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Development of a spectrophotometry method for the estimation of Ketotifen fumarate in bulk and the pharmaceutical tablet dosage form

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ABSTRACT

The developed and validation for the estimation of Ketotifen fumarate by using UV spectrophotometric method was a simple, accurate, precise and more sensitive. In comparison to previous literature survey, our method was shown to be more sensitive. The standard stock solutions were prepared using analytical grade methanol as per procedure and were scan at maximum absorbance 298nm. The concentration range of 10-100 ng/ml was found to be linear. The coefficient in the (r^2) was 0.9997. The regression equation was found to be y = 0.0278 X + 0.0184. According to the ICH guidelines, the developed method was validated and found to be accurate and precise. The validation parameters are the linearity, accuracy, precision, limit of deduction, limit of quantitation, robustness and ruggedness. The developed and the validated method can be successfully applied to the estimation of Ketotifen fumarate in bulk drugs and pharmaceutical dosage form for the routine analysis and monitoring of the quality of marketed drugs.

Keywords: Ketotifen fumarate, Validation, ICH guidelines, UV-Vis spectrophotometric method

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INTRODUCTION

Ketotifen is a second-generation H1-antihistamine and mast cell stabilizer. The chemical name of Ketotifen fumarate is 4-(1-Methyl-4-piperidylidene)-4H-benzo [4, 5]cyclohepta[1,2-b]thiophen-10(9H)-one hydrogen fumarate. Ketotifen fumarate is a finely crystalline powder with an empirical formula of $C_{23}H_{23}NO_5S$ and a molecular weight of 425.50 [1-3]. The structure of Ketotifen fumarate was shown in Figure 1. It is most commonly available as a salt of Fumaric Acid, Ketotifen fumarate, and is accessible in two forms. In its ophthalmic form, it is used to treat allergic conjunctivitis, or the itchy red eyes caused by allergies. In its oral form, it is used to prevent asthma attacks. Side effects include drowsiness, weight gain, dry mouth, irritability, and increased nosebleeds.

Only limited analytical method have been reported for the estimation of Ketotifen fumarate in biological fluids, pharmaceutical formulation, and UV visible spectrophotometry [4-15]. The significance of this work is to develop a simple, accurate, sensitive, precise and economic UV spectrophotometric method. We have proved our method is more reproducible and statistically validated.

MATERIALS AND METHODS

Materials

Ketotifen Fumarate was obtained as a gift sample from Zontron Pharmacetical Sdn Bhd, Sungai Petani, Malaysia. Distilled water, methanol, and other reagents were of analytical grade. UV-Vis spectrophotometer Beckman Coulter-DU800 from Straits Scientific (M)Sdn Bhd and 50 micron/1ml cuvette was used to obtain spectrum and absorbance measurement.

Method

Preparation of Stock solutions

Ketotifen fumarate 100mg standard was weighed and dissolved in 50 ml of Methanol in a 100 ml volumetric flask. The flask was kept for sonication for 10 minutes and volume was made up to the mark with Methanol (1000 mcg/ml) stock solution.

Selection of analytical concentration ranges

From the 1000 mcg/ml, pipette out 1 ml and placed into 10 ml volumetric flask. From the 100 mcg/ml, prepare 10, 20,40,60,80 and 100 ng/ml. Absorbance for this solution were measured at 298 nm under the spectra was shown in Figure 1. The standard solution analytical concentration ranges were found to be 10 to 100 ng/ml and this values were reported in Table 1.

Calibration curve for the Ketotifen Fumarate (10 – 100 ng/ml)

Appropriate volume of aliquots from standard Ketotifen fumarate stock solution was transfer to different volumetric flask of 10 ml capacity. The volume was adjusted to the mark with the methanol to obtain concentration of 10,20, 40, 60, 80, 100 ng/ml. Absorbance spectra of each solution against methanol as blank were measure at 298nm and the graph of absorbance against concentration were plot and shown in Figure 3. The regression equation and coefficient of determination was determined.

Sample preparation of determination of Ketotifen fumarate from tablet dosage form

Twenty tablets of a brand was weight and finely powdered with the help of mortar. The powder equivalent weight of Ketotifen fumarate was accurately weight and transferred to the volumetric flask. The flask was shaken and volume was made up to the mark with methanol. The above solution was centrifuged at 3000rpm for 7 minutes and carefully filtered through Whatmann filter paper. From this solution, 100mcg/ml was prepared and used for the estimation. To examine the absence of positive or negative interferences of excipients used in the tablet formulation, recovery studies were carried out.

Method Validation

The objective of method validation [16] is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. Accuracy was determined the recovery studies were carried out by adding the known amount of standard Ketotifen fumarate drug to the sample solution of the tablets. Precision for assay were determined by repeatability, interday and intraday precision for drug (each in 3 replicates). Ruggedness studies were carried out by changing the analysts. LOD and LOQ were performed and those values were presented in (Table 2).

RESULTS AND DISCUSSION

Linearity

6 point calibration graph were constructed covering a concentration range of 10-100ng/ml. 3 independent determination were performed at each concentration. The standard deviation of the slope and intercept were lobed. The determination coefficient exceeded 0.99.

Precision

The carried out predictability studies (n=6). Thus showing that equipment used for study worked correctly for the developed analytical method and is highly repetitive. For the intermediate precision, a study carried out by the same analyst working on 2 consecutive days (n=3). Both values were far below 5 %, the limit % set for the precision, and indicated a good method precision.

The optical characteristic such as beer's law limit, molar absorptivity and other parameter are presented in Table 2. The results of accuracy, precision and ruggedness studies were presented in Table 3-4 respectively.

Figure 1 Structure of Ketotifen fumarate

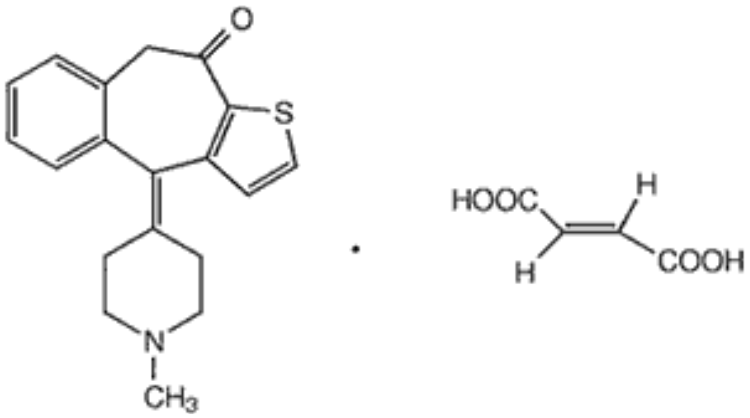

Figure 2 UV spectra of Ketotifen Fumarate at 298nm

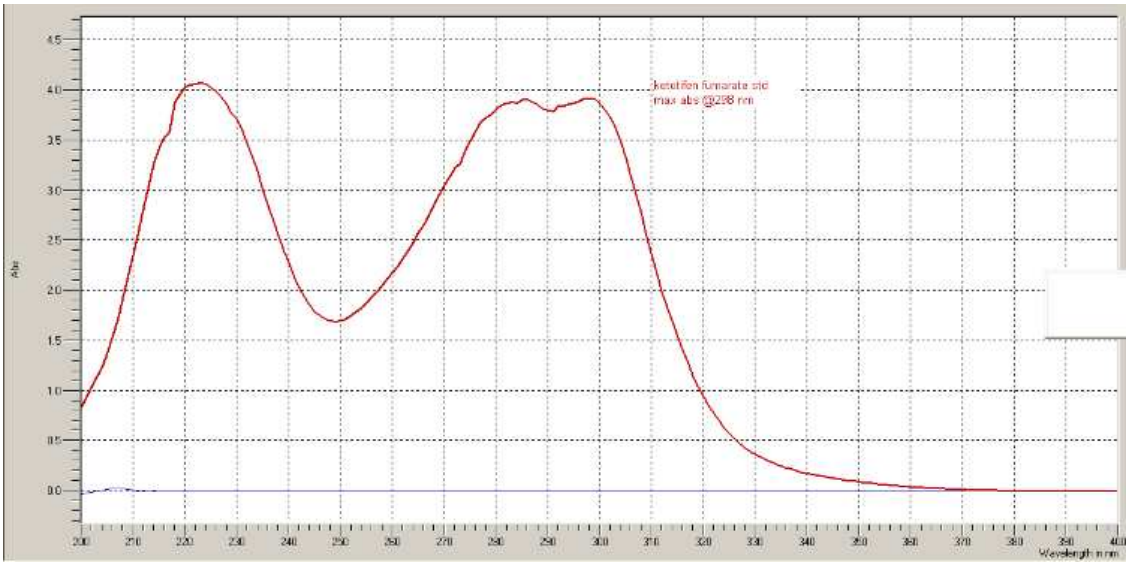

Table 1 Results if calibration curve at 298nm for Ketotifen fumarate by UV spectroscopy

SI. 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

Figure 3 Calibration curve for Ketotifen fumarate at 298nm by UV spectroscopy

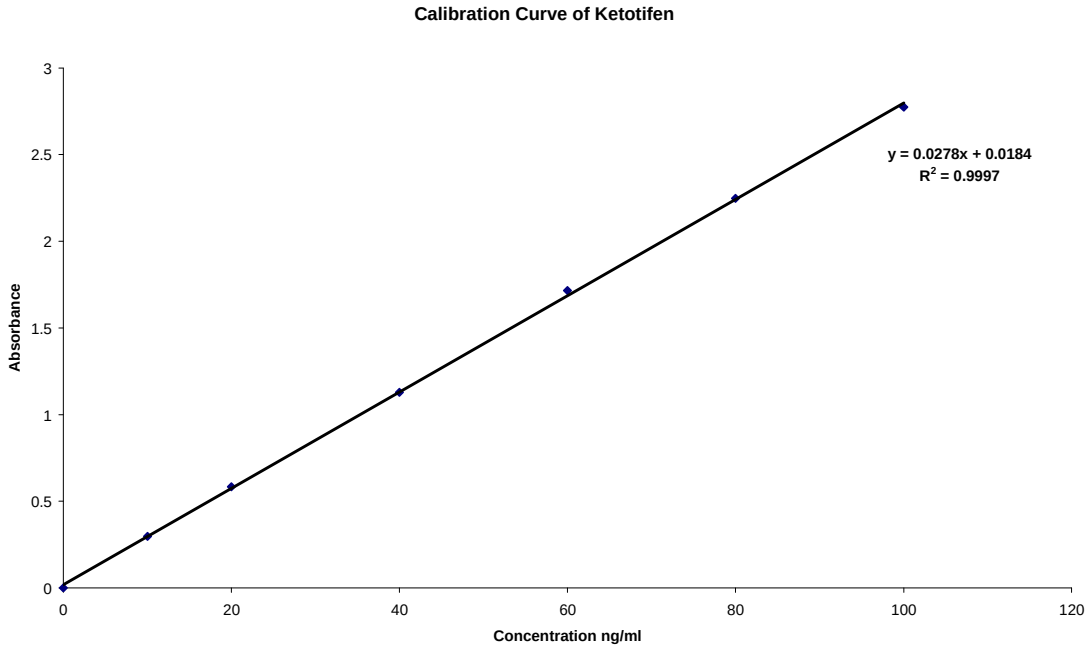

Table 2 Optical Characteristics of Ketotifen fumarate

PARAMETERS	RESULTS
Absorption maximum	298nm
Beer's law limit (ng/ml)	10-100 ng/ml
Correlation coefficient (r^2)	0.9997
Regression equation (y=mx + c)	y = 0.0278 X + 0.0184
Slope (m)	0.0278
Intercept (c)	0.0184
Limit of detection (ng /ml)	4
Limit of quantitation (ng/ml)	7

**Average of five determinations.*

Table 3 Accuracy results 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 Precision results of Ketotifen fumarate at 298nm

Conc. ng/ml	Inter-day Absorbance**±SD	Intra-day Absorbance**±SD
10	0.3068	0.3058
60	1.7250	1.7350
100	2.8305	2.8205

***Average of five determinations.*

CONCLUSION

Based on the results, it can be concluded that the developed method for the estimation of Ketotifen fumarate is simple, accurate, sensitive and reproducible.

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