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## ORIGINAL RESEARCH ARTICLE

# Advances in the application of chromatographic techniques in forensic toxicology fund

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## ABSTRACT

As knowledge and civilization advance, forensic science and technology are continuously evolving. Chromatography, a key analytical method known for its simplicity, speed, and high sensitivity, is extensively utilized worldwide for detecting toxic substances and drugs in forensic investigations. This paper offers a thorough analysis of chromatographic techniques from three key angles: the historical development of chromatography, its classification based on phase states, chromatographic separation mechanisms and stationary phase characteristics, and its various applications. Additionally, the paper forecasts future trends in chromatographic analysis technology, aiming to inspire new directions for the advancement and research of other forensic science technologies.

***Keywords:*** forensic science; chromatography; thin layer chromatography; gas chromatography; liquid chromatography

Poisons and drugs will not only do serious harm to human body, but also have an extremely adverse impact on society. They are a very important factor of social instability. Worldwide, a large number of poisoning cases are reported every year[1–4]. According to the relevant report data of China's drug control department, there were about 64,000 drug cases in China in 2020, involving 427,000 drug addicts[5]. With the continuous development of synthetic technology, new drugs are updated very quickly. Governments of all countries control[6]. In addition to traditional drugs, the Chinese government. In addition to the control of synthetic drugs, the control of new psychoactive substances has been gradually strengthened in recent years.

While strengthening the control, the laboratory detection and analysis technology of poisons and drugs is also developing with the upgrading of drugs. Compared with western developed countries, the research on poison and drug testing technology in China started late, but has DEVELOPED rapidly in recent years. Before the 1980s, thin layer chromatography (TCL) crystallization method was mostly used in the inspection of poisons and drugs in China. Infrared spectroscopy (IR). Ultraviolet (UV) inspection and chemical color development. With the increasing international exchanges in 1980, GC/MS was introduced into China, and the inspection of poisons and drugs in China entered a new stage. Since then, with the second-order mass

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spectrometry. With the successive introduction of ion chromatography, the inspection of poisons and drugs in China has entered a period of rapid development[7]. As an important analytical technique, chromatography is simple. Fast. With the advantages of high sensitivity, it has been widely used in the detection process of poisons and drugs in forensic science.

# 1. Principle, method and application characteristics of chromatographic analysis technology

## 1.1. Development process

Human beings began to use chromatography in 1903. Mikhail S. Tswett, a Russian botanist, used column adsorption chromatography technology to qualitatively and quantitatively separate plant pigments, and successfully expounded the principle of this method[8]. On this basis, American scholar Palmer et al.[9] applied it and involved a large number of chromatographic experimental results in their special report. In the 1930s, column chromatography separation technology began to be gradually applied to effectively separate and purify different kinds of organic compounds. Since the 1980s, modern chromatographic analysis has entered a new stage of development, which can analyze various components in complex systems. Elements and compounds with similar chemical properties are analyzed, and scientists have proposed many retention models[10,11].

## 1.2. Main principles

Chromatography, also known as chromatography, is used in analytical chemistry. Organic chemistry and other fields have a very wide range of applications, which mainly use the distribution between samples and stationary and mobile phases. Differences in forces such as adsorption or exchange. When the two phases move relatively, all kinds of substances to be measured are balanced for many times between the two phases to achieve the purpose of mutual separation.

## 1.3. Classification method

There are many kinds of chromatographic methods. According to different classification standards, chromatographic methods with different principles can be obtained. There are both differences and intersections between various methods.

### 1.3.1. Classification by two-phase state

According to the state of stationary phase and mobile phase, chromatography can be divided into four categories: gas-solid chromatography, gas liquid chromatography, liquid solid chromatography and liquid chromatography. The main analysis objects of gas chromatography and liquid chromatography are volatile organic compounds and various substances that can be dissolved in water or organic solvents respectively. Gas solid chromatography refers to the chromatographic separation method in which the mobile phase is a gas and the stationary phase is a solid substance. Activated carbon, silica gel can be used as stationary phase. Gas liquid chromatography refers to the chromatographic separation method in which the mobile phase is a gas and the stationary phase is a liquid. For example, the inert material diatomite coated with squalane can separate and determine trace methane in pure ethylene, acetylene, propane and other impurities. In liquid-solid chromatography, the stationary phase is a solid particle adsorbent with porous surface, such as alumina. Silica gel can competitively adsorb solute molecules and mobile phase molecules through the adsorption center on the surface, so as to achieve the dynamic balance of adsorption and desorption. The liquid-liquid chromatographic stationary phase includes an inert carrier and a stationary liquid coated on the inert carrier.

### 1.3.2. Classification according to chromatographic separation mechanism

According to the different physicochemical properties of chromatographic separation, it can be divided into adsorption chromatography, distributive chromatography, Ion exchange chromatography, Gel chromatography, etc. Adsorption chromatography takes the stationary phase as the adsorbent, and uses the adsorbent to separate and analyze the difference of adsorption capacity of different components. According to different mobile phases, it can be divided into gas-solid adsorption chromatography and liquid-solid adsorption chromatography. Partition chromatography is based on the different partition coefficients of different components between mobile phase and stationary phase, which can be divided into liquid-liquid partition chromatography and gas-liquid chromatography. Ion exchange chromatography is a chromatographic method that uses a material that can exchange ions as a stationary phase to separate ionic compounds. Such methods can be used for inorganic ions. Separation of various nucleic acid derivatives or amino acids. Gel chromatography is a method to separate components of different sizes by using gel to produce different retention effects, which is mainly used for the separation of larger molecules.

### 1.3.3. Classification by the nature of stationary phase

According to the geometric form of stationary phase, chromatographic methods can be divided into column chromatography, paper chromatography, thin layer chromatography, column chromatography mainly includes packed column chromatography and capillary column chromatography. Packed column chromatography is to put the stationary phase into the chromatographic column, while capillary column chromatography is to coat the stationary phase on the inner wall of the capillary. Paper chromatography uses paper as a carrier. Using the water or other substances adsorbed by the paper fiber as the stationary phase, the sample starts from one end of the paper, and uses the mobile phase to separate and analyze different components. Thin layer chromatography is a method of evenly spreading adsorbent on glass or plastic plate to form a thin layer and separating on the thin layer, which can be subdivided into adsorption method. Distribution method and ion exchange method, etc.

## 2. Application of chromatographic analysis technology in forensic toxicological analysis

### 2.1. Application advantages

In the field of forensic toxicology, chromatography is used to qualitatively or quantitatively detect the properties and contents of various components in the mixture. The quantity and content of samples in the case may be very small. The use of chromatographic technology reduces the requirements for the amount of samples. One analysis process usually requires only a slight increase in the order of magnitude. In the process of analysis, chromatographic analysis technology has high separation efficiency. Chromatographic columns can be used to separate and quantify dozens or hundreds of compounds with similar properties at the same time. By combining the separation and analysis of various components into one, the efficiency of the whole detection and analysis process is improved. Generally, the analysis of complex samples can be completed in a few minutes to dozens of minutes. Chromatography has high detection sensitivity. With the continuous development of signal processing technology and the continuous upgrading of detectors, trace substances of 10–9 g level can be detected by chromatography. If the samples are further processed in the early stage, the detection limit can be lower. In addition, the chromatographic analysis technology has good selectivity. Selecting the appropriate separation mode and detection method according to the research purpose can effectively screen out the target substances and eliminate other kinds of interferents.

## 2.2. Common methods

At present, thin layer chromatography is widely used in chromatographic analysis. Gas chromatography. Liquid chromatography. Researchers choose appropriate methods for analysis according to the purpose of the experiment or the nature of the sample.

### 2.2.1. Thin layer chromatography

Thin layer chromatography is an important experimental technique in the field of forensic toxicology. The experimenter puts the sample to be tested on the thin layer of the stationary phase, and after it is developed, compares its chromatogram with the specific shift value in the chromatogram of the reference substance, which can realize qualitative or quantitative analysis, and can be used to effectively separate fatty acids. Amino acids, alkaloids and other substances. Thin layer chromatography is easy to operate. Easy color rendering. But it is not suitable for quantitative analysis, and the separation effect of biopolymers is not ideal. Wang and Zhou[12] used TLC scanning to analyze the common drugs at the scene of drug driving, and selected compound licorice tablets as morphine. The results showed that the components of compound licorice tablets were effectively separated without interference between groups, and the color development effect was also good[13]. Chen et al.[14] detected MDPV, the main component of "bath salt", which involves methcathinones, by thin-layer chromatography. Using aluminum silica matrix thin-layer chromatographic plate, ethyl acetate methanol 25% ammonia (85:10:5) as the developing agent, under the irradiation of 254 nm ultraviolet light, the spots appear bright yellow, and become blue by Ninhydrin chromogenic agent. **Table 1** summarizes some applications of thin-layer chromatography in forensic toxicological analysis.

**Table 1.** Application of thin layer chromatography in forensic toxicological analysis.

| Object to be tested | Test method | Test material | Research results | Ref. |
|---|---|---|---|---|
| Heroin. Morphine. Twelve substances such as methamphetamine | TLC | Solid or powder | Twelve substances can be detected | [15] |
| Opioid drugs such as morphine | TLC | Urine | This method can be used to detect heroin abuse. The simultaneous presence of pethidine, dihydroetorphine, methadone and morphine does not interfere with the determination of morphine | [16] |
| Cocaine. Cocaine like, etc. | TLC + EASI-MS | Drug | Successfully distinguish cocaine and other substances mixture | [17] |
| Cocaine and dope | LC + PS-MS | Drug mixture | Various components were successfully separated, confirming the applicability of PS-MS | [18] |
| Cannabis drugs | TLC | Urine | Select the best condition for THC-COOH detection | [19] |

### 2.2.2. Gas chromatography

Gas chromatography is a major experimental technology in the field of forensic toxicology. By using helium or argon as carrier gas, the mixture is injected into the chromatographic column equipped with stationary phase, so as to realize the separation of different components. In recent years, gas chromatography has developed rapidly and has been widely used in laboratories at home and abroad. The separation efficiency of gas chromatography is high. One chromatographic column can separate complex samples with various properties and types, and the analysis speed is fast. It also has many advantages, such as high sensitivity and high selectivity. Gas chromatography is suitable for volatility. Substances with good thermal stability have general separation and analysis ability for other substances. At present, in the field of forensic toxicology, the samples used for the separation and detection of gas chromatography and GC-MS include drugs and their metabolites in in vitro drugs and biological samples. **Table 2** summarizes some applications of gas chromatography in forensic toxicological analysis in recent years.

**Table 2.** Application of gas chromatography in forensic toxicological analysis.

| Object to be tested | Extraction method/detection method | Test material | Research results | Ref. |
|---|---|---|---|---|
| Organic pesticides. Drugs. Sleeping sedative drugs | Micro bead extraction + GC | Blood | Establish a gas chromatography detection method and optimize the extraction conditions. The recovery can be more than 70%, and the relative standard deviation is less than 5% | [20] |
| Amphetamine. Methamphetmine and other drugs | Liquid liquid extraction + GC-MS | Hair | Within the batch. The precision between batches is 1.97%–9.18%, the accuracy is 94.28%–107.78%, the Extraction recovery is more than 85.00%, and the stability is good | [21] |
| Four amphetamine drugs | Solid phase microextraction + GC-MS | Urine | The linear relationship is good in the range of 50~2000 Ng/mL, the correlation coefficients are greater than 0.99, and the RSD is less than 10°. The quantitative limit of the method is 8.7~27.5 Ng/mL | [22] |
| Four organochlorine pesticides | Quechers + GC | Pesticide residues Retention | The minimum detection limit of the method is 0.00014–0.001, and the addition concentration is 0.05. 0.10. At 0.50 Mg/kg, the recovery is 88.8%–109.8% | [23] |
| Nicotine | Liquid extraction + DSP-GC-MS | Tobacco | The nicotine content is 1.21°% ~ 2.19%. It is clear that the best heating condition is heating from 50 ℃ to 300 ℃ at the speed of 60 ℃/min | [24] |

### 2.2.3. Liquid chromatography

Liquid chromatography is a practical experimental technique in the field of forensic toxicology. Compared with gas greater the concentration chromatography, high performance liquid chromatography can be applied to thermal instability. High boiling point. Among ionic substances, it has high separation efficiency. High sensitivity. It has a wide range of applications. It has many advantages, such as fast analysis speed, but its purchase cost and daily maintenance cost are high. With the increasing variety of poisons and drugs, gas chromatography can no longer meet the quantitative needs of test materials. Because of its higher sensitivity, liquid chromatography can significantly reduce its minimum detection limit. It is widely used by researchers because of its stronger applicability, which effectively improves the detection rate of drugs in the samples. **Table 3** summarizes some applications of liquid chromatography in forensic toxicological analysis in recent years.

**Table 3.** Application of liquid chromatography in forensic toxicological analysis.

| Object to be tested | Test method | Test material | Research results | Ref. |
|---|---|---|---|---|
| Six synthetic cannabinoids | LC-MS | Hair | The six substances have a good linear relationship between 3–200 ng/mg, the correlation coefficient is greater than 0.9901, the limit of quantitation is less than 3 ng/mg, the detection limit is less than 1 ng/mg, and the extraction recovery is 90.69%–97.88% | [25] |
| Three synthetic cannabinoids | HPLC-MS | Blood | Successfully established the detection and analysis method | [26] |
| Methcathinone | LC | Suspected drugs | The concentration of methcathinone showed a good linear relationship in the range of 0.5–1000 mg/mL, the detection limit was 0.063 mg/mL, and the average recovery was 118.2% | [27] |

## 2.3. Applicable objects

### 2.3.1. Suspected drugs in vitro

In vitro drugs are mainly studied after being seized by the public security investigation department. Methamphetamine (MA) is one of the most abused new drugs at home and abroad. As a synthetic drug, its main component is amphetamine compound, which has central nervous excitation and hallucinogenic effects, and belongs to a class of substances controlled by the United Nations Convention on psychotropic drugs[28]. Xia[29] established a method for rapid analysis of methamphetamine content in suspected drugs. The gas chromatograph and FID detector were used together. The results showed that this method could be used to detect methamphetamine in suspected drugs, and the linearity of the standard curve was good in the concentration range of 0.01~0.5 mg/mL. Yu et al.[30] used GC/MS to analyze the o-demethyltramadol in the "kartong" test material. Cap wood alkali. The results showed that the concentration of o-demethyltramadol in the sample was 24.5 mg/mL, with a good linear relationship ($r^2$ = 0.9993) and a detection limit of 0.1 μg/mL, the recovery is 99.6%~105.2% ($n$ = 5), and the intra day and intra day precision are less than 6.41%. In addition to traditional drugs and synthetic drugs, in recent years, with the emergence of a large number of new psychoactive substances in the market, the corresponding in vitro detection research is also increasing. Qian et al.[31] used GC-MS. Ultra high performance liquid chromatography quadrupole time of flight mass spectrometry (UPLC-Q-TOF MS) and infrared spectroscopy were used to qualitatively test the unknown fentanyl substances captured for the first time in China. The unknown samples were evenly ground and prepared into a 1 mg/mL solution with methanol. After ultrasonic centrifugation, the supernatant was taken for analysis. The results showed that when the retention time was 13.5 min, the main characteristic ion peak of the mass spectrum fragment of the unknown component was identified as carfentanil by NIST library search. He and Hu[32] successfully performed qualitative analysis of fluoroamine by GC/MS by establishing a gas chromatography-mass spectrometry analysis method of fluoroamine, and the results showed that the minimum detection limit of fluoroamine detected by this method was 10 μg/mL, and the linear relationship is good in the range of 0.2~1.0 mg/mL.

### 2.3.2. Detection of drugs and metabolites in biological samples

Common biological samples in forensic toxicology laboratories include blood. Urine and hair. Each kind of biological sample has its own advantages and disadvantages. Blood is a commonly used test material in the detection of poisons and drugs, and it contains high concentrations of drugs and metabolites. However, the blood extraction process is complex, and it is easy to cause certain safety hazards to operators. The detection time limit of blood is generally about 3 days. If the detection is not timely, the content of volatile substances such as ethanol in blood is easy to change to a large extent, resulting in wrong results. Meng et al.[33] established a GC/MS method for the detection of morphine drugs in blood, using V($CHCl_3$):V(isopropanol):V(n-heptane) = 50:17:33 as the extraction solvent, and derivatized by mstfa. The results showed that morphine. 6-monoacetylmorphine. The linear correlation coefficient of codeine is greater than 0.99, the minimum concentration of blood detection can reach 5 ng/mL, and the quantitative range is 10~1000 ng/mL. Drugs are generally excreted through urine after metabolism in the body. When drug suspects take drugs, relatively high levels of drug parasites and metabolites can be detected in their urine in a short time. However, the detection time limit of toxic substances in urine samples is relatively short, generally within 1 week, which is easy to lead to false negative results. In addition, because the collection of urine samples involves privacy, drug suspects need to collect them alone, which is prone to dilution. Risk of replacement or contamination. Peng et al.[34] established methamphetamine in urine. MDMA. Ketamine and other six common drug detection methods, and the detection results of various drugs were compared and analyzed. The results showed that the longer the retention time of drugs, the smaller the deviation between retention time and relative retention time,

the greater the concentration, and the more stable the deviation of ion ratio.

Compared with urine and blood samples, hair is stable. It has more advantages in judging the drug abuse situation of drug addicts within a few months, and its growth cycle has a certain regularity. Relevant research shows that[35], the growth cycle of hair is 0.6~1.4 cm/month[36]. The International Hair Testing Association recommends that the growth cycle of hair should be 1 cm/month. But it should be noted that hair dyeing. The intervention of external means such as perm will affect the test results. Meng[37] compared the characteristics of the hair of two types of drug abusers through experiments. The hair of heroin addicts was treated by methanol ultrasound, and liquid-phase extraction was carried out after adjusting the pH value. After the extract was evaporated, it was derivatized and analyzed by GC/MS; after alkaline digestion of the hair of methamphetamine users, small volume extraction was used, followed by derivatization and GC/MS analysis. The detection results showed that the minimum detection limit of opioid drugs was less than 3 μg/g, the minimum detection limit of four amphetamine drugs is 0.05 μg/g.

# 3. Trends and prospects of chromatographic analysis technology in forensic toxicology

## 3.1. Multi technology combination

Each analysis method has its own advantages and limitations. With the increasing variety of new compounds and the gradual complexity of structural types, a single analytical technology can not meet the requirements of accurate analysis. At this time, combined technology and instruments came into being. The combination of chromatography and other analytical methods can learn from each other and effectively improve the sensitivity of material analysis. Speed and identification ability have gradually developed into an important development direction of modern analytical chemistry. At present, the combined use of various chromatographic technologies and other technologies has become the mainstream in both foreign and domestic laboratories. Common chromatographic techniques include chromatography chromatography. Chromatography mass spectrometry and chromatography spectrum. Chromatography chromatography combination, also known as multidimensional chromatography, is the common use of many different types of chromatography, which can effectively improve the resolution of chromatography, but it is difficult to distinguish unknown substances. Chromatography mass spectrometry and chromatography spectrum can make up for this deficiency, and can analyze unknown components in multi-component mixtures quickly. Accurately obtain the analysis results and determine the molecular structure and molecular weight of the target. Commonly used chromatography-mass spectrometry and chromatography spectrum include GC-MS, GC-FTIR, LC-MS, LC-NMR, etc. In the study of Gicquel et al.[38], LC-MS/MS method was used to qualitatively test fluoroamine in biological samples, and lc-hrms and NMR were used to qualitatively and quantitatively test three powder samples. The results showed that the detection limit of fluoroamine in hair samples could reach PG level.

## 3.2. New technology development

Chromatographic technology is an important research field of modern instrumental analysis. Since its first use, the development of chromatographic analysis technology has become increasingly mature, but the research and exploration at the technical and application levels have never stopped. Stationary phase and mobile phase are the core components of chromatographic technology. Their research and innovation continue to expand the application field of chromatography, such as the use of chiral stationary phase can effectively separate and detect chiral chemicals. The monolithic column technology of the fourth generation of chromatographic packing has become a hot spot in the development of liquid chromatography in recent years. Monolithic column is the monomer of packing. Initiator. The porous structure column formed by in-situ

polymerization or solidification in the tube after mixing the pore forming agent. The porous structure makes the overall column have good permeability, effectively reduces the column pressure, improves the column efficiency and reproducibility, and can realize the rapid separation and analysis of the substances to be measured. In addition, how the detector can more sensitively capture effective signals is also a direction for researchers to explore. The gradual popularity of new detectors such as semiconductor laser fluorescence detectors has effectively improved the sensitivity of detectors.

### 3.3. Improvement of detection methods and processes

The identification work in the field of forensic toxicology is mostly carried out in accordance with the judicial identification standards or technical specifications, which will roughly describe the pretreatment and analysis methods of the tested materials, but most standards only involve the chromatography-mass spectrometry technology, and the specific selection process and use conditions of the detection and analysis methods and processes have no detailed and clear normative provisions, which need to be selected according to the laboratory testers or other relevant experience in the same field. With the continuous in-depth research and wide application of chromatographic analysis technology, more and more difficulties of detection and analysis technology have been broken through. It is believed that in the future development, it will be an important development trend to establish independent standards or technical specifications for the use of chromatographic analysis technology and constantly improve the forensic toxicology detection and analysis methods and processes.

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## Conflict of interest

The authors declare no conflict of interest.

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