Study on analytical detection technology of pesticide residue in agricultural product food safety

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Abstract. China is a large agricultural country, and the long-term use of pesticides in the development of agriculture has a certain toxic effect on human health and safety, which may cause cancer, teratogenicity, mutagenicity and other related symptoms. Technical analysis of pesticide residue monitoring is an important method to protect food safety, and the analytical technology of pesticide residue in agricultural products in China has made substantial progress. In this paper, the research progress of modern pesticide residue pretreatment technology and detection technology were described, as well as the development trend of pesticide residue detection technology. On this basis, the application of ultra performance liquid chromatography-tandem mass spectrometry (UPLC/MS-MS) technology was proposed to establish a highly sensitive and rapid method for the simultaneous detection of eight kinds of pesticide residues in agricultural products. In order to provide reference for the in-depth study of pesticide residues.

Keywords: Agricultural products, Food safety, Pesticide residues, Detection technology, tandem mass spectrum

1. Introduction
With the continuous improvement of people's living standards, the quality and safety of agricultural products are increasingly concerned, especially the pesticide residues in fruits and vegetables has become the focus of public concern. A large number of studies have shown that long-term consumption of fruits and vegetables with unqualified pesticide residues would have the risk of "teratogenicity, carcinogenicity and mutagenicity", causing significant damage to the human body [1]. Therefore, it is urgent to continuously improve the detection methods of pesticide residues and prevent and control the hazards of pesticide residues.

At present, governments all over the world have realized the harm of pesticide residues, and worked out the maximum residues to limit and standardize the use of pesticides. The Ministry of Agriculture has successively issued "Standards for Safe Use of Pesticides" and "Guidelines for Rational Use of Pesticides" [2]. Governments all over the world have taken measures to establish and improve food safety. At present, there are some common problems in food safety at home and abroad, but there are some differences between foreign countries and China in the causes of food safety hazards and the degree of harm caused.
Over the years of development, a relatively mature and complete system for pesticide residue analysis has been gradually formed and perfected in aspects such as relevant standards of detection methods and detection system [3]. With the development of high-efficiency pesticides, the continuous development of detection technology and concept optimization, there are strict requirements on the specificity and sensitivity of detection technology and pesticide residue analysis technology. The research and development of pesticide residue detection technology is of great significance in ensuring food safety.

2. Research progress of pesticide residue pretreatment technology and detection technology

2.1. Pretreatment technology of pesticide residue analysis

2.1.1. Solid phase extraction. Solid phase extraction (SPE) is a treatment technology developed in 1980. At present, the technology is very mature in the pretreatment of analysis in terms of commercial types and elite materials. It has the advantages of simplicity, rapidity, good reproducibility and high recovery rate. It is mainly treated by chromatographic analysis technology, and the target compound is adsorbed by solid adsorbent, separated from the sample, and then heated or eluted for adsorption, so as to achieve the separation purpose [4-5]. At present, SPE packing is common in the market, including inorganic materials (important sample pretreatment means), polymers and composites (effective sample enrichment and purification methods).

2.1.2. Ultrasonic extraction. Ultrasonic extraction is an effective method to extract the target by placing the sample and extractant in ultrasonic environment. Ultrasonic wave is a kind of high frequency sound wave, which vibrates more than 20,000 times per second. When ultrasonic waves vibrate in liquid, they produce cavitation, which can be used in gasification, coagulation, washing, extraction and other processes. When cavitation occurs, the air in the liquid is driven out to form a vacuum, which is the result of the action of huge sound pressure on the liquid. Ultrasonic extraction uses this energy to extract residual pesticides from various foods with solvents. Ultrasonic extraction is one of the most widely used extraction methods. Ultrasonic extraction is simple, rapid and can extract multiple samples at one time.

2.1.3. Accelerated solvent extraction technology. Accelerated solvent extraction is an automatic method for extracting solid or semi-solid samples at elevated temperature (50-200°C) and pressure (10.3-20.6 MPa), which has the advantages of less organic solvent consumption, rapid method and high recovery rate of components to be detected. Eight organochlorine pesticide residues in soil were extracted by ASE in Zhu Hongmei, and the recovery rate was 72.8%-93.2%[6]. Literature [7] used ASE to analyze 19 pesticide residues in fruits, and the results were satisfactory.

2.1.4. Supercritical fluid extraction. Supercritical fluid extraction technology is widely used in food pesticide residues. Supercritical fluid extraction technology uses the properties of supercritical fluid, such as high density, low viscosity, large diffusion coefficient, gas permeability and liquid distribution, to dissolve and separate the analyte from the matrix, and complete two steps of extraction and separation at the same time, with high separation efficiency, short operation period and no environmental pollution. Supercritical fluid extraction is fast, with little or no use of organic solvents.

2.2. Detection technique

2.2.1. Immunoassay method. The analysis method which combines antibody-antigen reaction with modern testing concept has the advantages of rapidity, agility and strong specificity, and integrates high sensitivity and immune response specificity, and only needs simple technology, equipment and professional training, which is the primary screening method of highly toxic pesticides. It is found that
immunoassay is more accurate, reliable, convenient and quick than conventional bioassay, and has become one of the most promising analytical techniques in pesticide residue analysis.

2.2.2. **Gas chromatography and its combined technology.** Gas chromatography is the most mature technology in the development of chromatography, but it has made new progress on the basis of conventional technology in recent years. The sensitivity of gas chromatography in pesticide multi-residue detection is improved by several orders of magnitude compared with the conventional method, and its application has made rapid progress at home and abroad. This capillary column with thick liquid film thickness and inner diameter can not only fill the capacity of the column, but also avoid adsorption or catalytic activation caused by the carrier, which is especially suitable for the determination of systems with low boiling point of solvents and high boiling point of detected substances [8].

2.2.3. **High performance liquid chromatography/liquid chromatography-mass spectrometry.** High performance liquid chromatography (HPLC) is suitable for analyzing organic compounds with high boiling point, which are volatile, unstable and easy to decompose when heated, large molecular weight and different polarities. At present, almost all carbamate pesticides in fruits and vegetables are determined by HPLC. Literature [9] has used this method to determine the residue of multi-effect pesticide in apples, and the recovery rate has reached the analysis requirements. In reference [10], 57 kinds of pesticide residues in vegetables and fruits were analyzed by LC-MS, and the recovery rate ranged from 70% to 100%.

2.2.4. **Enzyme inhibition method.** Enzyme inhibition method is a detection method which uses the phenomenon that the functional groups of enzymes are affected by certain substances, which leads to the decrease or loss of enzyme activity. It is mainly used for the detection of organic phosphorus pesticides and carbamate pesticide residues. Literature [11] used enzyme inhibition method to determine organophosphorus and carbamate pesticide residues in jujube. Literature [12], etc. analyzed the rapid detection method of enzyme inhibition rate of organophosphorus and carbamate pesticide residues in cowpea.

2.2.5. **Spectral technology.** Compared with chromatography, spectral technology has great application potential in pesticide residue detection. It can directly detect solid, liquid and gas samples, and has low requirements for sample pretreatment, little environmental pollution and fast analysis speed. For example, Fourier transform infrared spectroscopy can be used to determine pesticide residues simultaneously. Literature [13] studied the direct determination of pesticide residues in vegetables by mid-infrared attenuated total reflection spectroscopy, and put forward a feasible method for rapid detection of pesticide residues.

### 3. Application of pesticide residue analysis and detection technology in agricultural product food safety--Ultra performance liquid chromatography-tandem mass spectrometry

Seed coating agent is a kind of pesticide preparation, which is processed by pesticide technical drugs (insecticides, fungicides, etc.), some of which are also added with micro-fertilizer, hormone, film forming agent, dispersant, antifreeze and other additives. It can be coated on the surface of seeds directly or after dilution to form a protective film with certain strength and permeability [14]. The application of gas chromatography-mass spectrometry (GC-MS) to the determination of seed coating pesticides has been reported, but there is no report of multi-residue ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS/MS) for common seed coating pesticides.

In this paper, the pesticide residues of 8 kinds of coating agents in fruits, vegetables and grains were determined simultaneously by ultra-high performance liquid chromatography-tandem mass spectrometry, and the sample pretreatment method was optimized. In order to effectively reduce the interference of matrix, the standard curve was prepared by matrix extract and detected by monthly
UPLC/MS-MS-MS. The method is simple, rapid, high in recovery rate and low in organic solvent consumption. It can meet the requirements of detection technology of related seed coating pesticides at home and abroad.

3.1. Experimental part

3.1.1. Laboratory sample. All the samples needed in this experiment were purchased from supermarkets, which conformed to the sampling principle. The samples were crushed by a crusher and stored in a refrigerator at 4°C for use.

3.1.2. Instruments and reagents. Ultra-high performance liquid chromatography-tandem mass spectrometer; Type KQ-250 ultrasonic cleaner: Methanol; Acetonitrile; Water (deionized purified water). The purity of 8 kinds of coating pesticide standard materials was all over 97%. Prepare 1 000 mg/L standard stock solution with acetonitrile, and store it in the refrigerator at -18°C.

3.1.3. Sample extraction. Weigh 4.00 g of samples into a 40 mL plastic centrifuge tube with scale, add a small amount of methanol and water (1:1 by volume) solution to soak all samples, then let them stand for 35min, and then use the above methanol-water solution to make the volume to 22 mL. As the water content of vegetables and fruits is generally large, the above methanol-water solution can be directly used to make the volume to 22 mL; Samples of vegetables and fruits with very little water content can be treated as grain samples. The above sample solution was ultrasonically extracted for 35 min, then mixed evenly, and a small amount of extract was centrifuged at a rate of 15 000 r/min for 15 min, then filtered by 0.3μm microfiltration membrane to be detected.

3.1.4. Chromatographic conditions. Chromatographic conditions: chromatographic column: ACQUITY UPLC™ BEH C18, 1.8μm,2.4mm×55 mm; The mobile phase A: 4.5mmol/L ammonium acetate solution containing 0.2% formic acid; The mobile phase B is acetonitrile; The flow rate: 0.24mL/min; Column temperature is 350℃; Sample injection volume is 3.4μL; The temperature of sample chamber is 55℃. Gradient elution procedure is shown in Table 1:

| Time (min) | Flow mL/min | A% | B% | Linear type |
|-----------|-------------|----|----|-------------|
| 0         | 0.23        | 80 | 25 |             |
| 2.14      | 0.23        | 28 | 77 | 5           |
| 2.25      | 0.23        | 70 | 20 | 2           |
| 3.60      | 0.23        | 70 | 20 | 2           |

3.1.5. Mass spectrometry conditions. Ionization mode: ESI⁺ mode; Capillary voltage: 3.1kV; Ion source temperature: 120°C; Desolvent temperature: 350°C; Desolvent gas flow rate: 800L/h; Gas flow rate of cone hole: 55L/h; Photomultiplier voltage: 750V; Argon flow rate in collision chamber: 0.17L/h.

3.1.6. Quantitative analysis by Raman spectroscopy. In recent years, the chemometrics methods commonly used in Raman spectroscopy mainly include multiple linear regression, principal component regression, partial least square method, principal component analysis, artificial neural network and so on. In this paper, the multiple linear regression modeling method is mainly used. Multivariate linear regression (MLR) is also called inverse least square method, or P matrix method. In spectral analysis, the concentration matrix of samples with n calibration sets containing
components is $Y(n \times m)$, the spectrum is composed of $n$ samples and $p$ wavenumbers, the matrix is $X(n \times p)$, the regression coefficient matrix is $B$, and the concentration residual matrix is $E$, so the regression model of multi-component system is:

$Y = XB + E \quad (1)$

The least square solution is:

$B = (X^T X)^{-1} X^T Y \quad (2)$

It can be seen from formula (2) that as long as the concentration of some components in the sample is known and the spectral characteristic wavelength corresponding to the measured components is selected, the quantitative correction model can be established. The advantage of MLR is that for some uncomplicated systems, the concentration or content of the tested components can be calculated without considering the interference between components.

3.2. Results and discussion

3.2.1. Selection of sample pretreatment conditions. In the existing literature [15], organic solvents such as acetone, acetonitrile and ethyl acetate are mostly used to extract pesticide residues. In this experiment, the above solvents are used to extract the substances to be tested respectively. Through experiments, it is found that organic solvents cannot effectively extract benzimidazole compounds. Therefore, methanol was chosen as the organic solvent, and the extraction of target compounds with different proportions of water and methanol was studied, as shown in Figure 1.

![Figure 1](image-url)

**Figure 1.** Comparing the extraction effects of different proportions of water in the mixed solution, the order from No.1 to No.8 is carbenazim, thiabendazole, methomyl, thiamethoxam, pyriproxyfen, thiophanate methyl, carbofuran and metalaxyl.

The experimental results show that the volume ratio of methanol and water mixture is 1:1, and the extraction efficiency is the highest. At the same time, sample pretreatment steps are simplified, and
matrix interference is eliminated by this method with the help of MRM multi-reaction monitoring mode of tandem mass spectrometry [16].

3.2.2. Selection of instrumental analysis conditions

(1). Selection of chromatographic separation conditions. In this experiment, the ACQUITY UPLC™ BEH C18 column of Waters Company was used, and the influence of different ratio of mobile phase on the peak time and shape of each analyte was studied. The results show that different flow systems or proportions have a great influence on the ionization of the analyte. Adding 5.5mmol/L ammonium acetate into the mobile phase is helpful to eliminate the interference of sodium salt, while adding 0.2% formic acid is helpful to the formation of the parent ion peak of the analyte. Therefore, ammonium acetate and formic acid solution-acetonitrile are selected as the mobile phase in the experiment.

(2). Optimization of mass spectrometry conditions. Seed coating pesticide standard solutions with a concentration of 15μg/mL were used respectively, and the samples were injected directly. Through monitoring window observation, it is determined that the best position is when the response value of \([M + H]^+\) peak is the strongest. Fig. 2 shows the relationship between \([M + H]^+\) peak response intensity of methomyl and metalaxyl and cone voltage. The higher the peak response intensity, the higher the sensitivity and the lower the detection limit of mass spectrometry.

\[ \text{Figure 2. Effect of cone voltage on } [M + H]^+ \text{ peak intensity of methomyl and metalaxyl} \]

Under the selected conditions, the designated parent ions were scanned by sub-ions, and the collision energy and other parameters were optimized to obtain the fragment ions of each pesticide. Two or three sub-ions with strong response are selected as fragment ions detected by MRM mode.

(3). Linear relationship, recovery rate, precision and detection limit. In order to eliminate the influence of ion effect brought by matrix on quantitative determination results, a series of standard working solutions should be prepared with blank sample extracts, and matrix standard curves should be drawn for quantification. Compared with fruits, vegetables, wheat and other samples, soybean matrix is a sample with complex composition, so soybean is selected as the representative sample, and the linear relationship, recovery rate, precision and detection limit are analyzed and determined.
The determination was carried out under certain chromatographic and mass spectrometry conditions, and the standard curve of soybean matrix was drawn with the peak area versus concentration of each component. The results showed that 8 kinds of coating pesticides had good linearity in the concentration range of 1.4-2.8μg/L, which could meet the needs of quantitative analysis.

3.2.3. Sample determination

(1). Qualitative analysis. Sample solutions were determined by liquid chromatography-tandem mass spectrometry. If the retention time of the detected sample chromatographic peak is consistent with the corresponding analyte in the corresponding matrix standard solution, and the abundance ratio of the selected monitoring ions is consistent, it can be judged that the pesticide exists in the sample. According to the literature [17], the allowable deviation range of relative abundance is shown in Table 2.

| Relative abundance (%) | Allowable variation (%) |
|------------------------|------------------------|
| ≥50                    | 20                     |
| 20-50                  | 25                     |
| 10-20                  | 30                     |
| ≤10                    | 50                     |

(2). Quantitative analysis. In this method, LC-MS/MS adopts external standard calibration curve method for quantitative determination, which should ensure that the response values of pesticides in the tested samples are within the linear range. The method was applied to the determination of the residues of the above eight pesticides in 25 actual samples including apples, radish tomatoes, zucchini, Chinese cabbage and corn. A radish sample was found to contain carbendazim 2.1μg/kg and pyriproxyfen 2.4μg/kg. A zucchini sample contains carbendazim 3.5μg/kg; The carbendazim content in Chinese cabbage was between 2.8-4.5μg/kg.

4. Summary

With the vigorous development of modern technology, new technologies and equipment will be continuously improved and developed in pesticide residue analysis, and the technical analysis of pesticide residues is heading for the development direction of simple operation, high speed, high efficiency and high accuracy. In this study, UPLC/MS-MS was used to establish a highly sensitive and rapid method for simultaneous determination of pesticide residues in 8 kinds of coating agents in agricultural products. The method introduced in this paper can meet the detection requirements after being verified by matrix addition and recovery test and a large number of actual samples. Therefore, this method can be used for rapid and highly sensitive detection and analysis of multi-component pesticide residues in seed coating agents in fruits, vegetables, grains and other agricultural products. In the future development, with the continuous application of tandem mass spectrometry, this technology will be rich in higher sensitivity, new ion source technology, anti-interference ability and anti-pollution ability, etc., and play an important role in pesticide residue technology.

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