# APPLICATION FOR THE AUTHORISATION OF STEVIOL GLYCOSIDES PRODUCED BY ENZYMATIC CONVERSION IN AUSTRALIA AND NEW ZEALAND

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# Application for the Authorisation of Steviol Glycosides Produced by Enzymatic Conversion in Australia and New Zealand

## A. GENERAL REQUIREMENTS

In accordance with Section 3.1.1 (General Requirements) of the Food Standards Australia New Zealand (FSANZ) *Application Handbook* (FSANZ, 2025a), the following general information must be provided:

1. Format of the application;
2. Applicant details;
3. Purpose of the application;
4. Justification for the application;
5. Information to support the application;
6. Assessment procedure;
7. Confidential commercial information;
8. Other Confidential information;
9. Exclusive capturable commercial benefit;
10. International and other national standards;
11. Statutory declaration; and
12. Checklist.

Each point is addressed in the following subsections.

## A.1 Format of the Application

This application to amend the *Food Standards Australia New Zealand Food Code* ("the Code") is prepared pursuant to Guideline 3.1.1 – General Requirements and Guideline 3.3.1 – Food Additives of the FSANZ *Application Handbook* (FSANZ, 2025a), which require the following structured format to assess an application for a new food additive:

A. General information on the application;

B. Technical information on the food additive;

C. Information on the safety of the food additive; and

D. Information on dietary exposure to the food additive.

The application is presented in this format. At the start of each section (A to D) the information that must be addressed therein is specified in more detail. Additionally, an executive summary for the application is provided as a separate electronic document to this application. The application has been prepared in English and submitted electronically, as required by the FSANZ *Application Handbook* (FSANZ, 2025a).

## A.2 Applicant Details

Adorvia Biotechnology Co., Ltd is a subsidiary of Abiochem Biotechnology Co., Ltd and is a manufacturer of food ingredients. The contact details of, the company's representative, are listed below.


Company name: Adorvia Biotechnology Co., Ltd
Address: 5th Floor, Building 3, 1299 ZiYue Road
Shanghai, 200241, P.R. China

Telephone: [redacted]
Email: [redacted]


In addition, Trung Vo, of Intertek Health Sciences Inc., is involved in the preparation, submission, and stewardship of this application. His contact details are listed below.


Contact name: [redacted]
Address: Intertek Health Sciences Inc.
2233 Argentia Road, Suite 201
Mississauga, Ontario, Canada L5N 2X7

Telephone: [redacted]
Email: [redacted]


## A.3 Purpose of the Application

The purpose of this application is to amend the Code to authorise steviol glycosides with a high Reb M or Reb D content produced by enzymatic conversion as manufactured by Adorvia Biotechnology Co., Ltd ("Adorvia"). The food additive is produced using sucrose synthase, β-1,3-glycosyltransferase, and

β-1,2-glycosyltransferase obtained from genetically modified strains that are derivatives of *Escherichia coli* BL21 (DE3). These enzymes convert the naturally occurring steviol glycosides within an extract of *Stevia rebaudiana* Bertoni leaves to Reb M and Reb D. The production process is consistent with the process described in Annex 3 for enzyme-modified steviol glycosides by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (JECFA, 2021). The final product is a steviol glycoside preparation containing high amounts of Reb M or Reb D that meets JECFA's purity specifications and is similar to other steviol glycosides already permitted for use in a range of conventional food and beverage products in Australia and New Zealand under Schedule 15.

Currently, Schedule 3 of the *Australia New Zealand Food Standards Code* ("the Code") contains specifications for "*steviol glycosides produced by enzymatic conversion*" (S3—35), which includes Reb M and Reb D. This specification "*relates to a steviol glycosides preparation obtained from the leaves of the Stevia rebaudiana Bertoni plant*" and presents a range of permissible processes that exclude the use of Adorvia's steviol glycosides containing high amounts of Reb M or Reb D. This application, therefore, aims to amend the Code to encompass Adorvia's manufacturing process as another means to safely and effectively produce steviol glycosides containing high amounts of Reb M or Reb D. To that end, the following should be included in S3—35, to permit use of Adorvia's steviol glycosides from enzymatic conversion:

"by enzymatic conversion of purified stevia leaf extract to produce rebaudioside M or rebaudioside D using both of the following enzymes:

(i) UDP-glucosyltransferases sourced from *Escherichia coli* BL21 (DE3) containing the UDP-glucosyltransferase gene from *Solanum tuberosum* or *Stevia rebaudiana;* and

(ii) sucrose synthase (EC 2.4.1.13) sourced from *Escherichia coli* BL21 (DE3) containing the sucrose synthase gene from *Methylocaldum szegediense.*"

This application does not intend to change the purity specification (≥95% steviol glycosides) or propose an extension for the use of Reb M or Reb D in additional food products nor does it propose to increase the permitted quantities of these substances in permitted food products.

## A.4 Justification for the Application

### A.4.1 Technological Function for the Food Additive

Steviol glycosides obtained through extraction of the leaves of *S. rebaudiana* Bertoni or enzymatic conversion, such as the process described herein, are currently permitted for use as high-intensity sweeteners in Australia and New Zealand. These substances are valuable to the food and beverage industry to offer a sensory profile that is similar to sucrose for reduced-calorie or no-sugar food and beverage applications without compromising on consumers' taste expectations. The steviol glycosides with high content of Reb M or Reb D contain a total steviol glycoside content of ≥95%, which are similar to substances that are currently on the Australian and New Zealand marketplaces.

### A.4.2 Costs and Benefits for Industry, Consumers, and Government Associated with Use of the Food Additive

Steviol glycosides with high Reb M or Reb D content offers similar benefits to the food industry and consumers as other similar substances currently on the Australian and New Zealand marketplaces. Adorvia's steviol glycosides with high Reb M or Reb D content would be used within the same food and beverage

applications at the same use levels as other steviol glycosides as a sugar replacement. The intended food and beverage applications benefit consumers seeking reduced-calorie or no-sugar options in order to reduce their overall added sugar intake. The consumption of added sugar from food and beverages has been associated with weight gain and other chronic diseases, and therefore, the availability of reduced-calorie or no-sugar food and beverage products has direct implications on Australian and New Zealand public health, especially in consumers with specific medical conditions that require reduced sugar intake such as individuals with diabetes. Therefore, the permissibility of Adorvia's substance would provide the food and beverage industry with an alternative source of high purity steviol glycosides with high Reb M or Red D content aside from those that are currently permitted, which would promote healthy market competition and ultimately benefit the Australian/New Zealand consumer. Considering Adorvia does not intend to propose an extension for the use of this food additive in any additional food products, nor do they wish to propose to increase the permitted quantities of steviol glycosides in permitted food products, there is no perceived benefit or added cost to the government.

## A.5 Information to Support the Application

Technical information specific to Adorvia's steviol glycosides with high Reb M or Reb D content is provided in Section B. Information related to the enzymes used to produce the food additive, following the specific sections within the FSANZ Application Handbook related to process aids, is also provided. FSANZ have reviewed the safety of steviol glycosides, including specific rebaudiosides, within multiple applications, including but not limited to, A1157, A1176, A1183, and A1268, which relate to steviol glycosides produced by enzymatic conversion. Since the safety of steviol glycosides and specific rebaudiosides is well established by FSANZ, a short summary of the safety information of these substances is provided in Section C.

## A.6 Assessment Procedure

The most appropriate procedure to be adopted in assessing the application to be the General Procedure – Level 1. It is anticipated that this application will involve amending *Standard 1.3.1 – Food Additives* of the Code to modify the specifications outlined in Schedule 3 for steviol glycosides produced by enzymatic conversion (section S3–35). Adorvia does not request that the evaluation be expedited.

## A.7 Confidential Commercial Information (CCI)

Adorvia requests that certain proprietary information be considered confidential commercial information (CCI). General summaries of the proprietary data are provided within this application. Data requested to be treated as CCI have been removed and are provided [redacted]. In addition, [redacted]. All information presented in these appendices is requested to remain confidential as it holds significant commercial value to the company, including proprietary details on the manufacture of the final steviol glycoside product. Redacted, non-confidential versions of the annexes are also provided.

## A.8 Other Confidential Information

Adorvia requests that the identity of the companies that perform analysis testing (*i.e.,* stability, residue, *etc.*) remain confidential and that their identity not be disclosed to the general public. The identity and contact information for the companies and persons responsible for producing these data should be treated as confidential, as public disclosure of this information is not required for the safety assessment of this ingredient.

## A.9 Exclusive Capturable Commercial Benefit (ECCB)

Adorvia is currently not the only manufacturer of steviol glycosides with high Reb M or Reb D content. Therefore, the application would not confer exclusive capturable commercial benefit (ECCB) in accordance with Section 8 of the *Food Standards Australia New Zealand Act 1991*, as there are other companies who would likely benefit from approval of this application.

## A.10 International and Other National Standards

### A.10.1 The Joint FAO/WHO Expert Committee on Food Additives (JECFA)

At its 82nd meeting in 2016, the JECFA Committee conducted a re-evaluation of the safety, dietary intake, and specifications for steviol glycosides. The safety of steviol glycosides and the acceptable daily intake (ADI) of 0 to 4 mg/kg body weight, expressed as steviol, were confirmed. The Committee reviewed a new manufacturing process for rebaudioside A that used a genetically modified strain of *Yarrowia lipolytica* to re-express the steviol glycoside biosynthetic pathway. This led to the issuance of a new specification monograph for "*Rebaudioside A from Multiple Gene Donors Expressed in Yarrowia lipolytica*" in 2016. The purity requirement for rebaudioside A from genetically modified *Y. lipolytica* is no less than 95% total steviol glycosides on a dried basis.

Additionally, the Committee considered data demonstrating the shared metabolism of all steviol glycosides and established new specifications for "*Steviol Glycosides from Stevia rebaudiana Bertoni*" in 2017. This expanded the definition of steviol glycosides to include a mixture of compounds with a steviol backbone conjugated to various sugar moieties. The purity requirement for steviol glycosides from *S. rebaudiana* Bertoni is no less than 95% total steviol glycosides on a dried basis.

More recently, JECFA adopted a framework to develop specifications for steviol glycosides produced through 4 methodologies, including enzymatic modification (also referred to as enzymatic conversion) (JECFA, 2021). The JECFA framework for steviol glycosides has been ratified by the *Codex Alimentarius* into the *General Standard for Food Additives* (GSFA), and thus adoption on a global scale is currently underway (Codex, 2025). The specifications for steviol glycosides produced by enzymatic conversion share the same identity and purity (*i.e.,* ≥95% total steviol glycosides) requirements as steviol glycosides obtained from extraction of the leaves of *S. rebaudiana* Bertoni.

### A.10.2 United States

In the United States (U.S.), steviol glycosides have Generally Recognised as Safe (GRAS) status for use as general purpose sweeteners in foods. Over 80 GRAS notices have been submitted to the U.S. Food and Drug Administration (FDA) covering purified individual steviol glycosides, mixtures of steviol glycosides, and glucosylated steviol glycosides, all with a total steviol glycoside content of no less than 95%. The U.S. FDA has raised no objections to the GRAS status of steviol glycoside products for use as general purpose sweeteners in foods, recognising the general safety of these substances.

### A.10.3 Other Jurisdictions

Steviol glycosides are approved for use in a number of other jurisdictions, including the European Union (EU), Canada, Asia, Central/South America, Africa, and the Middle East. Further details of the regulatory approvals of steviol glycosides in Canada and the EU are presented in Sections C.4.3 and C.4.4, respectively. In several Asian countries, including Japan, China, Hong Kong, Indonesia, Malaysia, Myanmar, Pakistan,

Philippines, Singapore, Taiwan, Thailand, and Vietnam, steviol glycosides are approved as food additives or sweetening agents. Japan, for example, has authorised various stevia extracts for use. The Food Safety and Standards Authority of India (FSSAI) has also approved the use of steviol glycosides as a non-nutritive sweetener.

In Central/South American countries and several Middle Eastern and African nations, steviol glycosides, stevioside, *S. rebaudiana* leaves, and highly refined stevia extracts are permitted for use as low-calorie sweeteners. Additionally, these glycosides are recognised as food additives in Switzerland and Russia.

## A.11 Statutory Declaration

Signed Statutory Declarations for Australia and New Zealand are provided in Appendix B.

## A.12 Checklist

Completed checklists relating to the information required for submission with this application based on the relevant guidelines in the FSANZ *Application Handbook* are provided in Appendix C.

# B. TECHNICAL INFORMATION ON THE FOOD ADDITIVE

In accordance with Section 3.3.1 – Food Additives of the FSANZ *Application Handbook* (FSANZ, 2025a) the following technical information must be provided:

1. Nature and technological purpose of the food additive;
2. Information to enable identification of the additive;
3. Information on the chemical and physical properties of the additive;
4. Information on the impurity profile;
5. Manufacturing process;
6. Specifications for identity and purity;
7. Information for food labelling;
8. Analytical method for detection; and
9. Potential additional purposes of the food additive when added to food.

These points are addressed in the section that follows. In addition, to fulfil the requirements outlined in Guideline 3.3.2 – Processing Aids of the FSANZ *Application Handbook* FSANZ, 2025a), the following information on the enzymatic processing aids, including the production microorganisms, are presented:

1. Technical information on the processing aid;
2. Information related to the safety of an enzyme processing aid;
3. Additional information related to the safety of an enzyme processing aid derived from a microorganism; and
4. Additional information related the safety of an enzyme processing aid derived from a genetically modified microorganism.

## B.1 Nature and Technological Purpose of the Food Additive

### B.1.1 Technological Purpose

Adorvia's steviol glycosides produced by enzymatic conversion contain a high content of Rebaudioside (Reb) M or Reb D, and a total steviol glycoside content of ≥95%. The food additive is obtained through enzymatic conversion of steviol glycosides from an extract of *S. rebaudiana* Bertoni leaves. Adorvia's steviol glycosides fulfils the function as an intense sweetener and a flavour enhancer, consistent with the technological purposes listed in Schedule 14 – *Technological purposes performed by substances used as food additives*. This application is not intended to modify the currently permitted food and beverage uses of steviol glycosides as outlined in Schedule 15 of the Code.

### B.1.2 Sweetness Potency

Steviol glycosides produced by enzymatic conversion contain high levels of Reb M and/or Reb D, with a total steviol glycoside content of ≥95%. Based on its steviol glycoside distribution, it is expected that the food additive will have a sweetness intensity consistent with other steviol glycoside preparations already on the Australia and New Zealand marketplace (*i.e.*, 200 to 350 times sweeter than sucrose). The sweetness equivalence of steviol glycosides with high Reb M and/or Reb D content produced *via* enzymatic conversion was evaluated by Adorvia with a panel of trained tasters. The results indicate the sweetness intensity of steviol glycosides with high Reb M content to be approximately 312 times sweeter than a 5% sucrose solution and the sweetness intensity of steviol glycosides with high Reb D content to be approximately 250 times sweeter than a 5% sucrose solution. The full study report is provided in Appendix D.

### B.1.3 Stability

The shelf-life stability of steviol glycosides with high Reb M or Reb D content was investigated in 5 batches (Lot No. 1, 2, 3, 4, and 5) under accelerated conditions (40 ± 2°C; relative humidity: 75 ± 5%). A legend for the confidential lot numbers is provided in Appendix G. Samples were stored in sealed plastic bags for 3 months. Samples were analysed at timepoints 0, 1, 2, and 3 months of storage for the following parameters: appearance, total steviol glycoside and Reb M content, moisture, total plate count, mould and yeast, and *E. coli*. As shown in Table B.1.3-1, no significant changes in any of the tested parameters were identified throughout the storage period, indicating that steviol glycosides with high Reb M content produced by enzymatic conversion would have a similar shelf-life stability profile as other high-purity steviol glycoside preparations currently on the marketplace. Similarly, given the similarities in the chemical composition to steviol glycosides with high Reb D content produced by enzymatic conversion, and the fact that the manufacturing process is similar to the high Reb M preparation, it is expected that these results would extend to support the shelf-life stability of steviol glycosides with high Reb D content produced by enzymatic conversion. The study report is provided in Appendix E.

**Table B.1.3-1 Stability of Steviol Glycosides with High Reb M Content Produced by Enzymatic Conversion**

| Specification Parameter | Specification Limit | Timepoint (Months) | | | |
|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 |
| ***Lot No. 1*** | | | | | |
| Sensory | White to off-white powder | White powder | White powder | White powder | White powder |
| Rebaudioside M (anhydrous basis) (w/%) | ≥95.0% | 99.5 | 99.2 | 98.9 | 98.9 |
| Total steviol glycosides (anhydrous basis) (w/%) | ≥95.0% | 100.1 | 99.7 | 99.5 | 99.5 |
| Loss on drying (w/%) | ≤6.0% | 5.7 | 5.7 | 5.4 | 5.6 |
| Total plate count (CFU/g) | ≤1000 | <10 | NM | NM | <10 |
| Mould and yeast (CFU/g) | ≤100 | <10 | NM | NM | <10 |
| *Escherichia coli* (MPN) | ≤3 | <3.0 | NM | NM | <3.0 |
| ***Lot No.2*** | | | | | |
| Sensory | White to off-white powder | White powder | White powder | White powder | White powder |
| Rebaudioside M (anhydrous basis) (w/%) | ≥95.0% | 98.6 | 98.4 | 98.3 | 98.0 |

**Table B.1.3-1 Stability of Steviol Glycosides with High Reb M Content Produced by Enzymatic Conversion**

| Specification Parameter | Specification Limit | Timepoint (Months) | | | |
|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 |
| Total steviol glycosides (anhydrous basis) (w/%) | ≥95.0% | 99.3 | 99.2 | 99.1 | 98.8 |
| Loss on drying (w/%) | ≤6.0% | 4.7 | 4.7 | 4.5 | 4.7 |
| Total plate count (CFU/g) | ≤1000 | <10 | NM | NM | <10 |
| Mould and yeast (CFU/g) | ≤100 | <10 | NM | NM | <10 |
| *Escherichia coli* (MPN) | ≤3 | <3.0 | NM | NM | <3.0 |
| ***Lot No. 3*** | | | | | |
| Sensory | White to off-white powder | White powder | White powder | White powder | White powder |
| Rebaudioside M (anhydrous basis) (w/%) | ≥95.0% | 98.1 | 98.2 | 97.6 | 98.1 |
| Total steviol glycosides (anhydrous basis) (w/%) | ≥95.0% | 99.0 | 99.1 | 98.6 | 99.0 |
| Loss on drying (w/%) | ≤6.0% | 4.5 | 4.5 | 4.3 | 4.5 |
| Total plate count (CFU/g) | ≤1000 | <10 | NM | NM | <10 |
| Mould and yeast (CFU/g) | ≤100 | <10 | NM | NM | <10 |
| *Escherichia coli* (MPN) | ≤3 | <3.0 | NM | NM | <3.0 |
| ***Lot No. 4*** | | | | | |
| Sensory | White to off-white powder | White powder | White powder | White powder | White powder |
| Rebaudioside M (anhydrous basis) (w/%) | ≥95.0% | 97.3 | 98.2 | 96.8 | 96.8 |
| Total steviol glycosides (anhydrous basis) (w/%) | ≥95.0% | 98.3 | 99.2 | 97.9 | 97.8 |
| Loss on drying (w/%) | ≤6.0% | 4.3 | 4.3 | 4.0 | 4.3 |
| Total plate count (CFU/g) | ≤1000 | <10 | NM | NM | <10 |
| Mould and yeast (CFU/g) | ≤100 | <10 | NM | NM | <10 |
| *Escherichia coli* (MPN) | ≤3 | <3.0 | NM | NM | <3.0 |
| ***Lot No. 5*** | | | | | |
| Sensory | White to off-white powder | White powder | White powder | White powder | White powder |
| Rebaudioside M (anhydrous basis) (w/%) | ≥95.0% | 97.8 | 97.8 | 97.2 | 97.6 |
| Total steviol glycosides (anhydrous basis) (w/%) | ≥95.0% | 99.2 | 99.2 | 98.7 | 99.0 |
| Loss on drying (w/%) | ≤6.0% | 5.7 | 5.5 | 5.4 | 5.5 |
| Total plate count (CFU/g) | ≤1000 | <10 | NM | NM | <10 |
| Mould and yeast (CFU/g) | ≤100 | <10 | NM | NM | <10 |
| *Escherichia coli* (MPN) | ≤3 | <3.0 | NM | NM | <3.0 |

CFU = colony-forming units; MPN = most probable number; NM = not measured; Reb M = rebaudioside M.

## B.2 Information to Enable Identification of the Food Additive

Information to enable the identification of steviol glycosides produced by enzymatic conversion, including the chemical structure, the chemical name, the molecular weight and formula, and the common name, are presented below.

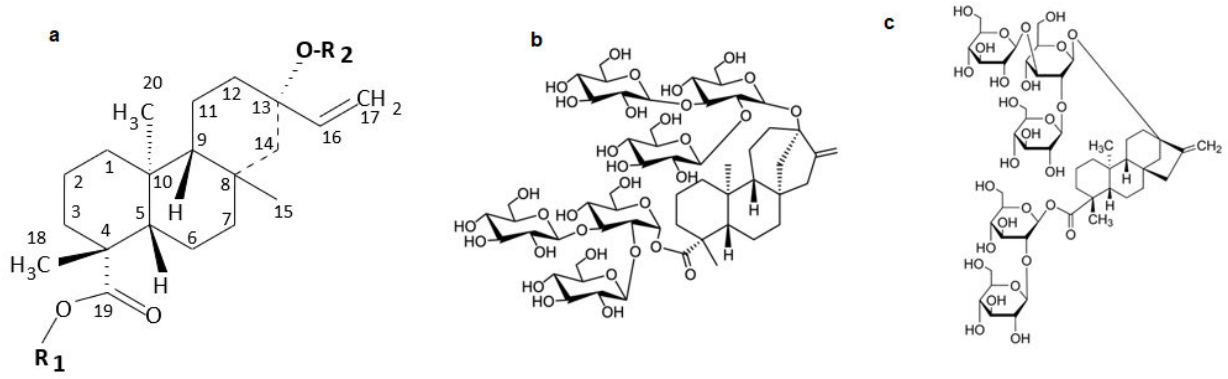
### B.2.1 Identity of Substance

Adorvia's steviol glycosides produced by enzymatic conversion contain a high content of Reb M or Reb D, and a total steviol glycoside content of ≥95%. The food additive is obtained through enzymatic conversion of steviol glycosides from an extract of *S. rebaudiana* Bertoni leaves. All steviol glycosides have the aglycone steviol as a backbone (see Figure B.2.1-1) with a number of sugar moieties ($R_1$ and $R_2$) attached to specific positions of the aglycone (C-13 and C-19). More than 60 steviol glycoside molecules found in stevia leaf extract differ only with respect to the type and number of glycoside units at positions $R_1$ and $R_2$. Reb M is an ent-kaurane diterpene glycoside with a steviol backbone. Unlike the other steviol glycosides, Reb M has two 2-O-ß-D-glucopyranosyl-3-O-ß-D-glucopyranosyl-ß-D-glucopyranosyl units, an ether at position C-13, and an ester at position C-19. Reb D is an ent-kaurane diterpene glycoside with a steviol backbone conjugated to 5 glucose units, an ether at position C-13, and an ester at position C-19. Details of the identity of the food additive are presented in Table B.2.1-1.

**Table B.2.1-1 Identity of Steviol Glycosides with High Reb M or Reb D Content Produced *via* Enzymatic Conversion**

| | |
|---|---|
| **Chemical Name** | Rebaudioside M: 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, 2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl ester<br><br>Rebaudioside D: 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, 2-O-β-D-glucopyranosyl-β-D-glucopyranosyl ester |
| **Common Name** | Steviol glycosides, enzyme-modified steviol glycosides, enzymatically produced steviol glycosides, steviol glycosides by enzyme modification |
| **Synonym** | Rebaudioside M, Rebaudioside D, Reb M, Reb D |
| **Chemical formula** | Rebaudioside M: $C_{56}H_{90}O_{33}$<br>Rebaudioside D: $C_{50}H_{80}O_{28}$ |
| **Molecular Weight** | Rebaudioside M: 1,291.29 Daltons<br>Rebaudioside D: 1,129.15 Daltons |
| **CAS number** | Rebaudioside M: CAS No. 1220616-44-3<br>Rebaudioside D: CAS No. 63279-13-0 |

**Figure B.2.1-1 Backbone Structure of (a) Steviol Glycosides and Chemical Structure of (b) Reb M and (c) Reb D**

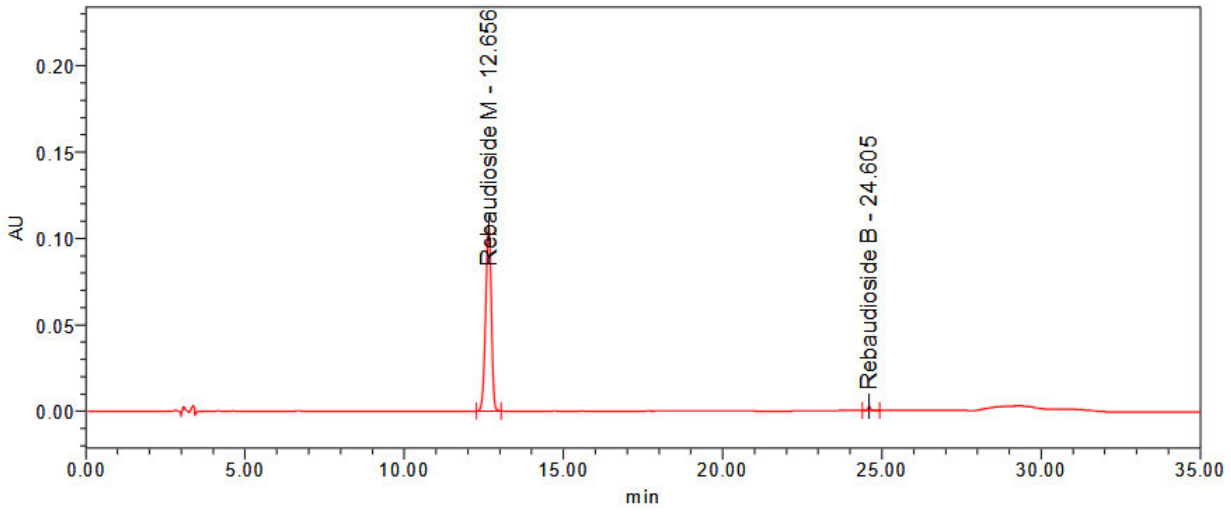



Reb D = rebaudioside D; Reb M = rebaudioside M.

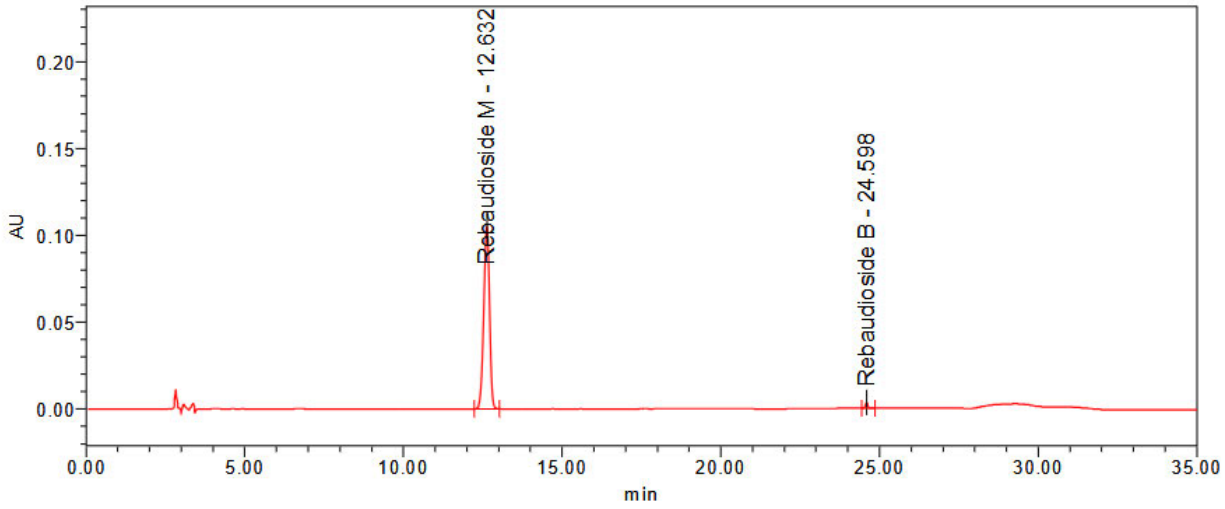
Adorvia's steviol glycosides produced by enzymatic conversion have been analysed by high-performance liquid chromatography (HPLC) following the method described by JECFA (2021, 2023). As shown in Figure B.2.1-2, 5 batches of steviol glycosides produced by enzymatic conversion with a high Reb M content showed a major peak at a retention time of approximately 12.6 minutes, corresponding to Reb M. Lesser peaks at retention time 19.1 and 24.6 minutes were observed, corresponding to rebaudioside A (Reb A) and rebaudioside B (Reb B), respectively.

**Figure B.2.1-2 High-Performance Liquid Chromatograms of Steviol Glycosides Produced by Enzymatic Conversion with High Reb M**

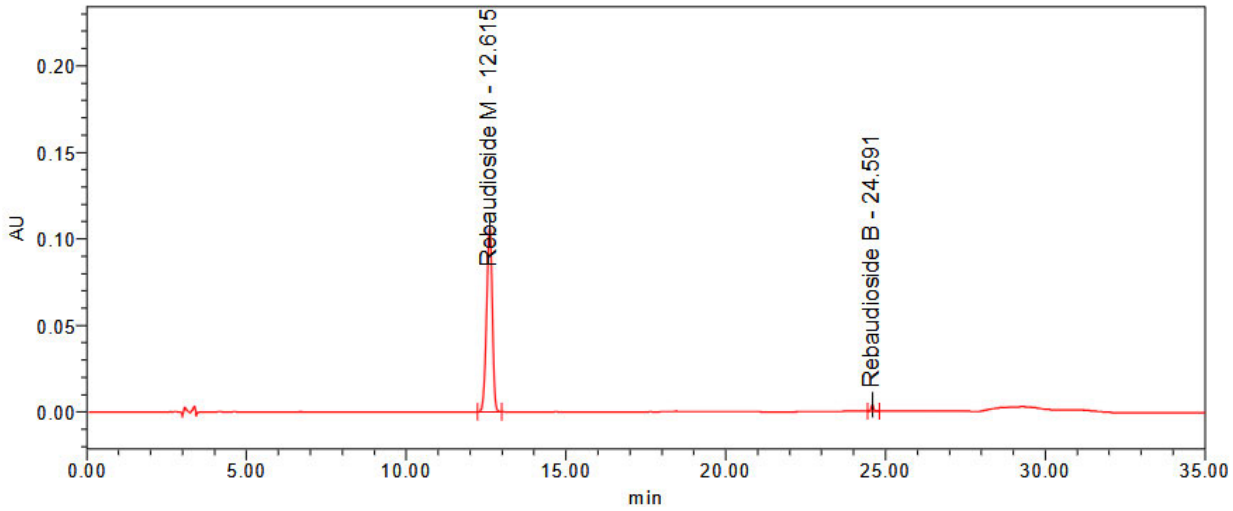
Lot No. 1



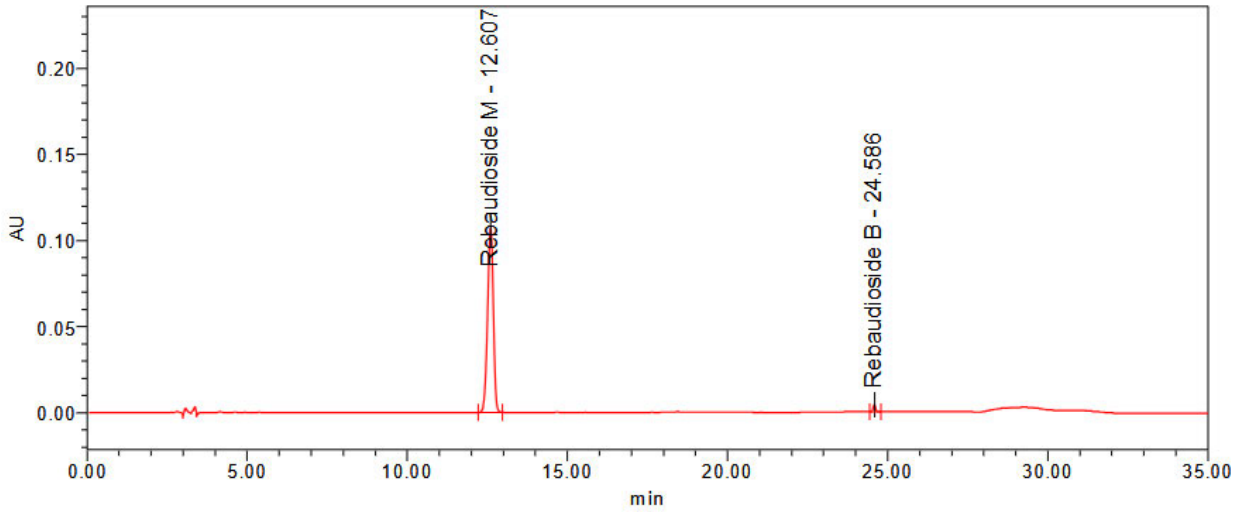

Lot No. 2



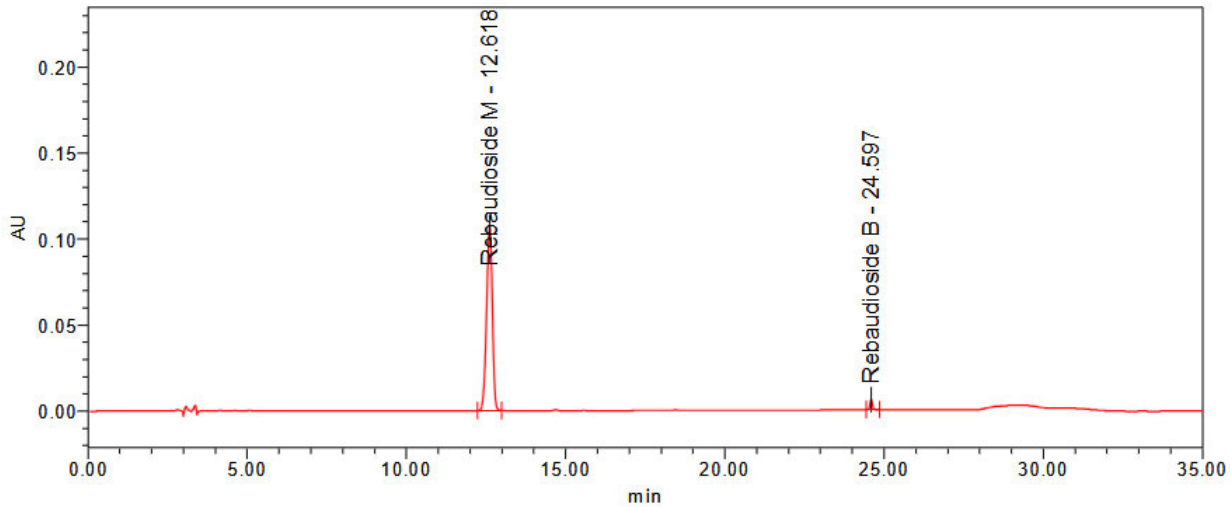

Lot No. 3




Lot No. 4



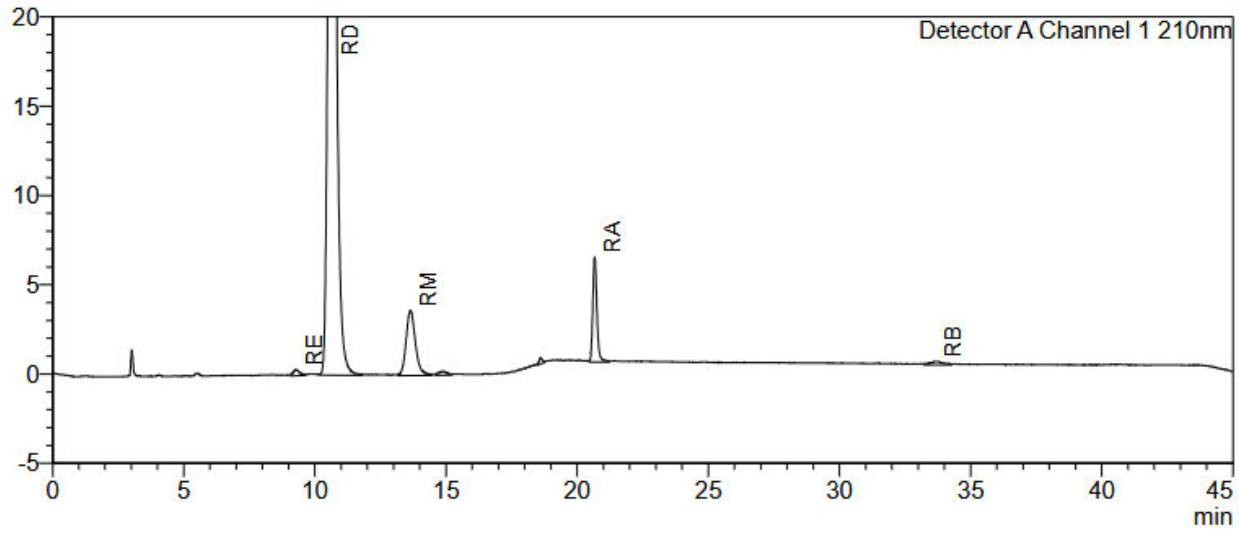

Lot No. 5



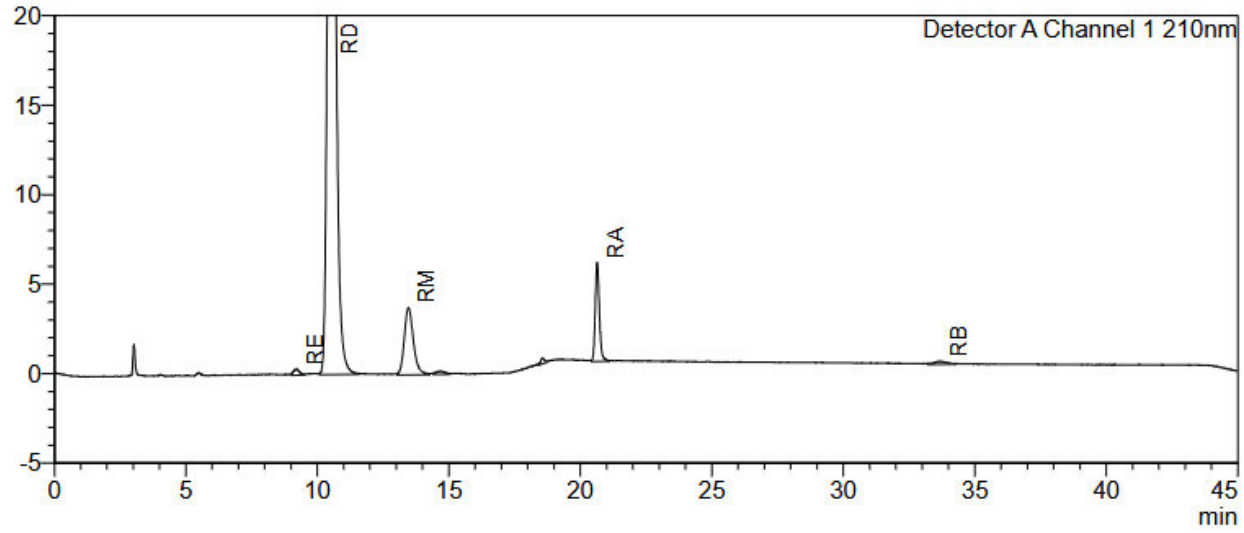

Similarly, as shown in Figure B.2.1-3, 5 batches of steviol glycosides produced by enzymatic conversion with a high Reb D content showed a major peak at a retention time of approximately 10.5 minutes, corresponding to Reb D. Lesser peaks at 9.2, 13.5, 20.5, and 37.5 minutes were observed, corresponding to rebaudioside E (Reb E), Reb M, and Reb A, respectively.

**Figure B.2.1-3 High-Performance Liquid Chromatography Chromatograms of Steviol Glycosides Produced by Enzymatic Conversion with High Reb D**

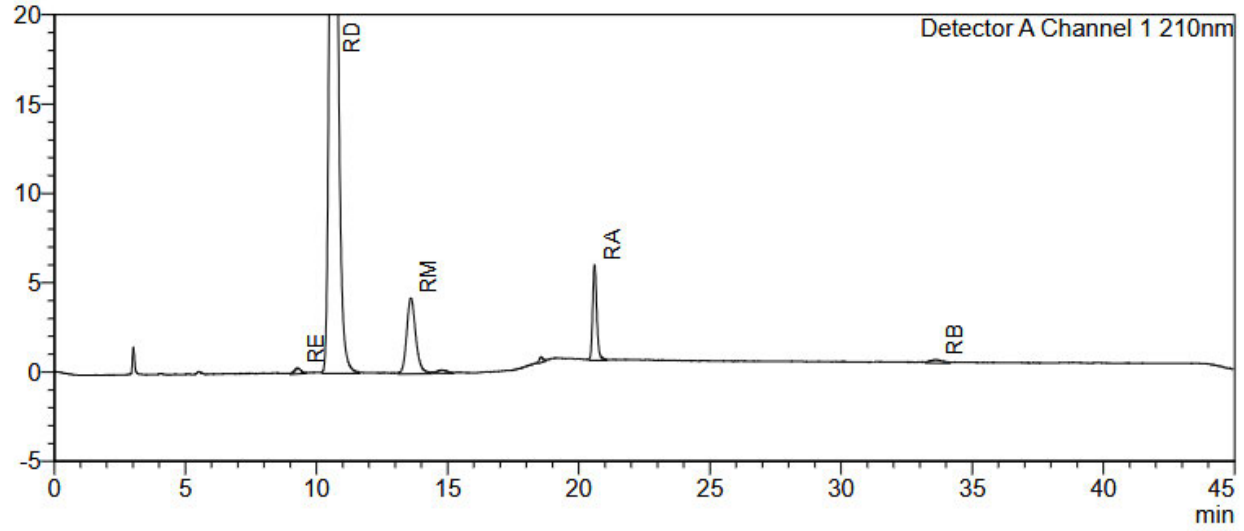
Lot No. 6



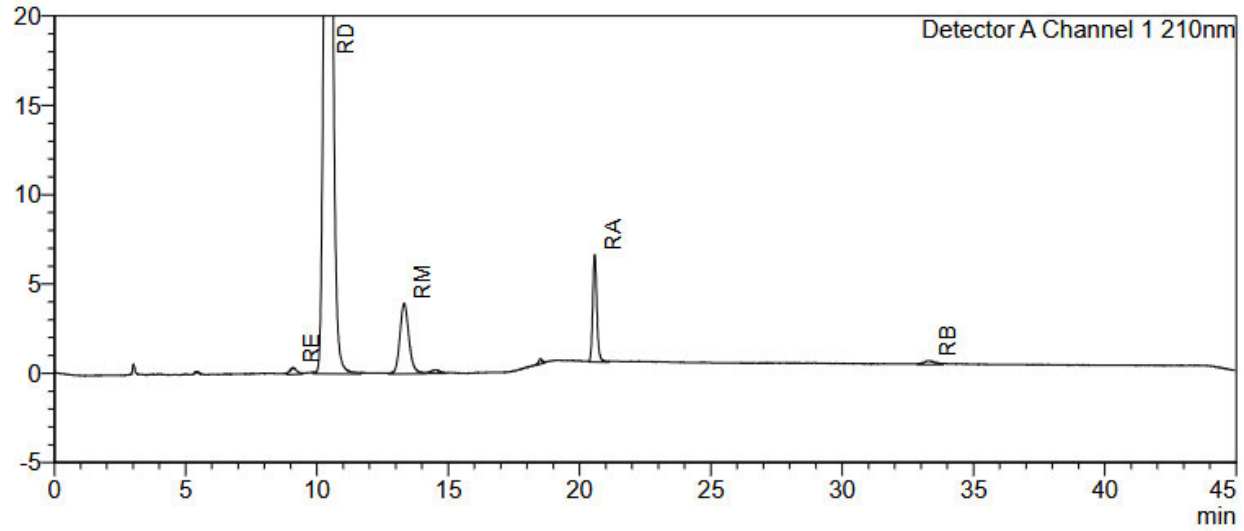
Lot No. 7




Lot No. 8

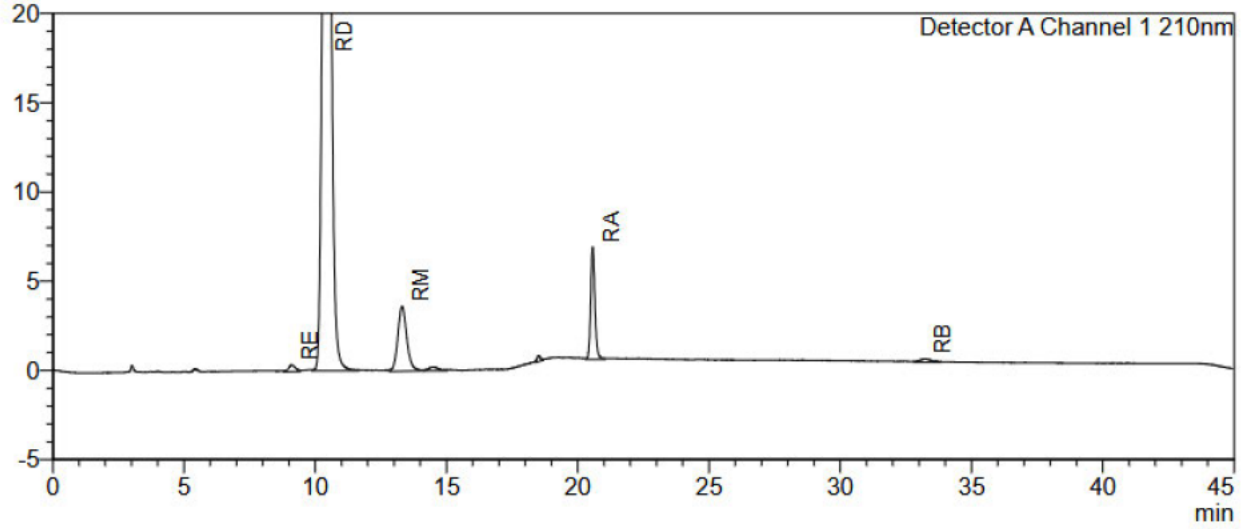



Lot No. 9




Lot No. 10

**Figure B.2.1-3 High-Performance Liquid Chromatography Chromatograms of Steviol Glycosides Produced by Enzymatic Conversion with High Reb D**




min = minutes; RA = rebaudioside A; RB = rebaudioside B; RE = rebaudioside E; RM = rebaudioside M; Reb D = rebaudioside D (also referred to as "RD" in the image).

## B.3 Information on the Chemical and Physical Properties

Steviol glycosides produced by enzymatic conversion are a white-to-off-white powder with a clean taste and no abnormal or off odour. Steviol glycosides are a group of compounds that share a similar molecular structure, where different sugar moieties are attached to the aglycone steviol (an *ent*-kaurene-type diterpene). Steviol glycosides include all compounds containing a steviol backbone conjugated to any number, combination, or orientation of saccharides (glucose, rhamnose, fructose, deoxyglucose, galactose, arabinose, and xylose (JECFA, 2020). Based on the similar chemical structure, all steviol glycosides share a common metabolic fate following consumption (Purkayastha *et al.,* 2016). Specifically, steviol glycosides are hydrolysed to steviol in the large intestine, which is subsequently absorbed and conjugated with glucuronic acid to form steviol glucuronide. The glucuronide metabolite is then excreted primarily *via* the urine in humans (Kraemer and Maurer, 1994; Koyama *et al*., 2003a,b; Geuns and Pietta, 2004 [unpublished]; Simonetti *et al*., 2004; Geuns *et al*., 2006, 2007; Wheeler *et al*., 2008; Roberts *et al.,* 2016).

The steviol glycoside distribution of 5 non-consecutive batches of steviol glycosides with high Reb M content, measured by HPLC, demonstrates that the food additive is comprised primarily of Reb M (average 98.6%) and lesser amounts of rebaudioside B (average 1.4%). The total steviol glycoside content is consistently greater than 95%. See Table B.3.1-1 below.

**Table B.3.1-1 Steviol Glycoside Distribution for 5 Production Batches of Steviol Glycosides with High Reb M Content Produced *via* Enzymatic Conversion**

| Steviol Glycoside | Manufacturing Lot No. | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | Mean |
| Rebaudioside B (%) | 0.9 | 1.2 | 1.4 | 1.5 | 2.1 | 1.4 |
| Rebaudioside M (%) | 99.1 | 98.8 | 98.6 | 98.5 | 97.9 | 98.6 |
| **Total Steviol Glycosides (%)** | **100.0** | **100.0** | **100.0** | **100.0** | **100.0** | **100.0** |

Reb M = rebaudioside M.

The steviol glycoside distribution of 5 non-consecutive batches of steviol glycosides with high Reb D content, measured by HPLC, demonstrates that the food additive is comprised primarily of Reb D (average 87.3%) and lesser amounts of other steviol glycosides, such as Reb M (average 6.9%), Reb A (average 4.7%), as well as Reb E and Reb B (average <0.5% each). The total steviol glycoside content is consistently greater than 95%. See Table B.3.1-2 below.

**Table B.3.1-2 Steviol Glycoside Distribution for 5 Production Batches of Steviol Glycosides with High Reb D Content Produced *via* Enzymatic Conversion**

| Steviol Glycoside | Manufacturing Lot No. | | | | | |
|---|---|---|---|---|---|---|
| | 6 | 7 | 8 | 9 | 10 | Mean |
| Rebaudioside E (%) | 0.306 | 0.329 | 0.315 | 0.352 | 0.354 | 0.331 |
| Rebaudioside D (%) | 87.761 | 87.552 | 86.678 | 87.413 | 87.303 | 87.341 |
| Rebaudioside M (%) | 6.507 | 6.82 | 7.779 | 6.719 | 6.83 | 6.931 |
| Rebaudioside A (%) | 4.749 | 4.653 | 4.558 | 4.829 | 4.812 | 4.720 |
| Rebaudioside B (%) | 0.290 | 0.297 | 0.265 | 0.297 | 0.284 | 0.287 |
| **Total Steviol Glycosides (%)** | **99.613** | **99.651** | **99.595** | **99.61** | **99.583** | **99.6104** |

Reb D = rebaudioside D.

## B.4 Information on the Impurity Profile

As described in Section B.6.1, Adorvia has established product specifications for steviol glycosides produced by enzymatic conversion that are consistent with the specifications in Schedule 3 of the Code for "steviol glycosides from *Stevia rebaudiana* Bertoni" (S3—35) and comply with the assay and impurity specifications for enzyme-modified steviol glycosides as established within Annex 3 of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) Framework for Steviol Glycosides (JECFA, 2021, 2023). Analyses for impurities such as residual protein, viable cells of the production strains, residual DNA of the production strains, and the processing contaminant isopropyl β-D-1-thiogalactopyranoside (IPTG) were conducted in batches of the final steviol glycoside products. In addition, 1 batch of the starting material was analysed for pesticides residues. These analyses are discussed further in Section B.6.

## B.5 Manufacturing Process

### B.5.1 Overview

Adorvia has developed an efficient production process for steviol glycosides with a high Reb M or Reb D content from highly purified Reb A (total steviol glycoside ≥95%) through *in vitro* multi-enzyme cascade reaction. In the first stage, genetically modified (GM) strains of *E. coli* BL21 (DE3) are fermented to produce 3 enzymes, which are then isolated for use in the enzymatic conversion process. In the second stage, the purified Reb A powder and sugar (glucose donor) are solubilised in water and mixed with the enzymes to generate a mixture of steviol glycosides containing more than >50% Reb M.

A summary of the enzymatic conversion process using the 3 enzymes is provided below.

1. β-1,2-glycosyltransferase facilitates the glucose transfer reaction in the presence of uridine diphosphate glucose (UDPG) or adenosine diphosphate glucose (ADPG). Reb A (SvG1G3) is converted to Reb D (SvG2G3), where an additional glucose unit is attached with a β-1,2- covalent

linkage at the C-19 position of the aglycone steviol. Note that Sv stands for steviol backbone, G1G3 for Reb A (as an example) indicates 1 glucose unit attached to C-19 position (R1), and 3 glucose units to C-13 position (R2) of steviol backbone.

2. In the presence of glycosyl donor UDPG or ADPG, β-1,3-glycosyltransferase facilitates the glucose transfer to Reb D (SvG2G3) to make Reb M (SvG3G3).

3. In the presence of sucrose, a glucose group is transferred onto uridine diphosphate (UDP) or adenosine diphosphate (ADP) by sucrose synthase, producing UDPG or ADPG. The reaction is reversible, and the UDP-UDPG cycle reaction can be repeated.

Details of the method of manufacture, including raw materials and processing aids used in the manufacturing process to generate the food additive and enzymes, are presented in Appendix A. Details of the production strain and construction of the production strain, and the enzymes involved in the production process are provided in Appendix F.

## B.5.2 Additional Information Regarding the Source Microorganisms and Enzymes Utilised as Processing Aids

### *B.5.2.1 Identity of the Enzymes*

The enzymes used as processing aids as part of the multi-enzyme cascade synthesis reaction to produce the final steviol glycoside products are sucrose synthase, β-1,3-glycosyltransferase, and β-1,2-glycosyltransferase. These enzymes are manufactured by Adorvia for the specific purpose of producing the company's steviol glycosides by enzymatic conversion; the enzymes will not be sold to another manufacturer.

β-1,2-glycosyltransferase facilitates the glucose transfer reaction in the presence of UDPG or ADPG. Reb A (SvG1G3) is converted to Reb D (SvG2G3), where an additional glucose unit is attached with a β-1,2-covalent linkage at the C-19 position of the aglycone steviol. In the presence of glycosyl donor UDPG or ADPG, β-1,3-glycosyltransferase facilitates the glucose transfer to Reb D (SvG2G3) to make Reb M (SvG3G3). Finally, in the presence of sucrose, a glucose group is transferred onto UDP or ADP by sucrose synthase, producing UDPG or ADPG. The reaction is reversible, and the UDP-UDPG cycle reaction can be repeated.

For each individual enzyme, the production strain is fermented at high density to obtain the fermentation broth. The fermentation broth is then concentrated by filtration and homogenised to release the intracellular enzymes. After that, the solution is filtered to remove the debris, thus obtaining the clear liquid containing the enzyme. Finally, this solution is ultrafiltered to remove undesired impurities, such as the production microorganism, resulting in the final liquid enzyme product.

### *B.5.2.2 Information on the Chemical and Physical Properties of the Enzymes*

Separate specifications for the 3 enzymes have been established and the analytical results from 3 batches of each enzyme are presented below in Tables B.5.2.2-1 to B.5.2.2-3. Certificates of analysis are provided in Appendix F. A legend for the confidential lot numbers is provided in Appendix G. These data demonstrate product consistency and the absence of the production microorganism in the final enzyme preparations. The enzymes are food-grade quality and conform to the recommended purity criteria established by the FCC (2024) and JECFA (2006a) and meet the requirements outlined in the JECFA (2021) monograph. The enzymes are exclusively used by Adorvia in the manufacture of steviol glycosides with High Reb M or Reb D.

As described in Section B.6.2.2, the enzymes are completely removed during the purification process and not present in the final steviol glycoside products (residual protein content below the LOQ of 20 mg/kg).

**Table B.5.2.2-1 Results of Batch Analyses for 3 Batches of Sucrose Synthase**

| Parameter | Specification | Method | Manufacturing Lot No. | | |
|---|---|---|---|---|---|
| | | | 1a | 1b | 1c |
| Activity | ≥10 U/mL | HPLC | 25 | 29 | 28 |
| Total viable count | <50,000 CFU/g | U.S. FDA BAM Chapter | <100 | <100 | <100 |
| *Salmonella* spp. | Not detected in 25 g | U.S. FDA BAM Chapter 5 | Not detected | Not detected | Not detected |
| *Escherichia coli* | Not detected in 25 g | U.S. FDA BAM Chapter 4 | Not detected | Not detected | Not detected |
| Total coliforms | ≤30 MPN/g | U.S. FDA BAM Chapter 4 | <10 | <10 | <10 |
| Antimicrobial activity | Negative | GB 4789.43 | Negative | Negative | Negative |
| Lead | ≤5 mg/kg | USP 43<2232> | Compliant | Compliant | Compliant |
| Total organic solids (%) | NS | ChP IV<0832/0841> | 0.23 | 0.27 | 0.25 |

CFU = colony-forming units; ChP = *Chinese Pharmacopeia*; GB = Chinese Standard; HPLC = high-performance liquid chromatography; NS = not specified; U.S. FDA BAM = United States Food and Drug Administration's *Bacteriological Analytical Manual*; USP = *United States Pharmacopeia*.

**Table B.5.2.2-2 Results of Batch Analyses for 3 Batches of β-1,2- glycosyltransferase**

| Parameter | Specification | Method | Manufacturing Lot No. | | |
|---|---|---|---|---|---|
| | | | 2a | 2b | 2c |
| Activity | ≥30 U/mL | HPLC | 36 | 32 | 35 |
| Total viable count | <50,000 CFU/g | U.S. FDA BAM | <100 | <100 | <100 |
| *Salmonella* spp. | Not detected in 25 g | U.S. FDA BAM Chapter 5 | Not detected | Not detected | Not detected |
| *Escherichia coli* | Not detected in 25 g | U.S. FDA BAM Chapter 4 | Not detected | Not detected | Not detected |
| Total coliforms | ≤30 CFU/g | U.S. FDA BAM | <10 | <10 | <10 |
| Antimicrobial activity | Negative | GB 4789.43 | Negative | Negative | Negative |
| Lead | ≤5 mg/kg | USP 43<2232> | Compliant | Compliant | Compliant |
| Total organic solids (%) | NS | ChP IV<0832/0841> | 0.28 | 0.24 | 0.25 |

CFU = colony-forming units; ChP = *Chinese Pharmacopeia*; GB = Chinese Standard; HPLC = high-performance liquid chromatography; NS = not specified; U.S. FDA BAM = United States Food and Drug Administration's *Bacteriological Analytical Manual*; USP = *United States Pharmacopeia*.

**Table B.5.2.2-3 Results of Batch Analyses for 3 Batches of β-1,3- glycosyltransferase**

| Parameter | Specification | Method | Manufacturing Lot No. | | |
|---|---|---|---|---|---|
| | | | 3a | 3b | 3c |
| Activity | ≥40 U/mL | HPLC | 45 | 51 | 49 |
| Total viable count | <50,000 CFU/g | U.S. FDA BAM | <100 | <100 | <100 |

**Table B.5.2.2-3 Results of Batch Analyses for 3 Batches of β-1,3- glycosyltransferase**

| Parameter | Specification | Method | Manufacturing Lot No. | | |
|---|---|---|---|---|---|
| | | | 3a | 3b | 3c |
| *Salmonella* spp. | Not detected in 25 g | U.S. FDA BAM Chapter 5 | Not detected | Not detected | Not detected |
| *Escherichia coli* | Not detected in 25 g | U.S. FDA BAM Chapter 4 | Not detected | Not detected | Not detected |
| Total coliforms | ≤30 CFU/g | U.S. FDA BAM | <10 | <10 | <10 |
| Antimicrobial activity | Negative | GB 4789.43 | Negative | Negative | Negative |
| Lead | ≤5 mg/kg | USP 43<2232> | Compliant | Compliant | Compliant |
| Total organic solids (%) | NS | ChP IV<0832/0841> | 0.17 | 0.17 | 0.19 |

CFU = colony-forming units; ChP = *Chinese Pharmacopeia*; GB = Chinese Standard; HPLC = high-performance liquid chromatography; NS = not specified; U.S. FDA BAM = United States Food and Drug Administration's *Bacteriological Analytical Manual*; USP = *United States Pharmacopeia*.

### *B.5.2.3 General Information on the Use of the Enzymes as a Food Processing Aid in Other Countries*

The sucrose synthase and glucosyltransferase enzymes described within this application are consistent with Annex 3 of the JECFA Framework for Steviol Glycosides, and are technologically similar to other sucrose synthases and glucosyltransferases used in the production of steviol glycosides by enzymatic conversion as listed in Schedule 18 of the Code.

### *B.5.2.4 Information on the Potential Toxicity of the Enzymes*

The Basic Local Alignment Search Tool (BLAST) program maintained by the National Center for Biotechnology Information was used to conduct a sequence alignment query of the amino acid sequence of each enzyme against downloaded protein sequences obtained from a curated database of venom proteins and toxins maintained by UniProt. Searches were conducted under the following conditions and criteria for significant sequence homology matches:

Default search parameters: word size of 6, Expect threshold of 0.05, BLOSUM62 scoring matrix with default gap costs and composition adjustments.

Structural homology/similarity criteria: identity >40%, E-value <0.001, bit-score >40.

No significant similarity to any toxins was identified from the sequence homology searches, indicating that the enzymes are not expected to pose any toxigenic concerns.

### *B.5.2.5 Information on the Potential Allergenicity of the Enzymes*

To investigate the possibility of allergenic cross-reactivity of the 3 enzymes used in the production of the food additive, an *in silico* assessment of sequence homology of the amino acid sequence of each enzyme against known allergens was conducted using the methodology described by FAO/WHO (2001), Codex Alimentarius (2003, 2009), and EFSA GMO Panel (2017). In accordance with these guidelines, the following sequence homology searches were conducted against the AllergenOnline database (Version 24)[1]:

[1] http://www.allergenonline.org.

1. A “sliding window” of 80–amino acid sequences (*e.g.*, segments 1-80, 2-81, 3-82, *etc*.) derived from each full-length amino acid sequence of the food enzyme (search parameters: minimum identity cut-off = 35%);

2. An 8–amino acid exact match; and

3. The full-length amino acid sequence (search parameters: FASTA36; E-value cut-off = 1 and maximum alignments of 20).

The 80–amino acid alignment searches were conducted using the FASTA search algorithm hosted by the AllergenOnline Allergenic Protein Sequence Search program[2] with default search parameters, and results were reviewed for any protein sharing ≥35% identity over a “sliding window” of 80 amino acids. Matches greater than 35% over 80 amino acids are suggestive of potential cross-reactivity with putative allergens; similarly, sequences sharing ≥35% identity over a window of 80 amino acids are common for many highly conserved proteins (Abdelmoteleb *et al.*, 2021). The FAO/WHO (2001), Codex Alimentarius (2003, 2009), and EFSA GMO Panel (2017) guidelines recommend searches with the 80–amino acid sliding window and 8–amino acid exact matches, and do not include a recommendation to conduct searches with the full-length sequence. However, given that structural similarity between folded proteins may be evaluated using the full-length amino acid sequences, as noted by Aalberse (2000), Goodman *et al*. (2008), and Abdelmoteleb *et al*. (2021), any matches identified from the 80–amino acid sliding window or the 8–amino acid exact match searches were further evaluated for the degree of significance and identity over the full sequence and only considered further in the present allergenicity assessment if the match also had a percent identity >50% over the full sequence.

No significant matches to any known allergens were identified in the full-length sequence alignment, 80–amino acid sliding window, and 8–amino acid exact match searches for any of the 3 enzymes. The available evidence indicates that the allergenic cross-reactivity potential of the enzymes is low. Furthermore, these enzymes are used as processing aids and residual levels of the enzymes have been demonstrated to be below the respective LOD within multiple production batches of the food additive (see Section B.6.2.2). Therefore, the allergenicity risk of the enzymes under their conditions of use is considered to be low.

#### *B.5.2.6 Origins and History of Use of the Source Microorganism*

The host organism used for production of the recombinant strains is *E. coli* BL21 (DE3). This strain is a common laboratory strain used for expression of biotechnology products. The strain was obtained from a commercial culture collection and therefore has an established and verifiable identity. The complete gene sequence of *E. coli* BL21 (DE3) was published in 2009 by Jeong *et al*. (2009), and comprehensive bioinformatic analyses of the organism are described by Studier *et al*. (2009). *E. coli* is also listed as an enzyme production microorganism in Annex 3 of the JECFA Framework for Steviol Glycosides (JECFA, 2021, 2023).

Like its close relative, *E. coli* K strain, the *E. coli* B strain is a model of bacterial physiology, metabolism and genomics, and has thus been widely used for metabolic engineering. In addition, the *E. coli* B strain is a well-established microorganism for the commercial production of therapeutic proteins and emerged as the preferred *E. coli* cell line because of favourable characteristics such as reduced acetate production, reduced enzyme activity, and higher rates of protein secretion compared to other lines (Baeshen *et al*., 2015; Rosano

[2] http://www.allergenonline.org/databasefasta.shtml.

*et al*., 2019). The BL21 (DE3) derivative has become the preferred host for recombinant protein production (Rosano *et al*., 2019).

*E. coli* BL21 (DE3) is not considered a human or animal pathogen. Pathogenicity studies conducted by Chart *et al*. (2000) with *E. coli* BL21 (DE3) showed lack of pathogenicity in mice, chicks, and sheep. The entire genome of *E. coli* BL21 (DE3) was characterised by Jeong *et al*. (2009), and the whole genome sequence (WGS) of the strain was compared in detail to the genomes of non-toxic, non-pathogenic strains *E. coli* K-12 (MG1655) and *E. coli* REL606 using bioinformatic analyses (Studier *et al*., 2009). No known *E. coli* enterotoxins or related pathogenic determinants were identified by the authors.

The production strains for the enzymes β-1,2-glycosyltransferase, β-1,3-glycosyltransferase, and sucrose synthase were identified as derivatives of *E. coli* BL21 (DE3) by WGS and bioinformatic analysis (see Appendix F). The 3 strains have been derived from the same parental microorganism using conventional genetic modification techniques. The purpose of the genetic modification was to enable the expression of the target enzymes through the insertion of a plasmid containing the corresponding genetic elements. The 3 *E. coli* hosts differ only on the specific enzyme-coding sequences present in the corresponding plasmid. The plasmids are not integrated into the chromosome.

#### *B.5.4.7 Pathogenicity/Toxigenicity of the Source Microorganism*

Searches for genes and/or genetic elements of concern were performed according to the *Statement on the requirements for whole genome sequence analysis of microorganisms intentionally used in the food chain* (EFSA, 2024). No virulence factors of concern were identified in any of the test strains. The strains carry 1 antimicrobial resistance (AMR) gene potentially conferring resistance to neomycin, ribostamycin, kanamycin A, gentamicin B, paromomycin, lividomycin, and gentamicin. The gene is widely used a marker gene and has been introduced into the test strain for this purpose. The results of the residual DNA testing in multiple batches of the final product confirm the absence of this AMR gene (limit of detection: 1 ng/g) (see Section B.6.2.4).

#### *B.5.4.8 Genetic Stability of the Source Microorganism*

The stability of the genetic material from each production strain was confirmed by demonstrating stable enzyme activity for each enzyme across multiple generations of cultivation. Further details are provided in Appendix A.

#### *B.5.4.9 Information on the Methods Used in the Genetic Modification of the Source Microorganism*

Details of the characteristics of the microbial production strains, the expression plasmids, and the techniques used to introduce genetic modifications are described in Appendix F. In addition, the results of the WGS analysis of the 3 microbial production strains are provided in Appendix F.

The absence of viable cells or DNA from the production strains in the final steviol glycoside products was confirmed using analytical methods according to the *Scientific Guidance for the submission of dossiers on Food Enzymes* (EFSA CEP Panel, 2021) (see Sections B.6.2.3 and B.6.2.4). Therefore, although the presence of an AMR gene sequence in the microbial production strains is considered a hazard, the risk associated to this hazard is considered absent (EFSA CEP Panel, 2021).

## B.6 Specification for Identity and Purity of the Food Additive

### B.6.1 Product Specifications for the Food Additive

The specifications for steviol glycosides produced by enzymatic conversion with high Reb M content or Reb D content are presented in Table B.6.1-1 and Table B.6.1-2, respectively. Certificates of analysis and methods of analysis are provided in Appendix G. Parameters for physical and compositional characteristics of the food additive, as well as appropriate limits for heavy metal impurities, have been established.

**Table B.6.1-1 Specifications for Steviol Glycosides with High Reb M Content Produced by Enzymatic Conversion**

| **Steviol Glycosides with High Reb M Content Produced by Enzymatic Conversion** | |
|---|---|
| Assay | Not less than 95% of total steviol glycosides, comprised primarily of rebaudioside M and other minor steviol glycosides, on the dried basis. |
| Description | White-to-light yellow powder, approximately between 200 and 350 times sweeter than sucrose (at 5% sucrose equivalency). |
| ***Identification*** | |
| Solubility | Freely soluble to slightly soluble in water |
| pH | Between 4.5 and 7.0 (1 in 100 solution) |
| ***Purity*** | |
| Total ash | Not more than 1% |
| Loss on drying | Not more than 6% (105°C, 2h) |
| Residual solvents | Not more than 200 mg/kg methanol<br>Not more than 5,000 mg/kg ethanol |
| Arsenic | Not more than 0.015 mg/kg |
| Lead | Not more than 0.2 mg/kg |
| Cadmium | Not more than 0.015 mg/kg |
| Mercury | Not more than 0.07 mg/kg |
| Total plate count | ≤1,000 CFU/g |
| Mould and yeast | ≤100 CFU/g |
| Total coliforms | ≤3 MPN/g |
| *Escherichia coli* | ≤3 MPN/g |
| *Staphylococcus aureus* | ≤10 CFU/g |
| Salmonella sp. | Not detected in 25 g |
| Residual protein | ≤50 mg/kg |

CAS = Chemical Abstracts Service; CFU = colony-forming units; h = hours; MPN = most probable number; Reb M = rebaudioside M.

**Table B.6.1-2 Specifications for Steviol Glycosides with High Reb D Content Produced by Enzymatic Conversion**

| **Steviol Glycosides with High Reb D Content Produced by Enzymatic Conversion** | |
|---|---|
| Assay | Not less than 95% of total steviol glycosides, comprised primarily of rebaudioside D and other minor steviol glycosides, on the dried basis. |
| Description | White-to-light yellow powder, approximately between 200 and 350 times sweeter than sucrose (at 5% sucrose equivalency). |
| ***Identification*** | |
| Solubility | Freely soluble to slightly soluble in water |

**Table B.6.1-2 Specifications for Steviol Glycosides with High Reb D Content Produced by Enzymatic Conversion**

| Steviol Glycosides with High Reb D Content Produced by Enzymatic Conversion | |
|---|---|
| pH | Between 4.5 and 7.0 (1 in 100 solution) |
| ***Purity*** | |
| Total ash | Not more than 1% |
| Loss on drying | Not more than 6% (105°C, 2h) |
| Residual solvents | Not more than 200 mg/kg methanol<br>Not more than 5,000 mg/kg ethanol |
| Arsenic | Not more than 0.015 mg/kg |
| Lead | Not more than 0.2 mg/kg |
| Cadmium | Not more than 0.015 mg/kg |
| Mercury | Not more than 0.07 mg/kg |
| Total plate count | ≤1,000 CFU/g |
| Mould and yeast | ≤100 CFU/g |
| Total coliforms | ≤3 MPN/g |
| *Escherichia coli* | ≤3 MPN/g |
| *Staphylococcus aureus* | ≤10 CFU/g |
| Salmonella sp. | Not detected in 25 g |
| Residual protein | ≤50 mg/kg |

CAS = Chemical Abstracts Service; CFU = colony-forming units; h = hours; MPN = most probable number; Reb D = rebaudioside D.

## B.6.2 Product Analysis

### *B.6.2.1 Batch Analyses*

Five independent, representative batches of steviol glycosides with a high Reb M or Reb D content produced by enzymatic conversion were analysed according to the specifications detailed in Section B.6.1 above. The results are presented below in Table B.6.2.1-1 and certificates of analysis are provided in Appendix G. A legend for the confidential lot numbers is provided in Appendix G. The results indicate that the manufacturing process for steviol glycosides with high Reb M or Reb D produced by enzymatic conversion yields a consistent product.

**Table B.6.2.1-1 Results of Batch Analyses for 5 Non-Consecutive Lots of Steviol Glycosides with High Reb M or Reb D Content Produced *via* Enzymatic Conversion**

| Proposed EU Specifications | | Manufacturing Lot No. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Parameter** | **Limit** | **Lot No. 1[a]** | **Lot No. 2[a]** | **Lot No. 3[a]** | **Lot No. 4[a]** | **Lot No. 5[a]** | **Lot No. 6[b]** | **Lot No. 7[b]** | **Lot No. 8[b]** | **Lot No. 9[b]** | **Lot No. 10[b]** |
| Assay[a] | Not less than 95% steviol glycosides[b] | 100.1 | 99.3 | 99.0 | 98.3 | 99.2 | 99.4 | 99.9 | 99.7 | 99.5 | 99.2 |
| Description | White to off-white powder | Pass | Pass | Pass | Pass | Pass | Pass | Pass | Pass | Pass | Pass |
| | Between 200 and 350 times sweeter than sucrose (at 5% sucrose equivalency) | See Section 2.2.1.3 | See Section 2.2.1.3 | See Section 2.2.1.3 | See Section 2.2.1.3 | See Section 2.2.1.3 | See Section 2.2.1.3 | See Section 2.2.1.3 | See Section 2.2.1.3 | See Section 2.2.1.3 | See Section 2.2.1.3 |
| ***Identification*** | | | | | | | | | | | |
| Solubility | Freely soluble to slightly soluble in water | Pass | Pass | Pass | Pass | Pass | Pass | Pass | Pass | Pass | Pass |
| pH | Between 4.5 and 7.0 (1 in 100 solution) | 5.7 | 5.7 | 5.7 | 5.6 | 5.6 | 5.7 | 5.7 | 5.6 | 5.6 | 5.8 |
| ***Purity*** | | | | | | | | | | | |
| Total ash | Not more than 1% | 0.02 | <0.01 | 0.02 | 0.02 | 0.01 | 0.05 | 0.01 | 0.04 | 0.01 | 0.01 |
| Loss on drying | Not more than 6% (105°C, 2 h) | 5.7 | 4.7 | 4.5 | 4.3 | 5.7 | 4.1 | 4.1 | 4.1 | 4.2 | 4.1 |
| Residual solvents | Not more than 200 mg/kg methanol | Not detected | Not detected | Not detected | Not detected | Not detected | Not measured | Not measured | Not measured | Not measured | Not measured |
| | Not more than | 425 | 534 | 301 | 518 | 294 | 238 | 238 | 246 | 251 | 252 |

**Table B.6.2.1-1 Results of Batch Analyses for 5 Non-Consecutive Lots of Steviol Glycosides with High Reb M or Reb D Content Produced *via* Enzymatic Conversion**

| Proposed EU Specifications | | Manufacturing Lot No. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Parameter** | **Limit** | **Lot No. 1[a]** | **Lot No. 2[a]** | **Lot No. 3[a]** | **Lot No. 4[a]** | **Lot No. 5[a]** | **Lot No. 6[b]** | **Lot No. 7[b]** | **Lot No. 8[b]** | **Lot No. 9[b]** | **Lot No. 10[b]** |
| | 5,000 mg/kg ethanol | | | | | | | | | | |
| Arsenic | Not more than 0.015 mg/kg | <0.015 | <0.015 | <0.015 | <0.015 | <0.015 | <0.015 | <0.015 | <0.015 | <0.015 | <0.015 |
| Lead | Not more than 0.2 mg/kg | <0.2 | <0.2 | <0.2 | <0.2 | <0.2 | <0.2 | <0.2 | <0.2 | <0.2 | <0.2 |
| Cadmium | Not more than 0.015 mg/kg | <0.015 | <0.015 | <0.015 | <0.015 | <0.015 | <0.015 | <0.015 | <0.015 | <0.015 | <0.015 |
| Mercury | Not more than 0.07 mg/kg | <0.07 | <0.07 | <0.07 | <0.07 | <0.07 | <0.07 | <0.07 | <0.07 | <0.07 | <0.07 |
| Residual protein | Not more than 50 mg/kg | <LOQ[c] | <LOQ[c] | <LOQ[c] | <LOQ[c] | <LOQ[c] | <LOQ[c] | <LOQ[c] | <LOQ[c] | <LOQ[c] | <LOQ[c] |
| ***Microbiological Analysis*** | | | | | | | | | | | |
| Total plate count (CFU/g) | ≤1,000 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 |
| Total coliforms (MPN/g) | ≤3 | <3.0 | <3.0 | <3.0 | <3.0 | <3.0 | <3.0 | <3.0 | <3.0 | <3.0 | <3.0 |
| Yeast and mould (CFU/g) | ≤100 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 |
| *Escherichia coli* (MPN/g) | ≤3 | <3.0 | <3.0 | <3.0 | <3.0 | <3.0 | <3.0 | <3.0 | <3.0 | <3.0 | <3.0 |
| *Salmonella* sp. | Negative in 25 g | Not detected | Not detected | Not detected | Not detected | Not detected | Not detected | Not detected | Not detected | Not detected | Not detected |
| *Staphylococcus aureus* (CFU/g) | ≤10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 |

CFU = colony-forming units; EU = European Union; h = hours; LOQ = limit of quantitation; MPN = most probable number; Reb D = rebaudioside D; Reb M = rebaudioside M.
[a] Steviol glycosides with high Reb M content produced by enzymatic conversion.
[b] Steviol glycosides with high Reb D content produced by enzymatic conversion.
[c] LOQ = 20 mg/kg.

**Table B.6.2.1-1 Results of Batch Analyses for 5 Non-Consecutive Lots of Steviol Glycosides with High Reb M or Reb D Content Produced *via* Enzymatic Conversion**

| Proposed EU Specifications | | Manufacturing Lot No. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Parameter | Limit | Lot No. 1[a] | Lot No. 2[a] | Lot No. 3[a] | Lot No. 4[a] | Lot No. 5[a] | Lot No. 6[b] | Lot No. 7[b] | Lot No. 8[b] | Lot No. 9[b] | Lot No. 10[b] |

#### *B.6.2.2 Residual Protein*

Three representative batches of steviol glycosides produced by enzymatic conversion were analysed for residual protein content. The analyses were conducted using a validated bicinchoninic acid (BCA) protein assay. According to this method, the residual protein content in all the analysed batches was below the limit of quantitation (LOQ). The test results and the method validation report are provided in Appendix H.

To allow for batch-to-batch variation and analytical uncertainty, specification limits for residual protein of not more than 50 mg/kg for steviol glycosides were established (see Section B.6.1).

#### *B.6.2.3 Viable Cells of the Production Strain*

The possible presence of viable cells of the production strains in the final steviol glycoside products was examined using a culture-based method according to the *Scientific Guidance for the submission of dossiers on Food Enzymes* (EFSA CEP Panel, 2021). The analysis was carried out for 5 batches of steviol glycosides with high Reb M or Reb D content (final product). No colonies corresponding to the enzyme production strains were observed and no other microbial growth was observed for any of the product batches. Further details on the analytical method, as well as the detailed results, are presented in Appendix H.

#### *B.6.2.4 Residual DNA of the Production Strain*

Five production-scale batches of steviol glycosides with high Red D content and 5 batches of steviol glycosides with high Reb M content were analysed for the presence of residual DNA from the production strain. Residual DNA was measured using a quantitative polymerase chain reaction (qPCR) method. Primers were designed to target 5 genes: *GT609*, *GT829*, *16S*, *GT109*, and *KanR*. The levels of residual DNA were below the LOD in all 5 batches of each product, indicating the absence of residual DNA from the production organism in the final products. The full study reports are provided in Appendix H.

#### *B.6.2.5 Process Contaminants*

Five batches of steviol glycosides with high Reb M content were analysed for residual levels of IPTG which is used as an inducer of enzyme expression in the manufacturing process of the 3 enzymes. The analyses were conducted using a validated method based on detection by high-performance liquid chromatography with mass spectrometry (HPLC-MS). The results demonstrate the residual IPTG to be below the LOQ. The certificate of analysis and the method validation report are provided in Appendix H. Considering that steviol glycosides with a high Reb D content are manufactured by the same process and employ similar filtration and purification techniques, it would be expected that residual levels of IPTG would be below the LOQ.

#### *B.6.2.6 Residual Pesticides*

One batch of the starting material, a stevia leaf extract containing high amounts of Reb A, was analysed for pesticide residues using the Quechers method (EN 15662:2008). This method covers a range of commonly used pesticides. The results demonstrate the pesticide residue to be below each respective LOQ, suggesting the absence of residual pesticides in the starting material, and ultimately the final steviol glycosides derived from the starting material. The certificate of analysis is provided in Appendix H.

## B.7 Information for Food Labelling

Steviol glycosides produced by enzymatic conversion is classified as a steviol glycoside under Schedule 3 and, as such, it would follow similar food labelling as for current steviol glycoside preparations. Steviol glycosides are considered intense sweeteners and flavour enhancers when added to various food products. Steviol glycosides have been assigned the INS number of 960. Adorvia's steviol glycosides produced by enzymatic conversion will be labelled under the functional class, sweetener, either as sweetener (960) or sweetener (steviol glycosides).

## B.8 Analytical Method for Detection

The analytical methods used to confirm that Adorvia's steviol glycosides produced by enzymatic conversion meet the established specifications (Section B.6.1) are internationally recognised (*e.g.*, Association of Analytical Communities [AOAC], United States Pharmacopeia [USP], JECFA). The rebaudioside content in the final product is quantified according to the JECFA 2021 HPLC method that has been validated for the quantification of rebaudiosides.

## B.9 Potential Additional Purposes of the Food Additive when Added to Food

As a high-intensity sweetener, Adorvia's steviol glycosides can be added to foods to replace the sweetness provided by sugars without significantly contributing to available energy. As such the food additive can be used by consumers to control caloric intake. Consumers following a weight-loss program looking to restrict their refined sugar intake or individuals with diabetes avoiding sugar consumption may also use the food additive for these purposes.

# C. INFORMATION RELATED TO THE SAFETY OF THE FOOD ADDITIVE

In accordance with Section 3.3.1 – Food Additives of the FSANZ *Application Handbook* (FSANZ, 2025a) the safety information outlined must be provided to extend the use of a currently permitted food additive.

1. Information on the toxicokinetics and metabolism of the food additive and, if necessary, its degradation products and/or major metabolites; and

2. Information on the toxicity of the food additive and, if necessary, its degradation products and major metabolites.

These points need only include reports of studies conducted since the last safety evaluation by FSANZ and are addressed in the section that follows.

Section 3.3.1 – Food Additives of the FSANZ *Application Handbook* (FSANZ, 2025a) states that, if available, safety assessment reports prepared by international agencies of other national government agencies should be provided. A summary of the safety assessment reports prepared by international agencies that have been published since the last safety evaluation by FSANZ is provided in the following section.

## C.1 Introduction

The safety of steviol glycosides has been well established through extensive risk assessments and safety evaluations conducted by scientific and regulatory authorities, including the U.S. FDA, Health Canada, FSANZ, EFSA, and JECFA. These assessments consistently conclude that steviol glycosides, including specific rebaudiosides such as Reb M and Reb D, share a common metabolic pathway. Following ingestion, these compounds are hydrolysed to steviol in the large intestine, absorbed, and subsequently conjugated with glucuronic acid to form steviol glucuronide, which is excreted in urine. FSANZ acknowledges this shared metabolic fate and has broadened the definition of steviol glycosides to encompass all glycosides present in the *S. rebaudiana* Bertoni leaf. Consequently, safety data generated for individual steviol glycosides can be applied to others as suitable surrogates due to their common metabolic processing.

FSANZ has evaluated the safety of steviol glycosides and specific rebaudiosides produced using various manufacturing approaches, including enzymatic conversion, across multiple applications (e.g., A1157, A1172, A1176, A1183, and A1268). More recently, Application A1318—concerning steviol glycosides produced via enzymatic conversion using enzymes derived from genetically modified *Escherichia coli* BL21—received a positive determination on 14 August 2025 (FSANZ, 2025b). In its approval report, FSANZ noted no safety concerns related to either the enzymes or the resulting steviol glycosides; the enzymes described in the present application are comparable to those assessed in A1318.

The following sections provide an overview of the established metabolic fate of steviol glycosides, summaries of safety evaluations by international regulatory bodies, and findings from studies published since the A1318 approval (August 2025). A comprehensive literature search was conducted through May 2026 using databases including Adis Clinical Trials Insight, AGRICOLA, AGRIS, Allied & Complementary Medicine™, BIOSIS® Toxicology, BIOSIS Previews®, CAB ABSTRACTS, Embase®, Foodline®: SCIENCE, FSTA®, MEDLINE®, NTIS, Toxicology Abstracts, and ToxFile®. The review was limited to full-text articles published in peer-reviewed scientific journals within these databases.

## C.2 Information on the Toxicokinetics & Metabolism of Steviol Glycosides

The metabolic fate of steviol glycosides is well characterised in the scientific literature and is summarised herein. steviol glycosides share a common absorption, distribution, metabolism, and excretion (ADME) profile due to their shared steviol backbone. Because of their β-glycosidic linkages, steviol glycosides are not hydrolysed in the upper gastrointestinal tract; instead, they are metabolised by the gut microbiota in the colon, resulting in the release of steviol (Wingard et al., 1980; Hutapea et al., 1997; Gardana et al., 2003; Koyama et al., 2003a,b; Geuns et al., 2003, 2007; Renwick and Tarka, 2008; Nikiforov et al., 2013; Purkayastha et al., 2016). Hydrolysis occurs in a stepwise manner, with the rate influenced by the structural complexity of each glycoside (Wingard et al., 1980; Koyama et al., 2003b). However, despite structural differences among steviol glycosides, *in vitro* studies using human fecal homogenates indicate that their overall hydrolysis rates are generally comparable (Purkayastha et al., 2014, 2015, 2016). Following microbial degradation, liberated steviol is absorbed into the portal circulation and distributed to tissues including the liver, spleen, adrenal glands, adipose tissue, and blood (Nakayama et al., 1986; Koyama et al., 2003b; Wang et al., 2004; Roberts and Renwick, 2008). In the liver, steviol undergoes conjugation with glucuronic acid to form steviol glucuronide. This metabolite, along with small amounts of unconjugated steviol or unmetabolised compounds, is eliminated primarily *via* urine and to a lesser extent *via* feces (Wingard et al., 1980; Nakayama et al., 1986; Kraemer and Maurer, 1994; Simonetti et al., 2004; Geuns et al., 2006, 2007; Roberts and Renwick, 2008; Wheeler et al., 2008).

As noted previously, this shared metabolic pathway is widely recognised by scientific and regulatory authorities, which have determined that safety data for individual steviol glycosides—such as stevioside and rebaudiosides D and M — can be extrapolated to support the safety of other high-purity steviol glycosides.

# C.3 Information on the Toxicity of Steviol Glycosides

## C.3.1 Toxicological Studies

### *C.3.1.1 Subchronic Toxicity*

The subchronic toxicity of steviol glycosides has been previously addressed in the safety evaluations by various regulatory bodies as discussed in Section C.4. The literature search did not identify any new systemic toxicity studies related to the safety of steviol glycosides or individual rebaudiosides.

### *C.3.1.2 Genotoxicity*

The genotoxicity of steviol glycosides has been previously addressed in the safety evaluations by various regulatory bodies as discussed in Section C.4. The literature search did not identify any new genotoxicity studies related to the safety of steviol glycosides or individual rebaudiosides.

### *C.3.1.3 Long-term Toxicity and Carcinogenicity*

The chronic toxicity/carcinogenicity of steviol glycosides has been previously addressed in the safety evaluations by various regulatory bodies as discussed in Section C.4. The literature search did not identify any new studies related to the safety of steviol glycosides or individual rebaudiosides.

### *C.3.1.4 Reproductive and Developmental Toxicity*

The reproductive and developmental toxicity of steviol glycosides has been previously addressed in the safety evaluations by various regulatory bodies as discussed in Section C.4. The literature search did not identify any new studies related to the safety of steviol glycosides or individual rebaudiosides.

# C.4 Safety Assessment Reports Prepared by International or National Agencies

The safety of steviol glycosides has been extensively assessed by numerous scientific and regulatory authorities, including FSANZ, the U.S. FDA, JECFA, EFSA, the European Commission’s Scientific Committee on Food (SCF), and Health Canada. Strong interest from both consumers and industry in their use as sweeteners has prompted a large body of safety evaluations, resulting in a robust and comprehensive safety database.

This body of evidence includes studies on the metabolic fate and pharmacokinetics of various steviol glycosides in both animals and humans, as well as investigations into acute, subchronic, and chronic toxicity, carcinogenicity, reproductive and developmental toxicity, and both in vitro and in vivo mutagenicity and genotoxicity. Clinical studies have also been conducted to further support safety. Early toxicological research focused primarily on stevioside, reflecting its abundance in *Stevia rebaudiana* leaves (Aze et al., 1991; Toyoda et al., 1997).

Given the shared metabolic pathway of steviol glycosides—namely their conversion to steviol—regulatory authorities, including FSANZ, have determined that safety conclusions can be applied collectively to all

steviol glycosides rather than assessed individually. A summary of recent evaluations and reports concerning steviol glycosides is presented below.

### C.4.1 Joint FAO/WHO Expert Committee on Food Additives (JECFA)

The safety of steviol glycosides has been thoroughly evaluated by JECFA at its 51st, 63rd, 68th, 69th, and 82nd meetings, held in 1998, 2004, 2007, 2008, and 2016, respectively (JECFA, 1999, 2006b, 2007, 2009b, 2017b). At earlier meetings, JECFA established a temporary acceptable daily intake (ADI) of 2 mg/kg body weight (expressed as steviol). This value was derived from a no-observed-adverse-effect level (NOAEL) of 970 mg/kg body weight/day (equivalent to 383 mg/kg body weight/day as steviol) identified in a 2-year rat study (Toyoda et al., 1997), with application of a 200-fold safety factor (JECFA, 2006b).

In 2008, following evaluation of additional animal and human data—particularly studies examining effects on blood pressure and glucose metabolism—the Committee concluded that the additional uncertainty factor of 2 was no longer necessary. As a result, JECFA established a full ADI of 4 mg/kg body weight (expressed as steviol) for steviol glycosides.

At its 82nd meeting in 2016, JECFA conducted a further review of the safety, dietary exposure, and specifications for steviol glycosides, reaffirming both their safety and the established ADI of 4 mg/kg body weight (expressed as steviol). The Committee also assessed a novel production method for rebaudioside A involving a genetically modified strain of *Yarrowia lipolytica* engineered to enhance expression of the steviol glycoside biosynthetic pathway. This evaluation led to the development of a new specification monograph titled “Rebaudioside A from Multiple Gene Donors Expressed in *Yarrowia lipolytica*” (JECFA, 2016, 2017b).

In addition, JECFA reviewed evidence supporting the common metabolic pathway shared by all steviol glycosides and updated its specifications for “Steviol Glycosides from *Stevia rebaudiana* Bertoni.” The revised definition encompasses mixtures of compounds containing a steviol backbone conjugated to varying numbers and combinations of sugar moieties—including glucose, rhamnose, xylose, fructose, arabinose, galactose, and deoxyglucose—in any configuration naturally occurring in the leaves of *Stevia rebaudiana* Bertoni. The purity specification remains unchanged, requiring not less than 95% total steviol glycosides on a dried basis.

More recently, JECFA adopted a framework for developing specifications for steviol glycosides produced through four methodologies, including enzymatic modification (also referred to as enzymatic conversion) (JECFA, 2021). This framework has been ratified by Codex Alimentarius and incorporated into the GSFA (Codex, 2023). Specifications for enzyme-modified steviol glycosides require the same identity and purity standards as those obtained from leaf extraction: the final product must contain no less than 95% total steviol glycosides. As outlined in Section B.6.1, Adorvia’s steviol glycosides meets or exceeds these identity and purity requirements and is manufactured in accordance with the processes described in Annex 3 of JECFA (2021). Therefore, no safety concerns are anticipated with respect to Adorvia’s food additive.

### C.4.2 United States Food and Drug Administration (FDA)

Steviol glycosides have been the subject of extensive regulatory review by the U.S. FDA over several decades. From the 1970s through the 1990s, the FDA received multiple food additive petitions for the use of stevia leaves or steviosides as alternative sweeteners. At that time, these petitions were not accepted due to insufficient technical and safety data. In 1991, the FDA issued an import alert restricting the entry of stevia leaves, steviosides, and foods containing stevia, citing inadequate safety information and concerns about the crude composition and low purity of these products.

The import alert was most recently updated in 2018 to permit the import of steviol glycosides with a purity of ≥95%, provided that documentation demonstrates compliance with this standard. Acceptable documentation includes an FDA “no questions” letter issued in response to a GRAS notice or Certificates of Analysis confirming adherence to established specifications (Perrier et al., 2018). This update reinforces that steviol glycosides marketed or imported into the United States must meet stringent purity and specification requirements.

A substantial number of GRAS notifications have been submitted to the FDA for high-purity steviol glycosides, including mixtures of glycosides, individual compounds such as stevioside, major and minor rebaudiosides (e.g., A, D, E, I, and M), and enzyme-modified steviol glycosides like the food additive described herein. In all cases, the FDA has consistently raised no safety concerns regarding these substances. To date, more than 80 GRAS notifications related to steviol glycosides have been submitted, reflecting the Agency’s position on the safety of high-purity steviol glycosides.

### C.4.3 Health Canada

Health Canada has expanded the definition of steviol glycosides to reflect their shared metabolic pathway. Initially, the definition was expanded to include rebaudioside M, recognizing that all steviol glycosides undergo hydrolysis to steviol, conjugation with glucuronic acid, and subsequent urinary excretion in humans. In 2017, Health Canada further extended the definition to encompass all steviol glycosides present in *Stevia rebaudiana* Bertoni owing to no safety concerns related to the use of these substances (Health Canada, 2016, 2017).

### C.4.4 European Food Safety Authority (EFSA)

EFSA have adopted positive scientific opinions on various high-purity steviol glycoside preparations obtained through enzymatic conversion, indicating that the Agency does not expect these high-purity steviol glycoside mixtures to present any safety concerns (EFSA FAF Panel, 2019, 2021, 2023, 2024, 2025). In its safety evaluation of steviol glycosides derived from *Stevia rebaudiana* Bertoni leaves, EFSA reviewed several short-term and subchronic toxicity studies in rodents. These studies demonstrated no adverse effects following dietary administration of steviol glycosides at concentrations up to 10% for four weeks or 5% for 13 weeks (EFSA ANS Panel, 2010). Across four short-term and subchronic rat studies, the highest tested doses were identified as the NOAELs. Based on a 90-day oral toxicity study in rats, EFSA established NOAEL values of 1,373 mg steviol equivalents/kg body weight/day for males and 1,533 mg steviol equivalents/kg body weight/day for females (EFSA ANS Panel, 2010).

Chronic toxicity was previously assessed by the Scientific Committee on Food in 1985 and 1999 (SCF, 1985; SCF, 1999) and later reviewed by EFSA in 2010. This evaluation included a chronic toxicity study in F344 rats, a combined chronic toxicity and carcinogenicity study in Wistar rats, and a carcinogenicity study in F344 rats (Yamada et al., 1985; Xili et al., 1992; Toyoda et al., 1997). Overall, these studies indicated no evidence of toxicity or carcinogenic potential. EFSA noted that tumor occurrences observed in the studies were typical for the species and strain and were considered negative for carcinogenicity. The NOAEL established in the two-year carcinogenicity study by Toyoda et al. (1997) served as the basis for determining the acceptable daily intake (ADI) for steviol glycosides.

# D. INFORMATION RELATED TO THE DIETARY EXPOSURE TO THE FOOD ADDITIVE

In accordance with Section 3.3.1 – Food Additives of the Food Standards Australia New Zealand *Application Handbook* (FSANZ, 2025a) the following dietary exposure information must be provided:

1. A list of the foods or food groups proposed to contain the food additive;

2. The maximum proposed level and/or concentration range of the food additive for each food group or food; and

3. For foods or food groups not currently listed in the most recent Australian or New Zealand National Nutrition Surveys (NNSs), information on the likely level of consumption (not applicable).

Each point is addressed in the following sections.

## D.1 Proposed Food Uses and Use Levels

The currently approved food uses and use levels for steviol glycosides in Australia and New Zealand, as described under Schedule 15, are presented in Table D.1-1 below (FSANZ, 2024). Adorvia intends to market the food additive for use as an intense sweetener under the same conditions of use as those presently authorised for steviol glycosides.

**Table D.1-1 Summary of Currently Permitted Food Uses and Use Levels for Steviol Glycosides in Australia and New Zealand**

| Category No. | Food Description | Steviol Glycoside Concentration (mg/kg) as Steviol Equivalents |
|---|---|---|
| 1.1.2 | Liquid milk products and flavoured milk | 115 |
| 1.2.2 | Fermented milk products and rennetted milk products | 175 |
| 3 | Ice cream and edible ices | 200 |
| 4.3.2 | Fruits and vegetables in vinegar, oil, brine, or alcohol | 160 |
| 4.3.4.1 | Low joule chutneys, low joule jams, and low joule spreads | 450 |
| 4.3.6 | Fruit and vegetable preparations including pulp | 210 |
| 5.1 | Chocolate and cocoa products | 550 |
| 5.2 | Sugar confectionary | 1100 |
| 6.3 | Processed cereal and meal products | 250 |
| 7.1.1 | Fancy breads | 160 |
| 7.2 | Biscuits, cakes, and pastries | 160 |
| 11.4 | Tabletop sweeteners | GMP |
| 13.3 | Formulated meal replacements and formulated supplementary foods | 175 |
| 13.4 | Formulated supplementary sports foods | 175 |
| 14.1.2.1 | Fruit and vegetable juices | 50 |
| 14.1.2.2.1 | Fruit drink | 200 |
| 14.1.2.2.2 | Low joule fruit and vegetable juice products | 125 |
| 14.1.2.2.3 | Soybean beverage (plain) | 100 (plain) |
| | Soybean beverage (flavoured) | 200 (flavoured) |
| 14.1.3 | Water based flavoured drinks | 200 |
| 14.1.4 | Formulated beverages | 200 |
| 14.1.5 | Coffee, coffee substitutes, tea, herbal infusions, and similar products | 100 |
| 20.2.0.1 | Custard mix, custard powder, and blancmange powder | 80 |
| 20.2.0.2 | Jelly | 260 |
| 20.2.0.3 | Dairy and fat based desserts, dips, and snacks | 150 (only dairy and fat based dessert products) |
| 20.2.0.4 | Sauces and toppings (including mayonnaises and salad dressings) | 320 |

GMP = Good Manufacturing Practice.

## D.2 Exposure Data

Adorvia's steviol glycosides with high Reb M or Reb D content is proposed for use as a sweetener under the same conditions as currently authorised for steviol glycosides in Australia and New Zealand. Since the food additive is intended to directly replace other steviol glycosides, the intake levels are expected to be the same as those already present in the market. Therefore, a separate intake assessment for Adorvia's steviol glycosides was not conducted for this application. It is important to note that use levels for steviol glycosides are expressed as steviol equivalents, not specified for any particular steviol glycoside. Instead, the levels are based on the total content of the aglycone (steviol) in the final food product resulting from the addition of any steviol glycoside meeting the appropriate specifications.

## D.3 Use of the Food Additive in Other Countries

In the U.S., the GRAS status of Adorvia's steviol glycosides, as described herein, has been notified to the FDA and filed under GRN 1294. The FDA issued a "no questions" letter. Adorvia's food additive is also approved for use in China (see Appendix I).

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