Research Article

Development and Validation of Stability Indicating Assay Method of Etodolac by using UV-Visible Spectrophotometer

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ABSTRACT

The objective of study was to study and develop a simple accurate, precise and cost effective UV-Vis spectrophotometric method for the estimation of Etodolac in bulk and pharmaceutical dosage form. The solvent used was methanol and the absorption maxima of the drug was found to be 226nm. A linear response was observed in the range of 2-20µg/ml with a regression coefficient of 0.999. The method was then validated for different parameters as per the ICH (International Conference for Harmonization) guidelines. This method can be used for the determination of Etodolac in quality control of formulation without interference of the excipients. Etodolac was subjected to stress degradation under different conditions recommended by ICH. The degradation studies was carried out by using the developed method.

Key words: Etodolac, stress degradation studies, validation, UV-Vis spectroscopy.

INTRODUCTION

Etodolac wavelength corresponding to maximum absorbances was noted which is its λ max i.e. at 226nm.

Molecular Formula: $C_{17}H_{21}NO_3$

Assay of Etodolac [1-5]

A quantity of powder equivalent to 50mg of Etodolac was taken in a 50ml volumetric flask and it was dissolved and diluted upto the mark with methanol. The resultant solution was ultrasonicated for 5 minutes. The solution was then filtered using Whatmann filter paper no. 40, from the filtrate appropriate dilutions were made in methanol to obtain the desired concentration (50µg/ml). This solution was then analyzed in UV and the result was indicated by % recovery.

MATERIALS AND METHODS

The drug Etodolac was obtained from Emcure Pharmaceutical, Bhosari-Pune. The instrument used for the present study was Shimadzu UV-1800 spectrophotometer. Methanol was used as a solvent (AR grade), NaOH (AR grade), HCl (AR grade) and H_2O_2 (AR grade) .These other chemicals were purchased from Merck Chemicals (Mumbai, India).

UV method development [1-8]

Preparation of stock solution

Standard stock solution of Etodolac was prepared by dissolving 10mg of Etodolac in 100ml of methanol which gives 100µg/ml solution.

Preparation of working solution

From the above stock solution 2ml was transferred into 10ml volumetric flask and The volume made was up to mark with methanol to give 20µg/ml. Etodolac was scanned with UV-Vis spectrophotometer in the range 200-400nm against methanol as blank and the wavelength corresponding to maximum absorbance was noted which is its □max i.e.at 226nm (fig.1).

Preparation of calibration curve

0.2 ml-2ml of 100µg/ml solution were diluted and the volume was made up to 10ml using methanol to produce 2-20µg/ml solutions respectively. The absorbance calibration curves were plotted by taking concentration on x-axis and absorbance on y-axis, which shows a straight line (fig.1). This straight line obeyed linearity in the concentration range of 2-20µg/ml. The correlation was found to be 0.999.

Acidic degradation [2-6]
2ml of stock solution of Etodolac and 5ml of 5 N HCl was added in 10ml of volumetric flask and the volumetric flask was kept at normal condition for 3 hour. After 3hour time interval, solution was neutralized and diluted with methanol in order to make the volume up to 10ml and the dilution was carried out to achieve the appropriate concentration (20µg/ml) (table no.2 and fig. no.2).

Alkali degradation [2-6]
2ml of stock solution of Etodolac and 5ml of 5N NaOH was taken in 10ml of volumetric flask, and the volumetric flask was kept at normal condition for 3hours. After 3hour time interval, solution were neutralized and diluted with methanol in order to make the volume up to 10ml,This solution was further diluted to achieve the appropriate concentration (20µg/ml) (table no.2 & fig.no.3).

Oxidative degradation [2-6]
2 ml of the stock solution of Etodolac and 5 ml of 6 % w/v of hydrogen peroxide was taken in 10 ml of volumetric flask, and volumetric flask was kept at normal condition for 3 hours. After 3 hour time interval, solution was diluted with methanol in order to make the volume up to 10 ml and This solution was further diluted to achieve the appropriate concentration (20µg/ml) (table no.2 & fig.no.4).

Dry heat induced degradation [2-6]
Etodolac sample was taken in a petri plate and exposed to a temperature of 50°C for 3hours in an oven. After 3hours, 10mg of the sample was diluted with methanol in order to make the volume up to 10ml. From this solution, dilutions were carried out to achieve the appropriate concentration (20µg/ml), and the solution was taken in cuvette for the UV-Vis Analysis (table no.2 & fig. no.5).

UV-Degradation at 254 nm [2-6]
10mg Sample of Etodolac was exposed to UV short (254nm) light for 3hours 10mg sample was dissolved in methanol and the volume was made up to 10ml. From this solution, appropriate dilution (20µg/ml) was carried out using methanol and taken in cuvette for the UV analysis.

UV-Degradation at 366 nm [2-6]
Sample of Etodolac was exposed to UV short (366nm) light for 3hour 10mg sample was dissolved in methanol and the volume was made up to 10ml. From this solution, appropriate dilution (20µg/ml) was carried out using methanol and taken in cuvette for the UV analysis.

Method validation

Linearity [2-3]
Various aliquots were prepared from the stock solution (100µg/ml) ranging from 2-20µg/ml. The sample were scanned in UV-Vis Spectrophotometer using methanol as blank. It was found that the selected drug shows linearity between the ranges of 2-20µg/ml (table no.1 and graph no.1).

Accuracy [2-3]
Solutions were prepared in triplicate at levels of 80%, 100%, and 120% of test concentration, using Etodolac working standard as per the method, and absorbance of each solution in triplicate was taken. The recovery result showed that the proposed method has an acceptable level of accuracy for Etodolac (in from 80% - 120% of test concentration) is from 99.51 % - 100.01 % (table no. 1 & 4).

Precision [2-3]
Precision of the method was demonstrated by intraday and interday variation studies. In intraday variation study six different solutions of same concentration 20µg/ml were analyzed three times in a day i.e. in the morning, afternoon and evening. In the interday variation studies, solution of same concentration 20µg/ml were analyzed three times for the three consecutive days and the absorbance results mean, standard deviation and % RSD was calculated (table no. 5 & 6).

Specificity [2-9]
10mg of Etodolac was spiked with 50 % (5mg), 100 % (10mg), and 150 % (15mg) the sample was analysed for % recovery of Etodolac.

Robustness [5-6]
Robustness of the method was determined by carrying out the analysis under different temperature condition i.e. at 23°C, 28°C and at 33°C. The respective absorbances of 20µg/ml were noted and the result was indicated as % RSD (table no.7).

Ruggedness [5-6]
Ruggedness of the method was determined by carrying out the analysis by different analyst and the respective absorbance of 20µg/ml was noted. The result was indicated as %RSD (table no.7).

Limit of Detection (LOD) [2-7]

The limit of detection (LOD) was determined by preparing solutions of different concentrations ranging from 0.1-0.5µg/ml. The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantified as an exact value (table no.1).

Limit of Quantification (LOQ) [2-7]

The LOQ is the concentration that can be quantified reliably with a specified level of accuracy and precision. The LOQ was calculated using the formula involving standard deviation of response and slope of calibration curve (table no.1).

RESULTS AND DISCUSSION

The developed method was found to be precise as the % RSD values for intraday and inter-day were found to be less than 2%. Good recoveries (99.97 % to 101.4 %) of the drug were obtained at each added concentration, indicating that the method was accurate. The method was also found to be specific indicated by the % recoveries ranging from 98.65 % - 101.16 %. The LOD and LOQ were found to be in sub-microgram level indicating the sensitivity of the method. The method was also found to be robust and rugged as indicated by the % RSD values which are less than 2 %. The results of assay show that the amount of drug was in good agreement with the label claim of the formulation as indicated by % recovery (101.4%). The summary of validation parameters of proposed spectrophotometric method is shown in table 1. The stress degradation studies showed that Etodolac undergoes degradation in acidic, alkaline, oxidation, dry heat and photolytic condition (39.50 %, 39.20 % 50.21 %, 20.76%, 9.92% respectively). Summary of the results of stress degradation studies of Etodolac are shown in the table no.2.

ACKNOWLEDGEMENTS

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Table 1: Summary of validation

PARAMETERS	Results
Linearity indicated by correlation coefficient	0.999
Precision indicated by %RSD	1.35%
Accuracy indicated by %Recovery	100.42%
Limit of Detection	1.1µg/ml
Limit of Quantification	3.33µg/ml
Range	2-20µg/ml
Linear regression Equation	0.995
Robustness indicated by %RSD	0.76%

Table 2: Summary of results of stress degradation studies

Stress condition	Time	Observation	%Degradation
Acidic degradation	4 hours	λ max shifted	39.50%
Alkali degradation	4 hours	λ max shifted	39.20%
6% Hydrogen peroxide	3 hours	λ max shifted	50.21%
Dry heat 50°C	3 hours	λ max shifted	20.76%
Photolytic	3 hours	λ max shifted	09.92%

Table 3: Optical Characteristics

Beer's law limit (µg/ml)	2-20 (µg/ml)
Correlation coefficient	0.999
Regression equation (Y*)	0.1306X-0.03
Slope(a)	0.1306X
Intercept (b)	0.03

Table 4: Accuracy reading of etodolac

No. of preparation	Concentration	Pure drug	% Recovery	Statistical results		
				Mean	SD	%RSD
S1	10	8	0.98			
S2	10	8	0.97	0.98	0.0002	1.03
S3	10	8	1.00			
S4	10	10	1.20			
S5	10	10	1.27	1.23	0.01	0.826
S6	10	10	1.24			
S7	10	12	1.47			
S8	10	12	1.51	1.49	0.01	0.666
S9	10	12	1.49			

Table 5: Precision

Concentration (µg/ml)	Absorbance			Statistical Analysis
10	1.76	1.78	1.74	Mean=1.75
10	1.76	1.76	1.74	SD=0.014
10	1.75	1.78	1.75	
10	1.74	1.76	1.75	%
10	1.75.	1.77	1.76	RSD=0.80
10	1.76	1.78	1.74	

Table 6: Intra assay precision

Concentration(µg/ml)	Absorbance 1	Absorbance 2	Absorbance 3	Average% RSD
10	1.76	1.78	1.74	
10	1.76	1.76	1.74	
10	1.75	1.78	1.75	Mean=1.75
10	1.74	1.76	1.75	SD=0.023
10	1.75.	1.77	1.76	%RSD=1.33
10	1.76	1.78	1.74	

Table 7: Result showing robustness and ruggedness method for etodolac

7.1 Analyst - 1

Concentration(µg/ml)	Absorbance	Statistical Analysis
10	1.75	
10	1.76	
10	1.77	Mean= 1.76
10	1.76	SD=0.01
10	1.75	%RSD=0.568%
10	1.76	

7.2 Analyst -2

Concentration(µg/ml)	Absorbance	Statistical Analysis
10	1.73	
10	1.74	
10	1.72	Mean= 1.773
10	1.75	SD=0.01
10	1.73	
10	1.74	%RSD=0.578%

7.3 Analyst -3

Concentration(µg/ml)	Absorbance	Statistical Analysis
10	1.70	Mean= 1.70 SD=0.0070 %RSD=0.4159%
10	1.70	
10	1.71	
10	1.70	
10	1.70	
10	1.71	

7.4 Temerature-23°C

Concentration(µg/ml)	Absorbance	Statistical Analysis
10	1.72	Mean= 1.72 SD=0.0057 %RSD=0.3356%
10	1.73	
10	1.72	
10	1.72	
10	1.73	
10	1.72	

7.5 Temperature- 28°C

Concentration(µg/ml)	Absorbance	Statistical Analysis
10	1.73	Mean= 1.1.74 SD=0.1414 %RSD=0.0081%
10	1.72	
10	1.76	
10	1.75	
10	1.74	
10	1.75	

7.6 Temperature-33°C

Concentration(µg/ml)	Absorbance	Statistical Analysis
10	1.70	Mean= 1.70 SD=0.000066 %RSD=0.003921%
10	1.70	
10	1.71	
10	1.70	
10	1.71	
10	1.70	

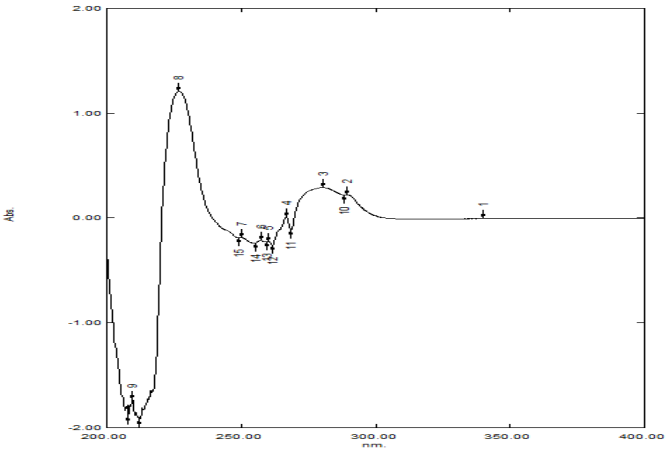


Fig. 1: Standard curve of Etodolac

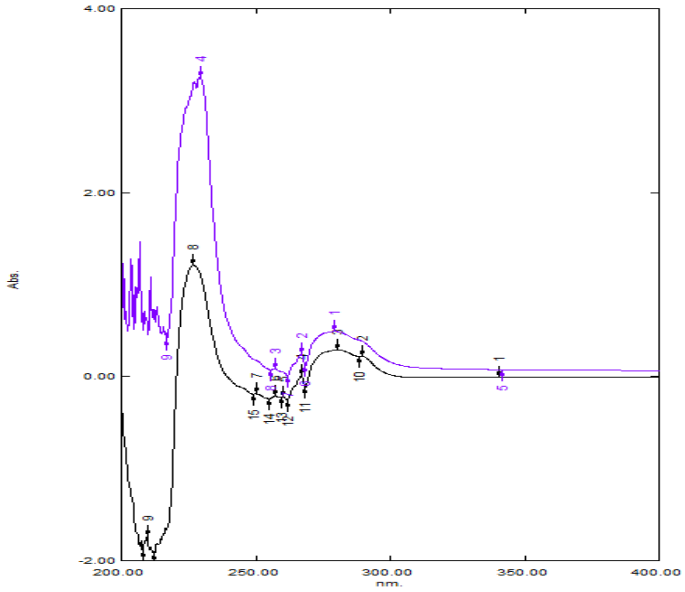


Fig. 2: Acidic degradation by HCl after 2hrs

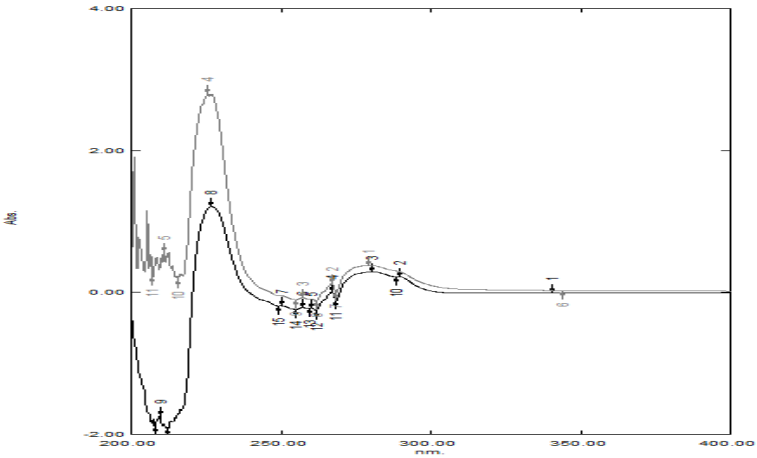


Fig. 3: Alkaline degradation by NaOH after 3hrs

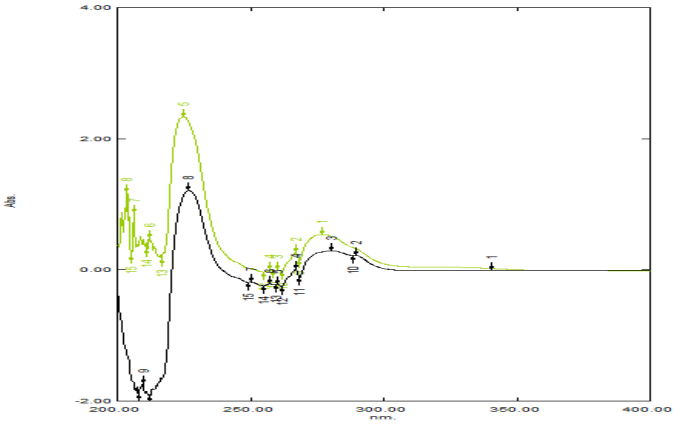


Fig. 4: Oxidative degradation after 3hrs

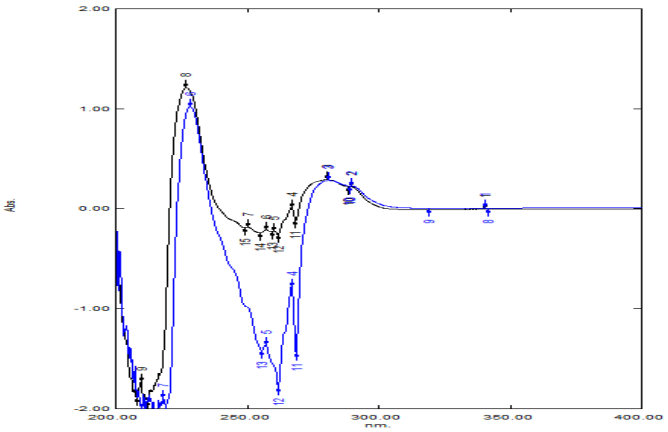


Fig. 5: Dry heat degradation after 3hrs at 50ºC

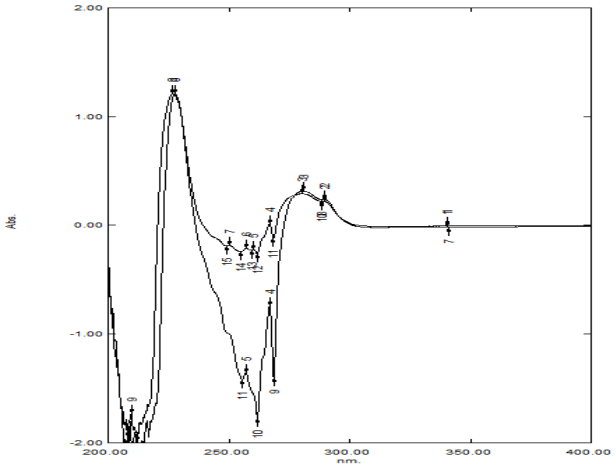
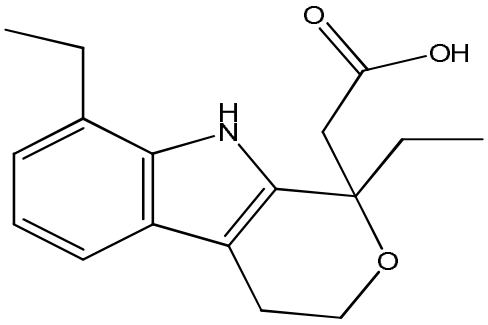
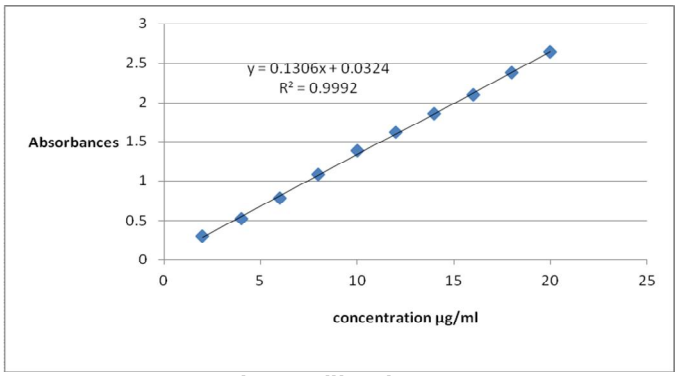


Fig. 6: UV degradation after 3hrs



2-(1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indol-1-yl)acetic acid

Fig. 7: Structure of Etodolac



Graph.1: Calibration curve

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