Mass Spectrometry: An Important Tool in Clinical Research

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Abstract: **Across the world, the analytical capabilities of mass spectrometry and its ability to accurately identify and quantify the minute amounts of compounds in complex sample matrices such as blood, urine and oral fluid are studied nowadays. Innovative mass spectrometry methods are being developed in place of conventional methods for a wide variety of research applications, including the accurate quantitation of trace levels of hormones, steroid panels, vitamin D, drugs of abuse, pain panels or immuno-suppressants. This paper consists of the literature on the applications of mass spectrometry in clinical research.**

***Keywords*--** **Mass spectrometry, clinical research, LC-MS, LC-MS/MS.**

I. INTRODUCTION

Today mass spectrometry has evolved as a very useful tool in the field of clinical research and its routine applications [1]. Mass spectrometry is a technique that measures ions in its gaseous state. When sample is introduced into an ion source, it ionizes and then separated in a mass analyzer according to their mass to charge ratio (m/z). Gas chromatography (GC) and liquid chromatography (LC) can be coupled with mass spectrometer and can be used for the purpose of qualitative and quantitative analysis. Nowadays GC-MS and LC-MS are the widely used instruments in the field of pharmaceutical research, clinical research and bio-medical research for the purpose of validation of quantitative analytical assays [2].

For a good GC-MS or LC-MS method development, proper sample preparation procedure and instrumentation (in terms of LC and MS) must be selected so that the results would be precise, accurate and reproducible. GC-MS instrument has limitation that only volatile compounds can be analyzed on it. Thermally unstable molecules can not be analyzed by the GC-MS application. Also the derivatization of the samples before injecting it in GC-MS is again the time consuming and lengthy procedure in GC-MS. Initially GC-MS technique was used for the biological samples but due to the above mentioned limitations, necessity of other techniques was arisen. In clinical samples, non-volatile and thermally labile molecules are found and to overcome from this problem, LC-MS technique was discovered. LC-MS is an appropriate separation technique for the polar, thermally labile and non-volatile molecules. It is also useful for the higher molecular weight compound; which was again the big issue in the case of GC-MS. LC-MS is the coupling of LC and MS which reduces the need of derivatization and other lengthy tedious procedure and save the analysis time. Now in recent time, clinical field and LC-MS are intertwined with each other. Because of the analytical power of the LC-MS, this technique has become very popular in analytical field especially in clinical and bio-medical research field. The aim of this paper is to give basic understanding of the clinical mass spectrometry and its applications to the clinical, bio-medical and bio-analytical research scientists.

II. SAMPLE PREPARATION

Sample preparation, also known as sample treatment/sample clean-up/sample extraction, is an integral part of bio-analytical method. In a clinical situation, the drug/metabolite/biomarker of interest is present in biological matrix which has a complex biochemical nature and comprises numerous components, viz. salts, acids, bases, proteins, cells, exogenous /endogenous small organic molecules like lipids and lipoproteins. However, the bio-chemical complexity of the matrix may differ from one to another (viz., tissue, whole blood, plasma/serum, urine, saliva etc.). In simple terms, sample preparation is a process which aims at selective isolation of the analyte of interest from the matrix, minimization/elimination of matrix components in the processed sample and, if required, concentration of the analyte of interest. It is therefore essential to ensure the quality of the sample itself, which makes sample preparation effective [3].

Therefore, the sample preparation procedure is a very critical part of this technique as clinical samples are the complex biological matrices and contain interferences that can leas to so-called matrix effect within the mass spectrometer. The internal standard method is most commonly used procedure for the sample preparation followed by the extraction to remove as much of the interferences as possible. Commonly used extraction methods are Liquid-Liquid Extraction (LLE), Solid-Phase Extraction (SPE), and simple protein precipitation with a solvent [3]. The ion suppression of the analyte, matrix effect and low response are the usually observed problems in mass spectrometry technique for the clinical samples if sample clean up is not sufficient. These all problem negatively affect the limit of detection (LOD), accuracy, precision of the assay. Today numbers of methods are developed in clinical research field to get the best optimum results and minimize the above mentioned problems as these are the most important parameters in analytical method validation required for all the clinical MS assays. The superior sample preparation and optimized chromatographic method are the two most effective ways of avoiding the ion suppression in clinical mass spectrometry.

Prepared sample is then injected into the liquid chromatographic system; where the different components of the sample are separated as per their polarity index before they enter into the mass spectrometer. In LC, the analytical column used is usually reversed phase; which means the silica based chemical modified packing material is being used as a stationary phase and more aqueous solvents are used as the mobile phase. The separation depends on the chemistry of the stationary phase means the selectivity (order of elution) of the analytical column depends on the polarity of the stationary phase. Less polar component will elute late on reversed phase column. The new generation columns are available in market now; which has the particle size less than 2µm which gives the better resolution and sharp peak shape; which are the most important criteria in assay methods.

III. INSTRUMENTATION

The block diagram is shown in Fig. 1. for the typical mass spectrometer coupled with liquid chromatography system. Different types of MS used in clinical research today depending on the application, as the ion source and mass analyzer are the core component of the MS system and due to the wide application of MS; continuous R&D is going on all over the world. After the chromatographic system, the sample is then introduced into the MS ion source. Ionization modes include electron ionization (EI), chemical ionization (CI), electro spray ionization (ESI), atmospheric pressure chemical ionization (APCI) and matrix assisted laser desorption ionization (MALDI). In the MS technique; ESI is the most commonly used ion source for the bio-molecules encountered in the clinical samples. In ion source; the component will be ionized and then in mass analyzer they will be separated according to their m/z charge ratio. Different mass analyzers are used as per the application such as, magnetic or electric sectors, time-of-flight (TOF), quadrupoles and two dimensional and three dimensional ion traps. The magnetic sector mass analyzer is used for the analysis of dioxins by high resolution MS instrument while the soft ionization technique ESI has led to eh use of quadrupoles mass analyzers, where the quadrupoles consists of four parallel rods or poles, generally of hyperbolic cross section through which ions are passed and separated.

After the discovery of tandem mass spectrometry (MS/MS) the specificity and sensitivity have been increased significantly in the clinical sample analysis. In this technique, two or more mass analyzers are placed in sequence and fragmentation of the ions is induced in a collision cell to give the structural information. This technique is especially very suitable for the complex biological samples. Today triple quadrupole MS/MS is very common in clinical and bio-analytical laboratories in which; there are first mass analyzing quadrupole, quadrupole collision cell and the third mass analyzing quadrupole. The LC-MS/MS technique has also been recommended by the American Endocrine Society in 2007 for the determination of endogenous steroid hormones over more traditional technologies such as immunoassays as this technique has greater sensitivity in conjunction with the improved specificity of MS/MS over immunoassays and therefore it is the preferred mode of analysis particularly in the clinical endocrinology field [4].

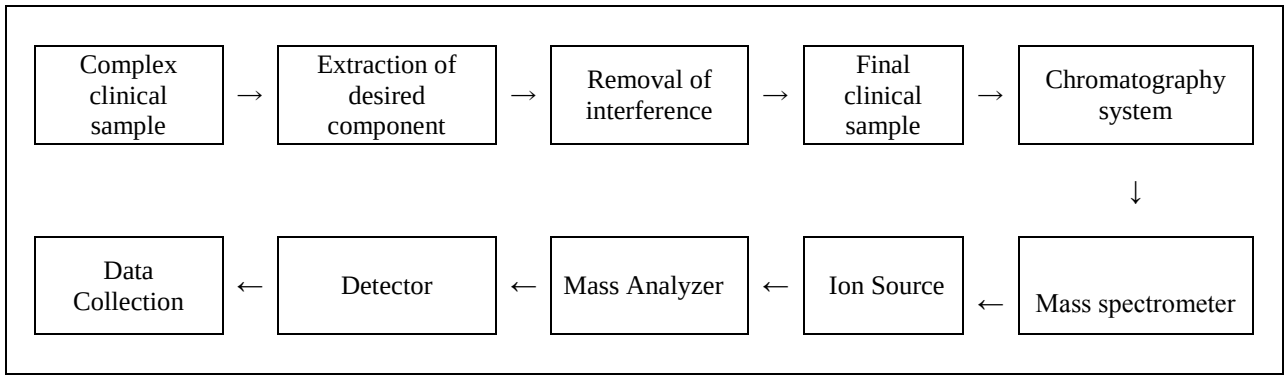

Fig. 1. Block diagram of LC-MS components.

IV. CLINICAL APPLICATIONS OF MASS SPECTROMETRY

An advancement of the clinical research field has been possible due to the entry of the MS technique with different types of chromatography. Because of the coupling of chromatography with MS, number of assay developments has been increased in the clinical research today. Again the steroid hormone analysis is well documented area in clinical research field. New born screening, multi-analyte drug monitoring, oncology drugs, anti-viral drugs, toxicant and drugs of abuse screening and endogenous peptide analysis etc. are the other area where clinical mass spectrometry technique is extensively used [5]. In the analysis of isomeric compounds using this technique, the possibility of false results is always there as the isomeric compounds will be eluted at the same retention time. To resolve this problem, the strong and efficient chromatography technique is required which can separate the isomers and isomeric impurities with a good resolution. After developing an efficient method, if those isomers are introduced into MS, one can interpret mass spectrum and the fragmentation pattern of each isomer. Fast chromatographic technique may not resolve the isomers and for that longer analysis time with complete isomer resolution should be selected.

As the world is overflowing with new technologies technique allows even the sample introduction in the chromatographic system without the extraction procedure in which; different types of online solid phase extraction (SPE) cartridges are connected before the analytical column; where the extraction takes place in that cartridge before it enters into the analytical column and from there it will be introduced into the MS. Steriods, thyroxine and isoprostanes are analyzed by this technique [6]. Online SPE cartridges are very efficient in removing the ion suppressing interferences as they can lower the response of the main analyte. The use of steroid panels is of a great interest for the diagnosis and treatment of complex endocrine disorders and multidimensional LC-MS/MS has been proven as a very useful technique for this challenging diagnostic clinical requirement. Thirteen different steroids have been successfully determined in protein-precipitated serum by this technique [7]. LC-MS can be used as a multiple sample injection system in which; more than one channels can be fed into one MS/MS and the beneficial part of this application is that we can use the LC-MS/MS system in highly efficient manner which; saves the analysis time and more out put can be obtained with this system so that we can get the full value of this costly instrument (which is one of the major drawback of this instrument).

Phenylketonuria (PKU) is a congenital disorder that renders phenylalanine hydroxylase dysfunctional. This enzyme is responsible for the conversion of phenylalanine to tyrosine. By measuring both the substrates from a newborn bloodspot by MS/MS with MALDI ionization and determining the ratio of concentrations it is possible to detect PKU in the newborn with extremely high sensitivity, specificity and reproducibility [8]. Above MALDI, ESI and APCI, triple quadrupole technology is also at the forefront of applied MS technology. High resolution mass spectrometry (HRMS) is not much discovered area in which; the accurate mass determination is possible for different metabolites as HRMS allows monitoring over a defined mass range by using triple quadrupole technique [9]. HRMS based on TOF and Fourier Transform instruments have similar cost to triple quadrupole instruments and those are also very effective tools. The accurate mass determination with HRMS technique is well studied for the screening applications [10]. Above all even further innovations, improvements and cost effectiveness is required in this technology to improve the results in quantitative analysis as HRMS technology is very accurate but it has limited linear range as compared to other MS techniques.

V. SUMMARY

The continuous research on MS applications in clinical research is going on across the world. We have quoted just few applications of clinical mass spectrometry as lots of papers are being published in this field every day by day, new online multi-dimensional chromatography year. This publication is our attempt to give the new direction to the clinical, bio-medical and bio-analytical research scientists in the field of clinical mass spectrometry. We strongly believe that in future MS will be an important research tool in the field of clinical research

VI. REFERENCES

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