IJAPB International Journal of Advances in Pharmacy and Biotechnology

Journal homepage: http://ijapbjournal.com/

Research Article

RP-HPLC Method Development and Validation of Rofecoxib in Bulk and Dosage Form

Yamini Kumara Tadikonda[1*], M. Dhanalakshmi[2]

[1*]*Department of Pharmaceutical Analysis, Pydah College of Pharmacy, Kakinada, AP, India.*
[2] *Department of Pharmaceutical Analysis, KLR Pharmacy College,Telangana1, India.*

ARTICLE INFO

Article history:
Received 15 May 2017
Received in revised form 24 May 2017
Accepted 27 May 2007
doi.org/10.38111/ijapb.20190502001

Keywords:
Rofecoxib, RP-HPLC,
Validation, ICH Guidlines.

ABSTRACT

A rapid and precise RP-HPLC method has been developed for the validated of Rofecoxib, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Symmetry C18 (4.6 x 150 mm, 5 µm) column using a mixture of Methanol and water (45:55 % v/v) as the mobile phase at a flow rate of 0.8 ml/min, the detection was carried out at 260 nm. The retention time of the Rofecoxib was 2.379 ±0.02 min respectively. The method produces linear responses in the concentration range of 24-120 mg/ml of Rofecoxib. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

1. Introduction

Rofecoxib is an anti-inflammatory, analgesic, and antipyretic effects of NSAIDs appear to result from the inhibition of prostaglandin synthesis used for the treatment of osteoarthritis, rheumatoid arthritis, acute pain in adults, and primary dysmenorrhea, as well as acute treatment of migraine attacks with or without auras. Although the exact mechanism of action has not been determined, these effects appear to be mediated through the inhibition of the COX-2 isoenzyme at the sites of inflammation with subsequent reduction in the synthesis of certain prostaglandins from their arachidonic acid precursors. Rofecoxib selectively inhibits the cyclooxygenase-2 (COX-2) enzyme, which is important for the mediation of inflammation and pain. Unlike non-selective NSAIDs, Rofecoxib does not inhibit platelet aggregation. It also has little to no affinity for COX-1. It is chemically denoted as 4-(4 methylsulfonylphenyl)-3-phenyl-5H-furan-2-one.Thest ructure of Rofecoxib was shown in figure 1. Literature review of Rofecoxib shown that there were several analytical methods like HPLC,[1-2] HPTLC[3] and only few methods were reported for RP-HPLC,[4-5] for the estimation of this drug in bulk and in its formulation. Hence the present work targeted to develop a new precise, accurate and sensitive RP-HPLC [6-10] method for the determination of Rofecoxib in API and formulation. The developed method validated as per ICH guidelines. [11-13]

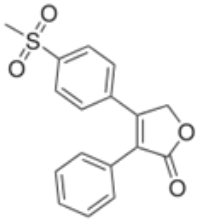

Fig 1: *Structure of Rofecoxib*

2. Materials & Methods

Chemicals & Reagents: Rofecoxib as pure standard reference drug was obtained from Sura labs, Hyderabad, India. Acetonitrile, Water and Methanol used were of HPLC grade and purchased from Merck specialties Private Limited, Mumbai, India.

* *Corresponding author.* Tel.:+91 9550169191.
E-mail address: dhanadlx@gmail.com

Instruments and chromatographic conditions: HPLC analysis was performed on chromatographic system of water 2695 separation module with empower software liquid chromatography comprising water 996 photo diode array detector , Column Apollo C18 (4.6×150mm) 5µ was used and an equipped with auto sampler. Derivative spectral and photometric absorbance measurements are done on UV spectrophotometer with software UV win, Lab India make 3092. 10mm path length quartz cells were used. Digital analytical balance Shimadzu make AUX 220 was used for weighing drug. The Symmetry C18 (4.6×150mm) 5µ column was used at 40ºC temperature. The mobile phase was considered was Methanol:water (45:55v/v). The flow rate was maintained at 0.8ml/min and wavelength was set to 260nm. Injection volume of 10µl was used.

Preparation of standard solution: To the weighed 10mg 0f Rofecoxib add 7ml of methanol in a 10ml of volumetric flask and make up to the mark with methanol. Pipette out 0.72 ml from the above stock solution intlo another 10ml volumetric flask and make up the volume with methanol up to the mark.

Procedure: Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization: Initially the mobile phase tried was methanol: Water and Acetonitrile: Water with varying proportions. Finally, the mobile phase was optimized to Methanol and Water in proportion 45:55 v/v respectively.

Optimization of Column: The method was performed with various C18 columns like ODS column, Xterra, and X Bridge C18 column. Symmetry C18 (4.6 x 150mm, 5□m) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Preparation of mobile phase: Accurately measured 450 ml (45%) of HPLC Methanol and 550 ml of HPLC Water (55%) were mixed and degassed in a digital ultrasonicator for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation: The Mobile phase was used as the diluent.

Preparation of Sample Solution: To the average weight of tablet and crush in a mortar and pestle and equivalent weight about 10mg of Rofecoxib was taken into a 10ml volumetric flask. And add about 7mL of Diluent and makeup the volume with the diluents. Further pipette 0.72ml of Rofecoxib above stock solution into a 10ml volumetric flask and makeup the volume with diluents.

Analytical method development: Trials showed that mobile phase with reverse phase C18 column gives symmetric and sharp peaks. After the optimization of chromatographic conditions, estimation of Rofecoxib as carried out by the developed RP-HPLC method. The chromatographic separation achieved using an Symmetry C18 (4.6 x 150mm, 5□m) column and column temperature was maintained at 40ºC. Mobile phase consists of a mixture of Methanol and Water in the ratio of 45:55% v/v. the mobile phase was set at a flow rate of 0.8ml/min and the volume injected was 10µl for every injection. The detection wavelength was set at 260nm. The sample and standard chromatograms for Rofecoxib were shown in figure 2 and 3 respectively.

Validation of the RP-HPLC method: RP-HPLC method was validated according to the International Conference on Harmonization guidelines (ICH Q2B). [14-15]

Linearity: The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. The standard calibration curve was constructed for different volumes of stock solutions of each were accurately transferred in to 10ml volumetric flasks and diluted to mark to yield a concentration range of 24 – 120µg/mL solutions of Rofecoxib. The calibration line was obtained by plotting the peak area against concentration of drug. Calibration curves were plotted with observed peak areas against concentration followed by the determination of regression equations and calculation of the correlation coefficients.

Precision: The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Intermediate precision: The intermediate precision was performed on different days by maintaining same conditions. It is also known as Ruggedness.

Repeatability: The repeatability was performed by injecting standard solution for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was calculated.

Accuracy: The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness. Accuracy may be determined by application of the analytical method to synthetic mixtures of the drug product components to which known amount of analyte have been added within the range of the method. Inject the three replicate injections of individual concentrations 50%, 100%, 150% were made under the optimized conditions. Recorded the chromatograms and measured the peak responses.

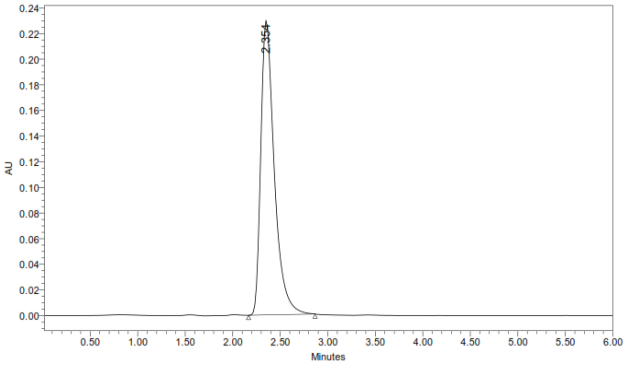

Fig.2: *Standard chromatogram of Rofecoxib*

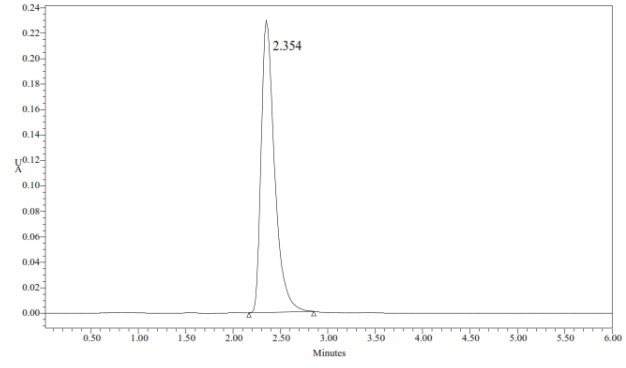

Fig.3: *Sample chromatogram of Rofecoxib*

System suitability: System suitability was carried out with five injections of solution of Rofecoxib in to the chromatographic system. From the chromatograms %RSD was calculated.

Specificity: ICH defines specificity as "the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically this might include impurities, degradants, matrix, etc. Specificity of a method was determined by testing standard substances against potential interferences. The method was found to be specific when the test solution was injected.

Limit of detection and limit of quantification: LOD and LOQ were calculated according to ICH recommendations where the approach is based on the signal-to-noise ratio. Chromatogram signals obtained with known low concentrations of analytes was compared with the signals of blank samples. A signal to noise ratio 3:1 and 10:1 was considered for calculating LOD and LOQ respectively.

$$LOD = 3.3 \times \sigma / s$$

$$LOQ = 10 \times \sigma / S$$

Robustness: Robustness of the method was verified by altering the chromatographic conditions like mobile phase composition, flow rate, detection wave length, etc. and the % RSD should be reported. Small changes in the operational conditions were allowed and the extent to which the method was robust was determined.

3. Results & Discussion

Specificity: Injected the three replicate injections of standard and sample solutions and calculate the assay by using formula:

$$\text{Assay} = \frac{\text{Sample Area X STD Wt X Sample Dilution X Purity X Tab Wt}}{\text{STD Area X STD Dilution X Sample Wt X Lable claim}}$$

The assay results for sample and standard were reported in **Table 1**. The % purity of Rofecoxib in pharmaceutical dosage form was found to be 99.7%.

Table 1: Peak results for Assay sample and standard of Rofecoxib

Name	RT	Area	Height	USP Tailing	USP Plate Count	Inj
Sample	2.354	2258820	243782	1.2	5639	1
	2.350	2258600	248236	1.2	6198	2
	2.354	2257284	247382	1.2	5928	3
Standard	2.354	2255919	248281	1.2	6582	1
	2.350	2255538	249382	1.2	5928	2
	2.354	2253363	241533	1.2	5291	3

Precision:

Intermediate precision: Intermediate precision determined by performing the System precision by different analyst and by different equipment. Calculated % RSD values were less than 2 and given in **Table 2.**

Repeatability: The data for repeatability of peak area measurement for Rofecoxib is based on five measurements of same solution of Rofecoxib. The % RSD value was found to be 0.4. The results were cited in **Table 3**.

System suitability: Five standard solutions of Rofecoxib were injected into chromatographic system and from the chromatograms %RSD, theoretical plates and peak symmetry were calculated. Results were reported in the **Table 3.**

Table 2: Results of Intermediate precision analyst 1 & 2 Day 1 & 2 for Rofecoxib

Peak Name	Analyst 1					Analyst 2				
	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
Rofecoxib	2.380	2236184	202188	5472	1.2	2.380	2236184	217363	5928	1.2
	2.383	2238020	201837	6193	1.2	2.383	2238020	218467	6183	1.2
	2.385	2239352	201273	5980	1.2	2.385	2239352	218346	5927	1.2
	2.385	2242466	203923	7163	1.2	2.385	2242466	221736	5163	1.2
	2.389	2244692	202938	6182	1.2	2.389	2244692	228361	4827	1.2
	2.389	2247654	201982	7684	1.2	2.346	2263431	217553	5019	1.2
Mean		2241395					2244024			
Std. Dev.		4333.851					9988.458			
% RSD		0.193355					0.445114			

***Table 3*: Results of repeatability & system suitability for Rofecoxib**

S. No Peak name	Repeatability					System Suitability				
	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
Rofecoxib	2.356	2259464	245362	5938	1.2	2.317	2274631	239458	5728	1.2
	2.356	2275915	248293	5827	1.2	2.302	2284721	239582	5093	1.2
	2.357	2282117	240795	5032	1.2	2.323	2238127	236493	5391	1.2
	2.358	2278675	230139	5978	1.2	2.343	2259349	249482	6139	1.2
	2.359	2282448	249605	6183	1.2	2.321	2204850	239452	5281	1.2
Mean		2275724					2252336			
Std.dev		9476.485					31827.08			
%RSD		0.416416					1.41307			

Limit of detection: Limit of detection is defined as lowest concentration of analyte that can be detected, but not necessarily quantified, by the analytical method. It is determined by the analysis of sample with known concentration of analyte and by establishing the minimum level at which the analyte can be reliably detected, and it was found to be 5.5 µg/ml of Rofecoxib.

Limit of quantification: Limit of quantification is the concentration that can be quantified reliably with a specified level of accuracy and precision. LOQ was found to be 16.7 µg/mL of Rofecoxib.

Robustness: The robustness was performed for the flow rate variations from 0.7 ml/min to 0.9ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Rofecoxib. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard and samples of Rofecoxib were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count. The results were reported in **Table 4**.

Table 4: Results for Robustness

Parameter used for sample analysis	Peak Area	RT	Theoretical plates	Tailing factor
Flow rate of 0.8 mL/min	3119086	2.379	5837	1.2
Flow rate of 0.7 mL/min	2640811	2.763	5361	1.2
Flow rate of 0.9 mL/min	2640354	2.234	5231	1.2
Less organic phase	2640758	2.765	4503	1.5
More organic phase	2640125	2.236	4491	1.5

Linearity and Range: Linearity and range estimated by constructing the calibration curve by taking concentration on X-axis and peak area on Y-axis of 24, 48, 72, 96 and 120 µg/mL solutions (prepared from standard stock solution). From the curve y-intercept is 34216 and slope is 31709. Calibration curve shown in Figure 5 and linearity values tabulated in Table 5.

Table .5: Linearity data for Rofecoxib

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33	24	791554
66	48	1647073
100	72	2283804
133	96	3058339
166	120	3839630

Accuracy: Accuracy was assessed by determination of the recovery of the method by addition of standard drug to the pre-quantified placebo preparation at 3 different concentration levels 50%, 100% and 150 %, taking into consideration percentage purity of added bulk drug samples. Each concentration was analyzed three times and average recoveries were measured. Sample recovery for each concentration was within the limit. Results of recovery were reported in Table 6.

Table 6: The accuracy results for Rofecoxib

% Conc.	Area	Amount (ppm)		% Recovery	Mean Recovery
		Added	Found		
50%	1172485	36	35.8	99.4	
100%	2314753	72	71.6	99.4	99.5%
150%	3480210	108	107.9	99.9	

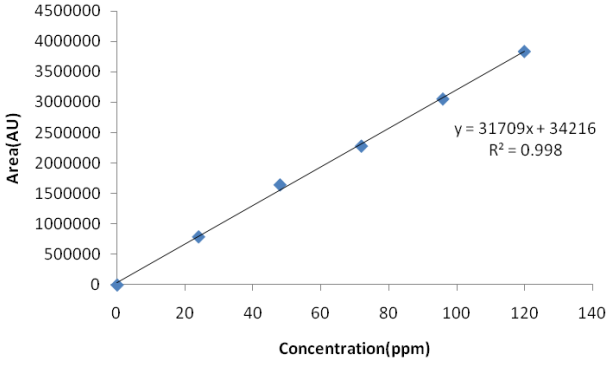

Fig 4: *Calibration curve for Rofecoxib*

4. Conclusion

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Rofecoxib in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Rofecoxib was freely soluble in ethanol, methanol and sparingly soluble in water. Methanol: water was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The % purity of Rofecoxib in pharmaceutical dosage form was found to be 99.7%. Correlation Coefficient (r) is 0.99, and the intercept is 34216. These values meet the validation criteria. The percentage recovery was found to be 99.5. the LOD and LOQ values of Rofecoxib were found to be 5.5µg/ml and 16.7µg/ml respectively. The tailing factor was found to be less than 2.0 and the number of theoretical plates (N) found to be more than 2000 for robustness. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Rofecoxib in bulk drug and in Pharmaceutical dosage forms.

5. Acknowledgement

Authors express their sincere thanks to The Principal and The Management of KLR Pharmacy College for providing necessary facilities to carry out the research work.

References

1. Sanjay P, Khan H (2005). HPLC method for simultaneous estimation of rofecoxib and tizanidine hydrochloride in tablets. Indian J. Pharm. Sci. 67(4): 504-05.
2. Natalia N, Rocio U, Luis F, Capitan V (2008). Determination of celecoxib, rofecoxib, sodium diclofenac and niflumic acid in human serum samples by HPLC with DAD detection. Chromatographia. 67: 1–2, 55–61.
3. Roosewelt C, Harikrishnab N, Muthuprasanna P (2007). Validated HPTLC method for simultaneous estimation of rofecoxib and ttizanidine hydrochloride in pure and tablet dosage form. Asian J. Chem.. 19(6): 4286-90.
4. Nagoji KEV, Vijayasrinivas S, Kiran Kumar M, Mathivanam N, Satish Kumar M, Rao MEB (2004). A new reverse phase high performance liquid chromatography method for analysis of rofecoxib in tablets. Indian J. Pharm. Sci. 129-31.
5. Dhanaraju MD, Varma DP, Magesh AR, Gunasekaram V. RP-HPLC method for simultaneous estimation of rofecoxib and tizanidine HCl in bulk and pharmaceutical dosage form. International Journal of Biopharmaceutics. 2010,1(2):46-50.
6. Andrea W, Brown P (1997). HPLC principle and practice, 1st ed. Academic press, 24-37.
7. Yuri K, Rosario L (2007). HPLC for pharmaceutical scientists, 1st ed. Wiley Interscience Inc. 15-23.
8. Snyder LR (1997). Practical HPLC method development, 2nd ed.. John Wiley and sons, 180-2.
9. Skoog DA, West DM, Holler FJ (1994). Introduction of Analytical Chemistry. Sounder College of Publishing, Harcourt Brace College Publishers. 1-5.
10. Sharma BK (1999). Instrumental method of chemical analysis Meerut. 175-203.
11. ICH Q2A, Validation of analytical methods, definitions and terminology. ICH Harmonized Tripartite Guideline. (1999).
12. Draft ICH guidelines on validation of analytical procedures definitions and terminology. Federal Register, Vol. 60. IFPMA, Switzerland, (1995), 1126.
13. Code Q2B, Validation of Analytical Procedures; Methodology. ICH Harmonized Tripartite Guidelines, Geneva, Switzerland, (1996), 1- 8.
14. Sahajwalla CG (2004). A new drug development, Vol. 141, Marcel Dekker Inc., New York, 421-6.
15. Breaux J, Jones K (2003). Understanding and implementing efficient analytical method development and validation. J. Pharm. Techn. 5:110-4