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e development of the bio analytical techniques brought a progressive discipline that the longer term holds many exciting opportunities to further improvement. e main impact of biolysis within the pharmaceutical industry is to get a quantitative measure of the drug and its metabolites. e purpose is to perform the pharmacokinetics, toxic kinetics, and bioequivalence and exposure response like pharmacokinetic/pharmacodynamics studies. Various bio analytical techniques are performed in bio analytical studies such as hyphenated techniques, chromatographic techniques, and ligand binding assays. is review extensively highlights the role of bio analytical techniques and hyphenated instruments in assessing the bio analysis of the drugs [1].

e eld of bio analysis has matured signi cantly from early studies in drug metabolism using many simple and advanced techniques, and in today's Bio analyst is well equipped to deal with the modern challenges. A bio analytical method may be a set of procedures involved within the collection, processing, storage, and analysis of a biological matrix for a compound. Bio analytical method validation (BMV) is the process used to establish that a quantitative analytical method is suitable for biochemical applications. Biolysis covers the quantitative measurement of Xenobiotic of drugs such as their metabolites, and biological molecules in unnatural locations or concentrations and Biotic like macromolecules, proteins, DNA, large molecule drugs, metabolites in biological systems. Bio analysis is a progressive discipline for which the future holds many exciting opportunities to further improve sensitivity, speci city, accuracy, e ciency, assay throughput, data quality, data handling and processing, analysis cost and environmental impact. e main impact of bio analysis within the pharmaceutical industry is to get a quantitative measure of the drug or its metabolites for the study of pharmacokinetics, toxic kinetics, bioequivalence and exposure-response like pharmacokinetic/ pharmacodynamics studies.

e focus of bio analysis within the pharmaceutical industry is to supply a quantitative measure of the active drug and/or its metabolite (s) for the aim of pharmacokinetics, toxic kinetics, bioequivalence and exposure–response (pharmacokinetics/pharmacodynamics studies).

e reliability of analytical ndings may be a matter of great importance in forensic and clinical toxicology, because it is in fact a prerequisite for proper interpretation of toxicological ndings. Unreliable results won't only be contested in court, but could also cause unjusti ed legal consequences for the defendant or to wrong treatment of the patient. In the last decade, similar discussions are happening within the closely related eld of pharmacokinetic (PK) studies for registration of pharmaceuticals [2].

As per Bioanalytical Method Validation (BMV) guidelines for industry, these guidelines are applied to bioanalytical methods that are used for the quantitative determination of medicine and their metabolites in biological matrices such as plasma, urine and preclinical studies. Bioanalytical method validation includes all of the procedures that demonstrate that a speci c method developed and used for quantitative measurement of analytes during a given biological matrix is reliable and reproducible. Validation of a bioanalytical method is that the process by which it's established that the performance characteristics of the tactic meet the wants for the intended bioanalytical application. ese performance characteristics are expressed in terms of bioanalytical method validation parameters.

Bioanalytical liquid chromatography-mass spectrometry may be a technique that uses liquid chromatography with the mass spectrometry. LC-MS is usually utilized in laboratories for the quantitative and chemical analysis of drug substances, drug products and biological samples. LC-MS has played a signi cant role in evaluation and interpretation of bioavailability, bioequivalence and pharmacokinetic data. rough LC-MS biological samples are determined throughout all phases of method development of a drug in research and internal control [3].

Method of study are being routinely developed, improved, validated, collaboratively studied and applied. Chromatographic separations are mainly required which depend on the samples to be analyzed. e chromatographic procedure is important for the systemic approach to LC-MS/MS method development. In most cases as desired separation can be achieved easily with only a few experiments. In other cases a considerable amount of experimentation may be needed.

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Collect the physicochemical properties of drug molecules from the literature.Determine solubility pro le, MS scanning and optimization, Mobile phase selection, Selection of extraction method and optimization, Selection of chromatographic method (based on solubility study, retention of compound)

Reversed Phase Chromatography: Reversed phase packing's like C18, C8 are the foremost popular and most generally used for reversed phase. In addition to those C4, C2 and phenyl bonded also are available. Reversed phase sorbents generally involves conditioning with an organic solvent (e.g. methanol) followed by an aqueous solvent (e.g. water).

Normal Phase Chromatography: Normal phase packing's include silica, amino and alumina. Normal phase packing generally requires conditioning with a non-polar solvent and elution is carried with polar solvents. Compounds which are with basic pH functional groups are retained by silica. However, polar compounds are irreversibly retained on a silica surface and during this case amino could also be used [4].

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Proper knowledge about the sample is important for an e cient method development. Some information regarding the analyte is important like

Number of compounds present, Molecular weights of compound, Sample Solubility, Drug Stability, Concentration range of compounds in samples of interest

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During the optimization stage, the initial sets of conditions that were evolved during the tactic development are improved and maximized in terms of resolution and peak shape, plate counts asymmetry, capacity, elution time, detection limits, limit of quantization, and overall ability to quantify the speci c analyte of interest. Optimization of a way can follow either of two general approaches like manual or computer driven. e manual approach includes varying one independent variable at a time, while holding all others constant, and recording the changes in response . e variables might include ow rates,mobile or stationary phase composition, temperature etc.

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Since most of the pharmaceutical compounds are polar in nature so reverse phase chromatography is normally tried rst in which a non-polar stationary phase is used. e mobile phase consists of water or bu er and organic phase (acetonitrile or methanol). Hence polar compounds get eluted rst and non-polar compounds are retained for a extended time. e stationary phases utilized in reverse phase chromatography are n-octadecyl (RP-18), n-octyl (RP-8), ethyl (RP-2), phenyl, cyano, diol and hydrophobic polymers. It is the rst choice for most samples; especially neutral or un-ionized compounds that dissolve in water-organic mixtures. Normal phase is tried if reverse phase fails where the sample could also be strongly retained with 100% acetonitrile as mobile phase [5,6].

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