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Journal of Pharmaceutical and Biomedical Analysis 44 (2007) 258–264
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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Short communication

# Rapid simultaneous determination for organophosphorus pesticides in human serum by LC–MS

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Received 13 November 2006; received in revised form 18 January 2007; accepted 19 January 2007
Available online 30 January 2007

**Abstract**

A simple and rapid method was developed for measuring 10 organophosphorus pesticides (acephate, methidathion, dichlorvos, fenthion, EPN, diazinon, phenthoate, malathion, fenitrothion, and cyanophos) in the serum of acute poisoning patients by LC/MS. Following deproteinization by acetonitrile, an aliquot of the biological sample was injected into a $C_{18}$ column using 10 mM ammonium formate-methanol as the mobile phase. Extraction recoveries were satisfactory and ranged between 60.0 and 108.1% in serum. The limits of detection (LODs) in serum ranged from 0.125 to 1 μg/ml, and the limits of quantitation (LOQs) ranged from 0.25 to 1.25 μg/ml. An excellent linearity was observed for these LOQs up to 8 μg/ml. Intra- and interassay precision and accuracy were satisfactory for most of the pesticides analyzed. In terms of temperature stability, of all the organophosphorus compounds analyzed, dichlorvos and malathion exhibited the most rapid degradations over 24 h at room temperature. Methidathion and diazinon remained relatively stable at all temperatures during the entire 4-week testing period. The present method was successfully applied to one actual case of acute poisoning. In conclusion, this method is simple, accurate, and useful for the determination of organophosphorus pesticides and should benefit both clinical and forensic toxicology.


*Keywords:* Organophosphorus pesticide; LC-APCI-MS; Simultaneous determination; Human serum; Validation; Application

## 1. Introduction

Organophosphorus compounds are toxic organic chemicals that act as acetylcholinesterase inhibitors. Since the Second World War, more than a 100 such compounds have been developed as insecticides. Poisoning from organophosphorus insecticides has occurred by accidental exposure through the skin and airways [1,2], self-ingestion from attempted suicides [3–5], and rarely, from deliberate homicide [6–8]. In the majority of organophosphate poisoning cases, the chemicals were consumed in order to commit suicide, and in Japan, the organophosphorus compounds most commonly used for this purpose are fenitrothion, malathion, and dichlorvos [9].

The mortality rate from organophosphate poisoning is high, and important factors contributing to this high mortality are delay in diagnosis and improper management. In contrast, early diagnosis and appropriate treatment are often lifesaving although the clinical course of organophosphate poisoning might be quite severe and necessitate intensive care management [10]. Thus, in such poisoning cases, rapid toxicological screening is necessary for correct diagnosis.

To date, the relationship between organophosphate concentrations in biological fluids (or blood) and the severity of a patient's reactions has not been thoroughly investigated [11]. This type of data is required to obtain a more accurate diagnosis for the prediction of the efficacy of clinical treatments, such as hemoperfusion and hemodialysis [12].

Many sample preparation procedures for the isolation organophosphates from urine [13] and plasma (blood) [13,14] have been published. Although liquid–liquid extraction [14] and solid-phase extraction [13] are the most commonly used techniques, acephate is very difficult to extract by these techniques due to the extremely polar nature of this compound. Thus, an alternative rapid and efficient extraction method for organophosphates, including acephate, is required.

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doi:10.1016/j.jpba.2007.01.036

There is some literature pertaining to the analysis of some organophosphorus pesticides in biological fluids or in autopsy specimens by using gas chromatography (GC) [13], GC–mass spectrometry (GC–MS) [14], or high-performance liquid chromatography (HPLC) [15]. Although GC provides high sensitivity, polar pesticides, including non-volatile, thermally labile pesticides, such as dichlorvos, cannot be analyzed without a preliminary derivatization step. However, HPLC can be used for the analysis of non-volatile compounds because preliminary derivatization is not required. In order to overcome this problem, liquid chromatography (LC)-based techniques have been successfully applied for the analysis of a wider range of pesticides. LC coupled to mass spectrometry (LC–MS) is more selective and can solve the problem of the determination of organophosphorus pesticides in groundwater [16]. Recently, LC–MS techniques have gained increasing popularity for the analysis of polar analytes in biological fluids, particularly for those compounds that are not readily amenable to GC or GC–MS analysis. Although the screening of 21 organophosphorus pesticides in serum by LC–MS was reported [17], validation – including the stability of organophosphates and interference – has not been thoroughly studied.

In this study, a simple and rapid LC-atmospheric pressure ionization-MS (LC-APCI-MS) method was developed, validated, and employed for the determination of 10 organophosphates in human serum; the organophosphates studied were those that are commonly used in Japan.

## 2. Experimental

### 2.1. Chemicals and reagents

Acephate, methidathion, dichlorvos, fenthion, EPN, diazinon, phenthoate, malathion, fenitrothion, and cyanophos were obtained from Wako Pure Chemical Industries (Osaka, Japan) (Fig. 1). Diazinon-$d_{10}$ and fenitrothion-$d_6$ were obtained from Hayashi Pure Chemical Ind., Co., Ltd. (Osaka, Japan). Ammonium formate, HPLC-grade acetonitrile, and methanol were obtained from Wako Pure Chemical Industries. Millex®-LH filters and Omnipore™ membrane filters were purchased from Millipore Corporation (Bedford, MA, USA). Deionized

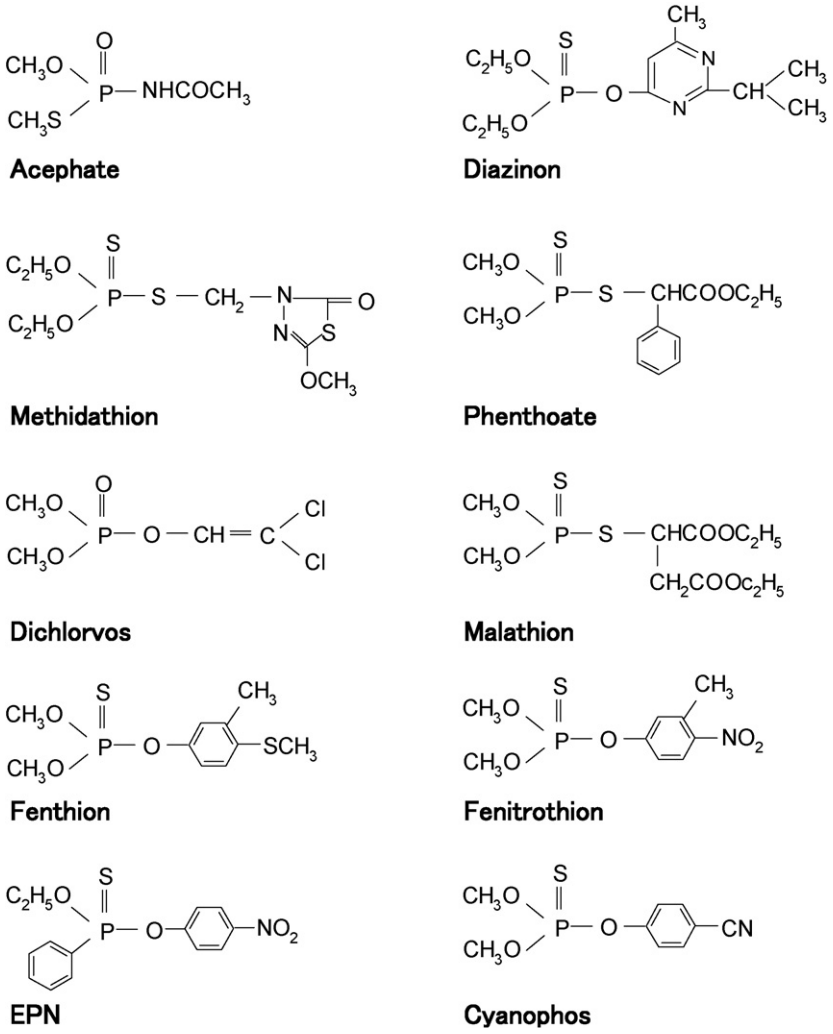


Fig. 1. Chemical structures of the organophosphorus compounds studied.

water was prepared using a MilliQ laboratory plant (Millipore).

### 2.2. Preparation of calibration standards and quality control samples

Primary stock solutions of each organophosphorus compound (1 mg/ml) were prepared in methanol. Working standard solutions of the compounds were prepared by combining the aliquots of each primary solution and diluting with methanol. The working solution for the internal standard (IS) (fenitrothion-$d_6$: 3 μg/ml, diazinon-$d_{10}$: 50 μg/ml) was prepared by diluting an aliquot of stock solution with methanol. All organophosphate solutions were stored at −20 °C in the dark when not in use.

Human serum calibration standards of organophosphate solutions (0.1, 0.25, 0.5, 1, 2, 5, and 8 μg/ml) were prepared by spiking the working standard solutions into a pool of drug-free human serum (200 μl). Quality control (QC) samples at 0.625, 2.5, and 7.5 μg/ml were prepared in bulk by spiking the appropriate working standard solutions into drug-free human serum (200 μl).

### 2.3. Sample preparation

Serum aliquots of 200 μl were added to each 5 μl of the IS solutions prepared in methanol. Diazinon-$d_{10}$ was used as the IS for acephate, methidathion, dichlorvos, fenthion, EPN, diazinon, phenthoate, and malathion, and fenitrothion-$d_6$ was used as the IS for fenitrothion and cyanophos (Table 2). For all samples, acetonitrile (200 μl) was added to the mixture. The resulting mixture was vortex-mixed for 1 min and then centrifuged at 3000 × *g* for 5 min. The supernatant was not evaporated to dryness under a stream of $N_2$ gas because some pesticides, such as dichlorvos, are thermally unstable [9]. The supernatant was filtered through a 0.45-μm Millex®-LH filter, and 20 μl of the filtrate was injected into the LC-APCI-MS.

### 2.4. LC–MS analysis

The HPLC system used a pump (LC-10A; Shimadzu, Kyoto, Japan), a detector (SPD-10A; Shimadzu), and data-processing equipment (Chromatopac C-R6A; Shimadzu). The analysis was performed on an XTerra MS C18 stainless steel cartridge column (2.1 mm × 100 mm, 3.5 μm; Waters, Milford, MA, USA) equipped with an XTerra MS C18 guard column (2.1 mm × 20 mm, 3.5 μm; Waters) at 50 °C. Gradient elution was used with solvents consisting of 10 mM ammonium formate in water (solvent A) and methanol (solvent B). The elution gradient was 0% B to 100% B (0–3 min), 100% B (3–9.5 min), and 100% B to 0% B (9.5–10 min) at a flow rate of 0.3 ml/min. The gradient solutions were filtered through a 0.45-μm Omnipore™ membrane filter before use.

The mass spectrometer used was a triple quadrupole QP8000α (Shimadzu) equipped with an APCI interface operating in the positive or negative mode. The parameters of the interface and detector were optimized in single MS full-scan mode (*m*/*z* 50–500) with direct injections of 10 μl of a 10 ng/μl standard solution. The following APCI inlet conditions were used. Nitrogen was used as a nebulizer gas with a flow rate of 2.5 l/min, the APCI probe temperature was 400 °C, the CDL temperature was 250 °C, and the detector voltage was 2.3 V. The peak-areas for all components were automatically integrated using CLASS 8000 Version 1.20 software (Shimadzu Chemical Laboratory Analysis System & Software, Kyoto, Japan).

### 2.5. Method validation

The method was validated by establishing linearity, intra- and interassay accuracy and precision, limits of detection (LODs) and quantitation (LOQs), recoveries, and stabilities.

Batches consisting of triplicate calibration standards for each concentration were analyzed on three different days for complete method validation. In each batch, three QC samples were assayed in sets of six replicates in order to evaluate the intra- and interday accuracy. The percentage deviation of the mean from the true values were expressed as relative error (R.E.) and standard deviation (S.D.) and served as measures of accuracy and precision. Calibration graphs of the organophosphorus compounds-to-internal standard peak-area ratios versus the theoretical concentration were constructed using linear least-squares regression analysis.

The absolute recovery and extraction efficiency (or relative recovery) were determined at three QC concentrations. The absolute recovery is eluted QC sample added IS, whereas the relative recovery added IS after eluting QC sample. The recoveries of the method were determined by expressing the mean concentrations obtained as a percentage of the spiked concentrations.

In order to evaluate the freeze–thaw cycle stability and temperature-matrix stability (at room temperature, 4°, and −30 °C), six replicates of three QC samples were prepared. The samples were either subjected to three freeze–thaw cycles or were stored before processing (at room temperature for 24 h, at 4 °C for 7 d, and at −30 °C for 4 weeks).

### 2.6. Application

A 43-year-old male attempted suicide by ingesting approximately 100 ml of both 5% fenitrothion and acephate emulsion. He was transferred to our emergency department approximately 2 h after ingesting the compounds. Blood samples were collected in dry heparin 3 h after ingestion, immediately placed on ice, centrifuged for 4 min at 3000 rpm at ambient temperature, and then immediately stored in a −20 °C freezer. After immediate thawing next day, serum aliquots of 200 μl were added to each 5 μl of the IS solutions (fenitrothion-$d_6$: 3 μg/ml, diazinon-$d_{10}$: 50 μg/ml) prepared in methanol. Following deproteinization by acetonitrile of 200 μl, the supernatant after centrifugation was filtered and 20 μl of the filtrate was injected into the LC-APCI-MS. The organophosphorus solutions were separated on an XTerra MS $C_{18}$ column using a linear gradient of methanol and ammonium formate (10 mM, pH 3.0).

## 3. Results and discussion

### 3.1. LC–MS

This report presents a simple screening procedure for 10 organophosphorus compounds. In order to simplify the process, a small quantity (200 μl) of acetonitrile was used for extraction without further evaporation to dryness because some pesticides, such as dichlorvos are thermally unstable [9]. The total extraction procedure took less than 10 min and did not require the use of a large amount of organic solvent. Following filtration, the extracted samples were injected into an LC-APCI-MS. The organophosphorus solutions were separated on an XTerra MS $C_{18}$ column using a linear gradient of methanol and ammonium formate (10 mM, pH 3.0). The retention times and ions obtained by LC are useful for the identification of each organophosphorus compound (Table 1). For quantification, molecular target ions of the 10 compounds were used in the positive and negative selected ion monitoring (SIM) modes; the *m/z* values are given in Table 1.

Figs. 2 and 3 show the typical LC-APCI-MS chromatograms. Because of the selectivity of both the procedure and the LC-APCI-MS, no interference was observed in the spiked blank serum or in the clinical samples.

Table 1
Retention times and ions observed for 10 organophosphate compounds using LC-APCI-MS

| Compound | Retention time (min) | Ion observed (*m/z*) | |
|---|---|---|---|
| | | Positive-ion mode | Negative-ion mode |
| Acephate | 3.72 | **143**, 184 | – |
| Methidathion | 5.42 | **144.9**, 338.2 | **157.0**, 286.85 |
| Dichlorvos | 5.17 | **221**, 338 | – |
| Fenthion | 5.85 | **279** | **262.95** |
| EPN | 5.99 | **294** | **293.85** |
| Diazinon | 5.88 | **305** | – |
| Phenthoate | 5.77 | **321**, 247.9 | – |
| Malathion | 5.55 | **331** | – |
| Diazinon-$d_{10}$ (IS) | 5.88 | **315** | – |
| Fenitrothion | 5.61 | – | **262** |
| Cyanophos | 5.33 | – | **228** |
| Fenitrothion-$d_6$ (IS) | 5.61 | – | **265** |

Bold: highest mass visible (*m/z*).

### 3.2. Linearity

Calibration by internal standardization was performed using linear regression employing 1/*x* weighting. The peak-area ratios of the target compounds and their respective internal standards were calculated for each standard curve. At least seven analyte concentrations were used for each standard curve. The limit of linearity was established by analyzing the increasing concentrations of the target compounds until one or more of the qualifying ion ratios exceeded the 20% limit or the determined concentration was greater than ±20% of the expected concentration.

For all the compounds, the calibration curves were linear up to 8 μg/ml (Table 2), and the mean results were within 20% of the expected concentration. The correlation coefficient ($r^2$) was >0.9838 for all compounds.

### 3.3. Sensitivity

The LOD was defined as the lowest concentration giving a response of three times the average baseline noise defined from five unfortified samples. The LOQ was determined as the lowest amount of a given pesticide that could be measured with an accuracy and CV of less than 20%. The determined LODs and LOQs are listed in Table 2.

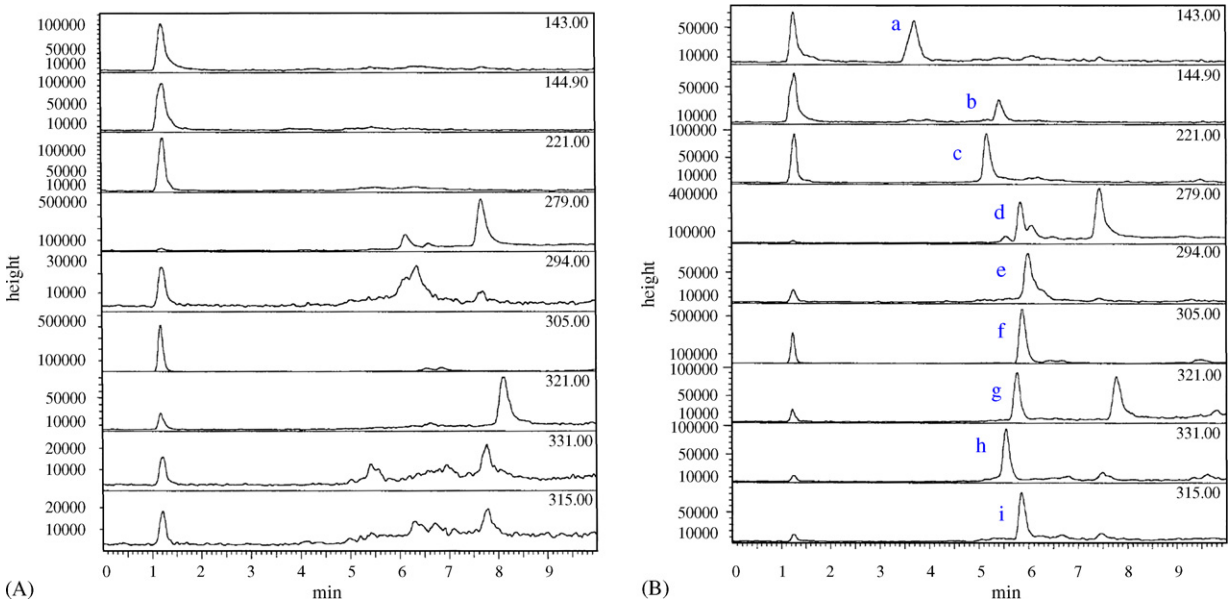


Fig. 2. LC-APCI-MS chromatograms obtained in the positive-ion mode. Selected ion chromatograms obtained by the analysis of blank serum (A) and blank serum spiked with organophosphate insecticides (each 7.5 μg/ml) (B). Peaks: a = acephate, b = methidathion, c = dichlorvos, d = fenthion, e = EPN, f = diazinon, g = phenthoate, h = malathion, and i = diazinon-$d_{10}$ (IS).

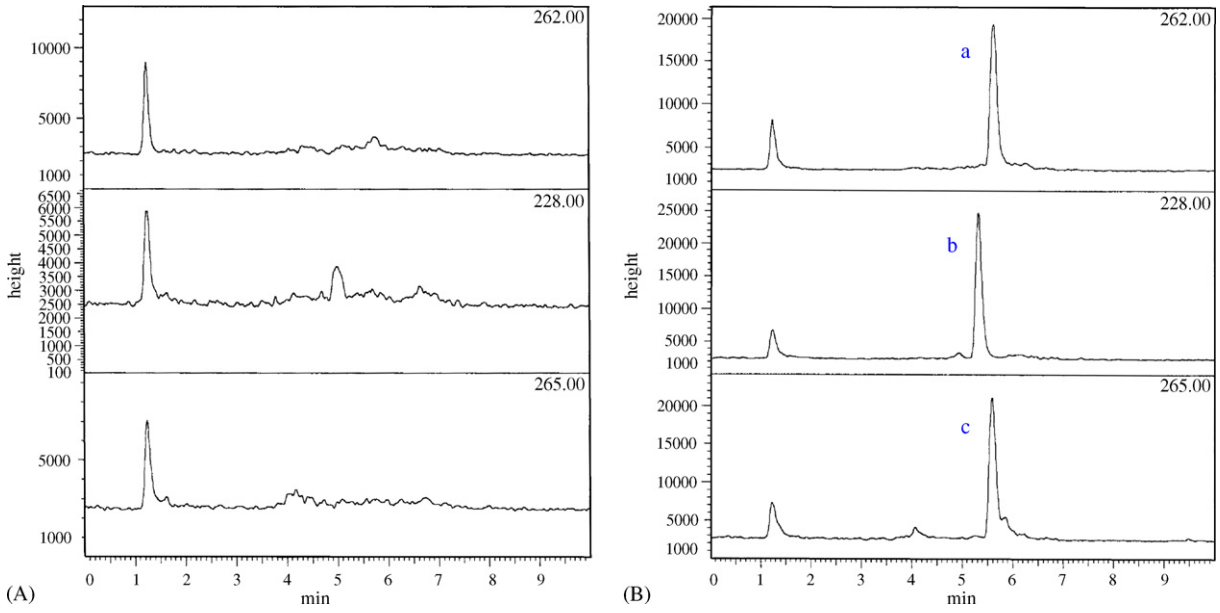


Fig. 3. LC-APCI-MS chromatograms obtained in the negative-ion mode. Selected ion chromatograms obtained by the analysis of blank serum (A) and blank serum spiked with organophosphate insecticides (each 7.5 μg/ml) (B). Peaks: a = fenitrothion, b = cyanophos, and c = fenitrothion-$d_6$ (IS).

### 3.4. Intra- and interbatch precision and accuracy

QC samples were prepared and analyzed in assay batches 1–3. Table 3 shows a summary of the intra- and interbatch precision and accuracy. The intrabatch accuracy (R.E.) ranged from 95.1 to 105.7% at three different concentrations with a precision (S.D.) of between 1.5 and 8.4%. The interbatch R.E. ranged from 96.3 to 108% with a S.D. of between 5.7 and 13.5% at three different concentrations. Both the R.E. and S.D. were satisfactory, with values of less than 15% for three QC serum samples. These

Table 2
Validation parameters for the target drugs

| Compound | Internal standard | LOD (μg/ml) | LOQ (μg/ml) | Linearity (μg/ml) | Regression line | Correlation coefficients |
|---|---|---|---|---|---|---|
| Acephate | Diazinon-$d_{10}$ | 0.25 | 0.375 | 0.375–8 | $y = 1.6649 \times -0.4819$ | 0.9838 |
| Methidathion | Diazinon-$d_{10}$ | 0.5 | 0.625 | 0.625–8 | $y = 0.9072 \times -0.4131$ | 0.9892 |
| Dichlorvos | Diazinon-$d_{10}$ | 0.5 | 0.625 | 0.625–8 | $y = 0.2448 \times -0.2455$ | 0.9972 |
| Fenthion | Diazinon-$d_{10}$ | 1 | 1.25 | 1.25–8 | $y = 0.3164 \times -0.2909$ | 0.9941 |
| EPN | Diazinon-$d_{10}$ | 0.375 | 0.5 | 0.5–8 | $y = 0.7222 \times -0.5127$ | 0.9904 |
| Diazinon | Diazinon-$d_{10}$ | 0.125 | 0.25 | 0.25–8 | $y = 7.3947 \times -0.5127$ | 0.9967 |
| Phenthoate | Diazinon-$d_{10}$ | 0.25 | 0.375 | 0.375–8 | $y = 0.9682 \times -0.0975$ | 0.9981 |
| Malathion | Diazinon-$d_{10}$ | 0.25 | 0.375 | 0.375–8 | $y = 1.3356 \times +0.4801$ | 0.9991 |
| Fenitrothion | Fenitrothion-$d_6$ | 0.125 | 0.25 | 0.25–8 | $y = 0.3837 \times +0.1703$ | 0.9978 |
| Cyanophos | Fenitrothion-$d_6$ | 0.125 | 0.25 | 0.25–8 | $y = 0.5595 \times +0.229$ | 0.9969 |

Table 3
Precision and accuracy of organophosphorus compounds in human serum quality control samples

| Compound | Accuracy ± standard deviation | | | | | |
|---|---|---|---|---|---|---|
| | Intrabatch (μg/ml) | | | Interbatch (μg/ml) | | |
| | 0.625 | 2.5 | 7.5 | 0.625 | 2.5 | 7.5 |
| Acephate | 103.5 ± 2.3 | 103.2 ± 2.9 | 97.2 ± 4.9 | 105.8 ± 6.5 | 98.1 ± 10.1 | 99.1 ± 8.4 |
| Methidathion | 98.4 ± 5.0 | 99.4 ± 6.2 | 95.1 ± 2.7 | 100.5 ± 8.8 | 103.8 ± 10.2 | 96.9 ± 10.4 |
| Dichlorvos | 100.6 ± 7.5 | 101.4 ± 6.3 | 101.5 ± 6.4 | 103.6 ± 10.2 | 101.9 ± 10.9 | 97.7 ± 11.1 |
| Fenthion | – | 101.8 ± 7.0 | 99.7 ± 7.4 | – | 105 ± 9.5 | 98.2 ± 8.5 |
| EPN | 98.3 ± 6.6 | 98.3 ± 6.6 | 105.7 ± 7.9 | 99.7 ± 9.8 | 98.5 ± 10.8 | 100.6 ± 13.5 |
| Diazinon | 101.6 ± 4.8 | 101.6 ± 7.0 | 98.1 ± 3.0 | 108 ± 7.6 | 104.8 ± 5.7 | 104.4 ± 8.1 |
| Phenthoate | 96.2 ± 3.5 | 96.2 ± 3.5 | 99.1 ± 5.6 | 101.4 ± 8.4 | 98 ± 8.5 | 96.3 ± 10.6 |
| Malathion | 99.5 ± 2.9 | 101.8 ± 3.2 | 97.7 ± 3.9 | 98.3 ± 10.4 | 99.1 ± 11.0 | 96.8 ± 8.3 |
| Fenitrothion | 101.7 ± 4.8 | 102.5 ± 8.4 | 102.4 ± 1.5 | 100.6 ± 10.4 | 97.6 ± 8.6 | 104.5 ± 6.4 |
| Cyanophos | 100 ± 5.5 | 98.4 ± 6.4 | 104 ± 5.9 | 105.1 ± 8.1 | 104.1 ± 9.4 | 105.4 ± 8.4 |

Table 4
Recoveries of organophosphorus compounds in human serum quality control samples

| Compound | Absolute recovery (%) | | | Relative recovery (%) | | |
|---|---|---|---|---|---|---|
| | 0.625 μg/ml | 2.5 μg/ml | 7.5 μg/ml | 0.625 μg/ml | 2.5 μg/ml | 7.5 μg/ml |
| Acephate | 103 | 94.2 | 99.2 | 92.6 | 72 | 87.6 |
| Methidathion | 104.3 | 94.1 | 106.8 | 97.7 | 81.3 | 94.5 |
| Dichlorvos | 104.8 | 107.1 | 99.3 | 108.1 | 87.9 | 95.4 |
| Fenthion | – | 92.7 | 103.7 | – | 80.7 | 83.9 |
| EPN | 97.1 | 95.9 | 93.7 | 60 | 108.1 | 82.7 |
| Diazinon | 106.8 | 94.6 | 107.2 | 84.9 | 73.8 | 94.7 |
| Phenthoate | 93.2 | 84.3 | 101.1 | 72.9 | 67.5 | 89 |
| Malathion | 102.3 | 82.3 | 100.6 | 94.4 | 60.1 | 85.8 |
| Fenitrothion | 89.7 | 105.8 | 87.9 | 64.4 | 81.1 | 101.1 |
| Cyanophos | 92.4 | 104.1 | 82.2 | 85.6 | 76.9 | 101.6 |

results indicate that the present method has satisfactory accuracy, precision, and reproducibility.

### 3.5. Recovery

The absolute recoveries were between 82.2 and 107.2%, and the relative recoveries were between 60 and 108.1% (Table 4). The apparent extraction recovery of organophosphorus compounds in serum was high—typically over 80% and approximately 60% for malathion. Nevertheless, using the present procedure, extraction recovery was repeatable for each concentration and good calibration curves were obtained. The CVs for the recovery of each compound and at each concentration were under 10%, and the average CV was $5.2 \pm 2.8\%$. Thus, protein precipitation with acetonitrile was successfully applied for the sample preparation of organophosphorus compounds from serum.

### 3.6. Interference

Although LC–MS is an extremely selective combination and interference is unlikely, we examined potential interference by drugs by adding compounds commonly present in clinical samples to three QC samples. The following compounds (0.5 and 1 μg/ml) were tested for interference with organophosphate quantification: promazine, promethazine, chlorpromazine, levomepromazine, propericiazine, thioridazine, perphenazine, nitrazepam, diazepam, estazolam, alprazolam, lorazepam, prazepam, lormetazepam, triazolam, etizolam, brotizolam, setiptiline, nortriptyline, desipramine, maprotiline, amitriptyline, imipramine, quetiapine, and zolpidem. Organophosphate quantification within 20% of the target was our criterion for dismissing potential interference. In no instance was interference observed in the reconstituted SIM chromatograms at the retention times of the analytes and the IS.

### 3.7. Stability

The stability of the processed samples was also evaluated (Table 5). Although three freeze–thaw cycles and storage (at 4 °C for 7 d and at −30 °C for 4 weeks) before analysis had little effect on the quantification, storage at room temperature for 24 h caused the decomposition of some compounds (dichlorvos and malathion).

A search of literature yielded only a single study that addressed the problem of organophosphate stability in fresh human blood [9]. This study reported that spiked fresh blood samples left standing at room temperature for 24 h exhibited a significant loss of analyte over the 24 h. Our expanded stability analysis included freeze–thawing and long-term storage of organophosphate in serum. The results are summarized as follows. When three QC samples were left on a benchtop at room temperature for 24 h there was an apparent loss of analyte with the exception of methidathion and diazinon; however, almost all

Table 5
Stabilities of organophosphorus compounds in human serum quality control samples

| Compound | Room temperature (μg/ml) | | | 4 °C (μg/ml) | | | Freeze (μg/ml) | | | Freeze and thaw (μg/ml) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0.625 μg/ml | 2.5 μg/ml | 7.5 μg/ml | 0.625 μg/ml | 2.5 μg/ml | 7.5 μg/ml | 0.625 μg/ml | 2.5 μg/ml | 7.5 μg/ml | 0.625 μg/ml | 2.5 μg/ml | 7.5 μg/ml |
| Acephate | 0.23 | 1.39 | 4.89 | 0.33 | 1.15 | 4.89 | 0.63 | 2.35 | 7.31 | 0.62 | 2.13 | 7.12 |
| Methidathion | 0.54 | 2.30 | 7.50 | 0.61 | 2.56 | 6.42 | 0.60 | 2.61 | 8.33 | 0.62 | 2.46 | 7.23 |
| Dichlorvos | 0.28 | 0.86 | 2.63 | 0.26 | 2.22 | 5.87 | 0.38 | 2.55 | 7.24 | 0.32 | 2.20 | 7.47 |
| Fenthion | – | 1.64 | 6.58 | – | 2.20 | 7.37 | – | 2.56 | 7.37 | – | 2.31 | 7.28 |
| EPN | 0.31 | 2.17 | 5.96 | 0.52 | 2.79 | 8.13 | 0.58 | 2.36 | 7.16 | 0.59 | 2.28 | 6.76 |
| Diazinon | 0.59 | 2.50 | 6.17 | 0.59 | 2.24 | 6.84 | 0.53 | 2.46 | 7.49 | 0.60 | 2.35 | 7.64 |
| Phenthoate | 0.39 | 2.46 | 6.86 | 0.65 | 2.53 | 6.48 | 0.61 | 2.15 | 7.07 | 0.55 | 2.13 | 7.16 |
| Malathion | – | 0.38 | 1.59 | 0.22 | 1.71 | 6.38 | 0.65 | 2.46 | 7.17 | 0.61 | 2.39 | 7.10 |
| Fenitrothion | 0.55 | 1.67 | 5.52 | 0.46 | 2.50 | 7.15 | 0.58 | 2.49 | 7.40 | 0.59 | 2.44 | 7.10 |
| Cyanophos | 0.59 | 1.38 | 5.19 | 0.61 | 2.44 | 5.50 | 0.51 | 2.03 | 6.29 | 0.61 | 2.39 | 7.28 |

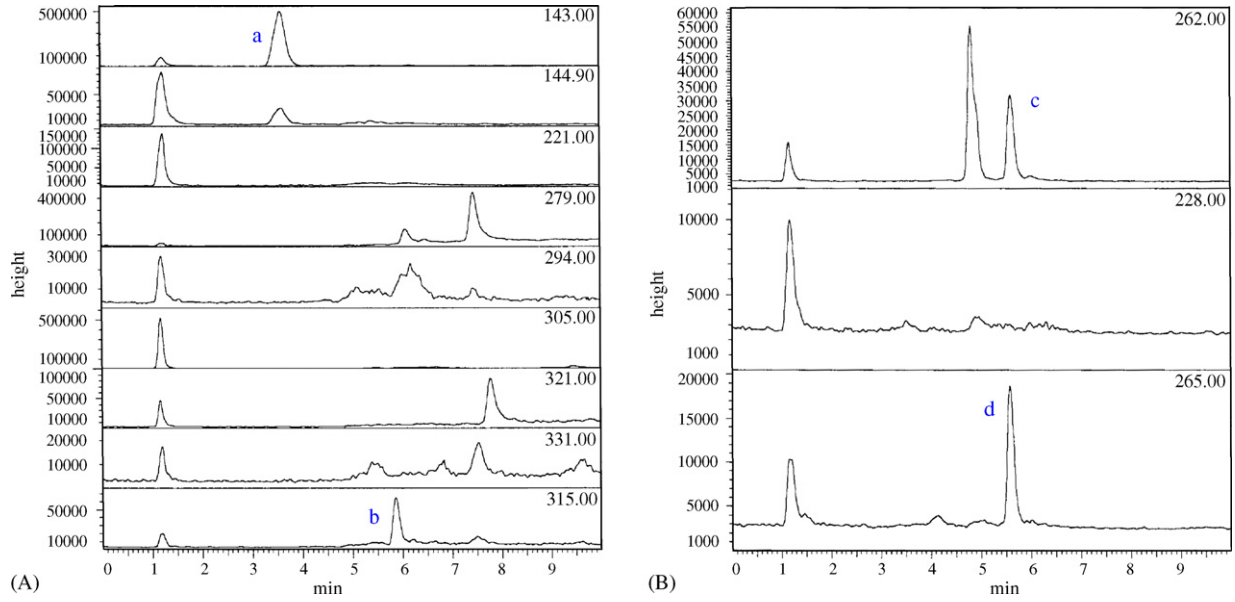


Fig. 4. Mass chromatograms of serum obtained from a clinical sample from a patient who ingested organophosphorus compounds. (A) positive-ion mode and (B) negative-ion mode. Peaks: a = acephate, b = diazinon-$d_{10}$ (IS), c = fenitrothion, and d = fenitrothion-$d_6$ (IS).

the analytes were stable through three freeze–thaw cycles and after 4 weeks storage at −30 °C (Table 5).

### 3.8. Application

In the present case, the determined serum concentrations of acephate and fenitrothion *on admission* were 7.2 and 4.5 μg/ml, respectively (Fig. 4).

## 4. Conclusion

A rapid and reliable LC–MS method has been described for the determination of organophosphorus pesticides in human serum using protein precipitation as the sample clean-up procedure. This method exhibited acceptable selectivity, sensitivity, precision, accuracy, linearity, recovery, and stability of the organophosphorus pesticides in the serum samples. The present method was successfully applied to the clinical toxicology screening of human blood samples after oral ingestion of organophosphorus pesticides.

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