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Research Article

Quantitative Determination of Ketotifen in Drug
dosage forms by Spectrophotometric method

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ABSTRACT

A new spectrophotometric method for the quantitative determination of ketotifen in pharmaceutical formulations, such as tablets and capsules, has been developed. This method is based on the formation of yellow complex as a result of the reaction between ketotifen and bromocresol purple (5',5"-dibromo-o-cresolsulfophthalein) with maximum absorption at 399 nm in acetone. The proposed method is valid according to the validation requirements of Ukrainian Pharmacopeia. According to the experimental data, the technique can be correctly reproduced and it is suitable for routine quality control.

Keywords: ketotifen, quantitative determination, spectrophotometric method, bromocresol purple.

1. INTRODUCTION

Ketotifen is known as a non-competitive, relatively selective antagonist of histamine (H₁-receptor) and stabilizer of mast cell. This drug inhibit the mediators, which released from mast cells and involved in hypersensitivity reactions. Decrease in activation of eosinophils and chemotaxis have also been demonstrated by ketotifen. Its properties, such as inhibition of the development of airway hyper-reactivity associated with activation of platelets by PAF (Platelet Activating Factor), inhibition of PAF-induced accumulation of eosinophils and platelets in the airways, suppression of the priming of eosinophils by human recombinant cytokines and antagonism of bronchoconstriction due to leukotrienes, can be contributed to its anti-allergic activity and its ability to affect on the underlying pathology of asthma. In modern clinical practice ketotifen is used for the treatment of asthma, rhinitis, skin allergies, and anaphylaxis¹.

Due to adverse events (potentiation of hypnotics, sedatives, other anti-allergy, alcohol) ketotifen is used for non-medical purposes, and in overdose has toxicological significance².

Based on the fact, that ketotifen drugs have fairly high demand in the pharmaceutical market, there is no doubt in the supplying desirability of worthy quality control of these drugs.

Thus development of accurate, express and available methods of active substances quantitative

determination in drug dosage forms of ketotifen is very important for modern pharmaceutical and toxicological analysis

According to the literature data, some methods of ketotifen analysis are known.

In one study a PVC membrane electrode for determination of ketotifen fumarate is reported, where ketotifen tetraphenylborate was used as ion exchanger. These are used for ketotifen determination using potentiometric titration in pure samples and its pharmaceutical preparations³.

Chemiluminescence method is described. It is based on the reaction of potassium hexacyanoferrate(III) with the mixture of calcein and ketotifen. A flow injection method was also established for the determination of ketotifen in the tablets⁴.

In other study method using chemiluminescence detection has been developed for the simple determination of ketotifen fumarate. The method is based on the catalytic effect of ketotifen fumarate in the chemiluminescence reaction of tris (1,10 phenanthroline) ruthenium(II), Ru(phen)₃²⁺, with Ce(IV) in sulfuric acid medium⁵.

Thin layer chromatography-densitometric determination method is described for the combination of ketotifen with pseudoephedrine or acetaminophen. The method employed thin layer chromatography aluminum plates precoated with silica gel G 60 F254 as the stationary phase.

Spectrodensitometric scanning integration was performed at 218 and 251 nm respectively⁶.

Another densitometric method for the determination of ketotifen hydrogen fumarate known⁷. Maximum wavelengths were 228 nm. It has been successfully applied to the analysis of substances and pharmaceuticals.

The methods of ketotifen analysis based on the reaction of the ketotifen with methyl orange to give ternary complexes are known. These complexes are readily extracted with organic solvent and absorbance was measured at 588 nm⁸.

In the present investigation we report the development of accurate, reproducible and sensitive spectrophotometric method based on the formation of ion-association complexes of ketotifen with bromocresol purple (BCP) in acetone. No interference of common excipients was observed in the assay of ketotifen in pharmaceutical formulations.

2. RESEARCH MATERIALS AND METHODS

2.1. Reagents

All chemicals of analytical or pharmaceutical grade were used throughout. Pure ketotifen fumarate substance was obtained from "Zdorovie" Pharmaceutical company's laboratory, Ukraine. BCP and acetone were obtained from «Macrochim», Ukraine. The dosage forms of ketotifen were obtained: from different firms - «Ketotifen-LCh» pills 1.0 mg from JSC «Lekchim-Kharkov», Ukraine, series 90613; «Ketotifen-Sopharma» pills 1.0 mg from JSC «Sopharma», Bulgaria, series 5010514; «Ketotifen» pills 1.0 mg from Ltd. «SI «GNCLS», Ukraine, series 121114; «Ketotifen-V» capsules 1.0 mg dosage forms JSC «Monpharm», Ukraine, series 11212.

2.2. Solutions

Pure ketotifen fumarate (equivalent 5 mg of ketotifen) was accurately weighed and transferred into a 100 ml calibrated flask, dissolved in acetone and diluted to the mark with acetone. The solution is stable at room temperature.

0.15% solution of BCP was prepared in acetone.

2.3. Apparatus

Analytic Jena UV-visible spectrophotometer model Specord 200 with 1 cm matched quartz cells was used for the absorbance measurements. The samples weighing were made with Kern electronic scales ABT-120-5DM.

2.4. Assay procedure

The aliquots of the solution containing 0.05-0.15 mg of ketotifen were transferred into a series of 10 ml calibrated flasks. 1 ml of BCP was added to each of

the calibrated flasks and diluted to the mark with acetone. The contents were shaken well and left at room temperature for a minute. The absorbance of the yellow colored species was measured at 399 nm, against corresponding reagent blank. A calibration graph was plotted.

2.5. Assay procedure for dosage forms

Twenty tablets were weighed and powdered. An amount of the powder equivalent to 0.6-1.9 mg of ketotifen was weighed into a 25 ml volumetric flask, 20 ml of acetone was added and shaken thoroughly for about 15–20 min. The content was diluted to the mark with acetone, mixed well and filtered through a filter paper to remove the insoluble matter. 2 ml of the filtrate was used for analysis using the procedure given above. The active substance content was calculated using the standard formulas⁹.

3. Results and discussion

3.1. Absorption spectra

Ketotifen reacts with BCP in acetone to give a soluble yellow coloured ion-association complex which exhibits an absorption maximum at 399 nm. Presumably the ion-pair complex is formed due to ketotifen excess electron density on the nitrogen atom and BCP donor proton. Under the experimental conditions, the reagent blank showed negligible absorbance as shown in Fig. 1.

3.2. Optimum reaction conditions

The optimum reaction conditions for quantitative determination of the ion-pair complex were established via a number of preliminary experiments. The optimum volume of the reagent was studied. It was observed that 1 ml of 0.15% of BCP was necessary for maximum colour development of the complex. When choosing a solvent the solubility of ketotifen, BCP and maximum absorbance values were taken into account. There was no appreciable change in absorbance of the product when the reactants addition order varied. Absorbance of the complex was found to be stable for more than 5 h. The temperature and time regimes in this case did not require correction because the reaction proceeds rapidly at room temperature. In optimal conditions Beer's law was obeyed in the concentration range of 5 - 15 $\mu\text{g ml}^{-1}$, detection limit is 0.74 $\mu\text{g ml}^{-1}$ ⁹.

3.3. Determination of some validation characteristics

According to requirements of Ukrainian Pharmacopoeia the following validation characteristics as precision, linearity, accuracy and robustness were determined¹⁰.

Linearity. Calibration graph was constructed by measuring the absorbance at seven concentration levels which showed linear response of absorbance in relation to concentration of ketotifen over the range of 5 - 15 $\mu\text{g ml}^{-1}$. The resulting dependence (Fig. 2) is described by linear regression equation $y=0.8024x-0.0183$, the correlation coefficient r and the residual standard deviation S_r , calculated by the method of the minimal squares, are 0.9998 and 0.0079 respectively.

Precision. Precision was determined from ketotifen samples at three different concentrations in the calibration range in three replicates. The relative standard deviation (RSD) was found to be between 1.06 and 1.56% for the dosage forms under study. The data is summarized in Table 1.

Accuracy. Accuracy was set for the drug dosage forms using standard addition method and suggested

the high accuracy of the proposed method. Recoveries were found to be between 99.52 and 100.82% (Table 2).

4. CONCLUSION

The proposed method for ketotifen determination has many advantages due to its rapidity, lower cost, instrumental simplicity and sensitivity. Unlike the gas chromatographic and HPLC procedures, the instrument is simple and is not of high cost. The reagents utilised in the proposed method are cheaper, readily available and the procedures do not involve any critical reaction conditions or tedious sample preparation. The wide applicability of the new procedure for routine quality control is well established by the assay of ketotifen in pure form, as well as in pharmaceutical preparations.

Table 1
Precision determination results for ketotifen dosage forms.

Drug dosage form	$\bar{X}_{(n=9)}$	S	RSD%	Δ_x	$A_s\%$
«Ketotifen-LCh» pills 1.0 mg (JSC «Lekchim-Kharkov», Ukraine)	1.032	$1.20 \cdot 10^{-2}$	1.16	2.17	3.20
«Ketotifen-Sopharma» pills 1.0 mg (JSC «Sopharma», Bulgaria)	1.035	$1.60 \cdot 10^{-2}$	1.56	2.87	3.20
«Ketotifen» pills 1.0 mg (Ltd. «SI «GNCLS», Ukraine)	0.987	$1.16 \cdot 10^{-2}$	1.17	2.20	3.20
«Ketotifen-V» capsules 1.0 mg (JSC «Monpharm», Ukraine)	1.005	$1.06 \cdot 10^{-2}$	1.06	1.96	3.20

Table 2
Accuracy determination results for ketotifen dosage forms.

Drug dosage form	$\bar{Z}_{(n=9)}$	RSD%	$\Delta \bar{Z}$	$\Delta \bar{Z} / \sqrt{n}$	$\bar{Z} - 100$
«Ketotifen-LCh» pills 1.0 mg (JSC «Lekchim-Kharkov», Ukraine)	99.52	1.93	3.55	1.18	0.48
«Ketotifen-Sopharma» pills 1.0 mg (JSC «Sopharma», Bulgaria)	100.82	4.46	8.37	2.79	0.82
«Ketotifen» pills 1.0 mg (Ltd. «SI «GNCLS», Ukraine)	100.69	2.50	4.66	1.55	0.96
«Ketotifen-V» capsules 1.0 mg (JSC «Monpharm», Ukraine)	100.35	4.71	8.75	2.92	0.35

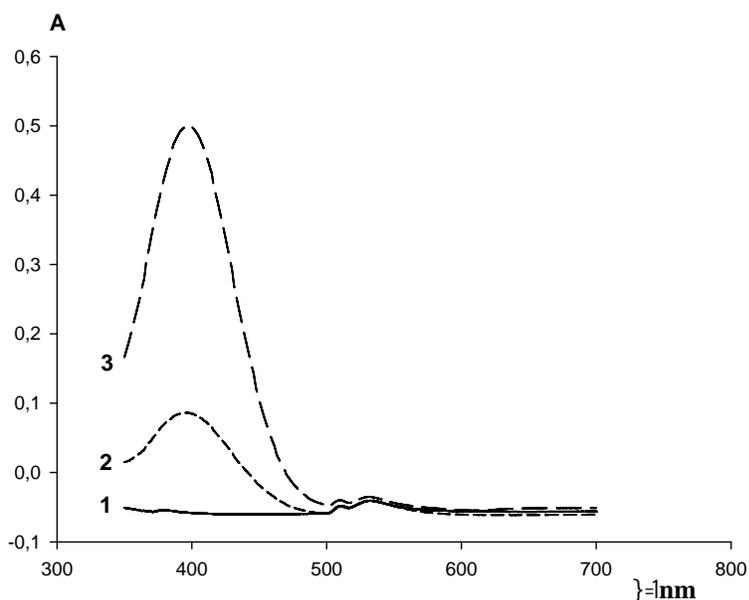


Fig. 1
Absorption of (1) ketotifen, (2) BCP and (3) their reaction product.

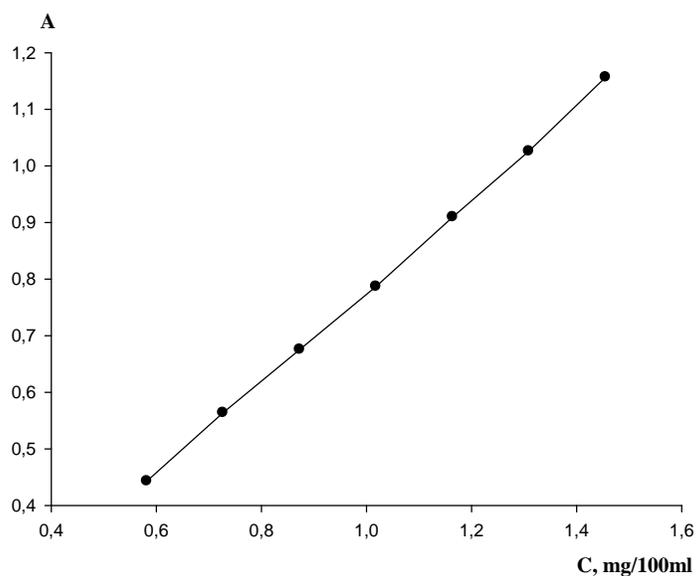


Fig. 2
Linear correlation between absorbance and concentration of ketotifen.

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