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Determination of Organophosphorus Pesticides (or Herbicides) in Soybean by Gas Chromatography (or High Performance Liquid Chromatography)

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Abstract: Organophosphorus pesticides can effectively control agricultural pests, improving the yield and quality of crops. However, the problem of pesticide residues is becoming more and more serious due to the extensive use of pesticides, potential teratogenicity and carcinogenicity pose a serious threat to consumers' health. In order to control the pollution of organophosphorus pesticide residues, besides strengthening the management of pesticide use, the key is to develop simple, fast and sensitive detection technology. Because soybean contains more oil and fat than cereals, it brings many difficulties to the analysis of pesticides. So far, there is no mature method in China. Based on the above background, the aim of this paper is to determine organophosphorus pesticides in soybean by gas chromatography. In this paper, the extractant and sample purifier of organic phosphorus in soybean were discussed, and the organophosphorus pesticides in soybean were extracted with acetone-dichloromethane mixed solvent, and Flori silica was used as purifying agent. The residues of four organophosphorus pesticides in soybean, dimethoate, methyl parathion and parathion, were determined by gas chromatography with flame photometric detector. Simple operation, high recovery and minimum detection concentration are as follows: dropping fear 0.03 mg/kg; dimethoate 0.04 mg/kg; methyl parathion 0.04 mg/kg; parathion 0.05 mg/kg.

1. Introduction

Organophosphorus pesticides are currently widely used in agriculture. They are mainly used for controlling plant diseases and insect pests. Because of their high insecticidal efficiency, low cost and wide range of control, they are well received by farmers [1]. There are many kinds of organophosphorus pesticides. According to the toxicity of the variety, it can be divided into low toxicity, poisoning and high toxicity. It can enter the human body through the respiratory tract, digestive tract and intact mucous membrane and skin, and combine with cholinesterase to form phosphorylcholine. Esterase, so that cholinesterase loses its role in catalyzing the hydrolysis of acetylcholine. Accumulated acetylcholine can cause muscarinic, nicotinic or central nervous system

effects on cholinergic nerves, causing harm to human body [2]. At present, organophosphorus pesticides have been widely used due to their high efficacy, low toxicity and low price. They are the most widely used insecticides and have become one of the three pillars of pesticides [3]. Since 2005, China's pesticide production has ranked first, and its application area ranks second in the world. Among them, organophosphorus pesticides play an important role. The use of pesticides plays an important role in controlling plant diseases and insect pests, protecting crop growth and increasing yield. However, its large-scale use can easily cause poisoning of humans and animals, and brings some food safety problems. Organophosphorus pesticides have relatively high effectiveness and persistence, and play an important role in the growth and development of crops. They are one of the most widely used pesticides in the world, accounting for 38% of the global pesticide use [4]. However, the actual use rate of OPs applied to crops is very low, and most of the OPs are not effectively utilized and remain in agricultural products, soils and water bodies, leading to serious environmental pollution problems, which in turn endanger human health [5].

OPs refers to organic compound pesticides containing phosphorus, which are widely used in agricultural and forestry production [6]. With the increase in the scale of production of agroforestry, the use of OPs is also increasing rapidly, making a great contribution to the development of agroforestry [7]. However, on the other hand, the effective use rate of applied OPs is very low. Most OPs enter the soil, water, atmosphere or residues in agricultural products through leaching, volatilization, accumulation, destroying fish and birds. And the mammalian endocrine system [8], causing serious environmental pollution problems [9]. At the same time, OPs, as a neurotoxin, can inhibit the activity of Acetylcholinesterase in the central nervous system, hinder the catalyzed hydrolysis of Acetylcholine, and block the transmission of neurotransmitters [10]. Long-term exposure to OPs in the human body, even low concentrations of OPs can impair the performance of various organs, such as defense response, reproduction, nerves, cardiovascular and respiratory systems, and increase the morbidity and mortality of patients with acute poisoning [11-12]. According to the World Health Organization, the number of pesticide poisonings has reached 3 million in recent years, causing more than 250,000 deaths. It can be seen that OPs not only seriously pollutes the ecological environment, but also affects human health [13].

High Performance Liquid Chromatography (HPLC) System for the Simultaneous Determination of Antioxidants in Oils Tert-Butyl Hydroquinone (TBHQ), Tert-Butyl Hydroxy Anisole (BHA) and 3,5-Di-tert-Butyl Hydrotoluene (BHT)[14]. Based on the coupling of experimental design and artificial neural network, Chen proposed a new method for optimal separation of antioxidants in oil. Experiments and optimization variables were designed using orthogonal design and artificial neural networks with extended Delta-bar-delta (EDBD) learning algorithms [15]. The response function (Rf) used is a weighted linear combination of two variables related to separation efficiency and retention time, from which the best conditions are obtained. The above antioxidants in rapeseed oil were separated and simultaneously determined by HPLC and 280 nm UV detection under optimized conditions. Linearity was obtained in the range of 10-200 cups/ml with recoveries of 98.3% (TBHQ), 98.1% (BHT) and 96.2% (BHA) [16], respectively. Zhu used static headspace-gas chromatography/mass spectrometry (HS-GC-MS) combined with accurate gravimetric analysis to analyze the volatile components in eucalyptus leaves. Accurate weight measurements obtained by time-of-flight mass spectrometry (TOF-MS) help determine the identification of volatiles in the analysis. 59 volatile components were identified in camphor, including cis-3-hexen-1-ol (5.6%), 3-hexen-1-ol, acetate (Z) (11.1%), β - Caryophyllene (15.4%), bicyclo germare (8.4%), trans-nerolidol (19.5%) and 9-oxaaryl alcohol (7.7%). The results show that the method of combining HS-GC-MS with accurate weight measurement can achieve reliable identification and has a wide range of applications in the analysis of volatile components in complex samples [17]. Harynuk presented a theoretical and proof of concept for a new operating mode for integrated

two-dimensional gas chromatography (GC x GC). In current GC x GC interface designs, the modulation period defines the separation time allowed in the second dimension. In the stop flow GC×GC mode, the flow in the main tower will briefly stop for a while. Therefore, the modulation period for the main column and the amount of time available for the second dimension separation become independent variables. This allows for the separation of the primary and secondary dimensions of the GC x GC system under more optimized conditions, thereby extending the time for the second size separation without sacrificing one size separation. With the further development of this technology, this new technology is expected to provide higher separation capacity and overall resolution [18].

Yan has established a simple method based on liquid chromatography coupled with electrospray tandem triple quadrupole mass spectrometry, which can be analyzed in selected reaction monitoring modes to analyze toxins in algae and water samples. Under the optimized liquid chromatography-mass spectrometry, twelve toxinswere effectively isolated in the selected reaction monitoring mode. Field acid, microcystins). The correlation coefficients of the calibration curves are all in the range of 0.9958-0.9998, indicating good linearity. The detection limit of the toxin in the method was less than 0.20 ng/mL, and the limit of quantification was in the range of 0.04 to 0.60 ng/mL. In addition to antitoxins, cylindrospermopsin and nodularmycin, the recovery of other toxins ranged from 55.45 to 140.85%. The relative standard deviation of daytime and intraday accuracy was 8.61% (n=5). High performance liquid chromatography (HPLC) / electrospray ionization (ESI) mass spectrometry (MS) methods have also been successfully used to analyze algae and water samples. Due to its unique selectivity and excellent sensitivity, the method developed is a tool for comprehensive analysis of 12 toxins at nanogram levels [19]. The identification of dry oil in art is an important step in the scientific research of man-made materials, which provides protectors and art historians with valuable information about the materials used and the painting techniques used. Hussein is committed to the analysis of the fatty acid composition in microscopic samples of art objects by GC-MS, and the traps and troubleshooting of the identification of dry oil. Hussein demonstrates that the ratio of palmitic acid to stearic acid (P/S) for each oil type and for dry oil identification depends on the sample dilution, so different dilutions of the same sample, in the case of nonlinear instrument response. Different P/S ratios can be obtained. This phenomenon may hinder the identification of dry oil and lead to erroneous interpretation. This is an important observation because in today, many times the P/S ratio is calculated based on the corresponding peak area ratio or using a single point calibration method. In these methods, the linearity of the instrument response cannot be controlled and ensured. In the case of analysis, the nonlinear instrument response is due to incomplete sample evaporation in the injector. Filling the glass liner with deactivated glass wool improves sample evaporation and ensures linearity of instrument response and P/S ratio independence after sample dilution [20].

In this paper, the extractant and sample purifier of organic phosphorus in soybean were discussed. The organophosphorus pesticide in soybean was extracted by acetone-dichloromethane mixed solvent, and Florissilite was used as a purifying agent. The gas chromatograph with flame photometric detector was used to analyze and determine the organophosphorus pesticide residues of DDT, Dimethoate, methyl parathion and parathion in soybean. The operation was simple, the recovery rate was high, and the minimum detection was carried out. The concentration was: drip fear 0.03 mg/kg; dimethoate 0.04 mg/kg; methyl parathion 0.04 mg/kg; parathion 0.05 mg/kg.

2. Proposed Method

2.1. Organophosphorus Pesticide Hazards

Organophosphorus pesticides are widely used because of their unstable chemical properties, easy

decomposition, short half-life, and difficulty in accumulation in crops, animals and humans. However, due to the increasing use of organic phosphorus, the frequency of use is getting higher and higher, and the application rate is getting larger and larger, which has become the most serious pesticide for contaminated food. At present, there are two main aspects of the use of organophosphorus pesticides in vegetables:

First, highly toxic, high-residue and even highly toxic organophosphorus pesticides are still used in vegetables, such as methamidophos, dichlorvos, malathion and methyl parathion, and due to the large and irregular use of organophosphorus pesticides. The residue limits for some pesticides in vegetables far exceed the national maximum residue limit. According to the statistics of pesticide residues in vegetables in some provinces and cities, the residual amount of methamidophos (highly toxic pesticides) was 0.005~0.572mg ŀg-1, and the detection rate and over-standard rate were both 5.9%; The residual amount of highly toxic pesticides is 0.0207~0.6723mg ŀg-1, the detection rate and over-standard rate are about 10%; the residual amount of parathion (high-toxic pesticide) is 0.090~0.996mg ŀg-1, the detection rate And the over-standard rate was 34.8%, 7.5%; the dichlorvos residue was 0.010~0.549mg ŀg-1, the detection rate and over-standard rate were 79%, 20%, respectively; the dimethoate residue was 0.017~0.138mg ŀg- 1, the detection rate and the over-standard rate were 73.5%, 15%; the malathion residue was 0.19~0.32mg ŀg-1, and the detection rate was 70%. From the above six organophosphorus pesticide residues, high-toxic pesticides are not allowed to be used on vegetables, but they are still detected in some areas and have a high rate of over-standard.

Second, the excessive pesticide residue has evolved from a single high-toxic pesticide in the past to a variety of pesticide residues. A single vegetable sample often detects multiple organophosphorus pesticides at the same time, and the proportion of multi-residue pesticide samples is still rising. The reason is that the producer applied the compound pesticide.

Consumption of agricultural products containing a large number of highly toxic and highly toxic pesticide residues can lead to acute poisoning of humans and animals. Long-term consumption of agricultural products with excessive levels of pesticide residues, although not causing acute poisoning, may cause chronic poisoning, leading to disease and even affecting the next generation. If you eat vegetables or fruits with serious pesticide residues, you may experience headache, dizziness, nausea, abdominal pain and other symptoms. In severe cases, you may experience difficulty breathing, paralysis, and coma. In severe cases, you may even threaten people's lives.

Due to the unreasonable use of pesticides, especially herbicides, it will cause different degrees of phytotoxicity, and even cause large-scale agricultural production or even production, which seriously affects agricultural production. After the pesticide is applied in the orchard, a part of the pesticide remains on the surface of the fruit tree leaf or fruit, and a part of it penetrates into the stratum corneum or tissue of the fruit tree, inhibiting or destroying the normal growth and development of the fruit tree, directly affecting the physiological metabolism of the fruit tree, causing different degrees of phytotoxicity. Acute phytotoxicity can cause spots, yellowing, chlorosis, withering, leaf rolling, defoliation, fruit drop. In leaves and fruits; chronic phytotoxicity can weaken photosynthesis of fruit trees, delay flower bud formation and fruit ripening, deterioration of taste and color, and even Lead to the death of fruit trees.

OPs are amide or thiol derivatives of phosphorus, phosphonic acid, thiophosphoric acid, thiophosphoric acid synthesized from esters, amides or thiol derivatives, and are widely used in the control of agricultural, domestic and parasitic and structural pests. OPs decompose rapidly when exposed to light and air, do not exist in the environment for too long, are not affected by bioaccumulation and biomagnification, and do not release toxic decomposition products. These characteristics make them widely used in modern agriculture and animals. Therefore, OPs are more popular than organochlorine pesticides such as DDT. However, when people who have sprayed OPs

or nearby waters are tested for OPs, they can still be detected. Therefore, it is not clear how long OPs can completely degrade in the natural environment.

OPs are one of the most toxic pesticides, and their extremely low concentrations in the environment can cause long-term damage to human health. According to the EPA classification, OPs are classified as toxicity class I (very toxic) or toxicity class II (poisoned). The toxicity of OPs is based on the inhibition of AChE activity, a key enzyme that is essential for the biological nervous system in the central nervous system of mammals and insects. In the cholinergic synapse, the enzyme can degrade choline, terminate the excitatory effects of neurotransmitters on the postsynaptic membrane, and ensure the normal transmission of neural signals in the body. And the enzyme has the activity of carboxypeptidase and aminopeptidase. AChE is involved in cell development and maturation and promotes neuronal development and nerve regeneration. When the activity of AChE is inhibited, it causes the neurotransmitters of mammals and insects to accumulate thiocholine (TCh), which interferes with muscle reactions and causes respiratory and myocardial damage and death. Although the level of pesticide exposure to a single compound or active ingredient does not exceed the level of risk to humans or the environment, the toxicity of OPs varies widely, depending on the chemical structure of the pesticide.

Due to the high toxicity of OPs, there is a chronic effect, and OPs will remain in food, drinking water and the environment. Therefore, the establishment of fast, reliable, sensitive and practical analysis devices and methods for OPs residue monitoring and detection has important practical significance for environmental protection, food safety and human health. Numerous analytical methods include gas chromatography(GC), liquid chromatography(LC), high performance liquid chromatography(HPLC), mass spectrometry(MS), capillary electrophoresis (CE), surface plasmon resonance (SPR), fluorescence spectroscopy, colorimetry And GC-MS have been used to analyze and detect OPs. Although these methods have been successfully applied to the detection of OPs, chromatography is time consuming and requires sample preparation and expensive equipment. For these reasons, only samples can be tested in the laboratory and cannot be detected online. Therefore, a simple, fast, sensitive, low-cost and reliable method for detecting low-concentration OPs needs to be developed.

2.2. Gas Chromatography (GC)

(1) Gas chromatography-mass spectrometry (GC-MS or GC-MS/MS)

The main application range of gas chromatography-mass spectrometry is consistent with gas chromatography, which is suitable for the detection of volatile substances. Mass spectrometry can overcome the false positive occurrence of gas chromatography alone, and has strong qualitative ability to confirm the target by mass spectrometry. Mass spectrometry has the characteristics of small matrix interference, high sensitivity and high selectivity, and is widely used in the fields of environment, textiles, pesticide residues and food. A method for the determination of ppm-level phosphorus compounds in organophosphorus pesticide samples by ion-pair extraction and GC-MS method was established. It is easy and accurate to determine whether 54 kinds of oxidized organophosphorus pesticides contain organophosphorus compounds. 92.2%-98.4%, relative standard deviation 0.7%-4.2%. Two kinds of sulfate, aniline and phenol organophosphorus pesticides were determined by gas chromatography-mass spectrometry. Ethyl acetate was used as an extractant and solvent in 40 min, and ultrasonic extraction was carried out to separate 16 kinds of oxidized organophosphorus pesticides. The 17 kinds of organic phosphorus of naphthol, phenylenediamine and benzenediol were analyzed by DB-WAX gas chromatography column, and the simultaneous detection of water-soluble and alcohol-soluble organophosphorus pesticides was realized. The internal standard method is beneficial to eliminate the difference caused by the change

of operating conditions and the difference of sample injection. The naphthalene is used as the internal standard to quantitatively detect the three isomers of phenylenediamine in organophosphorus pesticides. Gas chromatography-mass spectrometry has high sensitivity and specificity, but sometimes the sample needs to be derivatized. The benzaldehyde derivatization method is used to determine the p-phenylenediamine in the organophosphorus pesticide, the amino group is converted into the imine, and the organic phosphorus is improved. The stability of the substance enhances the response sensitivity of the instrument and is confirmed by mass spectrometry. The derivatization process is cumbersome and suitable for the detection of low-throughput samples. At present, some literature reports on the determination of pesticides in organophosphorus pesticides by gas chromatography-mass spectrometry, mainly using single quadrupole mass spectrometry, only full scan and selective ion scanning mode, and the detection of organophosphorus pesticide components and detection sensitivity are limited. The GC-MS/MS method is beneficial to the qualitative and quantitative determination of multi-component organophosphorus pesticides, which provides a basis for judging a large number of isomers in organophosphorus pesticides, greatly increasing the peak capacity and fully improving the detection sensitivity.

(2) High performance liquid chromatography (HPLC)

High performance liquid chromatography has the characteristics of strong separation ability and high sensitivity, and is not limited by the thermal stability and volatility of the analyte. The method has a wide application range. Many detectors such as ultraviolet detectors (UV), diode array detectors (DAD), etc., are also commonly used by researchers to separate and analyze organophosphorus pesticides. The 2007 edition of the "Hygienic Standard for Organophosphorus Pesticides" is the determination of eight oxidized pesticides by HPLC. The main components of oxidized organophosphorus pesticides are aromatic amine compounds, which can be better retained on conventional C18 column and C16 amide column, but aniline and phenolic substances will have different degrees of ionization under certain pH conditions. It is prone to problems such as poor retention performance and poor reproducibility. Using 50% methanol as mobile phase, m-benzenediol and 1-naphthol in organophosphorus pesticides were quantitatively analyzed on C18 column, which was well separated in 12min, with good reproducibility and high precision. The o-, m-p-phenylenediamine and benzenediol were separated, the detection limit was 0.05-0.16 μg/ml, and the effect of pH on the retention time of each component was investigated. Nine oxidized organophosphorus pesticides were analyzed on a C18 column. 13 kinds of aniline and phenolic oxidized organophosphorus pesticides were separated and detected by HPLC-DAD method at 280nm and 331nm. The effects of mobile phase species, pH and other factors were investigated. The detection limit was 0.2-2 mg/L, the method was verified in 5 laboratories. Twelve aniline compounds of phenylenediamines, aminophenols and diamine sulfates were simultaneously separated on the RP-AMIDEC16 column by using sodium heptane sulfonate as the mobile phase. Fourteen organophosphorus pesticide ingredients were simultaneously separated and determined using an amide C16 column. In order to reduce the matrix interference in organophosphorus pesticides, multiple liquid-liquid extractions were carried out with n-heptane, and four organophosphorus pesticides such as o-, m-, hydroquinone and m-aminophenol were detected by RP-HPLC-DAD method. And monitoring its stability, found that recovery, sensitivity, selectivity are better.

The use of a single chromatographic system can only qualitatively and quantitatively detect nearly 10 organophosphorus pesticides, which is far from meeting the needs of analysis and sanitation supervision of organophosphorus pesticides. Continue to use the C16 amide column to separate 22 organophosphorus pesticides in 50 minutes. The separation, except for the low concentration of sulfate, the relative standard deviation and recovery of the other components are

better. At present, most of the organophosphorus pesticides determined by liquid chromatography are concentrated in aniline and phenolic substances, and there are few reports on the detection of high molecular weight acidic and basic organophosphorus pesticides. A HPLC-DAD method for the simultaneous determination of 9 kinds of alkaline organophosphorus pesticides such as alkaline orange 31, basic violet 4, basic yellow 87 and rhodamine B in hair was established. The average recovery was 93.9%-102.5%, the relative standard deviation is 1.2%-3.5%, and the lowest detection concentration is 0.15-0.60 mg/kg.

Compared with gas chromatography, liquid chromatography has simplified the sample preparation. Because the matrix of organophosphorus pesticides is more complex, the background interference of chromatographic peaks is more, and the impurity response signal is stronger, although the extraction method has obtained a larger degree to some extent. Improvement, but for some structures, substances with similar properties can not be well separated by HPLC.

(3) High performance liquid chromatography-mass spectrometry (HPLC-MS or HPLC-MS/MS)

The combination of high separation performance of high performance liquid chromatography and strong qualitative ability of mass spectrometry has the characteristics of high separation efficiency, fast analysis speed, wide application range and good specificity, and can effectively avoid the derivatization of highly polar and thermally unstable compounds. This method has become one of the best means for qualitative and quantitative analysis of complex mixtures in the fields of drug analysis, food testing, and environmental testing. In 2004, HPLC (DAD)-MS (API) was used to determine 4-aminom-cresol and 5-amino-o-cresol. Computers were used to simulate possible metabolites in humans, and the two organophosphorus pesticides were tested. Metabolites were tested. Although HPLC/MS has sufficient sensitivity, it does not provide more complete structural information. HPLC-MS/MS, multi-stage mass spectrometry monitoring mode can obtain both molecular ion peaks and fragment ion peaks, which can be used for qualitative and quantitative analysis. It is the most commonly used method for the determination of organophosphorus pesticides by LC/MS. . The water was extracted with methanol, ethanol or ethyl acetate, extracted by n-hexane, purified by SPEC18 column, and 11 kinds of aniline and phenolic organophosphorus pesticides were determined by HPLC-MS/MS method to realize aniline and phenol. Simultaneous determination of isomers. Seven kinds of aminophenol compounds such as 3-diethylaminophenol and 2,3-dihydroxynaphthol in organophosphorus pesticides were simultaneously determined by UPHPLC-MS/MS multi-level monitoring and scanning. The method was fast. Analysis of high-throughput samples provides a reliable analytical platform.

Mass spectrometry can be used as both a detector and a mass spectrometer to confirm the target mixture. It is currently the best method for the comprehensive detection of organophosphorus pesticides. Compared with other methods, it has the characteristics of simple pre-treatment and high sensitivity. It is widely used in many fields such as chemical engineering, biology, medicine, environment. It provides a more accurate and rapid qualitative and quantitative method for the separation of complex mixtures.

In summary, high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) and gas chromatography-mass spectrometry (GC-MS/MS) have become one of the best means for the detection of organophosphorus pesticides in organophosphorus pesticides. Although this technology has been reported in the detection of the forbidden components of organophosphorus pesticides, the basic types of detection remain at about 10, which is difficult to better meet the daily supervision and testing of organophosphorus pesticides. Therefore, this project selected high performance liquid chromatography-mass spectrometry and gas chromatography-mass spectrometry to construct a method for simultaneous determination of various organophosphorus pesticides in organophosphorus pesticides.

3. Experiments

3.1. Instruments and Reagents

(1) Instrument

Agilent 7890A gas chromatograph (Agilent, USA); IKAT25 homogenizer (Germany); centrifuge (Beijing Jingli); IKARV10 rotary evaporator (Germany); vortex mixer, nitrogen blower and other conventional instruments.

(2) Reagents

N-hexane (chromatographically pure); acetone (chromatographically pure); sulfuric acid (analytical grade); organophosphorus pesticide standard (GSB-1401-2001, dichlorvos 78.2±9.8μg/ml, dimethoate 75.3±8.7μg/ml, methyl Parathion was 76.2±7.8 μg/ml, malathion was 77.7±7.5 μg/ml, and parathion was 76.6±7.3 μg/ml. 1.2 ml was diluted with acetone to 50 ml and stored under refrigeration conditions. C18 solid phase extraction column (2000mg/12ml, Aijieer); N-propyl ethylenediamine (PSA) solid phase extraction column (1000mg/6ml, Aijieer); Pesticarb solid phase extraction column (200mg/3ml, Ai Jer).

3.2. Experimental Preparation and Data Collection

(1) Sample preparation

1) Sample extraction

The soybeans are removed, mixed, pulverized, and cooled. Weigh 3.00g of soybean powder into a 50ml screw centrifuge tube, add 25ml of acetonitrile, vortex for 2min, centrifuge at 4000r/min for 4min, add the supernatant to a clean 50ml centrifuge tube, and add acetonitrile for 2 times (each time). 25 ml), the supernatant was combined and steamed at 35 °C to dry (solvent was acetone).

2) Sample purification

Degreasing The C18 solid phase extraction column was activated with 8 ml of acetonitrile. The extract was dissolved in 10 ml of acetonitrile and passed through a column at a flow rate of 1-2 drops/s; and then eluted with 15 ml of acetonitrile. The eluate was collected and placed in a 50 ml centrifuge tube, and nitrogen was blown to dry at 35 ℃. In addition to the impurities, the PSA-PC solid phase extraction column was activated with 8 ml of n-hexane + acetone (1+1, v+v), and the extract of the oil removal in (1) was dissolved in 5 ml of n-hexane + acetone (1+1). After the column, the flow rate is 1~2 drops/s; then it is eluted with 10ml of n-hexane + acetone (1+1). The eluent is collected and placed in a 15ml centrifuge tube. The nitrogen is blown to dry at 35 ℃, and 1ml of n-hexane is added. Acetone (1+1) was dissolved as a test solution.

(2) Gas chromatographic conditions

Column: Agilent DB-1701 (30m × 0.32mm × 0.25μm); injection volume: 1μl; injection method: no split; inlet temperature: 230 ℃; septum purge: 3ml / min; FPD detector Temperature: 240 ℃; H2 flow rate: 75 ml / min; air flow rate: 100 ml / min; tail blowing (N2): 60 ml / min; carrier gas (N2): 1.5 ml / min. Temperature programmed: 1 min at 120 ℃, 220 ℃ at 20 ℃ / min, held for 2 min, and then raised to 240 ℃ at 5 ℃ / min for 5 min.

(3) Calculation of results

The amount of pesticide residues in the sample is calculated as follows:

$$\omega(\mu g / g) = \frac{C_i \cdot V}{m} \times \frac{1}{1000} \quad (1)$$

Where: ω: pesticide content, unit μg / g (ie mg / kg); Ci: sample detection concentration, unit μg / L; V: sample volumetric volume, 1ml; m: solid sample quality; 1 / 1000: unit Conversion factor.

4. Discussion

4.1. Selection of Decontamination Solid Phase Extraction Column

(1) Selection of components of solid phase extraction column

The composition of the phase extraction column Carb is unchanged, and the purification effects of the equivalent NH2 and PSA components are compared. The organic phosphorus standard solution was added to the degreasing sample solution, and the spike removal test was performed at the level of 0.10 mg/kg. The color of the samples after the two solid phase extraction columns were compared, and the recovery rates of the four pesticides were recovered. The ratio = m actual × 100% / m theory (the same below), the comparison of the impurity removal effect of the solid phase extraction towers of different compositions is shown in Table 1.

Table 1. Comparison of impurity removal effects of solid phase extraction columns with different compositions

Solid phase extraction column composition	Carb(500mg)/PSA(500mg)	Carb(500mg)/NH_2 (500mg)
The average recovery rate/%(n=3)	86.3	70.2
Color	Colorless and transparent	Colorless and transparent

It can be seen from Table 1 that when the Carb composition is 500 mg, the PSA (500 mg) composite column and the NH2 (500 mg) composite column have comparable purification effects on the pigment, but the former has higher recovery rates for the four targets than the latter. Soybeans have high protein and fat content of about 36% and 18%, respectively. These samples are more effective in removing sugars, fatty acids, organic acids and some polar pigments by Carb/PSA column purification. PSA is a similar adsorbent to NH2. It is weaker than NH2 and has stronger capacity and ion exchange capacity than NH2 column. Therefore, the same capacity Carb/PSA column is selected for extraction.

(2) Determination of the capacity of the solid phase extraction column

The purification effects of the same degreasing-spiked sample solution were compared between Carb (500mg)/PSA (500mg), Carb (200mg)/PSA (500mg), PSA (500mg) and PSA (1000mg). The results showed that the recovery rate from low to high was Carb (500 mg) / PSA (500 mg) < Carb (200 mg) / PSA (500 mg) < PSA (1000 mg) < PSA (500 mg); PSA (1000 mg) > PSA (500 mg) > Carb (200 mg) / PSA (500 mg) and Carb (500 mg) / PSA (500 mg). It shows that the larger the capacity of the Carb column, the more the polar impurities are adsorbed and the more the organic phosphorus is adsorbed. Taking into account the recovery rate and color, Carb (200mg) / PSA (500mg) was finally determined as the optimal capacity of the impurity removal column.

4.2. Selection of Solvent for Washing

In GB/T19649, grain and solid phase extraction method is used to purify polar impurities such as pigments, and is eluted with acetonitrile + toluene (3+1). The solvent has good elution effect, but the boiling point of acetonitrile and toluene is high (acetonitrile 78 ℃). , toluene 110 ℃), rotary evaporation concentration time of more than 1h, the target loss; in addition, toluene belongs to the public security organs control reagents, the procurement process is cumbersome, long cycle, and the reagent is more toxic, the experimental personnel have long-term exposure to physical damage Big. In this study, the solvent acetonitrile and toluene were replaced with n-hexane and acetone.

Change the composition of the elution solvent: n-hexane + acetone (2+1), n-hexane + acetone (1+ 1), n-hexane + acetone (1 + 2), acetone, other methods of operation are unchanged. The results showed that the recovery of n-hexane + acetone (1+1) in the four elution solvents was higher and the color was the lightest as shown in Figure 1. Therefore, the solvent was selected as the elution

solvent when purifying impurities.

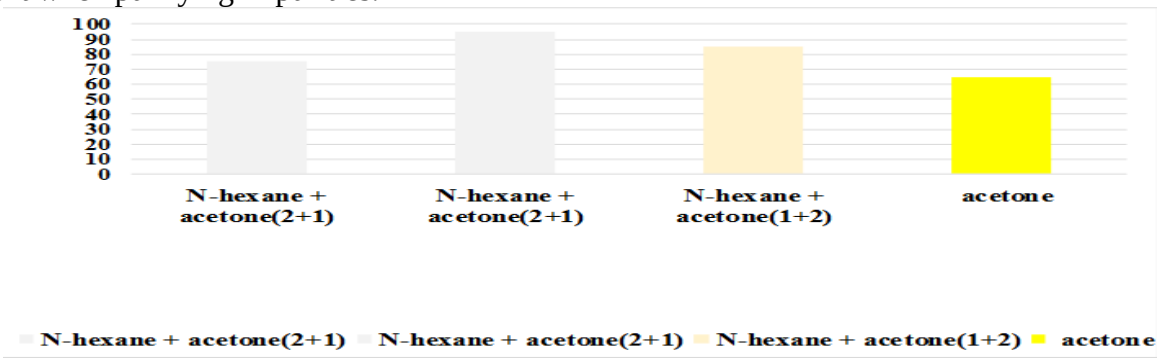

Figure 1. Effect of different elution solvents on recovery and color in the impurity removal process

4.3. Selection of Purification Method for Purifying Sample Liquid

The eluent in GB/T19649 needs to be re-spinned and concentrated, which has high requirements on equipment and technology, and the sample loss is also large. In the present study, the eluent is a low-boiling solvent, and the degreasing liquid after degreasing and impurity removal can be concentrated by nitrogen blowing. The operation time of the method is short, and the collection and concentration of the cleaning liquid are all performed by using a centrifuge tube, and the number of times of transfer is reduced, which is effective. Reduce target loss and increase organic phosphorus recovery as shown in Figure 2.

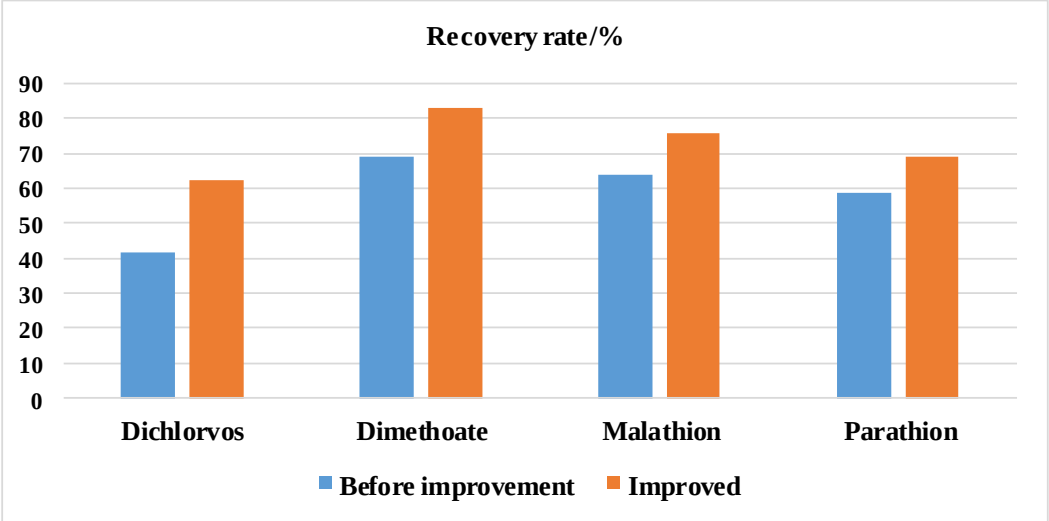

Figure 2 . Comparison of average recovery rates of pesticides before and after improvement

4.4. Linear Range, Correlation Coefficient, Detection Limit

The organic phosphorus standard can be used to protect the stock solution from acetone to a concentration of 15.0, 37.5, 75.1, 150.1, 375.4, 750.7 μg / L standard solution (due to the concentration of each component in the mixture is not exactly the same, the standard solution concentration is The concentration of dichlorvos is determined separately, and the standard curve is drawn with the concentration as the abscissa and the peak height as the ordinate and linear regression is performed. The correlation coefficient r=0.9988~0.9993, the results show that the linear relationship is good. The detection limit (LOD) of pesticides in the S/N=3 calculation method.

The relationship between linear concentration and retention time of various pesticides is shown in Figure 3. The detection limits, linear range, linear regression equation and correlation coefficient are shown in Table 2.

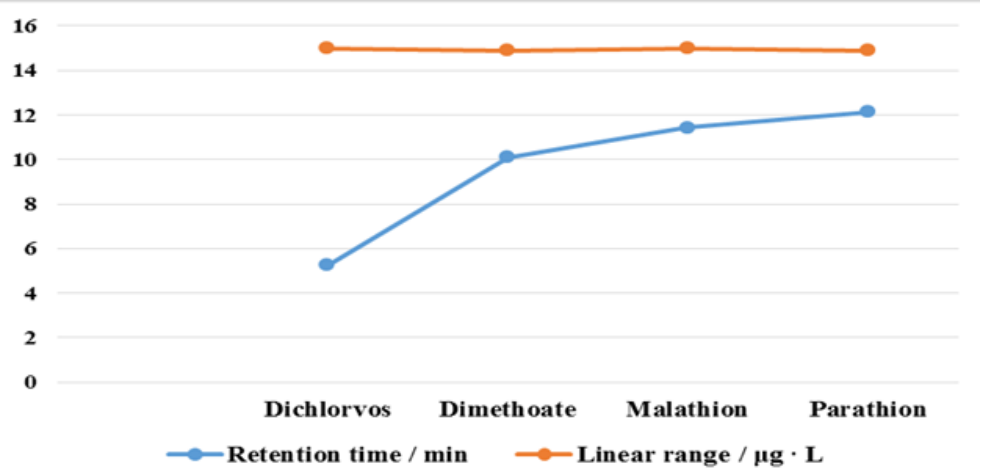

Figure 3. Linear concentration versus retention time

Table 2. Linear range of pesticides, linear regression equation, correlation coefficient and detection limit

Pesticide name	Retention time / min	Linear range / μg · L^{-1}	Linear equation	Correlation coefficient r	Detection limit (LOD) / μg · L^{-1}
Dichlorvos	5.255	15.0~750.7	y=5.78394x+50.59151	0.9993	5.0
Dimethoate	10.087	14.9~722.9	y=5.9114x+40.73099	0.9992	4.9
Malathion	11.422	15.0~745.9	y=4.39652x+11.65731	0.9993	5.0
Parathion	12.144	14.9~735.4	y=5.67012x+62.4949	0.9998	4.9

4.5. Recovery, Precision and Limit of Quantitation

Different volumes of organophosphorus standards were added to several equal amounts of soy samples and sample preparation was carried out as described in 3.2. Referring to the limit of four pesticides in GB2763 (see Table 3), the three-level spiked recovery test of 0.05, 0.10 and 1.00 mg/kg was carried out, and the feasibility of the method was investigated according to the spiked recovery rate. Each sample was repeatedly measured 6 times to obtain an average value, and the precision (i.e., coefficient of variation) was calculated. Soybean blank

The sample chromatogram is shown in Figure 4. The recovery and precision results are shown in Table 3.

It can be seen from Table 3 that the average recoveries of four organophosphorus pesticides in soybeans ranged from 62.8% to 93.4%, and the coefficient of variation was 1.70% to 3.50%. The recovery and precision of this method were within the required range. Considering the mixed concentration of the four pesticides, the national standard pesticide limit, the sample recovery rate and the precision, the limit of quantitation (LOQ) was determined by an intuitive method, and the LOQ was 0.05 mg/kg.

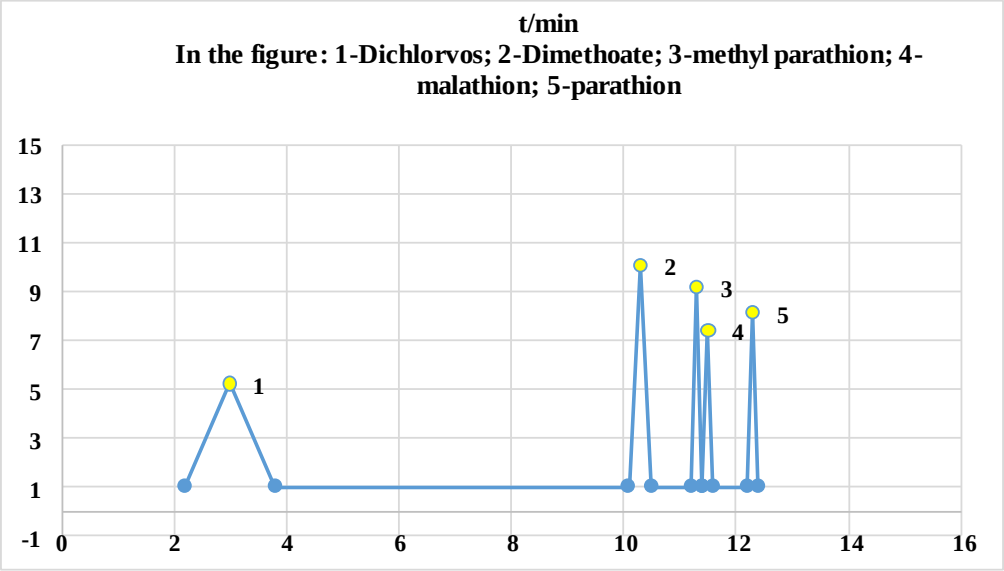

Figure 4. Blank sample of soybean sample spiked with gas chromatogram

Table 3. Spiked recovery, coefficient of variation and pesticide limit

Name	Scaling level /mg kg $^{-1}$	Recovery rate/%				Coefficient of variation/% (n=6)	Pesticide limit /mg kg $^{-1}$
		1	2	3	average		
Dichlorvos	0.04	65.1	64.3	60.0	63.8	3.50	⩽0.1
	0.10	64.3	66.7	64.2	64.5	2.53	
	1.00	73.4	68.5	70.4	70.3	2.11	
Dimethoate	0.04	76.5	82	79.2	80.1	3.12	⩽0.05
	0.10	76.7	83.4	88.6	84.5	2.78	
	1.00	93.2	99.7	87.3	94.3	2.85	
Malathion	0.04	70.6	75.6	72.2	72.3	2.46	⩽8
	0.10	82.0	84.8	80.2	83.5	1.95	
	1.00	94.5	84.4	88.5	89.7	1.70	
Parathion	0.04	69.6	70.8	70.0	69.7	2.97	⩽0.1
	0.10	78.9	79.3	76.6	78.6	2.73	
	1.00	84.5	90.2	85.1	86.9	2.12	

5. Conclusion

In this study, eight organophosphorus pesticides were synthesized using organophosphorus pesticide structural intermediates, dimethyl thiophosphoryl chloride as a public template. Both base parathion, phosphonamine and malathion have novel molecularly imprinted polymers with selective adsorption properties. The imprinted polymer has high adsorption capacity and adsorption rate, and the polymer is used as solid phase extraction adsorption. Agent, established a solid phase extraction-gas chromatography combined detection technology. Under optimal extraction conditions, the enrichment ratio of imprinted polymer to eight organophosphorus pesticides ranged from 25 to 480, and the precision (RSD) range of continuous enrichment for 5 times ranged from 1.50% to 4.09%, and the recovery rate was added. Between 80.11% and 97.70%.

In this study, the method of sample pretreatment was also explored, and a method for the determination of four organophosphorus pesticide residues in soybean by solid phase extraction-gas chromatography was established. The method can meet the requirements of GB2763-2016 for the maximum residue limit of edible soybean pesticides. . When the spiked recovery experiment was

carried out between 0.05 and 1.00 mg/kg, the average spiked recoveries of the four organophosphorus pesticides ranged from 62.8% to 93.4%, and the coefficient of variation was 1.70% to 3.50%.

In addition, the standard in this study is the direct purchase of mixed standards, only the pesticides with the national standard limit requirements, to avoid the cumbersome purchase and preparation process of a variety of standards, simplifying the actual testing process. In actual testing, if the hardware conditions are limited, the company may selectively increase the types of organophosphorus pesticides with similar properties on the basis of this research, or directly purchase pesticides with similar components and more types. The method has good recovery rate, reliable precision, low toxicity of reagents, strong operability of the method, and basically meets the daily testing needs of ordinary food enterprises.

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Data Availability

Data sharing is not applicable to this article as no new data were created or analysed in this study.

Conflict of Interest

The author states that this article has no conflict of interest.

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