Original

Determination of Acrylamide in Processed Foods by LC/MS Using Column Switching

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An LC/MS method was developed for the determination of acrylamide (AA) in processed or cooked foods. AA was extracted with a mixture of water and acetone from homogenized food samples after the addition of ¹³C-labeled acrylamide (AA-1-¹³C) as an internal standard. The extract was concentrated, washed with dichloromethane for defatting, and cleaned up on Bond Elut C18, PSA and ACCUCAT cartridge-columns, and then AA was determined by LC/MS in the selected ion recording (SIR) mode. For the LC/MS analysis, four LC columns were connected in-line and the flow of the mobile phase was switched according to a time-program. Monitoring ions for AA were *m/z* 72 and 55, and those for AA-1-¹³C were *m/z* 73 and 56. AA and AA-1-¹³C were determined without interference from the matrices in all samples. The recoveries of AA from potato chips, corn snack, pretzel and roasted tea spiked at the level of 500 ng/g of AA were 99.5–101.0% with standard deviations (SD) in the range from 0.3 to 1.6%. The limits of detection and quantification of the developed method were 9 and 30 ng/g for AA in samples, respectively.

The method was applied to the analysis of AA in various processed or cooked food samples purchased from retail markets. High levels of AA were found in potato chips and French-fried potato (467–3,544 ng/g). Fried and sugar-coated dough cakes (karinto) contained 374 and 1,895 ng/g. Corn snacks contained 117–535 ng/g of AA. Roasted foods (such as roasted sesame seed, roasted barley (mugi-cha), roasted tea (hoji-cha), coffee beans and curry powder) contained 116–567 ng/g of AA. Foods made from fish, egg and meat contained lower levels of AA than the plant-based foods. Foods containing much water showed a tendency to have low levels of AA compared with dry foods.

The proposed method was applicable to the analysis of AA in variety of processed foods.

Key words: acrylamide; ¹³C-labeled acrylamide; LC/MS; column switching; potato chips; French-fried potato; roasted tea

Introduction

In April 2002 the Swedish National Food Administration and researchers from the Stockholm University announced their findings that acrylamide (AA), a neurotoxic and probably carcinogenic chemical in humans, was present in many types of foods (especially potato chips, French-fried potato, biscuits, and cereals) prepared or cooked at high temperature^{*1}. Thereafter, the details of the findings were reported^{1), 2)}. AA is a chemical used for the synthesis of polyacrylamide or other chemical products, and it had not been found in normal foods. Confirmation of the finding was reported from Norway, Switzerland, the United Kingdom and the United States in the "Joint FAO/WHO Expert Consultation on Health Implications of Acrylamide in Foods" held in June 2002^{*2}.

The effect of AA in foods on human health has been

unknown and maximum levels for AA in foods have not been established. It is necessary to develop analytical methods of AA in foods and to determine AA in various foods for prospective risk assessment. Most of the analytical methods reported for analysis of AA in foods are based on derivatization followed by GC-ECD³⁾, GC/MS^{4)–6)} or LC/MS⁷⁾. We have reported an analytical method of AA in foods using GC/MS after bromination of AA⁸⁾. In recent reports, low levels of AA were determined by LC/MS/MS^{1), 2)}, but an LC/MS method without derivatization has not been fully reported^{*3}.

In the present study we devised an LC/MS system equipped with four columns, three pumps and two valves for column switching to determine AA in foods. The proposed method was applied to the analysis of AA in various processed or cooked foods including characteristic Japanese foods purchased from the retail markets.

^{*1} <http://www.slv.se/engdefault.asp>

^{*2} <http://www.li.se/dokument/news/nyheter/Acrylamid%20Expertrapport%20Final.pdf>

^{*3} The 51st Meeting of The Japan Society for Analytical Chemistry, Abstract, p. 157 (19–21 September, 2002)

Materials and Methods

1. Samples

Food samples for the recovery test and the examination of AA content were purchased from retail markets in Tokyo from May to September 2002. Those samples (after homogenization for solid samples) were stored at -30°C until analysis. Some cooked samples were analyzed immediately after cooking (such as French-fried potato, omelets, deep-fried fish and deep-fried meat). Tempura batter coatings were analyzed. Bread-crum coating of deep-fried fish and croquette were analyzed, as were both whole and bread-crum coating of fried cake of minced meats and deep-fried horse mackerel. Some samples tested were the same as those analyzed by GC/MS in the preceding paper⁸⁾.

2. Reagents

Standards: acrylamide, for electrophoresis (Kanto Chemical Co., Inc., Tokyo, Japan), purity 99.9%; internal standard, acrylamide-1- ^{13}C (AA-1- ^{13}C) (CDN Isotopes Co., Quebec, Canada), 99 atom% ^{13}C .

Stock standard solutions (100 $\mu\text{g}/\text{mL}$): 10 mg each of AA or AA-1- ^{13}C was exactly measured in 100-mL brown glass volumetric flasks and made up to 100 mL with water. The stock solutions were stored at 4°C .

Calibration standard solutions: Aliquots of AA standard solutions (equivalent to 0, 50, 100, 500, 1,000, 2,000 and 5,000 ng of AA) were mixed with 20 μL of internal standard solution (100 $\mu\text{g}/\text{mL}$ AA-1- ^{13}C) and made up to 10 mL with water.

Cartridge columns: Bond Elut C18 (500 mg/3 mL), Bond Elut Jr-PSA (500 mg) and Bond Elut ACCUCAT (600 mg/3 mL) were from Varian, Inc., CA, USA. Bond Elut C18 was conditioned with 2 mL of methanol and successively with 4 mL of water before use. The other columns were conditioned only with 4 mL of water before use.

Acetone: high quality for pesticide residue analysis (Kanto Chemical Co., Inc.).

Dichloromethane, methanol and water: HPLC grade (Kanto Chemical Co., Inc.).

3. Apparatus

A liquid chromatograph/mass spectrometer equipped with a column switching system was used. The construction of the apparatus is shown in Fig. 1. Four columns were used. Switching valves were inserted between column-1 and -2, and column -2 and -3.

Liquid chromatograph/mass spectrometer: model 2690 separation module liquid chromatograph (Waters, Co., Milford, MA, USA) coupled with a model Platform LCZ mass selective detector (Micromass UK Ltd., Altrincham, UK).

Degasser: ERC-3215 α (ERC Inc., Kawaguchi, Japan)

Switching valve: LabPRO (Rheodyne, Cotati, CA, USA) and EV700-100 (GL Sciences, Tokyo, Japan)

Pump: LC-10AD and LC-10ADvp (Shimadzu Co., Kyoto, Japan)

Homogenizer: Polytron (KINEMATICA AG, Littau, Switzerland)

Concentrator: Turbo Vap 500 (Zymark Co., Hopkin-

Fig. 1. Construction of the LC/MS system

Inj: injector; P1, P2, P3: pumps; C1, C2, C3, C4: columns; MSD: mass spectrometer

The pathway of mobile phase was (A) during the analysis except in the periods from 1.75 to 2.05 min (B) and from 4.66 to 5.15 min (C).

ton, MA, USA)

4. LC/MS conditions

Column: column-1, Hypercarb (50×2.1 mm i.d., particle size 5 μ m, Thermo Hypersil-Keystone, Runcorn, UK); column-2, Shodex MSPak GF-310 4B (50×4.6 mm i.d., particle size 6 μ m, Shoko. Co. Ltd., Tokyo, Japan); column-3, Atlantis dC18 (150×2.1 mm i.d., particle size 5 μ m, Waters Co., Milford, MA, USA); column-4, Develosil RPAQUEOUS-AR-3 (35×2.0 mm i.d., particle size 3 μ m, Nomura Chemical Co., Ltd., Seto, Japan)

Column oven temp.: 40°C for the column-1, -2 and -3, and room temperature for the column-4

Mobile phase composition: 0.1% acetic acid–methanol (95 : 5)

Flow rate; 0.25 mL/min

Sampling time from column-1 to column-2: 1.75–2.05 min

Sampling time from column-2 to column-3: 4.66–5.15 min

AA elution time from column-4: 8.5 min

Source block temp.: 120°C

Desolvation temp.: 350°C

Gas: nitrogen, flow rate, ca.300 L/hr

Capillary voltage: 3.50 eV

Ionization mode: positive ion electron spray

Measuring mode: selected ion recording (SIR)

Monitoring ion and corn voltage: m/z 72 (AA) and m/z 73 (AA-1- 13 C) at 24 V of corn voltage and m/z 55 (AA) and m/z 56 (AA-1- 13 C) at 40 V of corn voltage

Injection volume: 10 μ L

5. Sample preparation

A sample (5.0 g) was weighed into a 250-mL glass tube and 20–25 mL of water and 100 μ L of internal standard solution (100 μ g/mL AA-1- 13 C solution) were added to the sample. The mixture was allowed to stand for 30 min with occasional shaking. Acetone (50 mL) was added and the mixture was homogenized for 1 min with a homogenizer. The homogenate was filtered through a glass filter (GA-100), and the residue on the filter was washed with 30 mL of acetone. The filtrate was concentrated to less than 15 mL with a concentrator at 35°C. The concentrate was transferred to a 50-mL centrifugal tube, washed twice with 20 mL each of dichloromethane by shaking for 1 min and centrifuged at 2,700 rpm for 5 min. The water layer was made up to 15 mL with water.

An aliquot of the extract (3 mL, equivalent to 1.0 g of sample) was passed through a C18 cartridge column, followed by washing with 2 mL of water. All the eluate was collected and made up to 5 mL with water. An aliquot of the eluate (2 mL, equivalent to 0.4 g of sample) was passed through an ACCUCAT column connected to a PSA column, followed by washing with 2 mL of water. All the eluate was collected and made up to 4 mL with water to prepare the test solution.

6. Determination and confirmation of AA

AA was determined by LC/MS (SIR). The quantification was performed by comparison of the peak area ratios of m/z 72 (AA) and m/z 73 (AA-1- 13 C) for the test solution with those for the calibration standard. The peak area ratio of m/z 55 (AA) and m/z 56 (AA-1- 13 C) was used for confirmation. The recovery of internal standard was calculated from the peak areas of AA-1- 13 C in the test solution and the calibration standard.

The calibration standard (AA=0, AA-1- 13 C=200 ng/mL) contained a small quantity of AA as an impurity of AA-1- 13 C. Therefore, the limit of detection (LOD) was defined as 3 times the SD of the AA peak area of the calibration standard (AA=0, AA-1- 13 C=200 ng/mL). The limit of quantification (LOQ) was defined as 10 times the SD.

Results and Discussion

1. LC/MS conditions

In MS analysis, AA gave only a few small ions at any corn voltage. Ions m/z 72 (M+H) at corn voltage 24 V and m/z 55 (M–NH₂) at corn voltage 40 V were chosen for quantification and confirmation, respectively.

Some test solutions prepared by the proposed method still contained matrices interfering with the analysis of AA and AA-1- 13 C, and it was difficult to separate AA from interfering components completely by using one or two LC columns. Consequently, four LC columns having different properties were used with 2 switchings to obtain the best separation of AA. The fourth column was used to narrow the peak width of AA. Two switchings might be reduced to one by using other LC columns or longer ones.

Under these conditions, the analytical values obtained by using the ion m/z 72 were well correlated with the values obtained by using the ion m/z 55 in all samples. When the test solutions of roasted coffee bean, roasted tea, cocoa and meat products were monitored at m/z 55, some peaks were observed near the AA peak, but these peaks did not interfere with the analysis of AA.

The SIR chromatograms of the extracts of potato chips and roasted coffee bean are shown in Fig 2. The SIR chromatograms of the other foods were similar to

Fig. 2. SIR chromatograms of acrylamide (AA, m/z 72) and internal standard (AA-1- 13 C, m/z 73) in food samples

those of potato chips.

2. Extraction

AA is very soluble in water (2,155 g/L, 30°C), so in most cases AA is well extracted with water^{1)–8)} from samples. AA dissolved in water was hard to trap on any absorbent and the aqueous solution could not be concentrated without loss of AA. On the other hand, a sufficient amount of water should be used to extract AA entirely from samples, and it resulted in the dilution of AA in the test solution. Therefore, a mixture of water and acetone was used for the extraction, and acetone in the extract was removed by concentration before the clean-up procedure. Although acetone extraction without water gave good recovery of AA from spiked potato chips and the extract was clean compared with the water extract, acetone without water failed to extract AA contained originally in the sample.

3. Clean-up procedure

Some matrices in the food samples affected the ionization of AA in MS analysis even though they did not show interfering peaks with detection in SIR. Therefore, it was necessary to clean up the extract sufficiently for accurate analysis. The sample extract was defatted by washing with dichloromethane. Additional clean-up was examined using several cartridge columns, C18, PSA, ACCUCAT, Envi Carb (1,000 mg, Supelco), Oasis HLB (60 mg and 200 mg, Waters), and ISOLUTE Multi Mode (500 mg, International Sorbent Technology). No solid-phase trapped AA, so the columns were used to trap water-soluble matrices and to pass AA. The most suitable columns examined were C18, PSA and ACCUCAT. The solid-phase materials of C18 and ACCUCAT were the same as that of Multi Mode used in the LC/MS/MS method¹⁾. The sample extract was passed through these columns, followed by washing with water. The proposed clean-up procedure was sufficient to analyze AA in the potato products, but further clean-up was necessary for the other foods. No other suitable column was found, and ultrafiltration using a spin filter with 3 kDa cut off was not effective to remove the interference, so the LC/MS with a column switching system was applied.

4. Calibration curve and detection limit

The calibration curve was prepared using AA-1-¹³C as an internal standard. The calibration curve was linear in the range of 5 to 500 ng/mL of AA with a correlation coefficient r^2 of 0.999.

The purity of AA-1-¹³C used was 99 atom% ¹³C, so the calibration standard (AA=0, AA-1-¹³C=200 ng/mL) showed a response at m/z 72 derived from AA as an impurity of AA-1-¹³C. It corresponded to about 1% of the response at m/z 73 of AA-1-¹³C. The LOD was defined as 3 times the SD of the AA peak area of a calibration standard (AA=0, AA-1-¹³C=200 ng/mL). The LOQ was defined as 10 times the SD. The LOD on a sample mass basis was 9 ng/g and the LOQ was 30 ng/g. The LOD was similar to the LOD (10 ng/g) reported for the LC/MS/MS method^{1), 2)} and to the values reported in our preceding paper by GC/MS⁹⁾. Found values between the LOD and LOQ are indicated as trace (tr) in Table 2.

5. Recovery test and precision of analysis

The results of the recovery test are shown in Table 1. AA was added to potato chips, corn snack, pretzel and roasted tea leaves at the level of 500 ng/g. These samples were analyzed by the proposed method after the addition of an internal standard at the level of 2,000 ng/g. The recovery of the internal standard AA-1-¹³C was 67.9–82.8%. The control samples contained 50–1,008 ng/g of AA and the SD values of the found values were 0.4–13 ng/g (RSD: 0.16–2.4%). The mean value and SD of the corrected recoveries for these foods were 99.5–101.0% and 0.3–1.6%, respectively. The recovery and repeatability were acceptable, which suggests that the proposed method would have a good performance for the analysis of AA in foods.

6. AA contents in various processed foods

The analytical results of various commercial food samples are given in Table 2. The recoveries of AA-1-¹³C were calculated to confirm the propriety of each analysis, and were 43.8–86.3% (68.6±6.1%) for all samples. In some samples, the recoveries of internal standard might be reduced by decomposition during the analysis and/or inhibition of the ionization in MS analysis. AA in the standard solution was stable in the dark

Table 1. Recovery of Acrylamide from Spiked Foods

Sample	Main raw material	<i>n</i>	Added (ng/g)	Found (ng/g, mean±SD)	Recovery (% , mean±SD)
Potato chips	Potato	3	0	1,008± 1.6	99.8±0.3
			500	1,507± 1.3	
Corn snack	Corn	3	0	535±13	99.5±0.6
			500	1,033± 3.2	
Pretzel	Wheat	3	0	50± 0.4	99.9±1.6
			500	549± 8.0	
Roasted tea (Hoji-cha)	Tea	3	0	567± 3.5	101.0±1.3
			500	1,072± 6.5	

Table 2. Analytical Results of Acrylamide in Foods

Foods	Main raw material	Found values (ng/g)				
Storable foods						
Potato chips	Potato	3,544* 875*	2,175 467*	1,542*	1,385*	<u>1,008</u>
Potato snacks	Potato	57*	35*			
Dried mashed potato	Potato	nd*				
Bolo (small round cookie)	Potato	tr*				
Sweet potato snack	Sweet potato	112				
Deep-fried sweet potato (Imo-kenpi)	Sweet potato	336				
Corn snack	Corn	535	387*	238*	117*	
Cereals	Corn	122	113			
Pretzel	Wheat	56*	50	48*		
Biscuit, Cookie	Wheat	302	247	124		
Cracker	Wheat	302	227	53		
Fried and sugar-coated dough cake (karinto)	Wheat	1,895	374*	84		
Doughnut	Wheat	tr*				
Sponge cake (castilla), Cake (baum kuchen)	Wheat	nd	nd			
Rosted barley flour (mugi-kogashi)	Barley	236				
Rice cracker (senbei)	Rice	tr*				
Deep-fried rice cake (agemochi)	Glutinous rice	36	tr*			
Beans snacks	Immature pea	101				
Beans snacks	Wheat, soybean	83				
Roasted and ground bean (kinako)	Soybean	118	31*			
Roasted sesame seed	Sesame	197*	160	136	116	
Roasted peanuts	Peanuts	92	57			
Fried horsebeans	Horsebeans	120				
Roasted almonds	Almonds	324				
Roasted pistachio	Pistachio	34				
Roasted cashewnut	Cashewnut	tr				
Roasted walnut	Walnut	nd				
Apple chips	Apple	nd				
Banana chips	Banana	65				
Vegetable chips (string beans)	String beans	45				
Vegetable chips (pumpkin)	Pumpkin	55				
Vegetable chips (carrot)	Carrot	tr				
Vegetable chips (potato)	Potato	tr				
Bread-crumbs	Wheat	35	tr			
Precooked noodles (Instant noodles)	Wheat	163*	70	57	tr*	tr
Curry roux	Spice	116				
Curry powder	Spice	423				
Green tea	Tea	tr	nd			
Roasted tea (hoji-cha)	Tea	567	538*	519		
Tea	Tea	tr	nd			
Oolong tea	Tea	142	97	tr	nd	
Pu'er tea	Tea	nd				
Roasted barley (mugi-cha)	Barley	270	256*			
Roasted coffee bean	Coffee bean	231	153	151*		
Cocoa powder	Cacao beans	141	104			
Skim milk	Milk	tr				
Perishable foods						
French fries	Potato	784	693	565	512	
Sweet potato snacks (Daigaku-imo)	Sweet potato	34				
Bread	Wheat	tr	tr*	nd		
Boiled buckwheat noodles (soba)	Buckwheat	nd				
Boiled Japanese noodles (udon)	Wheat	nd				
Cooked rice	Rice	nd				
Soybean curd	Soybean	nd				
Toasted or fried soybean curd (yaki-tofu, ganmodoki, abura-age)	Soybean	nd	nd	nd		
Omelet (dashimaki-tamago)	Egg	tr				
Omelet	Egg	nd				
Egg roll (harumaki)	Wheat	tr				

Table 2. (Continued)

Foods	Main raw material	Found values (ng/g)				
Perishable foods						
Egg roll shell	Wheat	30				
Fried cake of minced meat	Wheat, meat	tr	nd			
Deep-fried chicken	Chicken, wheat	36	tr			
Baked mackerel (saba-shioyaki)	Mackerel	nd				
Batter coating (tempura)	Wheat	tr*	nd*			
Bread-crumb coating	Wheat	53*	32	tr	tr*	nd
Deep-fried horse mackerels	Horse mackerel, wheat	nd				
Tube-shaped fish paste cake (chikuwa)	Fish	nd	nd			
Fried fish paste (satsuma-age)	Fish	nd	nd			

*: The sample was the same as the one analyzed by GC/MS and reported in the preceding paper⁸⁾.

Figures underlined were the same as control values in the recovery test (shown in Table 1).

Each figure printed in boldface and italics was the same product produced on different days.

nd: not detected (<9 ng/g)

tr: trace ($9 \leq \text{tr} < 30$ ng/g)

Fig. 3. Correlation between acrylamide and water contents in foods examined

◆: potato chips; △: French-fried potato; ○: other foods

The water content was taken from "Standard Tables of Food Composition in Japan, fourth (1982) and fifth (2000) revised edition". For some foods, the values for similar foods were used.

at 4 or 10°C, but AA in some test solutions decreased by 20–30% in one day. The samples marked (*) were the same as those analyzed by the GC/MS method in the preceding paper⁸⁾. The values obtained by the LC/MS method correlated well with those obtained by the GC/MS method, and they were slightly larger (about 6.5%) than those obtained by GC/MS ($y = 0.934x + 2.998$, r^2 : 0.999). The analyses by the LC/MS method were performed one month after the analyses by GC/MS, so AA must be stable in these foods during this period.

High levels (exceeding 1,000 ng/g) of AA were found in potato chips, as also reported by Swedish researchers^{*1}. The levels of AA in potato chips were 467–3,544 ng/g. French-fried potato obtained from different fast-food shops contained almost equal levels of AA (512–784 ng/g). A fried and sugar-coated dough cake (karinto) which was indicated to have been fried twice contained 1,895 ng/g of AA, and other "karinto" contained 374 and 84 ng/g AA. Corn snacks contained 117–535 ng/g of AA. Roasted food products contained 116–567 ng/g of AA (such as roasted sesame seed,

roasted tea (hoji-cha), roasted barley (mugi-cha), coffee beans and curry powder). The AA levels in the staple foods were < LOD–70 ng/g (such as cooked rice, boiled noodles (soba and udon), bread and precooked (instant) noodles). An instant noodle sample that contained 163 ng/g of AA was a snack-type product, and it was taken without cooking. The foods made from fish, egg and meat contained lower levels of AA than the plant-based foods.

The products made by the same manufacturer on different days contained similar levels of AA (for potato chips, 1,008 and 875 ng/g; for corn snack, 535 and 387 ng/g; for pretzel, 50 and 48 ng/g; for roasted tea, 567 and 538 ng/g).

The variety of AA content in foods might result from the differences in raw materials and processing conditions such as temperature and heating time. It was reported that the Maillard reaction is involved in the formation of AA; the key precursors are asparagine and glucose, and water enhanced the reaction^{9), 10)}. The foods containing much water tended to have low levels of AA compared with the dry foods (Fig. 3). The water content might be one of the factors affecting the formation and/or decomposition of AA in foods. The foods fried or roasted at high temperature tended to have higher levels of AA than the foods boiled in water. The potato products tended to contain high levels of AA compared with similar products made from wheat or corn.

Conclusion

1) An LC/MS method for the analysis of AA in foods was developed. The method consisted of extraction with a mixture of water and acetone, clean-up by washing with dichloromethane and on C18, ACCUCAT and PSA cartridge-columns, and then LC/MS (SIR) using column switching.

2) The mean recoveries of AA from potato chips, corn snack, pretzel and roasted tea spiked at the level of 500 ng/g were 99–101% and the SD were 0.3–1.6%, when corrected based on internal standard AA-1-¹³C.

The LOD of AA in foods was 9 ng/g.

3) AA contents of various foods were analyzed by the developed method. AA was found in potato chips and French-fried potatoes at the levels of 467–3,544 ng/g, and in other foods except for one sample of “karinto” at the level of <LOD–567 ng/g.

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