International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 1 Jan-Feb 2022, pp: 1392-1397 www.ijprajournal.com ISSN: 2249-7781

Development and Validation of Uv-Visible Spectrophotometric Method for Etodolac

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Submitted: 25-02-2022 Accepted: 02-03-2022

ABSTRACT

Aim: To develop and validate a simple, precise and cost-effective UV-visible spectrophotometric method of Etodolac according to the ICH Q2 (R1) guidline.

METHOD: Spiked Etodolac solution was scanned over UV-visible range for its wavelength of maximum absorbance. Various calibration standards of Etodolac were prepared and absorbance was recorded at wavelength of maximum absorbance.Calibration curve of concentration vs. absorbance was plotted and linearity and range was calculated. Various analytical method validation parameters viz. accuracy, precision, LOD, LOQ, robustness, and ruggedness were calculated using QC standards.

Results: The maximum wavelength of Etodolac was found to be 279nm.The correlation coefficient over the concentration range of1-20 µg/mL was found to be 0.9998 .Developed UV method was found to be precise during the intra-day and inter-day study and shows percent relative standard deviation in the range of 0.0487, 0.1988, 0.3011, 0.3994, 0.5947, 0.7794, 0.974 respectively. Developed method was found to be robust and rugged for the intended use.

Conclusion: A simple, precise and costeffective UV-visible spectrophotometric method of Etodolac was developed. The said method was developed using economical percentage of organic phase in aqueous media as solvent. Said validated UV-visible method can be efficiently used for estimation of Etodolac in bulk as well as in plant extract.

KEYWORDS: UV-visible spectrophotometry, Etodolac, validation.

I. INTRODUCTION

Uv method developement of etodolac

Etodolac (E) is a nonsteroidal anti-inflammatory drug (NSAID) that exhibits anti-inflammatory, analgesic, and antipyretic activities in animal models. Etodolac (E) is a member of the pyrano carboxylic acid group of non-steroidal anti-inflammatory drugs (NSAIDs). Etodolac is insoluble in water and slightly soluble in simulated gastric fluid. Its therapeutic effects are due to its ability to inhibit prostaglandin synthesis. It is indicated for relief of signs and symptoms of rheumatoid arthritis and osteoarthritis. The therapeutic effectsof etodolac are achieved via inhibition of the synthesisof prostaglandins involved in fever, pain, swelling andinflammation. Etodolac is administered as a racemate. Similar to other NSAIDs, the anti-inflammatory effects of etodolac result from inhibition of the enzyme cyclooxygenase (COX). This decreases the synthesis of peripheral prostaglandins involved in mediating inflammation. Etodolac binds to the upper portion of the COX enzyme active site and prevents its substrate, arachidonic acid, from entering the active site.

The Molecular formula of Etodolac is C17H21NO3. The IUPAC7 Name of Etodolac is 2-{1,8-diethyl- 1H,3H,4H,9H-pyrano[3,4-b]indol-1-yl}acetic acid.

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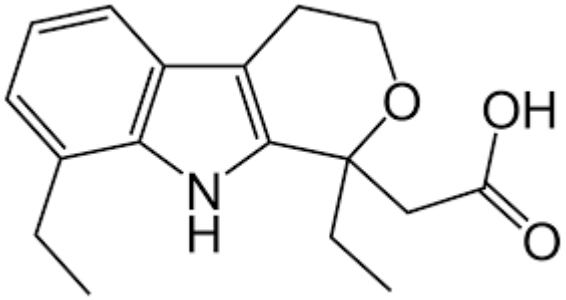

Fig.1 Chemical structure of ETODOLAC

II. METHODOLOGY:

INSTRUMENTS USED:

A double beam UV-visible spectrometer (UV-530, Jasco) with spectra manager software was used for the analysis. Quartz cells having 3 cm length with 1 cm path length were used for spectral measurement. Weighing balance (Vibra HT, Essae) with internal calibration mode was used for the accurate weighing purpose. Ultrasonicator(UCB-40, Spectralab) is also used .pH meter (LI-120, Elico)is also used to maintain the pH of solution.

Sr.No.	Instruments/Apparatus	Manufacturer/Model
1	UV-visible Spectrophotometer	UV-530,Jasco
2	Weighing balance	Vibra HT,Essae
3	Ultra sonicator	UCB-40,Spectralab
4	pH meter	LI-120,Elico

Table No.1 List of Instruments

Chemicals used:

ETODOLAC (ETO) was purchased from TCI Chemicals (India) Pvt. Ltd. All other chemicals of analytical grade were used for study.

Preparation of standard stock solution

Accurately weighed 10 mg of Etodolac was transferred into the calibrated volumetric flask and dissolved in 10ml mixture of Ethanol and water (70:30 v/v) to achieve a stock solution of 1000 µg/mL (Stock-I). Stock- I solution was suitably diluted with co-solvent system of Ethanol and water to achieve a solution of 100 µg/mL (Stock-II).

Determination of wavelength of maximum absorbance (λmax)

Stock-II solution was scanned using full scan mode with medium scanning speed for the entire range of UV and visible i.e. 200 to 800 nm with co-solvent system as a blank. After obtaining the spectrum, λmax was identified with the help of software. In order to achieve reproducible results, above method was repeated five times.

Preparation of calibration curve

Calibration curve was prepared by diluting the stock-II solution to achieve the seven different calibration standards representing 1, 2,4,8,10, 14, 20 µg/mL strength. Absorbance of each calibration standard was measured at pre-identified λmax 279 nm using fixed wavelength measurement mode. The calibration curve representing concentration vs. absorbance was plotted using Excel program of Microsoft Office 2010. Above mentioned procedure was repeated five times so that reproducible results can be obtained.

Method Validation

Developed UV method for the estimation of Etodolac was validated as per the ICH guidelines. Different parameters like linearity and range, accuracy, precision, robustness, ruggedness, limit of detection (LOD) and limit of quantitation (LOQ) were calculated using predefined calibration standards or quality control standards as described below.

Linearity and Range

Linearity of the proposed UV method was established using seven different calibration standards. After analysis of calibration standards, calibration curves in terms of absorbance vs. concentration plots were developed and subjected to linear least square regression analysis. R square value was considered to be important factor for establishing linearity of the proposed method. The interval between upper and lower concentration

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limit with acceptable linearity was reported to be the range of the proposed UV method.

Accuracy

The accuracy of the proposed UV method was evaluated using recovery studies after standard addition of analyte of interest. Three different solutions of Etodolac were prepared in triplicate at level of 80%, 100% and 120% of its predefined concentrations (3, 9 and 18 µg/mL). To the predefined concentrations, different amounts of Etodolac were added (standard addition method) and the accuracy was calculated on the basis of percent recovery. For calculating the percent recovery, following formula was used

%RC= (SPS-S/SP) × 100

Where,
SPS = Amount found in the spiked sample
S = Amount found in the sample
SP = Amount added to the sample
% RC = Percent recovery

Precision

The precision of the proposed UV method was established by performing intra- and inter-day UV analysis of quality control samples (3, 9 and 18 µg/mL). Apigenin solutions of 3, 9 and 18 µg/mL strength (n=5 for each concentration) were analyzed at morning, afternoon and evening time of three consecutive days. Deviation in the results was calculated in terms of % relative standard deviation (% RSD).

Robustness

Robustness of the developed UV method was established using different percentage of Ethanol in co-solvent system. Ethanol percentage in co-solvent system was intentionally adjusted to 75 and 25% and middle level quality control sample (9 µg/mL) of Etodolac was prepared using above mentioned co-solvent system separately. Samples (n=5) were analyzed at 279 nm for Etodolaccontent. The results were calculated in terms of % RSD.

Ruggedness

Ruggedness study of the method was carried out by analyzing triplicate samples of Etodolacsolution (9 µg/mL) using two different instruments (V-530, Jasco and BA-UV-2600, Bioage). Results were expressed in terms of % RSD.

Limit of Detection (LOD)

The LOD of the developed UV method was calculated by using following formula

LOD=3.3×SD/S
Where, SD= Standard deviation of Y-intercepts
S= Slope

Limit of Quantitation (LOQ)

The LOQ of the developed UV method was calculated by using following formula
LOQ= 10×SD/S
Where, SD= Standard deviation of Y-intercepts
S= Slope

III. RESULTS AND DISCUSSION

Determination of wavelength of maximum absorbance

Identification of wavelength of maximum absorbance is prerequisite for quantitative UV analysis. Solution representing absorbance value less than 1 is generally considered to be suitable for the determination of wavelength of maximum absorbance. Considering the prerequisite and the suitability, determination of maximum wavelength for Etodolac solution (9 µg/mL) was carried out using full scan mode of UV-Visible spectrophotometer (Fig). Full scan was processed using Jasco UV software and the λ_{max} was identified with the help of software. The λ_{max} was found to be 279 nm for Etodolac.

Preparation of calibration curve

Quantification of unknown samples by UV-Visible spectrophotometer or any other instrumental method of analysis needs a reproducible calibration curve and a mathematical equation stating correlation between concentration and the response. As compare to graphical method, above stated method is widely accepted and reproducible in nature. Considering the utility of quantitative analysis of Etodolac, calibration curve for Etodolac was developed using seven different calibration standards. The absorbance of different calibration standards at 279 nm was recorded using fixed wavelength mode. Calibration curve was repeated three times and the mean values ± standard deviation was reported as shown in Table 2 .

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Sr. No.	Concentration (µg/ml)	Absorbance
1	1	0.0487
2	2	0.1988
3	4	0.3011
4	8	0.3894
5	10	0.5947
6	14	0.7794
7	20	0.974

Table No.2 Calibration curve data of Etodolac

Method validation

Linearity and Range

Linearity and range are the key parameters of analytical method that demonstrates the limit within which the intended method is to be used for its optimum performance. Considering the prime importance of linearity and the range, seven point calibration curve of Etodolac covering a range of 1-20 µg/mL was plotted. Details of concentrations and the respective mean absorbance values are depicted in Table 2. Calibration curve when subjected to least square regression analysis yielded an equation; y = 0.0486x+0.0043 with correlation coefficient 0.9998 as shown in Figure 2. From the linearity study, it was revealed that, developed UV method was linear over the concentration range of 1-20 µg/mL.

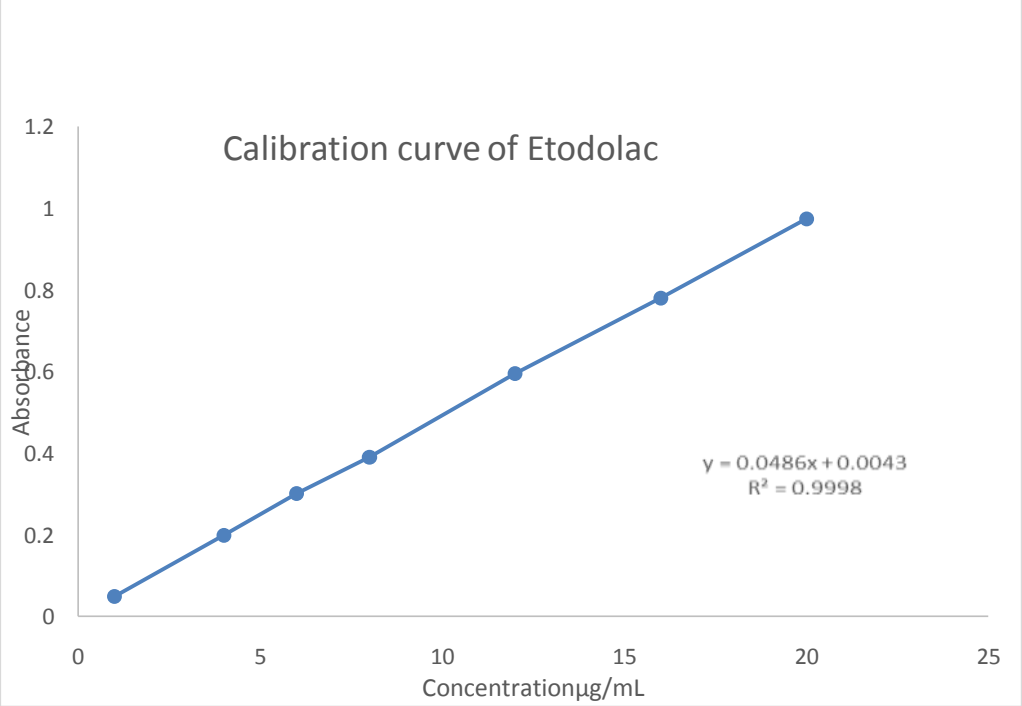

Fig No.2 Calibration curve of Etodolac

Accuracy

Accuracy is a measure of the closeness of the experimental value to the actual amount of the substance in the matrix. Accuracy is to be established over the entire calibration range of the analytical method so that at any point of determination, results obtained would be reliable. In case of UV method for Etodolac, accuracy was established using recovery studies. At 80 % standard addition, mean recovery of Etodolac was

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found to be 99.48% whereas at 100 and 120 % standard addition, it was found to be 99.99and 100.02% respectively. % RSD was found to be less than 2 for the Etodolac recovery studies as shown in Table 2. From the results of accuracy studies, it was observed that developed UV method is highly accurate as the percent recovery was in between 98 to 100% and the % RSD was well below 2%.

Table No 2: Accuracy data of UV method for Etodolac

S No.	Concentration (%)	Origin level (µg/mL)	Amount added (µg/mL)	% Recovery	Mean % Recovery	% RSD
1	80	1.5	1.496	99.73	99.48	0.7841
2	80	1.5	1.492	99.46		
3	80	1.5	1.489	99.26		
4	100	10	10.0154	100.15	99.99	0.5479
5	100	10	9.997	99.97		
6	100	10	9.985	99.85		
7	120	19.5	19.511	100.05	100.02	0.9741
8	120	19.5	19.497	99.98		
9	120	19.5	19.509	100.04		

Precision

Precision is a measure of degree of scatter. It expresses the reproducibility of the measurements. It is expected that an analytical method should generate outcomes that are reproducible. Precise analytical method leads to accurate results. Considering the importance of reproducible yet accurate results, intra- and inter-day precision of developed UV method was established at1.5,10 and 19.5µg/mL levels of Etodolac. The results in terms of mean absorbance values, percent assay and % RSD for the intra- and inter-day precision study are demonstrated in Table 3and Table 4 respectively. % RSD values of intra-day precision study were found to be in between 0.39 and 1.45 whereas those of inter-day precision study were in between 0.12 and 0.98. Overall, % RSD values of less than 2 demonstrated the precision of developed UV method.

Table No 3: Intra-day precision data of UV method for Etodolac

S No.	Conc. (µg/mL)	Morning			Afternoon			Evening		
		Mean	% Assay	%RSD	Mean	% Assay	%RSD	Mean	% Assay	%RSD
1	1.5	1.498	99.86	0.9741	1.489	99.26667	0.8952	1.491	99.40	0.971
2	10	10.01	100.10	0.3987	10.994	99.94	0.6284	10.12	101.20	1.458
3	19.5	19.514	100.07	0.8467	19.498	99.98974	0.7491	19.502	100.01	0.3984

Table No 4: Inter-day precision data of UV method for Etodolac

S No.	Conc. (µg/mL)	Day 1			Day 2			Day 3		
		Mean	% Assay	%RSD	Mean	% Assay	%RSD	Mean	% Assay	%RSD
1	1.5	1.502	100.13	0.651	1.495	99.66	0.4874	1.501	100.06	0.9654
2	10	9.994	99.94	0.974	9.989	99.89	0.3497	10.11	101.1	0.9874
3	19.5	19.497	99.98	0.478	19.507	100.03	0.1277	19.498	99.98	0.4517

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Robustness

Robustness of analytical method is the ability of a method to resist the change in its performance in spite of small, deliberate change in method parameters. It is an important parameter of analytical method as a small, un-intentional change in method parameters like solvent composition, buffer strength, pH etc. may occur during routine use and may hamper the performance of said method. It is expected that such change should not alter the performance of the analytical method. Therefore, robust analytical method is preferred. Robustness of proposed UV method was established by modifying the composition of co-solvent system. Change in Ethanol percentage (71 to 69 %) in co-solvent system did not affect the method performance. % RSD values were found to be in between 0.56 and 0.89 as shown in Table 5. % RSD values below 2 depicted that proposed UV method is robust in nature.

Table 5: Robustness data of UV method for Etodolac

S. NO	Concentration (µg/mL)	% Ethanol	Conc. (µg/mL)	% RSD
1	10	71	10.021	0.5648
2	10	69	9.994	0.8974

Ruggedness

Ruggedness of analytical method is the ability of a method to resist the change in its performance in spite of influential environmental factors like temperature. Rugged analytical methods are preferred as these methods are free from impact of environmental/external factors. In order to establish the ruggedness of proposed UV method, Etodolac solution was analyzed using two different UV-Visible spectrophotometers of two different labs. Sample analysis and data processing resulted into % RSD values between 0.89and 0.95. Results revealed that proposed UV method was rugged as it showed % RSD values less than 2 as shown in Table 6.

Table 6: Ruggedness data of UV method for Etodolac

S. No.	Concentration (µg/mL)	Instrument	Conc. (µg/mL)	% RSD
1	10	Jasco	10.011	0.8994
2	10	Bio-age	9.987	0.9597

Limit of Quantitation (LOQ) and Limit of Detection (LOD)

LOQ represents the lowermost concentration that can be analyzed with acceptable accuracy and precision. Generally, LOQ is the first calibration standard. LOD and LOQ of proposed UV method was found to be 0.8649 and 3.49 µg/mL respectively as shown in Table 7. Lower LOQ value indicated that proposed method would be sensitive enough to quantify the Etodolac content of samples at its lower level.

Table No 7: LOD & LOQ data for UV method for Etodolac

1	LOD	0.8649 µg/mL
2	LOQ	3.49 µg/mL

IV. CONCLUSION

A simple, accurate and precise UV-Visible spectrophotometric method for the estimation of Etodolac was developed and validated. Proposed method was found to be robust and rugged in nature and was successful.

ACKNOWLEDGEMENT

The extra-mural grant support of DST-DPRP, Govt.of India (Ref:-VI-D&P/626/2018-19/TDT) sanctioned to P.I. Dr. Sachin S. Bhusari for the proposed research work is highly acknowledged.