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Automated Solid Phase Extraction (SPE)-LC-MS/MS Method for the Determination of Acrylamide in Brewed Coffee Samples

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Abstract
Acrylamide is thought to be produced during the roasting process associated with coffee production. Acrylamide has been labeled as a probable human carcinogen. Due to the use of roasted coffee beans in making coffee and the high consumption of coffee world-wide, brewed coffee could be a source of daily exposure to acrylamide. Acrylamide determination has been shown to be challenging due to presence of coextractives in the final extract. Manual solid phase extraction followed by LC-MS/MS analysis has been reported as a successful method for the determination of acrylamide from brewed coffee samples. However, performing solid phase extraction manually can be tedious and time consuming and there is increasing demand for automation of these methods.

In this study, we show that a manual SPE method used for the determination of acrylamide in brewed coffee can be converted to an autosampler-compatible cartridge format and automated using a robotic autosampler controlled by user-friendly software. Calibration standards prepared in freshly brewed green coffee (unroasted) resulted in a linear calibration curve (r^2=0.99) from 1 ng/mL to 500 ng/mL. Precision of the automated SPE-LC/MS/MS method was calculated as CV = 1.7 %.

Introduction

Solid phase extraction (SPE) is one of the most popular sample preparation methods employed by chromatographers, based on the numerous published SPE methods found in the literature. Typically, a liquid sample is passed across an adsorbent bed to retain and concentrate target analytes and eliminate interference from the sample matrix. Alternatively, the adsorbent can be used to retain interferences while allowing the target analytes to pass through.

Previous work has shown that since standard SPE cartridge sorbents are used with the GERSTEL SPE Option, established manual SPE procedures can be transferred directly, automated, and conveniently controlled by the MAESTRO software [1].

A solid phase extraction procedure for the separation of acrylamide from brewed coffee has previously been shown to be effective for subsequent accurate determination by LC-MS/MS [2]. This SPE method takes advantage of both the ability of an adsorbent bed to retain and concentrate the target analytes and eliminate interference from the sample matrix as well as the ability of a second adsorbent bed to be used to retain interferences while allowing the target analytes to pass through.

For illustrative purposes, Figure 1 shows a graphical representation of a typical automated SPE extraction process. After optimization of the SPE sorbent selection, the manual SPE procedure was translated into an automated SPE method.

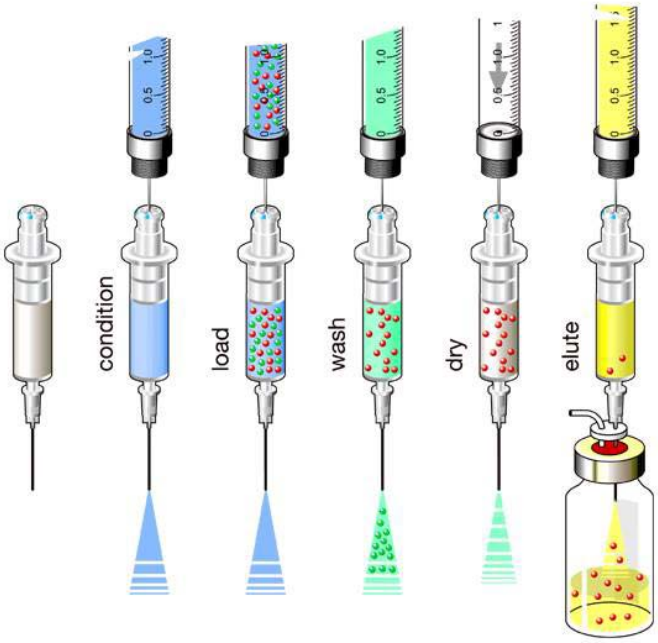

Figure 1. Graphical representation of a typical automated SPE procedure.

Experimental

Materials. Acrylamide (A3553-100G) was purchased from Sigma-Aldrich. D3-acrylamide (DLM-821-0.1), used as internal standard, was purchased from Cambridge Isotope Laboratories, Inc. Independent 1 mg/mL stock solutions of acrylamide and D3-acrylamide were prepared using 0.1 % formic acid in water. Intermediate acrylamide stock solutions were prepared by appropriately diluting the analyte stock solution with 0.1 % formic acid in water. A working internal standard stock solution was prepared at a concentration of 10 µg/mL using 0.1 % formic acid in water.

All ground coffee samples were brewed using an electric drip coffee maker. Ground coffee was added to a coffee filter (4.8 g/cup) and cold tap water was added to the water reservoir. After brewing, 10 mL of brewed coffee was transferred to a 10 mL glass screw top vial to be used for analysis.

Matrix matched calibration standards were prepared by making appropriate dilutions of the intermediate acrylamide stock solutions using freshly brewed green coffee (confirmed to have no acrylamide initially) to give the concentrations listed in Table 1. Following preparation, 10 mL of each standard was transferred to a 10 mL glass screw top vial to be used for analysis along with the other brewed coffee samples.

3 mL Strata-X SPE cartridges and 3 mL AccuCAT SPE cartridges, both in the GERSTEL SPE format, were placed in series onto the appropriate autosampler tray.

Instrumentation. All automated SPE PrepSequences were performed using a MultiPurpose Sampler (MPS XL) in the Dual Head configuration fitted with the GERSTEL SPE Option as shown in Figure 2. All analyses were performed using an Agilent 1290 HPLC with a Phenomenex Synergi Hydro-RP column (2.0 x 250 mm, 4 µm, 80 Å), an Agilent 6460 Triple Quadrupole Mass Spectrometer with Jet stream electrospray source and GERSTEL MPS XL autosampler configured with an Active Wash Station. Sample injections were made using a 6 port (0.25 mm) Cheminert C2V injection valve outfitted with a 10 µL stainless steel sample loop.

Figure 2. MultiPurpose Sampler (MPS XL) with GERSTEL SPE Option.

Analysis conditions LC.

Pump:	flowrate = 0.2 mL/min
Mobile Phase:	A - 5 mM formic acid in water
	B – acetonitrile
Gradient:	isocratic, 5 % B
Run time:	10 min
Injection volume:	10 µL (loop over-fill technique)
Column temp.:	45°C

Analysis conditions MS.

Operation:	electrospray positive mode
Gas temp.:	350°C
Gas flow (N_2):	5 L/min
Nebulizer pressure:	35 psi
Sheath Gas Heater:	250°C
Sheath Gas Flow:	11 L/min
Capillary voltage:	4000 V
Delta EMV:	+700 V

MS/MS parameters.

D3 – Acrylamide:	m/z 75.1 -> 58.1
Acrylamide:	m/z 72.1 -> 55.1
Dwell:	100 msec
Fragmentor voltage:	52 V
Collison energy:	8 V
Cell accelerator voltage:	7 V

Results and Discussion

The manual steps in the published SPE procedure [2] include:

1. A 3 mL/200 mg Phenomenex Strata-X SPE cartridge is conditioned using 3.5 mL of methanol followed by 3.5 mL of water.
2. 1.5 mL of brewed coffee sample is loaded onto the conditioned SPE cartridge and allowed to pass through the sorbent material, followed by 0.5 mL of water.
3. The cartridge is eluted with 1.5 mL of water and the eluate collected for further cleanup.
4. A 3 mL/200 mg AccuCAT SPE cartridge is conditioned with 2.5 mL of methanol followed by 2.5 mL of water.
5. 0.5 mL of the eluate collected from Strata-X cartridge is loaded onto the conditioned SPE cartridge and allowed to pass through the sorbent material to waste.
6. 0.8 mL of the eluate collected from the Strata-X cartridge is loaded onto the SPE cartridge and allowed to pass through the sorbent material and the eluate is collected.
7. 10 µL of the collected eluate from step 6. is injected for LC-MS/MS analysis.

We first determined that the electric drip coffee maker was free of acrylamide by taking the appropriate amount of cold tap water through a brewing process without the presence of ground coffee. The resulting water was then processed using the SPE procedure and analyzed using the LC-MS/MS method. No acrylamide was detected from the extracted water sample.

The manual SPE procedure was translated into a MAESTRO Prep Sequence and was demonstrated to successfully prepare sample extracts for subsequent analysis by LC-MS/MS. The Phenomenex Strata-X sorbent was used as a recommended alternative SPE sorbent since, during the course of method development, it was found that samples processed using the Strata-X sorbent resulted in higher peak area responses when compared to samples processed using the original SPE sorbent [2].

Figure 3 shows, (from left to right),

1) a brewed coffee sample,
2) the Phenomenex Strata-X, 3 mL/200 mg, SPE cartridge in the GERSTEL format after completing step 3 of the automated SPE procedure,
3) the eluate resulting from step 3 of the automated SPE procedure,
4) the Varian Bond Elut AccuCAT, 3 mL/200 mg, SPE cartridge in the GERSTEL format after completing step 6 of the automated SPE procedure, and
5) the eluate resulting from step 6 of the automated SPE procedure.

As shown in Figure 3, the automated SPE method provides a clear eluate for LC-MS/MS analysis.

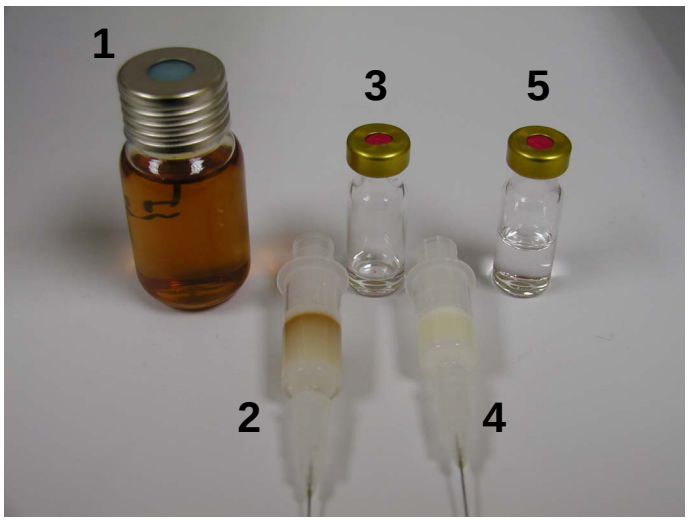

Figure 3. Representative picture showing an example brewed coffee sample at the various steps of the SPE procedure.

Figure 4 shows an overlay of the mass chromatograms for all calibration curve samples. The limit of quantitation was found to be 1 ng/mL acrylamide in brewed green coffee.

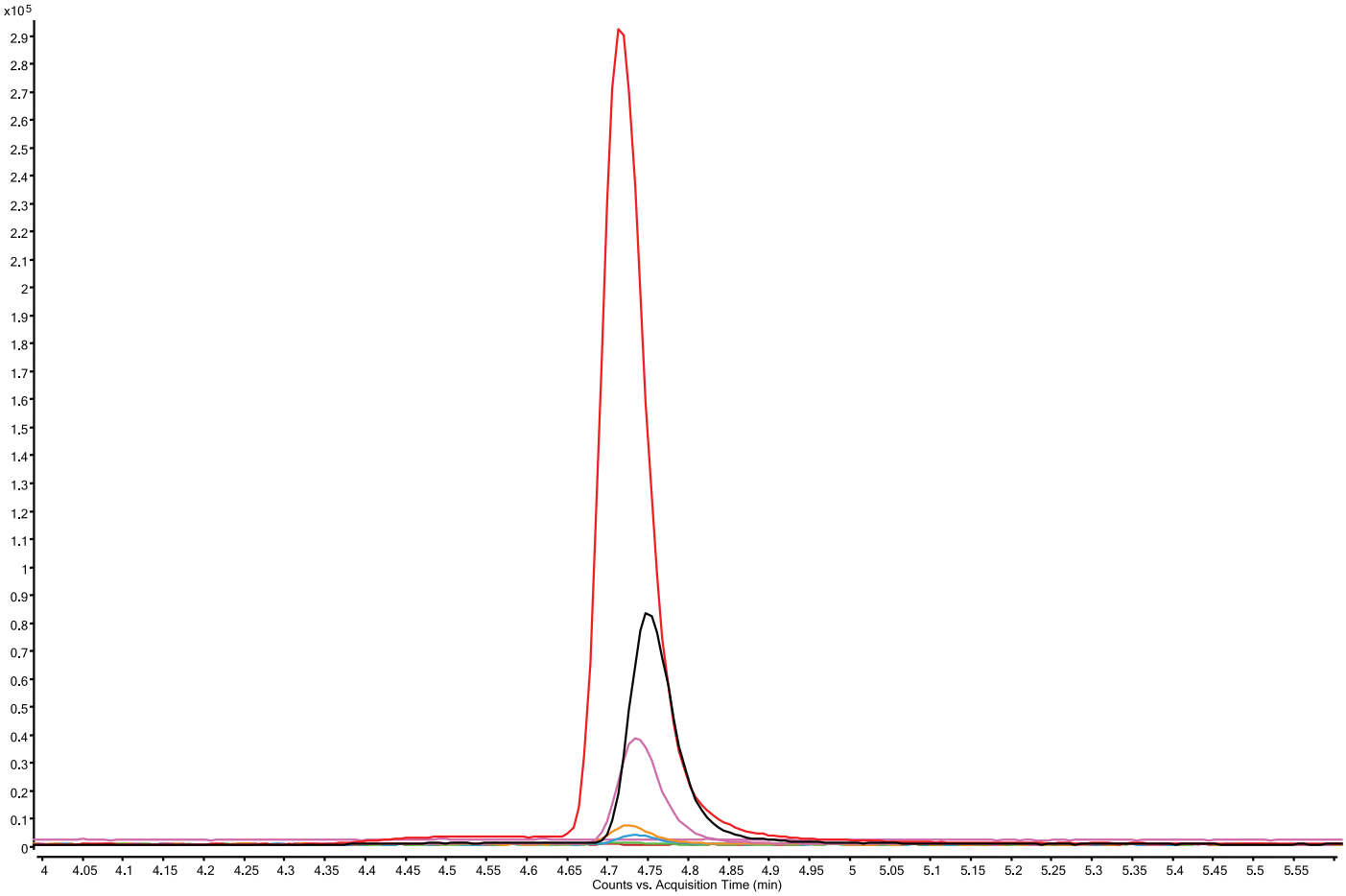

Figure 4. Overlay of mass chromatograms from calibration curve samples.

The calibration curve is shown in Figure 5. As can be seen, calibration standards prepared in freshly brewed green coffee (unroasted) resulted in a linear calibration curve (r^2=0.99) from 1 ng/mL to 500 ng/mL.

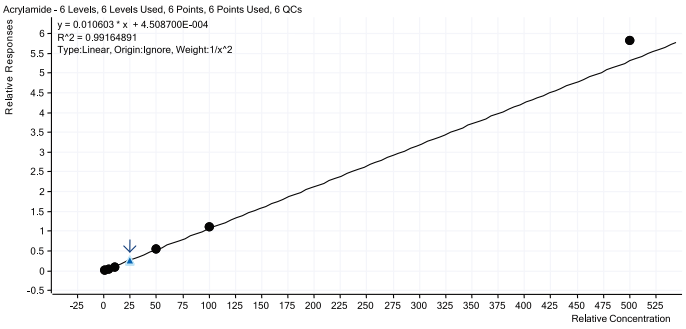

Figure 5. Acrylamide calibration curve.

Accuracy and precision of the automated SPE method was assessed using six independently prepared 25 ng/mL acrylamide QC samples. As shown in Table 1, the average % accuracy was found to be 97.9 % and the precision of the automated SPE-LC-MS/MS method was calculated as CV = 1.7 %.

Table 1. Automated SPE-LC-MS/MS: accuracy and precision results

	(Peak Area Ratio)	Calc. % Accuracy
Replicate 1	0.264	99.3
Replicate 2	0.267	100
Replicate 3	0.260	97.8
Replicate 4	0.255	96.1
Replicate 5	0.256	96.5
Replicate 6	0.259	97.7
mean	0.260	97.9
SD	0.00433	1.63
% CV	1.66	1.67

Figure 6 shows a comparison of a blank water sample, an extracted blank brewed green coffee sample, and an extracted 1 ng/mL brewed green coffee calibration standard. No acrylamide was detected for an extracted blank brewed green coffee sample.

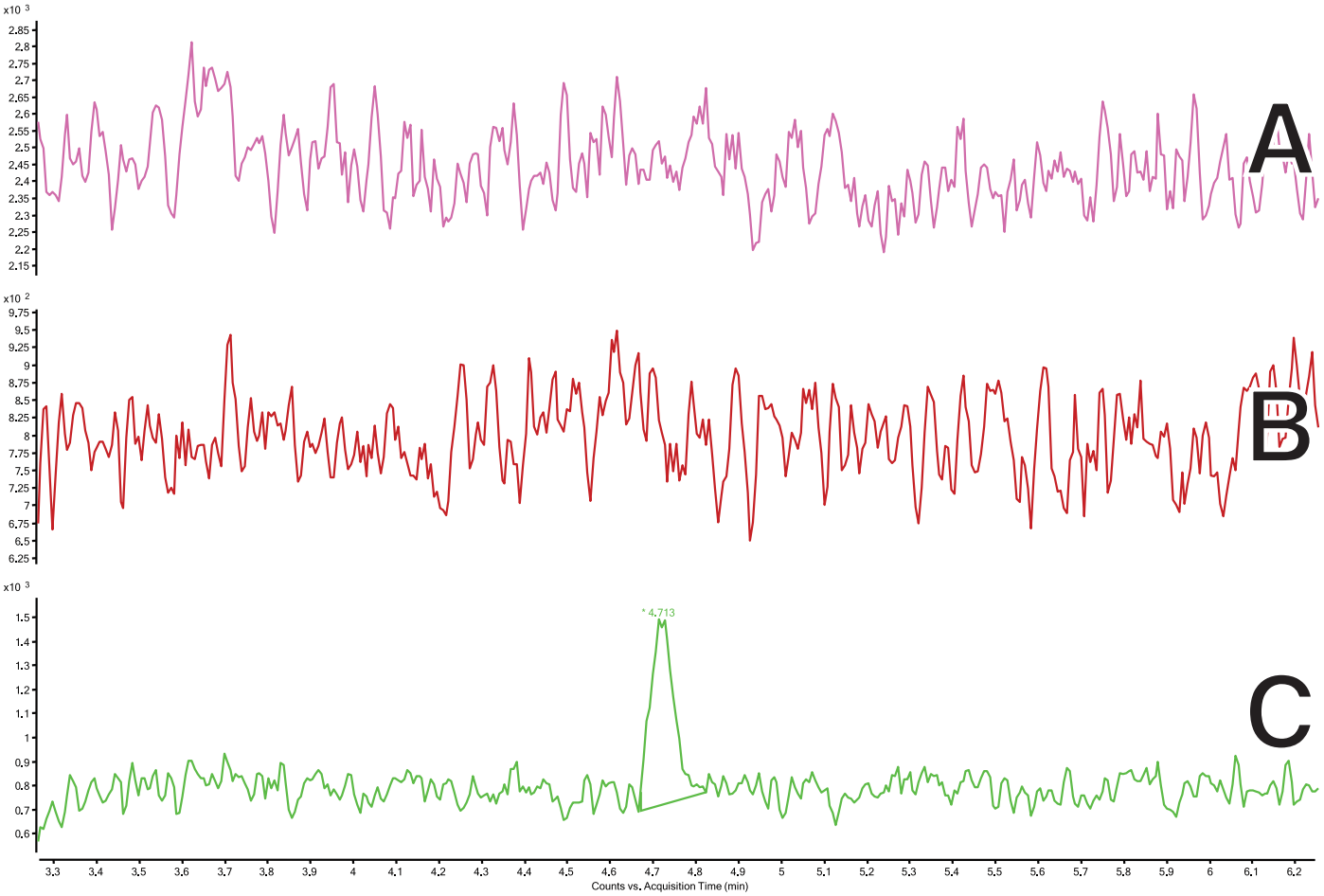

Figure 6. Comparison of blank water sample (A), extracted blank brewed green coffee sample (B), and extracted 1 ng/mL brewed green coffee calibration standard (C).

Figure 7 shows a comparison of an extracted 1 ng/mL acrylamide calibration standard in brewed green coffee to representative mass chromatograms from two (2) extracted blank brewed coffee samples (Brand A and Brand B). Acrylamide was observed to be present in both of these coffee samples. All samples were analyzed with and without the addition of internal standard.

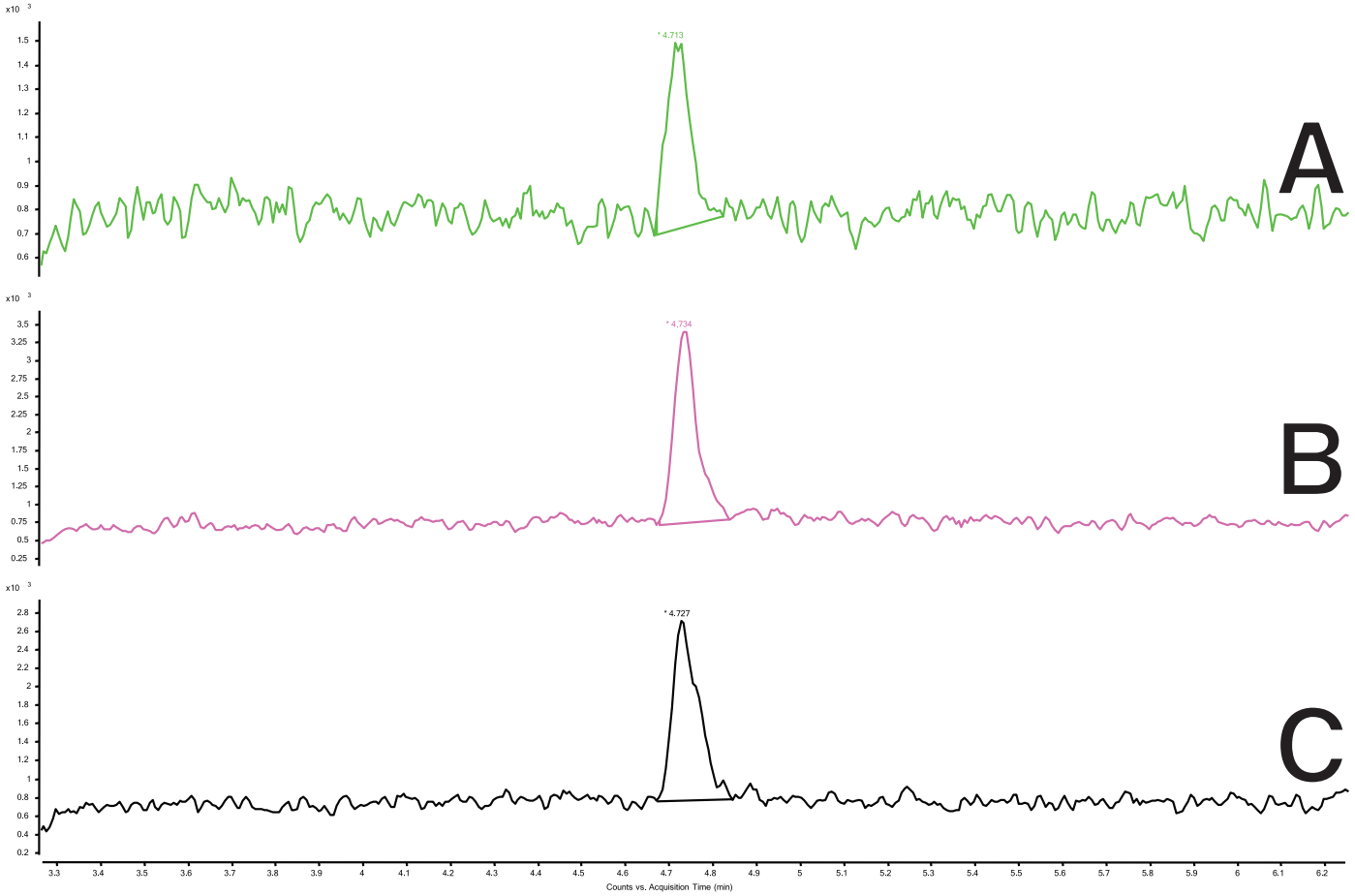

Figure 7. Comparison of extracted 1 ng/mL acrylamide calibration standard in brewed green coffee (A), extracted blank brewed brand A coffee sample (B), and extracted blank brewed brand B coffee sample (C).

To ensure that the SPE-LC-MS/MS method would result in accurate determination of the concentration of acrylamide in brewed coffee samples, independent of matrix differences, a standard was added to a blank brewed coffee sample, spiking it with 10 ng/mL acrylamide and then analyzing it in triplicate. The blank brewed sample was also analyzed in triplicate. As shown in Table 2, the resulting difference between the calculated acrylamide concentration from the extracted blank samples and that of the extracted spiked samples was found to be 11 ng/mL, indicating average recovery of 110 %.

Table 2. Results of acrylamide spiking experiment.

Sample Name	Acrylamide Response	D3-Acrylamide (ISTD) Response	Calc. Conc.	Blank Subtr. Calc. Conc.
Brewed coffee (Run 1)	9966	211092	4.84	-
Brewed coffee (Run 2)	8686	201278	4.40	-
Brewed coffee (Run 3)	7904	191375	4.19	-
Brewed coffee spiked with 10 ng/mL (Run 1)	28465	201476	15.1	10.7
Brewed coffee spiked with 10 ng/mL (Run 2)	29514	207499	15.2	10.8
Brewed coffee spiked with 10 ng/mL (Run 3)	31121	207502	16.1	11.6
			mean	11.01
			SD	0.522
			% CV	4.74
			% Recovery	110

A total of eleven (11) different commercial coffee samples were brewed and then analyzed in duplicate using the automated SPE-LC-MS/MS method. As shown in Table 3, acrylamide was detected in all but one of the brewed coffee samples examined.

Table 3. Brewed coffee analyses results.

Brewed Coffee Sample	Average Calc. Conc. [ng/mL]
Brand A	4.21
Brand B	3.45
Brand C	3.14
Brand D	2.17
Brand E	-
Brand F	2.76
Brand G	1.97
Brand H	5.63
Brand I	6.21
Brand J	2.42
Brand K	5.84

Conclusions

As a result of this study, we were able to show:

- A manual SPE procedure could be readily automated using the Dual Head GERSTEL MPS XL sampler and SPE sorbents in cartridges formatted for automation.
- A linear calibration curve resulting in an r^2 value of greater than 0.99 was achieved with a limit of quantitation for acrylamide of 1 ng/mL.
- The SPE-LC-MS/MS method proved to be accurate and precise. Accuracy data averaged 97.9 % (range: 96.1 % - 100 %) and a precision result of CV = 1.67 % for acrylamide in QC samples was achieved.
- Acrylamide was found in ten of the eleven brewed coffee samples analyzed using the automated SPE-LC/MS/MS method.

References

[1] F. Foster, J. Stuff, J. Whitecavage, and E. Pfannkoch, “Automation of Solid Phase Extraction Methods using a Robotic X-Y-Z Coordinate Autosampler with Software Control”, AppNote 3/2009, www.gerstelus.com

[2] D. Andrzejewski, J. Roach, M. Gay, and S. Musser, “Analysis of Coffee for the Presence of Acrylamide by LC-MS/MS” J. Agric. Food Chem. 2004, 52, 1996-2002.

G L O B A L A N A L Y T I C A L S O L U T I O N S

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