농약과학회지 제12권 제4호 (2008)
The Korean Journal of Pesticide Science Vol. 12, No. 4, pp. 315~322

ORIGINAL ARTICLE/RESIDUE AND SAFETY

Determination of Cyhalofop-butyl and its Metabolite in Water and Soil by Liquid Chromatography

Lina Hem, Jeong-Heui Choi, Xue Liu, Sathya Khay and Jae-Han Shim*

Natural Products Chemistry Laboratory, Division of Applied Bioscience and Biotechnology, College of Agriculture and Life Science, Chonnam National University, 300 Yongbong-dong, Buk-gu Gwangju 500-757, Republic of Korea

(Received September 18, 2008; accepted September 25, 2008)

Abstract

In this study, a simple, effective, and sensitive method has been developed for the quantitative residue analysis of cyhalofop-butyl and its metabolite cyhalofop acid in water and soil when kept under laboratory conditions. The content of cyholofop-butyl and cyhalofop acid in water and soil was analyzed by first purifying the compounds through liquid-liquid extraction and partitioning followed by Silica gel (adsorption) chromatography. Upon the completion of the purification step the residual levels were monitored through high-performance liquid chromatography (HPLC) using a UV absorbance detector. The recoveries of cyhalofop-butyl from three replicates spiked at two different concentrations ranged from 82.5 to 100.0% and from 66.7 to 97.9% in water and soil, respectively. The limit of detection and minimum detection level of cyhalofop-butyl in water and soil was 0.02 ppm and 10 ng, respectively. The recoveries of cyhalofop acid ranged from 80.7 to 104.8% in water and from 76.9 to 98.1% in soil. The limit of detection of cyhalofop acid was 0.005 ppm in water and 0.01 ppm in soil, while the minimum detection level was 2 ng both in water and soil. The half-live of cyhalofop-butyl was 4.14 and 6.6 days in water and soil, respectively. The method was successfully applied to evaluate cyhalofop-butyl residues in water and soil applied a.i. 30% emulsion, oil in water (EW) product.

Key words Cyhalofop-butyl, cyhalofop acid, half-life, HPLC-UV

Introduction

Herbicides play an important role in agriculture practices, and there are more than 300 herbicides. Throughout the world, most agricultural fields are widely treated with herbicides to control weeds, and the chemicals could pollute the aquatic environments by spray drift, leaching, runoff, or accidental spills, and herbicide residues are commonly found in surface waters although at a very low level [1].

Cyhalofop-butyl (CB) [(2R)-2-[4-(4-cyano-2-fluorophenoxy)-phenoxy]propanoic acid, butylester], is an aryloxyphenoxy-propionate (AOPP) herbicide for the post-emergence control of grasses in rice at application rate of a.i. 300 g/ha, at the two to four leaf stage to drained paddies [2,3,4]. In addition to this granule formulation which is applied to paddy water at about the 3 leaf stage, an EW (emulsion, oil in water) formulation has been intensively studied for foliar application at the late stage, i.e., the 5 leaf stage, as a "rescue" treatment [5]. However, some of the AOPP herbicides have a remarkable selectivity among graminaceous plants. For example, rice is very tolerant to cyhalofop-butyl [6]. The herbicide is formulated as an ester to facilitate the movement through the plant cuticle, but once in the plant, it rapidly hydrolyzed to cyhalofop-acid

*연락저자 : Tel. +82-62-530-2135, Fax. +82-62-530-0219
E-mail: jhshim@jnu.ac.kr

(CA), CA is the primary metabolite of CB and also the herbicidal active form [7].

Increasing attention has been paid to the adverse impact of pesticides on the environment, human health, and life-support systems. The potential of water contamination is high in areas where rice is cultivated under flooded conditions. The agrochemicals applied to aquatic environments such as paddy fields are a matter of concern for potential leaching and persistence in soil and water. During the draining phase, consisting of a forced movement of paddy water toward other field chambers and finally into surface water courses, agrochemicals can be mobilized and diffused into the environment. Conversely to nonflooded soils, the high volume of paddy water increases significantly the amount of agrochemicals in solution, and their possible adsorption into the paddy-field sediment is not favored [8].

Therefore, studies on the environmental fate of CB and its metabolite can provide data that will be highly relevant in evaluation the potential risks of this herbicide. Furthermore, the results of the environmental fate of this compound will provide a guide for the field application of CB and CA. Thus, the aim of the current investigation is to regularly monitor the residues through analytical method that combine short analysis time, sufficient selectivity, and sensitivity in order to better understand the environmental fate of CB and CA in water and soil when kept under laboratory conditions.

Materials and Methods

Solvent and reagents

Cyhalofop-butyl (97.4%), cyhalofop acid (99.9%) and the formulated product of 30% EW were kindly supplied by Dow AgroSciences (Seoul, Republic of Korea). Acetone, acetonitrile, benzene, ethyl acetate, methanol, dichloromethane, and *n*-hexane were LC grade and bought from Merck KGaA (Darmstadt, Germany). Sodium sulfate anhydrous and sodium chloride were analytical grade and purchased from Merck KGaA (Darmstadt, Germany). Silica gel (70-230 mesh, 60Å) and phosphoric acid were provided by Sigma (Missouri, USA) .

Standard preparation and standard curve

Cyhalofop-butyl

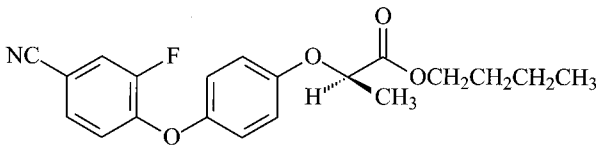
A stock solution of cyhalofop-butyl of 100 ppm was prepared by dissolving 10.27 mg of the standard reference sample in 100 mL of methanol. The stock solution was serially diluted with methanol to obtain concentrations of 0.5, 2, 3, 5, 10 ppm. 20 μL of each concentration solution was injected into the HPLC column and the calibration curve was prepared based on the peak height of each chromatogram.

Cyhalofop acid

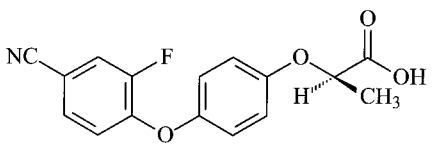
A stand stock solution (10 mg/100 mL) was prepared in methanol, the stock solution was serially diluted by methanol to prepare standard curve in 0.1, 0.5, 1, 2, 4 ppm. 20 μL of each concentration solution was injected into the HPLC column and the calibration curve was prepared based on the peak height of each chromatogram.

Treatment of water and soil with cyhalofop-butyl and cyhalofop acid

The trials were carried out with air-dried soil in the shade which was collected from Naju, Republic of Korea. This soil was put into experimental water baths, and the water was poured. Formulated product of CB diluted to 5000 times was applied to 15 experimental water baths (0.04 m^2) of the same size at the same rate of a.i. 0.0084 kg/10a. To avoid any variation in the concentration of



Cyhalofop-butyl



Cyhalofop acid

Fig. 1. Chemical structure of cyhalofop-butyl and cyhalofop acid.

applied herbicides (due to the evaporation of water), the experimental water bath was refilled with supplementary water every 3 days. The experimental baths treated with the herbicides were kept in the shade to protect herbicides against decomposition by solar light.

Physico-chemical properties of the soil

The physical and chemical properties of the experimental soil were determined as follows: pH was determined with a seven easy model pH meter (Mettler Toledo, USA), organic matter content was determined by the 1 *N* CH_3COONH_4 method (RDA, 1988).

Sampling

Water was collected using a beaker from three experimental baths after application of 30% EW of cyhalofop-butyl at 0 day (2 hrs), such that no soil residue came along from the bottom of the water bath. Later, the rest of the water was drained out, and the remaining soil was thoroughly mixed in a plastic bottle and collected. Similarly, samples were collected atl, 3, 5, 7, and 14 days from the other fifteen experimental baths after herbicides application. For each time point 3 experimental baths were used.

Sample Extraction Procedure and Purification

Water sample

Cyhalofop-butyl

Water sample (50 mL) was mixed with 100 mL of acetic acid-acetone (1:9, v/v) and shaken for 30 minutes. The sample was filtered through Celite under pressure using a Büchner funnel. The filtrate was transferred to a separatory funnel (1000 mL), 100 mL of distill water, 50 mL of saturated NaCl solution were added. The mixture was partitioned twice with dichloromethane (100 mL × 2). The combined dichloromethane layers were completely evaporated and reconstituted with 10 mL of *n*-hexane. The extract was purified by a glass column (11 mm × 400 mm) filled with silica gel. The column was washed with 50 mL of n-hexane-benzene (1:1, v/v), and eluted with 200 mL of benzene. The elute was evaporated under reduced pressure and re-dissolved in 2 mL of methanol and analyzed using HPLC-UVD.

Cyhalofop acid

Water sample (20 mL) was mixed with 100 mL of acetonitrile-1 M HCl (4:1, v/v) and shaken for 30 minutes. The sample was suction filtered through Celite using a Büchner funnel. The filtrate was transferred to a separatory funnel (1000 mL), 200 mL of distill water, 50 mL of saturated NaCl solution were added. The mixture was partitioned, reconstituted and purified the same way described for cyhalofop-butyl. The column was washed with 50 mL of ethyl acetate, and 10 mL of ethyl acetate-acetic acid (99:1, v/v), eluted with another 20 mL of ethyl acetate-acetic acid (99:1, v/v). The elute was evaporated reduced pressure and re-dissolved in 2 mL of methanol and analyzed using HPLC-UVD.

Soil sample

Cyhalofop-butyl

Soil sample were taken from the bottom of the experimental water bath after removing the water. 50 g of soil sample was placed into a conical flask and 100 mL of ethyl acetate-acetic acid (99:1, v/v), the mixture was shaken by a mechanical shaker for 30 minutes and then filtered. The filtrate was transferred to a separatory funnel (1000 mL), 200 mL of distilled water, 50 mL of saturated NaCl solution were added. The mixture was partitioned twice with dichloromethane (100 mL x 2). The combined dichloromethane layers were completely evaporated and

Table 1. Physico-chemical properties of the soil used for experiments

Sample	pH	Organic matters (g/kg)	Cation exchange capacity (me/100 g)	Distribution of particle size (%)			Soil Texture
				Clay	Slit	Sand	
Soil	5.3	18.3	12.9	36.0	50.9	12.1	SiCL

reconstituted with 10 mL of *n*-hexane. The extract was purified by a glass column (11 mm x 400 mm) filled with silica gel. The column was washed with 50 mL of *n*-hexane: benzene (1:1, v/v), eluted with 200 mL of benzene, and evaporated under pressure and re-dissolved in 2 mL of methanol and analyzed using HPLC-UVD.

Cyhalofop acid

20 g of soil sample was placed into a conical flask and 100 mL of acetonitrile-1 M HCl (4: 1, v/v) and the mixture was shaken for 30 minutes. The sample was filtered into a separatory funnel (1000 ml), 100 mL of distilled water, 50 mL of saturated NaCl solution were added. The mixture was partitioned, reconstituted, and purified the same way described for cyhalofob-butyl. The column was washed with 50 mL ethyl acetate and 10 mL of ethyl acetate-acetic acid (99:1), eluted with another 20 mL of ethyl acetate-acetic acid (99:1, v/v). The effluent was evaporated under pressure and re-dissolved in 2 mL of methanol and analyzed using HPLC-UVD.

Recoveries test

Cyhalofop-butyl

50 mL of water and 50 g of soil were spiked with 2 mL of 2 ppm and 10 ppm standard working solutions. The sample preparation was carried out with the method described above.

Cyhalofop acid

20 mL of water were spiked with 1 mL of 0.5 ppm and 4 ppm standard working solutions, and 20 g of soil was spiked with 2 mL of 0.5 ppm and 2 ppm standard working solutions. Sample preparation was carried out with the method described above.

HPLC equipment and chromatographic condition

A High performance liquid chromatography (Kontron Instruments 322 System) connected to a UV-VIS detector was used to identify cyhalofop-butyl and cyhalofop acid. Identification of the target analyte was carried out on μBondapak C18 column (3.9 × 300 mm column, Waters). A mixture of methanol-water-acetic acid (80:56:2, v/v/v), was used as the mobile phase for cyhalofop-butyl and a mixture of A and B (1:1.85, v/v), where the mixture of A was acetonitrile-phosphoric acid (1000:1, v/v) and the mixture of B was the mixture of water-phosphoric acid (1000:1, v/v), was used as the mobile phase for cyhalofop acid, with a flow rate of 1 mL/min. Under these conditions the retention time for cyhalofop-butyl and cyhalofop acid were 21.56 min and 17.57 min, respectively.

Results and Discussion

Calibration and linearity

The standard calibration curves were determined by injecting the standard solutions of cyhalofop-butyl and cyhalofop acid into the HPLC-UV system at five different concentration levels (25 μg-200 μg). From these injections the calibration curves were found to be linear with a

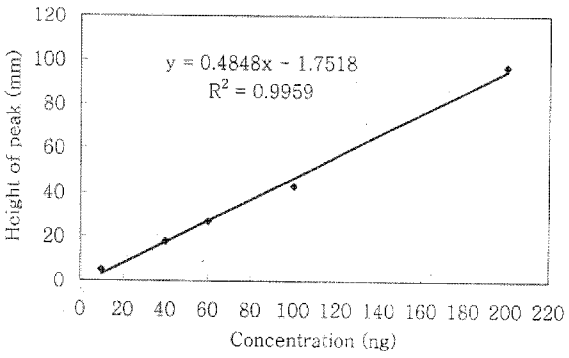


Fig. 2. Cyhalofop-butyl standard curve.

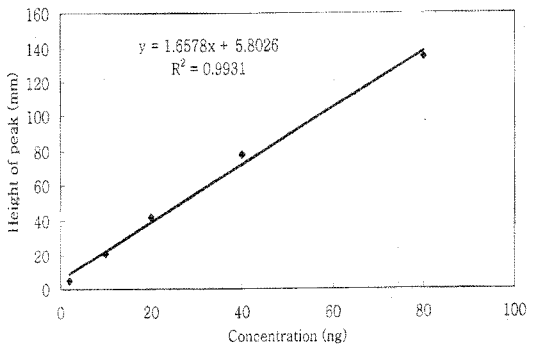


Fig. 3. Cyhalofop acid standard curve.

Table 2. Recoveries, limit of detection (LOD) and minimum detection level (MDL) obtained by HPLC analysis of cyhalofop-butyl in water and soil at two spiking levels (n=3)

Sample	Spiked concentration (mg/kg or L)	Recovery (%)				LOD (mg/kg or L)	MDL (ng)
		1	2	3	Average		
Water	0.08	88.9	100.0	83.3	90.7	0.02	10
	0.4	86.6	91.8	82.5	86.9		
Soil	0.08	83.3	88.9	66.7	79.6		
	0.4	97.9	74.2	87.6	86.6		

Table 3. Recoveries, limit of detection (LOD) and minimum detection level (MDL) obtained by HPLC analysis of cyhalofop acid in water and soil at two spiking levels (n=3)

Sample	Spiked concentration (mg/kg or L)	Recoveries (%)				LOD (mg/kg or L)	MDL (ng)
		1	2	3	Average		
Water	0.025	85.7	104.8	102.4	97.6	0.005	2
	0.2	86.7	80.7	83.0	83.5		
Soil	0.05	85.7	88.1	86.7	86.8	0.01	
	0.2	76.9	93.6	98.1	89.5		

correlation coefficient $r^2 > 0.99$ for both matrices (Fig. 2, Fig. 3).

Method performance

The recoveries from three replicates experiments of cyhalofop-butyl at two different concentration levels ranged from 82.5 to 100.0% in water and from 66.7 to 97.9% in soil, for cyhalofop acid at two different concentration levels ranged from 80.7 to 104.8% in water, and from 76.9 to 98.1% in soil (Table 2, 3). These recovery rates were satisfactorily high and highly reproducible, and confirmed the applicability of the method.

The limits of detection (LOD) were calculated by using minimum detection level (MDL) of analytical instrument. The LOD and MDL were found to be 0.02 ppm (mg/L or mg/kg) and 10 ng for water and soil (Table 2, 3). These results clearly indicate that this method is sufficiently sensitive to allow the measurement of the analyte in both water and soil. These levels are much lower than MRL which has been established by the Korea Food and Drug Administration (KFDA, 2008).

Selectivity was assessed by comparing the chromatograms of control water or soil with these matrices fortified with CB and CA. Endogenous peaks at the retention time of the analyte are not observed in any of the evaluated samples, indicating that, there was no obvious direct interference at the expected retention time. Representative chromatograms of control and spiked sample are shown in Fig. 4 and 5.

Table 4. Cyhalofop-butyl (CB) and -acid (CA) residues in experimental water and soil samples

Sample	Water (mg/L)		Soil (mg/kg)	
	CB	CA	CB	CA
Control	T[a]	0.126±0.005	T	T
1	T	0.115±0.000	T	0.015±0.002
3	T	0.098±0.003	T	T
5	T	0.074±0.000	T	T
7	T	0.028±0.001	T	T
14	T	T	T	T

[a] Trace meaning less than LOD

Dissipation pattern of cyhalofop-butyl and cyhalofop acid in water and soil

The dissipation pattern of cyhalofop-butyl and cyhalofop acid were evaluated following its application at a rate of a.i. 0.0084 kg/10a to water bath containing soil at the bottom. The residual amounts in the water and soil were measured at 0, 1, 3, 5, 7, and 14 days after fortification of the commercial products. It is worth noting that no quantifiable residues were observed in the control sample.

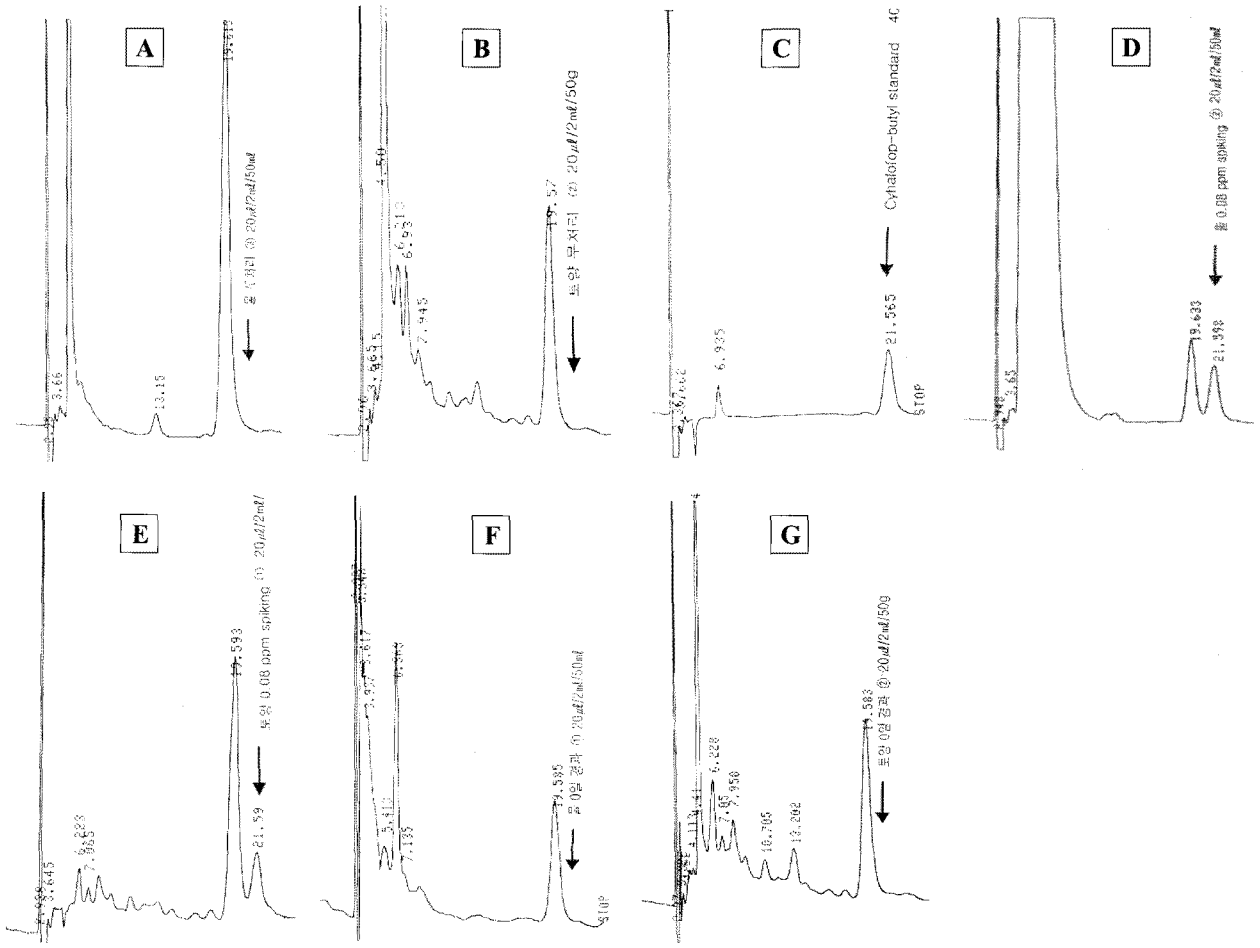


Fig. 4. Representative chromatograms of control water (A) and soil (B), cyhalofop-butyl standard (C, 2 mg/L), spiked water (D, 0.08 mg/L) and soil (E, 0.08 mg/kg) to controls for recovery, and water (F) and soil (G) sample at 0 day (2 hrs) treating the formulated product.

The result from these experiments showed that the amounts of cyhalofop-butyl and cyhalofop acid gradually decreased from the water and soil. Since fluorescent light (rather than solar light) was used as the irradiation source in this study, photodegradation might have had an effect on the rate of chemical degradation in this environment (Hu and Coats, 2007). Jackson and Douglas (1999) also reported that very rapid and extensive degradation of the parent ester of cyhalofop-butyl occurred in soil and sediment water systems, resulting in the formation of acid, amide and diacid metabolites [6].

Conclusion

A sensitive method based on LLE followed by SPE cleanup and final analyte determination by HPLC-UVD has been developed for the analysis of cyhalofop-butyl and cyhalofop acid in water and soil. Cyhalofop-butyl and cyhalofop acid has been efficiently determined with or without SPE cleanup using LC-MS/MS by other scientists (Choi *et al.*, 2001; Hall *et al.*, 2004; Hiemstra nad Kok, 2007; Wang and Wotherspoon, 2007). However, LC-MS/MS is not familiar to every laboratory and needs professional knowledge and experiences to operate it. The developed method could achieve good extraction efficiencies such as percent recoveries and RSD using general HPLC-UVD and cleanup procedure, preparative glass column chromatographic separation, without using commercial SPE cartridges. The recoveries in both matrices were within an acceptable range with

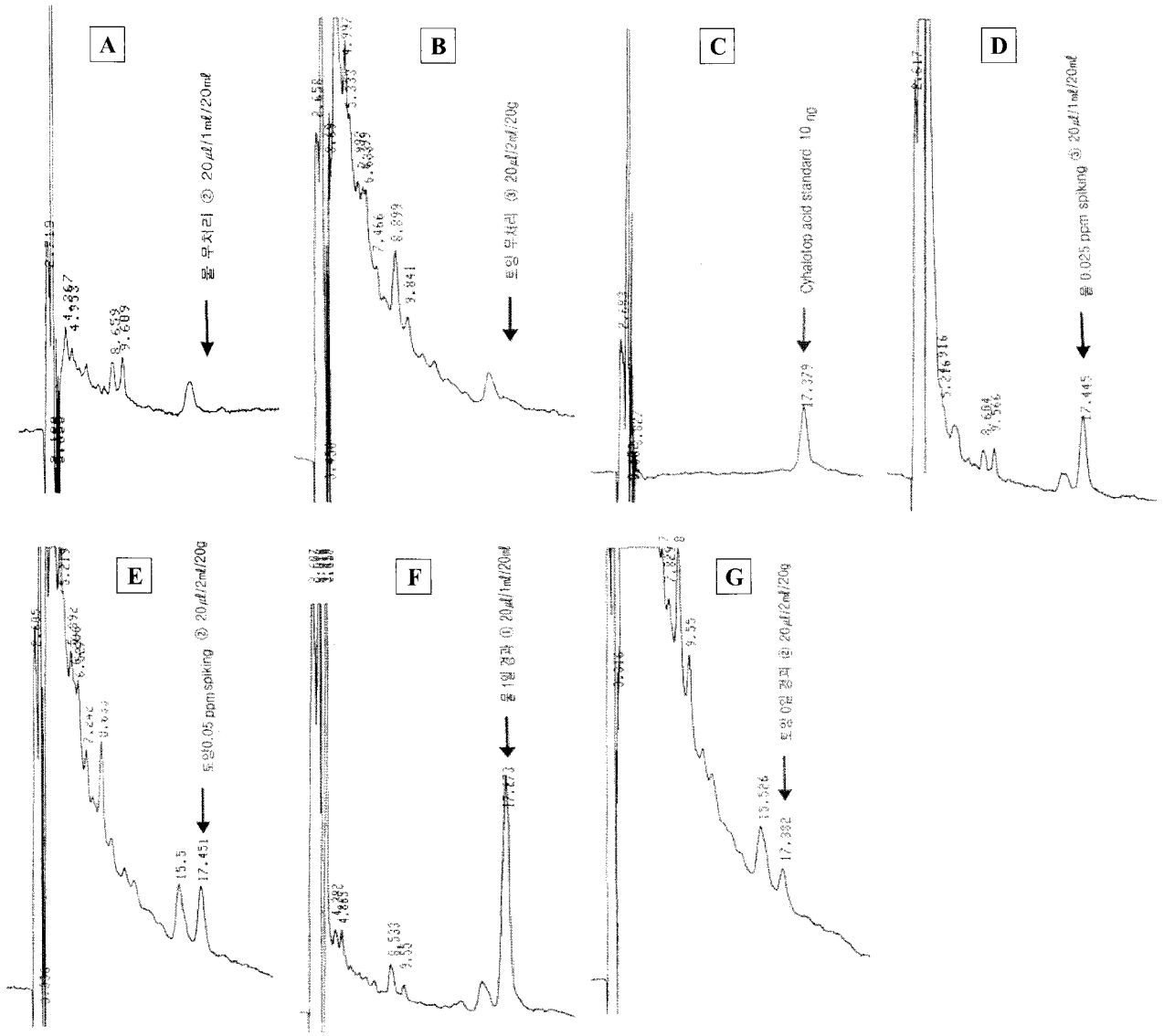


Fig. 5. Representative chromatograms of control water (A) and soil (B), cyhalofop acid standard (C, 0.5 mg/L), spiked water (D, 0.025 mg/L) and soil (E, 0.05 mg/kg) to controls for recovery, and water (F) and soil (G) sample at 0 day (2 hrs) treating the formulated product.

RSDs of ≤12%.

Literature Cited

Blasioli, S., I. Braschi, M. V. Pinna, A. Pusino and C. E. Gessa (2008) Effect of undersalted dissolved organic matter from composts on persistence, Adsorption, and Mobility of cyhalofop herbicide in soils. J. Agric. Food Chem. 56:4102~4111.

Choi, B. K., D.M. Hercales and A. I. Guser (2001) LC-MS/MS signal suppression effects in the analysis of pesticides in complex enviromental matrices. Fresenius. J. Anal. Chem. 369:370~377.

Choi, J.-H., M. I. R. Mamun, E.-H. Shin, H. K. Kim, A. M. Abd El-Aty and J.-H. Shim (2008) Determination of methoxyfenozide residues in water and soil by liquid chromatography: evaluation of its environmental fate under laboratory conditions. Toxicol. Res. 24(3):207~212.

Jackson, R. and M. Dougras (1999) An aquatic risk assessment for cyhalofop-butyl: A new herbicide for control of Barny arl grass in rice: Dow AgroScences, Letcobe Rege, Wantage, Oxfordshire, UK, Email: jacksonr@dow.com.

Kim, J.-S., J.-I. Oh, T. J. Kim, J. Y. Pyon and K. Y. Cho (2005) Physiological basis of differential phytotoxic activity between fenoxaprop-P-ethyl and cyhalofop-butyl-treated barnyardgrass.

Weed Biology and Management 5:39~45.
Mohammad, M., K. Itoh and K. Suyama (2008) Comparative effects of different families of herbicides on recovery potentials in Lemna sp. J. Pesti. Sci. 33(2):171~174.
Ottis, B. V., J. D. Mattrice and R. E. Talbert (2005) Determination of Antagonism between Cyhalofop-butyl and Other rice (Oryza sativa) Herbicides in Barnyardgrass (Echinochloa crus-galli). J. Agric. Food Chem. 53:4064~4068.
Pinna, W. V., I. Braschi, S. Blasioli, C. E. Gessa and A. Pusino (2008) Hydrolysis and Adsorption of cyhalofop-Butyl and Cyhalofop-acid on soil colloids. J. Agric. Food Chem. 56:5273~5277.
Ruiz-Santaella, J. P., A. Heredia and R. De Prado (2006) Basis of selectivity of cyhalofop-butyl in Oryza sativa L. Planta 223:191~199.
Takatori, S. S., M. Okihashi, Y. Okamoto, Y. Kitagawa, S. Kakimoto, H. Murata, T. Sumimoto and Y. Tanaka (2008) A rapid and easy multiresidue method for the determination of pesticide residues in vegetables, fruits, and cereals using liquid chromatography tandem mass spectrometry. Journal of AOAC International 91(4):871~883.
Wang, J. and D. Wotherspoon (2007) Determination of pesticides in apple by liquid Chromatography with electrospray ionization tandem mass spectrometry and estimation of meansurement uncertainty. Journal of AOA International 90(2):550~567.

LC를 이용한 물과 토양 중 Cyhalofop-butyl과 대사물질의 분석

헴리나 · 최정희 · 유학 · 카이 사티아 · 심재한*
전남대학교 농업생명과학대학 응용생물공학부 천연물화학실험실

요 약 실험실 내 조건에서, 물과 토양 중 cyhalofop-butyl과 그 대사물질인 cyhalofop acid를 위한 잔류분석법이 고감도에서도 간단하고 매우 효과적으로 개발되었다. 물과 토양 중 cyhalofop-butyl과 cyhalofop acid를 분석하기 위하여 액액분별추출과 silica gel chromatographic 정제를 수행하였으며 HPLC-UV를 이용하여 정성/정량하였다. Cyhalofop-butyl의 회수율은 2 가지 농도에서 3 반복 수행하여 각각 82.5-100.0%와 66.7-97.9%이었고, 검출한계와 최소검출량은 두 시료에서 모두 0.02 ppm과 10 ng이었다. Cyhalofop acid의 회수율은 물과 토양에서 각각 80.7-104.8%와 76.9-98.1%이었으며, 검출한계는 각각 0.005 ppm과 0.01 ppm이었고 최소검출량은 두 시료에서 모두 2 ng이었다. Cyhalofop-butyl의 반감기는 물과 토양에서 각각 4.14와 6.6 day였다. 개발되어진 본 시험법은 cyhalofop-butyl의 30% 유탁제를 처리한 물과 토양에서 그 잔류량을 분석하기 위하여 성공적으로 적용되었다.

색인어 Cyhalofop-butyl, Cyhalofop acid, 반감기, HPLC-UV