JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

# Analysis of Coffee for the Presence of Acrylamide by LC-MS/MS

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A variety of popular instant, ground, and brewed coffees were analyzed using a modified liquid chromatography−tandem mass spectrometry (LC-MS/MS) method specifically developed for the determination of acrylamide in foods. Coffee test portions were spiked with $^{13}C_3$-labeled acrylamide as an internal standard prior to their extraction and cleanup. Ground coffees (1 g) and instant coffees (0.5 g) were extracted by shaking with 9 mL of water for 20 min. Brewed coffee test portions (9 mL) were taken through the cleanup procedure without further dilution with extraction solvent. Coffee test portions were cleaned up by passing 1.5 mL first through an Oasis HLB (hydrophilic/lipophilic copolymer sorbent) solid phase extraction (SPE) cartridge and then a Bond Elut-Accucat (cation and anion exchange sorbent) SPE cartridge. The cleaned up extracts were analyzed by positive ion electrospray LC-MS/MS. The MS/MS data was used to detect, confirm, and quantitate acrylamide. The limit of quantitation of the method was 10 ng/g for ground and instant coffees and 1.0 ng/mL for brewed coffee. The levels of acrylamide ranged from 45 to 374 ng/g in unbrewed coffee grounds, from 172 to 539 ng/g in instant coffee crystals, and from 6 to 16 ng/mL in brewed coffee.

**KEYWORDS: Acrylamide; LC-MS/MS; coffee; FDA**

## INTRODUCTION

Acrylamide (2-propenamide) exists as both a monomer and a polymer; however, only the monomeric form has been shown to have neurotoxic properties in both animals and humans (*1*). It can be found in a variety of products such as dyes, gels for electrophoresis, contact lenses, food wraps, tobacco smoke, and grouts used in the construction industry. In 1994, the International Agency for Research on Cancer (IARC) labeled acrylamide a probable human carcinogen (*2*). Following the detection of acrylamide in blood drawn from Swedish workers, researchers in Sweden conducted extensive studies to determine the source of the exposure. They reported finding acrylamide in certain baked and fried foods such as potato crisps and French fries (*3*). The general findings are that arylamide will likely be found in foods that have been heated by means other than boiling. One possible pathway to the formation of acrylamide in some foods is likely via the Maillard reaction, which involves the reaction of an amino acid with a carbonyl compound in the presence of heat (*4*, *5*). It has been shown that the likely reactants which produce significant levels of acrylamide in foods are asparagine and glucose (*6*). Another possible route, not via a Maillard reaction, involves the reaction of asparagine with 2-deoxyglucose with the loss of carbon dioxide at temperatures >100 °C (*7*). In addition, a report by Yasuhara suggests that in lipid-rich foods the reaction of acrylic acid from acrolein and ammonia from the amino acid upon heating may also play an important role in acrylamide formation (*8*).

Although considerable controversy exists regarding the exposure levels relevant to carcinogenicity of acrylamide in humans, the reports on the presence of acrylamide in European food prompted the U.S. Food and Drug Administration (FDA) to analyze a variety of foods sold in the United States for the presence of acrylamide. Gas chromatography−mass spectrometry (GC-MS) methods have been used for the detection of acrylamide (*9*, *10*). The more sensitive of these methods require conversion of acrylamide to dibromopropionamide or 2-bromopropenamide derivatives prior to GC-MS analysis. This process is often incomplete and time-consuming. The currently favored analytical methods of choice for the analysis of underivatized acrylamide use high-performance liquid chromatography with detection by mass spectrometry (LC-MS). A number of papers have appeared using LC-MS or LC-MS/MS for the determination of acrylamide in specific food groups or a limited variety of foods (*11*−*14*). We used this platform to develop a single, simple LC-MS/MS method that works well for the detection and quantification of acrylamide in a wide variety of foods (*15*). The method entails room temperature aqueous extraction of acrylamide from the homogenized food, solid phase extraction (SPE) cleanup, and confirmation and quantitation by LC-MS/MS. It was successfully used to analyze >450 samples in duplicate from 35 different food groups.

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10.1021/jf0349634 
Published on Web 03/10/2004

During the course of our initial survey one food commodity, coffee, consistently proved to be difficult to analyze for the presence of acrylamide. Due to the use of roasted coffee beans in making coffee, the probability of significant levels of acrylamide being present was considered to be high. In addition, the high consumption of coffee by people living in the United States and in many other countries may make coffee a significant source of daily exposure to acrylamide. Recently, several studies have been published that include data on the levels of acrylamide in coffee (*16*, *17*). However, these studies present data for only a small variety of coffee and do not report any of the analytical difficulties associated with the analysis. We discuss some of the analytical problems associated with measuring acrylamide in coffee as well as report data from a larger variety of ground, instant, and brewed coffees.

## EXPERIMENTAL PROCEDURES

**Reagents.** *Solvents.* HPLC grade acetonitrile, methanol, 2-propanol, and water were obtained from EM Science, Gibbstown, NJ.

*Acids.* Formic acid and 99% glacial acetic acid were obtained from Cambridge Isotope Laboratories, Inc., Andover, MA.

*Chemicals.* Acrylamide 99% was obtained from Sigma-Aldrich, St. Louis, MO and $^{13}C_3$-labeled acrylamide 99% from Cambridge Isotope Laboratories.

**Apparatus.** *LC-MS System.* An Agilent 1100 HPLC (Agilent Technologies, Palo Alto, CA) equipped with a vacuum degasser, binary pump, autosampler, and temperature-controlled column oven was coupled to a Micromass Quattro Micro triple-quadrupole mass spectrometer (Waters/Micromass, Milford, MA) equipped with an electrospray source and Masslynx version 4.0 software. Agilent 1050 binary pump (Agilent Technologies) was used.

*Centrifuge.* The Allegra 21 Beckman Coulter centrifuge (Beckman Coulter, Palo Alto, CA) was equipped with a C0650 head.

*Analytical Column.* A Synergi 4$\mu$ Hydro-RP, 80 A, 250 × 2.0 mm, with a C18 2.0 mm guard column (Phenomenex, Torrance, CA), was used.

*SPE Cartridges.* Oasis HLB, 6 mL, 200 mg (Waters, Milford, MA), and Bond Elut-Accucat, 200 mg, 3 mL (Varian, Chicago, IL), cartridges were used.

*Sample Tubes.* Polypropylene 50 mL conical tubes with caps, and glass scintillation vials 20 mL with polypropylene caps (VWR Scientific Products, Buffalo Grove, IL) were used. Maxi-Spin filter tubes, 0.45 $\mu$m PVDF, were obtained from Alltech Associates, Deerfield, IL.

*Vials.* Amber glass autosampler vials 2 mL with septum screw caps were obtained from Agilent Technologies.

*Extraction Equipment.* A titer plate shaker was adapted to hold six 50 mL conical tubes (Labline Instruments Inc., Melrose Park, IL); the Fisherbrand Vortex Genie 2 was obtained from Fisher Scientific, Pittsburgh, PA.

**Standard Curve.** *Standard Solutions.* Labeled and unlabeled acrylamide were separately dissolved in 0.1% formic acid. A stock solution was prepared with 200 ng/mL (ppb) $^{13}C_3$-labeled acrylamide. A solution with 200 ng/mL $^{13}C_3$-labeled acrylamide was combined with native acrylamide at five concentrations between 8 and 3600 ng/mL, for the purpose of obtaining a daily response curve to verify instrument performance and to determine the relative response of labeled and unlabeled acrylamide. The acrylamide stock solutions are stable for long periods when stored in red glass at 7 °C. In December 2003, we prepared fresh $^{13}C_3$-labeled and $^{12}C_3$-unlabeled acrylamide stock solutions and then used these solutions to prepare a set of mixed standards. These portions were analyzed along with similar standards prepared in February 2003. The results showed no measurable degradation in the standards from February to December.

*Average Response Ratio.* To verify method performance, daily response curves were constructed relating the area ratio of unlabeled to labeled acrylamide (*m*/*z* 55/58) versus parts per billion of unlabeled acrylamide. Linearity was assessed from a five-point plot and judged to be acceptable if the linear correlation coefficient was >0.995. The average response ratio was calculated from the data and used to adjust the observed level of acrylamide to compensate for variations in instrument response.

**Sample Preparation.** *Extraction.* A carefully weighed amount of sample equivalent to ~1.0 g of ground coffee, 9 mL of brewed coffee, or 0.5 g of instant coffee was placed into a 50 mL centrifuge tube. Test portions were spiked with 1 mL (200 ng/mL) of $^{13}C_3$-labeled acrylamide internal standard. The test portions were then diluted with 9 mL of HPLC grade water for ground or instant coffee. Brewed coffee is an extract of coffee and, therefore, no additional water or extraction is needed. The centrifuge tubes were capped and shaken or vortexed for 30 s to mix contents. The extraction was completed by clamping the tubes containing the ground or instant coffee test portions in a rotating shaker and mixing the tube contents for an additional 20 min. The test portions were centrifuged at 9000 rpm (8422 rcf) for 15 min. A pipet was used to transfer a 5 mL aliquot of the clarified aqueous coffee to a Maxi-Spin, 0.45 $\mu$m PVDF filtration tube, and this tube was centrifuged at 6000 rpm (3743 rcf) for 5 min.

*Cleanup.* Oasis HLB columns were first conditioned with 3.5 mL of methanol followed by 3.5 mL of water. The solvents used for column conditioning were discarded. The cartridge was loaded with 1.5 mL of the filtered aqueous coffee extract. The extract was allowed to pass completely through the sorbent material and was followed with 0.5 mL of water. The column eluent was discarded. To elute the acrylamide, 1.5 mL of water was added to the sorbent bed and the eluent was collected in a 20 mL glass scintillation vial. Before the second SPE column (Bond Elut-Accucat, 200 mg, 3 mL) was conditioned, a mark was placed on the outside of the tube at a height equivalent to 1 mL of liquid above the sorbent bed. The cartridge was conditioned with 2.5 mL of methanol followed by 2.5 mL of water. The solvents used for conditioning were discarded. All of the eluent collected from the Oasis SPE cartridge was added to the Accucat column. The sample was allowed to pass through the column until it reached the mark previously placed on the outside; eluent from the column was then collected for analysis. The purpose of discarding the first 0.5 mL of sample was to avoid collecting residual water used to wash the column, which could dilute any acrylamide collected. The eluent was transferred to a 2 mL amber glass autosampler vial for LC-MS-MS analysis. The typical LC/MS retention time of acrylamide was 7.0 min at a flow rate of 0.2 mL/min.

*Coffee Brewing.* Coffee was brewed using an electric drip coffee maker. Ground coffee was added to the coffee filter, and cold tap water was added to the water reservoir. The ratio of grams of ground coffee used to cups of brewed coffee made was 4.8. This means 4.8 g of coffee was used for every cup of brewed coffee. After brewing, 9 mL of liquid was used for acrylamide analysis.

**Operating Conditions.** *HPLC System.* Standards and sample portion extracts (20 $\mu$L) were analyzed on an Agilent 1100 HPLC equipped with a vacuum degasser, binary pump, autosampler, and temperature-controlled column oven coupled to a mass spectrometer. The Synergi 4$\mu$ Hydro-RP 80 A 250 × 2.0 mm LC column was maintained at 35 °C. The mobile phase was 0.5% methanol in water. A mobile phase equilibration time of ~1.5 h is needed before analysis of standards or samples can begin. Too short an equilibration time will result in shifting acrylamide retention times. An Agilent 1050 binary pump was used to supplement the 1100 LC effluent with 0.1% acetic acid in 2-propanol at 0.05 mL/min via a tee connector prior to the mass spectrometer. This addition modifies the low mass ion composition so that the labeled and unlabeled acrylamide protonated molecules can be detected without interference from mobile phase ions *m*/*z* 74 and 76.

*MS/MS Conditions.* A Micromass Quattro Micro triple-quadrupole mass spectrometer equipped with an electrospray source was used for detection and quantification. The conditions were as follows: capillary voltage, 4.1 kV; cone voltage, 20 V; source temperature, 120 °C; desolvation gas temperature, 250 °C; desolvation gas flow, 710 L/h nitrogen; cone gas flow, 153 L/h nitrogen; and argon collision gas pressure to $6 \times 10^{-3}$ mbar for MS/MS. The collision energy (CE) was optimized for each multiple reaction monitored (MRM) transition. The LC-MS/MS run time was 10 min per sample.

*MS Calibration.* The mass scale is calibrated from 20 to 82, using ions at *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 in the background from the LC and supplemental flow.

*MS/MS Transitions.* An argon collision gas pressure of $6 \times 10^{-3}$ mbar was used for MS/MS. The collision energy for each monitored transition was optimized in MRM mode. 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. MS conditions were optimized by observing responses for loop injections of 100 pg of acrylamide.

## RESULTS AND DISCUSSION

Of the many types of foods analyzed using the FDA's LC-MS/MS method for determining acrylamide, coffee extracts were the most challenging due to the coextractives in the final extract. Some modification of the general method developed to analyze foods was needed to adjust for problems that occurred in the MS/MS analysis of the coffee extracts. The analysis of 1 g sample portions of ground coffee provided no problems in the detection of acrylamide. However, a 1 g portion of brewed coffee provided too little sample portion for the detection of acrylamide. Therefore, for brewed coffee, an increase in the sample portion to 9 mL (~8.8 g) was required. The dry instant coffee presented the opposite problem: a 1 g sample portion frequently resulted in a contamination on the trailing edge of the *m*/*z* 55 response of unlabeled acrylamide, which at times prevented confirmation and quantitation. This problem was eliminated by reducing the instant coffee sample portion to 0.5 g. Another method modification was required due to the loss of the acrylamide signal after repeated injections of coffee extracts onto the LC column. The loss of signal would at times be significant, and no response would be detected for either the labeled or unlabeled acrylamide. This problem was addressed by increasing the column oven temperature to 35 °C. Fortunately, the stable labeled internal standard serves as a quality control measure and permits the rapid diagnosis of this type of method failure. Finally, our original method used water with 0.5% methanol and 0.1% acetic acid as the mobile phase. We have since determined that eliminating the 0.1% acetic acid resulted in a 3-fold increase in the acrylamide response without affecting the chromatographic resolution of the column. After the analysis of a complete set of acrylamide standards and coffee extracts, it was important to thoroughly wash the column to prolong the life of the HPLC column and maintain good chromatographic resolution. The column was washed with 50:50 methanol/water for 2 h followed by 50:50 methanol/acetonitrile for up to 12 h.

Our goal in developing the analytical method for acrylamide in coffee was that it follow, as much as possible, the method developed for foods, which uses a 1 g sample size. The question of whether 1 g of sample was adequate to allow for a valid measure of the level of acrylamide in coffee was examined using ground coffee. Four 1 g and three 4 g portions of a ground coffee were extracted using proportionally larger amounts of water and internal standard for the 4 g sample. The average amount of acrylamide determined in the 1 g samples was 128 ng/g with a relative standard deviation (RSD) of 4.6%; the average amount found was 115 ng/g in the 4 g sample with a RSD of 0.9%. The determined levels are close enough to conclude that the smaller portion provides adequate sample from which to accurately determine the level of acrylamide present. We used the 1 g sample because we did not consider the RSD difference to be significant enough to alter our initial parameters.

MS analyses of coffee samples are challenging because of coextractives that make it through the cleanup steps and the inherent difficulties in confirming a low molecular mass (MW 71) compound in a complex matrix using mass spectrometry. To provide the selectivity needed to help lower or overcome the background signals, the MS experiment was set to acquire data in the MRM mode. In this mode only six collisionally induced transitions were monitored: three for the labeled acrylamide internal standard (*m*/*z* 75 > 75, 58, 29) and the corresponding three for the unlabeled acrylamide (*m*/*z* 72 > 72, 55, and 27).

Despite two SPE column cleanup steps, a LC separation, and the MS selectivity of MRM, a number of responses were observed both before and after the acrylamide response in the ion profiles monitored for coffee extracts. Chromatograms of the transitions monitored for a typical coffee extract are shown in **Figure 1**. The acrylamide retention time, typically 7 min, was pinpointed by the response for the spiked $^{13}C_3$-labeled internal standard. To ensure that coeluting compounds from the coffee extract did not interfere with the acrylamide signal, the responses for the three unlabeled transitions were compared to the comparable MS transitions for labeled standard. If the relative abundance values agreed to within ±10% of the standards, acrylamide was considered to be detected and no interference was present. This degree of allowed variability is stringent and was based on the FDA criteria for mass spectrometry guidelines for evaluating residues (*18*). Abundance values outside this window would be an indication of an interference or detection of a compound other than acrylamide. Unlike many other food matrices, determination of the amount of acrylamide in coffee extracts after only the first SPE (Oasis) cartridge cleanup was not feasible because of insufficient cleanup, which resulted in an interference on at least one of the transitions monitored for acrylamide.

As a means of eliminating unwanted signals from coextracted compounds, we investigated the effect of run time and its impact upon successive injections. By extending the LC-MS/MS run time to 25 min, a response on the *m*/*z* 55 ion was observed at ~23 min in all of the extracts (**Figure 2B**). This coextractive can affect the *m*/*z* 55 ion used for quantitation of acrylamide with repetitive injections. For example, if the analyses are run at 15 min intervals instead of 10 min, the tailing edge of the *m*/*z* 55 acrylamide response overlaps with the 23 min *m*/*z* 55 response from the previous injection (**Figure 2A**). This interference can preclude quantitation in sample extracts with low levels of acrylamide. By adjusting the injection interval to 10 min, this large interference in the *m*/*z* 55 channel could be eliminated and did not elute at the retention time of acrylamide in subsequent injections.

After elution of sample extract through the Bond Elut Accucat SPE cartridge, some acrylamide will remain on the cartridge. This residual acrylamide can be eluted with an additional 0.5 mL of water. For the coffee samples investigated, the level of acrylamide was high enough that collecting the residual acrylamide was not necessary. However, if the acrylamide levels are low, the additional wash may be needed. The total recovery of acrylamide for the method relative to the internal standard was 92 ± 6%.

The method's precision was also investigated in a study done with ground coffee. Three 1 g samples of the same ground coffee were extracted and analyzed each day for five consecutive days. The average acrylamide level over 5 days ranged from 118 to 134 ng/g. The RSD ranged from 2.4 to 6.0%. The low 5.1%

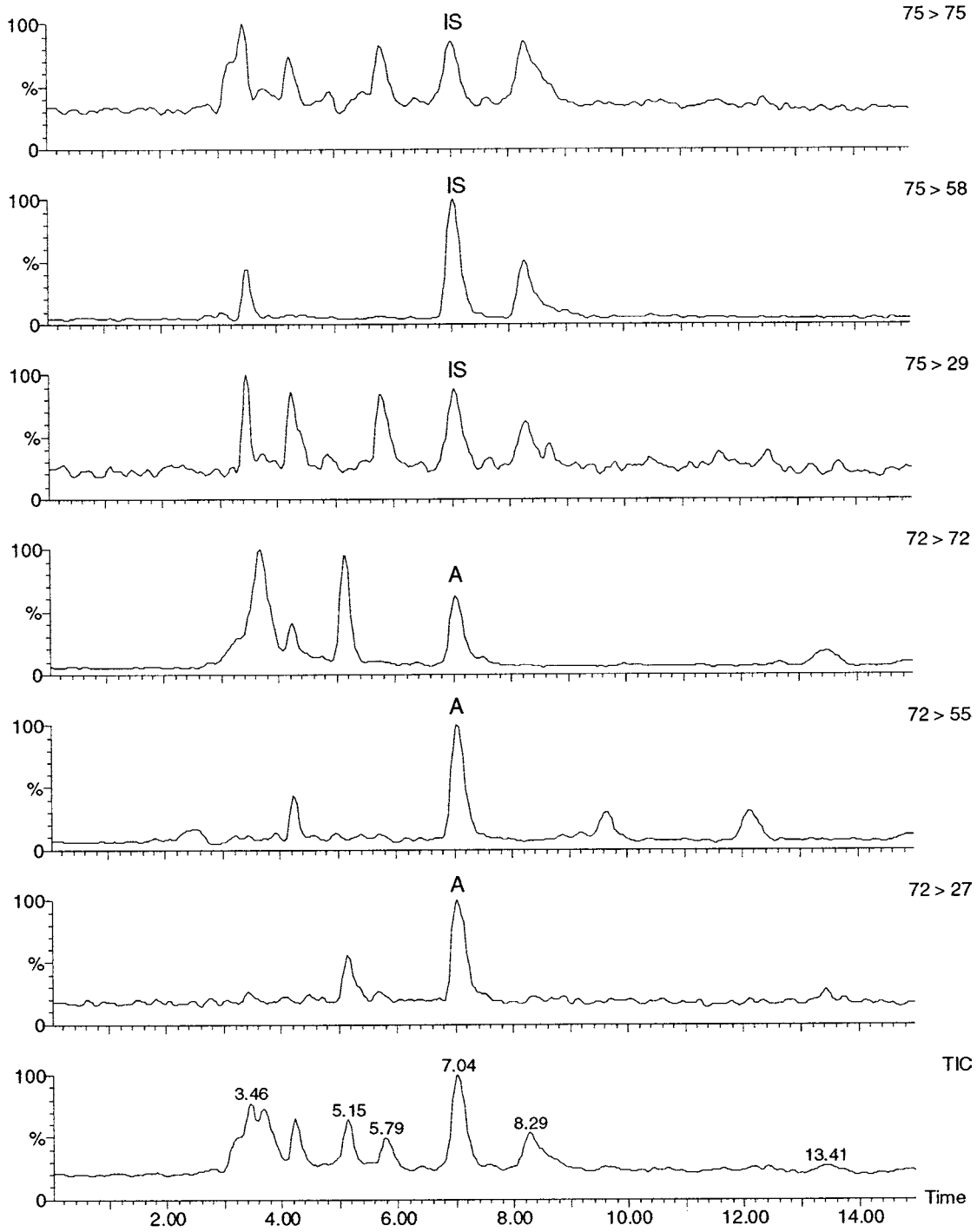


**Figure 1.** Total ion chromatogram (bottom) as well as individual ion chromatograms for a typical analysis of a coffee extract.

average RSD in the amount of acrylamide determined over 5 days demonstrates that the method has good precision.

The amount of acrylamide present in a coffee extract was calculated using the integrated area of the *m*/*z* 58 response recorded for the labeled internal standard and the unlabeled *m*/*z* 55 response. A calibration curve plotting the area ratio of *m*/*z* 55/58 versus acrylamide injected (8−3600 ng/mL) using a constant amount of $^{13}C_3$-labeled acrylamide was made prior to the analysis of sample extracts. A linear plot was always obtained, and correlation coefficients >0.995 were routinely observed. The standard curve data were used to determine an average relative response factor to compensate for possible variations in instrument response. This value was then used to adjust the calculated amount of acrylamide present in the sample.

Four criteria were used for confirmation of acrylamide in the coffee extracts. The signal for the analyte and internal standard must occur at the same retention time. Their signals must be separated from any other coextractives. The relative abundances of the signals recorded for the analyte (*m*/*z* 72, 55, and 27) must be within ±10% of the relative abundances recorded for the internal standard (*m*/*z* 75, 58, and 29). A 10:1 signal/noise ratio, determined by using Masslynx software, is required for the quantitation ion at *m*/*z* 55.

The results from the LC-MS/MS analysis of 30 varieties of locally purchased ground coffee from 9 manufacturers are shown in **Table 1**. The list includes caffeinated and decaffeinated coffees. The levels shown are an average of two analyses. The level of acrylamide detected ranged from 45 to 374 ng/g. The

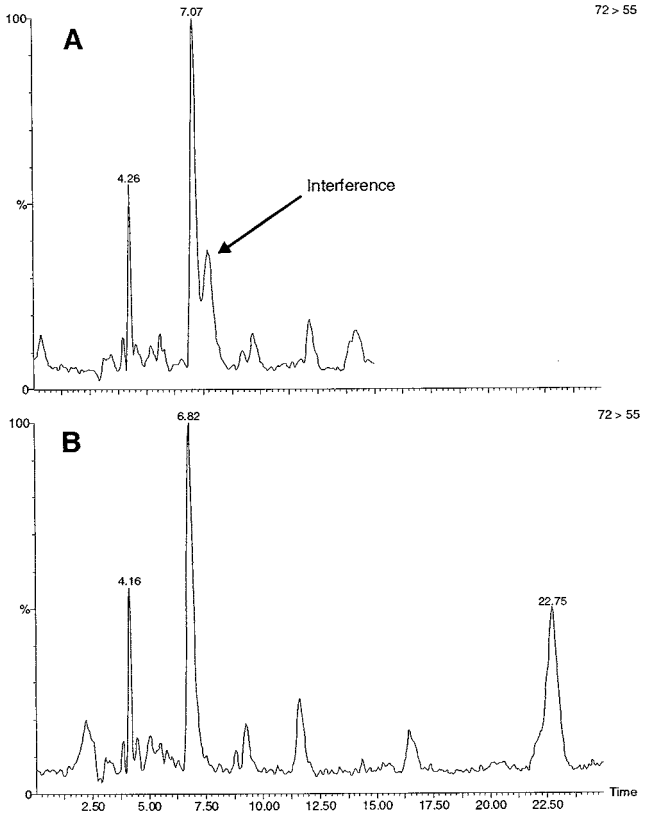


**Figure 2.** Selected ion chromatograms illustrating the effect of injection frequency on an interference in the *m*/*z* 55 channel. Chromatogram **A** shows the interference occurring on the shoulder of the acrylamide signal when injections were done at 15 min intervals. Chromatogram **B** shows the same interference occurring at 22.75 min when injections were done every 25 min.

data represent a very limited survey based on the analyses of only one or two containers for each listed manufacturer. The nanograms per gram levels shown in **Table 1** are the amount of acrylamide detected in only 1 g of ground coffee.

One difficulty in reviewing these numbers was converting the information into an exposure estimate on the actual amount of acrylamide consumed in a cup of coffee. In an effort to clarify this issue, we sought to provide a conversion factor that would allow an accurate estimation of extracted acrylamide in 300 mL (~12 oz) of brewed coffee. This factor was then used to generate the data for total micrograms of acrylamide in 300 mL of brewed coffee shown in **Table 1**. The conversion factor was determined by measuring the acrylamide level in four ground coffees and in the corresponding brewed coffee made from the same ground coffees. A conversion factor for each of the four ground/brewed coffees was calculated by dividing the level of acrylamide in the ground coffee by the amount determined in the corresponding brewed coffee. The four determinations were then combined to give an average conversion factor (26 ± 3). The measured level of acrylamide in each ground coffee listed in **Table 1** was then divided by this factor to get the estimated level (nanograms per gram) of acrylamide in brewed coffee and then multiplied by 295 g to give the amount of acrylamide in 300 mL of coffee (**Table 1**, column 2). Although the brewing process quantitatively transfers all of the acrylamide present in the ground coffee to the brewed coffee, factors such as differential water retention by the grounds and incomplete transfer of water from the brewing reservoir resulted in small deviations in the conversion factor.

**Table 1.** Average Level of Acrylamide in 31 Locally Purchased Ground Coffees and the Corresponding Estimated Level in 300 mL of Brewed Coffee

| manufacturer/brand | ng/g | μg/300 mL of brewed coffee[a] |
|---|---|---|
| Café Bustelo Dark Roast | 139 | 1.56 |
| Chock Full o' Nuts 100% Colombian | 246 | 2.80 |
| Chock Full o' Nuts All Method Grind | 205 | 2.33 |
| Chock Full o' Nuts Rich French Roast | 192 | 2.18 |
| Chock Full o' Nuts The Heavenly Coffee | 187 | 2.12 |
| Folgers Classic decaffeinated | 312 | 3.54 |
| Folgers French Roast | 319 | 3.63 |
| Folgers Classic Roast | 374 | 4.21 |
| Folgers Classic Roast (medium) | 352 | 3.98 |
| Folgers French Roast (mid-dark) | 197 | 2.24 |
| Folgers Classic Roast decaffeinated (medium) | 344 | 3.89 |
| Hills Brothers 100% Colombian | 64 | 0.74 |
| Hills Brothers | 169 | 1.92 |
| Maxwell House | 126 | 1.42 |
| Maxwell House Original | 251 | 2.86 |
| Maxwell House Signature Blend | 258 | 2.92 |
| Maxwell House Original Signature Blend | 171 | 1.95 |
| Maxwell House Original Signature Blend decaffeinated | 223 | 2.54 |
| Maxwell House French Roast | 185 | 2.09 |
| Maxwell House French Roast | 204 | 2.30 |
| Maxwell House Master Blend | 215 | 2.45 |
| Maxwell House Sanka decaffeinated | 298 | 3.39 |
| Medaglia D'oro Caffe Espresso | 165 | 1.86 |
| Melitta/Colombian extra fine grind | 266 | 3.02 |
| Starbucks Breakfast Blend | 161 | 1.83 |
| Starbucks French Roast | 97 | 1.09 |
| Starbucks French Roast | 150 | 1.68 |
| Starbucks House Blend | 151 | 1.71 |
| Starbucks Colombian | 163 | 1.86 |
| Yuban 100% Colombian | 70 | 0.79 |
| Yuban 100% Colombian | 45 | 0.50 |

[a] Estimated using a conversion factor determined from ground and brewed coffee.

The effect of variation in grind was also investigated. Four different brands of coffee were ground to the same uniform fine grind (0.59 mm) and analyzed. The acrylamide level determined for the fine grind was experimentally no different from the level determined from the irregular grind taken directly from the coffee can (data not shown). It was therefore unnecessary to grind all ground coffee samples to the same uniform size before extraction for valid comparison.

Approximately 6 months after quantitating acrylamide in the ground coffees, we selected three to be reanalyzed to determine if there was any detectable change in the level of acrylamide when ground coffee was stored in its original container at room temperature. For two coffees we measured a 40% decrease (from 191 to117 ng/g and from 172 to 103 ng/g) and in the third a 65% decrease (from 244 to 86 ng/g) in the level of acrylamide stored at room temperature. To examine if this loss was related to temperature, we reanalyzed the ground coffee used during proficiency testing of the general LC-MS/MS method developed for acrylamide in foods. The bulk of unused ground coffee from that study was stored in a freezer at −40 °C. The amount of acrylamide initially determined in this coffee after opening and homogenizing the sample was 109 ng/g. After 8 months of storage at −40 °C, the level of acrylamide was 113 ng/g. These results suggest that acrylamide loss occurs in ground coffee with time after the container is opened and stored at room temperature.

The data from the analysis of 12 different dry instant coffees are shown in **Table 2**. Duplicate analyses of each product gave average levels ranging from 169 to 539 ng/g. No correlation was apparent for the level of acrylamide with either the

**Table 2.** Average Level of Acrylamide in 12 Dry Instant Coffees and in 2.5 g of Instant Coffee Added to 300 mL of Boiling Water

| manufacturer/brand | ng/g | μg/300 mL[a] |
|---|---|---|
| Folgers Classic Roast | 458 | 1.14 |
| Folgers Classic decaffeinated | 259 | 1.04 |
| Maxwell House regular | 263 | 0.66 |
| Maxwell House decaffeinated | 172 | 0.43 |
| Nestle Classic | 471 | 1.17 |
| Safeway | 377 | 0.94 |
| Super G | 169 | 0.42 |
| Taster's Choice Gourmet Roast | 411 | 1.03 |
| Taster's Choice decaffeinated | 539 | 1.34 |
| Taster's Choice Vanilla Roast | 286 | 0.71 |
| Taster's Choice Hazelnut Roast | 263 | 0.66 |
| Taster's Choice Chocolate Roast | 266 | 0.66 |

[a] 2.5 g of instant coffee added.

**Table 3.** Average Level of Acrylamide in 9 mL of Brewed Black Coffee Purchased from Eight Retail Outlets and in Two Laboratory-Brewed Coffees along with the Corresponding Amount in 300 mL

| source | type | ng/mL | μg/300 mL[a] |
|---|---|---|---|
| Dunkin' Donuts A | regular | 10 | 2.95 |
| Dunkin' Donuts B | unknown | 8 | 2.36 |
| FDA Cafeteria | regular | 6 | 1.77 |
| FDA Cafeteria | decaffeinated | 6 | 1.77 |
| Folgers[b] | Classic Roast | 16 | 4.72 |
| Melitta[b] | Colombian | 11 | 3.24 |
| McDonalds | regular | 8 | 2.36 |
| Seven-Eleven A | French Roast | 6 | 1.77 |
| Seven-Eleven A | regular | 6 | 1.77 |
| Seven-Eleven B | unknown | 6 | 1.77 |
| Starbucks A | Lite Note | 11 | 3.24 |
| Starbucks A | Colombian | 7 | 2.06 |
| Starbucks B | unknown | 9 | 2.65 |

[a] 300 mL (295 g) of brewed coffee. [b] Laboratory-brewed.

manufacturer or the type of instant coffee. The flavored instant coffees (vanilla, hazelnut, and chocolate) all had essentially the same level of acrylamide, and decaffeinating did not appear to remove or lower the amount of acrylamide. A comparison of how many nanograms of acrylamide would be consumed in 300 mL (~12 oz) was made using a recipe of one rounded or slightly rounded teaspoon of instant coffee crystals per 300 mL of boiling water. This corresponded to ~2−3 g of dry instant coffee per cup. Using 2.5 g of instant coffee the range of acrylamide per 300 mL was between 0.42 and 1.34 μg.

Our study also included the analysis of brewed black coffee, purchased from eight retail outlets. In addition, two ground coffees from **Table 1** were used to make brewed coffee in our laboratory using an electric drip coffee maker with a grams of ground coffee to cups of brewed coffee ratio of 4.8. The results are shown in **Table 3**. The sample size analyzed was 9 mL (8.8 g). The level of acrylamide in the purchased coffee ranged from 6 to 11 ng/mL. In 300 mL, about the amount of coffee in a 12 oz cup, this would correspond to an acrylamide content of 1.77−3.24 μg and for 450 mL, about the amount in a 16 oz cup, 2.67−4.89 μg. The acrylamide levels in the laboratory-brewed coffee were 11 and 16 ng/mL, which are somewhat higher than the level in the retail brewed coffee. These levels, when compared to the purchased brewed coffee, were probably due to the use of a ground coffee with a higher acrylamide level and/or a greater amount of ground coffee in preparing the laboratory coffee.

While performing the brewing experiments, we became concerned about possible changes in acrylamide level as the brewed coffee heated in the carafe over time. To test this concern, we brewed Folgers Classic Roast coffee and analyzed a portion immediately after brewing and then again every hour for 5 h. We noted no change in acrylamide level in any of the time-sampled portions. Analysis of the grounds after brewing showed that no acrylamide remained in the grounds after brewing.

In conclusion, using a modified version of our validated method for the detection and quantification of acrylamide in foods, we found acrylamide present in a variety of popular instant, ground, and brewed coffees. Of all the food products analyzed in our laboratory for acrylamide, coffee extracts appeared to have the greatest number of coextractives. Even after cleanup, the extracts exhibited a number of responses before and after the acrylamide signal. Thus, maintaining good chromatographic resolution was an important control factor in avoiding contributions from unwanted coextracted material. The method's limit of detection for acrylamide was 10 ng/g for ground and instant coffees and 1 ng/mL in brewed coffee. The limit of detection was determined when the smallest ion monitored (*m*/*z* 27) reached a signal to noise ration of 3:1. On the basis of this study's findings, variability in acrylamide levels in coffees was found to be dependent on a number of factors, including coffee type, manufacturer, consumer preferences, and freshness of coffee. Finally, acrylamide proved to be quite stable in brewed coffee, as evidenced by the fact that no significant decrease in levels was observed after 5 h of heating.

## NOTE ADDED AFTER ASAP

In the original posting of this paper on March 10, the explanation of the calculation of the conversion factor was in error. In the left-hand column of the fifth page, the measured level of acrylamide in each coffee was divided (not multiplied) by this factor. The current posting (March 17) is correct.

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**Received for review August 26, 2003. Revised manuscript received January 30, 2004. Accepted February 6, 2004.**

JF0349634