In this issue: Recent Developments in the Clinical Application of Mass Spectrometry

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Mass spectrometry applications for toxicology

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ABSTRACT

Toxicology is a multidisciplinary study of poisons, aimed to correlate the quantitative and qualitative relationships between poisons and their physiological and behavioural effects in living systems. Other key aspects of toxicology focus on elucidation of the mechanisms of action of poisons and development of remedies and treatment plans for associated toxic effects. In these endeavours, Mass spectrometry (MS) has become a powerful analytical technique with a wide range of application used in the Toxicological analysis of drugs, poisons, and metabolites of both. To date, MS applications have permeated all fields of toxicology which include; environmental, clinical, and forensic toxicology. While many different analytical applications are used in these fields, MS and its hyphenated applications such as; gas chromatography MS (GC-MS), liquid chromatography MS (LC-MS), inductively coupled plasma ionization MS (ICP-MS), tandem mass spectrometry (MS/MS and MS^n) have emerged as powerful tools used in toxicology laboratories. This review will focus on these hyphenated MS technologies and their applications for toxicology.

Abbreviations (in alphabetical order)

***ADME**: absorption, distribution, metabolism, and elimination*
***APCI**: atmospheric pressure chemical ionization*
***API**: atmospheric pressure ionization techniques*
***CI**: chemical ionization*
***CID**: collision induced dissociation*
***DOA**: drugs of abuse*
***DRC**: dynamic reaction center*
***EI**: electron ionization*
***ESI**: electrospray ionization*
***FDA**: food and drug administration*
***FS**: full scan*
***FT-ICR**: fourier transform ion cyclotron resonance*
***FT-IT**: fourier transform ion trap*
***FWHM**: full width at half height*
***GC**: gas chromatography*
***GC-MS**: gas chromatography mass spectrometry*
***GLC**: gas-liquid chromatography*
***HR**: high resolution*
***IA**: immunoassays*
***ICP-MS**: inductively coupled mass spectrometry*
***IT**: ion trap*
***LC**: liquid chromatography*
***LC-MS**: liquid chromatography mass spectrometry*
***m/z:** mass to charge ratio*
***MALDI**: matrix assisted laser desorption ionization*
***MRM**: multiple reaction monitoring*
***MS**: mass spectrometry*
***MS/MS** and **MS^n**: tandem mass spectrometry*
***MW:** molecular weight*
***PAH**: polycyclic aromatic hydrocarbons*
***PK/PD**: pharmacokinetic/pharmacodynamics*
***Q1**: first quadrupole in MS instrument*
***Q2**: second quadrupole in MS instrument*
***Q3**: third quadrupole in MS instrument*
***QE** or **Q Exactive**: hydrid qudrupole-orbitrap mass spectrometer*
***QIT**: quadrupole ion traps*
***QTOF**: hybrid quadrupole time-of-flight mass spectrometer*
***RF**: radion frequency*
***SRM**: single reaction monitoring*
***TDM**: therapeutic drug monitoring*
***TOF**: time of flight*
***TQ-MS/MS**: triple quadrupole tandem mass spectrometer*
***WD**: waldenstrom's disease*
***2D**: two dimension*
***3D**: three dimension*

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INTRODUCTION

Toxicology can be thought of as the study of poisons, how poisonous encounters occur, how individuals respond to these encounters, and how to develop strategies for the clinical management of toxic exposures[1]. Poisons can be broadly defined as biologically active substances causing toxic effects in living systems. In essence, any biologically active molecule capable of altering normal physiology within a living system becomes a poison upon accumulation to quantities sufficient for a toxic effect[1]. For this reason, even therapeutic remedies can become poisons and toxic effects depend not only on the dose, but also on the overall pharmacokinetic and pharmacodynamic effects[2].

Since we are constantly surrounded by various chemicals, exposure can occur at home, work, or from the environment. The sheer complexity of possible poisons requires the use of sophisticated analytical tools and techniques to evaluate toxic exposures[3-6]. Toxic evaluations usually begin with qualitative or quantitative assessment in order to identify and/or quantify a toxic substance that could account for observed toxic syndromes (toxidromes) which are characteristic of different classes of poisons[7]. In addition, identification of the source for toxic

exposures is equally important. However, the overall role of laboratory testing is to identify and confirm the presence of a suspected poison and also to provide prognostic information when test results are able to predict clinical outcomes and/or help guide patient management.

In toxicology, the general analytical scheme for assessment of poisons in various matrices involves; 1) extraction, 2) purification 3) detection and 4) quantification (Scheme 1, A)[8]. The rise of modern analytical tools used by toxicology laboratories seems to have coincided with the chemical/industrial revolution (roughly 1850's to 1950's). A time which saw development of new liquid-liquid and solid-phase extraction methods along with qualitative or quantitative methods of detecting poisons based on their physical characteristics[8,9]. By the early twentieth century, chromatographic techniques using differential migration processes for separation of target molecules were developed by Mikhail Tsvet[9] and with the first versions of modern separation techniques such as liquid chromatography (LC) and gas-liquid chromatography (GLC or simply gas chromatography, GC) became routine in both analytical and preparative applications by mid-20th century[1,10,11]. At this time, labs also started to see the development of the first versions of modern mass spectrometers being used primarily for analysis of relatively pure materials[11-12].

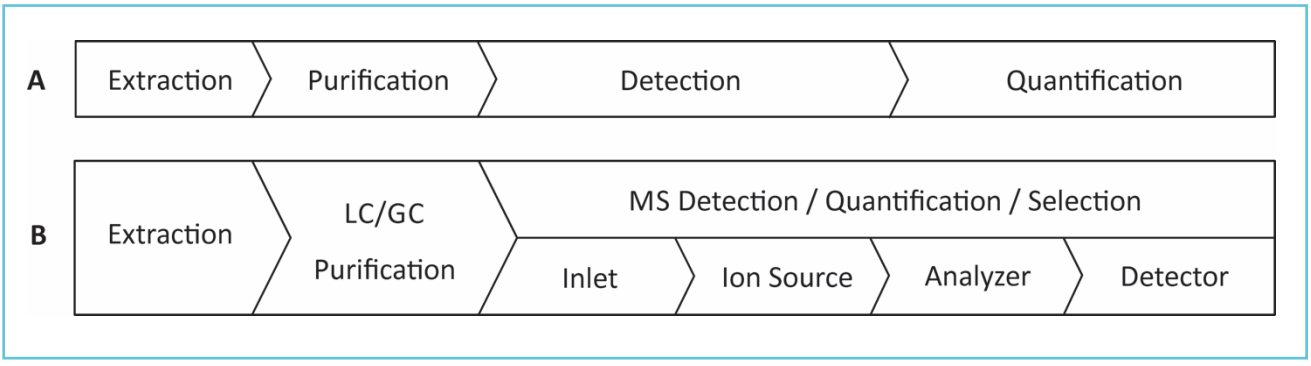
As MS, GC and LC technologies continued to advance in the second half of the 20th century, the more sophisticated methods used in modern toxicology laboratories started to emerge as amalgamations of separation and detection modes, creating new powerful analytical applications.

These included; high pressure liquid chromatography (HPLC), GC-MS, LC-MS, MS/MS and MS^n. These new technologies were initially used by research laboratories and later adopted into clinical laboratories[11,13]. To date, many of the modern analytical applications such as GC-MS and LC-MS still incorporate the same analytical scheme used by the earliest toxicology laboratories. But they are more stream-lined by combining multiple steps in the process with potential for automation (Scheme 1, B). This review will highlight current MS applications for Toxicology.

Mass spectrometry

Mass spectrometry is a quantitative technique which determines the mass-to-charge (m/z) ratio. In general, a mass spectrometer can be divided into four main components (Scheme 1, B): 1) a sample inlet, 2) an ion source, 3) a mass

Scheme 1 The analytical process for toxic compound evaluation in toxicology



**A) Steps involved in toxic compound isolation, identification and quantitation.*
B) GC-MS and LC-MS amalgamation of steps in the analytical process for toxic compound detection and quantitation.

analyzer, and 4) a detector. The sample inlet is where the sample enters the instrument before reaching the ion source. Ion sources are generally distinguished based on their underlining ionization technique[11,12]. The ionization technique used will determine the type of sample (e.g solid, liquid, vs gaseous samples) that can be analyzed in a given instrument and therefore also the type of chromatographic separation technique that should be coupled to the MS. Furthermore, the efficiency of sample ionization also determines in part the instrument's analytical sensitivity[11,12]. MS instruments in toxicology laboratories generally have LC or GC front ends, feeding into the instrument inlet either a liquid or gaseous sample for downstream ionization, analysis, detection, and quantitation (Figure 1, A-C)[3,4].

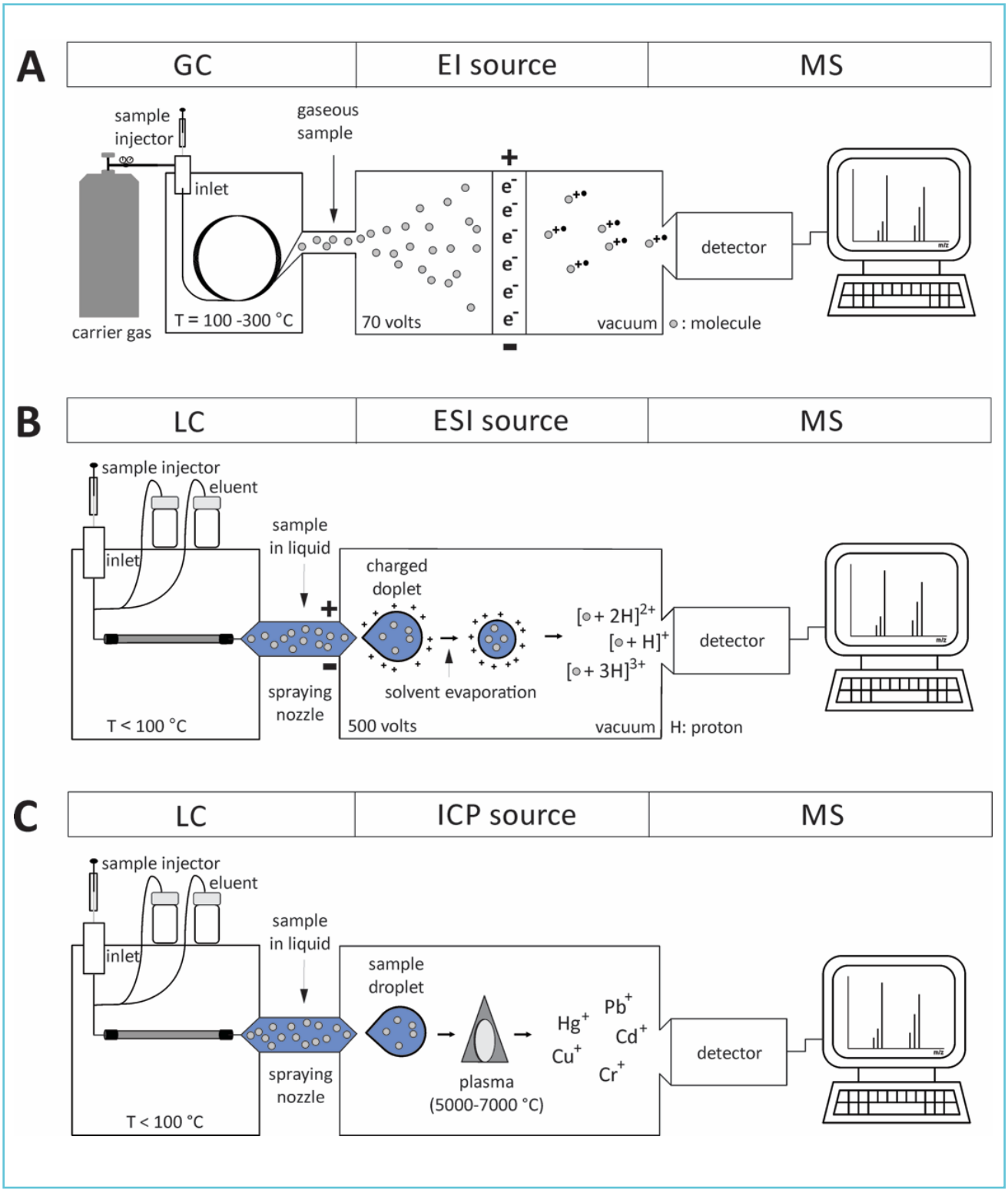
Common ionization techniques used by GC-MS include; electron ionization (EI) and chemical ionization (CI) for analysis of volatile and heat stable compounds (Figure 1A, GC-MS)[11]. For LC-MS, Atmospheric pressure ionization techniques (API) such as; electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) are used for non-volatile and heat labile compounds (Figure 1B, LC-MS). Inductively coupled plasma ionization (ICP) is another ionization method used for elemental analysis usually for metals determination using ICP-MS (Figure 1C, ICP-MS) and matrix assisted laser desorption ionization (MALDI) for ionization of solid samples for MS analysis. Since MALDI techniques are not commonly used in toxicology applications, these won't be discussed in much detail here. Furthermore, the focus will be on the more prevalent EI, ESI and ICP ionization techniques used for toxicology applications despite the fact that modern GC-MS and LC-MS instruments can usually switch between EI/CI and ESI/APCI ionization mechanisms, respectively[4,5,11].

Mass analyzers and MS performance

From the ion source, sample ions enter the mass analyzer. Mass analyzers are the heart of the instrument and determine key performance characteristics such as the instrument's mass resolution, accuracy, and range. The mass range is the analytical mass range of the instrument. The resolution determines the ability of the analyzer to resolve two adjacent masses on the mass spectrum and is defined by the full width of the mass peak at half height of the peak maximum (FWHM). For a given *m/z* value, the resolution can be expressed as a ratio of *m/z* to FWHM such that for an ion with *m/z* 1000 and peak width of 0.65 atomic mass unit (amu) at FWHM the resolution is 1538. Low resolution instruments have FWHM > 0.65 amu and high resolution instruments reaching FWHM < 0.1 amu. The mass accuracy of MS instrument refers to the error associated with a particular *m/z* measurement. High mass accuracy gives the ability to measure the true mass of an ion to more decimal points. For example if the true mass of target ion is 1000 *m/z* and the measured mass from the instrument is 1000.002 *m/z*. The mass accuracy can be expressed in parts per million based on the ratio of the difference between the true mass and the measured mass to that of the true mass. So a ratio of 0.002/1000 which equals 0.000002 or a mass accuracy of 2 ppm in this example.

Mass analyzers typically used in toxicology include; quadrupole, ion traps, time of flight (TOF) and sector[4,11,15,16]. Quadrupole analyzers use four parallel metal rods to create a variable electromagnetic field which allows ions within a particular *m/z* range to reach the detector in order to record the mass spectrum. Quadrupole analyzers are cheap and robust, but can typically only achieve resolution around 1000 and mass accuracies of 100 ppm[16].

Figure 1 **Simple representation of A) GC-MS; B) LC-MS; and C) ICP-MS instruments and the ionization process for EI, ESI, and ICP occurring prior to mass analysis and detection in the mass spectrometer**



Ion trap (IT) instruments include quadrupole ion traps (QIT), Fourier Transform Ion Cyclotron Resonance (FT-ICR) and orbitraps. QIT use 2D or 3D quadrupole fields to trap target ions in a confined space and the mass spectrum is acquired by scanning the radion frequency (RF) and direct current (DC) fields to eject selected ions for detection[11,12]. Resolution for QIT is about 1000 – 10,000 with mass accuracy > 50 ppm[16]. FT-ICR are ion trap that keep ions in cyclotron motion within the trap. *m/z* detection occurs through measurement of induced currents from changes in ion orbits when an RF field is applied. This, allows calculation of *m/z* values with high accuracy (resolution > 200,000 and accuracy 2-5 ppm)[11,12,16]. Orbitraps use a metal barrel to create an electrostatic field for trapping ions in cyclical motion. The detection method is similar to that use in FT-ICR traps but with lower resolution < 150,000 but similar mass accuracy to FT-ICR[16].

TOF mass analyzers use a fixed potential to accelerate ions through a drift tube. Since all ions in a given pulse will attain the same kinetic energy, ions accelerate according to their *m/z* value and the mass spectrum is collected based on the time it takes individual ions to strike the detector. TOF analyzers generally have a higher mass range than quadrupole and IT instruments with relatively high resolution (1000 - 40,000) and mass accuracy (> 5 ppm)[16].

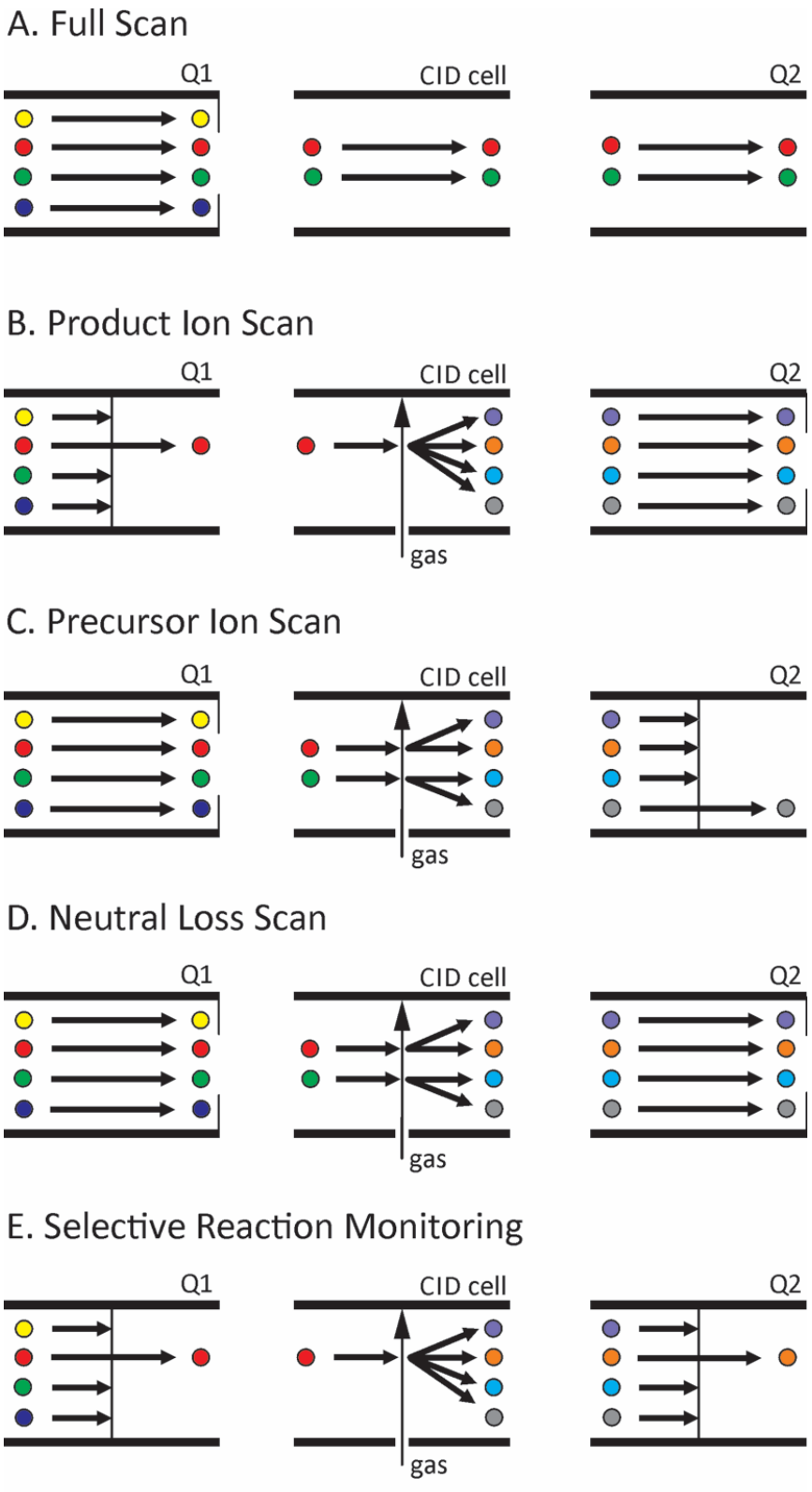
Sector analyzers are either magnetic sectors or double focusing (magnetic and electric) sectors. Similar to a TOF analyzer, magnetic sectors use a fixed potential to accelerate ions coming from the source such that ions attain the same kinetic energy but different momentum according to their *m/z*[16]. Accelerated ions are then passed through a magnetic field which guides ions through an arched path in order to strike the detector according to their momentum to charge ratio. By scanning the magnetic field strength, ions with different *m/z* are selected for detection. In magnetic sectors, resolution is limited by minor kinetic energy dispersions (ion velocities). A double focusing sector analyzer adds a electric field before or after the magnetic field to also focus ions according to their kinetic energy to charge ratios. Focusing ions of different velocities to the same point. This gives double focusing magnetic sectors relatively high resolution (100,000) and high mass accuracy (<1 ppm)[16].

In summary, the ion source, mass analyzer, and detector for a particular instrument all play a role in defining the instrument's analytical capabilities. It is also important to note that even though the basic design of MS instruments has stayed relatively unchanged over time, the performance capabilities of MS sources, analyzers, and detectors have continued to improve over time[4,11,13,15]. The strength of MS for Toxicology is the combined sensitivity and specificity that is needed to identify and quantify the toxic agents.

MS instruments

The versatility of MS analytical applications comes from the ability to couple different separation techniques in the front-end (i.e. GC or LC) and various analyzers either in tandem or hybrid configurations[4,5,11,12,15]. The type and arrangement in a given instrument not only determines its resolution, mass accuracy, and analytical range, but also the type of experiment(s) possible for analytical applications (Figure 2, A-E)[4,11,13,15]. In clinical applications, the MS instrument with most versatile capabilities is perhaps the triple quadrupole tandem mass spectrometer or TQ-MS/MS with three quadrupole analyzers arranged in tandem for MS/MS experiments[13]. The first quadrupole (Q1) selects ions that will enter the second quadrupole (Q2), a collision cell able to carry out collision induced dissociation (CID) of selected ions. From the collision cell, product ions enter the third quadrupole (Q3) which can guide selected ions into the detector. TQ-MS/MS instruments are capable of

Figure 2 **Analytical experiments possible with a TQ-MS/MS instrument**



performing full MS scans (FS, Figure 2A), multiple reaction monitoring (MRM, Figure 2, B-E), or single reaction monitoring (SRM, not shown) for analyte detection[13,3].

The MS/MS experiment involves selected fragmentation of target ions using CID followed by analysis of the products (Figure 2B, product ion scan)[13]. The target ion is often referred to as parent ion and CID fragments are referred to as product ions. In MS/MS experiments, MRM will follow the conversion of one parent ion to one product ion via CID (indicated as parent *m/z* > product *m/z*) or any experimentally feasible combination of parent and product ions given analytical capabilities of the instrument. MRM and SRM usually increases sensitivity based on improved signal to noise ratio, and the MS/MS offers increased specificity at the cost of decreased sensitivity since signal is lost at each round of fragmentation. Specificity improves when unique fragmentation patterns are able to distinguish co-eluting ions with identical exact mass as targeted molecule, but different chemical composition. In addition, MS/MS can also be used for structural determinations. A key advantage of the TQ-MS/MS instrument is the ability to do precursor ion scan (PI, Figure 3C) or neutral loss (NL, Figure 3D) reaction scans over a wide *m/z* range[4,11,13,15]. This application can use a single sample injection for rapid scanning of the full *m/z* spectrum in order to identify compounds with known functional groups that dissociate as detectable ions or neutral masses following CID.

Due to the tandem arrangement of quadrupole analyzers in the TQ-MS/MS, MS/MS is done sequentially in space between different analyzers. In IT instruments (QIT, Fourier transform ion trap or FT-IT, and orbitrap), MS/MS experiments are done in sequence over time based on the ability of the trap to retain selected ions following each round of CID[4,11]. MS/MS also occurs with high efficiency in IT instruments but one key limitation is the capacity to retain ions and *m/z* scanning speed[4,11]. 2D ion traps were designed to overcome the ion capacity problem and have a higher analytical range giving FS, SRM, and MRM capabilities over a wider *m/z* range compared to 3D ion traps[4,11]. The in-time MS/MS application of IT instruments means PI and NL screening experiments are not possible. However, MS^n experiments for structural determination of larger molecules are possible, usually with no more than three rounds of fragmentation due to loss of signal following each consecutive round of CID[4].

Over time, MS instruments have continued to improve in selectivity, mass accuracy, and resolution, along with formation of hybrid instruments with enhanced capabilities often designed to overcome limitations of available instrumentation. For example, one key limitation of TQ-MS/MS instruments is that the PI/NL scans cannot be performed in a single injection along with MS/MS acquisitions for targeted structural determination. The QTRAP is a hybrid TQ-IT instrument where the third quadruple is a linear IT, making possible the acquisition of PI, NL, and MS^n experiments in a single injection[4,11]. Other hybrid instruments are designed to couple more accurate mass determination with MS/MS or MS^n capabilities like the hybrid quadrupole time-of-flight (QTOF) instrument or quadrupole-orbitrap hybrid (QE or Q Exactive).

MS APPLICATIONS FOR TOXICOLOGY

To date, MS and its hyphenated applications (GC/LC/ICP-MS) have emerged as a powerful analytical tool for toxicology applications. GC-MS is generally used for analysis of volatile and heat stabile compounds, LC-MS for analysis of non-volatile and heat labile compounds, and ICP-MS for elemental analysis usually in metals determination[4,5,11,13,14,17]. Owing to the analytical versatility of MS methods with exceptional specificity,

sensitivity, dynamic range, and the ability to screen large numbers of unrelated compounds, MS applications are central for toxicological analysis of drugs and poisons. Current use includes drug analysis for targeted applications (e.g. in TDM and pain management), screening applications (e.g. in drugs of abuse (DOA), forensic toxicology, environmental toxicology, and clinical toxicology), and in pharmacokinetic/pharmacodynamics (PK/PD) research[5,11,14,15,17,18]. Here, we will focus on GC-MS, LC-MS, ICP-MS, and MS/MS capabilities and respective applications for toxicology.

Overcoming limitations of Immunoassays (IA) in TDM and drug screens

Since MS applications emerged at a time where IAs were already established in clinical laboratories, one driving force for the expansion of GC and LC-MS application in Toxicology has been efforts to overcome the limitations of IAs in drug analysis[13,19-22]. One limitation is IA are usually developed by manufacturers who seek FDA test approval based on commercial interests, with the end user having little control over this process. Another limitation is poor analytical specificity and analytical interferences[13,19-22]. The specificity of IA's developed for small drugs is usually limited to the detection of drug classes, but not necessarily individual drugs within a given drug class. This limitation could stem from the fact that antibodies generally recognize epitopes on large biomolecules, making the specificity of IAs poor for recognizing specific small molecules[13,22]. Currently, IA's are often used in first line screening for Toxicology since they can quickly identify a potentially negative sample, and are useful in identifying drug classes or specific drugs (i.e. benzodiazepines, opiates, amphetamines, cannabinoids, methadone, fentanyl, and phencyclidine), but suffer from high rates of false positive and false negative results due to a lack of specificity, cross reactivity, or interferences[4,21]. Since immunoassays are generally available as FDA approved tests on large automated analyzers, the common approach is to screen using an immunoassay first and then confirm positive results using GC-MS or LC-MS techniques which have superior sensitivity and specificity to identify specific molecules[4,21].

Drug analysis by GC-MS

Coupling of GC to MS provided an opportunity for development of routine applications with the specificity and sensitivity of MS (Figure 1A)[11,14,17,23]. GC is an analytical separation technique using a liquid or polymer stationary phase along with a gas mobile phase for separation of molecules based on partitioning between the stationary and gas phase. The process usually requires high temperature or temperature gradients (up to 350°C) in order to facilitate compound elution into the mobile gas phase (Figure 2A). The analytes are separated based on their column retention time, entering the MS in the gas phase for ionization usually with EI sources to facilitate MS detection. EI ionization uses the kinetic energy from a stream of high energy electrons (usually 70 eV) to strip electrons from analyte molecules at high temperatures, a process that produces a reproducible fragmentation patter from organic compounds (Figure 2A)[11]. For this reason, EI-GC-MS data is conducive to inter-laboratory spectral comparisons and extensive EI-GC-MS libraries have been generated for spectral matching based identification[11,23,24]. These libraries supplement "in-house" generated libraries and greatly increasing the ability to identify unknown compounds using GC-MS. This analytical advantage has made EI-GC-MS a premier tool for untargeted detection and quantitation of small molecules with MS specificity. EI-GC-MS is still used for general unknown screening applications using nearly any sample type[17,21,25]. Additionally, GC-MS is commonly used to confirm IA positive results in drug screens in

clinical toxicology[4,18,22,23]. One key limitation of GC-MS is the need to have volatile and heat stabile analytes, this means that some analytes require chemical derivatization in order to make the drugs sufficiently volatile for GC-MS analysis[23,25]. This limits GC-MS expansion to analysis of many drugs and adds additional steps and cost during sample preparation.

GC-MS applications for toxicology

GC-MS does have several advantages compared to its LC-MS/MS counterpart that include: efficient GC separation with higher chromatographic resolution and peak capacity, a homogeneous gas mobile phase (usually helium or hydrogen), optimization of separation conditions with precise electronic controls such as temperature programming, and the ability to search EI-MS databased for library based toxic compound identification[11,24]. Taken together with good MS sensitivity (1-10 µg/L) and specificity, a leading application of GC-MS is the general screening of unknown drugs or toxic compounds in doping control, environmental analysis, and clinical and forensic toxicology[24].

Therefore, in clinical toxicology, GC-MS is commonly used for screening blood and urine for acute overdose of prescription and over the counter medications in emergency room settings. This is specifically useful for drugs with toxic effects and known antidotes or therapies that can be initiated to treat the toxic effect[1,17,25]. It is also commonly used to perform drug screens for identification and/or quantitation of poisons in the clinical evaluation of toxindromes or in forensic investigations. Drugs commonly quantitated by GC-MS include; barbiturates, narcotics, stimulants, anesthetics, anticonvulsants, antihistamines, anti-epileptic drugs, sedative hypnotics, and antihistamines[24]. In environmental toxicology, GC-MS is used for the convenient screening of a wide range of toxic compounds such as; chloro-phenols in water and soil or polycyclic aromatic hydrocarbons (PAH), dioxins, dibenzofurans, organo-chlorine pesticides, herbicides, phenols, halogenated pesticides, and sulphur analysis in air[24]. One thing to mentions is most toxicology laboratories which can afford it are slowly replacing GC-MS with LC-MS as the method of choice for targeted drug screens for clinical and forensic toxicology applications[4,14,23]. Lastly, the higher specificity of MS detection compared to enzymatic spectrophotometric assays, GC-MS is sometimes used for identification and quantitation volatile substances (e.g. ethanol, methanol, acetone, isopropanol, and ethylene glycol) in body fluids such as blood and urine.

LC-MS applications for drug analysis

Due to the limitation of GC-MS for analysis of volatile and heat stable compounds, LC-MS applications have expanded MS applications to the direct analysis of non-volatile and heat labile molecules in toxicology laboratories (Figure 2B)[4,11,13,21,22,26]. The coupling of MS to LC was first possible when API-ESI sources became available in the 1990s, making it possible to ionize samples in the condensed phase and inject ions directly for MS analysis[11,12]. In contrast to EI used in GC, ESI is a soft ionization technique which does not induce fragmentation, instead, singly or multiply charged ions form from intact molecules due to proton transfer events (Figure 2B)[11,12]. ESI uses a capillary tube to flow solvent through a voltage potential before the solvent is sprayed into the MS vacuum as an aerosol[12]. Under vacuum, a heated gas (e.g. N_2) is used to dry the droplets and release gas phase ions for MS detection. The exact mechanism of ion formation by ESI is not fully understood, but the aerosol droplets are either negative or positively charged depending on the voltage applied and protonation/deprotonation events giving intact $[M+H]^+$ or $[M-H]^-$ ions for MS analysis (Figure 2B)[11,12]. To date, there seems to be no limit to

the size of molecule which can be ionized by ESI in biological samples[12]. Multiple protonation/deprotonation events also means ESI can yield more than one *m/z* peak from a single compound, a phenomenon that can either complicate the MS analysis or facilite measurements which improve precision or allow observation of *m/z* from targets with MW above the instrument range[12]. One inherent limitation of the ESI process, and therefore LC-MS, is the mass spectra of a given compound can vary depending on instrument conditions, including the capillary diameter, sample flow rate, and voltage applied[4,23]. The consequence is ESI mass spectra are instrument dependent, requiring the development of in-house derived spectral libraries for compound analysis[23,26]. Regardless, by overcoming key limitations of GC-MS, LC-MS has significantly expanded MS applications to targeted drug analysis of non-volatile and heat labile compounds such as drug metabolites[11,13-15,26].

The switch form GC-MS to LC-MS for analysis of toxin and drug metabolites in toxicology is notable[11,18,27-29]. One reason for this is that most drugs or toxicants entering the body undergo biotransformation by phase I (functionalization) and phase II (conjugation with hydrophilic endogenous molecules) metabolic reactions in order to facilitate elimination from the body[11,30]. The transformations often result with structurally diverse hydrophilic and heat labile metabolites with biological activities ranging from no pharmacological activity, to pharmacologically activity, to toxicity[15,23,29,30]. The nature of these drug metabolites, especially phase II metabolites, gives LC-MS a unique advantage for analysis of drugs and their metabolites using LC-MS, MS/MS and MS^n applications for identification, structural determination, and mapping PK/PD interactions during ADME [30]. To date, numerous studies have demonstrated that combined analysis of drug and metabolites greatly increases the ability to positively identify drug use using blood or urine samples[25]. Furthermore, urine has a much wider window of detection for detecting drug use, but extensive drug metabolism for urine excretion makes metabolite analysis more important for interpretation of results of urine drug analysis in pain management or DOA screening[18,25]. Lastly, LC-MS is also routinely used for targeted drug analysis in TDM, forensic toxicology, PK/PD pharmaceutical analysis, or in confirmation of compounds that do not work with GC-MS[4,18,25,31].

ICP-MS applications for analysis of toxic metals

ICP-MS was introduced for clinical use in 1980's for individual or multi-elemental metals analysis in toxicology[5,32]. The ICP source is designed for sample atomization and elemental analysis. Usually a peristaltic pump is used to inject aerosolized liquid samples into an argon plasma discharge at (5000-7000°C), but an LC can also be used for the separation of elements that require speciation (Figure 2C)[33]. The plasma vaporizes, atomizes, and effectively ionizes the sample for elemental analysis by MS. Advantages of LC-ICP-MS include the ability for metal speciation, multiple element measurements, and a wide dynamic range with accurate and precise trace metal measurements[34,35]. Detection limits for ICP-MS are commonly in the low ng/L range, giving an advantage in quantification of low levels of trace elements or toxic metals[5,35].

A key limitation of ICP-MS applications for metals analysis is polyatomic interferences[5,32,34]. These are interferences that result from the combination of two (or more) atomic ions from the sample matrix to form molecules which have the same *m/z* with analytical targets. One example is the combination of the argon plasma gas (40 Da) with a chloride ion (35 Da) or carbon (12 Da) from the biological matrix to produce ArCl (75 Da) and ArC (52 Da) ions. ArCl and ArC have the same *m/z* as arsenic and chromium,

two metals commonly incorporated into toxic metal surveys by ICP-MS[5]. To date, several ICP-MS applications have been developed in order to overcome isobaric or polyatomic interferences to improve specificity using collision/reactions cell applications. A dynamic reaction cell (DRC) uses a reactive gas in quadrupole ICP-MS instruments to overcome isobaric interferences from the plasma by reacting the gas with either the analyte (ion) of interest or isobaric compound (ion) in order to distinguish the two[5]. Equally, the quadrupole can act as a collision cell where a inert gas is introduced and will preferentially interact with polyatomic ions with larger radii, reducing their kinetic energy to allow resolution of polyatomic interferances from the analyte of interest through kinetic energy discrimination (KED). Lastly, collision induced dissociation (CID) in a triple quadrupole ICP-MS/MS can be used to break up polyatomic interferences prior to MS detection or a higher resolution instrument (e.g. double focusing sector ICP-MS) can be used to resolve polyatomic inteferences through accurate mass determination[5]. Owing to the high specificity, sensitivity, and reproducibility in elemental analysis by ICP-MS, this technique is now used in clinical laboratories for toxic metal and trace elements quantitation in a wide variety of samples, these include; whole blood, serum, plasma, urine and dry spots of these liquid samples (using laser ablation with ICP-MS). Sample collections in metal-free tubes are required for accurate determinations[5,34,35]. Other sample types used in forensic toxicology include; urine, hair, nail, tissue, and or other forensic materials.

Toxic metals and metal exposures

Metals represent some of the oldest toxicants known, with records of toxic metal exposures dating back to ancient times[1]. Nonetheless, many metals are also essential or trace elements with vital functions for life (i.e. cobalt, copper, iron, magnesium, selenium or zinc), but will become toxic with increased levels or pathologic metabolism like Cu in Wilson Disease (WD)[5]. Others like; thallium, arsenic, mercury, and lead, are poisons with no well-established physiological function. Other potentially toxic metals include: chromium, cadmium, platinum, nickel, aluminum, and gadolinium[5]. Metals exert their toxic effects through redox chemistry with biological targets, a process that might change the oxidation state of the metal and lead to formation of characteristic organometallic compounds[5,36]. Each metal has a specific mechanism of toxicity with different metal species varying in toxic effects. For this reason, metal speciation is an important aspect of clinical evaluations of toxic metal exposures[36]. Speciation involves identification and quantitation of different forms of a given chemical species. For example, chromium^{VI} (Cr^{VI}) is a powerful toxic oxidant whereas Cr^{III} is less toxic and plays a role in metabolism[5,36,]. Elemental mercury (Hg^{o}) has a lower toxicity than methyl mercury (MeHg), and arsenic is present in seafood as innocuous arsenocholine and arsenobetaine, but elemental arsenic is highly reactive and toxic to humans[5,36]. The different metal species can be distinguished through distinct; isotopic composition, oxidation state, or over-all molecular structure with speciationbeing essential in theevaluation of some toxic metal exposures[34-36]. Speciation with LC-ICP-MS effectively relies on LC separation of various metal species followed by MS detection. To date, methods have been developed for speciation of Hg, Arsenic, Cr and other[36].

Furthermore, isotopic fractionation by high resolution ICP-MS (HR-ICP-MS) or Q-ICP-MS can function as another method of metal identification. For example, lead isotopic ratios (^{206}Pb, ^{207}Pb, ^{208}Pb) may be useful to confirm the source of metal exposure in clinical toxicology or in forensic toxicology[5]. Studies have also shown $^{65}Cu/^{63}Cu$ isotopes ratios in dried urine

spots or serum can be used to classify treated and untreated Waldenstrom's disease (WD) patients when isotopically enriched sampes are administered[36-38]. For this reason, ICP-MS is a powerful tool for evaluation of metal exposures in forensic and clinical investigations with the ability to also use isotopic analysis to confirm the source of lead contamination. These distinctions are important since anthropogenic activities have introduced toxic metals such as lead (from gasoline) into the environment (air, water, and soil), the workplace, and consumer products such as food and pharmaceuticals[5,34-36]. Furthermore, metals are also used in implants for joint replacement (e.g cobalt, chromium, and titanium) and may leach-out during wear of the prosthetic device leading to the endogenous accumulation with potentially toxic consequences[36,39]. For these reasons, ICP-MS screening and speciation assays for toxic metals are commonly developed in order to evaluate toxic exposures in clinical toxicology, lethal exposures in forensic toxicology, and investigate environmental sources of metal exposure.

ICP-MS applications in clinical toxicology

ICP-MS is extensively used in multi-analyte toxic metal screens in whole blood, plasma serum and urine[5]. Blood and urine analysis is generally useful in assessing acute and chronic metal exposure with reference values available to aid with result interpretation from several geographical locations around the world[36]. Newer applications using dried blood or urine spots along with laser ablation for multi analyte metal analysis have also been described[38,40]. The multi-analyte ICP-MS metal panels can include up to dozens of targets including; lead, mercury, arsenic, cobalt, chromium, manganese, molybdenum, nickel, titanium, aluminum, and silver[5,36]. Lead is commonly evaluated in children due to its adverse effects on development[41]. Exposures can also occur from buildings with old lead water pipes, lead containing paint, or exposure from environment accumulation due to historic use of gasoline with tetraethyl lead[5,41]. Mercury exposure can occur from eating carnivorous fish which tend to contain high MeHg content as it accumulates up the food chain from environmental contamination. Exposures to mineral mercury leaching from dental amalgams has also been described[42]. Mineral mercury is usually measured in plasma and MeHg in whole blood to distinguish exposures from seafood and dental amalgams[5,36,42]. Arsenic is a substance that has been used in intentional poisonings, but accidental exposure can also occur through contaminated ground water[5,43]. Toxic levels of cobalt, chromium, manganese, molybdenum, nickel and titanium have been shown in people with various metal replacement joints or dental implants[5,39]. Aluminum is routinely quantified in plasma to monitor hemodialysis patients and it is also the subject of toxicological controversies associated with adverse effects from vaccines[5]. Historically, silver has been used as an effective bactericide but when taken in excess, exposures can result with development of argyria along with neurologic, hematologic, renal, or hepatic involvement with blood silver toxic levels as reported from cases of argyria[44-46].

ICP-MS applications in forensic toxicology

Deaths due to metal toxicity are uncommon and often unexpected, as a result, all unexplained deaths often prompt blood analysis for traditional metal poisons (e.g arsenic, thallium) toxic heavy metals (e.g arsenic, lead, cadmium, mercury) and other toxic metals (e.g aluminum, chromium, cobalt, molybdenum, nickel, vanadium or tungsten) or drugs (e.g contrast media). One advantage of forensic metals analysis by ICP-MS is the ability to use other sample types in addition to blood or urine[5]. For example, the use of laser ablation coupled with ICP-MS detection can allow the analysis of various samples

such as nail and hair in clinical or forensic toxicology analysis[5,40,47]. Blood and urine usually reflect exposure in the last days or hours[5]. Hair is a cumulative biomarker for longer term exposure compared to blood or urine. Each centimeter of hair represents one-month of exposure and can therefore be used to check for a longer window of exposure in clinical and forensic toxicology investigations. Hair can be used in conjunction with blood or urine results to differentiate a single exposure from chronic exposure by comparison with hair samples from a given growth period[5]. Alternatively, nails are another biomarker for forensic metals analysis by ICP-MS. Nails incorporate elements from blood during linear growth and thickening, providing a window of detection spanning 3 to 5 month for toxic metal exposure[5]. In clinical toxicology, nail collections are also considered non-invasive and contain more disulfide groups which help incorporate higher metal content, making it a preferred matrix for metals analysis for a longer window of detection when hair is not available due to balding or other reasons (e.g. religious reason)[5]. Lastly, tissue and biopsies for metals analysis by ICP-MS becomes important when blood and urine are not available and hair and nails are affected by external contamination, or when specific organs biopsies need to be checked for metal accumulation[5].

CONCLUSIONS

In summary, mass spectrometry (MS) is a powerful analytical technique able to distinguish ionizable chemical compounds or elements based on their *m/z* ratio in the gas phase. With exceptional sensitivity, accuracy, precision, and dynamic range, MS has emerged as an important tool in analytical determinations of poisons and their metabolites in clinical, forensic, and environmental toxicological evaluations. GC-MS is commonly used for general unknown screen (GUS) of poisons, drugs and their metabolites based on the capacity to identify a vast majority of chemical compounds using inter-laboratory EI-MS libraries. The limitation of GC-MS is that compounds need to be volatile or heat stable for compatibility with GC separation. This restriction often requires derivatization of non-volatile compounds for compatibility with GC separation and limits analysis of heat labile compounds which often includes drugs and their metabolites. LC-MS overcomes these limitations by using ESI to introduce ions from liquid samples into the MS for analysis of non-volatile and heat labile compounds. As such, LC-MS is slowly replacing GC-MS for the analysis of poisons, drugs, and their metabolites. Disadvantages of LC-MS include high cost and the inability to use inter-laboratory spectra for compound identification. To date, both GC/LC-MS are used in advanced laboratories along with MS/MS and MS^n applications for increased specificity in drug identification, drug metabolite analysis, and structural determination. Lastly, ICP-MS is commonly used for trace and toxic metal analysis in toxicology laboratories. A key advantage of ICP-MS is the ability to do multi-element panels in toxicological analysis along with the use of MS/MS, HR-MS, and DRC applications for resolving interfering compounds. Overall, MS is a versatile analytical tool with many useful applications and has the potential for automation. In general, trends for adopting MS applications for toxicology relies on the ability to multiplex quantitative and qualitative compound evaluations and hyphenated MS applications with higher mass resolution for increased analytical specificity.

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