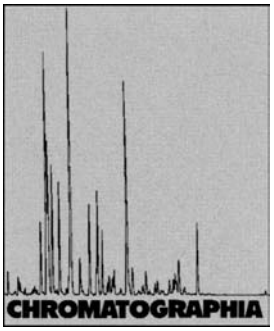
Multi-Residue Analysis of Some Polar Pesticides in Water Samples with SPE and LC–MS–MS



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Abstract

In the present study a multi-residue analytical method was developed for monitoring some polar pesticides such as acephate, methamidophos, carbofuran, isoproturon, dimethoate in water with SPE (solid-phase extraction) and LC–MS–MS. Acetochlor was taken as surrogate, and alachlor as internal standard. SPE with different types of columns was compared with LLE (liquid-liquid extraction). Further, the breakthrough volume for different pesticides was determined. The results showed that the selected pesticides can be determined very sensitively with LC–MS–MS. The minimum detectable quantity (MDQ) for each pesticide was about 1.0 ng. To date, SPE cartridge studies showed that the Oasis HLB cartridges were suitable for further studies. However, for Oasis HLB cartridge, different pesticide showed different breakthrough volume. The results showed that for acephate and methamidophos, the breakthrough volume was about 30 mL of water sample, much less than the breakthrough volume of other pesticides studied. Because of the higher vapor pressure and higher Henry's constant of methamidophos, dimethoate and carbofuran, much attention should be paid on their losses in the evaporation step of the experiment. This analytical method can be applied to determine pesticide contamination in environmental water samples.

Keywords

Column liquid chromatography–MS–MS
Solid phase extraction
Multi-residue analysis
Polar pesticides
Environmental water samples

Introduction

Regarding drinking water safety and environment protection especially in developing countries such as China where water shortage is a severe problem, it is necessary to monitor the water quality in the aspect of contamination with pesticides. For this purpose many analytical methods were established in the past years, nevertheless it is still difficult to determine the residue of some pesticides in water samples, such as methamidophos, acephate, carbofuran and isoproturon, for their higher polarity, although these pesticides are high toxic and widely used.

Liu et al. studied the multi-residue analysis of 26 pesticides in surface water with SPE and GC–NPD with a good recovery for most of the pesticides on a low spiking level, but the polar pesticides diuron, isoproturon, methamidophos, monocrotophos and omethoate were excluded [1]. In the studies of Bossi et al., a new LC–MS–MS method with SPE for polar pesticide was developed and validated for 53 pesticides, degradation products of pesticides and selected nitrophenols, including carbofuran, isoproturon, but not methamidophos and acephate [2].

SPE is now widely accepted as an alternative to laborious and time consuming LLE. For the analysis of polar pesticides, the SPE cartridges reported were including Oasis HLB, Chromabond HR-P, LiChrolut EN and C_{18} cartridges [2, 3, 4].

Ingelse et al. reported his results on polar pesticides in aqueous samples by direct injection with LC-MS and concluded that very polar pesticides could not be extracted from water using the usual SPE cartridges. The article pointed out that the high recovery for acephate reported by Lacorte could not be reproduced using similar SPE cartridges and sample volume [3, 5]. However, Frenich et al. reported high recoveries of methamidophos in 500 mL water samples with C_{18} cartridges [6]. In the latest report on the analysis of polar pesticides, methamidophos and acephate, in water samples, published by St-Amand and Girard good results were achieved with Oasis HLB cartridges [7]. In conclusion, previous information on analytical methods of polar pesticides in water samples were very confusing.

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Table 1. Quantitative ion and R_T of pesticides analyzed by LC–MS–MS

Pesticide	Precursor ion	Product ion	R_T min
Acephate	183.87	143.05	4.39
Acetochlor	270.01	224.00	10.33
Alachlor	270.18	238.30	10.37
Carbofuran	229.90	198.90	6.39
Dimethoate	221.90	165.15	7.77
Isoproturon	207.10	72.20	8.83
Methamidophos	141.94	94.00	4.02

The present study focused on the multi-residue analysis of selected polar pesticides, acephate, methamidophos, carbofuran, isoproturon and dimethoate in water samples and the development of an available method for the monitoring of pesticide contamination in water samples based on SPE with Oasis HLB cartridges and LC–MS–MS.

Experimental

Chemicals and Reagents

Pesticide standards, acephate, acetochlor (surrogate), alachor (internal standard), carbofuran, dimethoate, isoproturon, methamidophos (the purity was > 99.0 %) were from Ehrenstorfer (Germany). The quality of all used chemicals, acetonitrile, acetic acid, ethyl acetate, methanol, bidistilled water, Na_2SO_4, activated carbon, blue-gel were suitable for residue analysis and chromatography.

With Microlab 1000 dilutor (Microlab Hamilton), stock solutions of individual pesticides at 0.5 mg L^{-1} were prepared by exact weight and solution in methanol. The standard working solutions were obtained by appropriate dilutions with methanol.

SPE columns were 200 mg/3 mL Chromabond HR-P, filled with porous adsorptive resin based on polystyrene-divinylbenzene, and 500 mg/6 mL Oasis HLB, filled with a co-polymer of polydivinyl-benzene-co-*N*-vinylpyrrolidone.

LC-MS-MS Analysis

LC was carried out with Perkin Elmer Series 200 HPLC system, composed of two pumps and an autosampler with a 20 µL sample loop, a column oven and a Phenomenex Aqua (150 × 3.0 mm) reversed-phase HPLC column. The solvent gradient was A (methanol + 0.1% HAC) and B (water + 0.1% HAC) at a flow-rate of 0.5 mL min^{-1}. The initial composition was 100% B, followed by linear gradient to 50% B from 0 to 3 min and linear gradient to 100% A from 3 to 10 min, then changed to 100% B within 0.1 min and held for 5 min.

Mass analysis was performed with the MS–MS API 2000 (Applied Biosystems) spectrometer (Software version: Analyst 1.2). The operating parameters of the ESI-MS mode were optimised evaluating the sensitivity (signal-to-noise ration) and fragmentation for each pesticide. They were detected in the scan mode (m/z 50 to 369). For each pesticide, the precursor ion and product ion were chosen for quantitation and conformation (Table 1).

Sample Treatment and Water Analysis

Preparation of Water Samples

The water samples were filtered with prefolded filter paper, and a surrogate in methanol (0.5 mL of 0.5 mg L^{-1} acetochlor) was added, then SPE or LLE procedure were performed.

For Chromabond HR-P SPE studies: The conditioning of cartridge was carried out first with 3 mL of methanol and then 2×3 mL of bidistilled water. Then the water sample was passed through the cartridge under vacuum condition. The flow-rate was controlled by adjusting the vacuum. The cartridge (if necessary, it was stored at about −15 °C until analysis) was dried for 2 h (with an air-stream, using a reservoir filled with activated carbon and blue-gel), the analytes were eluted with 2 × 3 mL of methanol/ethyl acetate (1:1, *v*/*v*). The eluate (if necessary, it was stored at about −15 °C until analysis) was evaporated to dryness with weak nitrogen stream without disturbing the surface of the solution.

For Oasis HLB SPE studies: The conditioning of cartridge was carried out first with 5 mL of methanol and then 2 × 4 mL of bidistilled water. Then the water sample was passed through the cartridge. The cartridge (if necessary, it was stored at about −15 °C until analysis) was dried for 2 h (with an air-stream, using a reservoir filled with activated carbon and blue-gel), the analytes were eluted with 2 × 2 mL of methanol. The eluate (if necessary, it was stored at about −15 °C until analysis) was evaporated to dryness with weak nitrogen stream without disturbing the surface of the solution.

For LLE studies: The water sample was transferred into a 1000 mL separatory funnel, then 100 mL of ethyl acetate were added. It was shaken manually for 2 min. The water layer was collected in another separatory funnel, then another 50 mL of ethyl acetate were added to the water layer and shaken manually for 2 min. The ethyl acetate layers were combined, and passed through a filter with anhydrous Na_2SO_4 and evaporated to approximately 5 mL in a rotary evaporator at 40 °C. Then the concentrated solution was transferred to a 10 mL glass tube and evaporated to dryness with weak nitrogen stream without disturbing the surface of the solution.

Preparation of Injection Solution

The obtained sample was redissolved in 0.5 mL of acetonitrile, and the solution of the internal standard alachlor was added. The concentration of alachlor in the final solution was 500 µg L^{-1}. The sample was stored in the dark at about −15 °C until measurement.

Results and Discussion

Determination of pesticides with LC–MS–MS

Using the above chromatographic conditions, 50, 100, 500, and 1000 µg L^{-1} of mixed standard solutions were determined, respectively. The correlations and sensitivity for each pesticide is listed in Table 2. With LC–MS–MS traces of pesticides can be determined very sensitively. The MDQ can be decreased to 1.0 ng which cannot be achieved by GC–MS–MS.

Studies on SPE with Different Cartridges and LLE

The results of the recovery study with 0.5 μg L^{-1} of pesticides in 500 mL water samples with SPE (with Chromabond HR-P and Oasis HLB cartridges) and LLE showed that there were similar results except for the recoveries of dimethoate with the SPE (HR-P) method. LLE as a traditional method was very helpful to establish another method such as SPE, although it is solvent and time consuming and laborious (Table 3).

In comparison to SPE with different rates of loading sample, there was no significant difference of the recoveries between the two flow-rates, 20 mL min^{-1} and 50 mL min^{-1} (Table 3).

SPE with Oasis HLB was preferred to SPE with Chromabond HR-P from the results shown in Table 3. These results were very different from those of Ingelse et al. report where large volume (1 mL) water samples were directly injected into a RP18 HPLC column with "0" recovery of acephate and methamidophos [3]. The data agreed with the studies of Nogueira et al. and St-Amand and Girard, in which the divinylbenzene-co-*N*-vinylpyrrolidone solid phase showed the best effective recovery [7, 8].

For methamidophos and acephate, all methods tested in this study had lower recoveries because both are higher soluble in water and more polar than others. This can be demonstrated by their breakthrough volume with SPE cartridges which was less than 500 mL (Table 4).

Test of Breakthrough Volume

The breakthrough volume of isoproturon and dimethoate was more than 1000 mL, for carbofuran it was between 500 mL and 1000 mL. The breakthrough volume of acephate and methamidophos was less than 500 mL using Oasis HLB cartridges. Therefore with different sample volumes different recoveries were obtained as shown in Tables 4, 5 and Fig. 1.

From Table 4, 5 and Fig. 1, the breakthrough volume of acephate was 35 mL and of methamidophos 30 mL. Therefore, when acephate and methamidophos are to be detected in water samples with Oasis HLB cartridges, the sample volume should be less than 30 mL.

St-Amand and Girard obtained maximum recoveries of acephate and methamidophos, which were 90–95 % and 85–90 %, respectively, with Oasis HLB cartridges (3 mL/60 mg). But the breakthrough volume of methamidophos was only 5 mL and the LOD (limit of detection) was 30 μg L^{-1} [7]. In this case the method was not suitable for monitoring methamidophos contaminations in water.

In comparison of our results with those of St-Amand and Girard [7], it can be conducted that a higher content of the absorbent can help to enlarge the breakthrough volume.

Studies on Different Spiked Level of Pesticides

Because usually 500 mL of water samples were analyzed in monitoring studies, the analytes were spiked in 500 mL of water samples at three different concentrations and determined with Oasis HLB cartridges.

Table 2. Equation of linear range and sensitivity for each pesticide studied with LC–MS–MS

Pesticide	R^2	MDQ*
Acephate	0.9993	1.0ng
Acetochlor (surrogate)	0.9997	1.0ng
Carbofuran	0.9998	1.0ng
Dimethoate	0.9984	1.0ng
Isoproturon	0.9994	1.0ng
Methamidophos	0.9976	1.0ng

*The minimum detectable quantity

The results summarized in Table 6 show that the method is suitable for the monitoring of pesticides in water samples according to the European Union guideline with a maximum permission limit of 0.1 μg L^{-1} for drinking water.

Acephate and methamidophos could be detected with the method in water samples with concentrations of more than 1.0 μg L^{-1}, because of the low breakthrough volume and the higher limit of detection. Thus, it is not possible with this method to control drinking water contamination within the demanded limits (0.1 μg L^{-1}), but the obtained results are much lower than those (30 μg L^{-1} with a 5 mL water sample) reported by St-Amand and Girard [7].

Studies on Losses During Evaporation

The loss during evaporation is another problem to be considered, because of the

Table 3. Recoveries (%) of pesticides in 500 mL water samples with 0.5 μg L^{-1} spiked level with three methods

Method		Acephate	Acetochlor	Carbofuran	Dimethoate	Isoproturon	Methamidophos
SPE with HR-P[1]	Repeat1	11.2	88.6	76.4	7.2	100.0	5.3
	Repeat 2	10.9	78.2	76.2	2.8	94.8	–
	Mean	**11.1**	**83.4**	**76.3**	**5.0**	**97.4**	**5.3**
SPE with HLB[1]	Repeat1	15.4	88.9	94.7	81.7	110.1	4.9
	Repeat 2	9.1	85.0	111.3	77.3	106.7	5.3
	Mean	**12.3**	**87.0**	**103.0**	**79.5**	**108.4**	**5.1**
SPE with HLB[2]	Repeat1	10.4	87.2	110.1	69.8	105.7	5.1
	Repeat 2	11.4	93.3	98.3	77.7	111.4	5.5
	Mean	**10.9**	**90.3**	**104.2**	**73.8**	**108.6**	**5.3**
LLE	Repeat1	1.4	97.6	105.2	107.9	124.7	5.0
	Repeat 2	1.7	90.6	88.4	109.4	122.0	4.2
	Mean	**1.6**	**94.1**	**96.8**	**108.7**	**123.4**	**4.6**

Note:
1 The flow-rate of loading sample was 20 mL min^{-1}
2 The flow-rate of loading sample was 50 mL min^{-1}

Table 4. Recoveries of pesticides in different sample volumes with Oasis HLB SPE method

Sample volume mL	Spiked level μg L^{-1}	Recovery (%)	
		Acephate	Methamidophos
500	0.5	10.9	5.3
250	0.5	24.0	7.6
100	0.5	32.2	18.3
50	0.5	47.0	19.2
25	5.0	89.6	82.7
10	5.0	78.5	96.0
5	5.0	77.0	93.6

Table 5. Recoveries (%) of pesticides in two sample volumes with Oasis HLB SPE method

Sample volume mL	Acetochlor	Carbofuran	Isoproturon	Dimethoate
1000	89.3	44.7	95.9	86.2
500	90.3	104.2	108.6	73.8

Table 6. Recoveries of four pesticides at three spiked levels (%) with Oasis HLB SPE

Pesticide	0.5 μg L^{-1}	0.1 μg L^{-1}	0.05 μg L^{-1}
Acetochlor (surrogate)	90.3	97.7	92.9
Carbofuran	104.2	74.2	73.2
Dimethoate	73.8	82.0	63.5
Isoproturon	108.6	97.4	92.0

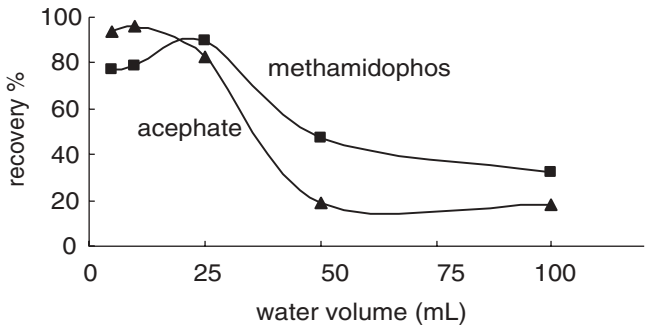

Fig. 1. Recoveries of Oasis HLB SPE for acephate and methamidophos in different volume of water samples

different effects of Henry's constant (Table 7). Higher vapor pressure and lower solubility could lead to higher Henry's constant and higher loss in the evaporation step. For most pesticides, Henry's constant determines the potential for loss of chemicals during evaporation steps in trace analysis.

To study the influence of the nitrogen stream on the loss of pesticide residue, the nitrogen stream increased two folds the above reported flow-rate. In Table 7 the recovery of the evaporation step for each pesticide and some of their physico-chemical properties are listed [9, 10, 11].

For example, dimethoate has a similar vapor pressure as acephate, but lower water solubility, therefore its Henry's constant was higher than that of acephate and the loss of dimethoate during the evaporation was likewise higher. In the series of 5 compounds tested, losses of methamidophos, dimethoate and carbofuran occurred easier during dryness while evaporating the solvent than losses of acephate. (Table 7). However, isoproturon seemed to be an exception. It has a higher Henry's constant shows, however, smaller losses than dimethoate and methamidophos. This is probably true because isoproturon has a much lower vapour pressure than other pesticides. In this case, vapour pressure played a more important role in the evaporation step.

Therefore, when the method is used, much attention should be paid to the evaporation step. The nitrogen stream should be weak and only cause a slight movement of the surface of the solution.

Conclusions

A multi-residue analytical method of the polar pesticides acephate, methamidophos, carbofuran, isoproturon, and dimethoate in water samples with SPE and LC–MS–MS was developed.

The comparison study between different SPE cartridges and LLE showed that Oasis HLB cartridges were suitable for the method developing. For different pesticides, Oasis cartridges showed different breakthrough volumes. For acephate and methamidophos, the breakthrough volume was about 30 mL of water sample, the LOD was 1.0 μg L^{-1}; 500 mL of breakthrough volume for carbofuran, and more than 1000 mL for isoproturon and dimethoate, and the LOD was below 0.05 μg L^{-1}.

Table 7. Recovery (%) of concentration step and their physico-chemical properties

Pesticide	Acephate	Carbofuran	Dimethoate	Isoproturon	Methamidophos
Repeat 1(%)	118.4	47.8	16.7	114.2	29.7
Repeat 2(%)	95.3	37.3	10.0	94.1	48.0
Vapor pressure (25°C) (mPa)	0.226	0.072	0.25	0.008	4.7
Solubility in water	790g L^{-1}	320 mg L^{-1}	23.8 g L^{-1}	65mg L^{-1}	> 200g L^{-1}
Henry's Constant (Pa m^3 mol^{-1}) 20 °C	1.0×10^{-8}	4.66×10^{-3}	1.2×10^{-6}	1.46×10^{-5}	$< 1.6 \times 10^{-6}$

It can be used for the monitoring of pesticide contaminations in environmental water samples because of its simple sample preparation, lower LOD, especially suitable for analyzing polar pesticides. But when pesticides with high vapour pressure and high Henry's constant were studied, much attention should be paid to possible losses when the evaporation step is performed.

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