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Original Article

REDOX REACTION BASED SPECTROPHOTOMETRIC ASSAY OF SOME DRUGS IN PHARMACEUTICALS

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ABSTRACT

Objectives: Pyridoxine hydrochloride (PRC) is used in the treatment of side roblastic anemia's and is also used in a variety of disorders including the treatment of depression. Dobutamine Hydrochloride (DOB) is used in the case of congestive heart failure to increase cardiac output and is also commonly used in the hospital setting as a pharmacologic stress testing agent to identify coronary artery disease. Linezolid (LZD) is a synthetic antibiotic used for the treatment of serious infections caused by gram-positive bacteria that are resistant to several other antibiotics. The main objective of our method is to develop a simple, accurate and sensitive spectrophotometric method for the assay of the above mentioned drugs in both tablet and pharmaceutical dosage forms.

Methods: The method is based on the red ox reaction of drugs with Folin Ciocalteu (FC) reagent in sodium carbonate medium and the resulting blue colored chromogen is measured at 755 nm.

Results: Beer's law is obeyed in the concentration range of 2.5–30 µg/ml (PRC), 1–10 µg/ml (DOB) and 2.5–70 µg/ml (LZD) respectively, with the corresponding molar absorptivity values of 7.145 X 10^3, 3.2080 X 10^4 and 6.299 X 10^3 l mol^{-1} cm^{-1}. The method is validated for accuracy, precision, LOD, LOQ, robustness and ruggedness as per the current ICH guidelines.

Conclusion: The validated method is successfully applied to quantify PRC, DOB and LZD in their commercial formulations with satisfactory results; hence the method is suitable for the determination of drugs in bulk and pharmaceuticals.

Keywords: Pyridoxine Hydrochloride, Dobutamine Hydrochloride, Linezolid Form-1, Folin Ciocalteu reagent, Spectrophotometry, Pharmaceuticals.

INTRODUCTION

Pyridoxine Hydrochloride (PRC), chemically (5-hydroxy-6-methylpyridine-3,4-dimethyl) dimethanol hydrochloride is used in the treatment of side roblastic anemia's; it is readily absorbed from the gastrointestinal tract following oral administration and is converted to the active forms, Pyridoxal phosphate and Pyridoxamine phosphate, which are stored mainly in the liver where there is oxidation to 4-pyridoxic acid and other metabolites that are excreted in the urine. It is involved in amino acid as well as carbohydrate and fat metabolism. It is used in a variety of disorders, including the treatment of depression. The survey of literature includes the methods are British Pharmacopoeia [1] (BP) (On CD-ROM: System stimulation Ltd. 3rd ed. London: Stationary office; 2000), Capillary electrophoresis [2], Zero-crossing [3], Chemi luminiscent [4], Derivative spectrophotometric and Differential derivative spectrophotometric [5, 6], High performance liquid chromatography [7, 8], Spectro fluorimetric and spectro photometric [9, 10] and spectrophotometry [11-13].

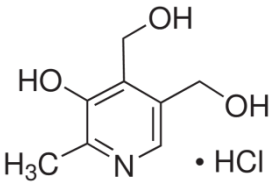
Dobutamine Hydrochloride (DOB), is chemically known as 4-(2-((1-methyl-3-(4-hydroxybenzene propyl) amido) ethyl-1,2-di-hydroxybenzene hydrochloric salt is a sympathomimetric with direct effects on β1-adrenergic receptors, giving it a prominent ionotropic effect on the heart. It also has some α and β2–agonist 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. British Pharmacopoeia [14] (BP) (Her majesty's stationary office. 1st ed. London; 2007), Capillary gas chromatography [15], voltammetry [16], Chemiluminescence [17], RP-HPLC [18] and Spectrophotometry [19-21].

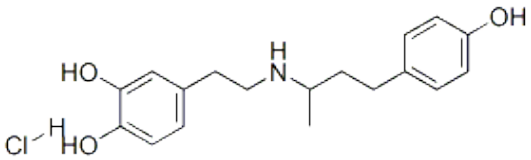
Linezolid (LZD), chemically (s)-N-[[3-[3-fluoro-4(4-morpholinyl) phenyl]-2-oxo-5-azolidinyl] methyl] acetamide was the first oxazolidinone to be developed and approved for clinical use. Linezolid is a synthetic antibiotic used for the treatment of serious infections caused by gram-positive bacteria that are resistant to several other antibiotics. Linzolid is active against most gram positive bacteria that cause diseases including streptococci, vancomycin-resistant enterococci and methicillin-resistant staphylococcus auras. The main indication of Linezolid is the treatment of severe infections caused by gram-positive bacteria that are resistant to other antibiotics; it should not be used against bacteria that are sensitive to drugs with a narrower spectrum of activity, such as penicillin's and cephalosporins. The literature survey includes British pharmacopeia method [22] (BP) (Her majesty's stationary office. 6th ed. London; 2010. p.410), Capillary electrophoresis [23], RP-HPLC [24], Derivative spectrophotometry [25], Spectrophotometry [26-28].

Visible spectrophotometry is by far the most widely used technique for the assay of the mentioned drugs. The above mentioned different methods suffer from one or more of the disadvantages such as drastic experimental conditions, use of organic solvent, longer standing time, poor sensitivity and narrow linear range. Folin Ciocalteu (FC) reagent has been widely used for the sensitive determination of a wide ranging phenol and amine organic compounds of pharmaceutical importance [31-36]. The main objective of the present work was to investigate the utility of FC reagent in the assay of drugs i.e., PRC, DOB and LZD. The method had sufficiently good accuracy and presented a simple and time saving assay of the mentioned drugs. The novelty of the method is the proposed method is used in drug formulations and also in quality control laboratories. The suggested method was further applied for

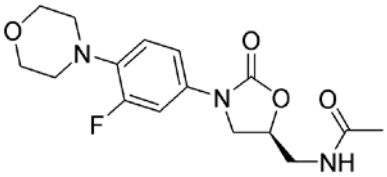
the determination of drugs in commercial pharmaceutical dosage forms, which were compared statistically with reference methods by means of t-test and F-test and were found not to differ significantly at 95% confidence levels. The procedure is characterized by its simplicity with accuracy and precision. The structures of studied drugs are.



Pyridoxine Hydrochloride



Dobutamine Hydrochloride



Linezolid

MATERIALS AND METHODS

Apparatus

A BL 198 Bio spectrophotometer (UV–VIS) with 1.0 cm matched quartz cuvettes was used for all absorbance measurements.

Reagents and solutions

Chemicals used were of analytical reagent grade. Double distilled water was used throughout the experiment. Pyridoxine hydrochloride, Dobutamine Hydrochloride (gift sample from Mylon, Brazil) and Linezolid (purchased from Cipla Ltd, Bangalore) were used as received.

Stock solutions of each drug containing 100 µg/ml were prepared by dissolving 10 mg of the respective drugs in 100 ml of water. The solutions were further diluted quantitatively according to their linearity range. The pharmaceutical preparations were purchased from a local market and analyzed.

Folin ciocalteu reagent

Aqueous solution of Folin Ciocalteu reagent (1:1 v/v) was prepared by mixing 50 ml of reagent (Merck, Mumbai, India) with 50 ml water.

Sodium carbonate

A 20% solution of sodium carbonate was prepared by dissolving 20 g compounds (S. D Fine Chem. Ltd., Mumbai, India) in 100 ml water.

Procedure for pharmaceutical formulation

Twenty tablets were weighed and ground into a fine powder. An accurately weighed quantity containing 10 mg of drugs (PRC and LZD) were transferred to a 100 ml volumetric flask, 60 ml water added, shaken well for 20 min and made up to mark with water, and then filtered. The solutions were further diluted according to their linearity range and analyzed by the recommended procedure. For the analysis of an injection (DOB), the requisite amount was transferred to a 100 ml volumetric flask and diluted with distilled water. The drug content in the diluted solution was determined by the recommended procedure.

Assay procedure

Into a series of 10 ml volumetric flasks, different aliquots of working standard solution of PRC (2.5–30 µg/ml), DOB (1–10 µg/ml) and LZD (2.5–80 µg/ml) were transferred to a series of 10 ml volumetric flasks. To each flask, 1.0 ml, 1.0 ml and 1.5 ml of 1:1 FC reagent, after 10 min, 1.0 ml, 1.5 ml and 1.0 ml of 20% sodium carbonate were added by means of micro burette. The flasks were stoppered and the contents were mixed well and kept at room temperature for 10 min. The volume was made up to the mark with water and the absorbance of each drug was measured at 755 nm against the corresponding reagent blank similarly prepared in the absence of drug.

RESULTS AND DISCUSSION

Folin Ciocalteu (FC) reagent is specially used for the determination of many phenolic compounds utilizing its liability to be reduced into blue colored product. Many drug substances such as Salbutamol [37], Minocycline [38], Trimetazidine [39], Gliclazide [40] and Isoniazid [41] have been determined on this basis. The structural features of PRC, DOB and LZD allowed the use of FC reagent for its assay.

The proposed method is based on the formation of a blue colored chromogen, following the reduction of phospho molybdo tungstic mixed acid of the FC reagent [30] by drugs, in the presence of sodium carbonate, which could be measured at 755 nm. The mixed acids in the FC reagent are the final chromogen and involve the following chemical species:

$3H_2O.P_2O_5.13WO_3.5MoO_3.10H_2O$

$3H_2O.P_2O_5.14WO_3.4MoO_3.10H_2O$

Drugs probably effects reduction of oxygen atoms from tungstate and/or molybdate in the FC reagent, there by producing one or more possible reduced species which have characteristic intense blue color. The method is based on the red ox reaction of drugs with Folin Ciocalteu reagent (FC) in sodium carbonate medium and the resulting blue colored chromogen is measured at 755 nm.

Spectral characteristics

The intensely blue colored product (molybdenum-tungsten mixed acid blue) formed in this method exhibited maximum absorption at 755 nm. The absorption spectra of the blue colored products against the reagent blanks are shown in [fig. 1].

Optimization for FC method

By varying one and keeping the other experimental parameters and amount of drug constant, the effect of FC reagent and Na_2CO_3 were tested. The maximum color intensity was obtained when 1.0–1.2 ml, 0.8–1.0 ml, 1.4–1.6 ml of F-C reagent and 2.8–3.0 ml, 1.4–1.5 ml, 2.4–2.6 ml of Na_2CO_3 were added to PRC, DOB and LZD respectively. The effect of volume of FC reagent and Na_2CO_3 for PRC [fig. 2A and 2B], DOB [fig. 3A and 3B], LZD [fig. 4A and 4B] is as shown below.

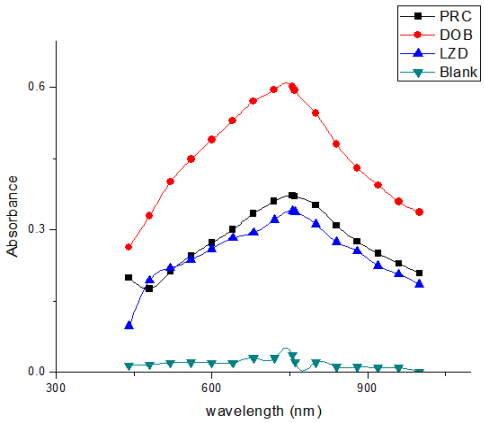


Fig. 1: **Absorption spectra of PRC (10 µg/ml), DOB (6 µg/ml), LZD (10 µg/ml) against reagent blank**

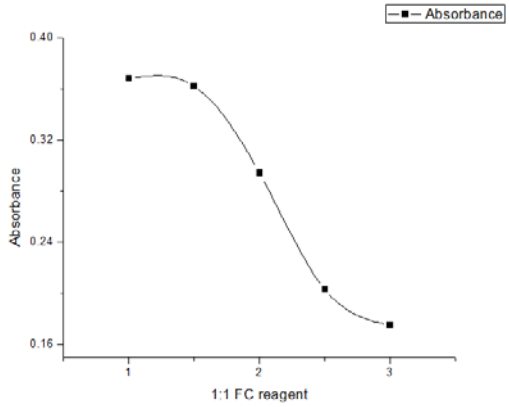


Fig. 2A: Effect of volume of FC reagent (1:1) on the reaction product with PRC (10 µg/ml) in Na_2CO_3 solution

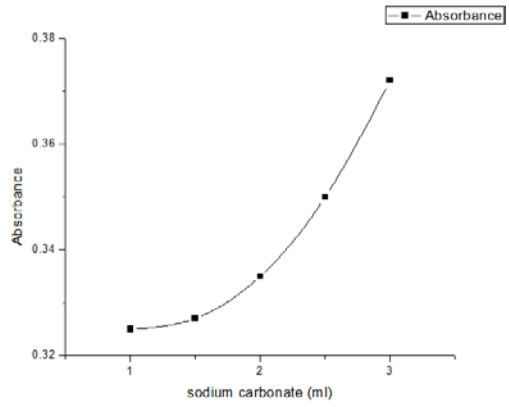


Fig. 2B: Effect of 20 % Na_2CO_3 solution on color formation with (10 µg/ml PRC)

Selection of reaction medium and optimization of the base

To select a suitable medium for the reaction, different aqueous bases such as sodium hydroxide, sodium carbonate or bicarbonate, sodium acetate, and sodium hydrogen phosphate were investigated. Better results were obtained with 20 % sodium carbonate.

Maximum color development was obtained in 10 min after mixing the reactants, and the color was stable for 12 h. The sequence of order of addition of the reactants had the significant effect on the absorbance value. So, the order used in the general procedure should be followed for maximum absorbance.

Validation of 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.

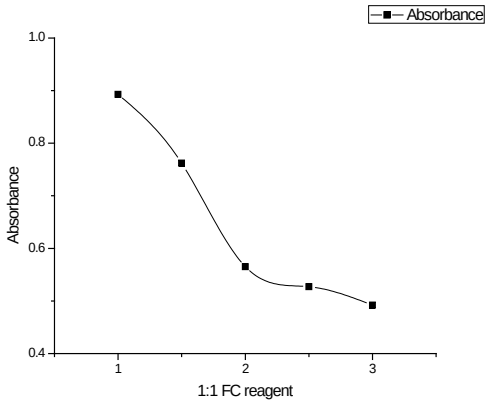


Fig. 3A: Effect of volume of FC reagent (1:1) on the reaction product with DOB (10 µg/ml) in Na_2CO_3 solution

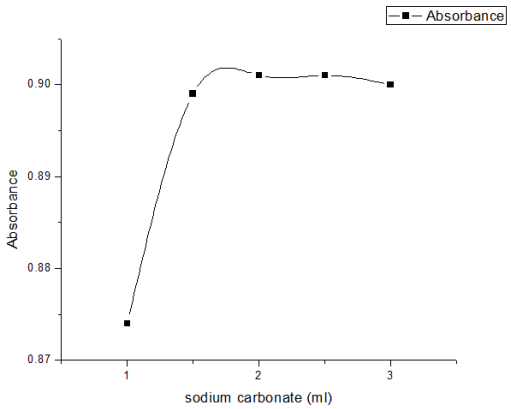


Fig. 3B: Effect of 20 % Na_2CO_3 solution on color formation with (10 µg/ml DOB)

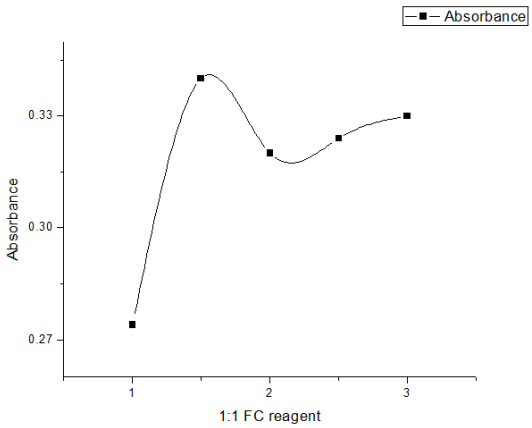


Fig. 4A: Effect of volume of FC reagent (1:1) on the reaction product with LZD (10 µg/ml) in Na_2CO_3 solution

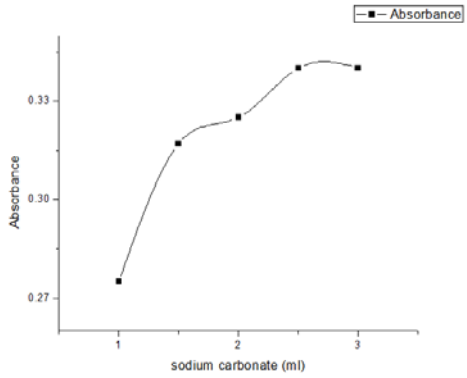


Fig. 4B: Effect of volume of 20 % Na_2CO_3 solution on color formation with (10 µg/ml LZD)

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.

Calibration graphs were constructed using standard solutions under optimum experimental condition. A linear relationship was observed between the absorbance and concentration of drugs from (2.5–30 µg/ml), (1–10 µg/ml) and (2.5–70 µg/ml) for PRC, DOB and LZD respectively. The molar absorptivity and sandell's sensitivity for each drug were calculated from Beer's law. Regression analysis of beer's law plots revealed good correlation. The limits of detection and limits of quantification were calculated. The sensitivity and regression parameters are shown in [table 1].

Table 1: Sensitivity and regression parameters

Parameters	Optical characteristics		
	PRC	DOB	LZD
color	blue	blue	blue
$\lambda_{max\ (nm)}$	755	755	755
Beer's law limit (µg/ml)	2.5-30	1-10	2.5-70
Molar absorptivity(l mol^{-1} cm^{-1})	7.145 X 10^3	3.2080 X 10^4	6.299 X 10^3
Sandell's sensitivity (µg cm^{-2})	0.0287	0.0015	0.0535
Limit of Detection [LOD](µg/ml)	0.8783	0.0659	0.5332
Limit of Quantification [LOQ](µg/ml)	0.2898	0.1999	1.6158
Regression equation[Y*]			
Slope [B]	0.0251	0.0956	9.507 X 10^{-3}
Intercept[A]	0.1093	0.0384	0.24671
Correlation coefficient [r]	0.9961	0.9998	0.9980
Relative standard deviation[b]	0.032	0.053	0.088

*Y= BX+A, where X is the concentration of the measured solution in µg/ml and Y is the unit for absorbance. [b]Average of five determinations (concentrations of 7, 5 and 18 µg/ml of pure drugs of PRC, DOB and LZD respectively)

Interference

In order to evaluate the suitability of the proposed method for the analysis of pharmaceutical preparations of the studied drugs, the interference of associated common excipients such as starch, talc, magnesium stearate and lactose were studied. The results indicated that none of the excipients studied interfered in quantitative analysis by the present method. The results are given in [table 2].

Table 2: Recovery of drugs from solutions with a 100 fold concentration of various additives present

Excipients	% [a]Recovery±% RSD		
	[b]PRC	[c]DOB	[d]LZD
Lactose	100.0±0.7	99.9±0.5	100.0±0.6
Starch	99.8±0.2	100.0±0.6	99.9±0.3
Talc	99.9±0.3	100.0±0.6	98.9±0.5
Magnesium stearate	99.5±0.5	99.8±0.4	99.6±0.2

[a] mean±RSD, n=3, [a] mean of three determinations, [b] concentration of PRC used–15 µg/ml, [c] concentration of DOB used–5 µg/ml, [d] concentration of LZD used–20 µg/ml

Precision and accuracy

The intraday precision (short term precision) of the drugs were analyzed by measuring the 5 independent samples at three different concentration levels (5, 15, 20 µg/ml) for PRC, (2, 4, 6 µg/ml) for DOB and (20, 40, 60 µg/ml) for LZD. Similarly the inter day precision (daily precision) was evaluated at the same concentration on five consecutive days (n = 5). The results are shown in [Table 3]. In order to check the validity of the proposed method, PRC, DOB and LZD were determined in some commercial formulations [Table 4] gives the results of the determination from which it is clear that there is no close agreement between the results obtained by the proposed methods and the label claim. The results were also compared statistically by a student's t-test for accuracy and variance ratio F-test for precision with those of the standard methods (BP method) at 95% confidence level. The calculated t-and F-values [Table 4] did not exceed the tabulated values (t = 2.44, F = 5.05) and indicated that there is no significant difference between the proposed method and the standard method.

The accuracy and validity of the proposed method was further ascertained by performing recovery studies. Pre-analyzed samples were spiked with pure drugs at three different levels and the total volume was found by the proposed method. Each determination was repeated five times. The recovery of the pure drug added was quantitative and co-formulated substances starch, talc, magnesium stearate and lactose did not interfere in the determination. The results of recovery study are compiled in [table 5].

Table 3: Evaluation of intraday and interday accuracy and precision

Formulation	[b]Amount taken (µg/ml)	[a]Amount found (µg/ml)	Intraday % Recovery±% RSD	Interday % Recovery±% RSD
	5.0	4.99	99.8±2.41	99.9±2.32
PRC	15.0	15.01	100.0±0.58	100.01±0.60
	20.0	19.98	99.99±0.55	100.0±0.57
	2.0	1.98	99.0±2.1	99.6±2.3
DOB	4.0	4.02	100.5±1.0	100.02±0.90
	6.0	5.99	99.8±0.39	99.99±0.41
	20.0	20.01	100.05±0.94	99.99±0.85
LZD	40.0	39.98	99.95±0.48	98.98±0.40
	60.0	59.99	99.98±0.37	99.8±0.31

[a] Mean value of five determinations

Table 4: Analysis of drugs in pharmaceutical formulations

Drug formulations	Label claimed	% Recovery±sd	
		[a]Proposed method	*Reference method (BP)
Benadon, tab (Piramal laboratories Ltd., India) (PRC)	40 mg	39.90±0.53 t =0.35 F=1.79	40.1±0.71
Dobier S, inj (Chandra bhagat Pharma, Pvt. Ltd., India) (DOB)	250 mg	250.01±0.97 t= 0.5 F=1.3	249.7±1.11
Alzolid,tab (Alembic chemical works Co Ltd., India) (LZD)	600 mg	597.4±0.89 t=1.57 F=1.49	598.8±1.09

*Mean of five determinations±Standard deviation(sd) 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.

Table 5: Results of recovery experiments by standard addition method

Formulation studied	Amount of drug taken in, µg	Amount of pure drug added, µg	*Total found, µg	%Recovery±%RSD
	5.0	2.5	7.52	100.2±1.23
Benadon (40 mg)	5.0	7.5	12.49	99.9±0.75
	5.0	12.5	17.51	100.0±2.74
	2.0	1.0	3.16	105.3±0.82
Dobier S(250 mg)	2.0	4.0	5.98	99.6±0.38
	2.0	7.0	9.11	101.2±0.35
	20.0	10.0	30.02	100.0±0.49
Alzolid (600 mg)	20.0	30.0	49.91	99.8±0.48
	20.0	50.0	69.96	99.9±0.48

* Mean value of five determinations

Robustness and ruggedness

Method robustness was studied by making small changes in the optimized experimental variables and their effect on the absorbance was evaluated by calculating the percentage RSD values. In order, to determine the method ruggedness, analyses were performed using three instruments and also by three analysts with the same instrument.

CONCLUSION

A simple, rapid, selective and sensitive method has been proposed for the assay of drugs and in pharmaceutical formulations. The method is based on the well-characterized and established red ox reaction and uses very common and inexpensive chemicals and easily accessible instrument.

The method is applied successfully to the assay of drugs in tablets and injection without interferences from the common excipients. The proposed method is suitable for PRC, DOB and LZD determination in bulk drug and pharmaceuticals; hence this method can be used in quality control laboratories.

CONFLICT OF INTERESTS

Declared None

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