APPNOTE

GERSTEL
MAKING LABS WORK

MACHEREY-NAGEL

GERSTEL AppNote 238

Automated Cleanup and LC-MS/MS Determination of Acrylamide in Ground Coffee and Cereal Coffee

Oliver Lerch[1], Marco Wolff[1], Hans Rainer Wollseifen[2], Torsten Kretschmer[2], Christian Falk[2]

[1]GERSTEL GmbH & Co. KG, Eberhard-Gerstel-Platz 1, 45473 Mülheim an der Ruhr, Germany
[2]MACHEREY-NAGEL GmbH & Co. KG, Valencienner Straße 11, 52355 Düren, Germany

Keywords

Acrylamide, Coffee, Cereal Coffee, SPE, LC-MS/MS, Lab Automation

Abstract

Acrylamide is a thermal process contaminant formed in foodstuff during heating at temperatures exceeding 120 °C. It is detected especially in French fries, potato chips, bread, and crispbread, as well as in coffee and coffee surrogates. The International Agency for Research on Cancer classifies it as probable human carcinogen and the EU has established indicative values for different foodstuffs.

This AppNote presents a partially automated analysis method based on DIN EN ISO 18862 for the determination of acrylamide in ground coffee and cereal coffee. Following the initial manual extraction steps, the final SPE cleanup was performed automatically by the GERSTEL MultiPurpose Sampler (MPS) using CHROMABOND ABC18 cartridges from MACHEREY-NAGEL. Subsequently the clean extracts were analyzed by LC-MS/MS.

The applicability of the method has been proven for three different coffee samples: Filter coffee, espresso, and cereal coffee. Automation of the complete workflow by the MPS and available modules is feasible but was not pursued within the scope of this work.

Introduction

Acrylamide (AA) is a thermal process contaminant formed in foodstuff during heating at temperatures exceeding 120 °C [1,2]. Its appearance was first reported by Tareke et al. in 2002 [3]. Acrylamide is classified in group 2A as a probable human carcinogen by the International Agency for Research on Cancer (IARC) [4] and high-level exposure is known to cause damage to the nervous system [1]. Extensive research has been undertaken to elucidate formation pathways in different foodstuffs and under differing processing conditions. The formation is dependent on pH, water content and foodstuff ingredients. Currently, the decarboxylation and deamination of the amino acid asparagine in the presence of reducing sugars – a first stage of the Maillard reaction – is considered the most important pathway of formation. Additionally, asparagine can be decarboxylated enzymatically and form acrylamide via the intermediate 3-aminopropionamide. Acrylamide can also be formed from other amino acids such as ß-alanine and aspartic acid via the intermediate acrylic acid. A further way of formation is the reaction between glycerol and fatty acids via acrolein as an intermediate. Figure 1 summarizes the most important pathways of formation [1,2].

APPNOTE

GERSTEL
MAKING LABS WORK
MACHEREY-NAGEL

GERSTEL AppNote 238

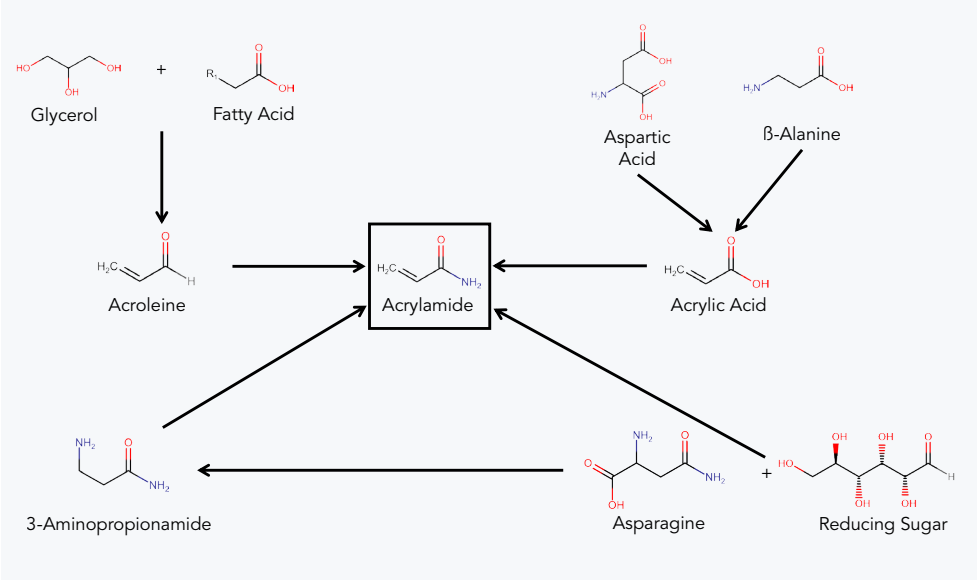

Figure 1: Formation pathways for acrylamide in foodstuff, adapted from [1]. The most relevant precursor is the amino acid asparagine.

High acrylamide levels of hundreds of µg/kg are found in French fries, potato chips, bread, and crispbread, as well as in coffee and coffee surrogates [5]. Although there is conflicting evidence about its potential harmfulness, the high level of concern is due to the presence in such foodstuffs that are widely consumed in large quantities. The European Food Safety Authority (EFSA) estimates the daily acrylamide intake between 0.4 and 1.9 µg/kg body weight. The European Union (EU) has issued recommendations for different foodstuffs. An indicative value of 450 µg/kg for roast coffee and 2000 or 4000 µg/kg for coffee substitutes, cereal based or others, was set [6].

Since the formation of acrylamide during food processing cannot be prevented completely, the main efforts are directed towards mitigation and reduction strategies, which again lead to strong demand for rugged and efficient analysis methods for different food classes. Among the various food groups, coffee is the one, for which limiting the formation of acrylamide is the most difficult. The contaminant is formed in the early stage of coffee bean roasting in very high amounts (some mg/kg). Fortunately, it is reduced by evaporation and further chemical reactions during the progress of roasting. Technical possibilities for reduction before or during roasting are limited. One idea is to develop coffee plants with low asparagine containing beans. With the European Union regulation, coffee producers are obliged to produce products with the lowest possible acrylamide levels and to demonstrate their commitment to reducing acrylamide levels.

Between 2010 and 2015, EFSA collected analysis data on acrylamide in coffee. An average of 522 µg/kg was found for ground coffee and 1499 for coffee substitutes [5]. This translates to beverage concentrations between 2 and 25 µg/L in filter coffee, 11 to 59 µg/L in espresso, 33 to 55 µg/L in espresso from capsules and 25 to 59 µg/L in decaffeinated espresso [1]. Generally, coffee prepared with Robusta beans contains approximately double the amount of acrylamide than coffee prepared only with Arabica species. Since acrylamide is a highly polar compound, mainly LC-MS/MS is employed for its determination in food matrices. In the early 2000s GC-MS methods were developed that either used a polar separation column or relied on derivatization, e.g. bromination [1,2,7]. As early as 2012, GERSTEL published AppNote [8] for the determination of acrylamide in brewed coffee based on automated SPE-LC-MS/MS.

When analyzing ground coffee and cereal coffee, efficient extraction of the matrix and subsequent matrix removal are necessary. The compound is usually extracted with aqueous solutions, followed by further cleanup with Carrez solutions (for deproteination) and solid-phase extraction. Non-polar solvents can further be used for degreasing. Such a workflow is described in DIN EN ISO 18862:2019-12 [9], a method which was accepted into the German "Amtliche Sammlung von Untersuchungsverfahren gemäß § 64 Lebensmittel- und Futtermittelgesetzbuch" as method BVL L 46.00-5.

In this AppNote, we describe an automated SPE cleanup method

according to DIN EN ISO 18862 combined with LC-MS/MS analysis. Initial extraction and cleanup steps were conducted manually, nevertheless their automation with GERSTEL equipment is feasible. All application work was conducted in the MACHEREY-NAGEL application laboratory in Düren, Germany, in close cooperation with GERSTEL.

Experimental

Materials and Solvents

All solvents and other reagents used were of analytical grade. Carrez I solution was prepared at a level of 10.6 g potassium ferrocyanide (II) trihydrate in 100 mL water; Carrez II solution at 21.9 g zinc acetate dihydrate in 100 mL water. Neat acrylamide (AA) and acrylamide-2,3,3 d_3 (AA-d_3) were purchased from LGC Standards (Wesel, Germany). From these, single stock (1000 µg/mL) and working solutions (10 µg/mL) in water were prepared. Coffee samples were purchased in a local supermarket.

Preparation of Samples

The following sample preparation steps were performed manually preceding the SPE-LC-MS/MS analysis:

- Weigh a 2 g sample into a 50 mL tube
- Add 2 mL n-hexane and shake
- Add 0.1 mL of internal standard working solution and a defined volume of analyte working solution (if needed) and mix briefly
- Add 20 mL of water and shake for 1 min
- Ultrasonicate for 15 min at 40 °C
- Centrifuge for 15 min at 4 °C and 4500 rpm
- Transfer 10 mL of aqueous supernatant into a 50 mL tube
- Add 1 mL each of Carrez I and II solution for protein precipitation and shake
- Wait for 5 min
- Centrifuge for 5 min at 4 °C and 4500 rpm
- Transfer the supernatant layer to a 20 mL volumetric flask
- Wash residue with 3 mL water and centrifuge again
- Transfer the supernatant layer into the same flask
- Wash residue with 3 mL water and centrifuge again
- Transfer the supernatant layer into the same flask
- Fill up the flask with water to a volume of 20 mL

For SPE cleanup a CHROMABOND® ABC18, 6 mL, 500 mg (MACHEREY-NAGEL, Düren, Germany, REF 730533MPS) was used. The cartridge was equipped for automated handling with the MultiPurpose Sampler (MPS, GERSTEL, Mülheim an der Ruhr, Germany), see figure 2.

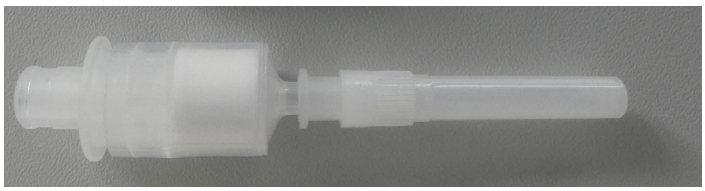

Figure 2: CHROMABOND® ABC18, 6 mL, 500 mg SPE cartridge (MACHEREY-NAGEL) equipped for automated handling with the GERSTEL MPS.

The following SPE cleanup was performed automatically by the MPS:

- Condition SPE cartridge with 5 mL methanol at 200 µL/s
- Condition SPE cartridge with 5 mL water at 200 µL/s
- Add 5 mL of clarified extract to the SPE cartridge at 100 µL/s and collect the eluate
- Wash the SPE cartridge with 3 mL water at 200 µL/s and collect the eluate
- Purge 30 s with a flow of nitrogen and collect the residual eluate
- Fill up the pooled eluate to a volume of 10 mL
- Inject an aliquot into the LC-MS/MS system

Instrumentation

An MPS fitted with SPE module, different trays for cartridges and vials, solvent reservoirs and a wash station was used for this application. Figure 3 shows the core part of the system – the SPE tray and SPE station. Conditioning and washing solvents and samples were transferred from solvent reservoirs to the SPE cartridge by a 2.5 mL autosampler syringe. An LC-MS/MS was used for determination of acrylamide in the prepared extracts.

GERSTEL
MAKING LABS WORK
MACHEREY-NAGEL

GERSTEL AppNote 238

Figure 3: SPE module and cartridge tray mounted on a MultiPurpose Sampler (MPS). All solvents are delivered by an automatically exchangeable syringe in the autosampler head.

Analysis conditions

A 10 µL aliquot of the extract was injected into the analysis system. Separation was performed on a NUCLEODUR® C18 Gravity-SB column, 100 mm length, 2 mm ID, 3 µm particle size (MACHEREY-NAGEL, REF 760606.20) with a gradient of 0.1% formic acid in water (eluent A) and acetonitrile (eluent B) and a flow rate of 0.25 mL/min at 30 °C. The gradient was as follows: Hold 0% B for 2 min, in 3 min to 100% B, hold for 2 min, in 2 min to 0% B, hold for 5 min. Detection was performed by a triple quadrupole mass spectrometer using the method parameters listed in table 1.

Table 1: Analytes with retention times, precursor- and product ions, and ion polarity in LC-MS/MS analysis.

Analyte:	Acrylamide	Acrylamide-d_3
Retention Time:	2.32 min	2.30 min
Precursor Ion:	72.10 Da	75.10 Da
Product Ion Quantifier:	55.00 Da	58.05 Da
Product Ion Qualifier:	44,2 Da	-
Product Ion Qualifier:	27.1 Da	-
Polarity:	Positive	Positive

Results and Discussion

Details of the extraction protocol for coffee samples were taken from DIN EN ISO 18862. SPE cartridges were conditioned, loaded, and washed according to the manufacturer´s recommendations. The MPS controls the flow by positive displacement, which is highly accurate leading to a highly repeatable workflow. The cleanup effect of the SPE cartridge it obvious from figure 4, resulting in adequate chromatograms and long maintenance intervals for both the separation column and the mass spectrometer.

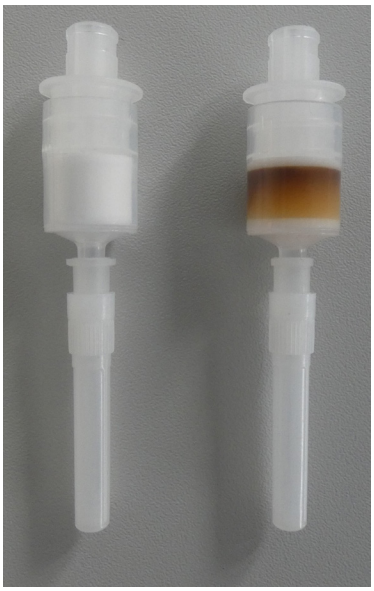

Figure 4: SPE cartridge before (left) and after (right) cleanup of a coffee extract.

GERSTEL AppNote 238

Three different coffee samples, namely ground filter coffee, ground espresso and cereal coffee, were examined. Standard addition calibrations were established by spiking coffee samples with different volumes of the standard working solution before sample preparation and analysis. The resulting calibration lines can be seen in figure 5a to c.

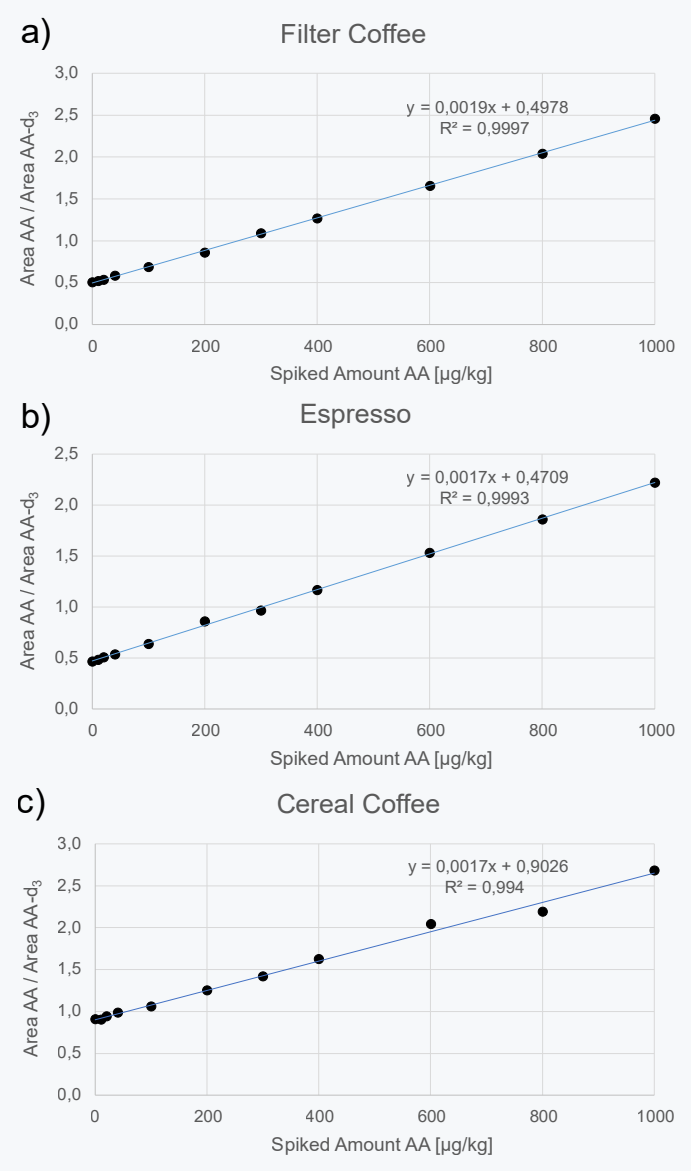

Figure 5: Standard addition calibration curves in the range of 10 to 1000 µg/kg added amount of acrylamide for a) filter coffee b) espresso and c) cereal coffee.

All correlation coefficients were larger than 0.999, except for cereal coffee which was 0.994. To verify method performance, samples of each coffee were spiked each three times at three different concentrations. Resulting concentrations were calculated and compared to the actual spiked amounts (see table 2). The trueness achieved was excellent - near 100% - for all filter coffee and espresso samples while for the two lower levels of the cereal coffee it was slightly less than perfect at 59 and 81% respectively.

Table 2: Trueness of analysis values for three different coffee samples at three different spiking levels, n=3.

Spiked Amount [µg/kg]	Filter Coffee, trueness [%]	Espresso, trueness [%]	Cereal Coffee, trueness [%]
50	104	99	59
300	100	102	81
600	100	98	95

No interfering matrix peaks or other chromatographic or mass spectral issues could be observed, and the sample preparation method worked ruggedly. Figures 6a-6c show representative chromatograms of all three samples at a spike level of 300 µg/kg.

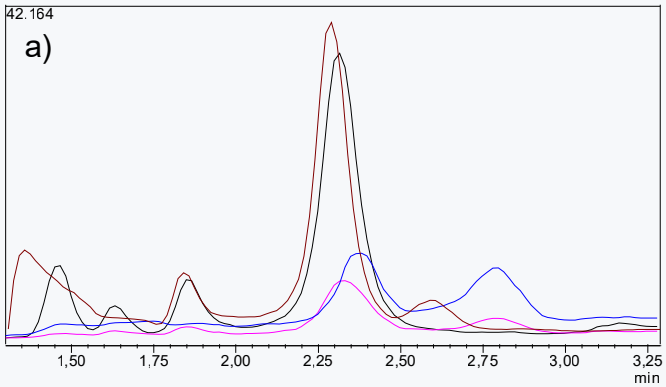

Figure 6: Chromatograms of a) filter coffee extract, b) espresso extract, and c) cereal coffee extract all spiked at 300 µg/kg. Shown are the quantifier traces 72.1 -> 55.0 (black) and two qualifier traces 72.1 -> 44.2 (blue) and 72.1 -> 27.1 (pink) for the native acrylamide and the quantifier trace 75,1 -> 58.05 (brown) for the internal standard acrylamide-d^3.

Conclusions

In this work, we have shown that the SPE cleanup step for the determination of acrylamide in coffee and cereal coffee can be automated conveniently by the GERSTEL MPS equipped with an SPE module using CHROMABOND® ABC18 cartridges from MACHEREY-NAGEL. The collected validation data show that the method works accurately and ruggedly with the slight exception of cereal coffee at very low concentrations. However, the concentrations of 50 and 300 µg/kg are not really relevant since the EU indicative value for cereal coffee is at 2000 µg/kg.

Although not used in this study, the MPS platform offers all necessary modules, such as, for example, a Vortex shaker, a centrifuge and an ultrasonic bath for comprehensive automation of the described DIN EN ISO 18862 workflow, which would render the analysis even more efficient. Samples would then just need to be weighed into a vial, and the vial sealed and placed on the MPS for automated sample preparation and analysis.

References

[1] C.M.D. Soares, R.C. Alves, M.B.P.P. Oliveira, *Acrylamide in Coffee: Influence of Processing, Chapter 69 in Processing and Impact on Active Components in Food*, Academic Press/Elsevier (**2015**), 575

[2] T. Kocadağlı, V. Gökmen, *Formation of acrylamide in coffee*, Current Opinion in Food Science 45 (**2022**) 100842

[3] E. Tareke, P. Rydberg, P. Karlsson, S. Eriksson, M. Törnqvist, *Analysis of acrylamide, a carcinogen formed in heated foodstuffs*, Journal of Agricultural and Food Chemistry 50 (**2002**) 4998

[4] https://monographs.iarc.who.int/list-of-classifications (acessed June 12th **2022**)

[5] European Food Safety Authority (EFSA), *Scientific Opinion on acrylamide in food*, EFSA Panel on Contaminants in the Food Chain (CONTAM), EFSA Journal 13 (**2015**) 4104

[6] COMMISSION RECOMMENDATION 2013/647/EU of 8 November 2013 on investigations into the levels of acrylamide in food, Official Journal of the European Union (**2013**) L 301/15

[7] M. Biedermann, S. Biedermann-Breml, A. Noti, K. Grob, P. Egli, H. Mändli, *Two GC-MS Methods for the Analysis of Acrylamide in Foods*, Mitteilungen aus Lebensmitteluntersuchung und Hygiene, 93 (**2002**) 638

[8] F.D. Foster, J.R. Stuff, E.A. Pfannkoch, *Automated Solid Phase Extraction (SPE)-LC-MS/MS Method for the Determination of Acrylamide in Brewed Coffee Samples*, GERSTEL Application Note No. 13/**2012**, www.gerstel.com

[9] DIN EN ISO 18862:2019-12, Coffee and coffee products - Determination of acrylamide - Methods using HPLC-MS/MS and GC-MS after derivatization