioside (96% pure) is presented in Table 9.1.

Table 9.1. 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)

No lethality was noted within 14 days after the 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

- 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.
- Mitsuhashi (1976) added up to 7% stevioside to the diets of F344 rats for 3 months and report no adverse effects.
- Akashi and Yokoyama (1975) dosed rats with up to 2,500 mg per kg bw per day for 3 months and reported no adverse effects.
- The Awney et al. (2011) study revealed toxicity in rats dosed at 15 and 1,500 mg per kg, 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;
    - 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

- 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 gains decreased in both sexes. Kidney weights were significantly lower in 5% males; ovary, kidney and brain weights were significantly increased in 5% females and there were decreased survival rates in males receiving 5%. However, stevioside was not carcinogenic at any level. The apparent NOAEL was the dietary level of 2.5%.
- 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%).
- 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) 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.
- No treatment-related increase in tumor incidence was seen in any of these studies.

E. Reproductive & Developmental Toxicity Studies

- 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).
- 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 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 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.
- 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.

- 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 50-60 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).
- A developmental study of 90% steviol in hamsters at 0, 250, 500, 750, or 1,000 mg per kg bw per day on days 6-10 of gestation resulted in a significant decrease in body weight gain and increased mortality (1/20, 7/20, and 5/12) at the three highest doses. No dose-dependent teratogenic effects were observed. The no observed effect level (NOEL) was 250 mg per kg bw per day for both maternal and developmental toxicity (Wasuntarawat et al., 1998).

F. Mutagenicity & Genotoxicity Studies

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

- Bacterial mutagenicity studies negative for mutagenic response:
  - Matsui et al. (1996)
  - Suttajit et al. (1993)
  - Klongpanichpak et al. (1997)
  - Matsui et al. (1996)
  - Pezzuto et al. (1985)
  - Medon et al. (1982)
- 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)
  - Sasaki et al. (2002)
- Mouse bone marrow/liver micronucleus studies negative for mutagenic response:
  - Oh et al. (1999)

One study was found to be positive and was conducted by Nunes et al. (2007a). The Nunes study revealed toxicity and was conducted on rat liver, brain, and spleen on rats dosed 4 mg per mL steviol glycosides in drinking water (estimated 80 to 500 mg per kg bw per day) for 45 days, which resulted in positive findings in all tissues – notably the liver. This study is considered to be an outlier in critical reviews conducted by Geuns (2007), Williams (2007), and Brusick (2008). These critiques were responded to by the authors (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.

- In two separate reviews by Carakostas et al. (2008) and Brusick (2008), the recent 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. Clinical Studies & Other Reports in Humans

In several studies, pharmacological and biochemical effects of crude extracts of stevia leaves and purified steviol glycosides have been investigated. The effects noted included glucose uptake, insulin secretion, and blood pressure. 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 of 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 9.2.

Table 9.2: 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

Author/ Year	Substance Tested	Total Daily Dose	Population Characteristics	Study Design and Duration	Noted Effects Safety parameter Results
					during the test and after overnight fasting in all volunteers.
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) + 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) + 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 ↓ 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 and 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
Gregersen et al. (2004)	Stevioside - 91% + 9% other stevia glycosides	Single dose 1 g stevioside or 1 g starch	12 patients with type 2 diabetes total	Acute paired cross-over study	↓18% glucose concentrations: Systolic and diastolic blood pressure unchanged. No adverse effects
Temme et al.	Stevioside 97%	750 mg/kg bw/day	4 male 5 female healthy	Short term study – 3	Blood chemistry, blood

AUTHOR/ YEAR	SUBSTANCE TESTED	TOTAL DAILY DOSE	POPULATION CHARACTERISTICS	STUDY DESIGN AND DURATION	NOTED EFFECTS SAFETY PARAMETER RESULTS
(2004)		(288 mg/kg bw steviol)	patients	days	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, Type 2 , 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 weeks 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	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)	1500 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

Part 2. Preparations That Are Primarily Rebaudioside A

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

1. Animal 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.

- 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).

- *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.
- 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} for 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.
- Sloter (2008a) examined the potential of rebaudioside A toxicity in rats up to 2,000 mg per kg bw per day

- Low levels of rebaudioside A were detected in peripheral blood of rats post administration of 2,000 mg per kg bw per day.
- Mean plasma concentrations of rebaudioside A of 0.6 µg per mL in plasma resulting in an estimated absorbed dose of 0.02% based on amounts calculated from urine collection.
- Mean fecal rebaudioside A and measured hydrolysis products, expressed as Total Rebaudioside A Equivalents, compared to daily administered dose results in an estimated dose recovery of approximately 84%.

2. 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 males and 11,728 mg per kg 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 Reb 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).

3. Mutagenicity & 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)
  - Rumelhard et al. (2016)
- Mouse lymphoma (L5178Y/TK+/) studies negative for mutagenic response:

  - Clarke (2006)
  - Williams and Burdock (2009)
  - Human lymphocyte study negative for mutagenic response: Rumelhard et al. (2016)
- Chromosome aberration studies negative for mutagenic response:
  - Human lymphocytes – Williams and Burdock (2009)
  - Chinese hamster lung fibroblasts – Nakajima (2000a)
- Mouse micronucleus studies negative for mutagenic response:
  - Krsmanovic and Huston (2006)
  - Williams and Burdock (2009)
  - Nakajima (2000b) (BDF1 mouse bone marrow)
  - 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)

4. Reproductive & Developmental Studies

- Curry et al. (2008) conducted a 2-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.

5. Clinical Studies on Rebaudioside A

A summary of the clinical studies conducted on rebaudioside A is presented in Table 9.3.

Table 9.3. 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	100 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	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

6. Safety of Rebaudioside A

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

- GRAS notifications 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 deemed to be safe as long as there is a combined purity of 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 slight 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 on 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 less 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
  - 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 Principal Metabolite: Steviol

A. Acute Toxicity Studies

- Toskulkac et al. (1997) administered single doses of steviol (90%) to various animals as follows:

- Rat, oral LD_{50} >15 g per kg
- 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 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) dosed groups of pregnant golden hamsters steviol (90%) at 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:
  - Klongpanichpak et al. (1997)
  - Procinska et al. (1991)
  - Compadre et al. (1988)
- 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:
  - Temcharoen et al. (2000) (rat)
  - Temcharoen et al. (2000) (mouse)
  - Matsui et al. (1996) (mouse)
  - 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:
    - 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
    - Terai et al. (2002) – Steviol was found to be mutagenic in Aroclor-induced rat liver S9 fraction.
    - 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.
    - 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.
- 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
    - 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.

Appendix 10 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 as a natural health product 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 was 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

To date, FDA has issued 57 “no questions” letters on GRAS Notices on rebaudioside A, rebaudioside D, rebaudioside M, or steviol glycosides, including those undergoing enzyme treatment (FDA, 2019).

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 10.1.

Table 10.1. 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)

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 (2017a) 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, 2017b).
- 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, 2019b).
- Most recently, 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, 2019a).

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 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 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 2009, France published its approval for the food uses of rebaudioside A with a purity of 97% (AFSSA, 2009a; AFSSA, 2009b).
- 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), 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).
- 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 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% Reb A or stevioside.
- 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 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).

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 (NewHope360, 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)

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 Reb M does not pose any “public health and safety issues” (FSANZ, 2015).
- On January 14, 2016, Reb M was approved for use “as a food additive in accordance with the current permissions for steviol glycosides” (FSANZ, 2016a).

- 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, 2016b).
- 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).

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).

Appendix 11 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 2-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 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 up to 200 mg per kg and in plain soy beverages up 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, 2016a).

- 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 reevaluate 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 2-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.

Appendix 12 GRAS Associates Expert Panel Report

The Generally Recognized as Safe (GRAS) Status of the Proposed Uses of High Purity Glucosylated Steviol Glycosides Preparations

July 16, 2019

Foreword

An independent panel of experts ("Expert Panel") was convened by GRAS Associates, LLC on behalf of their client, Daepyung Co., Ltd. (hereinafter "Daepyung"), to evaluate the safety and Generally Recognized as Safe (GRAS) status of Daepyung's proposed uses of STEVITEN FRESH and STEVITEN RICH 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 is generally available, and has been discussed in Part 6, as well as Appendices 9-11, of Daepyung's 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. The composite evidence from historical safe consumption and experimental studies collectively demonstrate the safety of the subject enzyme modified high purity steviol glycosides preparations for human food consumption.

The majority of the studies reviewed on steviol glycosides and steviol have been discussed in detail in previous GRAS submissions, including GRN 555, GRN 548, and GRN 536.

With regard to the safety documentation, the key pharmacokinetic data establish that steviol glycosides are not absorbed through the 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

[†] 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.

homogenates (Koyama et al., 2003a; 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 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). Recently, Roberts et al. (2016) noted in a persuasive argument using a chemical-specific adjustment factor (CSAF) that the ADI could be higher. 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 to 57 GRN submissions for steviol glycosides preparations, including 7 notifications regarding enzyme modified steviol glycosides.

The Expert Panel notes that Daepyung's STEVITEN FRESH is composed of 80-90% total unreacted steviol glycosides and glucosylated steviol glycosides, with the remaining 10-20% of the finished product comprised of unreacted maltodextrin. Similarly, Daepyung's STEVITEN RICH is composed of 85-95% total unreacted steviol glycosides and glucosylated steviol glycosides, with the remaining 5-15% of the finished product comprised of unreacted maltodextrin. Since the steviol glycosides extract raw material meets the minimum 95% total steviol glycosides specifications set forth by JECFA, and the finished STEVITEN products are a blend of the steviol glycosides and glucosylated steviosides derived from the raw material and unreacted maltodextrin, which in itself is GRAS, the Expert Panel has no safety concerns with the reported composition of the STEVITEN FRESH or STEVITEN RICH finished products.

The Expert Panel notes that Daepyung's manufacturing process for their high purity glucosylated glycoside 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, and GRN 662. The updated scientific literature review of steviol glycosides covering the time frame since GRN 662 was submitted through the present revealed no findings raising new safety concerns that would alter the previous GRAS determinations for similar enzyme modified steviol glycosides preparations.

The GRAS Associates Expert Panel convened on behalf of Daepyung has reviewed the proposed uses for STEVITEN FRESH and STEVITEN RICH. The highest 90th percentile consumption by any population subgroup of STEVITEN was calculated to be approximately 9.90 mg per kg bw per day (for STEVITEN FRESH), which is equivalent to 3.82 mg per kg bw per day steviol equivalents (calculated by a weighted sum estimate) for any population group, on a worst-case scenario basis. This estimated intake value is below the JECFA ADI of 4 mg per kg bw per day expressed as steviol equivalents. Therefore, STEVITEN FRESH and STEVITEN RICH 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,

END

Report of Chemical Characterization of Glucosylated Steviol Glycosides (STEVITEN FRESH)

File No. : DP-R-AD-STEVITEN-FRESH (0)
Date : 25. Feb. 2019

Prepared by : R.K. Kim /R&D
Prepared by : S.J. Kim /QA
Approved by : K.J. Kim /President

DAEPYUNG CO., LTD.

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1. Analysis of Glucosylated Steviol Glycosides by HPLC

Methods

HPLC Conditions

Instrument : Alliance 2695-2487(Waters)
Column : TOSOH, TSKgel Amide-80(250X4.6mm, 5um)
Column Temp : 30℃
Flow Rate : 0.85mL/min
Injection Volume : 20uL
Detection : uv@210nm
Mobile Phase A: Acetonitrile / 5%Water
B: Water / 5%Acetonitrile

• Gradient

Time(min)	% A	% B	Curve
0	89	11	-
31	77	23	6
33	77	23	6
34	68	32	6
35	77	23	6
37	70	30	6
38	77	23	6
70	77	23	6
80	89	11	6

Sample Preparation

Enzyme Treated Stevia extracts were prepared in 50% Acetonitrile/Water at a concentration of 20mg/mL.
Stevia extracts were prepared in 50% Acetonitrile/Water at a concentration of 20mg/mL.

Standard Preparation

Stevioside standard and Rebaudioside A standard was prepared in 50% Acetonitrile/Water at the following concentrations:

Stevioside 5, 10, 15, 20mg/mL
Rebaudioside A 5, 10, 15, 20mg/mL

Glucosylated Steviol Glycoside Components

Compound	m.w.	Empirical Formula	Level of Enzyme Glycosylation
Rubusoside	642	$C_{32}H_{50}O_{13}$	-
Steviolbioside	642	$C_{32}H_{50}O_{13}$	-
Dulcoside A	788	$C_{38}H_{60}O_{17}$	-
Rebaudioside B	804	$C_{38}H_{60}O_{18}$	-
Stevioside	804	$C_{38}H_{60}O_{18}$	-
Rebaudioside C	950	$C_{44}H_{70}O_{22}$	-
Rebaudioside F	936	$C_{43}H_{68}O_{22}$	-
Rebaudioside A	966	$C_{44}H_{70}O_{23}$	-
Rebaudioside D	1129	$C_{50}H_{80}O_{28}$	-
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
Diglucosyl Stevioside	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 Stevioside	1290	$C_{56}H_{90}O_{33}$	+3
Triglucosyl Rebaudioside A	1452	$C_{62}H_{100}O_{38}$	+3
Tetraglucosyl Stevioside	1452	$C_{62}H_{100}O_{38}$	+4
Tetraglucosy Rebaudioside A	1614	$C_{68}H_{110}O_{43}$	+4

Results

Table 1. Determination of the level of non-steviol glycosides components, by percent, in an enzyme treated stevia sample

The non- stevioside components were found to elute early in the analysis with retention times between 3.73 and 8.44 minutes. The chromatographic Purities of the samples were determined by normalizing the total peak area to 100%

1) Lot. 181128

Peak NO.	RT(min)	Area (%)	Total (%)
1	3.752	0.73	
2	4.148	1.54	
3	4.747	1.45	
4	6.418	0.21	
5	8.430	0.43	4.36

2) Lot. 181213

Peak NO.	RT(min)	Area (%)	Total (%)
1	3.737	0.73	
2	4.145	1.51	
3	4.746	1.38	
4	6.429	0.19	
5	8.437	0.42	4.23

3) Lot. 181219

Peak NO.	RT(min)	Area (%)	Total (%)
1	3.735	0.63	
2	4.145	1.53	
3	4.747	1.37	
4	6.448	0.19	
5	8.440	0.42	4.14

Table 2. The levels of Steviosides, by percent range, in non-enzyme treated and enzyme-treated stevia extracts.

To ensure product consistency, the level of stevioside and glucosylstevioside components was determined by HPLC analysis. The ranges determined for pre treated and post treated stevia extracts are found in Table 2

Component	Starting Material (%)	STEVITEN FRESH (%)
Rubusoside	2.0 ~ 3.5	0.0 ~ 0.6
Steviolbioside	0.0 ~ 1.0	0.5 ~ 1.5
Dulcoside A	0.0 ~ 1.0	0.5 ~ 1.5
Rebaudioside B	0.0 ~ 1.0	0.0 ~ 1.0
Stevioside	46.0 ~ 50.0	8.0 ~ 10.0
Rebaudioside C	5.5 ~ 6.5	2.0 ~ 3.0
Rebaudioside F	0.5 ~ 1.0	0.0 ~ 0.5
Rebaudioside A	35.0 ~ 40.0	7.0 ~ 9.0
Rebaudioside D	0.0 ~ 0.5	n.d
Monoglucosyl stevioside m/z 966	n.d	9.0 ~ 11.0
Monoglucosyl rebaudioside C m/z 1112	n.d	2.0 ~ 2.6
Monoglucosyl rebaudioside A m/z 1128	n.d	7.0 ~ 8.0
Diglucosyl stevioside m/z 1128	n.d	9.0 ~ 11.0
Diglucosyl rebaudioside C m/z 1274	n.d	1.5 ~ 2.5
Diglucosyl rebaudioside A m/z 1290	n.d	8.0 ~ 11.0
Triglucosy stevioside m/z 1290	n.d	8.0 ~ 13.0
Triglucosyl rebaudioside A m/z 1452	n.d	3.5 ~ 5.0
Tetraglucosyl stevioside m/z 1452	n.d	7.0 ~ 8.5
Tetraglucosy rebaudioside A m/z 1614	n.d	2.5 ~ 3.5
Unidenfied glucosylated m/z > 1614	n.d	5.0 ~ 6.0

Table 3. The level of steviol glycoside and non-stevioside components, by percent, in three lots of stevia extract before enzyme treatment.

The meaning 2.3 , 2.3 & 2.0 of Stevia extract (impurities) % are that steviol glydosides are min 95% Also the meaning of 4.4, 4.2 & 4.1 of Enzyme treated Stevia Extract (impurities) % are that steviol glydosides and Glucosylsteviosides (%) have been found to have greater than 95% purity

Therefore, the Glucosylated steviol Extract impurites(%)components were found to be present on levels less than 5%.

Lot.	Starting material impurities(%)	STEVITEN FRESH impurites(%)
181128	2.3	4.4
181213	2.3	4.2
181219	2.0	4.1

Table 4. The level of steviosides and non-steviosides, by percent, in three lots of enzyme treated stevia.

The same three lots of stevia extract were then enzyme treated, and subsequently tested for chromatographic purity on a molar basis. The results were obtained using the same method, and are shown in table 4. All Three enzyme treated stevia lots have been found to have greater than 95% purity, as demonstrated by HPLC chromatographic analysis.

Lot	Steviol glycosides and Glucosylsteviosides (%)	Glucosylated steviol glycosides impurites(%)
181128	95.6	4.4
181213	95.8	4.2
181219	95.9	4.1

Appendix A
Raw Data

Figure 1. HPLC Analysis of Stevia Extract Extract Before and after Enzyme Treatment

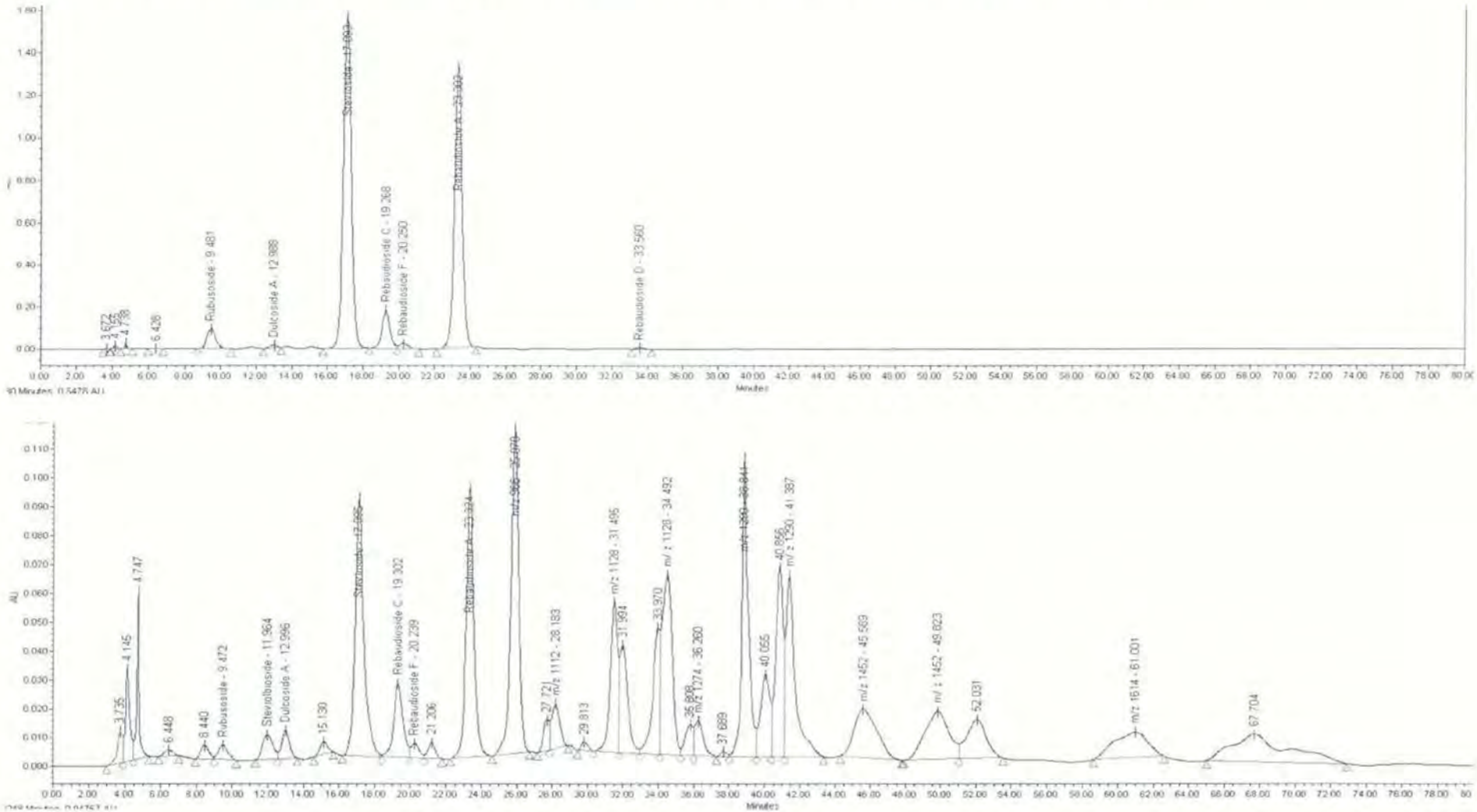
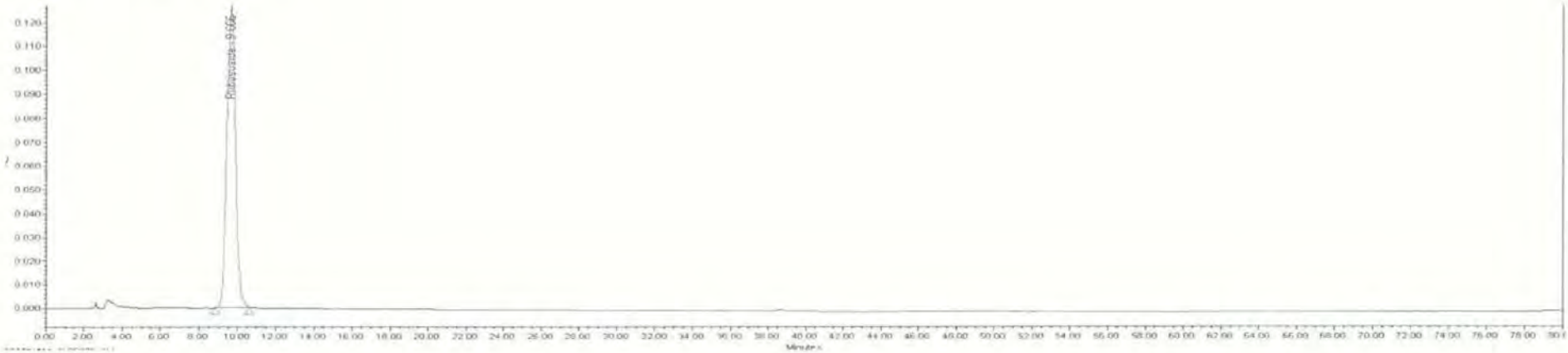
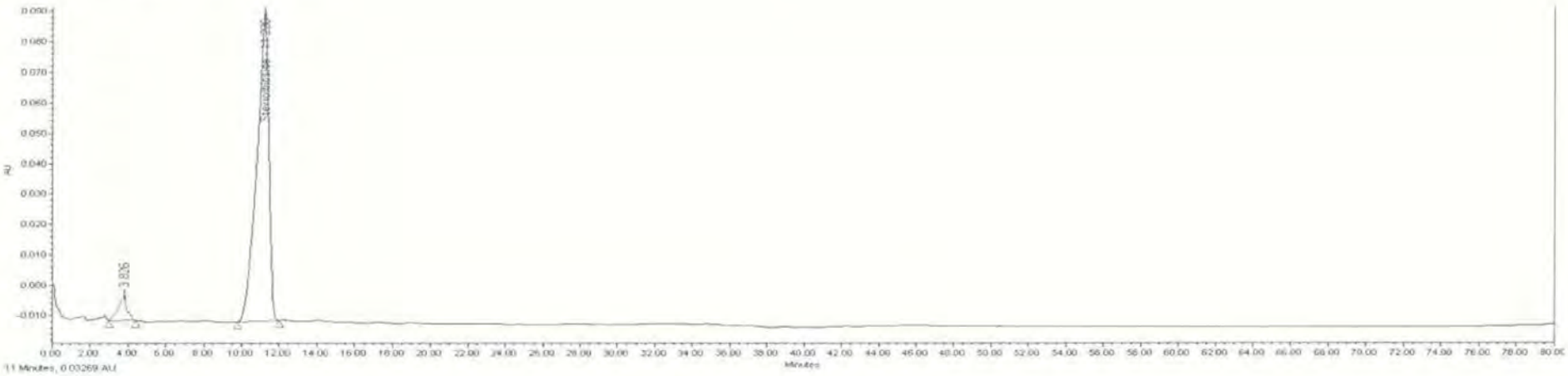


Figure 2. Spectral characteristics of Stevia Extract components(Standard)

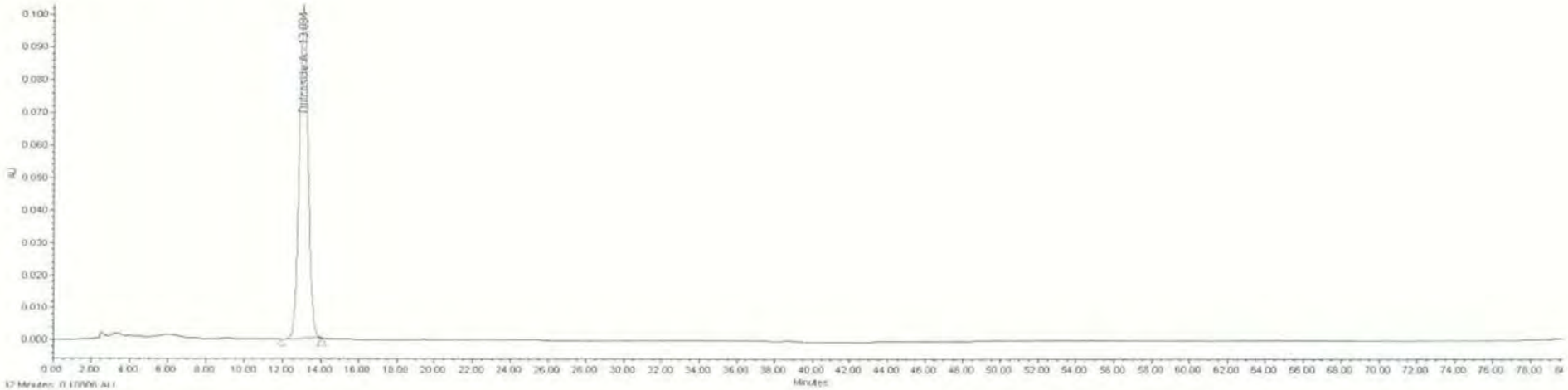
Rubusoside STD



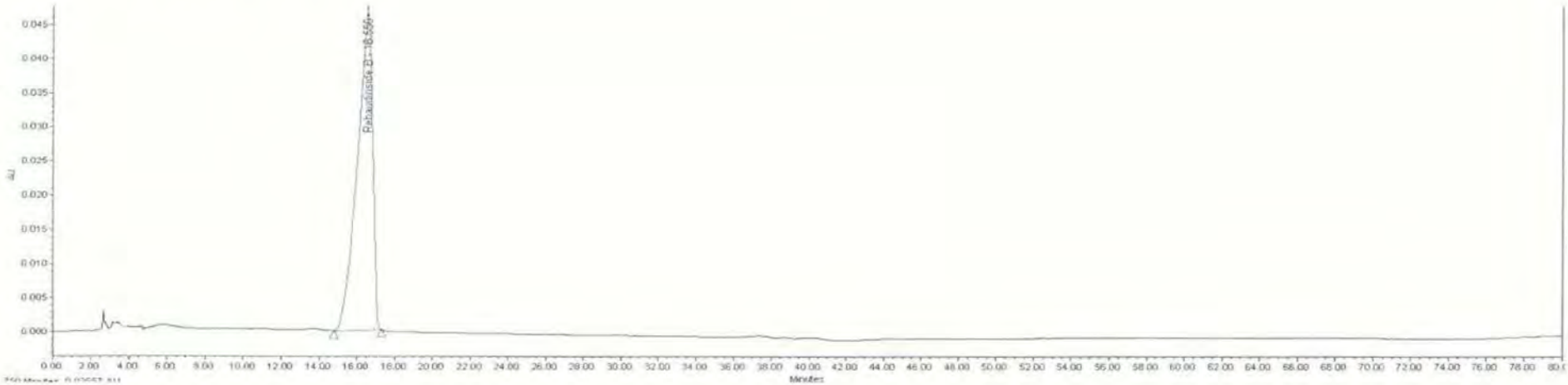
Steviolbioside STD



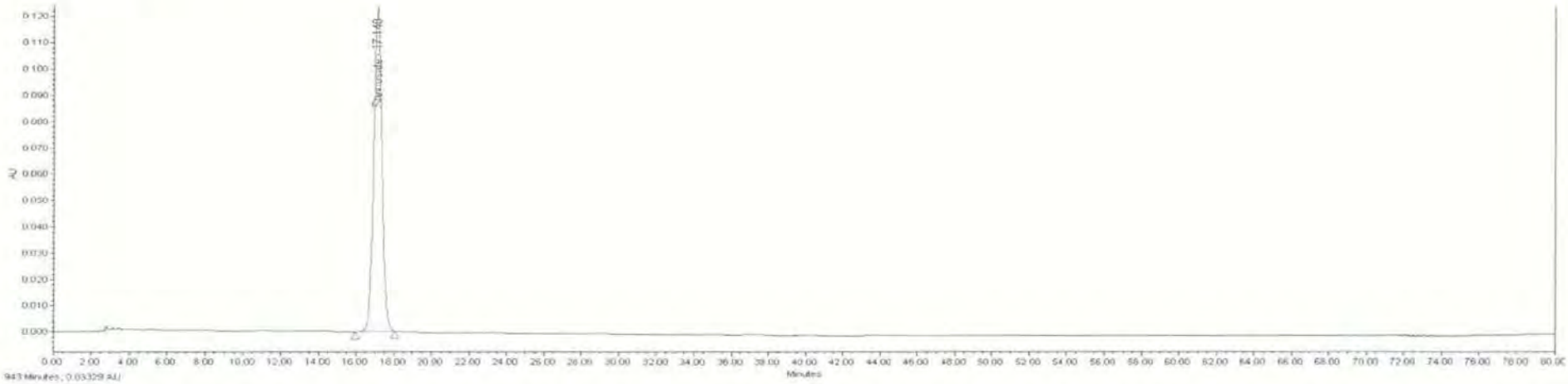
Dulcoside A STD



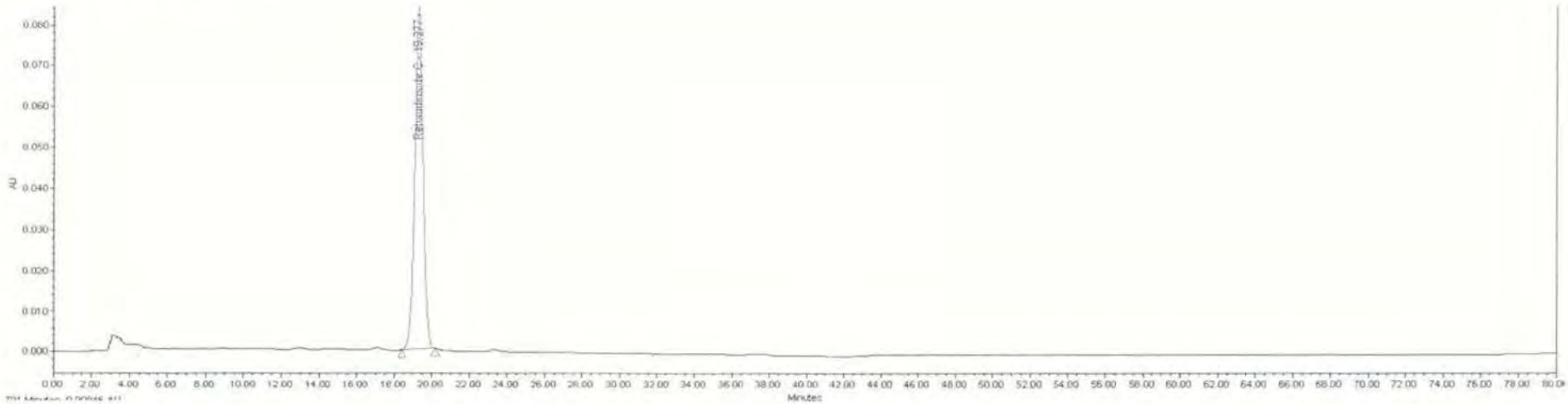
Rebaudioside B STD



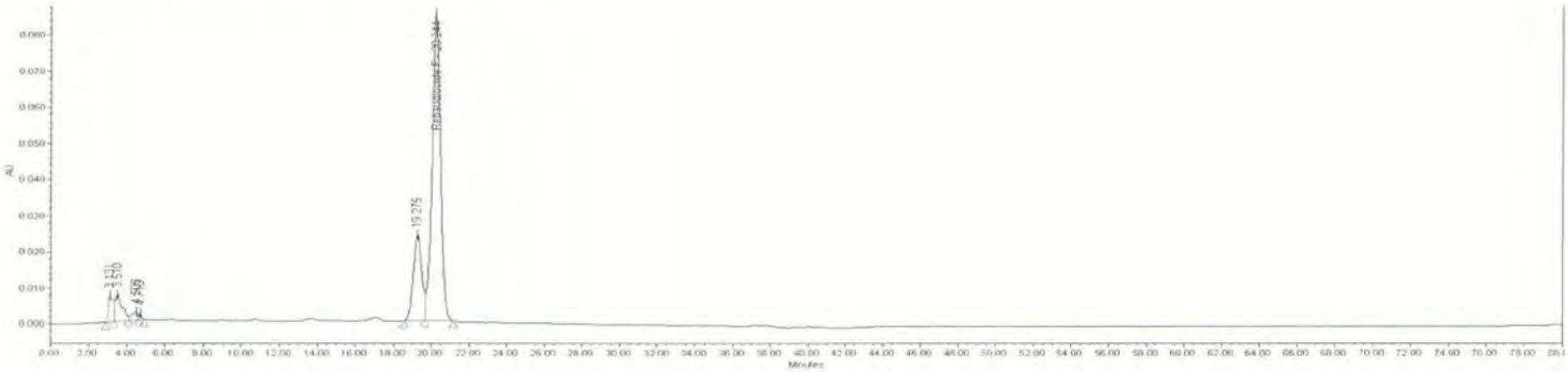
Stevioside STD



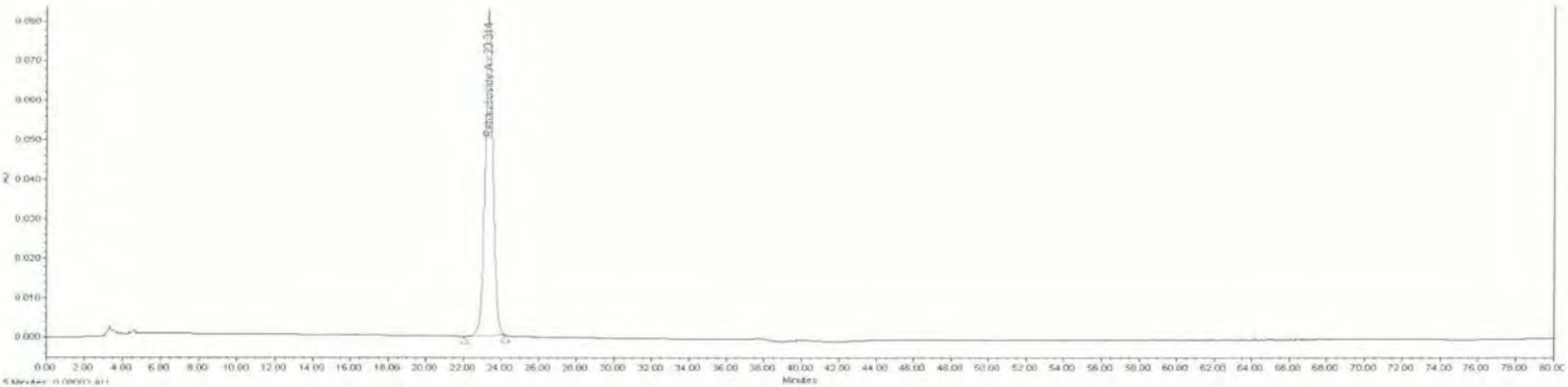
Rebaudioside C STD



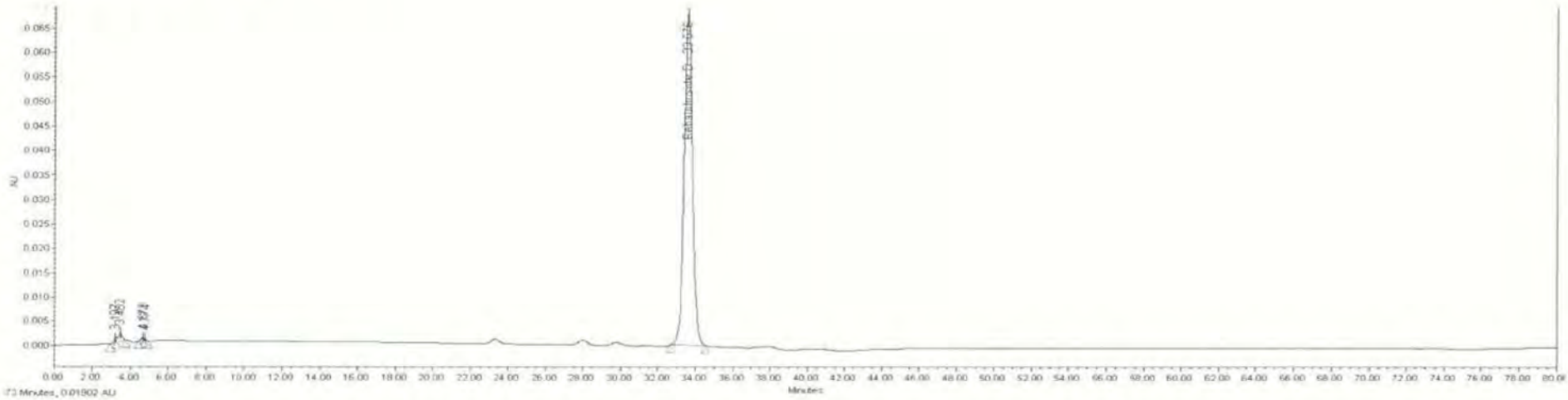
Rebaudioside F STD



Rebaudioside A STD



Rebaudioside D STD



Stevia Extract

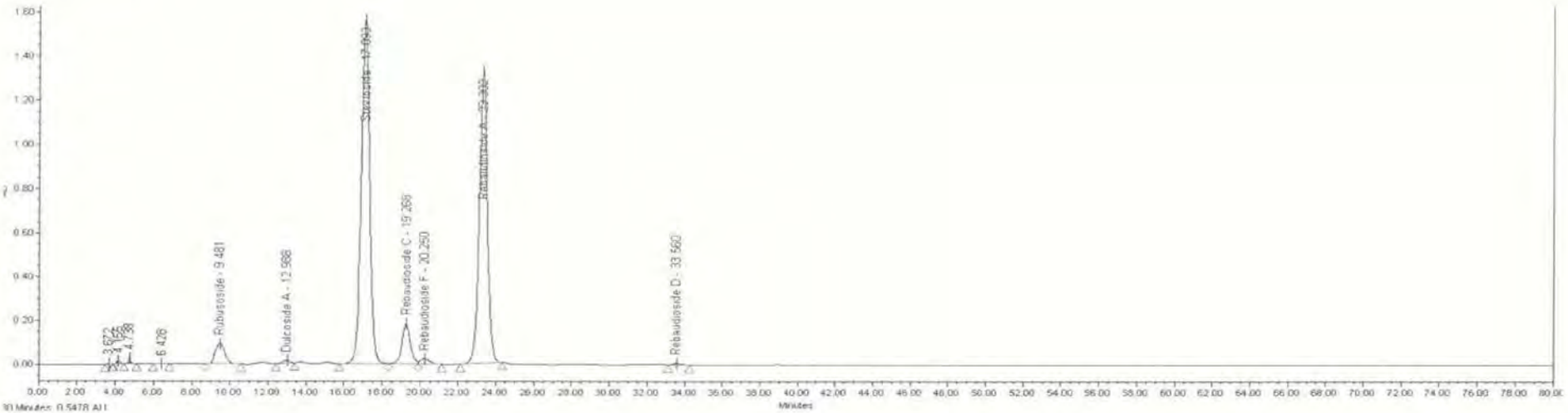
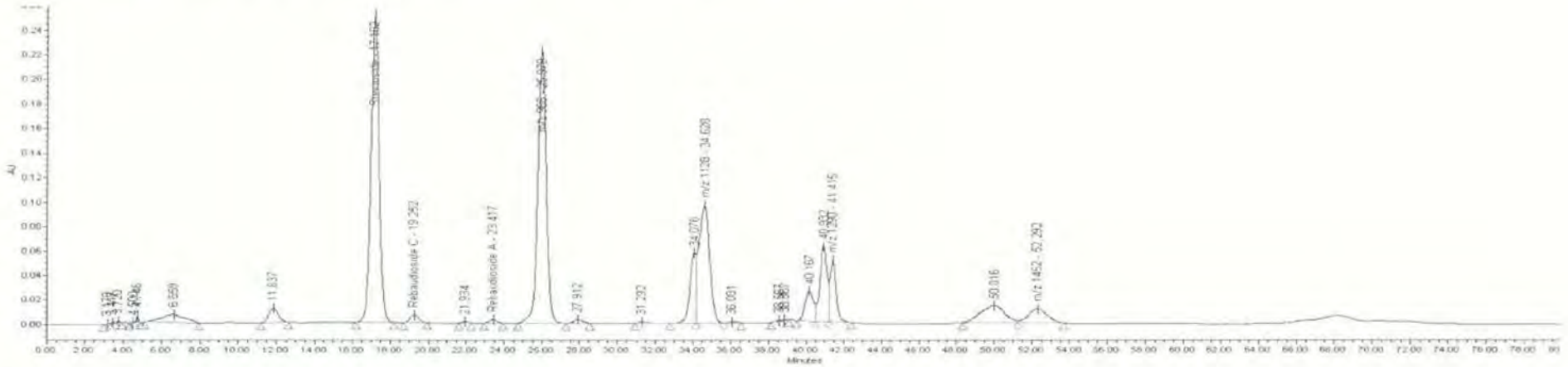
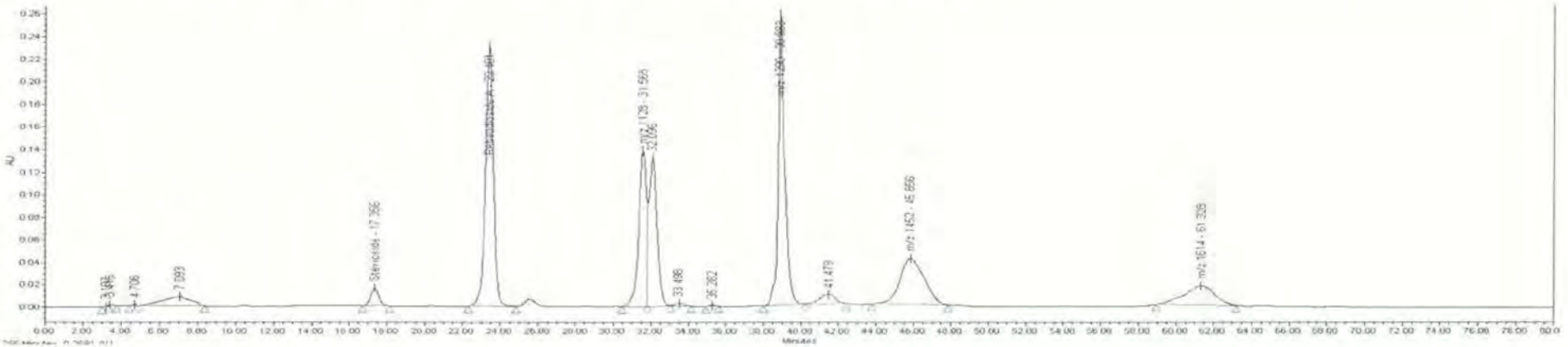


Figure 3. Spectral characteristics ofenzyme glycosylated Stevia components

Enzyme Treated Pure Stevioside(99% Stevioside → enzyme glycosylated Stevia)



Enzyme Treated Pure Stevioside(99% Rebaudioside A → enzyme glycosylated Stevia)



STEVITEN FRESH

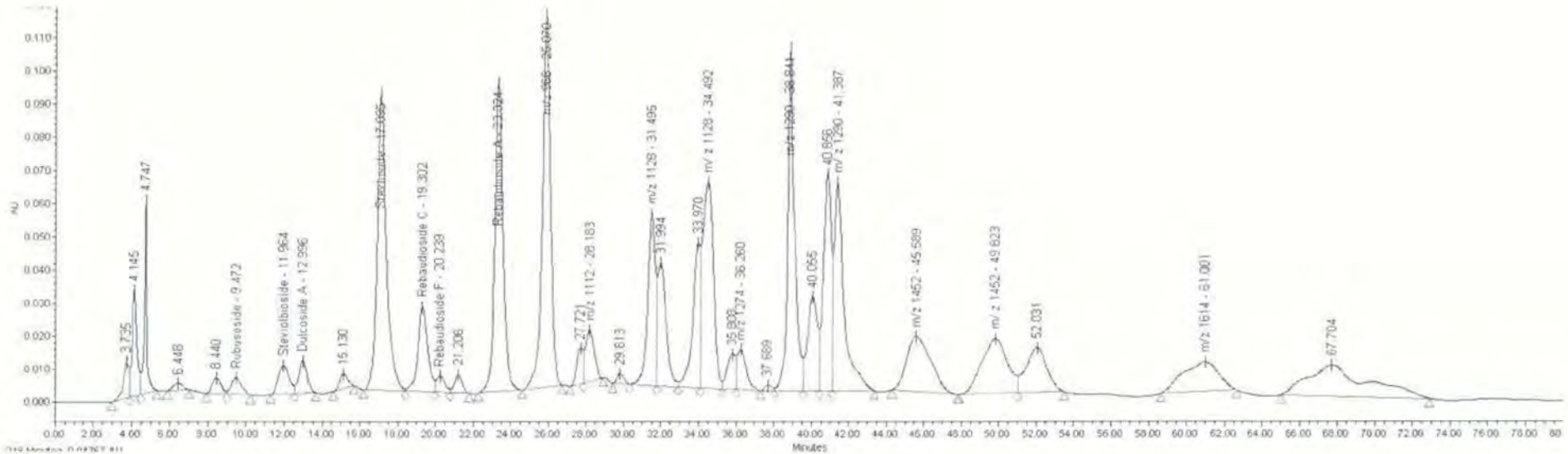


Figure 1. the chromatogram chart as above is shown that after 25 min, we can see different molecular weight which is glucosylated steviol glycoside.

Figure 2. The each nine standard chromatograms which are from "Japan Wako" and "The USA Chromadex" are to check position of stevia extract components

We can distinguish when stevia extract components are coming out

i) Rubusoside STD : about 9 min

ii) Steviolbioside STD : about 11 min

iii) Dulcoside A STD: about 13 min

iv) Rebaudioside B STD: about 16 min

v) Stevioside STD: about 17 min

vi) Rebaudioside C STD: about 19 min

vii) Rebaudioside F STD: about 20 min

viii) Rebaudioside A STD: about 23 min

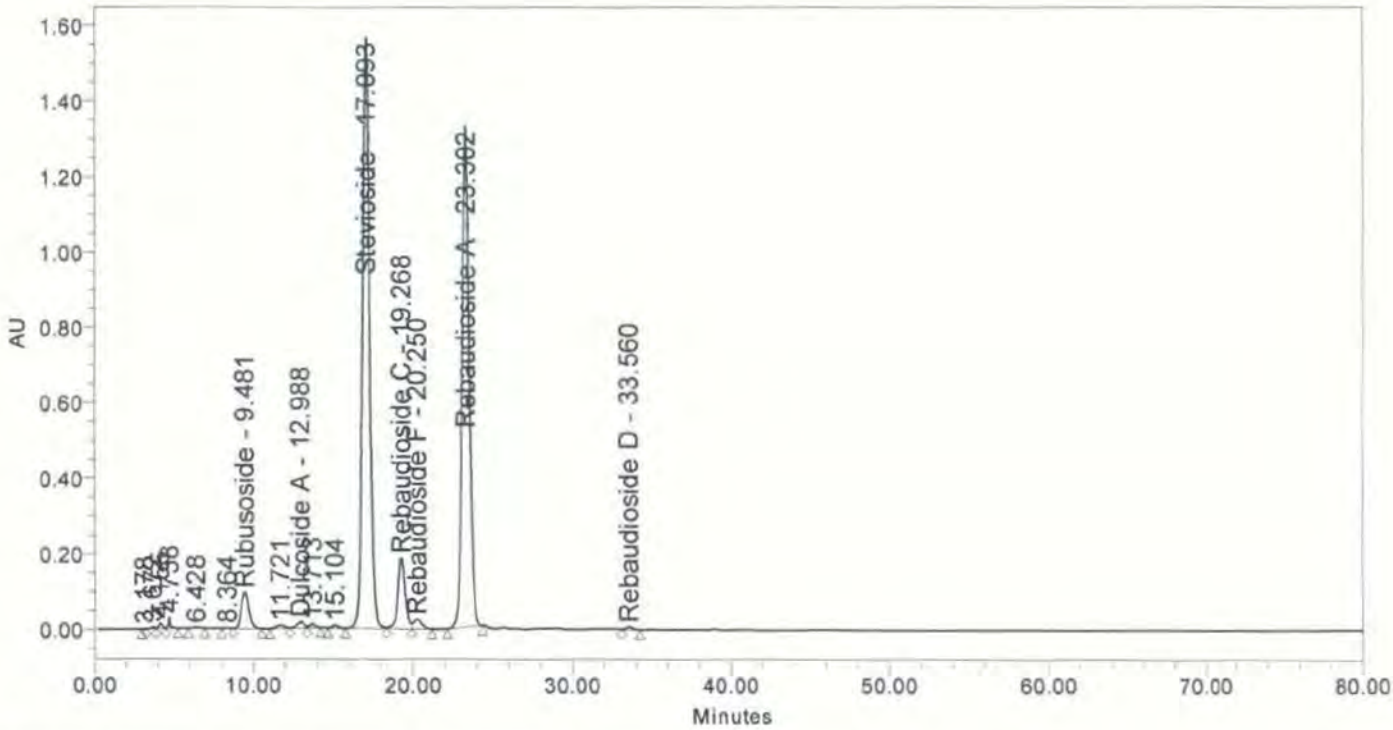
ix) Rebaudioside D STD: about 33 min

igure 3. The chromatogram of "Enzyme Treated Pure Stevioside99%, Rebaudioside A 99% " is similar with "The Chromatogram of STEVITEN FRESH". It means that STEVITEN FRESH is Enzyme Treated steviol glycosides

*Enzyme Treated Pure Stevioside 99%, Rebaudioside A 99% is treated from 99% contents by HPLC of Stevioside, Rebaudioside A.

SAMPLE INFORMATION

Sample Name:	Starting material	Acquired By:	System
Sample Type:		Sample Set Name:	GRAS
Vial:	10	Acq. Method Set:	G—AB—MS
Injection #:	1	Processing Method:	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	80.0 Minutes	Proc. Chnl. Descr.:	
Date Acquired:	2019-02-14 AM 1:24:11 KST		
Date Processed:	2019-02-18 PM 1:21:20 KST		

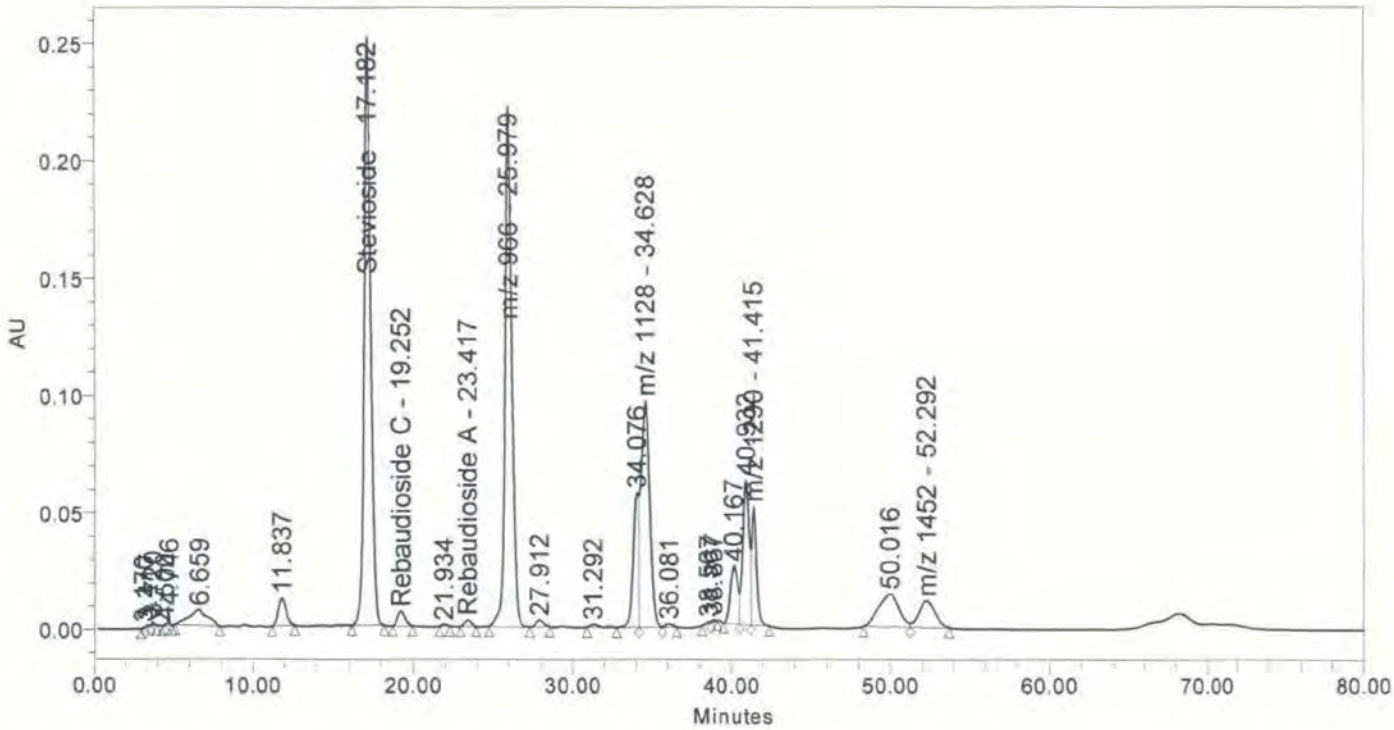


	Peak Name	RT	Area	% Area	Height
1		3.178	6025	0.01	563
2		3.672	77198	0.08	4491
3		4.155	231471	0.23	13711
4		4.738	235179	0.23	28951
5		6.428	63060	0.06	2000
6		8.364	22698	0.02	932
7	Rubusoside	9.481	3159184	3.14	95096
8		11.721	273555	0.27	7587
9	Dulcoside A	12.988	468574	0.47	15484
10		13.713	217715	0.22	8539
11		15.104	195753	0.19	7199
12	Stevioside	17.093	48824801	48.46	1566269
13	Rebaudioside C	19.268	5761282	5.72	187172

	Peak Name	RT	Area	% Area	Height
14	Rebaudioside F	20.250	844146	0.84	24738
15	Rebaudioside A	23.302	40149261	39.85	1326563
16	Rebaudioside D	33.560	219137	0.22	7277

SAMPLE INFORMATION

Sample Name:	Enzyme Treated Pure Stevioside	Acquired By:	System
Sample Type:		Sample Set Name:	GRAS
Vial:	26	Acq. Method Set:	G—AB—MS
Injection #:	1	Processing Methoc	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	80.0 Minutes	Proc. Chnl. Descr.:	
Date Acquired:	2019-02-15 AM 12:52:10 KST		
Date Processed:	2019-02-18 PM 1:27:46 KST		

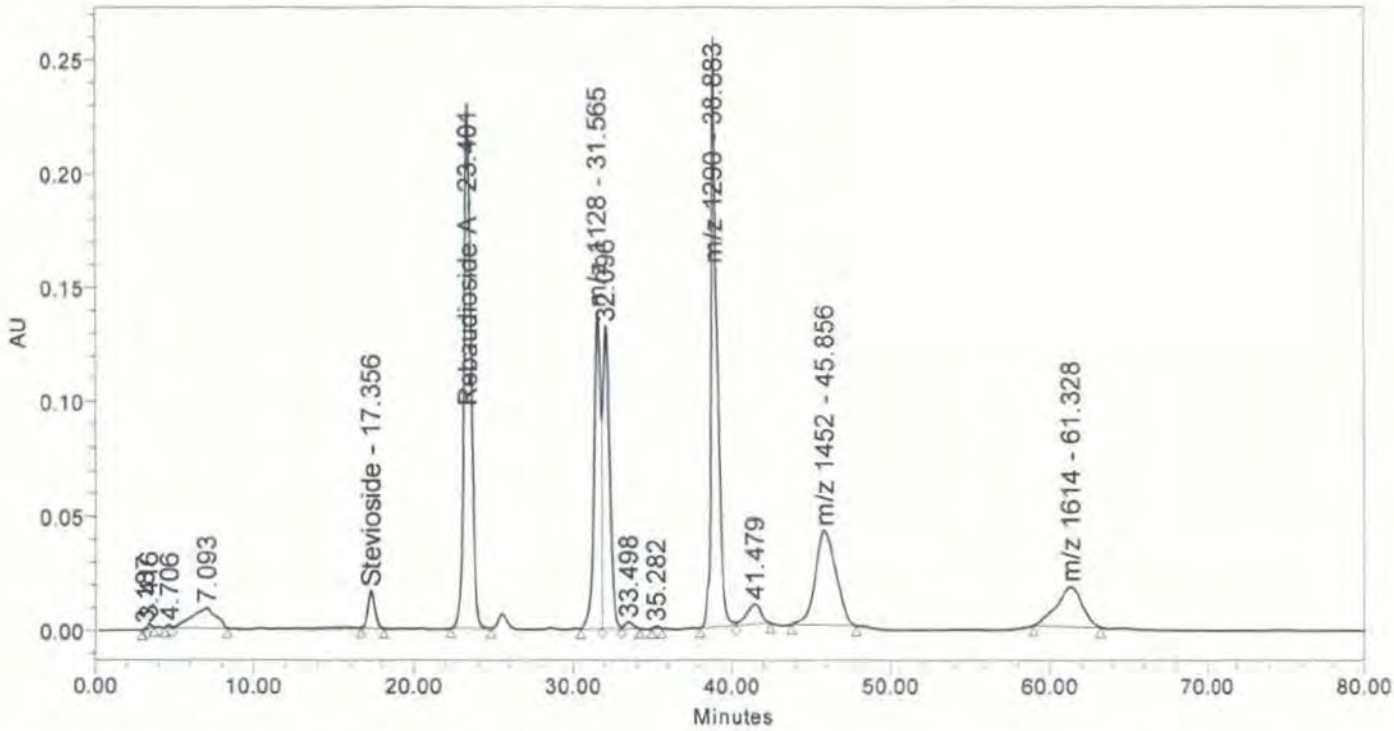


	Peak Name	RT	Area	% Area	Height
1		3.170	2885	0.01	317
2		3.417	9110	0.03	702
3		3.720	12475	0.05	1403
4		4.500	2141	0.01	323
5		4.746	35060	0.13	6553
6		6.659	547055	2.07	6754
7		11.837	408243	1.54	11903
8	Stevioside	17.182	7373465	27.89	251077
9	Rebaudioside C	19.252	201009	0.76	6304
10		21.934	18782	0.07	879
11	Rebaudioside A	23.417	77116	0.29	2904
12	m/z 966	25.979	6717819	25.41	221812
13		27.912	94357	0.36	2932

	Peak Name	RT	Area	% Area	Height
14		31.292	49135	0.19	1140
15		34.076	1267657	4.79	57618
16	m/z 1128	34.628	3904661	14.77	96795
17		36.081	38129	0.14	1252
18		38.567	14779	0.06	808
19		38.867	12833	0.05	875
20		40.167	784729	2.97	24841
21		40.932	1656164	6.26	61884
22	m/z 1290	41.415	1136797	4.30	50692
23		50.016	1270070	4.80	14219
24	m/z 1452	52.292	805146	3.05	11472

SAMPLE INFORMATION

Sample Name:	Enzyme Treated Pure	Acquired By:	System
Sample Type:	Rebaudioside A	Sample Set Name:	GRAS
Vial:	27	Acq. Method Set:	G—AB—MS
Injection #:	1	Processing Methoc	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	80.0 Minutes	Proc. Chnl. Descr.:	
Date Acquired:	2019-02-15 AM 2:13:30 KST		
Date Processed:	2019-02-18 PM 1:33:00 KST		

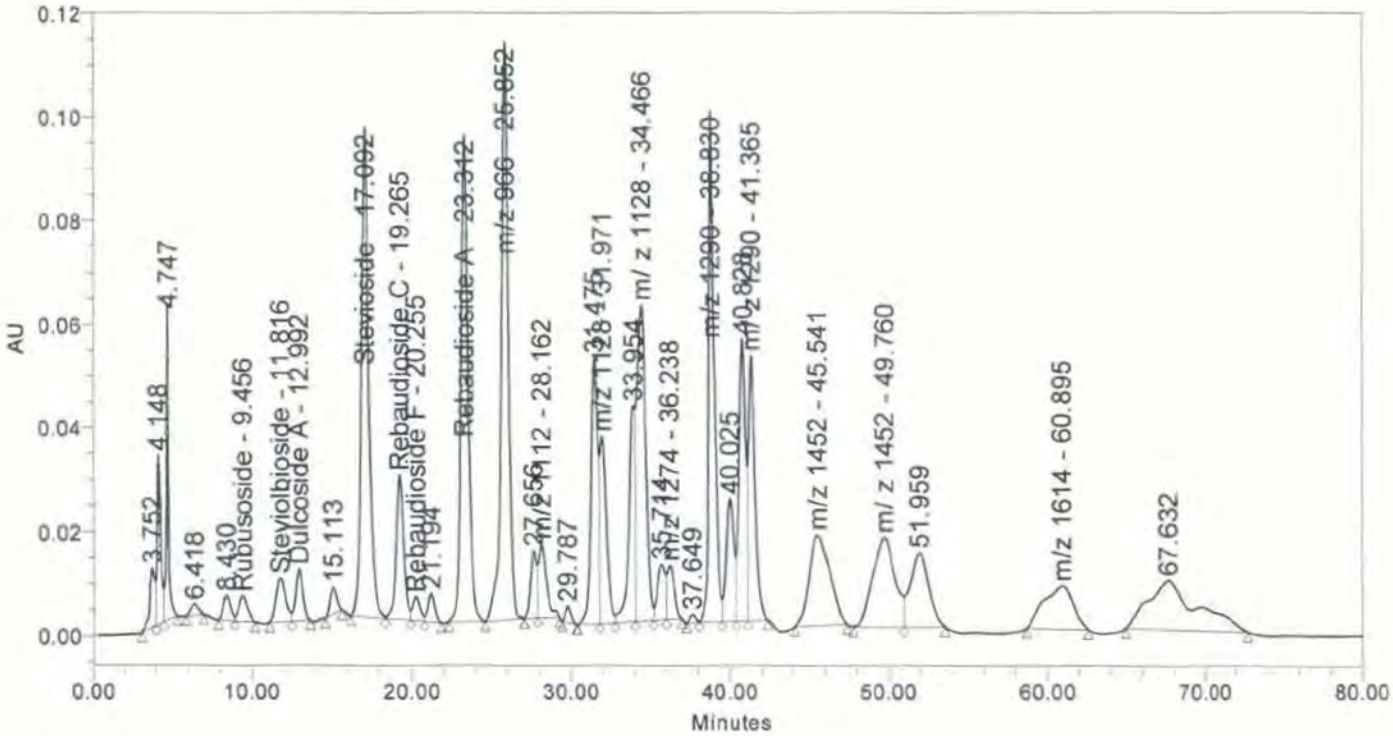


	Peak Name	RT	Area	% Area	Height
1		3.187	3792	0.01	383
2		3.416	28496	0.10	1931
3		4.706	14922	0.05	1197
4		7.093	896339	3.10	8794
5	Stevioside	17.356	469688	1.62	16186
6	Rebaudioside A	23.401	6769593	23.38	229559
7	m/z 1128	31.565	3984095	13.76	138374
8		32.096	3987869	13.77	132166
9		33.498	100452	0.35	2756
10		35.282	20337	0.07	953
11	m/z 1290	38.883	6426442	22.20	258190
12		41.479	549910	1.90	8681
13	m/z 1452	45.856	3726668	12.87	41405

	Peak Name	RT	Area	% Area	Height
14	m/z 1614	61.328	1975090	6.82	17428

SAMPLE INFORMATION

Sample Name:	STEVITEN FRESH	Acquired By:	System
Lot:	181128	Sample Set Name:	GRAS
Vial:	11	Acq. Method Set:	G—AB—MS
Injection #:	1	Processing Methoc	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	80.0 Minutes	Proc. Chnl. Descr.:	
Date Acquired:	2019-02-14 AM 2:45:18 KST		
Date Processed:	2019-02-18 AM 11:59:53 KST		

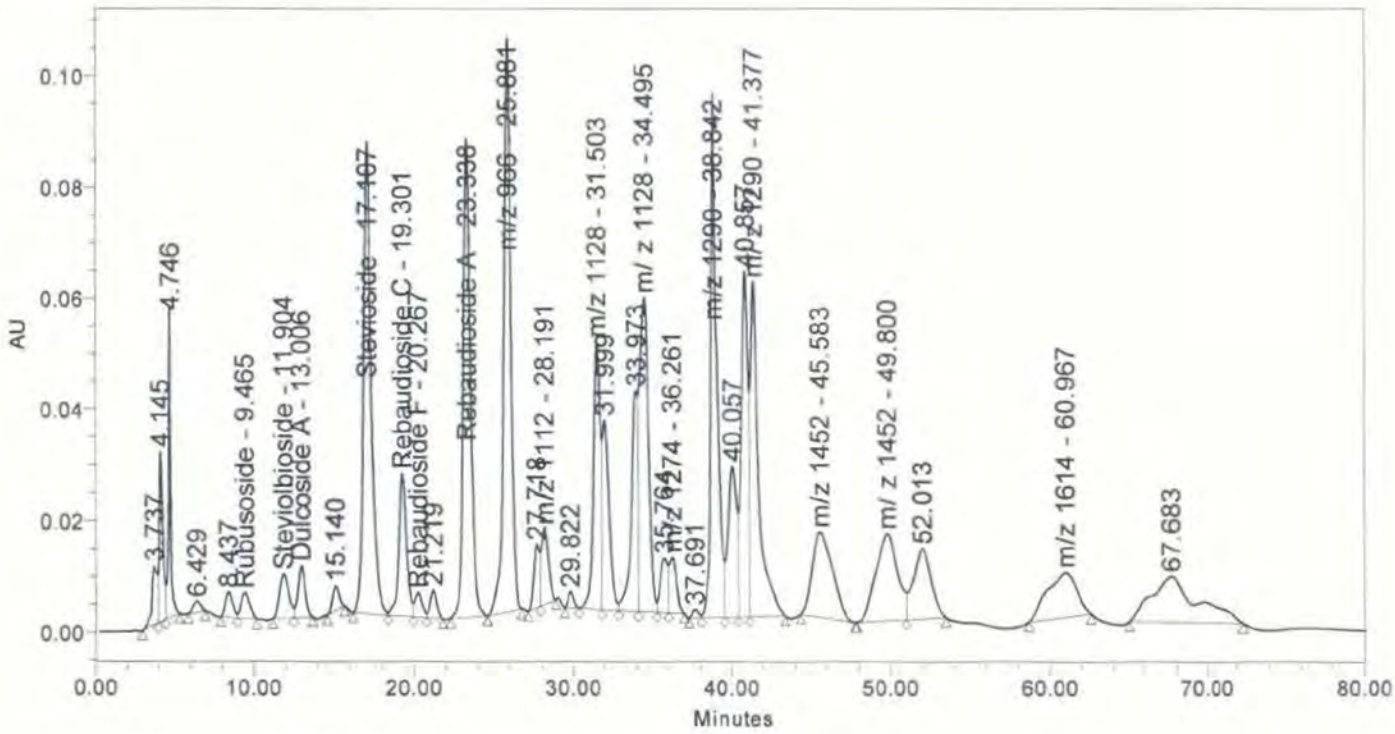


	Peak Name	RT	Area	% Area	Height
1		3.752	252710	0.73	11318
2		4.148	528968	1.54	32355
3		4.747	498081	1.45	58805
4		6.418	71569	0.21	2131
5		8.430	148397	0.43	4730
6	Rubusoside	9.456	181164	0.53	4797
7	Steviolbioside	11.816	348738	1.01	8344
8	Dulcoside A	12.992	311407	0.90	9948
9		15.113	146761	0.43	5089
10	Stevioside	17.092	3099382	9.00	94286
11	Rebaudioside C	19.265	896084	2.60	27698
12	Rebaudioside F	20.255	142038	0.41	4519
13		21.194	152021	0.44	5409

	Peak Name	RT	Area	% Area	Height
14	Rebaudioside A	23.312	2859828	8.31	93794
15	m/z 966	25.852	3519687	10.22	111307
16		27.656	361521	1.05	13070
17	m/z 1112	28.162	486593	1.41	14196
18		29.787	75376	0.22	3115
19		31.475	1523679	4.43	51273
20	m/z 1128	31.971	1051163	3.05	35924
21		33.954	1044237	3.03	41499
22	m/ z 1128	34.466	2464098	7.16	60830
23		35.714	362762	1.05	10674
24	m/z 1274	36.238	323086	0.94	10332
25		37.649	37240	0.11	1506
26	m/z 1290	38.830	2321387	6.74	98458
27		40.025	779277	2.26	23532
28		40.828	1520701	4.42	54308
29	m/ z 1290	41.365	1484350	4.31	51019
30	m/z 1452	45.541	1542342	4.48	17378
31	m/ z 1452	49.760	1658340	4.82	17152
32		51.959	1143978	3.32	14282
33	m/z 1614	60.895	1051563	3.05	8270
34		67.632	2037855	5.92	9479

SAMPLE INFORMATION

Sample Name:	STEVITEN FRESH	Acquired By:	System
Lot:	181213	Sample Set Name:	GRAS
Vial:	12	Acq. Method Set:	G—AB—MS
Injection #:	1	Processing Methoc	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	80.0 Minutes	Proc. Chnl. Descr.:	
Date Acquired:	2019-02-14 AM 4:06:25 KST		
Date Processed:	2019-02-18 PM 12:00:11 KST		

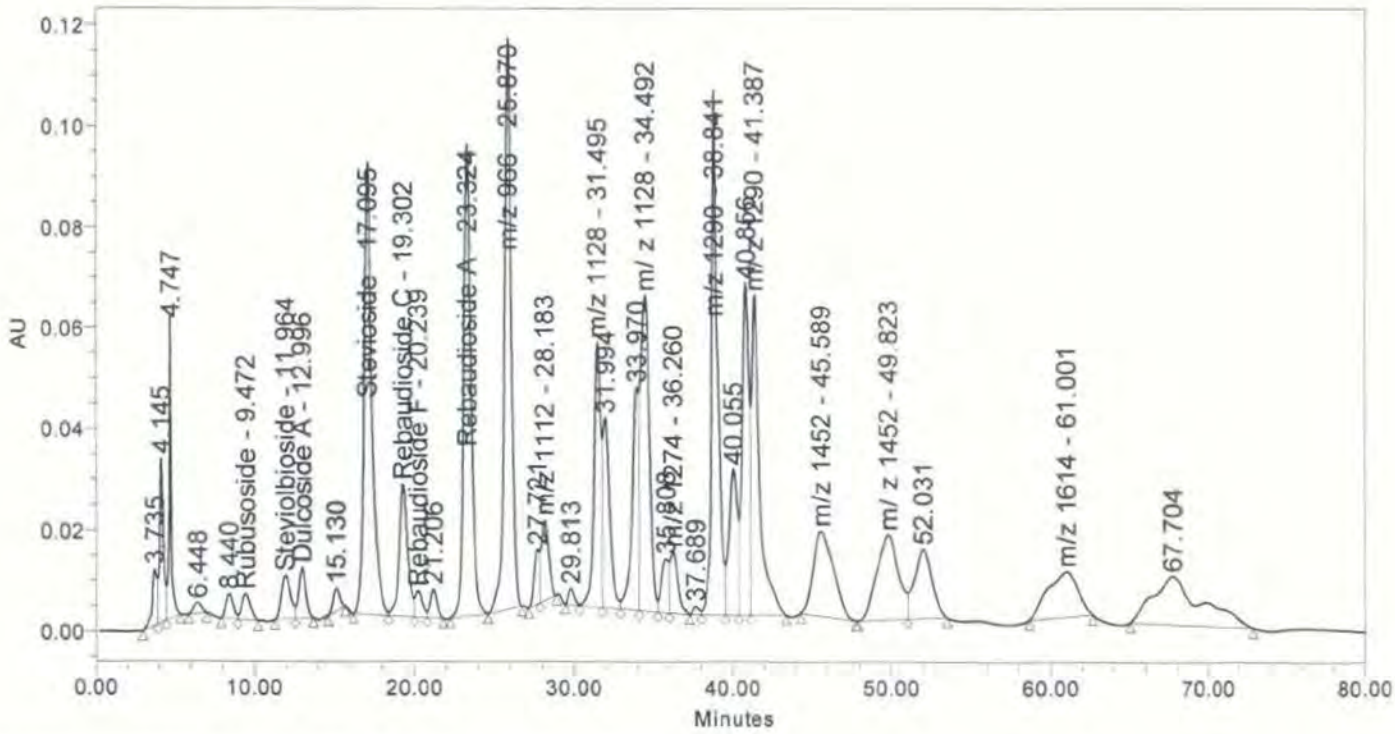


	Peak Name	RT	Area	% Area	Height
1		3.737	245161	0.73	10505
2		4.145	504281	1.51	30439
3		4.746	460704	1.38	54335
4		6.429	63881	0.19	1957
5		8.437	141429	0.42	4485
6	Rubusoside	9.465	169261	0.51	4564
7	Steviolbioside	11.904	316749	0.95	7866
8	Dulcoside A	13.006	283812	0.85	9179
9		15.140	128528	0.38	4396
10	Stevioside	17.107	2855012	8.55	84867
11	Rebaudioside C	19.301	840705	2.52	25481
12	Rebaudioside F	20.267	136248	0.41	4326
13		21.219	139835	0.42	4978

	Peak Name	RT	Area	% Area	Height
14	Rebaudioside A	23.338	2644392	7.92	85948
15	m/z 966	25.881	3214081	9.62	102962
16		27.718	285981	0.86	11338
17	m/z 1112	28.191	417519	1.25	13445
18		29.822	72564	0.22	2934
19	m/z 1128	31.503	1450407	4.34	47876
20		31.999	1001060	3.00	33794
21		33.973	1048049	3.14	39420
22	m/ z 1128	34.495	2289256	6.85	56316
23		35.764	313985	0.94	9743
24	m/z 1274	36.261	319441	0.96	9998
25		37.691	39030	0.12	1472
26	m/z 1290	38.842	2439815	7.30	94264
27		40.057	991723	2.97	27045
28		40.857	1870857	5.60	62083
29	m/ z 1290	41.377	2230071	6.68	60081
30	m/z 1452	45.583	1344779	4.03	15204
31	m/ z 1452	49.800	1464819	4.39	15518
32		52.013	973475	2.91	12523
33	m/z 1614	60.967	1031824	3.09	8008
34		67.683	1675101	5.01	8296

SAMPLE INFORMATION

Sample Name:	STEVITEN FRESH	Acquired By:	System
Lot:	181219	Sample Set Name:	GRAS
Vial:	13	Acq. Method Set:	G—AB—MS
Injection #:	1	Processing Methoc	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	80.0 Minutes	Proc. Chnl. Descr.:	
Date Acquired:	2019-02-14 AM 5:27:31 KST		
Date Processed:	2019-02-18 PM 12:00:24 KST		



	Peak Name	RT	Area	% Area	Height
1		3.735	230742	0.63	11074
2		4.145	562017	1.53	32343
3		4.747	503275	1.37	58262
4		6.448	71399	0.19	2102
5		8.440	152865	0.42	4820
6	Rubusoside	9.472	188748	0.52	5111
7	Steviolbioside	11.964	340053	0.93	8547
8	Dulcoside A	12.996	308673	0.84	9976
9		15.130	131541	0.36	4453
10	Stevioside	17.095	3090391	8.44	89584
11	Rebaudioside C	19.302	930801	2.54	25947
12	Rebaudioside F	20.239	159397	0.44	5051
13		21.206	160011	0.44	5640

	Peak Name	RT	Area	% Area	Height
14	Rebaudioside A	23.324	2877155	7.86	93293
15	m/z 966	25.870	3497169	9.55	112816
16		27.721	251189	0.69	10806
17	m/z 1112	28.183	512985	1.40	15732
18		29.813	80506	0.22	3209
19	m/z 1128	31.495	1577940	4.31	52066
20		31.994	1143251	3.12	37389
21		33.970	1167323	3.19	43909
22	m/ z 1128	34.492	2532154	6.91	62276
23		35.808	335532	0.92	10688
24	m/z 1274	36.260	428611	1.17	12158
25		37.689	42888	0.12	1631
26	m/z 1290	38.841	2667320	7.28	103862
27		40.055	1050773	2.87	28667
28		40.856	1929026	5.27	65632
29	m/ z 1290	41.387	2334014	6.37	63065
30	m/z 1452	45.589	1484844	4.05	16769
31	m/ z 1452	49.823	1582377	4.32	16764
32		52.031	1053142	2.88	13621
33	m/z 1614	61.001	1179038	3.22	8969
34		67.704	2064198	5.64	9581

2. Analysis of Glucosylated Steviol Glycoside by UV/LC

Glucose Standard Curve

Figure 4. Glucose Standard Curve

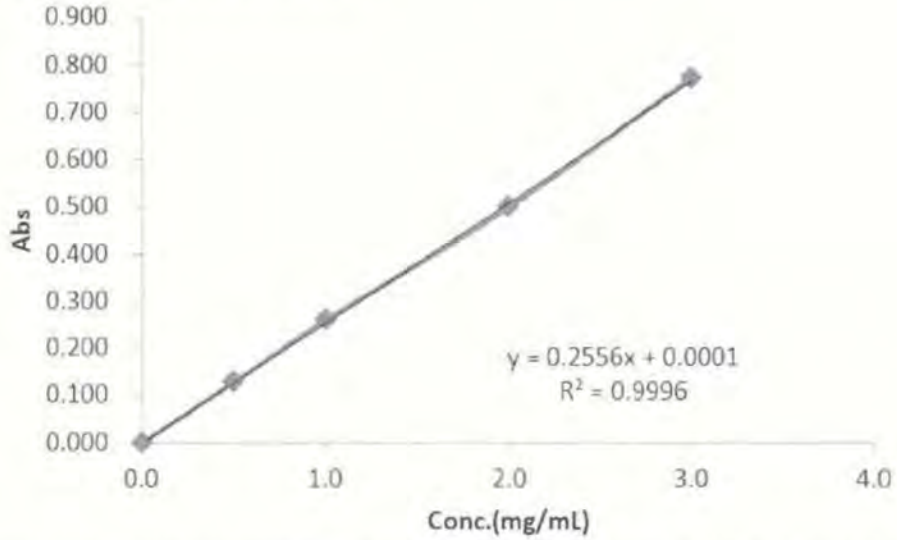


Figure out absorbance (abs) with each four glucose conc in table 5 and make calibration curve as above. Therefore it is reliable.

Y= 0.2556 X + 0.0001

Results

Table 5. Glucose Standard

Glucose Conc.(mg/ml)	Absorbance(abs)	Remarks
0.5	0.128	
1.0	0.261	
2.0	0.501	
3.0	0.772	

Table 6. STEVIEN FRESH

Lot	Amount of sample (g)	Moisture (%)	Absorbance (abs)	b* (mg/ml)	Y** (g)	Remarks
181128	1.0341	3.9	0.593	2.32	0.9938	
181213	1.0159	4.2	0.578	2.26	0.9732	
181219	1.0225	4.3	0.581	2.27	0.9785	

* b: Concentration(mg/ml) of D-glucose in the test solution

** Y: Dry basis weight(g) of the sample

X : Content (%) of ∝-Glucosyl residues = [(b X 200)/(Y X 1,000)] X 0.900 X 100

1) 181128

X (%)=[(2.32 X 200)/(0.9938 X 1,000)] X 0.900 X 100 =42.02

2) 181213

X (%) =[(2.26 X 200)/(0.9732 X 1,000)] X 0.900 X 100=41.82

3) 181219

X (%) =[(2.27 X 200)/(0.9785 X 1,000)] X 0.900 X 100=41.81

Methods

HPLC Conditions

Instrument : Alliance e2695-2489(Waters)

Column : TOSOH, TSKgel Amide-80(250X4.6mm, 5um)

Column Temp : 25 ℃ ± 5℃

Flow Rate : Adjust so that the retention time of rebaudioside A is about 21 min

Injection Volume : 20uL

Detection : uv@210nm

Mobile Phase : Mix HPLC-grade acetonitrile and water(80:20)

Procedure

Equilibrate the instrument by pumping mobile phase through it until a drift-free baseline is obtained. Record the chromatograms of the sample solution and of the standard solutions. The retention times relative to rebaudioside A(1.00) are:

0.45~0.48 for stevioside

0.25~0.30 for dulcoside A

0.63~0.69 for rebaudioside C

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

Calculate the percentage of each of the four steviol glycoside, in the sample from the formula:

$$\text{Content (\%) of steviol glycosides} = [W_S/W] \times [A_X/A_S] \times f_X \times 100$$

Where

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

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

A_S is the peak area for stevioside from the standard solution

A_X is the peak area for sample from the sample solution

f_X is the ratio of the formula weight of X to the formula weight of stevioside: 1.00(stevioside), 0.98(dulcoside A), 1.20(rebaudioside A), 1.18(rebaudioside C).

Results

Table 7. Total content (%) of ∝-Glucosyl steviol glycosides and unreacted steviol glycosides

Lot	Content (%) of steviol glycosides	Content (%) of ∝-Glucosyl residues	Remarks
181128	42.02	41.58	
181213	41.82	41.48	
181219	41.81	41.59	

Lot	Assay(%)*	Remarks
181128	83.6	
181213	83.3	
181219	83.4	

* Assay is Total content of ∝-Glucosyl steviol glycosides and unreacted steviol glycosides

※ Total content (%) of ∝-Glucosyl steviol glycosides and unreacted steviol glycosides
= Content (%) of steviol glycosides + Content (%) of ∝-Glucosyl residues

Table 8. Content (%) of ∝-glucosylsteviol glycosides

Lot	Assay (%)	Content (%) of unreacted steviol glycosides	Remarks
181128	83.6	7.3	
181213	83.3	7.5	
181219	83.4	7.5	

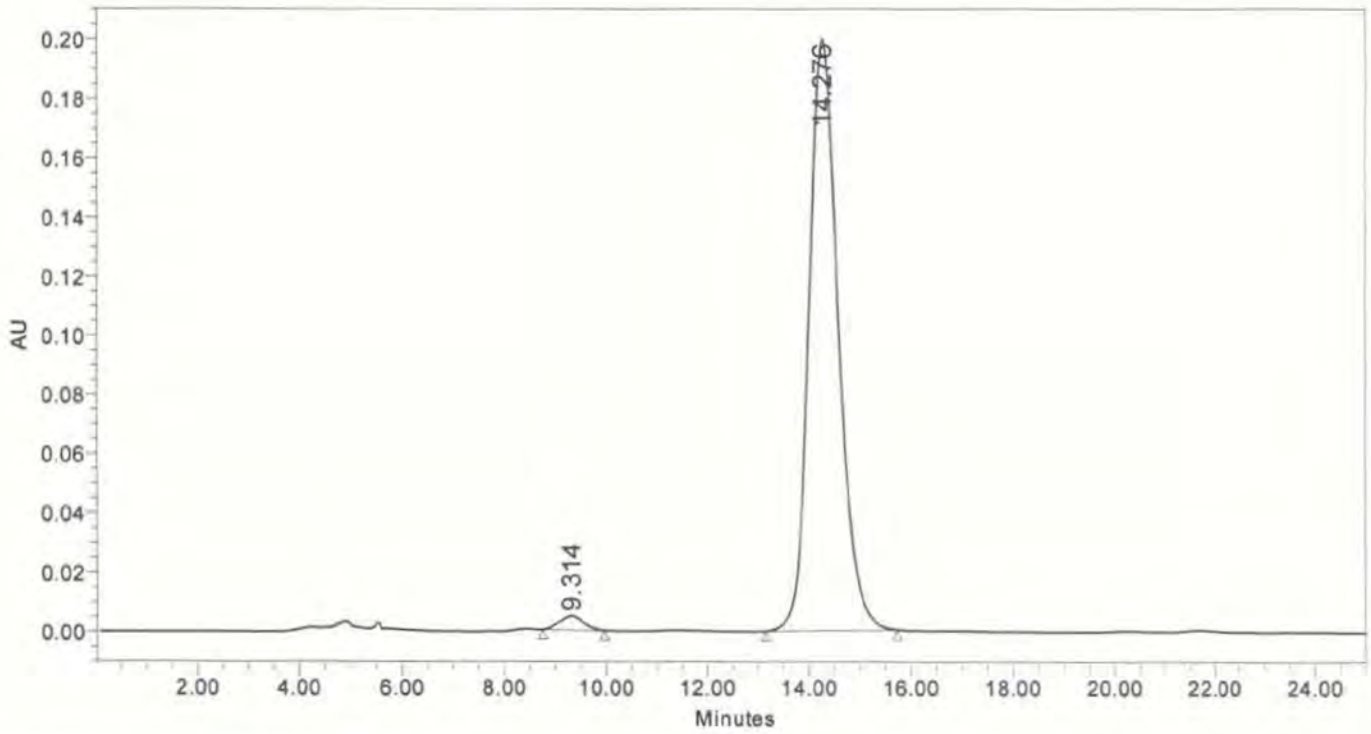
Lot	Content (%) of ∝-glucosylsteviol glycosides	Remarks
181128	76.1	
181213	75.8	
181219	75.9	

※ Content (%) of ∝-glucosylsteviol glycosides
= Content (%) of steviol glycosides + Content (%) of ∝-glucosyl residues − Content (%) of unreacted steviol glycosides

Appendix B
Raw Data

SAMPLE INFORMATION

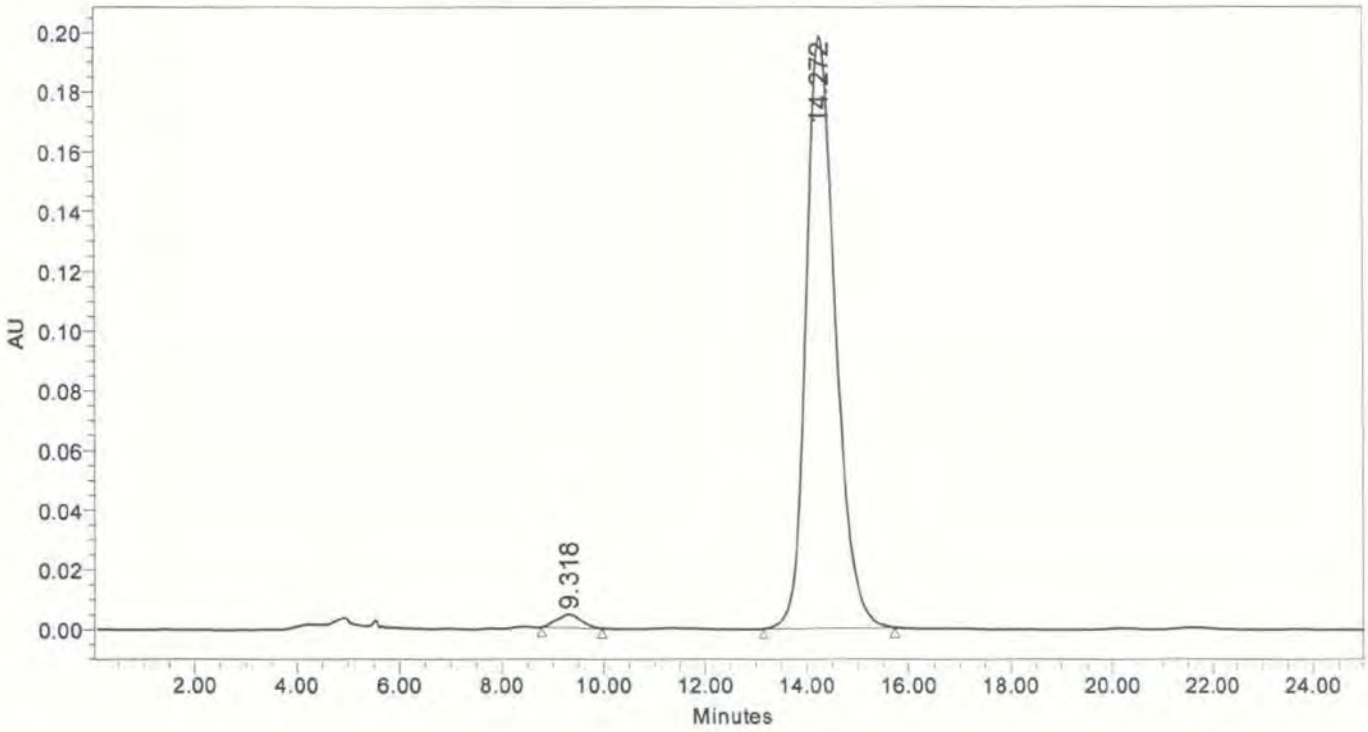
Sample Name:	Stevioside	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	GRAS
Vial:	1	Acq. Method Set:	함량 D—065— MS
Injection #:	1	Processing Methoc	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	25.0 Minutes	Proc. Chnl. Descr.:	
Date Acquired:	2019-02-18 PM 3:13:07 KST		
Date Processed:	2019-02-19 AM 11:02:41 KST		



	RT	Area	% Area	Height
1	9.314	155332	1.92	4774
2	14.276	7946135	98.08	199682

SAMPLE INFORMATION

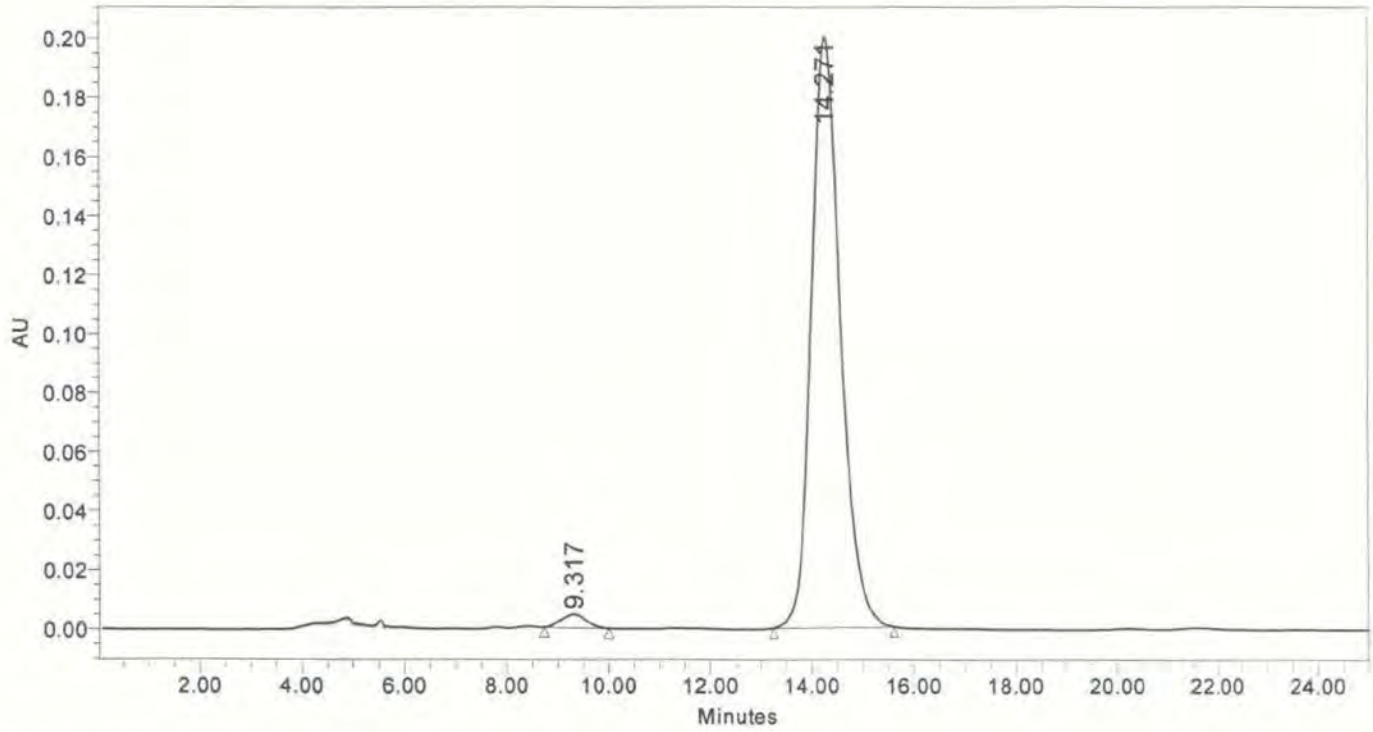
Sample Name:	Stevioside	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	GRAS
Vial:	2	Acq. Method Set:	함량 D—065— MS
Injection #:	1	Processing Methoc	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	25.0 Minutes	Proc. Chnl. Descr.:	
Date Acquired:	2019-02-18 PM 3:39:02 KST		
Date Processed:	2019-02-19 AM 11:02:57 KST		



	RT	Area	% Area	Height
1	9.318	149010	1.85	4511
2	14.272	7926953	98.15	198090

SAMPLE INFORMATION

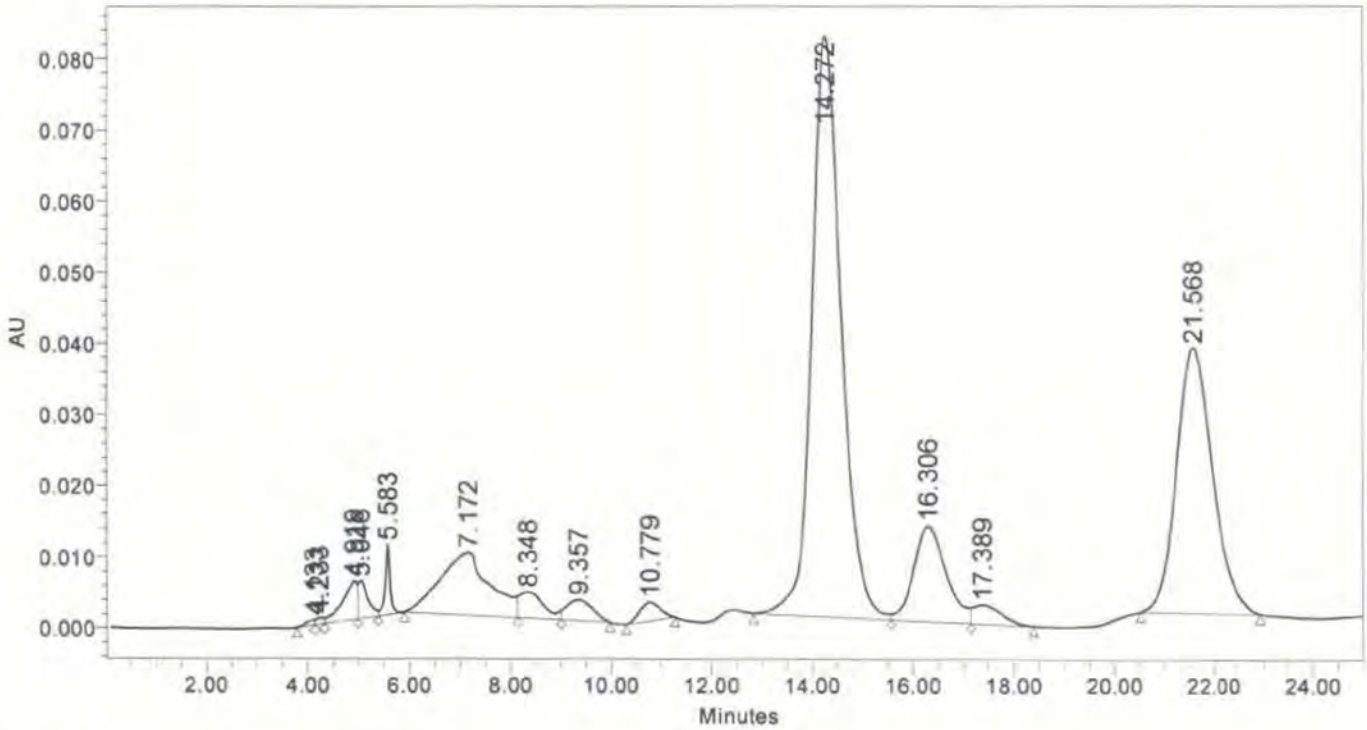
Sample Name:	Stevioside	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	GRAS
Vial:	3	Acq. Method Set:	함량 D—065— MS
Injection #:	1	Processing Methoc	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	25.0 Minutes	Proc. Chnl. Descr.:	
Date Acquired:	2019-02-18 PM 4:04:57 KST		
Date Processed:	2019-02-19 AM 11:04:26 KST		



	RT	Area	% Area	Height
1	9.317	156261	1.93	4535
2	14.271	7961143	98.07	199840

SAMPLE INFORMATION

Sample Name:	STEVITEN FRESH	Acquired By:	System
Lot:	181128	Sample Set Name:	GRAS
Vial:	4	Acq. Method Set:	함량 D—065— MS
Injection #:	1	Processing Methoc	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	25.0 Minutes	Proc. Chnl. Descr.:	
Date Acquired:	2019-02-18 PM 4:30:52 KST		
Date Processed:	2019-02-19 AM 11:08:43 KST		



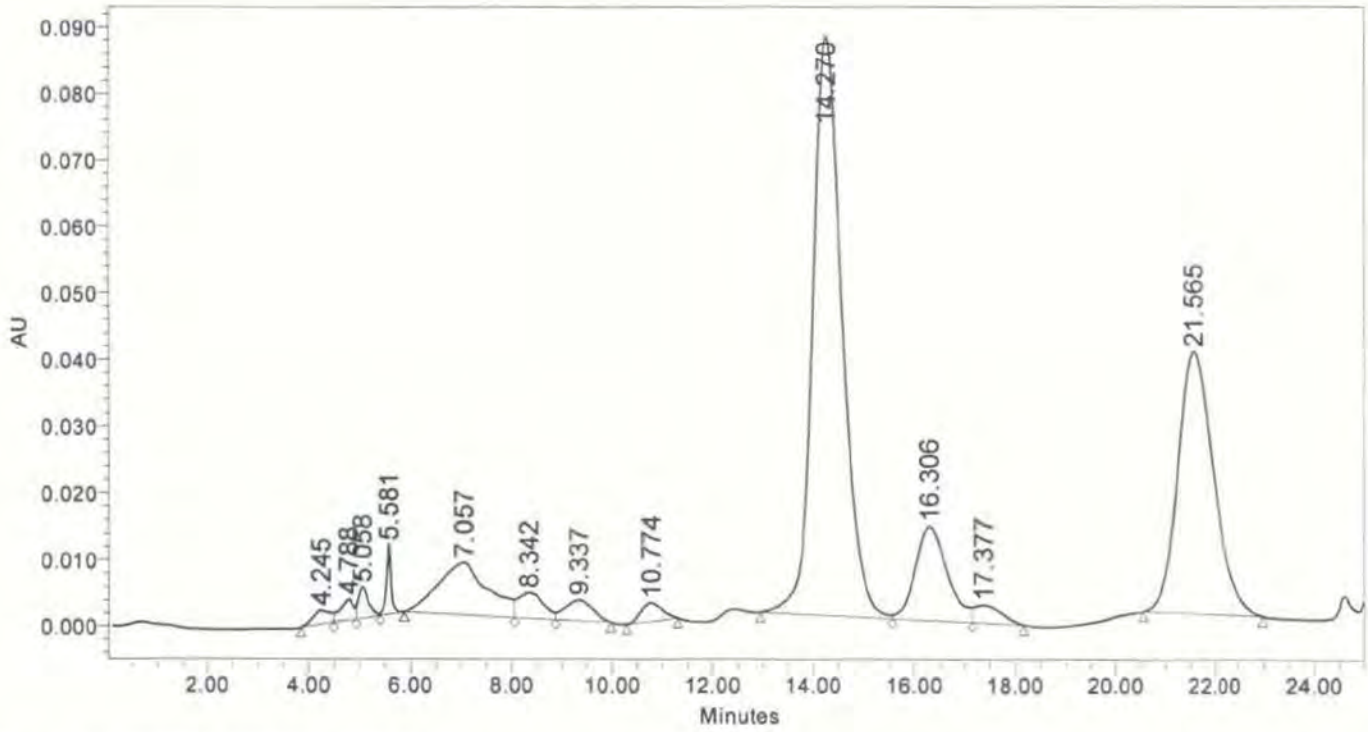
	RT	Area	% Area	Height
1	4.133	8382	0.12	761
2	4.233	10442	0.15	924
3	4.919	109073	1.55	5350
4	5.046	69505	0.98	5266
5	5.583	62915	0.89	9653
6	7.172	598044	8.47	8805
7	8.348	122225	1.73	3647
8	9.357	102390	1.45	2874
9	10.779	72814	1.03	2575
10	14.272	3296505	46.71	81522
11	16.306	600044	8.50	13442
12	17.389	104577	1.48	2677
13	21.568	1900007	26.92	37241

Empower 2 software

GRAS

SAMPLE INFORMATION

Sample Name:	STEVITEN FRESH	Acquired By:	System
Lot:	181213	Sample Set Name:	GRAS
Vial:	5	Acq. Method Set:	함량 D—065— MS
Injection #:	1	Processing Methoc	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	25.0 Minutes	Proc. Chnl. Descr.:	
Date Acquired:	2019-02-18 PM 4:56:47 KST		
Date Processed:	2019-02-19 AM 11:09:25 KST		



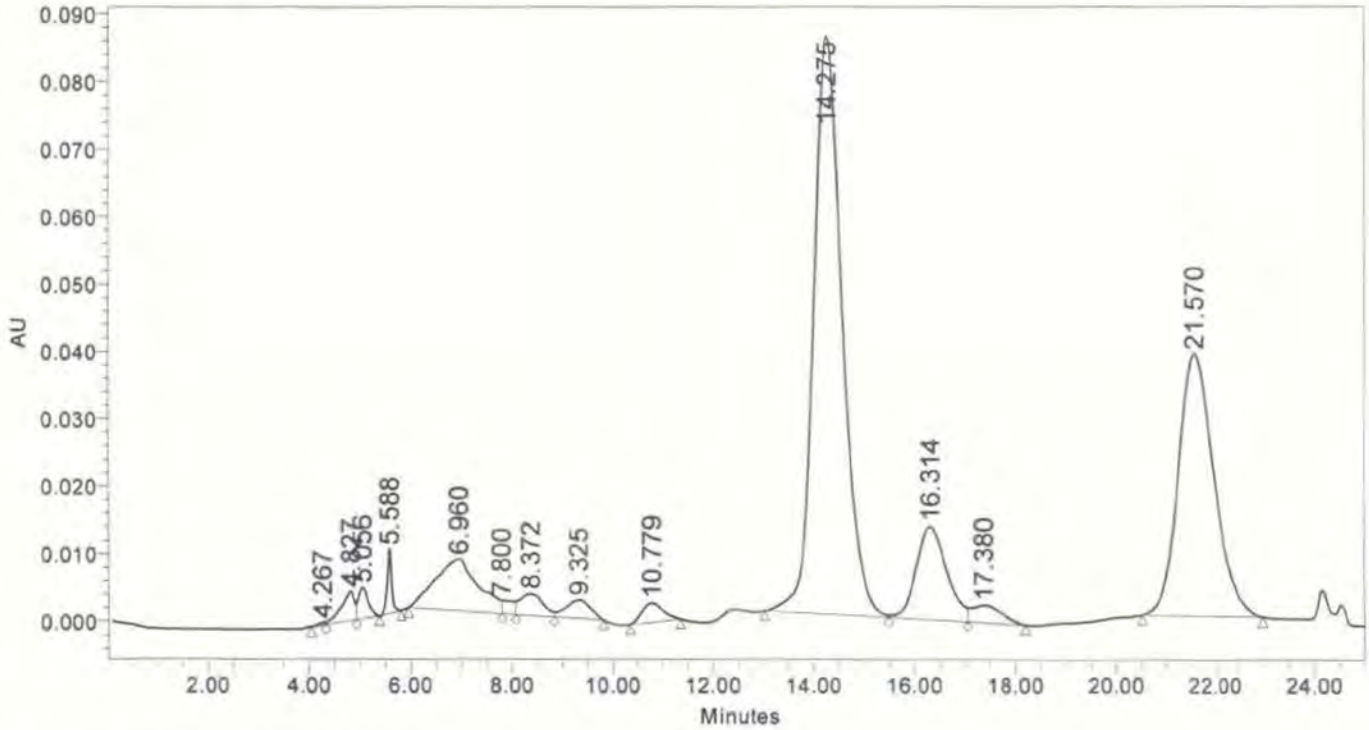
	RT	Area	% Area	Height
1	4.245	48486	0.66	2113
2	4.788	57818	0.79	3176
3	5.058	63822	0.87	4676
4	5.581	63214	0.86	10262
5	7.057	533032	7.29	7852
6	8.342	134157	1.84	3793
7	9.337	118082	1.62	3115
8	10.774	81250	1.11	2794
9	14.270	3493290	47.78	86853
10	16.306	622447	8.51	14131
11	17.377	99976	1.37	2696
12	21.565	1995317	27.29	39302

Empower 2 software

GRAS

SAMPLE INFORMATION

Sample Name:	STEVITEN FRESH	Acquired By:	System
Lot:	181219	Sample Set Name:	GRAS
Vial:	6	Acq. Method Set:	함량 D—065— MS
Injection #:	1	Processing Methoc	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	25.0 Minutes	Proc. Chnl. Descr.:	
Date Acquired:	2019-02-18 PM 5:22:42 KST		
Date Processed:	2019-02-19 AM 11:10:15 KST		



	RT	Area	% Area	Height
1	4.267	3344	0.05	342
2	4.827	78893	1.13	4338
3	5.056	61809	0.89	4609
4	5.588	55997	0.80	9436
5	6.960	451018	6.48	7671
6	7.800	30441	0.44	1923
7	8.372	100367	1.44	3157
8	9.325	90950	1.31	2687
9	10.779	81555	1.17	2824
10	14.275	3353077	48.14	85546
11	16.314	580983	8.34	13801
12	17.380	109046	1.57	2594
13	21.570	1967351	28.25	38816

3. Standard Curve

Result

Table 9. Standard Analysis

Standard	Conc.(mg/ml)	Retention Time(min)	Area	Remarks
Stevioside	5	17.153	30247554	
	10	17.113	55267784	
	15	17.144	80947221	
	20	17.171	109386339	
Rebaudioside A	5	23.351	24574770	
	10	23.394	44930455	
	15	23.420	64742431	
	20	23.418	81289585	

Figure out Area with each four stevioside Conc and Rebaudioside Conc in table 9 and make Figure 5 & 6 calibration curve as below. Therefore it is reliable.

Figure 5. Stevioside Standard Curve

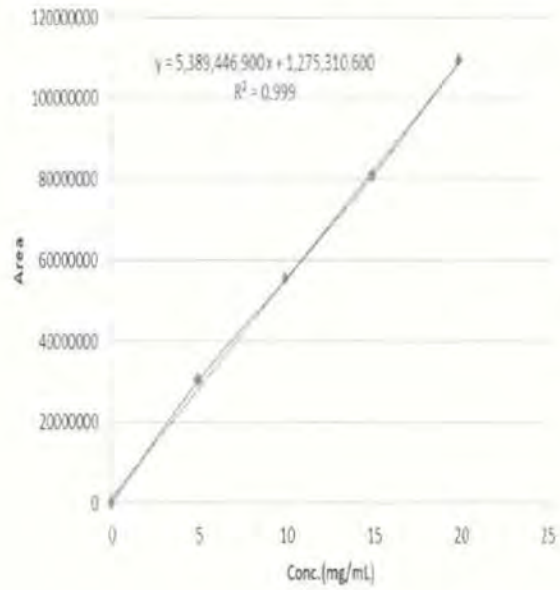
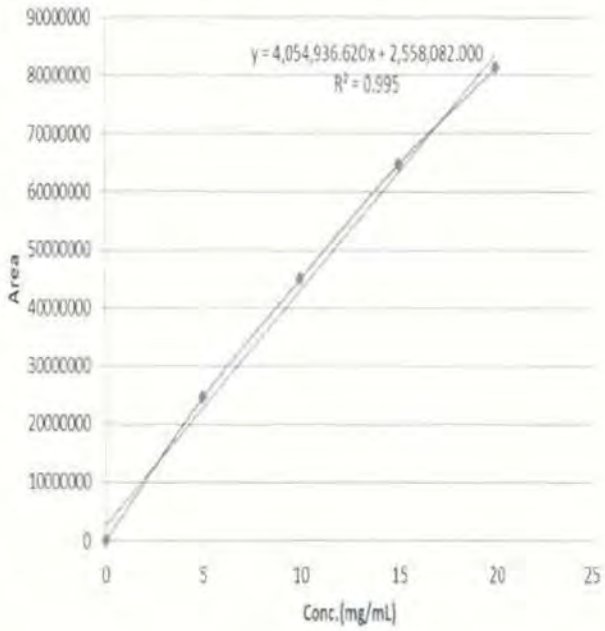


Figure 6. Rebaudioside A Standard Curve



Y = 5,389,446.9X + 1,275,310.6

R = 0.999

Y = 4,054,936.62X + 2,558,082

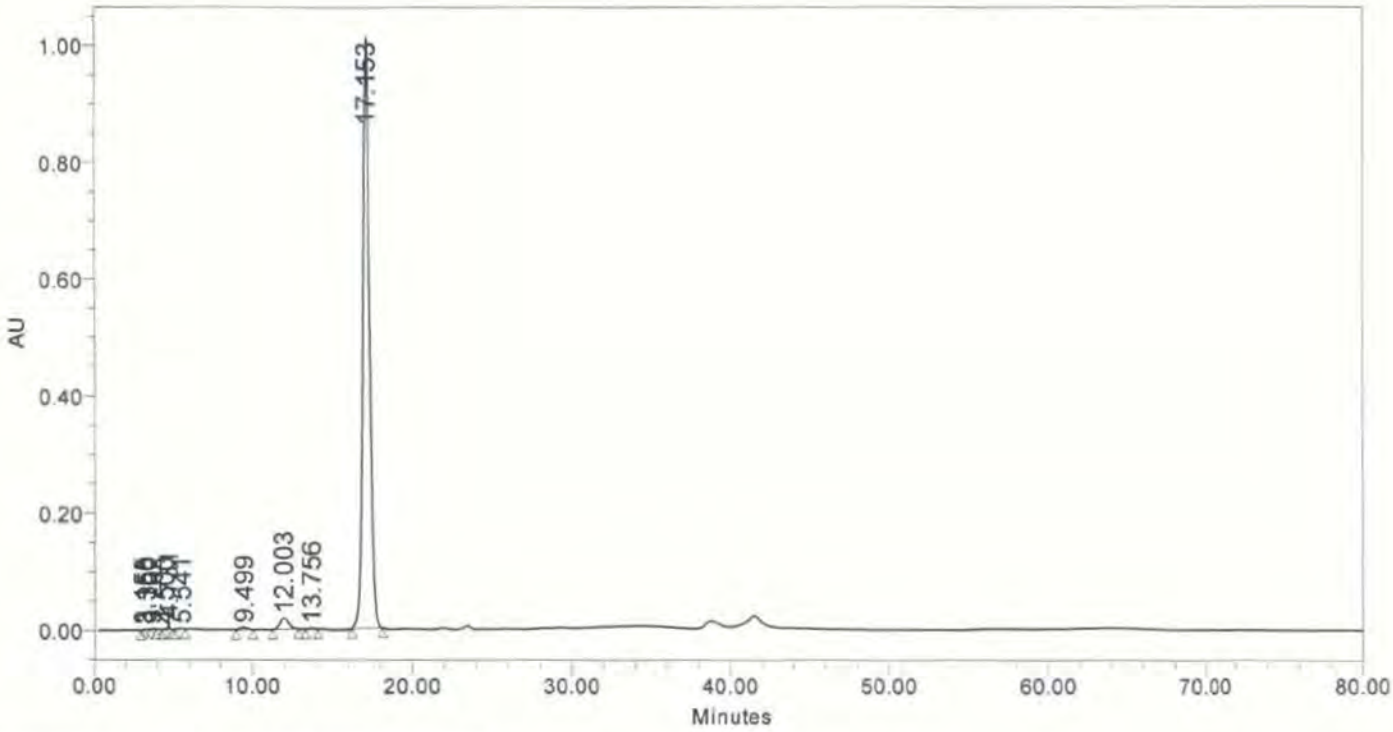
R = 0.997

Appendix C

Raw Data

SAMPLE INFORMATION

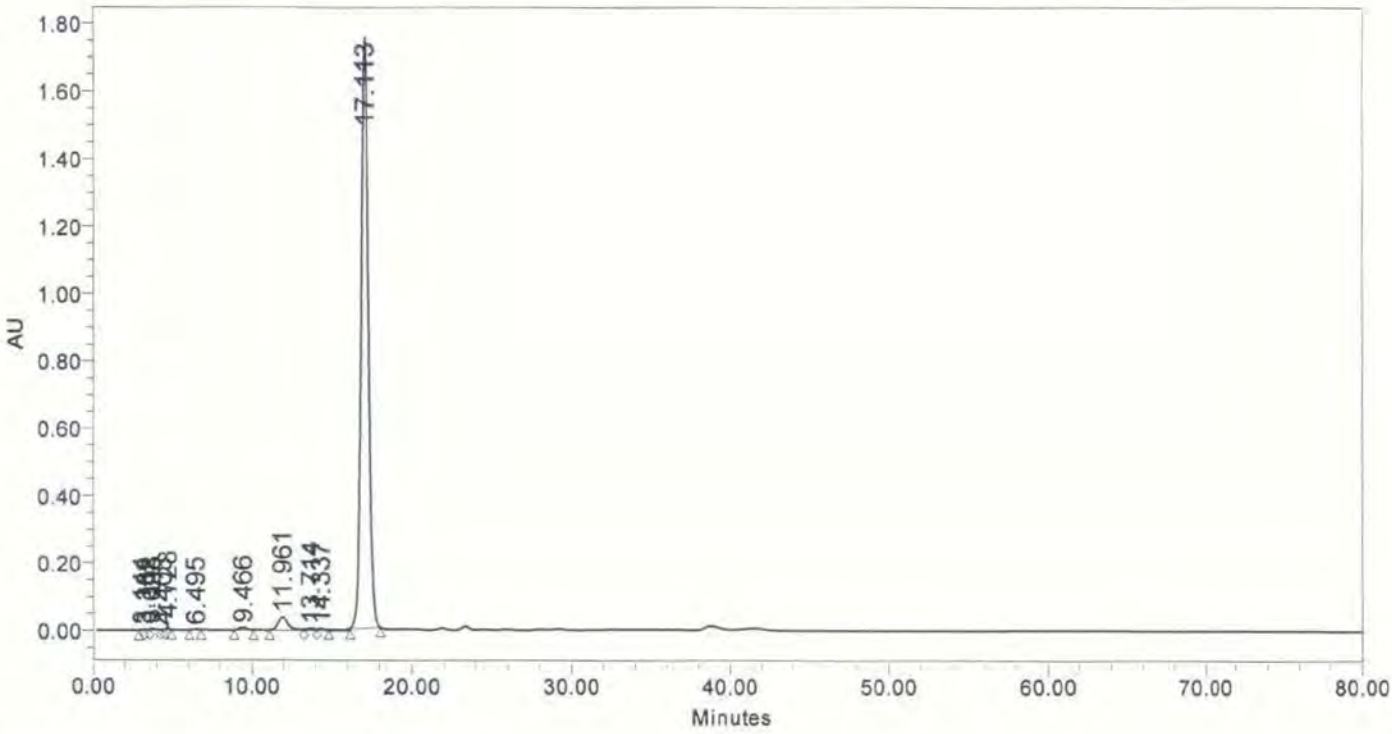
Sample Name:	STEVIOSIDE(5.0mg/mL)	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	GRAS
Vial:	18	Acq. Method Set:	G—AB—MS
Injection #:	1	Processing Methoc	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	80.0 Minutes	Proc. Chnl. Descr.:	
Date Acquired:	2019-02-14 PM 12:12:58 KST		
Date Processed:	2019-02-18 AM 9:46:46 KST		



	RT	Area	% Area	Height
1	3.155	6234	0.02	571
2	3.350	11908	0.04	776
3	3.702	9334	0.03	1088
4	4.500	3143	0.01	352
5	4.731	46086	0.15	9978
6	5.541	18634	0.06	1131
7	9.499	96703	0.31	3199
8	12.003	693874	2.23	18392
9	13.756	29912	0.10	1322
10	17.153	30247554	97.06	1010126

SAMPLE INFORMATION

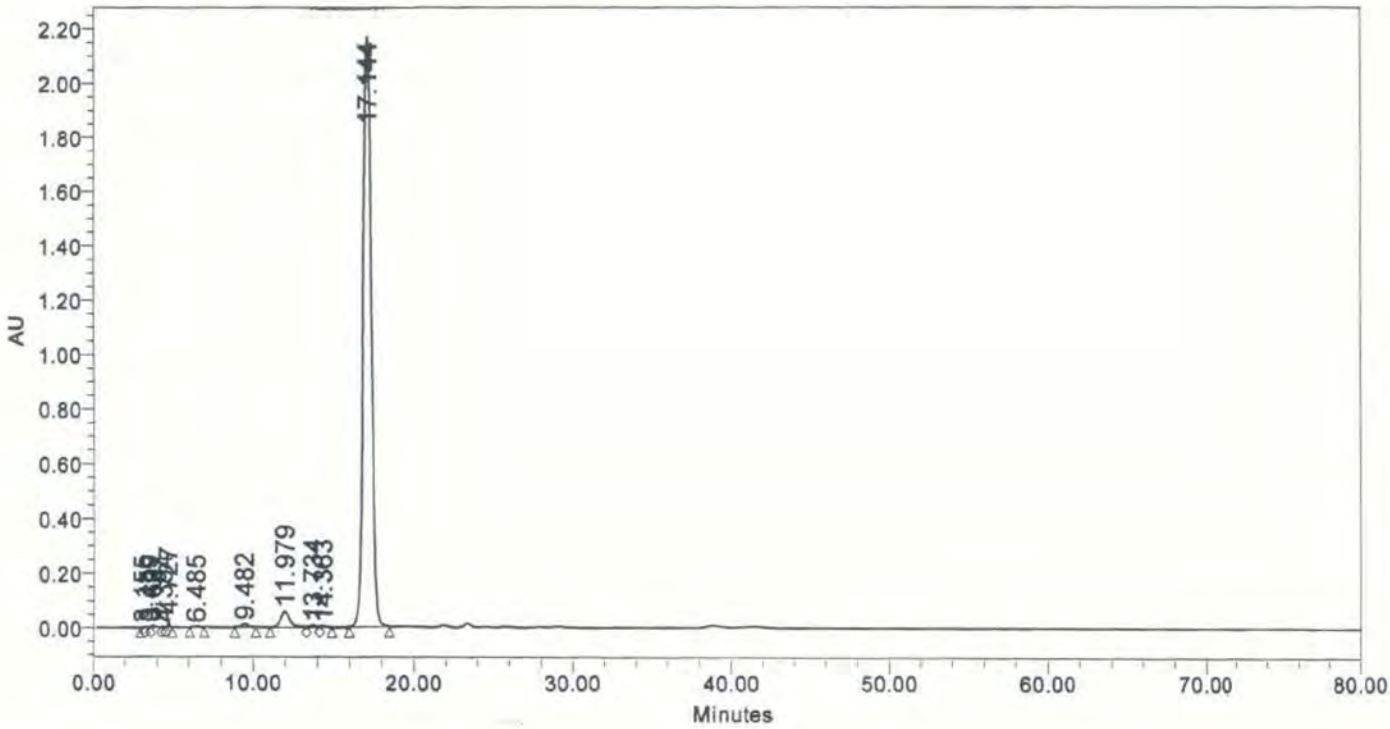
Sample Name:	STEVIOSIDE(10.0mg/mL)	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	GRAS
Vial:	19	Acq. Method Set:	G—AB—MS
Injection #:	1	Processing Methoc	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	80.0 Minutes	Proc. Chnl. Descr.:	
Date Acquired:	2019-02-14 PM 1:34:05 KST		
Date Processed:	2019-02-18 AM 9:47:11 KST		



	RT	Area	% Area	Height
1	3.144	14091	0.02	1283
2	3.382	36527	0.06	2159
3	3.698	78702	0.14	3868
4	4.403	16136	0.03	1341
5	4.728	99297	0.17	20129
6	6.495	28621	0.05	1207
7	9.466	204539	0.36	6509
8	11.961	1539641	2.68	37405
9	13.714	130378	0.23	4152
10	14.337	70091	0.12	2618
11	17.113	55267784	96.14	1751419

SAMPLE INFORMATION

Sample Name:	STEVIOSIDE(15.0mg/mL)	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	GRAS
Vial:	20	Acq. Method Set:	G—AB—MS
Injection #:	1	Processing Methoc	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	80.0 Minutes	Proc. Chnl. Descr.:	
Date Acquired:	2019-02-14 PM 2:55:12 KST		
Date Processed:	2019-02-18 AM 9:45:49 KST		



	RT	Area	% Area	Height
1	3.155	14863	0.02	1302
2	3.459	45754	0.06	2625
3	3.697	113305	0.14	5889
4	4.394	20475	0.03	1783
5	4.727	142312	0.18	29334
6	6.485	47660	0.06	1822
7	9.482	310828	0.40	9838
8	11.979	2295699	2.93	56736
9	13.734	195548	0.28	6219
10	14.363	106253	0.14	3910
11	17.144	80947221	95.79	2166115

SAMPLE INFORMATION

Sample Name:	STEVIOSIDE(20.0mg/mL)	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	GRAS
Vial:	21	Acq. Method Set:	G—AB—MS
Injection #:	1	Processing Method:	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	80.0 Minutes	Proc. Chnl. Descr.:	
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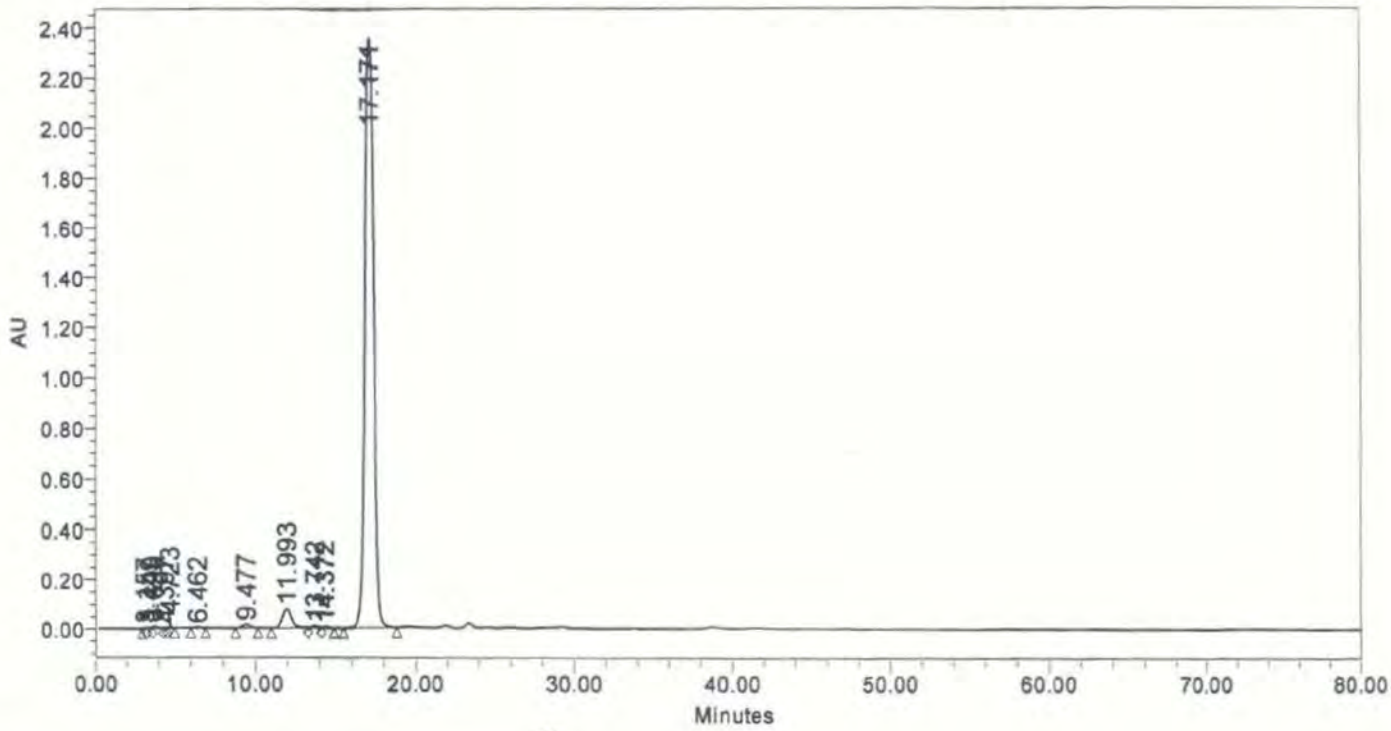
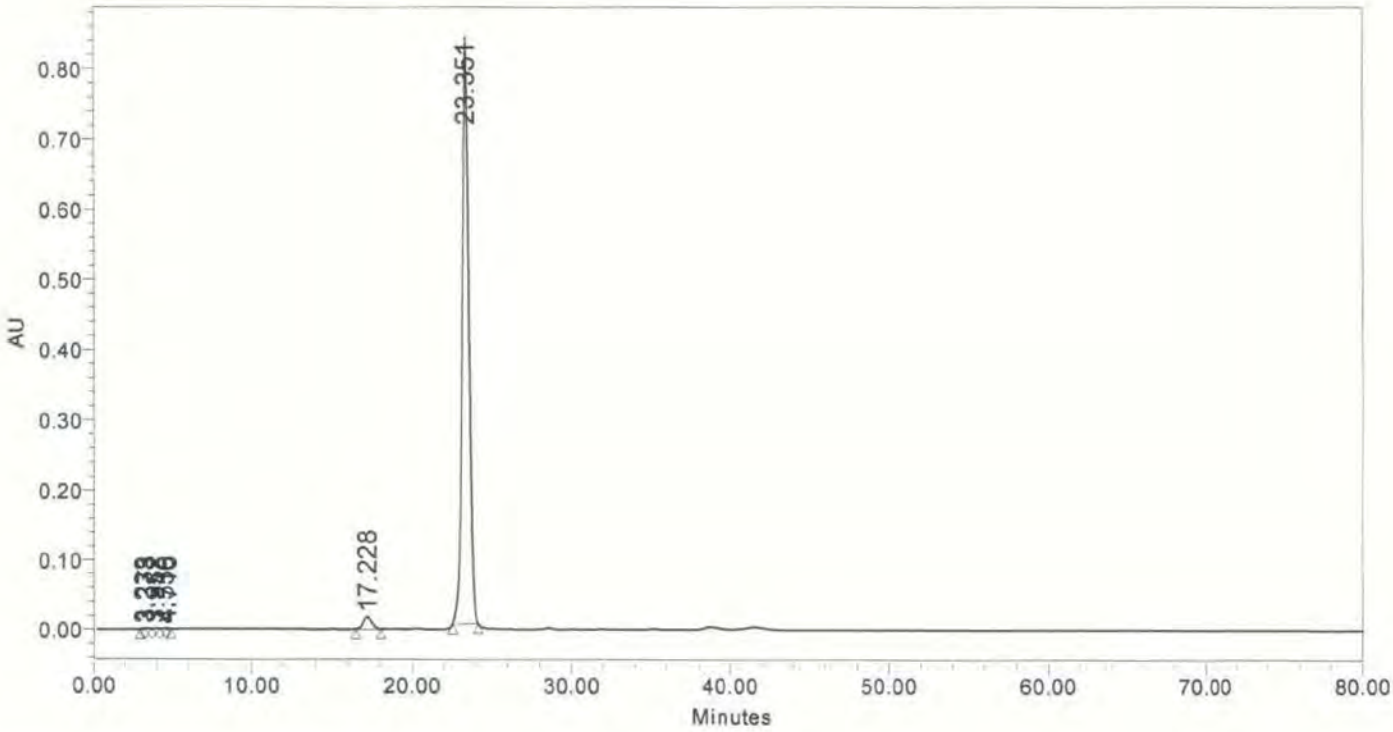
Date Processed:	2019-02-18 AM 9:41:59 KST		



	RT	Area	% Area	Height
1	3.157	19484	0.02	1714
2	3.449	54296	0.06	3045
3	3.699	144121	0.12	7738
4	4.397	26932	0.03	2393
5	4.723	195784	0.18	40016
6	6.462	64236	0.07	2362
7	9.477	424964	0.40	13380
8	11.993	3088985	2.97	77910
9	13.742	263473	0.18	8415
10	14.372	143274	0.10	5235
11	17.171	109386339	95.87	2352965

SAMPLE INFORMATION

Sample Name:	REBAUDIOSIDE A(5.0mg/mL)	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	GRAS
Vial:	22	Acq. Method Set:	G—AB—MS
Injection #:	1	Processing Metho	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	80.0 Minutes	Proc. Chnl. Descr.:	
Date Acquired:	2019-02-14 PM 7:27:34 KST		
Date Processed:	2019-02-18 AM 11:49:10 KST		



	RT	Area	% Area	Height
1	3.233	5393	0.02	683
2	3.378	24605	0.10	1349
3	3.952	13127	0.05	708
4	4.559	14678	0.06	913
5	4.716	8446	0.03	1105
6	17.228	628761	2.49	17536
7	23.351	24574770	97.25	837535

SAMPLE INFORMATION

Sample Name:	REBAUDIOSIDE A(10.0mg/mL)	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	GRAS
Vial:	23	Acq. Method Set:	G—AB—MS
Injection #:	1	Processing Methoc	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	80.0 Minutes	Proc. Chnl. Descr.:	
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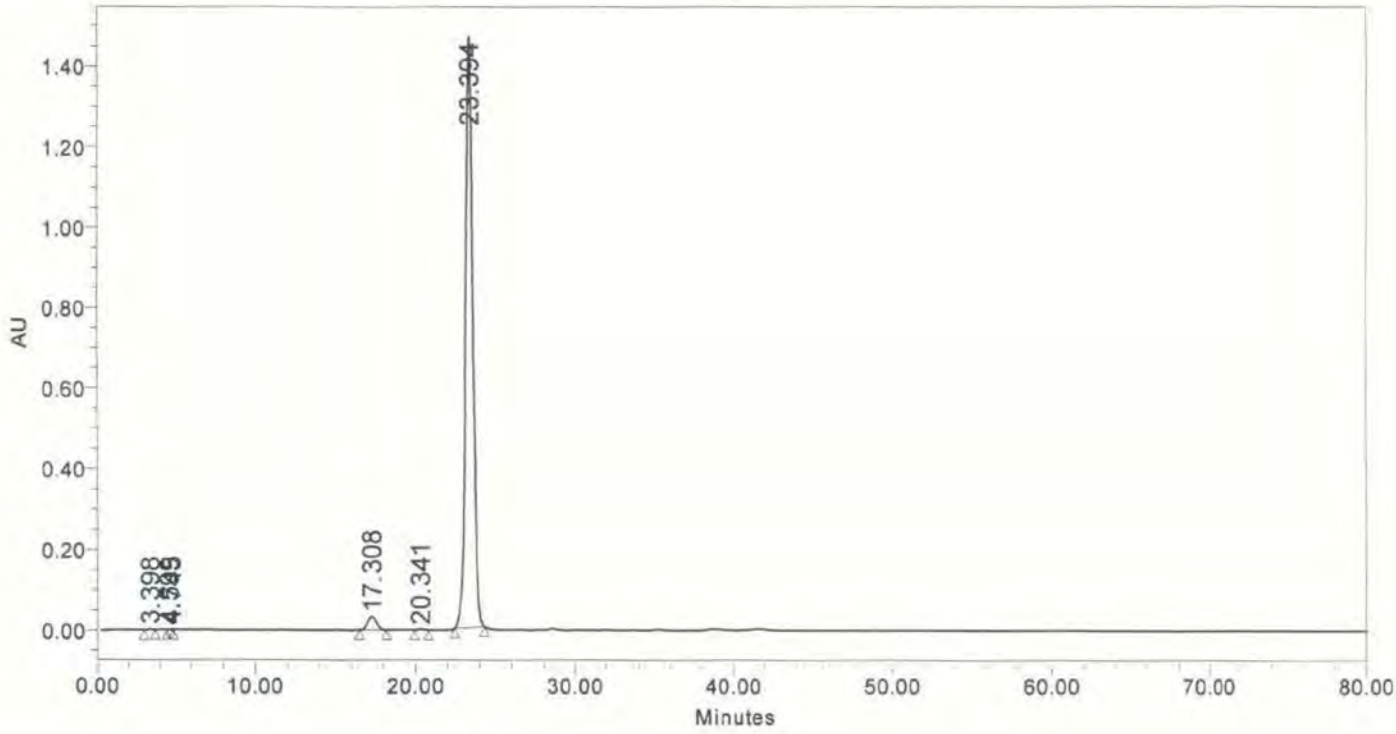
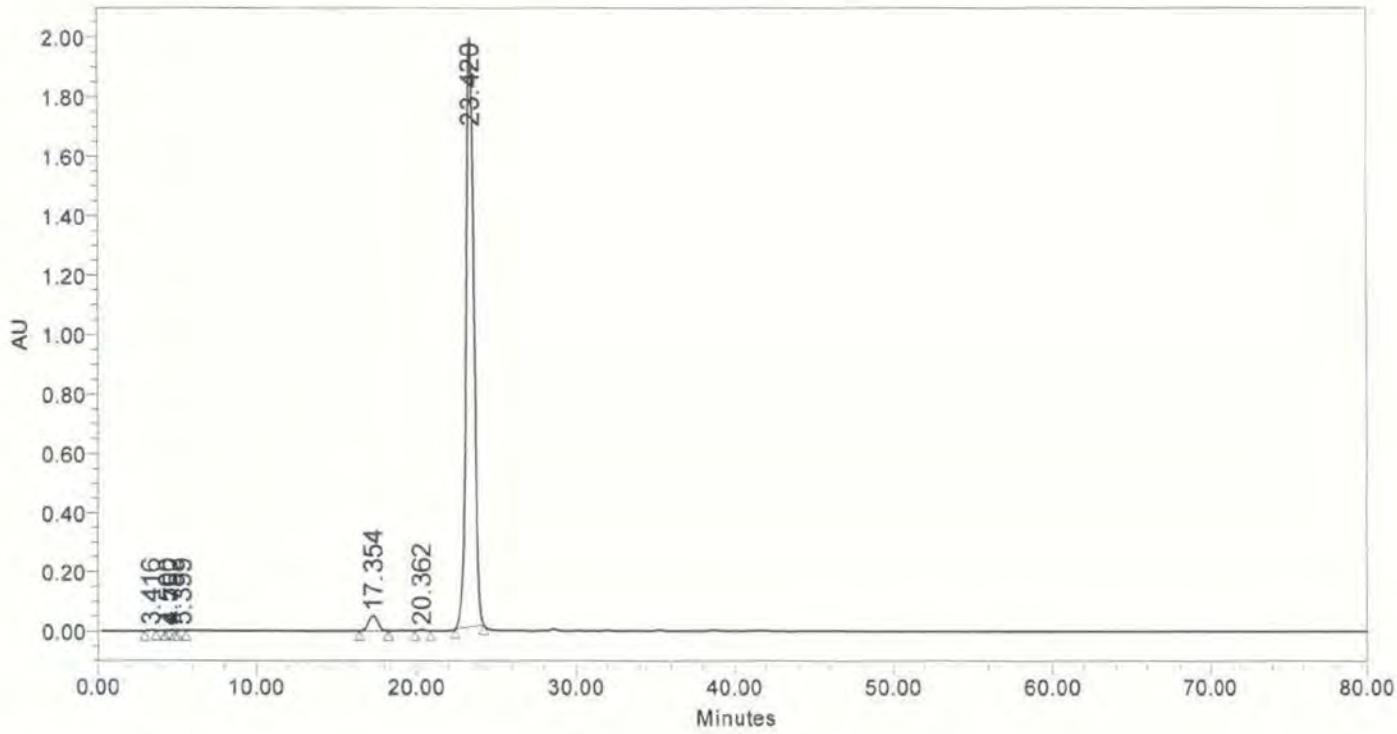
Date Processed:	2019-02-18 AM 11:48:16 KST		



	RT	Area	% Area	Height
1	3.398	30628	0.07	1629
2	4.599	4724	0.01	500
3	4.745	6677	0.01	1489
4	17.308	1189631	2.57	32456
5	20.341	41380	0.09	1616
6	23.394	44930455	97.24	1466044

SAMPLE INFORMATION

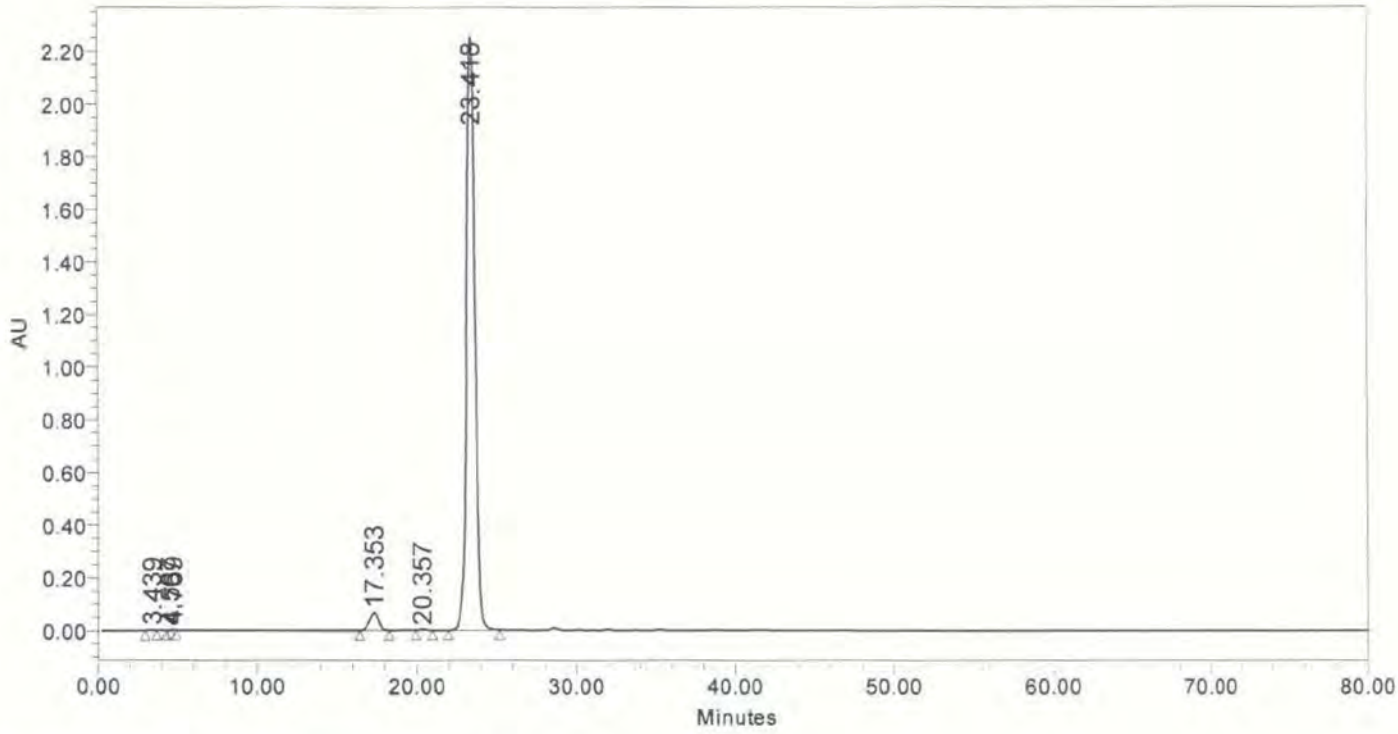
Sample Name:	REBAUDIOSIDE A(15.0mg/mL)	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	GRAS
Vial:	24	Acq. Method Set:	G—AB—MS
Injection #:	1	Processing Methoc	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	80.0 Minutes	Proc. Chnl. Descr.:	
Date Acquired:	2019-02-14 PM 10:09:46 KST		
Date Processed:	2019-02-18 AM 11:49:43 KST		



	RT	Area	% Area	Height
1	3.416	26613	0.04	1231
2	4.500	2636	0.00	436
3	4.752	17882	0.03	2550
4	5.399	11835	0.02	848
5	17.354	1881668	2.82	49545
6	20.362	70418	0.11	2607
7	23.420	64742431	96.99	1981808

SAMPLE INFORMATION

Sample Name:	REBAUDIOSIDE A(20.0mg/mL)	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	GRAS
Vial:	25	Acq. Method Set:	G—AB—MS
Injection #:	1	Processing Method	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	80.0 Minutes	Proc. Chnl. Descr.:	
Date Acquired:	2019-02-14 PM 11:31:05 KST		
Date Processed:	2019-02-18 AM 10:22:57 KST		



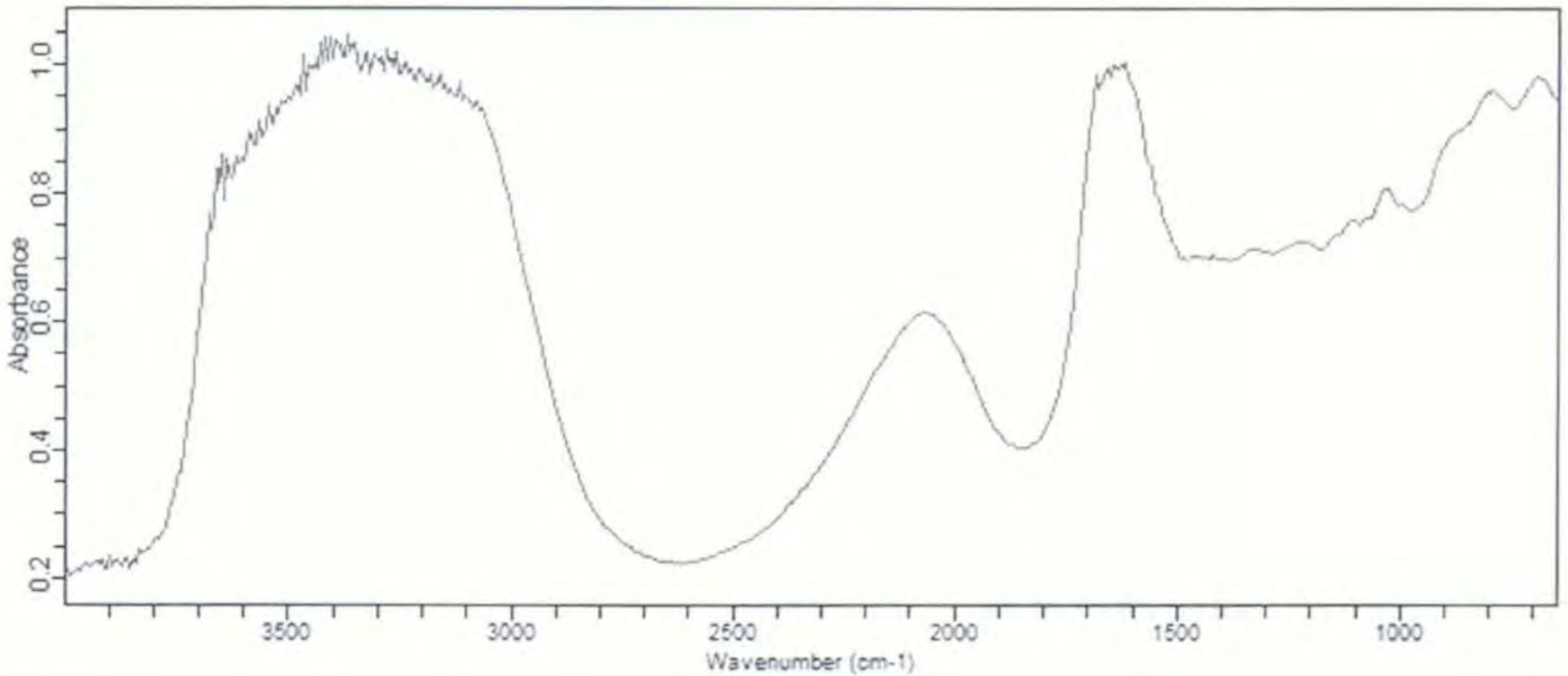
	RT	Area	% Area	Height
1	3.439	27445	0.03	1364
2	4.567	4335	0.01	587
3	4.769	19739	0.02	2916
4	17.353	2502823	2.98	65117
5	20.357	100564	0.12	3642
6	23.418	81289585	96.84	2250011

4. FTIR SPECTRUM OF ENZYME TREATED

Appendix C

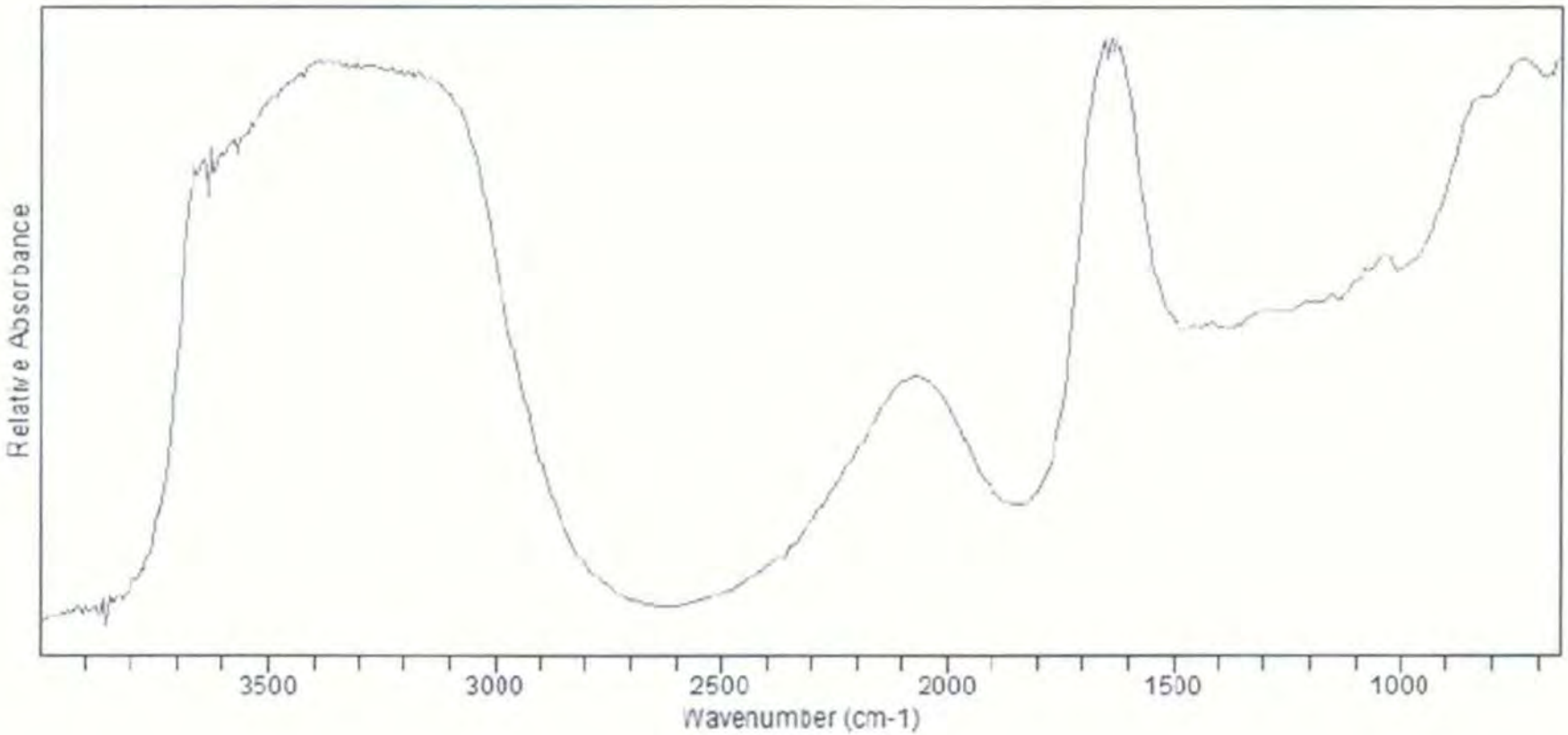
Raw Data

STEVITEN FRESH Lot. 181128



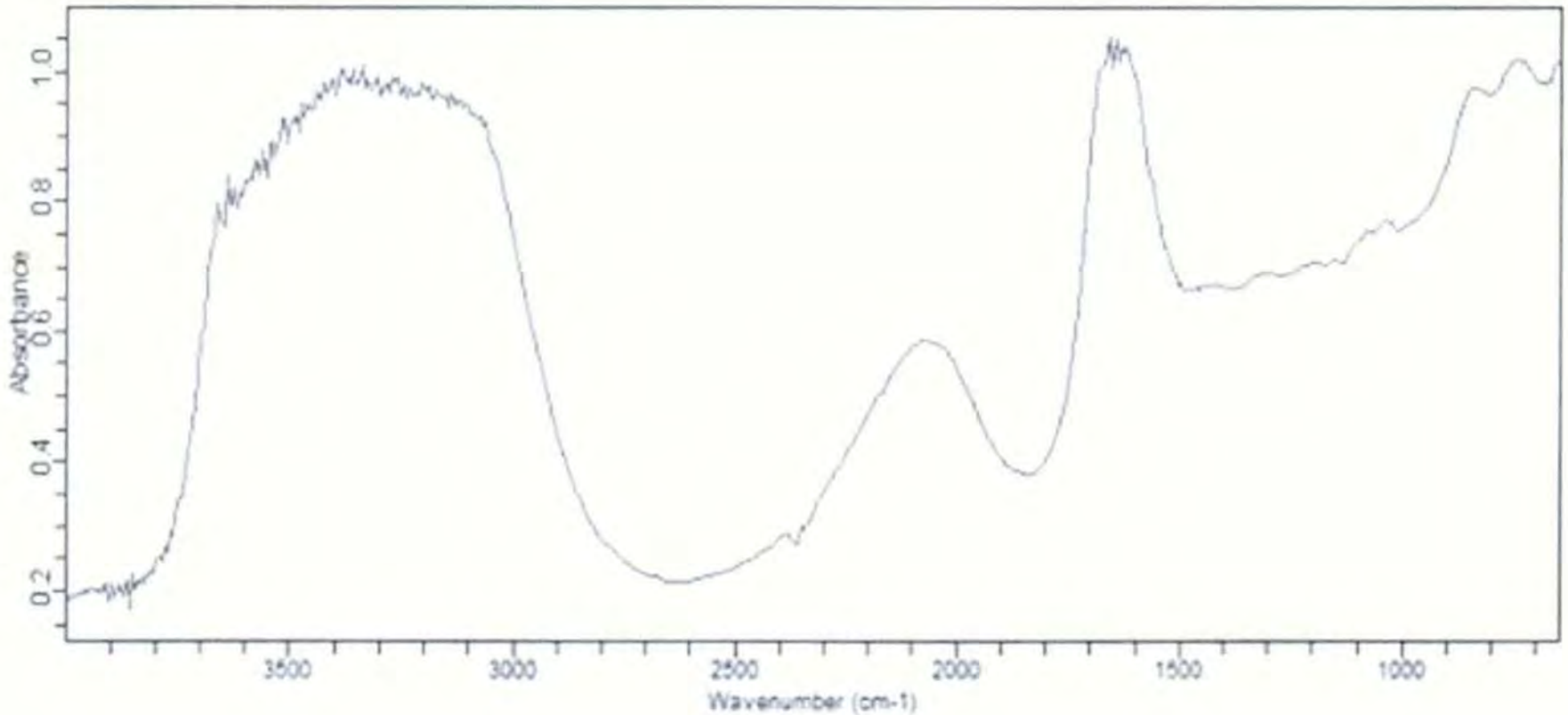
FTIR Spectrum of enzyme treated Lot. 181128

STEVITEN FRESH Lot. 181213



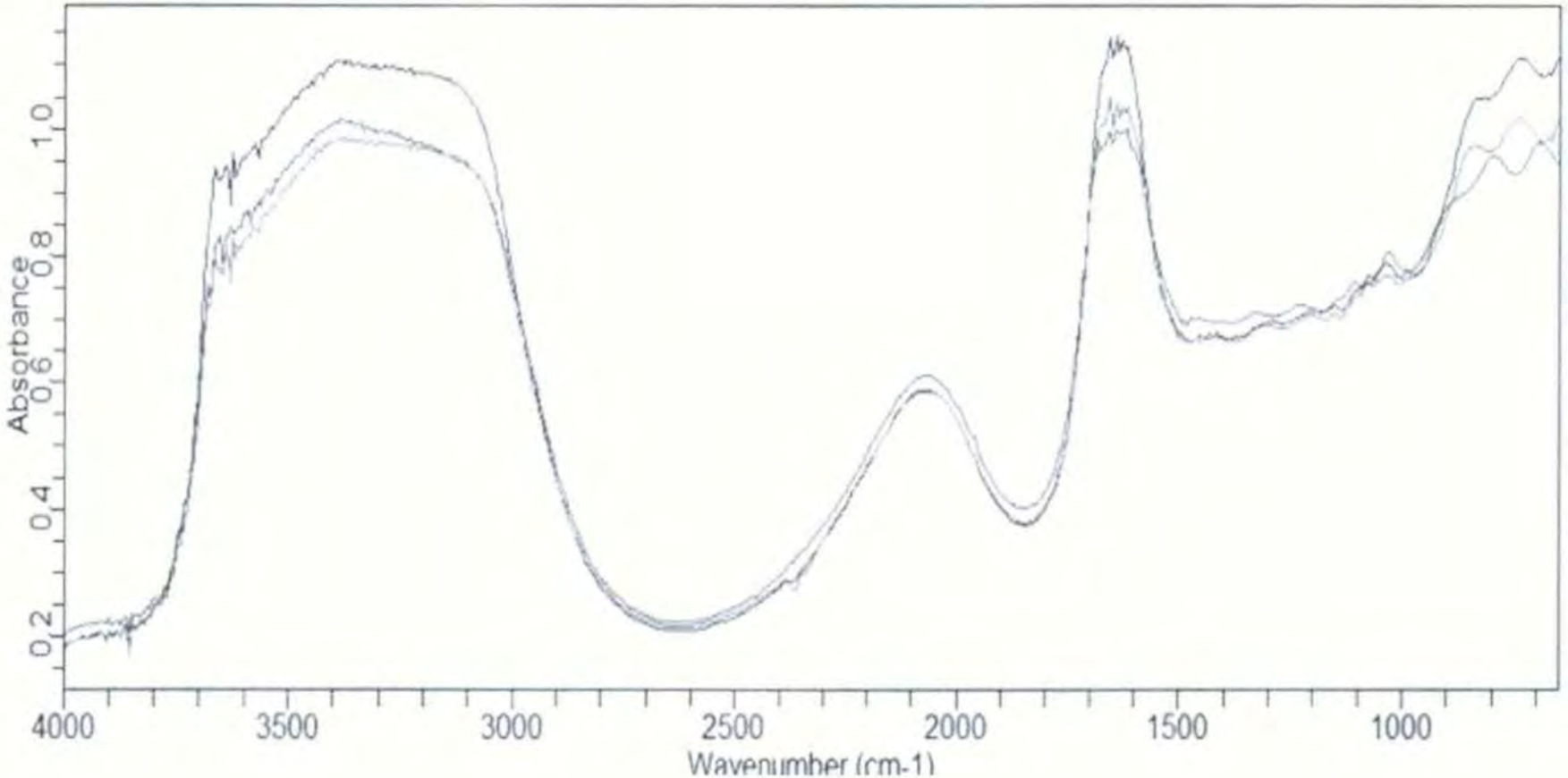
FTIR Spectrum of enzyme treated Lot. 181213

STEVITEN FRESH Lot. 181219



FTIR Spectrum of enzyme treated Lot. 181219

STEVITEN FRESH 3Lot. Overlay



5. A Comparison of the fingerprints of enzyme treated stevia, as analyzed by Ab Sciex LC/MS

Methods

UPLC Conditions

LC/MS system: NANOSPACE 5200 / Ab Sciex QTRAP 4500
Column: ACQUITY UPLC BHE Amide 1.7um, 2.1x150mm
Column Temp: 40℃
Flow Rate: 160 uL/min
Injection Volume: 1uL
Detection: uv@210nm
Mobile Phase A: Water, B: Acetonitrile

• Gradient

Time(min)	% A	% B	Curve
Init	30	70	-
10.0	30	70	6
25.0	50	50	6
26.0	70	30	6
35.0	70	30	6

MS Conditions

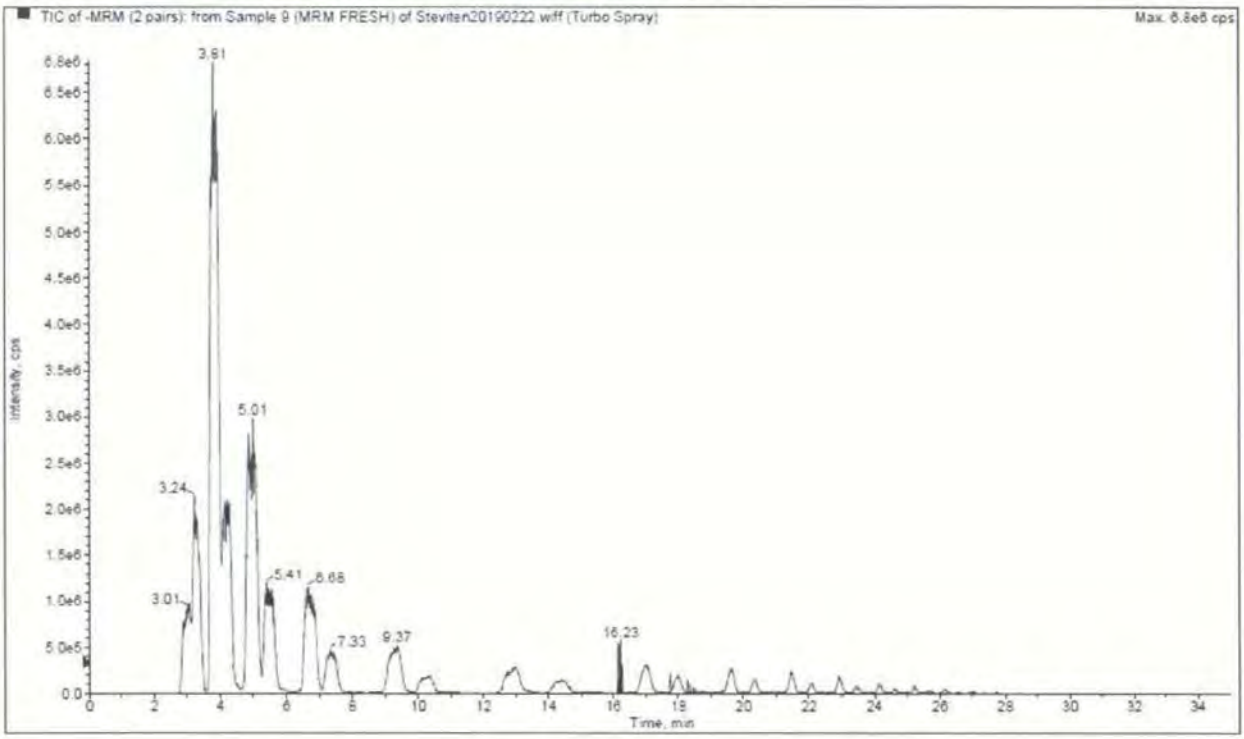
Ionization Mode : ESL negative
Curtain Gas : 30
IonSpray Voltage(IS) : 4500
Temperature : 400℃
Ion Source Gas : 50

- MS Scan Mode
  Mass Range : 500~2000Da
  Scan Time : 0.75s

- MS/MS Mode
  Mass Range : 100~2000Da
  Scan Time : 0.2s
  Collision Energy(CE) : 30

Appendix E
Raw Data

Figure 7. A Comparison of the finger prints of enzyme treated Stevia, as analyzed by Ab Sciex LC/MS (STEVITEN FRESH)



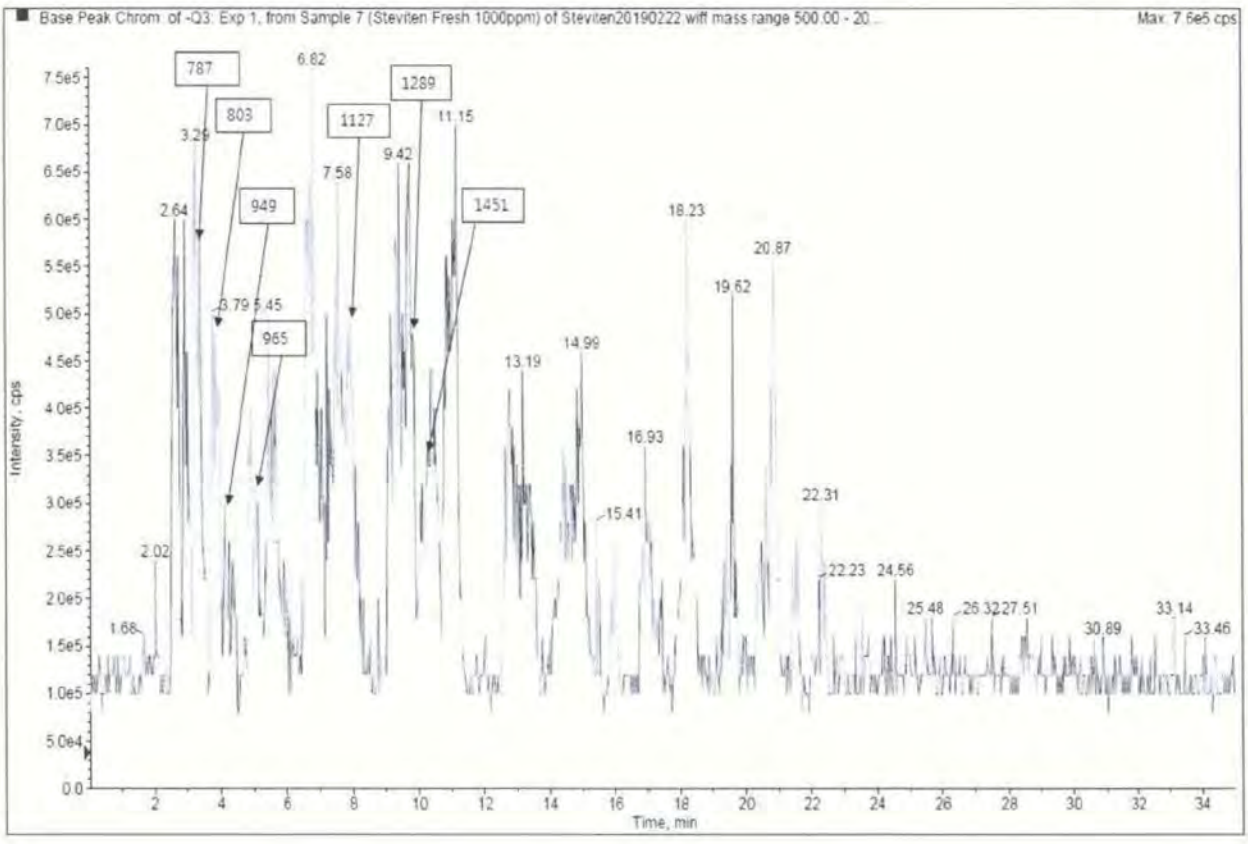
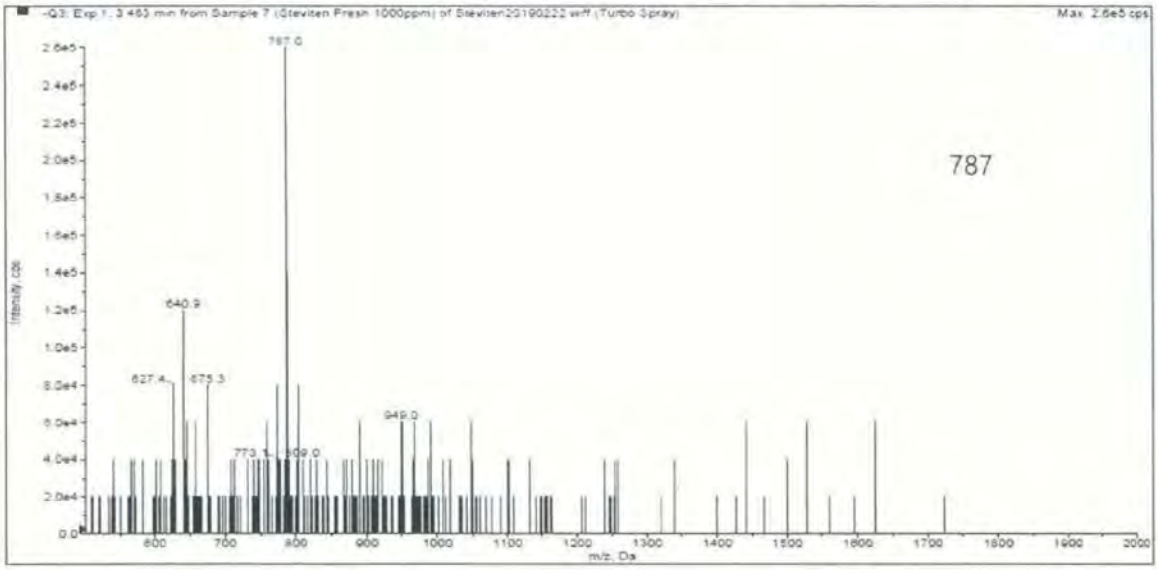
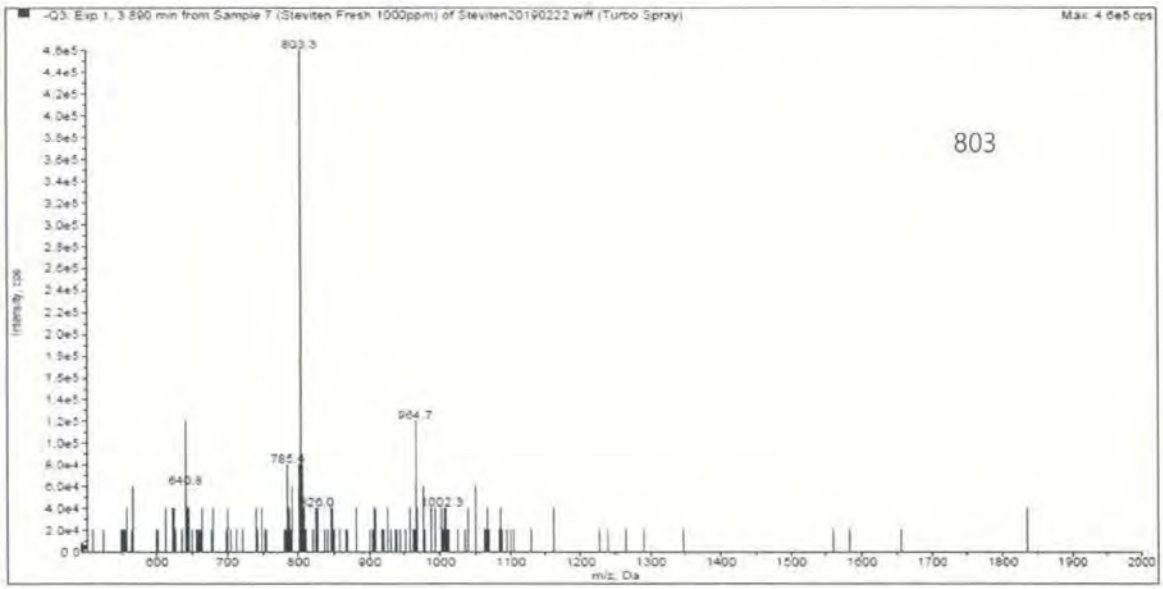
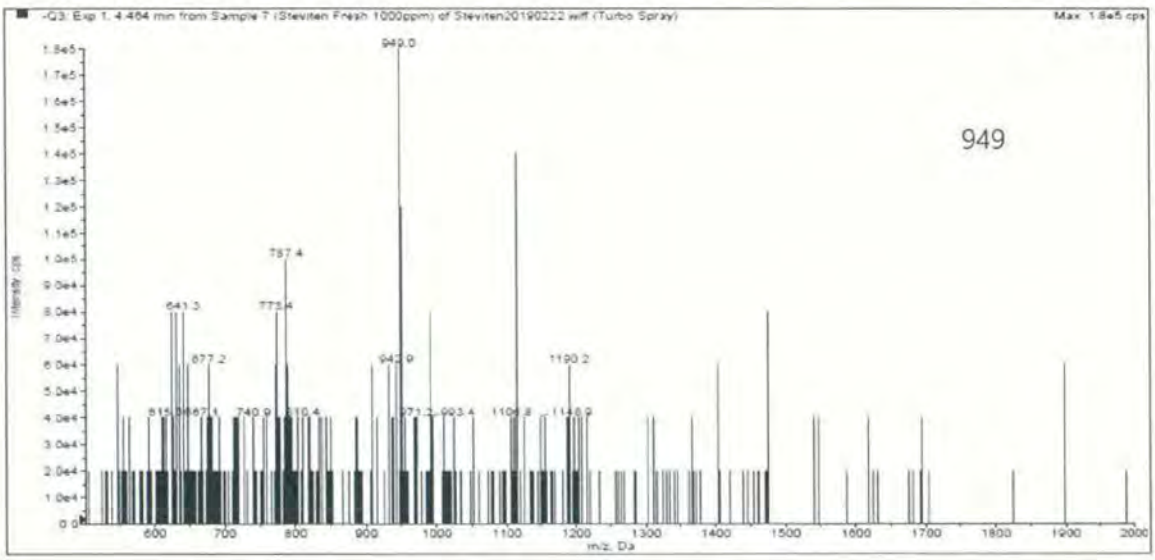
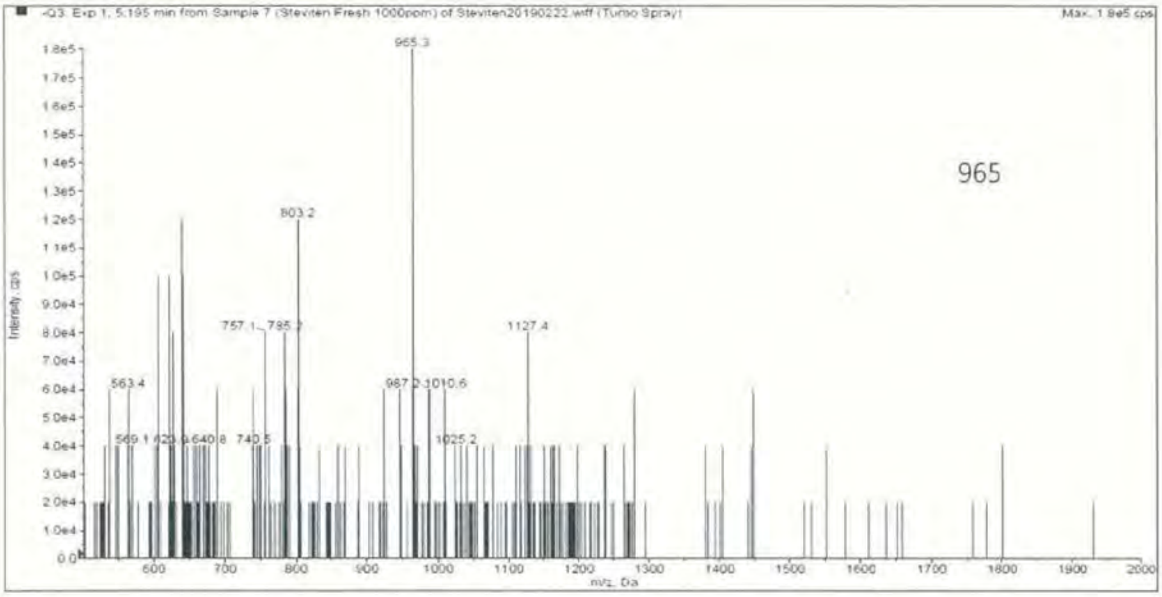


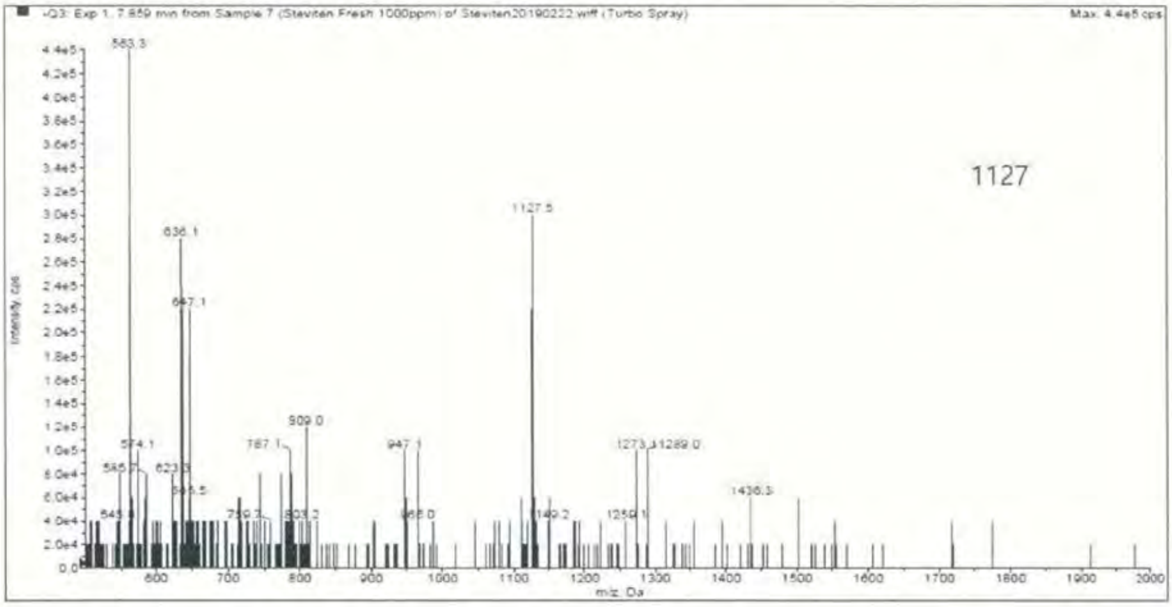
Figure 8. The molecular weigh of Steviol glucosides Componets

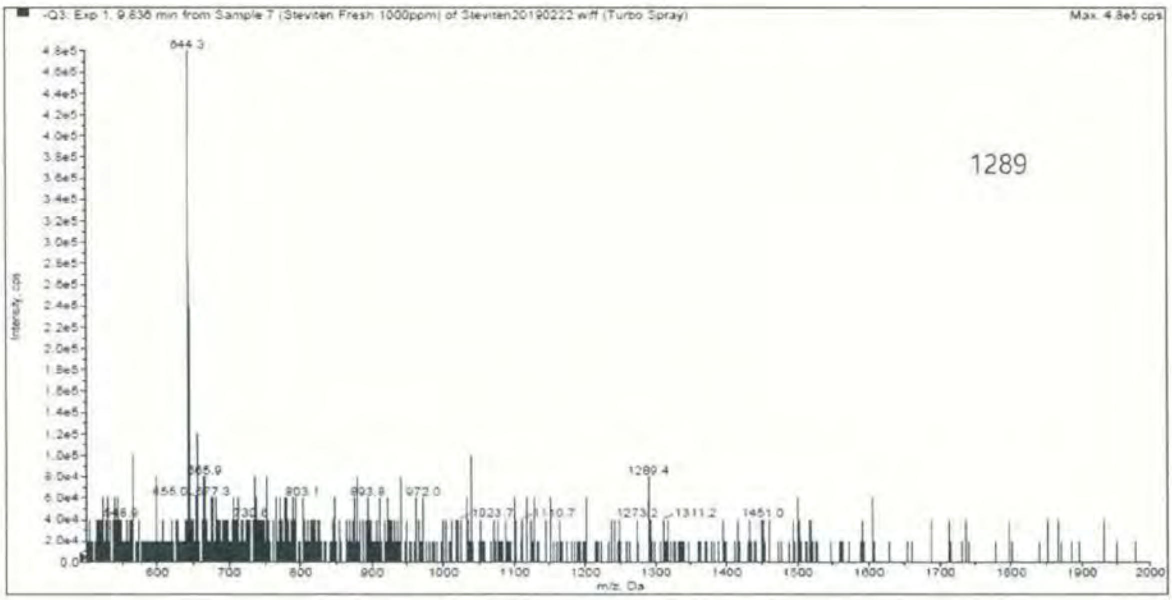







-Q3: Exp 1, 5.195 min from Sample 7 (Steviten Fresh 1000ppm) of Steviten20190222.wiff (Turbo Spray)
Max. 1.8e5 cps
965
965.3
803.2
757.1
785.2
1127.4
563.4
987.2
1010.6
569.1
640.8
740.5
1025.2
Intensity, cps
m/z, Da


-Q3: Exp 1, 7.859 min from Sample 7 (Steviten Fresh 1000ppm) of Steviten20190222.wiff (Turbo Spray)
Max. 4.4e5 cps
1127
563.3
1127.5
636.1
647.1
809.0
574.1
787.1
947.1
1273.1
1289.0
585.7
623.3
1436.3
779.3
803.2
966.0
1259.1
Intensity, cps
m/z, Da


-Q3: Exp 1, 9.636 min from Sample 7 (Steviten Fresh 1000ppm) of Steviten20190222.wiff (Turbo Spray)
Max. 4.8e5 cps
1289
644.3
1289.4
665.9
656.0
677.3
803.1
972.0
730.6
1273.2
1311.2
1451.0
Intensity, cps
m/z, Da

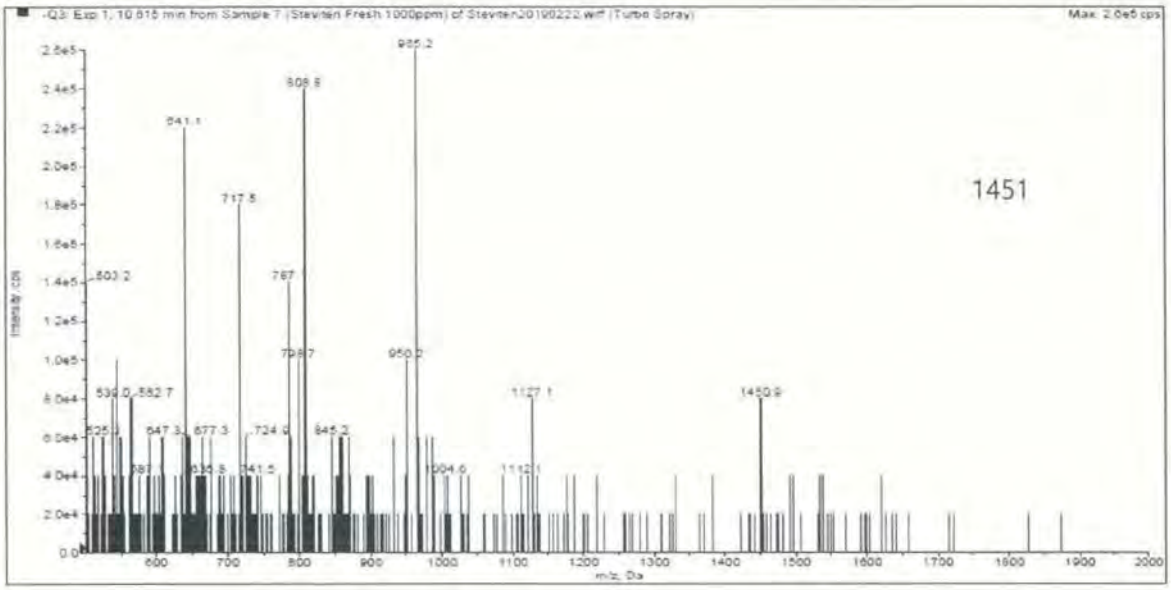


Figure 7. We know each position of steviol glycosides components by LC and also know molecular weight by Ab Sciex. Also we can see the same peak at the same time.

Figure 8. We can distinguish each component of steviol glycosides by molecular weight of Ab Sciex

Report of Chemical Characterization of Glucosylated Steviol Glycosides (STEVITEN RICH)

File No. : DP-R-AD-STEVITEN-RICH (0)
Date : 25. Feb. 2019

Prepared by : R.K. Kim /R&D
Prepared by : S.J. Kim /QA
Approved by : K.J. Kim /President

DAEPYUNG CO., LTD.

Table of contents

1. Analysis of Glucosylated Steviol Glycosides by HPLC

Methods

HPLC Conditions

Instrument : Alliance 2695-2487(Waters)
Column : TOSOH, TSKgel Amide-80(250X4.6mm, 5um)
Column Temp : 30°C
Flow Rate : 0.85mL/min
Injection Volume : 20uL
Detection : uv@210nm
Mobile Phase A: Acetonitrile / 5%Water
B: Water / 5%Acetonitrile

• Gradient

Time(min)	% A	% B	Curve
0	89	11	-
31	77	23	6
33	77	23	6
34	68	32	6
35	77	23	6
37	70	30	6
38	77	23	6
70	77	23	6
80	89	11	6

Sample Preparation

Enzyme Treated Stevia extracts were prepared in 50% Acetonitrile/Water at a concentration of 20mg/mL.
Stevia extracts were prepared in 50% Acetonitrile/Water at a concentration of 20mg/mL.

Standard Preparation

Stevioside standard and Rebaudioside A standard was prepared in 50% Acetonitrile/Water at the following concentrations:

Stevioside 5, 10, 15, 20mg/mL
Rebaudioside A 5, 10, 15, 20mg/mL

Glucosylated Steviol Glycoside Components

Compound	m.w.	Empirical Formula	Level of Enzyme Glycosylation
Rubusoside	642	$C_{32}H_{50}O_{13}$	-
Steviolbioside	642	$C_{32}H_{50}O_{13}$	-
Dulcoside A	788	$C_{38}H_{60}O_{17}$	-
Rebaudioside B	804	$C_{38}H_{60}O_{18}$	-
Stevioside	804	$C_{38}H_{60}O_{18}$	-
Rebaudioside C	950	$C_{44}H_{70}O_{22}$	-
Rebaudioside F	936	$C_{43}H_{68}O_{22}$	-
Rebaudioside A	966	$C_{44}H_{70}O_{23}$	-
Rebaudioside D	1129	$C_{50}H_{80}O_{28}$	-
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
Diglucosyl Stevioside	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 Stevioside	1290	$C_{56}H_{90}O_{33}$	+3
Triglucosyl Rebaudioside A	1452	$C_{62}H_{100}O_{38}$	+3
Tetraglucosyl Stevioside	1452	$C_{62}H_{100}O_{38}$	+4
Tetraglucosy Rebaudioside A	1614	$C_{68}H_{110}O_{43}$	+4

Results

Table 1. Determination of the level of non-steviol glycosides components, by percent, in an enzyme treated stevia sample

The non- stevioside components were found to elute early in the analysis with retention times between 3.73 and 8.50 minutes. The chromatographic Purities of the samples were determined by normalizing the total peak area to 100%

1) Lot. 181107

Peak NO.	RT(min)	Area (%)	Total (%)
1	3.739	0.82	
2	4.156	1.80	
3	4.749	1.59	
4	6.452	0.21	
5	8.489	0.25	4.67

2) Lot. 181203

Peak NO.	RT(min)	Area (%)	Total (%)
1	3.735	0.76	
2	4.150	1.58	
3	4.745	1.30	
4	6.450	0.20	
5	8.506	0.23	4.09

3) Lot. 181224

Peak NO.	RT(min)	Area (%)	Total (%)
1	3.736	0.83	
2	4.151	1.74	
3	4.747	1.56	
4	6.447	0.19	
5	8.509	0.23	4.55

Table 2. The levels of Steviosides, by percent range, in non-enzyme treated and enzyme-treated stevia extracts.

To ensure product consistency, the level of stevioside and glucosylstevioside components was determined by HPLC analysis. The ranges determined for pre treated and post treated stevia extracts are found in Table 2

Component	Starting Material (%)	STEVITEN RICH (%)
Rubusoside	2.0 ~ 3.5	0.0 ~ 0.6
Steviolbioside	0.0 ~ 1.0	0.5 ~ 1.5
Dulcoside A	0.0 ~ 1.0	0.5 ~ 1.5
Rebaudioside B	0.0 ~ 1.0	0.0 ~ 1.0
Stevioside	46.0 ~ 50.0	6.0 ~ 7.0
Rebaudioside C	5.5 ~ 6.5	1.0 ~ 2.0
Rebaudioside F	0.5 ~ 1.0	0.0 ~ 0.5
Rebaudioside A	35.0 ~ 40.0	5.0 ~ 6.0
Rebaudioside D	0.0 ~ 0.5	n.d
Monoglucosyl stevioside m/z 966	n.d	8.0 ~ 10.0
Monoglucosyl rebaudioside C m/z 1112	n.d	1.3 ~ 1.6
Monoglucosyl rebaudioside A m/z 1128	n.d	7.0 ~ 9.0
Diglucosyl stevioside m/z 1128	n.d	9.0 ~ 12.0
Diglucosyl rebaudioside C m/z 1274	n.d	1.5 ~ 2.5
Diglucosyl rebaudioside A m/z 1290	n.d	11.0 ~ 13.0
Triglucosy stevioside m/z 1290	n.d	9.0 ~ 14.0
Triglucosyl rebaudioside A m/z 1452	n.d	4.5 ~ 6.5
Tetraglucosyl stevioside m/z 1452	n.d	5.0 ~ 8.0
Tetraglucosy rebaudioside A m/z 1614	n.d	4.0 ~ 6.0
Unidenfied glucosylated m/z > 1614	n.d	4.0 ~ 7.0

Table 3. The level of steviol glycoside and non-stevioside components, by percent, in three lots of stevia extract before enzyme treatment.

The meaning 2.3 , 2.3 & 2.2 of Stevia extract (impurities) % are that steviol glydosides are min 95% Also the meaning of 4.7, 4.1 & 4.6 of Enzyme treated Stevia Extract (impurities) % are that steviol glydosides and Glucosylsteviosides (%) have been found to have greater than 95% purity Therefore, the Glucosylated steviol Extract impurites(%)components were found to be present on levels less than 5%.

Lot.	Starting material impurities(%)	STEVITEN RICH impurites(%)
181107	2.3	4.7
181203	2.3	4.1
181224	2.2	4.6

Table 4. The level of steviosides and non-steviosides, by percent, in three lots of enzyme treated stevia.

The same three lots of stevia extract were then enzyme treated, and subsequently tested for chromatographic purity on a molar basis. The results were obtained using the same method, and are shown in table 4. All Three enzyme treated stevia lots have been found to have greater than 95% purity, as demonstrated by HPLC chromatographic analysis.

Lot	Steviol glycosides and Glucosylsteviosides (%)	Glucosylated steviol glycosides impurites(%)
181107	95.3	4.7
181203	95.9	4.1
181224	95.4	4.6

Appendix A
Raw Data

Figure 1. HPLC Analysis of Stevia Extract Extract Before and after Enzyme Treatment

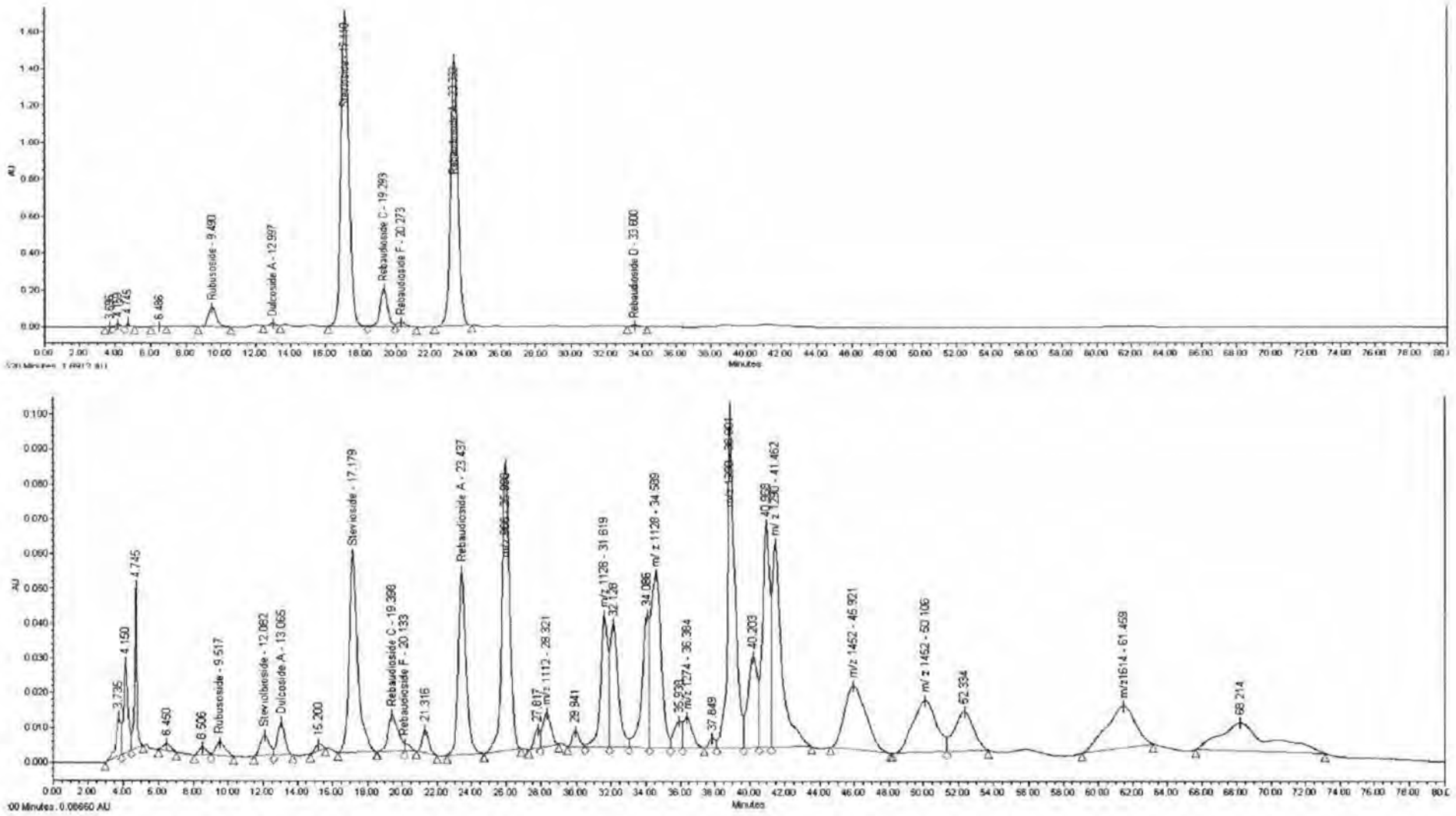
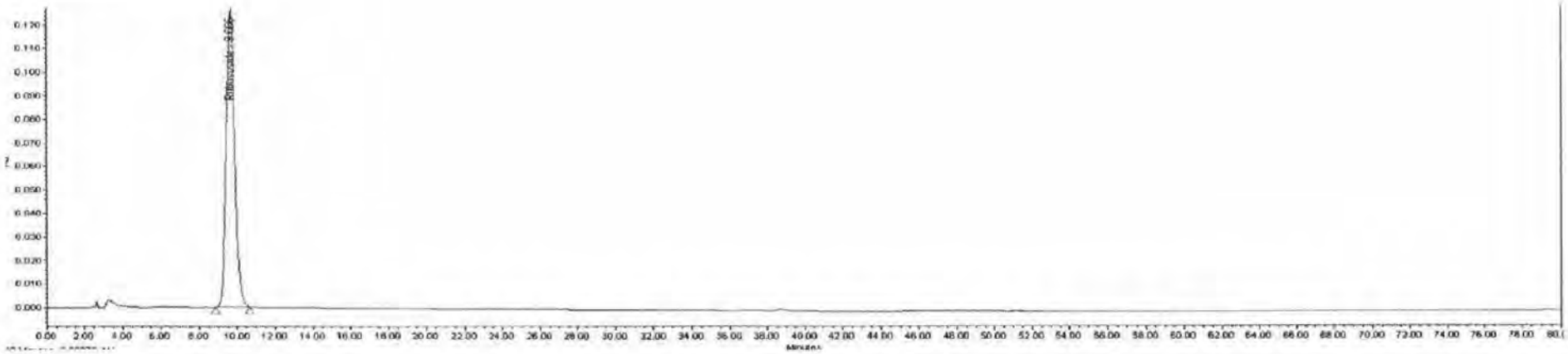
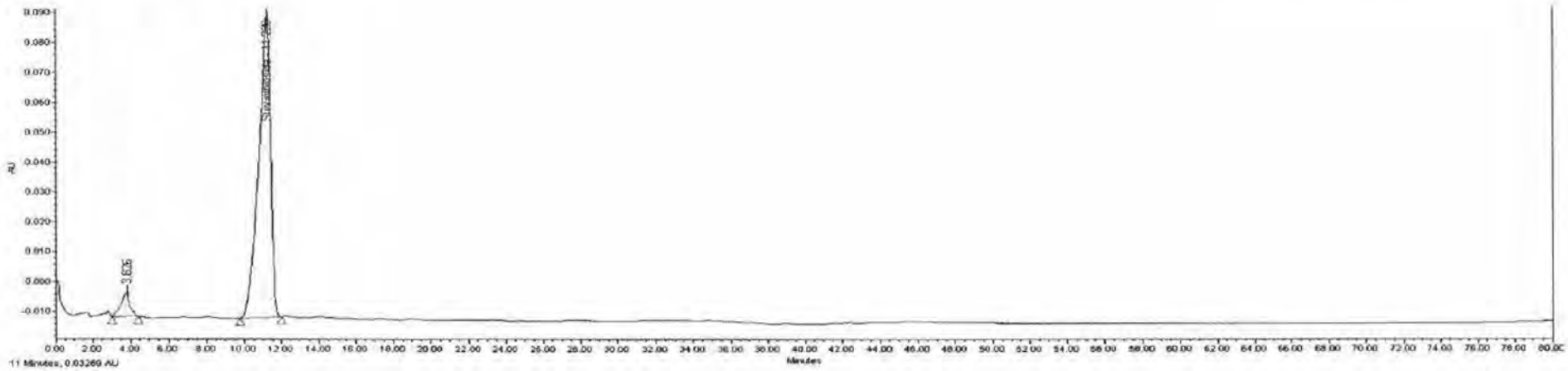


Figure 2. Spectral characteristics of Stevia Extract components(Standard)

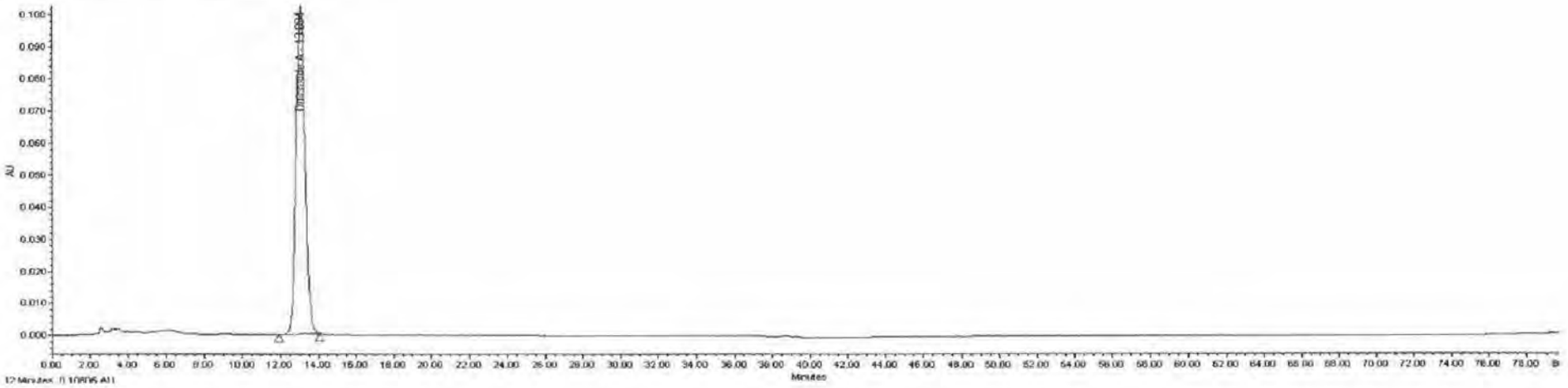
Rubusoside STD



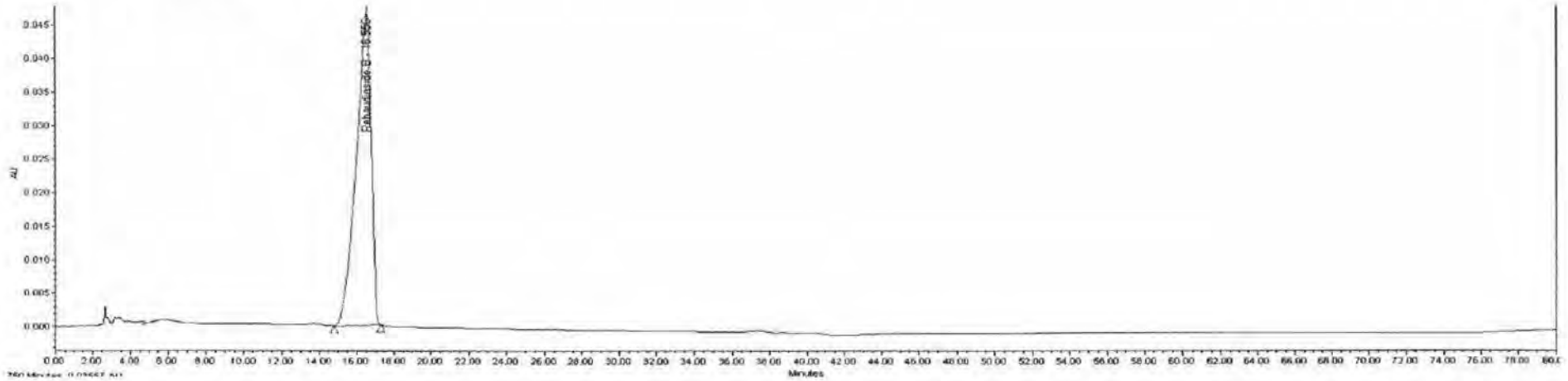
Steviolbioside STD



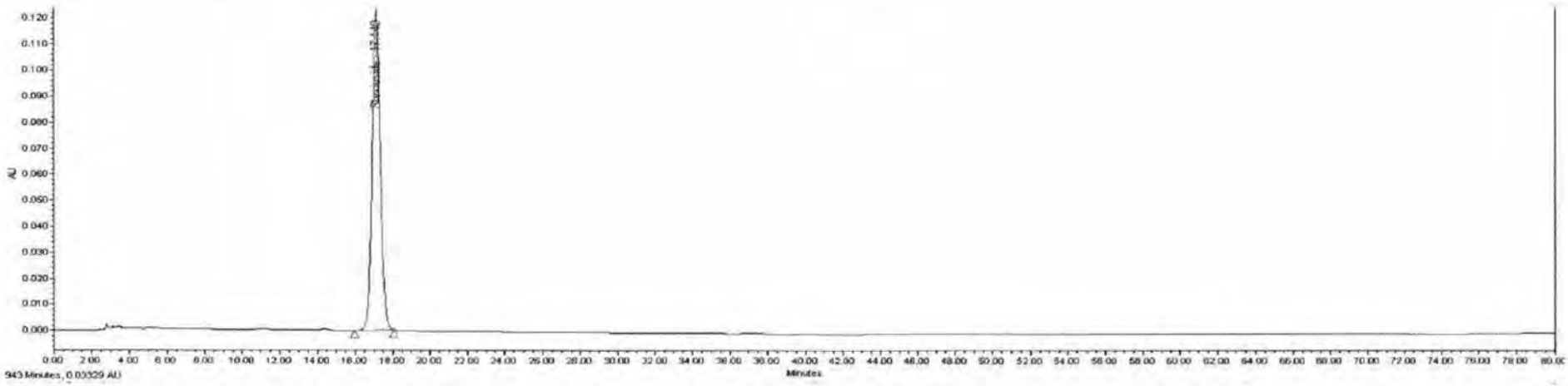
Dulcoside A STD



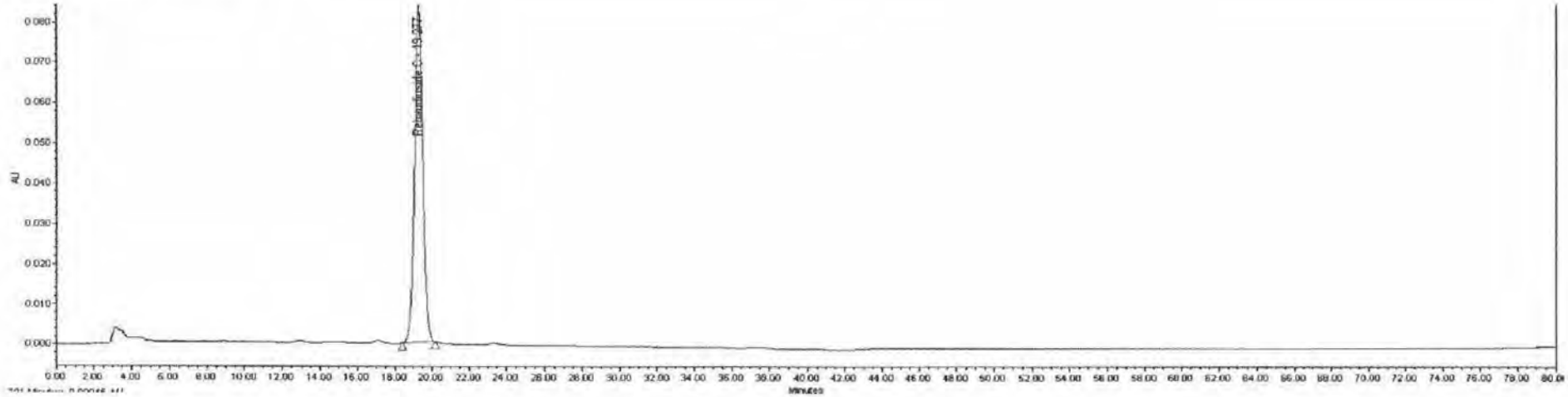
Rebaudioside B STD



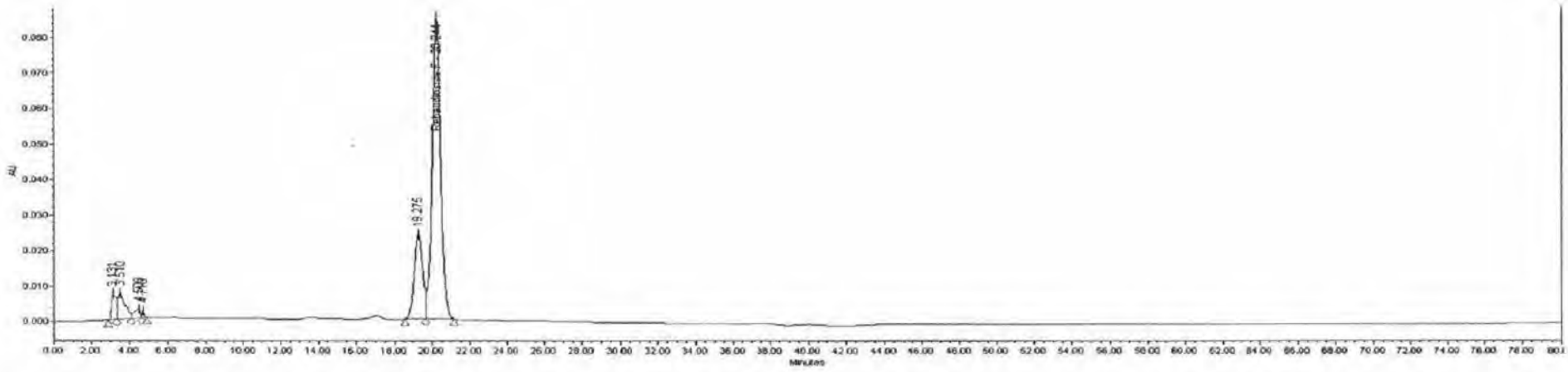
Stevioside STD



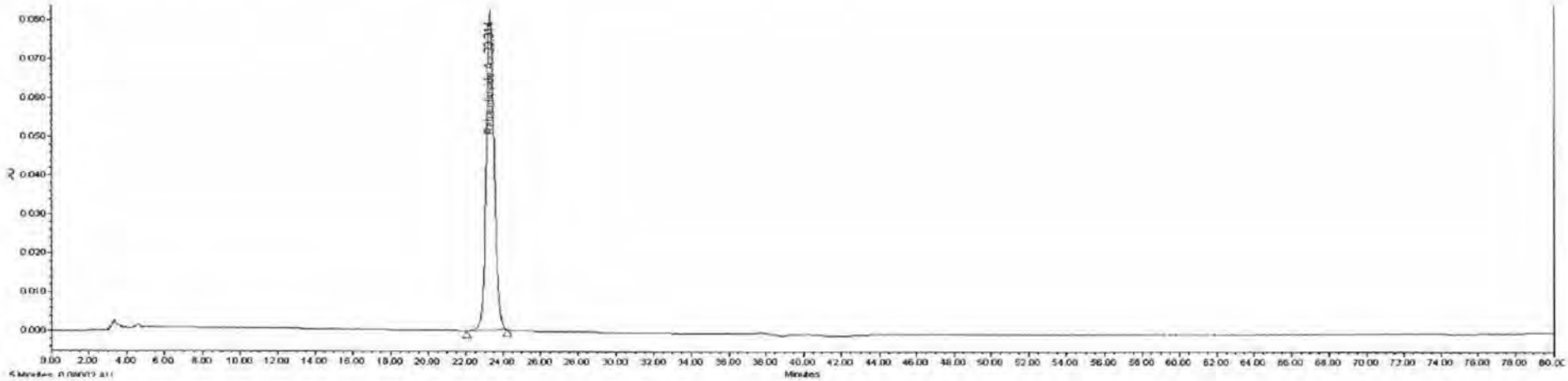
Rebaudioside C STD



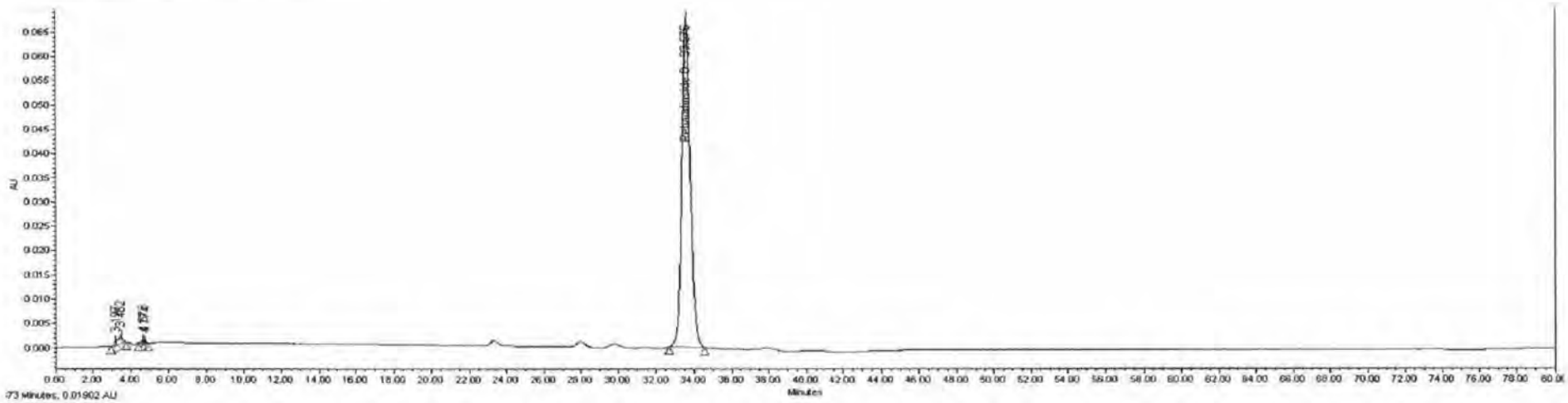
Rebaudioside F STD



Rebaudioside A STD



Rebaudioside D STD



Stevia Extract

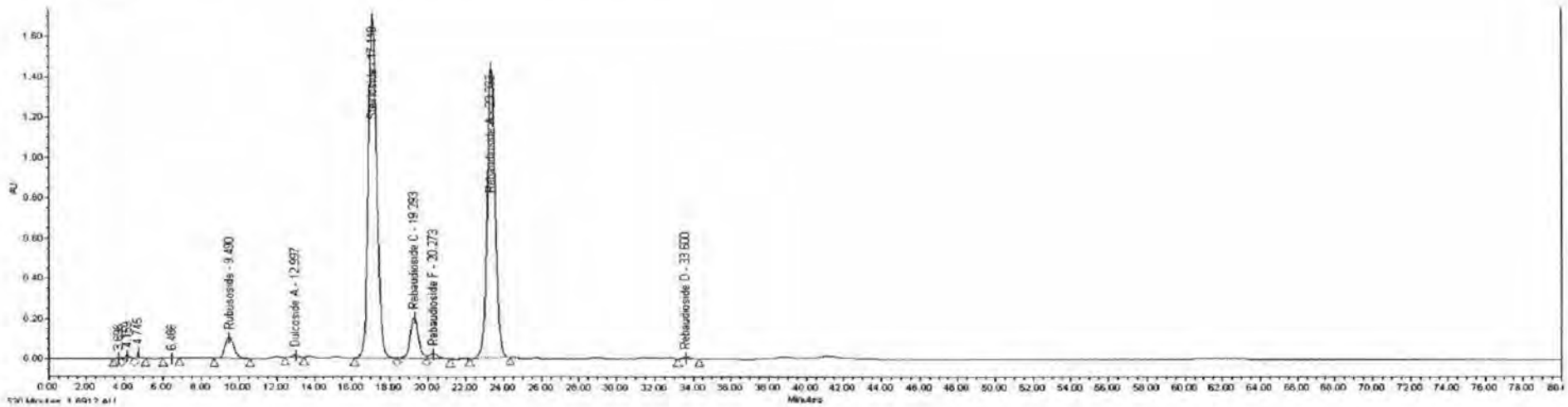
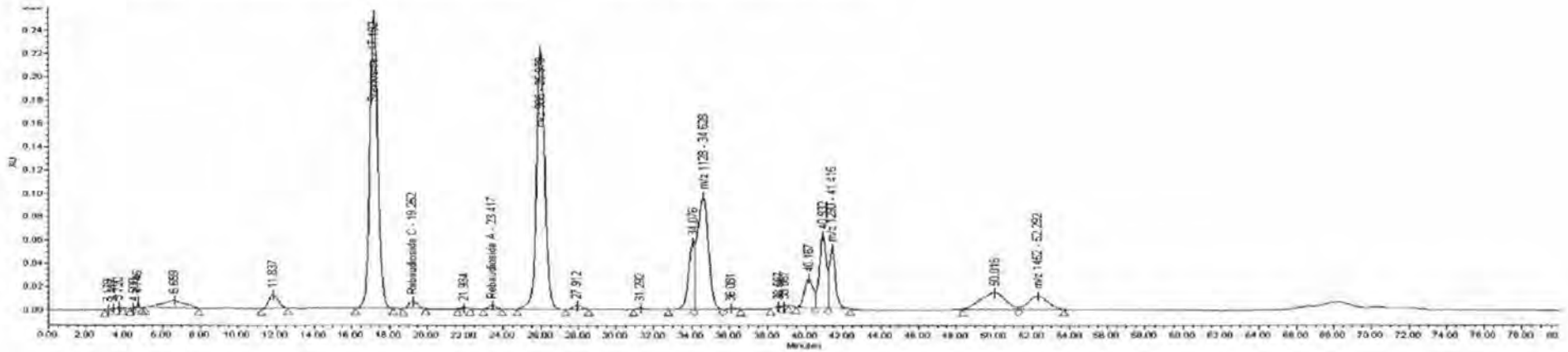
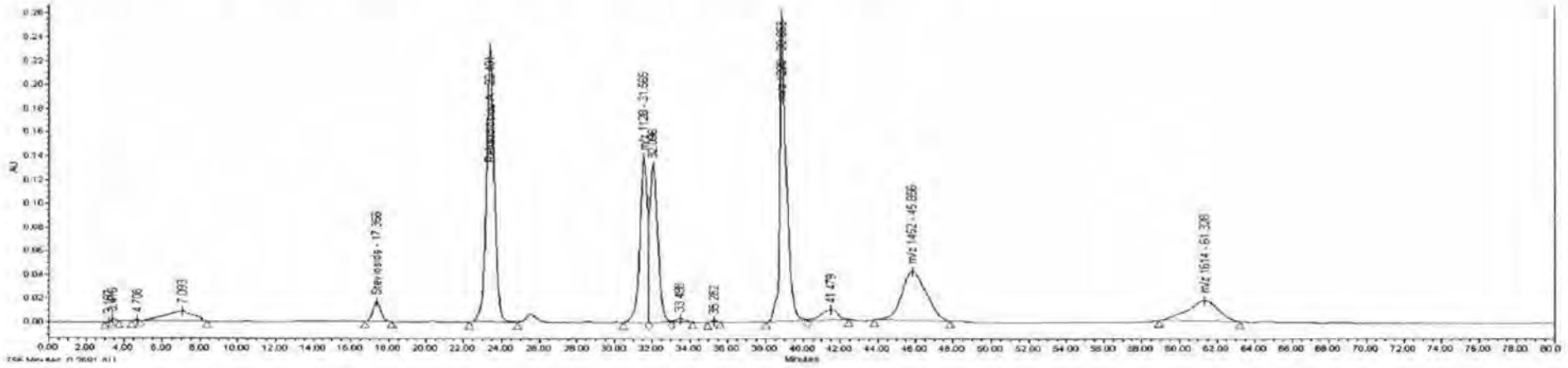


Figure 3. Spectral characteristics of enzyme glycosylated Stevia components

Enzyme Treated Pure Stevioside(99% Stevioside → enzyme glycosylated Stevia)



Enzyme Treated Pure Stevioside(99% Rebaudioside A→ enzyme glycosylated Stevia)



STEVITEN RICH

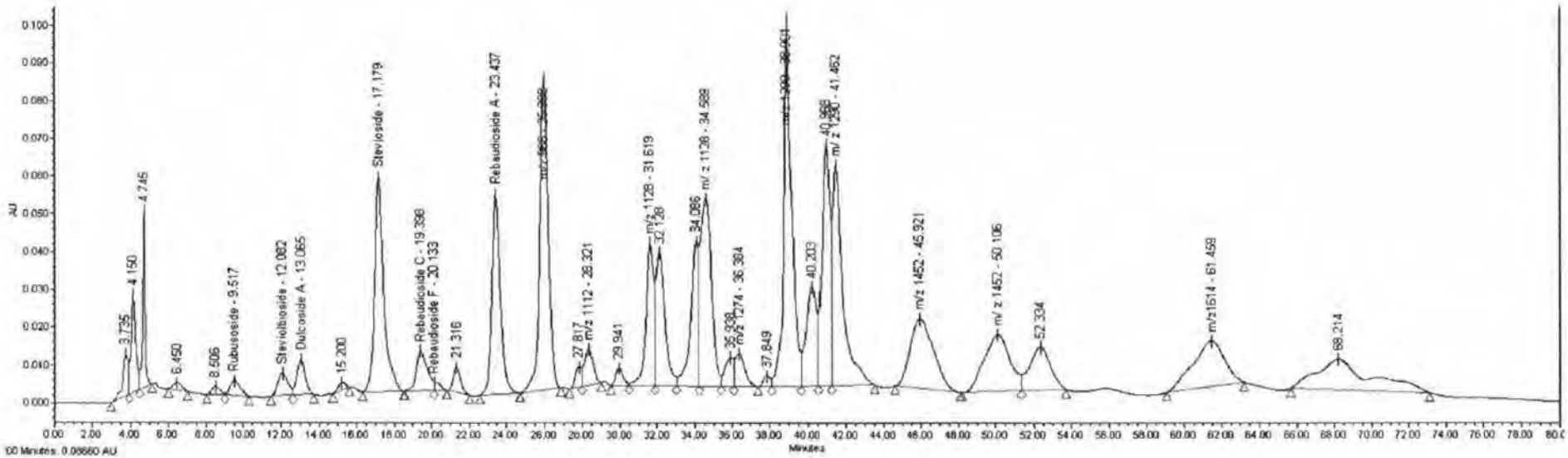


Figure 1. the chromatogram chart as above is shown that after 25 min, we can see different molecular weight which is glucosylated steviol glycoside.

Figure 2. The each nine standard chromatograms which are from "Japan Wako" and "The USA Chromadex" are to check position of stevia extract components

We can distinguish when stevia extract components are coming out

i) Rubusoside STD : about 9 min

ii) Steviolbioside STD : about 11 min

iii) Dulcoside A STD: about 13 min

iv) Rebaudioside B STD: about 16 min

v) Stevioside STD: about 17 min

vi) Rebaudioside C STD: about 19 min

vii) Rebaudioside F STD: about 20 min

viii) Rebaudioside A STD: about 23 min

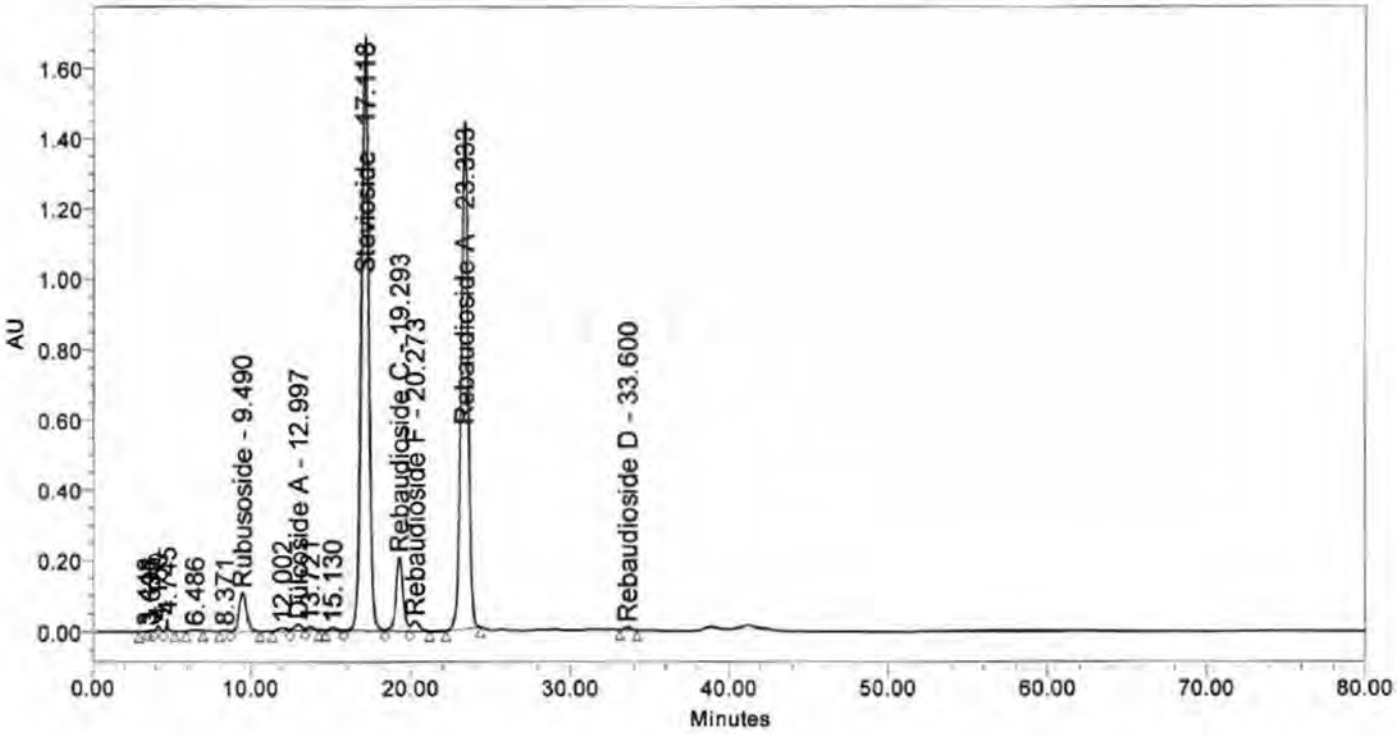
ix) Rebaudioside D STD: about 33 min

igure 3. The chromatogram of "Enzyme Treated Pure Stevioside99%, Rebaudioside A 99% " is similar with "The Chromatogram of STEVITEN RICH". It means that STEVITEN RICH is Enzyme Treated steviol glycosides

*Enzyme Treated Pure Stevioside 99%, Rebaudioside A 99% is treated from 99% contents by HPLC of Stevioside, Rebaudioside A.

SAMPLE INFORMATION

Sample Name:	Starting material	Acquired By:	System
Sample Type:		Sample Set Name:	GRAS
Vial:	14	Acq. Method Set:	G—AB—MS
Injection #:	1	Processing Methoc	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	80.0 Minutes	Proc. Chnl. Descr.:	
Date Acquired:	2019-02-14 AM 6:48:36 KST		
Date Processed:	2019-02-18 PM 1:16:24 KST		



	Peak Name	RT	Area	% Area	Height
1		3.448	27091	0.02	1505
2		3.696	56186	0.05	3706
3		4.159	263240	0.24	15236
4		4.745	229981	0.21	31739
5		6.486	65370	0.06	2019
6		8.371	26203	0.02	1076
7	Rubusoside	9.490	3471449	3.17	106411
8		12.002	313106	0.29	8631
9	Dulcoside A	12.997	546236	0.50	17519
10		13.721	244794	0.22	9681
11		15.130	225775	0.21	7924
12	Stevioside	17.118	52857743	48.32	1685634
13	Rebaudioside C	19.293	6336967	5.79	206925

	Peak Name	RT	Area	% Area	Height
14	Rebaudioside F	20.273	921634	0.84	27391
15	Rebaudioside A	23.333	43595983	39.86	1440813
16	Rebaudioside D	33.600	200897	0.18	7331

SAMPLE INFORMATION

Sample Name:	Enzyme Treated Pure Stevioside	Acquired By:	System
Sample Type:		Sample Set Name:	GRAS
Vial:	26	Acq. Method Set:	G—AB—MS
Injection #:	1	Processing Methoc	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	80.0 Minutes	Proc. Chnl. Descr.:	
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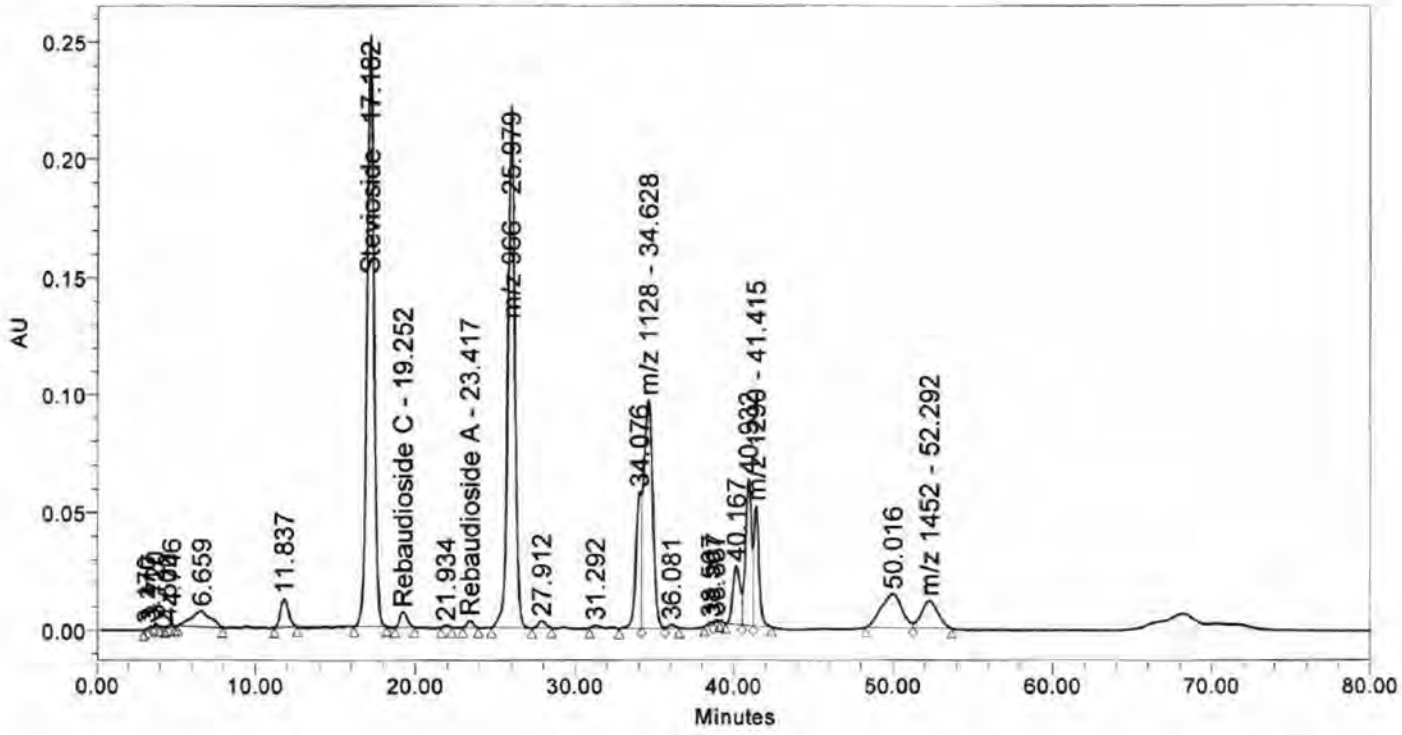
Date Processed:	2019-02-18 PM 1:27:46 KST		



	Peak Name	RT	Area	% Area	Height
1		3.170	2885	0.01	317
2		3.417	9110	0.03	702
3		3.720	12475	0.05	1403
4		4.500	2141	0.01	323
5		4.746	35060	0.13	6553
6		6.659	547055	2.07	6754
7		11.837	408243	1.54	11903
8	Stevioside	17.182	7373465	27.89	251077
9	Rebaudioside C	19.252	201009	0.76	6304
10		21.934	18782	0.07	879
11	Rebaudioside A	23.417	77116	0.29	2904
12	m/z 966	25.979	6717819	25.41	221812
13		27.912	94357	0.36	2932

	Peak Name	RT	Area	% Area	Height
14		31.292	49135	0.19	1140
15		34.076	1267657	4.79	57618
16	m/z 1128	34.628	3904661	14.77	96795
17		36.081	38129	0.14	1252
18		38.567	14779	0.06	808
19		38.867	12833	0.05	875
20		40.167	784729	2.97	24841
21		40.932	1656164	6.26	61884
22	m/z 1290	41.415	1136797	4.30	50692
23		50.016	1270070	4.80	14219
24	m/z 1452	52.292	805146	3.05	11472

Empower 2 SOFTWARE

GRAS

SAMPLE INFORMATION

Sample Name:	Enzyme Treated Pure	Acquired By:	System
Sample Type:	Rebaudioside A	Sample Set Name:	GRAS
Vial:	27	Acq. Method Set:	G—AB—MS
Injection #:	1	Processing Methoc	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	80.0 Minutes	Proc. Chnl. Descr.:	
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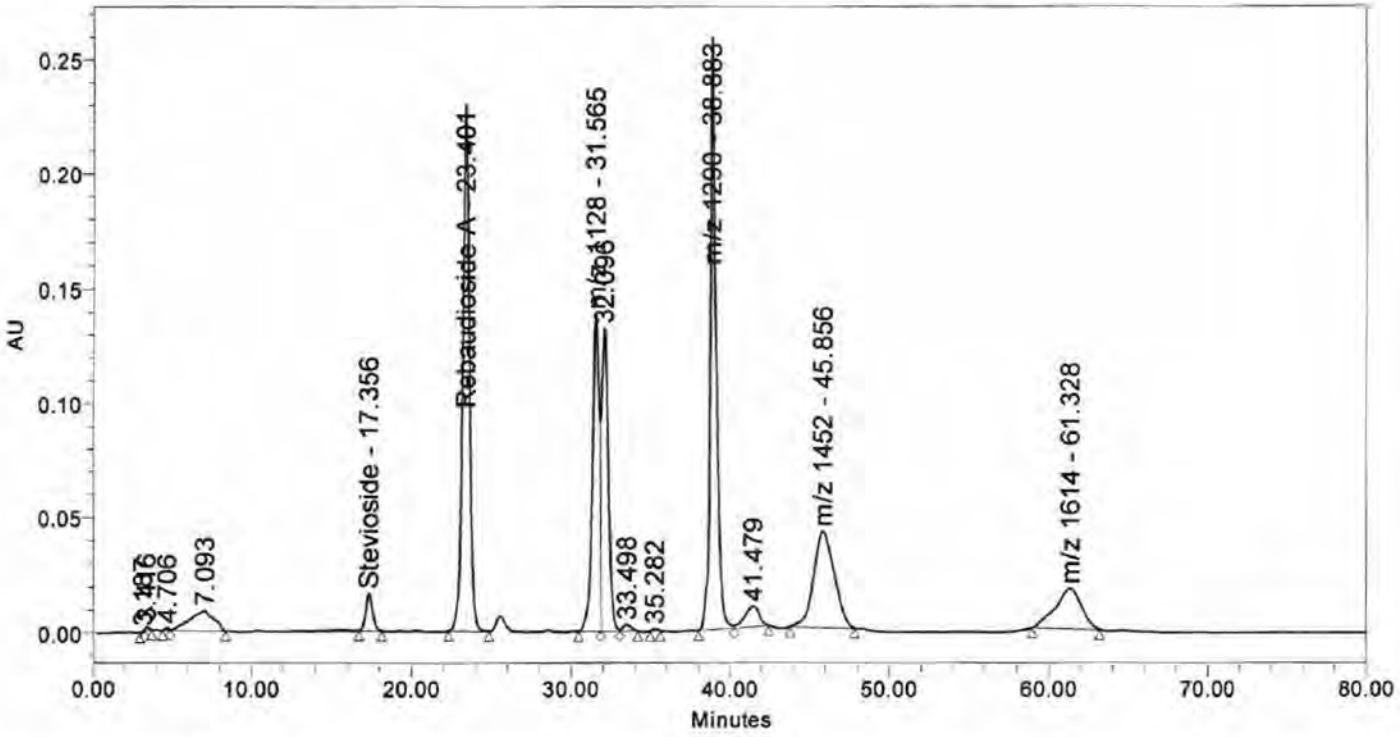
Date Processed:	2019-02-18 PM 1:33:00 KST		

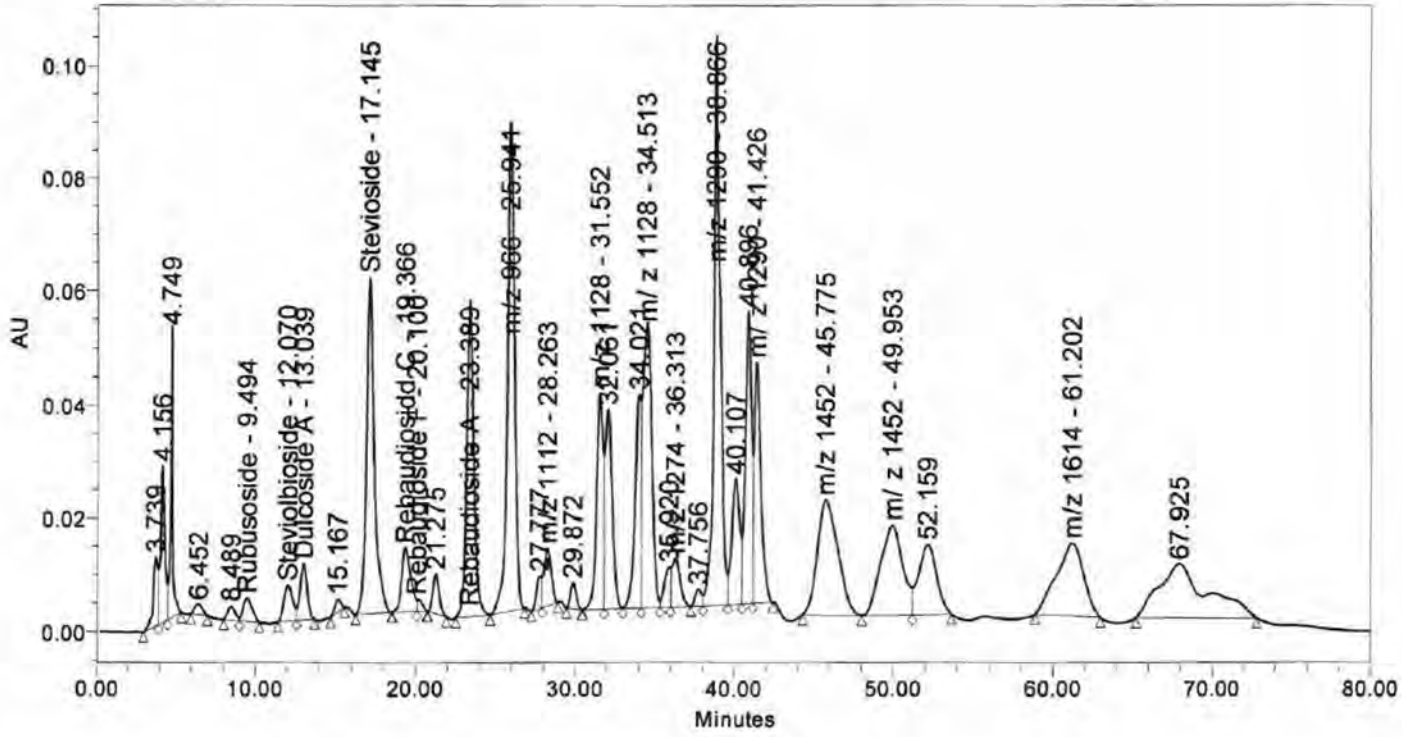


	Peak Name	RT	Area	% Area	Height
1		3.187	3792	0.01	383
2		3.416	28496	0.10	1931
3		4.706	14922	0.05	1197
4		7.093	896339	3.10	8794
5	Stevioside	17.356	469688	1.62	16186
6	Rebaudioside A	23.401	6769593	23.38	229559
7	m/z 1128	31.565	3984095	13.76	138374
8		32.096	3987869	13.77	132166
9		33.498	100452	0.35	2756
10		35.282	20337	0.07	953
11	m/z 1290	38.883	6426442	22.20	258190
12		41.479	549910	1.90	8681
13	m/z 1452	45.856	3726668	12.87	41405

	Peak Name	RT	Area	% Area	Height
14	m/z 1614	61.328	1975090	6.82	17428

SAMPLE INFORMATION

Sample Name:	STEVITEN RICH	Acquired By:	System
Lot:	181107	Sample Set Name:	GRAS
Vial:	15	Acq. Method Set:	G—AB—MS
Injection #:	1	Processing Methoc	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	80.0 Minutes	Proc. Chnl. Descr.:	
Date Acquired:	2019-02-14 AM 8:09:43 KST		
Date Processed:	2019-02-18 PM 12:00:40 KST		

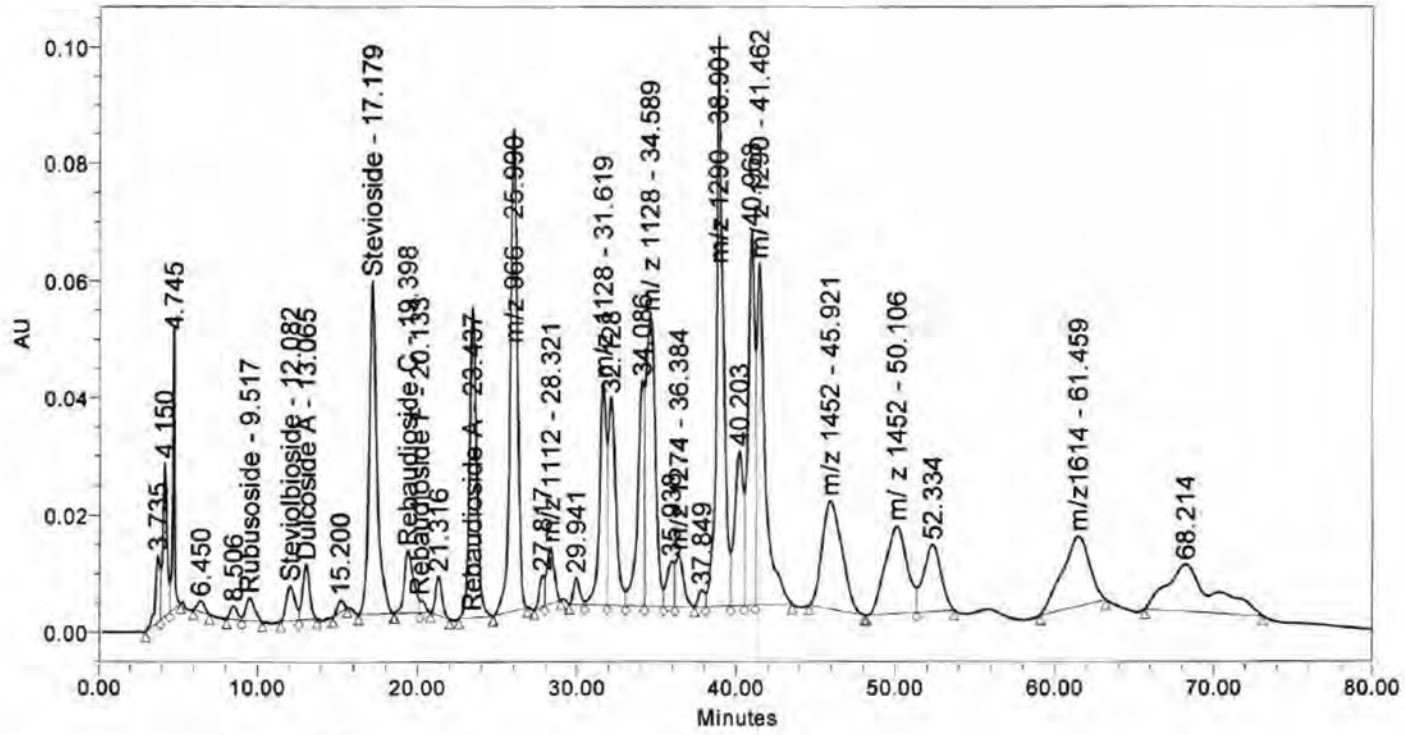


	Peak Name	RT	Area	% Area	Height
1		3.739	239843	0.82	12007
2		4.156	527241	1.80	27679
3		4.749	467620	1.59	50413
4		6.452	60247	0.21	1821
5		8.489	74413	0.25	2324
6	Rubusoside	9.494	153313	0.52	4018
7	Steviolbioside	12.070	232284	0.79	6128
8	Dulcoside A	13.039	297714	1.01	9796
9		15.167	53673	0.18	2062
10	Stevioside	17.145	1991464	6.78	59235
11	Rebaudiosidd C	19.366	444764	1.51	11244
12	Rebaudioside F	20.100	47926	0.16	2071
13		21.275	188174	0.64	6918

	Peak Name	RT	Area	% Area	Height
14	Rebaudioside A	23.389	1710898	5.83	55665
15	m/z 966	25.941	2672718	9.10	86444
16		27.777	127470	0.43	5739
17	m/z 1112	28.263	329487	1.12	10146
18		29.872	113670	0.39	4608
19	m/z 1128	31.552	1091465	3.72	38254
20		32.061	1085629	3.70	35307
21		34.021	937249	3.19	37856
22	m/ z 1128	34.513	2012676	6.86	49590
23		35.920	186030	0.63	6949
24	m/z 1274	36.313	325551	1.11	8532
25		37.756	83018	0.28	2938
26	m/z 1290	38.866	2703880	9.21	100650
27		40.107	770981	2.63	22116
28		40.896	1403104	4.78	51481
29	m/ z 1290	41.426	1266628	4.31	42520
30	m/z 1452	45.775	1815024	6.18	20330
31	m/ z 1452	49.953	1508715	5.14	15876
32		52.159	954876	3.25	12315
33	m/z 1614	61.202	1497705	5.10	12848
34		67.925	1983636	6.76	9443

SAMPLE INFORMATION

Sample Name:	STEVITEN RICH	Acquired By:	System
Lot:	181203	Sample Set Name:	GRAS
Vial:	16	Acq. Method Set:	G—AB—MS
Injection #:	1	Processing Methoc	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	80.0 Minutes	Proc. Chnl. Descr.:	
Date Acquired:	2019-02-14 AM 9:30:48 KST		
Date Processed:	2019-02-18 PM 12:00:57 KST		

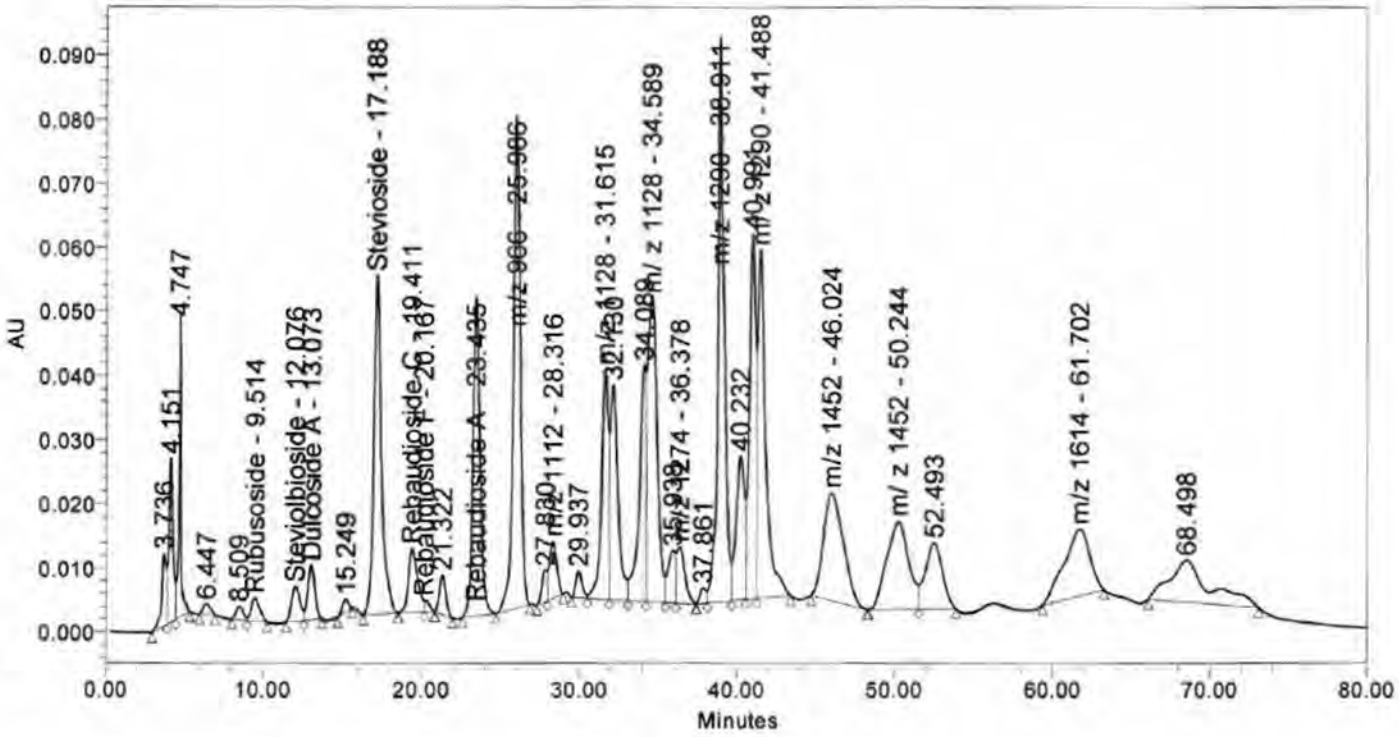


	Peak Name	RT	Area	% Area	Height
1		3.735	229673	0.76	11337
2		4.150	476814	1.58	26206
3		4.745	391609	1.30	47344
4		6.450	60280	0.20	1958
5		8.506	69660	0.23	2214
6	Rubusoside	9.517	145980	0.48	3869
7	Steviolbioside	12.082	219791	0.73	5890
8	Dulcoside A	13.065	284268	0.94	9372
9		15.200	51552	0.17	1978
10	Stevioside	17.179	1910437	6.34	56955
11	Rebaudioside C	19.398	427629	1.42	10609
12	Rebaudioside F	20.133	50106	0.17	2172
13		21.316	184791	0.61	6696

	Peak Name	RT	Area	% Area	Height
14	Rebaudioside A	23.437	1633885	5.42	52977
15	m/z 966	25.990	2551191	8.47	82166
16		27.817	119399	0.40	5364
17	m/z 1112	28.321	313027	1.04	9573
18		29.941	122790	0.41	4582
19	m/z 1128	31.619	1136965	3.77	37870
20		32.128	1173619	3.89	35363
21		34.086	1098876	3.65	38291
22	m/ z 1128	34.589	2060956	6.84	49544
23		35.938	247852	0.82	7641
24	m/z 1274	36.384	318926	1.06	8714
25		37.849	76437	0.25	2796
26	m/z 1290	38.901	2818857	9.35	97353
27		40.203	1003947	3.33	26230
28		40.968	1861396	6.18	63853
29	m/ z 1290	41.462	2217480	7.36	58360
30	m/z 1452	45.921	1588212	5.27	18354
31	m/ z 1452	50.106	1418021	4.71	14921
32		52.334	858558	2.85	11334
33	m/z1614	61.459	1397528	4.64	11905
34		68.214	1614178	5.36	8049

SAMPLE INFORMATION

Sample Name:	STEVITEN RICH	Acquired By:	System
Lot:	181224	Sample Set Name:	GRAS
Vial:	17	Acq. Method Set:	G—AB—MS
Injection #:	1	Processing Method:	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	80.0 Minutes	Proc. Chnl. Descr.:	
Date Acquired:	2019-02-14 AM 10:51:53 KST		
Date Processed:	2019-02-18 PM 12:01:09 KST		



	Peak Name	RT	Area	% Area	Height
1		3.736	228652	0.83	11297
2		4.151	480156	1.74	25611
3		4.747	430899	1.56	46147
4		6.447	52074	0.19	1647
5		8.509	63523	0.23	2039
6	Rubusoside	9.514	133007	0.48	3533
7	Steviolbioside	12.076	199888	0.73	5408
8	Dulcoside A	13.073	261200	0.95	8627
9		15.249	51334	0.19	1921
10	Stevioside	17.188	1772205	6.43	52876
11	Rebaudioside C	19.411	399146	1.45	10039
12	Rebaudioside F	20.167	40229	0.15	1739
13		21.322	167303	0.61	6209

	Peak Name	RT	Area	% Area	Height
14	Rebaudioside A	23.435	1514344	5.49	49549
15	m/z 966	25.986	2370927	8.60	76904
16		27.830	108216	0.39	5040
17	m/z 1112	28.316	297203	1.08	8953
18		29.937	115414	0.42	4245
19	m/z 1128	31.615	1087277	3.94	35657
20		32.130	1151658	4.18	33519
21		34.089	1090778	3.96	36735
22	m/ z 1128	34.589	2018708	7.32	47254
23		35.938	284030	1.03	8188
24	m/z 1274	36.378	325920	1.18	8880
25		37.861	63741	0.23	2387
26	m/z 1290	38.911	2493075	9.04	88138
27		40.232	835492	3.03	22529
28		40.991	1608055	5.83	57046
29	m/ z 1290	41.488	1967661	7.14	54387
30	m/z 1452	46.024	1475051	5.35	16836
31	m/ z 1452	50.244	1321993	4.80	13721
32		52.493	786479	2.85	10416
33	m/z 1614	61.702	1193023	4.33	10454
34		68.498	1180403	4.28	6671

2. Analysis of Glucosylated Steviol Glycoside by UV/LC

Glucose Standard Curve

Figure 4. Glucose Standard Curve

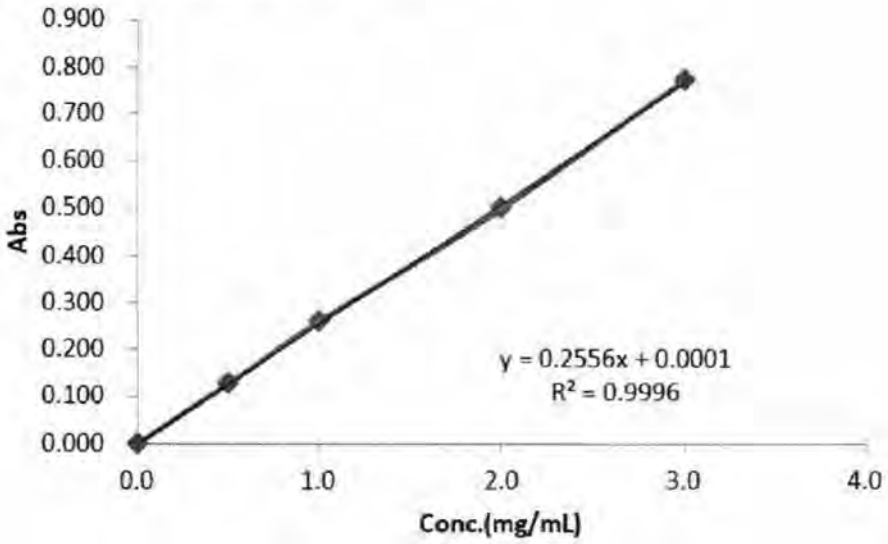


Figure out absorbance (abs) with each four glucose conc in table 5 and make calibration curve as above. Therefore it is reliable.

Y= 0.2556 X + 0.0001

Results

Table 5. Glucose Standard

Glucose Conc.(mg/ml)	Absorbance(abs)	Remarks
0.5	0.128	
1.0	0.261	
2.0	0.501	
3.0	0.772	

Table 6. STEVIEN RICH

Lot	Amount of sample (g)	Moisture (%)	Absorbance (abs)	b* (mg/ml)	Y** (g)	Remarks
181107	1.0179	3.9	0.621	2.43	0.9823	
181203	1.0208	4.2	0.615	2.41	0.9830	
181224	1.0257	4.3	0.626	2.45	0.9919	

* b: Concentration(mg/ml) of D-glucose in the test solution

** Y: Dry basis weight(g) of the sample

X : Content (%) of ∝-Glucosyl residues = [(b X 200)/(Y X 1,000)] X 0.900 X 100

1) 181107

X (%)=[(2.43 X 200)/(0.9823 X 1,000)] X 0.900 X 100 =44.51

2) 181203

X (%) =[(2.41 X 200)/(0.9830 X 1,000)] X 0.900 X 100=44.05

3) 181224

X (%) =[(2.45 X 200)/(0.9919 X 1,000)] X 0.900 X 100=44.44

Methods

HPLC Conditions

Instrument : Alliance e2695-2489(Waters)

Column : TOSOH, TSKgel Amide-80(250X4.6mm, 5um)

Column Temp : 25 ℃ ± 5℃

Flow Rate : Adjust so that the retention time of rebaudioside A is about 21 min

Injection Volume : 20uL

Detection : uv@210nm

Mobile Phase : Mix HPLC-grade acetonitrile and water(80:20)

Procedure

Equilibrate the instrument by pumping mobile phase through it until a drift-free baseline is obtained. Record the chromatograms of the sample solution and of the standard solutions. The retention times relative to rebaudioside A(1.00) are:

0.45~0.48 for stevioside

0.25~0.30 for dulcoside A

0.63~0.69 for rebaudioside C

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

Calculate the percentage of each of the four steviol glycoside, in the sample from the formula:

$$\text{Content (\%) of steviol glycosides} = [W_S/W] \times [A_X/A_S] \times f_X \times 100$$

Where

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

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

A_S is the peak area for stevioside from the standard solution

A_X is the peak area for sample from the sample solution

f_X is the ratio of the formula weight of X to the formula weight of stevioside: 1.00(stevioside), 0.98(dulcoside A), 1.20(rebaudioside A), 1.18(rebaudioside C).

Results

Table 7. Total content (%) of ∝-Glucosyl steviol glycosides and unreacted steviol glycosides

Lot	Content (%) of steviol glycosides	Content (%) of ∝-Glucosyl residues	Remarks
181107	44.51	47.99	
181203	44.05	48.15	
181224	44.44	47.96	

Lot	Assay(%)*	Remarks
181107	92.5	
181203	92.2	
181224	92.4	

* Assay is Total content of ∝-Glucosyl steviol glycosides and unreacted steviol glycosides

※ Total content (%) of ∝-Glucosyl steviol glycosides and unreacted steviol glycosides
= Content (%) of steviol glycosides + Content (%) of ∝-Glucosyl residues

Table 8. Content (%) of ∝-glucosylsteviol glycosides

Lot	Assay (%)	Content (%) of unreacted steviol glycosides	Remarks
181107	92.5	5.6	
181203	92.2	5.9	
181224	92.4	5.7	

Lot	Content (%) of ∝-glucosylsteviol glycosides	Remarks
181107	86.9	
181203	86.3	
181224	86.7	

※ Content (%) of ∝-glucosylsteviol glycosides
= Content (%) of steviol glycosides + Content (%) of ∝-glucosyl residues − Content (%) of unreacted steviol glycosides

Appendix B
Raw Data

SAMPLE INFORMATION

Sample Name:	Stevioside	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	GRAS
Vial:	1	Acq. Method Set:	함량 D—065— MS
Injection #:	1	Processing Methoc	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	25.0 Minutes	Proc. Chnl. Descr.:	
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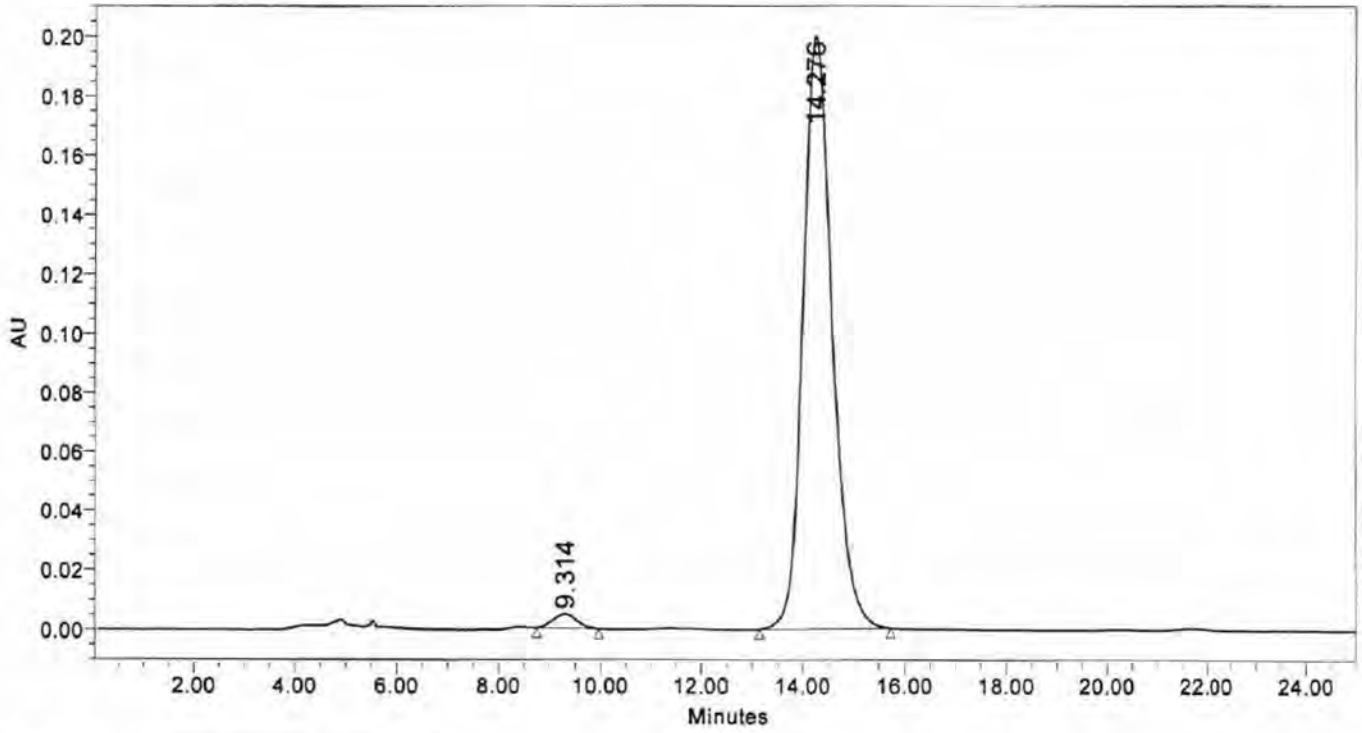
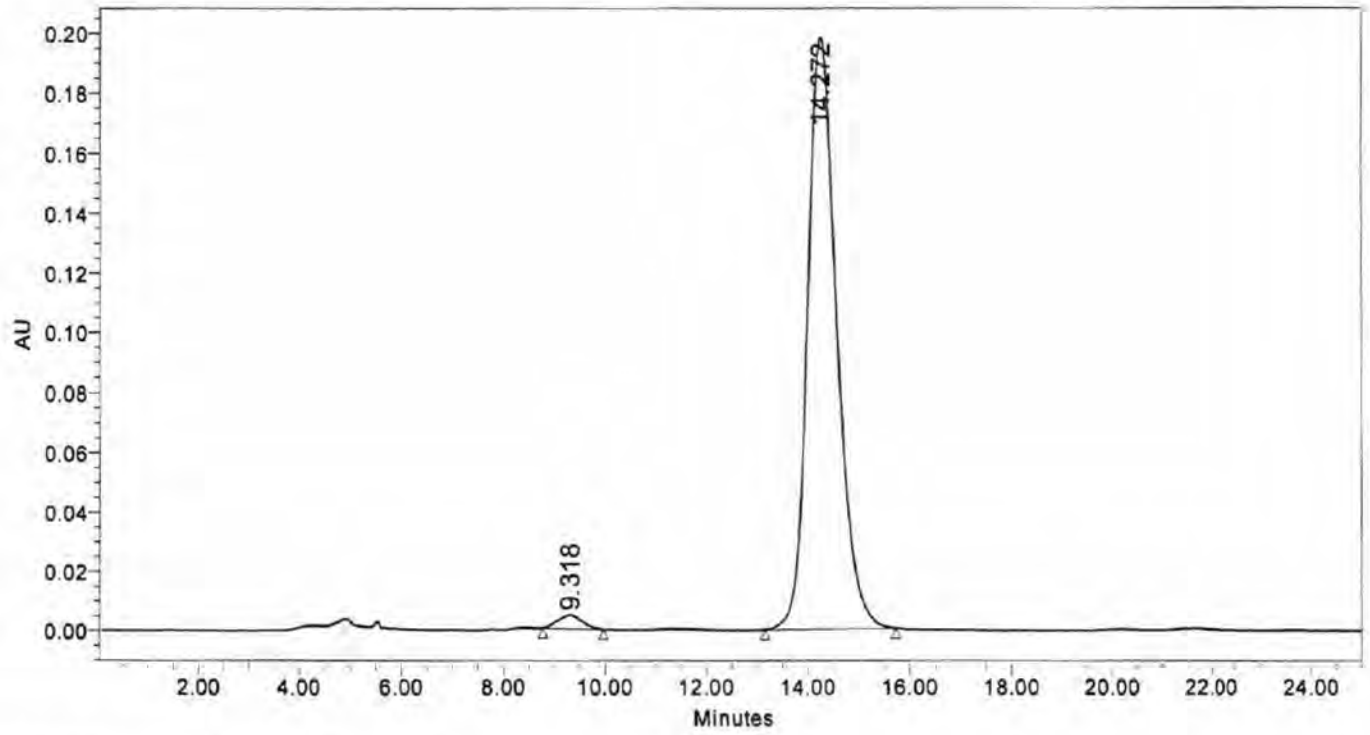
Date Processed:	2019-02-19 AM 11:02:41 KST		



	RT	Area	% Area	Height
1	9.314	155332	1.92	4774
2	14.276	7946135	98.08	199682

SAMPLE INFORMATION

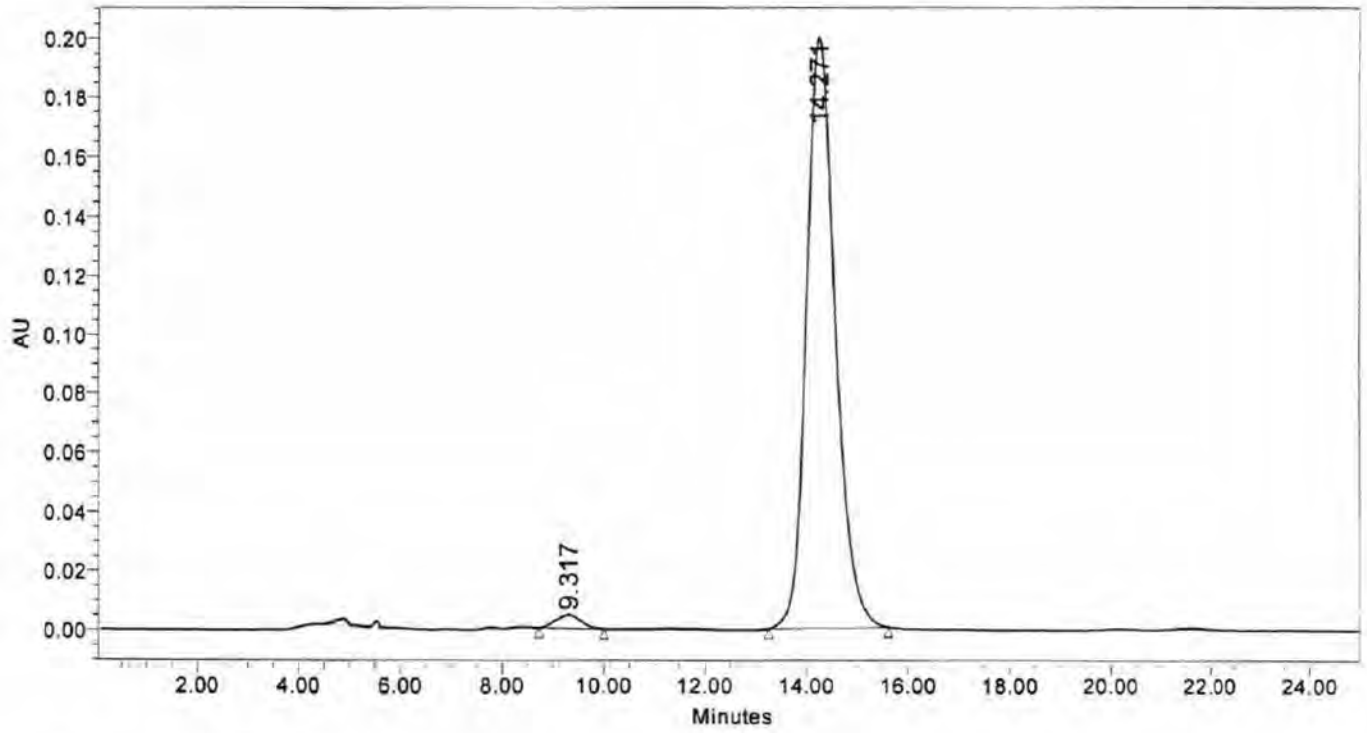
Sample Name:	Stevioside	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	GRAS
Vial:	2	Acq. Method Set:	함량 D−065− MS
Injection #:	1	Processing Methoc	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	25.0 Minutes	Proc. Chnl. Descr.:	
Date Acquired:	2019-02-18 PM 3:39:02 KST		
Date Processed:	2019-02-19 AM 11:02:57 KST		



	RT	Area	% Area	Height
1	9.318	149010	1.85	4511
2	14.272	7926953	98.15	198090

SAMPLE INFORMATION

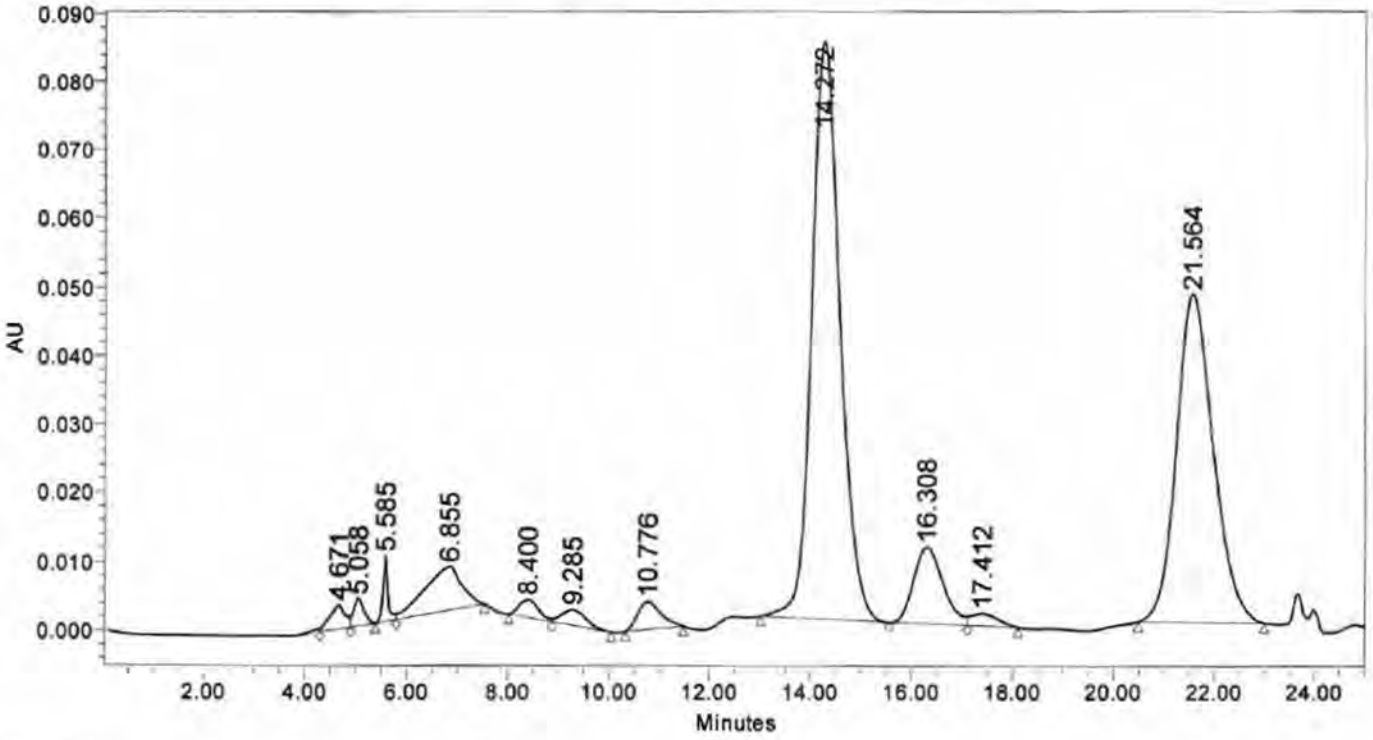
Sample Name:	Stevioside	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	GRAS
Vial:	3	Acq. Method Set:	함량 D—065— MS
Injection #:	1	Processing Methoc	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	25.0 Minutes	Proc. Chnl. Descr.:	
Date Acquired:	2019-02-18 PM 4:04:57 KST		
Date Processed:	2019-02-19 AM 11:04:26 KST		



	RT	Area	% Area	Height
1	9.317	156261	1.93	4535
2	14.271	7961143	98.07	199840

SAMPLE INFORMATION

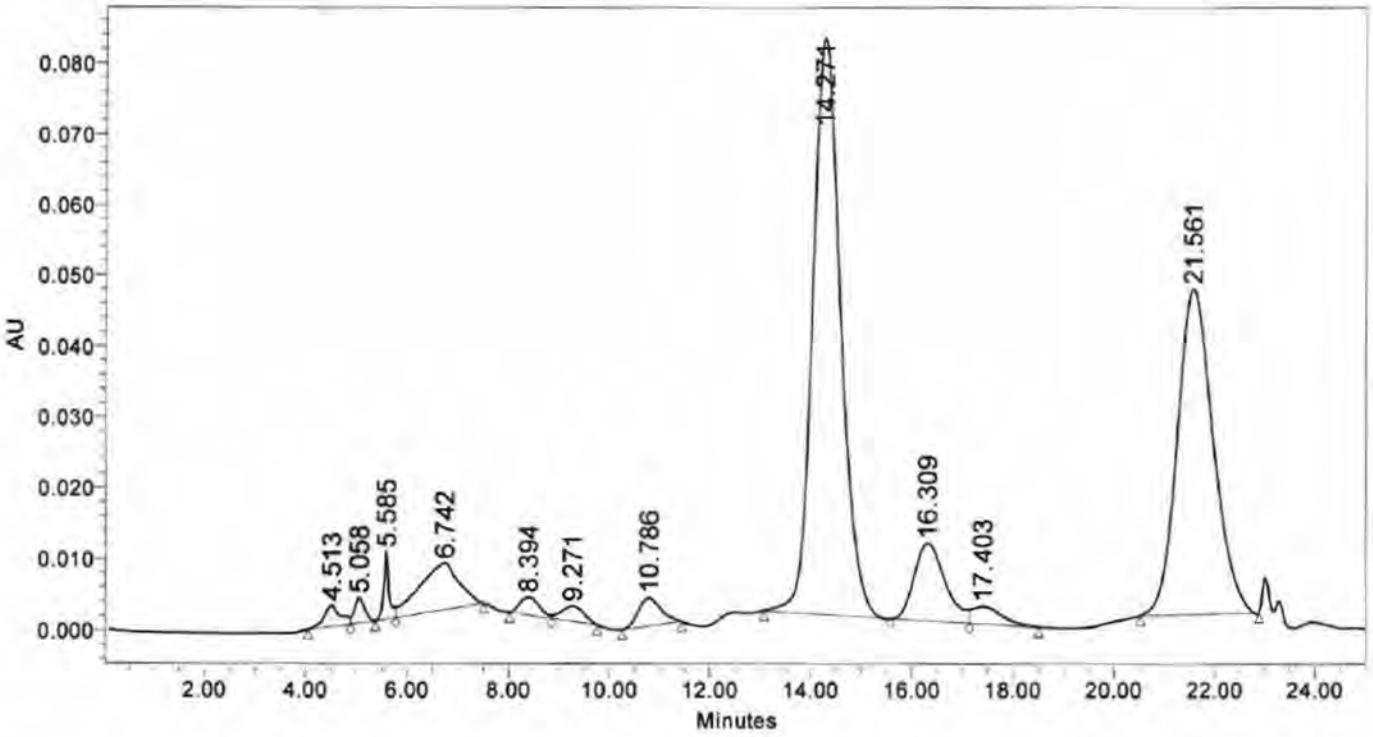
Sample Name:	STEVITEN RICH	Acquired By:	System
Lot:	181107	Sample Set Name:	GRAS
Vial:	7	Acq. Method Set:	함량 D−065− MS
Injection #:	1	Processing Methoc	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	25.0 Minutes	Proc. Chnl. Descr.:	
Date Acquired:	2019-02-18 PM 5:48:37 KST		
Date Processed:	2019-02-19 AM 11:10:58 KST		



	RT	Area	% Area	Height
1	4.671	72558	1.03	3516
2	5.058	56008	0.80	3969
3	5.585	63118	0.90	9388
4	6.855	326033	4.65	6316
5	8.400	69027	0.98	2593
6	9.285	76505	1.09	2284
7	10.776	129790	1.85	4053
8	14.272	3272759	46.66	84293
9	16.308	446876	6.37	11027
10	17.412	66711	0.95	1805
11	21.564	2435272	34.72	47848

SAMPLE INFORMATION

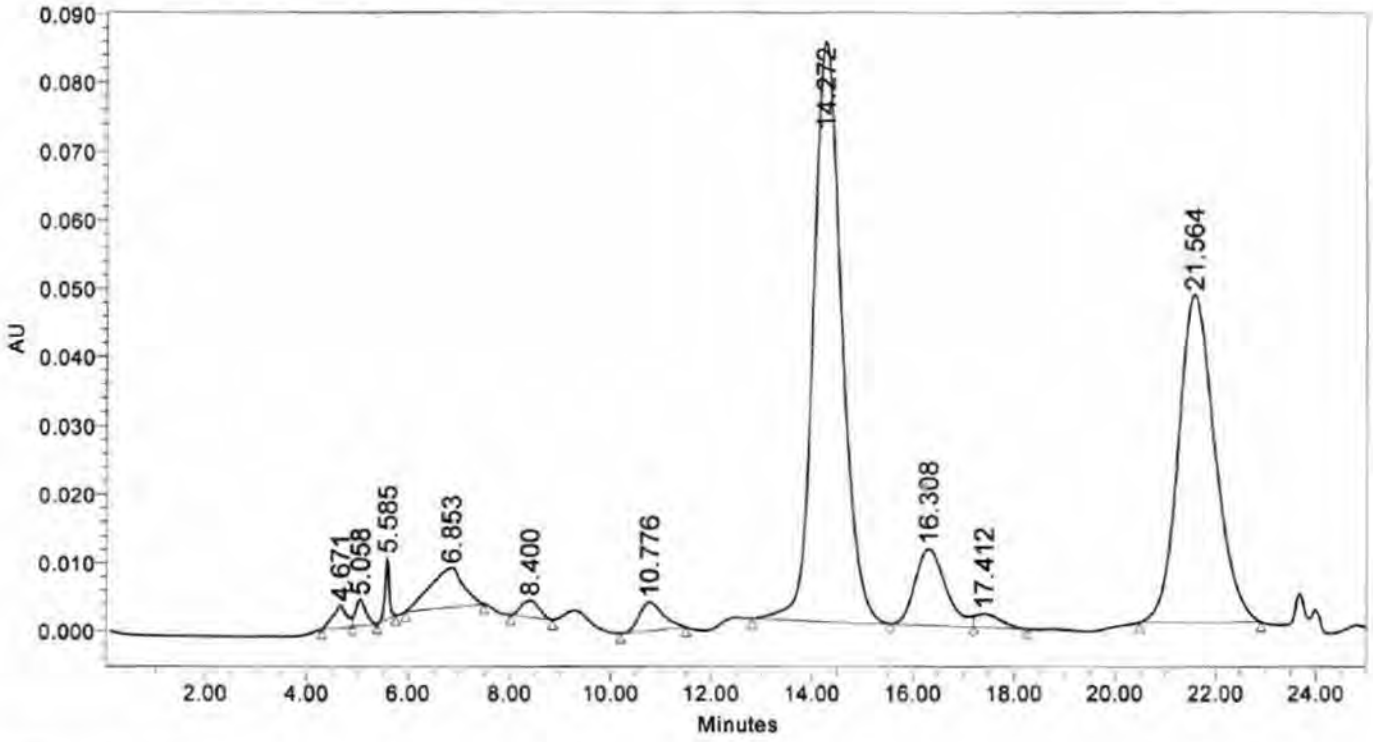
Sample Name:	STEVITEN RICH	Acquired By:	System
Lot:	181203	Sample Set Name:	GRAS
Vial:	8	Acq. Method Set:	함량 D—065— MS
Injection #:	1	Processing Methoc	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	25.0 Minutes	Proc. Chnl. Descr.:	
Date Acquired:	2019-02-18 PM 6:14:32 KST		
Date Processed:	2019-02-19 AM 11:11:40 KST		



	RT	Area	% Area	Height
1	4.513	64328	0.95	2955
2	5.058	47533	0.70	3493
3	5.585	66803	0.99	9322
4	6.742	352056	5.22	6453
5	8.394	65234	0.97	2450
6	9.271	63483	0.94	2081
7	10.786	122025	1.81	3898
8	14.271	3130042	46.39	81317
9	16.309	459322	6.81	10863
10	17.403	101405	1.50	2429
11	21.561	2275154	33.72	45784

SAMPLE INFORMATION

Sample Name:	STEVITEN RICH	Acquired By:	System
Lot:	181224	Sample Set Name:	GRAS
Vial:	8	Acq. Method Set:	함량 D—065— MS
Injection #:	1	Processing Method:	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	25.0 Minutes	Proc. Chnl. Descr.:	
Date Acquired:	2019-02-18 PM 5:48:37 KST		
Date Processed:	2019-02-19 AM 11:12:49 KST		



	RT	Area	% Area	Height
1	4.671	61151	0.89	3228
2	5.058	53174	0.77	3837
3	5.585	51336	0.75	8918
4	6.853	259575	3.77	5767
5	8.400	58754	0.85	2411
6	10.776	135539	1.97	4139
7	14.272	3310869	48.07	84546
8	16.308	471668	6.85	11224
9	17.412	69114	1.00	1971
10	21.564	2416789	35.09	47738

3. Standard Curve

Result

Table 9. Standard Analysis

Standard	Conc.(mg/ml)	Retention Time(min)	Area	Remarks
Stevioside	5	17.153	30247554	
	10	17.113	55267784	
	15	17.144	80947221	
	20	17.171	109386339	
Rebaudioside A	5	23.351	24574770	
	10	23.394	44930455	
	15	23.420	64742431	
	20	23.418	81289585	

Figure out Area with each four stevioside Conc and Rebaudioside Conc in table 9 and make Figure 5 & 6 calibration curve as below. Therefore it is reliable.

Figure 5. Stevioside Standard Curve

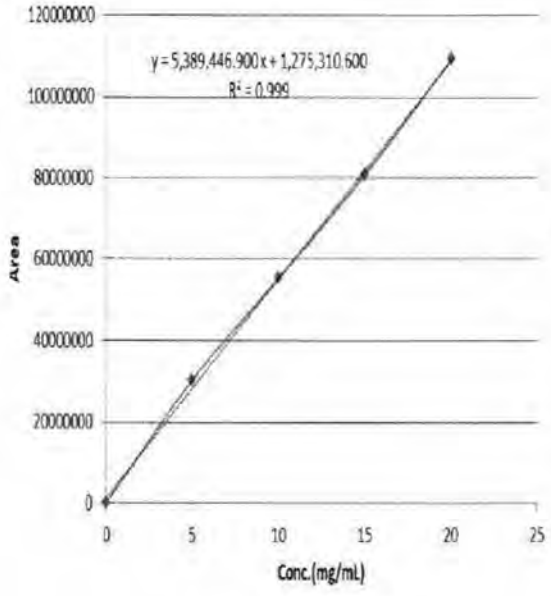
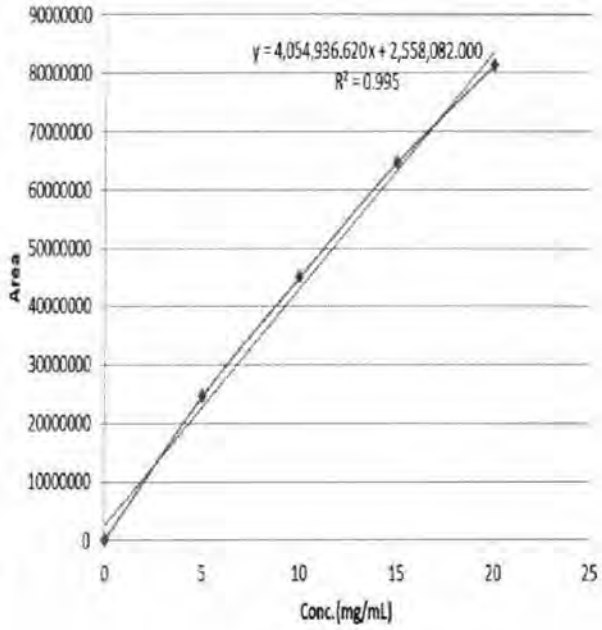


Figure 6. Rebaudioside A Standard Curve



Y = 5,389,446.9X + 1,275,310.6

R = 0.999

Y = 4,054,936.62X + 2,558,082

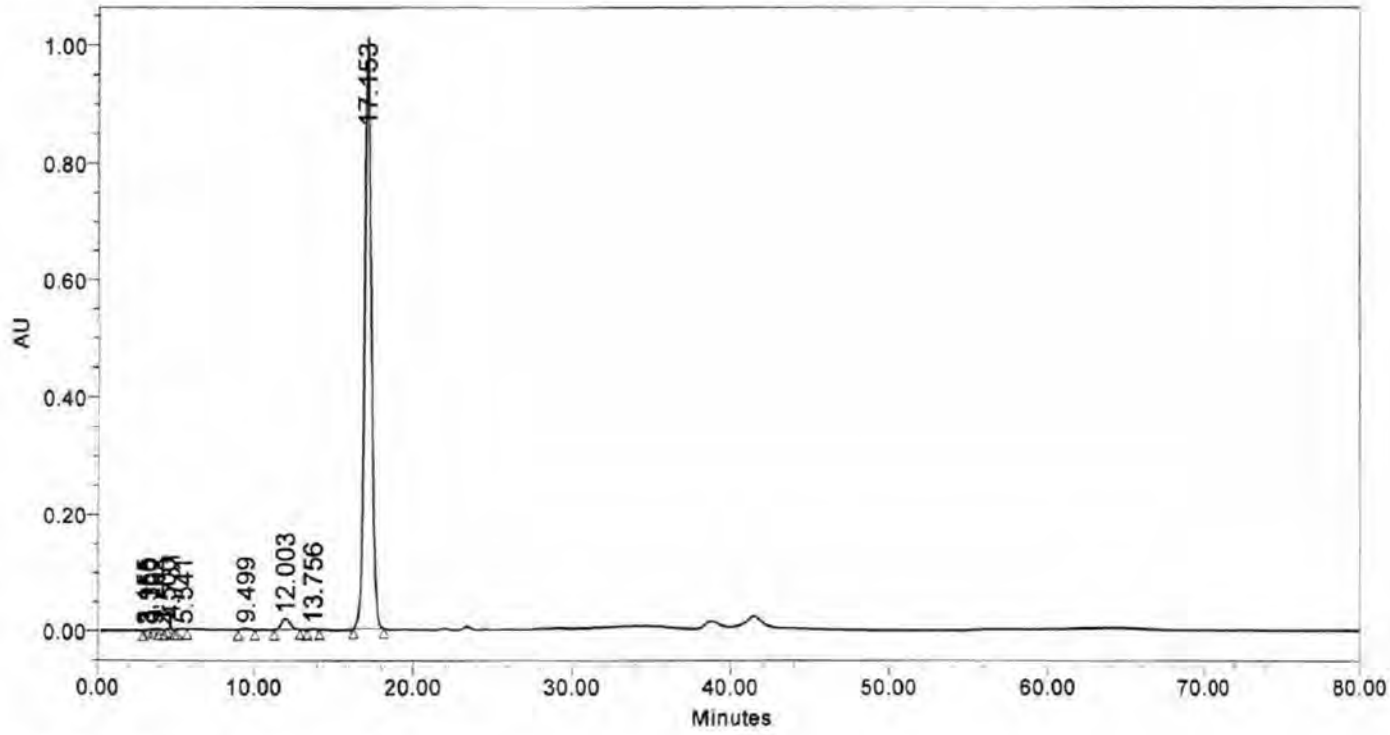
R = 0.997

Appendix C

Raw Data

SAMPLE INFORMATION

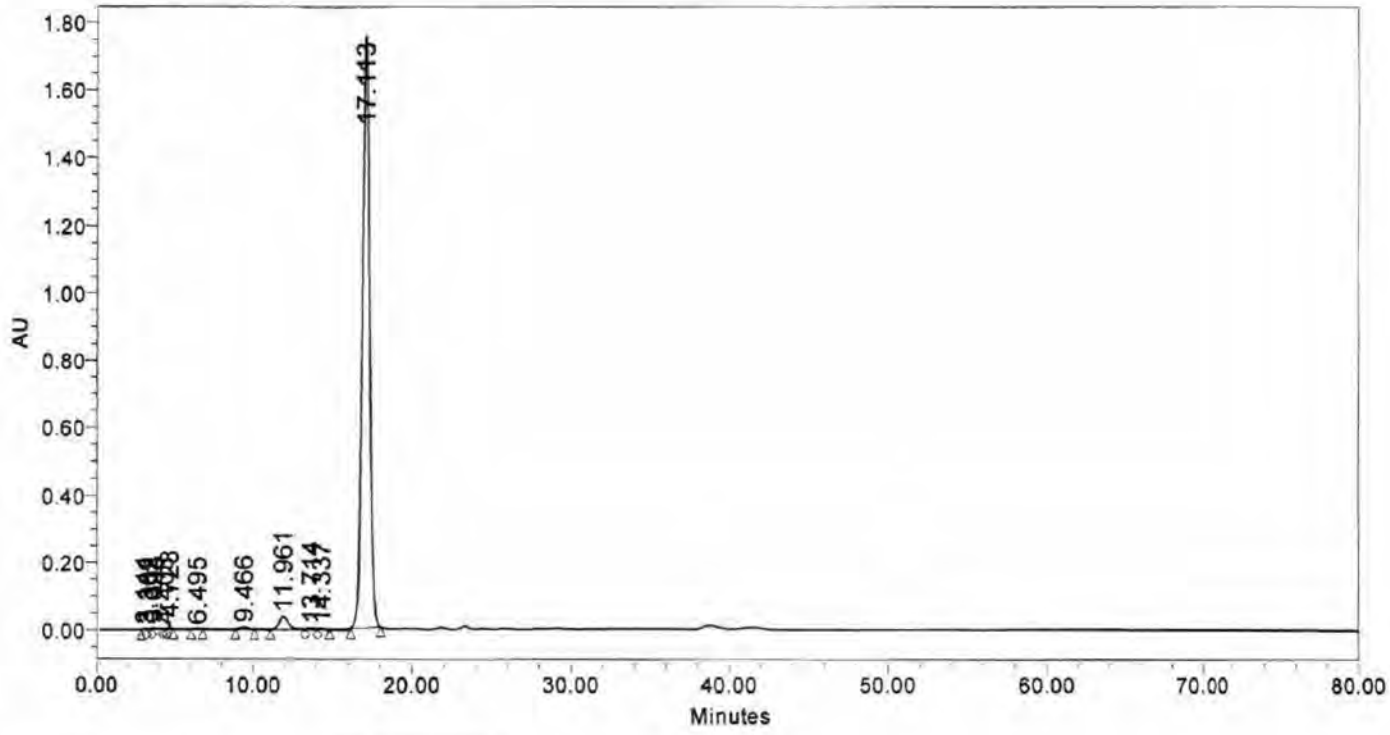
Sample Name:	STEVIOSIDE(5.0mg/mL)	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	GRAS
Vial:	18	Acq. Method Set:	G−AB−MS
Injection #:	1	Processing Methoc	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	80.0 Minutes	Proc. Chnl. Descr.:	
Date Acquired:	2019-02-14 PM 12:12:58 KST		
Date Processed:	2019-02-18 AM 9:46:46 KST		



	RT	Area	% Area	Height
1	3.155	6234	0.02	571
2	3.350	11908	0.04	776
3	3.702	9334	0.03	1088
4	4.500	3143	0.01	352
5	4.731	46086	0.15	9978
6	5.541	18634	0.06	1131
7	9.499	96703	0.31	3199
8	12.003	693874	2.23	18392
9	13.756	29912	0.10	1322
10	17.153	30247554	97.06	1010126

SAMPLE INFORMATION

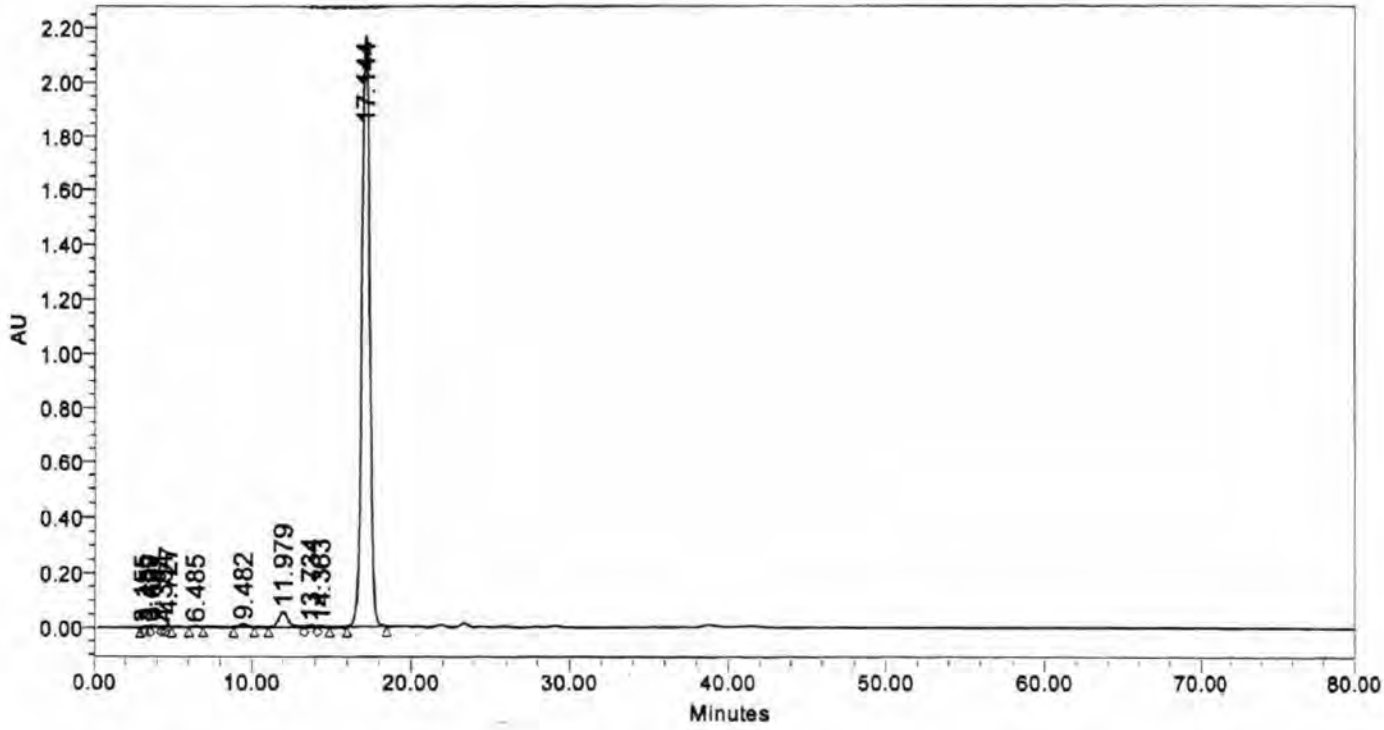
Sample Name:	STEVIOSIDE(10.0mg/mL)	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	GRAS
Vial:	19	Acq. Method Set:	G—AB—MS
Injection #:	1	Processing Methoc	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	80.0 Minutes	Proc. Chnl. Descr.:	
Date Acquired:	2019-02-14 PM 1:34:05 KST		
Date Processed:	2019-02-18 AM 9:47:11 KST		



	RT	Area	% Area	Height
1	3.144	14091	0.02	1283
2	3.382	36527	0.06	2159
3	3.698	78702	0.14	3868
4	4.403	16136	0.03	1341
5	4.728	99297	0.17	20129
6	6.495	28621	0.05	1207
7	9.466	204539	0.36	6509
8	11.961	1539641	2.68	37405
9	13.714	130378	0.23	4152
10	14.337	70091	0.12	2618
11	17.113	55267784	96.14	1751419

SAMPLE INFORMATION

Sample Name:	STEVIOSIDE(15.0mg/mL)	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	GRAS
Vial:	20	Acq. Method Set:	G—AB—MS
Injection #:	1	Processing Methoc	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	80.0 Minutes	Proc. Chnl. Descr.:	
Date Acquired:	2019-02-14 PM 2:55:12 KST		
Date Processed:	2019-02-18 AM 9:45:49 KST		



	RT	Area	% Area	Height
1	3.155	14863	0.02	1302
2	3.459	45754	0.06	2625
3	3.697	113305	0.14	5889
4	4.394	20475	0.03	1783
5	4.727	142312	0.18	29334
6	6.485	47660	0.06	1822
7	9.482	310828	0.40	9838
8	11.979	2295699	2.93	56736
9	13.734	195548	0.28	6219
10	14.363	106253	0.14	3910
11	17.144	80947221	95.79	2166115

SAMPLE INFORMATION

Sample Name:	STEVIOSIDE(20.0mg/mL)	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	GRAS
Vial:	21	Acq. Method Set:	G—AB—MS
Injection #:	1	Processing Methoc	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	80.0 Minutes	Proc. Chnl. Descr.:	
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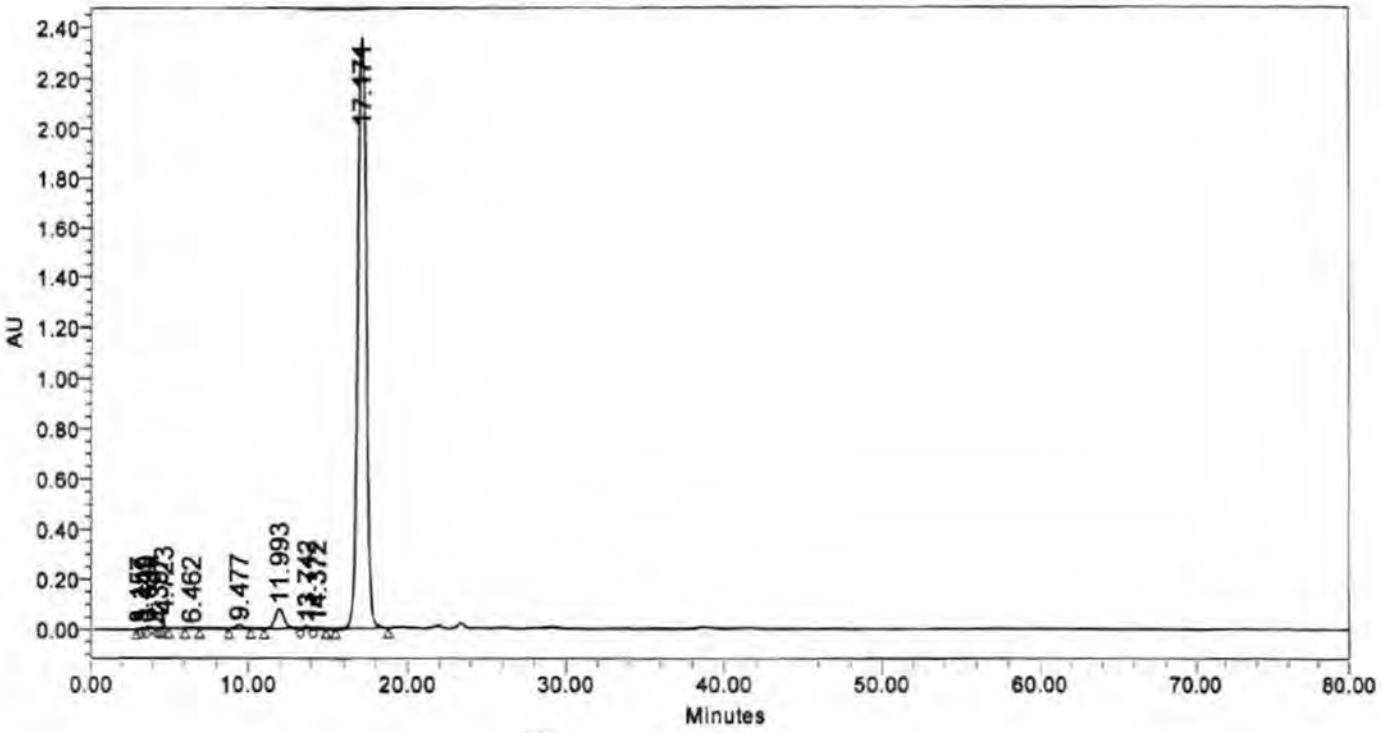
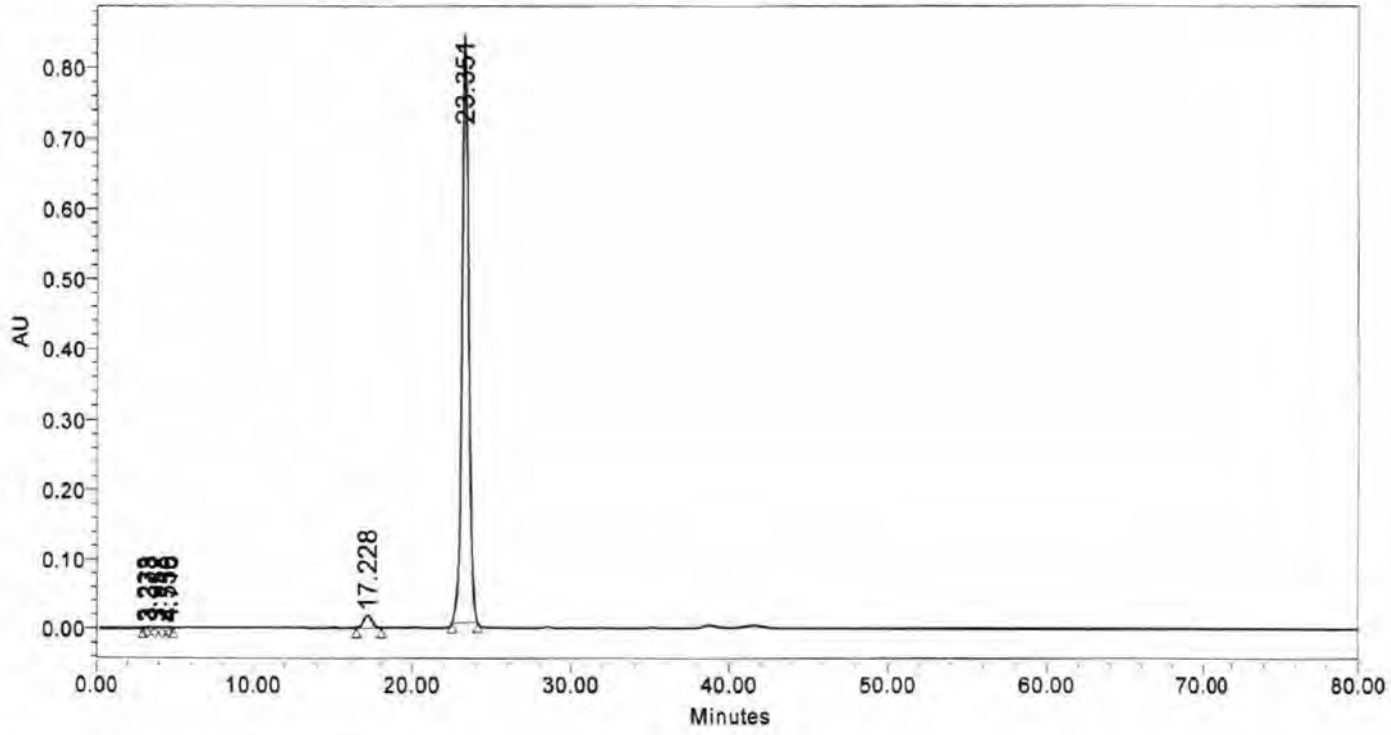


	RT	Area	% Area	Height
1	3.157	19484	0.02	1714
2	3.449	54296	0.06	3045
3	3.699	144121	0.12	7738
4	4.397	26932	0.03	2393
5	4.723	195784	0.18	40016
6	6.462	64236	0.07	2362
7	9.477	424964	0.40	13380
8	11.993	3088985	2.97	77910
9	13.742	263473	0.18	8415
10	14.372	143274	0.10	5235
11	17.171	109386339	95.87	2352965

SAMPLE INFORMATION

Sample Name:	REBAUDIOSIDE A(5.0mg/mL)	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	GRAS
Vial:	22	Acq. Method Set:	G—AB—MS
Injection #:	1	Processing Methoc	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	80.0 Minutes	Proc. Chnl. Descr.:	
Date Acquired:	2019-02-14 PM 7:27:34 KST		
Date Processed:	2019-02-18 AM 11:49:10 KST		



	RT	Area	% Area	Height
1	3.233	5393	0.02	683
2	3.378	24605	0.10	1349
3	3.952	13127	0.05	708
4	4.559	14678	0.06	913
5	4.716	8446	0.03	1105
6	17.228	628761	2.49	17536
7	23.351	24574770	97.25	837535

SAMPLE INFORMATION

Sample Name:	REBAUDIOSIDE A(10.0mg/mL)	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	GRAS
Vial:	23	Acq. Method Set:	G—AB—MS
Injection #:	1	Processing Methoc	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	80.0 Minutes	Proc. Chnl. Descr.:	
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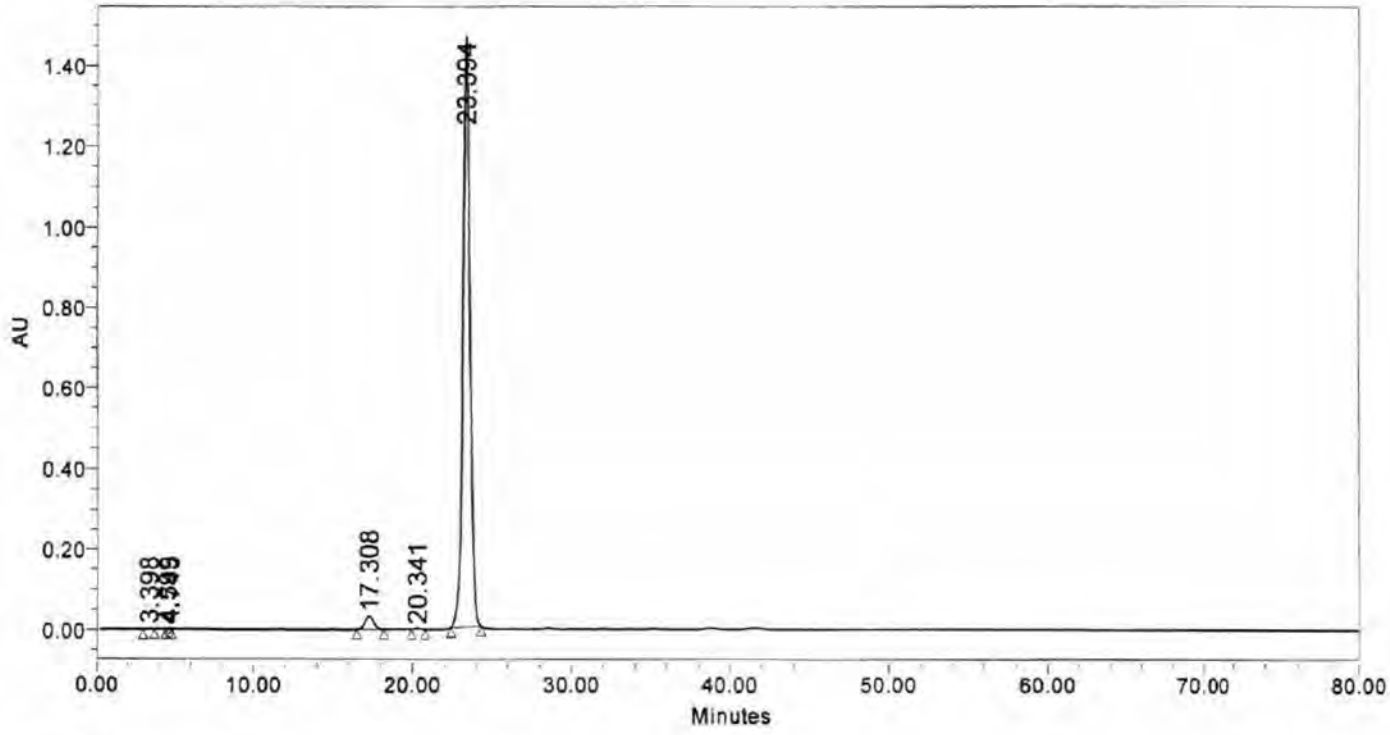
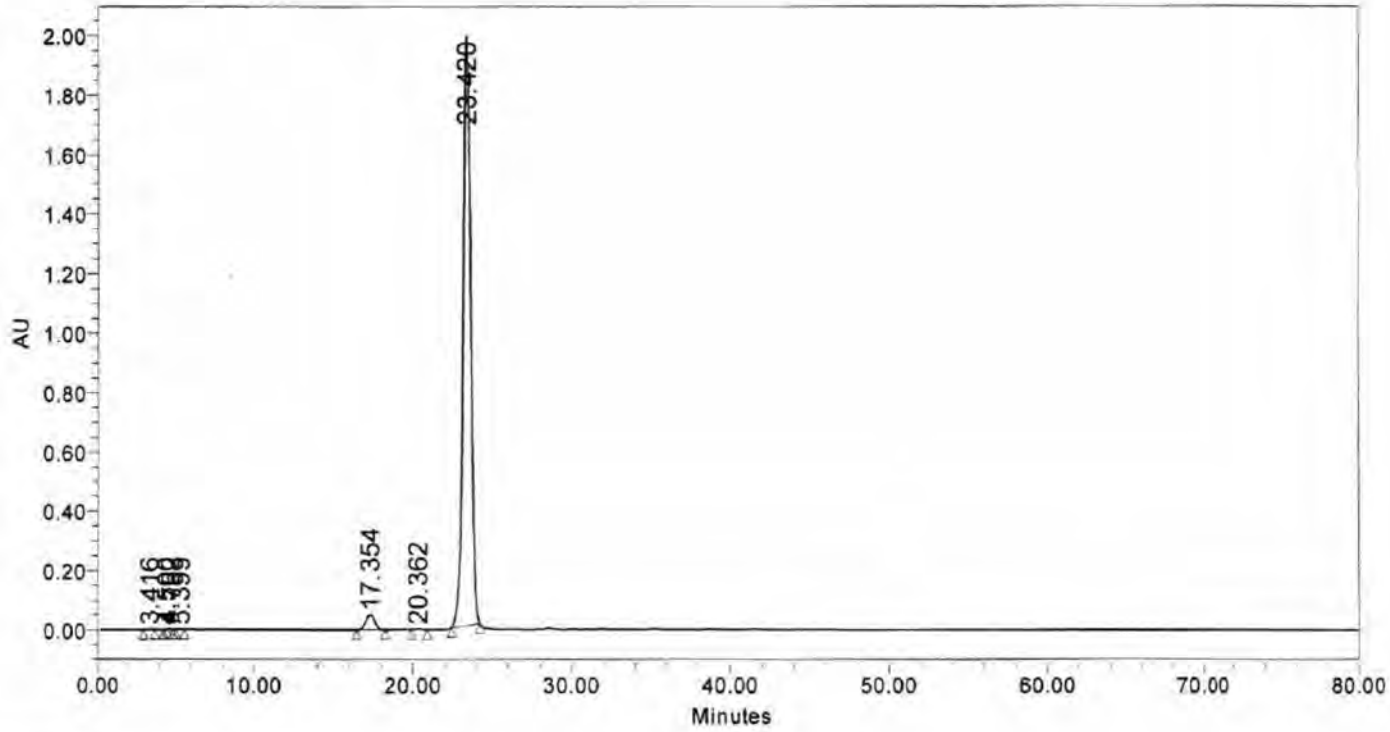
Date Processed:	2019-02-18 AM 11:48:16 KST		



	RT	Area	% Area	Height
1	3.398	30628	0.07	1629
2	4.599	4724	0.01	500
3	4.745	6677	0.01	1489
4	17.308	1189631	2.57	32456
5	20.341	41380	0.09	1616
6	23.394	44930455	97.24	1466044

SAMPLE INFORMATION

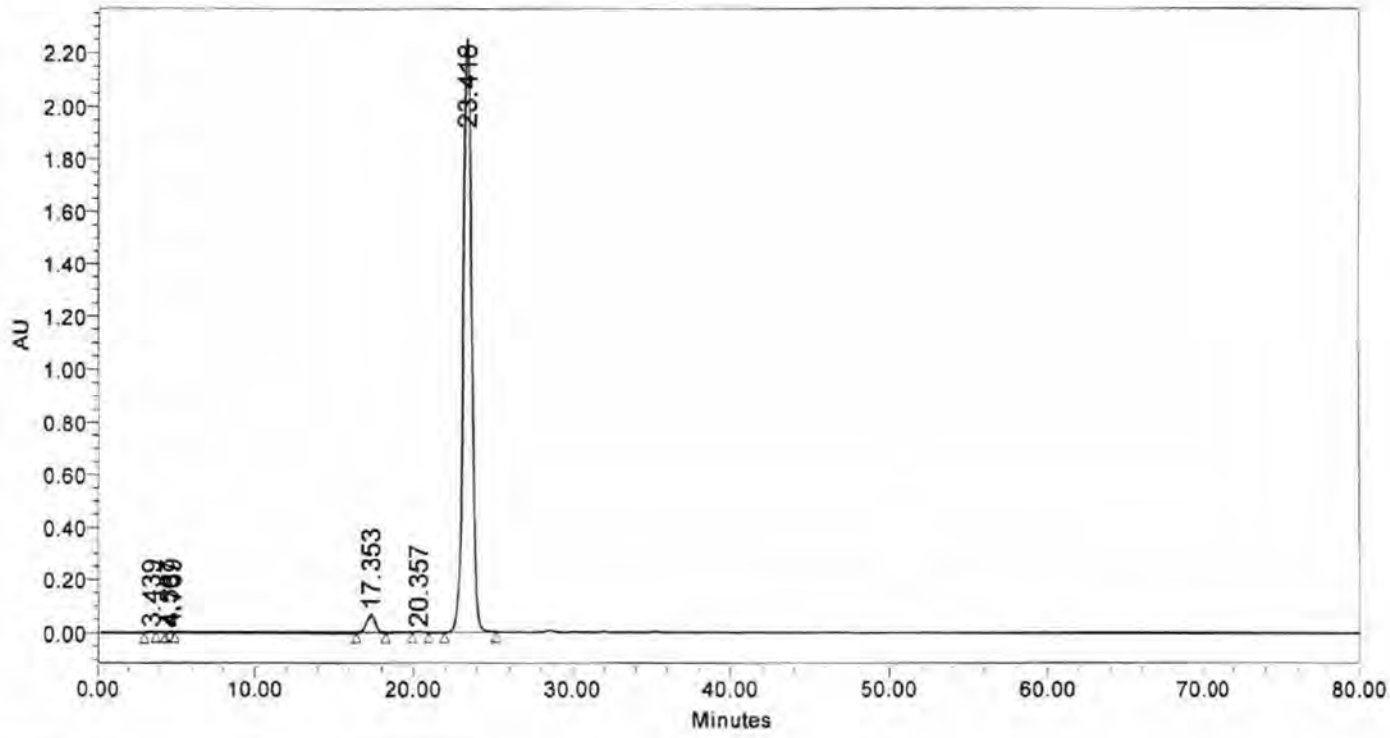
Sample Name:	REBAUDIOSIDE A(15.0mg/mL)	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	GRAS
Vial:	24	Acq. Method Set:	G—AB—MS
Injection #:	1	Processing Methoc	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	80.0 Minutes	Proc. Chnl. Descr.:	
Date Acquired:	2019-02-14 PM 10:09:46 KST		
Date Processed:	2019-02-18 AM 11:49:43 KST		



	RT	Area	% Area	Height
1	3.416	26613	0.04	1231
2	4.500	2636	0.00	436
3	4.752	17882	0.03	2550
4	5.399	11835	0.02	848
5	17.354	1881668	2.82	49545
6	20.362	70418	0.11	2607
7	23.420	64742431	96.99	1981808

SAMPLE INFORMATION

Sample Name:	REBAUDIOSIDE A(20.0mg/mL)	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	GRAS
Vial:	25	Acq. Method Set:	G—AB—MS
Injection #:	1	Processing Methoc	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	80.0 Minutes	Proc. Chnl. Descr.:	
Date Acquired:	2019-02-14 PM 11:31:05 KST		
Date Processed:	2019-02-18 AM 10:22:57 KST		



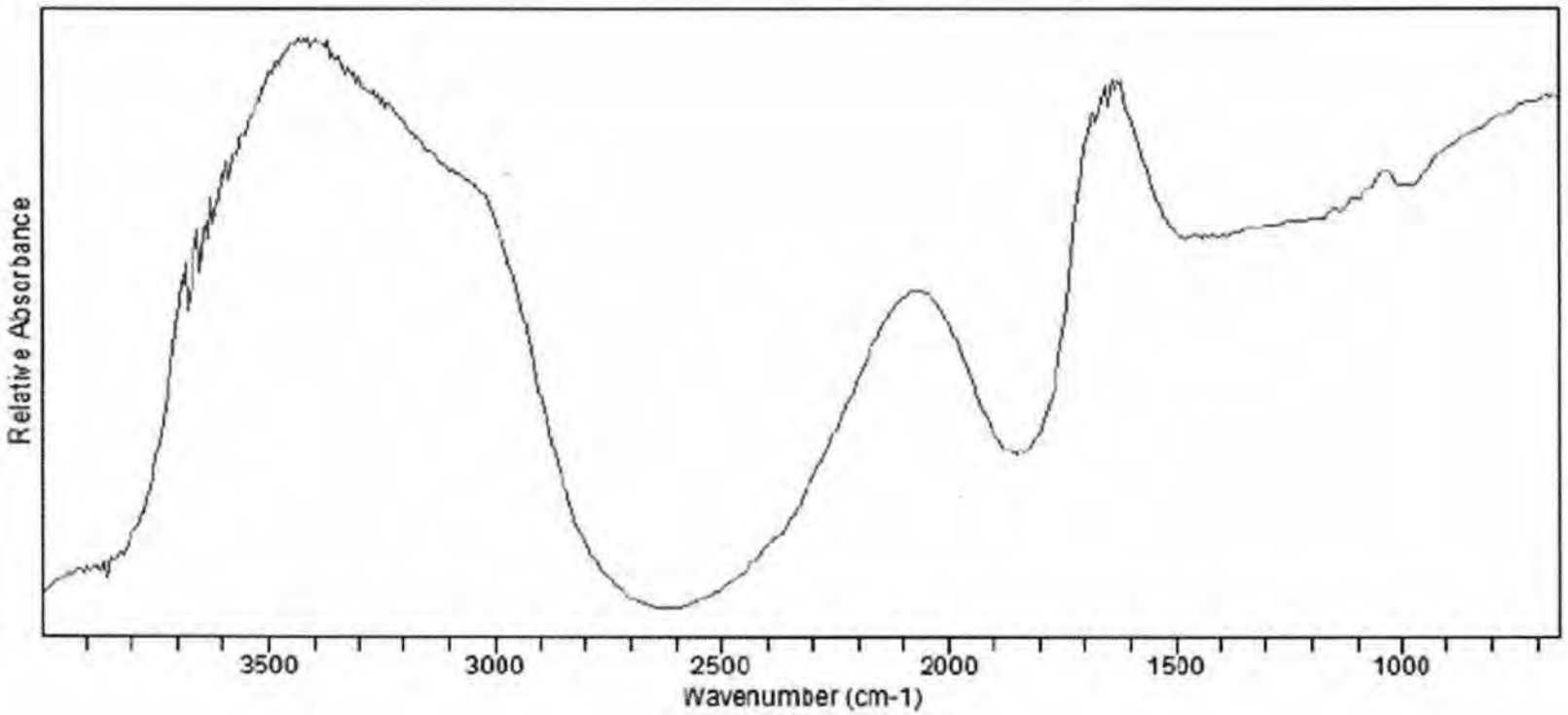
	RT	Area	% Area	Height
1	3.439	27445	0.03	1364
2	4.567	4335	0.01	587
3	4.769	19739	0.02	2916
4	17.353	2502823	2.98	65117
5	20.357	100564	0.12	3642
6	23.418	81289585	96.84	2250011

4. FTIR SPECTRUM OF ENZYME TREATED

Appendix C

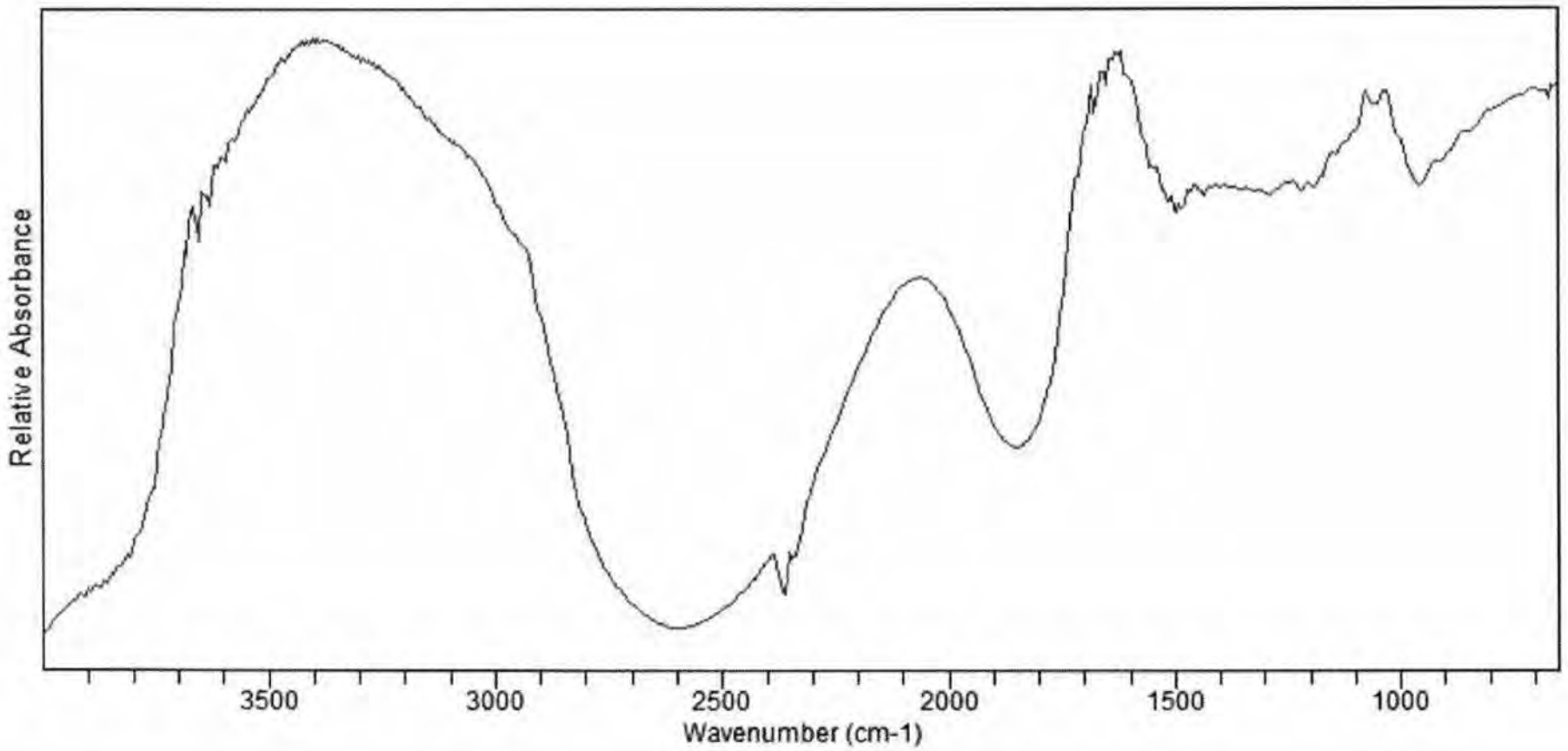
Raw Data

STEVITEN RICH Lot. 181107



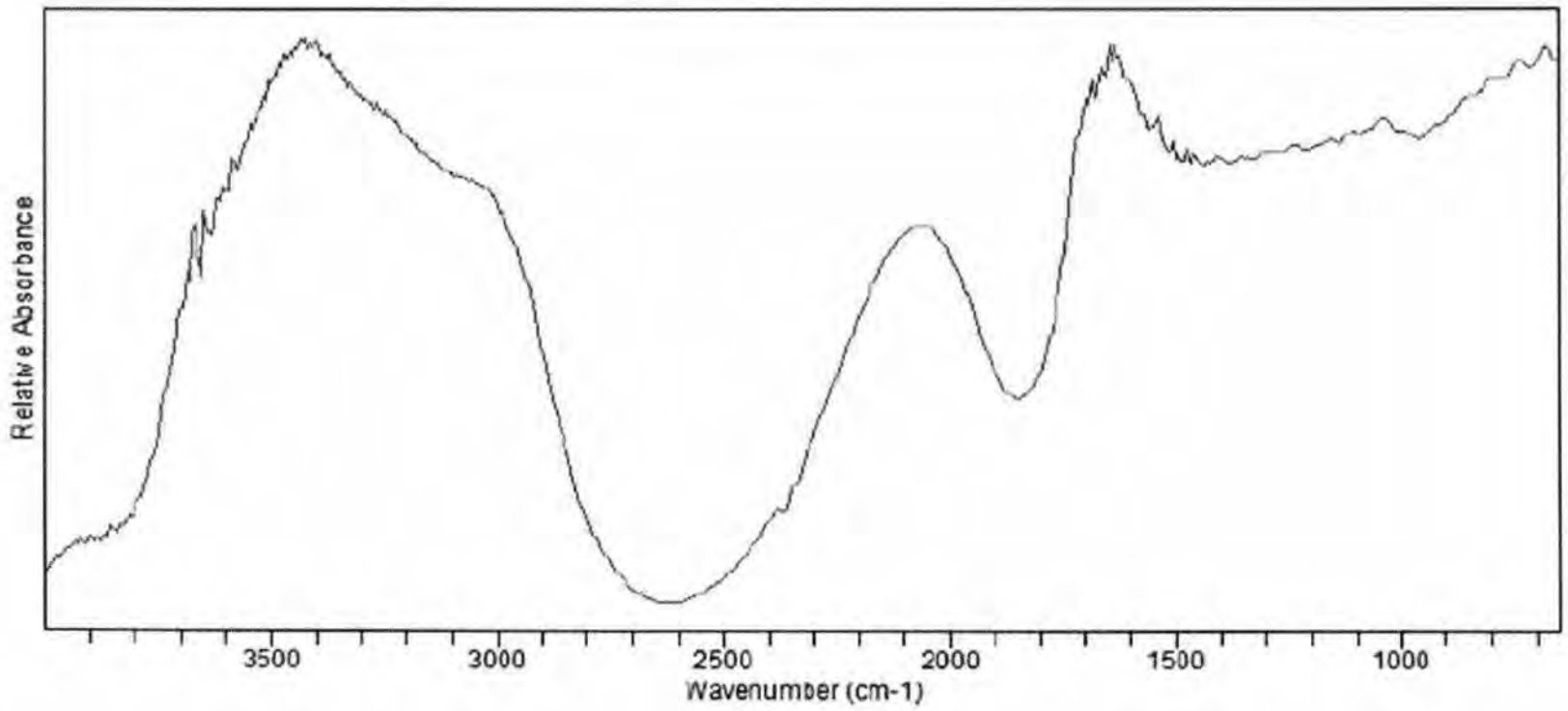
FTIR Spectrum of enzyme treated Lot. 181107

STEVITEN RICH Lot. 181203



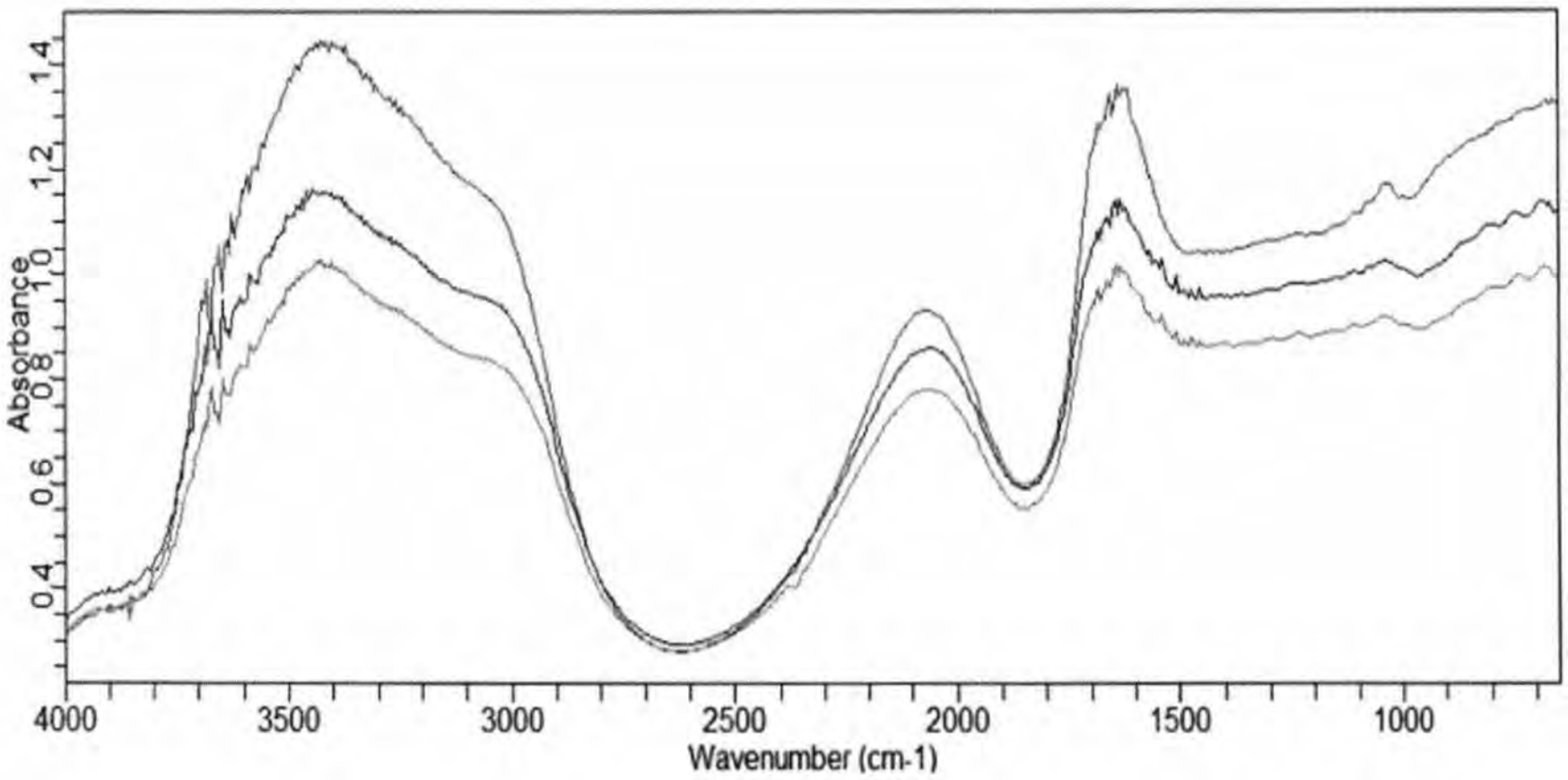
FTIR Spectrum of enzyme treated Lot. 181203

STEVITEN RICH Lot. 181224



FTIR Spectrum of enzyme treated Lot. 181224

STEVITEN RICH 3Lot. Overlay



5. A Comparison of the fingerprints of enzyme treated stevia, as analyzed by Ab Sciex LC/MS

Methods

UPLC Conditions

LC/MS system: NANOSPACE 5200 / Ab Sciex QTRAP 4500
Column: ACQUITY UPLC BHE Amide 1.7um, 2.1x150mm
Column Temp: 40℃
Flow Rate: 160 uL/min
Injection Volume: 1uL
Detection: uv@210nm
Mobile Phase A: Water, B: Acetonitrile

• Gradient

Time(min)	% A	% B	Curve
Init	30	70	-
10.0	30	70	6
25.0	50	50	6
26.0	70	30	6
35.0	70	30	6

MS Conditions

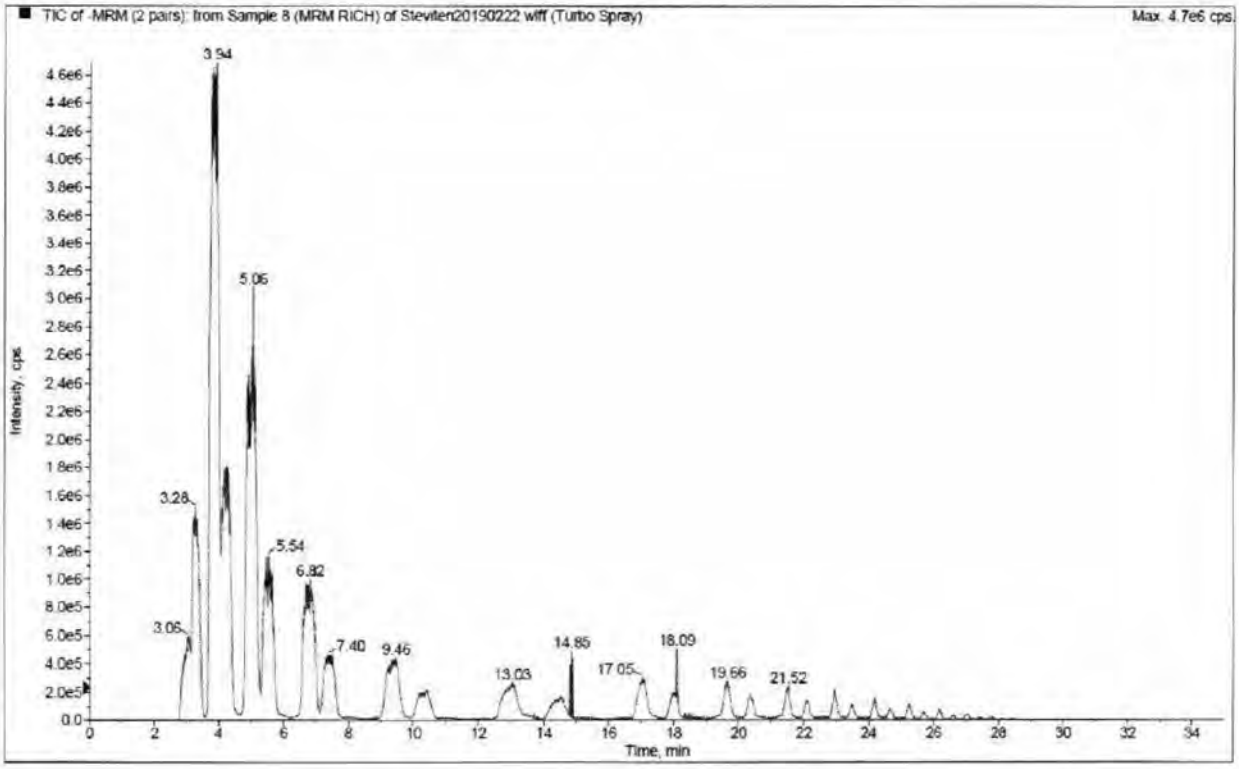
Ionization Mode : ESL negative
Curtain Gas : 30
IonSpray Voltage(IS) : 4500
Temperature : 400℃
Ion Source Gas : 50

- MS Scan Mode
  Mass Range : 500~2000Da
  Scan Time : 0.75s

- MS/MS Mode
  Mass Range : 100~2000Da
  Scan Time : 0.2s
  Collision Energy(CE) : 30

Appendix E
Raw Data

Figure 7. A Comparison of the finger prints of enzyme treated Stevia, as analyzed by Ab Sciex LC/MS (STEVITEN RICH)



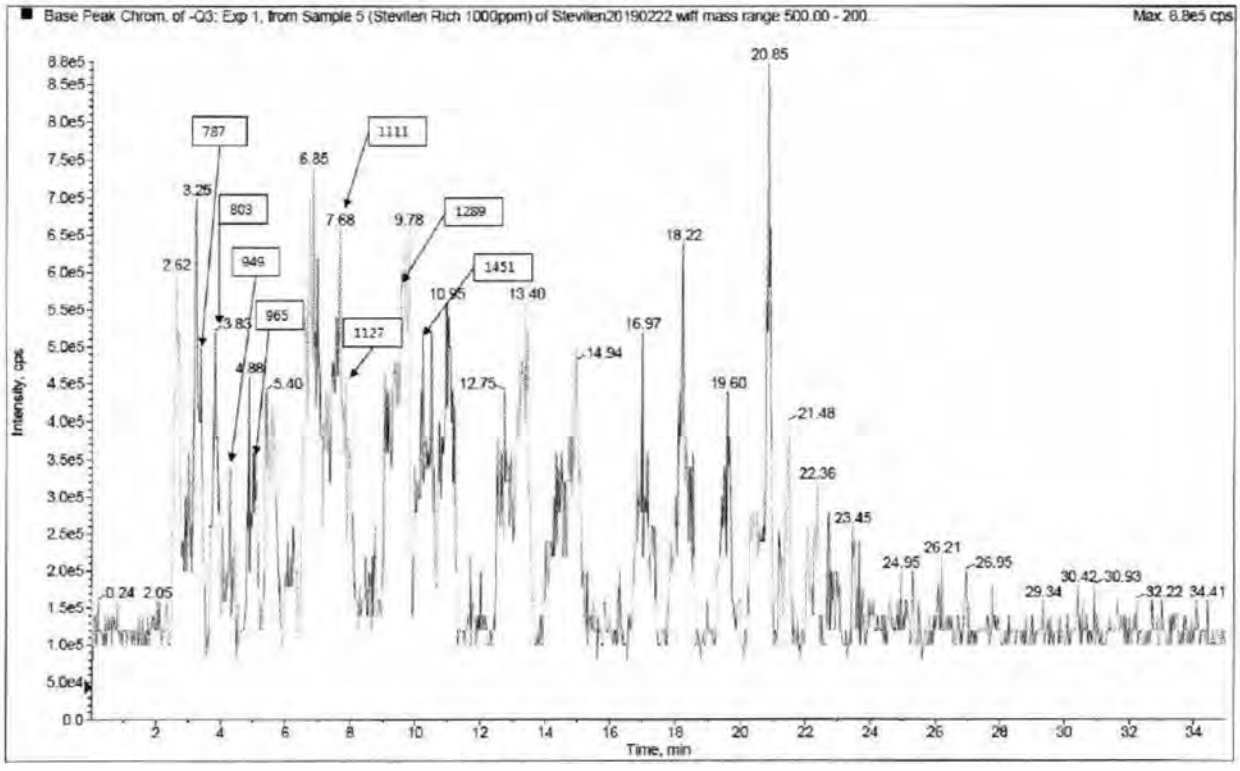
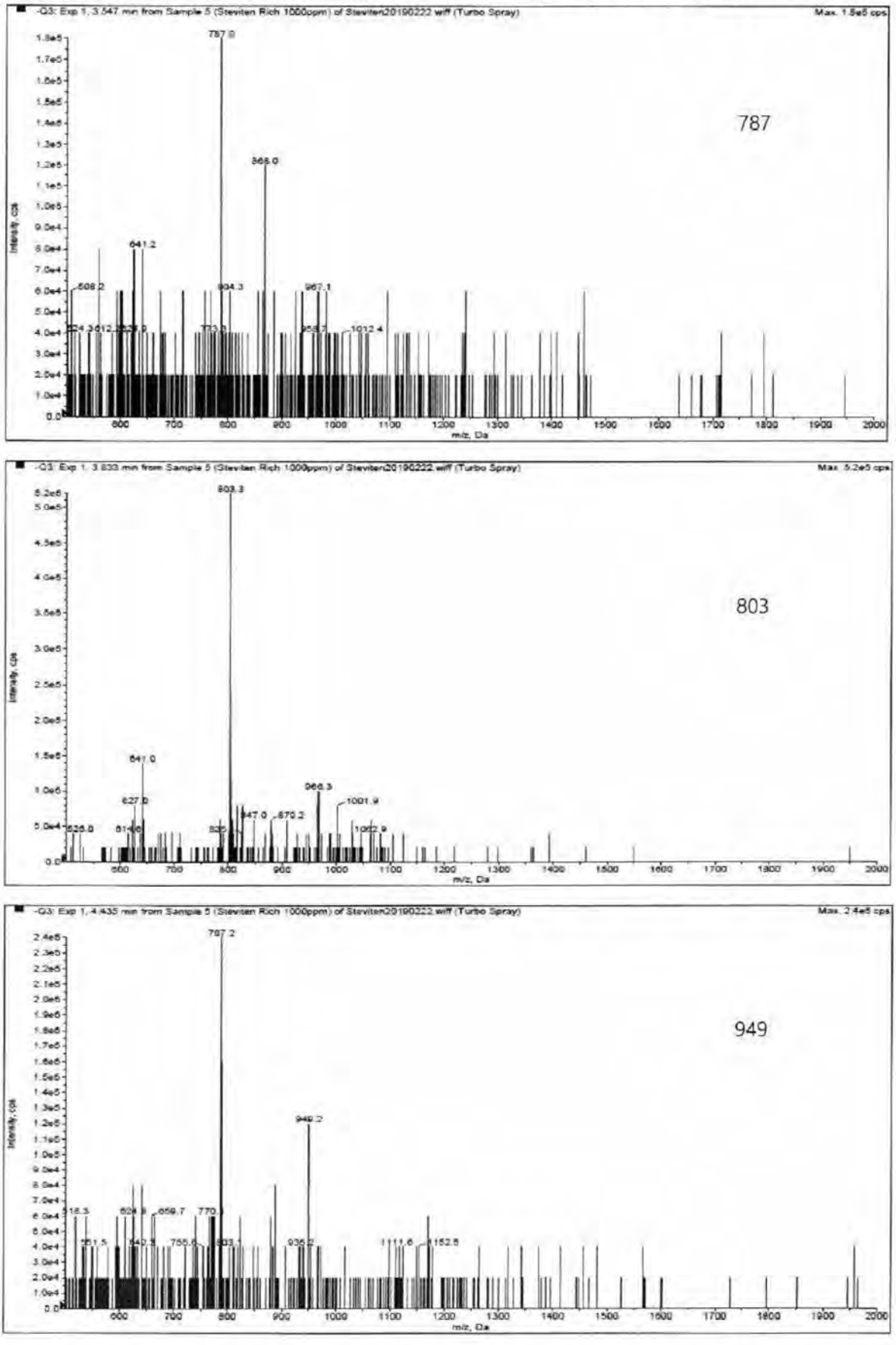
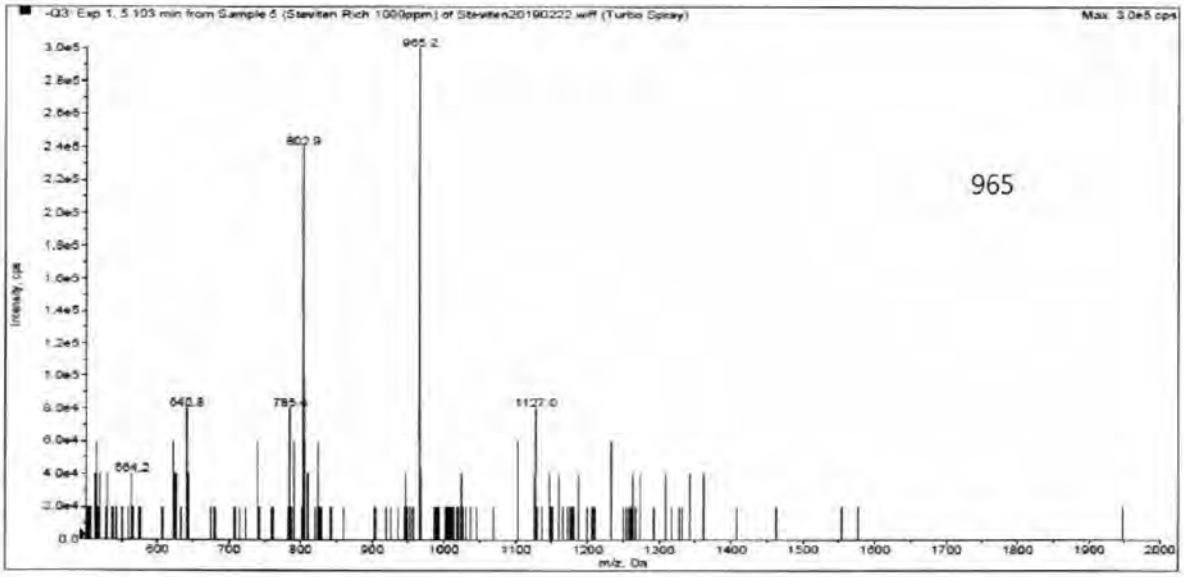
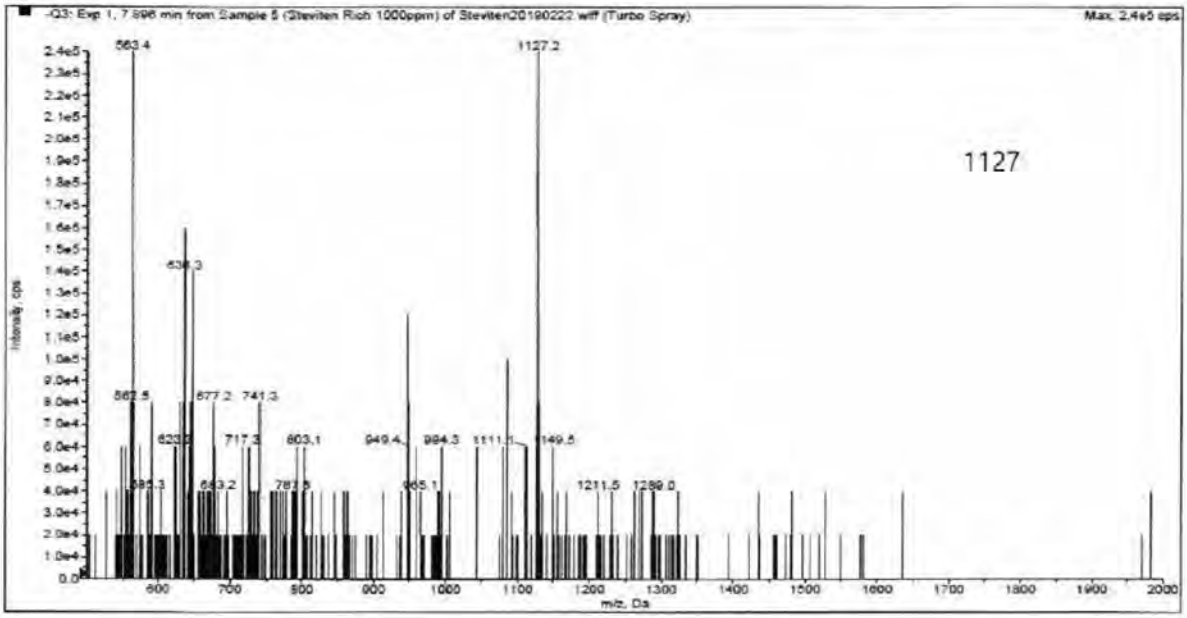


Figure 8. The molecular weigh of Steviol glucosides Componets




965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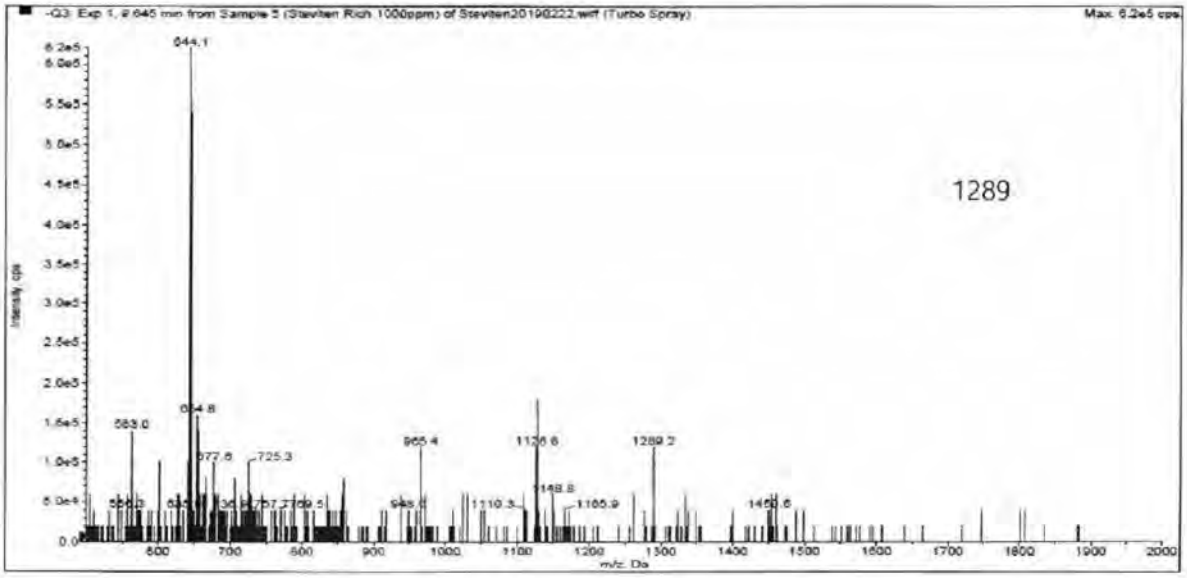
1289

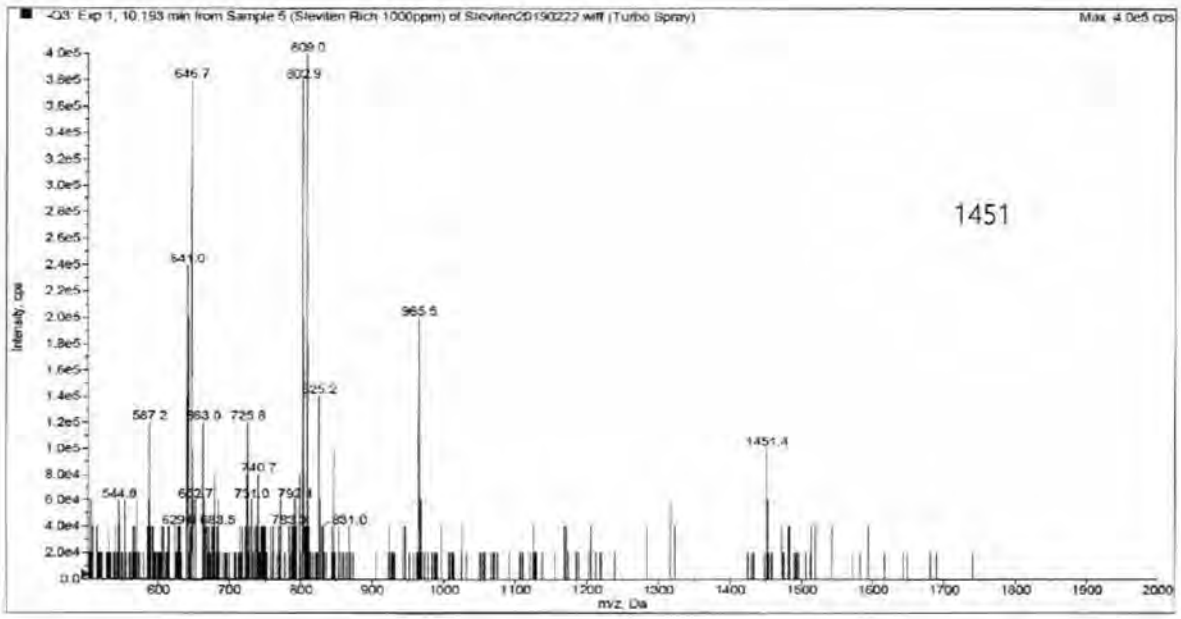

Figure 7. We know each position of steviol glycosides components by LC and also know molecular weight by Ab Sciex. Also we can see the same peak at the same time.

Figure 8. We can distinguish each component of steviol glycosides by molecular weight of Ab Sciex