Application of a Novel Automated Sample Preparation Platform for the Determination of Acrylamide in Instant Coffee and Analysis Using UPLC-MS/MS Analysis

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Introduction

Acrylamide is a small polar, highly water-soluble molecule formed when carbohydrate rich foodstuffs are cooked at high temperatures. Due to its neurotoxic and carcinogenic properties there has been an increased demand for reliable and accurate methods for acrylamide detection. By 2017, regulatory authorities established benchmark levels and recommended that companies continue to monitor and minimise the acrylamide levels in heat processed foods. This poster demonstrates the application of EN16618:2015, involving a two-step solid phase extraction (SPE) approach for the analysis of acrylamide in instant coffee followed by UPLC-MS/MS analysis. Subsequent method conversion to an automated sample preparation platform will be presented for assay speed, throughput, and overall workflow advantages.

Experimental

Reagents

Acrylamide, internal standard ($^{13}C_3$ acrylamide) were purchased from LGC Standards (Teddington, UK). Analytical reagents were from Sigma-Aldrich (Poole, UK). All solvents were HPLC grade from Rathburn Chemical Ltd (Scotland, UK) and Milli-Q (Merck Millipore, Germany) water used throughout. Instant coffee was purchased from local supermarkets.

Sample Preparation

Matrix Preparation:

2 g of instant coffee powder was dissolved with 250 mL of boiling tap water. After cooling 2 mL sample aliquots were removed for analysis.

Internal Standard: 10 µL of $^{13}C_3$ acrylamide (20 ng/µL) was added to the samples (total concentration 100 ng/mL) and left to stand for thirty minutes to equilibrate.

Solid Phase Extraction: EN16618:2015 specifies a dual SPE column approach to the extraction of acrylamide from various foodstuffs. Phase 1 is a flow through (no analyte retention) matrix scavenging protocol while phase 2 performs a catch and release to polish and allow concentration of the target analyte.

SPE Column 1: ISOLUTE® Multimode 1 g/6 mL (p/n: 904-0100-CG).
SPE Column 2: ISOLUTE® ENV+ 500 mg/6 mL (p/n: 915-0050-CG).

SPE processing was optimized using a 48-position positive pressure manifold (Biotage® Pressure+ 48: PPM-48) prior to method automation. Full SPE protocols are demonstrated in ***Table 1.***

Table 1. *Solid Phase Extraction Protocols.*

Step	SPE 1: ISOLUTE® Multimode		SPE 2: ISOLUTE® ENV+	
Condition	MeOH	2 mL	MeOH	5 mL
Equilibration	H_2O	2 x 4 mL	H_2O	5 mL
Sample load	Coffee Extract	** 2 mL	SPE 1 Extract ** 5 mL	
Wash 1	H_2O	** 3 mL	H_2O	4 mL
Dry	-		5 minutes	
Elution	-		MeOH:H_2O 60:40	2 mL

** Coffee load and H_2O wash collected in SPE 1 for further purification in SPE 2.

Biotage® Extrahera™ HV-5000 Automated Sample Preparation Platform

For increased throughput and reliability the sequential column SPE protocol was transferred to an automated sample preparation platform, the Extrahera™ HV-5000. The system is equipped with a 4 channel 5 mL pipetting head and utilises positive pressure processing functionality. Columns were processed in 24 position arrangement. The Extrahera™ HV-5000 platform is shown in ***Figure 1***.

Figure 1. *Biotage® Extrahera™ HV-5000 automated sample preparation platform.*

Post extraction: 10 µL of ethylene glycol was added to each of the extracts to avoid complete evaporation. Samples were evaporated at 40 °C using a gas flow step gradient on a TurboVap® LV evaporation system followed by reconstitution in 500 µL of Ultrapure H_2O for analysis.

UPLC/MS Conditions

Instrument: Waters ACQUITY UPLC (Waters Assoc., Milford, MA, USA).
Column: Restek Allure Acrylamide 5 µm (50 x 2.1 mm) (Thames Restek, High Wycombe, UK).
Mobile phase: 0.1% formic acid in both aq. (A) and MeOH (B).
Flow rate: 0.3 mL/min.
Column temp: 40 °C. **Injection volume:** 10 µL.
Gradient: Initial starting conditions of 100% A were held for 1.1 minute followed by a rapid linear gradient to 100% at 1.7 minutes. Initial conditions were resumed at 3 minutes.

Mass Spectrometry

Instrument: Waters Quattro Premier XE triple quadrupole mass spectrometer equipped with an ES interface for mass analysis (Waters Assoc., Manchester, UK). Positive ions were acquired in the MRM mode.

Desolvation Temp: 450 °C **Ion Source Temp:** 120 °C

Table 2. *MRM Parameters.*

Analyte	MRM	Cone Voltage (V)	Collision Energy (eV)
Acrylamide	71.9 > 55.2	23	8
Acrylamide-$^{13}C_3$	74.9 > 58.2	23	8

Results

Acrylamide is extremely volatile and known to suffer from evaporative losses during the dry-down step. Many protocols rely on partial evaporation to avoid this issue. The approach taken here was to use a "keeper" solvent to eliminate complete evaporation and allow maximum sample pre-concentration prior to analysis. ***Figure 2.*** demonstrates acrylamide evaporative effects with/without the use of ethylene glycol as the keeper solvent.

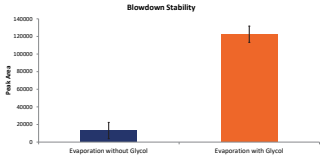

Figure 2. *Evaporation profiles with/without ethylene glycol.*

EN16618:2015 defines a dual SPE protocol previously optimized for the extraction of acrylamide from various foodstuffs. Initial extraction using the ISOLUTE® Multimode is designed for the retention of unwanted matrix components from the coffee while the acrylamide passes through unretained. The load fraction was collected with an additional water rinse. Subsequent purification was provided using a standard SPE catch and release mechanism with the ISOLUTE® ENV+ phase. ***Figure 3.*** demonstrates dual SPE workflow.

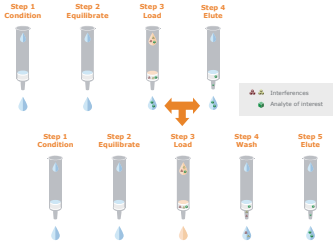

Figure 3. *Method EN16618:2015 dual SPE representation.*

The only adaption of this method was reduction of the equilibration volumes on the SPE protocols (6 mL > 4-5 mL) as outlined in ***Table 1.*** Comparison of recovery profiles, including breakdown for each SPE step (and total) for this modification and original are demonstrated in ***Figure 4***.

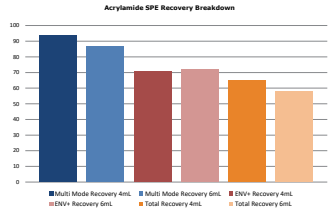

Figure 4. *Acrylamide SPE Recovery profiles.*

This optimized method was then automated using the Extrahera™ HV-5000 system. The system was optimized for pipetting, sample transfer and positive pressure processing for both SPE steps. Total process recoveries (two sample sets, n=4) for manual vs automated SPE processing are presented in ***Figure 5.***

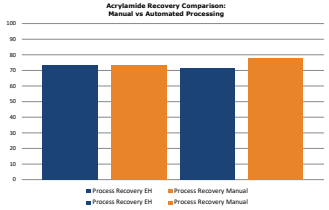

Figure 5. *Acrylamide recovery comparison of positive pressure processing and automated SPE.*

Figure 6. demonstrates calibration curve (5-500 ng/mL) performance for both manual positive pressure and automated protocols. Both procedures delivered excellent performance, coefficients of determination, $r^2 > 0.99$ and LOQs around 5 ng/mL. Precision and accuracy comparisons at low, mid and high QC concentrations for both protocols are detailed in ***Table 3.***

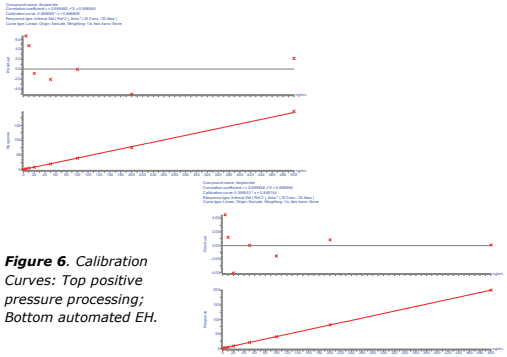

Figure 6. *Calibration Curves: Top positive pressure processing; Bottom automated EH.*

Table 3. *Processing precision and accuracy comparison.*

	Calculated Concentration (Accuracy)		% RSD (Precision)	
	PPM-48	EH HV-5000	PPM-48	EH HV-5000
Low QC (10 ng/mL)	9.5	9.70	10.0	8.4
Mid QC (50 ng/mL)	49.70	51.20	6.3	4.6
High QC (200 ng/mL)	208.85	202.85	2.3	4.2

Conclusion

- The poster presents an automatable approach to the EN16618:2015 method for the determination of acrylamide in coffee.
- Conversion of manual positive pressure processing to the automated system provided a walk away solution for this method, demonstrating excellent correlation.
- A walk away, two step SPE process capable of extracting 24 samples in 70 minutes is achievable.

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