Postcolumn Derivatization Liquid Chromatography/Mass Spectrometry for Detection of Chemical-Weapons-Related Compounds

William R. Creasy
EAI Corporation, Abingdon, Maryland, USA

Postcolumn derivatization for liquid chromatography/mass spectrometry (LC/MS) analysis was characterized for detection of some compounds related to chemical-weapons (CW) agents using an Atmospheric Pressure Chemical Ionization (APCI) source. The derivatizing reagents were added directly to the LC eluent flow, and the derivatization reactions occurred in the APCI source under typical operating conditions. The compound *S*-[2-(diisopropylamino) ethyl] methylphosphonothioic acid was methylated using the derivatizing reagent trimethylphenyl ammonium hydroxide (TMPAH). Methylphosphonic acid was doubly derivatized to form dimethyl methylphosphonate, although the signal for the derivatization product was very sensitive to the amount of TMPAH. Arsenic compounds related to the CW agent lewisite, including chlorovinyl arsonous acid and arsenic (III) oxide, were derivatized using 2-mercaptopyridine. The thiol group reacted readily with the arsenic (III) center and provided a significant improvement in sensitivity relative to the underivatized signal using APCI or electrospray ionization. Triethanolamine and ethyl diethanolamine were derivatized with benzoyl chloride, a commonly used LC derivatizing reagent for alcohols, to modify their mass spectra. Postcolumn derivatization using an APCI source gives an alternative for detecting some difficult-to-ionize compounds. It has the limitations that sensitivity was not always improved even though the major mass spectral peaks can be shifted; it is necessary to carefully select the reagent; and some reagents introduced strong interference peaks at specific masses in the spectrum and may suppress the ionization of some derivatized analyte ions. The reagent also produced contamination in the source, which had to be cleaned daily. (J Am Soc Mass Spectrom 1999, 10, 440–447) © 1999 American Society for Mass Spectrometry

Postcolumn derivatization is a common liquid chromatography (LC) method for improving the sensitivity of detectors to particular classes of analytes [1, 2]. It is often used with UV or fluorescence detection to add an absorbing or fluorescing group, respectively, to an analyte for which the detector has poor sensitivity. A typical disadvantage of this approach is that additional apparatus may be needed to heat and mix the LC eluent with the derivatization reagent to drive the reaction. This apparatus must be carefully designed to minimize peak broadening while providing sufficient time for the reaction to occur [1].

Mass spectrometry (MS) detection of LC separations has become a general detection method using atmospheric pressure ionization (API) techniques [3, 4]. However, there are some types of analytes that are difficult to detect by API-MS. These analytes include those with poor ionization efficiencies or low volatility, which cause them to have low ion signals. For example, compounds such as salts do not vaporize as the liquid solvent evaporates, but rather they form solid particles, so they are generally difficult to detect.

The API-MS detection for some low-sensitivity analytes may be improved by using chemical derivatization to increase the signal. Studies has been done using precolumn derivatization for electrospray ionization (ESI) LC/MS detection [5–8] and moving belt LC/MS [9].

Atmospheric pressure chemical ionization (APCI) sources are well-suited for on-line postcolumn derivatization. The APCI source nebulizes the liquid flow, and the sprayed liquid is heated to 300–400 °C in a drying gas flow to evaporate the liquid. The evaporation concentrates the analytes and reagents in the droplets, so less reagent is needed. Ionization is produced by a corona discharge at atmospheric pressure. The high temperature and corona drive derivatization reactions without any additional hardware, so peak broadening is not introduced by the experimental approach. The hardware modification is experimentally simple, be-

Address reprint requests to Dr. William Creasy, EAI Corporation, 1308 Continental Drive, Suite J, Abingdon, MD 21009. E-mail: wrcreasy@eaicorp.com

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cause it is only necessary to use a "T" in the tubing and a syringe pump to pump in the derivatization reagent into the flow of liquid from the LC.

Postcolumn derivatization is a possible approach for the analysis of small, inorganic anions. A number of anions that are difficult to analyze are produced from decontamination of chemical weapons compounds. The analysis of some chemical-weapons (CW) compounds [10, 11], particularly by LC/MS methods [12–17], has been studied. Some decontamination products are difficult to determine because of poor gas chromatography (GC) characteristics and low LC/MS sensitivity. Because the Chemical Weapons Convention [18] requires the destruction of existing chemical weapons, studies are currently underway to determine the optimal methods for chemically decontaminating a number of CW agents. The decontamination solutions can be complex, because they can contain high concentrations of caustic or reactive reagents along with a complex mixture of related products and byproducts. The number of compounds and the complexity of the matrix introduce some analytical difficulties [19].

Several derivatization reagents have been identified that can be used for postcolumn derivatization in LC/MS analysis. The study of a variety of compounds gives an indication of the general strengths and limitations of this approach. The technique has been used for analyzing decontamination solutions, although additional characterization of the methods is necessary to develop a standard operating procedure for routine sample analysis.

Experimental

LC/MS was done with a Hewlett-Packard 5989A "MS Engine" mass spectrometer with an Analytica of Branford atmospheric pressure chemical ionization (APCI) source or electrospray (ESI) source and HP 1090 HPLC. Postcolumn derivatization was done by adding derivatization solution with a Harvard syringe pump to the LC outlet tubing through a "T" connection in the tubing. The APCI vaporizer temperature was typically 375 °C, and the drying gas temperature was 300 °C. LC flow was 0.25 mL/min. Chromatography was done using a Zorbax Eclipse XDB-C18 150 × 2.1 mm column (HP Part No. 993700.902). LC runs were isocratic with 100% aqueous 0.001–0.05 M ammonium acetate in distilled (DI) water.

Trimethylphenylammonium hydroxide (TMPAH, CAS No. 1899-02-1) was from Fluka (Buchs, Germany) at a concentration of 0.1 M in methanol. 2-Mercaptopyridine (CAS No. 2637-34-5, 99%) was purchased from Aldrich Chemical (Milwaukee, WI) and used as a 0.1–0.3 M solution in HPLC grade acetonitrile (J. T. Baker, Phillipsburg, N.J.). Benzoyl chloride (CAS No. 98-88-4, 99+%) was purchased from Aldrich Chemical and used as a 0.1–0.2 M solution in acetonitrile.

Standards of 2-chlorovinyl arsenous acid (CVAA) and *S*-[2-(diisopropylamino)ethyl] methylphosphonothioic acid (DAEMPTA) were obtained from the Chemical Agent Standard Analytical Reference Material (CASARM) program of Edgewood Research, Development, and Engineering Center (ERDEC), Aberdeen Proving Ground, MD. NOTE: Researchers are cautioned that these are toxic compounds that must be handled with extreme caution and appropriate safety protective equipment. Other standard chemicals were commercially available.

Results

S-[2-(Diisopropylamino)ethyl] Methylphosphonothioic Acid (DAEMPTA)

DAEMPTA is a toxic minor product formed from base hydrolysis of the nerve agent *S*-[2-(diisopropylamino) ethyl] ethyl methylphosphonothioate (VX) [20]. It is further hydrolyzed to break the P–S bond to give nontoxic products. This compound must be detected in VX decontamination reaction solutions to demonstrate that it has been destroyed. The chemical structure of DAEMPTA has a zwitterion form in neutral or acidic pH:

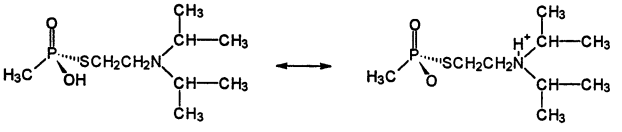

Diagram 1

DAEMPTA can be detected by LC/MS with APCI, but the sensitivity is less than that of VX (the O-ethyl ester), possibly because of poorer efficiency for forming positive ions for the zwitterion structure. Previous work has shown that GC analysis can be done on DAEMPTA using derivatization with TMPAH so that derivatization takes place in the hot GC injector to form a methyl ester [19]. Recent work by Crenshaw and Cummings [21] has shown that DAEMPTA can also be methylated using (trimethylsilyl)diazomethane. TMPAH has been used by Vouros and co-workers [22] for LC/MS derivatization using a moving belt interface.

To date, none of the methods for analyzing DAEMPTA in complex matrices have been completely satisfactory, although work is still in progress. Derivatization with GC analysis has not yet provided acceptable sensitivity in complex matrices in the presence of other acidic decontamination products. Positive ion APCI has been used to determine the compound using the $[M + H]^+$ ion of m/z 240 and MS/MS fragmentation, using a Finnigan TSQ-7000 tandem mass spectrometer [19]. On the HP 5989A instrument, degradation is observed in the source to form significant amounts of m/z 162. Figure 1, top panel, shows a mass spectrum of DAEMPTA with a prominent $[M + H]^+$ ion but also significant m/z 162 signal. This mass

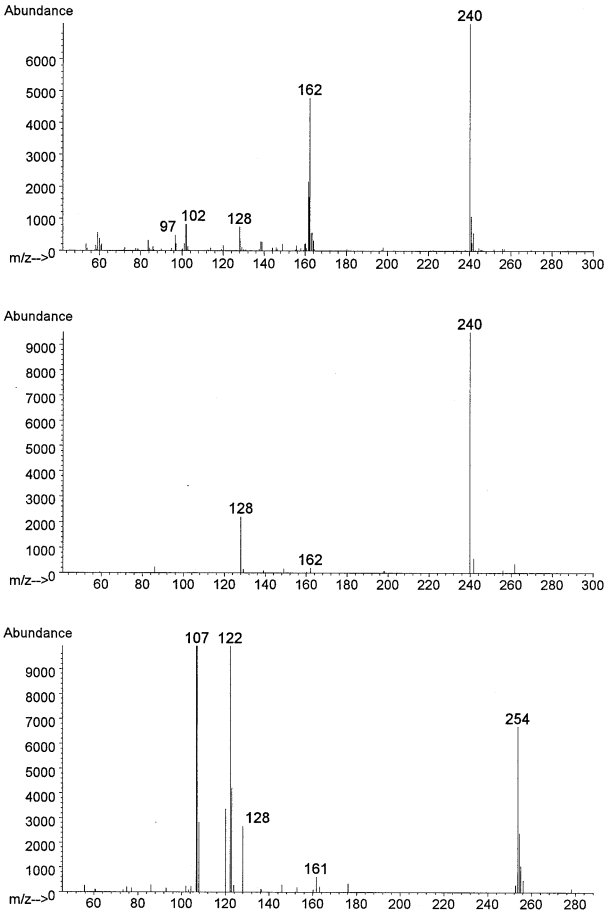

Figure 1. Mass spectra of *S*-[2-(diisopropylamino)ethyl] methylphosphonothioic acid, 100 μg/mL standard solution. Top panel: underivatized using positive ion APCI using a vaporizer temperature of 300 °C; middle panel: underivatized using positive ion ESI; bottom panel: derivatized with 8×10^{-3} M TMPAH after mixing with LC eluent. (The m/z 107 and 122 peaks are offscale, and both have peak signals of about 30,000.)

spectrum was taken with a vaporizer temperature of 300 °C, which is lower than the typical operating temperature. When the vaporizer temperature is increased from 300 to 375 °C, essentially no abundance of the m/z 240 ion is observed. This observation, along with previous experience with this approach, has shown that this approach may not be robust and reliable for a range of conditions. Positive ion ESI also gives signal for the m/z 240 ion which is comparable to APCI at low vaporizer temperatures, shown in Figure 1, middle panel. However, all of these methods have some difficulty in giving acceptable performance in decontamination matrices, which contain high salt concentrations and large amounts of ethyl methylphosphonic acid, which interferes or competes with the derivatization and detection of DAEMPTA.

Because of these difficulties, it is helpful to develop a variety of approaches for analyzing this compound. Because TMPAH was successful for derivatizing DAEMPTA in GC analysis, APCI-LC/MS analysis using postcolumn derivatization was done using TMPAH. The reaction forms the methyl ester ($[M + H]^+$ of m/z 254), eliminating the anionic site on the molecule. Figure 1, bottom panel, shows the mass spectrum of the methyl derivative formed with this approach. The derivatization with TMPAH has minimal dilution of the sample. For the derivatization, a solution of 0.1 M TMPAH is added at 15–25 μL/min to LC flow of 250 μL/min, giving only 6%–10% dilution of the LC flow. Thus, the sensitivity is comparable to APCI or ESI without derivatization. However, no standards of the methyl ester of DEAMPTA were available to determine the derivatization efficiency quantitatively, because this compound would be very toxic.

As additional work on sample cleanup and liquid chromatography of the samples is developed, the most effective ionization method will be selected based on the best fit to the sample preparation and the best sensitivity.

Methylphosphonic Acid (MPA)

Alkyl Methylphosphonic acids (AMPAs) are primary decontamination products of nerve agents, and MPA is the secondary decontamination product. AMPAs and MPA have been studied with a number of instrumental methods. They have been detected by GC with TMPAH derivatization [23, 24], trimethylsilyl derivatization [25, 26], tert-butyldimethylsilyl derivatization [27, 28], and pentafluorobenzyl derivatization [29]. They can be detected by LC/MS using ESI or APCI in positive or negative ion modes [12]. They have also been detected by CE with indirect UV detection [30] and CE/MS [31].

Methylphosphonic acid (MPA) can be detected directly using APCI or ESI. However, it is a divalent anion, so it may be less volatile than the monovalent AMPA ions. Formation of derivatives for GC analysis is sensitive to metal cations and pH of the solution [25, 27]. It is a reasonable test case for the detection of low-volatility inorganic ions using derivatization.

MPA was doubly derivatized in-source with APCI using TMPAH to form the dimethyl ester as the major analyte ion ($[M + H]^+$ of m/z 125), with a smaller peak for the single methyl ester ($[M + H]^+$ of m/z 111). Figure 2 shows the mass spectra of the underivatized and derivatized MPA solution. The underivatized MPA gives an $[M + H]^+$ of m/z 97, and no m/z 97 is observed in the derivatized spectrum. The CID mass spectrum of MPA was published previously by Black and Read [13].

The derivatization of MPA is very dependent on the amount of TMPAH added. The TMPAH was added as a 0.1 M solution in MeOH in the flow range of 0.5–5 μL/min to a flow of 250 μL/min of aqueous buffer. Too little TMPAH ($<10^{-4}$ M after mixing in the LC mobile phase) does not derivatize effectively. Too much ($>2 \times 10^{-3}$ M) and the signal disappears. Figure 3 shows the relative signal strength of the dimethyl ester of MPA (m/z 125) as a function of derivatization reagent flow.

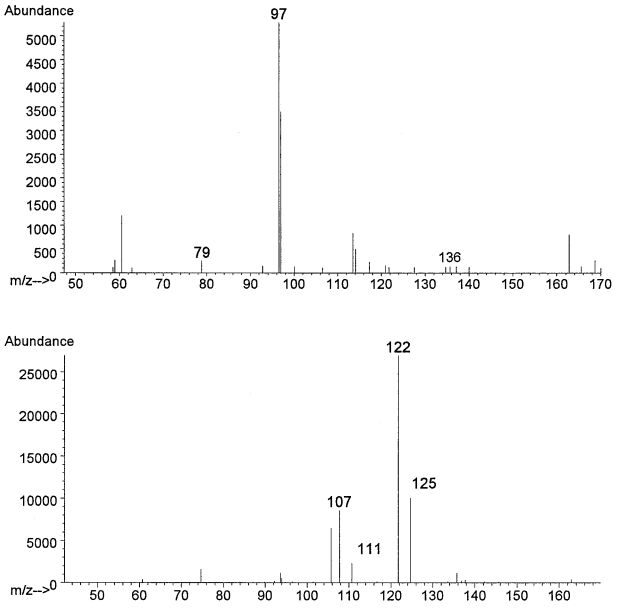

Figure 2. Mass spectra of methylphosphonic acid, 100 μg/mL standard solution. Top panel: underivatized; bottom panel: derivatized with 4×10^{-4} M TMPAH after mixing with LC eluent. In the bottom panel, the m/z 122 ion signal is offscale, and the peak signal is about 100,000.

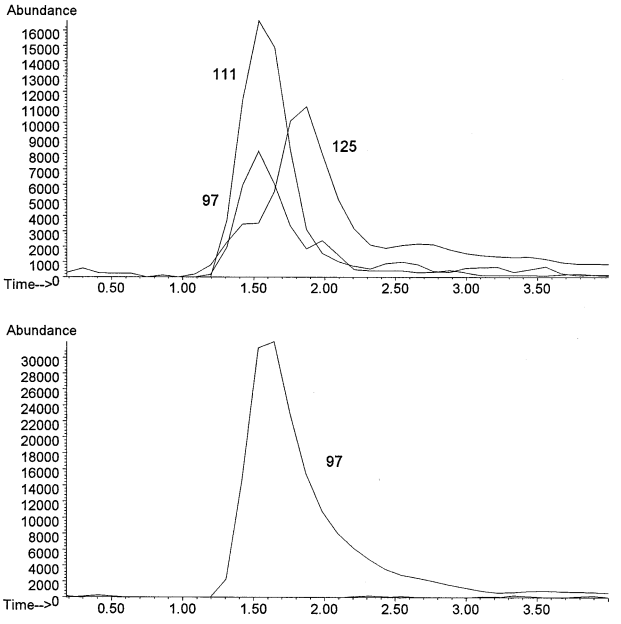

Figure 4. Chromatograph peak of derivatized vs. underivatized MPA, for a standard solution of 363 μg/mL in concentration. Top panel: Traces for m/z 97 (underivatized protonated MPA), m/z 111 (single methyl derivative), and m/z 125 (double methyl derivative). TMPAH flow was 1 μL/min of 0.1 M solution. Bottom panel: Trace for m/z 97 with no derivatizing solution added, but with the same chromatographic conditions.

This data were taken with a buffer concentration of 0.001 M ammonium acetate. With this buffer concentration, the addition of basic TMPAH strongly affects the pH of the solution. However, increasing the buffer concentration to 0.05 M ammonium acetate produced a nearly identical dependence on signal, even though the pH was well buffered in the range of 6–7 for all TMPAH concentrations. This result demonstrates that the decrease in signal is due to the TMPAH concentration and not the solution pH.

At high MPA concentrations (>200 μg/mL), there is incomplete derivatization, and the singly derivatized species and some underivatized protonated MPA are the predominant products, rather than doubly derivatized product. Figure 4 shows an extracted ion chromatogram of a solution of 363 μg/mL of MPA for deviatized and underivatized conditions. There is a distortion of the chromatographic peak shape, with the m/z 111 and 97 ions predominating in the highest concentration part of the peak, and the m/z 125 ion at the wings. The ion distribution shifts as the LC peak elutes, because the MPA concentration in the source changes. The relative signal for the m/z 125 ion can be increased by increasing the concentration of TMPAH, although the absolute signal decreases. Clearly, this effect would be a problem for quantitative work at high concentrations.

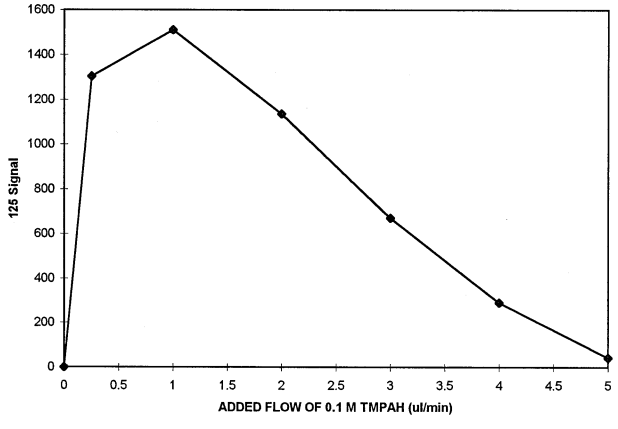

Figure 3. Dependence of the positive ion signal for the dimethyl ester of methylphosphonic acid on the added flow of TMPAH derivatizing reagent. Signal is in arbitrary units. Concentration of TMPAH solution is 0.1 M in methanol. LC flow is 250 μL/min of 0.001 M aqueous ammonium acetate. The standard solution of MPA was 145 μg/mL in concentration.

The dimethyl ester of MPA (dimethyl methylphosphonate, DMMP) is commercially available, so a calibration curve was obtained from standard solutions. The integrated signal for m/z 125 that was observed for the MPA double derivative, at low concentrations, is 5%–10% of the expected signal for the same concentration of DMMP standard. Some of this decrease in signal for MPA is due to signal suppression by the TMPAH. Analysis of a DMMP standard using the same flow of TMPAH (1 μL/min) gives a 30% of the signal compared to the signal with no TMPAH. So some of the signal decrease for the MPA double derivative is from signal suppression by the TMPAH, and some is because of derivatization reaction efficiency of <100%.

The reason for the strong dependence of signal on

the derivatization reagent concentration is probably because of the competition for charge between the N,N-dimethylaniline (DMA, $[M + H]^+$ of m/z 122), formed from decomposition of TMPAH, with the DMMP. DMA has a high proton affinity, so at high concentrations it is likely to scavange the charge in the APCI discharge and suppress ionization of DMMP. This effect was not a problem with the methyl ester of DAEMPTA, because that compound is also an amine and so has a higher proton affinity. This signal suppression indicates a limitation for the use of derivatizing reagents in APCI, and it is a particular problem for methyl derivatives, because the methyl group provides no added proton affinity to the analyte molecule.

Another issue that was studied was the affect of TMPAH derivatization on the sensitivity of MPA to cations in solution. It was hypothesized that methylation could improve the volatility of the analyte and decrease the probability of forming salt particles. However, it was found that, even with addition of TMPAH, the signal for MPA is sensitive to cations in solution. The presence of 200 ppm of Na^+ or Ca^{+2} decreases the signal for MPA, compared to the same standard with no cations. Thus, the use of postcolumn TMPAH derivatization is not a solution to the problem of detecting MPA in solutions with high salt content.

A few experiments were done to determine whether other AMPAs can be derivatized in-source with APCI using TMPAH. Isopropyl MPA was found to singly derivatize with TMPAH to form the monomethyl ester. There appeared to be no significant improvement in sensitivity.

Arsenic Compounds

The CW agent lewisite, 2-chlorovinylarsenic (III) dichloride, is decontaminated to form several arsenic compounds, including 2-chlorovinyl arsonous acid (CVAA) [32] and arsenic (III) oxide. CVAA can be detected with good sensitivity by derivatization with a dithiol compound, such as 1,3-propanedithiol, and GC detection [33]. Arsenic (III) oxide can be triply derivatized to a TMS derivative for GC analysis [34]. CVAA can be detected by LC with UV detection, and arsenic compounds can be detected with the best sensitivity by ICP/MS [35, 36] or other arsenic specific detection methods [37–42], although for these methods, identification of the compounds requires a chromatographic separation and retention time matching. Derivatization LC/MS can provide compound-specific information.

Without derivatization, the sensitivity for the arsenic compounds using LC/MS is not good. Arsenic (III) compounds such as arsenic (III) oxide are weak acids which do not strongly ionize in solution at low pH. The oxide gave an ion signal for m/z 107 and 123 at high concentrations using negative ion APCI, but the sensitivity was poor. Both negative ion APCI and ESI also gave ion signals for m/z 305 ($As_3O_5^-$), 321 ($As_3O_6^-$), 412 ($As_4O_7^-$), and possibly larger clusters, but also only at high concentrations.

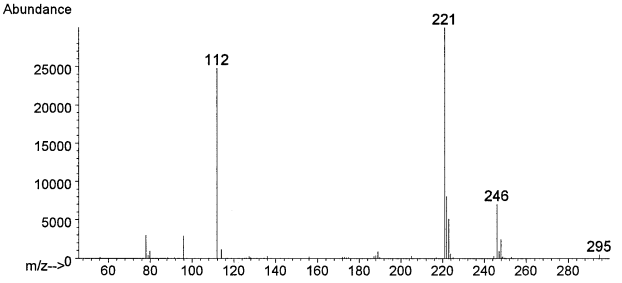

Figure 5. Mass spectrum of chlorovinyl arsonous acid (CVAA) derivatized in-source with 2-mercaptopyridine. Derivative peaks are m/z 246 and 248, and background peaks from the derivatizing reagent are at m/z 122 and 221. Derivatizing solution was 3.2×10^{-3} M after addition to the LC flow. The CVAA standard concentration was 132 μg/mL.

Postcolumn derivatization was used to improve the sensitivity. 2-Mercaptopyridine (Pyr–SH) was found to be a good postcolumn derivatizing reagent for As(III) compounds. The thiol group reacts readily with the As(III) center, analogous to GC methods [32]. Optimal concentrations of the reagent are $(1–5) \times 10^{-3}$ M of Pyr–SH after postcolumn addition and dilution. The signal is not strongly sensitive to the derivatizing reagent concentration. This method improves LC/MS sensitivity for CVAA and As(III) significantly, by an estimated 5–10 times.

Figure 5 shows a mass spectrum of a solution of CVAA with derivatization. CVAA could not be observed to give any signal in positive or negative ion APCI without derivatization at low concentrations. Figure 6 shows a comparison of the mass spectra of arsenic (III) oxide, underivatized using negative ion APCI, and derivatized with Pyr–SH.

Arsenic (III) oxide reacts to add two Pyr–SH molecules ($[M + H]^+$ is m/z 295). The most likely structure for this ion is a sulfonium ion:

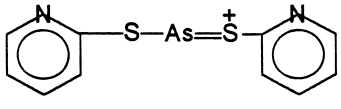

Diagram 2

CVAA adds one molecule of Pyr–SH ($[M + H]^+$ is m/z 246, with a m/z 248 ^{37}Cl isotope peak), also probably forming the sulfonium ion:

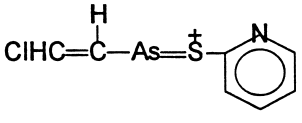

Diagram 3

Under some conditions, a double derivative of CVAA was observed with $[M + H]^+$ of m/z 357, which likely has a protonated structure. Pyr–SH produces a signifi-

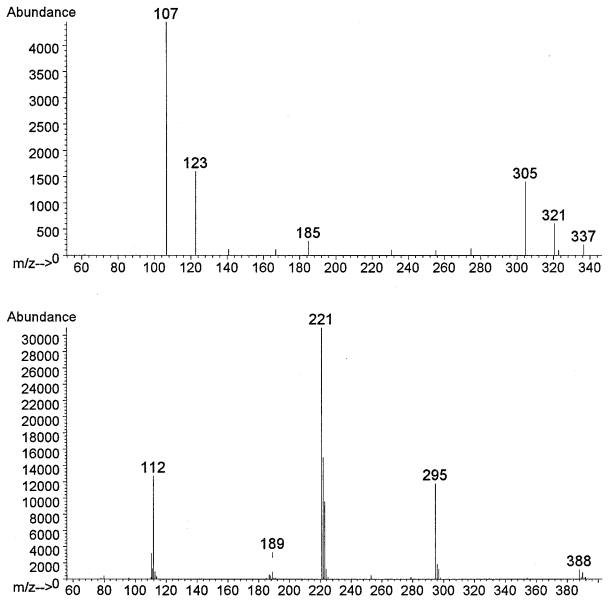

Figure 6. Mass spectra of arsenic (III) oxide. Top panel: 246 μg/mL standard without derivatization; bottom panel: 100 μg/mL standard with derivatization using 4×10^{-3} M 2-mercaptopyridine solution. In the bottom panel, the m/z 221 ion signal is offscale, and has an abundance of 80,000. The concentrations are given in terms of weight of As_2O_3.

cant background signal in the chromatogram at masses m/z 112 and 221 from the protonated thiol and disulfide, respectively.

Figure 7 shows a calibration curve of the m/z 295 signal for arsenic (III) oxide using postcolumn derivatization. The plot has good linearity, although it has a negative y intercept. The negative y intercept was confirmed in three repetitions of the calibration curve on different days, but the cause is not known. The negative y intercept gives a decreased sensitivity at low concentrations, particularly <10 μg/mL. In order to test the reproducibility of the signal for the derivative, multiple repetitions of an analysis of a single standard were done. For each repetition, the run time was 5 min, and 4×10^{-4} M of Pyr–SH was flowed into the source

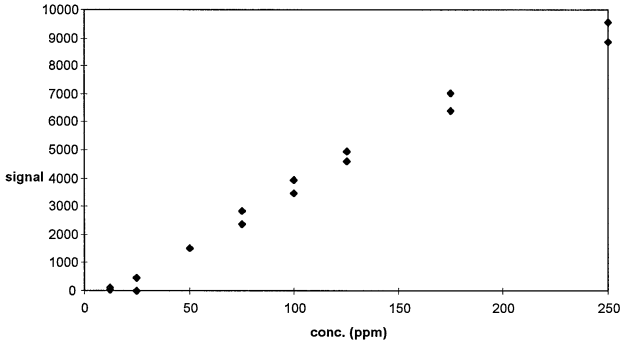

Figure 7. Calibration curve for arsenic (III) oxide derivatized in-source with 2-mercaptopyridine to form the double derivative at m/z 295. Derivatizing solution was 0.002 M after addition to LC flow. The concentrations are given in terms of weight of As_2O_3.

at a flow rate of 255 μL/min. For 15 repetitions, the relative standard deviation of the signal was 7.8%, and there was no trend showing decreasing signal during the runs.

The derivatization reaction produces sulfonium ions rather than protonated ions as a product, which raises the question of whether the pyridine or other amine functionality is necessary for the reagent. A second amine compound, 2-aminoethanethiol, was studied, and this compound also give analogous signals for the expected sulfonium ions for As(III) and CVAA. However, a compound that was not an amine, 2,3-dimercapto-1-propanol (also known as BAL, British Anti-Lewisite [43, 44]), but which is know to react well with arsenic (III) compounds, does not produce any signals for expected derivatization products. This compound is significantly more volatile than the amines. Another compound, 2,5-dimercapto-1,3,4-thiadiazole, was tried, but it was not very soluble in the aqueous mobile phase. Thus, the amine group on the derivatizing agent may be necessary to decrease the volatility and increase the solubility of the thiol reagent, which should improve the APCI ionization efficiency, even if the high proton affinity is not required to ionize the reaction product. However, such compounds have the disadvantage of producing strong background ion signals.

Alcohol Derivatization

Nitrogen mustards are CW agents that hydrolyze to produce the alcohols ethyldiethanolamine and triethanolamine. Sensitivity for the alcohols by LC/UV was improved by precolumn derivatizing with benzoyl chloride or 3,5-dinitrobenzoyl chloride, but this derivatization had to be done in anhydrous conditions with heating for 15–30 min at 60–100 °C. The derivatization modified the LC retention and increased the UV absorbance.

For LC/MS detection, benzoyl chloride was used for postcolumn, APCI derivatization. Derivatization occurred in hydrous conditions, so it was not necessary to use anhydrous LC mobile phases. Sensitivity was not affected, because ionization occurred at the amine rather than at the benzoate group. The parent mass shifted, which can remove the analyte from interferences, but the derivatizing reagent also introduced additional ions into the mass spectrum. Figure 8 shows the mass spectra of triethanolamine, with and without derivatization using benzoyl chloride. Without derivatization, the $[M + H]^+$ of m/z 150 is observed, which shifts to m/z 254 when singly derivatized. The CID mass spectrum of triethanolamine was published previously [13]. The mass spectra of ethyldiethanolamine are analogous, with an $[M + H]^+$ of m/z 134 without derivatization shifting to m/z 238 after derivatization.

Derivatization with 3,5-dinitrobenzoyl chloride was attempted to improve negative ion signal. No reaction products were observed in this case, even though this

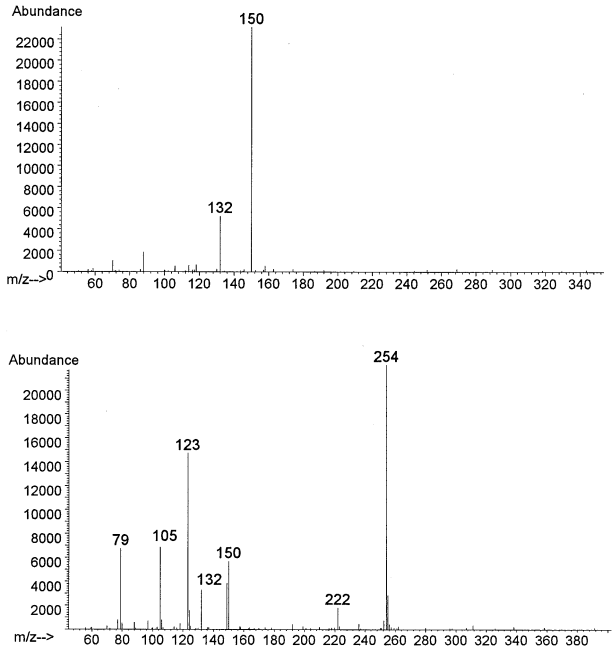

Figure 8. Mass spectra of 160 μg/mL standard of triethanolamine. Top panel: without derivatization; bottom panel, derivatization with 0.016 M benzoyl chloride after mixing with LC eluent. Ions of m/z 79, 105, and 123 are background ions from the benzoyl chloride.

reagent was successful for derivatizing the alcohols in solution. This may be because of poor derivatization or ionization efficiencies in the APCI source.

These results indicate that it is possible to derivatize alcohols in an APCI source with benzoyl chloride. It is still necessary to identify a derivatizing reagent with good reactivity and with a good proton affinity or electron affinity in order to detect nonamine-containing alcohols by APCI. An additional problem may be that many low-molecular-weight alcohols tend to be volatile, so they may evaporate in the APCI source before they have an opportunity to derivatize.

Discussion

LC/MS with postcolumn, in-source APCI derivatization can provide a method for the analysis of some types of difficult analytes. Postcolumn LC derivatization is experimentally simpler than GC derivatization, because the derivatizing agent is added to the postcolumn LC flow, requiring no additional sample preparation and no extra LC hardware in addition to the APCI source, aside from an extra syringe pump.

There are several cases in which postcolumn derivatization can be advantageous for LC/MS detection. DAEPMTA formed a methyl ester by using TMPAH, which eliminated the zwitterion character. MPA was also methylated. In the case of MPA, however, the ionization of the reagent competed with the ionization of the derivatized analyte, so the detection efficiency was not good. In addition, TMPAH produced abundant background ions in the mass spectrum which could potentially interfere with some analytes. The use of TMPAH also did not solve the general problem of the decrease of signal for MPA in the presence of cations in the sample solution.

For the detection of arsenic (III) compounds, derivatization with 2-mercaptopyridine significantly improved the sensitivity, compared to either positive or negative APCI or ESI without derivatization. This improvement is not surprising, because derivatization with related reagents improves sensitivity for GC analysis. Unlike TMPAH derivatization, there was no significant signal suppression by the derivatizing reagent. Because the derivatizing reagent and the derivatized analyte had similar functional groups, there was not any competition for ionization, and the signal strength was not strongly dependent on the concentration of reagent. The approach had reasonable long-term stability and could be used for quantitation.

Derivatization also offers the option of altering the major mass spectral peaks to avoid an interference. When derivatization is done postcolumn, the mass spectrum can be changed without changing the LC retention times and without extra sample preparation. This can provide an alternative confirmation or identification method for particular functional groups.

Another limitation of the postcolumn derivatization method is the relatively high load of reagent that is introduced into the APCI source, which can produce contamination of the electrodes and corona needle. This can require frequent (at least daily) cleaning. This could restrict the analysis of high sample volumes using this method, although small numbers of samples have been analyzed routinely. However, the problem of analyses that cause source contamination for APCI and ESI is of general concern to the instrument manufacturers. Many LC users are interested in methods that use nonvolatile buffers or additives, which can build up in the source. New developments in sources that are self-cleaning [45] or tolerant of nonvolatile buffers [46] should alleviate this problem.

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