DETERMINATION OF ACRYLAMIDE IN COFFEE BY LC-MS/MS

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INTRODUCTION

The roasting of coffee leads several chemical reactions, such as lipid oxidation, sugar decomposition and Maillard reactions to take place[1]. During the roasting process many components, which are essential for the flavour (such as aromatic acids) are created or altered leading to the distinct tastes of coffee, however acrylamide is also formed as an undesirable, unavoidable by-product[1].

Acrylamide is a small, polar molecule which can be easily extracted by hot water, suggesting the coffee brewing process allows for the extraction of acrylamide present in the coffee granules into the brew[2].

In 2015 the European Food Safety Authority (EFSA) published a risk assessment on acrylamide in food. The conclusion of this assessment was that acrylamide levels in food could lead to an increased risk of cancer, but no estimate on how much the risk is increased could be determined at that time. EU regulation 2017/2158[3], which came into force in April 2018, establishes mitigation measures and benchmark levels for reducing the presence of acrylamide in food. The benchmark levels set for roast coffee is 400 µg/kg and for instant coffee it is 850 µg/kg.

The analysis of acrylamide in processed foods has several analytical challenges to consider, which include:

- **Retention**: Acrylamide is a polar, low molecular weight compound which can create challenges for reversed phase C_{18} columns.
- **Matrix complexity**: A single sample cleanup is preferred to work for analysis of a range of complex processed food samples which greatly vary in composition.
- **Concentration range**: The method should be able to detect across a wide concentration range as the benchmark levels differ depending on the food type and can range from 40 µg/kg in baby food to 4000 µg/kg for coffee substitutes exclusively from chicory.

METHODS

Sample preparation and extraction:

Homogenized coffee samples are extracted using a modified QuEChERS method with 1g of sample taken for the extraction. Isotopically labelled internal standard (Acrylamide d3) is 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 is subjected to clean-up using dispersive SPE (dSPE). Extracts are evaporated to dryness and reconstituted in 0.1% formic acid in LCMS grade water, to provide a concentration step and solvent exchange into a weaker injection diluent. Full sample extraction details are available by request (**www.waters.com/acrylamide**).

LC conditions:

LC system:	UPLC I-Class
Column:	HSS C_{18} SB 1.8 µm

MS conditions:

System:	Waters Xevo TQ-S micro
Software:	MassLynx® v4.2
Ionization Mode:	ESI+

RESULTS AND DISCUSSION

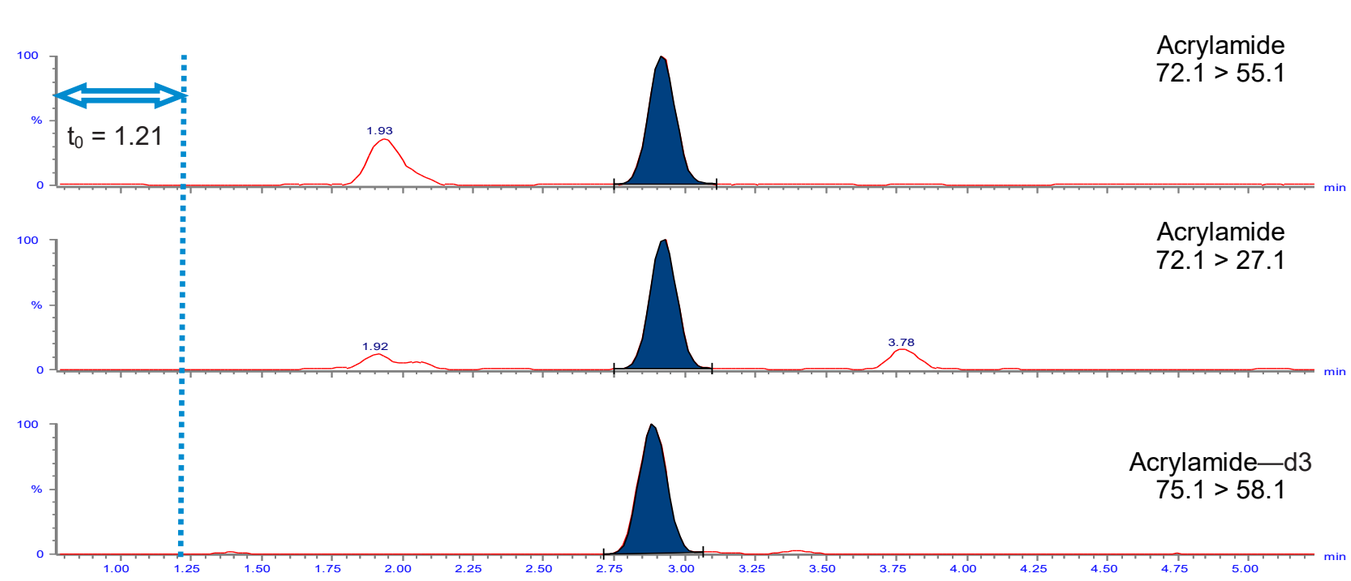

Figure 1. Chromatogram of a extracted, FAPAS coffee reference sample, measured at 244 µg/kg. The t_0 of the column ran at the 0.2 mL/min flow rate is indicated on the chromatogram, highlighting the excellent retention achieved with a simple LC gradient.

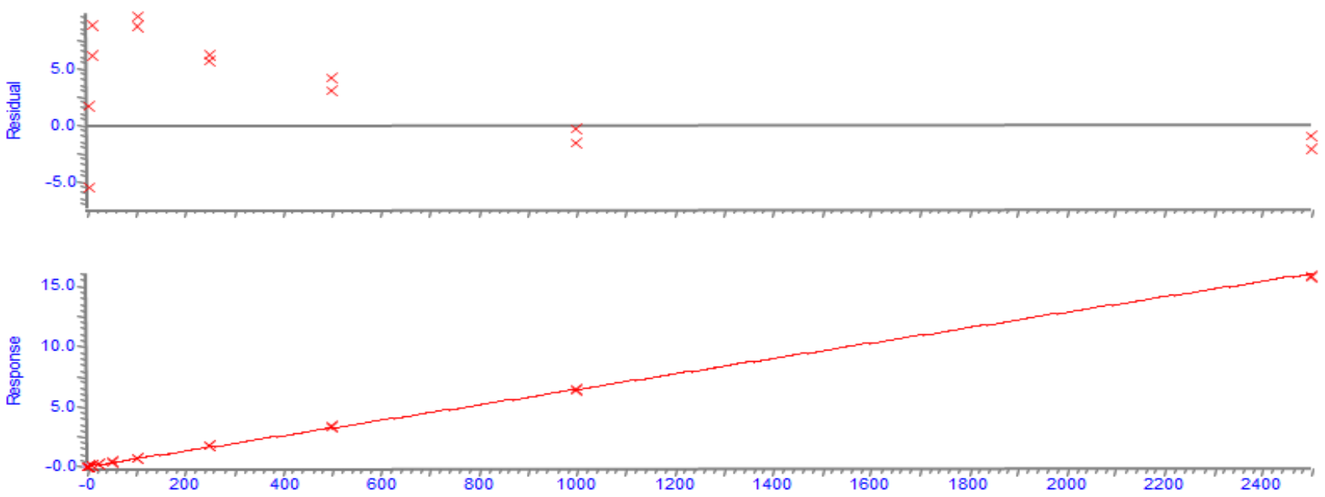

Figure 2. Calibration graph for acrylamide prepared in water (linear fit with 1/x weighting), r^2 = 0.999, all back calculated concentrations are within 20%.

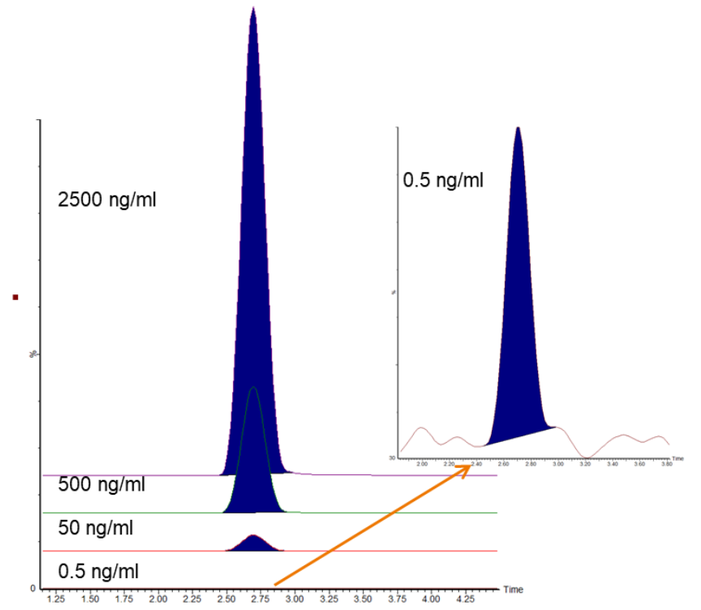

Figure 3. Example of quantifier ions achieved for the calibration range in Figure 2, thus showing excellent sensitivity and linearity (without saturation) over an extended **concentration range**.

Table 1. Results from the analysis of FAPAS test materials containing known amounts of acrylamide (n= 9)

	Coffee (TYG010RM)
Assigned value (µg/kg)	249
Measured value (µg/kg)	**244**
RSD (%)	4.6
Bias (µg/kg)	-2.0 %

CONCLUSIONS

- The modified QuEChERS approach shows excellent sensitivity and LC-MS/MS performance for the extraction, detection, identification, and quantitation of acrylamide in a selection of coffee samples.
- Validation of the method demonstrated excellent performance in terms of linearity, accuracy, precision and repeatability. These validation results further satisfied the criteria outlined in Commission Regulation (EU) 2017/2158.
- The method has been successfully tested on a range of processed food, including potato chips, fries, baby rusks, baby food and bread. By applying appropriate and simple cleanup, reliable quantitation was achieved against an solvent based calibration series. More example data can be found at **www.waters.com/acrylamide**.

References

1. Kocadağlı, T., Göncüoğlu, N., Hamzalıoğlu, A. and Gökmen, V. 2012. In depth study of acrylamide formation in coffee during roasting: role of sucrose decomposition and lipid oxidation. *Food & Function*, 3(9).
2. Guenther, H., Anklam, E., Wenzl, T. and Stadler, R. 2007. Acrylamide in coffee: Review of progress in analysis, formation and level reduction. *Food Additives and Contaminants*, 24(sup1)
3. Eur-lex.europa.eu. (2019). *EUR-Lex - 32017R2158 - EN - EUR-Lex*. [online] Available at: https://eur-lex.europa.eu/legal-content/GA/TXT/?uri=CELEX:32017R2158.

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