SPECIAL GUEST EDITOR SECTION

High-Performance Thin-Layer Chromatographic Analysis of Selected Organophosphorous Pesticide Residues in Tea

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The separation of 9 organophosphates (monocrotophos, quinalphos, triazophos, parathion-methyl, isofenphos-methyl, temephos, parathion, phoxim-ethyl, and chlorpyrifos) by high-performance thin-layer chromatography (HPTLC) with automated multiple development was studied. The HPTLC method was developed and validated for analysis of residues of phoxim-ethyl and chlorpyrifos in tea. The sample was extracted with acetonitrile and cleaned up by ENVI-CARB solid-phase extraction. The extract was directly applied as bands to glass-backed silica gel $60F_{254}$ HPTLC plates. The plates were developed with dichloromethane–hexane (1 + 1, v/v) in a glass twin-trough chamber. Evaluation of the developed HPTLC plates was performed densitometrically. The results indicated that the detection limits of phoxim and chlorpyrifos were 5.0×10^{-9} and 1.0×10^{-8} g, respectively. Recoveries of the pesticides from tea by this analytical method were 90.7–105.5%, and relative standard deviations were 7.3–13.5%. The precision and accuracy of the method were generally satisfactory for analysis of pesticide residues in tea.

Organophosphorous pesticides, which are widely used in agricultural practice, have adverse health implications, even at trace levels. These compounds are commonly found in agricultural products, mainly due to their use in the past but also due to the ongoing use of some of them in agricultural activities. Most reports of the analysis of organophosphorous pesticide residues in plant samples involved the use of gas chromatography (GC), high-performance liquid chromatography (HPLC), and biological enzyme methods (1–14). Tea is a plant matrix rich in natural constituents and may cause interference in the determination procedure. In many cases, searching for a fast and efficient method for enrichment and determination of organophosphorous residues from complex matrixes is still a challenge for many researchers.

In many analytical situations, modern quantitative high-performance thin-layer chromatography (HPTLC), when properly performed by well-trained analysts, has many advantages over HPLC or GC. These advantages include simplicity of operation, the availability of many sensitive and selective reagents for detection and confirmation without interference from the mobile phase, the ability to repeat detection and quantification at any time with changed conditions because fractions representing the entire sample are stored on the plate, in-system calibration for quantitative analysis, and cost-effectiveness because many samples can be analyzed on a single plate with low solvent usage.

In this study, we used the automated multiple development (AMD)–HPTLC and twin-trough chamber ascending developing systems to separate the organophosphates and to establish a method for determination of selected organophosphate pesticide residues in tea.

Experimental

Materials and Reagents

The dry tea was collected from the Yuexi tea factory in Anhui province, People's Republic of China (PRC). The samples were stored at 4°C in air-tight containers and ground to 40 mesh when required.

All the chemicals used in the experiments were of analytical grade.

The standards of monocrotophos (99.0%), quinalphos (99.0%), triazophos (99.0%), parathion-methyl (97.5%), isofenphos-methyl (90.9%), and temephos (99.0%) were provided by National Pesticide Quality Inspection Center (Beijing, PRC). Parathion (97.0%) and phoxim (97.0%) were provided by the Tianjin Pesticide Company Ltd (Tianjin, PRC). Chlorpyrifos (99.0%) was provided by the Zhejiang Xianju Pesticide Factory (Xianju, PRC).

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Table 1. Component proportions for each automated multiple development step in the 3 mobile phases systems

		System 1[a]			System 2[a]			System 3[a]			
Steps	Migration, mm	A	B	C	A	B	C	A	B	C	D
1	12	100			40	60		45	55		
2	14	90	10		35	65		35	65		
3	16	80	20		30	70		30	70		
4	18	70	30		25	75		25	75		
5	20	60	40		20	80		20	80		
6	22	50	50		15	85		15	85		
7	24	40	60		10	90		10	90		
8	26	30	70		5	95		5	95		
9	28	20	80			80	20		90		10
10	30	10	90			80	20		80	10	10
11	32		100			80	20		70	20	10
12	34		90	10		70	30		70	25	5
13	36		80	20		60	40		60	35	5
14	38		70	30		50	50		50	45	5
15	40		60	40		40	60		45	55	
16	42		50	50		30	70		35	65	
17	44		40	60		25	75		25	75	
18	46		30	70		20	80		20	80	
19	48		20	80		15	85		15	85	
20	50		10	90		10	90		10	90	
21	52						100		5	95	
22	54						100			100	
23	56						100				

[a] A = Acetone, B = dichloromethane, C = *n*-hexane, D = *t*-butyl methyl ether; all proportions are percentages.

Table 2. R_f values in different developing systems using automated multiple development and maximum absorption wavelengths

Pesticide	System 1	System 2	System 3	Wavelength, nm
Monocrotophos	0.35	0.20	0.25	221
Quinalphos	0.41	0.35	0.51	244
Triazophos	0.44	0.42	0.56	200
Isofenphos-methyl	0.50	0.50	0.61	200
Temephos	0.54	0.53	0.65	200
Parathion	0.58	0.55	0.71	287
Parathion-methyl	0.60	0.57	0.72	287
Phoxim	0.62	0.60	0.74	292
Chlorpyrifos	0.71	0.72	0.85	200

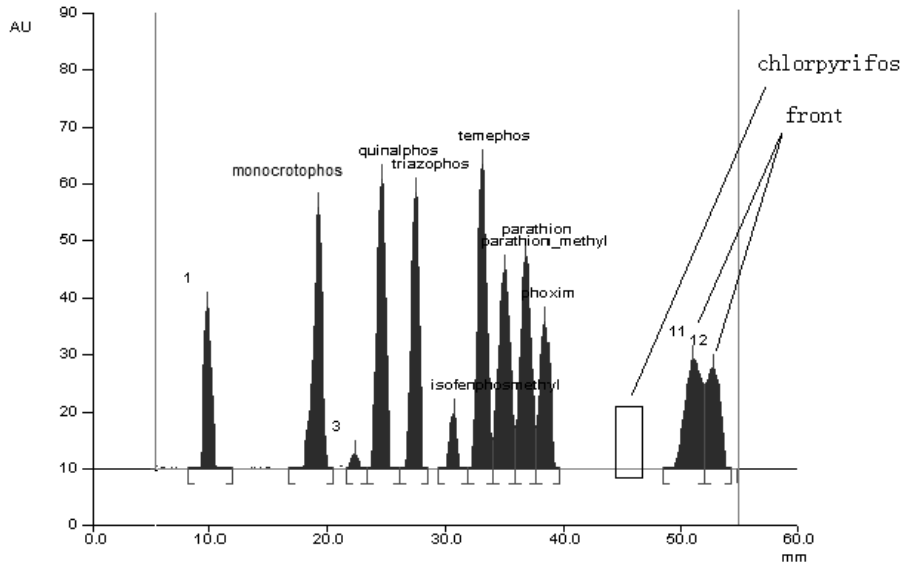

Figure 1. Chromatogram obtained using automated multiple development with system 3 (Table 1) scanned at 240 nm. Chlorpyrifos peak would appear at 202 or 280 nm. Peaks 1, 3, 11, and 12 are impurities.

Apparatus

(**a**) *Application device.*—Linomat 4 sample band applicator (CAMAG, Muttenz, Switzerland).

(**b**) *Syringe.*—100 µL (Hamilton, Bonaduz, Switzerland).

(**c**) *HPTLC chamber.*—Glass twin-trough chamber (20 × 10 × 4 cm; CAMAG).

(**d**) *AMD instrument.*—AMD 2 (CAMAG).

(**e**) *Densitometer.*—TLC Scanner 3 linked to winCATS software (CAMAG).

(**f**) *HPTLC plates.*—20 × 10 cm, 0.2 mm layer thickness, precoated with silica gel 60 F_{254} (Merck KGaA, Darmstadt, Germany).

(**g**) *Solid-phase extraction (SPE) columns.*—Supelclean ENVI-Carb, 3 mL (Supelco, Bellefonte, PA).

Preparation of Standard Solutions and Separation and Scanning of the Standards

(**a**) *Standard solutions.*—Standard solutions were prepared by dissolving 5 mg of accurately weighed standard

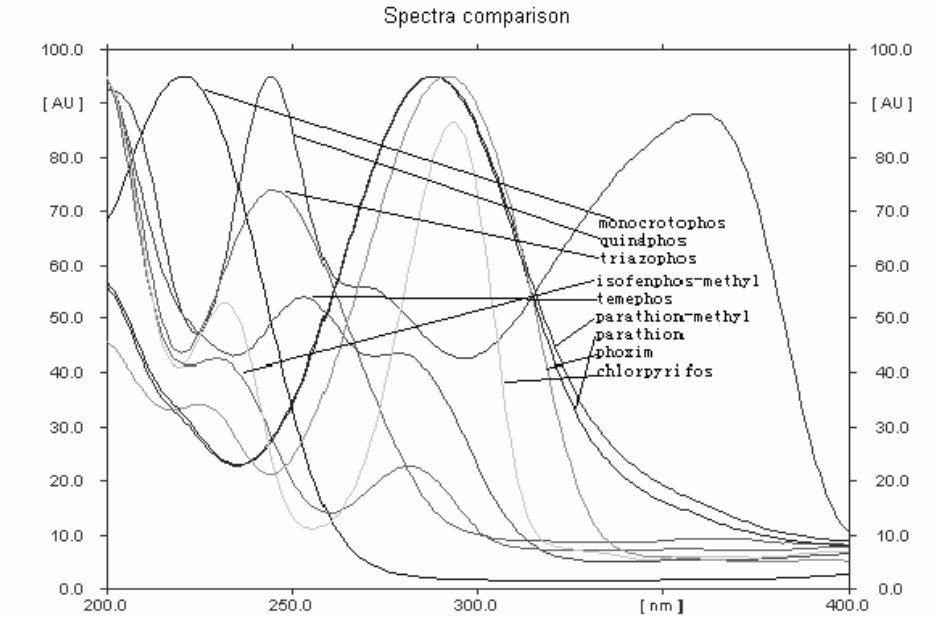

Figure 2. Spectra of the 9 organophosphates.

Table 3. Calibration data and method validation parameters for the quantification of 6 pesticides by HPTLC with twin-trough chamber development

Pesticide	Regression equation[a]	r	RSD of residuals[b]	LOD, ng[c]	LOQ, ng[d]	Instrument precision (CV), %[e]	Standard repeatability (CV), %[e]	Sample repeatability (CV), %[e]	Working range, ng
Phoxim	$Y = 0.647X + 5.859$	0.9969	3.12	5	10	0.89	1.61	1.08	10–200
	$Y = 3.448 + 0.391X - 0.000X^2$	0.9990	3.62	10	30	1.21	1.38	2.68	15–200
Chlorpyrifos	$Y = 0.3697X - 0.5131$	0.9914	3.72	10	30	1.63	1.33	1.85	30–250
	$Y = -2.029 + 0.358X - 0.001X^2$	0.9988	1.67	20	60	2.20	1.68	2.96	60–250
Quinalphos	$Y = 35.725 + 0.637X - 0.001X^2$	0.9992	2.13	5	10				15–200
				10	30	0.97	1.01	2.09	30–200
Triazophos	$Y = 3.112 + 0.547X - 0.001X^2$	0.9996	1.91	7	20				20–200
				15	50	1.02	1.34	1.94	15–200
Isofenphos-methyl	$Y = 6.232 + 0.282X - 0.000X^2$	0.9981	3.80	5	10				15–200
				10	30	0.868	1.25	1.55	30–200
Temephos	$Y = 4.575 + 0.749X - 0.001X^2$	0.9997	1.66	3	8				10–200
				8	20	1.66	1.52	1.11	8–150

[a] X = Amount of pesticide (ng), and Y = peak area.
[b] RSD = Relative standard deviation.
[c] LOD = Limit of detection.
[d] LOQ = Limit of quantitation.
[e] CV = Coefficient of variation; n = 6, 100 ng/spot.

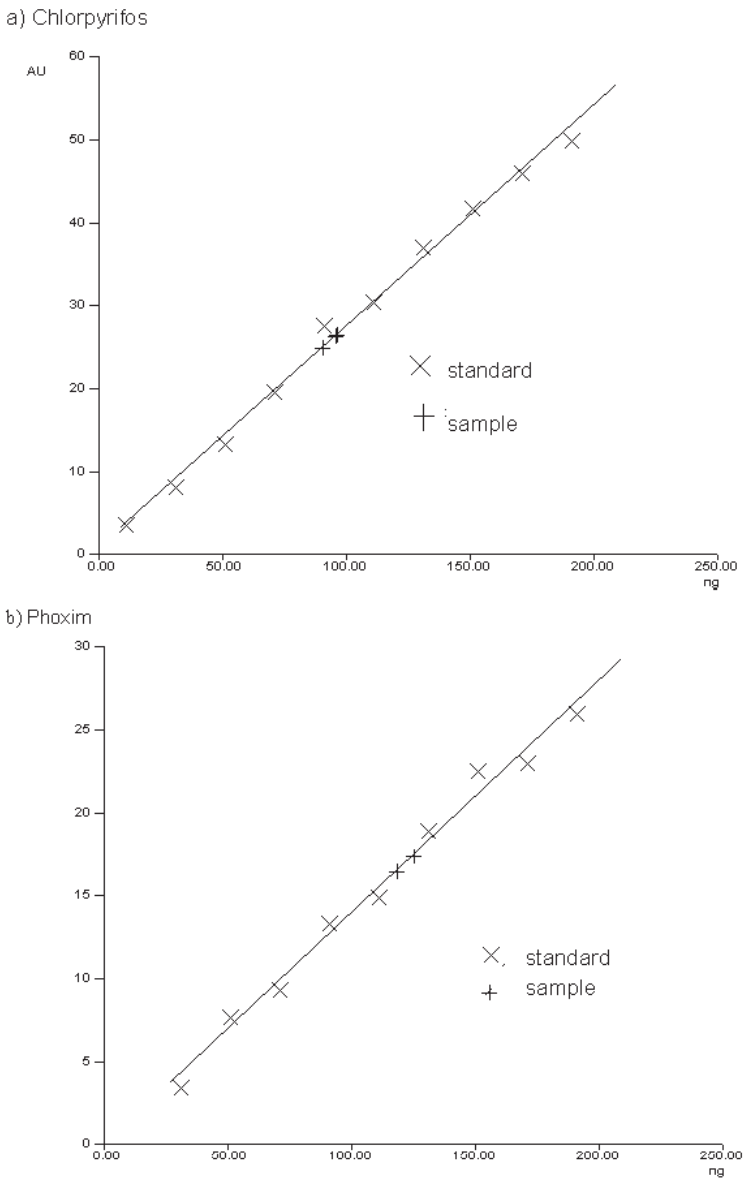

Figure 3. Calibration graphs for chlorpyrifos and phoxim.

Table 4. Validation data for the analysis of tea fortified with 1 mg/kg of the organophosphates

	Intraday recovery, % (n = 3)[a]								Recovery, %	
Pesticide	Day 1	CV, %	Day 2	CV, %	Day 3	CV, %	Interday recovery, % (n = 3)[b]	CV, %	High	Low
Phoxim	105.46	13.53	99.22	15.42	104.58	11.89	103.89	5.99	110.96	99.22
Chlorpyrifos	102.93	11.9	85.63	10.78	88.91	8.92	87.00	4.81	90.2	82.26
Quinalphos	87.8	7.94	90.7	9.60	109.10	6.57	89.20	20.48	109.10	73.2
Triazophos	110.22	13.53	105.46	17.64	91.21	8.94	100.04	9.01	110.21	93.03
Isofenphos-methyl	106.21	7.28	94.50	18.33	110.60	15.67	99.01	10.33	110.80	92.58
Temephos	98.36	6.28	88.60	12.97	72.02	10.34	85.02	14.03	98.36	75.38

[a] Samples analyzed 3 times each day.
[b] Samples analyzed on 3 consecutive days.

Table 5. Recoveries of 2 organophosphates at 3 levels of tea fortification

Pesticide	Level, mg/kg	Volume applied, μL	Recovery, %	CV, %
Phoxim	0.1	50	90.7	7.94
	0.4	20	105.46	13.53
	4.0	5	94.50	7.28
Chlorpyrifos	0.1	50	101.60	6.28
	0.4	20	102.93	11.89
	4.0	5	85.63	4.24

in methanol in a 5 mL volumetric flask. Each standard solution was then diluted 10-fold with 0.5 mL diluted to 5 mL. Stepwise dilution with the same solvent yielded solutions containing 1×10^{-5} and 1×10^{-6} g/mL. The proper volumes of each solution were combined to make a mixed standard solution when required.

(**b**) *Twin-trough chamber ascending development*.—The plates were developed in a twin-trough chamber with the mobile phase dichloromethane–hexane (1 + 1, v/v). The migration distance was 5 cm.

(**c**) *AMD development*.—The plates were developed for 21 steps with the mobile phase systems (Table 1) using AMD at 16 ± 2°C and 45 ± 10% relative humidity for a distance of 4 cm.

(**d**) *Scanning*.—The spots were scanned at 254 nm first; then the spectrum was scanned from 200 to 400 nm to find the maximum absorption wavelength.

Preparation of Sample Solutions

(**a**) *Sample solutions for AMD development*.—Dried powdered tea (2.5 g) was spiked by adding the mixed standard solution to furnish tea containing 0.1, 0.4, and 4.0 mg/kg phoxim and chlorpyrifos. Then the samples were balanced for 1 h and shaken for 1 min at intervals of 10 min to allow the spike solution to penetrate into the matrix.

(**b**) *Extract*.—The samples were shaken in a vibrator for 1 h after adding 30 mL acetonitrile (ACN). They were filtered before concentration to 1 mL. The SPE column was pretreated by rinsing with 3 mL petroleum ether–ACN (2 + 1, v/v). The extract was applied to it followed by 3 mL ACN. The eluate was collected and evaporated to dryness at room temperature under a stream of nitrogen gas. The residue was reconstituted in 1.0 mL methanol.

Procedures

(**a**) *Calibration graphs*.—Eight levels of each phoxim and chlorpyrifos standard solution were applied on an HPTLC plate. After development, the plate was dried in air. The peak areas were recorded, and calibration graphs were prepared by plotting peak areas vs the amount of pesticides.

(**b**) *Validation of the method*.—International Conference on Harmonization (ICH) guidelines (CPMP/ICH/381/95; CPMP/ICH/281/95) were followed for the validation of the analytical procedure. The method was validated for precision, repeatability, and accuracy. Instrumental precision was checked by repeated scanning of the mixture standards (50 ng) 6 times and expressed as coefficient of variation (CV). The repeatability of the method was confirmed by analyzing a 50 ng/spot mixed standard solution after application on the HPTLC plate [number of determinations (n) = 6] and expressed as CV. Variability of the method was studied by analyzing the mixture standards (50, 150, and 200 ng/spot) on the same day (intraday precision) and on different days (interday precision), and the results were expressed as CV. Accuracy of the method was tested by performing recovery studies at 3 spiked levels (0.1, 0.4, and 4.0 mg/kg). The recovery and average recovery were calculated. For the determination of limit of detection (LOD) and limit of quantitation (LOQ), different dilutions of the standard solutions were applied along with methanol as the blank and calculated on the basis of signal-to-noise ratio.

Results and Discussion

Separation of Nine Organophosphates

The mobile phase systems for AMD were composed of acetone, dichloromethane, and hexane (systems 1 and 2), and acetone, *t*-butyl methyl ether (TBME), dichloromethane, and hexane (system 3; Table 1). The R_f values in the different systems and the maximum absorption wavelengths of the organophosphates are given in the Table 2. The resolution using the mobile phase systems without TBME (system 1 or 2) was insufficient compared to system 3 (Figure 1). Most of the target organophosphates in the experiment have a similar structure, and TBME improved separation of the homologous mixture. The relative humidity was found to affect the organophosphate separation using AMD. The results of the separation were satisfactory at a relative humidity of 45 ± 10%. The resolution was insufficient when the relative humidity was >70%, especially for temephos, parathion, and phoxim. The study was conducted in the spring when the average relative humidity varied from 30 to 90%. It is suggested that the plates be put into a desiccator for at least 2 h after sample application when the relative humidity is too high.

The maximum absorption wavelength of each organophosphate is given in Table 2. The spectra of the 9 pesticides are shown in Figure 2.

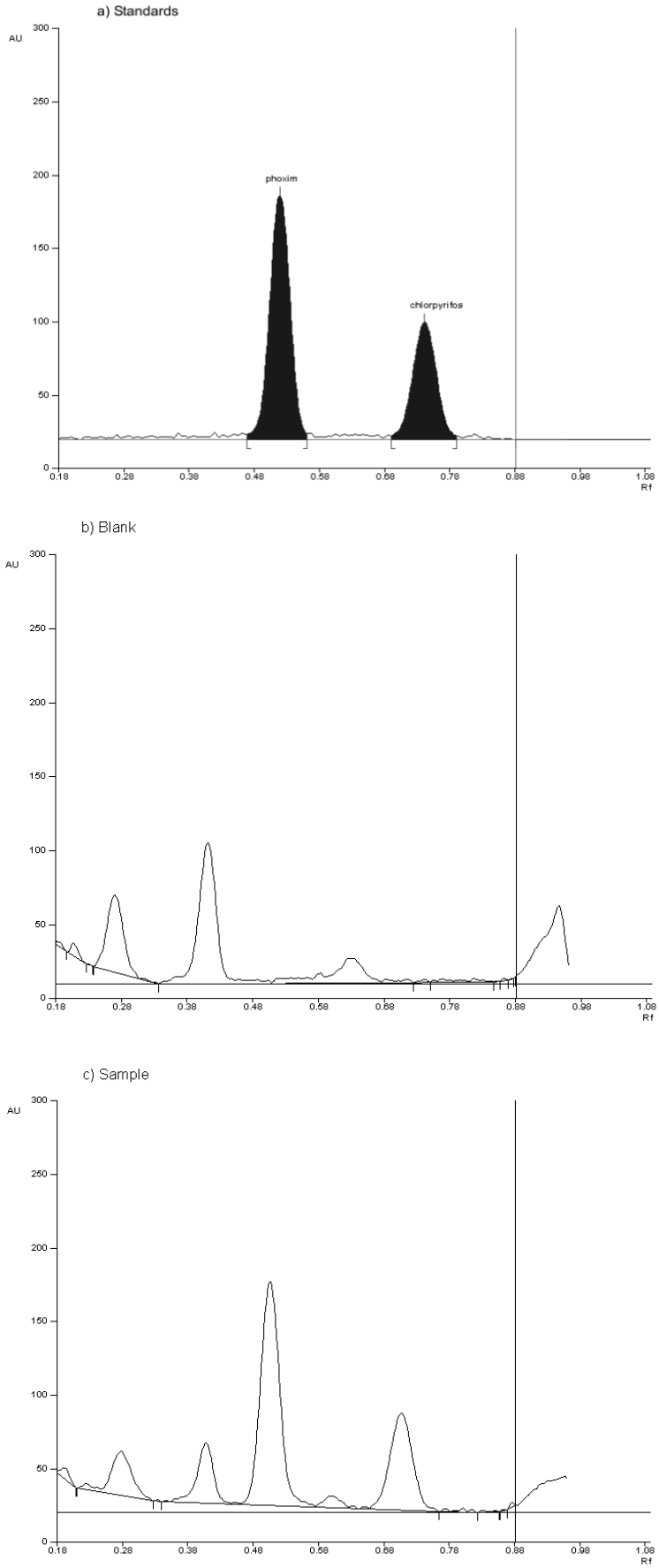

Figure 4. Chromatograms obtained in the determination test using twin-trough chamber ascending development.

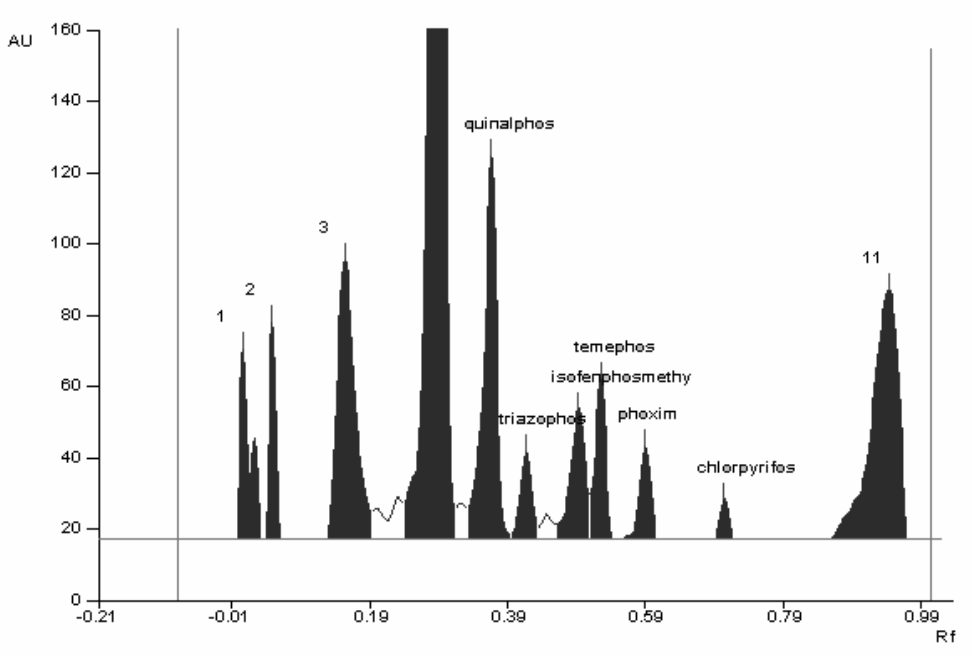

Figure 5. Chromatogram of the sample fortified with 6 organophosphates (1.0 mg/kg) using automated multiple development.

Determination of the Phoxim and the Chlorpyrifos Residues in the Tea

The organophosphates phoxim and chlorpyrifos are often used in a tea garden to control insects. Their residues in the tea were determined using HPTLC with twin-trough chamber ascending development. Calibration and validation data are listed in Table 3 for 6 pesticides. The LOD and LOQ for AMD were higher than with twin-trough chamber ascending development. The background noise of the stationary fluctuated greatly after the 21-step development in the AMD system. This affected the detection limits in the AMD system. Calibration graphs were also obtained (Figure 3a and b). The validation data for the tea fortifications at 0.1–4.0 mg/kg are given in Tables 4 and 5.

The method was suitable for the requirement of the pesticide residue analysis. The separation of the 2 targets and the impurities was complete using the twin-trough chamber with one ascending development step (Figure 4). However, the 6 organophosphates listed in Table 3 were not separated by the twin-trough chamber ascending development. We also tried analyzing the multiresidues of the 6 organophosphates in tea using the AMD system, but any attempts to avoid interferences in the separation were unsuccessful (Figure 5). Fortification levels <1.0 mg/kg could not be determined by the method.

Conclusions

Using HPTLC, we developed a separation method for 9 organophosphates using an AMD system, and an analytical method for 2 organophosphates (phoxim and chlorpyrifos) in tea using a twin-trough chamber ascending development system.

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