Application of Ambient Ionization Mass Spectrometry in Forensic Toxicological Analysis

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Abstract: Ambient ionization mass spectrometry(AIMS)is a mass spectrometry technology which could be used to analyze target analytes in samples under atmospheric pressure without or with simple sample pretreatment. With the advantages of simplicity, rapidness, non-destructiveness and wide application range, it is widely used in forensic toxicological analysis. This article gives a brief over- view on the ambient ionization(AI)technique, and the samples are divided into two types: in vivo test materials and in vitro test materials. The application of AIMS in the poison analysis of different types of test materials is summarized, and its application direction in forensic toxicological analysis is prospected.

Keywords: Ambient ionization mass spectrometry(AIMS); Mass spectrometry; Forensic toxicologi- cal analysis

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In forensic toxicology analysis, the nature of different cases and ways of committing crimes lead to a high diversity of samples as evidence, which may include blood. Urine. Saliva. Wash gastric juice. Hair. Drugs and latent fingerprints. Common instrumental analysis methods include spectral analysis [1]. Gas chromatography-mass spectrometry (GC-MS)[2-3] and liquid chromatography-mass spectrometry (LC-MS)[4], etc. Spectral analysis uses the absorption and emission of electromagnetic radiation to determine its structure and chemical composition, which often requires standard samples as a benchmark, so it is difficult to apply in practice. The sample pretreatment of GC-MS technology needs to be separated. The two steps of analysis are tedious, time-consuming and difficult to volatilize. Poor thermal stability. For substances with high polarity, GC-MS technology is also difficult to analyze.

In recent years, the technology of open ionization mass spectrometry (AIMS)based on direct ionization has developed rapidly. Under atmospheric pressure, this technology can carry out rapid analysis of the target with or without simple sample pretreatment, which is of great significance in food safety [5]. Drug analysis [6-7]. Environmental testing [8-10]. Analysis of daily necessities [11]. Forensic science [12-15] and other fields have been widely used. After a brief overview of open ionization (AI)technology, this paper focuses on the application of aims technology in the analysis

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of different types of samples, and prospects the development direction of this technology in the field of forensic toxicology analysis.

1 AI technology overview

In 2004, cooks et al. [16] proposed a new ionization method, desorption electrospray spray ionization (DESI), which is different from traditional ionization, and used it to detect metals. Polypeptides and proteins on the surface of polymers and minerals. Its spectrum is similar to that of conventional electrospray spray ionization (ESI). In contrast, desorption electrospray spray ionization mass spectrometry (DESI-MS)technology can complete sample analysis under non vacuum conditions. This is followed by real-time direct analysis (DART)[17]. Desorption atmospheric pressure chemical ionization (DAPCI)[18]. Open ultrasonic spray ionization (EASI)[19]. Matrix assisted laser desorption spray ionization (MALDISI)[20]. Desorption atmospheric pressure photoionization (DAPPI)[21]. Dozens of AI technologies including probe electrospray spray ionization (PESI)[22] have been proposed one after another. Compared with traditional instrumental analysis technology, AI technology has many advantages, such as no or simple sample processing. The ionization conditions are mild. Fast analysis speed and simple operation under non vacuum conditions. In actual analysis, it is usually based on the chemical structure of the tested sample. Physical and chemical properties and other factors to choose the appropriate ionization source.

According to different desorption methods, AI technology can be divided into three categories [23]: liquid extraction. Plasma desorption and laser ablation. The representative AI sources of three types of AI technology are shown in Figure 1. Liquid extraction ionization technology uses solvents to extract or desorb molecules from the surface of samples. It is usually used to analyze polar molecules ionized based on ESI mechanism, mainly including easi. PESI. DESI. Paper spray ionization (PSI). Contact spray ionization (TS)and extraction spray ionization (EESI), etc. Plasma desorption ionization technology has the same chemical principle as atmospheric pressure chemical ionization (APCI). The plasma produced by the discharge electrode interacts with the vaporized sample analyte to ionize, mainly including dart. DAPPI. DAP-CI. Low temperature plasma probe (LTP) and microwave plasma torch (MPT), etc. Since analytes need to be in the gas phase before ionization, rapid detection is limited to low molecular weight volatile compounds. Laser ablation technology uses a laser source to desorb the target analyte from the measured sample, and the ionization efficiency is low. Therefore, most laser based aims technologies are coupled with secondary ionization sources to improve the ionization efficiency and sensitivity, such as MALDI. Electro spray assisted laser desorption ionization (ELDI). Laser assisted desorption spray ionization (LADESI). Laser desorption spray ionization (LDESI) and laser denudation spray ionization (LAESI), etc. The latest development of laser ablation aims technology is the use of infrared laser sources to promote desorption and ionization, and the integration with various post ionization methods except electrospray spray. The combination of laser ablation and laser post ionization has the advantages of high horizontal resolution, high sensitivity and depth analysis while minimizing differential detection [24].

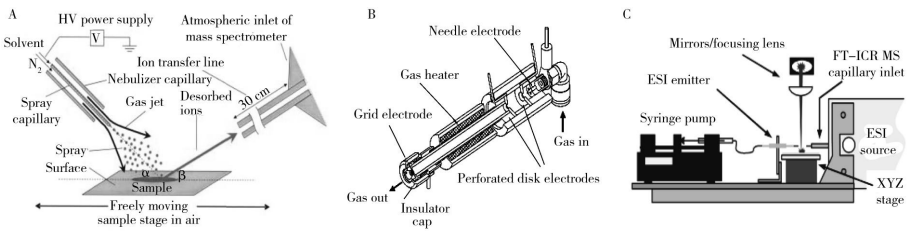

Figure 1 The schematic diagram of DESI(A)[16], clipped view of DART source(B)[17]and the schematic diagram of MALDESI(C)[20]

2 Application of aims technology in forensic toxicology analysis

In forensic toxicology analysis, forensic workers judge the nature of the case through the identification and analysis of relevant toxicants in the samples, provide investigation clues and evidence for the case, and provide basis for whether the parties bear legal responsibility. There are various types of samples involved in the analysis, which can be divided into in vivo samples and in vitro samples according to different types of samples. In vivo samples are also called biological samples, including blood. Urine. Saliva and other body fluids and hair and other tissues. The in vitro test material refers to the material that has not been metabolized by the human body. Samples absorbed and distributed, including various tablets found at the scene of the case. Latent fingerprints. Gastric lavage liquid and plant materials, etc. Aims technology is simple to operate because it does not need sample preparation and chromatographic separation. It is widely used in the field of forensic toxicology analysis due to its advantages such as short time-consuming.

2.1 In vivo test materials

2.1.1 Blood

Blood is commonly used in forensic toxicology analysis. Analyzing the concentration of drug poisons in blood is helpful to explain the cause of poisoning or death. In order to release the drug poisons bound with protein and prevent the interference of determination, it is traditionally necessary to deproteinize the blood samples, and the process is cumbersome. Time consuming. Aims technology can omit this step for direct analysis, which is fast. Simple. The advantage of no sample preparation makes this technology a feasible method for poison screening. Minakata et al. [25] used matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS)to conduct rapid quantitative analysis of four amphetamines in blood, and estimated the cause of death through their concentration in blood. Teunissen et al. [26] successfully established and verified a quantitative method based on paper spray mass spectrometry (PS-MS)for the non separation of 8 amphetamine compounds in whole blood. This method is fast. Sensitive. The selectivity is high, and the qualitative and quantitative analysis can be completed within 1.3 minutes. The detection limit of the method is 15~50 $ng \cdot mL^{-1}$, and the lower limit of quantification is lower than the typical physiological and toxicological level of amphetamine. Jett et al. [27] used psi technology and triple quadrupole mass spectrometry to quickly screen 134 common drugs and their metabolites in blood. Usui et al. [28] used probe electrospray spray ionization tandem mass spectrometry (PESI-MS/MS)to quickly and quantitatively analyze paraquat in serum. The method can directly ionize and determine paraquat, and the detection result can be obtained within 18 s, and

the detection limit is 0.004μG·L^{-1}, the lower limit of quantification is 0.015μg·L^{-1}. In the analysis of actual serum samples, there is no significant difference compared with the results of liquid chromatography tandem mass spectrometry (LC-MS/MS). PESI-MS/MS technology does not require continuous gas/Liquid flow, and the probe and sample plate are disposable, minimizing the risk of contamination. However, because this method does not involve chromatographic column separation, it is difficult to determine multiple targets at the same time, and it has been verified only in 9 poisoning samples, which makes this technology have certain limitations.

2.1.2 Urine

Urine often contains high concentrations of parent drugs and their metabolites, and their concentrations can be used as the basis for judging the abuse of drugs and poisons. Kauppila et al. [29] applied desi-MS to screen drugs and their metabolites in the urine samples of drug addicts, and detected amphetamines. Opium. Cannabinoids and benzodiazepines. Studies have shown that desi-MS technology is an effective tool for rapid screening of abused drugs and their metabolites from drug abusers' urine samples. Kennedy et al. [30] used paper spray tandem mass spectrometry (PS-MS/MS) and high-resolution mass spectrometry to conduct qualitative and quantitative analysis of fentanyl analogues and other abused drugs in the urine samples of drug abuse patients. 10μL urine samples are placed on paper spray cans containing internal standards and dried for direct analysis. The eight fentanyl analogues were linear in the mass concentration range of 0.5~50 ng·mL^{-1}, showing better quantitative results than LC-Ms.

Due to the high concentration of salt in urine. Due to the high content of endogenous organic matter, there may be matrix effect, which affects the signal intensity of the analyte. Using microextraction technology for simple sample pretreatment of urine samples can not only concentrate analytes, but also remove some impurities and weaken the matrix effect. Jagerdeo et al. [31] pretreated urine with packed adsorbent microextraction to concentrate analytes, and then analyzed cocaine and its metabolites in urine samples by real-time direct analysis time of flight mass spectrometry (DART-TOF MS). When the signal-to-noise ratio is 3:1, the bud base methyl ester. Benzoyl blastophylline. The detection limits of cocaine and coca ethylene were 22.9, respectively. 23.7. 4.0. 9.8 ng·mL^{-1}, with higher sensitivity than previous studies. The study also showed that if the appropriate internal standard was used, it was possible to realize the quantitative analysis of the tested substances. Ro- driguez-Lafuente et al. [32] used thin-film solid-phase microextraction as a sample pretreatment step for rapid screening and quantification of cocaine and methadone in urine, with a lower limit of quantification of 1μg·L^{-1}. Solid phase microextraction pretreatment process can preconcentrate analytes, improve sensitivity, and avoid residual salts in urine samples polluting ion sources.

2.1.3 Saliva

In recent years, saliva is easy to operate. Non intrusive. The advantage of low risk of adulteration or infection has become a new test sample, and the drug concentration in saliva is closely related to the drug concentration in blood [33]. Aims technology can analyze saliva samples directly and get test results quickly. Jhang et al. [34] established a new drug screening system for rapid screening and determination of 4-chloroamphetamine in saliva samples. The system can

switch between PS-MS and capillary electrophoresis mass spectrometry (CE-ESI-MS), which is simple. Sensitive. The detection limits of the two methods are 0.1 ppm and 0.25 ppm respectively. Pirro et al. [35] used contact spray mass spectrometry (TS-MS)to directly identify 14 common abused drugs from medical swabs. The direct analysis of swabs greatly simplifies the sampling and testing process of saliva, and provides a simple and fast method for drug detection. Wang et al. [36] used low temperature plasma probe mass spectrometry (LTP-MS)to screen and quantify 11 new psychoactive substances in saliva, used heat assisted desorption to improve signal strength, and performed tandem mass spectrometry to eliminate false positive signals and reduce noise. All analytes showed a good linear relationship, and the detection limit was equivalent to that of immunoassay. Morato et al. [37] used a new generation volume absorption micro sampling (vams)swab for sampling, and established a TS-MS method to simultaneously detect 30 common abused drugs (including opioids)in saliva. Benzodiazepines. Fentanyl derivatives. Methamphetamine. Cocaine. Substituted methylene dioxy phenylethylamine. Cathinone. Antidepressants and antipsychotics). This method requires only 10μL sample, the detection limit of complex drug mixture in biological matrix is mostly below 5 $ng \cdot mL^{-1}$.

2.1.4 Hair

Forensic hair analysis is considered to be the standard method to identify chronic drug users, which can show the use pattern and duration of drugs. With blood. Compared with urine and other samples, hair is easy to obtain. Stable. The advantages of long detection time limit are often used as the analysis object of forensic workers. Miki et al. [38] used MALDI-TOF MS to perform mass spectrometry imaging of methamphetamine in human hair. Studies have shown that the positive spots of methamphetamine with different intensities of hair interruption may indicate the drug abuse history of the tested person and the different blood drug concentrations after each administration. DeimLer et al. [39] analyzed the solution using laser ablation electrospray spray ionization tandem mass spectrometry (laesi-MS/MS). Scheduled drugs in hair and plants are analyzed. In the study, double-sided scotch tape was used to stick the hair sample to the microscope slide, and direct analysis was carried out after wetting with water. 10 $ng \cdot mg^{-1}$ morphine in the hair sample was successfully identified. Codeine and cocaine. Cuypers et al. [40] used matrix assisted laser desorption ionization mass spectrometry (MALDI-MSI)to study the effect of hydrogen peroxide treatment on cocaine incorporation in hair. Research shows that hydrogen peroxide bleaching will reduce the detectability of cocaine in hair. This discovery plays a very important role in forensic hair testing containing cocaine. In the test, attention should be paid to whether the tested hair has been oxidized to avoid wrong identification results.

2.2 In vitro test materials

2.2.1 Drugs

Aims technology can quickly and directly analyze complete samples, which has been proved to be suitable for the screening and qualitative analysis of abused drugs, and provides a fast and reliable analysis method for some illegal drugs and poisons found on site. Kauppila et al. [41] proved to understand the feasibility of atmospheric pressure photoionization mass spectrometry (Dappi-MS)for the direct analysis of illegal drugs in tablets, and successfully identified 3,

4-methylenedioxymethamphetamine from tablet drugs. Amphetamine. Fenazepam and buprenorphine. Steiner et al. [42] used the new technology of accurate mass time of flight real-time direct analysis mass spectrometry to quickly screen common abused drugs in forensic analysis. By analyzing 553 case samples, the use of this method as a screening tool was verified. Compared with GC-MS analysis, this technology is not limited by instrument temperature and time, and can detect more samples at the same time, with more detailed spectral information. Fedick et al. [43] combined paper surface enhanced Raman spectroscopy with PS-MS to quickly identify and confirm fentanyl and its analogues on site and help guide case investigation in real time. Burr et al. [44] integrated surface enhanced Raman scattering and PS-MS into a field analytical instrument operation platform for drug identification, and developed a dual-purpose plasma paper. The integrated system of surface enhanced Raman scattering paper spray ionization mass spectrometry (SERS-PS-MS)constructed in this study has achieved a chemical identification accuracy of 99.8% for blind measurement of 500 samples. The successful identification of synthetic cannabis isomer jwh-^{0}18 proves the analytical and identification ability of SERS, while PS-MS/MS cannot distinguish synthetic cannabis isomers.

Aims technology can directly take samples from the surface of tablet drugs and identify the authenticity of drugs by whether they contain active ingredients. Nyadong et al. [45] compared two-dimensional diffusion ordered 1H NMR spectroscopy with real-time direct analysis mass spectrometry (DART-MS). Desi-MS is combined to detect the chemical components of counterfeit antimalarial drugs. In 16 samples, only the expected active pharmaceutical ingredients were detected in 6 preparations, and sucrose was also detected. Lactose. Stearate. Dextrin, starch and other common organic excipients. Culzoni et al. [46] used dart-TOF MS technology to analyze alprazolam in routine drug identification and true and false drug identification cases. Many samples accurately detected the pseudomolecular ions and some fragments of alprazolam within the specified quality range.

2.2.2 Latent fingerprint

Latent fingerprints are the distribution of endogenous and exogenous chemicals in specific forms, which contain rich. Important forensic information, such as explosives that may have been in contact. Drug abuse and its metabolites. Latent fingerprint analysis is safe. High throughput and non-invasive detection of drug abuse poisons provides a potential way. Overlapping fingerprints left by different individuals are difficult to distinguish by optical methods, while mass spectrometry imaging can easily distinguish the formed imprints according to each person's unique chemical contact history [47-48]. The analysis of life characteristic components in fingerprints is conducive to the characterization of suspects and provides clues for case investigation [49]. IFA et al. [50] applied desi-MS technology to perform latent fingerprint imaging, and clearly identified the detailed features of fingerprints. In glass. Cocaine was successfully identified from latent fingerprints formed on ordinary planes such as paper and plastic. D_9 - tetrahydrocannabinol and high explosive RDX and other exogenous substances. Rowell et al. [51] used surface assisted laser desorption ionization time of flight mass spectrometry (SALDI-TOF MS)to detect methadone and its metabolites in the extracted latent fingerprint for the first time. Bailey et al. [52] adopted MALDI. Three open ionization surface mass spectrometry methods, Desi and Sims, were used to detect

cocaine and its metabolites in fingerprints. The results showed that MALDI and Desi were sensitive to cocaine in latent fingerprints. There was a good correlation between the test results of benzoyl bud alkaloid and aigonitine methyl ester and the test results of oral liquid. In addition, the low destructiveness of aims technology makes it possible to repeatedly analyze the same fingerprint.

2.2.3 Other in vitro test materials

Except drugs. In addition to the latent fingerprints, wash the gastric juice. Plant materials are also common in vitro samples in cases, real-time. The advantage of in-situ detection enables aims technology to obtain detection results in a very short time. Su et al. [53] used electrospray spray assisted laser desorption ionization mass spectrometry (eldi-MS)to realize the rapid detection of seven nonvolatile household pesticides in gastric juice. In the study, a metal probe was used to sample the gastric juice directly, and laser desorption was carried out on the probe. Under the irradiation of high-energy laser, the main proteins and peptides in the gastric juice were decomposed, and the detection sensitivity of pesticides was higher after eliminating the detection interference. Sampling. Desorption. The whole process of ionization and detection takes less than 30 s. Talaty et al. [54] used desi-MS technology to identify the genus Codonopsis (Corydalis). Alkaloids in Datura (Datura stramonium)and belladonna (belladonna)of Solanaceae were detected in situ. Tiny solvent droplets were sprayed onto the surface of plant tissues by electric spray for direct analysis, which was consistent with the results of tandem mass spectrometry. Longo et al. [55] used real-time direct analysis high-resolution mass spectrometry (DART-HRMS-MS)to quickly detect and quantify the enzyme scarring in plant materials. In order to show the ionization efficiency similar to that of the analyte, enzyme scarring-D_9 was selected as the internal standard for quantitative analysis, and the peak area ratio of the analyte to the internal standard was used to reduce the impact of environmental factors. The established method can effectively quantify the enzyme scarring in the range of 1100 ppm, and its existence can be confirmed in a few seconds.

3 Outlook

With the continuous maturity of AI technology, aims technology has become a powerful tool in forensic toxicology detection and identification, among which dart and Desi technology are the most widely used as early AI technology. Although at present, AI technology has developed from the initial few to dozens, but its sensitivity. There are still many challenges in terms of reproducibility and data complexity [56]. Many studies are devoted to improving the analytical performance of this technology, mainly including improving its sensitivity and reliability in qualitative and quantitative analysis, reducing the detection limit and realizing the quantitative analysis of compounds in trace or complex sample matrix. In recent years, aims technology has been applied to the analysis of nonpolar molecules and large biopolymers in complex samples.

A variety of analytes in natural samples usually lead to the complex mapping of aims mass spectrometry. The automation of analyte ion recognition and background ion removal will be a research direction in the future. With the improvement of chemical synthesis and other technologies, the types of poisons in forensic toxicology analysis are also increasing, and new poisons are pouring out. It is a long-term work to explore the most suitable object of each technology. Aims technology has direct advantages over traditional GC-MS and LC-Ms. The advantage of rapid analysis can

establish appropriate methods in the rapid screening of forensic drugs and poisons, and constantly expand the scope of drug and poisons bank. The on-site rapid detection technology based on small portable open ionization mass spectrometry has been applied in many fields [57-58], which is simple. Fast. The advantage of real-time analysis greatly improves the analysis efficiency. In the scene of a poison case, the application of a small portable open ionization mass spectrometer for on-site rapid analysis will win time for the case and point out the direction of investigation.

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