Spectrophotometric Determination of Ketotifen Fumarate in Pure and Pharmaceutical Preperations by Bromophenol Blue Reagent

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Abstract

A spectrophotometric reliable, rapid and sensitive method has been developed and validated for the determination Ketotifen fumarate . A method was described for the determination of Ketotifen Fumarate in pure form or pharmaceutical formulations, a colored ion-pair complex formation reaction among ketotifen fumarate and acid-dye bromophenol blue at pH 3.0 was used for the colorimetric determination of the drug. The complex formed was extracted into chloroform and the maximum absorbance of the solution was measured at 413 nm against blank. The calibration curve calculated obey Beer's law over the concentration range of 0.4-16 µg/ml and the regression equation was A=0.069x+0.036 (R^2=0.998). The recovery of the drug from a commercial tablet was 100.66-104.26 % of the label claim with a relative standard deviation of 0.867-1.472 %. The Sandell sensitivity values, limits of detection (LOD) and limit of quantification (LOQ) values have also been reported. No interference was observed from common excipients present in pharmaceutical formulations.

Keywords: Ketotifen fumarate, bromophenol blue, ion-pair complex, spectrophotometry

Introduction

Ketotifen fumarate [KTF; 10H-benzo(4,5)cyclohepta(1,2-b)thiophen- 10-one, 4,9-dihydro-4-(1-methyl-4- piperidinylidene)-(E)-2-butenedioate (1:1)] fig.1[1] is a nonspecific, oral mast cell stabilizer introduced in 1972.Its Histamine H1 Antagonists that selectively bind to but do not activate histamine H1 receptors, thereby blocking the actions of endogenous histamine. Included here are the classical antihistaminics that antagonize or prevent the action of histamine mainly in immediate hypersensitivity. They act in the bronchi, capillaries, and some other smooth muscles, and are used to prevent or allay motion sickness, seasonal rhinitis, and allergic dermatitis and to induce somnolence. [2-4] . Several methods have been reported for the determination of KTF in bulk and pharmaceutical formulations or biological samples, these methods include high performance liquid chromatography HPLC [5-7] , GC [8-11] , chemiluminescence [12] , atomic absorption spectrometry [13] , differential pulse polarographic method [14] , polymer membrane[15], capillary electrophoresis [16], flow

injection using PVC membrane selective electrodes [17] , carbon paste electrode [18] and square wave adsorptive stripping voltammetry [19].

A revision of the literature revealed that methods that have been developed for the analysis of KTF fumarate both in pharmaceutical or biological samples [5-19] are often based on instrumental methods and there are only few reports exist in literature specifically developed for the determination of in pharmaceutical preparation which are based on spectrophotometric methods [20-25].

On other hand ion pair extractive spectrophotometric methods have also been reported for the estimation of the drugs in pharmaceutical preparations which are based on colored complex of the drugs with reagents like bromophenol blue, bromocresol green , bromothymol blue and eriochrome black T [26-30]. So far, there has been no ion-pair extractive spectrophotometry method reported for an estimation of KTF fumarate with BPB reagent. The aim of present study was to develop and validate a simple method for the determination of KTF fumarate using spectrophotometric method which can be used as an alternative to the official method or other recommended procedures in quality control labs.

Materials and Methods

Apparatus

A double beam UV-Visible recording spectrophotometer (Cintra 5) with matched 1cm quartz cuvettes was used for absorbance measurements. PH-meter DW-9421 from Philips instrument, a Sartorius BL 210S balance, and a Pentium 4 computer (acer) was used for data processing.

Materials and Reagents

All chemicals used were of analytical reagent grade except , Ketotifen fumarate which was provide as standard powder from the state company for drug industries and medical appliances Sammara – Iraq (SDI) .

Standard KTF solution

A stock standard solution containing1000 $\mu g\ ml^{-1}$ of KTF was prepared in water by dissolving 1.3747 g of KTF in in 50 ml of water and diluting to 1000 ml by using volumetric flask . Working solution of 10 $\mu g\ ml^{-1}$ was freshly prepared by subsequent dilutions . Phthalate buffer, pH 3.0, was prepared by dissolving 2.04 g of potassium hydrogen phthalate in 100 ml of water and the pH was adjusted by using 0.1 M hydrochloric acid and NaOH [21]. A 0.04% w/v solution of Bromophenol Blue BPB was prepared by dissolving 0.04 g in 100 ml of water. Spectroscopic grade chloroform was for extractio Pharmaceutical preparations from local markets.

Analytical Procedure

Absorption Spectra

Fig. (2) shows the absorption spectra of the KTF-BPB ion-pair complex and of the reagent blank in chloroform. The absorption maximum of the ion-pair in chloroform is at 413 nm where the absorbance of the reagent blank is insignificant. Therefore, a wavelength of 413 nm was used for the examination of the conditions for the determination of KTF.

Calibration curve

Aliquots of the standard solution containing 2 to 75 µg KTF were transferred into a 125 ml separating funnel and to each one 0.5 ml of phthalate buffer (pH 3.0) . and1.0 ml of 0.04% w/v BPB reagent solution were added. The separating funnel was shaking gently with 5.0 ml of chloroform for 4 min. The two layers were allowed to separate, the absorbance was measured at 413 nm against a reagent blank which was prepared similarly. Calibration curve was plotted using absorbance-values versus concentration Fig.3.

Assay procedure for tablets

Twenty tablets were weighed and pulverized to a fine powder. An aliquot equivalent to about 1 mg of KTF was transferred into a 100-ml volumetric flask. A suspension of the drug with 5 mL ethanol and 50 ml water was shaken for 10 min and mixed well filtered using Whatman No.41 filter paper to a second100-ml volumetric flask. Final solution was diluted to 100 ml with water.

Results and Discussions

The proposed procedure is based on the reaction between KTF and BPB resulting in the formation of an ion-pair complex which could be extracted into chloroform and measured spectrophotometrically . The experimental conditions were optimized and the methods validated. The formation of the complex is shown in the reaction scheme1. given below.

Effect of pH

In order to confirm the optimum pH range 0.5 ml of phthalate buffer solution on the development and stability of the colored was used and the pH of the reaction mixture was adjusted exactly to values range between 2-5 with few drops of 0.1 N NaOH or 0.1 N HCl a plot of absorbance versus pH showed maximum color intensity and highly absorbance obtained at pH 3.0 Fig 4. On the other hand the absorbance decreased at pH above and below 3.0 . hence this pH was used in all the subsequent experimental work .

Effect of BPB Concentration

The formation colored complex was found to be affected by the concentration of BPB. To examine these, different concentrations of 1 ml BPB solution were added to a solution containing 1 ml of 20 $\mu g\, ml^{-1}$ of KTF. A gradual increase in the absorbance was observed up to0.04%, beyond which a plateau was obtained.. Hence, 1 ml of 0.04% BPB solution was maintained Fig.5.

Extraction Solvent and Shaking Time

Several organic solvents (chloroform, toluene, carbon tetra chloride ,1,2dichloroethane and dichloromethane) were examined for their ability to extract drug - BPB ion-pair complex. Among those organic solvents, chloroform was found to be the most suitable for quantitative extraction. An organic phase was required for times of 1 to 6 min produced constant absorbance, hence a shaking time of 4 min was chosen for use. The drug-dye complex in the aqueous phase was extracted with 5 ml of chloroform. The absorbance was measured each time under the optimum conditions and only one extraction was found to be adequate to achieve a quantitative recovery of the complex.

Reaction time and addition sequence

The effect of the reaction time was studied by preparation of KTF-PB colored complex and measured under the optimum conditions from 1 to 10 minutes and there were no significant changes in absorbance under the optimal conditions for the sequence of addition and the maximum absorption was for the sequence (Drug + Buffer +Reagent) .

Composition of ion-pair complexes

Anionic dyes such as BPB form ion-pair complex with the positively charged nitrogen containing molecule such as KTF. Each drug– dye complex, with two oppositely charged ions, behaves as a single unit held together by ions, behaves as a single unit held together by an electrostatic interaction. The suggested mechanism of KTF - BPB ion - pair complex formation is displayed in Scheme 1. The composition of the ion pairs associates was established by Job's method of continuous variation. In the present study, different amounts of KTF and BPB were added to each flask and extracted in the same manner as recommended procedure. The absorbance of formed KTF-BPB ion-pair complex was measured at 413 nm. The absorbance was plotted against [KTF]/[KTF]+[BPB] for Job's method .In Job's plot, the plot reached a maximum value at a mole fraction of 0.5, which indicated the formation of 1:1 (KTF-BPB) complex Fig.6 . The extraction equilibrium can be represented as follows:
KTF + (aq) + Dye - (aq) ↔ KTF + Dye - (aq) ↔ KTF + Dye (org) where KTF + and Dye- represent the protonated KTF and the anion of the BPB respectively.The subscript (aq) and (org) refer to the aqueous and organic phases . The absorbance of each solution was plotted against the mole fraction of the drug ,$V_{KTF}/V_{KTF}+V_{BPB}$ (Fig. 6).

Analytical data

Under the optimized experimental condition, calibration curve was constructed by plotting the absorbance at λmax against the concentration of KTF. Beer's law range, molar absorptivity, Sandell's sensitivity, regression equation, and correlation coefficient were determined for proposed method and are given in Table 1. A linear relationship was found between the absorbance at λmax and the concentration of the drug in the range of 0.4-15 μg ml^{-1} for KTF in the final measured with molar absorption coefficients of 2.94×10^4 l/mol.cm. Regression analysis of the Beer's law plots at λmax revealed a good correlation (R^2 = 0.998). The graph showed negligible intercept and were described by the regression equation, y =0.069 C + 0.036 .The high molar absorptivity of the resulting colored complex indicates and the high sensitivity of the method(Table 1).

Sensitivity and Validation of the method

The limit of quantification that can be determined was found to be 0.461 μg ml^{-1}. The limit of detection that can be reliably detected of 3 replicates was found to be 0. 162 μg ml^{-1} Samples of pure KTF was prepared and tested in 3 replicates using the proposed procedure. The complete set of validation assays was performed. The results are given in Table 1.

Application to dosage forms

The proposed method was successfully applied to the determination of KTF in commercial tablets. The applicability of the proposed method for assay of KTF in formulations was examined by analyzing various formulations and the results are tabulated in Table 2. three replicates determinations were made. Satisfactory results were obtained and were in a good agreement with the label claims for different batches. The results were reproducible with low RSD values less than (1.472 %). The accuracy of the method is indicated by the good recovery (100.66-104.26%).

Effect of additives and excipients

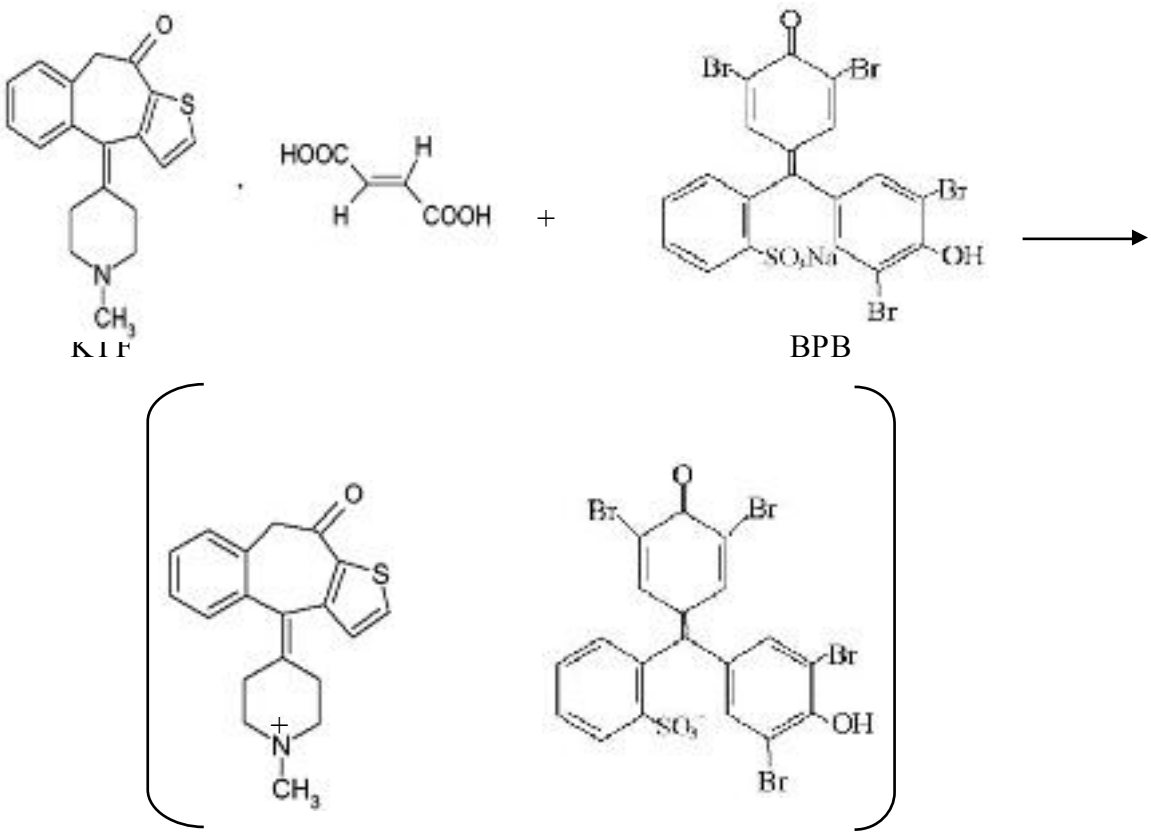
The results of analysis of the commercial formulation and the recovery study of drug suggested that commonly used additives and excipients (lactose, magnesium stearate, starch, sucrose, fructose and cellulose) do not interfere with the assay procedure. The proposed

method is sufficiently sensitive to permit determination of low concentration of KTF (0.162 μg/ml) did not interfere in the assay.

Conclusions

Unlike GC and HPLC techniques, spectrophotometry is simple and inexpensive. The importance of the technique also lies in the chemical reactions upon which the procedures are based rather than upon the sophistication of the instrument. This aspect of spectrophotometric analysis is of major interest in analytical pharmacy since it offers distinct possibility in the assay of a particular component in complex dosage formulations. The proposed methods require only dyes as reagents which are cheaper and readily available and the procedures do not involve any critical reaction conditions or tedious sample preparation. Moreover, method is simple, fast, accurate, adequately sensitive and free from interference by common additives and excipients. The present methods are superior with respect to both sensitivity and selectivity. The calculated ε values of the proposed method is 2.94×10^4 L/mol/cm . The present method, one of the characteristic features of green analytical chemistry. The wide applicability of the new procedures for routine quality control is well established by the assay of KTF in pure form and in pharmaceutical preparations.

In comparison with HPLC method the recoveries were 51.7–95.5% , precisions for the drugs in plasma were not greater than 9.5% [5] , that means the proposed method has a good recovery and precision .



Scheme1. KTF – BPB proposed complex

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Table (1): Sensitivity and regression parameters.

Parameter	KTF – BPB complex
λ_{max} (nm)	413nm
Color	yellow
Linearity range ($\mu g.mL^{-1}$)	0.4-15
Molar absorpitivites ($l.mol^{-1}.cm^{-1}$)	$2.94x10^4$
Regression equation	$A = 0.069$ [complex.$\mu g.mL^{-1}$] + 0.036
Calibration Sensitivity	0.036
Sandell's Sensitivity ($\mu g.cm^{-2}$)	0.01447
Correlation of Linearity (R^2)	0.998
Correlation coefficient (R)	0.9989
Detection limit LOD ($\mu g.mL^{-1}$)	0.162
Limit of quantifi cation (LOQ), $\mu g\ mL^{-1}$	0.461

Table (2): Application of proposed method.

Sample	Labeled amount (mg)	Conc. taken (µg.mL-1)	Conc.* Found (µg.mL-1)	Recovery %	R.S.D* [a] %	Accuracy [b]
Ketotifen fumarate 1mg/ tablet india	1	5	5.213	104.26	1.242	4.26
		10	10.253	102.53	1.138	2.53
Ketotifen fumarate 1mg/ tablet UAE	1	5	5.124	102.48	1.472	2.48
		10	10.066	100.66	0.867	0.66

[a]Relative standard deviation (%).

[b] (found – taken / taken) ×100.

n= 3

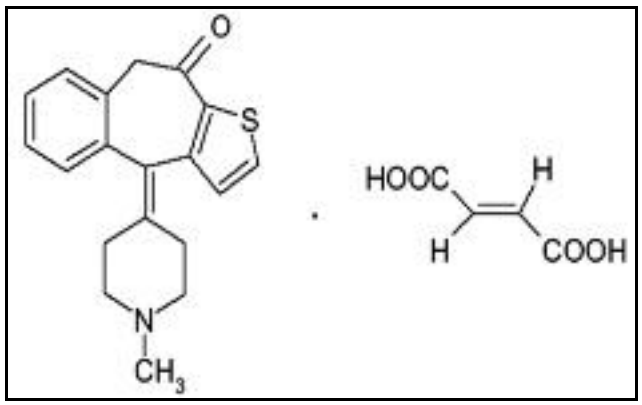

Fig.(1): Chemical structure of ketotifen fumarate (KTF) MWt. 425.5 gmol^{-1}

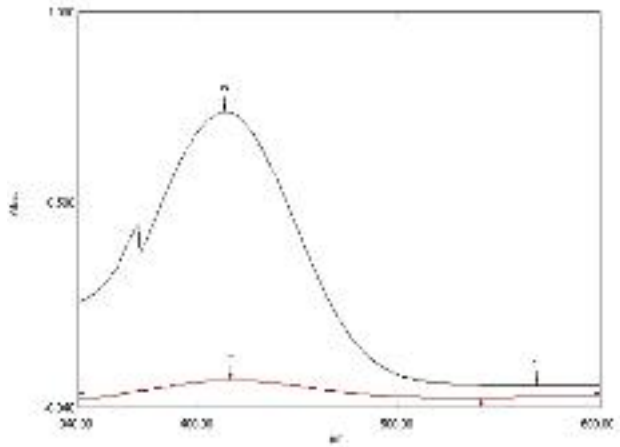

Fig. (2): The spectrum of 10µg/mL of ketotifen fumarate KTF against reagent blank and the reagent blank against chloroform.

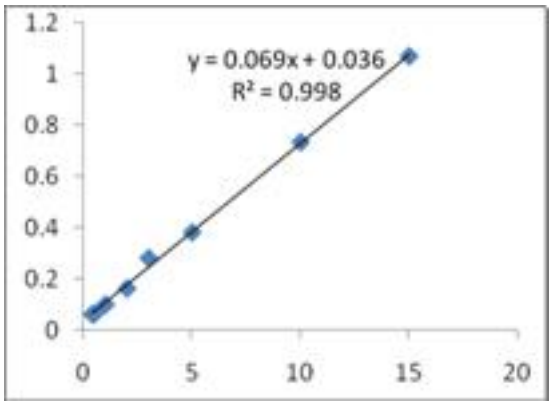

Fig.(3):Calibration curve of ketotifen fumarate KTF

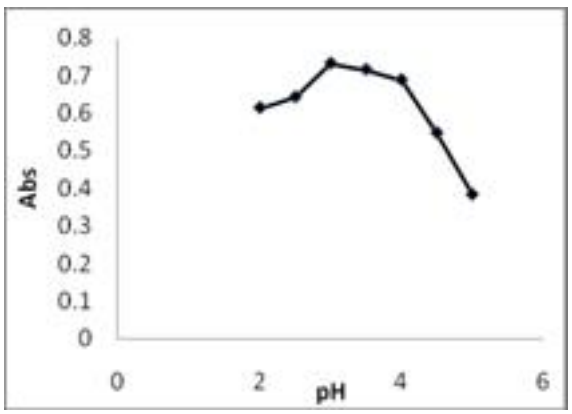

Fig.(4): The effect of pH on absorption of KTF 10µg/mL

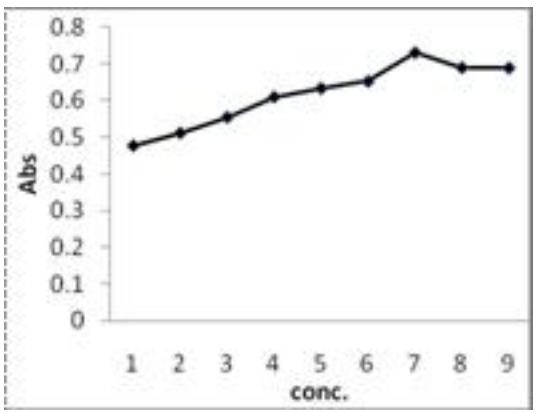

Fig.(5): The effect of reagent concentration(BPB) on absorption of KTF 10µg/mL

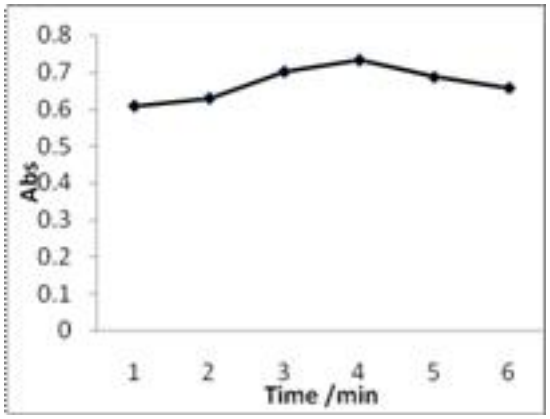

Fig.(6): The effect of shaking time of complex on absorption of KTF 10µg/mL

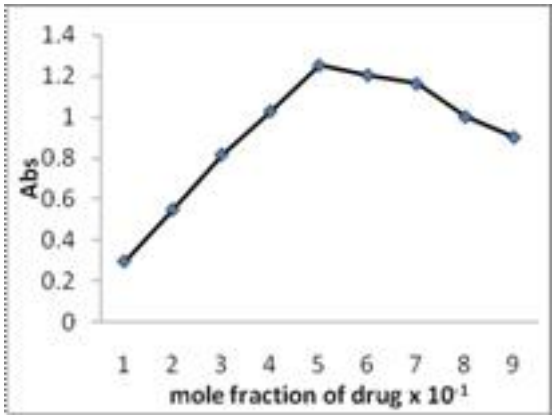

Fig.(7): Job's method of continuous variation plot for ion-pair complexe

التقدير الطيفي لعقار الكيتوتيفين فيوماريت بشكله النقي وفي المستحضرات الصيدلانية باستعمال البروموفينول الأزرق كاشفا

عمر صبيح الخزرجي

قسم الكيمياء ،كلية التربية ابن الهيثم ،جامعة بغداد

استلم البحث في :18 آيار 2011

قبل البحث في : 20 أيلول 2011

الخلاصة

استعملت طريقة طيفية حساسة وسريعة و مطورة لتقدير عقار كيتوتيفين فيوماريت . تم وصف الطريقة واستعمالها لتقدير العقار بصورته النقية وفي بعض المستحضرات الصيدلانية من خلال تكوين معقد اصفر اللون بين الكيتوتيفين فيوماريت وكاشف بروموفينول الازرق عند اس هيدروجيني =3 . المعقد اللوني المتكون استخلص باستعمال الكلوروفورم و قيست اعلى امتصاصية ضوئية له عند 413 نانومتر .منحنى المعايرة استجاب لقانون بير بمستوى تركيز من 0,4−15 ميكروغرام / مليليتر ، وكانت معادلة الخط المستقيم A=0.069x+0.036 ومعامل الترابط (R^2=0.998) . كانت نسبة الاسترجاعية للمستحضرات الدوائية على شكل حبوب بحدود 100,66 – 104,26 . كذلك حسبت قيم حساسية ساندل وكذلك الانحراف المعياري، وحد الكشف، و حد التقدير . لم يلاحظ اي تداخل للمضافات الموجودة في المستحضرات الصيدلانية على النتائج المستحصلة .

الكلمات المفتاحية :عقار الكيتوتيفين فيوماريت ، البروموفينول الأزرق ، معقدات المزدوجات الأيونية .