

# Optimization of the Pre-treatment Method for the Determination of 9 Organophosphorus Pesticide Residues in Cabbage by Gas Chromatography

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**Abstract.** The purpose of this study was to optimize the pre-treatment process for the detection of 9 organophosphorus pesticide residues in cabbage by gas chromatography, and to conduct a comparative experiment between this method and the standard method NY/T 761-2008 of the Ministry of Agriculture. The results show that the optimized pre-treatment method is to use 10 mL acetonitrile as the extractant, 150 mg N-propyl ethylenediamine (PSA) and 15 mg graphitized carbon black (GCB) as the purification agent, which can reduce the use of extractant and inorganic salt in the pre-treatment process, increase the recovery and detection limit, reduce the matrix effect and cost of instrument maintenance.

## 1 Introduction

Pesticides can be divided into insecticides, fungicides, herbicides, and acaricides according to the control objects. Insecticides account for about 65% of China's total pesticides, while organophosphorus pesticides (OPPs) account for more than 70% of total insecticides [1]. OPPs have the advantages of high efficiency and relatively low cost, which has become the most widely used pesticides in agriculture of China [2].

Whereas, misuse and excessive use of OPPs have aggravated pesticide residues in agricultural products [3]. Clinical data show that most OPPs are toxic to nerve cells, and this damage is irreversible, and there is a risk of teratogenicity and carcinogenesis [4]. Therefore, it is necessary to establish a highly selective and sensitive analytical method to detect pesticide residues, and provide technical support for the prevention and control of pesticide pollution and the standardized use of pesticides [5]. Gas chromatography (GC) is currently one of the most widely commonly used detection methods, especially fruit and vegetable matrix systems. Meanwhile, the selection of a suitable pre-treatment process is a prerequisite for the analysis of complex samples [6].

Cabbage is rich in nutrients and contains high-quality protein, cellulose, minerals, and vitamins. It is one of the important vegetables in China. However, during the planting

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period, the species of cabbage moth such as *Mamestra brassicae* L. occurred more frequently and were more difficult to eliminate [7]. Therefore, there has a lot of residues if a highly toxic OPP is selected. In this study, based on the optimization method for the detection of 9 OPPs by GC, cabbage was used as the research object to further optimize the pre-treatment process of extracting OPP residues. The optimized method was compared and discussed with the Ministry of Agriculture (MOA) standard method NY/T 761-2008 from the aspects of matrix standard curve, matrix spiking, and detection of actual samples.

## **2 Materials and methods**

### **2.1 Materials and chemicals**

Cabbage samples come from pollution-free farms and the sampling location of cabbage is in accordance with the provisions of the standard GB2763. Methamidophos, acetamidophos, epithion, phorate, monocrotophos, chlorpyrifos, fenthion, phosfolan, triazophos were obtained from Agro-Environmental Protection Institute, Ministry of Agriculture (Beijing, China). Acetone, n-hexane, ethyl acetate and acetonitrile were purchased from Merck (Darmstadt, Germany). Na<sub>2</sub>SO<sub>4</sub>, NaCl, n-propylethylenediamine (PSA), and graphitized carbon black (GCB) were purchased from Sinapharm Co., Ltd (Shanghai, China).

### **2.2 The detection conditions of GC**

In the preliminary research, the 7890A gas chromatograph (Agilent, USA) and Agilent DB-1701 (30 m×250 mm×0.25 μm) capillary chromatographic column were used to obtain the optimized detection conditions of GC for fast analysis of 9 OPPs followed as: the initial temperature of program heating of 150°C for 2 min, and then the temperature up to 250°C with a rate of 10°C/min for 14.5 min, the injection volume of 1.0 μL, splitless injection mode, the carrier gas flow rate of 1.0 mL/min, the detector temperature of 270°C, and the tail gas flow rate of 40 mL/min.

### **2.3 The optimization of pre-treatment method of cabbage**

#### **2.3.1 Pre-treatment procedures**

An Agilent DB-1701 capillary chromatography column was used to optimize the type of extractant, the volume of extractant, and the amount of purifying agent used in the pre-treatment process. Experimental procedures followed as: 5.0 g of homogenized cabbages was accurately weighed, then the extractant was added and separated the pesticide residues in cabbage. 2.0 g of MgSO<sub>4</sub> and 0.5 g of NaCl were added as water remover and shake for 5 min, then the supernatants were obtained after centrifuged at 4000 r/min for 5 min. 5.0 mL of supernatants were taken and added a certain amount of PSA and 15 mg of GCB. The mixed solutions were vortexed for 5 min to remove the impurities from cabbages and centrifuged at 4000 r/min for 5 min. 2.0 mL of supernatants were blown dry with nitrogen gas and re-dissolved in 1.0 mL of acetone for analysis by GC. The best conditions for pre-treatment are selected through the results of spike recovery and precision experiments.

### **2.3.2 Type of extractant**

The experiments were performed using ethyl acetate, acetone, acetonitrile, and n-hexane as the extractant respectively. By adding 9 organophosphorus standards for standard recovery and precision experiments to determine which extractant has the best extraction effect on OPPs in cabbage.

### **2.3.3 Volume of extractant**

The experiments were performed using 5.0 mL, 10.0 mL, and 15.0 mL of acetonitrile as the extractant respectively. By adding 9 organophosphorus standards for standard recovery and precision experiments to determine which volume of acetonitrile has the best extraction effect on OPPs in cabbage.

### **2.3.4 Amount of PSA**

The experiments were performed using 50 mg, 100 mg, 150 mg, 200 mg of PSA and 15 mg GCB as the purifying agent respectively. By adding 9 organophosphorus standards for standard recovery and precision experiments to determine which amount of PSA has the best purifying effect on OPPs in cabbage.

## **2.4 Comparative Experiment**

### **2.4.1 Matrix standard curve**

Cabbage without spraying pesticides was selected as the sample to perform the matrix standard curve experiment. The blank matrix solutions were obtained by the sample preparation method and added different volumes of organophosphorus mixed solutions, and then blew dry with nitrogen gas. A series of standard working solutions ranged from 0.02 µg/mL to 1.0 µg/mL were obtained through dissolving with acetone. The matrix standard curve experiments were performed according to the established method and the standard method NY/T 761-2008 of MOA. The matrix standard curves were obtained and compared.

The procedures of standard method NY/T 761-2008 of MOA followed as: 25.0 g of homogenized cabbages was accurately weighed, then 50 mL of acetonitrile was added and homogenized for 2 min. The filtrates were obtained through filtering with paper. 40 mL–50 mL of the filtrates was added 5–7 g NaCl and shook vigorously for 1 min, and stood for 30 min. 10 mL of supernatants were blown dry with nitrogen gas at 80°C water bath and re-dissolved in 5.0 mL of acetone for analysis by GC.

### **2.4.2 Matrix spike recovery**

Cabbage without spraying pesticides was selected as the sample to perform the matrix spike recovery experiment. The blank matrix solutions were obtained by the sample preparation method and added different volumes of organophosphorus mixed solutions to the concentrations of 0.02 µg/mL, 0.1 µg/mL, and 1.0 µg/mL. The matrix spike recovery experiments were performed triplicate according to the optimized method and the standard method NY/T 761-2008. The matrix spike recoveries were obtained and compared.

### 2.4.3 Detection and analysis of 9 OPPs in cabbage

The cabbage sprayed with OPPs was used as a sample and analyzed according to the optimized method and the standard method NY/T 761-2008. The efficacies of the two methods were compared in terms of detection values and detection limits.

## 3 Results and discussion

### 3.1 Selection of extraction solvent

In the analysis of pesticide residues, the pesticide residues are first extracted from the complex vegetable matrix, so it is important to choose the appropriate extractant. Appropriate extractant can extract pesticide residues from the complex sample matrix to the greatest extent, which not only saves time and improves extraction efficiency, but also improves the accuracy of pesticide residue analysis results [8]. Acetonitrile, ethyl acetate, acetone, and n-hexane were adopted to evaluate the effects of extractants on the spike recovery and precision of 9 organic phosphorus species in cabbage (Table 1). The recovery ranges of the 9 OPPs were: 89.4%–105.6%, 20.3%–83.3%, 89.7%–106.1% and 28.2%–86.3%, with precision ranges of 4.6%–6.7%, 6.9%–15.2%, 4.3%–8.2 %, and 7.3%–12.3% (n = 6). Acetonitrile and acetone have a good recovery effect on the extraction of 9 OPPs, but acetone easily dissolves the pigment in cabbage, which may cause subsequent purification difficulties. Acetonitrile is a good extraction solvent. After adding inorganic salts, acetonitrile can be well dispersed from water due to changes in the two-phase concentration, which is convenient for layering. Therefore, acetonitrile was selected to extract 9 OPPs from cabbage.

**Table 1.** Spike recovery and precision of nine OPPs in different extractants.

Organophosphorus	acetonitrile		ethyl acetate		acetone		n-hexane	
	Recovery (%)	Precision (%)	Recovery (%)	Precision (%)	Recovery (%)	Precision (%)	Recovery (%)	Precision (%)
Methamidophos	94.5	6.3	20.3	10.3	91.3	7.3	28.2	12.3
Acetamidophos	93.7	5.3	81.9	12.3	90.3	7.3	85.6	10.3
Epithion	98.2	5.9	79.6	6.9	95.9	6.9	80.2	8.9
Phorate	95.3	6.7	78.2	7.9	94.3	7.7	81.3	7.3
Monocrotophos	89.4	5.5	20.6	15.2	89.7	6.5	20.5	9.2
Chlorpyrifos	96.4	6.2	82.9	11.3	89.3	8.2	86.3	11.3
Fenthion	105.9	4.6	75.6	9.3	106.1	6.6	77.4	7.3
Phosfolan	102.6	4.8	83.3	8.6	94.3	7.8	80.9	9.6
Triazophos	95.2	5.3	75.3	11.6	93.2	4.3	76.9	10.6

### 3.2 Selection of extractant volume

The selection of the volume of extractant is also important [9]. The effects of different volumes of acetonitrile on the spike recovery and precision of 9 organic phosphorus pesticides in cabbage are shown in Table 2. When the volumes of acetonitrile were 5.0 mL, 10 mL, and 15 mL, the recovery ranges of 9 OPPs are 59.6%–75.5%, 88.2%–107.9%, and 86.7%–108.3%, the precision ranges from 8.3%–15.9%, 5.3%–9.8%, and 6.5%–10.3% (n = 6), respectively. When the volume of acetonitrile is 5.0 mL, the extraction effect is relatively poor. The reason may be that there are less extraction solvents and the OPPs in cabbage are not completely extracted. When the volume of acetonitrile is 10.0 mL and 15.0 mL, there is no obvious difference in extraction effect, and both can meet the experimental

requirements. Considering economic and environmental factors, the volume of acetonitrile was determined as 10.0 mL.

**Table 2.** Spike recovery and precision of nine OPPs in different volume of acetonitrile.

Organophosphorus	5.0 mL		10.0 mL		15.0 mL	
	Recovery (%)	Precision (%)	Recovery (%)	Precision (%)	Recovery (%)	Precision (%)
Methamidophos	75.5	10.3	93.6	6.3	92.3	10.3
Acetamidophos	63.7	8.3	92.9	6.3	89.9	7.3
Epithion	70.2	12.8	97.2	9.8	94.9	6.9
Phorate	69.3	15.9	96.2	6.9	90.3	7.7
Monocrotophos	74.4	10.5	88.2	5.5	86.7	6.5
Chlorpyrifos	62.4	9.2	93.1	7.2	90.9	8.2
Fenthion	64.9	10.6	107.9	5.6	108.1	6.6
Phosfolan	59.6	9.8	107.6	6.8	108.3	7.8
Triazophos	67.2	10.3	92.2	5.3	90.1	8.3

### 3.3 Selection of PSA quality

The effects of different quality of PSA on the spike recovery and precision of 9 OPPs in cabbage are shown in Table 3. When the amount of PSA is 50 mg, 100 mg, 150 mg, and 200 mg, the recovery of 9 OPPs ranges from 59.4%–70.5%, 77.3%–86.2%, 89.3%–105.9%, and 87.9%–110.9%, and the precision ranges from 6.9%–9.8%, 4.3%–8.2%, 3.8%–6.2%, and 7.3%–10.3% (n = 6). The results show that the recoveries of 9 OPPs are within the experimental requirements when the amount of PSA is 150 mg. When the PSA amount reaches 200 mg, some of 9 OPPs have a decreasing trend. This may be due to the increased adsorption of some OPPs when PSA is used in excess, thus the amount of PSA was determined to be 150 mg.

**Table 3.** Spike recovery and precision of nine OPPs in different quality of PSA.

Organophosphorus	50 mg		100 mg		150 mg		200 mg	
	Recovery (%)	Precision (%)	Recovery (%)	Precision (%)	Recovery (%)	Precision (%)	Recovery (%)	Precision (%)
Methamidophos	70.5	7.3	77.3	7.3	94.5	5.2	92.2	9.3
Acetamidophos	68.7	7.3	79.3	7.3	93.2	4.5	90.6	8.3
Epithion	69.2	6.9	78.9	6.9	96.3	6.2	94.1	8.9
Phorate	63.3	7.7	79.3	7.7	97.1	5.3	92.3	7.3
Monocrotophos	59.4	7.5	79.7	6.5	89.3	3.8	88.5	9.2
Chlorpyrifos	62.4	7.2	76.3	8.2	94.1	5.3	91.3	10.3
Fenthion	67.9	9.6	82.1	6.6	104.3	3.9	110.4	8.3
Phosfolan	68.6	9.8	84.3	7.8	105.9	4.6	110.9	8.6
Triazophos	67.2	8.3	86.2	4.3	91.2	5.4	87.9	8.6

### 3.4 Comparison of the optimization method and the standard method

The matrix standard curves determined by the established method and the standard method NY/T 761-2008 of MOA were obtained and the correlation coefficients ( $R^2$ ) of the linear equations of 9 OPPs in cabbage determined by the established method are not significantly different from that by the standard method NY/T 761-2008 of MOA, and the concentration ranges are a good linear relationship between 0.01–1.0  $\mu\text{g/mL}$ .

**Table 4.** Comparisons of matrix spike recoveries.

Organophosphorus	Recovery of the established method, %			Recovery of the standard method, %		
	0.02 µg/L	0.1 µg/L	1.0 µg/L	0.02 µg/L	0.1 µg/L	1.0 µg/L
Methamidophos	95.3	96.6	92.6	91.7	92.4	89.9
Acetamidophos	95.4	96.3	93.2	92.1	95.3	90.8
Epithion	99.2	99.1	97.2	95.7	96.5	94.7
Phorate	97.0	98.4	91.7	93.1	94.8	93.7
Monocrotophos	89.8	89.9	87.1	88.8	89.3	86.4
Chlorpyrifos	97.2	99.5	95.7	95.3	97.2	92.1
Fenthion	106.4	103.2	109.8	108.9	103.8	109.7
Phosfolan	107.8	102.3	105.7	107.9	105.6	110.2
Triazophos	95.3	96.2	97.3	94.2	95.1	93.9

The recoveries of the two methods are shown in Table 4. It can be seen from Table 4 that the recoveries of 9 OPPs in cabbage of the standard method of MOA is lower than the recoveries of the established method. The reason may be that the large volume of extractant and excessive amount of NaCl added in the standard method, resulting in the operation steps are complicated and the losses of 9 OPPs are relatively large. The cabbages sprayed with OPPs during the growth process were detected and analyzed (Table 5). It can be seen that all 9 OPPs can be detected. Compared with the standard method of MOA, the values detected by the established method have no significant difference, and the detection limits of the established method are lower than that of the standard method.

**Table 5.** Comparisons of the detection of 9 OPPs in cabbage.

Organophosphorus	Optimized method		Standard method	
	Value (mg/kg)	Limit (mg/kg)	Value (mg/kg)	Limit (mg/kg)
Methamidophos	0.04	0.0095	0.03	0.01
Acetamidophos	0.05	0.018	0.05	0.02
Epithion	0.01	0.019	0.01	0.02
Phorate	0.04	0.028	0.04	0.03
Monocrotophos	0.06	0.017	0.05	0.02
Chlorpyrifos	0.12	0.028	0.12	0.03
Fenthion	0.05	0.019	0.04	0.02
Phosfolan	0.05	0.028	0.05	0.03
Triazophos	0.04	0.009	0.04	0.01

## 4 Conclusion

In summary, the optimization of the sample pre-treatment process for detecting 9 organophosphorus pesticide residues in cabbage by GC has obtained a quick, simple and effective method for the determination of 9 OPPs in cabbage. Compared with the standard method of MOA, this optimized method has the advantages of less extractant dosage, higher recovery rate, lower detection limit, simple and convenient operation and high accuracy.

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