Liquid Chromatography–Tandem Mass Spectrometry

An Emerging Technology in the Toxicology Laboratory

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KEYWORDS

• LC-MS/MS • Toxicology application • Quadrupole mass spectrometer • MRM • ESI • APCI • APPI • Immunoassay

KEY POINTS

- Both licit and illicit opiates have effects on the immune and neurologic components of asthma inflammation and clinical disease as well as associated allergic responses.
- The end product of these interactions determines the clinical output of this complex interplay, with either worsening or improvement of asthma, and possible increase in allergic responses.
- In the last decade, Liquid Chromatography - Tandem Mass Spectrometry (LC-MS/MS) has seen enormous growth in routine toxicology laboratories.
- Major strengths of LC-MS/MS are improved specificity, flexibility and sample high throughput compared with other techniques.
- Technology advances in LC-MS/MS are taken place, such as automation, miniaturization, detector and LC improvements. Efforts in standardizing method development and forthcoming regulation will greatly impact the role of LC-MS/MS in toxicology laboratories.

INTRODUCTION

History of Liquid Chromatography–Tandem Mass Spectrometry

With roots stretching back more than 100 years, mass spectrometry (MS) is an analytical technique with both an interesting history and a promising future. MS was born from early studies of electromagnetism. It first gained importance in physics, where

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it was used to determine the existence of isotopes and the atomic weights of the elements.[1–4] Subsequently, MS was used on a massive scale for the separation of the isotopes of uranium as part of the Manhattan Project.[5] During the 1950s, MS became part of chemistry, being used to study small molecules, particularly by the petrochemical industry.[6] During that era, gas chromatography (GC)–MS was born,[7] and forecasted the path toward liquid chromatography–tandem mass spectrometry (LC-MS/MS) (or LC–mass spectrometry/mass spectrometry).

Instrumental developments in the field of MS accelerated throughout the latter half of the twentieth century. These developments included quadrupole, time-of-flight (TOF), and Fourier-transform MS.[8] The development and commercialization of thermospray,[9] atmospheric pressure chemical ionization (APCI),[10] and electrospray ionization (ESI)[11] in the 1980s enabled the successful interface of LC to mass spectrometers. The performance, sensitivity, and reliability of the instruments have been areas of active development. New mass analyzers continue to improve performance across the field, as have developments in ionization sources.

Although LC-MS is now commonly used in many clinical applications, the difficulties faced in interfacing a liquid chromatograph to a mass spectrometer were enormous. At the time of the first experiments in interfacing those techniques, most mass spectrometers operated at high vacuum, in the magnitude of 10 to 6 mm Hg (~10–4 Pa, 1 mm Hg = 133.3 Pa). Similarly, most liquid chromatographs were operated at flow rates near 1 mL/min. Because 1 mL of liquid water produces more than 1 L of gas at atmospheric pressure and the volume expands as pressure is reduced, vacuum systems were greatly challenged to deal with the volume of gas present at an LC/MS interface. A further challenge was that many mass spectrometers at the time operated at high voltage (kV), which is incompatible with high pressures.

A confluence of developments enabled the coupling of LC with MS. First, improvements in quadrupole mass spectrometers, which operate at lower voltages (hundreds of volts lower), made them viable candidates toward the maturation of LC-MS instruments. Next, improvements in vacuum systems made it possible to deal with the volume of gas generated in an LC-MS interface. Improvements in chromatography made the use of lower flow rates possible. In addition, improvements in interfaces reduced the volume of gas presented to the vacuum system. Simultaneously, Yost and Enke[12] introduced the triple quadrupole mass spectrometer. Another critical component was the use of computers to control mass spectrometers and chromatography systems, which also happened during this timeframe, making complex MS/MS scans possible.

These developments culminated in the introduction of the first commercial, dedicated LC-MS/MS instrument in 1989. The next year saw the publication of the first LC-MS/MS publication in the field of clinical chemistry.[13] From there, the field has grown enormously, as shown in **Fig. 1**.

Along the way, the field has been well-recognized. Nobel Prizes have been awarded to 6 practitioners in the field, starting with the 1906 Prize in Physics awarded to J.J. Thomson of Cambridge University, “In recognition of the great merits of his theoretical and experimental investigations on the conduction of electricity by gases,” which allowed him to invent the first mass spectrometer. His student, F.W. Aston, was awarded the 1922 Nobel Prize in Chemistry for the discovery of isotopes. In 1989, Hans Dehmelt and Wolfgang Paul shared half of the Nobel Prize in Physics for their development of the ion trap, which was fundamental in the development of quadrupole mass spectrometers. Most recently, John Fenn and Koichi Tanaka shared half of the 2002 Prize in Chemistry for “their development of soft desorption ionization methods for mass spectrometric analyses of biological macromolecules,” which meant electrospray for John Fenn and soft laser desorption for Koichi Tanaka.

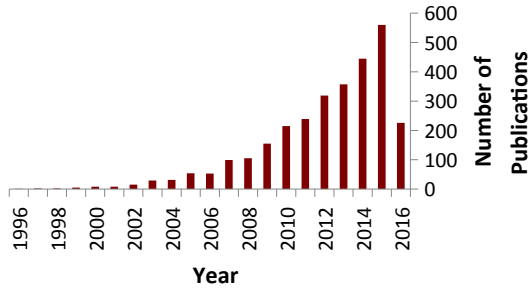

Fig. 1. Number of publications in the past decades in the field of clinical MS. The search was done on April 8, 2016 on PubMed, based on searching terms of "LC-MS MS" and "clinical."

Basic Principles of Tandem Mass Spectrometry

Fundamentally, mass spectrometers can be thought of as devices for weighing molecules. Tandem mass spectrometers, or MS/MS instruments, can be thought of as devices to perform chemistry on molecules, followed by weighing the results.[14] To accomplish these measurements, mass spectrometers work with charged molecules (ions) in vacuum. LC, on the other hand, works with molecules in solution. The transition of the sample into a charged, gas phase ion is the first step in MS followed by measurement.

Modern mass spectrometers consist of several discrete components: the ion source, mass analyzer, and detector, as shown in Fig. 2. The ion source often combines the interface between a chromatography system and the ionization process, as presented in later discussion. The mass analyzer separates the resulting ions. There are a variety of types of mass analyzers, including analyzers that separate different masses in space, like quadrupole and orbitrap analyzers, and analyzers that separate different masses in time, like the TOF analyzer. Multiple mass analyzers can be linked together with a collision cell to provide structural information from ions, as shown in Fig. 2, or, for analyzers that separate different mass in time, collisions can take place as part of a sequential experiment. Last, the ions must be detected. Two common detection methods are currently in use: one based on discrete particle impacts on an electron- or photomultiplier and the other based the detection of an image current.

There are 3 methods currently in wide use for transitioning molecules from the LC to the mass spectrometer: ESI, APCI, and atmospheric pressure photoionization (APPI). ESI and APCI are the most commonly used ionization methods, whereas APPI is a relative newcomer and has become a method of choice for many applications.

ESI uses a high voltage to create a fine aerosol of charged particles (Fig. 3A).[15] For larger chromatographic flow rates (above nanoliters per minute), aerosol production

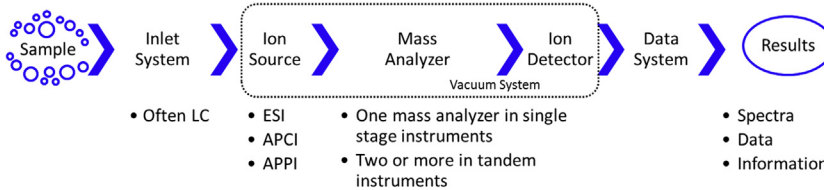

Fig. 2. Schematic diagram of a mass spectrometer. The 3 basic building blocks (inlet, mass spectroscopy, data system) of all mass spectrometers are shown. The ion source used depends on the inlet system and the analyte; other ion sources are used for non-LC inlet systems.

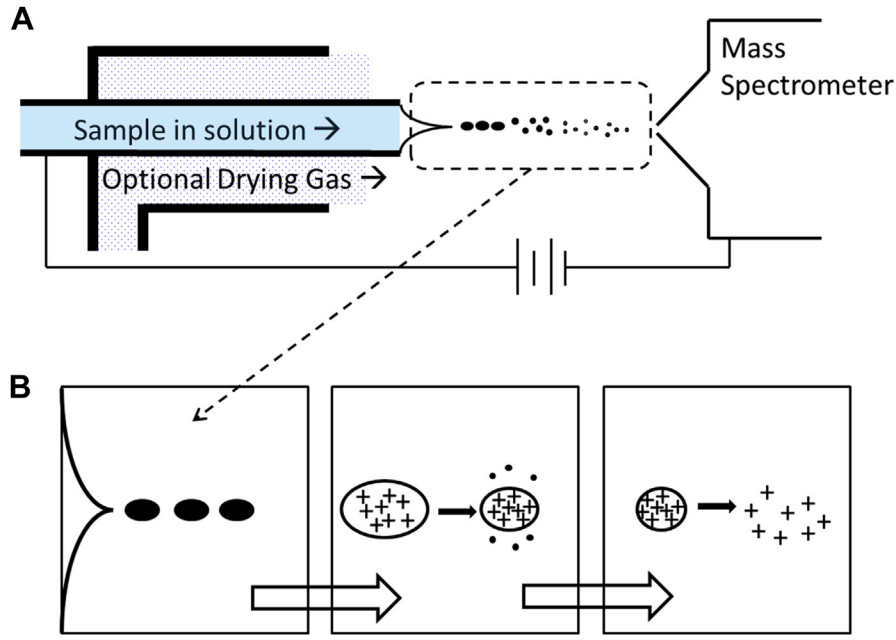

Fig. 3. ESI process. (*A*) Aerosol production. (*B*) Solvent evaporation/ion declustering. Electrospray produces ions through the application of a voltage to a liquid. The liquid disperses into charged droplets, which then decluster. A drying gas can be used to enhance the process, depending on the flow rate.

can be assisted by nebulization with a heated inert gas. Solvent molecules evaporate from the charged droplet until the Rayleigh limit (criterion for the minimum resolvable detail) is reached, after which the droplet undergoes Coulomb fission, essentially exploding and creating smaller charged droplets (Fig. 3B). This process continues until the droplets are small enough that, for small molecules, charged analyte ions evaporate from the droplet. For large molecules, the fission cycle continues until the droplet contains one charged analyte ion, which is then transferred into the mass spectrometer, resulting in multiple charges being associated with larger molecules.

APCI (Fig. 4) takes place in a heated nebulizer, with a corona discharge providing ionization.[16] The effluent from an LC flows into the ion source region. A concentric flow of gas assists with nebulization and the effluent flows through a region of high heat at the end of the APCI probe. This ionization procedure produces a thin "fog" of gas, which reacts with a corona discharge. Because most of the fog is the high-performance liquid chromatography (HPLC) solvent, an excess of reactant ions is formed from the solvent molecules, which then interact with the analyte molecules and ionize them. In positive ion mode, analyte molecules are ionized primarily by proton transfer from the reactant or by charge transfer. For proton transfer, the gas phase basicity of the analyte must be sufficient to abstract a proton from the reactant gas. For charge transfer, the ionization potential (IP) of the analyte must be sufficiently low for the analyte to lose an electron to the ionized solvent cloud. A third reaction pathway is via adduct formation, in which a positively charged species in the solvent cloud "sticks" to the analyte, forming a charged complex. In negative ion mode, the opposite reactions take place. Protons are abstracted from the analyte molecules to

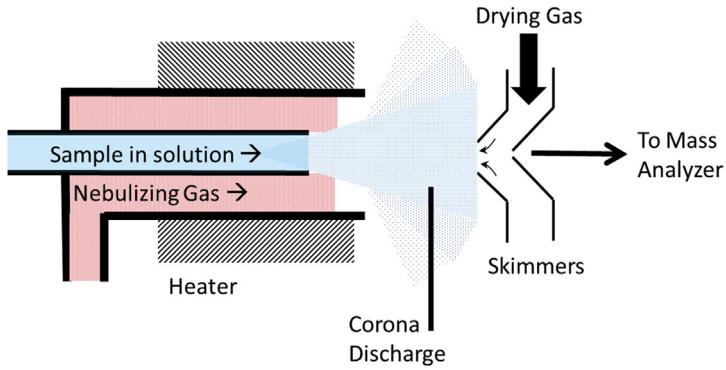

Fig. 4. APCI. APCI uses gas-phase ion-molecule reactions to produce charged analyte species. The effluent from an LC is converted to an aerosol, which is exposed to a corona discharge. Species produced by the discharge interact with the sample, producing ions that are detected by the mass spectrometer.

form negatively charged species, and electrons are transferred or negatively charged adducts are formed.

A variant of APCI uses short wavelength (UV) photons to ionize the analyte molecules in place of the corona discharge. This technique, known as APPI,[17] is most useful for analytes incorporating aromatic chemistry and has found utility in the field of clinical chemistry in the analysis of steroids. A krypton discharge UV lamp produces 10.6-eV photons that excite analyte molecules that undergo electron ejection to form cations. Ionization of analytes depends on the IP of the analyte; the IP of the analyte must be less than 10.6 eV. Most HPLC solvents have IPs greater than 10.6 eV and are not ionized. Deliberate addition of an ionizable species such as toluene or acetone can enhance the ionization by providing additional ionization pathways involving charge transfer or proton exchange. There is evidence to suggest that APPI is the least susceptible to ion suppression of the 3 atmospheric pressure ionization techniques, at least at low flow rates.[18] This phenomenon of reduced ion suppression is thought to be due to the lack of competition for charge in the ionization, because the photon flux is sufficient to promote ionization of all molecules present. The 3 ionization techniques are compared in **Table 1**, and the applicability of the techniques to different regions of chemical space is shown in **Fig. 5**.

APCI, APPI, and ESI take place at atmospheric pressure, which removes a great deal of the burden on the vacuum systems of the mass spectrometer. Because the analyte is charged at the end of the process, the ions can be focused through a small orifice into the mass spectrometer, which is operated at a higher vacuum. APCI is somewhat more robust and more energetic than ESI and works particularly well for less polar species. ESI works particularly well for more polar species. Because both techniques work predominantly by protonation/deprotonation, neither will work for species that do not exhibit some degree of gas-phase basicity (for the formation of positive ions by proton transfer) or gas-phase acidity (for the formation of negative ions by proton abstraction). In practice, that means that the analyte molecules must contain heteroatoms like nitrogen, oxygen, or sulfur to be successfully analyzed.

Mass spectrometers operate on charged species and measure the mass-to-charge ratio (m/Q or m/z) of analytes. Once molecules are charged, they can be influenced by electrostatic and magnetic fields. Because there are many ways to generate electrostatic and magnetic fields, there are many ways to manipulate ions and many different

Table 1
The advantages and disadvantages of electrospray ionization, atmospheric pressure chemical ionization, and atmospheric pressure photoionization ionization methods for clinical analysis

ESI	APCI	APPI
Softest ionization	More robust than ESI	Excellent for PAH
Capable of multiple charging (higher molecular weights)	More energetic ionization	Used for steroids
Most susceptible to ion suppression	Ionizes smaller, less polar molecules than ESI: steroids, benzodiazepines, carbamates	May require use of a dopant for ionization
Greatest coverage	Forms only singly charged ions	Can provide superior signal/noise ratio
Best compatibility with thermally labile species	Moderate susceptibility to ion suppression	Least susceptible to ion suppression
Most susceptible to ion suppression		

configurations for mass spectrometers. Subsequent focus is on triple quadrupole mass spectrometers. As suggested by the name, a triple quadrupole mass spectrometer consists of 3 quadrupole mass spectrometers joined together, usually inside one vacuum system. The first and third quadrupoles, typically called Q1 and Q3, respectively, are used to scan masses, while the middle quadrupole is used as a collision chamber. This arrangement is very versatile, and there are many different scan modes available, as shown in Fig. 6.[19,20] In the most basic mode, Q1 is scanned; there is no collision gas present in the collision chamber, and Q3 is set to pass all masses (or scans in sequence with Q1) (see Fig. 6A). This scanning mode results in a full scan mass spectrum, where all ions of the correct polarity that are produced in the source

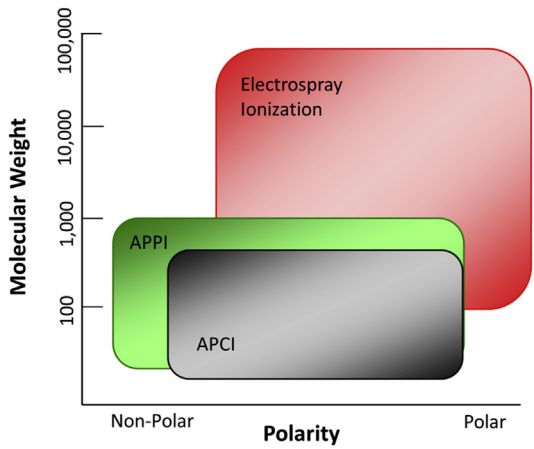

Fig. 5. Coverage map of ESI, APPI, and APCI in chemical space. APPI and APCI are more applicable to lower-polarity analytes of lower molecular weight, whereas ESI performs better for analytes with greater polarity. For ESI, a less-energetic ionization method is able to ionize higher molecular weight species than either APPI or APCI. There is considerable overlap between the techniques.

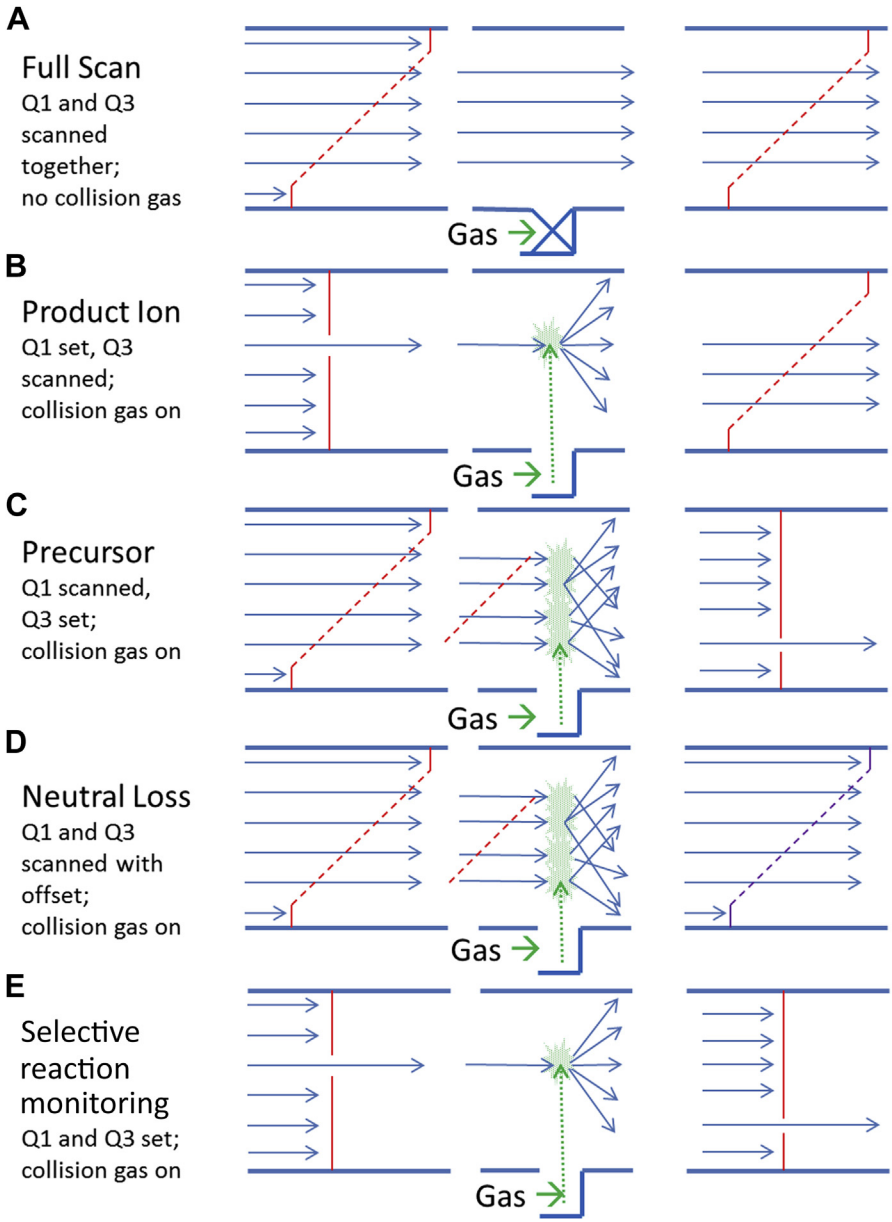

Fig. 6. The 5 main experiments performed with triple quadrupole mass spectrometers. (*A*) Full scan. (*B*) Product ion scan. (*C*) Precursor ion scan. (*D*) Neutral loss scan. (*E*) SRM. Note that collision gas is used for all experiments except full scan.

are observed in the mass spectrum, within the defined upper and lower limits of the scan. This scan mode is a useful scan mode, for example, for determining the molecular weights of species in a chromatogram. A second common scan mode is the product ion scan (see **Fig. 6**B). In this scan mode, one mass (actually a selected m/z ratio with a defined mass window) is selected in Q1 and allowed to collide with gas in the

collision quadrupole, and the resulting charged fragments are observed by scanning Q3. This scan mode is useful for determining the structure of an unknown species or for determining the product ions that are potential candidates for monitoring in other types of MS/MS scans. Fig. 6C shows the reverse of a product ion scan, the precursor ion scan. In this scan mode, ions are scanned in Q1 and allowed to fragment in the collision quadrupole, and only one fragment ion mass is allowed through by Q3. This scan is useful for determining all of the species in a sample that contain a common structural element and is used for determining, for example, the metabolites of a drug. The neutral loss scan is depicted in Fig. 6D and shows a scan mode that is complementary to the precursor ion scan. In this mode, Q1 and Q3 are offset, and the difference between the masses is set to determine the mass of a substructural group that is being observed. This scan mode is used to show, for example, all of the species in a sample that might contain a hydroxyl group, which would lead to loss of H_2O and which is observed by setting an offset of 18 Da between Q1 and Q3. The final common scan mode is selective reaction monitoring (SRM), shown in Fig. 6E. This method of scanning is the one typically used for quantitation. Q1 and Q3 are set to observe a preselected transition, and collision gas is present, resulting in a stable transition indicative of a particular species. An even more common variant of SRM is multiple reaction monitoring (MRM). In this mode, multiple selected product ions from a common precursor are observed to provide additional confirmation in the quantitation process. This scanning mode is the most popular method used in the toxicology laboratory for monitoring small molecule drugs. In all scan modes where collisions are used, the energy of collision is a variable that must be optimized and set.

APPLICATION OF LIQUID CHROMATOGRAPHY–TANDEM MASS SPECTROMETRY IN THE TOXICOLOGY LABORATORY

Traditional Toxicology Techniques

Before going to the in-depth discussion about the applications of LC-MS/MS in toxicology laboratories, an initial introduction to the traditional techniques that were popular in toxicology testing is warranted. Technology limitations of traditional techniques lead to the transition of using LC-MS/MS as the method of choice in clinical testing. The advantages and disadvantages of each technique are summarized in Tables 2 and 3.

Immunoassay

History and introduction Immunoassay (IA) was first developed to identify antigen-antibody complex formation. One example of the older IA method is the Ouchterlony double immunodiffusion assay developed in the 1940s, whereby both antigen and antibody diffuse through a semisolid gel independently to identify if the specific

Table 2
Comparison of the advantages of different toxicology techniques

	IA	GC-MS	LC-MS/MS
Fast turnaround	√	—	—
Specificity	—	√	√
Sensitivity	√	√	√
Ease of sample preparation	√	—	√
Ease of developing new test	—	√	√
High throughput	—	—	√

Table 3
Disadvantages of different techniques for clinical analysis

IA	GC-MS	LC-MS/MS
Lack of analytical specificity	Longer run times	More expensive instrumentation
Not suitable for testing large panel of analytes in one run	More complex sample preparation for larger molecules	Higher maintenance
Low requirement for qualified staff raises the concern of human error	Limited utility for large, polar, or thermally labile analytes	Ion suppression/matrix effect concerns
Not flexible for developing new assays		

antigen/antibody complex is formed.[21,22] In late 1950s, Yalow and Berson[23] first reported the development of a radioimmunoassay (RIA) for insulin that used radioactive isotopes. The radioactive labels used in this method emit gamma rays, which allow the quantitative detection of trace levels of insulin using a gamma counter. Since then, IAs have evolved considerably in the area of both research and clinical diagnostics. Depending on the detection methods, IA can be defined as RIA, enzyme immunoassay (EIA), fluorescent immunoassays, and chemiluminescent IAs.[24] The IAs most frequently used in clinical laboratories are quantitative or semiquantitative automatic IA analyzers with methods such as enzyme multiplied immunoassay technique (EMIT), fluorescent polarization immunoassay (FPIA), and chemiluminescent EIA.[25]

Primary use IA is widely used in the field of clinical toxicology primarily because of its ease of performance, minimal sample preparation requirement, and rapid turnaround time. IA is the dominant screening method at the point of care. The IA screening method is typically followed by the confirmation of individuals who test positive for drug use by other more specific assays such as MS.[26] According to the product guide 2015 from the College of American Pathologist, there are more than 20 manufacturers and 60 analyzers on the market serving the needs of clinical laboratories. Assays are available for blood, serum, or urine samples for the determination of ethanol, drugs of abuse, and therapeutic drug monitoring (eg, benzodiazepines, amphetamine and methamphetamine, cannabinoids). For example, Abbott Laboratories (Chicago, IL, USA) with its ARCHITECT analyzer and AXSYM analyzer, and Roche Diagnostic Laboratories (Nutley, NJ, USA) with its COBAS and INTEGRA analyzers, are 2 of the pioneers using this technique for monitoring a large variety of analytes.[24]

There are also IA test devices for point-of-care testing (POCT), which do not need large-scale instruments. These “devices” may be dipstick, cup, card, or cassette based.[27] The simple-to-use POCT device combines the sample collection and testing, thereby facilitating a fast turnaround time. However, the operator for this kind of assay needs to perform multiple steps, including sample collection, timing for the reaction end point, result interpretation, and data recording. One often-cited issue for these devices is that the resolution/interpretation of the result varies between different operators, because the positive readings are often solely dependent on visual signs, for example, change of color or absence of a line. On the other hand, laboratory IA instruments have the advantage of automation to capture data on a computer system, reducing the chance of human error.

Advantages The attributes of toxicology diagnostics consist of the following aspects: specificity, sensitivity, fast turnaround time, high throughput, ease of sample

preparation, and the flexibility to develop laboratory-based tests.[28] The major advantage of IA in the clinical testing is its rapid turnaround time. Since the 1980s, many companies have been devoted to developing fully automated IA systems for rapid and sensitive testing. It has been reported that, with an automated IA system, the analysis time of a total of 11 drugs (cyclosporine, tacrolimus, mycophenolic acid, valproic acid, digoxin, theophylline, carbamazepine, phenytoin, phenobarbital, vancomycin, and gentamicin) was 1.1 minutes and the time for reporting was 11 minutes, using the Viva-E Drug Testing System (Siemens, Palo Alto, CA, USA).[29] Moreover, most of the testing is performed using the homogeneous IA, which means that the assays are performed in solution without the need of excess sample preparation (phase separation, sample extraction, and so forth). Another advantage of IA is its sensitivity or limits of detection. With the development of this technique, nowadays both EMIT and FPIA assays can detect analyte levels in the nanomolar range, which fulfill the needs of most clinical diagnostics.

Disadvantages Analytical specificity is a major concern for the IA techniques. It is almost impossible to raise antibodies toward a single molecular structure, but rather, most antibodies are used to detect a family of compounds with the same chemical backbone, which may lead to false positive or false negative results. For example, cross-reactivity has been identified using IA for the detection of tricyclic antidepressants (TCA; eg, amitriptyline, imipramine, desipramine, and nortriptyline).[30] Positive results can be observed due to TCA, or a non-antidepressant drug cyclobenzaprine, due to the similarity of the 3-ring structure. When blood samples are tested, the monoclonal antibodies used in IA may bind nonspecifically with the proteins present in patient's serum and plasma, thereby producing false positive results.[31] IAs are limited to the assays for which the manufacturers can develop suitable antibodies. The average time for developing commercial IAs takes 2 to 5 years. Large discrepancies can be found in terms of sensitivity, result interpretation, cutoff values, and reference ranges from the kits and instruments designed by different manufacturers.[32–34] In addition, the throughput of IA is dependent on the manufacturer's assay kit; most of the time, individual IAs are necessary for the detection of each group of drugs, thus rendering it impractical to measure all the analytes of interest simultaneously. It is also worth noting that immunoanalyzers and assay kits are designed specifically for use with a certain matrix (urine or plasma). When modification is made for using a kit with a different matrix, the laboratory needs to thoroughly validate the method.[35] Ideally, clinical and laboratory staff need to be trained to know the limitations of IA and be aware of false positive/negative results related to this methodology. Both automated immunoanalyzers and POCT devices are easy to use, which leads to minimal technical requirements for the staff performing the test. When no specialist is present in the laboratory, results may not be interpreted properly.

Gas chromatography-mass spectrometry

Technology advancement of MS offers robust solutions that can be applied to clinical testing toward obtaining better specificity, accuracy, and sensitivity. Modern MS would not exist without the chromatographic systems used to provide separation of analytes. The 2 major chromatographies currently in use are GC and LC. Both use a stationary phase and a mobile phase, and they are distinguished by their mobile phases. GC uses an inert gas, typically helium, hydrogen, or nitrogen, as a mobile phase, whereas LC uses a liquid solvent system. The separation is affected by the analytes moving between the stationary phase and the mobile phase, in a process known as partitioning. LC and GC are used for different types of compounds and situations, and the 2 techniques are now very complementary. GC is used for smaller,

less polar molecules, and LC is used for larger or more polar molecules that are not amenable to GC (Fig. 7).

History and introduction GC is a robust technique that offers the ability to resolve volatile analytes from a complex biological matrix. MS provides the unambiguous identification of a compound based on its mass-to-charge ratio. In combination, GC and MS serve as a powerful and versatile analytical tool for both qualitative and quantitative purposes.[36] It was first reported in early 1950s that GC technology can be used for the separation of volatile compounds in a mixture.[37] In the mid 1950s, Roland S. Gohlke and Fred W. McLafferty at Spectroscopy Lab at Dow Chemical Co. worked in collaboration with Bill Wiley, Ian McLaren, and Dan Harrington at Bendix Labs, where they successfully produced the first direct-coupling GC-MS. Later, the technology of GC-MS started to quickly evolve with the introduction of capillary chromatographic columns and the advance of carrier gas separators to remove the GC carrier gas before introduction of a sample into the high-vacuum mass spectrometer. Nowadays, the modern GC-MS instrumentation is widely used as a high-resolution technique for analyte separation and identification. Applications of modern GC-MS include environmental analysis, forensics, clinical laboratory drug testing, and pharmacologic studies.

Advances in the ionization techniques promoted the variety of applications of GC-MS in the clinical testing. The electron ionization (EI) full-scan mode is the gold standard for comprehensive screening and systematic toxicologic analysis of drugs and metabolites.[38,39] Positive ion chemical ionization (PCI) is suitable for the identification of drug metabolites in biosamples.[40] PCI can give the molecular mass information of analytes when EI fails to produce a corresponding molecular ion.[41] Negative ion chemical ionization (NICI) can improve the sensitivity of analytes with electronegative moieties (eg, benzodiazepines) by several thousand-fold.[42]

Primary usage GC-MS has been used for drug monitoring for several decades, and it continues to be the definitive standard for toxicology laboratory confirmation analysis.[30] Because the detection of therapeutic drugs and drug of abuse can have serious consequences in patients' professional, social, and financial situations, it is generally accepted that positive results of certain drugs from screening procedures (eg, IA) need to be confirmed by a second method. GC-MS is one of the gold-reference methods for confirmation.

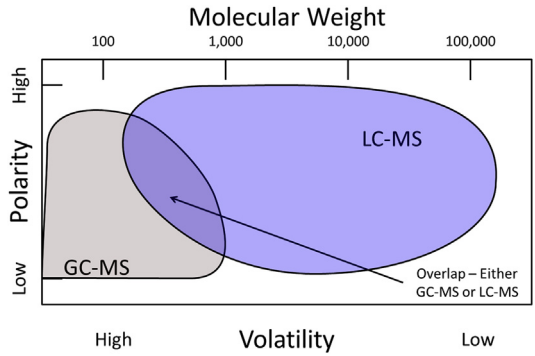

Fig. 7. Coverage map for GC-MS and LC-MS in chemical space. The coverage was illustrated based on volatility (lower x-axis), polarity (y-axis), and molecular weight (higher x-axis). GC-MS is useful for higher-volatility, lower-molecular-weight analytes, whereas LC-MS is useful for lower-volatility, higher-polarity analytes.

Another merit of this technique is that GC-MS can also be used for screening procedures for simultaneous detection of several drug classes.[43] Reliable screening methods have been developed for the detection of drugs of abuse[44,45]; therapeutic drug monitoring, including barbiturates, benzodiazapines, antidepressants, and morphine[46]; and pesticides.[47,48] There are several commercially available libraries for drugs, drug metabolites, poisons, and pesticides, providing universal spectra that can be applied on different GC-MS instruments from various manufacturers.[49]

Advantages Advances in technology have allowed the introduction of bench-top GC/MS instrumentation into clinical laboratories. GC/MS has been widely recognized for its reproducibility, specificity, and sensitivity to detect trace amounts of analytes. The long GC capillaries (~30 m) lead to better analyte separation from matrix and interferences compared with other chromatography techniques, which increases the specificity of targeted compounds. Sensitivity of GC-MS is significantly higher than IA for the detection of drugs and metabolites in relation to clinical toxicology testing. It is reported that the limit of detection (LOD) for opioids, tetrahydrocannabinol, and benzodiazepines ranges from 1 pg/mL to 0.1 ng/mL in biosamples using the GC-MS-NICI technique.[41] In addition, GC-MS offers improvement in specificity compared with the IA method, by combining high chromatographic resolution with full spectra information.

Similar to the IA screening method, GC-MS can also be configured for screening large panels of drugs in the same run, which is beneficial because IAs typically only screen one class of drug in one assay. In addition, toxicology laboratories can develop their own GC-MS applications for the less commonly tested drugs (eg, lysergic acid diethylamide [LSD]), for which IA methods are not available.

Disadvantages A major drawback for the GC-MS technology is its labor-intensive sample preparation procedures. Because of the nature of GC, GC-MS analysis is limited to small nonpolar analytes that are sufficiently volatile as well as thermally stable to vaporize at practical temperatures (the temperature in the GC injector and oven often do not exceed 300°C). For polar and thermally labile analytes, sample derivatization is the prerequisite to convert the analytes to volatile products before injecting into the gas chromatograph. The sample preparation steps may also include the cleavage of conjugates, extraction, and cleanup procedures; these factors contribute to the prolonged turnaround time and reduced throughput. In addition, GC-MS instruments are not available at most hospital laboratories; thus, positive IA samples need to be sent out to comprehensive or reference toxicology laboratories for GC-MS confirmation. In this case, the turnaround time can be delayed for days.

Liquid Chromatography–Tandem Mass Spectrometry Application in the Toxicology Laboratory

Although GC provides higher resolution separations, it comes with the cost of longer run times and only for thermally stable, volatile species. LC is more amenable to rapid separations and is optimized for larger analytes. Although there is much overlap, analyses are moving toward LC-MS/MS for speed, and in many cases, sensitivity. Many clinical methods were developed for GC and GC-MS before reliable LC-MS interfaces were available. LC, being more compatible with most clinical matrices and analytes and also using shorter run times with often-simpler sample preparation, is commanding an ever-growing share of the clinical laboratory landscape. Today, with its well-known merits of sensitivity, selectivity, and robustness, LC-MS/MS is serving the toxicology laboratories with fast, accurate, and comprehensive testing for nearly

all the analytes of interest in the field of drug-of-abuse and therapeutic drug monitoring. With proper sample preparation, LC-MS/MS can provide the solution for testing nearly all common biological fluids.

Common sample matrix and sample preparation methods

Urine, blood plasma, and oral fluid are the most commonly used matrices for clinical applications of LC-MS/MS. Because of the presence of endogenous components in different biomatrix fluids, especially in oral fluid and blood samples, ionization suppression or enhancement can occur, which is known as the "matrix effect." The precision as well as accuracy will be affected if the compounds of interest coelute with the matrix fluid. Sample cleaning also helps improve the sensitivity of the assay by achieving lower limits of detection. Therefore, the sample preparation is a very important step to ensure the quality of instrument performance for every assay.

The purpose of the sample preparation is to remove the interferences that can affect the detection of the target analytes and the lifetime of the column and the instrument. Also, the preparation steps serve to enrich the analytes of interests to be categorized within the method detectable range. **Table 4** shows the sample preparation options with their strength and shortcomings.

Workflow and high throughput are of key importance in the clinical laboratory, whereas the sample preparation is usually the "rate-limiting" step. The "dilute-and-shoot" method is gaining popularity in clinical applications by offering the quickest preparation, while reducing the matrix effect to a certain extent with simply diluting urine samples with mobile phases. When it comes to blood samples, cleanup is a prerequisite before the sample can be loaded for LC-MS/MS analysis. Simple methods like liquid-liquid extraction (LLE) and protein precipitation (PPT) are the 2 commonly used methods for sample cleanup. However, because LLE cannot remove phospholipids, which can cause formation of an emulsion during the extraction procedure, the recovery rate of analytes can be significantly affected. In addition, extraction solvents are typically nonpolar organics; therefore, it is expected that the recovery of polar drugs would be minimal. PPT is less selective than LLE, and it does not remove most of the interference component. An improvement to PPT is the commercially available protein removal plates, which removes the phospholipids and other interference components. The solid-phase extraction method (SPE) is perhaps the most

Table 4
Advantages and disadvantages of different sample preparation methods

Method	Advantages	Disadvantages	Suitable Matrices
Dilution-and-shoot	Simple, quick, and low cost	No cleanup and no selectivity	Urine
LLE	Simple, better cleanup than PPT	Difficult to automate, not suitable for highly polar analytes, solvent evaporation needed	Urine, plasma, serum, oral fluid
PPT	Simple, quick	No matrix interference removal, minimal selectivity, solvent evaporation needed	Whole blood, plasma, serum
SPE	Can be automated, best cleanup option, high reproducibility	Costly and method development can be difficult	Urine, whole blood, plasma, serum, oral fluid

powerful technique for sample preparation for clinical applications. SPE columns and kits are available for various analytes with selectivity in size or polarity. It is currently the best option for sample cleanup, because of its high recovery rate and reproducibility. SPE is particularly suitable for oral fluid sample preparation. Oral fluid contains extraction buffers, proteins, enzymes, and even oral swab tissues, and the analyte concentrations are 5 to 10 times lower in oral fluid than in a urine sample. Enrichment through SPE is essential to detect low concentration of drugs in oral fluid samples.

Drug monitoring for pain management drugs and drugs of abuse

MRM with ESI mode has been widely used in toxicology laboratories for developing in-house drug testing panels. MRM methods are commonly established on triple quadrupole mass spectrometers, where the first and third quadrupoles (Q1 and Q3) function as mass analyzers; the second quadrupole (Q2 or q) functions as a collision cell to introduce fragmentation of targeted ions through collision-induced dissociation. In order to develop an MRM method for a given analyte panel, each analyte needs to be "tuned" by direct infusion into the mass spectrometer to gain the best intensity, sensitivity, and selectivity. Although parameters vary from different mass spectroscopy manufacturers, the universally important parameters include the m/z for precursor ions (Q1) and transition ions (Q3), declustering potential (DP), and collision energy (CE). One or more Q3 masses could be chosen as both quantifier and qualifier to ensure a better identification of certain drug analytes. DP refers to the voltage applied to the orifice to prevent the target ions from being clustered with other ions in the matrix or solvent. CE is the voltage applied at the collision cell and determines the rate of acceleration when the drug ions enter Q2. The first step in method development is to optimize the values for each of these parameters because these values can be compounded as well as instrument dependent.

Two working examples for analysis of 72 pain management drugs and 52 psychiatric drugs are presented using MRM methods developed on a Sciex 4500 Q-Trap Mass Spectrometer (Sciex, Framingham, MA, USA) equipped with ESI. Drugs in the pain management panel and psychiatric panel were both separated through Shimadzu Nexera XR HPLC system (Shimadzu, Kyoto, Japan) and Restek Raptor Bi-phenol analytical column. For each drug panel, the methods were validated for accuracy, linearity, precision, LOD, limit of quantitation (LOQ), and carryover limits, which are all important aspects of clinical MS. Methods for both pain management and psychiatric drug panels were developed and validated according to standard procedures.

Chronic pain was defined by the International Association for the Study of Pain as "… an unpleasant sensory and emotional experience associated with actual or potential tissue damage…."[50] Currently, chronic pain has affected more than a quarter of million American lives leading to a cost of about $600 billion per year.[51–53] Pharmacologic therapies with pain-relieving medications (analgesics) are frequently recommended by physicians for pain management. There are 2 major categories of analgesics commonly available for a pain management program: opioid drugs and non-opioid drugs. Non-opioid drugs, such as aspirin and paracetamol, are mainly used for treatment of moderate levels of pain. Opioid drugs, such as codeine, morphine, and oxycodone, are usually recommended for treatment of severe pain. However, addiction to opioid analgesics, such as oxycodone or heroin, which share chemical similarities, has been frequently identified at various stages of a prescribed pain management program. Therefore, effective pain management drug monitoring via urine specimen assessment is essential to build the confidence for both physicians and pain patients alike, to affirm that patients are being compliant with the prescribed medications as well as to detect abused substances or illicit drugs. However, if the expected drugs

or metabolites are not present, this may be indicative of noncompliance, sample adulteration, or poor drug absorption, in addition to limitations of methodology, instrumentation, or detection sensitivities. An MRM method for pain management drug monitoring is presented in **Table 5**, which covers 32 drug categories that are widely prescribed to chronic pain patients as well as some of the commonly identified illicit drugs and abused substances. A comprehensive list of the drug category (see **Table 5**) includes amphetamine, benzodiazapines, opiates, opiate analogues, opioid, synthetic opioid, oxycodone, buprenorphine, fentanyl, methadone, gabapentin, heroin metabolite, methylphenidate, ketamine and norketamine, methylenedioxyamphetamines (MDA), muscle relaxant, phencyclidine (PCP), pregabalin, propoxyphene, sedative hypnotics, synthetic cannibinoids, tapentadol, tramadol, TCA, stimulant, cocaine metabolite, and bath salts, among others.

Psychiatric medication represents another category of prescription medications where drug monitoring has become of recent interest. There are 6 major psychiatric medication categories: antidepressants, antipsychotics, anxiolytics, depressants, mood stabilizers, and stimulants. Because of the high rates of poor compliance by the patients with mental health issues and the considerable genetic variability of metabolism of the psychiatric drugs, therapeutic monitoring of the psychiatric medication for many patients with psychiatric disorders has been proven valuable for improving the patients' compliance with the medication, avoiding toxicity, optimizing psychopharmacotherapy strategy, and discovering genetic polymorphism and pharmacokinetic mechanisms.[54,55] An LC-MS/MS method for monitoring of 52 psychoactive drugs using positive MRM mode is presented in **Table 6**. The present method covers 12 drug categories focusing on antidepressants (serotonergic), antidepressants (tricyclic), antidepressants (other), antiepileptics, antipsychotics, stimulants, muscle relaxants, alkaloids, ketamine, and methylphenidates.

Methodologies have been developed for the determination of analyte concentrations in urine using a dilute-and-shoot method. Urine aliquots were first separated by centrifugation, followed by hydrolysis with β-glucuronidase to remove β-D-glucuronic acid conjugates that were formed by human metabolism. The mixed urine samples were then diluted with a mobile phase gradient by a dilution factor of 4, followed by vortex and further centrifugation. The supernatant from the resulted urine sample was transferred into HPLC vials and loaded onto the column for MRM analysis. Total run time for this assay is 7 minutes using a gradient elution of mobile phase A (0.1% formic acid and 2 mM ammonium acetate in water) and mobile phase B (0.1% formic acid and 2 mM ammonium acetate in methanol). The gradient was increased from 5% to 95% mobile phase B over 5 minutes at a flow rate of 0.7 mL/min.

The precursor (Q1) and transition (Q3) ions for each of the 72 pain management drug and 52 psychiatric drug analytes, as well as their retention time, LOQ, and LOD, are listed in **Tables 5** and **6**, respectively. Calibration curves for each analyte covers the range from below the cutoff to above the commonly detected confirmation levels. Accuracy was achieved for all drug analytes within the entire calibration range. Coefficients of variation were less than 15% (data not shown). For all the drug analytes, the quantitation method showed linearity in the calibration range of $r > 0.99$. A representative chromatogram of all 72 pain management drug analytes identified using the ESI + mode MRM method is provided in **Fig. 8**. The total MRM detection window was 3 minutes. An overlay of MRM ion traces for both quantifier and qualifier transition ions of each drug is shown in different colors. The scheduled MRM algorithm provided by AB Sciex Analyst software allowed the monitoring of the MRM for each drug transition being triggered only within an appropriate time window flanking the retention

Table 5
List of 72 pain management drugs and their categories, precursor and transition ions, retention time, cutoff concentration, as well as method validation results for limit of detection and limit of quantitation

Group	Analyte	Q1 Mass (Da)	Q3 Mass (Da)	RT (min)	Cutoff (ng/mL)	LOD (ng/mL)	LOQ (ng/mL)
Adrenergic agonist	Ephedrine	166.115	91.1/115	1.35	25	0.35	1.07
Amphetamine	Amphetamine	136.1	91/119	1.49	100	1.02	3.08
	Methamphetamine	150.1	91.2/119.2	1.67	100	2.63	7.96
Bath salts	Mephedrone	178.3	145/144	1.91	3	0.15	0.47
	Methylone	208.1	160.1/132.1	1.74	3	0.13	0.39
	Methylenedioxypyrovalerone	276.2	126.1/135	2.41	3	0.17	0.50
Benzodiazapines	Diazepam	285.1	193.1/154	3.57	25	1.78	5.41
	Midazolam	326.1	291.1/222	2.96	25	0.35	1.08
	α-Hydroxyalprazolam	325.1	297/216.1	3.25	25	0.03	0.09
	Alprazolam	309.1	281/205.1	3.43	25	0.85	2.57
	7-Aminoclonazepam	286.1	222.1/121.1	2.39	25	0.51	1.55
	Flunitrazepam	314.1	268.2/239.2	3.38	25	0.56	1.70
	Flurazepam	388.2	315.1/134.1	2.76	25	0.40	1.22
	Lorazepam	321	275/229.1	3.06	25	0.61	1.84
	Nordiazepam	271.1	140/165.1	3.3	25	0.59	1.79
	Oxazepam	287.1	241/269.1	3.13	25	0.83	2.52
	Temazepam	301.1	255.1/177.1	3.39	25	0.83	2.50
	Triazolam	343	239/314.9	3.36	25	0.14	0.41
Buprenorphine	Buprenorphine	468.4	396.3/414.3	2.7	25	0.81	2.45
	Norbuprenorphine	414.3	101.1/165.1	2.42	25	3.51	10.64
Cocaine	Benzoylecgonine	290.2	168.1/105	2.27	25	0.46	1.40
Fentanyl	Fentanyl	337.2	105.1/188.1	2.69	3	0.14	0.42
	Norfentanyl	233.3	84.3/150.2	2.1	10	0.36	1.09
Gabapentin	Gabapentin	172.1	154.2/95.2	1.5	200	5.25	15.90
Heroin metabolite	6-MAM	328.1	165.2/211.2	1.7	10	0.70	2.12

Ketamine & norketamine	Ketamine	238.1	125/220.2	2.23	25	0.61	1.84
Methadone	EDDP (2-ethylidene-1,5-dimethyl-3, 3-diphenylpyrrolidine)	278.3	234.2/186.2	2.92	100	2.05	6.20
	Methadone	310.2	265.2/105	3.07	100	0.97	2.94
MDA	MDA	180.1	105/133	1.7	100	3.44	10.42
	MDEA	208.2	163.2/105.2	1.99	100	2.61	7.91
	3,4-Methylenedioxyamphetamine (MDMA)	194.1	163.2/105.2	1.84	100	0.98	2.97
Methylphenidate	Methylphenidate	234.1	84.1/91.1	2.24	25	0.32	0.96
Muscle relaxant	Carisoprodol	261.2	176.2/97.2	2.78	25	1.44	4.35
	Meprobamate	219.1	158.2/97.1	2.28	25	1.39	4.20
Nicotine	Cotinine	177.1	80/98.1	1.32	25	0.21	0.62
Opiates	Codeine	300.1	152.1/115.1	1.7	25	1.29	3.90
	Hydrocodone	300.1	199.1/128.1	1.84	25	1.12	3.39
	Hydromorphone	286.2	185/128	1.36	25	0.63	1.91
	Morphine	286.2	152/165	1.24	25	1.73	5.25
	Norcodeine	286.4	152.3/165.2	1.52	25	1.73	5.24
	Norhydrocodone	286.4	199.3/128.2	1.72	25	0.79	2.41
	Dihydrocodeine	302.4	199.2/128.3	1.66	25	0.42	1.26
Opiods & opiate analogues	Meperidine	248.2	220/174.1	2.27	25	0.46	1.40
Opioid, antagonist	Naloxone	328.1	212.2/253.1	1.62	25	2.03	6.15
	Naltrexone	342.16	212.2/267.2	1.8	25	0.86	2.60
Opioids, partial	Pentazocine	286.2	218.1/69.2	2.42	25	0.36	1.10
	Nalbuphine	358.3	340.3/272	2.06	25	0.85	2.58
	Butorphanol	328.3	310.3/131.2	2.49	25	0.22	0.66
Opioids, synthetic	Sufentanil	387.1	111.1/238	2.86	25	0.10	0.31
Other, antitussive, psychedelic	Dextromethorphan	272.1	171.1/215.1	2.78	25	1.34	4.06
	LSD	324.3	223.1/208	2.46	3	0.06	0.19

(*continued on next page*)

Table 5
(*continued*)

Group	Analyte	Q1 Mass (Da)	Q3 Mass (Da)	RT (min)	Cutoff (ng/mL)	LOD (ng/mL)	LOQ (ng/mL)
Oxycodone	Oxycodone	316.2	241/256	1.79	25	0.58	1.76
	Oxymorphone	302.1	227/198.1	1.28	25	0.74	2.24
	Noroxycodone	302.3	227.2/187.2	1.68	25	0.67	2.04
	Noroxymorphone	288.4	213.3/184.1	1.08	25	1.37	4.15
PCP	PCP	244.3	91/159.3	2.79	25	1.07	3.23
Pregabalin	Pregabalin	160.1	97/83	1.3	100	3.26	9.89
Propoxyphene	Propoxyphene	340.2	266.2/58.2	2.79	25	1.19	3.62
Sedative hypnotics	Zaleplon	306.2	236.3/264.2	3.3	25	0.91	2.75
	Zolpidem	308.2	235.1/236.1	2.61	10	0.41	1.24
	Zopiclone	389	245.1/217	2.37	25	2.10	6.36
Stimulant, synthetic	Phentermine	150.2	91.1/133.1	1.65	25	0.35	1.05
	Methcathinone	164.1	131.1/130	1.48	25	0.23	0.71
Synthetic cannibinoids	JWH-018N-Pentanoic acid	372.15	155/126.9	3.68	15	0.19	0.58
	JWH-073-4OH butyl	344.07	155/127	3.55	15	0.25	0.76
	JWH-073-N-butanoic acid	358.2	155/127	3.66	15	1.73	5.24
Tapentadol	Tapentadol	222.2	107.1/121.1	2.1	25	0.70	2.14
Tramadol	Tramadol	264.1	58.1/42.1	2.19	25	1.41	4.28
Tricyclic antidepressants	Nortriptyline	264.2	91/191.2	2.91	25	0.63	1.91
	Amitriptyline	278.2	91/191.2	2.94	25	0.67	2.04
	Imipramine	281.1	85.9/57.6	2.89	25	0.23	0.69
	Desipramine	267.2	72.1/193.2	2.86	25	0.54	1.62

time, thus avoided decreasing dwell times for each MRM transitions by reducing the numbers of concurrent MRMs being monitored. Target scan time was set as 0.2 seconds, and the MRM detection window was 30 seconds.

In summary, MRM methods of 2 large testing panels were presented as an example to demonstrate the benefit of using LC-MS/MS analysis to provide a fast and accurate tool for clinical testing. LC-MS/MS is one of the most suitable technologies for clinical toxicology applications toward rapid, highly selective, and robust testing of known drug analytes from human biofluids.

The advantages and disadvantages of liquid chromatography–tandem mass spectrometry

Advantages As shown in the above applications, LC-MS/MS offers superior specificity, sensitivity, and throughput, compared with other most commonly used techniques, such as IAs, UV-based chemical analysis, GC-MS, or conventional HPLC. LC-MS/MS offers much better specificity for a target molecule, because the quadrupole recognizes not only the original ionized molecule but also all the fragments derived from the original ionized molecule. Combined with the HPLC technique, the separation of the analytes is much easier and the retention time is another characteristic factor that enhances the selectivity of the mass spectrum.

The LC-MS/MS exhibits flexibility and versatility for the clinical laboratories to develop and validate new assays in house within a short time. LC-MS/MS assays, as laboratory developed tests, are highly attractive for target analytes where no commercial IAs are available. In contrast to GC/MS, which is limited to volatile molecules, LC-MS/MS has much wider range of applications because most biologically active molecules are polar, thermolabile, and nonvolatile. In addition, the LC-MS/MS sample preparation is simpler and does not require derivatization techniques.

A single LC-MS/MS run is able to provide a large number of quantitative or qualitative results. Thus, LC-MS/MS offers a far higher sample throughput. Another approach to increase the high throughput is to use the “multiplexed LC system.” The Thermo Fisher TLX4 online sample preparation system and the Sciex MPX system are 2 examples of such an endeavor. Basically, the concept of a multiplex system is to maximize the use of MS by introducing specimens to MS from multiple chromatographic systems in a staggered fashion. Multiplexing allows the “spare time” of MS, which is the LC starting time before the first analyte elutes and the end time after the last analyte elutes, to be used by detecting analytes on the second LC stream. Depending on the optimized chromatography separation condition for a give group of analytes, multiplex systems can generally increase the throughput to 1.5- to 2-fold.

Another advantage of using LC-MS/MS in clinical toxicology applications is the possibility of identifying adulterated samples. Usually, IA only provides information on the drug category with semiquantitative data, but does not specify the exact drugs. For example, a screen test only provides the information that the sample contains opioids or benzodiazepines, but does not specify exactly which opioid drugs or benzodiazepine drugs are detected. As the diversion of opioid medications by the patients to other people for use and sale is not rare, a more robust approach to drug testing can help identify drug adulteration of urine samples as a portal for drug diversion. LC-MS/MS gives quantitative data for prescribed drugs and their metabolites, thereby providing objective ancillary assistance to the clinician to help assess if the patient takes their medication regularly versus adulteration of the specimen. For example, ingested hydrocodone would normally convert hydromorphone as well as dihydrocodiene and norhydrocodone, all of which would likely

Table 6
List of 52 psychiatric drugs and their categories, precursor and transition ions, retention time, cutoff concentration, as well as method validation results for limit of detection and limit of quantitation

Drug Group	Analyte	Q1 Mass (Da)	Q3 Mass (Da)	RT (min)	Cutoff (ng/mL)	LOD (ng/mL)	LOQ (ng/mL)
Alkaloid	Mitragynine	399.2	174.1/159.1	1.7	25	2.67	8.08
Antidepressants, serotonergic	Trazodone	372.2	176.1/148.1	1.59	25	0.65	1.97
	Vilazodone	442.5	197.3/425	1.52	25	5.17	15.65
	Citalopram	325.2	109.1/262.2	1.56	25	0.71	2.15
	Desmethylmirtazapine	252	195/209	1.46	25	0.53	1.62
	Duloxetine	298.1	44.1/154.2	1.73	25	17.15	52
	Fluoxetine	310.1	44/148	1.59	25	1.87	5.67
	Mirtazapine	266.2	195.2/194.2	1.48	25	0.46	1.4
	Paroxetine	330.1	69.9/135.1	1.8	25	0.44	1.32
	Sertraline	306.1	159/275.1	1.8	25	1.32	4
	Venlafaxine	278.2	58/121.1	1.56	25	1.08	3.26
	O-Desmethylvenlafaxine	264.1	58.1/107	1.11	25	1.64	4.98
Antidepressants, tricyclic	Desipramine	267.2	72.1/193.2	1.7	25	0.88	2.65
	Imipramine	281	85.9/57.6	1.73	50	1	3.02
	Amitriptyline	278.2	91.0/191.2	1.83	25	1.38	4.19
	Clomipramine	315.2	86.1/58	1.83	25	0.5	1.51
	Doxepin	280.2	107.1/235.1	1.72	25	0.42	1.28
	Nortriptyline	264.2	91.0/191.2	1.73	25	1.33	4.02
Antidepressants, other	Bupropion	239.8	184/166	1.52	25	1.84	5.57
	Selegiline	188.3	91.1/119.1	1.2	25	0.62	1.88
Antiepileptics	Carbamazepine epoxide	253.1	210.2/180.1	1.79	25	1.04	3.16
	Carbamazepine	237.1	194.1/165	1.81	25	0.75	2.29
	Hydroxycarbamazepine	255.1	237/194.1	1.52	25	3.85	11.67
	Lamotrigine	256.02	211.1/157	1.38	25	5	15.16
	Oxcarbazepine	253.1	180.2/208.2	1.66	25	1.1	3.33

Antipsychotics	Ziprasidone	413.2	194.1/130	1.67	25	2.14	6.49
	Norquetiapine	296.12	210.1/139.1	1.66	25	0.79	2.41
	Olanzapine	313.1	256.2/198.1	1.22	25	0.72	2.19
	7-Hydroxyquetiapine	400.3	269/208	1.26	25	1.78	5.39
	9-Hydroxyrisperidone	427.2	207.2/110	1.48	25	0.7	2.11
	Aripiprazole	448.1	285.2/176.2	1.94	25	1.68	5.08
	Asenapine	286.2	165.1/229.1	1.78	25	0.6	1.83
	Clozapine	327.1	270.1/192.2	1.64	25	0.26	0.8
	Desmethylolanzapine	299.1	256.1/198.1	1.22	25	1.25	3.79
	Fluphenazine	438.3	171.1/143.2	1.86	25	0.8	2.43
	Haloperidol	376.1	123/358.2	1.59	25	0.43	1.3
	Iloperidone	427	261/190.1	1.71	25	0.41	1.25
	Lurasidone	493.7	166.3/220.2	2.59	50	0.61	1.86
	Quetiapine	384.2	221.2/253.2	1.66	25	1.89	5.73
	Risperidone	411.2	191.1/148.2	1.63	25	2.07	6.27
Ketamine	Ketamine	238.1	125/220.2	1.5	25	0.12	0.36
Methylphenidate	Ritalinic acid	220.1	84.1/56.2	1.22	25	1.04	3.16
Skeletal muscle relaxant	Cyclobenzaprine	276.2	215.1/216.1	1.73	25	1.49	4.51
Stimulant, synthetic	MDPV (Methylenedioxypyrovalerone)	276.2	126.1/135	1.42	25	1.51	4.59
	Mephedrone	178.3	145.0/144.0	1.14	3	0.08	0.25
	Methylone	208.1	160.1/132.1	1.03	3	0.51	1.54
	Methcathinone	164.1	131.0/130.0	0.89	25	0.35	1.05
Other, adrenergic agonist, antihistamine, antitussive, anxiolytic	Ephedrine	166.1	91.1/115.0	0.91	25	1.93	5.85
	Diphenhydramine	256.1	167/152.1	1.68	25	3.9	11.82
	Dextromethorphan	272.1	171.1/215.1	1.77	25	0.33	1
	Buspirone	386.3	122.1/95	1.77	25	1	3.03
Other, nicotinic agonist	Varenicline	212.091	169.1/168.1	0.93	25	2.62	7.95

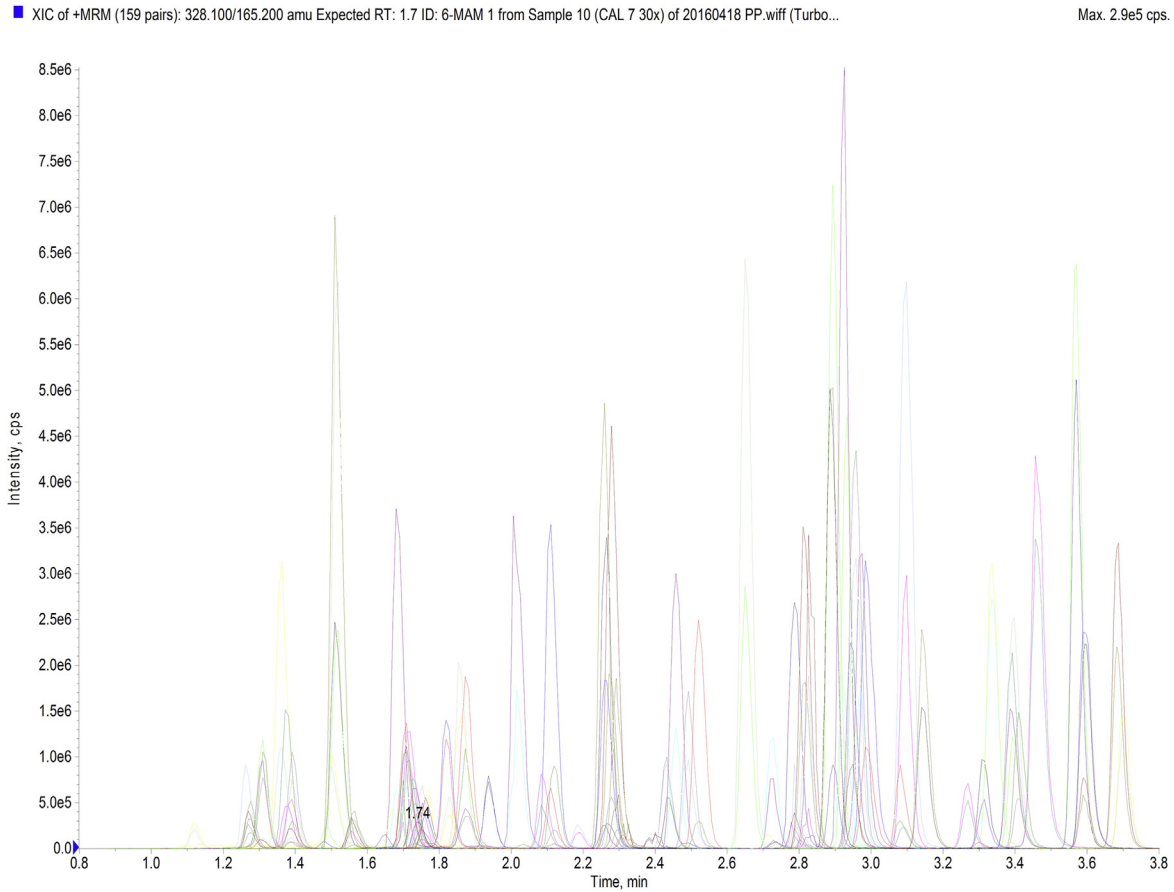

Fig. 8. Chromatogram of 72 pain management drug analytes identified using the positive MRM method. Overlaid MRM ion traces for both quantifier and qualifier transition ions are shown in different colors. MRM detection window was 3 minutes.

be detected in the urine with varying concentrations depending on time of ingestion, hydration status, liver and kidney function, among other factors. However, if hydrocodone is the only analyte detected in a urine specimen with a positive concentration that is over the high range cutoff value, whereas the expected metabolites (hydromorphone, dihydrocodiene and norhydrocodone) are negative or below the lower limits of detection cutoff, there would be a suspicion of specimen adulteration where the patient may have added hydrocodone directly into the urine sample, as a means of testing positive for the clinician while diverting the rest of the medication for economic incentives.

Disadvantages One major concern of the LC-MS/MS technique is ion suppression. Signal intensity obtained from a clean "standard" can differ significantly from human matrix samples, especially when the samples were not properly processed. Ion suppression presents a challenge in LC-MS/MS quantitation. However, using more selective extraction procedures for sample preparation and improving chromatographic retention to separate analytes from the highly polar matrix component can minimize the effect. Although LC-MS/MS offers the flexibility of developing assays to meet the clinical needs, the shortcoming of the flexibility is that method development, validation, and quality control may vary among different laboratories because most of the LC-MS/MS methods used in the clinical laboratories are often laboratory developed. Accuracy experiments are a prerequisite step in method validation to ensure the result variance is less than 20% from laboratory to laboratory. However, concern still exists regarding data discrepancies among different laboratories even with small variations like selecting different internal standards. The instrument itself is relatively more expensive compared with other technologies. Also, in addition to the expense of setting up and maintaining an LC-MS/MS laboratory, there is the understanding that highly qualified staff is required for method development, maintaining the instrument, as well as the day-to-day operation and data processing.

FUTURE PERSPECTIVES

MS technology has achieved an unprecedented maturation and development in the past few decades toward toxicology and other clinical applications. Many exciting new applications and developments are underway for clinical MS.[19] Advances in instrumentation, interfaces, software, and sample preparation techniques will all enable faster, better, and less-expensive testing. At the same time, integration of this technology into clinical automated systems shows promise toward moving the technology into a more automated setting. To reach that point, standardization of method development and validation processes, especially for laboratory developed tests, will need to take place.

Currently, most quantitative clinical analyses use tandem quadrupole mass spectrometers. The desire for increased sensitivity and robustness will drive instrument-related improvements, particularly in source design and ion transmission. Similarly, instrument development in LC continues, allowing faster chromatographic run times and greater resolution. Advances in both LC and MS will drive improvement in laboratory metrics, coupling decreased turnaround times with increased sensitivity.[56]

Although tandem quadrupoles will continue to be a dominant instrument in clinical chemistry analysis for some time, advances in instrumentation that enable the coupling of higher-resolution detection with the precise quantification available in triple quadrupoles will likely become available. Specifically, quadrupole TOF and

orbitrap mass spectrometers show great promise in adding the additional capability of high-resolution/accurate mass detection to clinical analyses, which will allow greater specification in the analysis.[57] High-resolution instruments provide both quantitative and qualitative analyses while acquiring high-resolution full scan or MS/MS data. Furthermore, high-resolution full scan data acquisition provides a more complete description of the content of a sample. The quantitative performance of current high-resolution instruments is reported to be similar to that of current triple quadrupole mass spectrometers.[58–60]

An additional venue for instrumental development is increased automation; this is taking place on several fronts simultaneously. The need for reduced cost drives advances in automation and the simplification of sample preparation, particularly for sample sets with relatively low numbers of samples. Integration of automated sample preparation with LC-MS/MS systems will reduce the effort and time required for sample preparation and will reduce the possibility of error as more sample information is passed electronically from one stage of analysis to the next. Similarly, the reduction of human intervention in the review of data will drive efficiency, as will the integration of data reporting from the analyst to the clinical laboratory information system. Bidirectional information transfer will also be a productivity tool. Ultimately, the integration of LC-MS/MS into clinical chemistry analyzers may provide access to the already-established technologies currently implemented for sample preparation and information handling.

Another pathway for MS that is heavily based on automation and miniaturization would be the introduction of MS systems in point-of-care venues for rapid testing of bodily fluids for therapeutic and illicit drugs, peptides, and hormones.[61] Before that goal can be accomplished, much work needs to be done to standardize the method development and validation processes, with a potential end of having US Food and Drug Administration (FDA)-approved tests using LC-MS/MS. The current guidelines provide an excellent framework for laboratory developed tests, but greater homogenization of methods between laboratories will provide more consistent results. The FDA is moving to increasing oversight on laboratory developed tests; any forthcoming regulation will greatly impact the role of LC-MS/MS in the clinical laboratory.

The future of MS in clinical analysis is promising and bright. Opportunities for research and improvement are constantly maturing. At the same time, the impact of LC-MS/MS in the health care community continues to grow, making this technology interesting and impactful.

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