Chapter 1

Mass Spectrometry in Clinical Laboratory: Applications in Therapeutic Drug Monitoring and Toxicology

Uttam Garg and Yan Victoria Zhang

Abstract

Mass spectrometry (MS) has been used in research and specialized clinical laboratories for decades as a very powerful technology to identify and quantify compounds. In recent years, application of MS in routine clinical laboratories has increased significantly. This is mainly due to the ability of MS to provide very specific identification, high sensitivity, and simultaneous analysis of multiple analytes (>100). The coupling of tandem mass spectrometry with gas chromatography (GC) or liquid chromatography (LC) has enabled the rapid expansion of this technology. While applications of MS are used in many clinical areas, therapeutic drug monitoring, drugs of abuse, and clinical toxicology are still the primary focuses of the field. It is not uncommon to see mass spectrometry being used in routine clinical practices for those applications.

Key words Clinical laboratory, Mass spectrometry, Liquid chromatography, Gas chromatography, Tandem mass spectrometry, Drugs, Therapeutic drug monitoring, Toxicology, Time-of-flight, Immunoassays

1 Introduction

Mass spectrometry, once considered a very specialized and expensive technology for routine use, has made its way in many clinical laboratories in recent years [1, 2]. This rapid growth has been made possible by developments in the technology, the advent of bench top systems, increased ease of operation, reduced capital investment, and more user-friendly software systems.

Therapeutic drug monitoring, testing for drugs of abuse, pain management, and forensic drug testing have been the early adaptors of this technology and still are the main driving force behind the fast growth of the field. Mass spectrometry has been introduced and utilized to overcome the inherent limitations of immunoassays from drug testing due to its high specificity. Enhancements in mass spectrometry continue to improve sensitivity and enable measurement of ever lower concentrations of analytes. GC-MS was the initial MS technique used in clinical laboratories, and introduction

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of LC-MS/MS enabled the analysis of many analytes that cannot be easily analyzed by GC and are not suitable for GC-MS. Methods have been reported on a wide array of analytes and the accumulation of experience in the community is available to help interested laboratories overcome the huddles to bring mass spectrometry to their practices to better serve our patients and for the betterment of health care.

The recent developments in mass spectrometry have made it a very attractive platform for clinical practice, yet apprehension due to the complexity of the technology, relatively high capital investment, personnel training, and the requirement for in-house method development and validation present challenges to the implementation of this technology in many routine clinical laboratories.

2 Clinical Applications

Immunoassays are commonly used methods for therapeutic drug monitoring and drugs of abuse in the clinical laboratory. Since immunoassays can cause false positive or false negative results due to lack of specificity or cross-reactivity, and immunoassays may not available for a number of drugs, MS has been used for confirmation of immunoassay results [3] and sometime used directly as screening methods. Measurement of small molecule drugs continues to push the development of the technology and to be one of the main driving forces for increasing applications of mass spectrometry in clinical practices. The focus of this volume is for therapeutic drug monitoring and toxicology. Drugs of abuse and therapeutic drugs commonly analyzed by mass spectrometry are listed in Table 1.

Testing for the screening and confirmation of inborn error of metabolism was another early adaptor of mass spectrometry and has played an important role in enhancing the applications of mass spectrometry [4, 5]. Recently, many developments have taken place in new fields of study, particularly endocrinology and hormone testing in clinical labs [6–8].

Although not yet widely found in clinical laboratories, applications of MS are expanding in the analysis of large molecules such as peptides, proteins, lipids, polysaccharides, and DNA [9–11]. Another emerging area is the application of matrix-assisted laser desorption/ionization (MALDI) mass spectrometry to rapid bacterial identification [12–14].

3 Fundamentals and Recent Developments of Mass Spectrometry-Based Analysis

A detailed description of mass spectrometry is beyond the scope of this chapter and only a brief description on the fundamentals of the technique is provided. Mass spectrometry is based on the ability to

Table 1
Drugs of abuse and therapeutic drugs commonly assayed by mass spectrometry

Drugs of abuse/other	Therapeutic drugs
• Amphetamines and related drugs • Barbiturates (amobarbital, butalbital, pentobarbital, phenobarbital, secobarbital, etc.) • Bath salts • Benzodiazepines (alprazolam, diazepam, lorazepam, midazolam, oxazepam, temazepam, clonazepam and their metabolites, etc.) • Buprenorphine • Cocaine and its metabolites • Cannabinoids • Cannabinoids, synthetic • Drug screening, broad spectrum • Ethanol use markers (Ethyl Glucuronide and Ethyl Sulfate) • Ketamine • Methadone and metabolites • Methamphetamine • Nicotine and metabolites • Opiates and opioids (morphine, codeine, hydrocodone, oxycodone, oxymorphone, 6-acetylmorphine, fentanyl, etc.) • Phencyclidine • Propoxyphene • Zolpidem	• Antidepressants (tricyclics and selective serotonin reuptake inhibitors) • Anticoagulants (dabigatran, rivaroxaban, apixaban, warfarin) • Anticonvulsants (lamotrigine, levetiracetam, 10-hydroxycarbazepine, topiramate, zonisamide) • Antipsychotics (haloperidol, fluphenazine, perphenazine, thiothixene) • Busulfan • Cardiac drugs (flecainide, mexiletine, propafenone, amiodarone) • Carisoprodol • 5-Fluorouracil • Ibuprofen • Immunosuppressants (cyclosporine, everolimus, mycophenolic acid, sirolimus, tacrolimus) • Indomethacin • Meprobamate • Methotrexate • Teriflunomide

influence the motion of charged particles with electric and magnetic fields. This allows separation of charged particles based on their mass-to-charge (m/z) ratios. A mass spectrometer can be thought of as an instrument that measures the masses of molecules that have been converted into ions.

3.1 Mass Spectrometry-Based Analysis Overview

Analytes need to be either positively or negatively charged (ionized) to be analyzed by a mass spectrometer. In addition, all other charged or potentially ionizable compounds in the sample can potentially be interferences for the analyte of interest. Samples need to be carefully treated before analysis and the sample preparation can be either very simple like "dilute and shoot" or very elaborate. Liquid-liquid or solid-phase extractions are commonly used. While samples can be introduced directly into a mass spectrometer, gas or liquid chromatographic systems for separation are typically used to first isolate the compounds of interest from the matrix.

After separation, the effluent from the chromatograph is ionized. The mass analyzer separates the ions formed based on their mass-to-charge ratio. At the end, the ions are "recorded" in the detector and reported out through the data analysis system.

A schematic diagram of a generic mass spectrometer with different options is shown in Fig. 1.

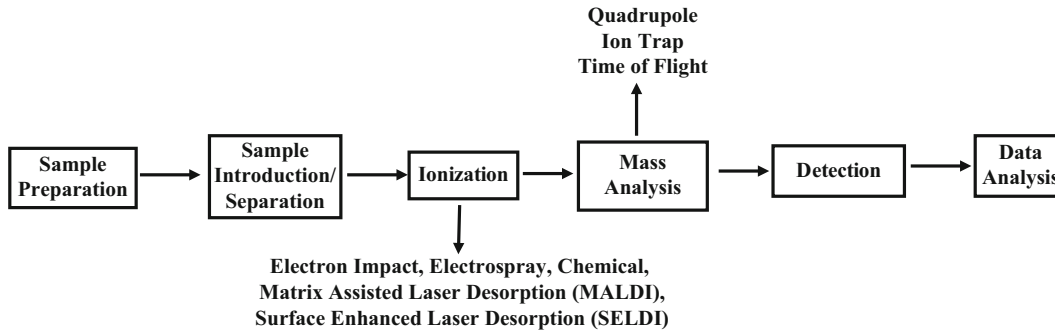

Fig. 1 Schematic diagram for a mass spectrometry analysis

3.2 GC-MS and LC-MS/MS

GC and LC are the two most common chromatography separation techniques coupled to mass spectrometry. GC has been used in clinical laboratories for several decades and LC has gained popularity in recent years mainly due to ease of sample preparation. Using GC-MS or LC-MS/MS, hundreds to thousands of drugs and toxins can be screened in a single analytical run [15]. GC-MS is suitable for analysis of small molecules that are volatile, nonpolar, and thermally stable. Analytes that are heat labile and difficult to derivatize are more suited for LC-MS/MS analysis. While both separation methods typically involves certain types of analyte extraction and possible concentration of the extract, GC often requires lengthy sample derivatization steps for compounds that are not volatile or thermally stable. Simple sample preparation and a broader array of analytes have enabled LC-MS/MS to gain popularity in clinical laboratories. Disadvantages of LC-MS/MS are less reproducible mass spectra, higher maintenance, and high cost.

3.3 Types of Mass Spectrometers

A variety of mass analyzers are used in clinical mass spectrometry. The most common types are single quadrupoles, triple quadrupoles, and time-of-flight (TOF) instruments with triple-quadrupole mass spectrometers being the most prevalent in clinical laboratories. Triple quadrupole mass spectrometers offer unique advantages. They are robust and can be used for multiple analytes. They have several scan modes available that are particularly applicable for clinical analyses. The most commonly used scan function is multiple reaction monitoring (MRM).

In an MRM assay, the compound of interest is identified based on the cleavage of a precursor ion to form a fragment ion. One or more fragment ions can be used. Two fragment ions are preferred to increase specificity of the analysis, in which case one fragment ion functions as qualifier ion and the other functions as quantifier ion. With an appropriate internal standard, MRM can generally be used for quantitative analysis.

Time-of-flight instruments bring an additional dimension of analysis due to its high-resolution enabling broad-spectrum screening and confirmation methods. The coupling of MALDI and TOF has enabled the emerging application of microorganism identification in microbiology laboratories.

4 Implementing Mass Spectrometry in a Clinical Laboratory

Implementation of mass spectrometry in a clinical laboratory is not an easy undertaking [16–23]. It is a major investment in both financial and human capital for an institution and requires careful planning and diligent execution for a successful outcome. Implementation is a multi-step process and a summary of key considerations is presented in Table 2.

The process typically starts with an assessment of clinical needs, instrument selection, and a financial justification, followed by several other essential tasks such as space planning, site preparation,

Table 2
Major steps in implementing mass spectrometry in a clinical laboratory

Clinical needs
• Is the primary consideration • Reduce turnaround time • Control over sample handling process and reduce handling errors
Financial considerations
• Key is to have an institutionally acceptable return on investment (ROI) • Benefits include bringing test in-house and reduce send-out costs • Primary investment is instrument itself – Capital investment or leasing options • Other investment considerations should include – Service contract – Infrastructure and space requirement, and may need renovation – Cost for interfacing to the LIS if desirable – Ongoing operating cost (e.g., high-grade reagents, special reagents, gas)
Instrument selection
• Based on intended analyses and economics • Site visit and communication with colleagues and vendors • Service availability and response time for service requests
Assay selection
• Based on type of instrumentation, analytes, and clinical needs • Literature search and communication with colleagues • Consider lab staff experience and training

(continued)

Table 2
(continued)

Infrastructure planning
• Space for instrumentation and HPLC • Gas supplies: compressor air, nitrogen gas dewars, or nitrogen generator • Ventilation and noise blocking • Lab space rearrangements (e.g., fixed vs. movable bench) • Dedicated electric system and uninterrupted power supply • IT support and data backup
Staff and personnel training
• Essential for a successful implementation • Is an ongoing process • Onsite training with manufacturers • Online training courses • Conferences workshops, symposia, and short courses
Method development and validation
• Meets CLIA requirements for high complex testing • Use highest grade reagents available (MS grade or at least HPLC grade) • Choose proper internal standards • Validation shall include – Precision – Accuracy – Analytical sensitivity – Analytical range and reportable range – Reference interval validation – Stability – Specificity and interference testing

installation, personnel training, and method evaluation. In total, the whole process can take from 6–9 months to 1 year. After that, in-house method development and validation bring another set of challenges. Mass spectrometry assays are considered high-complexity under CLIA and fall under the category of laboratory-developed tests. This section sheds some light onto these steps with the hope of saving the audience some headaches in the implementation phase.

4.1 Assay and Instrument Selection

A large body of evidence has indicated the value of mass spectrometry for clinical practice and many well-established tests are available for labs to consider [24–26]. For a lab new to mass spectrometry, clinical needs justification becomes a question of which tests to bring in-house and which methods to choose. Consultation with in-house physicians and advocates will ease decision making and increase acceptance.

While modern mass spec companies all provide high quality products, each vendor has its own unique "temperament." Consulting with colleagues and site visits can help narrow down the choices of vendors for further investigation. In addition, service availability and response time play an important role in decision making and should be considered very seriously.

4.2 Financial Justification

Financial justification is one of the initial challenges of bringing mass spectrometry technology to a laboratory. The financial justification can be approximated as a return on investment (ROI) estimation. The cost of instruments (ranges from low US$200,000 to US$4–500,000) and the savings from bringing tests in house on mass spectrometry are obvious considerations. Several other factors are not necessarily obvious for people who are new in this field and are important for the total financial estimation. These factors include the cost of an annual service contract, cost for infrastructure planning and space renovation, and operating costs. Each one of these cost can be executed with different options, which can make a large difference in the final financial commitment. While the typical practice has been to acquire instrumentation through the standard equipment capitalization process by purchasing the instrument upfront, reagent leasing and equipment leasing are other options. It is worth the time to go through the options for any given test and projected volume.

It is recommended to have a full-service contract for most laboratories to cover regular maintenance and repairs. Manufacturers often provide discounts for pre-purchased service contracts after the warranty period or a discount for purchasing multiple-year contracts.

Mass spectrometers require specific infrastructure elements, such as particular voltage/current requirements, high purity gas, ventilation and noise blocking system which are not necessarily in place at most institutions. Communication with the vendors is highly recommended to ensure that the infrastructure and space planning meet the vendor's specific requirements.

Besides the initial fixed cost, running an assay is relatively inexpensive. Nonetheless, several factors such as consumables for sample preparation, reagents, internal standards, quality controls, gas supplies, data analysis, and data reporting should be considered. MS-grade reagents can be expensive, and it is important to get the highest grade of reagents for MS assays. If they are not available, HPLC-grade reagents can sometimes be substituted. Internal standards are unique to mass spectrometry assays and can be very expensive.

The majority of mass spectrometry data still includes some manual processing, which is labor intensive. Many manufacturers are able and willing to assist in interfacing the data system to the laboratory information system (LIS), which can reduce the

reporting time significantly. If this is desirable, the cost for interfacing is another consideration.

Reimbursement rates for mass spectrometry assays are reasonable and the variable operating cost is relatively low. Despite the costs, the addition of mass spectrometric analyses to a laboratory in most cases can provide a reasonable ROI and can be financially justified.

4.3 Staff Training and Human Resources

Having properly trained staff for routine analysis and a skilled analyst for troubleshooting and method development are crucial to successful implementation. Hiring staff with proper training for mass spectrometry has been one of the major challenges to implementation of this technology in clinical laboratories. However, with dedicated instrument time and an experienced trainer, a good medical technologist can be trained to perform mass spectrometric analyses within a few weeks. When method development is involved, longer and more sophisticated training is required.

Training can be obtained in several ways. Instrument manufacturers can provide training during the installation, through their onsite live training classes or online tutorials. Many online training materials and tutorials are available from other organizations such as the American Association for Clinical Chemistry, and colleagues are always good resources. There is nothing, however, that can substitute for the experience gained from sitting in front of the instrument.

4.4 Method Development and Validation

Mass spectrometry assays are laboratory-developed tests, which require sophisticated validation processes. Most assays are developed in-house and standardization of the process is very important. The recent CLSI guideline is a good reference [27]. In addition to the typical validation steps for clinical assays including limit of detection, limit of quantitation, accuracy, analytical measurement range and clinical reportable range, reagent and analyte stability, reference range determination, and interference, mass spectrometric analyses require additional validation steps. A particular requirement of mass spectrometric analyses is an assessment of ion suppression. There are different ways to do ion suppression assessment, and compromise between the amount of sample cleanup and the level of ion suppression tolerable is often necessary. In many cases, the use of an internal standard can help correct for moderate levels of ion suppression.

The internal standard plays an important role in providing accurate results with mass spectrometry. The internal standard should be chemically similar to the analyte but with a different molecular weight. The heavy isotope labeled compounds with C13 and N15 have become the typical choice. A mass difference between the analyte of interest and the internal standard of at least 3 mass units is desirable, although a difference of at least 5 is preferred to completely reduce cross talk.

5 Conclusion

In recent years mass spectrometry has emerged as an important tool in laboratory medicine. While it has been applied in many clinical areas, therapeutic drug monitoring and toxicology remain as key applications and continue to be the driving force to push through challenges to increase the applications of mass spectrometry in clinical practices. Many methods have published in recent years in these areas and for analytes that were once considered unlikely for MS. Mass spectrometry remains a new, exciting, and sophisticated technology. Its implementation requires careful planning and diligent execution to ensure success in routine practices. This volume has collected many methods currently used in clinical laboratories with a sufficient level of detail to allow straightforward implementation. We hope that this will facilitate the adaption of this technology in clinical practices.

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