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Original Article

ENANTIOMERIC SEPARATION OF ETODOLAC IN A BULK DRUG SUBSTANCE BY REVERSE-PHASE CHIRAL LIQUID CHROMATOGRAPHY METHOD

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ABSTRACT

Objective: To develop novel, simple and rapid enantiomeric separation of Etodolac by reverse-phase high-performance liquid chromatographic method as per ICH guidelines.

Methods: The R-isomer and S-isomer were baseline resolved on a CHIRAL-AGP, (100 x 4.0 mm i. d, 5 µm) column using a mobile phase system containing 0.1 M sodium dihydrogen phosphate dihydrate pH 4.0 buffer: Isopropanol (85:15 v/v.) at detector wavelength 225 nm and column temperature 25 °C. The chromatographic resolutions between R-isomer and S-isomer were found three. The developed method was extensively validated according to ICH guidelines.

Results: Good linearity was observed for R-isomer over the concentration range of 300–3000 ng/ml, with the linear regression (Correlation coefficient R = 0.999) and proved to be robust. The limit of detection and limit of quantification of R-isomer was found to be 300 and 900 ng/ml, respectively for 10 µl injection volume. The percentage recovery of R-isomer was ranged from 98.0 to 102.0 in bulk drug samples of Etodolac. Etodolac sample solution and mobile phase were found to be stable for at least 48 hours. The proposed method was found to be suitable and accurate for the quantitative determination of R-isomer in bulk drugs.

Conclusion: A novel, simple and rapid enantiomeric separation of Etodolac by reverse-phase high-performance liquid chromatographic method was developed and validated as per ICH guidelines. The developed method can be used for the quantitative determination R-isomer in bulk drug materials in pharmaceutical industry.

Keywords: Etodolac, Reverse phase, Chiral HPLC, Validation, Solution and mobile phase stability.

INTRODUCTION

The Etodolac is widely used in the treatment pain or inflammation caused by arthritis or osteoarthritis. Etodolac is non steroidal anti-inflammatory drug (NASIDs). It works by reducing hormones that cause inflammation and pain in the body. In addition, it maintains a better physiological regulation of insulin secretion. Etodolac is racemic mixture of [+] S and [-]R-Etodolac. Etodolac chemically known as 1,8-Diethyl-1,3,4,9-tetrahydropyrano [3,4-b]indole-1-acetic acid, chemical structures show in (fig. 1), (R)-(-)–Etodolac show in (fig. 2) and (S)-(+)–Etodolac show in (fig. 3) It has been demonstrated in animal that the [+]S-form is biologically active.

Several different methods have been reported for qualitative and quantitative analysis of Etodolac. These include analysis of chiral non-steroidal anti-inflammatory drug [1], Direct high performance liquid chromatography separation of etodolac [2], RP-HPLC method for the quantization of etodalac in combined Dosage form [3], preparative resolution of etodolac enantiomers by preferential crystallization method [4], Optical resolution of drug by capillary electrophoretic techniques [5], Evaluation of the stero selective metabolism of the chiral analgesic drug etodolac [6], Exploration of an efficient method for optical resolution of etodolac [7], Enantio separation of Anti-Inflammatory Agent on chiral stationary phase [8], Determine Bioequivalence of S-Etodolac [9].

In the literature, there is no method for the separation of R-isomer and S-isomer of Etodolac in bulk drugs using reverse phase containing buffer 0.1M sodium dihydrogen phosphate dihydrate by high performance liquid chromatography. Normal-phase chromatography is the most popular mode of liquid chromatography at present for separation of isomer. Briefly the major disadvantages of normal phase HPLC lie in the highly non-polar nature of the mobile phase, the possibility of column inactivation by water, contamination by polar compounds and lower potential in terms of selectivity.

This report describes a reverse phase LC method for the rapid separation of R-isomer and S-isomer Etodolac. The developed HPLC method was validated for quantification of R-isomer in Etodolac as per ICH guidelines.

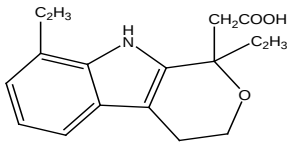

Fig. 1: Chemical structure of etodolac

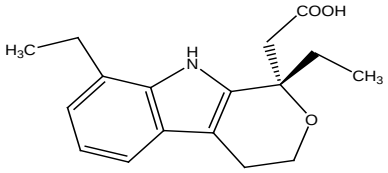

Fig. 2: Chemical structure of (R)-(-)–Etodolac

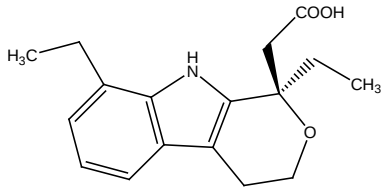

Fig. 3: Chemical structure of (S)-(+)-Etodolac

MATERIALS AND METHODS

Chemicals

Etodolac racemic, R-isomer and S-isomer were kindly gift by Enal lab Mumbai, Maharashtra, India. HPLC grade isopropanol purchased from Merck. AR grade Sodium dihydrogen phosphate dihydrate purchased from Merck. AR grade ortho phosphoric acid purchased from Rankem. HPLC grade methanol purchased from Merck.

Equipment

A shimadzu 2010 series LC system with UV detector and inbuilt auto injector were utilized for method development and validation. LC Solution software was used for data acquisition and system suitability calculations.

Sample preparation

Stock solutions of Racemic Etodolac (0.1 mg/ml) were prepared by dissolving the appropriate amount of the substances in methanol and diluent. The analyte concentration of S-isomer was fixed as 0.1 mg/ml. Working solutions of S-isomer and R-isomer were prepared in methanol and diluent.

Chromatographic conditions

The chromatographic conditions were optimized using a Chiral-AGP (100 x 4.0 mm i. d, 5 µm) column. The mobile phase was 0.1 M Sodium dihydrogen phosphate dihydrate pH 4.0 with ortho phosphoric acid: IPA (85:15, v/v). Diluents 0.1 M Sodium dihydrogen phosphate dihydrate pH 4.0 with ortho phosphoric acid. The flow rate was set at 1.0 ml/min. The column was maintained at 25 ºC and the detection was carried out at a wavelength of 225 nm. The injection volume was 10 µl.

Validation of the method

Method reproducibility

Method reproducibility was determined by measuring repeatability and intermediate precision (between-days precision) of retention times and peak areas for R–isomer and S-isomer.

In order to determine the repeatability of the method, replicate injections (n=6) of a 0.1 mg/ml solution containing S-isomer spiked with R-isomer (0.5 %) was carried out. The intermediate precision was also evaluated over three days by performing six successive injections each day.

Limit of detection and limit of quantification of R-isomer

The limit of detection defined as, the lowest concentration of analyte that can be clearly detected above the baseline signal, is estimated as three times the signal to noise ratio [10]. The limit of quantitation defined as, the lowest concentration of analyte that can be quantified with suitable precision and accuracy, is estimated as ten times the signal to noise ratio [10]. LOD and LOQ were achieved by injecting a series of dilute solutions of R-isomer.

The precision of the developed method for R-isomer at the limit of quantification was checked by analyzing six test solutions of R-isomer prepared at LOQ level and calculating the percentage relative standard deviation of an area.

Linearity of R-isomer

Detector response linearity was assessed by preparing six calibration sample solutions of R-isomer covering from 300 ng/ml (LOQ) to 3000 ng/ml (300 ng/ml, 600 ng/ml, 900 ng/ml, 1200 ng/ml, 1500 ng/ml and 3000 ng/ml), prepared in mobile phase from R-isomer stock solution.

Regression curve was obtained by plotting peak area versus concentration using the least squares method. Linearity was checked for 3 consecutive days in the same concentration range from the same stock solution. The percentage relative standard deviation of the slope and Y-intercept of the calibration curve was calculated.

Quantification of R-isomer in bulk sample

The Etodolac bulk sample gift by Enal Lab Mumbai, showed the presence of 0.20 % of R-isomer. Standard addition and recovery experiments were conducted to determine the accuracy of the present method for the quantification of R–isomer in bulk drug samples.

The study was carried out in triplicate at 0.2, 0.5 and 0.75 percent of the Etodolac target analyte concentration. The recovery of R-isomer was calculated from the slope and Y-intercept of the calibration curve.

Robustness

The robustness of a method is the ability of the method to remain unaffected by small changes in parameters such as flow rate, mobile phase composition and column temperature. To determine robustness of the method, experimental conditions were purposely altered and chromatographic resolution between R-isomer and S-isomer was evaluated.

The flow rate of the mobile phase was 1.0 ml/min. To study the effect of flow rate on the resolution of isomers, it was changed by 0.2 units from 0.8 ml/min to 1.2 ml/min. The effects of change in percent ethanol on resolution were studied by varying from-1 to+1 % while the other mobile phase components were held constant as stated in section 2.4. The effect of column temperature on the resolution was studied at 20 ºC and 30 °C instead of 25 °C while the other mobile phase components were held constant.

Solution stability and mobile phase stability

Stability of Etodolac in solution at analyte concentration was studied by keeping the solutions in tightly capped volumetric flask at room temperature on a laboratory bench for two days. Content of R-isomer was checked for six hours interval up to the study period.

Mobile phase stability was carried out by evaluating the content of R-isomer in Etodolac sample solutions prepared freshly at six hours interval for two days. Same mobile phase was used during the study period.

RESULTS AND DISCUSSION

Method development

The aim of this work is to separate the R-isomer and S-isomer of Etodolac using reverse phase HPLC within short run time, the analysis of Etodolac sample using reverse phase is time consuming. A 0.1 mg/ml solutions of isomeric mixture prepared in methanol and diluent were used in the method development. To develop a rugged and suitable LC method for the separation of Etodolac isomer, different mobile phases and stationary phases were employed in an attempt to separate the isomer of Etodolac. Various experiments were conducted to select the best stationary and mobile phases that would give optimum resolution and selectivity for the two isomer. The chromatographic separation was achieved on a Chiral AGP (100 x 4.0 mm i. d, 5 µm) column using a mobile phase system containing 0.1 M Sodium dihydrogen phosphate dihydrate pH 4.0: isopropanol (85:15, v/v).

The flow rate of the mobile phase was 1.0 ml/min. At 25 °C column temperature, the peak shape of Etodolac was found symmetrical.

In the optimized method, the typical retention times of R-isomer and S-isomer of Etodolac were about 3.6 and 4.2 minutes respectively. The isomeric separation of Etodolac is shown in system suitability chromatogram (fig. 4). Typical HPLC chromatogram of Etodolac bulk sample (100 µg/ml) spiked with R-isomer (0.2 %) shown in (fig. 5).

Validation results of the method

The system suitability test results are presented in (Table1). In the repeatability study, the relative standard deviation (RSD) was better than 0.5 % for the retention times of the isomers, 0.7 % for Etodolac peak area and 2.3 % for R-isomer peak area (table 2). In the intermediate precision study, results show that RSD values were in the same order of magnitude than those obtained for repeatability (table 2).

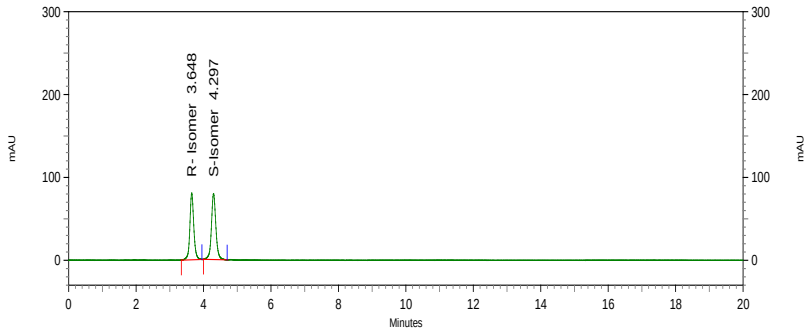

Fig. 4: Typical HPLC chromatogram of System suitability

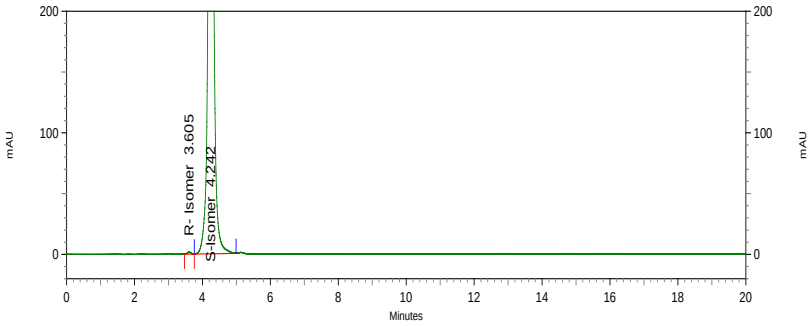

Fig. 5: Typical HPLC chromatogram of Etodolac bulk sample (100 µg/ml) spiked with R-isomer (0.2 %)

The limit of detection (LOD) and limit of quantification (LOQ) concentrations were estimated to be 300 and 900 ng/ml for R-isomer, when a signal-to-noise ratio of 3 and 10 was used as the criteria. The method precision for R-isomer at the limit of quantification was less than 3 % RSD (table 2).

Good linearity was observed for R isomer over the concentration range of 300–3000 ng/ml, with the linear regression equation y = 36.781X+569 (Correlation coefficient R = 0.999). Linearity was checked for R–isomer over the same concentration range for three consecutive days. The percentage relative standard deviation of the slope and Y-intercept of the calibration curve were 2.1 and 1.6 respectively (table 2).

Table 1: System-suitability report

Compound (n=3)	Rt	R_S	N	T
R-isomer	3.6	-	5100	1.1
S-isomer	4.3	3.0	5300	1.1

n =3 determinations, R_S-USP resolution, N-number of theoretical plates (USP tangent method), T-USP tailing factor

The standard addition and recovery experiments were conducted for R isomer in bulk samples in triplicate at 0.2, 0.5 and 0.75 percent of analyte concentration. Recovery was calculated from the slope and Y-intercept of the calibration curve obtained in linearity study and percentage recovery was ranged from 98.0 to 102.0 (table 3).

The chromatographic resolution of R-isomer and S-isomer of Etodolac peaks was used to evaluate the method robustness under modified conditions. The resolution between R-isomer and S-isomer of Etodolac was greater than 2.5 under all separation conditions tested (table 4), demonstrating sufficient robustness.

No significant change in the R-isomer content was observed in Etodolac sample during solution stability and mobile phase stability experiments. Hence Etodolac sample solution and mobile phase are stable for at least 48 hours.

Table 2: Validation results of the developed reverse phase method

Validation parameter	Results
Repeatability (n=6, % RSD)	
Retention time (R-isomer)	0.2
Retention time (S-isomer)	0.3
Area (R-isomer)	2.3
Area (S-isomer)	0.5
Intermediate precision (n=18, % RSD)	
Retention time (R-isomer)	0.3
Retention time (S-isomer)	0.4
Area (R-isomer)	2.6
Area (S-isomer)	0.6
LOD-LOQ (R-isomer)	
Limit of detection (ng/ml)	200
Limit of quantification (ng/ml)	600
Precision at LOQ (% RSD)	2.8
Linearity (R-isomer)	
Calibration range (ng/ml)	300-3000
Calibration points	6
Correlation coefficient	0.999
Slope (% RSD)	2.1
Intercept (% RSD)	1.6

n=3 determinations

Table 3: Recovery results of R-isomer in bulk drugs

Added (ng) (n=3)	Recovered (ng)	% Recovery	% RSD
2001	1962	98.1	2.6
5010	5102	101.8	2.2
7510	7502	99.9	2.4

Table 4: Robustness of the method

Parameter	USP resolution between R-isomer and S-isomer of Etodolac
Flow rate (ml/min)	
0.8	3.1
1.0	3.0
1.2	2.9
Column temperature (°C)	
20	2.1
25	3.0
30	2.8
Isopropanol percentage in mobile phase	
14	3.0
15	3.0
16	2.9

CONCLUSION

A novel, simple and rapid enantiomeric separation of Etodolac using reverse-phase 0.1 M Sodium dihydrogen phosphate dihydrate pH 4.0: IPA (85:15, v/v) mobile phase by high-performance liquid chromatographic method was developed and validated as per ICH guidelines. The method validation was carried out by using Chiral-AGP column. The developed method can be used for the quantitative determination of R-isomer in bulk drug materials in the pharmaceutical industry.

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CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Chang CG, Yi HT, Hai HH, Lu SY, Hui DJ, Su Z. Analysis of chiral non-steroidal anti-inflammatory drugs flurbiprofen, ketoprofen and etodolac binding with HSA. J Pharm Anal 2011;1(3):184-90.
2. Caccamese S. Direct high-performance liquid chromatography (HPLC) separation of etodolac enantiomers using chiral stationary phases. Chirality 1993;5(3):164-7.
3. Balan P, Carolin Nimila I, Lakshmi Prasanna M, Vanaja Rani M, Rajasekar S. RP-HPLC method development and validation of etodalac and paracetamol in combined dosage form. Asian J Res Chem 2011;4(7):1073-6.
4. Phan TD, Tran QT, Kyeong HK. Preparative resolution of etodolac enantiomers by preferential crystallization method. Arch Pharmacal Res 2009;32(10):1425-31.
5. Nishi H, Terabe S. Optical resolution of drug by capillary electrophoretic techniques. J Chromatogr A 1995;69:245-76.
6. Beckerscharfenkamp U, Blaschake GJ. Evaluation of the steroselective metabolism of the chiral analgesic drug etodolac. J Chromatogr B 1993;621:199-207.
7. Chou S, Tseng C, Chang L. Exploration of an efficient method for optical resolution of etodolac. J Chin Chem Soc 2001;48:229-34.
8. Xuejun Z, Zou L, Baochun S, Juanjuan C, Xiuzhu X. Enantioseparation of Anti-Inflammatory Agent on chiral stationary phase. J Anal Sci Methods Instrum 2012;2:18-22.
9. Menon S, Kadam N, Gursale A, Gokarn V, Palekar A. A Randomized, Crossover study to determine bioequivalence of S-Etodolac ER tablets versus etodolac ER tablets in healthy indian subjects. J Appl Res 2009;9(3):57-64.
10. ICH draft Guidelines on validation of analytical procedures. Definitions and Terminology. Federal Register IFPMA Switzerland 1995;60:11260-2.