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Multiresidue determination method for organophosphorus pesticides in serum and whole blood by gas chromatography–mass-selective detection

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Abstract

This paper describes a rapid, specific and sensitive method for the determination of 29 organophosphorus pesticides in blood and serum, involving a rapid solid-phase extraction procedure using Oasis HLB cartridges and gas chromatography coupled to mass-selective detection. The ionization was performed by electron Impact and acquisition in the single ion monitoring mode followed three specific ions per analyte. Extraction recoveries were satisfactory and ranged between 40 and 108% in blood and serum. Limits of detection ranged from 5 to 25 ng/ml and limits of quantitation (LOQs) ranged from 10 to 50 ng/ml, in blood and serum. An excellent linearity was observed from these LOQs up to 1000 ng/ml. Intra- and inter-assay precision and accuracy were satisfactory for most of the pesticides analyzed.

Keywords: Organophosphorus pesticides

1. Introduction

Among the insecticides, organophosphorus pesticides (OPs) are widely used and are frequently involved in deliberate and accidental human intoxication [1–4]. Rapid identification and quantification of the causal pesticide would provide useful information to clinicians for taking appropriate treatment decisions [5]. However to the best of our knowledge, no previously published method allowed the simultaneous determination in blood or serum of a large number of the organophosphorus pesticides most frequently encountered in intoxication cases [6,7]. Their volatility, good thermal stability and low polarity render OPs suitable for gas chromatographic analysis, particularly for their determination in biological matrices [8]. Gas chromatography (GC) or liquid chromatography (LC) coupled to mass spectrometry (GC–MS, GC–MS–MS or LC–MS) were used when a highly selective detection was required [9–12]. The detection and quantification limits of the most recently published techniques for a few organophosphate pesticides in blood and serum by GC–MS usually ranged from 50 to 100 μg/l [11].

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Moreover, no extraction procedures developed were able to determine a large number of organophosphate pesticides in blood.

Liquid–liquid extraction (LLE) is the most commonly used extraction procedure in blood. Only a few methods using solid-phase extraction (SPE) [13,14] or headspace solid-phase microextraction (HS-SPME) [11] have been reported for pesticide analysis. Most of these methods are complex and also time-consuming. To avoid any clean-up procedure, we looked for suitable SPE sorbents and in particular, we tested a polymeric-based sorbent formed by the macropolymer poly(divinyl), packed in Oasis HLB cartridges.

The aim of this study was to develop a method for the determination in blood and in serum of the 29 OPs mainly used by fruit growers in the Limousin region (France). Hence, we developed a new and sensitive method for the simultaneous determination of these OPs in human blood and serum, using a combination of rapid SPE and GC–MS.

2. Experimental

2.1. Reagents and materials

Vamidothion, dimethoate, ethoprophos, cadusaphos, mevinphos, phorate, terbuphos, fonophos, chlorpyriphos-methyl, chlorpyriphos-ethyl, fenithrothion, bromophos-methyl, isophenphos, malathion, parathion-methyl, fenthion, methidathion, parathion-ethyl, pirimiphos-methyl, pirimiphos-ethyl, quinalphos, phenamiphos, phosalone, ethion, phosmet, pyrazophos, azinphos-methyl, azinphos-ethyl and coumaphos were purchased from Cluzeau Info Labo (Libourne, France). A stock solution for each pesticide was prepared at 1 g/l in methanol. Acetonitrile (of Pestinorm grade), acetic acid, methanol and sodium acetate were purchased from Prolabo (Fontenay-sous-bois, France). All were of chromatographic purity. The working solutions were prepared at 10 mg/l, 1 mg/l and 100 μg/l in methanol. The calibrating standards solutions were prepared by appropriate dilution of working solutions in blank serum or blood. The internal standard (I.S.) solution of cyproheptadine was prepared at 50 mg/l in methanol. All stock and working solutions in methanol were stored at +4°C. Their stability was verified over a 3-month period by comparison with freshly prepared solutions. A pH 9.7, 0.1 *M* phosphate buffer was prepared by dissolving 6.81 g of potassium dihydrogenphosphate in 450 ml of deionized water, adjusting to pH 9.7 with 1 *M* potassium hydroxide, and making the total volume up to 500 ml with deionized water; the pH 7, 0.1 *M* phosphate buffer was prepared following the same process.

Blank human blood and serum were obtained from healthy donors collected and stored immediately at −18°C until analysis. These pesticide-free matrices were used to prepare matrix matched standards for calibration.

2.2. Gas chromatography–mass spectrometry

A Shimadzu GC 17A gas chromatograph, equipped with an AOC SPL 1400 automatic sampler and the split/splitless injector operated in the splitless mode, and coupled to a Shimadzu QP-5000 mass spectrometer (Touzart et Matignon, Courtaboeuf, France) was used. It was operated using Class 5000 GC–MS Shimadzu software (Touzart et Matignon). The analytical column was a Supelco PTE5, 30 m×0.32 mm I.D., 0.25 μm film thickness coated with a 5% biphenyl–95% dimethylsiloxane stationary phase (Supelco, St. Quentin-Fallavier, France). The chromatograph was programmed from an initial temperature of 60°C up to 280°C, at the rate of 10°C/min, and held at 280°C for 5 min. The total run time was 30 min. The temperatures of the injector and of the transfer line were 250°C and 280°C, respectively. Helium was used as the carrier gas (flow-rate: 2.1 ml/min). The mass spectrometer was operated in the electron impact (70 eV), selected ion monitoring (SIM) mode, with a total scan time of 1 s. For each analyte the most abundant and characteristic mass fragment for quantitation and two others for confirmation were chosen (Table 1). The compounds were subsequently identified by their relative retention times and by the ratios of their respective confirmation ions to their quantitation ion.

2.3. Extraction procedure

Blood and serum samples were treated differently to avoid protein interaction with pesticides. For

Table 1
Chromatographic relative retention times (RRTs), quantitation and confirmation ions selected and limits of detection (LODs) and quantitation (LOQs) for the GC–MS determination of 29 pesticides in serum and blood

Compound	RRT	Quant. ion, *m/z*	First confirmation ion		Second confirmation ion		Limits	
			m/z	Rel. int. (%)	*m/z*	Rel. int. (%)	LOD	LOQ
Azinphos-ethyl	1.09	132.0	160	73	373	73	5	10
Azinphos-methyl	1.06	104.0	160	45	–	45	5	10
Bromophos-methyl	0.84	331.0	329	66	333	66	5	10
Cadusaphos	0.67	159.0	158	69	213	69	5	10
Chlorpyriphos-ethyl	0.83	197.0	314	28	286	28	5	10
Chlorpyriphos-methyl	0.77	288.0	125	209	286	209	5	10
Coumaphos	1.12	362.0	226	98	210	98	5	10
Dimethoate	0.69	125.0	172	3	229	3	5	10
Ethion	0.95	231.0	384	3	153	3	25	50
Ethoprophos	0.64	158.0	242	9	200	9	5	10
Fenithrotion	0.80	277.0	260	66	125	66	25	50
Fenthion	0.83	278.0	169	48	153	48	5	10
Fonophos	0.72	246.0	137	219	–	219	5	10
Isophenphos	0.86	213.0	255	35	185	35	5	10
Malathion,	0.82	125.0	173	101	158	101	5	10
Methidathion	0.88	145.0	125	14	85	14	5	10
Mevinphos	0.53	127.0	164	7	192	7	5	10
Parathion-ethyl	0.83	291.0	97	371	109	371	25	50
Parathion-methyl	0.77	263.0	125	312	109	312	25	50
Phenamiphos	0.90	303.0	288	43	217	43	5	10
Phorate	0.68	260.0	231	146	121	146	5	10
Phosalone	1.05	182.0	367	10	184	10	25	50
Phosmet	1.04	160.0	161	13	317	13	5	10
Pirimiphos-ethyl	0.85	318.0	97	371	109	371	5	10
Pirimiphos-methyl	0.82	290.0	305	49	125	49	5	10
Pyrazophos	1.09	221.0	232	34	373	34	5	10
Quinalphos	0.87	146.0	298	4	157	4	5	10
Terbuphos	0.72	231.0	186	18	153	18	5	10
Vamidothion	0.59	87.0	119	20	146	20	5	10

Abbreviations: RRT, relative retention time; Quant. ion, quantitation ion; rel. int., relative intensity (%); LOD, limit of detection; LOQ, limit of quantitation.

whole blood, a 2-ml volume spiked with 2 μg of cyproheptadine as I.S. was placed in a screw capped glass tube and 2 ml acetonitrile added. The mixture was vortex-mixed and centrifuged at 3000 rpm (1600 *g*) for 5 min. The supernatant was concentrated to 1 ml at 40°C under a stream of nitrogen.

It was further treated like a serum sample. Serum samples (2 ml) (spiked with I.S.) or blood sample supernatants were deposited on an Oasis hydrophilic–lipophilic balanced copolymer (HLB) cartridge (3 ml/60 mg of sorbent) (Waters, Guyancourt, France), previously conditionned with 2 ml methanol and deionized water, successively. SPE was manually performed using a Vac Master SPE vacuum manifold purchased from J.T. Baker (Noisy-le-Sec, France). For the clean-up step, 2 ml of deionized water was added. After drying (20 min), the elution was carried out with 3 ml ethyl acetate. The eluate was evaporated to dryness under a gentle stream of nitrogen (at 40°C), and the residual was taken off by 100 μl ethyl acetate, and then 2 μl of this solution was injected into the GC–MS system.

2.4. Validation

All validation procedures were performed twice, using pesticide-free whole blood and serum. Calibration standards were prepared by adding 100 μl of appropriate dilutions of the mixture of the 29 compounds in methanol to 1.9 ml of blank serum or

blood to obtain the following concentrations limits of quantitation (LOQs) (10 or 50), 100, 200, 500 and 1000 ng/ml. Extraction recovery was evaluated by analyzing standards in triplicate at four concentration levels (10, 50, 200 and 1000 ng/ml) and by comparing the analyte/I.S. peak area ratios obtained with those of unextracted solutions.

The intra-assay precision was assessed at 10, 50, 200 and 1000 ng/ml by extraction and analysis on the same day of five fortified blood or serum samples for each level. For the intermediate (''inter-assay'') precision, a set of calibrating samples (10, 50, 200 and 1000 ng/ml) was analyzed each day for 5 days. The accuracy was determined by comparing the mean measured concentration to its theoretical value and expressed as mean relative error (MRE). Precision was expressed as relative standard deviation (RSD). The limit of detection (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 an RSD less than 20%. Calibration graphs of the pesticide-to-internal standard peak-area ratios versus theoretical concentration were constructed using linear least-square regression analysis.

3. Results and discussion

Fig. 1 and Fig. 2 show total ion current (TIC) chromatograms (for all selected ions) obtained, respectively, with a real serum sample from a patient intoxicated with ethyl-parathion (213 μg/l) and with

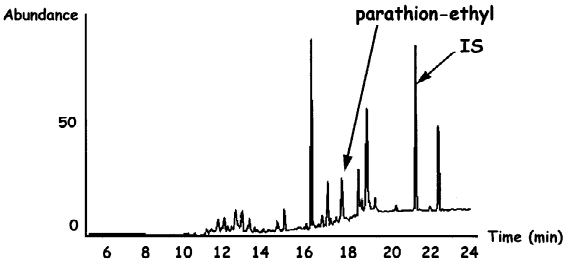

Fig. 1. Total ion current (TIC) chromatogram (for all selected ions) of a clinical serum sample collected after an intoxication by ingestion of parathion-ethyl (213 μg/l).

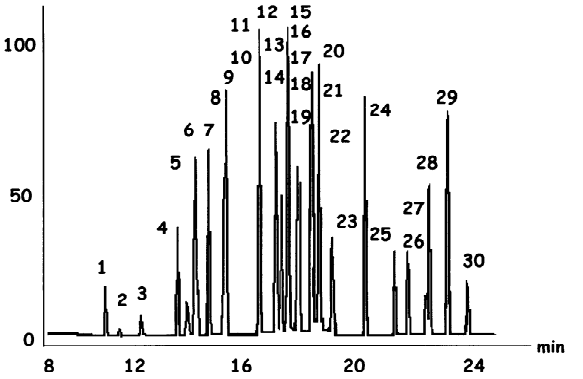

Fig. 2. Typical total ion current (TIC) chromatogram (for all selected ions) of blank human whole blood spiked at 2 μg/ml I.S. (24) and 200 μg/l of: (1) mevinphos, (2) vamidothion, (3) ethoprophos, (4) cadusaphos, (5) phorate, (6) dimethoate, (7) terbuphos, (8) fonophos, (9) chlorpyriphos-methyl, (10) parathion-methyl, (11) fenithrotion, (12) pirimiphos-methyl, (13) malathion, (14) chlorpyriphos-ethyl, (15) fenthion, (16) parathion-ethyl, (17) bromophos-methyl, (18) pirimiphos-ethyl, (19) isophenphos, (20) quinalphos, (21) methidathion, (22) phenamiphos, (23) ethion, (25) phosmet, (26) phosalone, (27) azinphos-methyl, (28) pyrazophos, (29) azinphos-ethyl and (30) coumaphos.

a 2 μg/l spiked whole blood sample. Average extraction recoveries ranged from 40 to 108% in blood and from 61 to 99% in serum (Table 2) with most of the standard deviations (SDs) less than 10%. We noticed differences in recoveries between blood and serum, presumably because of a loss of compound during the whole blood defecation step, though this has been optimized testing the influence of several reagents (such as methanol and acetonitrile) and of freezing. The use of acetonitrile, followed by a partial evaporation appeared to be the best solution. The crucial problem was to define optimal extraction conditions, in order to obtain satisfying recoveries for each of the 29 compounds. LLE and SPE procedures were tested and compared (results not shown). Various extraction solvents (such as methanol, acetonitrile, phosphate buffers at different pH values) were tried for LLE which revealed inefficient for the elimination of co-extractive interferences, despite our previous experience [15–17]. Indeed, SPE of pesticides from serum or blood is a useful alternative to classical LLE methods [13,14,18], though the major applications of SPE have been limited to plasma, serum and urine, because the presence of cells in blood samples tend

Table 2
Extraction recovery (%) of the 29 OPs analyzed in human blood and serum by GC–MS (five replicates at each concentration)

Compound	Theoretical concentration (μg/l)					
	LOQ (10 or 50)		200		1000	
	Blood, mean±SD	Serum, mean±SD	Blood, mean±SD	Serum, mean±SD	Blood, mean±SD	Serum, mean±SD
Azinphos-ethyl	75.0±5.4	93.6±1.6	73.1±3.4	96.2±2.3	101.8±3.2	96.5±0.2
Azinphos-methyl	76.8±7.9	82.7±13.3	77.2±8.9	90.1±10.3	96.6±5.3	92.1±1.6
Bromophos-methyl	64.2±7.4	87.6±3.4	45.3±0.2	95.1±4.3	55.1±1.1	86.2±0.7
Cadusaphos	80.4±9.5	95.7±0.8	80.8±9.7	66.9±5.5	58.5±0.1	92.7±2.6
Chlorpyriphos-ethyl	64.3±1.5	95.8±0.6	45.0±0.2	95.3±3.2	51.8±1.7	95.6±1.1
Chlorpyriphos-methyl	92.0±25.8	84.6±1.8	61.4±7.6	90.3±6.1	56.5±1.7	90.6±1.3
Coumaphos	53.2±5.1	88.0±3.4	51.1±2.6	87.0±2.3	107.9±12.3	96.83.7
Dimethoate	92.2±12.0	88.8±1.8	83.3±10.9	88.2±1.5	72.3±4.9	90.0±1.6
Ethion	40.8±6.4	88.8±1.2	39.5±2.6	97.9±2.6	57.5±1.8	94.7±0.9
Ethoprophos	92.7±18.5	98.0±1.0	84.4±6.0	78.6±6.2	61.5±1.0	96.8±3.3
Fenithrotion	62.3±8.2	85.5±4.2	70.8±2.7	95.4±3.0	67.9±3.0	91.5±1.3
Fenthion	49.3±3.8	88.5±3.7	53.9±0.5	90.7±3.8	50.4±1.4	89.7±1.2
Fonophos	69.8±5.9	97.6±2.8	73.3±6.2	78.7±7.1	51.9±0.5	90.9±1.3
Isophenphos	71.3±2.6	92.1±3.8	65.1±0.8	96.6±1.7	56.6±1.8	94.8±1.5
Malathion,	90.3±4.5	88.5±10.8	78.6±9.0	90.1±6.9	64.6±5.4	98.8±0.9
Methidathion	93.7±6.2	97.9±1.7	86.8±10.0	95.6±4.2	74.5±0.6	91.9±0.6
Mevinphos	70.2±13.5	70.5±19.5	95.9±6.4	55.5±8.8	57.1±1.3	88.2±3.7
Parathion-ethyl	53.8±6.8	91.4±1.1	62.9±0.6	98.5±2.0	59.6±0.6	97.1±2.0
Parathion-methyl	68.4±6.9	87.3±4.2	77.8±5.4	95.4±4.8	70.2±2.3	91.9±1.5
Phenamiphos	87.4±3.8	85.8±3.6	60.5±8.2	95.4±0.4	56.3±2.3	95.2±1.5
Phorate	57.4±6.3	92.2±5.1	77.5±11.4	61.3±10.0	41.2±2.3	83.2±1.8
Phosalone	52.7±5.1	92.6±1.4	56.4±2.6	97.7±2.2	98.1±0.3	95.9±0.5
Phosmet	76.6±6.7	97.5±0.9	73.3±3.6	97.8±2.0	98.9±10.3	94.0±1.2
Pirimiphos-ethyl	46.9±4.9	89.9±3.0	49.0±2.6	95.5±2.3	50.7±1.5	93.3±2.0
Pirimiphos-methyl	59.9±12.4	91.8±2.5	46.6±1.0	94.6±3.3	51.3±1.3	93.6±1.0
Pyrazophos	64.1±4.7	89.7±1.1	60.7±0.5	99.5±0.8	92.1±6.8	97.0±1.9
Quinalphos	68.3±6.7	98.6±0.9	65.4±2.2	95.4±3.0	56.5±0.3	96.8±0.6
Terbuphos	42.9±1.6	94.5±2.7	53.6±3.8	74.7±7.6	46.4±0.7	90.2±1.7
Vamidothion	66.0±17.6	81.01±4.7	70.2±2.8	89.5±2.8	45.0±3.6	91.4±4.3

Abbreviations: LOQ, limit of quantitation; SD, standard deviation.

to clog the column. We compared different apolar SPE phases such as octadecyl (C_{18}) and the HLB Oasis columns. These last columns yielded the highest recovery for the 29 compounds (Table 2) together. Several solvents (acetone, methanol, acetonitrile, ethyl acetate, and dichloromethane) were tested for elution and the best compromise between recovery and cleanliness was obtained with 2 ml ethyl acetate. Complete drying of the cartridge (for 20 min) was essential for quantitative elution of the analytes and to avoid the presence of water in the eluate.

The pH influence was also tested (using pH 9.5 phosphate buffer and pH 4.5 acetate sodium solution) resulted in low recovery. If the biological matrix is acidic or basic, pH value has to be readjusted to neutral (pH 7.4) with 0.01 *M* hydrochloride or sodium hydroxide solutions. Moreover, the influence of ionic strength seemed to be crucial: high molarity of buffer disrupts the retention mechanism on the HLB column polymer phase. Moreover, we observed that three other pesticides which are not listed in Tables 1–3 (dichlorvos, metadimophos and acephate) were not retained whatever the pH value.

Despite the absence of a clean-up procedure before GC–MS analysis, the present technique allows a good selectivity for the 29 pesticides, owing to the mass detector. Indeed, endogenous compo-

Table 3
Inter-assay accuracy and precision for the quantitation of 29 pesticides in human serum and blood, using GC–MS

Compound	Added (μg/l)	Blood sample			Serum sample		
		Found (μg/l)	Precision RSD (%)	Accuracy MRE (%)	Found (μg/l)	Precision RSD (%)	Accuracy MRE (%)
Azinphos-ethyl	10	10.6	16.3	6.4	9.6	10.0	−4.3
	1000	995.4	6.4	−0.5	1013.5	3.2	1.4
Azinphos-methyl	10	10.3	17.3	3.4	11.1	11.1	10.8
	1000	904.9	10.8	−9.5	969.0	1.8	−3.1
Bromophos-methyl	10	10.8	24.7	7.8	8.7	13.0	−13.3
	1000	1054.3	6.9	5.4	1053.0	8.1	5.3
Cadusaphos	10	12.1	19.2	21.4	10.5	12.2	4.5
	1000	1028.9	6.2	2.9	976.8	5.7	−2.3
Chlorpyriphos-ethyl	10	11.1	21.1	10.6	10.0	7.8	−0.3
	1000	1039.6	6.4	4.0	1023.3	4.4	2.3
Chlorpyriphos-methyl	10	10.9	21.4	10.6	9.0	13.1	−10.5
	1000	1011.0	2.3	1.1	1076.5	9.7	7.65
Coumaphos	10	9.5	15.0	−4.6	9.5	12.8	−5.5
	1000	1089.8	8.9	9.0	1085.8	8.5	8.6
Dimethoate	10	10.5	12.2	5.2	10.5	14.3	4.8
	1000	953.8	4.3	−4.6	1014.0	4.8	1.4
Ethion	50	44.9	18.1	−10.2	46.0	7.3	−8.0
	1000	1046.3	7.1	4.6	1031.8	3.8	3.2
Ethoprophos	10	11.3	13.4	13.0	10.3	17.0	3.0
	1000	1022.5	7.5	2.2	1014.8	12.3	1.5
Fenithrothion	50	41.6	3.2	−16.8	40.8	7.8	−18.4
	1000	1041.0	6.1	4.1	1090.0	9.0	9.0
Fenthion	10	9.0	23.5	−10.0	8.5	9.8	−15.3
	1000	1040.4	6.1	4.0	1012.3	3.6	1.2
Fonophos	10	10.7	11.7	7.0	9.8	4.6	−2.0
	1000	1074.2	5.5	7.4	994.5	3.1	−0.5
Isophenphos	10	10.2	10.2	1.6	8.8	3.5	−12.3
	1000	1056.1	4.8	5.6	1019.8	2.1	1.9
Malathion	10	11.3	11.7	12.6	9.9	24.5	−1.0
	1000	985.5	0.9	−1.5	1049.8	8.5	4.9
Methidathion	10	11.0	18.7	9.8	10.4	10.5	3.5
	1000	961.3	7.7	−3.9	1025.3	4.7	2.5
Mevinphos	50	50.6	12.6	1.3	45.4	21.9	−9.3
	1000	1073.6	7.5	7.4	1004.8	8.4	0.5
Parathion-ethyl	50	40.6	16.8	−18.7	38.9	10.4	−22.3
	1000	1086.0	6.6	8.6	1053.8	6.9	5.4
Parathion-methyl	50	39.5	3.9	−21.0	38.6	20.8	−22.8
	1000	1016.3	6.4	1.6	1032.5	6.8	9.9
Phenamiphos	10	11.0	16.4	9.8	8.3	10.6	−17.0
	1000	1083.0	1.9	8.3	1055.5	9.4	9.6

Table 3. Continued

Compound	Added (μg/l)	Blood sample			Serum sample		
		Found (μg/l)	Precision RSD (%)	Accuracy MRE (%)	Found (μg/l)	Precision RSD (%)	Accuracy MRE (%)
Phorate	10	10.5	8.1	5.0	10.2	20.0	1.5
	1000	1080.2	3.8	8.0	1003.8	6.3	0.4
Phosalone	50	45.0	19.5	−9.9	41.1	12.6	17.9
	1000	1030.9	7.2	3.1	1020.3	3.1	2.0
Phosmet	10	9.4	20.9	−6.4	10.9	15.6	8.8
	1000	966.7	10.9	−3.3	1008.8	4.3	0.9
Pirimiphos-ethyl	50	9.8	17.3	−2.2	9.5	2.6	−5.3
	1000	1120.0	3.0	12.0	1051.8	8.6	5.2
Pirimiphos-methyl	10	11.2	16.5	12.2	10.2	7.5	1.8
	1000	985.7	5.0	−1.4	1032.5	7.2	3.3
Pyrazophos	10	10.4	11.2	3.8	9.4	6.1	−6.3
	1000	1076.6	5.1	8.3	1046.3	7.6	4.6
Quinalphos	10	10.6	9.0	5.6	10.4	12.4	4.0
	1000	1002.4	5.0	0.2	1012.0	3.6	1.2
Terbuphos	10	10.4	16.5	3.6	8.9	5.6	−11.0
	1000	1082.6	5.1	8.3	1018.8	7.8	1.9
Vamidothion	10	10.1	12.8	0.6	9.8	27.7	−2.3
	1000	971.7	13.7	−2.8	986.5	9.5	8.7

Abbreviations: RSD, relative standard deviation; MRE, mean relative error.

nents (as cholesterol) and interference potentially present are not detected in the SIM mode. Moreover, this SPE procedure is easier and faster than liquid–liquid partitioning and produces relatively clean extracts. This SPE procedure is well suited for non-polar and semi-polar compounds such as most of the OPs and is applicable to urine and other biological matrices (gastric content, tissues, etc.). The results of the validation procedure are summarized in Tables 2 and 3. LODs are 5 ng/ml, and LOQs are 10 ng/ml in serum and blood for most of the compounds, except for ethion, parathion ethyl, parathion methyl, phosalone and fenithrotion (Table 1). They are lower than those obtained by previously published GC methods [8,9,11]. Moreover, these last methods often required additional time-consuming clean-up or derivatization steps.

The present method is repeatable and reproducible for all the compounds. For intra- and inter-assay precision, similar results were obtained with satisfactory RSD values. Hence, the inter-assay precision for the 29 analytes was excellent above their respective LOQs (Table 3). The calibration curve of each analyte was linear from its respective LOQ up to 1000 ng/ml, with correlation coefficient (r) between 0.998 and 0.999. The efficiency of this method was verified with clinical samples collected in intoxication cases (Fig. 1).

Hence, the present method, developed involves a selective SPE procedure and a specific GC–MS determination with satisfactory recoveries and LOQs among the lowest published to date for a multiresidue method in biological matrices using a single mass analyzing instrument. Clinical routine use confirmed that this method is suitable for the analysis of residual amounts of pesticides in biological fluids.

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