

POTASSIUM PERMANGANATE-TOLUIDINE BLUE DYE SYSTEM: A RELIABLE SPECTROPHOTOMETRIC PROTOCOL FOR THE EVALUATION OF DOPAMINE HYDROCHLORIDE AND METHYLDOPA

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Abstract: A sensitive and rapid indirect spectrophotometric approach was presented for the precise determination of dopamine hydrochloride and methyl dopa in raw materials and commercial preparations. The suggested procedure is based on the oxidation of the studied drugs with a surplus of potassium permanganate in an acidic milieu. The unconsumed oxidant was then involved in the quantitative oxidation of toluidine blue dye. The decrease in dye color was followed at 628 nm. A linear relationship was observed between the absorbance and the concentration of the studied compounds within the interval of 0.25-9 and 0.5-9 µg/mL with molar absorptivity of 1.75×10^4 and 2.05×10^4 for dopamine hydrochloride and methyl dopa, respectively. LOD values were established at 0.107 and 0.119 µg/mL, while LOQ values were 0.356 and 0.397 µg/mL for the two drugs, respectively. The recovery percentages were consistently near 100%, and the RSD was less than 1% for both drugs. The proposed procedure was successfully applied on pharmaceutical preparations. The results were in excellent agreement with the certified values.

Keywords: Spectrophotometric determination, dopamine hydrochloride, methyl dopa using, toluidine blue, oxidation.

Introduction

Dopamine hydrochloride (DPM) and methyl dopa (MTD) are classified as catecholamines (Fig. 1). Both are white to yellowish-white crystalline powders, with melting points of 240°C and 300°C for DPM and MTD, respectively [1,2]. DPM is systematically named as 4-(2-aminoethyl)benzene-1,2-diol hydrochloride [1]. It is prescribed as a safe alternative for patients to improve cardiac output and increase blood flow to vital organs (especially the kidneys) in cases of shock, heart failure, or severe hypotension. It works by stimulating specific receptors in the body to enhance cardiovascular function [3]. MTD is chemically known as (2S)-2-amino-3-(3,4-dihydroxyphenyl)-2-methylpropanoic acid [2] (also known by the brand name "Aldomet") and is a centrally acting antihypertensive medication that works by relaxing blood vessels in the brain. Its primary use is to treat high blood pressure; it is considered safe and is commonly used to treat preeclampsia, which is known as high blood pressure during pregnancy [4].

Due to the therapeutic significance of DPM and MTD, various methods have been reported for their determination. For DPM, these include spectrophotometric [5-8], electrochemical [9-11], and chromatographic techniques [12-14], whereas for MTD, spectrophotometric [15-17], electrochemical [18-20], and chromatographic methods [21-23] have been published.

Over the last two or three decades, spectrophotometric methods have undergone a remarkable evolution, driven by the development of various chromogenic reagents. These techniques have excelled in utilizing versatile chemical reactions to yield spectrophotometrically active colored species, via either direct or indirect analytical pathways [24]. The widespread use and importance of spectrophotometric methods are due to their simplicity, accuracy and cost-effectiveness [25].

Toluidine blue dye (also known as toluidine blue O, TB) is a basic dye belonging to the phenothiazine family (Fig. 1) [26]. It is used in different biological and medical applications, such as staining mast cells and other tissues [27, 28], detection of oral cancer [29], and detection of *Helicobacter pylori* [30]. Analysts' preference for using dyes is driven by their remarkable sensitivity, accuracy and cost-effectiveness, coupled with their time-efficient performance. Analytical

procedures fall into two main concepts: the formation of ion-association complexes [31] and dye-oxidant systems [32].

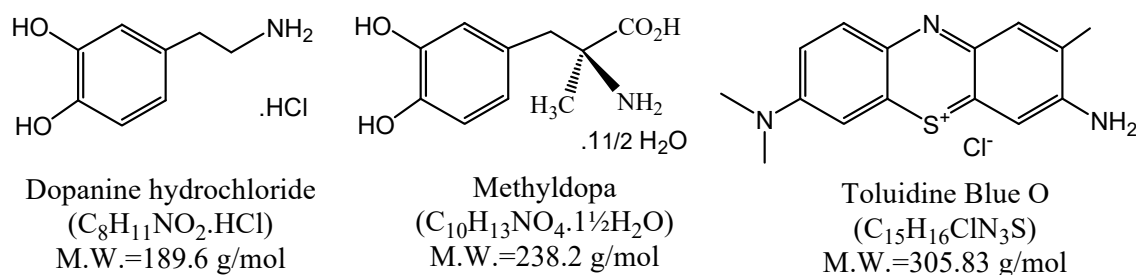


Fig. 1. Chemical structures of DPM, MTD and TB

The present study describes a straightforward and precise indirect spectrophotometric protocol for the estimation of two catecholamine drugs; DPM and MTD. The procedure relies on oxidizing the catecholamine using a specified surplus of potassium permanganate in an acidic solution, and subsequent determination of unreacted permanganate by bleaching of TB. The unreacted dye was monitored by absorbance measurement at 628 nm. The proposed method exhibited a good determination range along with robust statistical parameters and satisfactory agreement with the certified values of pharmaceutical forms.

Experimental part

All spectral measurements were conducted using a double-beam Shimadzu UV-1900i spectrophotometer provided with a 1 cm path length glass cuvettes. An ADAM electronic balance was used for all weighing procedures. Heating processes were maintained using an Elektro-mag water bath. All chemicals, drugs in bulk forms and reagents were of high purity and used as received without further purification. Stock and diluted solutions of drugs and chemical reagents were freshly prepared; appropriate dilutions were made using distilled water as illustrated in Table 1.

Table 1. List of the prepared solutions

Chemicals	Conc.	Preparation (Final dilution to 100 mL with distilled water)
DPM	50 µg/ml	Solubilization of 0.0050 g neat material in distilled water
MTD	50 µg/ml	Solubilization of 0.0050 g neat material in 2 mL of abs. ethanol
TB	50 µg/ml	Solubilization of 0.0050 g neat material in distilled water
KMnO ₄	0.001 mol/L	Solubilization of 0.0158 g neat material in distilled water
HCl	0.5 mol/L	Dilution of 4.14 mL of 12.07 mol/L acid with distilled water
H ₂ SO ₄	0.1 mol/L	Dilution of 0.546 mL of 18.3 mol/L acid with distilled water
Surfactants	0.1%	Solubilization of 0.1000 g neat material in distilled water

Adopted procedure for the calibration curve. Aliquots of (0.05-1.8) mL of DPM and (0.1-1.8) mL MTD (both 50 µg/mL) were pipetted into volumetric flasks of 10 mL capacity. Followed by 1 mL of sulfuric acid (0.1 M) was added to the flasks containing DPM, while 0.75 mL of hydrochloric

acid (0.5 M) was added to those of MTD. Afterward, 1 mL of KMnO_4 (1×10^{-3} mol/L) was added to each flask, and the mixture was allowed to stand for 3 min before adding 2 mL of TB (50 $\mu\text{g/mL}$). Subsequently, the solutions were adjusted to the mark. After mixing the flasks well and let them undisturbed for 10 min, the absorbance values were documented at 628 nm.

Pharmaceutical forms. The suggested method was applied to pharmaceutical forms supplied from the local market in the form of ampoules for DPM and tablets for MTD. DPM ampoules are labelled to contain 200 mg/5 mL. The solution of these was prepared by pipetting 5 mL from the ampoules into volumetric flasks of 100-mL capacity; the volumes were adjusted to the mark with distilled water, providing a 2000 $\mu\text{g/mL}$. A solution of 100 $\mu\text{g/mL}$ was prepared by dilution with distilled water. The latter was used to prepare other solutions. The procedure for MTD tablets was performed by weighing 10 tablets, which were smashed to fine powder. A portion equivalent to the weight of a single tablet (250 mg) was weighed and dissolved in 10 mL of ethanol with slight heating. After cooling to room temperature, the solution was transferred into a 250 mL volumetric flask. Distilled water was added to reach the calibration mark of the flask to yield a 1000 $\mu\text{g/mL}$ solution. The solution was filtered and employed for the formulation of the 100-mL mixture as well as additional preparations.

Results and Discussion

Preliminary study. TB is a basic dye, it has high stability, sensitivity and conspicuous color change during the oxidation reaction. This choice encouraged us to use it as a reagent for the indirect determination of DPM and MTD. As a preliminary step, it is necessary to obtain an absorbance spectrum for TB in the acidic medium under the existing laboratory conditions. Therefore, 2 mL of TB solution (50 $\mu\text{g/mL}$) was pipetted into individual volumetric flasks (10-mL capacity) with 1 mL HCl (1 mol/L). The total volume was adjusted to the graduation line with distilled water. After well mixing, the spectrum was performed and showed a maximum absorbance at 628 nm (Fig. 2).

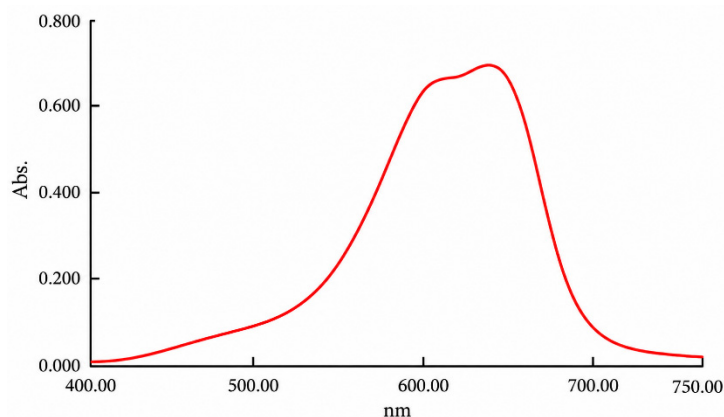


Fig. 2. Absorbance spectrum of 10 $\mu\text{g/mL}$ in acidic medium

Likewise, it is also necessary to construct a calibration graph for TB in the same medium condition to determine the topmost value of the linear response. Aliquots of increasing volumes of TB were pipetted into a series of 10 mL volumetric flasks. 1 mL of HCl (1 M) was added to each flask, and the mixture was diluted to the mark with distilled water. After well mixing, absorbance measurements were taken, and the obtained calibration graph was rectilinear (Fig. 3) in the range of 0.5-35.5 $\mu\text{g/mL}$. It is worth noting that one of the ideal features of dyes is providing a linear calibration graph within ranges and perfect coefficient of determination that impress analysts [5-7].

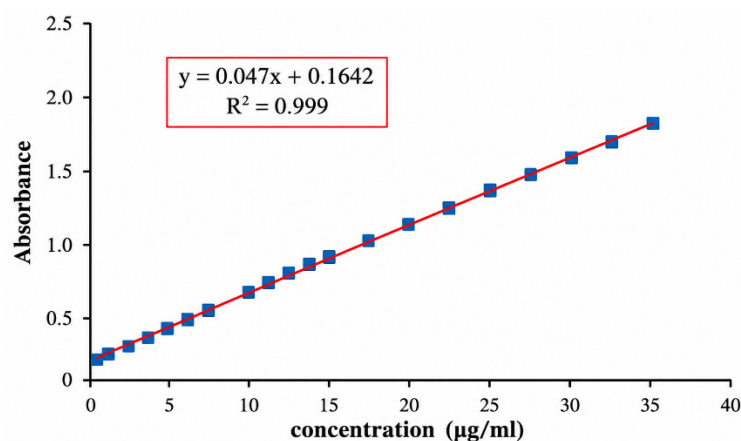


Fig. 3. Calibration graph of TB in acidic medium

Method optimization. Effect of oxidizing agent. As an initial step in the optimization process, the role of oxidizing species on the decolorization of TB ($10 \mu\text{g/mL}$) was investigated. Eight different types of oxidizing agents were explored.

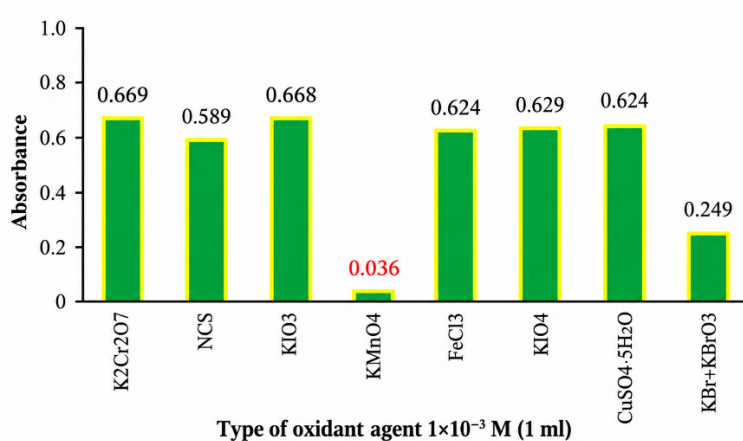


Fig. 4. Effect of oxidizing agent on TB decolorization

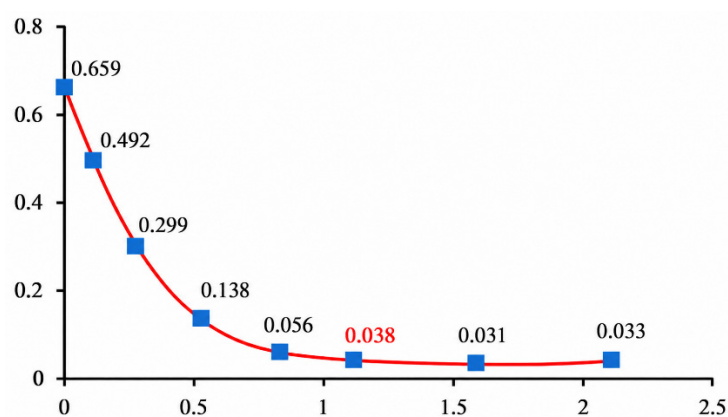


Fig. 5. Effect of KMnO₄ amount on TB decolorization

2 mL of TB solution ($50 \mu\text{g/mL}$) was transferred into a 10 mL volumetric flask, followed by the addition of 1 mL of HCl (1 mol/L). After that, 1 mL of oxidizing agent ($1 \times 10^{-3} \text{ mol/L}$) was added. The mixture was diluted up to the graduation line with distilled water. The flasks were shaken and the flasks were left to incubate for 10 min under ambient conditions. The measured absorbance was

recorded at 628 nm. The resulting data compiled in Fig. 4 demonstrate that KMnO₄ was the best oxidizing agent which gave the lowest absorbance. Otherwise, the effect of graduated volumes (0.1-2.0 mL) of KMnO₄ (1×10^{-3} mol/L) on TB bleaching was also investigated. The results in Fig. 5 presented that 1 mL is the best amount of KMnO₄.

Effect of acid. Most indirect spectrophotometric methods for the determination of drugs which are based on oxidation reactions involving the drug and dyes, must be carried out in an acidic medium, as the acid plays a crucial role in promoting the oxidation process. In our work, this section encompassed three aspects: type of acid, concentration of the chosen acid, and its optimal volume. According to Table 2, sulfuric acid (1 mol/L) provided the best absorbance for DPM whereas hydrochloric acid (1 mol/L) was the best for MTD. The optimal acid concentration and volume for sulfuric acid were 0.1 mol/L and 1 mL, and those for hydrochloric acid were 0.5 mol/L and 0.75 mL.

Table 2. Effect of acid type, concentration and volume

Drug	Acid type (1 mol/L)	Abs.	Acid conc. (mol/L)	Abs.	Acid vol. (mL)	Abs.
DPM	H ₃ PO ₄	0.332	0.03	0.375	0.1	0.328
			0.05	0.391	0.25	0.351
			0.07	0.402	0.5	0.382
			0.1	0.424	0.75	0.403
			0.3	0.409	1.0	0.435
	HCl	0.321	0.5	0.381	1.25	0.407
	HNO ₃	0.302	0.75	0.354	1.5	0.391
	CH ₃ COOH	0.285	1.0	0.361	1.75	0.351
					2	0.308
MTD	H ₃ PO ₄	0.295	0.03	0.394	0.1	0.358
			0.05	0.424	0.25	0.381
			0.07	0.431	0.5	0.416
			0.1	0.448	0.75	0.452
			0.3	0.421	1.0	0.418
	HCl	0.397	0.5	0.397	1.25	0.402
	HNO ₃	0.344	0.75	0.374	1.5	0.368
	CH ₃ COOH	0.308	1.0	0.303	1.75	0.328
					2	0.253

Effect of oxidation time: The time of drug oxidation is crucial, and it was investigated for both drugs. 1 mL of each drug was pipetted separately into 10 mL volumetric flasks. 1 mL of H₂SO₄ (0.1 mol/L) was added for DPM and 0.75 mL of HCl (0.5 mol/L) for MTD. 1 mL of KMnO₄ was also added to each flask. The solutions were allowed to stand for 1-15 min. After that, 2 mL of TB was added, and the total volume for each flask was adjusted to the graduation line with distilled water. Spectral measurements were performed for both drugs at 628 nm. Table 3 demonstrates that 3 min was the optimum duration.

Table 3. Effect of oxidation time

Oxidation time (min)	Abs.	
	DPM	MTD
1	0.385	0.401
2	0.402	0.409
3	0.439	0.458
4	0.410	0.403
5	0.384	0.393
7	0.341	0.371
10	0.315	0.354
15	0.286	0.331

Effect of temperature and stability. To study the temperature effect on absorbance and the stability of color, a 10 mL volumetric flask under the previously optimized addition and condition was

taken for both drugs. The effect of temperature was investigated for 20, 30 and 40 °C. The ideal temperature and time for color development for DPM and MTD were 20 °C (room temperature) and 10 min (Fig. 6). The colored solutions for the determination of both drugs were stable for more than 100 min at room temperature.

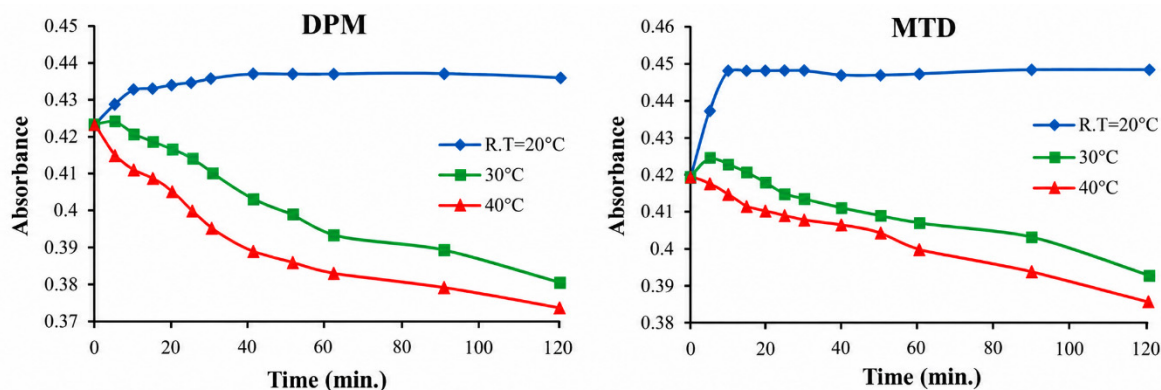


Fig. 6. Optimization of thermal conditions and stability on Abs.

Final absorbance spectrum: After determining all optimum conditions, the absorbance spectrum was recorded for TB in the presence of both drugs as shown in Fig. 7. To provide a useful overview, the established optimal conditions of the determination procedure are summarized in Table 4.

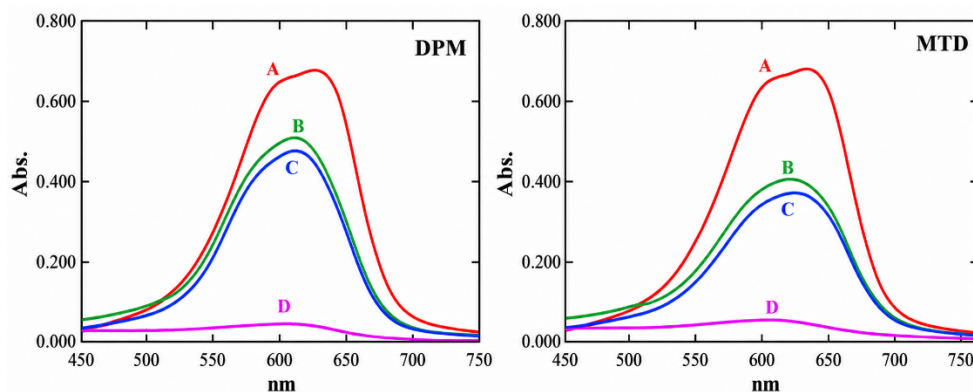


Fig. 7. Final spectrum of TB for determination of DPM and MTD A: Abs. spectrum of TB (10 µg/mL) containing: A: 10 µg/mL drug against blank. B: 5 µg/mL DPM and 4 µg/mL MTD against distilled water. C: 5 µg/mL DPM and 4 µg/mL MTD against blank. D: Abs. spectrum of blank vs distilled water

Table 4. Established optimal reaction conditions

Parameter	Value	
	DPM	MTD
TB (50 µg/mL) vol.	2	2
λ_{max} (nm)	628	628
Acid type	H ₂ SO ₄	HCl
Acid conc. (mol/L)	0.1	0.5
Acid vol. (mL)	1	0.75
KMnO ₄ (1×10 ⁻³) vol. (mL)	1	1
Temp. of the oxidation (°C)	20	20
Color development after addition (min.)	10	10

Stability duration (min)	120	120
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Calibration curve and analytical parameters. Under the optimum conditions, Calibration graphs were constructed for both drugs as presented in Fig. 8. The analytical parameters of the calibration graph are summarized in Table 5. According to the molar absorptivity and Sandell's sensitivity index, the results revealed high sensitivity, indicating an excellent linear relationship between absorbance and concentration.

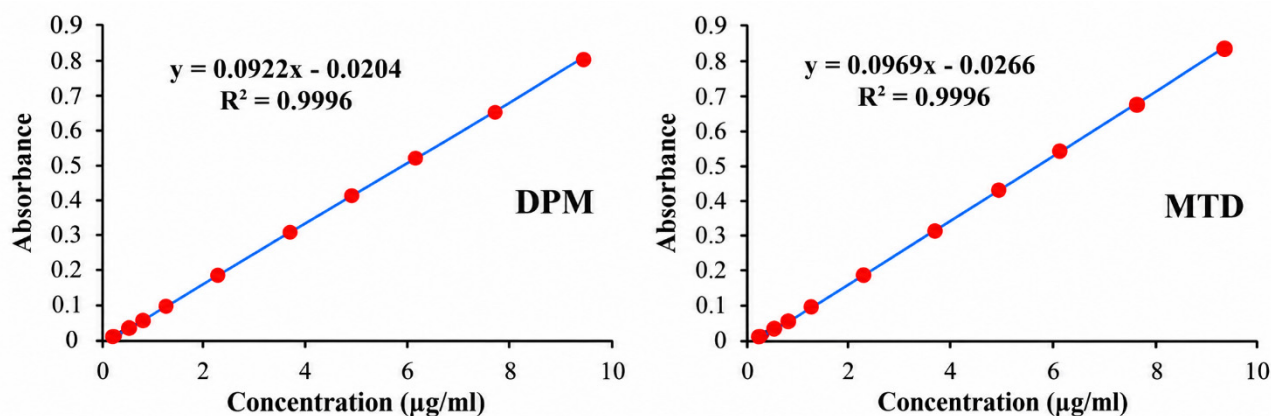


Fig. 8. Calibration graphs for determination of DPM and MTD

Table 5. Analytical parameter of determination method

Parameter	DPM	MTD
Linear scope (µg/ml)	0.25 - 9.0	0.5 - 9.0
Molar absorptivity (L/mol.cm)	1.75×10^4	2.05×10^4
Sensitivity index (Sandell) (ng/cm ²)	0.0108	0.0116
Limit of detection (µg/mL)	0.1067	0.1189
Limit of quantitation (µg/mL)	0.3558	0.3965
Slope	0.0922	0.0969
Intercept	0.0204	0.0266
Determination coefficient	0.9996	0.9996

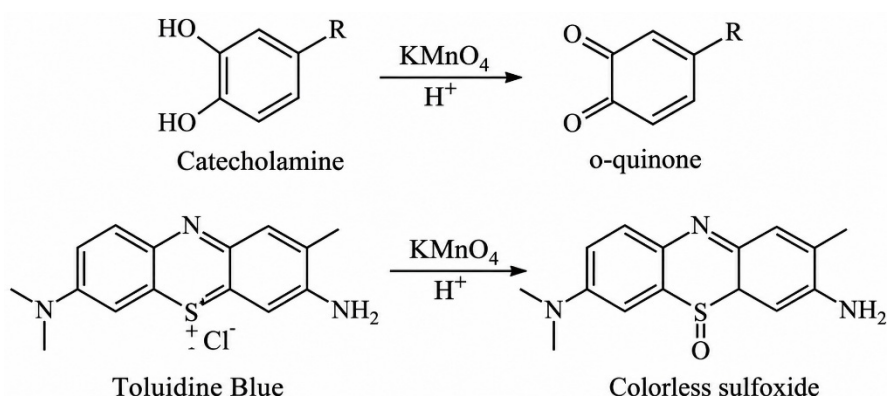
Method accuracy and compatibility: The precision and accuracy of the proposed approach were estimated by computing the percent recovery (Recovery %) and the coefficient of variation (RSD %). This estimation was performed using five replicate measurements for three concentrations of both DPM and MTD. As shown in Table 6, the resulting data revealed that the suggested approach achieves high repeatability.

Table 6. Reliability and method precision

Drug	Conc. (µg/mL)		Recovery* (%)	Average recovery (%)	RSD (%)
	Added	Detected			
DPM	2.5	2.44	97.96	99.63	1.31
	5.0	5.05	101.13		0.48
	7.5	7.48	99.81		0.32
MTD	2.5	2.53	101.46	100.51	1.38
	5.0	5.04	100.84		0.83
	7.5	7.44	99.23		0.29

*Average of five determinations.

Proposed mechanism of the reaction. Based on the empirical findings of the developed indirect estimation method and insights from established literature on dye–oxidant chemistry, a plausible reaction pathway is postulated. The mechanism initiates with the selective oxidation of the catecholamine core into its corresponding o-quinone derivative by potassium permanganate [33]. Upon the subsequent introduction of TB dye, the residual, unreacted oxidizing agent drives the bleaching process. This decolorization occurs via the oxidative disruption of the extended conjugated chromophore network that inherently governs the dye's intense color. The resulting transformation yields a colorless sulfoxide intermediate, manifested by a sharp, rapid attenuation in absorbance (Scheme 1). Under prolonged exposure, further exhaustive oxidation may proceed toward the complete fragmentation of the heterocyclic ring framework [6, 34, 35].



Scheme 1. Suggested mechanism

Application of the proposed method on pharmaceutical forms. Due to its high accuracy, the presented procedure was utilized for the determination of both drugs in commercial dosages as ampules for DPM and tablets for MTD. Three concentrations of each drug were taken: 2.5, 5.0 and 7.5 µg/mL. Table 7 illustrates the reliable results achieved by the suggested method.

Table 7. Determination of DPM and MTD in the dosage forms using the presented protocol

Dosage form	Labeled claim	Quantity present (µg/mL)		Obtained drug content* (mg)	Recovery * (%)	Average recovery (%)	RSD (%)
		Added	Detected				
DPM Injection	200 mg/5 mL	2.5	2.41	192.80	96.40	98.94	1.54
		5.0	5.03	201.22	100.61		0.46
		7.7	7.48	199.62	99.81		0.61
MTD Tablet	250 mg	2.5	2.53	252.62	101.05	99.90	1.49
		5.0	5.00	250.62	100.02		1.11
		7.7	7.39	246.57	98.63		0.26

*Average of five determinations.

The method of standard addition: To verify the performance and credibility of the presented protocol for the quantitation of the studied drugs free from any spectral or matrix interferences in dosage forms, the standard addition protocol was successfully applied to DPM and MTD. Two concentrations of each formulation were taken; 1 and 2.5 µg/mL. The data compiled in Fig. 9 and Table 8 confirm that the standard addition method is consistent with the presented method and has efficient selectivity.

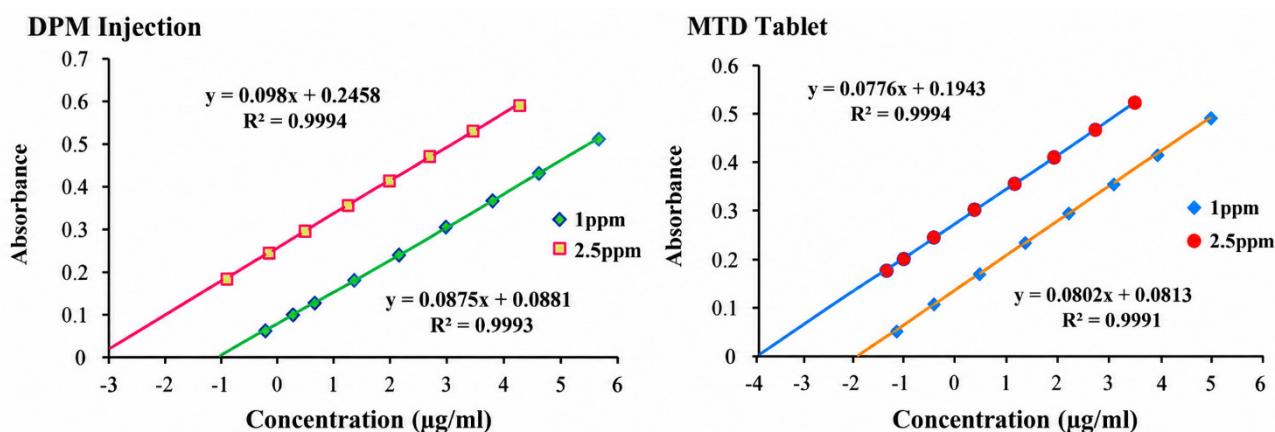


Fig. 9. The graph of standard addition for the estimation of DPM and MTD in their dosage forms

Table 8. Application of the standard addition method for the estimation of DPM and MTD in their dosage form

Dosage form	Labeled claim	Quantity added (µg/mL)	Recovery (%)	Drug content detected (mg)
DPM Injection	200 mg/ 5 mL	1	100.68	201.36
		2.5	100.32	200.64
MTD Tablet	250 mg	1	101.37	253.43
		2.5	100.15	250.38

As a concluding assessment of the presented method, it is useful to provide a brief comparison with previously published methods. As compiled in Table 9, the suggested method demonstrates augmented sensitivity based on the molar absorptivity. Moreover, a key advantage of this method is its simplicity, less time-consuming, and that it does not require pH adjustment or precise temperature control.

Table 9. Comparative evaluation of the proposed procedure with previously reported protocols

Parameter	Proposed procedure		Published method [36]	
	DPM	MTD	DPM	MTD
Type of reaction	Indirect (dye bleaching)	Indirect (dye bleaching)	Indirect (dye bleaching)	Indirect (dye bleaching)
Reagent used	Toluidine blue	Toluidine blue	Calcon dye	Calcon dye
Type of Oxidizing Agent	Potassium permanganate	Potassium permanganate	N-bromo succinimide	N-bromo succinimide
λ max (nm)	628	628	510	510
Medium of reaction	Acidic	Acidic	Acidic	Acidic
Linear scope (µg/ml)	0.25-9	0.5-9	1-36	2-40
Molar absorptivity (l/mol.cm)	1.75×10^4	2.05×10^4	4.3×10^3	3.2×10^3
Application	Injection	Tablet	Injection	Tablet

Conclusion

Spectrophotometric determination methods rank among the most precise analytical techniques for the quantification of various compounds in diverse fields, particularly pharmaceuticals, due to their importance to human health and the availability of samples for application. These analytical methods are characterized by their reliability and selectivity when a suitable reagent is available, as well as their simplicity and the elimination of sample extraction or preparation. Organic dyes have added value to determination methods; they serve as highly efficient colorimetric reagents,

characterized by exceptional chemical stability, a high molar absorptivity, a sharp absorbance peak, and compliance with Beer-Lambert's law within a good range of concentrations. This work describes the highly accurate determination of two catecholamine drugs; dopamine hydrochloride and methyl dopa in an aqueous acidic medium, demonstrating their successful compatibility with pharmaceutical formulation analysis. Furthermore, the proposed method has significant potential for the indirect determination of many drugs and oxidizable compounds, utilizing toluidine blue as a colorant, since it is a dye that exhibits almost total bleaching upon oxidation. The proposed method is characterized by its high sensitivity and low cost.

Conflicts of Interest

The authors declare no conflict of interest.

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