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Journal of Chromatography A, 1015 (2003) 119–127
JOURNAL OF CHROMATOGRAPHY A
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Determination of polar organophosphorus pesticides in vegetables and fruits using liquid chromatography with tandem mass spectrometry: selection of extraction solvent☆

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Received 7 June 2002; received in revised form 22 April 2003; accepted 23 April 2003

Abstract

A method based on liquid chromatography (LC)–mass spectrometry (MS)/MS was developed for sensitive determination of a number of less gas chromatography (GC)-amenable organophosphorus pesticides (OPs; acephate, methamidophos, monocrotophos, omethoate, oxydemeton-methyl and vamidothion) in cabbage and grapes. For extraction, several solvents were evaluated with respect to the possibility of direct injection, matrix-induced suppression or enhancement of response, and extraction efficiency. Overall, ethyl acetate was the most favourable solvent for extraction, although a solvent switch was required. For some pesticide/matrix combinations, reconstitution of the residue after evaporation required special attention. Extracts were analysed on a C18 column with polar endcapping. The pesticides were ionised using atmospheric pressure chemical ionisation on a tandem mass spectrometer in multiple reaction monitoring mode. The final method is straightforward and involves extraction with ethyl acetate and a solvent switch to 0.1% acetic acid/water without further cleanup. The method was validated at the 0.01 and 0.5 mg/kg level, for both cabbage and grapes. Recoveries were between 80 and 101% with R.S.D. $< 11\%$ ($n = 5$). The limit of quantification was 0.01 mg/kg and limits of detection were between 0.001 and 0.004 mg/kg.

Keywords: Vegetables; Fruits; Pesticides; Organophosphorus compounds; Acephate; Methamidophos; Monocrotophos; Omethoate; Oxydemeton-methyl; Vamidothion

1. Introduction

Organophosphorus pesticides (OPs) are a class of pesticides that generally act as cholinesterase inhibitors and are used as insecticides or acaricides in a wide variety of crops [1]. OPs vary widely in physico-chemical properties like water solubility, K_{ow}, vapour pressure, molecular weight and thermal stability. This paper focuses on very polar and/or thermolabile OPs, such as acephate, methamidophos, monocrotophos, omethoate, oxydemeton-methyl and vamidothion.

Within the EU, maximum residue levels (MRLs) have been established for the above-mentioned pesticides in many vegetables and fruits (except monocrotophos) ranging from 0.01 to 3 mg/kg [2]. For

☆ Presented at the Fourth European Pesticide Residue Workshop (EPRW 2002), Rome, 28–31 May 2002.

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doi:10.1016/S0021-9673(03)01209-3

vegetables and fruits intended for production of baby food, an MRL of 0.01 mg/kg is applicable for all pesticides [3]. This threshold level is also frequently applied for testing compliance with guidelines for organic production.

Majority of the OPs are easily analysed by gas chromatography (GC). Consequently, for residue analysis of OPs in vegetables and fruits, virtually all methods described in literature are based on gas chromatography using mass spectrometric (MS), (pulsed) flame photometric detection (FPD), or nitrogen phosphorus detection (NPD) (selected references: GC–MS [4,5], GC–FPD [6–9], GC–NPD [10]).

Due to their low molecular mass, very polar and/or thermolabile nature, difficulties are experienced with GC-based residue analysis of the OPs considered in this work, especially at low levels. Problems encountered include rapid deterioration of system performance [17], analyte losses in the inlet [11], large response differences between pesticides in extracts and the same concentration of pesticides in clean solvent (complicating quantification [7,12,13]), peak tailing [4], insufficient selectivity (matrix interferences) [14] and, probably due to all this, poor repeatability [9].

Liquid chromatography (LC)–MS is becoming a standard tool for pesticide residue analysis in vegetables and fruits [15]. Despite the limited robustness of GC-based methods for the polar OPs, no LC–MS-based methods have been described so far for these OPs (besides vamidothion in honey [16]). Recently, we described a method based on LC–MS/MS for the determination of polar OPs in water samples [17]. This method was found to be much more robust than the GC method that was used before. The promising results for water analysis encouraged us to adopt the method for residue analysis in vegetables and fruits. As the LC–MS/MS conditions were already established, the emphasis in this work was on sample preparation.

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. Stock solutions of the polar OPs were prepared in ethyl acetate, methanol, acetone and demineralised water. Dilutions were prepared in the same solvents to evaluate effect of the solvent on chromatography and for solvent-switching experiments. In the case of aqueous solutions, 0.1% acetic acid was added because OPs are more stable at acidic conditions. Calibration standards were prepared by dilution of the aqueous stock solution with 0.1% acetic acid in demineralised water. Matrix-matched calibration standards were prepared by dissolving the residue of blank extracts in calibration solutions in 0.1% acetic acid/water.

2.1. Sample preparation

One type of vegetable and one type of fruit were selected as model commodities for evaluation of the method. Cabbage was taken as an example of a vegetable having leaves with a relatively high wax content that may be co-extracted, grapes were selected as matrix with high sugar content. Organic cabbage (white head cabbage) and organic grapes were purchased in local shops. Samples were homogenised in a food cutter and subsamples of 25 g were extracted with 50 ml of solvent using an ultra-turrax for 2 min. In the case of ethyl acetate, 25 g of sodium sulphate was added, unless otherwise mentioned. If no clear liquid phase was obtained after settling, centrifugation was performed. In the case of solvent-switching, an aliquot of 1 or 2 ml of the organic extract was transferred into a glass tube and evaporated at 35 °C under a gentle flow of nitrogen gas. Residues were dissolved in a solution of 0.1% acetic acid in water with the aid of a vortex or ultrasonic bath (details are given in Section 3). Since water was used for reconstitution, the residue did not dissolve completely. Therefore, extracts were filtered through a PTFE filter (0.2 μm, Acrodisc) into the autosampler vial for LC–MS/MS analysis.

Whenever water is mentioned elsewhere in the paper, 0.1% acetic acid in water is meant.

2.2. Liquid chromatography–mass spectrometry

An HPLC pressure gradient pump system was used, consisting of two K1001 pumps and a high pressure

solvent mixer (Knauer, Germany). A Midas autosampler (Spark, The Netherlands) injected 20 μl (unless stated otherwise) onto a Phenomenex Aqua column (5 μm C18, 4.6 mm × 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: $t = 0$ min, 100% eluent A, $t = 3$ min 50% eluent A, $t = 10$ min 0% eluent A. Keep eluent at 0% A for 5 min, then in 1 min to 100% eluent A. Equilibration for 7 min at 100% eluent A before next injection. The flow rate was 0.7 ml/min.

Atmospheric pressure chemical ionisation (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 345 kPa. The nebulizer current was set at 2 μA, the CAD gas value was set at 2. The collision energy was in the range of 10–30 V (exact values in parentheses below) and was optimised for each compound. The following precursor → product ion pairs were monitored in MRM mode:

- methamidophos $m/z = 142 \rightarrow 94$ (19),
- acephate $m/z = 184 \rightarrow 143$ (11),
- omethoate $m/z = 214 \rightarrow 125$ (29),
- monocrotophos $m/z = 224 \rightarrow 127$ (21),
- oxydemeton-methyl $m/z = 247 \rightarrow 168$ (19),
- vamidothion $m/z = 288 \rightarrow 146$ (17).

The six transitions were measured continuously with dwell times of 200 ms.

For the evaluation of matrix-induced suppression or enhancement of the response, blank extracts were spiked with the analytes and quantified using standards of the same concentration in clean water. For the determination of extraction efficiency quantification was based on matrix-matched standards.

Quantification of sample extracts during validation was done using a calibration curve based on matrix-matched standards.

3. Results and discussion

Earlier, a method for the determination of polar OPs with HPLC–MS/MS was developed as part of a method for water analysis [17]. In this work, the aim was application of the LC–MS/MS method for analysis of vegetables and fruit, and the focus was on sample preparation. For sample preparation, classical solvent extraction using a blender or turrax was taken as a starting point, rather than alternative techniques like matrix solid phase dispersion (MSPD). Although good results have been reported with MSPD [18], the small amount of homogenised sample typically processed (as low as 0.5 g) is considered to put high demands on sample homogenisation with respect to the representativeness of the sub sample.

3.1. Choice of extraction solvent

First the extraction solvent was considered. For multi-residue analysis, two extraction solvents are used very frequently [4,19]: ethyl acetate with addition of sodium sulphate, and acetone followed by a partitioning in dichloromethane–petroleum ether (without addition of salt). Therefore, including these two extraction solvents in the evaluation was obvious. Three other solvents were considered. Acetone as such, i.e. without the partitioning step, water and methanol. Water because the analytes of interest are very well water soluble, the advantages of not having to use an organic solvent and the possibility of large volume injection on the analytical column [17]. Methanol was included as an alternative water miscible solvent to acetone.

3.2. Effect of solvent on injection band broadening

While water and methanol are common solvents for the introduction in reversed phase HPLC, acetone and ethyl acetate are not. However, if possible, direct injection of crude acetone or ethyl acetate extracts would be favourable with respect to straightforwardness of the method. Therefore, the effect of solvent on injection induced band broadening was also studied for these two solvents. Initially, 20 μl of standard solutions of the polar OPs in each of the four solvents (water, methanol, acetone, ethyl acetate) was injected. A 4.6 mm i.d. column and a methanol/water gradient starting at 5% of methanol, then rapidly increasing methanol to 50%, and a flow of 0.7 ml/min were used. The peak shape in water was used as reference.

With ethyl acetate, retention times decreased by 0.5–2 min, except for methamidophos for which no

retention time shift was observed. Peak shape was not much different compared to water injection, except for vamidothion for which unacceptable band broadening occurred. Direct injection of acetone solutions, even when limiting the volume to 10 μl, resulted in very broad peaks. For methanol, peak shape was acceptable for all polar OPs when injecting 10 μl, while band broadening started to occur for acephate and methamidophos at larger injection volumes. Dilution of methanol in water, five times, and increasing the injection volume to 100 μl, restored peak shape for the latter compounds. For acetone, dilution in water with corresponding increase in injection volume did not improve peak shape. It could be concluded that methanol can be injected without adverse effects on peak shape, ethyl acetate too, except for vamidothion, and that direct injection of acetone was not possible. In order not to exclude any of the target pesticides at this stage, direct injection of raw extracts was performed for aqueous and methanol extracts only, and not performed for ethyl acetate and acetone extracts. In the latter cases, a solvent switch to water was carried out before LC–MS/MS analysis.

3.3. Matrix effects

The occurrence of matrix effects in LC–MS is well known and has an impact on the quantification of the pesticides. Matrix effects can both reduce and enhance the response when compared to standards in neat solvents. Matrix effects depend on the instrument and interface used, the analytes, the matrix (amount of matrix per millilitre of extract) and the sample pre-treatment procedure (extraction solvent, used for clean up procedures).

As a part of the selection procedure of the extraction solvent, two commodities were selected for evaluation of matrix effects, cabbage and grapes. Blank extracts were prepared by extracting 25 g of homogenised sample with 50 ml solvent. This was done for each of the four solvents. In the case of ethyl acetate, the addition of sodium sulphate is common practise. Having in mind a combined extraction with GC-based methods, this was also done here. In the case of ethyl acetate and acetone extraction, an aliquot of the extract was evaporated to dryness and reconstituted in a standard solution of the OPs in water. For the water and methanol extracts, a small aliquot of a concentrated standard in water was added. In addition to these four extracts, a fifth extract was prepared by liquid–liquid partition of the acetone extract with equal amounts of dichloromethane and petroleum ether. The dichloromethane–petroleum ether phase was evaporated to dryness and reconstituted in a standard solution in water. In all cases, the concentration of OPs and the amount of matrix in the final extract was the same. The extracts were analysed with HPLC–MS/MS and the response was compared to standards in water (i.e. without matrix).

The extent to which matrix effects occurred, depended on the extraction solvent and the analyte. The least polar extraction solvent, dichloromethane–petroleum ether (obtained after partitioning with the initial acetone extract) showed no matrix effects for all six OPs (<10%). Some enhancement was observed when using ethyl acetate (up to approx. 20%). For water, methanol and acetone (crude extract, without partitioning), response enhancement was observed by a factor of 1.5–2 (less for methamidophos). As illustrated, the results are shown for acephate and methamidophos in Table 1.

The best way to compensate for matrix effects is the use of stable isotope internal standards, however, for most pesticides these are not available. It was considered that reducing the amount of matrix might reduce the enhancement observed. To verify this, standards were prepared in 10 times diluted sample extracts (i.e. 0.05 g/ml) and analysed. The matrix effects observed were generally similar to those of the undiluted extracts.

The trends observed for cabbage and grapes were very similar, supporting earlier observations that differences in matrix effects between commodities are

Table 1
Selected data on matrix-induced suppression or enhancement[a]

Extraction solvent	Acephate		Methamidofos	
	Cabbage	Grapes	Cabbage	Grapes
0.1% acetic acid/water	180	168	93	109
Methanol	159	145	89	99
Acetone	151	181	102	106
Acetone + DCM/PE partition	100	103	101	105
Ethyl acetate	97	108	97	106

[a] LC–MS/MS relative response (%) of standard prepared in extracts (0.5 g matrix/ml extract) relative to standard in water.

usually much smaller than the difference between any matrix and clean standard solutions.

Besides response enhancement, interferences were observed in the case of all three polar extraction solvents (water, methanol, acetone without the partitioning step) despite the use of MS/MS. The majority of the matrix eluted before the target pesticides. The interference was a big tailing peak starting at t_0 and then slowly coming down at 7 min. The pattern observed in the MS/MS chromatograms was similar to that observed in a single MS full scan. No real differences between grapes and cabbage were observed. The use of water as extraction solvent had a practical inconvenience that solid matter did not readily settle down and centrifugation was required before injection into the HPLC.

3.4. Extract evaporation and reconstitution

From the above, it became clear that extraction into an organic solvent was favourable with respect to matrix-effects in LC–MS, interferences in the MS/MS chromatogram, and from a sample handling point of view. On the other hand, a solvent switch, i.e. evaporation to dryness followed by reconstitution in mobile phase or water, is required in order to obtain acceptable peak shape in HPLC. Such a step may lead to losses of analytes by evaporation and/or incomplete reconstitution and needs to be investigated in detail. Evaporation of the extract leaves a layer of non-volatile matrix. This may be favourable (retains analytes and prevents them from evaporation) or results in problems, i.e. inclusion of the analytes in matrix that may not dissolve in eluent.

To check for possible losses during the evaporation step, a standard in ethyl acetate (without matrix) was evaporated to complete dryness (tube heater at 35 °C, gentle flow of nitrogen). The residue was dissolved in water. Losses were less than 10% (except for acephate, 19%), indicating that no unacceptable volatization of the pesticides occurred during the solvent switch.

To investigate different aspects of reconstitution of the residue, grape and cabbage extracts in ethyl acetate (0.5 g/ml) were spiked with the OPs. One millilitre of the spiked extracts were either evaporated to ‘just dry’, or left in the tube heater some extra time (arbitrarily 30 min was taken) which is more practical in a routine environment. Residues were then dissolved

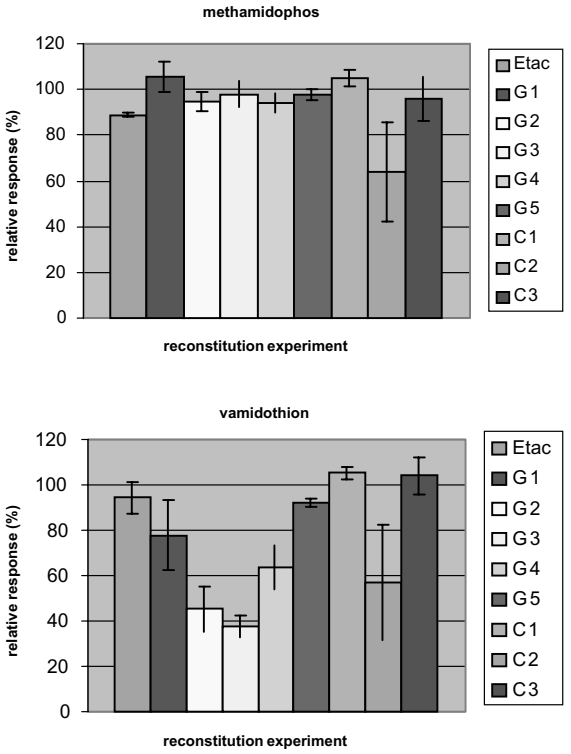

Fig. 1. Effect of conditions during solvent switch from ethyl acetate to water on recovery. For clarification of applied conditions see Table 2.

in water by just vortexing the tube, or by sonication in an ultrasonic bath for 5 min. In Table 2 all conditions tested are listed. The experiments were repeated five times.

In general, quantitative reconstitution was obtained (recovery 96–112%, R.S.D. = 2–10%). However, there were some exceptions. As an example, this is illustrated for methamidophos and vamidothion in Fig. 1. In the case of cabbage, low recoveries (57–65%) and large R.S.D.s (30–40%) were obtained for all six pesticides when extracts were evaporated to dryness + 30 min and then dissolved by vortexing only. With the aid of sonication, good recoveries and repeatabilities were again obtained. Evaporation until ‘just dry’ showed quantitative reconstitution irrespective of the use of vortex or sonication. A possible explanation of the phenomenon is that waxes from the leaves of cabbage incapsulate the pesticides when ‘over drying’ the extract. As the wax layer does not

Table 2
Conditions applied in reconstitution experiments for grape and cabbage extracts

Sample	Keeper	Evaporation (N_2/35 °C)	Reconstitution
EtAc	–	Just dry	Water/vortex
G1	–	Just dry	Water/vortex
G2	–	Just dry + 30 min	Water/vortex
G3	–	Just dry + 30 min	Water/ultrasonic bath
G4	–	Just dry + 30 min	First 100 μl methanol/vortex then 900 μl water
G5	100 μl 10% ethylene glycol in methanol	Just dry + 30 min	Water/ultrasonic bath
C1	–	Just dry	Water/vortex
C2	–	Just dry + 30 min	Water/vortex
C3	–	Just dry + 30 min	Water/ultrasonic bath

Relative response: response relative to standard solution in water; EtAc: ethyl acetate; G: grape; C: cabbage.

dissolve in water, only part of the pesticides will dissolve again.

The other exception was vamidothion. Besides the incomplete reconstitution in cabbage as described above, lower recoveries and larger bias were also obtained in the case of grape extracts. Results were acceptable when evaporating to 'just dry' but any additional time worsened the recoveries. Sonication did not improve this. Dissolving the residue first in 100 μl of methanol, and then adding water to 1 ml had a positive effect but the average recovery was still below 70%. No explanation could be given for the observation for this particular pesticide/matrix combination. Apparently, in order to improve the recovery of vamidothion, the extract should not be evaporated to complete dryness. To achieve this without having to closely watch the evaporation process, it was decided to add a keeper to the ethyl acetate extract. Ethylene glycol was chosen because it is very soluble in water and not volatile. A small amount of 10 μl only (100 μl of a 10% solution in methanol) was added to 1 ml of the extract before evaporation. The extract was evaporated to 'dryness' + 30 min and then dissolved in 1 ml water using sonication. This resulted in recoveries of over 90%. Since this procedure had no adverse effect on the recoveries of the other pesticide/matrix combinations, it was applied in further experiments.

3.5. Extraction efficiency

When using ethyl acetate or acetone/dichloromethane–petroleum ether for extraction, a partitioning of the polar OPs between an aqueous phase (water from the matrix) and an organic phase is involved. All the pesticides involved in this study are very polar and water soluble, $\log K_{ow}$ values are below zero (−0.22 to −0.89) except for vamidothion (0.12) [1]. To investigate the extraction yields, cabbage and grape samples were fortified with the OPs and extracted.

In the case of ethyl acetate extraction, 25 g of sample was extracted with 50 ml of ethyl acetate using a turrax. After settling, a solvent switch was carried out as described above for 1 ml of the raw extract. As mentioned before, the addition of sodium sulphate in ethyl acetate extraction is common practice in GC-based methods and therefore also done here. To verify whether the amount of salt was critical for extraction efficiency, additional experiments with no salt and 50% of the normally added 25 g were also performed.

In the case of acetone-based extraction, 15 g of sample was first extracted with 30 ml of acetone, then 30 ml of dichloromethane and 30 ml of petroleum ether were added and the mixture was turraxed again. After phase separation, 2 ml of the organic phase was evaporated and reconstituted in 1 ml water in order to obtain the same concentration analytes and amount of matrix per ml extract as for ethyl acetate extraction.

The results are presented in Table 3. The best recoveries were obtained for ethyl acetate with addition of 25 g of sodium sulphate. Reducing the amount of salt by 50% generally reduced recoveries, although they were still acceptable. Leaving out the salt resulted in recoveries below 50%. With the acetone/dichloromethane–petroleum ether procedure,

Table 3
Comparison of extraction efficiency of polar OPs ($n = 3$)

Pesticide	Recovery (%)			
	EtAc	EtAc + 12.5 g Na_2SO_4	EtAc + 25 g Na_2SO_4	Acetone, DCM/PE
Grapes				
Acephate	24	70	83	23
Methamidophos	21	65	96	12
Monocrotophos	50	92	100	50
Omethoate	18	69	87	25
Oxydemeton-methyl	15	82	86	33
Vamidothion	35	71	77	76
Cabbage				
Acephate	17	85	89	24
Methamidophos	18	70	81	13
Monocrotophos	43	96	110	39
Omethoate	18	87	100	22
Oxydemeton-methyl	15	87	104	24
Vamidothion	60	105	112	61
R.S.D.	3–8[a]	3–10	2–12	8–16

EtAc: ethyl acetate; DCM: dichloromethane; PE: petroleum ether (for details see text). Level of fortification: 0.05 mg/kg.
[a] Except vamidothion: R.S.D. = 32%.

all recoveries (except for vamidothion) were below 50%, i.e. similar to the results obtained for ethyl acetate without salt. Several modifications of the acetone/partitioning procedure have been described to improve the extraction efficiency for polar analytes, e.g. for methamidophos the addition of sodium chloride and partitioning with dichloromethane (without petroleum ether) [20]. This was not investigated here, since it became clear that the acetone and partitioning procedure had no advantages over ethyl acetate extraction and the latter was already in use in our laboratory for multi-residue analysis.

3.6. Validation

The final method was validated for cabbage and grapes, according to EU guidelines [21], by analysis of fortified samples at the 0.01 and 0.5 mg/kg level. The recoveries were determined based on standards prepared in the applicable matrix, to compensate for matrix effects (even though these were not very pronounced as was demonstrated earlier).

The results are presented in Table 4. Good recoveries and repeatabilities (meeting the EU guideline values of 70–110%, R.S.D. = 20%) were obtained

Table 4
Performance characteristics of LC–MS/MS method for polar OPs in cabbage and grapes as shown by recovery percentage

Pesticide	Fortification level 0.01 mg/kg ($n = 5$)		Fortification level 0.5 mg/kg ($n = 5$)		LOD* (mg/kg)
	Cabbage	Grapes	Cabbage	Grapes	
Acephate	82 (8)	95 (119)	83 (3)	91 (2)	0.004
Methamidophos	81 (3)	93 (6)	80 (7)	88 (3)	0.001
Monocrotophos	89 (2)	94 (8)	92 (3)	96 (4)	0.001
Omethoate	90 (6)	87 (5)	86 (4)	92 (5)	0.002
Oxydemeton-methyl	98 (6)	93 (6)	92 (5)	92 (4)	0.003
Vamidothion	99 (3)	101 (10)	98 (6)	100 (4)	0.001

R.S.D. values are shown in parentheses.
* S/N = 3.

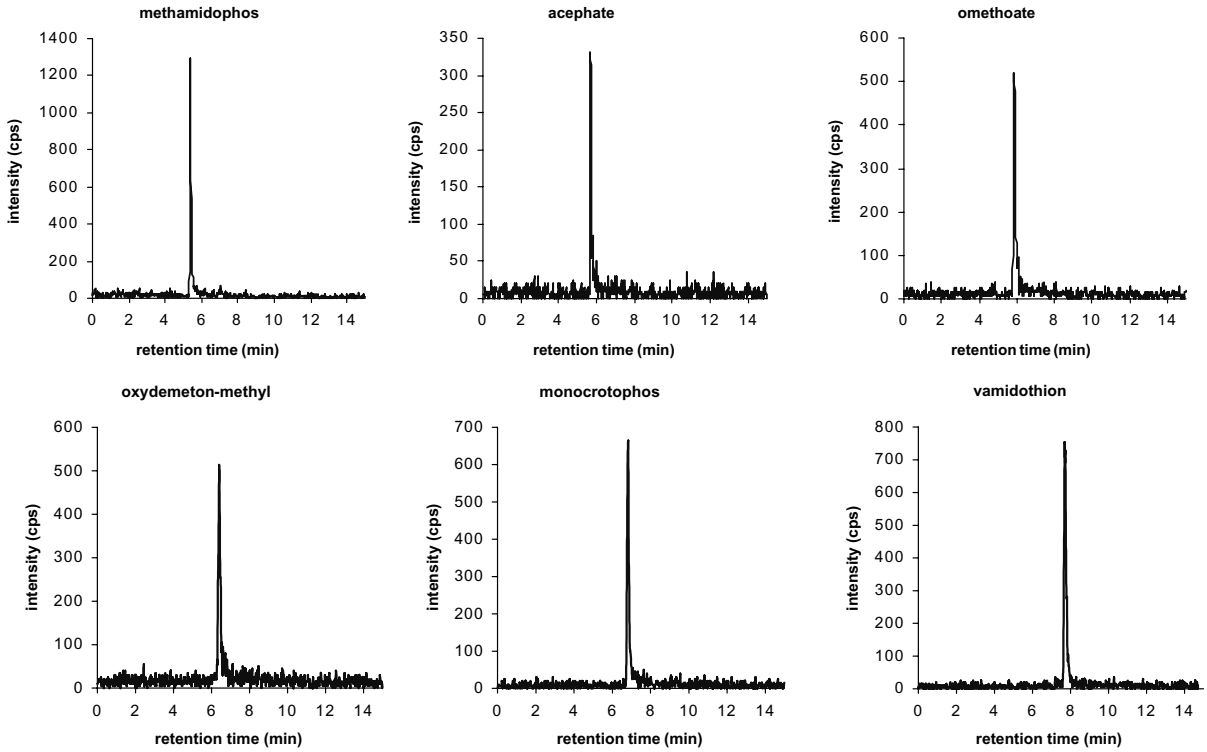

Fig. 2. Representative LC–MS/MS chromatograms for grapes fortified at 0.01 mg/kg for six polar OPs.

for all pesticides in both matrices. No peaks were detected in unfortified samples for any of the pesticide/matrix combinations. Representative LC–MS/MS chromatograms for grapes fortified at 0.01 mg/kg level are shown in Fig. 2. From these chromatograms, the limits of detection (LOD, defined as S/N = 3) were estimated to be in the range of 0.001–0.004 mg/kg.

To investigate whether standards in clean water can be used for quantification, a more exact determination of matrix effects was done by analysing standards of different concentrations in clean water and in the two matrices, and comparing the slopes of the calibration curves. All curves were linear over the range 0.004–0.45 mg/l (0.008–0.9 mg/kg), $R^2 > 0.998$. For each pesticide the slope of the calibration curve obtained for standards in clean water, grape extract and cabbage extract are included in Table 5. For acephate and methamidophos, enhancement up to 20% was

Table 5
Matrix effects observed in final method, comparison of slopes calibration curves

Pesticide	Slope				
	Water	Grapes	Relative %, grapes	Cabbage	Relative %, cabbage
Acephate	408	489	120	480	118
Methamidophos	1553	1822	117	1811	117
Monocrotophos	1101	1148	104	1135	103
Omethoate	1138	1159	102	1167	103
Oxydemeton-methyl	964	950	99	940	98
Vamidothion	1458	1290	89	1502	103

Grapes: standards in grape extract; cabbage: standards in cabbage extract.

observed, and matrix-matched calibration is recommended for accurate quantification. For the other OPs matrix effects were considered not significant and clean solvent-based standard could be used as well.

4. Conclusions

A method was developed for sensitive determination of a number of less GC-amenable organophosphorus pesticides. From the extraction solvents evaluated (water, methanol, acetone [with and without partition in dichloromethane–petroleum ether] and ethyl acetate), ethyl acetate was most favourable with respect to matrix effects, interferences in LC–MS/MS and extraction efficiency. For some pesticide/matrix combinations the use of a keeper and/or sonication was essential for quantitative reconstitution of the residue during the solvent-switch from ethyl acetate to water.

The limit of quantification, defined as the lowest level tested that still meets the EU guideline values for recovery and R.S.D. [21], was 0.01 mg/kg for all pesticides in both commodities. The sensitivity of the method is sufficient to enable testing of compliance with baby food regulations (i.e. 0.01 mg/kg for all pesticides [3]) and maximum residue limits established in The Netherlands and the EU (0.01 mg/kg or higher [2]).

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