International Journal of Current Pharmaceutical Research

ISSN- 0975-7066 Vol 17, Issue 1, 2025

Review Article

REVIEW ON SIZE EXCLUSION CHROMATOGRAPHY COUPLED WITH MASS SPECTROSCOPY

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Received: 28 Oct 2024, Revised and Accepted: 12 Dec 2024

ABSTRACT

The hyphenated technique was created by integrating the differentiating technique with directly spectroscopic technology to facilitate detection. It is discussed how SEC and electrospray ionization mass spectrometry (ESI-MS) can be combined, emphasizing the advantages this has for characterizing polymers. The capacity of SEC-ESI-MS to gather polymer structural information as well as to confirm polymer homologous transitions is first described. It's significant that the broad applicability of SEC-ESI-MS is underlined, for instance by its usage in the analysis of precise molecular weight distribution of polymers, the calculation of radical rate of polymerization coefficients, and the thorough exploration of radical photoinitiation systems. Thus, this momentous tutorial review intends to highlight the high potential of SEC-ESI-MS to emerge as a formidable and well-liked analytical method in polymer chemistry and to illustrate the capability of SEC-ESI-MS for the analysis of complicated polymers. The article addresses recent advancements in the uses of a variety of hyphenated techniques, such as the GC-MS method, LC-MS analysis, LC-FTIR, LC-NMR, CE-MS, and others.

Keywords: Hyphenated techniques, SEC-MS, Polymer analysis by SEC-MS, On-line coupling

DOI: https://dx.doi.org/10.22159/ijcpr.2025v17i1.6055 Journal homepage: https://innovareacademics.in/journals/index.php/ijcpr

INTRODUCTION

Hyphenated techniques combine chromatographic and spectroscopic technologies to maximize their respective advantages. Chromatography extracts pure or almost pure chemical components from a mixture. Spectroscopy generates selective information for identification by using standards or library spectra.

A few decades back, Hirschfeld has used the phrase "hyphenation" to characterize the online combination of a method for separation and several spectroscopy detection techniques. This technique is currently referred to as the hyphenated technique, which was developed through the combination of a method for separation and a spectroscopic detecting technique.

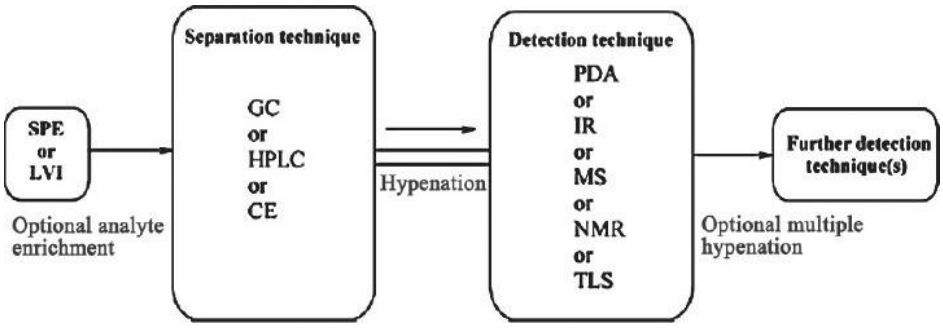

Fig. 1: Hyphenated techniques

SEC-MS was allegedly developed by a number of scientists who made significant contributions to the field. Scientists, including J. W. Jorgenson, R. J. Wells, and M. J. Heller launched the SEC-MS research effort in the 1980s. They were the first to employ electrospray ionization (ESI) to ionize large molecules and they demonstrated the feasibility of combining SEC with mass spectrometry using direct injection interfaces.

In the 1990s, scientists like R. B. Cole and R. D. Smith advanced the discipline by developing more intricate SEC-MS interface, such split flow interface and post-column flow splitting interactions. They also improved the technology's sensitivity.

Companies and models

The ACQUITY UPLC SEC-MS instrument is available from Waters Corporation and is used to analyze protein molecules, antigens, and other biomolecules.

For the examination of biomolecules, Agilent Technologies offers the 1260 Infinity II SEC system combined with their 6545XT AdvanceBio LC/Q-TOF mass spectrometer.

For the investigation of protein molecules, antibodies, and other biomolecules, Thermos Fisher Scientific provides the Vanquish Flex UHPLC system combined with their Q Enactive UHMR hybrids mass spectrometer.

The Nexera Bio UHPLC system and LCMS-9030 Q-TOF mass spectrometers are available from Shimadzu Corporation for the evaluation of peptides, proteins, and other biomolecules.

A maXis II ETD UHR-QTOF mass spectra is available from Bruker Corporation for the analysis of biomolecules.

Bio-Rad Laboratories offers the NGC Chromatography technology in conjunction with their Protein XPR36 protein-interacting array technology for the study of protein interactions.

Introduction of SEC-MS

Size exclusion chromatography (SEC), often known as gel-filtration chromatography or SEC, is a powerful technique for separating and characterizing biomolecules based on their size. It is a liquid chromatography method that is commonly utilized in biochemistry, evolutionary biology, and biotechnology research. SEC, when combined with tandem mass spectrometry (MS), provides critical information about the molecular mass and structural properties of biological macromolecules.

The separation of molecules based on their size and shape is the central concept of exclusion chromatography with size exclusion. The approach makes use of a porous stationary stage, which is typically comprised of cross-linked polymer beads with various pore diameters. These beads form a network of interconnected tubes through which the elements of the sample can flow. Smaller molecules can traverse the pores and interact more with the stationary phase, resulting in slower elution times, whereas larger molecules are rejected and elute faster.

The molecular weight and identification of the separated substances can be determined when SEC is combined with mass spectrometry. Mass spectroscopy is a very sensitive technique for ionizing substances and classifying them based on their mass-to-charge ratio. Eluted chemicals from the chromatographic column are analyzed using mass spectrometry through the combination of SEC and MS [1].

SEC and mass spectral analysis can be performed in conjunction to evaluate the distribution of molecular weight of a complex mixture of components of proteins such as molecules of protein, peptides, and nucleic acids. It explains the oligomeric state of the analytes, their presence of agglomerates, and their structural properties. The method can also be used to detect and describe contaminants or modifications in the sample, such as those generated by translational or chemical cross-linking.

In general, the combination of exclusion chromatography, size exclusion, and mass spectrometry (MS) provides a comprehensive method for characterizing biomolecules, providing significant information about their size, form, structure, and content. It is widely used in domains such as proteomics, pharmaceutical development, and structural biology, increasing our understanding of complex creatures and contributing in the identification of new medications [2].

Basic principles of SEC-MS

Size exclusion chromatography

Size limitations Chromatography is a technique for separating molecules depending on their size by the differentiating exclusions or inclusion of particles in permeable stationary phases. It is a robust and widely used method that delivers useful information about bio molecules distribution of size and structural characteristics.

Mass spectroscopy

The basic principle of mass spectrometry is the ionization, segregation, and ability to identify ions based on their proportion of mass to charge. By studying the resulting mass spectrum, experts may gain information about the weight of the molecule composition, and structure of the molecules under study. This concept underpins a broad spectrum of mass spectrometer a role in scientific research and is crucial to advancing the comprehension of many disciplines.

Overall, principle of size exclusion chromatography hyphenated with mass spectroscopy is the separation, authentication, and evaluation of biomolecules on the basis of their size, weight in molecules, and structural characteristics is possible using the SEC principle when combined with mass spectrometry. It is a strong technique for analyzing complicated biological materials, and it contributes to domains including the field of proteomics, biopharmaceutical expansion, and structural biology [3]

Instrumentation

Size exclusion chromatography

The components of size exclusion chromatography are:

- The mobile phase
- The pumps
- Sample injector
- The columns
- The pumps
- Detectors

Pumps

Pumps a solution of a polymer through the apparatus.

Viscosities of solutions produced by various types of polymers vary. Regardless of variations in viscosity, the pump output must be constant between analyses in order to compare data. Additionally, the precision of the solvent's flow rate might have a significant impact on some detectors. This constant inflow must be a crucial part of the apparatus.

Sample injector

Combines the polymer solution and enters the mobile phase.

If portion collecting is needed, the injector must have the capacity able to inject both tiny quantities (for calculating molecular weight) in addition to big volumes. The injector shouldn't alter the steady mobile phase flow. It should be able to automatically inject numerous samples when the volume of the sample is large [4].

Column

Separates sample parts from one another effectively.

Fast analysis and the highest level of separation are provided by high-efficiency columns. Every column must consistently deliver reproducible data over extended time periods intended for analytical and fractional collection purposes.

Detectors

Keeps track of the separation and reacts to the components as they come out of the column.

If components are to be received for additional analysis, detector have to be harmless to eluting components. The detectors must also be precise and have a broad linear range so that they can, if necessary, react to both small fragments and huge quantities of material [5-7].

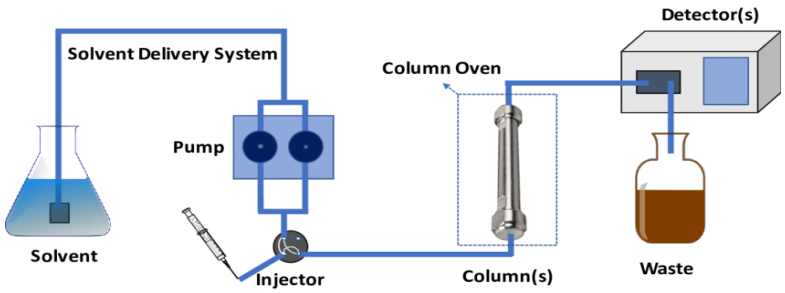

Fig. 2: Size exclusion chromatography

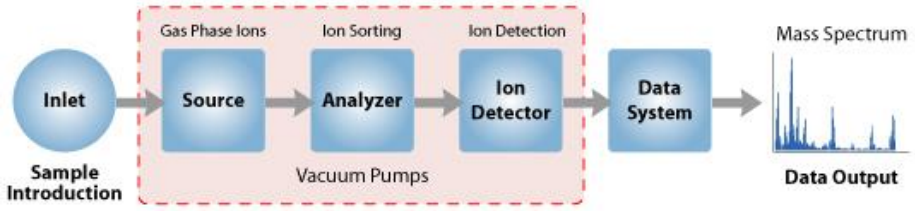

Fig. 3: Mass spectroscopy

Mass spectroscopy

The components of mass spectroscopy are:

- Ion source
- Mass analyzers
- Detectors

Ion source

In mass spectrometry, an ion source is a part or location where ions are created from a sample and then transferred to the mass spectrometer's chamber for analysis. The ion source has a significant impact on the performance and capabilities of a mass spectrometer.

The most frequent ionization sources are matrix-assisted laser desorption/ionization (MALDI), atmospheric pressure chemical ionization (APCI), and electrospray ionization (ESI). In addition to this, chemical ionization (CI) or negatively chemical ionization is another ionization source employed in MS.

Electron spray ionization

By delivering an intense electrical field to a liquid that is moving though a capillary tube that circulates with a weak flux, electrospray is created. Large charged droplets are created as a result, and they are then exposed to solvent evaporation. Ions are released from droplets as a result of coulombic repulsion, which is triggered by a spike in charge density brought on by the evaporation of the solvent. This is how ion formation works when utilizing this technique.

This method's detection sensitivity is only 10-8 µl, and a high volume is required to boost detection sensitivity. Non-volatile compounds can be ionized in liquids using ESI high mass samples, although this source of ionization has poor sensitivity, low fragmentation, and is unstable.

Matrix-assisted laser desorption/ionization (MALDI)

For big and/or labile molecules such as proteins, peptides, polymers, dendrimers, and fullerenes, MALDI is an ionization process. In this method, analytes are embedded. In a matrix that consumes energy at the laser's wavelength. In order to produce analyte ions, nitrogen ultraviolet, or UV, lasers (337 nm) are delivered over the matrix under vacuum. Three distinct models are proposed to account for the desorption of the matrix-sample components from the surface of the crystal because the process for ionization is unclear.

As a result of the laser's impact, there is quasi-thermal evaporation, which increases molecular mobility. The upper lattice the layers of the matrix are ejected as a result, causing desorption.

The matrix's structure is then expected to transmit protons to the sample molecules, charging the analyte [8, 9].

Mass analyzers

The mass spectrometer's analyzer separates ionized molecules based on their charge-to-mass ratios before sending the results to the detector, where they are detected and eventually transformed into a digital output.

Quadrupole mass analyzer

Due to their ease of use and ability to analyze a wide the mass band (10 to 4000 An. M. U./atomic mass units), the quadrupole and tripled quadrupole analyzers are the most widely used. The quadrupole provides superior linearity for quantitative analysis, good resolution (up to 4,000), excellent mass spectra, lightning-fast scanning (5000 A. M. U. per sec), and precise measurements of mass (0.1 to 0.2 A. M. U).

The underlying idea is to transitions through an axis that consists of four parallel, equally spaced poles, using electric fields to segregate them based on their mass-to-charge ratio. Based on the stability of their paths in the fluctuating electric fields that are supplied to the rods, ions become separated in a quadrupole.

In a quadrupole, there are two separate sets of metal rods. A radio frequencies (RF) current is provided between each pair of opposing rods after each pair is electrically connected to the other. The RF voltage is subsequently followed by a direct current voltage. Ions move between the rods of the quadrupole. For a specific ratio of voltages, only ions with a specific mass-to-charge ratio (m/z) will enter the detector. Other ions will crash with the rods because of their unstable paths. By continuously changing the applied voltage, this enables the user to choose of an ion with a specific m/z and enables the proprietor to scan for an array of m/z values.

Time of flight analyzer

This analyzer, often known as the TOF, is utilized in single MS systems. The TOF is connected to the quadrupole (QT of), another TOF (TOF-TOF), or a proton trap (QIT/TOF) in an MS/MS arrangement.

Time of Flight analyzer works on the theory that ions generate from an ion source and are then accelerated to high speeds by an electric field in the instrument's "drift tube."

The detector will use either a linear mode or a reflection mode to find the accelerated ions [10].

Detectors

All mass spectrometry, or MS, systems require some type of detector1 to convert the flow of size-separated ions into a measurable signal. Various kinds of detectors are used, depending on factors including dynamic range, data spatial retention as well as noise, and compatibility for the volume analyzer. Some of the often used types are array detectors, Faraday cups, photomultiplier translation dynodes, and electron multipliers (EM). This article talks about these detectors.

Electron multipliers

An electron multiplier is a sensitive detector based on the principle of secondary electron emission hypothesis. When an ion collides with the first dynode of the multiplier, several electrons are released. These electrons are accelerated and hit consecutive dynos, which leads to an order of electron multiplication. The final electron pulse is collected and amplified to offer a highly sensitive detecting method.

Faraday cup

FC detectors4 are relatively simple devices that cost little money. Their main benefit is that they can detect greater ion currents, whereas the EM cannot. The device's foundation is a hollow transmitting electrode coupled to the ground by a large resistance. Ions striking the collector's surface and producing an influx of

electrons from ground via the resistor amplifies the probability drop across the resistor. The elementary charge of a single ion is 1.6 x 10-19 °C. As, a result, a count rate of 1 × 106 °C/s (about the maximum practical limit for the use of EM detectors) would result in a voltage of 1.6 x 10-13A. The power amplifier must be capable to recognize a potential decrease of 16 mV even with a resistor of 1011 W or higher connected to ground. As a result, it will be more challenging to measure smaller currents since both the resistor and amplifiers circuitry's electronic and thermal noise will have a significant impact on accuracy. These parts are frequently housed in an evacuated, temperature-controlled chamber.

Photon multiplier dynode

In a photomultiplier conversion dynode detector, when ions strike a dynode, they first emit electrons. Photons are emitted when the produced electrons strike a phosphor screen. The photons get amplified in a cascade fashion after exiting the multiplier, comparable to the electron multiplier. The main advantage of using beams is that the detector's multiplier component can be retained vacuum-sealed to prevent contamination and greatly extend detector lifespan [11].

Coupling of SEC-MS

When SEC and MS are combined, the emitted constituents via the SEC are immediately injected into the mass spectrometer for analysis. This can be done via a variety of interactions, such as the electrospray ionization (ESI) depending on the analytes and the mass spectrometer being utilized.

One common technique is to use an ESI or MALDI sources for introducing the elution portions from SEC into a mass spectrometer after collecting them with a fraction collector. The collected mass spectra can provide details regarding the distribution of molecular weights of the separated species as well as structural characteristics including oligomeric states, modifications after translation, as well as other covalent changes.

Further improvements to the structural data can be made by tandem mass spectrometry testing on the fractions that were eluted by SEC-MS/MS. By selecting certain ions that are relevant within the mass spectrum, separating them, and then analyzing the fragmentation arrangements that occur, it is feasible to gain knowledge about the interconnection and organization of the subunits inside the molecules themselves along with their sequence.

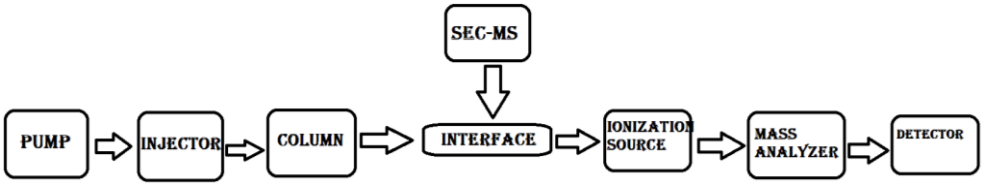

Fig. 4: Workflow of SEC-MS: integration of size exclusion chromatography with mass spectrometry

Interfaces

Direct injection interface

The SEC column effluent can be injected directly into the mass spectrometer's an ionisation sources without any additional sample preparation. The user interface is fast and simple to use, but it may result in a matrix effect and ions suppression that could reduce the precision and accuracy of the study [12].

Split flow interface

A chunk of the SEC the columns effluent is directed to waste at this contact, while the remaining amount gets pumped into the mass spectrometer's ionisation source. The precision and accuracy of the analysis are increased thanks to this interface's reduction in the quantity of matrix and impurities added to the ionisation source [13].

Post-column flow splitting interface

In this interface, the SEC column output is split into two separate streams and placed in a chamber that mixes with the additional stream. A make-up flow of the portable phase is delivered to a sample flow in the chamber of mixing to diluted the sample and boost the sensitiveness of the analysis. The diluted sample is then poured into the ionisation source of the mass spectrometer [14, 15].

Solid phase extraction interface

In this interface, the effluent from the SEC column is collected, and then the sample is concentrated while the matrix and contaminants are removed through a solid-phase extraction technique. The concentrated sample is then poured into the ionisation source of the mass spectrometer. This interface offers high selectivity and sensitivity while taking longer and requiring additional sample preparation [16, 17].

SEC and MS combined to find size variances

SEC and MS forced degradation analysis Investigations on forced degradation are frequently conducted as part of the creation of therapeutic mAbs and related products. The goals of these investigations are to comprehend the paths of compelled degradation and create consistency indicating tests to monitor deterioration. The conditions of forced deterioration experiments are typically harsh compared to actual storage, but this speeds up the development of relevant degradation products. In this regard, deterioration under thermal stress usually happens when temperatures are above 35 °C for a number of weeks. Due to the fact that various degradation pathways (including the development of aggregates and disintegration from peptide bond breakage) are sped up by elevated temperature, it is one of the most thoroughly studied tests for mAb-related products.

Case studies

SEC-MS analysis of attenuated monoclonal antibody concentrations

With the help of SEC and a direct mass spectrometer connection, antibody light and heavy chains can be separated.

The antibody light chains and heavy chain can be separated using mobile phases, including ACN, trifluoroacetic acids, and formic acid, respectively, after reduction by SEC.

The columns' performance was evaluated using a mobile phase that included 20% acetone, which 0.1% TFA, and 0.1% formic acid. The columns were run for a period of eighty minutes at an effective flow rate of 0.2 ml/min.

After reduction, the free light chain and free heavy chain of a recombinant monoclonal antibody are isolated from the thioether-linked light chain and heavy chain.

The greatest resolution for both heavy and light chain, the second-highest theoretical plate value for heavy chain, the highest theoretical plate value for light chain, and Tsk gel g3000swxl: Effective for protein separation.

Weight in molecules: 70,000–300,000.

Anti-drug conjugated (adc s) SEC-MS analysis

ADC analyses should be straightforward, quick, and accurate. SEC-MS is used to characterize antibody-drug conjugates at the unchanged molecular level.

The distribution of glycoforms and the normal drug-antibody proportion will be studied in 15 min.

To unite the cytotoxic qualities of small molecule drugs alongside the specificity of antibodies, Adc' S link the drug to an antibody.

It encourages the specificity and potency of chemotherapy medications.

Using the SEC-MS method, key quality traits of the pyrrole benzodiazepine-based adc'S are investigated. Sequence-selective Pbd 'S are DNA minor groove binding agents for cross-linking that have been proven to be exceedingly effective, even more potent than systemic chemotherapeutic drugs.

It enables the analysis of whole, fragile molecules like adc's.

The SEC-MS technique was developed in order to categorize pbd-based.

In native conditions, they used the compound ammonium acetate as a volatile ingredient in the chromatography mobile phase and very sensitive orbitrap MS detection. It was established that SEC-MS is a simple yet efficient technique for the quick and precise analysis of antibody-drug combination glycoprotein and average DAR. Because a variety of additional characteristics of quality are also available for examination, SEC-MS is a powerful technique for simultaneous analysis of many quality features of biopharmaceuticals like adcs.

Applications

Proteomics: To recognize and measure protein complexes and their components used to research post-translational changes, protein-protein interactions, and protein interactions.

Polymer analysis: To examine the distribution of polymers in a sample. Describe the polymer's molecular weight distribution, level of polymerization, which and branching structure.

Lipidomics: To examine the fatty acids and their complexes present in biological samples. Describe the structure, composition, and interactions between lipids and proteins in lipid membranes.

Metabolomics: To examine complexes of tiny compounds in biological samples. Give details on the makeup, structure, and interactions of metabolites with other molecules.

Drug discovery: To evaluate protein-ligand interactions and describe the drug's ability to connect to its target

CONCLUSION

The analytical capabilities are enhanced when SEC and MS are combined, giving researchers a powerful toolkit to investigate the complexities of polymers and biomolecules. This hyphenated technique continues to advance state-of-the-art research and makes a substantial contribution to our comprehension of the functional and structural features of various molecular entities.

SEC-MS was shown to be quick and accurate.

ACKNOWLEDGMENT

I need to recognize our cherished Principal prof. M. Sumakanth mam and staff of the Department of Pharmaceutical Analysis for giving the opportunity.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All authors have contributed equally

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Wilson ID, Brinkman UA. Hyphenation and hypernation the practice and prospects of multiple hyphenation. J Chromatogr A. 2003;1000(1-2):325-56. doi: 10.1016/s0021-9673(03)00504-1, PMID 12877178.
2. Exarchou V, Fiamegos YC, Van Beek TA, Nanos C, Vervoort J. Hyphenated chromatographic techniques for the rapid screening and identification of antioxidants in methanolic extracts of pharmaceutically used plants. J Chromatogr A. 2006;1112(1-2):293-302. doi: 10.1016/j.chroma.2005.11.077, PMID 16359690.
3. Fekete S, Beck A, Veuthey JL, Guillarme D. Theory and practice of size exclusion chromatography for the analysis of protein aggregates. J Pharm Biomed Anal. 2014 Dec;101:161-73. doi: 10.1016/j.jpba.2014.04.011, PMID 24816223.
4. Hong P, Koza S, Bouvier ES. Size exclusion chromatography for the analysis of protein biotherapeutics and their aggregates. J Liq Chromatogr Relat Technol. 2012;35(20):2923-50. doi: 10.1080/10826076.2012.743724, PMID 23378719.
5. Engelhardt H, Ahr G. Optimization of efficiency in size exclusion chromatography. J Chromatogr A. 1983 Dec 30;282:385-97. doi: 10.1016/S0021-9673(00)91616-9.
6. Ricker RD, Sandoval LA. Fast reproducible size exclusion chromatography of biological macromolecules. J Chromatogr A. 1996;743(1):43-50. doi: 10.1016/0021-9673(96)00283-x, PMID 8817873.
7. Bhavyasri K, Samreen Begum M. Sumakanth: 2-dimensional gas chromatography-mass spectroscopy: a review. Int J Pharm Sci Rev Res. 2022;76(1):140-50.
8. Bhavyasri K, Reddy ED, Rambabu D. Gas chromatography-mass spectrometry (GC-MS) and its applications a review. Int J Res Anal Rev. 2019;6(1):456-65.
9. Bhavyasri K, Mounika G. Capillary electrophoresis mass spectrometry (CE-MS) and its applications a review. Int J Sci Res Rev. 2019;8(2):3161-76.
10. Bhavyasri K, Fatima A, Swethasri R, Sumakanth M. Role of LC-MS in pharmacokinetic and pharmacodynamic studies in bioanalysis: a review. J Glob Trends Pharm Sci. 2021;12(1):8887-91.
11. Kusler JA, Mitsch WJ, Larson JS. Wetlands. Sci Am. 1994;270(1):64-70. doi: 10.1038/scientificamerican0194-64B.
12. Marcoux J, Champion T, Colas O, Wagner Rousset E, Corvaia N, Van Dorsselaer A. Native mass spectrometry and ion mobility characterization of trastuzumab emtansine a lysine linked antibody-drug conjugate. Protein Sci. 2015;24(8):1210-23. doi: 10.1002/pro.2666, PMID 25694334.
13. Cech NB, CG Enke. Practical implications of some recent studies in electrospray ionization fundamentals. Mass Spectrom Rev. 2001 Nov-Dec;20(6):362-87. doi: 10.1002/mas.10008.
14. Righetti PG, Verzola B. Folding/unfolding/refolding of proteins: present methodologies in comparison with capillary zone electrophoresis. Electrophoresis. 2001;22(12):2359-74. doi: 10.1002/1522-2683(200107)22:12<2359::AID-ELPS2359>3.0.CO;2-8, PMID 11519938.
15. Ehkirch A, Hernandez Alba O, Colas O, Beck A, Guillarme D, Cianferani S. Hyphenation of size exclusion chromatography to native ion mobility mass spectrometry for the analytical characterization of therapeutic antibodies and related products. J Chromatogr B Analyt Technol Biomed Life Sci. 2018 Jun 1;1086:176-83. doi: 10.1016/j.jchromb.2018.04.010, PMID 29684909.
16. Fussl F, Cook K, Scheffler K, Farrell A, Mittermayr S, Bones J. Charge variant analysis of monoclonal antibodies using direct coupled ph gradient cation exchange chromatography to high-resolution native mass spectrometry. Anal Chem. 2018;90(7):4669-76. doi: 10.1021/acs.analchem.7b05241, PMID 29494133.
17. Jones J, Pack L, Hunter JH, Valliere Douglass JF. Native size exclusion chromatography mass spectrometry: suitability for an antibody-drug conjugate drug to antibody ratio quantitation across a range of chemotypes and drug loading levels. MAbs. 2020 Jan-Dec;12(1):1682895. doi: 10.1080/19420862.2019.1682895, PMID 31769727.
18. Wei B, Han G, Tang J, Sandoval W, YT Zhang. Native hydrophobic interaction chromatography hyphenated to mass spectrometry for characterization of monoclonal antibody minor variants. Analytical Chem. 2019;91(24):15360-4. doi: 10.1021/acs.analchem.9b04467.
19. Shi RL, Xiao G, Dillon TM, Mc Auley A, Ricci MS, Bondarenko PV. Identification of critical chemical modifications by size exclusion

chromatography of stressed antibody target complexes with competitive binding. MAbs. 2021 Jan-Dec;13(1):1887612. doi: 10.1080/19420862.2021.1887612, PMID 33616001.

20. Liu H, Gaza Bulseco G, Faldu D, Chumsae C, Sun J. Heterogeneity of monoclonal antibodies. J Pharm Sci. 2008;97(7):2426-47. doi: 10.1002/jps.21180, PMID 17828757.
21. YU L, Remmele RL JR, He B. Identification of N-terminal modification for recombinant monoclonal antibody light chain using partial reduction and quadrupole time of flight mass spectrometry. Rapid Commun Mass Spectrom. 2006;20(24):3674-80. doi: 10.1002/rcm.2790, PMID 17117408.
22. H Shion. Development of integrated informatics workflows for the automated assessment of comparability for antibody-drug conjugates (ADCs) using LC-UV and LC-UV/MS. Waters Application Note. 720005366EN. 2015.
23. Shion H. High-performance vion ims qtof for monoclonal antibody intact and subunit mass analysis. Waters Application Note. 720005906EN. 2017.
24. Mc Pherson MJ, Hobson AD. Pushing the envelope: advancement of ADCs outside of oncology. Methods Mol Biol. 2020;2078:23-36. doi: 10.1007/978-1-4939-9929-3_2, PMID 31643047.
25. Kaur S, Bateman KP, Glick J, Jairaj M, F Kellie JF, Sydor J. IQ consortium perspective: complementary LBA and LC-MS in protein therapeutics bioanalysis and biotransformation assessment. Bioanalysis. 2020;12(4):257-70. doi: 10.4155/bio-2019-0279, PMID 32096432.
26. Kang L, Weng N, Jian W. LC–MS bioanalysis of intact proteins and peptides. Biomed Chromatogr. 2020;34(1):e4633. doi: 10.1002/bmc.4633, PMID 31257628.
27. Cong Y, Zhang Z, Zhang S, HU L, GU J. Quantitative MS analysis of therapeutic mAbs and their glycosylation for pharmacokinetics study. Proteomics Clin Appl. 2016;10(4):303-14. doi: 10.1002/prca.201500098, PMID 26548570.
31. Liu H, Gaza Bulseco G, Faldu D, Chumsae C, Sun J. Heterogeneity of monoclonal antibodies. J Pharm Sci. 2008;97(7):2426-47. doi: 10.1002/jps.21180, PMID 17828757.