

Fast Determination of Acrylamide in Food Samples Using Accelerated Solvent Extraction Followed by Ion Chromatography with UV or MS Detection

Introduction

Acrylamide, a known genotoxic compound, was recently detected in carbohydrate-rich fried or baked food samples by a Swedish research group, Tareke, et al.¹ The content of acrylamide was as high as several mg/kg for typical samples such as hash browns and french fries. Published methods for acrylamide include U.S. EPA Method 8032A that uses liquid extraction and GC-ECD for determinations in water² and a method by the German Health Agency (BGVV) that uses HPLC with UV detection for migration analysis of acrylamide from food packing materials.³

The method presented here consists of a fast, automated extraction method using accelerated solvent extraction.⁴ Samples were extracted in 20 min using pure water, water with 10 mM formic acid, or acetonitrile. The extracts were directly analyzed by ion chromatography (IC) using a 4-mm ion-exclusion column and both UV and MS detection. With this column, acrylamide is retained longer than on conventional reversed-phase columns, allowing separation from the many co-extractable compounds present in food samples. Results were obtained for acrylamide in french fries, potato chips, and crisp bread. The benefits of this method are simplicity, speed of analysis, and a degree of automation that allows the analysis of large numbers of samples with minimal labor.

Conditions

Extraction Conditions

Solvent:	Water, 10 mM formic acid, or acetonitrile
Temperature:	80 °C
Pressure:	1500 psi*
Heatup Time:	5 min
Static Time:	4 min
Number of Static Cycles:	3
Flush Volume:	60%
Purge Time (N2):	120 s

*Pressure studies indicate 1500 psi is the optimum extraction pressure for all accelerated solvent extraction applications.

Chromatographic Conditions

Column:	Thermo Scientific Dionex IonPac ICE-AS1, 4 × 250 mm, 7.5 µm, SP6003
Eluent:	3.0 mM formic acid in acetonitrile/water 30/70 (v/v)
Flow Rate:	0.15 mL/min
Inj. Volume:	25 µL
UV Detection:	2 02 nm
MS Detection:	ESI+: 3.0 kV, cone 50 V, probe temp. 300 °C, scan 50–250 <i>m/z</i> , SIM 72 <i>m/z</i>

Equivalent results can be achieved using the Thermo Scientific Dionex ICS-1100, Thermo Scientific Dionex ICS-1600, Thermo Scientific Dionex ICS-2100, Thermo Scientific Dionex ICS-4000, or the Thermo Scientific Dionex ICS-5000+ system.

Experimental

Extraction

Samples of 5 g were extracted using an accelerated solvent extraction system (Thermo Scientific Dionex ASE 100 or 200 accelerated solvent extraction system), with 34-mL cells for the Dionex ASE™ 100 accelerated solvent extraction system, and 33-mL cells for the ASE 200™ accelerated solvent extraction system.

Chromatography

Chromatographic analyses were performed on a Thermo Scientific Dionex DX-600 ion chromatograph, that included a GS50 gradient pump, PDA-100 photodiode array detector set at 202 nm, a Thermo Scientific MSQ single quadrupole mass spectrometer, and a Thermo Scientific Dionex AS50 Autosampler. A 250 × 4 mm i.d. Dionex IonPac™ ICE-AS1 analytical column (7.5- μ m cross-linked polystyrene divinylbenzene functionalized with sulfonate functional groups) was used to separate acrylamide from the matrix compounds. All measurements were made at 30 °C and all samples were filtered through 0.45- μ m filters. A 25- μ L sample loop was used for all the determinations. Data collection and the operation of all components in the system was controlled by Thermo Scientific Dionex Chromeleon 6.40 chromatography software.

Reagents and Standards

All reagents were analytical-grade. Formic acid was Suprapur® (Merck, Darmstadt, Germany), and acetonitrile was HPLC reagent-grade (Novachimica, Milano, Italy). Ultrapure water with conductivity <0.1 Ω S (DI water) was obtained from a Milli-Q® system (Millipore, Bedford, MA, USA). Working standard solutions of acrylamide were prepared by serial dilution of a 1000-mg/L stock standard solution.

Samples

French fries (Figure 1), potato chips, tortilla chips, wheat snacks with bacon flavor, and crisp bread were obtained from a local food store. Representative samples (5 g) were loaded into 34-mL extraction cells onto a glass fiber filter. Samples like wheat snacks with bacon flavor or bread samples, which have a tendency to dissolve or swell, were loaded into Soxhlet thimbles that were then placed in the extraction cells. Any void volumes were filled with glass beads (1-mm i.d.) to reduce the volume of the extraction solvent.

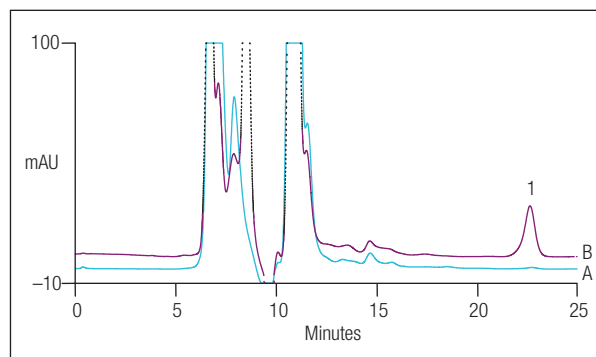


Figure 1. Comparison of french fry samples. Chromatographic conditions are listed under "Conditions". UV detection. (A) neat; (B) spiked with 40 μ g of acrylamide. Peak: 1. Acrylamide.

Results and Discussion

Accelerated Solvent Extraction

Pure water, water with 10 mM formic acid added, and acetonitrile were tested as the extraction solvent. Pure water extracts showed lower recoveries than the formic acid, but the formic acid extracts had lower stability. Extracts produced using acetonitrile were cleaner, as less material was coextracted from the sample matrix. The extraction temperature of 80 °C was chosen, because acrylamide starts to decompose at temperatures above 83 °C. With three extraction cycles of 4-min durations, a spiked french fries sample had a yield of 95% in the first extract and an additional 8% in the second extraction of the same sample using 10 mM formic acid.

Cleanup

The extract volume was determined using a volumetric flask. Afterward, the extracts were filtered using a 0.22- μ m nylon filter. Further cleanup using solid phase extraction or liquid extraction did not exhibit any significant improvements for the subsequent chromatographic analyses.

Analysis of Acrylamide Using IC/UV

The separation of acrylamide was performed using an IC system with a UV detector. Formic acid was chosen as the eluent instead of sulphuric acid because it is more compatible with MS detection. The amount of acetonitrile was optimized to 30% v/v to reduce the total run time and avoid interferences with matrix components.

Analysis of Acrylamide Using IC/MS

A Thermo Scientific MSQ single-stage quadrupole mass spectrometric detector was installed in series with the UV detector. The MS was operated in the positive electrospray (ESI+) ionization mode. Figure 2 shows a mass spectrum of acrylamide at a cone voltage of 50 V. The protonated molecular ion $[M+H]^+$ of acrylamide is detected at a mass-to-charge ratio (m/z) 72. In addition, a fragment ion is observed at m/z 55. This lower-intensity fragment ion can be used for confirmation while a more sensitive detection is achieved at m/z 72. Calibrations were performed using external standards in the range 0.01–1 mg/L. The corresponding calibration plots for both UV and MS detection show good linearity in the range 0.01–10 mg/L ($r^2 = 0.996$).

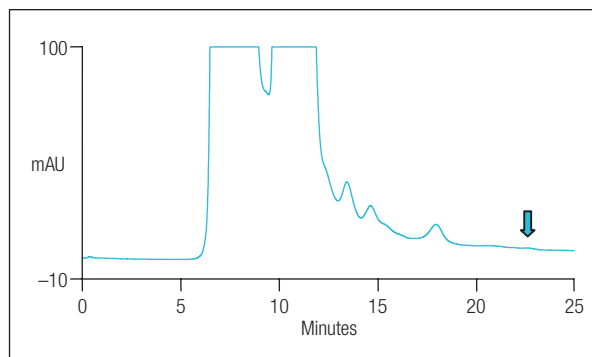


Figure 3. Chromatogram of a crisp bread sample with low acrylamide content. Chromatographic conditions are listed under “Conditions”. UV detection. Peaks: arrow indicates acrylamide retention time.

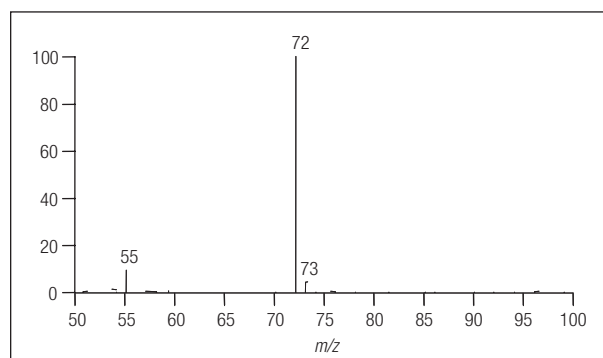


Figure 2. Mass spectrum of acrylamide in the range 50–100 m/z . Mass spectrometric conditions are listed under “Conditions”.

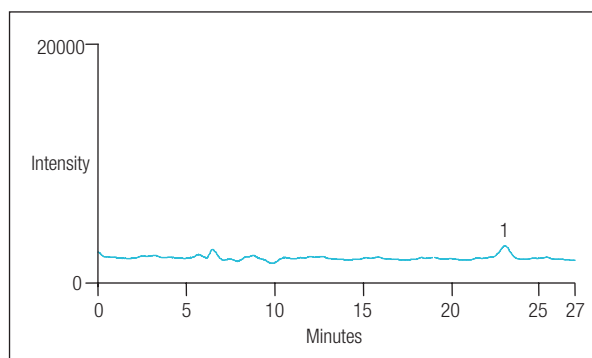


Figure 4. Chromatogram of a crisp bread sample with low acrylamide content. Chromatographic conditions are listed under “Conditions”. MS detection SIM mode. Peak: 1. Acrylamide 0.08 mg/Kg.

Conclusion

The accelerated solvent extraction method provides a fast and efficient extraction of acrylamide from various food samples. The extracted samples were analyzed directly using IC with UV or MS detection. Although UV detection is sufficient for most of the analyzed samples, MS detection offers a higher specificity and sensitivity, as shown in Figures 3–6. Results are summarized in Table 1. The required limits of determination of 50 $\mu\text{g}/\text{kg}$ acrylamide in food can be achieved with this method. This method is robust, selective, and relatively easy to perform.

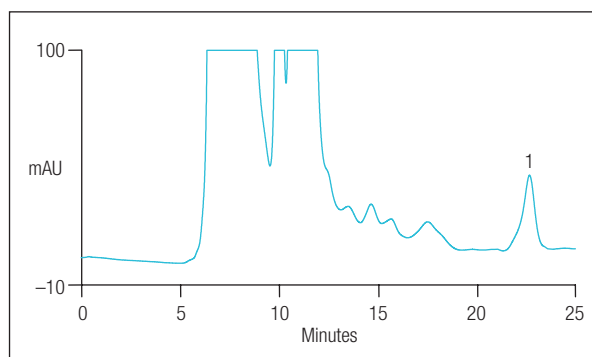


Figure 5. Chromatogram of a potato chips sample with high acrylamide content. Chromatographic conditions are listed under “Conditions”. UV detection. Peak: 1. Acrylamide 1.56 mg/Kg.

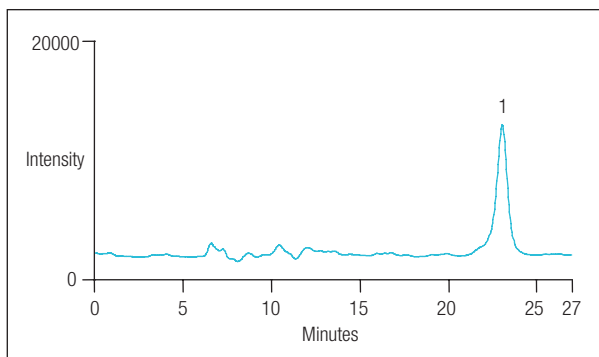


Figure 6. Chromatogram of a potato chips sample with high acrylamide content. Chromatographic conditions are listed under "Conditions". MS detection SIM mode. Peak: 1. Acrylamide 1.06 mg/Kg.

References

1. Tareke, E.; Ryberg, P.; Karlsson, P.; Eriksson, S.; Tornqvist, M., *J. Agric. Food. Chem.* **2002**, *50*, 4998–5006.
2. U.S. EPA Method 8032A; SW-846 Rev 1; U.S. Environmental Protection Agency, Office of Solid Waste, December 1996.
3. Acrylamide in food—serious problem or exaggerated risk? Results of BgVV Information Seminar, 29 Aug 2002; http://www.bgvv.de/cm/208/acrylamide_in_foods_serious_problem_or_exaggerated_risk.pdf.
4. Richter, B. E.; Jones, B. A.; Ezzell, J. L.; Porter, N. L.; Avdalovic, N.; Pohl, C. *Anal. Chem.* **1996**, *68*, 1033.

Table 1. Acrylamide Contents of Food Samples

Sample	Acrylamide UV* mg/kg	Acrylamide MS** mg/kg
Chips bacon flavored	n.d.	<0.05
Crisp bread	n.d.	0.08
Potato chips	1.57	1.06
Tortilla chips	n.d.	n.d.
French fries	0.11	0.10
French fries spiked	0.70 ^a	0.69 ^b

* 202 nm; ** SIM 72 m/z; ^a recovery 96.4%; ^b recovery 95.1%

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