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Validated RP-HPLC Method For The Simultaneous Determination Of Doxofylline In Bulk And Pharmaceutical Preparation

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ABSTRACT:

The simultaneous measurement of doxofylline in pharmaceutical formulations and bulk using a validated Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method is demonstrated. Methanol:phosphate buffer (pH 6.8, 30:70 v/v) is used as the mobile phase in a C18 column (250 × 4.6 mm, 5 µm particle size) at a flow rate of 1.0 mL/min. A wavelength of 254 nm was chosen for detection. The method's linearity, accuracy, precision, selectivity, sensitivity, and robustness were all validated in accordance with ICH requirements. Doxofylline was found to have a linearity range of 10–100 µg/mL and a correlation value (r^2) of 0.999. The technique showed outstanding accuracy, with recovery rates between 98.2% and 101.4%. The reproducibility of the approach was confirmed by the intraday and interday precision values being less than 2%. The reproducibility of the approach was confirmed by the intraday and interday precision values being less than 2%. The limit of quantitation (LOQ) was 1.0 µg/mL, while the limit of detection (LOD) was 0.3 µg/mL. Doxofylline was successfully analyzed using the validated RP-HPLC technique in both bulk and tablet formats. This technique provides doxofylline quality control in pharmaceutical preparations in a dependable, repeatable, and effective manner.

Keywords: doxofylline , Reverse Phase High-Performance Liquid Chromatography, pharmaceutical formulations

Introduction

Doxofylline

A xanthine derivative called doxofylline is frequently used as a bronchodilator to treat respiratory conditions, especially bronchial asthma, asthma, and chronic obstructive pulmonary disease (COPD). Although it shares a chemical makeup with theophylline, it is said to have a better safety record, particularly when it comes to less adverse effects including nausea, vomiting, and cardiac arrhythmias. Doxofylline improves airflow and lowers lung inflammation by relaxing the smooth muscles of the airways.

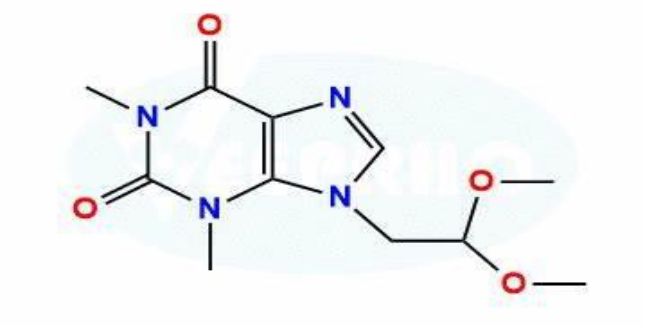

Fig 01 - structure of Doxofylline

Mechanism of Action:

- PDE, an enzyme that breaks down cyclic adenosine monophosphate (cAMP), is predominantly inhibited by doxofylline to produce its therapeutic effects. Doxofylline relaxes the muscles and widens the bronchi by blocking PDE and raising cAMP levels in the smooth muscle cells of the airways. Furthermore, by modifying several inflammatory pathways, doxofylline may provide anti-inflammatory properties that are advantageous for people with long-term obstructive airway illnesses.

Therapeutic Applications:

- Asthma: Doxofylline is used as a supplement to treat asthma, enhancing lung function and lowering symptoms such as wheeze and dyspnea.

- Chronic Obstructive Pulmonary Disease (COPD): By improving airflow and lowering the frequency of exacerbations, it helps manage COPD symptoms.
- Bronchitis: Doxofylline is sometimes administered to treat acute or chronic bronchitis by reducing inflammation and bronchospasm symptoms.

Pharmacokinetics:

- After oral treatment, doxofylline is quickly absorbed and reaches its peak plasma concentration in one to two hours. It is mostly metabolized in the liver, and most formulations allow for twice-daily administration due to its elimination half-life of about 12 hours.

Safety Profile:

- Doxofylline has less side effects than other xanthine derivatives, such as theophylline, which makes it easier for patients to tolerate. Headache, dizziness, and stomach pain are the most frequent, minor side effects. Doxofylline has a far decreased risk of major cardiovascular adverse effects, like arrhythmias, making it a better option for long-term treatment of respiratory conditions.

Dosage Forms:

- Depending on the severity of the illness and the patient's reaction to treatment, doxofylline is usually given in doses ranging from 400 mg to 800 mg daily. It comes in a variety of formulations, such as tablets, syrups, and oral solutions.

Analytical Methods

A procedure or series of operations used to ascertain a substance's composition, amount, or quality is known as an analytical method. It entails using scientific methods to determine, quantify, and describe a sample's chemical composition, physical attributes, or biological activity. In several disciplines, such as chemistry, biology, and pharmaceuticals, analytical techniques are essential for guaranteeing the efficacy, safety, and quality of goods and substances.

Analytical methods include, for example

- High-Performance Liquid Chromatography (HPLC), which is used to identify, separate, and quantify the constituents of a mixture.
- (RP-HPLC)One kind of liquid chromatography that is frequently used in analytical chemistry and biochemistry to separate, identify, and quantify components in a mixture is reverse phase high-performance liquid chromatography (RP-HPLC).
- Gas Chromatography (GC): Used to separate and analyze substances that evaporate without breaking down.
- Spectroscopy (UV-Vis, IR, NMR): This method is used to determine a compound's concentration and identify its chemical structure.

The Value of Analytical Techniques

- Quality assurance and control
- regulatory compliance
- purity and impurity determination
- stability testing
- precise active ingredient quantification
- method development and validation

RP-HPLC:

One kind of liquid chromatography that is frequently used in analytical chemistry and biochemistry to separate, identify, and quantify components in a mixture is called reverse phase high-performance liquid chromatography (RP-HPLC). It is known as the "reverse phase" because the mobile phase is polar (usually water or a buffer) and the stationary phase is non-polar (usually a C18 or C8 bonded silica column). When it comes to extracting hydrophobic substances from a combination, RP-HPLC is especially helpful.

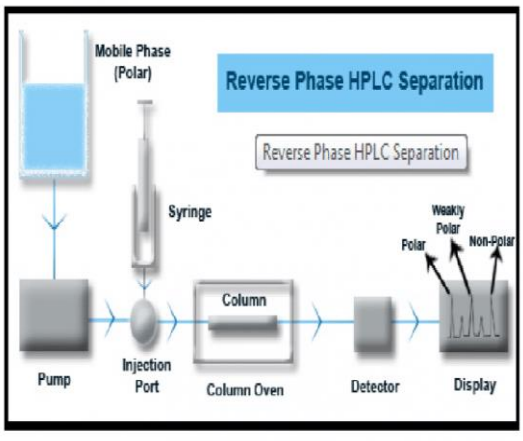

Fig 02 - RP HPLC Instrumentation **Fig 03 - RP HPLC**

Principle of RP-HPLC

A non-polar stationary phase (such a C18 column) is present in the chromatographic column into which the sample mixture is introduced in RP-HPLC. The chemicals are eluted with the aid of the mobile phase, which is often a blend of water and organic solvents such as acetonitrile or methanol. Based on their polarity and affinity for the stationary phase, the components of the sample are separated as a result of their interactions with it. Polar molecules move along the column more rapidly than hydrophobic ones, which elute longer due to their higher interaction with the stationary phase.

- Hydrophobic stationary phase: (C18, C8, or phenyl columns).
- Mobile Phase**:** A combination of polar solvents, such as buffers, methanol, acetonitrile, or water, is known as the mobile phase.
- Detector: Usually UV-Vis absorbance, but it can also be mass spectrometry, fluorescence, or refractive index.

RP-HPLC applications include:

- Pharmaceuticals: For the examination of pharmaceuticals, active pharmaceutical ingredients (APIs), and medication formulations.
- Environmental Testing: For the measurement of contaminants in soil, water, and air.
- Food and Drinks: To evaluate flavorings, preservatives, and additives.
- Clinical Research: To detect drug levels or biomarkers, biological samples (such as blood or urine) are analyzed.

Benefits of RP-HPLC

- High Resolution: Able to separate intricate compound mixes.
- Broad Range of Applications: Both polar and non-polar substances can be analyzed using this method.
- Sensitivity: High sensitivity as a result of the chemicals' efficient separation and detection.
- Quantification: Offers highly accurate quantitative results.

Method Development for Doxofylline Using RP-HPLC:

A number of important considerations must be made when creating an RP-HPLC method for the analysis of doxofylline, a bronchodilator used to treat respiratory disorders. These include choosing the right chromatographic conditions, which are essential for getting the best possible separation, resolution, and analytical accuracy. A comprehensive guide to choosing chromatographic settings for the development of the RP-HPLC method particularly for doxofylline can be found below.

1. The Stationary Phase Selection

One of the most crucial elements in the development of the RP-HPLC process is the stationary phase. The way it interacts with the components of the sample affects how the analytes are retained and separated.

Column Type: A C18 column (octadecylsilane), the most widely used stationary phase for RP-HPLC, is non-polar and perfect for separating moderately polar to non-polar substances such as doxofylline. If a less retentive phase is desired, a C8 column (octylsilane) may also be employed.

Particle Size: Although they may need more pressure, columns with smaller particle sizes (such as 3-5 μm) offer better separation and higher resolution. Doxofylline is frequently analyzed pharmaceutically using a column with a particle size of 5 μm.

2. Choosing the Mobile Phase

Because it facilitates the movement of the sample's constituent parts through the column, the mobile phase is essential to the separation process. Usually, it is a combination of water and an organic solvent, such as methanol or acetonitrile.

• Solvent Selection: Acetonitrile and water are frequently utilized as the mobile phase in the analysis of doxofylline. The way the components of the sample interact with the stationary phase depends on the polarity of the mobile phase.

• Mobile Phase pH: Doxofylline's ionization state and its interaction with the stationary phase can be affected by the pH, making it a significant factor. Weakly basic substances, such as doxofylline, are frequently better retained and resolved at a slightly acidic pH (3–4).

3. Rate of Flow

The analytical time and resolution are affected by the flow rate. While a low flow rate might lengthen analytical times and decrease throughput, an excessively high flow rate can result in poor separation.

Depending on the size and dimensions of the column, a typical flow rate for RP-HPLC analysis of doxofylline might be between 0.8 and 1.0 milliliters per minute.

4. Temperature of the Column

Resolution, retention durations, and the viscosity of the mobile phase can all be impacted by the column temperature. Pharmaceutical analysis RP-HPLC procedures typically employ column temperatures between 25°C and 40°C.

Since it provides a fair balance between resolution and analysis time, 30°C is a popular temperature setting.

5. Wavelength of Detection

The analyte's absorbance properties are used to determine the detection wavelength. In UV/Vis absorbance mode, doxofylline is best detected at a wavelength of 273 nm, where it absorbs substantially.

6. Volume of Injection

In order to prevent overloading the column and guarantee precise quantification, the injection volume should be optimized. Depending on the analyte concentration and detector sensitivity, a typical injection volume for pharmaceutical analysis could be between 20 and 50 µL.

7. Isocratic vs. Gradient Elution

The two main elution modes in RP-HPLC are gradient and isocratic.
Isocratic Elution: In this mode, the composition of the mobile phase doesn't change during the run. When assessing a single chemical, such as doxofylline, it is usually employed when the analyte separation is straightforward.
Gradient elution is a chromatographic run in which the composition of the mobile phase is altered. A more complicated method for separating combinations of chemicals with very differing polarity is gradient elution.

8. Method Validation

To guarantee the method's dependability and reproducibility after the chromatographic settings have been optimized, it should be tested using a number of parameters:

• Specificity: Doxofylline should be isolated from other substances or excipients in the formulation using a process that is specific.
• Linearity: Over a specified range of concentrations, the procedure should yield a linear response.
• Precision and Accuracy: When tested repeatedly, the procedure ought to yield precise and consistent results.
• Limit of Detection (LOD) and Limit of Quantification (LOQ): Doxofylline should be detectable and quantifiable at trace levels using this approach.

#Criteria for Validation

• Linearity: Making sure the procedure operates within the doxofylline concentration range that is necessary.
• Accuracy: Recovery studies to validate the method's dependability.
• Precision: repeatability and reproducibility within and between days.
• Selectivity: The method's capacity to separate doxofylline from other interfering substances and excipients.
Limits of quantification (LOQ) and detection (LOD) are examples of sensitivity.
• Robustness: Assessing the method's stability in the face of minor changes in the experimental setup.
• System Suitability: Examine variables such as resolution, theoretical plates, and tailing factor.

Applications of RP-HPLC for the Analysis of Doxofylline in Bulk:

1.Doxofylline Quantification in Bulk Drug Substance

Prior to being formulated into tablets or other pharmaceutical dosage forms, the concentration of doxofylline in bulk is frequently measured using RP-HPLC. A sensitive and accurate measurement of the drug's content can be obtained using RP-HPLC by selecting the right column, mobile phase, and detection wavelength.

2. Doxofylline Identification and Purity Testing

For confirming the identification and purity of bulk doxofylline, RP-HPLC is a great technique. The purity of the bulk medication can be evaluated by looking for extra peaks in the chromatogram, which can be used to identify impurities, degradation products, or related chemicals.

3. Doxofylline Stability Studies in Bulk Formulation

Stability investigations are carried out using RP-HPLC, which tracks doxofylline under various storage circumstances (such as temperature). It is possible to identify and measure the degradation products of doxofylline throughout time.

4. Bulk Doxofylline Determination for Formulation Development

When creating pharmaceutical formulations, RP-HPLC is utilized to track the amount of doxofylline present in bulk medicinal substances and assess the composition's uniformity. This aids in guaranteeing that the active ingredient is present in the formulation in the proper quantity.

5. Doxofylline Bulk Assay Method

A validated test method for assessing the potency of bulk doxofylline is developed using RP-HPLC. By using this technique, the bulk medication is guaranteed to fulfill the requirements needed to be used in pharmaceutical formulations.

6. How to Profile Impurities in Bulk Doxofylline

In bulk doxofylline, RP-HPLC is utilized to identify and measure degradation products and trace contaminants. Prior to the drug material being employed in the formulation process, this is crucial for guaranteeing its purity.

Challenges and Limitations of RP-HPLC :

1 .Matrix Effects-But like any analytical technique, RP-HPLC has drawbacks and difficulties that may impair its effectiveness. Matrix effects, solubility problems, and instrumental concerns are three major obstacles. These elements may have an effect on the accuracy, repeatability, and dependability of the results.

- Challenge:The term "matrix effects" describes the interference that might affect the chromatographic separation and quantification of the target analyte due to sample matrix, such as biological fluids, environmental materials, or intricate pharmaceutical formulations. In RP-HPLC analyses, matrix effects frequently result in decreased sensitivity, accuracy, and precision.
- Reason: Co-elution of Interfering Substances: Components in complex matrices frequently co-elute with the analyte, producing background noise or overlapping peaks. This may make it more difficult to identify and measure the target component.
- Effect: Decreased Accuracy and Sensitivity: Interfering chemicals can either amplify or decrease the analyte's signal, producing unreliable results.
- Remedies: 1. Sample Cleanup: Techniques including protein precipitation, liquid-liquid extraction (LLE), and solid-phase extraction (SPE) can lessen matrix interference.
- 2.Use of Internal Standards: By accounting for matrix effects, internal standards can increase quantification accuracy.

2. Solubility Issues

- Challenge: Solubility problems occur when the components of the analyte or sample are not very soluble in the mobile phase or need certain solvent conditions to dissolve. The accuracy of analysis may be impacted by partial dissolution, precipitation, or irregular sample concentrations caused by poor solubility.
- Causes :1.Insolubility in Common Solvents: Certain chemicals, particularly those with high polarity or hydrophobic properties, may not dissolve well in the frequently used mobile phases.
  2.Sample Matrix Composition: Complex matrices, such as biological samples, may include compounds that impair the analyte's solubility.
- Impact:1.Inconsistent Results: Incomplete sample dissolution due to poor solubility might lead to imprecise quantification and poor repeatability. 2.Systemic Precipitation: Insoluble substances may accumulate in the HPLC system, causing obstructions and column clogging.
- Remedies:1.Optimizing Solvent Composition: Improving solubility can be achieved by optimizing the mobile phase, which includes modifying pH or including co-solvents. 2. Use of Solubilizing Agents: Poorly soluble substances can become more soluble by adding surfactants or co-solvents (such as ethanol and dimethyl sulfoxide).3.Pre-Dissolution Techniques: Reproducibility can be increased by making sure the sample is fully dissolved prior to injection.

3. Instrumental Considerations

Challenge: The results of RP-HPLC analysis can be greatly impacted by instrumental limitations, including problems with detector sensitivity, column performance, pressure stability, and the calibration of the entire system. These problems may affect the chromatographic results' overall quality, resolution, and reproducibility.

Recent Developments and Innovations in RP-HPLC:

1. New Chromatographic Techniques

a. Liquid Chromatography with Ultra-High Performance (UHPLC)

- Innovation: Compared to conventional HPLC, Ultra-High-Performance Liquid Chromatography (UHPLC) is a major advancement. It enables faster separations with greater precision and sensitivity by using smaller particle sizes (less than 2 μm) and higher pressures (up to 15,000 psi).
- Use: UHPLC is very helpful for intricate investigations in environmental and pharmaceutical monitoring, where quick and accurate analysis is essential.

b. Chromatography in Two Dimensions (2D-HPLC)

- Innovation: Two chromatographic columns with distinct separation methods are combined in the hybrid technique known as 2D-HPLC. By enabling the two-stage resolution of complicated samples, enhancing peak resolution, and lowering the possibility of co-elution, this method improves separation capacity.
- Use: In pharmaceutical, food, and proteomics applications where careful separation of complex mixtures is required.

2. Green Chemistry Approaches

Challenge: There are serious health and environmental issues with the massive amounts of organic solvents used in RP-HPLC. The goals of green chemistry techniques are to use fewer solvents, produce less waste, and swap out dangerous solvents for safer ones.

a. Use of Green Solvent

- Innovation: Eco-friendly solvents including ionic liquids, supercritical fluids (like CO_2), and bio-based solvents (like ethanol) have become more popular. In comparison to conventional organic solvents, these solvents are non-toxic, biodegradable, and provide separation efficiencies that are on par with or superior.
- Advantage: Enhancing the sustainability of chromatographic analyses and lowering their negative effects on the environment. Costs of using and disposing of solvents are kept to a minimum.
- Use: As sustainability becomes more and more important, green solvents are especially helpful in environmental and medicinal analyses.

b. The reduction in size of HPLC systems

- Innovation: Smaller solvent and sample volumes are used in miniature HPLC systems, which save waste and expenses while preserving high separation efficiency.

- Advantage: This method produces high-quality chromatographic data while assisting in the development of more environmentally friendly analytical procedures. Field-based analysis is made possible by miniaturization for uses such as environmental monitoring.
- Use: Applied in both industrial and research settings, especially when minimal solvent consumption is essential or sample numbers are constrained.

3. Hybrid Analytical Methods

Challenge: Although RP-HPLC is a powerful technique, it can occasionally be limited in its capacity to properly characterize analytes, resolve complicated mixtures, and identify low-concentration molecules. To get around these issues, hybrid approaches combine RP-HPLC with other technologies like nuclear magnetic resonance (NMR) or mass spectrometry (MS).

a. HPLC-MS (HPLC and mass spectrometry combined)

- Innovation: For the identification of extremely sensitive and particular compounds, HPLC-MS has emerged as the industry standard. Combining mass spectrometry with HPLC improves the detection of low-concentration analytes and provides comprehensive molecular structure data.
- Advantage: It is perfect for complicated biological samples, medicines, and environmental analysis because to its increased sensitivity and specificity, particularly for trace analysis.
- Use: Proteomics, metabolomics, clinical diagnostics, and pharmacological analysis all make extensive use of this technique.

b. HPLC-NMR (HPLC coupled with Nuclear Magnetic Resonance)

- Innovation: In a single experiment, substances can be separated and their structures clarified by combining HPLC and NMR spectroscopy. Characterizing unknown compounds or verifying the identity of separated compounds are two advantages of this hybrid approach.
- Advantage: It is useful for analyzing complicated mixtures or unknown chemicals because it offers both chromatographic separation and comprehensive molecular structure information.
- Use: Applied to the study of natural products, pharmaceuticals, and the characterization of intricate chemical combinations.

Conclusion :

An efficient, dependable, and accurate approach for evaluating this pharmaceutical component is the Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for the simultaneous assessment of Doxofylline in bulk and pharmaceutical preparations. A crucial step in guaranteeing the established RP-HPLC method's accuracy, precision, and robustness is method validation. Specificity, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), and robustness are among the parameters included in this validation. These are crucial for verifying that the procedure yields reliable, consistent, and repeatable results under various circumstances and sample types.

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