INTERNATIONAL JOURNAL OF PHARMACEUTICAL, CHEMICAL AND BIOLOGICAL SCIENCES

Available online at www.ijpcbs.com

Research Article

SIMPLE AND ACCURATE ESTIMATION OF KETOTIFEN FUMARATE BY RP-HPLC

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ABSTRACT

The aim of this study was to develop and validate an isocratic reversed phase high-performance liquid chromatographic method for quantification of ketotifen fumarate (KF) in pharmaceutical solid dosage formulations. Simple, rapid, specific, accurate and precise reverse phase high performance liquid chromatographic method was developed for the estimation of ketotifen fumarate in Tablet dosage form. A Thermo $C_{18,}$ 250mm x 4.6mm id in Isocratic mode with mobile phase containing methanol: 10 mM ammonium acetate (30:70 %v/v pH: 3.5) was used. The flow rate was 1ml/min and effluents were monitored at 298nm. The retention time of ketotifen fumarate was 5 min. The concentration curves were linear in the concentration range of 5 to 150 ng/ml. The developed method was validated for specificity, precision, linearity, accuracy, ruggedness, robustness and solution stability. Recovery of ketotifen fumarate in formulations was found to be in the range of 97.36 % to 99.93 %. Proposed method was successfully applied for the quantitative determination of ketotifen fumarate in formulations.

Key words: RP-HPLC, Ketotifen Fumarate, Tablets, Validation and Chromatography.

INTRODUCTION

Ketotifen fumarate, 4,9-dihydro-4-(1-methyl-4-piperidinylidene)-10-H-benzo [4,5]cycloheptal- [1,2-b]thiophen-10-one fumarate, is used as an antiasthamatic agent[1] . Few analytical methods for estimation of ketotifen fumarate including spectrophotmetric[2-6]HPLC and[7-9]are reported. However, all these spectroscopic techniques have number of limitations to accurately determine the ketotifen in pharmaceutical dosage forms. On the other hand, RPHPLC is very simple, fast, highly reproducible and cost effective method to estimate ketotifen in pharmaceutical dosage forms. Additionally, the LC method can easily be utilized as a suitable analytical procedure for quality control and quantification of these metabolites in lichens, commonly used for various purposes in folklore and traditional systems of medicine of the Asian region. Here, we reported the development of simple, specific and sensitive and cost effective isocratic RP-HPLC method that will be used for the routine analysis of ketotifen fumarate in pharmaceutical dosage form. The important features and novelty of the proposed method included simple sample treatment with sonication of small amount of powder sample at ambient temperature, centrifugation, dilution; short elution time

with internal standard eluted prior to KF; good precision (R.S.D. less than 5%) and high recovery. The proposed method was optimized and validated as per the ICH guidelines[10].

Chromatographic conditions

HPLC chromatographic separation was performed on a Shimadzu liquid chromatographic system equipped with a LC-20AD solvent delivery system (pump), SPD-20A photo diode array detector, and SIL-20ACHT injector with 50µL loop volume. LC solution version 1.25 was applied for data collecting and processing (Shimadzu, Japan). The HPLC was carried out at a flow rate of 1.0ml/min using a mobile that is phase constituted of methanol -10mM ammonium acetate buffer (pH 3.0 adjusted with orthophosphoric acid (30:70, v/v)), and detection was made at 298.0nm. The mobile phase was prepared daily, filtered through a 0.45µm membrane filter (Millipore) and sonicated before use. A Thermo C18 column (25cm × 4.6mm i.d., 5µ) was used for the separation.

Preparation of stock solutions

Weighed accurately about 100 mg of ketotifen fumarate and was dissolved in 100 ml of methanol in to the 100ml light resistant volumetric flask. The final solutions containing 1.0 mg/ml. It was labeled and the solution was stored in a refrigerator below 8°C.

Sample preparation

Twenty tablets were accurately weighed. A quantity of powder equivalent to 1.0 mg of ketotifen fumarate and transferred in to 100ml light resistant flask and made up the required volume by using mobile phase. Pipetted out 5ml of resulting solution in to the 50 ml light resistant flask and made up the required volume by using mobile phase and sonicated for 15 min. Then finally filtered through 0.45m filter.

Optimization of the chromatographic conditions

The chromatographic conditions, especially the composition of mobile phase, were optimized through several trials to achieve good resolution and symmetric peak shapes for KF drug as a short run time. Different mobile phase ratio evaluated. Finally found that a mixture of methanol: ammonium acetate (10 mM pH 3.0) (30:70, v/v) could achieved the separation and finally adopted as the mobile phase.

C_{18} and C_8 columns were both tried for separation and resolution. It was found that the C_{18} offered more advantages over the C_8 column. Individual drug solutions were injected into the column at the concentration of 100 µg ml and both elution pattern and resolution parameters studied as a function of pH. In addition, UV spectra of individual drugs were recorded in the wavelength range from 210 to 300 nm. The pH effect showed that optimized conditions are reached when the pH value is 3.0, producing good and well shaped peaks for all drugs assayed. The choice to use a common wavelength set at 298 nm was considered satisfactory, permitting the detection of all drugs with adequate sensitivity. The data was analysed by the linear least squares fit method. The good chromatographic separation indicated that anyone of the drugs can be used as internal standard for the assay of the other drugs.

Method validation

The proposed method has been validated for the assay of ketotifen in formulation using following parameters:

Specificity

The specificity was established by preparing a ketotifen standard at 0.5% level of test concentration and injected 5 times into HPLC system as per the test procedure.

Linearity

Linearity was studied by preparing standard solution at different concentration levels. The linearity range was found to be 10-50mg/ml. The regression equation was found to be y = 0.0278X + 0.0184 with coefficient of correlation R^2 = 0.9997 (Figure1 and Table 1).

Precision

Precision was studied to find out intra and inter day variations in the proposed method of ketotifen fumarate at different concentration levels: 5 ng/ml, 50 ng/ml and

100 ng/ml on the same day and three different days respectively. The percentage % CV was calculated for intra and inter day precision and found to be less than 2%. The results of precision are presented in the table 2 and 2a.

Recovery Study

The recovery studies were carried out three times over the specified concentration range and the percentage recovery of ketotifen fumarate was found to be in the range of 97.36% to 99.93% and the results are presented in the Table 3 and Table 4.

Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD and LOQ for ketotifen fumarate were predicted basing on the parameters of standard error of estimate and slope, calculated from linearity of the response data of ketotifen fumarate 1.0 ng/ml and 5.0 ng/ml respectively.

Robustness

Robustness of the method was studied by changing the l max from 376 to 370 and the mobile phase composition of organic phase changed by ± 5% and pH± 2. The results showed that the retention time and peak area of ketotifen fumarate is remains almost unchanged and no significant degradation was observed.

Assay

The standard and sample solutions were injected three times separately; chromatograms and the peak areas were recorded. A representive chromatograms of sample has been given in Figure 2.

CONCLUSION

Proposed study describes new reverse phase high performance liquid chromatographic method for the estimation of ketotifen fumarate in formulations the method was validated and found to be simple, sensitive, accurate and precise. Percentage of recovery studies shows that, the method is free from interference of the other active ingredients and additives used in the formulation. The simplicity of the method allows for application in laboratories that lack sophisticated analytical instruments such as LC–MS/MS or GC–MS/MS that are complicated, costly and time consuming rather than a simple HPLC–UV method. Considering the possible worldwide development of counterfeit ketotifen fumarate, the proposed method could be useful for the national quality control laboratories in developing countries.

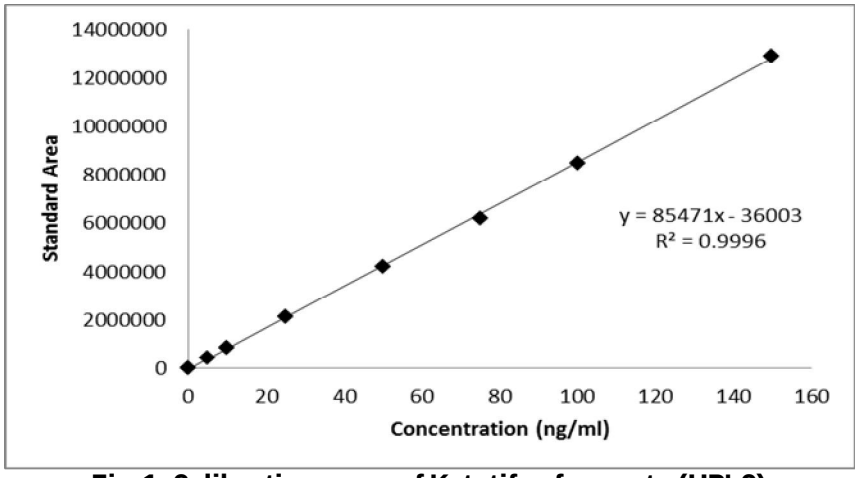

Fig. 1: Calibration curve of Ketotifen fumarate (HPLC)

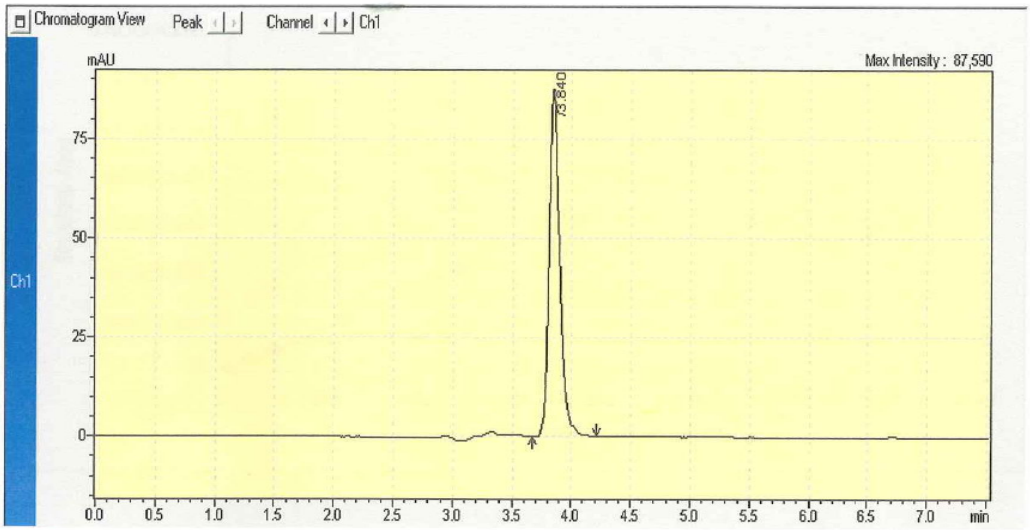

Fig. 2: Typical chromatogram of Sample ketotifen fumarate

Table 1: Results of calibration curve at 298nm for Ketotifen fumarate by UV spectroscopy

S. No.	Concentration (ng/ml)	Absorbance at 298nm
1	10	0.2966
2	20	0.5824
3	40	1.1276
4	60	1.7152
5	80	2.2474
6	100	2.7742

Table 2: Precision result of Ketotifen fumarate at 298nm

Table 2: Intraday result

	5ng/ml	50ng/ml	100ng/ml
	4.9819	49.8217	99.7989
	4.8912	49.9982	99.9915
	5.0017	50.0013	100.1011
	4.9972	48.1573	100.0917
	4.8852	49.9578	99.8972
Mean	4.9514	49.5873	99.9761
SD	0.058	0.803	0.129
% CV	1.176	1.619	0.129
% Accuracy	99.03	99.17	99.98

Table 2a: Interday result

	5ng/ml	50ng/ml	100ng/ml
	4.9985	49.7219	98.9815
	4.8997	49.9858	99.7297
	4.9152	48.9912	99.1253
	4.9915	49.9915	98.9989
	4.8792	49.1725	99.9872
Mean	4.9514	49.5873	99.9761
SD	0.058	0.803	0.129
% CV	1.176	1.619	0.129
% Accuracy	99.03	99.17	99.98

Table 3: Recovery studies of Ketotifen fumarate at 298nm

Amount of sample (ng/ml)	Amount of drug added (ng/ml)	Amount Recovered** (ng/ml)
10	1	10.51
10	2	12.14
10	3	12.98
10	4	14.01
10	5	14.98

**Average of five determinations.

Table 4: Accuracy result of Ketotifen fumarate at 298nm

	5 ng/ml	50 ng/ml	100 ng/ml
	4.8892	49.9515	99.9855
	4.7321	50.1002	99.9912
	4.9285	49.8913	100.1592
	4.8775	49.8875	99.8572
	4.9125	49.9972	98.9978
Mean	4.8679	49.9655	99.7981
SD	0.079	0.088	0.460
% CV	1.613	0.176	0.461
% Accuracy	97.36	99.93	99.80

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