LC-MS/MS Analysis of Emerging Food Contaminants

Quantitation and Identification Acrylamide in Starch-Rich Food

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Introduction

Acrylamide has been classified as 'probably carcinogenic to humans' by the International Agency for Research on Cancer (IARC) in 1994.[1]

Risk assessment of acrylamide evaluated by the European Union (SCF and CSTEE) demonstrated that the exposure of acrylamide to humans should be kept as low as possible with regard to the inherent toxic and carcinogenic properties of acrylamide.[2]

Also the California Office of Environmental Health Hazard Assessment (OEHHA) also included acrylamide on its Proposition 65 list of potential human carcinogens on January 1, 1990, and for known reproductive toxicity in February 25, 2011, and proposed establishing a maximum allowable dose level (MADL) of 140 micrograms per day.[3,4]

In early 2002, Swedish scientists discovered unexpected high levels of acrylamide in the blood of unexposed workers leading to the hypothesis that acrylamide might be present via dietary exposure.[5,6] The Swedish National Food Administration reported the presence of relevant amounts of acrylamide in starch-rich foods cooked at high temperatures, such as French fries, potato chips, baked goods, cereals, chocolate, and roasted coffee. These findings were soon confirmed by other research groups and, together with stakeholders, efforts were carried out to build greater understanding of acrylamide concerning the mechanism of its formation in foods, the risks associated for consumers, and possible strategies to lower acrylamide levels in foodstuffs.[7]

Mainly two methods (LC-MS/MS or GC-MS) are used for the analysis of acrylamide by laboratories all over the world. GC-MS after bromination offers adequate sensitivity with multiple ion confirmation. GC has a risk to produce false positive results because of acrylamide formation through the Maillard reaction in the hot GC injector (if the precursors, like asparagine and reducing sugars, are present in the extract). Determination of acrylamide using LC-MS/MS offers lower detection limits and avoids the time consuming derivatization step.[8,9]

Here we present a new method using LC-MS/MS with a simple solvent extraction followed by dilution to minimize any possible matrix effects, followed by fast LC separation and accurate and reproducible MS/MS detection using the AB SCIEX QTRAP® 4500 system with limits of quantitation down to low µg/kg (ppb) levels in food.

Experimental

Sample Preparation

The sensitivity and selectivity of the AB SCIEX QTRAP® 4500 systems allow minimal sample preparation for this analysis. Food samples were homogenized, and 1 g was simply extracted with 10 mL water, centrifuged, and finally diluted 10x with water to minimize possible matrix effects before LC-MS/MS analysis.

D_3-acrylamide was added to each extract as an internal standard.

LC

LC separation was achieved using the Shimadzu $UFLC_{XR}$ system with a Hypercarb 5 µm (100 x 2.1 mm) column with a gradient of water and methanol containing 0.1% formic acid at a flow rate of 0.5 mL/min. A short column wash with 90% methanol was performed after each analysis to maintain column

performance and increase method robustness. The injection volume was set to 20 µL.

MS/MS

The AB SCIEX QTRAP® 4500 was operated in Multiple Reaction Monitoring (MRM) mode. The Turbo V™ source was used with an Electrospray Ionization (ESI) probe in positive polarity. Three selective MRM transitions were monitored for acrylamide using the ratio of quantifier and qualifier ion for compound identification (Table 1).

LC-MS/MS data was processed using the MultiQuant™ software version 2.1.

Table 1. MS/MS Parameters for the detection of acrylamide using the AB SCIEX QTRAP® 4500 system

MRM	Q1/Q3	DP (V)	CE (V)
Acrylamide 1	72/55	35	15
Acrylamide 2	72/44	35	18
Acrylamide 3	72/72	35	5
D3-Acrylamide	75/58	35	15

Results and Discussion

First, limit of detection (LOD), limit of identification (LOI), linearity, and reproducibility were evaluated using injections of acrylamide ranging in concentration from 0.05 to 100 ng/mL using 10 ng/mL of internal standard.

Signal to noise (S/N) was calculated for each MRM transition using 3x standard deviation algorithm. By this approach, LOD for acrylamide was determined to be 0.02 ng/mL (S/N of quantifier MRM = 3) and the LOI to be 1 ng/mL (S/N of all three MRM > 3). The MRM ratio was automatically calculated using all standard injection resulting in an average ratio of 0.027 and 1.319, respectively (Figure 1).

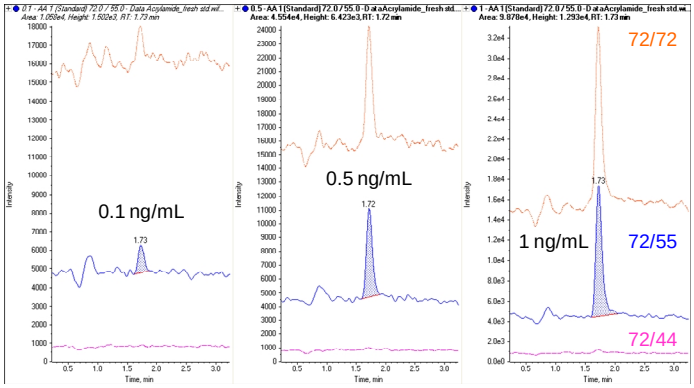

Figure 1. Injection of 0.1, 0.5, and 1 ng/mL of acrylamide, LOD was determined to be 0.02 ng/mL and LOI to be 0.5 ng/mL, and the average ratio of quantifier and qualifier ion was 0.027 and 1.319, respectively

Linearity was observed for the quantifier MRM transition from 0.05 to 100 ng/mL with accuracy values between 88.6 and 111.7% and for the qualifier MRM transitions from 0.5 to 100 ng/mL with accuracy values between 81.5 and 118.2%. All three calibration lines showed linearity with a regression coefficient of greater than 0.999 (Figure 2).

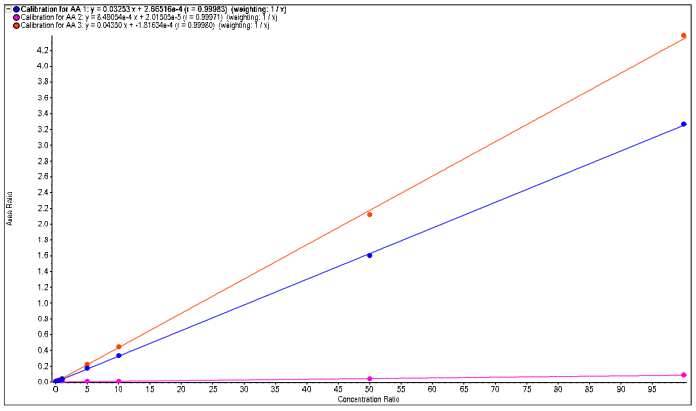

Figure 2. Linear range of the detection of acrylamide using D3-acrylamide as internal standard from 0.05 to 100 ng/mL with an r > 0.999 for all three MRM transitions

Repeat analysis was performed at 0.1 and 1 ng/mL resulting in a coefficient of variation (%CV) of 5.1% and 1.7%, respectively (Table 2).

Table 2. Reproducibility and accuracy over the entire linear range when quantifying Acrylamide using D_3-Acylamide as internal standard

Concentration (ng/mL)	# of injection	Accuracy (%)	% CV
0.050	1	111.7	-
0.100	3	103.2	5.1
0.500	1	88.6	-
1.000	3	94.3	1.7
5.000	1	106.7	-
10.00	1	101.7	-
50.00	1	98.6	-
100.0	1	100.4	-

This level of sensitivity allows the use of simple solvent extraction of food samples followed by 10x dilution to minimize possible matrix effects. Quantitation errors where further compensated through the use of internal standard.

Several food samples, including potato chips and roasted coffee, were purchased from a local store and analyzed by the method described. The MRM chromatograms of three samples are shown in Figure 3. Acrylamide findings corrected for dilution are presented in Table 3. Our results are in a similar range as reported by the FDA acrylamide survey and well below the 'indicative value' in potato chips and coffee as recommended by the EU.[9,10]

The quantifier MRM transition (72/55) was used to quantify the concentration of acrylamide in all samples. The MRM ratio of both qualifier to quantifier transitions were used to further identify acrylamide. MRM ratio tolerances of the guideline SANCO/10684/2009[11] were used.

Table 3. Reproducibility and accuracy over the entire linear range when quantifying Acrylamide using D_3-Acylamide as internal standard

Sample	Acrylamide concentration (µg/kg)	MRM Ratio 1 (0.013-0.041)	MRM Ratio 2 (1.055-1.583)
Potato chips A	260	0.016	1.086
Potato chips B	580	0.019	1.095
Roasted coffee	130	0.026	3.297

The ratio using the first qualifier (72/44) was well inside the tolerance of 50% for all samples. The ratio using the second qualifier (72/72) further identified the presence of acrylamide in potato chips. However, matrix interferences in the coffee extract resulted in an MRM ratio outside of the 20% tolerance.

These results highlight the difficulties often encountered when analyzing chemicals in challenging matrices, and reinforce and encourage analyzing as many as possible qualifier ions as possible to increase confidence in compound identification.

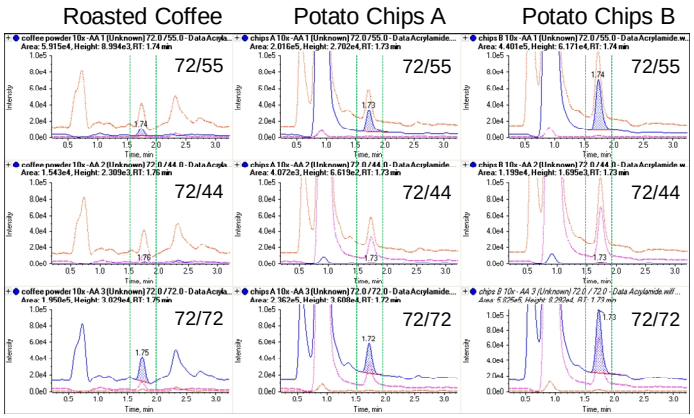

Figure 3. Quantitation and identification of acrylamide in store bought potato chips and roasted coffee

Additional cleanup steps, like the QuEChERS approach originally developed by Mastovska and Lehotay[12] or other solid-phase extraction (SPE) clean-up approaches, could also be used to further remove interfering matrix components from the extracts of highly complex food samples.

Summary

The method and data presented here showcase the fast and accurate solution for the quantitation and identification of acrylamide in food samples by LC-MS/MS. The AB SCIEX QTRAP® 4500 systems provide excellent sensitivity and selectivity for this analysis, with minimal sample preparation allowing maximized throughput for the analysis of many samples in a short time period.

Acrylamide was quantified in store bought potato chips and roasted coffee. Automatic MRM ratio calculation in MultiQuant™ software was used for compound identification.

Matrix interferences highlight the need for an internal standard to compensate any matrix effects and for multiple qualifier ions to be used to identify with high confidence in challenging matrices, such as roasted coffee.

The developed method can also be used for the detection of acrylamide in drinking water samples. Water samples can be injected directly. A slightly larger injection volume is recommendable to quantify and identify below 0.1 µg/L.

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