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Rapid Sample Preparation Method for LC–MS/MS or GC–MS Analysis of Acrylamide in Various Food Matrices

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A fast and easy sample preparation procedure for analysis of acrylamide in various food matrices was developed and optimized. In its first step, deuterated acrylamide internal standard is added to 1 g of homogenized sample together with 5 mL of hexane, 10 mL of water, 10 mL of acetonitrile, 4 g of $MgSO_4$, and 0.5 g of NaCl. Water facilitates the extraction of acrylamide; hexane serves for sample defatting; and the salt combination induces separation of water and acetonitrile layers and forces the majority of acrylamide into the acetonitrile layer. After vigorous shaking of the extraction mixture for 1 min and centrifugation, the upper hexane layer is discarded and a 1 mL aliquot of the acetonitrile extract is cleaned up by dispersive solid-phase extraction using 50 mg of primary secondary amine sorbent and 150 mg of anhydrous $MgSO_4$. The final extract is analyzed either by liquid chromatography−tandem mass spectrometry or by gas chromatography−mass spectrometry (in positive chemical ionization mode) using the direct sample introduction technique for rugged large-volume injection.

KEYWORDS: Acrylamide; sample preparation; liquid chromatography−mass spectrometry; gas chromatography−mass spectrometry; direct sample introduction

INTRODUCTION

Acrylamide is a neurotoxic compound classified as a probable human carcinogen and genotoxicant (*1*−*3*). Historically, acrylamide as a contaminant was only thought to be an issue in water and its potential exposure to humans was not of serious concern. However, the relatively recent discovery of acrylamide formation in certain fried and baked foods and its rather high levels (concentrations ranging up to 10 mg/kg) found in highly consumed food products, such as potato chips and fries (*4*), brought acrylamide to the forefront of interest among scientists, regulators, the industry, and consumer groups. For analytical chemists, this issue brought a new challenge in terms of the complexity and variability of food matrices to be analyzed and a demand for high sample throughput.

A traditional method used for water analysis employs conversion of acrylamide to a brominated product, which is extracted from water into a water-immiscible organic solvent and determined by gas chromatography (GC) with electron-capture or mass spectrometric (MS) detection (*5*, *6*). This method can be applied to foods (*7*), but the conversion process is rather time-consuming and often incomplete. A direct GC−MS analysis of acrylamide can be accompanied by several difficulties, which include a rather nonselective MS spectrum (due to the low molecular weight) and potential formation of acrylamide in the injection port from coextracted precursors (asparagine and reducing sugars) if present in the final extract (*8*). Also, the high solubility of acrylamide in water in comparison with organic solvents complicates sample preparation for GC.

One of the pitfalls in acrylamide analysis arises from the nature of the acrylamide formation in heated foods in the Maillard reaction (*9*, *10*). If the precursors (asparagine and reducing sugars) are present in the extract, procedures involving the use of elevated temperatures, such as hot GC injection or Soxhlet extraction (*11*−*13*), should be avoided. Another pitfall inherent to the analytical application is the lack of acrylamide-free (blank) food matrices that would have a matrix composition similar to that of the acrylamide-containing samples. The comparison of analytical results between samples and blanks is an important aspect to demonstrate method performance and qualitative identifications. Unfortunately, any attempt to prepare blanks would yield acrylamide in the sample or an incomplete representation of the sample because the Maillard reaction (a nonenzymatic browning process) results in a myriad of potential reaction products, including acrylamide.

Recent reviews (*8*, *14*−*16*) show that most laboratories employ liquid chromatography (LC)−MS for analysis of underivatized acrylamide in food samples. As in GC−MS, the molecular ion (*m/z* 72) produced in LC−MS does not provide a selective detection, thus tandem MS (typically with triple quadrupole instruments) is mostly performed after extract cleanup. The cleanup steps often employ solid-phase extraction (SPE), such as in a method developed by Roach et al. (*17*),

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which was used by the U.S. Food and Drug Administration (FDA) to conduct a survey of acrylamide levels in foods in the United States (*18*).

Our objective was to develop a simple and rapid sample preparation procedure for the analysis of acrylamide in various food matrices that would be directly compatible with both LC−MS/MS and GC−MS without any solvent exchange (evaporation) and/or derivatization prior to the determinative step. In the method development, we used elements from the QuEChERS (quick, easy, cheap, effective, rugged, and safe) method, such as dispersive-SPE cleanup, introduced by Anastassiades et al. for the analysis of pesticide residues in produce samples (*19*).

MATERIALS AND METHODS

Chemicals and Materials. Acrylamide (99%) and 2,3,3-d_3-acrylamide (98%) standards were obtained from Sigma (St. Louis, MO) and Cambridge Isotope Laboratories, Inc. (Andover, MA), respectively. Stock and working standards of acrylamide and d_3-acrylamide were prepared in acetonitrile (MeCN) or in deionized water with 0.1% formic acid. D-Glucose was purchased from Sigma, and L-asparagine was from Eastman-Kodak (Rochester, NY). MeCN, methanol (MeOH), and hexane were a high purity grade for residue analysis from Burdick & Jackson (Muckegon, MI). Deionized water was prepared by a Barnstead (Dubuque, IA) water purification system. Formic acid (98%) and anhydrous $MgSO_4$ were purchased from Fisher (Fair Lawn, NJ), and ACS-grade NaCl was from Mallinckrodt (Paris, KY). The $MgSO_4$ was baked for 5 h at 500 °C in a muffle furnace to remove phthalates and residual water. Ultrahigh purity He was employed in GC−MS as the carrier gas, and liquid-headspace supplied N_2 served as nebulizer, curtain, auxiliary, and collision gas in LC−MS/MS; both gases were obtained from Air Products (Allentown, PA).

Sorbents tested for dispersive-SPE included primary secondary amine (PSA) obtained from Varian (Harbor City, CA), C_{18} from J.T. Baker (Phillipsburg, NJ), graphitized carbon black (GCB) and polyamide resin Discovery DPA-6S from Supelco (Bellefonte, PA), Accucat from Varian (Harbor City, CA), and Oasis HLB from Waters (Milford, MA). PSA, C_{18}, DPA-6S, and GCB were purchased as bulk sorbents, whereas Accucat and Oasis HLB were packed in cartridges (200 mg/ 3 mL and 200 mg/ 6 mL, respectively). To remove interference, PSA sorbent was prewashed by water followed by acetonitrile and dried in the hood.

Food samples (potato chips, sweet potato chips, various crackers and snacks, peanut butter, chocolate, and chocolate flavored syrup) were purchased from local supermarkets. Check samples (water, peanut butter, chocolate, coffee, and two cereal samples) were prepared by the National Food Processors Association (NFPA), Washington, DC, for the participants of the "2004 Acrylamide in Food" workshop organized by the Joint Institute of Food Safety and Applied Nutrition (JIFSAN), College Park, MD.

Optimized Sample Preparation Procedure. The optimized sample preparation procedure entailed the following steps: (1) weigh 1 g of thoroughly homogenized sample into a 50 mL fluorinated ethylene propylene (FEP) centrifuge tube (Nalgene, Rochester, NY); (2) add d_3-acrylamide at 500 ng/g (100 μL of 5 μg/mL standard of d_3-acrylamide in deionized water with 0.1% formic acid); (3) add 5 mL of hexane using a solvent dispenser (in the case of high fatty matrices, such as peanut butter, vortex the tube thoroughly to solubilize/disperse the sample in hexane); (4) add 10 mL of deionized water and 10 mL of MeCN using solvent dispensers; (5) add 4 g of anhydrous $MgSO_4$ and 0.5 g of NaCl (preweighed as a salt mixture in a 20 mL vial); (6) immediately, seal the tube and shake vigorously for 1 min by hand to prevent formation of crystalline agglomerates and to ensure sufficient solvent interaction with the entire sample; (7) centrifuge the tube for 5 min at 3450 rcf (5000 rpm using a RT6000B centrifuge from Sorvall; Newtown, CT); (8) discard the hexane layer (see **Figure 1** for the solvent layer arrangement in a FEP tube after the centrifugation); (9) transfer 1 mL of the MeCN extract to a 2 mL minicentrifuge tube containing 50 mg of PSA and 150 mg of anhydrous $MgSO_4$; (10) mix (vortex) the extract with the sorbent/desiccant for 30 s; (11) centrifuge the tube at 3450 rcf for 1 min; and (12) place the supernatant into an autosampler vial for LC−MS/MS or GC−MS analysis.

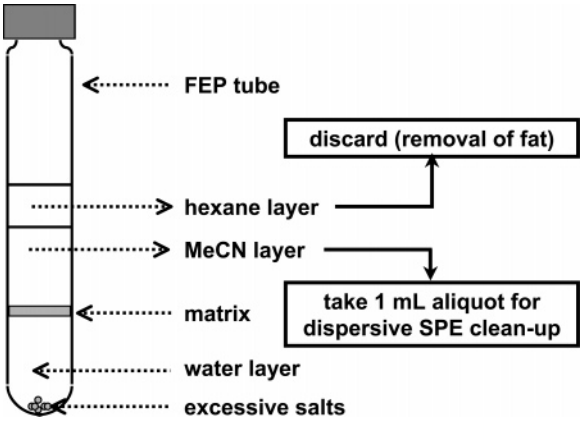

Figure 1. Schematic picture of the solvent layer arrangement in a FEP tube after the centrifugation of a food extract.

Check Sample Analysis. The check samples were prepared for the LC−MS/MS analysis using the optimized sample preparation procedure described above and, for comparison purposes, also by a method developed by Roach et al. at the U.S. FDA (*17*). The latter procedure consisted of the following steps: (1) weigh 1 g of thoroughly homogenized sample into a 50 mL FEP centrifuge tube; (2) add d_3-acrylamide at 500 ng/g; (3) add 5 mL of hexane to peanut butter and chocolate samples, and vortex the tube to dissolve/disperse the matrix; (4) add 10 mL of deionized water to all samples; (5) shake for 20 min in a shaker; (6) centrifuge the tube at 3450 rcf for 5 min (or longer if necessary); (7) discard the hexane layer; (8) filter a 5 mL aliquot of the water extract using 0.45 μm PVDF syringe filters (Millipore; Bedford, MA); (9) condition Oasis HLB SPE cartridges with 3.5 mL of MeOH followed by 3.5 mL of deionized water; (10) load the SPE cartridge with 1.5 mL of the filtered extract; (11) allow the extract to pass through the sorbent column followed by a 0.5 mL water wash (do not use vacuum in any of the SPE steps); (12) elute the cartridge with 1.5 mL of water, and collect the eluent for the second SPE cleanup using the Accucat cartridge; (13) mark the outside of the Accucat cartridge at the height of 1 mL of liquid above the sorbent bed; (14) condition the Accucat cartridge with 2.5 mL of MeOH followed by 2.5 mL of deionized water; (15) load the eluent collected from the Oasis HLB cartridge; (16) elute to the 1 mL mark, and then collect the remainder of the eluted portion; (17) transfer the eluate into an autosampler vial for LC−MS/MS analysis.

LC−MS/MS analysis. LC−MS/MS analysis was performed using an Agilent 1100 LC system with a binary pump, autosampler, column heater (kept at 25 °C), and degasser (Agilent Technologies; Palo Alto, CA) interfaced to an API 3000 triple quadrupole mass spectrometer (Applied Biosystems; Toronto, ON; Canada). Sample injection volume was 10 μL. A Phenomenex Aqua C18 column (150 × 3 mm; 5 μm particle size, 125 Å pore size) coupled to a C18 4 × 3 mm guard column (both from Phenomenex; Torrance, CA) was employed for the LC separation. During the method development, other LC columns were also tested including a Synergi Hydro-RP (250 × 2 mm; 4 μm particle size, 80 Å pore size) column from Phenomenex, a Prodigy ODS3 (150 × 3 mm; 5 μm particle size, 100 Å pore size) column from Phenomenex, and an Allure PFP Propyl (150 × 3.2 mm; 5 μm particle size, 60 Å pore size) column from Restek (Bellefonte, PA). A Valco (Houston, TX) divert valve was placed between the column outlet and MS source to eliminate the introduction of coextracted matrix components into the MS instrument prior and after acrylamide elution. The mobile phase was 99.5:0.5 water−MeOH (at 200 μL/min for 8 min) for elution of acrylamide (retention time 5.5 min) and 0.1% formic acid in MeCN−MeOH (50:50, v/v) for the postanalysis wash (at 500 μL/min for 7 min) followed by equilibration to initial conditions. The MS determination of acrylamide and d_3-acrylamide was performed in electrospray (ESI) positive mode (using the optimized MS instrument parameters obtained by the tuning) combined with monitoring of the most abundant MS/MS (precursor → product) ion transitions (dwell

time of 100 ms for each transition). For acrylamide, *m/z* 72 → 55, 44, and 27 were monitored, whereas *m/z* 75 → 58, 44, and 30 were used for d_3-acrylamide. Relative responses (peak areas) of acrylamide vs d_3-acrylamide (transitions *m/z* 72 → 55 and *m/z* 75 → 58, respectively) were used for the calibration and quantification purposes.

GC−MS Analysis. The GC−MS analysis of acrylamide was performed using a Saturn 2000 (Varian; Walnut Creek, CA) ion trap MS instrument equipped with a model 3800 gas chromatograph and a model 1079 temperature programmable inlet. Direct sample introduction (DSI) was used as an injection technique. In DSI, a 20 μL aliquot of the sample extract was placed in a microvial (a 1.9 mm i.d., 2.5 mm o.d., 15 mm long microvial from Scientific Instrument Services; Ringoes, NJ), which was then introduced into a 3.4 mm i.d. deactivated liner (Restek) using a manual DSI device (ChromatoProbe from Varian). As an alternative to DSI, large volume injection (LVI) into the same liner packed with a 7 mm piece of Carbofrit (Restek) was also evaluated. The GC−MS conditions were as follows: a Stabilwax-DB (Restek) capillary column of 20 m, 0.32 mm i.d., 1 μm film thickness (maximum temperature 220 C) connected to a 2 m, 0.15 mm i.d. deactivated retention gap; MS transfer line temperature 170 °C; MS ion trap temperature 200 °C; MS manifold temperature 50 °C; MeOH as a liquid chemical ionization (CI) reagent; data acquisition in full scan mode (*m/z* 50−85); GC analysis time 11 min (acrylamide retention time 9.3 min); inlet temperature, 100 °C held for 3.1 min, then a 200 °C/min ramp to 150 °C (held for 3 min), then a return to 100 °C (held till the end of the analysis); split vent open for the initial 3 min (split ratio 50:1), then closed for 3.35 min and then open again (split ratio 50:1 for 2.5 min, then 15:1 for the rest of the analysis); oven temperature program, 80 °C held for 6.35 min, then a 70 °C/min ramp to 200 °C (held for 2.94 min); GC carrier gas He; pressure program, 15 psi held for 2.9 min, then a 300 psi/min ramp to 30 psi (held till the end of the analysis).

RESULTS AND DISCUSSION

Sample Preparation Method Development and Optimization. Our objective was to develop a sample preparation method for analysis of acrylamide in various food matrices that would be compatible with both LC−MS/MS and GC−MS without any solvent exchange and/or derivatization prior to the determinative step. In this respect, MeCN represents a suitable extraction solvent because it should offer appropriate extraction selectivity and is compatible with both GC and reversed-phase LC analysis. Also, its immiscibility with hexane provides a simple cleanup option for removal of less polar, lipophilic matrix coextractives. Thus, the simplest approach would involve an extraction using MeCN combined with addition of hexane for extract defatting. However, as also found previously for other extraction solvents (*20*), an addition of water was necessary to facilitate acrylamide extraction from food samples (to swell the matrix). For example, only about 30% of acrylamide was extracted from 1 g of potato chips or peanut butter samples when using 10 mL of MeCN and 5 mL of hexane vs using the same solvent combination with the addition of 10 mL of water.

For GC analysis, the water present in the extract should be removed prior to injection. For pesticide residue analysis, Anastassiades et al. (*19*) demonstrated that a combination of anhydrous $MgSO_4$ and NaCl can induce a distinct phase separation between water and MeCN and induce most pesticides to partition into the upper MeCN layer (salting out mechanism). To test this approach and determine the optimum salt combination for acrylamide extraction, we added acrylamide and d_3-acrylamide (both at 50 ng/mL) to FEP centrifuge tubes containing 20 mL of water−MeCN mixture (50:50, v/v). Then, various combinations of $MgSO_4$ (2−5 g) and NaCl (0−4 g) were added to the tubes (the mixture must be shaken immediately after the addition of $MgSO_4$ to prevent formation of larger crystalline agglomerates in the presence of water). After vigorous shaking for 1 min and centrifugation, the volume of the MeCN layer was measured and the contents of acrylamide and d_3-acrylamide in the MeCN layer were determined by LC−MS/MS. The highest overall partition of acrylamide (>70%) was achieved when adding 4 g of $MgSO_4$ and 0.5 g of NaCl (see **Figure 2**). In real sample analysis, salts and other polar food components can slightly influence acrylamide partitioning but, as **Figure 2** demonstrates, the use of an isotopically labeled internal standard (d_3-acrylamide) provides an effective compensation for potential variability in acrylamide partitioning efficiency (≈100% relative acrylamide recovery vs d_3-acrylamide independent of the amount of salts added). Also, d_3-acrylamide compensates for potential biases from volumetric transfers and LC−MS or GC−MS signal variability caused by matrix effects and/or injection volume inaccuracies as discussed below.

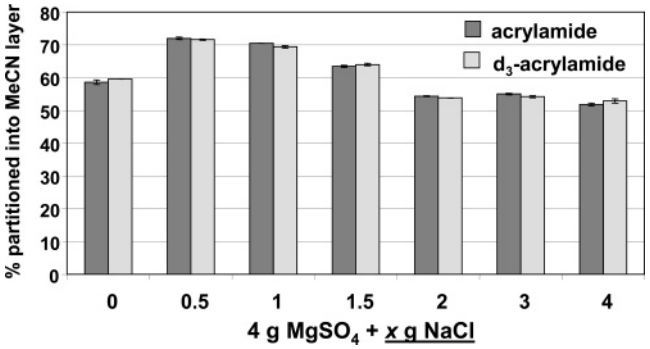

Figure 2. Partition of acrylamide and d_3-acrylamide (in %) into the MeCN layer in the experiments involving addition of 4 g of $MgSO_4$ and 0–4 g of NaCl to 50 ng/mL composite solutions of acrylamide and d_3-acrylamide in water-MeCN (50:50, v/v).

As starting points in the method development, we used a 1:10 sample to water ratio, a 1 g sample size, and a shaking time of 20 min, which were previously demonstrated to provide sufficient acrylamide extraction efficiency and reproducibility (*17*). It should be noted that any extraction procedure that involves only shaking requires thorough sample homogenization and disintegration to small particles using a food processor (or a mortar and a pestle) prior to the extraction (*19*, *20*). In our method, we added 10 mL of MeCN, 4 g of $MgSO_4$, and 0.5 g of NaCl to a 1 g sample and 10 mL of water in order to induce MeCN−water phase separation and force the majority of acrylamide into the MeCN layer. Other sample to solvent ratios and sample sizes were tested during the method optimization, but higher sample to solvent ratios (e.g., a 2 g sample, 10 mL of water, 10 mL of MeCN) resulted in difficult shaking due to the formation of a thick mixture and/or increased LC−MS signal suppression due to a higher level of matrix coextractives. Also, the total volume of the extraction mixture could not exceed ~30 mL in order to ensure an effective shaking in the 50 mL FEP tubes (*21*).

For matrices with a high fat content, such as peanut butter, it was necessary to add 5 mL of hexane to the sample to solubilize the fat and disperse solid particles prior to the addition of water and MeCN. We decided to add hexane to all samples independent of their fat content because acrylamide does not partition into hexane and the addition of hexane serves as a simple cleanup procedure for removal of less polar matrix components as mentioned earlier.

As for MeCN and the mixture of $MgSO_4$ and NaCl, they can be added to the sample at the same time as water. No significant differences between recoveries of acrylamide from potato chips and peanut butter samples were found when

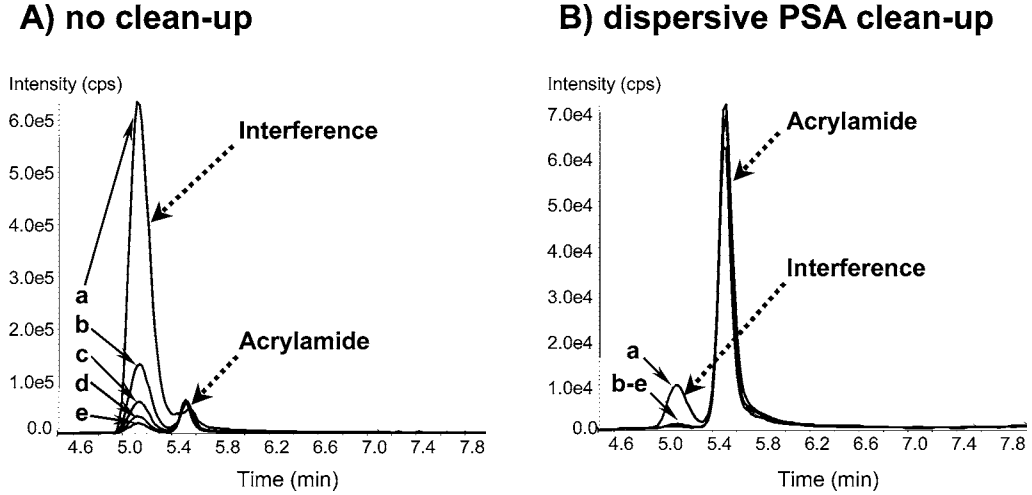

Figure 3. Overlays of LC–MS/MS chromatograms (*m/z* 72 → 55) of potato chip extracts prepared using different amounts of NaCl (*a* = 0 g, *b* = 0.5 g, *c* = 1 g, *d* = 1.5 g, and *e* = 2 g) in the partition step and analyzed (**A**) before and (**B**) after the dispersive-SPE cleanup with PSA sorbent.

comparing a procedure involving the addition of all components at the same time and shaking for 20 min vs a procedure involving the addition of water, shaking for 20 min, then addition of MeCN, and shaking for 20 min, followed by addition of $MgSO_4$ and NaCl and shaking for 20 min. Moreover, we determined that the shaking time can be reduced to 1 min because no significant difference was observed between 1 min vs 20 min shaking or even overnight swelling of the sample (potato chips) in water and 20 min shaking using the MeCN partition procedure. The relatively fast extraction is facilitated by vigorous shaking by hand and also by an increased temperature of the extraction mixture to 40−45 °C due to the exothermic hydration of $MgSO_4$ (*19*). We should note, however, that a longer swelling time may be needed for certain samples, such as those with burned surfaces.

After the extraction step, the mixture is centrifuged which results in formation of three liquid layers as shown in **Figure 1**. The middle MeCN layer contains the majority of acrylamide and d_3-acrylamide, whereas the upper hexane and the lower aqueous layers contain the least and the most polar matrix compounds, respectively. It should be noted that water-based only extraction employed in many other methods gives cloudy extracts that require filtration prior to the cleanup and/or determinative steps. The use of MeCN serves for deproteinization; thus the simple centrifugation step is sufficient to clarify the extract. Also, the elimination of filters minimizes potential in-lab contamination by acrylamide (we found acrylamide in water blanks filtered through nylon and polyethersulfone syringe filters).

Although the partition step was quite effective in removal of fat, salts, and some other unwanted components in LC and GC analysis, an additional cleanup procedure was desirable to remove a closely eluting matrix peak and to lower matrix effects in the LC−MS analysis. **Figure 3A** shows an LC−MS/MS chromatogram of a potato chip extract with a high level of an interfering matrix component in the acrylamide quantitation trace of *m/z* 72 → 55 when no NaCl was added during the extraction/partition step. The addition of NaCl decreases the polarity of the MeCN layer due to a more complete phase separation and, thus, controls extraction selectivity by reducing the content of more polar matrix coextractives in the extract as the amount of NaCl increases. The interference is presumably the amino acid valine, which was found in untreated aqueous extracts of potato chips and other food samples typically analyzed for acrylamide (*22*) and which is known to generate *m/z* 72 immonium ion.

Table 1. Comparison of the Check Sample Analysis Results Obtained by the Participating Laboratories (*n* = 18) with Results Obtained in Our Laboratory by the Comparison Method (*17*) and by the Presented Method, for Which the *Z* Scores Are Shown

check sample	mean (*n* = 18)	comparison method	our method	*Z* score
AA-1 cereal	22.0	18.0	21.5	–0.03
AA-2 peanut butter	103.3	91.5	101.5	–0.02
AA-3 chocolate[a]	120.5	154.0	216.5	+1.93
AA-4 coffee	157.8	136.5	144.5	–0.32
AA-5 cereal	29.5	19.5	22.0	–0.24
AA-6 water[b]	20.5		19.0	–0.42

[a] Results obtained by the participating laboratories for chocolate were rather variable (RSD = 49.8%). [b] Water sample was analyzed directly by LC–MS/MS.

The use of 0.5 g of NaCl lowered the interference level significantly but did not lead to its complete removal.

For a fast and simple cleanup, we employed a dispersive-SPE procedure that involves mixing of sorbent(s) with the extract in a tube to retain matrix interferants, but not the analytes (*19*). Several sorbents (and their amounts) were tested including PSA, C18, GCB, DPA-6S, Accucat, and Oasis HLB (see the Materials and Methods section for details). The PSA sorbent, which retains compounds containing carboxylic groups, was the most effective in decreasing the matrix suppression effect and mainly removing the interfering matrix component in the LC−MS/MS analysis (see **Figure 3B**). In the optimized dispersive-SPE procedure, a 1 mL aliquot of the MeCN layer is mixed with 50 mg of PSA sorbent and 150 mg of $MgSO_4$ in a minicentrifuge tube for 30 s. The addition of $MgSO_4$ removes residual water from the extract, which is important for GC−MS analysis, and it also serves for cleanup purposes (removal of compounds not soluble in dry MeCN). After centrifugation, the supernatant is placed in an autosampler vial for LC−MS/MS or GC−MS analysis.

LC−MS/MS Analysis. For LC separation, a Phenomenex Aqua C18 column was selected because its polar endcapping enabled operation with 100% aqueous conditions and provided acceptable retention and peak shape of acrylamide. In the final method, water with 0.5% MeOH addition was used as a mobile phase for acrylamide elution. Addition of 0.1% formic or acetic acid decreased the acrylamide signal by more than 50%, but we found that an acid addition was useful for rugged performance of the method. Thus, we added 0.1% formic acid to the

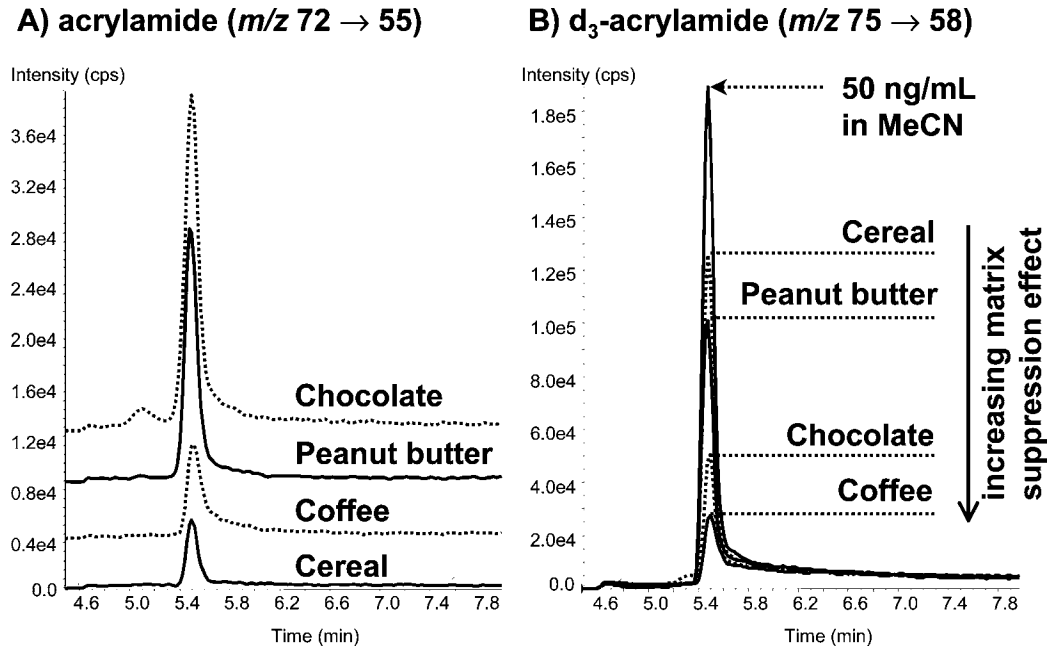

Figure 4. Overlays of LC–MS/MS chromatograms of (**A**) acrylamide (*m/z* 72 → 55) and (**B**) d_3-acrylamide (*m/z* 75 → 58) obtained in chocolate, peanut butter, coffee, and cereal check sample analyses. The internal standard d_3-acrylamide was added to all samples at 500 ng/g, which corresponds to 50 ng/mL in MeCN assuming 100% recovery (100% partition into the MeCN layer). The partition of d_3-acrylamide was ~75% for all tested check samples. The arrow indicates increasing matrix suppression effect (from cereal to coffee).

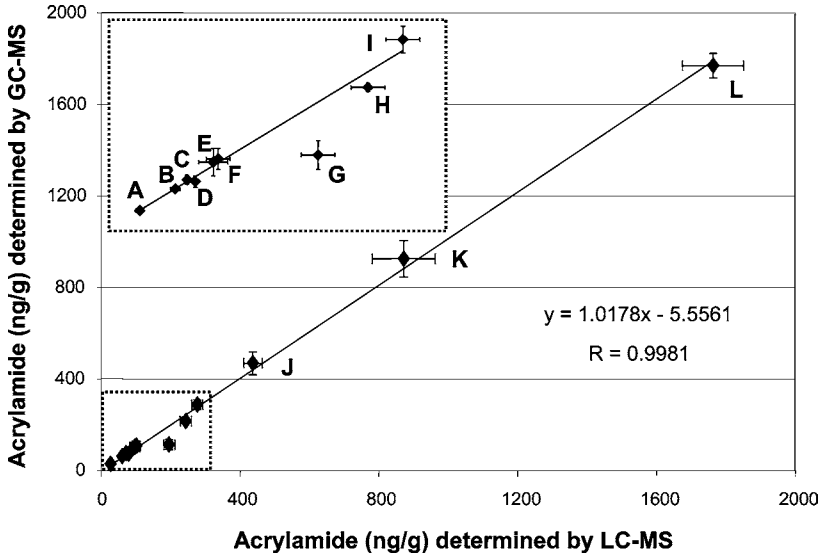

Figure 5. Correlation between acrylamide contents (in ng/g) determined by LC–MS and GC–MS techniques in potato chips (samples J, K, and L), sweet potato chips (I), corn-based snacks (C and F), crackers (D and H), peanut butters (B and E), chocolate (G), and chocolate flavored syrup (A).

postelution column wash with MeCN−MeOH (50:50, v/v). Some authors washed the column with a 100% organic mobile phase (up to 12 h) after a sequence of samples (*17*) to restore column performance but we prefered to perform a short column wash in each analytical run in order to maintain good peak shapes and intensities throughout the sequence.

The injection volume of 10 μL provided sufficient sensitivity and precision for the lowest calibration level (LCL) of 10 ng/g. Although it is unusual to inject a stronger solvent into a weaker reversed-phase mobile phase, the peak shapes and retention time consistency were good up to about 25 μL. At 10 ng/g, a 6.6% relative standard deviation of acrylamide to 500 ng/g d_3-acrylamide area ratio was obtained in intraday reproducibility experiments ($n = 4$). The calibration curves obtained by using relative peak areas of acrylamide vs d_3-acrylamide (transitions *m/z* 72 → 55 and *m/z* 75 → 58, respectively) were linear (regression coefficients >0.995) for the tested acrylamide concentration range of 10−2000 ng/g (1−200 ng/mL in MeCN). ESI matrix suppression effect (measured as a relative peak area of 50 ng/mL d_3-acrylamide injected in matrix extract vs MeCN) varied from 30−40% for coffee extracts to almost nonexistent for cereals (<10% difference in responses).

Analysis of Check Samples. Check samples (peanut butter, chocolate, coffee, two cereal samples, and water) were analyzed by LC−MS/MS in order to perform inter- and intralaboratory comparison of our sample preparation procedure with other methods. For the intralaboratory comparison, the check samples were also prepared by a method developed by Roach et al. (*17*) at the U.S. FDA (see Materials and Methods for details). **Table 1** shows the mean results obtained by 18 laboratories participating in the check sample testing round (*23*) and results obtained in our laboratory by the two different sample preparation

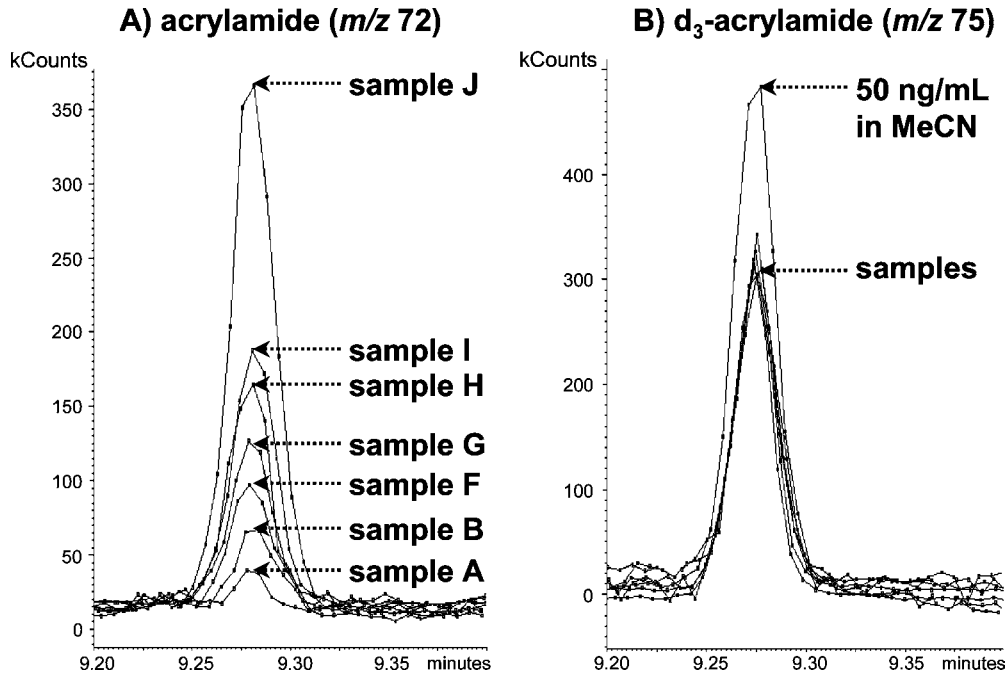

Figure 6. Overlays of DSI-GC–CI-MS chromatograms of (**A**) acrylamide (*m/z* 72) and (**B**) d_3-acrylamide (*m/z* 75) obtained in chocolate flavored syrup (sample A), peanut butter (sample B), corn-based snack (sample F), chocolate (sample G), crackers (sample H), sweet potato chips (sample I), and potato chips (sample J), and in a 50 ng/mL standard of d_3-acrylamide in MeCN. The internal standard d_3-acrylamide was added to all samples at 500 ng/g, which corresponds to 50 ng/mL in MeCN assuming 100% recovery (100% partition into the MeCN layer). The partition of d_3-acrylamide was ~75% for the tested samples.

methods. As the *Z* scores demonstrate, our method gave comparable results to other methods. It should be noted that the results reported for chocolate by the participants were rather variable (RSD = 49.8%) and that LC−MS analysis of chocolate samples should employ a more thorough cleanup (*24*). In the case of chocolate, the *Z* score was +1.93, thus close to the generally acceptable limit of +2.00. The result obtained for the chocolate sample by our method was higher vs the comparison method, which otherwise provided very similar results (correlation coefficient of 0.999) for the other 4 samples. More than a year later, we analyzed the same chocolate sample (stored in freezer) side-by-side by LC−MS/MS and GC−MS and determined mean acrylamide content of 196 ng/g vs 113 ng/g, respectively. This suggests the presence of an interfering coelutant in the LC−MS/MS chromatogram of acrylamide, which was not removed from the chocolate extract by the cleanup procedure. As discussed below, the GC−MS analysis offered a more selective acrylamide separation than LC−MS/MS and, thus, could tolerate a more complex chocolate extract.

Figure 4A shows LC−MS/MS chromatograms of acrylamide analysis in the check samples. **Figure 4B** compares responses of d_3-acrylamide obtained in the four different matrices. The partition of d_3-acrylamide was ~75% in all tested matrices (determined vs responses of d_3-acrylamide added at 50 ng/mL to d_3-acrylamide-free matrix extracts). The arrow in the figure indicates the increasing extent of the matrix suppression effect for coffee and chocolate as compared to cereal and peanut butter samples.

GC−MS Analysis Using Chemical Ionization and DSI for Large Volume Injection. As for the GC−MS analysis, our goal was to develop a method that would provide optimum selectivity and sensitivity for direct acrylamide determination in our extracts without a need for derivatization. For acrylamide detection, we used an ion trap GC−CI-MS with MeOH as a liquid CI reagent (note: Biedermann et al. (*25*) previously reported direct acrylamide analysis using GC−CI-MS with methane as a reagent gas). The GC separation was performed using a Stabilwax-DB capillary column, which contains a bonded, base-deactivated Carbowax polyethylene glycol stationary phase suitable for the GC analysis of underivatized amines and other basic, N-containing compounds. GC provides greater separation efficiency than LC; thus the MS/MS option in GC was not necessary to improve analytical selectivity. Also, low-volatile matrix components, such as valine, do not volatilize and interfere in the GC chromatogram. In the absence of matrix coelutants, the MS/MS approach lowers detectability; therefore all our GC−MS analyses were performed using single-stage MS detection.

The relatively short, wide-bore Stabilwax column was operated in a low-pressure (LP) GC setup with a short, narrow restriction capillary connected to the front of the analytical column to prevent extension of subambient pressure conditions to the injector (*26*−*28*). The restriction capillary also served as a retention gap protecting the analytical column and increasing sample loadability, which was also provided by a thicker film of the stationary phase (1 μm).

To improve sensitivity, we employed a large volume injection (LVI) technique called direct sample introduction (DSI). In DSI, up to ~30 μL of the extract is placed in a disposable microvial, which is then introduced into the GC inlet liner using a probe (*29*). This step can be automated by using an autosampler to inject a sample volume into the microvial placed in a liner (*30*), which is then inserted into the inlet (or a thermodesorption unit attached to the inlet). As in any LVI, the solvent should be evaporated and vented at conditions that provide fast and effective solvent removal without loss of analytes (it is preferable to leave about 1−2 μL of the solvent in the microvial serving as a keeper and/or aiding analyte focusing in the column). In our case, we introduced and evaporated 20 μL of the MeCN extracts at 100 °C and 15 psi, with the split vent open for 3 min. After the solvent venting step, the split vent was closed, the inlet was rapidly heated to 150 °C, and the

column head pressure was quickly ramped to 30 psi to provide a fast transfer of acrylamide to the column.

As opposed to other LVI techniques, the less volatile matrix components remained in the disposable microvial, which is removed from the inlet after each GC run. Thus, the DSI approach prevents contamination of the GC system by nonvolatile matrix coextractives, which normally leads to a shorter life of the analytical column, increased demand for sample cleanup, frequent need for system maintenance, and decreased ruggedness in GC (*28*). In addition to this advantage, the use of a microvial eliminates the need for trapping of the solvent in an LVI liner at relatively low temperatures and, thus, avoids excessive inlet and column cooling, which results in shorter cycle times. In comparison to an alternate LVI approach, a temperature of 50 °C was necessary to trap 10 μL of MeCN in the same liner packed with Carbofrit. The DSI injection volume of 20 μL provided a reliable LCL of 25 ng/g, which was sufficient for the various food samples that we analyzed. The calibration curves obtained by using relative peak areas of acrylamide vs d_3-acrylamide (*m/z* 72 and *m/z* 75, respectively) were linear (regression coefficients >0.995) for the tested acrylamide concentration range of 25−2000 ng/g (2.5−200 ng/mL in MeCN).

As mentioned in the Introduction, there is a concern related to the potential formation of acrylamide from its precursors in the injection port when direct GC acrylamide analysis without derivatization is performed. Thus, a key to avoid acrylamide formation in the inlet is to remove acrylamide precursors (asparagine and/or reducing sugars) from the extract prior to GC analysis. As mentioned earlier, the partition step in our method is very effective in terms of removal of polar coextractives. Also, the PSA sorbent should retain residual asparagine if it is present in the MeCN layer after the partition step. To test potential acrylamide formation in the GC system, we added 10 mL of MeCN, 4 g of $MgSO_4$, and 0.5 g of NaCl to 10 mL of 10 mM composite solution of asparagine and glucose in water (containing 500 ng/mL d_3-acrylamide). After 1 min of shaking and centrifugation, 20 μL of the MeCN layer was analyzed by DSI-GC−CI-MS before and after the PSA dispersive-SPE cleanup. No acrylamide peak was detected in either case.

To further confirm the validity of the GC−MS results, we performed side-by-side GC−MS and LC−MS/MS analyses of 12 different food sample extracts (prepared in replicates). **Figure 5** compares the results, showing a good correlation between the analyses (both the slope and correlation coefficient very close to 1) with the exception for the chocolate sample as discussed earlier. **Figure 6** shows DSI-GC−CI-MS chromatograms of acrylamide and d_3-acrylamide analysis in selected extracts. **Figure 6B** compares responses of d_3-acrylamide obtained in the matrix samples vs 50 ng/mL d_3-acrylamide in MeCN, which corresponds to 100% partition efficiency. The partition of d_3-acrylamide was ∼75% in all tested matrices (determined vs responses of d_3-acrylamide added at 50 ng/mL to d_3-acrylamide-free matrix extracts). As opposed to the LC−MS analysis, no significant matrix effects were observed in the DSI-GC−CI-MS approach (88−113% relative peak area of 50 ng/mL d_3-acrylamide injected in matrix extract vs MeCN).

In conclusion, the presented sample preparation procedure offers several advantages as compared to other published methods, including higher sample throughput and lower costs. It avoids time- and labor-intensive steps such as evaporation/solvent exchange, filtration, quantitative transfers, and/or multiple SPE cleanups using traditional cartridges. Also, potential contamination by acrylamide from labware is minimized due to the elimination of filters and the use of the FEP tube as the only reusable item. Moreover, the method is directly compatible with both LC−MS and GC−MS techniques, which provides orthogonal means of analysis for confirmation purposes. This is especially important in the acrylamide analysis, in which no true blank is available. The LC−MS/MS approach offers more sensitive determination, whereas the DSI-GC−CI-MS method provides more selective acrylamide analysis, which is a crucial factor in the case of highly complex food samples, such as chocolate or cocoa.

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