Research Article

Spectrophotometric Determination of Dobutamine Hydrochloride in Pharmaceutical Formulations

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ABSTRACT

Two simple and validated spectrophotometric methods were developed for the determination of Dobutamine Hydrochloride (DOB**)** in pharmaceutical dosage forms. Method A is based on the complexation of Fe (III**)** with sulpho salicylic acid, followed by the addition of drug, the bleaching of the formed complex absorption is measured at 515 nm. Method B is based on the reduction of Fe (III**)** by DOB in acidic medium and subsequent chelation of Fe (II) with Potassium ferricyanide and the resulting bluish green color is measured at 720 nm respectively. Under the optimum conditions, Beer's law is obeyed in the concentration range of 1 – 20 µg mL^{-1}, 0.4 – 3.0 µg mL^{-1} for methods A and B with molar absorptivity values 6.9494 X 10^{-4} and 1.1988 X 10^{5} L mol^{-1} cm^{-1} respectively. The limits of detection (LOD) and limit of quantification (LOQ) are also reported. The proposed methods were applied successfully to the determination of DOB in pure form and its tablets and no interference was observed from common excipients present in pharmaceutical formulations. Statistical comparison of the results of the proposed procedures with those obtained by the reference methods showed excellent agreement and indicated that no significant difference in accuracy and precision. The validity of the methods were established by recovery studies via Standard-addition technique with satisfactory results.

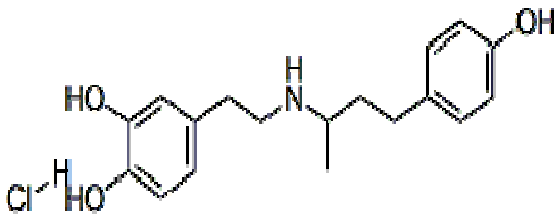
Keywords: Dobutamine Hydrochloride, Sulpho salicylic acid, Ammonium ferric sulphate, Potassium Ferricyanide, Pharmaceuticals, Spectrophotometry.

INTRODUCTION

Dobutamine Hydrochloride (DOB**)**, is chemically known as 4- (2-((1-methyl- 3-(4-hydroxybenzene propyl)amido)ethyl-1,2-di-hydroxybenzene hydrochloric salt, is a sympathomimetric with direct effects on β_1-adrenergic receptors, giving it a prominent ionotropic effect on the heart. It also has some α and β_2–agonist properties. Dobutamine is used in the case of congestive heart failure to increase cardiac output. It is indicated when parental therapy is necessary for ionotropic support in the short–term treatment of patients with cardiac decompensation due to depressed contractility, which causes the cardiac disease. The drug is also commonly used in the hospital setting as a pharmacologic stress testing agent to identify coronary artery disease.

The literature survey revealed that several analytical methods have been reported for the determination of DOB in pure drug, Pharmaceutical dosage forms and in biological samples using gas chromatography[1], Adsorption stripping voltametry[2], High performance liquid chromatography[3-5], Catalytic spectro fluorimetry[6], Flow–injection Chemiluminiscence[7] and Spectrophotometry[8-16].

While the present paper reports elegant methods for the spectrophotometric determination of DOB in pure and in pharmaceutical dosage forms. The first method is based on the bleaching of the complex and second method is based on the red ox and complexation reaction of drug with chelating agents. The structure of the studied drug is as shown below.



Dobutamine hydrochloride

MATERIALS AND METHODS

A systronics model 166 digital spectrophotometer with matched 1.0cm quartz cells were used for all absorbance measurements.

Analytical reagent grade chemicals and double distilled water were used throughout the experiment. DOB (gift sample from Mylon, Bangalore), Ammonium ferric sulphate, Sulpho salicylic acid (S. D. fine chem. Ltd, India) and Potassium ferricyanide (Glaxo laboratory, Mumbai, India).

Reagents and Solutions

Stock solution of Dobutamine hydrochloride containing 100 µg mL^{-1} were prepared by dissolving 10 mg of the respective drug in 100 mL of water. The solution was further diluted quantitatively according to their linearity range.

Preparation of Ammonium ferric sulphate (0.01M)

Prepared by dissolving 0.48g of Ammonium ferric sulphate in 1 mL of concentrated Hydrochloric acid and making the volume to 100 mL of water. The solution was further diluted quantitatively according to their linearity range.

Method A

***Preparation of Sulpho salicylic acid (SSA)* (0.01M)**

Prepared by dissolving 0.25g of SSA in water and diluted to 100 mL with water.

***Preparation of Acetic acid-sodium acetate buffer pH* (4.0)**

Prepared by mixing 40 mL of CH_3COOH (0.5M) and 10 mL of CH_3COONa (0.5M) in 50 mL standard flask.

Method B

***Preparation of Potassium ferricyanide* (0.01M)**

Prepared by dissolving 0.33g of Potassium ferricyanide in water and diluting to 100 mL with water.

***Preparation of H_2SO_4* (10M)**

Prepared by taking 55.5 mL of concentrated H_2SO_4 and diluting to 100 mL with water.

General procedure

Method A

Aliquots of varying standard solution of DOB (1-20 µg mL^{-1}) were transferred into a series of 10 mL calibrated flasks. To each flask add 0.5mL of ammonium ferric sulphate, 1.0 mL of SSA and 0.5 mL of acetic acid-sodium acetate buffer pH (4.0), heat it in a water bath for 5 min (70–80°C). After cooling the flasks to room temperature, the solutions were made up to the mark with distilled water. The absorbance of the solutions were measured against distilled water at 515 nm and the calibration graph was constructed by plotting absorbance vs. concentration for DOB. The order of addition of reagents is important to get the maximum absorbance.

Method B

Varying aliquots of standard solution (0.4–3 µg mL^{-1}) were transferred into a series of 10 mL calibrated flasks. To each flask add 1.0 mL of Potassium ferricyanide. After 10 min, add 1.5 mL ammonium ferric sulphate and 1.0 mL of 10M H_2SO_4. Then the flasks were further diluted and made up to the mark with distilled water and the absorbance of each of these were measured against reagent blank at 720 nm.

RESULTS AND DISCUSSION

In the method A, initially the reaction taking place between the Ammonium ferric sulphate [Fe(III)] and SSA. [i.e., Fe(III)] forms complex with SSA]. If the drug containing –OH group (electron rich), is added to the reaction mixture, the breaking of the complex occurs. i.e., the color of the complex is bleached. As the concentration of the drug increases, the color produced by the complex decreases. This is an indirect spectrophotometric method. The absorption spectra of DOB against distilled water is as shown in Figure 1. The unknown concentration of the drug can be calculated by knowing the absorbance at the λ_{max} using the regression equation.

Method B is based on the reaction of drug with Fe(III) in the presence of potassium ferricyanide in acidic medium to produce bluish green color with maximum absorption at 720 nm. The reaction involves the reduction of Fe(III) by DOB, which subsequently reacts with ferricyanide to form a bluish green color product in acidic medium. The proposed reaction mechanism for method B is as given below.

Drug + Fe(III) → Fe(II)

Fe(II) + Pot. Ferricyanide → Ferro ferricyanide complex

The absorption spectra of DOB against reagent blank is as shown in the Figure 2**.**

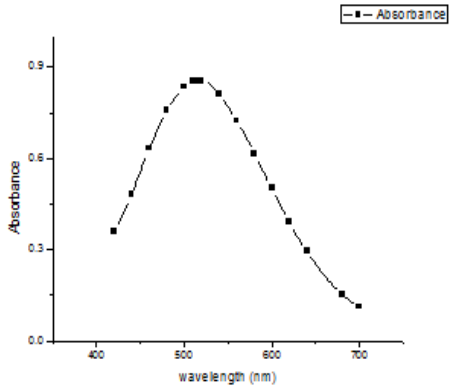


Figure 1: Absorption spectra of Dobutamine Hydrochloride against distilled water (2ppm) (method A)

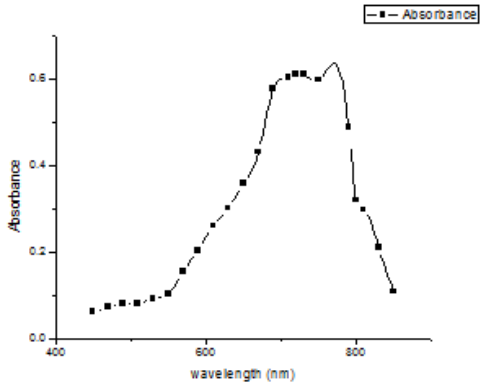


Figure 2: Absorption spectra of Dobutamine hydrochloride against reagent blank (2ppm)(method B)

Optimization of Reaction Variables

Method A

By varying one and keeping other experimental parameters and amount of drug constant, the effect of ferric ammonium sulphate, SSA, and buffer pH (4.0) were

tested (method A). The maximum color intensity was obtained when 0.5 mL of ferric ammonium sulphate, 1.0 mL of SSA and 0.5 mL buffer, were added to DOB. The effect of volume of ferric ammonium sulphate, SSA, and buffer on the drug is studied.

Effect of pH

The maximum color intensity was observed at pH 4 for DOB is as shown in the Figure 1(a). Effect of reaction time and temperature and stability of the colored product.

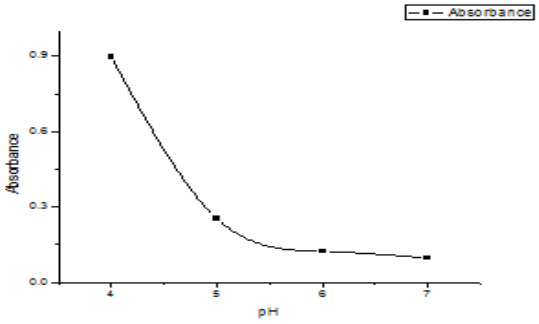


Figure 1(a): Effect of pH on DOB (2 µg mL^{-1})

Effect of Heating Time and Temperature

The optimum reaction time and temperature were determined by carrying out the reaction time and temperature were determined by carrying out the reaction at different temperatures (25 - 100°C) and the time intervals (0-30min). Satisfactory result, maximum color intensity and reproducible absorbance were obtained, when the reaction mixtures were heated to (70-80°C) for 5 min. The color development was stable for 2 days. The effect of time and temperature for DOB is as shown in Figure 1(b) and 1(c).

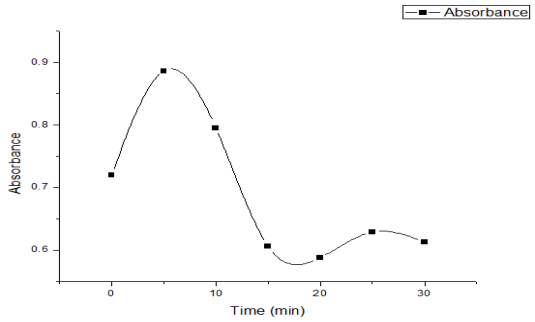


Figure 1(b): Effect of heating time on DOB (2 µg mL^{-1})

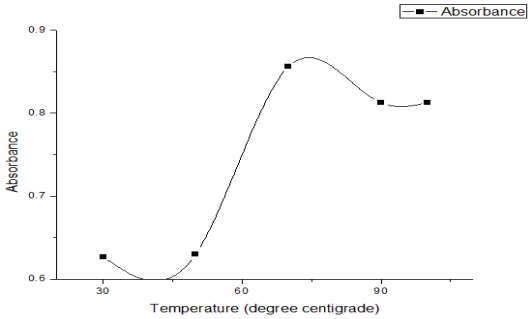


Figure 1(c): Effect of temperature on DOB (2 µg mL^{-1})

Effect of solvent

Water, Ethanol and acetonitrile were tested, water gave the best result for DOB.

Method B

To obtain the maximum color development the effect of Potassium ferricyanide and ammonium ferric sulphate were tested. The maximum color was developed when 1.0mL of pot. Ferricyanide and 1.5mL of ammonium ferric sulphate were added. Different concentrations of acid were tested. 1 mL of 10 M H_2SO_4 gives the maximum color development. The color was stable for 24 hours. The effect of volume of pot. Ferricyanide, ammonium ferric sulphate and H_2SO_4 on the drug is as shown in the Figures 2(a), 2(b) and 2(c).

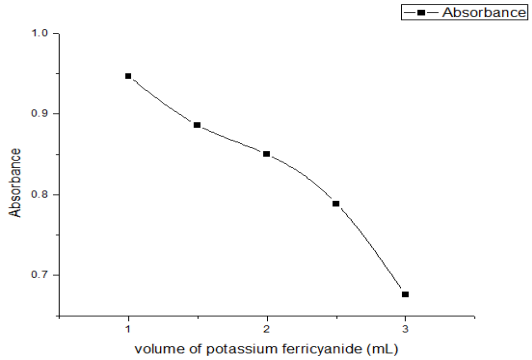


Figure 2(a): Effect of volume of Potassium ferricyanide on DOB (3 µg mL^{-1})

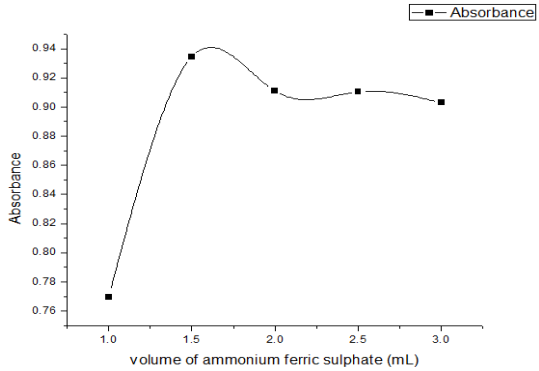


Figure 2(b): Effect of volume of Ammonium ferric sulphate on DOB (3 µg mL^{-1})

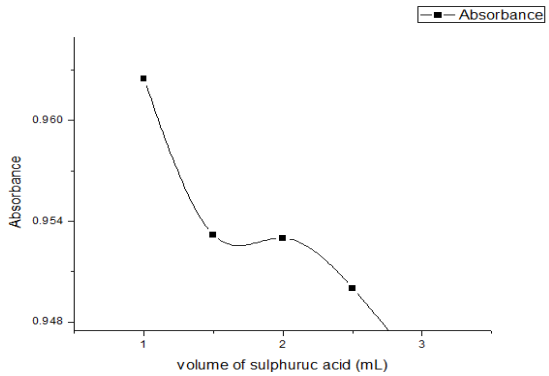


Figure 2(c): Effect of volume of 10M H_2SO_4 on DOB (3 µg mL^{-1})

Validation of the Proposed Method

The method was validated according to the procedures described in ICH guidelines (ICH Steering Committee. ICH harmonized tripartite guideline, 1996) for the validation of analytical methods.

Limits of detection (LOD) and Quantification (LOQ)

The Limits of detection (LOD) and Quantification (LOQ) were calculated according to the ICH guidelines using the formulae:

LOD = 3.3S/b, LOQ = 10S/b,

Where S is the standard deviation of blank absorbance values and b is the slope of the calibration plot.

The Beer's law range, Molar absorptivity, Sandell's sensitivity, Regression equation and correlation coefficient were determined for DOB.

A linear relationship was found within the range of 1–20 µg mL^{-1} (method A) and (0.4-3 µg mL^{-1}) (method B). Regression analysis of beer's law plots revealed good correlation. The limit of detection and limit of quantification were tested. The optimum characteristics and parameters are given in (Table 1).

Table 1: Optical characteristics and parameters of the studied drug

Parameters	Optical characteristics (DOB)	
	Method A	Method B
Color	Purple	Bluish green
λ_{max} (nm)	515	720
Beer's law limit (µg mL^{-1})	1-20	0.4-3
Molar absorptivity (L mol^{-1} cm^{-1})	6.9494 X 10^{-4}	1.1988 X 10^{5}
Sandell's sensitivity (µg cm^{-2})	4.8615 X 10^{-3}	2.8181 X 10^{-3}
Limit of Detection [LOD] (µg mL^{-1})	-1.1544	2.2875
Limit of Quantification [LOQ] (µg mL^{-1})	-3.4982	0.07643
Regression equation[Y*]		
Slope [B]	-0.0563	0.2966
Intercept[A]	0.9756	0.0646
Correlation coefficient [r]	-0.9918	0.9914
Relative standard deviation[b]	0.76	0.89

*Y= BX+A, where X is the concentration of the measured solution in µg mL^{-1} and Y is the unit for absorbance. [b]Average of five determinations (concentrations of 4, 8 and 12 µg mL^{-1} and 0.4, 1.2, and 2.4 µg mL^{-1} DOB for (method A) and (method B) respectively.

Interference Studies

The effect of common excipients used in the pharmaceutical preparation were studied by analyzing synthetic sample solutions containing the quantity of drugs as mentioned in Table 2 in presence of 100 fold more concentration of each excipients. The tolerance limit was defined as the concentration which gave an error of ± 3.0% in the determination of drug. The common excipients such as starch, dextrose, lactose, talc, magnesium stearate, had no effect in the analysis.

Table 2: Recovery of drugs from solution in presence of a with a 100 fold concentration of various additives used as excipients in formulation

Excipients	% Recovery ± %RSD[a]	
	Method A[b]	Method B[c]
Dextrose	99.8 ± 0.1	99.9 ± 0.2
Lactose	99.9 ± 0.2	100.0 ± 0.1
Sucrose	99.7 ± 0.3	99.8 ± 0.2
Starch	99.8 ± 0.4	99.7 ± 0.5
talc	99.9 ± 0.3	98.7 ± 0.4
Magnesium stearate	98.9 ± 0.2	99.7 ± 0.3

[a] Mean ± % R.S.D, n=3, [a]mean of three determinations

[b]concentration of DOB used – 4 µg mL^{-1}(Method A)

[c]concentration of DOB used – 1.2 µg mL^{-1} (Method B)

Precision Studies

The short term precision (intraday precision) of the drug was evaluated by measuring 5 independent samples at 3 different concentration levels (4, 8, 12 µg mL^{-1}) (Method A) and (0.4, 1.2 and 2.4 µg mL^{-1}) (Method B). Similarly the assay for daily precision (interday precision) at the same concentration level was repeated for 5 consecutive days (Table 3).

The available pharmaceutical dosage forms of the investigated drug was analyzed by the proposed method. The precision of the method was checked by taking five replicate measurements. The results obtained by the proposed and the reference methods for the dosage forms were compared statistically by means of F- and t-test and were found not to differ significantly at 95% confidence level are shown in Table 4.

The reliability and accuracy of the proposed methods were further ascertained through recovery studies using the standard addition method by adding different amount of standard drug to the preanalyzed dosage forms such that the cumulative amount after adding the drug did not exceed their linearity range (Table 5).

CONCLUSION

The proposed spectrophotometric methods for the determination of Dobutamine hydrochloride is fairly sensitive, simple, and economical with reasonable precision and accuracy. The optical parameters and statistical comparison justify these methods for application in routine drug estimation in pure and dosage forms.

Also, the procedures do not involve any critical reaction conditions or tedious sample preparation steps. So, the recommended methods are well suited for the assay and evaluation of drug in pharmaceutical preparation and can also be considered as a general method for the quantification of drug.

Table 3: Intra day and Inter day precision data of DOB

Formulation	Amount taken µg/mL	Intra-Day % Recovery ± % RSD[a]	Inter-Day % Recovery ± % RSD[b]
	4.0	101.2 ± 0.49	100.2 ± 0.48
DOB (method A)	8.0	99.8 ± 0.66	99.9 ± 0.66
	12.0	100.0 ± 1.14	99.99 ± 1.01
	0.4	100.25 ± 0.72	99.9 ± 0.69
DOB (method B)	1.2	99.16 ± 0.85	99.9 ± 0.89
	2.4	99.63 ± 0.29	100.0 ± 0.3

[a]Mean value of five determinations, [b]Mean of five determinations performed over a period of five days.

Table 4: Analysis of drugs in pharmaceutical formulations

Formulation studied	Label claimed mg[a]	Amount found by the proposed method ± SD, mg[a]	Reference method ± SD[a]*	%Recovery by the proposed method[b] ± RSD
DOB[c] (Method A)	**Dobier S (250 mg/mL) inj**	**249.7 ± 0.951**	**250.9 ± 0.832 [14]**	**99.88 ± 0.97**
		t = 2.32		
		F = 1.3		
DOB (Method B)	**Dobier S(250 mg/mL) inj**	**250.2 ± 0.975**	**249.7 ± 1.062 [13]**	**100.01 ± 0.89**
		t = 0.84		
		F = 1.38		

[a]Mean of five determinations ± Standard deviation. n=5; the t- and F-values obtained after comparison to the reference methods, which have the following theoretical values at 95% confidence limit t = 2.44 and F = 5.05. After adding two different amounts of pure drugs to the fixed concentration of pre analyzed pharmaceutical formulations, [c]DOB equivalent to 250 mg/mL (Chandra Bhagat pharma, Pvt. Ltd, India), References inside the brackets are the reported methods given under references.

Table 5: Results of recovery experiments by Standard Addition method.

Formulation studied	Amount of drug taken in inj, µg mL^{-1}	Amount of pure drug added, µg mL^{-1}	*Total found, µg mL^{-1}	Pure drug recovered % ± % RSD
Dobier S(250 mg/ mL) inj (Method A)	4	2.0	5.99	99.83 ± 0.75
	4	6.0	9.98	99.80 ± 0.963
	4	10.0	14.01	100.07 ± 0.66
Dobier S(250 mg/ mL) inj (Method B)	0.2	0.4	0.602	100.3 ± 0.91
	0.2	0.8	1.01	100.1 ± 1.10
	0.2	1.8	1.998	99.9 ± 0.66

*Mean value of five determinations.

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