MACHEREY-NAGEL
Chromatography application note

MN application note 07/2017

Determination of acrylamide in coffee

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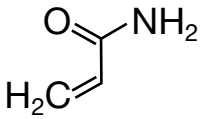
Abstract

This application note describes the determination of acrylamide in coffee using SPE for sample clean up. The eluates from SPE cleaning are finally analyzed by HPLC-MS/MS.

Introduction

The investigation of acrylamide in foodstuffs is frequently carried out within the context of general food control [1]. Recommendations on guideline values of acrylamide in foodstuffs have been published by the EU Commission and are taken into account during investigations [2]. Sample preparation methods have been established which allow efficient depletion of the matrix components from the coffee extracts. Clean up methods with classical SPE columns as well as dispersive SPE (QuEChERS) are used in coffee analysis.

In the following, several clean up procedures for the determination of acrylamide from coffee are compared. The depletion of the matrix is shown and the advantages of the respective clean up methods are discussed. The identification and the quantification of acrylamide in coffee were finally carried out by ESI mass spectrometry on NUCLEODUR® C_{18} Gravity column.



acrylamide

Figure 1: Compound of interest.

Sample pretreatment for solid phase extraction

- Add 2 mL of hexane to 2 g of coffee sample
- Shake
- Add 100 µL of an aqueous D_3-acrylamide solution (β = 1 µg/mL)
- Add 100 µL of an aqueous acrylamide solution (β = 1 µg/mL) for determining recovery rate
- Add 20 mL water and shake
- Ultrasonic extraction for 15 min at 40 °C
- Centrifuge the mixture at 4500 rpm, for 5 min at 4 °C
- Transfer the supernatant into a vial
- Add 1 mL of Carrez I solution and shake the mixture
- Add 1 mL of Carrez II solution and shake the mixture again
- Centrifuge the mixture at 4500 rpm, for 5 min at 4 °C
- Transfer the supernatant into a vial
- Add 2 mL water to the extracted coffee sample and shake it
- Centrifuge the mixture at 4500 rpm, for 5 min at 4 °C
- Combine the supernatants

Clean up with solid phase extraction

Column: CHROMABOND® ABC_{18}, 6 mL, 500 mg, (REF 730533)

Conditioning: 1 x 10 mL methanol, 1 x 10 mL water

Sample application: Sample was aspirated with low vacuum

Drying: 1 min with vacuum

Fill up sample extract with water to 20 mL for HPLC-MS

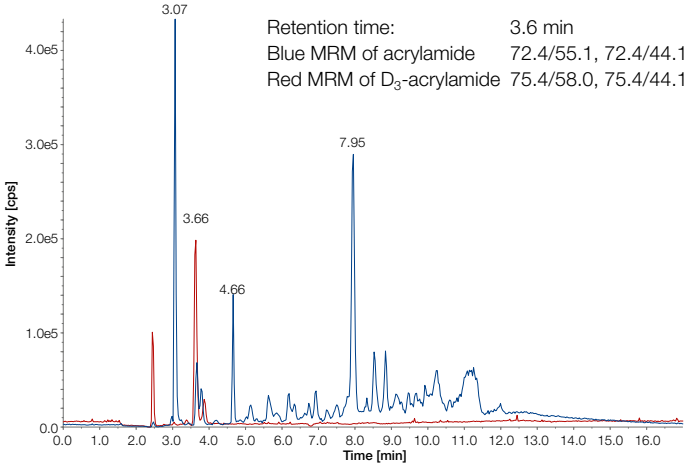


Figure 2: Chromatograms of HPLC-MS/MS analysis using a SPE column CHROMABOND® ABC_{18}, 6 mL, 500 mg for sample clean up.

Column: CHROMABOND® ABC_{18}, 6 mL, 1000 mg

Conditioning: 1 x 10 mL methanol, 1 x 10 mL water

Sample application: Sample was aspirated with low vacuum

Drying: 1 min with vacuum

Fill up sample extract with water to 20 mL for HPLC-MS

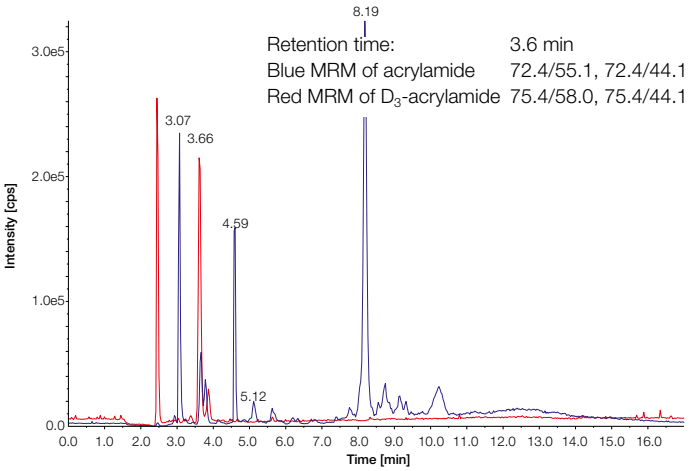


Figure 3: Chromatograms of HPLC-MS/MS analysis using a SPE column CHROMABOND® ABC_{18}, 6 mL, 1000 mg for sample clean up.

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Column: CHROMABOND® C_{18} ec, 6 mL, 500 mg, (REF 730014)
CHROMABOND® HR-XC, 6 mL, 500 mg, (REF 730955)

Conditioning: 1 x 10 mL methanol, 1 x 10 mL water

Sample application: Sample was aspirated with low vacuum

Drying: 1 min with vacuum

Fill up sample extract with water to 20 mL for HPLC-MS

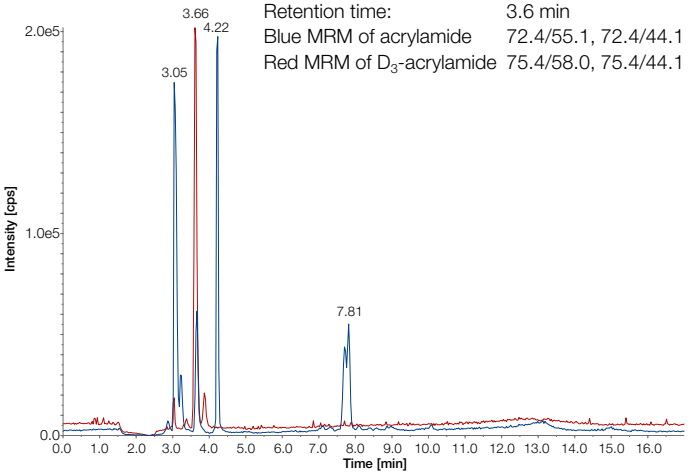

Figure 4: Chromatograms of HPLC-MS/MS analysis using a spe column CHROMABOND® C_{18} ec, 6 mL, 500 mg and CHROMABOND® HR-XC, 6 mL, 500 mg for sample clean up.

Sample pretreatment for dispersive solid phase extraction:

- Weigh out 1 g coffee sample into a 50 mL centrifuge tube
- Add 50 µL of an aqueous D_3-acrylamide solution (β = 1 µg/mL)
- Add 50 µL of an aqueous acrylamide solution (β = 1 µg/mL) for determining recovery rate
- Add 5 mL hexane and shake
- Add 10 mL water and shake
- Add 10 mL acetonitrile and shake
- Add QuEChERS extraction mix I (4 g $MgSO_4$, 1 g NaCl, 0.5 g NaH citrate • 1.5 H_2O, 1 g Na_3 citrate • 2 H_2O), (REF 730970)
- Shake vigorously for 1 min and cool down the mixture in an ice bath
- Centrifuge the mixture at 4500 rpm, for 5 min at 4 °C
- Transfer the acetonitrile supernatant into a vial

Cleanup for the combination of QuEChERS extraction mix I and QuEChERS cleaning mix XX (dispersive)

- Put 6 mL acetonitrile supernatant in a 15 mL centrifuge tube
- Add QuEChERS cleaning mix XX (1.20 g $MgSO_4$, 0.40 g CHROMABOND® Diamino), (REF 730658)
- Shake vigorously for 1 min and cool down the mixture in an ice bath
- Centrifuge the mixture at 4500 rpm, for 5 min at 4 °C
- Transfer the acetonitrile supernatant into a vial
- Dilute the extract 1:10 with water and filter through a syringe filter (CHROMAFIL® Xtra PTFE-20/13 pore size 0.2 µm, REF 729208)

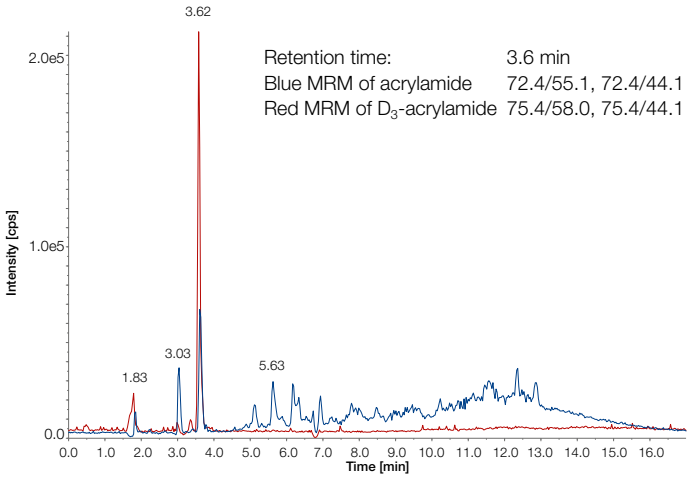

Figure 5: Chromatograms of HPLC-MS/MS analysis using QuEChERS extraction mix I and QuEChERS cleaning mix XX (dispersive) for sample clean up.

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Clean up for the combination of QuEChERS extraction mix I and Chromabond® Diamino (SPE in a cartridge)

Sample application: Aspirate slowly 6 mL of acetonitrile extract through SPE column (volume 3 mL, 500 mg) with low vacuum

Drying: 1 min with vacuum

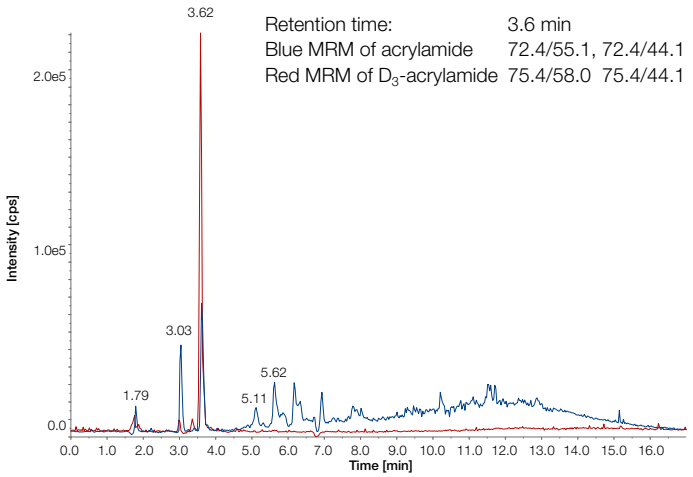

Figure 6: Chromatograms of HPLC-MS/MS analysis using QuEChERS extraction mix I and CHROMABOND® Diamino (6 mL, 500 mg cartridge) for sample clean up.

Sample application: Aspirate slowly 6 mL of acetonitrile extract through SPE column (volume 6 mL, 1000 mg) with low vacuum

Drying: 1 min with vacuum

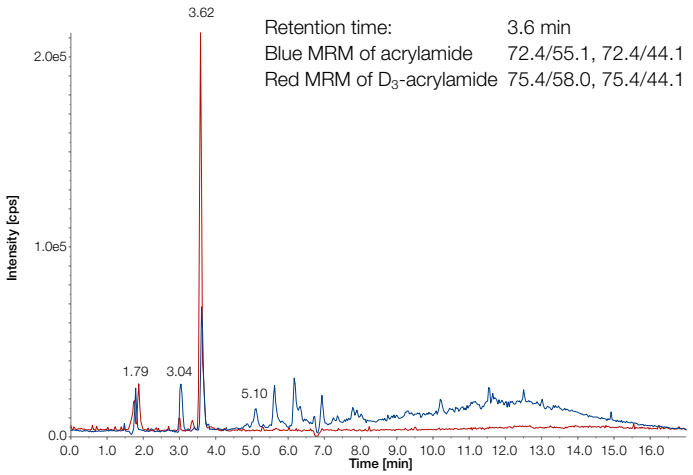

Figure 7: Chromatograms of HPLC-MS/MS analysis using QuEChERS extraction mix I and Chromabond® Diamino (6 mL, 1000 mg cartridge) for sample clean up.

Cleanup for the combination QuEChERS extraction mix I and QuEChERS cleaning mix XX (SPE in a cartridge)

Sample application: Aspirate slowly 6 mL of acetonitrile extract through SPE column (volume 6 mL) with low vacuum

Drying: 1 min with vacuum

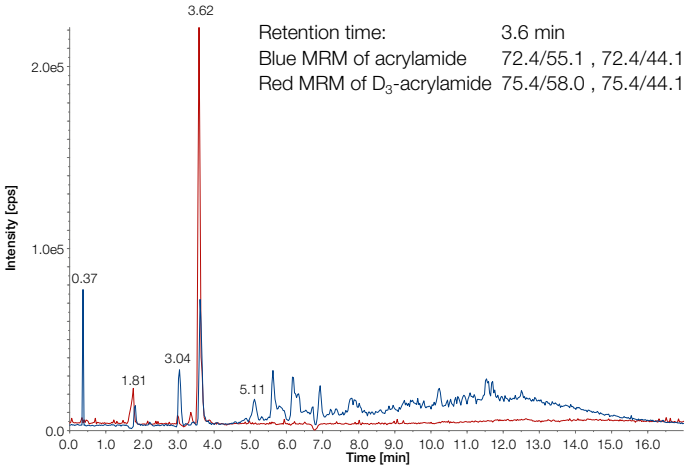

Figure 8: Chromatograms of HPLC-MS/MS analysis using QuEChERS extraction mix I and QuEChERS cleaning mix XX (SPE in a cartridge) for sample clean up.

Comparison UV spectra of purified coffee extracts

Comparison of extracts with clean up performed with octadecyl silica with an ion exchange function

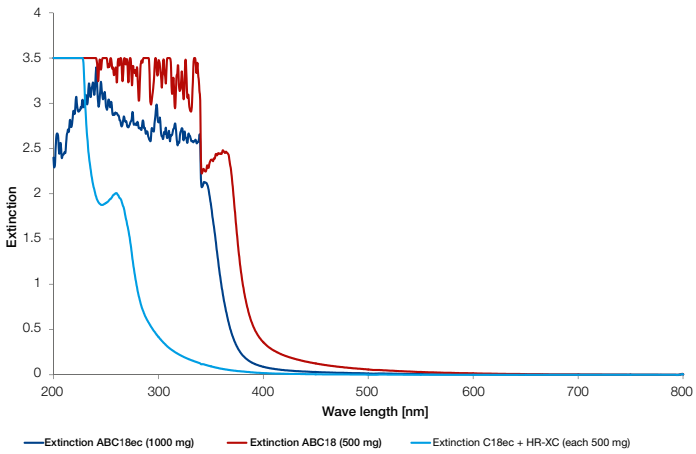

Figure 9: UV spectra, comparison of extracts with clean up performed with octadecyl silica with an ion exchange function.

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Comparison of extracts with clean up performed with CHROMABOND® Diamino

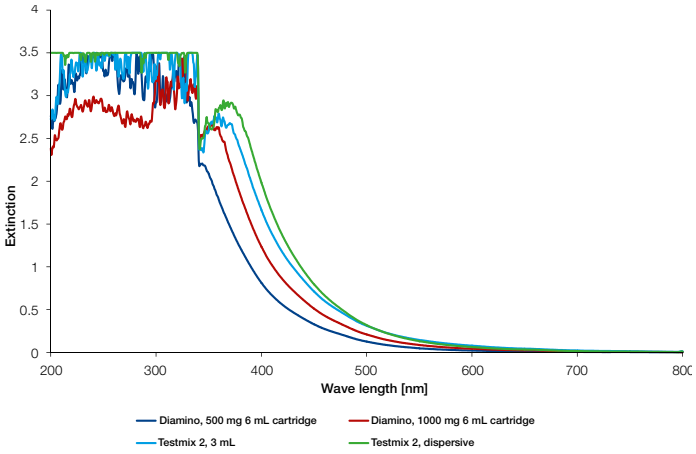

Figure 10: UV spectra, comparison of extracts with clean up performed with CHROMABOND® Diamino.

Comparison of extracts with clean up performed with different amounts of CHROMABOND® Diamino

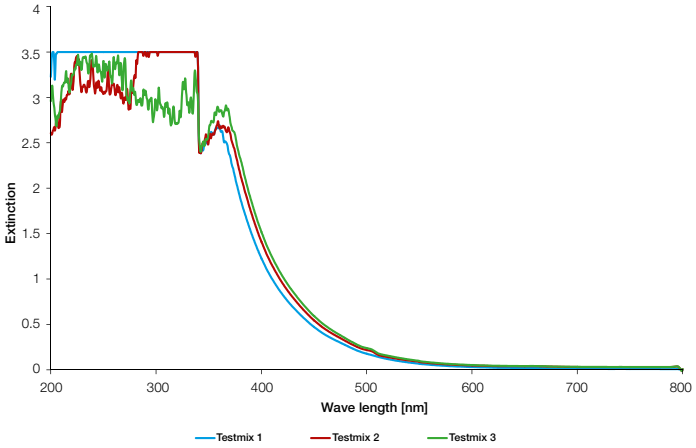

Figure 11: UV spectra, comparison of extracts with clean up performed with CHROMABOND® Diamino and different amounts of $MgSO_4$:
Testmix 1: $MgSO_4$ / CHROMABOND® Diamino, 4/1
Testmix 2: $MgSO_4$ / CHROMABOND® Diamino, 3/1
Testmix 3: $MgSO_4$ / CHROMABOND® Diamino, 2/1

Subsequent analysis: HPLC-MS / MS

Chromatographic conditions:

Column: EC 150/3 NUCLEODUR® C_{18} Gravity, 3 µm, (REF 760083.30)

Eluent A: 0.001 % formic acid in water

Eluent B: 0.001 % formic acid in methanol

Gradient: 10 % B in 10 min to 100 % B, back to 10 % B in 2 min, hold for 5 min

Flow rate: 0.25 mL/min

Temperature: 60 °C

Injection: 10 µL

MS conditions:

API 5500, ion source ESI, positive ionization mode, scan type MRM

Curtain gas 20 psig, ion spray voltage 5000 V, temperature 650 °C, nebulizer gas 60 psig, turbo gas 50 psig, CAD medium

MRM transitions

Analyte	$[M+H]^+$	Q_1 (Quantifier)	Q_2 (Qualifier)
Acrylamide	72.4	54.9	43.9

Table 1: MRM transitions for the analysis acrylamide.

Recovery rates

CHROMABOND Phase	Volume	Adsorbent weight	Recovery rate in %
CHROMABOND®ABC$_{18}$	6 mL	500 mg	97 ± 4
CHROMABOND®ABC$_{18}$	6 mL	1000 mg	99 ± 1
CHROMABOND® C $_{18}$ec / CHROMABOND® HR-XC	6 mL / 6 mL	500 mg / 500 mg	90 ± 0
CHROMABOND® Diamino	6 ml	500 mg	85 ± 0
CHROMABOND® Diamino	6 ml	1000 mg	85 ± 1

Dispersive solid phase extraction (first step)	Dispersive solid phase extraction (second step)	Recovery rate in %
extraction mix I	extraction mix XX performed in a polypropylene column (3 mL)	83 ± 2
extraction mix I	extraction mix XX	79 ± 5

Table 2: Comparison of recovery rates for presented clean up procedures.

Conclusion

The results show that the determination of acrylamide from coffee and especially the matrix depletion could be carried out successfully with all the tested products. By using classical SPE cartridges with octadecyl silica with an ion exchange function it was possible to recover more than 90 % of acrylamide. However, chromatograms of extracts of these phases contain more relevant matrix signals than the chromatograms of extracts which have been worked up with CHROMABOND® Diamino.

The UV spectra of purified sample extracts also show that a higher depletion of the sample matrix is possible by using a higher sorbent quantity or by using the combination of CHROMABOND® C_{18} ec and CHROMABOND® HR-XC.

The depletion of coffee matrix with the cleaning QuEChERS mix XX performed in a cartridge format as well as by dispersive SPE was very successful. These clean ups lead to good recoveries of approx. 80 %. The resulting chromatograms showed few relevant interfering signals, so that the integration of the acrylamide signals is simply possible. Further experiments with different diamino contents in the cleaning mix showed the diamino content is decisive for matrix depletion in coffee, the higher the better for the same recovery rates. CHROMABOND® C_{18} ec as an additional component in the cleaning mix did not lead to any improvement.

With regard to the recovery rates, to the analysis time, and to the depletion of relevant interfering signals of the different methods, the dispersive SPE has significant advantages over classical SPE.

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References

1. Determination of acrylamide in coffee and coffee products, § 64 L 46.00, 5.
2. COMMISSION RECOMMENDATION of 10.1.2011 for the investigation of the acrylamide content of foodstuffs.

Additional information

The following applications regarding “Determination of acrylamide in coffee” and further applications can be found on our online application database at *www.mn-net.com/apps*:

SPE:	MN Appl. No. 305572
SPE:	MN Appl. No. 306520
SPE (QuEChERS):	MN Appl. No. 306530
HPLC:	MN Appl. No. 127530

Product information

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

REF 729208, CHROMAFIL® Xtra PTFE-20/13
REF 760083.30, EC 150/3 NUCLEODUR® C_{18} Gravity, 3 µm
REF 730533, CHROMABOND® ABC_{18}, 6 mL, 500 mg
REF 730014, CHROMABOND® C_{18} ec, 6 mL, 500 mg
REF 730955, CHROMABOND® HR-XC, 6 mL, 500 mg
REF 730970, CHROMABOND® QuEChERS citrate extraction Mix I
REF 730658, CHROMABOND® QuEChERS Diamino clean up Mix XX
REF 730972 CHROMABOND® QuEChERS Mix III Diamino clean up Mix
REF 730562 CHROMABOND® Diamino, 6 mL, 500 mg
REF 730939 CHROMABOND® HR-X, 6 mL, 500 mg
REF 730223 CHROMABOND® centrifuge tubes with screw cap, 50 mL
REF 702293 Screw neck vials N 9, 1.5 mL
REF 702107 N 9 PP Screw cap, yellow, center hole, silicone white / PTFE red