Determination of Acrylamide in Starch-Based Foods by HPLC with Pre-Column Ultraviolet Derivatization

Zhiming Geng*, Peng Wang, and **Aiming Liu**
Institute of Agricultural Biotechnology, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, China

Abstract

A new method is developed for the determination of acrylamide in starch-based foods. The method included the extraction of acrylamide with water, defatting with hexane, derivatization with potassium bromate ($KBrO_3$) and potassium bromide (KBr), liquid–liquid extraction with ethyl acetate–hexane (4:1), and concentration. The final analyte (2-bromopropenamide, 2-BPA) is analyzed by high-performance liquid chromatography coupled with diode array detection for quantification and by gas chromatography coupled to mass spectrometry for confirmation. The chromatographic analysis is performed on an ODS–3 C_{18} column, and good retention and peak response of acrylamide are achieved under the optimal conditions. The limit of detection and quantitation are estimated to be 15 and 50 µg/kg, respectively. The recoveries of acrylamide from the commercial samples are spiked at levels of 50–1000 µg/kg, and range between 89.6 and 102.0%. These results show that this method should be regarded as a new, low-cost, and robust alternative for conventional investigation of acrylamide.

Introduction

Detection of high concentrations of acrylamide in heated starch-based foods by the Swedish National Food Administration in April of 2002 (1) raised worldwide concern because acrylamide was found to be carcinogenic in rodents (2–3) and was classified as a probable human carcinogen (4). The mechanism studies indicated that acrylamide formation occurs during the Maillard reaction of reducing sugars with asparagine at temperatures greater than 120°C, and the major reactants leading to the release of acrylamide are sugars and asparagine (5–7).

Analytical methods for acrylamide in foods have been extensively studied in recent years. To date, a great number of papers dealing with the analysis of acrylamide in cooked foods have been published. In these papers, the analytical methods are based mainly on mass spectrometry (MS) as the determinative technique, coupled with a chromatographic step either by liquid chromatography (LC) (8–12) or gas chromatography (GC) (13–18). As for the method sensitivity, the limits of quantitation (LOQ) ranged from 30 to 50 µg/kg for LC–MS, 4 to 30 µg/kg for GC–MS and LC–MS–MS, respectively. Hitherto, GC–MS and LC–MS–MS are regarded as the most useful and popular methods for the determination acrylamide in foods (19).

Besides LC or GC combined with an MS technique, some researchers reported analytical methods for the determination of acrylamide using simple LC or GC techniques; the advantages these methods are acknowledged as easy generalization, lower cost, and strong maneuverability compared with those combined with an MS technique (20–23). However, acrylamide is a very polar molecule, and its retention on conventional reversed-phase sorbent is poor, which results in an unsatisfactory separation between acrylamide and the co-extracts of the matrix in LC. Therefore, LC combined with ultraviolet detection (UV) or diode array detection (DAD) on conventional reversed-phase sorbent columns lacked the desired selectivity and could be applied only to those foods with a high-level acrylamide, such as potato-based food.

Bromination is most frequently applied in the derivative treatment during a GC-related analysis of acrylamide. The reaction product is 2,3-dibromopropionamide (Equation 1), which is less polar compared with the original compound and is detected as the final analyte (13,14,16). However, 2,3-dibromopropionamide (2,3-DBPA) has been found unstable under certain conditions and can be converted to the more stable derivative 2-bromopropenamide (Equation 2) on the inlet of GC or on the capillary column by dehydrobromination (25), which may yield poor repeatability and accuracy. Some researchers prefer to deliberately convert 2,3-DBPA to 2-bromopropenamide (2-BPA) by adding 10% of triethylamine to the final extract before injection (15,23,24).

2,3-DBPA:

$$Br_2 + H_2C = CH–CONH_2 \rightarrow H_2BrC–CHBr–CONH_2 \qquad \text{Eq. 1}$$

2-BPA:

$$H_2BrC–CHBr–CONH_2 \rightarrow HBrC = CH–CONH_2 + HBr \qquad \text{Eq. 2}$$

No matter whether 2,3-DBPA or 2-BPA is the target analyte, the advantage of acrylamide bromination is that a relative more volatile compound is produced, which improves GC characteristics. Bromination results in an increased selectivity, which compensates for a hard and time-consuming derivatization process. Because the bromination product is less polar than acrylamide, such a derivatization can also be used in an LC-based method to improve the retention of the analyte on conventional LC

*Author to whom correspondence should be addressed: email zmgeng@163.com

reversed-phase sorbents, and UV detection can be used as the determinative technique provided the bromination product absorbs within the UV wavelength range.

This paper successfully applies the bromination to an LC method for the determination of acrylamide in foods, and it is the first LC method dealing with acrylamide in such a way. Acrylamide is extracted from foods by water and converted to 2-bromopropenamide (2-BPA) by bromination and dehydrobromination prior to separation on a reversed-phase C_{18} column. 2-BPA is well resolved from matrix co-extractives and detected by DAD at 215 nm. The LOQ is 50 µg/kg, which is a little higher than those of GC–MS and LC–MS–MS methods and hinders its application to foods with acrylamide level less than 50 µg/kg. However, this method is a low cost technique with excellent maneuverability, which can be applied to a wide range of food category groups with acrylamide levels ranging from 50 to 2000–3000 µg/kg and easily adopted by most analytical laboratories. Therefore, the method may, after all, be accepted as a robust alternative for conventional investigation of acrylamide.

Experimental

Equipment

The HPLC system used was an Agilent 1100 (Waldbronn, Germany) consisting of a quaternary pump with vacuum degasser, a temperature controlled column oven, an autosampler, and a DAD. The chromatographic separations were performed on an ODS-3 C18 column (250 mm × 4.6 mm, Intersil, Japan). The GC–MS analysis was performed on a HP 6890 (Hewlett-Packard) gas chromatograph coupled with an HP 5973 benchtop mass selective detector (MSD), operated in selected ion monitoring (SIM) mode with positive electron impact (EI) ionization, and a HP5-MS capillary column (polysiloxane polymers, 30 m × 0.25 mm i.d., 0.25 µm film thickness, J&W Scientific, Agilent, CA) was used for separations. A HL-2070 multi-function food processor (Shanghai Herine Electric Appliance Co. Ltd., Shanghai, China) was used to pulverize and homogenize samples. A HL-2070 multi-function food processor (Shanghai Herine Electric Appliance Co. Ltd., Shanghai, China) was used to pulverize and homogenize samples. An HS2060A ultrasonic shaker (Kunshan Ultrasonic Instrument Co. Ltd., Kunshan, Jiangsu, China) was used for the extractions of the samples. A Refrigerated Centrifuge (Biofuge stratos, Germany) was used for the centrifugal separation. A RE-2000 rotary evaporator (Shanghai Yarong Biochemical Apparatus Co., Ltd, Shanghai, China) was used for concentrations. A Vortex mixer (Shanghai Qite Analytical Apparatus Co., Ltd, Shanghai, China) was used for thorough mixing of the solutions.

Chemicals and consumables

Acrylamide (99%) and $^2H_3$-labeled acrylamide (isotopic purity 98%) were purchased from Sigma-Aldrich (St. Louis, MO) and Cambridge Isotope Laboratories (Andover, MA), respectively. Methanol (HPLC-grade) was supplied by Merck (Darmstadt, Germany). All of other chemicals were of analytical grade. Water was purified with a Milli-Q system (Millipore, Bedford). AccuBOND Si (6 mL, 500 mg) solid-phase extraction (SPE) cartridges were supplied by Agilent Technologies (Santa Clara, CA).

Standards and reagents

The stock solution of acrylamide (1 mg/mL) and $^2H_3$-labeled acrylamide (0.1 mg/mL) were prepared by dissolving a suitable amount of the compounds in water. These solutions were then appropriately diluted with water to prepare working standards at 10 and 4 µg/mL, respectively. All stock solutions and working standards were kept at 4°C for a month. Carrez I solution was prepared by dissolving 15 g of potassium hexacyanoferrate in 100 mL of water, and Carrez II solution by dissolving 30 g of zinc sulfate in 100 mL of water.

Samples

All samples (fried potato chips, biscuits, and Chinese fried/baked foods) were purchased from a local supermarket. Before extraction, all the samples were pulverized and homogenized in a multi-function food processor. Certified reference test material (ERM-BD272 crisp bread) was obtained from Federal Institute for Materials Research and Testing (Berlin, Germany) to validate the robustness of the present method.

Extraction

The 2.00 g of the homogenized sample (1.00 g for fried potato chips) was weighed into a 50 mL centrifuge tube. For GC–MS confirmatory tests, the sample matrixes were additionally spiked with 125 µL of $^2H_3$-labeled acrylamide (4 µg/mL) as the internal standard, and the tubes were placed for 10 min in order that labeled acrylamide could adequately mix with sample matrix via osmotic effect. As for extraction of acrylamide, 18 mL of water was added along with Carrez I and Carrez II solutions (each 1 mL) to the tube. The tube was capped and shaken in an ultrasonic shaker for 15 min, and was centrifuged at 15,000 rpm for 10 min. To make a defatting process, the clear supernatant was transferred to a 60 mL separatory funnel, and 15 mL of hexane was added. The separatory funnel was shaken for 1 min, and then the upper organic layer was discarded, while the lower aqueous layer was collected for further use.

Bromination

Ten milliliters of the remaining lower aqueous layer and 0.6 mL sulfuric acid (10%, v/v) were sequentially added into a brown glass tube, and the tube was then placed into refrigerating cabinet for precooling (4°C, 15 min). An aliquot of derivatization reactants, including 1 mL of 0.1 mol/L potassium bromate ($KBrO_3$) and 2.5 g of potassium bromide (KBr) powder, was added to the pre-cooled solution. The tube was shaken with a vortex mixer briefly, and the reaction mixture was allowed to stand for 45 min at 4°C. The derivatization reaction was ended by adding 1 mL of 0.1 mol/L sodium thiosulfate solution. The mixture was transferred to a 60 mL separatory funnel and extracted with 15 mL of ethyl acetate–hexane (4:1, v/v). The organic phase was filtered into a 50-mL round-bottom flask through glass wool covered with ca. 2 g of calcinated sodium sulfate. The separate funnel and the filter were rinsed twice with 5 mL aliquots of ethyl acetate–hexane (4:1, v/v). Pooled fractions were evaporated to dryness on a rotary evaporator (40°C, 140 mbar). For the

HPLC–DAD analysis, the residue was dissolved in 500 µL of 0.01 mol/L sodium hydroxide and then neutralized with 500 µL of 0.01 mol/L phosphoric acid, and the final test solution was filtered through a 0.22-µm syringe filter before injection. For the GC–MS confirmatory test, the residue was dissolved in 5 mL of hexane followed by a SPE cleanup step.

Bromination of calibration standards

The 5, 10, 25, 50, 100, and 200 µL of acrylamide working standard solution (10 µg/mL) were transferred to six brown glass tubes, respectively, and 10 mL of distilled water and 0.6 mL sulfuric acid (10%, v/v) were then added to each tube. These solutions were brominated according to the procedure described herein for sample extracts. The final solutions were filtered through 0.22-µm syringe filters and transferred to autosampler vials which were stored at 4°C for two weeks.

HPLC–DAD analysis

An isocratic elution pattern was adopted for the separation of the analyte, and 7% (v/v) methanol in 0.025 mol/L sodium dihydrogen phosphate was used as the mobile phase. The column temperature was set at 25°C, the flow rate was maintained at 1.0 mL/min, while the detection was performed at 215 nm. The injection volume was 20 µL.

Confirmatory analysis by GC–MS

The hexane solution of the residue prepared by the aforementioned bromination step was submitted to an additional SPE cleanup step so that co-extractive interference could obviously be reduced prior to MS-based analysis. SPE cartridges were conditioned with 5 mL of acetone, followed by 5 mL of hexane. The 2.5 mL of the hexane solution was transferred onto the cartridge and allowed to pass through the sorbent material and discarded. Then, the cartridge was eluted with 5 mL of acetone, and the eluent was collected and evaporated to dryness on a rotary evaporator (40°C, 200 mbar). The residue was redissolved in 1 mL of redistilled ethyl acetate followed by adding 100 µL of triethylamine, and the solution was filtered through a 0.22-µm syringe filter. Finally, 1 µL of the final test solution was injected onto GC–MS for confirmatory analysis. Helium was chosen as the carrier gas at a flow rate of 1.0 mL/min. Following injection, the column was held at 60°C for 2 min, then programmed at 10°C/min to 200°C, and held for 5 min at 200°C (total runtime: 21 min). Injections by the autosampler were made in splitless mode with a purge activation time of 1.0 min and an injection temperature of 280°C. The GC–MS interface transfer line was held at 280°C. Under such conditions, the retention time of acrylamide and $^2H_3$-acrylamide derivatives was 6.2 min. Ions monitored were *m/z* 70 and 149 for 2-bromopropenamide, and *m/z* 110 and 153 for 2-bromo($^2H_2$)-propenamide.

Results and Discussion

Liquid-liquid extractions in sample treatment

As for the extraction of the sample, most LC methods employed water-extraction followed by solid-phase extractions (SPE) via various cartridges, and whether or not a defatting process was involved brought little change to the chromatogram. In this paper, a sample was also extracted with water, and the Carrez solutions were employed to precipitate protein like in the other papers (8,15,20). The extract was defatted by liquid-liquid extraction with hexane, followed by a reaction with bromination reagents, and the bromination product was extracted by a liquid-liquid extraction with a mixture of ethyl acetate and hexane. The merit of the defatting process was obvious. Without the defatting process, fat and other fat-soluble compounds could easily enter the organic phase with the brominaiton product during the second liquid-liquid extraction and pose interference peaks in the chromatogram. After bromination, acrylamide was converted to 2,3-DBPA, which was less polar and could be easily extracted by liquid-liquid extraction with some organic solvents such as ethyl acetate, acetone, etc. In the present paper, ethyl acetate, acetone, and their mixtures with hexane were tested in order to obtain the optimum solvent, which could extract the maximum of 2,3-DBPA with the minimum of the impurity, and the mixture of ethyl acetate and hexane (4:1, v/v) was chosen for its best performance of extraction.

In the whole procedure of sample treatment, two liquid-liquid extractions were performed before and after bromination, respectively, and yielded good outcomes of cleanup. Unlike other LC methods in which SPE via various cartridges were necessary in the cleanup step, the present method did not need any SPE and gave a clean chromatogram by the combination of two liquid-liquid extractions.

Bromination

Acrylamide bromination was well done in most GC-based methods with hydrobromic acid (HBr) and a saturated bromine–water solution (13–16). The derivative reaction was terminated by the addition of sodium thiosulfate to remove the excess bromine. In the present paper, an alternative technique derivatization with KBrO3 and KBr, which was proposed by Nemoto et al. (24), was employed with some modifications. Because the handling of elemental bromine was avoided, this derivatization technique was regarded as a safer derivative treatment of acrylamide (15,18).

In bromination of acrylamide, the product is 2,3-dibromopropionamide (2,3-DBPA), which is less polar compared to the original compound and is easily soluble in non-polar organic solvents like ethyl acetate and hexane. However, 2,3-DBPA was found unstable and could be converted to 2-bromopropenamide (2-BPA) on the inlet of GC or on the capillary column by dehydrobromination (25). Since the dehydrobromination might yield poor repeatability and accuracy, it was preferable to deliberately convert 2,3-DBPA to the stable 2-BPA prior to GC analysis. The conversion could be readily done by adding 10% of triethylamine to the final extract before injection (15,25). In this paper, it was found from three-dimensional (3D) chromatograms that both of 2,3-DBPA and 2-BPA had maximum absorptions, which appeared at 192 nm and 208 nm, respectively. 2-BPA was chosen as the target analyte because of its stronger UV absorption at the wavelength set in the paper (see Figure 1). Since adding of triethylamine led to many interfering peaks to the chromatogram, the conversion of 2,3-DBPA to 2-BPA by adding of triethylamine to

the final extract was not appropriate for the present method. A diluted sodium hydroxide solution (0.01 mol/L) was employed to convert 2,3-DBPA to 2-BPA, and the conversion was found to be accomplished instantaneously at room temperature with excellent reproducibility.

As for the bromination reaction time, bromination with hydrobromic acid (HBr) and a saturated bromine–water solution was frequently carried out from 1 h to overnight (19). In the present study, the relationship between the bromination yield and the reaction time was observed in order to obtain the least reaction time for bromination. With the same amount of the standard acrylamide (0.5 µg), bromination was allowed to stand for different time from 15 min to 12 h, and the target analyte (2-BPA) was then analyzed . The yields were obtained by comparing the areas of 2-BPA for different reaction times with the area of 2-BPA for 12 h. It was found that bromination was almost completed in half an h, and the yield varied little with the increase of reaction time from 45 min to 12 h. Therefore, 45 min was regarded as the least reaction time in which acrylamide bromination could be completed.

The reproducibility of bromination with $KBrO_3$ and KBr was also investigated. With the same amount of standard acrylamide (0.5 µg), bromination was performed in five replicates at five different days. The reproducibility was excellent with the values for relative standard deviation (RSD) of peak areas less than 3%.

Optimization of LC conditions

For the chromatographic separation of acrylamide based on LC, most researchers use reversed-phase chromatography. However, it is difficult to choose an appropriate mobile phase for a reasonable retention time because of the high polarity of acrylamide. Due to lack of desired selectivity, LC-based methods with reversed-phase chromatographic separation and UV detection seem to be unsuitable for the analysis of acrylamide in processed foods, especially for those foods with a low level of acrylamide. In the present study, such a predicament was changed when the acrylamide bromination was applied into the LC-based method. After bromination, 2,3-DBPA was produced and was finally converted to the target analyte (i.e., 2-BPA). Compared to acrylamide, 2-BPA was a less polar compound and its retention on reversed-phase sorbent was improved. Furthermore, the main functional groups (such as double C–C bond, carbonyl, and amino) of acrylamide were not changed, 2-BPA retained almost the same strength of absorption as acrylamide with a maximum absorption at 208 nm. Therefore, with pre-column bromination-derivatization, the performance of LC–UV or LC–DAD for the analysis acrylamide in foods could be improved dramatically.

In order to obtain the optimum mobile phase composition, methanol in water and methanol in some solutions (i.e., 0.025 mol/L sodium dihydrogen phosphate, 0.025 mol/L sodium tetraborate with pH being adjusted to 5 by phosphoric acid) were investigated. Methanol in 0.025 mol/L sodium dihydrogen phosphate was chosen as the mobile phase because of its best baseline stability and lowest background noise. The effect of methanol in the mobile phase on the retention of the target analyte was also observed within the range of 2–12% (v/v). The retention time was shortened while the peak height was increased sharply with the increase of methanol in mobile phase (see Figure 2). Thus, 7% (v/v, methanol in 0.025 mol/L sodium dihydrogen phosphate was finally selected because of the moderate retention time and the acceptable detection limit.

After the bromination, the original compound was finally converted to 2-BPA. In order to obtain the optimum detection wavelength, 2-BPA was detected at different wavelengths (i.e., 200 nm, 205 nm, 210 nm, 215 nm, and 220 nm) simultaneously. The detection wavelength was finally set at 215 nm for its stable baseline and satisfactory detection limit.

Under the conditions set in the paper, the retention time of the target analyte, 2-BPA, was about 13.8 min. A high repeatability of the retention time was obtained with RSD values lower than 5% for standards and different food matrixes. The extraction procedure followed by the acrylamide bromination gave a clear chromatogram with no interfering peaks appearing at the desired retention time (see Figure 3).

Calibration and method performances

Quantitation was performed on the basis of the calibration line of the peak area against the concentration, which was established by analyzing 2-BPA obtained from acrylamide standard within the amount range 0.05–2.0 µg. A good coefficient (R2) of 0.9992

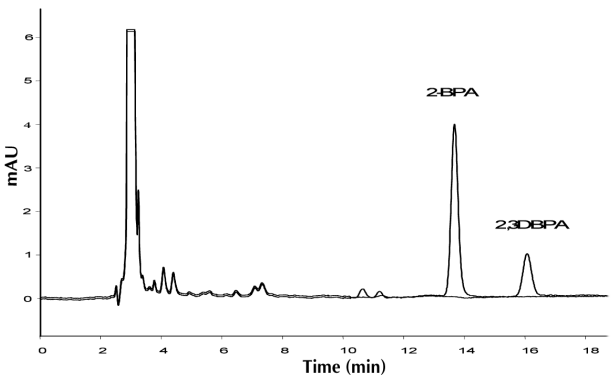

Figure 1. Overlapped chromatograms of 2-BPA and 2,3-DBPA, derived from the same amount of standard acrylamide (0.5 µg). HPLC conditions: column, ODS-3 C18 column (250 mm × 4.6 mm, Intersil, Japan); mobile phase, 7% (v/v) methanol in 0.025 mol/L sodium dihydrogen phosphate; flow rate, 1.0 mL/min; DAD detection at 215 nm; injection volume, 20 µL.

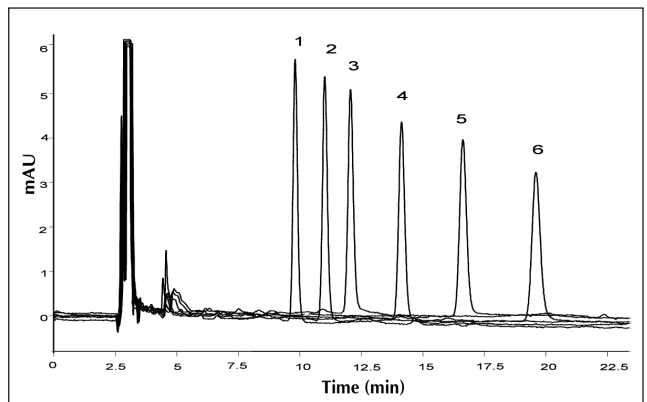

Figure 2. Overlapped chromatograms of 2-BPA obtained after changing ratio of methanol in mobile phase: 1, 12% (v/v); 2, 10% (v/v); 3, 8% (v/v); 4, 6% (v/v); 5, 4% (v/v); 6, 2% (v/v).

was achieved over the whole concentration range (50–2000 µg/L). The limit of detection (LOD) and the limit of quantitation (LOQ) were estimated to be 15 µg/kg and 50 µg/kg based on the signal-to-noise ratio of 3 and 10, respectively.

Table I lists the critical chromatographic parameters of methods for acrylamide based on LC–MS–MS, GC–MS and the present method, which indicated that the present method involved a sample treatment similar to GC–MS and a chromatographic separation similar to LC–MS–MS with DAD or UV as detection. Compared with the results of acrylamide analysis in other published papers, both the LOD and LOQ of the present method were a little higher than those of LC–MS–MS and GC–MS. However, it was enough to accurately quantify the acrylamide contaminant in conventional starch-based foods. In the present paper, acrylamide in commercial foods at the level range 94–1534 µg/kg was successfully analyzed as shown in Table II and Table III.

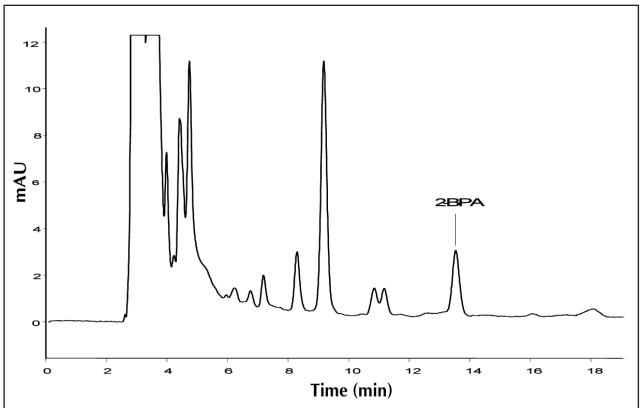

Figure 3. Chromatogram of biscuit (387 µg/kg). HPLC conditions as in Figure 1.

The optimized analytical method was validated by the intra- and inter-day determination of acrylamide in some representative foods [i.e., a biscuit, potato chip, and kaoshugan (a kind of Chinese baked food made from sweet potato)]. Table II summarizes the results of intra- and inter-day precision studies. The portions of representative foods were repeatedly analyzed three times each day in consecutive five days for the inter-day precision study, while the other portions were repeatedly analyzed five times within one day for the intra-day precision study. The precision of the method was excellent with RSD values ranged from 2.1 to 7.4%.

Recovery (n = 5) of the method was calculated by analyzing both spiked and non-spiked each of the starch-based foods. The average percentage recoveries of acrylamide from the samples spiked at levels of 50–1000 µg/kg ranged between 89.6 and 102.0% (see Table III). Five independent measurements of the

Table I. The Critical Parameters in Chromatographic Determinations for Acrylamide with LC–MS–MS, GC–MS and the Present Method*

	Internal standard	Derivatization regents	Chromatographic column	Chromatographic separation program	Target analyte	Detection	LOD/LOQ	Ref.
LC–MS–MS	$^{13}C_3$-acrylamide		Atlantis dC18 column, 210 mm × 1.5 mm (Waters, Milford, MA,)	Mobile phase: 10% methanol/0.1% formic acid in water with a flow speed of 0.2 mL/min. Temperature: 25°C	AA	MS–MS AA: *m/z* = 72, 55, 27; IS: *m/z* =75,58,30	LOD: 1–2 µg/kg LOQ: 4–5 µ/kg	18
	d_3-acrylamide		Bondapak C18 column, 300 mm × 0.9 mm (Waters, Milford, MA,)	Mobile phase: 0.1% acetic acid with flow rate of 0.6 mL/min, Temperature: not specified	AA	MS–MS AA: *m/z* = 72, 55; IS: *m/z* =75,58	LOD: 10 µg/kg LOQ: 20 µg/kg	12
GC–MS	$^{13}C_3$- acrylamide	KBr, HBr, saturated brominewater	ZB-WAX capillary column, 30 m × 0.25 mm i.d., 0.25 µm film thickness (Agilent)	Temperature Program: 65°C held for 1 min, ramped at 15°C/min to 170°C, 5°C/min to 200°C, 40°C/min to 250°C, held for 15 min.	2-BPA	MS 2-BPA: *m/z* = 149, 70; IS: *m/z* = 154, 110	LOD: 2 µg/kg LOQ: 5 µg/kg	15
	$^{13}C_3$-acrylamide	KBr, HBr, saturated brominewater	BPX-10 fused silica capillary column 30 m × 0.25 mm i.d., 0.25 µm film thickness; (SGE, Ringwood, Australia)	The temperature program: isothermal for 1 min at 65°C, increased at a rate of 15°C /min to 250°C, and isothermal for 10 min.	2,3-DBPA	MS 2,3-DBPA: *m/z* =152, 150, 106; IS: *m/z* = 155, 110)	LOD: 5 µg/kg LOQ: 15 µg/kg	13
Present method		KBr, $KBrO_3$, dilute H_2SO_4	ODS-3 C_{18} column, 250 mm × 4.6 mm (Intersil, Japan)	Mobile phase: 7 % (v/v) methanol in 0.025 mol/L sodium dihydrogen phosphate with a flow speed of 1.0 mL/min. Temperature: 25°C	2-BPA	DAD–UV (215 nm)	LOD: 15 µg kg- LOQ: 50 µg/kg	

* LOD = limit of detection; LOQ = limits of quantitation; 2-BPA = 2-bromopropenamide; AA = acrylamide; I.S. = internal standard.

certified reference material averaged to an acrylamide concentration of 942 µg/kg (certified value: 980 µg/kg, uncertainty: ± 90 µg/kg) with a RSD value of 3.8%. These results recommend the method to be valid for the sensitive determination of acrylamide in foods.

Confirmation by GC–MS

In the GC–MS methods with derivatization, both ^{13}C and ^{2}H labeled acrylamide can be applied as internal standards, and there is little difference in their monitored ions (26). In the present paper, GC–MS with ^{2}H labeled acrylamide as an internal standard was employed to analyze the final analyte (i.e., 2-BPA) for conformation. Since 2,3-dibromo($^{2}H_3$)propionamide looses one ^{2}H atom during dehydro-bromination, which results in an increased overlap of the mass spectra of the natural acrylamide and ($^{2}H_3$) acrylamide, some ions (e.g. *m/z* 108 and *m/z* 151) are not suitable for monitoring. Therefore, ions monitored were m/z 70 and 149 for 2-bromopropenamide, and *m/z* 110 and 153 for 2-bromo($^{2}H_2$)-propenamide.

Table II. Intra- and Inter-Day Precision Study of Acrylamide Levels Measured in Three Commercial Foods

	Potato chip	Biscuit	Kaoshugan*
Intra-day precision (n = 5)			
Acrylamide levels†			
Day 1	1534 ± 42	387 ± 20	190 ± 14
Inter-day precision (n = 3)			
Acrylamide levels			
Day 2	1510 ± 31	372 ± 14	178 ± 12
Day 3	1532 ± 36	396 ± 15	175 ± 12
Day 4	1579 ± 41	403 ± 21	198 ± 11
Day 5	1585 ± 38	381 ± 16	184 ± 13
Total mean (µg/kg)	1548	388	185
SD (µg/kg)	33	13	10

* A kind of Chinese baked food made from sweet potato.
† (mean ± SD, µg/kg)

Table III. Analysis of Acrylamide in Commercial Samples (n = 5)

sample	Concentration (means ± SD, µg/kg)	Added (µg/kg)	Determined (means ± SD, µg/kg)	Recovery (%)
Potato chip	1534 ± 42	–	–	–
		500	2013 ± 55	95.8
		1000	2522 ± 57	98.8
Crisp bread*	942 ± 36	–	–	–
		500	1438 ± 45	99.2
		1000	1921 ± 48	97.9
Biscuit	387 ± 20	–	–	–
		250	626 ± 33	95.6
		500	842 ± 41	91.0
Kaoshugan†	190 ± 14	–	–	–
		100	283 ± 20	93.0
		250	442 ± 32	100.8
Mahua‡	151 ± 9	–	–	–
		100	247 ± 18	96.7
		250	375 ± 23	89.6
Youtiao‡	94 ± 8	–	–	–
		50	145 ± 12	102.0
		100	192 ± 15	98.0

* Certified reference test material (ERM-BD272 crisp bread).
† A kind of Chinese baked food made from sweet potato.
‡ Two kinds of Chinese fried foods made from wheaten flour.

Conclusions

The present study developed an LC–DAD method for the determination of acrylamide in starch-based foods such as potato chips, biscuits, and Chinese fried/baked foods. To date, this is the first LC–DAD method dealing with contaminant analysis of acrylamide with pre-column ultraviolet bromination-derivatization. The sample preparation includes water extraction, defatting with n-hexane, derivatization with $KBrO_3$ and KBr, liquid–liquid extraction with ethyl acetate–hexane (4:1), and concentration. Unlike the poor retention of acrylamide on conventional LC reversed-phase sorbents, the target analyte (i.e., 2-BPA) is well resolved from the matrix co-extractives with a good retention. Furthermore, the method shows that no cleanup step of acrylamide derivative is necessary prior to LC–DAD analysis. The method presented in this study is sensitive enough for the analysis of acrylamide in conventional fried/baked foods, with the LOD and LOQ values at 15 and 50 µg/kg, respectively. Meanwhile, the present LC–DAD analytical method requires a relatively low-cost instrumentation compared with LC and GC with tandem MS methods already published in many journals, and can be applied for the investigation of acrylamide contaminant in heat-treated foods by many laboratories world wide easily.

In summary, the results of a series of accuracy, precision, and validation tests demonstrate that this method should be regarded as a new, low-cost, and robust alternative for conventional investigation of acrylamide. Further research is now being conducted to expand the applicability of the method to different food products.

Acknowledgements

Special thanks to Mr. Min Chen, Miss Rong Jiang, Dr. Xiangyang Yu, and Dr. Yan Shen for laboratory assistance. This work was financially supported by the Natural Science Foundation Council of Jiangsu province, China (Project no. BK2006166).

References

1. Swedish National Food Administration, Information about acrylamide in food, 24 April, 2002. http://www.slv.se
2. K. Hashimoto, J. Sakamoto and H. Tanii. Neurotoxicity of acrylamide and related compounds and their effects on male gonads in mice. *Arch. Toxicol.* **47:** 179–189 (1981).

3. M. Vanhoric and W. Moens. Carcinogen mediated induction of SV40 DNA amplification is enhanced by acrylamide in Chinese hamster CO60 cells. *Carcinogenesis* **4:** 1459–1463 (1983).
4. International Agency for Research on Cancer. IARC monographs on the evaluation of carcinogenic risks to humans, Vol. 60, Lyon, France, 1994, pp, 389-433.
5. C. Gertz and S. Klostermann. Analysis of acrylamide and mechanisms of its formation in deep-fried products. *Eur. J. Lipid Sci. Technol.* **104:** 762–771 (2002).
6. D.S. Mottram, B.L.Wedzicha and A.T. Dodson. Acrylamide is formed in the Maillard reaction. *Nature* **419:** 448–449 (2002).
7. R.H. Stadler, I. Blank, N. Varga, F. Robert, J. Hau, and P.A. Guy. Acrylamide from Maillard reaction products. *Nature* **419:** 449–450 (2002).
8. J. Rosén and K.E. Hellenäs. Analysis of acrylamide in cooked foods by liquid chromatography tandem mass spectrometry. *The Analyst* **127:** 880–882 (2002).
9. S. Riediker and R.H. Stadler. Analysis of acrylamide in food by isotope-dilution liquid chromatography coupled with electrospray ionization tandem mass spectrometry. *J Chromatogr. A.* **1020:** 121–130 (2003).
10. D. Andrzejewski, J.A.G. Roach, M.L. Gay and S.M. Musser. Analysis of coffee for the presence of acrylamide by LC-MS/MS. *J. Agric. Food. Chem.* **52:** 1996–2002 (2004).
11. Y. Zhang, J. Jiao, Y. Ren, X. Wu, and Y. Zhang. Determination of acrylamide in infant cereal-based foods by isotope dilution liquid chromatography coupled with electrospray ionization tandem mass spectrometry. *Anal. Chim. Acta* **551:** 150–158 (2005).
12. Y. Govaert, A. Arisseto, J. Van Loco, E. Scheers, S. Fraselle, E. Weverbergh, J. M. Degroodt, and L. Goeyens. Optimisation of a liquid chromatography–tandem mass spectrometric method for the determination of acrylamide in foods. *Anal. Chim. Acta* **556:** 275–280 (2006).
13. E. Tareke, P. Rydberg, P. Karlsson, S. Eriksson and M. Törnqvist. Analysis of Acrylamide, a Carcinogen Formed in Heated Foodstuffs. *J. Agric. Food Chem.* **50:** 4998–5006 (2002),
14. H. Ono, Y. Chuda, M. Ohnishi-Kameyama, H. Yada, M. Ishizaka, H. Kobayashi and M. Yoshida. Analysis of acrylamide by LC-MS/MS and GC-MS in processed Japanese foods. *Food Addit. Contam.* **20:** 215–220 (2003).
15. A. Pittet, A. Périsset and J.M. Oberson. Trace level determination of acrylamide in cereal-based foods by gas chromatography–mass spectrometry. *J. Chromatogr. A.* **1035:** 123–130 (2004).
16. C. M. D. Soares and J. O. Fernandes. MSPD Method to Determine Acrylamide in Food. *Food Anal. Methods* **2:** 197–203 (2009)
17. F. Tateo and M. Bononi. A GC/MS method for the routine determination of acrylamide in food. *Ital. J. Food Sci.* **15:** 149–151 (2003).
18. Y. Zhang, Y. Ren, H. Zhao, and Y. Zhang. Determination of acrylamide in Chinese traditional carbohydrate-rich foods using gas chromatography with micro-electron capture detector and isotope dilution liquid chromatography combined with electrospray ionization tandem mass spectrometry. *Anal. Chim. Acta* **584:** 322–332 (2007).
19. Y. Zhang, G. Zhang, and Y. Zhang. Occurrence and analytical methods of acrylamide in heat-treated foods: review and recent developments. *J. Chromatogr. A.* **1075:** 1–21 (2005).
20. V. Gökmen, H.Z. Senyuva, J. Acar, and K. Sarioglu. Determination of acrylamide in potato chips and crisps by high-performance liquid chromatography. *J Chromatogr. A.* **1088:** 193–199 (2005).
21. E. K. Paleologos and M.G. Kontominas. Determination of acrylamide and methacrylamide by normal phase high performance liquid chromatography and UV detection. *J. Chromatogr. A.* **1077:** 128–135 (2005).
22. H. Wang, A.W.M. Lee, S. Shuang, and M.M.F. Choi. SPE/HPLC/UV studies on acrylamide in deep-fried flour-based indigenous Chinese foods. *Microchem. J.* **89:** 90–97 (2008).
23. Y. Zhang, Y. Dong, Y. Ren, and Y. Zhang. Rapid determination of acrylamide contaminant in conventional fried foods by gas chromatography with electron capture detector. *J Chromatogr. A* **1116:** 209–216 (2006).
24. S. Nemoto, S. Takarasuki, K. Sakaki, and T. Maitani. Determination of acrylamide in food by GC/MS using 13C-labeled acrylamide as an internal standard. *J. Food Hyg. Soc. Jpn.* **43:** 371–376 (2002).
25. F. Andrawes, S. Greenhouse, and D. Draney. Chemistry of acrylamide bromination for trace analysis by gas chromatography and gas chromatography-mass spectrometry. *J. Chromatogr.* **399:** 269–275 (1987).
26. T. Wenzl, L. Karasek, J. Rosen, K.E. Hellenaes, C. Crews, L. Castle, and E. Anklam. Collaborative trial validation study of two methods, one based on high performance liquid chromatography–tandem mass spectrometry and on gas chromatography–mass spectrometry for the determination of acrylamide in bakery and potato products. *J. Chromatogr. A* **1132:** 211–218 (2006).

Manuscript received April 28, 2010;
revision received September 30, 2010.