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RP HPLC method for the determination of Etoricoxib in bulk and pharmaceutical formulations

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ABSTRACT

A simple, sensitive, precise and specific reverse phase high performance liquid chromatographic method was developed and validated for the determination of etoricoxib in bulk and tablet dosage forms. It was found that the excipient in the tablet dosage forms does not interfere in the quantification of active drug by proposed method. The HPLC separation was carried out by reverse phase chromatography on Shimadzu HPLC, 10-At detector with hypersil ODS C_{18} Column 250 X 4.6 mm (particle size of 5μ) and constant flow pump. Rheodyne injector with 20 μl loop with a mobile phase composed in the ratio acetonitrile: (0.05M) KH_2PO_4 buffer (50:50) at flow rate 1.8 ml /min. The detection was monitored at 283nm. The calibration curve for etoricoxib was linear from 0.5-85μg/ml and internal standard (Bromhexine) 10μg/ml were prepared by suitable dilutions of the stock solution with appropriate mobile phase. The interday and intraday precision was found to be within limits. The proposed method has adequate sensitivity, reproducibility and specificity for the determination of etoricoxib in bulk and its tablet dosage forms. LOD and LOQ for etoricoxib were found to be 0.193 and 0.450.Accuracy (recoveries: 99.8-100.4%) and reproducibility were found to satisfactory.

Keywords: Etoricoxib, RP-HPLC Method, Reverse phase chromatography, bromhexine Acetonitrile, Validation.

INTRODUCTION

Etoricoxib is a non steroidal anti inflammatory drug and highly COX-2 inhibitor [1-12]. Etoricoxib, 5-chloro-6'-methyl-3-[4-(methylsulfonyl)phenyl]-2,3'-bipyridine, produces dose dependent inhibition of COX-2 without inhibition of COX-1.It does not inhibit gastric

prostaglandin synthesis and has no effect on platelet function.COX-2 inhibition provides anti-inflammatory and analgesic effects[13-14]. It is used for symptomatic management of osteoarthritis [15-19], rheumatoid arthritis[20-24] primary dysmenorrhoea, postoperative dental pain, acute gouty arthritis[25], cancer treatment and prevention and migraine[26-31]. According to the literature survey it was found that few analytical methods such as Visible, UV, HPLC other methods were reported for etoricoxib(S.R. Shahi,et al 2008,M.J.Rose,et al 2002,,Robert Hartaman,et al[32-34].)The objective of the proposed methods to develop simple and accurate method for the determination of etoricoxib by RP-HPLC method in Pharmaceutical dosages forms.

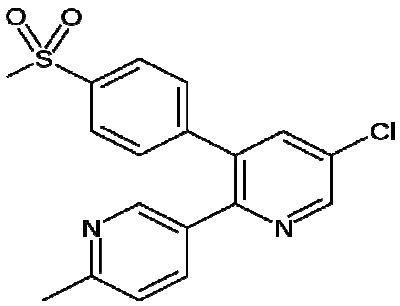


Fig-1 Etoricoxib

Chromatographic conditions

Chromatographic separation was performed on Shimadzu HPLC, 10-At detector with Hypersil ODS C_{18} Column 250 X 4.6 mm (particle size of 5µ) and constant flow pump. Rheodyne injector with 20 µl loop. The composition of the mobile phase is in the ratio acetonitrile: (0.05M) KH_2PO_4 buffer (50:50) was delivered at flow rate 1.8 ml /min. The mobile phase was filtered through a 0.45 µ membrane filter and sonicated for 15min. Analysis was performed at ambient temperature.Bromohexine was used as internal standard. Optimized chromatographic conditions are listed in **Table -1.**

Table-1. Optimized chromatographic conditions

Parameters	Method
Stationary phase (column)	Hypersil ODS C-18 250 x 4.6 mm, packed with 5 micron)
Mobile Phase	Acetonitrile: KH_2PO_4 Buffer
Flow rate (ml/min)	1.8
Run time (minutes)	10
Column temperature (°C)	Ambient
Volume of injection loop (µl)	20
Detection wavelength (nm)	283
Internal standard	Bromhexine
Drug RT (min)	3.083
Internal standard RT (min)	6.958

MATERIALS AND METHODS

T.D.Water (Triple distilled water), Acetonitrile HPLC grade (MERCK), Potassium dihydrogen Phosphate, (AR- Grade) etoricoxib

Preparation of standard drug and internal standard solutions

Stock solutions of the drug (pure) and internal standard were prepared by dissolving 25mg of etoricoxib in 25 ml of Acetonitrile (HPLC Grade, MERCK) and 25 mg of internal standard (Bromhexine) in 25 ml of mobile phase separately in 25ml volumetric flasks. Daily working standard solutions of etoricoxib were prepared between the range of 0.25-100µg/ml and internal standard (Bromhexine) 200µg/ml were prepared by suitable dilutions of the stock solution with appropriate mobile phase.

Preparation of sample solution

Twenty tablets were weighed to get the average weight and pulverized. The sample powder, equivalent to 25mg of active ingredient was extracted with acetonitrile sonicated for about 15 min and made to volume to get a stock solution of 1 mg/ml.This solution was filtered through a whatman filter paper. From this solution 0.02 to 0.04 ml were taken and it was further diluted to 10ml with mobile phase as under preparation of standard solutions to get different concentrations required.

Method Validation

Once the HPLC method development was over, the method was validated in terms of parameters like, precision, accuracy, linearity and range, LOD, LOQ, recovery studies, system suitability parameters etc. For all the parameters percentage relative standard deviation values were calculated. The proposed HPLC method was validated as per ICH guidelines.

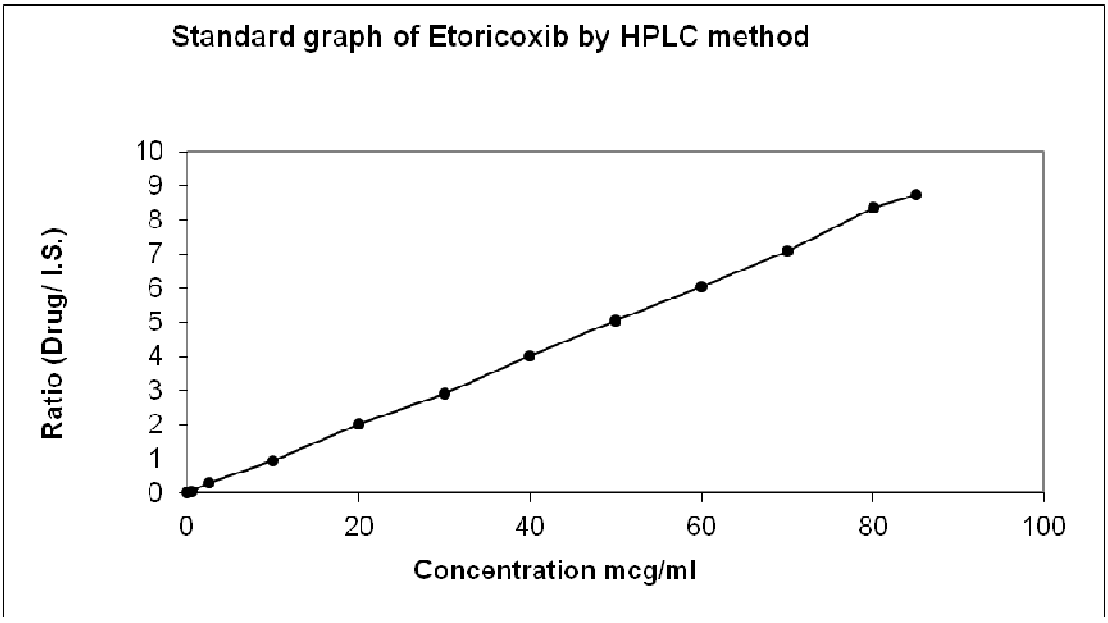


Fig-2- Linearity of Etoricoxib

Linearity and Range

The linearity of measurement was evaluated by analysing different concentrations of the standard solutions of the etoricoxib The Beer lamberts concentration was found to be between 0.5-85 µg/ml. Calibration curve was constructed by plotting average peak area against concentration and regression equation was computed. The results were shown in **Fig: 2**.The slope, intercept and correlation coefficient values were found to be 0.1021,-0.056 and 0.999

Precision

Precision was evaluated by carrying out three independent sample preparation of a single lot of formulation. The sample solution was prepared in the same manner as described in the sample preparation. Percentage relative standard deviation (% RSD) was found to be less than 1% for within a day and day to day variations, which proves that that method is precise.
Results were shown in **Table 3**.

Table-3 Intra – day and inter – day precision of etoricoxib standard

Drug	Theoretical concentration (µg /ml)	Intra-day concentration measured (µg/ml)		Inter-day concentration measured (µg /ml)	
		Mean (a)	RSD %	Mean (b)	RSD %
Etoricoxib	16	16.04	0.1568	16.046	0.1903
	20	20.01	0.09995	20.04	0.0499
	24	24.05	0.12474	24.033	0.1047

Analysis of etoricoxib in its Formulations

The amount of drug present in each pharmaceutical formulation was calculated through peak area ratio of component to that of internal standard by making use of the standard calibration curve (concentration µg/ml on X-axis and peak area ratios on Y-axis) the results were shown in **Table-4** The chromatogram was shown in **fig-3**

TABLE -4

Drug	Sample No	Label claim (mg/tab)	Amount estimated* (mg/tab)	% Label claim	% Deviation
Etoricoxib	1	60	59.7	99.5	0.5
	2	60	60.1	99.8	0.2
	3	60	60.3	99.5	0.5
	4	60	59.4	101.01	-1.1
	5	60	59.9	100.1	-0.1

**Each value is average of five determinations ± standard deviation*

Limit of Detection and Limit of Quantification

The limit of Detection (LOD) and limit of Quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The LOD for etoricoxib found to be 0.193. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). The LOQ was 0.450. It was concluded that the developed method is sensitive.

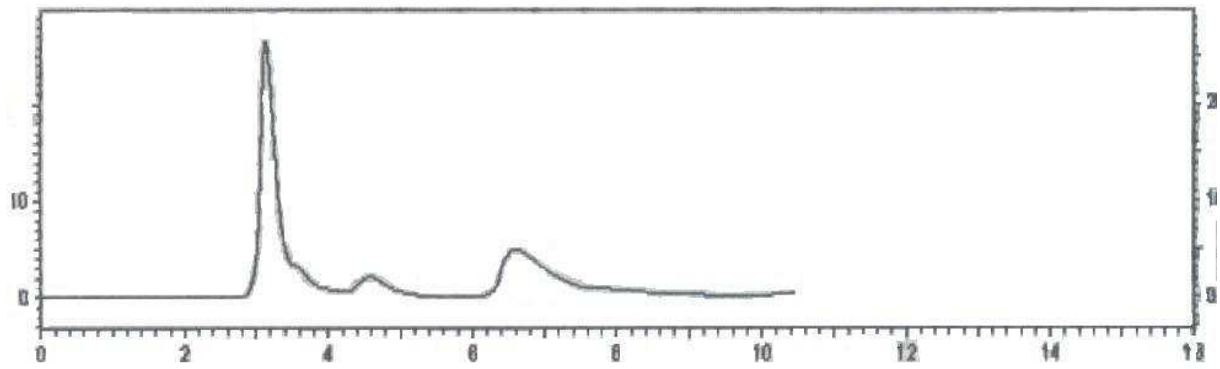
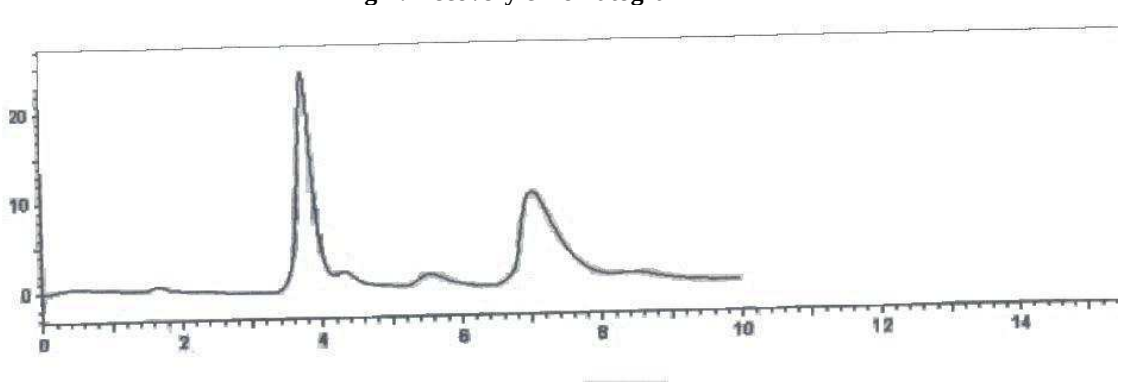


Fig-3 Assay of Etoricoxib

Table-5 Percentage Recovery

Drug	Amount Added (µg/ml)	Amount Recovered (µg/ml)	% Recovery
Etoricoxib	20	19.97	99.85
	30	30.01	100.03
	40	39.96	99.9

Fig-4: Recovery chromatogram



Recovery Studies

To determine the accuracy of proposed method recovery studies carried out by taking different amounts of bulk sample of etoricoxib within the linearity range were taken and added to the pre-analysed formulation. From that percent recovery values were calculated. Results were given below in **Table-5.** Recovery chromatogram was shown in **fig-4**

System suitability parameters

System suitability parameters can be defined as tests to ensure that the method can generate results of acceptable accuracy and precision. The requirements for system suitability are usually developed after method development and validation have been completed. The USP (2000) defines parameters that can be used to determine system suitability prior to analysis.

The system suitability parameters like Theoretical plates, Resolution (R), Tailing factor (T), LOD (µg/ml), LOQ (µg/ml) were calculated and compared with standard values to ascertain whether the proposed RP-HPLC method for the estimation of Etoricoxib in pharmaceutical formulations was validated or not. The results are recorded in **Table-6.**

TABLE-6

S.No	Parameters	Obtained Values
1.	Theoretical plates (N)	2340
2.	Resolution (R)	2.421
3.	Tailing factor (T)	1.6
4.	LOD (µg/ml)	0.172
5.	LOQ (µg/ml)	0.461

RESULTS AND DISCUSSION

From the optical characteristics of the proposed method it was found that the drug obeys linearity range within the concentration of 0.5-85µg/ml. From the results shown precision it was found that the percent RSD is less than 2%, which indicates that the method has good reproducibility. From the results shown in accuracy it was fond that the percent recovery values of pure drug from the preanalysed solutions of formulations were in between 99.85-100.03%, which indicates that the method is accurate. The system suitability parameters are within the specified limits and which refers the commonly used excipients and additives present in the pharmaceutical formulations did not interfere in the proposed method. The proposed method was found to be simple, precise, accurate and rapid for determination of etoricoxib from pure and its dosage forms. The mobile phase is simple to prepare and economical. The sample recoveries in all formulations were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation.

CONCLUSION

A convenient and rapid RP- HPLC method has been developed for estimation of etoricoxib in tablet dosage form. The assay provides a linear response across a wide range of concentrations. Low intra-day and interday % RSD coupled with excellent recoveries. Hence, this method can be easily and conveniently adopted for routine analysis of etoricoxib in pure form and its dosage forms and can also be used for dissolution or similar studies.

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REFERENCES

[1] Sengupta's cyclo oxygenase-2 .A new Therapeutic target agent. *Ind. Jou. Pharmaco*. **1991**: 31 Pg.322
[2] Cochrance Dj et al. *Drugs* **2002** 62 (18):2637-51.
[3] CIMS 85 April 2004 pg 32 continued on pg 55.
[4] Cims a move ahead in pain management pg24
[5] Fang yu jing et al medical progress **2003**; 33-39.
[6] COX-2 selective no steroidal anti inflammatory drugs. Do they really offer advantages? *Drugs* 2000 59(6) pg 1207-1216.
[7] Stichtenoth do Et. Al *Drugs* **2003** 63 (1) 33-45.
[8] Prescription list. Com
[9] Joshi U, J, et al selective cox-2 inhibitors a review Indian drugs 39 (7) July **2002** pg 355- 359
[10] Uma Maheswari R., Sathish Kumar D., Parthiban C.,Senivasan P., Krishnaveni A., Alagar Raja M., "The Antiseptic", vol. 101 No. 10 (ii) oct.**2004** pg 460-462.
[11] Friesen Rw, Brideau C, Chan CC, Charleson S, Deschenes D, Dube D, Ethier D, Firthin R, Gautheir JY Girard Y, Gordon R, Greig GM, Riendeau D, Savoie C, Wang Z, Wong E, Visco D, Xu LJ, Young RN. **1998**. *Bioorg Med chem. Lett* 8:2777-2782.
[12] Riendeau D, Percival MD, Brideau C, Charleson S, Dube D, Ethier D, Falgueyret J-P, Friesen RW,Gordon R, Greig GM, Guay J, Mancini J, Ouellet M, Wong E, Xu L-J, Boyce S, Visco D, Girard Y, Prasit P, Zamboni R, Gresser M, Ford-Hutchinson AW, Young RN, Chan C-C. **2001**. *J pharm Exp Ther* 296 (2): 558- 566.
[13] Lipsy p. *Am j Med*. **2001** 110(3A): IS-5S.
[14] Sengupta S. *Ind L Pharmac* **1999** 31:322-32
[15] Budavari, S., Eds., In; The Merck Index, 12th Edn., merck & Co. Inc,White house Station, NJ, **1994**, 691.
[16] Reynolods, J.E.F. Eds., In; Martindale, The Extra pharmacopoeia, 30th Edn., The pharmaceutical press, London, **1993**,1370.
[17] Manfred, E.W., In; Burger's Medicinal Chemistry and Drug Discovery, 5th Edn., Vol. III, John Wiley & Sons, Inc., New York, **1995**, 490.
[18] Kerala State Drug Formulary, Vol.1, Health and Family Welfare Department Government of Kerala, **1999**, 223.
[19] Rang H.P., Dale M.M., Ritter J.M., Moore P.K., pharmacology 5th Edition ; 432,**2003**.
[20] Hawkey C, Laine L, Simon T, Neaulieu A, Maldonado – Cocco J, Acevedo E, Shahane A, Quan H, Bolognese J, Mortensen E. **2000**. *Arthritis Rheum* 43 : 370 –377.
[21] Baumgartner Mg. **1997**. *Dtsch Apoth Ztg* 137:2157-2159.
[22] Emery P, Zeidler H, Kvien TK, Guslandi M, Naudin R, Stead H, Verburg KM, Isakson PC, Hubbard RC, Geis GS. **1999**. *Lancet* 354:2106-2111.
[23] Lipsky PE, Isakson PC. **1997**. *J Rheumatol Suppl* 49:9-14.
[24] Luog BT, Chong BS, Lowder DM. **2000**. *Ann pharmacother* 34:743-760.
[25] Goldenberg MM. **1999**. *Clin Ther* 21:1497-1513.
[26] Cannon GW, Caldwell JR, Holt P, McLean B, Seidenberg B, Bolognese J, Ehrich E, Mukhopadhyay S, Daniels B. **2000**. *Arthritis Rheum* 43:978-987.
[27] Clemett D, Goa KL. **2000**. *drugs* 59:957-980.
[28] Fung HB, Kirschenbaum HL. **1999**. *Clin Ther* 21:1131-1157.
[29] Goldenberg MM. **1999**. *Clin Ther* 21:1497-1513.

[30] Hawkey C, Laine L, Simon T, Neaulieu A, Maldonado – Cocco J, Acevedo E, Shahane A, Quan H, Bolognese J, Mortensen E. **2000**. *Arthritis Rheum* 43 : 370 –377.
[31] Baumgartner Mg. **1997**. *Dtsch Apoth Ztg* 137:2157-2159.
[32] Rose M.J., Agarwal N., Woolf E. J.M., Matuszewski B.K, *J.Pharm. Sci*.; **2002**, vol. 91, No-2,405-416.
[33] Robert Hartman, ahmed abrahim, Andrew Clausen, BingMao, Louis S. Crocker and Zhihong Ge., *Journal of Liquid Chromoto graphy and Related Technologies*, Vol.26, issue 15.
[34] S.R.Shahi, G.R. Agrawal, P.B. Rathi, *Rasayan J. Chem* Vol.1,No.2(**2008**), 390-394