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Spectrophotometric Methods for the Estimation of Isoxsuprine Hydrochloride in Bulk and Oral Dosage Form

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Abstract: *Four new, simple, sensitive and reproducible spectrophotometric methods have been developed for the estimation of isoxsuprine hydrochloride in tablet dosage form. Method A involves the determination of isoxsuprine hydrochloride by Q-absorbance ratio method at 269nm and 274nm; the Beer's concentration range was found to be 20-100 µg/mL. Method B and Method C involves the determination of isoxsuprine hydrochloride by first derivative spectrophotometry and second derivative spectrophotometry respectively. The normal spectrum was derivatized to first and second order derivative spectrum and the linearity was found to lie within the Beer's range for isoxsuprine hydrochloride. Method D involves the determination of isoxsuprine hydrochloride by Area under curve method (AUC) and the linearity was established. The correlation coefficient for all the four methods were found to be 0.999 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 could be adopted for routine analysis of bulk drug and its formulation.*

Keywords: *Area under curve (AUC), Beer's law, Derivative Spectrophotometry, Isoxsuprine hydrochloride, Q-Absorbance ratio*

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

II. Experimental Methods

2.1 Instrumentation

All spectral measurements were made on Shimadzu UV-Vis-spectrophotometer 1650 with 1cm matched quartz cell. All the solutions were freshly prepared with distilled water.

2.2 Preparation of Standard stock solution

It was prepared by dissolving 100mg of drug in 100mL standard flask and the volume was made up with water to produce 1000µg/mL.

2.3 Preparation of Sample solution

The average weight 20 Tablets of isoxsuprine hydrochloride was weighed and finely powdered. The powder equivalent to 10mg of isoxsuprine hydrochloride was taken in a 100 mL volumetric flask and shaken with water 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.

2.4 Assay

2.4.1 Method A: Q-Absorbance Method[5]

Q-Absorbance method depends on the property that, for a substance which obeys Beer's law at all wavelengths, the ratio of absorbance at any two wavelengths is a constant value independent of concentration or path length. The wavelengths selected for this method are 269nm and 274nm. The ratio of absorbance between these two wavelengths was calculated the difference in absorbances between their two wavelengths were calculated and plotted against concentration to determine the linearity; the concentration of sample was

determined by interpolation. The values obtained by the proposed method are represented by calibration graph (fig-1).

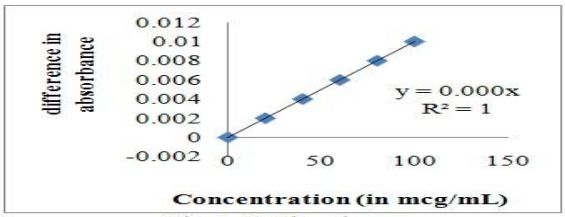

Fig 1: Q-Absorbance

2.4.2 Method B: First order derivative[6]

The standard stock solution of isoxsuprine hydrochloride was suitably diluted to give varying concentration ranging from 20-100µg/mL. The solutions were scanned in the range of 200-400nm and the primary spectrum was then derivatized to first order using derivative mode. The first derivative (D^1) spectrum is the plot of the rate of change of absorbance with wavelength against wavelength i.e., the plot of the slope of the fundamental spectrum against wavelength or dA/dλ vs. λ. The amplitude of the negative peak maximum at the zero crossing of the first order curve was measured in mm at 274nm. A calibration graph was obtained by plotting concentration versus amplitude. The sample solution was suitably diluted to get a concentration between 20-100µg/mL and the same procedure was adopted. The amplitude 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-2**).**

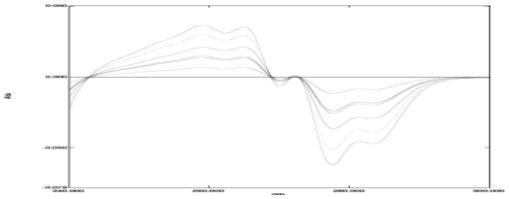

Fig 2: First order derivative

2.4.3 Method C: Second order derivative:[7]

The fundamental spectrum obtained for the above was then derivatized for the second order. The amplitude of the negative peak maximum was measured in mm at 274nm.The respective overlain spectra is shown in (fig-3).

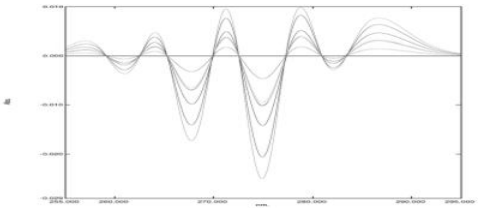

Fig 3 : Second order derivative

2.4.4. Method D: Area under the curve[8]

The AUC (area under curve) method involves the calculation of integrated value of absorbance with respect to the wavelength between the two selected wavelengths λ1 and λ2. The wavelength range is selected on the basis of repeated observations so as to get the linearity between area under curve and concentration.. The AUC for isoxsuprine hydrochloride (fig 4) was determined between 245 and 290 nm for both standard and sample. The calibration graph was plotted between AUC and concentration. The sample AUC was interpolated on the respective linearity chart of the AUC and the sample concentration was determined.

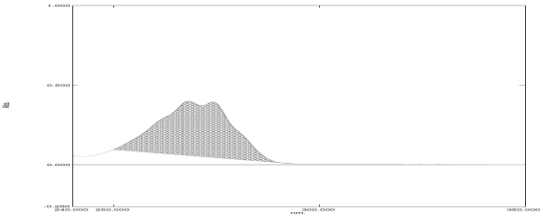

Fig-4: Area under the curve(AUC)

III. Recovery Studies

The recovery studies were carried out on spiked samples by adding predetermined amount of standard drugs to the respective sample. About 20, 40 and 100% of standard drugs were added to the sample solutions and the absorbance was measured. The percentage recovered was calculated. The recovery study was performed at three levels to confirm the precision and accuracy of the above said methods.

IV. Results And Discussions

Isoxsuprine hydrochloride was found to obey Beer's law in the concentration range of 20-100µg/mL. Isoxsuprine hydrochloride showed good linearity as indicated by correlation coefficient value of 0.999. The optical parameters of isoxsuprine hydrochloride with respect to all the three methods are presented in table no.1. The percentage of the individual drugs in the formulation according to the four methods were calculated and represented in the table no.2.

4.1 TABLE 1: Optical Parameters Of Isoxsuprine Hydrochloride By Uv Spectrophotometry

S.No	Parameters	Method A (Q-absorbance)	Method B (First derivative spectroscopy)	Method C (Second derivative Spectroscopy)	Method D (Area under Curve)
1	Wavelength range (nm)	274nm	274nm	274nm	274nm
2	Beer's law limit ($\mu g/mL^{-1}$)	20-100	20-100	20-100	20-100
3	Regression equation (y= mx+c)	0.00x+0.00	0.791x-0.238	1.077x+0.476	126.7x+152.6
4	Slope(m)	0.000	0.791	1.077	126.7
5	Intercept (c)	+0.000	-0.238	+0.476	+152.6
6	Correlation coefficient	1.0	0.999	0.999	0.999
7	Sandell's sensitivity ($\mu g\ cm^{-2}$/0.001A unit)	0.061224	---------	---------	---------
8	LOD (µg/mL)	-------	-4.15094	6.1682	16.9983
9	LOQ (µg/mL)	--------	-12.5786	18.6915	51.51028

4.2 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	Q-absorbance method	10mg	9.990	99.90	0.36000	0.207852	0.340042	0.36036
2	First order derivative spectroscopy		9.995	99.95	0.391706	0.226458	0.340042	0.391889
3	Second order derivative spectroscopy		10.098	100.98	1.044557	0.603093	0.340042	1.03442
4	AUC method		10.086	100.86	0.550485	0.317832	0.340042	0.545773

Each value is a mean of 3 determination

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 method were determined by recovery studies. The recovery studies were carried out on spiked samples and calculated for all the three methods. The percentages recovered were found to be in the range of 99-102% w/w (table.3) which showed that the excipients in the formulation did not interfere with the analysis

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4.3 TABLE 3: RECOVERY STUDIES

S.No	Methods	Label Claim	Amount of drug added(mg)	Amount of drug recoved (mg)	% Recovery
1	Q-absorbance method	10mg	2.00 4.00 10.00	1.98 3.97 19.98	99.00 99.25 99.95
2	First order derivative spectroscopy		2.00 4.00 10.00	2.01 4.01 10.05	100.5 100.25 100.5
3	Second order derivative spectroscopy		2.00 4.00 10.00	2.01 4.04 10.17	100.5 101.00 101.7
4	AUC method		2.00 4.00 10.00	2.03 4.01 10.03	101.5 100.25 100.00

Each value is a mean of 3 determinations

V. Conclusion

The percentage recovery of all the four methods lies between 99-102 %w/w. The correlation coefficient for all the four methods are 0.999 and the recovery studies indicates that there is no interference of other ingredients present in the formulation. Thus, these four methods are simple, precise, accurate, less time consuming and could be used for routine analysis.

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