—Regular Articles—

Simultaneous Analysis of 10 Pyrethroid Pesticides in Natural Medicines by GC/MS with Negative Chemical Ionization

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Many methods for the determination of pesticides residues in food have been reported. Although natural medicines should be confirmed as safe as food, only a few methods for the determination of pesticide residues in natural medicines have been reported. In this study, 10 pyrethroid pesticides were detected in natural medicines by GC/MS with negative chemical ionization (NCI). GC/MS with NCI can detect halogenated pesticides selectively, and thus is suitable for the detection of pyrethroid pesticides. This study indicates that GC/MS with NCI is useful for analyzing pyrethroid pesticides in natural medicines.

Key words——natural medicine; pyrethroid pesticide; negative chemical ionization; GC/MS

INTRODUCTION

Various illnesses have been treated with natural medicines. Natural medicines should be confirmed as safe as food because patients generally take natural medicines long term. In recent years, pesticides have often been detected from imported food. In Japan, a maximum residue level has been set for about 800 pesticides in food; however, most natural medicines are imported, and a maximum residue level has been set for only 2 pesticides in natural medicines,[1] and only a few analytical methods for the determination of pesticide residues in natural medicines have been reported.[2,3] Pyrethroid pesticides have been detected from natural medicines since their first detection in 2003, so pyrethroid pesticide residue in natural medicines has been a social problem in recent years. The current methods[4,5] for analyzing pyrethroid pesticides in natural medicines use GC/ECD and only 3 pyrethroid pesticides have been measured. In this study, we simultaneously analyzed 10 pyrethroid pesticides in natural medicines by GC/MS with NCI, which can detect halogenated pesticides selectively.

MATERIALS AND METHODS

Pesticide Standards Tefluthrin, Bifenthrin, Fenpropathrin, Cyhalothrin, Cypermethrin and Fenvalerate were purchased from Dr. Ehrenstorfer GmbH (Germany). Flucythrinate and Fluvalinate were purchased from Kanto Kagaku (Japan). Cyfluthrin, cis-permethrin and trans-permethrin were purchased from Wako Pure Chemical (Japan). Each compound was dissolved in acetone to make 0.1 or 0.05 mg/ml stock standard solution. Permethrin was a mixture of equal parts of cis-permethrin and trans-permethrin. Spiking solutions were prepared from stock standard solutions at 5 μg/ml. Working standard solutions were diluted with extracts of pesticide-free samples in order to prevent a matrix effect.

Reagents Acetone, hexane and sodium chloride were pesticide analysis grade from Wako Pure Chemical. Supelclean ENVI Florisil SPE Tubes 6 ml (1 g) (Florisil) were purchased from Supelco (USA).

Table 1. The Monitoring Ions Selected for SIM

Compound	Monitoring ion (m/z)	
	Major	Minor
Tefluthrin	241	205
Bifenthrin	205	241
Fenpropathrin	141	—
Cyhalothrin	205	241
Permethrin	207	35
Cyfluthrin	207	171
Cypermethrin	207	171
Flucythrinate	243	199
Fenvalerate	211	167
Fluvalinate	294	—

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Table 2. Linear Ranges and Correlation Coefficients of Standard Solutions Diluted with Extracts of Pesticide-free Samples

Compound	Rhei rhizoma		Phellodendri cortex		Puerariae radix	
	Range (ppb)	Correlation coefficient (γ)	Range (ppb)	Correlation coefficient (γ)	Range (ppb)	Correlation coefficient (γ)
Tefluthrin	5-1000	0.9973	5-1000	0.9996	5-1000	0.9981
Bifenthrin	5-1000	0.9986	5-1000	0.9998	5-1000	0.9985
Cyhalothrin	5-1000	0.9974	5-1000	0.9998	5-1000	0.9991
Fenpropathrin	5-1000	0.9979	5-1000	0.9999	5-1000	0.9995
Permethrin	10-1000	0.9971	10-1000	0.9998	50-1500	0.9966
Cyfluthrin	5-1000	0.9965	5-1000	0.9993	5-1000	0.9997
Cypermethrin	5-1000	0.9967	5-1000	0.9993	5-1000	0.9998
Flucythrinate	5-1000	0.9955	5-1000	0.9992	5-1000	0.9994
Fenvalerate	5-1000	0.9941	5-1000	0.9986	5-1000	0.9995
Fluvalinate	5-1000	0.9946	5-1000	0.9984	5-1000	0.9999

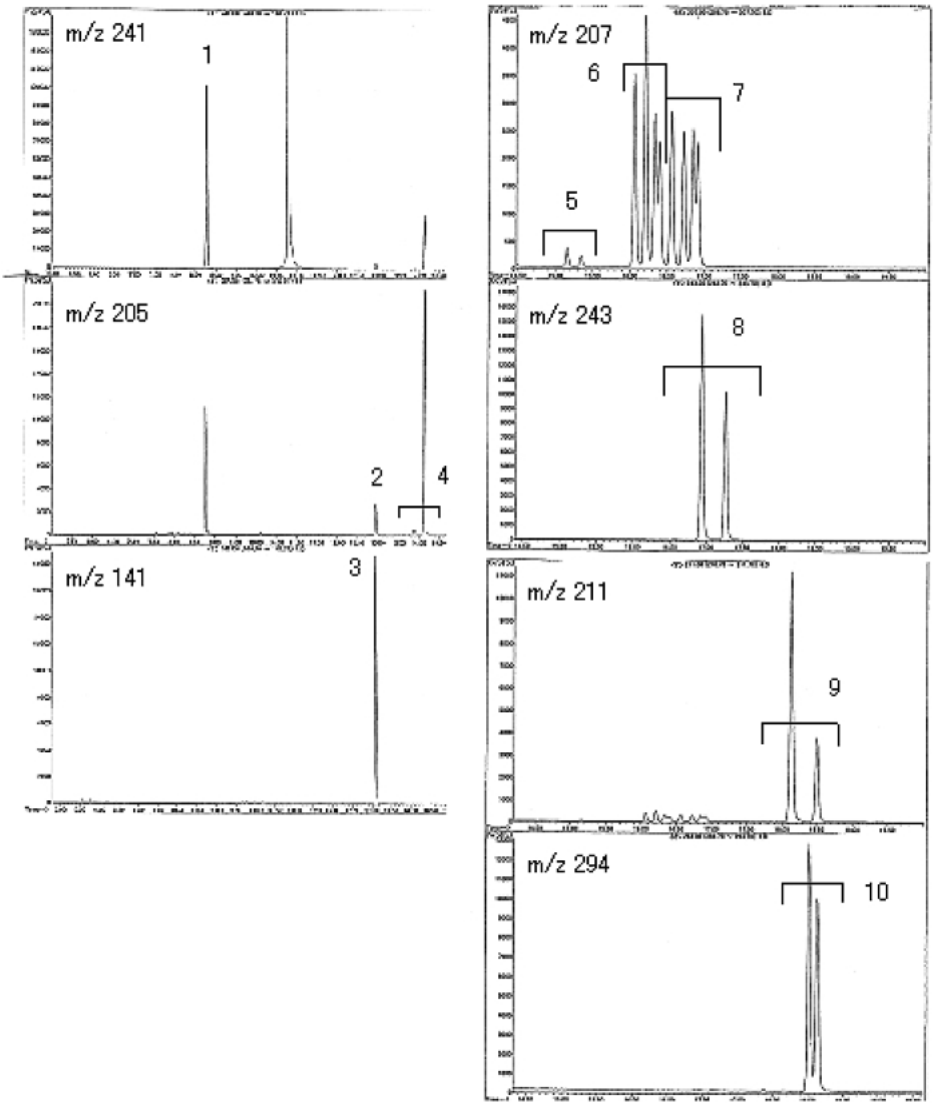

Fig. 1. SIM Chromatograms of Phellodendri Cortex Extract Fortified with Pesticides at 0.2 μg/ml
1: Tefluthrin, 2: Bifenthrin, 3: Fenpropathrin, 4: Cyhalothrin, 5: Permethrin, 6: Cyfluthrin, 7: Cypermethrin, 8: Flucythrinate, 9: Fenvalerate, 10: Fluvalinate.

Sample Preparation Puerariae radix, phellodendri cortex and rhei rhizoma were used in this study as Japan imports significant amounts of these 3 natural medicines. We confirmed that the concentrations of pesticide residues in these 3 natural medicines were below detectable levels with the proposed method. Natural medicines were powdered, 10 g was weighed out and 200—400 μl of spiking solution was added. After 30 minutes, 25 ml of acetone was added. After 1 hour, 50 ml of hexane was added and the mixture was homogenized for 2 min. The homogenate was filtered with filter paper (Toyo Roshi, Japan). The filtrate was washed 2 times with 25 ml of 5% NaCl solution, after washing with hexane. The obtained 10 ml hexane layer was loaded into a Florisil, and preconditioned with 5 ml acetone and 20 ml hexane. Pesticides were eluted with 15 ml of acetone-hexane (3 : 17). The eluate was evaporated and the residue was dissolved in 4 ml of acetone-hexane (3 : 17) for GC/MS analysis.

NCI Mode GC/MS A 5973MSD was connected to a GC 6890 (Agilent). GC conditions: column, HP-5ms capillary column 30 m×0.25 mm×0.25 μm (Agilent); helium carrier gas flow, 1.7 ml/min; injection temperature, 250℃; interface temperature, 250 ℃; ion source temperature, 200℃; ion mode, negative chemical ionization/selected ion monitoring mode; reaction gas, methane; oven temperature program: initial 50℃, 20℃/min to 240℃, then 4℃/min to 300℃ and held for 7 min; pulsed splitless injection at a volume of 1 μl by a HP 7683 auto injector (Hewlett Packard).

GC/ECD A Hewlett-Packard GC5890 series was used. The GC conditions were same as that of

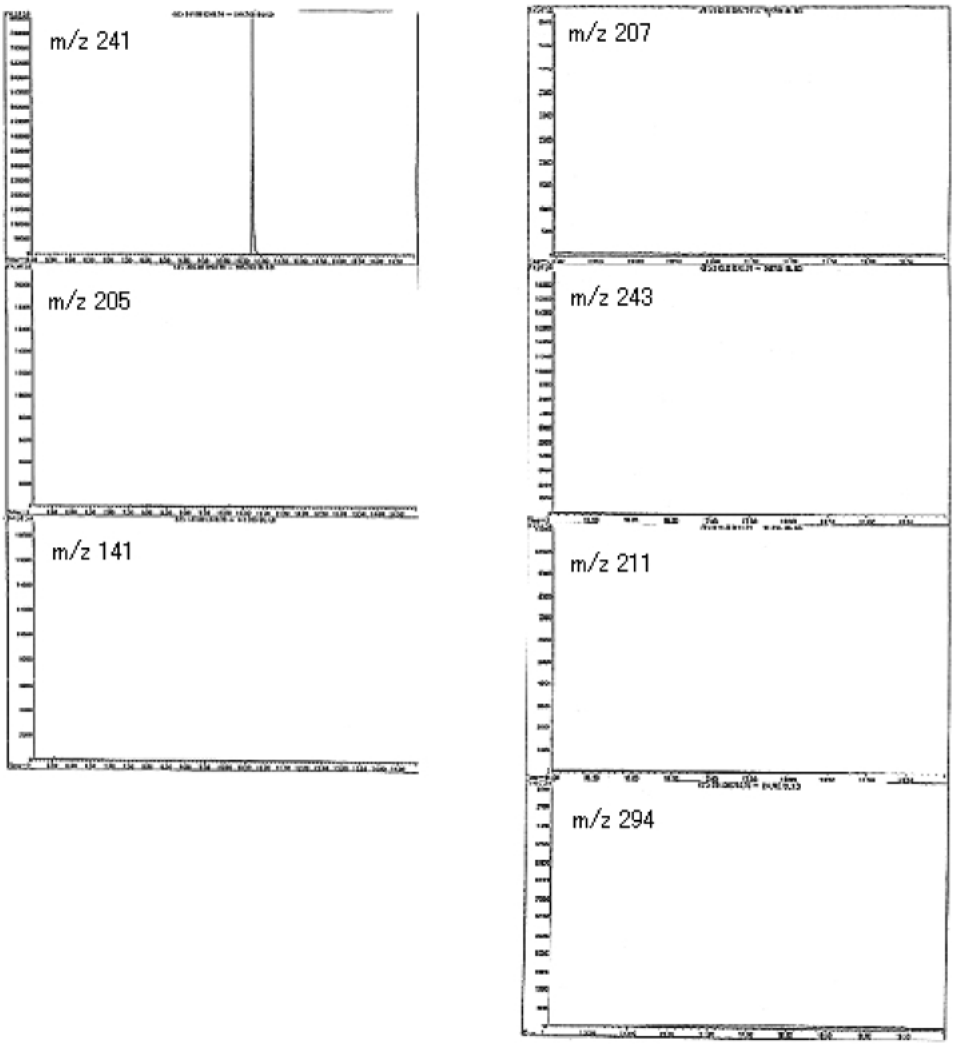

Fig. 2. SIM Chromatograms of the Phellodendri Cortex (Blank Sample)

NCI mode GC/MS.

RESULTS AND DISCUSSION

The monitoring ions selected for SIM detection are shown in Table 1. Matrix enhancement effects were sometimes observed in GC/MS. Standard solutions were therefore diluted with extracts of pesticide-free samples in order to prevent a matrix effect. The correlation coefficients of linearity can be seen in Table 2, which vary from 0.9941-0.9999. Standard solution diluted with extracts of 3 pesticide-free natural medicines showed good linearity. SIM chromatograms of the phellodendri cortex extract fortified with the pesticides are shown in Fig. 1. Pesticide peaks were clearly detectable. SIM chromatograms of the phellodendri cortex extract (blank sample) are shown in Fig. 2. There were no interfering peaks near the pesticide peaks. So GC/MS with NCI could detect pyrethroid pesticides selectively. GC/ECD chromatograms of the phellodendri cortex (blank sample) and standard solution diluted with hexane (0.1 ppm) are shown in Fig. 3. Too many interfering peaks were detected by GC/ECD. Figures 1 to 3 showed that GC/MS with NCI can detect pyrethroid pesticides more selective than GC/ECD. Tables 3 to 5 show the recovery results of 3 natural medicines, to which each pesticide was added at 0.1 and 0.2 ppm. The recovery rates of 10 pesticides were between 81% and 115% and most relative standard deviations (RSD) were less than 10 % at each spiked level. The recovery rates and RSD were satisfactory. The detection limits of 10 pesticides were below 10 ppb. The purpose of this study was to develop a selective method for the determination of pyrethroid pesticides in natural medicines.

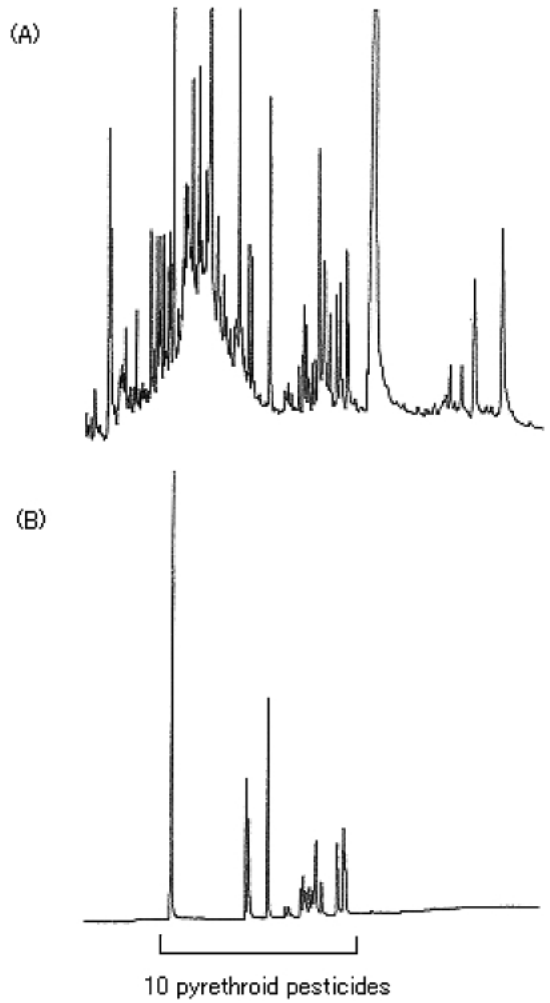

Fig. 3. GC/ECD Chromatograms of the (A) Phellodendri Cortex (Blank Sample), (B) Standard Solution Diluted with Hexane (0.1 ppm)

Table 3. Recovery of 10 Pyrethroid Pesticides in Rhei Rhizoma ($n=5$)

Spiked level (ng/ml)	10		20	
Compound	Average (%)	RSD (%)	Average (%)	RSD (%)
Tefluthrin	99.02	2.84	90.52	3.19
Bifenthrin	94.69	1.97	88.26	2.41
Cyhalothrin	96.69	2.61	84.95	3.13
Fenpropathrin	95.75	1.91	87.34	3.27
Permethrin	95.76	6.48	88.10	6.17
Cyfluthrin	96.11	4.34	82.39	3.24
Cypermethrin	95.70	3.99	83.11	3.58
Flucythrinate	94.67	3.52	82.13	3.79
Fenvalerate	92.74	3.54	82.64	4.71
Fluvalinate	90.75	2.94	81.26	4.12

Table 4. Recovery of 10 Pyrethroid Pesticides in Puerariae Radix ($n=5$)

Spiked level (ng/ml)	10		20	
Compound	Average (%)	RSD (%)	Average (%)	RSD (%)
Tefluthrin	100.50	3.25	98.94	6.01
Bifenthrin	94.66	4.38	93.69	4.69
Cyhalothrin	96.67	3.48	95.88	5.55
Fenpropathrin	94.28	5.87	99.85	4.79
Permethrin	106.11	7.42	100.29	12.17
Cyfluthrin	91.41	1.43	96.26	6.59
Cypermethrin	93.94	4.55	96.96	4.97
Flucythrinate	97.07	4.11	98.43	5.36
Fenvalerate	95.95	4.72	99.13	3.75
Fluvalinate	95.44	4.59	95.16	4.25

Table 5. Recovery of 10 Pyrethroid Pesticides in Phellodendri Cortex ($n=5$)

Spiked level (ng/ml)	10		20	
Compound	Average (%)	RSD (%)	Average (%)	RSD (%)
Tefluthrin	110.32	0.95	102.45	2.64
Bifenthrin	110.10	5.27	108.88	1.78
Cyhalothrin	107.08	2.01	104.01	1.94
Fenpropathrin	106.44	2.19	105.42	1.94
Permethrin	104.93	4.74	114.57	4.89
Cyfluthrin	106.22	2.23	106.66	2.46
Cypermethrin	108.21	2.41	107.78	2.82
Flucythrinate	107.64	2.25	106.86	2.64
Fenvalerate	107.65	1.84	110.53	1.95
Fluvalinate	109.56	1.90	110.60	2.94

This study indicates that GC/MS with NCI is useful for analyzing pyrethroid pesticides in natural medicines. This is the first report of an analytical method for the determination of pyrethroid pesticide residues in natural medicines by GC/MS with NCI.

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