

Analysis of steviol glycosides in low-calorie food

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Abstract

This application note describes the determination of steviol glycosides in low-calorie food. By using SPE for sample clean up it is possible to analyze steviol glycosides in difficult sample matrix. After clean up the eluates are analyzed by HPLC-UV.

Introduction

Due to a changed nutritional awareness, light products and calorie-reduced foods are becoming increasingly important in food trade. Artificial sweeteners have been in use for a long time and are currently discussed to be unhealthy and to be harmful to the environment. Using natural plant extracts for sweetening food gets popular. The plant, *Stevia rebaudiana* Bertoni, was originally known as novel food. In the meantime, the isolated steviol glycosides are permitted as food additives (sweetener E 960) and are added to many product groups such as beverages or fruit-containing spreads [1]. However, it must be noted that the ADI value of 4 mg/kg body weight and day is not exceeded, so that compliance with the prescribed maximum levels must be ensured [2]. This application note presents a method using SPE for sample clean up that allows to analyze steviol glycosides in difficult sample matrices. After clean up the eluates are analyzed by HPLC-UV on NUCLEODUR® PolarTec.

Compounds of interest

Steviolglycoside	Formula	Sweetening power (sucrose = 1)
Dulcoside	C ₃₈ H ₆₀ O ₁₇	30
Rebaudioside A	C ₄₄ H ₇₀ O ₂₃	200–300
Rebaudioside B	C ₃₈ H ₆₀ O ₁₈	150
Rebaudioside C	C ₄₄ H ₇₀ O ₂₂	30
Rebaudioside D	C ₅₀ H ₈₀ O ₂₈	221
Steviolbioside	C ₃₂ H ₅₀ O ₁₃	90
Stevioside	C ₃₈ H ₆₀ O ₁₈	150–250

Table 1: Steviol glycosides and its sweetening power.

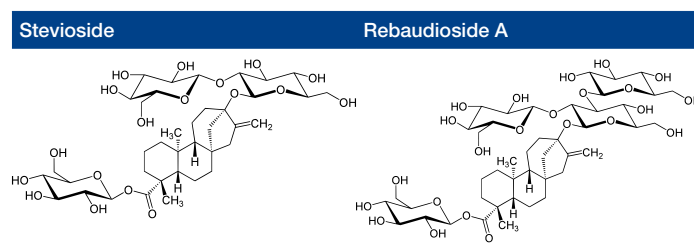


Figure 1: Major active compounds of sugar substitutes extracted from the leaves of the plant species *Stevia rebaudiana*, stevioside and rebaudioside A [3].

Sample preparation

Sample preparation was adapted for each sample matrix.

Solid phase extraction

Column type:

CHROMABOND® C₁₈ ec, 6 mL, 500 mg, (REF 730014)

Conditioning: 10 mL methanol, 10 mL water

Sample application: 5 mL sample extract, flow rate 5 mL/min

Washing: 10 mL methanol/water (40+60, v/v)

Elution: 5 mL methanol

Eluent exchange

Aliquots of 2 mL of the eluates from SPE are evaporated to dryness at 40 °C under a stream of nitrogen and then resolved in 2.0 mL water. Before subsequent analysis eluates are filtered with CHROMAFIL® Xtra PET-20/13 pore size 0.2 µm (REF 729222).

Subsequent analysis: HPLC-UV

Chromatographic condition

Column: EC 250/2 NUCLEODUR® PolarTec, 3 µm, (REF 760479.20)

Eluent: H₂O (ultrapure)/acetonitrile (66 + 34, v/v)

Flow rate: 0.35 mL/min

Temperature: 40 °C

Injection volume: 5 µL

Detection: UV: 200 nm



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Washing process in SPE

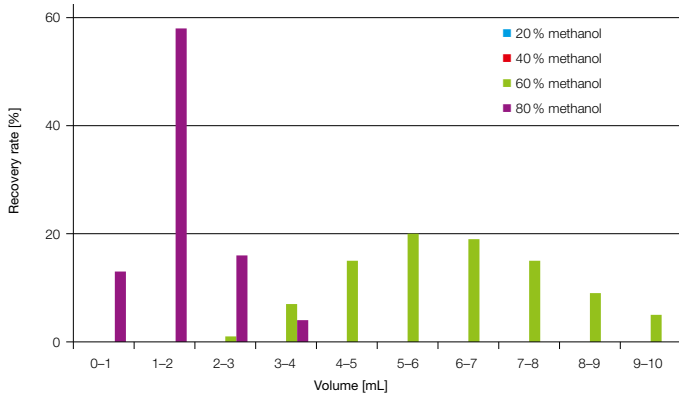


Figure 2: Influence of methanol content in wash-solution from CHROMABOND® C₁₈ ec.

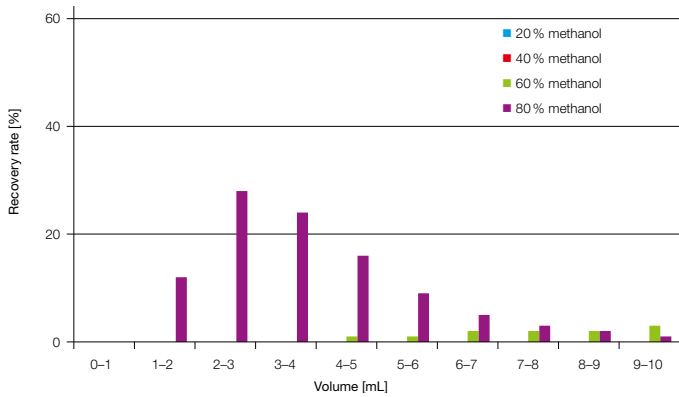


Figure 3: Influence of methanol content in wash-solution from CHROMABOND® HR-X.

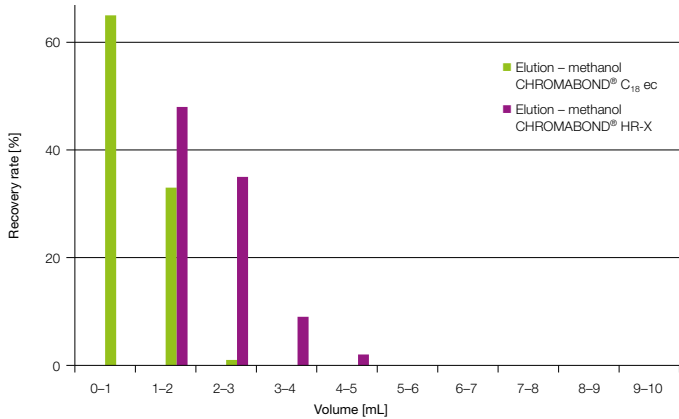


Figure 4: Elution profile after washing column with 10 mL methanol/water (40+60, v/v) from CHROMABOND® HR-X and CHROMABOND® C₁₈ ec.

Proofing loading capacity

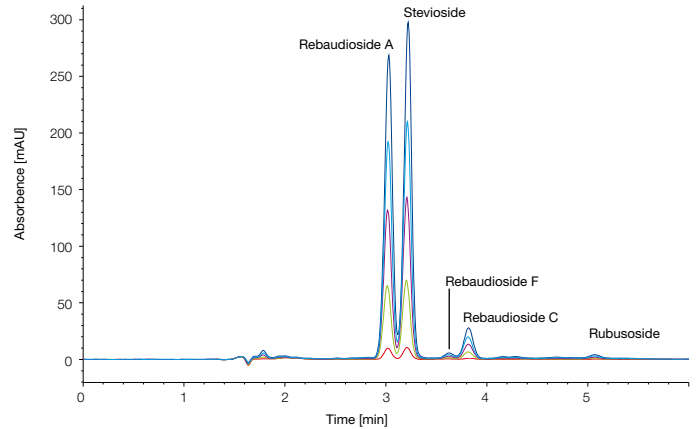


Figure 5: Chromatograms of solid phase eluates of different volumes of extracts of isolated steviol glycosides.

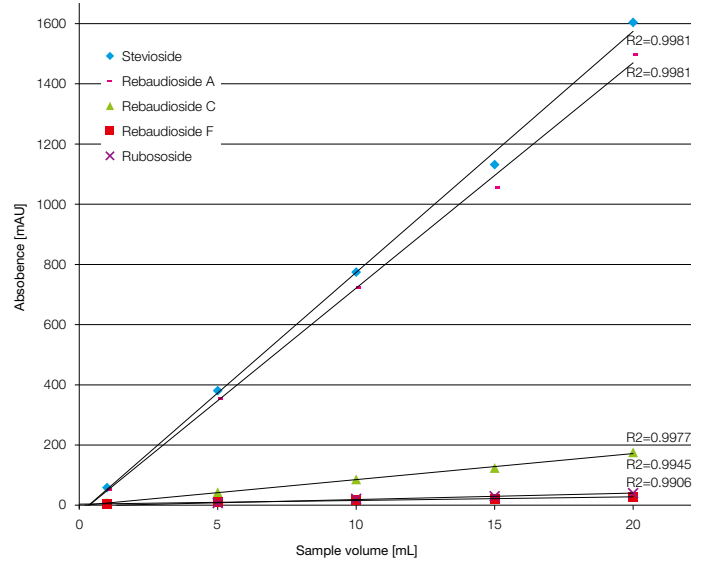


Figure 6: Proportionality between sample volumes for SPE and absorbance.

Control of extracts of isolated steviol glycosides

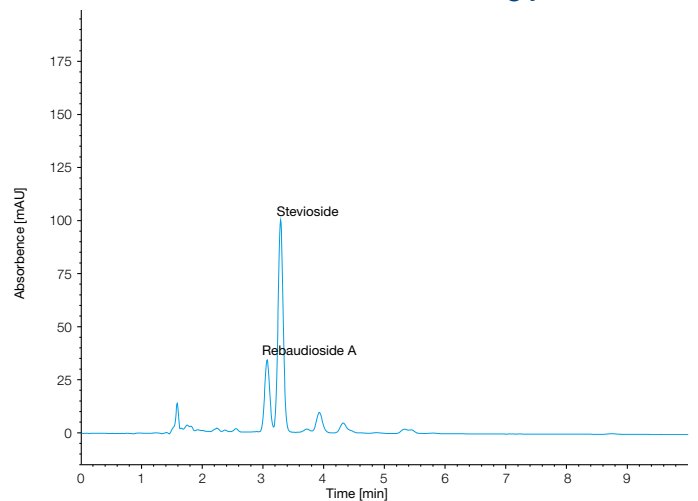


Figure 7: Chromatogram of an extract of isolated steviol glycosides, example A, $\beta = 100 \mu\text{g/mL}$ in eluent.

Analysis of steviol glycosides in low-calorie food

Control of extracts of isolated steviol glycosides

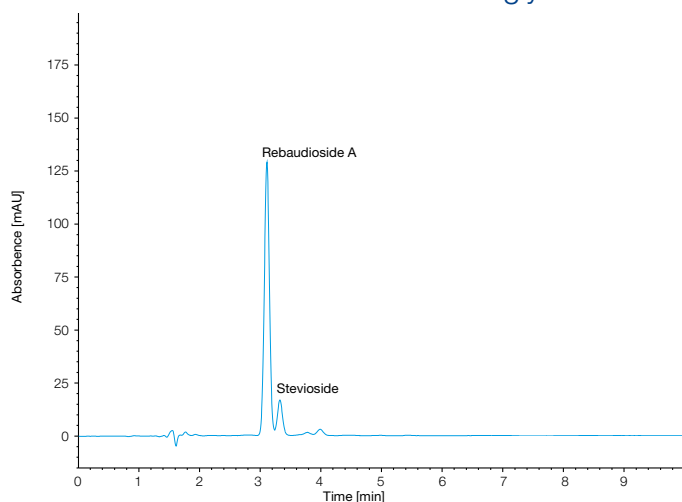


Figure 8: Chromatogram of an extract of isolated steviol glycosides, example B, $\beta = 100 \mu\text{g/mL}$ in eluent.

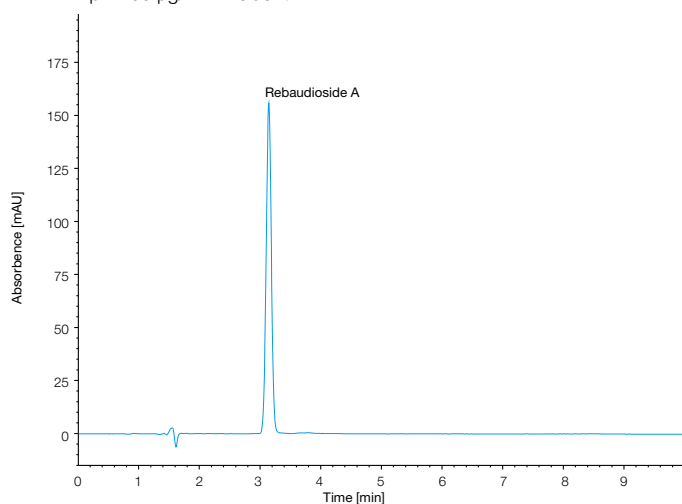


Figure 9: Chromatogram of an extract of isolated steviol glycosides, example C, $\beta = 100 \mu\text{g/mL}$ in eluent.

Control of low calorie-reduced foods

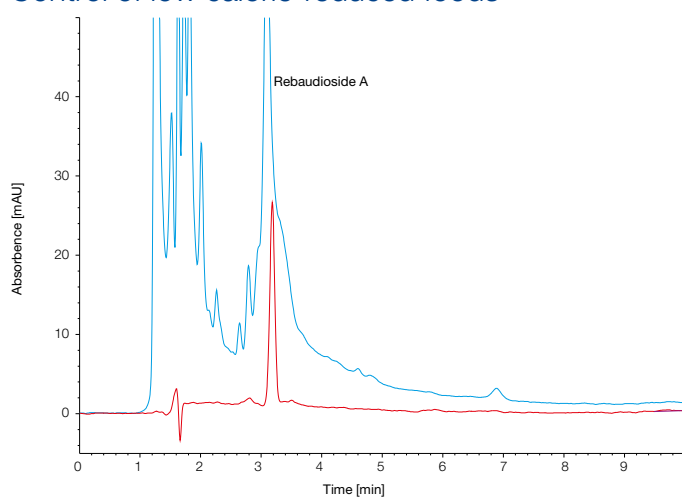


Figure 10: Clean up for licorice sweets, blue: chromatogram of a sample extract, red: chromatogram of a solid phase eluate.

Sample preparation

- Weigh out one piece licorice sweets in a centrifuge tube
- Add 20 mL water
- Solve sample for 15 min in an ultrasonic bath
- Centrifuge at 4500 rpm for 5 min at 4 °C
- Take 10 mL of sample extract for SPE (for direct HPLC injection: filter sample extract through a syringe filter (CHROMAFIL® Xtra PTFE-20/13 pore size 0.2 μm , REF 729208))

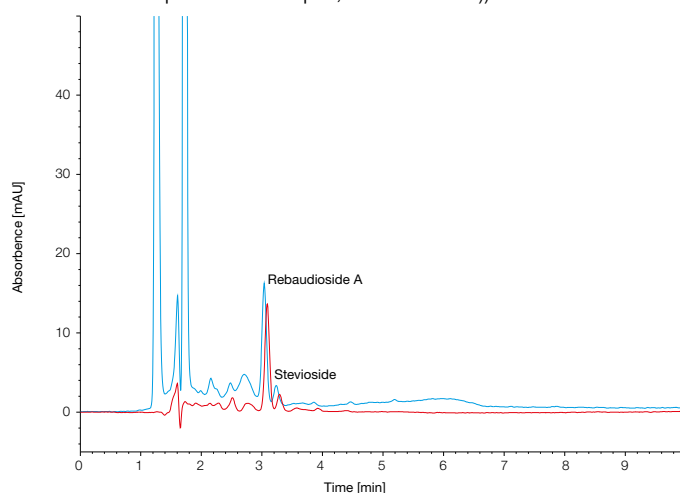


Figure 11: Clean up for Coca-Cola®, blue: chromatogram of a sample extract, red: chromatogram of a solid phase eluate.

Sample preparation

- Fill 20 mL of sample in a centrifuge tube
- Degasing sample 15 min in an ultrasonic bath
- Take 10 mL of sample extract for SPE (for direct HPLC injection: filter sample extract through a syringe filter (CHROMAFIL® Xtra PTFE-20/13 pore size 0.2 μm , REF 729208))

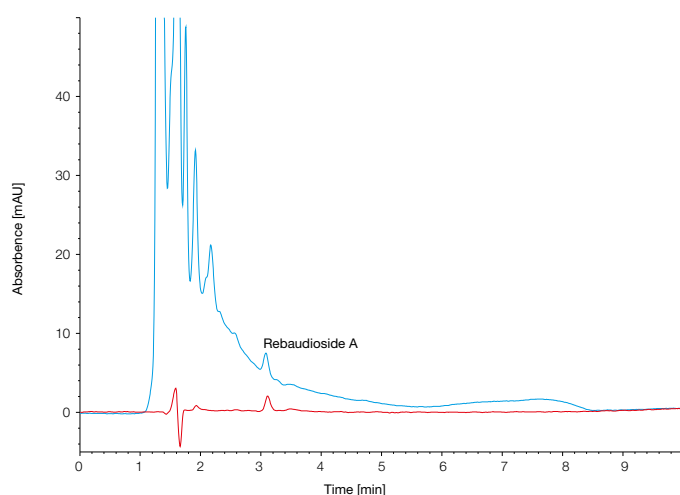


Figure 12: Clean up for strawberry fruit spread, blue: chromatogram of a sample extract, red: chromatogram of a solid phase eluate.

Analysis of steviol glycosides in low-calorie food

Sample preparation

- Weigh out 5 g of homogenized sample in a centrifuge tube
- Add 20 mL water
- Solve sample for 15 min in an ultrasonic bath
- Centrifuge at 4500 rpm for 5 min at 4 °C
- Take 10 mL of sample extract for SPE (for direct HPLC injection: filter sample extract through a syringe filter (CHROMAFIL® Xtra PTFE-20/13 pore size 0.2 µm, REF 729208))

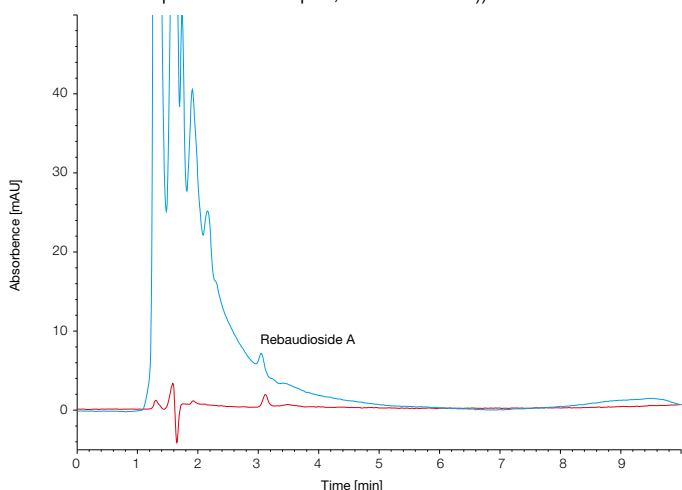


Figure 13: Clean up for multivitamin fruit spread, blue: chromatogram of a sample extract, red: chromatogram of a solid phase eluate.

Sample preparation

- Weigh out 5 g of homogenized sample in a centrifuge tube
- Add 20 mL water
- Solve sample for 15 min in an ultrasonic bath
- Centrifuge at 4500 rpm for 5 min at 4 °C
- Take 10 mL of sample extract for SPE (for direct HPLC injection: filter sample extract through a syringe filter (CHROMAFIL® Xtra PTFE-20/13 pore size 0.2 µm, REF 729208))

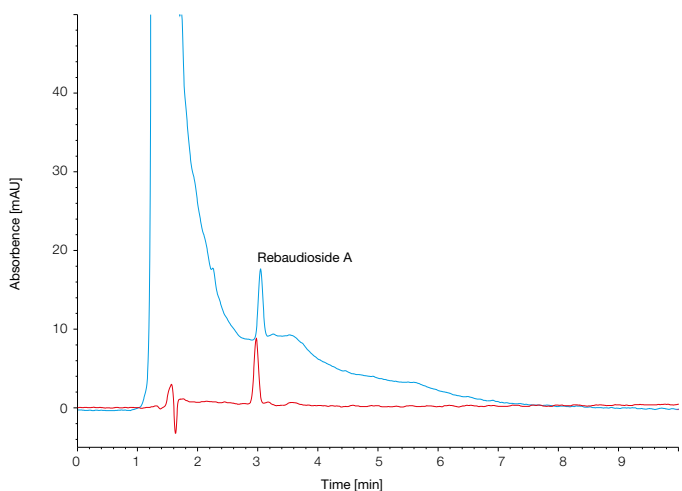


Figure 14: Clean up for tomato ketchup, blue: chromatogram of a sample extract, red: chromatogram of a solid phase eluate.

Recovery rate

Sample	CHROMABOND® C ₁₈ ec recovery rate in % (n = 3)
Tomato ketchup	95.0 ± 1.4
Licorice sweets	73.3 ± 3.1
Coca-Cola® life	92.3 ± 1.5
Multivitamin fruit spread	89.1 ± 8.9
Strawberry fruit spread	88.4 ± 0.9

Conclusion

The results of this work show that the solid phase extraction of steviol glycosides with CHROMABOND® C₁₈ ec and CHROMABOND® HR-X is very well suited. In particular, washing the stationary phase is different. CHROMABOND® C₁₈ ec shows less interaction with the analytes and the sample matrix in comparison to CHROMABOND® HR-X. Sample matrix could therefore be better removed by washing from the SPE column and the analytes could be eluted with smaller methanol volumes. The octadecyl modified silica phase shows a good loading capacity for all analytes. These properties could be useful for extracting individual steviol glycosides or for on-line SPE. The control of extracts of steviol glycosides or low-calorie food could be achieved.

This application note shows a simple and efficient chromatographic separation method for steviol glycosides using a NUCLEODUR® PolarTec column. The major compounds, rebaudioside A and stevioside, are separated with good resolution. The analytes were identified by HPLC-UV.

References

1. Scientific Opinion of the Panel on Food Additives and Nutrient Sources Added to Food on the Safety of Steviol Glycosides for the Proposal Uses As a Food Additive. EFSA J., (2010), 8(4), 1537.
2. U. Wölwer-Rieck, J. Agric. Food Chem., (2012), 60 (4), 886–895.
3. Cesar Gonzáles, María Tapia, Elevina Pérez, Dominique Pallet, Manuel Dornier, Main properties of steviol glycosides and their potential in the food industry: a review, Fruits 2014, (69), 127–141.

Additional information

The following applications regarding “Analysis of steviol glycosides in low-calorie food” and further applications can be found on our online application database at www.mn-net.com/apps:

SPE: MN Appl. No. 306540
HPLC: MN Appl. No. 128220

Product information

The following MACHERY-NAGEL products have been used:

REF 730014, CHROMABOND® C₁₈ ec, 6 mL, 500 mg
REF 730939, CHROMABOND® HR-X, 6 mL, 500 mg
REF 729222, CHROMAFIL® Xtra PET-20/13
REF 760479.20, EC 250/2 NUCLEODUR® PolarTec, 3 µm
REF 702293, Screw neck vials N 9, 1.5 mL
REF 702107, N 9 PP Screw cap, yellow, center hole, septum silicone white / PTFE red
REF 729208, CHROMAFIL® Xtra PTFE-20/13
REF 730223, CHROMABOND® centrifuge tubes 50 mL