JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Silylation of Acrylamide for Analysis by Solid-Phase Microextraction/Gas Chromatography/Ion-Trap Mass Spectrometry

ANTHONY F. LAGALANTE* AND MATTHEW A. FELTER

The Pennsylvania State University, Worthington Scranton Campus, 120 Ridge View Drive, Dunmore, Pennsylvania 18512

A method for quantitative analysis of acrylamide has been developed for use with headspace solid-phase microextraction (SPME). In the method, acrylamide undergoes silylation with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) to form the volatile *N,O*-bis(trimethylsilyl)acrylamide (BTMSA). Once formed, BTMSA is readily extracted from the headspace over the silylation reaction using a 100 μm poly(dimethylsiloxane) SPME fiber. A series of experiments was undertaken to optimize the amount of BSTFA, the silylation reaction temperature, the silylation reaction duration, and SPME sampling duration to maximize the analytical sensitivity for BTMSA. Acrylamide levels were quantified relative to a [$^{13}C_3$]-acrylamide internal standard using gas chromatography/ion-trap mass spectrometry (GC/MS) in the single ion monitoring mode. An analytical working curve was constructed and found to be linear over the 4 to 6700 ppb acrylamide range investigated with a limit of detection of 0.9 ppb. The native acrylamide levels of three commercial cereals were measured using the optimized analytical method. Quantitative standard additions of acrylamide to the cereal matrixes demonstrated complete recovery of the spiked acrylamide.

KEYWORDS: Acrylamide; BSTFA; carcinogen; cereals; derivatization; GC/MS; silylation; SPME

INTRODUCTION

In April 2002, researchers at Sweden's National Food Administration (NFA) and Stockholm University reported that unusually high levels of acrylamide (2-propeneamide) are formed when starch-based foods, such as potatoes, are fried or baked (*1*). However, when the same foods are analyzed in their raw state or after boiling, acrylamide is not detected. Acrylamide is considered a probable human carcinogen, a neurotoxicant, and a genotoxicant, and its discovery in foods has prompted worldwide attention (*2, 3*). Experimental evidence has concluded that acrylamide is formed when asparagine reacts with sugars above 120 °C in a Maillard reaction (*4, 5*). Different cooking temperatures and times, the amount of free asparagine, and the availability of sugars in foods contribute to the variability of acrylamide levels in foods. A targeted priority area for the Analytical Methods Working Group at JIFSAN is the development of independent and proficient methods of acrylamide analysis down to the 1−10 μg/kg (ppb) level in priority food matrixes (*6*).

Current analytical methods to measure low levels of acrylamide include liquid chromatography/tandem mass spectrometry (LC/MS/MS) and gas chromatography (GC) with detection by means of an electron capture detector (ECD) or a mass spectrometer (MS). To a large extent, LC/MS/MS has emerged as the preeminent method to analyze acrylamide as the highly polar acrylamide is very amenable to aqueous extractions from food matrixes. Along with acrylamide, water will coextract proteins and other high molecular weight compounds, and some degree of sample cleanup is necessary. Typically, proteins can be removed by Carrez precipitation, addition of acetonitrile, or filtration through a cutoff filter, while small coextractives are removed by solid-phase extraction (SPE) (*7*). One methodology to circumvent the coextraction of these high-molecular weight compounds is to sample acrylamide from the headspace above the extract using solid-phase microextraction (SPME). In headspace SPME, only low molecular weight, semi-volatile compounds will partition out of the aqueous extract into the headspace and thus the sampling of nonvolatile coextracts is avoided. Unfortunately, due to a high polarity, acrylamide does not partition out of the aqueous extract. Direct immersion of a polar polyacrylate SPME fiber into the aqueous extract to sample acrylamide is a less desirable situation, as nonvolatiles are likely to be sampled and introduced onto the GC. To achieve a headspace SPME technique for acrylamide, acrylamide must first be derivatized to decrease the aqueous solubility and subsequently sampled using headspace SPME.

Several GC methods have been published for trace analysis of acrylamide. These include direct analysis using positive ion chemical ionization (CI+) (*8*) or MS and ECD detection of derivatized acrylamide (*9*−*11*). For the purposes of chemical derivatization, acrylamide possesses two sites at which reactions

* Corresponding author. afl1@psu.edu; correspondence after August 2004: Department of Chemistry, Villanova University, 800 Lancaster Ave., Villanova, PA 19085-1699, anthony.lagalante@villanova.edu

10.1021/jf049759a CCC: $27.50
Published on Web 05/19/2004

can selectively occur; the amide group and the olefin double bond α to the amide carbonyl. Derivatization schemes to date have largely focused on increasing the volatility at the olefin double bond. The reported literature derivatizations for trace analysis of acrylamide are predominantly based on EPA Method 8032A (*12*). In aqueous solution, at acidic pH, the olefin double bond undergoes a dihalo-addition using Br^-/Br_2 to yield the 2,3-dibromopropionamide. The 2,3-dibromopropionamide is solvent extracted, concentrated, and analyzed by GC/ECD or GC/MS. Despite the labor involved, bromination is accepted due in large part to the sensitivity and selectivity of halogen-sensitive detectors, and bromination avoids thermal polymerization of acrylamide at elevated temperatures. However, above 180 °C, 2,3-dibromopropionamide is irreproducibly converted to 2-bromopropenamide at reactive at basic sites on the GC column and in the injection port liner. A weak base, such as triethylamine, is used to prevent the double dehydrohalogenation, forming instead the intermediate alkenyl halide as the major product (*10, 13, 14*).

To overcome limitations inherent in the bromination chemistry of acrylamide, a reproducible, quantitative GC/MS method was sought to quantify low levels of acrylamide in high-temperature cooked foods. The method developed enhances the volatility of acrylamide through silylation of the amide functionality. The silylated acrylamide is then amenable to headspace SPME concentration and subsequent quantitation by GC/MS.

MATERIALS AND METHODS

Chemicals and Materials. Acrylamide (Fisher Scientific, Pittsburgh, PA, >99% purity) and [$^{13}C_3$]-acrylamide (Cambridge Isotope Laboratories, Andover, MA, 99.2% ^{13}C purity) were refrigerated at 4 °C. *N,O*-Bis(trimethylsilyl)trifluoroacetamide (BSTFA, Aldrich Chemical Co., Milwaukee, WI) was stored in an amber vial capped with a PTFE/silicone septa. All solutions were prepared in residue grade acetonitrile (OmniSolv EMD Chemical, Gibbstown, NJ, >99.9% purity). The stock solutions were refrigerated at 4 °C for a maximum of 4 weeks. Cereals were purchased from a local supermarket and stored at room temperature.

Silylation of Acrylamide. All silylation reactions were prepared using volumetric pipets dispensed directly into 4 mL screw-top vials with PTFE/silicone septa (VWR Scientific, Pittsburgh, PA). The silylation optimization solution was 500 μL of a 1000 ppb acrylamide in acetonitrile and 10 μL of a 54.6 ppm [$^{13}C_3$]-acrylamide in acetonitrile internal standard (final concentration of 1070 ppb). BSTFA was drawn through a septum into a 250 μL gastight, glass syringe (Hamilton, Reno, NV) and added through the PTFE/silicone septa of the 4 mL vial to the acrylamide in acetonitrile solution. The 4 mL vial was placed in a preheated, aluminum dry-block (VWR Scientific). The silylation reaction temperature was controlled using an Omega CN7600 microprocessor temperature controller (Stamford, CT) coupled to a 100 Ω platinum RTD that controlled two 50 W resistive cartridge heaters embedded in the dry-block. The silylation reaction was stirred using a magnetic stirrer coupled to a Teflon-coated stir bar in the 4 mL vial. The derivatization variables that were optimized included the amount of BSTFA reactant used for silylation, the silylation reaction temperature, and the silylation reaction time.

SPME Sampling. A manual SPME holder (Supelco, Bellefonte, PA) fitted with a 100 μm poly(dimethylsiloxane) (PDMS) was used in all analyses. Prior to initial use, the fiber was conditioned according to the manufacturer's recommended procedures. Following completion of the silylation reaction, the 4 mL vial containing the silylation reaction mixture was placed in a water-jacketed beaker maintained at 50 °C by a Haake Model FJ circulating water bath. The water-jacketed beaker sat atop a magnetic stirrer that was coupled to the Teflon-coated stir bar in the 4 mL vial. The SPME fiber was exposed to the solution headspace above the stirred silylation reaction solution to concentrate the silylated acrylamide. The headspace sampling duration was optimized using conditions ascertained from the optimized silylation reaction.

GC/MS Analysis. Samples were analyzed on a Star software (Varian, Walnut Creek, CA) computer-controlled Varian 3900 gas chromatograph. The Varian 1177 injector was fitted with a Merlin Microseal septum and the injector temperature was maintained at 250 °C. The injector was operated in the splitless mode from 0 to 2 min during which the analytes were thermally desorbed from the SPME fiber and a 100:1 split after 2 min. Separation was achieved with a Varian CP-Sil 8 CB column (30 m, 0.26 mm i.d., 0.25 μm phase thickness). The initial oven temperature was 50 °C (0 min hold), ramped at 3 °C/min to 100 °C (0 min hold), and ramped at 25 °C/min to 250 °C. The helium carrier gas was electronic pressure controlled at a constant flow of 1.0 mL/min. Under these conditions *N,O*-bis(trimethylsilyl)acrylamide eluted at 9.3 min. The Varian 2100T ion-trap mass spectrometer was operated in EI+ mode (ionization energy 70 eV, multiplier 1400 V) with a scan range of 50−400 amu. The ion trap source and transfer line were maintained at 150 °C and 175 °C, respectively. The MS data accumulation was not initiated until 7 min to avoid detection of the large chromatographic peak due to the unreacted BSTFA.

Quantitation. Acrylamide was quantified using the method of internal standards using [$^{13}C_3$]-acrylamide. Derivatization of acrylamide and [$^{13}C_3$]-acrylamide with BSTFA results in the formation of *N,O*-bis(trimethylsilyl)acrylamide (BTMSA) and *N,O*-bis(trimethylsilyl)-[$^{13}C_3$]-acrylamide ([$^{13}C_3$]-BTMSA), respectively. Peak area determinations were made in the single-ion monitoring (SIM) mode using the most prominent ion in the mass spectrum of BTMSA. The concentration of acrylamide in an unknown was determined from the ratio of the chromatographic peak area of BTMSA to the chromatographic peak area of the known concentration of spiked [$^{13}C_3$]-BTMSA. A calibration curve was measured using a series of standard solutions of acrylamide in acetonitrile spanning the concentration range of 4 to 6700 ppb. The optimized derivatization and SPME sampling procedure was performed on 500 μL of each standard solution with 1070 ppb of the [$^{13}C_3$]-acrylamide internal standard.

Cereal Recovery Experiments. A percent recovery experiment was undertaken using commercial cereals. To evaluate the percent recovery, the level of acrylamide in a cereal matrix was determined before and after a 2705 ppb acrylamide spike. A portion of the cereal equal to the manufacturer's recommended serving size was ground into a fine powder with a commercial burr mill (Mr. Coffee). Between 1 and 2 g of the ground cereal, 10 mL of acetonitrile, and 200 μL of 54.6 ppm [$^{13}C_3$]-acrylamide internal standard were stirred for 1 h at ambient conditions in a 40 mL reaction vial. An aliquot of the supernatant acetonitrile phase was transferred to a 1.7 mL microcentrifuge tube and centrifuged at 5200 g for 10 min. A 500 μL volume of the centrifuged acetonitrile was transferred by pipet to a 4 mL reaction vial to undergo silylation using the optimized derivatization procedure and analysis by SPME/GC/MS. For the spiked cereal sample, a 2705 ppb spike (100 μL of 278 ppm acrylamide in acetonitrile) was added to the ground cereal, acetonitrile, and [$^{13}C_3$]-acrylamide internal standard solution prior to mixing for 1 h. The acrylamide levels in the native and spiked cereal samples were analyzed in triplicate.

RESULTS AND DISCUSSION

MS Analysis. The observed mass spectral fragmentation pattern for BTMSA and [$^{13}C_3$]-BTMSA is given in **Figure 1**. Generally, the parent molecular ion (M^+) is weak or nonexistent in silylated compounds at ionization energies of 70 eV (*15*). Instead, an intense peak corresponding to the loss of a methyl group from the parent compound usually serves to confirm the identity of the parent molecular mass. Indeed, for BTMSA and [$^{13}C_3$]-BTMSA, the molecular ion is not observed; however, rather prominent ions are observed corresponding to loss of a single methyl group at at *m/z* 200 and *m/z* 203 for BTMSA and [$^{13}C_3$]-BTMSA, respectively. The most intense ion in the mass spectrum results from the loss of the *N*-TMS group at *m/z* 128 and *m/z* 131 for BTMSA and [$^{13}C_3$]-BTMSA, respectively. The

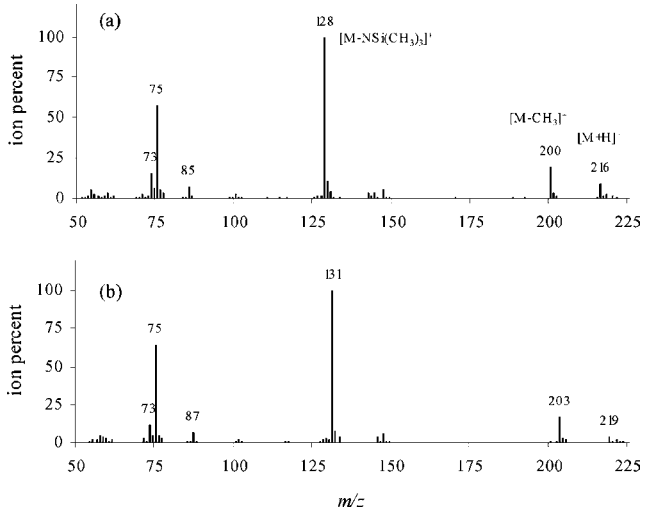

Figure 1. The observed mass spectral fragmentation patterns for (a) BTMSA and (b) [$^{13}C_3$]-BTMSA.

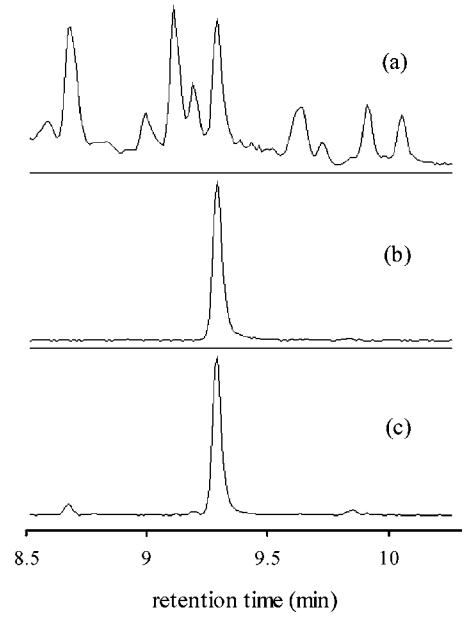

Figure 2. (a) The total-ion chromatogram, (b) SIM *m/z* 128 chromatogram, and (c) SIM *m/z* 131 chromatogram for a 1000 ppb solution of BTMSA and [$^{13}C_3$]-BTMSA.

m/*z* 128 ion fragment was selected for quantifying acrylamide levels in the SIM mode to maximize analytical sensitivity and the *m*/*z* 200 was used for confirmatory identification. [$^{13}C_3$]-BTMSA was quantified using SIM at *m*/*z* 131 with confirmatory identification using *m*/*z* 203. The resultant total-ion chromatogram and SIM chromatograms are depicted in **Figure 2**. In the SIM mode, the acrylamide and internal standard peak were free from any coeluting compounds and baseline resolved.

Optimization of the Acrylamide Silylation Reaction Conditions. Generally, there are two methods for derivatization employed with SPME: (1) derivatization of the analyte followed by headspace SPME of the derivatized analyte, or (2) direct solution immersion of the SPME fiber to first extract the analyte followed by on-fiber derivatization. Given the high water solubility of acrylamide, fiber-solution partition coefficients are likely to favor the aqueous solution. Although an on-fiber derivatization will most likely be feasible, the analytical sensitivity is expected to be poor due to the inability to extract and concentrate acrylamide at sufficiently low levels. Therefore, a

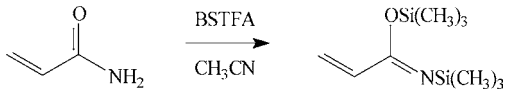

Figure 3. Silylation of acrylamide to form *N*,*O*-bis(trimethylsilyl)acrylamide (BTMSA).

solution-phase silylation of acrylamide was pursued to decrease the polarity of acrylamide and increase the fiber-headspace and headspace-solution partitioning for SPME sampling.

The silylation reaction of acrylamide to form BTMSA is depicted in **Figure 3**. In general, the ease of silylation decreases in the order: alcohols > phenols > carboxylic acids > amines > amides. Amides in general do not undergo any selective derivatization reactions because, in many respects, they can be considered derivatives of the amine and carboxyl groups. Silylation of primary amides has been carried out using trimethylchorosilane (TMCS) and triethylamine. Using a 1:1 molar ratio of amide to TMCS at ambient temperatures, the *N*-TMS amide is preferentially formed over the *O*-TMS derivative due to amide resonance stabilization of the silylated product. However, using an excess of TMCS and longer heating times the *N*,*O*-bis(TMS) amide is formed (*15*).

In this study, the silylation reaction was optimized in acetonitrile. The solubility of acrylamide in acetonitrile is 396 g/L at 30 °C while the solubility of acrylamide in water is 2155−2215 g/L (*16*). Even at high concentrations of acrylamide in foods (>1000 ppb), solubility limitations should not arise. In fact, the use of acetonitrile may be fortuitous to avoid extraction of proteins and other high molecular weight coextracts.

A series of experiments were undertaken to optimize the experimental variables of the silylation and subsequent SPME sampling. **Figure 4**a shows that the response of BTMSA maximizes at 20% (*V*/*V*) of added BSTFA/acetonitrile. Reactive hydroxyl groups will preferentially undergo silylation, and therefore the excess BSTFA is necessary to drive equilibrium toward the product BTMSA. For example, using a 1000 ppb sample of acrylamide in 500 μL of acetonitrile and adding 100 μL of BSTFA, the moles of BSTFA is 13.5 times the moles of acrylamide. A clear concern is that both *N*-(trimethylsilyl)-acrylamide and *N*,*O*-bis(trimethylsilyl)acrylamide are potential silylating agents and can undergo further silylation reactions to decrease the analytical sensitivity for acrylamide. The use of an excess of BSTFA also inhibits competitive silylation reactions that BTMSA may subsequently undergo. The optimization of the silylation reaction temperature of **Figure 4b** indicates that the maximum response of BTMSA occurs at 70 °C. Elevated reaction temperatures are likely to favor the *N*,*O*-bis(trimethylsilyl)acrylamide over *N*-(trimethylsilyl)acrylamide. However, a sharp decline in response is observed above 70 °C as the acrylamide may possibly undergo self-polymerization resulting in a loss in signal. In pure form, acrylamide is known to self-polymerize at its melting point (84.5 °C) (*16*). **Figure 4c** indicates that a reaction time of 1 h is sufficient for achieving equilibrium formation of the BTMSA. On the basis of these derivatization optimization studies, the optimized silylation conditions were chosen to be 500 μL of the acetonitrile solution containing the acrylamide to undergo silylation, 100 μL of BSTFA, a 70 °C silylation reaction temperature, and a 1 h reaction time. The only SPME variable that was optimized was the headspace sampling duration. **Figure 4d** indicates that a headspace fiber exposure time of 10 min is sufficient for BTMSA to achieve equilibrium partitioning into the fiber coating.

Calibration Curve and Limit of Detection. The concentration of acrylamide in **Figure 5** was expressed as a peak area

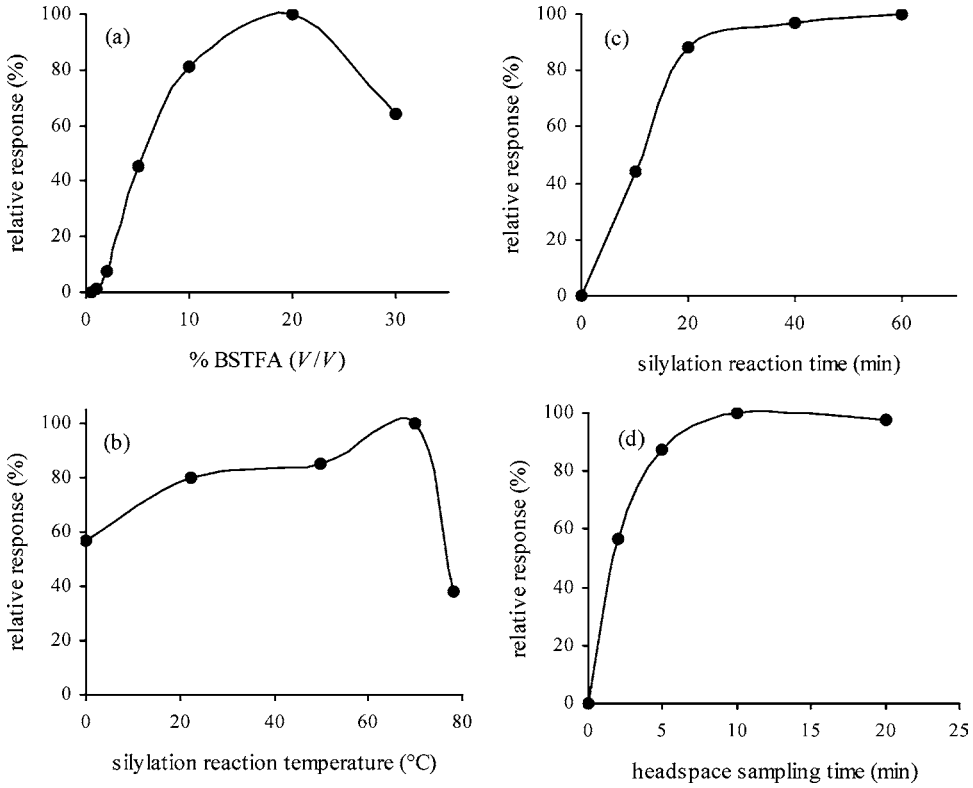

Figure 4. Optimization of the silylation and SPME variables to maximize the relative response of BTMSA. (a) Silylation conditions: 500 μL of 1000 ppb acrylamide, 70 °C, 60 min silylation. SPME conditions: 10 min headspace, 100 μm PDMS, 50 °C. (b) Silylation conditions: 500 μL of 1000 ppb acrylamide, 100 μL of BSTFA, 60 min silylation. SPME conditions: 10 min headspace, 100 μm PDMS, 50 °C. (c) Silylation conditions: 500 μL of 1000 ppb acrylamide, 70 °C, 100 μL of BSTFA. SPME conditions: 10 min headspace, 100 μm PDMS, 50 °C. (d) Silylation conditions: 500 μL of 1000 ppb acrylamide, 70 °C, 100 μL of BSTFA, 60 min silylation. SPME conditions: 100 μm PDMS, 50 °C.

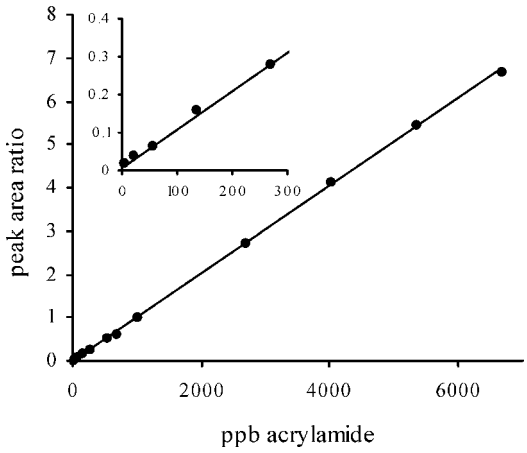

Figure 5. Calibration curve for acrylamide using a [$^{13}C_3$]-acrylamide internal standard. Inset is an expansion of the 0–300 ppb acrylamide range of the calibration curve.

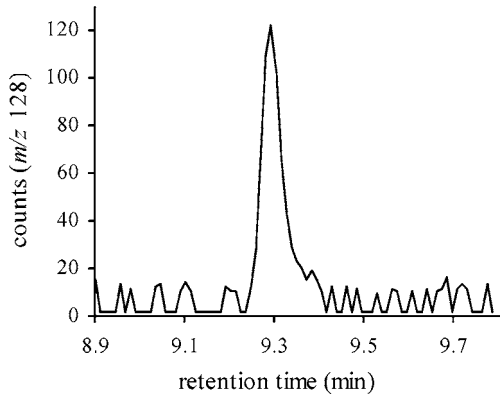

Figure 6. SIM (*m*/*z* 128) chromatogram of 4.33 ppb acrylamide.

ratio of the SIM area counts for acrylamide (*m*/*z* 128) to the SIM area counts for the [$^{13}C_3$]-acrylamide internal standard (*m*/*z* 131). Over the concentration range examined, the calibration curve was found to be linear ($r = 0.9998$, $\sigma_y = 0.002$, $n = 12$). The limit of detection (LOD) and limit of quantization (LOQ) for acrylamide were calculated from the calibration curve regression statistics. The LOD was defined as $3\sigma_b/m$ where σ_b is the standard deviation of the *y*-intercept and *m* is the slope. The LOQ was defined as $10\sigma_b/m$. Using these equations, the calculated LOD was 0.9 ppb and the LOQ was 3 ppb. A chromatogram of a 4.33 ppb acrylamide solution is shown in **Figure 6**. The potential of utilizing the selected-ion monitoring mode (*m*/z 200 + 128) of the Varian 2100T ion-trap as opposed to the single-ion monitoring mode (*m*/z 128) can further lower the LOD.

Cereal Recovery Experiments. Results of the analysis of acrylamide levels in spiked and nonspiked cereals are presented in **Table 1**. The values obtained demonstrate that the method possesses excellent precision with a relative percent deviation for the three cereals below five percent. The results obtained for native levels of acrylamide in the cereals (ng acrylamide per gram of cereal) examined are in general agreement with the range of acrylamide measured by the FDA for a wide variety of cereals (*17*). However, the FDA reported native levels of acrylamide in Rice Krispies (47 ppb) and Cheerios (266 ppb) determined by means of the LC/MS/MS method differ substantially from the results of **Table 1**. This is justifiable, as acrylamide levels will vary within cereal lots due to a variation

Table 1. Native Levels of Acrylamide in Commercial Cereals and the Percent Recovery of a 2705 ppb Spike of Acrylamide in the Cereals

sample	major ingredient	native acrylamide, ppb (RSD, %)	recovery, % (RSD, %)
Kellogg's Rice Krispies	rice	399 (4.4)	103 (5.3)
General Mills Cheerios	whole grain oats	172 (2.1)	102 (4.5)
General Mills Total	whole grain wheat	244 (4.4)	103 (4.6)

in cooking times, cooking temperatures, and the amount and distribution of free asparagine and sugar in the cereal. The extent of the cereal matrix effect on silylation can be assessed by examining the ratio of the counts for the [$^{13}C_3$]-acrylamide internal standard measured during the cereal analysis to the counts for an equivalent amount of [$^{13}C_3$]-acrylamide internal standard that undergoes silylation in pure acetonitrile. For Kellogg's Rice Krispies this ratio was approximately 1.0 indicating that the cereal matrix had a minimal effect on the analytical sensitivity for acrylamide. However, for Cheerios and Total, the ratio varied between 0.3 and 0.5, indicating there was a reduced sensitivity of acrylamide due to a matrix effect. Several possibilities exist to explain the reduced sensitivity observed. For instance, coextracts possessing functional groups that will preferentially undergo silylation over acrylamide will compete for partitioning onto the SPME fiber lowering the BTMSA headspace-fiber partitioning. Another scenario may be that a percentage of the spiked acrylamide and internal standard become bound within the cereal grain or coextracts, lowering the cereal−acetonitrile partitioning of acrylamide. Despite this matrix effect, the use of a [$^{13}C_3$]-acrylamide internal standard compensates for the cereal matrix effects and is essential to produce accurate, reproducible results. Inclusion of an SPE cleanup procedure prior to silylation is likely to reduce the matrix effects due to coextracts that potentially could reduce the analytical sensitivity.

Results from this study indicate that the silylation of the amide group offers a preferable method over bromination of the olefin double-bond toward enhancing the volatility of acrylamide. The silylated derivative is not reactive on-column, highly volatile, amenable to headspace SPME, and has a unique *m*/*z* molecular fragment for quantification by MS. The method LOD is comparable to the 1−10 ppb LOD typically reported by LC/MS/MS. The method developed herein can provide complementary results for acrylamide levels in side-by-side comparisons using independent techniques. When analyzing commercial foods, the use of a [$^{13}C_3$]-acrylamide internal standard is essential to account for matrix effects on the analyte signal. Further method developments are now being conducted in our laboratory to incorporate SPE cleanup prior to silylation as well as evaluation of the method for analysis of acrylamide levels in other high-temperature fried and baked foods.

ACKNOWLEDGMENT

The authors wish to express their gratitude to Robert E. Shirey at Supelco Inc. for providing helpful insight throughout the course of this study. Additionally, we thank Supelco Inc. for providing the SPME fibers used in the work and to Cambridge Isotope Laboratories for providing the [$^{13}C_3$]-acrylamide. M.A.F. thanks Penn State University for an undergraduate research stipend.

LITERATURE CITED

(1) Swedish National Food Administration. *Information About Acrylamide in Food*; April 24, 2002, http://www.slv.se.
(2) Health Implications of Acrylamide in Food. Report of a Joint FAO/WHO Consultation; Geneva, Switzerland, 25−27 June 2002,
(3) Friedman, M. Chemistry, Biochemistry, and Safety of Acrylamide. A Review. *J. Agric. Food Chem.* **2003**, *51*, 44504−4526.
(4) Mottram, D. S.; Wedzicha, B. L.; Dodson, A. T. Acrylamide is formed in the Maillard reaction. *Nature* **2002**, *419*, 448−449.
(5) Stadler, R. H.; Blank, I.; Varga, N.; Hau, J.; Guy, P.; Robert, M.-C.; Riediker, S. Acrylamide from Maillard reaction products. *Nature* **2002**, *419*, 449−450.
(6) Report of the Analytical Working Group. JIFSAN Acrylamide in Food Workshop, 28−30 October 2002, Chicago, IL.
(7) Reiediker, S.; Stadler, R. H. Analysis of acrylamide in food by isotope-dilution liquid chromatography coupled with electrospray ionization tandem mass spectrometry. *J. Chromatogr. A* **2003**, *1020*, 121−130.
(8) Biedermann, M.; Biedermann-Brem, S.; Noti, A.; Grob, K.; Egli, P.; Mändli, H. Two GC-MS Methods for the Analysis of Acrylamide in Foods. *Mitt. Lebensm. Hyg.* **2002**, *93*, 638−652.
(9) Nemoto, S.; Takatsuki, S.; Sasaki, K.; Naitani, T. Determination of Acrylamide in Foods by GC/MS Using ^{13}C-labeled Acrylamide as Internal Standard. *J. Food Hyg. Soc. Jpn.* **2002**, *43*, 371−376.
(10) Andrawes, F.; Greenhouse, S.; Draney, D. Chemistry of acrylamide bromination for trace analysis by gas chromatography and gas chromatography−mass spectrometry. *J. Chromatogr.* **1987**, *399*, 269−275.
(11) Gertz, C.; Klostermann, S. Analysis of acrylamide and mechanisms of its formation in deep-fried products. *Eur. J. Lipid Sci. Technol.* **2002**, *104*, 762−771.
(12) *Test methods for evaluating solid waste. Acrylamide by gas chromatography*; United States Environmental Protection Agency, SW 846 Method 8032A, 1996; EPA: Washington, DC.
(13) Raymer, J. H.; Sparacino, C. M.; Velez, G. R.; Padilla, S.; MacPhail, R. C.; Crofton, K. M. Determination of acrylamide in rat serum and sciatic nerve by gas chromatography-electron capture detection. *J. Chromatogr.* **1993**, *619*, 223−234.
(14) Bologna, L. S.; Andrawes, F.; Barvenik, F. W.; Lentz, R. D.; Sojka, R. E. Analysis of residual acrylamide in field crops. *J. Chromatogr. Sci.* **1999**, *37*, 240−244.
(15) Pierce, A. E. *Silylation of Organic Compounds. A Technique for Gas-Phase Analysis*; Pierce Chemical Co.: Rockford, IL, 1968; p 487.
(16) Daughton, C. G. *Quantiation of acrylamide (and polyacrylamide): critical review of methods for trace determination/formulation analysis and future-research recommendations*; The California Public Health Foundation, CGD-02/88, 23 June 1988, Berkeley, CA.
(17) FDA/CFSAN, Exploratory Data on Acrylamide in Foods, December 4, 2002, http://www.cfsan.fda.gov/~dms/acrydata.html

Received for review February 12, 2004. Revised manuscript received April 13, 2004. Accepted April 14, 2004. This work was funded in part by a Research Development Grant from the Pennsylvania State University and a Dr. Richard J. and Sally Matthews Research Award.

JF049759A