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New Validated Rp-HPLC Method for the Estimation of Dobutamine In Pharmaceutical Formulation

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ABSTRACT

A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for rapid assay of Dobutamine in Bulk and Pharmaceutical tablet Formulation. Isocratic elution at a flow rate of 1.5ml/min was employed on symmetry Shimadzu LC-20 AT$_{VP}$ Kromasil C-18 column at ambient temperature. The mobile phase consisted of Methanol: 0.01MPotassium di hydrogen Phosphate(60:40 v/v). The UV detection wavelength was 280nm and 20 µl sample was injected. The run time for Dobutamine is 6 min. The flow rate was found to be 1ml/min. Calibration graph was found to be linear at range 0.2mg/ml to 1.0mg/ml. The method was validated as per the ICH guidelines. The method was successfully applied for routine quality control analysis of pharmaceutical formulation. The HPLC method can be successfully applied for the routine quality control analysis of Dobutamine formulations, which could be the better choices compared to the reported methods of literature

Key words: Dobutamine, Rp- HPLC, UV detection, Recovery, Precise.

INTRODUCTION

Chemistry of Dobutamine [1] (Fig 1) revealed the chemical name as (*RS*)-4-(2-{[4-(4-hydroxyphenyl)butan-2-yl]amino}ethyl)benzene-1,2-diol. Dobutamine was developed by a Ronald Tuttle and Jack Mills, as a structural analogue of isoprenaline. [2] 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.[3] Primary side effects include those commonly seen for β_1 active sympathomimetics, such as hypertension, angina, arrhythmia, andtachycardia. Used with caution. [4] Dobutamine is administered as a racemic mixture consisting of both (+) and (−) isomers; the (+) isomer is a potent β_1 agonist and α_1 antagonist, while the (−) isomer is an $\alpha_1$agonist. [5] Dobutamine also has mild $\beta_2$agonist activity, which makes it useful as a vasodilator. [6] Dobutamine [7] is contraindicated in patients with idiopathic hypertrophic sub aortic stenos is and in patients who have shown previous manifestations of hypersensitivity to Dobutamine. H. Husseini etal [8], A sensitive and specific high-performance liquid chromatographic method with electrochemical detection was developed for measuring dobutamine in human plasma samples. Following an external standard method, 0.1 ml of EDTA—glutathione plasma was diluted on ice with 0.2 ml of a 5% trichloracetic acid solution. The mixture was centrifuged, filtered, and 30 µl were injected. Assessment was done by electrochemical detection. The assay was linear from 1 to 400 ng/ml plasma. For determination of dobutamine we also used a liquid—liquid extraction method routinely applied for plasma catecholamines. Liquid—liquid extraction requires application of 100–1000 µl of plasma. The standard curve was linear from 0.1 to 600 ng/ml. Absolute recovery of dobutamine was 90 ± 3% with the liquid—liquid extraction procedure and 91 ± 3% with the protein precipitation method. For both methods

dobutamine was separated on Nova-Pak C_{18} columns. The mobile phase used was 0.1 molar phosphate buffer—acetonitrile (80:20, v/v) with 1-octanesulfonic acid and triethylamine as ion-pair reagents. The pH was adjusted to 2.7.

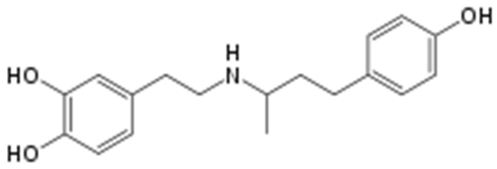


Fig 1: Structure of Dobutamine

Leflour, C etal [9], An isocratic reversed-phase high performance liquid chromatographic method has been developed for the determination of dobutamine in the plasma of dialysed patients. A solid phase extraction method with a Sep-Pak C_{18} cartridge was used to isolate the drug and isoxsuprine (internal standard) from plasma. The separation was carried out on an ODS-Hypersil column with 0.1 M phosphate buffer: acetonitrile: methanol (72 : 20 : 8 v/v/v) as the mobile phase. The recovery of dobutamine added to plasma by the extraction procedure was 87 ± 2.3% (mean ± SD). The accuracy and reproducibility of the method were within acceptable limits over the concentration range 0–1000 ng/ mL. Quantification was by fluorescence detection at 275 nm excitation and 310 nm emission wavelengths with a detection limit of 5 ng/ mL for dobutamine. This procedure was applied to ascertain the pharamacokinetics of dobutamine infusion in nine patients with cardiogenic shock and end-stage renal disease undergoing haemodialysis.

McKennon, D. W. etal [10], A sensitive and specific high-pressure liquid chromatographic method was developed for measuring dobutamine in human plasma samples. Nylidrin is employed as an internal standard. Following extraction and separation on a C_{18} reversed-phase column, the drug is detected by a fluorescence detector with an excitation wavelength of 195 nm and a 330-nm emission cutoff filter. The retention times of dobutamine and nylidrin are 5.2 and 19.2 min, respectively. The minimum level of sensitivity is 10 ng/ml. Reproducibility was ±5% over a 25–300-ng/ml range. Several drugs were screened for possible interference, but none interfered with the dobutamine analysis.

Knoll R, etal [11], a simple method for the routine determination of dobutamine in human plasma. With a relatively small amount of equipment--such as HPLC pump, RP 18-column, sample valve, fluorescence detector and an integrator--dobutamine levels could be determined quickly after a simple extraction procedure on Bond-Elut-CN-columns. In the case of critically ill patients suffering from different kinds of shock we found a wide variation of plasma levels in arterial blood samples. Even with an exact application of dobutamine in relatively low doses of about 2.5 micrograms/kg BW/min to 5 micrograms/kg BW/min, levels reached from 26.8 to 181 micrograms/ml. In our opinion, one reason for these variations might be a differing volume of distribution caused by varying intravascular volumes in critically ill patients. A further possibility might be a different degree of sulfoconjugation of dobutamine.

Therefore, the occasional occurrence of tachycardia under dobutamine therapy could be caused by relatively high plasma levels of free dobutamine.

Peak HPLC containing LC 20AT pump and variable wavelength programmable ***PDA detector*** and Rheodyne injector was employed for investigation. The chromatographic analysis was performed on a Kromasil C_{18} column 250 x 4.6 mm ID with 5 µ particle size and the column were maintained at ambient temperature. Degassing of the mobile phase was done using a Loba ultrasonic bath sonicator. A Denwar analytical balance was used for weighing the materials.

2.2 Chemicals and solvents

The reference sample of Dobutamine was obtained from Cipla, Mumbai. The Formulation was procured from the local market. Water, Methanol and potassium dihydrogen phosphate used were of HPLC grade and purchased from The chemicals were procured from E-Merck, India, Limited.

The mobile phase
The mobile phase was prepared by mixing Methanol and potassium di hydrogen phosphate and (60:40 v/v) by ultra bath sonicator for 30 min.

Preparation of solutions
Preparation of mobile phase solution
The mobile phase was prepared by mixing Methanol and 0.01M Potassium dihydrogen phosphate (60:40 v/v) by ultra bath sonicator for 30 min.

Standard Solution
Stock solution of Dobutamine was prepared by dissolving accurately weighed 10mg of drugs in 10ml Methanol (final concentration, 1000µg/ml). The prepared stock solutions were stored away from light. From the stock, standard solutions was freshly prepared during the day of analysis.

Preparation of working standard solution (A.P.I): From the stock solution 1.0 mg/ml solution was prepared.

Preparation of working standards for linearity
Solutions in the concentration range of 0.2-1.0mg/ml were prepared from the standard working solution.

Preparation of formulation sample solution
1.5mg of formulation powder was taken from DOBUCARD tablet powder (50mg formulation) and dissolved in 10ml of mobile phase and injected into HPLC and chromatogram was recorded. The amount of drug present in the 50mg formulation was calculated from linearity graph.

Method Development
For developing the method, a systematic study of the effect of various factors was undertaken by varying one parameter at a time and keeping all other conditions constant. Method development consists of selecting the appropriate wave length and choosing stationary and mobile phases. The following studies were conducted for this purpose:

Detection of wavelength
The spectrum of 10ppm solution of Dobutamine was recorded separately on UV spectrophotometer. The peak of maximum absorbance wavelength 280nm was observed.

Choice of stationary phase and mobile phase
Finally the expected separation and peak shapes were obtained on Kromasil C_{18} column 250 x 4.6 mm ID with 5 µ particle size.

Flow rate
Flow rates of the mobile phase were changed from 0.5-1.8 ml/min for optimum separation. It was found from experiments that 1.5 ml/min flow rate was ideal for elution of analyte.

Validation Procedure and Requirements
The analytical performance of the method of analysis was checked for specificity, System suitability, detection limit, and method precision.

Linearity and calibration
Linearity was assessed by performing single measurement at several analyte concentration varying quantities of stock standard solution diluted with the mobile phase to give a concentration of 0.2, 0.4, 0.6, 0.8, 1.0 mg/ml Injection was made at intervals of 13 min. The linearity was tested for the concentration ranging from 0.2mg/ml to 1.0mg/ml. The peak area ratio of the drug was plotted against concentration. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method.

Precision
Reproducibility was performed by injecting three replicates concentrations of standard and sample solutions which were prepared and analyzed by same analyst on same day. Inter-day variations in the peak area of drug solutions

and the amount of drug were calculated in terms of Percentage Relative Standard Deviation. The sample concentration is 1.0mg/ml.

Accuracy
Recovery assessment was obtained by using standard addition technique which was by adding known quantities of pure standards at three different levels in 80%, 100% and 120% to the pre analyzed sample formulation.

Assay
The estimation of drug in pharmaceutical dosage forms. DOBUCARD 5ml strength was evaluated for the amount of Dobutamine present in the formulation. Each sample was analyzed in triplicate after extracting the drug. The amount of drug present in formulation was calculated by comparing the mean peak area from standard.

Intermediate Precision or Ruggedness
Inter-day variations were performed by using six replicate injections of standard and sample solutions of concentrations which were prepared and analyzed by different analyst on three different days over a period of one week. Ruggedness also expressed in terms of percentage relative standard deviation.

Robustness
Robustness was carried out by varying two parameters from the optimized chromatographic conditions**.**

Specificity
The method was determined as specific by comparing test results obtained from analyses of sample solution containing excuse ingredients with that of test results those obtained from standard drug.

System Suitability Parameter
System suitability tests were carried out on freshly prepared standard stock solutions of Dobutamine and it was calculated by determining the standard deviation of Dobutamine standards by injecting standards in five replicates at 6 minutes interval and the values were recorded.

Dobutamine Analysis In Serum
Preparation of serum sample solution
From a local hospital blood was collected and serum was separated. 1ml of this serum was taken in a test tube and added 100µl of diltizem hydrochloride (1µg/ml) and 0.1ml of 1M NaOH and 5ml of dichloromethane and mixed about 20min in vortex mixer and centrifuged at 3000 rpm for 10min. From this centrifuged solution 4ml of organic layer was separated and evaporated to dryness to get residue. To this residue 100µl of 1M acetic acid and 3ml of n-Hexane and mixed for 5 min by vortex mixer and evaporated the organic layer and finally the remaining sample was injected into HPLC and chromatogram was recorded. The amount of drug present in the blood sample was calculated from linearity graph.

RESULTS AND DISCUSSION

The Reverse Phase High Performance Liquid Chromatography method was developed a stability indicating assay method. Pure drugs chromatogram was run in different mobile phases containing methanol, acetonitrile, THF, and different buffers like potassium dihydrogen phosphate, sodium dihydrogen phosphate, Ortho phosphoric acid in different volumes ratios. Different columns like C_8, C_{18}, phenyl, cyano with different dimensions were used. Then retention time and tailing factor were calculated. Finally Methanol and 0.01M Potassium dihydrogen phosphate in the volume of ratio 60:40 v/v (P^H: 3.5) and Kromosil C_{18} analytical column was selected which gave a sharp and symmetrical peak with 1.92tailing. Calibration graph was found to be linear at range 0.2mg/ml to 1.0mg/ml. five different concentrations of Dobutamine in range given above were prepared and 20µl of each concentration injected in HPLC as shown in the Figure no : 7.4.1.The slope (m) and intercept (c) obtained were found to be 282145.15 and 0.027684673.The correlation of coefficient (r^2) obtained was found to be 0.9999 as shown in the Table no : 7.4.01. It was observed that the concentration range showed a good relationship. The limit of detection for Dobutamine was found to be 10µg/ml and the limit of quantification was found to be 30µg/ml. It proves the sensitivity of method. The Percentage assay of Dobutamine in formulation was found to be 100.63%.as shown in the Table no: 7.4.01 and figure no: 7.5.1. The relative standard deviation value obtained was below 1 which indicates the precession of the method. The validation of the proposed method was further verified by recovery studies. The data was presented by

in the Table no: 7.4.02 and figure no: 7.4.2. The percentage recovery was found to be 102.89% which shows a good index of accuracy of the developed method. The amount of drug present in the human serum sample was calculated from the linearity graph was found to be 0.044mg/ ml as shown in Table no: 7.6.01 and Figure no: 7.6.1.

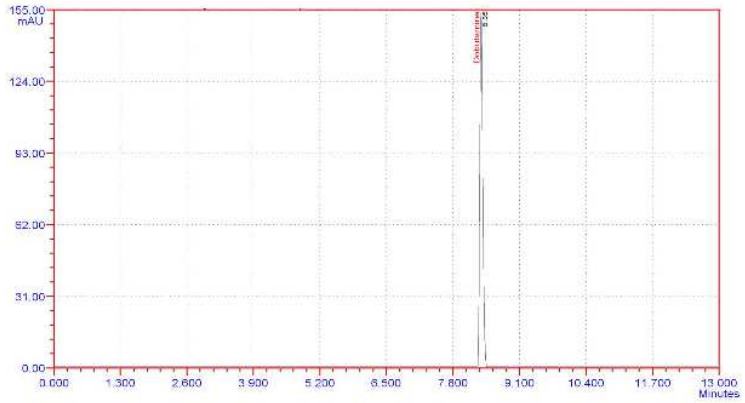
Table: 1 Optical Characterization Of Dobutamine

PARAMETERS	DOBUTAMINE
Linearity range(mg/ml)	0.2 – 1.0
Correlation coefficient (r)	0.9999
Slope(m)	282145.15
Intercept (c)	0.027684673
Limit of detection (LOD; µg/ml)	10
Limit of Quantification(LOQ; µg/ml)	30
Tailing factor	1.92
Retention time (min)	8.491
Theoretical plates	37064.92
(%) R.S.D	0.026
(%) Accuracy	102.89
(%) Formulation Assay	100.63
Serum analysis (mg/ml)	0.044

Table : 2 Recovery Data Of Dobutamine

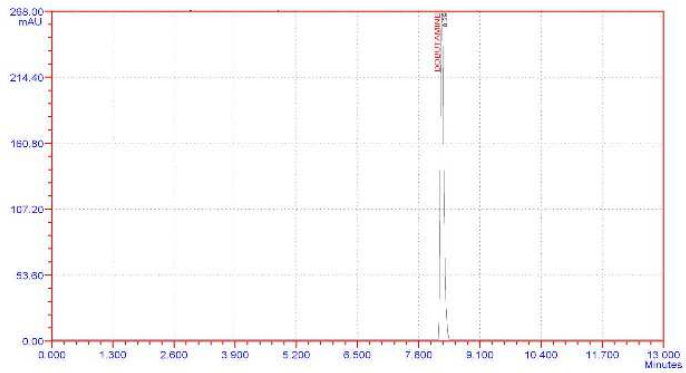
Pharmaceutical Formulation (Brand Name)	Labeled Amount (Mg)	Percentage Assay	Percentage Recovery
DOBUCARD	50 mg	100.63	102.89

**Average value of three different levels in triplicate*



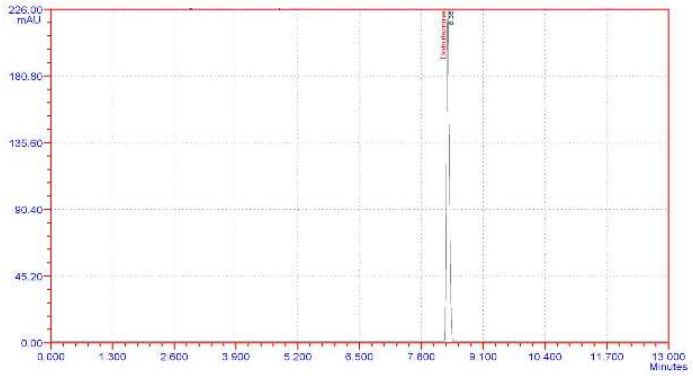
S.No	Name	Retain.T	Height	Area	Concentration	Tailing Factor	Theoretical plate
1	Dobutamine	8.353	13684	49808.1	100.000	1.21	104960
2	Sum		13684	49808.1	100.0000		

Fig:2 Chromatogram Of Dobutamine (Standard) and their values



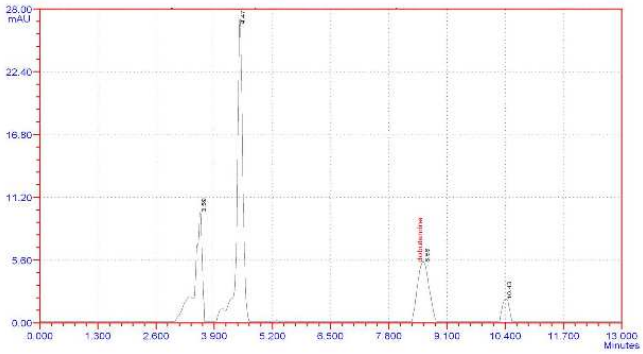
S.No	Name	Retain.T	Height	Area	Concentration	Tailing Factor	Theoretical plate
1	Dobutamine	8.261	27199	158050.8	100.000	0.01	40578
2	Sum		27199	158050.8	100.0000		

Fig:3 Chromatogram Of Dobutamine (Accuracy) and their results



S.No	Name	Retain.T	Height	Area	Concentration	Tailing Factor	Theoretical plate
1	Dobutamine	8.355	23404	105196.5	100.000	1.55	68864
2	Sum		23404	105196.5	100.0000		

Fig:4 Chromatogram Of Dobutamine (Formulation Assay) and their results



S.No	Name	Retain.T	Height	Area	Concentration	Tailing Factor	Theoretical plate
1		3.588	1355	23312.4	28.541	0.60	867
2		4.468	2897	38377.5	46.985	0.76	**2267**
3		8.583	618	12943.8	15.847	1.02	**3347**
4	Dobutamine	10.425	396	7046.8	8.627	1.03	**6841**
	Sum		5266	81680.4	100.0000		

Fig:5 Chromatogram Of Dobutamine (Serum) and their results

CONCLUSION

The RP-high performance liquid chromatographic method developed for the analysis of Dobutamine from their formulations was found to be accurate and precise. Thus, the proposed HPLC method can be successfully applied for the routine quality control analysis of Dobutamine formulations, which could be the better choice cooperative to the methods reported in the literature.

REFERENCES

[1] The Merck Index, 13th edition, Merck and company, INC, White House station, N J, **2001**, 597.
[2] R R Tuttle, J Mills, *Circ Res*, **1975**, 36, 185.
[3] H P, Rang, M M Dale, J M Ritter, R J Flower, *Rang and Dale's Pharmacology*, **2008**, 6.
[4] H Shen, *Pharmacolog* **2008,** 67.

[5] K Parker, L Brunton, L S Goodman, D. Blumenthal, I Buxton, (**2008**). Goodman & Gilman's manual of pharmacology and therapeutics. McGraw-Hill Medical. 159.
[6] F A Tibayan, A N Chesnutt, H G Folkesson, J Eandi, M A Matthay, *Am. J. Respir. Crit. Care Med*, **1997**, 156, 438.
[7] Y Sung-Hwa, *Archives Of Pharmacal Research* **2006**, 15, 191.
[8] H Hussein, V Mitrovic M Schlepper, *Journal of Chromatography: BiomedicalApplications*, **1993**, 620,164.
[9] C Leflour, T Dine, M Luyckx, C Brunet, B Gressier, M Cazin, H Robert, A Durocher, J C Cazin, *Biomedical Chromatography*, **1994**,8, 309.
[10] D W Mc Kennon, R E Kates, *Journal of Pharmaceutical Sciences*, **1978**, 67, 1756.
[11] R Knoll, M Brandl, *Journal of Chromatography: Biomedical Applications*,**1993**,620, 164.

TABLE :7.6.01
SERUM DATA OF DOUBTAMINE

Drug estimation in human serum by developed protocol:-

From linearity graph we can estimate amount of drug present in the sample.

Y = mx+c
Y = area
M = slope
X = concentration
C = intercept

$$\text{Concentration} = \frac{\text{area} - \text{intercept}}{\text{Slope}}$$

$$\text{Amount of DOUBTAMINE present in serum} = \frac{12943.8 - 0.02768467}{32613.14}$$

= 0.044mg/ ml