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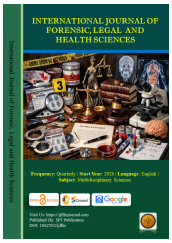

Review Article

Analytical Techniques for Detection of Abused Drugs in Biological Specimens: A Mini Review

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ABSTRACT

Drug abuse continues to be a major concern for public health and law enforcement agencies around the world. This requires development and application of reliable analytical techniques for accurate examination of abused drugs in various biological specimens. Blood, urine, hair and oral fluid are some of the routinely used biological specimens in forensic toxicology, each offering different advantages and limitations based on the purpose of analysis. Over the years, various analytical techniques have evolved from screening techniques to highly sensitive instrumental techniques. This literature review provides a summary of the routinely used techniques for the examination of drugs of abuse in biological specimens, particularly emphasizing on immunoassay, Gas Chromatography-Mass Spectrometry (GC-MS), Liquid Chromatography-Mass Spectrometry (LC-MS) and High-resolution Mass Spectrometry (HRMS). This review discusses principles, applications, strengths, limitation and recent advancement of these techniques, along with suitability of different biological samples for forensic toxicological examination. Comparative evaluation of these analytical techniques shows us how to make drug detection more accurate, sensitive and reliable.

Keywords: abuse, biological samples, sample extraction, analytical techniques, immunoassay

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INTRODUCTION

Drug abuse has become a major concern and threat to society around the world, placing burden on public health with rise in criminal activities. [1,2] As mentioned in the World Drug Report 2025, around 316 million individuals around the world use some kind of a drug excluding alcohol and tobacco. And the commonly abused drugs include cannabis, followed by opioids, amphetamines and cocaine. [3] The growing increase of multiple drug abuse and emergence of new psychoactive substances has complicated forensic toxicological examinations, creating the need for advancement in analytical techniques to ensure sensitive and reliable drug examination. [4] Detection of drugs of abuse plays a great role in forensics, workplace drug testing, anti-doping, and post-mortem analysis. [5] Confirming the presence of drugs and their metabolites is necessary for proving drug use, supporting legal investigations and ensuring accurate examination of toxicological samples. [6] After the drug enters the body, it undergoes absorption, distribution, metabolism and elimination (ADME). [7] Various biological matrices provide crucial information about the history of drug consumption. [8] However, each biological specimen has its own unique characteristics, advantages and limitations in detection of drugs of abuse which makes it suitable for forensic uses. The examination of drugs of abuse have become easier and more accurate with the advancement in analytical techniques. Some traditional techniques like immunoassay are still used as it provides rapid and reliable preliminary analysis. [9] However, advanced analytical techniques based on the principle of chromatographic separation such as GC-MS, LC-MS, and HRMS are used to examine drugs of abuse in biological specimens due to their

high sensitivity and reliability [10] The new technologies are further expanding the scope of forensic toxicological analysis. In recent years, many technologies have been developed for identification of drug of abuse in biological specimen. The properties of analyte, amount of sample and required sensitivity should be taken into consideration before selecting appropriate analytical technique. [11] Therefore, understanding the advantages and limitations is necessary for selecting appropriate analytical method for the purpose of examination. The present review discusses frequently used biological specimens, sample preparation methods, screening and confirmatory analytical methods, their principle, advantages and limitations for the examination of abused drugs in biological specimens.

BIOLOGICAL SPECIMENS USED FOR DRUG DETECTION

Drugs of abuse can be detected from various biological specimens including blood, urine, oral fluid, sweat, hair, nail, breast milk, placenta, meconium etc. [8, 11-14] The detection window, advantages and limitations of some commonly used biological samples are given below in Table 1.

Table 1. Shows detection window, advantages and limitations of some commonly used biological samples

Biological Specimen	Detection Window	Advantages	Limitation
Blood	Few hours to 24 hours (depends on the drug)	Best indicator of recent drug use	Invasive collection and short detection window
Urine	6 hours to 48 hours (extended period of detection on chronic uses)	High drug/metabolite concentration, non-invasive collection	Cannot determine current drug use
Oral fluid (saliva)	12 hours to 24 hours	Easy, rapid and non-invasive collection, indicates recent drug use	Drug concentration gets affected by saliva pH
Hair	Weeks to months (1cm= 1 month of drug consumption history)	Long term history of drug consumption, easy storage	Cannot detect very recent use, Hair colour or treatment may affect the results

Sample preparation techniques: Before the examination of drugs from biological specimens, the sample is prepared in order to isolate and extract drugs and their metabolites from the complex biological matrix reducing matrix interferences. The selection of an extraction technique should be based of type of sample, properties of the analyte, analytical method to be employed and the required sensitivity. [15-18] The most commonly used extraction techniques are summarized below in Table 2.

Table 2. Shows most commonly used extraction techniques

Technique	Principle	Common applications	Advantages	Limitation
Liquid-Liquid Extraction (LLE)	Analytes partition between two immiscible solvents	Urine, Blood, Plasma	Simple and inexpensive	Lengthy procedure and requires large volumes of organic solvent
Solid Phase Extraction (SPE)	Adsorption of analytes onto a solid sorbent followed by elution	Urine, Blood, oral fluid	High recovery, removes matrix interference	More expensive than LLE and cartridge dependent
Solid Phase Microextraction (SPME)	Extraction using a coated silica fibre without solvent	Blood, Urine, Saliva	Improved detection limits, solvent-free, Reduced analysis time	Limited extraction capacity
QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe)	Involves salting-out extraction followed by dispersive solid phase clean-up	Multiple drug testing	Easy, rapid, inexpensive and minimal solvent use.	Not suitable for all drug classes

Screening Immunoassay Tests: Immunoassay based techniques remains the commonly utilized screening techniques for detection of abused drugs in biological specimens as they provide cost effective and rapid preliminary results. However, these drugs identify drug classes than individual drug compounds. Also, these techniques may show cross-reactivity with structurally similar substances, resulting in false positives and false negatives. [19-22] The principle, advantages and limitations of some immunoassay-based detection techniques are summarised below in Table 3.

Table 3. Shows principle, advantages and limitations of some immunoassay-based detection techniques

Technique	Principle	Advantages	Limitation
ELISA (Enzyme-Linked Immunosorbent Assay)	Enzyme mediated colour change is produced when antigen-antibody reacts	High sensitivity, rapid, simple	Cross reactivity may produce false positive results
EMIT (Enzyme Multiplied Immunoassay Technique)	Enzyme activity changes when antibody binds to the target drug	Fully automated and rapid	Lower specificity
FPIA (Fluorescence Polarization Immunoassay)	Measures the changes in fluorescence polarization after antigen-antibody interaction	Rapid analysis and good precision	Cross reactivity may occur
RIA (Radioimmunoassay)	Uses radioactively labelled antigens to detect antigen-antibody interaction	Very high sensitivity	Uses radioactive materials, expensive

ANALYTICAL TECHNIQUES

After drug screening using immunoassays, analytical technologies have been commonly used since years for the confirmatory identification of the drugs and their metabolites in forensic toxicological analysis because of their high sensitivity, reliability and accurate results. Some of the highly utilized techniques are mentioned below:

Gas Chromatography-Mass Spectrometry (GC-MS) : GC-MS is frequently used in forensic toxicology to verify the existence of drugs and their metabolites. The method is very dependable for qualitative drug analysis because it combines the molecular identification strength of mass spectrometry with the separation power of gas chromatography. During the analysis, the volatile or derivatized compounds gets separated in the GC column according to their properties. After separation in the GC column, the analytes enter the mass spectrometer, where they undergo electron ionization. During this process, the molecules are ionized and further fragmented into ions. The generated mass spectra are compared with reference spectral libraries for the identification of the compounds. [23] For decades, GC-MS has served as a gold standard in forensic laboratories for the qualitative identification of drugs. It is used for examination of opioids, cannabinoids, amphetamines, cocaine, benzodiazepines, barbiturates, methadone and other drugs and their metabolites in biological specimens including blood, urine, oral fluid, nail and hair because of its high sensitivity, specificity, reproducibility and broad spectral libraries. [23-24] Many researchers have demonstrated the effectiveness of GC-MS for forensic analysis of drugs. Polettini et al. (1992) detected heroin and its metabolites in hair using GC-MS. [25] Meatherall (1999) developed a method for simultaneous detection of codeine, morphine, 6-acetylmorphine, hydrocodone, hydromorphone, oxycodone and oxymorphone in urine samples using GC-MS [26] Kusmiyati et al. (2023) performed qualitative analysis of morphine, heroin and codeine in urine samples using GC-MS. [27] UNODC has also recommended GC-MS for the examination of drug in forensic laboratories. [28] Besides its numerous advantages, it has few limitations. Non-volatile or thermally unstable compounds need chemical derivatization before analysis which increases sample preparation time. Also, the instrument is expensive and requires skilled personnel to operate and interpret. Despite its limitations, GC-MS continues to remain as a gold standard confirmatory technique for eamination of drug and their metabolites in biological specimens.

Liquid Chromatography-Mass Spectrometry (LC-MS): LC-MS is an advanced analytical technique for the confirmatory analysis of drug of abuse and their metabolites in forensic toxicology because it enables direct analysis of polar and thermally unstable compounds without any need for chemical derivatization. Thus, is regarded as a modern gold standard technique. [24] The commonly used LC-MS is LC-MS/MS, which consists of two mass analyzers, providing greater sensitivity and reliability. In the technique, analytes are first separated in liquid chromatography followed by detection using tandem mass spectrometry.

The routine application of LC-MS/MS includes the examination of opioids, benzodiazepines, antidepressants, cannabinoids, amphetamines and other substances and their metabolite in biological specimens such as “blood”, “urine”, “oral fluid” and “hair”. In a single run, the technology enables the simultaneous detection of several analytes. Hence, it is highly suitable for drug testing. LC-MS/MS is recently preferred over GC-MS, as it offers several advantages. It requires minimum sample preparation, does not require chemical derivatization for most of the analytes, provides sensitivity for polar and thermally unstable compound. Also, it can detect drugs present in very low concentrations. Consequently, LC-MS/MS has emerged as an ideal analytical technique for examining drugs and metabolites in biological specimens in forensic toxicological laboratories. Despite the advantages, the limitations of LC-MS/MS should be acknowledged. The instrumentation and maintenance costs are high, matrix effect may affect ionization efficiency and the accurate interpretation requires skilled analysts. Still, LC-MS/MS continues to be an excellent analytical technique due to the versatility it serves [29-31]

High-Resolution Mass Spectrometry (HRMS): LC-MS/MS is mass spectrometry with low resolution due to the determination using Quadrupole mass analyzers. HRMS consists of Time-of-Flight Mass Analysers (Tof-MS), providing highly accurate mass measurements up to 0.001 amu which helps in distinguishing compounds with similar molar mass but different structures. Thus, making it an advanced analytical technique used in examination of new psychoactive substances recently. The newly emerging psychoactive substances highlights the importance of HRMS in forensic analysis of drugs because many newly synthesized drugs are not available in the spectral libraries of conventional analytical methods. Further improvement in technologies such as “Quadrupole Time-of-Flight and Orbitrap mass spectrometers” have enhanced the capability of forensic laboratories in examination of complex drug mixtures. Despite having various advantages, the routine use of HRMS is limited due to its high maintenance and instrumentation costs, and requirement of skilled personnel for interpretation of mass spectral information. In addition, comprehensive spectral libraries are still under development which may limit its widespread application in routine forensic analysis. The future developments are expected to further integrate HRMS with artificial intelligence and automatic spectral interpretation which may make compound identification easier. With continuous advancement in analytical technologies for accurate compound identification, HRMS is expected to play an important role in toxicological investigations, particularly for the examination of new psychoactive substances in biological specimens. [24,32-34]

CONCLUSION

The reviewed literature reveals that no single analytical technique is universally suitable for the examination of all the abusive drugs across every biological matrix. To select the right analytical technique, one must consider the properties of the analyte, the biological specimen, and the resources available in the laboratory. For rapid initial screening, immunoassays still remain highly useful, on the other hand confirmation relies on the highly accurate and specific chromatographic techniques coupled with mass spectroscopy. Among the analytical techniques, GC-MS remains a reliable and widely accepted technique for routine forensic drug identification, while LC-MS/MS and HRMS have expanded analytical capabilities, particularly for polar compounds, complex drug mixtures, and new psychoactive substances. Advanced chromatographic instruments provide sensitivity and selectivity required for definite identification. At the same time, the rise in psychoactive substances and multiple drug abuse continues to create difficulties for laboratories. Future research needs to focus on developing such standards and methods which are fast and can detect multiple drugs in various biological samples. Over time, upgrading the analytical techniques and regularly updating spectral libraries will help in strengthening forensic testing, making toxicological results more reliable.

DECLARATIONS

Ethics Approval and Consent to Participate: Not applicable. This review is based solely on published literature and did not involve human participants or animals.

Consent for Publication: Not applicable**.**

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