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Spectrophotometric Determination of Isoxsuprine in Pure and Pharmaceutical Forms

P.Saritha*, T.Charan Singh[1] and C. Gyanakumari
Department of Chemistry, Osmania University, Hyderabad 500 007
[1]Department of Chemistry, G. Narayanamma Institute of Technology and Science, Hyderabad 500 008, India

Abstract*: Four simple, sensitive and accurate methods(I, II, III and IV) for the quantitative estimation of Isoxsuprine in both pure and pharmaceuticals have been developed, optimized and validated. Method I is based on the interaction of the drug (Isoxsuprine) with TCNE,whereas Method II on that with p-chloranil, while Method III on the same with iodine to form ion pair charge transfer complexes. The anionsof these reagents absorb attheir characteristic wavelengths (TCNE – 400nmand 420nm,p-chloranil –346nmand iodide - 366nm) whose absorbance linearly varies with the concentration of the drug and forms a basis for its estimation. Method IV is based on the interaction of the drug with bromothymol blue(BTB)to form chloroform extractable ion pair complex which absorbs maximally at 415nm whose absorbance also linearly varies with the concentration of the drug and forms a basis for its estimation. Factors affecting the absorbance are optimized and all the methods have been validated in terms of ICH guidelines.*

Key Words: *Isoxsuprine (ISX), TCNE (Tetracyanoethylene), p-Chloranil(p-CA), Iodine, Bromothymol blue (BTB), Spectrophometry.*

I. Introduction

Isoxsuprine hydrochloride (ISX) is chemically known as 4-Hydroxy-α-[1-[(1-methyl-2-phenoxyethyl)amino] ethyl] benzenemethanol hydrochloride "Fig.1". ISX is a vasodilator and causes direct relaxationof uterine and vascular smooth muscle. It is used in thetreatment of premature labor and as peripheralvasodilator. [1] Its quantitative determination is reported in US [2] and British pharmacopoeia. [3]Different analytical techniques have beenreported for the determination of ISX in bothpharmaceuticals and biological matrices. Theseinclude UV Spectrophotometry[4, 5] Ion selective electrode potentiometry,[6] Chemiluminescence,[7] High Performance Liquid Chromatography (HPLC),[8,10] Gas chromatography (GC),[11]GC-MS,[12]LC-MS,[13] Affinity Chromatography,[14] Polarography,[16] and Fluorimetry.[17] Recently its quantification using FC reagent[18] and a few πacceptors[19] have been reported by Basavaiah et al. Isoxsuprine was shown to form molecular complex with TCNE in chloroform [20]and this observation paved way that Isoxsuprine may be estimated by ion pair charge transfer complexation with some n and π acceptors as well with a sulphonate dye viz.,BTB. The results of the study arereported in the present communication.

II. Experimental

Instrumentation

The UV-Vis. spectra of the present study have been recorded on ELICO 210 double beam spectrophotometer, Thermo Nicolet 1000 and also on ELICO 159 UV-Visible single beam spectrophotometer using quartz cells of 10 mm path length. An Elico model Li-120 pH meter was used for pH measurement.

Chemicals

The chemicals used for the present study are TCNE, p-Choranil of Sigma, Iodine (BDH, Poole, UK) and AR grade BTB, Acetonitrile and 1,2–dichloroethane are of Spectrograde,Analytical gradeChloroform, andSodium acetate,Sodium tetraborate, Hydrochloric acid,ammonia solution are supplied by SD Fine Chemicals, Mumbai.Isoxsuprine was procured as gift sample from Hetero drugs pvt.Ltd., Hyderabad. The basic drug was regenerated from the aqueous solution by neutralization with ammonia solution.

Preparation of stock solutions

i) **TCNE** was recrystallized thrice from chlorobenzene and vacuum sublimed to get pure white crystals of TCNE. A stock solution of 100mg/100ml(w/v) ($7.8X10^{-3}$ M) in acetonitrile was freshly prepared.

ii) **P-Chloranil:** A solution of 1×10^{-3} M of p-CA was prepared by dissolving0.123 g in absolute ethanol anddiluted to 50 ml in calibrated flasks with the same solvent.

iii) **Borate buffer solution (pH 9):**0.05 M sodium tetraboratewas prepared in distilled water and adjusted to pH 9 by pHmeter.

iv) Iodine Solution:A solutionof4.0×10^{-3}M of iodine was freshly prepared in 1, 2- dichloroethane by dissolving 0.254g/50ml of the solvent to avoid errors due to the liberation of iodine and the BTB dye used as 0.025% solution in doubly distilled water.

v) Sodium acetate-hydrochloric acid buffersof pH 2.8 were prepared by mixing 50ml of 1.0 M sodium acetate solution with 49.50 ml of 1.0 MHCl solution and diluted to 250 ml with doubly distilled water. The pH of each solution was adjusted to an appropriate value with the aid of a pH meter.

III. Results And Discussion

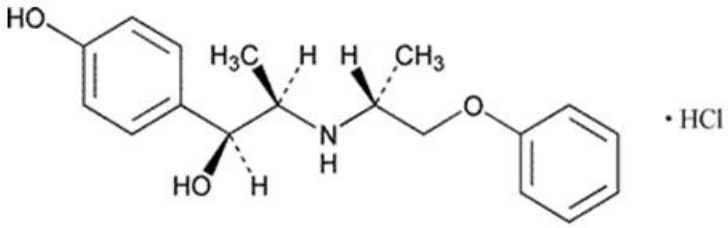
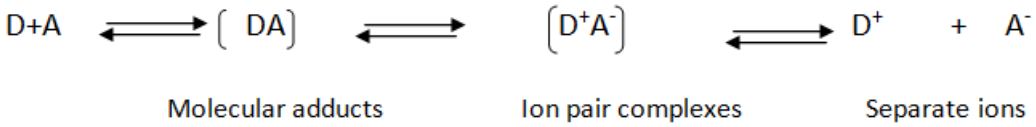


Fig.1 structure of Isoxsuprine

Interactions Interactions between the drug and the analytical reagents are shown below in "scheme.1"

Non polar solvents moderately polar solvents highly polar solvents



Scheme. 1

Donor and acceptor form molecular adducts in nonpolar solvents like hexane, ion pair complexes in moderately polar solvents like CH_2Cl_2 or $CHCl_3$ or CH_3CN and separate ions in highly polar solvent like water.

IV. Analytical Procedures

Method I

Different aliquots of the basic drug solutions in acetonitrile (1- 9ml) were transferred in to 10 ml standard flasks to this 1 ml of TCNE were added and the remaining volume was made up with same solvent. The contents were shaken for 15 sec. and the spectra of the solutions recorded"Fig. 2"

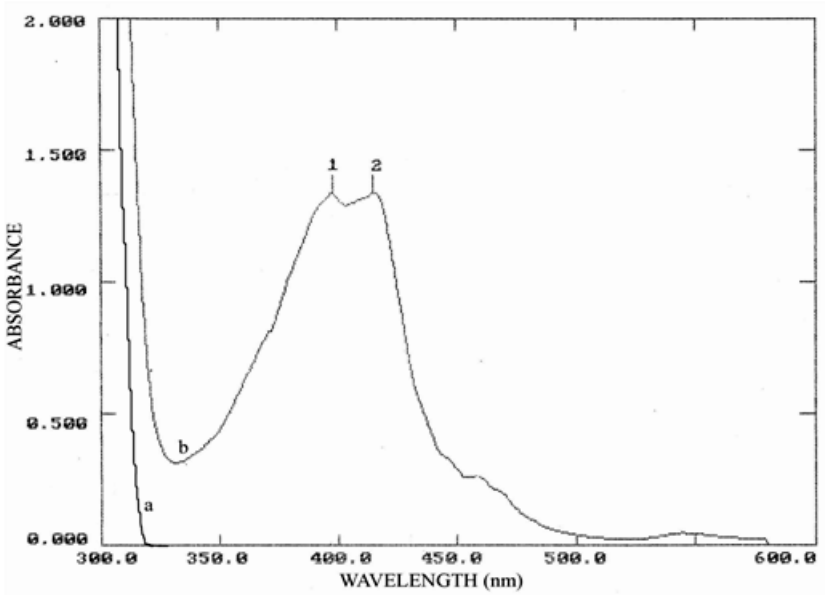


Fig .2 Absorption spectrum of reaction product of Isoxsuprine and TCNE

Method II

Aliquots of the working solution of Isoxsuprine were transferred into a series of 25 ml calibrated flasks. To this 0.5 ml of pH9 and 2 ml of 1×10^{-3}Mp-CA solution were added and diluted to the mark with double distilled water and left for 20mins at 40ºC. The absorbancewas measured at 346 nmagainst thereagent blank"Fig. 3"

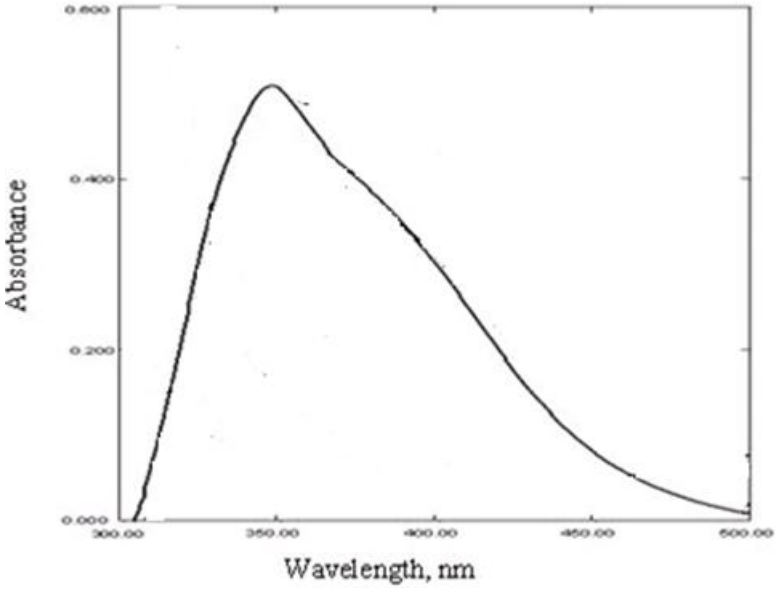


Fig .3 Absorption spectrum of reaction product of Isoxsuprine and p-CA

Method III

Different aliquots of the basic drug solutions in 1,2 dichloroethane (1-9ml)were transferred in to 10 ml standard flasks and 1ml of iodine in DCE was added and the remaining volume was made up with same solvent. The contents were shaken for 15 sec. and the spectra of the solutions recorded "Fig.4".

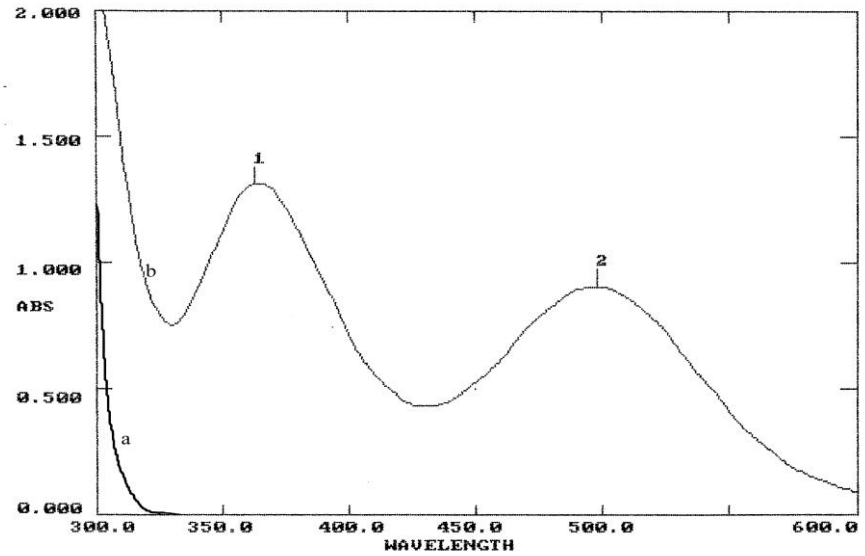


Fig .4 Absorption spectrum of reaction product of Isoxsuprine and iodine

Method IV

Different aliquots of drug solution were transferred into 125 ml separating funnel. To this 5 ml of buffer (pH 2.8), 5 ml of dye were added and total volume was made up to 20 ml with double distilled water. 10 ml of chloroform was added to the above contents and shaken for 5 mins, the two layers was separated after 5 mins. The yellow colored organic layer was collected which is stable for 3 hour. The absorbance of the solution is measured at 415 nm against blanksimilarly prepared"Fig. 5". The same procedure of analysis is followed either for assay of pure drug or for dosage form.

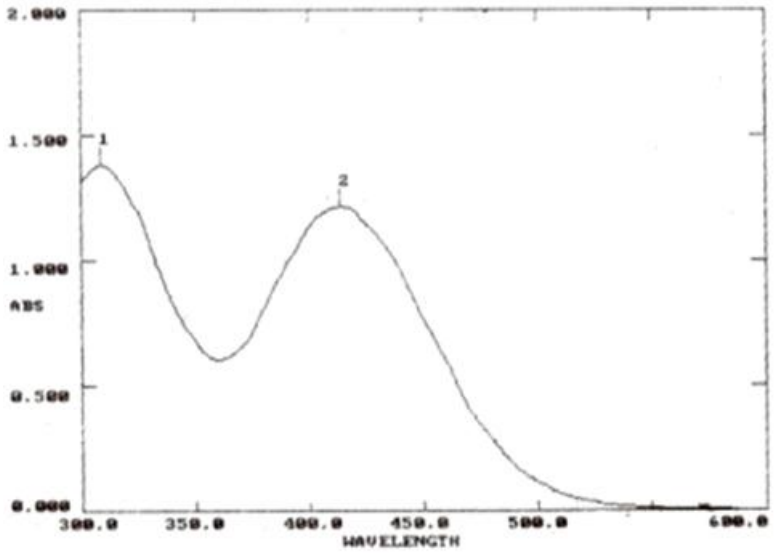


Fig .5 Absorption spectrum of reaction product of Isoxsuprine and BTB

Construction of calibration curves

The absorbance is divided by the corresponding concentrations of the drug called relative response is plotted on the y-axis and the corresponding concentrations on the x-axis. Parallel horizontal lines are drawn on the graph corresponding to95 percent and 105 percent of the horizontal line. The relative response values falling in this range only areselected for construction of calibration curves. The spectral and other statistical parameters are shown in "Table 1". The calibration curves for the four methods are shown in ("Fig 6". a,b,c,d).

Factors affecting the absorbance

Method I

i) **Volume of the reagent** - Different volumes of TCNE from 0.1ml to 2.4 ml were tried to optimize the concentration of the reagent and it is found that 1 ml is sufficient to produce maximum absorbance and hence it is used throughout the study

ii) **Time** - Yellow color were developed immediately after mixing the drug and the reagent and was stable for 255 min. after which it turned colorless

Method II

i) **Volumes of the reagent** - Similar trials were made to optimize the volume of p-chloranil and found to be 2 ml and used same throughout the study.

ii) **Temperature** - Temperature is varied between 25 to 50^{o}C and maximum absorbance was found at 40^{o}C and therefore was chosen for the study.

iii) **Time of heating** - Different time intervals were tried between 5 to 60minutes, and 20 mins is found to be sufficient for full absorbance and after one hour the solution turned opaque.

iv) **pH** - Acidic pH is well known to prevent the charge transfer and hence trials were made between pH 8 to 10, and pH 9 is found suitable for the study. Above pH 10 solutions turned black.

Method III

i) Volume of the reagent - Different volumes of iodine from 0.1ml to 2.0 ml were tried to optimize the concentration of the reagent and it is found that 1 ml is sufficient to produce maximum absorbance and hence it is used throughout the study.

ii) Time -The solutions turned brown immediately after mixing the drug with iodine and remained stable for an hour.

Method IV

i) Volume of the reagent - Different volumes of the BTB was tried between 1 to 10 ml and after 5ml no significant change in absorbance was observed and hence 5 ml was chosen for the study.

ii) Timeof shaking for extraction - Complete extraction of the drug for its quantification was found 20 minutes of shaking the contents

iii) **Effect of pH** - Effect of pH was studied in the range of1.5 to 3.5 and maximum absorbance was observed at a pH of 2.8 and hence was selected for the study.

Precision and accuracy studies

After construction of calibration curves each method was tested for precision and accuracy. Precision is determined by 6 replicate experiments and calculating the %RSD which is being less than 2 demonstrated the precision of the methods. Accuracy is determined by performing recovery studies taking drug samples of known concentrations falling in the Beer's law range. Excellent recovery showed the high accuracy ofthe methods developed. The results are presented in "Table. 2"

Analytical procedure for pharmaceutical formulations

Ten tablets 20 mg each of Isoxsuprine were ground in to fine powder, dissolved in 100 ml of distilled water, sonicated for complete dissolution. This solution was used for interaction with BTB. Ammonia solution was added to neutralize the solution and the precipitate was filtered through Whatman filter paper No 42 and the solid was dried and used as neutral base for interaction with TCNE, p-Chloranil and iodine in the solvents mentioned above. Recovery studies were performed using solutions of the drug of known concentrations falling in the Beer's law range. The recovery studies were statistically validated in terms of student t test and variance F test. Other validation parameters[21,22]are included in the "Table.3"

Fig.6 Calibration curves for estimation of Isoxsuprine

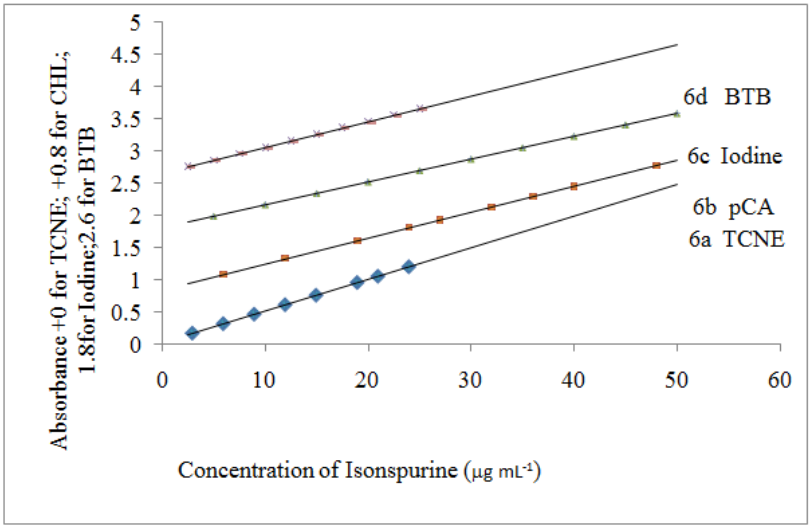


Table1.Spectral and analytical parameters for the determination of Isoxsuprine using different methods

Parameter/Reagent	TCN	p-Chloranil	Iodine	BTB
λmax (nm)	400&420	346	366	415
Beer's law limits (µg ml^{-1})	2.0-24	6.2-48	5-50	2.5-25
Molar Absorptivity (L Mol^{-1} cm^{-1})	19168	13725	18122	17075
Formation Constant (K, M^{-1})	1.724x10^{6}	1.436x10^{6}	1.27x10^{6}	1.25x10^{6}
Sandell Sensitivity (µg cm^{-2})	0.02	0.0283	0.0487	0.0181
Slope (a)	0.049	0.0203	0.0205	0.0402
Intercept (b)	0.0304	0.0363	0.0113	0.048
Correlation coefficient (r)	0.9996	0.999	0.9999	0.996
Limit of detection (µg ml^{-1})	0.27	0.27	0.55	0.3288
Limit of quantification (µg ml^{-1})	0.83	1.25	1.65	0.986
Regressionequation Y=b+ax	Y=0.030+ 0.049x	Y=0.0363+0.0203 x	Y=0.0113+ 0.0205x	Y=0.048+ 0.0402x

Table.2 Precision and Accuracy studies of the differentmethods developed for the assay of Isoxsuprine

Parameter/Reagent	TCNE	p-Chloranil	Iodine	BTB
Amount Taken ($\mu g\ ml^{-1}$)	4 8 12 16	4 8 12 16	4 8 12 16	4 8 12 16
Amount Found ($\mu g\ ml^{-1}$)	3.978 7.97 12.01 16.01	4.01 7.98 12.03 15.92	3.98 8.016 12.01 15.97	4.01 7.97 11.96 15.98
Recovery %	99.46 99.65 100.13 100.1	100.35 99.81 100.23 99.53	99.5 100.2 100.126 98.8	100.26 99.65 99.74 99.89
RSD %	0.33	0.38	0.32	0.272
Mean ± SD	99.84± 0.331	99.987± 0.38	99.91± 0.32	99.88± 0.27
Ref[18]Mean ± SD	99.356± 0.69	99.356± 0.69	99.356± 0.69	99.24± 0.44
t-test	1.37	1.73	1.59	2.25
F-test	0.23	0.31	0.215	0.38

Table.3 Application of proposed method for the analysis of Isoxsuprine in pharmaceutical form

Parameter/Reagent	TCNE	p-Chloranil	Iodine	BTB
Amount Taken ($\mu g\ ml^{-1}$)	4 8 12 16	4 8 12 16	4 8 12 16	4 8 12 16
Amount Found ($\mu g\ ml^{-1}$)	3.99 7.98 11.98 15.98	4.006 8.002 11.96 15.98	4.03 7.967 11.98 15.98	3.98 8.015 11.98 15.98
Recovery %	99.73 99.82 99.87 99.89	100.15 100.24 99.67 99.88	100.717 99.59 99.86 99.89	99.64 100.18 99.87 99.9
RSD %	0.072	0.2	0.4867	0.22
Mean ± SD	99.83± 0.071	99.93± 0.2	100.014± 0.48	99.9± 0.221
Ref[18]Mean ± SD	99.24± 0.44	99.24± 0.44	99.24± 0.44	99.24± 0.44
t-test	2.3	2.5	2.2	2.4
F-test	0.026	0.212	1.224	0.252

V. Conclusions

The proposed UV spectrophotometric methods are a simple, accurate, precise, rapid and economical for the estimation of ISX in bulk drug and tablet dosage form. The proposed methods use inexpensive reagents, solvents and instruments that are available in laboratories. Hence, these methods can be conveniently adopted for the routine analysis in quality control laboratories.

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