



Original article

HPLC method development and validation for rapid estimation of Etodolac related impurity-H in pharmaceutical dosage form



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ABSTRACT

Objective: The objective of the study was to develop simple, rapid RP-HPLC method for the estimation of Etodolac related impurity-H pharmaceutical dosage form.

Method: The chromatographic separation of Etodolac and its Impurity-H were done with a Kromasil C18, 150 × 4.6 mm, 5μ particle size analytical column using the mobile phase acetate buffer and acetonitrile taken in 55:45% v/v and the response was detected at 221 nm by using PDA detector. Flow rate was maintained at 1 ml/min and temperature was set at 30 °C.

Results: Retention time of Etodolac was 3.1 min and Impurity-H was 8.3 min. The tailing factor and plate count of Impurity-H were 1.06 and 8913 respectively. The developed method was validated as per ICH guidelines. Mean percentage recovery obtained was 108.9%. Beer's range was 2–12 μg/ml with correlation coefficient 0.999. The results of precision, LOD, LOQ, specificity and robustness were found within the limits.

Conclusion: The proposed method was found to be simple, precise, accurate and rapid for quantitative determination of Etodolac related impurity-H in Etodolac pure drug and pharmaceutical dosage form.

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1. Introduction

Etodolac ((RS)-2-(1, 8-Diethyl-4, 9-dihydro-3H-pyrano [3, 4-b] indol-1-yl) acetic acid) is a pyrene carboxylic acid derivative of non-steroidal anti-inflammatory drug.¹ Etodolac selectively inhibits the prostaglandin synthesis at the site of inflammation. It inhibits the cyclooxygenase-1 and 2 enzymes and thus decreases the production of prostaglandins.² Etodolac is widely used in the treatment for rheumatoid arthritis, osteoarthritis, postsurgical pains.² Etodolac related Impurity-H2-(7-ethylindol-3-yl) ethanol [Fig. 1] is a synthesis related impurity.¹ Balan et al, reported method development and validation of Etodolac in tablet dosage form by UV spectroscopy.³ Cosyns et al, reported sensitive high-performance liquid chromatographic method for the determination of Etodolac in serum.⁴ Claudio et al, reported gas chromatography–mass spectrometry determination of Etodolac in human plasma following single epicutaneous administration.⁵ Kiran et al, reported simultaneous estimation of Etodolac and thiocolchicoside in their combined marketed formulation by RP-HPLC.⁶ Krishnaiah et al, reported analysis of drug substances by using new concepts of HPLC

Etodolac and spiramycin.⁷ Patel et al, reported reversed phase-high-performance liquid chromatographic method for simultaneous estimation of tolperisone hydrochloride and Etodolac in a combined fixed dose oral formulations.⁸ Balan et al, reported RP-HPLC method development and validation of Etodolac and paracetamol in combined dosage form.⁹ Ammar et al, reported improvement of the Etodolac purity test by reversed phase-high-performance liquid chromatography.¹⁰

During the synthesis and purification of the drug there might be chance for impurities retained in the drug. Those may be toxic. So every impurity has its limit of existence according to ICH guidelines. So the work describes the simple method with rapid separation and estimation of Impurity-H in Etodolac pure drug and pharmaceutical dosage form.

2. Materials and methods

2.1. Instrumentation and materials

Chromatography was performed for rapid estimation of Etodolac related impurity-H in pharmaceutical dosage form Etova 200. Instrument used was Waters 2695 HPLC systems provided with Hamilton syringe, auto sampler and 2996 photodiode array detector. HPLC system was equipped with a column compartment with temperature control and an on-line degasser. Data acquisition

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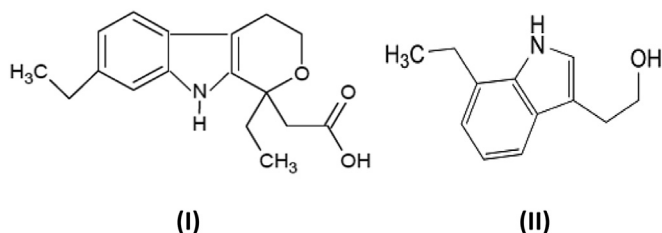


Fig. 1. Chemical structures of Etodolac (I) and Impurity-H (II).

and reporting were performed by Empower2 (Waters) chromatography software. Etodolac pure drug and Impurity-H were supplied by Spectrum Pharma Research Solutions, Hyderabad. HPLC grade acetonitrile, HPLC grade water and all other chemicals were obtained from Merck chemical division, Mumbai. HPLC grade water obtained from Milli-Q water purification system was used throughout the study. Marketed formulation Etova 200 mg was obtained from IPCA Laboratories limited.

2.2. Chromatographic conditions

The stationary phase used in this method was kromasil C18 (150 mm:4.6 mm:5 μ) column mobile phase composition used was acetate buffer and acetonitrile in the ratio of 55:45, flow rate was maintained at 1 ml/min, wavelength was set at 221 nm, temperature at 30 °C, injection volume is 5 μ l.

2.3. Preparation of buffer

0.77 g of ammonium acetate weighed was transferred to 1000 ml volumetric flask. 1/3rd part of the water of HPLC grade was transferred to volumetric flask and sonicated for 5 min. The flask was made up to the mark with HPLC grade water.

2.4. Preparation of mobile phase

Mobile phase composition was a mixture of solvent A and B. Solvent A is phosphate buffer. Solvent B is mixture of solvent A and acetonitrile in the ratio of 1:9. Diluent is a mixture of acetonitrile and water of HPLC grade in the ratio of 50:50.

2.5. Preparation of standard solution

5 mg of Etodolac drug was weighed accurately and transferred to 10 ml volumetric flask and dissolved in a small amount of diluent. The solution was sonicated for 5 min and the solution was made up with diluent to 10 ml which gives 500 ppm solution. 0.4 ml of the above solution was transferred to another 25 ml volumetric flask and was made up with the diluent to give 8 ppm solution.

2.6. Preparation of sample solution

Accurately weighed Etova tablet powder equivalent to 10 mg of Etodolac was transferred to 25 ml volumetric flask labelled 'Sample' and small amount of diluent was added to the flask. 4 mg of Impurity-H was weighed accurately and transferred to a 10 ml volumetric flask and made up to 10 ml with diluent after sonication. 0.5 ml of this solution was transferred to volumetric flask labelled 'Sample' and diluted to 25 ml with the diluent. The sample solution contains Etodolac of 400 ppm and Impurity-H of 8 ppm.

2.7. Validation

Validation of the method was done according to the ICH guidelines contains specificity, linearity, accuracy, precision, detection limit, quantification limit, robustness and system suitability.

3. Results and discussion

3.1. Optimization of developed method

Initially in the process of estimation of Etodolac related impurity-H in pharmaceutical dosage form Etova 200, reverse phase liquid chromatography separation was tried to develop using various ratios of methanol and water, acetonitrile and water as mobile phases, in which Etodolac and its Impurity-H did not responded properly, and the resolution obtained was not good. The organic content of mobile phase was also changed to optimize the separation of both drugs. pH of the mobile phase played important role in improving the tailing factor. Thereafter, acetate buffer:acetonitrile were taken in isocratic ratio of 55:45 and flow rate of 1.0 ml/min was employed. The resolution was improved by selecting the stationary phase Kromasil C18 column (4.6 \times 150 mm, 5 μ particle size) and the tailing of both peaks were reduced considerably and brought below 2. Detection was tried at various wavelengths from 205 nm to 280 nm to analyse both drug and Impurity-H. Both Etodolac and its Impurity-H showed maximum absorption at 221 nm of wavelength and that was selected as the detection wavelength for PDA detector. The retention times were found to be 3.1 min and 8.3 min for Etodolac and its Impurity-H, respectively. The chromatogram obtained was shown in the Fig. 2.

3.2. Method validation

3.2.1. System suitability

System suitability parameters such as number of theoretical plates, peak tailing, and retention time and resolution factor were determined. The results of the method were within limits. Plate count was more than 2000, tailing factor was below 2, resolution obtained was more than 2 and the results were summarized in Table 1.

3.2.2. Specificity

Blank, placebo, standard and sample with Impurity-H were injected separately and interference in the response was compared in the chromatograms. There was no interference of the other substances observed.

3.2.3. Linearity

400 ppm of Etodolac and Impurity-H stock solutions were prepared separately by dissolving 4 mg of drug and impurity into

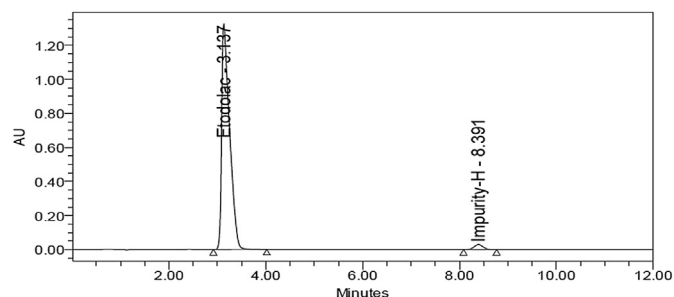


Fig. 2. Chromatogram of Etodolac spiked with 2% w/w Impurity-H.

Table 1
System suitability of Impurity-H.

Parameter	Etodolac	Impurity-H
Retention time	3.4 ± 0.3	8.4 ± 0.3
Plate count	4803	9025
Tailing factor	0.96	1.04
Resolution	4.5	4.5
RRF		0.99

Table 2
Linearity of Etodolac and Impurity-H.

Parameter	Etodolac	Impurity-H
Beer's range (ppm)	2 ppm	2 ppm
Slope	52576	52556
Y-intercept	32692	33816
Correlation coefficient (<i>r</i> ₂)	0.9999	0.9994
LOD (ppm)	0.13	0.09
LOQ (ppm)	0.4	0.28

two 10 ml volumetric flasks with diluent. From above stock solutions dilutions were made to get six concentrations 2, 4, 6, 8, 10, 12 ppm solutions each containing both Etodolac and Impurity-H. Six linear concentrations were injected and the calibration curve was obtained for both Etodolac and Impurity-H. Correlation coefficient of the Impurity-H was found to be 0.9994. The results were summarized in [Table 2](#).

3.2.4. Accuracy

400 ppm stock solution of Impurity-H was prepared by dissolving 4 mg of Impurity-H with diluent and made up to 10 ml in volumetric flask. Tablet powder equivalent to 10 mg of Etodolac weighed was taken into 25 ml volumetric flask labelled 50% spiked. 0.25 ml of solution from impurity stock was transferred to the volumetric flask labelled 50% spiked and made up to 25 ml with diluent. Tablet powder equivalent to 10 mg of Etodolac was weighed and transferred to a 25 ml volumetric flask labelled 100% spiked. 0.5 ml of solution from impurity stock was transferred to the volumetric flask labelled 100% spiked and made up to 25 ml with diluent. Tablet powder equivalent to 10 mg of Etodolac was weighed and transferred to a 25 ml volumetric flask labelled 150% spiked. 0.75 ml of solution from impurity stock was transferred to the volumetric flask labelled 150% spiked and made up to 25 ml with diluent. The sample solution was spiked with three different amounts of impurity. Here 2% (w/w) of Impurity-H was considered as 100% and sample was spiked with 50%, 100% and 150% of impurity i.e., 1% (w/w), 2% (w/w) and 3% (w/w), respectively. The average %Recoveries of the Impurity-H for 50%, 100% and 150% spiked were found to be 109.1, 108.6 and 108.8 respectively. Acceptance limit for related substances is 85–115%. The results were summarized in [Table 3](#).

3.2.5. Method precision

The sample solution was spiked with 2% (w/w) of Impurity-H and injected six times. In repeatability % amount was found to be 2.17% (w/w) and %RSD was calculated to be 0.95. In intermediate

Table 3
%Recovery data of Impurity-H.

%Spiking	(%w/w) found	%Recovery	%RSD
50%	1.06	109.1	0.21
100%	2.1	108.6	
150%	3.1	108.8	

Table 4
Precision of Impurity-H.

	Repeatability	Intermediate precision
Amount added (%w/w)	2.00	2.00
Avg. amount found (%w/w)	2.175408	1.910372
Standard deviation	0.020686	0.06784
%RSD	0.9509033	0.551149

Table 5
Robustness data of Impurity-H.

Parameter	%RSD
Flow rate	
Minus (0.9 ml/min)	0.250439
Plus (1.1 ml/min)	
Temperature	
Minus (25 °C)	0.238608
Plus (35 °C)	
Mobile phase	
Minus (50:50)	2.414595
Plus (60:40)	

precision % amount was found to be 1.91 and %RSD was calculated to be 0.55. Acceptance limit for related substances is NMT 5%. The results were summarized in [Table 4](#).

3.2.6. Limit of detection and the limit of quantification

Limit of detection (LOD) and limit of quantification (LOQ) of Etodolac related impurity-H was determined by calibration curve method. Solutions of Impurity-H were prepared in linearity range and injected as three sets. Average peak area of three sets was plotted against concentration. Detection limit and quantification limit were calculated by using following equations. $LOD = (3.3 \times Syx)/b$, $LOQ = (10.0 \times Syx)/b$.

Where *Syx* is residual variance due to regression; *b* is slope. The LOD and LOQ of the Impurity-H were calculated to be 0.09 ppm and 0.28 ppm respectively.

3.2.7. Robustness

In present study six small deliberate changes in method were made and these changes did not noticeably affect the system suitability parameters like retention time, area, resolution, plate count and tailing factor. The results were summarized in [Table 5](#).

4. Conclusion

A new HPLC method was developed and validated for estimation of Etodolac related Impurity-H in pharmaceutical dosage form. This method was fast, linear, accurate, and precise hence it can be employed for routine quality control of Etodolac related Impurity-H in drug testing laboratories and pharmaceutical industries.

Conflicts of interest

All authors have none to declare.

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