ELSEVIER

Journal of Chromatography A, 918 (2001) 67–78

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

# Determination of polar organophosphorus pesticides in aqueous samples by direct injection using liquid chromatography–tandem mass spectrometry

Benno A. Ingelse, Ruud C.J. van Dam, Rob J. Vreeken, Hans G.J. Mol*, Odile M. Steijger

*TNO Nutrition and Food Research, P.O. Box 360, 3700 AJ Zeist, The Netherlands*

Received 1 August 2000; received in revised form 19 February 2001; accepted 19 February 2001

**Abstract**

It was demonstrated that four out of six of the very polar organophosphorus pesticides (OPs), i.e. acephate, methamidophos, monocrotophos, omethoate, oxydemeton-methyl and vamidothion, could not be extracted from water using commonly available SPE cartridges. In addition, GC analysis on all six compounds was found to be troublesome due to their polar and thermolabile character. This initiated the development of an alternative highly sensitive and selective method for the determination of the above mentioned very polar OPs in water, based on LC–MS. Large volume (1 ml) water samples were directly injected onto an RP18 HPLC column with a polar endcapping. The latter was essential for obtaining retention and maintaining column performance under 100% aqueous conditions during the sampling. The compounds were ionized using atmospheric pressure chemical ionization and detected on a tandem mass spectrometer operated in multiple reaction-monitoring mode. The detection limits were in the range of 0.01–0.03 μg/l. Compared to conventional GC methods, the developed LC–MS procedure is very straightforward, fast and more reliable. This application demonstrates the applicability of LC–MS for analysis of polar OPs in surface, ground and drinking water, as a more favourable alternative to GC. 

*Keywords:* Water analysis; Pesticides; Organophosphorus compounds

## 1. Introduction

Organophosphorus pesticides (OPs) are a class of pesticides that generally act as cholinesterase inhibitors and are used for the control of spiders, mites, aphids, beetles, caterpillars etc. in a wide variety of crops [1]. However, OPs are toxic to all animals and humans, since all produce acetylcholinesterase. For evaluation of environmental waters and water resources for preparation of drinking water, highly sensitive methods for the determination of OPs in surface water, ground water and drinking water are required. In the European Union, a maximum allowable concentration of 0.1 μg/l for each individual (organophosphorus) pesticide in drinking water is in force. In the Netherlands, for aquatic ecosystems, the maximum allowable risk level for OPs varies from 0.0007 to 23 μg/l [2].

Organophosphorus pesticides vary widely in

*Corresponding author. Tel.: +31-30-694-4513; fax: +31-30 694-4077.

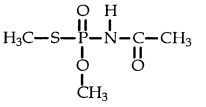
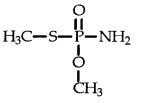
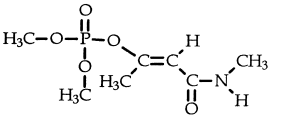
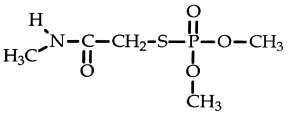
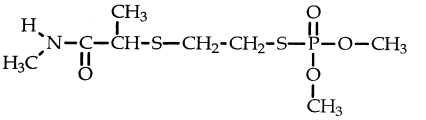
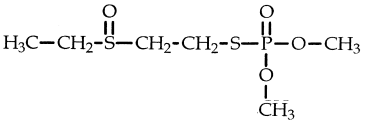
0021-9673/01/$ – see front matter 
PII: S0021-9673(01)00660-4

physico-chemical properties like water solubility, $K_{ow}$, vapour pressure, molecular mass and thermal stability. Many papers have described the determination of OPs in aqueous samples. Most OPs are easily analysed by GC and consequently GC methods are frequently cited, although LC–MS methods have also been reported [3–5]. Prior to chromatographic analysis, the OPs are usually extracted from the water sample by liquid–liquid extraction, (on-line) solid-phase extraction (SPE) or solid-phase micro extraction (SPME) and many successful applications have been described [6–9].

A small number of OPs with similar properties, however, is missing in the methods described in literature or is only incidentally taken into consideration, and includes: acephate, methamidophos, omethoate, vamidothion, oxydemeton-methyl and monocrotophos (selected physico-chemical properties and structures are presented in Table 1). These OPs have in common that they are thermally labile and/or very polar and therefore less GC-amenable. Moreover, especially acephate, methamidophos, monocrotophos and omethoate are extremely water soluble and, in contrast to most other pesticides that are quoted in literature as highly polar (e.g. oxamyl, acidic pesticides [10], dichlorvos, mevinphos, dimethoate [11]), not extractable (after the adequate pH adjustment) using the more common LLE or SPE procedures.

Liquid chromatography (HPLC) seems to be the obvious choice for the separation of the troublesome OPs mentioned above. Since these polar OPs have unsuitable UV-properties, mass spectrometric detection is favourable. LC–MS(–MS) has demonstrated its applicability for environmental analysis (e.g. [12–14]). In a few occasions, one or more of the very

Table 1
Properties of polar organophosphorus pesticides [1], [26]

| Name | MW | $S_{water}$ g/l | Log $K_{ow}$ $K_{oc}$ | Vap. press. mPa | Structure |
|---|---|---|---|---|---|
| Acephate | 183.2 | 790 | −0.89 2 | 0.226 |  |
| Methamidophos | 141.1 | >200 | −0.8 5 | 2.3 |  |
| Monocrotophos | 223.2 | Miscible | −0.22 6 | 0.29 |  |
| Omethoate | 213.2 | Miscible | −0.74 21–87 | 3.3 |  |
| Oxydemeton-methyl | 246.3 | Miscible | −0.74 27–138 | 3.8 |  |
| Vamidothion | 287.3 | 4000 | 0.12 8 | $9\times10^{-3}$ |  |

polar OPs have been included in the investigation. Lacorte et al. [15] studied in detail the effect of temperature and extraction voltage on the fragmentation of a number of organophosphorus pesticides, including acephate, methamidophos and vamidothion. Extraction of the OPs from 200 ml of ground water was briefly evaluated using SPE. Two types of 200 mg cartridges (ENV and LiChrolut) were studied. Recoveries were 125–154% for acephate, 72–83% for vamidothion and 24–31% for methamidophos which was attributed to losses during evaporation. No data on repeatability and limit of quantification for the overall method were given. Fernandez-Alba et al. [16] compared various sample handling and analytical procedures for monitoring of pesticides and metabolites in ground water. Acephate, methamidophos and omethoate were analysed with GC-IT-MS. SPE using $C_{18}$ disks or 500 mg ENV polymer cartridges resulted in no recovery at all for the mentioned polar OPs which was attributed to their high water solubility, this in contradiction to results presented in Ref. [15]. Molina et al. [11] compared several SPE sorbents for determination of a number of OPs including oxydemeton-methyl. Irrespective the sorbent used, good recoveries (88–130%) were obtained using LC–MS for analysis. The limit of detection was approx. 0.02 μg/l after 500-fold preconcentration by SPE. Norberg et al. [17] evaluated on-line SPE coupled to HPLC with diode array detection for determination of OPs in water. For most OPs good recoveries were obtained, but for omethoate and monocrotophos almost complete breakthrough occurred, despite the favourable ratio of stationary phase over sample volume with this approach.

To summarize, to our best knowledge, no successful method has been described until now for trace level determination of methamidophos, omethoate and monocrotophos in water, whereas the reported data on acephate, vamidothion and oxydemeton-methyl are very limited. In this work, first the limitations of conventional SPE and GC analysis are illustrated. The purpose of the work described in this study was to develop a rapid and straightforward method for the direct determination of the above mentioned polar OPs in water. A method based on HPLC with tandem mass spectrometry has been developed, validated and implemented for routine analysis of surface water, ground water and drinking water samples.

## 2. Materials and methods

Acephate, vamidothion, omethoate and monocrotophos were purchased from Brunschwig (Amsterdam, The Netherlands) and oxydemeton-methyl and methamidophos were obtained from C.N. Schmidt (Amsterdam, The Netherlands) and were of the highest analytical grade. All solvents were of HPLC grade. For HPLC analysis, stock solutions of the polar OPs were prepared in 0.1% acetic acid (HAc) in demineralized water. Calibration standards were prepared by dilution of the stock solution with 0.1% acetic acid in demineralized water. For GC analysis, stock solutions and dilutions from the stock were prepared in ethyl acetate. All solutions were stored in the dark at 2–10°C and found to be stable for at least 2 months.

For evaluation of the possibilities of SPE the following cartridges were used: SDB (styrene divinyl benzene polymer, 200 mg, Malinckrodt-Baker, Deventer, Netherlands), Oasis HLB ([poly(divinylbenzene-co-N-vinylpyrrolidone], 200 mg, Waters, Ettenleur, Netherlands), Envicarb (graphitized non-porous carbon, 100 $m^2/g$, 500 mg, Supelco, Zwijndrecht, Netherlands), Carbograph (graphitized carbon, 150 mg, Alltech, Hoogeveen, Netherlands).

### 2.1. Sample preparation

#### 2.1.1. SPE followed by GC-FPD analysis

The SPE cartridges were conditioned by rinsing with 2× approx. 5 ml of desorption solvent, 2× approx. 5 ml of methanol and approx. 10 ml of demi-water. Directly after the water, without letting the cartridge run dry, 100 ml of surface water sample, spiked with acephate, methamidophos, omethoate, oxydemeton-methyl and vamidothion, at the relatively high level of 50 μg/l, was applied to the SPE cartridge. After removal of excess water by air suction for approx. 5 min, the analytes were eluted with 2×5 ml of ethyl acetate or a mixture of dichloromethane/methanol (8:2 V/V). The volume was adjusted to 10 ml, i.e. no evaporative con-

centration was carried out, and this extract was subjected to GC-FPD analysis.

#### 2.1.2. Evaporative concentration/solvent switch followed by GC-FPD analysis

Five hundred ml of water sample was concentrated to approx. 3–5 ml at reduced pressure using a rotary evaporator at 50°C. The concentrated water was dissolved in 100 ml of ethyl acetate which was filtered through a filter containing anhydrous sodium sulfate. The ethyl acetate was concentrated to approx. 5 ml at reduced pressure using a rotary evaporator at 40°C (remaining traces of water evaporate azeotropically). The extract was quantitatively transferred into a calibrated tube and concentrated to 1 ml at 35°C under a gentle flow of nitrogen gas. This extract was subjected to GC-FPD analysis.

#### 2.1.3. Sample preparation for LC–MS–MS analysis

To 100 ml of the water samples, 100 μl glacial acetic acid was added. After filtering (0.4 μm pore size) the samples were directly injected onto the HPLC-column without further sample clean up or pre-concentration.

### 2.2. Gas chromatography–flame photometric detection

A Fisons (model 8000, Interscience, Breda, Netherlands) gas chromatographic system with split/splitless injection and FPD detection was used. GC conditions: injection volume, 4 μl, hot splitless injection (empty liner); column 30 m×0.32 mm ID, 0.25 μm DB1701; temperature program, 80°C (2 min)–20°C/min–160°C (5 min)–10°C/min–200°C (0 min)–20°C/min 260°C (5 min); FPD detection in phosphorus mode.

### 2.3. Liquid chromatography–mass spectrometry

An HPLC pressure gradient pump system was applied, consisting of two K1001 pumps and a high pressure solvent mixer (Knauer, Germany). A Midas autosampler (Spark, the Netherlands) injected 1000 μl onto a Phenomenex Aqua column (aqua 5 μm $C_{18}$, 4.6×150 mm, Torrance, CA). Eluent A consisted of $H_2O$: MeOH: HAc=94.9:5:0.1 and eluent B consisted of $H_2O$:MeOH:HAc=9.9: 90:0.1 (*V*:*V*:*V*). The gradient was as follows: $t=0$, 100% A; $t=3$ min., 50% A, $t=10$ min., 0% A. The flow-rate was 0.7 ml/min.

Atmospheric pressure chemical ionization (APCI) mass spectrometry was performed in the positive mode using an API 2000 (PE/Sciex, Foster City, CA). The nebulizer was heated at 400°C. All gases (curtain gas, nebulizer gas and auxiliary gas) were set at 50 p.s.i.. The nebulizer current was set a 2 μA, the CAD gas value was set at 2. The following precursor→product ion pairs were monitored in MRM mode: methamidophos $m/z=142\rightarrow94$ (19), acephate $m/z=184\rightarrow143$ (11), omethoate $m/z=214\rightarrow125$ (29), monocrotophos $m/z=224\rightarrow127$ (21), oxydemeton-methyl $m/z=247\rightarrow168$ (19), vamidothion $m/z=288\rightarrow146$ (17). The collision energy was in the range of 10–30 V (exact values between brackets) and was optimized for each compound.

Electro spray (turbo ion spray for this particular instrument) parameters used during evaluation of MS conditions, were as follows: turbo gas temperature 425°C, all gasses (curtain gas, turbo gas and auxiliary gas) were set at 50 p.s.i. The ion spray voltage was 5000 V. The CAD gas value was set at 3 p.s.i. The same precursor→product ion pairs as for APCI were monitored.

### 2.4. Quantification

With each batch of LC–MS analyses, seven calibration solutions with concentration ranging from approx. 0.025 to 2 μg/l of were injected before the sample extracts. From the responses, a calibration curve was calculated to evaluate linearity. It is a common phenomenon that the response of the MS-detector changes during sample analyses. Normally, this can be corrected by using a deuterated internal standard. However, as in this case these internal standards were not available, one of the calibration standards was again injected after each three samples and used to correct for the possible drift. The response of this calibration standard was a measure of the change of the detector response. Concentrations of samples were calculated using single-point calibration, relative to the average response of

the calibration standard injected before and after the sample.

For evaluation of ion suppression or enhancement due to the matrix, water samples were quantified using standards in 0.1% acetic acid/milliQ water of the same concentration.

## 3. Results and discussion

### 3.1. Limitations of SPE and GC

In literature there have been some controversial results with respect to the extraction of very polar OPs from water (e.g. [15,16]). To evaluate the possibilities of SPE, several types of cartridges designed for extraction of polar analytes, i.e. polymer and/or graphitized carbon based, were selected. A relatively small volume of a surface water sample (100 ml) was extracted and desorbed with ethyl acetate or, in case of cartridges containing graphitized carbon, with dichloromethane/methanol (8:2) as was recommended by Di-Corcia et al. [18]. The water was spiked with polar OPs at a high level (50 μg/l) so that evaporative concentration was not necessary for GC analysis and evaporative losses could be excluded as much as possible. The results are presented in Table 2. For both vamidothion and oxydemeton-methyl acceptable recoveries are obtained with several stationary phases, which is in agreement with data reported by others [11,15]. Acephate, methamidophos and omethoate, however, are hardly retained which, in agreement with Ref. [16], is attributed to breakthrough. The high recovery for acephate reported by Lacorte [15] could not be reproduced using similar polymer phases and sampling volumes. Evaporative loss as explanation for low recoveries for methamidophos, as suggested by Lacorte, is considered doubtful as for dichlorvos (which has a vapour pressure of 2100 mPa, almost 1000-fold higher than methamidophos) recoveries of approx. 70% are achieved (SDB), which is comparable with recoveries reported elsewhere [11]. In general, as one would expect, there is a relation between the log $K_{ow}$ and the extractability using SPE (the water solubility is less indicative). Compounds with log $K_{ow}$ values $>0$ like vamidothion and other OPs often quoted as polar such as dichlorvos (log $K_{ow}=1.9$, water solubility $=18$ g/l), mevinphos (0.27, miscible), and dimethoate (0.70, 24 g/l) can all be successfully extracted using SPE (e.g. SDB) cartridges. In contrast acephate, methamidophos and omethaote, which have a log $K_{ow}<0$, could not be retained. The exception in this respect is oxydemeton-methyl (log $K_{ow}=-0.74$).

Instead of concentrating the polar OPs using SPE, our in-house method employs evaporation of the water samples, at reduced pressure, to almost dryness, and reconstitution of the residual water in ethyl acetate. After evaporative concentration of the ethyl acetate extracts, the extracts are analyzed by GC with FPD or MS detection. Recoveries of 60–120% are usually obtained this way using matrix-matched calibration. The method is, however, time consum-

Table 2
Extraction efficiency of polar OPs using SPE[a]

| SPE cartridge | SDB | Oasis HLB | Envicarb | Envicarb | Carbograph |
|---|---|---|---|---|---|
| Desorption solvent (2×5 ml) | EtAc | EtAc | EtAc | DCM/MeOH | DCM/MeOH |
| | Mean recovery[b] (%) | | | | |
| Acephate | 1 | 0 | 7 | 4 | 2 |
| Methamidophos | 2 | 0 | 2 | 1 | 0 |
| Omethoate | 15 | 0 | 24 | 25 | 8 |
| Oxydemeton-methyl | 142 | 10 | 66 | 111 | 99 |
| Vamidothion | 77 | 64 | 77 | 74 | 153 |

[a] EtAc = ethyl acetate; DCM/MeOH = dichloromethane/methanol 8:2; Sample: 100 ml of surface water, fortified at 50 μg/l, details on SPE: see Experimental.

[b] $n=2$ except for carbograph ($n=1$).

ing. Moreover, the performance of the chromatographic system deteriorates rapidly during analysis of surface water extracts due to degradation and adsorption in the liner and the analytical column. This results in losses of analytes, severe peak tailing of the polar OPs and the response being very matrix dependent [19], which in turn complicates quantification, reduces sensitivity of the method and leads to very frequent maintenance (changing of liner and front section of analytical column). This is clearly demonstrated in Fig. 1. The upper chromatogram in Fig. 1 was obtained from the analysis of an extract of surface water fortified at 0.1 μg/l methamidophos, acephate and oxydemeton-methyl, injected on a ''clean'' GC system. Even on a clean system, oxydemeton-methyl breaks down in the inlet and is in fact determined indirectly by two of its degradation products. The lower trace represents the analysis of the same sample after the analysis of some 10–15 surface water extracts. Both system deterioration and response are strongly depended on the matrix.

The problems described above are typical for all six polar OPs included in this work. This combined with the difficulties during preconcentration was the reason for the development of a method based on large volume injection LC–MS. Other polar OPs like dichlorvos, mevinphos and dimethoate are much better GC amenable (although the response is, like for many other compounds, affected by the presence of matrix), and were therefore not included in the current work.

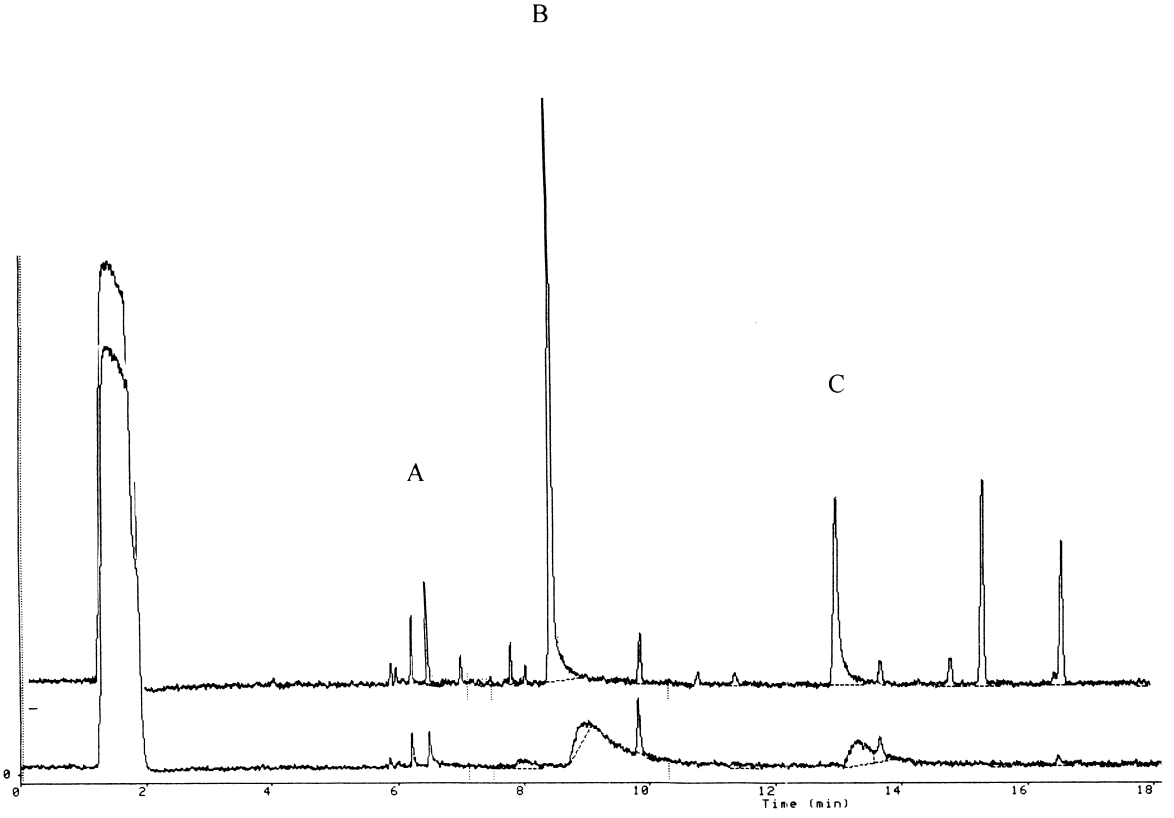



Fig. 1. GC-FPD chromatogram of an extract of surface water fortified at 0.1 μg/l of oxydemeton-methyl (A, the two peaks represent two transformation products), methamidophos (B) and acephate (C). The upper trace is obtained from an injection on a ''clean'' GC system, the lower trace is obtained after the analysis of 15 surface water extracts. Sample preparation: evaporative concentration/solvent switch, details and GC conditions: see Experimental.

## 4. Liquid chromatography

The polar OPs selected in this study, especially acephate and methamidophos are highly polar and highly water soluble compounds. In our first attempt to analyze these compounds using reversed-phase HPLC (RP-HPLC) on a standard ODS column, we did not obtain sufficient retention for the above mentioned compounds ($k \approx 1$). Consequently, the matrix components (mainly salts and humic acids) were interfering with the analytes and the MS response was strongly suppressed because of this. In another attempt, we applied the polymer based Shodex RSpak DE-413 column (Showa Denko k.k., CA). In earlier studies in our laboratory, this column showed good retention characteristics for polar compounds. After optimization of the LC parameters however, we found that retention was still insufficient ($k \approx 1.5$). The required limit of detection (0.1 μg/l) could be achieved; repeatability however did not meet the criteria set by the European Community for validation of residue analysis (i.e. $\leq$20%) [20]. This is most probably explained by matrix interferences of the various matrices, which differ between samples. The above examples stress the importance of optimizing retention, i.e. to separate the analytes from matrix compounds.

It is well known that non-volatile substances destabilize the electrospray process [21] and moreover contaminate the interface [22]. This limits the possibilities of using ion-pair chromatography (IPC). Some solutions to solve this problem have been described. Alonso et al. [23] determined anions (sulfonates) using a volatile ion pair reagent (triethylamine) and an orthogonal electrospray interface. In a recent study, Forngren et al. [24] applied a post-column ion-exchanger, thereby removing non-volatile counter-ions before entering the API-interface. They could continue their analyses for up to 3 h before regenerating the ion-exchange column. Although this technique seems promising, it cannot be operated in a continuous routine-way, without the use of a column-switching device.

Attempts in the current work to use ion-pair chromatography to increase retention failed due to the lack of volatile cationic ion-pair reagents. Although compatibility with non-volatile buffers and ion-pair reagents has been claimed by manufacturers of instruments with orthogonal or Z-shape designed interfaces, a more straightforward reversed-phase approach, without ion-pair reagents was preferred.

In the current study we applied a Phenomenex Aqua column. This column is endcapped with a hydrophilic reagent. This endcapping prevents the $C_{18}$ chains of the column from collapsing, even under 100% aqueous solutions. Moreover, the endcapping supplies additional selectivity, and, equally important in current application, retention for the most polar OPs. Resultantly, the retention factor of methamidophos (the fastest eluting compound) and acephate were 2.1 and 2.3 respectively.

A mixture of MeOH and water, with the addition of 0.1% acetic acid was chosen as eluent. Gradient elution was optimized with respect to peak shape and analysis time. Unlike the in-house GC method, both peak shape and retention time did not change during the analysis of (more than 50) samples.

As a part of this study, we examined the possibilities of using large volume injection as a tool to improve the detection limit of the method, as has been reported by others for more easy to retain carbamates [25]. In case a standard ODS column was applied, we found that large volume injection (500 μl) resulted in severe band broadening. The Phenomenex Aqua column however allowed injection volumes of 1 ml (the largest volume tested) without affecting peak shape and efficiency. Considering the volume of the column (approx. 1.5 ml), more than half the column volume is filled with water after injection. The surprisingly good performance, under these conditions is attributed to the polar endcapping, which prevents the column from collapsing under aqueous conditions.

### *4.1. Mass spectrometry*

For all polar OPs, $[M+H]^+$ was selected as the precursor ion. The product ions used for monitoring are given in the Experimental section. Postulated formulae of the product ions of organophosphorus pesticides have been discussed and described elsewhere [11,15]. As a part of this study, we compared the influence of the organic modifier on the detector response. We found that the response of the analytes was improved by a factor 2 when solvent mixtures of MeOH/water (50/50, *V*/*V*) were applied instead of

acetonitrile/water (50/50, *V*/*V*). This is explained by the fact that acetonitrile is a weaker proton donor than methanol (in aqueous phase, also likely in gaseous phase). Consequently, ionisation efficiency (in positive mode) is likely to be better with methanol. Besides being favourable for MS response, acetonitrile had an adverse effect on the chromatographic selectivity and was therefore no longer considered. The addition of 0.1% of acetic acid proved to be beneficial for the signal-to-noise ratios (*S*/*N*) of all polar OPs. Similar *S*/*N* values were found using propionic acid (0.1%) but *S*/*N* values worsened when formic acid (0.1%) or ammonium acetate (10 m*M*) was applied. The adverse effect of formic acid on the *S*/*N* can be explained from the high proton affinity values of the acid.

In order to optimize selectivity, tandem MS was applied for the detection of the polar OPs. Optimal selectivity obviously minimizes chemical noise and thus enables us to decrease the detection limits of the analytes in ''real'' samples. Signal to noise ratios (*S*/*N*) were compared for standard solutions of the polar OPs in water/MeOH mixtures (50/50, *V*/*V*) containing 0.1% HAc, in both positive and negative mode, using both ESI and APCI (after optimization of the source parameters). Positive mode electrospray proved to be the most sensitive technique for the analysis of the polar OPs in standard solutions. However, in real samples (surface water) strong signal suppression was observed. APCI is generally less sensitive to signal suppression due to matrix components. In our current application remarkably, even a slight signal enhancement was frequently observed. We therefore applied APCI (in positive mode) in the rest of this study. Again, the addition of 0.1% HAc gave the highest sensitivity.

### *4.2. Method validation*

Trueness and repeatability were determined by analyzing a fortified surface water sample in fivefold. This was performed at two different concentration levels, 0.05 μg/l and 10 times that concentration. For each of the polar OPs, Fig. 2 shows the MS–MS chromatograms of the surface water sample, fortified at the lower level. For each OP, the response obtained was compared with that obtained for a standard solution in milliQ water 0.1% acetic acid at the same concentration. The relative response (in % to the standard solutions) and the repeatability of the analysis are given in Table 3. The repeatability was generally better than 20% (exception monocrotophos at 0.05 μg/l, 23%) and is within the criterion set by the EU guideline for pesticide residue analysis [20]. In surface water before fortification, no peaks were detected in any of the MS–MS traces. As there is no sample pretreatment (apart from acidification and filtration) but just the injection of the sample, the response reflects matrix effects only and the measurement cannot be considered as a recovery. For this particular surface water, a response enhancement due to the matrix was observed and the average trueness found deviated 7–41% from the theoretical value. Therefore, for accurate quantification, the matrix effect should be compensated for.

To investigate the variability of the response (i.e. matrix effects) for different types of water, three other surface water samples taken from small ditches and canals at different locations in the Netherlands, two different ground water samples and local tap water were fortified with the polar OPs at the level of 0.5 μg/l. All samples were analysed on the same day. The response of each compound was compared with that of a 0.5 μg/l standard solution in milliQ water/0.1% acetic acid. The data obtained are presented in Table 4. Generally, a response enhancement was observed. For the drinking water and one of the groundwater samples no enhancement or even a slight suppression was observed. In most cases, the relative response was between 80 and 125%. Although matrix effects are not very pronounced for most samples, the quite strong enhancement in sample SW4 would result in too high concentrations reported. To some extent, the matrix effect is similar for the six OPs, but there are differences (e.g. in surface water sample SW4 128% for methamidophos and 185% for monocrotophos) and one internal standard would not be sufficient to compensate matrix effects for all analytes. For the most accurate results, standards should be prepared in the same matrix as the unknown samples. Obviously, the use of stable-isotope internal standards would be the best way to compensate for matrix effects.

Linearity of the method was evaluated using peak area obtained after analysis of seven standards solutions with concentrations in the range of approx.

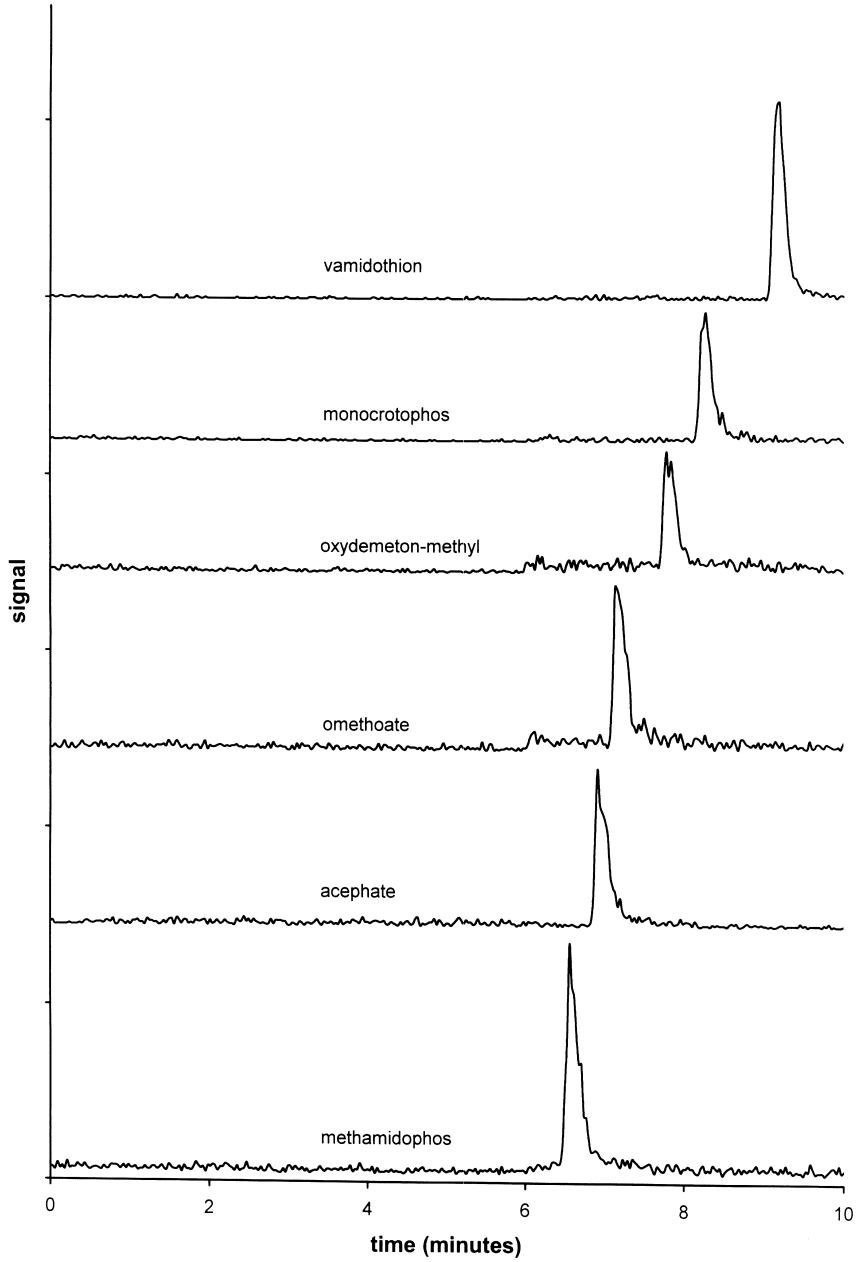



Fig. 2. MS–MS chromatograms of a surface water sample fortified with 0.05 μg/l of each of the polar organophosphorus pesticides. All experimental conditions as described in text.

0.025–2 μg/l. Linear calibration curves for all polar OPs were obtained, with a correlation coefficient $r^2 = > 0.99$ for acephate, methamidophos, omethoate and vamidothion and $r^2 > 0.98$ for monocrotophos and oxydemeton-methyl. The curves had no significant intercept ($P = 0.05$). Back calculation of the concentrations, from the area and the constructed curve, resulted in deviations that were generally less than 15%.

The limit of detection (LOD), defined here as the

Table 3
Trueness and repeatability for determination of polar OPs in surface water using large volume injection LC–MS–MS

| | 0.05 μg/l | | | 0.5 μg/l | | |
|---|---|---|---|---|---|---|
| | Trueness %[a] | | Repeatability RSD%[a] | Trueness %[a] | | Repeatability RSD%[a] |
| | Range | Average | | Range | Average | |
| Acephate | 108–149 | 120 | 14 | 109–129 | 118 | 8 |
| Methamidophos | 111–167 | 136 | 16 | 106–132 | 118 | 8 |
| Monocrotophos | 101–173 | 128 | 23 | 117–130 | 124 | 5 |
| Omethoate | 112–179 | 141 | 18 | 101–139 | 121 | 13 |
| Oxydemeton-methyl | 96–115 | 103 | 7 | 112–136 | 120 | 8 |
| Vamidothion | 91–143 | 110 | 19 | 95–116 | 107 | 8 |

[a] Trueness is response of analyte in surface water relative to that in a standard solution in milliQ at the same concentration.

concentration for which a signal-to-noise ratio of three is obtained, was estimated from the chromatograms obtained from fortified water samples at 0.05 μg/l. The LOD varied between 0.01 and 0.03 μg/l depending on the analyte and applied mass resolution. Lowering the resolution was beneficial for the $S/N$ of oxydemeton-methyl, however deteriorated the $S/N$ of methamidophos and acephate. In the chromatogram shown in Fig. 2, resolution is optimized for the latter compounds.

### 4.3. Application

After validation the method was applied for the routine analyses of surface and ground water samples. Fig. 3 shows a mass chromatogram of a surface water sample. In this particular sample, acephate was detected at a level of 0.2 μg/l. The trace of methamidophos (peak indicated with arrow, approx. 0.03 μg/l) can be expected since methamidophos has been identified as a major metabolite of acephate. The small peak before the methamidophos peak originates from an unknown compound in the surface water. Even though a selective MS–MS method has been developed, this last example shows the necessity of performing proper chromatography. If no, or hardly any chromatography is employed, false positive data could quite easily be obtained.

During the first 6 months after implementation more than 100 surface water samples have been analysed in batches of 10–20. The only problem encountered, once, was peak tailing. This was most pronounced for oxydemeton-methyl and caused by dirt built-up at the tip of the stainless steel sprayer tube of the APCI interface. This happened after continuous use of the instrument during several

Table 4
Matrix effects

| | SW1[a] | SW2 | SW3 | SW4 | GW1 | GW2 | DW |
|---|---|---|---|---|---|---|---|
| | % Response relative to standards in milliQ water[b] | | | | | | |
| Acephate | 118 | 112 | 112 | 140 | 90 | 132 | 118 |
| Methamidophos | 118 | 110 | 119 | 128 | 96 | 118 | 104 |
| Monocrotophos | 124 | 105 | 132 | 185 | 92 | 116 | 98 |
| Omethoate | 121 | 98 | 119 | 151 | 97 | 115 | 103 |
| Oxydemeton-methyl | 120 | 100 | 87 | 145 | 91 | 116 | 95 |
| Vamidothion | 107 | 102 | 123 | 167 | 80 | 104 | 90 |

[a] Is average value from surface water used in Table 3.
[b] Concentration in both standards in milliQ and water samples was 0.5 μg/l. All aqueous solutions contained 0.1% acetic acid. SW = surface water, GW = ground water, DW = drinking water.

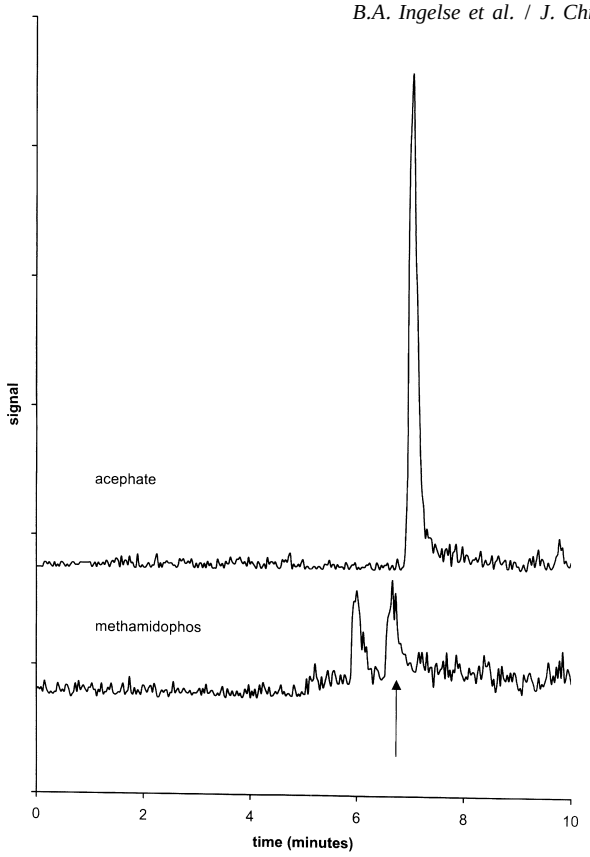



Fig. 3. MS–MS chromatogram of a surface water sample found positive on acephate. All experimental conditions and further explanation described in text.

months in which a variety of samples, including vegetables and fruits, were analysed. After replacement of the sprayer tube peak shapes were restored. This maintenance is minimal compared to that required in GC analysis.

## 5. Conclusion

Very polar OPs like acephate, methamidophos, and omethoate can not be extracted from water using the currently commonly available SPE cartridges. Although there is the possibility of a laborious procedure based on evaporation of the water for sample enrichment, a more efficient method would be highly desirable. In this work such a method, based on LC–MS–MS has been developed. The method is very straightforward, highly sensitive and selective. After filtration and acidification, water samples are directly injected onto a LC–MS system. When the APCI interface is applied, a slight enhancement of response was generally observed, and therefore matrix-matched calibration is recommended for accurate quantification. By direct injection of 1 ml, the LOD ($S/N = 3$) varied between 0.01 and 0.03 μg/l depending on the analyte and applied resolution. It may be possible to further lower the LOD by injection of even larger volumes, directly on the analytical column or by (on-line) SPE applying a pre-column packed with the same stationary phase. For the very polar OPs included in this work, LC–MS–MS proves to be a more favorable alternative to GC analysis. Currently, we have been investigating the applicability of this method for the determination of polar OPs in fruit and vegetable samples. Without clean up, the method proved to be suited for quantitative analysis down to the 0.01 mg/kg level. Details of this application will be described elsewhere.

## References

[1] C.D.S. Tomlin (Ed.), The Pesticide Manual, 11th ed., British Crop Protection Council, Surrey, UK, 1997.
[2] Integrale normstelling stoffen, Milieukwaliteitsnormen bodem, water, lucht. Ministry of Housing, Land-use planning and Environment (VROM), 1997.
[3] K. Iijima, M. Saka, Y. Odanaka, O. Matano, J. Pesticide Science 22 (1997) 17.
[4] H. Obana, K. Kikuchi, M. Okihashi, S. Hori, Analyst 122 (1997) 217.
[5] S. Lacorte, D. Barcelo, Anal. Chem. 68 (1996) 2464.
[6] M. Forcada, J. Bertran, F.J. Lopez, F. Hernandez, Chromatographia 51 (2000) 362.
[7] V. Lopez-Avila, R. Young, W.F. Beckert, J. High Res. Chromatogr. 20 (1997) 487.
[8] S. Hatrik, J. Tekel, J. Chromatogr. 733 (1996) 217.
[9] S. Lacorte, C. Molina, D. Barcelo, Anal. Chim. Acta 281 (1993) 71.
[10] A.C. Hogenboom, M.P. Hofman, D.A. Jolly, W.M.A. Niessen, U.A.Th. Brinkman, J. Chromatogr. A 885 (2000) 377.
[11] C. Molina, P. Grasso, E. Benfenati, D. Barcelo, J. Chromatogr. A 737 (1996) 47.
[12] G. D'Ascenzo, A. Gentili, S. Marchese, D. Perret, J. Chromatogr. A 800 (1998) 109.
[13] A.C. Hogenboom, W.M.A. Niessen, D. Little, U.A.T. Brinkman, Rapid Commun. Mass Spectrom. 13 (1999) 125.
[14] C. Aguilar, I. Ferrer, F. Borrull, R.M. Marce, D. Barcelo, Anal. Chim. Acta 386 (1999) 237.

[15] S. Lacorte, C. Molina, D. Barcelo, J. Chromatogr. A 795 (1998) 13.
[16] A.R. Fernandez, A. Aguera, M. Contreras, G. Penuela, I. Ferrer, D. Barcelo, J. Chromatogr. A 823 (1998) 35.
[17] J. Norberg, J. Slobodnik, R.J.J. Vreuls, U.A.Th. Brinkman, Anal. Methods Instrum. 2 (1995) 266.
[18] A. Di-Corcia, R. Samperi, A. Marcomini, S. Stelluto, Anal. Chem. 65 (1993) 907.
[19] H.G.J. Mol, M. Althuizen, H.-G. Janssen, C.A. Cramers, U.A.Th. Brinkman, J. High Res. Chromatogr. 19 (1996) 69.
[20] Guidance document on residue analytical methods, European Commission, Directorate General Health and Consumer Protection, SANCO/825/00 rev. 6, 20/06/00.
[21] K.L. Rundlett, D.W. Armstrong, Anal. Chem. 68 (1996) 3493.
[22] C. Crescenzi, A. Di Corcia, S. Marchese, R. Samperi, Anal. Chem. 67 (1995) 1968.
[23] M.C. Alonso, M. Castillo, D. Barcelo, Anal. Chem. 71 (1999) 2586.
[24] B.H. Forngren, J. Samskog, S.A. Gustavsson, N. Tyrefors, K.E. Markides, B. Langstrom, J. Chromatogr. A 854 (1999) 155.
[25] M. Honing, J. Riu, D. Barcelo, B.L.M. van Baar, U.A.Th. Brinkman, J. Chromatogr. A 733 (1996) 283.
[26] Metabolic Pathways of Agrochemicals, Part 2, Insecticides and Fungicides, T. Roberts, D. Hutson (Eds.), Royal Society of Chemistry, Cambridge, UK, 1999.