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A systematic Review on HPLC method Development and validation for the estimations of plant based and other antibiotics

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Abstract

This review discusses the development and validation of High-Performance Liquid Chromatography (HPLC) methods for the analysis of plant-based antibiotics and pharmaceutical compounds. HPLC is a widely used analytical tool in pharmaceutical research due to its high accuracy, sensitivity, and efficiency in separating, identifying, and quantifying drug components. The study highlights the significance of method development, considering factors such as molecular properties, solubility, and chromatographic conditions. The role of HPLC in ensuring drug safety, stability, and quality control is emphasized. Furthermore, the application of method validation ensures compliance with regulatory standards, enhancing the reliability of analytical results.

Key words : HPLC, Method Development, Method Validation, Plant-based Antibiotics, Pharmaceutical Analysis, Drug Stability, Quality Control, Chromatographic Techniques.

Metoprolol is used to treat high blood pressure either on its own or in conjunction with other drugs. Additionally, persistent (long-term) angina (chest discomfort) is treated with it. Metoprolol is also used to increase heart attack survivability. Metoprolol is also used to treat heart failure in combination with other drugs. Metoprolol belongs to a group of drugs known as beta blockers. In order to increase blood flow and lower blood pressure, it works by relaxing blood vessels and lowering heart rate. Untreated high blood pressure can harm the kidneys, heart, brain, blood vessels, and other bodily organs. High blood pressure is a frequent illness. Heart disease, a heart attack, heart failure, stroke, renal failure, blindness, and other issues can result from damage to these organs. Aside from medicine, altering your lifestyle can also aid in blood pressure regulation. These adjustments include cutting back on fat and salt in the diet, keeping a healthy weight, working out for at least 30 minutes every day, giving up smoking, and drinking alcohol sparingly. The medication flecainide is used to stop some potentially fatal irregular heartbeats. Flecainide belongs to the group of drugs known as antiarrhythmics. In order to stabilise the heart rhythm, it functions by slowing down electrical signals in the heart. Flecainide is available as an oral tablet. Typically, it is taken once every twelve hours. If they have negative effects or find that taking flecainide every 12 hours is not controlling their condition, some people may choose to take flecainide once every 8 hours. Take flecainide daily at around the same times. Pay close attention to the instructions on the label of your prescription and ask your chemist or doctor to explain anything you do not understand. Consume flecainide as prescribed.

HPLC Method Development and Validation:

In contemporary chemistry, high-performance liquid chromatography (HPLC) is a potent analytical instrument. It performs exceptionally well at locating, quantifying, and sorting the constituents of liquid-dissolved samples. Highly regarded for its accuracy in quantitative and qualitative evaluations, HPLC is widely used in the examination of pharmaceutical products and has made a substantial contribution to the field of analytical chemistry. An injection of a sample solution (stationary phase) is made into a porous column in high-performance liquid chromatography (HPLC). Next, a high-pressure liquid (mobile phase) is injected through the column. Because the sample's components are divided into stationary and mobile phases, they migrate through the column at varying rates. Separation is made possible by the elution that results at different times. The precision of HPLC stems from subtle component behaviours during partitioning, providing a reliable technique for examining a variety of substances in fields. A compound having a lower affinity for the stationary phase moves faster and covers a greater distance in high-performance liquid chromatography, whereas a molecule with a higher affinity moves more slowly and covers a shorter distance. The efficient separation and analysis of sample components is made possible by this differential migration. Pharmaceutical analysis

benefits greatly from HPLC's ability to effectively separate and quantify important drugs, reaction impurities, synthesis intermediates, and degradants. As a leading analytical instrument, HPLC is excellent for locating, quantifying, and separating various liquid-soluble sample components. Its accuracy is critical for both qualitative and quantitative drug product analysis, and it is essential for figuring out how stable a medication product is. As a technique that provides a thorough method for characterising pharmaceutical samples, HPLC is essential for guaranteeing the safety and quality of medicinal compositions (Fig. 1).

HPLC Method Development :

When techniques are developed effectively, laboratory resources are maximized and the goals necessary for each step of drug development are met. Method validation is the "process of demonstrating that analytical procedures are suitable for their intended use," and it is mandated by regulatory authorities at specific phases of the drug approval process. Knowing the physical and chemical properties of the drug makes it possible to choose from the extensive body of literature that is currently available the best high performance liquid chromatography method development. It is necessary to assemble information on the sample, such as its molecular mass, structure and functionality, pKa values, UV spectra, and solubility of the chemical. The need to eliminate insoluble contaminants by extraction (liquid or solid phase), derivatisation for detection, dilution, centrifugation, or other methods in order to regulate concentration (Fig. 2).

HPLC Method Validation :

The scientific soundness of the measurement or characterisation is demonstrated by the validation of an analytical method. Validation data must be supplied at every stage of the regulatory submission procedure. The validation procedure shows that an analytical technique measures the right material for the intended samples in the right amount within the right range. It enables the analyst to determine the technique's performance bounds and to comprehend the behaviour of the method. Establishing acceptance criteria and

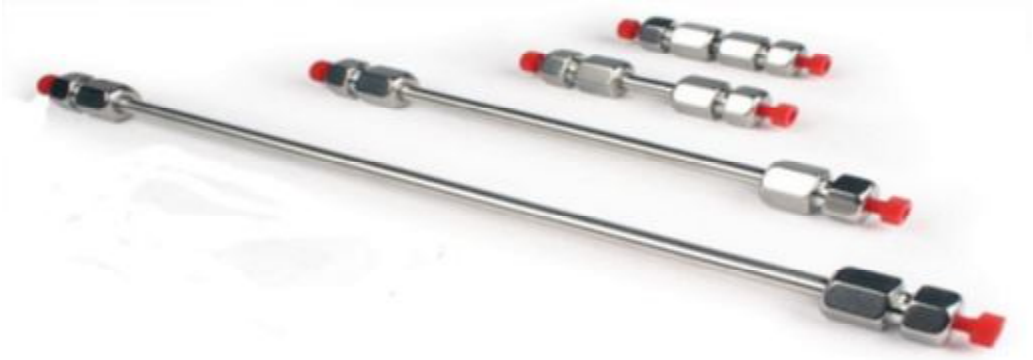

Figure 1. Columns with different length

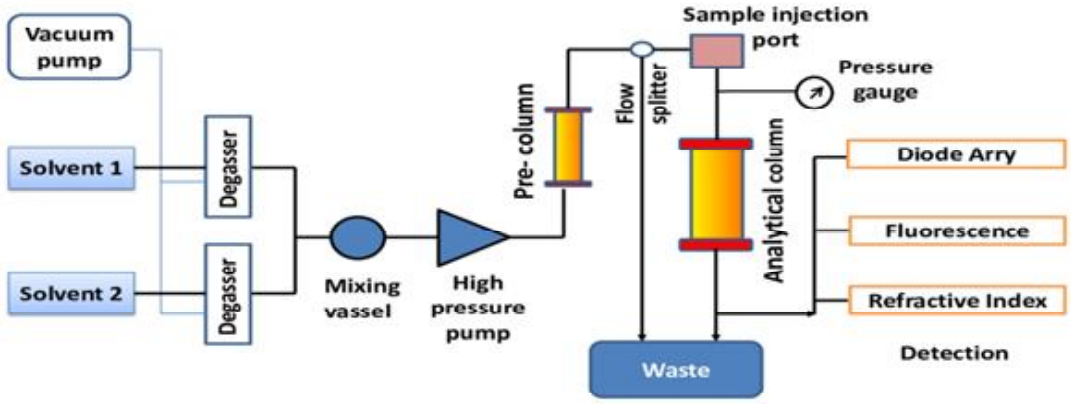

Figure 2: Schematic Diagram of HPLC

determining crucial parameters for method system suitability are the objectives. The International Organisation for Standardisation defines verification as "provision of objective evidence that a given item fulfils specified requirements," and defines validation as "verification, where the specified requirements are adequate for an intended use."

Various Validation Parameters :

Following are the different parameters for the HPLC Method Validation.

- ✓ Linearity
- ✓ System Suitability
- ✓ Accuracy
- ✓ Precision
- ✓ Robustness
- ✓ Ruggedness
- ✓ Limit of Detection
- ✓ Limit of Quantitation

Literature Review :

Dar *et. al.*[9] studied about Herein, a novel, rapid, reliable, simple method validation and simultaneous quantification of 11 bioactive compounds (mostly xanthones) have been described. International Conference on Harmonization guidelines were used for the analytical method validation. The calibration curves showed a good linear relationship ($r > 0.999$) within test range. Precision was evaluated by intra- and inter-day tests with relative standard deviation <2.79% and accuracy validation recovery of 74.16%–91.84%. On quantification study, the validated method described the high content of bioactive xanthone derivatives, including 1-hydroxy-3, 5-dimethoxyxanthone[16], 2-(allyloxy)-8-hydroxy-1, 6-dimethoxyxanthone[17] 1, 7, 8-trihydroxy-3-methoxyxanthone[18] and Coxanthone E [12] in *Codonopsis ovata*, which is advantageous given the numerous pharmacological and biological effects associated with these compounds, which mostly exhibit anti-cancerous, antioxidant, anti-inflammatory, anti-mutagenic and anti-obesity effects. The bulk abundance of these compounds can also be used for further modification to produce better lead molecules for drug discovery with low toxicity and high potency. The proposed

method makes it possible to simultaneously determine all bioactive compounds in one run and can be extended to marker-based standardization of herbal formulations in medicinal and pharmaceutical industries.[8]

Al Sheikh *et. al*.[2] studied about the pervasiveness of oral bacterial infections in diabetic patients, it is a serious health concern that may produce severe complications. We investigated 26 Ayurvedic medicinal plants traditionally used for treatment of the oral bacterial infections with the aim to look for new promising drug leads that can be further employed for herbal formulation design. The plants were grouped into three categories based on traditional usage. All plant extracts were examined for antibacterial, antibiofilm and antiquorum-sensing properties. The plants with significant activities including *Juglans regia*, *Syzygium aromaticum*, *Eruca sativa*, *Myristica fragrans*, *Punica granatum* and *Azadirachta indica* were further analyzed using HPLC-DAD-QToF and GC-MS. In silico and in vitro activity was evaluated for selected constituents. Finally, it could be concluded that eugenol and 2-phenylethylisothiocyanate are major contributors towards inhibition of bacterial biofilms and quorum sensing.[2]

Kantiani *et. al*.[13] studied about method for the detection of penicillins, cephalosporins and sulfonamides in animal feed using pressurised liquid extraction. This study presents the development and validation of a sensitive and fast (30 min extraction time and 10 min chromatographic run) method for the detection of penicillins, cephalosporins and sulfonamides in animal feed using pressurised liquid extraction and solid phase extraction as extraction and pre-concentration procedures, followed by liquid chromatography–quadrupole-linear ion-trap mass spectrometry. The developed method was validated showing limits of detection ranging from 0.12 (ampicillin) to 3.94 ng/g (amoxicillin), instrumental and analytical linearity coefficients above 0.99 in both standard and matrix-based solutions as well as relative recoveries ranging from 71% (cefoperazone) to 115% (cefazolin). Repeatability of the method was in the range of 1–9% (RSD %), whereas reproducibility ranged from 3% to 13% (RSD %). The developed and validated method was finally applied to the analysis of real feed samples. The results showed 10 out of 18 analytes to be present in at least one sample and all 14 samples to contain at least one analyte. Penicillin V, oxacillin, ceftiofur, cefoperazone, cefalexin, cefazolin, sulfamethoxypyridazine and sulfapyridine were not detected in any of the samples analysed. Considering the ban of antibacterials as growth promoters added in animal feed, this method is capable of detecting the low concentrations that could result from failure to comply with the regulations or on-site contamination.[13]

Tegegne *et.al*.[20] studied about high performance liquid chromatography (HPLC) method for the determination of thirteen selected pharmaceutical compounds in bulk and tablet dosage form. The aim of this study was to develop and validate a high performance liquid chromatography (HPLC) method for the determination of thirteen selected pharmaceutical compounds (metformin, amoxicillin, chloroquine, theophylline, trimethoprim, caffeine, norfloxacin, ciprofloxacin, acetylsalicylic acid, doxycycline hyclate, metronidazole, albendazole and cloxacillin) in bulk and tablet dosage form. Chromatographic

separation using a Kromasil C_{18} column, gradient elution with aqueous formic acid (0.1%), methanol and acetonitrile, a UV absorption wavelength of 250 nm and a mobile phase flow rate of 1 mL/min over a 22 min run time was optimized for complete separation of the selected target compounds. Calibration curve correlation coefficients ranged from 0.9985-0.9998 and the percentage relative standard deviations for repeated analysis was below 5%, indicating acceptable method precision. The limits of detection (LODs) and quantification (LOQs) ranged from 0.020-0.27 µg/L and 0.080-0.91 µg/L, respectively. The accuracy study yielded recoveries in the ranges 86.0-102% for pure compounds and 90.9-106% for compounds in tablet dosage form. The method is robust for small or deliberate changes to the chromatographic parameters and found to be appropriate for analysis of tablets for the determination of the thirteen pharmaceuticals.[20]

Kalshetti *et. al*.[12] Using a Phenomenex Luna C18 column (150 mm × 4.6 mm, 5 µm) as the stationary phase and a security guard cartridge C18 (4 × 3 mm) as the mobile phase, acetonitrile: methanol: phosphate buffer pH 7.5 (45:30:25) by isocratic elution at a flow rate of 1.0 ml/min, the RP-HPLC method has been developed for the separation and quantification of metoprolol succinate, Telmisartan, and Clinidipine. At 229 nm, the detecting process was done. For telmisartan, metoprolol succinate, and clinidipine, the eluted medicines have retention periods of 2.0, 2.8, and 6.8 minutes, respectively. For telmisartan, metoprolol succinate, and clinidipine, the regression coefficients are 0.997, 0.995, and 0.999, respectively. The approach is linear in the range of 10-80 µg / ml, 6.25-50 µg / ml, and 2.5-20 µg / ml. [12]

Pavan *et. al*.[17] A straight forward, precise, and reliable approach was created for the simultaneous measurement of Cilnidipine and Metoprolol in tablet dose form. A chromatogram (150 x 4.6 mm, 5µ) was run through an Altima. A mobile phase was pumped through the column at a rate of one millilitre per minute, comprising 0.1% OPA buffer and 45:55 v/v methanol. A constant 30°C was maintained. The ideal wavelength for cetiridipine and metoprolol was 225 nm. Metoprolol and cenidipine were shown to have retention times of 2.249 and 3.062 minutes, respectively. Metoprolol and cilnidipine assay results were 101.22% and 100.45%, respectively.[17]

Liliya *et. al*.[16] The development of analytical methods for medications belonging to the calcium channel blocker class was the search criterion. A scan of the literature covering the years 1990 to 2018 was conducted in order to keep the review thorough and up to date, as well as to highlight recent developments in the field of calcium channel blocker analysis techniques.[15]

Chaudhari *et. al*.[8] Metoprolol succinate and flecainide in a synthetic mixture can now be estimated with increased accuracy and precision thanks to a novel approach in high performance liquid chromatography. Using a 25 cm (4.6 mm x 250 mm, 5 um) ODS C18 column, the chromatographic separation was carried out at a specified temperature using a pH of 4 at a flow rate of 1 ml/min with UV detection at 222 nm. The buffer used was Buffer: Methanol: Acetonitrile (35:15:50). For

metoprolol succinate and flecainide, the approach yielded linear responses in the concentration range of 100-300 µg/ml (0.9993) and 50-150 µg/ml (0.99992), respectively.[10]

Rameshwar *et. al*.[18] With fewer drug extraction stages, the RP-HPLC method has been established together with a stability indicating attribute for the simultaneous estimation of metoprolol succinate and chlorthalidone in bulk and tablet dosage form. Utilising an Oyster ODS3, 150-4.6 mm column with a 5 µm particle size (Merck & Co.) as the stationary phase, the chromatographic analysis was carried out isocratically at a temperature of approximately 25°C with a flow rate of 1.0 mL/min. The eluent used was acetonitrile (650:350, v/v) at a wavelength of 225 nm, and the phosphate buffer had a pH of 2.3 (adjusted with 10% orthophosphoric acid). Acid, alkali, oxidative, photolytic, thermal, humidity, and other drug degradation processes were applied to individual drug components as well as combination medication products. The peaks resulting from these degradation processes were clearly distinguished from the peak of the active analytes.[18]

Ansari *et. al*.[4] For atenolol (ATE) and nifedipine (NIFE) in combination dosage form, a novel stability-indicating high performance liquid chromatography (HPLC) method has been developed and validated using various parameters. The mobile phase used to optimise the chromatographic conditions was MeOH:OPA (70:30) at a flow rate of 0.7 mL/min. A stationary phase consisting of a 4.6 × 250 mm column (C18) with a 5 µm particle size capacity was employed. At 233 nm, the detection was done. In the concentration range of 1 to 5 mcg/mL for NIFE and 20 to 100 mcg/mL for ATE, the response was shown to be linear.[1]

Doo-Yeoun Cho *et. al*.[10] were able to show that sarpogrelate is a strong and specific CYP2D6 inhibitor In vitro. In this instance, we assessed how sarpogrelate affected the pharmacokinetics and pharmacodynamics of metoprolol in individuals in good health.[9]

This stability indicates that metoprolol and cenidipine were estimated simultaneously using RP-HPLC. Phosphate buffer:acetonitrile at a 60:40, v/v ratio was found to be the optimal optimisation condition, with a flow rate of 1.0 mL/min for the mobile phase. Peaks were effectively separated using a BDS column (150 mm x 4.6 mm, 5 µm), with retention periods of 4.026 min for cenidipine and 2.679 min for metoprolol, respectively.[5]

Ansari *et. al*.[4] For the combined dosage form of atenolol and nifedipine, a new HPLC technique has been created and validated using various parameters. A MeOH:OPA (70:30) mobile phase was used to produce the chromatograms, with a flow rate of 0.7 ml/min. A stationary phase consisting of 4.6 x 250 mm C18 column with a particle size of 5 µm was employed. At 233 nm, the detection was done. For both nifedipine and atenolol, the concentration range of 1–5 mcg/ml and 20–100 mcg/ml, respectively, was observed to exhibit a linear response. For atenolol, the LOD and LOQ were determined to be 0.1415 and 0.4289, respectively, and for nifedipine, 0.1834 and 0.5558, respectively.[5,18]

Roman Grabic *et. al*. It was designed and proven to simultaneously determine over ninety drugs in water samples using a multi-residue technique. The new technique makes use of a single liquid chromatography–tandem mass spectrometry (LC–MS/MS) run following solid-phase extraction (SPE) sample enrichment. The drugs used in this procedure were selected for their potency (effect/concentration ratio) and fish bioaccumulation potential. Four types of chromatography Prior to mass spectrometry detection, columns were evaluated to maximise the separation (MS).[21]

High-Performance Liquid Chromatography (HPLC) has emerged as a vital analytical tool in pharmaceutical research, ensuring the accurate identification, quantification, and separation of various drug components. Its precision, sensitivity, and efficiency make it indispensable in drug development, quality control, and regulatory compliance. The present study highlights the importance of method development and validation in optimizing chromatographic conditions to achieve reliable and reproducible results. The effectiveness of HPLC is largely dependent on understanding the physicochemical properties of the drug, including molecular weight, solubility, UV spectra, and pKa values. Proper method development enables the selection of appropriate mobile and stationary phases, ensuring the effective separation of compounds while maintaining peak efficiency and resolution. Additionally, method validation is critical to ensure compliance with regulatory guidelines, making the analytical process robust and reproducible across different laboratories.

One of the key applications of HPLC in pharmaceutical research is in the analysis of plant-based antibiotics, which are gaining attention as potential alternatives to synthetic drugs. HPLC allows for the precise identification of active compounds in these natural formulations, ensuring their potency, stability, and safety. Furthermore, it plays a crucial role in determining impurities, degradation products, and formulation stability, all of which are essential for maintaining drug efficacy and patient safety. Overall, HPLC remains a gold standard in pharmaceutical analysis, providing a comprehensive approach to drug characteri-zation and quality assurance. Its ability to deliver precise and reliable results makes it an indispensable technique for drug discovery, formulation, and regulatory compliance. Future advancements in HPLC technology, including automation and improved detection techniques, will further enhance its applicability in pharmaceutical sciences, ensuring the continued development of safe and effective medications for global healthcare needs.

Conflict of Interest Declared None

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