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INVESTIGATING THE LIMITS OF LIQUID CHROMATOGRAPHY AND MASS SPECTROMETRY IN PHARMACEUTICAL ANALYSIS

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ABSTRACT

In the pharmaceutical industry, the technique of combining high performance liquid chromatography (HPLC) and mass spectrometry (MS) is one that is not only extremely useful but also absolutely necessary at virtually every stage of the drug development process. This includes biological target discovery, biological assays for high throughput screening, the characterization of the physicochemical properties of drug candidates, and drug metabolism and pharmacokinetics. Thanks to continuing innovations and discoveries in the areas of column technologies, LC/MS interfaces, and instrumentation, the field of LC/MS is continually expanding and branching out into new sectors. This growth and branching out is taking place in a variety of different domains. This article compares and contrasts the most current breakthroughs in the area of LC/MS interface ionisation techniques, such as ESI, APCI, APPI, and MALDI, as well as revolutionary hardware and software of mass spectrometry equipment, and discusses these developments in the context of attempts to find new drugs and create new treatments.

Keywords: *Chromatography, Mass Spectrometry*

INTRODUCTION

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 powerful analytical technique 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 technique of liquid chromatography-mass spectrometry, also known as LC-MS, has become standard practise. Because the recently developed API-based methods result in only mild ionisation, they can be supplemented for structural elucidation studies 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 applicable for the analysis of biological molecules, and the utilisation of tandem mass spectrometry and stable isotope internal standards makes it possible to expand highly sensitive and accurate methods by performing some method optimization in order to minimise the effects of ion repression. Method validation is an essential step in the drug development process, which comes between drug discovery and development.

As a result of today's global marketplace and the widespread production and distribution of food across the globe, consumers, governments, and food producers are becoming increasingly concerned about the quality and safety of the food they consume. There is a need for more stringent rules, as well as more diligent monitoring of foods by all parties involved in the food industry (regulators, vendors, and manufacturers), in order to protect the health of consumers. Chemical contaminants in food have been defined as "any chemical that was not intentionally added to food but was present from many potential sources." Examples of chemical contaminants in food include residues left over from the application of pesticides and veterinary medicines, chemicals that entered the food chain from the environment, chemicals that were formed during the processing of food, natural toxins, and accidental contamination at point sources. Adulteration of food is another way that contaminants can make their way into the food chain (intentional contamination).

Since the advent of mass spectrometry (MS) in analytical chemistry, numerous modifications have been made in an effort to enhance its level of performance. Ionizing, scanning, focusing, fragmenting, and detecting chemical structures are just some of the many techniques that have been developed throughout the years. In the early days of mass spectrometry (MS), the ability to detect individual precursor–product ion pairs was the primary factor that enabled the identification of various structures. This was accomplished by fragmenting molecules into smaller building pieces. In modern times, it is also possible to identify analytes based on the extremely precise masses obtained by high-resolution mass spectrometry (HRMS). Recent advances have made it possible to conduct studies that are more sensitive, selective, robust, and reproducible. Because of the latest generation of mass spectrometers' improved accuracy and repeatability, it is now possible to use these instruments not only for the identification of analytes but also for the quantification of such analytes. In this manner, MS has developed into a value that is well-established in various scientific fields, such as the pharmaceutical, (bio)chemical, (bio)medical, biological, and environmental sciences.

It is a common misconception that a mass spectrometer is nothing more than an alternate method of detection to UV/VIS, flame ionisation, fluorescence, and electrochemical analysis. A mass spectrometer's detection method, which involves analysing particular mass-to-charge ratios, also lends itself well to its application as a stand-alone approach for the separation of substances. However, the use of a mass spectrometer on its own remains extremely difficult because of the complexity of samples in emerging fields such as environmental, toxicological, and biomarker discovery studies. These studies require highly sensitive and selective measurements to avoid interferences, so using a mass spectrometer on its own is extremely difficult.

Due to the fact that mass spectrometry is both sensitive and extremely selective in comparison to other chromatographic detectors, coupling it to chromatographic procedures has always been seen as desirable. The linking of mass spectrometry and gas chromatography (also known as GC-MS) was first accomplished in the 1950s, and commercially viable equipment didn't appear until the 1970s. It is now common for clinical biochemistry laboratories to be equipped with relatively affordable and dependable GC-MS systems. These systems are indispensable in a number of fields that require the analysis of complex mixtures and the unambiguous identification of substances, such as the screening of urine samples for inborn errors of metabolism or drugs. The linking of mass spectrometry and liquid chromatography, also known as LC-MS, was an apparent extension; yet, development in this area was hampered for a number of years due to the relative incompatibility of existing MS ion sources with a continuous liquid stream. There were a few different interfaces developed, but because they were difficult to work with and lacked reliability, adoption by clinical laboratories was extremely low. Fenn's invention of the electrospray ion source in the 1980s brought about a

shift in this predicament's dynamics. Electrospray sources were swiftly created by manufacturers, resulting in the rapid development of devices that had a significant impact 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 yet 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 instruments had significantly increased, making it possible for clinical biochemistry laboratories to take advantage 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. There are a number of additional clinical applications for LC-MS, and the technique has a wider range of potential applications than GC-MS does. This is due to the fact that a wider variety of biological molecules can be analysed with LC-MS, as well as the increased prevalence of LC separation techniques in clinical laboratories. High specificity and the ability to deal with complicated combinations are the primary advantages offered by LC-MS in comparison to LC equipped with conventional detectors. These advantages are virtually identical to those offered by GC-MS.

Chromatography

Chromatography is a separation process that utilises a stationary phase in addition to a mobile phase in order to separate individual compounds from a mixture. The term chromatography comes from the Greek words chroma, which means colour, and graphein, which means writing; therefore, the term chromatography literally translates to "colour writing." Chromatography was initially invented in 1903 by a Russian botanist by the name of Mikhail Tswett. He was able to separate coloured plant pigments by using a calcium carbonate column.

OBJECTIVES

1. The Study Limits of Liquid Chromatography.
2. The Study and Mass Spectrometry in Pharmaceutical Analysis.

Chromatography in its many forms

1. According to the characteristics of both the fixed and mobile phases

- Chromatography of gases and solids
- Chromatography of gases and liquids
- Liquid-liquid chromatography (paper partition chromatography, column chromatography)

2. According to the fundamentals of chromatographic methodology and the different types of separation techniques

- In the chromatographic technique known as adsorption, the mobile (gaseous or liquid) phase is adsorbed onto the surface of the stationary (solid) phase. The affinity of the chemical towards the stationary

phase is the determining factor in the separation. Elution will go more slowly for those compounds that have a higher affinity for the stationary phase, whereas elution will proceed more quickly for those compounds that have a lower affinity for the stationary phase.

- In the chromatographic technique known as partition, the separation of substances is accomplished by dividing a solute between two different solvents. In this kind of chromatography, an immiscible liquid stationary phase is adsorbed to the surface of a solid adsorbent. This allows the liquid stationary phase to be separated from the mobile phase. The chromatography is performed in the same manner as has been described for adsorption column chromatography. The foundation for separation is provided by the fact that the partitioning of components of the sample between the mobile phase (the sample itself) and the stationary phase (liquid supported on a solid support) delays the elution of some of the sample's components.

- Chromatography based on ion exchange: The ions that are present in the solution (mobile phase) and the ions that are contained inside the ion exchange resin are able to engage in a process of reversible ion exchange with one another (stationary solid phase). There are two other subcategories that may be used to this method: cationic exchange chromatography and anionic exchange chromatography.

- Chromatography based on the principle of molecular exclusion: Gel filtration or gel permeation are two other names for this process. On the basis of their respective sizes, proteins, peptides, and oligonucleotides may be distinguished using this technique. The column is densely populated with non-porous inert spheres (column media). When a mixture of molecules of varying sizes was passed down the column, the smaller molecules would enter the pores of the spheres that make up the column media. This caused the elution process to take longer for the smaller molecules. On the other hand, the bigger molecules will be eluted from the spheres more quickly because they are unable to penetrate the pores of the spheres.

- Affinity chromatography is the most selective approach, and it is used to separate antibodies, proteins, and enzymes from the biological matrix. This technique was developed in the 1960s. It is predicated on the biological interactions that take place between two different molecules, such as an enzyme and its substrate, a receptor and its ligand, or an antibody and its antigen. Only the protein that is unique to its particular ligand in the stationary phase will bind to the protein in the mobile phase when the mobile phase, which contains a combination of proteins, antibodies, and enzymes, is passed through the stationary phase. Altering the ionic strength or pH of the solution will allow for the protein to be retrieved at a later time.

- Chiral chromatography is a kind of chromatography that is used to separate optical isomers of molecules, such as the levo and dextro forms.

3. Derived from many distinct types of analytical procedures

- Capillary electrophoresis
- Chromatography with conventional detectors
- Gas chromatography (GC)
- Liquid chromatography

- Capillary electrophoresis
- Chromatography with conventional detectors
- Gas chromatography (GC)
- Liquid chromatography
- Super critical fluid chromatography
- Hyphenated techniques (Mass-spectrometry)
- GC-MS and GC-MS/MS
- LC-MS and LC-MS/MS
- Supercritical Fluid Chromatography-MS (SFC-MS), Capillary Zone Electrophoresis-MS
- Supercritical Fluid Chromatography-MS (SFC (CZE-MS)

Liquid chromatography

Chromatography is the technique of separating the constituent components of a mixture depending on how those components interact with the stationary phase and the mobile phase in different ways. The Russian botanist Mikhail Tswett is credited with the invention of chromatography. In his work, Tswett described the process of separating plant leaf pigments in solution by passing the solution through a column of solid adsorbents. Tswett's discovery led to the development of modern chromatography. The most common physical arrangement for chromatography is called column chromatography. In this technique, the stationary phase is packed into a column, and the mobile phase is pushed through the column. High performance liquid chromatography, often known as HPLC, is a kind of column chromatography that utilises liquid as the mobile phase. Since its debut, HPLC has gained widespread use in the field of analytical research. In recent years, high-performance liquid chromatography (HPLC) has emerged as an essential technology for the separation of biomolecules due to its rapid separation speed, high resolving power, and compatibility with mass spectrometry.

The high-performance liquid chromatography (HPLC) method begins with the fundamental step of forcing the liquid mobile phase, which contains the sample, to pass under high pressure through the column that is packed with the appropriate matrix. This is accomplished by the compounds having varying degrees of contact with the column matrix, which results in the compounds being separated according to the amount of time it takes for each compound to transit through the column. A appropriate detector, such as a UV detector or mass spectrometer, is used to detect the signals given off by the molecules after they have been separated. HPLC depends on the following characteristics to separate target analytes: charge, hydrophobicity, affinity, solubility, and molecule weight.

Depending on these characteristics, chromatographic separations may be broken down into one of five primary categories, namely:

(1) Adsorption chromatography is a technique that uses the adsorption and desorption processes as its foundation.

(2) Partition chromatography is a kind of chromatography that is based on the process of the analytes being partitioned between the mobile phase and the stationary phase.

(3) Ion-exchange chromatography is a kind of chromatography that relies on the transfer of ions between ionic groups on the surface of the column and ions present in the mobile phase. Charge, ion size, and polarisation are all factors that influence ionic interactions. Additionally, the pH has an impact on the separation.

(4) Size-exclusion chromatography, commonly known as gel permeation or gel filtration chromatography, is a kind of chromatography that sorts substances according to their molecular weight and size. Because of the disparity in size, some molecules are able to fit within the pores of the stationary phase, which results in their being retained, but other molecules are unable to do so, and they are allowed to travel through the column without being retained. In this instance, the pore size is essential to the effective separation of the components.

(5) Affinity chromatography: in this form of separation, analytes attach to a ligand that is bonded to the stationary phase. The analytes are then eluted by the use of a chaotropic agent, through a change in pH, or through the use of a particular eluent.

Utilization of RPC in the process of separating proteins and peptides

RPC is a potent method for the investigation of peptides and proteins because it may produce great resolution under a broad variety of chromatographic settings. This makes it one of the most often used chromatographic techniques. Changing the characteristics and composition of the mobile phase allows for simple and straightforward manipulation of the chromatographic selectivity when using the RPC separation mode. In the purification of peptides and proteins on a laboratory scale, RPC is a technique that is used extensively. On the other hand, RPC has the potential to result in the permanent denaturation of protein samples, which decreases the likelihood of sample recovery in a biologically active state. For this reason, RPC is most often used for the separation of proteins and peptides, as well as quality control analysis, in situations when the recovery of functionally active protein is not necessary.

The pharmaceutical industry makes frequent use of RPC for the purpose of separating medicinal proteins and conducting analyses on those proteins. The octadecylsilane (C18) column is the one that is used in the analysis the majority of the time; however, the octyl silane (C8) column and the butyl silane (C4) column are also utilised. Before doing mass spectrometric analysis, Wang et al. showed that nano-RPLC is an essential approach for the single and multidimensional protein separation of complicated protein mixtures. RPC is also used in the process of analysing protein alterations such as deamidation and oxidation, both of which are regarded as protein impurities due to the fact that they oftenresult in the loss of the biological function that the protein has. Soreghan et al. presented a technique that identifies carboxylated proteins in aged mouse brain homogenates using reversed phase liquid chromatography and tandem mass spectrometry. The authors also documented the effects of a number of other chromatographic settings, such as the length of the alkyl chain present in the stationary phase, the temperature, and the ion-pairing agent, on the separation of proteins. When

proteins are digested by enzymes like trypsin or others, the resulting protein fragments typically contain about ten amino acids apiece. These protein fragments may also be separated via RPC, which results in the acquisition of valuable information about the protein sample. Peptide mapping is a technique that is often used in the process of determining which proteins are biotherapeutic. The glycosylation pattern of proteins may be determined by the use of RPC in conjunction with MS for the analysis of fragments produced by tryptic digestion. RPC has seen widespread use in bottom-up proteomics for the study of peptides, since it gives great resolution for peptides and has been used for this purpose.

Developments In Technology That Have Been Incorporated into Instruments

mass analyzers

UV detection is still an essential component of pharmaceutical research due to the fact that it is not just a method that is economical but also one that is very easily accessible, trustworthy, and easy. However, molecules that do not possess chromophores are unable to be detected, and the poor specificity of the approach is a serious restriction when taking into account intricate matrices. Because of this, MS became a significant force in the pharmaceutical sciences, which led to an exponential development in the number of publications in the field of analytical chemistry. As a result of this expansion, MS is now considered one of the most prestigious academic institutions in the world. As a consequence of this, the functionality and operation of the many distinct types of mass spectrometers that are presently available on the market have already been discussed in the appropriate scholarly literature in a significant amount of depth. One may find it beneficial to consult one of a number of in-depth assessments that give a side-by-side comparison of the benefits and drawbacks that are associated with the usage of specific instruments before choosing on a piece of equipment with which to carry out certain experiments.

These in-depth analyses may be found in a variety of different places online. It should be made clear that each mass spectrometer comes with its own unique set of advantages and disadvantages; as a result, it is difficult to evaluate the capabilities of many instruments side by side. When measured against the findings obtained using (U)HPLC-UV, the findings obtained through MS do not present a consistent picture. Instead, the results produced with MS are dependent on the equipment that was used to do the analysis, which means that the same sample might provide a variety of conclusions when analyzed by other types of mass spectrometers.

Because of this, the process of transferring a particular method from one laboratory to another is a difficult one that requires an intensive validation before it can be employed. Therefore, it is of the highest importance that a description be given of the many types of ionization and mass spectrometers that are now available. In addition, the specific form of research that will be carried out need to play a big influence in deciding the kind of mass spectrometer that will be utilized, and this ought to be the case regardless of whether or not the inquiry will be carried out at all. There is no use in drawing the comparison between a triple quadrupole (QQQ) instrument, which has a high scan rate and poor resolution, and a time-of-flight (TOF) instrument, which has the opposite specifications, due to the fact that they are often utilized for investigating different subjects. Because these techniques are so complementary to one another, the best use of them would be to employ both of them at the same time or to switch between the two strategies at regular intervals.

In spite of this, it is common practice to evaluate the capabilities of a number of different types of mass spectrometers in order to determine the conditions that are most suited for the analysis of certain substances.

For example, Viaene et al. showed that the best performing instrument in terms of selectivity, MEs, repeatability, accuracy, and sensitivity is depending on the molecule and even the concentration. They achieved this by contrasting the quantities of opioid medicines found in human plasma using equipment of the QQQ and QTOF varieties. Both a QQQ and a QTOF equipment were utilized in the research that Viaene and colleagues conducted. Surprisingly, the QTOF demonstrated a higher degree of sensitivity than the QQQ did for the majority of the pharmacological compounds that were examined during this inquiry. This was the case regardless of the kind of molecule that was being examined. The authors stated that this was mostly owing to the selection of the ions that were chosen for the aim of carrying out quantitative analysis. Opioids were detected using their precursor ions in the QTOF experiment, while the QQQ measurements required the opioids' product ions, which are in far lesser supply

Because of the fact that a more recent QTOF was compared with a QQQ from an earlier generation, it is feasible that the upgrades that have been made to this new instrument may be to blame for the enhanced sensitivity of the QTOF. This is because of the fact that the QTOF was compared with a QQQ from an earlier generation. As a result of the high cost of a mass spectrometer, many research institutes do not have the financial resources necessary to buy a range of pieces of equipment that are regarded as being at the cutting edge of their respective fields.

Additionally, if it is at all feasible to minimize the trouble of employing multiple pieces of equipment for the analysis of a single sample, it is in everyone's best interest to do so. Therefore, as a result of the recent development of hybrid devices that combine the benefits of numerous separate instruments, it is now feasible for researchers to make use of the same instrument for a range of different purposes. The QTrap and the QTOF are two examples that stand out as particularly notable in this regard. Both of these apparatuses are in fact triple quadrupoles; however, the third quadrupole in each of them has been replaced with either a linear ion trap or a TOF tube. As a direct result of this, the high speed and sensitivity of a conventional QQQ are connected, respectively, with either a higher resolution in the case of a QTOF or with MSncapability in the case of a QTrap.

When comparing these two instruments, it is essential to keep in mind that both of these devices are extremely good at recognizing and measuring the target chemical. This is something that needs to be kept in mind at all times. As a result, the QTOF may be utilized in HRMS; however, the duty cycle is limited because of its considerably slower scan speed, which is required when a large number of analytes have to be measured. Despite the fact that orbitrap mass analyzers have a slower acquisition speed than other types of mass analyzers, their use in the pharmaceutical industry is rising as a result of the extraordinarily high resolution that they give.

Clarifying proteome and metabolomic processes is the primary use for the resolving power of up to 500 000 full width at half maximum (FWHM) and an isotopic fidelity of up to 240 000 FWHM at m/z 200, both of which may be obtained with the most current version. A recent article written by Lin and colleagues gives a condensed overview of the several types of hybrid HRMS as well as the characteristics that are shared by all of them. Although it appears that the major focus of manufacturers is on the creation of instruments that are more accurate, speedier, and more durable and that have the maximum possible sensitivity and resolving power, there is also a tendency toward miniaturization (electronic supplementary material, figure S1).

There are already portable devices available on the market that can be utilized in the field. Despite the fact that miniaturized mass analyzers have lower sensitivity and resolving power due to the shorter residence time in these devices, there are currently mass analyzers that can be used in the field. The reduced sensitivity, on the other hand, is a drawback that cannot be solved for the vast majority of applications. Another key obstacle consists in locating an appropriate vacuum cleaner, which is necessary in order to gather measurements that are accurate, sensitive, and reproducible in nature. The precision of the measurements that can be achieved with these portable set-ups is also significantly influenced by the presence of changing environmental factors such as temperature and humidity. This is in contrast to the controlled environment of a laboratory, where measurements can be made with absolute certainty.

When discussing TOF tubes, the relevance of this point cannot be overstated. Despite this, portable technology represents a substantial improvement that warrants further effort and consideration from the relevant parties. They have a bright future ahead of them once improvements are made and new techniques are developed to solve the common problems that are now occurring. These miniaturized devices will, for example, have a significant potential in the field of clinical diagnostics, which requires methods that are both rapid and uncomplicated in order to carry out analysis on-site. These miniaturized devices will have both of these characteristics.

Techniques employing ionisation

Both the resolving power and the speed of the instrument are directly related to the kind of mass spectrometer and, more specifically, the type of mass analyzer that is utilized. The utilization of HRMS or conducting operations in the multiple reaction monitoring (MRM) mode have the potential to significantly cut down on the level of background noise that is visible in a spectrum. This will have a positive impact on the signal-to-noise ratio, which almost always leads to an increase in sensitivity. In spite of this, sensitivity is nonetheless particularly linked to the improved formation and directing of analyte production.

During the ionization process, only a certain fraction of the analytes are transformed into ions, and only a certain portion of those ions will finally make it to the mass analyzer. This is because only a certain portion of the analytes are turned into ions. In order to carry out a quantification in MS investigations that can be relied upon, it is of the greatest necessary that there be no variance in the percentage of ionised analytes throughout the various analytical runs that are done. This is because any variation in this proportion might throw off the results of the investigation. Depending on the nature of the research being done and the samples being collected, a certain type of ionization source will be used. The utilization of technologies that include ambient ionisation, which make it possible to do direct sample analysis with minimal or no sample pre-treatment at all, is a recent development that has lately acquired traction in the scientific community.

The fundamental advantage of using this approach is that it allows the measurements to be carried out with relative simplicity and rapidity. Ions are able to be generated in an uncomplicated manner from gaseous, liquid, or solid elements when they are exposed to air. In their respective studies, Huang et al., Ding & Duan, and Monge et al. offer a comprehensive study of ambient (plasma-based) ionization techniques, including desorption electrospray ionisation (DESI), direct sample analysis in real time (DART), and paper spray ionisation. These methods are utilized in the process of doing real-time analysis on samples. In addition to the mechanisms that are responsible for the operation of the system, they outline a variety of applications that are capable of making use of these techniques.

In spite of the fact that ambient ionisation mass spectrometry has the potential to be utilized quantitatively for point-of-care diagnostics, there are still issues with sensitivity and, more significantly, consistency when compared to chromatography-based mass spectrometry. In pharmaceutical analysis, ionization methods such as air pressure chemical ionization (APCI), atmospheric pressure photo-ionization (APPI), and in particular electrospray ionization (ESI) are hence still the methods of choice. When hyphenated with LC, these kinds of ionization have the potential to rapidly atomize the mobile phase, even under conditions in which the mobile phase is highly watery and at high flow rates. Even while it is theoretically feasible for soft ionization processes to generate multiple charged ions, the vast majority of the time they only generate singly charged ions instead.

CONCLUSION

This is made possible by the fact that electro spun nanofibers may be produced and used in this manner. This is conceivable due to the fact that the technique known as electro spinning enables the production of electro spun nanofibers. Nanofibers are very malleable and may be manipulated with relative ease. These nanofibers have a great degree of elasticity. Because of this, it is possible to conduct studies on both categories of chemicals at the same time if one were to make the necessary preparations. This is the first time that the desorption mechanism of the nanofiber-assisted laser desorption/ionization process has been explored in great depth. To the best of our knowledge, this is the first time that this has ever happened. In addition, a chemical thermometer was used in order to study both the efficiency of ion desorption and the transmission of internal energy. This study showed that it is beneficial to use liquids with a greater fluidity as the mobile phase when performing reversed phase separation on digested peptides and mutant proteins.

REFERENCES

1. Yamashita, M., Fenn, J.B. J. Phys. Chem. 1984, 88, 4451-4459.
2. Karas, M., Bachmann, D., Bahr, U., Hillenkamp, F. Int. J. Mass Spectrom. Ion Proc. 1987, 78, 53-68.
3. Fenner, N.C., Daly, N.R. Rev. Sci. Instrum. 1966, 37, 1068-1070.
4. Vastola, F.J., Pirone, A.J. Adv. Mass Spectrom. 1968, 4, 107-111.
5. Vastola, F.J., Mumma, R.O., Pirone, A.J. Org. Mass Spectrom. 1970, 3, 101-104.
6. Meuzelaar, H.L.C., Ten Noever de Brauw, M.C. Anal. Chem. 1978, 50, 985-991.
7. Wilkins, C.L., Weil, D.A., Yang, C.L.C., Ijames, C.F. Anal. Chem. 1985, 57, 520-524.
8. Coates, M.L., Wilkins, C.L. Biomed. Mass Spectrom. 1985, 12, 424-428.
9. Tanaka, K., Waki, H., Ido, Y., Akita, S., Yoshida, Y., Yhoshida, T. Rapid Commun. Mass Spectrom. 1988, 2, 151-153.
10. Tanaka, K. Angew. Chem. Int. Ed. 2003, 42, 3861-3870.
11. Karas, M., Bachmann, D., Hillenkamp, F. Anal. Chem. 1985, 57, 2935-2939.

12. Karas, M., Bachmann, D., Bahr, U., Hillenkamp, F. Int. J. Mass Spectrom. Ion Proc. 1987, 78, 53-68.

13. Karas, M., Hillenkamp, F. Anal. Chem. 1988, 60, 2299-2301.

14. Karas, M., Bahr, U., Ingendoh, A., Hillenkamp, F. Angew. Chem. 1989, 101, 805-806.

15. Karas, M., Ingendoh, A., Bahr, U., Hillenkamp, F. Biomed. Environ. Mass Spectrom. 1989, 18, 841-843.