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Determination of Butachlor and Pencycuron Residues in Vegetables and Rice: Application of the Macroporous Diatomaceous Earth Column

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ABSTRACT

Butachlor and pencycuron are commonly used pesticides in Taiwan, but still lack official methods for determining butachlor and pencycuron residues in agricultural products. An analytical method using a macroporous diatomaceous earth (MDE) column and florisil cartridge for cleanup procedure was developed for determination of butachlor and pencycuron in vegetables and rice. Butachlor and pencycuron were extracted from crops with acetone and the concentrated extract was transferred into the MDE column, eluted with *n*-hexane. The eluate was concentrated and applied on a florisil cartridge. The cartridge was washed with 5% diethyl ether in *n*-hexane (E/H), and then eluted with 15% E/H (fraction I) and 15% ethyl acetate in *n*-hexane (EA/H) (fraction II). Butachlor residue in fraction I was determined by GC-ECD. Pencycuron residue in fraction II was determined by HPLC-UV at 248 nm. Chinese mustard and rice samples were fortified with butachlor and pencycuron at levels of 0.25~0.75 ppm and analyzed. The recoveries of butachlor and pencycuron were between 84.9~94.9% and 88.3~94.8%, respectively. The detection limits of both pesticides in Chinese mustard and rice were 0.05 ppm.

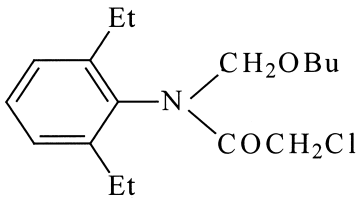
MDE liquid/liquid extraction cartridges provide a means of simplifying and speeding up multiple liquid/liquid extractions.

Key words: butachlor, pencycuron, vegetable, rice, macroporous diatomaceous earth column, florisil cartridge

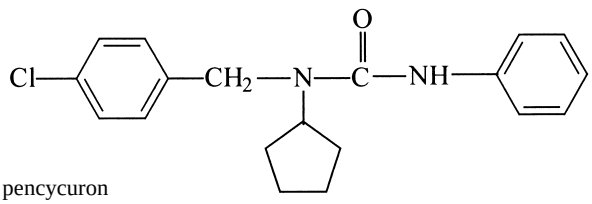
INTRODUCTION

Butachlor is a selective systemic herbicide. It can be degraded at 165˚C but is quite stable under UV light. It is soluble in most organic solvents and its solubility in water is 20 mg/L at 20˚C. Pencycuron, a stable fungicide, is not hydrolyzed even when kept at 25˚C under pH 4 for 280 days. The solubilities of pencycuron at 20˚C in water, methylene chloride, toluene, isopropanol, and *n*-hexane are 0.4 mg/L, 100-1000 g/L, 10-30 g/L, 1-10 g/L, and <10 g/L[1], respectively. The chemical structures of these two pesticides are shown in Figure 1[1]. The active group of butachlor is readily hydrolyzed in alkaline solution, where Cl group of butachlor is replaced by OH group. The herbicide activity under such conditions is therefore lost. According to the Tolerances for the Residues of Pesticides[3] set by the Department of Health, ROC, the tolerance levels for the residue of butachlor in both rice and lobule vegetables is 0.5 ppm, and residues of pencycuron in rice and lobule vegetables are 0.5 and 5.0 ppm, respectively. Butachlor is in great demand in the domestic market and is widely used as a pre-emergence herbicide in paddy rice fields. It is usually mixed with other pesticides, such as chlomethoxynil, bensulfuron-methyl, oxadiazon, or dymron to make a mixture to be both pre- and post-emergence herbicides as well as to inhibit both annual and perennial weeds from growing. Pencycuron is used to cure *Rhizoctonia solani* of paddy rice[4] and damping-off of crucifer. Pencycuron is in the top ten for domestic sales.

The residues of butachlor in agricultural products can be detected by gas chromatography (GC) equipped with ECD, MS, or NPD[5-7], and the residues of pencycuron can be detected using GC-MS[6] or high performance liquid chromatography (HPLC) equipped with a UV detector[8-9]. Liquid-liquid extraction by separatory funnel is traditionally used as a pretreatment method for the detection of residues of pesticides in agricultural products. This method, however, is time consuming and uses too much solvent. Iijima *et al*[6] applied a Macroporous Diatomaceous Earth Column (MDE column) instead of liquid-liquid extraction by separatory funnel to analyze the multiresidual pesticides in agricultural



(a) butachlor



(b) pencycuron

Figure 1. Chemical structures of (a) butachlor and (b) pencycuron.

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products. This method is worthy of trying. In this study, the MDE column and solid phase extraction (SPE) cartridge were used as tools for sample cleanup and the low toxic solvents were selected to research the analytical conditions. An analytical method needs to be developed which is time efficient, environmentally friendly, and has high recovery, satisfactory reproducibility, and high sensitivity in detection limits. The developed method could be a reference for related authorities to establish the standard inspection method.

MATERIALS AND METHODS

I. *Materials*

The Chinese mustard and rice samples were purchased from supermarkets or traditional markets. MDE column (Chem Elut, 20 mL) was made by Varian (USA) and florisil SPE cartridge (1000 mg, 6 mL) was purchased from J&W Scientific (CA, USA).

II. *Reagents*

Acetone and *n*-hexane used in this study were residual grades. Methanol and ethyl acetate was LC grade and diethyl ether was reagent grade. The standards of butachlor and pencycuron (of purity 99%) were purchased from Riedel-de Haen AG (Germany).

III. *Instruments and Analytical Conditions*

(I) *GC*

A Varian 3400 GC equipped with an ECD, a DB-1 or DB-5 capillary column (30 m × 0.53 mm i.d., J&W Scientific, CA, USA), and a Shimadzu C-R4A integrator was used in this study. The temperatures of oven, injection port, and detector were 210˚C, 250˚C, and 300˚C, respectively. The sample volume of 1 μL was injected. The flow rate of carrier gas, nitrogen, was 10 mL/min.

(II) *HPLC*

A Hitachi (Japan) HPLC system equipped with a Hitachi L-6200 pump, a HyPURITY^{TM} Elite C_{18} analytical column (25 cm × 4.6 mm i.d., 5 μm, Hypersil, Runcon, UK), a Hitachi L-4250 UV detector, and a Shimadzu C-R4A integrator was used. The UV detector was set at 248 nm. The mobile phase system was methanol: water (72: 28, v/v) pumped at 1.0 mL/min. The injection volume was 20 μL.

(III) *Gas Chromatography / Electron Impact Mass Spectrometer (GC / EIMS)*

A HP-5890 series II GC equipped with a HP 5970 B quadruple mass selective detector (MSD) and a J&W Rtx-5 analytical column (30 m × 0.25 mm i.d.) was used. A HP 59944A MS ChemStation software in HP 340C computer was used as the data analysis system. The oven temperature was programmed at 150˚C for 3 min followed by rising up to 250˚C at 10˚C/min. The injection port and MSD interface temperatures were 250˚C and 280˚C, respectively. The injection volume was 1 μL. The carrier gas was helium and the head pressure was set at 8 psi. The ionization energy was set at 70eV.

IV. *Methods*

(I) *Preparation of Standard Solutions*

Butachlor (100 mg) was accurately weighed into a 100-mL volumetric flask and *n*-hexane was then added to the volume to be a stock solution. A series of standard solutions were prepared by diluting the stock solution with *n*-hexane. Pencycuron (100 mg) was accurately weighed into a 100-mL volumetric flask and methanol was then added to the volume to make a stock pencycuron solution. The stock solution was then pipetted and diluted with methanol as needed to prepare a series of standard solutions.

(II) *Preparation of Sample Solutions*

1. Extraction

The rice sample was ground into powder and 10 g of which was then mixed with 18 mL of water and kept standing for 1 hr, then extracted with 100 mL acetone for 3 min. The Chinese mustard sample was homogenized and 20 g of which was then sampled and extracted with 100 mL of acetone for 3 min. The extraction solution was then filtered under suction. The residues and container were then washed with another 50 mL of acetone, which was then filtered. The filtrates were combined into an evaporation vessel and evaporated at 35~40˚C under vacuum. The concentrate (~18 mL) was applied onto a MDE column (Chem Elut, 20 mL, Varian, USA) and kept standing for 10 min allowing the concentrates evenly dispersed in MDE column. The concentrate in MDE column was eluted with 100 mL of *n*-hexane, evaporated to dryness, and then dissolved in 5 mL of *n*-hexane.

2. Solid Phase Extraction for Sample Cleanup

The above concentrate (2 mL) was loaded into a florisil cartridge (1000 mg, 6 mL, J&W Scientific, CA, USA), which was rinsed with *n*-hexane prior to applying samples. The concentrate in cartridge was then eluted stepwise with 10 mL of 5% diethyl ether in *n*-hexane (5% E/H), 15 mL of 15% diethyl ether in *n*-hexane (15% E/H), and 15% ethyl acetate in *n*-hexane (15% EA/H). The above eluents were separately collected. The eluent 15% E/H was evaporated to dryness, dissolved in 2 mL of *n*-hexane, and analyzed with GC-ECD for detection of butachlor. The eluent 15% EA/H was evaporated to dryness, dissolved in 2 mL of methanol, filtered through a 0.45 μm nylon membrane, and analyzed with HPLC-UV at 248 nm for detection of pencycuron. A flow

diagram for the analytical procedures is shown in Figure 2.

(III) *Identification and Quantification*

A series of concentrations of butachlor were prepared by diluting the butachlor stock solution with *n*-hexane and 1 μL of each was injected to GC. A series of concentrations of pencycuron were prepared by diluting the pencycuron stock solution with methanol and 20 μL of each was injected to HPLC. The standard curves in peak area verses concentration were plotted and the linear equations were calculated after a regression analysis. The sample and standard solutions were accurately taken and injected into GC or HPLC according to the analytical conditions as described. The peak areas of the butachlor or pencycuron in sample solutions were compared to those in standard solutions. The amounts of butachlor and pencycuron in test samples were thus calculated based on the standard curves.

(IV) *Recovery Test*

Butachlor and pencycuron standards were spiked into ground rice and homogenized Chinese mustard. The spiked samples were then kept in a hood for 1 hr to evaporate the solvent residues. The test samples with 0.25, 0.50, and 0.75 ppm butachlor and pencycuron were thus prepared. Each concentration of spiked samples was prepared in triplicate. A blank sample without standards was also prepared. The preparation of sample solution was as described. Recoveries for butachlor and pencycuron were calculated after GC or HPLC analysis.

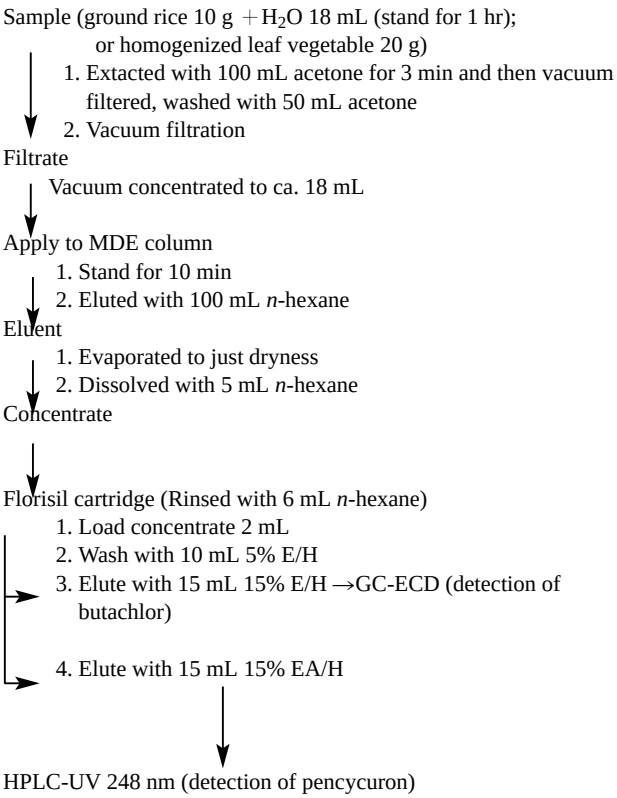

Figure 2. Analytical procedures for determiniing butachor and pencycuron residues in crops.

(V) *Detection Limit Test*

A suitable amount of butachlor and pencycuron was spiked to a homogenized sample. The test sample solution was prepared as described. The detection limit was estimated on the basis of signal to noise (S/N) ratio greater than 3.

RESULTS AND DISCUSSION

I. *Preparation of Sample Solutions*

(I) *The Elution of Butachlor and Pencycuron from MDE Column*

In this study, a macroporous diatomaceous earth (MDE) column, which is a commercialized liquid/liquid extraction cartridge, was used to replace the traditional separatory funnel for liquid/liquid extraction. MDE column is a polypropylene (PP) cartridge packed with highly pure and inert macroporous diatomaceous earth. The high surface area makes high efficiency in interaction between sample and extraction solvent without emulsion. MDE column is easy to use and the extraction process can be done without any suction, only relying on gravity. The column is packed with a phase-separation filtering material to protect the organic eluents from being contaminated with aqueous matrix. The advantages of using MDE column are as follows: 1. The device is simple and easy to use. 2. Several samples can be processed simultaneously. 3. Emulsion would not happen by using MDE column. 4. It is not necessary to dehydrate the eluent by anhydrous sodium sulfate. To bring the liquid/liquid extraction efficiency into full play, the sample was loaded into MDE column and kept standing for 10 min allowing sample solutory to evenly disperse in column. A stopcock was installed upon column to control the elution speed at 3~5 mL/min after applying extraction solution. This could increase recovery and reproducibility.

According to literature[6], *n*-hexane was used as eluting solvent for MDE column application. The recovery of standards was researched by collecting and analyzing each fraction of eluent (25 mL). The results are shown in Figure 3. The first fraction of eluent could give about 80% recovery for both butachlor and pencycuron. The standards could be fully recovered when 75 mL of eluent was collected. Practically, 100 mL of *n*-hexane was used as elution solvent and about 80 mL of eluent was collected that could result in a satisfactory recovery (Table 1). The MDE column operation was comparable to the traditional liquid/liquid extraction by using separatory funnel in terms of recovery. Moreover, MDE column is easy to use, time economical, and no emulsion problem. The eluent from an MDE column is clearer than when the extraction solution is processed by a separatory funnel.

(II) *Elution of Butachlor and Pencycuron from Florisil SPE Cartridge*

Standards were spiked to cartridge and different combinations of *n*-hexane (H), diethyl ether (E), and ethyl acetate (EA) were prepared to study the eluting conditions. Initially, the cartridge was eluted stepwise with 10 mL each of *n*-hexane, 5% EA/H, 15% EA/H, and 20% EA/H. Results are shown in Figure 4(a). They show that *n*-hexane was not capable of eluting butachlor and pencycuron; while 5% EA/H (10 mL) could completely elute out butachlor. Pencycuron, a higher polar compound as compared to butachlor, could not be completely eluted until 15% EA/H was applied. Rice and Chinese mustard were used as test samples to study the cleanup conditions. The cartridge was washed with 10 mL of *n*-hexane and eluted stepwise with 15 mL each of 5% EA/H and 15% EA/H and collected separately (cleanup condition A). The eluent 5% EA/H was evaporated under vacuum to dryness and reconstituted with *n*-hexane followed by injection to GC-ECD. A clean chromatogram without interference peaks was generated from rice sample. While many interference peaks appeared on the GC chromatogram from Chinese mustard sample. A small interference peak even appeared at the same retention time as butachlor (Figure 5). The cleanup condition was then modified to lower the eluting solvent polarity. Results showed that 3% EA/H could fully eluted out butachlor as shown in Figure 4(b); however, this condition was not capable of removing interference peaks. Based on the results showed in Figure 4(c), the cleanup condition was

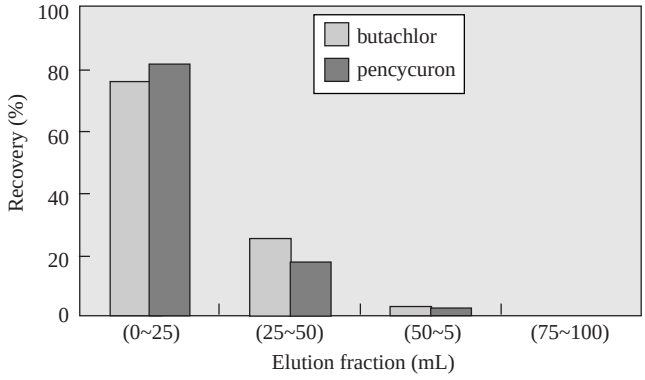

Figure 3. Elution profile of butachlor and pencycuron from MDE column by *n*-hexane.

Table 1. The average recoveries of butachlor and pencycuron spiked into rice and chinese mustards

Sample	Spiked level (ppm)	Recovery[a] (%)	
		butachlor	pencycuron
Rice	0.25	89.6 (3.5) [b]	91.8 (1.5)
	0.50	94.9 (1.8)	89.2 (1.2)
	0.75	91.2 (2.9)	88.3 (4.1)
Chinese mustard	0.25	89.9 (1.8)	90.4 (4.8)
	0.50	88.2 (4.4)	93.1 (2.8)
	0.75	84.9 (1.8)	94.8 (2.3)

[a]average of triplicate.
[b]value in the parenthesis is coefficient of variation (CV, %).

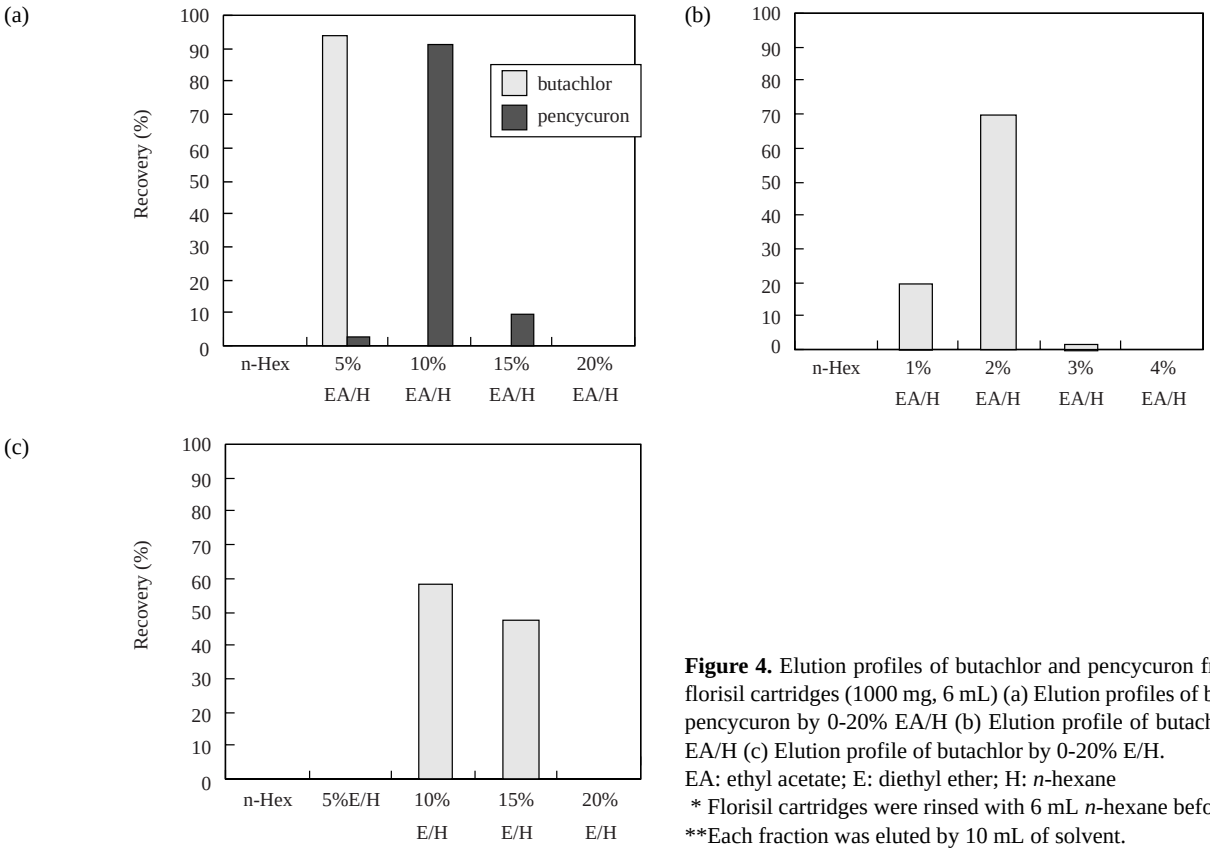

Figure 4. Elution profiles of butachlor and pencycuron from Sep-Pak florisil cartridges (1000 mg, 6 mL) (a) Elution profiles of butachlor and pencycuron by 0-20% EA/H (b) Elution profile of butachlor by 0-4% EA/H (c) Elution profile of butachlor by 0-20% E/H.
EA: ethyl acetate; E: diethyl ether; H: *n*-hexane
* Florisil cartridges were rinsed with 6 mL *n*-hexane before use.
**Each fraction was eluted by 10 mL of solvent.

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modified as follows: the cartridge was washed with 10 mL of 5% E/H and eluted stepwise with 15 mL each of 15% E/H and 15% EA/H (cleanup condition B). The interference impurities at the same retention time as butachlor were thus eliminated as shown in Figure 6. The eluent 15% EA/H was tested for pencycuron and showed no interference peaks appeared (Figure 7) indicating this cleanup condition was satisfactory.

The solvents, *n*-hexane, diethyl ether, and ethyl acetate, used in this study are all low in toxicity and capable of replac-

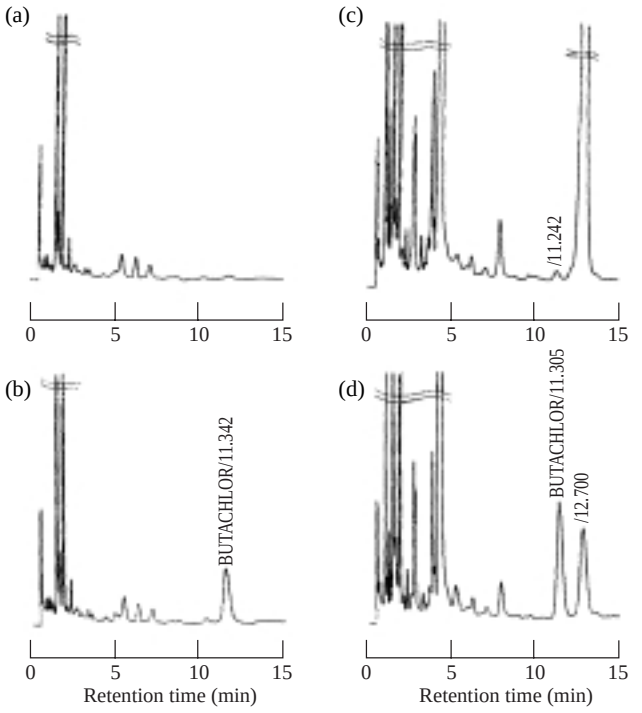

Figure. 5. GC chromatograms of (a) rice (b) rice spiked with 0.5 ppm butachlor (c) Chinese mustard (d) Chinese mustard spiked with 0.5 ppm butachlor (cleanup procedure A).
GC conditions : column: DB-5; oven temperature 210˚C; injector temp. 250˚C ; carrier gas: N_2, 10 mL/min; Detector: ECD.

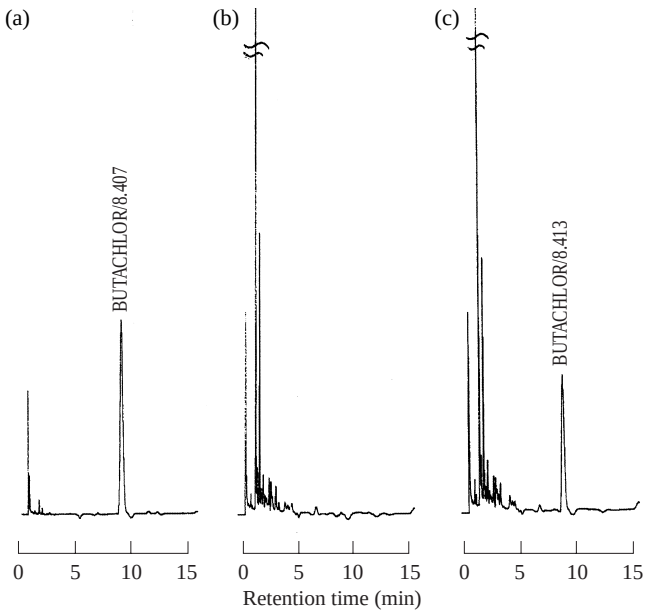

Figure 6. GC chromatograms of (a) butachlor standard (b) Chinese mustard blank (c) Chinese mustard spiked with 0.5 ppm butachlor (cleanup procedure B).
GC conditions : column: DB-1; oven temperature for 210˚C; injector temp. 250˚C ; carrier gas: N_2, 10 mL/min; Detector: ECD.

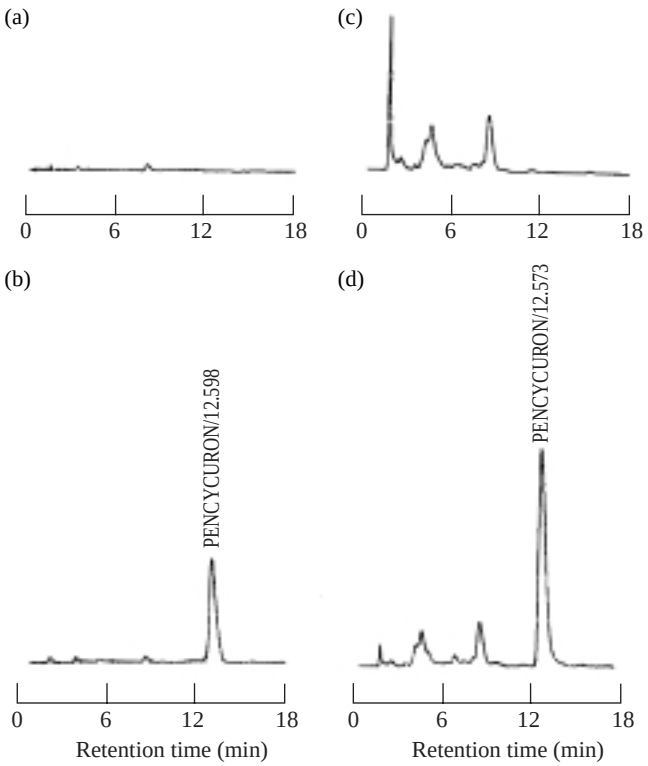

Figure 7. HPLC chromatograms of (a) rice blank (b) rice spiked with pencycuron (c) Chinese mustard blank (d) Chinese mustard spiked with pencycuron.
HPLC conditions: Column: HyPURITY Elite C_{18}; Mobile phase: MeOH: H_2O (72:28, v/v); Flow rate: 1.0 mL/min ; Detector: UV at 248 nm.

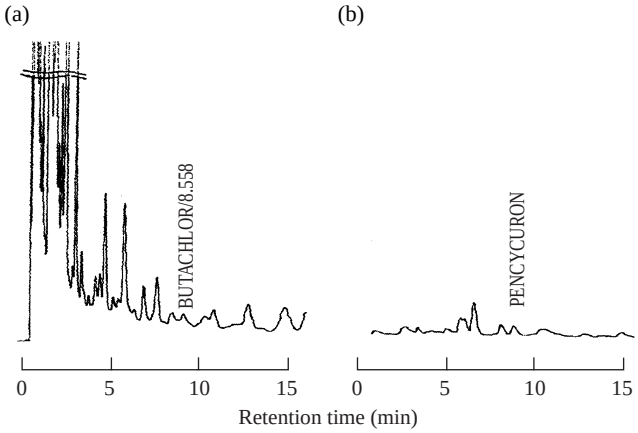

Figure 8. Chromatograms of the detection limit for (a) butachlor and (b) pencycuron in Chinese mustard (0.05 ppm).
GC conditions are shown in Fig. 6.
HPLC conditions are shown in Fig. 7.

ing chloroform and methylene chloride, the carcinogenic and highly polluting agents, for pesticide analysis.

II. *Recovery Test*

The recoveries of butachlor and pencycuron from rice and Chinese mustard spiked with 0.25~0.75 ppm standards are listed in Table 1. The recoveries of butachlor and pencycuron from rice were 89.6~94.9% with 1.8~3.5 coefficient of variation and 88.3~91.8% with 1.2~4.1% coefficient of variation, respectively. While the recoveries of butachlor and pencycuron from Chinese mustard were 88.2~89.9% with 1.8~4.4% coefficient of variation and 90.4~94.8% with 2.3~4.8% coefficient of variation, respectively. Above data shows both satisfactory recovery and reproducibility were achieved.

III. *Detection Limit Test*

The detection limits of both butachlor and pencycuron were all determined to be 0.05 ppm. Chinese mustard was used as test sample because the rice sample could generate

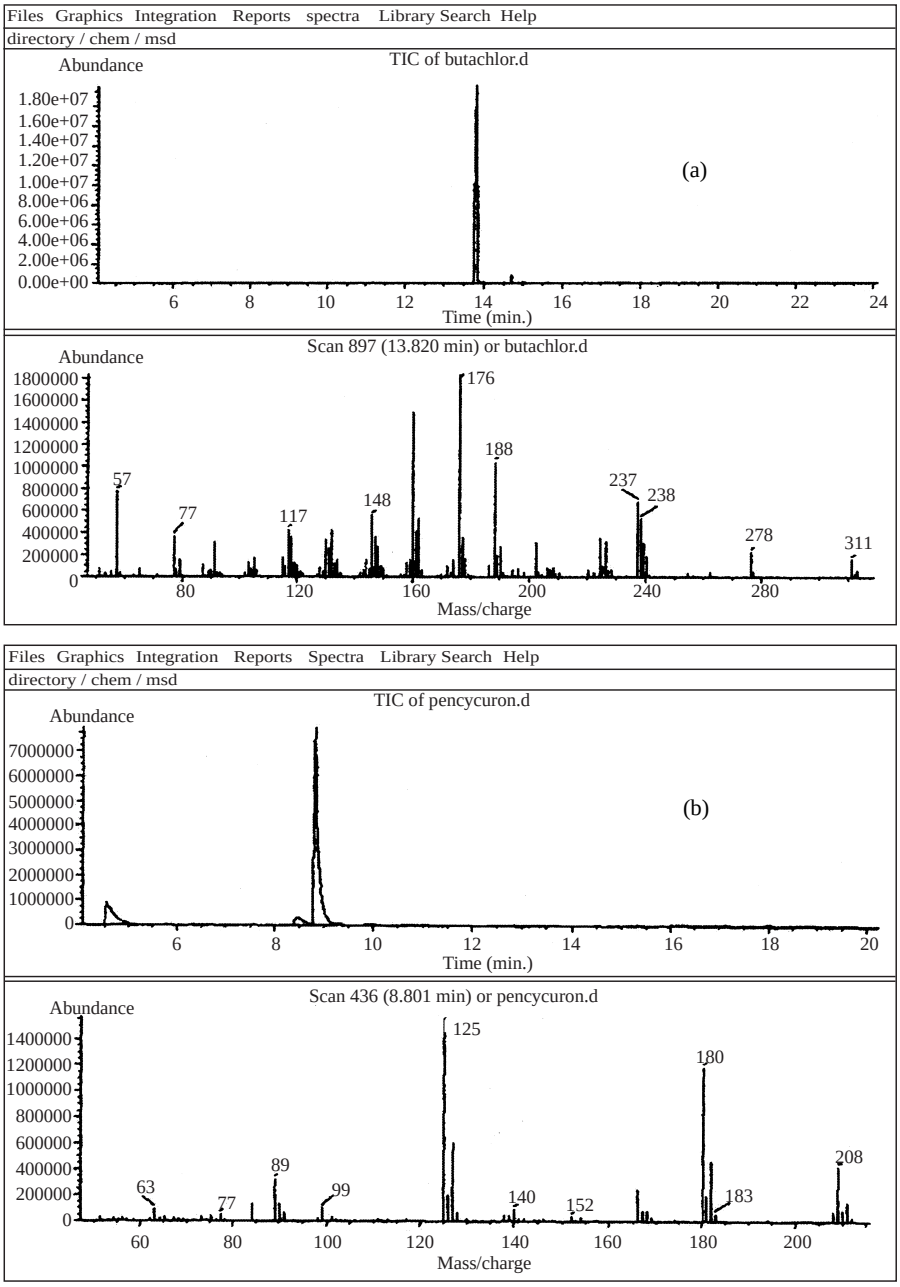

Figure 9. GC-MS spectrum of (a) butachlor and (b) pencycuron.
GC-MSD conditions: Column: Rtx-5; Initial temp.:150˚C; Initial time: 3 min; increasing temp. 10˚C/min, Final temp.: 270˚C; Injector port temp.: 250˚C; MSD interface temp.: 280˚C; Ionization energy: 70 eV.

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less interference peaks on chromatogram. Chromatograms of the detection limit for butachlor and pencycuron in Chinese mustard spiked with 0.05 ppm standards are shown in Figure 8. Based on S/N greater than 3, their detection limits were determined to be 0.05 ppm.

IV. *GC Analysis*

The GC-MS spectra of butachlor and pencycuron are shown in Figure 9. The fragment m/e 311 is the parent ion of butachlor indicating the molecule of butachlor is partly retained after impacted by electron. However, the parent ion of pencycuron can not be found on mass spectrum because it is heat-labile compound. The GC-MS spectra of standards can be used as references for further compound confirmation.

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應用多孔性矽藻土管柱於蔬菜及米中丁基拉草及賓克隆殘留量之分析

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摘　要

丁基拉草（butachlor）及賓克隆（pencycuron）分別係國內常用之殺草劑及殺菌劑，此兩種農藥於農產品中之殘留量並無公告之檢驗方法，本研究建立了應用多孔性矽藻土管柱（Macroporous Diatomaceous Earth Column; MDE column）及矽酸鎂固相萃取匣（florisil cartridge for solid phase extraction;）進行檢體之淨化以檢測農產品中丁基拉草（butachlor）及賓克隆（pencycuron）殘留量之方法。本檢驗係以丙酮為抽出溶媒，濃縮液通入多孔性矽藻土管柱，以正己烷沖提，再經矽酸鎂固相萃取匣淨化，先以5% 乙醚之正己烷溶液（diethyl ether/n-hexane; E/H）沖洗，續以15% E/H 及15% 乙酸乙酯之正己烷溶液（ethyl acetate/hexane; EA/H）沖提，分別收集15% E/H 沖提液（I）及15% EA/H 沖提液（II），沖提液（I）以氣相層析儀附電子捕獲檢出器（GC-ECD）偵測丁基拉草，沖提液（II）以高效液相層析儀附紫外光檢出器（HPLC-UV） 於波長248 nm 偵測賓克隆。添加丁基拉草及賓克隆各0.25~0.75 ppm 於米及小白菜中，丁基拉草之回收率為84.9~94.9% ，賓克隆之回收率為88.3~94.8%。本檢驗方法之最低檢出限量兩者皆為0.05 ppm 。

利用多孔性矽藻土管柱取代傳統之液、液分配萃取，及以矽酸鎂固相萃取匣進行檢體之淨化來檢測農產品中丁基拉草及賓克隆殘留量，回收率佳、再現性良好且操作簡易、快速，並具足夠之靈敏度，可作為相關單位訂定標準檢驗方法之依據，並進一步評估應用於農產品中農藥多重殘留之檢測。

關鍵詞：多孔性矽藻土管柱，蔬菜，米，丁基拉草，賓克隆，矽酸鎂固相萃取匣