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Rugged LC-MS/MS Survey Analysis for Acrylamide in Foods

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The described liquid chromatography−tandem mass spectrometry (LC-MS/MS) method for the detection of acrylamide in food entails aqueous room temperature extraction, SPE cleanup, and analysis by LC-MS/MS. The method is applicable to a wide variety of foods. [$^{13}C_3$]acrylamide is the internal standard. The limit of quantitation is 10 ppb (μg/kg). Data were obtained in duplicate from >450 products representing >35 different food types. The variability in analyte levels in certain food types suggests that it may be possible to reduce acrylamide levels in those foods.

KEYWORDS: Acrylamide; exploratory; FDA; food survey; liquid chromatography−tandem mass spectrometry; LC-MS/MS; method

INTRODUCTION

Acrylamide (2-propenamide, CAS Registry No. 79-06-1) is a potent cumulative neurotoxin in animals and man (*1*). Its biological activity can be attributed to the reactivity of its olefinic bond. Acrylamide metabolizes to an epoxide, glycidamide (2,3-epoxypropanamide), which reacts with DNA (*2*). Acrylamide binds to sulfhydryl groups on proteins and reacts with terminal valine groups, forming *N*-(2-carbamoylethyl)valine adducts. Cysteine and valine hemoglobin adducts have been used to estimate human exposure to acrylamide (*3*). Epidemiological studies have been unable to prove or disprove the human carcinogenicity of acrylamide, but it was found to cause cancer in animals at high dosage, so this neurotoxic compound is classified as a potential human carcinogen and genotoxicant (*4*).

In April 2002, researchers at the Swedish National Food Administration and Stockholm University reported to the press that they had found acrylamide in fried and oven-baked foods. Their conclusion that high-temperature cooking generated acrylamide in some foods led to a flurry of activity to identify the reasons for its formation in cooked food, the extent of its occurrence, the likely dietary exposure, and its possible risk to humans. Details of the Swedish method were not initially available so we chose to develop our own method for the analysis of acrylamide in food.

Existing assays for acrylamide relied on its conversion to 2,3-dibromopropionamide (*5*) or the more stable 2-bromopropenamide followed by analysis using gas chromatography or gas chromatography with mass spectrometric (GC-MS) detection (*6*). The extraction of the brominated product into an organic solvent from an aqueous extract permitted easy concentration of the derivatized analyte prior to its determination. Unfortunately, the derivatization was incomplete and time-consuming. A report of the use of reversed-phase high-performance liquid chromatography for the detection of underivatized acrylamide and glycidamide in rat plasma (*7*) provided a starting point for the development of assays for acrylamide in foods based on liquid chromatography with mass spectrometric detection. This soon became the analytical approach of choice.

The Swedish National Food Administration developed a liquid chromatography−tandem mass spectrometry (LC-MS/MS) assay for acrylamide in potato and cereal products over a range of 30−10000 μg/kg. The LC-MS/MS assay monitored five product ions of acrylamide and a single product ion of labeled acrylamide to identify and quantitate acrylamide in food extracts (*8*). The Stockholm University group used GC-MS and LC-MS/MS to further examine the origin of acrylamide during cooking. They reported moderate levels (5−50 μg/kg) in heated protein-rich foods and higher levels (150−4000 μg/kg) in carbohydrate-rich foods (*9*). Ahn and co-workers independently verified the Swedish findings with GC-MS and LC-MS/MS analyses of U.K. food products. Their cooking experiments showed that different cooking practices dramatically affect the acrylamide content of foods. Food browning was not the only factor. The method of cooking was also important. Grilling of potatoes produced higher levels of acrylamide than either frying or oven baking (*10*).

Several groups examined the mechanistic reasons for acrylamide formation in cooked food. Zyzak and co-workers used LC-MS to provide clear evidence for asparagine as a principal source of acrylamide in cooked food. They used three isotopically lableled forms of asparagine to show that asparagine and a carbonyl source generate acrylamide from asparagine in model foods with heating (*11*). There are four other reports linking acrylamide formation to the Maillard reaction between amino acids and reducing sugars (*12*−*15*). Stadler and co-workers show that *N*-glycosylasparagine is a direct precursor of acrylamide (*13*). Yaylayan and co-workers provide evidence that the ability

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of the open-chain form of this precursor to form oxazolidin-5-one is the key step that allows decarboxylation of asparagine with subsequent formation of acrylamide (*15*).

The present paper reports an analytical method for the detection and quantitation of acrylamide in foods in the United States. It entails room temperature aqueous extraction of acrylamide from food, solid-phase extraction (SPE) cleanup, and analysis by LC-MS/MS. The method is designed to be suitable for regulatory analyses. Isotopically labeled acrylamide is used as an internal standard. The limit of quantitation is 10 ppb of acrylamide. Recovery of added acrylamide is 95 ± 6%. Precision for replicate analyses in food products ranges from 1.5 to 3.9% RSD. Data obtained for >450 products analyzed represent >35 different food types. The observed bag-to-bag and lot-to-lot variability of acrylamide in potato chips of known source and type processed on the same production line by the same employee supports increased acrylamide formation with thermal aging of the cooking oil. The variability of acrylamide levels within selected food groups is exceeded by variability resulting from consumer cooking. This indicates that consumer preferences in food preparation are a factor in the occurrence of acrylamide in food.

EXPERIMENTAL PROCEDURES

Chemicals and Consumables. Acrylamide 99+%, formic acid, and 99% glacial acetic acid were obtained from Sigma-Aldrich (St. Louis, MO). $^{13}C_3$-Labeled acrylamide (99%) was obtained from CIL (Andover, MA). HPLC grade acetonitrile, methanol, 2-propanol, and water were obtained from Omnisolv, EM Science (Gibbstown, NJ). Certified disposable pipet tips, 50 mL polypropylene conical tubes with caps, and glass scintillation vials with polypropylene caps were obtained from VWR Scientific Products (Buffalo Grove, IL). Maxi-Spin filter tubes, 0.45 μm PVDF, Teflon manifold needles, and one-way stopcocks for SPE manifolds were obtained from Alltech Associates (Deerfield, IL). Oasis HLB 6 mL, 200 mg SPE cartridges were obtained from Waters (Milford, MA). Bond Elut-Accucat 200 mg, 3 mL SPE cartridges were obtained from Varian (Chicago, IL). Amber glass autosampler vials with septum screw caps were obtained from Agilent Technologies (Wilmington, DE). Synergi 4 μ Hydro-RP 80 A° 250 × 2.0 mm LC columns, C18 2.0 mm guard columns, and a guard cartridge kit were obtained from Phenomenex (Torrance, CA).

CAUTION: Acrylamide and $^{13}C_3$-labeled acrylamide are potent cumulative neurotoxins in animals and man and may be carcinogenic. Acetonitrile, formic acid, and methanol are hazardous. Avoid contact or inhalation exposure to these reagents.

Analytical Instruments. An Agilent 1100 HPLC equipped with vacuum degasser, binary pump, autosampler, temperature-controlled column oven, and 190−600 nm diode array detector was coupled to a Micromass Quattro triple-quadrupole mass spectrometer equipped with electrospray source. An Agilent 1050 binary pump used to supplement 1100 LC effluent prior to mass spectrometer with 1% acetic acid in 2-propanol.

Preparation of Standards. Acrylamide is stable in acid, unstable in base, and light sensitive. Accurately weighed solid portions of acrylamide and $^{13}C_3$-labeled acrylamide were dissolved in 0.1% formic acid in separate red glass volumetric flasks. An aliquot of the $^{13}C_3$-labeled acrylamide solution was diluted to prepare a spiking solution of 200 μg/kg (200 mg/mL) $^{13}C_3$-labeled acrylamide. Aliquots of the stock solutions of $^{13}C_3$-labeled acrylamide and acrylamide were combined to prepare working standard solutions of 200 μg/kg $^{13}C_3$-labeled acrylamide (200 ng/mL) with acrylamide at five concentrations between 8 and 3600 μg/kg. For example, 5.7857 mg of [$^{13}C_3$]acrylamide dissolved in 100 mL is 57.9 ng/μL. A 173 μL aliquot of this solution diluted to 10 mL would be 200 ng/mL or 200 μg/kg [$^{13}C_3$]acrylamide. An appropriate aliquot of acrylamide stock solution diluted to volume in the same flask creates a combined working standard solution of acrylamide in 200 μg/kg $^{13}C_3$-labeled acrylamide.

The stock solutions are stable for up to 6 months in red glass at room temperature. Working solutions of 40 μg/kg and less held in clear glass will noticeably degrade first. The combined working standards solutions were used to obtain a daily response curve to verify instrument performance and to determine the daily average relative response factor of acrylamide and $^{13}C_3$-labeled acrylamide. The relative response factors of the dilute standard solutions should be compared to the higher level solutions to decide when to prepare fresh working standards.

Sample Preparation. Serving size portions of solid foods are pulverized in a food processor or a variable-speed Waring blender prior to sampling. Liquid and powdered food products are sampled directly. Samples of 1.00 g were weighed into 50 mL centrifuge tubes, and 1 mL of 200 μg/kg $^{13}C_3$-labeled acrylamide internal standard and 9 mL water were added. Each tube was then capped and shaken by hand or vortex briefly to mix the contents of the tube. The tubes were clamped in a rotating shaker to mix the tube contents for 20 min. The tubes were centrifuged at 9000 rpm for 15 min with an Allegra 21 Beckman Coulter centrifuge using a C0650 head (Palo Alto, CA) or equivalent. A 5 mL aliquot of clarified aqueous layer was promptly removed by pipet for spin filtration. The pipet was inserted through the top oil layer, avoiding the bottom solids layer with the pipet tip when a portion of the aqueous phase was removed. The 5 mL aliquot was placed in a spin filtration tube and centrifuged at 9000 rpm for 2−4 min. If the filter clogged, a new filter tube was inserted, the unfiltered liquid was poured onto the new filter, and centrifugation was continued until most of the liquid had passed through the filter. Oasis HLB SPE cartridges were conditioned with 3.5 mL of methanol followed by 3.5 mL of water; the methanol and water portions were discarded. Each cartridge was loaded with 1.5 mL of filtered extract. The extract was allowed to pass through the sorbent material followed by 0.5 mL of water. Then the column was eluted with 1.5 mL of water, and the eluant was collected for Accucat SPE cleanup. The outside of the Accucat SPE cartridges was marked at the height of 1 mL of liquid above the sorbent bed, and then the A cartridges were conditioned with 2.5 mL of methanol followed by 2.5 mL of water. The methanol and water portions were discarded. All of the eluant collected from the Oasis SPE was loaded and eluted to the 1 mL mark before the remainder of the eluted portions was collected. These portions were transferred into 2 mL amber glass autosampler vials for LC-MS/MS analysis.

Analysis for Acrylamide in Foodstuffs by ESI-LC-MS/MS. The C18 2.0 mm guard column and Synergi 4 μ Hydro-RP 80 A 250 × 2.0 mm LC column were maintained at 26 °C. The mobile phase was 0.5% methanol/0.1% acetic acid in water. The capillary voltage was 4.1 kV. The cone voltage was 20 V. The source temperature was 120 °C. The desolvation gas temperature was 250 °C. The desolvation gas flow was 710 L/h nitrogen. The cone gas flow was 153 L/h nitrogen. The first quadrupole resolution setting was 15 V, and the third quadrupole resolution setting was 14 V.

The normal electrospray LC-MS background for 0.5% methanol/0.1% acetic acid in water contains ions at *m*/*z* 74 and 76. These ions interfere with the protonated molecule of the internal standard at *m*/*z* 75. Addition of 0.05 mL/min 1% acetic acid in 2-propanol to the LC effluent replaces these interfering ions with *m*/*z* 79 to permit detection of the internal standard parent ion.

The mass scales of the mass spectrometer were calibrated from 20 to 82 on *m*/*z* 22.98 (Na^+), 43.09 ($C_3H_7^+$), 61.10 ($C_3H_9O^+$), and 79.12 ($C_3H_{11}O_2^+$). These ions will be present when the LC flow of 0.2 mL/min 0.1% acetic acid and 0.5% methanol in water is combined with a supplemental flow of 0.05 mL/min 1% acetic acid in 2-propanol prior to the ion source.

The argon collision gas pressure was adjusted to 3×10^{-3} mbar for MS/MS. The collision energy was varied for each monitored transition in multiple reaction monitoring mode (MRM). The transitions monitored for acrylamide were 72 > 72 at 5 V, 72 > 55 at 10 V, and 72 > 27 at 19 V. The transitions monitored for labeled acrylamide were 75 > 75 at 5 V, 75 > 58 at 10 V, and 75 > 29 at 19 V. The dwell time for each monitored transition was 0.3 s with 0.02 s interchannel and interscan delay. The mass spectrometer tuning was optimized by observing responses for loop injections of 100 pg of acrylamide.

Twenty microliter volumes of extract or standard were injected for LC-MS/MS acrylamide analysis, and LC-MS/MS and UV data wer

acquired for 10 min. Acrylamide elutes near 7 min. The total time from one injection to the next injection was 12 min.

The column was washed for a minimum of 20 min with 50:50 methanol/acetonitrile after 48 samples or at the end of daily operations. Repetitive analyses of some foods such as coffee require more vigorous column washing. Mobile phase re-equilibration for analyses requires 1.5 h. The LC column should be replaced or reconditioned after 1000 extracts. Symptoms indicative of the need to recondition or replace the column are a 50% increase in analyte peak width and resultant decrease in sensitivity as indicated by disappearance of the 8 μg/kg standard response. The source inlet cones should be cleaned monthly.

A daily response curve was plotted of area response ratio for *m*/*z* 55/58 versus parts per billion acrylamide injected with a constant amount of $^{13}C_3$-labeled acrylamide. A five-point plot should be linear with a correlation coefficient >0.999. The average response ratio from the data points (μg/kg acrylamide/μg/kg labeled acrylamide) should be calculated and used to adjust the observed level of acrylamide to compensate for variation in instrument response.

Parts per billion of acrylamide should be calculated using the relationship that the ratio of the amount of internal standard to its response is equivalent to the ratio of the amount of acrylamide to the acrylamide response. This result is then divided by the weight of the test portion and the response ratio. Thus, if 200 ng of internal standard was added to a 1 g portion, then μg/kg acrylamide = (200 ng)(area *m*/*z* 55)/(area *m*/*z* 58)(g portion)(response factor). The limit of quantitation was defined as the level at which a 10:1 signal/noise ratio was observed for the analyte quantitation ion, *m*/*z* 55.

Confirmation of identity of the response is based on three criteria. First, the analyte and internal standard signals must occur at the same retention time. Second, their signals must be separated from those of the sample matrix (*16*). Third, the relative abundances of the signals recorded for the analyte at *m*/*z* 72, 55, and 27 must be within ±10% of the observed relative abundances recorded for the internal standard at *m*/*z* 75, 58, and 29 (*17*).

RESULTS AND DISCUSSION

The criteria for confirmation of identity in this method are stringent in comparison to those proposed by other laboratories. Brandl and co-workers use LC-MS/MS to record the concurrent transitions *m*/*z* 72>55 and *m*/*z* 75>58 as indications of the presence of acrylamide in low-moisture foods extracted with dichloromethane/ethanol in an accelerated solvent extraction (ASE) apparatus (*18*). The LC-MS/MS assay of Tareke and co-workers monitors transitions of *m*/*z* 72 to 54 and 55 for identification of acrylamide and monitors the transition *m*/*z* 75>58 for quantitation (*9*). Rosen and co-workers (*8*), Ahn and co-workers (*10*), and Becalski and co-workers (*14*) monitor the transition *m*/*z* 75>58 for quantitation and the transitions of *m*/*z* 72 to 72, 55, 54, 44, and 27 to confirm acrylamide identity. On the basis of a pending European Union directive for confirmation of drug residues in meat with LC-MS/MS, Rosen and co-workers suggest maximum tolerances for the relative intensities of these ions of ±50% for 54/55 and 44/55 and ±20% for 55/72 (*8*). Our ±10% reproducibility tolerance for LC-MS/MS data is consistent with mass spectrometry guidelines for regulatory analysis that were developed from U.S. FDA criteria for evaluating assays for carcinogenic residues (*19*).

The MS/MS technique imparts a measure of specificity to all of the above assays, but MS/MS data obtained from low-mass parent ions are subject to interference and can be misleading in the absence of sufficient internal standard data. The six monitored MS/MS transition profiles for a coffee extract in **Figure 1** show responses at various retention times in all six profiles. These data demonstrate that MS/MS analysis for acrylamide requires a prior chromatographic separation of the analyte from coextractive materials and unambiguous identification of the analyte under the conditions of analysis by comparison of the analyte response to that of the internal standard.

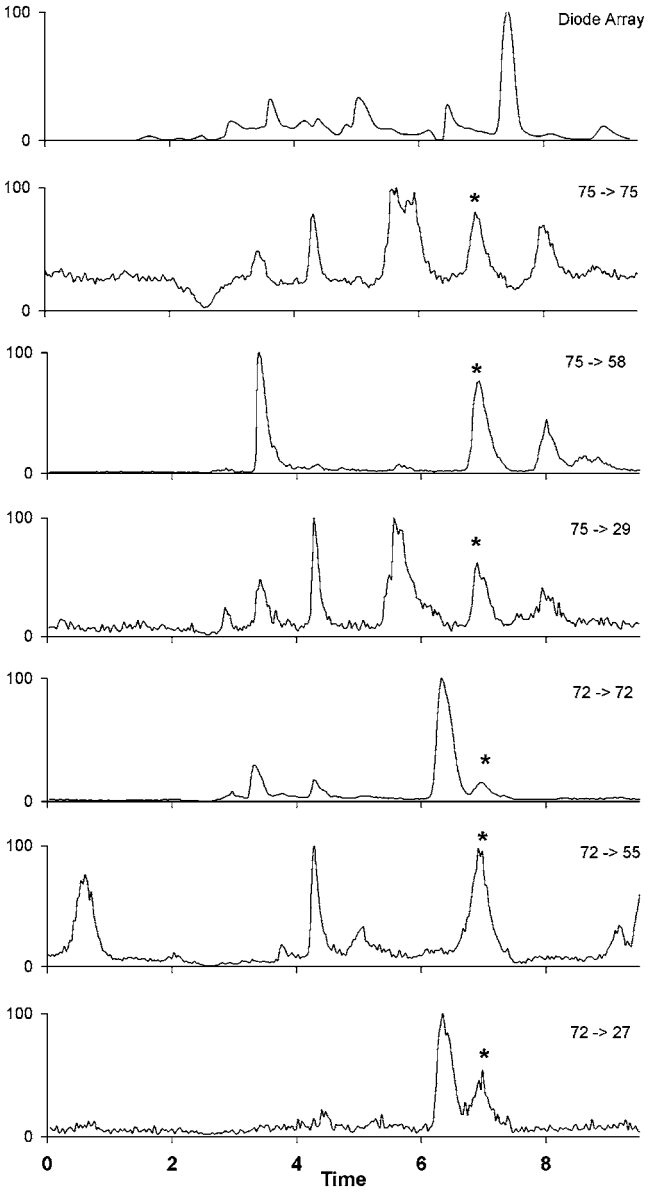

Figure 1. Raw data recorded for coffee extract. Top trace is the 190–600 nm summed signal recorded by the diode array detector. The next six profiles in descending order are *m*/*z* 75, 58, 29, 72, 55, and 29, respectively. The relative abundances of these transitions at the retention time of acrylamide for 75:58:29 are 100:42:6, and for 72:55:27 they are 100:46:6. Retention time of acrylamide at 35 °C column temperature is 6.94 min and is denoted by ∗.

Ahn and co-workers found carbohydrate foods such as pies and chocolate gave large matrix effects, whereas other foods gave extracts that were glutinous (*10*). We found coffee to be more troublesome and suggest that this difference is the result of our use of room temperature water extraction instead of more vigorous measures that would be expected to extract undesirable coextractives. The internal standard data corrected for any matrix effects in our extracts.

The internal standard data serve four direct purposes: location of acrylamide, verification of nondetection, quantitation, and confirmation of identity. Coelution of the internal standard with the analyte identifies the location of the analyte in the ion profiles. The presence of an internal standard response and the

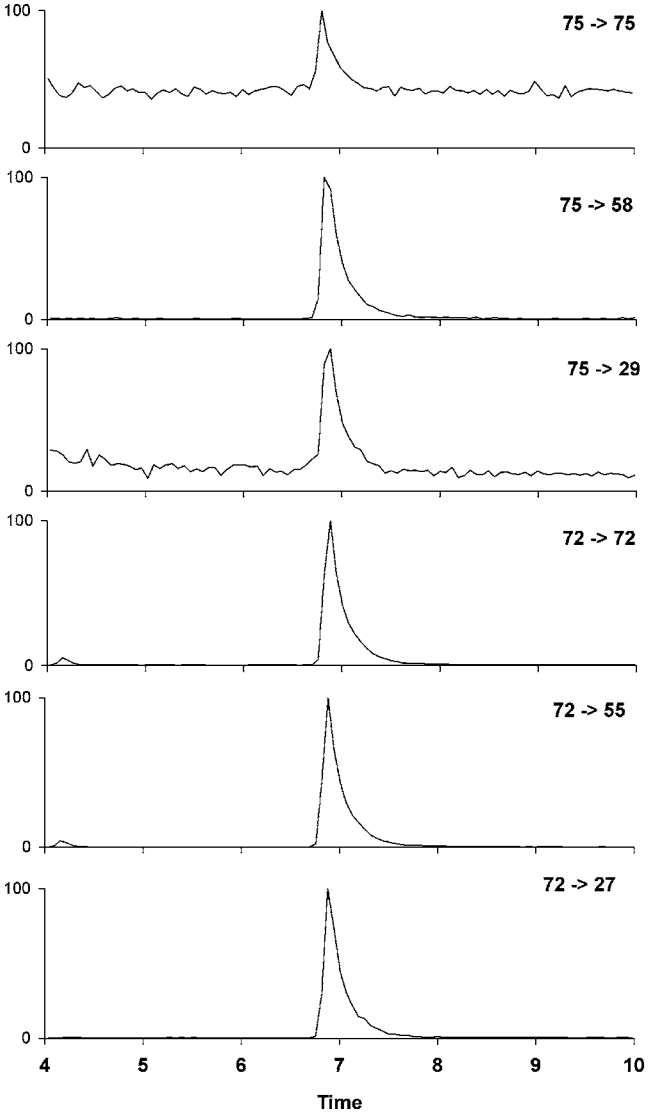

Figure 2. Raw data recorded for spiked beer extract at column temperature of 26 °C. Pronounced tailing is effect of beer on chromatography. The six profiles in descending order are *m*/*z* 75, 58, 29, 72, 55, and 29, respectively. The relative abundances of these transitions at the retention time of acrylamide for 75:58:29 are 100:46:8, and for 72:55:27 they are 100:48:7. Retention time of acrylamide in beer at 26 °C is 6.88 min.

absence of an acrylamide response are evidence for the absence of acrylamide in the test portion. The *m*/*z* 58 internal standard response and the *m*/*z* 55 acrylamide response are ratioed to quantitate the level of acrylamide in the test portion. A comparison of the relative abundances of *m*/*z* 72:55:27 and *m*/*z* 75:58:29 confirms the identity of acrylamide and the absence of interference in the quantitation when their relative abundances agree to within ±10%.

All four factors come into play when there are circumstances under which the sample alters the behavior of acrylamide in the LC-MS/MS. Beer shortens the LC retention time of acrylamide by ~0.2 min and skews its peak shape as shown for a spiked beer in **Figure 2**. The identical behavior of the internal standard locates the acrylamide response, and a relative abundance match confirms acrylamide identity.

Quality assurance is the indirect purpose of the internal standard. If the internal standard response in a sample set degrades or disappears, the data are demonstrating an analytical problem that must be corrected before the analyses can proceed. In contrast, the data would have been construed as nondetects or low results if the measurements were based on comparisons to external standard responses. For example, repetitive injections of coffee extracts into the LC-MS/MS at a column temperature of 26 °C may degrade the chromatography in <4 h. The column must be washed to restore its performance. Operating the column oven at 35 °C for coffee extracts allows completion of a typical day's work before stopping analyses to wash the column with 50:50 methanol/water for 2 h followed by 50:50 acetonitrile/methanol for up to 12 h. Low-sodium soy sauce is an example of a sample preparation problem. Sodium chloride is replaced by potassium chloride in the formulation of low-sodium soy sauce. The SPE cleanup was modified to its present form in order to recover internal standard from soy sauce. However, the present cleanup does not recover the internal standard from low-sodium soy sauce.

It is not possible to fully validate a method for every conceivable matrix. The [$^{13}C_3$]acrylamide internal standard is chemically identical to acrylamide and differs only in mass from acrylamide. Recovery of the internal standard provides both verification that the analyte can be recovered from the matrix and an internal correction for instrument performance or sample preparation effects. This makes it possible to be reasonably confident of analytical results for matrices other than those for which the method has been tested.

Extract Stability. Acrylamide is not stable in contact with raw plant tissue or soil microorganisms (*20*). Cooking halts the biodegradation of acrylamide so its stability in an extract is dependent on pH, exposure to light, reactive coextractives, and microorganisms. Extracts are analyzed within hours of their preparation. Some extracts have been reanalyzed within days or weeks of their initial analysis to study the repeatability of the results, but the long-term stability of acrylamide in the extracts of all of the foods surveyed is not known.

Linearity. The acrylamide response was linear over a range of 8−3600 μg/kg. Correlation coefficients better than 0.995 were obtained. However, linearity was best demonstrated by comparing the response factors obtained at each level. For example, over the course of a month, response factors were constant from level to level. The overall average was 0.98 ± 0.05, RSD 4.6%.

Lower Limit of Quantitation. The lower limit of quantitation was set at 10 μg/kg on the basis of compliance of all six monitored ions in standards data with the confirmation of identity criteria. The 8 μg/kg standards met these criteria. The 4 μg/kg standards usually met the criteria. The 2 μg/kg standards rarely met the identity criteria. Confirmed acrylamide responses of <10 μg/kg were reported as <10 μg/kg.

Recovery. Recovery of the method was demonstrated in three trials employing the method of standard addition. The matrix was crushed dry crackers provided by an outside agency for the first two trials. Acrylamide standard was added to each sample at four different levels. Total acrylamide was then determined for each level. When total acrylamide was plotted against added acrylamide, the *y*-intercept calculated by linear regression analysis was the incurred residue level in the unspiked sample. Subtracting the incurred residue from the total amount of acrylamide found indicated the spike recovery for each of the four spiked portions. The average recovery was 94.8 ± 5.6% for the eight determinations in the two experiments. These first two trials tested an initial version of the method that loaded 2 mL of extract onto the Oasis SPE cartridge, eluted the cartridge with 2 mL of water, and placed this 2 mL of eluate onto the Varian SPE cartridge. Eluting the cartridges with additional portions of water demonstrated that no acrylamide remained on the Oasis or Varian cartridges. The method recovery was

Table 1. Results of 5-Day Precision Study with 1 g Subsamples of Cereal, Bread Crumbs, Potato Chips, and Coffee

day of test	product composite	replicates analyzed	μg/kg acrylamide	SD[a]	RSD[b] (%)
1	cereal	4	219	2.6	1.2
2	cereal	3	214	11.2	5.2
3	cereal	3	211	14.6	6.9
4	cereal	3	232	12.8	5.5
5	cereal	3	234	6.4	2.7
summary	cereal	16	222	9.2	4.1
1	crumbs	4	64	1.9	3.0
2	crumbs	3	62	0.8	1.2
3	crumbs	3	62	0.6	0.9
4	crumbs	3	68	1.5	2.2
5	crumbs	3	65	0.6	0.9
summary	crumbs	16	64	2.4	3.8
1	chips	4	621	27.3	4.4
2	chips	3	597	28.9	4.8
3	chips	3	606	35.2	5.8
4	chips	3	638	4.7	0.7
5	chips	3	684	8.4	1.2
summary	chips	16	629	30.6	4.9
1	coffee	4	128	5.9	4.6
2	coffee	3	118	4.0	3.3
3	coffee	3	119	7.1	6.0
4	coffee	3	134	4.5	3.4
5	coffee	3	131	3.2	2.4
summary	coffee	16	126	6.4	5.1

[a] Standard deviation. [b] Relative standard deviation.

tested in its present form in a third trial in the same manner using 16 portions of a performance assessment sample of crushed dry cereal.

The method in its present form leaves half of the acrylamide on the Varian SPE cartridge. This residual acrylamide elutes within the next 0.6 mL of water passed through the cartridge. The split affects the absolute recovery of acrylamide, but the apparent recovery of acrylamide relative to the internal standard is unaffected. For example, the average apparent recovery of acrylamide was 92 ± 6% for 16 portions of a performance assessment sample of crushed dry cereal. The benefits of the split are that it results in a cleaner eluate and some flexibility in the analysis of difficult samples such as coffee.

Precision. Table 1 reports the results of a 5-day precision study in which portions of four foods were repeatedly analyzed three or more times each day. The tested products were bread crumbs, cereal, coffee, and potato chips. They contained incurred levels of acrylamide ranging from 62 to 684 μg/kg. The values for the RSD of each data set ranged from 0.9 to 6.9%.

Sample Size. The suitability of a 1 g sample size was tested against results from a 4 g sample size using the four products listed in **Table 2**. The 4 g portions were spiked with 4 mL of 200 ng/mL internal standard and 36 mL of water to retain the 10:1 solvent/sample ratio used in the extraction of 1 g portions with 1 mL of internal standard and 9 mL of water. The results in **Table 1** show effectively the same levels of acrylamide detected in the 1 and 4 g test portions with comparable variation between replicates.

Accuracy in Proficiency Testing. Assessment of method accuracy was based on the performance of the method in interlaboratory proficiency testing. The laboratory participated in three interlaboratory studies of acrylamide methodology. In a National Food Processors Association study, eight laboratories

Table 2. Effect of Sample Size on Acrylamide Level Detected

product composite	sample wt (g)	replicates analyzed	μg/kg acrylamide	SD	RSD (%)
cereal	1	4	219	2.6	1.2
cereal	4	3	220	6.4	2.9
bread crumbs	1	4	64	1.9	3.0
bread crumbs	4	3	65	1.7	2.6
potato chips	1	4	621	27.3	4.4
potato chips	4	3	552	14.3	2.6
coffee	1	4	128	5.9	4.6
coffee	4	3	115	1.1	0.9

used LC-MS/MS to analyze saltine crackers, fatfree saltine crackers, spiked saltine crackers, potato chips, acrylamide in water, and blank water for acrylamide. Data reported by five of the eight laboratories were in close agreement. The values from these five reports were averaged to assign acrylamide levels to the samples. The assigned levels and this laboratory's reported values were as follows: saltines, 148 ± 8 μg/kg, 137 μg/kg reported; fatfree saltines (only three reports), 26 ± 4 μg/kg, 30 μg/kg reported; spiked saltines, 154 ± 13 μg/kg, 129 μg/kg reported; potato chips, 224 ± 14 μg/kg, 219 μg/kg reported; water, 49 ± 4 μg/kg, 44 μg/kg reported; water blank, 0 reported. The U.K. Central Science Laboratory of the Department for Environment, Food and Rural Affairs (CSL) provided 37 participants with a crispbread for acrylamide analysis. The assigned value derived from the satisfactory respondent data of 32 participants was 1213 ± 178 μg/kg. This laboratory reported 1264 μg/kg without correction for recovery. The CSL then distributed a performance assessment sample of crushed dry cereal to 41 laboratories for acrylamide analysis. Their assigned value of 109 μg/kg was calculated from the most appropriate measure of the central tendency of the 35 respondents (*21*). This laboratory found 115 μg/kg that was reported as 124 μg/kg after correction for a 92% recovery. This result confirmed that use of the internal standard made correction for recovery unnecessary. The reported values of 1264 and 115 μg/kg were within 6% of their assigned values, whereas the recovery-corrected value differed from the assigned value of the trial by 12%.

Effect of Mobile Phase Composition. Water, 0.5% methanol in water, and 0.1% acetic acid/0.5% methanol in water were evaluated as mobile phases with California black olives, coffee, pretzels, and prune juice extracts. 2-Propanol containing 1% acetic acid was added postcolumn. Analyses with each mobile phase were performed on the same day and over a period of 40 days with no significant differences in results. Representative data are shown in **Table 3**. Acrylamide responses recorded with water or 0.5% methanol in water as the mobile phase are 2−3 times larger than those recorded with 0.1% acetic acid/0.5% methanol in water. The acrylamide response with water as the mobile phase has a longer retention time and is slightly asymmetrical in that it resembles a shark fin. The 0.5% methanol mobile phase results in chromatography for acrylamide that is comparable to 0.1% acetic acid/0.5% methanol in water. The increased acrylamide responses observed with a mobile phase of water or 0.5% methanol in water are thought to be a column effect rather than a source effect because acid enhances the ESI response of acrylamide.

Effect of Column Temperature. Pretzel data were obtained at column temperatures of 26, 35, and 40 °C to note the effect on the elution of coextractives that may interfere with the determination of acrylamide. Pretzel extracts contain a component that interferes with quantitation at 35 and 40 °C as shown

Table 3. Effect of Mobile Phase Composition[a] on Acrylamide Response

product	sample identity	area *m/z* 55	area *m/z* 58	μg/kg acrylamide	mobile phase	column temp (°C)
coffee	A	3079	3472	422	a	35
coffee	B	2904	2549	329	a	35
coffee	A	10548	11884	430	b	35
coffee	B	10517	9186	333	b	35
coffee	A	5176	6531	481	a	26
coffee	B	5348	5578	398	a	26
coffee	A	8620	9752	412	b	26
coffee	B	7862	6565	304	a	26
coffee	A	9279	10296	423	c	26
coffee	B	8666	7203	317	c	26
olives	C	71036	5705	2078	a	26
olives	D	70632	6741	1749	a	26
olives	C	146939	11767	1991	b	26
olives	D	152652	12675	1920	b	26
olives	C	173868	13801	2103	c	26
olives	D	163577	14446	1890	c	26
prune juice	E	9061	6167	267	a	26
prune juice	F	9128	6023	267	a	26
prune juice	E	14777	9940	258	b	26
prune juice	F	15094	9392	271	b	26
prune juice	E	14969	10235	265	c	26
prune juice	F	15843	9955	281	c	26
pretzels	G	2700	5704	89	a	26
pretzels	H	2563	5292	87	a	26
pretzels	G	7894	18123	84	b	26
pretzels	H	9112	17594	96	b	26
pretzels	G	6527	14240	86	c	26

[a] Compositions examined were (a) 0.1% acetic acid/0.5% methanol in water, (b) 0.5% methanol in water, and (c) 100% water.

in **Figure 3**. A column temperature of 26 °C is appropriate for food products other than coffee. Data for ground, brewed, and instant coffee portions were extensively examined prior to accepting a column temperature of 35 °C for the analysis of coffee. Lack of coeluting interferences was demonstrated by comparison of the relative abundances of ions recorded for acrylamide and labeled acrylamide. These coffee data will be reported elsewhere.

Exploratory Survey of Acrylamide Levels in U.S. Foods. Food groups were chosen for analysis if they were previously reported to contain acrylamide or if they represented a significant part of infant or young adult diet. Product sampling was not representative of all brands. Many of the food types are represented by only one product in the survey.

More than 35 different food types were collected for analysis. The products included baby foods, bagels, breads, crackers, crisps, coffee, doughnuts, French fries, infant formula, gravy, jelly, meats, nuts, potato chips, pastries, pies, pretzels, pudding, seasonings, soups, tortillas, canned and frozen vegetables, and canned fruits. Most products were analyzed as received. Some products were examined before and after cooking. The exploratory data on acrylamide levels in the foods the FDA tested were made available on the Internet at the following address: http://www.cfsan.fda.gov.

The FDA tested these foods to gather preliminary results about the presence of acrylamide in U.S. foods, not to evaluate levels in individual products. These samples cannot and should not be generalized or used to reach conclusions about acrylamide levels in particular brands of foods. These samples are also not statistically representative of specific foods or brands (*22*). The data reported in the listing are the average of two or more

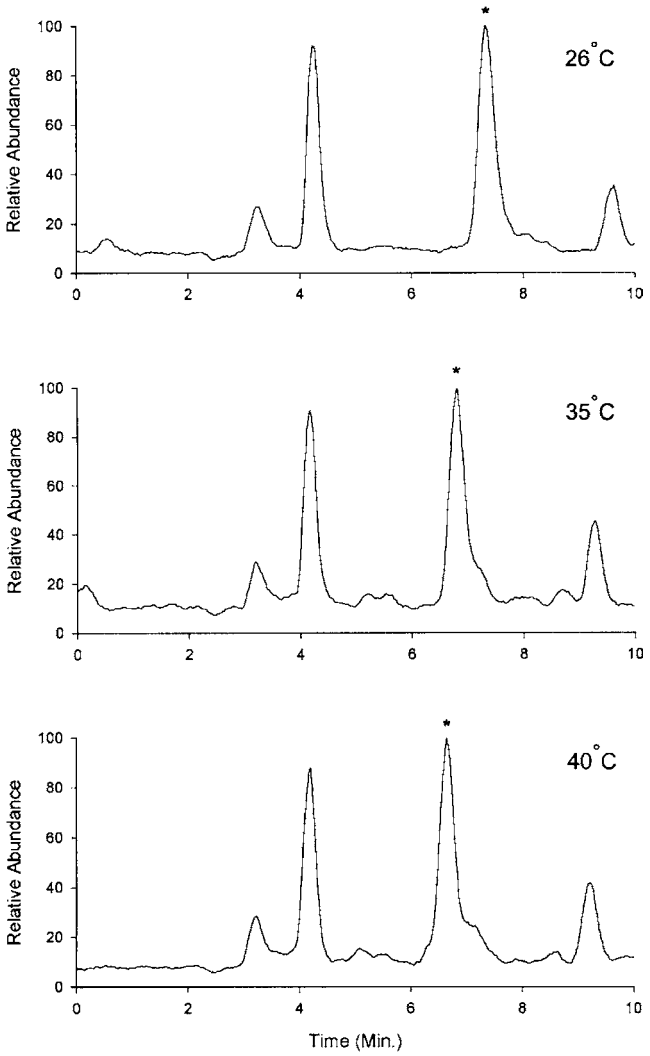

Figure 3. Smoothed *m/z* 55 data recorded for a pretzel extract at column temperatures of 26, 35, and 40 °C. Acrylamide responses are denoted by ∗. Acrylamide retention times are 7.33 min at 26 °C, 6.82 min at 35 °C, and 6.62 min at 40 °C with the 0.1% acetic acid/0.5% methanol in water mobile phase that is used in the method.

Table 4. Food Categories Found to Contain <10 μg/kg Acrylamide from a Limited Sampling of Available Products

beef bouillon	jelly, jam
caramel topping	marshmallows
chicken, raw, cooked	milk, condensed, powdered
dry baby cereal	nondairy creamer
fish, raw, canned	potatoes, raw, mashed
fruit, canned	soy sauce
gelatin	tapioca
gravy, canned	vegetables, canned, frozen
infant formula	vegetable protein

determinations for each product. **Table 4** lists some foods that contain little or no acrylamide.

Table 5 depicts the variability in the levels of acrylamide detected in a variety of food groups. Note that baby food is listed in **Table 5** even though baby cereal is listed in **Table 4** as containing negligible levels of acrylamide. The baby foods that contain little or no acrylamide are baby cereal and baby fruit (pureed fruit). There are other baby foods such as animal crackers and teething biscuits and prepared pureed meals that do contain acrylamide. Likewise, fish and chicken are listed in both **Tables 4** and **5**. The fish and chicken products in **Table 5**

Table 5. Examples of Acrylamide Level Variability in Some Tested Food Groups

food product	μg/kg acrylamide highest	lowest	median	brands tested
baby food	121	ND	107	25
bread	364	ND	34	23
cereal	1057	11	69	20
chicken, breaded	22	35	32	3
chips	2762	117	385	13
chocolate	909	ND	93	7
coffee, ground	374	37	185	47
cookies	432	36	199	7
crackers	620	26	121	12
fish, breaded	30	ND	12	13
fries (FF)[a]	1030	117	270	29
nuts, nut butters	457	ND	89	13
popcorn (MW)[b]	181	157	169	2
pretzels	309	31	107	25

[a] Cooked fries purchased from fast food restaurants. [b] Microwave-cooked popcorn.

are breaded items such as fish sticks. In those cases when the breading was analyzed separately from the fish and chicken, the acrylamide was found in the breading. Acrylamide was not present in tested fish and chicken.

Popcorn (MW) in **Table 5** refers to microwave popcorn. Microwave cooking does not produce acrylamide in all foods. Product packaging that includes a reflective material as a browning (microwave pizza) or popping (microwave popcorn) aid will produce cooking temperatures that generate acrylamide in the food (*23*).

Processing parameters other than temperature may be involved in the formation of acrylamide in foods. Chocolate, canned black olives, and prune juice are three products that warrant further study into the origins of acrylamide during their manufacture. Acrylamide is not stable in base (*5*). The various chocolate products in **Table 4** are an insufficient basis for a conclusion about the effect of base treatment on acrylamide. However, it is worthwhile to note that all of the chocolate products found to contain negligible amounts of acrylamide are labeled as "alkali processed" or "Dutch processed" cocoa, whereas two chocolate products containing ca. 300 and 900 μg/kg acrylamide are not labeled as alkali or Dutch processed. Canned California black olives contain high levels of acrylamide. Olives are prepared by brine and alkaline curing. Retorting California black olives during the canning step exposes the olives to temperatures that are known to form acrylamide, but the olive retorting environment differs from thermal processes that are known to produce acrylamide in other foods. It is not known if the ferrous gluconate used to fix the color of canned California black olives catalyzes acrylamide formation in this product. Sun-dried raisins do not contain detectable levels of acrylamide, but prunes do. This difference may be explained by the fact that prunes are produced by drying plums in forced-air ovens for up to 18 h. However, prune juice contains ~5 times more acrylamide than prunes. It is not known if the increased level of acrylamide in prune juice is the result of further acrylamide formation during heat treatment of the juice or simply accumulation of acrylamide in the juice.

Lot-to-lot and bag-to-bag variability of acrylamide level in potato chips was examined with the analysis of 23 bags of potato chips. There is measurable variation in detected levels from within lot and further variation between lots of potato chips. The lot-to-lot and bag-to-bag variability data are summarized in **Figure 4**. The potatoes for all lots were grown on the same farm and processed on the same production line by the same operator. Lots 4, 5, and 8 were cultivar A. Lots 3 and 6 were cultivar B. Lot 7 was cultivar C. The differences in acrylamide levels between lots 4, 5, and 8 correlated with the age of the oil used to cook the potato chips. Oil was added to the cooker during processing to offset oil absorbed by the chips. The oil thermally aged with use until it became necessary to clean the cooker and replace its oil charge to maintain product standards. The oil was approaching replacement in lot 4. It was replaced before lot 5. It was ready to be replaced at the time that lot 8 was produced.

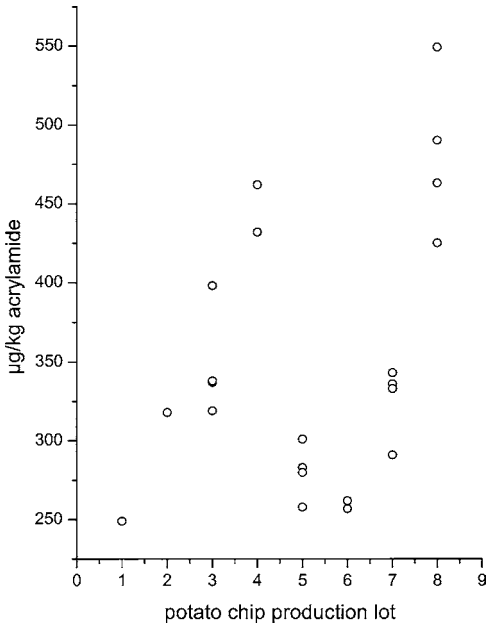

Figure 4. Lot-to-lot and bag-to-bag variability of acrylamide levels in potato chips of one manufacturer. Potatoes for all lots were grown on the same farm and processed on the same production line by the same operator. Lots 4, 5, and 8 are cultivar A. Lots 3 and 6 are cultivar B. Lot 7 is cultivar C. Lots are plotted in the order of their production dates. The differences in acrylamide levels between lots 4, 5, and 8 correlate with the age of the oil used to cook the potato chips.

The correlation between thermally aged cooking oil and increased acrylamide levels in potato chips suggests that changing the cooking oil more frequently is one way to reduce acrylamide levels in the potato chips. This consideration and alternative actions are currently under study.

In summary, there is considerable variability in acrylamide levels in prepared foods. A number of factors contribute to the variability, and the small number of samples tested could be misleading. The association of acrylamide levels in potato chips with thermal aging of the cooking oil suggests that it may be possible to reduce acrylamide levels in some foods through processing changes.

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