Simple Determination of 22 Organophosphorous Pesticides in Human Blood Using Headspace Solid-Phase Microextraction and Gas Chromatography with Mass Spectrometric Detection

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Abstract

A simple and rapid procedure for the determination of 22 organophosphorous pesticides (bromophos-ethyl, bromophos-methyl, chlorfenvinphos, chlorpyriphos, demethon-*S*-methylsulfon, diazinon, dichlorvos, dicrotophos, dimethoate, disulfoton, edifenphos, fenitrothion, fenthion, malathion, methidathion, mevinphos, monocrotophos, omethoate, parathion-ethyl, parathion-methyl, phosphamidon, and quinalphos) in human blood using headspace (HS) solid-phase microextraction (SPME) and gas chromatography (GC)–mass spectrometry (MS) is presented. The effects of various sample additions, incubation temperatures, absorption times, desorption times, and depths of fiber insertion into the injection port of the GC are optimized to enhance the sensitivity of the procedure. The recoveries of spiked blood samples are determined between 70% and 95% compared with samples prepared in water, and absolute recoveries are in the range between 0.1% and 19.6%. For quantitation in the single ion monitoring mode, linearity is established over concentration ranges from 0.025 to 5.0 µg/g with excellent coefficients of correlation (0.991–0.998). The detection limits are in the range between 0.01 and 0.3 µg/g. The time for analysis is 44 min per sample including extraction and GC–MS analysis. HS-SPME in combination with GC–MS is an effective method for the determination of organophosphorous pesticides in human blood and shows a great potential for use in rapid on-site analytical work, which is highly demanded in clinical and forensic toxicology.

Introduction

Organophosphorous pesticides are still widely used. Following metabolism in the organism these compounds cause many cases of acute accidental or suicidal poisonings by the inhibition of cholinesterase activity via phosphorylation by the oxygen analogue. A rapid identification of the causal pesticide would provide very useful information to clinicians for making treatment decisions in emergencies, which is also important in forensic cases. Most analytical methods for organophosphorous pesticides are useful for determination in plant, water, soil, or food (1–9). However, they are not directly applicable to biological samples taken in an emergency or during an autopsy. There is literature of the analysis of some organophosphorous pesticides in urine by gas chromatography (GC)–flame photometric detection (FPD) (10,11); in urine and plasma by GC–flame ionization detection (FID) and GC–nitrogen phosphorus detection (12,13); in gastrolavage, blood, and urine by GC–FID, GC–FPD, and GC–mass spectrometry (MS) (14); in a standard mixture, blood, lung, and liver by high-performance liquid chromatography (HPLC) (15,16); in blood by liquid chromatography–MS (17); and in blood by high-performance thin-layer chromatography (18).

Pawliszyn and coworkers (19,20) have developed a solid-phase microextraction (SPME) method that has been used as a variable solvent-free alternative to conventional liquid–liquid extraction and solid-phase extraction (SPE) procedures mainly for the environmental analysis of organic compounds. SPME in conjunction with analysis by GC has been demonstrated for a variety of classes of organic compounds, especially for volatile and semivolatile agents such as fuel-related hydrocarbons (21); polycyclic aromatic hydrocarbons (22); benzene, toluene, ethyl-benzene, and xylene (23); thinner compounds (24); organochlorine and organophosphorous pesticides from water samples and fruits (25–28); parathion and malathion from blood samples (29,30); and residual solvents in pharmaceutical products (31). It has also been demonstrated for different drugs such as clozapine (32), amphetamines (33–37), antidepressants (38), benzodiazepines (39,40), inhalation anesthetics (41,42), and barbiturates (43).

The proposed procedure combining headspace (HS) and SPME with GC–MS was applied for the detection of various organophosphorous pesticides in biological specimens.

Experimental

Materials

Bromophos-ethyl, bromophos-methyl, chlorfenvinphos, chlorpyriphos, demethon-*S*-methylsulfon, diazinon, dichlorvos, dicro-

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tophos, dimethoate, disulfoton, edifenphos, fenitrothion, fenthion, malathion, methidathion, mevinphos, monocrotophos, omethoate, parathion-ethyl, parathion-methyl, phosphamidon, and quinalphos were purchased from Riedel-de Haen (Seelze, Germany). Stock solutions of each compound were prepared at the 1000-µg/mL level in HPLC-grade methanol and stored in the refrigerator at 4°C. Working solutions were prepared by diluting the stock solutions to appropriate concentrations in methanol, and they also were stored in the refrigerator at 4°C. All other chemicals of HPLC or pro analysi quality were obtained from Merck (Darmstadt, Germany). Whole blood was purchased from the blood bank of the University Hospital of Bonn and tested by conventional GC–MS as to whether it was drug free. A manual SPME assembly with a replaceable extraction fiber coated with 100-µm polydimethylsiloxane was purchased from Supelco (Deisenhofen, Germany). The fiber was conditioned at 250°C for 1 h in the injector part of the GC according to the suppliers' instructions.

GC–MS

GC–MS analysis was performed using an HP Model 5890 Series II Plus (Hewlett Packard, Waldbronn, Germany) with an HP 5972 Mass Selective Detector. The system was equipped with a 30-m × 0.25-mm-i.d. fused-silica capillary column (Hewlett Packard, HP-5-MS, 0.25-µm film thickness). Data acquisition and analysis were performed using standard software supplied by the manufacturer. The column temperature was set at 120°C for 1 min, then it was programmed to increase up to 290°C at 10°C/min and hold for 1 min. The temperatures of the injection port and interface were set at 250°C, and the temperature of the detector was set at 290°C. The splitless injection mode was used, and helium with a flow rate of 1.0 mL/min was used as the carrier gas.

Electron impact (EI) mass spectra of the organophosphorous pesticides and azobenzene (the internal standard) were recorded by total ion monitoring. For quantitative determination, retention times and characteristic mass fragments were recorded, and the chosen diagnostic mass fragments were analyzed by monitoring the selected ions (SIM mode). The diagnostic mass fragments are presented in Table I. For quantitation, peak-area ratios of the target ion of the different organophosphorous pesticides and azobenzene (*m/z* 182) were calculated as a function of the concentration of the substance.

Table I. Diagnostic Mass Fragments of the Tested Organophosphorous Pesticides*

Substance	Mass fragments (*m/z*)
Azobenzene	77,105,152,**182**
Bromophos-ethyl	97,303,357,**359**
Bromophos-methyl	125,213,329,**331**
Chlorfenvinphos	81,**109**,267,323
Chlorpyriphos	97,**197**,286,314
Diazinon	**137**,152,179,199
Dichlorvos	79,**109**,145,185
Disulfoton	88,125,186,**274**
Edifenphos	**109**,173,201,310
Fenthion	109,125,169,**278**
Malathion	93,**127**,173,285
Methidathion	85,125,**145**,302
Mevinphos	109,**127**,164,192
Parathion-ethyl	97,**109**,139,291
Parathion-methyl	79,**109**,125,263
Quinalphos	90,118,**146**,157

* The target ion is indicated in bold face.

Sample preparation by HS-SPME

For sample preparation, 0.5-mL blood, azobenzene (1 mg/L, 10 µL), ammonium sulfate (0.2 g), and sulfuric acid (0.1M, 2 mL) were placed into a 10-mL HS vial and sealed rapidly with a silicon septum and an aluminum cap. The vial was immediately heated at 120°C for 15 min. The needle of the SPME device containing the extraction fiber was inserted through the septum and exposed into the HS of the vial. After 15 min, the needle was removed from the vial and inserted into the injection port of the GC–MS. The compounds absorbed on the fiber were desorbed by exposing the fiber in the injection port for 2 min and then analyzed.

Additions, incubation temperature, absorption time, desorption time, and depth of fiber insertion in the injection port

In order to gain optimal conditions in the sample preparation step, the effects of various additions, incubation temperatures, absorption times, desorption times, and depths of fiber insertion in the injection port were determined by testing two vials at each temperature and point. Samples with various additions of salt or other solutions containing 0.2-µg/g organophosphorous pesticide were prepared and analyzed as described previously. Furthermore, spiked blood samples were incubated at different temperatures (60°C, 70°C, 80°C, 90°C, 100°C, 110°C, 120°C, and 130°C) for 15 min to determine the optimal incubation temperature. In order to determine the absorption time, the fiber was exposed to the HS of the vials for different lengths of time (1, 2, 3, 4, 5, 7, 10, 15, and 20 min). The next step was to determine the most efficient desorption time. Therefore, the fiber was exposed to the injection port of the GC for 1.0, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 6.0, 7.0, and 8.0 min. Finally, the optimal depth of the fiber insertion in the injection port was determined by insertion with different depths (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, and 4.5 cm).

Calibration curves, limit of detection, and reproducibility

In order to determine calibration curves, blood samples spiked with organophosphorous pesticides at final concentrations ranging from 0.025 to 5 µg/g were prepared and analyzed using the described procedure. The calibration curves were obtained by plotting the peak-area ratio between the analytes and azobenzene. Azobenzene was used as the internal standard throughout the study according to Wylie and Uchiyama (44). The limit of detection was evaluated using a signal-to-noise ratio of 3.

The reproducibility was evaluated by analyzing six blood samples spiked with organophosphorous compounds at a concentration of 1 µg/g on the same day (repeatability) and over six consecutive days (reproducibility).

Absolute recovery

For the determination of the absolute recovery, blood samples (0.5 mL) spiked with 500 ng of each organophosphorous pesticide

were analyzed with the HS-SPME procedure, and the results were compared with a liquid injection of a methanolic solution (10 ng/2 µL).

Results and Discussion

Selectivity

The chromatograms of two blood samples spiked with test mixtures of organophosphorous pesticides (1 µg/g) in comparison with a nonspiked blood sample are demonstrated in Figure 1. The peaks were well-separated. Furthermore, also in the routine analysis no interferences were observed over a period of 2 years.

Additions and recovery

Recoveries were calculated by comparing the amount of drug detected in a blood sample to the amount detected in a water sample that was used as a standard (100%). The recoveries of organophosphorous pesticides in the presence of sulfuric acid were higher than that of distilled water (Table II). The addition of salt led to a further increase of sensitivity because of salting-out effects, whereas ammonium sulfate in comparison with sodium chloride supplied better results. The highest recoveries were achieved with a combination of sulfuric acid and ammonium sulfate.

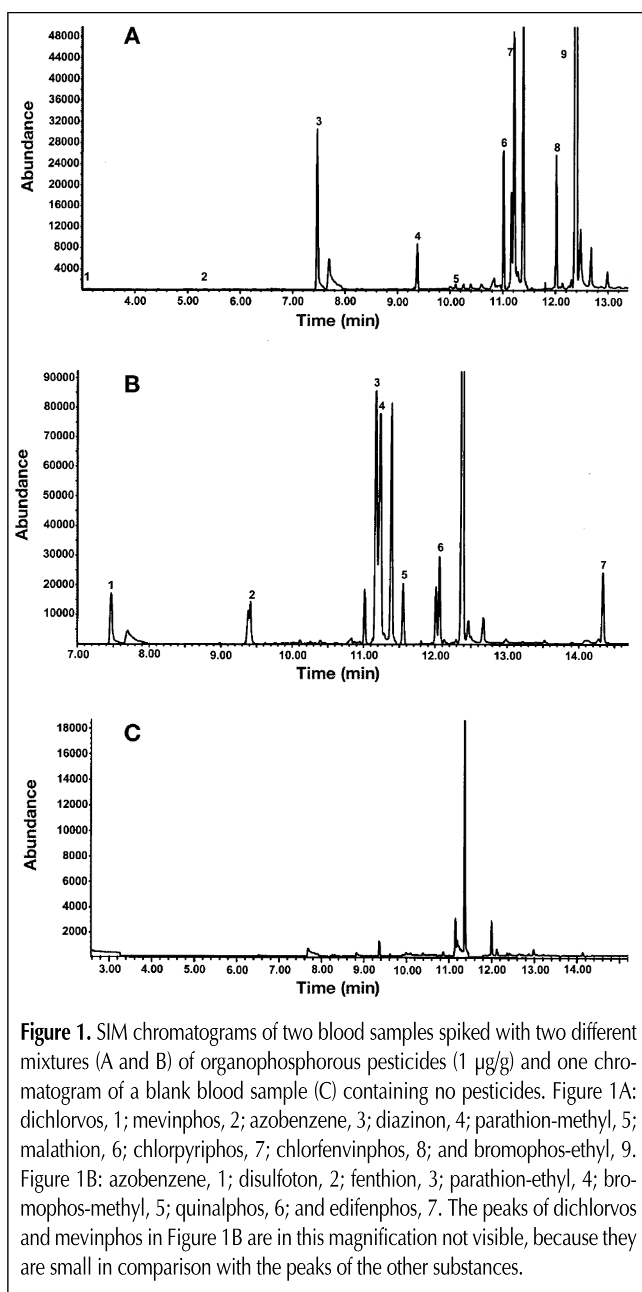

Figure 1. SIM chromatograms of two blood samples spiked with two different mixtures (A and B) of organophosphorous pesticides (1 µg/g) and one chromatogram of a blank blood sample (C) containing no pesticides. Figure 1A: dichlorvos, 1; mevinphos, 2; azobenzene, 3; diazinon, 4; parathion-methyl, 5; malathion, 6; chlorpyriphos, 7; chlorfenvinphos, 8; and bromophos-ethyl, 9. Figure 1B: azobenzene, 1; disulfoton, 2; fenthion, 3; parathion-ethyl, 4; bromophos-methyl, 5; quinalphos, 6; and edifenphos, 7. The peaks of dichlorvos and mevinphos in Figure 1B are in this magnification not visible, because they are small in comparison with the peaks of the other substances.

Incubation temperature

In order to determine the effect of temperature on the absorbed amount of analyte, vials were heated at different temperatures for 15 min. The highest sensitivity was reached at 120°C (Figure 2). Higher temperatures showed no advantages because substances were decomposed.

Absorption time

For HS-SPME it is necessary to reach an equilibrium between the gaseous phase and the solid phase of the fiber coating. Figure 3 shows the influence of different exposure times. At 10 min, equilibrium was reached. Longer absorption times did not achieve better results.

Desorption times

The compounds were released from the fiber by thermal desorption. The exposure of the fiber into the injection port for 2 min led to the best results.

Table II. The Effect of Different Additions on the Recoveries

	%Recovery		
Sample preparation	Malathion	Parathion-ethyl	Parathion-methyl
0.5 g H_2O	100	100	100
0.5 g H_2O + 2 mL 0.1M H_2SO_4	105.3	107.5	108.8
0.5 g blood + 2 mL 0.1M H_2SO_4	51.4	67.4	63.7
0.5 g blood + 2 mL 0.1M H_2SO_4 + 2.0 g NaCl	78.7	86.6	86.4
0.5 g blood + 2 mL 0.1M H_2SO_4 + 0.2 g $(NH_4)_2SO_4$	91.3	95.2	91.8

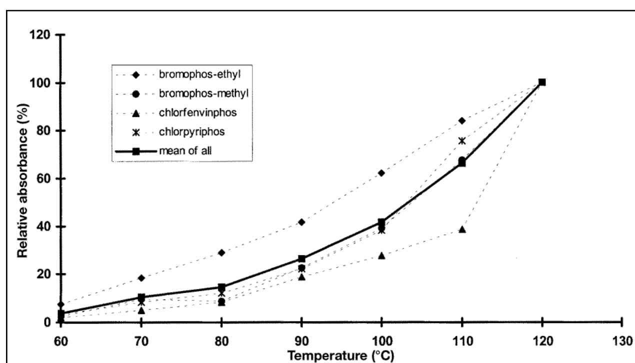

Figure 2. Effect of the incubation temperature on the amounts of organophosphorous pesticides extracted from spiked human blood samples (1 mg/kg). Exemplary shown at bromophos-ethyl, bromophos-methyl, chlorfenvinphos, chlorpyriphos, and the mean of all organophosphorous pesticides.

Depth of fiber insertion

We observed that the depth of fiber insertion into the injection port of the GC had an influence on the amount of analyte desorbed from the fiber. With a depth of fiber insertion of 3 cm, analysis was optimal (Figure 4).

Calibration curves, reproducibility, and limits of detection

The calibration curves showed a linear relationship at a concentration range from 0.025 to 5 µg/g with a coefficient of correlation from 0.991 to 0.998. The limits of detection for quantitative measurements in the SIM mode were in the range from 0.01 to 0.3 µg/g (Table III), and thus were below or in the concentration ranges described for intoxication (45–48). Using the EI mode, full-scan spectra were achieved for the 10- to 30-fold concentration of the organophosphorous compound present in the biological matrix. The coefficients of variation of the repeatability and the reproducibility determined with spiked blood samples were between 2.9% and 19.3% for repeatability and between 5.1% and 17.9% for reproducibility (Table III). Considering the coefficients of variation for the repeatability and reproducibility tests, the proposed method was found reproducible with the exception of disulfoton (the repeatability was 31.5% and the reproducibility 30.7%). The method was sufficient for a fast screening to discover intoxication with organophosphorous pesticides in clinical emergency analysis and forensic toxicology, which was the main goal of the development of this method.

Absolute recovery

The absolute recovery was calculated by the following formula:

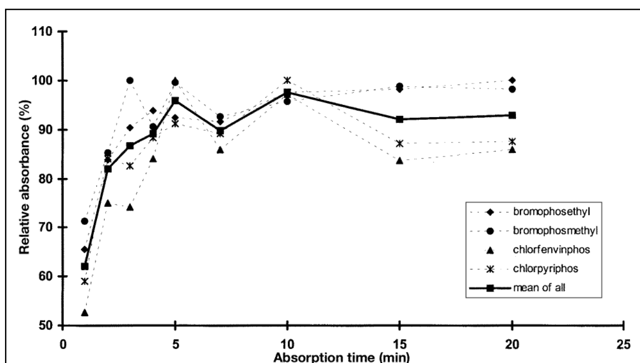

Figure 3. Effect of the absorption time on amounts of organophosphorous pesticides extracted from spiked human blood samples (1 mg/kg). Exemplary shown at bromophos-ethyl, bromophos-methyl, chlorfenvinphos, chlorpyriphos, and the mean of all organophosphorous pesticides.

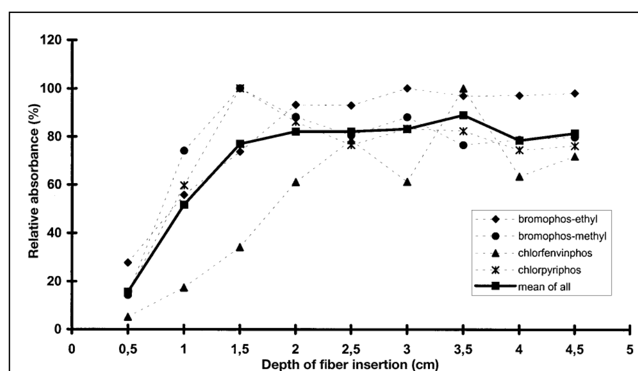

Figure 4. Effect of the depth of fiber insertion into the injector of the GC on amounts of organophosphorous pesticides extracted from spiked human blood samples (1 mg/kg). Exemplary shown at bromophos-ethyl, bromophos-methyl, chlorfenvinphos, chlorpyriphos, and the mean of all organophosphorous pesticides.

Table III. Validation data

		Intraday precision			Interday precision					
Substances	Spiked (µg/g)	Found (µg/g)	SD* (µg/g)	CV† (%)	Found (µg/g)	SD (µg/g)	CV (%)	R‡ calibration	LOD§ (µg/g)	Absolute recovery** (%)
Bromophos-ethyl	1	1.23	0.23	19.3	1.21	0.21	17.9	0.997	0.04	8.2
Bromophos-methyl	1	1.09	0.06	5.5	1.16	0.11	9.9	0.991	0.05	9.7
Chlorfenvinphos	1	1.09	0.06	5.3	1.10	0.07	5.9	0.998	0.05	1.7
Chlorpyriphos	1	1.20	0.14	11.4	1.29	0.21	16.8	0.994	0.03	8.3
Diazinon	1	1.10	0.06	5.0	1.19	0.11	9.4	0.991	0.01	2.9
Dichlorvos	1	1.32	0.20	15.4	1.37	0.23	17.6	0.991	0.10	0.1
Disulfoton	1	1.15	0.36	31.5	1.15	0.35	30.7	0.997	0.01	3.8
Edifenphos	1	1.28	0.14	11.5	1.29	0.15	12.3	0.987	0.30	10.6
Fenthion	1	1.13	0.04	3.7	1.36	0.07	5.7	0.995	0.01	2.7
Malathion	1	1.21	0.04	3.3	1.25	0.05	5.1	0.998	0.05	6.6
Methidathion	1	1.14	0.03	2.9	1.19	0.05	4.7	0.998	0.20	1.3
Mevinphos	1	1.25	0.07	6.3	1.31	0.09	7.8	0.992	0.10	0.1
Parathion-ethyl	1	0.98	0.04	4.1	0.97	0.07	6.9	0.993	0.02	4.7
Parathion-methyl	1	0.97	0.09	9.3	0.96	0.11	11.4	0.996	0.03	19.6
Quinalphos	1	1.05	0.10	9.5	1.06	0.12	11.0	0.998	0.01	1.3

* SD, standard deviation.
† CV, coefficient of variation.
‡ R, correlation coefficient.
§ LOD, limit of detection.
** The absolute recovery was determined by comparing the peak areas of the compounds of interest extracted by SPME (0.5 mL blood spiked with 500 ng of each organophosphorous pesticide) and those of a methanolic solution (10 ng/2 µL).

$$\%\text{Recovery} = \frac{(\text{Peak-area SPME} \times 100)}{(\text{Peak-area liquid injection} \times 50)} \quad \text{Eq. 1}$$

The results are shown in Table III.

The following organophosphorous pesticides could not be detected using the procedure described: demethon-*S*-methyl-sulfon, dicrotophos, dimethoate, fenitrothion, monocrotophos, omethoate, and phosphamidon. It has to be proven if a fiber with another coating is able to absorb these organophosphorous pesticides.

Poisoning by organophosphorous pesticides can occur in various situations, because on the one hand they are still widely used in commercial and domestic agriculture and on the other they are common for suicide. Conventional methods for the determination of such compounds require special sample cleanup such as liquid–liquid extraction (8,15), SPE (7,8,12,18), or liquid–liquid extraction using Extrelut columns (14, 17). These methods are labor-intensive, time-consuming, need large amounts of organic solvents, and as a result of matrix effects are often useless because they cause analyte loss during the preparation of the samples. Even with the most careful cleanup it is nearly impossible to achieve absolute clean samples, thus often impurity peaks are found in chromatograms that can sometimes cover the real peaks. With the HS-SPME method no matrix compounds are carried onto the GC column, thus the chromatograms did not show any interference. Another advantage was that the duration of life of the GC columns and the liners was increased and the need to clean the ion source was decreased, because they were not burdened with nonrelevant substances.

Conclusion

In conclusion, the described method is applicable for a large number of organophosphorous pesticides to get rapid information about a possible intoxication with these substances. HS-SPME in combination with GC–MS is a simple and rapid method for the determination of organophosphorous pesticides in human blood. In the last 12 months of using the described procedure, four cases of intoxication were detected that involved parathion-ethyl twice, malathion (Figure 5), and bromophos-ethyl.

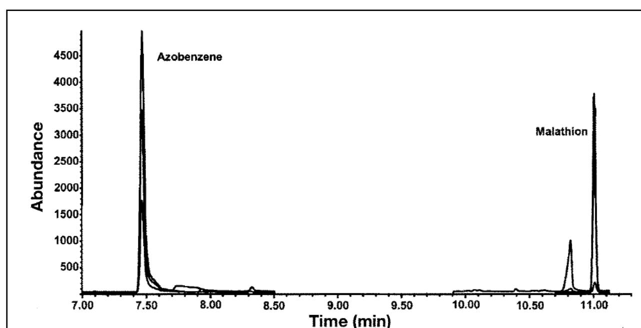

Figure 5. SIM chromatogram of a blood sample of a person intoxicated with malathion (1.13 µg/g). The ion traces are m/z 105, 152, and 182 (the target ion) for azobenzene and m/z 127, 173 (the target ion), and 285 for malathion.

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Manuscript accepted September 28, 2001.