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Clinical Applications of Mass Spectrometry in Toxicological Investigations: Advancing Therapeutic Drug Monitoring and Analysis

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Mass spectrometry (MS) has revolutionized therapeutic drug monitoring (TDM) and analysis in clinical toxicology. This review studies the recent advancements and applications of MS in toxicological investigations, highlighting its role in improving TDM precision and expanding the analyte detection scope. MS's versatility allows for precise drug quantification across various concentrations, enhancing patient care through personalized dosing regimens and monitoring drug efficacy and toxicity. MS-based assays provide superior specificity and sensitivity compared to traditional immunoassays, especially in complex matrices like blood, urine, and tissue samples. MS aids in identifying and quantifying novel psychoactive substances and designer drugs, addressing emerging challenges in clinical toxicology. The rapid adaptation of this substance to changing drug landscapes is a crucial aspect of its essential role in forensic and emergency toxicology. MS, alongside TDM, is increasingly being utilized in postmortem toxicology, aiding in thorough investigations into drug-related deaths, and contributing to forensic pathology and public health initiatives. Despite its widespread adoption, challenges such as standardization of methodologies, complex data interpretation, and cost-effectiveness persist. The integration of MS into clinical practice and its potential in toxicological investigations will be significantly enhanced by addressing these challenges. MS is crucial in postmortem toxicology, aiding in forensic pathology and public health interventions, but challenges like standardization, data interpretation, and cost remain. MS's application in toxicology is continuously evolving, providing exceptional capabilities in TDM, new psychoactive substances (NPS) detection, and forensic investigations. Future technological advancements are expected to enhance the clinical utility of MS, leading to improved patient outcomes and public health. MS continues to revolutionize clinical toxicology, offering exceptional capabilities in TDM, NPS detection, and forensic analyses, with continued advancements promising improved patient care and public health outcomes.

Keywords: Mass Spectrometry, Toxicological investigations, Therapeutic drug monitoring, Drug discovery, Insilico-methodologies

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1. Introduction

Pharmaceutical safety issues pose a global threat to human health, necessitating toxicological analysis and safety evaluation. Mass spectrometry imaging (MSI) has gained popularity as an instrument for predicting drug toxicity. It enables simultaneous quantitative, qualitative, as well as localization avoiding the need for intricate sample preparation and labeling procedures (Chen et al. 2023). Drugs of abuse recognition and quantification are among the urgent topics in forensic research, and the development of mass spectrometry technology has created novel possibilities for these examinations (Moore et al. 2008). One of the main factors for a high rate of Illicit activity is the extremely high global incidence of addiction and misuse of drugs (Beck, 2014). The primary emphasis in the advancement of novel analyzing procedures, with mass spectrometry (MS) serving as a crucial tool, has been on the extensive utilization of hallucinogens, natural drugs, psychotropic substances, and more lately "new psychoactive substances (NPS)" that are derived from the structures of certain formerly identified natural medications (Lee et al. 2016; Gwak and Almirall, 2015). Combining chromatography methods with mass spectrometry for toxicological analysis can screen unidentified medicines' metabolites. Tandem mass spectrometry or selected reaction monitoring enhances selectivity for target analysis when the signal/noise ratio is high (Mogollon et al. 2018; Cappelle et al. 2015).

The most commonly used method due to its higher specificity, selectivity, and detectability, but time-consuming when chromatographic separation and sample preparation are required (Cappelle et al. 2015; Chèze et al. 2008). Furthermore, because they can be used with little to no sample preparation, ionization mass spectrometry techniques like atmospheric solids analysis probe (ASAP), touch spray mass spectrometry (TS-MS), paper spray (PS), desorption atmospheric-pressure photoionization (DAPPI), low-temperature plasma (LTP), desorption electrospray ionization (DESI), and more recently laser diode thermal desorption (LDTD) in toxicological analysis have become more and more popular (Domin and Cody, 2014; Pirro et al. 2015; Wang et al. 2010).

Advancements in mass spectrometry are needed to detect substances with similar fragmentation patterns, as the matrix sample alone cannot provide relevant information for criminal investigations (Habala et al. 2016). Toxicology encompasses the examination of toxins, the mechanisms of dangerous events, and human responses to them, along with the development of strategies for the therapeutic management of toxic exposures (Langman and Kapur, 2006). Any biologically active compound that can change a biological system's normal physiology and reach sufficiently large concentrations to have a hazardous effect is considered a poison (Holsapple and Wallace, 2008). Because of this, even medicinal treatments have the potential to turn toxic, and the overall pharmacokinetic and pharmacodynamic effects as well as the dosage can have an impact on toxic effects (Holsapple and Wallace, 2008).

It is enclosed by chemicals all the time; thus, exposure might happen from the environment, at work, or at home. To assess toxic exposures, various analytical methods and instruments are necessary because of the intricate nature of possible toxins (Smith et al. 2007; Viette et al. 2012; Goullé et al. 2014; Shannon, Cox, and Baum, 1998). Toxic assessments involve quantitative or qualitative evaluations to identify and measure hazardous substances responsible for reported toxic syndromes (toxidromes) in various toxins (Holstege and Borek, 2012). LC-MS, a chromatographic technique with a mass detector, is widely used for forensic analysis of drugs, explosives, and chemical warfare agents. Its versatility, sensitivity, and accuracy surpass GC-MS. This covers LC-MS principles, ion sources, and analyzer types, discussing STA (Systematic Toxicological Analysis) and applications on crime site samples (Gahlaut, et al. 2014).

To ensure the organ's survival in the recipient, a lifetime of immunosuppressive medication is always administered after solid organ transplantation. Immunosuppressive medications must be used to protect the transplant and, ultimately, the patient's life. These medications are highly recommended for therapeutic drug monitoring (TDM) in Fig. 1, which modifies the appropriate dosage for each patient to prevent therapy-related side effects or rejection.

Without the appropriate analytical methods and equipment, this precisely regulated therapy would not be feasible. In practice, either chromatography techniques or immunoassays founded on concepts like fluorescence or colorimetric detection are employed. They all have to deal with the difficult, uniquely different matrix of whole blood. Attaining the necessary levels of quantification is challenging (Freudenberger et al. 2016).

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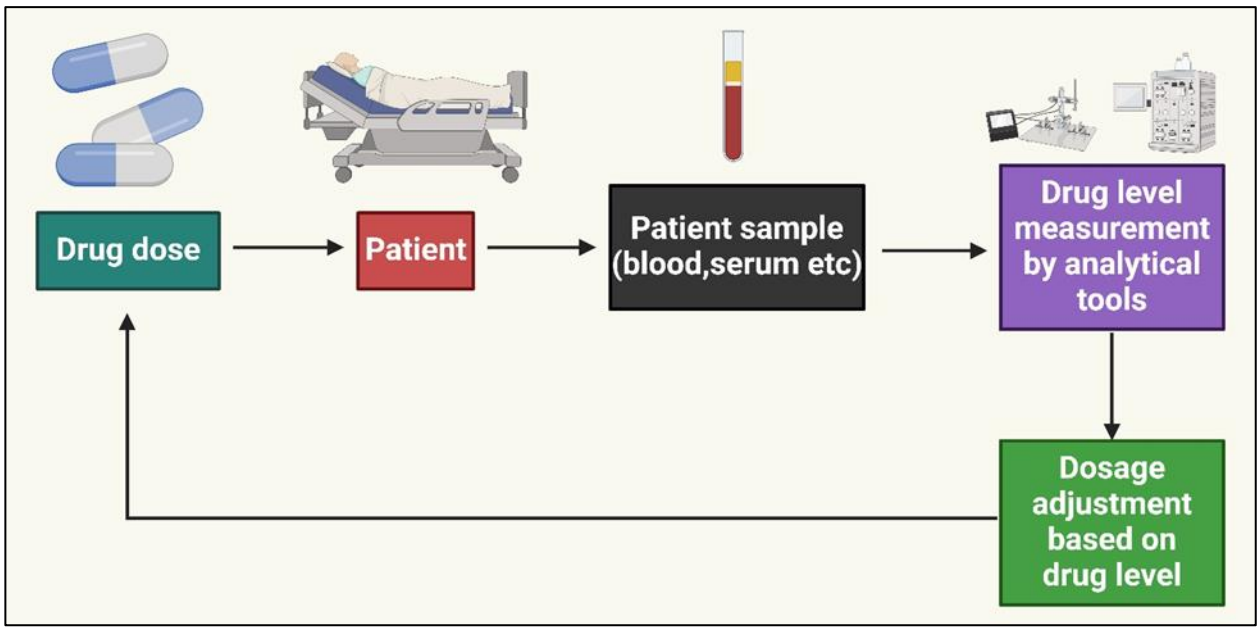

Fig. 1. The therapeutic medication monitoring procedure. The absorption, distribution, metabolism, excretion, and therapeutic efficacy of medication can vary post-consumption or treatment. Blood analysis can be used to determine if a patient is within a specific therapeutic window or has hazardous or subtherapeutic drug concentrations. Dosage modifications can enhance treatment effectiveness by adjusting drug concentrations, either at a timed or steady-state level.

The findings highlight MS's unmatched sensitivity, specificity, and adaptability in identifying a broad spectrum of medications, metabolites, and toxicants in diverse biological matrices (Moorthy et al. 2015). MS allows for precise and reliable measurement of small molecule medications as well as complex biologics, which helps with therapy optimization, dose adjustment, and adherence monitoring in clinical practice. This study also emphasizes the growing use of mass spectrometry in postmortem and forensic toxicology investigations, providing information on drug-related deaths, intoxications, and interactions. Utilizing cutting-edge mass spectrometry methods, like high-resolution mass spectrometry (HRMS) and tandem mass spectrometry (MS/MS), toxicologists can better interpret toxicological results in forensic settings, uncover new biomarkers, and clarify intricate drug metabolism pathways.

2. High-resolution mass spectrometry

High-resolution mass spectrometry (HRMS) is a method that accurately measures the mass of molecular ions in a sample, unlike the nominal mass method. This definition is provided by The Royal Society of Chemistry (Allen and McWhinney, 2019). The instrument resolution is typically used to describe the efficacy of a high-resolution mass analyzer. The "full width at half maximum" (FWHM) approach, which divides mass (m) by the peak width at 50% of the peak height (m/Δm50%), can be used to find an instrument's resolution. When m/Δm50% >10,000, a mass spectrometer is deemed capable of high-resolution analysis (Xian et al. 2012; Allen and McWhinney, 2019). High-resolution mass spectrometers, including Time-of-flight (TOF), orbitrap (OT) mass analyzers, and Fourier transform ion cyclotron resonance (FT-ICR) instruments, are widely available commercially using various technologies. A decrease in the price of devices like TOF and OT mass analyzers has led to an increase in the use of high-resolution mass analyzers in clinical laboratories in recent years (Jiwan, Wallemacq, and Hérent, 2011). Over the past two decades, MS has shown a growing usage in the field of environmental sciences to investigate the presence of organic pollutants. This has resulted in the emergence of various techniques, including gas chromatography (GC), high-resolution mass spectrometry (HRMS), tandem mass spectrometry (MS-MS), and liquid chromatography (LC). HRMS is particularly popular due to its applicability for both focused and broad assessment, allowing for retrospective analysis, pre- and post- and non-target analysis, and discovery of transformation and metabolite products. Most literature on HRMS has been obtained since 2005, reflecting its expansion and prevalence in this domain (Hernandez et al. 2012). High-resolution mass spectrometry (HRMS) is increasingly utilized for the examination of residual substances in foodstuffs due to its selective, sensitive, and rugged instrumentation. It offers

benefits like full-scan spectra, retrospective data analysis, compound-specific tuning, and structural elucidation. In quantitative multi-residue approaches, HRMS competes with traditional tandem mass spectrometry, but it still has hardware and software problems that may set tandem mass spectrometry aside (Kaufmann, 2012). Native MS is a recent addition to mass spectrometry, focusing on the analysis and characterization of macromolecules, particularly intact proteins and protein complexes in Fig. 2 (Tamara et al. 2021).

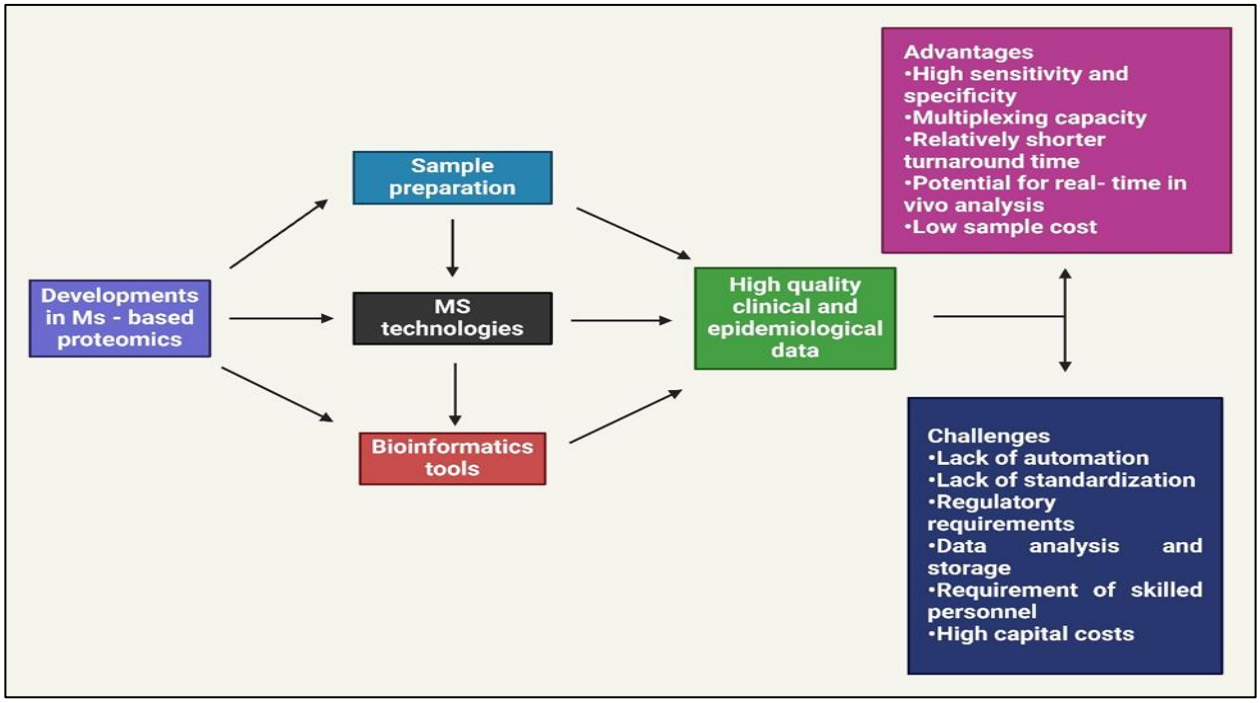

Fig. 2. Current advancements in sample preparation, MS technology, bioinformatic tools, and mass spectrometry-based clinical proteomics: key benefits and necessary considerations

3. Mass spectrometry's historical use in drug metabolism

The 1990s saw the development of commercially manufactured LC/MS instruments, high-resolution HRMS instruments, and data-mining technology, marking significant turning points in MS technology evolution and drug metabolism studies. ESI technology overcomes physical barriers in LC/MS, enabling direct liquid introduction into the gas phase, and enhancing analytical selectivity, sensitivity, and speed ideal for drug metabolic research (Lee and Kerns, 1999). However, the LC/MS platform now collects accurate HRMS and MS/MS datasets (Ma and Chowdhury, 2013; Zhu, Zhang, and Humphreys, 2011).

4. Translational difficulties for therapeutic drug monitoring

TDM is a clinical procedure that involves regular testing of specific medications to maintain consistent concentration in a patient's bloodstream. TDM is primarily used for drugs with limited therapeutic ranges, significant pharmacokinetic variability, challenging target concentrations, and drugs with both beneficial and negative effects (Azad et al. 2024). The foundation of TDM is the belief that there is a measurable correlation between the dosage and concentration of a drug. Within the bloodstream or plasma, there exists a relationship between the level of concentration and therapeutic effects. TDM starts at the time the drug is first administered and entails choosing a starting dosage that is suitable for the patient's age, weight, organ function, and clinical condition as well as any concurrent medication therapy. The dosage history, patient response, intended medical aims, and the sample time about the medication dose are all important considerations for interpreting concentration values. Using the right dosages of challenging-to-manage drugs to maximize therapeutic outcomes for patients in a range of clinical settings is the aim of therapy-directed medicine (Kang and Lee, 2009). Advancements, especially in the last half-century (Box 1), have demonstrated that pharmacological activity is directly connected with a drug's blood concentration. For this reason, concentration is a more appropriate measure of efficacy or toxicity than dosage (Praveen, 2024). TDM measures drug concentration in blood, plasma, or other biological fluids, aiming for a predetermined therapeutic range, and modifying drug dosage regimen accordingly. As a result, the specificity and sensitivity of the analytical method have a significant impact on the reliability of TDM. These tests are carried out in the modern clinical

TDM setting utilizing either immunoassays or chromatographic techniques along with specialized detectors (often mass spectrometers). However, there are certain practical drawbacks to these conventional techniques for the planned large-scale, dispersed TDM practice (Praveen et al. 2024). These include non-standard workflows, lengthy turnaround times, and expensive apparatus with intricate sample preparation.

In this sense, recent advancements in sensing technology present a special chance to get over these constraints and fully utilize TDM. The capabilities of these applications and their recent advancements have been thoroughly discussed (Shafiee et al. 2019; McKeating et al. 2016; Carlier et al. 2015; Meneghello et al. 2018). Personalized medicine and on-site care predate human civilization, with hunter-gatherer micro societies utilizing local knowledge and healers providing the best available medication. The Ebers Papyrus (1550 BCE), the Sushruta Samhita (600 BCE), the Sumerian clay tablets (2000 BCE), De materia medica (50–70 CE), the Shennong Bencao Jing (200 CE), and numerous other ancient documents are among the first examples of pharmacological therapy. The treatment concept remained largely unchanged for thousands of years until the 19th century, during the period of synthetic chemistry. The advent of scientific methods for manipulating the structure of organic materials led to the replacement of individualized tailored cures by mass-produced, industrialized "one-size-fits-all" products from neighborhood apothecaries (Praveen and Morales-Bayuelo, 2023). Advancements in electronics, data science, manufacturing technology, and process control have revolutionized the industry, challenging the fundamental assumption of automation. Dosage has been a crucial factor in treating patients nearing death due to toxicity or subtherapeutic exposure since ancient times.

Paracelsus once stated that everything is poison and nothing is without poison; only the dose makes a thing, not a poison. But Widmark made the first demonstration of this transition's monitoring and correlation in 1932. The 1960s saw the publication of the first PK study, establishing the field's significance due to doubts over the "one-size-fits-all" approach to blood concentration measurements for multiple medications. Another significant historical turning point was a 1965 publication that contained the first organized analysis of the significance of "monitoring of drugs". Innovations in chromatographic methods gave these studies even more impetus. Drug monitoring reached its zenith in the years that followed. Initially, MS, HPLC, and GC were used to measure the concentrations of different medications. The development of immunoassays, which transformed the idea by making assay execution more feasible, was another significant turning point (Ates et al. 2020). Pharmacokinetic, pharmacodynamic, and pharmacological methodologies and analyses must all be coupled when using TDM. It takes more than just measuring a patient's blood medication content and comparing it to a target range to utilize TDM appropriately. TDM is vital for developing safe and effective therapeutic drugs, as well as for personalizing these drugs. TDM can also be used to detect medication compliance issues in noncompliant patient instances. The patient's response, the intended clinical targets, the dosage history, and the sampling period about the dose are all important considerations when interpreting drug concentration results. With this data, the best dosage schedule for achieving the desired effect with the least amount of toxicity can be determined (Thomson, 2004; Borowitz, 1995).

5. Pharmacokinetics TDM of ISDs

TDM is a multidisciplinary clinical specialty to enhance the quality of treatment provided to patients by separately adjusting a drug's dose when clinical trials have demonstrated that doing so enhanced the result in a broad or specialized group of individuals., according to the definition given by the IATDMCT. A key component of TDM is the quantification of drug levels in the blood at predetermined sample times (PK monitoring), in addition to demographic, clinical, pharmacogenetic, and PD data. TDM is necessary anytime the dose-effect relationship precludes the use of a "one size fits all" approach to medication delivery. Drug concentrations must be closely monitored during immunosuppressive drug (ISD) therapy for several reasons: These medications have a restricted expected target range, which is typically expressed as the trough concentration. If the target range is missed, there could be serious consequences, including medication toxicity and/or overimmunosuppression that increase the risk of infection and cancer; on the other hand, there could be graft loss and impairment. It can be challenging to differentiate toxicodynamic effects from clinical disease (calcineurin inhibitor-induced nephrotoxicity from kidney transplant function impairment or BK virus nephropathy, for example). There is significant intraindividual and interindividual variability in the dose/exposure relationship.

Drug-drug, drug-disease, drug-food, and drug-environment interactions are just a few of the confounding factors that might alter dose/concentration relationships. Patients will require different adjustments for dosage and target range. ISD regimen adherence is vital and necessitates greater observation, particularly in patients who are teenagers or young children (Seger et al. 2016). ISDs are essential for both very low acute allograft rejection rates in transplant recipients and short-term patient survival. Therapy-related

adverse effects or treatment failure can be prevented by using customized medicine, which involves TDM to modify the dosage for each patient. This is necessary because of the narrow therapeutic index and high inter-patient pharmacokinetic variability of IDs. Effective TDM is required to accomplish this. However, without the appropriate clinical experience and analytical tools, it would not be feasible. This study aims to give a critical overview of the current clinical practices and analytical methodologies for the TDM of ISDs, as well as guidance for establishing reliable TDM. It also discusses some of the important practical elements of TDM (Seyfinejad and Jouyban, 2021).

6. Analytical validation

There are presently no established bioanalytical validation requirements for techniques based on dried blood samples. A few tests (such as freeze-thaw stability, which depends on storage and transportation conditions) might not be applicable as outlined in these guidelines, while others would need to be refined. It will also be necessary to assess a few more factors (Timmerman et al. 2011; Jager et al. 2014). As a result, during technique validation, a marginally higher number of samples will need to be examined. Before beginning any analytical validation, it is crucial to consider the method's required quality (Alffenaar et al. 2018). Despite FDA and EMA standards recognizing analytical performance requirements, DBS methodology may not always meet them. Based on scientific evidence, these requirements can be stringent, depending on the analyte and the method's intended use. Some have proposed applying biological variation-based acceptance criteria in this case, as is standard procedure in other clinical chemistry domains (Capiau et al. 2019).

7. Clinical validation

The DBS sampling method is only suitable for routine TDM care after being validated in a clinical study (Enderle et al. 2016; Zakaria et al. 2016; Hoogtanders et al. 2007; Kloosterboer et al. 2018). This would mean that blood, serum, or plasma analysis would partially replace standard venous whole-blood sampling. Clinical validation research involves analyzing paired DBS samples with venous blood, plasma, and/or serum samples. Clinical validation aims to demonstrate that DBS results can be used to compare data from traditional TDM methods, such as blood, serum, or plasma examinations. This guideline provides practical suggestions for clinical validation of a DBS test for TDM. Current recommendations for clinical validation are based on published studies using real finger prick blood-derived DBS, paired DBS and traditional matrix samples from at least 20 patients, and suitable statistical analysis (Kloosterboer et al. 2018; Veenhof et al. 2017; Zwart et al. 2018; Berm et al. 2016).

8. Structure for assessing data in support of TDM

It produced a framework to assess the evidence supporting TDM by altering the consensus recommendations for TDM of psychiatric medicines established by the AGNP (Arbeitsgemeinschaft für Neuropsychopharmacology und Pharmakopsychiatrie). This made it easier for the working group to concentrate on a standardized method for assessing the TDM evidence. An additional, higher degree of recommendation is the primary change we made for its use in cancer (Buko, 2017). A shift in a symptom-scale score is frequently used to measure clinical efficacy in psychiatry, but in cancer, it is important to distinguish between survival benefit—which is frequently necessary for FDA approval—and proof of activity, which is measured by tumor reduction. Phase II cancer trials' response rate doesn't indicate survival benefit in phase III trials, and randomized double-blind studies are considered best, but rarely conducted due to logistical difficulties. Furthermore, investigations with fixed dosages are preferred to establish the association between exposure and result (Nguyen and Fenn 2007).

9. Mass spectrometry technologies

9.1. Mass spectrometers

Triple quadrupole mass spectrometers are commonly used in clinical labs for quantitative analysis, utilizing metal rods to filter mass ions and transmit a specific m/z ion band. This selection usually has an accuracy of ~1 Da. A "triple quadrupole" is a set of quadrupoles that select for m/z twice, with the second quadrupole fragmenting the ions selected in the first filtering stage. Fragmentation enhances analytical precision as ionized compounds, including adducts, produce distinct fragmentation products despite their seemingly similar parent m/z. This characteristic is exploited by the third quadrupole, which chooses fragmentation products that arise. This kind of ion detection is referred to by one of two terms, selective reaction monitoring (SRM) or multiple reaction monitoring (MRM), depending on the number of mass changes being observed. Throughout an analysis, SRM detection just keeps an eye out for one transition. During an analysis, MRM detection keeps an eye out for several transitions—but only one at a time. In recent years, MRM-based techniques have been increasingly used to analyze protein digest peptide products, despite the majority of triple quadrupole MS being used for small molecule research (Grayson, 2011). The development of

software that simplifies the selection of transitions for peptide detection has significantly enhanced this method (Robb et al. 2000). Furthermore, the process of unique fragmentation is exploited by the method for establishing analytical specificity when using triple quadrupole mass spectrometers to screen for biomarkers (Pappin et al. 1993; Dingle and Butler-Wu, 2013; Zhang et al. 2004). The "precursor ion scan" scanning mode is frequently used for this research. The triple quadrupole searches for desired fragments in scanning mode, maintaining the last mass filter constant for a specific production arising from a specific collision energy. When targeting a known class of molecules (phosphates, sulfates, steroids, etc.) that yield identical fragments, this tactic can be helpful. Multiplex screening of several hundred possible biomarkers might be accomplished with the MRM test.

A smaller group of interesting biomarkers might be chosen based on the outcome and subjected to additional validation, most likely using immunoassays. MRM MS is cost-effective and faster, with hybrid instruments like Q-ToF, Triple-TOF, and Q-Orbitrap providing high resolution and fragmentation capabilities. These analyzers have the highest level of analytical specificity, which makes them indispensable for biomarker discovery research. However, they might be less sensitive to low-concentration analytes (less capacity to detect a low-concentration analyte) than a triple quadrupole analyzer. Hybrid mass spectrometers aid in biomarker discovery by analyzing chromatographic features for significant quantitative data and providing structural information for fragmentation events, either retrospectively or triggered. This analytical technique is used for shotgun proteomics, which involves breaking down proteins into peptides and sequencing them based on their MS/MS spectra, or for small molecule screening. The Triple-TOF enables sequential windowed acquisition of all theoretical ions (SWATH), a data-independent acquisition method, due to its lower duty cycle compared to Orbitrap-based analyzers (Kerian et al. 2014; Wu et al. 2013; Nemes and Vertes, 2007).

9.2. Fragmentation

The most popular technique for breaking up parent ions is collision-induced dissociation (CID), which is used in hybrid devices and triple quadrupole mass spectrometers. It entails putting an ion into an area where collision gas concentrations are high—nitrogen or argon—by applying an electrical potential to it. It can vary the applied potential to give varying levels of analytical specificity. CID is contrasted with other fragmentation techniques. Higher-energy collisional dissociation (HCD) uses a multipole collision cell for Orbitrap, eliminating low mass cutoff and allowing isobaric tag quantification, while electron transfer dissociation offers energy advantages. ETD makes it possible to sequence changed peptides more thoroughly and may help with the better characterization of glycosylated and phosphorylated peptides (Garg and Zhang 2016).

9.3. Ionization sources

Electrospray (ESI) revolutionized biological mass spectrometry by enabling the conversion of biologically generated compounds from liquid phase to gas phase, a traditional technique (Amirav, 2017). ESI discovery, created by John Fenn and Koicihi Tanaka, revolutionized biological mass spectrometry, earning them the 2002 Nobel Prize in Chemistry (Ojanperä et al. 2012). Atmospheric pressure chemical and photoionization are the primary methods for converting liquid phase molecules to gas phase, but are typically restricted to specialized compounds (Amirav, 2017). The low pre-analysis work-up associated with matrix-assisted laser desorption ionization (MALDI) is a plus. The standard procedure entails combining materials with a chemical matrix, then using a high energy laser to form ions. For the analysis of basic protein digests, this method (peptide mass fingerprinting) is incredibly practical (Thunig et al. 2012). Pre-analysis of complex specimens requires more time than simply connecting a liquid chromatograph system to an electrospray ionization source. MALDI has made significant progress in the field of clinical utility within the microbiology laboratory. For quickly screening cultivated organisms, MALDI is fast becoming the lead (Kauppila et al. 2011).

SELDI is a MALDI variant that uses a substrate to attach proteins to a surface, removing interferences. SELDI findings have led to the development of commercialized tests like OVA1, an IVDMIA for guiding exploratory surgery in women with abdominal masses, though used less frequently (Thunig et al. 2012). More recently developed direct atmospheric ionization devices show promise as an adjunct to conventional pathological tissue inspection methods. Typically, the tissue to be studied is sectioned, fixed, and stained with a variety of techniques (either immunology- or dye-based), which can be used to diagnose a patient when analyzed by a pathologist with training. MS interprets tissue segments in multiple dimensions, with direct tissue ionization providing molecular insight and specificity, eliminating the need for epitope binding or dye affinity (DelGuidice et al. 2020). While additional versions are also being developed quickly (Jagerdeo et al. 2015), the two most often used direct ionization procedures are Desorption Electrospray Ionization (DESI) (Wu et al. 2007) and Laser Ablation Electrospray Ionization (LAESI) (Wu et al. 2007).

10. Application to toxicology screening

It has been demonstrated that toxicology screening can help clinicians provide better care and treatment for their patients. Drug test findings in the emergency room seldom affect the way patients are treated during an emergency because of several reasons, such as delayed results, low sensitivity and specificity, and difficult interpretation (Cuypers et al. 2016). The National Academy of Clinical Biochemistry released guidelines in 2003 regarding the use of laboratory testing to assist patients who have been poisoned in the emergency room. The necessity of developing novel drug tests and enhancing quick drug detection methods in order to adapt to evolving drug abuse trends was emphasized here (Cuypers et al. 2016). The utilization of Q-TOF-MS in drug screening applications has shown promise in recent years as an analytical method to address many of the present and upcoming drug detection difficulties. Urine is the most common sample type used in clinical settings for drug screening nowadays, and immunoassay methods continue to be the most widely used technology. Because immunoassays are quick and simple to automate on standard biochemistry platforms, they are a helpful tool for screening a variety of medication classes. Despite these benefits, immunoassays have several intrinsic limitations that should be taken into account when applying the results in a clinical context.

One of the biggest issues with drug detection is its limited sensitivity and specificity, which can result in both false positive and false negative results. Inconsistent cross-reactivity profiles between different immunoassay techniques, varying cut-off levels for analyte detection, and immunoassay interferences, such as adulteration, are additional technical factors that could make clinical interpretation more difficult. GC-MS, which uses extensive universal chemical libraries to provide mass spectrometry's enhanced sensitivity and specificity, has been the gold standard for systematic toxicological analysis for decades. The fact that highly polar, non-volatile, or thermally unstable chemicals must be chemically modified to be analyzed by GC-MS can be a drawback of the technique, necessitating labor-intensive and intricate sample preparation procedures. The literature is replete with well-documented limitations of the immunoassay screening procedures now in use. Marin et al. conducted a study wherein 3571 urine samples that tested positive for amphetamine-type compounds by immunoassay (EMIT® II) yielded 389 false positive results (11.9%) upon confirmation by LC-MS-MS. The use of immunoassay-based medication screening in patients receiving chronic pain treatment for compliance monitoring was also examined in a recent study.

Comparing immunoassay methods for opiate detection against LC-MS-MS revealed 21% false negative results, suggesting that immunoassays may not be sensitive enough for medication compliance. In several therapeutic situations, the adoption of mass spectrometry-based methods for compliance monitoring was also advised. For instance, patients may include medication in their urine samples to mimic compliance. The parent medication was found in the sample, and mass spectrometry methods also revealed the absence of recognized urine metabolites that would have indicated the proper compliance. In cases of polypharmacy, it was also advised that patients identify unknown prescriptions or illicit drugs to identify potentially fatal drug interactions (Jannetto and Fitzgerald 2016)). Many of the limitations related to immunoassay and GC-MS have been resolved in recent years by applications that make use of Q-TOF-MS techniques. Because mass spectrometry has a high sensitivity and specificity by nature and may use large compound libraries, quick and thorough Q-TOF-MS screening methods are being used more frequently in forensic and therapeutic settings.

The requirement for individual laboratories to invest in the in-house production of spectral libraries has also been substantially eliminated by the availability of sizable commercial libraries created by vendors for use with their related platforms. Ultra-high performance liquid chromatography (UHPLC) and Q-TOF-MS have been routinely coupled, which has made sample preparation easier and made it possible to identify a wider variety of chemicals. According to Tsai et al. UHPLC-QTOF-MS was used to test for 62 drugs of abuse and their metabolites using a straightforward 5-fold dilution of urine with deionized water. Eight acidic compounds could be found in a 12-minute runtime in negative ionization mode and 54 basic compounds in a 15-minute runtime in positive ionization mode (Adaway et al. 2015).

11. Applications of MS in toxicology

MS and its hyphenated applications (GC/LC/ICP-MS) are powerful analytical tools in toxicology, analyzing volatile and heat-stabilized compounds, non-volatile and heat-labile compounds, and metals using ICP-MS (Hernandez et al. 2012; Kaufmann, 2012) MS applications are crucial for the toxicological investigation of pharmaceuticals and poisons due to their analytical adaptability, specificity, sensitivity, dynamic range, and ability to screen numerous unrelated substances. Drug analysis is currently used in PK/PD research as well as focused applications (e.g., TDM and pain

management) and screening applications (e.g., drugs of abuse (DOA), forensic toxicology in Table 1, environmental toxicology, and clinical toxicology) (Tamara et al. 2021; Lee and Kerns, 1999; Ma and Chowdhury, 2013; Zhu et al. 2011; Kang and Lee, 2009; Shafiee et al. 2019; McKeating et al. 2016; Carlier et al. 2015; Meneghello et al. 2018).

Table 1. Primary modifications and analysis techniques used in forensic toxicology analysis using mass spectrometry

Kind of MS analysis	*Mass spectrometry ionization methods combined with techniques for separation*	
	Advantages	*Disadvantages*
Laser diode thermal desorption	(i) The process is fully automated.	(i) It is not possible to simply switch between the positive and negative ionization modes. (ii) Before the liquid samples are moved towards the capillary surface, further sample preparation is required. The effects of interferences in complicated biological samples need to be investigated.
Negative chemical ionization	(i) Because of the stability of the electronegative moieties, it offers greater sensitivity at low concentrations (pg). (ii) Saves time by preventing incorrect interpretations of accurate results.	(i) Combining the approach with EI-MS yields better results by obtaining additional structural information. (ii) Methane is a typical reagent that is needed for the ionization in this procedure.
Touch spray	(i) Ionization facilitates the examination of solid or liquid samples without the need for pretreatment because the substrate (medical swabs) can be utilized as a tool for sample collection. (ii) Neat oral fluids can be directly and noninvasively sampled using the TS-MS.	(i) Of all the steps in the analytical process, the drying step for this substrate takes the longest.
Desorption atmospheric-pressure photoionization	(i) High salt content matrices do not provide a high ionization suppression.	(i) The biological matrix can affect the presence of high-suppression ionization. (ii) Sample preparation is frequently required to prevent ionization from being suppressed.
Atmospheric solids probe analysis	(i) It is simple to conduct solids and liquids analyses. (ii) During the analysis, this design permits a positive/negative switch.	(i) More research needs to be done on the effects of interferences in complicated biological samples. (ii) By reducing the study of high-molecule substances, this approach improves sensitivity when analyzing small-molecule medications.
Metal-assisted secondary ion mass spectrometry	(i) The target's distribution spectra can be obtained by coupling it to mass spectrometric imaging (MSI). (ii) When compared to the results obtained with LC-MS/MS as well as MALDESI, the limits of detection are lower. (iii) Sample preparation is not required to be done.	(i) There has been no quantitative analysis performed.
Low-temperature plasma	(i) Direct analysis can be carried out without sample preparation. (ii) The instrumentation is basic, and its setup allows for the use of air as the discharge gas and minimal discharge gas usage. (iii) It is possible to achieve high sensitivity and sensitivity without pretreating the samples.	(i) Only tiny organic compounds with low to moderate polarity are employed in this approach.

11.1. Addressing limitations of Immunoassays (IA) in TDM and drug screening

GC and LC-MS applications in toxicology emerged to overcome the limitations of IAs in drug analysis, as MS applications emerged during IAs' established presence in clinical laboratories. Manufacturers often design IAs for FDA test approval based on economic interests, leading to poor analytical specificity and interferences, which end users often have little input in (Hernandez et al. 2012; Kaufmann, 2012; Tamara et al. 2021; Lee and Kerns, 1999; Ma and Chowdhury, 2013; Zhu et al. 2011; Kang and Lee, 2009; Shafiee et al. 2019; McKeating et al. 2016; Carlier et al. 2015; Meneghello et al. 2018). When it comes to tiny pharmaceuticals, the precision of IAs is typically restricted to identifying drug classes rather than specific medications within a class. This restriction may arise from the fact that

antibodies typically identify epitopes on big macromolecules, meaning that IAs have low specificity when it comes to identifying particular tiny molecules (Hernandez et al. 2012; Meneghello et al. 2018). IAs are commonly used in first-line toxicology screening for identifying potentially negative samples and drug classes like phencyclidine, methadone, fentanyl, benzodiazepines, and amphetamines. The standard procedure involves screening using immunoassay, followed by confirmation using GC-MS or LC-MS techniques for specific molecule identification due to high false positive and false negative results. Immunoassays are typically available as FDA-approved tests on large, automated analyzers (Strathmann and Hoofnagle, 2011; Jannetto and Fitzgerald, 2016).

11.2. Drug analysis by GC-MS

The coupling of GC to MS enabled the construction of regular applications with the sensitivity and specificity of MS (Lynch et al. 2010; Yuan et al. 2015). GC is an analytical technique that separates molecules by partitioning them into stationery and gas phases, using a liquid or polymer stationary phase and a gas mobile phase. High temperatures, up to 350°C, are required for chemical elution into the mobile gas phase, facilitating MS detection by separating analytes and entering the gas phase using EI sources. EI ionization creates a consistent fragmentation pattern from organic compounds by utilizing high-energy electrons to remove electrons from analyte molecules at high temperatures (Lynch et al. 2010). Large EI-GC-MS libraries have been developed for spectrum matching-based identification, making EI-GC-MS data valuable for interlaboratory spectral comparisons (Lynch et al. 2010; Yuan et al. 2015). EI-GC-MS libraries enhance in-house libraries, improving GC-MS-based compound identification, and making it an effective method for untargeted detection and quantification of small compounds with MS specificity. EI-GC-MS is still utilized for broad unknown screening applications using various sample types (Jannetto and Fitzgerald, 2016; Yuan et al. 2015). GC-MS is frequently used to confirm positive results from drug screens in clinical toxicology (Adaway et al. 2015; Lynch et al. 2010; Yuan et al. 2015; Lynch et al. 2010). To function properly with GC-MS, some analytes require chemical derivatization to make them volatile and heat-stable (Yuan et al. 2015; Lynch et al. 2010).

11.3. Applications of GC-MS in Toxicology

GC-MS offers advantages like homogenous gas mobile phase, efficient separation, precise temperature programming, and library-based toxic compound identification through EI-MS databases, compared to LC-MS/MS (Chèze et al. 2008; Domin and Cody, 2014). GC-MS is widely used for drug screening, dangerous chemical identification, doping control, environmental analysis, and clinical and forensic toxicology due to its high specificity and MS sensitivity (Gahlaut et al. 2014). GC-MS is frequently used in emergency care settings to screen blood and urine for acute overdoses of prescription and over-the-counter medicines, particularly those with harmful side effects (Langman and Kapur, 2006; Shannon et al. 1998; Holstege and Borek, 2012). GC-MS is commonly used in forensic investigations and clinical evaluations for drug screenings, identifying and quantifying poisons like barbiturates, opioids, stimulants, anesthetics, anticonvulsants, antihistamines, and sedative-hypnotics (Smith et al. 2007). GC-MS is a useful tool for environmental toxicology screening hazardous compounds such as chlorophenols, PAH, dioxins, dibenzofurans, organo-chlorine pesticides, herbicides, phenols, halogenated pesticides, and sulfur analysis in air (Goullé et al. 2014). Most toxicology laboratories with financial means are transitioning from GC-MS to LC-MS for targeted drug screenings in clinical and forensic toxicology applications (Viette et al. 2012; Freudenberger et al. 2016).

12. Conclusions

MS has revolutionized TDM and analysis, providing precise quantification and broad analyte coverage. Future advancements should focus on standardization, technological improvements, and interdisciplinary collaboration for optimal patient care and public health. The integration of MS into clinical toxicology has significantly improved TDM and analytical investigations. MS provides precise drug quantification across various biological matrices, enabling personalized treatment strategies and accurate assessment of drug efficacy and safety. The tool's superior sensitivity and specificity, especially in challenging sample matrices, make it a crucial component in modern toxicology laboratories. MS aids in identifying and quantifying emerging psychoactive substances and designer drugs, thereby enhancing the understanding of the evolving clinical toxicology landscape. Future developments in mass spectrometry clinical applications in toxicological investigations present numerous opportunities for further advancement. The standardization of methodologies and data interpretation protocols is crucial for maintaining consistency and reliability across various laboratories. Technological advancements aimed at improving the sensitivity, speed, and throughput of MS instruments are expected to expand their utility in clinical practice. The demand for interdisciplinary collaboration between clinical toxicologists, analytical chemists, and forensic experts is increasing to challenge emerging challenges and enhance MS-

based methods in toxicological investigations. The widespread adoption of MS technologies in clinical settings will be significantly aided by efforts to enhance their cost-effectiveness and accessibility. Innovation and collaboration in MS can enhance the precision, efficiency, and clinical impact of toxicological investigations, ultimately improving patient care and public health outcomes. MS in toxicological investigations is expected to improve precision, sensitivity, and affordability, making it a crucial tool in TDM. Standardization and interdisciplinary collaborations will optimize data interpretation, and adapting to emerging challenges will enhance patient care.

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