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Analytical Methods

Cloud point extraction coupled with derivative of carbofuran as a preconcentration step prior to HPLC

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ABSTRACT

Carbofuran can be hydrolysed to from 2,3-dihydro-2,2-dimethyl-7-benzofuranol (BF). BF is coupled with 4-aminoantipyrene (AP) in presence of potassium ferricyanide ($K_3Fe(CN)_6$) to generate red coloured derivative(BFAP)having λmax 530 nm. Cloud point extraction (CPE) methodology and using surfactant Triton X-100 as extractant was applied as a preconcentration step prior to HPLC, the surfactant-rich phase containing BFAP was then analysed by HPLC in visible region. The high back ground absorbance of Triton X-100 in UV region was completely avoided. A new visible detection method with high-performance liquid chromatography (HPLC) has been developed for the determination of carbofuran. Using this method, we found that carbofuran residues could be determined with recoveries ranging from 80.4% to 84.5%, relative standard deviations in the range of 2.51–3.26% for three fortified rice levels, and the limit of detection as 5.0×10^{-4} cJ/cK.

1. Introduction

Carbofuran (2, 3-dihydro-2, 2-dimethyl-7-benzofuranyl methylcarbamate) is a broad spectrum insecticide widely used throughout China in agricultural crops, ornamental gardens and plants. Its use in rice combines both spraying upon the plant and application on the stored crop (Maroni, Colosio, Ferioli, & Fait, 2000). However, owing to its high toxicity for mammals, maximum residue limits (MRLs) of carbofuran have been established by different legislation. In the case of rice, MRLs is 0.2 mg/kg in China (First editor's office of China standard press, 2006) and 0.1 mg/kg in Japan (Edit committee of agriculture chemicals in foods, 2006). Hence it is essential to strengthen the control and improve the monitoring level. Many techniques have been reported for the determination of carbofuran, such as voltammetry (Rao, Loo, Sarada, Terashima, & Fujishima, 2002), gas chromatography (Rossi et al., 2001), high-performance liquid chromatography (HPLC) (Ting & Kho, 1991), gas chromatographic-mass spectrometric (Petropoulou, Gikas, Tsarbopoulos, & Sikos, 2006) and spectrophotometry (Jan, Shah, & Khan, 2003). These analytical methods entail an extraction step in which liquid–liquid extraction (Fenoll, Hellín, Martínez, Miguel, & Flores, 2007; Rissato, Galhiane, Almeida, Gerenutti, & Apon, 2007) and solid phase extraction (Blanco, Grande, & Gándara, 2002; D'Archivio, Fanelli, Mazzeo, & Ruggieri, 2007) are most commonly applied. However, in aforementioned extraction methods, solvents used are harmful to lab operators in particular and environment. So it is desirable to develop a facile, inexpensive and environment friendly method for extraction of carbofuran. Cloud point extraction (CPE) method is such an alternative method.

Aqueous solutions of many nonionic surfactants have the property of separating into two transparent liquid phases when heated above a certain temperature (cloud point temperature): one retains most of the surfactant (the surfactant-rich phase) and the other is an aqueous phase. The former, the surfactant-rich phase, can be utilised to preconcentrate target analytes. The technique is called cloud point extraction (CPE) (Paleologos, Giokas, & Karayannis, 2005; Stalikas, 2002). The CPE method is simple, economical, and atoxic. But the high background absorbance shown by many surfactants in the UV region can interfere with the determination of analyte by HPLC–UV system.

Many methods were proposed to overcome this weakness. For instance, some researches used surfactants that do not absorb at the working wavelengths of analytes (Saitoh & Hince, 1991). In other researches, the mobile phase that contains high methanol content was used in order that elution of surfactant might occur in a short period of time, which the background absorption of surfactant could be separated from the signal of analytes (Cordero, Pavón, Pinto, & Laespade, 1993). In addition, detections that are transparent to the surfactants under certain conditions were used for the determination of the analytes (Pinto, Pavón, & Cordero, 1994).

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We proposed another way to overcome this drawback. This method is based on the derivative reaction of pesticide where the analytes are detected in visible region which is transparent to surfactants. However, since many pesticides are colourless, a technique for yielding of coloured derivative of pesticide has to be applied in the determination of pesticides in visible region. This method is based on a colour product derived from pesticide. The derivative is detected in visible region and there is a linear relationship between the signal of the derivative and the concentration of pesticide.

When alkaline oxidising agents are used, phenols react with 4-aminoantipyrene (AP) to form intensely coloured compounds. Carbofuran can be hydrolysed into 2,3-hydro-2,2-dimethyl-benzofuran (BF) and methylamine in alkaline solution. The BF molecule has one free phenolic hydroxyl group and no substitute in the para position pare to the hydroxyl group. A red colour product (BFAP) is produced by the reaction of BF and AP. The maximum absorption wavelength of BFAP is at 530 nm; thus the background absorbance of surfactant was completely avoided. On the other hand, BFAP has a higher molar extinction coefficient as compared with carbofuran, because a linear relationship is established between the signals of BFAP and concentrations of carbofuran, the sensitivity of detecting carbofuran is also increased. A new CPE–HPLC–vis method was proposed for the determination of carbofuran.

2. Experimental

2.1. Reagents

Carbofuran (98%) was obtained from Jiangsu institute of pesticide (Jiangsu,China), Chemical-grade Triton X-100 (iso-octyl phenoxy polyethoxy ethanol) and HPLC-grade acetonitrile were obtained from Lingfeng Reagent Co. Ltd. (Shanghai, China). Sodium sulphate anhydrous (Na_2SO_4), sodium dihydrogen phosphate (NaH_2PO_4), sodium hydrogen phosphate (Na_2HPO_4), phosphoric acid (H_3PO_4), 4-aminoantipyrene (AP), potassium ferricyanide ($K_3Fe(CN)_6$), sodium hydroxide(NaOH) and sodium tetraborate ($Na_2B_4O_7{\cdot}10H_2O$) were purchased from Shanghai Chemical Reagent Company (Shanghai, China), all other reagents were of analytical grade.

The 1.0% solution of AP was prepared in 10% ethyl alcohol; carbofuran stock solution (100 mg/l) was prepared with acetonitrile; pH of $Na_2B_4O_7$–NaOH buffer solution (0.10 mol/l) was at 9.5.

2.2. Apparatus

A chromatographic system (Waters, Milford MA USA) consisted of a delivery pump (Model 515), a UV–vis absorbance detector (Model 2487) and an injector with a 20 μl loop. The stationary phase column was a LiChrospher C_{18} column (250 mm × 4.6 mm), particle 5 μm, (Hanbon Reagent Company, Jiangsu, China). Agilent 1100 LC/MSD mass spectrometer (Agilent, USA) 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. UV-1700 (Shimadzu, Japan) spectrophotometer was used for absorption measurements. A thermostat (Tongzhou Instrument Plant, Jiangsu, China) was used to maintain the desired temperature within ±1.0 °C.

2.3. General procedure

2.3.1. Hydrolysis of carbofuran and formation of coloured reaction

Eight millilitres aliquots of solutions containing carbofuran were placed in a 15 ml centrifugal vial. 0.60 ml of 0.60 mol/l NaOH solution was added and kept for 10 min for complete hydrolysis to form BF. Then 0.6 ml of 0.6 mol/l HCl solution and 0.25 ml of $Na_2B_4O_7$–NaOH buffer solution were added to maintain the pH of the solution at 9.5. 0.25 ml of 1.0% AP and 0.30 ml of 2.0% $K_3Fe(CN)_6$ were added to form BFAP. The reaction mechanism of BFAP was shown in Fig. 1.

2.3.2. Cloud point extraction of BFAP

In a typical CPE experiment, 0.8 ml of 50% Triton X-100 and 1.8 g of Na_2SO_4 were added to BFAP solution obtained as 2.3.1 procedure, After Na_2SO_4 was completely dissolved, the solution was kept for 10 min for isolating two phases and then complete separation of two phases was achieved by centrifugation at 3500 rpm for 10 min. The aqueous phase was sucked out by the aid of a syringe and the surfactant-rich phase was left in the centrifugal vial with volume of the surfactant-rich phase was a little smaller than 0.20 ml, LC mobile phase was added to reduce its viscosity and the final volume of the phase was diluted to 0.30 ml. The preconcentration factor is about 33.

2.3.3. Procedures for treating rice sample

Rice was pulverised and sieved to obtain samples with particle sizes up to 200 meshes. Rice powder (1.0 g) was precisely weighed

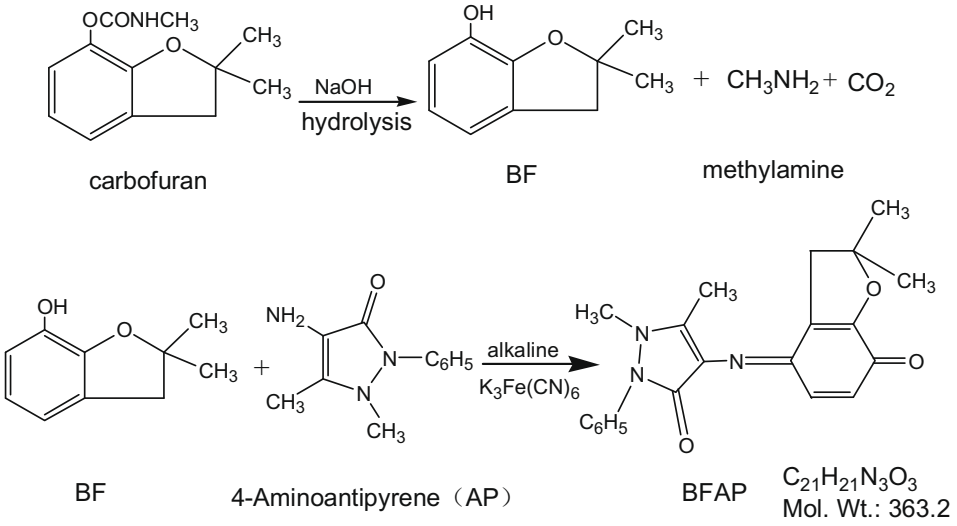

Fig. 1. Reaction mechanism of carbofuran with derivatising reagents.

and placed in 15 ml centrifuge tube; 10 ml of 1.0% (v/v) Triton X-100 solution was added. The tube was kept in the ultrasonic cleaning bath for 20 min at 50 °C. Then, the mixture was centrifuged at 2000 rpm for 10 min and the supernatant fluid was filtered through 0.45 μm membrane. After 8.0 ml of the filter liquor were treated in the same procedures as described in Sections 2.3.1 and 2.3.2, 20 μl of the surfactant-rich phase were analysed by HPLC–vis method.

2.3.4. LC-MS experiment

LC-MS experiment was carried out on Agilent 1100 LC/MSD mass spectrometer, with the MSD equipped with ESI source. The ionisation mode was positive. The interface and MSD conditions were as follows: dry gas (N_2) 9.5 l/min, dry gas temperature 350 °C, gas pressure (N_2) 40 psi, spray capillary voltage 4000 v, ion transfer voltage 70 v and scan range 105–800 m/z.

2.3.5. HPLC–vis experiment

After the two phases was separated, the surfactant-rich phase was diluted to 0.3 ml and then 20 μl of the surfactant-rich phase was injected into chromatographic system, a mobile phase was acetonitrile: 5.0×10^{-3} mol/l Na_2HPO_4–NaH_2PO_4 buffer solutions (pH 7.4) (60:40, v/v) and was filtered through 0.45 μm membrane. A flow rate was at 0.70 ml/min and the detection was performed at wavelength 530 nm.

3. Results and discussion

3.1. Colour reaction of carbofuran

3.1.1. Hydrolysis conditions of carbofuran

Amount of NaOH solution should be sufficient for the complete hydrolysis of carbofuran, but not excessive in order not to interfere with the coloured reaction process. A satisfactory result was obtained when 0.60 ml of 0.60 mol/l NaOH solution was added and kept for 10 min.

3.1.2. Colour reaction

When alkaline oxidising agents are used, AP reacts with some phenols such as the BF to form intensely coloured compounds, so AP can be used as a sensitive reagent for detecting the phenols.

Fig. 1 shows that the hydrolysis product (BF) of carbofuran is one of the phenols. Conditions formed the coloured derivative of BF were investigated. When pH of solution was at 9.5 and concentrations of AP and $K_3Fe(CN)_6$ solution were 0.025% and 0.06%, respectively, the absorptivity of the coloured derivative (BFAP) was the highest. The absorbance spectrum for the above solution was recorded in UV–vis region, the maximum absorption wavelength of BFAP was at 530 nm.

3.2. Structure verification of BFAP

The reaction mechanism of BFAP was shown in Fig. 1, analogous to that of propoxur phenol (Venkateswarlu & Seshaiah, 1995). However, there are not works concerning the structure verification. LC-MS was used to verify structure of BFAP. Fig. 4a is its chromatogram, and corresponding mass spectrum was shown in Fig. 2. The peak at m/z 386.2 (M + 23) is most likely molecule. Leading to the molecule proposal below $C_{21}H_{21}N_3O_3$ (molecular weight 363.2), therefore, the structure of BFAP was verified and reaction mechanism shown in Fig. 1 was proved.

3.3. Optimisation experiments of CPE

3.3.1. Effect of Triton X-100 concentration

A series of solutions containing 4.0×10^{-2} mg/l carbofuran, Na_2SO_4 (18%, w/v) and different concentrations of Triton X-100 were treated described in Sections 2.3.1 and 2.3.2. The influence of Triton X-100 concentration on extraction efficiency was investigated by measuring recoveries of BFAP. The results indicated that the extraction efficiencies of BFAP increased up to 4% (v/v) with the increase of Triton X-100 concentration, and then remained constant when the concentration of Triton X-100 was 4–8% (v/v). The maximum recovery was 90% for BFAP. However, the surfactant-rich phase volume depends on Triton X-100 concentration, the increase of which can induce the decrease of the preconcentration factor due to the increase in the final volume of surfactant-rich phase. To obtain the higher detection signal, 4% (v/v) Triton X-100 was chosen.

3.3.2. Effect of pH

In order to prevent the decomposition of BFAP, it must be kept in alkaline solution. Therefore, effect of pH on the extraction efficiency of BFAP was studied over the pH range 7.5–11. The experimental results indicated that the extraction efficiencies remained relatively constant over the pH range 9.5–11. In following experiments, pH was controlled at 9.5.

3.3.3. Effect of salt type and concentration

The effects of some salts, such as Na_2CO_3, NaCl, $Na_3C_6H_5O_7$ (sodium citrate) and Na_2SO_4, on the CPE behaviour were investigated. It was found that the presence of Na_2SO_4 induced the phase separation at room temperature and resulted in extraction of maximal efficiency. For this reason, the effect of Na_2SO_4 was further studied, as shown in Fig. 3, the recovery of BFAP increased when the concentration of Na_2SO_4 increased up to 18% (w/v) and then remained constant above that. Thus, 18% (w/v) Na_2SO_4 was chosen.

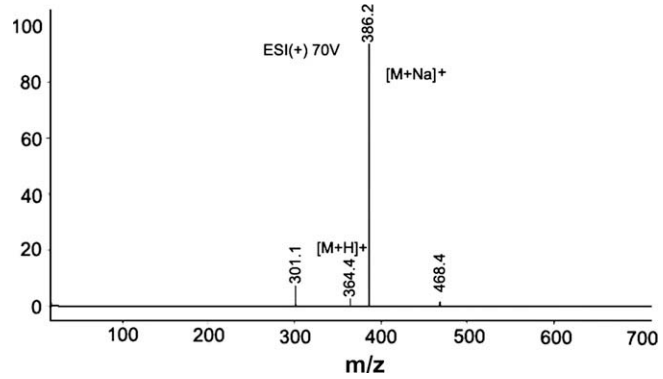

Fig. 2. The mass spectrum of the BFAP, the LC-MS condition as described in Section 2.

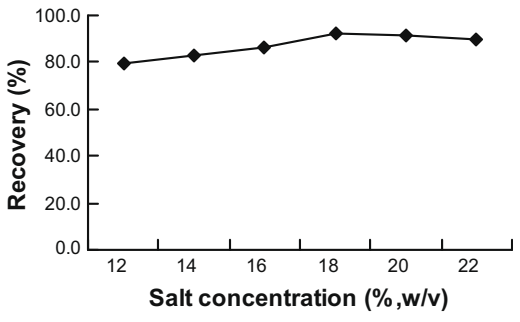

Fig. 3. Effect of salt concentration on recovery when using 4% (v/v) Triton X-100, CPE conditions: room temperature, 10 min, pH 9.5.

3.3.4. Effect of equilibration temperature and time

It was also proved by experiments that the recovery of carbofuran remained constant in temperature between 20 and 30 °C whereas beyond 30 °C, the recovery gradually decreased. Therefore, room temperature is adequate for the CPE procedure. The dependence of recovery upon equilibration time was also investigated for a time interval of 10–40 min. The results showed no significant effect on the recovery. Therefore, 10 min of equilibration time was finally selected.

3.4. Analytical characteristic

3.4.1. Verification of the method

A series of standard carbofuran solutions was treated as Sections 2.3.1 and 2.3.2 and then injected into HPLC system under optimised conditions. A linear relationship between the peak area of BFAP and carbofuran in the concentration range of 2.0×10^{-3}–0.30 mg/kg was confirmed, with 0.9997 correlation coefficient. Limit of detection was 5.0×10^{-4} mg/kg by a signal-to-noise ration of 3/1.

In order to check repeatability of the CPE method for the preconcentration of BFAP, the standard solutions at three different concentrations (1.0×10^{-2}, 4.0×10^{-2} and 0.10 mg/kg) were treated and determined, and nine replicates were analysed at per concentration level. Average recovery and relative standard deviation varied from 87.9% to 91.7% and from 1.23% to 2.56%, respectively.

3.4.2. Determination of carbofuran in rice samples

The rice samples were treated and detected as Sections 2.3.3 and 2.3.5. No signal corresponding to carbofuran was detected in any of the samples analysed. Fig. 4c shows the corresponding chromatogram.

Rice samples were fortified with 5.0×10^{-3}–0.15 mg/kg levels with carbofuran. The fortified samples were treated as explained in Section 2.3.3. The surfactant-rich phase was injected into HPLC system as the conditions in Section 2.3.5. Fig. 4b shows the corresponding chromatogram; the retention time of BFAP was about 7.63 min and the peak was separated from those scattered peaks.

The average recoveries of spiked samples with added amount of 5.0×10^{-3}, 2.0×10^{-2}, and 0.15 mg/kg of carbofuran were 84.5%, 82.6% and 80.4%, respectively and the corresponding relative standard deviation for three replicate determinations were 2.85%, 2.51% and 3.26%, respectively.

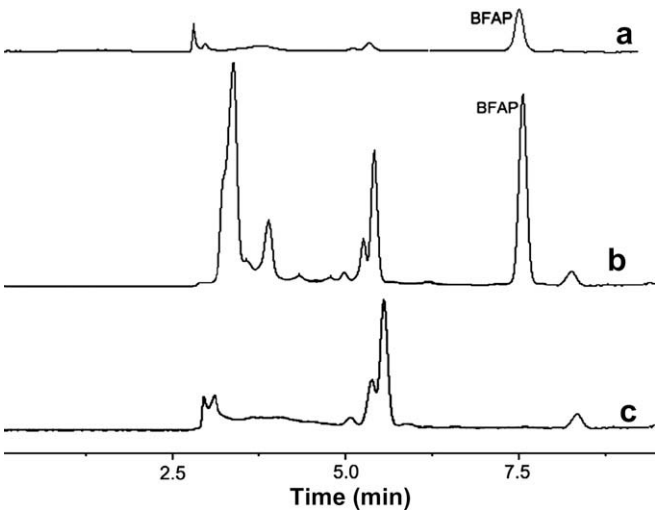

Fig. 4. Chromatogram obtained for the injection of a BFAP solution after treated as 2.3.1. (a) Chromatograms obtained for the injection of a surfactant-rich phase of a spiked rice sample (b) and of a rice sample (c) after cloud point preconcentration. Chromatographic conditions as described in Section 2.

4. Conclusions

The colour derivative of carbofuran was prepared in a single step. The extraction process did not require any organic solvents. The present method for the determination of carbofuran in rice sample was found to be facile, sensitive, inexpensive and environmental-friendly. The coloured analytes are detected in visible region, in which the background absorption of surfactant may not interfere with the determination of the analytes. On the other hand, when the determination is carried out by HPLC–UV system, the response of the coloured derivate is higher than that of original compound.

This CPE method for extraction of carbofuran proved to be an efficient step prior to the determination with HPLC–vis detection. The method can be used to determine other pesticides which could be hydrolysed into the phenolic compounds.

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