Screening Five High-toxic Organophosphorus Pesticides in Chinese tea Using Gel Permeation Chromatography-GC-MS Method

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Abstract. A gas chromatography - mass spectrometry (GC-MS) analysis method for detecting five kinds of high-toxic pesticides in Chinese tea was established. Gel permeation chromatography (GPC) was used as a sample preparation method. Purified samples were analyzed by GC-MS, detected in selected ion monitoring mode, and quantitated using the external standard method. The results showed a good linearity within the range from 0.005 mg/L to 0.500 mg/L. The average recovery rates of three spiked levels were 93.86% to 106.31%. All relative standard deviations (RSDs) were less than 10%. The detection limit was 0.0005 to 0.0020 mg/kg. This method is stable and reliable, simple to operate, and suitable for detection and analysis of trace organophosphorus pesticides in Chinese tea.

1. Introduction

With the widespread application of five kinds of high-toxic organophosphorus pesticides including methamidophos, methyl parathion, parathion, monocrotophos and phosphamidon in agricultural production, the problem of pesticide residues in agricultural products has received increasing attention. Since January 1, 2007, a complete prohibition of domestic sales and use of these five kinds of high-toxic organophosphorus pesticides has been implemented in China [1]. However, there are still a minority of farmers over-depending on methamidophos for its fast insecticidal effect, who continue to buy and use high-toxic pesticides such as methamidophos. To ensure the safety of Chinese tea, the detection and analysis means of five kinds of high-toxic organophosphorus pesticide residues in Chinese tea must be strengthened.

In recent years, gas chromatography - mass spectrometry (GC-MS) technique has been widely used in the detection of pesticide multi-residues. However, their high technical requirement for sample pretreatment has become the bottleneck in the application of the method. Conventional purifying method consumes a large amount of organic solvent, and its procedures are more complicated, with larger analysis error. Gel permeation chromatography (GPC) has obvious advantages with respect to the separation and purification of samples rich in fat, pigments and other macromolecules [2-4]. GPC sample separation is a physical process that can well separate macromolecules such as proteins, pigments and fat and small molecules such as pesticides, and the consumption of organic solvent for GPC is decreasing as the development of column, and it is simple to operate, with small analysis error. In this work, the present study used GPC techniques for sample pretreatment. Meanwhile, GC-MS was used for quantitative and qualitative analysis of five high-toxic organophosphorus pesticide residues in Chinese tea, in order to establish a GPC-GC-MS method for detection of five pesticide residues in Chinese tea.

2. Materials and Methods

2.1 Instruments and reagents

Gel permeation chromatography (GPC Ultra), Trace DSQⅡ GC/MS (Finnigan, USA); acetone (HPLC grade, Chengdu Kelong Chemical Reagent Factory).

Five kinds of high-toxic pesticide standards (with the concentration of 100 mg/L, Agro-Environment Protection Institute of the Ministry of Agriculture) were diluted with acetone to a concentration of 0.005 mg/L, 0.001 mg/L, 0.050 mg/L, 0.250 mg/L and 0.500 mg/L for making the mixed standard curve.

2.2 Instrument Parameters

2.2.1 Conditions for GPC

Standard chromatographic column packing materials (biology gel 3), mobile phase: ethyl acetate: cyclohexane (1:1, V/V). The extracting solution was concentrated to 5.0 mL by GPC prior to sample injection for separation and purification at 5.0 mL/min. Effluent collection period was from 1300 s to 2000 s. The collected fluid was concentrated to the constant volume of 5.0 mL.

2.2.2 Conditions for GC-MS

Conditions for GC: Chromatographic column: TG-5MS, 30 m × 0.25 mm × 0.25 μm, helium as carrier gas, flow rate of 1 mL/min, in splitless mode, Inlet temperature: 250 ℃; Programmed temperature: Starting from 40℃ and holding for 4 min, raising to 170℃ at 10℃/min and holding for 5 min, then raising to 250℃ at 10℃/min and holding for 8 min.

Conditions for MS: Ion source: EI source in positive ion mode; Temperature: 250 ℃; Transmission line temperature: 280 ℃; Mass range m/z: 20-500au; Solvent delay: 5 min.

A full scan within the range from 20 au to 500 au was first performed in a full scan mode to determine the retention time and major ions of each pesticide.The selected ion monitoring mode was then performed. The monitoring ions of five kinds of high-toxic pesticides were 94 for methamidophos, 127 for monocrotophos, 127 for phosphamidon, 109 for methyl parathion and 291 for parathion (Table 1).

Table 1 Retention time and characteristic ions of five high-toxic organic phosphorus pesticides

Retention time/min	Pesticides	Identification ions, m/z	Quantitative ions, m/z
6.37	Methamidophos	94,95,141	94
9.81	Monocrotophos	127,192,97,109	127
10.39	Phosphamidon	127,264,138,72	127
10.72	Methyl parathion	109,125,2631	109
11.67	Parathion	291,109,97,139	291

2.3 Experimental Methods

The tea sample weighed 10 g was transferred into a 100 mL colorimetric tube, which was added with 50 mL of acetone and shaken well and stood for overnight. After filtration, the filtrate was collected in a 250 mL round-bottom flask, which was added with 25 + 25 mL of acetone for repeated extraction. After filtration, the filtrates were merged and rotarily concentrated to almost dryness. It was added with 15 mL of ethyl acetate: cyclohexane (1:1) and thoroughly dissolved and injected into GPC for purification (the sampling injection was concentrated to a constant volume of 5.0 mL by GPC for purification by column). The collected fluid was concentrated to a constant volume of 5.0 mL for GC-MS analysis.

3. Results and Analysis

3.1 Regression Equation, Linear Range and Detection Limit

A series of mixed standards of five pesticides prepared in Section 2.1 were injected for GC-MS analysis. Ion current graph was selected as Figure 1. The linear range was from 0.005 mg/L to 0.500 mg/L (Table 1). The sampling detection limits of five pesticides were calculated by comparing the ratio of signal to noise: 0.003 mg/L for methamidophos, 0.002 mg/L for monocrotophos, 0.001 mg/L for phosphamidon, 0.004 mg/L for methyl parathion, and 0.003 mg/L for parathion. As the weight of sample in 10g, the method detection limits of five pesticides were 0.0015 mg/kg for methamidophos, 0.0010 mg/kg for monocrotophos, 0.0005 mg/kg for phosphamidon, 0.0020 mg/kg for methyl parathion, and 0.0015 mg/kg for parathion.

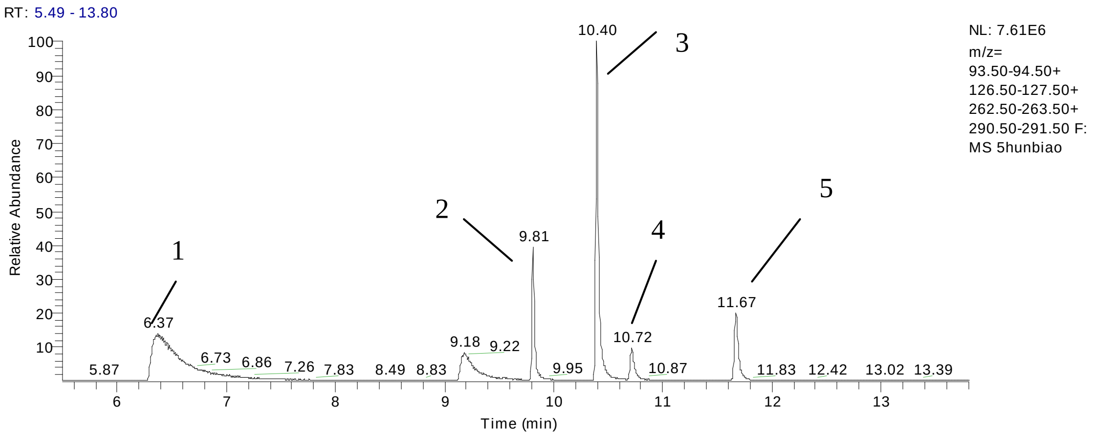

Fig. 1 SIM chromatogram of the standard samples of five organic phosphorus pesticides

1: Methamidophos; 2: Monocrotophos; 3: Phosphamidon; 4: Methyl parathion; 5: Parathion.

Table 2 Standard curve equations of the standard samples of five organic phosphorus pesticides

Pesticides	Linear range /(mg/L)	Regression equation	Correlation coefficient/r	LOD/ (mg/kg)
Methamidophos	0.005~0.500	$y = 1247678x+123$	0.9981	0.0015
Monocrotophos	0.005~0.500	$y = 486291x-54$	0.9989	0.0010
Phosphamidon	0.005~0.500	$y = 473866x-112$	0.9992	0.0005
Methyl parathion	0.005~0.500	$y = 166473x+432$	0.9995	0.0020
Parathion	0.005~0.500	$y = 312598x-11$	0.9996	0.0015

3.2 Recovery and Precision of the Method

A certain volume of mixed standard solution was added into a blank sample, which was treated and measured according to the method described above in Section 2.3 and quantitated with the external standard method. The recovery of standard addition was repeatedly calculated for six times. The results were shown in Table 3. The average recovery rates of three spiked levels were 99.68% to 106.31%, 94.14% to 98.11%, and 93.86% to 98.21%, respectively. RSDs were less than 10%.

Table 3 Recovery and precision of the method (n = 6)

Pesticides	added concentration/(mg/kg)	Recoveries/%	RSD/%
Methamidophos	0.01	99.68	6.37
	0.10	95.67	9.32
	0.50	94.74	8.67
Monocrotophos	0.01	102.65	9.68
	0.10	96.32	6.33
	0.50	94.10	6.98
Phosphamidon	0.01	100.14	8.66
	0.10	98.11	9.10
	0.50	93.86	7.74
Methyl parathion	0.01	102.55	7.32
	0.10	96.19	9.78
	0.50	96.36	8.14
Parathion	0.01	106.31	8.69
	0.10	94.14	10.21
	0.50	98.21	9.68

3.3 Sample Analysis

From the results obtained from the analysis of 20 tea samples that had been treated in accordance with the method described above in Section 2.3, it was seen that a sample from 20 tea samples was detected with phosphorus amine pesticide residues, with the concentration of 0.11 mg/kg.

4. Summary

In the present study, a detection method for five high-toxic pesticide residues in tea samples by GC-MS after GPC pretreatment was established and used for measurement of 20 commercially available tea samples. The linear range, precision and recovery of standard addition the method were investigated. The results showed a good linearity within the range from 0.005 mg/L to 0.500 mg/L. The recovery of standard addition was between 93.86% and 106.31%. The detection limit was 0.0005 to 0.0020 mg/kg. It is indicated that the detection method is more suitable for detection and analysis of trace pesticide residues in Chinese tea.

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