DETERMINATION OF ACRYLAMIDE IN COFFEE BY LC-MS/MS

Waters

THE SCIENCE OF WHAT'S POSSIBLE.™

JD De-Alwis, Euan Ross, Joanne Williams, Simon Hird
Waters Corporation, Wilmslow, SK9 4AX, UK

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:	Waters ACQUITY UPLC I-Class
Column:	Waters ACQUITY UPLC HSS C_{18} SB 1.8 µm
Column temperature:	30^0C
Sample temperature:	10^0C
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:	Available on request. (**www.waters.com/acrylamide**)

MS conditions:

System:	Waters Xevo TQ-S micro
Software:	MassLynx® v4.2
Ionization Mode:	ESI+
Acquisition mode:	MRM
Capillary voltage:	0.5 kV
Cone voltage:	20V
Cone gas flow:	50 L/Hr
Desolvation temperature:	600°C
Desolvation gas flow:	1000 L/Hr
Source Temperature：	150°C

MRM Transitions:

Compound	MRM transition	Collision Energy (eV)	Retention time (min)
Acrylamide	72.05 > 55.10	12	2.91
Acrylamide	72.05 > 44.10	10	
Acrylamide	72.05 > 27.15	10	
Acrylamide d3	75.00 > 58.10	15	2.88

RESULTS

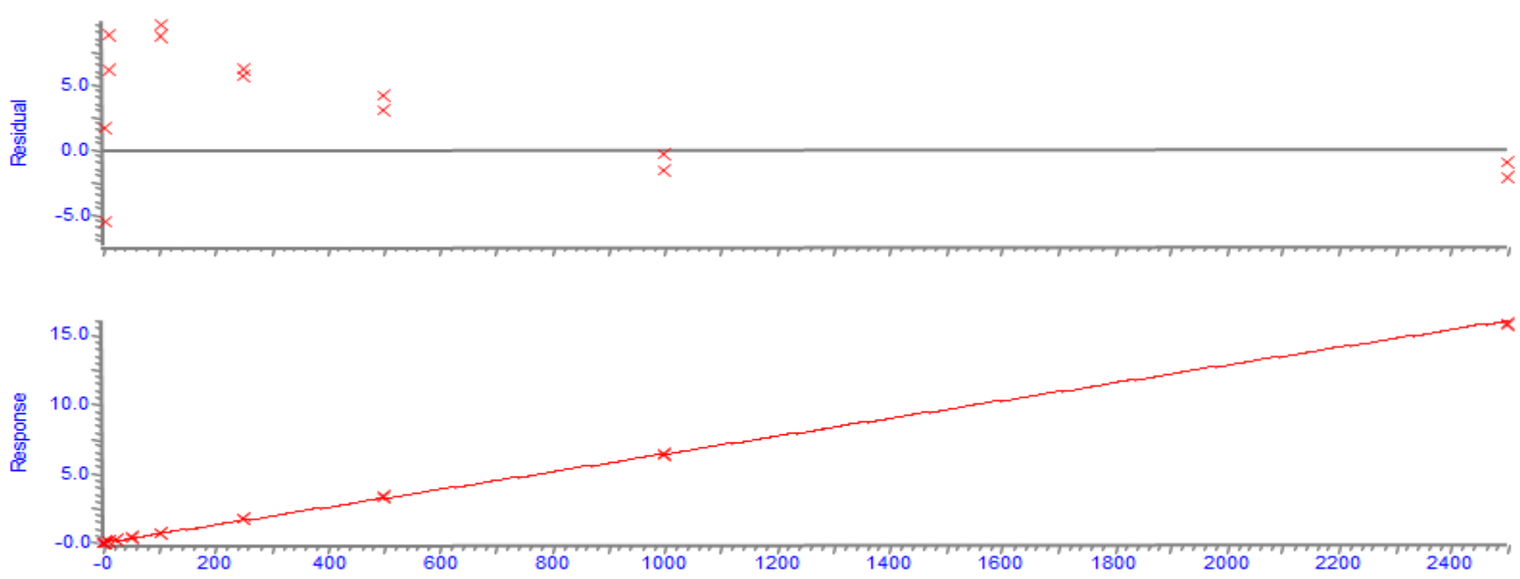

Figure 1. Calibration graph for acrylamide prepared in water (linear fit with 1/x weighting), r^2 = 0.999, all back calculated concentrations are within 20%.

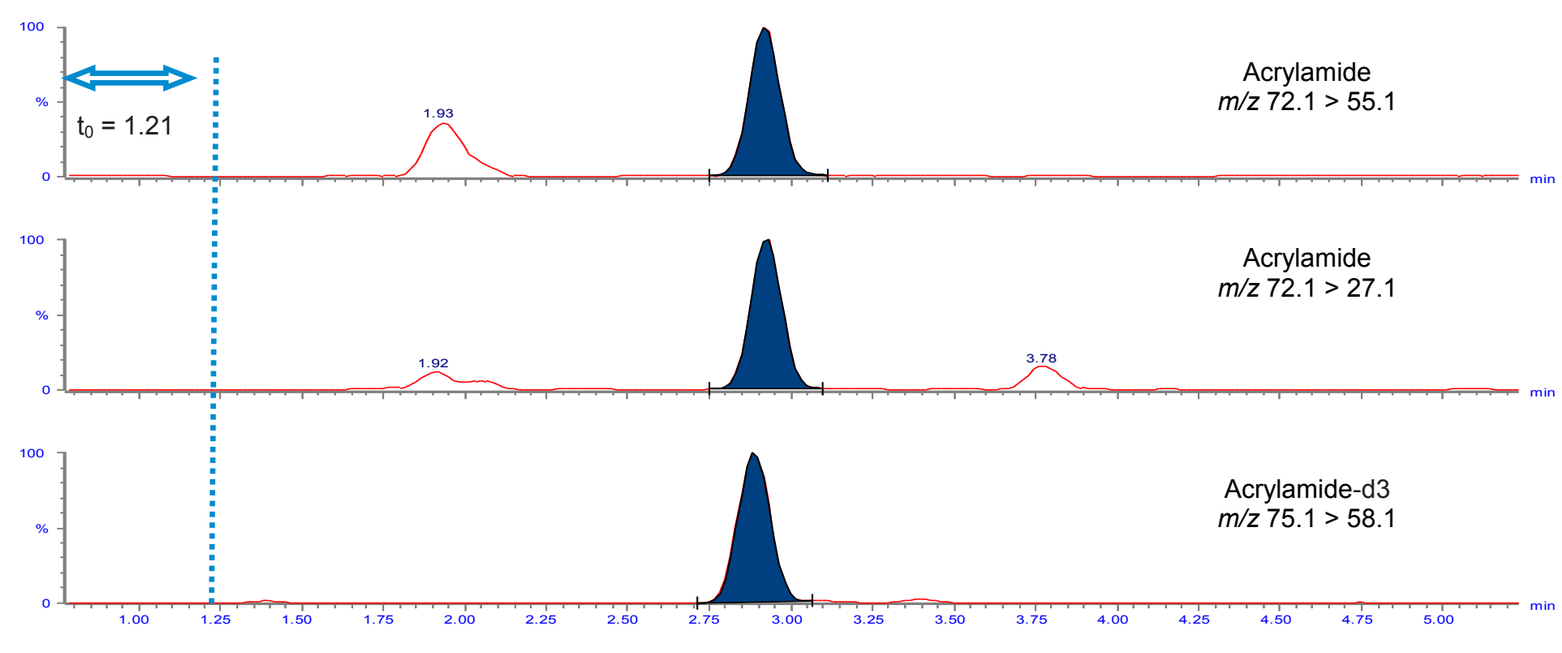

Figure 2. Chromatogram of a extracted, FAPAS coffee reference sample, measured at 244 µg/kg. The t_0 of the column ran at the 0.2 mL/min flow rate is indicated on the chromatogram, highlighting the excellent retention achieved with a simple LC gradient.

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

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

CONCLUSION

- **The modified QuEChERS approach showed excellent sensitivity and LC-MS/MS performance for the 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, in accordance with 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. 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), p.970. (Accessed 30 January 2019)
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), pp.60-70. (Accessed 30 January 2019)
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 [Accessed 30 Jan. 2019].

Scan QR Bar Code for More Information

#AnalyticalFoodies
Because we love food analysis

TO DOWNLOAD A COPY OF THIS POSTER, VISIT WWW.WATERS.COM/POSTERS