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Determination of Butocarboxim Residue in Agricultural Products by HPLC with Post-Column Derivatization System

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ABSTRACT

A method using high performance liquid chromatography (HPLC) for the determination of butocarboxim in agricultural products was developed. Butocarboxim was extracted from samples with acetone and the extract solution was concentrated. The residue was dissolved in sodium chloride solution and partitioned with *n*-hexane. The aqueous phase was collected followed by extracted with dichloromethane, which was then evaporated to a volume of 2 mL prior to passing through an aminopropyl cartridge for sample clean-up. Determination of butocarboxim residue in crops was carried out by HPLC equipped with a post-column derivatization system and a fluorescence detector. HPLC separation was performed on a Lichrospher 60 RP-Select B column eluted with a mobile phase of acetonitrile-water (25:75, v/v). Butocarboxim was hydrolyzed at 90°C under alkaline conditions and subsequently reacted with *o*-phthaldialdehyde (OPA) / 2-mercaptoethanol reagent via a post-column reactor to generate a fluorophore, which was then detected with a fluorescence detector at Ex 340 nm and Em 455 nm. Average recoveries from radishes and bamboo sprouts, which were spiked with 0.1~0.3 and 0.2 ppm butocarboxim, respectively, were in the range of 81.9~82.6%. The detection limit was 0.05 ppm. No butocarboxim residue was detected in 10 commercial products including radishes, carrots, and bamboo sprouts.

Key words: **agricultural products, butocarboxim, aminopropyl cartridge, high performance liquid chromatography (HPLC), post-column derivatization system, fluorescence detection.**

INTRODUCTION

Butocarboxim, which belongs to the class of systemic insecticides, is an isomer of aldicarb. Its potency is comparable to that of aldicarb, while its toxicity to mammals is much less than aldicarb [1,2]. The tolerance level of butocarboxim in root crops is set at 0.1 ppm according to the Department of Health in Taiwan[3]. The chemical structure presented in Figure 1 reveals butocarboxim is a carbamate pesticide. Analysis of carbamate pesticides was routinely performed using an official method entitled "Method of Test for Pesticide Residues in Foods-Multiresidue Analysis" [4]

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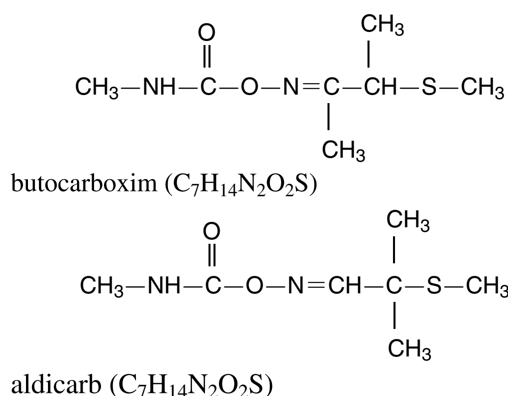


Figure 1. Chemical structures of butocarboxim and aldicarb.

announced by the Department of Health. Using high performance liquid chromatography (HPLC) equipped with a post-column reaction system where the compounds of interest are derivatized with *o*-phthaldialdehyde (OPA) / 2-mercaptoethanol reagent under high temperature and alkaline conditions, the analytes are separated and derivatized to fluorophors, which are then detected with a fluorescence detector. This official method is capable of detecting 8 carbamate pesticides (not including butocarboxim) and 4 of their metabolites simultaneously. The analytical technique comprising of post-column reaction and fluorescence detection has been widely used to analyze carbamate pesticides. Analysis of butocarboxim using a post-column reaction system has also been reported[5~8]. However, a solid phase extraction (SPE) for sample clean-up has not yet been applied in routine multiresidue analysis. Analysis without sample clean-up could result in higher noise background or interference peaks appearing on the chromatogram. A pre-column derivatization followed by HPLC[9] or Gas Chromatography (GC)[2,10] detection for butocarboxim analysis has also been reported in the relevant literature. However, the pre-column derivatization procedure is too complex to be applicable for a routine analysis. Furthermore, butocarboxim is a thermal labile compound, which is not suitable to be analyzed by GC. The purpose of this study was to develop a reliable HPLC method using SPE for sample clean-up and a post-column derivatization system followed by a fluorescence detection for rapid analysis of butocarboxim in wide varieties of crops. The established method is expected to be adopted as an official method for related authorities to detect butocarboxim in crops.

MATERIALS AND METHODS

I. *Materials*

Test samples of radishes (*Raphanus sativus* L.), carrots (*Daucus carota* L.), Co-ba *(Zizania latifolia* Turcz) and bamboo sprouts (*Leleba edulis* Odashima and *Sinocalamus latiflorus* McCluse) were purchased from traditional markets.

II. *Reagents*

Residue grade acetone, *n*-hexane, and dichloromethane, and LC grade methanol and acetonitrile were used in this study. Sodium sulfate anhydrous, sodium chloride, sodium hydroxide, OPA, disodium tetraborate, and 2-mercaptoethanol were reagent grade. The butocarboxim standard (of purity 99%) was obtained from Riedel de Haen AG (Germany).

III. *Methods*

(I) *Preparation of Standard Solution*

The butocarboxim standard (100 mg) was accurately weighed into a 100-mL volumetric flask and methanol was then added to the volume as a stock solution. Standard solution was prepared by diluting the stock solution to the appropriate concentrations as needed.

(II) *Sample Preparation*

1. *Extraction*

A mixture of sample (20 g) and acetone (80 mL) was homogenated for 3 min and then filtered. The residue and container were rinsed with 40 mL of acetone. The acetone extracts were combined

and concentrated at 35~40°C using a vacuum rotary evaporator. The residue was dissolved in 50 mL of 10% sodium chloride solution and partitioned with 30 mL of *n*-hexane in a separation funnel for removal of co-extracts. Aqueous phase was collected and extracted with 70 mL of dichloromethane twice. The dichloromethane was combined, dehydrated with anhydrous sodium sulfate, and evaporated to dryness at 35~40°C under vacuum to obtain the sample residue.

2. *Sample Clean-up*

The above sample residue was dissolved in 2 mL of dichloromethane and applied on a aminopropyl cartridge (500 mg, 3 mL, J. T. Baker, USA), which was rinsed with 5 mL of dichloromethane in advance. The cartridge was eluted with 10 mL of dichloromethane and the eluate was then evaporated to dryness under vacuum. The dry residue was dissolved in 1 mL of acetonitrile and filtered through a 0.45 μm nylon membrane disc prior to HPLC analysis.

(III) *Reagent Preparation*

1. *0.05 N Sodium Hydroxide Solution*

An equal amount of sodium hydroxide in water (1:1, w/w) was left standing for 10 days. Twenty-seven mL of supernatant was diluted with water to a volume of 100 mL and 10 mL of which was continuously diluted with water to a volume of 1000 mL to make up a 0.05 N sodium hydroxide solution.

2. *0.05 N Sodium Borate Solution*

Disodium tetraborate ($Na_2B_4O_7 \cdot 10\ H_2O$) (19.1 g) was accurately weighed and dissolved in de-ionized water by heating. After cooling down, the solution was diluted with de-ionized water to a volume of 1000 mL.

3. *OPA Solution*

OPA (500 mg) was accurately weighed and dissolved in 10 mL of methanol, which was then diluted with 0.05 N sodium borate solution to a volume of 1000 mL. The final solution was filtered through a 0.45 μm membrane and then spiked with 1 mL of 2-mercaptoethanol to make an OPA solution.

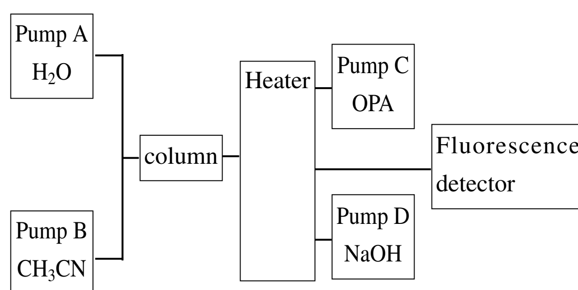


Figure 2. System configuration of HPLC with post-column reaction.

(IV) *HPLC Conditions*

Shimadzu (Japan) HPLC equipped with the following devices was used (Figure 2):

1. Solvent delivery system (Model L6200 pump): to deliver mobile phase, sodium hydroxide solution, and OPA solution.

2. Post-column reactor: including a thermal static device kept at 90°C, a sodium hydroxide solution reaction loop (2 m x 0.5 mm i.d., stainless steel), and a OPA solution reaction loop (2 m x 0.5 mm i.d., stainless steel).

3. RT-551 Spectrofluorometric detector: set at Ex 340 nm and Em 455 nm. Analytical column was Lichrospher 60 RP-Select B (5 μm, 250 x 4.0 mm i.d., Merck). A mobile phase of acetonitrile : water (25/75, v/v) pumped at 1.0 mL/min flow rate was used. Both sodium hydroxide and the OPA solution were pumped at 0.5 mL/min flow rate. Sample injection volume was 20 μL.

(V) *Identification and Quantification of Butocarboxim*

After sample preparation as described above, 20 μL of sample solution was injected into the HPLC device. Butocarboxim was tentatively identified by comparing the retention time of the peak in the sample with that of the standard. The calibration graph was obtained by linear regression of peak areas of compound versus concentrations

over the range from 1 to 8 μg/mL. The butocarboxim content in samples is expressed as ppm.

(VI) *Recovery Test*

A recovery test was carried out in triplicate for each concentration and performed by spiking 0.1~0.3 ppm and 0.2 ppm butocarboxim in radishes and bamboo sprouts, respectively. Preparation of the spiked sample as well as blank sample was as described above.

(VII) *Test for Limit of Detection*

The limit of detection was calculated on the basis of a signal-to-noise ratio of 3, after an appropriate level of butocarboxim standard was spiked to blank homogenate, prepared as described above, and then analyzed by HPLC.

RESULTS AND DISCUSSION

I. *Sample Preparation*

(I) *Extraction*

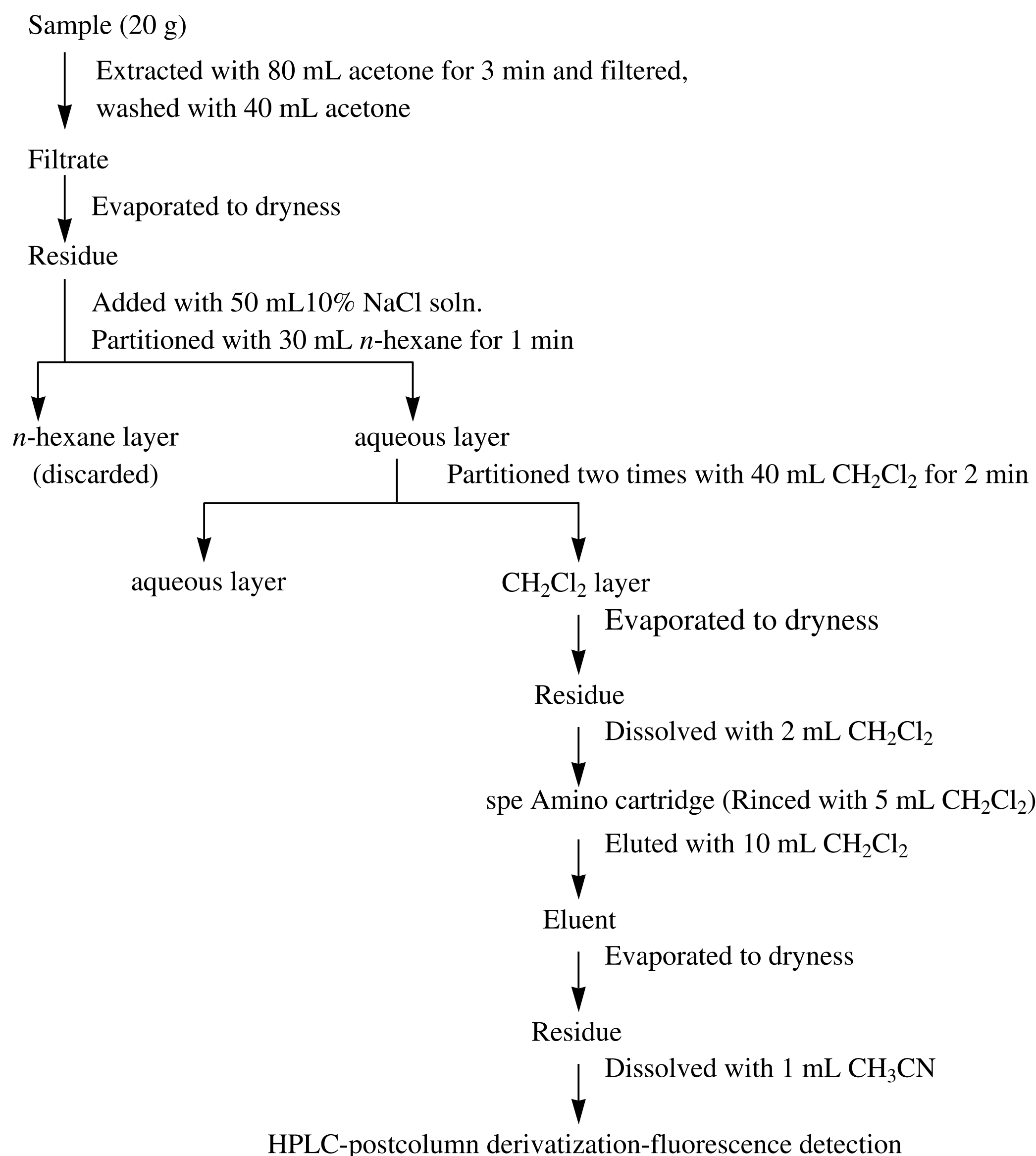


Figure 3. The analytical procedure for determining butocarboxim residue in crops.

Methanol : water (1:1, v/v) [8] and acetone [4-7, 10] have been reported to be the initial extraction solvents for butocarboxim residue analysis in crops. In this study, radish samples were spiked with 0.1 ppm butocarboxim and extracted with acetone, acetonitrile, methanol or methanol/water (1:1. v/v) solution to compare the extraction efficiency of the solvent. Because a severe emulsion effect was observed during partitioning with the methanol/water (1:1, v/v) extract and the methanol extract could result in too many interference peaks on the HPLC chromatogram, the above two solvents were not suitable as extraction solvents. Acetonitrile could yield the highest butocarboxim recovery and less interference. However, the Immediately Dangerous to Life or Health Concentration (IDLH) of acetonitrile (4000 ppm) is much lower than that of acetone (20000 ppm). Acetone was therefore used as an extraction solvent instead of acetonitrile due to its lower toxicity. The procedure for sample preparation is schematically presented in Figure 3. Sample partition with *n*-hexane for removal of co-extracts was not considered in the early stage of this research, nor in the literature[1-10]. Preliminary study showed that the preparation of bamboo sprout samples without *n*-hexane treatment could lead to precipitation in the test solution and low butocarboxim recovery. Sample preparation with *n*-hexane partitioning could prevent precipitation, increase butocarboxim recovery and improve resolution on the HPLC chromatogram as well.

(II) *Solid Phase Extraction*

Solid phase extraction was not officially used as a clean-up procedure for carbamate pesticide analysis according to the method announced by the Department of Health[4]. However, the sensitivity and accuracy in identification and quantification of the target compound could be affected by the interference when post-column reaction combined with HPLC-fluorescence detection is used. Solid phase extraction for sample clean-up was therefore tested in this study to remove interference from radish and carrot extracts. Of the florisil, silica, and aminopropyl cartridges tested, the aminopropyl cartridge gave the best butocarboxim recovery (ca. 100%) and sample clean-up efficiency. Using the silica cartridge could only yield ca. 60% recovery after eluted with the dichloromethane/methanol solution. Figure 4 shows the HPLC chromatograms of radishes spiked with butocarboxim with or without *n*-hexane partition followed by passing through an aminopropyl cartridge. As can be seen, an aminopropyl cartridge was capable of removing interference from the co-extract and thus gave a chromatogram with a clean background. Application of an aminopropyl cartridge on the sample preparation could also increase the life of HPLC column, instrument stability, and accuracy in results as well.

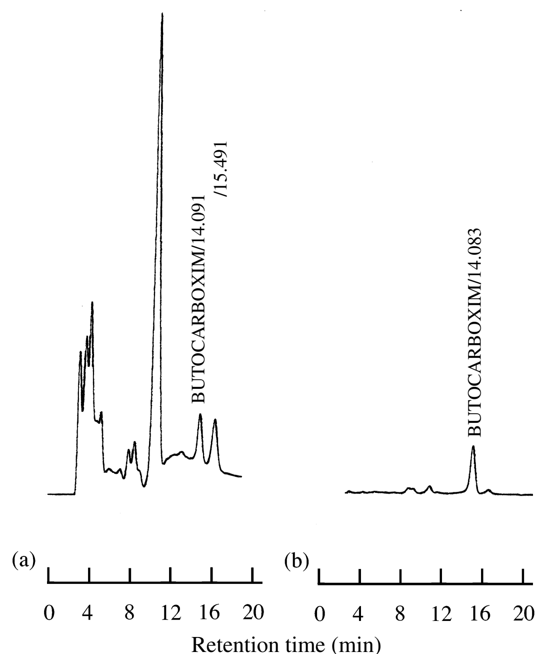
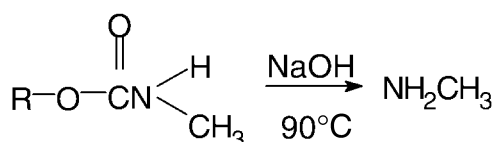


Figure 4. HPLC chromatograms of radish spiked with butocarboxim (a) before (b) after *n*-hexane partition and aminopropyl cartridge cleanup.
HPLC Column: Lichrospher 60 RP-Select B; Mobile phase: CH_3CN: H_2O (25:75, v/v), flow rate: 1.0 mL/min ; NaOH soln. flow rate: 0.5 mL/min; OPA soln. flow rate: 0.5 mL/min; Reactor temperature: 90°C; Detector: Fluorescence with excitation at 340 nm and emission at 455 nm.

II. *HPLC Conditions*

(I) *Principle*

After separation by the HPLC column, the carbamate pesticide is hydrolyzed in the reaction loop under alkaline conditions while at an elevated temperature to form a methyl amine compound, which is then reacted with OPA / 2-mercaptoethanol reagent to give a carbamate derivative. The resulting product is a fluorophore and



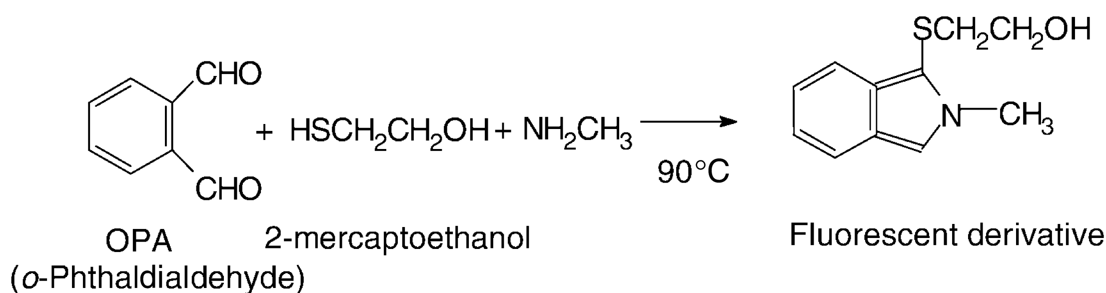


Figure 5. The reaction scheme for forming fluorescent derivative of carbamate.

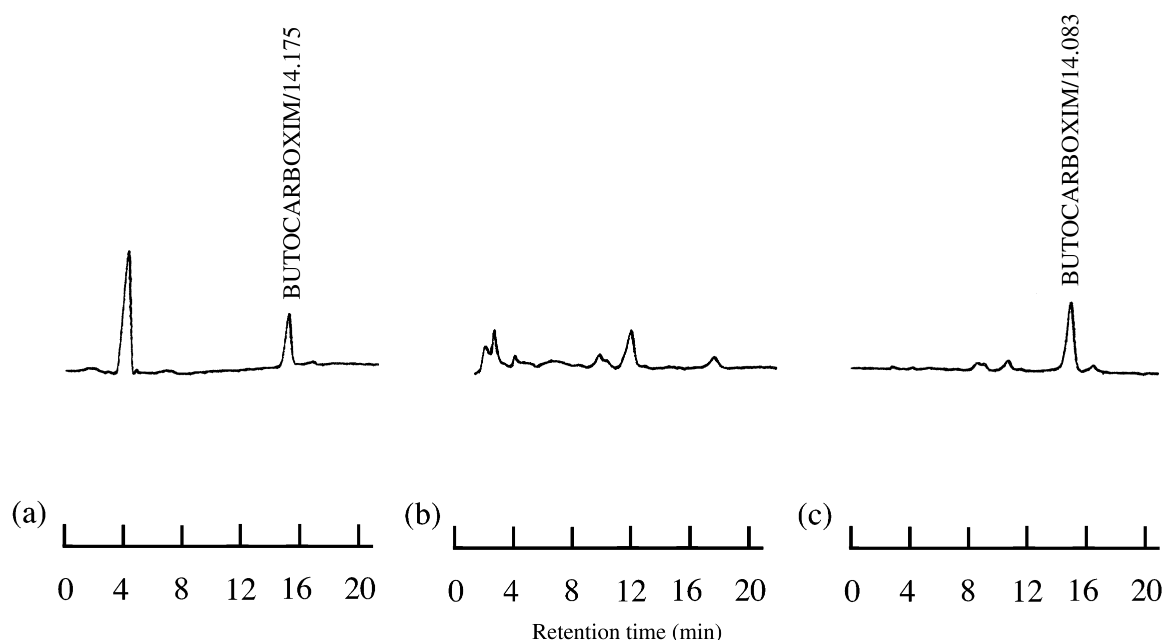


Figure 6. HPLC chromatograms of (a) butocarboxim standard (b) radish blank (c) radish spiked with 0.2 ppm butocarboxim.
HPLC conditions are shown in Fig. 4.

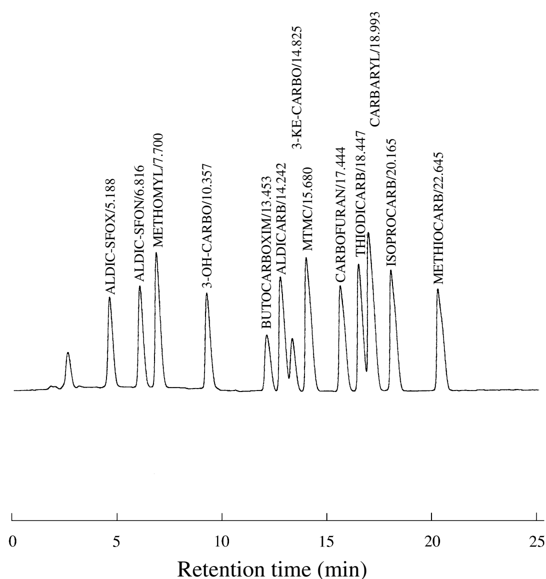


Figure 7. HPLC chromatograms of 9 carbamate pesticides and four of their metabolites.
HPLC Column: Lichrospher 60 RP-Select B; Mobile phase: gradient, CH_3CN: H_2O (12:88, v/v) → 30 min → CH_3CN: H_2O (30:70, v/v), flow rate: 1.5 mL/min ; NaOH soln. flow rate: 0.5 mL/min; OPA soln. flow rate: 0.5 mL/min Reactor temperature: 90°C; Detector: Fluorescence with excitation at 340 nm and emission at 455 nm ; Each standard conc.: 1.0 μg/mL.

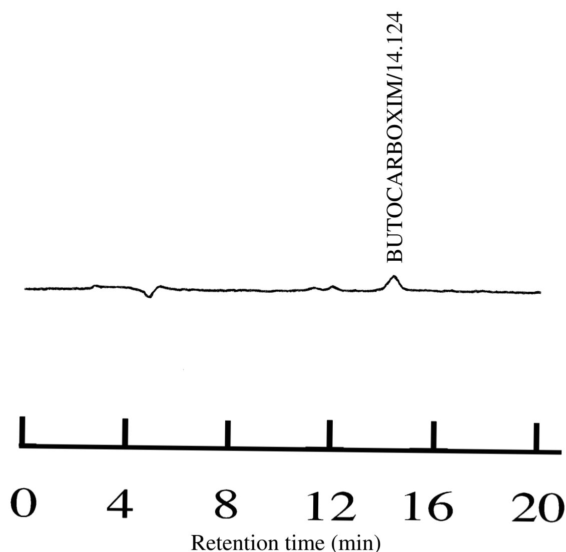


Figure 8. HPLC chromatogram of the detection limit for butocarboxim (0.05 ppm butocarboxim in bamboo sprout).
HPLC conditions are shown in Fig. 4.

Table 1. Recoveries of butocarboxim in spiked crops

Sample	Spiked level (ppm)	Recovery [a](%)
Radish	0.1	82.6 (9.0)[b]
	0.2	81.9 (3.5)
	0.3	82.3 (4.5)
Bamboo sprout	0.2	82.5 (4.4)

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

can be detected using a fluorescence detector, which is much more sensitive and selective in terms of compound detection. The derivatization reaction is schematically shown in Figure 5.

(II) *Mobile Phase Selection*

1. *Single Residue Analysis for Butocarboxim Detection*

A various ratios of acetonitrile to water as mobile phases pumped at 1.0 mL/min were tested to separate butocarboxim peak from other interference peaks. The retention times of butocarboxim were found to be 9.6 and 14.1 min, respectively, when the mobile phases of acetonitrile : water (30:70, v/v and 25:75, v/v) were used. The later mobile phase was capable of yielding a satisfactory resolution between the butocarboxim peak and its adjacent peaks as shown in Figure 6. A mobile phase consisting of acetonitrile: water (25 : 75, v/v) was thus selected for butocarboxim analysis in this study.

2. *Multiresidue Analysis*

A mixture of 8 carbamate pesticides and 4 of their metabolites and butocarboxim could be well separated using a gradient mobile phase of acetonitrile / water starting from a composition of 12/88 to 70/30 in 30 min as shown in Figure 7. This condition could be used for multiresidue analysis.

III. *Recovery*

Butocarboxim recoveries from radishes and bamboo sprouts are presented in Table 1. Recoveries from radishes spiked with 0.1~0.3 ppm butocarboxim were at the range of 81.9~82.6% with a coefficient of variation of 3.5~9.0%. Average recovery from bamboo sprouts spiked with 0.2 ppm butocarboxim was 82.5% with 4.4% coefficient of variation. A satisfactory recovery as well as reproducibility was obtained using this proposed method.

IV. *Limit of Detection*

The limit of detection was determined to be 0.05 ppm (Figure 8), which is lower than the tolerance level, indicating the proposed method is sensitive enough to be an official method for butocarboxim detection.

V. *Investigation of Commercial Products*

Commercial root vegetables including 3 radish, 2 carrot, 2 Co-ba, and 3 bamboo sprout samples were investigated. No butocarboxim residue was found in the above tested samples.

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以高效液相層析及後置反應裝置檢測農產品中佈嘉信殘留量

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摘　要

本研究建立了以高效液相層析法檢驗農產品中佈嘉信 (butocarboxim)之殘留量。本檢驗法係以丙酮為抽出溶媒，抽出液經減壓濃縮至無溶媒，加入食鹽水，以正己烷振搖去雜質後並以二氯甲烷萃取，經胺基固相萃取匣(aminopropyl cartridge)淨化，以高效液相層析儀配合後置反應裝置及螢光檢測器進行分析。佈嘉信經層析管Lichrospher 60 RP-Select B，移動相乙腈：水＝25:75 (v/v)分離後，於90℃、鹼性下水解後，和鄰苯二甲醛 (*o*-phthaldialdehyde) / 乙硫醇 (2-mercaptoethanol) 試劑衍生螢光物質，以螢光檢測器於激發波長340 nm及放射波長455 nm偵測。添加佈嘉信於蘿蔔中0.1～0.3 ppm，平均回收率為81.9～82.6%，添加於麻竹筍中0.2 ppm，平均回收率為82.5%。本檢驗法之最低檢出限量為0.05 ppm。以建立之檢驗方法分析市售檢體，蘿蔔、紅蘿蔔、茭白筍、烏腳綠竹筍及麻竹筍等十件皆未檢出佈嘉信。

關鍵詞：農產品，佈嘉信，胺基固相萃取匣，高效液相層析，後置反應，螢光檢測。