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Food Chemistry 101 (2007) 401–409
Food Chemistry
www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods

An improved LC-MS/MS method for the quantitation of acrylamide in processed foods

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Received 3 March 2005; received in revised form 24 October 2005; accepted 25 October 2005

Abstract

An improved liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed for the determination of acrylamide in processed foods. The homogenized samples, spiked with $^{13}C_3$-acrylamide as an internal standard, were extracted with water and centrifuged. D_5-3-chloropropanediol as a recovery standard was added to 1-ml aliquots, and the sample was purified with a C_{18}-cartridge column. The extract was directly analyzed using LC-MS/MS without derivatization. The ion transitions of 72–55 *m/z* (acrylamide), 75–58 *m/z* ($^{13}C_3$-acrylamide), and 116–98 *m/z* (d_5-3-chloropropanediol) were found to be the most reliable for the identification and quantification of acrylamide in multiple reaction monitoring. The limit of quantification for acrylamide, defined as a signal-to-noise ratio of 10:1, was 2 μg/kg. The use of d_5-3-chloropropanediol minimized the effects of variation in the sample matrixes and increased the quality of analysis. This method could be applied to the quantification of acrylamide in processed foods.

Keywords: Acrylamide; $^{13}C_3$-Acrylamide; d_5-3-Chloropropanediol; LC-MS/MS; Food processing

1. Introduction

Many recent studies have indicated that acrylamide, which is designated as a probable human carcinogen, is formed in diverse foods when they are processed and/or cooked such as by roasting and frying (Ahn et al., 2002; Nemoto, Takarasuki, Sakaki, & Maitani, 2002; Swedish National Food Administration, 2002). Usually, it is not detectable in food before processing, but is present in a range of cooked foods (Rydberg et al., 2003). Many reports have suggested that acrylamide formation is particularly likely in carbohydrate-rich foods (Hofmann, 1998; Yaylayan, Machiels, & Istasse, 2003), especially produced by the reaction between asparagine and glucose (Becalski et al., 2004; Mottram, Wedzicha, & Dodson, 2002; Stadler et al., 2002). Acrylamide can also be produced from acrylic acid or acrolein, which results from sugars and glycerols (Gertz & Klostermann, 2002). However, the exact chemical mechanisms involving amino acids and reducing sugars in acrylamide formation in baked and/or fried foods have yet to be elucidated.

Precise analysis methods should be used to determine the content of acrylamide in various processed foods and foodstuffs. High-performance liquid chromatography (HPLC) with an ultraviolet detector has been used to determine acrylamide in tap water (EPA, 1994). A gas chromatography electron-capture detector (GC/ECD) with derivatization procedures has also been used for the determination of trace amounts of acrylamide in water (EPA, 1996; Hashimoto, 1976). These methods are very

Abbreviations: 2-BPA, 2-bromopropenamide; CV, coefficient of variation; 2,3-DBPA, 2,3-dibromopropionamide; ESI, electrospray ionization; ESI+, electrospray positive ionization; GC/ECD, gas chromatography electron-capture detector; GC/MS, gas chromatography/mass spectrometry; HPLC, high-performance liquid chromatography; LC-MS/MS, liquid chromatography-tandem mass spectrometry; MRM, multiple reaction monitoring.

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0308-8146/$ - see front matter © 2005 Published by Elsevier Ltd.
doi:10.1016/j.foodchem.2005.10.025

useful for simple aqueous solutions, but are not suitable for measuring trace amounts of acrylamide in a complex matrix such as processed food. Therefore, determining analysis methods for measuring trace levels of acrylamide with accuracy, speedy, and repeatability is urgently required. Most of the analysis methods reported for the analysis of acrylamide in water and processed foods are based on derivatization of bromination followed by GC/ECD (Andrawes, Greenhouse, & Draney, 1987; Arikawa & Shiga, 1980; Castle, 1993; Castle, Campos, & Gilbert, 1991; EPA, 1996; Nemoto et al., 2002; Takata & Okamoto, 1991; Takatsuki, Nemoto, Sasaki, & Maitani, 2003). During the derivatization procedure, the ethylenic double bond in acrylamide is substituted with potassium bromide or potassium bromate to produce 2,3-dibromopropionamide (2,3-DBPA). Derivative methods can usually be improved by increasing the resolution of the capillary column and the sensitivity of the detector. However, 2,3-DBPA has been found to be unstable, and some of the derivative can be converted to the more stable 2-bromopropenamide (2-BPA) at the inlet of the gas chromatograph or in the column (Andrawes et al., 1987; Nemoto et al., 2002). Furthermore, this method is known to have other problems, such as poor repeatability and accuracy, and a low recovery yield (<60%). Nemoto et al. (2002) reported that GC/ECD and gas chromatography/mass spectrometry (GC/MS) exhibited improved repeatability using dehydrobromination of triethylamine, which converted 2,3-DBPA to the more stable 2-BPA before analysis. Researchers have also studied the use of liquid chromatography-tandem mass spectrometry (LC-MS/MS), and GC-chemical ionization mass spectrometry (GC-CI/MS) to remove problems in both the derivative reaction and dehydrobromination (Andrzejewski, Roach, Gay, & Musser, 2004; Roach, Andrzejewski, Gay, Nortrup, & Musser, 2003; Robert et al., 2004; Stadler et al., 2002). However, an external quantitative analysis revealed poor reproducibility and recovery yield. To overcome these problems, methods using internal standards have been studied. 2-Methylacrylamide and $^{13}C_3$-acrylamide are used as internal standards in GG/MS analysis (Becalski et al., 2004; Lagalante & Felter, 2004; Mottram et al., 2002; Nemoto et al., 2002; Tareke, Rydberg, Karlsson, Eriksson, & Törnqvist, 2002), and $^{13}C_3$-acrylamide and D_3-acrylamide are available for LC-MS/MS (Rosén & Hellenäs, 2002; Rosén, 2002). Tareke et al. (2002) reported that an internal standard could reduce the discrepancies between GC/MS and LC-MS/MS. However, the FDA has recommended the LC-MS/MS using $^{13}C_3$-acrylamide for the determination of acrylamide in food, because GC/ECD or GC/MS methods can exhibit problems in reproducibility and detection limits resulting from derivation efficiencies (EPA, 1996; Nemoto et al., 2002). The LC-MS/MS method employing $^{13}C_3$-acrylamide is easier than GC/ECD and GC/MS, but the problems of poor recovery efficiency of both acrylamide and $^{13}C_3$-acrylamide during the extraction and purification of a sample matrix have not been solved.

In the present solvent system, 3-chloropropanediol (3-CPD) is water-soluble and has very similar retention time with acrylamide and $^{13}C_3$-acrylamide but a different mass spectrum. However, some food samples such as soy sauce contains 3-CPD in the sample matrix. This interference could completely be avoided so we used d_5-3-chloropropanediol (D_5-3-CPD) as a recovery standard. D_5-3-CPD is a reliable, sensitive and fast analytical method for the determination of acrylamide in processed foods.

The objectives of this study were to develop an LC-MS/MS method for the determination of acrylamide and to improve the reliability of analysis methods applied to food samples by determining the recovery efficiency using both recovery and internal standards.

2. Materials and methods

2.1. Food samples

Samples of rice, bread, corn chips, potato chips, biscuits, candy, and coffee were purchased from retail markets in Kyonggido, Korea. The samples were stored at <4 °C until analysis.

2.2. Reagents

All reagents were of analytical grade unless otherwise stated. Acrylamide (99.9%) and formic acid were purchased from Sigma Chemical (St. Louis, MO). Methanol and acetic acid were purchased from Merck (Darmstadt, Germany). $^{13}C_3$-labelled acrylamide (99.0%) and d_5-3-chloropropanediol (99.4%) were purchased from Cambridge Isotope Laboratory (Andover, MA) and used as internal and recovery standards, respectively. High-purity water was obtained from an ultrapure water system (Human Science, Seoul, South Korea).

2.3. Standard solutions

The stock solutions of acrylamide, $^{13}C_3$-labeled acrylamide, and d_5-3-chloropropanediol were prepared in distilled water at concentrations of 1000 μg/ml. The standard solutions were protected from light and stored in a refrigerator at 4 °C.

2.3.1. Internal standard solution

A 1.0-ml aliquot of the $^{13}C_3$-labeled acrylamide stock solution was diluted to 1000 ng/ml, and 2 ml of the resulting solution was added to each sample before extraction.

2.3.2. Recovery standard solution

A working recovery standard (20 μg/ml) was prepared by dilution of the 1000 μg/ml d_5-3-chloropropanediol standard solution with water. A 2-μl aliquot of the 20-μg/ml working recovery standard solution was added to 1 ml of each sample and mixed thoroughly prior to purification with a C_{18} cartridge.

2.3.3. Calibration standard solution

Using a microsyringe, 0.1, 5, 25, 100, 200, 300, 400, and 500 μl of acrylamide stock solution were transferred to a series of 10-ml volumetric flasks, and 20 μl of the internal standard and 20 μl of the recovery standard were added together to each flask and then diluted to the correct volume with water. All standard solutions were stored at 4 °C, and then stood at room temperature for about 30 min prior to analysis. The maximum storage time for the working standard solution was 1 month. We freshly made the calibration standard every time before use.

2.4. LC-MS/MS analysis

Sample analysis was conducted with an HPLC-Sykam S2100 Solvent Delivery System (Sykam, Germany) coupled to MS/MS with an electrospray ionization (ESI) source (Quattro Micro, Manchester, UK). The software used to operate the device and perform spectral analysis was MassLynx 4.0. A schematic diagram of MS/MS is shown in Fig. 1. The samples were separated by the Aqua C_{18} HPLC column (2 × 250 mm), packed with 5-μm particles (Phenomonex, Torrance, CA), using the mobile phase with aqueous 0.2% acetic acid and 1% methanol, at a flow rate of 0.2 ml/min for 14 min. The volume of each sample injected was 20 μl. The electrospray positive ionization (ESI+) source had the following settings: capillary voltage of 4.2 kV, source temperature of 120 °C, desolvation temperature of 240 °C, desolvation gas flow rate of 650 l/h with nitrogen, and an argon gas pressure of 2.5 mbar (used as the collision gas). Acrylamide was determined by multiple reaction monitoring (MRM). MRM was performed by monitoring the 72- to 55-*m/z* transition for acrylamide, the 75- to 58-*m/z* transition for $^{13}C_3$-acrylamide, and the 116- to 98-*m/z* transition for d_5-3-chloropropanediol. In all MRM transitions, the dwell and inter scan delay times were 1 and 0.2 s, respectively.

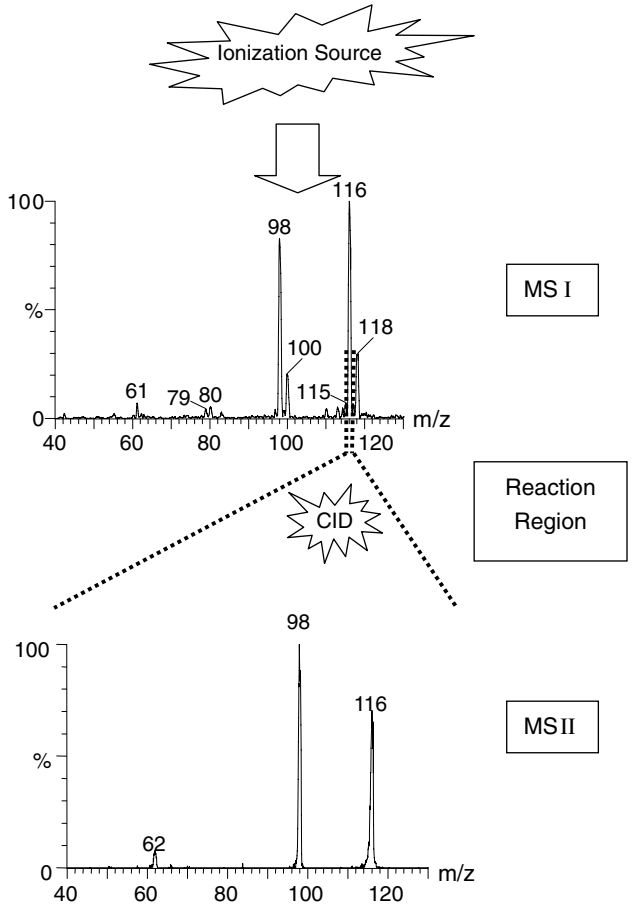

Fig. 1. Schematic diagram of MS/MS (MS I: full scan spectrum, MS II: daughter-ion scan spectrum of the 116-*m/z* ion, CID: collision-induced dissociation cell).

2.5. Sample preparation

A portion of each sample equal to the recommended serving size of the manufacturer was crushed and homogenized, and then 10 g of it was placed in a 250-ml beaker to which 2 ml of an internal standard and 98 ml of water were added. Samples were well mixed for 20 min and the aqueous phase was centrifuged at 9000 rpm for 10 min. Then, a 1-ml aliquot was pipetted into a 10-ml polypropylene graduated conical tube with a cap. Recovery standard solution (2 μl) was added into the extracted sample and mixed well. A C_{18} solid-phase extraction cartridge (Sep-Pak Plus, Waters, Milford, MA) was activated with 5 ml of methanol followed by 5 ml of water, and then used to purify an aliquot of the sample. The eluent was discarded. The acrylamide residues in the cartridge were eluted with 2 ml of water and collected. All eluent from the previous step was passed through a 0.45-μm membrane and collected for LC-MS/MS analysis. The extracts of the samples were directly analyzed by LC-MS/MS.

3. Results and discussion

All materials have specific mass spectra that can be used to identify them. For quantification at low concentrations, however, 3–5 representative ions of the compounds should be selected because complete spectra are not available due to increased matrix interferences increasing the noise level. It is very important to select ions that are structurally relevant to the target compound but have no interfering substances or background signal. Therefore, in the present study, $^{13}C_3$-acrylamide and d_5-3-chloropropanediol were selected to display the characteristics of acrylamide.

3.1. Mass spectrum of acrylamide

An LC-MS/MS method was developed to analyze acrylamide in foods without involving a derivative procedure of acrylamide. Fig. 2(a) shows the ESI+ mass spectra of acrylamide. The ions of 72, 55 and 56 *m/z* contain structurally relevant information on acrylamide in the ESI+ mode. The mass spectrum of acrylamide show a protonated

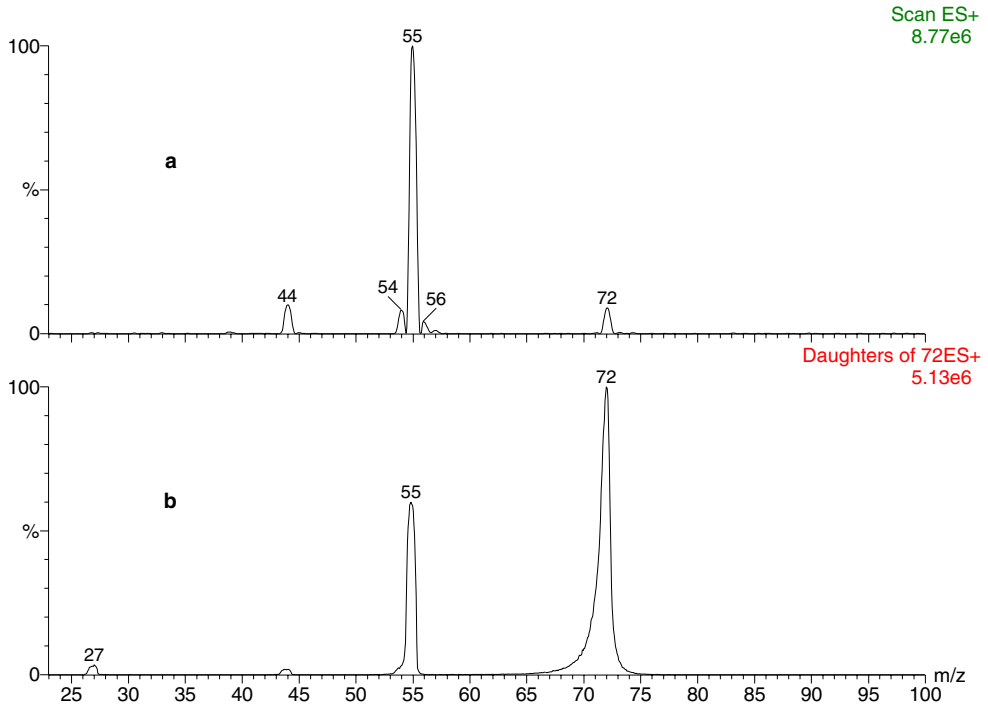

Fig. 2. ESI+ mass spectra of acrylamide. (a) Scan spectrum and (b) daughter-ion scan spectrum of the $[M + H]^+$ ion at 72 *m/z*.

molecule ($[M + H]^+$) at 72 *m/z*. This molecular ion produced abundant ions at 55 *m/z* resulting from the loss of a protonated amine in acrylamide, which could be explained by the α-cleavage effects. The abundance of 56-*m/z* ion was evaluated as 3% of that of 55-*m/z* ion, which can be explained by the isotope rule of carbon. Amide and ethenyl groups produced ions of 44 and 27 *m/z*, respectively. One of the most important aspects when analyzing low-molecular-weight compounds using LC/MS or LC-MS/MS, is minimizing the interference ions that increase the background signal from the mobile phase and samples. One method is to use MRM, which is one of the functions of MS/MS; acrylamide was also identified and quantified by MRM. The daughter-ion scan spectrum was obtained from the characteristic ion at 72 *m/z* including information on the protonated acrylamide molecule ion ($[M + H]^+$), as in Fig. 2(b). Characteristic ions appeared at 27, 44, and 55 *m/z*. The daughter ion at 55 *m/z* was used for quantification and identification. The cone voltage and collision energy for the increasing-intensity transition from 72 to 55 *m/z* were 13 V and 45 eV, respectively. Acrylamide was identified and quantified by MRM using the selected 72- to 55-*m/z* ion transition.

3.2. Mass spectrum of $^{13}C_3$-acrylamide

The $[M + H]^+$ ion of $^{13}C_3$-acrylamide appeared at 75 *m/z* in LC-MS/MS in the ESI+ mode. Fig. 3(a) shows the mass spectra of $^{13}C_3$-acrylamide. The 58-*m/z* ion, representing the base peak of $^{13}C_3$-acrylamide, resulted from the loss of a protonated amine group due to α-cleavage effects. However, the 59-*m/z* isotope ion did not appear, because isotope carbons are substituted for all carbons in acrylamide. An amide group including one atom of isotopic carbon produced a 45-*m/z* ion, and an ethenyl group including two atoms of isotopic carbon produced a 29-*m/z* ion, which could be explained by the α-cleavage effects. The daughter-ion scan spectrum of the 75-*m/z* ion, representing the $[M + H]^+$ ion of $^{13}C_3$-acrylamide, is shown in Fig. 3(b). The daughter-ion scan spectrum was obtained from the characteristic 75-*m/z* ion including information on the $[M + H]^+$ ion of $^{13}C_3$-acrylamide. The characteristic ions appeared at 29, 45, and 58 *m/z*. The daughter ion at 58 *m/z* produced by the loss of an amino group from $^{13}C_3$-acrylamide was used for quantification and identification. The cone voltage and collision energy for the increasing-intensity transition from 75 to 58 *m/z* were 13 V and 45 eV, respectively. $^{13}C_3$-acrylamide was identified and quantified by MRM using the selected 75- to 58-*m/z* ion transition.

3.3. Mass spectrum of D_5-3-chloropropanediol

Fig. 4(a) shows the mass spectra of d_5-3-chloropropanediol. Characteristic ions appeared at 79, 80, and 98 *m/z*. D_5-3-chloropropanediol exhibited characteristic ions that were separated by 2 amu: 116 and 118 *m/z* in LC-MS/MS in the ESI+ mode. D_5-3-chloropropanediol exhibited characteristic lines separated by 2 amu, corresponding to ^{35}Cl and ^{37}Cl (Lee, 1998). For a monochlorinated compound, the ^{35}Cl:^{37}Cl ratio will always be 3:1. D_5-3-chloropropanediol, used as a recovery standard, exhibited an

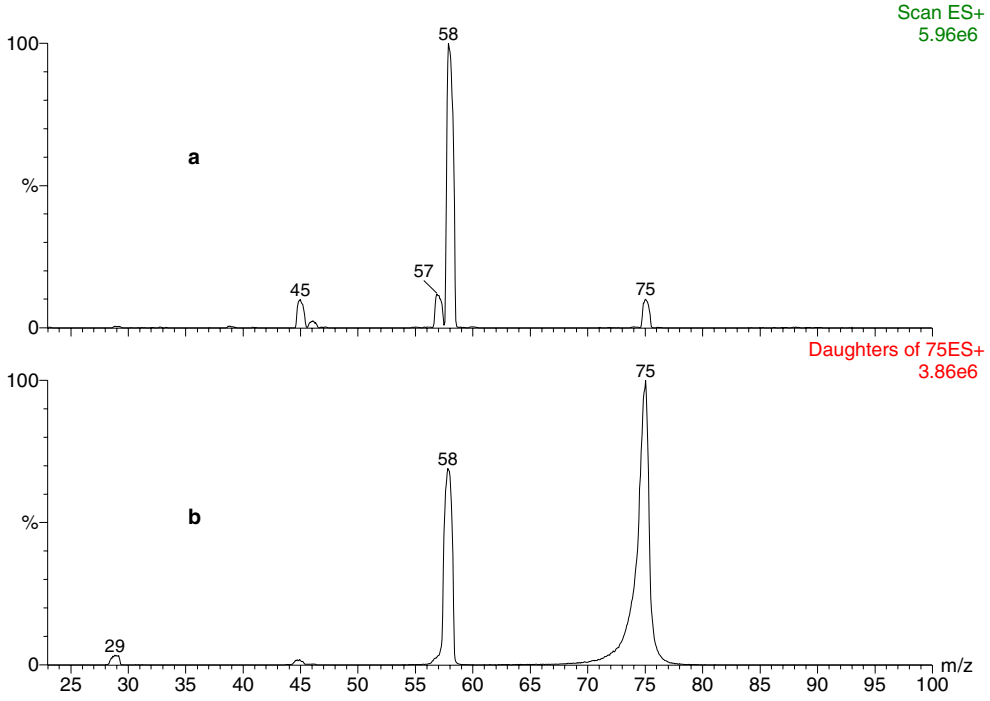

Fig. 3. ESI+ mass spectra of $^{13}C_3$-acrylamide. (a) Scan spectrum and (b) daughter-ion scan spectrum of the $[M + H]^+$ ion at 75 *m/z*.

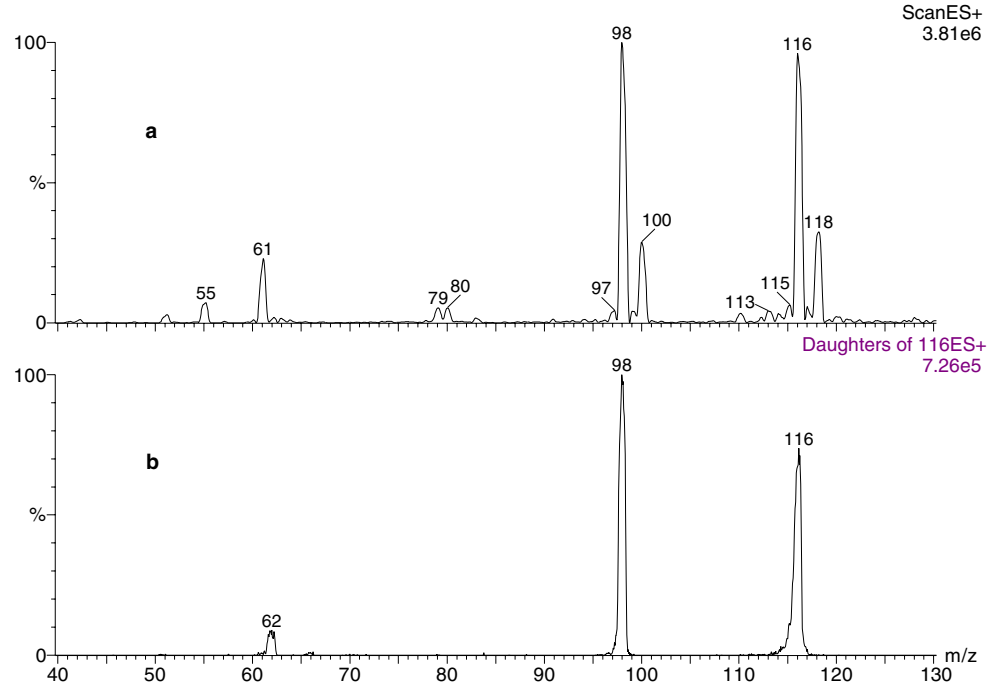

Fig. 4. ESI+ mass spectra of d_5-3-chloropropanediol. (a) Scan spectrum and (b) daughter-ion scan spectrum of the $[M + H]^+$ ion at 116 *m/z*.

isotopic pattern characteristic of the presence of a chlorine atom. The abundance ratio of 116 *m/z* ($[M + H]^+$) to 118 *m/z* ($[M + H + 2]^+$) was 3:1. The 98-*m/z* ion was attributable to the loss of water from d_5-3-chloropropanediol. Therefore, the abundance ratio of 98 *m/z* ($[M + H - H_2O]^+$) to 100 *m/z* was also 3:1. The daughter-ion scan spectrum was obtained from the characteristic ion at 116 *m/z* including information on the $[M + H]^+$ ion of d_5-3-chloropropanediol, Fig. 4(b). The daughter ion at 98 *m/z*, was used for quantification and identification. This ion indicates the structural information of the loss of water from the molecular ion. The cone voltage and collision

energy for the increasing-intensity transition from 116 to 98 *m/z* were 12 V and 7 eV, respectively. D_5-3-chloropropanediol was identified and quantified by MRM using the selected 116- to 98-*m/z* ion transition.

The three compounds mentioned above could be identified and quantified, simultaneously by selecting their unique associated ions. In particular, the identification and quantification of acrylamide and $^{13}C_3$-acrylamide, which could not be separated using an HPLC column, were performed accurately on the basis of mass separation. The limits of detection and quantification were obtained when the signal-to-noise ratio was more than 3 times and more than 10 times (peak to peak), respectively. In the present study, the limits of detection and quantification of acrylamide were 0.1 and 2 μg/kg, respectively, but the values varied with the sample matrix and its homogeneity. However, the method using d_5-3-chloropropanediol as a recovery standard decreased the matrix effects and increased the reliability of the analysis. The retention time mostly decreased when run overnight because of column contamination. However, $^{13}C_3$-acrylamide always had the same retention time as acrylamide. Therefore, the problem of a variable retention time was easily overcome by using a method that employed both an isotopic internal standard and a recovery standard.

3.4. Conditions for separation

The chromatograms of acrylamide, $^{13}C_3$-acrylamide and d_5-3-chloropropanediol are shown in Fig. 5. The acrylamide peak could be identified easily in the chromatograms despite changes in the retention time, because this peak always overlapped that of $^{13}C_3$-acrylamide used as an internal standard. The reliability of data was improved by calculating the relative retention time with d_5-3-chloropropanediol used as a recovery standard. A methanol contents of <0.5%, lead to rapid contamination of the analysis column and a poor peak shape for d_5-3-chloropropanediol. In contrast, a methanol contents of >2%, lead to short elution time for acrylamide and overlapping with contaminants of the samples. Some of the contaminants and acrylamide exhibited similar mass spectra (e.g., the 72- to 55-*m/z* ion transition), which interfered with sample quantification when the concentration of acrylamide was low. It was inferred that the unknown compounds had structures and creation mechanisms similar to those of acrylamide. However, acrylamide, $^{13}C_3$-acrylamide and d_5-3-chloropropanediol were appropriately separated in a column using an aqueous mobile phase containing 1% methanol. The samples were analyzed using an electrospray probe in the positive ionization mode. It is usual for organic acids to be added into the mobile phase to improve the ionization yields of compounds in this mode. However, the concentration of acid should be optimized in order to avoid a decrease in the signal-to-noise ratio due to matrix effects. In this study, acetic acid at 0.2% was selected for improving the ionization efficiencies of the three compounds in LC-MS/MS. The results indicate that the aqueous mobile phase containing 1% methanol and 0.2% acetic acid is appropriate for the identification and quantification of acrylamide in processed foods.

3.5. Calibration

Quantification of acrylamide was performed through comparison with a calibration curve, as shown in Fig. 6.

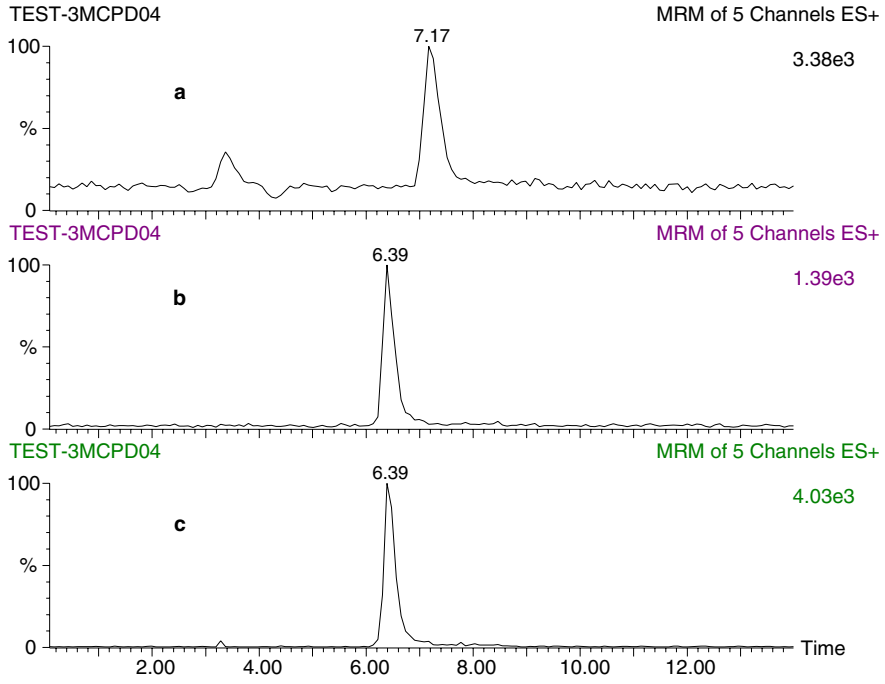

Fig. 5. Chromatograms of d_5-3-chloropropanediol (a), $^{13}C_3$-acrylamide (b) and acrylamide (c).

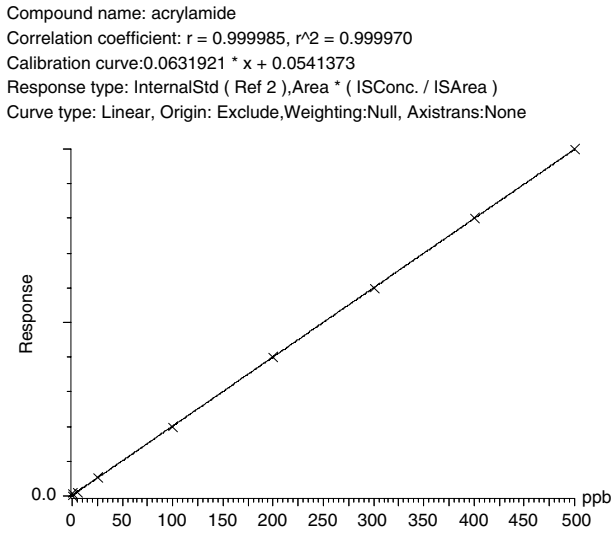

Fig. 6. Calibration curve for acrylamide (S: standard, IS: internal standard).

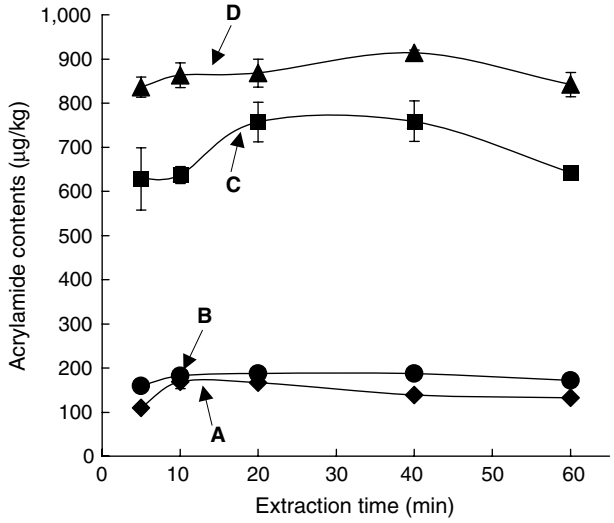

Fig. 7. The extraction effects of acrylamide in food samples (A and B: wheat-flour snacks; C and D: potato chips). Values are mean ± SD (n=3).

The calibration curve was computed using the ratio of the area of the acrylamide (72–55 m/z) peak to that of the $^{13}C_3$-acrylamide (75–58 m/z) peak. The correlation coefficient was $r = 0.99$. The recovery efficiency in each sample was based on the ratios of the areas of d_5-3-chloropropanediol (116–98 m/z) peak to that of the $^{13}C_3$-acrylamide (75–58 m/z) peak.

3.6. Extraction time

One of the most important factors influencing analysis speed, repeatability, and accuracy was the suitable extraction time. A long extraction allows the formation of secondary products or decomposition of target compounds. For determining the optimal extraction times of acrylamide in processed foods, four samples with differing acrylamide concentrations and physical properties were tested. We removed lipidic layer from potato chips by mixing 10 g of sample with 98 ml of water and centrifuging at 9000 rpm. Upper lipidic layer was discarded and water layer was used for analysis. The results are shown in Fig. 7. The acrylamide concentrations in snack samples (fried foods made from wheat flour) did not vary after 10 min of extraction. However, the acrylamide concentrations in the potato chips did vary with the extraction period, increasing up to 20 min and then decreasing after 40 min. Therefore, 20 min of extraction of acrylamide in processed foods appears to be optimal.

3.7. Recovery yields

The recovery yields of acrylamide are dependent on the constituents and concentrations of target compounds in samples (Bologna, Andrawes, Barvenik, Lentz, & Sojka, 1999; Rydberg et al., 2003). Therefore, for the recovery test, four samples made from different materials (rice, wheat, corn and potato) were selected. The recovery yields of acrylamide in the steamed rice and bread, which have relatively low acrylamide and high moisture contents, were estimated by adding 10 and 300 μg/kg acrylamide, respectively. For the fried foods, which have relatively high acrylamide and low moisture contents, 500 μg/kg acrylamide was added. Table 1 lists the recovery yields of acrylamide in different food matrixes, which were determined as 97–102%. In this study, the recovery efficiencies were higher than those of Arikawa and Shiga (1980) and Yasuhara et al. (2003), which were >80% and 85%, respectively. Takatsuki et al. (2003) reported that the recovery yields of $^{13}C_3$-acrylamide used as an internal standard were >68%. In order to decrease this serious discrepancy, d_5-3-chloropropanediol was added as a recovery standard prior to purification after extraction of acrylamide in samples, and the loss of $^{13}C_3$-acrylamide was calculated in each of the samples. Whilst the absolute amounts of acrylamide, $^{13}C_3$-acrylamide and d_5-3-chloropropanediol fluctuated during the process of purification, the relative proportions of these three compounds remained relatively constant. This made it possible to estimate whether $^{13}C_3$-acrylamide was lost during the analysis. This indicates that where the recovery yields of $^{13}C_3$-acrylamide are lower than 90%, the analysis

Table 1
The recovery yields of acrylamide using the LC-MS/MS method

Food sample	Acrylamide contents (μg/kg)			Recovery yield (%, w/w)[a]
	Before spiking	Spiked amount	After spiking	
Rice	4	10	14	100.0
Bread	33	300	331	99.3
Corn chips	110	500	597	97.4
Potato chips	1367	500	1878	102.2

[a] Recovery yield (%) $= \frac{\text{After spiking} - \text{Before spiking}}{\text{Spiked amount}} \times 100$.

Table 2
The repeatability of the LC-MS/MS method

Food sample	Acrylamide[a] (μg/kg)	CV[b] (%)
Bread	33.0 ± 4.9	9.0
Candy	34.3 ± 3.1	4.5
Coffee	237.7 ± 6.3	3.1
Biscuits	714.2 ± 42.8	5.2
Potato chips	1376.7 ± 8.8	0.4

[a] Each sample was split into five aliquots after homogenization, and then analyzed during the same day. Values are mean ± SD ($n = 5$).
[b] CV: coefficient of variation.

results for acrylamide should be corrected by repeating the preparation. Because $^{13}C_3$-acrylamide is the compound that is most physicochemically similar to acrylamide, the loss of $^{13}C_3$-acrylamide during a purification procedure should be measured. Using d_5-3-chloropropanediol as a recovery standard was suitable as the index compound for estimating the extraction and purification of acrylamide in samples. Therefore, the reliabilities of data from samples with complex matrixes can be determined by using this technique.

3.8. Repeatability

Five food groups were selected to compare various analysis errors based on variable sample matrixes and acrylamide concentrations. The repeatability of samples using the current method was tested, and the results are listed in Table 2. The acrylamide content in the five food groups ranged from 33 to 1377 μg/kg, which are detectable levels for normal processed foods (Roach et al., 2003). The repeatability of the five replications of bread, for which homogenization is difficult, was the lowest, with a coefficient of variation (CV) of 9%. The CVs of candies and liquid-phase coffee were 4.5% and 3.1%, respectively. The CV of potato chips was the lowest, at 0.4%. The repeatability was clearly dependent on residual concentrations and the characteristics of samples.

4. Conclusion

An LC-MS/MS method has been developed for the analysis of acrylamide in processed foods using both internal and recovery standards. Samples were extracted for up to 20 min and analyzed by LC-MS/MS using the mobile phase of methanol, acetic acid, and water (1.0/0.2/98.8, v/v/v). The ion transitions of 72–55 m/z (acrylamide), 75–58 m/z ($^{13}C_3$-acrylamide), and 116–98 m/z (d_5-3-chloropropanediol) were found to be the most reliable for identification and quantification purposes. The recovery yield of acrylamide was >97%, and the repeatability was high with a CV of <9%. Therefore, this method could be used to minimize the effects of variations in the matrix and hence be applicable to the determination of acrylamide in a variety of processed foods.

References

Ahn, J. S., Castle, J., Clarke, D. B., Lloyd, A. S., Philo, M. R., & Speck, D. R. (2002). Verfication of the findings of acrylamide in heated foods. *Food Additives and Contaminants, 19*, 1116–1124.

Andrawes, F., Greenhouse, S., & Draney, D. (1987). Chemistry of acrylamide bromination for trace analysis by gas chromatography and gas chromatography-mass spectrometry. *Journal of Chromatography, 399*, 269–275.

Andrzejewski, D., Roach, J. A. G., Gay, M. L., & Musser, S. M. (2004). Analysis of coffee for the presence of acrylamide by LC-MS/MS. *Journal of Agricultural and Food Chemistry, 52*, 1996–2002.

Arikawa, A., & Shiga, M. (1980). Determination of trace acrylamide in the crops by gas chromatography. *Bunseki kagaku, 29*, T33–T39.

Becalski, A., Lau, B. P.-Y., Lewis, D., Seaman, S. W., Hayward, S., Sahagian, M., et al. (2004). Acrylamide in french fries: influence of free amino acids and sugars. *Journal of Agricultural and Food Chemistry, 52*, 3801–3806.

Bologna, L. S., Andrawes, F. F., Barvenik, F. W., Lentz, R. D., & Sojka, R. E. (1999). Analysis of residual acrylamide in field crops. *Journal of Chromatographic Science, 37*, 240–244.

Castle, L. (1993). Determination of acrylamide monomer in mushrooms grown on polyacrylamide gel. *Journal of Agricultural and Food Chemistry, 41*, 1261–1263.

Castle, L., Campos, M. J., & Gilbert, J. (1991). Determination of acrylamide monomer in hydroponically grown tomato fruits by capillary gas chromatography-mass spectrometry. *Journal of the Science of Food and Agriculture, 54*, 549–555.

EPA Method 8316., (1994). Acrylamide, acrylonitrile and acrolein by high performance liquid chromatography.

EPA Method 8032A., (1996). Acrylamide by gas chromatography.

Gertz, C., & Klostermann, S. (2002). Analysis of acrylamide and mechanisms of its formation in deep-fried products. *European Journal of Lipid Science and Technology, 104*, 762–771.

Hashimoto, A. (1976). Improved method for the determination of acrylamide monomer in water by means of gas-liquid chromatography with an electron-capture detector. *Analyst, 101*, 932–938.

Hofmann, T. (1998). Acetylformoin – A chemical switch in the formation of colored Maillard reaction products from hexoses and primary and secondary amino acids. *Journal of Agricultural and Food Chemistry, 46*, 3918–3928.

Lagalante, A. F., & Felter, M. A. (2004). Silylation of acrylamide for analysis by solid-phase microextraction/gas chromatography/ion-trap mass spectrometry. *Journal of Agricultural and Food Chemistry, 52*, 3744–3748.

Lee, T. A. (1998). Halogenated hydrocarbons. In *A beginner's guide to mass spectral interpretation* (pp. 47–59). New York, USA: Wiley.

Mottram, D. S., Wedzicha, B. L., & Dodson, A. T. (2002). Acrylamide is formed in the Maillard reaction. *Nature, 419*, 448–449.

Nemoto, S., Takarasuki, S., Sakaki, K., & Maitani, T. (2002). Determination of acrylamide in food by GC/MS using ^{13}C-labeled acrylamide as an internal standard. *Shokuhin Eiseigaku Zasshi, 43*, 371–376.

Roach, J. A. G., Andrzejewski, D., Gay, M. L., Nortrup, D., & Musser, S. M. (2003). Rugged LC-MS/MS survey analysis for acrylamide in foods. *Journal of Agricultural and Food Chemistry, 51*, 7547–7554.

Robert, F., Vuataz, G., Pollien, P., Saucy, F., Alonso, M. I., Bauwens, I., et al. (2004). Acrylamide formation from asparagine under low-moisture Maillard reaction conditions. 1. Physical and chemical aspects in crystalline model systems. *Journal of Agricultural and Food Chemistry, 52*, 6837–6842.

Rosén, J. D., (2002). Acrylamide in food: Is it a real threat to public health? American council on science and health. Available from http://www.acsh.org.

Rosén, J., & Hellenäs, K. E. (2002). Analysis of acrylamide in cooked foods by liquid chromatography tandem mass spectrometry. *Analyst, 127*, 880–882.

Rydberg, P., Eriksson, S., Tareke, E., Karlsson, P., Ehrenberg, L., & Tornqvist, M. (2003). Investigation of factors that influence the acrylamide content of heated foodstuffs. *Journal of Agricultural and Food Chemistry, 51*, 7012–7018.

Stadler, R. H., Blank, I., Varga, N., Robert, F., Hau, J., Guy, P. A., et al. (2002). Acrylamide from Maillard reaction products. *Nature, 419*, 449–450.

Swedish National Food Administration, (2002). Information about acrylamide in food Uppsala, Sweden. April. Available from http://192.71.90.8/engakrylanalysresultat.htm.

Takata, K., & Okamoto, T. (1991). A method for determination of acrylamide in environmental samples by gas chromatography using bromination dehydrobromination. *Journal of Environmental Chemistry, 1*, 559–565.

Takatsuki, S., Nemoto, S., Sasaki, K., & Maitani, T. (2003). Determination of acrylamide in processed foods by LC/MS using column switching. *Shokuhin Eiseigaku Zasshi, 44*, 89–95.

Tareke, E., Rydberg, P., Karlsson, P., Eriksson, S., & Törnqvist, M. (2002). Analysis of acrylamide, carcinogen formed in heated foodstuffs. *Journal of Agricultural and Food Chemistry, 50*, 4998–5006.

Yasuhara, A., Tanaka, Y., Hengel, M., & Shibamoto, T. (2003). Gas chromatographic investigation of acrylamide formation in browning model systems. *Journal of Agricultural and Food Chemistry, 51*, 3999–4003.

Yaylayan, V. A., Machiels, D., & Istasse, L. (2003). Thermal decomposition of specifically phosphorylated D-glucose and their role in the control of the Maillard reaction. *Journal of Agricultural and Food Chemistry, 51*, 3358–3366.