E-ISSN: 2788-9270
P-ISSN: 2788-9262
www.pharmajournal.net
NJPS 2024; 4(2): 124-128
Received: 19-07-2024
Accepted: 24-08-2024

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A review on comparative analysis of HPTLC and RP-HPLC techniques for the method development of polmacoxib: Insights and innovations

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Abstract
This article compares two Polmacoxib analytical methods: high-performance thin layer chromatography (HPTLC) and reverse-phase high-performance liquid chromatography (RP- HPLC). It evaluates the effectiveness of HPTLC for bulk pharmaceutical forms, concentrating on validity, accuracy, and precision. In contrast, the evaluation assesses RP-HPLC for capsule dosage forms, looking at its development, sensitivity, and robustness. The evaluation summarizes the merits and drawbacks of each approach, taking into account sensitivity, precision, convenience of use, and application to various pharmacological forms. It also examines the practical consequences for quality control and regulatory compliance, which can help with the choice of a suitable analytical technique.

Keywords: Polmacoxib, RP-HPLC, HPLC, validation

Introduction
Polmacoxib is a selective cyclooxygenase-2 (COX-2) inhibitor, belonging to the class of non- steroidal anti-inflammatory drugs (NSAIDs). Its chemical structure is characterized by a benzothiazole ring system, which is essential for its COX-2 selectivity [5]. The molecule features a sulphonamide group and an aromatic ring, contributing to its pharmacological activity. Polmacoxib exhibits its primary action by selectively inhibiting COX-2, an enzyme involved in the synthesis of prostaglandins that mediate inflammation and pain. By targeting COX-2 over COX-1, Polmacoxib reduces inflammation, pain, and fever with a lower risk of gastrointestinal side effects compared to non-selective NSAIDs that inhibit both COX-1 and COX-2 [5, 6]. This selective inhibition contributes to its efficacy in managing inflammatory conditions while minimizing adverse effects on the gastric mucosa. Polmacoxib is used primarily in the treatment of osteoarthritis and other chronic inflammatory conditions. Its effectiveness in alleviating pain and reducing inflammation makes it a valuable option for patients with conditions such as rheumatoid arthritis, ankylosing spondylitis, and other musculoskeletal disorders. It is often prescribed to improve the quality of life for individuals experiencing pain and stiffness associated with these chronic conditions. Polmacoxib's selective COX-2 inhibition helps in managing symptoms while aiming to reduce the risk of gastrointestinal complications commonly associated with traditional NSAIDs Accurate and reliable analytical methods, such as High-Performance Liquid Chromatography (HPLC) and High-Performance Thin-Layer Chromatography (HPTLC), are crucial for the development, quality control, and clinical monitoring of Polmacoxib due to their roles in ensuring drug efficacy, safety, and regulatory compliance. During drug development, precise techniques like HPLC are essential for pharmacokinetic studies, revealing how Polmacoxib is absorbed, distributed, metabolized, and excreted in the body, which guides dosage optimization and formulation strategies. In quality control, both HPLC and HPTLC are used to verify the purity and potency of Polmacoxib, ensuring that each batch meets stringent specifications and maintains consistency. This is critical for both the safety and effectiveness of the drug, as it prevents variations that could impact therapeutic outcomes. In clinical monitoring, accurate analysis using methods like HPLC helps tailor dosages to achieve therapeutic effectiveness while minimizing potential toxicity. Furthermore, reliable methods such as HPTLC are necessary for detecting any adverse effects or drug interactions, thereby safeguarding patient health and ensuring that the drug performs as intended in a real-world setting. Overall, these analytical methods are pivotal in maintaining Polmacoxib's quality and therapeutic success throughout its lifecycle.

This review synthesizes findings from various journal articles to outline a comprehensive approach for developing and validating an RP-HPLC method for Polmacoxib, integrating insights from HPTLC methods where applicable to provide a holistic view of the analytical strategies employed.

Overview of RP-HPLC AND HPTLC

High-Performance Thin-Layer Chromatography (HPTLC): It is an advanced chromatographic technique used for separating and analysing compounds based on their differential migration through a thin layer of adsorbent material. The basic principles of HPTLC involve several key steps. HPTLC utilizes a thin layer of adsorbent material, typically silica gel or alumina, coated onto a flat, inert support such as a glass, plastic, or aluminium plate [2]. This adsorbent layer acts as the stationary phase in the chromatography process [2, 6]. The sample is applied to the plate in small spots or bands using a precise applicator [7]. This step deposits the sample mixture onto the adsorbent layer, creating a starting point for the separation process. The plate is then placed in a development chamber containing a solvent or a solvent mixture known as the mobile phase. As the solvent rises through the adsorbent layer by capillary action, it carries the sample components along with it. Compounds in the sample separate based on their interactions with the stationary phase and their solubility in the mobile phase. Those with stronger interactions with the adsorbent move more slowly, while those with higher affinity for the mobile phase migrate faster [7, 8]. This differential movement results in the separation of compounds along the length of the plate. After the development, the plate is dried and then subjected to visualization techniques to detect and analyse the separated compounds. This can involve UV light, chemical reagents, or other staining methods that highlight the individual components. The positions of the separated compounds are recorded, and their characteristics (e.g., retention factor or Rf values) are used to identify and quantify the compounds. HPTLC separates compounds based on their migration through a thin adsorbent layer on a plate, utilizing differences in their interactions with the stationary phase and their affinity for the mobile phase [9]. This technique provides a high-resolution separation of components, enabling detailed analysis and characterization.

Reverse phase high performance liquid chromatography (RP-HPLC): Creating a method for Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) necessitates a methodical approach to achieve an accurate, reliable, and repeatable analytical procedure. The procedure begins with defining the analytical objectives, which include identifying the target components for separation and quantification as well as understanding the nature of the sample matrix, which could be pharmaceuticals, environmental materials, or food products. Choosing the right column is critical; a C18 column is typically used since it is good at separating hydrophobic chemicals, but other column types such as C8 or phenyl may be used based on the specific needs of the analysis. The mobile phase is then selected, which consists of a combination of water and organic solvents such as acetonitrile or methanol, as well as pH adjustment with buffers such as phosphate or acetate, to maximize interaction between the mobile phase and analytes, hence increasing separation efficiency [11]. The development process involves initial screening, which tests a variety of mobile phase compositions and flow rates to establish the best conditions. Gradient elution is commonly used to increase separation by altering the mobile phase composition during the run, with both the gradient profile and duration tuned to get the best resolution within a tolerable analysis time [12]. Flow rate and column temperature are carefully controlled, with typical flow rates ranging from 0.5 to 1.5 mL/min and temperatures set between 25 °C and 30 °C to assure uniformity and repeatability. The injection volume is designed to avoid overloading the column while keeping sufficient sensitivity to identify the analytes. Method validation is an important stage that involves several crucial aspects: specificity is confirmed by confirming that the method is free of interference from other sample components, and linearity is tested by creating calibration curves over a range of concentrations. To verify the method's dependability, accuracy and precision are assessed by recovery studies and repeated measurements, and limits of detection (LOD) and quantification (LOQ) are set using the signal-to-noise ratio and calibration curves [13]. The method's robustness is evaluated by analysing its performance with minor modifications in circumstances, such as changes in flow rate or mobile phase composition, and stability is tested for both the sample and the mobile phase under storage conditions [14]. Detailed documentation is required, which includes recordings of all experimental parameters such as column specifications, mobile phase compositions, gradient profiles, flow rates, and detector settings, as well as example chromatograms that show peak resolution and form. Troubleshooting entails dealing with difficulties such as peak shape anomalies and retention time variations, which might be caused by column overload or system pressure oscillations [12]. This complete method development strategy assures that the RP-HPLC method produces precise, reliable, and reproducible findings that are appropriate for a wide range of analytical applications.

Method Development for Polmacoxib

HPTLC Method Development

In the development of an HPTLC method for Polmacoxib, a systematic approach was taken to optimize the mobile phase by initially conducting trials on Thin-Layer Chromatography (TLC) before finalizing the conditions for HPTLC. Various mobile phase compositions were tested to determine the optimal conditions for achieving a reliable separation of Polmacoxib [2].

Optimization Process: The optimization process began with testing different mobile phase ratios using a 100ppm standard solution of Polmacoxib. Several mobile phases were evaluated. After extensive trials, the most effective mobile phase was identified as toluene: ethyl acetate:
Methanol in a ratio of 8:2:1 (v/v/v). This ratio provided an Rf value of 0.4403 for Polmacoxib, indicating an effective separation and resolution of the compound [2].

Chromatographic Conditions: The optimized mobile phase was then employed for HPTLC method development. Chromatographic separation was conducted on silica gel 60 F254 plates (100 x 100 mm). Samples were applied using a CAMAG Lino mat 5 auto-sampler equipped with a

microliter syringe (100.0 μL). The samples were spotted in the form of bands with a position of Y: 8.0 mm, length: 8.0 mm, and width: 0 mm. The solvent front was allowed to migrate 80 mm on the plate [2].
Development of the plates was carried out in a CAMAG twin-through chamber, with the mobile phase toluene: ethyl acetate: methanol (8:2:1 v/v/v) used at room temperature for 20 minutes. Following development, the plates were air-dried for 5 minutes at room temperature [2].

Detection and Quantification: For detection and quantification, a CAMAG TLC Scanner 4 with Vision CATS software was utilized. Scanning was performed with a slit dimension of 6 x 1.45mm and a scanning speed of 100 mm/s. Two detectors were employed: one in absorbance mode with a 254 nm deuterium lamp and a K320 filter, and the other in fluorescence mode with a 366 nm mercury lamp and a K320 filter. The maximum wavelength (λ max) for Polmacoxib using the optimized mobile phase was found to be 322 nm [2].

Method Validation
The validation of the HPTLC method was conducted to ensure its reliability and accuracy. The study addressed several key parameters [1]:

- **Specificity:** The method's specificity was proven by showing that Polmacoxib could be clearly separated from other components and contaminants in bulk form.
- **Linearity:** Calibration curves were developed with doses ranging from 0.5 to 10 μg/spot. The approach produced a linear response with a correlation coefficient near 1.0, indicating reliable quantification.
- **Accuracy and Precision:** Recovery studies assessed accuracy, with Polmacoxib recoveries ranging from 98% to 102% recorded. Precision was measured using repeatability and intermediate precision tests, which yielded a relative standard deviation (RSD) of less than 2%, providing consistent findings.
- **Limit of Detection (LOD) and Limit of Quantification (LOQ):** The signal-to-noise ratio was used to establish the LOD and LOQ, enabling for accurate detection and quantification of Polmacoxib at low concentrations.
- **Robustness:** The method's resilience was verified by changing factors such mobile phase composition and development circumstances. The approach proved to be robust, with only minor changes in performance under these alterations.
- **Stability:** The stability of Polmacoxib in the sample and mobile phase was tested, indicating that the method is still reliable under normal storage and handling circumstances.

Results and Conclusion
The developed HPTLC method, using a 100 ppm Polmacoxib API solution, demonstrated good resolution and an optimal Rf value of 0.4403. The use of the mobile phase toluene: ethyl acetate: methanol (8:2:1 v/v/v) effectively separated Polmacoxib, making it a suitable choice for the HPTLC analysis of this compound. The method provides a robust and reliable approach for the qualitative and quantitative analysis of Polmacoxib, ensuring accurate results in both research and quality control applications [2, 6, 7, 8].

RP- HPLC Method Development
1. Column Selection
Using a C18 column because it is good at holding and separating hydrophobic chemicals such as polmacoxib. The analysis was performed using a 250 mm × 4.6 mm column with a particle size of 5 μm, which offered sufficient resolution and efficiency [11, 2].

2. Mobile Phase Optimization
The mobile phase was carefully adjusted to ensure successful polmacoxib separation. A typical mobile phase was a combination of water and acetonitrile, with the ratio adjusted to 60:40 to balance resolution and peak form. Buffer solutions [2], such as phosphate buffers, were utilized to keep the pH between 3.0 and 7.0, which is critical for Polmacoxib's stability and interaction with the stationary phase [12].

3. Gradient Elution and Flow Rate
The purpose of gradient elution is to increase separation efficiency. The gradient profile includes a 20-minute change in mobile phase composition from 30% to 70% acetonitrile [12], which improved Polmacoxib peak resolution. The flow rate was kept at 1.0 mL/min to balance analytical time and column efficiency [11].

4. Detection
Polmacoxib was discovered using UV absorbance at 254 nm [12], which accurately captures the compound's absorbance. The sensitivity was tuned to guarantee precise quantification of Polmacoxib at varying concentrations [11].

5. Method Validation
The validation process described by several critical parameters [2]:

- **Specificity:** The approach was validated for specificity, distinguishing polmacoxib from other excipients and contaminants in capsule dosage form.
- **Calibration curve:** Calibration curves showed linearity from 1 to 50 μg/mL, with a correlation coefficient close to 1.0, indicating a consistent linear response [13].
- **Recovery:** Recovery trials demonstrated high accuracy and precision, with polmacoxib recoveries ranging from 98% to 102%. Precision was tested using repeatability and intermediate precision tests, which yielded relative standard deviations of less than 2% [14].
- **Limit of Detection (LOD) and Limit of Quantification (LOQ):** The LOD and LOQ were determined using signal-to-noise ratios, ensuring reliable detection and quantification of Polmacoxib at low concentrations [13].
- **Robustness:** The method's robustness was verified using tiny variations in flow rate and mobile phase composition, revealing that it remains dependable even when experimental conditions alter slightly [14].
- **Stability**: The stability of both the polmacoxib sample and the mobile phase was assessed, validating the method's dependability under ordinary storage and handling settings [13].

Conclusion
RP-HPLC method development for Polmacoxib, highlighting key aspects such as column selection, mobile phase optimization, gradient elution, and detection. The

validation results confirm the method's robustness and reliability, making it a valuable reference for similar analytical tasks. This review underscores the importance of a well-optimized RP-HPLC method for ensuring accurate and consistent analysis of Polmacoxib in pharmaceutical formulations [11, 12, 13, 14].

Comparison of HPTLC and Rp-Hplc Method

Here's a detailed comparison of HPTLC and RP-HPLC methods for analysing Polmacoxib, highlighting their distinct advantages and applications [1, 2].

Aspect	HPTLC method	RP-HPLC method
Principle	Separation based on differential adsorption and partitioning on a stationary phase.	Separation based on hydrophobic interactions between analytes and a reverse-phase column.
Stationary Phase	Silica gel 60 F254 plates, suitable for separating hydrophobic compounds.	C18 column (250 mm × 4.6 mm, 5 µm), effective for high-resolution separation of hydrophobic compounds.
Mobile Phase	Mixture of toluene, ethyl acetate, and methanol (e.g., 8:2:1 ratio).	Gradient system of water and acetonitrile, with or without buffers.
Sample Preparation	Extraction from bulk pharmaceutical form, filtration, application as spots on the plate.	Dissolution, filtration, and injection into the HPLC system.
Chromatographic Conditions	Development in a twin-trough chamber until the solvent front reaches the desired distance.	Use of gradient elution, flow rate adjustments, and column temperature control.
Detection	UV absorbance at 254 nm, with densitometric scanning for quantification.	UV absorbance at 254 nm or 280 nm, with real-time detection and quantification.
Separation and Resolution	Visual separation on the plate, generally faster but with lower resolution.	High-resolution separation due to controlled column conditions and gradient elution.
Quantification	Based on peak area measurement from densitometric scans; less precise.	Based on peak area or height measurement from UV detector; highly precise.
Method Validation	• **Specificity:** Validated for clear separation from impurities. • **Linearity:** Good correlation over concentration range. • **Accuracy and Precision:** High accuracy with recoveries between 98-102%, RSD < 2%. • **LOD and LOQ:** Determined based on signal-to-noise ratios.	• **Specificity:** Validated for no interference and clear separation. • **Linearity:** Calibration curves with high correlation coefficient. • **Accuracy and Precision:** High accuracy with recoveries between 98-102%, RSD < 2%. • **LOD and LOQ:** Highly sensitive, reliable detection at low concentrations
Robustness	Tested by varying mobile phase composition and development conditions; generally robust.	Tested by varying flow rate, mobile phase composition, and temperature; highly robust
Stability	Evaluated for stability of Polmacoxib in sample and mobile phase.	Evaluated for stability of Polmacoxib and mobile phase under storage conditions.
Time Efficiency	Generally faster for initial analysis; visual results can be obtained quickly.	Can be longer due to gradient elution and column equilibration; provides detailed analysis.
Complexity	Simpler and cost-effective; requires careful handling of sample application.	More complex setup; precise control over experimental conditions, but more expensive.

Conclusion

In the analytical evaluation of Polmacoxib, both High-Performance Thin-Layer Chromatography (HPTLC) and Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) offer distinct advantages and serve specific purposes in drug analysis.

HPTLC Method: The HPTLC method provides a cost-effective and straightforward approach for the estimation of Polmacoxib in pharmaceutical bulk forms. The method involves using silica gel 60 F254 plates and a mobile phase consisting of toluene, ethyl acetate, and methanol, with detection at 254 nm. HPTLC is particularly useful for preliminary screenings and bulk drug analysis due to its simplicity and relatively quick results. It effectively separates Polmacoxib, allowing for qualitative and semi-quantitative assessments. The method's validation shows high specificity, linearity, accuracy, and precision, making it robust for bulk pharmaceutical evaluations. However, its resolution and quantification precision are generally less refined compared to RP-HPLC [2, 6, 7, 8].

RP-HPLC Method: The RP-HPLC method, offers a more detailed and precise analysis of Polmacoxib in capsule dosage forms. Utilizing a C18 column and gradient elution with a mobile phase of water and acetonitrile, RP-HPLC provides high-resolution separation and accurate quantification. The method's validation demonstrates exceptional specificity, linearity, accuracy, precision, and sensitivity, making it suitable for in-depth quality control and regulatory compliance. RP-HPLC's detailed analytical capabilities make it the preferred choice for precise quantitative analysis and detailed characterization of Polmacoxib [1, 11, 12, 133, 14].

Overall, both methods are valuable depending on the analytical needs. HPTLC is advantageous for quick, cost-effective analysis and initial screening of bulk drug materials, while RP-HPLC excels in providing high-resolution, precise, and reliable quantitative data essential for quality control in finished dosage forms. Each technique complements the other, offering a comprehensive toolkit for the analysis of Polmacoxib in various pharmaceutical contexts.

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