KNAUER

Application Note

Fast online SPE purification of Stevia plant extracts

Category	Food analysis
Matrix	Plant
Method	HPLC
Keywords	Stevioside, steviol glycoside, online SPE, HPLC Plus
Analytes	Stevioside, rebaudioside A
ID	**VFD0093N** , November 2011

Summary

Sample cleanup via solid phase extraction (SPE) and similar methods are time-consuming and cost-intensive procedures. Especially the purification of complex plant extracts requires special care. In this application a robust and sensitive online SPE sample preparation method was introduced for the determination of steviol glycosides. The method starts with a filtered plant extract. The purification step and determination of the steviol glycosides require less than 13 minutes. With recovery rates of 107% for stevioside and 99% for rebaudioside A and relative standard deviations of 0.4% for stevioside and 0.9% for rebaudioside A (n=10) the sensitivity and suitability of the processing method is demonstrated. Equipped with KNAUER switching valves a KNAUER PLATINblue HPLC PLUS system can be used in continuous operation. This sample preparation method is therefore especially interesting for high throughput analysis, e.g. in process monitoring or quality control.

Introduction

Steviol glycosides are responsible for the sweet taste of stevia plant leaves. These compounds range in sweetness from 75 up to 450 times sweeter than sucrose. The taste profile is a pure sweet one without the typical bitter aftertaste of some well known sweetener. Furthermore steviol glucosides are almost free of calories and show no fermentation. In addition these glucosides inhibit the formation of caries and plaque.

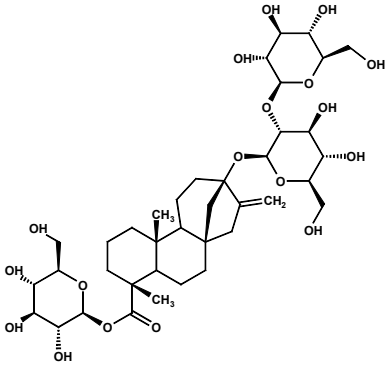
The extraction of steviol glycosides from Stevia leaves are accompanied by many possible impurities such as pigments, sugars and other plant material. These contaminants can disturb significantly the safe qualitative and quantitative determination of the target analytes. Typically a series of purification steps are needed before the analysis can start.[1] These purification steps are normally very time-consuming, error-prone and cost-intensive and sometimes even lead to loss of the analytes.

If it was possible to combine the sample preparation and the following determination with an HPLC system, this would be a time-saving and cost-effective solution allowing high throughput analysis for monitoring and for quality control. To realize this concept requires the HPLC system to do a few more tasks than just measuring the sample. As a first step the sample has to be transferred to an SPE cartridge and purified on it. After switching the SPE cartridge in the injection flow path of the analyzing HPLC system the target analyte has to be flushed from the cartridge. Finally the cartridge needs to be cleaned and prepared for the next sample preparation. Of course this process must be very stable and sensitive for the steviol glycosides.

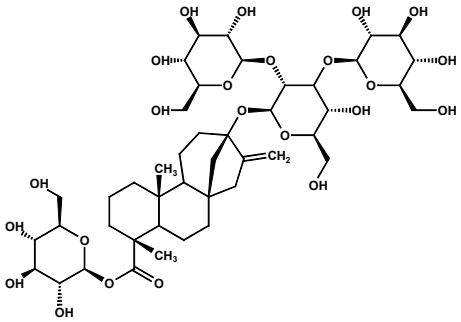
Experimental: Sample extraction

0.8 g of dried and pulverized stevia plant leaves are mixed with 30 ml acetonitrile / water (70:30), sonicated and heated at 70 °C for 15 minutes. Then the extract is filtrated through a 0.45 µm filter.

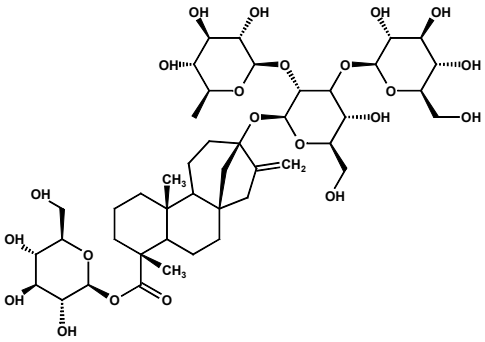
Chemical structures



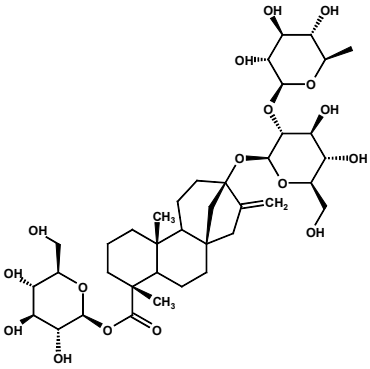
Stevioside



Rebaudioside A



Rebaudioside C



Dulcoside A

Method parameters

Extract purification

Step 1	Injection of 10 µl extract
Step 2	Sample transport from injection part to SteviaClean cartridge and cleaning step with acetonitrile/water 90:10 (v/v) for 2 minutes and at a flow rate of 2 ml/min.

Sample analysis

Step 3	Switching the SteviaClean cartridge into the determination part of the HPLC Plus system for 30 seconds and starting the PDA acquisition immediately after switching.
Column	Eurospher 100 NH_2, 5 µm, 150 x 3mm
Mobile phase	Acetonitrile/Water 80:20 (v/v)
Flow rate	1.0 ml/min
Column temperature	35°C
Detection	UV, 210 nm

SteviaClean cartridge cleaning and conditioning (during sample analysis)

Step 4	After switching back, flush with water for 4 minutes at a flow rate of 3 ml/min.
Step 5	Flushing 4 minutes with acetonitrile/water 90:10 (v/v) at a flow rate of 3 ml/min and then at 0.5 ml/min flow rate until the method ends

Results

As shown in figure 1 the online SPE purification works very fast and robust with the KNAUER SteviaClean cartridge. By combining this cleanup procedure with a previously developed HILIC - HPLC separation method, a complete purification and determination run can be accomplished within 13 minutes.[2] For standard impurity level plant extracts the SteviaClean cartridge can be used up to 10 times without any penetration of matrix components or losses of stevioside and rebaudioside A. During the following determination the SteviaClean cartridge was flushed with the listed mobile phases. The slightly shifted retention times between the two runs result from the extra dead volume of the cartridge. For 10 measurements the relative standard deviation of the peak area was 0.4% for stevioside and 0.9% for rebaudioside A. The recovery rate was 107% for stevioside and 99% for rebaudioside A.

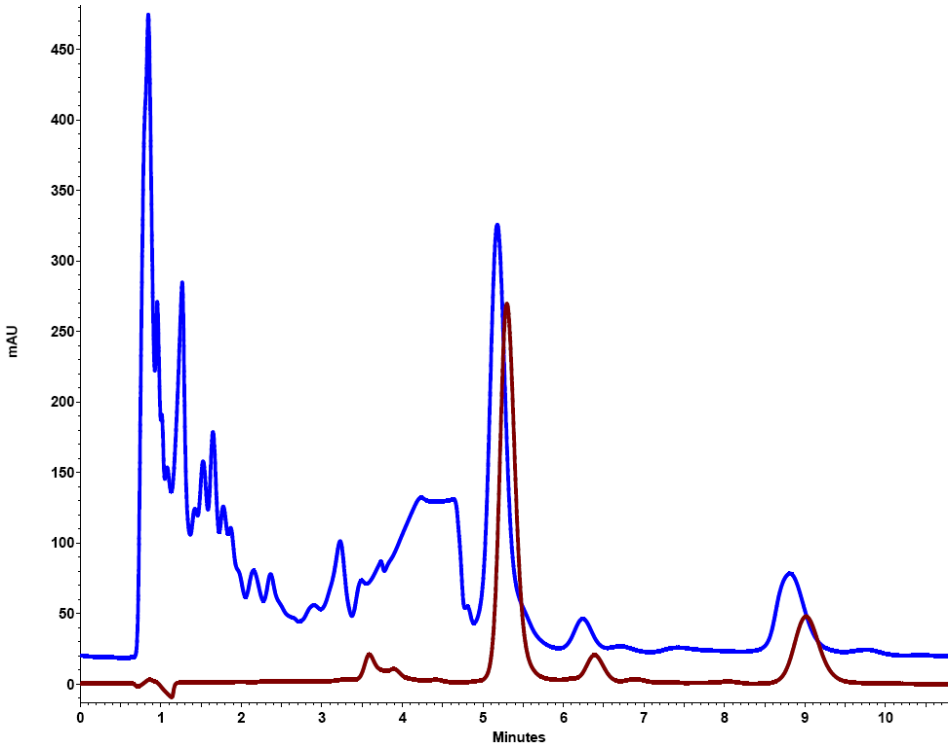


Fig. 1

Overlay of non purified extract (blue) and purified extract (red)

Substance	Standard deviation of peak area over 10 measurements	Recovery rate over 10 measurements (compared to non-cleaned sample)
Stevioside	0.4 %	107 %
Rebaudioside A	0.9 %	99 %

Conclusion

This online SPE purification method applying KNAUER SteviaClean SPE cartridges in combination with a KNAUER PLATINblue HPLC Plus system is able to reliably purify and determine a stevia plant extract in less than 13 minutes. With the two 6/1 port 1/32" switching valves in the KNAUER Column Thermostat T-1 it is possible to install up to six KNAUER SteviaClean cartridges for the automated determination of up to 60 samples with an overall run time of approximately 15 hours. Therefore a very suitable method is provided for high throughput analysis.

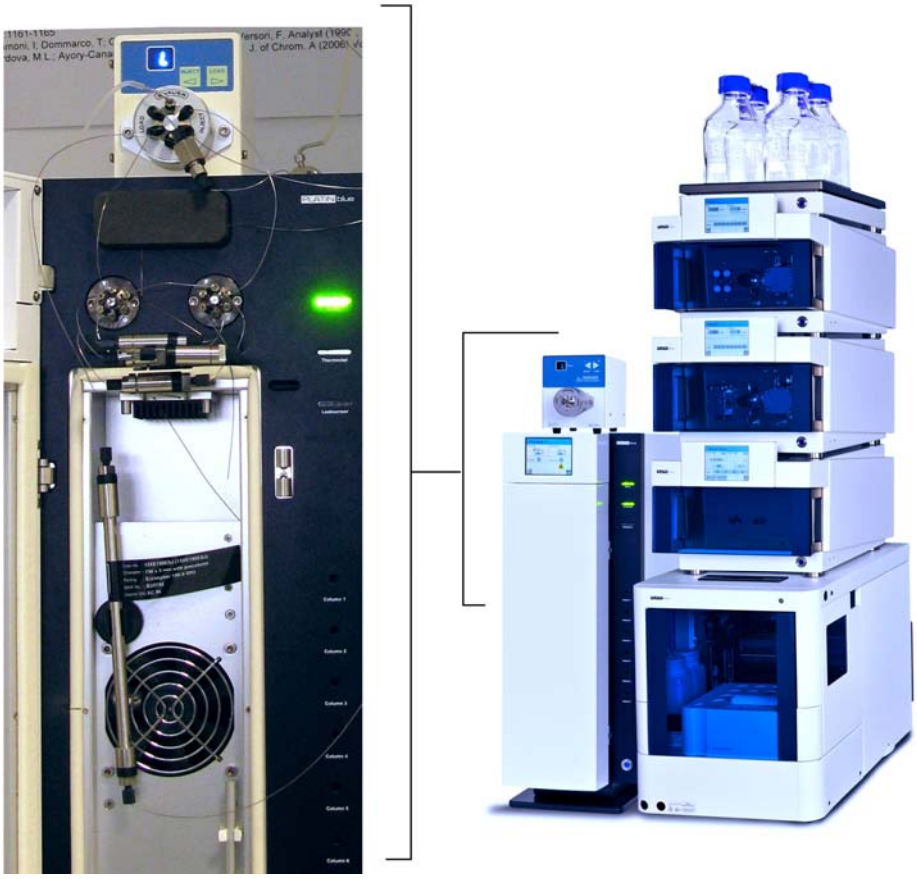


Fig. 2
KNAUER PLATINblue HPLC Plus system for online SPE purification with five SteviaClean cartridges)

Physical properties of recommended column

Eurospher NH_2 packing material is an excellent choice for very polar substances in HILIC mode and can also be used as a weak anion exchanger. Furthermore this phase is suitable for normal phase – and polar organic modes.

Stationary phase	Eurospher 100 – 5 NH_2	
USP code	L8	
Pore size	100 Å	
Particle size	5 µm	
Form	spherical	
Surface area	350 m^2 / g	
% C	3	
Endcapping	no	
Dimensions	150 x 3 mm	
Parameter limits	maximum temperature	60 °C
	maximum pressure	400 bar
	pH range	2 – 8
Order number	15CE190ESJ	

Recommended instrumentation

The SPE purification and the following analysis were performed on a KNAUER PLATINblue HPLC Plus system comprising of two Pump P-1 (one for analysing and one for transport), Autosampler AS-1, Column Thermostat T-1 with two integrated 6/1 port 1/32" valves, Detector PDA-1, Assistant with two 1/8" solvent selection valves and one separate two way 1/16" valve. Other configurations are also available. Please contact KNAUER to configure a system that's perfect for your needs.

Description	Order No.
Pump P-1, incl. 10 ml pump head with degasser	A60016
Pump P-1, incl. 10 ml pump head with SmartMix	A60015
Assistant with two 1/8" solvent selection valves	A6700V001
Smartline two way 1/16" switching valve	A1369-1
Autosampler AS-1	A63500
Column Thermostat T-1 with two integrated 6/1 port 1/32" valves	A63412
Detector PDA-1	A62031
10 mm flow cell	A64150
ChromGate software PLATINblue edition	A65111

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