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Spectrophotometric Approach for the Determination of Dobutamine Hydrochloride Using Diazotization-Coupling Reaction

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طريقة طيفية لتقدير الدوبتامين هيدروكلوريد باستخدام تفاعل الازوتة والاقتران

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الملخص:

تم اقتراح طريقة طيفية بسيطة ودقيقة وقابلة للتكرار لتقدير الدوبتامين هيدروكلورايد ((DBفي شكله النقي وفي المستحضرات الصيدلانية وذلك باستخدام تفاعل الاقتران الأزوي باستخدام كاشف جديد مشتق من الميثوتريكسات. تعتمد الطريقة المقترحة على ازوتة الميثوتريكسات باستخدام نترات الصوديوم ($NaNO_2$)في وسط حامضي عند درجة حرارة (0–5°C)، تليها خطوة اقتران الكاشف المؤزوت مع المركب الدوائي الدوبتامين في وسط قلوي من هيدروكسيد الأمونيوم ((NH_4OHلتكوين صبغة ازوية صفراء مستقرة تُظهر أقصى امتصاص عند الطول الموجي 466 نانوميتر. تم ضبط الطروف المثلى بما في ذلك نوع وحجم الحامض، تركيز وكمية الكاشف، نوع وحجم القاعدة، مدة التفاعل، اختيار المذيب، وترتيب الإضافة.

وضمن الظروف المثلى المستحصلة تم رسم المنحنى القياسي حيث أظهرت الطريقة علاقة خطية ضمن مدى تركيز يتراوح من 2 إلى 60 ميكروغرام/مل، بمعامل ارتباط ((R^2قدره 0.9943، وكانت قيمة الامتصاصية المولارية تبلغ 1.10×10^5 لتر·مول$^{-1}$·سم$^{-1}$. كما تم تحديد حد الكشف (LOD) وحد التقدير الكمي ((LOQبقيمة 0.0231 و 0.0702 ميكروغرام/مل على التوالي. تم اختبار دقة وموثوقية الطريقة المقترحة من خلال حساب نسبة الاسترجاع والانحراف القياسي النسبي، حيث أظهرت الدراسة ان متوسط نسبة استرجاع قدره 100.68% وانحراف معياري نسبي ((RSDأقل من 6.42%. تم تطبيق الطريقة المقترحة بنجاح في التحليل الكمي للدوبتامين في الأشكال الصيدلانية (الحقن)، حيث اظهرت دقة ممتازة دون حدوث أي تداخل من مواد السواغ. كما تم دراسة نسبة التفاعل بين الكاشف المؤزوت والدواء حيث أظهرت النتائج ان نسبة للتفاعل هي 1:1 (دوبوتامين: ميثوتريكسات).

الكلمات الدالة: التحليل الطيفي، التحليل الدوائي، ميثوتريكسات، دوبوتامين هيدروكلورايد، تفاعل الاقتران الأزوي.

Abstract

A simple, rapid, and sensitive spectrophotometric method has been suggested for the determination of Dobutamine hydrochloride (DB) in pure forms and pharmaceutical formulations using an azo-coupling reaction with an innovative reagent derived from methotrexate. The developed method is based on the diazotization process of methotrexate using NaNO2 in an acidic medium at (0–5°C), followed by a coupling step with Dobutamine in an alkaline medium (NH4OH) to yield a stable yellow azo dye showing a maximum absorbance at 466 nm. Experimental factors—including the type and volume of acid,

diazotizing agent, base, reaction duration, solvent selection, and order of addition—were practically optimized. Under the established optimal conditions, the method demonstrated linearity in the concentration range 2–60 µg/mL, with a correlation coefficient (R^2) of 0.9943 and a molar absorptivity of 1.10×10^5 $L \cdot mol^{-1} \cdot cm^{-1}$. The limits of detection (LOD) value and quantification (LOQ) value were determined to be 0.0231 µg/mL and 0.0702 µg/mL, respectively. Accuracy and precision of the proposed spectrophotometric method were confirmed through recovery studies, yielding a mean recovery of 100.68% and a relative standard deviation value (RSD) of less than 6.42%. The proposed approach was successfully applied to the quantitative analysis of Dobutamine in pharmaceutical formulations (Injection), demonstrating excellent accuracy and negligible interference from common excipients. A stoichiometric ratio of 1:1 (Dobutamine –Methotrexate) was confirmed.

Keywords: Spectrophotometric analysis, Drug Analysis, methotrexate, Dobutamine Hydrochloride, Azo-Coupling Reaction.

Introduction

Dobutamine hydrochloride (DB) is a cardio-selective adrenergic agonist that primarily stimulates β_1-adrenergic receptors[1]. Chemically, it is named as 4-(2-((1-methyl-3-(4-hydroxyphenyl)propyl)amino)ethyl)benzene-1,2-diol hydrochloride, and it is commonly offered as a white crystalline powder. It is easily soluble in methanol (Me) and distilled water, slightly soluble in absolute ethanol, and practically insoluble in DMSO and chloroform. Its molecular formula is $C_{18}H_{23}NO_3$•HCl, with a molecular weight of 337.84 g/mol[2]. The compound is unstable under alkaline conditions, and it should be stored away from light and requires proper storage and handling precautions[3].

From a chemical viewpoint, Dobutamine is a synthetic catecholamine derivative and presents as a racemic mixture of two enantiomers (±). The positive isomer is mainly responsible for the positive inotropic effect through selective β_1-adrenergic receptor stimulation in cardiac tissue[4]. Pharmacodynamically, dobutamine selectively activates β_1-adrenergic receptors in the myocardium, resulting in enhanced myocardial contractility and increased heart rate. It also exhibits minor vasodilatory effects via β_2-receptor stimulation and minimal α_1-receptor activity. Its catechol structure renders it vulnerable to oxidative degradation; therefore, newly prepared solutions must be protected away from light and kept at acidic to neutral pH to ensure chemical stability[5].

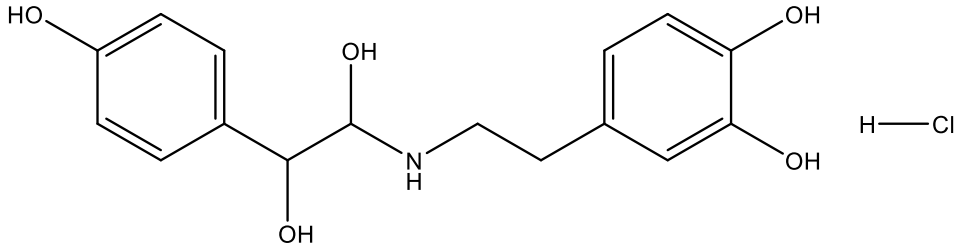
Clinically, Dobutamine is designated for the short-term management of cardiac decompensation, predominantly in acute heart failure and cardiogenic shock. It was advanced to offer increased myocardial contractility with fewer peripheral vascular effects compared to dopamine. Its rapid onset and short plasma half-life (approximately 2 minutes) require continuous intravenous infusion. This pharmacokinetic profile makes it a preferred agent in intensive care settings, where precise titration and rapid reversibility are essential[3].

Adverse effects of Dobutamine are primarily related to its adrenergic stimulation, including tachycardia, arrhythmias (notably atrial fibrillation and premature ventricular contractions), and dose-dependent blood pressure variability. Additional side effects may include chest pain,

dyspnea, headache, nausea, anxiety, and tremors. The drug is contraindicated in obstructive hypertrophic cardiomyopathy and should be used with caution in patients with atrial fibrillation, acute coronary syndromes, uncontrolled hypertension, or severe aortic stenosis. Notably, concomitant use with monoamine oxidase inhibitors (MAOIs) may result in serious drug interactions and should be avoided[6, 7].

The literature shows that a number of analytical approaches have been described for the determination of DB in pure forms and pharmaceutical dosage, such as Voltammetry[8-10], HPLC[11-13], FI Chemiluminescence[14, 15], and Spectrophotometry methods[3, 16-19].

Due to its narrow therapeutic index and critical role in cardiac care, the improvement of cost-effectiveness, sensitive, and precise analytical techniques for Dobutamine quantification in pharmaceutical forms and biological fluids is needed. Chromatographic methods such as High-performance liquid chromatography (HPLC) and Gas chromatography (GC) remain the most widely used techniques. However, it needs a high-purity solvent and an expert technician to run an experiment. Spectrophotometric methods offer simplicity and speed, though they generally lack the sensitivity and specificity of chromatographic techniques. So, the objective of this research is to improve a precise and cost-effective spectrophotometric method that could be applied for routine drug analysis and testing the quality of pharmaceutical products. The method should be free from interferences caused by excipients and should have high sensitivity and reproducibility.



4-[2-[[2-(4-Hydroxyphenyl)-1,2-dihydroxyethyl]amino]ethyl]benzene-1,2-diol hydrochloride

Chemical Formula: $C_{16}H_{20}ClNO_5$
Molecular Weight: 341.79

Instruments and Chemicals Used

Instruments:

- A Shimadzu UV-1800 PC, UV-Visible Double Beam Spectrophotometer, was employed, operated via the UV Probe 2.42 software, to carry out spectrophotometric measurements, determine the absorption band, and optimize the experimental conditions.
- Ultrasonic dissolution of samples was achieved using an ultrasonic cleaner (Power Sonic 40) supplied by Lab Tech, Korea.

Reagents and Chemicals

- Dobutamine Hydrochloride manufactured by VEM Company-Turkey
- In this research, a novel azo reagent obtained from the Al-Hikma Company-Gordan was used (Methotrexate. The diazotization of the methotrexate was achieved by reacting it with sodium nitrite (NaNO2) in an acidic medium at a low temperature (0-5°C). Sulfamic acid was added to remove the excess.
- Sodium nitrite was supplied by BDH, while all other chemical reagents were obtained from Fluka. All solvents used were of analytical reagent grade, and ultrapure distilled water was utilized throughout the experimental procedures.

Preparation of Solutions

Dobutamine solution

A Dobutamine solution with a concentration of 500 µg/mL was prepared by diluting 40 mL of a 1250 µg/mL Dobutamine standard solution with distilled water to the 100 mL mark in a volumetric flask.

Reagent solution (Methotrexate)

A Methotrexate solution with a concentration of 150 µg/mL was prepared by diluting 0.6 mL of the pure compound (2500 µg/mL) with distilled water in a 100 mL volumetric flask.

Optimization of Experimental Conditions

1-Study of the effect of the amount of diazotized reagent.

The effect of varying reagent volumes following the diazotization process was studied. Sodium nitrite was added to an acidic medium containing acetic acid (1 M), and the mixture was kept at 0–5°C for 10 minutes. Subsequently, Sulfamic acid (1%) was added to eliminate excess nitrous acid, followed by the addition of the pharmaceutical compound and ammonium hydroxide solution (2 M) to adjust the medium to alkaline conditions. The absorbance of the resulting colored product was measured at 466 nm against corresponding blank solutions (Figure 1).

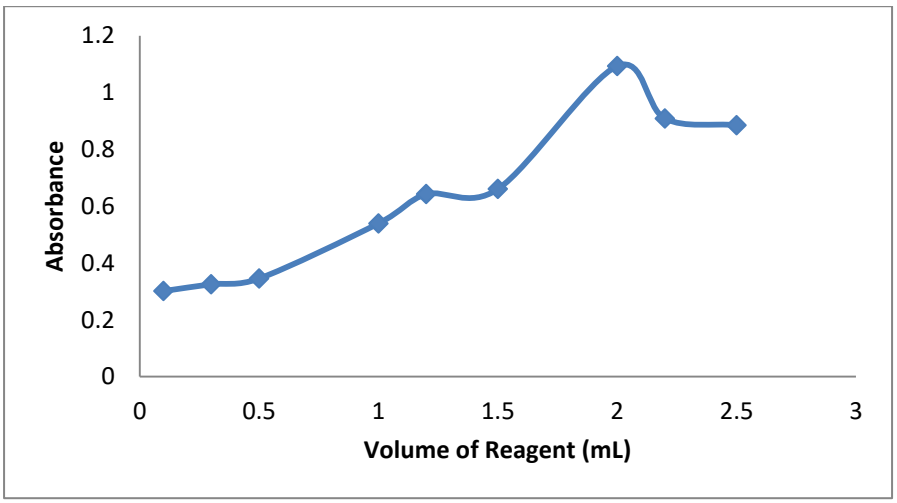


Figure 1: Study of the effect of reagent volume on the reaction

2-Study of Acid Type

The effect of acid type on the diazotization of methotrexate was evaluated using various acids at a concentration of 1 M and a volume of 1 milliliter, and the absorbance was measured at 466 nm. As shown in Figure 2, acetic acid produced the highest absorbance, confirming its efficiency as the selected reaction medium.

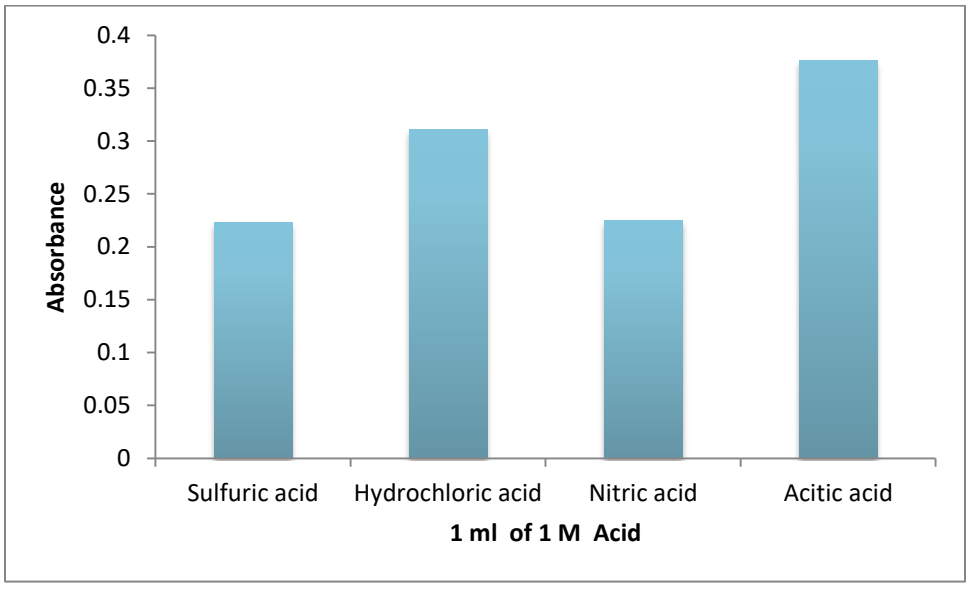


Figure 2: Study of the effect of the type of acid

3-Study of Acetic Acid Volume

The effect of acetic acid volume at a concentration of 1 M (0.2–2.0 mL) was studied to determine the optimal volume for the diazotization of Dobutamine. After adding the reagent and sodium nitrite, diazotization was carried out at 0–5°C for 10 minutes, followed by the addition of Sulfamic acid, then Dobutamine and ammonium hydroxide. The volumetric flasks were then completed to the mark with distilled water. Absorbance was measured at 466 nm after 10 minutes at room temperature. As shown in Figure 3, 1 mL of acid gave the highest absorbance and was therefore adopted in subsequent experiments.

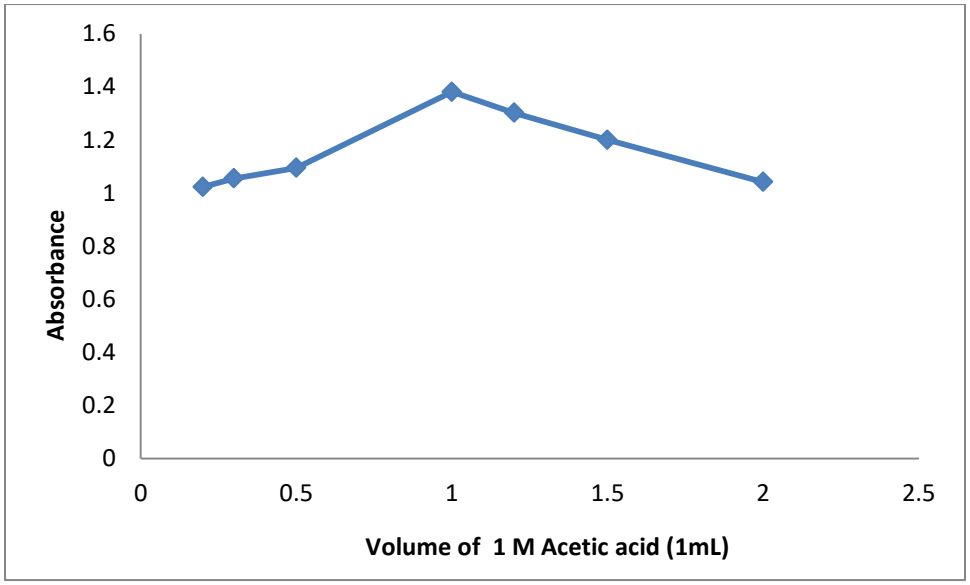


Figure 3: Study of the effect of acid volume on the reaction

4-Study of Sodium Nitrite Volume

The effect of sodium nitrite volume (2%) on the efficiency of Methotrexate reagent diazotization was evaluated using incremental volumes (0.2–2.5 mL) in a medium containing 1 mL of the reagent and 1 mL of acetic acid (1 M). After completing the diazotization at 0–5°C and removing excess nitrous acid, Dobutamine and ammonium hydroxide were added, and absorbance was measured at 466 nm. As shown in Figure 4, a volume of 1 mL of sodium nitrite yielded the highest absorbance and was adopted for subsequent experiments.

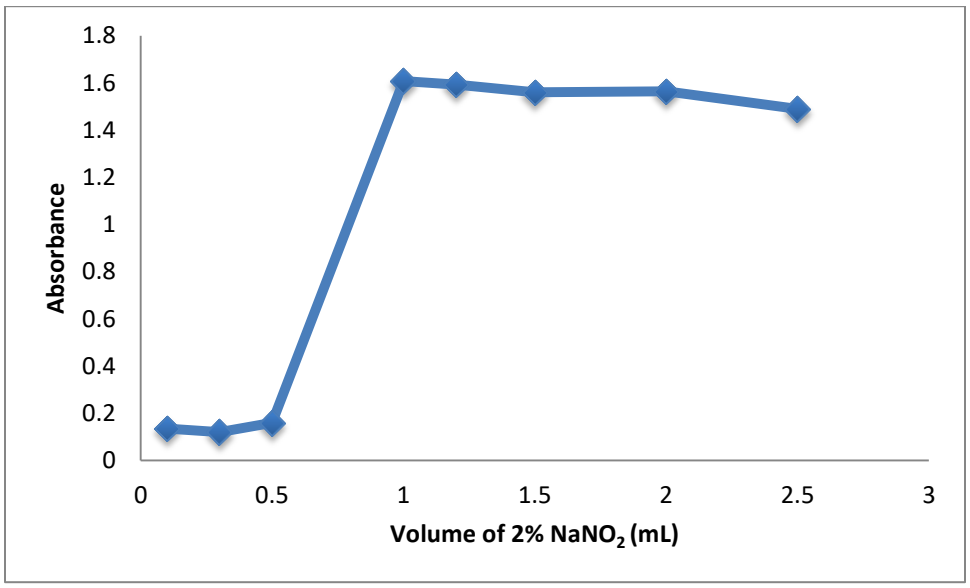


Figure 4: Study of the effect of sodium nitrate volume on the reaction

5-Study of Sulfamic Acid Volume

The effect of Sulfamic acid volume (1%) on the removal of excess nitrous acid during the diazotization of methotrexate was evaluated using volumes ranging from 0.2 to 2.0 mL. The results showed that 0.2 mL was optimal, yielding the highest absorbance at 466 nm, indicating its efficiency in enhancing spectral response and complex stability, as shown in Figure 5.

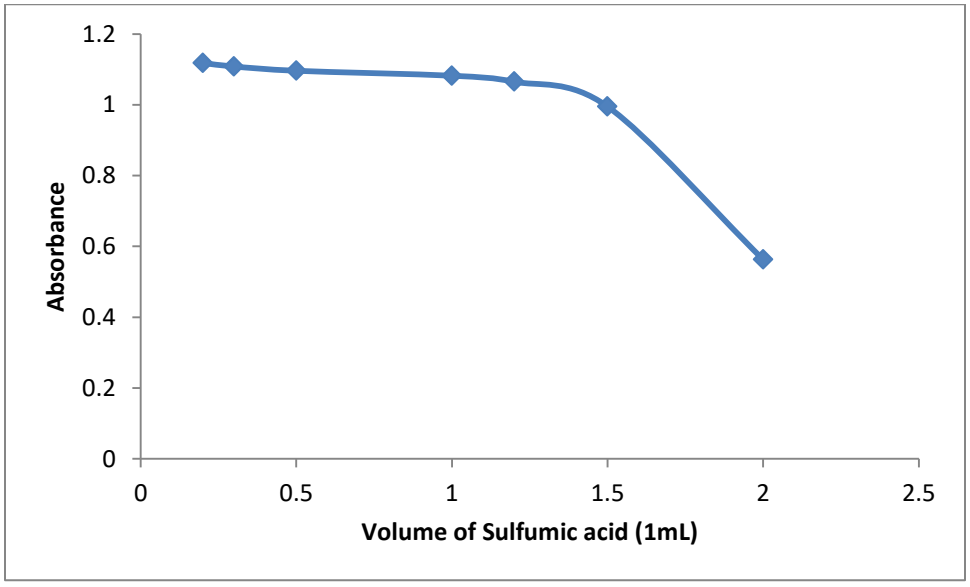


Figure 5: Study of the effect of Sulfamic acid volume on the reaction

6-Study of the Type of Base

The effect of different bases at a concentration of 2 M on the coupling reaction with Dobutamine was studied. As shown in Figure 6, ammonium hydroxide gave the highest absorbance, indicating its efficiency as the optimal basic medium, and it was adopted in the subsequent stages of the study.

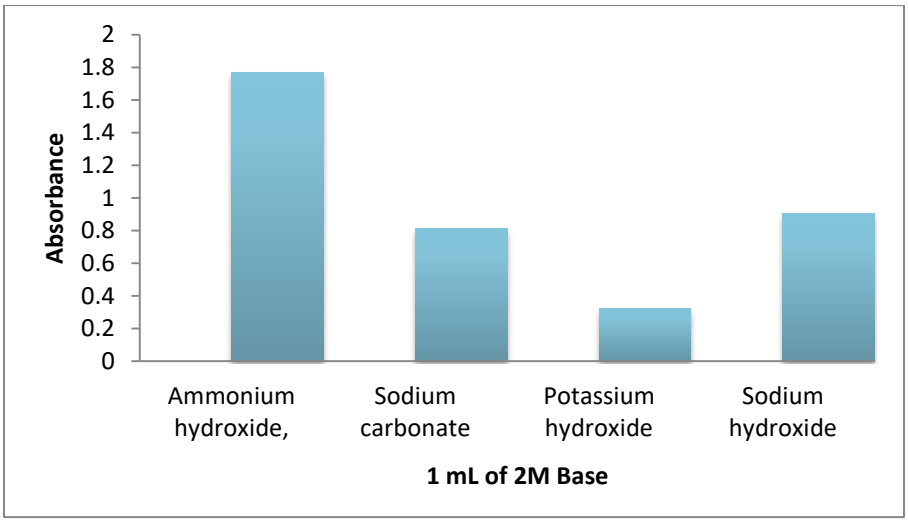


Figure 6: Study of the effect of the type of Base

7-Study of Ammonium Hydroxide Volume

The effect of ammonium hydroxide volume (2 M) on the absorbance of the coupling product was evaluated. As shown in Figure 7, a volume of 2.0 mL yielded the highest absorbance, indicating its efficiency in providing an optimal basic medium for complex stability and color intensity.

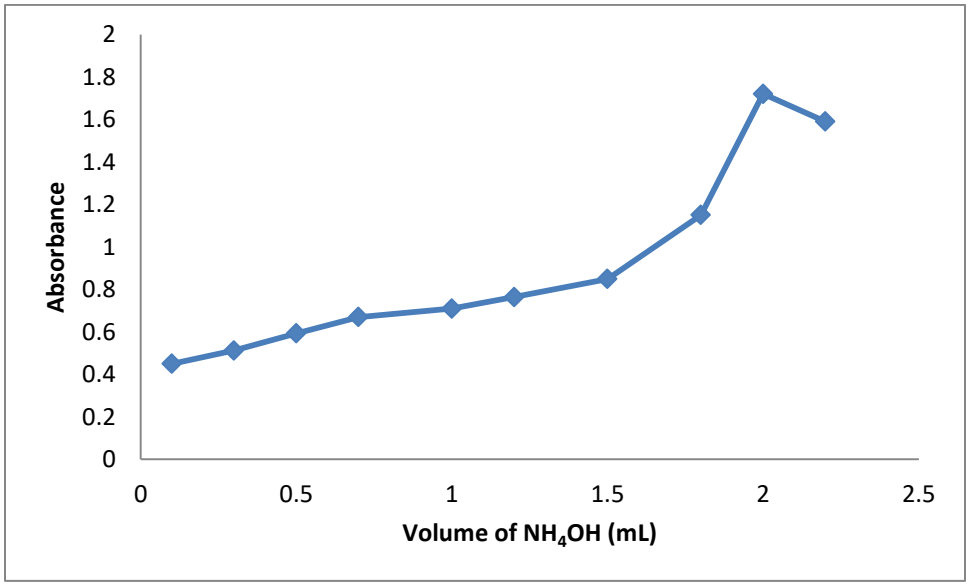


Figure 7: Study of the effect of NH_4OH volume on the reaction

8-Study of the Optimal Time for Completion of the Diazotization Reaction.

The study aimed to determine the optimal time for the diazotization of the reagent (methotrexate) at low temperatures (0–5°C). The optimal volume of the reagent was added along with sodium nitrite and hydrochloric acid, and the reaction time varied from 0 to 30 minutes. Then, Sulfamic acid, Dobutamine hydrochloride, and ammonium hydroxide were added, and the volume was completed to 10 mL. Absorbance was measured at 466 nm after 10 minutes at room temperature. As shown in Figure 8, the results indicated that 10 minutes is the optimal time for diazotization, yielding the highest absorbance.

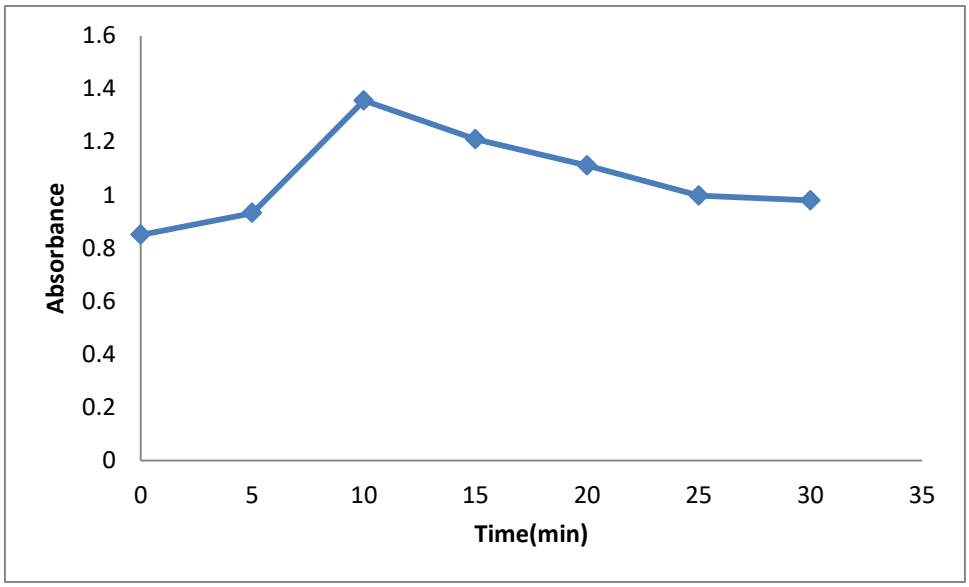


Figure 7: Diazotization Reaction Time

9-Study of the Effect of Solvent Type

The effect of the solvent type used in the final dilution step on the absorbance of the coupling reaction product was investigated by comparing distilled water with several organic solvents under the optimized reaction conditions. After completing all reaction steps, each solvent was used to make up the volume in volumetric flasks, and absorbance was measured at 466 nm. The results in Table 1 showed that distilled water yielded the highest absorbance, which is attributed to its high polarity, suitability for ionic interactions, chemical stability, and lack of interference with reaction components. These properties make distilled water the preferred solvent for spectrophotometric applications**.**

Table 1: The effect of the type of solvent used for dilution

Solvent	Water	Ethanol	Methanol	Aceton	Acetonitrile
Absorbance	**1.664**	1.596	1.681	1.517	**1.638**
λ max(nm)	**466**	430	428	428	**426**

10-Study of Dye Stability and the Effect of Temperature

The effect of temperature (0–30°C) on the absorbance and stability of the coupling product was studied to determine the optimal thermal conditions. As shown in Figure 9, room temperature (20°C) was found to be the most suitable, giving the highest absorbance and maintaining good stability for over 90 minutes without significant degradation.

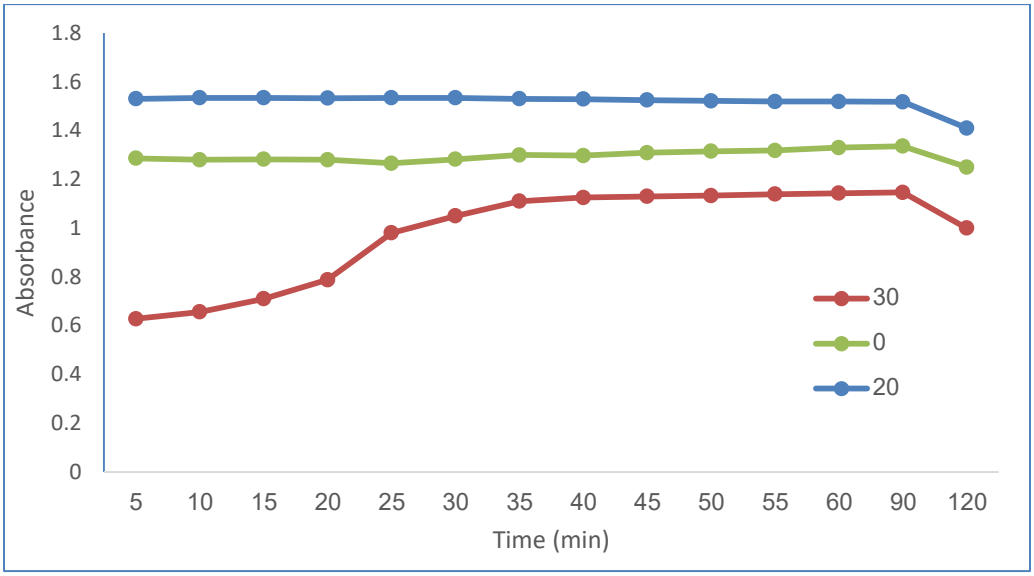


Figure 9: The effect of temperature and time

10- Study of the Effect of Surfactants

The effect of surfactants at a 1% concentration on the absorbance of the colored complex was evaluated. As shown in Figure 10, they did not enhance absorbance or color stability and were therefore excluded from subsequent experiments.

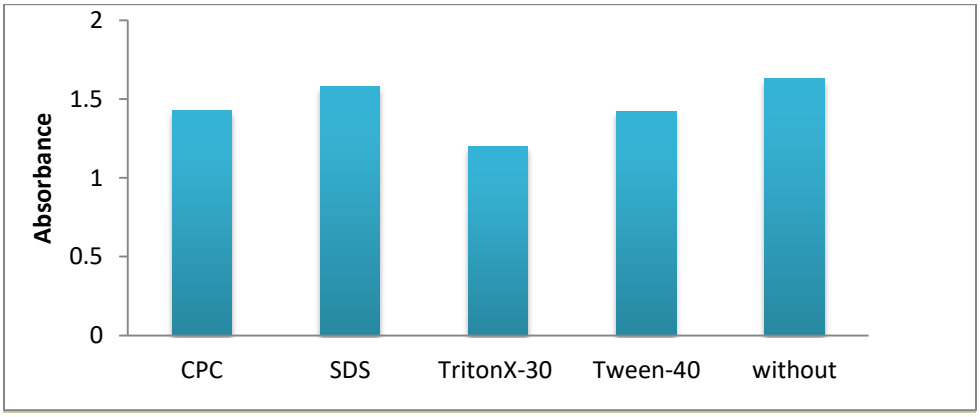


Figure 10: The effect of surfactants

11- Study of the Effect of Addition Sequence

The effect of the addition sequence of reaction components on the absorbance of the resulting colored complex was investigated to identify the optimal order that ensures maximum reaction efficiency and dye stability. Various sequences were tested under the previously optimized conditions as shown in Table 2. The results indicated that sequence (1) produced the highest absorbance at the specified wavelength, highlighting its effectiveness in enhancing the reaction outcome. Consequently, this sequence was adopted in subsequent experiments to ensure consistency and accuracy in spectrophotometric measurements.

Table 2: Effect of order of addition

Order number	Reaction component	Absorbance
1	**R+N+A+S+D+B**	**1.143**
2	**N+R+A+S+B+D**	**0.148**
3	**A+R+N+S+D+B**	**0.746**
4	**R+N+S+A+D+B**	**0.724**

R=Methotrexate, **A**=Acetic acid, **N**=Sodium nitrite, **S**=Sulfamic acid, **D**=Dobutamine, **B**=Ammonium hydroxide

Table 3 summarizes the optimal reaction conditions that yielded the highest absorbance of the colored complex. These conditions were adopted as the basis for applying the proposed analytical method in the determination of the drug compound in its pharmaceutical formulation.

Table 3: Optimal reaction conditions

Experimental Conditions	Dobutamine
max (nm)λ	**466**
ml (ml)/ Methotrexate 150 µg	**1**
$NaNO_2$ 2% (ml)	**1**
CH_3COOH 1M (ml)	**1**

Sulfamic acid 1% (ml)	**0.2**
NH_4OH 2M (ml)	**2**
Temperature (C˚)	**20 (R.T)**
Diazotization Time (min)	**10**
Development Time (min)	**10**
Stability period (min)	**90**

12- Final Absorption Spectrum

The final absorption spectrum of the azo dye resulting from the coupling of diazotized Methotrexate reagent with Dobutamine hydrochloride in an alkaline medium (NH_4OH) was recorded under the optimized reaction conditions. The colored complex exhibited maximum absorbance at 466 nm, as shown in Figure 11, in comparison with the blank solution and distilled water.

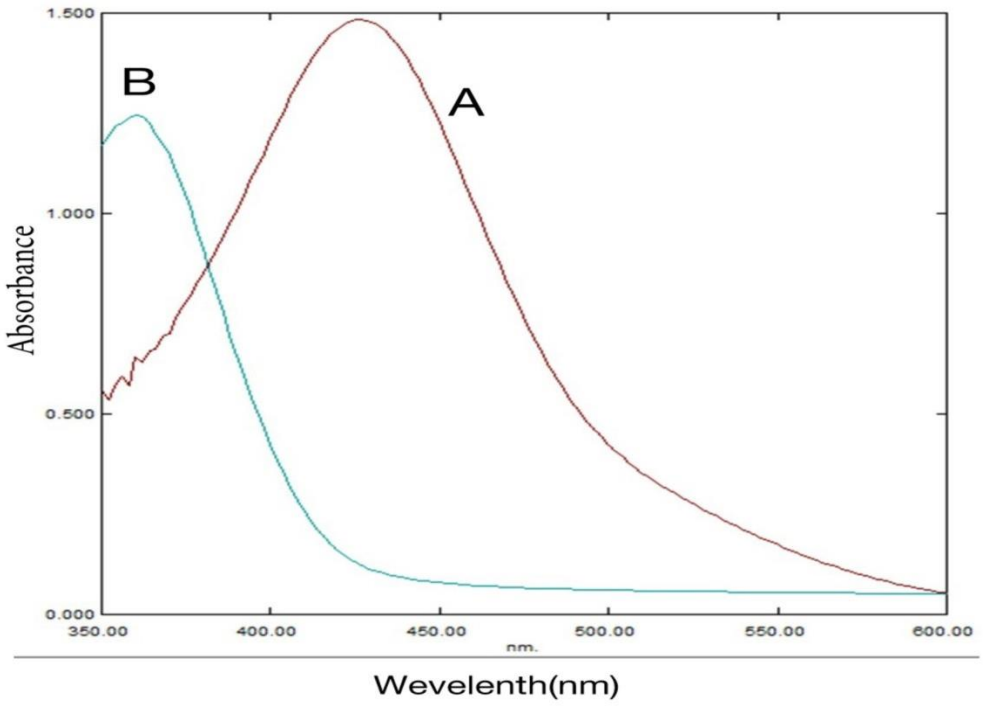


Figure 11: Absorption spectrum of the azo dye

A= Sample Vs Blank, B=Blank Vs water

13- Calibration Curve for Dobutamine Determination

A calibration curve was constructed for the determination of Dobutamine hydrochloride using diazotized methotrexate as the chromogenic reagent, under optimized reaction conditions.

Varying volumes of Dobutamine solution (0.04–1.2 mL) were added, and absorbance was measured at 466 nm. As shown in Figure 12, the curve exhibited a linear relationship within the range of 2–60 µg/mL with a correlation coefficient (R^2) of 0.9943. The molar absorptivity was found to be 1.10×10^5 $L\bullet mol^{1}\bullet cm^{1}$, indicating the high sensitivity and efficiency of the proposed method.

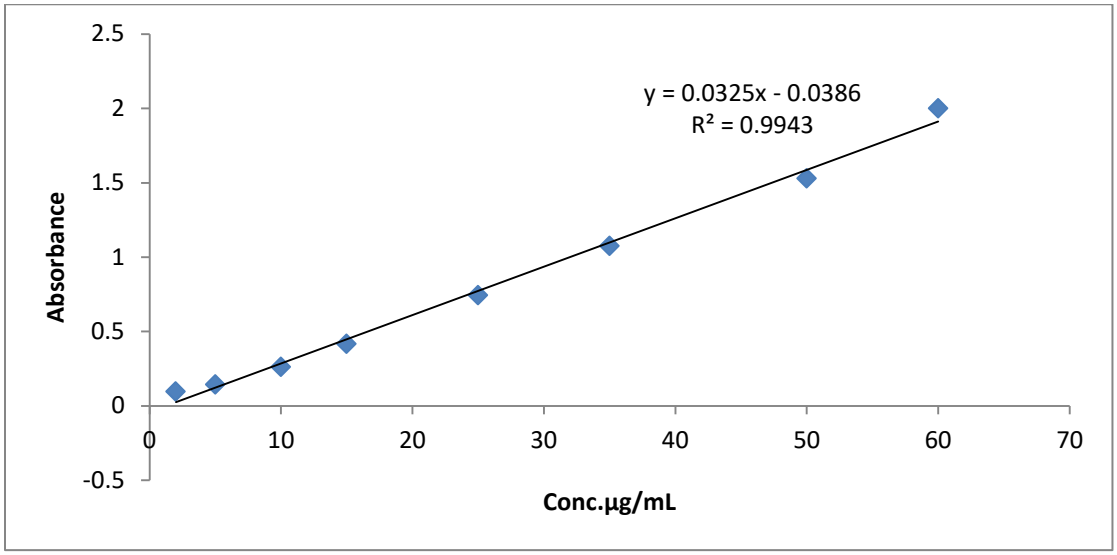


Figure 12: Calibration Curve

Parameter	Dobutamine
Linearity range (µg/ml)	**2_60**
Molar absorptivity ($l.mol^{-1}.cm^{-1}$)	**1.10×10^5**
Sandell Sensitivity ($\mu g/cm^2$)	**3.09×10^{-2}**
LOD* (µg/ml)	**0.0231**
LOQ* (µg/ml)	**0.0702**
Intercept	**0.0385**
Slope	**0.0325**
Determination coefficient (R^2)	**0.9943**

Table 3 presents the statistical values obtained.

14-Applications

Accuracy and Precision of the Proposed Method

The accuracy and precision of the method were evaluated using five measurements at four different concentrations of Dobutamine hydrochloride. The results showed an average recovery

of 100.68% and a relative standard deviation (RSD) below 6.42%, confirming the method's high accuracy, precision, and suitability for analytical applications**,** as shown in Table 4.

Table 4: Accuracy and Precision Results

Drug	**Cons. of drug (µg/ml)**		**Recovery* (%)**	**Average recovery (%)**	**RSD* (%)**
	Added	**Found**			
Dobutamine. HCl	2	4.011	103.4	100.68	**6.42**
	5	5.53	99.29		**6.36**
	25	24.17	100.0		**0.81**
	50	47.98	99.96		**0.56**

Application of the Proposed Method to the Pharmaceutical Preparation

The proposed method was applied for the determination of Dobutamine hydrochloride in its pharmaceutical dosage form, specifically an injectable solution, as described below. As shown in Table 5, the pharmaceutical product manufacturer.

Pharmaceutical preparation	**Declared composition**	**Company**
Dobutamine-Vial	**250 mg Dobutamine. HCl**	**TAJ PHARMA**

Analysis of Dobutamine Hydrochloride Injection

A stock solution of the injection was prepared at a concentration of 2,500 µg/mL, then diluted to obtain a 250 µg/mL solution. Different volumes (1, 0.5, 0.1, 0.04 mL) were taken to prepare concentrations of 50, 25, 5, and 2 µg/mL, respectively, under the optimized experimental conditions. The results presented in Table 6 demonstrate the high accuracy of the proposed method.

Table 6: Application for drug analysis

Pharmaceutical preparation	**Certified value**	**Amount present (µg/ml)**		**Drug content found* (mg)**	**Recovery(%)***	**Average Recovery (%)**
		Added	Found			
Dobutamine_ Vial TAJ PHARMA	250mg	2	4.00	254.37	101.75	**100.10**
		5	5.69	244.2	97.68	
		25	23.86	251.08	100.43	
		50	48.04	251.35	100.54	

Standard Addition Method

The standard addition method was employed to evaluate the accuracy and selectivity of the proposed analytical method for the determination of Dobutamine hydrochloride in its pharmaceutical formulation and to confirm the absence of interference from excipients. The analysis was conducted at two different concentrations under the established optimal conditions. The results, as presented in the table, demonstrated the high accuracy and specificity of the method, with no significant interference from formulation components. Furthermore, the obtained values showed acceptable statistical agreement with the labeled content, confirming the method's reliability for routine pharmaceutical analysis.

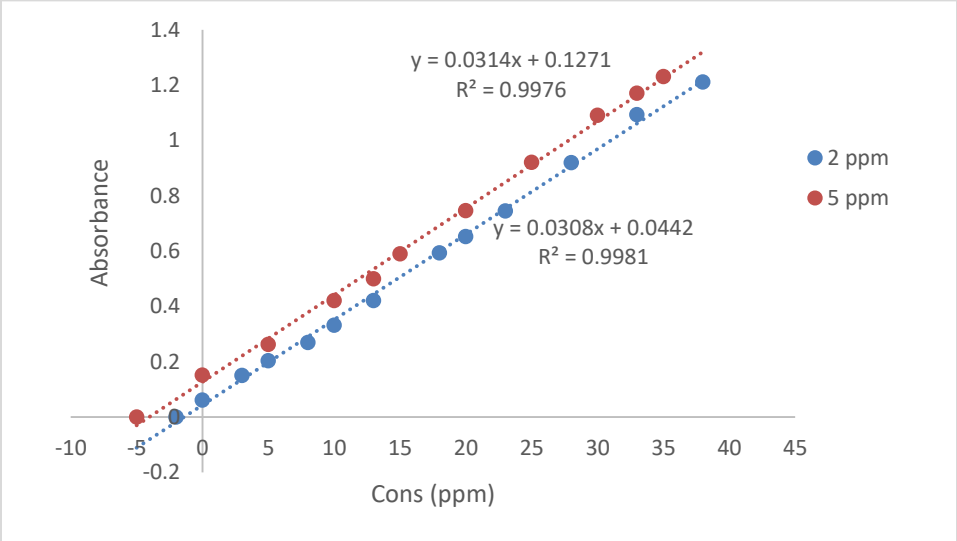


Figure 13: Standard addition Curve for the determination of Dobutamine.

Table 7: Statistical values related to the standard addition method.

Continuous Variation Method (Job's Method)

Pharmaceutical preparation	**Certified value**	**Amount present (µg/ml)**		**Recovery (%)**	**Drug content found (mg)**	
		Added	Found		Present method	**Standard addition method**
Dobutamine _ Vial TAJ PHARMA	250 mg	2	0.628	95.54	254.37	**238.85**
		5	0.729	96.0	244.2	**240.0**

To evaluate the stoichiometric ratio between the azo reagent (Methotrexate) and the drug compound (Dobutamine), the continuous variation method was applied using solutions of equal concentrations (3.32×10^{4} M) with a fixed total volume of 1 mL for the Dobutamine-Methotrexate mixture in a final volume of 10 mL. Absorbance was measured at 466 nm against corresponding blanks. Results shown in Figure 14 indicate a 1:1 stoichiometric ratio between Dobutamine and the reagent.

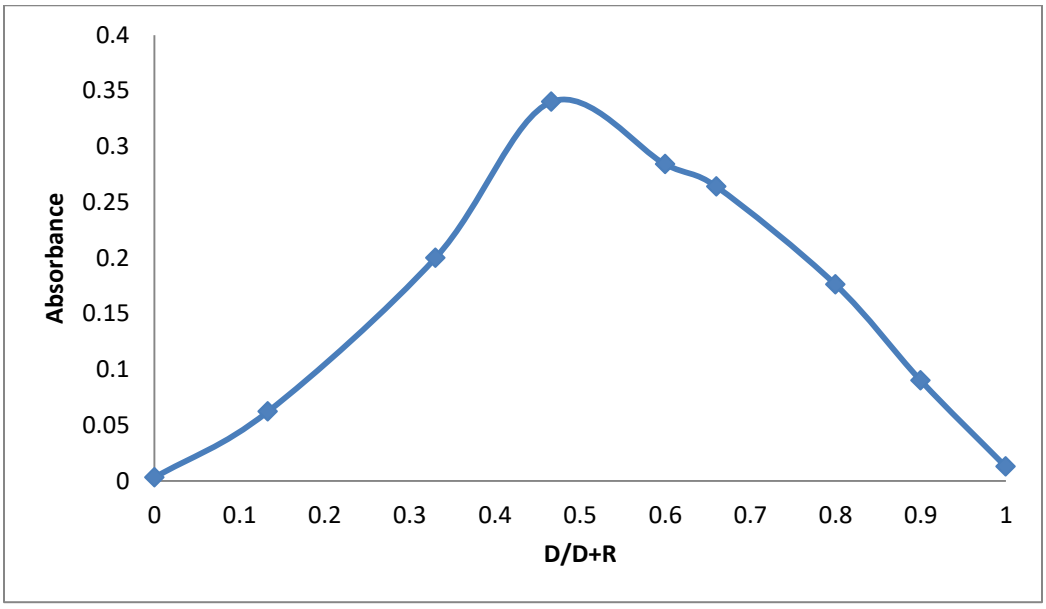


Figure 14: Job's Method

Mole Ratio Method

The molar ratio method was employed to validate the complex formation ratio between Dobutamine and the diazotized reagent (Methotrexate) previously determined by Job's method. A fixed volume of Dobutamine (1.0 mL, 3.32×10^{4} M) was reacted with increasing volumes of the reagent of the same concentration within the range of 0.0–1.5 mL. After adjusting the total volume appropriately, absorbance was measured at 466 nm against corresponding reagent blanks. The results, shown in Figure 15, exhibited spectral behavior consistent with Job's method, confirming a 1:1 stoichiometric ratio and supporting the reliability of both methods under the established experimental conditions.

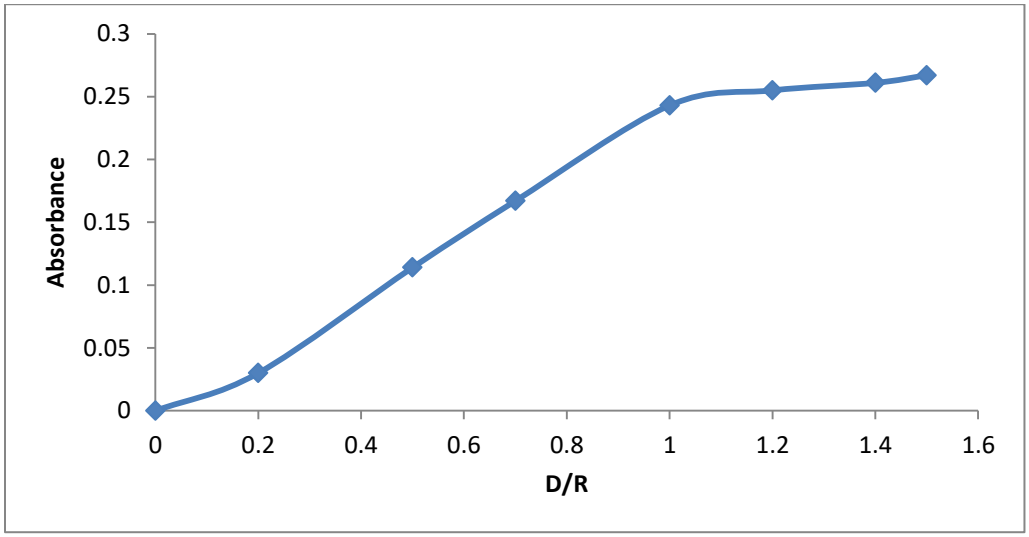


Figure 15: Mole Ratio Method

Stability Constant of the Formed Complex

The stability constant of the 1:1 complex formed between Dobutamine and the diazotized Methotrexate reagent was determined spectrophotometrically by preparing equimolar solutions, along with solutions containing a fixed concentration of Dobutamine and an excess of the diazotized reagent, to assess the complex's stability.

Table 8: Stability constant calculation

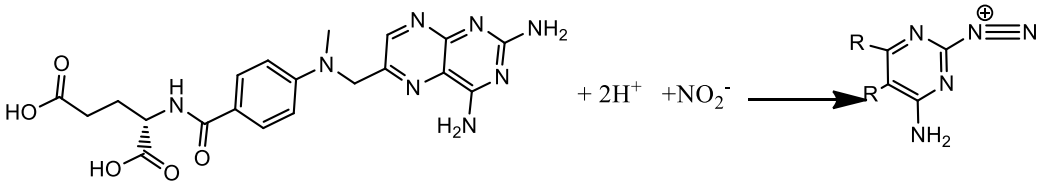
Compound	Conc.(mol.l^{-1})	Absorbance		α	Average of K_{st} (l.mole^{-1})
		As	Am		
Dobutamine.HCl	$10^{-5}\times1.6$	0.165	0.278	0.406	7.39×10^{3}
	$10^{-5}\times3.32$	0.286	0.596	0.520	
	$10^{-5}\times4.98$	0.448	0.896	0.500	

Proposed Chemical Reaction

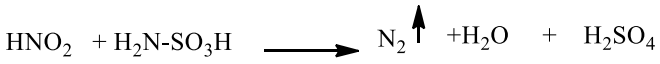
Under the optimized conditions, methotrexate as a reagent undergoes a diazotization reaction in the presence of sodium nitrite and hydrochloric acid. In acidic medium (HCl), the primary aromatic amine group of the reagent reacts with the nitrous acid (generated in situ from $NaNO_2$ and HCl) to form an extremely reactive diazonium salt. To confirm complete removal of unreacted nitrous acid or excess nitrite ions—which could inhibit the next step or cause side reactions—a 1% solution of Sulfamic acid was added. Sulfamic acid acts as a scavenger, reacting with the residual nitrous acid to produce nitrogen gas and sulfate.

Once the diazonium salt is generated and stabilized, it is directly exposed to a coupling reaction with Dobutamine in an alkaline medium (NH_4OH). The phenolic functional group in Dobutamine was activated under basic conditions, allowing it to work as an efficient nucleophile and readily couple with the diazonium group. This results in the formation of a stable yellow-colored azo dye.

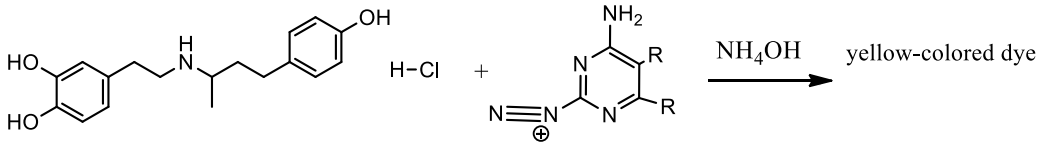
The intensity of the yellow color corresponds to the concentration of Dobutamine and is measured using a spectrophotometer at a maximum absorption wavelength of 466 nanometers. The chemical transformation and overall process of dye formation are illustrated in Scheme 1, which outlines the step-by-step reaction pathway from methotrexate diazotization to azo dye formation.



The excess nitrite was removed by 1% sulfamic acid.



The diazonium salt is then coupled with dobutamine in a basic medium to form a yellow-colored dye, which is measured at 466 nanometers.



Scheme 1: Proposed Chemical Reaction

Comparison of the Developed Method with an Existing Method

The proposed spectrophotometric method, based on diazotization and coupling using diazotized methotrexate, was compared with two previously reported methods (NQS and Fe–Ferricyanide). The results demonstrated the superiority of the current method in terms of sensitivity (ε = 1.10×10^5 $L\cdot mol^{-1}\cdot cm^{-1}$), lower limits of detection (LOD = 0.0231 µg/mL) and quantification (LOQ = 0.0702 µg/mL), a wide linear range (2–60 µg/mL), and high precision and recovery (RSD < 4.26%, Recovery > 100.68%). Additionally, it offers simple execution, low cost, and excellent dye stability, making it a reliable choice for the determination of Dobutamine in pharmaceutical formulations.

Table 9: Comparison of the recommended method with the published methods

Analytical parameters	Present method	Literature method (Roopa et al., 2015)
λmax(nm)	466	**720**
Reagent	Methotrexate	**Ferric-Ferricyanide**
Linearity (µg /ml)	2-60	**0.4 – 3**
Molar absorptivity ($l.mol^{-1}.cm^{-1}$)	1.10×10^5	**8.7×10^3**
Sandell Sensitivity (µg/cm2)	3.09×10^{-2}	**26.0**
Recovery (%)	%100.68	**%95.2**
LOD (µg/ml)	0.0231	**0.06**
LOQ(µg/ml)	0.0702	**0.19**

Conclusion

A simple, rapid and cost-effectivness spectrophotometric method was advanced for the microdetermination of Dobutamine, based on the diazotization of methotrexate as a reagent and its subsequent coupling with Dobutamine in an alkaline medium, resulting in the formation of a stable yellow azo dye with maximum absorbance at 466 nm. The method obeyed Beer's law over the concentration range of 2–60 µg/mL, with a molar absorptivity of 1.10×10^5 $L\cdot mol^{-1}\cdot cm^{-1}$ and Sandell sensitivity of 3.09×10^2 µg/cm². The results revealed high accuracy (recovery of 100.68%) and good precision (RSD < 6.42%). The method was effectively applied to pharmaceutical formulations, confirming its effectiveness and applicability for routine analysis.

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