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THE BOUNDARIES OF LIQUID CHROMATOGRAPHY AND MASS SPECTROMETRY IN PHARMACEUTICAL ANALYSIS

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ABSTRACT

Several qualitative and quantitative applications of LC/MS have been recorded in the pharmaceutical industry. These applications may be broken down into four basic disciplines: synthetic organic chemistry, combinatorial library parallel synthesis, bioanalysis in support of ADME, and proteomics. There is no question about the significance of any of these individual domains. This article also discusses other analytical procedures that may be used in conjunction with LC/MS to increase the amount of analytical information that can be gleaned from the data. Since the application of LC/MS is now rather widespread and is only anticipated to become even more widespread in the not too distant future, this research concentrates almost entirely on recent developments that have been documented in the literature with specific publications.

Keywords: *Pharmaceutical Industry, Boundaries of Liquid*

INTRODUCTION

Effects Of the Matrix

Suppression of ions and augmentation of ions

Molecules are capable of being studied with an extremely high level of precision thanks to the development of brand-new mass spectrometers with high levels of resolution. Due to the excellent resolving power of mass analyzers, these instruments are often utilised with minimum or even without any previous sample preparation. This is possible because interference masses may be separated using the mass analyzers. Indeed, the great specificity of the technology that is now available will produce a reduction in the MEs that are generated by comparable masses. Regrettably, ion suppression and/or enhancement that was introduced during the ionisation of the sample does not always appear in the chromatogram.

As a result, Taylor referred to MEs as the 'Achilles heel' of quantitative MS. The evaluation of MEs in atmospheric pressure ionisation mass spectrometry, which is still regarded as the gold standard for quantitative pharmaceutical analysis, has been the subject of a great number of research and reviews that have been carried out and published over the course of many years. Different possibilities have been proposed in these publications; nevertheless, the precise mechanism of ion suppression or augmentation has not yet been elucidated.

Multiple variables, such as the properties of the analyte, the kind of matrix, the sample preparation technique, and the chromatographic settings, may all have an effect on MEs. It is quite probable that these characteristics have a synergistic impact on the ionisation; yet, it cannot be ruled out that MEs may be assigned to just one cause. This is because it is not possible to rule out the possibility of several causes. During the APCI process, molecules first enter the gas phase and are then subjected to chemical ionisation. During the ESI process, the analytes get ionised when they are allowed to escape from a charged droplet when the droplet evaporates. Because of this, it is generally believed that ESI is more prone to ion suppression, particularly for polar and, as a result, less volatile chemicals that continue to be trapped in the matrix droplets. In addition, reactions have the ability to neutralise some of the ions that are present in the gas phase. When it comes to ESI, it is well knowledge that co-eluting matrix components have a negative impact on the amount of ionisation that may be achieved. In instance, the presence of fewer or non-volatile co-eluting solutes will make droplet formation more difficult and thus slow down the depletion of the solvent. An unfavourable rivalry between medicines and co-eluting substances for the excess of charge to be ionised may also emerge. This competition is for the ionisation of the pharmaceuticals. As a result, the structure and, therefore, the physicochemical characteristics of both the analytes and the matrix components have a significant impact on MEs.

Because mass spectrometry is more sensitive and more specific than other chromatographic detectors, coupling it with chromatographic methods has long been seen as desirable. This is owing to the fact that MS may be coupled with chromatographic techniques. The connection of mass spectrometry and gas chromatography (also known as GC-MS) was first accomplished in the 1950s, and commercially viable equipment did not appear until the 1970s. It is currently common for clinical biochemistry labs to be equipped with very affordable and dependable GC-MS systems. These systems are useful in a number of fields that need the analysis of complicated mixtures and the unambiguous identification of substances, such as the screening of urine samples for inborn errors of metabolism or pharmaceuticals.

The linking of mass spectrometry with liquid chromatography, also known as LC-MS, was an apparent extension; yet, development in this field was hampered for a number of years owing to the relative incompatibility of current MS ion sources with a continuous liquid stream. There were a few different interfaces created, but since they were difficult to work with and lacked reliability, adoption by clinical labs was very low. Fenn's invention of the electrospray ion source in the 1980s brought about a shift in this predicament that had previously existed. Electrospray sources were swiftly created by manufacturers, resulting in the fast development of devices that had a significant influence on protein and peptide biochemistry. In 2002, Fenn and Koichi Tanaka were jointly awarded the Nobel Prize for their work on the development of matrix aided laser desorption ionisation. This is another another MS ionisation technology that is particularly helpful for the investigation of biological molecules.

By the middle of the 1990s, both the cost and performance of LC-MS equipment had significantly increased, making it possible for clinical biochemistry labs to take use of the new technology. The field of biochemical genetics was one of the first to do so, and one of the most important early applications was the examination of dried blood spot samples taken from neonates to look for a variety of inherited metabolic disorders.3 There are a number of additional clinical uses for LC-MS, and the method has a wider range of potential applications than GC-MS does. This is due to the fact that a wider variety of biological molecules may be studied with LC-MS, as well as the increased prevalence of LC separation techniques in clinical labs. High specificity and the capacity to deal with complicated combinations are the primary advantages offered by LC-MS in comparison

to LC equipped with conventional detectors. These advantages are almost identical to those offered by GC-MS. In 2003, a piece titled "Applications of Electrospray Mass Spectrometry" was published in The Clinical Biochemist Reviews. The present study focuses on the fundamentals of LC-MS, the practical factors that need to be taken into account when setting up LC-MS experiments, and a review of some of the most important applications in clinical biochemistry, with a particular emphasis on applications involving small molecules.

OBJECTIVES

1. The Study Boundaries of Liquid Chromatography And Mass Spectrometry.

2. The Study Synthetic Organic Chemistry, Combinatorial Library Parallel Synthesis, Bioanalysis.

Mass Spectrometry Instrumentation

In order to function, mass spectrometers must first transform the analyte molecules into a charged (ionised) state. This is followed by the examination of the ions and any fragment ions that are formed as a result of the ionisation process, based on the ratio of their mass to their charge (m/z). It is possible to do ionisation and ion analysis using a variety of different methods, which has led to the development of a large number of distinct kinds of mass spectrometers that use varying configurations of these two processes. In actual practise, some configurations are far more adaptable than others, and the following descriptions concentrate on the primary kinds of ion sources and mass analysers that are likely to be used in LC-MS systems inside clinical labs.

Source Of Ionisation Generated by Electrospray

Fenn transformed ESI into a reliable ion source that was able to interface with LC. He also showed the applicability of ESI to a number of significant groups of biological compounds.1 Electron Spin Ionisation works well with molecules that are only moderately polar, and as a result, it is ideally adapted to the investigation of a wide variety of metabolites, xenobiotics, and peptides. A tiny spray of charged droplets is formed by nebulizing liquid samples as they pass through a metal capillary while the capillary is kept at 3 to 5 kV. The liquid samples are pushed through the capillary. In order to reduce the risk of contamination, the capillary is often positioned in a direction that is either orthogonal to, or off-axis from, the entry to the mass spectrometer. The combination of heat and dry nitrogen results in the fast evaporation of the droplets, and during this process, any electrical charge that is still present on the droplets is transferred to the analytes.5 After that, the ionised analytes are moved into the high vacuum of the mass spectrometer using a succession of tiny apertures and focusing voltages. Switching between these two modes while an analysis is being carried out is possible. The ion source and subsequent ion optics may be controlled to detect either positive or negative ions, and either mode can be used to detect ions.

When operating under typical circumstances, ESI is referred to be a "soft" ionisation source. This designation indicates that the analyte receives a comparatively low amount of energy, and as a result, only a little amount of fragmentation takes place. When compared to other MS ion sources, such as the electron impact source that is often used in GC-MS, which results in considerable fragmentation, this aspect of MS stands out. It is feasible to boost ESI "in-source" fragmentation by raising voltages inside the source to increase the number of collisions with nitrogen molecules. Doing so will result in increased fragmentation. In LC-MS analysis, this has been utilised to detect components that have similar structural properties.

For example, the glycans in glycopeptides may be fragmented in-source to yield 204 m/z reporter ions. This technique has been put to use to identify components. This property has been used in order to define the structure of glycans by identifying glycopeptides within tryptic digests of protein.6 Although beneficial for certain analytes, in-source fragmentation is restricted for others. In order to produce the substantial fragmentation that is necessary for structural investigations and tandem MS, more reliable fragmentation procedures, such as collision-induced dissociation (see below), are needed.

Ions with a single charge are produced most often by molecules that are less than 500 daltons in size and have a single functional group that may carry an electrical charge. When the ion source is used in the positive ion mode, this may result in the analyte gaining a proton (M+H+), whereas when the ion source is operated in the negative ion mode, this can result in the analyte losing a proton (M-H). When salts are present, adduction of cations and anions may take place. Some examples of this are M+NH4+, M+Na+, and M+K+. Other examples include M+formate and M+acetate. Larger molecules and molecules that include several charge-carrying functional groups, such proteins and peptides, have the potential to display multiple charging, which may lead to the formation of ions such as M+2H2+, M+3H3+, and so on. This ends up producing an envelope around proteins consisting of ions that have varying charge states. On mass spectrometers that scan up to just 4000 m/z, this feature may be exploited to correctly detect analytes with large molecular weights including proteins up to 100 kDa. In point of fact, the detection of ions with m/z values higher than this is a rare occurrence.

Although ESI is the most common approach for obtaining ions from biological molecules, it is possible that molecules with a neutral or low polarity, such as lipids, would not be ionised very well using this technique. Following is a description of two different ionisation procedures that have been developed for similar analytes.

Source Of Chemical Ionisation Produced by Atmospheric Pressure

As is the case with ESI, the process of atmospheric pressure chemical ionisation (APCI) involves pumping liquid through a capillary until it becomes nebulized at the very end. In the vicinity of the tip of the capillary, a corona discharge occurs, which first ionises the gas and solvent molecules that are present in the ion source. These ions subsequently react with the analyte, which results in charge transfer and ionisation of the analyte. Small, thermally stable compounds that are difficult to ionise using ESI are ideal candidates for this method's use.7,8 For instance, free steroids do not ionise very effectively when subjected to ESI because the molecules that make up free steroids are largely non-polar, neutral, and lack any functional groups that are able to transport a charge. The sensitivity of the LC-MS analysis of free steroids has therefore been improved with the use of APCI.9–11 Single-charged ions predominate in APCI, in contrast to the phenomenon of multiple charging that is seen in ESI. There are single ion sources that are available, and they are able to switch between APCI and ESI. The method has also been used in the study of lipids and vitamins that are fat-soluble.

The combination of liquid chromatography and mass spectrometry, abbreviated as LC/MS, is rapidly becoming the instrument of choice for liquid chromatographers. It is a strong analytical method that combines the detection specificities of mass spectrometry with the resolving power of liquid chromatography. As a result, it is also known as tandem mass spectrometry. With the development of electrospray ionisation (ESI), which provides a straightforward and dependable user interface, the method of liquid chromatography-mass spectrometry, often known as LC-MS, has become standard practise. Because the recently established API-based approaches result in only moderate ionisation, they may be supplemented for structural elucidation investigations either by invoking fragmentation-induced collisions in the interface itself or by resorting to LC-

tandem MS, which is achieved with the assistance of a triple quadrupole system. It is useful for the analysis of biological molecules, and the utilisation of tandem mass spectrometry and stable isotope internal standards makes it possible to expand very sensitive and accurate procedures by doing some method modification in order to avoid the impacts of ion repression. Method validation is an essential step in the drug development process, which comes between drug discovery and development.

The results of the LC/MS analysis may be used to derive information on the molecular weight, structure, identity, and amount of certain components of the sample. The selectivity, specificity, LOD, LOQ, linearity, range, accuracy, precision, recovery, stability, roughness, and robustness of liquid chromatographic investigations are determined by the study.1,2. 1.4.3 Research and development of new pharmaceuticals

During the drug discovery stage, the objective is to produce a unique lead candidate that has desirable pharmaceutical qualities (such as effectiveness, bioavailability, and toxicity) so that it may be tested in preclinical studies.

Screening for activity is performed on potential lead compounds that are either found in natural product sources or taken from the huge database of a synthetic amalgam library. The lead compounds that are identified in the field via screening efforts are then optimised through tight cooperation with exploratory metabolic programmes and drug safety analyses. It was estimated in 1997 that the synthesis and screening of around one hundred thousand different compounds is normally necessary in order to uncover a single high-quality lead molecule.

The procedure of determining the identity of a lead compound might take anywhere from two years to four years. Additional one to two years may be required for the lead that was generated as a consequence. Target identification, lead identification, and lead optimisation are the key types of analyses that occur throughout the drug development stage. 3-9. 1.4.4 Applications of LC/MS and Advances in Its Development for Use in Discovery Chemistry Accessing Compound Identity and Purity

The mission of the medicinal chemist is to create drug-like compounds that have a high affinity and activity for the purpose of testing in the clinic. This is accomplished by constructing a comprehensive knowledge of the structure–activity and structure–property correlations (SAR, SPR) via an iterative process of molecular design, synthesis, and hypothesis testing (regarding either the activity or the characteristics of the molecules). The objective of the analytical scientist is to put into place LC/MS systems that will provide the utmost capabilities for labs that are under pressure to meet throughput requirements. The LC/MS open access service has become the standard approach to monitor the progress of synthetic reactions in real time and/or validate the identification and purity of compounds from structure–activity relationship investigations. The analysis is carried out by chemists with little training on sample submission. Purification of compounds may take anywhere from 25 to 50 percent of a medicinal chemist's time in the lab, thus optimising the purification process can take up to half of that.

A great number of pharmaceutical businesses have established labs for the purification of their products in order to construct a technological platform that combines various procedures, technologies, techniques, and data management systems. Centralised purification facilities that have experimental expertise in analytical and medicinal chemistry and a high degree of automation are becoming an increasingly potent line of attack for sustaining small-scale and large-scale achiral and chiral purification at various levels of throughput. The main

technique in this situation is liquid chromatography with UV and/or MS-guided collection, which is able to provide thousands of purified samples every week.

Drug Discovery Environment

The requirements and preferences of the discovery scientists have to be satisfied for the information obtained by pharmacological profiling to be of any value. Research into the creation of new drugs is becoming an increasingly difficult and complex area. The initial emphasis is always on tried and proven tactics, and in order for new strategies to be adopted, they need to first demonstrate their efficacy. In the process of designing a pharmaceutical profiling programme, it is essential to take into consideration the following four aspects of discovery research:

The primary emphasis is placed on ligands with a high affinity. The need for information in order to make sound judgements. The need for quickness. Both in terms of variety and quantity. It has been shown that the usage of high-performance liquid chromatography coupled with mass spectrometry (HPLC–MS) or tandem mass spectrometry (HPLC–MS–MS) is the preferred analytical approach for the majority of the tests that are used in the different phases of the discovery of novel drugs. The method of screening compound libraries is one way to describe the new drug development process. Once hits are identified, they are chosen and tweaked to become leads, which are then optimised until a molecule is found that has the potential to be further developed into a drug candidate. For the purpose of analysing freshly synthesised compounds before they are added to a chemical library, HPLC–MS and HPLC–MS–MS are useful analytical tools. The purpose of these assays is to determine whether or not the proper chemical was synthesised and whether or not the purity level is high enough to be utilised in the library. In the second step, different physical and chemical characteristics of these novel chemical entities (NCEs), such as physiological solubility, permeability, and chemical stability, are evaluated. HPLC–MS is often employed for these tests since it is able to provide accurate results. Additionally, there is a battery of drug metabolism and pharmacokinetics (DMPK) tests that are carried out as a component of new drug discovery.

These tests measure the absorption, distribution, metabolism, and excretion (ADME) properties of the new chemical entity (NCE), in addition to the pharmacokinetic (PK) parameters of the molecule. For the most part, the measurement step in these experiments is performed using HPLC–MS or HPLC–MS–MS. This review will offer an overview of the different ways in which LC–MS (which will be used as a term that incorporates both HPLC–MS and HPLC–MS–MS) may be used in the process of discovering novel drugs. Specifically, this overview will focus on HPLC–MS. This study will also offer an introduction into the numerous kinds of mass spectrometers that may be chosen for the multiple jobs that can be conducted utilising LC–MS as the analytical instrument.

Fundamentals Of LC–MS Analysis

The auto sampler, the high-performance liquid chromatography (HPLC) system, the ionisation source (which interfaces the LC to the MS), and the mass spectrometer are the components that make up an LC–MS system. In an ideal scenario, each of these components would be managed by its own individual computer system. Due to the fact that HPLC is such a common method, it will not be detailed more here. It is important to remember that in order to interface HPLC with MS, there are certain limitations placed on the flow rate as well as the mobile phases that may be used. A typical example of phase inversion As the mobile phase, HPLC systems

that are coupled to MS would use a mixture of water and either methanol or acetonitrile as their solvent of choice. The mobile phase modifiers are subject to certain constraints, such as the need that they be volatile in the vast majority of instances. To enhance the chromatography of the analytes of interest, mobile phase modifiers are compounds that are added to the mobile phase. Their primary purpose is to do so. Ammonium acetate, acetic acid, and formic acid are some examples of the kinds of mobile phase modifiers that are often used. There are a number of studies that centre their attention on the HPLC parameters that are significant in LC–MS analyses. Between the high-performance liquid chromatography (HPLC) eluant and the mass spectrometer, there are many different kinds of ionisation sources that may serve as the interface. Electrospray ionisation (ESI) and atmospheric pressure chemical ionisation (APCI) are the two kinds of sources that are considered to be the most universal. Both of these source types are currently considered to be standard equipment on mass spectrometers that are used for LC–MS applications.

Electrospinning Technology

Electrospinning has been acknowledged as an effective method for the production of polymer nanofibers in recent years. In recent years, electrospinning has been used to effectively transform a variety of polymers into ultrafine fibres. Nanofibers can only be created from a viscoelastic material since only such a material can withstand significant deformations and yet have sufficient cohesion to withstand the pressures that are generated during the pulling process. Electrospinning may be traced back more than seven decades earlier to the release of a series of patents that describe an experimental configuration for manufacturing polymer filaments using an electric force. These patents describe an experimental setup for electrospinning. In the year 1952, Vonnegut and Neubauer were successful in producing streams of highly electrified droplets that were uniform and around 0.1 millimetres in diameter. They came up with a straightforward machine for producing electrical atomization.

In 1955, Drozin conducted research on the process of dispersing a variety of liquids into aerosols by applying high electric potentials to the system. He discovered that some liquids, when subjected to the appropriate circumstances, resulted in the liquid being expelled from the capillary in the form of a highly dispersed aerosol consisting of droplets of a size that was generally consistent. In addition to this, he caught several phases of the dispersion. In 1966, Simons received a patent for an equipment that could use electrospinning to manufacture non-woven textiles that were very thin and very light in weight, and which included a variety of designs.

He discovered that the fibres that formed in solutions with a low viscosity tended to be more elongated and delicate, while the fibres that formed in solutions with a higher viscosity were generally continuous. Baumgarten developed a device in 1971 that was capable of electrospinning acrylic fibres with diameters ranging from 0.05-1.10 micrometres. Adjusting the flow rate of an infusion pump allowed for the droplet to be kept at a fixed size as it rotated freely while being supported by a capillary tube made of stainless steel. While a high voltage was delivered to the capillary tube, the fibres were collected on a metal screen that was connected to the ground. In recent years, the electrospinning technique has acquired increasing attention due to the increased interest in nanotechnology. This is due to the fact that ultrafine fibres or fibrous structures of different polymers can be readily manufactured using this process. The diameters of these structures may be as small as submicrons or nanometers. The electrospinning method is quite similar to the one that was described by Baumgarten. The electrospinning equipment consists of a high voltage power source, a syringe pump, and a grounded collector.

During the electrospinning process, a polymer solution is injected via a needle attached to a syringe that has been charged to a high voltage (usually 10-30 kV). The flow rate of the injection is precisely regulated. When the optimal voltage is reached, the electrostatic repulsion between the liquid droplet and the needle tip is strong enough to overcome the surface tension of the droplet, resulting in the formation of a Taylor cone. After this, a charged liquid jet is extended out from the Taylor Cone and travels towards the collector by a whipping process. Along the way, the solvent evaporates and the fibre elongates before being deposited on the collector.

To this day, nanomaterials have emerged as having good qualities for analytical chemistry applications. This is mostly owing to the fact that they have a significant surface area to volume ratio and that a broad array of chemical and morphological modification techniques are available. Electrospinning has emerged as the most adaptable approach for the manufacture of nanofibrous materials out of all the options now accessible. Electrospun nanofibers have found widespread use in analytical separation, either as sorbent material for solid phase extraction methods or as the material for membrane separation techniques. These nanofibers have been employed in both of these capacities.

Additionally, colorimetric analytical devices based on electrospun nanofiber were included into the system for the purpose of detection.159 In more recent times, the Olesik group has developed the use of electrospun nanofibers as the substrate for surface-assisted laser desorption/ionization in order to circumvent the drawbacks of the matrix that are inherent in the conventional MALDI process.160,161 Electrospun nanofibers have never before been used as substrates for matrix-free laser desorption/ionization mass spectrometry, but this is the first time that it has been done.

CONCLUSION

It is possible to create a significant amount of these nanofibers. Because of the even distribution of samples throughout the surface and the lack of interference from the background, this approach has a greater degree of repeatability than the conventional MALDI does. In particular, when it comes to the identification and analysis of extremely small molecules, this provides it an advantage over more common types of the MALDI approach. In order to evaluate how much of an effect the surface has on the laser desorption and ionization process, many nanoparticles with varied degrees of laser absorption power were encased in polymeric nanofibers. This was done so that the amount of the surface's influence could be evaluated. Different quantities of laser absorption power were applied to these nanoparticles.

REFERENCES

1. Beavis, R.C. Org. Mass Spectrom. 1992, 27, 653-659.
2. Hillenkamp, F., Karas, M. Int. J. Mass Spectrom. 2000, 200, 71-77.
3. Cotter, R.J. Time-of-Flight Mass Spectrometry: Instrumentation and Applications in Biological Research, American Chem. Soc., Washington, DC, 1997.
4. Siuzdak, G. The Expanding Role of Mass Spectrometry in Biotechnology (2nd), MCC Press, San Diego, 2006.
5. Montaudo, G., Lattimer, R.P. Mass Spectrometry of Polymers, CRC Press, Boca Raton, 2001.

6. Murgasova, R., Hercules, D.M. Int. J. Mass Spectrom. 2003, 226, 151-162.

7. Pozniak B.P., Cole R.B. J. Am. Soc. Mass Spectrom. 2007, 18, 737-748.

8. Fernández De La Mora J. Annu. Rev. Fluid Mech. 2007, 39, 217-243.

9. Cole, R.B. Electrospray and MALDI Mass Spectrometry: Fundamentals, Instrumentation, Practicalities, and Biological Applications (2nd). Wiley. 2010.

10. Banerjee, S., Mazumdar, S. Int. J. Anal. Chem. 2012, 2012, 1-40.

11. Smith, R.D., Bruce, J.E., Wu, Q., Lei, Q.P. Chem. Soc. Rev. 1997, 26, 191-202.

12. Gross, J.H. Mass spectrometry: a textbook. Springer Science & Business Media, 2006.

13. Dreisewerd, K., Schürenberg, M., Karas, M., Hillenkamp, F. Int. J. Mass Spectrom. Ion Proc. 1995, 141, 127-148.

14. Nordhoff, E., Ingendoh, A., Cramer, R., Overberg, A., Stahl, B., Karas, M., Hillenkamp, F., Crain, P.F. Rapid Commun. Mass Spectrom. 1992, 6, 771-776.

15. Berkenkamp, S., Menzel, C., Hillenkamp, F., Dreisewerd, K. J. Am. Soc. Mass