

SOLUTIONS BY



# Automated Detection of Acrylamide in Water

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**Keywords:** DIN 38413-6; German drinking water ordinance, TrinkwV; Austrian drinking water ordinance, TWV; United States National Primary Drinking Water Regulations (US NPDWR); US EPA Method 8032A

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## Introduction

River water and drinking water are regularly checked for their quality. For the purpose of this monitoring, samples are taken according to DIN 38413-6 and tested for acrylamide by means of solid phase extraction. Due to its carcinogenic potential, acrylamide was included in the drinking water ordinance of 21 May 2001 as a new parameter in the substance list (annex 2, part I, serial no. 1). The acceptable limit was set to  $0.1 \mu\text{g} / \text{L}$ . The necessity for examination arises through the use of polyacrylamides as flocculants in drinking water treatment as well as through their use in the paper industry. In principle, there is always the possibility of releasing acrylamide in water, since during the technical production of polyacrylamide traces of the monomeric acrylamide remain in the end product.

The following shows how samples are prepared by means of solid phase extraction (Solid Phase Extraction - SPE), both manually and automatically using the FREESTYLE SPE robotic system for subsequent analysis. Due to the option of using the system around the clock and the sequential, unattended processing of the samples, a very high sample throughput can be achieved with automated processing. The user's workload is thereby considerably reduced, and more time for other tasks becomes available. [1]



## 2 Method Development

### 2.1 Chemicals

- Water: Ultra-pure water produced by Milli-Q Integral
- Methanol (for SPE): for analysis, Merck
- Methanol (for LC-MS): ULC/MS Biosolve
- Sodium thiosulfate pentahydrate, p.a., Merck
- Formic acid 99 % ULC/MS Biosolve
- Acrylamide, 4 x cryst.  $\geq 99.9\%$

### 2.2 Sample material

Prepare a 1 L solution: 1 mL of a methanolic acrylamide standard (1  $\mu\text{g}$  / mL) plus 1 mL of an aqueous sodium thiosulfate / pentahydrate solution (100 mg / mL) made up to 1 L using distilled water. Of this solution use 100 mL as sample (n=6).

### 2.3 Solid phase extraction (manual)

SPE - Steps	CHROMABOND® Carbon A, 6 mL, 1000 mg
Conditioning	8 mL methanol 8 mL water
Loading	sample loading at 20 mL/min
Washing	1 mL water
Drying	15 minutes with N <sub>2</sub>
Elution	5 x 2 mL methanol

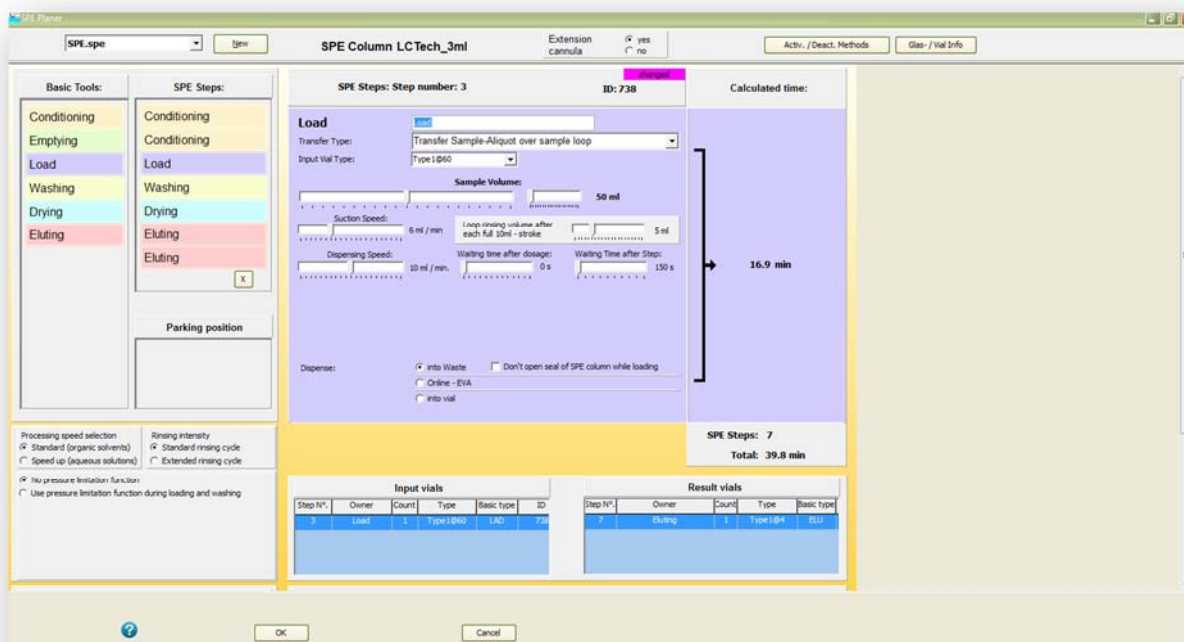
## 2.4 Solid phase extraction (automated by LCTech)

### 2.4.1 FREESTYLE robotic system

The LCTech FREESTYLE robotic system is used for automated sample preparation. This system consists of the basic building block FREESTYLE BASIC, which can be equipped with various modules, depending on the user requirements. Equipped with the SPE module, FREESTYLE processes all methods in the field of solid phase extraction. The solid connection of the SPE column with the robotic arm enables the column to be moved to anywhere on the platform. In addition, this feature also allows a controlled pressure application of up to 4 bars, which is particularly important for applications in which the column may become blocked through suspended particles. All SPE standard column formats (1, 3, 6, 8, 15 mL) or LCTech glass columns (up to 15 mL) can be used for automated processing with FREESTYLE SPE.

### 2.4.2 Processing with FREESTYLE SPE

The manual solid phase extraction can be easily transferred to the FREESTYLE SPE robotic system for automated sample preparation. The easy-to-use software of the system provides a method editor, which makes method development on the system fast and uncomplicated. Drag-and-drop can be used to define the general structure of the method within seconds. By moving bars, parameters such as volume and speed can be specified.



# APPLICATION NOTE | AN0015

The following steps are fully automated processed by FREESTYLE.

SPE steps	Fully automated
Conditioning	8 mL methanol, 10 mL/min.
Conditioning	11 mL water, 10 mL/min.
Loading	50 mL sample, 10 mL/min.
Loading	50 mL sample, 10 mL/min.
Drying	15 min., nitrogen
Elution	10 mL methanol/acetonitrile 1:1, 10 mL/min.
Drying	20 mL air, 20 mL/min

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

**FREESTYLE™**  
AUTOMATISIERTE PROBEVORBEREITUNG

LCTech FreeStyle - Report on Methods: SPE Date: 28.10.2016 Time: 16:10:32

Name: Acrylamid.spe		SPE Column: LCTech_6ml.col	
Extension cannula:	yes		
Processing speed selection:	Standard (organic solvents)		
Rinsing intensity:	Standard rinsing cycle	wo. Extra Cleaning after Load	
Use pressure limitation function during loading and washing:	no		
Step: Konditionieren_MeOH		Basic type: Conditioning Step: - ID: 628	
Volume: 8 ml	Suction Speed: 10 ml/min Repetitions: 0 Waiting Time after Dosage: 10 sec.	Dispensing Speed: 10 ml/min Waiting Time after Step: 5 sec.	Port: 1 MeOH Dispense: Into Waste
Step: Konditionieren_Wasser		Basic type: Conditioning Step: - ID: 629	
Volume: 11 ml	Suction Speed: 10 ml/min Repetitions: 0 Waiting Time after Dosage: 10 sec.	Dispensing Speed: 10 ml/min Waiting Time after Step: 5 sec.	Port: 8 H2O Dispense: Into Waste
Step: Laden		Basic type: Load - Transfer Sample-Allquot over sample loop Step: - ID: 630	
Volume: 50 ml Vial Type: Type1@60 without rinsing of vial	Suction Speed: 10 ml/min Waiting Time after Dosage: 0 sec.	Dispensing Speed: 10 ml/min Waiting Time after Step: 150 sec.	Dispense: Into Waste
Step: Laden		Basic type: Load - Transfer Sample-Allquot over sample loop Step: - ID: 631	
Volume: 50 ml Vial Type: Type1@60 without rinsing of vial	Suction Speed: 10 ml/min Waiting Time after Dosage: 0 sec.	Dispensing Speed: 10 ml/min Waiting Time after Step: 150 sec.	Dispense: Into Waste
Step: Trocknen		Basic type: Drying - Nitrogen drying by defined time Step: - ID: 632	
Drying time with nitrogen 900 sec.		Dispense: Into Waste	
Step: Elution		Basic type: Eluting Step: - ID: 633	
Volume: 2.5 ml	Suction Speed: 10 ml/min Repetitions: 3 Waiting Time after Dosage: 60 sec.	Dispensing Speed: 10 ml/min Waiting Time after Step: 60 sec.	Port: 7 MeOH/ACN 1:1 Dispense: Into vials Number of vials: 1 Vial Type: Type1@16
Step: Trocknen		Basic type: Drying - Drying by defined air volume Step: - ID: 634	
Air volume: 20 ml	Suction Speed: 100 ml/min	Dispensing Speed: 20 ml/min	Dispense: stay on actual position

Method report

## 2.5 Eluent exchange

- The eluate (from manual or automated clean-up) is transferred into a round cuvette (nano reaction vial, OD: 16 mm, 20 pcs., P/N: 91680).
- Rinsing with 1 mL methanol.
- The temperature on the thermoblock (Vario 4; P/N: 919300) is set to 40 °C.
- Sample concentration under a gentle stream of nitrogen to a volume of 1 mL, then transferred and filled to a volume of 1 mL with water.
- The eluate is then injected.

## 2.6 Measurement using LC-MS/MS

The following measurement was performed on a Dionex Ultimate 3200 HPLC system in HPG configuration with an AB Sciex API 3200 MS detector. The following configuration was used:

Column	EC 150/3 NUCLEODUR® C18 Gravity, 3 µm
Eluent A	0.001 % formic acid in water
Eluent B	0.001 % formic acid in methanol
Gradient	10 % B to 100% B (10 min); 100% B to 10% B (2 min); 10% B (hold 5 min)
Flow rate	0.25 mL/min
Injection volume	10 µL
Column temperature	60 °C
Detection	MS/MS, AB Sciex API 3200 ion source: turbo spray (ESI) scan type: MRM polarity: positive curtain gas: 15 psig ion spray voltage: 5000 V temperature: 650 °C gas 1 (nebuliser): 60 psig gas 2 (turbo gas): 50 psig CAD gas: 6 psig

## 2.7 Configuration FREESTYLE

1.	FREESTYLE BASIC	P/N	12663-12
2.	FREESTYLE SPE	P/N	12668
3.	Rack for solvent supply	P/N	13156
5.	Column adapter for 6 mL SPE columns with jam strips	P/N	14613
6.	Caps for 6 mL columns (reusable)	P/N	14881
7.	Rack for up to 18 SPE columns	P/N	13946
8.	Tray for 16 mL vials	P/N	11933
9.	Frame for trays (100 mm)	P/N	11915
10.	Tray for 60 mL flasks	P/N	12106
11.	Frame for trays (120 mm)	P/N	12103
12.	Adapter for SPE columns (reusable)	P/N	13382
13.	Screw thread bottle (16 mL)	P/N	V0016
14.	Screw thread bottle (60 mL)	P/N	F060
15.	Sealing Cap (60 mL)	P/N	V0024-SL
16.	Seals for screw thread bottles (60 mL)	P/N	V0025-D

## 2.8 Consumables by MACHEREY-NAGEL

1.	EC 150/3 NUCLEODUR® C18 Gravity, 3 µm	P/N	760083.30
2.	CHROMABOND® Carbon A PP-columns, 6 mL, 1000 mg	P/N	730167

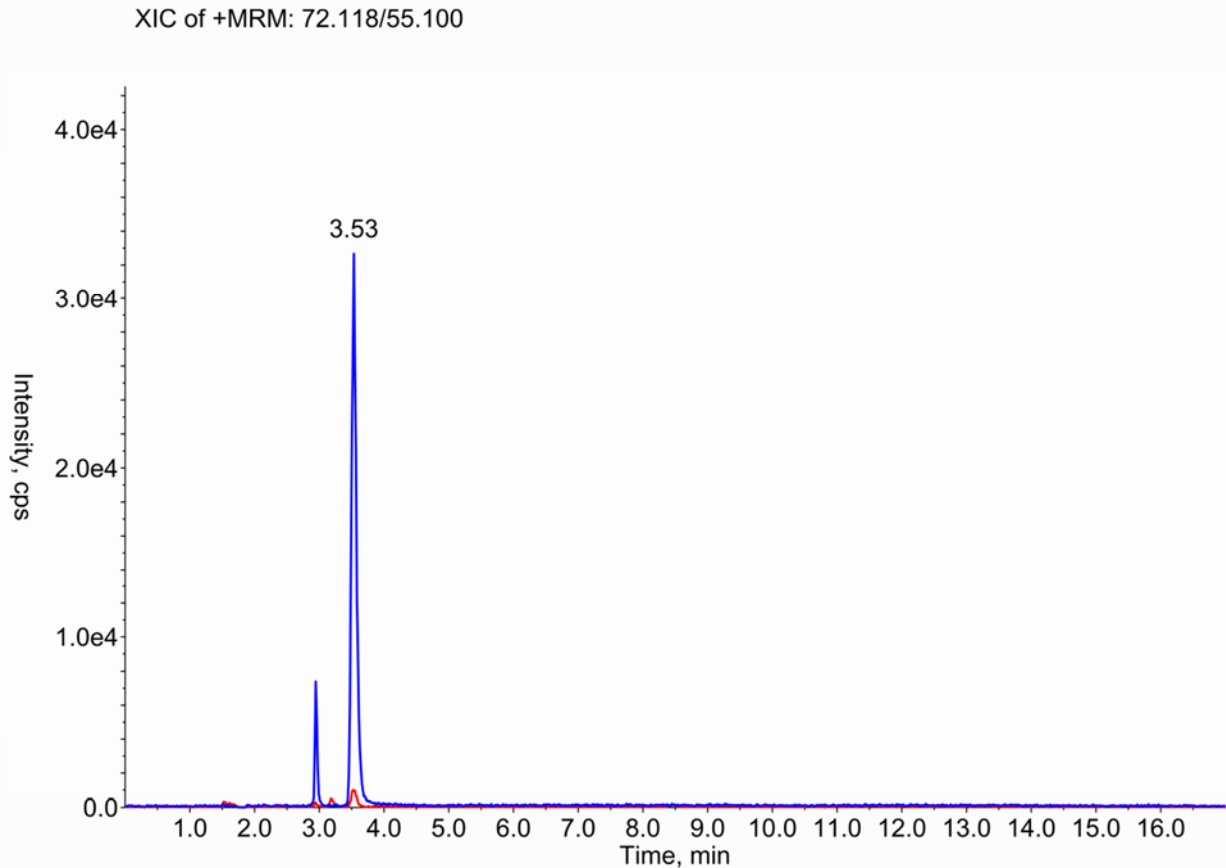


## 3 Results

### 3.1 Recovery rates (automated with FREESTYLE SPE)

Analyte (n=6)	Mean Value (%)	RSD (%)
Acrylamide	87	4

### 3.2 Chromatogram



### Summary

The FREESTYLE SPE determines acrylamide in water samples reliably and in norm-conformity to DIN 38413-6 by means of fully automated SPE with high recoveries as well as high reproducibility.

Unattended, the system processes samples 24 hours a day, 7 days a week.

## 4 Sources

[1] Validation document for DIN 38413-6, determination of acrylamide processes via high performance-liquid chromatography and mass spectrometric detection (HPLC-MS/MS)

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