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Development and Validation of RP-HPLC Method for Estimation of Polmacoxib in Marketed Formulation

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ABSTRACT

The pain medication polmacoxib has less cardiovascular adverse effects and a faster beginning of relief from osteoarthritis symptoms compared to celecoxib. It is also safer for the gastrointestinal tract than typical nonsteroidal anti-inflammatory drugs (NSAIDs). Among all known NSAIDs, it is the lowest daily dose. When it comes to both short-term and long-term stability assessment, HPLC is a multipurpose instrument. Unfortunately, there is only one documented HPLC method for the Polmacoxib assay, and official monographs are not available. While the current approach takes 15 minutes to operate, it is possible to reduce this time for a single medicinal compound by adjusting the mobile phase composition, decreasing the column length, and increasing the flow rate. This study aims to estimate Polmacoxib in capsule dose form using a speedy, accurate, and easy-to-understand RP-HPLC approach that indicates stability. From initial solution production through validation and implementation in the analysis of commercial product batches, this research intends to explore liquid chromatographic operations. The goal is to learn how different variables including mobile phase ingredients, buffer acidity, analytical column dimensions, temperature, and flow rate influence sensitivity and separation. In order to ensure that the method is suitable for analysing Polmacoxib drug samples that are available for purchase, it will undergo validation in accordance with ICH criteria for linearity, accuracy, robustness, recovery, LOD-LOQ, and solution stability. Our research shows that the RP-HPLC method was developed by combining a short column with a mobile phase. The proportions of water to acetonitrile and methanol were 40:60 by volume/volume, respectively. The technique, which only took eight minutes to complete, was found to be faster and cheaper than the one described in the article. The approach was confirmed to be an easy-to-use, fast, and accurate analytical methodology in accordance with the standards set by the International Council for Harmonisation (ICH). There was further confirmation that it was accurate, sensitive, precise, robust, and stable-indicating.

1. INTRODUCTION:

A number of osteoarthritis conditions have been studied with polmacoxib, including hip, knee, localised primary, and osteoarthritis of the knee. One novel nonsteroidal anti-inflammatory drug (NSAID) that inhibits both COX-2 and CA-I/II is polmacoxib [1, 2]. Its two-pronged approach lessens the negative impact of COX-2 inhibition on the cardiovascular system. The IUPAC name for polmacoxib is 4-(3-(3-fluorophenyl)-5,5-dimethyl-4-oxo-4,5-dihydrofuran-2-yl) benzenesulfonamide, and its chemical formula is C18H16FNO4S (Figure 1)[3,4]. Synovial fluid has almost no CA, inflammatory

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tissues have low CA levels but high COX-2 expression. Osteoarthritis pain and inflammation can be alleviated with polmacoxib's complete inhibition of COX-2 in inflammatory joint tissues[5]. An essential function of erythrocytes is to store polmacoxib and deliver it to areas with low CA activity, like arthritic joints, in a protected form. Whole blood Polmacoxib concentrations are 85–100 times greater than CA-free plasma concentrations (6). Low systemic exposure is maintained while sustained medication doses are delivered to CA-deficient inflammatory tissues through this tissue-specific transport route. Consequently, polmacoxib is most effective when used to inflammatory osteoarthritic joints, and it has less of an impact on the cardiovascular system and the digestive tract (7). It appears that other bodily compartments are spared from prolonged exposure to polmacoxib because its residence period is longer in inflamed osteoarthritic joints than in blood. The majority of its excretion occurs in the faeces, while a little quantity is eliminated by the urine [8].

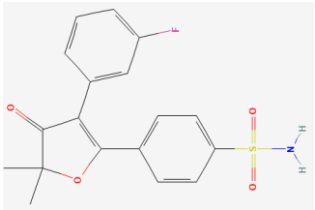

Figure 1: Chemical Structure of Polmacoxib

2. MATERIALS AND METHODS:

2.1 Drugs and Chemicals:

Polmacoxib capsules (Poliexar Capsule, 2 mg label claim) are procured from the Abbott, while Methanol (HPLC grade) and Acetonitrile (HPLC grade) procured from the Rankem and water being HPLC grade procured from the Merck.

2.2 Instruments:

Shimadzu (Prominence – i LC2030 3D Plus liquid chromatography), Analytical balance (Mettler Toledo, Model: ML204/01).

2.3 Methodology (By HPLC)

Mode of elution	Isocratic
Column	Agilent Eclipse XBD-C18 (150 mm × 4.6 mm ID x 5 µ particle size)
Mobile phase	Water: Methanol 35:65 %v/v
Flow rate	1.2 mL/min
Wavelength	236 nm
Injection Volume	50 µL
Column Temp	40ºC
Run Time	8 minutes

2.3.1 Preparation of mobile phase:

Water 350 ml and methanol 650 ml mixed well to make 1 L of mobile phase. Sonicated to degas and filtered through 0.45 µ particle size filter under vacuum to remove any particulate matter[9].

2.3.2 Preparation of Stock and Standard solution:

Weigh accurately 20 mg of polmacoxib API and transfer the weighed polmacoxib powder into 100-mL volumetric flask. Add 30 mL of methanol to the flask and sonicate to dissolve the content. Once the API is dissolved, add more methanol to the flask until the volume reaches the mark of the flask. Aliquot of 1 mL polmacoxib stock was diluted with the help of diluent to 10 mL which yields a concentration of 20 µg/ml of polmacoxib[10].

2.3.3 Sample Preparation:

Ten Poliexar hard gelatin capsules with label claim of 2 mg/capsule were emptied onto a buffer paper. Average weight was computed. Then weight equal to 2 mg of powder was taken into 10 ml volumetric flask. 5 ml methanol added to the flask and sonicated for 20 minutes to extract the content. Then added more methanol to the flask until the volume reaches the mark of the flask. The solution was filtered through 0.45 µ syringe filter. Aliquot of 1 mL clear filtrate was diluted with the help of diluent to 10 mL which yields a concentration of 20 µg/ml of polmacoxib[11].

2.3.4 Validation of the UFLC method:

Adjusting several UFLC settings (FDA, 1995, 1997, 2000, 1994, 1987; USP, 2000) confirmed the reliability of the UFLC approach[12]. Calibration plot least-squares linear regression analysis verified the UFLC method's linearity[13], the limits of detection and quantification for the medicines mentioned were determined to be three and five epochs, respectively, above and below the baseline noise, The process adhered to the guidelines established by the United States Pharmacopoeia (USP, 2000)[14], specificity[15], precision[16] accuracy[17], robustness[18] and ruggedness[19] were determined.

2.3.5 Quantitative Analysis:

The quantitative examination of medications used standard evaluation methods, confirming drug determination by comparing peak regions in both conventional and plasma samples (20, 21). Limits of detection and quantification were determined using a range of drug concentrations. Microsoft Excel was used for statistical analysis, and medication dosages were determined using an equation. Standardization using tyrosine allowed for determining % recoveries and identifying method flaws.

3. RESULTS AND DISCUSSION:

3.1 Chromatogram analysis of Polmacoxib Standard and in Marketed Formulation:

After multiple trials with mobile phases (Water:

Journal of Molecular Science
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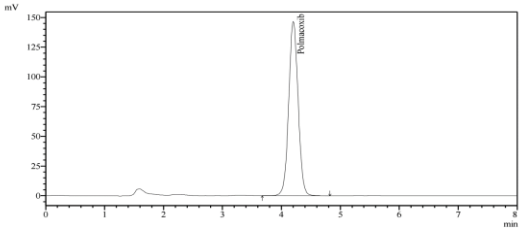
Methanol 35:65 %v/v) was chosen due to better resolution and symmetrical peaks. Standard and Sample drug showed absorbance at 238 and 240 nm, respectively making it the detection wavelength. An optimized chromatogram showed separation of Standard and Sample drug at different RTs (Figure 2). For Standard and Sample drug, the retention (k), separation (α), and resolution (Rs) factors were determined (Table 2).

A. Chromatogram of Standard Polmacoxib:



B. Chromatogram of Sample Polmacoxib in Marketed Formulation

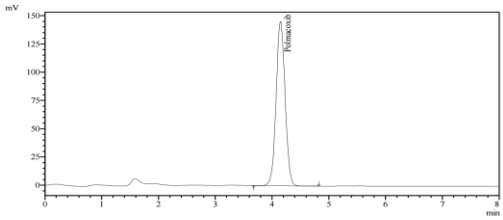


Figure 2: Chromatogram of Polmacoxib Standard & in Marketed Formulation

Table 1: Tailing factor (T), separation factor (Rs), resolution factor (Rs), and retention time (Rt) for Polmacoxib Standard & in Marketed Formulation in Optimized Condition

S.No	Drugs	Rt	k	α	Rs	Tfactor
1.	Standard	4.197	2.30	1.08	2.55	1.20
2.	Marketed Formulation	4.165	2.40	1.09	2.53	1.19

3.2 Validation:

For Polmacoxib Standard and in Marketed Formulation, the linearity of the calibration curves was tested over concentration ranges of 4.038-6.058 µg/mL and 4.099-6.149 µg/mL, respectively. The peak area vs. concentration was calculated using this method. Table 2 shows that the Polmacoxib Standard & in Marketed Formulation has a Lowest Detectable Dose (LOD) of 0.273 and a Limit of Quantitative Determination (LOQ) of 0.228 and a LOQ of 0.224. Polmacoxib Standard & in Marketed Formulation stated precision was assessed at three distinct concentrations (0.001, 0.005, and 0.025 mgmL-1) as shown in Table 3. For each of the three concentrations, five separate chromatographic runs were carried out. For the computations performed, the confidence intervals ranged from 98.277 to 100% and the root-mean-square deviation ranged from 0.322 to 0.722. Table 4 shows that various concentrations of the reported compounds were used to validate the accuracy of the HPLC method. We looked at 0.1, 0.05, and 0.25 millimoles per litre as our concentrations. There were five chromatographic runs out of a total of five. We found out how precise their measurements were by analysing peak area data from five copies of these molecules. Table 5 shows that the absolute error levels varied between 0.15 and 0.5.

Table 3: Results for Linearity (n=3) for Polmacoxib Standard & in Marketed Formulation in Optimized Condition

Parameters	Standard Polmacoxib	Sample Polmacoxib
Slope	985421	978560
R^2	0.999	0.998
Linearity Range	4.041-6.060 µg/ml	4.099-6.151 µg/ml
LOD	0. 280 µg/ml	0.290 µg/ml
LOQ	0.231 µg/ml	0.234 µg/ml

Table 4: Results for Precision (n=3) for Polmacoxib Standard & in Marketed Formulation in Optimized Condition

Parameters	Standard Polmacoxib	Sample Polmacoxib
Intraday Precision (% RSD)	0.81	0.79
Interday Precision (% RSD)	0.89	0.83

Table 5: Results for Precision (n=3) for Polmacoxib Standard & in Marketed Formulation in Optimized Condition

Recovery Level	Standard Polmacoxib			Sample Polmacoxib		
	Amount added	Amount found	% Recovery	Amount added	Amount found	% Recovery
80 %	50	49.14	98.28	50	49.95	97.90
90 %	60	59.99	99.06	60	59.01	98.32
100 %	70	69.89	99.34	70	69.90	99.87
Mean Recovery	99.89 % w/w			99.69 % w/w		

Table 6: Results for Robustness for Polmacoxib Standard & in Marketed Formulation in Optimized Condition

Parameters	Standard Polmacoxib	Sample Polmacoxib
With flow rate 0.9 ml	0.55	0.56
With flow rate 1.0 ml	0.88	0.89

Journal of Molecular Science
Volume 35 Issue 3, Year of Publication 2025, Page 539-542
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4. CONCLUSIONS:

The RP-HPLC method was developed using a short column and mobile phase, with a Water: Methanol 35:65 %^{v}/v ratio. The method was found to be more economical and less time-consuming than the published one, with a total run time of 8 minutes. The method was found to be stable-indicating, sensitive, precise, robust, and accurate, as per the ICH guidelines, and was validated as a simple, rapid, and precise analytical method.

5. CONFLICT OF INTEREST:

None.

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