

SCHEDULE 3

Regulation 4(6)

Amendment to the Annex to Commission Regulation (EU) No 231/2012 for the re-numbering of rebaudioside M produced via enzyme modification of steviol glycosides from Stevia E 960c(i) (formerly E 960c) and for the addition of a specification for rebaudioside M, AM and D produced via enzymatic conversion of highly purified steviol glycosides from Stevia leaf extracts (E 960c(ii))

1. In the appropriate place, insert the following entry—

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

Synonyms	
Definition	<p>Steviol glycosides produced via enzymatic conversion of highly purified steviol glycosides (rebaudioside A or stevioside) from Stevia leaf extracts are composed predominantly of rebaudioside M, rebaudioside D, and rebaudioside AM.</p> <p>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 <i>Stevia rebaudiana</i> Bertoni plant using UDP-glucosyltransferase and sucrose synthase enzymes produced by genetically modified strains of <i>Escherichia coli</i> (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.</p> <p>Viable cells or DNA of <i>Escherichia coli</i> (pPM294, pFAH170, and pSK041) must not be detected in the food additive.</p>
Chemical name	<p>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</p> <p>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</p> <p>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</p>

Status: This is the original version (as it was originally made).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

Synonyms			
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	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 ² .		