

Review Article

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Clinical laboratory use of liquid chromatography mass spectrometry

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Abstract: Mass spectrometric methods, which transition from chemistry laboratories to clinical laboratories in the basic field, are frequently used in areas such as metabolic analysis, endocrinology, drug research, protein studies and toxicology. Although it is preferred more than immunoassay methods in large-volume laboratories, in certain analyses, it is thought to be an important keystone in clinical laboratory practice. It has found more space in the laboratory area, with users increasing their device experience and working on the applications of problematic tests. It has the potential to take part in metabolic mapping and multiplex analysis, especially in proteomics, with its performance.

Keywords: analysis; clinical use; lab; liquid chromatography; mass spectrometer.

Introduction

Mass spectrometry (MS) is one of the techniques that has recently come into use in clinical laboratories. MS techniques provide higher sensitivity, specificity and accuracy than other techniques used in clinical laboratories. To ensure high sensitivity and accuracy, samples must be pre-treated before analysis. Since pre-processing processes are often done manually, they are error-prone and time-consuming. The use of MS in clinical laboratories will intensify with the pre-processing steps automated with the ever-evolving technology. The goal of the developed techniques is to use them in the routine laboratory in the clinical biochemistry field. In this process, it should be

noted that MS methods are still in a transitional phase. The development processes in routine clinical chemistry, immunoassay auto analyzers and whole blood count devices continue in MS devices.

Mass spectrometers commonly used in clinical laboratories appear as;

- (1) Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS),
- (2) Gas chromatography with mass spectrometry (GC-MS),
- (3) Inductively coupled plasma mass spectrometry (ICP-MS) and
- (4) Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF).

Since liquid chromatography coupled with tandem MS (LC-MS/MS) is the most widely used MS technique in biochemistry laboratories, this review mainly focuses on the routine use of LC-MS/MS devices.

MS technique based on matrix-assisted laser desorption ionization (MALDI) is mostly used in clinical microbiology laboratories. Significant progress has been made in the diagnosis of infectious agents with the development of rapid methods for the identification of gram-positive, gram-negative and fungi with MALDI-TOF MS analyses [1]. In addition, MALDI-TOF MS analyzes are used in pharmacogenomics, pharmacoepigenetics, and pharmacomicrobiota studies [2]. Gas chromatography MS analyzes (GC-MS) are widely used to detect toxic materials in food, therapeutic drug monitoring, analysis of volatile organic compounds, metabolomics, natural gas and petroleum analysis [3]. GC-MS is also widely used in steroid analysis [4]. ICP-MS methods are used for the detection of elements in the various matrix. MS methods using the ion trap techniques are used in mainly protein studies [5].

The clinical laboratory journey of the MS method

The MS technique was firstly used in sterol metabolism in the 1930s with the discovery of deuterium. The first

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commercially available GC-MS device was put in use in 1965. It can be seen that the first study on multiplex urine steroid analysis was published in 1966. 1971 is the year that quadrupoles were put into use. Steroid hormone analysis with stable isotope labelled internal standards began in 1974. Production of silica columns in chromatography technique, development of solid-phase extraction method, development of thermospray ionization technique, and the introduction of triple quadrupole devices are seen as developments between 1980 and 1990.

Mass spectrometry techniques have been awarded 5 Nobel Prizes during their development process. Joseph John Thompson, also known as the inventor of the electron, was one of the first users of mass spectrometry and was awarded the Nobel Prize in Physics in 1909. Francis William Aston was awarded the Nobel Prize in Chemistry in 1922 for discovering stable isotopes. The 1989 Nobel Prize in Physics was awarded to Wolfgang Paul, who discovered the ion trapping technique. The 2002 Nobel Prize winners in Physics are Koichi Tanaka, who introduced the MALDI technique, and John Bennett Fenn, who discovered the soft electron spray ionization (ESI) technique (Figure 1).

Difference between MS methods from other routine analysis techniques

Commonly used methods in clinical laboratories include the techniques such as photometry, atomic absorption spectroscopy, potentiometry, molecular-based detection

and immunoassay methods. Separation techniques such as electrophoresis and chromatography are also widely used in clinical laboratory analysis. LC-MS/MS technique is a method that enables the detection of the desired molecule according to the mass/charge ratio (m/z) after chromatographic separation. Since LC-MS/MS is based on a separation technique, the molecule to be analyzed must be separated from its matrix by pre-treatment. MS technique is based on the principle of ionizing and measuring the molecule separated from the matrix. Blood, serum or plasma, urine, and cerebrospinal fluid (CSF) have different matrices, and the desired molecule must be separated from these matrices. For the separation process, techniques such as extraction, derivatization and often liquid or gas chromatography are used as pre-treatment steps.

LC-MS/MS is a method that determines both the mass and structure of the analyte to be examined. After chromatographic separation, the first compounds are ionized, the ions selected in the first mass analyzer are filtered and transferred to the fragmentation unit. After fragmentation, the product ions are formed and filtered again in the third mass analyzer, then sent to the detector for analysis. In tandem MS application, only selected ions can pass, while others are removed. First, the disease metabolite to focus on is determined by identifying the ions to be analyzed using the multiple reaction monitoring (MRM) mode. Secondly, mass and structure are determined with high specificity using mass spectrometry.

Since MS analyses used in routine laboratories are mostly performed with “in house” developed methods, it is necessary to know the molecule’s structure well. Although it is unnecessary to know the exact structure of the

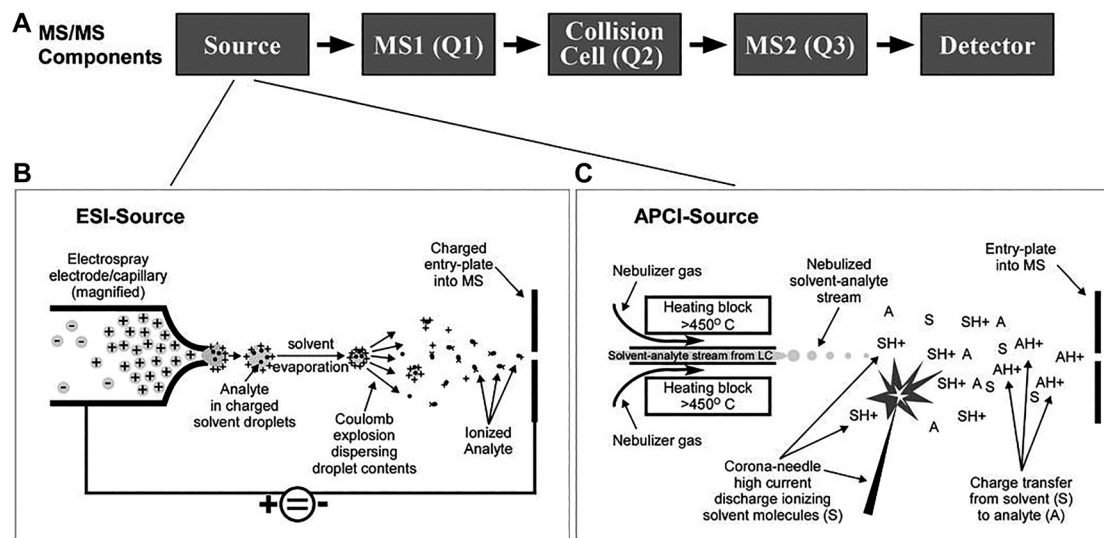


Figure 1: Ionization techniques in mass spectrometry. With permission from ref. [6].

molecule to be analyzed in other routine analyses, knowing the structure of the molecule to be analyzed is very important for using the MS technique. Which processes will be used in extraction, derivatization, and chromatographic separation can only be determined by knowing the molecule's structure. *In vitro* diagnostic vendors often wait for the clinical utility of a diagnostic biomarker to be accepted before implementing the new kits. In laboratories with in-house developed mass spectrometric tests, there may be problems due to laboratory to laboratory variability for the same analyte. There is currently limited assay standardization for MS-based methods. In the absence of a ready-to-use kit, the method development and validation process should be carried out very carefully [7]. Even the MS device with the most sensitive detector cannot give accurate results in a sample that has not been meticulously prepared during the pre-processing stage. Stable isotopes of the molecule are used as internal standards in MS analyses. Stable isotopes are molecules whose molecular weight is increased by adding a neutron to the nucleus of the atom but whose charge is not changed. Thus, since the physical property (charge) of the molecule does not change, it will show the same behaviour as the molecule we are looking for in the analysis but will have a different molecular weight. C^{13} for C^{12} , H^2 for H^1 , N^{15} for N^{14} , O^{17} and O^{18} for O^{16} appear as stable isotopes used. Deuterium (H^2) and C^{13} are the most frequently used stable non-radioactive isotopes for internal standards.

LC-MS/MS applications in clinical chemistry

Often clinical MS applications are used in small molecule (molecular weight <1,000 Da) analysis. These molecules; small organic molecules that appear as amino acids, organic acids, simple carbohydrates, steroids, fatty acids, and metabolites. In recent years, with the development of ion sources, it has become possible to analyze more polar and larger molecules such as peptides, proteins, oligonucleotides and DNA.

Applications in Clinical Laboratories;

- Metabolism, especially hereditary metabolism disorders.
- Endocrinology, steroid hormones, amino acid derivative hormones, biological amines.
- Toxicology, clinical (especially therapeutic drug monitoring) and forensic toxicology.

Hereditary metabolic diseases

Hereditary metabolic disease screening was carried to a different dimension when tandem mass spectrometry was first described in newborn screening in 1990. Hereditary metabolic disease scanning is often called newborn tandem MS screening. There has been a significant increase in the number of detectable amino acid-fatty acid metabolism diseases and organic acidemias with the use of the tandem mass method. Newborn screening is performed from dried blood spots impregnated with filter paper, taken at the 72nd hour after birth. The amino acids that are frequently checked in newborn screening by mass spectrometry are arginine, citrulline, leucine, methionine, phenylalanine, and tyrosine. While phenylalanine levels are checked for phenylketonuria, hyperphenylalaninemia and pterin deficiencies, tyrosine levels are detected for tyrosinemias, fumarylacetoacetate deficiency, and tyrosine aminotransferase deficiency [8].

Amino acids and acylcarnitines in the samples are analyzed by derivatization with methyl or ethyl esters in the tandem MS method. While most scanning programs with tandem MS use this method, the analysis is simplified by removing derivatization in new high-resolution devices. Every laboratory that studies metabolic diseases should determine the threshold values. In tandem MS scans, the level of the relevant amino acid is generally expected to be 5 times higher than normal to be considered abnormal amino acid metabolism [9]. A disease suspected by screening should be confirmed with appropriate diagnostic tests such as amino acid, organic acid, enzymatic or genetic analyses [10].

Tandem MS analysis has very high accuracy in the detection of phenylketonuria, tyrosinemia type II, MSUD, arginosuccinate lyase deficiency, propionic acidemia, methylmalonic acidemia, isovaleric acidemia, medium-chain fatty acid catabolism disorder. It is also highly reliable in many other organic acidemias, very long-chain lipid oxidation deficiencies, carnitine acylcarnitine transport deficiencies, and multiple acyl-CoA dehydrogenase deficiencies. It gives very reliable results in the detection of many other organic acidemias, long-chain lipid oxidation deficiencies, carnitine acylcarnitine transport deficiencies, and multiple acyl-CoA dehydrogenase deficiencies. Reliability is low in detecting long-chain fatty acid oxidation deficiency, carnitine palmitoyltransferase I deficiency, short-chain fatty acid oxidation deficiency, homocystinuria, fumarylacetoacetate deficiency. Similarly, the reliability of the analysis by mass spectrometry technique

is low in glutaric aciduria, MSUD variants, ornithine transcarbamylase, carbamoyl phosphate synthetase and N-acetyl glutamate synthetase deficiencies [11]. Reliability depends much more on the characteristics of the molecule that is analyzed in specific diseases and the nature of the disease. In some diseases, metabolites that must be detected may not be accurately quantified with mass spectrometry, such as carbohydrates, or there may be an easier screening methodology, such as cystic fibrosis. The false positivity rate in tandem MS analyses is given as 0.2–0.3%. Although a clear rate has not been determined for each disease, these systems have a very high sensitivity. The rate of patients diagnosed with screening is higher than those diagnosed clinically. Since no chromatographic separation is used before mass spectrometry in newborn screening with the tandem MS technique, it cannot separate isomeric amino acids or molecules with the same mass (leucine 131 D, isoleucine 131 D, hydroxyproline 132 D ...). Before measuring isomeric amino acids, chromatographic separation is required [12].

LC-MS/MS in endocrinology

Although the trials were first started on steroids from blood content analysis with MS, its use in clinical laboratories was delayed until after 2000 due to the inadequacy of technology. The current clinical laboratory MS use in hormone analysis is carried out in hormones with small-molecule structures. We come across both multiplex [13–15] and single analysis studies [16] for MS analyses of steroid hormones, free T₃, free T₄, and biogenic amines. The very small amount of free forms of these hormones in the circulation and the presence of more than 99% bound to proteins can cause problems in immunoassay analysis. MS analysis is advantageous because of the small molecular weight, similar structures, low levels and hydrophobic physical properties of these hormones.

Low molecular weight

The molecular weights of clinically significant steroid hormones range from 270 to 368 Da [17]. These molecules weighing less than 1,000 Da are extremely small molecules to create an immune response. In order to create antibodies for immunoassay analysis, hapten formation can be achieved by adding additional peptide structures to these

molecules, thus giving immunogenic properties. Steroid hormones with molecular weights close to each other also have the same molecular weight. Steroid hormones with the same molecular weight are;

- (1) Testosterone and Dehydroepiandrosterone 288.42.
- (2) 17 OH Progesterone and 11-deoxycorticosterone 330.46.
- (3) 11 deoxycortisol and corticosterone 346.46.
- (4) Cortisol and 18-OH corticosterone 362.46.
- (5) Cortisone and Aldosterone have a molecular weight of 360.46.

For the analysis of steroid hormones with the same molecular weight, a double MS/MS detector should be used instead of a single MS detector. It should not be forgotten that even MS methods are open to interference in steroid hormone measurements. There are two ways to solve it analytically to reduce interference. The first is to detect different fragmentation products by using two mass detectors (MS/MS) instead of a single mass detector. The second way is to provide chromatographic separation of molecules in the LC section. A similar problem can be seen in the analysis of 25 OH Vit D₃ with 3-epi-25 (OH) D₃ because both molecules have the same molecular weight. It has been observed that the 3-epi-25 (OH) D₃ form is higher in childhood. Although the function of epi forms of steroid hormones is not fully known, they appear as molecules that will cause serious interference in immunoassay analyzes. 25OH D₃ and 25 OH D₂ interferences are close to 100% in immunoassay kits [18].

Similar structure

Steroid hormones are very similar in structure. Differences in the presence or number of keto groups, double bonds, and OH groups in steroid hormones cause the formation of different steroid hormones. Interference is frequently encountered in immunoassay analyzes of very similar structures. Interference of steroid hormones with high serum concentration (cortisol ~300 nM) to hormones with lower concentration but similar in structure (11-deoxycortisol ~5 nM) may affect the results. Similarly, a high concentration of DHEA-SO₄ (~7,000 nM) interferes with E₂ (~0.5 nM), 17 OHP (~2–3 nM), testosterone (~10 nM), and progesterone (~1 nM) and these interferences should be taken into account in immunoassays. In addition to the interference effects of endogenous steroids, steroid metabolites appear as other interfering molecules [19].

Lipophilic character

Hormones with hydrophobic physical properties need carrier protein in circulation. Steroid-binding globulins, cortisol-binding globulin, thyroxine-binding globulins and albumin play a role in this transport. Hormone binding globulins can alter the measurable analyte concentration in the sample by removing or blocking them. Hormones have an important place in measuring binding kinetics to these proteins [20]. Changes in the level of these proteins are affected by many conditions such as polymorphic protein structures, liver diseases, and nephropathies with proteinuria. The binding of cortisol by cortisol-binding globulin can be inhibited by denaturation of this protein or by the addition of a blocking agent. Free fatty acid levels that increase as a result of heparin administration may change free T4 levels by binding to specific binding proteins [21]. If pre-extraction is performed with diethyl ether before the analysis in immunoassays in hormone analysis, the correlation with LC-MS/MS analyses increases [22].

Heterophilic antibodies

Heterophilic antibodies consist of natural or autoantibodies that bind with low affinity to molecules with low antigenic properties. In immunoassays, heterophilic antibodies bind to the binding and signal producing antibody, enzyme and cause false-positive results. This interference in immunoassays usually occurs by non-competitive mechanisms [23, 24]. Again, human antimouse antibodies (HAMA), human anti-animal antibodies have serious interferences on cardiac markers, thyroid hormones, drugs and tumor markers. Mass spectrometric methods do not show such interference.

Since the LCMSMS method is accepted as a reference method, correlation studies performed by comparing the commercial immunoassay kits produced with the MS method seem to be a common validation method. It is observed that the result compatibility of new generation immunoassay commercial kits with MS methods is increasing [25, 26]. In the UK, the use of MS techniques in steroid analysis is becoming more and more common. UK NEQAS for steroid hormones proficiency testing schemes changed their target value from all laboratories trimmed mean to LC-MS/MS [27].

Clinically important biogenic amines are bioactive endogenous compounds which play a significant physiological role. Serotonin, dopamine, noradrenalin, adrenalin and their metabolites, vanillylmandelic acid,

5-hydroxyindolacetic acid can be considered useful biomarkers for the diagnosis, prognosis, therapy and follow-up of several neuroendocrine and cardiovascular diseases. MS can quantify and identify biogenic amines based on a mass-dependent transition between precursor and product ion. Electrospray ionization is mainly used ionization source for MS analysis to determine biogenic amines. Literatures demonstrated that LC-MS/MS have a predominant position in biogenic amines investigations due to high selectivity and sensitivity [28–30]. With the strong sensitivity and specificity values in the analysis of biogenic amines, especially metanephrine analyzes can be performed with MS. Metanephrine and normetanephrine analyzes, which are frequently encountered with problems in chromatographic analyses, can be measured effectively with the MS technique [28]. Analysis of porphyrins and 5-aminolevulinic acid (ALA), and porphobilinogen (PBG) in physiological liquids can be performed by mass spectrometry [31].

Analysis of large molecules such as peptides and proteins by MS methods cannot be used in daily routine practice. The routine use of measurements made by fragmentation of proteins and analysis of the protein-specific peptide fragment of interest is very limited. In patients with suspected thyroglobulin antibodies or heterophile antibodies, serum thyroglobulin levels can be measured in Mayo clinical laboratories [32]. Techniques such as liquid-liquid, SPE, immunoaffinity-MS, immobilized metal affinity chromatography, and protein precipitation are used for extraction, and ESI and MALDI techniques are frequently used for MS ionization [33].

LC-MS/MS in toxicology

Analytical toxicology is the section that identifies and measures the levels of drugs and xenobiotics in biological samples and their products resulting from their metabolism. The scope of analysis is broadly wide, including poison and drug abuse screening, forensic, environmental and occupational toxicology and therapeutic drug monitoring. The scope of this analysis is intertwined. It covers applications in the emergency department and general hospital. The matrix of biological samples taken for analysis may be found in an even more mixed form in the post-mortem state. In this case, the analytical method should be more sensitive, selective and reliable [34].

In a global point of view, MS is a specialized technique for the clinical laboratory. It has the potential to be

included in the laboratory in the future. Even though literature research shows that mass spectrometry applications in clinical laboratories have increased in the last 10 years, there is not enough information about the content of the applications and how they are applied. The devices do not have records in a database, and data from health-care practitioners and financial institutions are insufficient. Considering the total laboratory test load, the rate of performing the analyzes with MS is low. The reason for its high use, especially in the field of clinical toxicology, is the limited number of alternative analysis methods in this field. For example, 70% of the participants who participated in external quality control tests in the field of therapeutic drug monitoring reported that they analyzed the tests by MS method [35].

The MS method is considered the “gold standard” in drug analysis and clinical toxicology in terms of its sensitivity, specificity and applicability of applications to various fields. MS method-based drug analyzes are used for many reasons, such as detecting drug concentrations in the blood in clinical toxicology, determining the causes of death in forensic cases, and detecting illegal substance use and levels in traffic controls [36].

Although immunoassay-based drug screening and detection kits are ready-to-use kits that give rapid results, and work in automated systems, they do not cover the measurement of drugs investigated in clinical toxicology and their circulating metabolites. Although many immunoassay kits have limited specificity in certain drug groups, they only serve to detect the drug. Again, these commercial kits require certain cut-off values in drug analysis. This is due to their low sensitivity. In addition, they tend to cross-react with some drug-independent compounds. For this purpose, MS in clinical toxicology is used for confirmation after immunoassay-based screening tests. In addition, it is useful for both identification and quantitative analysis of substances that are not found in the immunoassay test panel [37].

The most important advantage of the use of immunoassay-based commercial systems in clinical toxicology is the short time to result. In a period of 30 years, many companies have turned to develop immunoassay-based kits that provide fast results for drug measurements. Most of these kits use homogeneous immunoassays and can report the levels of 11 drugs (cyclosporine, tacrolimus, mycophenolic acid, valproic acid, digoxin, theophylline, carbamazepine, phenytoin, phenobarbital, vancomycin and gentamicin) in less than 1.5 min. However, these kits perform analyzes directly from the sample matrix without any pre-treatment (extraction, protein precipitation, etc.). In the enzyme multiplied immunoassay technique (EMIT)

and Fluorescence polarization immunoassay (FPIA) analyses developed recently, detection can be made sensitively at nanomole levels. Problems in analytical specificity are the main shortcomings of immunoassay-based kits. Specifically, synthesizing antibodies belonging to the molecule itself is a very difficult process. Although the basic chemical structure of many of the substances detected in toxicology is the same, there are very small group differences. These small differences can lead to false positive or negative results. When these similar drug molecules are tested, the antibodies found in the immunoassay may bind to the drug-like molecule in the patient sample with a non-specific nature to be measured, causing erroneous results. The average time to market for an immunoassay test is 2–5 years. The antibody design of this test belongs entirely to the manufacturer. Therefore, many technical features such as analytical sensitivity, cut-off values, interpretation of results, etc., may vary in kits of different companies in kits produced based on immunoassay. Again, these commercial kits are designed for only a certain sample type, and their applicability to all body fluids is limited. Therefore, clinical laboratories using these systems may have to follow validation procedures for different types of sample matrices. LC-MS/MS systems provide higher specificity and sensitivity than commonly used methods such as immunoassay, chemical measurement with UV detector, GC-MS and classical HPLC. The reason for this is that the substance analyzed in the quadruple in the LC-MS/MS system is detected not only in itself but also in the ionized fragments of this molecule. When paired with the classic HPLC system, physical separation of the analyzed substances provides additional selectivity. So much so that substances can be detected by their retention time [38].

Mass spectrometric methods for many classes such as immunosuppressive drugs [39, 40], antivirals [41, 42], antiepileptic drugs [43], chemotherapeutic drugs [44, 45], antibiotics [46, 47], antidepressants and antipsychotics [48, 49] has been published.

Another important area in clinical and forensic toxicology is the investigation of unidentified drugs or poisons. For this purpose, the first method to be applied is screening. Depending on the type of case analyzed and the clinical signs of poisoning, the screening method should contain more than 10,000 potentially toxic compounds. Analytical method selection and application in toxicological screening are completely different from clinical laboratory applications. If the potential substance in the sample is known, a comprehensive molecule screening can be performed if the targeted screening is unknown. LC-MS/MS analysis with MRM has now become the

standard for targeted screening involving many substances. With this method, it is possible to quantify after detection. It is the specificity, and the number of scans displayed that determine the quantitation. With high-resolution liquid chromatography-mass spectrometry, the selectivity is increased as the ions can be observed two times more. Another superior feature of the high-resolution MS (HR-MS) technique is its ability to perform targeted-selective quantitation or general screening. The analysis of isobaric substances with increased solubility obtained with the help of this technique can be performed. In other words, the interference of endogenous biomolecules with the same mass that interferes during the analysis is reduced. With the help of this technique, the mass and structure of the main and fragmented fragments can be determined, as well as by mapping the structures and masses of potential poisons from libraries [50]. With the use of the ionization process in the mass spectrometry technique, the experience in this field has increased. Interferences caused by co-eluting compounds during the analysis, such as ion suppression or enhancement, have begun to be minimized. Systematic toxicological screening can be performed in GC-MS with the use of libraries to which these compounds are matched. In the GC-MS method, non-volatile, polar and thermolabile compounds cannot be analyzed without a long derivatization procedure. Diode array HPLC can provide applications for compounds that are difficult to analyze by gas chromatography. However, in toxicology, some compounds have low absorbance in UV light. Therefore, depending on this defect of the detector, detection may not be possible. It is an alternative method for the analysis of compounds that can not be analyzed by LC-MS/MS. Molecules can be detected by comparing ionization patterns from libraries loaded in the instrument, although this is not standardized [34]. The use of matrix-assisted laser desorption ionization (MALDI) time-of-flight (TOF) for bacterial identification in the microbiological field has significantly shortened the time to results. With MALDI, analysis of tissue proteins, drugs and their circulating metabolites, lipids and other components can be performed [51]. MS systems are not common in laboratories due to high expenses and maintenance costs. Also, the most important point is to have experienced qualified personnel for MS platforms. For future aspects of tandem mass spectrometry: Parameters such as retention times, fragmentation of molecules, and molecule-molecule interactions, which are analyzed in mass spectrometry systems, are stored in various libraries by experts. However, the part that is open in this area and can be considered a new area can be considered follows.

In mass spectrometry systems, hundreds of molecular analyzes can be performed multiplex from a sample. If the chemical properties, fragmentation patterns, and ionization types of these molecule classes are integrated into deep learning-based framework systems after prioritized machine learning, the optimal ionization and analysis characteristics of the substances to be analyzed in the future can be determined within a map.

Studies in the field of proteomics in mass spectrometry systems can be considered the main cornerstone in terms of recognizing these structures, especially in biological systems. MS methods have come a long way in the analysis of relatively small and stable molecules in shape. However, we see that this distance is quite limited in dynamic protein analyzes. It is clear that MS methods have a long way to go for routine analysis of proteins. Not only the structural diversity of proteins but also their functional variability challenges MS analytical systems. Missing proteins, proteins having unknown functions, alternative splice isoforms, post-translational modifications, and lack of proper quality control are the main problems to be solved. Proteins synthesized from the same gene but in which different forms are used for completely different clinical situations are used in the routine laboratory. For example, calcitonin is used in the follow-up of calcium metabolism and medullary carcinoma of the thyroid; its prohormone procalcitonin is used in bacterial inflammation. While calcitonin is secreted from thyroid tissue and adipose, we see that procalcitonin is secreted from almost all parenchymal tissues in response to inflammation. One of the main post-transcriptional modifications is protein phosphorylation which can be detected with Western Blotting but has little clinical practice use. After the completion of tissue-based proteomics studies, the use of MS for the analysis of changes in the level of clinically significant proteins that are secreted or shed from tissues may be possible in clinical practice.

Conclusions

The use of mass spectrometry in the clinical laboratory is becoming more common day by day. The lack of commercial kits on alternative platforms and the problems due to interference in current analyses are why mass spectrometry is preferred. Despite this, device costs and experience are the biggest problems. In the future, it is envisaged that it will take place in laboratories in the form of a desktop, ready-to-use auto analyzers.

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