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Identification of iso- and *n*-propylphosphonates using liquid chromatography–tandem mass spectrometry and gas chromatography–Fourier transform infrared spectroscopy

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Abstract

Organophosphorus nerve agents and their precursors, specifically listed in the schedules of chemicals in the Annex to the Chemical Weapons Convention (CWC), include analogues with C_1–C_3 alkyl groups on phosphorus. The Organisation for the Prohibition of Chemical Weapons (OPCW) requires designated laboratories to unequivocally identify isomeric propyl groups bonded to phosphorus in analytes that may be present in samples submitted for analysis. Homologous series of isomeric pairs of dialkyl iso- and *n*-propylphosphonates, alkyl iso- and *n*-propylphosphonochloridates, and alkyl iso- and *n*-propylphosphonofluoridates, have been analysed by liquid chromatography–ion trap tandem mass spectrometry and/or by gas chromatography–Fourier transform infrared spectroscopy. The results show that *P*-propyl isomers can be reliably differentiated by collision induced dissociation (CID) of selected fragment ions and by their infrared P=O stretching and C–H deformation frequencies.

Keywords: Warfare agents; Organophosphorus compounds; Phosphonates; Propylphosphonates

1. Introduction

The Chemical Weapons Convention (CWC) prohibits the development, production, stockpiling and use of chemical weapons [1], and is administered by the Organisation for the Prohibition of Chemical Weapons (OPCW) based in The Hague, The Netherlands. An annex to the CWC lists certain toxic chemicals and their precursors in three schedules, according to the risk they pose to the Convention. An important component of the CWC is a verification regime, which may include inspections of declared or suspect CW facilities. Under certain circumstances samples may be collected from these facilities and analysed in off-site laboratories. The OPCW maintains a network of designated laboratories for this purpose, and requires these laboratories to participate in one proficiency test per year. Laboratories are required to identify a number of spiked chemicals according to a strict set of criteria.

Nerve agents constitute one of the most important groups of chemical warfare (CW) agents. They fall into three classes, defined in Schedule 1A of the CWC by generic structures **1**–**3**. A number of key precursors are included in Schedules 1 and 2 of the CWC, including dialkyl alkylphosphonates and alkyl alkylphosphonochloridates (mostly Schedule 2B.4, chlorosarin and chlorosoman 1B.11) related to generic structures **1** and **2**. Dialkyl alkylphosphonates can be used as precursors and are often present in nerve agents as by-products of the production process. Due to their stability they are often used as spiking chemicals in proficiency tests. The alkyl substituent on phosphorus in **1** (G agent series) and **2** (V agent series) may be methyl, ethyl, isopropyl or *n*-propyl. The criteria for unequivocal identification, stipulated by the Technical Secretariat of the OPCW, require the iso- and *n*-propyl isomers to be

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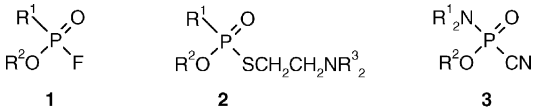
unequivocally identified. The *O*-alkyl substituents, which may contain from 1 to 10 carbon atoms and therefore include a very large number of isomers, need to be identified only in terms of the molecular formula. Differentiation of *P*-propyl substituents has become an important feature of OPCW proficiency tests. Although *P*-propyl isomers may be differentiated by GC retention time, and in many cases by EI mass spectra if standards or reference spectra are available, they are difficult to identify if standard data are not available. They can be readily differentiated by NMR spectroscopy but only if concentration and matrix permit. An inability to differentiate *P*-propyl isomers in the screening phase of a proficiency test usually requires the laboratory to synthesise alternative isomers, adding considerably to the pressures of time in these tests.



Two techniques that can differentiate isomeric propylphosphonates are liquid chromatography–tandem mass spectrometry (LC–MS–MS) and gas chromatography–Fourier transform infrared spectroscopy (GC–FT-IR). LC–MS–MS, using either atmospheric pressure chemical ionisation (APCI) or electrospray ionisation (ESI), provides an important screening and identification procedure for the hydrolysis products of nerve agents (and other CW agents) and dialkyl alkylphosphonates [2,3]. Although simple ESI or APCI spectra do not generally discriminate *P*-propyl isomers, van Baar et al. [4] demonstrated clear differentiation of iso- and *n*-propylphosphonic acids using electrospray tandem mass spectrometry, by collision induced dissociation (CID) of the protonated molecules. Vibrational frequencies in IR are also sensitive to small changes in the structural environment [5–7]. In particular, P=O stretching and C–H deformation frequencies of the *P*-alkyl group are sensitive to the nature of the alkyl group bonded to phosphorus. These IR bands therefore offer an additional opportunity for differentiating *P*-propyl isomers.

This paper extends the method of van Baar to dialkyl propylphosphonates and alkyl propylphosphonochloridates, and describes the application of GC–FT-IR to differentiate *P*-propyl isomers on the basis of P=O and C–H vibrational frequencies. The methods are validated by comparing the LC–MS–MS and/or GC–FT-IR spectra of a homologous series of 15 isomeric pairs of dialkyl propylphosphonates, and 5 isomeric pairs each of alkyl phosphonochloridates and alkyl phosphonofluoridates. The availability of a number of isomeric pairs of *O*-propyl analogues also allowed a comparison of LC–MS spectra as a means of differentiating *O*-propyl isomers.

2. Experimental

2.1. Materials

Fifteen pairs of isomeric dialkyl propylphosphonates, and five pairs each of alkyl propylphosphonochloridates and alkyl propylphosphonofluoridates, were synthesised by the Organic Chemistry Group, Dstl, Porton Down. Purities were generally >95% and structures were confirmed by ^{1}H NMR. For LC–MS, ammonium formate was purchased from Fluka (Gillingham, UK) and HPLC-grade methanol was from Aldrich (Gillingham, UK); water from a Milli-Q system (Millipore, UK) was used for all dilutions and mobile phase.

2.2. LC–MS and LC–multiple MS (MS^n)

2.2.1. LC conditions

A Hewlett-Packard (HP) series II 1090 LC was fitted with a Columbus (Phenomenex, Macclesfield, UK) C_{18} column, 150 mm × 2 mm i.d., particle size 5 μm. A linear gradient elution was performed at a flow rate of 0.2 ml/min, with a mobile phase of 0.02 M ammonium formate in water (solvent A) and 0.02 M ammonium formate in methanol (solvent B). The gradient employed was: 5% B (0–5 min), 5% to 90% B (5–10 min), held at 90% B (10–15 min), returned to initial composition 5% B (15–20 min), and equilibrated at 5% B (20–25 min). Injections of 20 μl were used for all analyses.

2.2.2. Sample infusion

Solutions in water (10 μg/ml) were infused from a 250 μl syringe (Unimetrics), at a flow rate of 2.0 μl/min, into a 0.2 ml/min flow of 95% 0.02 M ammonium formate in water plus 5% 0.02 M ammonium formate in methanol.

2.2.3. MS conditions

MS analysis was performed using a Finnigan LCQ ion trap LC–MS system fitted with an atmospheric pressure ionisation (API) interface, operated in positive APCI mode. The discharge current was set at 5 μA, capillary temperature at 150 °C and vaporiser at 400 °C. The sheath gas flow was maintained at 80 (arbitrary units) and auxiliary gas at 20 (arbitrary units). Mass spectra were collected with a scan range *m*/*z* 50–400 and a source CID of 5%. MS–MS product ion spectra of the precursor ions, *m*/*z* 125 (±1 *m*/*z*) and *m*/*z* 139 (±1 *m*/*z*) were obtained with a source CID of 20% and an ion trap CID of 12%. MS^3 product ion spectra were obtained with the source CID disabled and the ion trap CID set at 12%.

Confirmatory analysis was performed using similar conditions on a Finnigan TSQ 700 triple sector quadrupole LC–MS–MS system. The discharge current was set at 5 μA, capillary temperature 150 °C and vaporiser 400 °C. MS–MS product ion spectra were collected using the precursor ions *m*/*z* 139 or *m*/*z* 125, with source CID offset −20 V, Q_2 offset −25 V and argon pressure 0.107 Pa. LC conditions were as outlined above.

Table 1
Product ions of *m/z* 125 for 10 isomeric pairs of dialkyl propylphosphonates (RO)PrP(O)OR′ (source CID 20%, ion trap CID 12%)

P-Pr isomer	R	R′	t_R (min)	Mol. mass	% Relative abundance				
					Precursor *m/z* 125	$-H_2O$ *m/z* 107	$-C_2H_4$ *m/z* 97	$-C_3H_6$ *m/z* 83	$-C_3H_6-H_2O$ *m/z* 65
iso	Et	Et	19.00	180	100	10	15	59	18
n	Et	Et	18.96	180	100	4	0	0	0
iso	iso-Pr	Et	19.73	194	100	16	24	90	24
n	iso-Pr	Et	19.71	194	100	0	0	0	0
iso	*n*-Pr	Et	19.69	194	100	7	18	73	65
n	*n*-Pr	Et	19.66	194	100	3	0	0	0
iso	*n*-Bu	Et	20.38	208	100	13	24	70	15
n	*n*-Bu	Et	20.32	208	100	6	0	0	0
iso	iso-Pr	iso-Pr	20.27	208	100	9	28	74	20
n	iso-Pr	iso-Pr	20.23	208	100	5	0	0	0
iso	*n*-Pr	iso-Pr	20.32	208	100	16	20	82	20
n	*n*-Pr	iso-Pr	20.32	208	100	6	0	0	0
iso	*n*-Pr	*n*-Pr	20.37	208	89	15	26	100	65
n	*n*-Pr	*n*-Pr	20.34	208	100	3	0	0	0
iso	*n*-Bu	iso-Pr	20.92	222	100	14	20	86	16
n	*n*-Bu	iso-Pr	20.94	222	100	5	0	0	0
iso	*n*-Bu	*n*-Pr	20.97	222	99	18	27	100	65
n	*n*-Bu	*n*-Pr	20.91	222	100	99	0	0	0
iso	*n*-Bu	*n*-Bu	22.08	236	100	17	20	89	24
n	*n*-Bu	*n*-Bu	22.03	236	100	4	0	0	0

2.3. Hydrolysis of alkyl propylphosphonochloridates

Alkyl propylphosphonochloridates are generally too reactive to be analysed under reversed phase LC conditions. However, they are readily hydrolysed to the corresponding alkyl propylphosphonic acid by addition of water to a solution of the alkyl propylphosphonochloridate in hexane. Water (1 ml) was added to a solution of the chloridate (10 μg) in hexane (1 ml) and the mixture vortexed for 1 min. The alkyl propylphosphonic acid partitioned predominantly into the water layer and was analysed by LC–MS using conditions as described above.

2.4. GC–FT-IR

The instrumentation consisted of a Hewlett-Packard 5890 Series II gas chromatograph (GC) interfaced to a HP 5965B infrared detection (IRD) system. The GC was equipped

Table 2
Product ions of *m/z* 139 for five isomeric pairs of alkyl methyl propylphosphonates (RO)PrP(O)OMe (source CID 20%, ion trap CID 12%)

P-Pr isomer	R	t_R (min)	Mol. Mass	% Relative abundance				
				Precursor *m/z* 139	$-H_2O$ *m/z* 121	$-C_2H_4$ *m/z* 111	$-C_3H_6$ *m/z* 97	$-C_3H_6-H_2O$ *m/z* 79
iso	Me	16.84	152	100	11	0.3	6	49
n	Me	16.85	152	100	5	0	0	1
iso	Et	18.21	166	100	15	0.4	9	53
n	Et	18.25	166	100	11	0	0	6
iso	iso-Pr	19.18	180	100	19	2	7	59
n	iso-Pr	18.96	180	100	9	0	0	2
iso	*n*-Pr	19.06	180	100	18	2	7	67
n	*n*-Pr	19.03	180	100	7	0	0	1
iso	*n*-Bu	19.78	194	100	24	3	12	66
n	*n*-Bu	19.74	194	100	10	0	0	2

with a HP Ultra-2 (5% phenyl, 95% methyl silicone) capillary column, 25 m × 0.32 mm i.d., 0.52 μm film thickness, plus a 2.5 m × 0.25 mm intermediate polarity retention gap (Restek). The carrier gas was helium at a constant pressure of 9×10^4 Pa; sample introduction was by cool-on-column injection of 2.5 μl. The GC oven temperature was held at 40 °C for 5 min, increased at 10 °C/min to 280 °C, and held at 280 °C for 7.5 min. The GC–IRD interface was operated at 280 °C and the flow cell at 260 °C. The optical resolution was 8 cm^{-1} with a Coadd factor of 4. A narrow band mercury/cadmium/telluride (MCT) detector was used with a range of 4000–750 cm^{-1}.

3. Results and discusion

3.1. LC–MS–MS

3.1.1. Identification of P-propylphosphonates

Under the LC conditions used, isomeric (*P*-Pr) dialkyl propylphosphonates were not reliably differentiated by LC retention time (Tables 1 and 2) or by their APCI mass spectra. Under some chromatographic conditions, the isopropyl isomers elute marginally faster than the *n*-propyl isomers but, in the absence of pairs of standards, retention time is not a reliable indicator. The simple APCI spectra (source CID set

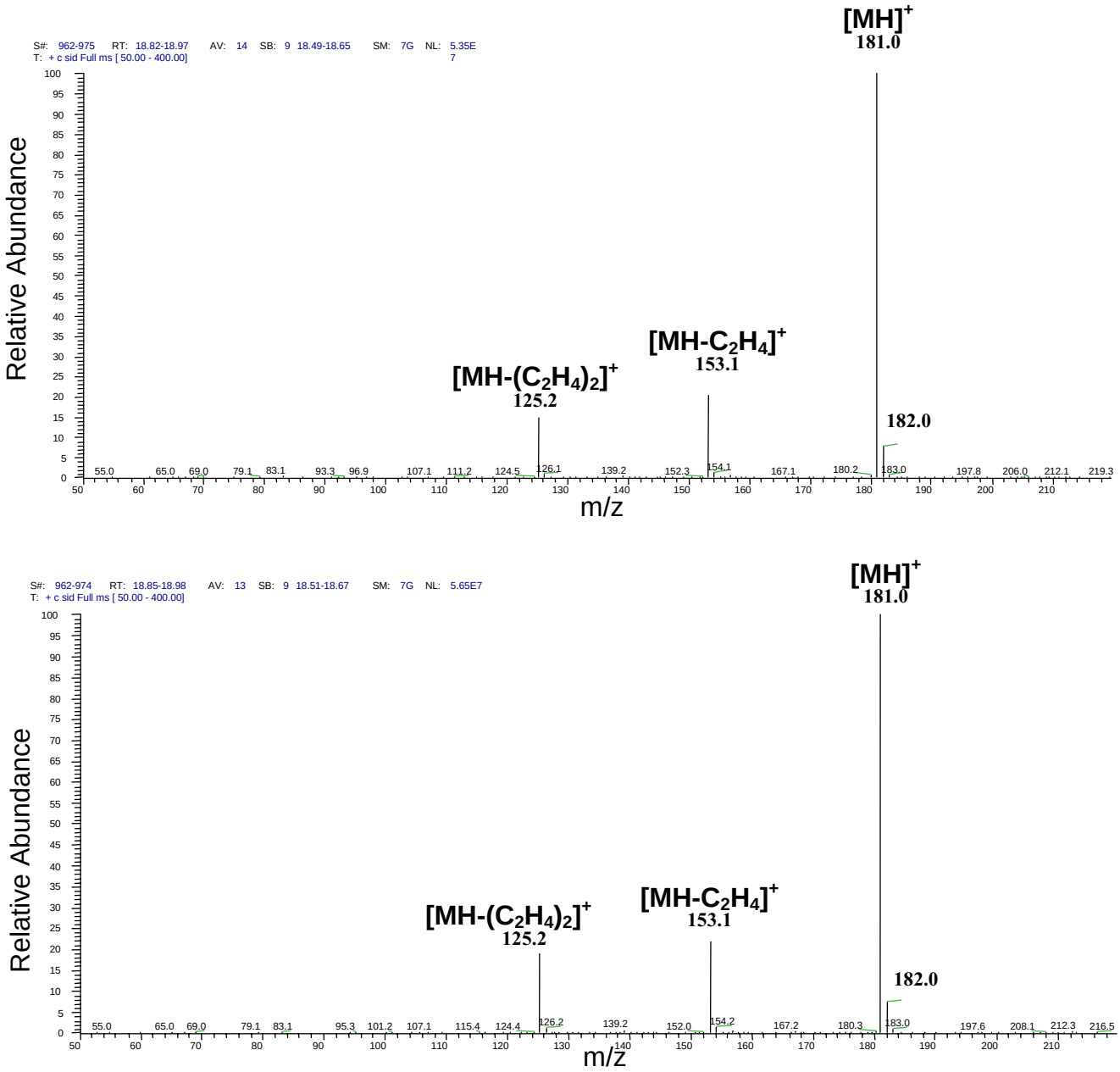


Fig. 1. APCI-MS spectra of diethyl isopropylphosphonate (upper) and diethyl *n*-propylphosphonate (lower) (Finnigan LCQ ion trap, in-source CID 5%).

at 5%) were characterised by abundant protonated molecules and fragment ions resulting from sequential loss of alkene from cleavage of *O*-alkyl groups (C_2–C_{10}). These fragmentations arise from McLafferty-type rearrangements. With alkyl methyl propylphosphonates, only the larger *O*-alkyl group is cleaved. In OPCW proficiency tests, simple APCI or ESI spectra allow a very rapid tentative identification of the analyte in terms of the number of carbon atoms in the alkyl groups attached to phosphorus and oxygen, but they do not discriminate isomers. Fig. 1 shows the APCI mass spectra of isomeric diethyl propylphosphonates.

Using ESI, van Baar et al. [4] demonstrated that iso- and *n*-propylphosphonic acids could be differentiated by the CID spectra of the protonated molecules $[PrP(O)(OH)_2 + H]^+$. The CID spectrum of the protonated molecule of the *P*-isopropyl isomer showed a strong product ion, *m*/*z* 83 $[MH–C_3H_6]^+$, resulting from P–C cleavage with loss of propene. Deuterium labelling experiments showed this to involve a 1,4-hydrogen shift from one of the isopropyl methyl groups. This product ion was not observed with the *n*-propyl isomer. The isopropyl isomer also showed an intense product ion from further loss of H_2O, *m*/*z* 65 $[MH–C_3H_6–H_2O]^+$, which was weak for the *n*-propyl isomer. In contrast, CID of the protonated molecule of the *n*-propyl isomer gave an abundant product ion from loss of water, resulting from P–O cleavage; this ion was weak for the isopropyl isomer.

Preliminary experiments, using direct infusion rather than LC, showed that similar APCI-MS–MS spectra could be observed for dialkyl propylphosphonates. The fragment ion resulting from sequential loss of alkene groups, *m*/*z* 125, $[MH–C_nH_{2n}–C_mH_{2m}]^+$, is isobaric (presumably identical) with the protonated molecule of propylphosphonic acids. It is a major product ion observed in the CID spectra of the protonated molecules, either in-source or in the ion trap. Collision energies for both source and ion trap MS–MS were investigated by infusion of diethyl iso- and *n*-propylphosphonates. In-source CID was set at 20% to promote fragmentation of the protonated molecule to provide a moderately abundant ion *m*/*z* 125. For CID of this ion, ion trap collision energies below 10% proved to be ineffective for isomer identification due to insufficient fragmentation. Values greater than 10% demonstrated a clear difference in the MS–MS spectra for the iso- and *n*-propyl isomers, although higher percentage CID values resulted in a loss of intensity in the total ion current. The variation of product ion intensities with collision energy is shown in Fig. 2. Optimum differentiation of isomers was observed with CID set between 10 and 13%; 12% CID was selected for the analytical method. Similar observations were made for product ions of *m*/*z* 139 $[MH–C_nH_{2n}]^+$ in alkyl methyl propylphosphonates.

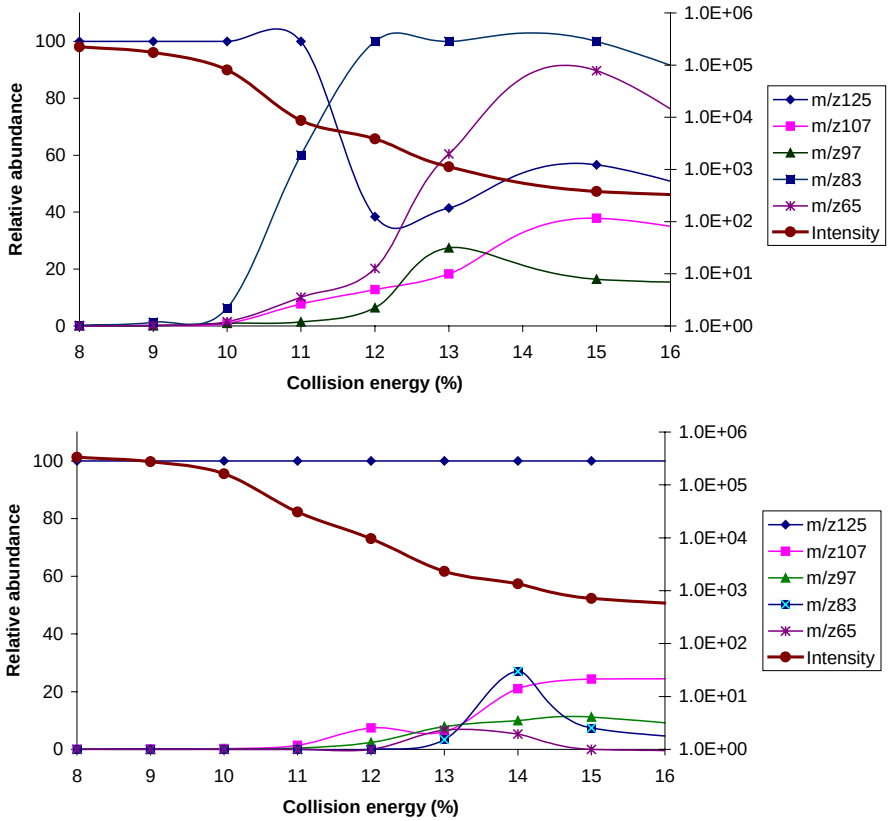


Fig. 2. Relative abundance of product ions of *m*/*z* 125 vs. collision energy for diethyl isopropylphosphonate (upper) and diethyl *n*-propylphosphonate (lower) (Finnigan LCQ ion trap, APCI).

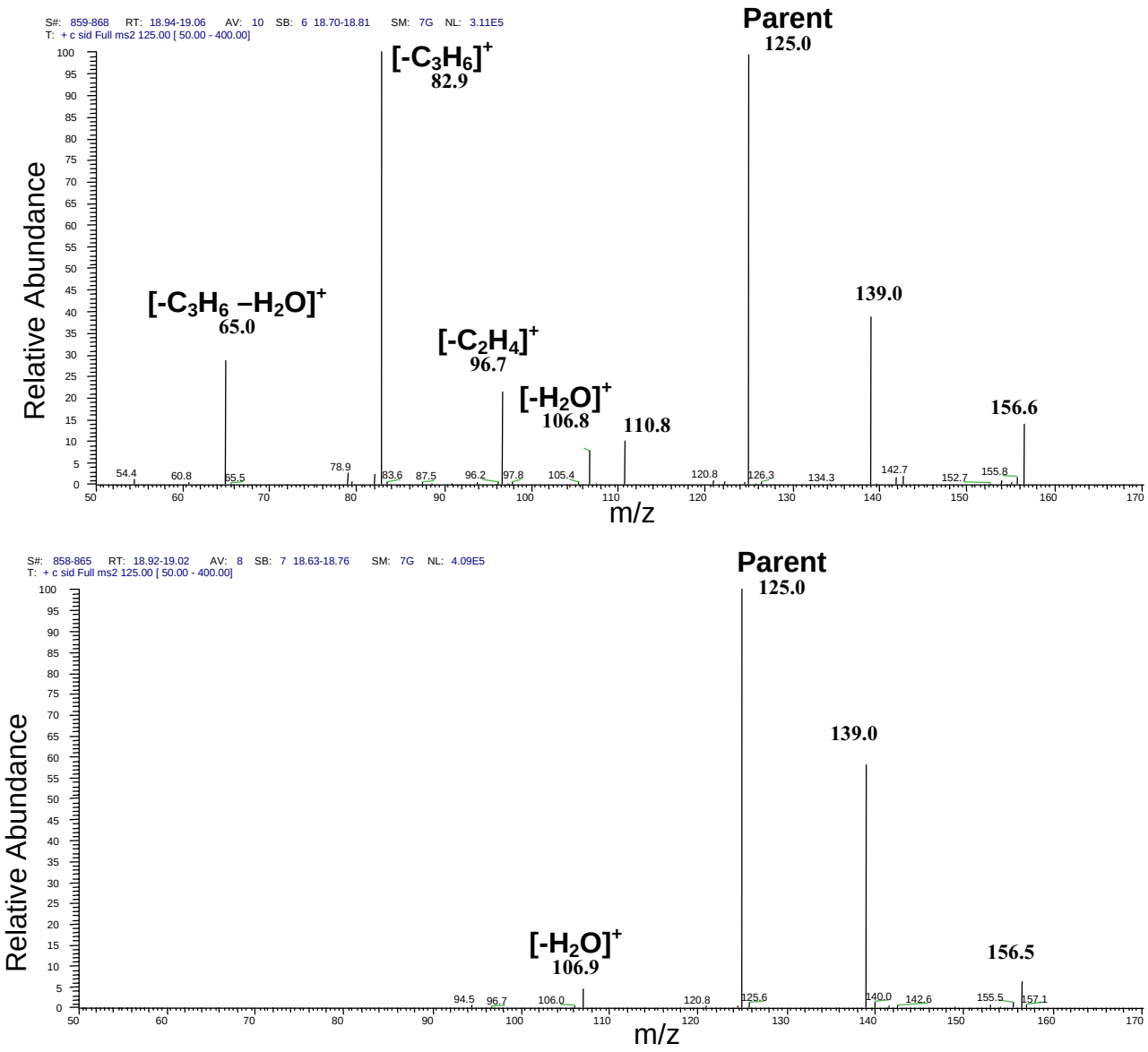


Fig. 3. APCI-MS–MS product ion spectra of *m*/*z* 125 $[MH–(C_2H_4)_2]^+$ for diethyl isopropylphosphonate (upper) and diethyl *n*-propylphosphonate (lower) (Finnigan LCQ ion trap, in-source CID 20%, collision energy 12%).

The isolation of *m*/*z* 125 in the ion trap and CID produced a number of product ions, mostly similar to those observed by van Baar for the protonated molecules of propylphosphonic acids. With the isopropyl isomers, the most abundant product ion, *m*/*z* 83, $[125\text{-}C_3H_6]^+$ resulted from loss of propene; moderately abundant product ions were also observed at *m*/*z* 65 $[125\text{-}C_3H_6–H_2O]^+$, from loss of propene and water, and at *m*/*z* 97 $[125\text{-}C_2H_4]^+$ from loss of ethene. A weaker product ion was observed at *m*/*z* 107 from loss of water $[125\text{-}H_2O]^+$. In contrast, very little fragmentation of *m*/*z* 125 was observed for the *n*-propyl isomers under these conditions, a weak ion at *m*/*z* 107 from loss of water being the single significant product ion in most cases. The product ions of *m*/*z* 125, observed for 10 isomeric pairs of dialkyl propylphosphonates, are shown in Table 1; product ion spectra for isomeric diethyl propylphosphonates are shown in Fig. 3. The only notable differences from the CID spectra of the protonated molecules of propylphosphonic acids reported by Van Baar (using a triple quadrupole system) were the generally weaker ions resulting from loss of ethene in the ion trap. With alkyl methyl isopropylphosphonates, CID of *m*/*z* 139 provided analogous product ions at *m*/*z* 121, $[139\text{-}H_2O]^+$, *m*/*z* 111, $[139\text{-}C_2H_4]^+$ (weak), *m*/*z* 97 $[139\text{-}C_3H_6]^+$ and *m*/*z* 79, $[139\text{-}C_3H_6–H_2O]^+$; *m*/*z* 79 was much more abundant than *m*/*z* 97. The *n*-propyl isomers showed very little fragmentation of *m*/*z* 139 under the CID conditions selected, loss of water being the main product ion observed in most cases. The product ions for *m*/*z* 139, observed for five isomeric pairs of alkyl methyl propylphosphonates, are shown in Table 2; product ion spectra for

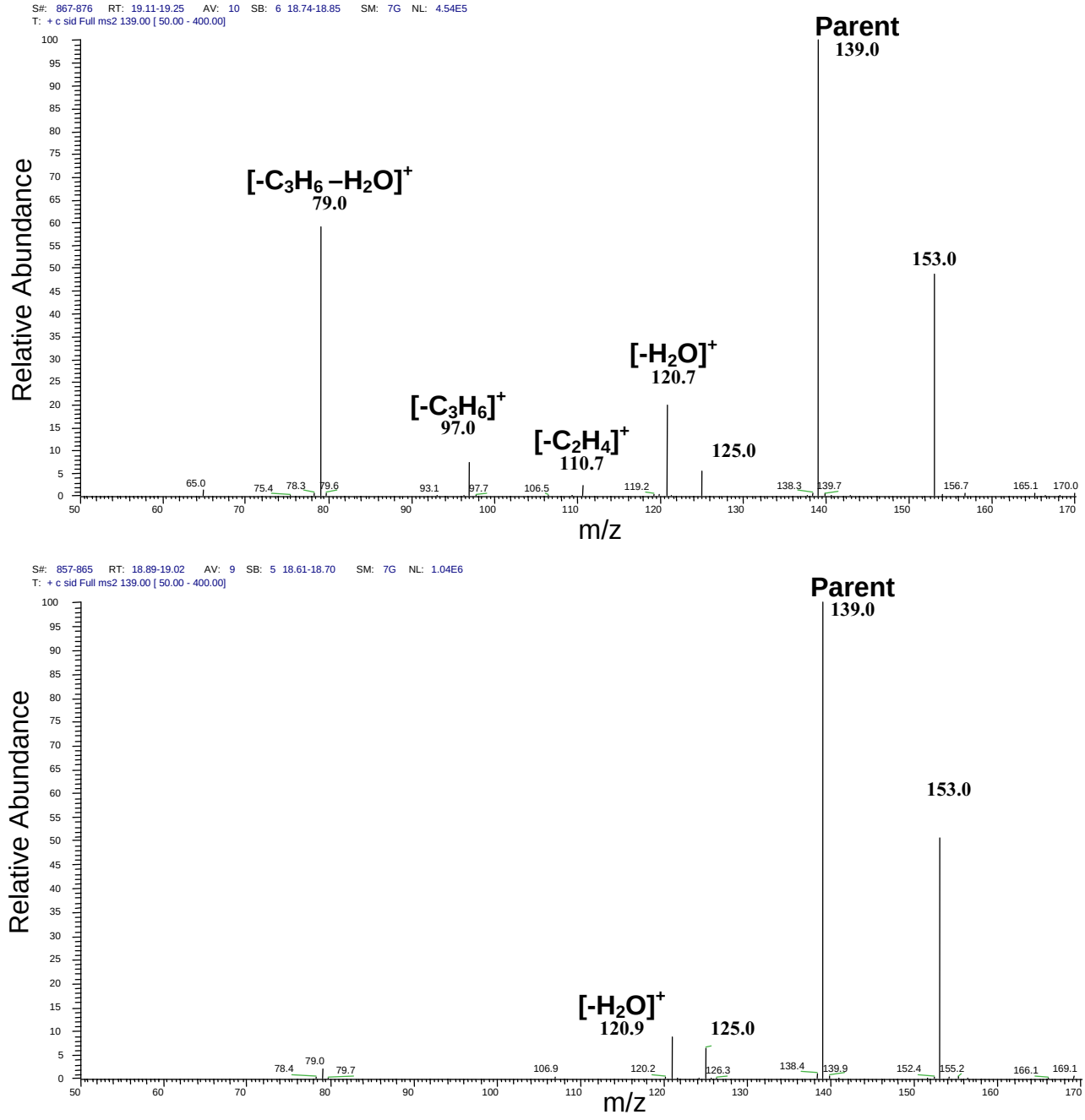


Fig. 4. APCI-MS–MS product ion spectra of *m/z* 139 $[MH–(C_3H_6)]^+$ for methyl isopropyl isopropylphosphonate (upper) and methyl isopropyl *n*-propylphosphonate (lower) (Finnigan LCQ ion trap, in-source CID 20%, collision energy 12%).

isomeric methyl isopropyl propylphosphonates are shown in Fig. 4.

Weak ions (*m/z* 111 from 125 or *m/z* 125 from 139) were observed in the product ion spectra from isopropyl isomers, apparently from loss of CH_2 from the respective precursor ions. These probably result from adducts within the ion trap. Bell et al. [8] suggested that a similar ion observed in the product ion spectrum of the protonated molecule of dimethyl methylphosphonate resulted from addition of background water in the ion trap to a product ion resulting from loss of MeOH. Ions observed in the product ion spectra at *m/z* values higher than the precursor ion (*m/z* 139 and 157 from *m/z* 125, and *m/z* 153 from *m/z* 139) are also presumed to arise from adducts, probably with background methanol. These ions were not observed with a triple sector quadrupole instrument, in which the residence time within the collision cell is considerably shorter. An alternative to promoting in-source CID and performing CID (MS–MS) on fragment ions *m/z* 125 or 139 is to isolate MH^+ and perform MS^3. Similar product ion patterns were observed for the isomers.

MS^3 on the protonated molecules achieves somewhat greater selectivity without loss of sensitivity, but is only appropriate for ion trap systems.

3.1.2. Identification of O-propyl isomers

The availability of a number of isomeric *O*-propyl alkyl propylphosphonates allowed a comparison of full scan APCI spectra obtained with source CID set at 5%. These conditions are sufficient to reduce solvent clusters but produce relatively little fragmentation. The *O*-propyl isomers showed consistently different relative abundances of the protonated molecule and the fragment ion $[MH–C_3H_6]^+$. The latter has been postulated to result from a rearrangement involving γ hydrogen transfer [9]. In all of the *O*-isopropyl

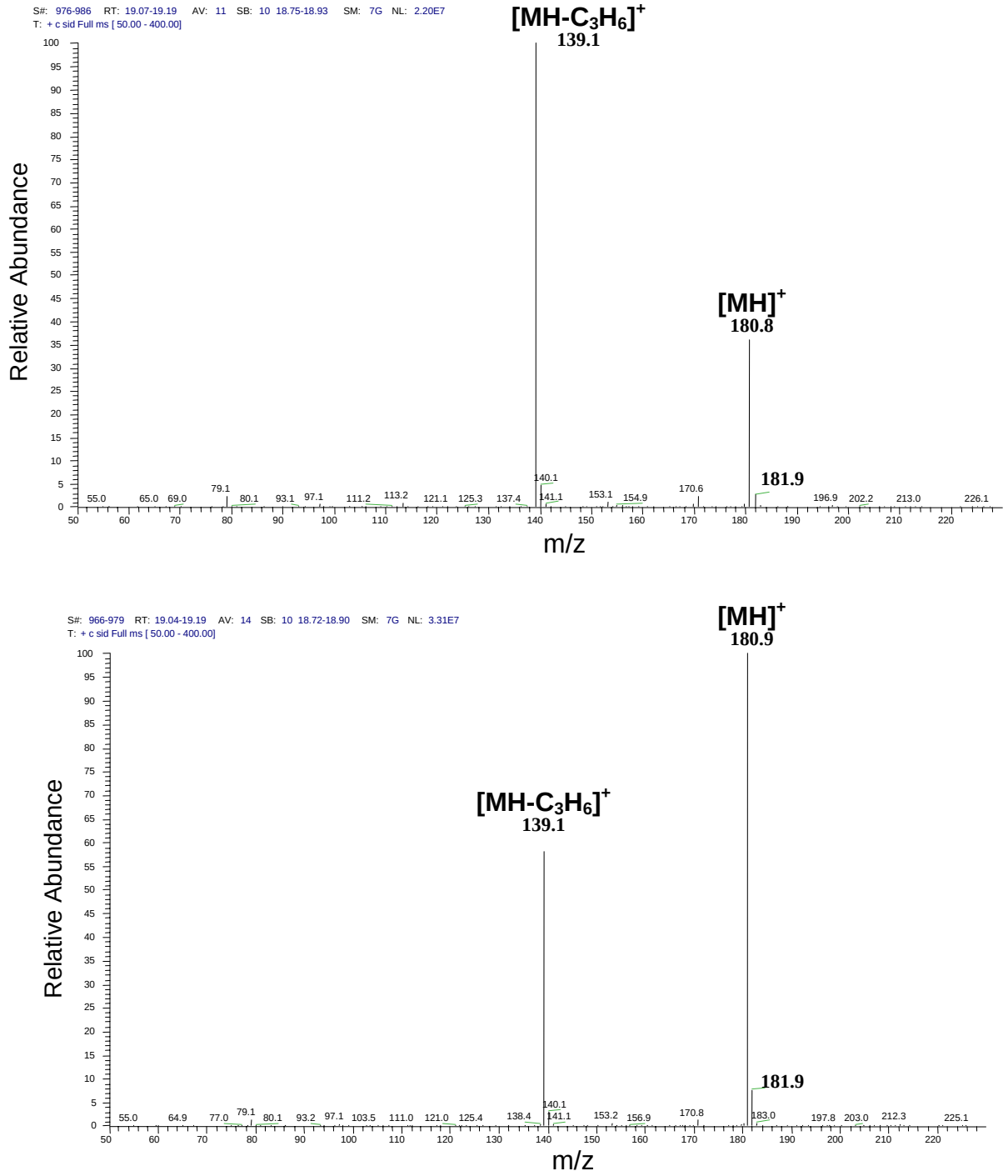


Fig. 5. APCI-MS spectra of methyl isopropyl isopropylphosphonate (upper) and methyl *n*-propyl isopropylphosphonate (lower) (Finnigan LCQ ion trap, in-source CID 5%).

Table 3
Major ions in the APCI spectra of isomeric (*O*-propyl) propyl alkyl propylphosphonates (PrO)PrP(O)OR (source CID 5%)

P-Pr isomer	R	*O*-Pr isomer	Ions *m/z* (% relative abundance)		
			MH^+	$-C_nH_{2n}$	$-C_nH_{2n}-C_mH_{2m}$
iso	Me	iso	181 (35)	139 (100)	–
iso	Me	*n*	181 (100)	139 (60)	–
n	Me	iso	181 (20)	139 (100)	–
n	Me	*n*	181 (100)	139 (87)	–
iso	Et	iso	195 (31)	153 (100)	125 (28)
iso	Et	*n*	195 (100)	153 (51)	125 (19)
n	Et	iso	195 (31)	153 (100)	125 (41)
n	Et	*n*	195 (100)	153 (52)	125 (22)
iso	iso-Pr	iso	209 (77)	167 (63)	125 (100)
iso	iso-Pr	*n*	209 (76)	167 (100)	125 (52)
iso	*n*-Pr	*n*	209 (100)	167 (23)	125 (21)
n	iso-Pr	iso	209 (52)	167 (47)	125 (100)
n	iso-Pr	*n*	209 (62)	167 (100)	125 (73)
n	*n*-Pr	*n*	209 (100)	167 (29)	125 (37)
iso	*n*-Bu	iso	223 (92)	181 (100)	125 (53)
iso	*n*-Bu	*n*	223 (100)	167 (23)	125 (25)
n	*n*-Bu	iso	223 (93)	181 (100)	125 (76)
n	*n*-Bu	*n*	223 (100)	167 (24)	125 (32)

isomers, with one exception, the fragment ion from loss of C_3H_6 had a greater relative abundance than the protonated molecule; in *n*-propyl isomers the reverse was the case. This is consistent with the greater number of γ hydrogens (six) in the *O*-isopropyl isomers. The single exception was the analogue where both *O*-alkyl groups were isopropyl, which readily lost both isopropyl groups to give an abundant ion *m/z* 125. The APCI spectra for methyl isopropyl isopropylphosphonate and methyl *n*-propyl isopropylphosphonate are compared in Fig. 5. Data for 18 isomers are given in Table 3.

3.1.3. Alkyl propylphosphonochloridates

Alkyl propylphosphonochloridates are generally too reactive to be analysed by LC–MS. The addition of water to alkyl propylphosphonochloridate solutions in hexane resulted in an efficient (>80%) conversion to alkyl propylphosphonic acids, which partition preferentially into the aqueous phase. LC–MS–MS analysis of the aqueous solutions differentiated the *P*-propyl isomers in a manner similar to that described for dialkyl propylphosphonates. Ion trap CID spectra of *m/z* 125, $[MH-C_nH_{2n}]^+$, or *m/z* 139, MH^+ in the case of isomeric methyl propylphosphonochloridates, showed differences similar to those observed with dialkyl propylphosphonates. Product ion spectra for three isomeric pairs are shown in Table 4.

3.1.4. Validation

The differentiation of isomeric dialkyl propylphosphonates by their MS–MS spectra was validated by the analysis of 15 pairs of isomers. Five individual dialkyl propylphosphonates shown in Tables 1 and 2 were analysed without prior knowledge of the structures. In each case, the *P*-propyl isomer was correctly identified. The method has also been applied retrospectively to samples from OPCW proficiency tests, again without the analyst knowing the structure of

Table 4
Product ions of *m/z* 125 or *m/z* 139 for three isomeric pairs of alkyl propylphosphonic acids (RO)PrP(O)OH, derived from hydrolysis of alkyl propylphosphonochloridates

P-Pr isomer	R	% Relative abundance				
		Precursor *m/z* 125 or 139	$-H_2O$ *m/z* 107 or 121	$-C_2H_4$ *m/z* 97 or 111	$-C_3H_6$ *m/z* 83 or 97	$-C_3H_6-H_2O$ *m/z* 65 or 79
iso	Me	100*	5	2	14	72
n	Me	100*	3	0	0	2
iso	iso-Pr	39	9	6	100	1
n	iso-Pr	100	4	0	0	2
iso	*n*-Pr	35	14	4	100	19
n	*n*-Pr	100	3	1	0	1

* Denotes precursor ion *m/z* 139.

the analyte. *O*-*n*-Propyl *n*-propylphosphonic acid was correctly identified in a water sample. The method also tentatively identified *n*-propyl *n*-propylthiophosphonic acid in this sample. The abundance of the protonated molecule, *m*/*z* 183, was greater than that of the fragment ion *m*/*z* 141, $[MH–C_3H_6]^+$, suggesting an *O*-*n*-propyl substituent. CID of *m*/*z* 141 showed only very weak loss of propene, also indicative of a *P*-*n*-propyl substituent.

3.2. GC–FT-IR

The identification of organophosphorus compounds containing a single *P*-alkyl substituent can best be carried out by concentrating on the spectral features arising from the phosphoryl (P=O) group and the *P*-alkyl group. P=O stretching and symmetrical and asymmetrical C–H deformations associated with the P–C group occur in the frequency range 1200–1500 cm^{-1}.

3.2.1. The P=O group

The position of the P=O stretching band is dependent upon the electronegativity of the substituents coupled to phosphorus [5–7]. In studies involving >1500 compounds, Thomas [6,7] derived an equation for the calculation of the P=O stretching frequency for condensed phase spectra, $\nu_{P=O} = 930+40\Sigma\pi$. The value of π is assigned to the other phosphorus substituents and is derived from P=O frequency data of compounds which best fit the equation. Using this formula, the theoretical values of the P=O stretching frequency for iso- and *n*-propylphosphonates in condensed phase lie between 1222 and 1282 cm^{-1}, with *P*-isopropyl < *P*-*n*-propyl, and diester < chloridate < fluoridate. If these values hold true for vapour phase spectra then *P*-propyl isomers should be differentiated.

In two recent studies [10,11], involving analogues of the nerve agent sarin and dialkyl alkylphosphonates respectively, P=O stretching frequencies were compared for a range of methyl-, ethyl-, isopropyl- and *n*-propyl-phosphonyl compounds. McGarvey et al. [10] obtained data from methyl-, ethyl- and *n*-propyl compounds, and a limited number (four) of dialkyl isopropylphosphonates. The only significant difference observed was in the P=O stretching frequency at 1251 cm^{-1} for *P*-isopropyl-, contrasting with 1261–1268 cm^{-1} for the other compounds studied. The study also showed that for the entire range of phosphonofluoridates, P=O stretching frequencies lay between 1302 and 1324 cm^{-1} but with no correlation through the series of compounds as predicted by Thomas. Kireev et al. [11] reported that the vapour phase spectra for ethyl-, iso- and *n*-propylphosphonates gave a broad band covering both P=O stretch and symmetrical C–H deformations, in contrast to condensed phase spectra where individual bands are seen. They attributed the band maximum to the P=O stretch and demonstrated a significant difference between iso- and *n*-propyl isomers, which is used in a substructure library search routine.

The values for P=O stretching frequencies, observed in this present study for 15 isomeric pairs of dialkyl alkylphosphonates, and five pairs each of alkyl propylphosphonochloridates and fluoridates, were in the range 1248–1313 cm^{-1}; they are listed individually in Tables 5–7. This range of frequencies is higher than the theoretical condensed phase spectra as predicted by Thomas. However, these values are in accordance with Nyquist [12], who modified the π values of Thomas to account for the higher frequencies found in the vapour phase, where intermolecular interactions are reduced. The results demonstrate a consistent difference in the P=O stretching frequencies for the dialkyl

Table 5
Diagnostic vibrations (cm^{-1}) for 15 isomeric pairs of dialkyl propylphosphonates (RO)PrP(O)OR

R	R′	P=O stretch		C–H deformations			
		Isopropyl	*n*-Propyl	Isopropyl		*n*-Propyl	
				Asym.	Sym.	Asym.	Sym.
Me	Me	1259	1273	1470	~1285	1464	~1240
Me	Et	1255	1271	1471	~1285	1464	~1240
Et	Et	1252	1268	1471	~1285	1462	~1240
Me	*n*-Pr	1255	1271	1469	~1285	1464	~1240
Me	iso-Pr	1253	1268	1469	~1285	1464	~1240
Et	*n*-Pr	1252	1268	1470	~1285	1464	~1240
Et	iso-Pr	1251	1267	1469	~1285	1464	~1240
n-Pr	*n*-Pr	1252	1268	1469	~1285	1465	~1240
n-Pr	iso-Pr	1251	1267	1469	~1285	1465	~1240
iso-Pr	iso-Pr	1248	1265	1468	~1285	1464	~1240
Me	*n*-Bu	1255	1271	1470	~1285	1465	~1240
Et	*n*-Bu	1252	1268	1470	~1285	1465	~1240
n-Pr	*n*-Bu	1252	1268	1469	~1285	1466	~1240
iso-Pr	*n*-Bu	1250	1267	1469	~1285	1466	~1240
n-Bu	*n*-Bu	1252	1268	1470	~1285	1466	~1240
Mean		1253	1269	1470	~1285	1465	~1240

Table 6
Diagnostic vibrations (cm^{-1}) for five isomeric pairs of alkyl propylphosphonochloridates (RO)PrP(O)Cl

R	P=O stretch		C–H deformations (cm^{-1})			
	Isopropyl	*n*-Propyl	Isopropyl		*n*-Propyl	
			Asym.	Sym.	Asym.	Sym.
Me	1290	1288	1468	~1259	1464	1238
Et	1287	1286	1470	~1259	1463	1238
n-Pr	1287	1286	1470	~1259	1465	1240
iso-Pr	1287	1285	1468	~1259	1464	1238
n-Bu	1287	1286	1470	~1259	1467	1239
Mean	1288	1286	1469	~1259	1465	1239

Table 7
Diagnostic vibrations (cm^{-1}) for five isomeric pairs of alkyl propylphosphonofluoridates (RO)PrP(O)F

R	P=O stretch		C–H deformations (cm^{-1})			
	Isopropyl	*n*-Propyl	Isopropyl		*n*-Propyl	
			Asym.	Sym.	Asym.	Sym.
Me	1311	1313	1471	~1270	1463	1246
Et	1309	1308	1471	~1270	1463	1247
n-Pr	1309	1307	1471	~1270	1466	1247
iso-Pr	1307	1305	1471	~1270	1466	1244
n-Bu	1309	1307	1472	~1270	1467	1245
Mean	1309	1308	1471	~1270	1465	1246

iso- and *n*-propylphosphonates, sufficient to allow assignment of structure. P=O stretching frequencies for *P*-isopropyl were in the range 1248–1259 cm^{-1} (mean 1253 cm^{-1}) and for *P*-*n*-propyl in the range 1265–1273 cm^{-1} (mean 1268 cm^{-1}). The spectra for the fifteen pairs of isomers are displayed as an overlay in Fig. 6 and demonstrate a remarkable consistency throughout the range of compounds analysed. However, spectra for isomeric chloridates and fluoridates, overlaid in Figs. 7 and 8, showed no significant difference in P=O stretching values. With these compounds, the major influence on the frequencies is probably exerted by the chlorine and fluorine substituents.

3.2.2. The phosphorus-alkyl substituent

Very little information is available for assigning C–H symmetrical deformations in compounds with a *P*-alkyl group higher than ethyl. Thomas [7] states that for *P*-methyl a characteristic, sharp medium intensity band is observed at about 1300 cm^{-1}, the exact frequency being dependent on the other substituents on phosphorus. *P*-ethyl exhibits weak absorption bands close to 1250 cm^{-1} which may result in splitting of the P=O band. Insufficient information is available for higher *P*-alkyl substituents. Söderström [13] described the characterisation of *P*-alkyl groups in the condensed phase, with examples of C–H deformations for *P*-methyl, *P*-ethyl, *P*-isopropyl and *P*-*n*-propyl substituents, but drew no firm conclusion for differentiating the propyl isomers. McGarvey et al. [10] observed a shoulder, 5–10 cm^{-1} on the lower frequency side, of the P=O stretching band in alkyl isopropylphosphonofluoridates and a weak band at 1244–1245 cm^{-1} for the alkyl *n*-propylphosphonofluoridates, allowing differentiation of the two isomers.

The results of this study for C–H symmetrical and asymmetrical deformations are also presented in Tables 5–7, and as overlaid spectra covering the range 1150–1550 cm^{-1} in Figs. 6–8. As with P=O stretching, the bands for C–H

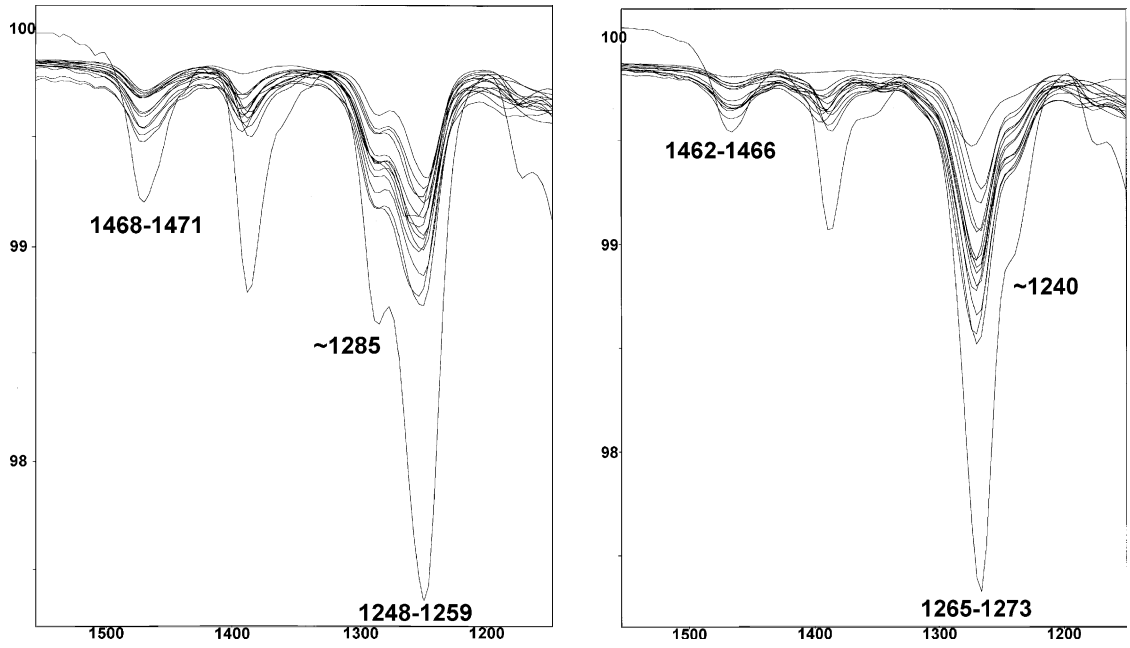


Fig. 6. Overlaid GC–FT-IR spectra of 15 dialkyl isopropylphosphonates (left) and 15 dialkyl *n*-propylphosphonates (right).

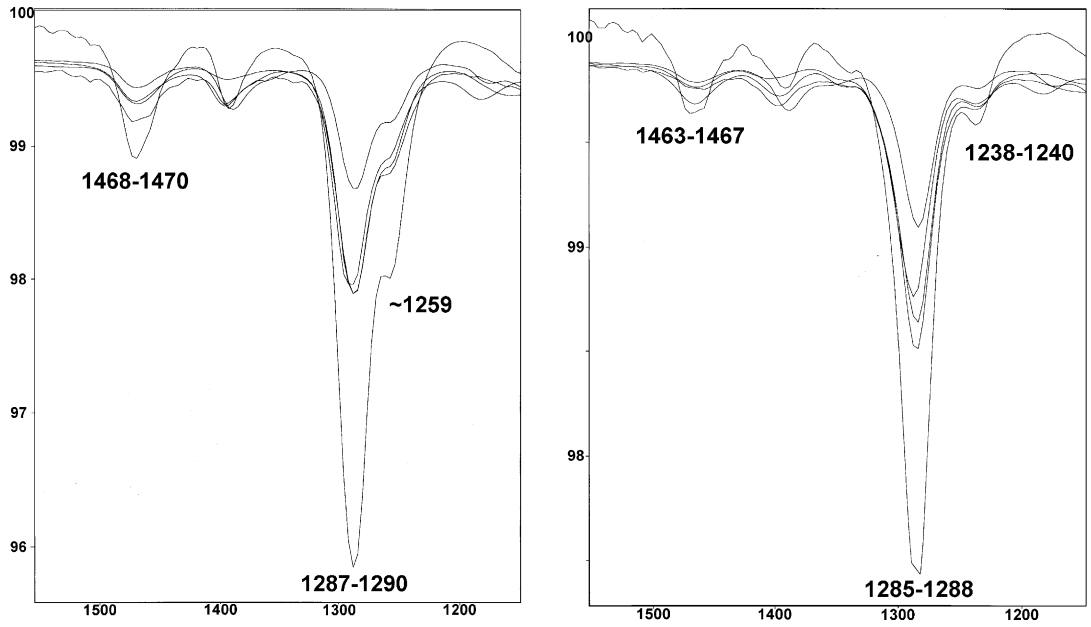


Fig. 7. Overlaid GC–FT-IR spectra of five alkyl isopropylphosphonochloridates (left) and five alkyl *n*-propylphosphonochloridates (right).

deformation showed remarkable consistency over the series of compounds analysed. The symmetrical C–H deformations associated with the P–C group can be seen as resolved or partially resolved bands (shoulders) on the side of the P=O stretching band. The dialkyl propylphosphonates presented in Fig. 6 show partially resolved bands (shoulders) at ∼1285 cm^{-1} for the isopropyl isomers and ∼1240 cm^{-1} for the *n*-propyl isomers. The asymmetrical C–H deformations for the whole range of compounds studied show values for the isopropyl isomers at 1468–1472 cm^{-1} and for the *n*-propyl isomers at 1462–1467 cm^{-1}. This is a small but still significant difference when coupled with the other spectral information from each compound. The chloridates presented in Fig. 7 also showed a consistent difference. The isopropyl isomers had a partially resolved band at ∼1259 cm^{-1} whereas the *n*-propyl isomers exhibited a fully resolved band at 1238–1240 cm^{-1}. The fluoridates, presented in Fig. 8, show a similar pattern where the isopropyl compounds have a partially resolved band (shoulder) at ∼1270 cm^{-1} and the *n*-propyl compounds have a completely resolved band at 1244–1247 cm^{-1}.

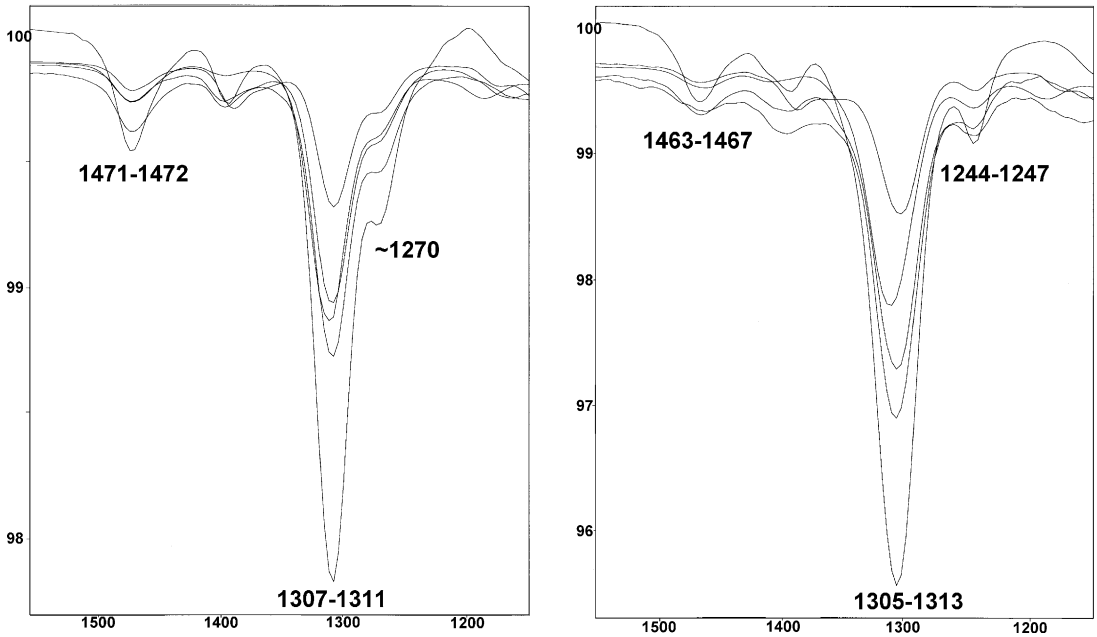


Fig. 8. Overlaid GC–FT-IR spectra of five alkyl isopropylphosphonofluoridates (left) and five alkyl *n*-propylphosphonofluoridates (right).

4. Conclusions

APCI tandem mass spectrometry has been shown to provide a robust method for the identification of isomeric dialkyl propylphosphonates, and alkyl propylphosphonochloridates after hydrolysis to alkyl propylphosphonic acids. The iso- and *n*-propyl isomers are clearly differentiated by their collision induced fragmentation pathways. This extends methodology reported by van Baar et al. [4], which differentiated iso- and *n*-propylphosphonic acids using ESI tandem mass spectrometry. *O*-Propyl isomers can also be differentiated, but on the basis of the relative abundance of protonated molecules and a common fragment ion rather than divergent fragmentation pathways. The methodology can readily be incorporated into LC–MS screening procedures for hydrolysis products of CW agents and dialkyl alkylphosphonates. Modern software allows monitoring of full scan data and simultaneous collection of MS–MS product ion spectra for selected precursor ions. This form of data collection allows simultaneous screening of samples for polar CW analytes of relevance to the CWC, and preliminary identification of *P*-propyl and *O*-propyl isomers, thereby reducing analysis time. In particular, it allows synthesis to be directed at the identified isomer for confirmation.

Additional evidence for the structure of isomeric propylphosphonates can be obtained using GC–FT-IR. Dialkyl propylphosphonates, alkyl propylphosphonochloridates and alkyl propylphosphonofluoridates show consistent differences in the spectral pattern between 1150 and 1550 cm^{-1}, allowing confident differentiation of *P*-propyl isomers.

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References

[1] Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and on their Destruction, Technical Secretariat for the Organisation for the Prohibition of Chemical Weapons, The Hague, 1997.
[2] R.W. Read, R.M. Black, J. Chromatogr. A 862 (1999) 169.
[3] R.M. Black, R.W. Read, J. Chromatogr. A 759 (1997) 79.
[4] B.L.M. van Baar, A.G. Hulst, E.R.J. Wils, J. Mass Spectrom. 33 (1998) 1104.
[5] L.J. Bellamy, The Infrared Spectra of Complex Molecules, vol. 1, third ed., Chapman and Hall, London, 1975.
[6] L.C. Thomas, Interpretation of the Infrared Spectra of Organophosphorus Compounds, Heyden & Son, London, 1974.
[7] L.C. Thomas, in: R. Belcher, D.M.W. Anderson, (Eds.), The Identification of Functional Groups in Organophosphorus Compounds (An International Series of Monographs, No. 7), Academic Press, London, 1974.
[8] A.J. Bell, D. Despeyroux, J. Murrell, P. Watts, Int. J. Mass Spectrom. Ion Processes 165–166 (1997) 533.
[9] M.W. Wensing, A.P. Snyder, C.S. Harden, J. Mass Spectrom. 30 (1995) 1539.
[10] D.J. McGarvey, J.R. Stuff, B.R. Williams, H.D. Durst, Spectrosc. Lett. 33 (2000) 795.
[11] A.F. Kireev, I.V. Rybal'chenko, V.I. Savchuk, V.N. Suvorkin, V.I. Kholstov, J. Anal. Chem. 55 (2000) 837.
[12] R.A. Nyquist, The Interpretation of Vapor-Phase Infrared Spectra, Sadtler Research Laboratories, Philadelphia, 1984.
[13] M.T. Söderström, in: J.R. de Haseth (Ed.), Proceedings of 11th International Conference on Fourier Transform Spectroscopy, Athens, GA, August 1997, AIP Press, New York, 1998, p. 457.