World Journal of Pharmaceutical research

Volume 2, Issue 6, 3016-3024. Research Article ISSN 2277 – 7105

UTILISATION OF OXIDATIVE COUPLING REACTIONS FOR THE ESTIMATION OF DOBUTAMINE

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Article Received on
18 August 2013,

Revised on 25 Sept. 2013,
Accepted on 30 October 2013

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ABSTRACT

Three simple spectrophotometric methods (A-C) for the assay of Dobutamine (DBU) in pure state and in formulations have been developed based on the oxidative coupling reaction of DBU, with N,N-dimethylamino-paraphenylenediamine (DMPD) in the presence of Chloramine–T (CAT) (method A), 4-aminophenazone (4-AP) in the presence of IO_4^- (method B) and 3-methyl benzothiazolinone hydrazone (MBTH) in the presence of cerium IV (Ce IV) (method C). Regression analysis of Beer-Lambert plots showed good correlation in the concentration ranges 2.0-20.0 µg/ml, 1.0-8.0 µg/ml and 2.0-10.0 µg/ml for methods A, B and C respectively. The results of analysis have been validated statistically and by recovery studies.

Keywords: Visible spectrophotometry, Dobutamine, 4-aminophenazone, 3-methyl benzothiazolinone hydrazone, Chloramine.

INTRODUCTION

DBU is a synthetic catecholamine used in the treatment of heart failure and cardiogenic shock. Its primary mechanism is direct stimulation of β_1 receptors of the sympathetic nervous system. Dobutamine is used to treat acute but potentially reversible heart failure, such as which occurs during cardiac surgery or in cases of septic or cardiogenic shock, on the basis of its positive inotropic action.Dobutamine can be used in cases of congestive heart failure to increase cardiac output. It is indicated when parenteral therapy is necessary for inotropic support in the short-term treatment of patients with cardiac decompensation due to depressed contractility, which could be the result of either organic heart disease or cardiac surgical

procedures. It is not useful in ischemic heart disease because it increases heart rate and thus increases myocardial oxygen demand. Chemically it is 1,2-benzenediol, 4-[2-[3-(4-hydroxyphenyl)-1-methylpropyl] amino]ethyl]. A few numbers of methods such as Spectrophotometric[1-5] and HPLC[6-20] were reported for the estimation of DBU. Literature survey revealed that few visible spectrophotometric methods (Sastry et al, 2002), are reported for its quantitative determination in bulk drug and pharmaceutical formulations. During the course of our efforts to develop sensitive visible spectrophotometric methods, it was observed that the analytically useful phenolic hydroxyl group in DBU has not been properly exploited. Three visible spectrophotometric methods (A, B and C) based on the oxidative coupling reaction of DBU with the reagents such as DMPD–CAT (method A), 4-AP - IO_4^- (method B) and MBTH–Ce(IV) (method C) have been developed. All the methods are applicable to the determination of DBU in bulk form and in formulations.

EXPERIMENTAL

Instruments

Spectral and absorbance measurements were made on Systronics UV- Visible Spectrophotometer 117 with 10mm matched quartz cells. An Elico LI-120 digital pH meter was used for pH measurements.

Reagents

All the chemicals used were of analytical grade. All the solutions were prepared freshly in doubly distilled water. Aqueous solutions of 2.39×10^{-3}M DMPD (Merck) and 7.11×10^{-4}M CAT (Loba) were prepared for method A. Aqueous solutions of 2.46×10^{-2}M 4-AP (Ferack) and 4.68×10^{-3}M IO_4^- (BDH) were prepared for method B. Aqueous solutions of 8.58×10^{-3}M MBTH (Loba) and 1.58×10^{-2}M Ceric ammonium sulphate in 0.36 N H_2SO_4 (BDH) were prepared for method C.

Preparation of drug solutions

A 1 mg/ml solution was prepared by dissolving 100 mg of pure DBU in 100 ml of distilled water and this stock solution was diluted stepwise with distilled water to obtain the working standard solutions of concentrations 200µg/ml for method A, 50 µg/ml for method B and 20 µg/ml for method C. Sample solutions for formulations (tablet or injection) were prepared exactly in the same manner as given under the standard solutions with prior filtration before making up to volume and analyzed as described for pure samples.

PROCEDURES

Method A

Aliquots of DBU solution (0.5 – 2.0 ml, 200 µg/ml) were transferred into a series of 25 ml calibrated tubes. Then 1.0 ml of DMPD solution and 1 ml of CAT solution were added and the tubes were kept aside for 15 min. The solutions in each tube were made up to the mark with methanol. The absorbance was measured at 520 nm against a reagent blank prepared in a similar way. The amount of DBU was computed from its calibration graph.

Method B

Aliquots of standard DBU solution (1.0-3.0 ml, 50 µg/ml) were transferred into a series of 25 ml calibrated tubes. Then 2.0 ml of 4-AP and 5.0 ml of $NaIO_4$ solutions were added and kept aside for 5 min. The volume was made up to the mark with distilled water. The absorbance was measured at 530 nm against a similar reagent blank. The amount of DBU was computed from its calibration graph.

Method C

Aliquots of standard DBU solution (1.0 – 3.0 ml, 20 µg/ml) were transferred into a series of 10 ml calibrated tubes. The total volume in each tube was brought to 3.0 ml with distilled water. One ml each of MBTH and ceric ammonium sulfate were added and the tubes were kept aside for 5 min. at room temperature. The solutions in each tube were made up to mark with distilled water and the absorbances were measured after 5 min. at 640 nm. against a reagent blank. The amount of DBU was computed from its calibration graph.

RESULTS AND DISCUSSION

The optimum conditions for the colour development of method were established by varying the parameters one at a time in each method, keeping the others fixed and observing the effect produced on the absorbance of the coloured species.

The optical characteristics such as Beer's law limits, molar absorptivity for each method are given in Table 1. The precision of each method was found by measuring absorbances of six replicate samples containing known amounts of drug and the results obtained are incorporated in Table1. Regression analysis using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient (r) for each method and are presented in Table 1. The accuracy of each method was ascertained by comparing the results by proposed and reference methods (UV) statistically (Table 2). This comparison shows that

there is no significant difference between the results of proposed methods and those of the reference ones. The similarity of the results is obvious evidence that during the application of these methods, the additives and excipients that are usually present in tablets do not interfere in the assay of proposed methods. As an additional check of accuracy of the proposed methods, recovery experiments were performed by adding a fixed amount of the drug to the preanalysed formulations. The amount of drug found and the % recovery was calculated in the usual way.

The proposed methods are applicable for the assay of drug (FOR) and have the advantage of wider range under Beer's law limits. The decreasing order of sensitivity and λ_{max} among the proposed methods are C>B>A respectively. The proposed methods are simple, selective and can be used in the routine determination of DBU in bulk samples and formulations with reasonable precision and accuracy.

Table. 1: Optical characteristics, precision and accuracy of the proposed methods for Dobutamine

Parameters	**Method A**	**Method B**	Method C
λ_{max} (nm)	520	535	640
Beer's Law limits (µg/ml)	2 – 25	1 –10	2 – 12
Molar absorptivity (l $mol^{-1}cm^{-1}$)	9.456 x 10^3	2.628 x 10^4	2.284 x 10^4
Sandell's sensitivity (µg/cm^2/ 0.001 absorbance unit)	0.032	0.011	0.013
Regression Equation (y = a + bc)[a] Slope (b)	0.0313	0.0874	0.0754
Intercept (a)	-0.0003	0.0008	0.0018
Correlation coefficient (r)	0.9999	0.9998	0.9999
Standard Error of Estimation (S_e)	0.0016	0.0028	0.0013
Relative Standard Deviation (%)[b]	0.2364	0.4554	0.3727
% Of range error (confidence limit) (i) 0.05 level	0.198	0.381	0.312
(ii) 0.01 level	0.292	0.563	0.461
% Error in bulk sample	-0.075	0.172	-0.1320

[a] Y=a+bc, where c is the concentration in µg/ml.

[b] Average of six determinations considered.

Table . 2 : Assay and recovery of DBU in pharmaceutical formulations

Sample	Labelled amount (mg)	Amount found by Proposed Methods*			Amount found by reference method[1]	%Recovery by Proposed methods**		
		A	B	C		A	B	C
Tablet I	1	0.99± 0.001	0.99± 0.002	1.00± 0.004	0.99± 0.001	99.83± 0.15	99.73± 0.45	99.81± 0.004
Tablet II	1	1.00± 0.003	1.00± 0.005	0.99± 0.007	1.00± 0.002	100.38± 0.66	100.37± 1.00	99.94± 0.032
Tablet III	1	0.99± 0.005	0.99± 0.005	1.00± 0.011	0.99± 0.005	99.55± 0.56	99.81± 0.50	100.16± 0.79
Tablet IV	1	0.99± 0.010	1.00± 0.006	0.99± 0.009	1.00± 0.007	99.20± 1.09	100.29± 0.64	99.72± 0.11

*Average ± standard deviation of six determinations.

**After adding 3 different amounts of the pure labelled to the pharmaceutical formulation, each value is an average of 3 determinations.

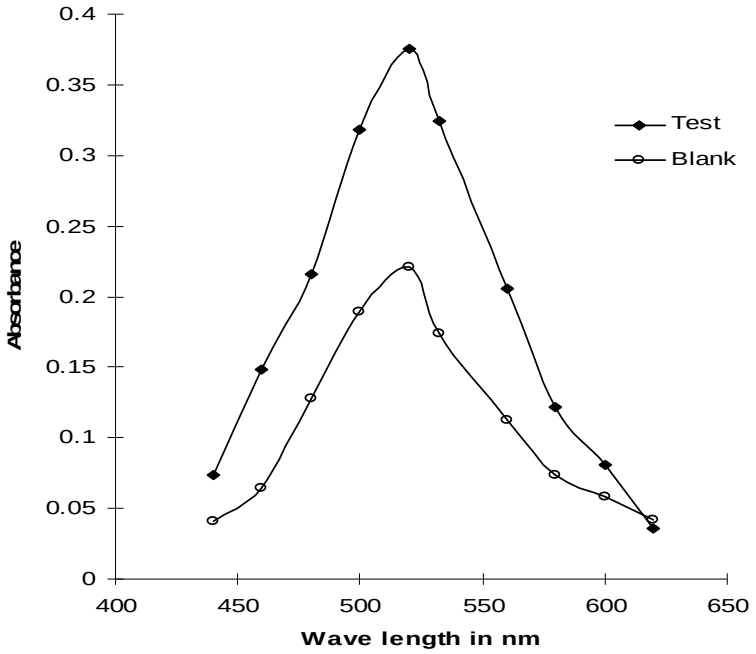

Fig.1 Absorption spectra of the DBU- DMPD–CAT system (♦♦) (concentration of DBU: 2.276 x 10^{-2}, DMPD: 1.91 x 10^{-4} M, CAT: 2.84 x 10^{-5} M) and reagent blank vs. chloroform (o-o).

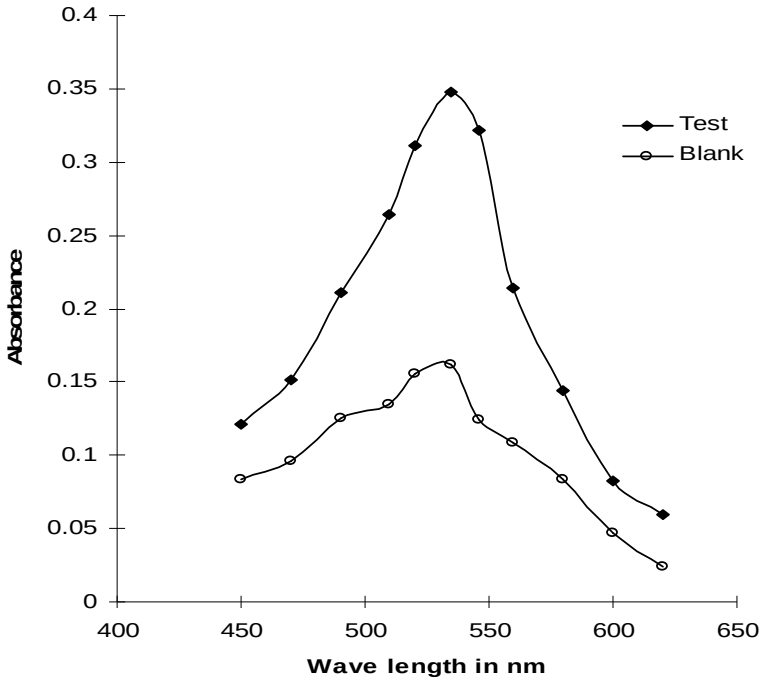

Fig.2 Absorption spectra of the DBU-4-AP - IO_4^- system (♦♦) (concentration of DBU: 1.300 x 10^{-2}, 4-AP: 1.96 x 10^{-3} M, IO_4^- : 9.36 x 10^{-4} M) and reagent blank vs. chloroform (o-o).

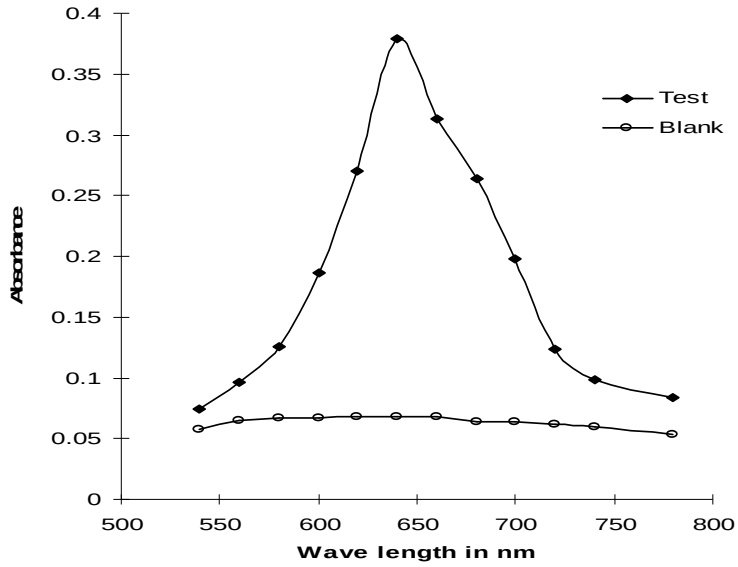

Fig.3 Absorption spectra of the DBU- MBTH–Ce(IV) system (♦♦) (concentration of DBU : 1.300 x 10^{-2}, MBTH : 8.56 x 10^{-4} M , Ce IV : 7.90 x 10^{-4} M), reagent blank vs. chloroform (o-o).

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