



Spectrophotometric Determination of some Drugs using Oxidation Reduction Reactions

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Abstract

A spectrophotometric method is proposed for the determination of some drugs containing amino group such as mesalazine, metoclopramide and dopamine in pharmaceutical formulations. It was simple, precise, accurate, rapid, and based on the oxidation of each drug with chromate as an oxidizing agent in the presence of 1N hydrochloric acid. Then indigo carmine is reacted with residual chromate in the presence of a catalysis factor (sodium oxalate). Increasing in absorbance's value of the color system is proportional to the amount of the three drugs which is measured at the selected wavelength of 610 nm.

The proposed method is obeying Beer's law in the ranges of (1-40, 2-44 and 2-52) ppm for the concentration of mesalazine, metoclopramide and dopamine respectively. Molar absorptivity was 0.191×10^4 , 0.449×10^4 and 0.191×10^4 $\text{L.mol}^{-1}.\text{cm}^{-1}$ mesalazine, metoclopramide and dopamine respectively. While, Sandell's sensitivity index of 0.0806, 0.0667 and 0.0806 $\mu\text{g.cm}^{-2}$ mesalazine, metoclopramide and dopamine respectively.

The proposed spectrophotometric method has a good recovery when it is applied for the determination of the three drugs in pure form and pharmaceutical doses.

Keywords: Spectrophotometric oxidation-reduction reaction, Mesalazine, Metoclopramide, Dopamine

1. Introduction

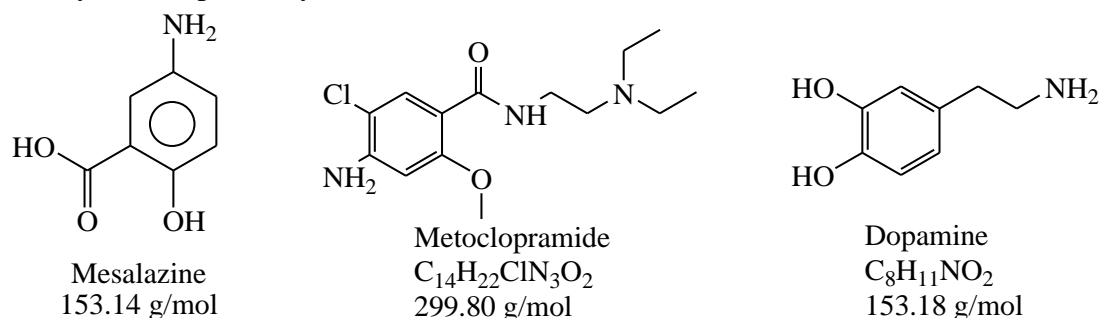
Mesalazine and metoclopramide are used for treating of digestive system diseases, drugs containing amino group as well as dopamine **Scheme 1**. Metoclopramide is one of the medications groups which is known as dopamine-receptor antagonists, therefore these drugs have been chosen in this study.

Mesalazine (MEZ), also named as 5-amino-2-hydroxybenzoic acid, is white color for powder and crystals [1]. it is relatively insoluble in some organic solvents like ether and chloroform, while it is solubility increased in a diluted solution of bases and acids [2]. Some biological terms like enzymes are inhibited to be produced by MEZ, such as synthetase and cyclo-oxygenase [3]. also, MEZ played an important role as an activating factor of the

platelet. MEZ as an anti-inflammatory drug is used for treating the common disease of inflammatory bowel and Crohn's disease [4, 5].

Metoclopramide (MET) is a medication chemically known as 4-amino-5-chloro-N-(2-diethylaminoethyl)-2-methoxybenzamide [1]. and used for stomach and esophageal problems. Also MET is used to treat hyperemesis gravidarum by pregnancy's women as a second choice. There is a relation between MET and DOP, MET belongs to medications group which is known as dopamine-receptor antagonists. People with Parkinson's disease must be noticed closely when they are used the medication DOP as an antagonist to treat the emesis. Patients are not recommended to take MET if they have been taken antipsychotics drugs [6,7].

Dopamine (DOP) chemically named as 4-(2-Aminoethyl) benzene-1,2-diol[1]. DOP is synthesized in the brain, plants and most animals. It is a chemical send signal to nerve cells when it is released by other nerve cells, dopamine's pathways in the brain are controlling the release of various hormones. Any dysfunctions of the dopamine system are associated with important diseases in the nervous system such as Parkinson's disease; it is causing motor impairment and tremor. The important function of dopamine is a neurotransmitter in the brain. Other functions increase the excretion of sodium and output urine from kidneys. It reduces insulin production as well as reduces gastrointestinal motility, protects intestinal mucosa, reduces the activity of lymphocytes in the pancreas and in both digestive system and immune system respectively [8-11].



Scheme 1. Chemical structure of MEZ, MET and DOP.

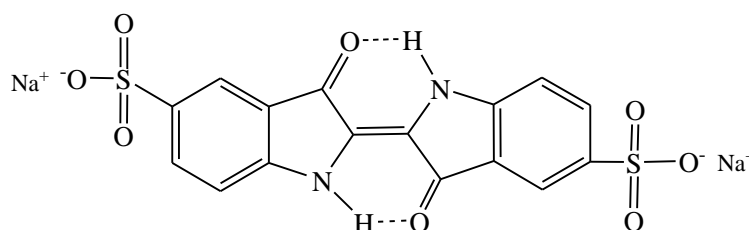
Several techniques, including spectral methods, were used to estimate three drugs as documented in the literature. MEZ was evaluated in its pure form and in its pharmaceutical preparations using various organic reagents such as 1, 2-Naphthoquinone-p-sulphonate, 4-dimethylaminocinnamaldehyde [12]. o-chloranil [13]. 1, 5-diphenylcarbazine [14]. N-(1-naphthyl) ethylenediamine and 8-hydroxyquinoline [15]. Also, inorganic reagents are used to determine MEZ like sodium nitroprusside with hydroxylamine HCl [16]. and ferric nitrate [2]. Another method depends on ultra violet region to determine MEZ [17,18].

Spectrophotometric methods are also used for the determination of MET with its degradation products [19]. as well; others applied for the determination of MET in bulk and pharmaceutical preparations [20-22]. MET has been determined in pharmaceuticals and spiked human urine using diazotization reaction [23-26]. Sequential injection is used for spectrophotometric determination of MET [27]. Development and validation of UV-spectroscopic method are used for assaying of MET in bulk and injectable dosage form [28]. Batch and cloud point extraction spectrophotometric is used for the determination of DOP [29]. A spectrophotometric method is used for determination of DOP in various samples such as bulk and injectable forms [30, 31]. Other used charge transfer reaction with bromanil to determine DOP [32]. Or, we used a spectrophotometric evaluation of DOP and progesterone

in breast cancer serum [33]. Standard addition methods are used for determination of DOP and levodopa in tablets and ampoules [34].

DOP can be determined spectrophotometrically in biological and pharmaceutical samples [35]. Flow injection analysis has been used for the determination of methyl dopa and DOP in pharmaceutical preparations [36]. also, DOP was determined in various samples such as pharmaceutical, banana, urine and serum samples by potassium ferricyanide-Fe (III)[37]. Another method is used for the determination of DOP in the microliter scale using the microfluidic system based on polymeric technology [38].

The organic reagent which used in this work is named indigo carmine **Scheme 2**. indigo carmine (INC) also named as 5,5'-indigodisulfonic acid disodium salt. It is an oxidation-reduction indicator, prepared by sulphonation process of indigo. INC is rapidly dissolved in water, and it is safe to use as a colorant of food as well as a dye in the industry of capsules [39].



Indigo Carmine

M.wt = 466.36 g/mol

$C_{16}H_8N_2Na_2O_8S_2$

Scheme 2. The chemical structure of INC.

2. Experimental

2.1 Equipment

Double beam JascoV-630-Uv-visible spectrophotometer with a pair of a plastic 1-cm matched cells were used for measuring absorbance while a of HANNA instrument pH 211 microprocessor pH meter was used for pH measurements.

2.2 Drug Stock Solution, (1000 µg/ml)

0.1 g of MEZ was weighed and dissolved in 10 ml of ethanol absolute and diluted with distilled water using a 100 ml volumetric flask. While (MET & DOP) was prepared by dissolving the same weight (0.1g) of pure dopamine hydrochloride (Fluka) or metoclopramide (SDI) in distilled water then diluted it to the mark with the same solvent in 100 ml volumetric flask. Standard solutions were prepared by suitable dilution of the stock standard solution.

2.3 Chromate Ion Solution, (8.6×10^{-4} M) Solution

0.0167 g of potassium chromate (Fluka) was dissolved in distilled water. Then the final volume brings to 100 ml in an amber color volumetric flask, which is being stable more than 1 month [24].

2.4 INC solution, (1×10^{-3} M)

0.1165 g of indigo carmine dye (BDH) was weighed, dissolved then completed by distilled water to a 250 ml in a dark volumetric flask in order to be stable for 3 days.

2.5 Catalyst solution of sodium oxalate, (0.1 M)

1.34 g of sodium oxalate (Fluka) was dissolved in distilled water to prepare 0.1 M of this solution which is diluted by distilled water using a 100 ml dark volumetric flask.

2.6 Hydrochloric Acid Solution, (1N).

A suitable appropriate dilution of (21.85 ml) of concentrated hydrochloric acid (density=1.16) with distilled water in 250 ml volumetric flask to prepare 1 N of HCl.

2.7 Pharmaceutical Formulations (tablet)

Ten tablets of pharmaceutical formulations for MEZ, MET and DOP were crushed and mixed well then, an equivalent weighed to 0.01 g of drugs were dissolved, filtered then diluted to 100 ml with a clean and dry volumetric flask.

2.8 Pharmaceutical Preparation (Ampoule)

Plemazol, 10mg/2ml (SDI Co. Iraq) 2 ml ampoules each containing 0.01 g of MCP were transferred to 100 ml volumetric flasks and completed the volume with distilled water. Dopamine hydrochloride ampoule (200mg/5ml): one ampoule of dopamine hydrochloride was diluted to 100 ml in a volumetric flask with distilled water. Then 5ml from the above solution was diluted to 100 ml in a volumetric flask with distilled water to obtain (100 ppm).

3. Results and Discussion

Different analytical parameters which were affected on the optimum conditions of the color development for 100 µg of each drug in 25 ml as a final volume, then the absorbance intensity was measured at 610 nm and optimum pH from 3.2 to 3.4 for the proposed method.

3.1 Effect of Type and Quantity of Acid

Various quantities (0.1-2.0 ml) of an acids such as (HCl, H₂SO₄, HNO₃, and CH₃COOH), were used to determine the optimum pH for the oxidation-reduction reaction between chromium ion and drugs, HCl is considered to be optimum in this study because of the best absorbance values so that, (0.1-2.0 ml) of 1M hydrochloric acid was studied. **Figure 1.** shows that use 1 ml of hydrochloric acid (1 M) was optimum due to a high value of absorbance and the more stable colored dye. This scale was adopted in subsequent experiments.

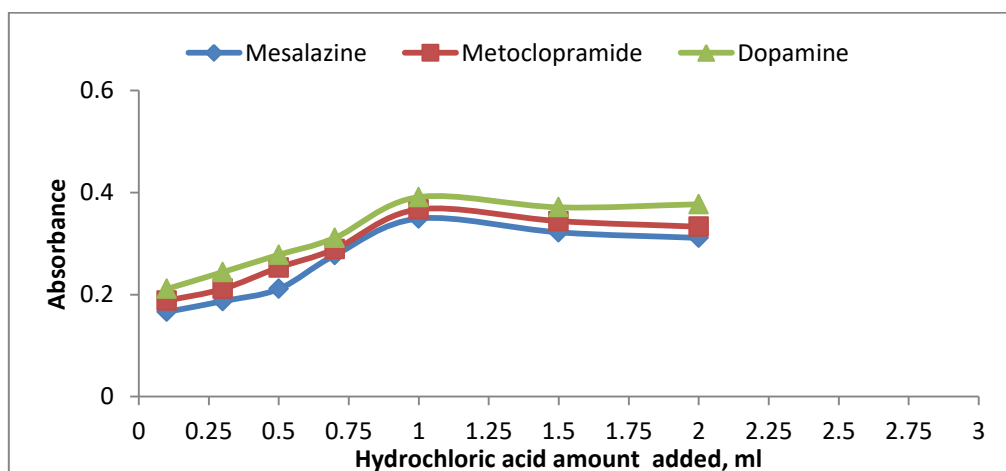


Figure 1. Study the effect of hydrochloric acid.

3.2 Study the Effect of Catalyst

Sodium oxalate was used as a promoting the activation of Cr (VI) oxidation's system [40]. Thus, different quantities of (0.1 M) $C_2O_4^{2-}$ solution (1.0-3.0) ml were tested with (100) μg of drugs in final volume of 25ml. It is observed from **Figure 2.** that using (2.0) ml of $C_2O_4^{2-}$ solution was optimum amount because it gives the best stability and absorbance, therefore, it used in subsequent experiments.

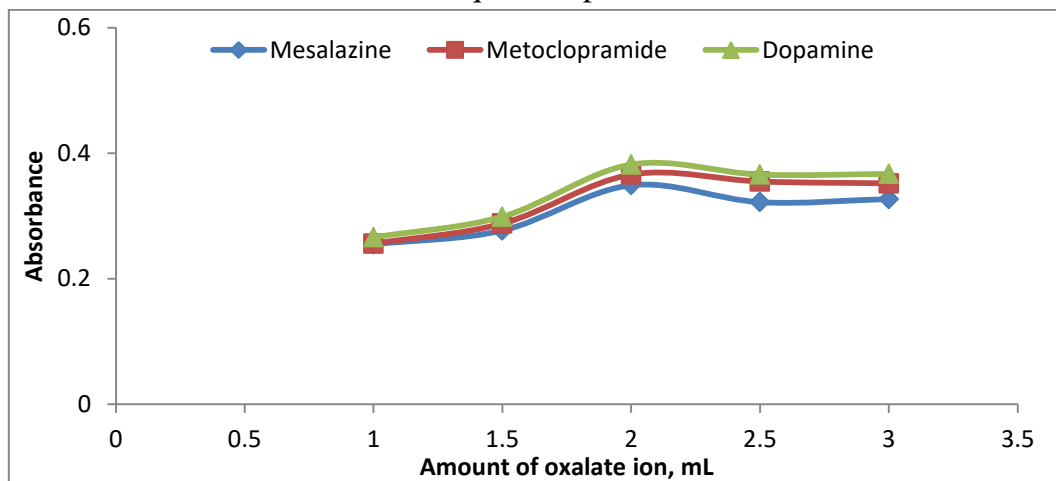


Figure 2. Effect of catalyst.

3.3 Study the Amount of Chromate Ion

To choose the suitable amount of chromate (VI) which oxidized the drugs, various amounts (0.5-3.0) mL of (8.6×10^{-4} M) chromate and (100-1000) μg of drugs solution in final volume 25 ml were studied. Resulted from this study that 1.5 ml of Cr (VI) was considered to be optimum because the higher value of determination coefficient (0.9909, 0.9947 and 0.9966) for MEZ, MET and DOP respectively, for this reason it selected for the subsequent experiments

3.4 Study the Effect of Reagent's Amount

The addition of indigo carmine as a reagent on the reaction mixture to produce the bluish color. This was investigated by different amount of (0.5-2.0) ml 1.0×10^{-3} M of the dye, the experimental results indicated that (1.0) ml of indigo carmine reagent was optimum volume (determination coefficient (R^2) 0.9935, 0.9954 and 0.9964), for MEZ, MET and DOP respectively, and fixed for the subsequent experiments

3.5 Study the Effect of Addition's Order

Different orders were studied to check the best orders of addition. **Table 1.** indicates that the first order for each drug is considered to be optimum and selected for the subsequent experiments for MEZ, MET and DOP because it gave the highest absorbance value, that means the addition of oxidant agent to the drugs must followed by catalyst in presence of acid medium to complete the oxidation process, then, the reagent must be added at last.

Table 1. Order effect of addition.

Reaction components	Order number	Absorbance
MEZ		
D + O + C+ H + INC	I	0.334
D + H + O + C + INC	III	0.281
D + INC + O + H + C	IV	0.089
D + C + O + H + INC	II	0.262
MET		
D + O + C+ H + INC	I	0.361
D + H + O + C + INC	III	0.296
D + INC + O + H + C	IV	0.110
D + C + O + H + INC	II	0.271
DOP		
D + O + C+ H + INC	I	0.390
D + H + O + C + INC	III	0.287
D + INC + O + H + C	IV	0.121
D + C + O + H + INC	II	0.298

D= MEZ or MET or DOP, O=Oxidant, H=Hydrochloric acid, INC=Indigo carmine, C=Catalyst.

3.6 Study the Stability Time

The intensity of the absorbance for various quantities of MEZ, MET and DOP at 610 nm in the reaction mixture was measured at various time intervals. **Figure 3.** shows the absorbance when it is measured immediately as well as after two hours is seemly to be constant.

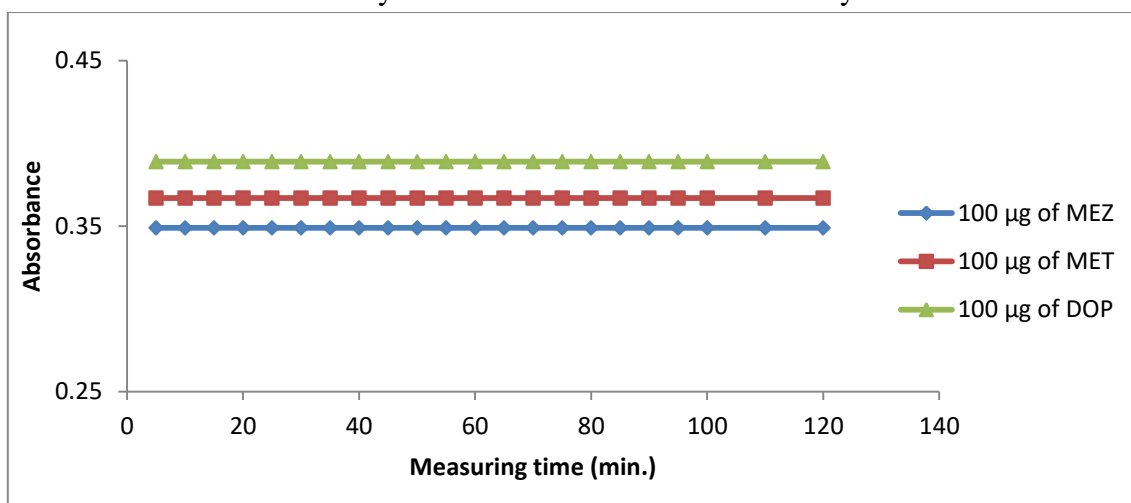


Figure 3. Study the stability of the resulting dye.

3.7 The Final Absorption Spectrum and the Calibration Graph of the Three Drugs

Drugs (MEZ, MET and DOP) were treated according to the optimum conditions, the final spectrum and calibration are shown in **Figure 4,5.** While **Table 2.** Indicates some analytical variables of the present method. A linear calibration graph was measured at 610 nm are obtained over the concentrations (1-40, 2-44 and 2-52) ppm for MEZ, MET, and DOP respectively. Molar absorptivity of 0.191×10^4 ; 0.449×10^4 and 0.191×10^4 L. mol⁻¹.cm⁻¹ for MEZ, MET, and DOP respectively, and Sandell's index sensitivity 0.0806; 0.0667 and 0.0806 µg.cm⁻² for MEZ, MET, and DOP respectively.

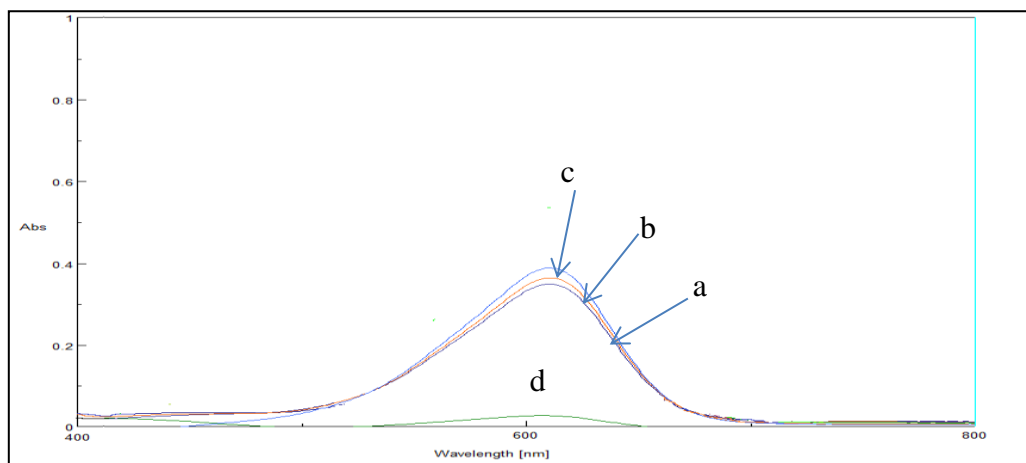


Figure 4. Final absorption spectra of 100 µg of (a) MEZ, (b) MET and (c) DOP/25 ml measured against a reagent blank (d) blank against distilled water.

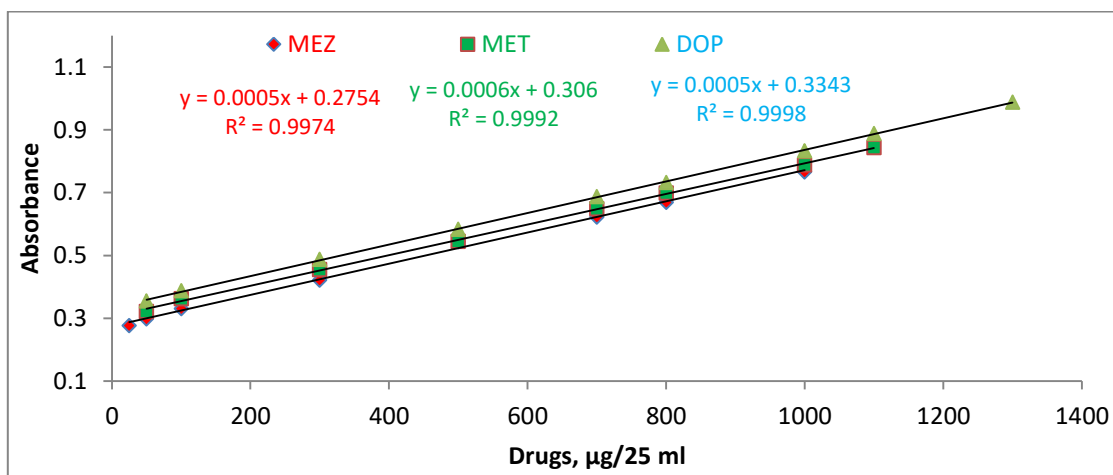


Figure 5. Calibration's graph of drugs (MEZ, MET and DOP).

Table 2. Analytical parameters of a proposed method.

Analytical variables	Values
Molar absorptivity (L.mol ⁻¹ .cm ⁻¹)	0.191×10 ⁴ MEZ; 0.449×10 ⁴ MET; 0.191×10 ⁴ DOP
Color's stability (minutes)	120.0
Sandell's sensitivity	0.0806 MEZ; 0.00667MET; 0.0806DOP
Regretion equation	y = 0.0005x + 0.2754 MEZ; y = 0.0006x + 0.306 MET; y = 0.0005x + 0.334 DOP
Coefficient of determination	0.9974 MEZ; 0.9992 MET; 0.9998 DOP
Nature of the resulted dye	1 : 1

3.8 Method's Accuracy and its Precision

Average of five determinations of MEZ, Met and DOP were determined at various quantities (100, 300 and 500) µg/25ml for each one to check and test the accuracy as well as the precision for the calibration curve. The results are tabulated in **Table 3**. Are reliable.

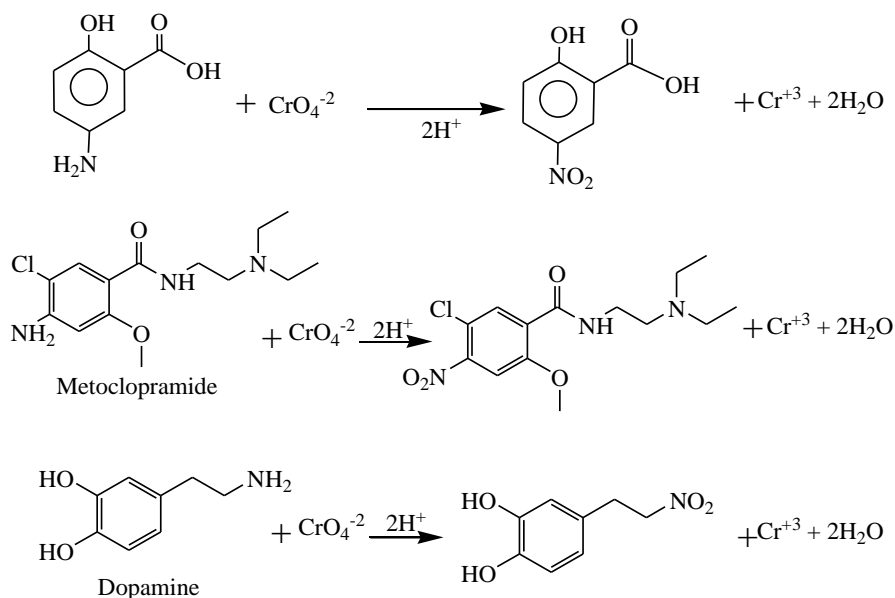
Table 3. Accuracy and precision.

Quantity of MEZ measured, µg/25ml	Recovery %*	RSD, %*
100.0	99.74	±0.4082
300.0	99.59	±0.3247
500.0	99.32	±0.2698
Quantity of MET measured, µg/25ml	Recovery %*	RSD, %*
100.0	100.54	±0.5290
300.0	100.21	±0.2901
500.0	100.36	±0.2895
Quantity of DOP measured, µg/25ml	R, %*	RSD, %*
100.0	99.70	±0.4748
300.0	100.23	±0.3729
500.0	99.81	±0.2895

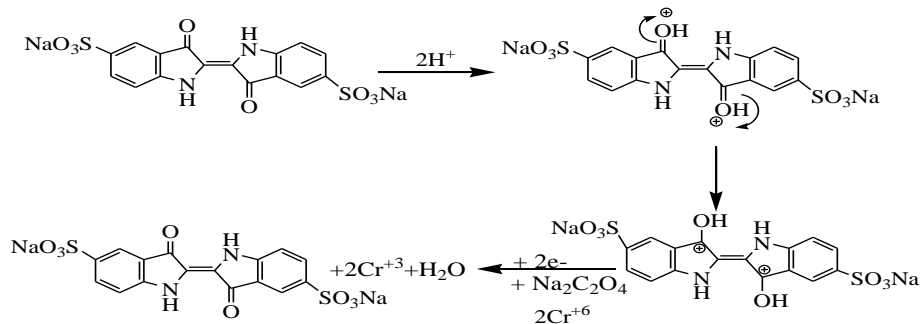
* Average for 5 measurements.

3.9 Mole Ratio

Continuous variations (Job's method) have been used for the determination the reaction ratio of the three drugs with chromate. The experimental results indicated that 1:1 belong to ratios of MEZ, MET and DOP to chromate respectively. Depending on this ratio, the reaction's mechanism is suggested as below **Scheme 3**. [44,45].



The probable reaction mechanism **Scheme 4**. Has been suggested between the oxidizing agent (chromate VI) and the reagent (indigo carmine dye) in the presence of catalyst agent (sodium oxalate) in an aqueous solution of hydrochloric acid [41]. as follows:



3.10 Study the Effect of Interferences

To study the selectivity and efficiency of the present method, the effect of some foreign materials that are usually present in dosage forms (gum acacia, lactose, menthol, starch and glucose) were added in different amounts (100, 500, 1000) to (100) μg of drugs/25 ml. There is no effect of foreign substances in the proposed method as it was obtained from **Table 4**.

Table 4. Effect of interferences.

Recovery (%)									
Drugs, μg	MEZ, 100 μg			MET, 100 μg			DOP, 100 μg		
Interferences, μg	100	500	1000	100	500	1000	100	500	1000
Acacia	99.70	99.40	99.10	99.72	99.73	100.27	98.97	98.73	98.50
Glucose	100.29	100.59	99.70	100.28	100.54	100.82	99.74	99.48	99.23
Lactose	100.59	99.70	100.29	99.44	99.73	99.72	100.77	100.25	100.26
Menthol	100.30	100.90	99.40	99.17	99.45	98.89	100.25	100.51	100.26
Starch	100.60	100.59	99.69	100.28	100.27	100.55	100.52	100.51	100.77

3.11 Application of the Proposed Method

The experimental results in **Table 5**. indicate that a good value of recovery, relative error as well as the measured quantity of each drug when the present method applied on the pharmaceutical preparations for MEZ, MET and DOP.

Table 5. Application of the method.

MEZ	μg MEZ present/25ml	μg MEZ measured/25ml	R*, %	R.E*, %
Pentasa tablet, 500 mg (Ferring, Germany)	200	199.48	99.74	-0.2600
	400	398.34	99.58	-0.4150
	600	596.89	99.48	-0.5183
Awasalazine tablet, 400 mg, (Awamedica, Iraq)	200	197.91	98.95	-1.0450
	400	396.67	99.16	-0.8325
	600	595.85	99.31	-0.6916
MET	μg MET present/25ml	μg MET measured/25ml	R*, %	R.E*, %
Plemazol, 10mg/2ml (SDI Co. Iraq)	200	199.01	99.50	-0.4950
	400	399.21	99.80	-0.1975
	600	598.03	99.67	-0.3283
Meclodin, 10mg (CID Co. Egypt)	200	197.53	98.76	-1.235
	400	394.49	98.62	-1.3775
	600	591.13	98.52	-1.4783
DOP	μg DOP present/25ml	μg DOP measured/25ml	R*, %	R.E*, %
Dopamine hydrochloride (200 mg/5 mL) miser. Co Egypt	200	198.64	99.32	-1.3500
	400	398.54	99.63	-1.6400
	600	597.20	99.53	-1.5466
Dopamine hydrochloride injections (Biologici Italy Lab., Novate, Milano - Italy): 200 mg/5 mL)	200	197.30	98.65	-0.6800
	400	393.44	98.36	-0.3650
	600	590.72	98.45	-0.4666

* Average of five determinations.

Table 6. Shows the calculated value of t-test at the 95% confidence level for five degrees of freedom [42]. Did not exceed the theoretical values (t-tabulated is 4.95) when the present method is compared with literature methods [43, 21, 30].

Table 6. T-test values of MEZ, MET and DOP.

Pharmaceutical preparations	Values of t-test
Pentasa tablet, 500 mg (Ferring, Germany)	± 1.8185
Plemazol, 10mg/2ml (SDI Co. Iraq)	± 1.1821
Dopamine hydrochloride injections (Biologici Italy Lab., Novate, Milano - Italy): 200 mg/5 mL)	± 0.6469

3.12 The Comparison of the Proposed Method with Literature

Several analytical variables those have been explained in **Table 7**. shows the comparison between the proposed and some methods in the literature.

Table 7. Proposed and literature method's comparison.

Analytical variables	Present method	methods in Literature		
		MEZ [40]	MET [21]	DOP [30]
Reaction	Oxidation - reduction	Oxidative coupling	azo coupling reaction	Complex formation
λ_{\max} (nm)	610	530	459	470
Reagent	Indigo Carmin	Pyrocatechol	Orcinol	DCQ
Beer's law range ($\mu\text{g. mL}^{-1}$)	1-40 MEZ; 2-44 MET; 2-52 DOP	0.4-10	0.6-12	4-45
Molar absorptivity ($\text{L.mol}^{-1}.\text{cm}^{-1}$)	1900 MEZ; 4490 MET; 1900 DOP	3685	19044	1938.76
Medium	HCl	H_2SO_4	NaOH	-----
Color's stability (minutes)	120.0	65.0	24 hrs.	-----
Nature of the resulted dye	1: 1	1: 1	-----	1: 4
Method's application	Pharmaceutical formulation	Pharmaceutical preparation	Pharmaceutical preparation	Bulk and Injectable Forms

DCQ: 2,6-dichloroquinone 4-chloroimide.

The results in **Table 7**. Showed that the present method is somewhat sensitive to apply for the pharmaceutical preparations of each drug (MEZ, MET and DOP).

4. Conclusion

In this work, a spectrophotometric method was proposed which is described as an accurate, simple and fast. It was used to estimate three drugs containing the active amino group (MEZ, MET and DOP) using oxidation-reduction reaction. This method is obeying Beer's law in the ranges of 1-40, 2-44 and 2-52 ppm for the concentration of MEZ, MET and DOP respectively. Molar absorptivity was 0.2×10^4 , 0.5×10^4 and 0.2×10^4 $\text{L.mol}^{-1}.\text{cm}^{-1}$ MEZ, MET and DOP respectively. The proposed method was applied successfully to the determination of those three drugs in pure and their pharmaceutical preparations.

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