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Development and validation of fixed-time method for the determination of isoxsuprine hydrochloride in commercial dosages forms

Nafisur Rahman* and Nasheed Afaq

The main aim of this work was to develop a kinetic spectrophotometric method for the quantitative analysis of isoxsuprine hydrochloride in commercial tablets. The method is based on the reaction of isoxsuprine hydrochloride (ISx) with hydroxylamine hydrochloride and ammonium cerium (IV) nitrate in sulphuric acid medium at room temperature which resulted in the formation of yellow-coloured product peaking at 380 nm. The reaction is followed spectrophotometrically by measuring the absorbance as a function of time. Fixed time method ($\Delta A = A_4 - A_2$, where A_2 and A_4 refer to absorbance measurements taken at 2 and 4 min, respectively) was adopted for constructing the calibration curve which was found to be linear over the concentration range of 30–80 $\mu g mL^{-1}$ with molar absorptivity of 5.95×10^3 L mol^{-1} cm^{-1}. The method has been applied successfully to the determination of isoxsuprine hydrochloride in tablets. Statistical comparison (point and interval hypothesis tests) of the results showed that there is no significant difference between the proposed method and reference method.

Keywords: fixed-time method; isoxsuprine hydrochloride; hydroxylamine hydrochloride; ammonium cerium (IV) nitrate; pharmaceutical analysis.

Introduction

Isoxsuprine hydrochloride ((ISx), 4-Hydroxy-α-[1-[(1-methyl-2-Phenoxy-ethyl) amino] ethyl] benzene methanol, is an orally and parenterally active peripheral and cerebral vasodilator (Scheme 1). It is used for the treatment of cerebral vascular insufficiency, dysmerrtiea and premature labour.[1] It caused isotropic and chromotropic effect on heart.

The drug is official in *Martindale: the extra pharmacopoeia*.[2] The assay procedure is cited in the monograph of the *British Pharmacopoeia*[3] which describes a UV-spectrophotometric method. In order to assure the quantity of ISx in dosage forms, several analytical methods have been reported which include high performance thin layer liquid chromatography (HPTLC),[4] high performance liquid chromatography (HPLC),[5,6] gas chromatography,[7] flow injection analysis,[8–10] iodometrics titration,[11] voltammetery,[12] conductometry,[13] spectrophotometry and flourometry.[14] UV-visible spectrophotometry, due to its low cost, and inherent simplicity, is the technique of choice in the research laboratories, hospitals, and pharmaceutical industries, even today. Some spectrophotometric methods have been developed for the determination of ISx in bulk and pharmaceutical preparations. The quantification of drug was done based on the reaction of ISx with Fe (III) and 1,10-phenanthroline,[15] chloranil,[16] Fe (III) and subsequent complexation of Fe (II) with 2, 2′-bipyridine,[17] sodium nitrite in acidic medium followed by alkalization,[18] diazotized sulphanilic acid,[19] 4-aminoantipyrine in the presence of alkaline oxidizing reagent,[20] Fast red B salt in alkaline medium, eosin and Pb (II) in acetate buffer in presence of non-ionic surfactant, potassium-ferricyanide and phthalophenone in alkaline medium,[21] eosin and Cu (II)[22] and dyes such as fast green (FCF) or Orange II.[23] Few kinetic spectrophotometric methods have been reported for determination of ISx in pharmaceutical preparations.[24,25] Moreover, some specific advantages of kinetic methods, such as selectivity, can be expected because of the measurement of evolution of the absorbance as a function of reaction time.

There is still a need for a sensitive kinetic spectrophotometric method for determination of ISx that can be adopted for routine analysis of pharmaceutical samples. In the present investigation, a kinetic spectrophotometric method based on the reaction of ISx with hydroxylamine hydrochloride and ammonium cerium (IV) nitrate in sulphuric acid medium is described and the proposed method has been extended to the determination of ISx in tablets.

Experimental

Instrumentation

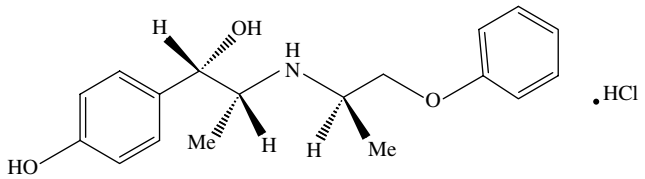
All absorption spectra were recorded using Elico UV-visible spectrophotometer (Elico Ltd, Model No. SL-164, Hyderabad, India) with matched quartz cells. Absorbance was measured using spectronic 20 D+ spectrophotometer (Milton Roy Co., New York, USA).

Chemicals

All chemicals and reagents used were of analytical grade

* *Correspondence to: Dr Nafisur Rahman, Reader, Department of Chemistry, Aligarh Muslim University, Aligarh-202002 (U.P.) India.*
E-mail: cht17nr_amu@yahoo.com

Department of Chemistry, Aligarh Muslim University, Aligarh-202002 (U.P.) India



Scheme 1. Structural formula of isoxsuprine hydrochloride.

(i) 1.82×10^{-2} M Ammonium cerium (IV) nitrate (Qualigens Fine Chemicals Ltd., Mumbai, India) solution was freshly prepared in distilled water.
(ii) 1.43×10^{-1} M Hydroxylamine hydrochloride (Ranbaxy Fine Chemicals Ltd., New Delhi, India) solution was prepared in distilled water.
(iii) 2.55 M sulphuric acid (Sd. Fine-Chemicals Ltd., Mumbai, India)

ISx as a reference standard was obtained from Farmenta Biotech Ltd., Thane, India (Batch No. ISOX 02-02) and used as received. Tablet formulations of ISx such as duvadilan (Solvay Pharma Ltd, Mumbai, India) and Tdilan (Juggat Pharma, Bangalore, India) were purchased from local drug stores. Each tablet labeled to contain 20 mg ISx.

A standard solution of ISx (1 mg mL^{-1}) was prepared in distilled water.

Procedure for the determination of ISx

Aliquots of the standard solution of ISx (1 mg mL^{-1}) corresponding to 300–800 µg were transferred into 10 mL volumetric flasks. To each flask, 0.9 mL of 1.43×10^{-1} M hydroxylamine hydrochloride was added followed by 1.5 mL of 2.55 M sulphuric acid and left for 1 min after shaking. Then, 1.7 mL of 1.82×10^{-2} M ammonium cerium (IV) nitrate was added and volume was completed with distilled water. The absorbance of the resulting solution was measured at 380 nm against the reagent blank as a function of time. The change in absorbance (ΔA) between the time t_1 (2 min) and t_2 (4 min) was computed and plotted against the initial concentration of ISx. The amount of ISx in a given sample can be computed either from the calibration graph or corresponding regression equation.

Assay of ISx tablets

To determine the ISx in tablet formulations, the contents of five tablets were finely powdered and stirred with 25 mL distilled water and left stand for 10 min, then filtered through Whatmann filter paper No. 42. Filtrate was diluted to volume in a 100-mL volumetric flask with distilled water. The content of drug in each sample was determined using the proposed procedure and B.P. method as a reference.[3]

Limits of detection (LOD) and quantitation (LOQ)

The following expressions were used to calculate LOD and LOQ values:

$$\text{LOD} = 3.3 \times S_0/b \quad \text{and} \quad \text{LOQ} = 10 \times S_0/b$$

Where S_0 is standard deviation of the intercept and b is the slope.

Evaluation of bias

The point and interval hypothesis tests[26] have been used to evaluate the bias. In interval hypothesis test, the criterion for acceptance of the proposed method is that when the true mean is within ±2.0% of that of the reference method, i.e.

$$0.98 < \mu_2/\mu_1 < 1.02 \quad (1)$$

which can be generalized to

$$\theta_L < \mu_2/\mu_1 < \theta_U \quad (2)$$

where θ_L and θ_U are lower and upper acceptance limits, respectively. The limits of this confidence interval can be calculated using the following quadratic equation:

$$\theta^2\left(\overline{x_1^2} - S_p^2 {t_{tab}}^2/n_1\right) + \theta(-2\overline{x_1}\overline{x_2}) + \left(\overline{x_2^2} - S_p^2 {t_{tab}}^2/n_2\right) = 0 \quad (3)$$

where $\overline{x_1}$ and $\overline{x_2}$ are mean values based on n_1 and n_2 measurements, respectively. S_p is the pooled standard deviation and t_{tab} is the tabulated one-sided t-value, with $(n_1 + n_2 - 2)$ degrees of freedom at 95% confidence level.

Results and Discussion

It has been reported[27] that the ammonium cerium (IV) nitrate in the presence of hydroxylamine hydrochloride forms stable orange-coloured complexes with aromatic hydroxyl compounds. The colour was completely developed in 30–60 min and these complexes absorbed maximally at 380 nm. ISx possesses phenolic group in its moiety and hence reacts in a similar fashion, with ammonium cerium (IV) nitrate in the presence of hydroxylamine hydrochloride at room temperature resulting in the formation of coloured complex which absorbs maximally at 380 nm. The intensity of the colour increases with time and hence a kinetic spectrophotometric method has been developed for the determination of ISx in commercial dosage forms. The absorption spectrum is shown in Figure 1.

Optimization of variables

Various parameters affecting the formation of the coloured product were examined and optimized by carrying out a series of experiments.

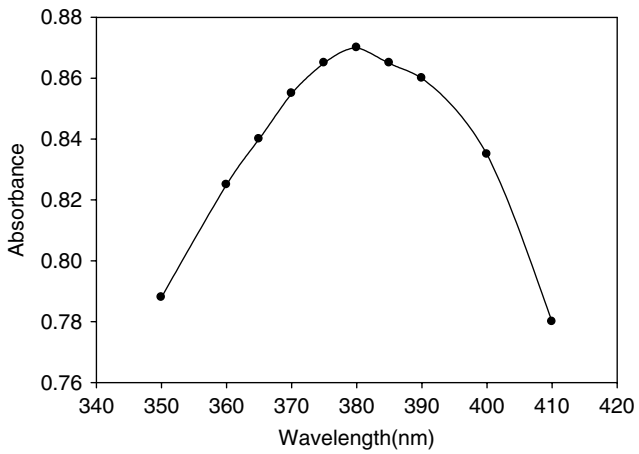


Figure 1. Absorption spectra of complex of isoxsuprine hydrochloride.

Effect of hydroxylamine hydrochloride concentration

The effect of the concentration of hydroxylamine hydrochloride on the absorbance of the coloured product was investigated over the concentration range of 8.58×10^{-3} M–1.72×10^{-2} M; keeping the concentrations of sulphuric acid (0.56 M), ISx (50 μg mL^{-1}) and ammonium cerium (IV) nitrate (3.09×10^{-3} M) constant. It was observed that the maximum and constant absorbance was obtained in the concentration range 1.14×10^{-2} M–1.43×10^{-2} M. Therefore, 1.29×10^{-2} M hydroxylamine hydrochloride was used as an optimum concentration.

Effect of sulphuric acid concentration

The influence of the concentration of sulphuric acid on the formation of product was investigated in the range of 0.13–0.46 M. It was observed that the maximum absorbance was obtained with 0.31 M sulphuric acid and remained constant upto 0.46 M. The optimum value of sulphuric acid was chosen as 0.38 M for the determination process.

Effect of ammonium cerium (IV) nitrate concentration

The effect of Ce (IV) concentration on colour development was studied in the concentration range of 9.1×10^{-4}M–3.64×10^{-3} M; keeping the concentrations of ISx (50 μg mL^{-1}) and sulphuric acid (0.38 M) and hydroxylamine hydrochloride (1.29×10^{-2} M) constant. It was found that increasing the concentration of Ce (IV) resulted in a subsequent increase in absorbance up to 2.73×10^{-3} M and remained constant up to 3.64×10^{-3} M. Therefore, 3.09×10^{-3} M Ce (IV) was chosen as an optimum concentration for further studies.

Validation

The proposed method has been validated for specificity, linearity, accuracy, precision, LOD, and LOQ.

Selectivity

The selectivity of the proposed spectrophotometric method was ascertained by analyzing the pure ISx in presence of tablet formulations such as starch, lactose, magnesium stearate, talc, and avisil. It was found that common excipients present in tablet formulations did not cause any significant interference.

Linearity

Under the optimized experimental conditions, the absorbance of each solution was measured as a function of time at 380 nm. The change in absorbance (ΔA) between the times t_1 (2 min) and t_2 (4, 6,8,10, or 12 min) was computed and plotted against the initial concentration of ISx. It was found that the most acceptable linearity was obtained when the change in absorbance (ΔA) between 2 min and 4 min ($\Delta A = A_4 - A_2$) was plotted against the initial concentration of ISx. Therefore, this fixed time was selected for the assay procedure. The linear dynamic range, molar absorptivity, regression equation, correlation coefficient, LOD, LOQ, and standard deviations of intercept (S_a) and slope (S_b) are summarized in Table 1. The small figures (i.e. S_a and S_b) obtained refer to the high precision of the method.

Table 1. Optical performance and regression characteristics of the proposed methods

Parameters	Proposed Methods
λ_{max} (nm)	380 nm
Stability (h)	1 hr
Linear dynamic range (μg mL^{-1})	30–80
Molar absorptivity (L mol^{-1} cm^{-1})	5.95×10^3
Linear regression equation	$\Delta A = 4.51 \times 10^{-3}$ C − 1.022
S_a	1.703×10^{-2}
S_b	3.12×10^{-4}
Correlation coefficient (r)	0.9952
Detection limit (μg mL^{-1})	6.76
Quantitation limit (μg mL^{-1})	20.49

[a] With respect to A = a + bC, Where C is the concentration (μg mL^{-1}) and A is absorbance.
[b] Confidence interval of the intercept at 95% confidence level.
[c] Confidence interval of the slope at 95% confidence level.

Accuracy and precision

The accuracy and precision of the proposed method was ascertained by determining the ISx content in pure form at two concentration levels: 35.0 and 75.0 μg mL^{-1}. Each concentration level was analyzed five times within a day as well as for five consecutive days. The results are presented in Table 2. The percent relative error and RSD were less than ±2.5% and 1.91%, respectively.

Robustness

The robustness of the proposed method was evaluated by slight variation of the experimental parameters such as:

- room temperature ±3 °C
- volume of 2.55 M H_2SO_4, 1.5 ± 0.3 mL
- volume of 1.43×10^{-1} M hydroxylamine hydrochloride, 0.9 ± 0.1 mL
- volume of 1.82×10^{-2} M Ce (IV), 1.7 ± 0.3 mL

To assess the robustness, the active drug content in Tidilan tablet was determined. The quality control sample solution containing 60 μg mL^{-1} ISx was analyzed five times. The percent recovery ± RSD (100.20 ± 0.50) was found to be appreciable. This concluded that the proposed method is robust.

Applicability of the proposed method

The proposed method was further applied to the determination of ISx in commercial dosage forms. The analysis of same batch

Table 2. Test of precision (intra-day and inter-day assays) for determination of ISx in pure form

Parameters	Intra-day assay		Inter-day assay	
Amount taken (μgmL^{-1})	35.000	75.000	35.000	75.000
Amount found (μgmL^{-1})	35.399	74.689	34.132	74.432
Standard deviation(μgmL^{-1})[a]	0.517	0.668	0.651	0.705
Relative standard deviation (%)[a]	1.462	0.894	1.907	0.947
Relative error (%)[a]	1.14	−0.415	−2.48	−0.757

[a] Mean for five independent analyses.

Table 3. Application of the proposed method to the determination of ISx in tablets

Formulations	Proposed method		Reference method		t & F- values[b]	θ_L[c]	θ_U[c]
	Recovery (%)	RSD[a] (%)	Recovery (%)	RSD[a] (%)			
Duvadilan tablet	100.06	0.85	100.18	0.81	t = 0.164 F = 1.041	0.982	1.02
Tidilan tablet	100.12	0.44	100.19	0.70	t = 0.209 F = 2.542	0.989	1.01

[a] Mean for five independent analyses.
[b] Theoretical *t* ($\nu = 8$) and *F*-values ($\nu = 4, 4$) at 95% confidence level are 2.306 and 6.39, respectively.
[c] In Pharmaceutical analysis, a bias, based on recovery experiments, of ±2% is acceptable ($\theta_L = 0.98$ and $\theta_U = 1.02$).

Table 4. Comparison with other reported methods

Reagent	Linear dynamic range (μg mL^{-1})	Molar absorptivity (L mol^{-1} cm^{-1})	Analysis time	Remarks	Reference
4-Aminoantipyrine	1–18	1.20×10^4	–	Sensitive and rapid	20
p- N, N-Dimethyl phenylenediamine & Chloramine-T	10–40	2.1×10^3	7 min	Tedious, requires extraction with isobutanol	28
Fe (III) and 2, 2′-Bipyridine	20–100	1.01×10^3	15 min heating at 100 °C	Tedious and time consuming	17
Fast Green & Orange-II	0.4–4 and 2.0–20	3.32×10^4 and 9.04×10^3	2 min at RT	Sensitive but tedious and requires extraction with organicsolvent	23
4-Chloro-7-nitrob-enzo-2-oxa-1, 3-diazole (NBD-Cl)	2–20	–	30 min	Tedious, require heating at controlled temp, and costly reagent	25
Sodium nitrite and Copper acetate	8–96	–	25 min heating at 100 °C	Tedious to perform	29
Ammoniun cerium (IV) nitrate and hydroxylamine HCl	30–80	5.95×10^3	4 min at RT	Sensitive, easy to perform and cost-effective reagent	Present method

RT = Room temperature

of samples was also carried out using UV spectrophotometric method.[3] The results of the proposed method were compared with those obtained by reference method in terms of mean recovery, RSD, θ_L, θ_U, t and F values (Table 3). No significant difference was noticed between the proposed method and reference methods regarding accuracy and precision as revealed by *t*- and F- test. The interval hypothesis test has also been performed to compare the results at 95% confidence level. It was observed that true bias of all samples is less than ±2% indicating the compliance of the regulatory authorities.[29]

The performance of the proposed method was compared with other existing spectrophotometric methods. The data presented in Table 4 revealed that the linear dynamic range of the proposed method is suitable for the determination of ISx in tablets and its sensitivity is comparable with other existing methods. Moreover, the analysis time is less as compared to other methods and also does not require extraction of complex into organic solvent. The method is simple requiring only cheaper reagents.

Conclusions

The proposed method provides simple, accurate, and reproducible analysis of ISx in commercial dosage forms. The method has high molar absorptivity (5.95×10^3 L mol^{-1} cm^{-1}) with acceptable linear dynamic range (30–80 μg mL^{-1}) and high tolerance limit for excipients found in dosage forms. The interval hypothesis test proved that the proposed method has acceptable bias of ±2% indicating the method is accurate and precise.

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