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A Rapid and Simple Method for the Determination of Ethephon Residue in Agricultural Products by GC with Headspace Sampling

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ABSTRACT

Ethephon quickly decomposes to ethylene under alkaline and high temperature conditions. In this research, we developed a rapid and simple method suitable for routine analysis of ethephon residues in agricultural products using gas chromatography (GC) equipped with a flame ionization detector (FID). This method was based upon the headspace sampling and determined the ethylene formed. Average recoveries from apples, tomatoes, grapes, kiwifruits and sugarcane, which were spiked with 1~3 ppm ethephon, were in the range of 88.3~98.6% and the coefficient of variation were ranged of 2.2~7.5%. The detection limit was 0.1 ppm.

Key words: agricultural products, ethephon residue, gas chromatography (GC), flame ionization detector (FID), headspace sampling, ethylene

INTRODUCTION

Ethephon (2-chloroethylphosphonic acid) is available commercially as 'Ethrel', 'Florel', 'Cerone', 'Prep', 'Bromoflor', 'Flordimex', 'Camposan', 'Etheverse', and 'Tomathrel'[1]. Ethephon is permitted to be applied to 'large berries', 'fruit vegetables', 'small berries', 'pome fruit' and 'sugarcane' groups of foods, and the tolerance level is set at 2 ppm according to the "Pesticide Residue Limits in Foods" announced by the Department of Health in Taiwan[2]. It is a plant growth regulator with systemic properties, penetrates into the plant tissues, and is translocated and progressively decomposed to ethylene[1], which is a kind of plant gas hormone which affects the growth processes of plants, including seed germination, fruit maturation, flower wilt, etc. Ethylene is widely used as a ripening accelerator in the post-harvest of fruits. The sources are pure ethylene gas, or gas generated from an ethylene generator, or ethephon. Ethephon was found to be the most effective nongaseous ethylene-releasing chemical[3].

Ethephon is stable in aqueous solutions having pH values less than 3.5 and decomposition occurs with the liberation of ethylene at higher pH levels[1]. It is also sensitive to UV irradiation[1]. The gas chromatographic procedures developed earlier for ethephon residue analysis with a flame photometric detector determined the methylated phosphonic acid compound[4]. Simplified extraction and clean-up procedures described in recently published papers are time-consuming[4]. Rapid and simple methods have been developed suitable for routine analysis of ethephon residues in agricultural products based upon the quantity of ethylene released from ethephon at pH values of 12-14, thus omitting tedious extraction, clean-up and derivatization steps[3,5,6].

Ethephon is decomposed to ethylene and dihydrogen phosphate under alkali and high temperature conditions. Indirect methods for determination of ethephon by quantity of liberated ethylene or titration dihydrogen phosphate with sodium hydroxide can be used. Sequence reactions during determination of ethephon in pesticide formulations according to the CIPAC (Collaborative International Pesticide Analytical Council) titrimetric method are noted (Figure 1). A rapid colorimetric method was also reported to determine ethephon residue in watermelons[7].

Reports about indirect determination of ethephon in crops by quantity of liberated ethylene were applied to fruits and cereals[3,5,6]. The conditions of ethylene-forming reaction (shaking method, incubation temperature and time) were not the same in those reports. Most of the methods developed used a headspace sampler, which is not popular in laborato-

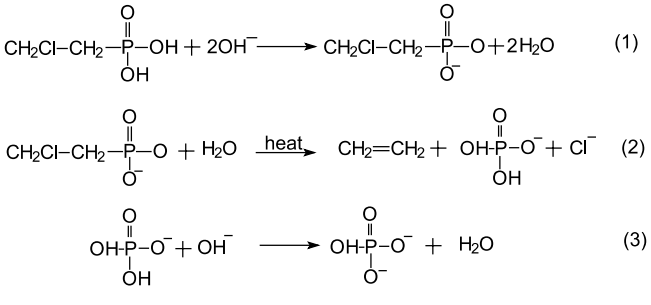

Figure 1. Sequence reactions during determination of ethephon in pesticide formulations according to CIPAC titrimetric method: neutralization (equation 1), thermal decomposition of ethephon (equation 2), and titration of dihydrogen phosphate formed (equation 3).

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ries. The purpose of this study was to establish a reproducible GC method using a headspace syringe instead of a headspace sampler for rapid analysis of ethephon residue in a wide range of crops. The established method is expected to be adopted as an official method for related authorities to detect ethephon residue in crops.

MATERIALS AND METHODS

I. *Materials*

Test samples including kiwi fruits, tomatoes, grapes, apples and sugarcane were purchased from traditional markets.

II. *Reagents*

Acetone was residue grade. Hydrochloric acid and potassium hydroxide were reagent grade. The ethephon standard (of purity 99%) was obtained from Riedel de Haen AG (Germany).

III. *Methods*

(I) *Preparation of Standard Solution*

Accurately weighed ca. 100 mg ethephon standard, transfered to a 100 mL volumetric flask, diluted to volume with 0.1 N HCl solution and mixed as a stock solution. Standard solution was prepared by diluting the stock solution to 100 μg/mL with 0.1 N HCl solution.

(II) *Preparation of Samples for Ethylene Determination*

Samples were homogenized with a Waring blender, and 5 grams of the slurry were weighted in a 24 mL headspace vial (amber, open-top closures, Teflon/silicone septum). A mixture of 4 mL of water, 1 mL of acetone and 1 mL of 30% KOH solution were added to the vial and sealed immediately. The reaction vials were shaken at 150 rpm in a reciprocal shaking bath (shaker bath BT-350, YIHDER, Taiwan) at 60˚C for 3 hr. After standing to room temperature, gas samples of 1 mL were removed from the headspace with a headspace syringe (fitted with valve and special Luer probe with side pole) and analyzed by gas chromatography.

(III) *Calibration Curve*

Nine mL of water and 1 mL of acetone were piped into reaction vials, a standard solution of 25~200 μL (ethephon 2.5~20 μg) was added, and then 1 mL of 30% KOH solution was added, the vial was immediately closed very tightly. The reaction vials were shaken at 150 rpm in a reciprocal shaking bath at 60˚C for 3 hr. After standing to room temperature, gas samples of 1 mL were removed from the headspace by the use of a headspace syringe and analyzed by gas chromatography. A calibration curve was drawn based on the ethylene peak area and the ethephon content (μg).

(IV) *GC Conditions*

Shimadzu (Japan) GC equipped with a flame ionization detector (FID, H_2 flow rate = 70 mL/min, air flow rate = 700 mL/min) and a straight liner was used. Column was GS-Q (30 m x 0.53 mm i.d., J&W Scientific, CA, USA). The column oven temperature was held at 40˚C for 5 min and then programmed to 120˚C at 30˚C/min. Both temperatures of injector and detector were 250˚C. The injection volume of headspace gas was 1 mL. The carrier gas was nitrogen at a flow rate of 5 mL/min.

(V) *Identification and Quantification of Ethephon*

After sample preparation as described above, 1 mL of headspace gas was injected into the GC device. Ethephon was tentatively identified by comparing the retention time with that of the standard. The ethephon content was calculated with the following equation:

Ethephon (ppm) = C/M

Where C is the content of ethephon in sample reaction vial calculated by calibration curve (μg) and M is weights of sample (g).

(VI) *Recovery Test*

A recovery test was carried out in triplicate for each concentration and performed by spiking 1~3 ppm of ethephon in kiwi fruits, tomatoes, grapes, apples and sugarcane, respectively. Preparation of the spiked sample as well as blank sample was as described above.

RESULTS AND DISCUSSION

I. *The Conditions of Ethylene-Forming Reaction*

In this study, we chose an appropriate size of reaction vial (24 mL) with screw and open-top cap to perform the ethylene-forming reaction. After reaction, the gas of the headspace can be removed directly by a headspace syringe and analyzed by GC. In the sample preparation procedures, adding 4 mL of water and 1 mL of acetone in 5 g of homogenized sample was not only to reduce the viscosity of the sample slurry and increase reaction efficiency, but also helpful to perform uniform recovery between different samples. Ethephon is a systemic pesticide and penetrates plant tissues. Investigation by Hunter *et al*. in 1978 showed that spiked samples without acetone in the reaction system yielded recoveries depending on the nature of fruit tissue and that the phenomenon disappeared in the presence of acetone. It is further speculated that systemically incorporated ethephon in field-treated crops becomes more extensively eluted from cell constituents when applying this solvent system.

In order to achieve optimum and reproducible ethylene production, continuous shaking during reaction is very important. Some papers omit shaking or shaking intermittently during reaction[5,6,8]. Following this research could not

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produce reproducible results, and a long reaction time was necessary. Incubation temperatures exceeding 60˚C appear to be inadvisable since loss of ethylene must be considered due to elevated vapor pressure inside the injection bottle. The reaction efficiency, and thereby the concentration of ethylene available for identification, depends on the completeness of the degradation of the original compound and, to the same extent, on the insolubility of the gas in the liquid phase. To ensure the completeness of the degradation of ethephon in the reaction vials, the temperature was set at 60˚C and the vials shook at 150 rpm. The relationship between incubation time and ethylene releasing was analyzed. Figure 2 shows a plot of ethylene peak areas from headspace GC analysis versus time of incubation at 60˚C and 150 rpm shaking for ethephon standard and ethephon spiked samples. As Figure 2 indicates, the decomposition of ethephon standard solution was very rapid and completed in 30 min, but the liberation of ethylene from decomposition of ethephon in kiwi fruits was much slower. The recovery of ethephon was high, over 85%, and reached a maximum for 3 hr of reaction.

II. *GC Conditions*

(I) *The Number and Volume of Injection*

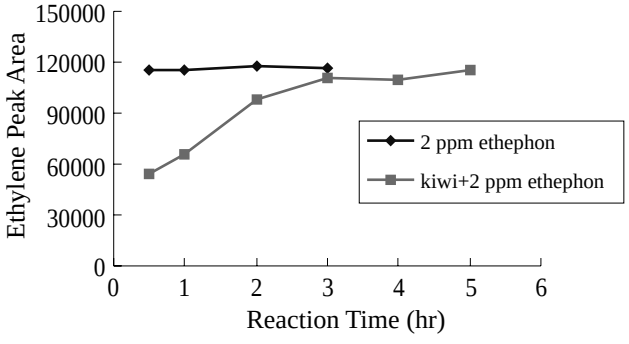

Figure 2. Ethylene-releasing curves for ethephon standard and kiwifruit samples spiked with ethephon standard during reaction at shaking bath (60˚C, 150 rpm).

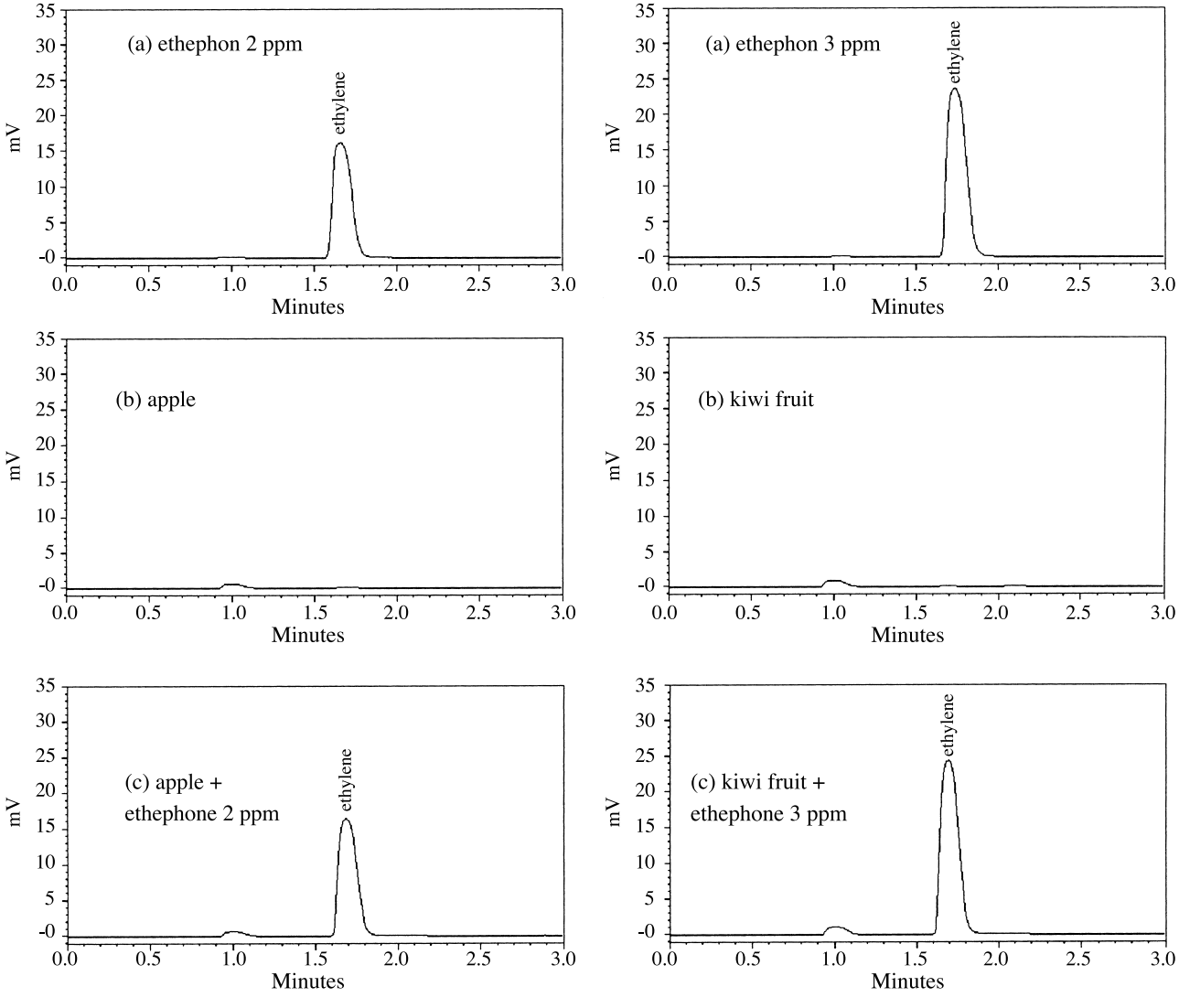

Figure 3. GC chromatograms of apple and kiwi fruit samples spiked with ethephon: (a) ethephon standard (b) unspiked sample (c) sample spiked with ethephon.

GC column: GS-Q; temperature for oven: 40˚C (4 min), 30˚C/min, 120˚C (8 min); injector temp.: 250˚C; detector temp.: 250˚C; carrier gas: N_2, 5 mL/min; detector: FID.

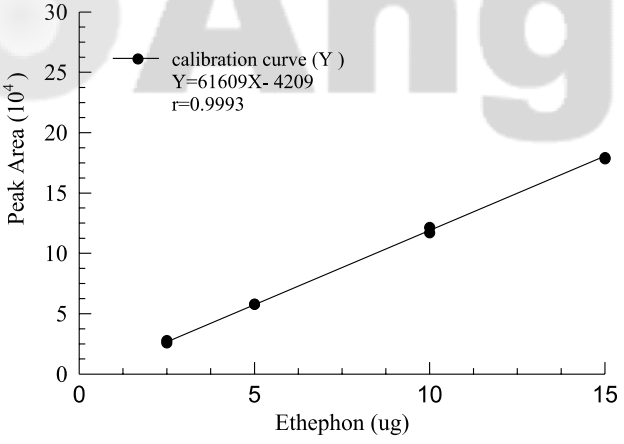

Figure 4. Calibartion curve for ethephon.

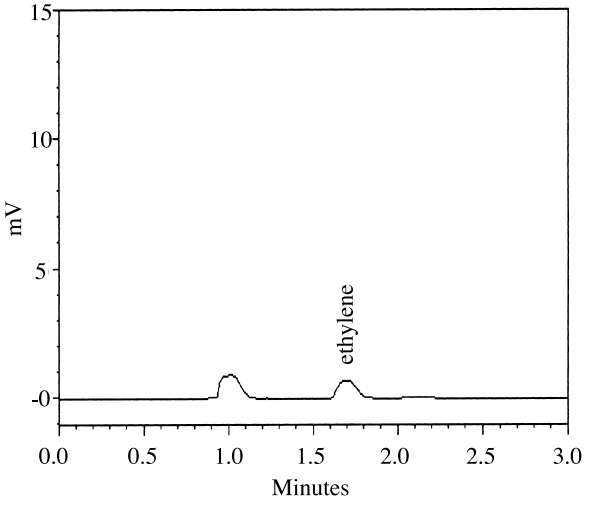

Figure 5. GC chromatogram of kiwifruit sample spiked with 0.1 ppm ethephon.

GC conditions are shown in Fig. 3.

Using the method described above, a headspace of ca. 14 mL was left in the reaction vials. Injecting 1 mL gas of headspace in a reaction vial caused redistribution of ethylene in the headspace and influenced quantification of ethephon in the next injection from the same vial. It's important that only a single injection be used for analyzing samples and standards. In our study, an additional injection would cause 6~7% recovery off. Headspace aliqots of more than 1 mL result in a severe peak broadening, and due to the partial pressure of the water-vapor in the headspace, can lead to disturbances in the separation column.

(II) *Column*

Ethylene (C_2H_4) is a kind of light hydrocarbon. A PLOT (porous layer open tubular) column like GS-Q packed with porous divinylbenzene homopolymer can separate air, carbon dioxide, C_1 and C_2. In our study, this type of column can eliminate the interference of carbon dioxide from respiration of plant cells during ethylene formation reaction. Figure 3 shows the GC chromatograms of ethephon in apple and kiwifruit samples. An ethylene peak (retention time was ca 1.7 min) was separated from carbon dioxide peak (retention time was ca 1.0 min). The quantity of carbon dioxide releasing from respiration of plant cells depends on the type of crops and environmental conditions (temperature, pH value, etc.).

Table 1. Recoveries of ethephon in spiked crops

Sample (Crop type)	Spiked level (ppm)	Recovery[a] (%)
Kiwi fruit	1	93.7 (3.2) [b]
(Large berries)	2	90.4 (2.2)
	3	93.8 (3.7)
Tomato	1	95.9 (2.4)
(Fruit vegetable)	2	93.0 (4.0)
	3	98.6 (2.6)
Grape	1	94.4 (5.1)
(Small berries)	2	93.0 (2.8)
	3	96.9 (2.4)
Apple	1	88.3 (4.6)
(Pome)	2	91.6 (7.5)
	3	96.4 (5.6)
Sugarcane	1	93.8 (2.6)
(Sugarcane)	2	93.2 (6.4)
	3	90.9 (3.3)

[a] means of triplicate.

[b] value in the parenthesis represents the coefficient of variation (CV, %).

Although the ethylene peak appeared in 2 min for GC-FID analysis at 40˚C column temperature, raising the temperature up to 120˚C for 8 min was necessary to eliminate acetone vapor remaining in the column and to maintain good performance of the column.

III. *Calibration Curve and Recovery Test*

By using the method described above, the regression coefficient of the calibration curve was determined to be 0.9993 (Figure 4). Ethephon recoveries from apples, tomatoes, grapes, kiwifruits and sugarcane are presented in Table 1. Recoveries from apples, tomatoes, kiwifruits and sugarcane spiked with 1~3 ppm ethephon were in the range of 88.3~98.6% with a coefficient of variation of 2.2~7.5%. A satisfactory recovery as well as reproducibility was obtained using this proposed method.

IV. *Limit of Detection*

The limit of detection was set at 0.1 ppm (Figure 5), which is much lower than the tolerance level, indicating the proposed method is sensitive enough to be an official method for ethephon detection. The established method was sensitive enough to determine ethephon residue in crops less than 0.1 ppm. Ethylene occurs widely as an endogenous hormone during the ripening of fruits, and interferes with the measurement of the residues of ethephon. Ernst and Anderegg[(5)] suggested that concentrations of ethylene smaller than the amount equivalent to 0.1 ppm of ethephon should not be considered as residues without further confirmation.

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利用氣相層析儀及氣體採樣快速檢測農產品中益收生長素殘留量

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摘　要

益收生長素（ethephon）在鹼性及高溫之條件下會快速分解產生乙烯（C_2H_4），測定乙烯之生成量可間接定量益收生長素，本研究即利用此一原理進行反應並以氣相層析附火焰離子檢出器（gas chromatography-flame ionization detector, GC-FID）測定反應生成之乙烯來定量農產品中益收生長素殘留量。添加益收生長素於蘋果、葡萄、番茄、奇異果及甘蔗中1~3 ppm檢體濃度，回收率爲88.3~98.6%，變異係數爲2.2~7.5%。本檢驗法之最低檢出限量爲0.1 ppm。

關鍵詞：農產品，益收生長素，氣相層析，火焰離子檢出器，乙烯