



Spectrophotometric estimation of Methyldopa and Isoprenaline in pure form and pharmaceutical preparations

Hind Khalid Salman

Khalida Mohammed Omar

University of Mosul, College of Science, Department of Chemistry, Mosul, Iraq.

Article Information

Article history:

Received: September 20,2023

Reviewer: December 4,2023

Accepted: December 28,2023

Available online

Keywords:

*Spectrophotometry,
Catecholamine, Methyldopa,
Isoprenaline, p-Nitroaniline*

Correspondence:

Abstract

Two simple, rapid, highly sensitive, and accurate spectrophotometric methods were utilized to estimate catecholamine drugs methyldopa and isoprenaline. Oxidative coupling reaction was used to estimate methyldopa using ferric nitrate as an oxidizing agent and coupled with mesalazine reagent in an alkaline medium of sodium hydroxide solution producing a blue color measured at λ_{max} 578 nm, linearity of calibration curve was in concentration range 2 -28 $\mu\text{g/ml}$ with a correlation coefficient of 0.9986, molar absorptivity and Sandell's index were calculated, equal to $0.566 \times 10^4 \text{ l.mol}^{-1}.\text{cm}^{-1}$ and $0.042 \mu\text{g.cm}^{-2}$ respectively, limit of detection and quantification values were equal to 0.06509 and 0.2169 $\mu\text{g/ml}$, respectively. Isoprenaline was estimated using a azo-coupling reaction using a diazotized p-Nitroaniline reagent coupled with isoprenaline in an alkaline medium of sodium hydroxide solution and 5rt presence of TritonX-100(1%) surfactant solution. The orange-red azo dye produced which measured at λ_{max} 510 nm., calibration curve linearity was in concentration range between 2 -20 $\mu\text{g/ml}$ with a correlation coefficient of 0.9985, molar absorptivity, Sandell's index were calculated and equal to $2.3434 \times 10^4 \text{ l.mol}^{-1}.\text{cm}^{-1}$ and $0.0105 \mu\text{g.cm}^{-2}$ respectively, LOD and LOQ were equal to 0.02119 and 0.07064 $\mu\text{g/ml}$, respectively. These methods proved successful in estimating methyldopa and isoprenaline in pure forms and pharmaceutical preparations.

التقدير الطيفي لأدوية الكاتيكول امين المثيل دوبا والإيزوبرينالين في الصورة النقية وفي المستحضرات الصيدلانية

هند خالد سلمان خالدة محمد عمر

جامعة الموصل كلية العلوم قسم الكيمياء

الملخص:

تم استخدام طريقتين طيفيتين بسيطتين وسريعتين وعاليتين الحساسية ودقيقتين لتقدير دوائي الكاتيكول امين المثيل دوبا الإيزوبرينالين. تم استخدام تفاعل الاقتران التأكسدي لتقدير المثيل دوبا باستخدام نترات الحديد كعامل مؤكسد واقتترانه بكاشف ميزالازين في وسط قاعدي من محلول هيدروكسيد الصوديوم ليعطي ناتج أزرق اللون وتم قياس الامتصاص عند الطول الموجي 578 نانومتر وكانت خطية منحنى المعايرة في نطاق التركيز 2-28 مايكروغرام/مليلتر مع معامل ارتباط 0.9986 وتم حساب قيم الامتصاص المولاري وحساسية ساندل وكانا يساويان 0.566×10^4 لتر. مول⁻¹. سم⁻¹ و 0.042 ميكروغرام. سم⁻² على التوالي، كانت قيم حد الكشف وحد التقدير الكمي تساوي 0.06509 و 0.2169 مايكروغرام/مليلتر على التوالي. تم تقدير الإيزوبرينالين باستخدام تفاعل الاقتران الأزوي باستخدام كاشف البار-نيتروأنيلين المؤزوت مقترناً مع الإيزوبرينالين في وسط قاعدي من محلول هيدروكسيد الصوديوم بوجود محلول TritonX-100 (1%) ليتم إنتاج صبغة الأزو البرتقالية الحمراء التي تم قياس امتصاصها عند الطول الموجي 510 نانومتر، وكانت خطية منحنى المعايرة في نطاق التركيز بين 2-20 مايكروغرام/مليلتر مع معامل ارتباط 0.9985، وتم حساب قيم الامتصاص المولاري وحساسية ساندل وكانا 2.3434×10^4 لتر. مول⁻¹. سم⁻¹ و 0.0105 ميكروغرام. سم⁻² على التوالي، كانت حد الكشف وحد التقدير الكمي تساوي 0.02119 و 0.07064 مايكروغرام/مليلتر على التوالي. وقد أثبتت الطريقتان نجاحهما في تقدير مستويات المثيل دوبا الإيزوبرينالين في أشكالهما النقية وفي المستحضرات الصيدلانية.

الكلمات الدالة: الطيف الضوئي، الكاتيكول امين، المثيل دوبا، الإيزوبرينالين، بار-نيتروأنيلين.

Introduction

Methyldopa (MED) and Isoprenaline hydrochloride (ISP) belong to catecholamine drugs which are known as vicinal-diols aromatic compounds that own amines linked to a benzene ring. Containing two hydroxyl groups (catechol) [1]. MED belongs to the drug category α_2 -adrenoceptor agonist and is chemically known as [(2S)-2-Amino-3-(3,4-dihydroxyphenyl)-2-methylpropanoic acid sesquihydrate (L-methyldopa sesquihydrate)], its molecular weight 238.2 g/mole with the chemical formula ($C_{10}H_{13}NO_4 \cdot 1\frac{1}{2}H_2O$) as shown in Fig.1 [2]. MED acts in the central nervous system as an α_2 -adrenergic agonist where neurons take it up and undergo decarboxylation and hydroxylation reactions to form the pseudo transmitter α -methyl norepinephrine which binds to α -2 receptors and thus induce elastic relaxation and lowers blood pressure [3]. MED tablet is widely used in clinical practice as an antihypertensive drug for moderate to severe hypertension, especially hypertension caused by kidney dysfunction as well as hypertension in women through pregnancy [4]. Still, the drug has many side effects such as drowsiness, liver problems, damage to red blood cells, and allergic symptoms to the drug [5] as well as dizziness, headache, dry mouth, and depression [6].

Isoprenaline hydrochloride (ISP) belongs to the drug category beta-adrenoceptor agonist in the heart, bronchi, skeletal muscle, and gastrointestinal tract and was approved for use by the FDA in 1982 as a non-selective beta-adrenergic receptor agonist and a trace amine-associated receptor agonist [7]. ISP is widely used to treat cardiac arrest, heart block, and slow heart rate, and rarely, it is used to treat asthma [8]. Stimulation of beta-adrenergic receptors by ISP in the heart produces positive inotropic effects (increases contractility), increases heart rate, increases conduction rate through the atrioventricular node, and increases myocardial relaxation during diastole, so these effects increase systemic blood pressure and reduce diastolic blood pressure through their vasodilatory effects [9]. ISP shown in Fig.2 is available in the form of injections, tablets, oral suspension, and in the form of inhalation spray and the drug has the molecular formula $C_{11}H_{17}NO_3 \cdot HCl$ and a molecular weight of 247.72 g/mol [10]. ISP drug has many side effects, when used in high doses it undergoes a process of auto-oxidation which leads to the formation of free radicals that stimulate the oxidation of fats which causes the destruction and damage of the cardiac muscle cell membrane [11]. Also, excessive stimulation of beta receptors using isoprenaline leads to physiological disorders such as high blood pressure, atherosclerosis, myocardial infarction, and myocardial necrosis [12].

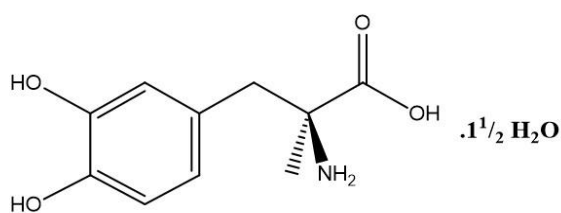


Fig.1 Chemical structure of MED

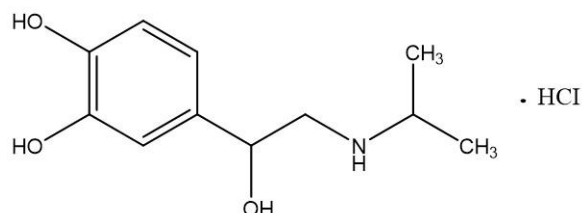


Fig.2 Chemical structure of ISP

According to the literature MED is estimated by several different methods such as spectrophotometric [13,14,15,16], chromatography [17,18,19], amperometry [20], voltammetry [21,22,23], spectrofluorometry [24]. ISP is also estimated by several different methods but most of them are voltammetry such as [25,26,27,28,29], chromatography [30,31,32], chemiluminescence [33], spectrofluorometry [34], spectrophotometric [35,36]. However, several of these methods require expensive equipment and versed operators, therefor in the present paper we develop two simple and sensitive methods to determine methyl dopa and isoprenaline spectrophotometrically by oxidative coupling and diazotization coupling methods, respectively.

EXPERIMENTAL

Apparatus

Absorption spectra and absorbance measurements were measured using a Shimadzu UV-Visible 160 spectrophotometer with a 1.0 cm light path, the weight was performed using BEL-type sensitive balance, and for pH measurement HANNA PH 211 was used.

Chemicals and reagents

The BDH and Fluka companies equip all chemicals and reagents with high purity while methyl dopa and isoprenaline are bought from (SDI) company.

Methyl dopa solution (100µg/ml)

This solution was prepared by dissolving 0.0100g of pure MED in 5 ml of ethanol with heating and stirring until dissolving, then diluting the solution to 100 ml with distilled water in a volumetric flask and keeping the solution in a dark bottle.

Isoprenaline hydrochloride solution (100µg/ml)

ISP solution was prepared by dissolving 0.0100g of the pure drug in distilled water then diluting the solution to 100 ml with distilled water in a volumetric flask and keeping the solution in a dark bottle.

Mesalazine reagent solution (1×10^{-2} M)

The solution of Mesalazine was prepared by dissolving 0.0153g in 5 ml ethanol and 20 ml distilled water with heating and stirring until dissolving, then diluting it to 100 ml with distilled water in a volumetric flask.

Diazotized p-Nitroaniline solution DPNA (1×10^{-2} M)

This solution was prepared by dissolving 0.138g of PNA in 20 ml hydrochloric acid solution 1.0 M and the volume completed to 80ml with distilled water, then the solution was heated with stirring until dissolving, after that, the solution was transferred to 100 ml volumetric flask and cooled in an ice bath until the temperature reach (0-5 °C), then 0.069g of

sodium nitrite added to the solution with stirring for 5 minutes followed by transfer the diazotized solution to a dark bottle and kept in the refrigerator for one day [37].

Ferric nitrate solution (1×10^{-2} M)

This solution was prepared by dissolving 0.024g of ferric nitrate in distilled water with stirring until dissolving and then diluting it to 100 ml with distilled water in a volumetric flask.

Sodium hydroxide solution (1.0 M)

This solution was prepared by diluting an ampole of NaOH 10M (100 ml) in a 1000 ml volumetric flask with distilled water, then the solution was stored in a plastic container.

Methyldopa tablet solution (100 μ g/ml)

Five tablets from each one alone (Methyldopa /Iraq, 250 mg MED), (Methyldopa /UK, 250 mg MED), and (Kadomet/Egypt, 250 mg MED) were weighed, powdered, and mixed. An exact amount of the powder equal to 0.0100g of MED was dissolved in 5 ml of ethanol, heating and stirring until dissolving, then filtered in a conical flask and completed in a volumetric flask with 100 ml distilled water.

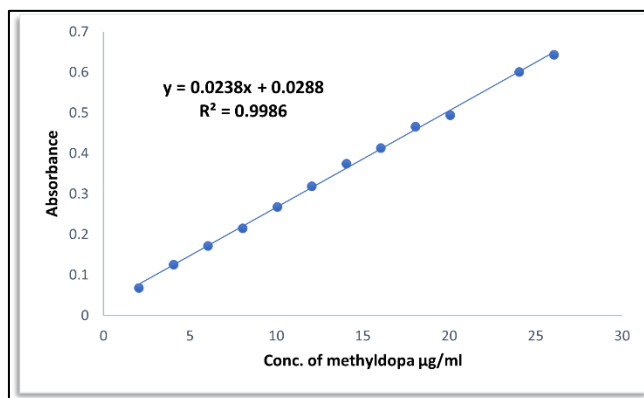
Isoprenaline hydrochloride Injection solution (20 μ g/ml)

Five ampoules of (Isoprenaline Injection IP, 2mg/1ml, Samarth company, India) were mixed. A 2 ml was transferred from the solution to a 50 ml volumetric flask and diluted with distilled water to get a concentration of 80 μ g/ml, and from it was prepared 20 μ g/ml.

A. Estimation of methyldopa by oxidative coupling reaction using mesalazine and Ferric nitrate

General recommended and calibration graph procedure

Various volumes of standard solutions of methyldopa (100 μ g/ml) transfer to a series of 10 ml volumetric flasks to cover the range of concentration from (2 -28 μ g/ml), then 1.0 ml of mesalazine (0.01M) is added and followed by 0.5 ml of ferric nitrate (0.01 M) after that waiting for 5 minutes to complete the oxidation reaction of MED, after that was adding of 2.5 ml of sodium hydroxide (1.0 M) and waiting for 10 minutes and diluted to the mark with distilled water and measured the absorbance of the colored product at 578 nm. against reagents blank. The calibration graph was linear over the concentration range of 2-26 μ g/ml, and negative deviation from Beer's law was noticed at concentrations higher than 26 μ g/ml (Fig. 3). The value of molar absorptivity was calculated and equal to 0.566×10^4 l.mol⁻¹.cm⁻¹ and Sandell's index equal to 0.042 μ g.cm⁻², The detection limit (LOD) and the quantification limit (LOQ) [38] were 0.06509 and 0.2169 μ g/ml, respectively.



(Fig. 3) Calibration graph for estimation of MED

RESULT AND DISCUSSION

A.1 Principle of the method

An oxidative coupling reaction for the estimation of methyl dopa involves a coupling reaction of MED and mesalazine using ferric nitrate as an oxidizing agent and an alkaline medium to produce a colored product.

A.2 Selection of the better oxidizing agent

The best oxidizing agent was studied by adding 1.0 ml of the available oxidizing agents in concentration 0.01M of each one into a 10 ml volumetric flask which contained 1.0 ml MED and 1.0 ml of mesalazine 0.01M, then the volumes were completed with distilled water to the mark. The solution was left to stand for 5 min to complete the oxidation and then 1 ml of 1M NaOH was added, then the solution was left for another 5 min. The absorbance was measured against the reagents blank, and the results in (Fig 4) and (Tablet 1) showed that ferric nitrate is a better oxidizing agent that gives a better lambda max with a better absorbance value.

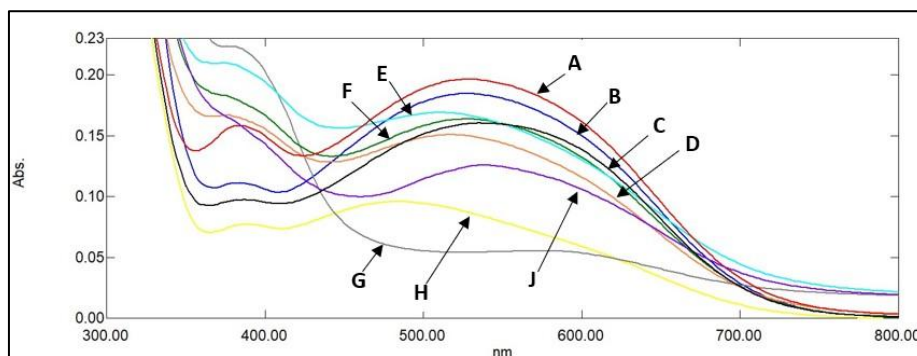


Fig.4: Selection of the best oxidizing agent

Table 1: Selection of the best oxidizing agent

Oxidizing agents (1×10^{-2} M)	Absorbance	$\lambda_{\max}$, (nm)
Ferric nitrate (A)	0.194	528
Sodium persulfate (B)	0.185	526

Ammonium ferric nitrate (C)	0.160	532
Potassium iodate (D)	0.151	518
Chloramine T (E)	0.169	510
potassium periodate (F)	0.164	528
N-chlorosuccinimide (G)	0.347	330
Ferric chloride (H)	0.160	532
N-bromosuccinimide (J)	0.126	540

A.3 Selection of the best concentration of the reagent and oxidizing agent

To select the best concentration of mesalazine and ferric nitrate, in a 10ml volumetric flask containing 1.0ml of MED, 1.0 ml of mesalazine was added in two different concentrations, and then 1.0 ml of ferric nitrate also in two concentrations was added, the solution left to stand for 5 min. And then 1ml of NaOH was added and after 5 min. The absorbance was measured at 528 nm. against a reagent blank, the result in (Table 2) shows that 1×10^{-2} M was the best concentration for both the reagent and oxidizing agent.

Table 2: Selection of the best concentration of the reagent and oxidizing agent

Conc. of Mesalazine (M)	Conc. of ferric nitrate (M)	Absorbance	$\lambda_{\max}$, (nm)
1×10^{-2}	1×10^{-2}	0.196	528
1×10^{-2}	1×10^{-3}	0.174	524
1×10^{-3}	1×10^{-2}	No colour appeared	
1×10^{-3}	1×10^{-3}	No colour appeared	

A.4 Selection of the best volume of the ferric nitrate and mesalazine

After choosing the best concentrations, it has been studied the best volume of the ferric nitrate, the results in (Table 3) show that 0.5ml is the best volume that completes the oxidation reaction and gives the best linearity for the reaction. In addition, it was kept using 1.0 ml of mesalazine in subsequent experiments because it gave the best absorbance as shown in (Fig.5).

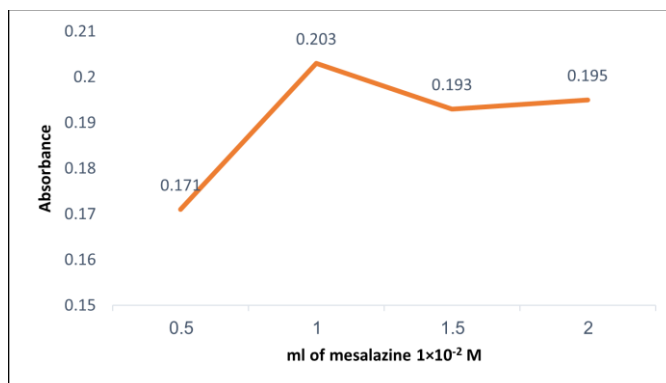


Fig.5: Selection of the best volume of the mesalazine

Table 3: Selection of the best volume of the ferric nitrate

ml of ferric nitrate (1×10^{-2} M)	Absorbance / μ g of methyldopa in 10 ml					R^2
	50	100	150	200	250	
0.3	0.104	0.187	0.243	0.352	0.402	0.9891
0.5	0.134	0.199	0.252	0.342	0.393	0.9931
0.8	0.128	0.193	0.240	0.332	0.379	0.9900
1	0.123	0.193	0.271	0.299	0.342	0.9677
1.5	0.0649	0.164	0.242	0.294	0.328	0.9610

A.5 Selection of the best base

Different kinds of strong and weak bases were studied, and the results shown in (table 4) that sodium hydroxide is an ideal medium for the reaction. In addition, added 2.0 ml of 1M NaOH is accompanied by Bathochromic shift (red shift), and added 2.5 ml of NaOH gives a higher absorbance, which was chosen as the better amount in subsequent experiments as shown in (Table 5).

Table 4: Selection of the best base

Base solution 1 ml of 1 M	NaOH	KOH	NaHCO ₃	Na ₂ CO ₃	NH ₄ OH
Absorbance	0.198	0.169	0.062	0.081	0.053
pH	12.70	12.61	9.77	10.60	9.34

Table 5: Selection of the best volume of base

ml of 1M NaOH	0.5	1	1.5	2	2.5	3	3.5
Absorbance	0.160	0.196	0.219	0.247	0.258	0.255	0.250
$\lambda_{\max}$ (nm)	528	528	528	578	578	578	578

A.6 Effect of time on the reaction

This effect was studied by adding 1.0ml of MED and 1.0ml of mesalazine, followed by adding 0.5ml of ferric nitrate, and waiting for a different time (3-20) min. before the addition of

the base, then added 2.5ml of NaOH and waited for a different time (5-30) min. (Table 6) shows that 5 min. is the best time for oxidation of MED, and 10 min. was the best time for completing the reaction.

Table 6: Effect of time on the reaction

Standing time before addition NaOH, min.	Absorbance/ Standing time after addition of NaOH and before dilution, min.				
	5	10	15	20	30
3	0.250	0.254	0.252	0.250	0.250
5	0.254	0.266	0.265	0.264	0.264
10	0.248	0.269	0.270	0.271	0.270
15	0.249	0.271	0.270	0.271	0.270
20	0.249	0.267	0.266	0.268	0.268

A.7 Effect of temperature on the reaction

The effect of temperature on the reaction was studied with different degrees (5-50 °C). The results in (Table 7) show that the best absorbance value was taken at room temperature (24°C ±2).

Table 7: Effect of temperature on the reaction

Temp. °C	5	10	24±2	40	50
Absorbance	0.233	0.245	0.268	0.247	0.238

A.8 Effect of addition sequence of the reaction component

Several experiments with different sequences of the ferric nitrate were conducted to get a better oxidation reaction, the result in (Table 8) shows that the sequence followed was the best so, it was continued.

Table 8: Effect of addition sequence of the reaction component

Sequence number	Reaction component *	Absorbance
I	D + M + F + B	0.264
II	D + F + M + B	0.259
III	M + D + F + B	0.255
IV	M + F + D + B	0.258

*Methyl dopa (D), Mesalazine (M), Ferric nitrate (F), NaOH (B)

A.9 Effect of surfactants on the reaction

The effect of different four types of surfactants (positive, negative, and neutral), which are (CTAB, SDS, CPC, (1×10^{-3} M), and triton X-100 (1%), studied their effect on the

absorbance intensity of the colored resulting. The results obtained explain that the absorbance reduces after the addition of these surfactants, so they are neglected.

A.10 Studied the stability time of the resulting product

The stability of the blue color of the resulting product was studied for two different concentrations of methyldopa (10 and 20) $\mu\text{g/ml}$ by measuring the absorbance for different periods, the reading values were observed to be stable for at least 60 min. as shown in (Fig. 6).

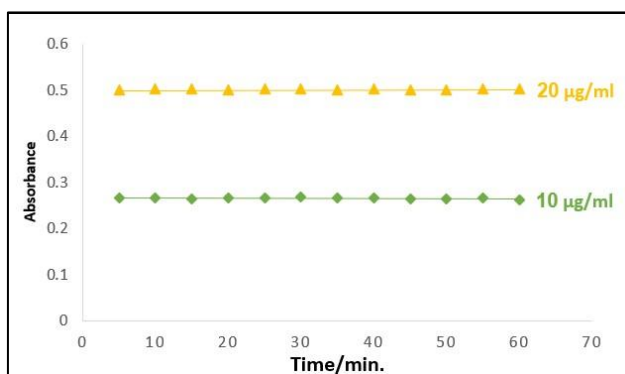


Fig.6: the stability time of the resulting product

A.11 Effect of additives excipients on the reaction

Several types of drug excipients that were added when manufacturing the pharmaceutical preparations (Glucose, Starch, Arabic Gum, Sucrose, Talk, and Lactose) were studied under the optimal conditions by adding different quantities of these additives (200, 500, 1000) to 100 $\mu\text{g}/10\text{ ml}$ of methyldopa. The results show no significant interference from these compounds indicating the selectivity and efficiency of the suggested method for its applications in pharmaceutical preparations.

A.12 Final absorption spectrum for the estimation of MED

Methyldopa was treated according to the proven optimal conditions, the final absorption spectrum in (Fig 7) shows the spectrum for 10 $\mu\text{g/ml}$ MED versus the distilled water and versus the blank, the spectrum of the blank versus the distilled water.

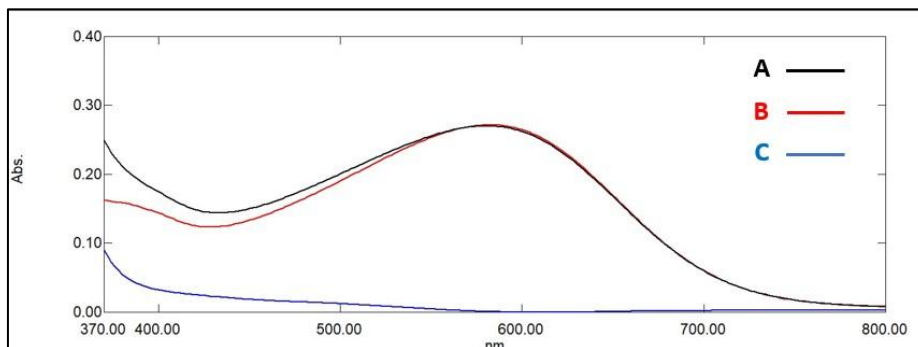


Fig. 7: Final Absorption Spectrum for 10 µg/ml MED

(A) Sample versus water, (B) Sample versus blank, (C) Blank versus distilled water

A.13 Accuracy and precision for the estimation of methyldopa

The accuracy and precision of the estimation of MED were calculated by applying the previously described approved working method for two different concentrations (10 and 20) µg/ml. The recovery and relative standard deviation were calculated, and the results were included in (Table 9) which showed that the proposed method was satisfactory.

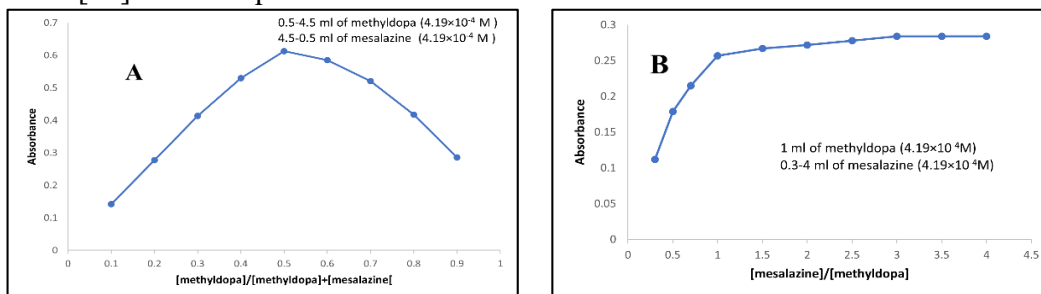
Table 9: Accuracy and precision for the estimation of methyldopa

Amount of methyldopa µg/ml		Recovery, %*	Relative error, %*	Relative standard deviation, %*
Taken	Found			
10	10.014	100.15	0.15	0.4260
20	19.98	99.91	-0.09	0.2305

*Average of five determinations

A.14 Nature of the reaction between MED and mesalazine

To understand the mole ratio of the reaction between the MED and mesalazine under the optimized conditions previously described, applying the variation method (Job's method) and molar ratio method [39] used the same concentration for each MED and mesalazine (4.19×10^{-4} M) and the results in (Fig.8) shows that the ratio of the reaction was 1:1 for MED and mesalazine, also applied slope ratio method by drawing two calibrations between MED and mesalazine in the same conc. (4.19×10^{-4} M), (Fig. 9) shows the drawing of the two standard curves, where the result of dividing the slope of the first curve by the slope of the second curve confirms the ratio of the reaction was 1:1. The stability constant value of the product was also calculated [40]. It was equal to 4.249×10^5 l/mol.

**Fig.8: (A) Job's method (continuous variation), (B) Mole ratio method**

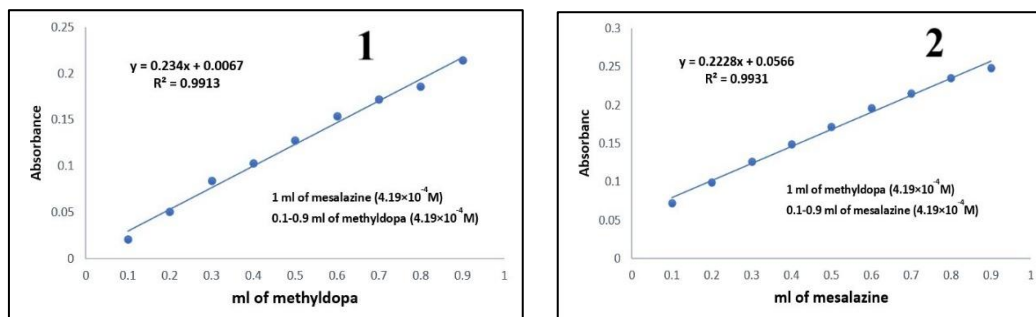
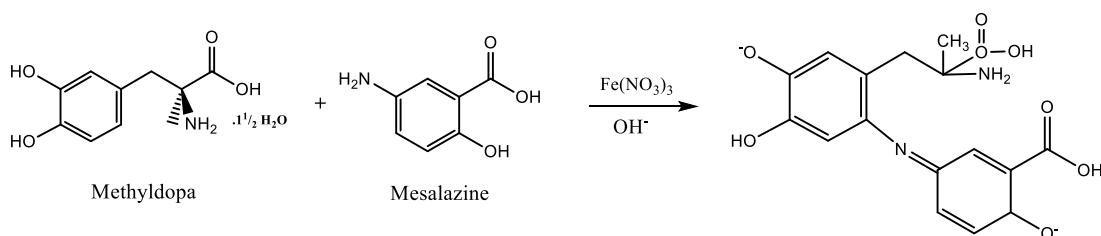


Fig. 9: Curves of slope ratio between MED and mesalazine

Therefore, according to the obtained result shown in Figures 8 and 9, the suggested reaction is explained below:



The stability Constant (K_s) of the Formed color has been studied for two concentrations, and the average of K_s values is equal to 4.249×10^5 l/mol. This indicates that the colored product has high stability.

A.15.1 Application part of the reaction between MED and mesalazine

The proposed method for the determination of methylodopa was applied in its available samples dosage form tablets [(Methylodopa, 250mg, Iraq) and (methylodopa, 250mg, UK) and (Kadomet, 250mg, Egypt)] with two different concentrations (10, 20) $\mu\text{g/ml}$. The results in (Table 10) evidence the success of the proposed method for determination of MED.

Table 10: Application of the determination of methylodopa

Pharmaceutical Preparations	Amount of Methylodopa $\mu\text{g/ml}$	Recovery, %*	Relative error, %*	Relative stander deviation, %*
Methylodopa, Tablet 250mg, Iraq	10	101.11	1.11	0.7380
	20	100.56	0.56	0.2624

Methyldopa, Tablet 250mg, UK	10	98.80	-1.2	0.3159
	20	99.75	-0.25	0.1697
Kadomet, Tablet 250mg Methyl., Egypt	10	103.82	3.82	0.6081
	20	102.52	2.52	0.1102

*Average of five determinations [41]

A.15.2 Appraisal of the suggested method by applied standard addition method

Applied the standard addition method to prove the efficiency and effectiveness of the suggested method for the determination of MED in its pharmaceutical preparations and prove there are no interferences of the drug with additives existing in the pharmaceutical preparations. The results in (Fig.10) and (Table 11) indicate that the standard addition method is approved with the suggested method.

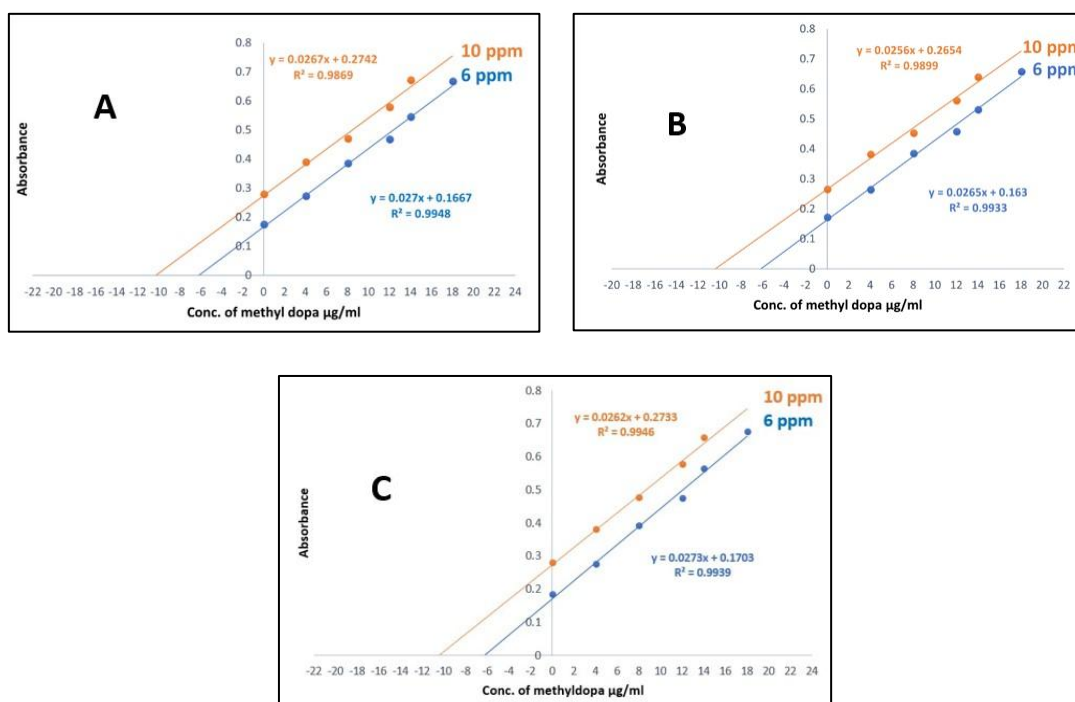


Fig. 10: Standard addition curves for determination of MED preparations

(A) Methyldopa,250mg/tablet, Iraq (B) Methyldopa,250mg/tablet, UK (C) Cadomet,250mg/tablet, Egypt

Table 11: Determination of MED in pharmaceuticals by standard addition method

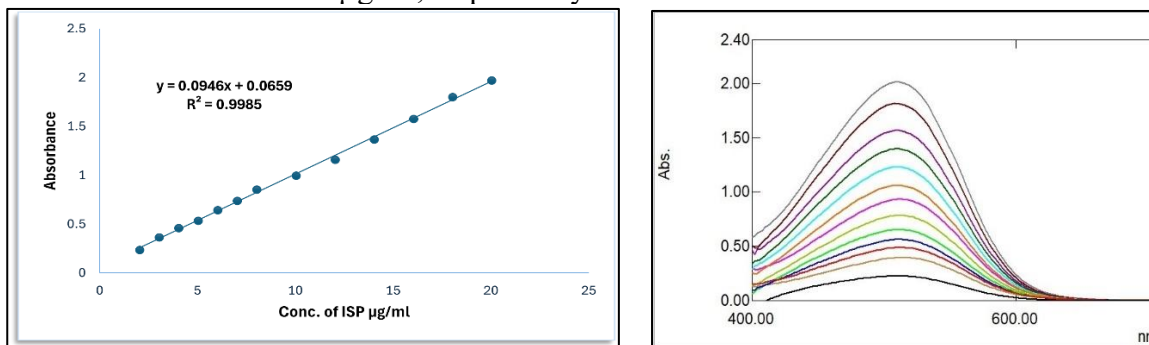
Pharmaceutical Preparation	Amount of methyldopa taken (µg/ml)	Amount of methyldopa measured (µg/ml)	Recovery %
Methyldopa, Tablet 250mg, Iraq	6	6.17	102.90
	10	10.26	102.69

Methyldopa, Tablet 250mg, UK	6	6.15	102.51
	10	10.36	103.67
Kadomet, Tablet 250mg Methyl., Egypt	6	6.23	103.96
	10	10.43	104.31

B. Estimation of isoprenaline by Azo-coupling reaction by using p-Nitroaniline reagent

General procedure and calibration graph for isoprenaline

Various volumes of standard solutions of isoprenaline ($100\mu\text{g/ml}$) transfer to a series of 10 ml volumetric flasks to involve the range of concentration from (2 -22 $\mu\text{g/ml}$), then 1.0 ml of diazotized p-Nitroaniline (1×10^{-2} M) was added and waiting for 5 minutes to complete the coupling between ISP and DPNA, after that adding of 1.5 ml of sodium hydroxide (1.0 M) and 1.5 ml of TritonX-100(1%) solutions, then diluted to the mark with distilled water and measured the absorbance of the azo dye at 510 nm. against reagents blank. The calibration graph in (Fig. 11) was linear over the concentrations range of 2-20 $\mu\text{g/ml}$, and negative deviation from Beer's law was noticed at concentrations more than 20 $\mu\text{g/ml}$. The molar absorptivity value was calculated and equal to $2.3434\times 10^4 \text{ l.mol}^{-1}.\text{cm}^{-1}$ and Sandell's index was equal to $0.0105 \mu\text{g}.\text{cm}^{-2}$, The detection limit (LOD) and the quantification limit (LOQ) [38] were 0.02119 and 0.07064 $\mu\text{g/ml}$, respectively.

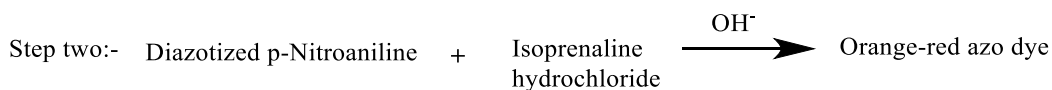
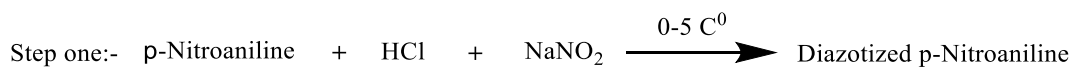


(Fig. 11) Calibration graph for estimation of ISP

RESULT AND DISCUSSION

B.1 Principle of the method for estimation of ISP

The first step involves the reaction of p-Nitroaniline with sodium nitrate in an acidic medium of hydrochloric acid in low temperatures (0-5 °C) to form the corresponding diazonium salt. The second step forming diazonium salt reacts with the isoprenaline in an alkaline medium to produce orange-red azo dye.



B.2 Selection of the best volume of the diazotized p-Nitroaniline

The best volume was chosen by adding different volumes of DPNA (0.02 M) to different concentrations of isoprenaline and waiting (3-5) minutes, then adding 1.0 ml of 1.0 molar sodium hydroxide and studying its effect on the linearity of the reaction. The results in (Table 12) showed that using 1.0 ml is the optimal volume, so it was adopted in subsequent experiments.

Table 12: Selection of the best volume of the diazotized p-Nitroaniline

ml of DPNA	Absorbance / μg of ISP in 10 ml						R^2
	30	50	80	100	120	150	
0.5	0.265	0.409	0.601	0.675	0.842	0.956	0.9904
0.8	0.289	0.444	0.676	0.756	0.971	1.061	0.9818
1	0.309	0.473	0.673	0.787	0.979	1.146	0.9962
1.2	0.264	0.469	0.710	0.774	1.001	1.175	0.9882
1.5	0.258	0.426	0.692	0.765	0.984	1.108	0.9850

B.3 Selection of the best base used

Different kinds of strong and weak bases were studied, and the results in (Table 13) that sodium hydroxide is an ideal medium for the reaction. In addition, different concentrations of NaOH were studied and the result in (Table 14) shows that 1 M of NaOH is the best concentration. And 1.5 ml is the best volume that gives the higher absorbance so, it was chosen as the better amount in subsequent experiments as shown in (Table 15).

Table 13: Selection of the best base

Base solution 1 ml of 1 M	NaOH	KOH	NaHCO ₃	Na ₂ CO ₃	NH ₄ OH
Absorbance	0.786	0.697	Turbid	Turbid	No color appears
pH	12.61	12.6			

Table 14: Selection of the best concentration of NaOH

Conc. of NaOH (M)	0.5	1	2	3	4	5
Absorbance	0.752	0.789	0.756	0.678	0.629	0.687

Table 15: Selection of the best volume of NaOH

Volume of NaOH (1M), ml	0.5	1	1.5	2	2.5
Absorbance	0.748	0.790	0.810	0.802	0.801

B.4 Studied the time required for the conjugation between DPNA and ISP

The time required for the complete conjugation between the DPNA and isoprenaline before adding sodium hydroxide was studied. The results listed in (Table 16) showed that the best time for completing the coupling is 5 minutes.

Table 16: Effect of the time on the absorbance value for resulting azo dye

Time, min.	Immediately	3	5	8	10
Absorbance	0.780	0.795	0.809	0.811	0.810

B.5 Effect of addition sequence of the reaction component

Several experiments were studied with different sequences of reaction components to obtain the best absorption value for the resulting azo dye and the results shown in (Table 17) show that the sequence I followed in the previous experiments is the best, so it was continued in the subsequent experiment.

Table 17: Effect of addition sequence of the reaction component

Sequence number	Reaction component *	Absorbance
I	ISP + DPNA + B	0.811
II	DPNA + ISP + B	0.806
III	B + DPNA + ISP	0.152

*Isoprenaline (ISP), Diazotized p-Nitroaniline (DPNA), NaOH (B)

B.6 Effect of surfactants on the color of resulting azo dye

The effect of using different types of surfactants (negative, positive, and neutral) was studied in different sequences after the best addition sequence was fixed in the previous experiment by adding 1.0 ml of these agents to the previously fixed reaction components. The results listed in (Table 18) show that adding the agent (SDS) had no effect while adding the agents (CPC, CTAB) led to a red shift towards higher wavelengths but with a decrease in absorption values. While the addition of TritonX-100 gave a higher absorption value with a higher wavelength when added after sodium hydroxide, note that the absorption value without adding factors is 0.805 at a wavelength 490 nm., so it was approved in subsequent experiments. In addition, 1.5 ml of TritonX-100 is the best volume that gives the higher absorbance so, it was chosen as the better amount in subsequent experiments as shown in (Table 19).

Table 18: Effect of surfactants

Sequence addition of surfactant*	Absorbance/1.0 ml of surfactant added			
	SDS $1 \times 10^{-3} \text{M}$	CPC $1 \times 10^{-3} \text{M}$	CTAB $1 \times 10^{-3} \text{M}$	TritonX-100(1%)
	$\lambda = 490$	$\lambda = 500$	$\lambda = 500$	$\lambda = 510$
ISP + S + DPNA + B	0.802	0.353	0.447	0.631
ISP + DPNA + S + B	0.798	0.360	0.452	0.639
ISP + DPNA + B + S	0.806	0.624	0.559	0.920

*Isoprenaline (ISP), Diazotized p-Nitroaniline (DPNA), NaOH (B), Surfactant (S)

Table 19: Selection of the best volume of the TritonX-100(1%)

ml of TritonX-100(1%)	0.5	1	1.5	2	2.5
Absorbance	0.611	0.917	0.986	0.593	0.560

B.7 Studied the stability time of the resulting azo dye

The stability of the resulting red azo dye color was studied for three different concentrations of ISP (5, 10, and 14) $\mu\text{g/ml}$ by measuring the absorbance for different periods, the reading values were observed to be stable for at least 60 min. as shown in (Fig. 12).

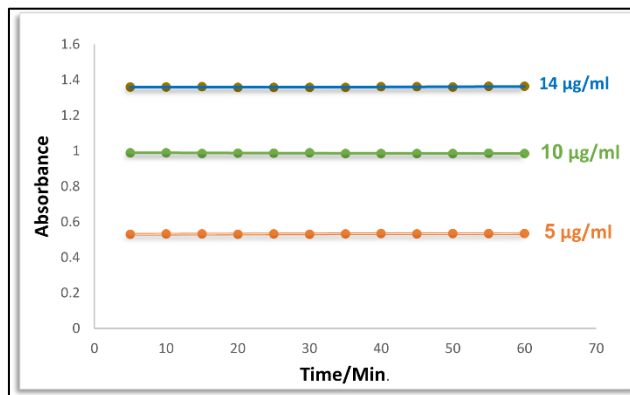


Fig. 12: The stability of the resulting azo dye

B.8 Final absorption spectrum for the estimation of ISP

Isoprenaline was treated according to the proven optimal conditions, the final absorption spectrum in (Fig. 13) shows the spectrum for 10 $\mu\text{g/ml}$ ISP versus the distilled water and versus the blank, the spectrum of the blank versus the distilled water.

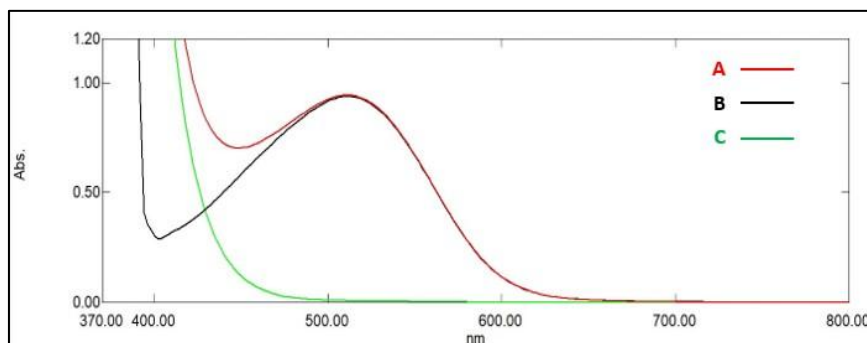


Fig. 13: Final Absorption Spectrum for 10 µg/ml ISP

(A) Sample versus water, (B) Sample versus blank, (C) Blank versus distilled water

B.9 Accuracy and precision for the estimation of isoprenaline

The accuracy and precision of the estimation of ISP were calculated by applying the previously described approved working method for three different concentrations (5, 10, and 14) µg/ml. The recovery and relative standard deviation were calculated, and the results were included in (Table 20) which showed that the proposed method was satisfactory.

Table 20: Accuracy and precision for the estimation of isoprenaline

Amount of ISP µg/ml		Recovery, %*	Relative error, %*	Relative standard deviation, %*
Taken	Found			
5	5.001	100.03	0.03	0.3089
10	10.01	100.10	0.10	0.1509
14	13.96	99.71	-0.29	0.3293

*Average of five determinations

B.10 Nature of the reaction between isoprenaline and Diazotized p-Nitroaniline

To understand the mole ratio of the reaction between ISP drug and DPNA under the optimized conditions previously described, applying the variation method (Job's method) and molar ratio method [39] used the same concentration for each ISP and DPNA (4.03×10^{-4} M) and the results in (Fig.14) shows that the ratio of the reaction was 1:1 for ISP and DPNA, also applied slope ratio method by drawing two calibrations between ISP and DPNA in the same conc. (4.03×10^{-4} M), (Fig. 15) shows the drawing of the two standard curves, where the result of dividing the slope of the first curve by the slope of the second curve confirms the ratio of the reaction was 1:1.

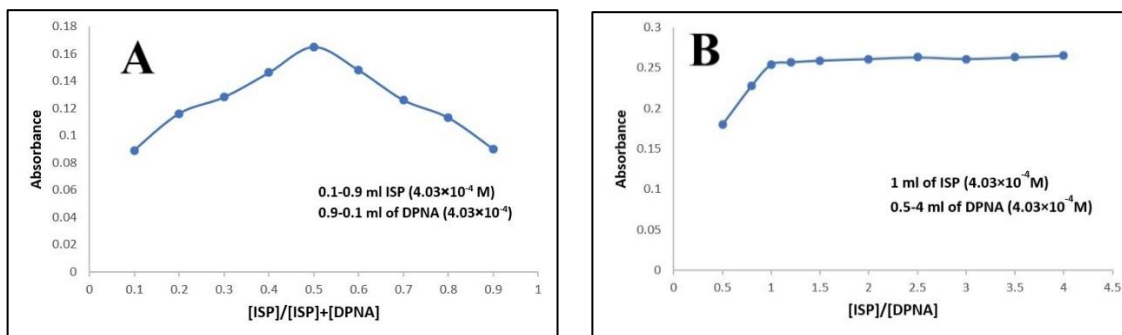


Fig. 14: (A) Job's method (continuous variation), (B) Mole ratio method

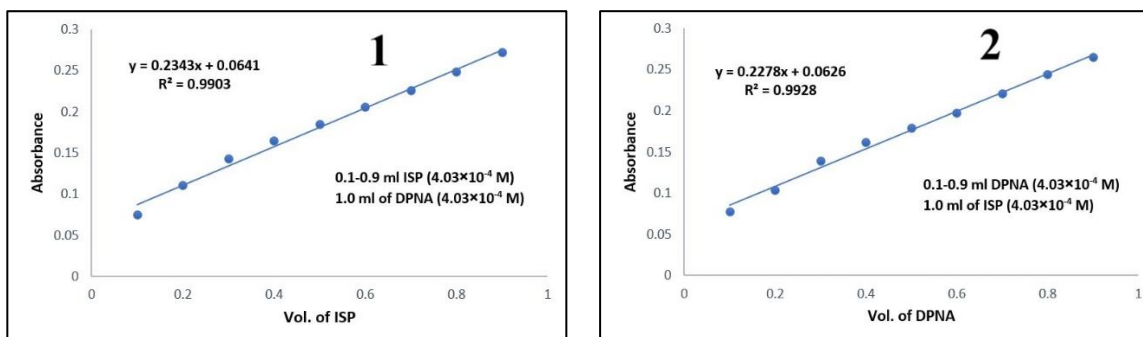
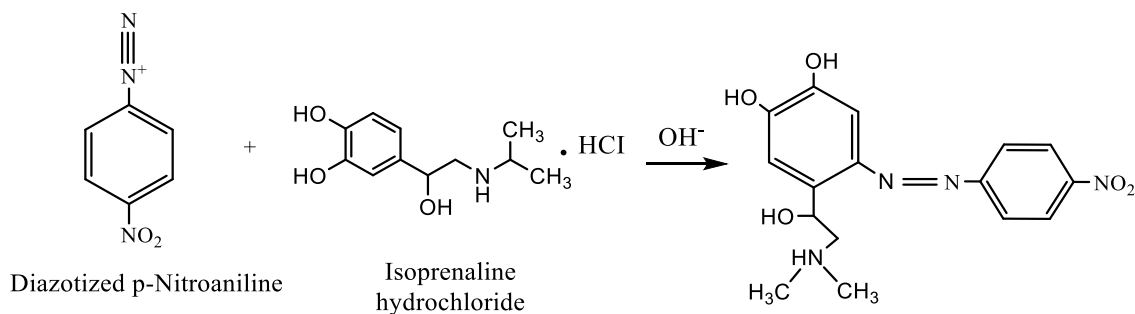


Fig. 15: Curves of slope ratio between ISP and DPNA

Therefore, according to the obtained result shown in Figures 14 and 15, the suggested reaction is explained below:



The stability Constant (K_s) of the Formed color has been studied for two concentrations, and the average of K_s values is equal to 8.8935×10^4 l/mol. This indicates that the color of the azo dye has high stability.

B.11.1 Application part of the reaction between isoprenaline and Diazotized p-Nitroaniline

The proposed method for the determination of isoprenaline hydrochloride was applied in its available sample dosage form injection (Isoprenaline Injection IP, 2mg/1ml, India) with three concentrations (5, 10, and 14) $\mu\text{g/ml}$. The results in (table 21) evidence the success of the proposed method for determination of ISP.

Table 21: Application part of the determination of isoprenaline hydrochloride

Pharmaceutical Preparations	Amount of ISP $\mu\text{g/ml}$	Recovery, %*	Relative error, %*	Relative stander deviation, %*
Isoprenaline Injection IP 2mg/1ml Samarth, India	5	99.62	-0.38	0.3100
	10	98.99	-1.01	0.1522
	14	101.51	1.51	0.3284

*Average of five determinations [41]

B.11.2 Appraisal of the suggested method by applied standard addition method

Applied the standard addition method to prove the efficiency and effectiveness of the suggested method for the determination of isoprenaline hydrochloride in its pharmaceutical preparations and prove there are no interferences of the drug with additives existing in the pharmaceutical preparations. The results in (Fig.16) and (Table 22) indicate that the standard addition method is approved with the suggested method.

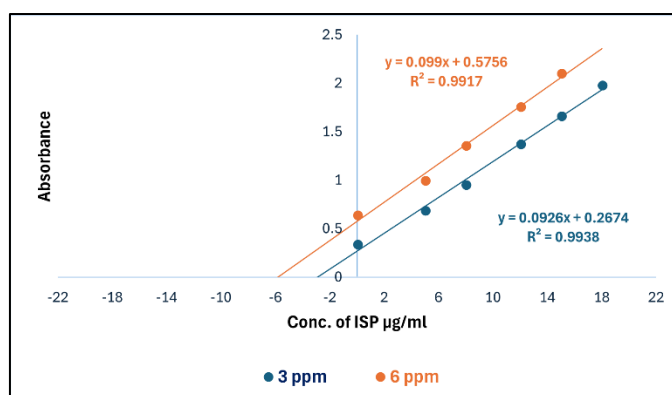


Fig. 16: Standard addition curve for determination of ISP preparations
Isoprenaline Injection IP, 2mg/1ml, India

Table 22: Determination of ISP in pharmaceuticals by applied standard addition method

Pharmaceutical Preparation	Amount of ISP taken ($\mu\text{g/ml}$)	Amount of ISP measured ($\mu\text{g/ml}$)	Recovery %
Isoprenaline Injection IP 2mg/1ml Samarth, India	3	2.887	96.25
	6	5.814	96.90

Conclusion:

In this manuscript, two spectrophotometric methods were developed for the estimation of methyl dopa and isoprenaline, both methods were characterized by being fast with high sensitivity, simplicity, and good accuracy and precision, without the need for control of temperature or extraction steps. MED determination by using oxidative coupling reactions with mesalazine as a reagent and ferric nitrate as an oxidizing agent and the absorbance of the colored product was measured at λ max 578 nm. ISP was determined by azo-coupling reaction with the diazotized p-Nitroaniline reagent in the presence of sodium hydroxide and TritonX-100(1%) solutions and the orange-red azo dye producing measured at λ max 510 nm. The two methods were applied successfully for the determination of methyl dopa in its pharmaceuticals (tablets) and isoprenaline pharmaceuticals (injection) with excellent results and recoveries.

Reference

1. Faris,A.J. and Hasan,M.A. (2023). Development of spectrophotometric method for the determination of catecholamines in pure and pharmaceutical formulations, using pyromellitic dianhydride reagent by charge transfer reaction. *Egyptian Journal of Chemistry*, 66(13),307 – 318.
2. British pharmacopoeia. (2016). The stationary office, London, Vol II 255-257.
3. Agustin B. Actis Dato, Valeria R. Martinez, Jorge O. Velez Rueda, Enrique L. Portiansky, Verónica De Giusti, Evelina G. Ferrer and Patricia A.M. Williams. (2024). Improvement of the cardiovascular effect of methyl dopa by complexation with Zn(II): Synthesis, characterization and mechanism of action. *Journal of Trace Elements in Medicine and Biology*, 18, 127327.
4. Rensong Wang, Chen Chen, Wenge Yang, Peng Zhou, Fu Zhu, Hanhan Xu, Guoxing Hu, Wei Sun, Weiliang Shen, and Yonghong Hu, (2022). Solubility determination, model correlation and preferential solvation of methyl dopa in binary mixed solvents from 278.15 K to 323.15 K. *Journal of Molecular Liquids*, 363, 119838.
5. Azar Dehnavi and Ahmad Soleymannpour. (2020). Highly sensitive voltammetric electrode for the trace measurement of methyl dopa based on a pencil graphite modified with phosphomolibdate/graphene oxide. *Microchemical Journal*, 157, 104969.
6. Mahin Baladi, Mahnaz Amiri, Hamid Akbari Javar, Hadi Mahmoudi-Moghaddam, and Masoud Salavati-Niasari. (2022). Green synthesis of perovskite-type TbFeO₃/CuO as a highly efficient modifier for electrochemical detection of methyl dopa. *Journal of Electroanalytical Chemistry*, 915, 116339.
7. Sun,R. He,H. Yuan,L. Wan,Y. Sha,J. Li,L. Jiang,G. Li,Y. Li,T. and Ren,B. (2021). Thermodynamic analysis and molecular simulation of solid–liquid phase equilibrium of isoprenaline hydrochloride in eleven pure solvents at saturation. *The journal of chemical thermodynamics*,160, 106411.
8. Abdelrahman,D. Habotta,O. Taher,E.S. Elashry,E. Ibrahim,I.A. Abdeen,A. Megahed,A. Ibrahim,R. Answer,H. Mihaela,O. Olga,R. Alwutayd,K.M. Al-Serwi,R.H. El-Sherbiny,M. Sorour,S.M. and El-Kashef,D.H. (2024). Suppression of NLRP3

- inflammasome orchestrates the protective efficacy of tiron against isoprenaline-induced myocardial injury. *Frontiers in Pharmacology*, 15.
9. Manjula,N. and Chen,S. (2021). One-pot synthesis of rod-shaped gadolinia doped zinc oxide decorated on graphene oxide composite as an efficient electrode material for isoprenaline sensor. *Composites Part B: Engineering*, 211, 108631.
 10. The United States pharmacopeia (2018). The national formulary, vol I 2259-2262.
 11. El-Sherbiny,A.M. Attia,A.A. Elfadeel,S.M. and Hassan,M.S. (2021). A study of the cardio-protective effect of pioglitazone on isoprenaline- induced myocardial infarction in male albino rats. *The Egyptian Journal of Hospital Medicine*, 85(1),2816-2822.
 12. Anamalley,R Rajassageran,L. Apparoo,Y. Jauri,M.H. Kamisah,Y. Yunos,N.M. and Zainalabidin,S. (2022). Repeated administration of low dose isoprenaline on the rat's cardiovascular system. *Sains Malaysiana*, 51(7), 2147-2157.
 13. Humeidy,I.T. Salman,S.A. And Hashim,K.K. (2020). Spectrophotometric determination of methyl dopa with 2, 6-diaminopyridine reagent using oxidative coupling reaction. *Journal of Engineering Science and Technology*, 15(3), 1824- 1839.
 14. Dhamra,M.Y and Al-Sabha,T.N. (2020). Spectrophotometric Method For Indirect Determination Of Antihypertensive Drugs In Pharmaceuticals. *Egyptian Journal of Chemistry*, 63(10), 3767–3777.
 15. Salman,S.D. and Khalaf,H.S. (2024). Spectrophotometric Estimation of Methyl dopa Drug in pure and pharmaceutical formulations. *Egyptian Journal of Chemistry*, 67(8), 451 – 457
 16. Bichan,M.J.K. and Abdoon, F.M. (2019). A novel spectrophotometric determination of methyl dopa through ternary complexation procedure using Fe(iii), Mn(ii), and Co(ii) with 2-aminopyridine. *Asian Journal of Pharmaceutical and Clinical Research*,12(3),366-371.
 17. Vlase,L. Mihu,D. Popa,D.S. Popa,A. Briciu,C. Loghin,F. Ciortea,R. and Mihu,C. (2013). Determination of methyl dopa in human plasma by LC/MS-MS for therapeutic drug monitoring. *Studia Universitatis Babes-Bolyai Chimia*, 58(1),31-41.
 18. Emara,S. Masujima,T. Zarad,W. Kamal,M. Fouad,M. and EL-Bagary,R. (2015). A combination of isocratic and gradient elution modes in HPLC with the aid of time-overlapping process for rapid determination of methyl dopa in human urine. *Journal of Liquid Chromatography & Related Technologies*,38(2),153–162.
 19. Shu-Fang Li, Hai-Long Wu, Yong-Jie Yu, Yuan-Na Li, Jin-Fang Nie, Hai-Yan Fu, and Ru-Qin Yu. (2010). Quantitative analysis of levodopa, carbidopa and methyl dopa in human plasma samples using HPLC-DAD combined with second-order calibration based on alternating trilinear decomposition algorithm. *Talanta*, 81(3), 805-812.
 20. Upan,J. Themsirimongkon,S. Saipanya,S. and Jakmunee,J. (2019). Gold nanoparticles decorated on carbon nanotube modified screen-printed electrode for flow injection amperometric determination of methyl dopa. *Chiang Mai Journal of Science*, 46(3),537-546.
 21. Tajik,S. Beitollahi,H. and Biparva,P. (2018). Methyl dopa electrochemical sensor based on a glassy carbon electrode modified with Cu/TiO₂ nanocomposite. *Journal of the Serbian Chemical Society*, 83(7–8),863–874.
 22. Najafi,K. Asadpour-Zeynali,K. Mollarasouli,F. (2020). Preparation of a magnetic nanosensor based on cobalt ferrite nanoparticles for the electrochemical determination of

- methyldopa in the presence of uric acid. *Combinatorial Chemistry & High Throughput Screening*, 23(10),1023-1031.
23. Borji,S Beitollahi,H. and Nejad,F.G. (2023). Evaluating the Electrochemical Detection of Methyldopa in the Presence of Hydrochlorothiazide Using a Modified Carbon Paste Electrode and Voltammetric Analysis. *Topics in Catalysis*, 67,773–784.
 24. El-Adl,S.M. El-Sadek,M.E. and Saeed,N.M. (2018). Spectrofluorometric determination of lisinopril dihydrate and methyl- dopa in bulk and pharmaceutical formulation by using dansyl chloride. *Journal of Pharmacy and Pharmacology Research*, 2(1), 001-014.
 25. Mazloun-Ardakani,M. Rajabzadeh.N. Dehghani-Firouzabadi,A. Benvidi,A. Mirjalili,B.B. and Zamani,L. (2016). Development of an electrode modified on the basis of carbon nanoparticles and reduced graphene oxide for simultaneous determination of isoproterenol, uric acid and tryptophan in real samples. *Journal of Electroanalytical Chemistry*, [760](#),151-157.
 26. Mohammadi,S.Z. Tajik,S. Badri,Y. Tezerji,L.S. Mousazadeh,F. and Farzan,A. (2024). Sensitive determination of the cardiac drug isoproterenol in the presence of acetaminophen using modified electrode with multiwall carbon nanotube/ZnCo-Zeolite imidazole frameworks and ionic liquid. *Diamond and Related Materials*, 148,111445.
 27. Manjula,N. Chen,T. Chen,S. Yu,J. Hao,Q. and Lei,W. (2022). One step construction of crystal rod like Bi₂O₃/ZnO nanocomposite for voltammetry determination of isoprenaline in pharmaceutical and urine sample. *Microchemical Journal*, 172,B, 106894.
 28. Sasikumar,R. Kim,B. and Ishfaq,A. (2023). Active-site-rich binary metal oxides integrated organic–inorganic hybrid nanocomposite: Electrochemical simultaneous detection of multi-drugs of isoprenaline and resorcinol in real samples. *Microchemical Journal*, 187, 108375.
 29. Palakollu,V.N. Chiwunze,T.E. Gill,A.A. Thapliyal,N. Maru,S.M. and Karpoormath,R. (2017). Electrochemical sensitive determination of isoprenaline at β -cyclodextrin functionalized graphene oxide and electrochemically generated acid yellow 9 polymer modified electrode. *Journal of Molecular Liquids*, 248, 953-962.
 30. Gaddala,V.S. Dachuru,R.S. and Tella,E.D. (2020). A validated LCMS method for the analysis of isoproterenol – a β adrenoreceptor agonist in spiked human plasma. *International Journal of Pharmaceutical Sciences And Research*, 11(9), 4567-4574.
 31. Zhou,J. Yin,H. Ma,H. Wei,S. Wen,E. Zhang,W. and Dang,H. (2017). An efficient and selective analytical method for the quantification of a β -adrenoceptor agonist, isoproterenol, by LC-MS/MS and its application to pharmacokinetics studies. *Journal of Liquid Chromatography & Related Technologies*, 40(13), 699-705.
 32. Camañas,R.V. Mallols,J.S. Lapasió,J.T. and Ramis-Ramos,G. (1995). Analysis of pharmaceutical preparations containing catecholamines by micellar liquid chromatography with spectrophotometric detection. *Analyst*, 120,1767-1772.
 33. Zhou,G. Zhang,G. and Chen,H (2002). Development of integrated chemiluminescence flow sensor for the determination of adrenaline and isoprenaline. *Analytica Chimica Acta*, 463, 257–263.
 34. Wang,Z. Zhang,Z. Fu,Z. Fang,L. Luo,F. Chen,D. and Zhang,X. (2003). Mushroom tissue-based flow-injection fluorescence system for the determination of isoprenaline. *Analytica Chimica Acta*, 494,63–70.

35. Sakr,M. Hanafi,R. Fouad,M. Al-Easa,H. and El-Moghazy,S. (2019). Design and optimization of a luminescent Samarium complex of isoprenaline: A chemometric approach based on Factorial design and Box-Behnken response surface methodology. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 208,114-123.
36. El-Shabouri,S.R. Hussein,S.A. and Abdel-Alim,A.M. (1988). Simple and rapid spectrophotometry method for determination of adrenaline and isoprenaline. *Journal of Association of Official Analytical Chemists*,71(4),764–767.
37. Othman,N.S. and Othman,N.H. (2013). Spectrophotometric determination of teicoplanin via coupling with diazotized p-nitroaniline. *Rafidain Journal of Science*, 24(5),42-51.
38. Shrivastava, A. and Gupta, V. B. (2011). Methods for the determination of limit of detection and limit of quantitation of the analytical methods. *Chronicles of Young Scientists*, 2(1): 21-25.
39. De Levie, R. (1997). Principles of quantitative chemical analysis. McGraw-Hill Science, Engineering & Mathematics
40. Miller, J., & Miller, J. C. (2018). Statistics and chemometrics for analytical chemistry. Pearson education.
41. Christian, G.D. ; Dasgupta, P.K. and Schug, K.A. (2014). Analytical Chemistry, 7 th Edn., John Wiley & Sons, Inc., USA, pp. 84-87, 90,91, 429.