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ARTICLE

Determination of Four Carbamate Pesticides in Corn by Cloud Point Extraction and High-Performance Liquid Chromatography in the Visible Region Based on Their Derivatization Reaction

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A highly sensitive method for the determination of arprocarb (AC), carbofuran (CF), isoprocarb (IC), and fenobucarb (FC) is proposed. The method is based on alkaline hydrolysis of the four carbamate pesticides, and the resultant hydrolysis products are reacted with 4-aminoantipyrene (AP) to give four red color products. The colored compounds are enriched and separated by cloud point extraction (CPE) method, and the coacervate phase containing the compounds is determined with a high-performance liquid chromatography (HPLC) system in the visible region. AC, CF, IC, and FC were determined on the basis of a linear correlation between the signals of the colored compounds and the concentrations of the pesticides. The method is applied to determine the four pesticides in corn samples; the limits of detection are 2.0×10^{-4} mg L^{-1} for AC, CF, and IC and 5.0×10^{-4} mg L^{-1} for FC, with recoveries ranging between 84.8 and 93.0%, at spiking levels of 5×10^{-3}, 2×10^{-2}, and 0.2 mg kg^{-1}, respectively.

KEYWORDS: Carbamate pesticides; cloud point extraction (CPE); derivatization reaction; high-performance liquid chromatography (HPLC); corn sample

INTRODUCTION

Carbamates have played an important role in the increasing agricultural productivity. However, carbamates are considered to be hazardous to the environment and human health, and they are on the priority list released by the U.S. Environmental Protection Agency (EPA) (*1*). Carbamate pesticides are only allowed to be used in food crops and stored grain in China. Therefore, the determination of carbamates in water (*2*), plants (*3*,*4*), soils (*5*,*6*), body fluids (*7*), etc., is of major importance for human health protection and environmental containment. The evaluation of trace levels of carbamates in agricultural products is nowadays a priority objective to ensure food quality and to protect consumers against possible health risks.

Many techniques have been developed for analyzing carbamates. The typical characteristics of carbamates are high polarity, solubility in water, and thermal instability. Therefore, analysis by gas chromatography (GC) is difficult. Liquid chromatography (LC) is mainly used in the analysis of carbamates; the detectors used include UV (*3*−*5*, *8*) and fluorescence (*9*, *10*). The UV detector presents a relatively low sensitivity to carbamate pesticides, and the nonfluorescence of several carbamate pesticides complicated their fluorescence detection. Excellent sensitivity is achieved with GC-MS (*11*) and LC-MS (*12*). However, the instruments are expensive. Electrochemical methods (*13*) and immunoassay techniques (*14*) were also applied to determine carbamates. However, difficulties are encountered when other nontargets adsorb at the surface of the working electrode, resulting in poor repeatability.

To enhance sensitivity, liquid−liquid extraction (LLE) (*15*), supercritical fluid extraction (SFE) (*9*), solid-phase extraction (SPE) (*16*), solid-phase microextraction (SPME) (*17*), microsolid-phase extraction (μ-SPE) (*18*), and microwave-assisted extraction (MAE) (*4*, *5*) are applied to extract the analyte from the matrix. LLE and SPE methods are somewhat tedious or unfriendly due to the use of nonaqueous solvent. SFE and MAE were suitable extraction means for carbamates in sediment samples. However, a sample cleanup using SPE cartridges is required after SFE and MAE. The cleanup steps are extensive and laborious. SPME give satisfactory results, but the fibers used in SPME are expensive and fragile. Furthermore, heating is required to facilitate the extraction, and, thus, this technique is not suitable for carbamate extraction for its thermal instability. The extraction efficiency of μ-SPE is satisfactory except the extraction procedure is tedious (> 90 min).

Cloud point extraction (CPE) methodology can offer an interesting alternative to the extraction systems due to many advantages, such as reduced consumption of organic solvent, disposal cost, and extraction time. The CPE technique has been widely used for the extraction of metal ions (*19*), biological materials (*20*), polycyclic aromatic hydrocarbons (*21*), polychlorinated compounds (*22*), and drugs (*23*). HPLC is most commonly used for the determination of many chemical and biological species after the CPE process, and the most convenient detector is a UV detector.

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Many carbamate pesticides can be extracted with the CPE method. However, they cannot be directly determined with the CPE-HPLC-UV method due to the intense absorption of surfactant in the UV region. This problem can be possibly solved by using surfactants that do not absorb at the working wavelengths used in chromatography (*24*) or employing cleanup procedures (*25*). However, these methods are somewhat inconvenient. We propose another simple way to overcome this drawback. This method is to make the working wavelength red shift, which is based on the formation of colored products derived from the pesticides.

In this work, carbamate pesticides arprocarb (AC), carbofuran (CF), isoprocarb (IC), and fenobucarb (FC) were investigated. The pesticides hydrolyze into different phenols in alkaline solution. The phenols reacted with AP to form intensely colored compounds in the presence of an alkaline oxidizing agent. Subsequently, the colored derivants were extracted from the matrix solution by using the CPE method. Finally, the surfactant-rich phase containing target analytes was separated and determined by HPLC-UV at 510 nm. The derivatization method could also increase the detectability of carbamate detection in HPLC analysis. The new method has been applied to determine the four carbamate pesticides in spiked corn sample.

INSTRUMENTATION AND REAGENTS

Instrumentation. The chromatographic system was a Waters 515 pump connected to a Waters 2487 UV−vis absorbance detector, with a 20 μL loop (Waters USA). The stationary phase was a LiChrospher C_{18} column (5 μm, 250 mm×4.6 mm) (Hanbon Reagent Co., Jiangsu, China). An Agilent 1100 LC/MSD mass spectrometer (Agilent USA) was equipped with a pump, photodiode array detector, and quadrupole mass filter. A centrifuge (model TDL 40B) from Anke Instrument Plant (Shanghai China) was used to separate surfactant solutions into two phases. A UV-1700 (Shimadzu Japan) spectrophotometer was used for absorption measurements. A thermostat from Tongzhou Instrument Plant (Jiangsu, China) was used to maintain the desired temperature within ±1.0 °C. Ultrasonication (ultrasonic frequency = 28 kHz, power = 80 W) from Kunshan Ultrasonic Instrument Plant (Jiangsu, China) was used for degassing of the mobile phase and extraction of corn samples.

Reagents. Arprocarb (97% purity), carbofuran (98%), isoprocarb (98%), and fenobucarb (97.6%) were obtained from Jiangsu Institute of Pesticide (Jiangsu, China), and their stock standard solutions of 100 mg L^{-1} were prepared with acetonitrile. Reference solutions were kept at 4 °C until use. Corn samples were purchased from Suguo supermarket (Jiangsu, China). Triton X-100 (iso-octyl phenoxy polyethoxy ethanol) was of analytical grade and purchased from Shanghai Lingfeng Reagent Co. (Shanghai, China). 4-Aminoantipyrene (AP) and potassium ferricyanide ($K_3Fe(CN)_6$) were purchased from Guangdong Guanghua Chemical Factory (Guangdong, China). The 1.0% (w/v) solution of AP was prepared in 10% (v/v) ethyl alcohol; 2% (w/v) $K_3Fe(CN)_6$ was prepared with distilled water before use. HPLC-grade acetonitrile and ethyl alcohol were obtained from Nanjing Reagent Plant (Jiangsu, China). The other reagents used were all of analytical grade and brought from Nanjing Reagent Plant (Jiangsu, China).

EXPERIMENTAL PROCEDURES

Derivatization Procedure. Aliquots of 5 mL of solution containing each carbamate pesticide of 1.0 mg L^{-1} were taken in a 15 mL centrifugal vial; the pesticides were hydrolyzed by adding 1.0 mL of 0.6 mol L^{-1} NaOH solution, shaking, and holding for 10 min at 25 °C. After that, 1.0 mL of 0.6 mol L^{-1} HCl solution was added to counteract the NaOH. The pH of 0.10 mol L^{-1} buffer solution of $Na_2B_4O_7$−NaOH was adjusted to 9.5. Then, 0.5 mL of $Na_2B_4O_7$−NaOH buffer solution was added, followed by 0.2 mL of 1.0% (w/v) AP solution and 0.3 mL of 2% (w/v) $K_3Fe(CN)_6$ solution, with continuous shaking. The concentrations of AP and $K_3Fe(CN)_6$ were 0.025% (w/v) and 0.075% (w/v), respectively. The solution was kept for 5 min for full color development. Therefore, four colored compounds corresponding to AC, CF, IC, and FC were formed.

CEP Procedure. Aliquots of 10 mL of the solution containing four colored compounds, 4% (v/v) Triton-100, and 18% (w/v) Na_2SO_4 were shaken. After Na_2SO_4 was completely dissolved, the two phases were separated by centrifugation for 10 min at 3500 rpm. The volume of the surfactant-rich phase was usually 0.1 mL. The supernatant aqueous phase was carefully removed with a syringe, and the surfactant-rich phase was left in a centrifugal vial. The HPLC mobile phase was added to the surfactant-rich phase, and the final volume was 0.4 mL. The concentration factor was 25.

Preconcentration of Corn Samples. Standard solutions containing four carbamate pesticides were added to 10.0 g corn samples. The spiked corn samples were pulverized and sieved to obtain samples with particle sizes up to 0.075 mm. Aliquots of 1.0 g of corn powder were placed in 15 mL centrifuge tubes, and 8 mL of 1.0% (v/v) Triton-100 solution was added. These samples were kept in ultrasonic cleaning for 20 min. Then, the mixture was centrifugated at 3000 rpm for 10 min, and the supernatant fluid was filtered through a 0.45 μm membrane. After 5 mL of the filter liquor had been treated as derivatization and CPE procedures, 20 μL of the surfactant-rich phase was analyzed by a HPLC-Vis method.

HPLC Experiment. The mobile phase contained 5 mmol L^{-1} Na_2HPO_4−NaH_2PO_4 buffer solution (pH 7.4 ± 0.1) and acetonitrile (40:60, v/v). The flow rate was 0.6 mL min^{-1}. The mobile phase was filtered through a 0.45 μm membrane before use. Twenty microliters of the surfactant-rich phase was injected into the HPLC system, and the detection wavelength was set at 510 nm. To remove the Triton-100 absorbed in the C_{18} column after the analyte was eluted, a cleanup step with 100% methanol over 20 min was performed after injection each day. According to the linear relationship between the signals of the derivates and the concentrations of the carbamate pesticides, the amounts of AC, CF, IC and FC were obtained.

LC-MS Experiment. An LC-MS experiment was carried out on an Agilent 1100LC/MSD mass spectrometer, with the MSD equipped with ESI source. The ionization mode was positive. The HPLC conditions followed the HPLC system conditions described above. The interface and MSD conditions were as follows: dry gas (N_2), 9.5 L min^{-1}; dry gas temperature, 350 °C; gas pressure (N_2), 40 psi; spray capillary voltage, 4000 V; ion transfer voltage, 70 V; and scan range, m/z 105−800.

RESULTS AND DISCUSSION

Preparation of Colored Compounds and Their Characteristics. *Hydrolysis and Color Reaction Conditions*. It was found that 1.0 mL of a 0.6 mol L^{-1} NaOH solution was sufficient for the complete hydrolysis of the four carbamate pesticides. The conditions effected on the color reaction, such as pH value and concentrations of AP and $K_3Fe(CN)_6$, were studied. The derivatization reaction was carried out in alkaline media. Therefore, the pH studies were performed in the range of pH 7.0−11.0 with concentrations of AP and $K_3Fe(CN)_6$ in the range of 0.005−0.05% (w/v) and 0.015−0.15% (w/v), respectively. It was found that the highest change in absorbance was obtained at pH 9.5, when concentrations of AP and $K_3Fe(CN)_6$ solutions were 0.025% (w/v) and 0.075% (w/v). Larger amounts of AP could disturb the detection of corn samples.

Structures and Characteristics of Colored Compounds. Carbamate pesticides are easily hydrolyzed to form different phenols in alkaline solution (*13*). AP can react with some of the phenols when an alkaline oxidizing agent, such as $K_3Fe(CN)_6$, is used (*26*). Correspondingly, AC, CF, IC, and FC were hydrolyzed into 2-isopropoxycyclohexa-2,5-dienol (YD), 2,2-dimethyl-2,3,4,7-tetrahydrobenzofuran-7-ol (BF), 2-isopropylcyclohexa-2,5-dienol (LD), and 2-*sec*-butylcyclohexa-2,5-dienol (BD), respectively. YD, BF, LD, and BD reacted with AP, respectively, and four relevant products were obtained. The four colored products were (*Z*)-4-(3-isopropoxy-4-oxocyclohexa-2,5-dienylideneamino)-1,5-dimethyl-2-phenyl-1,2-dihydropyrazol-3-one (YDAP), (*E*)-4-(2,2-dimethyl-7-oxo-2,3-dihydrobenzofuran-4(7*H*)-yildeneamino)-1,5-dimethyl-2-phenyl-1,2-dihydropyrazo-1,3-one (BFAP),

(*Z*)-4-(3-isopropyl-4-oxocyclohexa-2,5-dienylideneamino)-1,5-dimethyl-2-phenyl-1,2-dihydropyrazol-3-one (LDAP), and (*Z*)-4-(3-*sec*-butyl-4-oxocyclohexa-2,5-dienylideneamino)-1,5-dimethyl-2-phenyl-1,2-dihydropyrazol-3-one (BDAP), respectively. The reactions to obtain the colored compounds are shown in **Figure 1**. The structural formulas and other analytical characteristics of the four colored compounds are shown in **Table 1**.

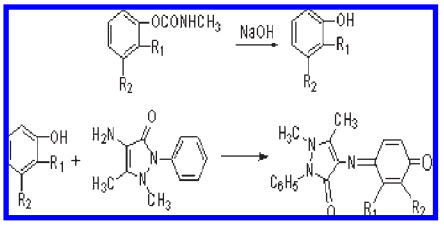

Figure 1. Reaction formula of formation of the color compounds.

Table 1. Characteristics of the Color Derivatives

Pesticides	Derivants	Structure of derivants	Molecular formula Molecular mass λ max
Arprocarb (AC)	YDAP		$C_{20}H_{21}N_3O_3$ 351.2 500nm
Carbofuran (CF)	BFAP		$C_{21}H_{21}N_3O_3$ 363.2 530nm
Isoprocarb (IC)	LDAP		$C_{20}H_{21}N_3O_2$ 335.2 490nm
Fenobucarb (FC)	BDAP		$C_{21}H_{23}N_3O_2$ 349.2 490nm

The molecular weights of the four colored products derived from AC, CF, IC, and CF were verified by LC-MS. The corresponding peaks of the molecular ion (M + 23) were at *m*/*z* 374.2, 386.2, 358.2, and 372.2, respectively. The results were in agreement with the conjecture of four colored compound structures.

Solvent type, solvent strength (volume fraction of organic solvent in the mobile phase and pH of the buffer solution), and flow rate were varied to determine the chromatographic conditions giving the best separation. The maximum absorbances of the colored products derived from AC, CF, IC, and FC were at 500, 530, 490, and 490 nm, respectively. Therefore, it was concluded that 510 nm was the most appropriate wavelength for simultaneous analysis of the four colored products of the pesticides. The best results were obtained by using a mobile phase of 5 mmol L^{-1} Na_2HPO_4−NaH_2PO_4 buffer solution (pH 7.4 ± 0.1) and acetonitrile (40:60, v/v), with a flow rate of 0.6 mL min^{-1}.

The chromatograms of four carbamate pesticides of 1 mg L^{-1} and Triton-100 of 4% (w/v) in the UV region (at 230 nm) were obtained. The four carbamate pesticides were eluted in the order AC, CF, IC, and FC with retentions of 4.2, 5.6, 8.8, and 11.0 min, respectively. It was found that the background absorbance of Triton-100 could overlap the chromatographic peak of the four carbamate pesticides at 230 nm. Therefore, the four pesticides cannot be detected with the CPE-HPLC method in the UV region.

The chromatograms of Triton-100 and four derivants at 510 nm are shown in **Figure 2**, chromatograms a and c, respectively. It can be seen that the retention times of the four derivants (YDAP, BFAP, LDAP, and BDAP) were 6.3, 7.6, 10.3, and 12.1 min, respectively, and Triton-100 had no signal at 510 nm. Chromatograms b and c of **Figure 2** show that the color derivants have higher molar absorptivities as compared with their corresponding carbamate pesticides.

Optimization of CPE Conditions. *Triton-100 Concentration*. The variation of extraction efficiency with Triton-100

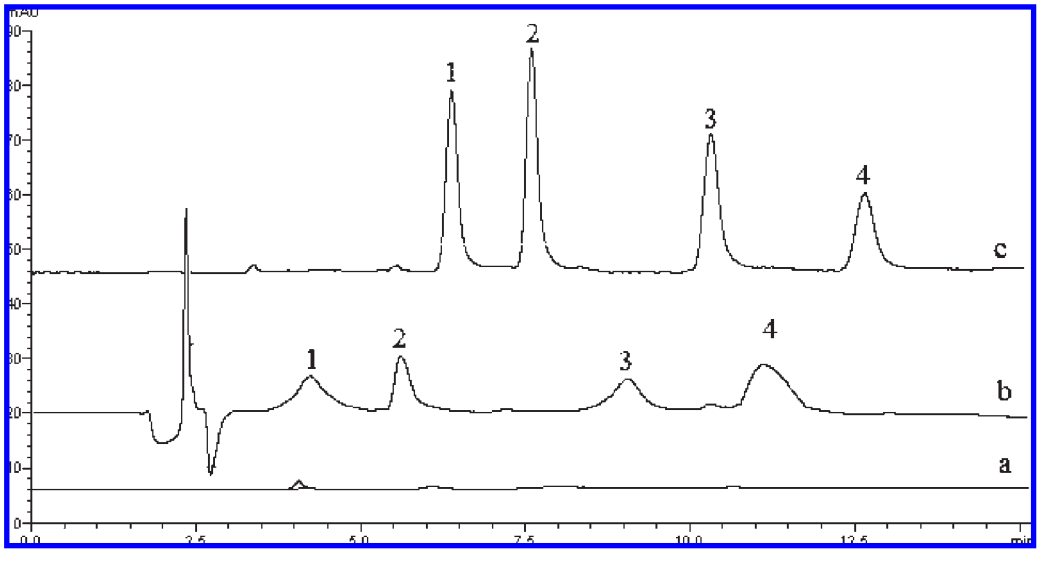

Figure 2. HPLC-UV−vis at 510 nm. Samples: 1 mg L^{-1} AC, 1 mg L^{-1} CF, 1 mg L^{-1} IC, and 1 mg L^{-1} FC; 4% Triton-100: (**a**) Triton-100; (**b**) four carbamate pesticides (1, AC; 2, CF; 3, IC; 4, FC); (**c**) four derivants (1, YDAP; 2, BFAP; 3, LDAP; 4, BDAP).

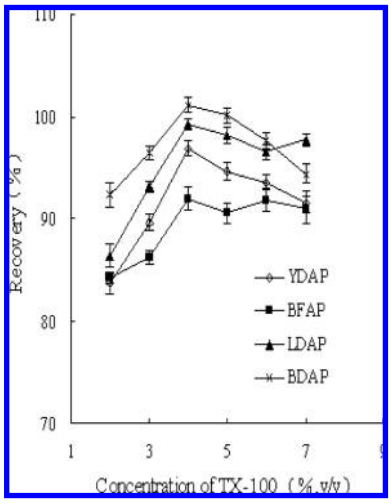

Figure 3. Effect of concentration of TX-100 on extraction efficiency. CPE conditions: 25 °C, equilibration for 10 min, 18% (w/v) Na_2SO_4, pH 9.5.

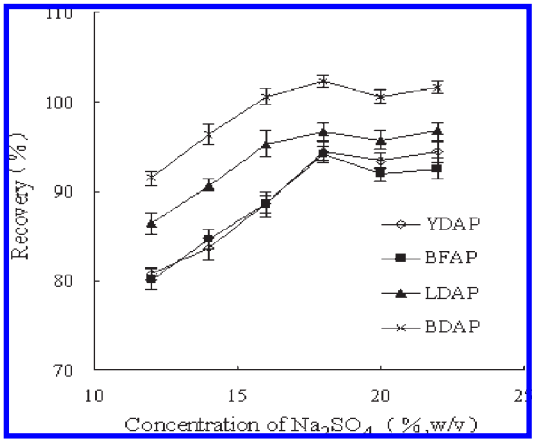

Figure 4. Effect of concentrations of Na_2SO_4 on the extraction efficiency. CPE conditions: 25 °C, equilibration for 10 min, 4% (v/v) Triton-100, pH 9.5.

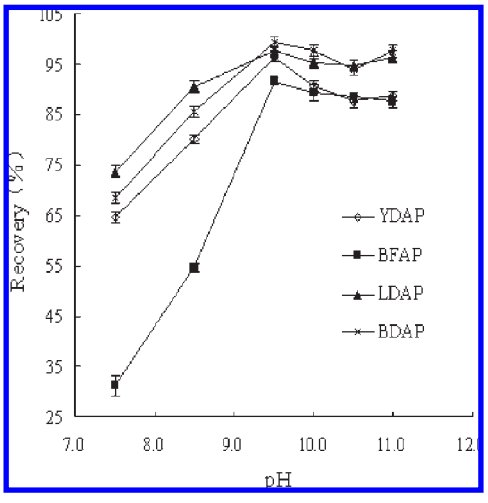

Figure 5. Effect of pH on extraction efficiency. CPE conditions: 25 °C, equilibration for 10 min, 4% (v/v) Triton-100, 18% (w/v) Na_2SO_4.

concentration was examined within the range of 2−7% (v/v). As shown in **Figure 3**, at a lower concentration of surfactant, the extraction efficiency of the four carbamate pesticides was low. Content quantitative extraction was observed for a Triton-100 concentration of 4% (v/v), and the recovery remained constant above that. However, the volume of the surfactant-rich phase increases approximately from 0.08 to 0.17 mL with increasing concentration of Triton-100 from 2 to 7% (v/v). To avoid the decrease of the concentration factor, a Triton-100 concentration of 4% (v/v) was used in the following experiments.

Salt Type and Concentration. The CPE behavior was also determined as functions of salt types and concentrations. Addition of salt can accelerate phase separation and lower the cloud point of the surfactant solution. Besides, the fractional coacervate phase volume decreases with salt concentration. It is explained that more water goes to the dilute phase due to a salting-out effect. The relevant electrolytes are usually in high concentrations (*27*, *28*). Different salts (Na_2SO_4, NaCl, $(NH_4)_2SO_4$, Na_2CO_3, and $C_6H_5Na_3O_7$) were tested. The CPE systems had different behaviors with salt type. When Na_2SO_4 was added to the system, the highest recovery efficiency was obtained, and the solution was separated into two phases at room temperature.

Figure 4 shows the effect of Na_2SO_4 concentration on the CPE procedure of the four derivants. The extraction efficiencies of four derivants increased as the concentration of Na_2SO_4 increased from 12 to 22% (w/v). Further increase in concentration of Na_2SO_4 had no significant effect on the extraction efficiency. Thus, a Na_2SO_4 concentration of 18% (w/v) was employed for further experiments.

Temperature and Centrifugation Time. When the CPE procedure was processed at the equilibration temperature of the surfactant, the best extraction efficiency was achieved. On the other hand, addition of salt can lower the equilibration temperature. A temperature range of 20−60 °C was studied. Maximum extraction efficiency was observed in the range of 25−30 °C; beyond 30 °C, a significant decrease of the efficiency was obtained, probably due to the instability of the derivants. Accordingly, 25 °C was used in the CPE procedure.

The equilibration time was also investigated in the rage of 5−20 min at 3500 rpm. It was found that the maximum extraction efficiency was presented between 10 and 20 min. Therefore, 10 min was chosen as the optimum equilibration time.

pH. The derivants of the four carbamate pesticides must be kept in alkaline solution to prevent decomposition. Therefore, a pH range of 7.5−11 was studied. As can be seen from **Figure 5**, the recovery increased rapidly when the pH increased to 9.5, especially for BFAP, and then remained constant in the pH range of 9.5−11. Thus, the pH of the extraction solution was maintained at 9.5 by adding $Na_2B_4O_7$−NaOH buffer solution in the derivatization procedure.

Analytical Characteristic. *Analytical Figures*. After the standard solutions containing different concentrations of carbamate pesticides were treated according to the derivatization and CPE procedure, 20 μL of the surfactant-rich phase was injected into the chromatographic system as described for the HPLC experiment. The analytical characteristic data of the present system for the four carbamate pesticides are summarized in **Table 2**. A linear calibration graph for the peak areas of derivatization versus concentrations of carbamate pesticides was obtained for concentrations in the range from 8.0×10^{-4} to 0.50 mg L^{-1} for AC, CF, and IC and from 2.0×10^{-3} to 0.50 mg L^{-1} for FC. The limit of detection calculated from a signal/noise ratio (s/n = 3) was 2.0×10^{-4} mg L^{-1} for AC, CF, and IC and 5.0×10^{-4} mg L^{-1} for FC.

The limits of detection using HPLC with UV detection (*8*), fluorescence detection (*9*), and MS detection (*12*) were 5, 1.6, and 0.3 μg L^{-1}, respectively. The results in the current method were lower than or similar to those values obtained by other methods reported.

Table 2. Analytical Features of the Method

pesticide	LOD[a] (mg L^{-1})	LOQ[b] (mg L^{-1})	linear range (mg L^{-1})	regression eq[c]	correl coeff
arprocarb	2×10^{-4}	8×10^{-4}	8×10^{-4}–0.5	$Y = 2.3 \times 10^6 X + 9.1 \times 10^2$	0.9948
carbofuran	2×10^{-4}	8×10^{-4}	8×10^{-4}–0.5	$Y = 3.5 \times 10^6 X + 2.7 \times 10^2$	0.9987
isoprocarb	2×10^{-4}	8×10^{-4}	8×10^{-4}–0.5	$Y = 1.9 \times 10^6 X + 2.1 \times 10^2$	0.9977
fenobucarb	5×10^{-4}	2×10^{-3}	2×10^{-3}–0.5	$Y = 4.2 \times 10^5 X + 2.9 \times 10^2$	0.9990

[a] LOD, limit of detection calculated as 3 times the signal-to-noise ratio. [b] LOQ, limit of quantization calculated as 10 times the signal-to-noise ratio. [c] X is the concentration (mg L^{-1}) and Y is the peak area of HPLC.

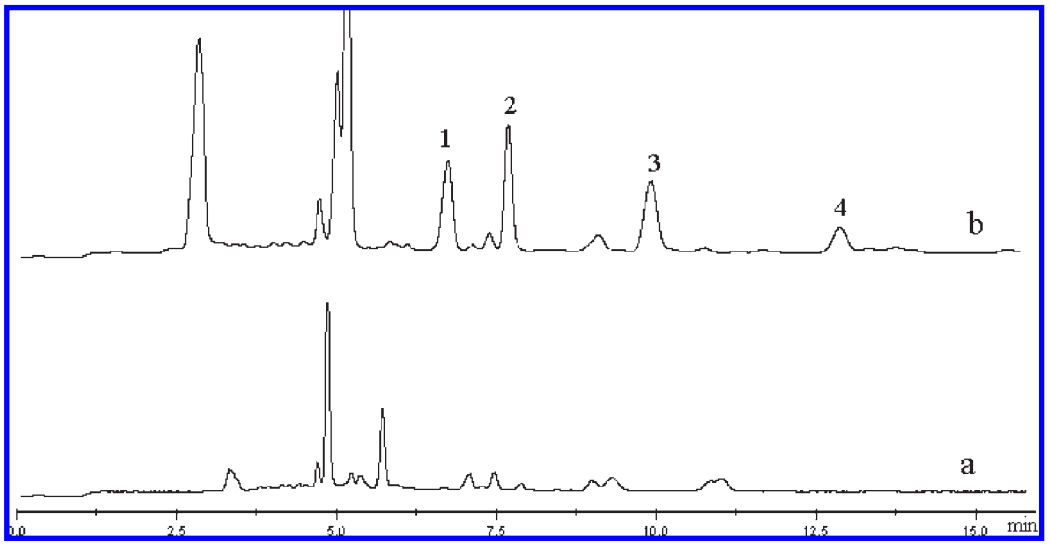

Figure 6. Chromatograms of samples: (**a**) blank corn sample; (**b**) spiked corn sample (1 mg L^{-1}) (1, YDAP; 2, BFAP; 3, LDAP; 4, BDAP).

Table 3. Analytical Results of Corn Samples

pesticide	added (μg kg^{-1})	CPE found (μg kg^{-1}) (mean ± SD, n = 5)	LLE[a] found (μg kg^{-1}) (mean ± SD, n = 5)	CPE recovery (%) (mean ± RSD, n = 5)
arprocarb	2	1.64 ± 0.07	nd[b]	82.3 ± 3.5
	20	17.77 ± 0.45	nd	88.8 ± 2.3
	200	182.67 ± 4.04	179.64 ± 3.54	91.4 ± 2.0
carbofuran	2	1.69 ± 0.08	nd	84.2 ± 3.8
	20	18.00 ± 0.53	nd	90.0 ± 2.6
	200	188.33 ± 2.08	178.83 ± 4.76	94.2 ± 1.1
isoprocarb	2	1.69 ± 0.09	nd	84.7 ± 4.0
	20	18.27 ± 0.60	nd	91.3 ± 3.0
	200	181.67 ± 3.79	178.87 ± 3.76	90.8 ± 1.9
fenobucarb	5	4.64 ± 0.14	nd	86.6 ± 2.7
	20	17.87 ± 0.35	nd	89.3 ± 1.8
	200	179.34 ± 4.16	180.25 ± 4.41	89.7 ± 2.0

[a] LLE, liquid–liquid extraction. The preconcentration (LLE) of corn samples was according to the repored methods of NY/T 761-2008. HPLC system conditions: mobile phase, acetonitrile/water (60:40, v/v); flow rate, 0.6 mL min^{-1}; wavelength, 230 nm; injection volume, 20 μL. [b] nd, not detected.

Application of the Method in Corn Sample. No residues of AC, CF, IC, and FC were found in corn samples. Corn samples were spiked with four carbamate pesticides at levels of 5×10^{-3}, 2×10^{-2}, and 0.2 mg kg^{-1}, respectively. The spiked samples were treated according to the CPE procedure, and five replicates per concentration level were analyzed.

After CPE, the chromatograms obtained from blank corn sample and corn sample spiked at 0.1 mg kg^{-1} are shown in **Figure 6a**,**b**, respectively. It can be seen from **Figure 6** that the matrix of corn sample did not interfere with the determination of the four carbamate pesticides.

The levels of recovery and relative standard deviation (RSD) are given in **Table 3**. The average recoveries of the spiked standards for the analytes were 82.3–94.2% with average RSDs of 1.2–4.0%. The CPE method was compared with the LLE method. As summarized in **Table 3**, there was no significant difference between the proposed method and the reference method (NY/T 761-2008).

In China, the maximum residue limits (MRL) of the carbamate pesticides (AC, CF, IC, and FC) in corn are 0.5, 0.2, 0.2, and 0.3 mg kg^{-1}, respectively (*29*). The new analysis method may meet the above-mentioned standard.

The following conclusions can be obtained from the present work.

The CPE-HPLC-Vis method has been shown to be very attractive for the detection of carbamate pesticides: (1) Compared with the absorbance maximum of the four carbamate pesticides (λ = 230 nm), the wavelength positions of derivants are in the visible region. In this case, the background absorbance of Triton X-100 does not overlap with the peak of targets. Therefore, the surfactant-rich phase was directly analyzed with the HPLC system in the visible region. (2) The color derivants are determined by UV–vis detector; the absorptive signals of the derivants are much higher than those of the original carbamate pesticides and a higher concentration factor can be obtained by CPE. Therefore, the sensitivity of the new method is much higher than that of determinating carbamate pesticides directly. (3) Organic solvent was not used in procedures of sample treatment and CPE; the analysis method is friendly to both the environment and operators.

Many pesticides can be hydrolyzed to form phenols. Some of these phenols can react with AP to form colored derivants, and the pesticides can be determined by using the new method. However, pesticides that can yield the same phenols cannot be simultaneously analyzed by the HPLC system.

ABBREVIATIONS USED

AC, arprocarb; CF, carbofuran; IC, isoprocarb; FC, fenobucarb; YD, 2-isopropoxycyclohexa-2,5-dienol; BF, 2,2-dimethyl-2,3,4,7-tetrahydrobenzofuran-7-ol; LD, 2-isopropylcyclohexa-2,5-dienol; BD, 2-*sec*-butylcyclohexa-2,5-dienol; AP, 4-aminoantipyrene; CPE, cloud point extraction; HPLC, high-performance liquid chromatography; YDAP, (*Z*)-4-(3-isopropoxy-4-oxocyclohexa-2,5-dienylideneamino)-1,5-dimethyl-2-phenyl-1,2-dihydropyrazol-3-one; BFAP, (*E*)-4-(2,2-dimethyl-7-oxo-2,3-dihydrobenzofuran-4(7*H*)-yildeneamino) 1,5-dimethyl-2-phenyl-1,2-dihydropyrazo-1,3-one; LDAP, (*Z*)-4-(3-isopropyl-4-oxocyclohexa-2,5-dienylideneamino)-1,5-dimethyl-2-phenyl-1,2-dihydropyrazol-3-one; BDAP, (*Z*)-4-(3-*sec*-butyl-4-oxocyclohexa-2,5-dienylideneamino)-1,5-dimethyl-2-phenyl-1,2-dihydropyrazol-3-one.

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