



## Determination of Methimazole in Pharmaceutical Preparation and Human Serum by Square Wave and Differential Pulse Polarographic Methods

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### ABSTRACT

**Objectives:** In this study, the polarographic behaviour of methimazole was studied using cyclic, square wave and differential pulse polarographic methods. **Methods:** The influence of several variables (including nature of the buffer, concentration, scan rate, drop size, etc.) was examined in square wave and differential pulse polarographic methods for methimazole. The best square wave and differential pulse polarographic responses were obtained in 0.1 M sulfuric acid. The peak currents were measured with a static mercury drop electrode at 0.10 V versus Ag/AgCl. **Results:** The linearity was established over the concentration range of 5-80 µg/mL for both methods in supporting electrolyte and human serum. The methods were validated and applied to the determination of methimazole in a commercial tablet. There was no significant differences between the results obtained by square wave and differential pulse polarographic methods. Square wave and differential pulse polarographic methods are also available and applicable for the determination of mentioned substance in human serum. No electroactive interferences from the endogenous substances were found in the serum samples. Mean recovery was 99.9%. **Conclusion:** They were concluded that the developed methods were accurate, sensitive, precise, reproducible and useful for the quality control of methimazole in pharmaceuticals and spiked serum.

**Keywords:** Differential pulse polarography; Methimazole; Serum; Square wave polarography; Tablet

### INTRODUCTION

Methimazole (2-mercapto-1-methylimidazole) (**Figure 1**) used in treatment of hyperthyroidism by the production of thyroxin, a hormone excreted by the thyroid gland, inhibits the formation of thyroid hormones.<sup>1</sup> It is absorbed by the gastrointestinal tract and acts as an immunosuppressive agent in graves disease<sup>2-4</sup>.

Several analytical procedures have been described for the determination of methimazole including thin layer chromatography<sup>1</sup>, coulometry<sup>5</sup>,

conductometry<sup>6</sup>, high-performance liquid chromatography with ultraviolet detection<sup>7</sup>, spectroscopy<sup>8-10</sup>, electrochemistry with a silver-silver sulphide solid-state electrode<sup>11</sup>, liquid chromatography with amperometric detection at a nafion/indium hexacyanoferrate film modified electrode<sup>12</sup> capillary zone electrophoresis with amperometric detection at a carbon electrode<sup>13</sup> potentiometric and voltammetric methods<sup>14</sup>.

Only a limited number of articles have been published in the literature on the use of chemically modified glassy carbon electrodes for the determination

of methimazole including acetylene black/chitosan film modified glassy carbon electrode<sup>15</sup>, multi-walled carbon nanotube modified glassy carbon electrode (GCE)<sup>16</sup> and a carbon paste electrode modified with a schiff base complex of cobalt<sup>17</sup>.

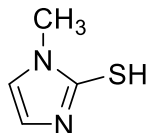


Figure 1. Chemical structure of methimazole

There are some problems encountered in using such methods. Spectrophotometric methods suffer from low range. Chromatographic methods are relatively slow and expensive and they require derivatization or time consuming extraction procedures. Thus, the use of simpler, faster and less expensive, but still sensitive electrochemical techniques can be considered as a useful alternative. The polarographic techniques present some advantages in relation to many other analytical techniques. Progress obtained with pulse techniques have increased the range of practical applications of polarography by enabling determinations of electroactive species at lower concentrations. When compared to chromatography, the polarographic procedures have several advantages such as their low cost and short time required for analysis. On the other hand, electroanalytical methods offer useful applications in kinetic and equilibria studies, much more than HPLC which often can perturb equilibria in the reaction mixture.

The development of a new method capable of determining drug amount in pharmaceutical preparations or biological fluids is important. Electroanalytical techniques have been used for the determination of a wide range of drug compounds with the advantages that there are, in most, instances no need for derivatization and that these techniques are less sensitive to matrix effects than other analytical techniques. Additionally, application of electrochemistry includes the determination of electrode mechanism. Redox properties of drugs can give insights into their metabolic fate or their in vivo redox processes or pharmacological activity. Despite the analytical importance of the electrochemical behaviour and oxidation mechanism of methimazole, no report has been published on the polarographic study of the electrochemical oxidation of methimazole in pharmaceuticals and spiked human serum.

The goal of this work was the development of new polarographic methods for the direct determination of methimazole in pharmaceutical preparation and spiked human serum samples without any time-

consuming extraction or evaporation steps prior to drug assay. This paper describes fully validated, simple, rapid, selective and sensitive procedures for the determination of methimazole employing square wave polarography (SWP) and differential pulse polarography (DPP) at mercury electrode. Also, this work was also aimed to study the polarographic behavior and oxidation mechanism of methimazole using cyclic, SWP and DPP techniques.

## MATERIAL AND METHODS

### Chemicals, reagents and analytical conditions

Methimazole (99.0% purity) was obtained from Sigma (St. Louis, MO, USA). Thyromazol tablets were purchased from the local pharmacy (Erzurum, Turkey). A stock solution of 100 µg/mL was prepared by dissolving the compound in 0.1 M sulfuric acid. Standard solutions were prepared by serial dilution of the stock solution with selected supporting electrolyte. The calibration curve for SWP and DPP analysis were constructed by plotting the peak current against the methimazole concentration. The ruggedness and precision were checked at different days, within day and between days. Relative standard deviations were calculated to check the ruggedness and precision of the method<sup>18</sup>. The precision and accuracy of analytical methods are described in a quantitative fashion by the use of relative errors (bias %). One example of relative error is the accuracy, which describes the deviation from the expected results. All solutions were kept in the dark in a refrigerator and were used within several hours to avoid hydrolysis. However, voltammograms of the sample solutions recorded 48 h after preparation did not show appreciable change in assay values.

Voltammetric measurements were obtained with Gamry Potentiostat Interface 1000 controlled with software PHE 200 and PV 220. A three electrode cell system was used a static mercury drop electrode system (BAS 100 W/B), including a hanging mercury drop working electrode, a platinum-wire auxiliary electrode and an Ag/AgCl (KCl 3M, BAS) electrode as the reference electrode. All pH measurements were made with Model 538 pH meter (WTW, Austria), calibrated with standard buffers (Fixanal, Riedel-deHaen, Germany) at room temperature. All measurements were carried out at ambient temperature of the laboratory (22-25 °C).

For analytical application, the following parameters being employed: SWP pulse amplitude 25 mV, frequency 15 Hz, potential step 4mV; DPP pulse amplitude 50 mV, pulse width 50 ms, scan rate 20 mV/s.

**Table 1. Regression data of the calibration curves for quantitative determination of methimazole**

Parameters	SWP		DPP	
	Supporting electrolyte	Serum	Supporting electrolyte	Serum
Measured potential (mV)	25	22	28	24
Linearity (µg/mL)	5-80	5-80	5-80	5-80
Slope	0.0240	0.0243	0.0263	0.0238
Intercept	1.241	2.715	3.944	2.343
R	0.996	0.995	0.997	0.994
S <sub>a</sub>	0.012	0.014	0.011	0.012
S <sub>b</sub>	0.244	1.643	0.049	2.483
LOD (µg/mL)	0.50	0.60	0.40	0.50
LOQ (µg/mL)	1.50	1.80	1.20	1.50
Precision (RSD%)	2.32	3.02	3.26	3.46
Accuracy (% relative error)	-2.28	2.31	2.49	2.51
Repeatability of peak current (RSD%) <sup>a</sup>	1.79	2.48	1.76	2.68
Repeatability of peak potential (RSD%)	1.02	2.08	1.24	2.19
Reproducibility of peak current (RSD%)	2.44	3.64	3.06	3.21
Reproducibility of peak potential (RSD%)	2.03	2.16	3.21	3.14

RSD: Relative standard deviation, <sup>a</sup>Average of six replicate determinations, S<sub>a</sub>: Standard deviation of intercept of regression line, S<sub>b</sub>: Standard deviation of slope of regression line, R: Coefficient of correlation, LOD: Limit of detection, LOQ: Limit of quantification

### Procedure for pharmaceutical preparations

A total 10 tablets of Thyromazol, each containing 5 mg of methimazole were accurately weighed and powdered. An amount of this powder corresponding to one tablet methimazole content was weighed and accurately transferred into 100 mL calibrated flask and 50 mL of 0.1 M sulfuric acid was added and then the flask was sonicated to 10 min at room temperature. The flask was filled to volume with 0.1 M sulfuric acid. The resulting solutions in both the cases were filtered through Whatman filter paper no 42 and suitably diluted to get final concentration within the limits of linearity for the respective proposed method. The drug content of methimazole tablet was calculated from the current potential curve.

### Analysis of spiked serum samples

Drug free human blood, obtained from healthy volunteers (after obtaining their written consent), was centrifuged (5000 rpm) in 10 min at room temperature and separated serum samples were stored frozen until assay. An aliquot volume of serum sample was fortified with methimazole dissolved in 0.1 M sulfuric acid to achieve final concentration of 5-80 µg/mL. Acetonitrile removes serum proteins more effectively, as the addition of 0.5 mL volume of acetonitrile is sufficient to remove the proteins. After vortexing for 30 s, the mixture was then centrifuged for 10 min at 5000 rpm.

Then, the supernatant was taken carefully. Appropriate volumes of this supernatant were transferred into the volumetric flask and diluted up to the volume with 0.1 M sulfuric acid. The analyses were carried out using a standard addition method. The concentration of methimazole was varied in the range of 5-80 µg/mL in human serum sample.

## RESULTS AND DISCUSSION

### Effect of supporting electrolyte

Polarographic response of methimazole was studied in various supporting electrolytes at pH 2-12. Methimazole yielded a single oxidation wave in supporting electrolytes such as 0.25 M phosphate buffer between pH 2 and 10, 0.04 M Britton-Robinson buffer between pH 2 and 12 and 0.1 M sulfuric acid were used as the supporting electrolytes. However, a best-defined oxidation peak was obtained in 0.1 M sulfuric acid. Therefore, this work was selected the 0.1 M sulfuric acid as supporting electrolyte.

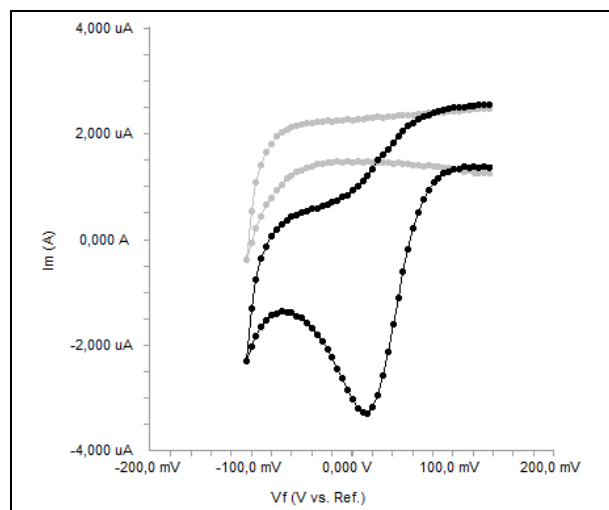
### Effect of supporting electrolyte concentration

The effect of the total concentration of sulfuric acid was tested over the range 0.025-0.40 M. The peak current increased gradually upon the sulfuric acid concentration increasing from 0.025 to 0.15 M and then reached a current plateau until 0.40 M, while the peak

potential  $E_p$  shifted positively from -40 to 120 mV. Therefore, 0.1 M concentration of the sulfuric acid was selected in this experiment.

### Electrochemical behavior of methimazole

Successive cyclic polarograms of methimazole obtained in 0.1 M sulfuric acid at a scan rate of 100 mV/s are shown in **Figure 2**.



**Figure 2.** Cyclic voltammogram for the oxidation of 50 µg/mL methimazole in 0.1 M sulfuric acid at hanging mercury drop working electrode, scan rate: 0.1 V/s.

The cyclic voltammogram of 50 µg/mL methimazole exhibits a single anodic peak. The study of the influence of scan rate shows that the peak current changes linearly with scan rate. The role of adsorption is further supported by the sharp form of the main anodic peak and by the dependence of the peak current on scan rate ( $v$ ). For diffusion current the plot of  $\log i_p$  as a function of  $\log v$  could have a slope of 0.5 and for a purely adsorption current a slope of 1.0. The regression of  $\log i_p$  vs  $\log v$  gave a slope value of 0.51, indicating that the oxidation current is of diffusional nature. On the other hand, as scan rate was increased from 10 to 1000 mV/s, the peak potential shifted toward more positive potential as expected for an irreversible oxidation process.<sup>16</sup> The value of  $an$ , product of transfer coefficient and number of electrons transferred in the rate-determining step, was determined from treatment ( $\log i_p$  vs  $E$ ) of the polarographic curves. The value obtained (0.43) shows the total irreversibility of the electron transfer process. It was also demonstrated by the linear relationship obtained between the peak potential ( $E_p$ ) and the logarithm of scan rate in the range 10-1000 mV/s. Based on the polarographic behavior of methimazole, a quantitative method was developed. To select the best electrochemical method,

the anodic peak obtained by cyclic, SWP and DPP were compared with each other. In order to develop a voltammetric method for determination of the methimazole, we selected the SWV and DPV techniques, since the peaks were sharper and better defined at lower concentration of methimazole than those obtained by cyclic and linear sweep voltammetry with a lower background current, resulting in improved resolution. SWV and DPV are effective and rapid electroanalytical techniques with well-established advantages, including good discrimination against background currents and low detection and determination limits<sup>19-21</sup>.

### Validation of the method

The validation was carried out by establishing specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), ruggedness, recovery according to ICH Q2B recommendations<sup>22</sup>.

### Specificity

Excipients (corn starch, magnesium stearate, lactose, sodium lauryl sulfate, polyethyleneglycol, titanium dioxide, carboxymethylcellulose, hydroxypropylmethylcellulose and talc) were added to the drug for recovery studies, according to the manufacturer's batch formulas for 5 mg methimazole per tablet. The mean percentage recovery of 50 µg/mL methimazole showed no significant excipient interference; thus the procedures were able to assay methimazole in the presence of excipients, and hence it can be considered specific.

### Linearity

Standard solutions were prepared as 5-80 µg/mL (5, 10, 20 - 80 µg/mL) for SWP and DPP (**Figures 3,4**) respectively.

Calibration curves were constructed for methimazole standard by plotting the concentration of compound versus peak current responses. The calibration curves were evaluated by its correlation coefficients. The correlation coefficients ( $r$ ) of all the calibration curves were consistently greater than 0.99. The linear regression equations were calculated by the least squares method using Microsoft Excel® program and summarized in **Table 1**.

### Accuracy and precision

Accuracy of the assay methods was determined for both intra-day and inter-day variations using the six times analysis of the quality control (QC) samples. Repeatability refers to the use of the analytical procedure within a laboratory over a short period of time that was evaluated by assaying the QC samples during the same day. Intermediate precision was

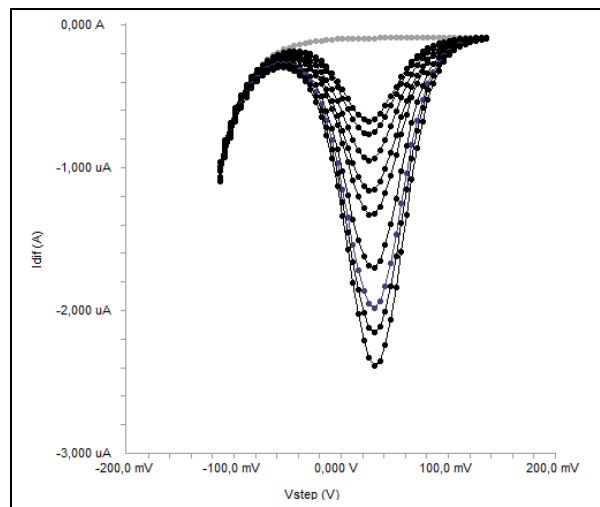


Figure 3. Square wave polarograms for different concentrations of methimazole in 0.1 M sulfuric acid (5, 10, 20, 30, 40, 50, 60, 70 and 80 µg/mL).

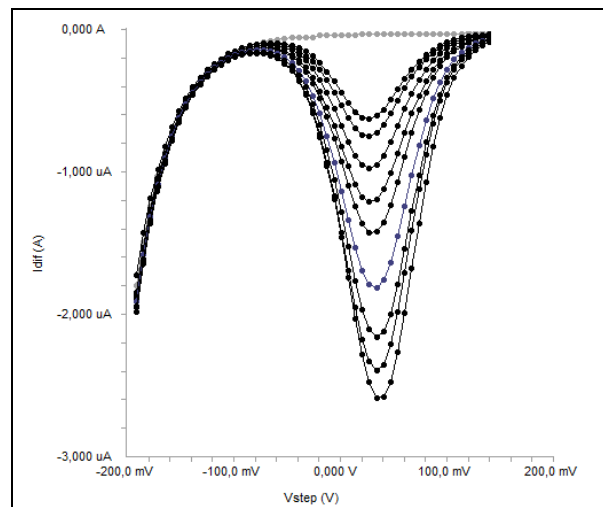


Figure 4. Differential pulse polarograms for different concentrations of methimazole in 0.1 M sulfuric acid (5, 10, 20, 30, 40, 50, 60, 70 and 80 µg/mL).

Table 2. Recovery of methimazole in pharmaceutical preparations by proposed methods

Pharmaceutical preparation	Added (µg/mL)	Found ± SD	SWP		Found ± SD	DPP	
			Recovery (%)	RSD <sup>a</sup> (%)		Recovery (%)	RSD <sup>a</sup> (%)
Thyromazol (35 µg/mL)	5	4.8 ± 0.20	96.0	4.17	5.1 ± 0.18	102.0	3.53
	15	14.5 ± 0.29	96.7	2.00	14.8 ± 0.25	98.7	1.69
	35	35.6 ± 1.12	101.7	3.14	35.2 ± 1.67	100.6	4.74

SD: Standard deviation of six replicate determinations, RSD: Relative standard deviation, <sup>a</sup> Average of six replicate determinations

Table 3. Recovery of methimazole in spiked human serum

Method	Added (µg/mL)	Intra-day			Inter-day		
		Found ± SD	Recovery (%)	RSD <sup>a</sup> (%)	Found ± SD	Recovery (%)	RSD <sup>a</sup> (%)
SWP	20	20.3 ± 0.29	101.5	1.43	20.5 ± 0.34	102.5	1.66
	40	39.4 ± 0.63	98.5	1.60	39.5 ± 0.63	98.8	1.59
	60	58.4 ± 1.92	97.3	3.29	61.1 ± 1.82	101.8	2.98
DPP	20	20.4 ± 0.38	102	1.86	20.3 ± 0.48	102	2.36
	40	39.6 ± 0.72	99.0	1.82	39.7 ± 0.78	99.3	1.96
	60	59.1 ± 2.14	98.5	3.62	59.2 ± 2.11	98.7	3.56

SD: Standard deviation of six replicate determinations, RSD: Relative standard deviation, <sup>a</sup> Average of six replicate determinations



assessed by comparing the assays on different days (3 days). The intra-day accuracy ranged from -2.28 % to 2.51% and precision from 2.31% to 3.46%. The results obtained from intermediate precision (inter-day) also indicated a good method precision (Table 2).

#### Limits of detection (LOD) and quantification (LOQ)

The LOD and LOQ of methimazole by the proposed methods were determined using calibration standards. LOD and LOQ values were calculated as  $3.3 \sigma/S$  and  $10 \sigma/S$ , respectively, where  $S$  is the slope of the calibration curve and  $\sigma$  is the standard deviation of y-intercept of regression equation ( $n=3$ )<sup>22</sup>. The LOD and LOQ values of the methods were summarized in Table 1.

#### Ruggedness

In this study, the SWP and DPP determination of methimazole were carried out by a different analyst in same instrument with the same standard. The results showed no statistical differences between different operators suggesting that the developed method was rugged.

#### Stability

To evaluate the stability of methimazole, standard solutions were prepared separately at concentrations covering the low, medium and higher ranges of calibration curve for different temperature and times. These solutions were stored at room temperature, refrigeratory (4 °C) and frozen (-20 °C) temperature for 24h and 72h. Stability measurements were carried out with SWP and DPP method. The results were evaluated comparing these measurements with those of standards and expressed as percentage deviation and methimazole was found as stable at room temperature, 4 and -20 °C for at least 72h.

#### Recovery

To determine the accuracy of the SWP and DPP methods and to study the interference of formulation additives, the recovery was checked as three different concentration levels. Analytical recovery experiments were performed by adding known amount of pure drugs to pre-analyzed samples of commercial tablet form. The recovery values were calculated by comparing concentration obtained from the spiked samples with actual added concentrations. These values are also listed in Table 2.

#### Analysis of spiked serum samples

The optimized procedure was successfully applied for the determination of methimazole in protein-free spiked human serum samples. Acetonitrile was tried as a serum precipitating agents. No extraction steps other than the centrifugal protein separation were required prior to the assay of drug. Figures 5 and 6

illustrate the response of successive standard additions of methimazole.

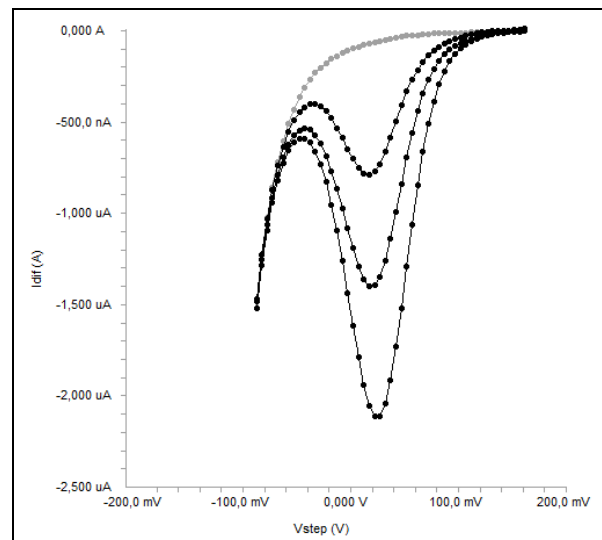


Figure 5. Square wave polarograms obtained for different concentrations of methimazole in spiked serum (blank, 10, 40 and 70 µg/mL).

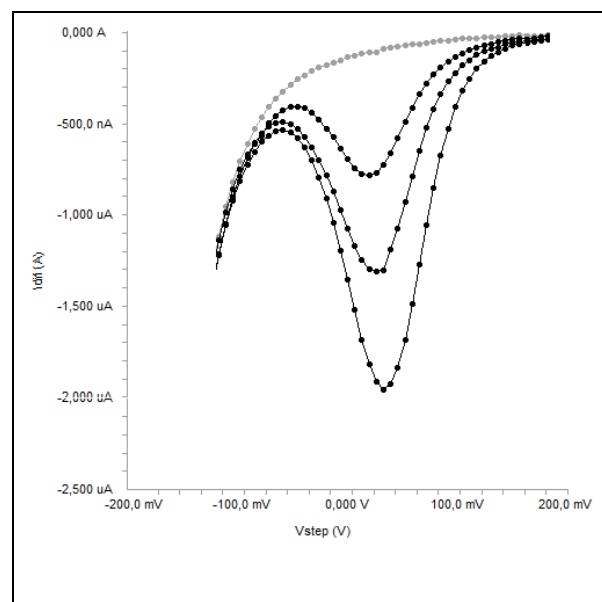


Figure 6. Differential pulse polarograms obtained for different concentrations of methimazole in spiked serum (blank, 10, 40 and 70 µg/mL).

Calibration equation parameters and necessary validation data were shown in Table 1. Obtained recovery results of spiked human serum samples were given in Table 3.

**Table 4 Comparison of the proposed and reported methods for determination of methimazole**

Parameters	SWV	DPV	Reported method <sup>16</sup>	Reported method <sup>17</sup>
Mean (recovery %)	100.1	99.9	98.6	96.9
SD	0.718	1.623	-	-
% RSD	0.717	1.728	2.64	4.50
Variance	0.515	2.634	-	-
SE	0.294	0.665	-	-
t-test (2.228) <sup>a</sup>	0.918	-	-	-
F- test (5.1) <sup>a</sup>	3.97	-	-	-

SD: Standard deviation of six replicate determinations, RSD: Relative standard deviation, SE: Standard error, <sup>a</sup>Theoretical values, Theoretical values at  $P=0.05$ ,  $H_0$  hypothesis: no statistically significant difference exists between three methods (the proposed SWV and DPV methods and the reported method stated in the 16<sup>th</sup> reference),  $F_1 > F_c$ :  $H_0$  hypothesis is accepted ( $P > 0.05$ )

**Table 5. Comparison of analytical parameters of proposed method with previously reported in the literature**

Electrode construction	Linear range (µM)	LOD <sup>a</sup> nM	Reproducibility (%RSD)	Reference
Acetylene black/chitosan film modified glassy carbon electrode	0.1-20	20 nM	3.10	15
Multi-walled carbon nanotube modified glassy carbon electrode	0.1-500	30 nM	2.64	16
Carbon paste electrode modified with a Schiff base complex of cobalt	1.0-100	500 nM	<4.5	17
Mercury drop electrode	10-80 µg/mL (0.043-0.701 µM)	0.50 µg/mL (437.9 nM)	2.76	<b>(Proposed work)</b>

<sup>a</sup> LOD: Limit of detection

The recovery results of methimazole in serum samples were calculated from the related linear regression equations, which are given in **Table 1**. The LOD and LOQ values were also calculated and shown in Table 1. Repeatability and reproducibility of peak potential were also shown in **Table 1**.

#### Comparison of the methods

Voltammetry has been recently proposed as a promising new analytical method for electrochemical detection of drugs. Owing to the high sensitivity, low cost, simplicity of instrumentation and short analysis time voltammetric techniques are important methods for pharmaceutical analysis<sup>23,24</sup>.

SWP and DPP methods were applied for the determination of the commercial tablets (**Table 2**). The results show that high reliability and reproducibility of two methods. The best results were statistically compared using the t-test. At 95% confidence level, the

calculated t-values do not exceed the theoretical values (**Table 4**).

Therefore, there is no significant difference between SWP and DPP methods. The relative standard deviation (RSD) was 1.73% using the proposed methods for the polarographic analysis of thyromazol tablets. The validity of the proposed procedures applied to thyromazol tablets was also assured by the recovery of standard additions. A mean recovery of 99.2% with RSD of 4.74 was obtained. The results of the drug analysis obtained from the proposed methods are in close agreement with the claimed value. At the same time, the results obtained are also comparable with the results obtained from liquid chromatography and capillary zone electrophoresis.<sup>12,13</sup> Also, the results obtained using the proposed method in this study are well compared with several electrochemical methods for the determination of methimazole as shown in **Table 5**.

However, the results obtained from the proposed methods indicate that the methods are more precise and accurate for the determination of methimazole in drug samples. SWP and DPP are effective and rapid electroanalytical techniques with well-established advantages, including good discrimination against background current and low detection limits<sup>21</sup>. Two calibration graphs from the bulk solution of methimazole according to the procedures described above were constructed by using SWP and DPP. And, the methods are requiring less than 2 min to run samples.

## CONCLUSION

Two novel electro-analytical methods involving SWP and DPP at dropping mercury electrode were proposed to determine methimazole content in pharmaceuticals and spiked plasma. The SWP and DPP methods presented for the quantitative determination of methimazole allowed the accurate determination and was found to be rapid, simple and highly sensitive. The main advantage of such a procedure is the possibility to determine the concentration of the active component directly from the methimazole formulations and spiked serum samples without any previous treatment, such as extraction, clean-up, derivatization or pre-concentration which are tedious, time consuming and also polluting. Therefore, the proposed methods can be used effectively, without separation for routine analysis of methimazole in pure form, its formulations and human serum.

## Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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