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New spectrophotometric method for determination of isoxsuprine hydrochloride in tablets

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ABSTRACT

A new simple, rapid, precise, highly specific and economical spectrophotometric method has been developed for determination of isoxsuprine hydrochloride in pharmaceutical dosage forms. The phenol moiety of isoxsuprine hydrochloride is coupled with diazotized sulfanilic acid, resulting in development of yellowish orange colored complex with an absorption maximum at 443.7nm. Beer Lambert's law is followed in the concentration range 10-28 μg/mL. Limits of detection (LOD) and limits of quantitation (LOQ) are 16.25 and 49.24 μg/mL respectively. The effect of experimental variables such as concentration of color producing reagents and stability of color were investigated to optimize the method. The method was suitably validated following ICH norms and commonly accepted guidelines for validation of analytical procedures considering the parameters of linearity, accuracy, precision (repeatability and intermediate precision), sensitivity (Sandell's sensitivity, LOD and LOQ), selectivity and robustness. Statistical analysis was performed to determine percent relative standard deviation (%R.S.D.), standard deviation (S.D.), standard error (S.E.), and t-values at 95% confidence.

KEYWORDS

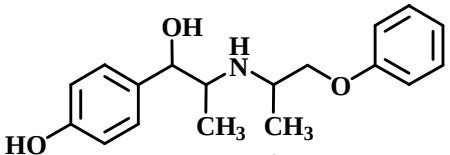
Spectrophotometry;
Isoxsuprine;
Colorimetry;
Diazotization;
Validation.

INTRODUCTION

Isoxsuprine is chemically described as 4-hydroxy-α-[1[(1-methyl-2-phenoxyethyl) amino] ethyl] benzene methanol, used as a peripheral vasodilator[1] (Figure 1).

The literature reports few methods for its determination in pharmaceutical preparations. These include stability indicating HPLC method for the determination of isoxsuprine in dosage forms[2], and high-performance liquid chromatography of two peripheral vasodilators, nylidrin hydrochloride and isoxsuprine hydrochloride, in pharmaceutical dosage forms[3], spectrophotometric determination of ritodrine and isoxsuprine hydrochlorides using 4-aminoantipyrine[4] and spectrophotometric determination of some adrenergic drugs using 2,6-dichloroquinone-chlorimide[5].

To best of our knowledge, no spectrophotometric method has yet been reported for the determination of isoxsuprine alone in pharmaceutical dosage forms. The



Isoxsuprine

present study was hence conducted with the objective of developing simple, rapid, precise and economical spectrophotometric method for the analysis of isoxsuprine. The method was suitably validated and all optimization parameters were considered.

EXPERIMENTAL

Apparatus

A UV/Vis spectrophotometer(GBC Cintra 10, Australia) with 10mm matched quartz cells was used for experiments.

Reagents

The chemicals used were of analytical grade. Sodium hydroxide and sodium nitrite were purchased from Qualigens and sulfanilic acid was purchased from Lobachemie. Purified water was used for the experiments.

Reagent preparation

Reagent A consisted of 0.1% w/v sulfanilic acid in 0.1 N HCL. Reagent B consisted of 1% w/v sodium nitrite in water. Reagent C consisted of 0.1N NaOH.

Preparation of standard solutions

Standard isoxsuprine solution was prepared by dissolving 10mg (accurately weighed) of isoxsuprine hydrochloride standard in 100mL of water, yielding a stock solution of 100µg/mL (A).

General procedure and construction of calibration curves

In this method, reagent A(1.0mL) was added to stoppered 10mL volumetric flasks(in triplicates), followed by placing the flasks in ice cold water, subsequent cooling and addition of 1.0mL of reagent B and then 1.0, 1.2 …..2.8mL of standard stock solution(A) were transferred into a series of volumetric flasks, to maintain alkalinity 1.0ml of reagent C was then added to each of these flasks and volume made up with water. The absorbances of the yellowish orange colored solutions formed were measured at 443.7nm against the reagent blank and the calibration curve was plotted.

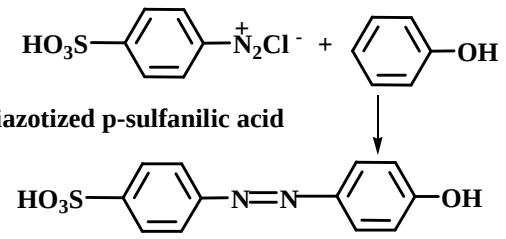
Procedure for commercial tablets

Twenty tablets(Label claim, 10mg of isoxsuprine hydrochloride per tablet) were weighed and powdered. A quantity of the powder equivalent to 10mg drug was extracted with successive 10mL portions of water, filtered and the volume was made upto 100mL with water. This was then suitably diluted, and the general procedure was then followed as described above. The absorbances were measured accordingly.

RESULT AND DISCUSSION

Isoxsuprine hydrochloride contains a phenol moiety, which can undergo coupling with diazotized sulfanilic acid. The diazotization of sulfanilic acid takes place upon its reaction with sodium nitrite in ice cold conditions. The coupling of phenol with the diazotized product is favored by alkaline conditions. The reaction takes place according to the following proposed SCHEME[6].

Optimum reaction conditions



The reaction conditions were optimized spectrophotometrically[7-8]. All the parameters were optimized by carrying out each study in triplicates.

Beer Lambert's law limit

The reaction was investigated over the concentration range 1-100µg /mL. Beer Lambert's law was found to be obeyed in the concentration range 10-28µg /mL.

Quantity of reagents

Optimum quantity of reagent A, and B was found to be 1.0mL each while that of reagent C was found to be 0.8mL.

Effect of time on color stability

The color developed upon reaction was found to be stable for seven hours.

Validation of the proposed procedures

Full Paper

The proposed method was suitably validated following ICH norms and commonly accepted guidelines for validation of analytical procedures[9-13].

Linearity

Under the optimal experimental conditions the absorbance values were found to be proportional to drug concentrations over the ranges stated in TABLE 1. Good linearity was manifested as depicted by the value of the correlation coefficients (r) as evident from TABLE 1.

Accuracy

The accuracy of the proposed procedures was assessed by calculating the recovery of the drug spiked (80%, 100%, and 120% of the test concentration analyzed) in common tablet excipients(starch, talc, lactose, HPMC, MCC, magnesium stearate)[14]. The results are presented in TABLE 2.

Precision

The precision of the method was evaluated by calculating the relative standard deviation of the assay results of a given drug concentration in six replicates (TABLE 2). Interday precision and intraday precision studies were also carried accordingly(TABLE 2).

Sensitivity

The limit of detection and quantitation as well as Sandell's sensitivity[15] for the proposed procedures is tabulated in TABLE1.

$$\text{LOD} = \frac{3.3 \times \text{S.D}}{\text{m}} \text{ and } \text{LOD} = \frac{10 \times \text{S.D}}{\text{m}}$$

where S.D. and m are the standard deviation and the slope of the calibration line, respectively.

Selectivity

Interference from excipients was studied by preparing a synthetic blend consisting of standard drug and commonly used excipients. Standard solution was prepared from this blend (as already described for method A and method B) and calibration curve was plotted accordingly. The experimental data(absorption coefficients, correlation coefficients, slope, intercept) were comparable with that for standard drug without excipients. These data along with those for recovery study rule out any interaction of excipients in the analysis of isoxsuprine hydrochloride by the proposed method.

TABLE 1 : Optical characteristics

Parameter	Colorimetric method
λ_{max}	443.7
Beer's law limits (μg/mL)	10-28
Molar absorptivity (L/mole/cm)[a]	1.089×10^5
Sandell's sensitivity (μg/cm^2/0.001 absorbance unit)	0.0031
Regression equation (y = mx+c)	
Slope (m)	0.0301
Intercept (c)	0.0362
Correlation coefficient (r)	0.9997
Limit of detection (LOD) (μg/mL)	16.25
Limit of quantitation (LOQ) (μg/mL)	49.24

[a]Determined from mean absorption coefficient which is average of ten determinations each performed in triplicate

TABLE 2 : Validation data

Study	Colorimetric method		
	Mean	S.D.	% R.S. D.
Accuracy (% drug recovery)			
80 % level[α]	99.29	0.47	0.48
100 % level[α]	98.96	0.37	0.38
120 % level[α]	98.62	1.18	1.20
Precision	S.E.	S.D.	% R.S.D.
Repeatability[b]	0.02	0.04	0.40
Inter day precision[b]	-	0.04	0.40
Intra day precision[b]	-	0.02	0.20
Robustness (stress study)[α]	-	% Assay	% Degradation
Control sample	-	98.18	-
HCl treated sample	-	85.95	12.23
NaOH treated sample	-	83.07	15.11
H_2O_2 treated sample.	-	70.47	27.71
Heat treated sample.	-	92.2	5.98
UV treated sample.	-	88.68	9.50

[α]Performed in triplicate, [b]Denotes average of three determinations including six replicate each

Robustness

To study the robustness of the proposed method, the sample solutions were stressed under varying conditions. Effects of acidic, alkaline, oxidative, thermolytic and photolytic conditions were studied. The data incorporated in TABLE 2 show the robustness of the procedure adopted.

Statistical analysis

The proposed method as applied for commercial formulation was statistically analyzed[16-18]. Percent relative standard deviation(%R.S.D.), standard deviation (S.D.), standard error (S.E.), and t-values at 95% con-

TABLE 3 : Statistical analysis

Statistical analysis for determination of isoxsuprine in tablets								
Method	**Tablet**	**Amount taken (mg)**	**Amount found (mg)[a]**	**S.D.**	**%R.S.D.**	**S.E.**	**'t' calculated**	**'t' theoretical**
A	Duvadilan	10	10.0413	0.04	0.40	0.02	2.0650	2.571

[a]Average of six determinations. Theoretical 't' values at 95% confidence level for (n-1) degrees of freedom 't' (0.05, 5) = 2.571, n = 6. Low values of S.D., S.E., and % R.S.D. indicate the precision of the methods. As calculated 't' values were less than theoretical 't' values, the results of analysis were in good agreement for each tablet.

fidence level were calculated and are tabulated in TABLE 3.

CONCLUSION

No spectrophotometric method has been reported in literature for analysis of isoxsuprine as a single drug in commercial formulations.

The method described here requires no additional extraction process and is simple, sensitive, accurate, precise, economical and easily applicable to commercial formulations.

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Supplementary information available

The supplementary information available includes the absorption spectrum of isoxsuprine. It also graphically illustrates the various parameters considered while optimizing the reaction conditions.

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