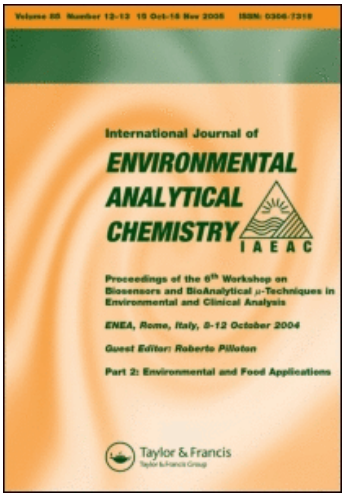

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Optimization of high-performance liquid chromatography and solid-phase extraction for determination of organophosphorus pesticide residues in environmental samples

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OPTIMIZATION OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND SOLID-PHASE EXTRACTION FOR DETERMINATION OF ORGANOPHOSPHORUS PESTICIDE RESIDUES IN ENVIRONMENTAL SAMPLES

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High-performance liquid chromatography with solid-phase extraction (HPLC-SPE) was optimized for the analysis of three organophosphorus pesticide residues in water, apples and vegetable samples. Octadecylsilica disks (47-mm diameter) were used for solid-phase extraction. The parameters that affect both separation and extraction of methyl parathion, parathion and phoxim, such as mobile-phase composition, ionic strength, temperature, pH, and breakthrough volume, were investigated. The application of optimized HPLC-SPE to environmental samples gave reproducible results with low detection limits of $5\,\mu g\,L^{-1}$ for methyl parathion and parathion and $2.5\,\mu gL^{-1}$. Precisions of less than 8, 9 and 12% were obtained for water, spinach and apple samples, respectively.

Keywords: High performance liquid chromatography; Solid-phase extraction; Organophosphorus pesticides

INTRODUCTION

Pesticide use in agriculture has greatly improved food production worldwide. However, a series of risks have followed. After their release into the environment, pesticides may have different fates. Sprayed pesticides may be carried in the air and end up in other parts of the environment, such as water and soil, while those applied directly to the soil may be washed off to nearby water bodies or percolate down to ground waters [1–3]. Studies have shown pesticides present in many rivers and lakes around the world [4].

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These pesticides undergo degradation, which usually produces less-harmful breakdown products. However, in some cases it may produce more-toxic products. Degradation of parathions and phoxim to form oxon is a good example. The half-lives of degradation processes vary, with some being resistant to degradation by any means and persisting in the environment for months or even years. Most pesticides, by their nature, are designed to kill or adversely affect living organisms. Thus, they pose a threat to humans and animals as well as to the general environment. Some studies have shown that highly soluble pesticides and degradation products tend to be leached and thus may contaminate ground water [5]. Organophosphate pesticides have been reported to cause toxicological problems to humans, animals and other water fauna because of cholinesterase inhibition effects [6–10].

Owing to their impact on the environment, several organizations and regulatory bodies have adopted priority lists and imposed legislation to protect the quality of drinking and surface waters, as well as food. Thus the analysis and determination of pesticide residues in the environment are of particular importance.

Pesticides form a large group of compounds with widely differing structures and biological activities. These diversities pose great challenges in developing methods for multiresidue analysis. The determination of pesticide residues at low concentrations and in diverse matrices requires not only highly selective detection techniques but also efficient extraction and cleanup methods. Analytes of interest are often present at trace levels concentrations or in complex matrices. Sample preparation is thus necessary to extract, clean and concentrate them prior to analysis.

There are several sample preparation techniques, with the emphasis being directed towards minimizing the use of toxic organic solvents, attaining higher extraction selectivity to minimize matrix interference, protecting the environment, saving labor through ease of automation and generation of good results [7,11]. Some of these sample preparation methods include: solid-phase extraction (SPE), solid-phase micro-extraction (SPME), purge and trap, distillation, conventional liquid–liquid (L–L) extraction, accelerated solvent extraction, solvent microextraction, supercritical fluid extraction (SFE) [1,12,13]. SPE, SFE, SPME and L–L extraction are the most commonly used for pesticide residues sample preparation [1,14–16].

Because of environmental concerns, analytical procedures that do not require, or that minimize, use of organochlorine solvents such as dichloromethane, banned in 1993, are desirable [12]. The L–L extraction method minimizes volumes of organic solvents, most of which are toxic. It is slow, labor intensive, not amenable to automation, has low theoretical plate numbers and is affected by emulsion masking [12,15,18,19]. SPME is seldom in equilibrium and does not allow trace enrichment, unless the phase ratios are very small (less than 0.001) [1, 20]. The SFE method is often limited in handling large numbers of samples in an automated system. Henion and co-workers give an extensive review on the development of SPE to its present status [15]. SPE has several advantages over the other methods used in preparation of pesticide residue samples [1,21–25]. It requires fewer steps and hence it saves both labor and cost, and effective in trace enrichment [12, 26]. High enrichment factors of over 1250-fold can be obtained [27].

SPE formats such as cartridges or disk formats can be used for sample storage and transportation of volatile or labile pesticide residue samples. It can also be used or in cases where otherwise large volumes of sample would have to be transported. Such application is water sampling in the remote fields where the pesticides residues

may be present in trace levels. The analytes are trapped in the disk and only disks are sent to the laboratory instead of large amounts of water [17, 20, 26, 28, 29].

High-performance liquid chromatography (HPLC) is one of the most important innovations in recent years. It is widely used in trace analysis of pesticides, food, environmental samples and clinical chemistry. It is a versatile method for separation and analysis of compounds that are difficult to separate or thermally unstable, especially for labile pesticide residue samples [14, 30]. Gas chromatography (GC) has also been widely used. However, with the inclusion of thermally labile and polar pesticides and their degradation products, HPLC has gained considerable acceptance compared to GC [1–5,11].

The use of small particle-size sorbents in the packing of the HPLC column provides high plate numbers, which, compounded with the ability to judiciously vary the mobile phases, enables separation of difficult-to-separate compounds to be attained. The combination of SPE and HPLC is thus a versatile method for analysis of organophophorus pesticides in various sample matrices.

The accuracy and precision of analysis are dependent on sample preparation, instrumental performance and the operator. Sample preparation often takes up to 60% of analytical time.

The optimization of HPLC and SPE and the determination of organophosphorus pesticide residues in environmental samples have been studied and the parameters influencing the determinations have been investigated. Satisfactory results are obtained by using the methods presented.

EXPERIMENTAL

Instruments

HPLC system (Shimadzu LC-6A), equipped with: LC-6A high-pressure solvent delivery pump, UV-Vis spectrophotometric detector (Shimadzu SPD-6AUV); chromatogram integrator system (Shimadzu C-R4A); CTO-6A column box; SCL-6B system control panel (Shimadzu); 7125 Six-way valve sample injector. Standard 47-mm disk extraction manifold System (3 MN Co., St. Paul, MN); SHZ-D (III) model circulatory water pump (Shenyang, China); rotary vacuum evaporator; electric shaker; digital pH Meter with glass electrodes (PHS-3B, Shanghai, China).

Reagents

Methyl parathion, parathion and phoxim standards (purity > 99.5%) were obtained from Sigma. Methanol, ethyl acetate, hexane and acetone were analytical-reagent grade and sodium chloride were of reagent grade. All were supplied by Beijing Chemical Industry Co. Double-distilled water was used, unless otherwise stated. Stock standard solutions were prepared at 1 mg/mL concentrations were accurately prepared from the original standards in 10-mL volumetric flasks using methanol. They were stored in the dark at 4°C. A mixed working standard of the three pesticides at 0.1 mg/mL was freshly prepared by appropriate dilution of the stock solutions.

Samples and Sample Preparation Procedures

Apple Samples

Apples were purchased from local markets at harvesting season and stored at 4°C but were analyzed within three days of collection. One kilogram of apples was macerated to homogeneity in an electric food mixer. Triplicate samples of 20 g each were weighed into round-bottom flasks, drenched with 30 mL of acetone and then shaken for 20 minutes on a linear electric shaker. This extract was removed and the residue re-extracted with an equal volume of acetone to ensure complete extraction. The combined extracts were filtered through a Whatman filter paper wetted with acetone in a Buchner funnel. The filtrate was evaporated to dryness in a vacuum evaporator in a water bath maintained at 50 ± 0.5°C. The residue was taken up with 5 mL methanol. It was then evaporated to near dryness, diluted to 200 mL with water in a 500-mL flask then stirred for two minutes followed by solid-phase extraction and analysis by HPLC with UV detection.

For comparative and method validation purposes, a set of 20-g triplicate samples, each spiked at 0.25 mg kg^{-1} with the three pesticides were extracted and analyzed using the same procedure as above with one set partitioned only with hexane.

Vegetable Samples

Spinach vegetables were grown in the demonstration garden of Northeast Normal University. Fresh samples were collected and analyzed within two days. Spinach vegetable samples collected prior to application of pesticides were used as the blank. Samples were collected after pesticide application. 500 g were prepared and analyzed using the same procedure as for the apples but using ethyl acetate for the extraction.

Water Samples

Other than double-distilled water, tap and surface runoff waters (from the garden) were used as environmental samples. Samples were stored at 4°C but analyzed within two days of collection. Tap and surface runoff water were filtered through 0.5 μm Gelman (Ann Arbor, MI), nylon membrane. 0.5% methanol was added to maintain conditioning of the disk sorbent during percolation of water samples. It has been noted that C_{18} sorbent tend to lose its adsorption capacity with percolation of large volumes of water samples [31–33].

RESULTS AND DISCUSSION

Wavelength Optimization

The optimal wavelengths for determination of the three organophosphorus pesticides methyl parathion, parathion and phoxim were determined using double-distilled water spiked with their working standards. All other chromatographic conditions were kept constant while varying the wavelength from 200 to 320 nm. The wavelength with the strongest absorption for each of the three pesticides was selected.

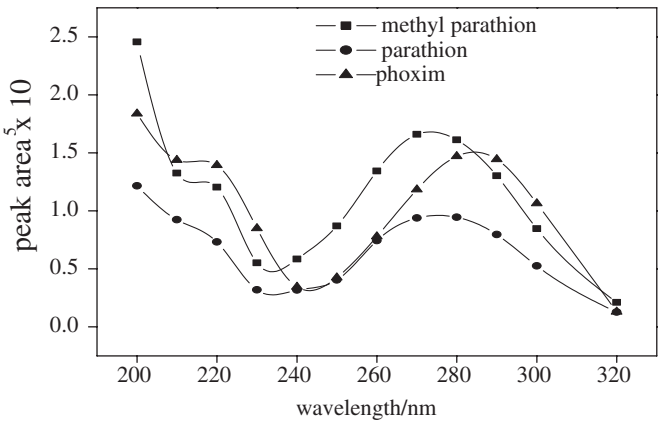

FIGURE 1 Absorption spectra of the three organophosphorous pesticides.

From Fig. 1, it can be seen that the wavelengths below 240 nm gave higher absorption peaks but there were interfering peaks. At wavelengths above 260 nm there were no interfering peaks. Maximum and well-resolved peaks were obtainable at 280 nm, which was therefore chosen as the wavelength at which analysis of the three organophosphorus pesticides was done.

Optimization of Mobile-phase Composition and pH

From our experiments, a methanol/water mixture at flow rate 0.7 mL/min was found to be the most suitable mobile phase for chromatographic analysis of the three organophosphorus pesticides. At fixed mobile-phase flow rates and wavelength (0.7 mL/min and 280 nm respectively), the mobile-phase composition was varied and the effect on the peak areas and retention times was investigated.

From Fig. 2, it can be seen that an increase in the proportion of methanol causes an increase in the peak area and a sharp decrease in retention time, though to differing extents for each pesticide. It is most pronounced with phoxim. At methanol compositions below 55% the retention time is too long, which is wasteful of analytical time while more than 85% produces a loss in resolution. A methanol/water composition 70 : 30 (v/v) gave the best separations in a reasonably short analysis time. The effect of pH of the mobile phase on separation was also investigated. The results are shown in Fig. 3. No adjustment of mobile-phase pH was thus necessary in further work.

Optimization of Column Temperature

Temperature plays an important role in separations, especially in liquid chromatography processes. It affects intermolecular interactions between the stationary phase and the analyte, thus affecting separations. This process becomes more effective with decrease in temperature. To optimize temperature, the retention times and peak areas of the three organophosphorus pesticides were measured over a temperature range of 25–65°C. Results show that temperature has a significant effect on retention time for the three pesticides. By successively increasing column temperature from 25°C to

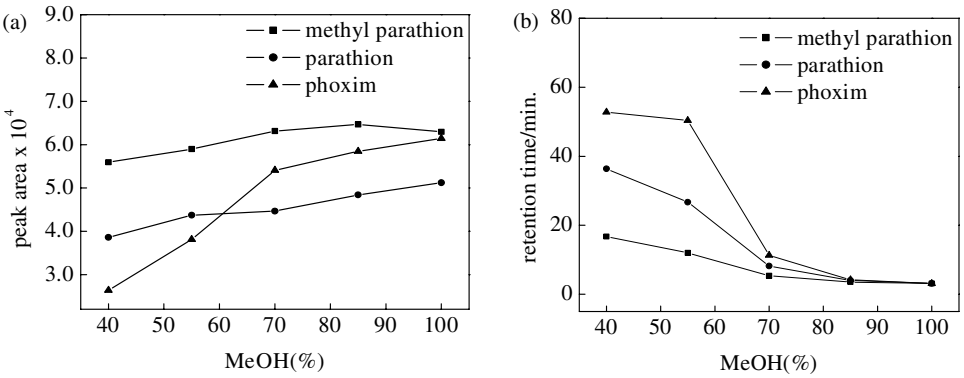

FIGURE 2 Effect of methanol composition in mobile phase on absorption (a), and retention (b) of methyl parathion, parathion and phoxim pesticides.

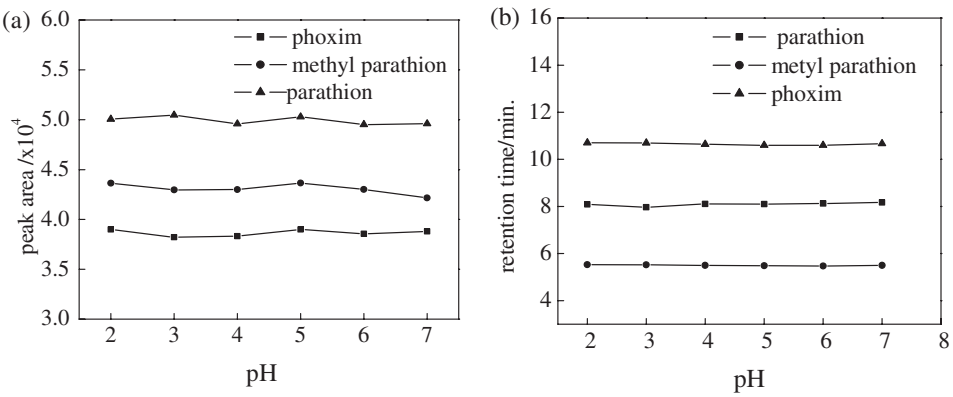

FIGURE 3 Effect of mobile-phase pH on absorption (a), and retention (b) of methyl parathion, parathion and phoxim pesticides.

65°C, it was possible to shorten the retention time of the three pesticides remarkably from 6.50, 10.10, 12.50 to 4.20, 6.50, 7.50 minutes, respectively, for methyl parathion, parathion and phoxim and hence reduce the analytical time. Considering the effect of high temperatures on column life, 35°C was chosen as an optimum working column temperature throughout this work.

Optimization of Solid-phase Extraction (SPE)

Octadecylsilica disk formats (47-mm diameter) were used for solid-phase extraction. Ethyl acetate and methanol were used for disk elution and activation respectively. Optimized flow rates of 30 mL/min gave high recoveries within a reasonably short analysis time. Procedures in disk conditioning include activation, conditioning, washing, sample elution and drying. During the conditioning and washing steps it is necessary to ensure that the disks are not allowed to become dry.

The amount of methanol required for optimum elution of trapped pesticides was determined by pre-concentrating a series of double-distilled water samples fortified at

TABLE I Percentage recoveries of pesticides using different volumes of elution solvent, after C_{18} disk extraction of fortified double-distilled water samples

Volume of elution solvent (mL)	*Recovery* (%)		
	Methyl parathion	*Parathion*	*Phoxim*
5	86.2	79.6	81.5
10	95.8	96.7	95.1
15	97.5	96.8	95.2
20	95.6	96.5	94.1
25	95.7	96.6	94.9
30	95.7	96.5	94.8

TABLE II Percentage recoveries of pesticides from different volumes of fortified double-distilled water after extraction with C_{18} disk

Sample volume (mL)	*Recovery* (%)		
	Methyl parathion	*Parathion*	*Phoxim*
150	98.91	97.08	96.24
200	98.56	98.15	95.98
250	99.05	97.25	96.62
300	99.24	97.12	96.75
350	99.13	97.99	95.84
500	99.06	97.75	95.87
750	98.13	95.06	85.07
1000	94.03	89.23	82.65

the same concentration with the pesticides. The disks were then eluted with different volumes of methanol and the recoveries were compared. The results are given in Table I. 10 mL was chosen as a minimum volume of elution solvent since no significant difference was achieved for volumes higher than this.

Sample Capacity of the Disk

The breakthrough volume estimation was done by preconcentrating a series of double-distilled water samples spiked with the same amount of pesticides so as to give the same signal. The results are shown in Table II.

Effect of Ionic Strength on Recovery

Increase in ionic strength is generally expected to increase recoveries by decreasing the solvent–solute interaction. Di Corcia *et al.* [34] observed that this effect become more pronounced with increase in polarity of the analyte. This ionic-strength effect of the sample solution was investigated for the three pesticides using recoveries from double-distilled water samples containing sodium chloride from 5 to 30 g/L. The results are shown in Table III. No significant effect on the recoveries were noted, though phoxim showed a slight increase of about 0.5% with 15 g/L sodium chloride. Thus, no sodium chloride was added in further work.

TABLE III Percentage recoveries of the pesticides from double-distilled water with different concentrations of sodium chloride

Amount of sodium chloride added (g/L)	*Recovery* (%)		
	Methyl parathion	*Parathion*	*Phoxim*
0	95.86	97.95	97.99
5	96.50	98.12	98.28
10	96.40	98.05	98.24
15	96.46	98.06	98.76
20	96.51	98.07	98.74
25	96.48	98.06	98.76
30	96.46	98.08	98.76

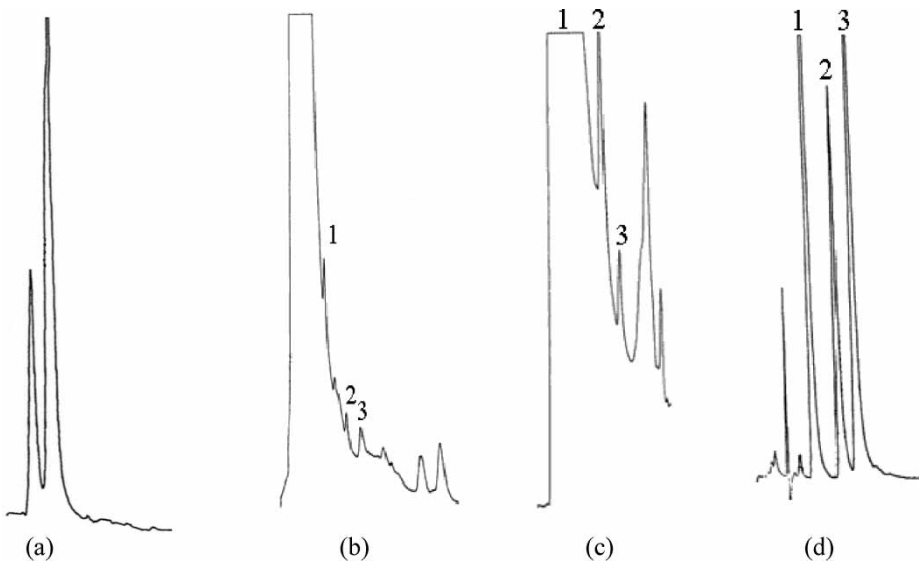

FIGURE 4 Chromatograms apple sample for the three pesticides after different levels of extraction-clean up process. Blank sample (a), fortified samples ethyl acetate extracted (b), extracted with ethyl acetate, partitioned with hexane and then cleaned once with SPE (c), extracted with ethyl acetate, partitioned with hexane and cleaned twice with SPE (d). (1) Methyl parathion (2) parathion (3) phoxim.

Analysis of Environmental Samples

The method performance was validated by construction of standard calibration curves using samples of double-distilled water spiked with the three pesticides at concentrations of 5, 10, 15, 20, 25, 30, 35, 40 and 50 μg/L. Regression curves were constructed from peak area (A) and concentration (C, μg/L). The results showed good linearity for pesticides over the tested concentration range. The precision (RSDs) were 4.7, 7.1 and 4.13% for methyl parathion, parathion and phoxim, respectively.

Figure 4 shows extraction of the three pesticides in fortified apple matrices. Figure 4(a) show that the extracts from market samples of apples had no traces of the pesticide residues under investigation. Thus they could be used as blank samples for the rest

of the work. Figure 4(b), showed that ethyl acetate is not effective in extracting these pesticides in apple matrices; the recovery was low and matrix interference was high. Hence a less polar solvent is needed for such extraction. Partitioning with hexane proved a better method. However, matrix interference was still high (see Fig. 4c). Solvent exchange followed by a double solid-phase extraction was more useful in extraction and preconcentration from these difficult matrix conditions (see Fig. 4d).

Vegetable sample matrices were more complex because of coloring matter. When double solid-phase extraction was applied after extraction with ethyl acetate it was possible to minimize this effect. Graphitized carbon has also been used to eliminate coloring interference [32].

Calibration Curves, Detection Limits and Precision

Good linearity was obtained in the concentration range 0.01–1.0 mg/kg. However, there were slight deviations at concentrations above 0.5 mg/kg of fortified blank samples analyzed. This could be attributed to degradation during the maceration process and matrix effects, which tend to be severe at higher pesticide concentrations. Other authors have explained this observation as well [32, 35]. The linear correlation coefficients for spinach were lower than those of apples. This may be due to the slightly polar matrices, which may have retained some pesticides during the solvent-exchange step prior to SPE cleanup, especially in the case of methyl parathion. Calibration curves obtained with pure methanol matrix were linear over the tested calibration range of 0.01–1.0 mg/kg. The correlation coefficients for the three organophosphorus pesticides were higher than 0.9800.

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