Application and Research of Analytical Chemistry in Food Inspection

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Abstract. Analytical chemical methods in food production are widely used in food safety testing. High performance liquid chromatography is a type of chemical analysis method that is also commonly used. To this end, the paper will use the application of high-performance liquid chromatography in food detection as an entry point to study the application of analytical chemistry in food detection. High performance liquid chromatography (UPLC) is characterized by stability, ease of use, variable sensitivity and selectivity. It shows better chromatographic resolution, more sensitive analysis, shorter analysis time, and can reduce solvent consumption. This article provides a brief overview of the application of UPLC in the rapid detection of dairy additives, food additives, contaminants, mycotoxins, and illegal chemical additives.

1. Introduction

"The people put food first, food first", food safety is the focus of consumers' attention, which directly affects consumers' health. Food safety is also an interdisciplinary field that specifically discusses ensuring food hygiene and food safety, reducing hidden diseases, and preventing food poisoning during food processing, storage, and sales. Therefore, food safety is very important. Food safety includes both production safety, business safety, results safety, and process safety, both current safety and future safety. Food safety means that the food is non-toxic and harmless, meets nutritional requirements, and does not cause any acute, subacute, or chronic harm to human health. In recent years, despite the rapid development of the food industry and the continuous improvement of food safety management, there are still many problems in my country's food safety. Clenbuterol incident, melamine incident, Sudan red incident, malachite green incident and the current African swine fever incident have caused consumers to worry about the food safety environment. Therefore, the rapid detection of food safety hazards has become a guarantee for food safety the important task is to provide a strong guarantee for consumers' health. The use of efficient, reliable, and rapid analysis and detection technology to detect illegal additives or harmful substances in food, and at the same time to judge according to the corresponding national standards, severe penalties for unqualified food and its manufacturers, is of great significance to ensure food safety.

Chromatography, also known as "chromatographic analysis", "chromatographic analysis", is a separation and analysis method, which has a very wide range of applications in analytical chemistry, organic chemistry, biochemistry and other fields. Chromatography uses the selective distribution of different substances in different phase states, eluting with the mixture in the
mobile phase relative to the stationary phase. Different substances in the mixture will move along the stationary phase at different speeds, and finally achieve the effect of separation. Chromatography technology has many advantages: high separation efficiency, wide application range, fast analysis speed, low sample consumption and high sensitivity. The most widely used in food analysis is high performance liquid chromatography (HPLC). Let L denote the total resolution, and the separation efficiency of the liquid chromatography column is expressed by the following formula:

\[ R = \frac{1}{8} \left( \frac{a - 1}{a} \right) \left( \frac{k}{k+1} \right)^n \]  

In the formula, \( k \) represents the distribution coefficient, which is the ratio of the amount of the sample in the stationary phase to the amount of the sample in the mobile phase. \( a \) represents the separation factor, which is the ratio of the partition coefficients of the two components.

2. Application experiment of high-performance liquid chromatography (analytical chemistry) in food additives

Food additives can be divided into natural food additives and chemically synthesized food additives according to their sources. Currently commonly used chemically synthesized food additives, which may have some toxicity, may also be converted into other carcinogenic, teratogenic and mutagenic toxic substances in food. In addition, illegal additives (cyclamate, lemon yellow, sunset yellow, carmine) are flooding the market, causing greater safety risks. Therefore, it must be regularly monitored. The analysis of food additives by HPLC is a fairly mature method, such as gradient elution analysis of synthetic colorants in food, and isocratic analysis of food preservatives (benzoic acid, sorbic acid, dehydrocafestol acid, p-hydroxybenzoic acid) Esters, sweeteners (sodium saccharin, glycyrhrizin, etc.), antioxidants, etc. The paper uses colorants as an entry point to study the application of high-performance liquid chromatography in food additives [1].

2.1. Materials and methods

2.1.1. Materials and equipment. High performance liquid chromatograph, polyamide SPE solid phase extraction cartridge 500mg/3mL, solid phase extraction instrument, nitrogen blowing instrument, vortex instrument. Methanol, chromatographically pure; formic acid, analytically pure; ammonia, analytically pure; ammonium acetate, chromatographically pure; commercially available dry red wine. Lemon yellow, amaranth red, carmine red, sunset yellow, alluring red, bright blue, purity> 97.5%. Standard stock solution: Weigh 0.1g (accurate to 0.1mg) of 6 kinds of synthetic colorants into a 100mL volumetric flask, dissolve it with 90mL of water, dilute to the mark with methanol, and prepare a standard solution with a mass concentration of 1000mg/L; mix Standard working solution: Measure each standard stock solution from 1mL to 100mL volumetric flask, make up to volume with water, and prepare a mixed standard working solution with a mass concentration of 10mg/L.

2.1.2. Test method. For general samples, such as cakes, beverages, pickles, meat products, etc., add about 10 times the volume of petroleum ether to remove oil, repeat this step 3 times. Add about 8 volumes of saturated sodium chloride solution for ultrasonic extraction for about 20 minutes, then add
2 volumes of methanol for purification, centrifuge, take the supernatant, pass the membrane, and distract on the machine.

For foods with heavy interference such as wine, sauce, candy and other substrates, it needs to be purified by polyamide column. Take 2g of the sauce sample and dilute it to 10mL with water. After crushing the candy, dissolve it in warm water, bring the volume to 10mL, and centrifuge to take the supernatant. Wine samples can be directly subjected to solid phase extraction. Activate the small polyamide column with 3mL methanol and 3mL water in turn, take 5mL sample and put it on the column overnight, control the flow rate at 1.5mL/min ~ 2mL/min, and use 3mL methanol-formic acid-water (volume ratio 2:2:6) 3. Rinse the solid-phase extraction column with 3mL of water, discard all the effluent, vacuum dry and elute with 7mL of 10% ammonia-methanol solution, collect the eluent, blow dry at 40°C with nitrogen, add 1mL of water to mix, filter membrane After filtration, liquid phase analysis was performed [2].

2.2. Research results

2.2.1. Chromatographic detection conditions. Although the six synthetic colorants described in the study have a maximum absorption at 254nm, and the GB/T5009.35-2003 liquid chromatography signal is also selected at 254nm, but when performing specific sample detection, this wavelength is not the specific absorption wavelength of the colorant. Many substances absorb at this wavelength, thus forming a large number of interference peaks, which raises the chromatographic baseline, see Figure 1. The maximum absorption wavelength in the visible light region is selected for signal collection. Although the signal response of the standard product is reduced, the interference of the above impurity peaks can be effectively avoided during sample detection, but the sensitivity is greatly improved.

![Figure 1. Liquid chromatogram of negative samples at 254nm wavelength](image)

2.2.2. Quantitative analysis. Dilute the mixed standard solutions of lemon yellow, amaranth, carmine, sunset yellow, allure red, and brilliant blue into standard solutions of different concentrations, and draw a standard curve with the peak area as the ordinate and the mass concentration as the abscissa under the chromatographic conditions. The results showed that the chromatographic peak areas and corresponding concentrations of lemon yellow, amaranth red, carmine, sunset yellow, allure red, and brilliant blue showed a good linear relationship. The linear correlation coefficients exceeded 0.99, and the detection limit was 0.05 mg/kg. The test was carried out in accordance with the above method. The recovery rate test was performed at the three detection levels of low detection limit, 2 times detection
limit and 10 times detection limit, and each recovery test was repeated 6 times. The results are shown in Table 1. It can be seen from the table that the method has good reproducibility and the recovery rate is between 86.2% and 97.5%.

Table 1. Solid phase extraction-liquid chromatography method for detecting the recovery rate of synthetic colorants in food

| Scalar | Lemon yellow | Amarant | Carmin | Sunset yellow | Temptation red | Bright blue |
|--------|--------------|---------|--------|--------------|----------------|------------|
|        | average value |         |        |              |                |            |
| 0.0    | 0.0454       | 0.0431  | 0.0442 | 0.0437       | 0.0438         | 0.0471     |
|        | Recovery rate/% |        |        |              |                |            |
| 0.0    | 90.77        | 86.21   | 88.36  | 87.48        | 87.6           | 94.12      |
|        | standard deviation |        |        |              |                |            |
| 0.0    | 0.56         | 0.82    | 0.94   | 1.03         | 0.92           | 0.83       |
|        | average value |         |        |              |                |            |
| 0.0    | 0.0933       | 0.0907  | 0.0934 | 0.0941       | 0.0908         | 0.0912     |
| 0.1    | Recovery rate/% |        |        |              |                |            |
| 0.1    | 93.35        | 90.68   | 93.44  | 94.12        | 90.78          | 91.15      |
|        | standard deviation |        |        |              |                |            |
| 0.1    | 0.33         | 0.61    | 1.74   | 0.52         | 0.4            | 0.64       |
|        | average value |         |        |              |                |            |
| 0.1    | 0.487        | 0.478   | 0.48   | 0.48         | 0.47           | 0.471      |
| 0.5    | Recovery rate/% |        |        |              |                |            |
| 0.5    | 97.5         | 95.51   | 96.05  | 96.07        | 94.01          | 94.22      |
|        | standard deviation |        |        |              |                |            |
| 0.5    | 0.12         | 0.28    | 0.21   | 0.27         | 0.33           | 0.46       |

2.3. Research results

The test used trans high-performance liquid chromatography to detect artificial colorants in food. After the samples were extracted and purified by solid phase extraction, the interference of impurities in wine, sauce and other samples was significantly removed. The gradient of methanol-ammonium acetate solution eluted. The maximum absorption wavelength in the visible light region is selected for signal collection, which effectively avoids the interference of impurity peaks and improves the detection sensitivity. The method has good reproducibility and is suitable for daily rapid testing in conventional laboratories.

3. High-performance liquid chromatography (analytical chemistry) detection experiment in biological toxins

The HPLC method uses an immunoaffinity column or C18 column to extract, purify, concentrate, and separate aflatoxin M1 in the sample, and then detects it by an HPLC tandem detector. The immunoaffinity column utilizes the specific adsorption characteristics of antigen and antibody. The affinity column can only specifically and selectively adsorb aflatoxin, while other impurities pass through the column smoothly, and then use the eluent to elute the aflatoxin Down. This method greatly simplifies the sample preparation process, and can use high-performance liquid chromatography to simultaneously quantify and characterize aflatoxin M1, has the advantages of simple operation, high sensitivity, good stability and so on, and has been widely used.

3.1. Materials and methods

3.1.1. Materials and equipment. The test sample is a brand of infant milk powder. Shimadzu LC-20AT high-performance liquid chromatograph with RF-20A fluorescence detector; KQ-500 ultrasonic
cleaning machine, Kunshan Ultrasonic Instrument Co., Ltd.; centrifuge, speed 7000 r/min; nitrogen blower; aflatoxin M1 immunity Affinity column, Beijing Teller Technology Co., Ltd.; aflatoxin M1 standard solution, purity ≥98%.

3.1.2. Method. Chromatographic conditions. Chromatography column: C18 (250mm×4.6mm, 5μm); mobile phase: acetonitrile + water (25+75); flow rate: 1.0ml/min; column temperature: 40℃; fluorescence detector: 365nm excitation wavelength, 435nm emission wavelength; Injection volume: 10μl. The standard solution stock solution (concentration: 0.5 μg/ml) was appropriately diluted with 10% acetonitrile to prepare standard solutions with concentrations of 2.0, 4.0, 6.0, 8.0, and 10.0 ng/ml, which were injected and analyzed. The results showed that Aflatoxin M1 has a good linear relationship in the concentration range of 2-10ng/ml. The linear equation is as follows:

\[
Y = aX + b \\
a = 2.4e - 0.03 \\
b = 0.71 \\
R^2 = 0.9918475 \\
R = 0.9951945
\]

Connect the immunoaffinity column under a 50ml glass syringe, add 50ml of accurate milk sample to the glass syringe, connect it with a vacuum pump, and control the sample to flow through the column at a stable flow rate of 2 ~ 3ml/min. Inject 10ml of pure water to rinse the immunoaffinity column. Discard all effluent and drain the affinity column. Add 4.0ml of chromatographic grade acetonitrile for elution, collect all the eluent in a glass test tube, and then blow nitrogen to near dryness at 30℃, and bring the volume to 1ml with water for LC determination.

3.2. Results

3.2.1. Working curve. Prepare a standard working solution of aflatoxin M1 with concentrations of 2.0, 4.0, 6.0, 8.0, and 10.0 ng/ml for analysis. The standard curve is shown in Figure 2.

3.2.2. Detection limit. A series of standard solutions with different concentrations were prepared by appropriately diluting the standard solution stock solution, and the samples were analyzed. The signal-to-noise ratio of 3:1 was used as the detection limit, and the detection limit of aflatoxin M1 was 0.4 μg/ kg. Take the standard solution and inject 6 times continuously. The results show that the RSD of
aflatoxin M1 is 2.60%, and the precision is good. Six parallel samples of each sample were operated according to the method under the preparation of the test solution. The results showed that the RSD of aflatoxin M1 was 6.0%, and the repeatability was good [3].

3.2.3. Recovery rate test. Take 10g of aflatoxin M1 negative milk powder, add 0.04ml of aflatoxin standard solution with a concentration of 0.5μg/ml, and operate according to the method under the preparation of the test solution. The test results are shown in Table 2.

| Background value // ng | Scalar // ng | Measured value // ng | Recovery rate // % | RSD // % |
|------------------------|-------------|----------------------|-------------------|---------|
| 0                      | 20.00       | 19.29                | 96.4              | 0.50    |
| 0                      | 20.00       | 19.24                | 96.2              |         |
| 0                      | 20.00       | 19.44                | 97.2              |         |
| 0                      | 20.00       | 19.46                | 97.3              |         |
| 0                      | 20.00       | 19.26                | 96.3              |         |
| 0                      | 20.00       | 19.28                | 96.4              |         |

3.3. Results discussion
This test established a method for the determination of aflatoxin M1 in dairy products by high-performance liquid chromatography. This method is based on the specificity of the immunoaffinity column for the adsorption of aflatoxin, and uses the good separation effect of HPLC to achieve a sensitive and accurate detection effect; at the same time, this method is simple and easy, and the stability and reproducibility of the detection results are both Better, suitable for the detection of large quantities of samples.

4. High performance liquid chromatography (analytical chemistry) in pesticide residue test
Sulfa drugs are a class of synthetic antibacterial drugs that are widely used in animal husbandry and aquaculture. They play a significant role in reducing animal morbidity, mortality, improving the quality of aquatic animals and promoting livestock growth. The study found that after taking antibiotics, a small part remained in the human body and animals, and most of them were excreted in the form of original drugs and metabolites through the faces and urine of patients and animals, and entered the environment, affecting the soil, surface and groundwater, and various organisms. Harmful, resulting in a large number of drug-resistant strains, and ultimately cause potential harm to humans through bioaccumulation and food chain transmission.

4.1. Materials and methods
Accurately weigh 10 mg of sulfamethazine (SAD), sulfadiazine (SDZ), and sulfamethazine (SM2) standards, dissolve them in acetonitrile and dilute to a 10 mL volumetric flask. The concentration is 1 Keep the mg/mL stock solution in the refrigerator at 4°C until use. When in use, the mobile phase is gradually diluted to prepare a series of working solutions. After all samples have undergone micro-extraction of the dispersion, the resulting extract must be filtered with a 0.2 μm filter before injection. Filter the water sample through a 0.45μm filter membrane, weigh the homogenized chicken liver sample Sg in a 50 mL pointed bottom stopper centrifuge tube, add 10 mL of 50 mM phosphoric acid: acetonitrile solution with a volume ratio of 30:70, 3.5g Anhydrous sodium sulphate, ultrasonic extraction for 15 min, centrifugation at 4500 r/min for 8 min. Transfer the supernatant (acetonitrile extraction layer) to a centrifuge tube containing 150 mg C18, 300 mg neutral alumina and 200 mg amino adsorbent powder, vortex for 1 min, and centrifuge at 4000 r/min for 5 min. 0.45 gm filter membrane, take 1 mL of the filtrate as a dispersant for the micro-extraction of the dispersion liquid in the next step [4].
4.2. Research results

Existing research on dispersion liquid-liquid microextraction generally extracts the target analyte from the liquid sample, because the liquid sample is beneficial to the formation of the ternary emulsion system during the dispersion liquid-liquid microextraction. The most critical entry point for applying liquid-liquid microextraction to the solid sample pre-treatment is to first extract the target analyte from a complex solid matrix, and the choice of extractant must also meet the subsequent dispersion liquid-liquid microextraction process. Conditions make it a complete and effective process. In this paper, the method of dispersive solid phase extraction (DSPE) was used to extract and purify the sample, combined with DLLME for extraction and enrichment pre-treatment, and the pre-treatment method of dispersion liquid microextraction was successfully applied to veterinary drug residues in chicken liver Analysis.

The target analyte—sulpha drugs usually contain primary amino groups and sulphonamide groups and are acid-base amphoteric, and can be dissolved in acidic or alkaline solutions. Most sulpha drugs have high polarity and usually use organic solvents Extract it. The more commonly used extractants mainly include acetonitrile, methylene chloride and their mixed solvents. Studies have shown that adding a small amount of acid solution (phosphoric acid) to acetonitrile can improve the extraction efficiency of drugs in various matrices. In this paper, the effect of the concentration of phosphoric acid added to acetonitrile on the extraction effect was studied, and 25 mM, 50 mM, 75 mM, and 100 mM phosphoric acid were selected for extraction experiments. The results showed that when 50 mM phosphoric acid was added, the extraction effect was significantly improved. Therefore, 50 mM phosphoric acid was added to acetonitrile for extraction. Under the condition that the optimal phosphoric acid concentration is fixed at 50 mM, the volume ratio of phosphoric acid in acetonitrile is optimized. The results are shown in Figure 3: As the volume ratio of phosphoric acid increases, the extraction efficiency increases, and finally at 50mM phosphoric acid: The best extraction efficiency is achieved when acetonitrile is 30:70. So in the end, 50 mM phosphoric acid: acetonitrile (30:70) was selected as the extractant. The experimental results show that 50 mM phosphoric acid: acetonitrile (30:70) solution was used as the extraction solvent for chicken liver samples [5].

![Figure 3. Effect of phosphoric acid efficiency at different volume ratios](image-url)
The experimentally optimized DSPE-DLLME method was used to analyse chicken liver samples purchased from supermarkets and poultry markets. The chicken liver samples were homogenized, and 5g each was taken for pre-treatment under optimized DSPE-DLLME conditions. High-performance liquid chromatography detection showed that the contents of the four sulphonamides in these two chicken livers were lower than the detection limit of this method. The two chicken liver samples were spiked and recovered at two levels, and the determination was repeated six times, and the average value was taken to verify the recovery rate and stability of the method. The recovery rate of standard addition is between 70.2%-91.4%, and the relative standard deviation is less than 5.35%. The actual sample spiked chromatogram is shown in Figure 4.

4.3. Discussion
In this experiment, a pre-treatment method combining dispersive solid phase extraction and dispersing liquid microextraction was established, and reversed-phase high-performance liquid chromatography was used to determine sulfamethanoyl, sulfathiazole, sulfamethazine, and sulfamethazine in different chicken liver samples. Four methods of sulphonamide veterinary drug residues. The method has the advantages of simple operation, fast, good reproducibility, high sensitivity, less sampling volume, wide linear range, less amount of organic solvent, and low price of materials and drugs. In addition, the liquid-liquid micro-extraction method commonly used in the analysis of liquid samples is improved to be applicable to the analysis of solid samples, which expands the application range of the method and can be used for the detection of actual samples [6].

5. Conclusion
With the rapid development of the food industry, food additives have greatly promoted the technological progress and technological innovation of the food industry, and have become an indispensable part of the modern food industry. With the increase in the variety of food additives, the safety of its use will surely attract more and more attention. This is bound to promote the continuous development of its detection methods. High-performance liquid chromatography has become an important method for the detection of food additives due to its simple operation, accurate results, rapid analysis, good reproducibility, and the ability to measure multiple components simultaneously. With the continuous development and progress of science and technology, high-performance liquid chromatography instruments are also constantly updated and developed, and the combination of various detection technologies is becoming more and more common, including liquid chromatography techniques.
(LC) and mass spectrometry (MS) and nuclear magnetic Integration of resonance spectrometer (LC-MS-NMR), gas chromatography-liquid chromatography combined (GCHPLC), solid phase microextraction-liquid chromatography combined (SPME-HPLC), focused microwave assisted extraction-liquid chromatography The use of (FAME-HPLC), etc., has greatly expanded the scope of application of HPLC and improved the level of substance detection. In the future, HPLC will play an important role in the field of food additive inspection and escort the healthy and sustainable development of the food industry.

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