INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES
[ISSN: 0975-4725; CODEN(USA): IJPS00]
Journal Homepage: https://www.ijpsjournal.com

OPEN ACCESS

Review Article

Recent Advances in RP-HPLC Linked Bioanalytical Assay with Applications: An Overview

Pratiksha Rajguru*[1], Kiran Mali[2], Dr. R. D. Chakole[3], Kajal Bansode[4], Kiran Ukey[5], Rugved Sathwane[6], Vinayak Rasal[7]

[1,2,4,5,6,7]*Research Student, Government College of Pharmacy Karad, Shivaji University Kolhapur, Maharashtra, India-41514.*
[3]*Associate Professor, Government College of Pharmacy, Chh. Sambhajinagagr, Maharashtra, India- 431005.*

ARTICLE INFO

Published: 25 June 2025
Keywords:
Chromatography, HPLC, Bio-analytical study, RP-HPLC, Plasma analysis
DOI:
10.5281/zenodo.15739458

ABSTRACT

Background: Chromatography has been a pillar of separation techniques for many years. Variation is the basis of the separation process in the rates at which a solvent (mobile phase) passes through a medium (stationary phase). Chromatography comes in various forms, each with its separation technique and stationary and mobile phases. However, the separation of biological material is difficult. Among chromatographic techniques, reverse-phase liquid chromatography is the preferred technique to detect the analyte concentration in plasma at therapeutic range. Bioanalytical techniques measure the concentration of drugs, metabolites, and +biomarkers in physiological fluids. So, the invention of a bio-analytical technique using RP-HPLC is a breakthrough for the chemical profiling of different medications. Need: Bioequivalence and bioavailability studies significantly rely on RP-HPLC. It helps to identify trace levels of numerous medications and metabolites in biological samples and to evaluate how well a medication is absorbed, distributed, metabolized, and eliminated from the body. Application: Bioanalytical methods involving RP-HPLC are widely used today, particularly in the pharmaceutical, clinical, and biotechnological industries, drug discovery, etc. The main objective of this study is to outline the most recent innovation in bioanalytical methods utilizing RP-HPLC for various synthetic and natural drugs. This article discusses the application of RP-HPLC in the development of bioanalytical methods. Conclusion: The effective creation of a bio-analytical method based on RP-HPLC for several drugs was presented in the paper. This established a benchmark for analysts to use the created techniques for various in vivo investigations.

***Corresponding Author:** Pratiksha Rajguru
Address: *Research Student, Government College of Pharmacy Karad, Shivaji University Kolhapur, Maharashtra, India-41514.*
Email ✉: Pratiksharajguru2000@gmail.com
Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

INTRODUCTION

Analytical chemistry copes with the chemical profiling of compounds by improving the identification of compounds with quantitative investigation. The compounds studied may be elements, samples, or mixtures [1]. Drug discovery, method development, and innovations of drug delivery systems heavily rely on analytical technique development and validation, and hence analytical chemistry serves as a milestone in the pharmacy sector. The most potent analytical method available to contemporary chemists is most likely chromatography. The word "chromatography" originates from the words "chromo" (color) and "graph," which means to write; in other words, during measurement or analysis, color groups are formed by the separation of different compounds [2]. Its ability is what gives it the strength to quantitatively identify different components in mixtures by using a single analytical procedure [3, 4]. Achieving an acceptable separation at an appropriate timeframe is the ultimate objective of using chromatography. To achieve this objective, a variety of chromatography techniques have been established. More than any other chromatographic technique, High-Performance Liquid Chromatography is frequently utilized to identify, quantify, and purify the parts of mixtures as well as to separate them because of its great precision, sensitivity, and adaptability. Numerous substances, including those that are challenging to study using other techniques, can be effectively separated and analyzed thanks to it. HPLC is frequently used in industries like pharmaceuticals, food, and environmental testing because of its quick, repeatable results and adaptability in combining with different detectors [5, 6].

RP-HPLC:

In the historical background, Howard and Martin (1950) investigated an alternate method named reversed-phase mode as a modern method of separation of substances in HPLC [7]. The chromatographic method known as Reverse phase liquid chromatography [RP-HPLC] is often employed to separate polar and non-polar substances based on their hydrophobicity [8, 9]. The original purpose of RP-HPLC is to separate physiologically significant chemicals in an open column. RP-HPLC has advanced quickly since Halasz and Sebastian introduced chemically bonded packing in 1969, even though its application was limited in the absence of stability of stationary phase packings [10]. According to estimates, more than 65 percent (maybe even 90 percent) of all HPLC separations are performed in this mode. This is due to the method's ease of use, adaptability, and scope, which permit it to deal with chemicals with a variety of molecular masses of molecule and polarities [11-13].

Theory of RP-HPLC:

The dissolved substance in RP-HPLC comes into contact with both the stationary and mobile phase, which is usually an immobilized hydrophobic ligand, to facilitate separation. Although there is significant debate regarding its exact nature, it is widely believed that hydrophobic binding interactions are primarily driven by positive entropy influence. The first stage of mobility conditions in RP-HPLC is mostly water-based, suggesting a degree of organized water structure around the molecule of solute and immobilized ligand. The amount of hydrophobic surface exposure to solvent is decreased as the solute binds to the hydrophobic ligand that has been immobilized, leading to a favorable increase in binding. Compared to bulk water, water near hydrophobic zones is thought to be more ordered. When hydrophobic patches come into contact,

some of the "structured" water is displaced, which ultimately enhances the system's total entropy. When using reversed-phase chromatography, moieties of solutes with distinct levels of hydrophobicity can adsorb or desorb on a stationary phase, i.e., hydrophobic is crucial for achieving separations [14-15].

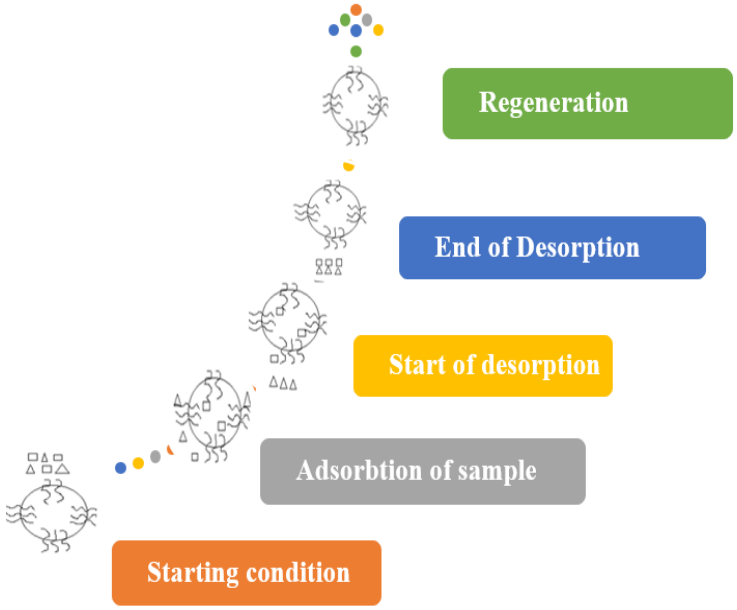

Fig. no. 1 Principle of Reverse-Phase High-Performance Liquid Chromatography

RP-HPLC enlightens the path over conventional HPLC in different ways. It is possible to separate ionic and ionizable compounds with a great deal of flexibility using selective equilibria like ion pair and ion suppression. The stationary phases are chemically bound, which makes the columns stable and the separations repeatable. Due to the small surface energies of bound phases, re-equilibration time is minimal and analyses are quick (typically requiring just 5–10 column volumes to reach equilibrium). In reverse-phase liquid chromatography, physical-chemical characteristics, including hydrophobicity, dissociation constant, and complexation constant, can be measured. It states the challenges to comprehend how research, product development, and quality control in the pharmaceutical, biomedical, and life sciences might appear today if reversed-phase liquid chromatography had not been created. For the aforementioned reasons, this field currently makes extensive use of RP-HPLC [16]. Though there are so many advantages of RP-HPLC, it also possesses challenges like PH Range Limitation, Presence of Unreacted Silanol Groups, and Complex Retention Mechanism. To overcome these challenges, some approaches were adopted as

1. The issue with unreacted Silanol groups can be addressed by treating the silica surface using a silylating chemical such as trimethylchlorosilane, removing multiple retention mechanisms, and increasing column-to-column repeatability of retention times.

2. The intricacy of the retention system is an area of ongoing research, with progress being made in many laboratories.

3. Because separations can be carried out directly within the specified pH range or by secondary

chemical equilibria such as ion pairing, the pH range restriction does not affect the adaptability of RPLC. A method based on bioanalysis for the simultaneous measurement of pharmaceuticals was developed utilizing RP-HPLC in blood plasma. Due to the excellent sensitivity and specificity, RP-HPLC techniques are well-suited for identifying trace amounts of medications and molecules in complex biological media, including urine, serum, and plasma. The RP-HPLC technique can produce results rapidly, which is essential when pharmacokinetic research requires time-sensitive data. According to studies, RP-HPLC techniques may recover 95–100% of different medicines, demonstrating efficient extraction and quantification. Therefore, with its excellent sensitivity, specificity, and capacity to evaluate intricate biological materials, RP-HPLC is a potent instrument in the development of bio-analytical methods. Over the last thirty years, significant advancement has been made in the creation and verification of the bioanalytical approach. At various points in time, the Food and Drug Administration (FDA), European Medicine Agency (EMA), China Food and Drug Administration (CFDA), Centre For Drug Evaluation and Research (CDER), Global CRO Council (GCC), European Bioanalytical Forum (EBF) and Anvisa (Brazil) the collaborated to control and coordinate the validation and development of bioanalytical technique. There are three workshops conducted on bio-analytical methods as follows:

Table 1. AAPS /FDA Workshop of bio-analytical method development [17]

Event	Venue	Report	Organizers	Key Focus
Dec 3-5, 1990	Arlington, Virginia.	Pharmaceutical Research and four other journals published this report. Focus on bio-analytical method validation and development for pharmacokinetics and studies of bioavailability and bioequivalence.	AAPS, FDA, FIP, HPB, AOAC	Analytical method development, including the definition of assay and method used in pharmacokinetics, bioavailability, and bioequivalence studies.
Jan 2000	-	"Bioanalytical Method Validation: A re-examination with ten years of development" – the report. Guidance of the FDA on the creation and validation of bioanalytical methods in May 2001.	AAPS, FDA	Review and progress of validation of bioanalytical method over the decade.
May 1–3, 2006	Arlington, Virginia	Several suggestions for advancing the development and validation of bioanalytical methods. White paper on validating bio-analytical procedures released.	AAPS, FDA	Suggestions on achieving effective validation of the bioanalytical technique.

Bioanalytical Method Development:

The development of bioanalytical techniques is among the bottlenecks for drug development [18]. The Growth of delicate, reliable, and selective techniques that are bioanalytical for statistical analysis of medication and its metabolite is essential to successful drug development. Investigation of New Drug Application (NDA), Abbreviated New Drug Application on toxicokinetic and pharmacokinetic study needs the information obtained from these methods [19]. The suggested bioanalytical techniques must be properly planned, adequately verified, and recorded to a high enough standard for usage in drug analysis to produce reliable results. Preparation of the sample, analyte separation, and analyte detection are the three essential, interrelated components that comprise the development of a method [20, 21].

Sample Preparation:

Preparation of the sample is necessary for the unambiguous identification, validation, and measurement of the analyte since it affects almost all subsequent assay stages [22,23]. The following are some objectives of sample preparation-

- ✓ Removal of matrix components
- ✓ The analyte concentration needed to reach the analytical instrument's detection limits.
- ✓ The analyte solvent or solution is changed to make it suitable for injection into a chromatographic apparatus as the
- ✓ mobile phase.
- ✓ Elimination of substances that can clog the chromatographic tube or contaminate the detector's interface
- ✓ Analyte stabilization to prevent enzymatic or hydrolytic breakdown

Traditional sample preparation methods, which involve numerous processes, are still quite time-consuming and labor-intensive. As a result, during the previous decade, numerous innovative techniques for sample preparation have been created. Newly developed sample preparation methods include solid phase microextraction, liquid-liquid microextraction, pressurized liquid extraction, extraction using restricted access material, microextraction using packed solvent, molecularly imprinted polymer, monolith spin extraction, turbulent chromatography, salting out liquid-liquid extraction, stir bar sorptive extraction, and others in addition to widely used conventional and automatic SPP, LLE, PP techniques. [22].

a) **Protein Precipitation (PP):**

Plasma and blood samples can be extracted via protein precipitation. This involves the denaturation of proteins through various substances, including salts (like ammonium sulfate), organic solvents (including acetone, acetonitrile, methanol, and acids (like perchloric and trichloroacetic acid). The analyte can be extracted into the precipitating solvent by centrifuging the solvent after denaturation. To minimize interference from external substances or endogenous molecules, solid-phase extraction (SPE) is often combined with protein precipitation. Methanol is commonly chosen as the organic solvent because it produces a pure supernatant that may be directly introduced into LC-MS/MS. Other precipitation options besides acids and organic solvents include salts [24].

b) **Liquid-Liquid Extraction (LLE**):

This process typically involves dispersing analyte molecules between the Biphasic system and extracting the analyte from one phase into another. After extraction, after being separated from the hydrophilic phase, the organic layer evaporated to obtain the dry form of the sample, often using nitrogen gas. Direct polar analyte injection is

feasible and has been extracted into the aqueous phase onto a reverse phase column [25]. This method is not suitable for thermolabile substances due to the high temperatures involved during evaporation [26].

c) **Solid phase Extraction (SPE):**

Sample loading, conditioning, cleaning, and elution are the four main processes of solid phase extraction. In this, a column is activated by using an organic solvent, solvating the functional group of the sorbent, and moistening of packing material. Water or an aqueous buffer is introduced to provide a suitable mechanism of adsorption. For sample loading methods like gravity feed, pumping, and vacuum aspiration, pH adjustment was adopted. In the washing step, it retains the analyte while eliminating matrix interferences. Lastly, in elution, analyte-sorbent interactions were distributed using the appropriate solvent to minimize residual interferences [27].

Biological matrices used in bioanalysis:

Examining a variety of biological matrices is necessary for bioanalytical studies, including plasma, serum, blood, hair, breast milk of humans, urine, perspiration, saliva, tissue, and cerebrospinal fluid. Furthermore, every matrix has unique challenges. As an example, urine has a lot of salt in it, but plasma has more phospholipids [28, 29]. Traditionally, bioanalysis makes considerable use of biofluids including serum, blood, plasma, saliva, tissue, and urine. Hair, breast milk of humans, and excrement have also been utilized as biological specimens in recent times. In post-mortem research, hair exhibits a high degree of deterioration because it is a solid, sturdy matrix that is manageable and rarely interfered with when being collected [30]. Breast milk from humans is a great indicator of drug use and environmental contaminants [31]. It is well-recognized that the excretion of drugs and metabolites in breast milk presents a serious risk to nursing mothers. The easiest way to evaluate a drug's pharmacokinetics is to measure its concentration in the blood, serum, or plasma. Given that a drug in the plasma and the tissues is in a state of dynamic equilibrium, changes in the concentration of medication in the plasma will correspond with changes in the concentration of medication in the tissues [32]. Since it frequently takes time to process or purify biological samples, ideal storage conditions for biological samples must be determined. Oxidation-sensitive samples can be effectively preserved in airtight containers. Drugs that are sensitive to moisture can be mostly dehydrated via lyophilization or freeze-drying [33]. In sample preparation, pre-treatment of both plasma and serum samples is not required if the analyte is protein-bound. One of the following approaches can be used in such circumstances.

- By adding acids or bases at concentrations of 0.1M or higher, the sample's pH is adjusted to pH 9. Separate the resulting supernatant and use it as the sample for extraction.
- Protein from biological fluid can be precipitated using a polar solvent such as acetonitrile, methanol, or acetone in a 1:2 ratio by centrifugation, and the supernatant is used for the extraction.
- Centrifugation, homogenization, and hydrolysis of the conjugate are pre-treatment techniques used if the analyte is protein-bound. [34].

Further, RP-HPLC method development for Bioanalytical study includes several crucial steps. These procedures all work together to guarantee that the resulting technique is reliable, precise, and repeatable for measuring medications or metabolites in highly complex biological matrices.

From the literature survey of drugs to the extraction of drugs from biological matrices, the selection of internal standards and methods with the initial process parameters starts. After analyzing the analytical method in aqueous standards and then in the biological matrix, after optimization. At the end, the developed method validation is performed [35, 36]. The fact that bioanalytical techniques and processes are always evolving and becoming better, and that they frequently represent the state of the art. It's equally important to emphasize that every bioanalytical method has unique characteristics that differ depending on the analyte. In these cases, it could be necessary to create unique validation standards for every analyte [37]. Bioanalytical method validation involves various types and levels that must be understood to meet the basic requirements of the process. [38, 39] All criteria affecting the quality of the data including selectivity, accuracy, precision, recovery, linearity, calibration model, and the limit of detection, lower limit of detection, stability, reproducibility, and ruggedness are included in fundamental parameters for validation of chemical assay [40, 41, 42, and 43]. The chromatographic conditions involved in RP-HPLC are summarised in Table 2

Table no. 2 chromatographic conditions for RP-HPLC

Mobile phase	**A linear gradient of solvent A (involving mobile phase) and solvent B (involving an organic solvent like acetonitrile)**
Stationary phase	A nonpolar stationary phase, like a C8 or C18 linear alkyl chain, covalently bonds to column material.
pH	The acidic mobile phase, usually at low pH, is used to study peptides.
Flow rate	Commonly in the range of 1- 2ml/min.
Temperature	Maintained at 40°C or higher
Detection	UV detection at wavelengths 200-400nm is used for drug analysis.

The assay of compounds using RP-HPLC techniques with bioanalytical method development.

The development of RP-HPL methods is an essential step in bioanalytical testing, which is used to examine both synthetic and natural medications. The separation, identification, and quantification of Active Pharmaceutical Ingredients (APIs) in intricate biological matrices are common uses for this technology. Clinical and pharmaceutical research relies heavily on RP-HPLC because it guarantees precise and dependable analysis by optimizing parameters including detection wavelength, flow rate, stationary phase composition, and mobile phase.

Natural products: From the discovery to the development stage of natural active compounds, the pharmacokinetic characterization of a chemical molecule generally includes sample preparation and bioanalysis method validation [45].

Hypering, Quercetin, Quercitroside, Myricitrin: This is the first publication to concurrently identify the four main compounds of Folium Rhododendron Micranthi using the HPLC method. Using a gradient mobile phase made up of 1% acetic acid and acetonitrile at a flow rate of 1.0 mL/min and a detection wavelength of 355 nm, the best separation and detection conditions were obtained on a C18 analytical column. Within test ranges, all calibration curves displayed strong

linear regression (r > 0.9993). Analyte recovery was higher than 99.4%, and repeatability of relative standard deviations was less than 2.3%. According to the findings, Folium Rhododendri Micranthi contained 0.166%, 0.303%, 0.299%, and 0.053% of myricitrin, hyperin, quercitroside, and quercetin, respectively [46].

Curcumin and quercetin: Two significant flavonoids with excellent anti-inflammatory, antioxidant, antidiabetic, analgesic, and anti-cancer properties are curcumin (CUR) and quercetin (QUE). The two medications were simultaneously estimated in the plasma of rats using a bioanalytical technique developed using RP-HPLC. Fisetin served as the internal benchmark. A Glacial acetic acid (GAA) of 2% and acetonitrile (ACN) 1ml/min of flow rate was used for gradient elution. All three phytoconstituents' chromatograms were captured at a detection wavelength of 392 nm. The method of protein precipitation was used to extract the medications from the samples of plasma. Fisetin, QUE, and CUR were shown to have retention times (Rts) of 4.2, 5.5, and 12.1 minutes, respectively. It was found that the devised approach was linear in the 2–10 mg/mL range, with regression coefficients (r2) for QUE and CUR of 0.9998 and 0.9993, respectively. In plasma samples, the Limit of Detection (LOD) and Limit of Quantification (LOQ) were determined to be 0.18 and 0.54 mg/mL for QUE and 0.35 and 1 mg/mL for CUR, respectively [47].

Docetaxel and Curcumin: According to FDA standards, the current HPLC approach for the simultaneous assessment of Docetaxel and Curcumin in rat plasma is evaluated with an internal reference that is straightforward, sensitive, accurate, robust, and repeatable. The HPLC apparatus of Agilent 1260 Infinity was used to analyze the samples under isocratic conditions, taking advantage of a Capcell Pak C8 column (4.6 mm × 150 mm, 5 µm). Acetonitrile and triple-distilled water (40/60, v/v) at a 1ml/min flow rate made up the mobile phase. Curcumin and docetaxel were measured simultaneously in the eluent by monitoring it at 230 nm. This new approach will be used to conduct more research on the pharmacokinetics following oral delivery of a unique DTX/CCM-loaded oral product to rats [48].

Fisetin: Fisetin, a significant phyto-flavonoid, has excellent antioxidant, Parkinson's disease, and anti-cancer properties. A C-18 reverse phase column in conjunction with reverse phase ultra-fast liquid chromatography was used to design and evaluate a bioanalytical method for estimating the amount of fisetin in the rat's plasma. An internal standard was quercetin. Acetonitrile and orthophosphoric acid (0.2% v/v) in a 30:70 v/v ratio made up the mobile phase. The chromatogram of both substances was detected at a wavelength of 362 nm, and the flow rate was set at 1 mL/min. Drugs were extracted from plasma samples using the protein precipitation method. Fisetin and quercetin were discovered to have retention periods of 8.3 and 16.9 minutes, respectively. Pharmacokinetic and biodistribution studies of the medication in bulk or fisetin found in different pharmaceutical or neutraceutical formulations can be investigated further using the established approach. Additionally, pharmaceutical organizations can utilize it for routine quality control analysis to estimate fisetin [49].

Folic acid: This research work uses methylparaben as an internal reference, which highlights the development of a bioanalytical method for determining the folic acid amount in rat plasma. On an ODS C18 column that is inert (4.6 x 100 mm, 5 µm), chromatographic separation was

carried out using an isocratic elution system potassium phosphate buffer-based mobile phase: methanol having a ratio of 75:25 v/v. With a column temperature of 40°C and a pressure of 88–90 bars, the flow rate of the mobile phase was maintained at 1 ml/min. Folic acid was detected at a maximum wavelength of 283 nm. Folic acid and Methylparaben were found to have retention times of 6.4 and 13. This study may serve as a reference for some bioanalytical method development investigations of both new and existing drug candidates. Validation of the folic acid bioanalytical method by using various parameters by international regulatory criteria is also included in this work [50].

Gallic acid: A bioanalytical approach was also created to assess the gallic acid amount by RP-HPLC utilizing the gradient elution method in the rat plasma. For the development of this bioanalytical approach, a 4.6*150 mm Zorbax SB C18 5µ column was utilized. The produced mobile phase was filtered using a Millipore 0.45 µm filter. At 30 °C, the column temperature was maintained. The flow rate of the mobile phase was set at 1.0 milliliters per minute. At 271 nm, a PDA-style detector was employed. Acetonitrile (ACN) with 0.08 percent formic acid (B) and water with 0.1 percent formic acid (A) made up the mobile phase. It was determined that the developed approach detailed in this report was firmly within a reasonable range. In the future, this technique can be effectively applied to pharmacokinetic, bioequivalence, and therapeutic drug monitoring studies in clinical laboratories to estimate Gallic acid either by itself or in conjunction with another analyte or marker that is present in bulk or an extract that contains different phytoconstituents [51].

Ellagic acid: RP-HPLC was used in the current investigation to develop an innovative, dependable, and economical bioanalytical approach for measuring EA in Wister rat plasma. To measure the quantity of EA in plasma, this study used columns from Zorbax SB (C18, 5 µm, 4.6 × 150 mm; Santa Clara, CA, USA) and Ascentis (C18, 5 µm, 100 × 4.6 mm; Bellefonte, PA, USA). The temperature of the column was kept at 30 °C. The flow rate of the mobile phase was set at 1.0 milliliters per minute. At 254 nm, a PDA-style detector was employed. The gradient chromatographic techniques were used to conduct the tests. The mobile phase consisted of 0.1% formic acid in water (A) and ACN with 0.08% formic acid (B). This currently developed and validated RP-HPLC method satisfies all validation requirements, and the approach demonstrated no matrix effect on RT [52].

Xanthohumol: This is a bioactive chemical found in beer that has a tangible impact on beverages. XH is derived from the female Hops plant, which is a member of the Fabaceae family. This study describes how different researchers created a bioanalytical technique for detecting xanthohumol in rat plasma, urine, and feces. After researching every technique, I've discovered that they're all costly and take longer to analyze the samples. Thus, by ICH M10 requirements, this research has concentrated on the development and validation of the straightforward, reliable, economical, and time-efficient RP-HPLC bioanalytical method using UFLC. The isocratic elution method was employed with a run time of 10 min. The mobile phase ratio of 0.1% v/v OPA (A): Methanol (B) was 15:85 v/v at a flow rate of 0.8 mL/min and an injection volume of 20 µL. A wavelength of 370 nm was used to record the chromatograms of curcumin and XH. Curcumin and XH had retention times of 5.8 and 7.4 minutes, respectively. The method of protein precipitation was used to extract the spiked XH from plasma.

All results of the validation parameter fall inside the recognized boundaries [53].

1. Synthetic drug:

Cetirizine, Levocetirizine: The method used to analyse pharmaceuticals in plasma after oral delivery of Levocetirizine (5 mg) with Cetirizine (10 mg) in Pashtun smokers and non-smokers from various districts in Khyber Pakhtunkhwa, Pakistan, is studied in this paper. Cetirizine and Levocetirizine levels in human plasma were determined using the reverse-phase high-performance liquid chromatography method, which was created and validated. Methanol was used to extract the drug from human plasma, and 97% of the drug was recovered. On an ACE Generix 100-5, (250 × 4.6 mm, 5 µm) C18 RP column, the pharmaceuticals were separated using a mobile phase consisting of distilled water and acetonitrile in a ratio of 41:59 v/v at 1.5 ml/min flow rate, 3.39 ± 0.02 min of retention time having a wavelength of detection 230 nm, and column oven temperature of 26°C [54].

Remogliflozine Etabonate: This article details how the LC-MS method has been applied to ascertain the concentration of Remogliflozine in rat plasma. Furthermore, techniques for measuring REM levels in bulk and tablet formulations using RP-HPLC and HPTLC have been published. However, there is currently no technique for employing RP-HPLC to measure REM in human plasma. This research aims to develop a novel, accurate, and reliable RP-HPLC technique for measuring REM in human plasma. The analysis of the Chromatogram was carried out at 224 nm on a THERMO C18 (250×4.6 mm, 5 µm) column that uses a mobile phase made up of a methanol-0.1% acetic acid combination (80:20 v/v) with a flow rate of 1 mL/min. The linearity, precision, accuracy, and robustness of the approach were all confirmed. Forced degradation tests were carried out under acidic/alkaline hydrolytic, peroxide, and photolytic conditions by ICH recommendations. It was found that the created RP-HPLC technique was linear, accurate, precise, and robust; as a result, it could be effectively applied for routine drug quantification and stability monitoring in biological samples [55].

Cinnarizine and Domperidone: Simultaneous estimation of these drugs in biological fluid, particularly in plasma, is necessary to comprehend the pharmacokinetic characteristics of this drug combination. To achieve this objective, a bioanalytical method based on RP-HPLC was developed and verified in which Irbesartan (IRB) is used as an internal standard (IS) for the detection of these medications in rat plasma. The protein precipitation technique was used to extract IS and (CIN & DOM), the analytes from plasma samples. A C18 Sunfire TM analytical column (5 µm, 250 mm × 4.6 mm) is used to perform chromatographic separation. A mobile phase consisting of acetonitrile and methanol, which is isocratic in 30:70 ratios, is used at a 1 ml/min flow rate. A UV detector was used to record the detection of all three components at a wavelength of 270 nm. With a total run time of 10 minutes, DOM, CIN, and IS were eluted at 3.2, 4.5, and 6.1 minutes, respectively [56].

N-nitroso dimethylamine: Control of N-nitrosamine impurity is crucial for ensuring the safety of pharmaceutical products. The authors of the in vivo study that was published on ranitidine, one of these compounds, have now retracted it, indicating that ranitidine may be converted in vivo to N-nitrosodimethylamine (NDMA), a likely human carcinogen. It was necessary to characterize NDMA production using a bioanalytical approach. A bioanalytical technique was thus developed and validated. For this, the analyte is extracted by using liquid-liquid

extraction and mixed with an internal standard. The temperature of the column was kept at 25°C. For a gradient elution, a mobile phase made up of 0.1% formic acid and 10mm ammonium formate in water (A), and 100% acetonitrile (B) was employed at a 0.4 ml/min flow rate. It was discovered that the developed method for NMDA was validated within an acceptable range [57].

Selenium nanoparticles: This study gives a general overview of SeNPs, their possible use in treating Alzheimer's disease, and the primary bioanalytical methods used in this area. The primary bioanalytical methods utilized in cellular research about AD will be presented in this section. These methods fall into three subgroups:

a) Cytotoxicity tests
b) Accumulation and investigation of intracellular effects
c) Evaluation of oxidative stress.

Table no. 3 bioanalytical methods of selenium nanoparticles used in cellular research [58]

cytotoxicity test	Accumulation and intracellular effect	Oxidative stress
• UV-VIS method • flow cytometry	• Microscopy techniques • Fluorescence spectroscopy • Inductively coupled plasma techniques	• Fluorescence microscopy • Flow cytometry • Fluorescence spectroscopy

Pyrimethamine: A liquid oral version of PYR was created to enhance the treatment of kids with Congenital Toxoplasmosis; it was modified for a small number of dosages and for infants who are unable to swallow capsules. For this reason, this study was published. To investigate the stability of the PYR suspension, a stability-indicating method and a dosing method were required. A Kinetex Core-shell C18 column, thermostated at 40°C, connected to a diode array (λ=230 nm, 280 nm, scan from 190 to 400 nm) and an acetonitrile/methanol/KH2PO4 buffer gradient (pH 3, 10 mm) was used for the HPLC analyses. By ICH guidelines, the quantification process was linear, specific, accurate, and precise. Using this HPLC-UV approach, a stability study of the formulation under development will be conducted [59].

Escitalopram: The invention and validation of the bioanalytical method for estimating the amount of Escitalopram in human plasma using the RP-HPLC method is the basis of this review study. Extraction is done by the solid phase method. Using 0.2% ortho-phosphoric acid and acetonitrile in a 65:35% v/v ratio as the mobile phase. Samples were separated on an Enable C18 column at a flow rate of 1.0 ml/min at room temperature. The detection was done at 240 nm. By ICH requirements, all analytical validation parameters were established in this research study. This study concludes that this approach is appropriate for routine quantitative analysis in pharmaceutical dosage forms and can be applied to pharmacokinetic and toxicological investigations of Escitalopram oxalate [60].

Amlodipine Besylate: The goal of this research work is to use RP-HPLC to estimate amlodipine besylate in the plasma of humans in a straightforward, accurate, and precise bioanalytical manner that complies with US FDA

regulations. The method used for chromatographic separation was conducted utilizing a Shimadzu LC-2010 CHT with LC solution software and a UV detector, and the Shimadzu ATY 224 electronic balance was used for weighing. The "Waters" C18 column was used for the separation process [61].

Table no. 4 Details of chromatographic parameters

Sr. no.	Condition/Parameter	Details
1	Column	Waters C18 5µm (4.6 x 250mm)
2	Mobile phase	Buffer: ACN: Methanol (20:70:10 v/v/v)
3	Flow rate	1.0 ml/min
4	Column temperature	28 °C
5	Volume of injection	20 µl
6	Detection wavelength	360 nm

Applications:

The development of bioanalytical techniques utilizing RP-HPLC is essential for the examination of different drugs. To ensure the safety and effectiveness of medications in therapeutic applications, RP-HPLC is frequently used for pharmacokinetic investigations, bioequivalence testing, and quality control.

Table No. 5 Summary of Applications of Analytical Techniques

Method	Drug	Conclusion	Detector	Ref
RP-HPLC	Sorafenib	Reduced solvent usage, short retention period, accurate peak symmetry, robustness, and precision	1260 Diode Array Detector.	62
	Metformin	Optimal retention period of 4.4 min. And strong chromatographic peak separation and resolution.	UV-detector, 233 nm	63
	Dapagliflozin	The developed technique is a good assay and purity method that can be used to analyze dapagliflozine in various formulations.		64
	Ribavirin	Regarding specificity, precision, linearity, quantification limit, detection limit, and accuracy, a reverse phase HPLC isocratic technique has been designed and verified in compliance with ICH criteria regarding the quantitative measurement of ribavirin in tablets.	Shimadzu SPD-20A Prominence UV-VISIBLE detector	65
	secnidazole	The created approach is proven to be Lambert-Bear's Law compliant, specific, straightforward, accurate, reproducible, dependable, linear, and proportional.	Alliance 2695 with a 2487 dual wavelength detector	66
	Darunavir ethanolate	Because the procedure was straightforward, sensitive, accurate, and exact, it can be used regularly to determine DRV in pharmaceutical and bulk materials.	SPD-10 Avp UV detector	67
	Mesalamine	As demonstrated above, the method's accuracy, reproducibility, repeatability, linearity, precision, and selectivity all attest to its dependability. The comparatively short run time (10 minutes) allows for quick sample quantification in regular and quality-control tablet evaluation.		68

	Sofasbuvir	The technique offers notable benefits over previously documented methods in terms of reduced retention time, selectivity, and accuracy.	Photo Diode Array detectors	69
	Aliskiren	TThe system suitability experiments were conducted, all parameters were merged with simplicity, and the devised RP-HPLC method was validated.	SPD-10AVP UV-Visible detector	70
	silodosin	The verified approach was determined to be straightforward, accurate, and exact.	UV detector	71
	quercetin	The new approach is suitable for routine quercetin quantification and quality control as well as in vivo animal research due to its short chromatographic run time (13 min).	UV2075plus intelligent UV detector	72
	Meropenem	The selected method for the validation of Meropenem by RP-HPLC was found to be specific under the study	UV detection of 300 nm.	73
	Daclatasvir dihydrochloride	It was determined that a straightforward, accurate, quick, precise, repeatable, and reasonably priced RP-HPLC method with a high correlation coefficient was created during the current investigation for the measurement of Daclatasvir dihydrochloride in pharmaceutical solid dosage forms.	UV detection at 308 nm.	74
	Curcumin and piperine	For the simultaneous measurement of curcumin and piperine, a straightforward, precise, and economical reversed-phase HPLC approach was created in this study.	UV visible detector	75
	Ropinirole HCL	According to the study's findings, the suggested RP-HPLC method for estimating Ropinirole hydrochloride is straightforward and precise for both prescription dosage forms and bulk	UV detector at 245nm	76
	Ofloxacin and tinidazole	Two formulation components can be routinely analyzed using this created approach.	PDA detector	77
Bioanalytical method	Sofosbuvir	The extraction process recommended is simpler, faster, more reliable, and more sensitive than earlier techniques.	photodiode array detector model 2996	78
BAn ioanalytical method by RP-HPLC	ticagrelor	The RP-HPLC method that was developed turned out to be straightforward, accurate, exact, and sensitive for estimating Ticagrelor in human plasma.	Detection wavelength of 254 nm.	79
Bioanalytical method by HPLC MS /MS	Verapamil and enlapril	In conclusion, a highly sensitive, specific, repeatable, quick, and high-throughput LC-MS/MS assay was developed and validated to measure verapamil and enalapril in human plasma in the presence of enalaprilat, by regulatory criteria.		80

The bioanalytical method by RP-HPLC	Cefixime and dicloxacillin	The technique is suitable for routine therapeutic drug monitoring and emphasizes measuring the levels of Cefixime and dicloxacillin in the bloodstream.	UV/Vis detection at 225 nm	81
	Latanoprost	The results confirm accuracy, linearity, and precision, and the HPLC method is relatively simple.	UV detector at 210 nm	82
	Velpatasvir and Sofosbuvir	The developed bioanalytical technique is simple, precise, and reproducible. It is applicable for measuring Velpatasvir and Sofosbuvir in plasma.	UV detector wavelength is 254 nm.	83
Bioanalytical method by LC-MS	Metaxalone	A highly sensitive, specific, and repeatable isocratic LC approach, combined with a mass detection method, is considered superior due to the widely acknowledged advantages of LC over other techniques.	UV spectroscopy with LC Chromatography method (HPLC-UV) , GC with flame ionization detection, and GC with mass detection.	84
The bioanalytical method by RP-HPLC	Chlorthalidone and clonidine	The technique is effective for regular therapeutic drug monitoring and specifically measures the blood levels of the medications Chlorthalidone and cilnidipine.	248 nm with a photodiode array (PDA) detector.	85
The bioanalytical method by RP-UFLC	Herbal quercetin	The technique can function as a stand-alone element or in combination with other methods for food product analysis, pharmacokinetics, and bioequivalence profiling.	Photodiode array detector	86
Bioanalytical method	Metoprolol and medium	An LC-MS/MS assay with good sensitivity, specificity, repeatability, speed, and throughout was created and verified to measure Metoprolol and meldonium in human plasma, as required by regulations.	ZORBAX HILIC Plus	87
Bioanalytical method	lenvatinib	For clinical laboratory pharmacokinetic and therapeutic drug monitoring, the method is straightforward, quick, accurate, precise, and appropriate.	photodiode array detector	88
Bioanalytical method by HPLC	Deferasirox	Deferasirox can now be detected in human plasma using a fast, sensitive, specific, accurate, and reliable bioanalytical method, thanks to recent advancements.	Younglins Acme 9000 LC system was equipped with a UV detector	89
The bioanalytical method by RP-HPLC	levocetirizine	The current procedure was found to be quick and simple based on the results from HPLC investigations.	UV-PDA detector set at 232 nm	90
The bioanalytical	Thiocolchicoside and lornoxicam	Thiocolchicoside and Lornoxicam were analyzed simultaneously in human plasma and pharmaceutical dosage forms using a	PDA detection at 295 nm	91

method by RP-HPLC		validated isocratic HPLC assay, ensuring accuracy and precision.		

Summary of challenges:

The Bioanalytical method development is a complicated process that calls for an agile methodology. Researchers may thus encounter several difficulties when creating and approving biological analytic techniques. Among the most frequent issues mentioned by FDA reviewers are:

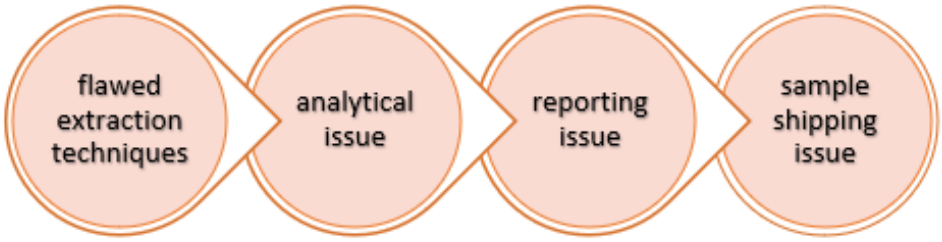

Figure 2: Challenges in bioanalytical method development

a) **Flawed extraction techniques:** A crucial step in bioanalysis is extraction, which entails both getting rid of any contaminants and getting the material ready for examination. A variety of extraction processes are employed.

Typical varieties consist of, but are not restricted to:

Liquid extraction, protein precipitation, and extraction in the solid phase (SPE).

b) **Analytical Issue:** Problems may come up during the sample analysis phase in addition to the sample preparation phase.

- **The matrix**: Common matrices such as plasma, serum, urine, and tissues have varying consequences for sample clean-up and interference accounting.

- **The quantity of samples**: Developing an optimal approach may depend critically on the quantity of samples and analysis.

- The quantity of analytes that need to be measured will expand the extent of the method development procedure and make the separation more complex.

- **Analyte Pharmacokinetic Profile**: By knowing the state's pharmacokinetic profile, the scientist can identify lower quantization levels.

c) **Reporting issues**: Method validation and analytical reports are the two primary report formats produced by bioanalytical labs. These might exist as independent documents or as appendices.
The following aspects should be covered in the analytical report: The title page, Statement on GLP adherence, and quality control. Table of study summaries Description of the method Substances Standards for preparation and quality control Procedures for receiving and storing samples

d) **Problems with sample shipping**: The necessary protocols to be adhered to for gathering biological samples should be described by investigators. Additionally, the guidelines for sample management must be

unambiguous. To maintain their integrity, the samples might be kept frozen for transportation or kept at a particular temperature. Additionally, they can require storage for a predetermined amount of time.

CONCLUSION:

The many techniques and validation processes that are encountered when analyzing various study samples are covered in this article. We have examined crucial facets of creating and approving bioanalytical techniques to raise the standard and increase acceptability in this area of study. The simple sample preparation technique of this method and very short chromatographic time enable the quick processing of several samples in pharmacokinetic research. The process satisfied the US FDA's validation requirements. As a result, the method described can be used for pharmacokinetic studies and therapeutic medication monitoring in humans. High accuracy and good precision were found when results were validated in compliance with ICH and EMA requirements. Recent developments in several areas, such as sample preparation, separation techniques, matrix effect reduction, and particular recommendations for bioanalytical method validation, all of which are covered in this review, support the idea that RP-HPLC has emerged as a preferred method for bioanalysis of small molecules.

REFERENCES

1. Gupta S, Vera P, Mishra AP, Omar N, Mathura R. A Review on Novel Analytical Method Development and Validation by RP-HPLC Method. Indian Journal of Forensic Medicine & Toxicology. 2021 Sep 5; 15(4):3479-86.
2. Skoog DA, West DM, Holler FJ, Crouch SR. Fundamentals of analytical chemistry. Fort Worth: Saunders College Pub.; 1996.
3. Scott RPW: Principles and Practice of Chromatography. Chrom Ed Book Series, 2003; 1-2.
4. Chatwal GR. Instrumental methods of chemical analysis. Himalaya Publishing House; 2022 Mar 10.
5. Harwood LM, Moody C. Experimental organic chemistry: principles and practice. (No Title). 1989 Apr.
6. Akshata M. G, Bhupendra M. M, Amitkumar R. D, Sunil P. P, Review on: RP-HPLC, HBRP Publication, Journal of Advances in Experimental Therapeutics and Neurotherapeutics 2024 Dec 2; 21-27.
7. Howard, G. A., and Martin, A. J. P. (1950) Biothem. J. 46, 539-548. 7. Halasz, I., and Sebastian, I [cross reference]
8. Snyder LR, Kirkland JJ, Dolan JW. Introduction to modern liquid chromatography. John Wiley & Sons; 2011 Sep 20.
9. Ahuja S. Overview of modern pharmaceutical analysis. In Separation Science and Technology 2011 Jan 1 (Vol. 10, pp. 1-9). Academic Press.
10. Halasz, I., and Sebestian, I. (1%9) Angew. Chem. 8, 453-454. [Cross reference].
11. Willard HH, Merritt LL, Dean JA, Settle FA. Instrumental methods of analysis, CBS Publishers and Distributors. New Delhi. 1986; 580:626.
12. Connors AK. A Text Book of Pharmaceutical Analysis. A Wiley Interscience publication, 3rd edition 2005; 373-400
13. Ahuja S. High-pressure liquid chromatography. Comprehensive Analytical Chemistry. 2006 Jan 1; 47:485-559.
14. Dorsey JG, Cooper WT: Retention mechanisms of bonded-phase liquid chromatography. Anal. Chem. 66th edition 1994; 857A-867A

15. Stanford CW: Physical chemistry of macromolecules 1961.
16. Poole CF, Poole SK. The column in liquid chromatography. The Essence of Chromatography; Elsevier: Amsterdam, the Netherlands. 2003:267-429.
17. Deshpande MM, Pasture VS, Mohan M, Chaudhari SR. Practical approach for development and validation of bioanalytical method: A review. Inventi Rapid: Pharm Analysis & Quality Assurance. 2016; 2017(1):1-8.
18. Moein MM, El Beqqali A, Abdel-Rehim M. Bioanalytical method development and validation: Critical concepts and strategies. Journal of Chromatography B. 2017 Feb 1; 1043:3-11.
19. Pushpa Latha E, Sailaja B. Bioanalytical method development and validation by HPLC: a review. J Appl Pharm. 2014 Dec; 1:1-9.
20. Pushpa Latha E, Sailaja B. Bioanalytical method development and validation by HPLC: a review. J Appl Pharm. 2014 Dec; 1:1-9.
21. Chandramouli R, Kumar P, Bibhishan KV. Analytical method development and validation for pre-clinical analysis. Journal of Pharmaceutical Sciences and Research. 2010 Dec 1; 2(12):795.
22. Nováková L, Vlčková H. A review of current trends and advances in modern bio-analytical methods: chromatography and sample preparation. Analytical chimica acta. 2009 Dec 10; 656(1-2):8-35.
23. Chapter 2 Fundamental Strategies for Bioanalytical Sample Preparation [Cross Reference].
24. Charade M, Welankiwar A, Kumar J, Chakole R. Bioanalytical method development and validation. Int J Adv Pharm Anal. 2013; 3:90-4.
25. Wal P, Kumar B, Bhandari A, Rai AK, Wal A. Bioanalytical method development–Determination of drugs in biological fluids. Journal of pharmaceutical science and technology. 2010; 2(10):333-47.
26. Rahman A. Bioanalytical Method Development, Validation, and Techniques Used for Pharmacokinetic Studies Using LC-Ms/Ms. Contemporary Investigations and Observations in Pharmacy. 2012; 1(2):63-71.
27. Kirthi A, Shanmugam R, Prathyusha MS, Basha DJ. A review of bioanalytical method development and validation by RP-HPLC. Journal of global trends in pharmaceutical sciences. 2014; 5(4):2265-71.
28. Vaghela A, Patel A, Patel A, Vyas A, Patel N. Sample preparation in bioanalysis: a review. International Journal of Scientific & Technology Research. 2016 May; 5(05):6-10.
29. Murugan S, Elayaraja A, Chandrakala K, Ramaiah P, Vulchi C. A review of method development and validation by using HPLC. International journal of novel trends in pharmaceutical sciences. 2013 Jul 10; 3(4):78-81.
30. Reid E, Wilson ID. Analysis for drugs and metabolites, including anti-infective agents. In International Bioanalytical Forum 1989: Guilford, England) 1990. Royal Society of Chemistry.
31. European Medicine Agency (EMA) –July 2011,1- 23 [cross reference]
32. Vaghela A, Patel A, Patel A, Vyas A, Patel N. Sample preparation in bioanalysis: a review. Int J Sci Technol Res. 2016;5(5):6-10..
33. Pushpa Latha E, Sailaja B. Bioanalytical method development and validation by HPLC: a review. J Appl Pharm. 2014 Dec; 1:1-9.
34. LK T, NT R, UN M. A review on bioanalytical method development and validation. Asian J Pharm Clan Res. 2016; 9(3):6-10.
35. Mujewar IN, Bhusnure OG, Jag tap SR, Gholve SB, Giram PS, and Savangikar AB. A review of bioanalytical method development

and various validation stages involved in method development using RP-HPLC. Journal of Drug Delivery and Therapeutics. 2019 Aug 25; 9(4-s):789-95.

36. Chauhan A, Singh S, Chandarana C, Prajapati P. A Comprehensive Review on Bioanalytical Method Development and Validation for Pharmaceuticals.
37. Wood R. How to validate analytical methods. TrAC Trends in Analytical Chemistry. 1999 Sep 1; 18(9-10):624-32.
38. Charade M, Welankiwar A, Kumar J, Chakole R. Bioanalytical method development and validation. Int J Adv Pharm Anal. 2013; 3:90-4.
39. Taleuzzaman M, Gilani SJ, Ali S, Hafeez A, Nagarajan K. Bio-Analytical Method Validation-A Review. Austin J Anal Pharm Chem. 2015; 2(5):1053.
40. http://www.fda.gov/downloads/Drugs/Guidances/ucm 070107.pdf [cross reference]
41. S.kargerGmbh and Freiburg. SOP 12: Validation of Bioanalytical methods, Standard Operating Procedure for Clinical Trials of the CESAR, Oncology, 2003 ;(supply 6):52-55. [Cross reference].
42. Tiwari G, Tiwari R. Bioanalytical method validation: An updated review. Pharmaceutical methods. 2010 Oct 1; 1(1):25-38.
43. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/08/WC500109686.pdf
44. Tiwari G, Tiwari R. Bioanalytical method validation: An updated review. Pharmaceutical methods. 2010 Oct 1; 1(1):25-38.
45. Ayumiati I, Saputri FA, Muchtaridi M. Sample preparation and bioanalysis validation for natural product samples. International Journal of Applied Pharmaceutics. 2021 Mar 7; 13(2):33-47.
46. Xiuling Yang, Xiaowei Zhang, Zhifang Yuan, Xiaona Li, Lantong Zhang, Lifang Fan, Simultaneous Determination of Myricitrin, Hyperion, Quercitroside, and Quercetin in Folium Rhododendron Micranthi by RP-HPLC, Journal of Chromatographic Science, Volume 47, Issue 8, September 2009, Pages 714–717, https://doi.org/10.1093/chromsci/47.8.714
47. Khursheed R, Wadhwa S, Kumar B, Gulati M, Gupta S, Chaitanya MV, Kumar D, Jha NK, Gupta G, Prasher P, Chellappan DK. Development and validation of RP-HPLC-based bioanalytical method for simultaneous estimation of curcumin and quercetin in rat plasma. South African Journal of Botany. 2022 Sep 1; 149:870-7.
48. Kim DW, Yousaf AM, Li DX, Kim JO, Yong CS, Cho KH, Choi HG. Development of RP-HPLC method for simultaneous determination of docetaxel and curcumin in rat plasma: Validation and stability. Asian Journal of Pharmaceutical Sciences. 2017 Jan 1; 12(1):105-13.
49. Kumar R, Kumar R, Khursheed R, Awasthi A, Khurana N, Singh SK, Khurana S, Sharma N, Gunjal P, Kaur J, Corrie L. Development and validation of RP-HPLC method for estimation of fisetin in rat plasma. South African Journal of Botany. 2021 Aug 1; 140:284-9.
50. CHAUDHARY A, SHRANGARE G, BHUSARI S. Bioanalytical method development and validation of folic acid from rat plasma using reverse-phase high-performance liquid chromatography. Journal of Research in Pharmacy. 2023 Jul 1; 27(4).
51. Mane VA, Killedar SU, More HA, Gaikwad AS, Tare HA. A novel RP-HPLC gradient elution technique for bioanalytical method development and validation for estimating Gallic acid in waste rat plasma. Int J App Pharm. 2023 Mar 7; 15(2):153-60.
52. Mane V, Anorak N, Ghorpade V, Shinde T, Kolekar A, Gore S. Development and

Validation of a Bioanalytical Method for Estimating Ellagic Acid in Wistar Rat Plasma by RP-HPLC Using a Gradient Elution Technique. BIO Integration. 2024 Aug 1; 5(1):975.

53. Harish V, Almalki WH, Alshehri A, Alzahrani A, Alzarea SI, Kazmi I, Gulati M, Tewari D, Chellappan DK, Gupta G, Dua K. Bioanalytical Method Development, Validation and Stability Assessment of Xanthohumol in Rat Plasma. Molecules. 2022 Oct 21; 27(20):7117.
54. Khattak M, Iqbal Z, Jan A, Ali N, Nasir F, Shahbaz N. Development and Validation of Rapid and Cost-Effective RP-HPLC/UV Method for Determination of Cetirizine/Levocetirizine in Blood Plasma of Smokers and Non-Smokers: a Two Cohort Comparative Study. Journal of Chromatographic Science. 2024 Nov 14:bmae058.
55. Waditake PD, Kolhe MH, Mate MK, Bhor RJ, Bhalerao PS, Mhaske MP. Stability Indicating Bioanalytical Method Development and Validation for Estimation of Remogliflozin Etabonate by RP-HPLC in Human Plasma. International Journal of Pharmaceutical Investigation. 2024 Oct 1; 14(4).
56. Vij M, Dand N, Kumar L, Ankalgi A, Wadhwa P, Alshehri S, Shakeel F, Ghoneim MM, Alam P, Wani SU. RP-HPLC-Based Bioanalytical Approach for Simultaneous Quantitation of Cinnarizine and Domperidone in Rat Plasma. Separations 2023, 10, 159 [Internet]. 2023
57. De Palma R, Patel V, Florian J, Keire D, Selaya D, Strauss DG, Rouse R, Matta MK. A Bioanalytical Method for Quantification of N-nitrosodimethylamine (NDMA) in Human Plasma and Urine with Different Meals and Following Administration of Ranitidine. Journal of Pharmaceutical Sciences. 2023 May 1; 112(5):1315-23.
58. Vicente-Zurdo D, Rosales-Conrado N, León-González ME. Unraveling the in vitro and in vivo potential of selenium nanoparticles in Alzheimer's disease: A bioanalytical review. Talanta [Internet]. 2024; 269(125519):125519. Available from: http://dx.doi.org/10.1016/j.talanta.2023.125519
59. Muhammad S, Ribier Z, Benoit G, Bereave J. Stability indicating RP-HPLC method development and validation for the determination of pyrimethamine in an oral pediatric suspension. Journal of Pharmaceutical and Biomedical Analysis Open [Internet]. 2024; 4(100035):100035. Available from: http://dx.doi.org/10.1016/j.jpbao.2024.100035
60. Deepthi V, Dharuman J, Neetha K. Bioanalytical method development and validation for the estimation of Escitalopram in human plasma using RP-HPLC method. Int. J. Creative Res. Thoughts…2022 Nov.
61. Jain HK, Mane RR. Bioanalytical Method Development and Validation for Estimation of Amlodipine Besylate in Human Plasma Using RP-HPLC. Int. J. PharmTech Res. 2016;9:233-9.
62. Sankar PR, Viswanath A, Eswarudu MM, Babu PS, Divya B, Neelima K, Devi KR. Development and validation of RP-HPLC method for the determination of sorafenib in the pharmaceutical dosage form. Int. J. Pharm. Sci. Rev. Res... 2021 Jul; 69:15-23.
63. Arayne MS, Sultana N, Zuberi MH. Development and validation of RP-HPLC method for the analysis of metformin. Pak J Pharm Sci. 2006 Jul 1; 19(3):231-5.
64. Debata J, Kumar S, Jha SK, Khan A. A New RP-HPLC method development and validation of dapagliflozine in bulk and tablet dosage form. Int J Drug Dev Res. 2017; 9(2):48-51.

65. Babu GR, Rao AL, Rao JV. A rapid RP-HPLC method development and validation for the quantitative estimation of ribavirin in tablets. Int J Pharm Sci. 2015; 7:60-3.
66. Farooqui NA, Smith AA, Sharma HK, Manavalan R. Analytical method development and validation of secnidazole tablets by RP-HPLC. Journal of Pharmaceutical Sciences and Research. 2010 Jul 1; 2(7):412-6.
67. Patel BN, Suhagia BN, Patel CN. RP-HPLC method development and validation for estimation of Darunavir ethanolate in tablet dosage form. International Journal of Pharmacy and Pharmaceutical Sciences. 2012; 4(3):270-3.
68. Moharana AK, Banerjee MR, Sahoo CK, Kanta NA. Development and validation of RP-HPLC method for mesalamine. Asian J. Pharm. Clin. Res. 2011; 4:71-3.
69. Rani JS, Devanna N. A new RP-HPLC method development and validation of simultaneous estimation of Sofosbuvir and Velpatasvir in pharmaceutical dosage form. Int. J. Eng. Technol. Sci. Res. 2017 Nov; 4:145-52.
70. Swamy GK, Rao JV, Kumar JR, Kumar UA, Bikshapathi DV, Kumar DV. Analytical method development and validation of aliskiren in bulk and tablet dosage form by RP-HPLC method. Journal of Pharmacy Research. 2011 Mar; 4(3):865-7.
71. Goud VM, Rao AS, Ranjan SP, Shalini SD, Sowmya S, Bhoga B. Method development and validation of RP-HPLC method for assay of Sildosin in pharmaceutical dosage form. Inter. J. Pharma. Sci. 2013 Mar; 3:194-6.
72. Sanghavi N, Bhosale SD, Malode Y. RP-HPLC method development and validation of Quercetin isolated from the plant Tridax procumbens L. Journal of Scientific and Innovative Research. 2014; 3(6):594-7.
73. Negi V, Chander V, Singh R, Sharma B, Singh P, Upadhaya K. Method development and validation of meropenem in pharmaceutical dosage form by RP-HPLC.
74. Shah SH, Nasiri MI, Sarwar H, Ali A, Naqvi SB, Anwer S, Kashif M. RP-HPLC method development and validation for quantification of Daclatasvir dihydrochloride and its application to the pharmaceutical dosage form. Pak. J. Pharm. Sci. 2021 May 1; 34(3):951-6.
75. Khismatrao AN, Bhairy SR, Hirlekar RA. Development and validation of RP-HPLC method for simultaneous estimation of curcumin and piperine. Int J Appl Pharm. 2018; 10(5):43-8.
76. Sreekanth N, Rao B, Mukkanti K. RP-HPLC method development and validation of Ropinirole hydrochloride in bulk and pharmaceutical dosage forms. Int J Pharm Pharm Sci. 2009; 1(1):186-92.
77. Dharuman J, Vasudevan M, Somasekaran KN, Dhandapania B, Ghodea PD, Thiagarajana M. RP-HPLC method development and validation for the simultaneous estimation of Ofloxacin and tinidazole in tablets. Int. J. Partech Res. 2009 Apr; 1(2):121-4.
78. Madhavi S, Rani AP. Bioanalytical method development and validation for the determination of Sofosbuvir from human plasma. Int J Pharm Pharm Sci. 2017 Mar; 9(3):35-41.
79. D'cruz D, Babu A, Joshy E, Aneesh TP. Bioanalytical method development and validation of ticagrelor by RP-HPLC. Int J Appl Pharm. 2017 May 1; 9:51-4.
80. Logoyda L. Bioanalytical method development and validation from the simultaneous determination of verapamil and enalapril in the presence of enalaprilat by HPLC MS/MS. Int J Appl Pharm. 2018; 10:19-27.
81. Bhinge SD, Malipatil SM, Sonawane LV. Bioanalytical method development and validation for simultaneous estimation of

Cefixime and dicloxacillin by RP-HPLC in human plasma. ActaChimicaSlovenica. 2014 Sep 1; 61(3).

82. Patel P, Patel P, Giram P. Bioanalytical method development and validation for Latanoprost quantification in pharmaceutical ophthalmic microemulsion formulation by RP-HPLC. J Anal Bioanal Tech. 2015 Nov 1; 6(284):2.
83. Phani RC, Prasad KR, Mallu UR. Bioanalytical method development and validation for simultaneous determination of Velpatasvir and Sofosbuvir in spiked human plasma. Asian Journal of Chemistry. 2017; 29(11):2565-9.
84. Kandasamy K, Gowdra VS, Nammalvar H, Govindarajan AK. Bioanalytical method development, validation, and quantification of Metaxalone in rat plasma by liquid chromatography-tandem mass spectrometry. J Bioanal Biomed S. 2012; 6:2.
85. Eswarudu MM, Rao AL, Vijay K. Bioanalytical method development and validation for simultaneous determination of Chlorthalidone and cilnidipine drugs in human plasma by RP-HPLC. International journal of research in pharmacy and chemistry. 2019; 9(1):33-44.
86. Shanmugam R, Gowthamarajan K, Priyanka DL, Madhuri K, Karri N. Bioanalytical method development and validation for herbal quercetin in nanoformulation by RPUFLC in rabbit plasma. Journal of Bioequivalence Bioavailability. 2013; 5:191-6.
87. Horyn M, Logoyda L. Bioanalytical method development and validation for the determination of Metoprolol and meldonium in human plasma. Pharmacia. 2020 Jul 31; 67(2):39-48.
88. Veni GK, Ajitha A, Abbulu K. Bioanalytical method development and validation of lenvatinib by RP-HPLC method. International journal of pharmaceutical sciences and research. 2020; 27:3313-9.
89. Kalshetti MS, Patil RY, Karale RA, Kulkarni AA. Bioanalytical method development for estimation of Deferasirox in human plasma. Int. J. Pharm. Pharm. Sci. 2015; 7(9):399-402.
90. Jain N, Jain DK, Jain R, Patel VK, Patel P, Jain SK. Bioanalytical Method Development and Validation for the Determination of Levocetirizine in Pharmaceutical Dosage Form and Human Plasma by RP-HPLC. Journal of Applied Pharmaceutical Science. 2016 Oct 29; 6(10):063-7.
91. Kumar P, Shukla S, Subudhia BB, Ganure AL. Bioanalytical method development and validation for the simultaneous estimation of Thiocolchicoside and lornoxicam in human plasma and in pharmaceutical dosage form by RP-HPLC. Intern. J. Pharm. Pharm. Sci. 2012;4:251-9.

HOW TO CITE: Pratiksha Rajguru*, Kiran Mali, Dr. R. D. Chakole, Kajal Bansode, Kiran Ukey, Rugved Sathwane, Vinayak Rasal, Recent Advances in RP-HPLC Linked Bioanalytical Assay with Applications: An Overview, Int. J. of Pharm. Sci., 2025, Vol 3, Issue 6, 4293-4313. https://doi.org/ 10.5281/zenodo.15739458