한국환경농학회지 제23권 제4호 (2004)
Korean Journal of Environmental Agriculture Vol. 23, No. 4, pp. 251-257

High-Performance Liquid Chromatographic Determination of Cyclosulfamuron Residues in Soil, Water, Rice Grain and Straw

Young-Deuk Lee* and Chan-Hyeok Kwon

Division of Life and Environmental Science, Daegu University, Gyeongsan 712-714, Korea

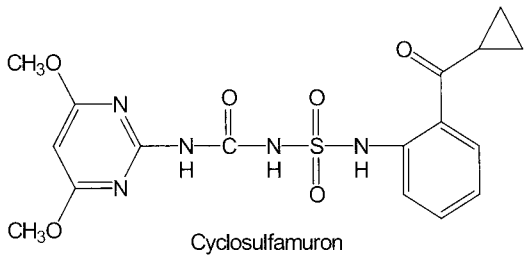
(Received November 19, 2004. Accepted December 13, 2004)

ABSTRACT : Analytical methods were developed to determine cyclosulfamuron residues in soil, water, rice grain and straw using high-performance liquid chromatography (HPLC) with ultraviolet absorption detection. In these methods, cyclosulfamuron was extracted with aqueous Na_2HPO_4/acetone and acetone/methanol mixture from soil and rice samples respectively. Liquid-liquid partition coupled with ion-associated technique, Florisil column chromatography, and solid-phase extraction (SPE) were used to separate cyclosulfamuron from interfering co-extractives prior to HPLC analysis. For water sample, the residue was enriched in C_{18}-SPE cartridge, cleaned up *in situ*, and directly subjected to HPLC. Reverse-phase HPLC under ion-suppression was successfully applied to determine cyclosulfamuron in sample extracts with the detection at its λ_{max} (254 nm). Recoveries from fortified samples averaged 87.8±7.1% (n=12), 97.3±7.2% (n=12), 90.8±6.6% (n=6), and 78.5±6.7% (n=6) for soil, water, rice grain and straw, respectively. Detection limits of the methods were 0.004 mg/kg, 0.001 mg/L, 0.01 mg/kg and 0.02 mg/kg for soil, water, rice grain and straw samples, respectively.

Key words: Cyclosulfamuron, residue analysis, HPLC determination, soil, water, rice.

INTRODUCTION

Cyclosulfamuron [1-[[2-(cyclopropylcarbonyl)phenyl]sulfamoyl]-3-(4,6-dimethoxypyrimidin-2-yl)urea] is a sulfonylurea herbicide that selectively controls a wide range of weeds during rice cultivation[1-3]. Its herbicidal activity which is known to be mainly via inhibition of acetolactate synthase and/or acetohydroxyacid synthase, is quite high enough to control annual and perennial weeds by applying only 45~60 g active ingredient per ha to the paddy field[4,5]. Even if the residue level would be far low in accordance with the reduced application rate, the residue arising from drift or carryover may still cause side effects on non-target organisms or succeeding crops due to its high biological activity[6-8]. An evaluation on the soil behavior as well as terminal residues in the crop harvest of cyclosulfamuron should be also prerequisite using the reliable analytical method as other herbicides were similarly estimated. Extremely low application rate of cyclosulfamuron, however, gives rise to practical difficulties to develop an analytical methods for its residues in crop and environmental samples. Its initial residue is presumed to be *ca.* 0.05 mg/kg level on the basis of 10 cm soil depth and this figure just reaches the detection limit of conventional analytical method for pesticide residues[9]. Since the residue decreases as time elapsed, a highly sensitive method to detect minute residues, preferably one tenth of the initial residue or less, is required to precisely track the residue in the soil environment[10]. The method performance should be extended to meet residue tolerances in rice harvest and stream water. Although some analytical methods for sulfonylurea herbicides are available[11-16], no specific method for cyclosulfamuron residues has been reported. The present paper describes analytical methods for cyclosulfamuron



Cyclosulfamuron

*Corresponding author:
Tel: +82-53-850-6753 Fax: +82-53-850-6759
E-mail: ydpechem@daegu.ac.kr

residues in paddy soil, water, rice grain and straw samples using high-performance liquid chromatography (HPLC). The methods were developed not only to achieve high sensitivity but to fulfill method reliability and readiness for analytical operation.

MATERIALS AND METHODS

Chemicals

Analytical standard of cyclosulfamuron (99.9% 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℃ for at least 6 months. Working solutions for fortification and HPLC calibration were freshly prepared in acetonitrile and acetonitrile/water (50/50, v/v), respectively, whenever necessary. Florisil (60~100 mesh, pesticide residue grade) was purchased from Aldrich Chemical (USA) and activated at 130℃ for more than 5 h prior to use. Solid-phase extraction (SPE) tubes of Supelclean C18™ (6 mL, 1 g packing) and Supelclean SCX™ (3 mL, 0.5 g packing) were obtained from Supelco (USA). Acetonitrile and deionized water were HPLC grade. All other solvents were pesticide residue grade or reagent grade freshly redistilled in glass. All other reagents were reagent grade unless specified.

Soil, water, and rice samples

Rice plants, Ilpoom and Palgong varieties, were grown in Kyungsan and Youngchun paddy fields located in Kyungbuk Province, respectively, where no cyclosulfamuron had been applied during the whole cultivation period. Bulk soil samples were collected from each field to the soil depth of 10 cm during the cultivating season. Paddy water was also sampled from standing water in the fields. At maturity rice plants were harvested from Kyungsan field. Grain and straw parts were separated from the plants and air-dried. Composite samples were prepared in compliance with the instructions in Korean Test Guidelines for Pesticide Persistence[10]. Soil samples were air-dried, and passed through 2-mm sieve prior to use. Physicochemical characteristics of soils are shown in Table 1. Paddy water was briefly filtered to remove plant debris before use. Rough rice grains were husked and finely pulverized to pass 40-mesh sieve using Wiley mill. Straw samples were chopped, air-dried, and also finely ground to pass 40-mesh sieve.

Table 1. Physicochemical characteristics of soils

Soil designation	Soil texture	Soil separate (%)			pH	OM (%)	CEC (cmol(+)/kg)
		Sand	Silt	Clay			
Kyungsan	SiCL	12.0	57.1	30.9	5.7	1.9	15.5
Youngchun	SiL	22.7	63.3	14.0	5.4	2.1	14.8

Extraction and cleanup of samples

Soil. To a 25 g of paddy soil sample (oven-dry basis) was added 2 mL of 2% aqueous disodium phosphate. After brief shaking and standing for 10 min, 100 mL of acetone was added, extracted for 1 h at 200 rpm, and suction-filtered. The flask and filter cake were washed with fresh 50 mL of acetone, and the washings were combined. The filtrate was evaporated to remove acetone at 40℃ *in vacuo* and the residue was quantitatively transferred into a 250-mL separatory funnel. The flask washed with 25 mL of ethyl ether, 25 mL of *n*-hexane and 40 mL of 2% aqueous disodium phosphate, and all the washings were transferred into the separatory funnel. After vigorous shaking for 1 min, the aqueous phase was transferred into another separatory funnel. The organic layer was extracted again with 20 mL of 2% aqueous sodium phosphate. To the combined aqueous layer, 6 *N* hydrochloric acid was added to adjust pH to 3.0±0.1 and then vigorously extracted with three 40 mL portions of *n*-hexane. Hexane phases were combined and evaporated just to dryness at 40℃. The residue was dissolved in 5 mL of acetonitrile and thoroughly mixed with 45 mL of deionized water for SPE cleanup. A SPE C18 cartridge was installed in a vacuum manifold (Supelco. USA) and activated by eluting with 10 mL of methanol and 5 mL of water in turn. The sample extract was loaded into the cartridge at the speed of 5 mL/min. The cartridge was eluted with 15 mL of acetonitrile/water (30/70, v/v) mixture and the fraction was discarded. The cartridge was then eluted with 10 mL of acetonitrile/water (60/40, v/v) mixture and the fraction was collected in a 10-mL volumetric flask for HPLC determination.

Water. Paddy water sample was suction-filtered through 0.45 ㎛ membrane filter prior to SPE extraction. To a 100 mL portion of the filtrate was added 1 mL of acetonitrile. A SPE C18 cartridge was installed and activated in a same manner of soil SPE. The water sample was applied to the cartridge at the speed of 5 mL/min. The cartridge was eluted with 15 mL of acetonitrile/water (30/70, v/v) mixture and the fraction was discarded. The cartridge was then eluted with 10 mL of acetonitrile/water (60/40, v/v) mixture and the fraction was collected for HPLC analysis.

Rice. A portion of grain (20 g) or straw (10 g) sample was extracted with 150 mL of acetone/methanol/water (2/2/1, v/v/v) mixture or 150 mL of acetone/methanol/water (1/1/1,

v/v/v) mixture for 1 h at 200 rpm and suction-filtered. The flask and filter cake were washed with fresh 40 mL of the extracting solvent, and the washings were combined. Total volume of the filtrate was adjusted to 200 mL. A 100 mL aliquot equivalent to half of grain or straw sample was transferred into a 1-liter separatory funnel, and sequential addition of 30 mL of saturated NaCl and 300 mL of water was followed. The aqueous layer was extracted with two 50 mL portions of dichloromethane. Dichloromethane phases were combined and evaporated just to dryness at 40℃. The residue was dissolved in 50 mL of *n*-hexane and transferred into a 250-mL separatory funnel. Dissolution was repeated with 40 mL and 20 mL portions of 2% aqueous disodium phosphate and all the rinsate was transferred into the funnel. The funnel was then vigorously shaken, stood until two layers completely separated, and the organic layer was discarded. To the aqueous layer, 6 *N* hydrochloric acid was added to adjust pH to 3.0±0.1 and then vigorously extracted with three 40 mL portions of *n*-hexane. Hexane phases were combined and evaporated just to dryness at 40 ℃. The residue was dissolved in 5 mL of dichloromethane for Florisil column chromatography. A chromatographic column (11 mm i.d. × 40 cm) was plugged with glass wool, dry packed with 5 g of activated Florisil 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 acetonitrile and the fraction was discarded. The column was then eluted with 40 mL of methanol/acetonitrile mixture (10/90, v/v). The eluate was collected and concentrated just to dryness. For the grain sample, the residue was dissolved in 10 mL of acetonitrile/water (50/50, v/v) mixture prior to HPLC analysis. The straw extract was reconstituted with 10 mL of acetonitrile/water (20/80, v/v) mixture, and 1 *N* hydrochloric acid was added to adjust pH to 2.0±0.1 for further SPE cleanup. In the vacuum manifold, a SPE SCX cartridge was installed and activated by serial elution of 3 mL of methanol and 3 mL of water (pH 2.0±0.1). The sample extract was loaded into the cartridge at the speed of *ca.* 2 drops/sec. The cartridge was eluted with 10 mL of methanol/water (20/80, v/v) mixture and the fraction was discarded. The cartridge was then eluted with 10 mL of methanol/water (60/40, v/v) mixture and the fraction was collected for HPLC determination.

High-performance liquid chromatography

High-performance liquid chromatography (HPLC) was performed using a HPLC system consisted of Waters (USA) 510 pump, 486 tunable UV/VIS absorbance detector, Rheodyne (USA) 7125 injector and Hewlett Packard (USA) 3396 Series II integrator. Nova-Pak C18 (3.9 mm i.d. × 150 mm, 4 ㎛ spherical, Waters, USA) was used as the analytical column. Operating parameters used for the determination of cyclosulfamuron residues are as follows; mobile phase, acetonitrile/0.05 M monosodium phosphate (50/50, v/v), isocratic; flow rate, 1.0 mL/min; detection, UV absorption at 254 nm, 0.004 AUFS; sample size, 50 ㎕; chart speed 0.5 cm/min. Under these conditions, retention time of cyclosulfamuron was 5.7 min.

Validation of analytical methods

Recovery experiments were run on control soil, water, rice grain and straw samples to validate analytical methods used for cyclosulfamuron residues. Prior to extraction, series of control samples were fortified at specified concentrations by spiking corresponding standard solutions in acetonitrile. After standing for 2 h, analytical procedures mentioned above were performed to produce quality assurance data.

RESULTS AND DISCUSSION

Various combined herbicides containing cyclosulfamuron are available for weed control in rice paddies. When commercial granular or suspension concentrate formulations are assumed to use at the recommended dose, its application rate is in the range of 0.0045~0.006 kg/10a[5]. This figure corresponds to *ca.* 0.05 mg/kg level of the initial residue on the basis of 10 cm soil depth. According to the Pesticide Fate Test Guidelines, the behavior of the herbicide in the soil environment should be evaluated until 90% of the initial residue is dissipated[10]. In this respect, the residue analytical method should have capability to detect 0.005 mg/kg of the residue at least. Since the detection limit of conventional analytical method is around 0.05 mg/kg[15,16], tenfold higher sensitivity is required. Therefore, the study was mainly focused on enhancing method sensitivity along with specificity while minimal analytical operation was maintained.

Cyclosulfamuron is an ionizable compound showing its pKa value of 5.04. In aqueous neutral and slightly alkaline solutions, the compound exists in the anionic form through

the loss of one of the urea hydrogen atom. Its log P value varies drastically as pH changes, ranging from 2.05 (pH 5) to 0.7 (pH 8). Vapor pressure is record to be 0.022 mPa at 20℃[3]. Cyclosulfamuron showed physicochemical properties similar to other sulfonylurea herbicides. It is known that sulfonylureas are not directly amenable to gas-liquid chromatography (GLC) because of their low volatility and thermal instability. Although GLC has been used in conjunction with various derivatization techniques, these approaches have not become widely accepted, owing to poor performance and troublesome procedure[11-14]. In this study, therefore, HPLC was involved to determine the cyclosulfamuron residue.

When reverse-phase HPLC using an octadecylsilyl column was employed, cyclosulfamuron showed typical characteristics of ionizable compound. Under the mobile phase of acetonitrile/water mixture, cyclosulfamuron was quite early eluted as given in Table 2. As water contents increased, cyclosulfamuron was retained but peak broadening became much severer than that usually found in neutral compounds. The extraordinary broadening strongly indicated that cyclosulfamuron co-existed as ionized/unionized forms in the column. When an acidic ion suppressor, monosodium phosphate, was added to the mobile phase, cyclosulfamuron showed a sharp symmetrical peak with threefold more retention as overall polarity of the compound reduced[17].

Since cyclosulfamuron is not readily oxidized nor reduced and had no fluorophore, ultraviolet absorption detector was the only choice among common HPLC detectors. When a cyclosulfamuron standard solution prepared in the HPLC mobile phase was scanned using Hewlett Packard (USA) 8452A photodiode array spectrophotometer, maximum absorption was found at 254 nm with extinction coefficient of 2.2×10^4 $cm^{-1}M^{-1}$. These λ_{max} and absorptivity seemed to be high enough to provide selectivity and sensitivity for the usual determination of pesticide residues[17-18]. Since tenfold higher sensitivity was required for the cyclosulfamuron residue, however, more concentration of sample extract was needed. As a result, much possibility of interference by co-extractives absorbing nearby ultraviolet region was expected. The study was, therefore, mainly conducted to develop efficient but simple cleanup methods for purification of sample extracts.

Considering the weak acid character of cyclosulfamuron, an attempt was done at partition step to remove interfering co-extractives. According to Henderson-Hasselbalch equation, pKa - pH = log[HA]/[A^-], ratio of unionized/ionized forms could be controlled by adjusting pH of the aqueous solution. As pKa of cyclosulfamuron is 5.04, acidification of the solution below pH 3 may lead organic-soluble unionized form to be predominant. Conversely, most of cyclosulfamuron exists in the ionic form above pH 7 as confirmed by log P change at different pH[4]. If the sample

Table 2. Effect of mobile phase on the chromatographic behavior of cyclosulfamuron in Nova-Pak C18 column

Mobile phase[a]	Retention time (min)	k'
I	1.39	0.29
II	2.48[b]	1.29[b]
III	5.72	4.29

[a]I, acetonitrile/water (50/50, v/v); II, acetonitrile/water (30/70, v/v); III, acetonitrile/0.05 M monosodium phosphate (50/50, v/v), [b]Peak broadening found.

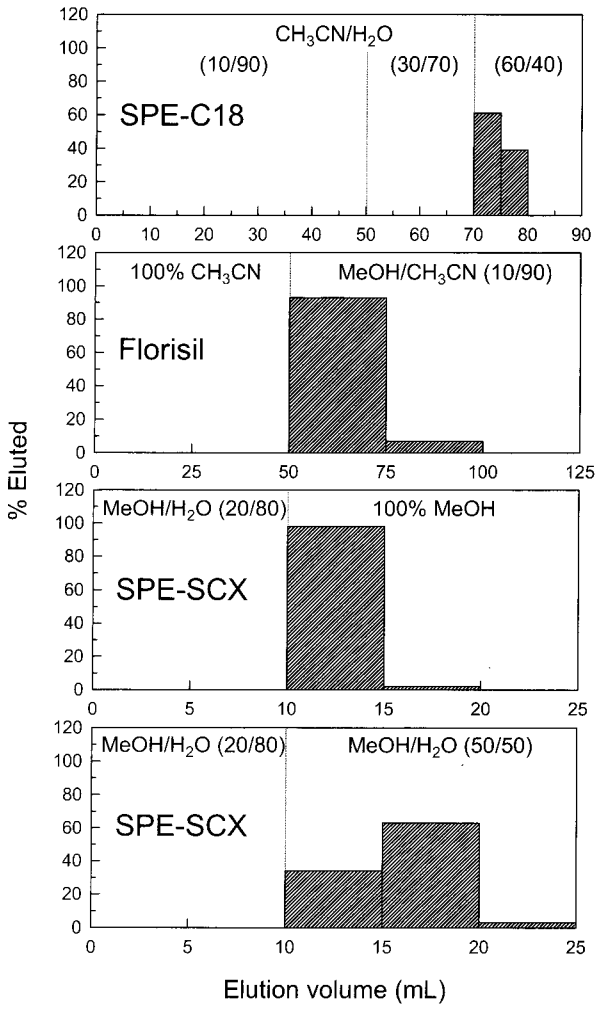


Fig. 1. Elution profiles of cyclosulfamuron on SPE C18, Florisil and SPE SCX columns.

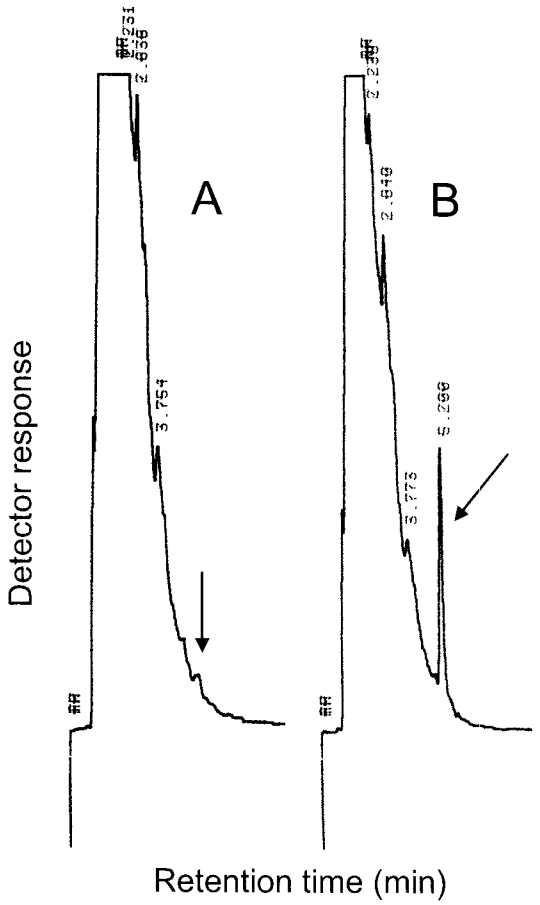


Fig. 2. Typical HPLC chromatograms of extracts from soil samples. A, control; B, fortified with cyclosulfamuron at 0.04 mg/kg.

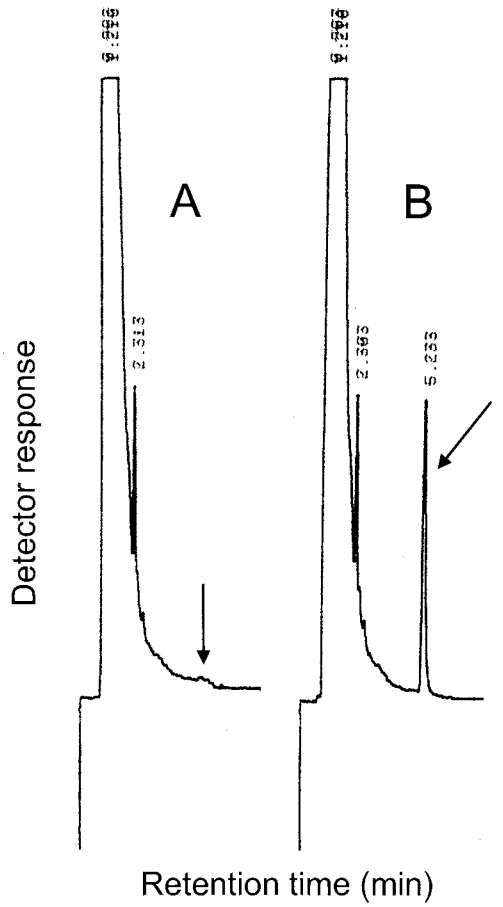


Fig. 3. Typical HPLC chromatograms of extracts from water samples. A, control; B, fortified with cyclosulfamuron at 0.01 mg/L.

extract is partitioned with organic solvent under slightly alkaline condition, ionized cyclosulfamuron still remains in the aqueous phase while organic-soluble co-extractives are removed. On the other hand, unionized cyclosulfamuron could be recovered from the acidified extract with organic solvent as water-soluble interferences discarded. Using this ion-associated partition technique, most of co-extractives from samples were effectively washed off.

Reversed partition, adsorption and cation-exchange chromatographic methods were selectively employed to further purify the extracts. Elution profiles of cyclosulfamuron on chromatographic media were optimized as shown in Fig. 1. Reversed partition chromatography using SPE C18 cartridge was applied to cleanup soil extracts. Acetonitrile/water mixtures with decreased polarity were sequentially eluted to sharply fractionate the cyclosulfamuron eluate as well as to discard co-extractives from the extract. SPE C18 was also used to extract water sample. Cyclosulfamuron in the water sample was trace-enriched in the retaining region of high water contents and eluted from the cartridge by decreasing the eluant polarity. Adsorption chromatography was carried out on Florisil column to purify extracts of rice grain and straw. Coloring co-extractives including plant pigments were effectively removed. Cation-exchange chromatography using SPE SCX was coupled with Florisil column to cleanup straw extracts in which severe interference was observed.

Typical HPLC chromatograms of soil, water, rice grain and straw extracts are shown in Fig. 2, 3, and 4, respectively. The proposed method produced clean HPLC chromatograms for soil, water and grain samples. Chromatograms of straw samples were rather complicated but quite acceptable for quantitation. Based on 3% full scale deflection (S/N >10), detection limits of the proposed method were 0.004 mg/kg, 0.001 mg/L, 0.01 mg/kg and 0.02 mg/kg for soil, water, rice grain and straw samples, respectively. Sensitivity for soil samples was high enough to detect at least one tenth of the initial residue which resulted from the cyclosulfamuron application in rice paddies. The method thuscould be utilized to investigate the behavior of cyclo-

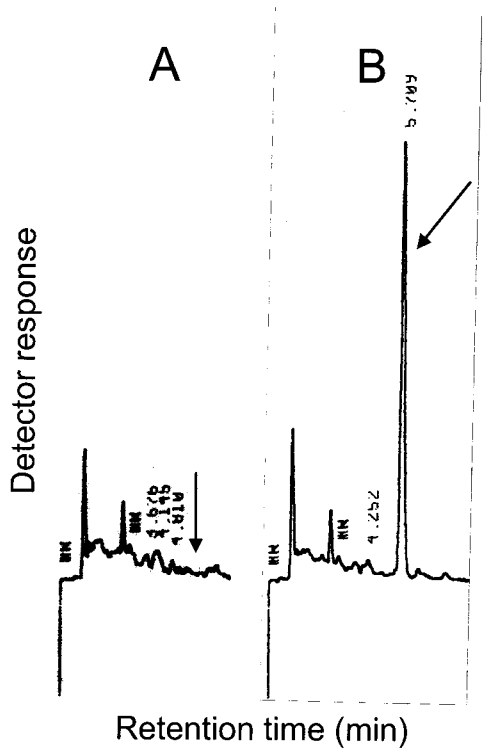


Fig. 4. Typical HPLC chromatograms of extracts from rice grain samples. A, control; B, fortified with cyclosulfamuron at 0.2 mg/kg.

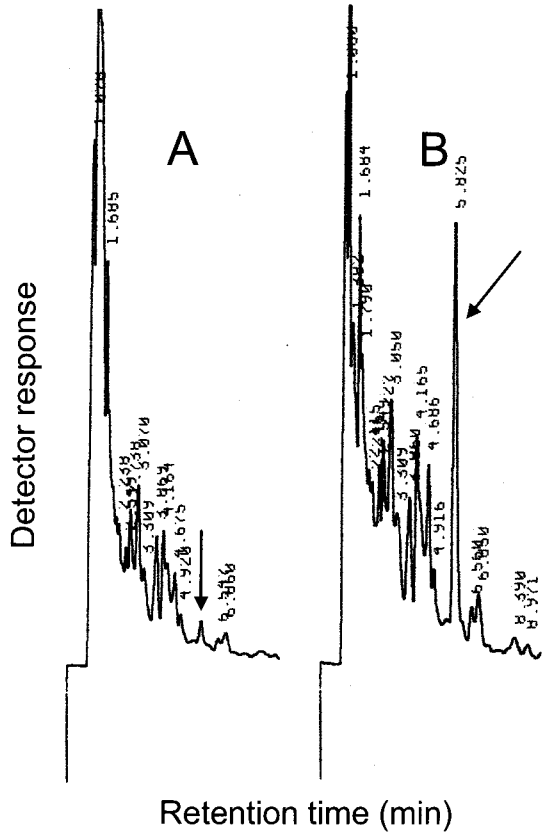


Fig. 5. Typical HPLC chromatograms of extracts from rice straw samples. A, control; B, fortified with cyclosulfamuron at 0.4 mg/kg.

esulfamuron in the soil environment until 90% of the applied dose was dissipated. Even if no tolerances have been stablished yet for rice and water[19-20], the method detectability would be expected to suffice the lowest monitoring level compared with tolerances of other sulfonylurea herbicides.

Percent recoveries generated during the validation of analytical methods are presented in Table 3. Recoveries averaged 87.8±7.1% (n=12), 97.3±7.2% (n=12), 90.8±6.6% (n=6), and 78.5± 6.7% (n=6) for soil, water, rice grain and straw samples, re-spectively. All the methods were successfully validated as measured by mean recoveries of more than 70% by 6 to 12 replicates per sample type. The coefficient of variation (CV) over all types of samples were less than 10%, indicating that high reproducibility of methods confirmed precision of analytical data for cyclosulfamuron residues in environmental and rice samples in spite of complicated analytical procedure.

The proposed method also 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 Administration[10]. Analytical procedures do not require any

Table 3. Recovery of cyclosulfamuron from fortified samples

Sample	Fortification (mg/kg)[a]	Recovery±SD (%)[b]	Detection limit (mg/kg)[a]
Kyungsan soil	0.04	91.5±9.0	0.004
	0.2	88.7±5.0	
Youngchun soil	0.04	84.1±7.9	0.004
	0.2	87.0±8.2	
Kyungsan water	0.01	102.2±0.7	0.001
	0.1	104.1±1.6	
Youngchun water	0.01	87.4±5.0	0.001
	0.1	95.6±0.9	
Rice grain	0.2	89.0±6.7	0.01
	1.0	92.7±7.3	
Rice straw	0.4	82.6±7.0	0.02
	2.0	74.3±3.4	

[a]mg/L for water samples, [b]Mean values for triplicate samples with standard deviations.

special apparatus or instruments but consist of currently available techniques familiar to the residue analyst as well. Sample preparation time, which is inevitably concomitant with enhancement of method sensitivity, takes longer than other residue analytical method. The speed of analysis is still comparable to other method as estimated that one experienced person can analyze 6 samples per day. Therefore, authors suggest that the proposed method could be sufficiently applied to the routine analysis of cyclosulfamuron residues in soil, water and rice samples.

ACKNOWLEDGMENTS

This research was supported in part by the Daegu University Research Grant, 2002.

REFERENCES

1. Murai, S., Hasui, H., Kawai, K., Kimpara, M. and Suzuki, M. (1993) AC 322,140 - a new herbicide for use in transplanted paddy rice in Japan, Proceedings of the 14th Asian-Pacific Weed Science Society Conference, Brisbane, Australia, p.154-158.
2. Quakenbush, L. S., Rodaway, S. J., Tecle, B., Brady, T. E., Lapade, B., Marc, P. and Condon, M. E. (1993) AC 322,140 - a new broad-spectrum herbicide for selective weed control in transplanted and water-seeded rice, Proceedings of the 10th Australian Weeds Conference, Brisbane, Australia, p.36-40.
3. Rodaway, S. J., Tecle, B. and Shaner, D. L. (1993) Mechanisms of selectivity of AC 322,140 in paddy rice, wheat, and barley, Proceedings of the Brighton Crop Protection Conference, Brighton UK, p.239-246.
4. Tomlin, C. (2003) *The Pesticide Manual* (13th ed.), p.222-223, British Crop Protection Council, Surrey, UK.
5. Agricultural Chemicals Industrial Association (ACIA) (2004) *The Pesticide Use Guidelines*, p.357, ACIA, Korea.
6. Hassall, K. A. (1990) *The Biochemistry and Uses of Pesticides*, pp. 463~488, MacMillan Press, London, UK.
7. Rouchaud, J. R., Neus, O., Cools, K. and Bulcke, R. (1999) Flupysulfuron soil dissipation and mobility in winter wheat crops, *J. Agric. Food Chem.*, 47, 3872-3878.
8. Vega, D., Cambon, J P. and Bastide, J. (2000) Triflusulfuron-methyl dissipation in water and soil, *J. Agric. Food Chem.*, 47, 3733-3737.
9. AOAC (2000) Pesticide and industrial chemical residues. In *Official method of analysis* (17th ed.), AOAC International, Arlington, VA, USA.
10. Rural Development Administration (RDA) (2001). Test guidelines for pesticide persistence. In *Criteria and Guidelines for Pesticide Registration*, RDA Notification No. 2000-23 Annex 8, Korea.
11. Ahmad, I. (1987) Capillary column gas chromatographic determination of trace residues of the herbicide chlorsulfuron in agricultural runoff water, *J. Assoc. Off. Anal. Chem.* 70, 745-748.
12. Ahmad, I. and Crawford, G. (1990) Trace residue analysis of the herbicide chlorsulfuron in soil by gas chromatography-electron capture detection, *J. Agric. Food Chem.* 38, 138-141.
13. Klaffenbach, P., Holland, P. T. and Lauren, D. R. (1993) Analysis of sulfonylurea herbicides by gas-liquid chromatography. 1. formation of the thermostable derivatives of chlorsulfuron and metsulfuron-methyl, *J. Agric. Food Chem.* 41, 388-395.
14. Klaffenbach, P. and Holland, P. T. (1993) Analysis of sulfonylurea herbicides by gas-liquid chromatography. 2. determination of chlorsulfuron and metsulfuron-methyl in soil and water samples, *J. Agric. Food Chem.* 41, 396-401.
15. Slates, R. V. (1983) Determination of chlorsulfuron residues in grain, straw, and green plants of cereals by high-performance liquid chromatography, *J. Agric. Food Chem.* 41, 113-117.
16. Powley, C. R. and Bernard, P. A. (1998) Screening method for nine sulfonylurea herbicides in soil and water by liquid chromatography with ultraviolet detection, *J. Agric. Food Chem.* 46, 514-519.
17. Snyder, L. R. Kirkland, J. J. and Glajch (1997) *Practical HPLC method development* (2nd ed.), p.60-76 and 292-317, John Wiley, New York, USA.
18. Lee, Y. D. and Kwon, C. H. (1999) Multiresidue analysis of eight acaricides in fruits, *Agric. Chem. Biotechnol.* 42 (4), 191-196.
19. Codex Committee on Pesticide Residues (1999) In *List of Maximum Residue Limits for Pesticides in Food and Animal Feeds*, Codex Alimentarius Commission, FAO/WHO, Rome, Italy.
20. Korean Food and Drug Administration (2002) The Code of Food, p.69-70