

DETERMINATION OF METHYL PARATHION IN VEGETABLES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Organophosphorus pesticides are one of the most widely used insecticides, which are mainly used in grain, vegetables and fruits. Methyl parathion is a kind of organophosphorus pesticide, which belongs to nerve agent. It can cause different degrees of poisoning to human and livestock and cause serious environmental pollution. Therefore, it is of great significance to establish an effective method for detecting methyl parathion residues in agricultural products. The determination of methyl parathion is often carried out by gas chromatography, but because of the strong polarity and thermal instability of methyl parathion, gas chromatography brings certain difficulty. The High Performance Liquid Chromatography (HPLC) method was researched for the testing of Methyl Parathion residues in vegetables, and the chromatographic conditions for sample extraction, purification and detection were screened and optimized. In vegetables, the matrix is complex, the pesticide residue is low, and there are many interference factors. The main residual components are not easy to separate, enrich and purify, so the detection of related pesticides is not accurate. After lots of experimental exploration, the chromatographic conditions by acetonitrile extraction agent, methanol:water (73:27) as mobile phase and UV detection wavelength choosing 270 nm was selected finally. What's more, QuEChERS (Quickly, Easy, Cheap, Effective, Rugged, Safe) method, a new pretreatment technology for pesticide residue detection in agricultural products developed in the world recent years, was used for pretreatment of three kinds of vegetables, and PSA and GCB were selected as purifying agents for sample pretreatment ultimately. The experimental results displayed that the chromatographic peak area of Methyl Parathion exhibiting a good linear relationship with its concentration in the 0.05 μM ~20 μM range, and the standard curve equation is $Y=4833.5x-32.64$, the correlation coefficient is 99.96%. The average recoveries of Methyl Parathion in three kinds of vegetables (Lettuce, Cucumber and tomato) were between 87.38% and 114.12% at the three spiked levels of 0.5, 2 and 8 μM , and the relative standard deviation (RSD) was between 1.72% and 6.2%. This method has the good points of simple operation, accurate and reliable, and is suitable for the detection of MP pesticide residues in various vegetables.

Key words: Methyl Parathion, pesticide residue, High Performance Liquid Chromatography.

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Introduction. The use of pesticides can largely protect crops from pests and diseases, which has become an indispensable part of production materials in modern agriculture (Jiang, et al., 2016; Kumar et al., 2018; Qiao, Qian et al., 2018). Organophosphorus pesticides are widespread pesticides in agricultural production in China (Li et al., 2014; Ng et al., 2015; Yu. et al., 2016), which have the advantages of high efficacy, variety, and easy degradation (Khan et al., 2021). Therefore, Methyl parathion is widely used in the control of various crop pests due to its low price and wide range of efficacy (Chen et al., 2021; Eddleston et al., 2020; Liao et al., 2016). However, Long-term abuse and illegal use of pesticides not only have seriously threatened the ecological environment (Anakwue, 2019; Fang et al., 2021; Kumar et al., 2018; Saethre et al., 2012; Wang et al., 2020; Wei et al., 2022; Zhao et al., 2013), but also causes great harm to human's health by inhibiting the activity of cholinesterase in nervous system, even fatal or carcinogenic (Huang & Huang, 2012; Kalipci et al., 2010). So, it is very meaningful to build a reliable and efficient detect method for pesticide residues (Liu et al., 2014; Lu et al., 2018). Organophosphorus pesticide residual can be detected by gas chromatography, but most of the organophosphorus pesticides have strong polarity and thermal instability, which brings certain difficulties to gas chromatography analysis

(Chen, 2010). HPLC method has combined the advantages of common liquid chromatography and gas chromatography, and then developed after appropriate improvement. It has the function of separating and preparing water-soluble substances by liquid chromatography at room temperature, and the characteristics of gas chromatography (i.e., high pressure, high speed, high resolution, and high sensitivity) and good repeatability, small sample quantity, easy to multiple measurements (Ashraf et al., 2022; Gao et al., 2017; Harshit et al., 2017; Rana et al., 2011; Vichapong et al., 2016).

In this study, High Performance Liquid Chromatography (HPLC) was utilized to determine MP in different vegetables. This method has the good points of simple operation, accurate and reliable, and is suitable for the detection of MP pesticide residues in various vegetables (Chen, & Li, 2013; Pang et al., 2011; Tan, et al., 2009; Wang, Chen, Cao, & Li, 2015).

Materials and methods. *Experimental Instruments and Reagents.*

Experimental instruments were:

UPLC-PDA (I-Class PLUS, Waters Corporation, Milford, MA, USA);

Vortex Shaker (IKA Vortex 3, Germany);

Millipore filter system (Millipore Corporation, Bedford, MA, USA);

Centrifuge (Sigma 3K 30, Germany).

In research were used such reagents as:

HPLC-grade acetonitrile (MREDA Technology Inc, USA);

Methanol (Dikma Beijing, China);

Primary secondary amine (PSA, Tianjin Bona Aijer Technology Co., LTD); Graphitized carbon black (GCB, Hangzhou Micron Pie Technology Co., LTD);

NaCl (Sinopharm Chemical Reagent Co. LTD).

Chromatographic conditions. Chromatographic column was Shim-Pack: VP-ODS; Mobile phase was methanol and water, and the ratio was determined by experiment results; The flow rate was 1.0mL/min; The sample size was 5 μ L; Quantitatively determined by calculating the peak areas.

Preparation of standard solution. Methyl parathion pesticide stock solution is dissolved in acetonitrile to 20 μ M/L, stored at -18 $^{\circ}$ C for use. The standard reserve solution was diluted step by step to obtain standard solutions with concentrations of 0.05 μ M, 0.1 μ M, 0.5 μ M, 1 μ M, 5 μ M and 10 μ M, respectively.

Sample preparation. Lettuce, cucumber, and tomato were obtained from the Plant factory in Henan Institute of Science and Technology, and vegetables can be grown without any pesticides there. Each vegetable sample was washed and dried, and then cut them into pieces and put them into a homogenizer for homogenization. 10 g sample were weigh into a 50 ml centrifuge tube; 2 g NaCl and 10 mL acetonitrile are added, blended, and vortex for 3 min, then centrifuged for 5min at 3,500 rpm. An aliquot of 2 ml was transferred from the supernatant to a new clean 2 ml centrifuge tube containing a certain amount of PSA and GCB purification material. The samples were again vortexed for 3min and then centrifuged at 10,000 rpm for 5 min. Afterwards, 2 ml supernatant was taken and filter them through 0.02 μ M filter membrane before sampling.

Generally, vegetable samples without any pesticides were taken, a certain amount of standard mixture was added, and the determination was repeated for 3 times according to the given chromatographic conditions.

Results. 1. **Selection of detection wavelength.** The UV spectrum of aromatic compounds has characteristic

absorption at 254 nm, Through the wavelength scanning of the diode array detector, the pesticide had the maximum absorption at 270 nm, so 270 was selected as the detection wavelength.

2. **Selection of Mobile phase.** Methanol-Water was used as mobile phase, and different ratios were set for screening. Experimental results showed that V-methanol: V-water (73:27) was the best pesticide separation, meanwhile, the interference of blank samples was minimal, and the analysis time was short.

3. **Linear relation and limit of detection.** Under the optimal liquid-mass separation conditions, a series of standard solutions with different concentrations of MP were prepared for determination, and the standard spectrum of MP (which concentrations is 10 μ M) is shown in Fig.1. Then the standard curve of mass concentration was drawn based on the peak area of each component. The results show that the linear range of MP is 0.05~20 μ M, and the standard curve equation is $Y = 4833.5x - 32.64$, the correlation coefficient is 99.96%, which can meet the needs of quantitative analysis.

4. **Precision and recovery rates.** Fig. 2 and Fig. 3 were respectively the control check chromatogram and spiked chromatogram of cucumber sample. The relative standard deviation and spiked recovery results were shown in the Table 1.

Table 1

Determination of spiked recovery rate of vegetable samples

Sample	Added(μ M)	Recovery rates (%)	RSD(%)
Lettuce	0.5	109.84%	2.61
	2	114.11%	4.2
	8	103.3%	1.72
Cucumber	0.5	102.23%	6.2
	2	108.58%	3.51
	8	104.82%	2.89
Tomato	0.5	89.67%	2.47
	2	87.38%	4.86
	8	90.31%	5.83

Discussion. Under optimized experimental conditions, methyl parathion has strong absorption

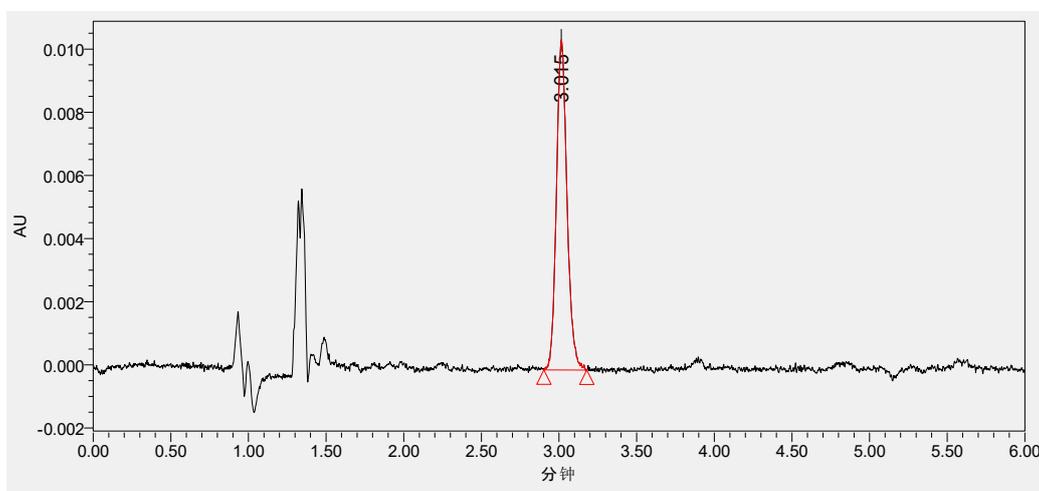


Fig. 1. HPLC chromatogram of Methyl Parathion standard

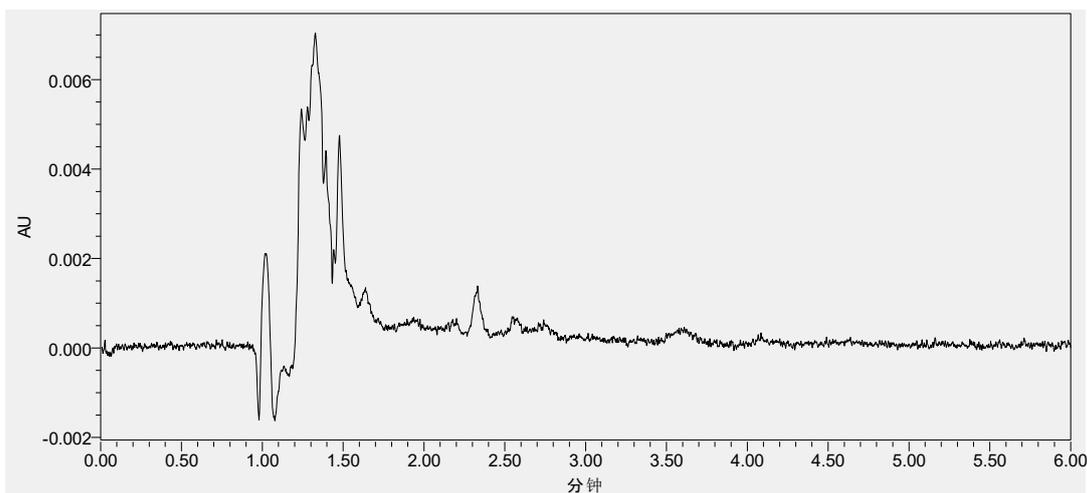


Fig. 2. The control check Chromatogram of cucumber sample

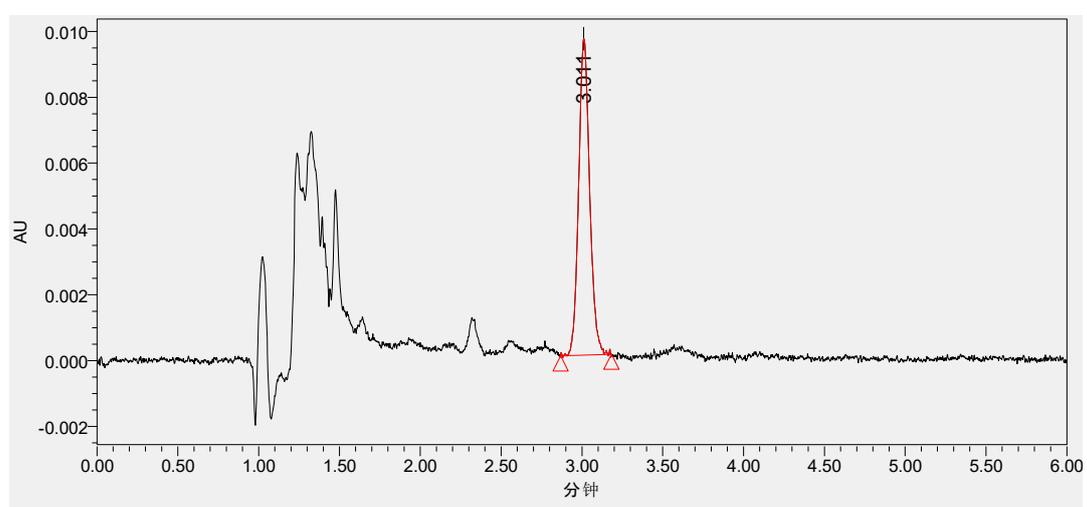


Fig. 3. The spiked chromatogram of cucumber sample

at 270nm, so 270nm was chosen as the detection wave length. In the experiment, the separation conditions of methanol-water in mobile phase were optimized with different proportions and different flow rates, and finally methanol: water = 73:27 was selected. QuEChERS (Quickly, Easy, Cheap, Effective, Rugged, Safe) method, a new pretreatment technology for pesticide residue detection in agricultural products developed in the world recent years, was used for pretreatment of three kinds of vegetables (Gonzalez-Gomez, et al. 2022), and PSA and GCB were selected as purifying agents for sample pretreatment ultimately. In the standard recovery experiment, there was a linear relationship between the standard addition amount of methyl parathion and the chromatographic analysis results (peak area) in the concentration range of 0.05~20 μ m, that is, in the above concentration range of sample extraction, purification, determination and other steps can make the target to be tested with a stable recovery rate. In the studies that have been done, Liu et al. (2012) established a detection method in dry cabbages by ultrasonic extraction-gas chromatography for the 12 organophosphorus insecticides

residues detection, and the results showed that the concentration range of linearity was 0.01~1.00 μ g/mL for methyl parathion detection, and the percent recoveries was 104.5%~110.5%, coefficients of variation was 2.4%~2.6%, and the detection limits was 0.016mg/kg. (Liu Chao et al., 2015) Developed a LC-MS/MS method for methyl parathion in three kinds of tea based on novel HPLC column Inertsil ODS-3 with methanol and 0.1% acetic acid as mobile phase, combined with MS. The experiment results showed a linear relationship in the range of 0~2.0 ng/mL, and the detection limit was 0.47ng/kg. The adding standard recovery was 83.5%~106.3%. Muckoya V. A. with colleagues (Muckoya et al., 2020) presented a simple and rapid method for determination of 11 kinds of pesticides in wastewater matrices, A chemometric approach for the optimisation of vortex-assisted dispersive liquid-liquid microextraction experimental conditions prior to liquid chromatography-mass spectrometry detection was applied. The results showed that LOD was 0.67 μ g/L, and spiked recovery rate was 97.68~115.15 for the Methyl parathion detection. Therefore, compare to the previous work, our research results are of progressive significance.

Conclusions. For this article, a HPLC method was established for the determination of MP in three vegetables. The linear range of the method was 0.05~20 μM . The recoveries rate and RSD of lettuce, cucumber and tomato were in the range of 87.38%~114.12%. HPLC method can effectively separate and determine MP pesticide residues in vegetables, and the operation is relatively simple and quick, the result is stable and accurate, which is suitable for the determination of MP in many vegetables.

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Визначення метилпаратіону в овочах методом високоефективної рідинної хроматографії

Фосфорорганічні пестициди є одними з найбільш широко використовуваних інсектицидів, які головним чином використовуються на зернових, овочевих культурах та фруктах. Метилпаратіон – це різновид фосфорорганічного пестициду, який відноситься до нервово-паралітичної речовини. Він може спричиняти різного ступеня отруєння людини та худоби, а також викликати серйозне забруднення навколишнього середовища. Тому велике значення має встановлення ефективного методу виявлення залишків метилпаратіону в сільськогосподарській продукції. Визначення метилпаратіону часто проводиться за допомогою газової хроматографії, але через сильну полярність і термічну нестійкість метилпаратіону цей метод визначення має певні труднощі. Для тестування залишків метилпаратіону в овочах був досліджений метод високоефективної рідинної хроматографії (ВЕРХ), а також хроматографічні умови для екстракції, очищення та виявлення зразків. В овочевій продукції досить складно визначити залишкову кількість пестицидів. Основні залишкові компоненти нелегко розділити, збагатити і очистити, тому виявлення супутніх пестицидів не є точним. Дослідним шляхом було встановлено такі оптимальні умови для проведення хроматографічних досліджень: співвідношення метанолу до води (73:27) як рухомої фази та довжина хвилі виявлення ультрафіолету 270 нм. Більше того, метод QuEChERS (Швидко, Легко, Дешево, Ефективно, Надійно, Безпечно) – нова технологія попередньої обробки для виявлення залишків пестицидів у сільськогосподарських продуктах, розроблена в світі останніми роками, була використана для

попередньої обробки трьох видів овочів, а PSA та GCB були обрані, у підсумку, як очисні агенти для попередньої обробки зразків. Результати експерименту показали, що хроматографічна площа піку метилпаратіону демонструє пряму лінійну залежність з його концентрацією в діапазоні 0,05 мкМ~20 мкМ, а стандартне рівняння кривої дорівнює $Y=4833,5x-32,64$, коефіцієнт кореляції становить 99,96%. Середній вміст метилпаратіону в трьох видах овочів (салат, огірок і помідор) становив від 87,38% до 114,12% при трьох рівнях визначення 0,5, 2 і 8 мкМ, а відносне стандартне відхилення (RSD) становило від 1,72% до 6,2%. Цей метод має переваги простоти роботи, точності та надійності та підходить для виявлення залишків пестицидів (метилпаратіону) у різних овочах.

Ключові слова: Метилпаратіон, залишки пестицидів, високоефективна рідинна хроматографія.