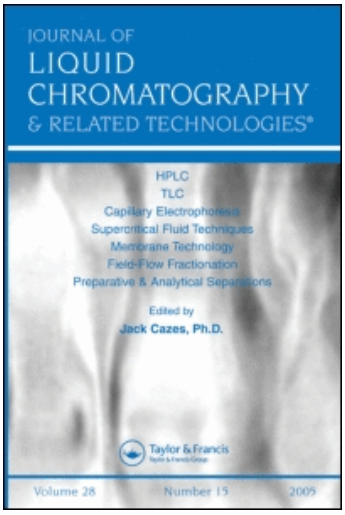

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Development of High-Performance Liquid Chromatography–Electrospray Mass Spectrometry with Size-Exclusion Chromatography for Determination of Acrylamide in Fried Foods

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ABSTRACT

A method by liquid chromatography–electrospray mass spectrometry (LC/MS) with a size-exclusion chromatography column for the analysis of acrylamide in fried foods, is reported. In the mass spectral analysis using an electrospray MS, the signal at m/z 72, which was assigned to the $[M+H]^+$ ion, was observed as the main peak. The main m/z signal showed a maximum at the fragmentor voltage of 100 V. The retention time for the elution of acrylamide from the size-exclusion chromatography column ranged from 15 to 20 min, and the eluate was analyzed by LC/MS

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with a reversed-phase column. A linear response was found for the acrylamide standard tested within the concentration range of 5.0–1000 ng/mL, with correlation coefficients (r) greater than 0.99. Sample preparation was performed by means of solvent extraction, which gave acrylamide recoveries higher than 90% with relative standard deviations less than 10%, with ^{13}C-labeled acrylamide standard.

Key Words: Acrylamide; Liquid chromatography mass spectrometry; Size-exclusion chromatography; Fried food.

INTRODUCTION

Recently, a group of Swedish researchers reported that some fried or baked starchy foods, such as potato chips and french fries, contain a high concentration of acrylamide.[1] That study made the news worldwide[2,3] The U.S. Food & Drug Administration and the European Commission are currently conducting investigations to confirm the acrylamide report. Various researchers from all parts of the world, including Japan, have started investigations to determine acrylamide in cooked foods. However, only a few methods are available for the identification and determination of acrylamide in foods. Therefore, the development of a sensitive, selective, and accurate method for the evaluation of acrylamide in foods is required. In the present study, we describe the development of a novel and simple analytical technique for the determination of acrylamide in foods using high-performance liquid chromatography–electrospray mass spectrometry with size-exclusion chromatography as a very useful tool for the determination of various compounds in biological and food samples.[4–8] Thus, it was used in combination with gel permeation chromatography (GPC) for the separation of acrylamide and clean-up of impurities compounds in foods.[9,10]

EXPERIMENTAL

Reagents

Acrylamide standards were purchased from Kanto Chemical Industries, Ltd. (Tokyo, Japan). Acrylamide-^{13}C surrogate standard was purchased from CDN Isotopes Co. (Quebec, Canada). HPLC-grade methanol, as the mobile phase was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Purified water was obtained using a Milli-Q gradient A10 Elix system (Millipore, Bedford, MA).

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Food Samples

Fried food samples (potato chips and French fries) were obtained from convenience stores and fast-food restaurants in Japan.

Liquid Chromatography–Mass Spectrometry with Gel Permeation Chromatography Measurements

Liquid chromatography/mass spectrometry measurements were performed using an Agilent 1100 MSD-SL system (Agilent Technologies, Palo Alto, USA). The MS interface was used for electrospray ionization (ESI). An Agilent pump was used to deliver a flow to elute the sample from a GPC column and to perform the separation on an analytical column. A Shimadzu LC-10AS pump (Shimadzu, Kyoto, Japan) was used to deliver a flow through the analytical column for stability chromatography. A Mightysil RP-18 GP (100×2.0 mm, 5 μm) analytical column with a guard column (Mightysil RP-18 GP, 5×2.0 mm, 5 μm) from Kanto Chemical Industries, Ltd., was used for separation. The GPC column (Mspak GF-310 4E, 4.6×250 mm from Shodex, Co., Tokyo, Japan) was used for clean up. The injection volume was 20 μL. Liquid chromatography separation was carried out using water/methanol (80/20, v/v). The working conditions for electrospray MS were as follows: the drying nitrogen gas temperature was set at 350°C and the gas was introduced into the capillary region at a flow rate of 12 L/min; the capillary was held at a potential of 3500 V relative to the counter electrode in the positive ion mode. The fragmentor voltage was fixed at 100 V for acrylamide during the chromatographic run. When working in the selected ion monitoring (SIM) mode, m/z 72 (acrylamide) and 73 (acrylamide-1-^{13}C) ions were monitored.

The column-switching LC/MS system, as depicted in Fig. 1, was used for the direct injection of extracted samples. After the sample was injected by an

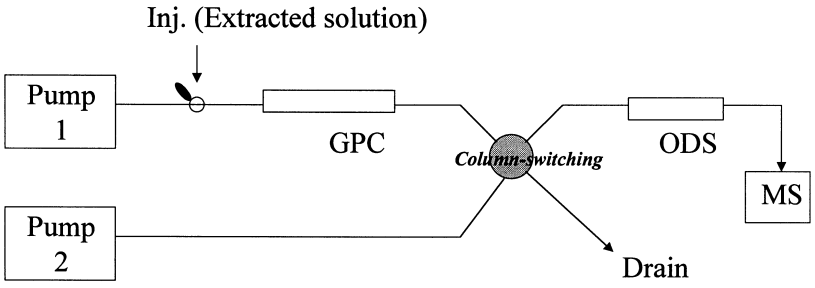

Figure 1. Schematic of the column-switching LC/MS with GPC system.

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auto-sampler, it was loaded onto the GPC column by the mobile phase at a flow rate of 0.2 mL/min controlled by pump 1. This column was allowed to drain during the 15-min run, during which the sample was cleaned on-line in the column. Impurities compounds in foods were eluted, whereas acrylamide was retained on the GPC column. After 15 min, the switching valve was changed (see Fig. 1). This configuration connected the GPC column to the analytical column and the MS detector in the flow path of LC pump 2 from the 15th to the 20th min of the run. The column temperature was maintained at 40°C for LC. The separation was carried out using the mobile phase at a flow rate of 0.2 mL/min. The eluate from the analytical column was directed to the MS without fractionating. After eluting for 10 min, the switching valve was returned to its original position. The run time for the assay was 25 min.

Sample Preparation

A fried food sample was cut into pieces by a cutter. One gram of sample was spiked with 0.1 mL of aqueous solution of the internal standard (final concentration was 0.1 μg/mL). This sample solution was incubated at room temperature for 30 min. The extraction of acrylamide from the sample was carried out by adding 10 mL of water and ultrasonication for 10 min. The mixed sample was centrifuged (3000 rpm for 15 min). The upper layer was transferred into a tube, and filtered (0.45 μm). The obtained sample solution was measured by the LC/MS.

RESULTS AND DISCUSSION

Optimal Liquid Chromatography/Mass Spectrometry with Gel Permeation Chromatography System

In the MS analysis using ESI–MS with flow-through injection analysis of standard solutions, signals representing m/z 72 and 73 ions were observed as the main peaks for acrylamide ($[M+H]^+$) and acrylamide-1-^{13}C ($[M+H]^+$), respectively. The most important parameter affecting the determination of acrylamide by LC/MS is the fragmentor voltage. In order to establish the optimum fragmentor voltage for the detection of acrylamide, the m/z 72 signal was plotted against the fragmentor voltage (Fig. 2). The optimal fragmentor voltage was 100 V for acrylamide ($[M+H]^+$). After the sample solution was injected, it was loaded onto the GPC column by pump 1. The sample solution was cleaned on-line in the GPC column. The acrylamide fraction was introduced by means of the column switching valve (Fig. 3). The background peaks in the blank solution were negligible.

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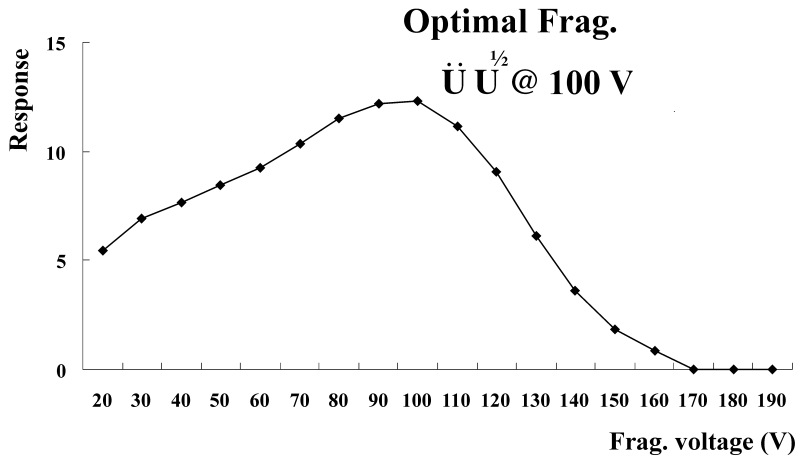

Figure 2. Effect of fragmentor voltage on the signal intensity of the $[M + H]^+$ ion of acrylamide. Carrier solution: water/methanol (80/20, v/v), Flow rate: 0.2 mL/min, MS detection conditions: ESI, the drying nitrogen gas temperature set at 350°C, the capillary region at a flow rate of 12 L/min, the capillary at a potential of 3500 V, and detection the positive ion mode.

The limits of quantification (LOQ) and of detection (LOD) were calculated as follows:

$$\text{LOD} = 3\left(\frac{\text{Signal peak area}}{\text{noise level}}\right)$$

$$\text{LOQ} = 10\left(\frac{\text{Signal peak area}}{\text{noise level}}\right)$$

For quantitation of acrylamide, the peak ratio of standard to internal standard solution, the stable isotope-labeled internal standard, was calculated. The calibration curve was obtained from the peak ratio vs. concentration plot using HP ChemStation software from Agilent Technologies. A linear response was obtained and found to be linear within the concentration range of 5.0–1000 ng/mL, with correlation coefficients (r) greater than 0.999. In addition, LOQ and LOD are 1.0 and 5.0 ng/mL, respectively.

Recovery and Sample Blank

Conventionally, the preparation of food samples for the detection of contaminants involves solvent extraction followed by all sorts of clean up

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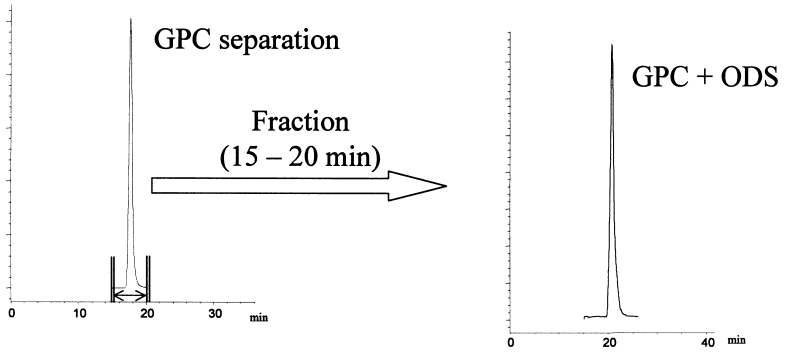

Figure 3. Chromatograms of acrylamide obtained by column-switching LC/MS with GPC system. Mass spectrometry detection mode: SIM (m/z 72), positive, and fragmentor voltage 100 V.

processes. In this study, however, only conventional solvent extraction is used with no clean up procedure. The use of methanol and water as extraction solvents was examined. A high background level was observed in the extraction process with methanol [Fig. 4(A)]. However, good clean up and detection was realized by using water as the extraction solvent [Fig. 4(B)].

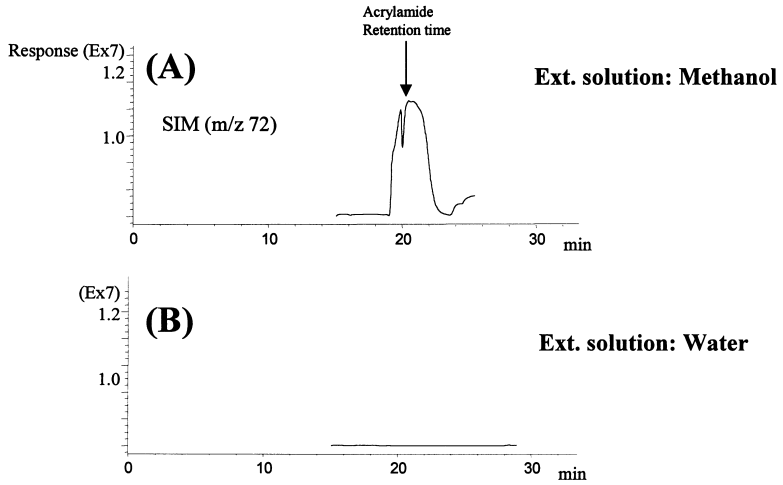

Figure 4. Chromatograms of acrylamide extracted using different solvents. Mass spectrometry detection mode: SIM (m/z 72), positive, fragmentor voltage 100 V. Extraction solvent: methanol (A) and water (B).

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Table 1. Recovery of acrylamide in fried foods.

Sample	Added conc. (ppb)	Avg. recovery (%) (S.D., $n = 3$)
Potato chips	100	97.3 (5.1)
	2,000	96.7 (7.3)
French fries	100	93.0 (4.0)
	2,000	102.2 (3.2)

Therefore, we selected water as the extraction solvent, and applied the combination of only solvent extraction and the addition of the internal standards for LC/MS with the GPC system.

The recovery of acrylamide in fried food samples using water as the extraction solvent was calculated as follows:

$$\text{Avg. recovery (\%)} = \left[\frac{S_1 + S_2 + S_3}{3}\right] \times 100$$

where S_n = spiked concentration for detection/blank concentration in sample.

The blank fried food samples already contained acrylamide at concentrations of 1.5 ppm (potato chips) and 200 ppb (French fries). The recovery and the relative standard deviation (RSD) for fried food samples spiked with standard and stable-isotope labeled internal standards are shown in Table 1. Recovery of 93.0–102.2% with a RSD of 3.2–7.3% was achieved. Thus, this extraction system is useful for the simple and rapid pretreatment of acrylamide in fried food samples.

ABBREVIATIONS

LC/MS	Liquid chromatography mass spectrometry
GPC	Gel permeation chromatography
ESI	Electrospray ionization

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