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SHORT COMMUNICATION

New chiral reverse phase HPLC method for enantioselective analysis of ketorolac using chiral AGP column

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KEYWORDS

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Abstract A simple, specific, precise, sensitive and rapid reverse phase-HPLC method was developed for determination of ketorolac enantiomers, a potent nonnarcotic analgesic in pharmaceutical formulations. The method was developed on a chiral AGP column. Mobile phase was 0.1 M sodium phosphate buffer (pH 4.5): Isopropanol (98:2, v/v), at a flow rate of 1 mL/min with run time of 15 min. Ultraviolet detection was made at 322 nm. The linearity range was 0.02–10 μg/mL for each of the enantiomers. The mobile phase composition was systematically studied to find the optimum chromatographic conditions. Validation of the method under the conditions selected showed that it was selective and precise and that the detector response was linear function of ketorolac.

1. Introduction

Ketorolac tromethamine is a pyrrolizine carboxylic acid derivative used for its analgesic activity [1], with cyclooxygenase inhibitory activity. It is approved for the treatment of mild to moderately severe post-operative pain. The drug is available as a racemic mixture of an equimolar ratio of R (+) and S (−) stereoisomers (Fig. 1) [2]. Enantiomers of chiral drugs can present differences in pharmacological activity or efficacy. Enantioselective analytical techniques have therefore become more and more important in the field of drug analysis. Enantiomeric separations of ketorolac were performed by liquid chromatography (LC), using an indirect method; with nonchiral stationary phases [3–6]. Chiral stationary phases for the separation of the enantiomers are made of immobilized proteins such as human serum albumin [7], α1-acid glycoprotein [8,9], or amylose tris (3, 5-dimethylphenylcarbamate) [10]. The methods reported for chiral separation of ketorolac have either long analysis time or are less sensitive [11]. The aim of this study was to develop a simple, specific, sensitive and precise reverse phase chiral high performance liquid chromatographic method for determination of ketorolac enantiomers (Fig. 1) in

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bulk and pharmaceutical formulations using the chiral α1-acid glycoprotein (AGP) column with photo diode array (PDA) detector.

2. Materials and methods

2.1. Chemicals

Ketorolac enantiomers were purchased from TRC, Canada. Methanol (MeOH), isopropanol (IPA), and acetonitrile (ACN) used were procured from Merck, India. Sodium di-hydrogen phosphate (NaH_2PO_4), sodium hydroxide and phosphoric acid of AR grade were also obtained from Merck, India.

2.2. Instrumentation

Quantitative reverse phase high performance liquid chromatography (RP-HPLC) was performed on isocratic ultra fast liquid chromatography (Shimadzu) with PDA detector. The column used was chiral-AGP column (100 mm × 4.0 mm I.D., particle size 5 μm, Chrom Tech Ltd., Sweden). AG-135 analytical electronic balance and pH meter were from METTLER-TOLEDO and EUTECH respectively.

2.3. HPLC conditions and chiral column

Mobile phase was filtered before use through a 0.22 μm membrane filter. Then it was degassed in a bath sonicator for 20 min and pumped from the solvent reservoir to the column at a flow rate of 1 mL/min to equilibrate the system. Injection volume was optimized to 50 μL. The back pressure yielded was 735.49–931.63 Kg m/s^2. The run time was set for 15 min and eluents were monitored at 322 nm. The column used was made of AGP, a glycoprotein containing 183 amino acids and carbohydrate moiety in the form of N-linked glycans [12], having isoelectric point of 2.7–3.8 [13]. The carbohydrate moiety is believed to be involved in binding of compounds. The enantioselective properties of chiral-AGP may be affected by several chromatographic parameters such as the buffer nature, the pH value of the buffer, the organic modifier (nature and percentage), the buffer concentration, the presence of charged additives in the mobile phase and the temperature [14].

2.4. Preparation of sample and method development

Sodium dihydrogen phosphate 0.1 M was used in the mobile phase. The optimized pH of the mobile phase was developed by working in the range of 4–7. Different organic modifiers, namely, isopropanol, acetonitrile and methanol were used and the one which gave better results was used in concentration range of 2–10% of mobile phase. The order of elution was determined based on their retention time by separately injecting pure enantiomers of ketorolac. Primary stock solution of 100 μg/mL ketorolac racemic mixture was prepared by dissolving 5 mg of racemic ketorolac in a 50 mL volumetric flask containing methanol and the volume was made by methanol. Working standard solutions were prepared by suitable dilution of primary stock solutions with mobile phase.

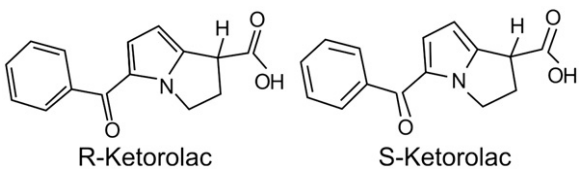

Figure 1 Chemical structure of ketorolac enantiomers.

2.5. Method validation

2.5.1. Calibration curve

It was obtained by preparing three sets of the drug solutions in mobile phase containing ketorolac racemates at a concentration of 20 ng/mL–10 μg/mL; 50 μL of these drug solutions were injected into column, and the peak area and retention time were recorded. The regression curve was constructed by plotting the peak areas against the concentration of racemates in μg/mL.

2.5.2. Precision and accuracy

Minimum of nine determinations was done over three concentration levels in triplicate [15]. Percentage relative standard deviation (% RSD) of peak area and the percentage of bias were calculated for precision and accuracy, respectively.

2.5.3. Limits of detection (LOD) and quantification (LOQ)

LOD and LOQ were calculated on the basis of response and slope of the regression equation. They were calculated from the formula $3.3\sigma/S$ and $10\sigma/S$ respectively where 'σ' is standard deviation of the y-intercept of the regression line and 'S' is slope of the calibration curve.

2.5.4. Solution stability

Stability of drug solution was studied by keeping the solution in a tightly closed volumetric flask in the refrigerator for 48 h. Content of each enantiomer was checked at regular intervals up to the study period.

3. Results and discussion

The variables which altered the enantioselectivity when using chiral AGP column were chosen and the effect of each variable was studied. The variables selected were pH of buffer, organic modifier and organic modifier concentration. Here, the effect of individual variable was studied by keeping all other chromatographic conditions constant.

Table 1 Effect of pH on selectivity, resolution and capacity factor.

Mobile phase	α	R_s	k	k^1
95:5 (0.1 M NaH_2PO_4,pH 4 :IPA)	1.3	1.9	3.6	4.7
95:5 (0.1 M NaH_2PO_4,pH 4.5 :IPA)	1.4	1.8	3.5	4.8
95:5 (0.1 M NaH_2PO_4,pH 5.5 :IPA)	2.0	1.8	0.5	0.9

α—Selectivity; R_s—Resolution; k—Capacity factor for R-enantiomer; k^1—Capacity factor for S-enantiomer; IPA—Isopropanol.

3.1. Method development

3.1.1. Effect of pH

Increase in the pH increased the negative charge on column. Retention of ketorolac, decreases, and it elutes much faster, which means decreased value of capacity factor as shown in Table 1. The opposite thing occurred by decreasing the pH. Optimized pH was found to be 4.5 by several trails.

Table 2 Effect of organic modifier on selectivity, resolution and capacity factor.

Mobile phase	α	R_s	k	k^1
95:5 (0.1 M NaH_2PO_4,pH 4.5:IPA)	1.4	1.8	3.5	4.8
95:5 (0.1 M NaH_2PO_4,pH 4.5:MeOH)	1.3	1.4	6.1	7.6
95:5 (0.1 M NaH_2PO_4,pH 4.5:ACN)	1.2	1.3	6.6	8.0

α—Selectivity; R_s—Resolution; k—Capacity factor for R-enantiomer; k^1—Capacity factor for S-enantiomer; IPA—Isopropanol; MeOH—Methanol; ACN—Acetonitrile.

Table 3 Effect of organic modifier concentration on resolution and capacity factor.

Mobile phase	R_s	k	k^1
98:2 (0.1 M NaH_2PO_4,pH 4.5:IPA)	2.3	5.1	6.1
95:5 (0.1 M NaH_2PO_4,pH 4.5:IPA)	1.8	3.5	4.8

R_s—Resolution; k—Capacity factor for R-enantiomer; k^1—Capacity factor for S-enantiomer; IPA—Isopropanol.

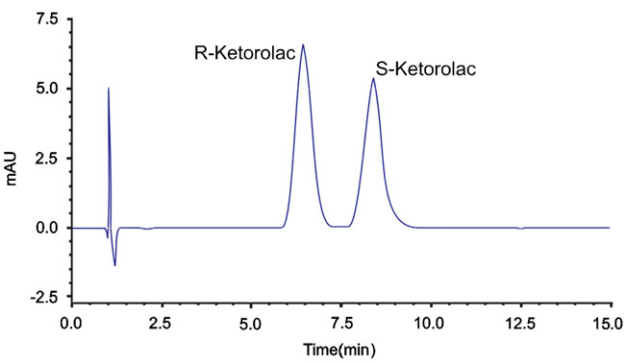

Figure 2 Chromatogram of 3 μg/mL of racemic ketorolac showing the two completely resolved peaks of R and S enantiomers. Mobile phase: 98:2 (0.1 M NaH_2PO_4, pH 4.5 :IPA), flow rate 1 mL/min.

3.1.2. Effect of organic modifier

The use of organic modifier also played a major role. IPA gave optimized values of resolution, selectivity and capacity factor as shown in Table 2.

3.1.3. Effect of organic modifier concentration

The maximum amount of organic modifier to be used was 20% of mobile phase, as the concentration increased the resolution and the retention time decreased. Optimized ratio was found to be 98:2 (buffer: IPA) as it gave better resolution, as shown in Table 3.

3.2. Method validation

The optimized mobile phase was found to be 98:2 (0.1 M NaH_2PO_4, pH 4.5: IPA). The order of elution was, that R (+) enantiomer eluted faster than S (−) enantiomer and their retention times was 6.4 and 8.4 min respectively (Fig. 2). During development of the method on the chiral AGP column, it was shown that the method was selective because the enantiomers were separated to baseline (R_S = 2.3). The validation parameters of the optimized method are given in Table 4, the linearity range of each enantiomer solution of ketorolac was found to be 20 ng/mL–10 μg/mL and the LOD and LOQ values for each enantiomer was found to be 5 ng/mL and 15 ng/mL, respectively. The RSD for repeatability (within-day precision, n = 3) and RSD for reproducibility (between-day precision, n = 3) for each enantiomer at three different quantification concentrations were found to be less than 2%. These results confirmed the good precision of the method and the % bias less than 2% at each concentration (n = 3) indicated that the method was accurate. The correlation coefficients for the relationships between concentration and detector response showed that the response was linear in the range examined. By changing the flow rate there was a change in retention time only but the resolution and selectivity were not significantly affected. Therefore, the method was robust and there was no significant change in the content of enantiomers after 48 h. So, the drug solution was stable for 48 h.

4. Conclusion

A simple reversed-phase HPLC method for analysis of the enantiomers of ketorolac has been developed and validated.

Table 4 Validation parameters of each enantiomer.

Concentration of each enantiomer (μg/mL)	Repeatability (n = 3, % RSD)		Intermediate precision (n = 3, % RSD)		Accuracy (n = 3, % bias)	
	R-Ketorolac	S-Ketorolac	R-Ketorolac	S-Ketorolac	R-Ketorolac	S-Ketorolac
0.05	1.7	1.9	1.9	1.9	1.3	1.8
5.00	1.4	1.0	1.6	1.8	1.5	1.7
10.00	0.7	1.1	1.0	1.4	1.0	1.2

RSD: Relative standard deviation.

Use of chiral AGP and the approach to optimize the chromatographic conditions in order to obtain better resolution, selectivity and reduced time of analysis were described by studying the effect of each variable. The optimized chromatographic conditions gave baseline separation with resolution of approximately 2.2 for the enantiomers within 10 min. The method is linear, precise, accurate and sensitive, thus being suitable for daily direct enantioselective analysis of ketorolac. The method is also very robust and enables quality-control analysis of enantiomer composition and purity with large sample throughput.

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