

QUANTITATIVE DETERMINATION OF ACRYLAMIDE IN FOOD ON THE EXAMPLE OF COFFEE USING 2D-LC-ESI-MS/MS

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Introduction

Acrylamide (2-propenamide) was first detected in 2002 by a Swedish working group in various starch-containing, thermally processed foods like French fries or potato crisps [1]. Legal limits for the content of acrylamide in foodstuffs have not yet been set at both national and European levels. However, there is a recommendation from the European Commission of 08.11.2013 defining signal values for ten food groups [2]. In 2018, benchmark values for the content of acrylamide in various foods were then set in accordance with Regulation (EU) 2017/2158 [3]. A comprehensive opinion on acrylamide in foodstuffs, which discusses in particular the mutagenic and carcinogenic effects of acrylamide, was published by the Federal Institute for Risk Assessment (BfR) on 29.06.2011 [4]. The procedure presented here describes a method approach for the extraction as well as the measurement by 2D-LC-ESI-MS/MS of acrylamide in different food groups, here on the example of coffee. One goal was the development of a simple and uniform sample preparation procedure, which could be applied to all food matrices. Furthermore a powerful 2D-LC-ESI-MS/MS method was established in order to achieve a maximum of chromatographic resolution as well as a minimum of ionic suppression and thus a lower limit of quantification.

Method

Sample Preparation

- weigh 1.0 g homogenized sample into 30-mL centrifuge tube
- add 50 µL internal standard (acrylamide-d3) and wait 2 minutes
- add approx. 2 g glass beads
- add 4.0 mL water and wait for 1 min
- add 8.0 mL acetonitrile and shake for 1 min
- add 4 mL *n*-heptane with the help of a dispensett
- shaking for 10 min on horizontal shaker
- add ca. 5 g of QuEChERS-salt-mixture (MgSO₄/NaCl, 4/1, w/w), shake for 60 sec
- immediately shaking by hand
- ultrasonication for 5 min at 55 - 60 °C
- immediately shaking by hand
- centrifuge at 4,000 x g for 3 min
- transfer of ca. 1.5 mL middle phase (acetonitrile) into a Eppendorf cap for dSPE
- add 100 mg PSA, 150 mg water-free MgSO₄ and shake for 30 sek
- centrifuge at 10,000 g for 20 sek (benchtop centrifuge)
- transfer 1 mL of cleaned acetonitril phase into HPLC-vial
→ LC-ESI-MS/MS measuring

Schematic 2D LC Approach

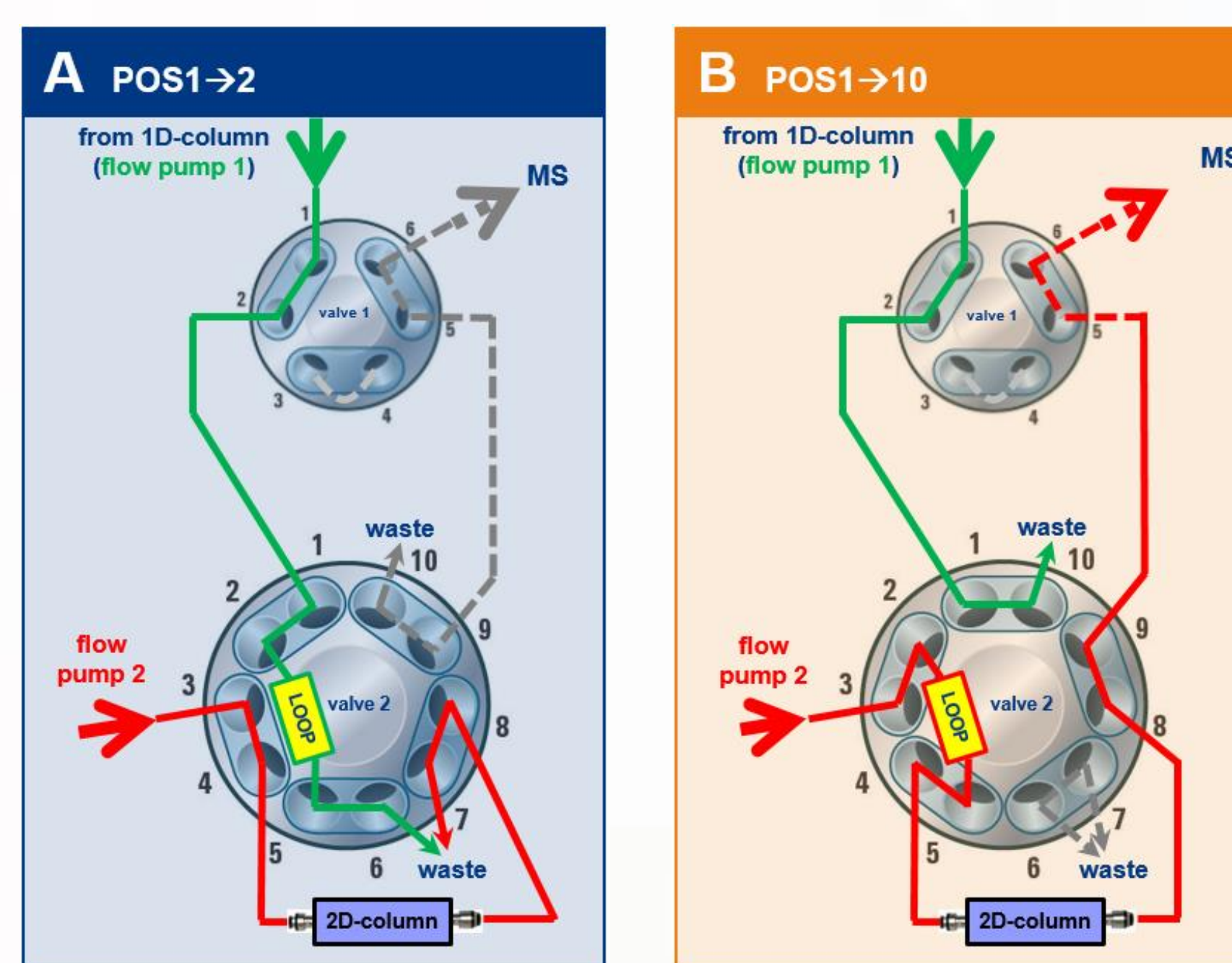


Figure 1:
Schematic illustration of valve positions for 2D-LC-method.

A: The flow runs from the 1D column (green) to valve 1 which is in position 1→2 for the entire duration of the method. The flow runs from here to valve 2 and there into position 1. From here it runs via position 2 into the sample loop (yellow), which is connected to position 5. After the acrylamide has eluted from the 1D column (retention time approx. 4.1 min) and then entered the loop, valve 2 switches to position 1→10 at this precise moment.

B: After switching valve 2 to position 1→10, the flow from pump 2 (red) is routed via the loop so that the content is transported to the 2D column here. Due to a different chemical selectivity of the 2D column, further impurities co-eluted with the first column are separated. The acrylamide eluted then after a total time of approx. 7.8 minutes.

LC Conditions

LC-System	Agilent® 1200 or 1290 UHPLC	Flow col. 1	400 µL/min
Column 1D-LC	RP-column with aromatic selectivity	Flow col. 2	600 µL/min
Column 2D-LC	RP-column with polary modified C18 material	Eluent A	H ₂ O + 0.1% FA
Pre-Column	Phenomenex® Security Guard Ultra C18	Eluent B	ACN + 0.1% FA
		Col. Oven	40 °C
		Post-Split	waste/source (1/1, v/v)
Duration	11 min, total	Injection vol.	8 µL

ESI-MS/MS Conditions

MS-System	Agilent® Ultivo QqQ
Ion Source	AJS ESI (positive mode), capillary voltage 2000 V, gas temp. 120 °C, gas flow 12 L/min, nebulizer 45 psi, sheath gas temp. 240 °C, sheath gas flow 6 L/min, nebulizer voltage 400 V
Precursor Ions	[M+H] ⁺ adducts
Product Ions	Acrylamide: 72/55 and 72/44; D3-Acrylamide: 75/58 and 75/44

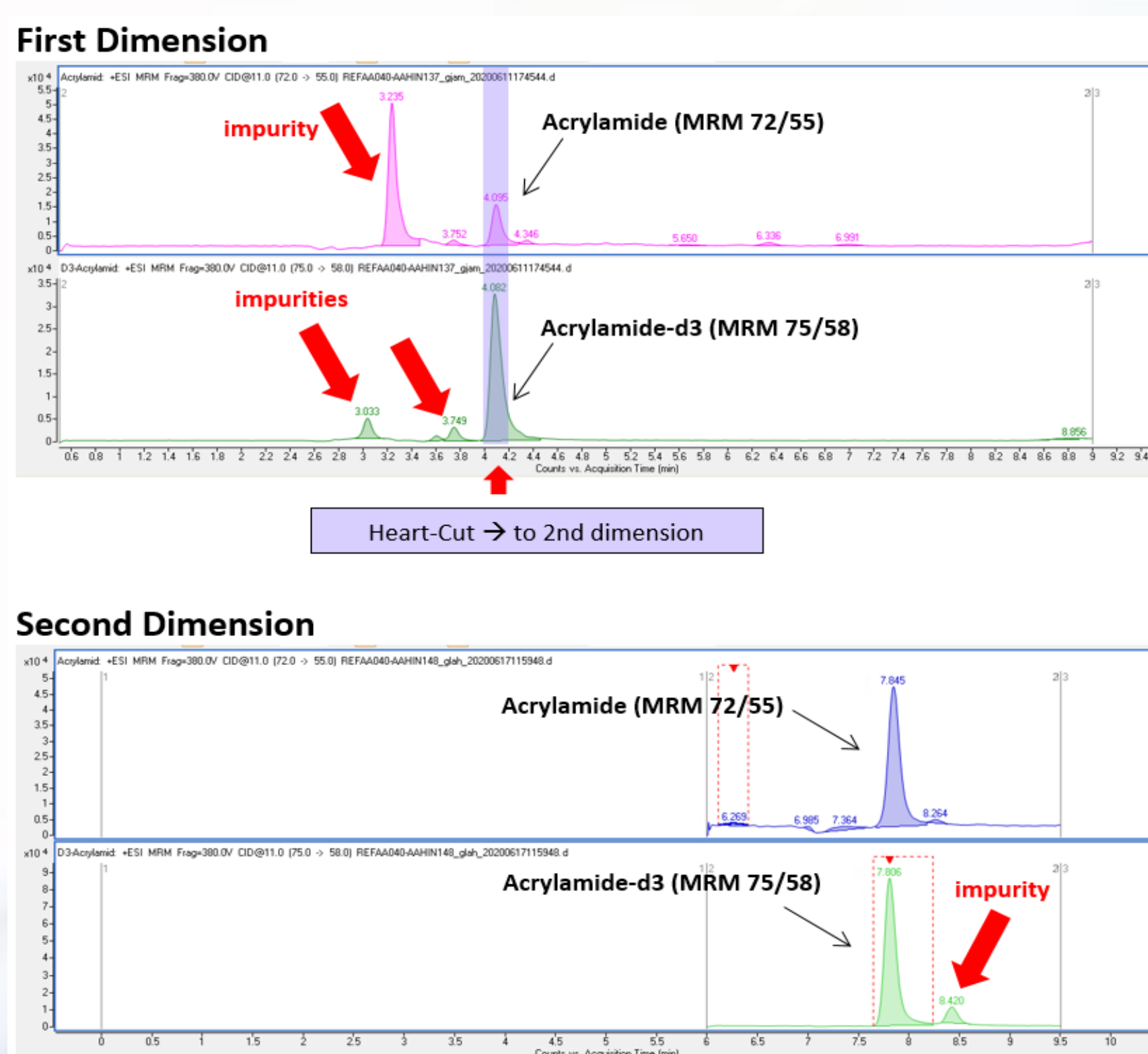


Figure 2:
Chromatograms of 1D- chromatogram (above) and 2D-chromatogram (below) of instant coffee extract. The upper trace is the MRM transition to acrylamide (*m/z* 72/55), the lower trace (green) represents the MRM transition to the internal standard of acrylamide-d3 (*m/z* 75/58).

In the 1D chromatogram, acrylamide and the internal standard elutes at approx. 4.1 minutes. It is easy to see that interfering substances (red arrows) elute with the same mass transitions (or are split off from the matrix due to in-source fragmentation) at earlier points in time. The 1D LC flow in the violet hatched area from 4.0 to 4.2 min (corresponds to 80 µL) is briefly stored using a 2D loop (see figure 1 in yellow) and then transported to the 2D column by switching the 2D valve. Acrylamide elutes here at approx. 7.8 min. The additional separation mechanism already mentioned under figure 1 takes place here. In the example shown, a matrix-typical contamination can be separated for instant coffee on the trace of the internal standard at approx. 8.4 min (red arrow).

Results of Validation

Validation Data

	Roasted Coffee	Instant Coffee
repeatability, coefficient of variation [%]	2.8	8.3
reproducibility, coefficient of variation [%]	5.4	6.1
LOQ [µg/kg]	30	30
linearity (r ²)	>0.995	>0.995

Trueness (proficiency test results 2021)

Matrix	proficiency test provide	proficiency test no.	month/year	assigned value [µg/kg]	range [µg/kg]	measured value [µg/kg]	z-score
instant coffee	FAPAS	30107	JAN 2021	561	365.2 – 756.8	510	-0.5
roasted coffee	BVL	621	JUL 2021	149.5	106.9 – 192.1	155	0.3
roasted coffee	DRRR	2010392, 210846, 1	NOV 2021	240.0	188.3 – 291.6	270	1.2
instant coffee	DRRR	2010392, 210846, 2	NOV 2021	465	281.9 – 684.4	550	0.9
instant coffee	FAPAS	30117	DEC 2021	603	395 – 811	570	-0.3
roasted coffee	Bipea	004-0294	DEC 2021	55.1	27.5 – 82.7	47	-0.6

Conclusion

The presented method is suitable for the quantitative determination of acrylamide in roasted and instant coffee but also for matrices like potato crisps, biscuits, infant food or cornflakes and shows good results for repeatability, reproducibility, linearity and trueness. The LOQs were in the range of 30 µg/kg for both coffee types. All proficiency tests in 2021 for roast and instant coffee were passed, with z-scores ranging from -0.6 to 1.2. The method presented here shows a simple and inexpensive sample processing and purification. The subsequent two-dimensional chromatography has a high chromatographic resolution and efficient separation performance with a short running time at the same time. The trueness and precision can be rated as very good. With 15,000 to 18,000 samples per year, the robustness is also documented.

References

- Tareke, E. *et al.*: Analysis of Acrylamide, a Carcinogen Formed in Heated Foodstuffs; J Agric Food Chem, 50 (17), 2002.
- European Commission; Commission Recommendation on investigations into the levels of acrylamide in food, (2013/647/EU), 08.11.2013.
- European Commission; Commission Regulation (EU) 2017/2158 laying down mitigation measures and benchmark values for the reduction of acrylamide levels in food", 11.04.2018.
- Opinion no. 043/2011 of the Federal Institute for Risk Assessment (BfR) from 29.06.2011.

