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QUANTIFICATION OF METHIMAZOLE IN BULK DRUGS AND PHARMACEUTICAL FORMULATIONS USING MULTIVARIATE UV-VISIBLE SPECTROPHOTOMETRIC CALIBRATION

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ABSTRACT

This study aims to establish an UV-Visible spectroscopic technique to develop an easy, sensible, and reproducible method for Methimazole by applying a multivariate regression equation. Recommended technique depends on the equation of the linear regression performed by taking absorbance at five distinct wavelengths. Methimazole's maximum absorbance value was obtained at 252 nm. Graph obtained from concentration 35 - 65µg/mL resulted in linear curve and the regression coefficient was obtained as 0.999. %RSD values for Intra-day, as well as inter-day precision, was obtained as 0.3032 and 0.299. The assay value determined was between 99.84% - 100.18% w/w.

Keywords: Methimazole, Anti-thyroid agent, Multivariate calibration, UV-Visible spectroscopy, Assay, ICH guidelines

INTRODUCTION

Hyperthyroidism is treated with an anti-thyroid drug Methimazole **[1] (Figure 1)**. Methimazole chemically known as 1-methyl-1H-imidazole-2-thiol **[2]**. Methimazole is a white crystalline powder that has a boiling point of 417.15k, the molecular weight of Methimazole is 114.17 g mol^{-1} and the molecular formula is $C_4H_6N_2S$ **[3]**. It acts by reducing the synthesis of thyroxine T4 and tri-iodothyronine T3 hormones by affecting the reaction between thyroglobulin to protein and peroxidase enzyme causing depletion. Another condition called Graves' disease

can also be treated with Methimazole **[4]**. Methimazole is stated in United States pharmacopeia and it is consumed orally **[5]**. Based on the review of literature various methods have been observed such that UV-Visible spectroscopy (UV) **[6-10]** High-performance liquid chromatography (HPLC) **[3-4, 11-13]**, Liquid chromatography-mass spectroscopy (LC-MS) **[14]**, High performance thin layer chromatography (HPTLC) **[15]**, Electrochemistry coupled to mass spectrometry **[16]**, Gas chromatography-mass spectroscopy (GC- MS) **[17]**.

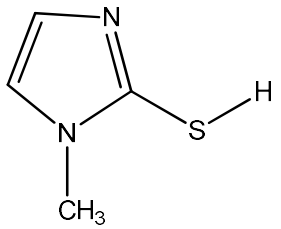

Figure 1: Chemical structure of Methimazole

The technique which is recommended was highly accurate and reliable with greater certainty, therefore Methimazole is evaluated directly using UV-visible technique. Compared to other methodology this technique is employed for the analysis of the various pharmaceutical preparations and it is considered to be direct, and affordable and we can obtain a quick result than any other technique. A specific outcome was obtained from a multivariate standardization procedure and a dependent variable 'm' is obtained by the conversion from the outcome, this analytical technique provides good sensitivity, resolving power, efficiency, and affordability of Methimazole and gives validated quantification. The analyte absorbance (X) is measured for 5 various concentrations at 5 distinct wavelengths, (λ = 246, 249,252, 255, and 258nm); the following formula can then be applied for any preferred wavelength **[18-25]**.

$A_{\lambda 246} = a\ X\ C_x + \ k_1$ ---------- (1)

$A_{\lambda 249} = b\ X\ C_x + \ k_2$ ---------- (2)

$A_{\lambda 252} = c\ X\ C_x + \ k_3$---------- (3)

$A_{\lambda 255} = d\ X\ C_x + \ k_4$---------- (4)

$A_{\lambda 258} = e\ X\ C_x + \ k_5$----------(5)

Whereas absorbance of the analyte is denoted as A_λ, the analyte's slope of the linear regression functions are a, b, c, d, and e; the corresponding intercepts were k1, k2, k3, k4, k5 at five wavelengths and the analyte's concentration is denoted as Cx. The selected five wavelengths equation (1-5) listed above summarised in the following formula:

$A_T = a\ X\ C_x + \ b\ X\ C_x + \ c\ X\ C_x + \ d\ X\ C_x + \ e\ X\ C_x + \ K_T$-------- (6)

Equation above is further condensed as

$A_T = C_x\ (a + b + c + d + e) + \ K_T$-- (7)

For the five selected wavelengths K_T and A_T the summation total of the intercept and absorbance of the regression equation. The following formula computes the concentration of the analyte X.

$$C_x = \frac{A_T - K_T}{(a + b + c + d + e)}$$

MATERIALS AND METHODS

Chemicals and reagents

- Distilled water
- Methimazole was obtained as gift sample from Ideal Analytical and Research Institute, Pondicherry.
- Methimazole was obtained as a marketed tablet formulation METHIMEZ (Label claim -10 milligram), manufactured by Sun pharmaceutical industries Ltd and purchased at a medical store.

Instruments used

- UV-Visible double beam spectroscopy (LAB INDIA 3092)
- Ultrasonic sonicator
- Microbalance

Analytical method development

Choice of the solvent

Methimazole is freely soluble in water. So, water is used for the further dilution of the sample and standard stock solution.

Standard stock solution preparation

Methimazole standard stock solution prepared by solubilizing 10 mg of drug substance into a 100 mL volumetric flask and distilled water is used to make up the volume. This standard stock solution was used to make aliquots of solutions with concentration ranging from (35 - 65µg/mL).

Determination of λ_{max}

The maximum absorbance of Methimazole is determined from the solution prepared by dissolving the standard stock solution with water to 10 µg/mL. Prepared solutions were scanned over the range of 200 - 400 nm in the UV-Visible region. The maximum absorbance of the Methimazole obtained at 252 nm **(Figure 2)**.

Sample solution preparation

Preparation of sample solution is done by taking thirty tablets of Methimazole, accurately weighed, and powdered. The weight corresponding to 10 mg was taken and transferred to a 50 mL standard flask, sonicated for 15 minutes, dissolved, and makeup to the mark with water and this solution is used for further analysis.

Method Validation

This technique has been validated for sensitivity, precision, accuracy, and linearity accordance to the ICH guidelines **[26]**.

Linearity

Linearity was obtained for the concentration range 35 – 65 µg/mL from standard stock solution. These solutions were scanned over wavelengths from 246 nm, 249 nm, 252 nm, 255 nm, and 259 nm respectively, to reduce instrumental deviations and improve the correlation **(Figure 3, Table 1)**. The graph is plotted as concentration against absorbance and calibrations were achieved.

Table 1: UV Calibration data at five distinct wavelengths

Concentration (µg/mL)	Absorbance				
	246 nm	249 nm	252nm	255 nm	258 nm
35	0.303	0.320	0.324	0.312	0.289
40	0.391	0.410	0.415	0.401	0.364
45	0.480	0.501	0.508	0.491	0.444
50	0.569	0.596	0.601	0.567	0.529
55	0.653	0.684	0.693	0.663	0.611
60	0.741	0.777	0.789	0.756	0.684
65	0.834	0.866	0.877	0.840	0.766

[#]**Average of 5 determinations; UV= Ultra violet**

The limit of detection and quantification was determined by the sensitivity of the method using the formula below.

$LOD = 3.3\ \sigma/S$ …….... (8)

$LOQ = 10\ \sigma\ /S$..... (9)

Hereby, lowermost concentration of standard deviation (SD) is σ and the standard curve of the slope is denoted as S.

Precision

For measuring the intra-day and inter-day precision, the solution of 10 µg/mL was prepared and scanned for six times. The intra-day precision was measured within a day and the inter-day precision was measured in six various days.

Accuracy

The recovery study for recommended technique were concluded at 80%, 100%, and 120% by applying the standard addition technique, and using this % recovery was calculated. The solutions for the recovery study were prepared from both standard and sample stock solutions by taking constant volume of standard and various volumes of sample solutions. The absorbance of these solutions were measured at the absorbance maxima.

Assay

The amount of Methimazole present in the tablet calculated by measuring absorbance at 252 nm from the extracted tablet solution.

RESULTS AND DISCUSSION

The Methimazole maximum absorbance obtained at 252 nm with water as the solvent as shown in **Figure 2**.

Within the concentration range between 35 - 65 µg/mL this technique was found to be linear. An excellent linear correlation is obtained from the calibration plots with R^2=0.9995 - 0.9999. The % relative standard deviation for precision obtained as 0.3032 and 0.299. The obtained values of detection and quantification of limit are 0.285 and 0.866 µg/mL, respectively. Hence the values lie within validation parameters and is in accordance to the ICH guidelines limitations.

Linearity

Linearity scanned for the concentration range of 35 - 65 µg/mL for the wavelengths 246 nm, 249 nm, 252 nm, 255 nm and 258 nm respectively, it is depicted in **Figure 3**, and the calibration curves are represented from **Figures 4 to 8**. The % relative standard

deviation has low values that determines technique is accurate and reliable for each wavelength. The calculation of detection and quantification of limit has been done and results were depicted in **Table 2**.

Precision

The recommended technique is unique, reliable and accurate as shown by low standard deviation values. The intra-day precision in addition to inter-day precision values obtained as 0.3032 and 0.299, respectively. At each wavelength precision values should be within limits less than 2%. **(Figure 9, 10)**.

Recovery

The Methimazole % recovery was found between 99.84% to 100.18% w/w, according to the ICH guidelines. The acceptable range of % recovery was from 97 - 103 % w/w **(Figure 11, Table 3)**.

Assay

Methimazole maximum absorbance was measured at 252 nm for the tablet formulation by using UV-Visible spectroscopy. The amount and assay percentages were obtained as 9.98 mg and 99.87 % w/w, further % Relative standard deviation values are depicted in **Table 4**.

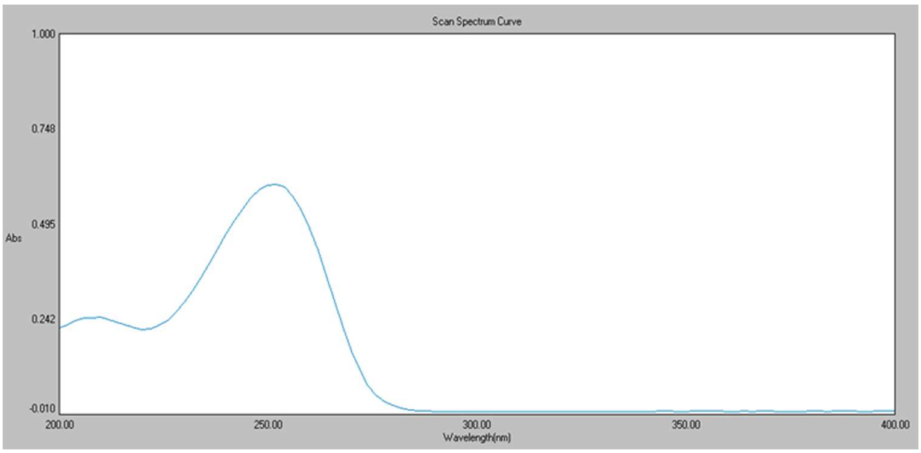

Figure 2: UV spectrum of Methimazole (10 µg/mL), λ_{max} at 252 nm

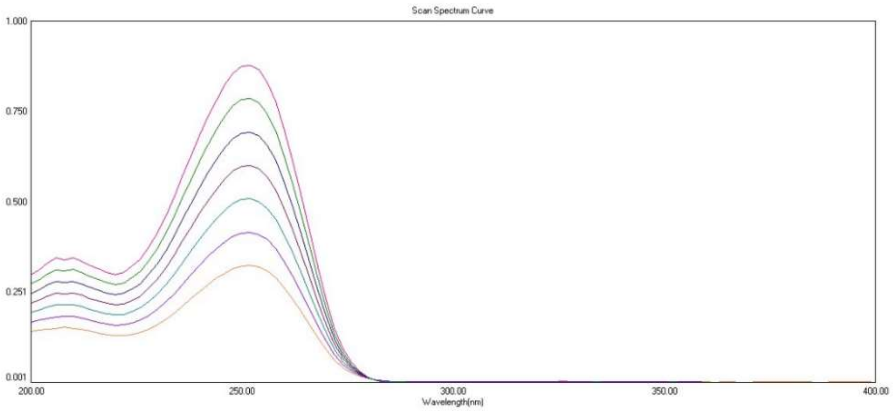

Figure 3: UV Spectrum of Methimazole showing linearity at 252 nm

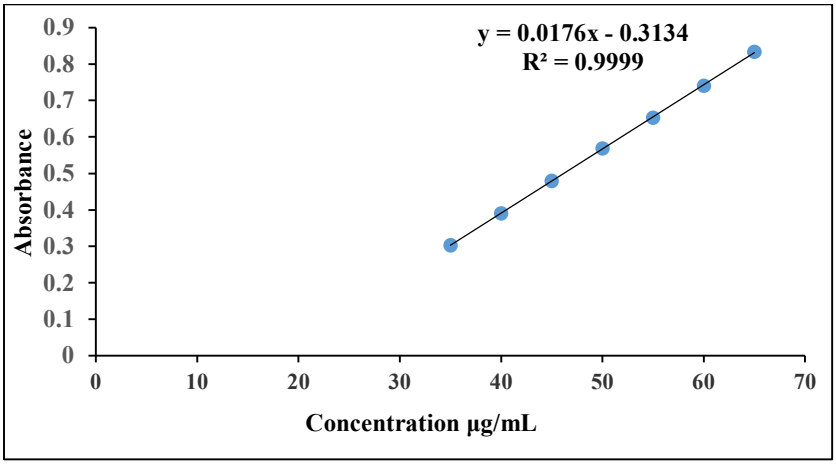

Figure 4: Calibration curve at 246 nm

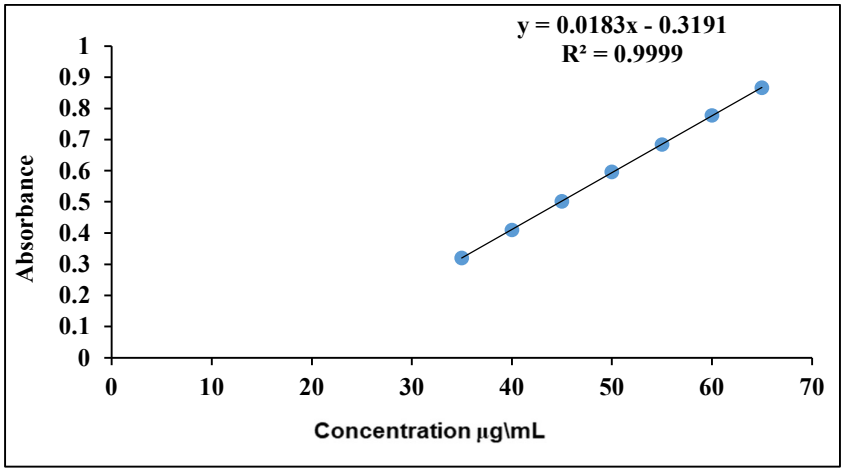

Figure 5: Calibration curve at 248 nm

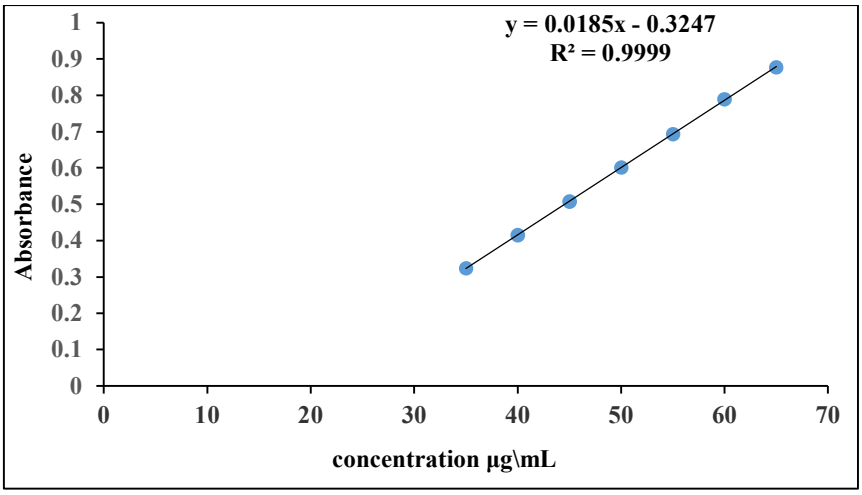

Figure 6: Calibration curve at 252 nm

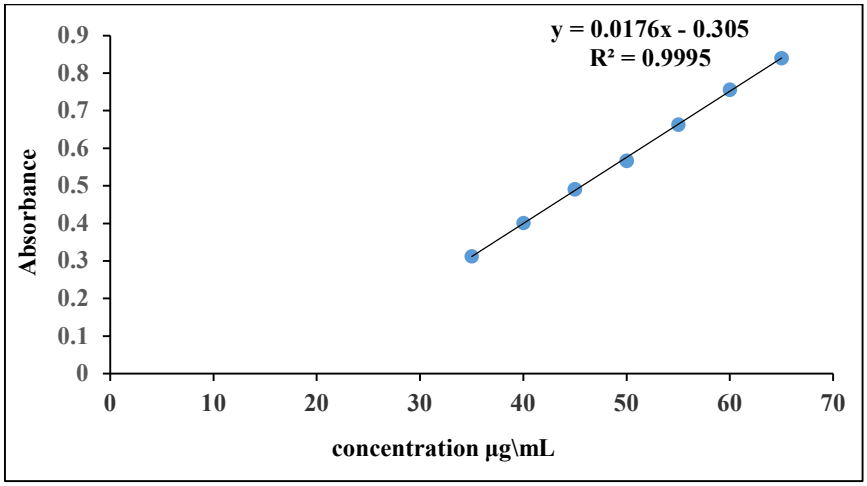

Figure 7: Calibration curve at 255 nm

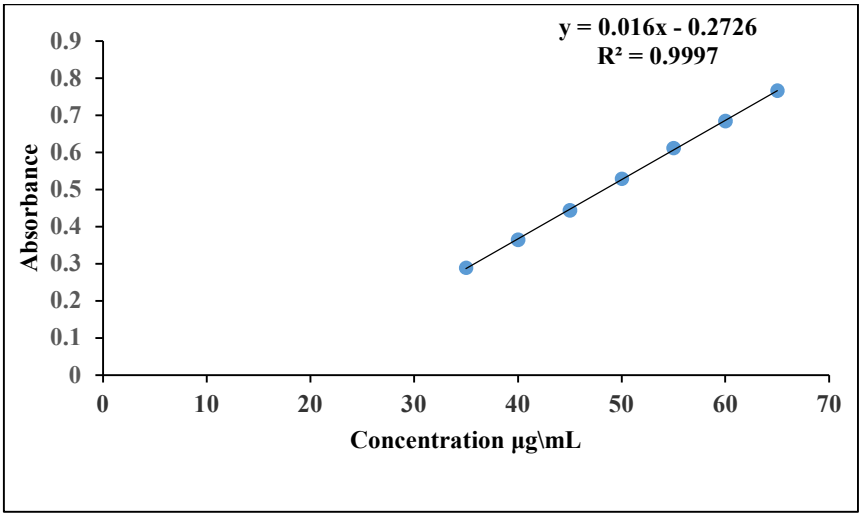

Figure 8: Calibration curve at 258 nm

Table 2: Linearity data with LOD and LOQ at selected five wavelengths

Wavelength (nm)	Regression equation	R2	LOD (µg/mL)	LOQ (µg/mL)	% RSD
246	y = 0.0176x - 0.3134	0.9999	0.3865	1.1713	0.363
249	y = 0.0183x - 0.3191	0.9999	0.2797	0.8476	0.260
252	y = 0.0185x - 0.3247	0.9999	0.2858	0.8661	0.266
255	y = 0.0176x - 0.305	0.9995	0.8678	2.6297	0.804
258	y = 0.016x - 0.2726	0.9997	0.6611	2.0034	0.608

*nm = nanometre; µg/mL = Microgram per millilitre

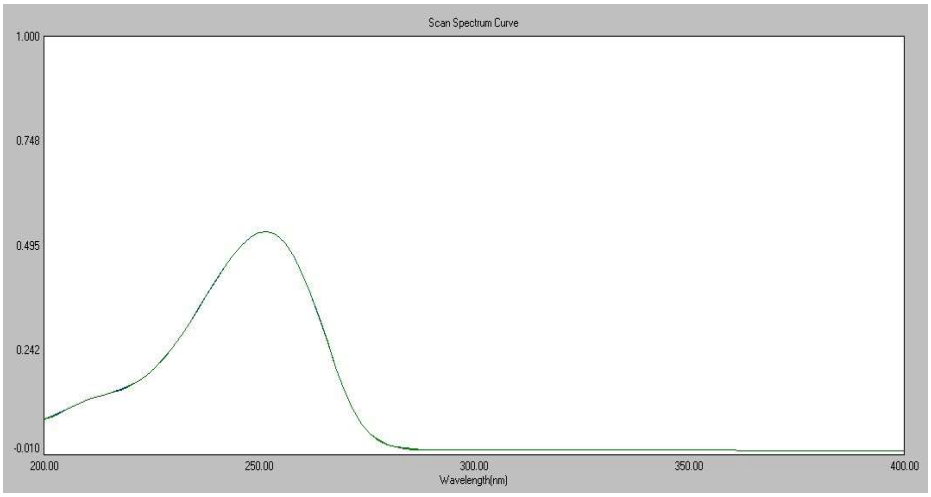

Figure 9: UV spectra showing intraday precision

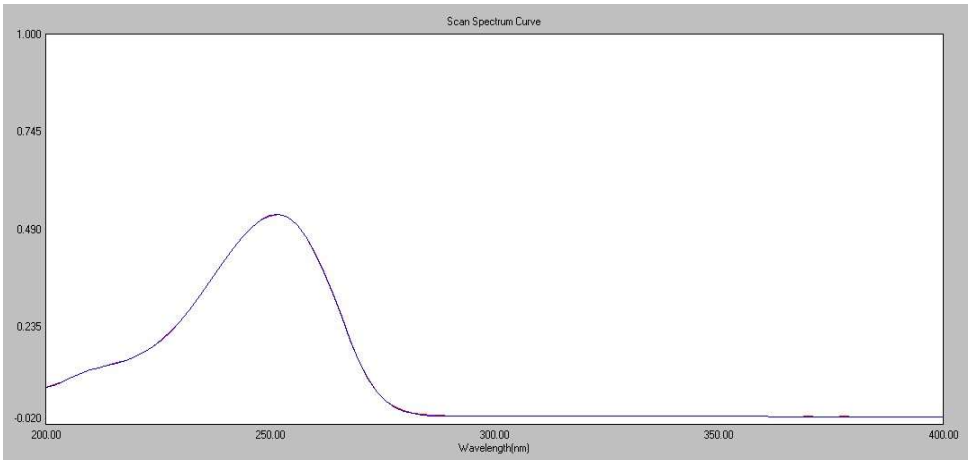

Figure 10: UV spectra showing interday precision

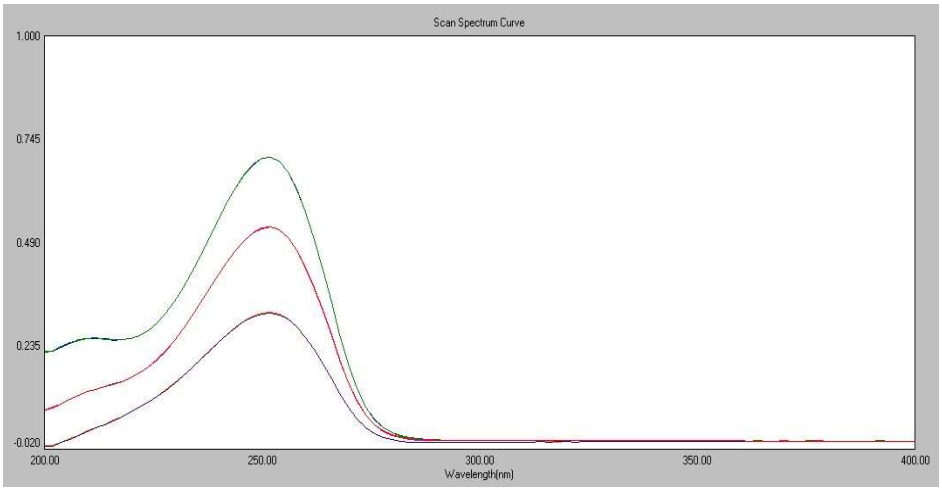

Figure 11: UV Spectrum showing accuracy of Methimazole

Table 3: Recovery Studies

Wavelength (nm)	Amount present (µg/mL)	Amount added (µg/mL)	Absorbance	Amount recovered (µg/mL)	% Recovery
246 nm	30	10	0.392	40.02	100.05
		20	0.568	49.98	99.96
		30	0.742	59.95	99.92
249 nm	30	10	0.408	39.95	99.98
		20	0.595	50.1	100.20
		30	0.776	59.98	99.97
252 nm	30	10	0.414	39.96	99.90
		20	0.602	49.92	99.84
		30	0.788	59.96	99.93
255 nm	30	10	0.402	40.01	100.03
		20	0.568	49.96	99.92
		30	0.757	60.07	100.12
258 nm	30	10	0.363	40.07	100.18
		20	0.528	49.95	99.90
		30	0.685	59.92	99.87

Table 4: Assay of Methimazole

Label claim (mg)	Amount obtained (mg)	% Assay
10	9.98	99.8
10	9.97	99.7
10	10.01	100.1
Average	9.98	99.87
SD		0.20817
% RSD		0.20844

CONCLUSION

It is concluded that the recommended technique is more accurate, precise, reproducible, cost-effective, and highly reliable than the conventional UV-Visible Spectrophotometry for Methimazole assay. According to the Quality Guidelines of ICH, this method has been validated and they are within the range of the limits. This method was found simple than complicated HPLC and HPTLC methods and it can be used for the routine quality control assay of Methimazole bulk drugs and pharmaceutical dosage forms.

ETHICAL STATEMENT

This study does not involve experiments on animals or human subjects.

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article exists.

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