STEVIOL GLYCOSIDES

Prepared at the 69th JECFA (2008), published in FAO JECFA Monographs 5 (2008), superseding specifications prepared at the 68th JECFA (2007), published in FAO JECFA Monographs 5 (2008). An ADI of 0 - 4 mg/kg bw (expressed as steviol) was established at the 69th JECFA (2008).

SYNONYMS INS no. 960

DEFINITION The product is obtained from the leaves of *Stevia rebaudiana* Bertoni. The leaves are extracted with hot water and the aqueous extract is passed through an adsorption resin to trap and concentrate the component steviol glycosides. The resin is washed with a solvent alcohol to release the glycosides and product is recrystallized from methanol or aqueous ethanol. Ion exchange resins may be used in the purification process. The final product may be spray-dried.

Stevioside and rebaudioside A are the component glycosides of principal interest for their sweetening property. Associated glycosides include rebaudioside C, dulcoside A, rubusoside, steviolbioside, and rebaudioside B generally present in preparations of steviol glycosides at levels lower than stevioside or rebaudioside A.

Chemical name

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

C.A.S. number

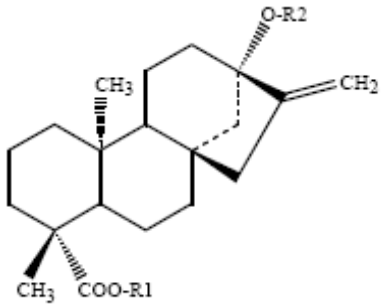
Stevioside: 57817-89-7
Rebaudioside A: 58543-16-1

Chemical formula

Stevioside: $C_{38}H_{60}O_{18}$
Rebaudioside A: $C_{44}H_{70}O_{23}$

Structural Formula

The seven named steviol glycosides:



Compound name	*R1*	*R2*
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)
Rubusoside	*β*-Glc	*β*-Glc
Steviolbioside	H	*β*-Glc-*β*-Glc(2→1)
Rebaudioside B	H	*β*-Glc-*β*-Glc(2→1) | *β*-Glc(3→1)

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

Formula weight

Stevioside: 804.88
Rebaudioside A: 967.03

Assay

Not less than 95% of the total of the seven named steviol glycosides, on the dried basis.

DESCRIPTION

White to light yellow 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
Stevioside and rebaudioside A	The main peak in the chromatogram obtained by following the procedure in Method of Assay corresponds to either stevioside or rebaudioside A.
pH (Vol. 4)	Between 4.5 and 7.0 (1 in 100 solution)

PURITY

Total ash (Vol. 4)	Not more than 1%
Loss on drying (Vol. 4)	Not more than 6% (105°, 2h)
Residual solvents (Vol. 4)	Not more than 200 mg/kg methanol and not more than 5000 mg/kg ethanol (Method I in Volume 4, General Methods, Organic Components, Residual Solvents)
Arsenic (Vol. 4)	Not more than 1 mg/kg Determine by the atomic absorption hydride technique (Use Method II to prepare the test (sample) solution)
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an AAS/ICP-AES 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 Volume 4 (under "General Methods, Metallic Impurities").

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

Standards
Stevioside, >99.0% 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 solutions
(a) Accurately weigh 50 mg of dried (105°, 2 h) stevioside standard into a 100-ml volumetric flask. Dissolve with mobile phase and dilute to volume with mobile phase.
(b) Repeat with previously dried rebaudioside A standard.

Sample solution
Accurately weigh 60-120 mg of dried (105°, 2 h) sample into a 100-ml volumetric flask. Dissolve with mobile phase and dilute to volume with

the mobile phase.

Chromatography Conditions

Column: Supelcosil LC-NH_2 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 rebaudioside A is about 21 min.
Injection volume: 5-10 µl
Detector: UV at 210 nm
Column temperature: 40°

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 0.12-0.16 for rubusoside
0.25-0.30 for dulcoside A 0.35-0.41 for steviolbioside
0.63-0.69 for rebaudioside C 0.73-0.79 for rebaudioside B

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

Calculate the percentage of each of the seven steviol glycosides, *X,* in the sample from the formula:

$$\%X = [W_S/W] \times [f_X A_X/A_S] \times 100$$

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 of *X* for 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), 0.80 (rubusoside), 0.80 (steviolbioside), and 1.00 (rebaudioside B).

Calculate the percentage of total steviol glycosides (sum the seven percentages).