

Development and Validation of RP-HPLC Method for the Dissolution and Assay of Etoricoxib in Pharmaceutical Dosage Forms

Birbal Singh², Rita Santhakumar², Indu Bala³, Shyam Baboo Prasad¹, Surajpal Verma^{1*}

¹*School of Pharmaceutical Sciences, Lovely Professional University, Phagwara- 144411, Punjab, India; Tel: +919878464676; Fax: +91 1824501900.*

²*Ranbaxy Research Laboratories Ltd. Gurgaon, Haryana, India.*

³*Chandigarh College of Pharmacy, Landran, Mohali, Punjab, India.*

Available Online: 1st January, 2015

ABSTRACT

A simple, accurate, precise, sensitive and reproducible reverse phase high performance liquid chromatography method has been developed for the quantitative determination of etoricoxib in pharmaceutical dosage forms. The assay was performed on Inertsil ODS-4 column as stationary phase. The mobile phase containing 0.01M sodium perchlorate monohydrate and acetonitrile in the ratio of 48:52 v/v. The detection of analyte performed by using UV detector at wavelength maxima 235 nm. The column oven temperature was maintained at 30° C. Percentage recovery found within the limit ranges 98.9-99.5%. The calibration curve was linear over the concentration range of 70-130% and correlation coefficient was found to be 0.99. Peak purity had passed in the sample solution for all degradation conditions, no interference from placebo observed under stressed conditions. Sample solution was found to be stable at 10°C for about 21 hours. In dissolution study the method was linear over the concentration range of 20-120% and correlation coefficient was found to be 0.99. Percentage recovery was found to be 99.3-100.8%. Filter equivalency was found within the limits for both standard solutions. The method was further validated with respect to linearity, accuracy, precision and robustness according to ICH guidelines. The method was statistically evaluated and can be applied for routine control analysis of etoricoxib in tablet formulations.

Key Words: Etoricoxib, filter equivalency, RP-HPLC, ICH, linearity, accuracy and precision.

INTRODUCTION

Etoricoxib (5-chloro-2-[6-methyl pyridine -3-yl]-3-[4-methyl sulfonyl phenyl] pyridine) is a non steroidal anti inflammatory drug and highly COX-2 inhibitor administered orally as an analgesic and anti-inflammatory drug^{1, 2}. It is used for the treatment of osteoarthritis, rheumatoid arthritis and gouty arthritis. It inhibits the synthesis of prostaglandins by inhibiting cyclooxygenase-2 enzyme⁴⁻⁶. It is an off-white crystalline powder, freely soluble in alkaline aqueous solution and relatively insoluble in water⁷. It is available in market as tablet dosage forms (strength 60, 90, 120 mg) and is not official in pharmacopoeia. It is active at low dose and has major side effect as gastric toxicity⁸.

It was determined in human plasma using HPLC-MS/MS. Spectrophotometric and capillary zone electrophoresis methods were also reported for the determination of etoricoxib. In the present study proposed a relatively simple, reliable, reproducible RP-HPLC method for the

determination of etoricoxib in pharmaceutical dosage forms & validated the developed method according to ICH guidelines⁹.

MATERIALS AND METHOD

Chemical and Reagents: Pharmaceutical grade etoricoxib drug was provided as gift sample from Ranbaxy Research Laboratories Ltd. (Haryana, India). The tablet formulation of etoricoxib with strength of 120 mg/ tablet collected from product development research department of Ranbaxy. HPLC grade acetonitrile (Merck) was used and all other chemicals used were of analytical grade.

Instrument used:

Quantitative HPLC was performed on a binary gradient HPLC (Agilent), Electronic weighing balance (Sartorius, Mettler Toledo), Dissolution apparatus (Distek system 2100 C), Column (Inertsil ODS-4, 250 x 4.6 mm, 5µm) was used for separation.

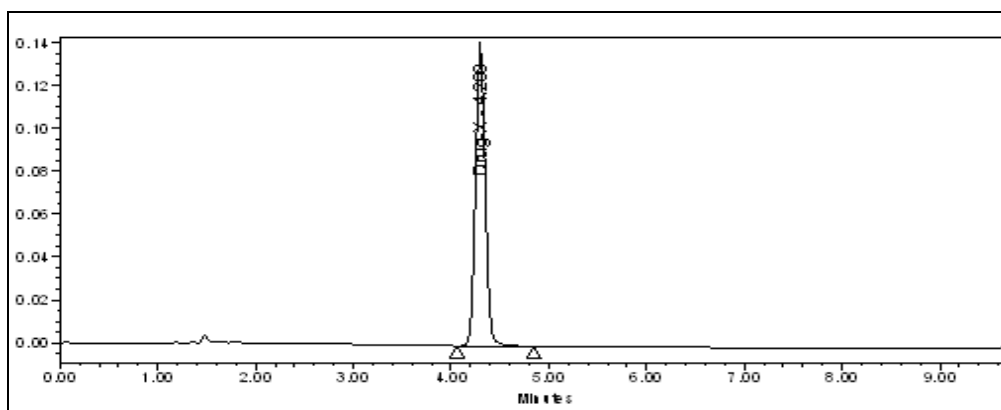


Fig 1 Chromatogram of system suitability

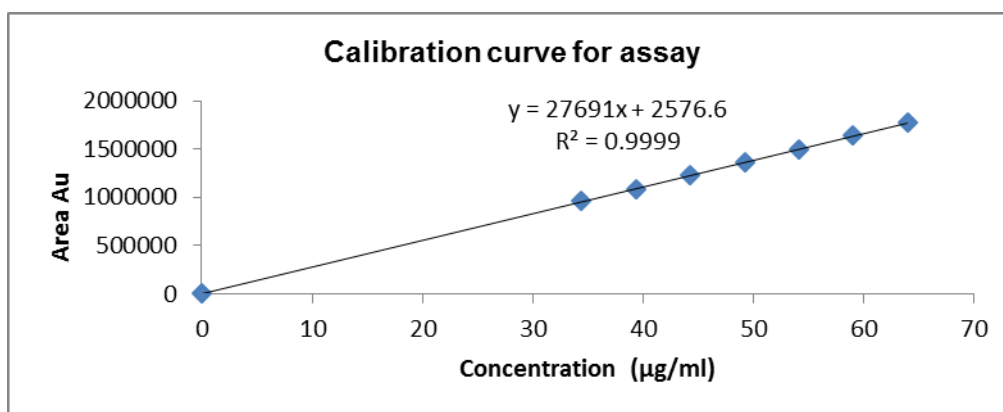


Fig 2 Calibration curve for assay

Chromatographic parameters: Quantitative HPLC with chromatographic conditions as column oven temperature 30°C, flow rate 1.5 mL/min., UV detector at wavelength maxima 235 nm, Injection volume 10 µL, Run time 10 min. and sample tray temperature 10°C were used to get best separation.

Table 1: System suitability data

Name	Retention Time	Area	USP Tailing	USP Plate Count
Etoricoxib	4.299	968182	1.1	8766

Preparation of mobile phase: The mobile phase was prepared with sodium perchlorate monohydrate (0.01M): acetonitrile in the ratio 48:52 v/v.

Preparation of diluent solution: The diluent solution containing sodium perchlorate monohydrate (0.01 M): acetonitrile in the ratio 50:50 v/v.

Preparation of standard solution: Standard solution was prepared by mixing 49.05 mg API into 100 ml volumetric flask, 70 ml diluent was added and the solution was sonicated. Volume was made up with diluent. Five ml standard solution was diluted with 50 ml diluent, mixed and filtered through 0.45 µm nylon filter. The concentration of standard solution was 48 µg/mL.

Preparation of sample solution: 20 tablets was taken and crushed to powder form. Weigh the powder equivalent to

120mg of etoricoxib. Transferred into 250 mL volumetric flask, 180 mL of diluent was added and sonicated for 20 minutes with intermittent shaking. The volume was made up with diluent and mixed well. The 5 mL solution was diluted with 50 mL diluent, mixed and filtered through 0.45 µm nylon filter.

Preparation of spiked sample solution: Impurity A and Impurity B were qualitatively added in small amount to sample solution into a 2 ml HPLC vial. The solution was sonicated to dissolve the impurities.

RESULTS AND DISCUSSION

Method development

By hit and trial method following chromatographic parameters provided best results for the analysis of etoricoxib:

Column	:Inertsil ODS-4, (250mm x 4.6mm), 5µm
Flow	:1.5 mL/min.
Wavelength (UV)	:235 nm
Injection volume	:10 µL
Column oven temperature	:30 °C
Run time	:10 min.
Sample tray temperature	:10 °C
Elution	:Isocratic

Method validation

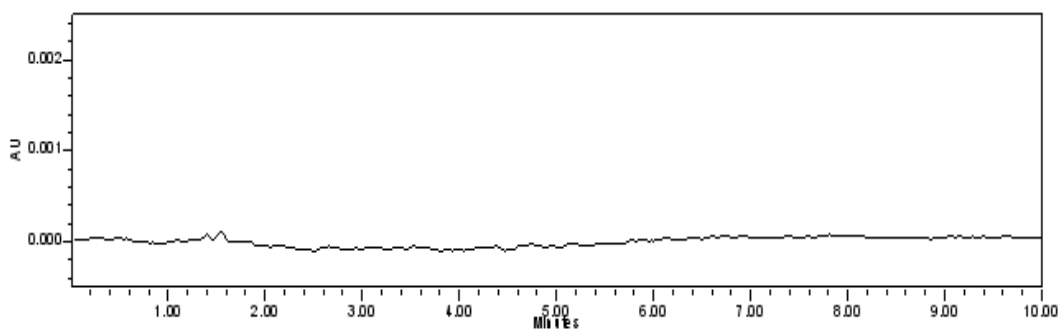


Fig 3 chromatogram of Placebo solution

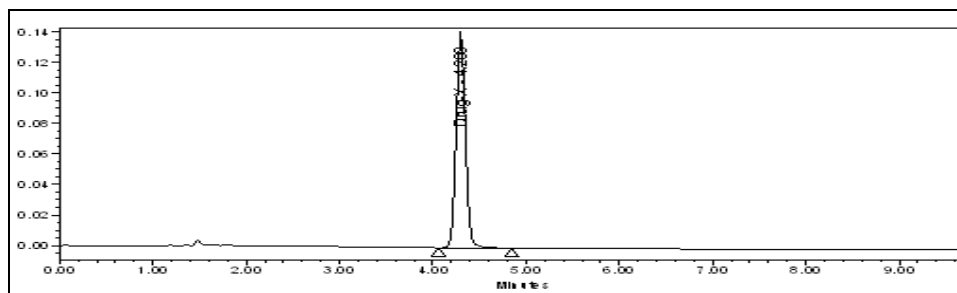


Fig 4 Chromatogram of standard solution of etoricoxib drug

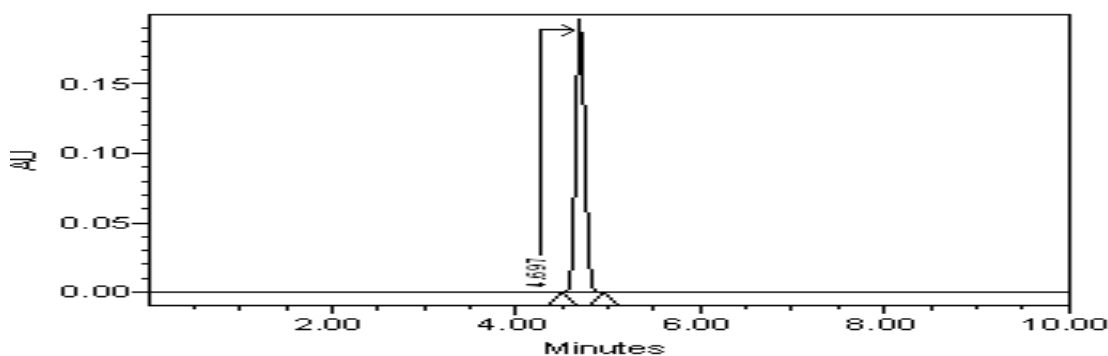


Fig 5 Chromatogram of sample solution of etoricoxib

System suitability

Standard solution was prepared, analyzed and chromatograms studied for different parameters such as tailing factor, resolution and USP plate count.

Linearity and range

Etoricoxib obeys Beer's law in the concentration range of 34.44-63.96 µg/ml with regression equation $y = 27691x + 2576$ and correlation coefficient 0.99985

Specificity

Specificity of method determined by injecting one injection of each blank, placebo, standard solution, sample solution, spiked sample and individual impurity solutions. The chromatograms examined for any interference from placebo and blank at main RT.

Accuracy and precision

Accuracy for the method was calculated by recovery study. Recovery sample was prepared by spiking known

amount of drug of different concentration (1 µg/ml, 2 µg/ml and 3 µg/ml) into different dilution and calculate the % recovery. Precision data was compiled by calculating %RSD for System precision and method precision.

Filter compatibility study:

Standard solution and sample solution was prepared as per method requirement and filtered through nylon, Glass nylon, PVDF and PTFE filter. The filtered solutions were compared with the unfiltered samples.

Stability study

Sample solution was prepared at the concentration level of 48 µg/ml and stability for analytical solution at 10°C was performed. Sample solution was found to be stable at 10°C 21 hrs.

Degradation Study

Table 2: Summary of method development and validation of assay

Parameter	Result	Discussion
Linearity and range	Correlation coefficient for etoricoxib = 0.99985	The method shows a good linearity parameter in the range of 70-130µg/ml
Specificity	No interference from the placebo at the retention time of etoricoxib peak and it also passes the peak purity test.	All the results shows that the method is specific for etoricoxib.
Accuracy	% recovery values with the method were 99.4%, 99.5%, 98.9%	% recovery value was within the limit (98%-102%)
Precision	% RSD for system precision was 0.829% and for method precision-was0.216%	%RSD should not be more than 2.0 % for replicate injection of standard solution it was found within limit for this method
Filter compatibility test	The ratio of filtered area with respect to unfiltered area for sample solution is 0.995 and for standard solution is 0.997	The ratio of area of filtered with respect to unfiltered solution should be (0.99-1.01)
Stability	Absolute difference between area counts of n th time point and the area counts of initial time point should be NMT 2 % for any two consecutive injections	Sample solution was found stable at 10°C 21 hrs
Degradation	No Interference from placebo was observed under any stress condition. etoricoxib peak purity passes in all above degradation conditions.	No Interference from placebo was observed under any stress condition. etoricoxib peak purity passes in all above degradation conditions.

In order to establish whether analytical method for the assay was stable, etoricoxib tablets were subjected to stress conditions to conduct forced degradation studies. Stress studies were carried out under the conditions of acid, base, peroxide, reduction and light exposure. Etoricoxib and placebo kept in oven at 105° C for thermal degradation study.

Table 3: Recovery sample preparation data

S. No	Recovery-Sample Name	Amount Spiked(mg)	Amount Recovered (in mg)	% Recovery
1	Recovery (100% level)-1	120.65	119.743	99.3
2	Recovery (100% level)-2	121.25	122.084	100.8
3	Recovery (100% level)-3	119.99	119.944	100.1
			Mean	100.1
			SD	0.75
			%RSD	0.7

Method development and validation for Dissolution

The rate and extend in which the amount of drug substance over a period of time is called dissolution. It is expressed as percentage release of drug substances present dosage form. Liquid chromatography (LC) is an analytical chromatographic technique that is useful for separating ions or molecules that are dissolved in a solvent. If the sample solution is in contact with a second due to difference in adsorption, ion exchange, partitioning or size. These differences allow the mixture of components to be separated from each other by using

these differences to determine the transit time of the solute through a column.

Chromatographic parameters

Column	:Inertsil ODS-4, (250-mm x 4.6-mm), 5µm
Flow	:1.5 mL/min.
Wavelength (UV)	:235 nm
Injection volume	:10 µL
Column oven Temperature	:30 °C
Run time	: 10 min.
Sample tray temperature	: 10 °C
Elution	: Isocratic

Dissolution Parameters

Medium	: Simulated Gastric Fluid pH1.2, 900 mL
Apparatus	: USP Apparatus II (Paddle)
Rotational speed	: 50 rpm
Temperature	: 37°C ± 0.5°C
Time	: 10, 20, 30, 45, 60 min,

Preparation of buffer (Simulated Gastric Fluid pH 1.20, without Enzymes)

The maximum solubility of Etoricoxib was found in the simulated gastric fluid media pH 1.2 without enzymes so standard stock solution was prepared first in SGF media then further dilution in dissolution media. Simulated gastric fluid media was prepared using 14 g of sodium chloride and 49 mL of hydrochloric acid into 7 L distilled water. Adjust the pH to 1.2 ± 0.05 with (10% w/v) orthophosphoric acid.

Preparation of standard solution

Standard solution was prepared using 68 mg of working standard into a 100 mL volumetric flask, added about 70

Table 4: Linearity solution preparation

Linearity Solution	Vol. of Stock solution	Dilution	Further Dilution	Conc. ($\mu\text{g/mL}$)	Area Counts	
Linearity 1	5	100	7	50	4.67	137777
Linearity 2	5	100	5	10	16.67	482607
Linearity 3	5	100	7	10	23.34	677234
Linearity 4	5	100	8	10	26.68	768534
Linearity 5	5	100	9	10	30.01	861608
Linearity 6	5	100	-	-	33.35	945784
Linearity 7	11	200	-	-	36.68	1028221
Linearity 8	6	100	-	-	40.02	1138766
					Slope	28047
					Intercept	13849
					CC	0.99971

mL of simulated gastric fluid pH 1.2 and was sonicated to be dissolved. The volume was made up with SGF and mix well. 5 mL of standard solution was diluted upto 100 mL with diluent and filtered through 0.45 μm Nylon filter.

Preparation of Sample Solution

Sample solution was prepared by using 1 tablet in each dissolution vessel. At the Specified time withdraw 10 mL of the aliquot from each vessel (with equal replacement of dissolution medium). Sample solution was diluted 5 ml into 20 mL with dissolution medium and filtered through 0.45 μm nylon filter.

Preparation of test (Sample) Solution for stability study at room temperature

Sample solution was prepared using one tablet in each four 1000 ml volumetric flask, added 900 ml respective media mixed well, sonicate for 60 minutes. After sonication withdraw 10 mL of the aliquot from each volumetric flask and diluted 5 ml of the solution to 20 ml with respective media. Filter the sample solution through 0.45 μm nylon filter. As same placebo solution was prepared in respective media.

Method validation

Specificity study: Specificity of the method was

Table 5: Summary of method development and validation of dissolution

Parameter	Result	Discussion
Linearity	Correlation coefficient for Etoricoxib = 0.99971	The proposed method gives linear result with a wide range of concentration of drug (Limit-not less than 0.99)
Specificity	No interference from the placebo at the retention time of etoricoxib peak. Peak purity of etoricoxib passes.	There was no interference from placebo at the retention time of etoricoxib peak. Peak Purity of etoricoxib was passed in the chromatograms of sample solutions.
System Precision	%RSD - 0.8%	RSD should not be more than 2.0 % for replicate injection of standard solution.
Method Precision	RSD = 0.2%	RSD should not be more than 10 %.
Accuracy	Recovery for n = 3 [100 % level] Preparation -1 = 99.3 % Preparation -2 = 100.8 % Preparation -3 = 100.1%	% Recovery value should be within 95%-105%
Filter compatibility test	Standard solution ratio of filtered with respect to unfiltered solution was found within limit Sample solution ratio of filtered with respect to unfiltered solution was found within limit	For standard solution ratio of filtered with respect to unfiltered solution (0.985-1.015) For sample solution ratio of filtered with respect to unfiltered solution (0.985-1.015)
Stability	Absolute % difference between area counts of n th time point and the area counts of initial time point should be NMT 3 % for any two consecutive injections.	Sample solution found stable at 10 °C for about 20 hrs.

determined by injecting placebo solution in the system. Amount equivalent to 290.82 mg was dissolved in 900 ml dissolution medium in a 1000 ml volumetric flask, and sonicated for 60 min. 5 ml of placebo solution was diluted upto 20 ml with dissolution medium, filtered through 0.45µm nylon filter.

Accuracy: Recovery studies were carried out by spiking known amount of drug to test sample which corresponds to 100% label claim in triplicate. Standard solutions were prepared with known amount of API and placebo in dissolution medium. Standard solutions were injected to HPLC system in triplicate to carryout recovery studies. Peak areas were noted to calculate the amount of drug sample recovered.

Linearity: The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in sample within a given range.

The range of analytical method is interval between the upper and lower levels of analyte that have been demonstrated to be determined within a suitable level of precision, accuracy and linearity.

Filter Equivalency

Preparation of Standard Solution for filter study: Standard solution filtered through 0.45µm nylon filter, 0.45µm glass nylon filter, 0.45µm PVDF filter, 0.45µm PTFE filter and inject unfiltered.

Preparation of test Solution for filter study: Test solution was prepared using one intact tablet in 1000 ml volumetric flask, added 900 ml dissolution media, sonicate for 60 min with intermittent shaking. Test solution was further diluted 5 ml into 20 ml with dissolution medium, filtered through different filters for filter compatibility study 0.45µm nylon filter, 0.45µm glass nylon filter, and 0.45µm PVDF filter and 0.45µm

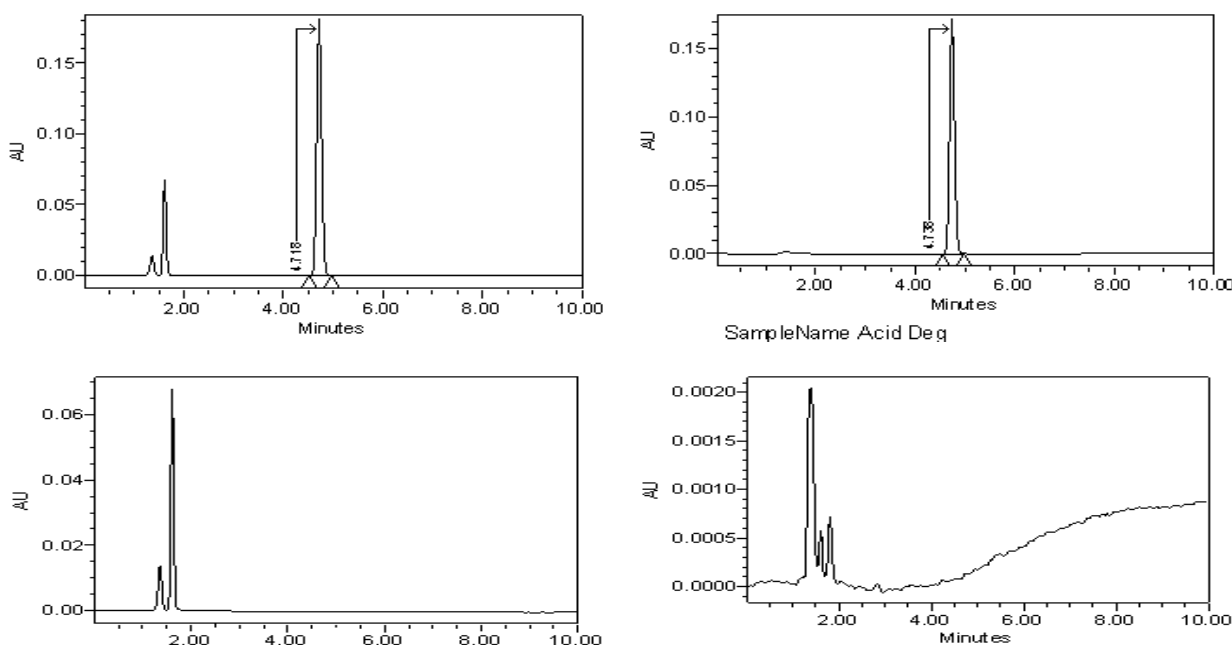


Fig 6 Chromatograms of Degradation studies

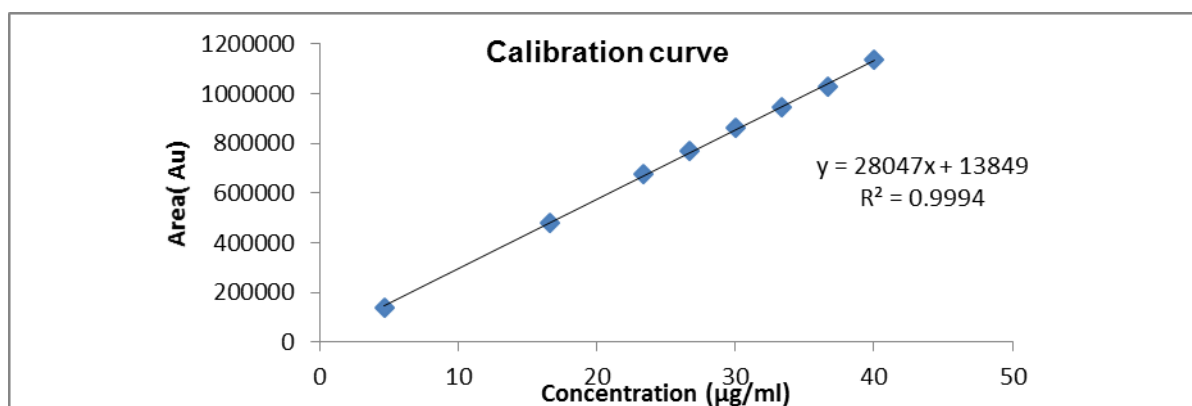


Fig 7 Calibration curve of linearity study

PTFE filter. Test solutions were centrifuged at 3000 rpm for 5 min

Stability study: Sample solution was loaded for SIAS at 10°C.

System suitability: Standard solution was prepared and analyzed. Chromatograms were studied for different parameters such as tailing factor, resolution and theoretical plates to see that whether they complies with recommended limit or out of recommended limit.

Etoricoxib obeys beer's law in the concentration range of 34.44-63.96 µg/ml with regression equation $y = 27691x + 2576$ and correlation coefficient 0.99985. Percentage recovery values were found 99.4, 99.5 and 98.9. Etoricoxib peak purity was passed in all above condition and Sample solution was found stable at 10°C 21 hrs. The method is well accurate, precise, linear, reproducible and stable.

CONCLUSION

The proposed method was found to be simple, precise, accurate, rapid and economical for the determination of Etoricoxib in pharmaceutical dosage forms. The sample recoveries in all formulations were in good agreement with their respective label claims suggesting that no interference of excipients from the formulations. The Validation performed on the in-house (Ranbaxy Research Laboratory) developed method in accordance to the ICH guidelines and method was found to be validated with acceptable accuracy, precision, linearity, and specificity. Hence this method can be conveniently adopted for routine analysis of Etoricoxib. Based on above data, it is concluded that method is suitable for intended use.

ACKNOWLEDGEMENTS

Authors are grateful to Ranbaxy Research Laboratories Ltd., India for providing the necessary research facilities.

REFERENCES

1. The Merck Index, An Encyclopedia of Chemical, Drugs and Biologicals, Maradele j.O. Neil. Eds, 14th Ed., Published by Merck Research Lab, Division of Merck and Co. Inc., Whitehouse station, NJ 2006, Monograph No. 3887 , 662.
2. ICH Guidelines. Q2A: Validation of Analytical Procedure, Methodology: Geneva; 1995.
3. Indian Pharmacopoeia. Government of India, ministry of health and family welfare: The Indian Pharmacopoeia Commission, Ghaziabad; 2010.
4. Topalli S, Chandrasekhar TG, Annapurna M, Validated RP-HPLC method for the assay of Etoricoxib (A Non-Steroidal Anti-Inflammatory Drug) in Pharmaceutical Dosage Forms. E-Journal of chemistry. 2012: 9(2), 832-838.
5. Brautigam L, Nefflen JU, Geisslinger G, Determination of etoricoxib in human plasma by liquid chromatography–tandem mass spectrometry with electrospray ionization. Journal of Chromatography B.2003: 788, 309–315.
6. Ramakrishna NVS, Vishwottam KN, Wishu S, Koteswara M, Validated liquid chromatographic ultraviolet method for the quantitation of Etoricoxib in human plasma using liquid–liquid extraction. Journal of Chromatography B. 2005: 816, 215–221.
7. Starek M, Review of the applications of different analytical techniques for coxibs research. Talanta. 2011: 85, 8-27.
8. Bagade SB, Meshram DB, Tajne MR. Estimation of Etoricoxib in tablet Dosage form by RP-HPLC using Internal Standard with Emphasize on Specificity parameter Method. Oriental Journal of Chemistry. 2011; vol. 27 (2): pg. 697-701.
9. Snyder LR, Kirkland JJ, Glajch JL. Practical HPLC method development, Wiley Interscience Publication, John Wiley & Sons Inc, 2nd ed. 1998; pg. 205-15