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Applikationsbericht

Determination of Acrylamide in Processed Foods using ACQUITY UPLC I-Class and Xevo TQ-S micro

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Abstract

This application note demonstrates a new LC-MS/MS method, using the ACQUITY UPLC I-Class System, coupled with the Xevo TQ-S micro has been developed for the determination of acrylamide. This method offers a rapid, cost-effective approach for quantifying acrylamide in processed food matrices including potato chips, coffee, bread, and baby food.

Benefits

Rapid cost effective approach for quantifying acrylamide in processed food matrices including potato chips, coffee, bread, and baby food

Introduction

Acrylamide is a highly polar, water soluble compound with many uses in industrial processes and in the

production of textiles. Acrylamide is also a food contaminant which can be formed during food production by high temperature (+120 °C) cooking.[1] The main chemical reaction that causes this is known as the Maillard Reaction.[2] The toxicological properties of acrylamide have been extensively studied and include neurotoxicity, genotoxicity, carcinogenicity, and reproductive toxicity. Acrylamide has been classified as a Group 2A carcinogen by the International Agency for Research on Cancer (IARC).[3]

Since acrylamide is present in a wide range of everyday foodstuffs this health concern applies to all consumers, with children being the most exposed age group on a body weight basis. French fries, potato chips, cookies, and coffee exert the highest contribution to dietary exposure of acrylamide to humans. There have been recent reports of acrylamide levels in excess of 1000 µg/kg in fried potato products.[4]

In June 2015, the European Food Safety Authority (EFSA) published its first full risk assessment of acrylamide in food.[1] Experts from EFSA's Panel on Contaminants in the Food Chain (CONTAM) reconfirmed previous evaluations that acrylamide in food potentially increases the risk of developing cancer for consumers in all age groups.[1]

So far, there are no maximum levels in Europe for acrylamide in food. The food industry has undertaken a lot of work to identify and implement measures to reduce acrylamide levels in food. This includes developing guidance on ways to limit acrylamide formation in a variety of foods and processes. EU regulation 2017/2158, which came into force in April 2018, establishes mitigation measures and benchmark levels for reducing the presence of acrylamide in food.[5] The new legislation will require food business operators to put in place simple, practical steps to manage acrylamide within their food safety management systems.

In this application note, we report the results for an internal validation of a method for the determination of acrylamide in a range of representative foodstuffs, using Waters ACQUITY UPLC I-Class System with the Xevo TQ-S micro Tandem Quadrupole Mass Spectrometer.

Experimental

Sample Preparation and Extraction

Homogenized food samples were extracted using a modified QuEChERS Acrylamide Starter Kit for UPLC-MS/MS, (p/n: 176004417 <https://www.waters.com/nextgen/us/en/shop/standards--reagents/176004417-

acrylamide-starter-kit-lc-ms.html>) method with 1 g of sample taken for the extraction. Isotopically labelled internal standard (Acrylamide d3) was added to all samples prior to extraction in order to correct for any variability during extraction, clean-up and LC-MS/MS analysis.

The supernatant from the modified QuEChERS extracts were subjected to clean-up using Dispersive SPE (dSPE) Tubes which contained 300 mg of primary secondary amine (PSA) sorbent and 900 mg of MgSO4 (Acrylamide Starter Kit for UPLC-MS/MS, p/n: 176004417 <https://www.waters.com/nextgen/us/en/shop/standards--reagents/176004417-acrylamide-starter-kit-lc-ms.html>). Extracts were evaporated to dryness and reconstituted in 0.1% formic acid in LC-MS grade water, to provide a concentration step and solvent exchange into a weaker injection diluent. For more information of the available consumables kits used for this acrylamide application note please visit waters.com/acrylamide. Full sample extraction details are available by request (www.waters.com/acrylamide < https://www.waters.com/waters/form.htm?id=135008043&alias=ALIAS_acrylamide_FOOD&changedCountry=Y&lset=1&local >).

The performance of the method was assessed using the criteria in Commission Regulation (EU) 2017/2158. The trueness and precision of the method were assessed via measurement of spiked samples and FAPAS test materials (potato chips and coffee) used as reference materials. The portions of the representative commodities were spiked at 50 and 200 µg/kg with the exception of the baby foods, which were spiked at 40 µg/kg for the lower spike level. The calibration standards were prepared over the range of 0.5 to 2500 ng/mL in water. Replicate injections (n=15) at two concentration levels were run between bracketed calibration curves to assess the precision and trueness of the LC-MS/MS method.

UPLC conditions

UPLC system:	ACQUITY UPLC I-Class
Column:	ACQUITY UPLC HSS C_{18} SB, 1.8 µm, 2.1 × 100 mm Acrylamide Starter Kit for UPLC MS/MS, (p/n: 176004417)
Column temp.:	30 °C

Sample temp.:	10 °C
Injection volume:	5 µL (partial loop with needle overfill)
Flow rate:	0.2 mL/min
Mobile phase A:	Water with 0.1% formic acid (LCMS grade)
Mobile phase B:	Methanol (LCMS grade)
Gradient:	Full gradient conditions available on request waters.com/acrylamide

MS Conditions

MS system:	Xevo TQ-S micro
MS software:	MassLynx v4.2
Ionization mode:	ESI+
Acquisition mode:	MRM
Capillary voltage:	0.5 kV
Cone voltage:	20 V
Cone gas flow:	50 L/hr
Desolvation temp.:	600 °C
Desolvation gas flow:	1000 L/hr

Source temp.: 150 °C

Compound	MRM translation	Collision energy (eV)	Retention time (min)
Acrylamide	72.05 > 55.10	12	2.69
Acrylamide	72.05 > 44.10	10	–
Acrylamide	72.05 > 27.15	10	–
Acrylamide d3	75.00 > 58.10	15	2.66

*Table 1. MRM transitions.**

**MRM transitions that showed the best selectivity were used. Data were acquired using MassLynx Software (v4.2) and processed using TargetLynx XS. The optimum dwell time was set automatically using the Autodwell function.*

Results and Discussion

Optimization of the LC-MS/MS method was accomplished by evaluating various columns, mobile phase compositions, gradients, and MS transitions. The conditions detailed in the experimental section provide the best overall performance of those tested.

The performance of the method has been assessed using the criteria outlined in Commission Regulation (EU) 2017/2158. Validation of the method demonstrated excellent performance for the determination of acrylamide in a range of representative foodstuffs. The results show that the method is applicable for checking compliance with the benchmark levels for many of the foods specified in the Regulation (EU) 2158/2017: potato chips, bread, coffee, baby biscuits, and baby food (Table 2).

	Chips	Bread	Coffee	Baby food	Baby biscuits
Benchmark level (µg/kg)	750	50	400	40	150
Required LOQ (µg/kg)	50	20	50	20	50
Required LOD (µg/kg)	15	6.0	15	6.0	15

Table 2. Benchmark levels for the presence of acrylamide in foodstuffs and associated required values for LOD and LOQ.

The ACQUITY UPLC HSS C_{18} SB Column provides excellent retention and peak shape for acrylamide. The use of tri-functional bonding provides mechanical robustness to the ligands, allowing for the use of this stationary phase in small particle size column chemistries (sub-2-µm), thus facilitating the benefits of UltraPerformance Liquid Chromatography (UPLC) for this application, such as increased chromatographic resolution and short run times. Figure 1 shows the chromatogram for acrylamide in a standard in water at 2 ng/mL (equivalent to 4 µg/kg in sample extract).

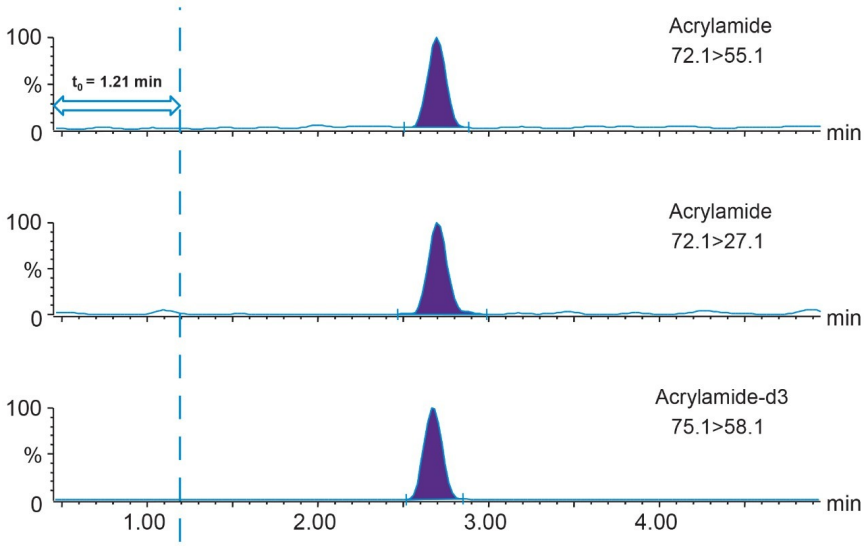

Figure 1. Chromatogram for acrylamide in water at 2 ng/mL (equivalent to 4 µg/kg in sample extract).

The linearity of response was evaluated using a bracketed calibration over a suitable concentration range (0.5 to 2500 ng/mL), as shown in Figure 2. Matrix-matching was not used due to difficulty of locating acrylamide-free samples to use as blanks. Acrylamide-d3 was used as an internal standard to correct for any variability through the whole method, including any LC-MS/MS matrix effects. The coefficients of determination (r^2>0.999) and residuals (<10%) were all excellent.

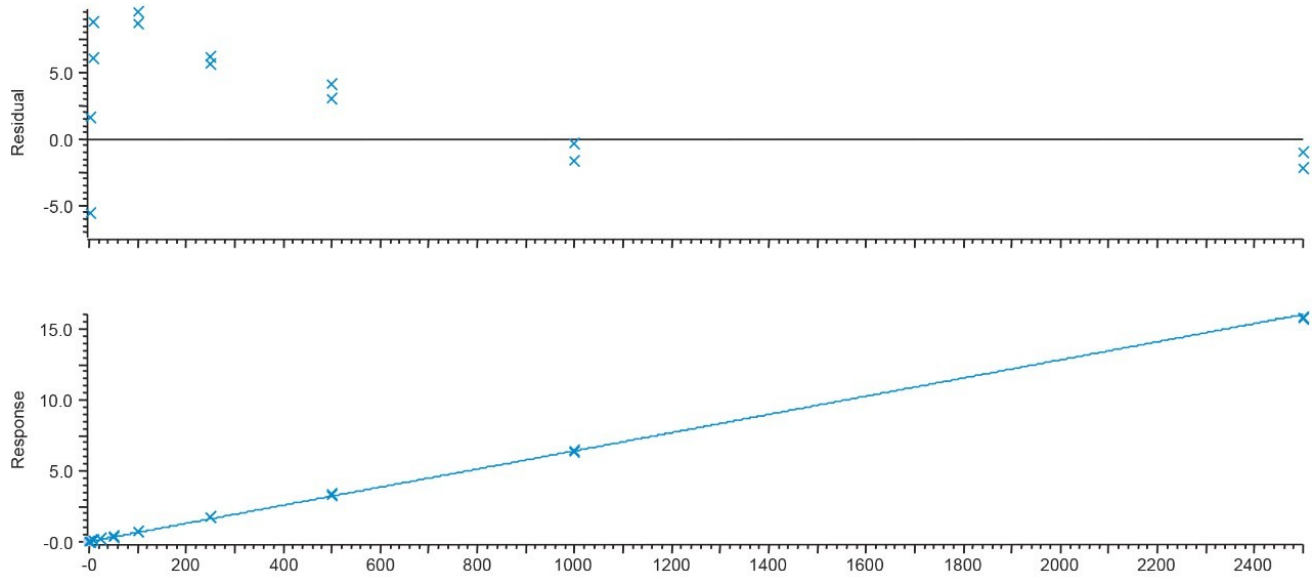

Figure 2. Calibration graph for acrylamide prepared in water (linear fit with 1/x weighting).

Figure 3 shows the detection of acrylamide in samples of representative commodities spiked at the relevant benchmark level (Table 1). Figure 4 shows the detection of acrylamide in baby biscuits at 14 µg/kg. The method demonstrates excellent sensitivity and selectivity and is suitable for checking compliance with LODs/LOQs derived from EU benchmark levels. The application solution also has the potential for analysis at lower concentrations, which is useful for due diligence testing.

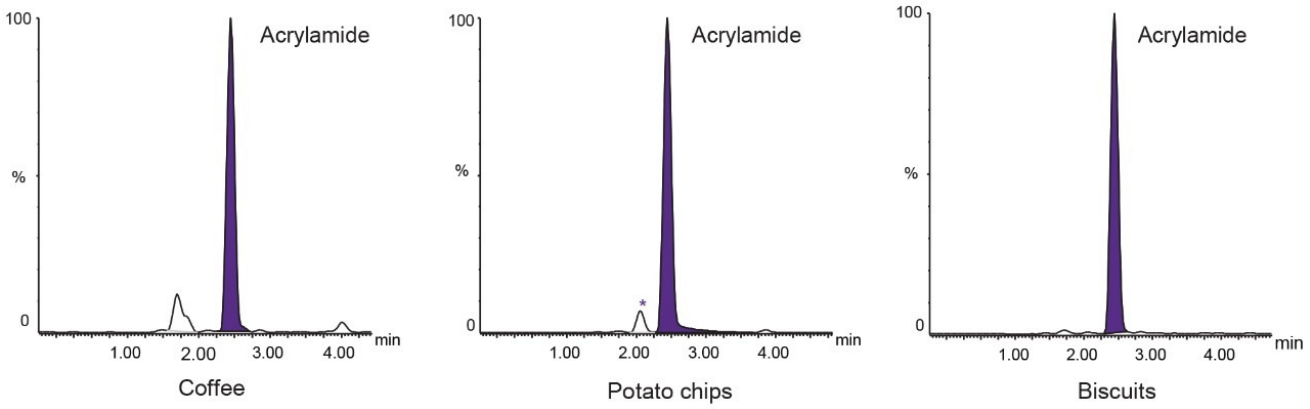

Figure 3. Chromatograms for acrylamide spiked into various commodities at the benchmark level.

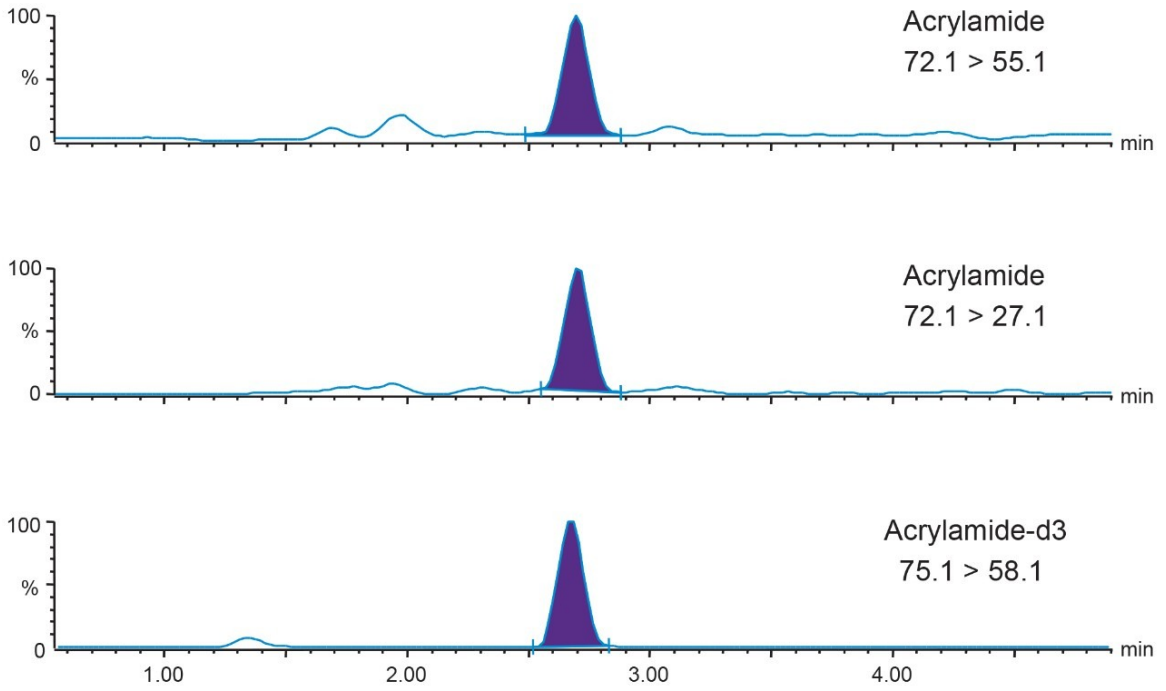

Figure 4. Chromatograms for acrylamide detected in baby biscuits at 14 µg/kg.

The rapid cleanup method proved to be effective even for difficult matrices such as potato chips and coffee, as shown by the lack of isobaric interference. An example of a potato chips sample with and without cleanup is shown in Figure 5. Alternative, more selective cleanup steps, such as SPE using Oasis MCX, could equally be

used to remove interfering matrix components from complex food samples. Excellent sensitivity and selectivity also facilitate a reduction in the mass of laboratory sample taken for extraction, which helps to keep the contamination of the LC-MS/MS system to a manageable minimum.

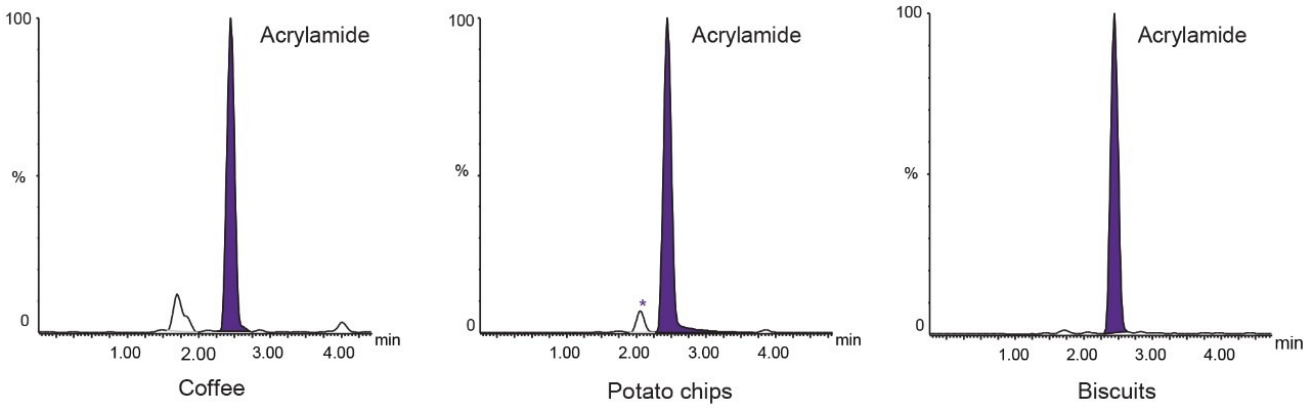

Figure 5. Chromatograms from the analysis of potato chips spiked with acrylamide, before and after cleanup.

Internal validation of the method demonstrated excellent performance for the quantification Internal validation of the method demonstrated excellent performance for the quantification of acrylamide in a range of representative foodstuffs (Table 3). Using acrylamide-d3 as an internal standard, accuracy of the method at both spiked concentrations was excellent and met the performance criteria; measured recoveries were within the range 86% to 107% (acceptance criteria 75–110%) and repeatability (RSDr) between 1.6 and 5.5%. Regulation (EU) 2158/2017 uses values derived from the Horwitz Equation to assess repeatability. However, as the Horwitz Equation gives unacceptable high values for mass fractions lower than 100 µg/kg, the repeatability of the method has been assessed using more challenging acceptance criteria in use elsewhere (e.g. ≤20% RSD).[6] The %RSDs achieved with this method were significantly lower than the SANTE acceptance criteria (<6%) and consistent among various matrices.

	Potato chips	Bread	Coffee	Baby food	Baby biscuits
Lower level spike					
Recovery (%)	97	95	99	86	90
RSD (%)	4.6	1.8	4.4	1.6	3.3
Higher level spike					
Recovery (%)	97	88	100	96	107
RSD (%)	5.5	2.2	4.0	3.8	4.3

Table 3. Measured recovery and repeatability from the analysis of representative commodities, spiked with acrylamide at two concentrations (n= 5 at each concentration, internal standard corrected).

Reference materials were analyzed to further evaluate the performance of the analytical method (Figure 6). The measured values from the analysis of FAPAS coffee and potato chips reference materials agreed well with the assigned values with good precision (Table 4). Commission Regulation (EU) 2158/2017 does not contain any criteria to support the identification of acrylamide, but the ion ratios and retention times from the analysis of the reference materials agreed well with the reference values derived from the spiked samples and all were well within the tolerances typically used for residue analysis (e.g. ≤20% and ±0.1 min).[6]

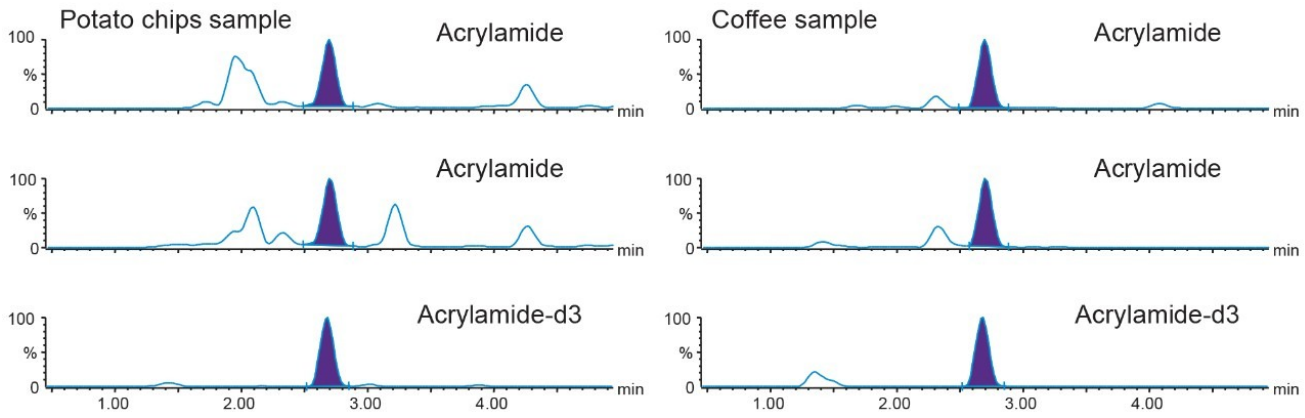

Figure 6. Chromatograms from the analysis of FAPAS test materials known to contain acrylamide.

	Coffee (TYG010RM)	Potato chips (TET043RM)
Assigned value (µg/kg)	249	625
Measured value (µg/kg)	244	597
RSD (%)	4.6	3.0
Bias (µg/kg)	-2.0%	-4.5%

Table 4. Results from the analysis of FAPAS test materials containing known amounts of acrylamide (n= 9).

Conclusion

The aim of this study was to evaluate the performance of the ACQUITY UPLC I-Class System coupled with the Xevo TQ-S micro for the determination of acrylamide in processed foods. In-house verification of this approach showed excellent sensitivity for the detection, identification, and quantification of acrylamide. The modified QuEChERS method provided effective extraction and cleanup of acrylamide and can be applied to many food matrices. The results from matrix reference standards demonstrate that the analytical methodology is accurate, reproducible, precise, and rugged. The Xevo TQ-S micro provided exemplary performance in terms of linearity and calibration range. The trueness and precision of the method determined at various QC levels gave excellent results for bias and %RSD.

References

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6. European Union (2017). Document No. SANTE 11813/2017. Guidance Document on Analytical Quality Control and Method Validation Procedures for Pesticides Residues Analysis in Food and Feed (Online). https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_ wrkdoc_2017-11813.pdf. Accessed 3 Oct. 2018.

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