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Deuterated internal standards for gas chromatographic–mass spectrometric analysis of polar organophosphorus pesticides in water samples

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Abstract

It is essential to know whether drinking water contains any pesticides up to concentrations close to the European Union limit of 0.1 μg/l, in order to protect the population. Mass spectrometry (MS) using a suitable internal standard (I.S.) should improve the quantitative analysis of pesticides, the I.S. circumventing loss of compounds during the analytical procedure and correcting the analytical variability. In this study we verified this assumption in GC–MS, synthesising specific internal standards for four organophosphorus pesticides with poor stability, comparing the performances with and without the I.S.

Keywords: Internal standards; Water analysis; Organophosphorus compounds; Pesticides

1. Introduction

In recent years pesticides have been used on an increasingly wider scale throughout the world [1]. As a consequence, ground water has become contaminated in many cases, and we have to check whether drinking water contains pesticides in order to protect the population. Detection of pesticides in water up to concentrations close to the European Union (EU) limit of 0.1 μg/l is therefore an important task. However, pesticides involve compounds belonging to a range of chemical classes, with different behaviour and analytical problems.

Mass spectrometry (MS) has been proposed as a validating technique for identifying and quantifying pesticides and pollutants, on account of its high sensitivity in determining compounds on the basis of their physico–chemical characteristics [2]. The US Environmental Protection Agency (EPA) uses MS for an increasing number of quantitative applications. The availability of suitable internal standards (I.S.s) that enhance the quantitative analysis of pesticides, greatly increasing its accuracy and precision, will improve the diffusion of MS methods.

Gas chromatography (GC) and liquid chromatography (LC) coupled with MS permit compounds labelled with stable isotopes to be used as I.S.s, because their properties are very similar to those of the substances to be analysed. Deuterated I.S.s are used mainly to compensate for sample loss during clean-up and to correct the deviations of injection volumes and variability in detector response. They

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may be particularly useful when labile or volatile compounds have to be analysed. In earlier studies our group studied pesticides characterized by low stability listed by the EU as priorities for investigation [3–5]. Within this frame, in a previous work [6] we synthesized deuterated fenamiphos and its metabolites to be used as I.S.s in quantitative analysis of spiked water.

Dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate (trichlorfon) is an organophosphorus pesticide [7] characterised by polarity and thermolability, included in a list of 11 pesticides prioritized for investigation in Europe. Trichlorfon can be easily transformed in an alkaline medium to dichlorvos (2,2-dichlorovinyldimethyl phosphate), which is considered the first transformation product in environmental matrices [8]. Methods for the analysis of these compounds have been proposed since 1960 [9–11], but only recently have LC–MS methods been proposed capable of detecting a concentration of 0.1 μg/l [12–16]. No sensitive GC–MS method exists.

Demeton-*O*-methyl and demeton-*O* are used as acaricides [17] and as systemic and contact insecticides in mixtures with *S*-2-ethylthioethyl-*O*,*O*-dimethyl phosphorothioate (demeton-*S*-methyl) and its ethyl ester analogue demeton-*S* [17]. In vitro thermal rearrangement of demeton-*S*-methyl and demeton-*S* to the corresponding thiono analogues demeton-*O*-methyl and demeton-*O* has been reported [18] and can occur in water due to environmental processes or under analytical conditions. In these cases the use of a deuterated standard may clear the way to obtaining suitable accurate analytical methods.

As part of a project for monitoring organophosphorus agrochemicals in the environment [19], we were interested in the analysis of trichlorfon, dichlorvos, demeton-*O*-methyl and demeton-*O* (Fig. 1) in water. Using an easy and efficient synthesis procedure we prepared unlabelled and deuterated standards of these pesticides and used them for the analysis of these pollutants in water by GC–MS in the selected ion monitoring (SIM) mode. Other authors in the past indicated *O*-phenyldimethylthiophosphinate and 2-naphthyldimethylthiophosphinate as suitable I.S.s for quantitative analysis of organophosphorus pesticides in general [17]. In this study we evaluated the improvement of the analytical procedure achieved by using deuterated I.S.s.

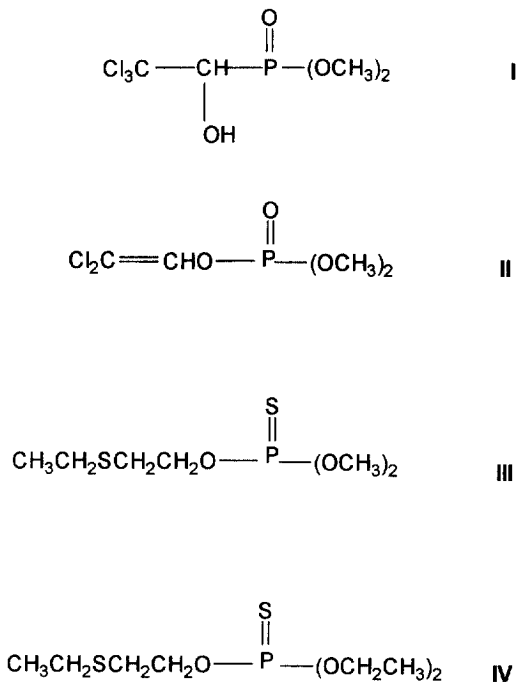

Fig. 1. Formulae of trichlorfon (I), dichlorvos (II), demeton-*O*-methyl (III) and demeton-*O* (IV).

Different extraction procedures were investigated. For solid-phase extraction (SPE) [20,21] we tested several extractive phases in order to find the most suitable one for each pesticide.

2. Experimental

2.1. Chemicals and materials

2.1.1. Unlabelled compounds

Trichlorfon was purchased from the Institute of Organic Industrial Chemistry (Warsaw, Poland), dichlorvos from Riedel-de Haën, and demeton-*O* from Dr. Ehrenstorfer (Augsburg, Germany). Demeton-*O*-methyl was synthesized in our laboratories (see Section 2.1.2.1).

2.1.2. Deuterated compounds

Melting points were determined on a Kofler hot-stage apparatus and were not corrected. Identities and quantities of products synthesized were established by MS and ^{31}P nuclear magnetic resonance (NMR). ^{31}P NMR spectra were recorded with a

Table 1
Chemical and analytical data for the deuterated standards of trichlorfon, dichlorvos, demeton-*O*-methyl and demeton-*O*

Standard	Isotopic purity (%)	^{31}P NMR
I-d_6	>99	18.40
II-d_6	>99	−2.90
IIIa-d_6	>99	71.80
IIIb-d_{10}	>99	68.20

Isotopic purity was determined by GC–MS analysis as reported in Section 2.2.

Bruker AC 250 spectrometer. Tetramethylsilane (TMS) (internal) and phosphoric acid (external) were used as standards and C^2HCl_3 as solvent. Chemical shifts are reported in ppm in Table 1 and MS analyses in Fig. 2. The solvents used for synthesis and extraction were specific for pesticide residue analysis and were purchased from Merck (Darmstadt, Germany) and Carlo Erba (Milan, Italy) and were employed without purification. Flash chromatography for purification of the synthesized products was done with silica gel 60 (63–200 μm) and thin-layer chromatography (TLC) was run on silica gel 60 F_{254} plates from Merck, using distilled solvents. Demeton-*O*-methyl (IIIa in Fig. 3) and demeton-*O* (IIIb in Fig. 3) were prepared as previously reported [22].

2.1.2.1. Synthesis

[$^2H_2$]Diazomethane. To a mixture of $CH_3CH_2O^2H$ (10 ml, 99.5 atom% ^2H) and ^2H_2O (10 ml, 99.8 atom% ^2H), 300 mg of sodium was added, under stirring and ice cooling. After 1 h a solution of *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide (2.5 g) in anhydrous ether (15 ml) was added at 65°C, under stirring, over a period of 20 min by using an apparatus for diazomethane preparation. The combined ethereal distillates contain deuterated diazomethane which was used immediately in the following reaction.

[$^2H_6$]Trichlorfon (I-d_6). A solution of 1.1 g of phosphoric acid in 5 ml of ^2H_2O (99.8 atom% ^2H), was evaporated under vacuum and the residue dissolved in 10 ml of ether. Diazomethane was added dropwise until a persistent yellow solution was obtained. The solvent was evaporated under vacuum

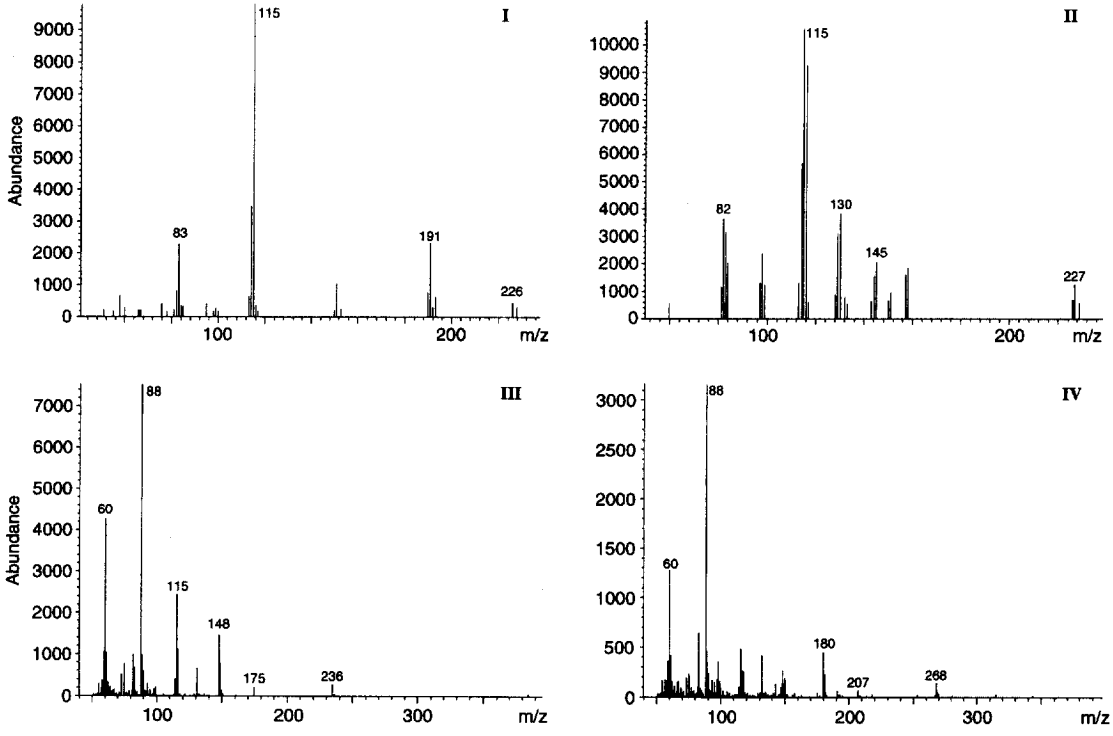

Fig. 2. Mass spectra of the four deuterated analogues of dichlorvos (I), trichlorfon (II), demeton-*O*-methyl (III) and demeton-*O* (IV).

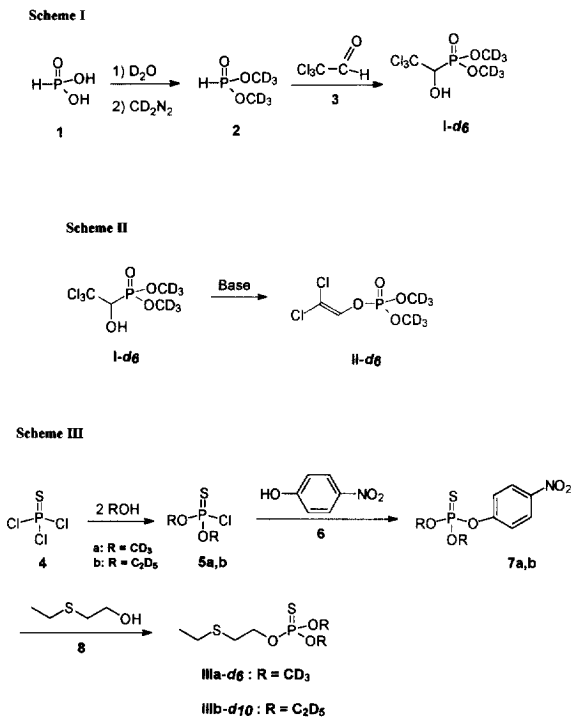

Fig. 3. Schemes of synthesis of the deuterated analogues of trichlorfon (scheme I), dichlorvos (scheme II), demeton-*O*-methyl and demeton-*O* (scheme III). D=Deuterium.

and the resulting [$^2H_6$]dimethyl hydrogenphosphite was treated dropwise with freshly distilled chloral (from 2.5 g of chloral hydrate distilled over sulfuric acid). The reaction mixture was kept below 25°C and stirred for 4 h. The mixture was then dissolved in benzene and washed with water (3×20 ml), dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. Flash chromatography (SiO_2; hexane–EtOAc, 2:8) yielded the pure product as a white solid (m.p. 77–79°C). MS *m/z* of mean ions and relative abundance are: 115 (100), 130 (36), 227 (12).

[$^2H_6$]Dichlorvos (II-d_6). A solution of I-d_6 in 100 ml of phosphate buffer at pH 10 was stirred at room temperature for 3 h. The mixture was extracted twice with methylene chloride (30 ml), and the organic layers were dried over anhydrous Na_2SO_4 and concentrated in vacuo to give pure II-d_6 as an oil. MS *m/z* of mean ions and relative abundance are: 115 (100), 191 (24), 226 (4).

Phosphorothioic acid *O,O*-hexadeuterodimethyl *O*-(4-nitrophenyl) ester (7a). To a stirred solution of thiophosphoryl chloride (23.4 mmol, 3.96 g) in dry benzene (45 ml) at 0°C a methanol solution (45 ml) of [$^2H_3$]sodium methoxide [from 46.8 mmol of sodium and 50 ml of $C^2H_3O^2H$ (99.8 atom% ^2H)] was added dropwise over 3 h. The resulting mixture was stirred for 3 h at room temperature then concentrated in vacuo to a thick slurry. The residue was partitioned between 50 ml of toluene and 30 ml of water. The organic phase was washed with water, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The resulting crude *O,O*-hexadeuterodimethyl chlorophosphorothionate was dissolved in 50 ml of dry ethanol and added dropwise to an ethanolic solution (100 ml) of sodium *p*-nitrophenate (23.4 mmol, 3.77 g). The reaction mixture was refluxed for 1 h, cooled to room temperature and the resulting suspension was filtered and the solvent concentrated in vacuo. The crude residue was dissolved in toluene (80 ml) and the organic layer was washed with water, dried (Na_2SO_4), and concentrated in vacuo. Flash chromatography (SiO_2; hexane–EtOAc, 9:1) yielded 7a as a pure oil: ^{31}P NMR (C^2HCl_3): 66.0 (m).

[$^2H_6$]Demeton-*O*-methyl (IIIa-d_6). To a solution of 7a (3.8 mmol, 1 g) in cyclohexane (30 ml) 2-hydroxyethyl ethylsulfide (0.8 g, 7.6 mmol) in the same solvent (10 ml) was added followed by aqueous NaOH (7.5 ml of a 50% solution in water) dropwise over 10 min. The reaction mixture was stirred at 50°C for 144 h. After cooling to room temperature the organic layer was washed twice with acidic water, dried (Na_2SO_4), filtered, and concentrated in vacuo. Flash chromatography (SiO_2; hexane–EtOAc, 9:1) afforded the pure product as an oil.

Phosphorothioic acid *O,O*-decadeuterodiethyl *O*-(4-nitrophenyl) ester (7b). The above procedure was followed as described except that *O,O*-decadeuterodiethyl chlorophosphorothionate was employed. Flash chromatography (SiO_2; hexane–EtOAc, 9:1) gave the pure compound as an oil: ^{31}P NMR (C^2HCl_3): 68.1 (m). MS *m/z* of mean ions and relative abundance are: 88 (100), 115 (32), 236 (3).

[$^2H_{10}$]Demeton-*O* (IIIb-d_{10}). The above procedure was followed as described except that compound IIIb-d_{10} (7 mmol, 200 mg) was employed. Flash chromatography (SiO_2; hexane–EtOAc, 9:1) afforded the pure product as an oil. MS *m/z* of mean ions are: 88 (100), 180 (14), 268 (4).

2.1.3. Solid-phase extraction

LiChrolut EN extraction cartridges (200 mg, 3 ml) were obtained from Merck. C_{18} Isolute cartridges (500 mg, 6 ml) were obtained from International Sorbent Technology (Hengoed, Mid-Glamorgan, UK). Chem Elut-Hydromatrix was purchased from Varian (Harbor City, CA, USA). The solvents used for extraction and analysis were specific for pesticide residue analysis and were purchased from Merck and Carlo Erba.

2.2. Instrumental analysis

The four pesticides were analysed in water by GC–MS. The samples extracted were analyzed by a HP 5890 GC instrument with a mass-selective detector HP 5971 (Hewlett-Packard, Palo Alto, CA, USA). The GC oven was fitted with an EASY 1701 from Analytical Technology (Cernusco sul Naviglio, Milan, Italy) 25 m×0.25 mm I.D., 0.25 μm film thickness.

For each compound two methods were developed, in Scan and SIM modes.

For trichlorfon helium pressure was kept at 40 kPa and the injector at 180°C. The oven was started at 80°C, then up to 190°C at 15°C/min, then to 240°C at 6°C/min.

For dichlorvos the helium pressure was kept at 40 kPa, the injector at 240°C. The oven was programmed as follows: 3 min at 60°C, from 60 to 180°C at 15°C/min and then 2 min at 180°C.

For both demeton-*O*-methyl and demeton-*O* the pressure was kept at 50 kPa, the injector at 240°C and the oven was programmed as follows: 1 min at 80°C, from 80 to 180°C at 10°C/min, from 180 to 260°C at 30°C/min and then 2 min at 260°C.

Mass-to-charge values selected for the four compounds and their deuterated analogues for the SIM option are reported in Table 2.

Table 2
SIM mass-to-charge values chosen for each analyte in GC–MS

Compound	*m/z* (unlabelled)	*m/z* (deuterated)
Trichlorfon	110–221	116–227
Dichlorvos	109–185	115–191
Demeton-*O*-methyl	109–230	115–236
Demeton-*O*	170–258	180–268

2.3. Extraction

2.3.1. Sample preparation

Tap water samples of 100 ml, adjusted to pH 3.5, were spiked at 5 μg/l with each of the four pesticides separately. The compounds were successively extracted from the water matrix with liquid–liquid extraction (LLE) or SPE with various sorbents; supercritical fluid extraction (SFE) was used for two of these compounds.

At first, recoveries were tested adding the I.S. after the extraction and before the evaporation step. The concentration of I.S. in the sample was equal to that of the unlabelled compound (5 μg/l) for trichlorfon, demeton-*O*-methyl and demeton-*O*; a lower concentration of I.S. (around 1.5 μg/l) was used for dichlorvos. The extract obtained was then evaporated to a final volume of 100 μl under a gentle stream of nitrogen and 1 μl of the final extract was analyzed in the GC–MS system.

Recoveries with and without the I.S. were compared. Three replicates using the I.S. and three without were done for each pesticide on the same day. This comparison was possible for trichlorfon, dichlorvos and demeton-*O*-methyl, but not for demeton-*O* because the deuterated analogue of this compound, previously synthesised (several months before) and used for the preliminary LLE replicates, underwent degradation.

2.3.2. Liquid–liquid extraction

LLE was used for trichlorfon, demeton-*O*-methyl and demeton-*O*. Three successive aliquots of 30 ml of solvent were used to extract 100 ml of sample. Ethyl acetate was used to extract trichlorfon, hexane for demeton-*O*-methyl and demeton-*O*.

2.3.3. Solid-phase extraction

LiChrolut EN and C_{18} cartridges were used. LiChrolut EN cartridges were conditioned at atmospheric pressure with 5 ml of acetonitrile and 5 ml of distilled water. They were then transferred to a Supelco (Bellefonte, PA, USA) vacuum apparatus for loading the samples under vacuum at a flow of 13 ml/min. The cartridges were dried under vacuum for 15–20 min and eluted with two 3-ml aliquots of acetonitrile, maintaining a contact time of 3 min between solvent and sorbent.

For the other sorbents the scheme of the procedure was basically the same. C_{18} cartridges were conditioned with 5 ml of ethyl acetate and activated with 5 ml of methanol, loaded at a flow-rate of about 15 ml/min and dried under vacuum for about 25 min, then eluted with 5 ml of ethyl acetate without vacuum. Three replicates were done for each extraction.

2.3.4. Supercritical fluid extraction

SFE was carried out on a Isco CO2-SFX 220 Model using CO_2 with a 4.5 purity (Sapio, Milan, Italy), spiking mineral water with the analytes (at 5 μg/l) and collecting 1 ml of the water onto a sodium sulphate or 10 ml Chem Elut filled extraction cartridge. The trapping solvent was ethyl acetate, gently heated to avoid ice formation at the end of the restrictor; the working pressure was 51 711 kPa, temperature 60°C, the volume of CO_2 was of 30 ml for trichlorfon and 60 ml for demeton-*O*-methyl, the flow 2 ml/min.

The procedure adopted after extraction was the same as after SPE (see Section 2.3.3).

2.4. Derivatization

We derivatized trichlorfon as previously described [23]. Briefly, the extract was dried, then derivatized at room temperature for 50 min with 100 μl of a mixture of pyridine–acetic anhydride (1:4) for GC analysis of trichlorfon as the acetyl derivate. The extract was dried again and loaded to a final volume of 100 μl of ethyl acetate; 1 μl of the extract was injected in the GC–MS system.

2.5. Recovery

For the procedure with the I.S., the percentage recovery was calculated using the relative response factor (ratio of the areas of the two selected *m/z* for the two equimolar standards).

For the procedure without the I.S., the percentage recovery was calculated using an external standard. Mass-to-charge values for the unlabelled and deuterated compounds considered for the recovery calculation were *m/z* 110 and 116 for trichlorfon, 185 and 191 for dichlorvos, 109 and 115 for demeton-*O*-methyl, 170 and 180 for demeton-*O*.

3. Results

3.1. Synthesis of the analytical standards

Deuterated analytical standards were prepared in good quantities and with high isotopic purity (Table 1) by easy and rapid procedures. Hexadeuterated trichlorfon (I-d_6, Fig. 3, scheme I) and dichlorvos (II-d_6, Fig. 3, scheme II) were prepared starting from phosphoric acid in which the unlabelled methyl group was introduced by esterification with deuterated diazomethane, prepared using a conventional non-deuterated reagent in deuterated water and ethanol (see Section 2.1.2.1).

Deuterated trichlorfon was prepared (Fig. 3, scheme I) by further condensation with anhydrous chlorine following the general procedure reported [24]; deuterated dichlorvos was prepared in quantitative yields from I-d_6 by reaction in basic aqueous solution (Fig. 3, scheme II).

Deuterated demeton-*O*-methyl (IIIa-d_6) and demeton-*O* (IIIb-d_{10}) were prepared (Fig. 3, scheme III) following the general procedure previously reported for the synthesis of the unlabelled standards [17], starting from the corresponding deuterated *O,O*-dialkyl chlorophosphorothionate (5a,b) (obtained by reaction of thiophosphoryl chloride with deuterated methanol or ethanol) with *p*-nitrophenol to give compounds 7a,b. Final *trans*-esterification with the ethylthioethanol (8) gives compounds III_a-d_6 and IV-d_6.

Mass spectra of the four deuterated standards synthesized are reported in Fig. 2.

[$^2H_6$]Dichlorvos (I) shows a molecular ion at *m/z* 226, 6 u more than the molecular ion of dichlorvos, indicating the presence of the correct number of deuterium atoms in the molecule. Two other deuterated ions at *m/z* 115 and 191 derive from loss of Cl_2CCHO and HCl, respectively. These ions are, as expected, 6 u more than the corresponding ions of dichlorvos. For trichlorfon (II) the molecular ion cannot be seen, but the presence of the correct number of deuterium atoms is demonstrated by the *m/z* 227, which derives from a HCl loss. The deuterated ions with *m/z* 145 and 115 are due to loss of CCl_3 and Cl_3CCHOH, respectively. The ion with *m/z* 130, corresponding to a $PO(OC^2H_3)_2$ loss, is the same as the *m/z* in the unlabelled compound,

showing that the deuterium atoms are all in the correct positions.

For deuterated demeton-*O*-methyl (III) the molecular ion with *m/z* 236 is shifted 6 u in comparison with the unlabelled demeton-*O*-methyl, indicating the presence of the correct number of deuterium atoms. The deuterated ions with *m/z* 175, 148 and 115, deriving from loss of CH_3CH_2S, $CH_3CH_2SCH_2CH$ and $CH_3CH_2SCH_2CH_2S$, respectively, show the deuterium atoms all in the correct positions. The ion with *m/z* 88, corresponding to the $HOPS(OC_2^2H_5)_2$ loss, is the same as the ion in the unlabelled compound and gives further confirmation of the presence and position of the deuterium atoms.

For demeton-*O* (IV) the molecular ion with *m/z* 268 shows a shift of 10 units, demonstrating that the correct number of deuterium atoms is present. The deuterated ions with *m/z* 207 and 180, due to loss of CH_3CH_2S and $CH_3CH_2SCH_2CH$, respectively, confirm that the deuterium atoms are all positioned correctly. Similarly, the ion with *m/z* 88, common to demeton-*O*-methyl, being the same as the ion of the unlabelled compound, shows that the deuterium atoms are all correctly positioned.

3.2. GC–MS analysis

Good calibration curves for the four compounds ($0.996<R<0.999$) were obtained in GC–MS analysis in the SIM mode, injecting 1 μl of each solution at different concentrations, obtained by dilution of a solution with a concentration of a few ng/μl, with a fixed concentration of the specific deuterated standard (from 100 pg/μl to a few ng/μl depending on the I.S. considered).

The limit of detection (LOD) varied from 100 pg for dichlorvos, demeton-*O*-methyl and demeton-*O* to 25 pg for derivatized trichlorfon.

3.3. Recoveries from water

Selected ion current profiles of the unlabelled and deuterated compounds adopted for calculating the recovery of trichlorfon, dichlorvos and demeton-*O*-methyl are presented in Fig. 4. Table 3 presents the recoveries for the four compounds using the different extraction methods; all the results refer to extraction followed by addition of the specific I.S. before

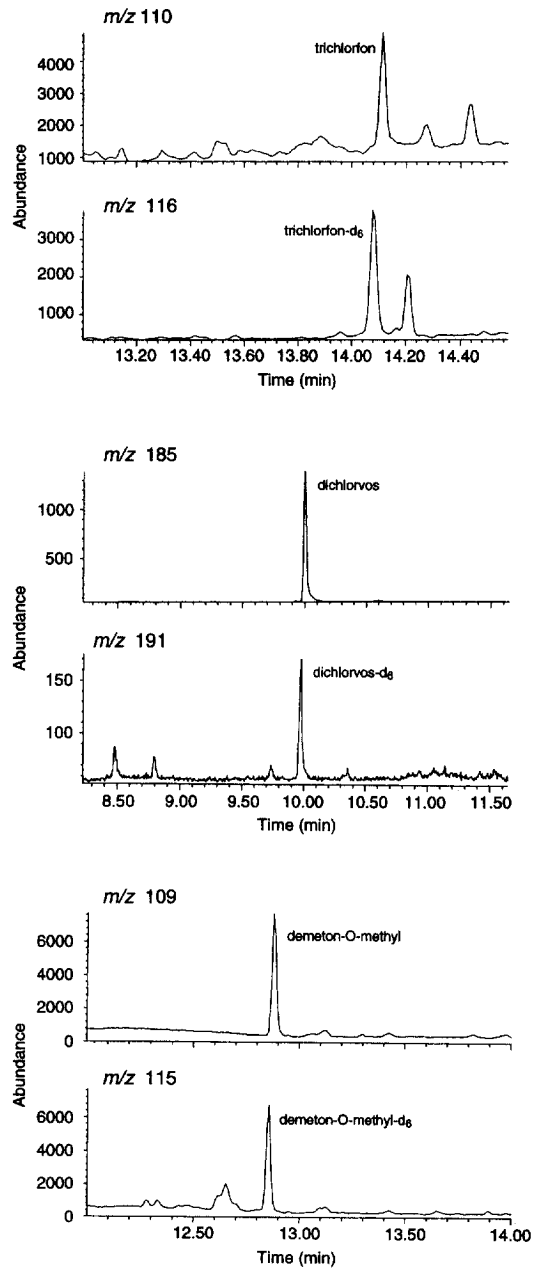

Fig. 4. GC–MS selected ion current profiles of masses of unlabelled and deuterated analogue adopted for calculating the recovery of trichlorfon, dichlorvos and demeton-*O*-methyl.

evaporation. For trichlorfon, SFE provided good results, comparable to LLE. Recovery with C_{18} was around 50%, and LiChrolut EN proved to be the most suitable phase (see Table 3). For dichlorvos

Table 3
Recovery (S.D., %) of trichloron, dichlorvos, demeton-*O*-methyl and demeton-*O* with different extraction techniques, with and without I.S.

	Trichloron		Dichlorvos		Demeton-*O*-methyl		Demeton-*O*
	With I.S.	Without I.S.	With I.S.	Without I.S.	With I.S.	Without I.S.	With I.S.
LLE	85.2 (±37.5)	–	–	–	84.1 (±3.3)	58.5 (±10.4)	68.0 (±4.4)
C_{18}	53.9 (±13.3)	–	84.7 (±3.4)	–	–	–	–
LiChrolut EN	90.5 (±7.4)	44.7 (±23.9)	77.1 (±8.7)	39.7 (±11.8)	50.8 (±4.2)	–	–
SFE	83.3 (±13.8)	–	–	–	106.7 (±58.2)	–	–

extractions with LiChrolut EN and C_{18} were both good. For demeton-*O*-methyl extractions with LiChrolut EN were tried but results were not good (recovery was around 50%). Demeton-*O*-methyl is in fact one of a group of organophosphorus compounds that are difficult to analyze. Other sorbents (ENV$^+$ from Isolute and SDB from Baker) were also tested for this compound, adopting the same procedure used for LiChrolut EN, but results were worse. SFE was used as well, but the standard deviation was high. LLE remained the best solution for extracting this compound. LLE with hexane also gave adequate recovery for demeton-*O* (see Table 3).

A comparison of the two procedures – with and without the I.S. – for trichlorfon, dichlorvos and demeton-*O*-methyl is presented in Table 3. The mean difference of the recoveries obtained in the two cases, averaged on the three compounds, was large (36.3%).

4. Discussion

The differences between recoveries obtained using the I.S., added before the evaporation step, and without it are indeed large and can be explained by the fact that the I.S., mimicking the behaviour of the analyte, overcomes evaporative loss and degradation during analysis, which are the main problems affecting the stability of the compound after extraction. Evaporation is a critical step because of the related evaporative losses and hydrolysis. Evaporative loss is a particular problem for a volatile compound like dichlorvos [25], where addition of the I.S. as a control overcomes this difficulty.

Stability problems during analysis are reported for trichlorfon [26] and for the isomer of demeton-*O*-methyl, demeton-*S*-methyl [14]. For trichlorfon several precautions (like avoiding glass wool and keeping the injector at low temperatures) have been adopted, in accordance with a previous study [28], but the I.S. helped overcome degradation during analysis. For demeton-*O*-methyl the I.S. helped to cope with polarity and thermolability, which normally cause compound loss.

Among the sorbents tested, LiChrolut EN provided very good recoveries for both trichlorfon and dichlorvos; for demeton-*O*-methyl there were problems probably due to the low stability of the compound. Other authors [14] found poor recoveries for its isomer demeton-*S*-methyl with all the sorbents except LiChrolut EN, which gave 70% recovery.

The difference in recoveries using LiChrolut EN and C_{18} found for trichlorfon, but not for dichlorvos (see Table 3), may be due to the higher solubility in water of trichlorfon (154 g/l in water at 25°C in comparison with 10 g/l in water of dichlorvos at 20°C). LiChrolut EN does have hydrophilic properties, while C_{18} is hydrophobic, so the first is more efficient because of the enrichment of more polar molecules [27].

Results obtained for trichlorfon and dichlorvos appear better than those reported for LC–MS using the same sorbents [14], but no real comparison can be made because of the different matrices used in the two studies (tap water in this case and groundwater in that). The same authors [14] confirmed that recoveries from different matrices can differ widely. Results for dichlorvos are comparable to those with carbon black cartridges in high-performance liquid chromatography [28].

SFE gave good results for trichlorfon, comparable with those with LLE and with a lower standard deviation, not far from those with SPE. Thus, this technique can be considered a useful alternative. For demeton-*O*-methyl SFE gave instead highly variable

results, because of conversion of the compound to its demeton-*S*-methyl isomer.

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