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Short communication

Application of high-performance thin-layer chromatography for the detection of organophosphorus insecticides in human serum after acute poisoning

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Abstract

We developed a rapid and simple method for identifying 25 commonly used organophosphorus insecticides in human serum using high-performance thin-layer chromatography (HPTLC). These organophosphates were separated on plates with three different developing systems within 6–18 min and detected by means of ultraviolet radiation and coloring reactions with 4-(4-nitrobenzyl)pyridine-tetraethylenepentamine reagent (NT reagent) or palladium chloride reagent ($PdCl_2$ reagent). Each organophosphate was accurately identified by means of the $R_F \times 100$ value and the spot color in three systems. The detection limits of dichlorvos, fenitrothion, malathion, methidathion, parathion and trichlorfon in serum by the liquid–liquid extraction method were 1.1, 0.12, 0.12, 0.05, 0.6 and 0.1 μg/ml, respectively. These sensitivities may be sufficient to detect those organophosphates in patient serum after acute poisoning.

Keywords: Organophosphorus insecticides; Insecticides

1. Introduction

Various kinds of organophosphates are widely used as excellent insecticides. The easy availability and rapid action of these compounds are the main reasons for their use in suicides. The various acute toxicities of organophosphates are achieved by inhibiting cholinesterase activity. The antidotes for organophosphate poisoning are atropine and pralidoxime (2-PAM), a cholinesterase reactivator. However, pralidoxime is not equally useful against all organophosphates; e.g., it is most effective in poisoning caused by parathion [1]. Thus, the rapid identification of the causal insecticides would provide very useful information to clinicians for making treatment decisions in suicide-attempt emergencies.

Among various chromatographic methods, high-performance thin-layer chromatography (HPTLC) is comparatively simple, rapid and convenient for identifying many chemicals. We previously reported a rapid and sensitive method for the detection of benzodiazepines and zopiclone in serum using HPTLC [2]. There have been a number of reports on identification of organophosphates using thin-layer chromatography (TLC) (e.g., [3]). However, there are few reports on simple screening methods of organophosphorus insecticides by HPTLC [4]. Here

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we show a simple and rapid HPTLC method for identifying 25 commonly used organophosphates in human serum in poisoning emergencies. In this study, we decided to use a combination of three developing solvent systems from various systems used in TLC of organophosphates in order to obtain the most excellent discriminating power.

2. Experimental

2.1. Apparatus and reagents

The HPTLC system consisted of a CAMAG horizontal development chamber (Muttenz, Switzerland), silica gel 60 F254 HPTLC plates (Merck Art. 5628, 10×10 cm) and RP-18 WF254S HPTLC plates (Merck Art. 13124, 10×10 cm) (E. Merck, Darmstadt, Germany). The solid-phase extraction system consisted of C_{18} Bond Elut columns (50 mg capacity; Varian, Harbor City, CA, USA) and the VAC ELUT SPS 24 (Analytichem International, Harbor City, CA, USA). The gas chromatography (GC) system consisted of a GC-14B (Shimadzu) connected to a flame photometric detection (FPD) instrument, a DB-1 capillary column (15×0.53 mm, 1.5 mm; J&W Scientific, Folsom, CA, USA), and a Chromatopac C-R6A data processor (Shimadzu, Kyoto, Japan). Drug-free lyophilized serum was purchased from Bio-Rad (Anaheim, CA, USA) to prepare the control and standard organophosphate sera. Pirimiphos-methyl was obtained from Imperial Chemical Industries (Blacknell, UK), pyridafenthion was from Sumitomo (Osaka, Japan), and the other organophosphates and all other chemicals were from Wako (Osaka, Japan).

2.2. Standard organophosphate serum preparation

Stock solutions of organophosphate solutions were prepared in a concentration of 10 μg/ml in acetone. To prepare the standard drug serum, 0.1 ml of the stock solution was transferred into a test tube and evaporated under a stream of nitrogen gas at room temperature. The residue was reconstituted in 1 ml of drug-free serum, and further diluted with the drug-free serum if necessary.

2.3. Extraction

Both the solid–liquid and liquid–liquid methods were performed to extract organophosphates from serum. In the former method, the C_{18} Bond Elut column was pretreated by rinsing with 2 ml of methanol and 2 ml of water. The standard serum (1 ml) was directly applied to the Bond Elut column followed by washing with 2 ml of water. Each organophosphate was collected into a conical tube by eluting with 1 ml of *n*-hexane–ethyl acetate (75:25, v/v). The eluents were evaporated to dryness at room temperature under a stream of nitrogen gas.

In the liquid–liquid extraction, 1 ml of saturated NaCl solution was added to the standard serum (1 ml), and the mixture was shaken with 3 ml of *n*-hexane–ethyl acetate (75:25, v/v) for 10 min. After centrifugation at 1800 *g* for 5 min, the organic phase was transferred into a glass tube and evaporated to dryness at 25°C in vacuo.

The residue was reconstituted in 10 μl of acetone, and a 1-μl aliquot was applied 1 cm from the side of the HPTLC plate using a 1 μl Hamilton microsyringe (Hamilton, Reno, NV, USA). The drug-free serum was used as a blank.

2.4. Development

We selected the following three developing solvent systems on the basis of their discriminating power: system 1, *n*-hexane–acetone (80:20, v/v) [5]; system 2, toluene [3]; system 3, methanol–water (70:30, v/v) [6]. Silica gel 60 F254 HPTLC plates were used for systems 1 and 2, and RP-18 WF254S HPTLC plates were used for system 3. The plates were developed in a sandwich configuration in CAMAG development chambers, from both sides towards the middle of the plate, and then dried at room temperature. The migration distance was 5 cm.

2.5. Detection

The separated spots on the plates were first placed under UV radiation at the wavelength of 254 nm. Next, each plate was treated with 4-(4-nitrobenzyl)pyridine-tetraethylenepentamine reagent (NT reagent) or palladium chloride reagent ($PdCl_2$ reagent) to visualize the spots, and their R_F values

were measured. The irradiated plate was sprayed heavily with 2% 4-(4-nitrobenzyl) pyridine in acetone. After acetone was evaporated, the plate was placed in 110 for 5 min and sprayed lightly with 10% tetraethylenepentamine acetone solution until the maximum spot intensity was obtained. Alternatively, the irradiated plate was sprayed with a 0.5% solution of $PdCl_2$ in 10% HCl, dried in the air and kept on heating.

2.6. Recovery test using gas chromatography

The recovery of the extraction procedure was determined using gas chromatography (GC). Helium was used as both the carrier gas (21–22 ml/min) and the make-up gas (20 ml/min). The flow-rates of air and hydrogen gas were 11.9 ml/min (80 kPa) and 3.1 ml/min (60 kPa), respectively. The GC injection and detector temperatures were both 240°C. The initial column temperature was 150°C; it was increased by 5°C/min to 173°C, by 3°C/min to 200°C, and finally by 7°C/min to 240°C (3 min). The organophosphate concentration was calculated from the peak heights obtained from the internal standard (parathion for salithion, and salithion for the others) and the extraction sample.

3. Results and discussion

Organophosphates were developed and separated on the HPTLC plates within 6–18 min in the three systems. Table 1 shows the $R_F \times 100$ values of 25 organophosphates in each HPTLC system. These $R_F \times 100$ values were similar to those reported by TLC [3–6]. The mean values for each standard deviation (S.D.) of the $R_F \times 100$ values of the 25 organophosphates examined in systems 1, 2 and 3

Table 1
$R_F \times 100$ values and S.D. of organophosphates in three development systems

Compound	System 1		System 2		System 3	
	$R_F \times 100$	S.D.	$R_F \times 100$	S.D.	$R_F \times 100$	S.D.
Acephate	0	0	0	0	88	1.0
Chlorpyrifos	55	1.3	87	1.1	35	0.8
Chlorpyrifos-methyl	48	1.1	82	0.8	45	1.1
Cyanophos	24	1.5	34	3.1	64	1.5
Diazinon	44	2.5	11	1.9	50	1.6
Dichlofenthion	55	0.6	86	1.3	35	2.9
Dichlorvos	18	1.2	3	0.5	73	1.4
Dimethoate	3	0.3	0	0	78	0.9
Disulfoton	53	2.6	62	2.7	47	0.9
EPN	36	2.5	66	2.4	42	1.5
Ethion	45	1.5	67	1.5	35	2.6
Fenitrothion	30	1.7	56	2.4	58	1.2
Fenthion	38	1.3	64	1.8	51	1.0
Formothion	11	1.1	7	1.6	77	0.9
Isoxathion	36	1.3	49	1.5	47	0.5
Malathion	28	2.1	16	2.4	63	1.4
Methidathion	25	0.9	22	2.5	66	1.0
Parathion	37	1.4	60	1.1	54	1.0
Phenthoate	33	1.2	42	2.2	58	1.8
Phosalone	26	1.9	36	2.4	50	1.0
Phosmet	16	2.3	21	2.0	64	0.7
Pirimiphos-methyl	45	1.7	35	0.9	48	1.3
Pyridafenthion	12	1.0	2	1.0	56	1.4
Salithion	32	1.2	62	1.1	68	0.9
Trichlorfon	0	0.5	0	0	81	0.8

Each $R_F \times 100$ value is the mean of 10 determinations.
Developing agents: System 1, *n*-hexane–acetone (80:20, v/v); system 2, toluene; system 3, methanol–water (70:30, v/v).

were 1.4, 1.7 and 1.2, respectively, suggesting good reproducibility for all three HPTLC systems.

Discriminating power is defined as the probability that two randomly selected substances can be separated in the chromatographic system. The values of discriminating power were calculated according to a previous report [7]. When calculated using the error factor of 10, the values of the discriminating powers were 0.683, 0.817 and 0.647 in systems 1, 2 and 3, respectively. The effective discriminating power for HPTLC analysis of organophosphate was given by the combination of systems 1 and 2 (0.943) or systems 2 and 3 (0.940). The value for all chromatographic systems combined was 0.960, suggesting that the present method is useful for detecting and identifying various organophosphorus insecticides.

The colors of these organophosphates by the three detection systems are shown in Table 2. The intrinsic responses to UV radiation and the coloring reactions with the NT and $PdCl_2$ reagents were different among the organophosphates. It has been reported that NT reagent is equally sensitive for thio- and nonthio-organophosphorus insecticides [8], and that its detection limit is 0.03–20 μg per spot for organophosphates on TLC [5]. Except for diazinon and EPN, all of the organophosphates examined showed blue spots. Twelve organophosphates were not detected by UV radiation. Dichlorvos and trichlorfon were not detected by the reaction with $PdCl_2$ reagent, because these organophosphates are nonthio-organophosphates [3,6].

The detection limits (the lowest concentration at which the spot was visible on the plate) of HPTLC for the organophosphates are shown in Table 3. The detection limits of UV radiation for the standards in acetone were 10–100 ng per spot. The detection limits of NT reagent reaction for the 25 organophosphates were 5–300 ng per spot, which were lower than those of the $PdCl_2$ reagent reaction except for disulfoton. The present HPTLC is thus superior in sensitivity to conventional TLC reported previously [5]. In addition, since the developed spots demonstrated strong color in proportion to the quantity of organophosphate spotted, the quantitative estimation can be made using densitometry.

The recovery ratios of important organophosphates from serum during the extraction procedure are shown in Table 4. Dichlorvos and trichlorfon in the solid-phase extraction showed recovery ratios lower than those in the liquid–liquid extraction. Because dichlorvos and trichlorfon are soluble in water, this difference may be due to the loss during washing with water in the solid-phase extraction method. The detection limits of dichlorvos, fenitrothion, malathion, methidathion, parathion and trichlorfon in serum were 1.1, 0.12, 0.12, 0.05, 0.6 and 0.1 μg/ml in the liquid–liquid extraction method, respectively. These sensitivities may be sufficient to detect these organophosphates in patient serum after acute poisoning.

The HPTLC system yielded a better separation and more rapid development than conventional TLC. We found that HPTLC is more sensitive and discriminatory than conventional TLC in detecting organophosphorus insecticides. This method would

Table 2
Colors of organophosphates developed by the HPTLC system

Compound	Detection method		
	UV	NT	Pd
Acephate	neg.[1]	Blue	Brown
Chlorpyrifos	neg.	Blue	Pale brown
Chlorpyrifos-methyl	neg.	Blue	Pale brown
Cyanophos	Pale gray	Blue	Pale brown
Diazinon	Gray	Pale red	Brown
Dichlofenthion	neg.	Pale blue	Pale brown
Dichlorvos	neg.	Blue	neg.
Dimethoate	neg.	Blue	Brown
Disulfoton	neg.	Pale blue	Brown
EPN	Gray	Yellow	Brown
Ethion	neg.	Blue	Brown
Fenitrothion	Gray	Blue	Pale brown
Fenthion	Gray	Blue	Brown
Formothion	neg.	Blue	Brown
Isoxathion	Gray	Blue	Pale brown
Malathion	neg.	Blue	Brown
Methidathion	Gray	Blue	Brown
Parathion	Gray	Blue	Pale brown
Phenthoate	Pale gray	Blue	Brown
Phosalone	Pale gray	Blue	Brown
Phosmet	Gray	Blue	Brown
Pirimiphos-methyl	Blue–gray	Blue	Pale brown
Pyridafenthion	Gray	Blue	Brown
Salithion	neg.	Blue	Brown
Trichlorfon	neg.	Blue	neg.

Detection method: UV, UV radiation (254 nm); NT, reaction with 4-(4-nitrobenzyl) pyridine-tetraethylenepentamine reagent; Pd, reaction with palladium chloride reagent.
[1] neg.: Fluorescence or color not observed.

Table 3
Detection limits of organophosphates

Compound	Detection limit in acetone solution (ng/spot)		
	UV	NT	Pd
Acephate	neg.[a]	10	50
Chlorpyrifos	neg.	30	100
Chlorpyrifos-methyl	neg.	5	100
Cyanophos	100	5	100
Diazinon	30	50	50
Dichlorvos	neg.	30	neg.
Dichlofenthion	neg.	100	100
Dimethoate	neg.	5	10
Disulfoton	neg.	300	50
EPN	30	50	50
Ethion	neg.	50	30
Fenitrothion	50	10	100
Fenthion	10	5	50
Formothion	neg.	5	50
Isoxathion	30	10	100
Malathion	neg.	10	50
Methidathion	50	5	30
Parathion	50	50	100
Phenthoate	100	5	50
Phosalone	100	10	50
Phosmet	50	5	30
Pirimiphos-methyl	30	30	100
Pyridafenthion	50	50	50
Salithion	neg.	10	50
Trichlorfon	neg.	5	neg.

Detection method: UV, UV radiation (254 nm); NT, reaction with 4-(4-nitrobenzyl) pyridine-tetraethylenepentamine reagent; Pd, reaction with palladium chloride.
[a] neg.: Not detected by 300 ng.

permit an easy detection and identification of organophosphates in insecticide poisoning emergencies, and provide a helpful information on pralidoxime therapy to physicians.

References

[1] Drug Evaluations, American Medical Association, Philadelphia, PA, 6th ed., 1989, pp. 66–67.
[2] K. Otsubo, H. Seto, K. Futagami, R. Oishi, J. Chromatogr. B 669 (1995) 408.
[3] S.N. Tewari, S.P. Harpalani, J. Chromatogr. 130 (1977) 229.
[4] C. Marutoiu, M. Vlassa, C. Sarbu, S. Nagy, J. High Resolut. Chromatogr., Chromatogr. Commun. 10 (1987) 465.
[5] H. Mori, T. Sato, H. Nagase, Y. Sakai, S. Yamaguchi, Y. Iwata, R. Hashimoto, Jpn. J. Toxicol. Environ. Health 40 (1994) 101.
[6] N. Tsunoda, Jpn. J. Toxicol. Environ. Health 32 (1986) 447.
[7] A.H. Stead, R. Gill, T. Wright, J.P. Gibbs, A.C. Moffat, Analyst 107 (1982) 1106.
[8] R.R. Watts, J. Assoc. Off. Agr. Chemists. 48 (1965) 1161.

Table 4
Recovery ratios of important organophosphates with solid-phase and liquid–liquid extraction methods ($n=5$)

Compound	Solid-phase extraction		Liquid–liquid extraction	
	Recovery (%)	C.V. (%)	Recovery (%)	C.V. (%)
Dichlorvos	27.6	14.4	63.8	16.0
Fenitrothion	86.0	4.0	94.5	3.9
Malathion	84.5	3.3	95.8	2.3
Methidathion	101.8	2.9	101.3	4.4
Parathion	82.2	1.3	85.5	4.2
Trichlorfon	67.2	10.1	101.5	6.1