

SCHEDULE 2

Regulation 4

Amendments to the Annex to [Commission Regulation \(EU\) No. 231/2012](#) concerning the specification of steviol glycosides (E 960a) (formerly E 960) and for the addition of a specification for rebaudioside M produced via enzyme modification of steviol glycosides from Stevia (E 960c)

1. In the entry for steviol glycosides, for the heading “E 960 STEVIOL GLYCOSIDES” substitute—

“E 960a STEVIOL GLYCOSIDES FROM STEVIA”.

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

“E 960c REBAUDIOSIDE M PRODUCED VIA ENZYME MODIFICATION OF STEVIOL GLYCOSIDES FROM STEVIA

Synonyms			
Definition	<p>Rebaudioside M is a steviol glycoside composed predominantly of rebaudioside M with minor amounts of other steviol glycosides such as rebaudioside A, rebaudioside B, rebaudioside D, rebaudioside I, and stevioside.</p> <p>Rebaudioside M is obtained via enzymatic bioconversion of purified steviol glycoside leaf extracts (95% steviol glycosides) of the <i>Stevia rebaudiana</i> Bertoni plant using UDP-glucosyltransferase and sucrose synthase enzymes produced by the genetically modified yeasts <i>K. phaffii</i> (formerly known as <i>Pichia pastoris</i>) UGT-a and <i>K. phaffii</i> UGT-b that facilitate the transfer of glucose from sucrose and UDP-glucose to steviol glycosides via glycosidic bonds.</p> <p>After removal of the enzymes by solid-liquid separation and heat treatment, the purification involves concentration of the rebaudioside M by resin adsorption, followed by recrystallisation of rebaudioside M resulting in a final product containing not less than 95 % of rebaudioside M. Viable cells or the DNA of the yeasts <i>K. phaffii</i> UGT-a or <i>K. phaffii</i> UGT-b must not be detected in the food additive.</p>		
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		
Molecular formula	Trivial name	Formula	Conversion factor
	Rebaudioside M	C ₅₆ H ₉₀ O ₃₃	0.25
Molecular weight and CAS No	Trivial name	CAS Number	Molecular weight (g/mol)
	Rebaudioside M	1220616-44-3	1291.29
Assay	Not less than 95% rebaudioside M on the dried basis		
Description	White to light yellow powder, approximately between 200 and 350 times sweeter than sucrose (at 5% sucrose equivalency)		
Identification			

Status: This is the original version (as it was originally made).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

Synonyms	
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 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
Residual protein	Not more than 5 mg/kg
Particle size	Not less than 74µm (using a mesh #200 sieve with a particle size limit of 74 µm)".