

Original Article

NOVEL SELECTIVE SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF METHIMAZOLE IN PURE FORM AND IN PHARMACEUTICAL FORMULATION

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Received: 16 Sep 2019, Revised and Accepted: 12 Dec 2019

ABSTRACT

Objective: To develop and validate new, selective spectrophotometric colorimetric analytical methods for the quantification of methimazole in its pure form and in its pharmaceutical preparations.

Methods: Method A is based on the oxidation of methimazole with potassium permanganate in alkaline medium, the manganate ion produced was measured at λ_{\max} = 610 nm. Method B is a kinetic determination of methimazole using fixed-time method based on the oxidation of methimazole using known excess of cerium (IV) nitrate in acidic medium and assessing the unreacted Ce (IV) by adding a fixed amount of methyl orange and measuring the absorbance of the resultant solution at λ_{\max} = 507 nm which is equivalent to the unreacted methyl orange. The reaction conditions and analytical parameters are investigated and optimized. Method validation was carried out according to ICH guidelines in terms of linearity, LOD, LOQ, precision, and accuracy.

Results: Beer's law is obeyed in the range of 1.50–15.00 $\mu\text{g/ml}$ for method A and 0.25–3.00 $\mu\text{g/ml}$ for method B. The developed methods were subjected to the detailed validation procedure. The proposed spectrophotometric methods were applied for the determination of the methimazole in its pure form and in its pharmaceutical formulation. The percentage recoveries were found to be 100.82 % and 99.85 % in the pharmaceutical formulation for the two proposed methods, respectively.

Conclusion: Both developed spectrophotometric methods, considered as green analytical chemistry, were found to be novel, highly selective and can be applied for the quality control of methimazole in its pure form and in its pharmaceutical formulation based on the simplicity, applicability of the parameters, accessibility of the reagents employed and reasonably low time of analysis.

Keywords: Methimazole, Spectrophotometric methods, Potassium permanganate, Cerium (IV) nitrate, Methyl orange

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INTRODUCTION

Methimazole is an imidazole drug used in the management of hyperthyroidism. In the USP, methimazole has been assayed by silver nitrate precipitation method [1]. Various methods based on different analytical procedures have been described in literature for the determination of methimazole including Ultraviolet-Visible (UV-Vis) spectrophotometry [2-7], derivative spectrophotometry [8], Raman spectroscopy [9, 10], resonance light scattering spectroscopy [11], chemiluminescence [12-14], fluorescence [15, 16], capillary electrophoresis [17], immunoassay [18], molecularly imprinted biomimetic sensing [19], Thin Layer Chromatography (TLC) [20] and High Performance Liquid Chromatography (HPLC) [21-25]. Methimazole has been also determined using electrochemical methods by using different chemically modified electrodes through introducing various electron mediators on its surface [26-33].

The UV-Vis spectrophotometer is a simple, low-cost instrument. From literature, there are no visible spectrophotometric methods reported for the determination of methimazole using Potassium permanganate nor Cerium (IV) nitrate which encouraged us to develop these methods. In alkaline medium, potassium permanganate is reduced to manganate on which absorbs maximally at 610 nm, whose absorbance is proportional to the concentration of the drug under study [34-36]. However, in acidic medium Cerium (IV) nitrate is one of the most important oxidants. The use of cerium (IV) as an oxidant is either based on the decrease in its yellow colour or indirect measurement of excess cerium (IV) by a reaction with a colour developing dye or reagent. The first method being measured in the UV-region gives rise for several interferences by UV interfering molecules while adopting the second method and measuring in the visible region eliminates such interferences [37-40].

The aim of the present work was to report new, simple and environment-friendly spectrophotometric methods for the

determination of methimazole in pure and in pharmaceutical formulations that could be considered superior in comparison with the previously reported methods.

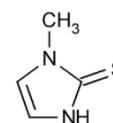


Fig. 1: Structure of methimazole

MATERIALS AND METHODS

Chemicals and materials

Methimazole was certified to contain 98.9 % (Pharmaline, Lebanon).

Tapazole® tablets (Pharmaline, Lebanon), labeled to contain 5 mg methimazole were purchased.

All chemicals and reagents used were of analytical grade; Ammonium Cerium (IV) nitrate (AnalaR), H₂SO₄ (Avonchem, 98 %), KMnO₄ (Fluka), Methyl orange (Aldrich chemicals, dye content ≈ 75 %), NaOH (Scott Science UK, purity 98-99 %), distilled water was produced in-house.

Instrumentation

Jasco V-730 double beam UV-Vis Spectrophotometer interfaced with a computer programmed with Jasco Spectra Manager software that was used for spectrophotometric measurements, spectral acquisition, and elaboration. A pair of 1 cm quartz cells were used to measure the absorption spectra.

Preparation of solutions

Standard stock solution

Methimazole standard stock solution having the concentrations of 500 µg/ml was prepared using distilled water as a solvent.

Working standard solution

A calculated volume of methimazole standard stock solution was diluted with distilled water to obtain a concentration of 50 µg/ml for the working standard solution.

Reagents

Sodium hydroxide solution (NaOH 0.5 M) was prepared by accurately transferring 20g of NaOH into a 1 L volumetric flask. The pellets were dissolved and diluted to volume with distilled water.

Potassium permanganate solution (KMnO₄ 0.005 M) was freshly prepared by accurately transferring 79 mg of KMnO₄ into a 100-mL volumetric flask. The powder was dissolved and diluted to volume with distilled water.

Sulphuric acid solution (H₂SO₄ 0.5 M) was prepared by diluting 27.18 ml of concentrated sulfuric acid (98 %) with distilled water in a 1 L volumetric flask.

Ammonium Cerium (IV) nitrate solution (50 mg %) was freshly prepared by accurately weighing 50 mg of Ammonium Cerium (IV) nitrate. The powder was dissolved and diluted to volume with 0.5 M H₂SO₄ in a 100-mL volumetric flask.

Methyl orange solution (5 mg %) was freshly prepared by accurately weighing 6.7 mg of methyl orange with dye content 75 %. The powder was dissolved and diluted to volume with distilled water in a 100-mL volumetric flask.

Recommended procedure

Method A

Aliquots of methimazole working standard solution were transferred into a series of 10-ml volumetric flasks to cover the concentration range stated in table 5. A volume of 1.5 ml of 0.5 M NaOH followed by 2 ml of 0.005 M KMnO₄ was added to each flask, mixed well and completed to volume with distilled water. The absorbance values of the resultant mixtures were measured at 610 nm after 2 min against a blank prepared similarly.

Method B

Aliquots of methimazole working standard solution were transferred into a series of 10-ml volumetric flasks to cover the concentration range stated in table 5. To each volumetric flask, 1 ml of 50 mg % Ammonium Cerium (IV) nitrate solution was added. The solutions were mixed well and set aside in darkness for 30 min with occasional swirling. Afterward, 1.2 ml of 5 mg % methyl orange solution was added to each volumetric flask. After 5 min, the volume was diluted to the mark with distilled water. The absorbance value of each solution was measured against distilled water as blank at 507 nm.

Application to a pharmaceutical preparation

Ten Tapazole® tablets were accurately weighed and finely powdered. For Method A, a weight equivalent to 5 mg methimazole was dissolved in 10 ml methanol, filtered using Whatman No. 41 filter paper, a volume of 10 ml distilled water was added, the resulting solution was then evaporated using a 70 °C set water bath to a volume of 5 ml. The solution was then quantitatively diluted with distilled water to obtain a solution within the calibration range for the analysis. For Method B, a weight equivalent to 5 mg methimazole was dissolved with distilled water and diluted to 100 ml with distilled water. The obtained solution was then filtered using Whatman No. 41 filter paper. A convenient aliquot was then considered for the analysis using the proposed method. The amount of methimazole present in the tablet was determined by fitting the resulting absorbance value into the calculated regression equation.

Method validation

Under the optimized experimental conditions, the developed methods were validated in accordance to the procedures described in ICH guidelines for the following parameters [41]: Linearity, limit of detection (LOD), limit of quantitation (LOQ), precision and accuracy.

Linearity, concentration range, the limit of detection and limit of quantitation

Linearity was determined in the concentration range of 1.5-15 µg/ml and 0.25-3 µg/ml for methimazole in method A and B respectively.

LOD and LOQ were calculated according to the ICH guidelines by using the following formula [41]:

$$\text{LOD} = 3.3 S_{y/x}/b$$

$$\text{LOQ} = 10 S_{y/x}/b$$

Where " $S_{y/x}$ " is the standard deviation of the regression line/residuals and "b" is the slope of the calibration curve.

Accuracy and precision

In order to assess the accuracy of the proposed methods, the % recovery of methimazole using the standard addition method at three different concentration levels within the linearity range was determined.

The precision of the proposed methods was assessed by intra-day and inter-day variation studies. The precision was expressed in the form of a percentage relative standard deviation (%RSD). During intra-day studies, three sample solutions of each concentration were analysed on the same day whereas inter-day studies were determined by analysing three sample solutions of each concentration for three consecutive days.

RESULTS AND DISCUSSION

Method A

The oxidation reaction between methimazole and KMnO₄ in alkaline medium yields the green-colored manganate radical which absorbs maximally at 610 nm (fig. 2).

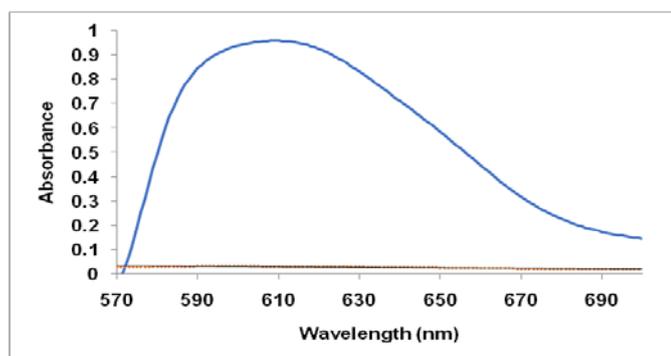


Fig. 2: Absorption spectrum of 15 µg/ml of methimazole with 2 ml 0.005 M KMnO₄ and 1.5 ml 0.5 M NaOH

Optimization of reaction conditions

Experimental conditions affecting color development were optimized by fixing all conditions and changing one variable at a time. It was found that 2 ml of 0.005 M KMnO_4 and 1.5 ml of 0.5 M NaOH were adequate for stable and maximum absorbance.

Mechanism of the reaction

The stoichiometry of the reaction between Methimazole and KMnO_4 in alkaline medium was determined by adopting the limiting logarithmic method [42]. Two sets of experiments were performed. The first set of experiments was carried out using increasing concentrations of methimazole (8.76×10^{-6} – 1.3×10^{-4} M) at fixed permanganate concentration (1.5×10^{-3} M). The second set of

experiments was carried out using increasing concentrations of permanganate (2.5×10^{-4} – 1.5×10^{-3} M) at fixed methimazole concentration (1.3×10^{-4} M). The logarithms of the absorbance values were plotted against the logarithms of the methimazole and KMnO_4 concentration in the first and second set of experiments, respectively. Two straight lines were obtained. The slopes of the fitting lines were calculated (fig. 3).

For the calculation of the ratio of the reaction between Methimazole and KMnO_4 in alkaline medium, the slope of KMnO_4 curve was divided by the slope of the drug curve. It was found that the ratio was 1.439:1.13 pointing out to a ratio of 1:1 (KMnO_4 to Methimazole). Accordingly, the reaction pathway is suggested to proceed as [7]:

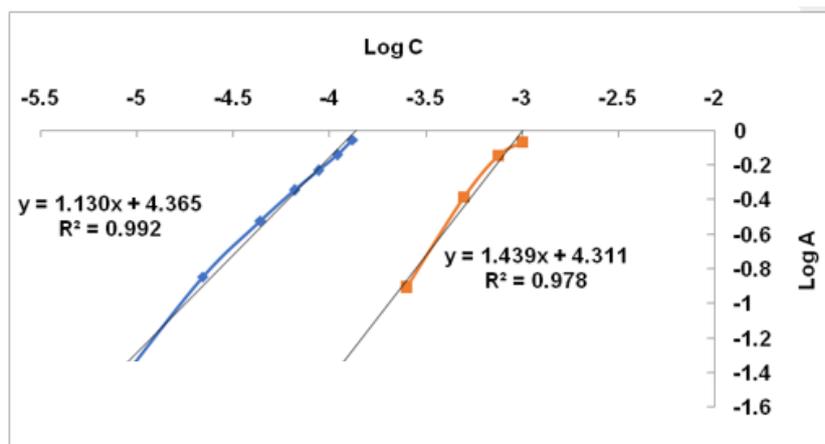


Fig. 3: Limiting logarithmic plot for molar reactivity of methimazole with potassium permanganate

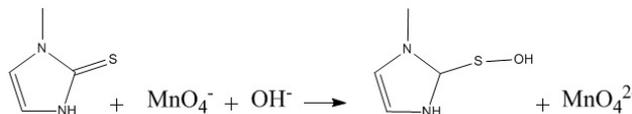


Fig. 4: Suggested mechanism for the oxidation of methimazole by KMnO_4 in alkaline medium

According to the suggested mechanism of oxidation, the method is based on the presence of the thiol group which is oxidized by the $\text{MnO}_4^-/\text{OH}^-$ system, therefore the absence of this group will retard the reaction pathway. So, it can be concluded that this method can be considered as a stability-indicating method for methimazole determination (7).

Kinetics of the reactions

It was evident from fig. 5 that the reaction was rapid and became more or less constant after 2 min pointing out to the difficulty to determine the kinetics of the reaction.

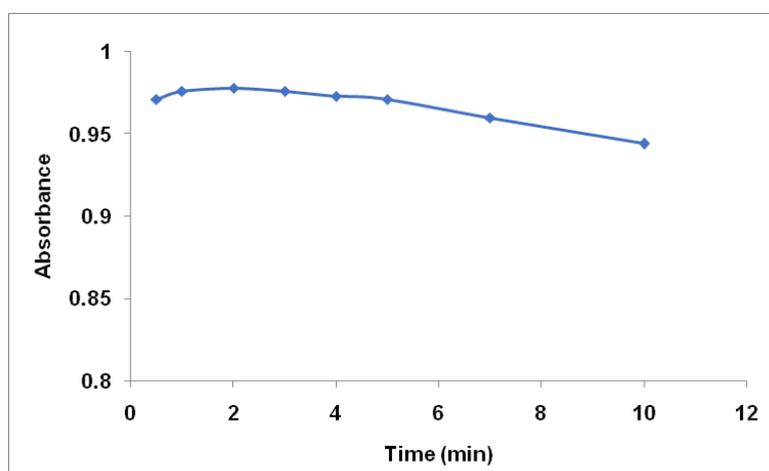
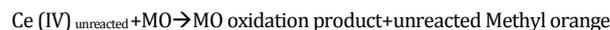
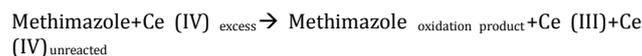


Fig. 5: Effect of time on the absorbance value of the reaction product of 15 $\mu\text{g/ml}$ methimazole at 610 nm

Method B

In this method, Methimazole was allowed to react with a known excess of Ce (IV) nitrate in acidic medium. The unreacted Ce (IV) was reacted with excess methyl orange and the residual methyl orange was determined by absorbance measurement at λ_{\max} 507 nm [40]. In the presence of increasing amounts of the oxidant Ce (IV), the absorbance values of methyl orange decreases. Once the oxidation reaction is assured complete; the unreacted Ce (IV) amount is inversely proportional to the methimazole amount in the sample. According to the scheme below, with increased methimazole concentration, the amount of unreacted Ce (IV) bleaching methyl orange was less and thus the solution absorbance value increases linearly with methimazole concentration (fig. 6).

The proposed reaction mechanism scheme of the indirect determination of methimazole by oxidation with Ce (IV) is shown below [40]:



Optimization of reaction conditions

It was found that 1 ml of 50 mg % cerium (IV) nitrate in acidic medium followed by 1.2 ml of 5 mg % methyl orange solution after 30 min at room temperature were optimum to get the stable and maximum color intensity.

Kinetics of the reactions

The rate of the reaction was dependent on methimazole concentration. Under the optimum conditions described above, the absorbance-time curve was constructed with various drug concentrations in the range of 1-3 $\mu\text{g/ml}$. Both Ce (IV) and methyl orange were kept at their optimal concentration of 5 mg % and 0.6 mg %, respectively.

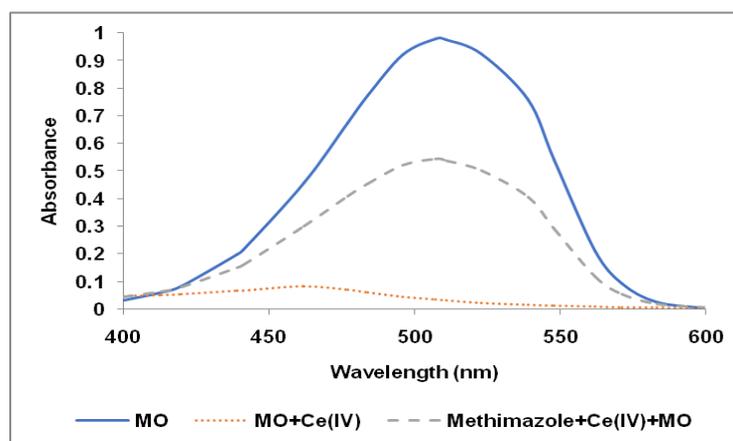


Fig. 6: Absorption spectra of solutions containing (—) 0.6 mg% methyl orange (MO); (---) 0.6 mg% MO+5 mg% Ce (IV) and (---) 0.6 mg% MO+5 mg% Ce (IV)+1.7 $\mu\text{g/ml}$ methimazole

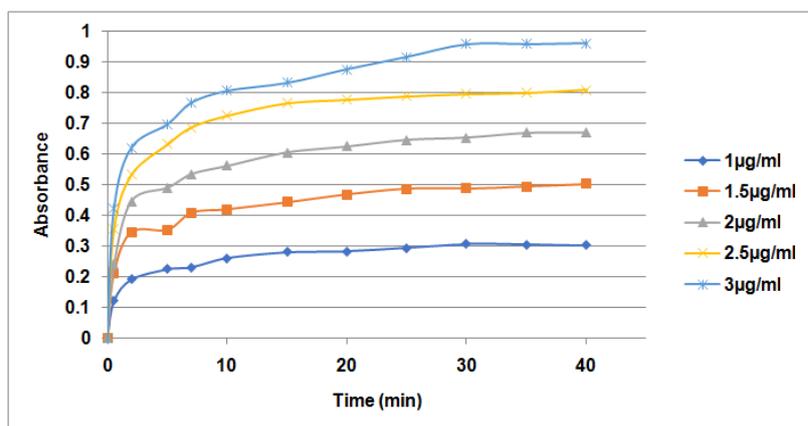


Fig. 7: Absorbance-time curve for the oxidation reaction of methimazole (1-3 $\mu\text{g/ml}$) by Ce (IV) in acidic medium

From the graphs shown (fig. 7), the rate increases with increasing methimazole concentration, indicating that the reaction rate obeys the rate law equation stating:

$$\text{Rate} = k' [\text{C}]^n$$

Where k' is the pseudo-order rate constant and n is the order of the reaction.

From the graphs shown (fig. 7), the rate of the reaction may be estimated by the variable-time method, measured as $\Delta A/\Delta t$ where A is the absorbance value and t is the time in seconds. Taking

logarithms of rates and concentrations, the equation is transformed into:

$$\log \text{rate} = \log k' + n \log C$$

Regression of $\log (\text{rate})$ versus $\log [C]$ by least square method generates the calibration equation of:

$$\log \text{rate} = 1.024 \log C + 1.424 \quad r = 0.997$$

With $k' = 26.55 \text{ s}^{-1}$, correlation coefficient (r) = 0.997 and the reaction is pseudo first order ($n \approx 1$) with respect to methimazole (fig. 8).

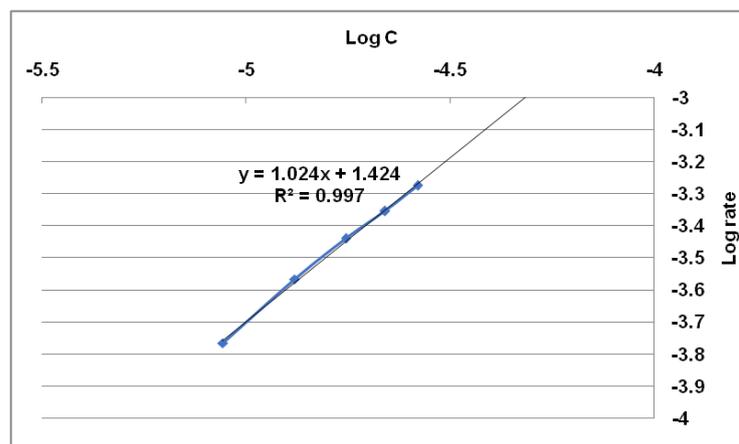


Fig. 8: Log rate versus Log C of methimazole

Under the optimized conditions, the quantitation of methimazole would result in pseudo-first-order reaction rate with respect to its concentration. However, the reaction is pseudo-zero order with respect to Ce (IV) and methyl orange as methimazole concentration was determined using an excess amount of Ce (IV) and methyl orange, 5 mg % and 0.6 mg %, respectively.

Therefore, the rate will be directly proportional to methimazole concentration in a pseudo-first-order rate equation as follows:

$$\text{Rate} = k' [C]^n$$

Where k' is the pseudo-first-order rate constant.

This equation was the core equation for several experiments that were conducted to obtain methimazole concentration. For this reason, the rate constant, initial rate, fixed absorbance, and fixed-time methods were performed. Based on the applicability, the sensitivity, the correlation coefficient (r) and the intercept, the most suitable analytical method was selected.

Rate constant method

It was performed by plotting graphs of logarithms of absorbance values versus time for different methimazole concentrations in the

range of 1-3 $\mu\text{g/ml}$. rectilinear graphs were obtained having a slope of the line $k' / -2.303$. Pseudo-first-order rate constants corresponding to different methimazole concentration were calculated from slopes and are presented in table 1.

Regression of methimazole concentration versus k' gave the following equation:

$$k' = 98.46 C - 0.019 \quad r = 0.257$$

The value of the correlation coefficient (r) indicates poor linearity which is probably due to inconsistency of k' making the method not applicable.

Initial rate method

The initial rate of reaction would follow a pseudo order rate constant and obey the following rate equation:

$$\vartheta = \frac{\Delta A}{\Delta t} = k' [C]^n$$

It was determined by measuring the initial tangents of the absorbance-time curves slopes at different concentration levels (table 2).

Table 1: Values of k' , calculated from slopes of log A versus time graphs multiplied by (-2.303) for different methimazole concentrations

Concentration (M)	k' (s ⁻¹)
8.76×10^{-6}	-0.01863
1.31×10^{-5}	-0.01652
1.75×10^{-5}	-0.0187
2.19×10^{-5}	-0.01565
2.63×10^{-5}	-0.01691

Table 2: Values of slopes of initial tangents for different methimazole concentrations

Concentration (M)	Slope (s ⁻¹)
8.76×10^{-6}	0.00597
1.31×10^{-5}	0.01055
1.75×10^{-5}	0.01162
2.19×10^{-5}	0.01745
2.63×10^{-5}	0.02077

A calibration curve was constructed by plotting the logarithm of the initial rates ($\Delta A / \Delta t$) versus the logarithm of the concentration, and gave the equation:

$$\log \vartheta = 1.103 \log C + 3.367 \quad r = 0.971$$

The value of the correlation coefficient (r) indicates poor linearity suggesting that the first step is too fast and not rate-determining, in addition, the graphs were not easy to plot.

Fixed absorbance method

It was performed by measuring the time required for methimazole concentration to reach an absorbance of a pre-selected value (0.45) as it gives the widest calibration range. The reciprocals of time ($1/\Delta t$) were plotted against the initial concentration of methimazole. The regression equation was obtained (table 3).

Table 3: Values of 1/Δt taken at fixed absorbance (0.45) for different methimazole concentrations

Δt (s)	1/Δt (s ⁻¹)	Conc. (M)	Regression equation	Regression coefficient
990	0.00101	1.31 × 10 ⁻⁵	$\frac{1}{\Delta t} = 2033C - 0.026$	r = 0.989
126	0.007937	1.75 × 10 ⁻⁵		
60	0.016667	2.19 × 10 ⁻⁵		
36	0.027778	2.63 × 10 ⁻⁵		

The value of the correlation coefficient indicates poor linearity, and therefore this method cannot be used.

Fixed time method

It was performed by measuring the absorbance value of the reaction solution containing different methimazole concentrations at a pre-

selected fixed time, which was accurately determined. Calibration graphs of absorbance value versus initial concentration of methimazole were established at fixed times of 2, 5, 10, 15, 20, 25, 30 and 35 min (table 4).

Table 4: Regression equations at different fixed times for methimazole in the concentration range of 0.25-3 μg/ml

Time (min)	Regression equation	Correlation coefficient
2	y = 0.208x+0.011	0.983
5	y = 0.244x-0.008	0.987
10	y = 0.279x-0.004	0.990
15	y = 0.285x+0.015	0.982
20	y = 0.298x+0.009	0.989
25	y = 0.308x+0.009	0.993
30	y = 0.320x-0.001	0.999
35	y = 0.322x+0.001	0.995

The slope is increasing with time while the intercept is decreasing. The most acceptable value of the correlation coefficient was obtained after a fixed time of 30 min; hence the time of 30 min was chosen as the most suitable time for measurement and calibration construction. Therefore, the fixed time method was the kinetic method selected to calculate the concentration of methimazole through its oxidation.

Method validation

Linearity, concentration range, the limit of detection and limit of quantitation

Under the optimum reaction conditions, the standard calibration curve was constructed. The linear regression equations were

obtained by the least-squares method; a linear correlation was found between the absorbance value at λ_{max} and methimazole concentration.

Linearity parameters including the correlation coefficient, intercept and slope for the calibration data are summarized in table 5. For accurate determination, sensitivity parameters such as apparent molar absorptivity and Sandell's sensitivity values, as well as the limits of detection and quantification, were calculated as per the current ICH guidelines [41] and illustrated in table 5.

Statistical data suggest the methods to be highly sensitive; reflected by the high molar absorptivity and low Sandell sensitivity values.

Table 5: Assay parameters for the spectrophotometric determination of methimazole

Parameter	Method A	Method B
λ _{max}	610 nm	507 nm
Beer's law limits (μg/ml)	1.5-15	0.25-3
Molar absorptivity(L/molcm)	6.78798×10 ³	3.6597 × 10 ⁴
Sandell sensitivity	0.0168	0.0032
LOD(μg/ml)	0.466	0.05
LOQ(μg/ml)	1.413	0.15
Regression equation		
Intercept (a)±SD	-0.00815	-0.00126
Slope (b)±SD	0.059455	0.3205
Correlation coefficient (r)	0.9993	0.9998
S _a	0.0078	0.0045
S _b	0.0008	0.0023
S _{y/x}	0.0084	0.0048

Accuracy and precision

The accuracy of the methods was determined by calculating the % recovery of methimazole using the standard addition method at three different concentration levels within the linearity range. Percentage recoveries were then calculated and summarized in table 6 for method A and in table 7 for method B. The obtained % recoveries show that the method has adequate accuracy.

The precision of the method was expressed in the form of inter-and intra-day as percentage relative standard deviation (% RSD) for a series of measurements. The precision of the method was verified by

repeatability and intermediate precision studies. Repeatability studies were performed by analyzing three different standard solutions, three times each on the same day (intra-day analysis). The intermediate precision of the methods was checked by repeating studies on three different days (inter-day analysis). The results obtained in table 6 for method A and table 7 for method B show good precision of the method.

Method application

The applicability of the proposed methods was tested in the pharmaceutical formulation Tapazole® tablet. Satisfactory

percentage recovery was obtained with low % RSD (table 8). This indicates high accuracy and precision in the determination of methimazole in its commercial tablets. Table 8 represents a statistical comparison between the assay of methimazole in its tablets by the proposed methods and a reference method [21] by

means of Student's t-test for accuracy and F-tests for precision at 95% confidence level. The calculated t and F values did not exceed the theoretical values, indicating any significant difference in accuracy and precision between the proposed method and the reference method [21].

Table 6: Accuracy and precision data for the spectrophotometric determination of methimazole in pure form with KMnO₄ in alkaline medium

	Methimazole taken ($\mu\text{g/ml}$)	Methimazole found ($\mu\text{g/ml}$)	% RSD	% Recovery	% Error
Inter-day	5	5.01	6.67	100.20	0.20
	10	10.22	2.29	102.20	2.20
	15	14.95	2.01	99.67	0.33
Intra-day	5	5.17	3.65	103.40	3.40
	10	10.05	0.87	100.50	0.50
	15	15.05	1.36	100.33	0.33

Table 7: Accuracy and precision data for the determination of methimazole in pure form using the proposed spectrophotometric method

	Methimazole taken ($\mu\text{g/ml}$)	Methimazole found ($\mu\text{g/ml}$)	%RSD	% Recovery	% Error
Intra-day	1	1.017	2.906	101.70	1.70
	1.5	1.537	2.367	102.47	2.47
	2	2.050	2.010	102.50	2.50
Inter-day	1	1.006	1.299	100.60	0.60
	1.5	1.544	3.008	102.93	2.93
	2	2.073	3.518	103.65	3.65

Table 8: Determination of methimazole in pharmaceutical preparation using methods A and B

	Method A	Method B	Reference method [21]
^a Mean % Recovery \pm SD	100.82 \pm 0.28	99.85 \pm 1.00	101.78 \pm 1.79
^b %RSD	0.28	1.00	1.77
^c Error	0.82	0.15	1.78
	t-value= 0.294381	t-value= 0.233565	
	F-value= 0.135296	F-value= 0.475271	

^amean \pm SD for five determinations, ^bRelative standard deviation, ^cRelative error, ^{**}Theoretical values of t-and F-test at P=0.05 are 2.13 and 5.05, respectively

CONCLUSION

Two spectrophotometric colorimetric methods have been developed and validated for the analysis of methimazole. The quantification of methimazole through these two methods accomplishes the requirements of specificity, precision, and accuracy in order to be used for the quality control of pharmaceuticals. These methods can be applied for the quality control of methimazole based on the simplicity, applicability of the parameters, accessibility of the reagents employed and reasonably low time of analysis. The proposed methods based on redox reaction were able to quantify the studied drug in its pharmaceutical formulation without the interference from commonly used excipients.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

Malak Y. Al Bathish designed and performed the experiments and the measurements. Azza A. Gazy was involved in planning and supervised the work. Malak Y. Al Bathish and Azza A. Gazy processed the experimental data along with the calculations, designed the fig. and interpreted the results. Malak Y. Al Bathish, Azza A. Gazy and Marwa K. El Jamal revised the results and drafted the manuscript.

CONFLICTS OF INTERESTS

The authors declare that there are no conflicts of interest.

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