

STEVIOLE GLYCOSIDES (TENTATIVE)

Information required for commercially available products on

- analytical data on distribution and concentrations of all component steviol glycosides, including those that are not identified in these tentative specifications
- method of analysis for the determination of all component steviol glycosides, including those that are not identified in these tentative specifications
- the nature and concentration of the non-steviol glycosides fractions
- the quantities of residual solvents from purification steps of the manufacturing process
- the hydrolytic stability of the steviol glycosides in acidic foods and beverages

New tentative specifications prepared at the 63rd JECFA (2004), published in FNP 52 Add 12 (2004). A temporary ADI of 0-2 mg/kg bw (expressed as steviol) was established at the 63rd JECFA (2004).

DEFINITION

Steviol glycosides are obtained by extracting leaves of *Stevia rebaudiana* Bertoni with hot water followed by solvent purification of the water-soluble extract. Ion exchange resins may also be used during the purification process. Stevioside and rebaudioside A are the principal steviol glycosides of the specified material. Rebaudioside C and dulcoside A are secondary steviol glycosides. Other steviol glycosides may also be present.

Chemical name

The following are the chemical names for the principal and secondary steviol glycosides:

Stevioside: 13-[(2-O-β-D-glucopyranosyl-β-D-glucopyranosyl) oxy] kaur-16-en-18-oic acid β-D-glucopyranosyl ester

Rebaudioside A: 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy] kaur-16-en-18-oic acid β-D-glucopyranosyl ester

Rebaudioside C: 13-[(2-O-α-L-rhamnopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy] kaur-16-en-18-oic acid β-D-glucopyranosyl ester

Dulcoside A: 13-[2-O-α-L-rhamnopyranosyl-β-D-glucopyranosyl]oxy] kaur-16-en-18-oic acid β-D-glucopyranosyl ester

C.A.S. number

The following are the C.A.S. numbers for the principal and secondary steviol glycosides:

Stevioside: 57817-89-7

Rebaudioside A: 58543-16-1

Rebaudioside C: 63550-99-2

Dulcoside A: 64432-06-0

Chemical formula

The following are the chemical formulas for the principal and secondary steviol glycosides:

Stevioside: $C_{38}H_{60}O_{18}$

Rebaudioside A: $C_{44}H_{70}O_{23}$

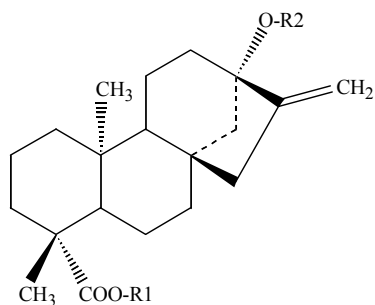
Rebaudioside C: $C_{44}H_{70}O_{22}$

Dulcoside A: $C_{38}H_{60}O_{17}$

Structural formula

The following are the structural formulas for the principal and

secondary steviol glycosides:



<u>Compound name</u>	<u>R1</u>	<u>R2</u>
Stevioside	β -Glc	β -Glc- β -Glc(2→1)
Rebaudioside A	β -Glc	β -Glc- β -Glc(2→1) β -Glc(3→1)
Rebaudioside C	β -Glc	β -Glc- α -Rha(2→1) β -Glc(3→1)
Dulcoside A	β -Glc	β -Glc- α -Rha(2→1)

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

Formula weight	The following are the formula weights for the principal and secondary steviol glycosides:		
	Stevioside:	804.88	Rebaudioside C: 951.03
	Rebaudioside A:		967.03
			Dulcoside A: 788.88

Assay Not less than 95% of total steviol glycosides. The sum of the percentages of stevioside and rebaudioside A is not less than 70%.

DESCRIPTION White crystalline powder, odourless or having a slight characteristic odour. About 200-300 times sweeter than sucrose.

FUNCTIONAL USES Sweetener

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Freely soluble in water and in ethanol

Stevioside and rebaudioside A The material contains not less than 70% of stevioside and rebaudioside A as identified and determined in the Method of Assay.

PURITY

Ash (Vol. 4) Not more than 1%
Test 3 g of the sample (Method I)

<u>Loss on drying</u> (Vol. 4)	Not more than 4% (105°, 3h)
<u>Residual solvents</u>	Information required
<u>Arsenic</u> (Vol. 4)	Not more than 1 mg/kg
<u>Lead</u> (Vol. 4)	Not more than 1 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in FNP 5, "Instrumental methods".

METHOD OF ASSAY

Determine the percentages of the steviol glycosides by high pressure liquid chromatography (Volume 4).

Standards: Stevioside, >99.3% purity and rebaudioside A, >97% purity (available from Wako pure Chemical Industries, Ltd. Japan).

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

Standard solution: Accurately weigh 50 mg of dried (105°, 3h) stevioside standard into a 100 ml volumetric flask and dilute to volume with mobile phase.

Sample solution: Accurately weigh 60 – 120 mg of the sample into a 100 ml volumetric flask. Dissolve in the mobile phase and dilute with the mobile phase to volume.

Conditions:

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

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 solution.

The relative retention times of dulcoside A and rebaudioside C with respect to stevioside are 0.68 – 0.76 and 1.15 - 1.23, respectively. To obtain the retention time of rebaudioside A, use the rebaudioside A standard.

Measure the peak areas of stevioside, rebaudioside A, rebaudioside C and dulcoside A from the sample solution. Measure the peak area of stevioside from the standard solution.

Calculate the percentage of stevioside, dulcoside A, rebaudioside A and rebaudioside C from the formulas:

$$\begin{aligned}
 \% \text{ stevioside} &= \frac{[Ws/W] \times [Aa/As]}{[Ws/W] \times [Ab/As]} \times 100 \\
 \% \text{ dulcoside A} &= \frac{[Ws/W] \times [Ab/As]}{[Ws/W] \times [Ac/As]} \times 100 \\
 \% \text{ rebaudioside A} &= \frac{[Ws/W] \times [Ac/As]}{[Ws/W] \times [Ad/As]} \times 100
 \end{aligned}$$

100

$$\% \text{ rebaudioside C} = \left[\frac{W_s}{W} \right] \times A_d \times \left[\frac{1.18}{A_s} \right] \times 100$$

where

W_s = weighed amount (mg) of stevioside in the standard solution

W = weighed amount of sample (mg)

A_s = Peak area of stevioside from the standard solution

A_a = Peak area of stevioside from the sample solution

A_b = Peak area of dulcoside A from the sample solution

A_c = Peak area of rebaudioside A from the sample solution

A_d = Peak area of rebaudioside C from the sample solution

The factors 0.98, 1.20, and 1.18 for, respectively, dulcoside A, rebaudioside A, and rebaudioside C are the ratios of their formula weights to that of the formula weight of stevioside.

Calculate (1) the % of steviol glycosides (sum the four percentages) and (2) the sum of the percentages for stevioside and rebaudioside A.