Extraction of Acrylamide from Coffee Using ISOLUTE® SLE+ Prior to LC-MS/MS Analysis

This application note describes a Supported Liquid Extraction (SLE) protocol for the extraction of acrylamide from coffee using ISOLUTE® SLE+ columns with LC-MS/MS detection.

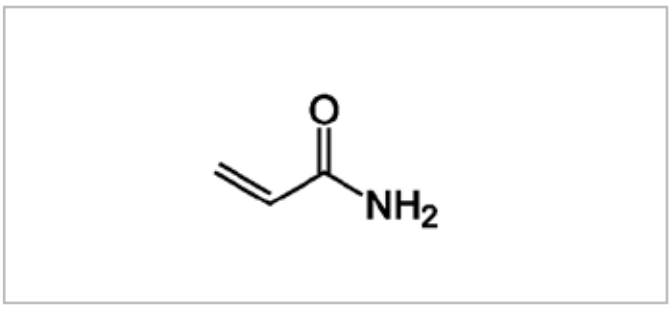


Figure 1. Structure of Acrylamide

Introduction

The method described in this application note achieves high recoveries of acrylamide in coffee. The method is sensitive enough to measure levels as low as 1 ng/mL in coffee (solution), 25 ppm in ground coffee (solid) or 125 ppm in instant coffee (solid, traditional or decaffeinated) and gives good selectivity from what is a challenging matrix.

ISOLUTE SLE+ products provide clean, rapid, robust and efficient extraction solutions for a wide range of analytes.

Analyte

Acrylamide

Sample Preparation Procedure

Format:	ISOLUTE® SLE+ 1 mL Columns, part number 820-0140-C
Sample Pre-treatment:	Coffee was prepared in the same way that it would normally be consumed. In the case of ground coffee, 60 g of ground coffee was percolated with 1500 mL of boiling water. For instant coffee 2 g of instant coffee powder was dissolved in 250 mL of boiling water. This resulted in solutions containing coffee 'solid' concentrations of 40 mg/mL for ground coffee and 8 mg/mL for instant coffee. Once prepared the coffee was left to reach room temperature.
Calibration Line Preparation:	A 128 ng/mL acrylamide coffee over-spiked solution was prepared by diluting 25.6 µL of a 10 µg/mL aqueous acrylamide solution to 2 mL with control coffee. This was then serially diluted seven times by transferring 0.8 mL, diluting with 0.8 mL of control coffee, mixing, and then transferring 0.8 mL of this mixture; repeating the procedure until a solution with an over-spiked level of 1 ng/mL had been reached. 0.625 mL aliquots were transferred to wells containing 10 µL of a 4 µg/mL $^{13}C_3$ acrylamide solution in water and 12.75 µL of a saturated solution of ammonium hydroxide in water.

Biotage®

Supported Liquid Extraction

Sample work-up: Samples (0.625 mL) were transferred to tubes containing 10 µL of a 4 µg/mL $^{13}C_3$ acrylamide solution in water and 12.75 µL of a saturated solution of ammonium hydroxide in water. The tube was briefly shaken and then 0.5 mL of the mixture transferred to a 1 mL capacity ISOLUTE® SLE+ column.

Sample loading: Load pre-treated sample (0.5 mL) onto each well. Apply a pulse of vacuum (VacMaster-10 or 20 Sample Processing Manifold, 121-1016 or 121-2016) or positive pressure (Pressure+ Positive Pressure Manifold, PPM-48) to initiate flow. Allow the sample to absorb for 5 minutes.

Analyte Elution: Elute with ethyl acetate: tetrahydrofuran, (1 : 1, v/v, 2x 2.5 mL) and allow to flow under gravity into a tube already containing 2 µL ethylene glycol. Apply vacuum or positive pressure to elute any remaining extraction solvent.

Post Elution: Dry the volatile constituents of the eluate in a stream of air or nitrogen using an SPE Dry (ambient, 20 to 40 L min^{-1}), (SD-9600-DHS or SD2-9600-DHS) or TurboVap LV, (C103198 or C103199) (15 bar at ambient for 1 hr). Reconstitute in water (200 µL).

HPLC Conditions

Instrument: Waters Acquity

Column: Phenomenex Hydro, 4 µm 50 x 2 mm C18 column with a C18 guard cartridge and on-line filter

Mobile Phase: A: 0.1% formic acid in water
B: 0.1% formic acid in methanol

Flow rate: 0.3 mL min^{-1}

Injection: 10 µL

Gradient: Initial 100 % A, hold till 0.6 min
linear ramp to 100 % B over 0.25 min (0.85 min), hold 1.65 min (2.5 min)
linear ramp to 100 % A in 0.01 min (2.51 min), hold 2.49 min (5 min)

Column temperature: 40 °C

Sample temperature: 20 °C

Table 1. Typical retention times for acrylamide using the LC-MS/MS method described

Compound	Retention time (min)
Acrylamide	1.02
Acrylamide $^{13}C_3$	1.02

MS Conditions

Ions were selected in order to achieve maximum sensitivity using multiple reaction monitoring

Instrument: Waters Quattro Premier

Ionization mode: ES+

Desolvation temp.: 450 °C

Source temp: 120 °C

Table 2. Positive Ion Mode - MRM Parameters

MRM transition	RT	Compound ID	Cone, V	CE, V
71.9 – 55.2	1.0	Acrylamide	23	8
74.9 – 58.2	1.0	Acrylamide $^{13}C_3$	24	9
Dwell = 0.2 sec, Inter-channel delay = 0.005 sec				

Results

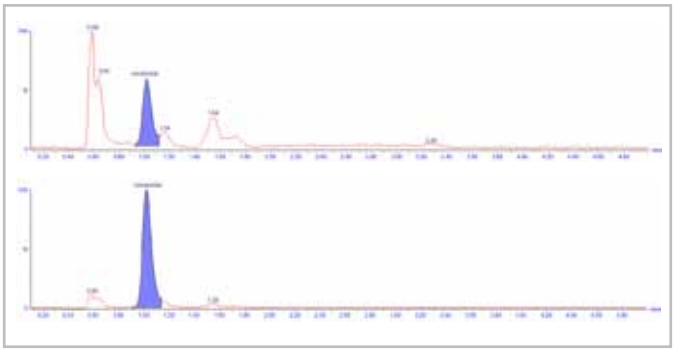


Figure 2. Extracted ion chromatograms in positive ion mode using ISOLUTE® SLE+ procedure (sample: 500 µL ground coffee, not spiked (process derived levels only) and over-spiked with 128 ng/mL acrylamide)

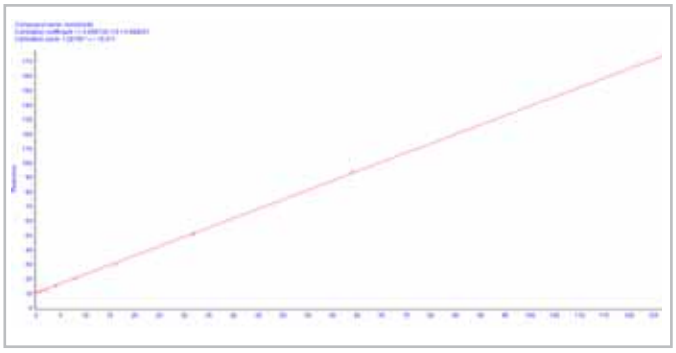


Figure 2. Typical calibration curve for Acrylamide in ground coffee, expressed on a linear scale

Table 3. Performance and recovery data for acrylamide

Matrix	Recovery %	% RSD(n=6)
Fresh roast coffee	81	8.2
Instant coffee	82	5.5
Instant decaffeinated coffee	73	3.7

Recovery and RSD calculations based on extractions of blank matrix spiked at 64 ng/mL without using an internal standard. The blank acrylamide response was subtracted from both extracted and fortified quantities prior to calculating both recovery and RSD.

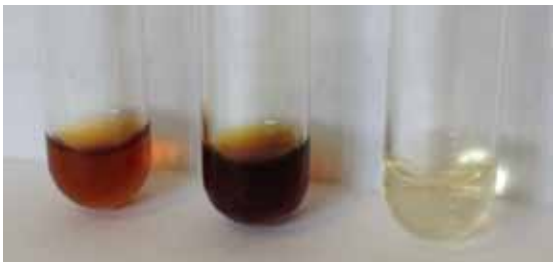
Table 4. Analyte performance from ground coffee

Analyte	r^2
Acrylamide	0.998

r^2 calculations were based on line including a 'zero' standard, over-spiked standards between 1 to 128 ng/mL and applying a weighting factor of 1/x.

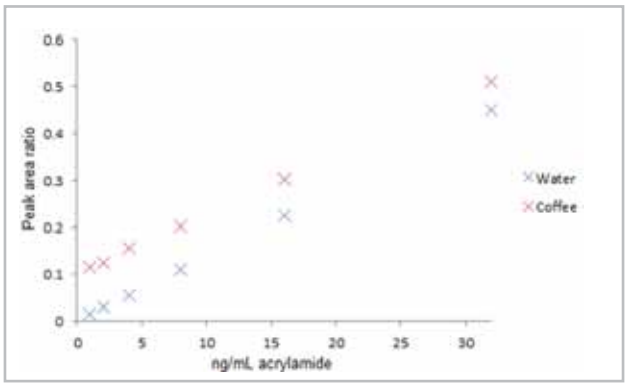
Additional Notes

» The addition of the ammonia solution results in the coffee changing from a mid-brown to a darker brown appearance. Although the sample added to the ISOLUTE SLE+ column is darker as a result of the basification, the final extract appears visibly cleaner than with untreated coffee. The image below shows 0.5 mL untreated ground coffee (left), coffee combined with 2% concentrated ammonia solution (middle) and the SLE extract diluted to an identical volume with water (right).



» The majority of the coffee dyes are removed by being trapped on the SLE material. The image below shows an unused 1 mL SLE column (left) compared to a column that has undergone a full extraction including the removal of acrylamide (right).

» A calibration line extracted in water gave similar properties to that extracted in coffee but without the intercept due to the lack of any process derived acrylamide. Preparing a calibration line in this solvent could be applied for ultra low level acrylamide determinations.



» Ethylene Glycol was added in a small quantity prior to the extraction step to avoid the evaporated sample drying completely. Without this additive being present the majority of the acrylamide would be lost at this stage.

» A 100% aqueous mobile phase was required to give retention to the polar analyte. This required a column that was designed to work under these conditions and the method included a relatively long equilibrium time between samples.

Ordering Information

Part Number	Description	Quantity
820-0140-C	ISOLUTE® SLE+ 1 mL Sample Volume Columns	30
121-1016	Biotage® VacMaster-10 Sample Processing Manifold	1
121-2016	Biotage® VacMaster™-20 Sample Processing Manifold	1
PPM-48	Biotage® PRESSURE+ 48 Positive Pressure Manifold	1
SD-9600-DHS-NA	Biotage® SPE Dry Dual Sample Concentrator System, 110V	1
SD2-9600-DHS-EU	Biotage® SPE Dry Dual Sample Concentrator System, 220V	1
C103199	TurboVap ®LV	1

For the latest application notes and more information about ISOLUTE® SLE+ visit www.biotage.com

EUROPE
Main Office: +46 18 565900
Toll Free: +800 18 565710
Fax: +46 18 591922
Order Tel: +46 18 565710
Order Fax: +46 18 565705
order@biotage.com

NORTH AMERICA
Main Office: +1 704 654 4900
Toll Free: +1 800 446 4752
Fax: +1 704 654 4917
Order Tel: +1 704 654 4900
Order Fax: +1 434 296 8217
ordermailbox@biotage.com

JAPAN
Tel: +81 3 5627 3123
Fax: +81 3 5627 3121
jp_order@biotage.com

CHINA
Tel: +86 21 2898 6655
Fax: +86 21 2898 6153
cn_order@biotage.com

To locate a distributor,
please visit our website at
www.biotage.com

Part Number: AN796