ISSN: 0975-766X
CODEN: IJPTFI
Available Online through
www.ijptonline.com
Research Article

SPECTROFLUORIMETRIC QUANTIFICATION OF ISOXSUPRINE HYDROCHLORIDE IN BULK AND ORAL DOSAGE FORM

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Received on 20-01-2013 *Accepted on 18-02-2013*

Abstract

Simple and sensitive spectrofluorimetric method have been developed for the estimation of isoxsuprine hydrochloride in bulk and oral dosage form Method A involves the determination of isoxsuprine hydrochloride by direct fluorimetric method in the concentration range was 0.4–2.0µg/mL; Method B involves the determination of isoxsuprine hydrochloride by oxidation with alkaline potassium permanganate fluorimetric method in the concentration range was 0.02– 0.1µg/mL. The correlation coefficient for the methods were found to be 0.999 & .0998 , and the developed methods were analyzed for specificity, limit of detection (LOD), limit of quantification (LOQ), linearity of response, precision and accuracy. Thus the proposed methods are highly specificity & sensitive and could be adopted for routine analysis of bulk drug and its formulation.

Keywords: Spectrofluorimetry, oxidation with alkaline potassium permanganate, Limit of detection (LOD), Limit of quantification (LOQ)

Introduction

Isoxsuprine[1] (ISX), 4-Hydroxy-α-[1-[(1-methyl-2-phenoxy-ethyl)amino]ethyl benzenemethanol, is a vasodilator that produces the effects of β-adrenoreceptor stimulation and α-adrenoreceptor antagonism; the former effect is more predominant.

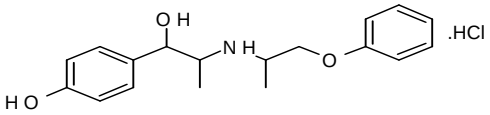
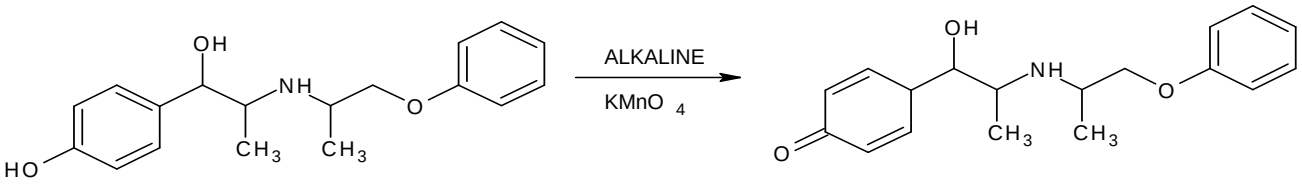


Fig-1: Structure of isoxsuprine hydrochloride.

It is used in the treatment of cerebral and peripheral vascular diseases. It is also used to arrest premature labour. Several analytical methods inclusise of colorimetric method[2], MS-MS identification[3] in post administration equine urine, spectrophotometric methods[4] have been reported for the determination of isoxsuprine hydrochloride in raw material, dosage forms and biological fluids[5]. Literature survey revealed that few sophisticated analytical methods have been reported for the estimation of isoxsuprine hydrochloride. The present work aims to devise a novel method by spectrofluorimetry which has not been reported till date.

Chemical reaction:



(isoxsuprine) (oxidised form of isoxsuprine)

Materials and method:

All the solutions were freshly prepared with distilled water.

1. Sodium hydroxide solution (1×10^{-4} M)
2. Potassium permanganate solution (5×10^{-3} was prepared in 0.0001M NaOH)
3. Bulk material: sample of isoxsuprine hydrochloride was gifted from Juggat pharmaceutical Ltd.
4. Dosage form: Isoxsuprine hydrochloride was purchased from local market.

Experimental Methods

Instrumentation

The fluorimetric measurements were made on Bioteck – synergy H4 hybrid reader instrument (model no : 252129) with Gen5 software.

Method A- Direct fluorimetric method [8]

Preparation of calibration graph

It was prepared by dissolving 100mg of drug in 100mL standard flask and the volume was made up with water to produce 1000µg/mL. This solution was further diluted with water to give varying concentration ranging from 0.4-

2.0µg/mL. The solutions were scanned in the range of 200-900nm and the relative fluorescence was measured an emission wave length (λ_{em}) of 305nm with an excitation wave length (λ_{ex}) at 270nm. A calibration graph was obtained by plotting fluorescence intensity versus concentration.

Assay

Weighed 20 tablets of isoxsuprine hydrochloride and ground to fine powder. Accurately weighed tablet powder equivalent to 1mg of isoxsuprine hydrochloride was taken in a 100 mL volumetric flask and shaken with water to dissolve the active ingredient and made up to volume to produce 100µg/mL. The solution was then filtered, first few ml of the filtrate was discarded and the filtrate was used for further analysis. The sample solution was suitably diluted to get a concentration between 0.4-2.0µg/mL and the same procedure was adopted. The fluorescence intensity obtained for the sample was then interpolated on the calibration graph and the concentration of isoxsuprine hydrochloride in the sample was then determined. The spectrum for this method is shown in (fig-2**).**

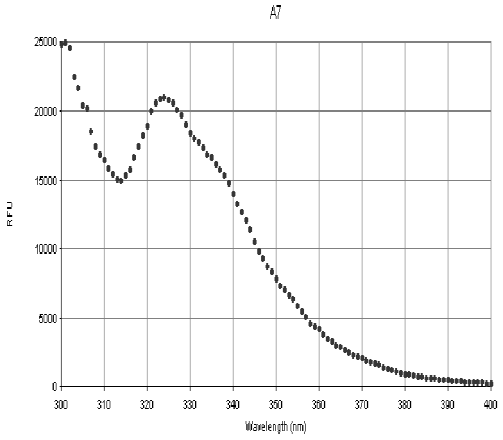


Fig-2: Direct fluorimetric method.

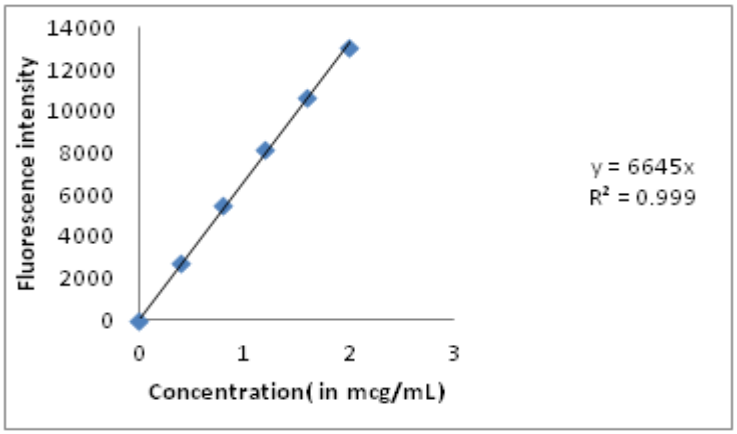


Fig 3: Calibration graph of fluorescence intensity versus concentration.

Method B- For oxidation with alkaline potassium permanganate fluorimetric method [8]

Preparation of calibration graph

It was prepared by dissolving 100mg of drug in 100mL standard flask and the volume was made up with water to produce 1000µg/mL. This solution was further diluted with water to give varying concentration ranging from 0.02-0.10µg/mL; 1.0 ml of alkaline potassium permanganate solution was added and the solution were scanned the range of 200-900nm. The relative fluorescence intensity was measured an emission wave length (λ_{em}) of 302nm with an

excitation wave length(λ_{ex}) at 270nm. A calibration graph was obtained by plotting fluorescence intensity versus concentration.

Assay

Weighed 20 tablets of isoxsuprine hydrochloride and ground to fine powder. Accurately weighed tablet powder equivalent to 1mg of isoxsuprine hydrochloride was taken in a 100 mL volumetric flask and shaken with 0.0001M NaOH to dissolve the active ingredient and made up the volume to produce 100µg/mL. The solution was then filtered, first few ml of the filtrate was discarded and the filtrate was used for further analysis. The sample solution was suitably diluted to get a concentration between 0.02-0.10µg/mL and the same procedure was adopted. The fluorescence intensity obtained for the sample was then interpolated on the calibration graph and the concentration of isoxsuprine hydrochloride in the sample was then determined. The overlain spectrum for this method is shown in (fig-4**).**

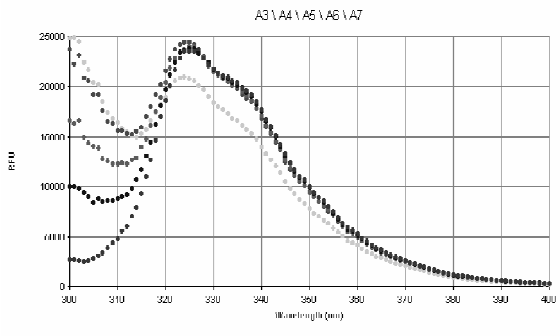


Fig 4: Oxidation with potassium.

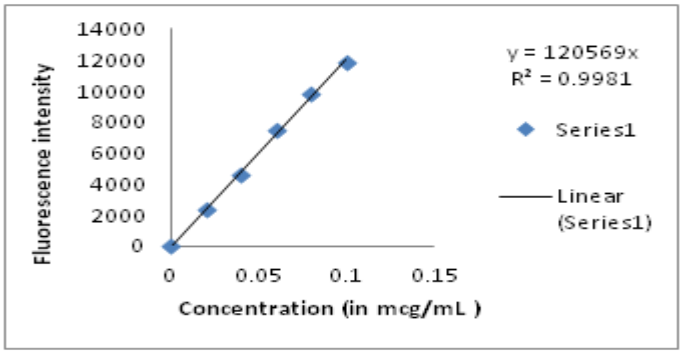


Fig 5: Calibration graph of fluorescence intensity permanganate fluorimetric versus concentration method.

Recovery studies:

The recovery studies were carried out on spiked samples by adding predetermined amount of standard drugs to the respective sample. About 50 and 100% of standard drug was added to the sample and the absorbance was measured. The percentage recovery was calculated. The recovery study was performed at two levels to confirm the precision and accuracy of the above said method

Results and Discussions

Isoxsuprine hydrochloride was found to exhibit fluorescence in the concentration range of 0.4-2.0µg/mL by direct fluorimetric method & oxidation with alkaline potassium permanganate method in the concentration range was 0.02-

0.10µg/mL. Isoxsuprine hydrochloride showed good linearity as indicated by correlation coefficient value of 0.999 &0.998 respectively. The optical parameters of isoxsuprine hydrochloride with respect to all the two methods are presented in table no.1. The percentage of the individual drugs in the formulation according to the two methods were calculated and represented in the table no.2.

Table-1: Optical Parameters of Isoxsuprine Hydrochloride by Spectrofluorimetry.

S.No	Parameters	Method A (Direct fluorimetric method)	Method B (oxidation of alkaline KMnO4 fluorimetric method)
1	Wavelength range (nm)	305	302
2	Fluorescence concentration range ($\mu g/mL^{-1}$)	0.4-2.0	0.02-0.1
3	Regression equation(y= mx+c)	6645X+0.00	12056X+0.00
4	Slope(m)	6645	12056
5	Intercept (c)	0.00	0.00
6	Correlation coefficient	0.999	0.998
7	LOD (µg/mL)	0.301504	-0.00404
8	LOQ (µg/mL)	0.913674	-0.01225

Table-2: Result of Tablet Assay.

S.No	Methods	Label claim	Amount found by proposed method (mg)	% Label Claim	SD	SE	(95%) CI	%RSD
1	Direct fluorimetric method	10 mg	10.069	100.69	0.030551	0.017639	0.340042	0.03034
2	Oxidation of alkaline $KMno_4$ fluorimetric method		9.968	99.68	0.045092	0.026035	0.340042	0.045234

Each value is a mean of 3 determinations

The results of the analysis showed that the amount of drugs were in good agreement with the label claim of the formulation. The accuracy of the proposed methods were determined by recovery studies. The recovery studies were

carried out on spiked samples and calculated for all the two methods. The percentages recovered were found to be in the range of 99 – 100 % w/w (table.3) which showed that the excipients in the formulation did not interfere with the analysis.

Table-3: Recovery Studies.

S.No	Methods	Label claim	Amount of drug added(mcg)	Amount of drug recovered (mcg)	% Recovery
1	Direct fluorimetric method	10mg	0.02	0.0198	99.0
2	Oxidation of alkaline $KMnO_4$		0.04	0.0398	99.25

Each value is a mean of 3 determinations

Conclusion

The percentage recovery of the two methods lies between 99 - 100 %w/w. The correlation coefficient for the two methods was found to be 0.999 & 0.998 and the recovery studies indicate that there is no interference of other ingredients present in the formulation. Thus, these two methods are simple, precise, accurate, less time consuming,specific, sensitive and could be used for routine analysis.

Acknowledgement

The authors are thankful to the sri ramachandra innovis-sophanicated instrumentation laboratiers (crf-sil) , porur full support to carry out the study.

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