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Research Article

DETERMINATION OF ETODOLAC BY RP-HPLC METHOD

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ABSTRACT

A simple, rapid, economic, accurate and precise reverse phase high performance liquid chromatography method for analysis of Etodolac was developed and validated according to ICH guidelines. The quantification of the drug was carried using photodiode array detector. RP-C18 column was used in isocratic mode, with 60:40 ratio of Acetonitrile: Methanol as a mobile phase. The flow rate was set to 1.0 ml/min. The UV detection was carried out at wavelength 226 nm wavelength was selected for analysis. The method was validated by performing recovery study.

KEYWORDS: Reverse phase high performance liquid chromatography**,** Etodolac, Acetonitrile, photodiode array, isocratic mode, recovery study.

INTRODUCTION

Etodolac is chemically as 1, 8-Diethyl-1, 3, 4, 9-tetrahydropyrone (3, 4-b) indole -1-acetic acid. It is a racemic mixture of [+] S enantiomers and [-] R enantiomers[1].Etodolac is belongs to pyranocarboxylic acid class of non-steroidal anti-inflammatory drug, developed in the 1970s [2].It is used for rheumatoid arthritis and osteoarthritis, postoperative pain and inflammation [3], and used as analgesic, anti-inflammatory agent, antipyretic and cyclo-oxygenase inhibitor. The mechanism of action of Etodolac is mainly prostaglandin synthetase enzyme inhibition. The enzyme inhibited by NSAIDs is the Cyclo-oxygenase enzyme. Etodolac attached to the upper part of the COX enzyme active site and avoid its substrate, arachidonic acid, from entering the active site. Cyclo-oxygenase present in two separate entities, one is Cox-1 and other is Cox-2[4, 5]. Etodolac is official in the United States Pharmacopoeia and British Pharmacopoeia [6].

Literature survey revealed that only few methods have been reported for the determination of Etodolac. Some methods have been reported in combination of other drugs like paracetamol, thiocolchicoside etc. A simple stability indicating analytical method development and validation for the simultaneous estimation of Paracetamol and Etodolac using RP-HPLC method in bulk and pharmaceutical dosage form[7], method development and validation of Etodolac by visible spectroscopy[8],development and validation of HPLC method for estimation of Etodolac in rat plasma[9], development and validation of UV- spectrophotometric and RP-HPLC method for the simultaneous estimation of Paracetamol and Etodolac in marketed formulation[10] these methods were reported. The present research work describes a simple, precise and accurate RP-HPLC method for the determination of Etodolac

MATERIALS AND METHODS

Apparatus

An Isocratic HPLC Jasco PU- 4180 with Photo diode Array detector and RP-C18 column was used. A Rheodyne injector with a 20 µL loop volume was used for the injection of sample. The HPLC system was equipped with Empower software for data processing.

Chemicals and Reagents

Pure drug Etodolac was procured as gift sample from IPCA Laboratories, Mumbai. Water, Acetonitrile and Methanol were of HPLC grade.

Selection of wavelength

Initially method development work was initiated by taking UV visible spectra from 200-400 nm. The standard stock solutions were prepared separately by dissolving pure drug Etodolac in a solvent (mobile phase) followed by dilution with the same solvent. After optimization of all conditions for UV analysis, this has been performed to know the wavelength maxima of Etodolac, so that the same wavelength can be utilized in HPLC UV detector to estimate the Etodolac.

Mobile phase preparation

600 mL (60%) of Acetonitrile (ACN) and 400 mL (40%) of Methanol (MeOH) were mixed well using mixing vessel and degassed in ultrasonic water bath for 15 min. The solution was filtered through 0.45 µm under vacuum using Ultrapore Filtration assembly

Preparation of stock solution

Reference solution: 25 mg of Etodolac standard was transferred into 25 mL volumetric flask, dissolved and make up to volume with mobile phase to obtain concentration of 1mg/mL. Further dilution was done by transferring 1 mL of the above solution into a 10 mL volumetric flask and subsequent dilutions were made to obtain 100µg/mL (as stock) with mobile phase.

Standard drug preparation for the analysis

Dilutions were made in so prepared stock solution by using micropipette to withdraw 1mL and followed by dilution with mobile phase to have resultant solution having concentration of 10 µg/mL

Chromatographic conditions

The mobile phase containing Acetonitrile: Methanol (60:40) was optimized for study of Etodolac. The mobile phase was filtered through 0.45 µ membrane filter and then ultrasonicated for 15 min. The flow rate was set to 1.0 mL/min. The 226 nm wavelength was selected for analysis. All determinations were performed at constant column temperature (30 ± 2ºC).

VALIDATION STUDY [11, 12, 13]

Linearity

The linearity of an analytical process is its ability to get test results which are directly proportional to the concentration of analyte in the sample. For this study the solutions were prepared in the range of 2, 5, 10, 15, 20 & 25 µg/mL and injected. Regression coefficient was calculated by plotting a graph between concentration of the test solution on X-axis and response of the corresponding solutions on Y-axis.

Accuracy

Accuracy is the degree of concurrence between the true value and the measured value. To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts of pure drug Etodolac (80%, 100% and 120%) were taken and further added to the pre-analyzed formulation of concentration 10 µg/mL. From that percentage recovery values were calculated.

Precision study

Precision –Repeatability / intraday

The closeness of agreement between sequences of measurements represents the precision of the developed method. The precision of every method was discovered separately from the retention times and peak areas obtained by actual determination of six replicates of a fixed amount of drug Etodolac. The percent relative standard deviation was calculated for Etodolac

Intermediate Precision

To evaluate the intermediate precision (interday) of the method, precision was performed on different day. It is also known as Ruggedness. The standard solutions prepared in the precision were injected on the other day, for six times and measured the area for all six injections in HPLC. The Percent RSD for the area of six replicate injections was found to be within the specified limits. The percent relative standard deviation was calculated. Acceptance criteria: The Percent relative standard deviation of Etodolac, from the six units should be not more than 2.0 %

System suitability

System suitability which makes certain that the system is suitable and reproducible for current analytical run, it is one of the important tests defined in the first American Association of Pharmaceutical Scientists (AAPS/FDA) bio analytical workshop. To verify whether analytical system is working properly, this study was evaluated by injecting the standard drugs of Etodolac six times into HPLC system. The RSD of the parameters like theoretical plates, peak area, retention time and asymmetric factor were calculated.

Acceptance criteria
1. The % RSD for the retention times of principal peak from 6 replicate injections of each Standard solution should be not more than 2.0 %
2. The % RSD for the peak area responses of principal peak from 6 replicate injections of each standard Solution should be not more than 2.0%.
3. The number of theoretical plates (N) for Etodolac, peaks is NLT 3000.

Specificity

The specificity study was carried on optimized formulation liquisolid compact tablet of Etodolac to check interference of various excipients on retention time of drug. 3 injections of sample prepared using tablet and 3 injections of pure drugs were injected. The solution was injected to the chromatographic system to evaluate the possible interfering peaks.

Acceptance criteria: Chromatograms of sample and standard should be same with near Retention time.

Robustness

The robustness verifies the capacity of the method to remain unchanged by small deliberate changes in various parameters and its ability to provide reproducible data during actual sample analysis. Small alterations in the chromatographic conditions have been made to find out the robustness of the method. Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1mL/min), temperature (±2°C) and organic phase content in mobile phase (± 2%) studied to determine the robustness of the method are also in favor of (% RSD < 2 %) the developed RP-HPLC method for the analysis of Etodolac.

Acceptance criteria
The Tailing Factor of Etodolac standards should be NMT 2.0 for Variation in Flow.

LOD and LOQ

Limits of Detection [LOD] and Quantification [LOQ] were calculated directly from the calibration plot.
Limit of detection it is also called detection limit, the smallest amount or concentration of analyte in the test sample that can be consistently distinguished from zero.
Limit of quantification is the lowest amount or concentration of analyte that can be concluded with an acceptable repeatability and trueness.

Both the values are calculated by using following formulas;

$$\text{LOD} = 3.3\,\frac{\sigma}{S}$$

$$\text{LOQ} = 10\frac{\sigma}{S}$$

Where σ is the standard deviation of intercept and S is the slope of the calibration plot.

RESULTS AND DISCUSSION

Selection of wavelength

UV spectrum of Etodolac solution showed maximum absorbance at 226 nm. The wavelength of 226 nm was chosen as suitable detection wavelength because of clear flat baseline.

Linearity

The linearity of calibration curve was evaluated using linear regression analysis. The calibration curve was constructed by plotting a graph of peak mean area versus concentration. Linear regression equation was found to be y = 3989x + 1724 (R^2=0.999) which meet the analytical method validation acceptance criteria. Hence linearity of method was proved over the concentration range of 0 - 25 µg/mL. (Table2).

Accuracy

The accuracy of method was assessed by recovery studies on samples. The good recovery values for accuracy study determine that method is accurate as shown in Table 3. The average % recovery of Etodolac was found to be within the limits and that are 99.58 - 99.95 respectively.

Precision

The intra-day and inter-day precision of method was expressed as RSD value. The RSD values for intra- and inter-day were found to be less than 2%, thus indicating the good precision of the method. It is shown in Table 4 and Table 5.

System Suitability

The RSD of the parameters like theoretical plates, peak area, retention time and asymmetric factor were calculated and shown in Table 6.

Specificity

The specificity study results are as shown in Table 7 indicates that the chromatograms of sample and standard are having matching retention times.

Robustness

Robustness of the method was determined by carrying out the analysis under different temperature condition i.e. 28ºC, 30C, 32ºC (Table8)

LOD and LOQ

Table 9 highlights the observations and predictions from detection and quantification study.

Calculation of Limit of detection (LOD) = 3.3 × (106/3989) = 0.0876 µg/mL
Calculation of Limit of quantification (LOQ) = 10 × (106/3989) = 0.26 µg/mL

TABLE 1: STATISTICAL PARAMETER FOR THE CALIBRATION GRAPH OF ETODOLAC BY RP-HPLC METHOD

Parameter	RP-HPLC (Etodolac)
Beer law range (µg/mL)	2 - 25
Slope	3989
Intercept	1724
Coefficient of Correlation	0.999
Limit of detection (LOD) µg/mL	0.0876
Limit of quantitation (LOQ) µg/mL	0.26

TABLE 2. PEAK AREA OF ETODOLAC

Concentration (µg/mL)	Peak area
0	0
2	10144
5	22327
10	43261
15	61857
20	79946
25	101697

TABLE 3: ACCURACY STUDY DATA

Std. Concentrations in ppm	Peak area observed	Recovery added in ppm	Total concentration	% Recovery added	Peak area observed	Concentration observed in ppm	% Mean	% RSD
10	43515	8	18	80	73257	18.00	99.75	0.036
					73310	17.94		
					73281	17.93		
10	43620	10	20	100	81109	19.90	99.58	0.095
					81198	19.92		
					81264	19.93		
10	43724	12	22	120	89489	22.00	99.95	0.07
					89384	21.97		
					89510	22.00		

TABLE 4: INTRADAY/ REPEATABILITY DATA OF ETODOLAC

Sr. No.	Standard Concentrations (ppm)	Peak area observed	Concentration observed in ppm	% RSD
1	10	43238	10.40712	0.31
2	10	43198	10.39709	
3	10	43201	10.39784	
4	10	43522	10.47832	
5	10	43234	10.40612	
6	10	43345	10.43394	

TABLE 5: INTERDAY/ INTERMEDIATE PRECISION OF ETODOLAC

Sr. No.	Day	Session	Std. Concentration (ppm)	Peak area observed	Concentration observed in ppm	%RSD
1	1	Morning	10	43265	10.41389	0.043152
2		Afternoon	10	43308	10.42467	
3		Evening	10	43267	10.41439	
4	2	Morning	10	43274	10.41614	
5		Afternoon	10	43297	10.42191	
6		Evening	10	43284	10.41865	

TABLE 6: SYSTEM SUITABILITY STUDY

Sr. No.	Peak Name	tR	Area	Height	Area %	Height %	NTP	Symmetry Factor
1	STD1	2.341	43265	4746	100	100	1321	0.856
2	STD2	2.332	43314	4761	100	100	1327	0.826
3	STD3	2.339	43295	4765	100	100	1357	0.830
4	STD4	2.335	43146	4768	100	100	1324	0.857
5	STD5	2.326	43245	4765	100	100	1341	0.838
6	STD6	2.345	43451	4761	100	100	1354	0.827

TABLE 7: SPECIFICITY STUDY

Sr. No.	Peak Name	tR	Area	Height	Area %	Height %	NTP	Symmetry Factor
1	10 ppm	2.386	43324	4714	100.000	100.000	1289	0.816
2	10 ppm	2.345	43465	4716	100.000	100.000	1291	0.820
3	10 ppm	2.374	43354	4751	100.000	100.000	1298	0.811
Optimized tablet Liquisolid compact								
4	Unknown	2.305	45025	4654	100.000	100.000	1398	0.815
5	Unknown	2.355	43660	4682	100.000	100.000	1384	0.828
6	Unknown	2.335	44658	4675	100.000	100.000	1387	0.824
Pure drug Etodolac at 1 ml/min, mobile phase composition 60:40 (ACN:MeOH) & at 30 ^{0}C								
7	10 ppm	2.386	43324	4714	100.000	100.000	1289	0.816

TABLE 8: ROBUSTNESS STUDY AT DIFFERENT FLOW RATE, TEMPERATURE AND MOBILE PHASE COMPOSITION ACN: MEOH

Flow rate						
	0.9 mL/min		1 mL/min		1.1 mL/min	
	43658	10.51241	43324	10.42868	43638	10.5074
	43624	10.50389	43465	10.46403	43645	10.50915
	43673	10.51617	43354	10.4362	43635	10.50664
Average	43651.67	10.51082	43381	10.44297	43639.33	10.50773
SD	25.10644	0.006294	74.27651	0.01862	5.131601	0.001286
RSD		0.062939		0.186203		0.012864
Temperature						
	28ºC		30ºC		32ºC	
	43715	10.5267	43324	10.42868	43658	10.51241
	43735	10.53171	43465	10.46403	43675	10.51667
	43740	10.53297	43354	10.4362	43680	10.51792
Average	43730	10.53046	43381	10.44297	43671	10.51567
SD	13.22876	0.003316	74.27651	0.01862	11.53256	0.002891
RSD		0.033163		0.186203		0.028911
Mobile Phase % ACN: MeOH						
	58:42		60:40		62:38	
	43601	10.49812	43324	10.42868	43764	10.53898
	43631	10.50564	43465	10.46403	43715	10.5267
	43648	10.5099	43354	10.4362	43756	10.53698
Average	43626.67	10.50455	43381	10.44297	43745	10.53422
SD	23.79776	0.005966	74.27651	0.01862	26.28688	0.00659
RSD		0.059658		0.186203		0.065898

TABLE 9: OBSERVATIONS AND PREDICTIONS FROM DETECTION AND QUANTIFICATION STUDY

Sample	Y axis Intercept
PEAK 1- 10 ppm	1724
PEAK 2 - 10 ppm	1685
PEAK 3 - 10 ppm	1524
AVG	1644.33333
S.D.	106.020438

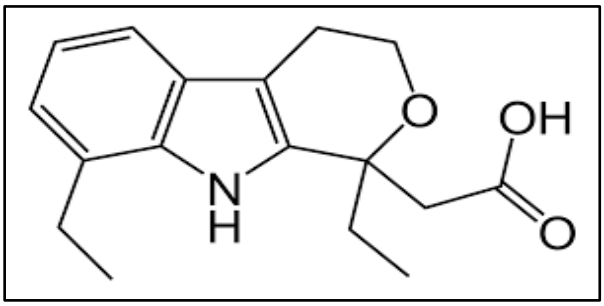

Fig 1: Structure of Etodolac

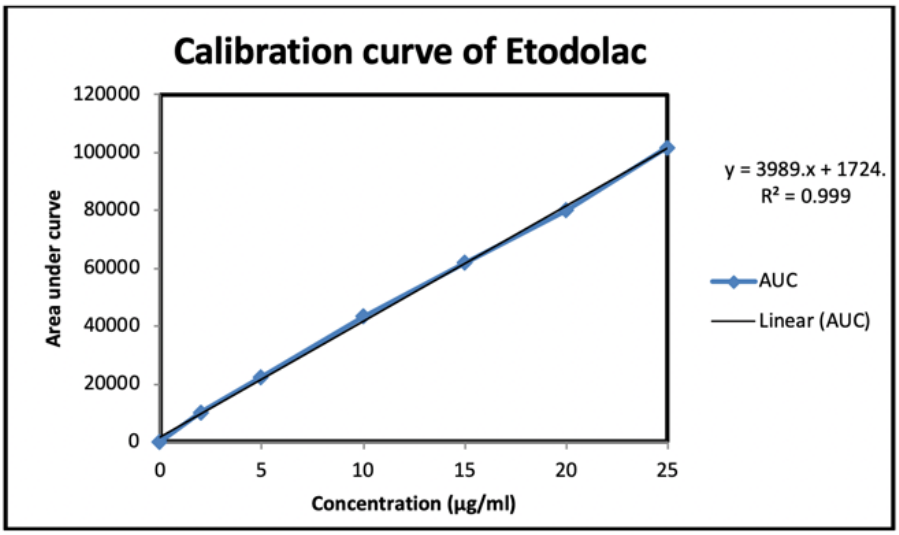

Fig 2: Linearity plot for etodolac

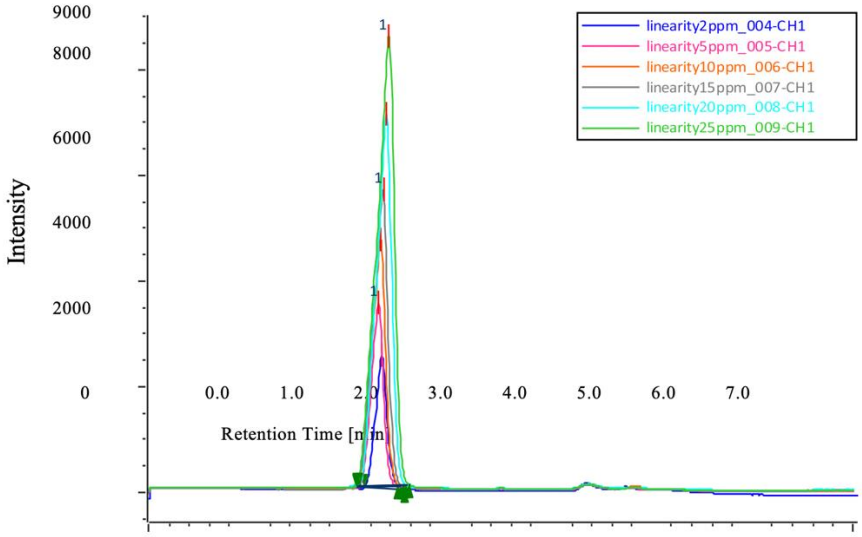

Fig 3: Linearity Overlay Chromatogram

CONCLUSION

The RP-HPLC method was developed and validated for determination of Etodolac. The selected method was found to be sensitive, accurate and reproducible for analysis of Etodolac

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ABBREVIATIONS

RPHPLC = Reverse phase high performance liquid chromatography
SD = Standard deviation
RSD = Relative standard deviation
LOD = Limit of detection
LOQ =Limit of quantitation
ppm = Part per million
AUC =Area under curve

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