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Simultaneous Determination of 16 Pyrethroid Residues in Tea Samples Using Gas Chromatography and Ion Trap Mass Spectrometry

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Abstract

Pyrethroids are widely used in tea production, and pesticide residues in brewed tea are becoming a major issue. Thus, an appropriate control method of pyrethroid residues in tea samples has to be developed and used to reduce the potential health hazard from consumption of pyrethroids. A method is described here for the simultaneous determination of 16 pyrethroid residues in tea samples. The insecticides were extracted using acetone and then underwent cleanup through a florisil column. Analysis was performed by gas chromatography with ion trap mass spectrometry (GC–IT-MS) in MS–MS mode. Retention time and specific ions were used for identification. Recoveries at spiked levels (0.001–0.2 µg/g) for the 16 pyrethroids ranged from 71.3% to 106.3%, and the coefficient of variation was less than 17% in each case. The limits of detection were from 0.001 to 0.05 µg/g. The proposed method was successfully applied to determine pyrethroid residues in 25 brewed made tea samples. It was found that there were bifenthrin, cyfluthrin, lamda-cyhalothrin, cypermethrin, dicofol, fenpropathrin, fenvalerate, fluvalinate, and tetramethrin residues in different samples with levels ranging from 1.18–3071.29 µg/kg.

Introduction

China is the second largest tea exporter in the world with the largest plantation area. Tea crops are often attacked by several pests, such as the tea leaf roller (Caloptilia theivora), Homona magnanima Diakonoff, and Toxoptera aurantii (1).

Pyrethroids provide quick control of insects at low rates and are effective against various insects and mites. The relatively low mammalian toxicity and improved stability in outdoor environments account for the increasing use of pyrethroids in agriculture. Also, synthetic pyrethroids have a short safety interval due to relatively loose requirements of maximum residue limits and are widely used in tea plantation (2,3). Besides, pyrethroids can be produced as formulations with organochlorine or organophosphorus pesticide, which broadens the spectrum of pest control (4–6).

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However, some pyrethroid insecticides have shown slow metabolism in the environment and are often applied with synergists, which enhance the toxicity of pesticides and counteract the degradation (7). Pyrethroid residues in crops and environments are frequently reported. Some reports have pointed out that long-term exposure to pyrethroids can result in developmental neurotoxic and immunotoxic effects (8–10). Tea is a popular drink and directly consumed by soaking the leaves in hot water. The detection of pyrethroid residues in tea is very important for human health. Many countries and international organizations have set the maximum residue limits (MRLs) for different pyrethroid pesticides in tea (Table I). China has banned the use of dicofol, fenvalerate, and fenpropathrin in tea plantations because of the potential hazard of these pesticides to humans (11).

Table I. The Conditions of GC and Ion Trap

GC Conditions				
Injection Mode	Spitless			
Injection Volume	1 µL			
Injection Temp.	280°C			
Carrier Gas	He			
Flow	1.0 mL/min			
The Programmed Oven Temp.	**Temp. (°C)**	**Rate (°C/min)**	**Hold (min)**	**Total (min)**
	60		2.00	2.00
	150	25.0	0.00	5.60
	250	6.0	0.00	22.27
	300	4.0	5.23	35.00
Ion Trap Conditions				
Emission Current	50 µamps			
Multiplier	1750 V			
Target TIC	5000 counts			
Max Ionization Time	65000 µsec			
Prescan Ionization Time	1500 µsec			
Background Mass	45 *m/z*			
Trap Temp.	220°C			
Transfer Line Temp.	260°C			
Manifold Temp.	45°C			

Many methods for measuring pyrethroids have been used in the context of agricultural chemical determination, such as immunoassays, capillary electrophoresis, liquid chromatography (LC), gas chromatography (GC), and so on (12–22). There are halogen atoms in most pyrethroid molecules and electron capture detector (ECD) provides rapid, sensitive, and low-cost detection for pyrethroids. Nakamura et al. (23) detected natural pyrethrins and 12 synthetic pyrethroids in vegetables, fruits, grains, beans, and green tea leaves using GC–ECD; the recoveries at fortified levels (250–1000 µg/kg) were 60–103.5%. Similar reports on pyrethroid detection by GC–ECD can often be seen in various matrices including, water, soil, sediments, milk, meat, vegetables, and fruits (24–31).

While ECD is currently used in routine analysis, the overlapping compounds could not be determined independently using GC–ECD in multi-residue analysis, which needs to be resolved using mass spectrometry (MS). Tea isn't an easy matrice. Natural contents in tea leaves, like colored materials (theocin and saponin), can be co-extracted in sample preparation, and MS is always needed for definite result confirmation (32). Huang (33) developed a multi-residue analytical method using GC–MS (selective ions mode) for 102 pesticides (containing 8 pyrethroid insecticides) in tea, and the LOQs ranged from 47 to 500 µg/kg depending on the compounds. Bishnu et al. (34) detected cypermethrin and deltamethrin residues in tea ecosystems in the hills in India, and non-synthetic pyrethroid residues were also detected.

The present paper describes the establishment of an analytical method using GC–IT-MS for 16 synthetic pyrethroids in tea and the application of this method in brewed tea samples. The goal of the study is to propose the effectiveness of IT-MS in multi-residue detection of pyrethroids in routine applications, and to assess the extent of pyrethroids residue contamination in Chinese tea.

Experimental

Chemicals

All pesticide standards were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). A stock standard solution was prepared with cyclohexane to 1000 mg/L, and the working concentration of mixed standards was 10 mg/L. All solutions were stored at –20°C until use. All solvents used in this study were of pesticide residue grade. Anhydrous sodium sulfate and florisil were heated at 650°C for at least 6 h to remove any possible background pesticide contamination. Florisil was deactivated using water (5%, m/m) and stored in a desiccator until use. Other reagents were of analytical grade or better.

Tea samples

All tea samples were obtained from the Tea Quality Supervision and Examination Station in Beijing, China. The tea samples were ground and then filtered through a 40 mesh sieve.

Approximately 2 g tea powder was soaked in 15 mL hot water (90–100°C) for 2 h and then extracted using 30 mL acetone. The tubes were vortexed for 1 min and ultrasonicated for 20 min. After that, more acetone was added to a total volume of 50 mL. The extracted solution (20 mL) was mixed with aqueous solution sodium chloride (15%, m/m) and then partitioned with 25 mL hexane. The liquid–liquid partition process was repeated once, and the upper solution was combined for evaporation. The residues were reconstituted with 5 mL petroleum ether.

Cleanup was carried out using a sandwich glass cartridge (40 cm × 12 mm), which was packed with 1 g anhydrous sodium, 5 g florisil, and 2 g anhydrous sodium sulfate from bottom to top. The cartridge was conditioned with 20 mL petroleum ether and loaded with the extracted solution. Another 20 mL petroleum ether was used to rinse the cartridge, and the retained fraction was eluted with 100 mL petroleum ether–diethyl ether (85–15, v/v).

Table II. The Main Parameters and MRLs in Tea of Sixteen Compounds

Insecticide	Molecular weight	Time segment	Retention window (min)	Retention time(min)	Precursor ions (m/z)	Waveform type	Excitation amplitude	Quan ions (m/z)	Ref ions (m/z)	MRLs (43) µg/g
Dicofol	370.5	1	15.00–16.38	16.279	139	Non-resonant	47	139	111,140	20(EU),ND(U.S)
Fenson	268.7	2	16.38–16.70	16.434	141	Non-resonant	30	140	138,139	
Allethrin (I, II)	302.4	3	16.70–17.70	17.036,17.209	123	Non-resonant	36	121	121,81	
Chlorfenson	303.2	4	17.70–19.00	18.533	175	Non-resonant	39	110	111,129	0.1(EU)
Bifenthrin	422.9	5	19.00–22.75	22.642	181	Non-resonant	70	165	165,166	5(EU), 25 (Japan)
Tetramethrin-I	331.4			22.662	164	Resonant	1.20	93	135,93	
Tetramethrin-II	331.4	6	22.75–22.90	22.810	164	Non-resonant	60	107	93,91	
Fenpropathrin	349.4	7	22.90–23.20	22.994	265	Resonant	0.63	210	236,172	0.02(EU)
Phenothrin (I, II)	350.4	8	23.20–23.90	23.437,23.667	123	Non-resonant	44	81	121,95	0.1 (EU), 10 (Japan)
lamda-Cyhalothrin	449.9	9	23.90–24.90	24.387	197	Resonant	0.33	161	177,141	15 (EU,Japan), 3 (China)
Permethin(I, II)	391.3	10	24.90–26.50	25.872,26.132	183	Resonant	0.56	168	155,181	0.1 (EU), 20 (China)
Cyfluthrin (I, II, III, VI)	434.3	11	26.50–27.45	26.946,27.168 27.265,27.359	163	Non-resonant	35	163	161,127	20 (Japan)
Cypermethrin (I, II, III, VI)	416.3	12	27.45–29.00	27.575, 27.822,27.911,28.012	163	Resonant	0.72	127	125,91	0.5 (EU), 20 (China)
Flucythrinate (I, II)	451.4			27.935,28.338	199	Non-resonant	32	157	171,197	20 (China,Japan), 0.1 (EU)
Fenvalerate	419.9	13	29.00–29.53	29.046	225	Resonant	0.56	147	119,199	2 (China), 1 (Japan), 0.05 (EU)
Fluvalinate (I, II)	502.9	14	29.53–30.00	29.647,29.811	250	Resonant	0.65	200	200,215	10 (Japan)
Deltamethrin	505.2	15	30.00–31.5	31.111	253	Resonant	0.32	172	172,174	10 (FAO, China, Japan), 5 (EU)

The cleaned extracts were condensed to 200 µL for analysis.

Fortified tea samples were prepared by adding a certain volume of mixed standard solution (10 mg/L) to the weighted samples and allowing them to stand overnight before extraction. Six replicates were prepared at each spiked level. Recoveries and limits of detection (LOD) of the method were obtained by spiking tea samples at 1–200 µg/kg. The LOD was based on the peak height value 3 times the baseline noise and a coefficient of variation of 6 repeated analyses less than 20%.

Analysis by GC–IT-MS

The equipment used was as follows: Gas chromatograph, Varian GC 3800 with 1079 injector (Varian Inc., Walnut Creek, CA); mass spectrometer, Varian Saturn 2000 ion trap mass spectrometer; workstation, Varian Saturn GC–MS workstation, version 6.9; analytical column, VF-5ms, low bleed capillary column, 30 m × 0.25 µm × 0.25 mm. The conditions of GC and ion trap are listed in Table I.

Table III. Recoveries and LODs of Sixteen Compounds in Tea Sample (*n* = 6)

Insecticides	Spiked level (µg/kg)	Average % Recoveries	CV (%)	LOD (µg/kg)
Dicofol	1	73.2	3.65	1
	10	82.5	1.79	
Fenson	5	77.6	2.45	5
	50	79.4	3.64	
Allethrin	10	75.7	6.13	10
	100	84.2	4.23	
Chlorfenson	10	83.5	4.65	10
	100	87.4	6.21	
Bifenthrin	10	76.3	7.63	10
	100	84.3	5.42	
Tetramethrin	10	76.2	8.32	10
	100	80.2	10.6	
Fenpropathrin	5	85.1	3.23	5
	50	93.5	2.51	
Phenothrin	5	82.5	5.25	5
	50	92.6	6.22	
lamda-Cyhalothrin	10	89.7	8.65	10
	100	92.1	7.62	
Permethin	10	81.3	16.84	10
	100	87.4	6.33	
Cyfluthrin	50	83.1	11.51	50
	200	86.4	7.32	
Cypermethrin	50	80.2	15.94	50
	200	90.3	9.51	
Flucythrinate	10	106.3	14.21	10
	100	93.2	6.52	
Fenvalerate	50	72.1	8.32	50
	200	104.2	4.87	
Fluvalinate	50	71.3	8.22	50
	200	85.1	9.56	
Deltamethrin	50	78.3	11.42	50
	200	79.5	6.37	

The parameters of mass spectrometry for each compound were optimized and are listed in Table II. The GC–MS–MS method was set up by dividing the runtime into 15 segments based on the retention times of the 16 compounds. In general, one compound was set in one time segment unless the retention time was too close to be divided.

Results and Discussion

Optimization of Sample Preparation

Acetone can effectively extract the targeted compounds from matrix. The coefficient of variation of the method was < 17% and recoveries ranged from 72.3% to 106.3% for each compound in our tests (Table III). Because of the complexity of the tea extracts, the cleanup step is necessary before analysis. Some conventional sample preparation in analysis of pyrethroids residues including liquid-liquid partitioning (35) or solid-matrix partitioning (36) and/or gel permeation chromatography (37) are used in literature. Adsorption chromatography like florisil, silica gel, or aluminium oxide was used most frequently (13). The extracts of tea were cleaned-upe by a florisil cartridge here to remove amounts of colored substances through changing the polarity of elution solution. Satisfactory purified results were obtained and no remarkable matrix effects were found in our work.

Optimization of instrument parameters

Pyrethroids belong to lipophilic substances with high boiling points, and low/midpolarity capillary columns were usually used for their separation by GC (13). VF-5ms column, as a low polarity column, was used here with the selected temperature program. The injection temperature was 280°C to ensure all compounds can be vaporized transiently after injection. The slow heating rates from 150°C to 280°C in the programmed temperature process ensured the effective separation of similar structured pyrethroids. A typical chromatograph is shown in Figure 1.

Theoretically, ion trap mass detector can be performed with high "n" of MS^n, although high "n" values result in little signals that can't be detected. We used electron impact mode with MS–MS acquisition. After the isolation step, the precursor ion could be subjected to excitation to generate the characteristic product ions. Full-scan EI (electron impact ion source) mass spectra of each compound were recorded in the electron impact mode with ionization energy of 70 eV, under automatic gain control (AGC). The base peak of the mass spectra was chosen as the parent ion for each compound, but another higher intensity peak (whose $m/z > 100$) was used as the precursor ion when the m/z of the base peak was less than 100. The ion with higher m/z was chosen when the peak intensities were close.

A low target value (5000 counts) was set to minimize the 'space-charge' effects in ion trap mass spectrometer. The filament emission current was set at 50 µA, and the multiplier voltage was raised from its software-optimized value according to a 400 V_{p-p} offset. The optimization of experimental conditions for dissociation of ions generated from each compound was facilitated. Two excitation methods (38) (resonant and non-resonant method) were tested for individual ions in each compound to

Table IV. Summary of the Detected Levels of Targeted Compounds in Different Category of Tea for 25 Samples*

	Detected concentrations in different variety (µg/kg)				
Insecticide	Green tea	Dark tea	Black tea	Oolong tea	Scented tea
Dicofol	1.18–3.67	5.12–1730.05	2.32–11.07	9.74–110.08	1.62–21.34
Fenson	–	–	–	–	–
Allethrin –	–	–	–	–	
Chlorfenson	–	–	–	–	–
Bifenthrin	14.25–110.77	39.82–42.12	–	75.85	233.30–3071.29
Tetramethrin	13.58	10.91	–	84.08–908.26	17.25–117.92
Fenpropathrin	–	6.58–12.25	–	–	5.50
Phenothrin	–	–	–	–	–
Lamda-cyhalothrin	–	–	–	11.12	–
Permethin	11.92	–	–	–	–
Cyfluthrin	–	34.23	–	29.21	18.75
Cypermethrin	20.13–187.65	42.98–69.41	39.02–43.45	32.84–49.23	29.54–32.38
Flucythrinate	–	–	–	–	–
Fenvalerate	–	13.18	–	–	–
Fluvalinate	–	21.14	–	–	–
Deltamethrin	–	–	–	–	–

*Note: "–" not detectable.

obtain the best response. McLuckey et al., (39) showed that the ratio of ion abundance in mass spectrum depended on the quantity of samples injected into the system (self-ionization phenomenon). We used the mass spectra of a more dilute mixture solution (200 µg/L for cypermethrin and cyfluthrin, 100 µg/L for other compounds) to provide useful diagnostic peaks. The retention time, ion fragments, and ratio of ion abundance were used for peak identification. The pyrethroid pesticides, such as cypermethrin and cyfluthrin, used in this study were racemic, and four peaks were found for these pesticides under the conditions used. The combined area of peaks was used for quantitation of these compounds. Table III shows the LODs of the 16 compounds. The LOD varied from 1 µg/kg to 50 µg/kg depending on the compound. The LODs here are similar to the data obtained using quadrupole mass spectrometer (11,25,32), which exhibits the applicability of GC–IT-MS in pyrethroid analysis.

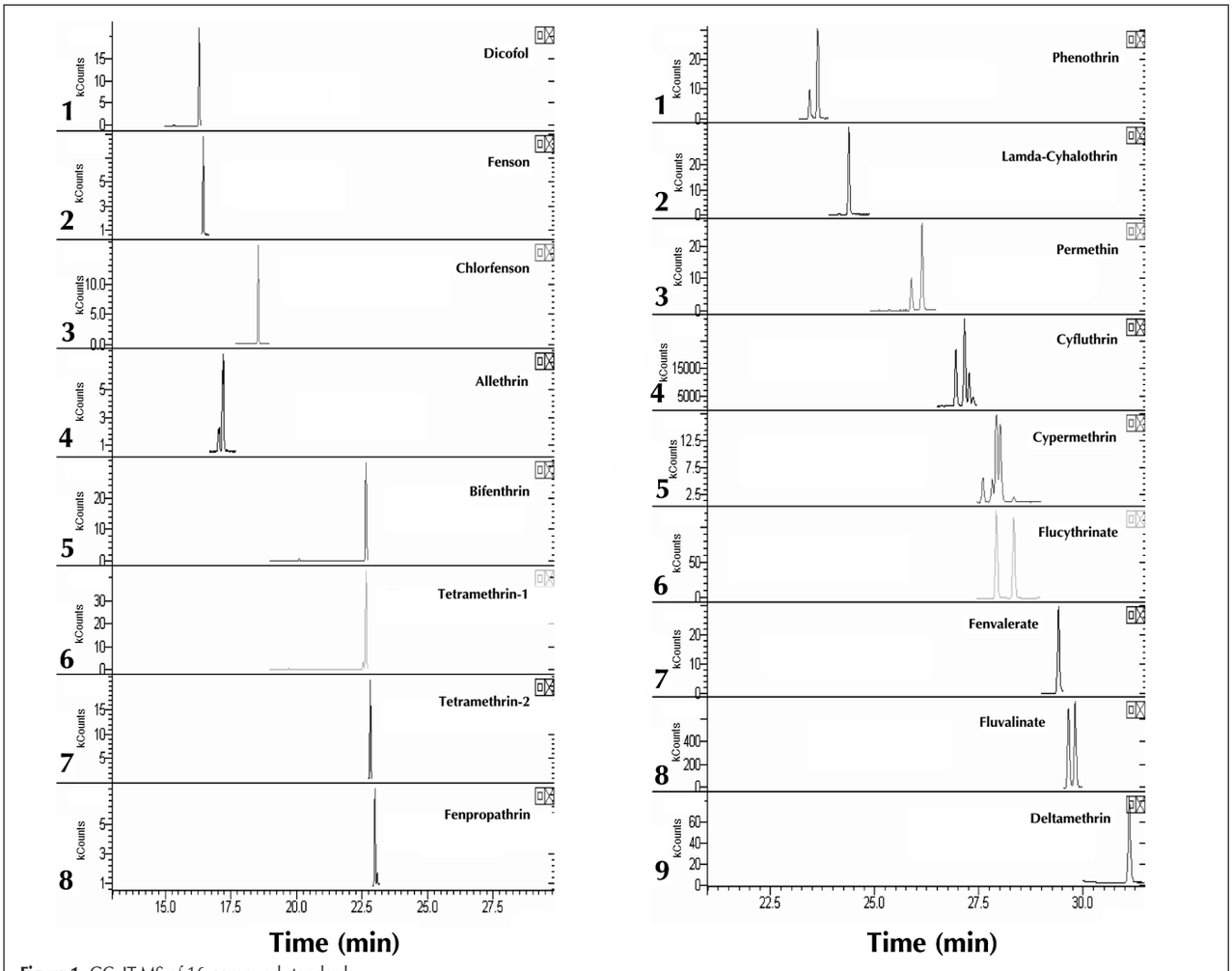

Figure 1. GC–IT-MS of 16 compund standards.

Detection of pyrethroid residues in tea samples

Twenty-five tea samples (8 green tea, 5 scented tea, 3 oolong tea, 5 dark tea, and 4 black tea samples) produced in 2008 were used in this study, and the detection results are shown in Table VI. Seventeen samples (6 green tea, 4 scented tea, 2 black tea, 3 dark tea and 2 oolong tea samples) were found to contain cypermethrin residues with residual levels ranging from 20.13–187.65 µg/kg. Eleven samples were found to contain bifenthrin residues with concentrations ranging from 14.25 to 3071.29 µg/kg (4 green tea, 3 scented tea, 3 dark tea and 1 oolong tea samples). All samples were found to contain dicofol residues with levels ranging from 1.18 to 1730.05 µg/kg. Tetramethrin residues were found in 1 green tea sample (13.58 µg/kg), 1 dark tea sample (10.91 µg/kg), 2 scented tea samples (17.25, 117.92 µg/kg), and 2 oolong tea samples (84.08, 908.26 µg/kg). Three samples were found to contain fenpropathrin residues, 2 dark tea samples (6.58, 12.25 µg/kg), and 1 scented tea sample (5.50 µg/kg). A lamda-cyhalothrin residue was found in one oolong tea sample (11.12 µg/kg). A permethrin residue was detected in one green tea sample (11.92 µg/kg). Cyfluthrin residues were found in one scented tea (18.75 µg/kg), one oolong tea (29.21 µg/kg), and one dark tea sample (34.23 µg/kg). A fenvalerate residue was found in one dark tea sample (13.18 µg/kg) and fluvalinate was found in one dark tea sample (21.14 µg/kg).

The MRLs of some pyrethroids in tea were relatively loose (see Table II). Pyrethroid residues in tea seemed a common problem. Although the use of dicofol, fenvalerate, and fenpropathrin have been banned in tea plantations in China, the results of this study showed that abuse of these pesticides may exist in some regions. Cypermethrin and bifenthrin were the most common residues in tea (positive ratio was 68% and 66%, respectively).

Tea is consumed by infusing the processed tea leaves in boiling water. The studies on leaching residues of pyrethroids into the tea brew (40) revealed that the rate of leaching of compounds depends on the solubility and octanol–water partitioning coefficients (Kow). Most pyrethroids are less water soluble with high Kow values (e.g., log Kow is 6.9 for cypermethrin). Manikandan et al. (40) tested the leaching of residues of deltamethrin, fenpropathrin, and lamda-cyhalothrin from black tea to brew and none of the targeted compounds were detectable in the tea brew. Tsumura et al. (41) reached the same conclusion through analyzing the residues of pyrethroids and 3-phenoxybenzoic acid (PBA, the ester cleavage metabolite of pyrethroids) from the infusion of contaminated tea leaves, but PBA residues were found in the infusion. Tsumura et al. thus thought that the acid part of the moiety of pyrethroid may be infused in tea for drinking. There often are halogens in the acid moiety of pyrethroids, which may pose more hazards on the human health.

In addition, considering that overuse of these pesticides would produce pyrethroid-resist populations of insects (42), the controlled application of these pyrethroids is essential.

Conclusion

A multi-residue method based on GC–IT-MS in MS–MS mode was developed to detect pyrethroid residues in tea samples. The tea samples were extracted with acetone, liquid–liquid extracted with n-hexane and underwent further cleanup by florisil solid phase extraction. Good recoveries (71.3–106.3%) at different spiked levels and acceptable CV (< 17%) showed the ruggedness of the analytical method. The evaluation of pyrethroid residues in 25 tea samples showed that this method has good practicability for the analysis of these residues in tea and in other herbal matrices.

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