Extraction of Acrylamide from Coffee and Potato Chips (Crisps) using Supported Liquid Extraction (SLE) prior to LC-MS/MS Analysis

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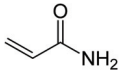
Introduction

Acrylamide (structure shown in ***Figure 1.***) analysis has increased in recent years due to its neurotoxic and carcinogenic properties. It is a small polar analyte with limited functionality leading to difficulties with extraction, purification and chromatography from various matrices. There are many methods for acrylamide measurement in the literature, most involving dual solid phase extraction procedures. Approaches such as reversed phase and mixed-mode SPE have been used to selectively retain specific matrix components, or porous graphitic carbon as a more retentive phase to bind acrylamide. These are expensive, complicated and time consuming. This poster demonstrates a novel, sensitive, cost effective and rugged method for the analysis of acrylamide in coffee and potato chips (crisps) using supported liquid extraction prior to LC-MS/MS analysis. We will demonstrate extraction from coffee down to a concentration of 1 ng/mL and in potato chips to a level of 10 ng/g (ppb).

Figure 1. *Structure of Acrylamide*



Experimental

Reagents

Ammonium hydroxide (28% conc), ethyl acetate and tetrahydrofuran were purchased from Fisher Scientific (Loughborough, UK). Ethylene glycol and formic acid were purchased from Sigma Chemical Co. (Poole, UK). Acrylamide, internal standard ($^{13}C_3$ acrylamide) and methanol were purchased from LGC Standards (Teddington, UK). Milli-Q water was used throughout (Millipore, Watford, UK). Coffee and potato chips (crisps) were purchased from local supermarkets.

Sample Preparation

ISOLUTE® SLE+ Procedure (*Figure 2.*)
Columns: ISOLUTE® SLE+ 1 mL capacity; 820-0140-C.

Matrix Pre-treatment:
Coffee: Cold coffee (instant and ground from bean) samples (500 µL) were pre-treated with 10 µL NH_4OH.
Potato chips: Potato chips (crisps) were finely crushed using a pestle and mortar. 1 g (±1 %) were transferred to a 15 mL screw-cap centrifuge tube. Water (10 mL) was added to each tube and mixed on a rotating mixer for one hour. After mixing, the tube was centrifuged at 3000 x g for 12 minutes

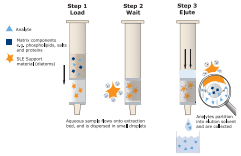
Internal standard: 10 µL of $^{13}C_3$ acrylamide was added to the samples and left to stand for thirty minutes in order to equilibrate with the matrix.

Sample Application:
Coffee: 500 µL was applied to the ISOLUTE® SLE+ column.

Potato chips: 650 µL from the aqueous layer was applied to the ISOLUTE® SLE+ column.

Analyte Extraction: 2 x 2.5 mL of EtOAc:THF (1:1, v/v). Each aliquot was allowed to flow under gravity for 5 minutes before applying a pulse of vacuum for 10-20 seconds to completely remove the final aliquot.

Figure 2. *Schematic of ISOLUTE® SLE+ Supported Liquid Extraction procedure.*



Post Extraction: 2 µL of ethylene glycol was added to the collection vessels to avoid complete evaporation. Extracts were evaporated under ambient temperature and reconstituted in 200 µL of H_2O for analysis.

HPLC Conditions

Instrument: Waters Acquity UPLC (Waters Assoc., Milford, MA, USA).
Column: Phenomenex Hydro 50 x 2 mm, 4 µm (Macclesfield, UK).
Mobile Phase: 0.1% formic acid (aq) and 0.1% formic acid/MeOH.
Gradient: 0% B held for 0.6 min; at 1 min 100% B, held until 2.5 min; 2.6 min initial starting conditions resumed.
Flow Rate: 0.3 mL/min.
Injection Volume: 10 µL.
Column Temperature: 40 °C.

Mass Spectrometry

Instrument: Quattro Premier XE triple quadrupole mass spectrometer (Waters Assoc., Manchester, UK) equipped with an electrospray interface for mass analysis.
Desolvation Temperature: 450 °C
Ion Source Temperature: 120 °C
Collision Gas Pressure: 3.5 x 10^{-3} mbar

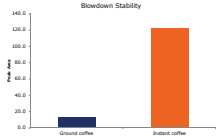
Table 1. *Waters Quattro Premier XE parameters.*

Analyte	MRM Transition	Cone Voltage (V)	Collision Energy (eV)
Acrylamide	71.9 > 55.2	23	8
$^{13}C_3$ Acrylamide	74.9 > 58.2	24	9

Results

Initial experiments focussed on the evaporation step for sample concentration. Acrylamide is extremely volatile and suffers from evaporative losses during blow down. Almost complete loss of the analyte was observed during sample pre-concentration. The use of 2 µL of ethylene glycol eliminated this issue when drying at ambient temperatures as demonstrated in ***Figure 3.***

Figure 3. *Reconstitution recovery of acrylamide with and without 2 µL ethylene glycol in collection vessel.*



The main challenge in assessing method sensitivity was that process derived acrylamide present in the food samples prevented the ability to run a true matrix blank. This was particularly the case for potato chips where the level was equivalent to approximately 40% of the total calibration scale. ***Figure 4.*** demonstrates incurred levels of acrylamide in purchased potato chips.

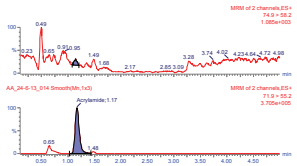
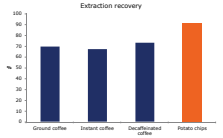


Figure 4. *Acrylamide levels in un-spiked potato chips (bottom), internal standard transition (top).*

Extraction was investigated using numerous organic solvents with varying degrees of polarity (data not shown). The combination of ethyl acetate and tetrahydrofuran gave the best combination of good recoveries while maintaining extract cleanliness. Extraction recoveries for coffee (ground, instant caffeinated and decaffeinated) ranged from 67 to 73% while potato chips returned values > 90%, as demonstrated in ***Figure 5.*** RSDs below 10% were obtained for all matrices using the optimized procedure.

Figure 5. *Blank-corrected extraction recoveries of acrylamide in three coffee types and potato chip extract.*



Figures 6 and 7. illustrate pigment removal from coffee samples using the optimized extraction procedure. The addition of NH_4OH resulted in ionization of the pigments increasing water solubility and causing them to remain in the aqueous phase on the column, while acrylamide extracted into the organic solvent.

Figure 6. *Coffee processed SLE+ column (top), unused SLE+ cartridge (bottom).*

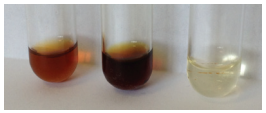
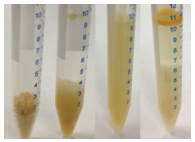


Figure 7. *500 µL ground coffee solution (left), 500 µL ground coffee solution with 10 µL NH_4OH, coffee SLE+ extract reconstituted in 500 µL water (right).*

Figure 8. demonstrates the potato chip matrix extraction procedure.

Figure 8*. 1 g of dry crushed potato chips (left), added to water prior to rotating (centre left), after rotating (centre right) and after centrifugation (right) showing the upper oil layer, middle aqueous layer and lower potato starch layer*



The performance of the coffee assay is illustrated by ***Figure 9.*** The calibration curve was constructed with process derived levels as an intercept. Calibration lines were linear over the range 1-128 ng/mL for prepared coffee solution, being equivalent to 25-3200 ppb for solid ground coffee or 8-1024 ppb for solid instant coffee (regular or decaffeinated).

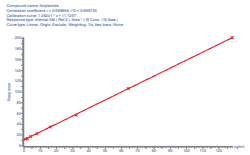
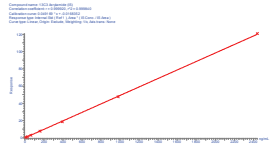


Figure 9. *Calibration performance of acrylamide in coffee.*

Calibration lines were linear over the range 10-2440 ppb for potato chips. Because of the high levels of process derived acrylamide present in potato chips (up to 40% of the calibration range) the sensitivity of the method could only be estimated by performing the quantitation in reverse. For these experiments spiked unlabelled acrylamide was combined with process derived levels as a surrogate internal standard while extracting different levels of spiked $^{13}C_3$ acrylamide. Excellent coefficients of determination with r^2 greater than 0.99 were obtained using this reverse calibration approach as illustrated in ***Figure 10.***

Figure 10. *Calibration performance of acrylamide in potato chips.*



Conclusion

- A method has been developed which measures acrylamide quickly, easily and cost effectively from two challenging matrices at highly sensitive levels.
- Despite acrylamide being a relatively polar molecule excellent separation was demonstrated between this and matrix interferences on the ISOLUTE® SLE+ material.

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