IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

Applications of MS in Pharmaceutical Analysis: A Review

[1]S. B .Ombase, [2]R. B. Pandhare, [3]K. D. Nagrale, [4]M. S. Charde, [5]R. D. Chakole

Department of Pharmaceutical Chemistry,

Government College of Pharmacy, Vidyanagar Karad, Dist: Satara,

Pin: 415124, Maharashtra, India,

***For correspondence**

Ms. Sonal Baburao Ombase

Government College of Pharmacy, Vidyanagar, Karad,

Dist: Satara, Maharashtra, India, 415124

Abstract:

The mass-to-charge ratio of ions is measured in mass spectrometry. It is now a crucial analytical technique in biological research and is capable of characterising a wide range of biomolecules, including sugars, proteins, and oligonucleotides. A overview of mass spectrometry's history is covered in this review, along with an introduction to the technology's fundamental concepts. Examples of new research articles as well as an overview of some ongoing applications are given. The methods now employed to identify, measure, and describe proteins and peptides are next discussed. Mass spectrometry has a wide range of uses, and as technology advances, this list is only going to get longer. As the strength of The first outcome of a mass spectral signal inversely correlated with the ratio of matching element, mass spectroscopy is also helpful in quantitative elemental analysis. Additionally, it is a non-invasive technique that enables in vivo research on humans. Recent studies have examined the potential uses of mass spectrometers in the biomedical field. Additionally, chromatographic methods like LC-MS, GC-MS, and LC/MS/MS utilise it as a sensitive detector. The technique's usefulness in pharmaceutical and biological studies has been greatly increased by recent hyphenated technology advancements.

Keywords: Mass spectroscopy, Biomedical, Chromatography, etc.

Introduction:

The definition of a mass spectrometer may seem simple it is an instrument that can ionize a sample and measure the mass-to-charge ratio of the resulting ions. However, the versatility of this function has allowed it to become a vital tool in a wide range of fields, including biological research. This versatility arises from the fact that mass spectrometers can give Qualitative and quantitative information on the elemental, isotopic, and molecular composition of organic and inorganic samples [1]. The device is currently employed in a variety of fields, including pharmaceutical (drug discovery, pharmacokinetics, and drug metabolism), clinical (neonatal screening, haemoglobin analysis, and drug testing), environmental (water quality, food contamination, and pollutant identification), and geological (oil composition), sports (drug testing for doping in performers), forensic (poison and drug metabolite determination), mineral processing (mineral identification of rare earth and Parts per quadrillion of metals and biotechnology (proteins, peptide analysis) related fields.[2] Mass spectrometers can give qualitative and quantitative information the elemental, isotopic, and molecular composition of organic and inorganic samples.[1]

The most scientific approach for figuring out a compound's molecular mass and elemental breakdown is mass spectroscopy. In this method, an intense electron beam is used to target molecules. The molecules are ionised and divided into numerous fragments, some of which have a certain mass-to-charge ratio, or m/e ratio. The unstable positively charged molecule M+ may break down into daughter ions, represented by M+1, with energies about 10 and 70 eV depending on the strength of the bond. There is a mass spectrum produced when the detector records the ions that were divided in the analyser under the influence of an electric and magnetic field.[2]

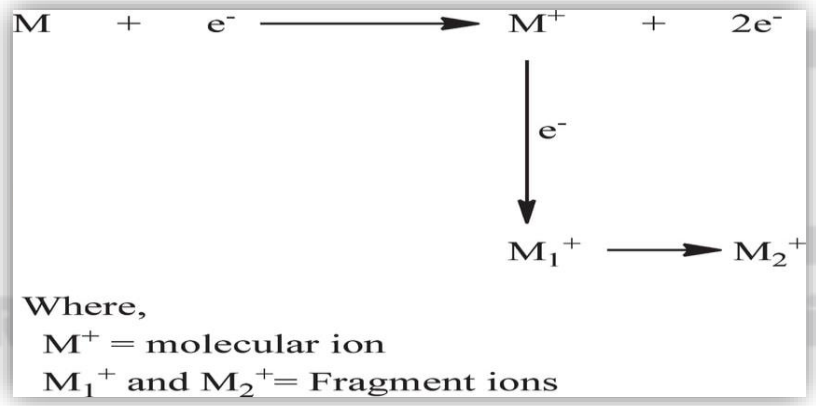

Figure 1. Molecular ionisation caused by an electron bombardment.[3]

Components of Mass spectrometer:

1. Sample inlet

2. Ion source

3. Mass analyser

4. Ion detector

5. Data output

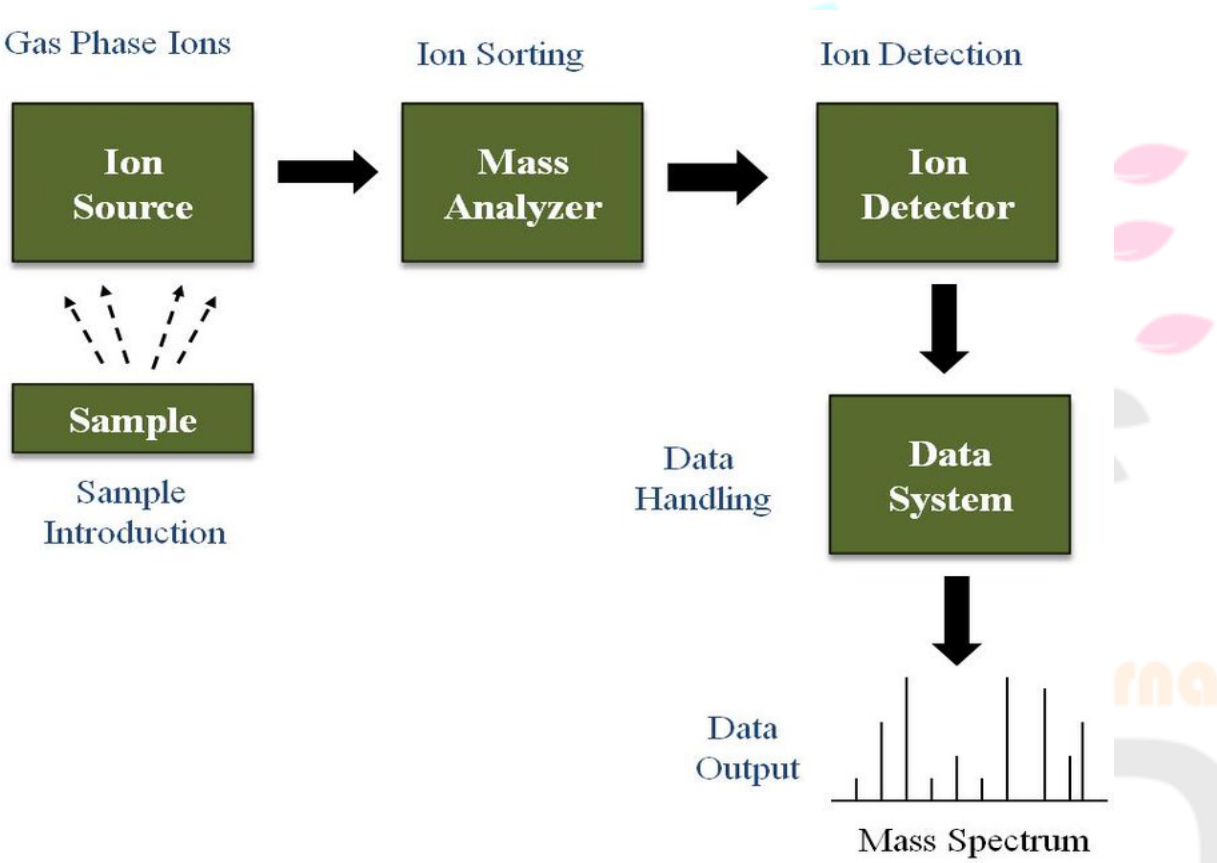

Figure 2. Components of mass spectrometer.[4]

1. Sample inlet-The sample is inserted into the vacuum inside the ion production chamber of the mass spectrometer using the inlet procedure.

2. Ion sources-In the ion production chamber, neutral sample molecules are ionised before being accelerated into the mass analyser tube.

3.Mass analyser-The mass analyser tube is the component on which a range of the mass spectrometer depends particularly. This section separates developed ions into groups determined by their mass-to-charge ratio (m/e), either physically or temporally, as gas is moved from the input system to the.

4. Ion detectors-Ion collector chambers are used to obtain and detect the separated ions. After that, the signal is sent to a data collection system to be analysed. Between the ion generating chamber, analyser tube, and ion

collection, a powerful vacuum is introduced. The vacuum system gives low pressure, which lessens the chance of ion-molecule reaction, ion dispersion, and ion neutralisation.[2]

5. Data output-The ion detected in Detectors will give the graph in the data system.

Applications of Mass Spectroscopy:

1. Biological Research:

IRMS is often utilised in projects utilising stable isotopic tracers in biomedical research. These investigations include feeding or injecting substances containing elements with aberrant stable isotope ratios into a target system. IRMS is then used to record any changes in the element's isotopic ratio in the system. A nutrient's in vivo absorption, retention, and utilisation can be studied utilising tracers with stable isotopes. Additionally, stable isotope tracers can be utilised as a clinical tool for disease detection or to examine energy expenditure [28]. Faraday cups are used as the ion detectors while magnetic sectors serve as the mass analyser's in IRMS. The ion source is typically a thermal ionisation or electron impact source.

2. Small Organic Molecule:

The atomic mass, structural formula, molecular makeup, or concentration of the analyte can all be determined using the mass spectrometric examination of organic substances. The formula and molecular makeup can be ascertained manually or by compared to a database of references of spectra based on the measured m/z ratio and their peak intensities. For instance, Lavermicoccaeal employed MS to find novel anti-fungal substances made by the bacterium Lactobacillus plantarum strain 21B, which is used to make sourdough bread. Separated culture filtrate components were examined for anti-fungal activity. Then, using gas chromatography-MS, the individuals with the greatest activity levels were described. The substances that were present were identified by comparing the spectra to those in a library of MS spectra.In the high-throughput examination of chemicals made in synthetic libraries, especially those made to find new medicines, MS is also a useful tool. Both structural data as well as information describing a molecule's binding affinity can be obtained if a mass-spectrometer is coupled to an LC apparatus with the right columns. By identifying the main metabolites from in vitro studies and measuring them in vivo to ascertain pharmacokinetic parameters, MS can also be used to research medication metabolism. In this trial, healthy participants between the ages of 1 and 12 received the influenza drug oseltamivir. Then, using MS, the levels of oseltamivir and one of its analogues were tracked over time in the plasma and urine. The pharmacokinetic information gathered made it possible to create dose regimens for kids that were suitable.

3. Macromolecules:

The research of proteins, peptides, DNA, fatty acids, and lipids are all included in the MS analysis of macromolecules. To "Protein Characterization," we defer to the subject of applications to the research and analysis of proteins and peptides. The study of modified oligonucleotides that might not be suitable with the present enzymatic procedures applied to sequence DNA has been the main focus of oligonucleotide analysis research.Using an ESI source connected to a Fourier transform-ion cyclotron resonance analyser, McLafferty

and colleagues were able to arrange oligonucleotides up to 100 nucleotides in length. MS may be employed as well to examine DNA methylation and other changes. It is common practise to employ ESI or MALDI as an ionisation source in macromolecular research. There are numerous mass analyser's utilised, including ion trap and TOF. Tandem MS is frequently used to clarify structural information or sequencing data.[1]

4. Protein Quantification:

Knowing the amount of protein expression as well as the proteins displayed by an organism is frequently necessary for proteomic research. The comparison of the levels of protein expression in various systems is a frequent research technique. In the past, metabolic labelling of a variety of proteins in a single system, such as cell lysates, with a heavy element like 15N, was used to do quantitative studies of the protein content using MS. The approach, though, is only applicable to tissues and cells that can be metabolically labelled. The ICAT approach is a new labelling technique that eliminates this restriction.[1]

5. Pharmaceutical Analysis:

MS, and particularly LC-MS, have been employed extensively in pharmacological research. Quantitative metrics are essential in toxicological research whether it comes to clinical examinations, residue profiling, doping control assessments, or any indication of medicines in foods and beverages. MS is used to quantify medications and related compounds, such as phytonutrients and products of decomposition, in the emerging field of environmental science, notably water studies. Additionally, MS is employed in drug development, biopharmacy, radiopharmacy, and pharmacokinetic investigations. In reality, when complicated matrices make a trustworthy spectrophotometric measurement impossible, greater than 85% of the time, pharmaceutical product quantitation is performed using MS. Because of this, the application of MS for quantitative pharmacological analysis is a topic that receives a lot of attention in the literature. Table S2 in the electronic supplementary material gives a basic summary of recently published review papers that cover a variety of subjects. Considering that imaging, proteomics, and metabolomics are extremely specialised disciplines of study. [5]

6. Veterinary Drug Analysis:

Mass spectrometry (MS) is now widely employed as a confirmation technique for the traditional biological methods of measurement based on RIA, ELISA, or biosensors, and provides quick diagnosis of compliant vs questionable material (i.e., screening stage). In fact, LC or gas chromatography with MS is a clear and effective instrument of choice for recognising and quantifying residues in feed and food. Today, LC-MS-based analytical methods have replaced GC-MS-based methods, even though they should be viewed as complementary. The favoured methodologies used to be based on GC-MS when considering substances belonging to Groups A1 (stilbenes), A2 (thyrostats), A3 (steroids), A4 and A5. Today's critics argue against the necessity of a derivatization phase (such as silylation or acylation) before GC separation, but they likely downplay or even ignore the unparalleled chromatographic separation seen on capillary GC. But it's a truth that LC-MS methods offer a general strategy that can be used with the greatest variety of veterinary medications.[6]

7. MALDI MSI in Pharmaceutical Research and Development:

A vital tool for label-free bioanalysis of the spatial distribution of biomolecules, medications, and other xenobiotics in tissue slices is matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI MSI). Recent developments in instrumentation, collecting samples, multimodal processes, quantification, analytical standardisation, and 'big data' processing have made MALDI MSI widely used in pharmaceutical research. These advancements have allowed the technology to be used in drug discovery processes other than drug disposition studies, most notably in the study of pharmacodynamic biomarkers and in toxicology.[7]

8. Identifying Pharmaceutical Transformation Products in Water:

The amount of OH produced from O3 in most water-treatment applications is typically modest, thus AOPs use a mixture of O3 and hydrogen peroxide (O3/H2O2) to boost the concentration of OH and remove more recalcitrant chemicals .AOPs also include a variety of methods (such as H2O2/UV, O3/UV, -radiolysis, TiO2 photocatalysis, and photo-assisted Fenton) for creating oxygen species that is highly reactive that can directly degrade organic contaminants found in drinking water as well as waste water. Additionally, it is thought that naturally occurring abiotic breakdown processes (such photolysis under sunshine) considerably improve the elimination of pharmaceutical waste in surface waters. Once more, it is anticipated that the hydroxyl radicals, particularly in photosensitized oxidation, would play a significant role. [8]

9. Drugs and Biomedical Analysis:

In the last decade, liquid chromatography–tandem mass spectrometry (LC–MS/MS) has become one of the major tools for bioanalytical analysis [1]. Among pharmaceutical companies, >70% of the samples today are analyzed with these instruments. LC–MS/MS techniques frequently provide specific, selective and sensitive quantitative results often with reduced sample preparation and analysis time relative to other commonly employed techniques. The atmospheric pressure ionization (API) includes electrospray and atmospheric pressure chemical ionization (ESI and APCI). They are complementary and suitable to analysis of a great diversity of drugs.[9]

10. Imaging, Pharmaceutical Research and Development:

Understanding drug pharmacology, toxicity, and distribution in great detail is crucial for pharmaceutical research and development (R&D). Drug compounds must reach target receptors at the site where they act at an unbound concentration high enough to offer effectiveness, but not so high that they trigger a harmful reaction, in order to have an impact. When determining the efficacy or toxicology of a new chemical entity in vivo, additional assays are necessary. Plasma concentration measurements have traditionally been employed as a surrogate for the concentration of drug in tissues, but this does not always accurately represent the levels within particular organs or sub-compartments. The two-dimensional (and three-dimensional) molecular distribution of physiological chemicals, medicines, lipids, proteins, peptides, and drug delivery systems in biological tissue is visualised using the label-free multiplex approach known as mass spectrometry imaging (MSI). As a result, the method has the capacity to gather data on pharmacodynamics and biomarkers in addition to drug and metabolite distribution, which can be incredibly useful at various stages of the drug development process.[10]

11. Identifying Pharmaceutical Biotransformation Products in The Environment:

Pharmaceuticals' effects are chronic instead of immediately hazardous since they exist in trace amounts in the environment. The time period of exposure to the non-target organism, susceptibility to the chemical, and bioavailability all play a role in their effects [16]. Contrary to human drug metabolism, which must be thoroughly investigated before drugs have been authorised, microbial degradation of such substances, their transformation pathways, and products have only recently attracted interest. The enzymatic reactions in waste and environmental fluids are typically distinct to those in mammals due to the various enzyme systems involved. This presumption was validated by Jjemba, who demonstrated that pharmaceuticals with high rates of target organism metabolism (and thus low rates of excretion) may have a low environmental (bio)degradability by nature. However, this may only have applied to a small portion of the biological transformation products, which were easily discovered by targeted searches of metabolites. Other investigations have revealed the presence of comparable molecules resulting from biochemical biotransformation and human metabolism.[11]

12. Food Related Analysis:

Mass spectrometry (MS) has a significant role to play in the development of food science. The role of mass spectrometry and similar techniques in food analysis for quality control has been steadily growing over the past few years. This significant extension of the role of MS in food-related studies has been made possible by developments in The creation of new interfaces as a result of liquid chromatography-mass spectrometry (LC-MS) connection has increased the possibilities and automation of many processes.The key to this progress in recent years has unquestionably been the considerable advancements in ionisation techniques that have a wide range of applications and great sensitivity for the analysis of high-polar and high-molecular mass chemicals of food concern. New ionisation methods like electrospray (ESI) and matrix-assisted laser desorption ionisation (MALDI), combined with instruments with tandem MS (MS-MS) capabilities, have had a significant impact on quadrupole, magnetic sector, or time-of-flight (TOF) instruments.[12]

13. Identification and Structural Studies of Flavonoids Glycosides:

The current advancement of so-called "soft" ionisation techniques has enhanced the use of MS in the investigation of flavonoid glycosides. This family of compounds includes polar, thermally labile, and non-volatile substances. Underivatized flavonoid glycosides were not amenable to chemical ionisation (CI) or electron impact (EI), with electron energies ranging from 10 to 100 eV. Both techniques require derivatization of the hydroxyl groups (methylation,trimethylsilylation, and acetylation) and the analyte to be in the gas phase for ionisation. For derivatized mono- and di-glycosides, little structural data about the sugar or the aglycone could be gleaned. Desorption ionisation methods made it possible to analyse flavonoid glycosides without the need for derivatization. [13]

14. Theory of Error for Target Factor Analysis:

A concept of error for target factor analysis is created based on the earlier-discussed theory of error for abstract factor analysis. The theory explains how the error in the target test vector interacts with the error in the data matrix. It is discovered that the apparent error in a target test equals the vector sum of the real errors in the

target and anticipated vectors. Without requiring any prior knowledge of the error in the data matrix or the target vector, the theory can anticipate the sizes of these errors. To evaluate the validity and deservingness of a target vector, an assurance function and a spoil function are created. We give examples using mass spectrometry, nuclear magnetic resonance spectrometry, and model data.[14]

15. Analysis of Chinese Medicine:

To enable monitoring and/or quality control (QC) of dynamic changes, process analytical technology (PAT) is frequently used to measure the physical and chemical compositions of produced or natural products in processes. The U.S. Food and Drug Administration's proposed regulatory framework included a definition of the PAT in 2004. The expansion of the traditional Chinese medicine (TCM) industry has considerably benefited from the advancement of PATs in recent years. PATs are frequently employed in the quality control (QC) of TCM pharmaceutical operations, including quality evaluations of basic ingredients, monitoring concoction processes, quantifying legitimate formulations, and continuous evaluations of extraction procedures. A requirement for guaranteeing the security, effectiveness, and calibre of Chinese herbal goods is the development of QC technology for pharmaceutical procedures. "Quality by design" is replacing "quality by test" as the guiding principle for TCM medications. This is in line with the objective of PATs, according to which the drug quality is taken into account during the design phase rather than only by testing the finished product. Due to this, the TCM process analysis has moved from an offline to an online format. [15]

16. Clinical Laboratory:

1 Mass spectrometric (MS) methods for any analyte analysis may often be broken down into three steps: (1) preparing a sample, (2) separation using chromatography (if necessary), and (3) Using mass spectrometry. Each of these processes is covered in detail in the textbooks 1-3 and journal articles cited throughout. Laboratory and Clinical Standards Institute's resources are also recommended for readers seeking further in-depth advice on mass spectrometry's application in clinical laboratories.4,5 This article's goal is to give a succinct, layperson's introduction to mass spectrometry's use in clinical laboratories.[6]

17. Application in Toxicology:

Numerous clinical laboratories provide immunoassay-based toxicologic screening techniques. LC-MS/MS or GC-MS confirmatory testing, and even fewer offer GC-MS comprehensive drug screenings. Cutoffs for screening assays are set to offer enough specificity at a reasonable level of sensitivity. They are qualitative tests. However, most screening assays are not intended to achieve a definitive identification (e.g., morphine vs. hydromorphone), rather to detect a class of substances (e.g., opioids). Confirmatory testing is typically quantitative and carried out utilising a sensitive and focused approach, like SIM or MRM. When a higher level of sensitivity or knowledge of the true identity of the molecule is required, confirmatory testing, such as that carried out using LC-MS/MS, is essential.[16]

18. Lipid Analysis:

As a result, the molecules with protonated ions ([M + H]+) or extended molecular ions (such as [M + NH4]+, [M + Na], and [M + K]+) are the cations that are typically detected in these spray ionisation and desorption procedures. If the molecule preferentially produces negative ions, deprotonated molecular anions. At the same time that these remarkable ionisation techniques were being created, tools were also becoming available to transmit product ions using effective collision cells (radio frequency-only quadrupole fields), impart energy to break covalent bonds within ions using collisional activation and collision-induced dissociation (CID), and then reevaluate the product ions by a second mass spectrometer (MS/MS). It is possible to see ([M H]) and included anion ([M + Cl] and [M + acetate]). These CID advancements were incorporated into the triple quadrupole mass spectrometer, which was found to have a variety of MS/MS operation modes, including neutral loss scanning, product ion scanning, precursor ion scanning, and selected reaction monitoring (SRM; also known as multiple reaction monitoring), that were well suited for the analysis of lipids. With the development of the second generation time-of-flight analyser's (8), ion-trapping technology of the ion cyclotron resonance cell, and the orbitrap mass spectrometer (9), high resolution mass measurement of molecular ion species and product ions following CID became routinely possible. Due to the significant demand for these tools in proteomic research, much of this instrumental development took place in the commercial manufacturing sector. However, these methods were excellent for lipid analysis, and it was now conceivable to use MS and MS/MS to tackle complex issues. [17]

19. Imaging Mass Spectroscopy In Drug Development:

A thorough understanding of the innovative drug candidates' absorption, distribution, metabolism, excretion, and toxicity (ADMET) characteristics is necessary for drug development. Characterising the tissue distribution of the drug candidate is crucial to comprehending other aspects of drug research because most medicines or metabolites are not uniformly distributed. For therapeutic drugs to have the desired pharmacological impact, they must be efficiently delivered to the specified target site (Lanao and Fraile 2005; Mouton et al. 2008). Additionally, the buildup of the parent substance or its metabolites in unanticipated organs may result in unintended secondary pharmacology and toxicity (Castellino et al. 2011, Pellegatti and Pagliarusco 2011). Compound distribution studies are crucial for assessing environmental contaminant risk as well as mechanistic research and preclinical safety.[18]

20. Direct Drug Analysis:

Mass spectrometry (MS), which offers excellent specificity, selectivity, and sensitivity in the molecular identification of a variety of tiny analytes, particularly illicit and medicinal medicines, currently plays a vital part in drug detection from oral fluid. However, its usage has been restricted to the analytical laboratory and is relatively slow when coupled to chromatography, whether it be gas chromatography (GC) or liquid chromatography (LC). In spite of these developments, measurement costs are still high, the preparation of biological fluids can be time-consuming, and analysis need specialised workspaces. In situ screening is typically quite advantageous, and because of this, immunoassay instruments are commonly employed for on-site testing.

These devices are portable, inexpensive, and quick, however because to their low specificity, additional samples are needed for validation using recognised hyphenated MS techniques.[19]

Conclusion:

In these chapter, we review the basic principle of mass spectroscopy , as well as its application. Mass spectrometry is a very sensitive method that can examine even microscopic amounts of the chemical. This skill is used for a variety of tasks, including forensic, clinical, pharmaceutical, and phytochemical studies. This method not only clarifies the composition of the compounds but also offers information on their molecular formula and isotopic abundance. This complex technique might be combined with several chromatographic techniques because to the availability of interphases.

Acknowledgments:

The authors are thankful to AICTE New Delhi for providing the financial support during M. Pharm tenure. Also, thankful to the Principle of Government College of Pharmacy, Karad for providing required facilities.

References:

1 .Finehout, Erin J., and Kelvin H. Lee. "An introduction to mass spectrometry applications in biological research." Biochemistry and molecular biology Education 32.2 (2004): 93-100

2. Baghel, Uttam Singh, et al. "Application of mass spectroscopy in pharmaceutical and biomedical analysis." Spectroscopic Analyses-Developments and Applications (2017): 105-121.

3.https://www.researchgate.net/figure/Ionization-of-molecule-by-electron-bombardment_fig1_32163632.

4.https://www.researchgate.net/figure/Components-of-a-mass-spectrometer_fig2_6033979

5. Loos, Glenn, Ann Van Schepdael, and Deirdre Cabooter. "Quantitative mass spectrometry methods for pharmaceutical analysis." Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences 374.2079 (2016): 20150366.

6. Le Bizec, Bruno, Gaud Pinel, and Jean-Philippe Antignac. "Options for veterinary drug analysis using mass spectrometry." Journal of Chromatography A 1216.46 (2009): 8016-8034.

7. Schulz, Sandra, et al. "Advanced MALDI mass spectrometry imaging in pharmaceutical research and drug development." Current opinion in biotechnology 55 (2019): 51-59.

8. Kosjek, Tina, and Ester Heath. "Applications of mass spectrometry to identifying pharmaceutical transformation products in water treatment." TrAC Trends in Analytical Chemistry 27.10 (2008): 807-820.

9. Zhong, Dafang, et al. "Applications of liquid chromatography–tandem mass spectrometry in drug and biomedical analyses." Clinica chimica acta 313.1-2 (2001): 147-150.

10. Swales, John G., et al. "Mass spectrometry imaging and its application in pharmaceutical research and development: A concise review." International Journal of Mass Spectrometry437 (2019): 99-112.

11. Kosjek, T., et al. "Mass spectrometry for identifying pharmaceutical biotransformation products in the environment." TrAC Trends in Analytical Chemistry 26.11 (2007): 1076-1085.

12. Careri, Maria, Federica Bianchi, and Claudio Corradini. "Recent advances in the application of mass spectrometry in food-related analysis." Journal of Chromatography A 970.1-2 (2002): 3-64.

13. Stobiecki, Maciej. "Application of mass spectrometry for identification and structural studies of flavonoid glycosides." Phytochemistry 54.3 (2000): 237-256.

14. Malinowski, Edmund R. "Theory of error for target factor analysis with applications to mass spectrometry and nuclear magnetic resonance spectrometry." Analytica Chimica Acta103.4 (1978): 339-354.

15. Hu, Wangmin, et al. "Online Pharmaceutical Process Analysis of Chinese Medicine Using a Miniature Mass Spectrometer: Extraction of Active Ingredients as An Example." Journal of Pharmaceutical Analysis (2023).

16. Strathmann, Frederick G., and Andrew N. Hoofnagle. "Current and future applications of mass spectrometry to the clinical laboratory." American journal of clinical pathology 136.4 (2011): 609-616.

17. Murphy, Robert C., and Simon J. Gaskell. "New applications of mass spectrometry in lipid analysis." Journal of Biological Chemistry 286.29 (2011): 25427-25433.

18. Karlsson, Oskar, and Jörg Hanrieder. "Imaging mass spectrometry in drug development and toxicology." Archives of toxicology 91 (2017): 2283-2294.

19. Pirro, Valentina, et al. "Direct drug analysis from oral fluid using medical swab touch spray mass spectrometry." Analytica Chimica Acta 861 (2015): 47-54.