Determination of acrylamide in coffee and coffee products using SPE clean-up and HPLC-MS/MS (ISO 18862:2016)

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Application benefits

- Successful determination of acrylamide in coffee and coffee products according to ISO 18862:2016
- High recovery rates and an effective SPE clean-up were achieved with CHROMABOND® ABC18, a special octadecyl modified silica phase with ion exchange functionality
- Fast and sensitive HPLC analysis on a NUCLEODUR® C18 Gravity-SB column

MN products

REF 730533
CHROMABOND® ABC18, 6 mL, 500 mg

REF 730223
CHROMABOND polypropylene (PP) centrifuge tubes, 50 mL, screw cap, empty

REF 760606.20
EC 100/2 NUCLEODUR® C18 Gravity-SB, 3 µm

REF 702293
Screw neck vials N 9, 1.5 mL

REF 702107
N 9 PP Screw cap, yellow, center hole, silicone white / PTFE red

MN application numbers

SPE: 306750
HPLC: 128980

Keywords

Acrylamide, coffee, SPE, LC-MS/MS, ISO 18862:2016

Introduction

It is well known that in 2002, for the first time, Swedish scientists succeeded in proving that acrylamide was formed as a by-product of the browning reaction (Maillard reaction) when some starchy foods such as French fries, crisps and crispbread had been heated. When carbohydrate rich foods contain the amino acid asparagine, larger quantities of acrylamide are formed at temperatures of over 120 °C (see Figure 1). Even more acrylamide is formed at 170–180 °C.

At that time, the dangers of acrylamide were still unknown. Therefore, the precautionary principle was applied for the contaminant acrylamide to minimize the risks for human health. The plan was to reduce the acrylamide concentrations gradually in Germany and Europe. Some years later, a reliable risk assessment on the potential dangers to human health was published in 2015 [1]. It described and confirmed the carcinogenic effect of the substance.

The European Commission adopted a Regulation establishing mitigation measures and benchmark levels for the reduction of the presence of acrylamide in food on November 20th, 2017 [Regulation (EU) 2017/2158] [2]. This regulation has directly been applied in all Member States since April 11th, 2018. As a result, the food industry had to optimize their procedures to minimize acrylamide levels in foodstuffs according to the described indicative values. At present, there are many methods for the determination of acrylamide in coffee, which recommend a special SPE sample clean-up [3].

This application note describes the determination of acrylamide in coffee using CHROMABOND® ABC18 columns for sample clean-up. The identification and the quantification of acrylamide in coffee were finally carried out by ESI mass spectrometry on NUCLEODUR® C18 Gravity-SB column.

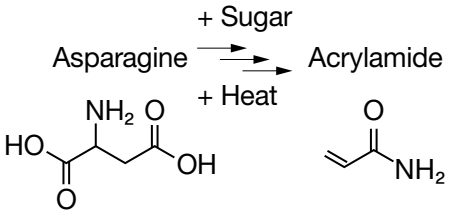

Figure 1: Formation of acrylamide from asparagine.

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Sample pretreatment

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Extraction

1. Weigh 2.0 g homogenized sample into an empty 50 mL centrifuge tube (REF 730223)
2. Add 2.0 mL hexane and shake the mixture
3. Add 0.1 mL of internal standard solution (10 µg/mL acrylamid-D3 in methanol) and 0.1 mL of standard solution (for matrix-matched calibration)
4. Shake the mixture
5. Add 20 mL water
6. Shake the mixture for 1 min
7. Put the mixture for 15 min into an ultrasonic bath at 40 °C
8. Centrifuge the mixture for 15 min at 4500 rpm at 4 °C
9. Transfer 10 mL of aqueous layer to an empty 50 mL centrifuge tube

Clarification of extract

1. Add 1 mL of each clearing reagents* and shake the mixture
2. Wait 5 min and centrifuge 5 min at 4500 rpm at 4 °C
3. Transfer supernatant layer into 20 mL flask
4. Wash residue with 3 mL water and centrifuge again
5. Transfer supernatant layer into the same flask
6. Wash residue with 3 mL water and centrifuge again
7. Transfer supernatant layer into the same flask
8. Fill up the flask up with water to a volume of 20 mL

* Clearing reagents:
Carrez I: Solve 10.6 g potassium ferrocyanide (II) trihydrate in 100 mL of water
Carrez II: Solve 21.9 g zinc acetate dihydrate in 100 mL of water

Solid phase extraction clean-up

1. Column: CHROMABOND® ABC18, 6 mL 500 mg (REF 730533)
2. Conditioning: 5 mL methanol, 5 mL water
3. Sample application: 5 mL of clarified extract (collect sample solution)
4. Washing step 1: 3 mL water (collect wash solution)
5. Drying step: 1 min with vacuum
6. Pool collected sample solution and collected wash solution and fill up with water to a volume of 20 mL.

Analysis by HPLC-MS / MS

MN Appl. No. 128980

Chromatographic conditions

Column	EC 100/2 NUCLEODUR® C18 Gravity-SB, 3 µm (REF 760606.20)
Eluent A	0.1 % formic acid in water
Eluent B	acetonitrile
Gradient	hold 0 % B for 2 min, in 3 min to 100 % B, hold 100 % B for 2 min, in 2 min to 0 % B, hold 0 % B for 5 min
Flow rate	0.25 mL/min
Temperature	30 °C
Injection volume	10 µL
MS conditions	Shimadzu LCMS 8050
Acquisition mode	SRM
Interface	ESI
Polarity	positive
Interface voltage	4 kV
Interface temperature	300 °C
DL temperature	200 °C
Nebulizing gas flow	2.5 L/min
Heating gas flow	10 L/min
Heat block	400 °C
Drying gas flow	10 L/min
CID gas	230 kPa

MRM transitions

Analyte	Q1 Mass (Da)	Q3 Mass (Da) qualifier	Q3 Mass (Da) quantifier	Retention time (min)
Acrylamide	72.10	44.20	55.00	2.32
Acrylamide-D3	75.10	44.15	58.05	2.30

Table 1: MRM transitions and retention times of acrylamide and acrylamide-D3.

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Matrix-matched calibration

Figure 2 a

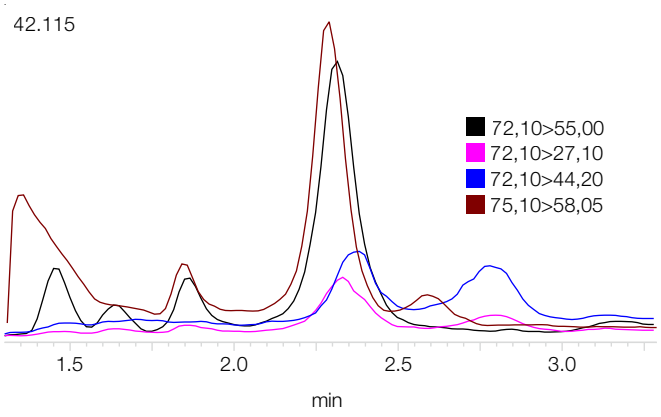

Figure 2 b

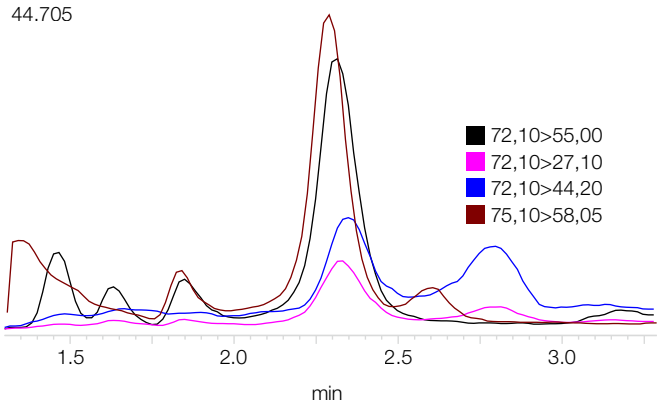

Figure 2 c

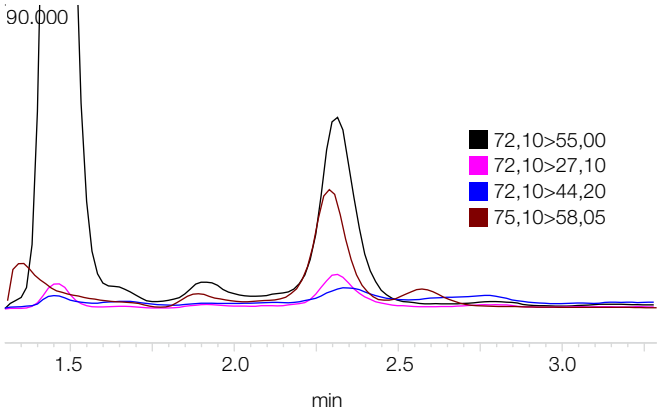

Figure 2 a-c: Chromatogram of samples: a = coffee, b= espresso, c = cereal coffee.

Figure 3 a

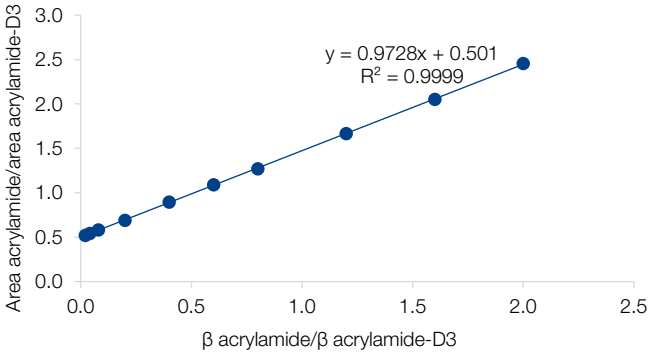

Figure 3 b

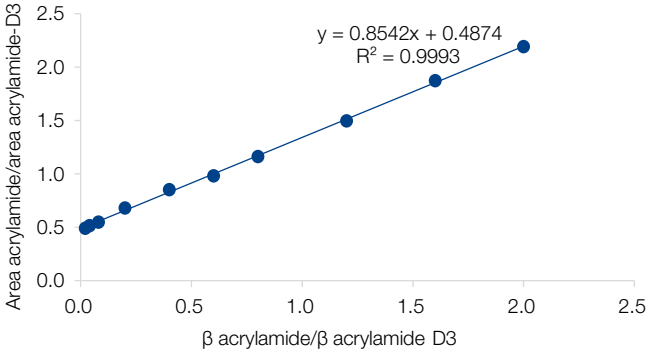

Figure 3 c

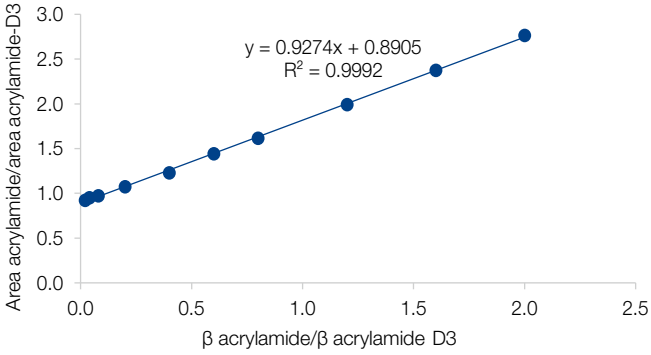

Figure 3 a–c: Matrix-matched calibration was carried out in a range from 10 ng/g up to 1000 ng/g for each sample matrix: a = coffee, b = espresso, c = cereal coffee.

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Recovery rates

Standard addition level of acrylamide	Recovery rate of roasted coffee (%)	Recovery rate of espresso coffee (%)	Recovery rate of cereal coffee (%)
50 ng/g sample	109	86	66
300 ng/g sample	100	101	102
600 ng/g sample	100	100	100

Table 2: Recovery rates for the presented SPE clean-up using CHROMABOND® ABC18 columns.

Conclusion

This application note presents the reliable and successful determination of acrylamide from coffee and coffee products according to regulative recommendations. By using CHROMABOND® ABC18 SPE columns, it was possible to achieve high recovery rates for acrylamide from three matrices with good reproducibility.

The used SPE phase, a special octadecyl (C18, RP18, ODS) silica phase with ion exchange (IEX) function shows effective matrix reduction with ionic and hydrophobic interaction mechanisms.

The work shows that the indicative values for coffee and coffee products could be verified with good recovery rates by the presented method. The identification and the quantification of acrylamide in coffee were finally carried out by ESI mass spectrometry on NUCLEODUR® C18 Gravity-SB column.

References

[1] Scientific Opinion on acrylamide in food, EFSA Panel on Contaminants in the Food Chain (CONTAM), European Food Safety Authority (EFSA), Parma, Italy, EFSA Journal 2015;13(6):4104.

[2] Regulation (EU) 2017/2158 — mitigation measures and benchmark levels for the reduction of the presence of acrylamide in food.

[3] Determination of acrylamide in coffee and coffee products, § 64 L 46.00, 5.

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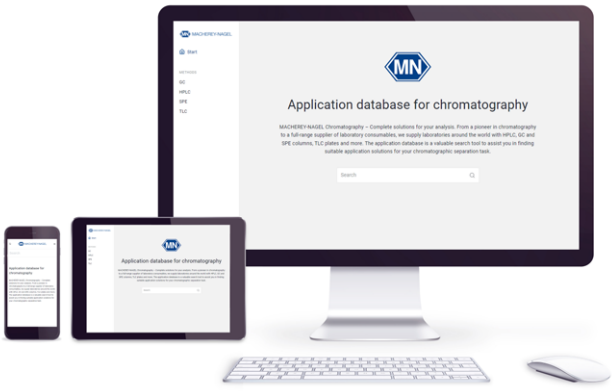

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