Acrylamide analysis in ground coffee using a novel extraction method

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This technical note describes a method for the analysis of acrylamide in coffee with good matrix spike recovery and removal of known acrylamide interferences. The sensitivity of the SCIEX QTRAP 4500 system enabled an injection volume of 5 µL, while still achieving a calculated limit of quantitation (LOQ) of 45 µg/kg in coffee, below the target LOQ of 50 µg/kg specified in the AOAC Standard Method Performance Requirements (SMPR).[1] The mean absolute recovery in the matrix spikes was 76% and the mean internal standard (ISD)-normalized apparent recovery was 100% (5.3% CV, Figure 1). A novel sample preparation method was developed for the complex coffee extract, which consisted of a QuEChERS method with solid-phase extraction (SPE) cartridge clean-up. Spikes with 3 potential acrylamide interferences (N-acetyl-β-alanine, lactamide and 3-aminopropanamide) showed that these compounds were concurrently lost during sample preparation and chromatographically separated from the acrylamide. The method was applied to the analysis of 5 purchased ground coffees.

Key benefits of acrylamide analysis in coffee using the QTRAP 4500 system

- **Method accuracy and sensitivity:** The AOAC SMPR criteria were met with a mean absolute recovery of 76% and calculated LOQ of 45 µg/kg

- **Removal of known acrylamide interferences:** Sample preparation procedure did not significantly retain the interferences and they were chromatographically separated from the acrylamide peak

- **Good analyte retention and void volume separation:** The Phenomenex Luna™ Omega SUGAR column showed a retention factor (k') of 0.61 (retention time ~3.5 min) within the 9-min runtime

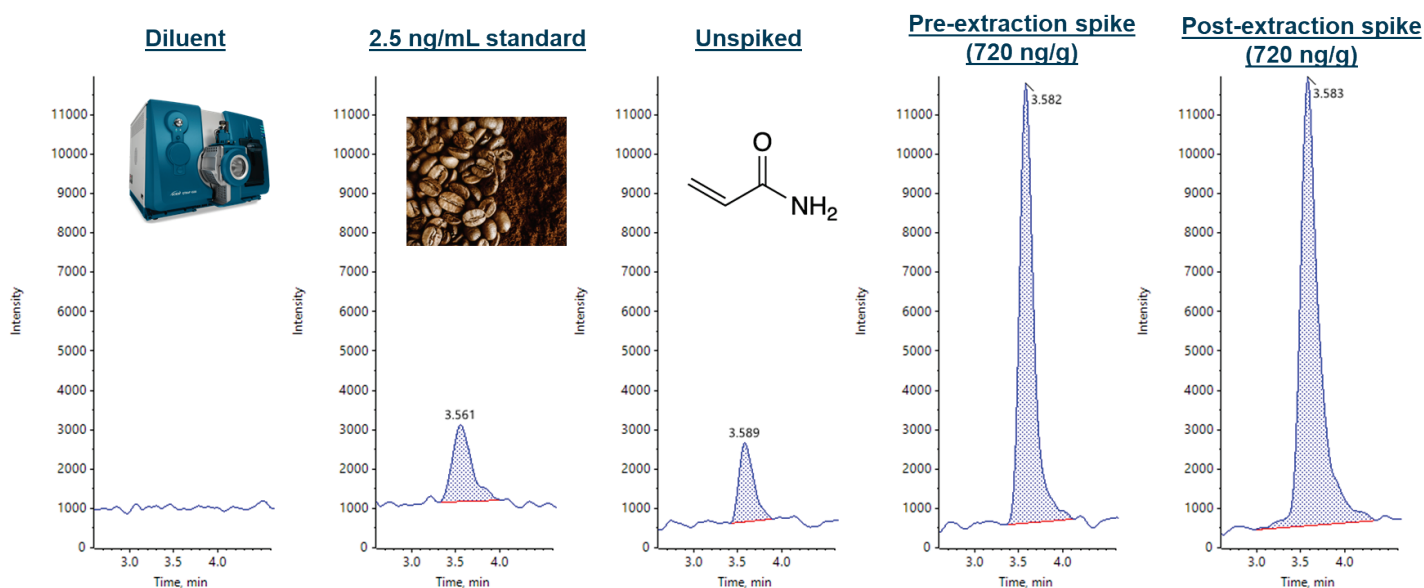## Pooled coffee matrix spikes

Figure 1. Representative extracted ion chromatograms (XICs) of acrylamide in blank diluent, 2.5 ng/mL solvent standard and pooled coffee matrix spikes. The XICs shown are from the quantifier transition (m/z 72.3/55.0). All y-axes are scaled to the same height for comparison. Pooled coffee matrix spikes represent the unspiked (blank) coffee. The pre-extraction and post-extraction spikes were performed at 720 ng/g. Similar area counts were achieved for the pre- and post-extraction spikes, demonstrating good absolute recovery in the pooled coffee.

Introduction

In 1994, the International Agency for Research on Cancer (IARC) classified acrylamide as carcinogenic to humans.[2] Acrylamide forms as a byproduct during food processing, especially when cooking starchy foods such as roasted coffee, French fries, potato chips, and cereals at high temperatures.[3] Excessive consumption of coffee on a regular basis can lead to high-level exposure to acrylamide, which can cause adverse effects in humans. Therefore, acrylamide levels in processed food products must be reliably quantified. This technical note presents a robust method for analyzing acrylamide in a complex coffee matrix using the QTRAP 4500 system.

Methods

Standard preparation: The acrylamide neat standard was purchased from Supelco (Sigma Aldrich) and the neat acrylamide-d_3 internal standard was purchased from Toronto Research Chemicals. Initial and intermediate stock solutions were prepared in LC-MS grade water. Final calibration standards were prepared in a solution of 90:10 (v/v), acetonitrile/water to final concentrations ranging from 2.5–500 ng/mL. The final acrylamide-d_3 concentration was 25 ng/mL in the calibration standards.

Procedural recoveries in the spiked coffee matrix: Five different ground coffee samples were purchased and pooled for the matrix spikes. Prior to the spiking experiments, the pooled coffee sample was analyzed to quantify the background acrylamide levels. The pooled coffee sample contained approximately 230 μg/kg of acrylamide. As prescribed by the AOAC SMPR document, matrices with high inherent acrylamide levels were spiked at 3–5x the background level. Therefore, the acrylamide spike concentration was 720 ng/g. The acrylamide-d3 internal standard was spiked at 1500 ng/g.

The potential impact of 3 known acrylamide inferences (*N*-acetyl-β-alanine, lactamide and 3-aminopropanamide) was evaluated by spiking 1 mL of LC-MS grade water before and after the extraction protocol was completed. The pre-extraction level was 150 ng/mL for acrylamide and 1500 ng/mL for the interferences.

Sample preparation: A 1 g sample of the pooled ground coffee was weighed into a 50 mL plastic centrifuge tube. Then, 20 mL of water was added and the resulting solution was vortexed for 20 minutes and centrifuged at 4500 rpm for 10 minutes. After centrifugation, 3 mL of the water layer was collected for SPE clean-up using Phenomenex Strata™ X cartridges (33 μm, polymeric reversed phase, 500 mg/6 mL, P/N: 8B-S100-HCH). Cartridges were conditioned with 5 mL of methanol followed by 5 mL of water. The 3 mL aqueous coffee extract was loaded and discarded. The acrylamide was eluted with 2 aliquots of 3 mL of water and collected in a clean 50 mL tube. Further cleanup was performed using a QuEChERS method by adding 3 mL of acetonitrile, 2 g of MgSO$_4$ and 2 g of NaCl to the aqueous SPE eluent. The solution was vortexed for 10 minutes and centrifuged at 4500 rpm. After centrifugation, 1 mL of the upper acetonitrile layer was collected and filtered using a Phenomenex 0.22 μm PTFE (hydrophobic) syringe filter (P/N: AF8-7702-12) prior to LC-MS/MS analysis. The sample preparation efficiency is visually shown in Figure 2.
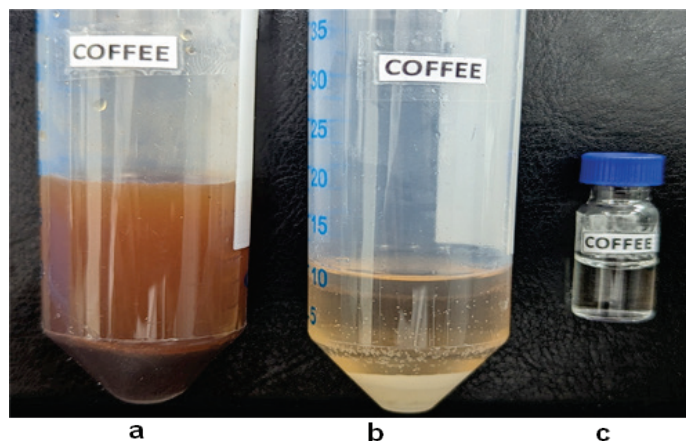

Figure 2. Representative coffee sample showing the method clean-up efficiency. a) The coffee matrix extract before the clean-up procedure. b) The extract after the addition of acetonitrile, MgSO$_4$ and NaCl. The water (lower) and acetonitrile (upper) layers are separated. c) The cleaned acetonitrile after centrifugation and syringe filtering.

Chromatography: Chromatographic separation was performed using the ExionLC AD system with a Phenomenex Luna Omega 3 μm SUGAR column (150 x 4.6 mm, P/N: 00F-4775-E0). Table 1 lists the gradient conditions used with a flow rate of 0.8 mL/min. Mobile phase A was 5mM ammonium formate in water (pH 3.22) and mobile phase B was acetonitrile. The injection volume was 5 μL and the

column oven temperature was set to 30°C. The autosampler cooler temperature was set to 10°C and 0.5 mL of rinsing solution was used for needle washing.

Table 1. Gradient program for the analysis of coffee using the QTRAP 4500 system.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0.0	2	98
4.0	2	98
4.2	50	50
6.5	50	50
7.0	2	98
9.0	2	98

Mobile phase A: 5mM ammonium formate in water (pH 3.22)
Mobile phase B: Acetonitrile
Rinsing solution: 50:50 (v/v), water/acetonitrile

Mass spectrometry: Samples were analyzed on the QTRAP 4500 system with electrospray ionization in positive ion mode. Acquisition was performed using multiple reaction monitoring (MRM) mode with 2 selective MRM transitions (Table 2). During the interference experiments, a pseudo-MRM was used for 3-aminopropanamide for additional confirmation. The source and gas parameters and compound-dependent MRM parameters used are presented in Table 3.

Table 3. Optimized source and gas parameters for the analysis of acrylamide in coffee using the QTRAP 4500 system.

Parameter	Value
Polarity	Positive
Ion source gas 1	60 psi
Ion source gas 2	50 psi
Curtain gas	35 psi
Source temperature	500°C
Ion spray voltage	5500 V
CAD gas	8 psi

Data processing: Data were processed using SCIEX OS software, version 2.1.6. For the post-spiked samples, spiking occurred in the final extract of the extracted blank sample. Assessment of the method recovery was performed using the raw acrylamide area count that was not normalized to the acrylamide-d3 internal standard response. This was considered the absolute recovery, as the internal standard presumably corrected for any loss during sample preparation. Pre- and post-extraction spikes were prepared in 5 replicate samples (n=5). Concentrations were blank-corrected using the unspiked matrix levels.

Table 2. Optimized compound-specific MRM parameters for the analysis of acrylamide in coffee using the QTRAP 4500 system.

	Q1 (m/z)	Q3 (m/z)	DP (V)	CE (V)	CXP (V)
Acrylamide_1	72.3	55.0	38	15	8
Acrylamide_2	72.3	27.2	38	33	8
Acrylamide-d_3	75.1	58.1	42	16	16
N-Acetyl-β-alanine_1	132.0	114.1	29	10	8
N-Acetyl-β-alanine_2	132.0	72.0	29	18	6
Lactamide_1	90.1	44.0	40	25	12
Lactamide_2	90.1	45.0	40	19	12
3-Aminopropanamide_1	89.0	72.0	36	17	6
3-Aminopropanamide_2	89.0	44.1	36	30	5
3-Aminopropanamide_3	89.0	89.0	36	5	10

Sensitivity, accuracy, precision and linearity of the solvent calibration standards

The performance of the QTRAP 4500 system for acrylamide analysis was evaluated through triplicate injections of the solvent calibration standards prepared in 90:10 (v/v), acetonitrile/water. The curve was linear across the range of 2.5–500 ng/mL with an r^2 of >0.996 and mean accuracies ranging between 97% and 108% (Table 4). The method showed excellent accuracy (101%) and precision (3.9% CV) with good peak shape at the LOQ of 2.5 ng/mL (Figure 1).

Table 4. The LOQ, linear dynamic range (LDR), correlation coefficient, accuracy range and precision range for acrylamide analysis using the QTRAP 4500 system. Injections were performed in triplicate.

LOQ (ng/mL)	LDR (ng/mL)	Correlation coefficient (r^2)	Accuracy (%)	Precision (%CV)
2.5	2.5–500	0.996	97-108	1.2-9.0

Investigation of acrylamide interferences: Chromatographic separation and removal during sample preparation

The Phenomenex Luna Omega SUGAR column provided good retention for acrylamide and separation from the void volume, while also separating the 3 interferences (3-aminopropanamide, lactamide and *N*-acetyl-β-alanine). A mixed standard containing acrylamide (100 ng/mL) and the 3 interferences (500 ng/mL) was injected to investigate potential coelution. As shown in Figure 2, the optimized chromatographic conditions resulted in ~1.5 min separation from lactamide (RT=5 min) and ~3.5 min separation from *N*-acetyl-β-alanine (RT=8 min). The 3-aminopropanamide

was not detected during any injection. It is postulated that the 2 amine functional groups resulted in very high retention for this compound on the column.

In an additional experiment, the 3 interference chemicals were spiked into the pooled coffee sample and processed through the sample preparation. Figure 3 shows the results of the pre- and post-extraction spikes for *N*-acetyl-β-alanine and lactamide. The *N*-acetyl-β-alanine was completely lost during the sample preparation, whereas only about 10% of the lactamide was retained. Overall, these results demonstrate that the interferences did not impact the acrylamide peak. That is, the chemicals were concurrently unretained during sample preparation and chromatographically separated from the target acrylamide peak.
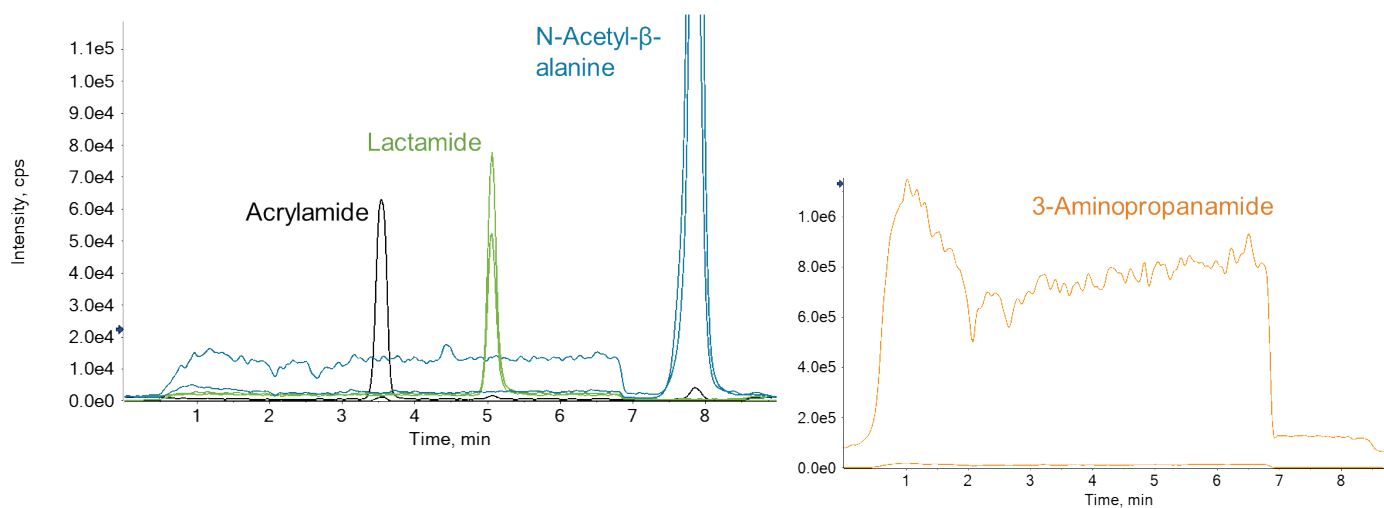

Figure 2. Overlaid XICs of acrylamide and the 3 interference compounds (lactamide, *N*-acetyl-β-alanine and 3-aminopropanamide) in a solvent standard. Quantifier and qualifier transitions are shown for all analytes. The results demonstrate that acrylamide was chromatographically separated from the 3 interferences by the Phenomenex Luna Omega SUGAR column. The XIC for 3-aminopropanamide is shown on a separate panel due to differences in the response amplitude. The pre-spike concentrations were 150 ng/mL for acrylamide and 1500 ng/mL for the interference compounds.
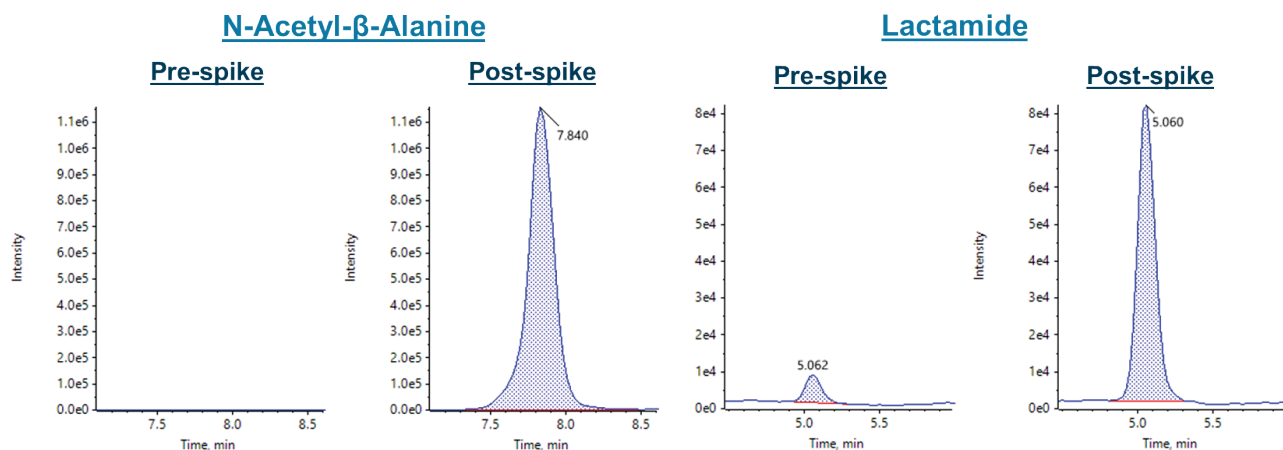

Figure 3. XICs for *N*-acetyl-β-alanine and lactamide spiked pre- and post-extraction. The *N*-acetyl-β-alanine was not retained during sample preparation, whereas approximately 10% of the lactamide was retained.

Recovery and precision in matrix spikes

Analysis of the pooled coffee sample showed incurred (blank) acrylamide levels of 230 µg/kg. According to the AOAC SMPR, the coffee matrix spikes (n=5) were performed at 720 µg/kg, at 3x the background concentration. Recovery values were blank-corrected to the mean unspiked acrylamide response and the results are shown in Table 5. The mean ISD-normalized apparent recovery was 100% (5.3% CV). The raw, non-ISD normalized pre- and post-spike mean recoveries were 88% (5.2% CV) and 115% (6.3% CV), respectively. The mean absolute recovery was 76%, representing the ratio of the non-ISD normalized pre- to post-extraction spike recoveries. Representative chromatograms of the coffee blank, pre- and post-extraction spikes are shown in Figure 1.

As per the AOAC SMPR, the LOQ was calculated by extrapolating the signal-to-noise (S/N) ratio in the blank coffee to a concentration corresponding to a S/N ratio of 10. The peak-to-peak S/N ratio for the blank coffee was approximately 60 and therefore the LOQ was conservatively set to 45 µg/kg.

Table 5. Mean recovery and %CV for acrylamide spikes in ground coffee. Matrix spikes were performed at 720 µg/kg due to background levels in the pooled coffee sample. Results are shown for the quantifier transition.

Apparent recovery (%)		Post-spike recovery (%)	Absolute recovery (%)
Raw recovery (%CV)	ISD-normalized recovery (%CV)	Raw recovery (%CV)	
88 (5.2)	100 (5.3)	115 (6.4)	76

Analysis of market samples

Five different coffee samples were purchased from a local store, processed through the extraction protocol and quantified against a single solvent calibration curve. Four of the 5 samples exhibited acrylamide concentrations greater than the solvent-based LOQ, at levels ranging from 179 ng/g to 245 ng/g (Table 6). Interestingly, the unroasted coffee was the only sample that did not contain detectable acrylamide levels.

Table 6. Acrylamide concentrations in 5 ground coffee products.

Sample	Concentration (ng/g)	%CV (n=3)
1	314	11
2	246	3.0
3	179	5.5
4 (Unroasted)	Not detected	Not detected
5	215	5.2

Conclusions

This technical note demonstrated:

- An LOQ of 2.5 ng/mL in the solvent-based standard using the QTRAP 4500 system

- Excellent accuracy (101%) and precision (3.9% CV) at the 2.5 ng/mL LOQ level

- Good retention of acrylamide using the Phenomenex Luna Omega SUGAR column and a retention factor (k') of 0.61 using the 9-min runtime

- A 76% mean absolute recovery of the matrix spikes and calculated LOQ of 45 µg/kg

- Effective removal of 3 known acrylamide interferences during the developed sample preparation procedure. These interferences were chromatographically separated from the acrylamide peak.

References

1. *Standard Method Performance Requirements* (SMPRs®) for Acrylamide in Potato Products, Baby Food, Bread, Other Cereal and Bakery Products, Cocoa Products, Coffee, Tea, Herbs and Spices (Including Their Extracts and Mixtures), Dry Pet Food, and Nuts. 2023 AOAC International. AOAC SMPR® 2022.006.

2. World Health Organization, International Agency for Research on Cancer. 1994. Lyon, France. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 60: Some industrial chemicals.

3. U.S. FDA. Acrylamide. Process Contaminants in Food. February 25, 2022.