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Cloud point extraction coupled to spectrophotometry for the determination of carbaryl in vegetables การสกัดแบบจุดขุ่น ร่วมกับ สเปกโทรโฟโทเมทรี สำหรับการตรวจวัดคาร์บาริล ในตัวอย่างผัก

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ABSTRACT

This research presents cloud point extraction (CPE) using Triton X-114 (TX-114) as the extractant for preconcentration of carbamate pesticide, carbaryl, in vegetable samples and then quantitative determination by spectrophotometry. In spectrophotometry, carbaryl was investigated based on diazotization reaction. Conditions for CPE and diazotization reaction were optimized. The conditions of CPE were 1.5% w/v TX-114, 1% w/v NaCl, equilibrated at 55°C and centrifuged at 3,500 rpm for 10 min. For the diazotization reaction, the extract from CPE was hydrolyzed in alkaline medium (pH > 12) and derivatized with 0.6 mmol L⁻¹ 2,4-dimethoxyaniline (DMA) and measured the absorbance at 510 nm. The proposed method gave acceptable recoveries and the detection limit of 0.10 mg L⁻¹. The results were comparable to those obtained from HPLC (*t*-test, p = 0.05).

บทคัดย่อ

งานวิจัยนี้ได้ใช้เทคนิคการสกัดแบบจุดขุ่น (CPE) โดยใช้ไทรทัน X-114 (TX-114) เป็นสารสกัดในการเพิ่มความ เข้มข้นของคาร์บาริล ซึ่งเป็นสารกำจัดแมลงกลุ่มคาร์บาเมทในตัวอย่างผัก แล้วหาปริมาณด้วยวิธีสเปกโทรโฟโทเมทรี ซึ่ง ตรวจวัดคาร์บาริล โดยอาศัยปฏิกิริยาไดเอโซไทเซชัน ได้ศึกษาสภาวะที่เหมาะสมของทั้ง CPE และวิธีสเปกโทรโฟโทเมทรี พบว่า สภาวะที่เหมาะสมของ CPE คือ TX-114 เข้มข้น 1.5% และ NaCI เข้มข้น 1%โดยน้ำหนักต่อปริมาตร อุณหภูมิ 55 °C ปั่นเหวี่ยงที่ 3,500 รอบต่อนาที เป็นเวลา 10 นาที สำหรับปฏิกิริยาไดเอโซไทเซชัน นำสารที่สกัดได้ จาก CPE มาไฮโดร ไลซ์ในสารละลายเบส (พีเอช > 12) และทำปฏิกิริยากับสารละลาย 2,4-dimethoxyaniline เข้มข้น 0.6 มิลลิโมลต่อลิตร แล้ว วัดค่าการดูดกลืนแสงที่ 510 nm วิธีที่นำเสนอนี้ให้ก่าร้อยละการกลับคืนที่ยอมรับได้ และขีดจำกัดการตรวจวัด 0.10 มิลลิกรัมต่อลิตร ผลการทดลองที่ได้ไม่แตกต่างอย่างมีนัยสำคัญกับเทคนิค HPLC ที่ความเชื่อมั่น 95%

Key Words : Carbamate pesticides, Carbaryl, Spectrophotometry คำสำคัญ : สารกำจัดแมลงกลุ่มคาร์บาเมท คาร์บาริล สเปกโทรโฟโทเมทรี

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Introduction

Carbamate compounds were introduced as pesticides in the early 1950s and are still used extensively as pesticides (Cheng et al., 2007). Carbamates are compounds with chemical structure R-O-C(O)-N(CH₃)-R', where R is an alcohol, an oxime or a phenol and R' is a hydrogen or a methyl group. There are approximately 30 commercially available carbamate pesticides in the markets.

Carbamates are toxic substances of human being, neurotoxic effect as reversible inhibitors of the enzyme cholinesterase (Diez et al., 2006). One of the most commonly used carbamate is carbaryl (1-naphthyl-N-methylcarbamate). Several authorities set regulation for the contamination of carbaryl in food such as Thai agricultural commodity and food standard established the maximum residue limits (MRLs) for carbaryl in some vegetable samples at $3.0 - 5.0 \text{ mg kg}^{-1}$ (TACFS, 2008), while Asian Ministry on Agriculture and Forest established for the MRLs of cabbage and leafy vegetables at 5.0 mg kg^{-1} and 10.0 mg kg^{-1} , respectively (ASEAN cooperation, 2002).

In order to investigate the contamination of pesticides in food, it needs sensitive analytical methods as well as effective methods for sample preparation. The widely used techniques for the analysis of carbamates are HPLC and CE (Cheng et al., 2007). The common detector for both techniques is UV-detector. While, mass spectrometer (MS) is used to improve sensitivity. However, the mentioned instruments are expensive and sophisticated. Several simple methods like spectrophotometry have been investigated for the determination of carbamate pesticides. The spectrophotometric methods are usually based on the derivatization of the pesticides with appropriate labeling reagents to form clearly strong color solutions that can be easily applied for visible spectrophotometric detection.

The derivatization of pesticides is generally performed via diazotization reaction to form azo-dye compounds which provide high molar absorptivity (\mathcal{E}) with 3-4 orders of magnitude when compare to their native forms. Several reagents are used as derivatizing reagents such as *p*-dimethylphenylene diamine (Sastry et al., 1987), trimethylaniline (Alvarez-Rodriguez et al., 1997).

The sample preparation for the analysis of pesticides from vegetables and fruits are consisted of (i) extraction and (ii) clean up and/or preconcentration of the extracts. The conventional extraction method using organic solvents is the most commonly used method to extract the pesticides from vegetables. While, liquidliquid extraction (LLE) and solid-phase extraction (SPE) are among the popular methods for clean up and preconcentration steps.

Recently, cloud point extraction (CPE) has been introduced as an alternative technique to LLE and SPE (Chen et al., 2009). CPE uses surfactant to extract the target analytes from aqueous phase. None toxic organic solvents are required in CPE. Quick Easy Cheap Effective Rugged and Safe (QuEChERS) method is another sample preparation technique, it has been recognized as an effective sample preparation for various food matrices (Koesukwiwata et al., 2008).

The present work is aimed at the development of a simple method using Vis-spectrophotometry for the The **11**th Khon Kaen University 2010 Graduate Research Conference

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determination of carbaryl in vegetable samples. CPE and QuEChERS are chosen as the sample preparation

methods. This work emphasizes on optimization of CPE

and spectrophotometry to improve the sensitivity for the

Materials and methods

Instruments

Absorbance measurements and spectra recording were performed on a Spectrophotometer (Agilent 8453 UV-Vis spectroscopy system, Germany). A 1-cm thick quartz cell was used throughout the experiments. Reciprocal shaker (Vision Scientific Co., Ltd., Korea) was used for extraction of the vegetables. Centrifuge instruments (Biomed group Co., Ltd., Thailand and Kokusan type H-11N, Biomed group, Co., Ltd., Japan) were used for phase separation. Vortex (Scientific Industries, INC., USA) was used for mixing the samples or analytes in solvent. Rotary evaporator (R-200 Buchi, Switzerland) was used for eliminated organic solvent. The chromatographic instruments consisted of a water 600 controller Dual pump (Waters, USA), a Rheodyne injector with 20 µL sample loop and Waters 2996 Absorbance detector. The Empower software was used for data acquisition. A Chromolith Speed ROD (C18 monolithic 50 mm x 4.6 mm) column was used.

Chemicals and reagents

All reagents were of analytical reagent grade. Carbaryl was purchased from Riedel-deHaen (Germany). The stock 1,000 mg L^{-1} of carbaryl was prepared in methanol. Triton X-114 (TX-114) was purchased from

Acros (USA). 2,4-dimethoxyaniline (DMA) from Fluka (Japan). Sodium nitrite (NaNO2) from Riedel-deHaen (Germany). Concentrated HCl, sodium acetate (CH₂COONa), sodium hydroxide (NaOH) and magnesium sulphate (MgSO₄) were purchased from Carlo Erba (France). Sodium sulphate anhydrous (Na₂SO₄) was purchased from Fluka (Japan). Sodium chloride (NaCl) was purchased from Ajex Finechem (Australia). Carbograph and PSA were purchased from Vertical Chromatography Co., Ltd. (Thailand).

Diazotization reaction

Standard solution or sample solution from CPE was hydrolyzed and mixed with DMA, NaNO₂, and HCl. The absorbance was measured at 510 nm. A blank reagent solution was performed in the same manner without carbaryl spiking. All experiments were consecutively studied in triplicate.

Sample preparation

Vegetable samples including cucumber, cabbage, kale, long bean, and mustard were taken from local markets in Khon Kaen university. The edible part of the sample (~500 g) was cut into 1- cm pieces and blended using a commercial food mixer. A sample portion (15.0000 g) was placed in a 50 mL centrifuge tube. Aliquot of 25.00 mL 1% v/v acetic acid in 99% v/v acetonitrile was added and shaken 250 rpm for 1 hour. After shaking, the extract was added with Na₂SO₄ anhydrous, MgSO₄ and CH₃COONa, vortex immediately and then centrifuged for 10 min at 2,500 rpm and transferred aliquot of upper layer into a 50 mL tube containing Carbograph and PSA, The tube was shaken

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immediately and solution was filtered. The filtrate was then evaporated using a rotary evaporator (at 50 $^{\circ}$ C water bath) to eliminate acetonitrile. The solution was diluted with water to 10.00 mL prior to the extraction by CPE. For spiked samples, the 1.0 mg kg⁻¹ carbaryl was added to the represent samples and equilibrated at room temperature for 30 min before extraction.



Results and discussion

Spectrophotometry of carbaryl

The diazotization of carbaryl involves two steps, including hydrolysis and coupling steps, which the reactions are shown in Fig. 1.

Coupling



Fig. 1 Diazotization reaction of carbaryl

Cloud point extraction procedure

Standard or sample solutions (10.00 mL) were mixed with NaCl and TX-114 solution in a centrifuge tube. The solution was shaken and kept in a thermostat water bath at 55 °C for 20 min and then centrifuged at 3,500 rpm for 10 min to complete the phase separation. The solution was kept in an ice bath for 10 min. The aqueous phase (upper part) was withdrawn, while the surfactant rich phase (lower phase) was measured the volume using a 10.00 mL syringe. A surfactant rich phase (~400 μ L) was then diluted with methanol to decrease viscosity before analysis. First step, carbaryl was hydrolyzed with NaOH to form naphtholate anion, while DMA was mixed with NaNO₂ and HCl to form diazonium ion. Second step, the naphtholate anion was mixed with diazonium ion to form azo dye. The orange-red color derivative with the maximum absorbance at 510 nm was obtained.

To obtain high signal of absorbance for diazotization, the effect of the concentrations of DMA, NaNO₂, HCl and NaOH on the derivatization of carbaryl (3.0 mg L^{-1}) was investigated. The studied concentrations of DMA, NaNO₂ and HCl were 0.2 to1.0 mmol L^{-1} , 0.1 to 1.0 mmol L^{-1} and 2.0 to 10.0 mmol L^{-1} , respectively. While concentration of NaOH was studied according to pHs ranged from 2.0 to 14.0. The influence

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of the studied parameters on the absorbance of the derivative is shown in Fig. 2. The optimum concentrations for the formation of diazonium ion (see Fig. 2 (A-C)) are 0.6 mmol L^{-1} , 0.2 mmol L^{-1} and 2.0 mmol L^{-1} for DMA, NaNO₂ and HCl, respectively. The optimum condition for hydrolysis of carbaryl is using NaOH at pH higher than 12 (see Fig. 2 (D)).



Fig. 3 The stability of carbaryl derivative



Fig. 2 Optimization for derivatization of carbaryl:

- (A) Concentration of DMA: conditions; NaNO₂ 0.5 mmol L^{-1} , HCl 5.0 mmol L^{-1} and pH 12
- (B) Concentration of NaNO₂: conditions; DMA 0.6 mmol L⁻¹, HCl 5.0 mmol L⁻¹ and pH 12
- (C) Concentration of HCl: conditions; DMA 0.6 mmol L⁻¹, NaNO₂ 0.2 mmol L⁻¹ and pH 12
- (D) pH: conditions; DMA 0.6 mmol L^{-1} , NaNO, 0.2 mmol L^{-1} and HCl 2.0 mmol L^{-1}

The stability of the derivative was also studied by leaving the derivative to stand for over 2 hours and measured the absorbance at 510 nm every 20 min. The results are shown in Fig. 3, indicated that the derivative was stable for 2 hours. In CPE, the analytes from a large bulk aqueous solution is extracted into a less surfactant rich phase that resulting a higher preconcentration. The parameters influenced CPE including concentration of surfactants, concentration of sodium chloride, and equilibration (time and temperature) were studied. TX -114 was used as the extractant and its concentrations were studied in the The **11**th Khon Kaen University 2010 The **11** Graduate Research Conference

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range 0.5 to 2.5 % w/v while, NaCl was studied at the concentrations ranging from 1.0 to 5.0 % w/v, and equilibration temperature was studied from 25° C to 55 $^{\circ}$ C. The effects of TX-114 concentration and equilibration temperature are shown in Fig. 4. The optimum conditions for CPE are 1.5 % w/v TX-114, 1.0 % w/v NaCl and equilibration temperature at 55 $^{\circ}$ C.



Fig. 4 Optimization for CPE of carbaryl (3.0 mg L^{-1}) when (A) is concentration of TX-114 (% w/v) and (B) is equilibration temperature (°C); (*n*=3)

Analytical performance of CPE coupled to

spectrophotometry

Under the optimum conditions, linear range was investigated by detecting the carbaryl derivative at different concentrations using matrix match calibration plot, five concentration levels of carbaryl ranging from 0.5 to 7.0 mg L⁻¹ were prepared in the sample matrix and were analyzed. The calibration plots were constructed using absorbance versus concentration of carbaryl. Limit of detection (LOD) and limit of quantitation (LOQ) were calculated as carbaryl concentration giving a signal equal to the blank signal plus 3 and 10 standard deviation of the blank (n=5), respectively (Santalad et al., 2008).

The typical absorption spectra obtained from the study of calibration plot are shown in Fig. 5. Table 1 summarizes the linear equations and the recoveries obtained from CPE-spectrophotometric and HPLC methods. The results (Table 1) indicated that good linearity with the correlation coefficients higher than 0.99. Using CPE and spectrophotometric method, the LOD and LOQ obtained were 0.10 mg L⁻¹ and 0.50 mg L^{-1} , respectively. While the spectrophotometric method without CPE gave the LOD and LOQ of 1.00 mg L^{-1} and 5.00 mg L^{-1} . It is clearly seen that CPEspectrophotometric method has high sensitivity enough for the detection of carbaryl residue at MRL level. The accuracy was investigated in term of recovery by analyzing the spiked samples (1.0 mg kg⁻¹ carbaryl), while the precision was examined by analyzing the spiked samples in five replicate. The recoveries obtained were in the range 80 to 90 % with the standard deviation less than 10.



Fig. 5 Absorption spectra of spiked cucumber with different concentrations of carbaryl

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 Table 1 Calibration curves and recoveries of carbaryl in

vegetable samples obtained by CPE; (n=5)

Sample	Linear equation	R ²	Recovery
			(%)
			±SD
Spectrophotometry			
Cucumber	Y = 0.165X + 0.036	0.998	90.33±8.1
Cabbage	Y = 0.160X + 0.094	0.999	86.81±6.3
Kale	Y = 0.086X + 0.036	0.990	82.43±3.9
Long bean	Y = 0.082X + 0.032	0.998	85.53±7.2
Mustard	Y = 0.096X + 0.013	0.995	80.22±9.9
*HPLC			
Cucumber	Y = 1848743X + 2563671	0.995	88.80±2.1
Cabbage	Y = 1770791X - 231937	0.996	84.23±6.1
Kale	Y = 1765881X - 109696	0.999	83.54±3.9
Long bean	Y = 1452102X + 576203	0.993	81.43±5.4
Mustard	Y = 1557534X + 211546	0.998	80.44±4.8

*Conditions for HPLC; monolithic column, mobile phase: MeOH/H₂O (50/50 v/v), flow rate: 0.5 mL min⁻¹, injection volume: 20 μ L, detection wavelength: 220 nm.

The results obtained from the proposed method were compared to those obtained from HPLC method based on *t* –test performed at a 95% confidence level. The results indicated that there is no significant difference between the proposed spectrophotometry and HPLC. In addition, under the optimum condition only carbaryl gave colored derivative with high molar absorptivity ($\varepsilon > 1 \times 10^4$ L mol⁻¹ cm⁻¹), while the other carbamates could not form derivative with DMA. Therefore, the proposed method is selective for carbaryl.

Determination of carbaryl in vegetable samples

Five samples of five varieties of vegetables including, cucumber, cabbage, kale, long bean, and mustard were studied. No contamination of carbaryl was observed in any of the vegetables studied.

Conclusion

This study demonstrates a simple spectrometric method using 2,4-dimethoxyaniline as the derivatizing reagent for the determination of carbaryl. The method consists of CPE and spectrophotometry. CPE is an effective preconcentration method providing about 10 time preconcentration compared to the method without CPE. Therefore, the proposed method has a potential to detect the carbaryl residue in vegetables (MRLs = 3.0-10.0 mg kg⁻¹). In addition, the recoveries obtained from the propose method and HPLC were not significantly different. (*t*- test, p = 0.05).

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