APPLICATION NOTE

Liquid Chromatography/ Mass Spectrometry

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Analysis of Acrylamide in Potato Chips by Isotopically Standard Addition UHPLC-MS/MS

Introduction

Acrylamide is an amide-type organic compound that can be formed by cooking or processing food at elevated temperatures (especially starch-rich compounds such as potatoes and cereals). Acrylamide is formed mainly in food by the reaction of the aminoacid asparagine with reducing sugars (particularly glucose and fructose) as part of the Maillard reaction. It can also be formed by reactions containing 3-aminopropionamide. The formation of acrylamide occurs mainly in conditions of high temperatures (generally higher than 120 °C) and low humidity.

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Acrylamide is classified as "probable carcinogen to humans" (Group 2A) by the International Agency for Research on Cancer (IARC), based on animal studies. At this time, it is not clear that these results can be extrapolated to humans.

Acrylamide is also a component of tobacco smoke, and an intermediate agent in the synthesis of polyacrylamides, substances used as flocculants in water treatment and in the paper industry. It is found in certain foods after preparation or processing at high temperatures, such as cooking, roasting or frying.

Although it is likely that acrylamide has been part of our diet since we began cooking food, the safety concerns posed by this substance since its discovery in food in 2002 have prompted the world experts to recommend reducing their presence in our diet. Regulation (EU) 2017/2158 of the Commission was published, establishing mitigation measures and reference levels to reduce the presence of acrylamide in foods.

Experimental

Standard Solutions

Stock solution of acrylamide (0,20 mg/mL) was prepared by dissolving 20 mg of the compounds in 100 mL of methanol. The standard working solutions were prepared by serial dilution sample, calibration levels were: 0,5 – 1,0 – 5,0 - 10 - 50 - 100 µg/L in methanol/water 50/50, and containing 100 µg/L of isotopically C_{13}-acrylamide.

Sample Preparation Procedure

All samples were previously homogeneized. Acrylamide extraction was as follows:

1. Analytically weigh 1,0 ± 0, 1 g crushed potato chips.
2. Add 100 µl of 10 mg/L standard solution of acrylamide-C13.
3. Add 25 mL of 0.1% formic acid in methanol solution and mix on a shaker for 15 minutes.
4. Centrifugation for 5 min. (3000 rpm).
5. Take 1 mL of extract and add 1 mL of water for SPE.
6. Condition C18 SPE tube (6 mL, 500 mg) with 2 mL methanol and 2 mL water/methanol 50/50.
7. Use vacuum for 5 min. dry C18 phase.
8. Apply 2 mL of extracted potato chip solution to SPE tube. Allow sample solution to pass through tube with only gravity flow and recover the extract for HPLC/MS analysis. Final sample dilution was 50 times.

HPLC

Acrylamide was separated on a PerkinElmer QSight® LX50 HPLC system using a UHPLC BEH C18 column (2.1 x 50 mm, 2.7 µm). Column temperature was set at 40°C. Mobile phase consisted of water containing 0,05% formic acid (A), and methanol with 0,05% formic acid (B). The flow rate was 0,30 mL/min and the elution gradient is shown in Table 1. The injection volume was 5 µL.

Table 1. HPLC conditions.

Time, min	A, %	B, %	Flow, mL/min
0	95	5	0,30
0,2	95	5	0,30
10	0	100	0,30
12,5	0	100	0,30
12,6	95	5	0,30
15	95	5	0,30

MS Conditions

Detection of acrylamide was carried out on a PerkinElmer QSight 210 triple quadrupole mass spectrometer equipped with an electrospray ionization source operating in positive ion mode and multiple reaction monitoring mode (MRM). Mass spectrometer conditions were as follows: *ElectroSpray*, 5000 V; *Source Temp*, 300 °C; *HSID Temp*, 275 °C; *Drying Gas*, 100 mL/min; *Nebulizer Gas*, 300 mL/min. A summary of the monitored ions and the optimized MRM parameters for the analytes is shown in Table 2.

Data acquisition and processing was performed using the PerkinElmer Simplicity™ 3Q software.

Method validation was conducted at two levels of concentration, 50 and 250 µg/kg, by characterizing linearity, selectivity, accuracy (recovery) and intermediate precision.

The validation was carried out along three sequences of analysis between different days (5 trials per day and for each level), obtaining a total of 15 results per level.

Results

The selectivity of the method was evaluated by adding a known concentration of acrylamide equivalent to the limit of quantification to a matrix target (25 µg/kg). No matrix effect was observed by comparing solvent solution and extract solutions (the variation of the mean of the responses of 10 trials between both samples was less than 10%). Matrix concentration in sample extracts is very low (20 mg/ml).

Table 2. MRM conditions.

Compound	Precursor Ion	Fragment Ion	Collision Energy, V	Dwell Time, ms
Acrylamide 1	72	54	15	100
Acrylamide 2	72	55	15	100
Acrylamide-C13	73	56	15	100

Recovery data for analyte spiked in matrices are summarized in Table 3. Very good recoveries were obtained (from 85 to 109%).

Figure 1 shows the linearity plot for standard solutions over a range of 0,5-100 µg/L (internal standard).

Figures 2 and 3 show MS chromatograms of 0.5 and 5 µg/L acrylamide calibration solutions.

Figure 4 shows MS chromatogram from potato chip spiked with 250 µg/kg of acrylamide (final extract theoretical concentration of 5,0 µg/L).

Table 3. Summary of validation results.

Validation parameter	50 g/kg	250 g/kg
Mean recovery, %	85,1	108,6
Intermediate precision, %	7,4	3,6
U, (k=2)	15%	8%

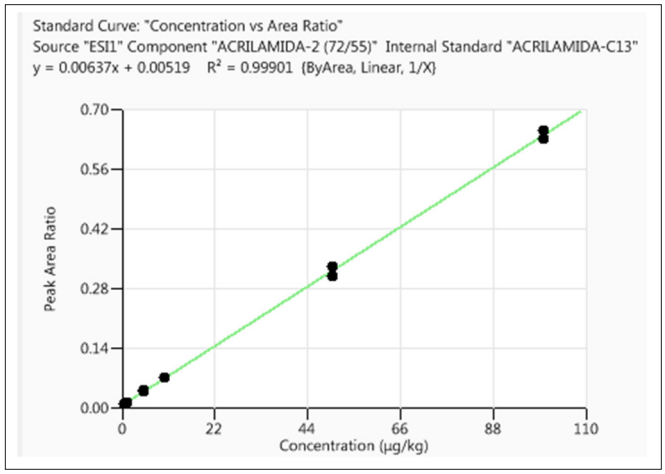

Figure 1. Acrylamide internal standard calibration from 0,5-to 100 µg/L (R^2=0.998, residuals<10%).

The method was in compliance with the technical requirements set by Regulation (EU) 2017/2158 (recovery 75-110%, RSD < Horwitz modified, LOQ ≤ 50 µg/kg).

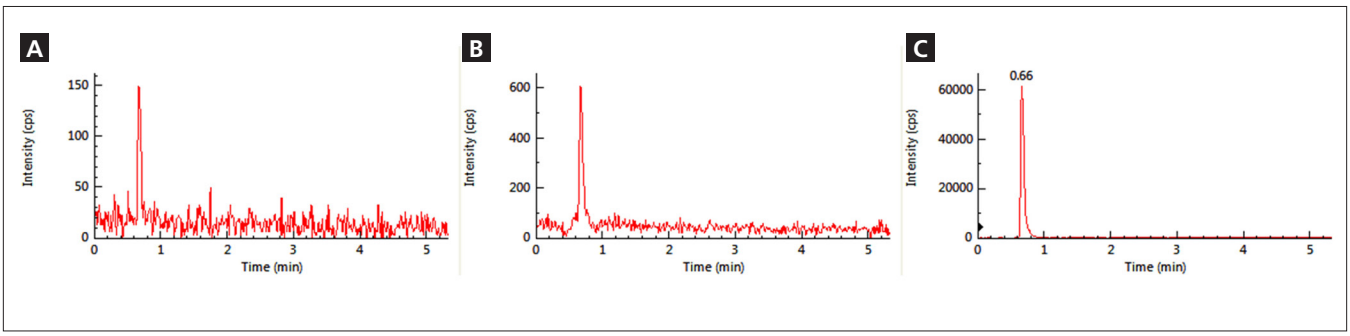

Figure 2. Chromatograms for acrylamide standard (0,5 ug/L) and internal standard. a) Acrylamide 1 with MRM transition 72/54; b) Acrylamide 2 with MRM transition 72/55; c) Acrylamide-C13 with MRM transition 73/56.

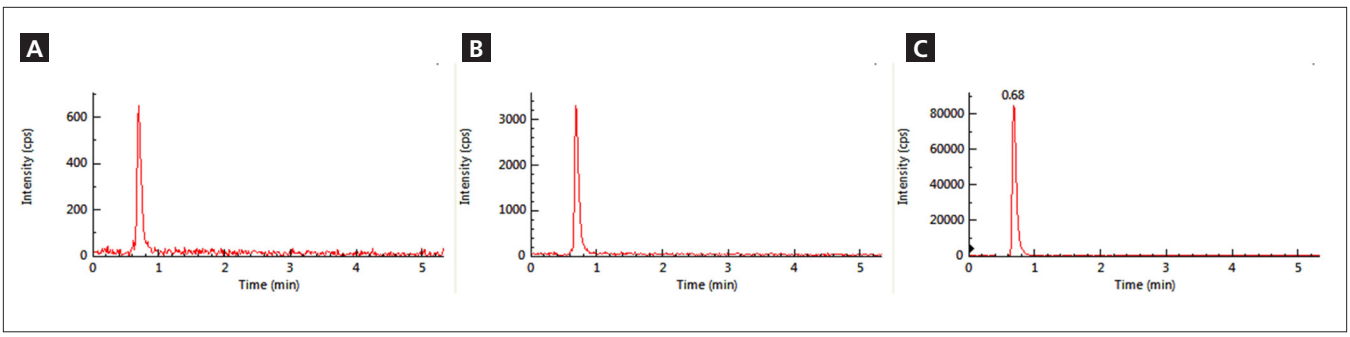

Figure 3. Chromatograms for acrylamide standard (5 ug/L) and internal standard. a) Acrylamide 1 with MRM transition 72/54; b) Acrylamide 2 with MRM transition 72/55; c) Acrylamide-C13 with MRM transition 73/56.

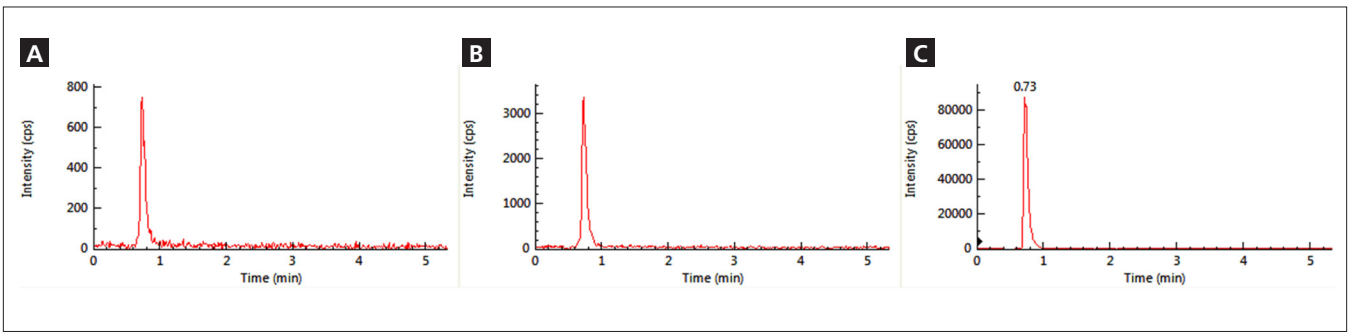

Figure 4. Chromatograms for acrylamide and internal standard from spiked potato chip sample. a) Acrylamide 1 with MRM transition 72/54; b) Acrylamide 2 with MRM transition 72/55; c) Acrylamide-C13 with MRM transition 73/56.

Conclusions

We have developed a rapid, sensitive and reproducible HPLC-MS/MS method for analysis of acrylamide in potato chips, using a simple sample preparation procedure. The LOQ is 25 µg/kg.

The QSight mass spectrometer provides a robust platform for analysis of trace acrylamide levels in compilance with European Regulation. This method can also be applied to other types of regulated matrices such as bread, biscuits, breakfast cereals, and more.

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