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New Stevia Extract Reference Material

Analysis of Steviol Glycosides using HPTLC

Extracts from the leaves of the *Stevia rebaudiana* plant have a long tradition of being used as a sweetener. Stevia extracts are approximately 300 times sweeter than sucrose while only having a negligible effect on blood glucose and has therefore been increasingly used as an alternative to artificial sweeteners.

Regulations

Stevia is FDA approved as a dietary supplement and one of the active constituents, rebaudioside A, is considered “Generally Recognized As Safe (GRAS)”. The European Community has allowed the use of steviol glycosides as food additives since December 2011, and WHO defined the acceptable daily intake of steviol glycosides to be 4 mg per kg body weight.[1]

Analysis

HPTLC (High-Performance Thin-Layer Chromatography) is a fast and efficient tool to create molecular fingerprints of complex chemical mixtures. It is therefore particularly well suited for the analysis of plants and plant-derived products.

A new Stevia extract reference material is now available which is provided with comprehensive documentation, including a quantitative value for the major component Stevioside as well as qualitative confirmation of various other constituents (Rebaudiosides A, B, C and D, Dulcoside A, Rubusoside, Steviolbioside, Stevioside). In addition to HPLC with assigned peak identities, the certificate of analysis includes HPTLC fingerprint results.

HPTLC fingerprint method for the analysis of *Stevia rebaudiana* leaves [2, 3]

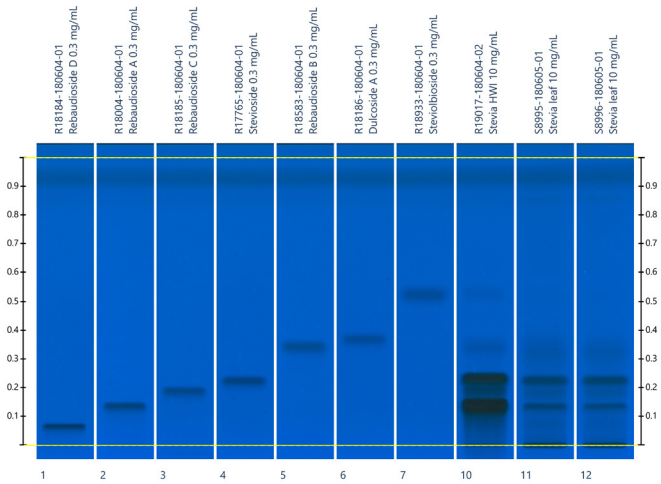
Samples

Extract: 50 mg were suspended in 50 mL of methanol and sonicated for 10 min. The suspension was centrifuged, and the supernatant used.

Leaf: 0.5 g of powdered leaf were suspended in 30 mL of water and boiled for 10 min. The solution is filtered into a 50 mL volumetric flask and the volume is made up with water.

Chromatogram after derivatization under UV 366 nm

Track 1: rebaudioside D
Track 2: rebaudioside A
Track 3: rebaudioside C
Track 4: stevioside
Track 5: rebaudioside B
Track 6: dulcoside A
Track 7: steviolbioside
Track 8: Stevia extract reference material
Track 9: *Stevia rebaudiana* leaf 1
Track 10: *Stevia rebaudiana* leaf 2



In the fingerprint of the dry *Stevia rebaudiana* leaf extract (track 8), zones corresponding in color and position to those of the standards Rebaudioside A, C, Stevioside, Rebaudioside B and Dulcoside A (which are co-eluting), and teviolbioside are seen. The fingerprint is similar to those of *S. rebaudiana* leaf (tracks 9 and 10). Rebaudioside D is only seen in the fingerprint of the leaf, particularly under UV 366 nm (very faint zone).

Description	Quantified Components	Qualitatively Confirmed Components	Cat. No.	Package Size
Stevia extract	Stevioside	Rebaudiosides A, B, C and D, Dulcoside A, Rubusoside, Steviolbioside, Stevioside	**06295001**	500 mg

Table 1: Stevia extract reference material

Description	Format / cm	Pack Size/ No. Plates	Cat. No.
HPTLC Silica Gel 60 F_{254}	20 x 10	50	**1056420001**

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Cat. No.	Product	Package Size
90378	Dulcoside A	10 mg
92273	Isosteviol	10 mg
38462	Rebaudioside A	10 mg
49747	Rebaudioside B	10 mg
30987	Rebaudioside C	10 mg
19189	Rebaudioside D	10 mg
19345	Steviol	10 mg
59754	Steviolbioside	10 mg
50956	Stevioside	10 mg

Table 3: Analytical Standards for *Stevia rebaudiana* constituents

SigmaAldrich.com/stevia

The complete listing of our entire phytochemical standards range:

SigmaAldrich.com/medicinalplants

References:

[1]: WHO Food Additives Series 54; 2006; page 117

[2]: Wald JP, Morlock G. Quantification of steviol glycosides in food products, Stevia leaves and formulations by planar chromatography, including proof of absence for steviol and isosteviol. Journal of Chromatography A, 2017.

[3]: CAMAG Application Note A-117.1: Densitometric quantification of two steviol glycosides in Stevia leaf dry extract, www.camag.com

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