Research of food additives and their detection technology

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Abstract: Given the critical role food additives play in both public health and biomedicine, it's essential to prevent microbial contamination in food processing, which necessitates the careful use of additives to safeguard health. In order to avoid microbial contamination during food preparation and consumption, manufacturers need to add food additives. However, the overdose of food additives would affect health. Food additives need to be detected and controlled. Four commonly used detection methods, namely high-performance liquid chromatography, capillary electrophoresis, thin layer chromatography and electrochemical analysis, and two combined detection methods, namely LC-MS/MS and GC-MS/MS, are listed in this paper. High separation efficiency, selectivity, and sensitivity are all advantages of high-performance liquid chromatography. But the analysis time is longer than that of gas chromatography. The operation of thin-layer chromatography is simple and the color is convenient. The combined detection techniques of LC-MS/MS and GC-MS/MS were compared. This article summarized the advantages and disadvantages of the commonly used detection method and combined detection methods. It is concluded that the precision of the combined method is higher, but the equipment and process of detection are complicated.

1. INTRODUCTION

In order to avoid microbial contamination during food preparation and consumption, food additives need to be added. These additives not only serve technical purposes such as pH control, viscosity, stability, and uniformity but also carry biological functions that are increasingly relevant in the field of biomedicine, including their role in stability, degradation process inhibition, and extending shelf life, which can have direct implications for pharmaceutical preservation and delivery systems. [1][2].

The Food Agriculture Organization and the European Union adopt food additives classification based on the types of foods that may be added [3]. This classification is crucial for ensuring the safety and efficacy of these additives in various applications, including those in biopharmaceuticals where the integrity and bioavailability of active compounds are paramount. Food additives are classified into 25 categories based on their commercial application, with around 230 distinct chemicals. Their use in the processing, packing, and transporting processes is not only to increase product quality, durability, and stability but also to modify color, odor, and flavor qualities, which are essential considerations in the formulation of pharmaceutical products for patient compliance and therapeutic efficacy.

However, some additives can promote the toxicity of consumers and cause bad flavor in food, namely acidity regulator, color, antioxidant and preservative. The food business needs adhere to national and international quality standards, establish the circumstances under which food additives may be used, and maintain food safety via stringent quality control. The use of food additives necessitates strict food safety policies, as some additives may endanger consumers' health. The cumulative consumption of food additives has been shown to promote allergies, diabetes, obesity, and metabolic problems [4]. Therefore, each country has its own set of laws that encourage proper quality control in industrialized foods and identifies and quantifies food additives used or created in industrial processes. For illegal food additives, which are used to cover up the degradation process or insufficient operating procedures in some industries, they also need to be carefully monitored.

2. RELATED WORKS

The hydrogen flame ionization detector (FID) in gas chromatography has high selectivity for several common antioxidants such as BHA, BHT, TBHQ, etc. Zong Wanli used WBI injection port and capillary chromatography column J&WDB1701 (30m) × 0.53mm × one μ m) The FID detector detects the content of three antioxidants (BHT, BHA, TBHQ) in instant noodles. Linear range BHT and BHA are 10-200 μ G/mL, TBHQ between 25-500 μ G/mL. The method is fast, simple, and has good reproducibility. The average recovery rate is over 90%, and the RSD is all less than 3.0%. Farajzadeh et al. also detected the three antioxidants mentioned above in milk using a FID detector, and obtained detection limits and quantification limits (LOQ) of 0.76-1.16ng/mL and 2.66-3.96ng/mL, respectively. GC-MS/MS has higher sensitivity and stronger anti-interference ability than single-stage mass

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spectrometry, making it a good alternative to high-resolution mass spectrometry. Zhang et al. developed an analytical method based on gas chromatography-triple quadrupole tandem mass spectrometry to detect 23 spices in tobacco. Firstly, dichloromethane was used as the extraction solvent for ultrasound assisted extraction. Combined with GC-MS/MS technology, satisfactory results were obtained for the analysis of tobacco samples. The linear range of 23 spices ranges from 0.2 to 500.0 μ Between g/L, the correlation coefficient R2 ≥ 0.9963, and the detection limit and quantification limit of the method are 0.1-2.0, respectively μ G/L and 0.4-6.0 μ Between g/kg, the recovery rates of three different levels of spiked (LOQ, 2LOQ, 4LOQ) ranged from 61.2% to 93.8%, and the relative standard deviation (RSD) (n=6) was less than 7.8%. Shen extracted four essence from infant formula with Oasis HLBC18-60mg solid phase extraction column, and detected them with GC-MS/MS, and the LOQ (LOQ, S/N=10) of these four essence was 10 μ G/kg, the average spiked recovery rate measured at different concentrations is between 82.8% -107.5%, with a relative standard deviation (RSD) of ≤ 8.9%. This technology has high sensitivity and precision in detecting infant formula foods, while also providing potential possibilities for detecting other foods.

3. THE NECESSITY OF FOOD ADDITIVES DETECTION

Food additives needed to be added, which ensure the food safety. However, the overdose of food additives would affect human health. The detection of food additives was necessary.

Sweeteners are food additives in the modern food processing industry. Excessive use of aspartame and saccharin can cause harm to the human body. Ibrahim I. Gosadi investigated a sample of 302 Saudi diabetic patients and found that when asked about patients' attitudes toward artificial sweeteners, only 25% of the samples agreed that their use helps reduce calorie intake, while 35% believed that artificial sweeteners are damaging to the health [5].

The excessive melamine and clenbuterol in food would cause the harm to the human body, the local government and country subsequently formulated policy on melamine and clenbuterol, and made strict regulations on the use and detection. In 2008, there was a melamine-contaminated milk powder incident in China, resulting in a large number of infants and young children with urinary tract stones. Some of these youngsters developed renal problems and even died [6]. Studies have found that melamine is used to synthesize melamine formaldehyde resins for the production of laminates, coatings and molding compounds, such as tableware and kitchenware [7]. Nitrogen is released as a flame retardant during combustion or combustion when mixed with resin. Melamine's high nitrogen concentration made it a cheap and efficient non-protein nitrogen source for ruminants as early as the 1950s.

Food safety accidents caused China and the world to pay much attention to food safety issues. In order to avoid similar incidents, China has enacted a slew of food-safety measures. The Ministry of Health's Health Inspection Bureau issued a notification on the management restriction of melamine in dairy products, stipulating a nationwide standard of up to 2.5 mg/kg in liquid milk, milk powder and any other item having more than 15% milk. Simultaneously, a strong food safety law was formulated and a food safety system was established [8].

Clenbuterol is commonly utilized in the therapeutic therapy of people and animals as a bronchodilator and decongestant [9]. Clenbuterol, on the other hand, has been misused as a growth promoter for livestock, reducing fat accumulation and increasing muscle mass [10]. According to a 2013 research, clenbuterol buildup in animals and milk can transfer to humans through the food chain and induce food poisoning symptoms such as heart palpitations and muscle tremors [11]. Therefore, many countries have strictly prohibited the use of clenbuterol as a feed additive for food animals [9]. Maximum residual limits for Clenbuterol in milk, muscle, liver, and kidney have been defined by the FAO at 0.05 g/l, 0.2 g/kg, and 0.6 g/kg, respectively.

Synthetic food additives have rapidly supplanted natural food additives, resulting in a slew of difficulties such as food additive misuse, overuse, and even toxicity. Obviously, food additives can provide people with a lot of sensory pleasure and commercial convenience, but they can also pose a risk to their health. As a result, quantitative examination of food additive levels is extremely important [5].

4. DETECTION METHOD

4.1. High performance liquid chromatography (HPLC)

HPLC employs liquid as the mobile phase and injects mixed solvents of different polarities and different proportions into the chromatographic column through a flow pump, so that different components are separated in the chromatographic column and enter the detector for detection.

Box-Behnken is designed to optimize HPLC conditions and simultaneously determine five additives and caffeine in soft drinks. Inertsil OctaDecylSilane- (ODS-) 3V reverse phase chromatography column (250 × 4.6 mm, 5 μm) is used as the stationary phase. Recovery rate ≥ 95.75%, the best pH value of this method is 6.0, the flow rate is 1.0 mL/min, and the flow ratio is 95%. The limit of detection (LOD) and limit of quantification (LOQ) were 0.10–0.19 μg/mL and 0.33–0.63 μg/mL, respectively. This approach was used effectively to evaluate the presence of five food additives and caffeine in soft drinks at the same time. Using HPLC, an efficient, precise, and dependable approach was created. It is concluded that small changes in the flow ratio have a direct effect on the separation. The research provides a sensitive and effective approach for detecting potassium sorbate, sodium benzoate, carmine, allure red, ponceau 4R and caffeine in soft drinks [12].

Sun and others have researched a fast and sensitive analysis analytical technique for determining different food additives in beverage samples that combines high

performance liquid chromatography with diode array detection (HPLC-DAD) and chemometric methodologies. Although the peak overlap and variable interference in the actual beverage, the chromatogram and spectral dimensions, the second-order calibration approach based on the linear alternating triplet decomposition (ATLD) algorithm can be used. The detection limit for all additives is 1.40-165.1 ng/ml, while the quantification limit is 4.20 500.2 ng/ml. The recovery rate of standard additives is 87.3 ~ 103% (except the gourmeters), and the RSD is less than 10.2% [13].

When compared to the findings produced by the traditional HPLC approach, the HPLC-DAD method was faster, more sensitive, and adaptable, and it can be used to evaluate food additives and monitor quality in a variety of complicated beverages. Figure 1 shows a schematic representation of the HPLC analytical technique for food additives, which consists of a solvent supply system, a sample system, a separation system (chromatographic column), a detection system, and a data processing and recording system. Before HPLC analysis, it should check whether it is a suitable chromatographic column and mobile phase, and wait for the column to reach equilibrium and finally discharged with the eluent.

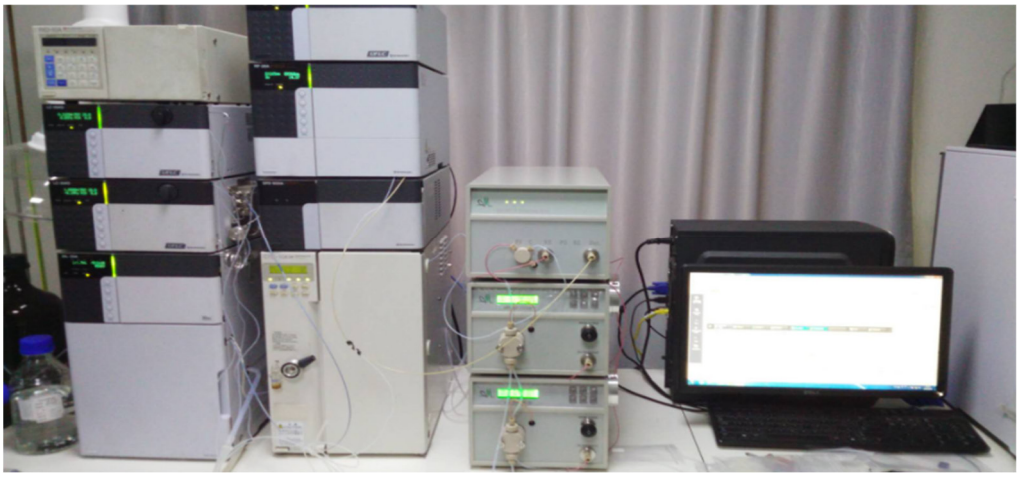

Figure 1. The analytical method of HPLC for detection of food additives

4.2. CE capillary electrophoresis

The principle of capillary electrophoresis is that the elastic quartz capillary is used as the separation channel, and the driving power is a high-voltage direct current electric field. Differences in mobility and distribution behavior of the components in the sample allow for separation.

L. Del Giovine and A. Piccioli Bocca researched a capillary electrophoresis technique to analyze the same food coloring in ice cream. The capillary column used is 50 cm×70 lm I.D., and the electrophoresis buffer is a 25 mM sodium phosphate and 25 mM sodium borate pH=8 combination. The results demonstrated that the three dyes were separated in a short period of time. The advantages of CE method were fast, sensitive and simpler than previous analysis techniques. The actual cost of analysis is low and it is the best initial screening tool. This method outperforms HPLC in terms of versatility, cost-effectiveness, and full automation for the detection of colors in ice cream. CZE, on the other hand, only detects three synthetic colours at low amounts in handmade and industrial ice cream and popsicles [14].

In order to execute rules governing the quantities of additives permitted in food, efficient technologies for extracting and analyzing these chemicals are required. Paula Jane Vickers and others used capillary electrophoresis to carry out research on the detection method of tartrate additives in food. A neutral polyacrylamide-coated capillary, a high ionic strength mobile phase, and reverse EOF are used in the experiment. The detection limit is 10mg/ml. The electrolyte column adjustment time is 4 minutes. Studies have proved that capillary electrophoresis is suitable for the successful separation and detection of appropriate levels of tartrate in the spiked sample matrix. The method has good reproducibility, can well separate matrix components and tartrate, and accurately analyze the spiked concentration of tartaric acid. However, for jam, further research on extraction is needed. The losses were most likely caused by the baking process, according to samples of Madeira cakes and digestive biscuits. For this sort of study and long-term operating repeatability, it is advised that tests on the service life of polyacrylamide-coated chromatographic columns be conducted in the future [15].

Capillary electrophoresis is an efficient, fast method, and the cost is very low. However, due to the small diameter of the capillary, the optical path is too short, resulting in low sensitivity.

4.3. Electrochemical analysis method

The discipline of examining the occurrence of a charged interface established by two types of conductors and the changes that occur on it is known as electrochemistry. Batteries or high-voltage electrostatic discharge can be used to interact with electricity and chemical processes.

Jorge Hoyos-Arbeláez and colleagues conducted research on the antioxidant activity of food and drinks using electrochemical analysis. The study focuses on the four most often used electrochemical procedures (cyclic voltammetry, differential pulse voltammetry, square wave voltammetry, and chronoamperometry). Some strategies based on synthetic free radical capture are used in the study. According to research, electrochemical techniques have been utilized to mine AC in various food and beverage samples. The use of costly reagents in spectrophotometry is harmful to the environment, the reaction time is unpredictable, the sample pretreatment time is lengthy, and the accuracy and sensitivity are low. Electrochemical technology is becoming a substitute. The electrochemical method has excellent correlation with other analytical methods. The advantages of electrochemical technology were fast, simple, economical, and highly sensitive. It can also be applied to multiple methods in the same equipment. Therefore, it is easier to obtain diverse information [16].

Synthetic colorants are widely utilized in the food and beverage industries, particularly in drinks. Excessive use of synthetic colorants may be hazardous to one's health. Yun Yang et al. Carried out electrochemical analysis to determine the presence of synthetic food colorants in drinks. This article compares the characteristics of three kinds of materials (precious metal nanomaterials, carbon materials and polymer materials), and discusses the future research direction of this field. From 2010 to 2018, the design of the sensor was mainly based on carbon materials. Carbon materials have been employed in the electrochemical detection of synthetic colorants, especially in the design of carbon-based nanocomposites. The electrochemical analysis method has the advantage of being simple and fast. Molecular imprinting technology can detect synthetic colorants more specifically, but the entire preparation process is cumbersome. At present, synthetic colorant sensors can detect at most two beverages at the same time, but beverages usually contain multiple colorants. Therefore, the future research direction is to construct sensors that can detect multiple colorants at the same time, and to improve the stability, specificity and repeatability of electrochemical sensing [17].

The electrochemical analysis method has high accuracy and wide measurement range; the instrument is relatively simple, the price is low, the debugging and operation of the instrument are simple, and it is easy to realize automation; the sensitivity is high, and the minimum detection limit can reach 10-12mol/L. However, the selectivity of electrochemical analysis is generally poor.

4.4. Thin layer chromatography (TLC)

TLC is a simple and efficient chromatographic process that takes little time. It is commonly used to detect contaminants in compounds. TLC may be used to track the development of a reaction as well as separate the same components in a mixture [18].

In Ceara, Brazil, Andrade et al. examined the synthetic food colorings in commercially made carbonated orange and grape soft drinks. Extracts of lemon yellow (E102), amaranth (E123), sunset yellow (E110), and brilliant blue were detected using TLC (E133). The findings revealed that using identification and quantification methods for quality control of synthetic pigments in soft drinks is suggested. However, a kind of food coloring used in grape soft drinks is greater than the legally authorized level, enabling vulnerable people to consume these goods, especially when the labeling is inaccurate. This method has been successfully used to determine the food coloring components indicated on the labels of soft drinks [19].

Baranowska et al. used the TLC method to analyze food colors, sweeteners and preservatives. Patent Blue V, Quinoline Yellow, Brilliant Blue FCF, Lemon Yellow, Azoranthine, Ponceau 4R, Curcumin, Indigo Carmine, Cochineal, Methyl Violet, Mixed Carotene, Pure Caramel, Erythrosium Red B, and orange S are separated on silica gel G using isopropanol—(12.5%) ammonia water as the mobile phase. Aspartame, acesulfame K, sodium cyclic amine, and benzoic acid are separated over a thin layer of silica gel G using a mobile phase of ethanol-isopropanol-(12.5%) ammonia water, 10 + 40 + 1 (v/v). Food additives in 23 different types of foaming and non-foaming drinks are analyzed using these chromatographic technologies [20].

TLC has many advantages, including convenient operation, simple equipment, and easy color development. However, its separation effect on biopolymers is not ideal.

5. COMBINED DETECTION METHOD

5.1. LC-MS/MS detection method

The molecular ion peak is used to determine which substance it is in the LC-MS/MS process. Methods of qualitative and quantitative analysis should be combined.

Deuterated vitamin D standards are often used as internal standards for LC-MS/MS analysis of vitamin D3 and 25-hydroxy vitamin D3 in foods. Petra et al. quantified vitamin D3 and 25-hydroxyvitamin D3 in foods using LC-MS/MS detection techniques. DAD/UV and MS/MS detectors are used in the experiment. The use of isotope-labeled vitD3-[d6] post-column perfusion may be used to assess the influence of various eluent additions on ion suppression in various food matrix extracts. As a result, it was found that a large amount of ions suppressed the phenomenon. In contrast, carbon-labeled internal standards can achieve 100% recovery and stable chromatographic analysis. The analysis of vitamin D in foods needs to be standardized to improve the accuracy of the test, particularly for meat and egg products.

Sulfite is a food ingredient that helps to minimize oxidation and browning in a number of foods. Katherine S. Carlos and colleagues investigated the presence of sulfite in food and drinks [21]. The LC-MS-MS approach for detecting food additives is depicted in Figure 2. The LC-MS-MS instrument contains the paper spray system VeriSpray, multi-channel liquid phase Transcend, Vanquish Charger, Online SPE system EQuan Plus which can greatly improve the efficiency of instrument analysis.

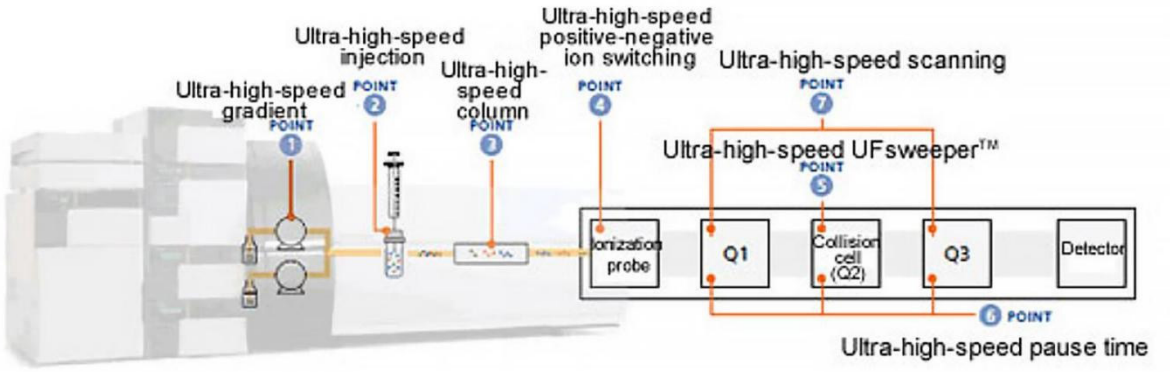

Figure 2. LC-MS-MS method for additives detection.

The study employed four distinct methods to determine the sulfite content of ten different commercial sulfite products (LC-MS/MS method, spectrophotometry, test strip method, and spot measurement method). The findings of LC-MS/MS and spectrophotometry are the most equivalent among these approaches. The test strip is only reliable if the SO2 concentration is more than 50 mg/kg (ppm). The sulfite detection kit shown the most potential as a rapid test technique, although it produced high percent RSD values for various study matrices and required the analyst to use a spectrophotometer. Before doing the test, the individual matrix should be extensively investigated to ensure that it is acceptable for the specific matrix and sulfite content evaluated. According to research, the methodologies provided by these fast analysis kits lack the accuracy and reproducibility of the LC-MS/MS approach over a wide range of concentrations. They can be used by consumers or food processors to assess foods with high sulfite content. They cannot, however, alert consumers of the presence of sulfites at amounts near the regulation label level [22].

LC-MS/MS has high sensitivity, high accuracy, good repeatability, and it can be used for quantitative and qualitative detection.

5.2. GC-MS/MS detection method

GC-MS uses gas chromatography as a sampling system for mass spectrometry to separate complex chemical groups, and mass spectrometer as a detector for qualitative and quantitative analysis.

The GC-MS-MS approach for detecting food additives is depicted in Figure 3. The GC-MS-MS instrument was composed by Agilent 7890B gas chromatograph, 5977B mass spectrometer, G3452-60552 ECD detection and a SSM1810 solid-state modem. Food contact materials (FCM) manufactured of plastic incorporate a variety of additives. These substances can pass through the material and into the meal, affecting the health of customers. Luka Znidersic et al. used the GC-MS/MS method to determine the additives in food contact materials. In the GC-MS/MS method, a gas chromatograph 7890A tandem mass spectrometer 7000B equipped with a multi-purpose autosampler MPS is employed. The carrier gas is helium (>99.999 percent), and the flow rate is 1.4 mL/min. The transmission line has a temperature of 280°C, whereas the quadrupole has a temperature of 150°C. Ionization is an electron impact of 70 eV. Record the total ion chromatogram in the m/z range 35-700. Use the mass spectrum library NIST to identify compounds through spectral comparison [23]. Compared with GC-MS, GC-MS/MS has higher resolution.

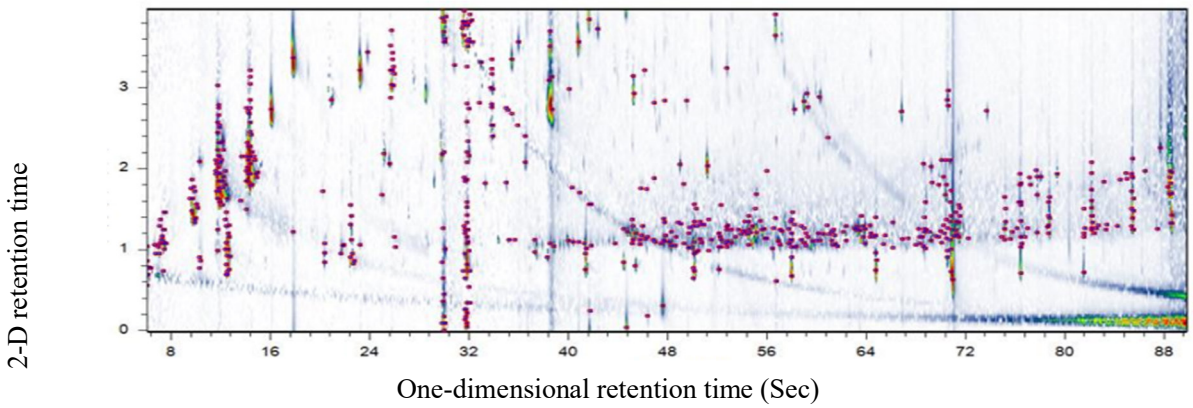

Figure 3. GC-MS-MS method for detection of food additives.

6. CONCLUSIONS

Through the comparative analysis of four kinds of commonly used detection methods, namely, high-performance liquid chromatography (HPLC), capillary electrophoresis method, thin layer chromatography (TLC) and electrochemical analysis method, the author found that the high performance liquid chromatography (HPLC) method with liquid as mobile phase, flow through the pump injected with different polarity and proportion of mixed solvent in the chromatographic column, make each component in the chromatographic column separation, reach the detector for testing. The procedure is effective, precise, and dependable. The separation channel in capillary electrophoresis is a flexible quartz capillary, and the driving force is a high-voltage direct current electric field. The separation is performed by variations in the mobility and distribution behavior of the components in the sample. Capillary electrophoresis is a more sensitive, low-cost, and completely automated approach than HPLC. The discipline of investigating the occurrence of a charged interface established by two types of conductors and the changes that occur on it is known as electrochemical analysis. Batteries or high-voltage electrostatic discharge can be used to interact with electricity and chemical processes. The electrochemical analysis method is simple and fast. TLC was convenient to operation, simple equipment, and easy color development. This article also mentions two combined detection methods, LC-MS/MS and GC-MS/MS. These two methods are more accurate. Through the comparison of combined use and conventional detection methods, the limitation of the current method is that the mechanism of the detector is complicated and needs to be further optimized.

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