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

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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. 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 US EPA Method 8032A that uses liquid extraction and GC–ECD for determinations in water and a method by the German Health Agency (BGVV) that uses HPLC with UV detection for migration analysis of acrylamide from food packing materials.

The method presented here consists of a fast, automated extraction method using accelerated solvent extraction (ASE). Samples were extracted in 20 min using pure water or water with 10 mM formic acid. The extracts were directly analysed 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 coextractable 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 labour.

CONDITIONS
Extraction Conditions
Solvent: Water or 10 mM formic acid
Temperature: 80 °C
Pressure: 10 MPa
Heat-up time: 5 min
Static time: 4 min
Number of static cycles: 3
Flush volume: 60%
Purge time (N2): 120 s

Chromatographic Conditions
Column: 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: 254 nm
MS detection: ESI+: 3.0 kV, cone 50 V, probe temp. 300 °C, scan 50–250 m/z, SIM 72 m/z

EXPERIMENTAL
Extraction
Samples of 5 g were extracted using an accelerated solvent extraction system, (ASE 100 or ASE 200, Dionex, Sunnyvale,
Samples such as wheat snacks with bacon flavour or bread samples, which have a tendency to dissolve or swell, were loaded into Soxhlet thimbles that were then placed in the extraction cells.

California, USA) with 34 mL cells for the ASE 100, and 33 mL cells for the ASE 200.

Chromatography
Chromatographic analyses were performed on an DX-600 ion chromatograph, (Dionex, Sunnyvale, California, USA) that included a GS50 gradient pump, PDA-100 photodiode array detector set at 202 nm, an MSQ™ single quadrupole mass spectrometer and an AS50 autosampler. A 250 × 4 mm i.d. IonPac® ICE-AS1 (Dionex, Sunnyvale, California, USA) analytical column (7.5 µm cross-linked polystyrene divinylbenzene functionalized with sulphonate 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 µm filters. A 25 µL sample loop was used for all the determinations. Data collection and the operation of all components in the system was controlled by 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 ΩS (DI water) was obtained from a MILLI-Q® system (Millipore, Bedford, Massachusetts, USA). Working standard solutions of acrylamide were prepared by serial dilution of a 1000 mg/L stock standard solution.

Samples
French fries, potato chips, tortilla chips, wheat snacks with bacon flavour and crisp bread were obtained from a local food store. Representative samples (5 g) were loaded into 34 mL extraction cells onto a glass fibre filter. Samples such as wheat snacks with bacon flavour 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.

RESULTS AND DISCUSSION
ASE Extraction
Pure water and water with 10 mM formic acid were tested as the extraction solvent. Pure water extracts showed lower recoveries than the formic acid, but the formic acid extracts had lower stability. 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.

Clean-up
The extract volume was determined using a volumetric flask. Afterward, the extracts were filtered using a 0.22 µm 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 single-stage quadrupole mass spectrometric detector (Thermo Finnigan MSQ, Dionex, Sunnyvale, California) 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 (r² = 0.996).

CONCLUSIONS
The ASE method provides a fast and efficient extraction of acrylamide from various food samples. The extracted samples were analysed directly using IC with UV or MS detection. Although UV detection is sufficient for most of the analysed 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 µg/kg acrylamide in food can be achieved with this method. This method is robust, selective and relatively easy to perform.

REFERENCES