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Determination of Pyrethroid Pesticides in Cinnamomi Cortex

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(Received August 26, 2008; Accepted October 7, 2008)

In Japan, maximum residue levels (MRL) have been set for eight pesticides (α-BHC, β-BHC, γ-BHC, δ-BHC (BHCs), p,p′-DDE, o,p′-DDT, p,p′-DDD, p,p′-DDT (DDTs)) in 14 crude drugs (below 0.2 ppm as total of BHCs, below 0.2 ppm as total of DDTs). There are fears that pesticides present in crude drugs for which MRL are set will be changed from BHCs and DDTs to other pesticides with MRL setting as the turning point. There are few surveys of pyrethroid pesticide in crude drugs distributed in Japan. The actual situation of pyrethroid pesticides in crude drugs distributed in Japan after setting MRL is not unclear and should be clarified. Although a method to analyze permethrin, cypermethrin and fenvalerate in 11 crude drugs was reported, it is not adequate because the recovery rates of permethrin, cypermethrin and fenvalerate from Cinnamomi cortex were very low and the method, including liquid-liquid partition is difficult. In this study, we developed a method using solid-phase extraction to analyze permethrin, cypermethrin and fenvalerate in Cinnamomi cortex with acceptable recovery rates. The sample solution was determined by gas chromatography/mass spectrometry with negative chemical ionization. The recovery rates of permethrin, cypermethrin and fenvalerate from Cinnamomi cortex were between 87.9 and 90.7%. Five samples of Cinnamomi cortex were analyzed according to the proposed method. No samples contained permethrin, cypermethrin and fenvalerate over detection limits. The proposed method could analyze permethrin, cypermethrin and fenvalerate in all crude drugs for which MRL are set.

Key words——crude drug; pyrethroid pesticide; cinnamomi cortex; gas chromatography/mass spectrometry

INTRODUCTION

Various illnesses are treated with crude drugs. Crude drugs should be confirmed to be as safe as food because patients generally take crude drugs over a long term. In Japan, MRL have been set for eight pesticides (BHCs and DDTs) in 14 crude drugs, including Cinnamomi cortex (Table 1).[1] MRL were set for Ginseng radix, Ginseng radix rubba and Sennae folium in 1998, and the other 11 crude drugs (11 CD) in 2006. We reported previously that BHCs in Ginseng radix and Sennae folium decreased with age, with MRL setting as the turning point.[2,3] Organochlorine pesticides in 11 CD were analyzed in 2003.[4] One or more pesticides (α-BHC, β-BHC, γ-BHC, δ-BHC, p,p′-DDE, o,p′-DDT, p,p′-DDD, p,p′-DDT) were detected from all 11 CD, so it is possible that BHCs or DDTs in 11 CD with MRL set in 2006 decreased, with MRL setting as the turning point. There are fears that pesticides present in crude drugs will change from BHCs and DDTs to other pesticides. There are few surveys of pesticide residues in crude drugs distributed in Japan. In particular, there are very few surveys of pyrethroid pesticide in crude drugs[4–7], and the latest surveys were reported in 2004. The actual situation of pyrethroid pesticide in crude drugs distributed in Japan after setting MRL is not unclear and should be clarified. Permethrin, cypermethrin and fenvalerate have been detected from crude drugs,[4–6] and were analyzed in 11 crude drugs (including Cinnamomi cortex)[4,5] and 6 crude drugs[6] for which MRL are set. The project of the Ministry of Health, Labour and

Table 1. 14 Crude Drugs for Which an MRL Is Set

CINNAMOMI CORTEX
GINSENG RADIX
GINSENG RADIX RUBBA
SENNAE FOLIUM
ASTRAGALI RADIX
POLYGALAE RADIX
GLYCYRRHIZAE RADIX
ASIASARI RADIX
CORNI FRUCTUS
PERILLAE HERBA
ZIZYPHI FRUCTUS
AURANTII NOBILIS PERICARPIUM
ERIOBOTRYAE FOLIUM
MOUTAN CORTEX

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Welfare (MHLW project) is the largest.[4,5] An analytical method using gas chromatography with electron capture detection (GC/ECD)[4,5] was reported in the MHLW project. This method is not adequate because the recovery rates of permethrin, cypermethrin and fenvalerate from Cinnamomi cortex are very low (between 31 and 50%),[4] and the method, including partition between hexane and 10% sodium chloride solution and between acetonitrile and hexane is difficult. Cinnamomi cortex is a very important crude drug and is imported in immense quantities (about 2000 tons/year),[1] and it is therefore important to ensure its safety. In this study, we developed a method using solid-phase extraction to analyze permethrin, cypermethrin and fenvalerate in Cinnamomi cortex with acceptable recovery rates. We previously reported that GC/mass spectrometry (MS) with negative chemical ionization (NCI) could analyze pyrethroid pesticides more selectively than GC/ECD.[8] We therefore analyzed pyrethroid pesticides by GC/MS with NCI in this study.

MATERIALS AND METHODS

Pesticide Standards Pesticide standards were obtained from Dr. Ehrenstorfer G.m.b.H. and Riedel de Haën (Germany). Each compound was dissolved in hexane to make 0.5 mg/ml of standard stock solution. Spiking solutions were prepared from standard stock solutions at 5 μg/ml. Working standard solutions were diluted with extracts of crude drugs to prevent a matrix effect.

Reagents Acetone, hexane, toluene, acetonitrile, sodium chloride and anhydrous sodium sulfate of pesticide analysis grade and activated charcoal were purchased from Wako Pure Chemical Industries (Japan). Supelclean ENVI Florisil SPE Tubes (6 ml, 1 g, Florisil) were purchased from Supelco (USA). Microcrystalline cellulose was purchased from Funakoshi Co. Ltd (Japan). Microcrystalline cellulose and activated charcoal were mixed in equal amounts and 1 g was weighed into a polypropylene tube (inside diameter; 12 mm) to make an activated charcoal mini-column.

Sample Preparation The sample preparation method was according to the previous report.[9] Crude drugs were crushed, 10 g was weighed out, and 400 μl of spiking solution (each pesticide: 2 μg) was added. After 30 min, 40 ml of acetonitrile was added and, following 30-min incubation, the mixture was homogenized for 1 min. Sodium chloride (1 g) and anhydrous sodium sulfate (4 g) were added. The mixture was shaken and centrifuged for 10 min at 3000 rpm. Twenty milliliters of the acetonitrile layer obtained were loaded into the activated charcoal mini-column preconditioned with 20 ml of acetonitrile-toluene (3:1). Pesticides were eluted with 20 ml of acetonitrile-toluene (3:1). The eluate and pass-through solutions were mixed and evaporated. The residue was dissolved in 5 ml of toluene and loaded into the Florisil mini-column preconditioned with 5 ml of acetone-hexane (3:17) and 5 ml of toluene. Pesticides were eluted with 25 ml of acetone-hexane (3:17). The eluate and pass-through solutions were mixed and evaporated. The residue was dissolved in 5 ml of acetone-hexane (3:17) for GC/MS analysis.

NCI Mode GC/MS GC/MS conditions were according to the previous report.[9] A 5973MSD was connected to a GC6890 (Agilent, USA). GC conditions: column, DB-1701 capillary column 30 m$\times$0.25 mm$\times$0.25 μm (J&W Scientific, USA); helium carrier gas flow, 1.7 ml/min; injection temperature, 200°C; interface temperature, 270°C; ion source temperature, 180°C; ion mode, NCI/selected ion monitoring (SIM) mode; reaction gas, methane; oven temperature program, 50°C for 1 min, 25°C/min to 100°C, and then 5°C/min to 270°C and held for 10 min; injection mode, splitless; injection volume, 2 μl.

RESULTS AND DISCUSSION

Analysis The monitoring ions selected for SIM detection are shown in Table 2. SIM chromatograms of Cinnamomi cortex extract fortified with pesticides are shown in Fig. 1. Pesticide peaks were clearly detectable. Each pesticide was calculated using total of the peak area. Many crude drugs are dry matter, so it is considered that the extract of crude drugs is subject to a matrix enhancement effect in GC/MS analysis. When working standard solution was diluted with acetone-hexane (3:17), the recovery rates were very high in Cinnamomi cortex (between 140.1 and 200.7

Table 2. Monitoring Ions Selected for SIM

Compound	Monitoring ion (m/z)	
permethrin	207	35
cypermethrin	207	171
fenvalerate	211	213

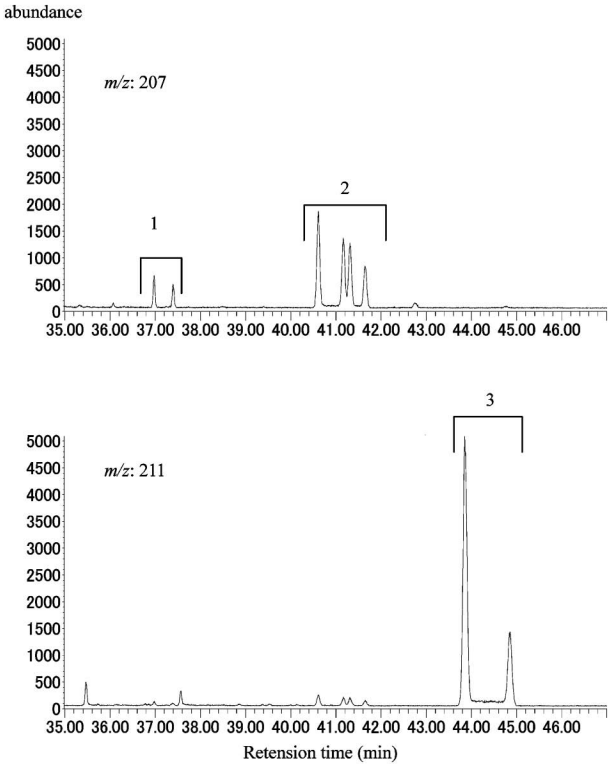

Fig. 1. SIM Chromatograms of Cinnamomi Cortex Fortified with Pesticides at 0.2 ppm
1: permethrin, 2: cypermethrin, 3: fenvalerate.

%). There are some methods to prevent matrix enhance effect like effective sample clean-up after the extraction, improvement of the chromathographic system and dilution of working standard solutions with extracts of crude drugs. To prevent the matrix enhancement effect, we diluted working standard solutions with extracts of crude drugs.[8,9] When working standard solutions were diluted with extracts of Cinnamomi cortex, the recovery rates were satisfactory (Table 3). It is considered that diluting working standard solution with an extract of Cinnamomi cortex overcome the matrix enhancement effect. This procedure was simple and could prevent the matrix enhancement effect. This result indicates that diluting working standard solutions with extracts of crude drugs are useful for analyzing permethrin, cypermethrin and fenvalerate in crude drugs.

Recovery tests were conducted three times for Cinnamomi cortex. Intra-day recovery rates of permethrin, cypermethrin and fenvalerate were between 87.9 and 90.7%, and for RSD were between 4.1 and 8.2% (Table 3). Inter-day recovery rates of permethrin, cypermethrin and fenvalerate were between 88.0 and 91.2%, and for RSD were between 1.1 and

Table 3. Recoveries of Permethrin, Cypermethrin and Fenvalerate in Cinnamomi Cortex

Compound	intra-day		inter-day[a)]	
	Average (%)	RSD (%)	Average (%)	RSD (%)
permethrin	90.2	4.1	91.2	2.9
cypermethrin	90.7	6.6	88.5	2.2
fenvalerate	87.9	8.2	88.0	1.1

[a)] Inter-day recovery study was carried out over 3 working days.

Table 4. Linear Ranges and Correlation Coefficients of Standard Solutions Diluted with Extract of Cinnamomi Cortex

Compound	Range (ppb)	Correlation coefficient (γ)
permethrin	50–1000	0.9950
cypermethrin	50–1000	0.9928
fenvalerate	50–1000	0.9944

2.9% (Table 3). The recovery rates and RSD were satisfactory for residue analysis. The recovery rates of the proposed method are much better than those of the MHLW project and it does not use difficult liquid-liquid partition; therefore, the proposed method seems useful for analyzing permethrin, cypermethrin and fenvalerate in Cinnamomi cortex. The correlation coefficients of linearity can be seen in Table 4, and vary from 0.9928–0.9950. Standard solution diluted with extract of Cinnamomi cortex showed good linearity. The detection limit of permethrin was 20 ppb (concentration in the injected solution; peak height was over 3 times the noise level in the major ions), and the detection limits of cypermethrin and fenvalerate were below 10 ppb.

Application of the GC/MS Method for Determination of Pyrethroid Pesticide in Cinnamomi Cortex Distributed in Japan Five samples of Cinnamomi cortex were analyzed according to the proposed method. Cinnamomi cortex was obtained in Japan (year: 2007, locality: 4 samples, China; 1 sample, Indonesia). No samples contained permethrin, cypermethrin and fenvalerate over detection limits. This result suggests that Cinnamomi cortex currently distributed in Japan does not contain high levels of permethrin, cypermethrin and fenvalerate.

Recovery Test Using 13 Other Crude Drugs We attempted to apply the proposed method to 13 other crude drugs for which MRL are set. Working standard solutions were respectively diluted with each ex-

Table 5. Recovery of Permethrin, Cypermethrin and Fenvalerate in Other 13 Crude Drugs ($n=3$)

Compound	permethrin		cypermethrin		fenvalerate	
	Average (%)	RSD (%)	Average (%)	RSD (%)	Average (%)	RSD (%)
GLYCYRRHIZAE RADIX	93.4	7.0	93.3	4.7	90.0	2.1
GINSENG RADIX	109.2	5.3	110.4	8.1	112.5	7.2
GINSENG RADIX RUBBA	104.9	3.8	103.9	4.2	103.2	4.2
SENNAE FOLIUM	102.3	2.3	95.8	2.6	94.6	3.0
ASTRAGALI RADIX	87.6	9.3	85.4	6.0	84.2	4.1
POLYGALAE RADIX	80.6	7.1	83.6	7.1	83.4	10.0
ASIASARI RADIX	83.5	13.0	89.0	11.7	93.8	12.3
CORNI FRUCTUS	97.6	6.1	96.7	6.8	96.2	5.8
PERILLAE HERBA	81.7	4.8	89.2	10.0	88.1	11.1
ZIZYPHI FRUCTUS	88.2	4.7	86.4	4.8	79.7	20.0
AURANTII NOBILIS PERICARPIUM	91.1	8.8	96.5	8.4	107.3	8.4
ERIOBOTRYAE FOLIUM	92.9	7.9	95.8	9.2	97.5	7.9
MOUTAN CORTEX	94.2	5.0	90.9	1.4	87.8	1.0

tract of crude drugs. The recovery tests were conducted three times for 13 crude drugs. The recovery rates of permethrin, cypermethrin and fenvalerate were between 79.7 and 112.5%, and most RSD were less than 10% (Table 5). The recovery rates and RSD were satisfactory. The proposed method can therefore be used to analyze permethrin, cypermethrin and fenvalerate in all 14 crude drugs for which an MRL is set with good recovery rates and RSD.

CONCLUSION

A method for determining permethrin, cypermethrin and fenvalerate in Cinnamomi cortex was developed and gave satisfactory recovery rates and RSD values for residue analysis. Five samples of Cinnamomi cortex were analyzed according to the proposed method and no samples contained high levels of permethrin, cypermethrin and fenvalerate. The proposed method could analyze permethrin, cypermethrin and fenvalerate in all 14 crude drugs for which MRL are set with acceptable recovery rates. The proposed method can be routinely applied because permethrin, cypermethrin and fenvalerate in all 14 crude drugs for which MRL are set can be analyzed using a common analytical method.

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