DETERMINATION OF ACRYLAMIDE IN COFFEE BY LC-MS/MS

JD De-Alwis,[1] Euan Ross,[1] Joanne Williams[1] and Kenneth Rosnack[2]

[1] Waters Corporation, Stamford Avenue, Wilmslow, SK9 4AX, UK.
[2] Waters Corporation, 34 Maple St, Milford, MA 01757, USA.

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

INTRODUCTION

The roasting of coffee is an essential process, promoting several chemical reactions, which result in the distinctive flavors and taste. Such reactions include lipid oxidation, sugar decomposition and Maillard reactions, where key flavor components, such as aromatic acids are created or altered. However, another compound that can often be formed is acrylamide– 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 provides ideal conditions for the extraction of acrylamide present in the coffee granules into the brew. [2]

Acrylamide's toxicological properties have been extensively studied and it is classified as a group 2A carcinogen by the International Agency for Research on Cancer (IARC). 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.

Acrylamide hit the headlines again internationally in March 2018, when a judge in California ruled acrylamide fell under the State's Proposition 65 labeling requirements. At the same time, the European Union (EU) regulation 2017/21583 came into force, establishing 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. [3]

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 were extracted using a modified QuEChERS method with 1g 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 was subjected to clean-up using dispersive SPE (dSPE). Extracts were 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 [For more information, scan the QR code below or visit **www.waters.com/acrylamide**]

Figure 1. Example of extracts and chromatography achieved for ground coffee beans, following QuEChERS extraction without any cleanup and with dSPE cleanup, respectively. Applying cleanup **removes matrix complexity**, yielding cleaner extracts and more selective chromatography. The integrated peak show acrylamide.

LC conditions:

LC system:	ACQUITY UPLC I-Class
Column:	ACQUITY UPLC HSS C_{18} SB 1.8 µm
Column temperature:	30°C
Sample temperature:	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
Mobile phase B:	Methanol

MS conditions:

System:	Xevo TQ-S micro
Software:	MassLynx v4.2
Ionization Mode:	ESI+
Capillary voltage:	0.5 kV
Cone voltage:	20 V
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
	72.05 > 44.10	10	
	72.05 > 27.15	10	
Acrylamide d3	75.00 > 58.10	15	2.88

RESULTS AND DISCUSSION

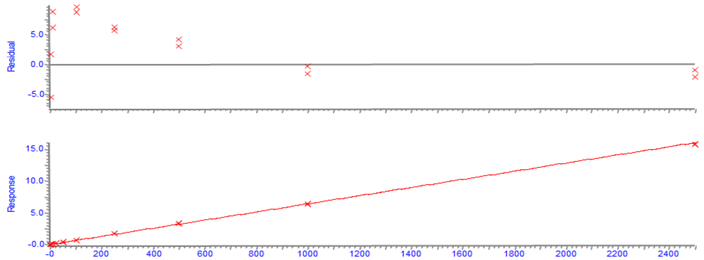

Figure 2. Calibration graph for acrylamide prepared in water (internal standard corrected) over the range of 0.5 to 2500 ng/ml with a linear fit and 1/x weighting, yielding r^2 = 0.999. All back calculated (residuals) concentrations are within 20%.

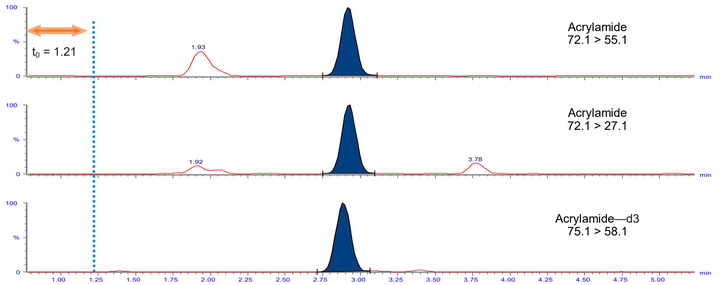

Figure 4. Chromatogram of an extracted, FAPAS coffee reference sample, measured at 244 µg/kg. The t_0 of the column ran at the 0.2 mL/min flow rate is indicated on the chromatogram, highlighting the **excellent retention** achieved with a simple LC gradient.

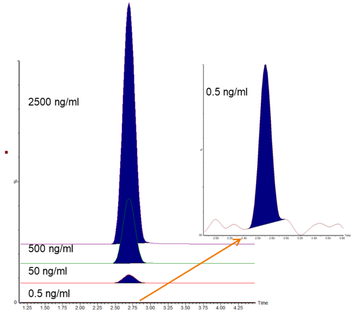

Figure 3. Example of quantifier ions achieved for the calibration range in Figure 2, thus showing excellent sensitivity and linearity (without saturation) over an extended **concentration range**.

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 %

Scan QR Bar Code for More Information

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. Kocadağli, 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.

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