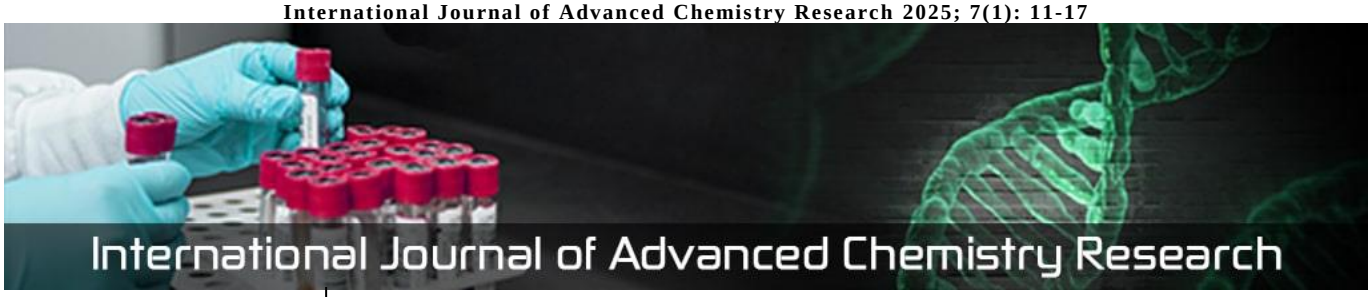

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Development of a sensitive spectrophotometric method for the quantification of ketotifen fumarate in aqueous solutions via diazotization of 4-aminoantipyrene

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Abstract
This study introduces a novel, rapid, and highly sensitive spectrophotometric method for the quantification of Ketotifen fumarate in aqueous solutions. The presented method involves the diazotization of 4-aminoantipyrene utilizing sodium nitrate in an acidic medium, followed by the subsequent coupling of the diazonium compound with Ketotifen in a basic environment. The resultant orange azo dye demonstrates a peak absorbance at 483 nm and possesses solubility and stability in aqueous solution for over 60 minutes. The absorbance of the dye exhibits a linear response within the concentration range of 1–10 µg/mL, with a molar absorptivity of 11,943.84 $L \cdot mol^{-1} \cdot cm^{-1}$ and a Sandell's sensitivity index of 0.0259 µg/mL. The method conveys exceptional accuracy, with results ranging between 98% and 100.16%, and has been successfully utilized for the analysis of pharmaceutical formulations.

Keywords: Spectrophotometry, diazotization reactions, ketotifen fumarate, 4-aminoantipyrene

Introduction
Ketotifen fumarate is characterized by its antihistaminic properties and allergic effects. It is known chemically as 4-(1-methyl-4-piperidinylidene)-4H-benzo-[4,5]-cyclohepta-[1,2-b] thiophene-10-(9H)-one hydrogen fumarate (Fig. 1), with a molecular formula of C19H19NOS. C4H4O4 and a molecular weight of 425.50 g/mol. Ketotifen fumarate appears as a white, odorless crystalline powder [1]. In addition to treating allergic disorders like rhinitis and conjunctivitis, it is used to avoid asthma and alleviate hay fever and urticaria symptoms [2].

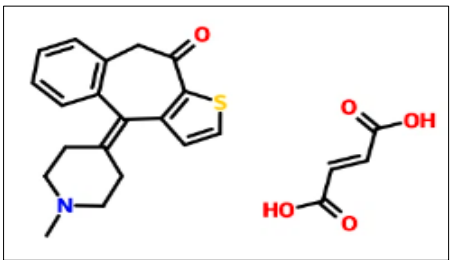


Fig 1: Structure of Ketotifen fumarate

Various methods have been reported for the estimation of ketotifen. Ketotifen fumarate has been measured in liquid pharmaceutical dosage forms using an isocratic reverse-phase high-performance liquid chromatography (HPLC) method [3-6]. Furthermore, potentiometric sensing instruments have been created to detect ketotifen fumarate [7, 8]. Additionally, Nagam S. T. and Manhl H. I. established the determination of ketotifen fumarate in pure and pharmaceutical preparations using turbidimetric measurements (0-180°) using the Ayah

6SX1-T-1D Solar Cell CFI Analyzer [9]. Two spectrophotometric approaches and titration methods have also been described for the determination of ketotifen fumarate [10, 11]. Additional techniques include the use of Nanosensors [15, 16], turbidity measurement [14], and fluorometry [12, 13].

To identify and determine ketotifen hydrogen fumarate, the goal of this study was to create a sensitive, easy, and economical spectrophotometric method based on the diazotization of 4-amino antipyrine using sodium nitrate with a solution of hydrochloric acid that produces diazonium salt.

2. Materials and Methods

Ketotifen fumarate ($C_{23}H_{23}NO_5S$) was obtained from Samarra Drug Industry (SDI) Iraq. Hydrochloric acid (37%), Nitric acid HNO_3, Sulfuric acid, Phosphoric acid, Acetic acid, Potassium hydroxide, Sodium hydroxide, Calcium hydroxide and Sodium carbonate were obtained from Merck company. Sodium bicarbonate was obtained from B.D.H company.

Shimadzu UV Spectrophotometer (UV-1800) Japanese made with 1 cm quartz cells, KERN ABS 120 -4N sensitive balance Singapore made.

2.1 Standard Drug Solutions: The standard stock solution of Ketotifen (100 ppm) was prepared by dissolving 0.0138 g of Ketotifen fumarate in 100 mL of distilled water. Subsequently, a range of concentrations (1–27 µg/mL) was prepared by performing serial dilutions.

2.2 Reagents and Reduced Solutions

The reagent 4-aminoantipyrine and the reducing agent sodium nitrite solutions were prepared by dissolving 0.2 g and 0.069 g, respectively, in 100 mL of distilled water to obtain a concentration of 0.01 M. The solutions were stored in a dark bottle to prevent degradation.

2.3 Base Solutions

The solutions of bases, including sodium hydroxide, potassium hydroxide, calcium hydroxide, sodium bicarbonate, and sodium hydrogen carbonate, were prepared at 1 M by weighing an appropriate amount of each base and dissolving it in 100 mL of distilled water.

2.4 Acid Solutions

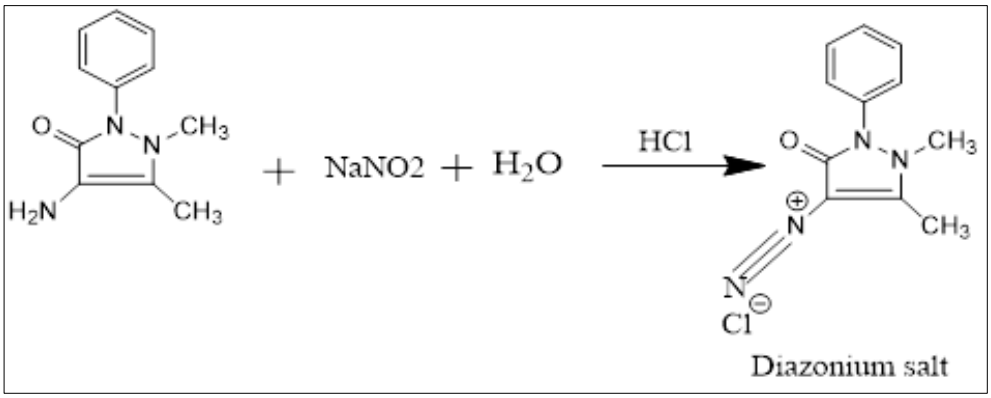
Acid solutions (hydrochloric acid, nitric acid, phosphoric acid, sulfuric acid, and acetic acid) were prepared at a concentration of 0.1 M by diluting the appropriate volumes in 100 mL of distilled water.

3. Results and Discussion

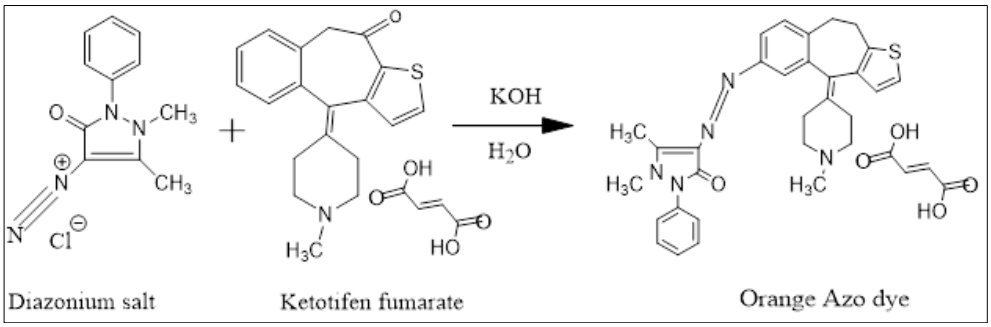
For the subsequent experiments, 100 µg/mL of Ketotifen was prepared in a final volume of 25 mL. The absorbance was measured at a wavelength of 483 nm.

3.1 Principle of the method

This method involves the diazotization of 4-aminoantipyrine using sodium nitrate in the presence of hydrochloric acid to produce a diazonium salt:



The resulting diazonium salt then couples with Ketotifen under basic conditions using potassium hydroxide, resulting in the formation of an orange dye.



3.2 Study of the optimum reaction conditions

To find the ideal circumstances for producing a pigment with the highest color intensity, a number of characteristics were measured.

3.3 Study of selecting the best acid

Both weak and strong acids were tested in order to determine which was best for the diazotization process. The best color intensity was obtained when 1 mL of 0.1 M

hydrochloric acid (HCl) was tested. Consequently, HCl was selected as the best acid for this study.

Table 1: The Best Acid for the Method

Acid used 0.1 M	Absorbance	Color
HCl	0.303	Bright orange color
H_2SO_4	0.013	Very light-yellow color
H_3PO_4	0.102	Very light-yellow color
HNO_3	0.040	Very light-yellow color
CH_3COOH	0.043	Very light-yellow color

3.4 Study of selecting the best volume of HCL

Increasing quantities of 0.1 M hydrochloric acid ranging from 0.1 to 1.4 mL were tried in order to determine the ideal hydrochloric acid volume for the diazotization process.

Table 2: The Best Volume of 0.1 M HCl

Volumes of HCl 0.1 M	Absorbance	Color
0.1 mL	0.087	Colorless
0.2 mL	0.183	Colorless
0.3 mL	0.177	Colorless
0.4 mL	0.149	Very light-yellow color
0.5 mL	0.032	Very light orange color
0.6 mL	0.250	Very light orange color
0.7 mL	0.345	Very light orange color
0.8 mL	0.132	Very light orange color
0.9 mL	0.390	Dark orange color
1 mL	0.411	Very light orange color
1.2 mL	0.555	Very light orange color
1.4 mL	0.683	Very light orange color

3.5 Study of selecting the best base

The optimal base for the diazotization process was determined by examining both weak and strong bases. Since 1 mL of 1 M potassium hydroxide (KOH) displayed the strongest color intensity, it was selected as the most suitable base for this investigation.

Table 3: The Best Base for the Method

Base used 1 M	Absorbance	Color
NaOH	0.081	Colorless
KOH	0.515	Bright orange color
Ca $(OH)_2$	0.044	Very light-yellow color
Na_2CO_3	0.075	Very light-yellow color
$NaHCO_3$	0.064	Very light-yellow color

3.6 Study of selecting the best volume of KOH

To adjudge the optimal volume of potassium hydroxide (KOH) for the diazotization operation, volumes of 1 M KOH were sampled, assorting from 0.1 to 1.4 mL.

Table 4: The Best Volume of 1 M KOH for the Method

Volumes of KOH 1 M	Absorbance	Color
0.1 mL	0.233	Very light orange color
0.2 mL	0.272	Very light orange color
0.3 mL	0.300	Very light orange color
0.4 mL	0.222	Very light orange color
0.5 mL	0.563	Dark orange color
0.6 mL	0.354	Very light orange color
0.7 mL	0.379	Very light orange color
0.8 mL	0.323	Very light orange color
0.9 mL	0.270	Very light orange color
1 mL	0.347	Very light orange color
1.2 mL	0.250	Very light orange color
1.4 mL	0.233	Very light orange color

3.7 Study of selecting the best volume of 4-aminoantipyrine (0.01 M):

To find the optimal volume of 4-aminoantipyrine for the diazotization process, growing volumes of 0.01 M 4-aminoantipyrine, ranging from 0.1 to 1.4 mL, were examined.

Table 5: The Best Volumes of 4-Aminoantipyrine (0.01 M) for the Method

Volumes of 4-aminoantipyrene 0.01 M	Absorbance	Color
0.1 mL	0.072	Very light orange color
0.2 mL	0.129	Very light orange color
0.3 mL	0.148	Very light orange color
0.4 mL	0.179	Dark orange color
0.5 mL	0.108	Very light orange color
0.6 mL	0.113	Very light orange color
0.7 mL	0.049	Very light orange color
0.8 mL	0.126	Very light orange color
0.9 mL	0.163	Very light orange color
1 mL	0.081	Very light orange color
1.2 mL	0.055	Very light orange color
1.4 mL	0.177	Very light orange color

3.8 Study of selecting the best volume of sodium nitrate (0.01 M):

To decide the ideal volume of sodium nitrate utilized as the diazotization specialist, expanding volumes of 0.01 M sodium nitrate (NaNO2) were tried, going from 0.1 to 1.4 mL.

Table 6: The Best Volumes of $NaNO_2$ (0.01 M) for the Method

Volumes of $NaNO_2$ 0.01 M	Absorbance	Color
0.1 mL	0.050	Very light orange color
0.2 mL	0.043	Very light orange color
0.3 mL	0.066	Very light orange color
0.4 mL	0.118	Very light orange color
0.5 mL	0.122	Very light orange color
0.6 mL	0.186	Very light orange color
0.7 mL	0.180	Very light orange color
0.8 mL	0.236	Dark orange color
0.9 mL	0.211	Very light orange color
1 mL	0.182	Very light orange color
1.2 mL	0.149	Very light orange color
1.4 mL	0.209	Very light orange color

3.9 Study of the effect of time on absorbance

The previously determined ideal conditions were used to determine the ideal reaction time for the diazotization process. The resulting diazonium salt maintained its stability for more than 60 minutes, during which time the orange dye's absorbance didn't change.

Table 7: The Optimal Time for the Method

Time (minutes)	Absorbance	Color
5	0.134	Orange color
10	0.238	Orange color
15	0.319	Orange color
20	0.300	Orange color
25	0.229	Orange color
30	0.152	Orange color
35	0.166	Orange color
40	0.112	Orange color
45	0.109	Orange color
50	0.107	Orange color
55	0.098	Orange color
60	0.092	Orange color
65	0.089	Orange color
70	0.088	Orange color

3.10 study of the addition sequence on the results: The table below provides an overview of the various addition sequences that were assessed during the diazotization and coupling processes:

Table 8: The Best Order of Addition for the Method

Order of addition	Absorbance
Reagent +$NaNO_2$ + HCl +KET+KOH	0.309
$NaNO_2$ +Reagent +HCl +KET+ KOH	0.179
$NaNO_2$ +HCl+ Reagent+ KET+ KOH	0.241
KET +Reagent+$NaNO_2$ +HCl+ KOH	0.181
KET+ Reagent +HCl+NaNO2 + KOH	0.069
HCl + Reagent+$NaNO_2$+KOH+ KET	0.141
HCl+ KET + Reagent + $NaNO_2$ + KOH	0.122

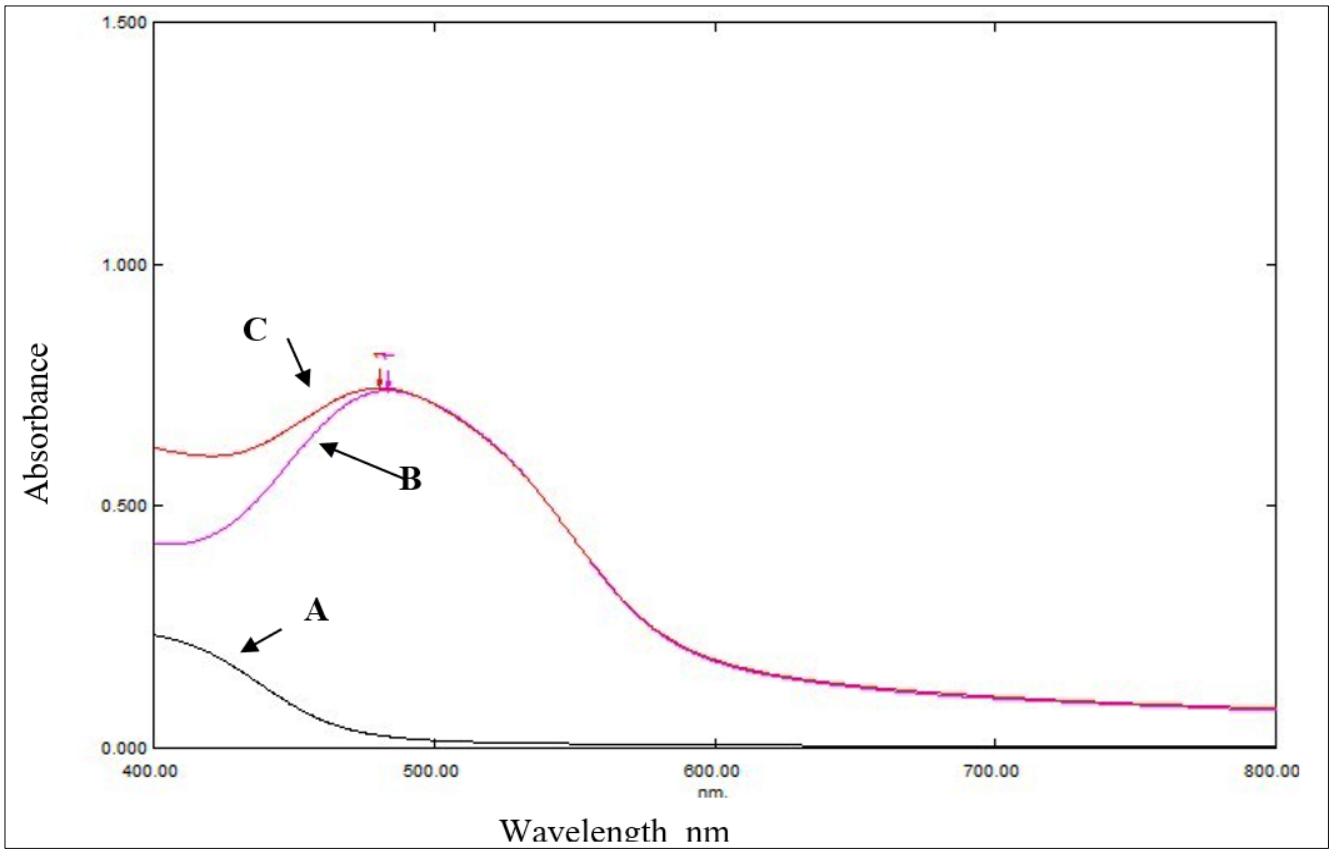
From the sequences tested, it was determined that adding the diazotization agent first resulted in the fastest reaction. This was followed by the addition of acid, then the Ketotifen drug, and finally potassium hydroxide. The first sequence was selected as it yielded the highest absorbance and color intensity.

Table 9. Summary of Ideal Conditions for Ketotifen

Drug Compound	λ max	Temp (°c)	Stability period (min)	Reagent Vol	NaNO2 Vol.	NaOH 1M Volume	HCl 0.1M Volume	Suitable solvent
Ket	483 nm	25 ˚C	15 min	0.4 mL	0.8 mL	0.5 mL	0.9 mL	Distilled Water

3.11 Final absorption spectra

The absorption spectrum generated by the orange dye exhibited the highest absorbance at 483 nm, measured against a blank. The orange dye formed from the coupling of the diazonium salt with Ketotifen in a basic medium. The blank, measured against distilled water, showed absorbance at 316 nm, as depicted in the figure below.



A: Blank against distilled water B: Orange dye against distilled water C: Orange dye against blank

Fig 1: Absorption Spectrum of Ketotifen at 483 nm by UV-Visible Technique

3.12 Procedure and calibration graph

To establish the calibration graph, 25-mL volumetric flasks were used. To each flask, 0.4 mL of 0.01 M 4-amino antipyrine reagent was added. Following this, equal volumes of the diazotized agent (0.01 M $NaNO_2$) were added to each flask, which were then shaken thoroughly. A series of escalating amounts of Ketotifen, ranging from 0.2 to 7 mL (1 to 27 µg/mL), were then added to each flask after 0.9 mL of 0.1 M HCl. Following the addition of 0.5 mL of 1 M potassium hydroxide to each solution, the flasks were well shaken. After filling the volumes to the proper level with distilled water, they were left to stand for fifteen minutes. At 483 nm, the solutions' absorbance was measured in comparison to the blank. The calibration graph showed an apparent molar absorptivity of 11,943.8436 L/mol.cm and linearity limits ranging from 1 to 10 µg/mL.

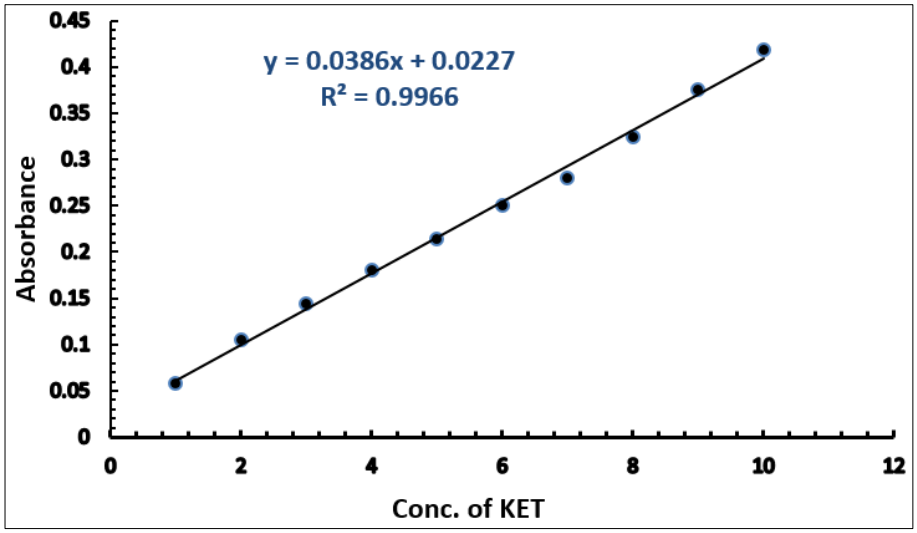


Fig 2: The Calibration Curve of Ketotifen (KET) at 483 nm

Table 10: Calibration Graphs for the Determination of Ketotifen by This Method

Drug	Slope	The calibration coefficient R^2	Linearity range (µg/mL)	Molar absorptivity (L/mol.cm)	LOD (µg/mL)	LOQ (µg/mL)	Sandel Index (µg/mL)
KET	0.0386	0.9966	1-10	11943.8436	0.2689	0.8963	0.0259

3.13 Accuracy and Precision

To assess the accuracy and precision of the proposed method within the linearity limits, Ketotifen (KET) was quantified at three different concentrations. The results indicated that the method is both highly accurate and precise.

Table 11: Recovery, Average Percentage, and Precision of the Method

Amount taken µg/mL	Abs	Amount found µg/mL	Rec%	Avg of Rec%	RSD
3	0.134	2.88	96%	98%	1.94%
3	0.137	2.96	98.7%		
3	0.139	3.01	100%		
4	0.181	4.01	102%	100.16%	1.751%
4	0.177	3.99	99.9%		
4	0.175	3.94	98.9%		
5	0.214	4.95	99%	99%	1.44%
5	0.212	4.90	98%		
5	0.218	5.05	101%		

3.14 Nature of dye: To investigate the nature of the association between Ketotifen and the resulting diazonium salt, Job's method was employed. The results confirmed a 1:1 ratio for the formation of the orange azo dye.

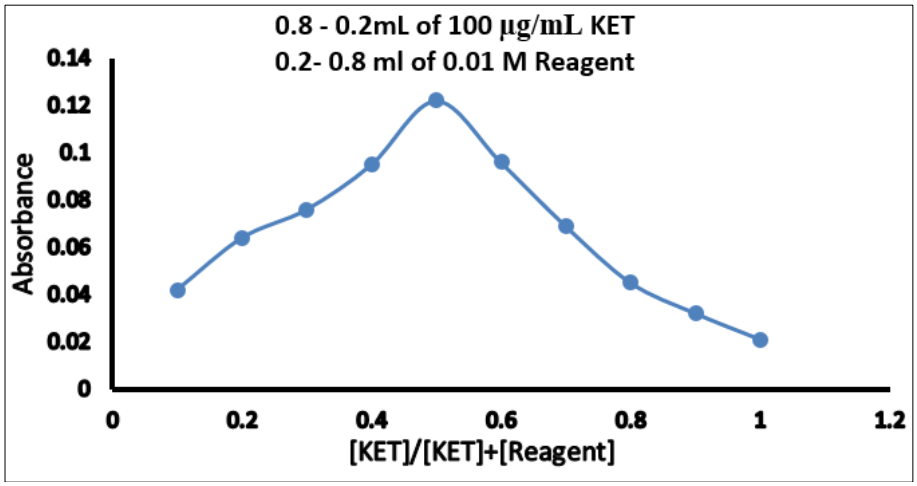


Fig 3: Application of Job's Method

The proposed formula is as follows

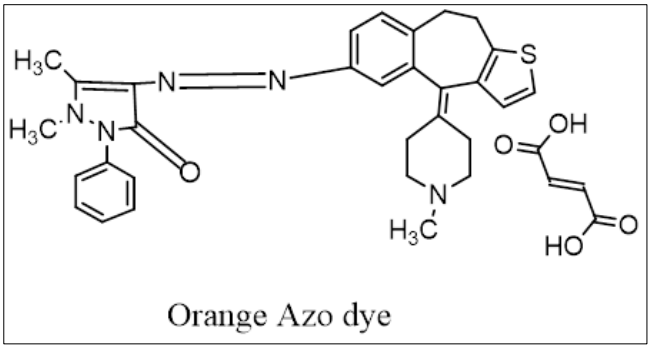


Fig 4: Orange Azo dye

3.15 The effect of interference

The impact of certain interfering compounds on the drug formula was studied by adding varying amounts to a solution containing 100 µg/mL of Ketotifen in 25 mL volumetric flasks.

Table 12: Summary of the Effect of Excipients

Interferences	Conc. Taken µg/mL	Abs	Conc. Found µg/mL	Rec%
Glucose	3	0.140	3.03	101%
	5	0.220	5.111	102%
	7	0.288	6.87	98%
Lactose	3	0.145	3.16	105%
	5	0.225	5.2	104%
	7	0.282	6.71	95.8%
Sucrose	3	0.135	2.9	96.97%
	5	0.223	5.1	103%
	7	0.285	6.79	97%
Starch	3	0.142	3.69	103%
	5	0.218	5.05	101%
	7	0.284	6.76	96%

The results clearly demonstrate that the excipients do not significantly affect the absorption of Ketotifen using the proposed method.

Table 13: Pharmaceutical Preparations Used, Their Concentration, and Origin

Drug	Pharmaceutical content	Concentration	Company
Ketotifen	Astomed	1 mg	Medico Labs.Homs – Syrya

4. Application of the method on pharmaceutical dosage

The proposed method was successfully applied to a pharmaceutical preparation of Ketotifen. Five grains of the drug were accurately weighed, ground, and filtered. A solution of 50 µg/mL pharmaceutical preparation was prepared in a 50 mL volumetric flask and brought to volume with distilled water. The results are presented in the table below.

Table 14: Pharmaceutical Application Dosage Used

Amount taken	Amount taken		Rec%	Avg. of Rec%
	Abs	Conc		
3	0.138	0.298	99.5%	100.9%
4	0.181	4.1	102%	
5	0.211	4.87	97%	
6	0.265	6.27	104%	
7	0.300	7.2	102%	

5. Conclusion

The diazotization and conjugation method were successfully adapted for the UV-Vis technique to quantify Ketotifen. This method demonstrated a linear relationship in the concentration range of 1-10 µg/mL, with a molar absorptivity of 11,943.8436 L/mol.cm and a Sandell sensitivity of 0.0259 µg/mL. The method proves effective for the quantification of Ketotifen in pharmaceutical formulations, yielding high accuracy and precision. Additionally, the limits of detection (LOD) and quantification (LOQ) values indicate enhanced sensitivity.

6. Comparison of the Proposed Method with Literature Methods

The method proposed for estimating Ketotifen fumarate was compared with other oxidative coupling reaction methods, and the results are presented in Table 15.

Table 15: This comparison highlights the differences and advantages of the proposed method in relation to those found in the literature.

Analytical Parameter	Literature Method [17]	Literature Method [18]	Present method
Reagent	Acid–dye bromophenol	Bromothymol blue (BTB)	4-amino antipyrine
Beers law	(0.4-16) µg/ml	(0.3-25) µg/ml	(1-10) µg/ml
λ max	413 nm	417nm	483 nm
Molar absorption coefficient	29400 L/mol.cm	15780.93 L/mol.cm	11943.8436 L/mol.cm
Solvent	Chloroform	Chloroform	Distilled water
Recovery	102%	95%	99%
RSD	1.1797	1.469	3.1103
Sandell index	0.01447 µg/ml	0.019608 µg/ml	0.0259 µg/ml
Pharmaceutical preparation	Tablets	Tablets, Syrup	Tablets

7. Acknowledgment

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8. Conflict of Interest

The authors declare that there is no conflict of interest.

9. Funding: None.

10. Ethical Clearance

No laboratory experiments have been conducted on animals.

11. References

1. Cartwright AC. The British pharmacopoeia, 1864 to 2014: medicines, international standards and the state. Routledge; c2016. https://doi.org/10.4324/9781315614182
2. Alwan A, Abou Yousif Z. Iraqi Drug Guide. 1st ed. NBSD, Iraq; c1990. p. 60.

3. Ali I, Alam SD, Raja R, Khan SA, Anjum R, Jain AK, *et al*. Advances in simple and chiral-HPLC methods for antiallergic drugs and chiral recognition mechanism. Analytica. 2023;4(1):66–83. https://doi.org/10.3390/analytica4010007
4. Vegesna S, Prasada Rao CMM. Bio-method development and validation of ketotifen fumarate in rabbit plasma using RP-HPLC. Journal of Advanced Zoology. 2024;45(2):1359-1376. https://doi.org/10.53555/jaz.v45i2.4243
5. Salih MA, Aziz DF, Ali SI. Determination of ketotifen fumarate in syrup dosage form by high-performance liquid chromatography. Kurdistan Journal of Applied Research. 2020;2020:63-72. https://doi.org/10.24017/science.2020.ICHMS2020.7
6. Yousuf M, Ahmad M, Usman M, Naeem M, Khan BA, Khan MK. Development and validation of HPLC method for simultaneous determination of ketotifen fumarate and salbutamol sulfate in bulk and tablet dosage forms. Main Group Chemistry. 2022;21(1):19–27. https://doi.org/10.3233/MGC-210064
7. Issa YM, Khater MM, Mohamed SH. Solid contact sensors for determination of the antihistaminic drug ketotifen fumarate. Sensing in Electroanalysis. 2012;7:373–391. https://hdl.handle.net/10195/58364
8. Zhang X-L, Ma H-Y, Wang Y-N, Wang G, Gao C-Y. Determination of ketotifen fumarate based on the chemiluminescence reaction of luminol with potassium permanganate. Phys Test Chem Anal (Part B: Chem Anal). 2015;7:029. http://dx.doi.org/10.21123/bsj.2019.16.2.0353
9. Al-Awadie NST, Ibraheem MH. Quantitative determination of ketotifen fumarate in the pure form and tablets via the use of turbidity by reaction with cerium (IV) sulfate using AYAH 6SX1-T-1D CFI analyser. International Journal of Research in Pharmacy and Chemistry. 2016;6(3):411–431.
10. Abbas FR. Two derivative spectrophotometric methods for the simultaneous determination of 4-aminoantipyrine in presence of its acidic products. Ibn AL-Haitham Journal for Pure and Applied Sciences. 2018;31(2):86–96. https://doi.org/10.30526/31.2.1960
11. Raghu MS, Basavaiah K, Prashanth KN, Vinay KB. Titrimetric and spectrophotometric methods for the assay of ketotifen using cerium (IV) and two reagents. International Journal of Analytical Chemistry. 2013;2013:697-651. https://doi.org/10.1155/2013/697651
12. Turkie NS, Munshid HQ. New fluorometric method for the determination of ketotifen fumarate using continuous flow injection analysis via ISNAG-fluorimeter. Baghdad Science Journal. 2019;16(2):353-360. http://dx.doi.org/10.21123/bsj.2019.16.2.0353
13. Al-Banaa MAK. New mode for the determination of ketotifen fumarate using low-pressure mercury lamp via continuous flow injection analysis. Systematic Reviews in Pharmacy. 2020;11(2):58. https://doi.org/10.5530/srp.2020.2.58
14. Al-Awadie NST, Saleem AJ. New turbidimetric method for determination of ketotifen fumarate in pharmaceutical drugs using line-continuous flow injection manifold design of KTF-ammonium molybdate (Am) system. International Journal of Pharmaceutical Research. 2020;12(2):395–401. https://doi.org/10.31838/ijpr/2020.12.02.0046
15. Saad AM, El-Deen AK, Nasr JJM. Carbon dots-adorned silver nanoparticles as a straightforward sustainable colorimetric sensor for the rapid detection of ketotifen in eye drops and aqueous humor. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2024;314:124204. https://doi.org/10.1016/j.saa.2024.124204
16. Sumar MO, Samadi N, Irandoust M, Narimani S. One-step green synthesis of highly luminescent carbon dots from beef and application as selective and sensitive nanosensor for ketotifen fumarate detection in human serum and urine samples. Analytical and Bioanalytical Chemistry Research. 2022;9(1):123-132. https://doi.org/10.22036/abcr.2021.296649.1659
17. Al-Khazrajy O. Spectrophotometric determination of ketotifen fumarate in pure and pharmaceutical preparations by bromophenol blue reagent. Ibn AL-Haitham Journal for Pure and Applied Sciences. 2016;24(3):827.
18. Mahmood AK. Spectrophotometric determination of ketotifen drug in pure form and pharmaceutical preparation using bromothymol blue via ion-pair formation. Al-Nahrain Journal of Science. 2013;16(4):1-10. https://doi.org/10.22401/JNUS.16.4.01