QUANTITATIVE DETERMINATION OF ACRYLAMIDE IN FOOD USING 1D- AND 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]. Furthermore, the European Commission is planning new benchmark values for acrylamide for 2018. 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 [3]. The procedure presented here describes the development and validation of a method approach for the extraction of acrylamide from different food groups as well as its measurement by 1D- and 2D-LC-ESI-MS/MS. The method is a further development of the method published in 2004 by Hoenicke *et al*. [4]. One goal was the development of a simple and uniform sample preparation procedure, which could be applied to all food matrices. Furthermore, in addition to a simple measurement using 1D-LC-ESI-MS/MS, a powerful 2D method was established in order to achieve a minimum of ionic suppression and thus a lower limit of quantification (5 instead of 30 µg/kg).

Method

Sample Preparation

1. weigh 1.2 g homogenized sample into 30-mL centrifuge tube
2. add 50 µL internal standard (c(D_3-acrylamide) = 30 mg/L) and wait 2 minutes
3. add ca. 2 g glass beads
4. add 6 mL acetonitrile and shake for 1 min (protein precipitation)
5. add 6 mL water
6. add 6 mL *n*-heptane with dispensett
7. shaking for 5 min on horizontal shaker
8. ultrasonication for 5 min at 55 - 60 °C
9. add ca. 3 g of QuEChERS-salt-mixture (MgSO4/NaCl, 4/1, w/w) and shake for 60 sec
10. centrifuge at 4000 x g for 3 min
11. transfer of ca. 1.5 mL middle phase (acetonitrile) into a HPLC-Vial

→ LC-ESI-MS/MS measuring

LC Conditions

LC-System	Agilent® 1200 or 1290 UHPLC
Column 1D-LC	RP-column with aromatic selectivity
Column 2D-LC	RP-column with polar modification
Pre-Column	Phenomenex® Sec. Guard Ultra C18
Column Oven	40 °C
Inj. Vol.	6/12 µL (1D/2D-LC)
Eluent A	H_2O + 0.1% FA
Eluent B	acetonitrile + 0.1% FA
Flow	400 µL/min (binary)
Post-Split	waste/source (50/50, v/v)
Duration	6/8 min (1D/2D-LC)

ESI-MS/MS Conditions

MS-System	Sciex® API4000/5500 QQQ or Agilent 6495b QQQ
Ion Source	ESI (positive mode), Voltage: 5500 V, Temp: 400 °C, CUR: 30 psi, CAD: medium, Gas 1: 60 psi, Gas 2: 40 psi
Precursor Ions	$[M+H]^+$ adducts
Product Ions	Acrylamide: 72/55, 72/44 and 72/27; D3-Acrylamide: 75/58, 75/44 and 75/30

Column Oven Valve Positions for 1D- and 2D-LC

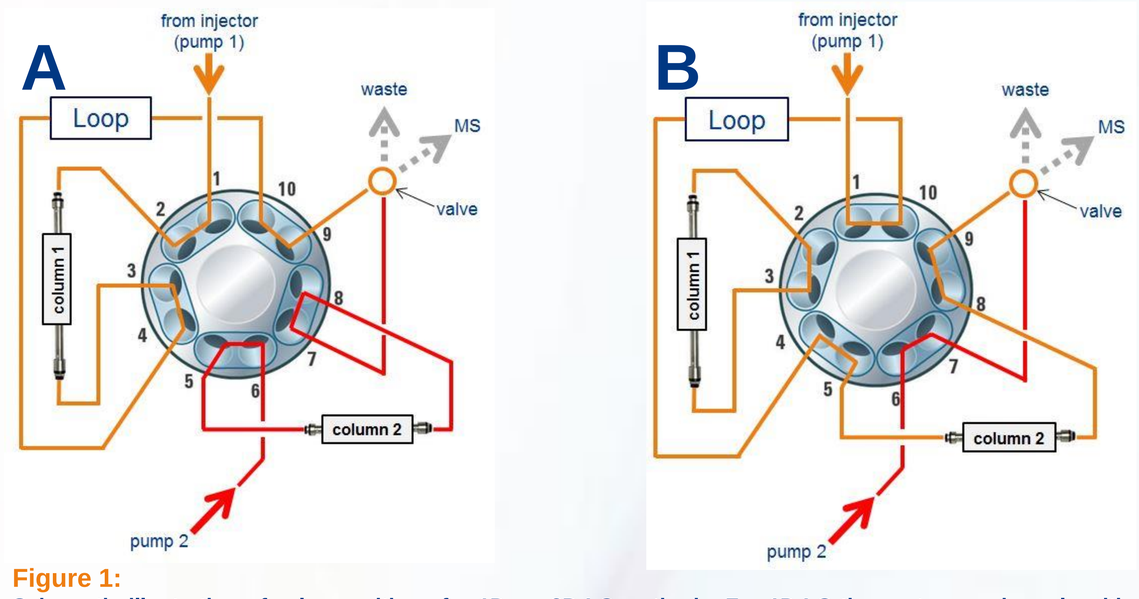

Figure 1:
Schematic illustration of valve positions for 1D- or 2D-LC-methods. For 1D-LC the system works only with orange circuit on column 1 and pump 1 (figure A). For 2D-LC the sample separation starts with position A on column 1. After trapping of the eluting acrylamide peak in a loop, the valve switches to figure B and than back to A for further clean-up on column 2. Literature source for picture of 10-port/2-position valve: [5].

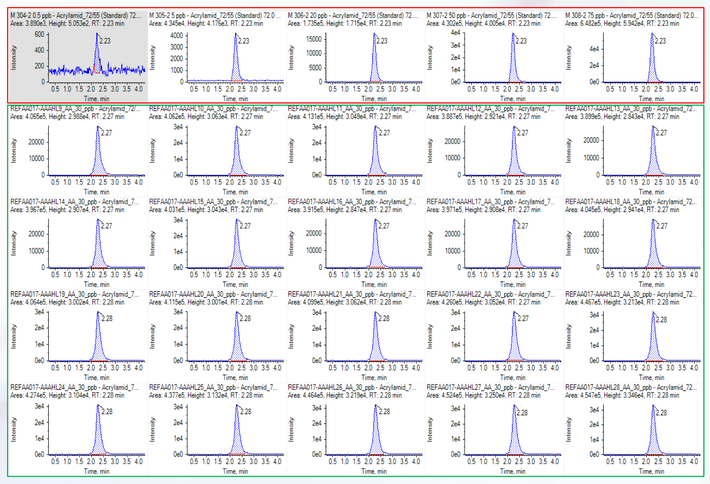

Figure 2:
Chromatograms of 2D-LC-ESI-MS/MS-method. The top five peaks (red frame) shows the MRM-signal of acrylamide in matrix-free standard solutions. The chromatograms below (green frame) shows measurements from extracts of unspiked and spiked instant coffee samples. The additional orthogonal selectivity of the Hypercarb® column leads to an improved separation of interfering matrix components and thus to a better peak shape and signal intensity due to lower ion suppression.

Results of Validation

Repeatability

Matrix	1D-LC-Method C_V (n = 6) [%]	2D-LC-Method C_V (n = 6) [%]
Cookies	2.95	2.67
Instant Coffee	4.53	0.77
Potato Crisps	1.31	2.52
Baby Food	3.02	3.37
Roasted Coffee	2.66	1.78
Cornflakes	2.92	4.45

Accuracy (Proficiency Test Results)

Matrix	FAPAS No.	Month/Year	assigned value X_A [µg/kg]	2D-LC measured value X_M [µg/kg]	1D/2D-LC z-score
Instantcoffee	3061	MAR 2016	700	615.6	0.8
Crisbread	3063	APR 2016	124	120.7	0.1
Potato Crisps	3065	JUN 2016	1270	1103	1.0
Biscuit	3067	SEP 2016	54.3	65.4	-0.8
Roasted Coffee	3069	JAN 2017	255	230	-0.5
Biscuit	3075	SEP 2017	323	300	-0.4

Conclusion

The presented method is suitable for the quantitative determination of acrylamide in various thermally processed food products. The method was validated for the matrices potato crisps, biscuits, instant coffee, roasted coffee, baby food and cornflakes. It shows good results for repeatability, accuracy and linearity. The LOQ´s were in the range of 30 µg/kg (1D-LC-method) or 5 µg/kg (for 2D-LC-method), respectively. All measurements of FAPAS proficiency test material since october 2015 would have been passed with z-scores ≤ 1.5 with the here presented method. Another advantage is that all matrices can be processed identically and in less time than the usual standard method (DIN EN 16618 2015).

References

[1] Tareke, E. *et al*.: Analysis of Acrylamide, a Carcinogen Formed in Heated Foodstuffs; J Agric Food Chem, 50 (17), 2002.

[2] European Commission; Commission Recommendation on investigations into the levels of acrylamide in food, (2013/647/EU), 08.11.2013.

[3] Stellungnahme Nr. 043/2011 des Bundesinstitut für Risikobewertung (BfR) vom 29.06.2011.

[4] Hoenicke, K. *et al*.: Analysis of acrylamide in different foodstuffs using liquid chromatography–tandem mass spectrometry and gas chromatography–tandem mass spectrometry; Anal Chim Acta, 520 (1-2), 2004.

[5] Website from Agilent Technologies, www.agilent.com, 09.10.2017





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