

Determination of Monocrotophos Residues by HPLC

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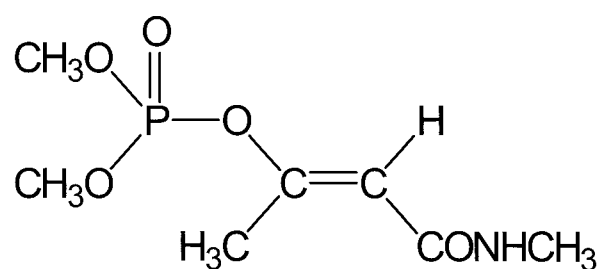
ABSTRACT: An analytical method was developed to determine monocrotophos residues in apple, citrus, and soil using high-performance liquid chromatography (HPLC) with ultraviolet absorption detection. Monocrotophos was extracted with acetone from apple, citrus and moist soil samples. The extract was concentrated, added with saline water, and subjected to *n*-hexane washing to remove nonpolar co-extractives. Dichloromethane partition was then followed to recover monocrotophos from the aqueous phase. Silica gel column chromatography was employed to further purify the extract prior to HPLC determination. Reverse-phase HPLC using an octadecylsilyl column was successfully applied to separate and quantitate the monocrotophos residue in sample extracts at the wavelength of 230 nm. Overall recoveries of monocrotophos from fortified samples averaged $95.3 \pm 2.1\%$ ($n=6$), $97.0 \pm 0.7\%$ ($n=6$), and $92.8 \pm 4.3\%$ ($n=12$) for apple, citrus, and soil, respectively. The proposed method was quite reproducible and sensitive enough to replace the troublesome gas-liquid chromatographic analysis for monocrotophos residues.

Key words: Monocrotophos, residue analysis, HPLC determination, apple, citrus, soil.

INTRODUCTION

Monocrotophos [dimethyl (*E*)-1-methyl-2-(methylcarbamoyl) vinyl phosphate] is a typical organophosphate insecticide or acaricide which has been widely used to control insects and mites during fruit cultivation. Its systemic action along with contact poison enabled the insecticide to effectively control chewing as well as sucking insects especially aphids^{1,2}. However, chronic toxicity of this insecticide represented by acceptable daily intake (ADI) value of 0.0006 mg/kg seems not so low to ensure safety of the residue and, accordingly, maximum residue limits (MRLs) are being strictly regulated in the range of 0.05~1.0 mg/kg for raw agricultural products^{3,4}. Regarding the situation that fruits are consumed in large quantity and particularly in raw state, evaluation of the residue level of monocrotophos is of considerable importance to ensure safety of the harvest. Development of the highly reliable methods is, therefore, required not only to evaluate the residue levels in the harvest but to precisely track its persistence in soil environment^{5,6}. As monocrotophos shows sufficiently high vapor pressure, the compound

has been routinely included as an analyte in multiresidue analytical methods for organophosphorus insecticides using gas-liquid chromatography (GLC)^{7,10}. Affected by highly polar nature and thermally labile carbamoyl group, however, erratic reproducibility along with peak tailing during GLC analysis has been reported even in the official method^{7,8}. The present paper describes a new analytical method for monocrotophos residues in apple, citrus and soil samples using high-performance liquid chromatography (HPLC). The method was developed not only to achieve reliability higher than current methods but to fulfill required sensitivity and readiness for analytical operation.



Monocrotophos

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MATERIALS AND METHODS

Chemicals

Analytical standard of monocrotophos (99.4% pure) was kindly supplied by BASF Agro Co., Korea. Stock standard solution of 1000 mg/L was prepared in acetonitrile. The stock solution was stable at 4°C at least for 6 months. Acetonitrile and deionized water were HPLC grade. All other solvents were pesticide residue grade or reagent grade freshly redistilled in glass. Silica gel (70–230 mesh, CC grade) was purchased from Merck, Germany and activated at 130°C for more than 5 h prior to use. All other reagents were reagent grade unless specified.

Fruit and soil samples

At maturity apple (Fuji variety) and citrus (Koongchun variety) samples were harvested in bulk from orchard fields located in Chilgok, Kyungbuk Province and Seokwipo, Jeju Province respectively, where no monocrotophos had been applied during the whole cultivation period. Composite fruit samples were prepared in compliance with the instructions in Korean Test Guidelines for Pesticide Persistence¹¹. Apple fruit was minced after removing and discarding the hilum and ovary portions while whole citrus fruit including peel and flesh was chopped and blended. Each representative sample was stored frozen at -20°C until analyzed. Bulk soil samples were also collected from two orchard fields, Kyung-san and Chilgok in Kyungbuk Province, to the soil depth of 10 cm during the cultivating season, air-dried, and passed through 10-mesh sieve before use. Physicochemical characteristics of soils are shown in Table 1.

Extraction and partition

A 25-g portion of apple or citrus fruit sample was weighed into a 500-mL homogenizer cup, and 100 mL of acetone was added. The mixture was macerated at 10,000 rpm for 2 min in a high-speed homogenizer (Nihonseiki Kaisha AM-8, Japan). A 25-g portion of soil sample was moistened with 25 mL of distilled water. After standing for 10 min, 100 mL of acetone was added and extracted for 1 h on a gyrotatory shaker at 200 rpm. The homogenate was

suction-filtered through the filter paper (Toyo No. 6, Japan) on a porcelain Büchner funnel. The cup and filter cake were washed with 50 mL of fresh acetone, and the rinsate was combined with the previous filtrate. The filtrate was evaporated *in vacuo* at 40°C to remove acetone. The concentrate was quantitatively transferred into a 250-mL separatory funnel, and sequential addition of 50 mL of *n*-hexane and 50 mL of saturated NaCl solution in water was followed. After vigorous shaking for 1 min and standing until two layers clearly separated, the upper hexane phase was discarded. The aqueous phase was then vigorously extracted with two 50 mL portions of dichloromethane. The lower dichloromethane phase was dried over 20 g anhydrous sodium sulfate layer, collected in 250 mL distilling flask, and evaporated just to dryness *in vacuo* at 40°C. The residue was dissolved in 10 mL of dichloromethane and subjected to silica gel column chromatography.

Silica gel column chromatography

A chromatographic column (11 mm i.d. × 40 cm) was plugged with glass wool, dry packed with 5 g of activated silica gel and topped with *ca.* 2 cm layer of anhydrous sodium sulfate. The column was pre-washed by passing 25 mL of dichloromethane through it until the solvent level reached the top of sodium sulfate layer. The dichloromethane extract from the partition step was poured into the column and the column wall was rinsed twice with 2 mL portions of dichloromethane. When the liquid drained to sodium sulfate layer, the column was eluted with 50 mL of acetone/dichloromethane mixture (20/80, v/v) and the fraction was discarded. The column was then eluted with 50 mL of acetone/dichloromethane mixture (55/45, v/v) and the fraction was collected. The eluate was concentrated just to dryness and the residue was reconstituted with 10 mL of acetonitrile/water mixture (8/92, v/v) for HPLC determination.

High-performance liquid chromatography

High-performance liquid chromatography (HPLC) was performed using a HPLC system equipped with two Waters (USA) 510 pumps, 680 gradient controller, 486 tunable UV/VIS absorbance detector, Hewlett Packard (USA) 1100 autosampler, column oven and 3396 Series II integrator. Nova-Pak C18 (3.9 mm i.d. × 150 mm, 4 μm spherical, Waters, USA) was used as the analytical column. Operating parameters used for the determination of monocrotophos residues are as follows; mobile phase, acetonitrile/water (8/92, v/v), isocratic; flow rate, 1.0 mL/min; column temperature, 30°C; detection, UV absorption at 230 nm, 0.008 AUFS;

Table 1. Physicochemical characteristics of soils

Soil designation	Soil texture	Soil separate (%)			pH	OM (%)	CEC (cmol(+)/kg)
		Sand	Silt	Clay			
Kyungsan	L	40.9	43.1	16.0	5.4	3.4	17.8
Chilgok	SL	52.7	36.3	11.0	5.5	2.5	13.0

sample size, 30 μ L; chart speed 0.5 cm/min. Under these conditions, retention time of monocrotophos was 9.6 min.

Validation of the analytical method

Recovery experiments were run on control apple, citrus, and soil samples to validate the analytical method proposed for monocrotophos residues. Prior to extraction, series of control samples were fortified with monocrotophos standard solution in acetonitrile at specified concentrations. After standing for 2 h, analytical procedures mentioned above were carried out to produce quality assurance data.

RESULTS AND DISCUSSION

Monocrotophos has been routinely analyzed as a component in multiresidue method for organophosphorus insecticides using GLC with nitrogen-phosphorus or flame-photometric detection (FPD)⁷⁻¹⁰. A preliminary study was conducted to investigate whether GLC with FPD could be applied to determine the monocrotophos residue. Under the condition of column temperature 190°C, isothermal, and helium 15 mL/min as carrier gas, monocrotophos on SPB-608 capillary column (0.53 mm i.d. \times 30 m, 0.5 μ m film thickness) showed a skew peak with backward tailing. During six consecutive injections of 1 ng monocrotophos standard producing *ca.* 50% full scale deflection, large variation in peak area was observed with 6.5% of relative standard deviation. Earlier studies also indicated that erratic recovery and poor reproducibility were found as affected by its high polarity and thermal lability^{7,8}. Although a trifluoroacetyl derivatization of carbamoyl moiety had tried to improve peak tailing and response variation, this method was only adequate for confirmatory purpose because of instability of the derivative¹⁰. Taken in conjunction with the log P value of 0.6 and high water solubility, monocrotophos seemed to be just the compound more preferably analyzed by HPLC rather than GLC in spite of its moderate vapor pressure, 0.29 mPa.

When reverse-phase HPLC using an octadecylsilyl column was employed, monocrotophos showed a sharp symmetrical peak under the mobile phase of acetonitrile/water mixture. In the range of 5% to 20% of acetonitrile contents in water, its capacity factor, varied from 2.3 to 34.8 respectively, linearly increased almost double as acetonitrile contents decreased. This indicated that monocrotophos remained unionized as neutral form in the mobile phase of acetonitrile/water mixture and, as a result, there was no need for ion-suppression¹². As monocrotophos is not readily oxidized nor reduced and had no fluorophore, ultraviolet absorption

detector was the only choice among common HPLC detectors. When a monocrotophos standard solution in acetonitrile/water mixture was scanned using Hewlett Packard (USA) 8452A photodiode array spectrophotometer, λ_{max} was found at 216 nm. Although extinction coefficient at λ_{max} , 1.34×10^4 cm⁻¹M⁻¹, seemed to be high enough to residue analysis, its chromophore unfortunately absorbed only such short wavelength that much possibility of interference by co-extractives absorbing nearby ultraviolet region was expected¹³. With minimal loss of sensitivity, longer wavelength at 230 nm with extinction coefficient of 0.97×10^4 cm⁻¹M⁻¹ was used for measurement. However, there might be still low selectivity in the determination step and thus rigorous purification of sample extracts was required. The study was, therefore, mainly focused on the development of efficient but simple cleanup methods.

Considering the polar nature of monocrotophos, an attempt was done at partition step to remove nonpolar co-extractives. From the acetone extract diluted with saline water, monocrotophos was not partitioned into hexane phase but all extracted into dichloromethane phase. Employing a washing step with hexane, 96% and 91% of co-extractives from apple and citrus samples were effectively removed, re-

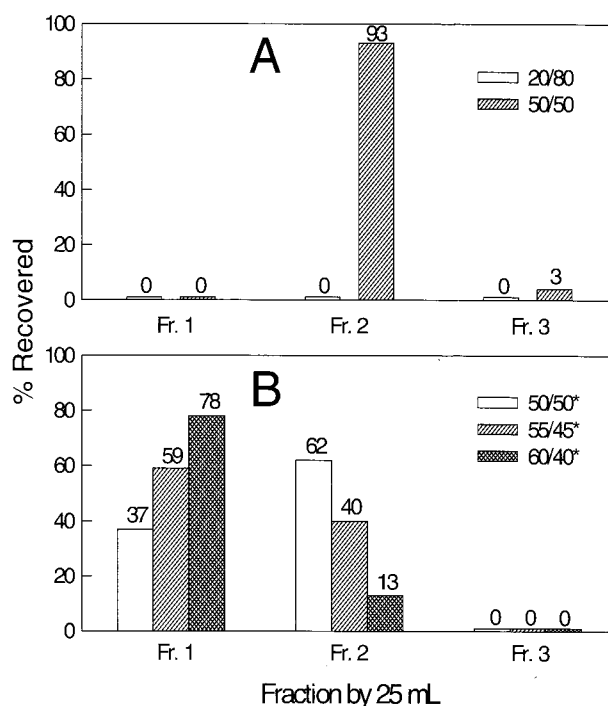


Fig. 1. Elution profile of monocrotophos on silica gel column. A, directly eluted with acetone/dichloromethane mixtures (v/v); B, pre-eluted with 50 mL of acetone/dichloromethane mixture (20/80, v/v) and then eluted with acetone/dichloromethane mixtures (v/v).

spectively. Adsorption chromatography was applied to further purify the extracts. Elution profile of monocrotophos on silica gel column is shown in Fig. 1. Using acetone/dichloromethane mixtures as eluting solvents, the monocrotophos eluate could be sharply fractionated from the extract. Coupled with the proposed partition and adsorption chromatography, fruit and soil extracts were purified to provide nearly colorless solutions for HPLC determination.

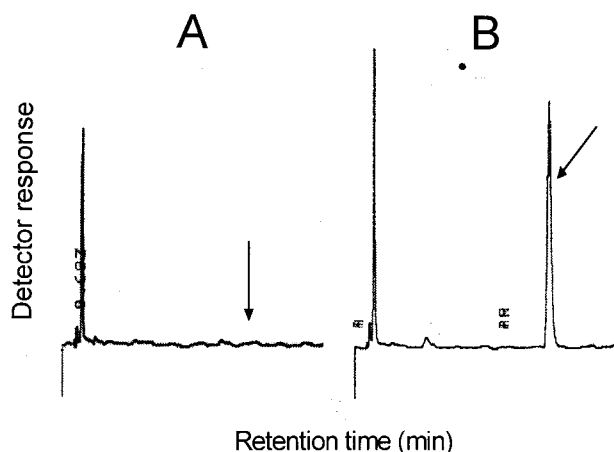


Fig. 2. Typical HPLC chromatograms of extracts from apple samples. A, control; B, fortified with monocrotophos at 0.2 mg/kg.

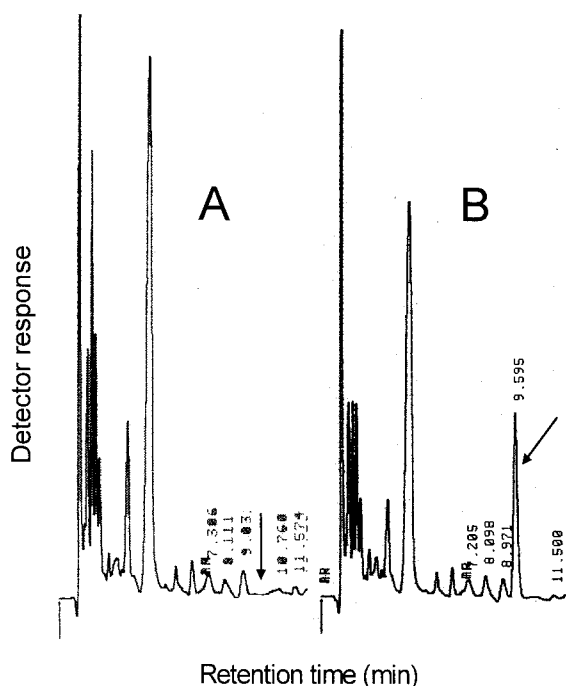


Fig. 3. Typical HPLC chromatograms of extracts from citrus samples. A, control; B, fortified with monocrotophos at 0.2 mg/kg.

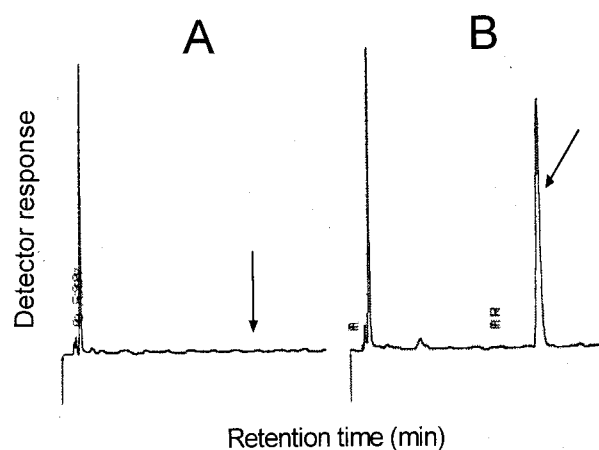


Fig. 4. Typical HPLC chromatograms of extracts from soil samples. A, control; B, fortified with monocrotophos at 0.2 mg/kg.

Typical HPLC chromatograms of apple, citrus and soil extracts are shown in Fig. 2, 3, and 4. The proposed method produced very clean HPLC chromatograms for apple and soil samples. Chromatograms of citrus samples were rather complicated but quite acceptable for quantitation. Detection limits of the proposed method were 0.02 mg/kg for fruit and soil samples based on 3% full scale deflection (signal to noise ratio > 10). These sensitivities were sufficiently high to detect at least 1/2~1/50 of MRLs (0.05~1 mg/kg) established for monocrotophos in fruits^{3,4}. Method detectability were also high enough to track the behavior of monocrotophos in the soil environment as well as to evaluate its terminal residues⁶.

Percent recoveries generated during the validation of analytical methods are presented in Table 2. Recoveries averaged 95.3±2.1% (n=6), 97.0±0.7% (n=6), and 92.8±4.3% (n=12) for apple, citrus, and soil samples respectively. The analytical method was successfully validated as measured by mean recoveries of more than 70% by 6 to 12 replicates per sample type. Coefficients of variation (CV) over all types of samples were less than 10%, indicating that the method could be reproducibly applied to analyze monocrotophos residues in fruit and soil samples¹¹.

In this study, a simple method known as peak purity test was applied to confirm the monocrotophos residues in fruit and soil samples¹⁴. According to Beer-Lambert Law, absorbance of a compound at two specified wavelengths are solely dependent upon extinction coefficients while light path and its concentration are constant. Extinction coefficient is another physical characteristic inherent to a compound, other than chromatographic behavior, and thus can

Table 2. Recovery and detection limit of monocrotophos residues

Sample	Fortification (mg/kg)	Recovery \pm SD (%) ^{a)}	Detection limit (mg/kg)
Apple	0.2	94.6 \pm 2.8	0.02
	1.0	96.1 \pm 1.0	
Citrus	0.2	97.0 \pm 1.1	0.02
	1.0	97.0 \pm 0.3	
Kyungsan soil	0.2	86.1 \pm 3.5	0.02
	1.0	94.6 \pm 0.1	
Chilgok soil	0.2	95.1 \pm 1.2	0.02
	1.0	95.3 \pm 0.4	

^{a)}Mean values for triplicate samples with standard deviations.

be used as a confirmatory tool. Since peak area in a chromatogram is the sum of absorbances measured at unit time, difference in peak areas between two wavelengths is proportional to the difference in extinction coefficients if a peak purely represents an analyte. When determination of a sample is performed under identical HPLC operating condition, ratio of relative peak area to relative extinction coefficient measured at two wavelengths should be 1.00 in the absence of interference. Therefore, deviations from this value will provide the degree of interference caused by co-extractives. Considering absorption characteristics of monocrotophos, confirmatory wavelengths were set at 216 and 220 nm. Applied to fruit and soil samples fortified with 0.2 mg/kg of monocrotophos each, the ratios obtained at confirmatory wavelengths ranged 0.98–1.10 showing more than 90% peak purity as indicated in Table 3. As a result, monocrotophos residues could be confirmed by the peak purity test at limits of 0.02 mg/kg. Even though this method is qualitatively inferior to using more sophisticated instruments such as liquid chromatography/mass spectrometry, it has merits of simplicity and *in situ* confirmatory operation.

Table 3. Confirmation of the monocrotophos residue by monitoring at different wavelength

Sample	Wavelength (nm)	Relative PA/ ϵ ^{a)}
Apple	216	1.04
	220	0.98
Citrus	216	1.07
	220	1.10
Soil	216	1.03
	220	1.03

^{a)}Peak area (PA)/extinction coefficient (ϵ) relative to values at 230 nm.

The proposed method satisfies criteria of the analytical method for pesticide residues, which are more than 70% of recovery, less than 10% of CV, and more sensitive than 0.05 mg/kg of detection limit, on Test Guidelines for Pesticide Persistence notified by Rural Development Administration¹¹⁾. Analytical procedures do not require any special apparatus or instruments but consist of currently available techniques familiar to the residue analyst as well. The method is so rapid to operate that one experienced person can analyze 12 samples per day. The final solution prepared for HPLC was so well purified that one HPLC guard column could allow more than a hundred injections of sample extracts with no chromatographic deterioration. Therefore, authors suggest that the proposed method could be sufficiently applied to the routine analysis of monocrotophos residues in rice and soil samples.

ACKNOWLEDGMENTS

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