

Quantitative Spectrophotometric Estimation of Dapoxetine Hydrochloride in Pure Form and Pharmaceutical Formulations Using MBTH Reagent

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Abstract

A novel simple, selective, rapid and cost effective visible spectrophotometric method was established for the determination of dapoxetine hydrochloride in bulk and in tablet dosage forms. This technique works on the principle of the oxidation of Dapoxetine HCl with acid in presence of 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) through mediation by ferric ions in weak acidic medium formed by HCl. It forms a stable blue-violet colored compound with wavelength maxima at 612 nm. The factors influencing color development were systematically optimized including the type of acidic medium, the volume of acid, the oxidant, the volume of oxidant, the coupling reagent, the volume of reagent, the order of addition, the reaction time and the temperature. Hydrochloric acid, ferric chloride, and MBTH gave the highly analytical response among the tested variables. Under the optimized conditions, Beer's law is obeyed over the concentration range of 2.0–20.0 $\mu\text{g mL}^{-1}$, with the regression equation $A = 0.0489C + 0.0618$ and a coefficient of determination of 0.9983. The limit of detection and limit of quantification are 0.171 and 0.518 $\mu\text{g mL}^{-1}$, respectively. The method showed best accuracy and precision, with recoveries close to 100% and relative standard deviation values below 2%. The proposed method is successfully applied to the determination of dapoxetine hydrochloride in commercial tablet formulations without significant interference from common excipients. Therefore, the procedure is suitable as the simple visible spectrophotometric alternative for routine pharmaceutical quality-control analysis.

Keywords: Dapoxetine Hydrochloride; Oxidative Coupling; MBTH; Pharmaceutical Analysis; Spectrophotometry.

1. Introduction

Dapoxetine hydrochloride is a selective serotonin reuptake inhibitor used in pharmaceutical dosage forms as a short-acting therapeutic agent. Chemically, the dapoxetine hydrochloride compound can be described as a naphthyloxy phenylpropylamine analog whose molecular formula is represented as $\text{C}_{21}\text{H}_{24}\text{ClNO}$ and whose molecular mass is 341.9 g mol^{-1} [1]. The presence of tertiary amine group, naphthoxy group, and aromatic rings in the chemical structure can account for some of its spectral and oxidation properties [1]. Dapoxetine is listed in drug databases as a hydrochloride salt used in marketed pharmaceutical formulations, and its analytical determination remains important for quality-control laboratories [2]. Several analytical methods have been reported for dapoxetine hydrochloride in pharmaceutical and biological samples. High-performance liquid chromatography coupled with tandem mass spectrometry has been applied for the determination of dapoxetine hydrochloride in human plasma and bioequivalence studies [3]. LC-MS/MS methods have also been used for simultaneous analysis of dapoxetine with other drugs in biological matrices [4]. Other bioanalytical and separation approaches, including mass spectrometric and capillary electrophoresis methods, have also been described for dapoxetine determination [5,6]. Chromatographic procedures, including HPLC-UV and stability-indicating HPLC methods, have been reported for dapoxetine determination in pharmaceutical dosage forms and impurity studies [7,8]. HPTLC methods have also been used for quantitative determination of dapoxetine either alone

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or in combination with other drugs [9]. Spectrophotometric methods remain attractive for routine pharmaceutical analysis because they are simple, rapid, inexpensive, and suitable for laboratories that do not have access to advanced chromatographic or mass spectrometric instruments [10–12]. However, direct UV methods may suffer from low selectivity when excipients or co-formulated drugs absorb in the same ultraviolet region. Therefore, visible spectrophotometric methods based on chromogenic reactions may provide better selectivity and easier visual monitoring. Oxidative coupling reactions are widely used in pharmaceutical analysis because they convert weakly absorbing drug molecules into intensely colored products measurable in the visible region [13–15]. In such reactions, an oxidizing agent activates the coupling reagent or the analyte to generate a reactive intermediate, which then reacts with the drug to form a colored chromogen. MBTH is one of the useful chromogenic reagents in oxidative coupling reactions because it can produce colored products with several pharmaceutical compounds under suitable oxidation conditions [13–15]. Recent studies have also expanded the analytical monitoring of dapoxetine using electrochemical and sensor-based approaches, including nanomaterial-modified electrodes, paper-based microfluidic sensing, and electrochemical platforms for drug formulations and water samples [16–19]. These investigations substantiate the persistent vigilance of analysis towards dapoxetine and highlight the demand for the development of simple, economical, and accurate spectrophotometric methodologies for its estimation. The present work proposes an acidic oxidative coupling spectrophotometric method for dapoxetine hydrochloride determination using MBTH as the coupling reagent, ferric chloride as the oxidizing agent, and hydrochloric acid as the acidic medium. The novelty of the method lies in using an acidic visible oxidative coupling system for dapoxetine hydrochloride with systematic comparison of three acids, three oxidizing agents, and three coupling reagents. The method was optimized and validated according to accepted analytical validation parameters, including linearity, accuracy, precision, detection limit, quantification limit, and pharmaceutical application [20,21].

2. Materials and Methods

2.1. Apparatus

The absorbances were measured with the help of a T92+ double beam UV–Visible spectrophotometer (China) fitted with 1.0 cm matched quartz cuvettes. The control and data analyses were done through the UV Probe 2.34 software running on the Dell computer under Windows 7 (China). Weighing of the reference substance and tablet powder was done with the help of the Sartorius BL210SAG sensitive balance (Germany). Solution acidity was measured using a Jenway 3310 pH meter (China), and heating, when required, was carried out using an HPL-248 heater (China). All glassware was cleaned, rinsed with distilled water, and dried before use. All measurements were performed at room temperature unless otherwise stated.

2.2. Reagents and Chemicals

Dapoxetine hydrochloride standard material was used as the active pharmaceutical ingredient. MBTH, ferric chloride hexahydrate, potassium periodate, N-bromosuccinimide, hydrochloric acid, sulfuric acid, acetic acid, N,N-dimethyl-p-phenylenediamine, and 4-aminoantipyrine were of analytical grade. Distilled water was used throughout the study. Commercial dapoxetine hydrochloride tablets labeled to contain 30 mg dapoxetine hydrochloride per tablet were used for application studies.

2.3. Preparation of Dapoxetine Hydrochloride (DXH) Stock Solution

A stock solution of dapoxetine hydrochloride, $1000 \mu\text{g mL}^{-1}$, was prepared by dissolving 0.1000 g of pure dapoxetine hydrochloride in a small volume of distilled water containing 1.0 mL of 0.1 M hydrochloric acid, followed by dilution to 100 mL with distilled water in a volumetric flask. A working standard solution of $250 \mu\text{g mL}^{-1}$ was prepared by suitable dilution of the stock solution with distilled water.

2.4. Preparation of 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) Solution

A 1.0×10^{-3} M MBTH solution was prepared by dissolving the appropriate amount of MBTH in distilled water and diluting to 100 mL in a volumetric flask. The solution was freshly prepared and protected from direct light.

2.5. Preparation of Ferric Chloride Solution

A 5.0×10^{-3} M ferric chloride solution was prepared by dissolving the required amount of ferric chloride hexahydrate in distilled water and diluting to 100 mL. The solution was freshly prepared before use.

2.6. Preparation of Acidic Media

Hydrochloric acid, sulfuric acid, and acetic acid solutions were prepared at a concentration of 0.1 M and tested as acidic media for the oxidative coupling reaction.

2.7. Tablet Sample Preparation

The tablets were precisely weighed and reduced to powder form. A measured amount of the powder equivalent to 30.0 mg of dapoxetine hydrochloride was weighed and dissolved in a 100 mL volumetric flask. Distilled water along with 1.0 mL of 0.1M Hydrochloric acid up to 50 mL was added into the flask and mixed for 15 minutes. The mixture was then filtered through the Whatman Filter paper, and the filtrate was diluted to the mark. The dilutions were done in order to attain concentrations within the calibration range.

2.8. General Analytical Procedure

Aliquots of DXH working solution within the final concentration range of 2.0 – 20.0 $\mu\text{g mL}^{-1}$ were pipetted into a set of 25 mL volumetric flasks. To each flask 1.0 mL 0.1 M HCl, 1.0 mL ferric chloride solution and 1.0 mL MBTH solution were added in the best sequence. The solutions were combined and let them stand at 25 °C for 10 min. Then, the volume was made up to the mark with distilled water. The absorbance of the produced bluish violet chromogen was read at 612 nm against a reagent blank which was prepared in the same manner but without the drug.

3. Results and Discussion

3.1. Analytical Principle of the Method

The methodology is based on a tentative oxidative coupling in acidic medium. Ferric chloride is an oxidant and could oxidize MBTH to an electrophilic reactive intermediate, which couples immediately with DXH to yield a bluish-violet chromogen. The resulting product exhibits maximum absorption at 612 nm. The depth of the color was found to increase linearly with dapoxetine concentration in the investigated linear range enabling its quantitation by visible spectrophotometry.

Table 1 Selection of the Acidic Medium

Acidic medium	Concentration	λ_{max} (nm)	Absorbance
Hydrochloric acid	0.1 M	612	0.608
Sulfuric acid	0.1 M	478	0.465
Acetic acid	0.1 M	387	0.305

Hydrochloric acid showed the maximum absorbance and also the color was more clear bluish violet. Sulfuric acid resulted in a satisfactory response with an acceptable absorbance but it was lower than that of the hydrochloric acid. Acetic acid gave a weak response, probably due to insufficient acidity and less efficient oxidative coupling. Therefore, hydrochloric acid was selected as the optimum acidic medium.

Table 2 Effect of Hydrochloric Acid Volume

HCl volume (mL)	Approximate pH	Absorbance at 612 nm
0.3	3.10	0.426
0.5	2.75	0.498
0.8	2.42	0.581
1.0	2.25	0.609
1.3	2.05	0.590
1.5	1.90	0.563
2.0	1.65	0.512

The absorbance increased with increasing acid volume up to 1.0 mL. Higher acid volumes decreased the response, possibly due to excessive protonation of reactive sites or decreased coupling efficiency. Therefore, 1.0 mL of 0.1 M HCl was selected.

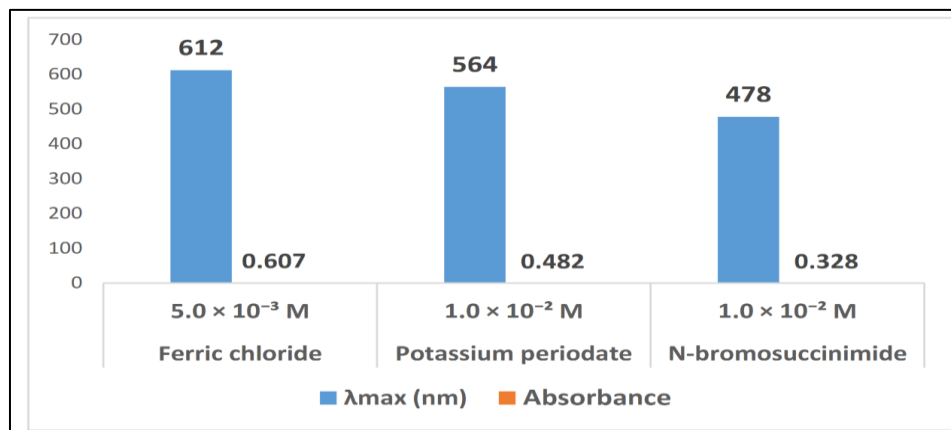


Figure 1 Selection of Oxidizing Agent

Ferric chloride produced the highest and most stable absorbance. Potassium periodate gave an acceptable but lower response, while N-bromosuccinimide produced a weak color with lower stability. Therefore, ferric chloride was selected as the best oxidizing agent.

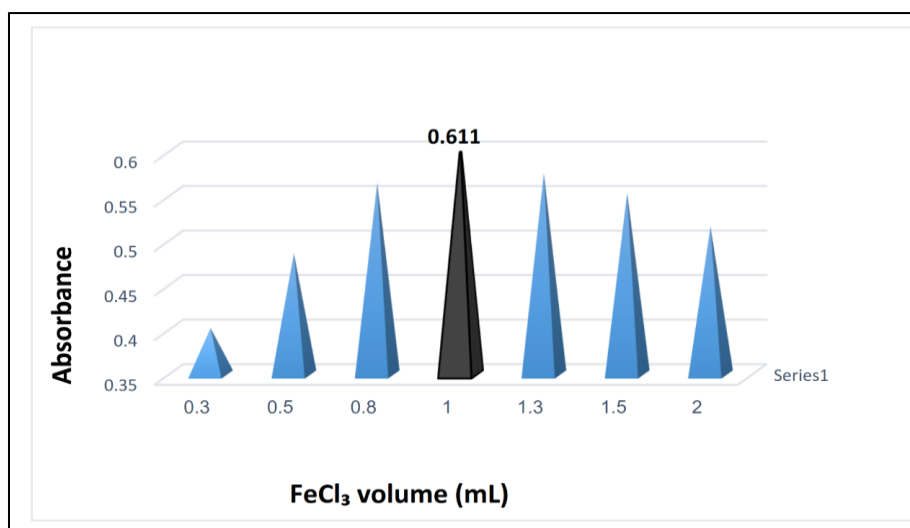


Figure 2 Effect of Ferric Chloride Volume

The absorbance reached a maximum at 1.0 mL of ferric chloride solution. Further increase in oxidant volume decreased the absorbance, probably due to over-oxidation of the chromogenic product. Therefore, 1.0 mL was selected.

Table 3 Selection of Coupling Reagent

Coupling reagent	Concentration	λ_{max} (nm)	Absorbance
MBTH	1.0×10^{-3} M	612	0.609
N,N-dimethyl-p-phenylenediamine	1.0×10^{-3} M	485	0.438
4-aminoantipyrine	1.0×10^{-3} M	423	0.282

MBTH was the best coupling reagent, giving the highest absorbance and better color stability. N,N-dimethyl-p-phenylenediamine gave an acceptable response, while 4-aminoantipyrine gave a weak response under acidic conditions. Therefore, MBTH was selected.

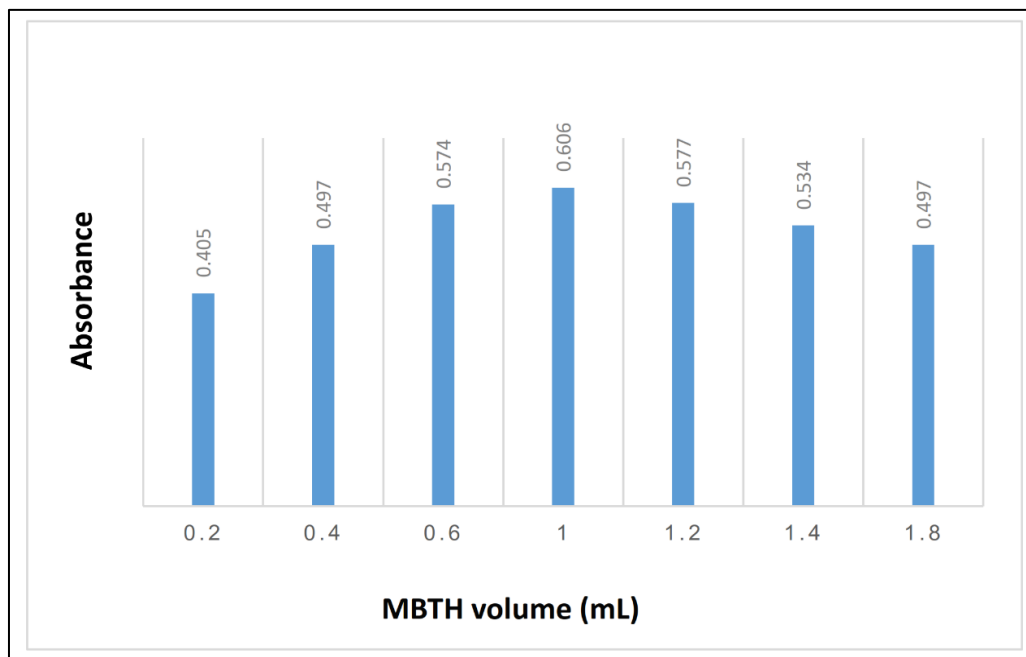


Figure 3 Effect of MBTH Volume

The maximum absorbance was obtained with 1.0 mL of MBTH solution. Larger volumes caused a slight decrease in absorbance, possibly due to increased blank response. Therefore, 1.0 mL was selected.

Table 4 Effect of Order of Addition

Order No.	Order of addition	Absorbance
1	D + HCl + FeCl ₃ + MBTH	0.608
2	D + MBTH + FeCl ₃ + HCl	0.545
3	D + FeCl ₃ + MBTH + HCl	0.566
4	MBTH + FeCl ₃ + D + HCl	0.520
5	HCl + MBTH + FeCl ₃ + D	0.493
6	FeCl ₃ + HCl + D + MBTH	0.558

D = dapoxetine hydrochloride(DXH).

The best absorbance was obtained when DXH was first mixed with hydrochloric acid, followed by ferric chloride and then MBTH. This order was adopted in the final procedure.

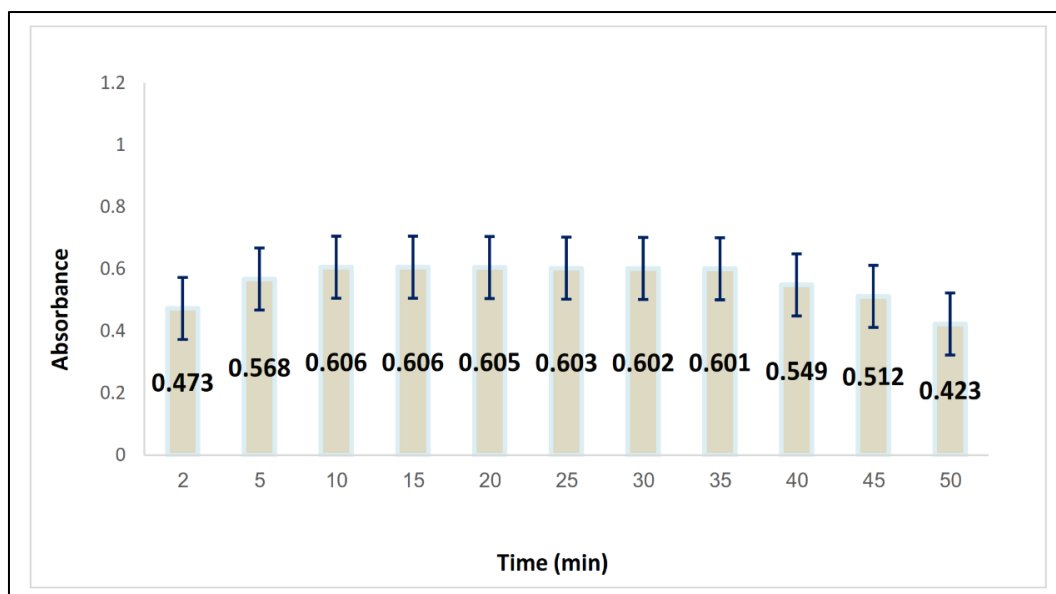


Figure 4 Effect of Reaction Time

The absorbance increased rapidly up to 10 min and then remained nearly constant between 10 and 35 min, indicating acceptable short-term stability of the formed chromogen. At 35 min, the absorbance was gradually reduced. Thus, 10 min was considered as the optimal reaction time for routine determination since a high response was obtained in a short analysis time.

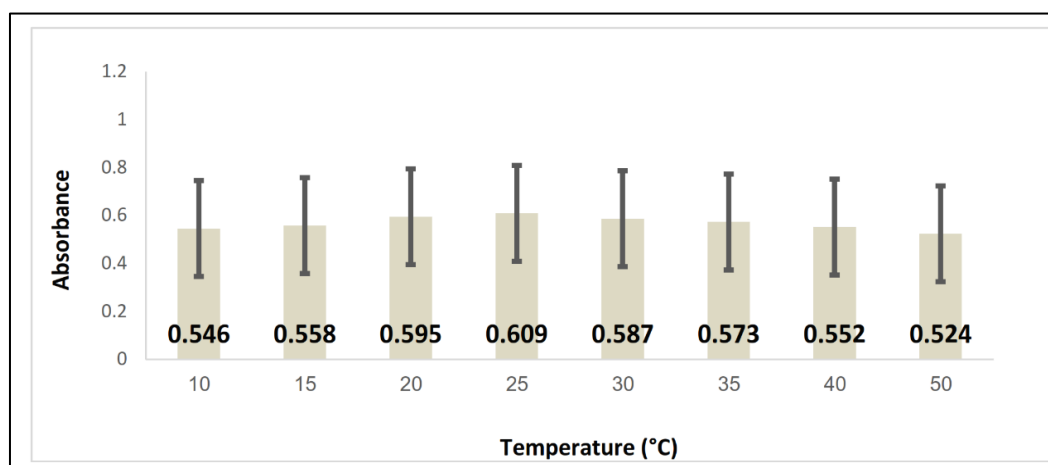


Figure 5 Effect of Temperature

The maximum absorbance was observed at 25 °C. Increasing the temperature led to a gradual decline of the absorbance, which could be attributed to an instability of the colored product, at least in part. Thus, the room temperature was chosen.

Table 5 Summary of Optimum Experimental Conditions

Parameter	Optimum condition
Drug	Dapoxetine hydrochloride
Reaction type	Acidic oxidative coupling
Acidic medium	HCl
HCl volume	1.0 mL

Oxidizing agent	FeCl ₃
FeCl ₃ volume	1.0 mL
Coupling reagent	MBTH
MBTH volume	1.0 mL
λ_{max}	612 nm
Reaction time	10 min
Temperature	25 °C
Final volume	25 mL
Blank	Reagent blank

3.2. Calibration Curve and Linearity

Equal portions of the standard solution of dapoxetine hydrochloride were processed as per general procedure under the optimized conditions. The reading of absorbance was taken at 612 nm against reagent blank. A good linear correlation was observed between the absorbance and concentration in the range 2.0–20.0 $\mu\text{g mL}^{-1}$.

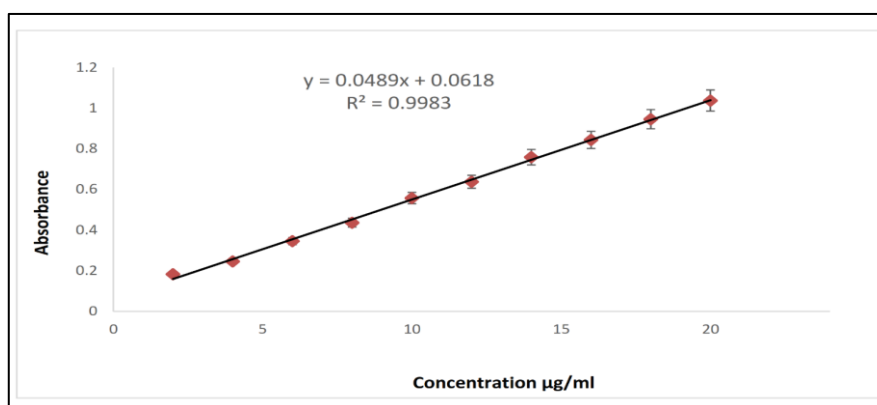


Figure 6 Calibration Data for Dapoxetine Hydrochloride

The regression equation was: $A = 0.0489C + 0.0618$

where A and C are the absorbance and concentration of dapoxetine hydrochloride in $\mu\text{g mL}^{-1}$, respectively. The R^2 was found to be 0.9983 which showed good linearity for the routine application in pharmaceutical.

Table 6 Analytical Parameters of the Proposed Method

Parameter	Value
λ_{max}	612 nm
Linearity range	2.0–20.0 $\mu\text{g mL}^{-1}$
Regression equation	$A = 0.0489C + 0.0618$
Coefficient of determination	0.9983
Molar absorptivity	$1.67 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$
Sandell sensitivity	$0.0204 \mu\text{g cm}^{-2}$
LOD	$0.171 \mu\text{g mL}^{-1}$
LOQ	$0.518 \mu\text{g mL}^{-1}$

3.3. Accuracy and Precision

The accuracy and precision of the method was assessed by testing three different levels of dapoxetine hydrochloride. Each concentration was injected 6 times. The percentage of recovery, relative error and relative standard deviation were calculated.

Table 7 Accuracy and Precision for Dapoxetine Hydrochloride in Pure Form

Taken ($\mu\text{g mL}^{-1}$)	Found ($\mu\text{g mL}^{-1}$)	Recovery (%)	RE (%)	RSD (%)
4.00	4.04	101.00	+1.00	1.32
8.00	7.97	99.63	-0.38	1.18
12.00	12.15	101.25	+1.25	0.92

Obtained recovery values are close to 100%, and the RSD values are below 2%, indicating good accuracy and precision of a proposed method in pure form.

3.4. Job's Method of Continuous Variation

Stoichiometric ratio between DXH and MBTH was investigated use Job's method of continuous variation. Equimolar solutions of DXH and MBTH are mixed in different mole fractions while keep a total molar concentration constant. The maximum absorbance is obtained at the mole fraction of 0.5, indicating a 1:1 ratio between dapoxetine hydrochloride and MBTH under a select reaction conditions.

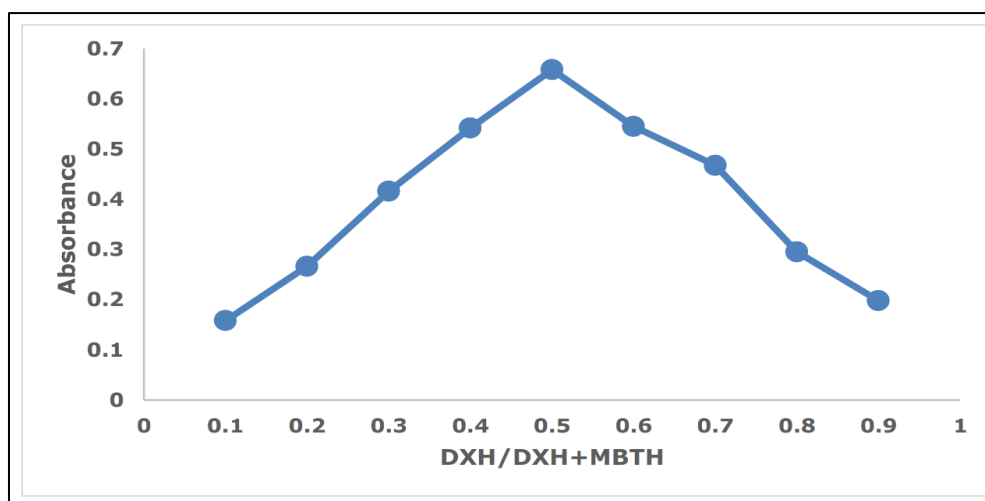


Figure 7 Job's Method for Stoichiometric Ratio Determination

3.5. Application to Pharmaceutical Tablets

The aforementioned technique was successfully applied to the analysis of dapoxetine hydrochloride in tablet dosage forms. Sample solutions were prepared according to the methodology described above and analyzed under optimum conditions.

Table 8 Direct Determination of Dapoxetine Hydrochloride in Tablets

Taken ($\mu\text{g mL}^{-1}$)	Found ($\mu\text{g mL}^{-1}$)	Recovery (%)	RE (%)	RSD (%)
6.00	5.96	99.33	-0.67	1.44
10.00	10.12	101.20	+1.20	1.06
14.00	13.82	98.71	-1.29	0.88

Results show acceptable recovery and precision, indicate that common tablet excipients didn't significantly interfere with a propose method.

3.6. Standard Addition Method

The established spiking method was used to verify the method of determination, and to determine potential interference in tablet excipients. The amount of the added standard recovered was calculated from the difference between the total detected concentration and the concentration of the sample.

Table 9 Standard Addition Results for Dapoxetine Hydrochloride in Tablets

Sample taken ($\mu\text{g mL}^{-1}$)	Standard added ($\mu\text{g mL}^{-1}$)	Total found ($\mu\text{g mL}^{-1}$)	Added recovery (%)	RSD (%)
6.00	4.00	10.03	100.75	0.96
6.00	8.00	13.94	99.25	1.10
6.00	12.00	18.08	100.67	0.84

The recovery values obtained by standard addition were close to 100%, confirming the suitability of the proposed method for tablet analysis and the absence of significant matrix interference.

3.7. Comparison with Reported Methods

The proposed technique was compared with a few existing methods for the determination of DXH. Although chromatography and electrochemical methods are very sensitive, the proposed visible spectrophotometric method is easy to perform and economical enough for routine laboratory use.

Table 10 Comparison of the Proposed Method with Selected Reported Methods

Method	Matrix	Linear range	Detection wavelength / signal	Main advantage	Reference
HPLC-MS/MS	Human plasma	ng mL^{-1} level	MS/MS detection	High sensitivity	[3]
LC-MS/MS	Human plasma	ng mL^{-1} level	MS/MS detection	Bioanalytical application	[4]
UV spectrophotometry	Tablets	$\mu\text{g mL}^{-1}$ level	UV region	Simple but less selective	[10]
HPTLC	Tablets	$\mu\text{g band}^{-1}$ level	Densitometry	Useful for mixtures	[9]
Electrochemical/sensor-based methods	Tablets and water	μM level	Current/opto response	Sensitive but needs electrode or sensor preparation	[16]
Proposed method	Tablets	$2.0\text{--}20.0 \mu\text{g mL}^{-1}$	612 nm	Simple acidic visible method	

4. Discussion

The designed acidic oxidative coupling reaction is the basis for a new simple visible spectrophotometric method for the determination of dapoxetine hydrochloride. The choice of hydrochloric acid as the best acidic medium was significant due to its maximum absorbance and the most stable bluish-violet shade. The feebler response found with acetic acid may be due to lack of sufficient acidity, sulfuric acid on the other hand gave tolerable response but the color intensity

was much less. This indicates that the acidity of the reaction medium has a great influence on the generation and stabilisation of the coloured compound. Ferric chloride among the other tested oxidizing agents yielded the highest peak probably due to its mild oxidation of MBTH in acidic media and it does not over oxidize the resulted chromogen. K periodate exhibited lower absorbance, and N-bromosuccinimide afforded negligible response, which may be due to less selective oxidation or partial reactions. The result of comparing coupling reagents indicated that MBTH was the best reagent for this reaction. An adequate response was observed with N,N-dimethyl-p-phenylenediamine, while under acidic conditions 4-aminoantipyrine was weak, probably due to the superiority of use under alkalinity. The Reaction time study The increase in absorbance was rapid up to 10 min and stable (constant) up to 35 min (after decrease); consequently, 10 min was employed for a fast and sufficiently stable determination. The calibration curve demonstrated satisfactory linearity in the range 2.0–20.0 $\mu\text{g mL}^{-1}$ and the regression equation was $A = 0.0489C + 0.0618$ with $R^2 = 0.9983$. The LOD and LOQ were calculated as $3.3\sigma/S$ and $10\sigma/S$, respectively, where σ is the standard deviation of the blank response and S is the slope of the calibration curve. The accuracy and precision findings reveal that the recoveries were close to 100% and the RSD was less than 2%, which is considered suitable for use in pharmaceutical quality control. The standard addition experiments indicated that usual excipients contained in the matrix of the tablet did not cause any significant interference. In contrast to HPLC-MS/MS and electrochemical techniques, this spectrophotometric method is simpler and more cost-effective technique and does not require utilization of sophisticated instrumentations or complex sample pretreatment. It may not offer best in class potential for bioanalytical or trace environmental analysis, but it is helpful for routine assay of dapoxetine hydrochloride in pharmaceutical dosage forms.

5. Conclusion

A US oxidative coupling reagent was developed in acidic medium for determination of dapoxetine hydrochloride in bulk and pharmaceutical preparations. The described procedure is based on the reaction of dapoxetine hydrochloride with 3-methyl-2-benzothiazolinone hydrazone (MBTH) in the presence of ferric chloride and HCl to produce a bluish violet coloured chromogen which has an absorption maximum at 612 nm. The proposed method was linear in the chosen concentration range with the correlation coefficient of 0.9993. The newly developed and validated procedure was applied for the estimation of venlafaxine in tablet. In this study the reaction parameters for the coupling reaction with three acids, three oxidants and three coupling reagents were examined. The best system composed of HCl, FeCl_3 and MBTH were selected among the different composites tested. The method is inexpensive, simple, and appropriate for routine quality-control analysis of dapoxetine hydrochloride tablets.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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