We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Mass Spectrometry Coupled with Chromatography toward Separation and Identification of Organic Mixtures

Asmae Bouziani and Mohamed Yahya

Abstract

Mass spectrometers can provide information about molecular composition and chemical structure. However, with complex mixtures, superpositions and even suppression of signals may occur. On the other hand, Chromatography is an ideal technique for separating complexes but is often insufficient for compound identification. Hence, coupling both techniques in order to eliminate the limitations of each technique makes perfect sense. In this contribution, a brief description of mass spectrometry coupled with chromatography in the gas and liquid phase will be discussed to explain the advantages of coupling the two methods. The ionization techniques are also reported and followed by application areas of these techniques. Finally, the recording and treatment of the results are reviewed.

Keywords: Gas Chromatography, Liquid chromatography, Ionization, EI, CI, ESI, APCI, MALDI

1. Introduction

One of the shortcomings of mass spectroscopy (MS) is the identification of a complex mixture. However, to overcome this limitation, MS could be coupled with a separation technique such as liquid chromatography (LC) or gas chromatography (GC). The sample injected into the MS ought to be separated first. The injected samples could be in the liquid phase for LC/MS or the gas phase for GC/MS. The injection of the sample into MS could be done in two ways: either the sample is collected and then analyzed off-line, or the MS is linked to the chromatograph, and the mass spectrum is obtained as the mixture is eluted [1–4]. Though the primary benefit of the separation technique coupling with MS is the obtention of a spectrum that allows identifying the separated product, it is not the only advantage that may be attained. The detector must display the following properties:

- The products separated before the detector need to stay separated, meaning that the detector does not interfere with the chromatographic resolution.
- Highly sensitive.
- Can detect all product eluted.

- Provide enough information about the structure to be able to identify the compounds eluted.
- Selectivity: allows the identification of a specific product in the mixture.
- The output signal must be proportional to the concentration.
- The response factor must be constant or at least foreseeable.
- The performance/cost ratio must be as small as possible.
- Do not damage the product.
- The deconvolution of chromatographic peaks needs to be possible.

The last parameter is important because of the possibility that one chromatographic peak may correspond to two products.

In this contribution, the MS coupling with GC and LC will be discussed, focusing on the ionization techniques used for the coupling. The most important application of the GC–MS and LC–MS are also given in brief. Finally, the recording and treatment of the outcome are reviewed.

2. Mass spectrometry coupled with gas chromatography

A complex mixture can be separated via GC, and MS can identify these compounds. Hence combining these two techniques can be advantageous. Moreover, GC and MS can both run in the gas phase making the linking straightforward, the performance stable, and good reproducibility.

The GC separates and introduces molecules into the MS via direct injection or after heating. The separation depends on the difference of the thermodynamic properties (boiling points and selective absorption in the stationary phase) and the difference in the distribution in the stationary phase and the mobile phase (carrier gas). In this case, MS acts as a detector, which includes an ionization source, mass analyzer, and electron multiplier tubes. First, the analyzed molecules are injected into MS via GC, and the ionization source ionizes them into gaseous ions, then they enter into the mass analyzer. The separation of ions occurs based on the variance of the mass-to-charge ratios, and then the separated ions reach the electron multiplier, which produces an electrical signal and giving a 3D output of the analyzed molecules. A schematic figure of the main parts of GC–MS is given in **Figure 1**.

Figure 1.
The schematic of the components of GC–MS.

2.1 Coupling GC with MS

2.1.1 Open coupling

In the open-coupling system, the chromatographic column is connected to the MS with a T-shaped tube encompassing a smaller diameter tube (**Figure 2**). A deactivated fused silica or platinum capillary also leads to this tube and goes into the source of the MS. In order to evade condensation, the capillary needs to be kept under a vacuum and heated. The pressure inside the T-shaped tube must be equal to the atmospheric pressure, so the tube is closed at the edges but not sealed. The oxygen can oxidize the eluted molecules; in order to avoid that, helium is used. The diameter of the tube that enters the MS is essential. It needs to supply an adequate flow with regards to the gaseous conductance and pumping capacity. Hence the diameter of the capillary is 0.15 mm, and the length is 50 cm heated to 250°C will carry 2.5 ml/min of the eluted gas into the source. In practice, this is enough to pump everything coming out of a capillary column. The eluted molecules are not enhanced in an open-coupling system. The experiment is carried under the typical chromatographic environments, with one end of the column is under atmospheric pressure. The advantages of this system are the easiness of the column changing and the simplicity of the settings (no unique settings are needed). This system is generally used when no enhancement is required.

2.1.2 Direct coupling

In this system, the capillary column enters the spectrometer source directly through a set of vacuum-sealed connections. No pumping is needed since the capillary is essentially very lengthy. The column inside diameter of 0.25 mm with a length of 15 m minimum is needed (**Figure 3**). The major downside of this system is not permitting the solvent's removal, and the column change is complex. When the column is sufficiently long, the chromatography is conducted between an atmospheric and vacuum at the opening and the other end of the column, respectively.

2.2 The ionization techniques

The ionization techniques such as electron impact (EI), Chemical ionization (CI), and field ionization (FI) have been accessible for several years, which makes the GC–MS the oldest coupling technique [5]. The ionization occurs inside the

Figure 2.
 Schematic representation of an open-coupling system.

Figure 3.
 Schematic representation of a direct coupling system.

instruments for the three mentioned techniques, namely in the high vacuum of the MS. For the hydrophobic and small hydrophobized molecules to be analyzed with GC–MS, the MS must vaporize undecomposed analytes [6]. The gas is led into the ionization chamber of MS via the outlet of the GC separating capillary. The MS must be kept under a vacuum because of the capillary columns, which operate at low flow rates; for this reason, the carrier gas that emerges from the GC column into the chamber of ionization needs to be pumped out.

2.2.1 Electron impact ionization

A hot-cathode discharges electrons through the EI, resulting in an electron beam-forming at the ionization chamber between the glowing cathode and the capture anode (**Figure 4**). Once the molecules go through the electron beam, an electron is bumped out of the molecule's surface, which gives a radical cation. The obtained ions at an electron energy of 70 eV are unstable and deteriorate rapidly, generating characteristic fragments that are automatically identified through the spectrum libraries. The GC–MS allows easy and reliable identification as well as the quantification of the molecules existing in the user database. Currently, NSIT 20 Mass Spectral Library has 350.643 carefully evaluated spectra.

2.2.2 Chemical ionization

The CI is similar to the EI, except that the reactant gas molecules are ionized and not the analyte molecules (**Figure 5**). Ammonia, methane, or isobutane may be used as a reactant gas. The charge transfer due to the deprotonation (negative ion mode) or protonation (positive ion mode) occurs between the analyte molecules and the ionized reactant gas. The negative CI is particularly very sensitive. The detection of a quantity of octafluoronaphthalene corresponding to 200,000 molecules was successfully reached in 1992 when McLafferty and Michnowicz used negative CI [7]. The CI generates fewer fragment ions contrary to EI.

2.2.3 Field ionization

The FI almost does not generate any fragments. A high voltage is applied to a carbon-activated metal fiber in the source chamber. The excavating of separate

Figure 4.
Schematic representation of EI source.

Figure 5.
 Schematic representation of CI source.

electrons from the analyte molecules occurs due to the high field strengths that form at the tips of the branches of the carbon dendrites [8, 9]. The FI is a less sensitive ionization technique compared to the EI and CI (**Figure 6**).

2.3 Domaine of GC: MS applications

Coupling GC with MS opened the door to several applications [10]. In this contribution, we will limit to the most important ones.

- **Environmental monitoring:** The major application of GC–MS is monitoring environmental pollutants. Dibenzofurans, herbicides, dioxins, phenols, sulfur, and chlorophenol are all detected via GC–MS in air, soil, and water.
- **Medicine:** the detection of numerous congenital metabolic diseases is possible due to GC–MS usage for the screening tests. If the subject has a genetic metabolic disorder, a specific compound is detected in the urine.
- **Food:** GC–MS can analyze aromatic compounds present in food or beverages, including ester, alcohols, and fatty acids. It is mainly used to detect contamination or spoilage. Oils, perfumes, and essential oils also can be analyzed.

Figure 6.
 Schematic representation of FI source.

- Forensic: especially forensic toxicology, GC–MS finds a wide application identifying poisons and steroids (anabolic steroids) in biological samples and anti-doping labs.
- Pharmaceutical: The GC–MS is used primarily for the identification of impurities in the active pharmaceutical ingredients. Furthermore, in the field of medicinal chemistry, it can be used to characterize the synthesized compounds.
- Biological: Narcotics, alcohols, and drugs can be detected in the body fluid via GC–MS. Moreover, it allows the detection of pollutants and metabolites in serum.
- Geochemical research: GC–MS finds a vital application in geochemical research because of the structured mass spectral peaks and low volatile sample analyzability. The atmosphere of Venus was analyzed using GC–MS.
- Chemical war: The detection of chemical warfare agents in public places is performed using GC–MS.
- Industrial: Aromatic solvent and inorganic gases can be analyzed via GC–MS to detect impurities in cosmetics.

3. Mass spectrometry coupled with liquid chromatography

High-performance liquid chromatography (HPLC) is an innovative type of LC used in various fields, including food analysis and pharmaceuticals. It is primarily beneficial for low or non-volatile organic compounds that are not suitable for GC. The main difference between HPLC and LC is the solvent's mobility. In the case of LC, the solvent moves by force of gravity, while in HPLC, it moves under high pressure obtained through pumps. The use of the pumps ensures the overcome of the pressure drop in the column and reducing the separation time. The combined technique between MS and HPLC is generally identified as LC–MS (**Figure 7**).

LC coupling with MS is more complicated than with GC because of the need to generate gas-phase ions for the MS. Furthermore, the necessity to eliminate the elution solvent is another downside of LC–MS. In the case of water, if the column used has a small diameter permitting a maximum flow rate of 0.1 ml min^{-1} , which is equal to 0.1 g min^{-1} of water, generating a flow rate of $135 \text{ cm}^3 \text{ min}^{-1}$ of gas at atmospheric pressure. This flow is too high to be injected under a vacuum into a source. In order to overcome this downside, numerous methods are used [11–13].

Figure 7.
Schematic representation of LC–MS.

3.1 Ionization and ions source

The coupling of HPLC and MS became possible with the installation of electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) in the commercial apparatus [14, 15].

3.1.1 Electrospray ionization

Electrospray ionization (ESI) consists of pressing the analytes present in the solution through a capillary. The charged droplets form when a high voltage is applied (between 1.5 and 5 kV) [16]. The charge density improves with the elimination of solvents from the droplets via continuous evaporation. In addition, the surface area increases due to splitting droplets into smaller droplets at a specific charge density (Coulomb explosion). At the end of this process, the remaining microdroplets emit single ions, or the droplets only contain single solvated ions that will be entirely desolvated upon further drying [17–19]. The transfer of the ions into the high vacuum of the MS is carried out via a capillary or small hole in the front plate through electric fields (**Figure 8**).

ESI is applicable for various compounds such as proteins and peptides, oligosaccharides, bio-organic molecules, polymers, and non-covalent complexes.

3.1.2 Atmospheric pressure chemical ionization

APCI has attracted considerable attention due to its ability to produce ions from solution and analyzing rather nonpolar compounds. Like electrospray, the liquid analyte is directly injected into the ionization chamber via an APCI probe (**Figure 9**). The analyte solution is submitted to a nebulization to produce fine droplets of aerosol spray, which will undergo rapid heating in the nitrogen stream and then emerge at the end of the probe as a stream of a vaporized analyte. In the area of the corona discharge needle, the reagent ions are formed. The analyte molecules react with these ions and form protonated or deprotonated analyte ions that are singly charged [20, 21].

Generally, the transfer of proton happens in the positive mode to generate $[A+H]^+$ ions. However, the negative mode may also occur, and the M^- and $[A-H]^-$ are formed from electron transfer or proton loss, respectively. During ionization, the solvent clusters and high gas pressure influence the reagent ions resulting in reduced fragmentation and intact quasi-molecular ions. The process is considered more energetic than ESI, which results in the absence of multiple charging [22].

3.1.3 Matrix-assisted laser desorption ionization

The matrix-assisted laser desorption ionization (MALDI) is another ionization technique, which permits high molecular weight molecules injection into the

Figure 8.
Schematic representation of ESI.

Figure 9.
Schematic representation of APCI.

gas phase as intact ions. MALDI technique gives desorbed analyte with a relative mass of 300KDa. In MALDI, the analytes are crystallized using an excess matrix compound (DBA, Sinapic acid, etc). Then the crystallized analyte is carried into the high vacuum of the MS and irradiated via laser. Finally, the analyte molecules are carried into the gas phase after the matrix evaporated the absorbed laser energy (**Figure 10**). The transfer of protons between the matrix and analyte molecules is responsible for ionization [23–25]. The downside of this technique is the connection to the chromatography, which needs to be indirect either manually or through robotics. Currently, MALDI is limited to scanning applications where a matrix sprayed sample is scanned in two-dimension via a laser beam to get a mass distribution to produce false-color images [26, 27].

No fragmentations due to ionization are obtained when ESI, APACI, and MALDI are used, hence the “soft” reference. Furthermore, because of their covered polarity and molecular weight array, ESI and MALDI are perfect for bio-molecules analysis.

Figure 10.
Schematic representation of MALDI.

ESI and MALDI, in particular, are ideal for bioanalytics (proteins, peptides, etc.) due to their covered polarity and molecular weight range.

3.2 LC: MS domaine of applications

The LC–MS found application in numerous fields. In this section, the most crucial area will be discussed.

- Forensic: LC–MS could be used to determine toxicity in food and beverages, also in drug analysis. The LC–MS can detect trace amounts of toxins in numerous materials [28].
- Doping: LC/ESI-MS in negative mode can detect doping agents such as 4-Methyl-2-hexanamine in the urine [29, 30].
- Environmental: Phenyl urea-based herbicides are detected via LC–MS as well as trace amounts of carbaryl in food [31].
- Pharmacology: LC–MS is used to quantify and elucidate the structure of drugs in biological samples (urine, saliva, plasma, etc). It can also be used for the study of the metabolism of drugs [30].

4. Outcomes recording and treatment

Regardless of GC–MS or LC–MS, an online data system is present, containing an acquisition processor, a magnetic recorder, and a computer.

4.1 Outcomes recording

As a function of time, the spectrometer offers two series of outputs: the number of ions detected and, at the same time, the mass of these ions is given. The mass of each ion emerges with a particular distribution over some time, as displayed in **Figure 11**. Thus, the number of ions detected can be computed from the area under the curve, whereas the centroid of the peak displays the ion's mass. The mass determination is effectuated via the acquisition processor, where the signal related to the number of ions accumulates quickly.

For instance, in 1 s, a spectrometer covers 500 mass, which means in 2 ms 1 mass. For this period, eight measurements of the number of ions ought to be conducted, meaning 0.25 ms assigned for each sample. In other words, 4000 samples ought to be measured per second, and the frequency of the sampling is 4 kHz. The ions detector's current goes through a resistance 4000 times a second, and at the end of the resistance, the acquisition processor is responsible for reading the potential difference relative to the number of ions detected and then digitalize it. The obtained output value corresponds to the y axis of the mass spectrum. The x-axis value corresponds to the reading of the mass indicator. The bar graph is the result of an algorithm that permits the processor to define the limits of the peak and centroid. The number of ions corresponds to the sum of the values read within these limits, whereas the ion's mass corresponds to the interruption of the indicator value at the centroid. A representative obtained bar graph is given in **Figure 12**.

In the case of a broader mass range scanning or high-resolution usage, increasing the sampling speed is needed, increasing the data points per unit time.

Figure 11.
Schema of a chromatogram.

Figure 12.
MS spectra.

One of the essential characteristics of the data acquisition process is the dynamic range, connected in part to the signal digitization possibilities. For instance, an ion detector may identify one to a million ions that reach the detector at once.

Its dynamic range, the largest to the lowest measurable signal ratio, is equivalent to 10^6 . In an ADC with 16 bits, the numerical obtained values are between 1 and 2^{16} . Therefore the dynamic range is considerably lower than that of the detector. However, this problem can be overcome by reading different value ranges consecutively.

4.2 Instrument monitor and treatment of outcomes

The subsequent operations are possible because of the newly available programs.

4.2.1 Outcomes acquisition management

The operators can select numerous parameters such as the scan mode or the selected-ion monitoring mode, the array of the scanned masses, low or high

resolution, primarily due to the acquisition program. The acquisition processor settings are arranged to correspond to the data supplied by the operator regarding the analysis to be performed. The parameter offered by the operator wholly controls the recent apparatus. Automatic injectors allow the performance of numerous successive chromatographic analyses with no interference from the operator. The most current systems allow the programming of tuning modifications or Change in the type of measurement. For instance, the operator can program the system to measure the spectrum in a negative mode if an ion with a given m/z value is spotted at a set retention time. The MS was revolutionized with these options.

4.2.2 Interpretation of the outcomes

The operator can intervene and modify the parameters at any time due to the interactive program. Additionally, the following operations may be done:

- Reconstruction of the ion chromatogram based on the sum of the intensities of the ions detected.
- The chromatogram can be enlarged or vertically amplified to highlight the low-intensity peaks.
- Multiple spectra can be displayed on the screen. Therefore, the comparison of spectra, one at the beginning and the other at the end of elution, is possible.
- Detecting compounds that may not be noticeable on the chromatogram.

Coupling an MS to chromatography leads to an enhanced dynamic range of the chromatography as well as an improved resolution.

4.2.3 Other programs

Other programs can be used; they are given below:

- Individual program: Can be used to draw spectra with several formats, comparing spectra, and 2D or 3D spectra drawing.
- A subtraction program is used to eliminate the background noise from a spectrum or highlight the variations between 2 spectra.
- Library search programs: for the identification of the obtained spectrum.
- Labeling a mass to an elemental composition can be effectuated by limiting the search to acceptable chemical formulas. For instance, a mass of 40 Da can be ascribed to C_2H_5O and CHO_2 . Again, low or high resolution can be used for calculation. The downside of the low resolution is that the number of possibilities is too high.
- The calculated isotopic abundances can be compared with experimental values.

Utility programs can be used to extract spectra from analysis and then delete the others.

5. Conclusions

In summary, it can be said that the most popular separation techniques (GC, HPLC) can be coupled with MS applying suitable ionization techniques. Coupling essentially removes current constraints of the single methods; thus, chromatography coupled with MS has become crucial in many analytics fields. Mainly in the area of bioanalytics, “proteomics” has launched an entirely different area of work over the past 20 years.

Conflict of interest

The authors declare no conflict of interest.

Author details

Asmae Bouziani^{1*} and Mohamed Yahya²

¹ Chemical Engineering Department, Middle East Technical University, Ankara, Turkey

² Faculty of Science, Department of Chemistry, Gazi University, Yenimahalle, Ankara, Turkey

*Address all correspondence to: asmae@metu.edu.tr

IntechOpen

© 2021 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

References

- [1] Kostianen R, Kotiaho T, Kuuranen T, Auriola S. Liquid chromatography/atmospheric pressure ionization - Mass spectrometry in drug metabolism studies [Internet]. Vol. 38, Journal of Mass Spectrometry. John Wiley & Sons, Ltd; 2003 [cited 2021 Jun 10]. p. 357-72. Available from: www.interscience.wiley.com
- [2] Gelpí E. Interfaces for coupled liquid-phase separation/mass spectrometry techniques. An update on recent developments. J Mass Spectrom [Internet]. 2002 Mar 1 [cited 2021 Jun 10];37(3):241-53. Available from: www.interscience.wiley.com
- [3] Mastovska K, Lehotay SJ. Practical approaches to fast gas chromatography-mass spectrometry. J Chromatogr A [Internet]. 2003 [cited 2021 Jun 10];1000:153-80. Available from: www.elsevier.com/locate/chroma
- [4] Klampfl CW. Review coupling of capillary electrochromatography to mass spectrometry. Vol. 1044, Journal of Chromatography A. Elsevier; 2004. p. 131-144.
- [5] Gohlke RS, McLafferty FW. Early gas chromatography/mass spectrometry. J Am Soc Mass Spectrom [Internet]. 1993 [cited 2021 Jun 14];4(5):367-71. Available from: [https://link.springer.com/article/10.1016/1044-0305\(93\)85001-E](https://link.springer.com/article/10.1016/1044-0305(93)85001-E)
- [6] Hubsehmman H-J. Subject Index. In: Handbook of GC/MS [Internet]. Wiley-VCH Verlag GmbH; 2007 [cited 2021 Jun 14]. p. 575-83. Available from: <https://onlinelibrary.wiley.com/doi/full/10.1002/9783527612857.indsub>
- [7] Fred W McLafferty JAM. State-of-the-art GC/MS. Chemtech. 1992;22(3): 182-189.
- [8] Gross JH. Field Ionization and Field Desorption. In: Mass Spectrometry [Internet]. Springer Berlin Heidelberg; 2004 [cited 2021 Jun 14]. p. 355-80. Available from: https://link.springer.com/chapter/10.1007/3-540-36756-X_8
- [9] Bursey MM. H. D. Beckey. Principles of field ionization and field desorption mass spectrometry. Pergamon Press, Oxford, 1977. Biol Mass Spectrom [Internet]. 1978 Jul 1 [cited 2021 Jun 14];5(7):iii-iii. Available from: <https://onlinelibrary.wiley.com/doi/full/10.1002/bms.1200050710>
- [10] Medeiros PM. Gas Chromatography - Mass Spectrometry (GC-MS). In: White WM, editor. Encyclopedia of Geochemistry: A Comprehensive Reference Source on the Chemistry of the Earth [Internet]. Cham: Springer International Publishing; 2018. p. 530-5. Available from: https://doi.org/10.1007/978-3-319-39312-4_159
- [11] Niessen WMA. State-of-the-art in liquid chromatography-mass spectrometry. Vol. 856, Journal of Chromatography A. Elsevier; 1999. p. 179-197.
- [12] Ramarao NT, Vidyadhara S, Sasidhar RLC, Deepti B, Yadav RS. Development and Validation of LC-MS/MS Method for the Quantification of Chiral Separated R-Bicalutamide in Human Plasma. Am J Anal Chem. 2013;04(02):63-76.
- [13] Ardrey RE. Liquid Chromatography - Mass Spectrometry: An Introduction [Internet]. John Wiley & Sons; 2003. (Analytical Techniques in the Sciences (AnTs) *). Available from: <https://books.google.com.tr/books?id=f1QiHP3wsAcC>
- [14] Holčapek M, Jirásko R, Líska M. Recent developments in liquid chromatography-mass spectrometry and related techniques. Vol. 1259, Journal of Chromatography A. Elsevier; 2012. p. 3-15.

- [15] Thomson BA. Atmospheric pressure ionization and liquid chromatography/mass spectrometry - Together at last. *J Am Soc Mass Spectrom* [Internet]. 1998 [cited 2021 Jun 15];9(3):187-93. Available from: [https://link.springer.com/article/10.1016/S1044-0305\(97\)00285-7](https://link.springer.com/article/10.1016/S1044-0305(97)00285-7)
- [16] Yamashita M, Fenn JB. Electrospray ion source. Another variation on the free-jet theme. *J Phys Chem* [Internet]. 1984 Sep 1;88(20):4451-9. Available from: <https://doi.org/10.1021/j150664a002>
- [17] Nguyen S, Fenn JB. Gas-phase ions of solute species from charged droplets of solutions. *Proc Natl Acad Sci* [Internet]. 2007 Jan 23;104(4):1111 LP – 1117. Available from: <http://www.pnas.org/content/104/4/1111.abstract>
- [18] Chiarinelli J, Bolognesi P, Avaldi L. Ion optics simulation of an ion beam setup coupled to an electrospray ionization source, strengths, and limitations. *Rev Sci Instrum*. 2020 Jul;91(7):73203.
- [19] Majuta SN, DeBastiani A, Li P, Valentine SJ. Combining Field-Enabled Capillary Vibrating Sharp-Edge Spray Ionization with Microflow Liquid Chromatography and Mass Spectrometry to Enhance 'Omics Analyses. *J Am Soc Mass Spectrom* [Internet]. 2021 Feb 3;32(2):473-85. Available from: <https://doi.org/10.1021/jasms.0c00376>
- [20] Thurman EM, Ferrer I, Barceló D. Choosing between atmospheric pressure chemical ionization and electrospray ionization interfaces for the HPLC/MS analysis of pesticides. *Anal Chem*. 2001 Nov;73(22):5441-5449.
- [21] Byrdwell WC. Atmospheric pressure chemical ionization mass spectrometry for analysis of lipids. Vol. 36, *Lipids*. American Oil Chemists Society; 2001. p. 327-46.
- [22] Cai SS, Syage JA. Comparison of atmospheric pressure photoionization, atmospheric pressure chemical ionization, and electrospray ionization mass spectrometry for analysis of lipids. *Anal Chem*. 2006 Feb;78(4):1191-1199.
- [23] Karas M, Gö M, Unger Schäfer J. Ionization in matrix-assisted laser desorption/ionization: singly charged molecular ions are the lucky survivors. Vol. 35, *JOURNAL OF MASS SPECTROMETRY* *J. Mass Spectrom*. 2000.
- [24] Wilkendorf LS, Bowles E, Buil JB, Van der Lee HAL, Posteraro B, Sanguinetti M, et al. Update on matrix-assisted laser desorption ionization-time of flight mass spectrometry identification of filamentous fungi. *J Clin Microbiol*. 2020 Dec;58(12):1263-1283.
- [25] De Cesare V, Moran J, Traynor R, Knebel A, Ritorto MS, Trost M, et al. High-throughput matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry-based deubiquitylating enzyme assay for drug discovery. *Nat Protoc*. 2020 Dec;15(12):4034-4057.
- [26] Zaima N, Hayasaka T, Goto-Inoue N, Setou M. Matrix-assisted laser desorption/ionization imaging mass spectrometry. Vol. 11, *International Journal of Molecular Sciences*. Molecular Diversity Preservation International; 2010. p. 5040-5055.
- [27] Angel PM, Caprioli RM. Matrix-assisted laser desorption ionization imaging mass spectrometry: In situ molecular mapping. *Biochemistry*. 2013 Jun;52(22):3818-3828.
- [28] Di Stefano V, Avellone G, Bongiorno D, Cunsolo V, Muccilli V, Sforza S, et al. Applications of liquid chromatography-mass spectrometry for food analysis. Vol. 1259, *Journal of Chromatography A*. Elsevier B.V.; 2012. p. 74-85.
- [29] Pitt JJ. Principles and applications of liquid chromatography-mass

spectrometry in clinical biochemistry.
Clin Biochem Rev. 2009 Feb;30(1):19-34.

[30] Zhang T, Watson DG. A short review of applications of liquid chromatography mass spectrometry based metabolomics techniques to the analysis of human urine. Vol. 140, Analyst. Royal Society of Chemistry; 2015. p. 2907-2915.

[31] Pérez-Magariño S, Revilla I, González-Sanjosé ML, Beltrán S. Various applications of liquid chromatography-mass spectrometry to the analysis of phenolic compounds. J Chromatogr A. 1999 Jun;847(1-2):75-81.