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Solid-phase microextraction method for gas chromatography with mass spectrometric and pulsed flame photometric detection: studies of organoarsenical speciation

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Abstract

The development, optimization, and application of a novel method for arsenic speciation based on capillary gas–liquid chromatography with simultaneous quadrupole ion-trap mass spectrometric (MS) detection and pulsed flame photometric detection (PFPD) is described. The method couples the sensitive arsenic-selectivity of PFPD with the structure elucidation capability of molecular MS detection for the determination of trace levels of unknown organoarsenicals in complex matrices. The conditions that affect the PFPD response in the presence of interfering species were optimized using the sequential Simplex algorithm for three key factors: gate delay (18.3 ms), gate width (9.1 ms), and combustion gas composition (16.6 ml/min H_2). Complete discrimination in the PFPD of the arsenic signal from interfering S-, C-, and OH-emitting species that are problematic in existing methods was achieved. Additionally, a revised interpretation of our previously reported mechanism [J. Chromatogr. A 807 (1998) 253] for the dithiol derivatization and subsequent GC–MS determination of dimethylarsinic acid is presented.

Keywords: Detection, GC; Organoarsenic compounds; Monomethyl arsonous acid; Dimethyl arsinic acid

1. Introduction

Recently we described a method for the determination of several organoarsenic acids that combined chemical derivatization with solid-phase microextraction (SPME) and gas chromatography–mass spectrometry (GC–MS) [1]. For polar and/or thermally-labile analytes, analyte derivatization prior to SPME–GC can enhance detectability and improves chromatographic resolution [2]. We examined a series of dithiol compounds for derivatization of the arsenicals, and the best results were obtained with 1,3-propanedithiol (PDT). The derivatization procedure, fiber type, and extraction time were then optimized. Using this method, the limit of detection for 2-chlorovinylarsonous acid was improved 400-fold compared to conventional solvent extraction methods.

We sought to further improve the selectivity of our existing method by incorporating in parallel an arsenic-specific detector and thereby make the method more robust for determining unknown arsenicals

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in complex environmental samples. Of the various methods for arsenic-specific detection in GC, the most sensitive are based on flame atomic emission spectroscopy, with reported detection limits in the sub-part per billion (ppb) range [3,4]. For example, Estes et al. have described an atomic emission detection (AED) method for GC in which triethylarsine was measured over a 500-fold range to a detection limit of 155 pg (on-column), with the detector sensitive to 6.5 pg/s [5]. Problems with this type of approach are primarily in discharge tube erosion and a lack of selectivity, though these have been largely overcome for several elements other than arsenic [6].

An alternative to atomic spectroscopic detection for GC that is simpler, less expensive, and possesses comparable figures of merit was developed by Amirav et al. [7] — pulsed flame photometric detection (PFPD). PFPD improves the conventional FPD by exploiting the time-dependence of the photon emission process using an electronically-gated photon detector [8]. Initial conditions for arsenic detection by GC–PFPD have been reported using a relatively long gate delay (>10 ms) in conjunction with a high-pass optical filter (>695 nm). Arsenic was measured successfully at low (sub-ppb) levels with high sensitivity (<5 pg/s) [9].

The goal of the work reported herein was to develop a method that would allow us to unequivocally identify trace levels of unknown organoarsenicals in complex matrices. We sought to improve upon the reported conditions for determining organoarsenicals using PFPD by focusing not only on optimizing the arsenic response but also greatly improving the degree of interference rejection. Isolation of the arsenic response from those signals emitted by carbon- or sulfur-containing species is critical when the analyst is confronted with complex environmental samples containing trace-level arsenicals. Because GC methods for arsenicals are most often based on thiol derivatization, the detector must be ''blind'' to co-eluting or overlapping sulfur-containing compounds (e.g. impurities, non-As reaction products). Hence, the ability to not only detect arsenic emission with high sensitivity but also to eliminate the contribution from sulfur-emitting species in particular is paramount. We thus describe in the following pages the development, optimization, and application of the method to the determination of organoarsenicals in freshwater sediment.

2. Experimental

2.1. Reagents

All chemical reagents used were analytical reagent grade or better. Reagent water (18 MΩ cm) was prepared using a NanoPure filtration system equipped with an ultraviolet lamp (Barnstead-Thermolyne, Dubuque, IA, USA). Arsenic standards, including arsenious acid, arsenic acid, dimethyl arsinic acid (DMAA), and triphenylarsine (TPA); benzothiophene (BTP); and 1,3-propanedithiol were obtained from Aldrich (Milwaukee, WI, USA). Monomethyl arsonic acid (MMAA) was synthesized in our laboratory by the Meyer reaction, as modified by Quick and Adams [10]. All glassware and plasticware were acid-washed after use for at least 48 h in 5% (v/v) analytical reagent-grade nitric acid to remove background contamination. Arsenic-containing standards with concentrations less than 1 mg/l were prepared on the day of use. All aqueous standards were stored at 4°C.

2.2. Instrumentation

Organoarsenicals were determined by gas–liquid chromatography using a Varian GC–MS system (Walnut Creek, CA, USA) which consisted of the following components: Model 3800 capillary gas–liquid chromatograph (DB-5MS column, 30 m×0.25 mm with 0.25-μm film, J&W Scientific, Folsom, CA, USA) with Model 1079 split/splitless injector; SPME apparatus (Supelco, Bellefonte, PA, USA); electron impact ionization source (70 eV); PFPD performed in the arsenic mode using a high-pass optical filter (Schott RG695 nm, BES Optics, Warwick, RI, USA) and Model R5070 photomultiplier tube (PMT; Hamamatsu, Bridgewater, NJ, USA) set to 610 V with a 200-mV trigger level; Saturn 2000 quadrupole ion-trap mass spectrometer (10–650-*m*/*z*

range, unit resolution). The automatic gain control (AGC) of the MS system was used throughout this study. Automated library searching was performed using the National Institute of Standards and Technology (NIST) Mass Spectral Database (version 3.0). The effluent from the column was divided 1:1 (PFPD:MS) through an outlet splitter system (Model OSS-2, Scientific Glass and Engineering, Austin, TX, USA) using deactivated fused-silica (50 cm × 0.25 mm I.D. to the PFPD system and 25.0 cm × 0.10 mm I.D. to the MS system), based on a similar method [11]. The mobile phase was ultra-high purity (99.999%) helium (Praxair, Milwaukee, WI, USA) at a constant linear velocity of 42 cm/s through electronic flow control.

2.3. GC program

Two GC methods were used; one for the optimization study (syringe injection) and the other for the analytical study (SPME introduction). For optimization work using TPA (10.0 ng on-column) and BTP (9.3 ng on-column), 1-μl injections were made using a 1:100 split ratio; the injection port was held at 250°C. The column was held for 2.0 min at 135°C, then programmed at 50°C/min to 300°C, where it was held for 2 min. For the SPME work, the injection port was 250°C for the fiber desorption step (splitless). The initial column temperature was 45°C for 5 min, then programmed at 20°C/min to 160°C, followed by 8°C/min to 210°C and finally 50°C/min to 300°C, where it was held for 5 min. The transfer line between the GC system and the MS system was maintained at 170°C for all experiments. Mass spectra were obtained by scanning from m/z 35 to 400 with a 0.7-s scan time.

2.4. Software

GC instrument control and data acquisition were performed on a Pentium personal computer (Dell, Optiplex GX1, Dallas, TX, USA) using Saturn System software version 5.21 and PFPD analysis software version 1.0 (Varian). Sequential Simplex optimization was performed using MultiSimplex version 2.04 (MultiSimplex, Karlskrona, Sweden).

3. Results and discussion

3.1. Method development

We sought to improve upon on our previous SPME–GC–MS work [1] in two ways: (i) to develop a more accurate understanding of the MS response to the major methylated oxyanions of arsenate, and (ii) to incorporate an optimized method for parallel arsenic-specific detection. In our previous work with DMAA, we were unable to determine the exact structure of the thiol-derivatized reaction products of DMAA observed in the mass spectrum. Because of the popularity of the thiol-derivatization approach [12–23], we initially focused on a reinterpretation of the mechanism. The DMAA–PDT mass spectrum has major peaks at m/z 197 and m/z 211. We postulated previously that DMAA derivatized with the dithiol by the elimination of a methyl group to form $CH_3AsH_2S–S(CH_2)_3$, and then formed the cyclic dithiaarsenoline product (M–H), $CH_3As^+HS–S(CH_2)_3$ at m/z 197. Because self-chemical ionization (CI) processes were clearly occurring in the ion-trap mass spectrometer (i.e. ions larger than M^+), we suggested that the peak at m/z 211 was not the molecular ion but was in fact an artifact of the ion trap, formed as a result of CI by transfer of a methyl group (and displacement of H). However, we continued to question our mechanistic explanation and chose to investigate it further as part of the overall method optimization discussed herein.

We presently hypothesize that DMAA initially reacts with the strongly reducing thiol [present at ~1% (v/v)] by producing the trivalent methylarsine, CH_3AsH_2. Methylarsine then will undergo immediate cyclization with PDT to form the pentavalent $(CH_3)_2AsH_2S–S(CH_2)_3$. This neutral, volatile, and thermally-stable molecule is then chromatographed and produces $(CH_3)_2As^+HS–S(CH_2)_3$ at m/z 197 by EI ionization (M–H). The parent molecular could also gain $–CH_3$ by displacing H (i.e., self-CI), and thereby produce the species observed at m/z 211, $(CH_3)_2As^+S–S(CH_2)_3$. To test this hypothesis, we employed the tandem MS function of the ion trap. By selecting either m/z 196–198 or m/z 210–212 during the parent ion scan, and selecting full scan (m/z 35–400) during the daughter ion scan, we found that the parent DMAA-thiol product was

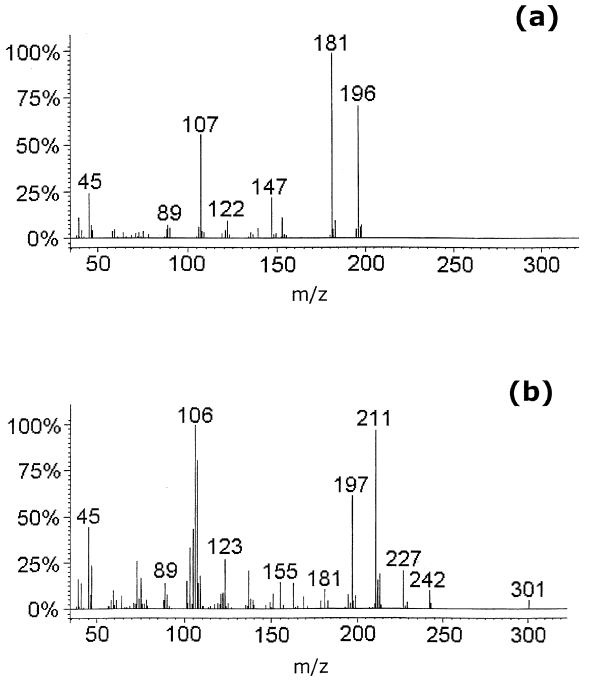

Fig. 1. Mass spectra of (a) MMAA-PDT and (b) DMAA-PDT. For conditions, see text.

observed only when m/z 196–98 was selectively sampled in the mass analyzer (Fig. 1a). No molecular ions or fragments were observed when we selected m/z 210–212 during the parent ion scan. This indicates that m/z 211 is indeed a self-CI product of m/z 197, supporting our original mechanism [1]. Minor species are also produced by self-CI at higher m/z values when m/z 196–198 was the parent ion range (e.g., at m/z 301, M+103). We also studed the reactivity of MMAA (not available in the previous study) with PDT and found that the results were inconsistent with the DMAA-PDT mechanism. If MMAA underwent reaction with PDT but did not lose its methyl group in the process, one would expect to observe an identical mass spectrum [i.e., in both cases, the reaction product would be $CH_3AsH_2S–S(CH_2)_3$]. However, the observed mass spectrum for MMAA-PDT (Fig. 1a) is different than that observed for DMAA-PDT (Fig. 1b), with major species at m/z 181 and m/z 196 [presumably for $As^+S–S(CH_2)_3$ and $CH_3As^+S–S(CH_2)_3$, respectively]. Curiously, self-CI products at higher m/z values were not observed. Furthermore, two separate

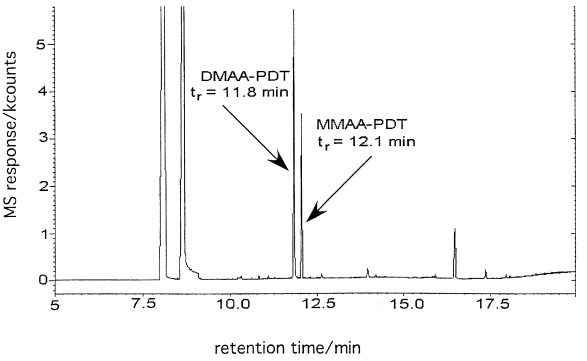

Fig. 2. Separation of MMAA and DMAA using the SPME–GC–MS method. The PDT-derived products are indicated for each compound. For conditions, see text.

zones are observed in the chromatogram (Fig. 2), i.e., MMAA-PDT (t_R = 12.1 min) and DMAA-PDT (t_R = 11.8 min). On the other hand, if MMAA lost its methyl group by reaction with PDT, it would be difficult to explain the presence of the (methylated) species at m/z 196 in the observed spectrum (Fig. 1a). Further work is in progress in our laboratory to understand these observations.

We then turned our attention to developing and optimizing the parallel PFPD method. We first studied the time-dependence of the PFPD emission to several organoarsenicals. Arsenic possesses the longest delay in emission for elements that have been studied using PFPD [9]. In Fig. 3, the time-resolved signal for triphenylarsine (TPA) is shown as an example. The emission for a sulfur compound (benzothiophene, BTP) is essentially complete at 10 ms. Compared to the temporal signal for S_2^*, the arsenic emission is not only of greater magnitude, but can be readily separated with an electronic emission-time gate applied to the PMT. Without the use of an optical cut-off filter (>695 nm), the sulfur emission is of equivalent delay, and of greater magnitude. With the filter, a gate delay of 10 ms will block the sulfur signal, allowing the photons from the excited arsenic to be detected by the PMT. The arsenic signal reaches a peak at ~11 ms, and continues until >15 ms. The separation of arsenic emission from that of carbon and OH is also depicted in Fig. 3. Carbon and OH have virtually undelayed emission, and their luminescence is complete in less that 4 ms. These emission times are typical for

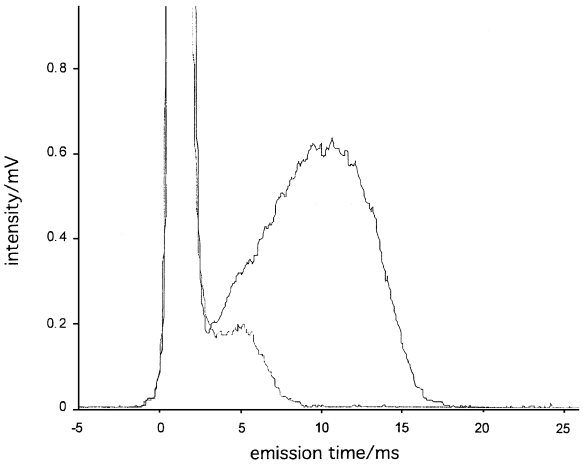

Fig. 3. Emission profiles for arsenic (top) and sulfur (bottom) in the PFPD system. Arsenic (as TPA) and sulfur (as BTP) chemiluminescent emission were measured. For conditions, see text.

volatile As-, S-, and OH-containing compounds that we and others have studied. Additionally, the energy of the emitted photons can also provide selectivity. For instance, the arsenic emission is characterized by a broad ''white light-type'' spectrum (~350–900 nm, with a maximum at ~450 nm), while sulfur emission is primarily at energies higher than 700 nm [8]. Therefore, by using an appropriate combination of gate delay, gate width, and wavelength discrimination, a selective method for arsenic should be straightforward to construct. Other factors that may affect the PFPD response to arsenic, such as combustion gas ratios, flame structure, and detector operating conditions, were of interest as well. We therefore chose to investigate a multivariate optimization for these along with the spectral and temporal domain factors to create an improved method that would provide enhanced selectivity for determining environmental levels of arsenicals.

3.2. Method optimization

There are many physical and chemical factors that can influence the figures of merit for an analytical method. We therefore initially employed a saturated fractional factorial experimental design matrix to identify the key factors, assuming a first-order model [24]. The factors chosen (and two ''dummy'' factors) were placed in an orthogonal matrix using coded factor space (Table 1). The ''dummy'' factors, injector temperature and mobile phase flow, did not affect the behavior of the PFPD system [9]. A total of eight experiments using combinations of these factors were conducted using TPA and the average response ($n=3$) was determined. Main effects were most prominent for gate delay ($\beta_{GD}=3.0\cdot10^4$), combustion gas composition ($\beta_{CG}=2.7\cdot10^4$), and gate width ($\beta_{GW}=1.9\cdot10^4$), where β_i is the parameter coefficient for a given factor. The other factors had negative β values. The gate control and flame composition were the most influential factors in the performance of PFPD. Because the temperature of the flame affects the stability of the excited state species that is formed, combustion gas composition was also identified as an important factor. This factor was important when the flame was at its highest temperature (24 ml/min H_2 and 23 ml/min air); the nearly 1:1 ratio apparently provides optimal combustion conditions.

Table 1
Factor screening matrix

Factor	Low	High	C/D
Combustion gas flow	H_2-rich	Air-rich	D
Detector temperature	200°C	300°C	C
Combustor diameter (I.D.)	2 mm	3 mm	D
Gate delay	2 ms	16 ms	C
Gate width	2 ms	20 ms	C
Injector temperature	250°C	300°C	C
Mobile phase flow	1 ml/min	3 ml/min	C

Continuous (C) or discrete (D) values are indicated.

In deciding the relative importance of a given factor, it is imperative to examine the interaction effects as well as the main effects. Therefore, we subjected the three key factors to a sequential Simplex optimization procedure [24]. For the response function, we chose to use the peak area for TPA minus the sum of the peak areas for the solvent (*n*-hexane) and BTP, allowing us to measure the degree of interference from C- and S-emitting species, that is:

$$Response = [PA_{TPA} - (PA_{BTP} + PA_{HEX})]$$

We began the optimization using recommended values for operating the PFPD system in the As

mode [9]. Fig. 4 shows the progression of the optimization; a total of 19 iterations were performed ($n=3$). Peak areas were determined for TPA ($t_R = \sim$ 5.5 min), BTP ($t_R = \sim 2.5$ min) and *n*-hexane ($t_R = \sim 1$ min). It is important to note that because the method used for the optimization study had a much steeper oven temperature program than the analytical method, peak tailing was observed in the chromatogram. Because the peak tailing did not affect resolution of the compounds in the test mixture, the benefit of reducing the run time by nearly 60% was justified. The initial conditions produced a decidedly non-selective response of the detector to As-, S-, and C-containing species. By optimizing the arsenic response relative to that of sulfur and carbon, virtually infinite selectivity was obtained (number 19 in Fig. 4). Clearly, some sensitivity was sacrificed to this achieve this goal (e.g. iterations 14 and 16 in Fig. 4). This is also obvious when one considers only the response depicted in Fig. 3, where the optimal value appears to be ~10 ms. In fact, for applications where the S-selectivity is not as important, one would be well-advised to use the 10-ms gate delay with a ~10-ms temporal gate width (emission window). Nevertheless, the detector sensitivity for the final method conditions is 4 pg/s, in contrast to the original published sensitivity of 1 pg/s [9]. The optimized levels for the factors were 16.3 ms for gate delay, 9.1 ms for gate width, and 16.6 ml/min for combustion gas flow. The air flows to the PFPD system were 10.0 and 17.0 ml/min.

Unlike atomic spectroscopic methods, the method described herein is not only useful for identifying but also for elucidating the structures of unknown arsenic compounds, both volatile and thiol derivatized non-volatile, in complex matrices such as sediments [25]. The efficient separation of the thiol-derivatives of the major alkylated protonated oxyanions (MMAA, DMAA) as well as a representative arsine (TPA) is observed. Other arsenicals (arsine, MMA, DMA) can be determined by this approach using purge and trap sample introduction rather than SPME

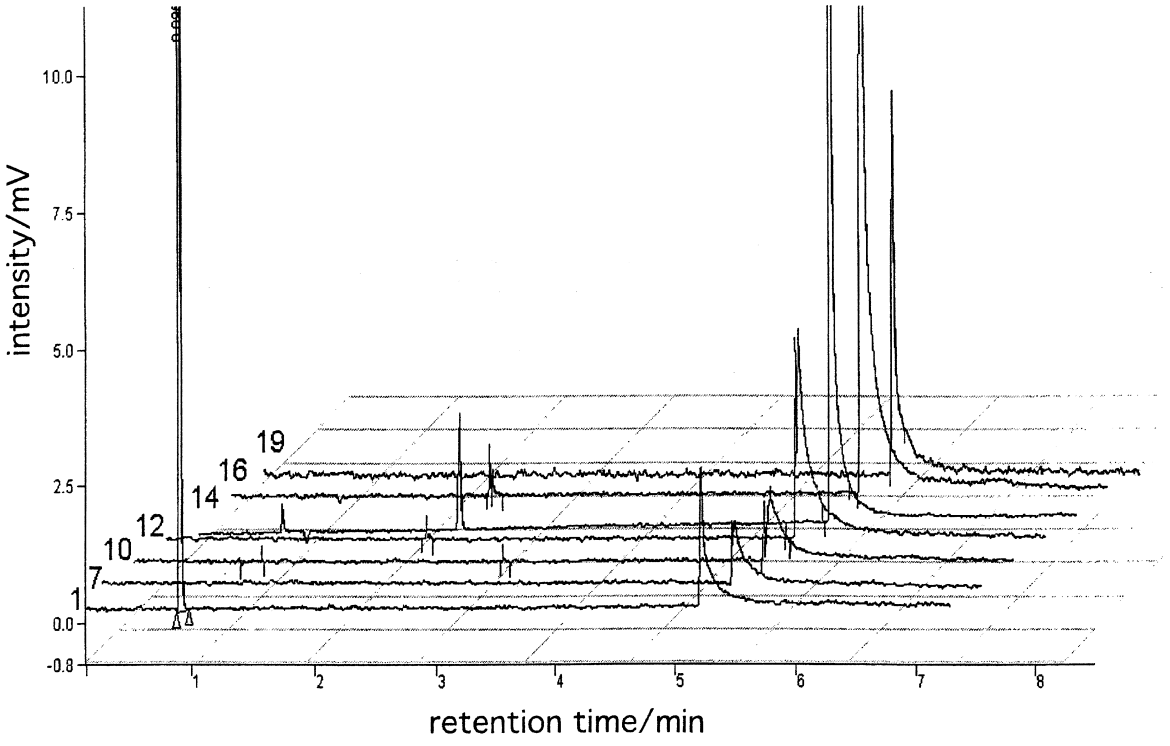

Fig. 4. Comparison of consecutive GC–PFPD chromatograms generated during Simplex optimization study. Seven representative chromatograms of the 19 trials that were performed are depicted. For conditions, see text.

[15]. Our work thus improves the existing method for determining unknown arsenic species by GC–PFPD. Additionally, the method complements other GC–MS methods for arsine determination, most notably the recent work of Pantsar-Kallio and Korpela [26] by adding an optimized capability to simultaneously determine the arsenic-containing peaks in the chromatogram, thereby making structure elucidation of unknowns by parallel molecular MS easier (e.g. using NIST mass spectral databases). The methodology is flexible in that it may be extended to the study of other volatile metals and metalloids in the environment.

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