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Original Contribution

HPLC DETERMINATION OF KETOPROFEN IN TABLET DOSAGE FORMS

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ABSTRACT

PURPOSE: A simple, specific, precise and accurate reversed phase liquid chromatographic (RP-LC) method has been developed for the determination of ketoprofen in tablet dosage form.

METHODS: The chromatographic separation was achieved on a LiChrosorb C18, 250 mm x 4.6 mm, 5 μm column at a detector wavelength of 230 nm and a flow rate of 1.0 ml/min. The mobile phase was composed of methanol, 0.1M ammonium acetate buffer pH 6.9, acetonitrile and tetrahydrofuran (73:20:5:2 *v/v/v/v*). The retention time of ketoprofen was 3.49 min. The method was validated for the parameters like specificity, linearity, precision, accuracy, limit of quantitation and limit of detection.

RESULTS: The method was found to be specific as no other peaks of impurities and excipients were observed. The square of correlation coefficient (R^2) was 0.9999 while relative standard deviations were found to be <2.0%.

CONCLUSIONS: The proposed RP-LC method can be applied for the routine analysis of commercially available formulations of ketoprofen.

Key words: Liquid chromatography, validation, ketoprofen, quality control

INTRODUCTION

Ketoprofen (2-(3-benzoylphenyl)-propionic acid) is a potent non-steroidal anti-inflammatory drug (NSAID) used for the treatment of a wide range of painful and inflammatory illness (1). Like the most NSAIDs, ketoprofen is advantageous because it lacks addictive potential and does not result in sedation or respiratory depression. Ketoprofen is a white or almost white crystalline powder having empirical formula $C_{16}H_{14}O_3$ with molecular weight of 254.3 and melting point 94˚ to 97˚C. It has pKa of 5.94. It is practically insoluble in water, freely soluble in alcohol, acetone, and dichlormethane (2).

Several types of analytical procedures have been proposed for the analysis of ketoprofen in pharmaceutical formulations. The procedures include capillary zone electrophoresis (3), UV-spectrophotometry (4-8), high-performance liquid chromatography (9-13), flow injection technique with hemiluminiscence (14, 15), flow injection with UV-detection (16), polarography (17), micellar elecrokinetic chromatography (18), electrochemical methods (19, 20) and quantitative Fourier transformation infrared spectrophotometry (21). European Pharmacopoeia recommended acid-base titration for analysis of ketoprofen in substance, UV-spectrophotometry for its determination in capsules as well as liquid chromatography for assay in gel.

The aim of this paper is to develop a specific, precise and accurate chromatographic method that could be applied in quality control for the determination of ketoprofen in capsules in respect of European Pharmacopoeia and ICH requirements.

MATERIALS AND METHODS

Chemicals and Reagents

Ketoprofen RS (Sigma-Aldrich) was used as standard. Profenid capsules containing 50 mg

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active substance were obtained commercially. LC-grade methanol, acetonitrile and tetrahydrofuran were supplied from Merck (Germany). All other chemical reagents were of analytical grade.

Instrumentation and chromatographic conditions

Chromatographic separation was performed on modular HPLC system LC-10A Shimadzu (Japan) arranged with a LC-10A pump, solvent degasser DGU-3A, Rheodyne injector, column oven CTO-10A, SPD-M10A diode array detector and communication bus module CBM-10A. Separation was achieved isocratically with a LiChrosorb C18, 250 mm x 4.6 mm, 5 µm column eluted with a mixture of methanol, 0.1M ammonium acetate buffer pH 6.9, acetonitrile and tetrahydrofuran (73:20:5:2 *v/v/v/v*) as the mobile phase at flow rate of 1 ml/min. Detection was carried out by absorbance at 230 nm. The analysis was carried out at an ambient temperature and injection volume was 20 µl.

Preparation of standard solutions

Twenty mg of accurately weighed standard ketoprofen was dissolved and made up to mark with mobile phase in a 100 ml volumetric flask, to get primary stock solution of 200 µg/ml. Serial dilutions were made to obtain 5, 10, 25, 50, 75 and 100 µg/ml using mobile phase. All solutions were filtered through 0.45 µm membrane filter prior to use.

Sample preparation

A commercially available capsule formulation containing ketoprofen 50 mg was analyzed using this method. The content of 20 capsules was taken and powdered. The powder equivalent to 50 mg of ketoprofen was accurately weighed and transferred into a 100 ml volumetric flask. To this, 70 ml of mobile phase was added and sonicated for 10 min with occasional shaking to disperse and dissolve the contents. The volume was made up to 100 ml with the same diluent to give 500µg/ml. This solution was filtered through 0.45 µm membrane filter and diluted suitably using mobile phase to obtain 50 µg/ml solutions.

RESULTS AND DISCUSSION

The **Fig. 1** showed typical chromatogram obtained from analysis of standard solution using the proposed method. The retention time observed –3.49 min permits a rapid determination of the drug, which is important for routine analysis.

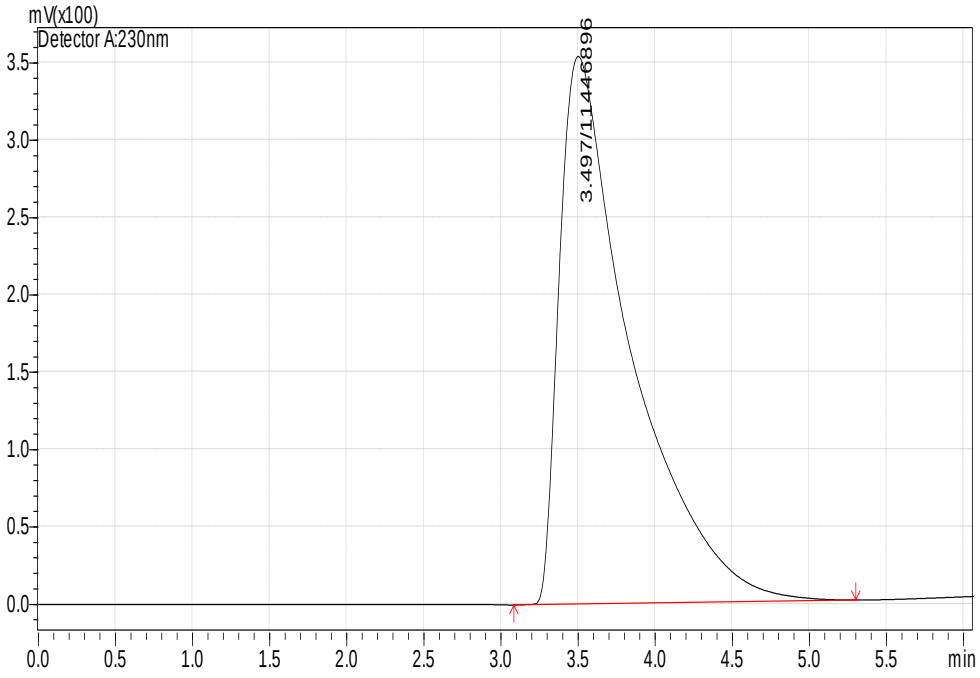

Fig. 1. Chromatogram obtained from ketoprofen RS

System suitability parameters for this method were reported in **Table 1**.

Table 1. *System suitability parameters for the method*

Parameter	Ketoprofen
Retention time (min) ± % RSD	3.49±0.09
Tailing factor ± % RSD	0.82±0.15
Theoretical plates ± % RSD	7895±0.45
LOQ	0.5 μg/ml
LOD	0.1 μg/ml

Validation study

The proposed method was validated as per ICH guidelines with respect to specificity, linearity, precision and accuracy.

Specificity

The specificity of the method was determined by checking the interference with the components from placebo. No interference was observed for any of the components like excipients of both drugs.

Calibration and linearity

Calibration curve was constructed in the range of 5.00-100.0 μg/ml for ketoprofen to encompass the expected concentration in measured samples. The corresponding linear regression equation was *y=14587.2x-1256.1* with square of correlation coefficient R^2 of 0.9999. An excellent correlation existed between the peak areas and concentration of ketoprofen.

Precision

The precision of the method was evaluated by performing six independent determinations of the test sample preparation and calculating RSD (%). The RSD value measured during assessment of precision was <2.0% for ketoprofen, confirming the method is precise **(Table 2)**.

Table 2. *Precision of the method*

№	Amount found, mg/tablet	Statistical data	
1.	50.02		
2.	49.95	Mean	49.66
3.	49.26		
4.	50.17	SD	0.434
5.	49.30		
6.	49.24	%RSD	0.87

Accuracy

To determine the accuracy of the method, the recovery was checked at three different concentration levels – 50, 100 and 150 %. Values of analytical recovery experiments were listed in **Table 3**.

CONCLUSION

The newly developed LC method is specific, precise, accurate and rapid. The analytical procedure is suitable for quality control of pharmaceutical preparation containing ketoprofen.

Table 3. *Results from study of accuracy*

Drug	Level (%)	Theoretical concentration (μg/ml)	Observed concentration (μg/ml)	Mean recovery (%) ± SD	RSD (%)
Ketoprofen	50	24.90	24.56	99.11±1.31	1.32
			24.43		
			25.06		
	100	49.25	49.52	99.81±0.642	0.64
			49.10		
			48.87		
	150	74.72	73.95	99.63±0.620	0.62
			74.85		
			74.51		

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