

SCHEDULE 2

Regulation 3(6)

Insertion of entry in the Annex to Commission Regulation (EU) 231/2012 for E 960c(ii) for Rebaudioside M, AM and D Produced via Enzymatic Conversion of Highly Purified Steviol Glycosides from Stevia Leaf Extracts

“E 960c(ii) REBAUDIOSIDE M, AM AND D PRODUCED VIA ENZYMATIC CONVERSION OF HIGHLY PURIFIED STEVIOL GLYCOSIDES FROM STEVIA LEAF EXTRACTS

Synonyms
Definition

Steviol glycosides produced via enzymatic conversion of highly purified steviol glycosides (rebaudioside A or stevioside) stevia leaf extracts are composed predominantly of rebaudioside M, rebaudioside D, and rebaudioside AM.

Rebaudiosides D, M and AM are produced via enzymatic conversion of highly purified steviol glycoside (rebaudioside A or stevioside) extracts (95% steviol glycosides) obtained from *Stevia rebaudiana* Bertoni plant using UDP-glucosyltransferase and sucrose synthase enzymes produced by genetically modified strains of *Escherichia coli* (pPM294, pFAH170, and pSK041) that facilitate the transfer of glucose from sucrose and UDP-glucose to steviol glycosides via glycosidic bonds. After removal of the enzymes by solid-liquid separation and heat treatment, the purification involves concentration of the steviol glycosides by resin adsorption, followed by recrystallisation of the steviol glycosides resulting in a final product containing not less than 95 % of total steviol glycosides, including one or more of rebaudiosides D, M and AM.

Viable cells or DNA of *Escherichia coli* (pPM294, pFAH170, and pSK041) must not be detected in the food additive.

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

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

Rebaudioside AM: 13-[(2-*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

Molecular formula

Trivial name	Formula	Conversion factor
Rebaudioside M	C ₅₆ H ₉₀ O ₃₃	0.25
Rebaudioside D	C ₅₀ H ₈₀ O ₂₈	0.29
Rebaudioside AM	C ₅₀ H ₈₀ O ₂₈	0.29

Molecular weight and CAS No

Trivial name	CAS Number	Molecular weight (g/mol)
Rebaudioside M		
Rebaudioside D		
Rebaudioside AM		

Status: This is the original version (as it was originally made). This item of legislation is currently only available in its original format.

	Rebaudioside M	1220616-44-3	1291.29
	Rebaudioside D	63279-13-0	1129.15
	Rebaudioside AM	2222580-26-7	1129.15
Assay	Not less than 95 % of steviol glycosides on the dried basis, including one or more of rebaudiosides D, M and AM.		
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 solvent	Not more than 5000 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		
Residual protein	Not more than 5 mg/kg''		
