

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Application of Mass Spectroscopy in Pharmaceutical and Biomedical Analysis

Uttam Singh Baghel, Atamjit Singh,
Deeksha Singh and Manish Sinha

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.70655>

Abstract

Mass spectrometry (MS) is a powerful analytical tool with many applications in pharmaceutical and biomedical field. The increase in sensitivity and resolution of the instrument has opened new dimensions in analysis of pharmaceuticals and complex metabolites of biological systems. Compared with other techniques, mass spectroscopy is only the technique for molecular weight determination, through which we can predict the molecular formula. It is based on the conversion of the sample into ionized state, with or without fragmentation which are then identified by their mass-to-charge ratios (m/e). Mass spectroscopy provides rich elemental information, which is an important asset to interpret complex mixture components. Thus, it is an important tool for structure elucidation of unknown compounds. Mass spectroscopy also helps in quantitative elemental analysis, that is, the intensity of a mass spectra signal is directly proportional to the percentage of corresponding element. It is also a noninvasive tool that permits *in vivo* studies in humans. Recent research has looked into the possible applications of mass spectrometers in biomedical field. It is also used as a sensitive detector for chromatographic techniques like LC-MS, GC-MS and LC/MS/MS. These recent hyphenated technological developments of the technique have significantly improved its applicability in pharmaceutical and biomedical analyses.

Keywords: mass spectrometry, pharmaceutical, biomedical, phytochemical, structure elucidation

1. Introduction

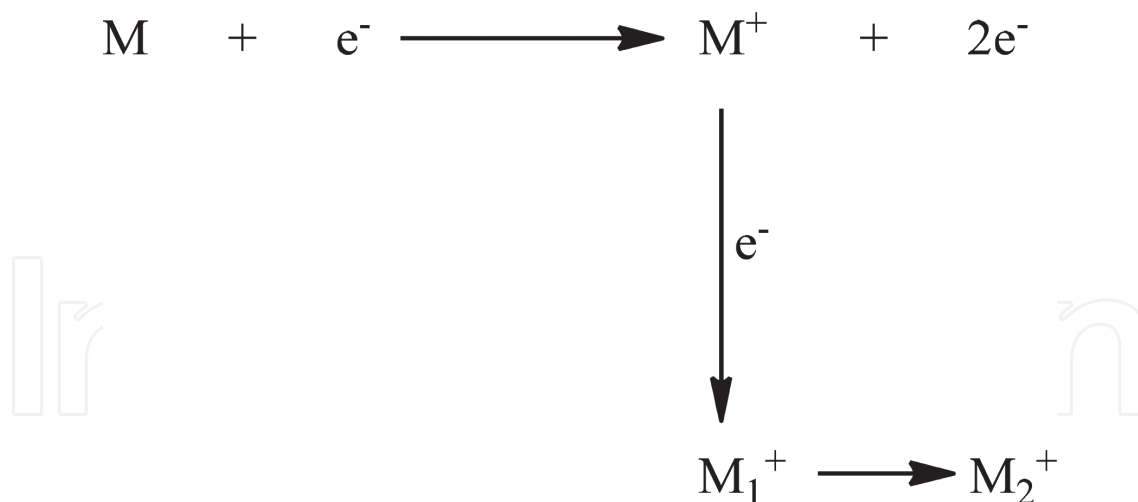
Mass spectrometry (MS) is an advanced technique for determining the molecular weight of a compound. The first mass spectrometer was developed in 1912 by J.J. Thompson. The instrument

has now a wide range of applications in pharmaceutical (drug discovery, pharmacokinetics, drug metabolism), clinical (neonatal screening, hemoglobin analysis, drug testing), environmental (water quality, food contamination, pollutant determination), geological (oil composition, hydrocarbon fraction determination in petroleum industry), metallurgy (determination of rare earth metals and metals at ppq (parts per quadrillion)), sports (dope test of drugs in athletes), forensic (poison and drug metabolite determination) and biotechnology (proteins, peptide analysis) like fields.

2. Principle

The mass spectroscopy is based on the positive ion generation. For its most popular model, the electron impact ionization with magnetic sector analyzer, the sample under investigation is converted into vapor phase and bombarded with electrons having energy sufficient to knock out one electron from it (>10 eV) to produce a positively charged ion called molecular ion or parent ion which is denoted by M^+ .

Positively charged molecule M^+ is often unstable, and with increase in energy (10–70 eV) according to bond strength, they break into fragments called fragment or daughter ion which is denoted by M^{+1} . Ions formed are separated in analyzer under the influence of electric and magnetic field and are recorded by the detector to give rise a mass spectrum (Figure 1).



Where,

M^+ = molecular ion

M_1^+ and M_2^+ = Fragment ions

Figure 1. Ionization of molecule by electron bombardment.

3. Components of mass spectrometer

Mass spectrometer mainly consists of following components:

1. Inlet system
2. Ion generation chamber
3. Analyzer tube
4. Ion collector
5. Data collection system

The inlet system transfers the gaseous form of sample into the vacuum of the ion generation chamber of mass spectrometer. In the ion generation chamber, neutral sample molecules are ionized and then accelerated into the mass analyzer tube. The mass analyzer tube is the most important part on which a range of the mass spectrometer depends. This segment separates generated ions, either in space or in time, according to their mass-to-charge ratio (m/e). Once the ions are separated, they are collected and detected in ion collector chamber. Then, the signal is transferred to a data collection system for data investigation. The high vacuum is applied between the ion generation chamber, analyzer tube and ion collector. The vacuum system is maintaining the low pressure which minimizes the chances of ion-molecule reaction, scattering and neutralization of the ions (Figure 2).

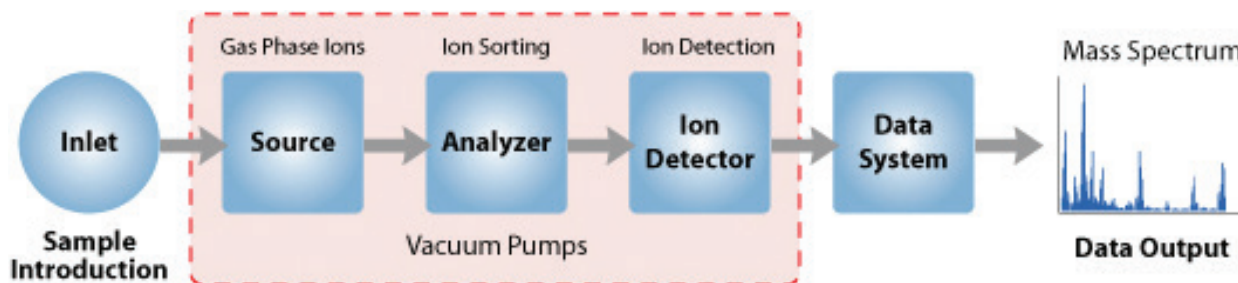


Figure 2. Components of mass spectrometer.

4. Applications

4.1. Phytochemical analysis

Mass spectroscopy is widely employed in phytochemical analysis due to its capability to identify and measure metabolites having very low molecular weight at very low concentration ranges below nanogram per milliliter (ng/mL). Therefore, it is considered as trace analysis methodology. A variety of analyte separation techniques like capillary electrophoresis, gas chromatography and high-performance liquid chromatography are

united with mass spectroscopy for simultaneous separation and determination of analytes called CE-MS, GC-MS and HPLC-MS, respectively. Mass spectrometers like quadrupole or quadrupole-time-of-flight (Q-TOF) are frequently employed in combination along with gas chromatographic system. Several phytoconstituents are volatile and thermolabile, and they can be analyzed by electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI). ESI is commonly employed in HPLC-MS and CE-MS. Fourier transform ion cyclotron resonance (FT-ICR), orbitrap and TOF are emerged as high-performance mass analyzers that are able to screen metabolites with fraction of seconds due to their high resolution. Combination of TOF with one (Q-TOF) or two quadrupoles (Qq-TOF) is emerged as hybrid mass spectrometers that are able to cover unlimited mass range with high scan rates up to 10^6 u/s and high resolving power. Analytes having high molecular weight and temperature sensitive can be efficiently analyzed by HPLC coupled with atmospheric pressure ionization-mass spectrometer (API-MS) [1]. Some of the recent research articles depicting the application of mass spectrometry for the phytochemical analysis are listed in **Table 1**.

S. no.	Analytical technique	Sample source	Analytes	Reference
1	HPLC-ESI-MS	<i>Leontopodium</i> species (Asteraceae)	Fatty acids, sucrose, diterpenes, sesquiterpene	[2]
2	GC × GC-MS	Essential oil of <i>Pelargonium graveolens</i>	α-Pinene, myrcene, limonene, citrinellal, geraniol	[3]
3	GC-MS	Methanolic fruit extract of <i>Momordica charantia</i>	Vitamin E, gentisic acid, 1-pentadecyne	[4]
4	GC-MS	Extracts of <i>Aerva lanata</i>	(R)-(+)- γ -Valerolactone, 5,14-di (N-butyl)-octadecane, 9-octadecenoic acid, 2-propynoic acid	[5]
5	GC-MS	Ethanol extract of <i>Azolla microphylla</i>	(R)-(+)- γ -Valerolactone, 5,14-di (N-butyl)-octadecane, 9-octadecenoic acid, 2-propynoic acid	[6]
6	UHPLC-ESI-MS	<i>Rhizopus microsporus</i> var. <i>oryzae</i> challenged soya bean seedlings	Prenylated isoflavonoids and isoflavonoids like daidzein, genistein, glycitein	[7]
7	HPLC-ESI-MS/MS	<i>Radix astragali</i>	Calycosin, calycosin-7-O- β -D-glycoside, formononetin, formononetin-7-O-glycoside	[8]
8	HPLC-MS/MS	<i>Glycyrrhiza uralensis</i> Fisch. extract	Glycyrrhizic acid, liquorice saponin G2, liquiritin, licuraside, ononin, glycycomarin	[9]
9	LC/MS/MS	Dried plums	Hydroxycinnamics, including acids, esters and glycosides; hydroxybenzoic acids and one flavonoid	[10]
10	UHPLC-MS	<i>Licorice</i> root extract in 70% ethanol, ethanol and ethyl acetate	Prenylated flavonoids	[11]

Table 1. Applications of mass spectroscopy in phytochemical analysis.

4.2. Structure elucidation

Mass spectroscopy has major use in structure elucidation of compounds. Mass spectrum is produced in the form of bar graph which is interpreted by using the following peaks.

1. *Base peak*: It is the most intense peak of the mass spectrum. It has 100% abundance.
2. *Molecular ion peak*: It has highest mass-to-charge ratio (m/e) in mass spectrum created due to loss of one electron from molecule, and its m/e value roughly represents the molecular weight of compound.
3. *Isotopic peaks*: Isotopic peaks are often seen in the mass spectrum due to the presence of isotopes. These are usually one or two mass units higher or lower than the ion peak. It can be represented by $m + 1$ and $m + 2$ for one and two molecular weight higher isotopes and *vice versa* for lower weight isotopes. Intensity of the peak depends on the natural abundance of isotopes. These are often used as characteristic of a compound for identification.
4. *Fragment ion peak*: Peak of fragment ion in mass spectrum is called fragment ion peak. Fragmentation pattern is characteristic for a particular organic compound. So we can confirm the compound by comparing with the library of fragmentation pattern of reference compounds.
5. *Metastable ion peak*: This ion is formed from the disintegration of fragment ion in the analyzer tube of mass spectrometer. It is generated due to loss of kinetic energy of ion during acceleration from ionization chamber to analyzer tube. This ion appears in the spectrum at m/e ratio, which depends upon the mass of original ion from which it is formed (m_1^+) and fragment ion mass (m_2^+). Metastable ion peaks usually appear at nonintegral values of m/e ratio and are often seen as broad peaks. This is used for the confirmation of proposed fragmentation pattern of a molecule.

$$m^* = \frac{(m_2^+)^2}{(m_1^+)}$$

Here, m^* is the mass of metastable ion observed in mass spectrum.

There are some rules which are employed in interpretation of mass spectra in structure elucidation process. These are:

1. *Nitrogen rule*: Nitrogen rule gives very useful clue about the presence or absence and number of nitrogen atoms. It is divided into two parts:
 - i. If m/e value of molecular ion peak is odd number, then it may contain odd number of nitrogen atoms.
 - ii. If m/e value of molecular ion peak is even number, then it may or may not contain even number of nitrogen atoms.

2. *Hydrogen deficiency index (HDI)*: Number of pairs of hydrogen required to saturate the compound is called hydrogen deficiency index (HDI). Hydrogen deficiency index is also called unsaturation index which gives the information about the number of π -bonds and/or rings present in a molecular structure.

Steps for determination of hydrogen deficiency index:

1. Make the correction in the predicted base formula with respect to the elements obtained from other spectral data, and the correction requires addition or removal of hydrogen atoms, which depends upon the type of element added.
 - A. For group V elements (N, P, As, Sb, Bi): Addition of one hydrogen atom is required with each added element.
 - B. For group VI elements (O, S, Se, Te): There is no need of addition of any hydrogen atom in the formula.
 - C. For group VII elements (F, Cl, Br, I): The removal of one hydrogen atom is required with each added element.
2. After specific needed corrections, compare molecular formula of unknown compound with the formula of saturated hydrocarbon.
3. Calculate the difference in hydrogen atoms between two formulas and corresponding pairs of hydrogen atom.

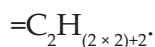
Interpretation of hydrogen deficiency index:

1. If hydrogen deficiency index is one, there must be one double bond or one ring present in the structure.
2. If hydrogen deficiency index is two, there must be triple bond or two double bonds, two rings, or one double bond and one ring present in the structure.
3. Similarly, benzene ring has hydrogen deficiency index four because it contains three double bonds and one ring in it.
4. Any substance with hydrogen deficiency index four or more possibly contains a benzenoid ring in it, and the compounds with hydrogen deficiency index less than four cannot contain such type of ring.

We can understand this application by the following examples:

Example 1: Calculate the HDI of C_2H_4 .

C_2H_4 has two carbon atoms. Therefore, base molecular formula will be:



Hydrogen atoms in predicted base molecular formula = 6.

Hydrogen atoms in actual molecular formula of compound = 4.

Difference in hydrogen atoms between two formulas (6–4) = 2 (which corresponds to one pair of hydrogen atom).

Hydrogen deficiency index is 1, because one pair of hydrogen atom is required to saturate the compound.

Example 2: Calculate the HDI of C_6H_6 .

C_6H_6 has six carbon atoms. Therefore, base molecular formula will be:

$$=C_nH_{2n+2}$$

$$=C_6H_{(2 \times 6)+2}$$

$$=C_6H_{14}$$

Hydrogen atoms in predicted base molecular formula = 14.

Hydrogen atoms in actual molecular formula of compound = 6.

Difference in hydrogen atoms between two formulas (14–6) = 8 (which corresponds to four pairs of hydrogen atom).

Hydrogen deficiency index is 4, because four pairs of hydrogen atoms are required to saturate the compound.

Fragmentation pattern is also an important component of mass spectra from which qualitative analysis of compounds can be done, and it is also useful in elucidation of structural arrangement of compound. From Beynon table, one can predict the possible elemental arrangement or composition of particular mass and determine the molecular formula of compound. The following examples from the literature support the present application of mass spectroscopy.

The structure of flavonoid monoglycosides like genistein-7-O-glucoside, genistein-4'-O-glucoside, 2'-hydroxygenistein-7-O-glucoside and apigenin, isolated from shoot of lupin (*Lupinus luteus* L.), was elucidated by using LSI-MS and EI-MS with double-focusing reversed geometry between mass spectrometer [12].

The analysis of sulfated heparin-like glycosaminoglycan oligosaccharides was done with the help of tandem mass spectroscopy (MS/MS) using quadrupole ion trap mass spectrometer and quadrupole orthogonal acceleration time-of-flight mass spectrometer, and their fragmentation pattern was also studied. This study suggested the use of tandem mass instruments like Q-TOF and metal cations in mass spectroscopy of heparin-like glycosaminoglycan oligosaccharides [13].

A one-step complete analysis method was developed for galacto-oligosaccharide mixtures obtained in lactose transgalactosylation using β -galactosidase from *Aspergillus oryzae* based on ion-mobility spectrometry-tandem mass spectrometry (IMS-MS/MS) with electrospray ionization [14].

The characterization of commercial prebiotic galacto-oligosaccharide mixture was done with linear ion-trap mass spectrometer coupled with high-performance anion-exchange chromatography (HPAEC) using electrospray ionization combination with 1H NMR and ^{13}C NMR [15].

The synthesis of a novel sequence of di- and tri-organotin (IV) compounds which contains germanium having the general formula $R_{4-n}SnL_n$ along with characterization was performed by elemental analysis, FT-IR, multinuclear NMR (1H , ^{13}C , ^{119}Sn) and mass spectrometry by double-focusing mass spectrometer [16].

The characterization of polyisobutylenes was done by various mass spectrometry techniques like tandem mass spectroscopy with MALDI-TOF and ESI-QIT. The primary structure was determined by multistage mass spectrometric analysis, the presence of specific functional groups (e.g., OH or OCH_3) was confirmed, and also differentiation between isomeric functional groups was done [17].

4.3. Peptide and protein sequence/structure analysis

Mass spectroscopy has an important application in analysis of sequence of amino acids in proteins and peptides, that is, analysis of structure of proteins and peptides, and this is employed increasingly. This can be performed by stepwise hydrolysis accompanied with chromatography. Peptides are converted into amino alcohols which are volatile in nature. These amino alcohols derivatized and analyzed in mass spectrometer which aids sequence analysis. However, sequencing of underivatized peptides as in fast atom bombardment mass spectrometry (FABMS) is also employed. New techniques like MALDI and tandem mass spectroscopy are also in trend [18, 19]. Various examples from the literature support the present application.

The peptide sequence was analyzed using combination of gas-phase ion/ion chemistry and tandem mass spectrometry. The quadrupole linear ion trap with electrospray ionization and chemical ionization was also utilized in the analysis to characterize the primary structure of intact proteins [20].

Similarly, RNA polymerase II (Pol II) transcription initiation complex structure was analyzed by cross-linking and mass spectroscopy. They employed linear ion trap quadrupole (LTQ)-orbitrap spectrometer and recorded Fourier transform mass spectrometer (FTMS) spectra at 100,000 resolutions. The cross-linking/MS was used as an integrated structure analysis tool for large multi-protein complexes [21].

A new procedure was revealed which enabled selective sequencing and detection of serine-, threonine- and tyrosine-phosphopeptides at very low level of femto mole in protein digests with electrospray mass spectroscopy (ES-MS) using quadrupole mass spectrometer [22].

Another similar study revealed a method for determination of amino acid sequence of fractions of peptides from apolipoprotein B by tandem mass spectrometry. In this, triple quadrupole mass spectrometer along with LSI-MS was employed [23].

4.4. Clinical studies

Implementation of mass spectroscopy in clinical laboratory resulted in significant advancements. Sometimes greater degree of sensitivity is required when analyte quantity is too low and mass spectroscopy due to its higher sensitivity marks a valuable place in clinical analysis [24]. In

any disease condition, the chemistry of body changes which results in the changes in products in body fluids and excretion products can be detected for the diagnosis purpose by chromatographic instrument like gas chromatography equipped with mass spectroscopy [18]. Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) is now in trend that is used to directly analyze and image pharmaceutical compounds in intact tissue [25].

Organic acidurias are an inherited disorder of metabolism in man, and gas chromatography coupled with mass spectroscopy is used to define an organic aciduria involving isovaleric acid. This technique is used in diagnosis and characterization of inborn errors of organic acid metabolism [26]. A simplified method was developed for clinical diagnosis of organic aciduria with gas chromatography–mass spectrometry (GC–MS) using quadrupole mass spectrometer. The urine samples were analyzed from patients, and acids were identified like methylcitric acid, margaric acid and glutaric acid [27].

Matrix-assisted laser desorption/ionization imaging mass spectrometry is emerged as a potent instrument for the investigation of small molecules and proteins in biological systems by *in situ* analysis of tissue sections [28]. A new technique was developed for the identification of proteins on tissue using tryptic digestion followed by matrix-assisted laser desorption/ionization imaging mass spectrometry with tandem mass spectrometry analysis. They used MALDI-TOF for their study [29]. Likewise, the level of antitumor drug SCH 226374 was determined in mouse tumor tissue using MALDI-QqTOF mass spectrometer. In whole brain homogenates, the concentration of drug was determined at nanogram levels with high-performance liquid chromatography/tandem mass spectrometry using triple quadrupole mass spectrometer [25].

Matrix-assisted laser desorption ionization-Fourier transform ion cyclotron resonance (MALDI-FTICR) is also an efficient technique for imaging of drugs and metabolites in tissue. A method based on MALDI-FTICR for imaging of olanzapine in kidney and liver as well as imatinib in glioma was described [30].

There is dramatic amplification in interest and implementation of clinical mass spectrometry for testing of vitamin D. LC–MS/MS method enables to distinguish between vitamin D₂ and vitamin D₃ and also provides information on the vitamin D epimeric form, both of which are not currently possible with existing immunoassays. Mass spectroscopy is the favored method for endocrine disorders analysis, for example, steroid analysis which requires technical competence, skill and experience for the needed improvement [24].

Mass spectroscopy can also be used for the investigation of bile acids in biological fluids. Bile has bile acids as the major constituents which are synthesized in liver and secreted in gall bladder or in intestine. Bile acids have many vital physiological functions like lipid absorption and cholesterol homeostasis. Under normal healthy condition, only small quantities of bile acids are found in peripheral circulation and urine, but in hepatobiliary and intestinal diseases it will get affected. This occurs due to disturbances in synthesis and pharmacokinetics in the body. Hence, the evaluation of bile acid can become useful for investigating the liver or intestinal functions along with the diagnosis of various diseases such as cholestasis, colon and liver cancer. The complex structure and low concentration of bile acids in biological fluids make

their analysis technically difficult. For many years, GC–MS has been used but LC–MS has also been used for qualitative analysis of bile acids. A rapid, accurate, sensitive and reproducible method was developed using liquid chromatography–electrospray tandem mass spectrometry (LC–MS/MS) to investigate conjugated and total bile acids in samples of human bile and mixture of bile acid standards. The results coincide with the results obtained by the GC–MS technique. This method has important advantages over others because of the high specificity, sensitivity and selectivity of tandem mass spectrometry [31].

Mass spectroscopy has also application in clinical microbiology. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been freshly adapted for the recognition of whole microorganisms from their colonies on media or directly from cultures in blood and urine. This technique can precisely identify even those bacteria which are difficult by conventional methods and that day is not far away when this technology will complement the conventional microbiologic identification methods. In this technique, a portion of an isolated colony is loaded in the instrument and the comparison of spectrogram is done to a library by a proprietary algorithm to identify the organism. The ease of use, ability to run large numbers of isolates per batch, simplicity of setup, automation, rapid turnaround time and low reagent costs are the major advantages. By optimization, the cost of operation reduces to one-tenth that of conventional method with automated biochemical testing platforms. A Swiss study has done a comparison between MALDI-TOF MS system and conventional methods for the identification of 1371 routine bacteria and yeast isolates. MALDI-TOF MS provided identifications for 98.5% of the isolates, including 93.2% at the species level and 5.3% at the genus level. Of the species-level identifications, 95.1% matched conventional identifications. Important deficiencies in present MALDI-TOF MS platforms include misclassification such as of *Shigella* as *Escherichia coli* and *Streptococcus pneumoniae* as *Streptococcus mitis* and, additionally, poor performance with polymicrobial samples. In some cases, instruments have identified only one organism without indicating the presence of others. Despite the need for improvement, mass spectrometry will become popular in the near future with fast turnaround times, ease of use and potential operational cost savings [32].

4.5. Pharmaceutical analysis

Mass spectroscopy emerged as a powerful tool for various operations in pharmaceutical field mainly in drug development. New methods and instruments in mass spectroscopy are developed at a very high rate. Mass spectroscopy now becomes an irreplaceable tool in all types of drug discoveries due to its high sensitivity, speed, versatility and selectivity [33].

Mass spectroscopy is widely used for detection of impurities in samples. Likewise, the use of LC–MS for multidimensional evaluation of impurities during drug development is described. They used peptide drugs as an example and used ion trap mass spectrometer with electrospray ionization in their method [34]. Similarly, it can also be used for detecting the purity profile of active pharmaceutical ingredients (API), that are, MK-0969, an M3 antagonist; MK-0677, an oral-active growth hormone secretagogue and API-A, a cathepsin K inhibitor. The elucidation of impurity structure was made by utilization of LC–MS using quadrupole ion trap mass spectrometer equipped with an electrospray ionization or an

atmospheric pressure chemical ionization (APCI) interface [35]. A protocol for qualitative and quantitative analysis of pharmaceutical compounds by MALDI-TOF mass spectrometry was described. Two drugs lopinavir and ritonavir were analyzed and described that HIV protease inhibitors can successfully be quantified in peripheral blood mononuclear cells using MALDI-TOF mass spectrometry [36].

Matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) emerged as a valuable tool in direct analysis of pharmaceutical formulations. MALDI-MSI can be used for direct analysis of homogeneity of the active drug compound throughout the excipients contained in tablets. A direct analysis in real-time ion source coupled with a time-of-flight mass spectrometer (DART-MS) method for screening of pharmaceutical formulations was developed. A library of compounds were analyzed using mass spectra data collected by DART-MS operated in switching mode at 20, 60 and 90 V settings. This library consisted of 17 commonly encountered drugs in parenteral pharmaceutical formulations, that is, surgical analgesic: fentanyl, hydromorphone and morphine; anesthetic: baclofen, bupivacaine, ketamine, midazolam, ropivacaine and succinylcholine; and a mixture of other drug classes: caffeine, clonidine, dexamethasone, ephedrine, heparin, methadone, oxytocin and phenylephrine [37].

4.6. Forensic applications

In forensic study, sample is in minute quantity; therefore, high sensitivity is required for analysis. Mass spectroscopy coupled with gas chromatography emerged as an indispensable tool in forensic field as well as LC-MS has also wide utility in forensic study. In forensic studies, the use of mass spectroscopy is becoming significant because of increase in the demand to investigate use of illegal drugs through analyzing body fluids and tissues. The sample for forensics in the case of drug abuse is mainly urine, hair and blood. Some of the drugs in routine analysis include opiates, cocaine, marihuana, lysergic acid diethylamide (LSD) and amphetamines. However, cases of murders or death due to poisoning and drug overdose are also the prime targets for these drug candidates' analysis [38].

A liquid chromatographic thermospray tandem mass spectrometric method was developed for quantitative analysis of some drugs having hypnotic, sedative and tranquilizing properties, that is, benzodiazepine, thioxanthene, butyrophenone, methadone and diphenylbutylpiperidine in whole blood. A triple-stage quadrupole mass spectrometer was used in the analysis at a very low detection limit of 0.05–0.5 ng/ml [39]. Similarly, a method was developed for determining common drugs of abuse in body fluids using liquid chromatography-atmospheric pressure chemical ionization mass spectrometry (LC-APCI-MS). Drugs analyzed were opiate agonists (morphine, morphine-3-glucuronide, morphine-6-glucuronide, 6-monoacetylmorphine, codeine, codeine-6-glucuronide, dihydrocodeine, dihydromorphine, buprenorphine, methadone, tramadol, and ibogaine), cocaine and its metabolites (benzoylecgonine and ecgonine methyl ester) and lysergic acid diethylamide in serum, blood, urine and other biological matrices by using single quadrupole instrument [40]. Determination of 11-nor-9-D-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) in urine [41], alfentanil, fentanyl and its derivatives with other opioid drugs like morphine, buprenorphine, codeine,

heroin, methadone, naloxone, naltrexone, tramadol, pentazocine, pethidine and others in hair [42] has been done by LC-MS and LC-MS/MS. Analysis of methadone and its metabolites with other illicit drugs like cocaine, phencyclidine, heroin and 6-acetylmorphine in hair by GC-MS is another successful application of mass spectroscopy [43].

4.7. Metabolites analysis

Determination of metabolic pathway and different metabolites of a drug or xenobiotics is very important to assess its different parameters of pharmacokinetics. Drug metabolic reactions can be divided into two parts: 1. Phase I or functionalization reactions and 2. Phase II or conjugation reactions. Both of these transformations involve changes in the molecular weight. These changes can be accurately measured by mass spectrometer.

In structural characterization by mass spectrometry, the exchange of labile hydrogen with deuterium (H/D exchange) in small organic molecules has been widely used and this occurs in solution containing functional groups which have labile hydrogen(s) such as -SH, -OH, -N(R)H, -NH₂ and -COOH. During biotransformation, attachment of polar functional groups occurs, which causes changes in the number of exchangeable hydrogens. The number of exchangeable hydrogens in metabolites can give additional information to facilitate structural elucidation. This approach was applied to differentiate sulfoxide and sulfone metabolites from the isomeric mono- and di-hydroxylated metabolites, respectively. For example, the H/D exchange method was used for the drug metabolism studies of denopamine and promethazine in which N- or S-oxide was easily distinguished from the hydroxylated metabolites. A triple-stage quadrupole mass spectrometer equipped with electron impact (EI), FAB, APCI, ESI and thermospray (TSP) systems was utilized in the study [44].

Oxidation of a tertiary amino group to form an N-oxide is an important biotransformation pathway for many drugs and xenobiotics. N-oxide metabolites have the same elemental composition as those metabolites resulting from hydroxylation. Differentiation by mass spectrometry is a challenging task because these analytes exhibit the same *m/z* for their protonated or deprotonated molecules and their product ion mass spectra are usually very similar. Deoxygenation of N-oxides during atmospheric pressure chemical ionization represents potential way to differentiate N-oxides from hydroxylated metabolites. 6-OH desloratadine and N-oxides can be clearly differentiated as the major fragment ion from N-oxides was due to the loss of an oxygen atom while the prominent fragment ion from 6-OH desloratadine was due to loss of H₂O [45].

Stable isotope-labeled (²H, ¹³C, ¹⁵N, ¹⁸O, ³⁴S and others) xenobiotics can facilitate metabolite detection and identification by mass spectrometry, especially when radiolabeled parent drug is not available [46–48]. Custom-designed isotopic clusters resulting from the mixture of natural and synthetically enriched isotopes can greatly facilitate the detection and identification of metabolites. For example, the detection and identification of ribavirin metabolites in rats was done with the aid of stable isotope labeled drug [49]. Similarly, a fast and sensitive liquid chromatography–tandem mass spectrometry method was developed for simultaneous determination of acetaminophen and its glucuronide and sulfate metabolites (APAP-GLU and APAP-SUL) in small plasma volumes. The tandem triple quadrupole mass spectrometer equipped with an electrospray ionization source was used in the study [50].

5. Conclusion

Mass spectrometry is a very sensitive technique which can analyze even minute quantities of the molecule. This ability is utilized for various purposes like phytochemical, clinical, pharmaceutical and forensic analyses. This technique, not only elucidates the structure of the compounds, but also provides the information of molecular formula and the isotopic abundance of particular molecular formula. The availability of interphases made it possible to hyphenate this sophisticated technique with the different chromatographic techniques. This opened the new horizons of its applicability. The variations and permutation combinations of different ionization techniques with the different analyzers provide the analysis of diversified chemical entities at the femtogram level. The uniqueness of the technique of making fragments of the compound under investigation provides valuable structural information. This is helpful in the study of metabolite, peptide sequencing and macromolecules. This information is directly applicable in pharmaceutical and biomedical analysis. The development of double and triple quad techniques and their application have definitely uplifted the level of research and analysis in biomedical field, and this chapter gives the update on the topic.

Author details

Uttam Singh Baghel^{1*}, Atamjit Singh², Deeksha Singh³ and Manish Sinha²

*Address all correspondence to: drusb1985@yahoo.com

1 Department of Pharmaceutical Chemistry and Analysis, Kota College of Pharmacy, Kota, India

2 Department of Pharmaceutical Chemistry, Laureate Institute of Pharmacy, Kangra, India

3 Primary Health Centre, Meenabadoda, Sawai Madhopur, India

References

- [1] Seger C, Sturm S. Analytical aspects of plant metabolite profiling platforms: Current standings and future aims. *Journal of Proteome Research*. 2007;**6**:480-497
- [2] Safer S, Cicek SS, Pieri V, Schwaiger S, Schneider P, Wissemann V, Stuppner H. Metabolic fingerprinting of *Leontopodium* species (Asteraceae) by means of ¹H NMR and HPLC-ESI-MS. *Phytochemistry*. 2011;**72**:1379-1389
- [3] Shellie RA, Marriott PJ. Comprehensive two-dimensional gas chromatography-mass spectrometry analysis of Pelargonium Graveolens essential oil using rapid scanning quadrupole mass spectrometry. *The Analyst*. 2003;**128**:879-883
- [4] Singh R, Kumar A, Giri DD, Bhuvaneshwari K, Pandey KD. Gas chromatography-mass spectrometry analysis and phytochemical screening of methanolic fruit extract of Momordica Charantia. *Journal of Recent Advances in Agriculture*. 2012;**1**:122-127

- [5] Thangavel A, Balakrishnan S, Arumugam A, Duraisamy S, Muthusamy S. Phytochemical screening, gas chromatography-mass spectrometry (GC-MS) analysis of phytochemical constituents and anti-bacterial activity of *Aerva Lanata* (L.) leaves. *African Journal of Pharmacy and Pharmacology*. 2014;**8**:126-135
- [6] Kannappan P, Bhaskaran KS, Sundaram S, Gopalakrishnan VK. Quantitative phytochemical analysis, in vitro antioxidant potential and gas chromatography-mass spectrometry studies in ethanolic extract of *Azolla Microphylla*. *Asian Journal of Pharmaceutical and Clinical Research*. 2016;**9**:318-323
- [7] Simons R, Vincken J, Bohin MC, Kuijpers TFM, Verbruggen MA, Gruppen H. Identification of prenylated pterocarpan and other isoflavonoids in *Rhizopus* spp. elicited soya bean seedlings by electrospray ionisation mass spectrometry. *Rapid Communications in Mass Spectrometry*. 2011;**25**:55-65
- [8] Qiu F, Tong Z, Gao J, Wang M, Gong M. Rapid and simultaneous quantification of seven bioactive components in radix *Astragali* based on pressurized liquid extraction combined with HPLC-ESI-MS/MS analysis. *Analytical Methods*. 2015;**7**:3054-3062
- [9] Fan R, Li N, Jiang X, Yuan F, Gao Y. HPLC-DAD-MS/MS identification and HPLC-ABTS+ on-line antioxidant activity evaluation of bioactive compounds in liquorice (*Glycyrrhiza uralensis* Fisch.) extract. *European Food Research and Technology*. 2014;**240**:1035-1048
- [10] Fang N, Yu S, Prior RL. LC/MS/MS characterization of phenolic constituents in dried plums. *Journal of Agricultural and Food Chemistry*. 2002;**50**:3579-3585
- [11] Simons R, Vincken JP, Bakx EJ, Verbruggen MA, Gruppen H. A rapid screening method for prenylated flavonoids with ultra-high-performance liquid chromatography/electrospray ionisation mass spectrometry in licorice root extracts. *Rapid Communications in Mass Spectrometry*. 2009;**23**:3083-3093
- [12] Franski R, Bednarek P, Siatkowska D, Wojtaszek P, Stobiecki M. Application of mass spectrometry to structural identification of flavonoid monoglycosides isolated from shoot of lupin (*Lupinus Luteus* L.). *Acta Biochimica Polonica*. 1999;**46**:459-473
- [13] Zaia J, Costello CE. Tandem mass spectrometry of sulfated heparin-like glycosaminoglycan oligosaccharides. *Analytical Chemistry*. 2003;**75**:2445-2455
- [14] Carevic M, Bezbradica D, Banjanac K, Milivojevic A, Fanuel M, Rogniaux H, Ropartz D, Velickovic D. Structural elucidation of enzymatically synthesized galacto-oligosaccharides using ion-mobility spectrometry-tandem mass spectrometry. *Journal of Agricultural and Food Chemistry*. 2016;**64**:3609-3615
- [15] Coulier L, Timmermans J, Bas R, Dool RVD, Haaksman I, Klarenbeek B, Slaghek T, Dongen WV. In-depth characterization of prebiotic galacto-oligosaccharides by a combination of analytical techniques. *Journal of Agricultural and Food Chemistry*. 2009;**57**:8488-8495

- [16] Khosa MK, Mazhar M, Ali S, Dastgir S. Synthesis, characterisation, and structural elucidation by spectral investigation (FT-IR, multinuclear NMR, mass spectrometry) of biologically active organotin(IV) compounds containing germanium. *Turkish Journal of Chemistry*. 2010;**34**:387-398
- [17] Wollyung KM, Wesdemiotis C, Nagy A, Kennedy JP. Synthesis and mass spectrometry characterization of centrally and terminally amine-functionalized polyisobutylenes. *Polymer Chemistry*. 2005;**43**:946-958
- [18] Beckett AH, Stenlake JB. (2007), *Practical pharmaceutical chemistry*. 4th ed. India: CBS publishers and distributors; 2007. 493 p.
- [19] Yerger AL, Coorssen JR, Backlund PS, Blank PS, Humphrey GA, Zimmerberg J, Campbell JM, Vestal ML. De novo sequencing of peptides using MALDI/TOF-TOF. *Journal of the American Society for Mass Spectrometry*. 2002;**13**:784-791
- [20] Syka JEP, Coon JJ, Schroeder MJ, Shabanowitz J, Hunt DF. Peptide and protein sequence analysis by electron transfer dissociation mass spectrometry. *Proceedings of the National Academy of Sciences of the United States of America*. 2004;**101**:9528-9533
- [21] Chen ZA, Jawhari A, Fischer L, Buchen C, Tahir S, Kamenski T, Rasmussen M, Lariviere L, Bukowski-Wills JC, Nilges M, Cramer P, Rappsilber J. Architecture of the RNA polymerase II-TFIIF complex revealed by cross-linking and mass spectrometry. *The EMBO Journal*. 2010;**29**:717-726
- [22] Carr SA, Huddleston MJ, Annan RS. Selective detection and sequencing of phosphopeptides at the femtomole level by mass spectrometry. *Analytical Biochemistry*. 1996;**239**:180-192
- [23] Hunt DF, Yates III JR, Shabanowitz J, Winston S, Hauer CR. Protein sequencing by tandem mass spectrometry. *Proceedings of the National Academy of Sciences of the United States of America*. 1986;**83**:6233-6237.
- [24] Strathann FG, Hoofnagle AN. Current and future applications of mass spectrometry to the clinical laboratory. *American Journal of Clinical Pathology*. 2011;**136**:609-616
- [25] Reyzer ML, Hsieh Y, Ng K, Korfmacher WA, Caprioli RM. Direct analysis of drug candidates in tissue by matrix-assisted laser desorption/ionization mass spectrometry. *Journal of Mass Spectrometry*. 2003;**38**:1081-1092
- [26] Chalmers RA, Lawson AM. Organic acids in man: The analytical chemistry, biochemistry and diagnosis of the organic acidurias. *Analytical Biochemistry*. 1982;**127**:458-460
- [27] Nakagawa K, Kawana S, Hasegawa Y, Yamaguchi S. Simplified method for the chemical diagnosis of organic aciduria using GC/MS. *Journal of Chromatography B*. 2010;**878**:942-948
- [28] Zaima N, Hayasaka T, Goto-inoue N, Setou M. Matrix-assisted laser desorption/ionization imaging mass spectrometry. *International Journal of Molecular Sciences*. 2010;**11**:5040-5055

- [29] Groseclose MR, Andersson M, Hardesty WM, Caprioli RM. Identification of proteins directly from tissue: *in situ* tryptic digestions coupled with imaging mass spectrometry. *Journal of Mass Spectrometry*. 2007;**42**:254-262
- [30] Cornett DS, Frappier SL, Caprioli RM. MALDI-FTICR imaging mass spectrometry of drugs and metabolites in tissue. *Analytical Chemistry*. 2008;**80**:5648-5653
- [31] Perwaiz S, Tuchweber B, Mignault D, Gilat T, Yousef IM. Determination of bile acids in biological fluids by liquid chromatography-electrospray tandem mass spectrometry. *Journal of Lipid Research*. 2001;**42**:114-119
- [32] Mitsuma SF, Mansour MK, Dekker JP, Kim J, Rahman MZ, Tweed-Kent A, Schuetz P. Promising new assays and technologies for the diagnosis and management of infectious diseases. *Clinical Infectious Diseases*. 2013;**56**:996-1002
- [33] Feng WY. Mass spectrometry in drug discovery: A current review. *Current Drug Discovery Technologies*. 2004;**1**:295-312
- [34] Ermer J. The use of hyphenated LC-MS technique for characterisation of impurity profiles during drug development. *Journal of Pharmaceutical and Biomedical Analysis*. 1998;**18**:707-714
- [35] Zhou L, Mao B, Reamer R, Novak T, Ge Z. Impurity profile tracking for active pharmaceutical ingredients: Case reports. *Journal of Pharmaceutical and Biomedical Analysis*. 2007;**44**:421-429
- [36] Van Kampen JJ, Burgers PC, De Groot R, Luider TM. Qualitative and quantitative analysis of pharmaceutical compounds by MALDI-TOF mass spectrometry. *Analytical Chemistry*. 2006;**78**:5403-5411
- [37] Poklis JL, Mohs AJ, Wolf CE, Poklis A, Peace MR. Identification of drugs in parenteral pharmaceutical preparations from a quality assurance and a diversion program by direct analysis in real-time AccuTOFTM-mass spectrometry (DART-MS). *Journal of Analytical Toxicology*. 2016;**40**:608-616
- [38] Foltz RL. Recent applications of mass spectrometry in forensic toxicology. *International Journal of Mass Spectrometry and Ion Processes*. 1992;**118/119**:237-263
- [39] Verweij AMA, Hordijk ML, Lipman PJJ. Liquid chromatographic-thermospray tandem mass spectrometric quantitative analysis of some drugs with hypnotic, sedative and tranquillising property in whole blood. *Journal of Chromatography B: Biomedical Sciences and Applications*. 1996;**686**:27-34
- [40] Bogusz MJ, Maier RD, Kruger KD, Kohls U. Determination of common drugs of abuse in body fluids using one isolation procedure and liquid chromatography--atmospheric-pressure chemical-ionization mass spectrometry. *Journal of Analytical Toxicology*. 1998;**22**:549-558
- [41] Breindahl T, Andreasen K. Determination of 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid in urine using high-performance liquid chromatography and electrospray

- ionization mass spectrometry. *Journal of Chromatography B: Biomedical Sciences and Applications*. 1999;**732**:155-164
- [42] Gergov M, Nokua P, Vuori E, Ojanpera I. Simultaneous screening and quantification of 25 opioid drugs in post-mortem blood and urine by liquid chromatography–tandem mass spectrometry. *Forensic Science International*. 2009;**186**:36-43
- [43] Goldberger BA, Darraj AG, Caplan YH, Cone EJ. Detection of methadone, methadone metabolites, and other illicit drugs of abuse in hair of methadone-treatment subjects. *Journal of Analytical Toxicology*. 1998;**22**:526-530
- [44] Ohashi N, Furuuchi S, Yoshikawa M. Usefulness of the hydrogen–deuterium exchange method in the study of drug metabolism using liquid chromatography-tandem mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis*. 1998;**18**:325-334
- [45] Ramanathan R, AD S, Alvarez N, Blumenkrantz N, Chowdhury SK, Alton K, Patrick J. Liquid chromatography/mass spectrometry methods for distinguishing N-oxides from hydroxylated compounds. *Analytical Chemistry*. 2000;**72**:1352-1359
- [46] Branfma AR, McComish MF, Bruni RJ, Callahan MM, Robertson R, Yesair DW. Characterization of diaminouracil metabolites of caffeine in human urine. *Drug Metabolism & Disposition*. 1983;**11**:206-210
- [47] Baillie TA, Halpin RA, Matuszewski BK, Geer LA, Chavez-Eng CM, Dean D, Braun M, Doss G, Jones A, Marks T, Melillo D, Vyas KP. Mechanistic studies on the reversible metabolism of rofecoxib to 5-hydroxyrofecoxib in the rat: Evidence for transient ring opening of a substituted 2-furanone derivative using stable isotope-labeling techniques. *Drug Metabolism Disposition*. 2001;**29**:1614-1628
- [48] Weidolf L, Covey TR. Studies on the metabolism of omeprazole in the rat using liquid chromatography/ion spray mass spectrometry and the isotope cluster technique with [34S]omeprazole. *Rapid Communications in Mass Spectrometry*. 1992;**6**:192-196
- [49] Chowdhury SK, editor. *Identification and Quantification of Drugs, Metabolites and Metabolizing Enzymes by LC-MS*. 1st ed. Amsterdam: Elsevier; 2005. 277 p
- [50] Gicquel T, Aubert J, Lepage S, Fromentry B, Morel I. Quantitative analysis of acetaminophen and its primary metabolites in small plasma volumes by liquid chromatography-tandem mass spectrometry. *Journal of Analytical Toxicology*. 2013;**37**:110-116

