Extraction of Acrylamide from Fried Potato Chips (Crisps) 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 potato chips / crisps using ISOLUTE SLE+ columns with LC-MS/MS detection.

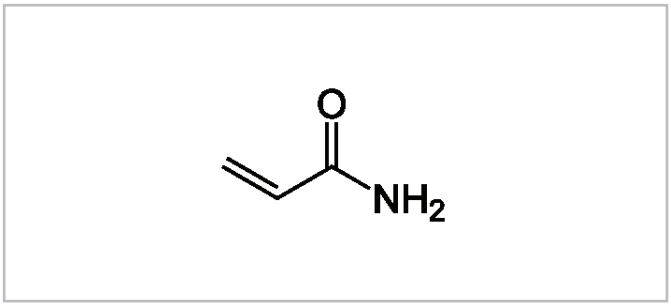

Figure 1. Structure of Acrylamide

Introduction

The method described in this application note achieves high recoveries of acrylamide in fried potato chips (crisps). The method is sensitive enough to measure levels as low as 10 ppb in a popular brand and flavor and has also been tested in flavored varieties that were both machine and hand fried.

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: A suitable sample of crisps (e.g. 25 g) was finely crushed to a consistent sample using a pestle and mortar before being transferred to an airtight container. When required, between 0.99 and 1.01 g of crushed crisps was accurately weighed into a 15 mL screw capped centrifuge tube. This was spiked with 10 µL of internal standard solution. The crisps were then left for approximately 30 minutes to allow the solvents to evaporate and the acrylamide and internal standard to soak into the crisps.

Water (10 mL) was added to each tube.

The tubes were rotated for at least one hour at a relatively slow speed e.g. 20 rpm, before centrifugation at 2875 g for 12 minutes. A 0.65 mL aliquot of the aqueous layer was removed taking care not to take up any of the thin upper oil layer.

Supported Liquid Extraction

Sample loading: Load pre-treated sample (0.65 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, 2 x 2.5 mL) and allow to flow under gravity into a tube containing 2 µL ethylene glycol in each well. 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 a SPE Dry (SD-9600-DHS or SD2-9600-DHS) (40 °C, 20 to 40 L min^{-1}) or TurboVap 96 (C103198 or C103199) (15 bar at 40 °C 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	1.16
Acrylamide $^{13}C_3$	1.16

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

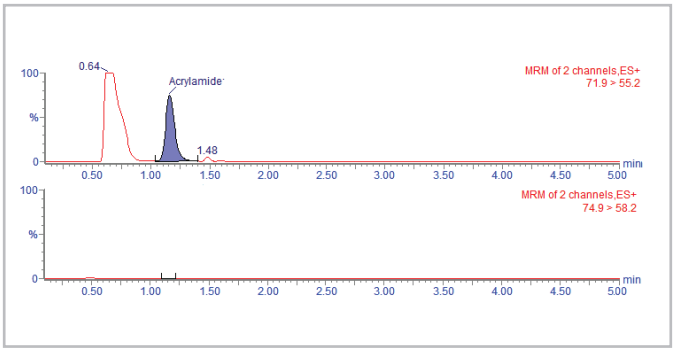

Figure 2. Extracted ion chromatograms in positive ion mode using ISOLUTE® SLE+ procedure (sample: 650 µL crisp extract, not spiked (process derived levels only)). Top trace = Acrylamide, Bottom trace = $^{13}C_3$ Acrylamide on an equivalent scale.

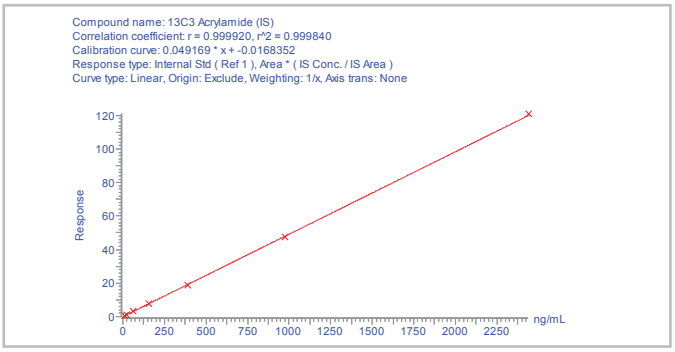

Figure 3. Calibration curve for $^{13}C_3$ Acrylamide in ground coffee, expressed on a linear scale. See additional notes below.

Table 3. Performance and recovery data for acrylamide and internal standard

Analyte	Recovery %	% RSD(n=6)
Acrylamide	90	6.3
$^{13}C_3$ Acrylamide	89	2.7

Recovery and RSD calculations based on extractions of blank matrix spiked at 1280 ng/mL without using an internal standard. The average blank response was subtracted from both extracted and fortified quantities prior to calculating both recovery and % RSD.

Table 4. Analyte performance from potato chips (crisps).

Analyte	r^2
Acrylamide	0.9998

r^2 calculations were based on a 'reversed' calibration line. The analyte was the internal standard $^{13}C_3$ acrylamide and the internal standard was acrylamide containing process derived and manually overspiked levels. Standards ranged from 10 to 1280 ng/g applying a weighting factor of 1/x.

Additional Notes

» Sensitive acrylamide calibration could not be directly demonstrated in crisps due to the presence of large levels of process produced analyte within the matrix giving a substantial intercept to the calibration line and inferior precision at low levels as a result. To demonstrate that analysis of acrylamide and its tri ^{13}C equivalent was sensitive and consistent the calibration line was prepared of the internal standard, $^{13}C_3$ acrylamide instead. Spiked acrylamide was added to the process produced acrylamide already present within the crisps and used as the internal standard. See **Figure 3**.

» A calibration line extracted without crisps gave greater recoveries however the ratio of acrylamide to $^{13}C_3$ acrylamide was broadly similar so this could be investigated as a possible calibration line for low level acrylamide analysis.

» The method was shown to work in salted and flavored potato chips (crisps) and also those that were labeled as "hand fried".

» 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.

Biotage®

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

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