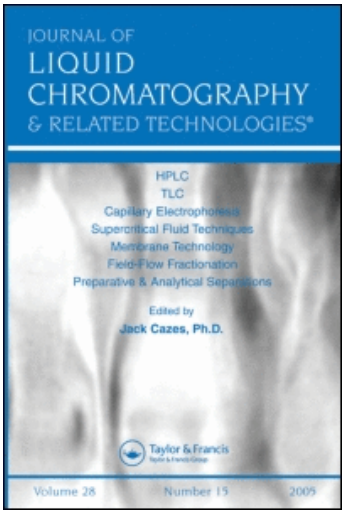

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PRE-COLUMN DERIVATIZATION REACTION COUPLED WITH ULTRASONIC WAVE EXTRACTION FOR THE TRACE ANALYSIS OF ATRAZINE AND SIMAZINE IN SOIL AND CROPS BY LIQUID CHROMATOGRAPHY, ULTRAVIOLET DETECTION

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ABSTRACT

Combined with ultrasonic wave extraction, a pre-column derivatization reaction high performance liquid chromatographic ultraviolet detection procedure was developed for the determination of atrazine and simazine in soil and crop. The 4-(2-phthalimidyl) benzoyl chloride (PIB-Cl) was used as a pre-column derivatization reagent for high performance liquid chromatography (HPLC). The ultrasonic wave technique was applied to the extraction for trace atrazine and simazine in soil and crop. The clean-up and second-time concentration procedures, which was indispensable in the conventional analytical methods for soil and crop analysis because of the complexity of the samples, were replaced by derivatization reaction between PIB-Cl and analytes. The optimum absorption wavelength of 345 nm and the molar absorption coefficients of 10^5 level for the derivatives were found.

The derivatization reaction and chromatographic separation conditions were optimized systematically. The detection limits of 1.1 ng/g for atrazine and 1.0 ng/g for simazine were obtained with the recoveries of 90-95% for soil and crop samples. On the basis of the practical application to 5 soil and 3 crop samples, this method was compared with the conventional GC-MS method. The reliability of this procedure was verified.

INTRODUCTION

Atrazine and simazine are mainly released into the environment by agricultural and industrial processes. As a result, these contaminants end up in soil, crop, and water. In order to detect them at the very low levels encountered in real samples, many analytical methods were developed.[1-4] In recent years, several procedures, using solid-phase extraction (SPE) technique[5, 6] and lamina extraction disks,[7] have been applied for the identification and quantitation of atrazine and simazine in surface, ground, and drinking water. The detection limits at the μg/L, even ng/L level, could be obtained. Less often, methods however, have been found to improve the analytical methods of atrazine and simazine in soil and crop samples because of the complexity of these samples. The enzyme-immunoassay of atrazine and simazine was also mainly developed for water samples.[8-10] Difficulty in optimizing the extraction conditions, when strong interactions occur between the analytes and the matrix, was found for the supercritical fluid extraction (SPE) technique used for real soil samples analysis.[11] No standard analytical method for atrazine and simazine in soil and crop was found in recent EPA sampling and analysis methods. So, the conventional extract, concentration, clean-up, second-time concentration, and gas chromatography nitrogen-phosphorus detector (GC-NPD) or mass spectrometer (MS) detector was often applied to the trace analysis for the soil and crop samples.

The ultrasonic wave extraction (UWE), pre-column derivatization (PCD) high performance liquid chromatography ultraviolet detection (HPLC-UV) procedure, from another point of view, was developed to improve the analytical method. There were three characteristics for this method. The first aspect was low detection limit. The ultraviolet absorption investigation indicated that atrazine and simazine derivatives of PIB-Cl have strong ultraviolet characteristics at the wavelength of 345 nm with the molar absorption coefficients of 1.07×10^5 and 1.21×10^5 L • cm^{-1} • mol^{-1} respectively. The detection limit at ng/g level can be obtained for soil and crop samples. The second aspect is fast analytical procedure for soil and crop samples. UWE technique was applied to replace the conventional oscillation extraction or Soxhlet extraction technique. Moreover, the time-consuming clean-up and second-time concentration processes were replaced by the derivatization reaction because a lot of co-extracts from soil and crop samples no longer

responded at the wavelength of 345 nm. Therefore, the pretreatment time for soil and crop samples was decreased greatly. The third aspect is that only atrazine and simazine can be determined under the detection conditions mentioned in this paper. The other triazine that contains -NH group(s) would be able to be detected if the absorption wavelength is adjusted for them.

In our laboratory, derivatization reagent, 4-(2-phthalimidyl) benzoyl chloride (PIB-Cl) was applied to react with atrazine and simazine for their determination. The derivative reaction and chromatographic separation conditions for atrazine and simazine were investigated and optimized. The repeatability, precision, and recovery for this procedure were examined. The analytical data of five agricultural soils and three crop samples, analyzed by this method, were compared with the conventional GC-NPD method.

EXPERIMENTAL

Apparatus and Materials

The HPLC measurements were carried out on a Shimadzu LC-6A instrument equipped with Shimadzu spectrophotometric SPD-1 detector. The stainless-steel column used (25 cm × 4.6 mm I. D.) was packed with Du Pont ODS chemically bonded phase, particle size 10 μm, and was pre-tested by the manufacturer. The wavelength of 345 nm was selected for all measurements. Acetonitrile-water (75/25, v/v), as mobile phase, is adjusted to pH 6 with 0.1% H_3PO_4 solution. The derivatives were eluted with the mobile phase at a flow-rate of 0.8 mL/min. The molar absorption coefficients for PIB-Cl derivatives of atrazine and simazine were measured on a Shimadzu DU-650 spectrophotometer. The extraction operation for soil and crop was conducted on a BEIJING CX-250 ultrasonic wave device with 40KHZ.

Gas chromatography was carried out on a Hewlett-Packard Model 5700A instrument equipped with Model 18789A NPD and Model 3392A reporting integrator. The chromatographic systems employed were 2.1 m × 4 mm I.D. column packed with 3% OV-1 on 100-120 mesh Gas-Chrom Q; helium flow-rate, 35 mL/min; injection port 250°C; detector 300°C; column 170°C.

Atrazine and simazine were purchased from Ciba-Geigy (Basel, Switzerland). PIB-Cl was synthesized in our laboratory. Milli-Q pure water device was used. All organic solvents were of analytical-reagent grade and purified by redistillation.

Soil samples from an agricultural area (Hebei Province, China) were collected from the surface (top 10-cm freeze-dried and sieved through 120-μm mesh. The composition of the soil was 7-10% clay, 25-35% silt, 58-68% sand,

and 0.88-1.55% organic matter; the pH was 7.8-8.0. For crop samples the whole watermelon plants were collected from an atrazine-accident field (Hebei Province, China) freeze-dried and sieved through 120-μm mesh.

Synthesis of PIB-Cl

PIB-Cl was synthesized with o-phthalaldehyde and 4-aminobenzoic acid using a literature method[12] and examined by Mass Spectrometry [PIB-Cl, fine colorless needles, m.p. > 230°C. Calc. for $C_{15}H_{10}NO_2Cl$: 66.4%C, 3.7%H, 5.2%N; found: 66.6%C, 3.8%H, 5.2%N. M.S. m/z, 271(M+)] and by IR [740cm^{-1} C-Cl, 1300cm^{-1} 1370cm^{-1} C-N, 1600cm^{-1} C_6H_6, 1690cm^{-1} C=O].

The examination showed that the synthesized product PIB-Cl was in good agreement with its molecular formula and structure.

UWE-PCD-HPLC-UV Procedure

One hundred grams of soil or crop sample and 50.0 mL of methanol were added to a 300.0-mL plug-Erlenmeyer flask. The mixture was treated with ultrasonic wave for 5 minutes and filtered with microporous funnel (4G). The extraction process was repeated three times. The combined extract was prepared for concentration.

The soil or crop extract was filtered through 5 cm of anhydrous Na_2SO_4 in a sintered glass filter column. A 50-mL of fresh methylene chloride was used for washing the column. The extract was evaporated to about 3 mL on a rotary evaporator at 35°C in a water bath with vacuum. The concentrated extract was dried under a gentle stream of nitrogen. Then it was transferred to a graduated bottle and diluted to 2.0 mL with benzene quantitatively.

One milligram of PIB-Cl and 100 mg of catalyst were added to the graduated bottle. The mixture was shaken gently for 25 min at 20°C in a water bath and centrifuged at 4000 rpm. Then, 10 μL of clear liquid was injected onto the column.

Conventional Extraction GC-NPD Procedure

The conventional extraction GC-NPD procedure was carried out as literatures methods.[13-15] Briefly, the soil and crop samples were collected, freeze-dried, sieved through a 120-μm mesh and blended with methanol. The mixture was shaken for 1.5h at 270 rpm on an oscillating machine.

The extracts were filtered, evaporated, dried with nitrogen, and cleaned up on a deactivated Florisil column. The analytes were eluted from the column with 3% methanol in benzene. The fractions were evaporated to 1 mL. Then the 5 μL of clear liquid was injected onto the column.

RESULTS AND DISCUSSION

Optimization of Derivatization Conditions

A systematic study of the conditions for derivatization was performed with temperature being varied between 5 to 40°C and the reaction time between 20 min to 60 h. The optimum conditions were a reaction time of 25 min at 20°C.

A mixture of the solids NaOH-Na_2CO_3 (1:1, w/w, 80 mesh) was prepared as the catalyst for the derivatization reaction. The maximum yield was achieved when the mole ratios of PIB-Cl to atrazine and simazine were >30 and the catalyst to atrazine and simazine ratio was >3800. In order to ensure the maximum yield, 1.0 mg of PIB-Cl (PIB-Cl/analytes more than 60) and 100 mg of catalyst (mole ratio more than 8000) were applied to the analytes at the ng/g level in every derivatization procedure.

In order to select the optimum solvent for derivatization reaction, acetone, acetonitrile, methylene chloride, tretramethylene oxide, and benzene were tested. Benzene, treated with sodium metal to remove the trace water in it, was the best solvent because of the high solubility of reactants and derivatives. Besides, benzene was also a safe solvent for chromatographic column because of the very low solubility of the catalyst in it. Moreover, the derivatives were stable for more than 10 days if kept in benzene at room temperature.

Chromatographic Conditions

The derivatives were scanned using Shimadzu DU-650 spectrophotometer. The optimum ultraviolet absorption wavelengths of 300 nm for PIB-Cl, of 235 nm for atrazine and simazine, and of 345 nm for the derivatives were found. So the absorption wavelength of 345 nm was selected for all measurements. When soil and crop extracts, without derivatization, were injected onto the chromatographic column no peak was found at the wavelength of 345 nm. When the derivatization reaction occurred, many co-extracts did not react with PIB-Cl under the above derivatization condition. So, they did not interfere with the chromatographic separation. Therefore, the time-consuming clean-up and second-time concentration processes were omitted with no practical problem.

Table 1

Dependence of Capacity Factors on the Acetonitrile Concentration

Acetonitrile %:	95	90	80	70	60	50	40
Atrazine	2.6	3.4	6.4	10.5	16.6	21.0	26.8
Simazine	2.4	3.0	3.4	9.2	13.2	19.6	22.0

At the same time, the molar absorption coefficients of 1.07×10^5 L • cm^{-1} • mol^{-1} for atrazine derivative and 1.21×10^5 for simazine derivative were measured. On the basis of the high molar absorption coefficients, the low detection limits at ng/g level for analytes can be obtained.

Aqueous acetonitrile, methanol, and tretramethylene oxide were tested for the chromatographic separation. Aqueous acetonitrile was found to be the optimum mobile phase for the separation and determination of the derivatives. The dependence of capacity factors on acetonitrile concentration in the mobile phase is shown in Table 1. The capacity factors of both the UV labeled atrazine and simazine increased and the separation between the labeled atrazine and simazine was improved with the acetonitrile concentration decreased.

When the acetonitrile concentration was 60-80%, PIB-CI, the labeled atrazine and simazine were easy to separate from each other. When the acetonitrile concentration was lower than 60%, the target peaks extended. For real samples a 75% aqueous acetonitrile was found to be the optimum composition for the separation among PIB-CI, the UV labeled atrazine and simazine with no overlapping peaks. When the pH of the mobile phase was adjusted with 0.1% H_3PO_4 from 5.5 to 7.5, no apparent changes had taken place for the chromatographic retention volume of PIB-CI, the UV labeled atrazine and simazine. However, the mobile phase of pH 6 was easy to get better peaks shapes. Therefore, a 75% aqueous acetonitrile of pH6 was selected as the mobile phase for the determination of real samples. The typical chromatograms of UWE-PCD-HPLC-UV procedure for standard, soil and crop samples are shown in Figure 1.

Performance of HPLC-UV Detection

Soil and crop samples were spiked with atrazine and simazine in order to determine the repeatability, precision and recovery. Several characteristics of this method are given in Table 2.

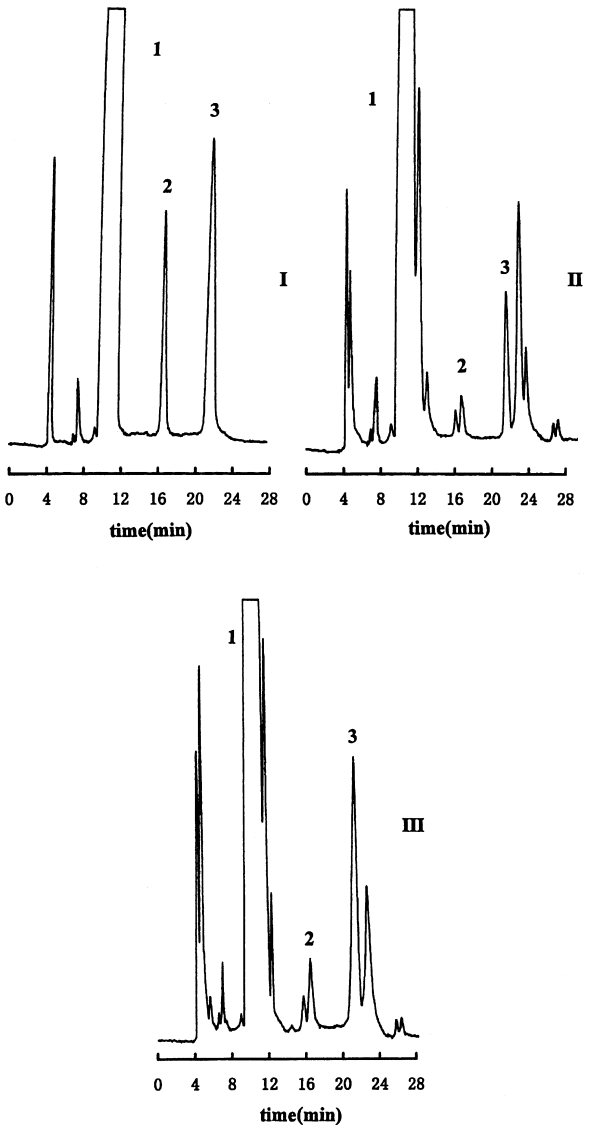

Figure 1. The Typical Chromatograms of Standard, Soil and Crop Samples by UWE-PCD-HPLC-UV Procedure. I = a standard mixture of atrazine (98.2 ng/g) and simazine (61.0 ng/g); II = soil sample (1#); III = crop sample (1#). Condition: *Du Pont* ODS column(250x4.6 mm); Mobile phase, 75% aqueous acetonitrile; Flow-rate, 0.8 mL/min.; UV detection, 345nm; Injection volume, 10 μL. Peaks: 1= PIB-Cl; 2 = simazine derivative; 3 = atrazine derivative.

Table 2

Data for Atrazine and Simazine in Ultraviolet Detection

	Soil		Crop	
	Atrazine	**Simazine**	**Atrazine**	**Simazine**
Spike (ng/g)	30	30	30	30
S.D. (n = 4)	2.5	2.2	2.8	3.0
R.S.D. (%)	6.3	6.5	7.2	7.6
Recovery (%)	91	90	95	92

Detection Limit (S/N = 3)	Atrazine	1.1 ng/g
	Simazine	1.0 ng/g
Linear Range	Atrazine	0.12-300 ng
	Simazine	0.11-300 ng
Regression Equation	Atrazine	y = 1.2808 x + 0.11, R = 0.998
	Simazine	y = 1.5318 x + 0.16, R = 0.999

The standard deviations (S.D.) were 2.2-3.0 and relative standard deviations (R.S.D.) were less than 8% (n=4). The recoveries of 90-95% were obtained for real samples. The regression equations of peak height (cm) and injection concentration (μg/g) were determined with correlation coefficients of 0.998. According to the extraction ratio in pretreatment, when the signal-to-noise ratio was 3, the detection limits of 1.1 ng/g for atrazine and 1.0 ng/g for simazine could be obtained.

The conventional oscillation extraction technique was difficult to smash the fine soil and crop granular thoroughly. Thus, the analytes within the granular could not be dissolved completely by extraction solvent. The Soxhlet extraction technique was a time-consuming process (more than 8h). It may be possible for analytes to be decomposed under the condition of high temperature and long time reflux operation. However, the UWE technique not only smashed the soil and crop granular but also avoided the long time high temperature operation. Therefore, the high recovery could be gained.

Using derivatization reaction, the analytes were connected with strong ultraviolet absorption matter PIB-Cl. As a result, the derivatives with the molar absorption coefficients at 10^5 level were formed. So the sensitivity was improved. The improvement of the sample pretreatment not only increased sensitivity but also shortened analytical time. For soil and crop analysis UWE-PCD-HPLC-UV procedure, i.e., ultrasonic wave extraction, concentration,

Table 3

Comparison Between HPLC-UV and GC-NPD Procedures

	Soil					Crop		
	1	**2**	**3**	**4**	**5**	**1**	**2**	**3**
HPLC-UV (ng/g)	45.1	33.4	58.3	36.4	53.8	85.7	106.6	117.4
GC-NPD (ng/g)	48.6	35.6	54.5	35.1	50.4	90.8	101.3	125.8
R. D. (%)	7.5	6.4	6.7	3.6	6.5	5.8	5.1	6.9

derivatization, and HPLC-UV, spent less time than the conventional GC-NPD procedure, i.e., extraction, concentration, clean-up, second-time concentration and GC-NPD. Compared with oscillation extraction (3h) or Soxhlet (8 h) extraction, the ultrasonic wave extraction was a time-economy process (30 min).

Compared with the clean-up and second-time concentration process (about 100-200 min), the derivatization reaction was also a time-economy process (20 min). As a result, each soil or crop sample can be analyzed within 100 min by this procedure.

On the basis of the good repeatability, low detection limits, and high recoveries, the residues of atrazine and simazine in soil and crop samples can be analyzed by this procedure quantitatively.

Comparison of HPLC-UV and GC-NPD Data

In order to examine the reliability of the UWE-PCD-HPLC-UV procedure, 5 soil and 3 crop samples from agricultural field were analyzed by this procedure in our laboratory and, simultaneously, by the conventional off-line extraction GC-NPD procedure in another laboratory. The analytical data are shown in Table 3.

The analytical results achieved by this procedure were in good agreement with the results by GC-NPD. The R.D. of the two methods was less than 8% (n=4). The detection limits for these two methods were at same level.

Moreover, the UWE-PCD-HPLC-UV procedure spent less time than the conventional GC-NPD procedure. So, the UWE-PCD-HPLC-UV procedure was reliable.

CONCLUSIONS

The potential of the UWE-PCD-HPLC-UV procedure was demonstrated by the successful analysis of soil and crop samples. Atrazine and simazine were used as model compounds for pesticides that contain -NH group(s). Ultrasonic wave technique was successfully applied to the extraction process both to raise the recovery and to shorten the extraction time. On the basis of the conventional sample pretreatment procedure, the clean-up and second-time concentration process was replaced by a derivatization process. The derivatives with strong ultraviolet absorption properties could be detected by the HPLC-UV system. Detection limit at the ng/g level and recoveries in the range 90-95% for real samples can be obtained. By means of the practical application to 5 soil and 3 crop samples, the analytical results of these two methods were compared. The reliability of the UWE-PCD-HPLC-UV procedure was verified.

However, there were two disadvantages for this procedure. First, the automated on-line analysis of this procedure is still not carried out and this procedure is still a relative time-consuming procedure for soil and crop analysis. Secondly, PIB-Cl was not commercially available.

In spite of some disadvantages, the UWE-PCD-HPLC-UV procedure, with its high sensitivity and selectivity, is the first step to search for automated on-line analysis for soil and crop samples. If ultraviolet derivatization detection is connected with SPE technique, the detection limit will be possible to be decreased greatly for soil and crop analysis. If ultraviolet derivatization detection is combined with SFE, it may be possible to achieve a fast and sensitive technique for soil and crop analysis. If a new derivatization reagent that reacts with analytes rapidly is found, it is likely to accomplish an automated on-line analysis for soil and crop samples.

In a word, the UWE-PCD-HPLC-UV procedure was an alternative to the conventional GC-NPD or GC-MS method for the determination of trace atrazine and simazine in soil and crop.

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