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Automated Detection of Artificial Sweeteners

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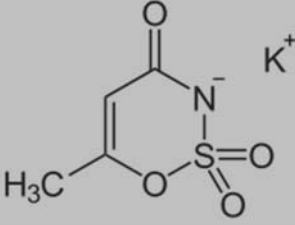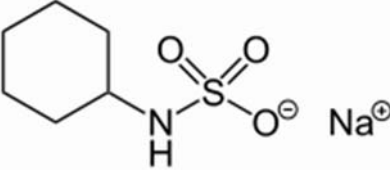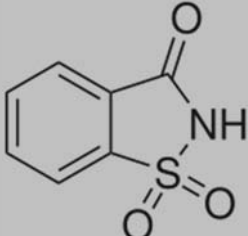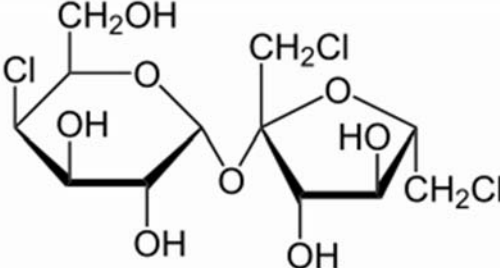
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1 Introduction

World-wide, artificial sweeteners are used to replace sugar in numerous foods. Artificial sweeteners are hardly or even not at all metabolised by the human body, and consequently enter our municipal waste water. However, purification processes applied in sewage treatment plants usually fail to remove acesulfame, cyclamate, saccharin and sucralose and hence find their way into the groundwater. Thus, these named substances also serve as indicators for water quality. Due to this fact, this application note is limited from the seven most common to the four above-mentioned environmentally relevant sweeteners.

The following shows how samples are prepared for subsequent analysis by means of solid phase extraction (SPE), both manually and automatically using the FREESTYLE XANA robotic system. Due to the option of using the system around the clock, parallelisation of individual processing steps as well as the simultaneous processing of several samples, a very high sample throughput can be achieved. The user's workload is thereby considerably reduced, and more time for other tasks becomes available.

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1.1 Analytes

Analyte	Structural formula	Chemical formula	Molecular weight
Acesulfame-K		$C_4H_4KNO_4S$	201,24 g·mol^{-1}
Cyclamate		$C_6H_{12}NNaO_3S$	201,22 g·mol^{-1}
Saccharin		$C_7H_5NO_3S$	183,19 g·mol^{-1}
Sucralose		$C_{12}H_{19}Cl_3O_8$	397,63 g·mol^{-1}

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2 Method Development

2.1 Chemicals

- Formic acid 99 %, ULC/MS Biosolve B.V.

- Acetonitrile, ULC/MS Biosolve B.V.

- Ammonium hydroxide solution ≥ 25 %, eluent additive for LC-MS, Fluka

- Double distilled water, Milli-Q by Millipore

2.2 Standards

- Sucralose ≥ 98% (HPLC) SIGMA (Life Science)

- Acesulfame K, puriss. ≥ 99 % (HPLC) Fluka

- Saccharin sodium salt dihydrate for synthesis, Merck Schuchardt

- Sodium cyclamate, 99,9 % SUPELCO

2.3 Sample material

The sample material used consists of 1 L tap water, which is spiked with 50 ng / L of each analyte.

2.4 Solid phase extraction (manual)

SPE - Steps	CHROMABOND® HR-XAW 45 µm, 3 mL, 200 mg
Conditioning	3 mL methanol 3 mL water
Loading	sample loading at 5-10 mL/min
Washing	4 mL water
Drying	air via syringe
Elution	3 mL methanol 3 mL methanol + 1 % NH_3

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2.5 Solid phase extraction (automated)

2.5.1 FREESTYLE XANA

The LCTech robotic system FREESTYLE XANA is particularly suitable for automated sample preparation of large volume water samples of 1 to 10 L. A very high sample throughput can be achieved as a result of its innovative design which enables parallelisation of individual processing steps and simultaneous processing of up to 3 samples.

Working mechanism of FREESTYLE XANA
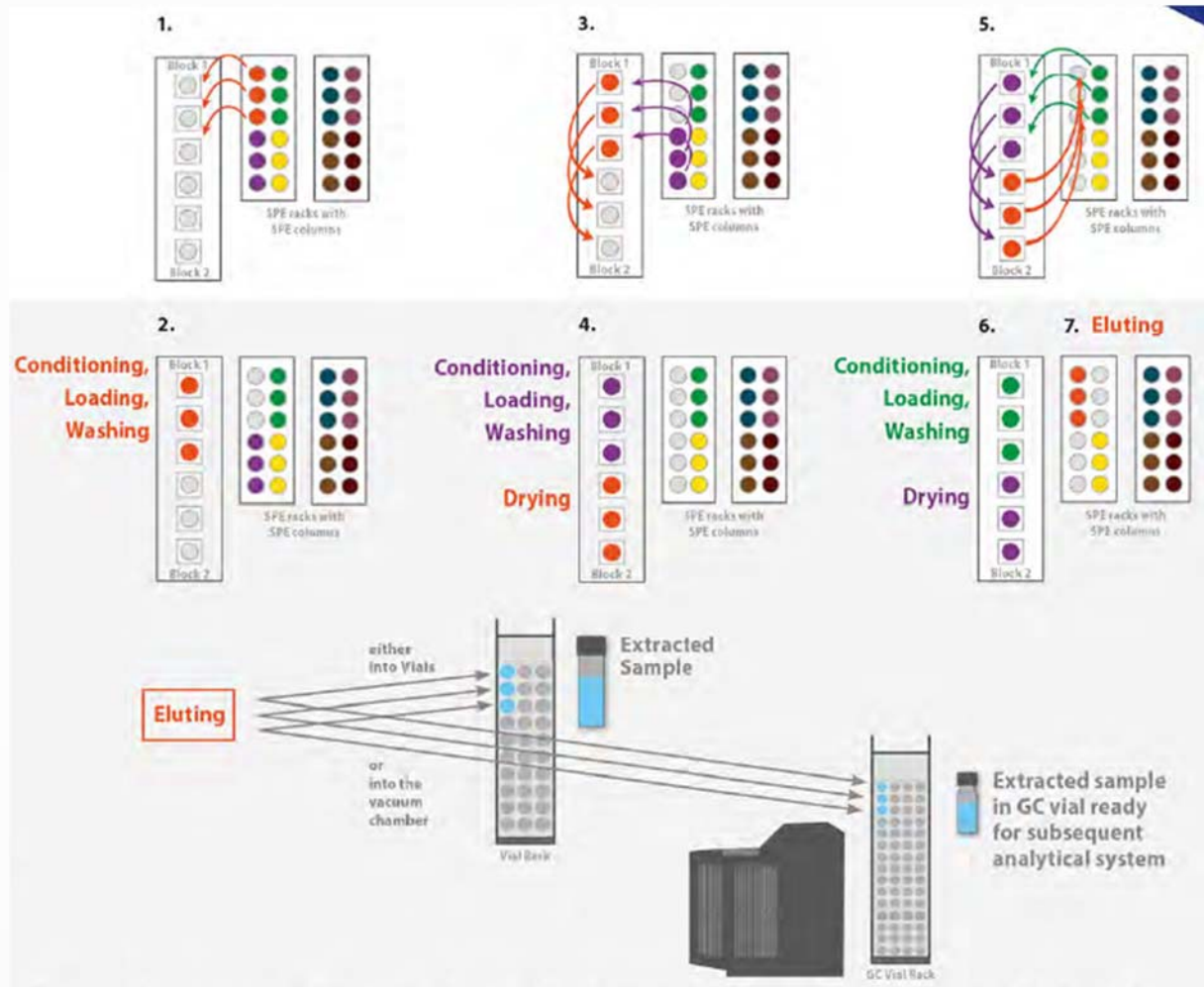
1.   The SPE gripper moves up to 3 columns from the SPE rack into block 1 of the working station:

2.   Simultaneous conditioning, loading and washing of the 3 columns.

3.   The SPE gripper transfers the 3 processed columns from block 1 into block 2 of the working station. Then the SPE gripper takes the next columns (up to 3) out of the SPE rack and places them into block 1 of the working station.

4.   Block 1: Conditioning, loading and washing of up to 3 columns simultaneously.
        Block 2: Drying of the first 3 columns.

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5.   The SPE gripper returns the 3 dried columns of block 2 to the SPE rack.
        Then, the SPE gripper moves 3 loaded and washed columns from block 1 into block
        2 of the working station. The SPE gripper takes the next columns (up to 3) from the
        SPE rack and places them into block 1 of the working station.

6.   Block 1: Conditioning, loading and washing of up to 3 columns simultaneously.
        Block 2: Drying of up to 3 columns simultaneously.

7.   The first 3 columns are sequentially eluted into vials or into the vacuum chamber of
        the EVAporation module. After EVAporation to the desired end volume, samples are
        filled into GC vials. The water samples are now prepared and ready for subsequent
        analysis.

Working station on the FREESTYLE platform

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The sample rack of the FREESTYLE XANA offers space for 24 x 1 L sample containers. Loading of the pull-out sample rack with sample containers is effortless, since each position can be easily accessed.
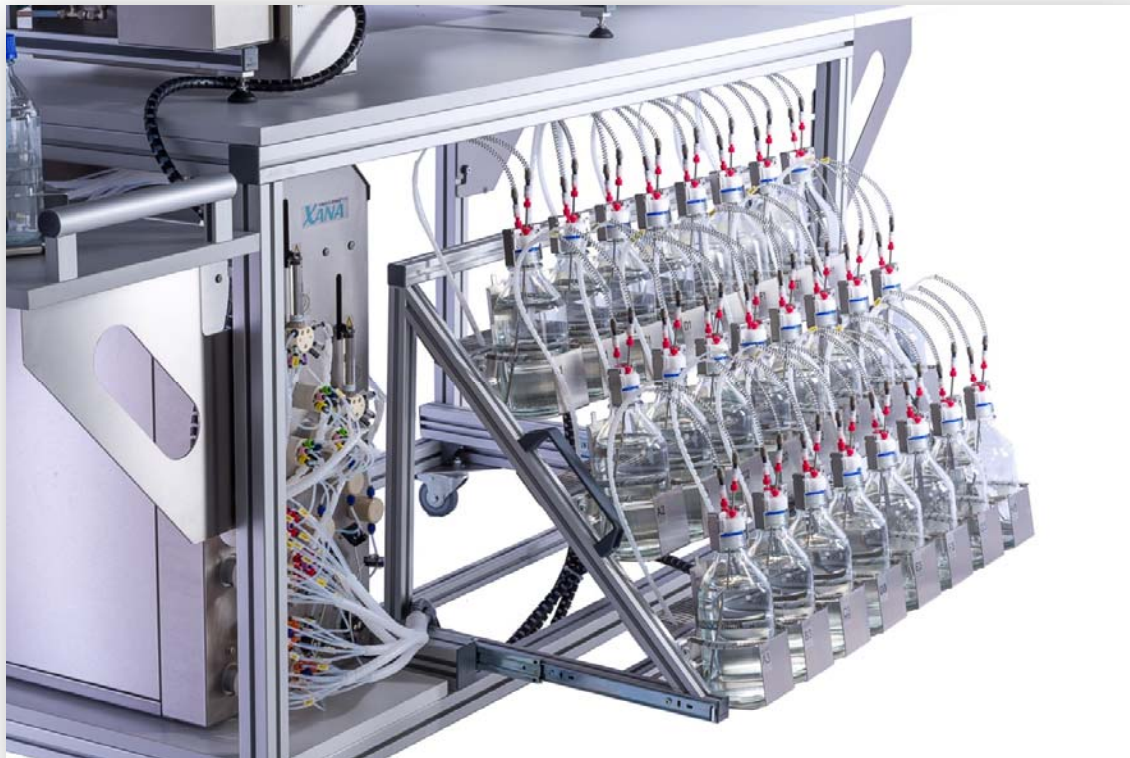
Each sample container is fitted with a single-hand operated closure, which holds the suction capillary at an angel. This capillary position in combination with an inclined position of the sample bottle in the rack maximises the sample load by minimising residues in the sample bottle.

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2.5.2 Automated processing with FREESTYLE XANA

The manual method of solid phase extraction can be easily transferred to the FREESTYLE XANA robotic system for automated sample preparation.

The easy-to-use system software offers a building set of ready-made functions, which make method development on the system fast and uncomplicated.

The following steps are fully automated processed by FREESTYLE XANA.

SPE steps	Fully automated
Conditioning	3 mL methanol, 10 mL/min. 3 mL water, 10 mL/min.
Loading	1000 mL sample, 8 mL/min.
Washing	4 mL water, 8 mL/min.
Drying	1 min., nitrogen
Elution	3 mL methanol, 2 mL/min. 3 mL methanol + 1% NH_3, 2 mL/min
Drying	20 mL air, 20 mL/min

Elution

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The detailed method parameterization on the FREESTYLE XANA is shown in the method report below.

Method report

2.6 Dilution

- The respective eluates are filled to 5 mL using methanol.

- Thereafter, 100 µL each are taken out and filled to 1 mL using water/acetonitrile (95/5 v/v) + 0.1 % formic acid.

- Then, all eluates are injected.

2.7 Measurement using LC-MS/MS

The measurement was performed on an Agilent 1290 Infinity II system with the following configuration:

Configuration	Designation
Pump	G7120A
Autosampler	G7167B
Oven	G7116B

Column	EC 100/2 NUCLEOSHELL® RP 18plus, 2.7 µm
Eluent A	0.1% formic acid in water
Eluent B	0.1% acetonitrile in water
Gradient	5 % B to 55 % B (5 min); 55 % B to 95 % B (1 min); 95 % B (hold for 2 min); 95 % B to 5 % B (0.1 min); 5 % B (hold for 4.9 min)
Flow rate	0.3 mL/min
Injection volume	20 µL
Column temperature	30 °C
Detection	*MS/MS, AB Sciex QTRAP 5500* ion source: turbo spray (ESI) scan type: MRM polarity: negative curtain gas: 15 psig ion spray voltage: 4500 V temperature: 650 °C gas 1 (nebuliser): 40 psig gas 2 (turbo gas): 50 psig CAD gas: 3 psig

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2.8   Configuration FREESTYLE XANA

1.	FREESTYLE BASIC	P/N	12663-12
2.	FREESTYLE SPE	P/N	12668
3.	Rack for solvent delivery	P/N	13156
4.	FREESTYLE XANA	P/N	15082
5.	Column adapter for water extraction (3 mL)	P/N	14892
6.	Caps for columns (3 mL)	P/N	14862
7.	Frame for trays (100 mm)	P/N	11915
8.	Tray (4 mL)	P/N	11926
9.	Adapter for SPE-columns	P/N	13382
10.	Screw thread bottle (4 mL)	P/N	V0004

2.9   Consumables by MACHEREY-NAGEL

1.	EC 100/2 NUCLEOSHELL® RP 18plus, 2.7 µm	P/N	763234.20
2.	CHROMABOND® HR-XAW (45 µm) PP-columns, 3 mL, 200 mg	P/N	730748P45

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3 Results

3.1 Recovery rates

Analyte (n=6)	Mean Value (%)	RSD (%)
Acesulfame-K	68 %	6 %
Cyclamate	91 %	4 %
Saccharin	93 %	3 %
Sucralose	103 %	5 %

**Recovery Rate (%) in Relation to Reference Sample (n=6)**
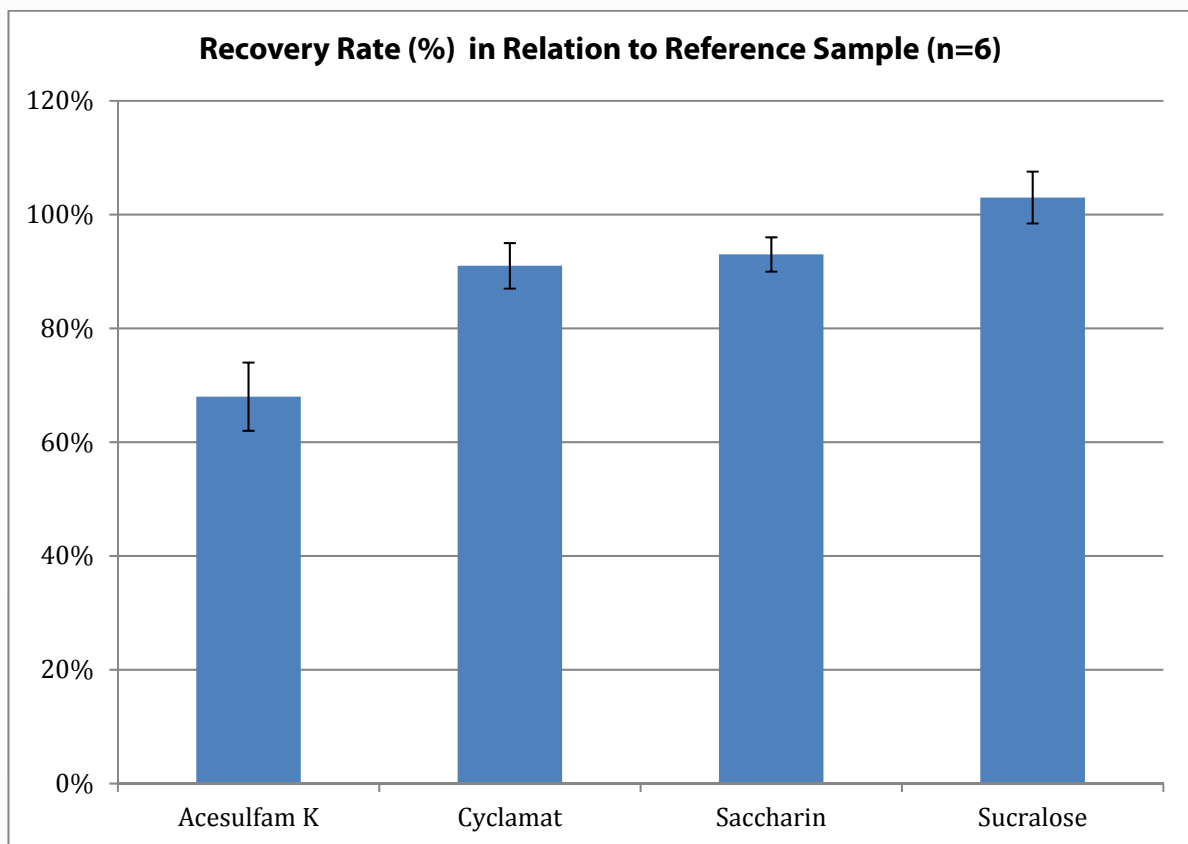
Summary

FREESTYLE XANA detects reliably and with high reproducibility artificial sweeteners in water samples using fully automated and parallel SPE concentration.

Unattended, the system processes samples 24 hours a day, 7 days a week.
Through parallelisation of individual processing steps and simultaneous processing of several samples, a very high sample throughput can be achieved.

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3.2 Chromatograms

Acesulfame — 2.04 min

Cyclamate — 3.32 min

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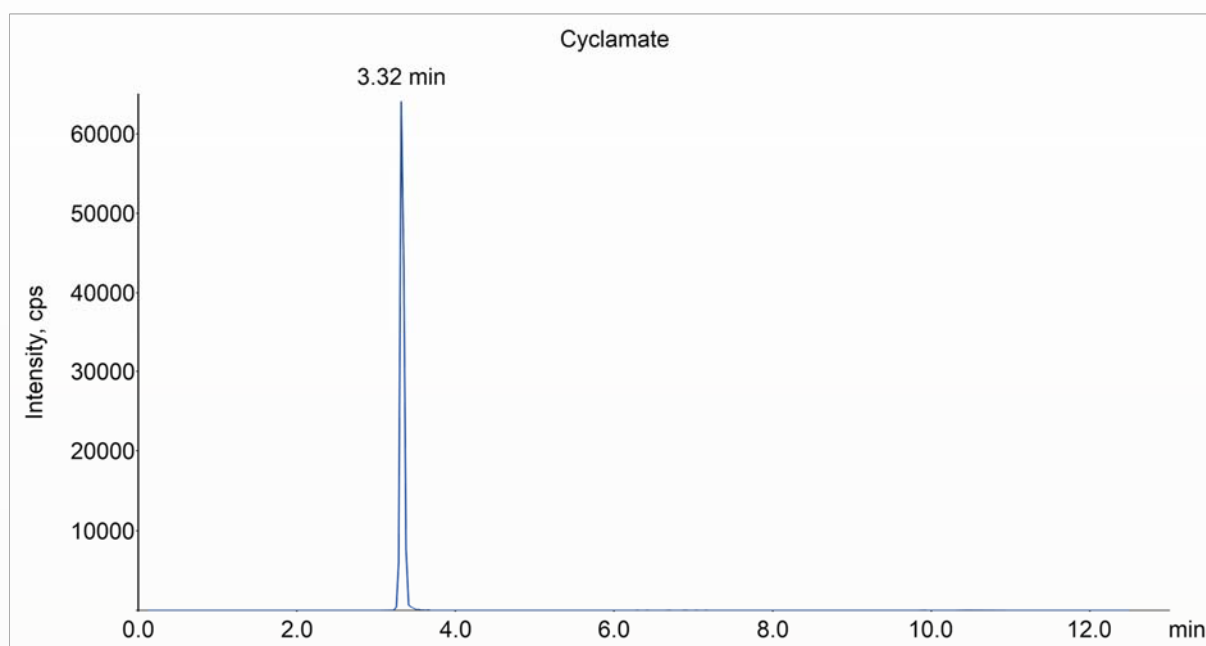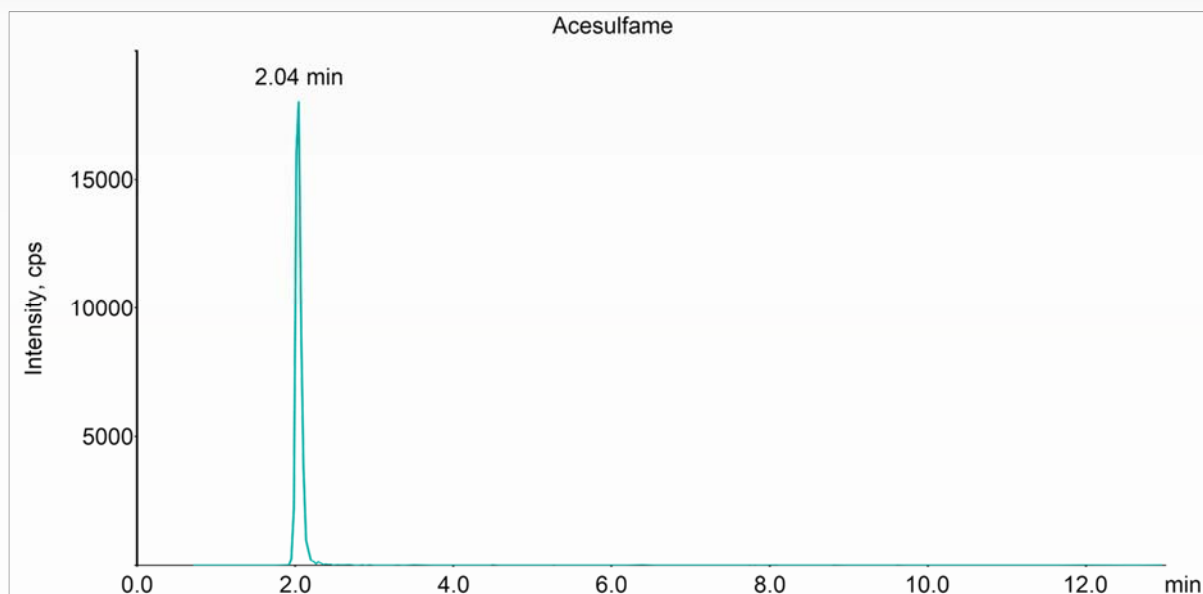
Saccharin
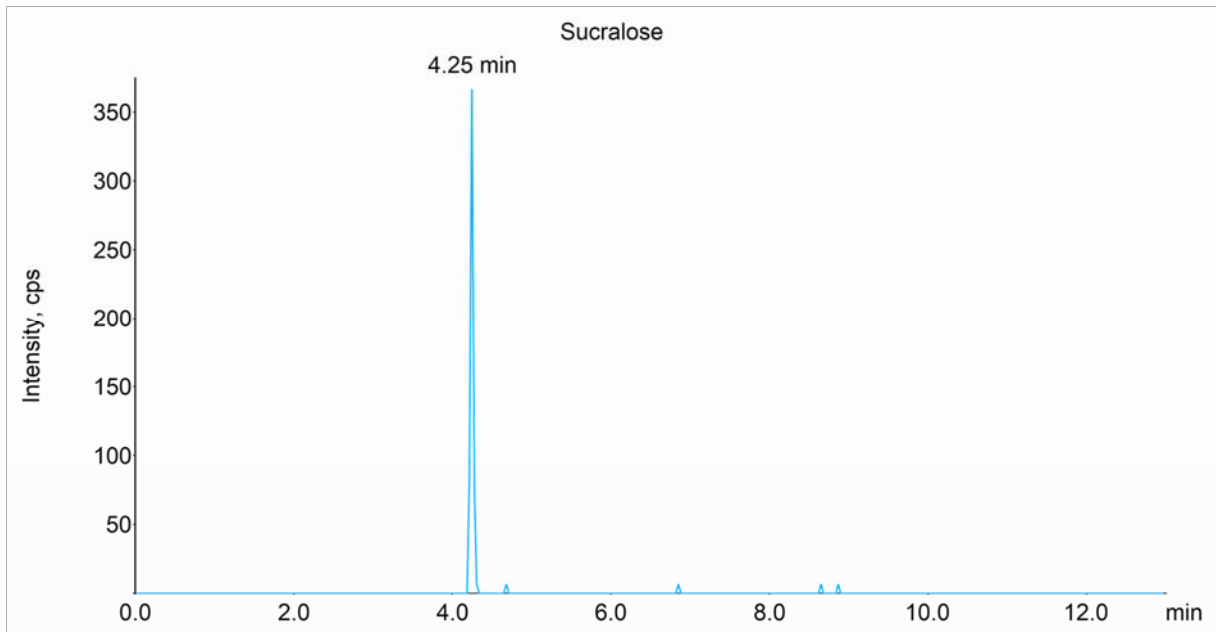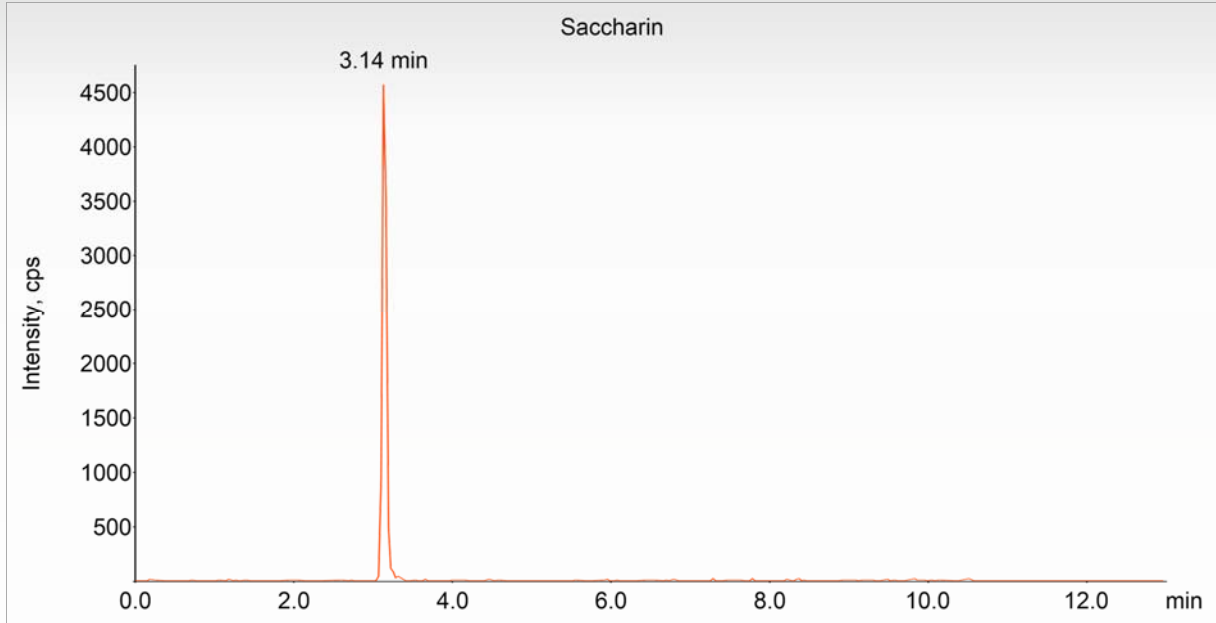
Sucralose

Retention Time	Analyte	Polarity	Quantitative Transfer [m/t]	Qualitative Transfer [m/t]
2.04 min	Acesulfame-K	(-)	162.0/82.1	162.0/77.9
3.14 min	Saccharin	(-)	182.0/42.1	182.0/105.9
3.32 min	Cyclamate	(-)	178.0/80.0	178.0/79.0
4.25 min	Sucralose	(-)	395.0/35.2	395.0/395.1

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