

Spectrophotometric Determination of Ketotifen Drug in Pure form and Pharmaceutical Preparation Using Bromothymol Blue Via Ion-Pair Formation

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Abstract

The ion-pair formation method has been applied for the spectrophotometric determination of Ketotifen, in bulk sample and in dosage form. The method is accurate, simple and sensitive depending on the extraction of the formed ion-pair with bromothymol blue (BTB) as a chromogenic reagent in chloroform, use phthalate buffer of pH 3.5. The formed complex show absorbance maxima at 417 nm against reagent blank. The calibration graph is linear in the ranges of 0.3-25 $\mu\text{g}\cdot\text{mL}^{-1}$ with detection limit of 0.213 $\mu\text{g}\cdot\text{mL}^{-1}$. The results show the absence of interferences from the excipients on the determination of this drug. The proposed method have been applied successfully for the determination of Ketotifen in pharmaceutical preparations.

Keywords: Spectrophotometric, Ketotifen, Ion-Pair, BTB.

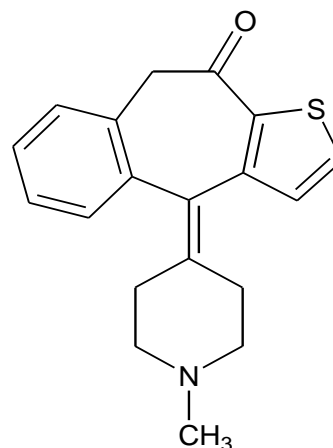
Introduction

Ketotifen, (Scheme 1), 4,9 – dihydro-4 - (1-methyl - 4 -piperidinylidene) – 10 - H-benzo (4,5) cycloheptal - (1,2-b) thiophen-10-one, (molecular weight = 309.43g.mole⁻¹)⁽¹⁾. It has antialergic properties and anti histaminic action^[2]. It is prescript for prevention of asthma, symptomatic relief of hay fever and urticaria; also used in the treatment of allergic conditions such as rhinitis and conjunctivitis^(2,3).

Several methods have been reported for estimation of ketotifen including; HPLC[4-6], potentiometry^(7,8), voltammetry⁽⁹⁾, coulometric titration methods⁽¹⁰⁾ and Gas chromatographic-mass spectrometric method⁽¹¹⁾.

Spectrophotometry⁽¹²⁻¹³⁾; is most convenient techniques because of their inherent simplicity, adequate sensitivity, low cost and wide availability in all quality control laboratories.

The present work describes the utility of BTB reagent, (molecular weight= 624.39g.mole⁻¹) for spectrophotometric determination of Ketotifen in pure form as well as in their dosage form based on ion-pair formation between the cited drug and BTB. In addition, the optimization of chemical dependent variables of affecting absorbance have been studied.



Scheme (1) The chemical structure of Ketotifen.

Experimental Parts

Apparatus

A Cintra 5 spectrophotometer with 1 cm quartz cells was used for absorbance measurements. PH-meter DW-9421 from Philips instrument, a Sartorius BL 210S balance, and a Pentium 4 computer (DELL) was used for data processing.

Materials and Methods

All Chemicals used were of analytical reagent grad unless otherwise is mentioned. Ketotifen fumarate standard powder (purity 99.8%) were kindly provided by the State Company for Drug Industries and Medical Appliances, Samara-Iraq (SDI).

Bromothymol blue (Aldrich), 0.1% (w/v), (1.602x10⁻³M) solution prepared by dissolving

0.1 g of the dye in 5 mL of methanol and then the solution was diluted to a final volume of 100 mL with distilled water. Working solutions were freshly prepared by subsequent dilutions.

Hydrochloric Acid (Aldrich), ~ 0.1 M, a 0.85 mL of concentrated hydrochloric acid (37%, sp.gr1.18) was added to 50 mL distilled water and diluting to the mark in a 100 mL calibrated flask.

Potassium Hydroxide (fluka), ~ 0.1 M, prepared by dissolving 0.5600 g of potassium hydroxide in 25 mL distilled water and diluted to 100 mL in volumetric flask with distilled water.

Phthalate buffer 0.2M solution was prepared by dissolved 4.08 g of potassium hydrogen phthalate (MERCK) in 25 mL distilled water and diluted to 100 mL in volumetric flask with distilled water, the pH was adjust to 3.5 by using few drops of 0.1M HCl and/or 0.1M KOH⁽¹⁴⁾.

Ketotifen standard solution 250 µg.mL⁻¹ (0.808x10⁻³M)

It was prepared by dissolving weighed amount of the drug equivalent to 25 mg of ketotifen in 10 mL ethanol and diluting to 100mL in a volumetric flask with distilled water. Working solutions were freshly prepared by subsequent dilutions.

General recommended Procedure

0.5ml of ketotifen standard solution were transferred into a series of 50 mL separating funnels; so that the final concentration is in the range of (0.5-25) µg.mL⁻¹, to each funnel 0.5 mL of phthalate buffer pH 3.5 and 0.5 mL of 0.045% BTB reagent solutions were added. The separating funnels were shaken vigorously with 5 mL chloroform for 2 mints. The two phases were then allowed for clear separation and the absorbance of the yellow colored organic phase was measured at 417nm against a reagent blank prepared similarly without addition of ketotifen. The calibration graph was constructed by plotting the measured absorbance of the organic phase against the drug concentration.

Job's method procedure⁽¹⁵⁾

Transfer aliquot volume of 2.060x10⁻⁴ M ketotifen standard solution into a series of

50 mL separating funnels, to each funnel 0.5 mL of phthalate buffer pH 3.5 and aliquot volume of 2.060x10⁻⁴ M BTB solution were added, in such a way that the total volume of the drug and BTB solution are constant but the molar ratio varies systematically (1:9, 2:8, 7:3, and so forth). Then each solution were extracted and analyzed as in general recommended procedures.

Analysis of ketotifen in pharmaceutical preparations^(16,17)

I. In Tablets

The content of 50 tablets were mixed well and a certain amount of fine powder was accurately weighted to give an equivalent to 25 mg of ketotifen was dissolve in 50 mL of ethanol, swirled, leaved to stand for 5 mints and diluted to 100 mL in a volumetric flask with distilled water. The solution was filtered by using Whatman filter paper No.41 to avoid any suspended or un-dissolved material before use, and the first portion of the filtrate was rejected. Working solutions were freshly prepared by subsequent dilutions with distilled water, and analyzed by the recommended procedure.

II. In Syrup

An accurately measured volume of the mixed five bottles of the drug equivalent to 20 mg of ketotifen; was quantitatively transferred into 200 mL volumetric flask and diluted to the mark with distilled water. Working solutions were freshly prepared by subsequent dilutions and analyzed by the recommended procedures.

Results and Discussion

Ion pair complex formation reaction have been used as the basis for the development of simple and sensitive spectrophotometric method for the determination of many pharmaceutical compounds⁽¹⁸⁻²¹⁾.

In this study ketotifen react with BTB in acidic buffer to give yellow color chloroform soluble ion-pair complex, which exhibits absorption maxima at 417 nm against their reagent blank; (Fig.(1)). The experimental conditions were optimized and the method validated.

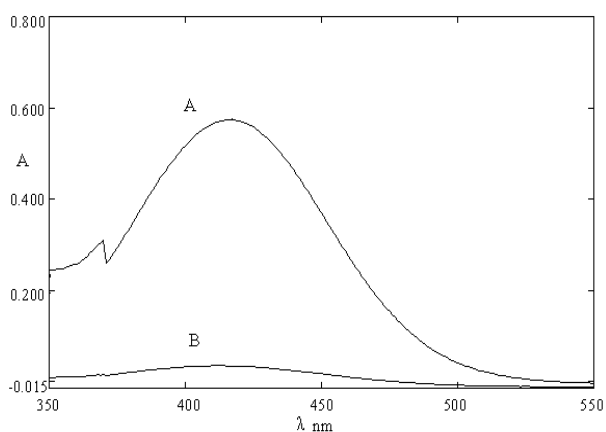


Fig. (1) Absorption spectra of (A) $10 \mu\text{g.mL}^{-1}$ ketotifen -BTB ion-pair complex, (B) Reagent blank against chloroform. Under optimum experimental conditions, (0.5 mL phthalate buffer, pH 3.5 and 0.5mL of 0.045% BTB reagent).

Effect of pH

The effect of pH on extraction of $10 \mu\text{g.mL}^{-1}$ of ketotifen was shown in (Fig.(2)), 0.5mL of the cited drug was mixed with 0.5mL of phthalate buffer. The pH was then adjusted to a value between (2.0 - 4.5). with few drops of 0.1M KOH or 0.1M HCl. It was noticed that maximum color intensities and constant absorbance values were found at pH 3.5. The absorbencies decrease at pH above or below the optimum value. Hence, a pH of 3.5 was used in all the subsequent experimental work.

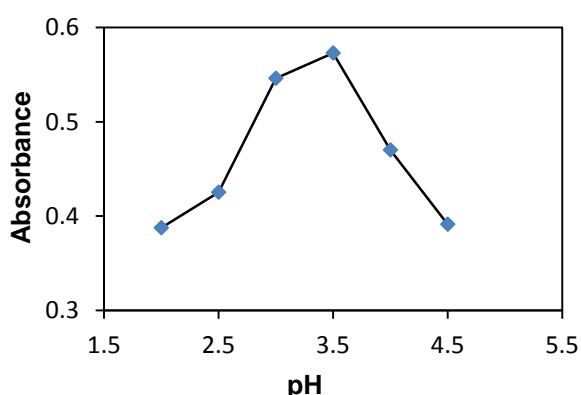


Fig. (2) Effect of pH on the Absorbance of $10 \mu\text{g.mL}^{-1}$ ketotifen; 0.5mL of 0.05% BTB.

Effect of reaction time

The optimum reaction time for the reaction of ketotifen with BTB was studied at ambient temperature (25 ± 2). It was found the reaction was instantaneous. Hence the product attained

maximum and constant absorbencies immediately after the cited drug have been mixed with BTB and the developed color, remained strictly unaltered for at least 24 hours in drake place.

Effect of reagent concentration

The influences of reagent concentration on the absorbance of complex are illustrated in(Fig.(3)), 0.5 mL of 0.045 % solutions of BTB were found to be enough to develop the maximum color intensities of complex, after which no more increase in absorbance values was obtained; therefore, the cited concentration of BTB solution were used.

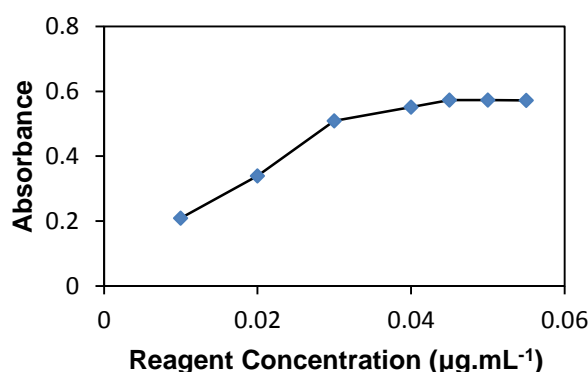


Fig. (3) Effect of Reagent Concentration on the Absorbance of $10 \mu\text{g.mL}^{-1}$ of ketotifen; pH3.5 and 0.5mL BTB.

Effect of the order of mixing

The effect of order of addition of the reactant was also studied. It was found that best result were obtained by placing the cited drug, the buffer and finally the reagent instead of any other orders of addition.

Effect of shaking time

The optimum shaking times for the complete extraction of the formed ion pair complex with chloroform were studied for the period of 1-5 minutes (Table (1)). It was found that the optimum shaking times for complete extraction of ketotifen ion pair complex, at room temperature 2 minutes.

Table (1)
Effect of shaking time on extraction of
 $10 \mu\text{g}\cdot\text{mL}^{-1}$ ketotifen; 0.5mL of 0.045% BTB,
 $\text{pH}(3.5)$.

Shaking time (minute)	Absorbance
1	0.5702
2	0.5731
3	0.5622
4	0.5461
5	0.5456

Effect of the extraction solvent

Several organic solvents, such as, toluene, carbon tetrachloride, benzene, 1,2-dichloroethane, Dichloro methane and chloroform were examined for their ability to extract the drug-BTB ion-pair complex. The latter was found to be the most suitable solvent in terms of extraction efficiency (Table (2)). Also it was observed that only a single extraction with 5 mL portion of chloroform was adequate to achieve a quantitative recovery of the complex.

Table (2)
Effect of type of extraction solvent on
absorbance of $10 \mu\text{g}\cdot\text{mL}^{-1}$ ketotifen; 0.5mL of
 0.045% BTB, $\text{pH}(3.5)$.

Extraction solvent	Absorbance
Toluene	0.0362
Carbontetrachloride	0.0340
Benzene	0.0111
1,2-Dichloro ethane	0.3478
Dichloro methane	0.1989
Chloroform	0.5731

Stoichiometry of the complex

The stoichiometry of the reaction was studied by Job's method of continuous variation (Fig.(4)). The result showed that 1:1 ratio were formed; through the electrostatic

attraction between the positive protonated ketotifen with the anion of BTB (Scheme (2)).

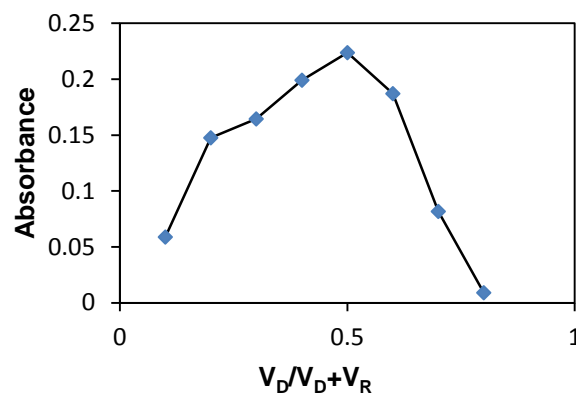
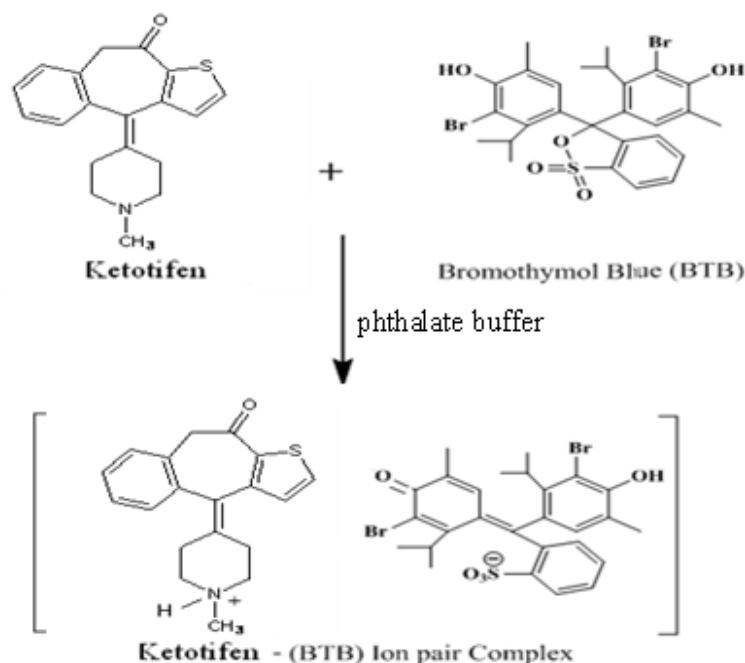


Fig. (4) Continuous variation of ketotifen -
BTB ion pair complex,
(each one $2.060 \times 10^{-4} \text{ M}$).



Scheme (2) Proposed reaction pathway between: ketotifen–BTB ion pair complex. Under optimum experimental conditions, (0.5mL phthalate buffer, pH 3.5 and 0.5mL of 0.045% BTB reagent).

Calibration graph

Employing the experimental conditions, (as in general recommended Procedure). linear calibration graph was constructed by plotting absorbance values as a function of drug concentration (Fig.(5)), which show that Beer's law was obey in the concentration range of $0.3\text{-}25\ \mu\text{g}\cdot\text{mL}^{-1}$ with a correlation coefficient ($R= 0.9993$) and detection limit of $0.213\ \mu\text{g}\cdot\text{mL}^{-1}$.

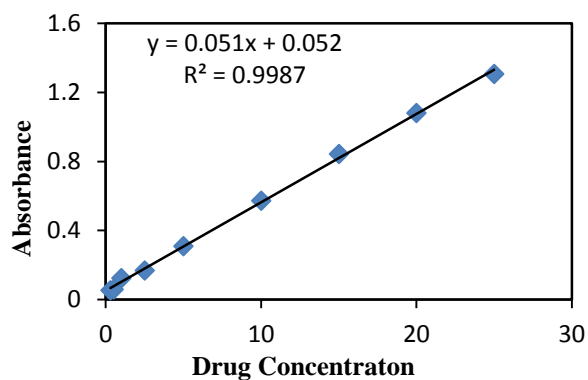


Fig. (5) Calibration graph of ketotifen-BTB ion pair complex. Under optimum experimental conditions, (0.5mL phthalate buffer, pH 3.5 and 0.5mL of 0.045% BTB reagent).

Spectral characteristics of the proposed method

Under optimum experimental conditions of the proposed method, the regression plot showed linear dependence of absorbance signals on the concentrations of the studied drug in the range given. The regression equations, correlation coefficients, molar absorptivities, detection limits and sandell sensitivities in addition to other parameters are given in (Table (3)).

Table (3)
Spectral characteristics and statistical data of the regression equations for determination of ketotifen using ion-pair and charge transfer complexes formation.

<i>Parameter</i>	<i>Ion-Pair Complex Formation</i>
λ_{\max} (nm)	417
Color	Yellow
Linearity range ($\mu\text{g.mL}^{-1}$)	0.3 – 25
Molar absorptivities ($\text{l.mol}^{-1}.\text{cm}^{-1}$)	15780.93
Regression equation	$A = 0.051[\text{Ke. } \mu\text{g.mL}^{-1}] + 0.052$
Calibration Sensitivity	0.020
Sandell's Sensitivity ($\mu\text{g.cm}^{-2}$)	19.608
Correlation of Linearity (R^2)	0.9987
Correlation coefficient (R)	0.9993
Detection limit ($\mu\text{g.mL}^{-1}$)	0.213

Accuracy and precision

The accuracy of the proposed method was evaluated by analyzing five replicate analyses of three different amounts of the drug (within Beer's law) by calculating the relative error percentage. The analytical results obtained from this investigation are summarized in (Table (4)). The values of the mean error ($x_i - \mu$) were less than the values of indeterminate error ($\pm ts/\sqrt{n}$), indicating that no significant differences between the mean and the true

values; at 95% confidence level. The precision was determined in each case by calculating the percentage relative standard deviation (RSD %) for five determinations at each of the studied concentration limit and were found to be in the range of 1.347-1.679%. It can be considered to be very satisfactory. The proposed method was compared statistically with other methods found in the literature and the results are shown in (Table (5)).

Table (4)
Evaluation of accuracies and precisions of the proposed procedure.

<i>Drug Conc.n ($\mu\text{g.mL}^{-1}$)</i>		<i>Rel. Error %</i>	<i>R.S.D %</i>	<i>$x_i - \mu$</i>	<i>$\pm ts/\sqrt{n}$</i>
<i>Taken</i>	<i>Found*</i>				
2.5	2.519	0.760	1.679	+0.019	0.049
10	9.951	0.490	1.347	-0.049	0.154
20	20.258	0.013	1.381	+0.258	0.321

*Average of five determinations.

$t = 2.571$ for $n=5$ at 95% confidence limit.

Table (5)
Analytical Parameters for the analysis of ketotifen by the proposed and others methods.

Ref. No.	methods	λ_{max} (nm)	Linear range $\mu\text{g.mL}^{-1}$	Correlation Coefficient (R)	Recv. %	RSD %
-	Proposed method	417	0.5-25	0.9993	98.4-100.6	1.347-1.679
12	spectrophotometry Ion-pair	423	5.15-61.91	0.9836-0.9892	97.6	0.48
22	spectrophotometry Atomic absorption	469.5	2.5-22.5	-	100.11	0.43
		588	10-80		99.94	0.72
23	spectrophotometry ^a Chemiluminescence	-	6.0×10^{-9} - 2.0×10^{-7}	-	96.0-103.0	1.9-2.1
7	Potentiometric ^b methods	-	1.0×10^{-5} - 1.0×10^{-2}	0.9998	98.28-102.47	1.2-4.0
24	Potentiometric ^b methods Flow injection	-	5.6×10^{-6} - 1.0×10^{-2}	-	99.5	1.4
			1.0×10^{-5} - 1.0×10^{-2}		99.2	1.2
6	HPLC	302	0.6-30	-	100.5	1.2

a: g.mL⁻¹, b: Molar Concentration.

Interferences Study

Before proceeding with analysis of dosage form interferences study were carried out. The results showed that no interferences were found in the presence of 500 μg of the studied

excipients (lactose, sucrose, starch, glucose, magnesium stearate, sodium citrate, and sodium chloride) in the determination of ketotifen, (Table (6)).

Table (6)
Percent Recovery for 10 $\mu\text{g.mL}^{-1}$ of ketotifen in the presence of 500 $\mu\text{g.mL}^{-1}$ of Excipients using ion-pair and charge transfer complexes formation.

Excipients	Conc. Fund ($\mu\text{g.mL}^{-1}$)	Recovery%
lactose	9.879	98.790
Sucrose	10.219	102.190
Starch	9.902	99.020
Glucose	9.963	99.630
Magnesium Stearate	10.172	101.720
Sodium Citrate	10.122	101.220
Sodium Chloride	9.898	98.980

**Average of three determinations.*

Analysis of dosage forms

The applicability of the proposed method for the determination of ketotifen in commercial dosage form was examined by analyzing of their content of the active ingredient by the proposed method (ion-pair complex formation). The results given in (Table (7)) reveal that the recoveries were in the range of, reflecting high accuracy and

precision of the proposed method as indicated by low Percentage relative standard deviation (RSD%) value. The recommended method was statistically compared with standard method (2), no significant differences were found between the calculated and theoretical values of t - test and F- test at 95% confidence limit (Table (8)).

Table (7)
Spectrophotometric determination of ketotifen in pharmaceutical compounds using Ion –Pair Complex Formation.

Ion–Pair Method	Drug Conc. ($\mu\text{g.mL}^{-1}$)		Recovery%	R.S.D.* %
	Taken	Found*		
Zaditen ^R Tablets ^a	2.5	2.485	99.400	1.728
	10	9.925	99.250	1.505
	20	20.301	101.505	1.642
Gloditen Tablets ^b	2.5	2.568	102.720	1.853
	10	10.276	102.760	1.889
	20	20.483	102.415	2.254
Ketonil ^R Tablets ^c	2.5	2.458	98.320	1.888
	10	9.889	98.890	2.517
	20	20.331	101.655	2.412
Ketonil ^R Syrup ^d	2.5	2.558	102.320	1.922
	10	10.264	102.640	2.461
	20	20.451	102.255	2.333

*Average of five determinations.

a Ketotifen 1mg / tablet Novartis/Swiss.

b Ketotifen (as fumarate) 1 mg/ tablet Global/UAE.

c Ketotifen fumarate (equivalent to 1 mg Ketotifen/ tablet) RAM/ Jordan.

d Ketotifen fumarate (equivalent to 1 mg Ketotifen / 5 mL) RAM/ Jordan.

Table (8)
t- and F-values for analysis of Ketotifen in pharmaceutical compounds.

Sample no.**	Conc. Taken	Proposed Method		Standard Method		t- Value ^a	F- value ^b
		Conc. Found*	R.S.D. (%)	Conc. Found*	R.S.D. (%)		
1	2.5	2.485	1.728	2.516	1.550	0.756	1.216
2	10	9.925	1.505	10.200	1.216	2.083	1.334
3	20	20.301	1.642	20.242	1.511	0.184	1.234

* Average of five determinations.

** Ketotifen 1mg / tablet Novartis/Swiss.

a - Theoretical value for t-test, for N=2, at 95% confidence limit is (4.303).

b - Theoretical values for F-test, for N=(1.1), at 95% confidence limit is (161.45).

Conclusions

The utility of BTB reagent for the spectrophotometric determination of Ketotifen was established. The method based on ion-pair formation between the cited drug and BTB as a chromogenic reagent. The proposed method was found to be accurate, simple and sensitive. It was satisfactorily applied to the determination of ketotifen in pharmaceutical product samples.

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0.213 مايكروغرام/ مل. أظهرت الدراسة أيضاً أن الطريقة المقترحة خالية من تأثير المتداخلات المعروفة والتي تتواجد عادة في المستحضرات الصيدلانية، وقد أمكن تطبيق الطريقة بنجاح لتقدير الكيتوتيفين في بعض تلك المستحضرات.

الخلاصة

لقد تم استخدام طريقة طيفية لتقدير الكيتوتيفين في عينات نقية وبعض المستحضرات الصيدلانية، اعتماداً على تكوين معقد الأزواج الأيوني. كانت الطريقة دقيقة، بسيطة وحساسة تعتمد بالاساس على استخدام الكلوروفورم في أستخلاص معقد الأزواج الأيوني المتكون بين العقار قيد الدراسة مع الكاشف بروموتايمول الأزرق من وسط مائي ویدالة حامضية 3.5، لقد اظهر المعقد المتكون للكيتوتيفين أعظم امتصاص له عند الطول الموجي 417 نانومتر مقابل محلول الخلب، واظهر منحنى المقايسة علاقة خطية لمدى من التراكيز (0.3 - 25) مايكروغرام / مل ويحد كشف قدره