Review Article

Qualification and Calibration of Hyphenated Techniques Liquid Chromatography: Mass Spectroscopy and Gas Chromatography - Mass Spectroscopy

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ABSTRACT

A growing discipline has emerged in the pharmaceutical industry to obtain a quantitative life of a medication and its metabolites as a result of the invention of bio analytical methods. They developed a combination of instruments to evaluate the bio analysis of the medication. The term "LC-MS" refers to a bio-analytical technique that combines the mass analysis capabilities of mass spectrometry with the physical separation capabilities of liquid chromatography. The first mention of this combination dates back to 1967, & the initial LC-MS system was released in the 1980s.The main objectives of this are an overview of the concept, method development, & method validation. The main objectives of this are an overview of the concept, method development, & method validation. A quick summary of an introduction to HPLC's stationary phase is provided. The efficient development and validation of analytical techniques is an essential component of pharmaceutical research and development. Several crucial components which are essential to succeed in these industries will also aid in regulatory compliance. Experience, both the total experience of the department's validation development and validation department and the level of experience among the individual scientists, is one of these characteristics. A solid supervision and instruction program is another essential component for the creation and validation of methods that are successful. Businesses need to maintain a proper level of competency in this essential sector in order to produce medications that have been proven effective as well as secure. The most crucial instrument for identifying and measuring volatile and semi-volatile organic chemicals in complicated mixtures is gas chromatography-mass spectrometry. The quantification of contaminants in drinking water and wastewater, as well as the quantitation of pharmaceuticals as well as the metabolites they produce in blood and urine, are all often done using GC-MS. In contrast to LC-MS, GC-MS is utilized to detect residual solvents.

Keywords: Gas Chromatography, Liquid Chromatography, Mass Spectrometry, Qualification, Calibration, Validation, Analytical Techniques.

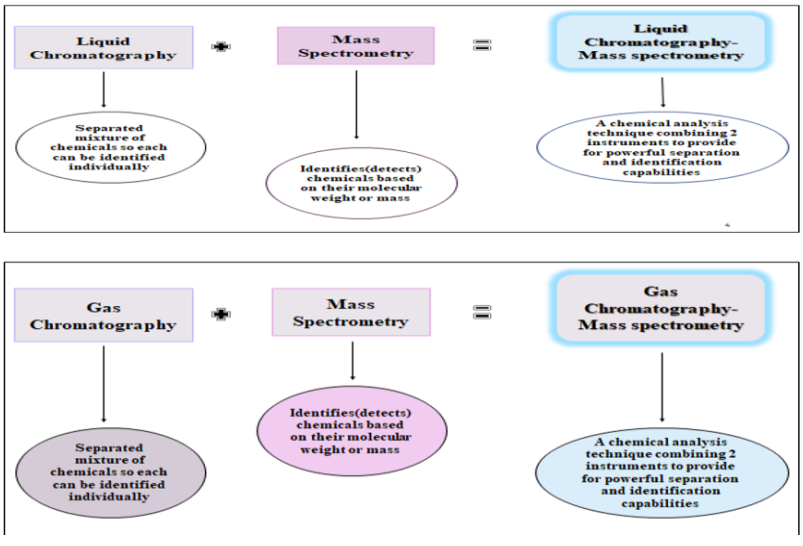
INTRODUCTION

Liquid Chromatography (LC) method

A separation technique called Liquid Chromatography (LC) is used to separate the various parts of a mixture. A sample is mass transported between a polar mobile phase and a non-polar stationary phase in this procedure.[1-2]

Mass Spectrometry or (MS)

Atoms or molecules are made ionized in mass spectrometry (MS) to make it easier to separate and detect them based upon their molecular weights and charges (the mass to charge ratio). MS is employed in many different fields, including as atomic physics and biochemistry.[1]



METHODOLOGY AND RESULTS

Gas Chromatography combined with Mass Spectrometry[5-9]

Two primary parts make up a GC-MS instrument: a gas chromatograph and a mass spectrometer. By employing a gas chromatograph to separate chemical mixtures into their individual components, GC-MS follows with an MS detector to identify and quantify those components on a molecular level. It is amongst the most precise as well as efficient instruments to analyze samples of volatile organic substances.

Qualification:

In order to demonstrate that equipment can perform regularly as intended and in accordance with pre-set acceptance criteria, as laid out in the manufacturer's recommendation/design qualification standards and guidelines, qualification is the creation, execution, & documenting of tests on the equipment. It also serves to verify the working capability of the equipment. Before using any equipment, instruments, facilities, or areas, they must first be qualified.

A few of the phases in qualification are User Requirement Specifications (URS), Factory Acceptance Tests (FAT), Site Acceptance Tests (SAT), Design Qualification (DQ), Installation Qualification (IQ), Operational Qualification (OQ), and Performance Qualification. The user, with support from the equipment's manufacturer or supplier, a certified qualification team & an engineering practitioner, must correctly qualify all new and used facilities, systems, and instruments.

Before moving on to the validation phase for the manufacturing process, you require qualified employees, equipment, instrument, facilities, region, systems, or software.

Calibration:

The process of determining the functional relation between the measurable readings and analytical quantities is referred to as calibration. Each instrument used throughout the inquiry needs to be properly calibrated in order for the data it produces to be trustworthy and used by others. Calibration is carried out to make sure that a process or piece of equipment is performing as intended. It must be performed on a regular basis in order to recognize equipment drift and recover accuracy. As a weighing balance is calibrated on regular intervals against its certified value, calibration is performed at all times against the standard reference.

The quality system includes calibration and qualification. Product quality may suffer if one of them drifts. To help with the enhancement of the system safety, and regulatory requirements as well as product quality, it must be maintained over a long period of time.

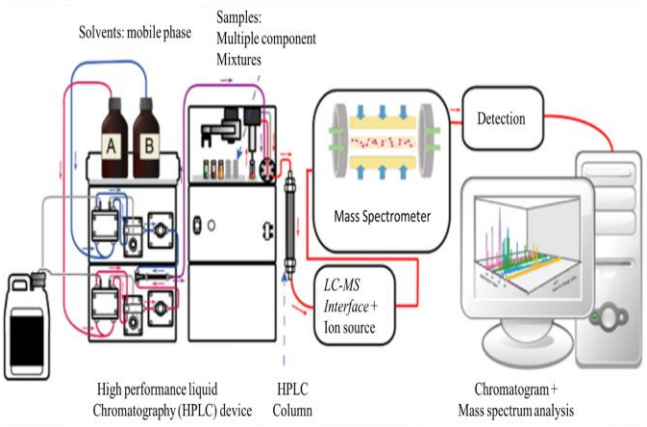


Figure 2: Instrumentation for LC-MS

LC-MS Qualification in and Calibration[13-20]

Qualification Parameters

The calibration, fine-tuning, system compatibility test, and complete validation of a particular LC-MS at the final stage of the process influence its daily performance.

1) Parameters for Calibration:

The instrument parameters that are utilized to calibration are those which are constant across all instrument types, such as

(a) **Peak Width**

(b) **Profile Scan and Peak Shapes**

(c) **A Massive Assignment:** It is carried out with particular MS calibrates. Calibrates ought to be precisely described reference items. Documentation of these compounds' certification and handling is recommended.

(d) **Resolution vs. Sensitivity:** Ion intensity and width of peak are compromised in order to achieve mass resolution.

2) Tuning Parameters:

Tuning parameters are the aspects of an instrument whose values can vary based on the type of experiment being run. A constant stream of the desired analyte's tuning solution must be introduced into the MS through a manual, semiautomatic, as well as automatic tuning procedure.

Three methods exist for doing this:

- By directly injecting the fluid using syringe pumps (direct infusion),
- By using the syringe pump to feed the sample to the LC's effluent.
- By injecting the sample in to the LC's effluent using a loop injection nozzle Flow Injection Analyses (FIA).

For fine-tuning syringe pumps experiments with low flow rates, the first method is effective. These second and third techniques are beneficial for LC studies with greater flow rates. The optimum parameters, which impact the signal quality, vary depending on the brand and model of the instrument. Examples include the following variables: the

source temperature, the ionization voltages, the gases, the ion pathway potentials collision power, the solution, or the mobile-phase flow rate.

3) System Suitability Testing:

System appropriateness enables system performance to be assessed through analysis of a predetermined solution before the analytical batch is conducted. The performance of the entire analytic system, the chromatographic procedure, and also the mass spectrometer's sensitively to the target compounds should all be assessed during system suitability testing. Analytical methods are the primary component of operational details for a specific analysis in several LC-MS SOPs.

This is particularly helpful for quantitative analysis since the analytical processes require detailed knowledge of the instrument settings and particular calibrations that could be required for a given analyte. As a result, daily system checks are provided through system suitability testing.

4) Validation:

Validation is the final step to make sure an LC-MS system performs as expected. In addition to calibrating equipment, tuning, testing, and reviewing them, validation entails assessing documentation to accuracy, completeness, and conformity with Standard Operating Procedures. Validation entails four distinct steps:

- Instrument and computer executing it are both subject to computer system validation, abbreviated CSV.
- Verification of the analytical method applied with that device.
- System appropriateness testing, which verifies expected performance by putting both the tool and the procedure through their paces.
- Quality Assurance/Quality Control evaluation of sample analysis information gathered using such a system.

Calibration: -

Depending on the needed level of mass accuracy, LC-MS should be calibrated. When accurately measuring the mass of peptides and proteins, for instance, instrument calibration is required to be checked every day. Small molecule quantitative analysis, however, calls for less frequent calibration.

1. MS General Tuning and Calibration Practice

By injecting a calibration solution, the mass analyzing device should be regularly calibrated on an ongoing schedule. It frequently makes use of an ESI (Electrospray Ionization Source). The mixture ought to produce ions that cover the entirety of the instrument's mass spectrum, or else at least a portion of the spectrum of mass that will be used in subsequent analyses.

When employing ESI mode, the software package that uses the most recent LC-MS technology available on the market provides an automated methodology to fine tune and even calibrate. To enhance the parameters that affect ion detection, manual or semiautomatic techniques are still required for older devices and/or particularly specific applications.

The mass spectrometer in an LC-MS device is tuned as well as calibrated in three phases:

a. MS calibration,

b. Ion source along with transmission optimization

c. Maximizing the detection of one or more particular ions by fine tweaking.

2. Calibration of a quadrupole mass filter

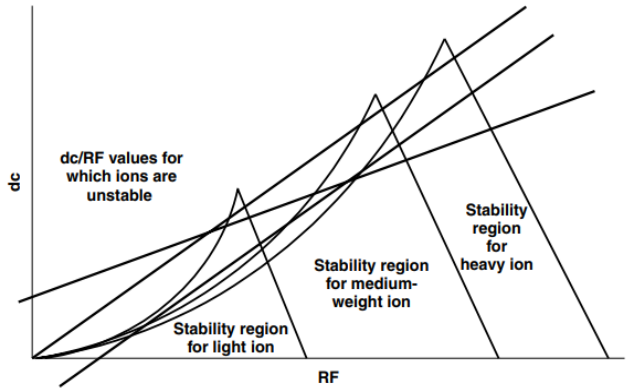


Figure 3: Sensitivity vs. Resolution

The quadrupole mass filter has various scan lines.

Calibration Curves: -Up to three calibration curves may be needed for a quadrupole mass spectrometer:

a. A calibration for Static

b. A calibration for scanning

c. A calibration for the scan speed compensation

3. Solutions for Calibration

Any mass spectrometer's mass scale must be calibrated, so it's critical to locate reference substances that work with the specific ion source. Perfluorocarbons, which are often used as calibrants for chemical and electron ionization (EI and CI), are useless in the ESI mode.

The right calibration materials for LC-ESI-MS

- not have an impact on memory;
- not contaminate the source with non-volatile substance; and
- Be appropriate in both the positive-ion as well as negative-ion mode.

The principal calibrants used / currently in use to calibrate ESI-MS can be divided into the following categories:

Perfluoroalkyl triazines, proteins, cluster of alkali metal salt, poly-ethers, water group, and salts of acetate are all examples of polymers.

In conclusion, a variety of calibrants are currently used, depending on the instrument's manufacturer and/or the application. The most often employed substances include polypropylene or polyethylene glycols, ultramark 1621, a phosphazines, mixtures of caffeine, a tiny peptide (MRFA), & myoglobin, or different combinations of peptides and proteins.

4. APCI Source Calibration and Tuning

Generally speaking, operating and optimizing an APCI connection is extremely straightforward. In comparison to, example, ESI, less focus is given to optimizing important interface variables including the flow of liquid point, The solvent setup, nebulizer in addition to auxiliary gas supply, and probe status, & vaporizer temperature.

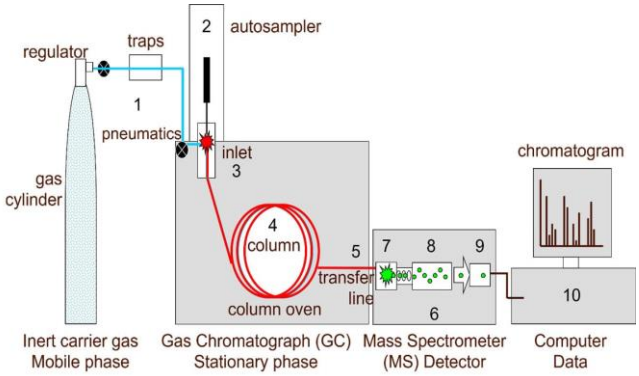


Figure 4: GC-MS Instrumentation[8]

Table 1: Calibration Method for GC-MS[10 &20]

Check parameters	Acceptance criteria
Mass Sensitivity	S/N Ratio > 60:1
Repeatability-RT GC-MS/MS	% RSD MAX 0.5 %
Repeatability-Area GC-MS/MS	% RSD MAX 5 %
Detector linearity	$R^2 \geq 0.99$
Split Ratio	$R^2 \geq 0.99$
Carry-over	Height should be less than 0.4%

Sensitivity

Examine the self-calibration and auto-tuning, and keep the calibration data. Make up a standard octa-fluoronapthalene (OFN) using a suitable sample vial at a concentration of 1 pg/ul in so much octane. Run the GC-MS and inject octa-fluoronapthalene. Verify the OFN's mass spectrum and signal-to-noise ratio.

The acceptance Criteria: S/N greater than 60 octa-fluoronapthalene for a molecular ion of 10 ppb at m/z 272 or EI Sim.

Table 2: Repeatability

INSTRUMENT	INFORMATION	ACCEPTANCE CRITERIA
GC-MS	Lindane is extracted for GC-MS calibration. You can also use other GC compounds with proven purity as your reference material. Prepare the stock solution at 100 g/ml. Retention times as well as area are replicated five times, and the% RSD is calculated.	Retention time's % RSD should be less than 0.5%. Area's % RSD should be less than 0.5%.

Table 3: Linearity of the Detector

Instrument	Linearity
GC-MS	Then, inject the lindane calibration solution with a 20, 40, 60, 80, or 100 ng/ml concentration. Place the concentration at the X axis along with the area through the Y axis to create a linearity graph.

Acceptance Criteria: The coefficient of correlation, or r2, obtained from the graph of linearity for different levels must not be less than or equal to 0.99.

Split Ratio

Maintain track of a chromatogram for each split ratio by injecting the sample with identical quantity of a standard solution at different split ratios, which include 1:10, 1:50, or 1:100. Create a linearity graph with the ratio in the X axis and the area in the Y axis.

Acceptance Criteria: The ratio's linearity graphs must provide a correlation coefficient r2 that cannot be less than 0.99.

Head Space

Calculate the percentage RSD of both the duration of retention and the retention area after injecting one milligram per litre of hexane into oil five times.

Criteria for Acceptance: Retention time's % RSD should be less than 0.5%. Area's % RSD should be less than 0.5%.

Formula for RSD:
RSD = STDED*100/Average

Frequency- Once every six months.

Table 4: Carry Over (GC-MS, GC-FID and GC-ECD)

Vial No.	Position given for reference	Sample details
1.	10	Solvent
2.	11	Std. in Solvent
3.	12	Solvent

Calculate the carryover percentage using the equation given below.

Where Carry Over (%) = Hco×100/Hi

Peak height for the vial's HCo 12 analyte.

Peak analyte height measured from vial 11.
Acceptance Criteria: Height shouldn't be more than 0.4%.

CONCLUSION

Liquid chromatography is capable of separating delicate and complicated natural mixtures, in which the chemical makeup needs to be thoroughly established (for example, fluids from biological organisms, environmental samples, and pharmaceuticals), making the incorporation of MS with LC systems desirable. Additionally, the analysis of volatile explosive residues can be done using LC-MS. Using gas chromatography and mass spectrometry together is a flexible way to separate, evaluate, and identify unidentified chemical substances.

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REFERENCES

1. De Hoffmann, Edmond; Stroobant, Vincent (2002). Mass Spectrometry (Principles and Applications) (2nd Ed.). Wiley. pp. 157–158. ISBN 0-471-48566-7.
2. Pitt, James J. "Principles and Applications of Liquid Chromatography-Mass Spectrometry in Clinical Biochemistry". Clin Biochem Rev. 2009;30(1):19–34. PMC 2643089. PMID 19224008.
3. R. K. Boyd, J. D. Henion, M. Alexander, W. L. Budde, J. D. Gilbert, S. M. Musser, C. Palmer and E. K. Zurek, J. Am. Soc. Mass Spectrom., 1996;7:211–218.
4. Guidelines for Writing an SOP for Mass Spectrometry, prepared by the Measurements and Standards Committee of the American Society for Mass Spectrometry, Sept. 1, 1997 (available from www.asms.org/mssop.h).
5. Adlard ER, Handley AJ (2001). Gas chromatographic techniques and applications. London: Sheffield Academic. ISBN 978-0-8493-0521-4.
6. Barry EF, Grob RE (2004). Modern practice of gas chromatography. New York: Wiley- Interscience. ISBN 978-0-471-22983-4.
7. Eiceman GA (2000). "Gas Chromatography". In Meyers RA (Ed.). Encyclopedia of Analytical Chemistry: Applications, Theory, and Instrumentation. Chichester: Wiley. p. 10627. ISBN 0-471-97670-9.
8. Sparkman DO, Penton Z, Kitson FG (17 May 2011). Gas Chromatography and Mass Spectrometry: A Practical Guide. Academic Press. ISBN 978-0-08-092015-3.
9. Jones M. "Gas Chromatography – Mass spectrometry". American Chemical Society. Retrieved 19 Nov 2019.
10. Holmes JC, Morrell FA. "Oscillographic Mass Spectrometric Monitoring of Gas Chromatography". Applied Spectroscopy. 1957;11(2):86-89. DOI: 10.1366/000370257774633394. ISSN 0003-7028.
11. Sparkman DO, Penton Z, Kitson FG (17 May 2011). Gas Chromatography and Mass Spectrometry: A Practical Guide. Academic Press. ISBN 978-0-08-092015-3.
12. Gohlke RS. "Time-of-Flight Mass Spectrometry and Gas-Liquid Partition Chromatography". Analytical Chemistry. 1959;31(4):535-541. DOI:10.1021/ac50164a024. ISSN 0003-2700.
13. Shah VP, Midha KK, Findlay JWA, Hill HM, Hulse JD, McGilveray LJ, et al. Bioanalytical Method Validation—A Revisit with a Decade of Progress, Synthesis of Conference held in Arlington, VA; December 3-5, 1990 and report published in pharmaceutical research 1992;9:588-592. Pharmaceutical Research 2000;17(12):1551-1557
14. Shah VP. The history of bioanalytical method validation and regulation: Evolution of a guidance document on bioanalytical methods validation. The AAPS Journal. 2007;9(1):E43-E47. Article 5
15. L. Jiang and M. Moini, J. Am. Soc. Mass Spectrom, 1992;3:842–846.
16. W. M. A. Niessen, Liquid Chromatography–Mass Spectrometry, Chromatographic Scientific Series, Vol. 79, Marcel Dekker, New York, 1999.
17. Rosen J, Hellenas KE. Analysis of acrylamide in cooked foods by liquid chromatography tandem mass spectrometry. Analyst. 2002; 127(7):880-2.
18. Choi BK, Hercules DM, Gusev AI. Effect of liquid chromatography separation of complex matrices on liquid chromatography–tandem mass spectrometry signal suppression. Journal of Chromatography A. 2001 Jan 12; 907(1- 2):337-42.
19. Shen H.W., Yu A.M. Conference report: New analytical technologies for biological discovery. *Bio-analysis.* 2010; 2:181–184. DOI: 10.4155/bio.09.175
20. Cech NB, Enke CG. Practical implications of some recent studies in electrospray ionization fundamentals. Mass Spectrom Rev. 2001; 20(6):362-87. DOI: 10.1002/mas.10008, PMID 11997944.
21. 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.
22. 33. Bhavyasri K, Begum S and Sumakanth M: 2-Dimensional gas chromatography-Mass spectroscopy: a review, International Journal of Pharmaceutical Sciences Review and Research 2022;76(1): 140-150. 34.
23. Bhavyasri K, Reddy DE and Rambabu D: Gas chromatography-mass spectrometry (GC-MS) and its applications – a review. International Journal of Research and Analytical Reviews, 2019; 6(1): 456-465.
24. Khagga Bhavyasri, Amina Fatima, R. Swethasri and Sumakanth M: Role of LC-MS in Pharmacokinetic and Pharmacodynamic

Studies In Bioanalysis: A Review, Journal of Global Trends in Pharmaceutical Sciences 2021;12(1):8887-8891.

25. Dr. K. Bhavyasri, Samreen Begum, Dr. Mogili. Sumakanth: 2-Dimensional gas chromatography-Mass spectroscopy: a review, International Journal of Pharmaceutical Sciences Review and Research 2022;76(1):140-150.
26. Dr.K. Bhavyasri, E. Divya Reddy, Dr.D. Rambabu: Gas chromatography- mass spectrometry (gc-ms) and it's applications – a review, International Journal of Research and Analytical Reviews 2019;6(1):456-465.
27. Bhavyasri K, Ch. Dhana Lakshmi and Swethasri R: Surface Enhanced Raman Spectroscopy. Panacea Journal of Pharmacy and Pharmaceutical Sciences 2019;8(1):01- 07.
28. Bhavyasri K and Mounika G: Capillary Electrophoresis - Mass Spectrometry (CE-MS) and it's applications – A Review, International Journal of Scientific Research and Reviews 2019; 8(2):3161-3176.
29. Sri BK, Samreen Begum, R. Swethasri and Mogili. Sumakanth: Raman Spectroscopy in Reverse Engineering, J Pharmacy Science and Research 2022;14(7):776-782. 26.
30. Bhavyasri K, Ch. Dhana Lakshmi and Swethasri R: Surface Enhanced Raman Spectroscopy. Panacea Journal of Pharmacy and Pharmaceutical Sciences 2019;8(1):01-07.
31. Swethasri R, Bhavyasri K and Soundarya P: Thin layer chromatography surface enhanced Raman spectroscopy, Universal Review Journal 2019;8(4):1374-1379.
32. Skoog, D.A.; Holler, F.J.; Crouch, S.R "Principles of Instrumental Analysis" 6th ed. Thomson Brooks/Cole Publishing: Belmont, CA 2007.
33. Guijo A, Hartmann F, Wudy A. Introduction to gas chromatography-mass spectrometry. Methods Mol Biol 2013; 1065(1):27-44. DOI: 10.1007/978-1-62703-616-0_3; PMID: 23996356. 15. Honour JW. Gas chromatography-mass spectrometry. Methods Mol Biol. 2006;324:53-74. DOI: 10.1385/1-59259-986-9:53; PMID: 16761371.
34. Bhavya Sri K, Begum S, Swethasri R, Mogili. Sumakanth: Raman spectroscopy in reverse engineering. J Pharm Sci Res. 2022; 14(7):776-82.
35. Swethasri R, Bhavyasri K, Soundarya P. Thin layer chromatography surface-enhanced Raman spectroscopy. Universal Review-Journal. 2019;8(4):1374-9.
36. Bhavyasri K, Sindhu K, Rambabu D. A Review: Liquid Chromatography-Nuclear Magnetic Resonance spectroscopy (LC-NMR) and its applications. 2019;9:18-24.