Application Note

Food Testing & Agriculture

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Quantification of Acrylamide in a Variety of Food Matrices by LC/MS/MS Triple Quadrupole

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Abstract

This Application Note describes a method for the quantitation of acrylamide in food matrices using an Agilent 1290 Infinity II LC coupled to an Agilent 6470A triple quadrupole LC/MS system with Agilent MassHunter workstation software. The method was applied to raw potato, potato chips (three different brands), processed black olives, raw and roasted salted almonds, raw hazelnut, roasted salted pistachios, and ready-to-drink coffee, achieving limits of quantification (meeting acceptable recovery, precision, and signal-to-noise (S/N) criteria) of 2.5 ng/g.

Introduction

Acrylamide has been discovered to be present in cooked food, especially in heat-processed products such as potato chips, and roasted and bakery products[1,2]. Therefore, acrylamide content is commonly monitored in certain processed foods. Figure 1 gives the structure of acrylamide.

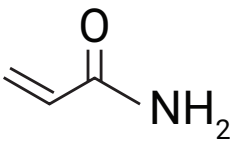

Figure 1. Structure of acrylamide.

To provide identification and quantification of acrylamide in a variety of food matrices, this study developed and evaluated a fast, high-throughput, and sensitive method based on UHPLC/MS/MS. The isotopically labeled internal standard acrylamide-$^{13}C_3$ was added to compensate for matrix effects. Method criteria for data acceptance were established.

Experimental

Equipment

All experiments in this study were performed using an Agilent 1290 Infinity II LC consisting of an Agilent 1290 Infinity II multisampler (G7167B), an Agilent 1290 Infinity II high speed pump (G7120A), and an Agilent 1290 Infinity II multicolumn thermostat (G7116B) coupled to an Agilent 6470A triple quadrupole LC/MS system (G6470A). Instrument control, data acquisition, qualitative and quantitative data analysis, and reporting were done using Agilent MassHunter workstation software.

Chromatographic conditions

Parameter	Setting
Analytical Column	Porous graphitic carbon, 100 × 3 mm, 5 µm
Column Oven	60 ±2 °C
Injection Volume	5 µL
Run Time	8 minutes
Autosampler	5 ±2 °C
Mobile Phase A	0.1% AcOH in water
Mobile Phase B	0.1% AcOH in methanol
Seal Wash	60:40 ACN:IPA
Needle Wash	30:70 MeOH:H_2O

Gradient settings

Time (min)	Flow (mL/min)	%A	%B
0	0.25	90	10
5.5	0.25	90	10
6.0	0.25	10	90
8.0	0.25	10	90
8.1	0.25	90	10
13.0	0.25	90	10

MS parameters

Parameter	Setting
MS Acquisition	MRM
Stop Time	8 minutes
Ion Source Type	Agilent Jet Stream electrospray ionization (AJS ESI positive)
Drying Gas Temperature	325 °C
Drying Gas Flow	12 L/min
Nebulizer	60 psi
Sheath Gas Heater	350 °C
Sheath Gas Flow	12 L/min
Capillary	2,500 V
Nozzle Voltage	1,000 V
Precursor Ion and Production Ion Resolution	Unit
Compound-Specific Conditions	See Table 1

Samples and standards

The study matrices included raw potato, potato chips (three different brands), processed black olives, raw and roasted salted almonds, raw hazelnut, roasted salted pistachios, and ready-to-drink coffee. Acrylamide native and isotope labeled standards ($^{13}C_3$, IS) were obtained from Cambridge Isotope Laboratories, Inc. (Tewksbury, MA, USA). The samples and standards were stored refrigerated at 5 °C.

Method

Description

Below is the detailed description of sample preparation. Table 1 gives the analyte-specific LC/MS conditions.

1. Weigh 1 g of sample (weigh 10 g for coffee drinks).
2. Spike with target analyte solution and analyte IS spike solution.
3. Add 10 mL of water, and vortex (no water needs to be added for coffee drinks).
4. Add 10 mL of ACN; shake for 10 minutes.
5. Add 1.3 to 1.5 g 4:1 (w:w) $MgSO_4$:NaCl salt (Agilent QuEChERS pouch, p/n 5982-5550); vortex.
6. Centrifuge at 4,000 rpm for six minutes.
7. Draw 1 mL of upper organic layer to a 2.0 mL microcentrifuge tube.
8. Add 1 mL of water (dilution factor (DF) of 20); vortex.
   Or
   Draw 2 mL of the upper layer into a 15 mL centrifuge tube; evaporate the sample to dryness.
9. Add 1 mL of water to the tube (DF of 5); vortex.
10. Filter through a 0.2 µm Agilent Captiva premium syringe filter (p/n 5190-5132).
11. Ready for LC/MS injection using positive ESI mode.

Table 1. Analyte-specific LC/MS conditions: precursor-to-product ion transitions, fragmentor, collision energies (CE), cell accelerator voltage (CAV), and retention times (RT).

Compound	Type	Precursor Ion (*m/z*)	Product Ion (*m/z*)	RT (min)	Dwell Time (ms)	Fragmentor (V)	CE (V)	CAV (V)
Acrylamide	Target	72.1	55	3.8	200	80	5	5
Acrylamide	Target	72.1	44	3.8	200	80	5	5
Acrylamide	Target	72.1	27	3.8	200	80	8	5
Acrylamide IS	IS	75.1	58	3.8	200	80	5	5

Evaluation procedure

Due to most matrices in this study containing different amounts of acrylamide, the method performance was evaluated by analyzing a representative raw potato sample (as a matrix blank) together with four replicates of low QC spiking levels at 2.5, 5, and 10 ng/g depending on the dilution factor in the sample preparation.

Method performance was also verified by analyzing additional sample matrices (potato chips, processed black olives, raw and roasted salted almonds, raw hazelnut, roasted salted pistachios, and ready-to-drink coffee) with QC spiking at 500 ng/g. To further confirm the method reproducibility, roasted salted almonds and roasted salted pistachios were analyzed in four replicates.

The quantitation was performed using an isotopically labeled internal calibration curve with 1/x weight.

Evaluation criteria

Specificity:

- The relative error (RE%) of retention time of each analyte peak to the average of standard peaks is less than 2 %.
- The ion ratio is within the tolerance of 30 %.

Linearity and range:

- The calibration curve has R^2> 0.99.
- The residual of each working standard is within ±15 %.
- The calibration standards should bracket the analyte concentration level.

Precision:

- RSDs from at least three replicates are ≤20 %.

Accuracy:

- Spike recovery is within 70 to 130 %.

Results and discussion

Sample extraction approach

Salt-assisted water and acetonitrile layer partition/extraction proved to be an easy and effective approach for acrylamide analysis in various matrices. Most ionic interferences would stay in the aqueous layer while acrylamide is extracted into the organic layer. The acetonitrile layer can then be further diluted with water or dried down/reconstituted with water to achieve the lower limit of quantitation (LOQ) if needed. Figure 2 demonstrates the supernatant cleanness after salt-assisted (mixture of $MgSO_4$ and NaCl) liquid-liquid partition (10 mL of H_2O:10 mL ACN) comparing to that of direct water (20 mL) extraction.

Specificity

Multiple reaction monitoring (MRM) was used for acrylamide detection. Monitoring MS/MS transitions with evaluation of the ratio of their relative intensities and RT of analyte peaks enables the target analyte to be distinguished from potential interferences in quantitative analysis. Figure 3 shows an example of an extracted ion chromatogram of a 10 ng/mL working standard in ultrapure water. Figure 4 shows that a reagent blank spiked with internal standard is free of analytes in water.

Matrix effect

Interfering substances in the matrix may be observed, and affect MS ionization, causing ion suppression or enhancement.

Matrix effect in this study was evaluated as:

Matrix effect = Response of IS in matrix extract/response of IS in neat standard – 1

A negative value means ion suppression, and a positive value means enhancement. Currently, there is no guideline for matrix effect due to the variation in method and instrument performance. However, matrix effects should be considered when setting up LOQ for it to pass the criteria of recovery, %RSD, and S/N (~10 for LOQ).

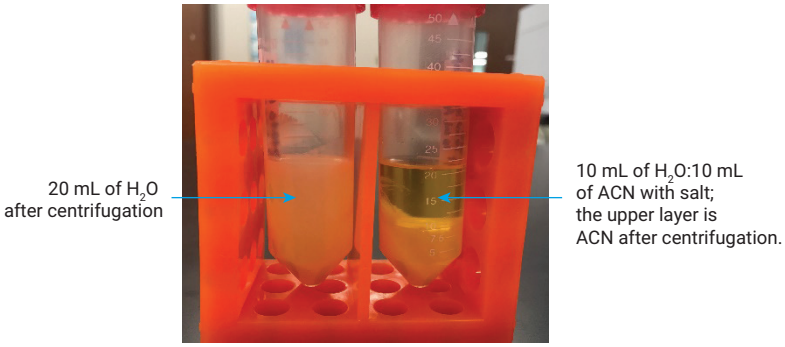

Figure 2. Comparison of the effect of extraction by 1:1 H_2O:ACN with salt-assisted partition and by direct water extraction.

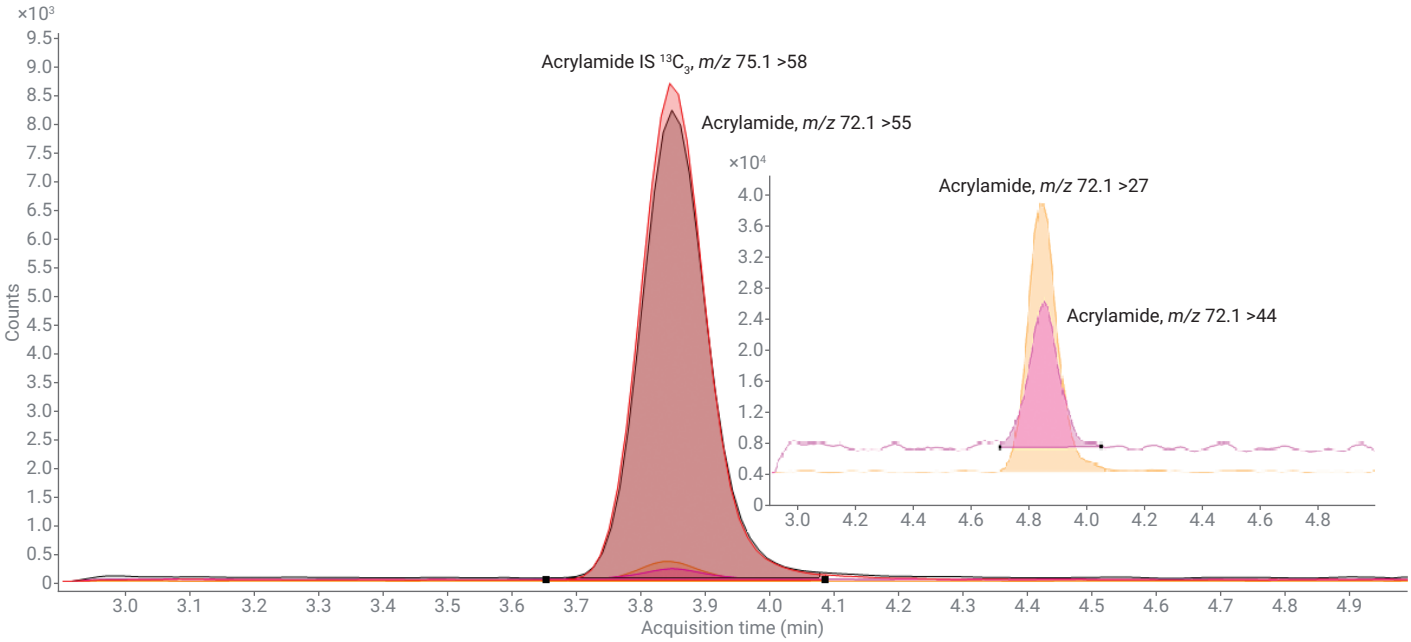

Figure 3. Extracted ion chromatogram of acrylamide working standard at 10 ng/mL with IS at 10 ng/mL in water.

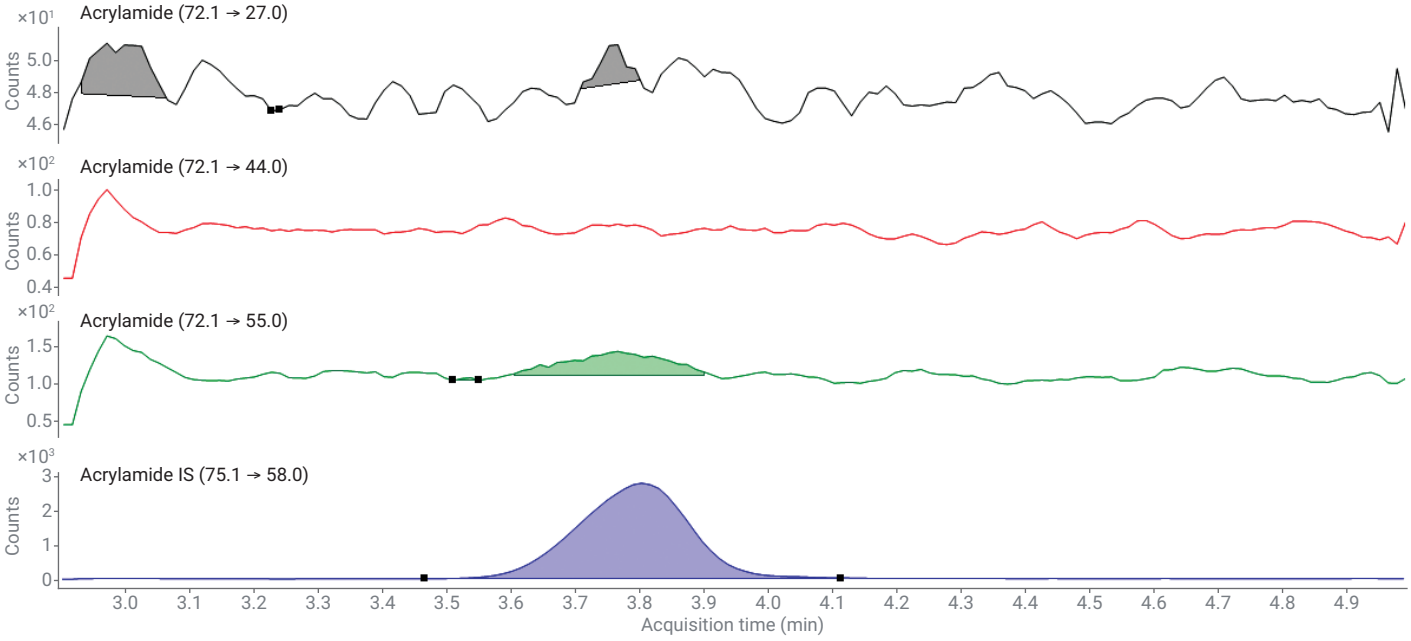

Figure 4. Extracted ion chromatogram of a reagent blank.

Although all the sample matrices in this study experienced ion suppression (see Figure 5 and Table 4 in the Sample tests section), the LOQ was set up to me*et all* the criteria.

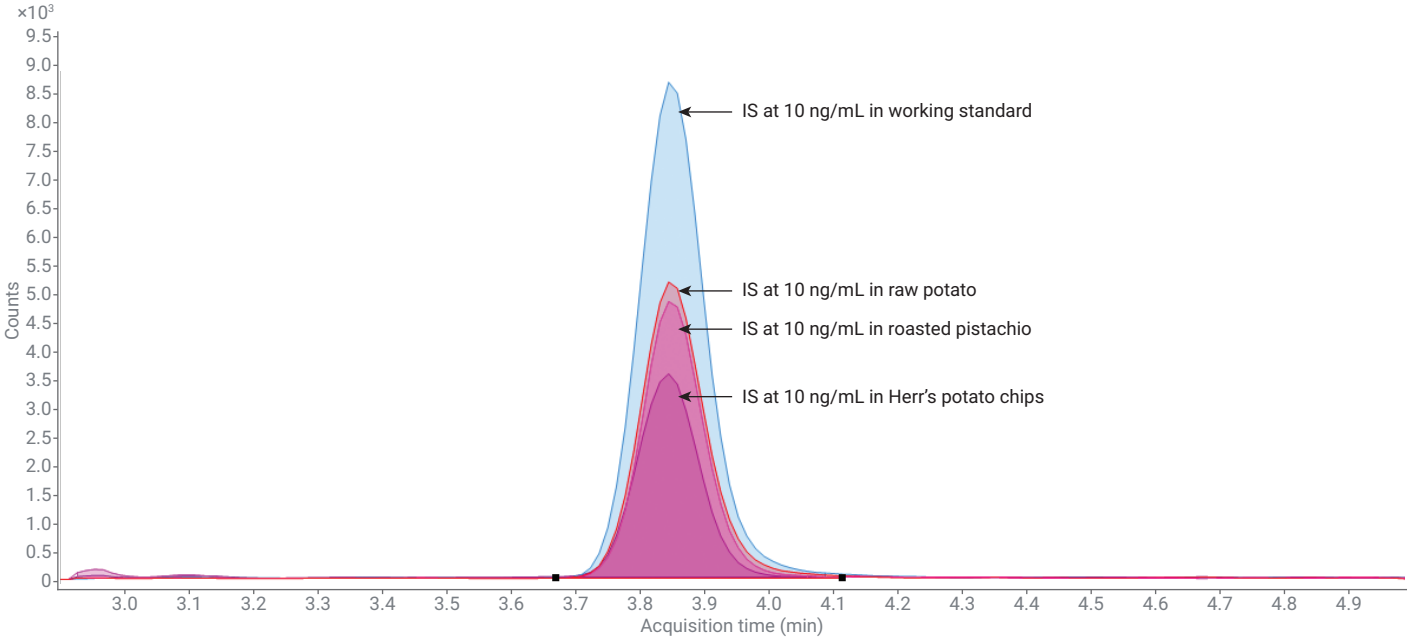

Figure 5. Comparison of acrylamide internal standard response in matrix extracts to that in neat standard.

Range and linearity

The method was evaluated over the range of 0.1 to 200 ng/mL.

To evaluate the linearity of the method, eight working standard (WS) solutions of acrylamide containing internal standard acrylamide $^{13}C_3$ were made at 0.1, 0.5, 1, 5, 10, 50, 100, and 200 ng/mL. The calibration curve residuals were ≤15 % for WS1 to WS8. The linearity was determined using an isotopically labeled internal calibration with a 1/x weighting factor. The coefficient of determination (R^2) values were >0.99. Figure 6 gives an example of the calibration curve.

Accuracy and precision

Accuracy was determined by fortifying samples before extraction with the analyte standard solution at levels of 2.5, 5, and 500 ng/g with a dilution factor of 5 in sample preparation and 10 and 500 ng/g with a dilution factor of 20 in sample preparation. The results were corrected using internal standards, which were fortified at 10 ng/mL for all samples. Accuracy for samples that contain acrylamide (see Table 4) was based on spiked/unspiked values as determined by internal standard.

The precision was evaluated by analysis of fortification of raw potato at levels of 2.5, 5, and 10 ng/g in four replicates. The precision was further verified by analysis of two unspiked native samples of roasted salted almonds and pistachios in four replicates.

Acceptable acrylamide recoveries (within 70 to 130 %) and %RSD (≤20 %) were obtained for all replicates. Table 2 shows the accuracy and precision results at 2.5, 5, 10, and 500 ng/g. Table 3 shows the precision results of the two native samples.

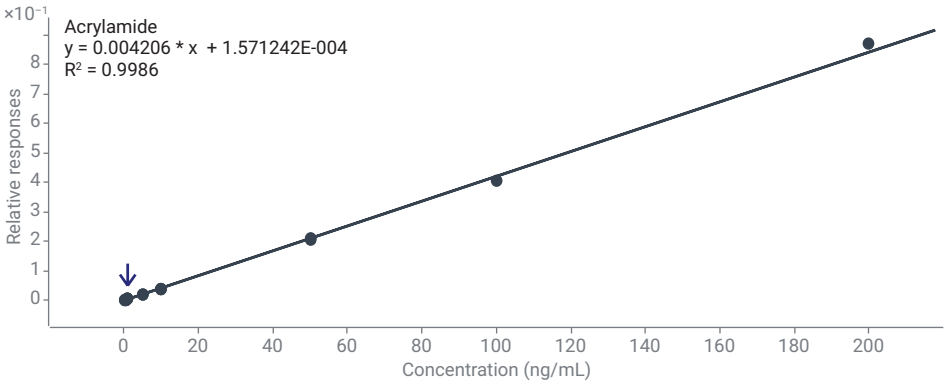

Figure 6. Calibration curve for working standards.

Table 2. Accuracy (spike recovery, %) and precision (%RSD) at 2.5, 5, and 10 ng/g and accuracy at 500 ng/g.

Spike Level	Matrix	2.5 ng/g	5 ng/g	10 ng/g		500 ng/g	
Dilution Factor		5	5	5	20	5	20
Replicates		n = 4	n = 4	n = 1	n = 4	n = 1	n = 1
Recovery at LOQ Level (Average) (%)	Raw potato	89.1	98.3		85.5		
RSD at LOQ Level (%)		19.3	11.0		10.7		
Recovery for Different Matrices (%)	Raw almond			115	102	92.0	104
	Raw hazelnut					94.6	107
	Roasted almond					95.0	104
	Roasted pistachio					86.1	95.3
	Black olive pitted					103	126
	Lay's Baked with sea salt					87.4	103
	Herr's Kettle salt and vinegar					97.7	90.4
	Cheese Crunch					106	103
	Coffee, ready to drink						96.7

Table 3. Precision (%RSD) of unspiked roasted almond and roasted pistachio.

Sample Identification	Dilution Factor	Sample Result (ng/g)
Roasted Almond 1	20	529.9
Roasted Almond 2	20	565.3
Roasted Almond 3	20	594.7
Roasted Almond 4	20	579.7
	Average	567.4
	Std. dev.	27.73
	RSD (%)	4.9
Roasted Pistachio 1	5	13.3
Roasted Pistachio 2	5	11.5
Roasted Pistachio 3	5	13.4
Roasted Pistachio 4	5	11.5
	Average	12.4
	Std. dev.	1.07
	RSD (%)	8.6

LOQ

Figure 7 shows each extracted ion chromatogram of acrylamide and IS at the lowest standard level of 0.1 ng/mL, and LOQ corresponding level of 0.5 ng/mL.

Raw potato was used to demonstrate the establishment of method LOQs because most matrices included in this study contain acrylamide. Raw potato was evaluated at fortified levels of 2.5 and 5 ng/g with a sample DF of 5, and 10 ng/g with a sample DF of 20. The acceptable recovery and RSD% were obtained for all spiking levels (Table 2). Figure 8 shows an example of the raw potato with spiking levels of 2.5 and 5 ng/g having an S/N >10 with qualifier ion transition. The matrix effect of raw potato is similar with that of other matrices. The LOQ for this method is set at 2.5 ng/g with DF of 5 and 10 ng/g with DF of 20.

LOQ in sample calculation:

0.5 ng/mL × 5 mL (DF)/1 g = 2.5 ng/g

0.5 ng/mL × 20 mL (DF)/1 g = 10 ng/g

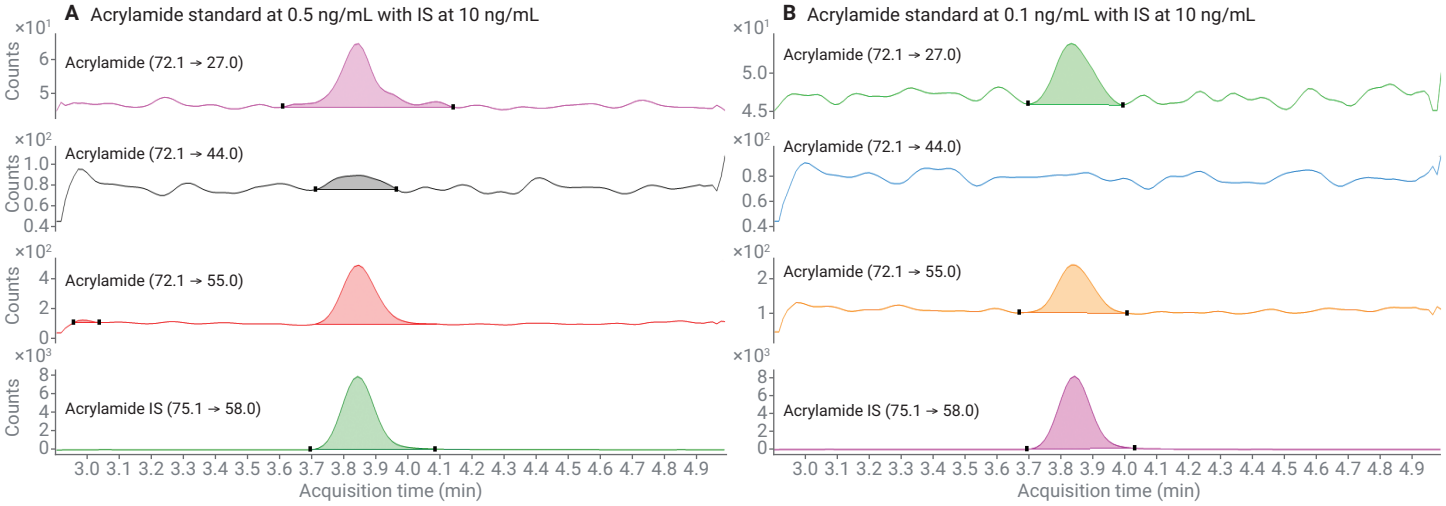

Figure 7. Extracted ion chromatogram of acrylamide working standard at 0.1 ng/mL (B) and 0.5 ng/mL (A, calibration level corresponding to LOQ) with IS at 10 ng/mL in water.

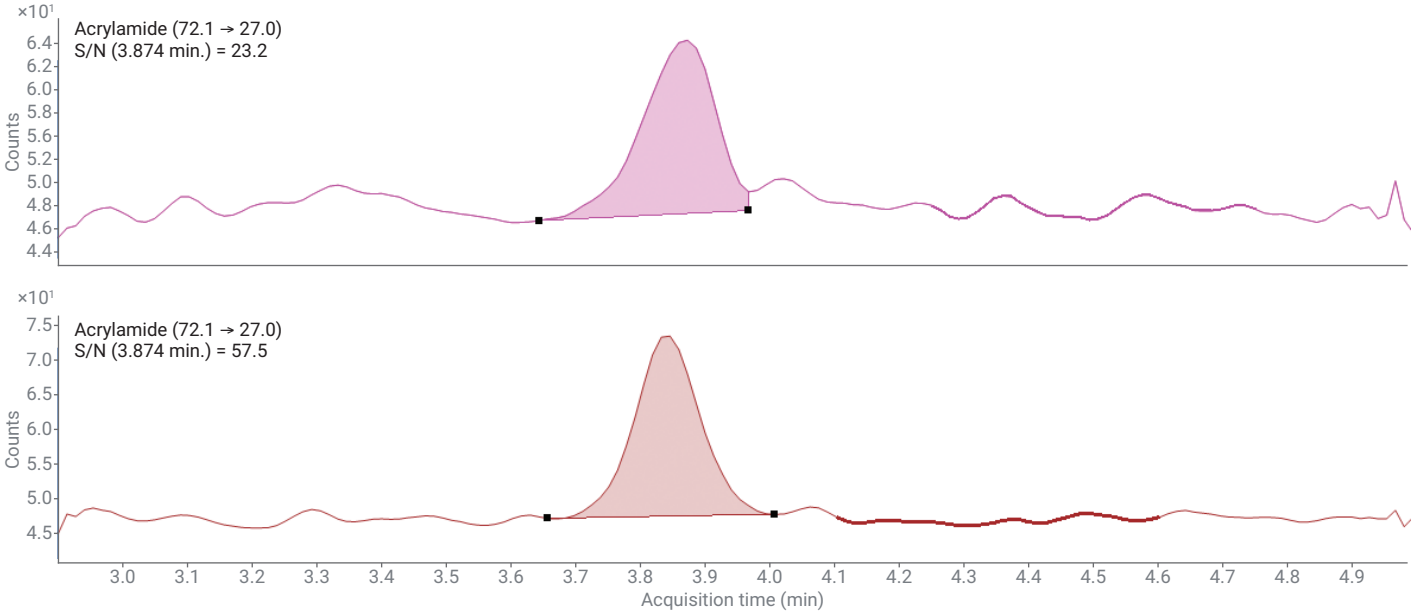

Figure 8. The S/N at 2.5 (LOQ level) and 5 ng/g in raw potato with the sample dilution factor at 5.

Sample tests

The evaluated method was applied to various sample matrices, including raw potato, potato chips (three different brands), processed black olives, raw and roasted salted almonds, raw hazelnut, roasted salted pistachios, and ready-to-drink coffee. Table 4 shows the sample results and matrix effect.

Conclusions

A rapid, high-throughput, and sensitive UHPLC-MS/MS method for the identification and quantification of acrylamide in a variety of food sample matrices was presented, using a 1290 Infinity II LC coupled to an 6470A triple quadrupole LC/MS system with MassHunter workstation software. The evaluation demonstrated that the method can achieve adequate specificity, linearity, accuracy, and precision.

Table 4. Sample results and matrix effect for various samples.

Sample	Dilution Factor (20)		Dilution Factor (5)	
	Sample Result (ng/g)	Matrix Effect	Sample Result (ng/g)	Matrix Effect
Reagent Blank (Water)	<10 ng/g	–0.36	<2.5 ng/g	–0.53
Nuts Samples				
Raw Almond	<10 ng/g	–0.34	<2.5 ng/g	–0.61
Raw Hazelnut	<10 ng/g	–0.50	<2.5 ng/g	–0.66
Roasted Almond	567.4	–0.42	483.7	–0.73
Roasted Pistachio	14.8	–0.58	12.5	–0.59
Olive Sample				
Black Olive Pitted	687.9	–0.33	615.8	–0.77
Chips Samples				
Raw Potato	<10 ng/g	–0.47	<2.5 ng/g	–0.54
Lay's Baked With Sea Salt	595.0	–0.44	593.4	–0.68
Herr's Kettle Salt and Vinegar	657.2	–0.53	635.8	–0.66
Cheese Crunch	232.2	–0.48	247.6	–0.69
Coffee Sample				
Coffee, Ready to Drink	11.6	–0.54		

References

1. Krishnakumar, T.; Visvanathan, R. Acrylamide in Food Products: A Review. *J. Food Process Technol.* **2014**, *5*, 1–9.
2. Granby, K.; Fagt, S. Analysis of Acrylamide in Coffee and Dietary Exposure to Acrylamide from Coffee. *Anal. Chim. Acta.* **2004**, *520*, 177–182.

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