Simultaneous Determination of Procymidone, Pyridaben and Beta-Cypermethrin Residues in Tea Solution by GC–ECD

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A sensitive and effective method for the simultaneous quantitative determination of procymidone, pyridaben and beta-cypermethrin residues in tea solutions was developed and validated using a gas chromatograph (GC) equipped with an electron capture detector (ECD). The analysis of the three pesticides in tea solutions involved extraction with petroleum ether–ethyl acetate (3:1, v/v), cleanup using a Florisil solid-phase extraction cartridge and subsequent determination by GC–ECD. Recovery studies were carried out at three spiked levels (0.05, 0.1 and 0.5 mg/kg). The overall average recoveries using this method in green teas and black teas at the three concentration levels ranged from 85.63 to 105.80% with relative standard deviations in the range of 1.29–4.97% ($n = 5$) for all analytes. The quantification limits of procymidone, pyridaben and beta-cypermethrin were 0.025, 0.038 and 0.030 mg/kg, respectively, which were lower than the maximum residue limits (MRLs) of 0.1 mg/kg procymidone, 0.05 mg/kg pyridaben and 0.5 mg/kg beta-cypermethrin in tea samples established by European Union legislations. This study provides a theoretical basis for China to draw up MRLs for procymidone, pyridaben and beta-cypermethrin in tea solutions.

Introduction

Tea is an old and popular beverage consumed worldwide, valued for its specific aroma and flavor as well as its health-promoting properties (1–3). However, tea drinking can also represent a significant potential source of human exposure to pesticides, which are unavoidably or improperly used for protection against pests and putrescence during plant cultivation and product-manufacturing processes (4, 5).

To make drinking of tea convenient, black and green tea have been transformed into a liquid beverage. After the manufacturing process, the liquid tea beverage in water at ambient temperature can be consumed directly. Currently, seven million tons of liquid tea beverage are consumed every year in China (6). The high consumption rate and significant health risk as a result of pesticide residues in teas have caused regulatory agencies around the world to establish stringent maximum residue limits (MRLs) to monitor pesticide residues in tea and related products. MRL is the maximum concentration of a pesticide residue that is legally permitted or recognised as acceptable in or on a food, agricultural commodity or animal feedstuff, as set by Codex or a national regulatory authority (7, 8). In the European Union (EU) and Japan, 436 and 252 MRLs, respectively, have been established (9, 10). However, fixed safety limits for many pesticide residues in tea solution have not yet been established.

The analysis of most pesticide residues is generally carried out in a sequence of steps, which include target extraction from the sample matrix, cleanup and concentration, and then chromatographic separation and quantification (11–13). Solvent selection is essential for efficient extraction (14). Because tea samples contain complex components, including pigments, alkaloids and polyphenols, the analysis of pesticide multi-residue in tea is usually difficult owing to matrix interference and complicated extraction procedures (15). A literature survey revealed the use of acetonitrile, ethyl acetate and cyclohexane–ethyl acetate for the extraction of pesticide residues in tea (15–19). Numerous methods have been published on the determination of procymidone, pyridaben and cypermethrinin in tea and tea solutions using gas chromatography (GC) (20–23) and GC–mass spectrometry (MS) (24) with florisil, Envi-Carb + NH2, or alumina + graphitized carbon solid-phase extraction (SPE) cartridges, or no cleanup (23). However, methods for multi-residue analysis of pesticides in tea solution are still limited (16, 23, 25).

The aim of this work was to develop a rapid, efficient and sensitive method for the analysis of three pesticide residues, including procymidone, pyridaben and beta-cypermethrinin, in liquid tea beverage samples.

Materials and Methods

Chemicals and reagents

Analytical standards of procymidone (99.5%), pyridaben (99.0%) and beta-cypermethrin (99.0%) were obtained from Beijing Helishun Technology Co. (Beijing, China). Hexane (chromatography grade) was from Burdick & Jackson (Seoul, Korea). Analytical grade petroleum ether was purchased from Hangzhou Petrochemical Co. (Hangzhou, China). Analytical grade ethyl acetate and acetone were purchased from Hangzhou Shuanglin Chemical Reagent Co. (Hangzhou, China). Analytical grade acetonitrile was purchased from Shanghai Lingfeng Chemical Regent Co. (Shanghai, China). Florisil and PSA (primary secondary amine) SPE cartridges (500 mg/6 mL) were purchased from Beijing Zhenxiang Industry & Trade Co. (Beijing, China). C18 SPE cartridges (500 mg/6 mL) were purchased from Agela Technologies (Tianjin, China).

Instrumentation and GC–ECD analytical conditions

A Trace GC Ultra gas chromatograph (Thermo Finnigan; Waltham, MA) with electron capture detector (ECD) was used

for the quantification and confirmation of the three target compounds. A capillary column VF-35MS (30 m × 0.25 mm i.d., 0.25 μm film thickness), supplied by Varian Company, was employed with nitrogen (purity 99.999%) as carrier gas at a constant flow rate of 2.0 mL/min. Nitrogen was also used as makeup gas at a constant flow rate of 30 mL/min. Detector and base temperatures were set at 300 and 280°C, respectively. The column temperature was programmed as follows: 80°C for 0 min and directly to 220°C at 50°C/min, then held for 1 min, 220°C directly to 280°C at 50°C/min, and then held for 8 min. The injector port was maintained at 280°C and 1.0 μL was injected in the splitless mode.

Standards

A standard stock solution of the three pesticides (100 mg/L) was prepared in hexane. The standard solutions required for preparing a calibration graph (0.01, 0.02, 0.05, 0.1, 0.2 and 0.5 mg/L) were prepared from the stock solution using serial dilution with hexane. The mixture standard solutions of the three pesticides (1 and 10 mg/L) used for sample spiking were also prepared in acetone. All solutions were stored in a refrigerator in the dark at 4°C. No degradation in the working standard solutions was observed for three months.

Sample preparation and clean-up

Green tea solution (Kangshifu) and black tea solution (Kangshifu) samples were obtained from Hangzhou Dingjin Food Co. (Hangzhou, China). Blank tea samples were fortified at three concentration levels by adding appropriate amounts of the pesticide standard solutions. Twenty milliliters of fortified tea samples were weighed into a graduated cylinder with stopper. A 50-mL mixture of petroleum ether–ethyl acetate (3:1, v/v) was added, shaken vigorously for 1 min and allowed to stand for 20 min. The supernatant (25 mL) was concentrated to dryness using a rotary evaporator at 40°C. Resulting residues were dissolved with petroleum ether (2 mL) for purification by Florisil cartridges. A Florisil cartridge was conditioned with petroleum ether (5 mL). A 2-mL extract solution was loaded onto the cartridge and collected in a flask. Analytes were eluted with 6 mL petroleum ether–acetone (3:2, v/v), and the eluate was collected and brought to dryness using a rotary evaporator at 40°C. The dry residue was dissolved in 5 mL hexane for GC–ECD analysis.

Recovery studies

Recovery was determined through experiments using a fortified blank matrix with mutually independent replicates at the three concentration levels (0.05, 0.1 and 0.5 mg/L) of procymidone, pyridaben and beta-cypermethrin. Five determinations were carried out on the green tea and black tea concentrations. Before the extraction step, the standard in the acetone was added into the fortified samples, followed by standing for 30 min, and then processed according to the previously described procedure.

Precision and accuracy

Tests of intra-day precision and accuracy of the method were performed using five parallel samples at three different concentration levels (0.05, 0.1 and 0.5 mg/kg) prepared as described previously. Five replicate determinations at three concentration levels (0.05, 0.1 and 0.5 mg/kg) were analyzed on three different days by three analysts for inter-day reproducibility.

Results and Discussion

Optimization of extraction and cleanup

In the present experiments, petroleum ether, petroleum ether–ethyl acetate, ethyl acetate and acetonitrile were studied based on their extraction efficiencies. Acetonitrile extraction showed low recovery (47.95%) for procymidone and poor parallelisms (>10%) for all three pesticides. Petroleum ether recovery of beta-cypermethrin (131.78%) was also very high. Thus, petroleum ether–ethyl acetate (3:1, v/v) extraction was selected because it showed 101.19–105.09% recovery for all three pesticides (Figure 1). A similar extraction of ethyl acetate–cyclohexane (9:1, v/v) has been reported previously (15).

The SPE technique, as a classic and reliable residue analytical method employed to purify matrix and samples concentration, was conducted to obtain high sensitivity (16). To elute all analytes and reduce impurities as much as possible, petroleum ether–acetone (3:2, v/v) was chosen as the appropriate eluent. Tea matrix contains high amounts of polyphenols, methyl xanthines such as caffeine, and purines, as well as different phenolic acids, making it difficult to purify (15). The primary aim of the cleanup step was to remove as many co-extractives
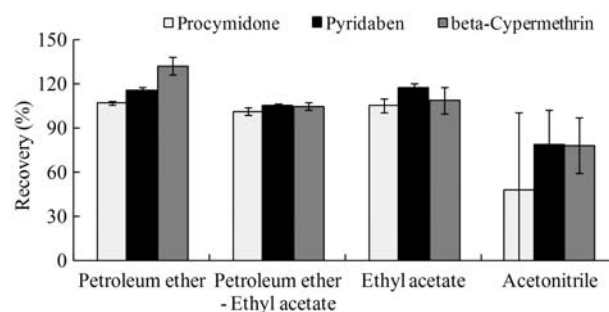

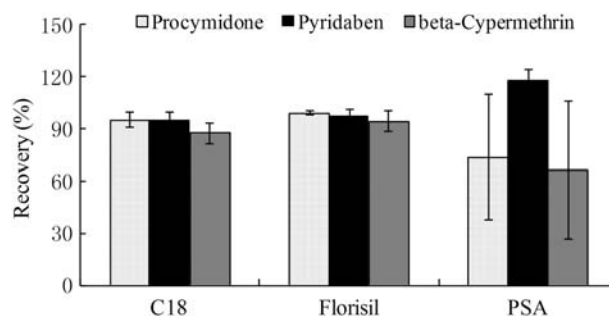**Figure 1.** Recovery test of four candidate extraction solvents (n = 3).

Figure 2. Recoveries of procymidone, pyridaben, and beta-cypermethrin with petroleum ether–acetone as eluent at concentrations of 0.1 mg/L (n = 3).
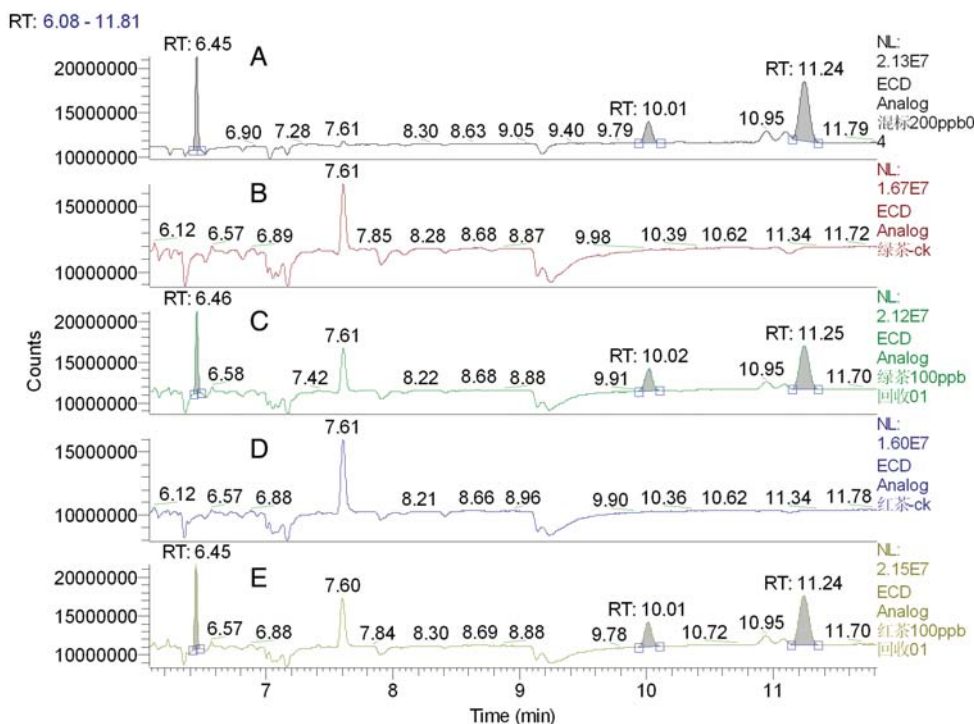
Figure 3. GC−ECD chromatograms: a standard of the three pesticides (0.2 mg/L) (A); the three pesticides in blank green tea solution (B); the three pesticides in a spiked sample of green tea solution (0.1 mg/kg) (C); the three pesticides in blank black tea solution (D); the three pesticides in a spiked sample of black tea solution (0.1 mg/kg) (E).

as possible from the extract using different sorbents. The most commonly used sorbents include C18, PSA, NH2, SAX and/or Florisil SPE cartridges (26–29).

Figure 2 shows that poor recoveries (<75%) and parallelisms (>10%) were obtained for procymidone and beta-cypermethrin with the PSA SPE cartridges for cleanup. The C18 SPE column cleanup required a longer time (introduced process, elution process) than the Florisil SPE column. Acceptable results for each analyte were achieved using the Florisil SPE cartridge. In addition, the Florisil SPE cartridge had higher cleanup efficiency, which was found to be a major advantage because it was able to separate the analytes completely from a large number of pigments, alkaloids and polyphenols in teas. Therefore, recovery and purification were solved using the Florisil SPE cartridge. Under the previously described conditions, the chromatograms of blank tea samples are shown in Figures 3B and 3D, while those of the spiked tea samples at 0.1 mg/kg are shown in Figures 3C and 3E. All figures demonstrated that the method had good selectivity. Therefore, the Florisil SPE cartridge was chosen to purify the teas.

Linearity

A series of pesticides standard solutions was analyzed using GC−ECD. Linear regression analysis was also performed. The calibration range was linear from 0.01 to 0.2 mg/L (besides pyridaben from 0.01 to 0.5 mg/L), and the equations of the standard curve were as follows:

$y = 77693887.48x + 346530.89$ ($R^2 = 0.9973$) for procymidone, $y = 509366790.49x + 253911.06$ ($R^2 = 0.9966$) for

pyridaben, and $y = 233809758.54x + 215893.83$ ($R^2 = 0.9997$) for beta-cypermethrin, where y = peak area and x = concentration (mg/L).

Limits of detection and quantitation

The limits of detection (LODs) for the three pesticides were considered to be the concentrations that produced a signal-to-noise (S/N) ratio of 3, and the limits of quantitation (LOQs) were defined as an S/N ratio of 10. The LODs were estimated from the chromatogram corresponding to the lowest point used in the standard curve. In this work, the LOD was estimated to be 0.006, 0.015 and 0.009 mg/kg for procymidone, pyridaben and beta-cypermethrin from five replicate extractions, respectively, containing the three pesticides at low concentration levels (Table I). The LOQ corresponded to the lowest fortification level for the three pesticides and was found to be 0.025, 0.038 and 0.030 mg/kg for procymidone, pyridaben and beta-cypermethrin from five replicate extractions, respectively (Table I). The MRLs of 0.1 mg/kg

Table I

LODs and LOQs of the Three Pesticides

Pesticide	LODs (mg/kg)	LOQs (mg/kg)
Procymidone	0.006	0.025
Pyridaben	0.015	0.038
beta-Cypermethrin	0.009	0.030

Table II
Intra-Day Precision and Accuracy for the Three Pesticides in Tea Solutions ($n = 5$)

Analyte	Green tea						Black tea					
	0.05 mg/kg		0.1 mg/kg		0.5 mg/kg		0.05 mg/kg		0.1 mg/kg		0.5 mg/kg	
	Accuracy (%)	Precision (%)	Accuracy (%)	Precision (%)	Accuracy (%)	Precision (%)	Accuracy (%)	Precision (%)	Accuracy (%)	Precision (%)	Accuracy (%)	Precision (%)
Procymidone	101.34	3.85	98.49	2.24	96.53	2.81	101.84	1.77	92.81	3.80	98.82	1.29
Pyridaben	105.80	2.89	102.82	3.65	101.81	4.32	103.95	2.49	100.03	3.73	102.46	1.30
beta-Cypermethrin	90.58	3.44	89.62	1.61	103.77	1.41	87.05	3.38	85.63	4.97	102.18	2.84

*Note: for intra-day assay, five parallel samples at each different concentration level were prepared by the same analyst on the same day.

Table III
Inter-Day Precision and Accuracy for the Three Pesticides in Tea Solutions ($n = 15$)

Analyte	Green tea						Black tea					
	0.05 mg/kg		0.1 mg/kg		0.5 mg/kg		0.05 mg/kg		0.1 mg/kg		0.5 mg/kg	
	Accuracy (%)	Precision (%)	Accuracy (%)	Precision (%)	Accuracy (%)	Precision (%)	Accuracy (%)	Precision (%)	Accuracy (%)	Precision (%)	Accuracy (%)	Precision (%)
Procymidone	101.56	2.74	99.22	2.69	97.15	4.69	102.94	2.06	97.62	5.18	101.44	2.91
Pyridaben	104.96	2.77	102.83	3.48	100.39	4.50	104.96	2.44	101.23	3.63	104.28	2.32
beta-Cypermethrin	90.12	5.69	89.23	5.14	101.86	4.34	91.79	6.17	89.93	6.12	102.46	3.69

*Note: for inter-day assay, five replicate determinations were carried out at each of the three concentration levels on three different days.

procymidone, 0.05 mg/kg pyridaben and 0.5 mg/kg beta-cypermethrin were established by the EU Commission. Because the LOQs were lower than the MRLs, the proposed method was suitable for the analysis of the three pesticide residues in tea solution samples.

Recovery

The average recoveries of procymidone, pyridaben and beta-cypermethrin following the analytical procedure were in the range of 92.81–101.84%, 100.03–105.80% and 85.63–103.77%, respectively, which were within the ranges expected for residue analysis (Table II). A variation was observed between the recoveries for different analytes. This may be because the recovery was related to the structure of the analyte and the combinative intensity of all analytes with the tea solution matrix.

Precision and accuracy

Tests of intra-day precision and accuracy of the method were performed using five parallel samples at three different concentration levels (0.05, 0.1 and 0.5 mg/kg) prepared as described previously. All samples were analyzed on the same day together with the daily calibration. Accuracy and precision values were determined at each level. Results for all samples are listed in Table II.

Five replicate determinations at three concentration levels (0.05, 0.1 and 0.5 mg/kg) were analyzed on three different days by three analysts for inter-day reproducibility. The concentration of the validation samples was calculated with the equation of the daily calibration curve. The summarized results are presented in Table III.

As indicated by the results, the relative standard deviations (RSDs) ranged from 1.29 to 4.97% within a day ($n = 5$) and 2.06 to 6.17% in three days ($n = 15$). The precision and stability of the proposed method were acceptable.

Fang *et al.* studied the determination beta-cypermethrin and pyridaben residues in tea solution, which involved dichloromethane liquid–liquid extraction three times, and no cleanup (23). In this study, the relatively simple pretreatment process, clean separation, less use of organic solvents, high recovery and good reproducibility indicated that the method was suitable for analysis of the three pesticide residues in tea solution. This work also provided evidence for multi-residue analysis of pesticides in tea solution.

Conclusions

A chromatographic method based on GC–ECD for the determination of three pesticides in tea liquid beverage has been developed and validated. The pretreatment of the three pesticides in tea solutions involved extraction with petroleum ether–ethyl acetate (3:1, v/v) and clean-up by Florisil SPE. The developed method showed satisfactory validation parameters in terms of linearity, recovery, accuracy, LOD, LOQ, and intra-day and inter-day repeatability. The average recoveries of the three pesticides ranged from 85.63 to 105.80%. The uncertainty associated with the analytical method, expressed as RSD, was lower than 4.97%. The LOQs of procymidone, pyridaben, and beta-cypermethrin were 0.025, 0.038 and 0.030 mg/kg, respectively. This method is simple and rapid, and all the required equipment is readily available in most analytical chemistry laboratories.

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