MACHEREY-NAGEL
Chromatography application note

MN application note 08/2017

Determination of residues of sweeteners in fresh water

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Abstract

This application note describes the determination of residues of sweeteners from fresh water in lower µg/L range using SPE for sample clean up und analyt concentration. After eluent exchange, the eluates from SPE are finally analyzed by HPLC-MS/MS.

Introduction

In water analytics the investigation of residues of sweeteners gains more and more importance. With the consumption of reduced calorie products or with the use of cosmetic products these compounds reach the environment for the most part of them unchanged. Hence, sweeteners are a good indicator for anthropogenic pollution in water, or for the entry of surface water in drinking water. In the environmental analytics the investigation of sweeteners is common. Hence, efficient analytic methods are necessary for determination of residues of sweeteners in very low concentrations, which allows to separate and to quantify the compounds of interest.

Sample Preparation using HR-X for SPE

Sample pretreatment

Addition of 25 mL 1 mol/L KH_2PO_4 buffer pH 2.5 to 1 L water sample solution

Addition of 1 mL of standard solution (c = 50 µg/mL for each of the respective standards).

Solid phase extraction

Column type:

Chromabond® HR-X polypropylene columns (45 µm), 3 mL, 200 mg, (REF 730931P45)

Conditioning:

5 mL methanol, 5 mL KH_2PO_4 buffer (25 mmol/L, pH 2.5)

Sample application:

with a flow of 5–10 mL/min

Washing:

4 mL water

Elution:

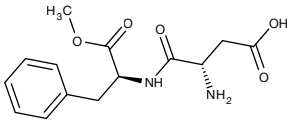
3 mL methanol, 3 mL methanol + 1 % NH_3

Eluent exchange:

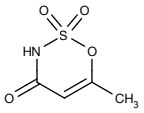
Eluent exchange is performed manually. Eluates from the SPE are evaporated to dryness at 40 °C under a stream of nitrogen and then redissolved in water – acetonitrile (95:5, v/v).

Compounds of Interest

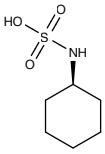
Aspartame



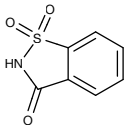
Acesulfame



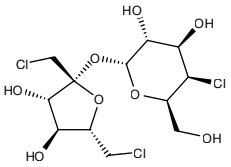
Cyclamate



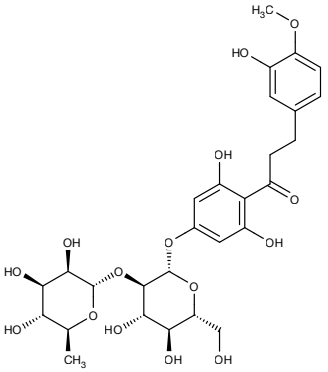
Saccharin



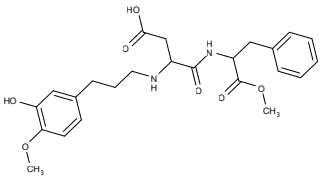
Sucralose



NHDC



Advantame



Neotame

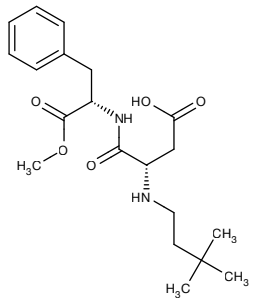


Figure 1: Overview of analyzed sweeteners.

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Determination of residues of sweeteners in fresh water

Subsequent analysis: HPLC-MS/MS

Chromatographic conditions

Column:

EC 100/2 NUCLEOSHELL® RP 18plus 2.7 µm, (REF 763234.20)

Eluent A:

H_2O (ultrapure) + 0.1 % formic acid

Eluent B:

acetonitrile + 0.1 % formic acid

Gradient:

5–55 % B in 5 min, 55–95 % B in 1 min, 95 % B for 2 min, 95–5 % B in 0.1 min, 5 % B for 4.9 min

Flow rate:

0.3 mL/min

Temperature:

30 °C

Injection volume:

10 µL

MS conditions for neotame and advantame:

API 3200 (AB Sciex), ion source ESI, positive ionization mode, curtain gas 15 psig, ion spray voltage 4500 V, temperature 650 °C, nebulizer gas 40 psig, turbo gas 50 psig, CAD 3.0 psig

MS conditions for the other analytes:

API 3200 (AB Sciex), ion source ESI, negative ionization mode, curtain gas 15 psig, ion spray voltage –4500 V, temperature 650 °C, nebulizer gas 40 psig, turbo gas 50 psig, CAD 3.0 psig

MRM transitions

Analyte	$[M-H]^-$	$[M+H]^+$	Q_1 (Quantifier)	Q_2 (Qualifier)
Acesulfame	162.0		82.1	77.9
Aspartame	293.1		200.1	261.1
Cyclamate	178.0		80.0	79.0
Neohesperidin	611.2		303.1	125.0
Saccharin	182.0		42.1	105.9
Sucralose	395.0		35.2	359.1
Advantame		459.2	102.1	252.2
Neotame		379.3	172.2	85.2

Table 1: MRM transitions for analyzed sweeteners.

Chromatograms

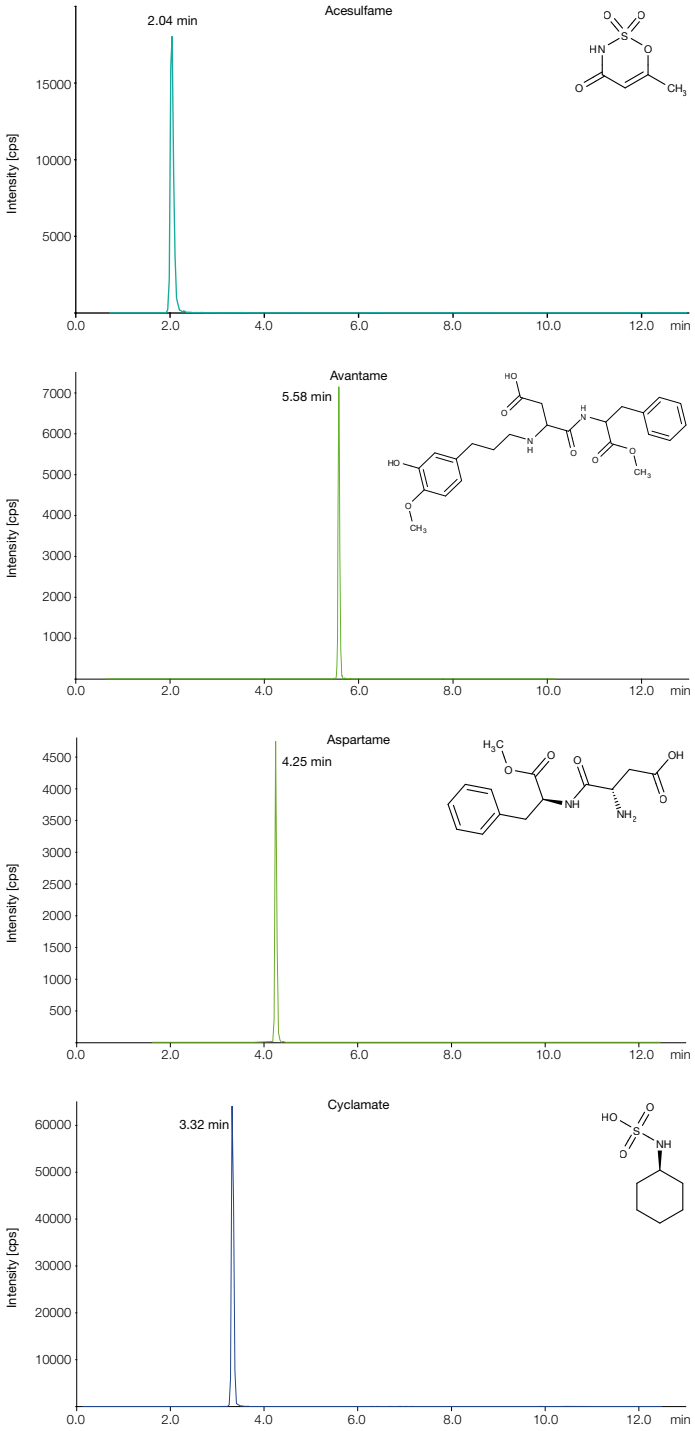

Figure 2: Chromatograms of HPLC-MS/MS analysis, first part (acesulfame, advantame, aspartame, cyclamate).

Determination of residues of sweeteners in fresh water

Chromatograms

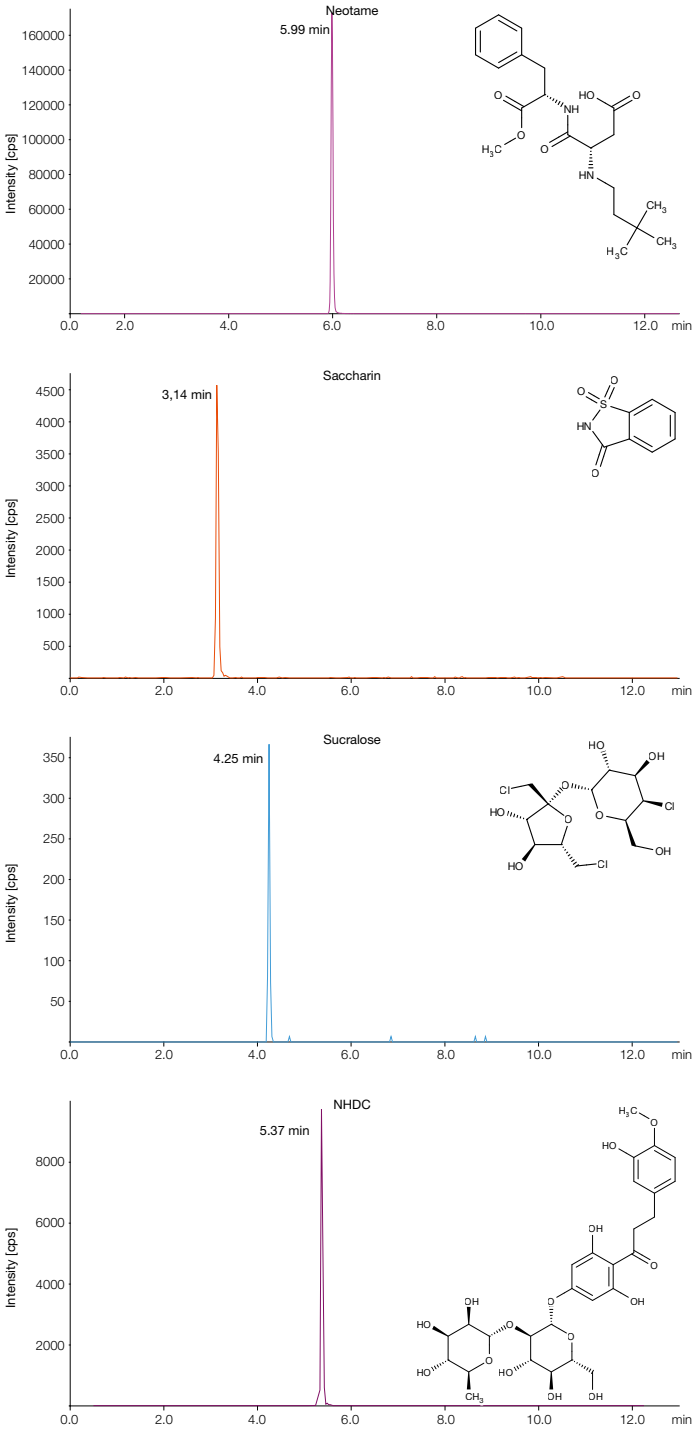

Figure 3: Chromatograms of HPLC-MS/MS analysis, second part (neotame, saccharin, sucralose, NHDC).

Calibration curves

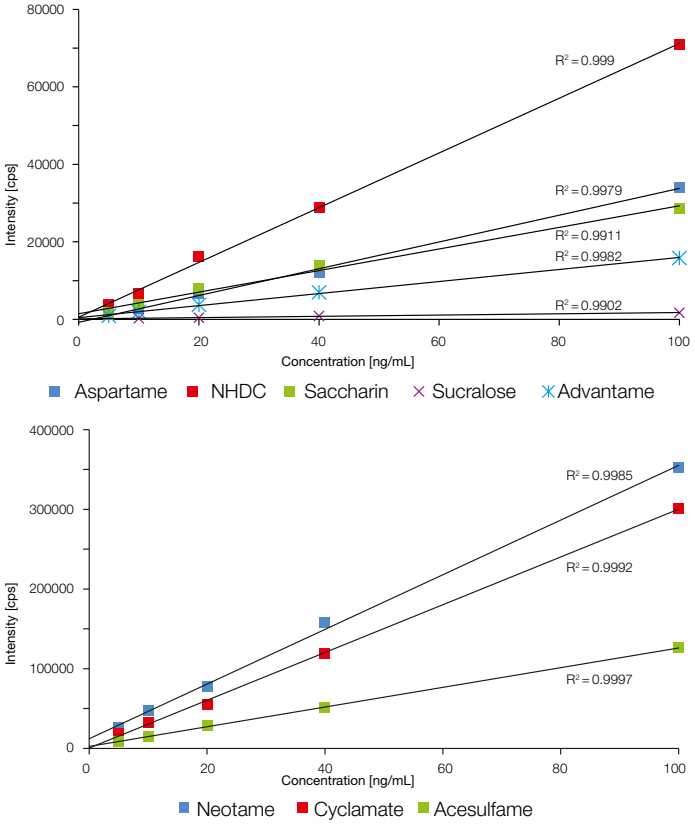

Figure 4: Calibration curve of sweeteners, linear range from 5 ng/mL to 100 ng/mL.

Tables

Analyte	Recovery rate [%]
Acesulfame	8
Aspartame	86
Cyclamate	15
NHDC	84
Saccharin	57
Sucralose	70
Advantame	95
Neotame	92

Table 2: Recovery rates for sweeteners analyzed with CHROMABOND® HR-X, particle size 45 µm, pH 2.5, 200 mg.

Analyte	Recovery rate [%]
Acesulfame	84
Aspartame	66
Cyclamate	100
NHDC	2
Saccharin	108
Sucralose	101
Advantame	60
Neotame	86

Table 3: Recovery rates for sweeteners analyzed with CHROMABOND® HR-XAW, particle size 45 µm, 200 mg.

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Conclusion

Sweeteners are a heterogeneous group of analyt molecules. They show clearly different polarities which is important for their enrichment.

This application note shows that enrichment by ionic interactions works much better for the analytes acesulfame and cyclamate using CHROMABOND® HR-XAW.

However, utilizing the hydrophobic interactions of CHROMABOND® HR-X the recovery rates of less polar analytes like advantame, neotame or NHDC are much higher.

Additional information

The following applications regarding „The Determination of residues of sweeteners in fresh water“ and further applications can be found on our online application database at *www.mn-net.com/apps*

SPE (with CHROMABOND® HR-X): MN Appl. No. 306010

SPE (with CHROMABOND® HR-XAW): MN Appl. No. 306020

HPLC: MN Appl. No. 127390

Product information

The following MACHEREY-NAGEL products have been used in this application note:

REF 730931P45, CHROMABOND® HR-X (45 µm), 3 mL, 200 mg

REF 730748P45, CHROMABOND® HR-XAW (45 µm), 3 mL, 200 mg

REF 763234.20, EC 100/2 NUCLEOSHELL® RP 18, 2.7 µm

REF 702923 Screw neck vials N 13, 4 mL

REF 702052 N 13 PP Screw cap, black, closed top, septum silicone white / PTFE red

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

Image credits