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**TITLE**CAPILLARY COLUMN GAS CHROMATOGRAPHIC-TANDEM MASS SPECTROMETRIC ANALYSIS OF
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Capillary column gas chromatographic-tandem mass spectrometric analysis of phosphate esters in the presence of interfering hydrocarbons

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Abstract

Daughter, parent and constant neutral loss spectra, and multiple reaction ion monitoring data were all evaluated for the detection and confirmation of phosphate esters during capillary column GC-MS-MS analysis with a hybrid tandem mass spectrometer. Constant neutral loss and parent modes involve scanning of the sector, thus reducing the benefits of higher sector resolution, while daughter and multiple reaction ion monitoring data may be acquired with higher sector resolution. The benefit of higher sector resolution was demonstrated for the detection and confirmation of phosphate esters in the presence of diesel exhaust extract components at levels several orders of magnitude above that of the phosphate esters. Detection limits for daughter operation were approximately the same as those obtained during capillary column GC-MS analysis of standards under electron impact ionization, and *S/N* ratios in excess of 100:1 were observed during multiple reaction ion monitoring of the diesel exhaust extract spiked at the 200 pg level with phosphate esters.

1. Introduction

The widespread agricultural and industrial use of organophosphorus compounds as pesticides, fertilizers and fire retardants has led to the development of numerous instrumental methods for the detection and confirmation of these compounds in environmental samples. Some organophosphorus compounds, including pesticides and chemical warfare agents or their decomposition products, are toxic and for this reason highly specific methods are required for

the trace detection of these compounds in the environment. Mass spectrometry (MS) and in particular, capillary column gas chromatography (GC)-MS, while generally accepted as the technique of choice for the confirmation of many organophosphorus compounds, has limitations in the presence of chemical interferences. The selectivity of tandem mass spectrometry (MS-MS), particularly when interfaced to a chromatographic separation technique, may overcome difficulties associated with the confirmation of target compounds in the presence of chemical interferences.

This drive for increased specificity for the trace analysis of toxic organophosphorus compounds

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in environmental and other samples has resulted in a number of applications involving the use of MS-MS. Recent pesticide studies include a paper detailing the MS-MS data for 26 organophosphorus pesticides [1], the use of MS-MS for the determination of pesticide residues in foods [2,3] and the demonstration of high-performance liquid chromatography-MS-MS with thermospray ionization for the analysis of organophosphorus pesticides [4]. Similar investigations have been conducted with organophosphorus chemical warfare agents to support the detection and confirmation of these compounds under the United Nations Chemical Weapons Convention. The ammonia chemical ionization (CI) daughter spectra of four organophosphorus chemical warfare agents have been reported [5] and the specificity of capillary column GC-MS-MS has recently been demonstrated for the confirmation of organophosphorus chemical warfare agents in matrices similar to those expected during battlefield sampling [6,7]. In the latter two cases the MS-MS analyses were performed under low-resolution conditions with a hybrid instrument. Higher sector resolution with a hybrid instrument should decrease chemical interferences over those observed during analysis under low-resolution hybrid or triple quadrupole operation. The potential advantages of higher resolution during daughter and multiple reaction ion monitoring (RIM) operation and the relative merits of parent and constant neutral loss operation have not been previously investigated for the capillary column GC-MS-MS confirmation of organophosphorus compounds.

Mass-analysed ion kinetic energy mass spectra have been acquired for dimethyl methylphosphonate and trimethyl phosphite [8] and the daughter spectra of m/z 110 for trimethyl phosphate and m/z 110 and 138 for triethyl phosphate have been published [9,10]. The MS⁵ spectra for triethyl phosphate was obtained during multiple-stage MS analysis [11] and a number of other organophosphorus compounds, including phosphate esters, have been characterized during atmospheric-pressure ionization MS-MS [12-14]. Most recently a homologous series of n -C₁

to n -C₄ phosphate esters (trimethyl phosphate, triethyl phosphate, tri- n -propyl phosphate and tri- n -butyl phosphate), often employed as chemical simulants for chemical warfare agents, were characterized by MS-MS. Daughter spectra were obtained for all the principal electron impact ionization (EI) ions of each phosphate ester and optimal collisional-activated dissociation (CAD) cell conditions were established by stepping the CAD cell energy from 1 to 100 eV (laboratory scale) in 1-eV increments with residual air and argon target gases [15].

Previous CAD cell conditions [15], were incorporated in the present study designed to evaluate the capabilities of a hybrid tandem mass spectrometer for the confirmation of organophosphorus compounds. A complex hydrocarbon matrix, similar to that expected during battlefield sampling, was used to evaluate the relative merits of daughter, parent, constant neutral loss and RIM for the confirmation of organophosphorus compounds in the presence of significant chemical interferences.

2. Experimental

2.1. Standards

Trimethyl phosphate, triethyl phosphate, tri- n -propyl phosphate and tri- n -butyl phosphate were provided by the Defence Research Establishment Suffield Organic Chemistry Laboratory. Standard solutions used for capillary column GC-MS-MS analysis were prepared in dichloromethane (BDH, Omnisolv).

The phosphate esters were added to a complex extract of diesel exhaust emissions collected on a Canadian C2 respirator canister. Two aliquots of this diesel extract were spiked with the phosphate esters such that 1- μ l GC injections of the spiked extracts resulted in the loading of either 2.5 ng or 200 pg of phosphate ester. This matrix, described previously [6], contains numerous hydrocarbons at high concentrations and was selected to evaluate the sensitivity and selectivity

of GC-MS-MS for the analysis of phosphate esters.

2.2. Instrumental

Capillary column GC-MS-MS analyses were performed with a VG AUTOSPEC-Q (EBEQQ geometry) hybrid tandem mass spectrometer equipped with a Hewlett-Packard Model 5890 gas chromatograph. A 15 m \times 0.32 mm I.D. DB-1701 J & W capillary column (0.25 μ m film thickness) was used for all GC-MS and GC-MS-MS analyses with the following temperature program: 40°C (2 min hold) 10°C/min to 280°C (5 min hold). All GC injections were cool on-column using an injector of our own design [16].

The EI-MS operating conditions were as follows: source pressure, $3 \cdot 10^{-6}$ Torr (1 Torr = 133.322 Pa); source temperature, 200°C; electron energy, 70 eV; and electron emission, 100 μ A. EI mass spectra were obtained using a VG EI/CI source at a resolution of 1000 (10% valley definition) and an accelerating voltage of 8 kV. Mass spectral data were collected from 250 to 50 u at a scan rate of 1 s/decade.

In an initial study, all four phosphate esters were introduced through the heated septum reservoir and breakdown curves were obtained for all the principal ions at three different CAD cell pressures over 1 to 100 eV [15]. The best compromise between sensitivity and spectral content were obtained with a CAD cell argon pressure of $8 \cdot 10^{-7}$ – $9 \cdot 10^{-7}$ Torr and an energy of 25 eV (laboratory scale). This argon pressure reduced the intensity of the perfluorokerosene (PFK) ion at m/z 219 to 50% of its original intensity under residual air CAD cell pressures. A typical daughter spectrum for m/z 219 at 30 eV (with no detectable signal below 12 eV) under this CAD cell condition gave the following ion ratios: m/z 219: m/z 131: m/z 69 = 1:0.25:0.15. Daughter spectra were obtained under these CAD cell conditions for the molecular ion or a high-mass EI fragmentation ion for each of the four phosphate esters during capillary column GC-MS-MS analysis. The quadrupole was operated at unit resolution and scanned from 250 to

50 u at 1 s/scan and the sector resolution was set at a value in the 1000 to 3400 (10% valley definition) range.

Multiple reaction ion monitoring GC-MS-MS data were obtained for tripropyl phosphate by monitoring the m/z 183 to m/z 141 and m/z 183 to m/z 99 transitions under the chromatographic and CAD cell conditions described above. Each transition was monitored for 80 ms with a 40-ms delay. Resolution of the sector was either 1000 or 2500 (10% valley definition).

Parent spectra for diagnostic phosphate ester EI fragmentation ions were obtained under identical chromatographic and CAD cell conditions used for daughter spectra acquisition. Spectra were obtained by scanning the sector from 250 to 50 u at 0.7 s/decade.

Constant neutral loss spectra were obtained for the loss of 30 u (corresponds to the loss of CH₂O; trimethyl phosphate detection), 28 u (corresponds to loss of C₂H₄; triethyl phosphate monitoring), 42 u (corresponds to loss of C₃H₆; tripropyl phosphate monitoring) and 56 u (corresponds to loss of C₄H₈; tributyl phosphate monitoring) by scanning the sector and quadrupole over 250 to 50 u at 1.5 s/scan. Chromatographic and CAD cell conditions were identical to those used during daughter spectra acquisition.

Daughter, parent or constant neutral loss spectra for all four phosphate esters were obtained in a single chromatographic analysis by monitoring for trimethyl phosphate during the first 6 min and each of the remaining phosphate esters over subsequent 3-min intervals (triethyl phosphate followed by tripropyl phosphate and tributyl phosphate).

3. Results and discussion

3.1. General

Hybrid tandem mass spectrometers are typically operated in one of the following four modes during capillary column GC-MS-MS analysis:

Scan mode	Sector operation	Quadrupole operation
(a) Daughter scan	Transmit specified ion (parent ion)	Scan over a mass range
(b) Parent scan	Scan over a mass range	Transmit specified ion (daughter ion)
(c) Constant neutral loss	Scan over a mass range	Quadrupole scanning linked to the sector scanning such that at any point in time the quadrupole transmits lower mass ions equal to the neutral loss mass
(d) Reaction ion monitoring (RIM)	Transmit specified ion (parent ion)	Transmit specified ion (daughter ion)

The first three modes result in the acquisition of MS data over a given mass range and as such provide a high level of confirmation, particularly if the acquired mass spectra contain three or more ions. The fourth mode, RIM, like selected ion monitoring during capillary column GC-MS, results in the acquisition of single (or multiple) ion data. This results in an increase in sensitivity, generally at the expense of identification certainty.

Two modes, daughter and RIM, require transmission of ions of a selected mass through the sector of the hybrid instrument. By increasing the resolution of the sector it is possible to resolve a target ion from other ions of the same nominal mass. This advantage of the hybrid geometry, which generally leads to a reduction in chemical interferences, is not possible with triple quadrupole tandem mass spectrometers and may be advantageous for the analysis of target compound(s) in complex matrices.

Parent operation, a technique often used for the detection of compounds that fragment to form a specific daughter ion (*e.g.*, compounds with a certain functional group), generally does not have equivalent utility for target compound analyses in complex samples. The nominal (daughter) mass transmitted through the quadrupole could easily be formed from ions, other than those of interest, in a complex sample matrix. This would result in the acquisition of numerous parent spectra, making it difficult to resolve the target compound unless the daughter mass was unique to the compound and was not common to the matrix.

Constant neutral loss can be quite specific provided the neutral loss is unique to the target compound(s) and not to extraneous sample com-

ponents. However, both parent and constant neutral loss operation gain little from increased resolution as the sector is scanned as opposed to being locked on a particular mass (where the resolution of the sector determines the width of the mass window).

3.2. Phosphate ester applications

A concern of the United Nations Organization for the Prohibition of Chemical Weapons is the ability to retrospectively detect and positively identify the presence of organophosphorus chemical warfare agents and related compounds in samples collected in support of peacekeeping or peacemaking operations. Under these scenarios the likelihood of collecting extraneous compounds increases, particularly where diesel powered vehicles would be in use. Collected airborne samples would contain a large amount of hydrocarbon material similar to the diesel exhaust samples collected previously [6,7]. This complex matrix was therefore selected to evaluate the suitability of capillary column GC-MS-MS with a hybrid instrument for the detection and confirmation of phosphate esters.

Fig. 1 illustrates the capillary column GC-MS total-ion-current chromatogram obtained for the diesel exhaust extract spiked with 2.5 ng of each phosphate ester under full scanning EI conditions, the most commonly employed mode of operation for the analysis of environmental samples. The four phosphate esters were effectively masked by the presence of high levels of chemical interferences and EI ions characteristic of hydrocarbons were acquired at the retention times of the phosphate esters. Only tributyl phosphate, which elutes following the bulk of the

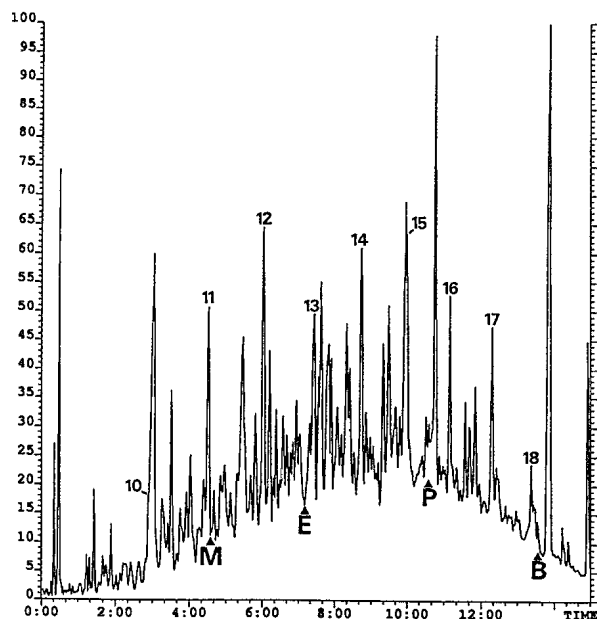


Fig. 1. Capillary column GC-MS (EI) total-ion-current (250 to 50 u) chromatogram obtained for the diesel exhaust extract spiked with 2.5 ng of trimethyl phosphate (M), triethyl phosphate (E), tripropyl phosphate (P) and tributyl phosphate (B). *n*-Alkane carbon numbers are indicated above the appropriate sample component and the retention time of phosphate esters are indicated by letter. Time in min.

hydrocarbon envelope, exhibited characteristic ions in approximately the same ratio as observed during standard analysis. However, background ions were equivalent to or greater than the signal of m/z 155 and m/z 211, which would suggest a sample detection limit of approximately 25 ng ($S/N = 10$). Sample detection limits for the other phosphate esters would be much higher.

As in conventional MS analyses, the monitoring of higher-mass ions during MS-MS analyses is preferred as chemical background decreases with mass. For this reason, the daughters of the molecular ion or highest-mass EI fragmentation ion (in the absence of molecular ion data) were evaluated for the detection and confirmation of phosphate esters spiked into the diesel exhaust extract at the 2.5-ng level (Fig. 2). Monitoring of the daughters of the molecular ions (even mass) of trimethyl phosphate and triethyl phosphate resulted in the detection of both compounds with minimal chemical interference. Considerably more chemical interference

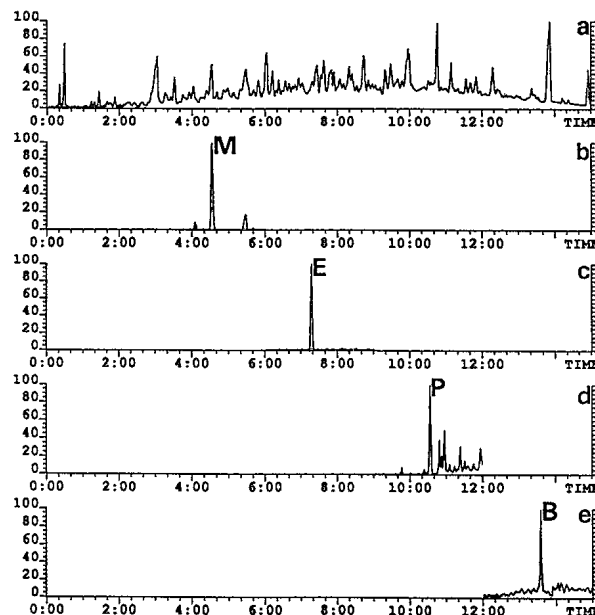


Fig. 2. (a) Capillary column GC-MS (EI) total-ion-current (250 to 50 u) chromatogram obtained for the diesel exhaust extract spiked at the 2.5-ng level with each phosphate ester. CAD chromatograms for daughters of (b) m/z 140 [M^+ for trimethyl phosphate (M)], (c) m/z 182 [M^+ for triethyl phosphate (E)], (d) m/z 183 [$[M - C_3H_5]^+$ for tripropyl phosphate (P)] and (e) m/z 211 [$[M - C_4H_7]^+$ for tributyl phosphate (B)] obtained with a sector resolution of 1000. Time in min.

was noted during the acquisition of daughter data for the higher-mass fragmentation ions (odd mass) of tripropyl phosphate and tributyl phosphate. The observed reduction in chemical interference during monitoring of even mass ions (generally odd electron species) and the reduction of chemical interferences with increased mass were consistent with generally accepted MS ion abundances. Fig. 3 illustrates the CAD spectra obtained for the daughters of m/z 140 (M^+ for trimethyl phosphate), m/z 182 (M^+ for triethyl phosphate), m/z 183 ($[M - C_3H_5]^+$ for tripropyl phosphate) and m/z 211 ($[M - C_4H_7]^+$ for tributyl phosphate) obtained with a sector resolution of 1000. The daughter spectra obtained were identical to those obtained with standards, even in the presence of numerous chemical interferences, and contained the minimum of three ions, considered essential for target compound confirmation.

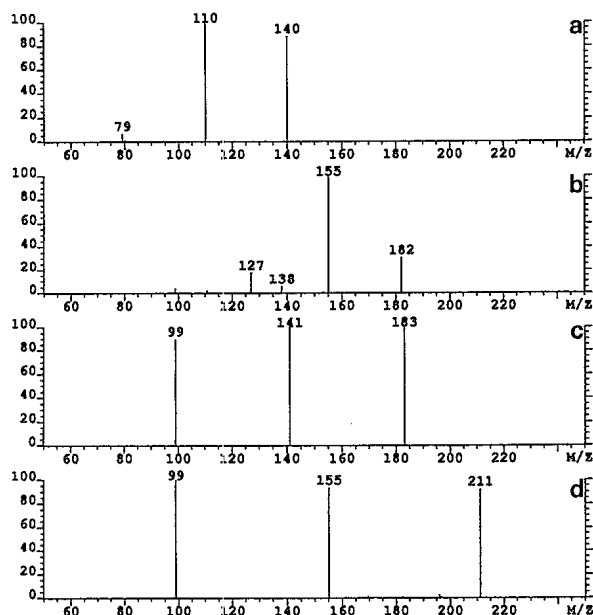


Fig. 3. Daughter spectra of (a) m/z 140 for trimethyl phosphate, (b) m/z 182 for triethyl phosphate, (c) m/z 183 for tripropyl phosphate and (d) m/z 211 for tributyl phosphate obtained during capillary column GC-MS-MS analysis of diesel exhaust extract spiked at the 2.5-ng level with each phosphate ester (see Fig. 2).

The highest level of chemical interferences were noted during acquisition of the daughters of m/z 183.08 ($[M - C_3H_5]^+$ for tripropyl phosphate). The bulk of the chemical interferences were likely due to hydrocarbon or amine (from amines on the charcoal bed material used for diesel exhaust sampling) ions with the following general formulae, $[C_xH_y]^+$ or $[C_xH_yN]^+$. By increasing the sector resolution to 2400 (10% valley definition), a significant reduction in chemical interference was observed for the daughters of m/z 183.08 (Fig. 4). Similar reductions in chemical interference were observed for the other three phosphate esters upon increasing the sector resolution to 2400, the practical upper limit for reliable automated operation. Above this resolution (e.g., 3400) manual operation was required to ensure transmission of the desired mass.

The sensitivity of daughter operation, based on 2.5-ng injections of phosphate ester standards, was found to approach typical EI full

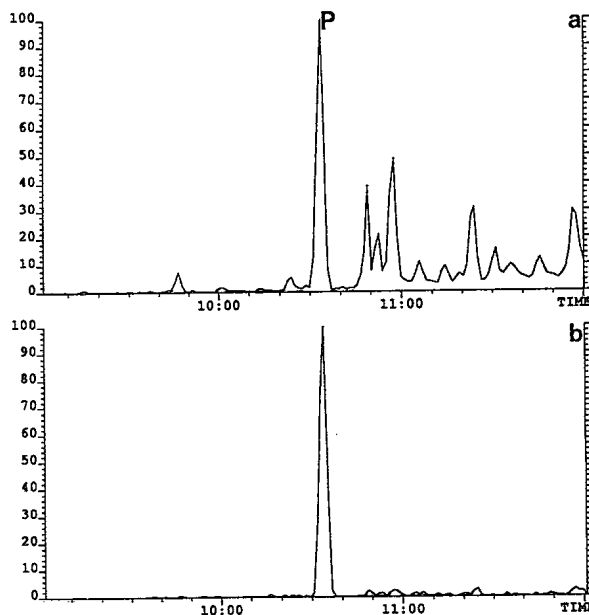


Fig. 4. CAD chromatograms for daughters of m/z 183.08 ($[M - C_3H_5]^+$ for tripropyl phosphate (P)) obtained with a sector resolution of (a) 1000 and (b) 2400. Time in min.

scanning detection limits (e.g., 100 to 500 pg). Lower levels of phosphate esters may be confirmed by multiple RIM (minimum of two transitions) in a mode analogous to selected ion monitoring (SIM). However, RIM sensitivity suffers over that routinely quoted for SIM due to quadrupole transmission of some neutrals formed in the CAD cell.

Multiple RIM was evaluated for the diesel exhaust extract spiked with 200 pg of tripropyl phosphate, as chemical interference was greatest for this phosphate ester (see Fig. 2). Fig. 5 illustrates the RIM chromatograms for the m/z 183 to m/z 141 transition (loss of C_3H_6) with a sector resolution of 1000 and 2400. Chemical interferences eluting after tripropyl phosphate were reduced with increased resolution and similar S/N ratios in excess of 100 were observed for tripropyl phosphate at both sector resolutions. During the same analysis the m/z 183 to m/z 99 transition, due to sequential losses of C_3H_6 , was also monitored to meet confirmation requirements (monitoring of a minimum of three ions). Increased resolution was of little benefit as

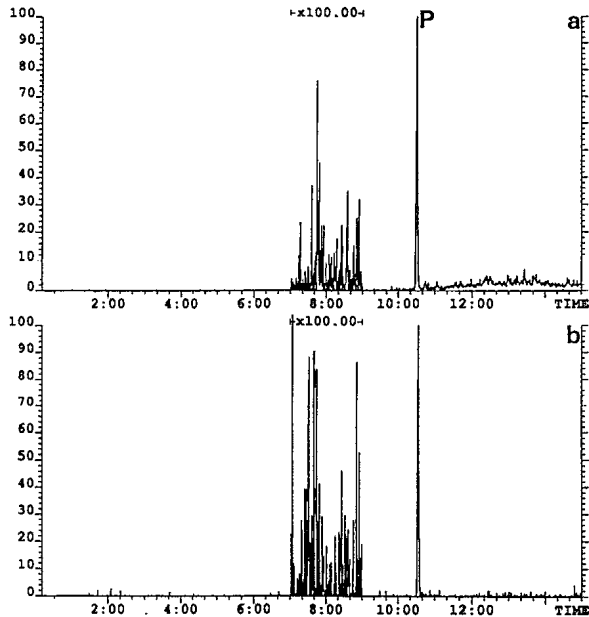


Fig. 5. Reaction ion monitoring chromatograms obtained for m/z 183 to m/z 141 transition during analysis of diesel exhaust extract spiked with 200 pg of tripropyl phosphate with a sector resolution of (a) 1000 and (b) 2500. Time in min.

this transition was not observed in the daughter spectra of typical extract sample components.

Constant neutral loss can be specific if the neutral loss monitored is not common to the chemical background. Neutral losses of CH_2O (for trimethyl phosphate) and C_xH_{2x} (where $x = 2, 3$ or 4 for the remaining phosphate esters) were selected for evaluation as they were commonly observed during characterization of these compounds. Fig. 6 illustrates the constant neutral loss spectra obtained during GC-MS-MS analysis of a 5-ng standard. The spectra obtained were consistent with the acquired daughter data, with the observed ions being those that would fragment in the CAD cell to give rise to a lower mass ion due to loss of a CH_2O or C_xH_{2x} .

The specificity of constant neutral loss for phosphate esters was evaluated by spiking the diesel exhaust extract at the 2.5-ng level. While the spiked components appeared to be resolved from chemical interferences, this was not the case. All the acquired constant neutral loss spectra were heavily influenced by coeluting

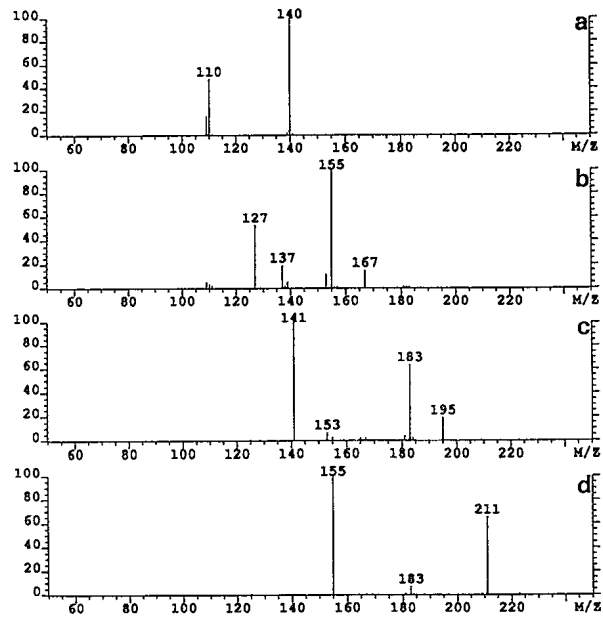


Fig. 6. Constant neutral loss spectra for (a) trimethyl phosphate (loss of 30 u), (b) triethyl phosphate (loss of 28 u), (c) tripropyl phosphate (loss of 42 u) and (d) tributyl phosphate (loss of 56 u) obtained during capillary column GC-MS-MS analysis of a standard containing 5 ng of each phosphate ester.

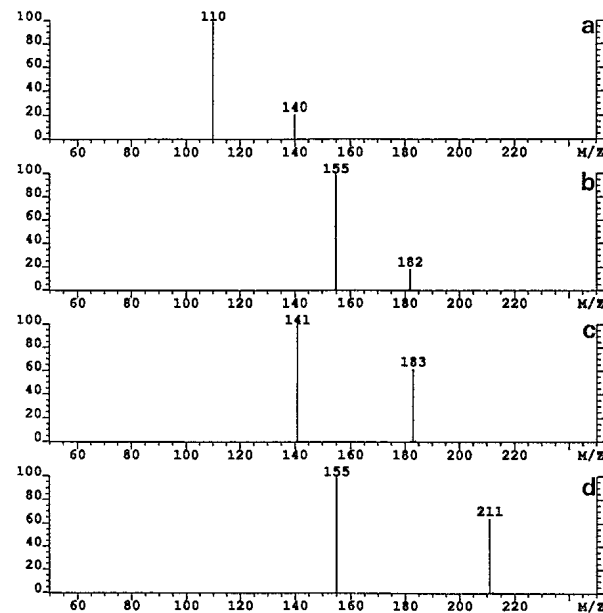


Fig. 7. Parent spectra of (a) m/z 110 for trimethyl phosphate, (b) m/z 155 for triethyl phosphate, (c) m/z 141 for tripropyl phosphate and (d) m/z 155 for tributyl phosphate obtained during capillary column GC-MS-MS analysis of a standard containing 4 ng of each phosphate ester.

hydrocarbon(s). The spectra closest to that obtained with a standard was that at the retention time of trimethyl phosphate. The loss of CH_2O would be less likely than C_xH_{2x} for a hydrocarbon matrix, but the possibility of constant neutral loss of C_2H_6 remains and likely contributed to the chemical background in the acquired spectra.

Parent spectra of higher-mass EI fragmentation ions at m/z 110 ($[\text{M} - \text{CH}_2\text{O}]^+$ for trimethyl phosphate), m/z 155 ($[\text{M} - \text{C}_2\text{H}_3]^+$ for triethyl phosphate), m/z 141 ($[(\text{PrO})\text{P}(\text{OH})_3]^+$ for tripropyl phosphate) and m/z 155 ($[(\text{BuO})\text{P}(\text{OH})_3]^+$ for tributyl phosphate) were obtained during GC-MS-MS analysis of a 4-ng standard (Fig. 7). However, the parent data obtained following spiking of the diesel exhaust extract with 5 ng of each phosphate ester were heavily influenced by coeluting interferences and were not interpretable.

4. Conclusions

Daughter, parent and constant neutral loss spectra, and multiple reaction ion monitoring data were all evaluated for the detection and confirmation of phosphate esters during capillary column GC-MS-MS with a hybrid tandem mass spectrometer. Constant neutral loss and parent modes involve scanning of the sector, thus negating the potential benefits of higher sector resolution. Both parent and constant neutral loss were evaluated as possible techniques for the detection and confirmation of phosphate esters in the presence of chemical interferences, similar to those expected during battlefield sampling. Neither technique was specific to the phosphate esters and resulted in the detection of considerable chemical hydrocarbon content.

Daughter and multiple reaction ion monitoring data may be acquired while operating with higher sector resolution. This benefit was demonstrated for the detection and confirmation of phosphate esters in the presence of hydrocarbon interferences at levels several orders of magnitude above that of the phosphate esters. De-

tection limits for daughter operation were approximately the same as those obtained during capillary column GC-MS analysis of standards under electron impact ionization, and S/N ratios in excess of 100 were observed during multiple reaction ion monitoring of the same diesel exhaust extracts spiked at the 200 pg level. Both of these modes of operation were highly specific for the detection and confirmation of these chemical warfare agent simulants in presence of numerous chemical interferences.

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