MACHEREY-NAGEL
Chromatography application note

MN application note 10/2017

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.

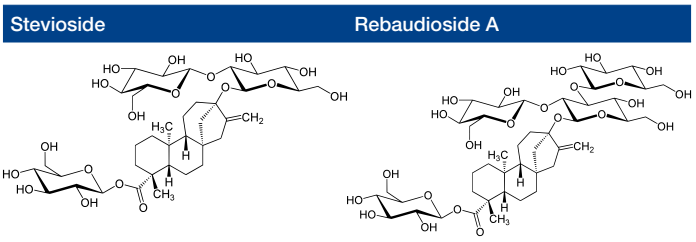

Figure 1: Major active compounds of sugar substitutes extracted from the leaves of the plant species Stevia rebaudiana, stevioside and rebaudioside A [3].

Sample preparation

Sample preparation was adapted for each sample matrix.

Solid phase extraction

Column type:

CHROMABOND® C_{18} 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 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 conditions

Column:

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

Eluent:

Water (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

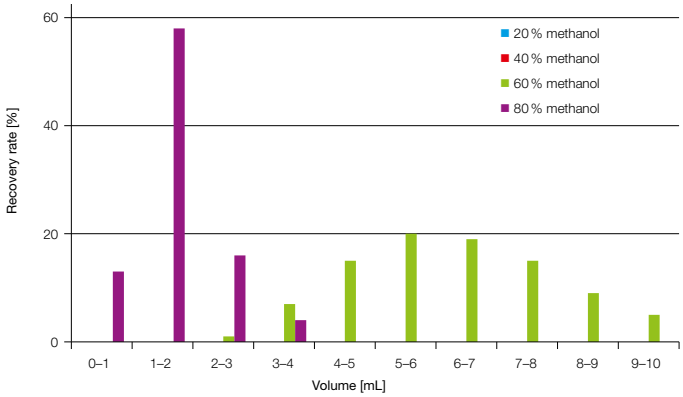

Figure 2: Influence of methanol content in wash-solution from CHROMABOND® C_{18} ec.

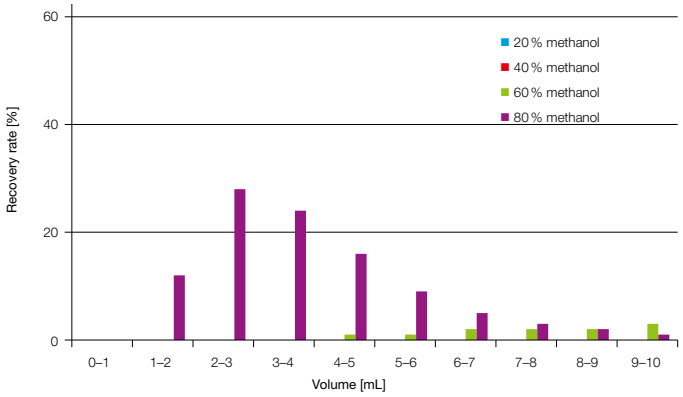

Figure 3: Influence of methanol content in wash-solution from CHROMABOND® HR-X.

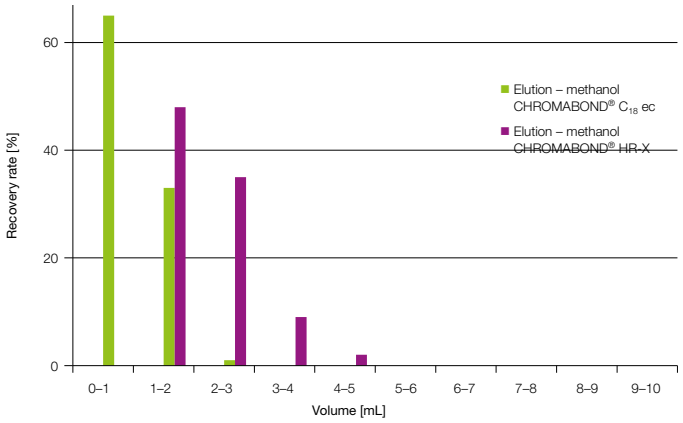

Figure 4: Elution profile after washing column with 10 mL methanol – water (40+60, v/v) from CHROMABOND® HR-X and CHROMABOND® C_{18} ec.

Proofing loading capacity

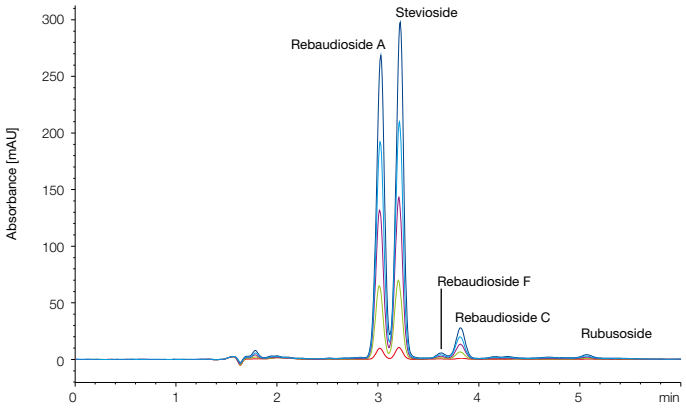

Figure 5: Chromatograms of solid phase eluates of different volumes of extracts of isolated steviol glycosides.

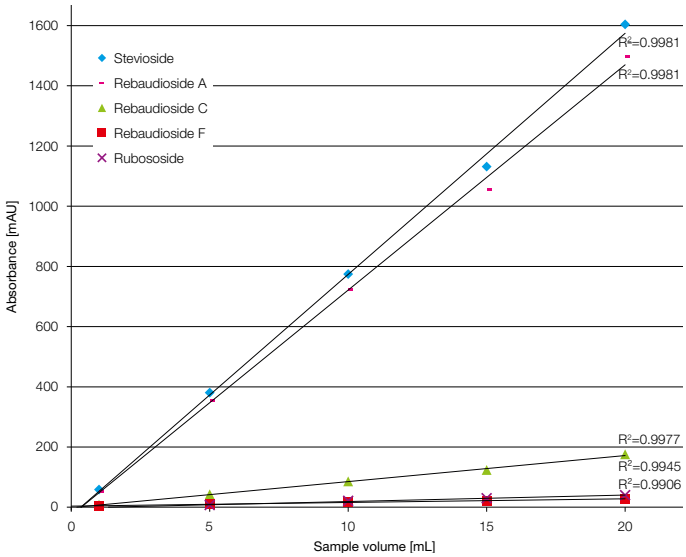

Figure 6: Proportionality between sample volumes for SPE and absorbance.

Control of extracts of isolated steviol glycosides

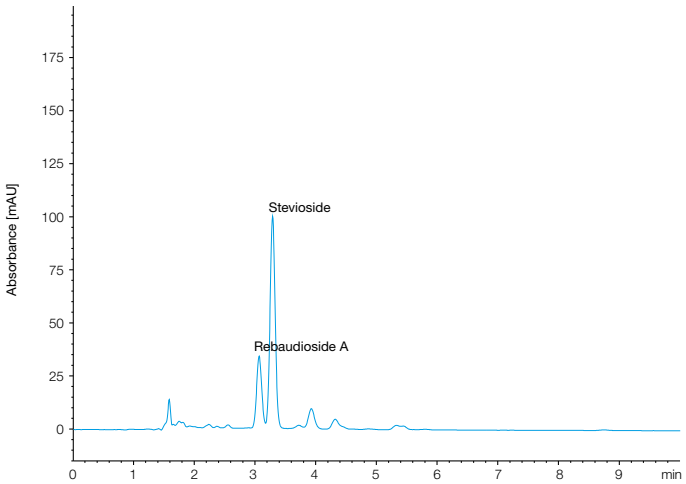

Figure 7: Chromatogram of an extract of isolated steviol glycosides, example A, β = 100 µg/mL in eluent.

Analysis of steviol glycosides in low-calorie food

Control of extracts of isolated steviol glycosides

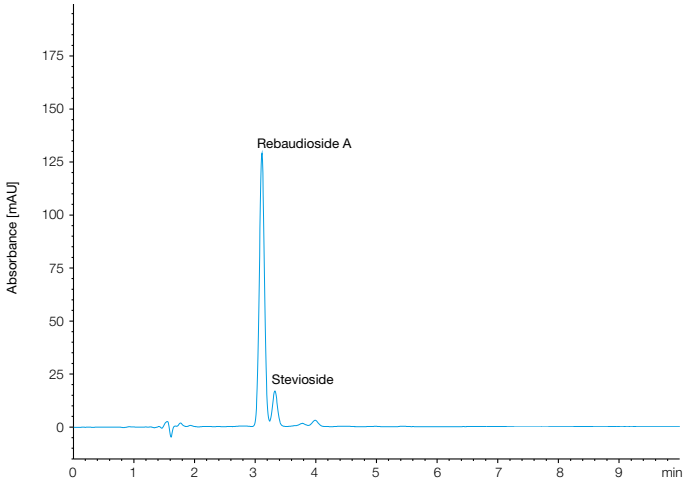

Figure 8: Chromatogram of an extract of isolated steviol glycosides, example B, β = 100 µg/mL in eluent.

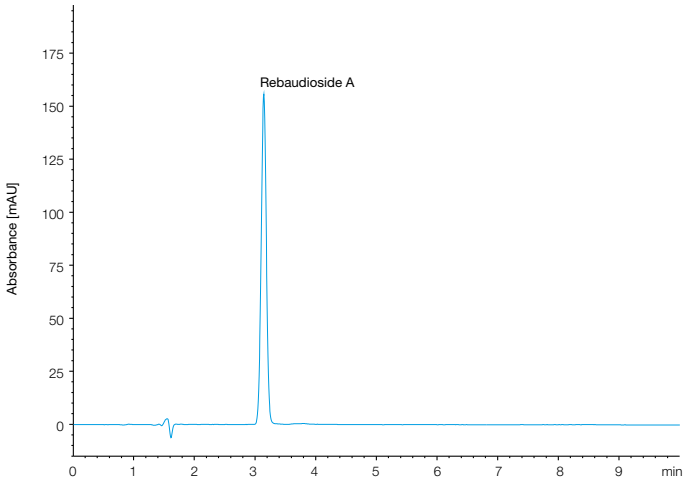

Figure 9: Chromatogram of an extract of isolated steviol glycosides, example C, β = 100 µg/mL in eluent.

Control of low calorie-reduced foods

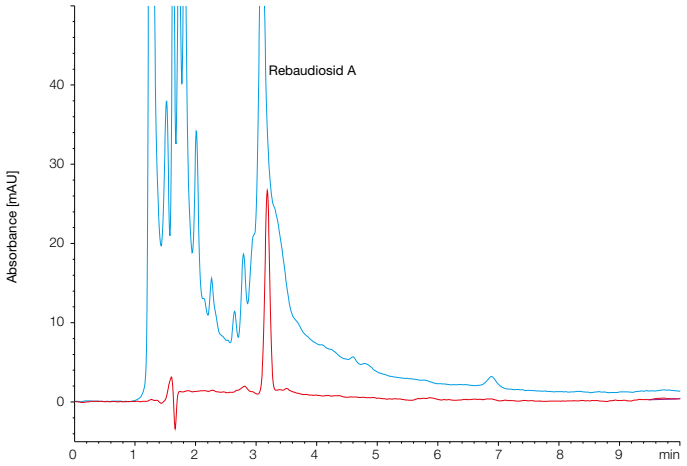

Figure 10: Clean up for licorice sweets, blue: chromatogram of a sample extract, red: chromatogram of a solid phase eluate.

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))

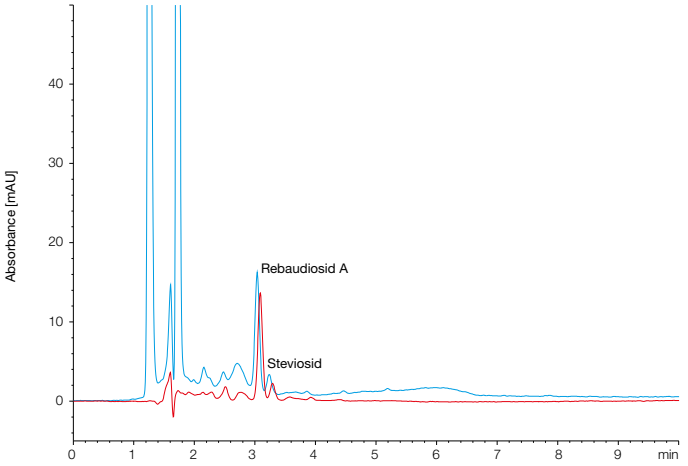

Figure 11: Clean up for Coca-Cola®, blue: chromatogram of a sample extract, red: chromatogram of a solid phase eluate.

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))

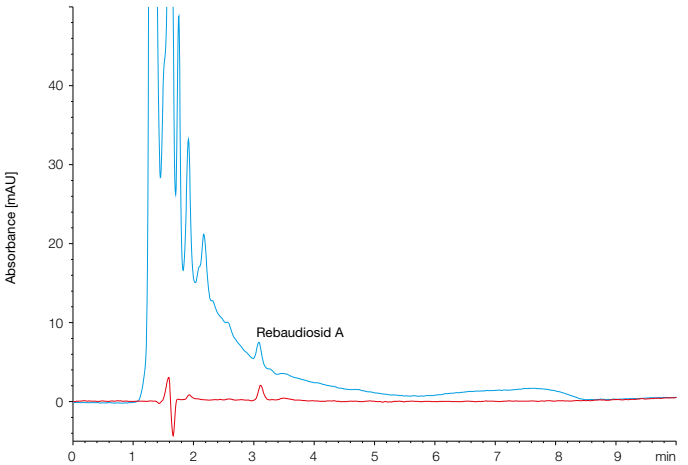

Figure 12: Clean up for strawberry fruit spread, blue: chromatogram of a sample extract, red: chromatogram of a solid phase eluate.

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Sample preparation

- Weigh out 5 g of homogenized sample in a centrigue 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))

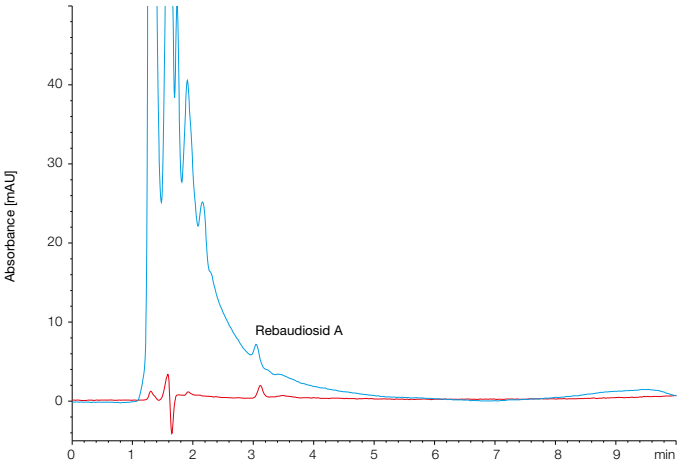

Figure 13: Clean up for multivitamin fruit spread, blue: chromatogram of a sample extract, red: chromatogram of a solid phase eluate.

Sample preparation

- Weigh out 5 g of homogenized sample in a centrigue 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))

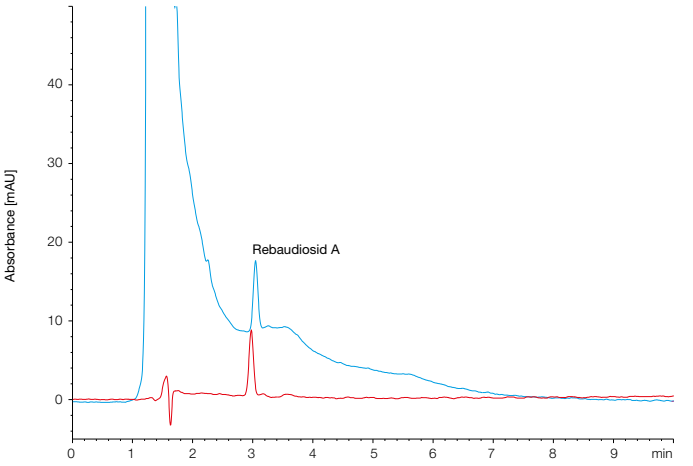

Figure 14: Clean up for tomato ketchup, blue: chromatogram of a sample extract, red: chromatogram of a solid phase eluate.

Recovery rate

Sample	CHROMABOND® C_{18} ec recovery rate [%] (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® C_{18} ec and CHROMABOND® HR-X is very well suited. In particular, washing the stationary phase is different. CHROMABOND® C_{18} 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 MACHEREY-NAGEL products have been used:

REF 730014, CHROMABOND® C_{18} 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