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High Performance Liquid Chromatographic and Ultra Violet Spectroscopic Determination of Etoricoxib in Pharmaceutical Formulations

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A simple, reliable and reproducible high performance liquid chromatographic (HPLC) and UV spectroscopic methods (UV method) were developed for the analysis of etoricoxib formulation. The HPLC analysis was performed using BDS-Hypersil C-8 (150 × 4.6 mm, 5 µm, Thermo electron corporation, USA) column. The mobile phase used was water : acetonitrile : methanol (50 : 25 : 25, v/v/v) at a flow rate of 1.25 mL/min with ultraviolet detection at 284 nm at 20 ± 1ºC. UV spectroscopic analysis was performed at 284 nm. Extraction of etoricoxib from tablet was carried out using methanol. Linearity was observed in the concentration range from 5-50 µg/mL using HPLC. For UV spectroscopic analysis the linearity range was 5-35 µg/mL (at 284nm). The results, obtained by the two methods in pharmaceutical preparation were compared. There were no significant differences between the mean values and the precision. These validated methods are suitable for the determination of etoricoxib in pharmaceutical tablet formulations.

Key Words: Etoricoxib, HPLC, UV spectroscopy, Pharmaceutical preparation.

INTRODUCTION

Etoricoxib (Arcoxia®), (5-chloro-2-(6-methyl pyridin-3-yl)-3-(4-methylsulfonyl phenyl)pyridine) (Fig. 1), is a relatively new non-steroidal anti-inflammatory drug with high selectivity in cyclooxygenase-2 inhibitory activity. Etoricoxib is indicated to relieve the signs and symptoms of osteoarthritis, rheumatoid arthritis and acute pain, chronic back pain, dysmenorrhoea, ankylosing spondylitis and acute gouty arthritis. Non-steroidal anti-inflammatory drugs (NSAIDs) have been used for decades for the treatment of pain and inflammation. Conventional NSAIDs are very effective in treating pain; however, they have a poor tolerability profile, as

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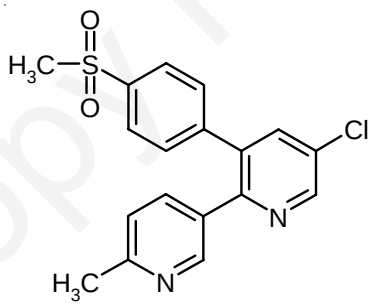
they are associated with an increased risk of damage to the gastrointestinal tract. Recently, introduced etoricoxib selectively inhibits cyclooxygenase-2 and hence cause little or no gastrointestinal side effects[1-4].

Dosage form of etoricoxib is available in international market. A search of the literature revealed that a few HPLC methods have been developed to determine etoricoxib in biological fluids[5-10]. Nevertheless, no isocratic HPLC method/UV-visible spectroscopic method have been reported for the determination of etoricoxib in formulations. Determination of etoricoxib with its impurities in raw material has been reported utilizing gradient elution technique[11]. However, this method uses YMC-AQ column as the stationary phase, which is not as common as the one used in this work.

The aim of this study was to develop and validate a simple and fast LC and UV method, through evaluation of the parameter of linearity, precision and accuracy, detection and quantitation limits, robustness and specificity to determine etoricoxib in pharmaceutical formulations.

This paper reports two simple, economical, accurate, reliable and reproducible analytical methods for the estimation of etoricoxib in formulation. The procedure is applied successfully for the analysis of the commercial tablets, purchased from international market and the results are compared statistically.



Etoricoxib

Fig. 1. Chemical structure of etoricoxib

EXPERIMENTAL

The HPLC method was performed on a Dionex® HPLC (Dionex-Softron GmbH, Munchen, Germany) system equipped with a quaternary pump (model P580), an automated sample injector (model ASI-100) holding 100 µL loop, a column oven (model STH-585), a photodiode array detector (UVD-340S) and a data system (Chromoleon Version 6.2) was used. The separation of the compounds was made on a BDS Hypersil C-8 Column (150 × 4.6 mm, 5 µm, Thermo electron corporation, USA) at temperature 20 ± 1°C. The mobile phase (water: acetonitrile: methanol; 50

: 25 : 25, v/v/v) was prepared and filtered through 0.45 μm membrane filter (Millipore, Milford, MA). The mobile phase flow rate was 1.25 mL/min. The sample volume was 10 μL and the run time was 10 min.

For initial development and ruggedness Merck-Hitachi (Lachrome®) HPLC equipped with quaternary gradient pump (Lachrome® 7100), variable UV-Visible detector (model-7400), Rheodyne 6-port sample injector (Model 7725i; Rheodyne, Rohnert Park, CA, USA) with a 10 μL sample loop, was used. The signals were acquired and analysed using PC Nelson 1022 computer integrator.

UV-Visible-NIR spectrophotometer (Jasco, Tokyo, Japan, model V570) with automatic wavelength accuracy of 0.1 nm and 10 mm matched quartz cuvettes with spectra manager software (Ver. 1.53) was used for all absorbance measurements. Ruggedness studies were performed on Ati-Unicam spectrophotometer (Unicam Limited, Cambridge, UK). (a) Etoricoxib reference material (99.63%) (Fig.1) was obtained as gift from the Orchid Chemical and Pharmaceutical Ltd., Chennai, (India), whereas the pharmaceutical formulation containing etoricoxib was obtained commercially. (b) Etoricoxib tablets labelled to contain 120 mg of the drug and the following excipient; pre-gelatinized starch, lactose, micro-crystalline cellulose. (c) Acetonitrile and methanol of HPLC grade and *o*-phosphoric acid of analytical grade were purchased from Fisher Scientific (Fairlawn, New Jersey, USA). Double distilled deionized water was used for HPLC.

Method development

Different solvent systems were used to develop sensitive, reliable and rugged high performance liquid chromatographic and spectrostroscopic method. The criteria employed for assessing the suitability of a particular solvent system for the drug was cost, analysis time, and sensitivity of the assay, preparatory steps and use of the same solvent system for extraction of the drug from the formulation excipient for estimation of the drug content.

Preparation of standard curve

HPLC method: A standard curve was prepared to evaluate the linearity of the proposed method. The stock solution (1 mg/mL) was prepared using an amount of powder equivalent to 100 mg of etoricoxib that was transferred to a 100 mL volumetric flask and diluted with methanol. 10 mL of stock solution was transferred to 100 mL volumetric flask and the volume was made with mobile phase to obtain a solution of 100 μg/mL. Aliquots of this working solution were transferred to 20 mL volumetric flasks and diluted with mobile phase. The final concentration obtained were 5, 10, 20, 25, 30, 40, 50 μg/mL. Each solution was prepared in triplicate from the solution sited above. These solutions were filtered through a 0.45 μm membrane filter before column injection. Each solution was injected

in triplicate. The stock solution was stored in amber coloured tubes/ volumetric flask wrapped with aluminium foil between 4-8ºC.

The mobile phase (water: acetonitrile: methanol; 50 : 25 : 25, v/v/v) was prepared, filtered through 0.45 µm membrane filter, was delivered at 1.25 mL/min for column stabilization. During this period the baseline was monitored. The calibration standard solutions were injected serially on to a HPLC system under the specified chromatographic conditions described previously. The data were acquired and analyzed by the chromatographic software. The peak areas were plotted against corresponding concentrations, linear relationship was obtained (Table-1). Chromatogram parameters, retention time and asymmetry factor were standardized.

UV method: The stock solution was prepared using an amount of powder equivalent to 100 mg of etoricoxib that was transferred to a 100 mL volumetric flask and diluted with methanol. 10 mL of stock solution was transferred to 100 mL volumetric flask and the volume was made with methanol to obtain a solution of 100 µg/mL. From this working solution, various concentrations were made to obtain solution of 5, 10, 15, 20, 25, 30 and 35 µg/mL. These solutions were added to the quartz cell and the calibration curve were prepared using methanol as blank at 284 nm. The absorbance values were plotted against corresponding concentration. Linear relationships were obtained (Table-1). The working solution were prepared freshly everyday. The stability of the drug in the methanol was also investigated.

Preparation of sample solutions

HPLC method: 20 Tablets (amount declared 120 mg etoricoxib per tablet) were weighed and triturated in an agate mortar, pounded and finally a portion of sample composite equivalent to 10 mg of etoricoxib was accurately weighed into a 100 mL volumetric flask. The drug was extracted with 50 mL methanol by sonicating for 10 min with occasional stirring. The volume was made up to 100 mL with the solvent. Then it was mixed, centrifuged and filtered though 0.45 µm membrane filters.

Aliquot of this solution were diluted in mobile phase at concentration of 20 µg/mL for HPLC. These solutions were chromatographed and the area of the each peak was determined. The concentration of etoricoxib(C), were calculated by placing the peak area (Y) into the regression equation, which was obtained from the standard solution (Table-2).

UV Method: The aliquot (sample solution 100 µg/mL as described above) were diluted in methanol at concentration of 10 µg/mL. Absorbance values were recorded at 284 nm against methanol. The concentration of the etoricoxib (C) was calculated by placing the absorbance value (Y') into the regression equation, which was obtained from the standard solutions (Table-2).

TABLE-1
CHARACTERISTIC PARAMETERS OF THE CALIBRATION EQUATION FOR THE PROPOSED HPLC AND UV METHOD FOR THE DETERMINATION OF ETORICOXIB

Parameters	HPLC (284 nm)	UV (284 nm)
Calibration range (μg/mL)	5-50	5-35
Detection limit (μg/mL)	0.25	0.5
Quantitation limit (μg/mL)	1.0	1.0
Regression equation*		
Slope (b)	0.5080	0.049147
Standard deviation of the slope (S_b)	0.002961	0.000132
Relative standard deviation of the slope (%)	0.58	0.27
Confidence limit of the slope[#]	0.4987 - 0.5174	0.04779- 0.04882
Intercept (a)	0.053688	0.002488
Standard deviation of the intercept [#]	0.10255	0.00199
Correlation coefficient (r^2)	0.99972	0.99991

*Y = a + bC where C is the concentration of etoricoxib and Y is the peak area
#95 % confidence limit

TABLE-2
RESULTS OF THE DETERMINATION OF ETORICOXIB IN TABLETS USING HPLC AND UV SPECTROSCOPY

Method	Sample (mg) Etoricoxib	Experimental amount [a] (mg)	Purity	CV (%) (intraday)	CV (%) (interday)[b]
HPLC	120	121.54(1.02)	101.29		
		119.78(0.51)	99.82		
		119.23(1.05)	99.36		
		120.65(1.86)	100.54		
		117.96(0.31)	98.30		
		121.01(0.69)	100.84	1.09	1.11
UV	120	120.38(0.14)	100.32		
		119.97(0.39)	99.98		
		120.22(0.99)	100.18		
		121.87(0.43)	101.56		
		119.30(0.79)	99.41		
		118.55(0.32)	98.79	0.93	0.53

[a]Mean of three determination, CV (%) are listed in brackets,
[b]3 days

Method validation

In this work, the method was validated by determination of linearity, precision, accuracy, detection and quantitation limits, ruggedness and specificity[13, 14].

Linearity: The calibration curve was obtained with seven concentration of standard solution, (5-50 μg/mL for HPLC method and 5-35 μg/mL for UV method). The solutions were prepared in triplicate. The linearity was evaluated by linear regression analysis, which was evaluated by the least square regression method.

Precision: The precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability was evaluated by assaying samples at same concentration and during the same day. The intermediate precision was studied by comparing the assay on different days (3 d). Six sample solutions (20 µg/mL) for HPLC and (10 µg/mL) for UV method were prepared and assayed.

Accuracy: The accuracy was determined by recovery of known amount of etoricoxib reference standard added to the samples at the beginning of the process. For the HPLC method, an accurately weighed amount of powder equivalent to 20 mg of etoricoxib was transferred to 100 mL volumetric flask and dissolved in methanol (final concentration of 200 µg/mL). Aliquots of 2.0 mL of this solution were transferred into 20 mL volumetric flasks containing 1.0, 2.0 and 3.0 mL of etoricoxib standard solution (100 µg/mL) and mobile phase was added to make up to volume to give a final concentration of 25, 30 and 35 µg/mL. For the UV method, an accurately weighed tablet triturate equivalent to 10 mg of etoricoxib was transferred to 100 mL volumetric flask and dissolved in methanol (final concentration of 100 µg/mL). Aliquots of 2.0 mL of this solution were transferred into 20 mL volumetric flasks containing 1.0, 2.0 and 3.0 mL of etoricoxib standard solution (50 µg/mL) and methanol was added to make up to volume to give a final concentration of 12.5, 15 and 17.5 µg/mL. All solutions were prepared in triplicate and assayed. The percentage recovery of added etoricoxib standard was calculated using the equation proposed by AOAC[12].

Specificity: Series of five solutions of the drug in 20 µg/mL for HPLC method were prepared from the working solution meant for method validation and analyzed.

Ruggedness: The ruggedness of the method was determined by varying the analyst, instrument (ATI-Unicam spectrophotometer and Merck-Hitachi HPLC) and different column of same make for LC method.

Limit of detection and limit of quantitation: The parameters LOD and LOQ were determined on the basis of response and slope of the regression equation for UV and signal to noise ratio in case of HPLC method.

Stability: The stability of etoricoxib in methanolic solution was studied by HPLC method. Sample solution of etoricoxib (50 µg/mL) were prepared in triplicate and stored at 4º and 25ºC for 24, 48 and 72 h in amber coloured tubes wrapped with aluminium foil. The stability of these solutions was studies by performing the experiment and looking for the change in the chromatographic pattern compared with freshly prepared solution.

RESULTS AND DISCUSSION

HPLC method: The developed HPLC method was applied for the determination of etoricoxib. To optimize the chromatographic conditions various combination of water, acetonitrile and methanol (50:50:0, 70:30:0, 40:60:0, 50:25:25, 60:0:40, 30:0:70, 20:0:80, v/v/v) were tested. Mobile phase selection was based on peak parameters (height, asymmetry, tailing) run time, preparation time and cost. The mobile phase water:acetronitrile: methanol (50:25:25, v/v/v) was selected.

Before an analytical method is applied to quality control, it is necessary to validate the method. The validation ensures that the procedure is suitable for its intended purpose. The type of method and its respective use to determine which parameter should be evaluated. It is the responsibility of the analyst to select the parameters considered relevant for each method[15].

To access linearity, a calibration curves for etoricoxib were constructed by plotting concentration versus peak area and showed good linearity in the 5-50 µg/mL range. The representative linear equation was y = 0.5080 × + 0.002961, with correlation coefficient (r = 0.999) highly significant for the method (Table-1). The LOD and LOQ were found to be 0.25 µg/mL and 1.0 µg/mL respectively. The validity of the assay was verified by means of the ANOVA. According to ANOVA there are linear regression ($F_{calculated} > F_{critical}$; P = 0.01) and there are no deviation from linearity ($F_{calculated} < F_{critical}$; P = 0.01).

The precision of the method was determined by repeatability (intra-day) and intermediate precision (inter-day) and was expressed as CV (%) of a series of measurement. The experimental values for the estimation of etoricoxib in samples are presented in Table-2. The result obtained shows CV (%) of 1.09 % indicating good intra-day precision. Inter-day variability was calculated from assay on 3 days and shows a mean CV (%) of 1.11 %. The accuracy of the method was determined and the mean recovery was found to be 100.46 % (Table-3) indicating an agreement between the true values and the values found.

Fig. 2 shows a typical chromatogram obtained from the analysis of a standard and sample solution of etoricoxib using proposed method. As shown in this figure, etoricoxib was eluted forming symmetrical peak, well separated from the solvent front. The retention time observed (*ca.* 4.8min) allows a rapid determination of the drug, which is important for routine analysis. The specificity test demonstrated that there was no interference in the drug peak. The chromatogram obtained through the injection of the placebo solution did not contain any other peak at the retention time of etoricoxib. The chromatogram peak purity tools show that the peak was 100 %. Thus, it was shown that the peak at 4.8 min was not due to any interference from the excipients in the formulation. In this method, the internal standard was not used as there was no extraction or separation step involved.

TABLE-3
EXPERIMENTAL VALUES OBTAINED IN THE RECOVERY TEST FOR ETORICOXIB BY PROPOSED METHODS

Method	Sample concentration (µg/mL)	Concentration of added standard (µg/mL)	Recovery (%) ± CV (%)
HPLC	20.0	5.0	101.18 ± 0.62
	20.0	10.0	100.16 ± 1.09
	20.0	15.0	100.04 ± 1.01
UV-284	10.0	2.5	99.72 ± 1.17
	10.0	5.0	100.43 ± 0.88
	10.0	7.5	101.20 ± 0.73

*Mean of three determinations.

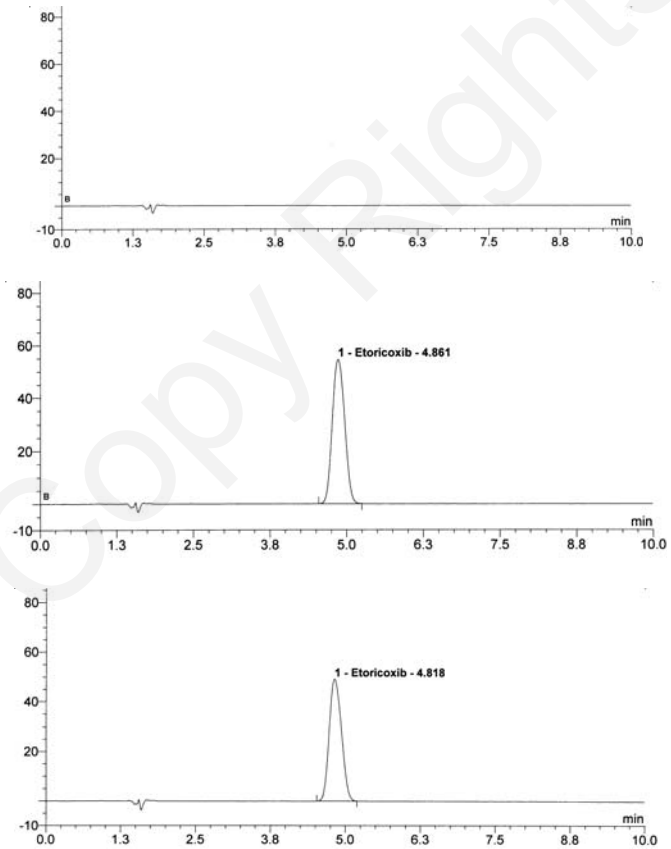


Fig. 2. Chromatogram of placebo solution, b. etoricoxib (25µg/mL) sample solution and c. standard solution. Chromatographic conditions: mobile phase water: acetonitrile: methanol (50:25:25, v/v/v); flow rate of 1.25 mL/min; BDS-C-8 (150 × 4.6 mm, 5 µm); UV detection at 284 nm, temperature 20 ± 1ºC; injection volume 10 µL

The ruggedness of the method was evaluated by changing the analyst or the HPLC instrument or column of same make. These changes did not produce any significant change in the chromatographic data, except that the Dionex HPLC expressed the area count in term of 1.000 while the Lachrome HPLC equipped with integrator expressed the area count in term of 1,000.

UV method : The proposed UV method allows a rapid and economical quantitation of etoricoxib in tablets without any time-consuming sample preparation. The λ_{max} 284 nm was chosen for the analysis to avoid possible interference from the tablet excipients. Calibration curves were constructed in the range of expected concentrations (5-35 µg/mL). Beer's law is obeyed over this concentration range. The representative equation of analysis was $y = 0.049147x \pm 0.000132$ with a correlation coefficient of 0.999 (Table-1). In case of UV spectroscopy the limit of detection was 0.50 µg/mL. The samples were analyzed using methanol as solvent. Fig. 3, shows the UV spectra of the reference drug and Tablet extract in methanol.

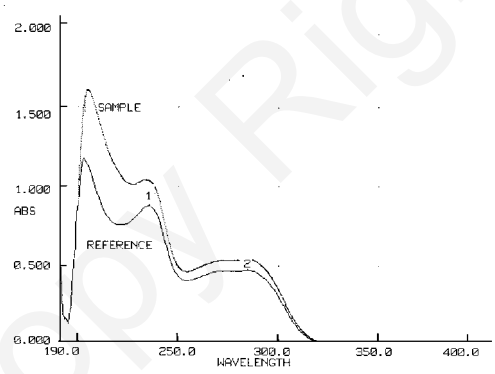


Fig. 3. UV spectra of etoricoxib reference (10.0 µg/mL) and tablet exract sample (12.5 µg/mL) in methanol showing λ_{max} (1)235 and (2) 284 nm

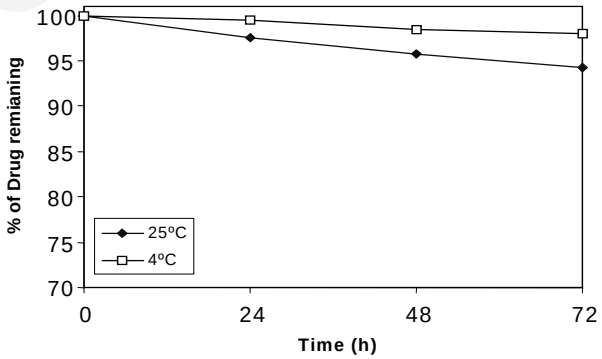


Fig. 4. Curve of etoricoxib solution (50 µg/mL solution in mobile phase) stability stored at 4º and 25ºC during 24, 48 and 72 h

Table-2 shows the experimental values obtained for the determination of etoricoxib in samples indicating a satisfactory intra-day variability (CV% of 0.93 %) and inter-day variability (CV% of 0.53 %). A good accuracy of the method was verified with a mean recovery of 100.45 % (Table-3).

Comparison between HPLC and UV method: The proposed analytical methods were compared using statistical analysis. The Student's t-test was applied and does not reveal significant difference between the experimental values obtained in the sample analysis by the two methods. The calculated t-value (t_{calc} = 0.25) was found to be less than the critical t-value (t_{crit} = 2.23) at 5 % significance level.

Stability: The stability of etoricoxib in mobile phase was evaluated to verify that any spontaneous degradation occured when the samples were prepared and stored. Fig. 4 shows the stability profile at 4º and 25ºC for 24, 48 and 72 h. The results were expressed at percentage of drug remaining. The data obtained showed that sample solution were stable during 24 h when stored at 4º and 25ºC with a degradation less than 5 %. Etoricoxib was less stable at 25ºC with degradation of 5.7 % after 72 h.

Conclusion

Developed high performance liquid chromatographic and ultraviolet spectroscopic methods are simple, reliable and reproducible for the analysis of etoricoxib in pharmaceutical formulations. The reported method can be used successfully for the effective qualitative and quantitative analysis of etoricoxib in tablet or other pharmaceutical formulation. However, The UV spectroscopic method is simpler and more economical than the HPLC.

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