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Analytical Methods

Application of the standard addition method for the determination of acrylamide in heat-processed starchy foods by gas chromatography with electron capture detector

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Abstract

A gas chromatography electron capture detector (GC-ECD) using the standard addition method was developed for the determination of acrylamide in heat-processed foods. The method entails extraction of acrylamide with water, filtration, defatting with *n*-hexane, derivatization with hydrobromic acid and saturated bromine-water, and liquid–liquid extraction with ethyl acetate. The sample pretreatment required no SPE clean-up and concentration steps prior to injection. The final extract was analyzed by GC-ECD. The chromatographic analysis was performed on polar columns, e.g. Supelcowax-10 capillary column, and good retention and peak response of the analyte were achieved under the optimal conditions. The qualification of the analyte was by identifying the peak with same retention time as standard compound 2,3-DBPA and confirmed by GC–MS. GC–MS analysis confirmed that 2,3-DBPA was converted to 2-BPA nearly completely on the polar capillary column, whether or not treated with triethylamine. A four-point standard addition protocol was used to quantify acrylamide in food samples. The limit of detection (LOD) was estimated to be 0.6 μg/kg on the basis of ECD technique. Validation and quantification results demonstrated that the method should be regarded as a low-cost, convenient, and reliable alternative for conventional investigation of acrylamide.

Keywords: Acrylamide; Gas chromatography; Electron capture detection; Derivatization; Standard addition method; Heat-processed starchy foods

1. Introduction

The discovery of acrylamide in the human foods (Ahn et al., 2002; Becalski, Lau, Lewis, & Seaman, 2003; Rosén & Hellenas, 2002; Tareke, Rydberg, Karlsson, Eriksson, & Törnqvist, 2000, 2002) led to surveys exploring the levels of that potentially hazardous chemical (IARC, 1994), and spurred a search into suitable analytical procedures for its determination in foodstuffs. The potential presence of acrylamide in foods was initially investigated, in a tomato and mushroom matrix, by employing derivatization of acrylamide (bromination of a double bond) and subsequent GC–MS detection (Andrawes, Greenhouse, & Draney, 1987; Castle, 1993; Castle, Campos, & Gilbert, 1991; Gertz & Klostermann, 2002). Later, Rosén and Hellenas (Rosén & Hellenas, 2002), developed a LC-MS/MS-based method, and soon a few more variants of that procedure appeared in the literature (Ono et al., 2003; Swiss Federal Office of Public Health, 2002; Takatsuki, Nemoto, Sasaki, & Maitani, 2003; US FDA, 2003). Also, several groups reported using GC–MS techniques for determination of acrylamide without derivatization (Biedermann, Biedermann-Brem, Noti, & Grob, 2002; Rothweiler & Prest, 2003; Tateo & Bononi, 2003). During the past two years, many more papers and reviews were published about the occurrence and analytical methods of acrylamide in heat-treated foods (Castle & Eriksson, 2005; Eberhart et al., 2005; Kim, Hwang, & Lee, 2006; Pittet, Périsset, & Oberson, 2004; Ren, Zhang, Jiao, Cai, & Zhang, 2006; Senyuva

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& Gökmen, 2006; Wenzl, Beatriz de la Calle, & Anklam, 2003; Zhang, Zhang, & Zhang, 2005).

Although MS is chosen as a main technique for GC and HPLC-based analysis, there is still a need to develop a reliable, sensitive, rapid, and low-cost analytical method for the determination of acrylamide without using MS (Zhang et al., 2005). Recently, Schieberle et al. developed a HPLC/fluorescence method for the determination of acrylamide (Schieberle, Köhler, & Granvog, 2005). Regretefully, the method could not be applied to food samples because of the interference of food matrix. Gökmen et al. developed a sensitive reverse-phase HPLC-DAD method for acrylamide analysis in potato-based processed foods, and the rapid and convenient measurement was successfully achieved (Gökmen, Senyuva, Acar, & Sarioglu, 2005). But the method required a relatively complex sample pretreatment including extraction of acrylamide with methanol, purification with Carrez I and II solutions, evaporation and solvent change to water, and clean-up with a Oasis HLB solid-phase extraction (SPE) cartridge. It was also found that the sensitivity of HPLC method was lower than GC and MS-based methods.

Analysis for acrylamide by bromination and GC determination was relatively advanced even before acrylamide was discovered in heated foods, because of the need to test drinking water, waste waters, crops, and biological samples for acrylamide (Bologna, Andrawes, Barvenik, Lentz, & Sojka, 1999; Castle, 1993; Castle et al., 1991; Poole, 1981; US EPA, 1996). Bromination affords an analyte that can be analyzed more easily at trace levels than acrylamide itself. Recently, Zhang et al. (Zhang, Dong, Ren, & Zhang, 2006; Zhang, Ren, Zhao, & Zhang, 2007) developed a GC-ECD method for identification and quantification of acrylamide in conventional fried complex food matrixes such as potato crisps, potato chips, and fried chicken wings. The method showed a lower limit of detection than MS-based methods. During our study, we also found that GC-ECD is a very sensitive method for the determination of acrylamide in foods.

Nowadays, the commonly used methods for acrylamide quantification include external standard and internal standard method. For determination of acrylamide in foods, an external quantitative analysis revealed poor reproducibility. Zhang et al. (Zhang et al., 2006) used external method for calibrating sample extracts in their study and suggested an improvement of quantitative method by introducing an internal standard, which is the most commonly used quantification method for acrylamide. The usually used internal standards for the determination of acrylamide include isotope-labeled internal standard (e.g. $^{13}C_3$-acrylamide or $^{2}H_3$-acrylamide, etc.) and non-isotope-labeled internal standards (e.g. methacrylamide, etc.). The isotope-labeled internal standards are the most ideal internal standard, but can only be used in MS-based analysis. It was reported that satisfactory repeatability of the results of the analysis could not be achieved until isotopically labelled acrylamide was used. This could be due to the differing stability of the compounds. Another reason for the large coefficient of variation (CV) might be the incomplete derivatization of structurally different internal standards. It was also reported that a large difference in the reaction kinetics of the bromination reaction of acrylamide and methacrylamide existed. So a long bromination reaction time was required when methacrylamide was used as internal standard (Castle & Eriksson, 2005; Wenzl et al., 2003).

The method of standard addition is also an accurate quantification method and especially useful when the matrix of the sample is very complex and the extraction yields strongly vary (Basilicata et al., 2005; Ito & Tsukada, 2001). The quantification was performed by the method of standard addition as follows: a set of GC peak area of the analyte obtained for each sample (one for unspiked portions and three for portions spiked with different levels of standard solutions) were plotted as y-axis, while amounts of standard substances in the portions were plotted as x-axis. A calibration curve was prepared by linear regression method. The absolute value for the x-axis obtained from the calibration curve, when the value of the y-axis was equal to zero, was calculated as the amount of the analyte in unspiked portion of the sample. In 1993, Castle reported the determination of acrylamide monomer in mushrooms by GC–MS using standard addition method, but the detailed quantificaton methods was not described in the article (Castle, 1993).

The objective of the present study is to develop a low-cost, convenient, sensitive, and accurate quantitative method for the determination of acrylamide in heat-processed starchy foods by GC-ECD using standard addition method. Meanwhile, we observed the stability of 2,3-DBPA in different capillary column using GC–MS analysis.

2. Materials and methods

2.1. Chemicals

Acrylamide (99.5%) was purchased from labor Dr. Ehrenstorfer-Schäfers (Augsberg, Germany). 2,3-Dibromopropionamide (2,3-DBPA) ($\geqslant$99%) was purchased from Baoding Lucky Chemical Co. Ltd., (Hebei province, China). All of other solvents and chemical such as potassium bromate, hydrobromic acid ($\geqslant$40.0%), bromine (Br_2), *n*-hexane were of analytical grade and obtained from Chengdu Kelong Chemicals Factory (Sichuan province, China). Methanol, ethyl acetate were HPLC grade and obtained from Tianjin Kermel Chemical Reagents Development Centre (Tianjin, China). Potassium bromide, sodium sulfate anhydrous were purified by calcinations in a crucible at 600 °C (muffle furnace) for 4 h and stored in tightly closed containers at room temperature. Triethylamine was purchase from Tianjin Damao Chemical Reagent Factory (Tianjin, China). Bidistilled, deionized and 0.20 μm filtered water was used throughout the experiments.

2.2. Standards and reagents

Stock solution of acrylamide (100 μg/ml) was prepared by dissolving acrylamide in methanol. Working solution of acrylamide was prepared by diluting the stock solution to concentration of 10 μg/ml with distilled water. Stock solutions and working solutions were kept at 4 °C in dark for a month. 2,3-DBPA solution (2.5 μg/ml) was prepared by dissolving 2,3-DBPA in ethyl acetate. The saturated bromine-water solution (ca. 3.0%) was prepared by solving bromine (~8 ml) in 500 ml water until precipitation became visible.

2.3. Samples

Experiments were conducted with a series of commercial products, which were mainly some heat-processed starchy foods such as fried instant noodles, non-fried instant noodles, potato chips, and biscuits. All of the foods were purchased from retail outlets in China and stored at room temperature.

2.4. Sample preparation

Samples were pulverized and homogenized in CNS-8161 multi-function food processor (Shunde Caina Electric Appliance Co. Ltd., Guangdong province, China) prior sampling. For every sample, four aliquots (10 g of sample each one) were weighed into four 100 ml volumetric flasks, aliquots of 0, 0.5, 1.0, 2.0 ml of acrylamide working solution (10 μg/ml) was added to each flask, respectively. Then distilled water was added to volume. After mixing in a mixer for 30 min, the mixture was filtered. A portion (25 ml) of the filtrate was transferred into separatory funnel and 25 ml *n*-hexane was added. After thorough hand mixing for 2 min, the aqueous phase was transferred into a 100 ml Erlenmeyer flask. The calcinated potassium bromide (7.5 g) was dissolved into the separatory aqueous phase with stirring, and the pH of the solution was adjusted to value between 1 and 3 by the addition of a few drops (~0.4 ml) of hydrobromic acid. Then 8 ml of saturated bromine-water solution was added to flask whilst stirring. The flask was covered with aluminum foil and transferred into an ice bath where reaction was allowed to take place for 1 h in the dark. After the reaction was completed, the excess bromine was decomposed by adding a few drops (~0.4 ml) of 1 M sodium thiosulfate solution until the yellow color disappeared. Then the mixture was extracted with 25 ml of ethyl acetate by shaking for 1 min. After phase separation, the organic phase was taken and dried over sodium sulfate. The solution was finally filtered through a 0.45 μm microfilter into an autosampler vial for GC analysis.

For the intentional conversion of 2,3-DBPA to 2-bromopropenamide (2-BPA), 10% triethylamine was added to the filtered sample.

2.5. GC-ECD analysis

GC-ECD was used for the quantification of acrylamide in the tested samples. Pretreated samples were analyzed on a GC-2010 chromatographic system from Shimadzu Corporation (Kyoto, Japan) coupled with a micro-electron capture detector connected on line. 2,3-DBPA solution was used for the qualification of acrylamide in tested samples. Sample volume of 5 μl (solvent ethyl acetate) was injected on-column with AOC-20i automatic injector system onto a Suplecowax-10 capillary system (polyethyleneglycol, 30 m × 0.25 mm i.d., 0.25 μm film thickness, Supelco Inc., PA, USA). Separations were performed using nitrogen as the carrier gas. Following injection, the column was held at 60 °C for 1 min, then programmed at 20 °C/min to 220 °C and held for 3 min at 220 °C, then at 30 °C/min to 250 °C and held for 5 min at 250 °C. The GC-ECD sample injector interface temperature and detector interface temperature were both held at 260 °C.

In order to observe the stability of 2,3-DBPA in capillary column, RTX-1 (30 m × 0.25 mm i.d., 0.25 μm film thickness, Restek, USA) and DB-5 (30 m × 0.25 mm i.d., 0.25 μm film thickness, Agilent Technologies, Palo Alto, CA, USA) capillary columns were also used.

2.6. Quantification

Quantification was performed by the method of standard addition as follows: a set of GC peak areas of the analyte obtained for each sample (one for unspiked portions and three for portions spiked with different levels of standard solutions) were plotted as *y*-axis, while concentrations of acrylamide added in the portions (0, 0.05, 0.1 and 0.2 μg/ml for unspiked and three spiked portions, respectively) were plotted as *x*-axis. A calibration curve was prepared by the least-squares method. The acceptance criteria for a calibration curve is a correlation coefficient (R^2) greater than 0.995. The absolute value for the *x*-axis obtained from the calibration curve, when the value of the *y*-axis was equal to zero, was calculated as the amount of acrylamide in unspiked portion of the sample.

2.7. Confirmatory analysis by GC–MS

2,3-DBPA solutions and brominated sample extracts prepared by aforementioned pretreatment steps were analyzed by GC–MS. For GC–MS analysis, 1 μl of the test solution was injected onto GC–MS, which was performed on an Ultra GC gas chromatograph coupled with TRACE DSQ II benchtop mass selective detector (MSD) operated in full scan mode with positive electron impact (EI) ionization. The GC column was a DB-5 MS and DB-WAX MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness, Agilent Technologies, Palo Alto, CA, USA), and the carrier gas was helium at 1 ml/min. Following injection, the column was held at 65 °C for 1 min, then programmed at 15 °C/min to 230 °C and held for 5 min at 230 °C.

Injections by an AI300 Autosampler (1 μl) were made in splitless mode with an injection temperature of 260 °C. The ion source temperature was 230 °C, The GC–MS interface transfer line was held at 280 °C. The extract ions were m/z 106, 108, 150, 152 for 2,3-DBPA and 106, 108, 149, 151 for 2-BPA.

3. Results and discussion

This work describes a quantitative analytical method for the low level determination of acrylamide in heat-processed foods by a modified GC-ECD using the standard addition method. The proposed method is comparable to the US EPA method for analysis of acrylamide in water (US EPA, 1996) and the method proposed by Zhang et al. (Zhang et al., 2006; Zhang et al., 2007), but modification have been made particularly regarding sample pretreatment (without solid phase extraction clean-up and concentration) and the qualitative and quantitative methods (using 2,3-DBPA for qualification and standard addition method for quantification).

3.1. Sample pretreatment

A simplified pretreatment method was developed in this study. The modified sample pretreatment steps include extraction of acrylamide with water, defating with *n*-hexane, followed by bromination of the acrylamide. The reaction product (2,3-DBPA) is extracted with ethyl acetate, dried over sodium sulfate, and then directly analyzed by GC-ECD without further concentration. The simplified sample pretreatment procedures could shorten the experimental time and improve the repeatability of the method.

3.2. Bromination

The advantage of acrylamide bromination is that a more volatile compound is produced, which leads to improved GC characteristics (less polar), removal of potentially interfering co-extractive, and higher sensitivity when detected with ECD. When GC–MS method was used for the analysis of acrylamide, the brominated derivatization was also commonly undertook.

In the present method, conversion of acrylamide to 2,3-DBPA is performed according to the protocol originally described by Hashimoto (Hashimoto, 1976), which involves addition of potassium bromide, hydrobromic acid, and a saturated bromine-water solution. The excess of bromine is then removed by addition of sodium thiosulfate until the solution becomes colorless. Under these conditions, the yield of 2,3-DBPA is constant and >80% when the reaction time is more than 1 h (US EPA, 1996). Our observations were consistent with the report (data not shown).

It was reported that under certain conditions, 2,3-DBPA could be converted to the more stable derivative 2-BPA on the inlet of the GC or directly on the capillary column (Andrawes et al., 1987; Castle, 1993; Zhang et al., 2006). But the reports about the dehydrobromination of 2,3-DBPA disagreed. Andrawes et al. reported that dehydrobromination of 2,3-DBPA took place in the front end of the packed FFAP column and both the mono- and di-bromoderivatives were detected in a midly polar DB-5 capillary column. On an inert FFAP capillary column, the derivative does not decompose to 2-BPA nor is it eluted in a symmetrically shaped peak (Andrawes et al., 1987). Castle reported that the major brominated derivative of acrylamide detected in DB-17 capillary column was in fact 2-BPA, suggesting that the dehydrobomination had occurred either in the injection port of the GC–MS or on column (Castle, 1993). Gertz et al. reported that on DB-5 MS column, a transformation of 2,3-DBPA to 2-BPA does not take place, so it is unnecessary to transform dibrominated compound to the more stable 2-monobromopropenamide by adding triethylamine (Gertz & Klostermann, 2002). In the study by Zhang et al. (Zhang et al., 2006), acrylamide was derivatized with bromination and detected by GC-ECD in HP-INNOWax capillary column, and 2-BPA rather than 2,3-DBPA was chosen as the quantitative analyte because the peak response of 2-BPA was nearly 20 times higher than 2,3-DBPA.

In our study, we found 2,3-DBPA showed a single symmetrically shaped peak in GC-ECD analysis (Fig. 1). By comparing the chromatographs of brominated derivatives from blank and acrylamide standard, we found one peak that have same retention time as 2,3-DBPA in the derivatives from acrylamide standard and did not find other additional chromatographic peak which did not exist in blank (Fig. 2).

When using RTX-1 and DB-5 capillary columns for GC-ECD analysis, 2,3-DBPA standard was eluted as two peaks, which implies 2,3-DBPA was unstable in non-polar or mildly polar columns. After treatment with trietheylamine, only one peak was detected in the tested columns. When using SuplecoWax-10 column, whether or not treated with triethylamine, only one peak was detected and the retention time of the peak remained unchanged, which implied 2,3-DBPA was converted to 2-BPA completely on SuplecoWax-10 column even when triethylamine was not added. This result was not consistent with the report by Zhang et al. (Zhang et al., 2006). In the study by Zhang et al. (Zhang et al., 2006; Zhang et al., 2007), the untransformed 2,3-DBPA on INNOWax column was detected.

In order to confirm the conversion of 2,3-DBPA to 2-BPA on column, GC–MS analysis was used. When using DB-5 MS capillary column, 2,3-DBPA standard showed two brominated derivative peaks as in GC-ECD analysis, which were identified as 2-BPA and 2,3-DBPA, respectively. When triethylamine was added to 2,3-DBPA standard, 2,3-DBPA was transformed to 2-BPA.

When using DB-WAX MS capillary column, whether or not the sample was treated with triethytlamine, we could only detect one derivative peak as in GC-ECD analysis. The single derivative peak was identified as 2-BPA (Fig. 3). This observation by GC–MS confirmed 2,3-DBPA

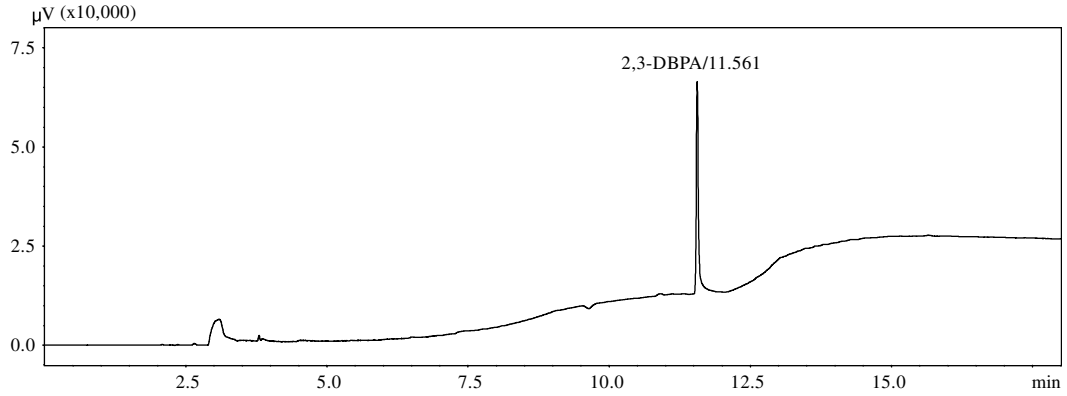

Fig. 1. GC-ECD chromatograph of 2,3-DBPA (2.5 μg/ml) in SuplecoWax-10 capillary column. Retention time of 2,3-DBPA: 11.561 min.

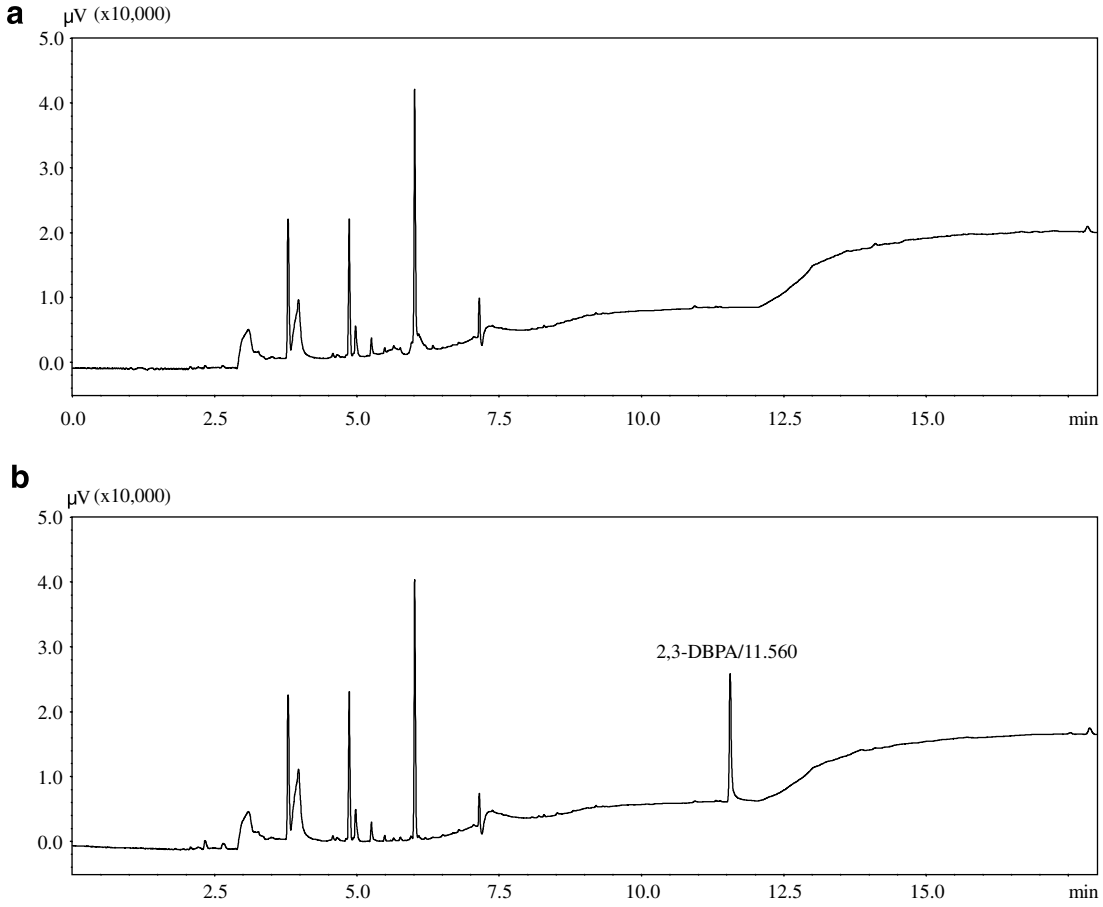

Fig. 2. GC-ECD analysis of the brominated derivatives in SuplecoWax-10 capillary column: (a) blank (water); (b) acrylamide standard (0.2 μg/ml).

was converted to 2-BPA completely on DB-WAX capillary column, which suggested 2,3-DBPA was also converted to 2-BPA completely on SuplecoWax-10 capillary column during GC-ECD analysis. So when using polar capillary columns, e.g. DB-WAX or SuplecoWAX-10, the brominated derivative of acrylamide can be analyzed directly without treatment with triethylamine. But when using non-polar or mildly-polar capillary columns, the conversion to the monobromo derivative has to be carried externally by the use of triethylamine.

It had been reported that the dehydrobomination of 2,3-DBPA had occurred either in the injection port of the GC–MS or on column (Castle, 1993; Zhang et al., 2006; Zhang et al., 2007). Robarge et al. reported that the more stable 2-BPA was created in situ from the thermal decomposition in the injector (Robarge, Phillips, & Conoley, 2003). We found that the decomposition of 2,3-DBPA took place mainly on the columns. Because using the same injection port, 2,3-DBPA converted to 2-BPA completely on DB-WAX column while only a small portion of 2,3-DBPA

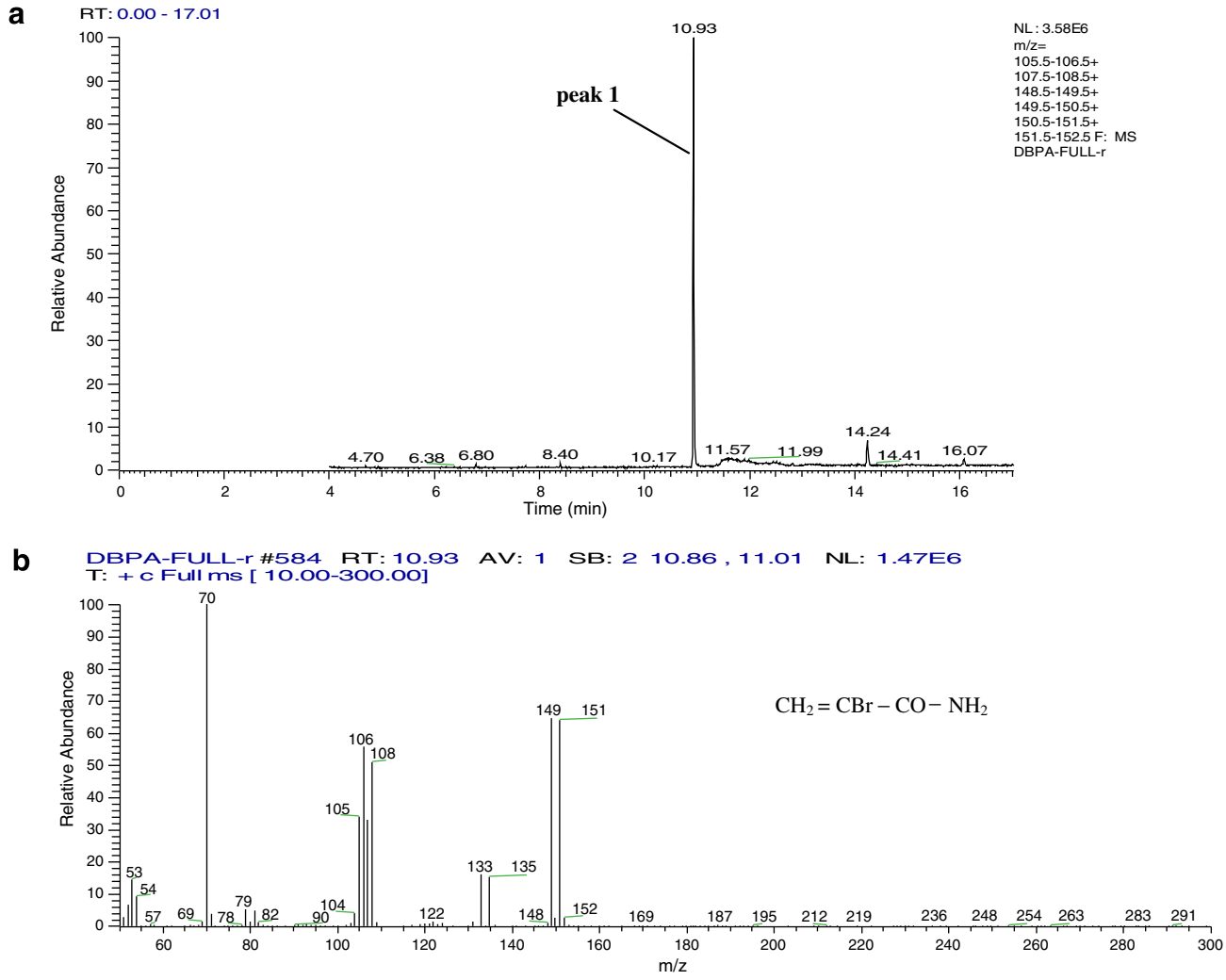

Fig. 3. (a) The extract ion chromatogram of 2,3-DBPA standard (5.0 μg/ml) without adding triethylamine, peak 1 (RT 10.93 min): brominated derivative. (b) Mass spectra for peak 1 in A. Column, DB-WAX MS capillary column.

converted to 2-BPA on DB-5 column. Although increasing the temperature of GC inlet could increase the intensity of 2,3-DBPA dehydrobromination on DB-5 column, but most of 2,3-DBPA was still stable. Meanwhile, decreasing the temperature of GC inlet, 2,3-DBPA still decomposed completely on DB-WAX column. So the conversion of dibromo derivative to monobromo derivative occur mainly on columns and probably depends on the nature of capillary columns to a greater extent.

3.3. Choice of GC column and detection sensitivity of GC-ECD

In the present study, we chose a capillary column with a high-polarity characteristics of solid phase. When the GC analysis for the sample extracts was performed on Supleco-Wax-10 or DB-WAX capillary column, many co-extractives could be detected due to no clean-up procedures were performed. But acrylamide response could be well detached from such unidentified co-extractives under the optimized chromatographic conditions (Fig. 4), which lay down the basis of GC-ECD method for determination of acrylamide in complex food matrix without clean-up pretreatment. While using non-polar and mildly polar columns, the target analyte could not well detached from the interference peaks. So polar capillary columns were more suitable choice.

When using SuplecoWax-10 and DB-WAX capillary column, dibromo derivative can be analyzed directly without adding triethylamine. The sample without adding of triethylamine had less impurity interference. When using non-polar and mildly polar capillary columns, it will be necessary to achieve dehydrobromination intentionally by the use of triethylamine.

Triethylamine was now accepted as an ideal derivative reagent for the conversion of 2,3-DBPA to 2-BPA and 2-BPA was regarded as more stable than 2,3-DBPA (Andrawes et al., 1987). But our study showed that the 2,3-DBPA sample untreated with triethylamine was also very stable during storage.

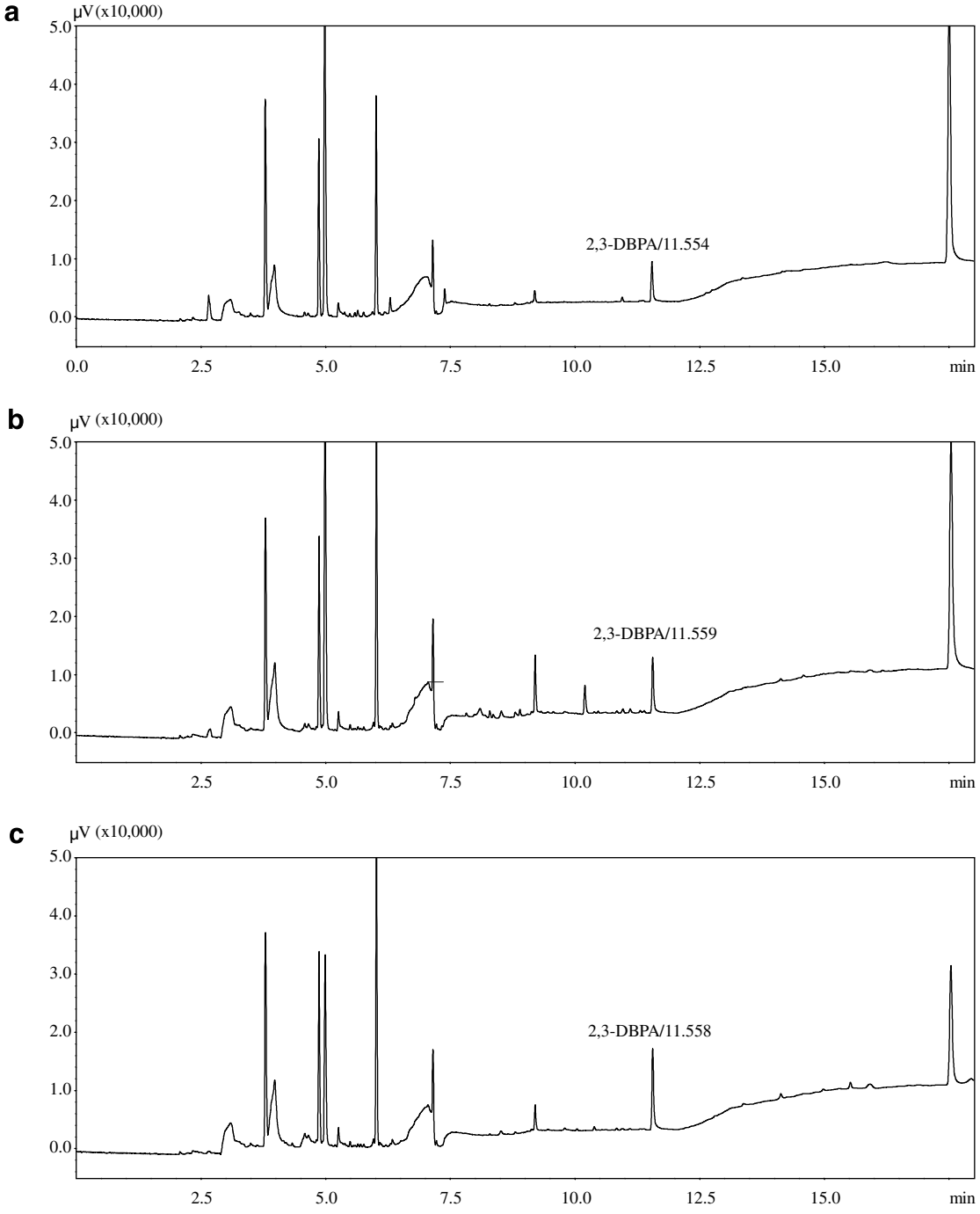

Fig. 4. GC-ECD analysis of acrylamide in heat-processed foods. (a) instant noodles (574 μg/kg); (b) biscuits (777 μg/kg); (c) potato chips (1176 μg/kg).

GC-ECD method is a sensitive method for the determination of acrylamide. The US EPA method 8032A estimated the detection limit of 0.032 μg/L in an aqueous matrix (US EPA, 1996). Zhang et al. reported that the limit of detection (LOD) was 0.1 μg/kg. we also found that GC-ECD method had a very low LOD. The LOD calculated from the measurement of standard solution derivatives is estimated at 0.6 μg/kg (signal-to-noise ratio of 3), and the limit of quantitation (LOQ) at 2.0 μg/kg (signal-to-noise ratio of 10). If the extracted samples were concentrated before analysis, a lower LOD and LOQ could be achieved. The lower LOD of GC-ECD made it possible to directly analyze the extracted sample without further evaporation concentration.

3.4. Qualification and quantification

Both accurate qualification and quantification are very important for the determination of acrylamide in foods. In present study, the analyte, which was identified as

2-BPA, could be well separated from co-extractives, so it is easy to identify the analyte. The retention time of the analyte in different samples was very stable, with the variability less than 0.1%. In addition, the standard addition could be a help in identifying the target analyte because only the target peak increased with the standard addition.

In this study, the quantification was performed by the method of standard addition. A calibration curve was prepared by linear regression method. The amount of the analyte in unspiked portion of the sample was calculated from the calibration curve. An overlapping chromatogram of samples spiked and unspiked with acrylamide are shown in Fig. 5. Results showed that the response of ECD was linearly changed with the concentration of acrylamide added. Excellent linearity was obtained with typical values for the correlation coefficient (R^2) between 0.999 and 1.000 (Fig. 6). A correlation coefficient of at least 0.995 generally indicates acceptable characterization of the curve.

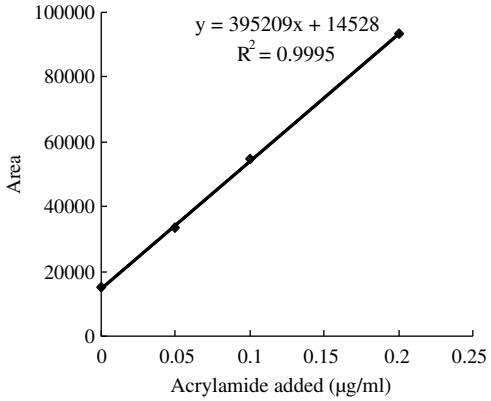

Fig. 6. Calibration curve for brominated samples by GC-ECD using the standard addition method. A, B, and C = samples spiked with 500, 1000, and 2000 μg/kg of acrylamide, respectively; D = sample unspiked with acrylamide. Equation of straight line $y = 395209x + 14528$, $R^2 = 0.9995$. "Zero-point" acrylamide concentration ($[\text{acrylamide}]_c$) is obtained as follows $y = 0 : x = -0.03676$ and $[\text{acrylamide}]_c = 0.03676$ μg/ml.

3.5. Method performance

3.5.1. Repeatability

The method was validated by replicate analysis of four different samples (including fried instant noodles, non-fried instant noodles, biscuits, and potato chips) under repeatability and intermediate reproducibility conditions (Table 1). The samples were first analyzed six times in parallel so as to get information about within-day variation. Good repeatability was obtained for four above-mentioned samples. The acrylamide content in the four food groups ranged between 54 and 1021 μg/kg, with repeatability relative standard deviations (R.S.D.(r)) ranged between 3.9% and 7.1%. Additional precision data were obtained by duplicate analysis of the same products on four different days, which gave a low intermediate reproducibility relative standard deviations (R.S.D.(iR)) ranged between 3.8% and 7.7%.

3.5.2. Recovery yields

The recovery yields of the method was determined in four tests employing the method of standard addition ranging from 50 to 1000 μg/kg. Four different samples (fried instant noodles, non-fried instant noodles, biscuits, and potato chips) were selected. The recovery yields of acrylamide in the fried instant noodles and non-fried instant noodles, most of which have relatively low acrylamide, were estimated by adding 50 μg/kg acrylamide. For the biscuits and potato chips, most of which have relatively high acrylamide, 500 and 1000 μg/kg acrylamide were added. Table 2 lists the average recovery yields of acrylamide in different

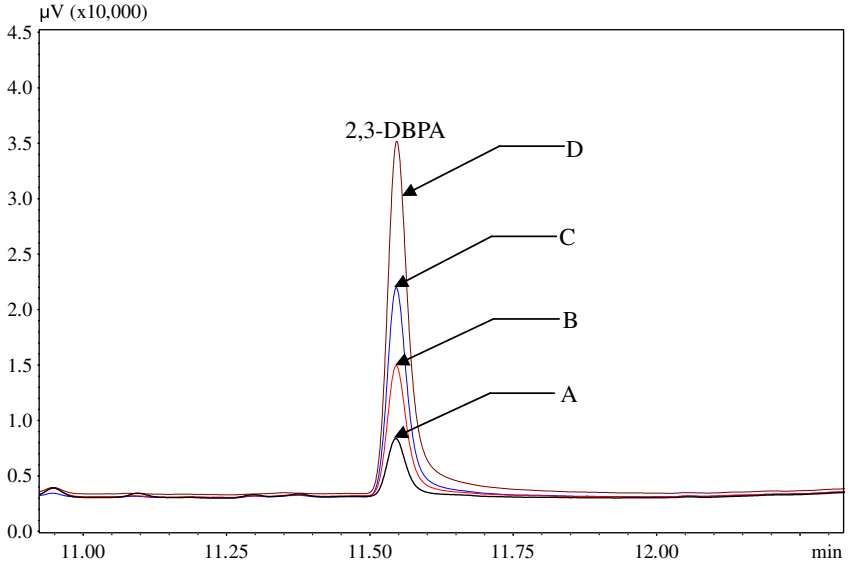

Fig. 5. The overlapping chromatograph of samples spiked and unspiked with acrylamide. A: sample spiked with no acrylamide standard; B, C, D: sample spiked with 0.05, 0.1, 0.2 μg/ml acrylamide standard, respectively.

Table 1
Acrylamide levels measured in two commercial cereal products under repeatability and intermediate reproducibility conditions

	Fried instant noodles	Non-fried instant noodles	Biscuits	Potato chips
Repeatability conditions (n=6)				
Acrylamide levels (mean ± SD, μg/kg)				
Day 1	54 ± 3.3	56 ± 4.0	487 ± 23	1021 ± 40
R.S.D.(*r*)(%)	6.1	7.1	4.6	3.9
Intermediate reproducibility conditions				
Acrylamide results (μg/kg)				
Day 2	49, 53	50, 51	493, 470	984, 1017
Day 3	56, 52	57, 52	499, 516	1068, 1043
Day 4	48, 53	61, 57	458, 491	976, 1015
Day 5	54, 57	57, 61	458, 475	1043, 1087
Mean (μg/kg)	53	56	482	1029
S.D.(*iR*)	3.1	4.3	21	39
R.S.D.(*iR*)(%)	5.9	7.7	4.3	3.8

Table 2
The recover yields of acrylamide using the GC-ECD method ($n = 3$)

Food sample	Acrylamide contents (μg/kg)			Recovery (%)	RSD (%)
	Before spiking	Spiked amount	After spiking		
Fried instant noodles	54	50	97	93.3	2.9
Non-fried instant noodles	56	50	98	92.5	5.1
Biscuits	487	500	942	95.4	3.9
Potato chips	1021	1000	2051	101.5	4.4

food matrixes. Excellent percentage recovery yields (92.5–101.5%) were obtained with acceptable variation (RSD%: 2.9–5.1%) according to the present method.

4. Conclusion

The present study developed a GC-ECD method for identification and quantification of acrylamide in heat-processed starchy foods using standard addition method. It required a relatively low-cost instrumentation to perform when compared to MS detection-based methods already published, and can be adopted by many laboratories worldwide easily. The sample preparation is simple and rapid, without SPE clean-up and concentration prior to GC-ECD analysis. The method presented in this study is sensitive enough for the analysis of acrylamide in heat-processed foods with the LOD and LOQ valued at 0.6 and 2.0 μg/kg, respectively. The standard addition methods could be another suitable quantification method for the determination of acrylamide in heat-processed foods.

The choice of capillary columns was very important for the GC-ECD analysis. It was recommended that polar capillary columns (e.g. SuplecoWax-10 and DB-WAX capillary column, etc.) were used when using GC-ECD for the analysis. Because the dibromo derivative could be stably transformed to monobromo compound on the columns, the derivatized samples can be analyzed directly without adding triethylamine. The derivatized samples untreated with triethylamine were also stable during storage. There exists less impurity interference when triethylamine was not added. But when using non-polar or mildly-polar capillary columns for GC or GC–MS analysis, the conversion to the monobromo derivative has to be carried externally by the use of triethylamine.

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