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A simple kinetic spectrophotometric method for the determination of isoxsuprine in dosage forms

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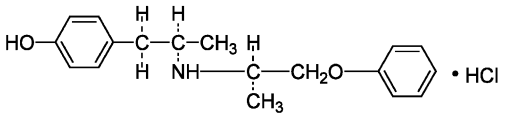
Abstract

A simple and sensitive kinetic method was developed for the determination of isoxsuprine in pharmaceutical preparations. The method is based upon a kinetic investigation of the oxidation reaction of the drug with alkaline potassium permanganate at room temperature for a fixed time of 30 min. The absorbance of the coloured manganate ion was measured at 610 nm. Alternatively, the decrease in the absorbance of potassium permanganate after addition of the drug was measured at 525 nm. The absorbance–concentration plots in both procedures were rectilinear over the range of 0.5–4 μg ml^{-1} ($r = 0.9998$) with a minimum detectability of 0.05 μg ml^{-1} (1.48×10^{-7} M). The different experimental parameters affecting the development and stability of the colours were carefully studied and optimized. The determination of isoxsuprine by the fixed concentration and rate constant methods is also feasible with the calibration equations obtained but the fixed time method has been found to be more applicable. Both procedures were applied to the determination of isoxsuprine in formulations. The results obtained were in good agreement with those obtained using a reference method. The proposed method was also adopted to detect isoxsuprine in spiked human plasma at its therapeutic level of concentration (0.4 μg ml^{-1}). A proposal of the reaction pathway was postulated.

Keywords: Kinetic determination; Isoxsuprine; Potassium permanganate; Pharmaceutical analysis

1. Introduction

(±)-(αR*)-*p*-hydroxy-α-[IS*)-1-[[(IS*)-1-methyl-2-phenoxyethyl]amino]ethyl] benzyl alcohol-hydrochloride (isoxsuprine), is a vasodilator that produces the effects of β-adrenoceptor stimulation and α-adrenoceptor antagonism; the former effect is the predominant. It is used in the treatment of cerebral and peripheral vascular diseases. It is also used to arrest premature labor [1].



Structrural formula of isoxsuprine HCl.

Several analytical methods have been reported for the determination of ISX in raw material, dosage forms and biological fluids. A good guide to the analytical methods of this drug published up to 1997 is found in the comprehensive review written by Belal et al. [2]. The more recent publications include spectrophotometry [3–5], polarography [6] and HPLC [7,8].

The literature is still poor in analytical procedures based on kinetics specially for pharmaceuticals or biological fluids. However, some specific advantages in the application of kinetic methods can be expected [9]: such as, selectivity due to the measurement of the evolution of the absorbance with the time of the reaction instead of the measure of a concrete absorbance value.

The aim of the present work was to study the reaction between isoxsuprine and alkaline potassium permanganate kinetically in an attempt to evaluate the drug in dosage forms. The results obtained were promising.

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2. Experimental

2.1. Apparatus

UV–Vis 1601, Shimadzu recording Spectrophotometer (P/N 206-67001) equipped with kinetic accessory and provided with temperature controlled cell (TCC-240A), thermoelectrically temperature, recording 0–1.0 absorbance, wavelength (nm) 610, factor = 1, number of cells 1, reaction time 40 min, cycle time 0.1.

2.2. Reagents and materials

The following reagents were used:

- Potassium permanganate (Merck, Darmstadt, Germany): 5×10^{-3} M aq. solution, freshly prepared.
- Sodium hydroxide (BDH, UK): 0.5 M aq. solution.
- Acetone (Aldrich, Germany).
- ISX HCl (Batch No. 191401) was purchased from Sigma, (Saint Louis, MO, USA). Tablets containing the drug: duvadilan tablets, labelled to contain 20 mg each (batch no. 198) and vascular tablets, labelled to contain 20 mg each (batch no. 798140) were obtained from commercial sources.
- Standard solutions.

A stock solution was prepared by dissolving 20.0 mg of ISX HCl in 100 ml of distilled water and further diluted with the same solvent as appropriate.

2.3. Procedures

2.3.1. Recommended general procedures

2.3.1.1. First method. Prepare a standard solution that contains 20.0 μg ml^{-1}. Transfer aliquots of this solution accurately measured, into 10 ml volumetric flask; add 1 ml of 0.5 M NaOH followed by 2.5 ml of 5×10^{-3} M $KMnO_4$, shake the mixture well. Allow the reaction mixture to stand for 30 min. Make up to the volume with water. Measure the absorbance of the resulting solution at 610 nm against a reagent blank solution prepared simultaneously. Plot the values of the absorbance against the final concentration in μg ml^{-1} to get the calibration graph. Alternatively, derive the regression equation.

2.3.1.2. The second method. Transfer aliquots of the standard solution into 10 ml volumetric flasks. Add 1 ml of 0.5 M NaOH followed by 0.6 ml of 7.6×10^{-3} M $KMnO_4$, then shake the mixture. Let to stand for 30 min. Make up to the volume with distilled water. Measure the absorbance of the solution at 525 nm. Plot the absorbance value versus the final content of the drug to get the calibration graph. Alternatively, derive the regression equation.

2.3.2. Procedure for the tablets

Weigh and pulverize 10 tablets. Transfer a weighed quantity of the powder equivalent to 10 mg of ISX HCl into a small flask. Shake with 3×30 ml of C_3H_6O for 10 min, then filter into a conical flask. Wash the beaker and filter with few ml of C_3H_6O and pass the washings to the same flask. Evaporate the C_3H_6O using a rotatory evaporator at 50 °C till dryness. Dissolve the residue in 3×30 ml of water and filter, if necessary, into 100-ml volumetric flask, then complete to the mark with water. Proceed as described above. Measure the absorbance at 610 or 525 nm as mentioned above. Determine the nominal content of the tablets either from a previously plotted calibration graph or using the corresponding regression equation.

2.3.3. Procedure for detection of isoxsuprine in spiked human plasma

To 1 ml of spiked plasma, add 3 ml of MeCN, mix well, then centrifuge for 10 min. Transfer the clear supernatant to a 10 ml volumetric flask and repeat the extraction with MeCN. Complete to the mark with MeCN. Proceed as described under Section 2.3.1.1 or Section 2.3.1.2. Measure the absorbance at 610 or 525 nm as mentioned above.

3. Results and discussion

3.1. Kinetics and optimization of the parameters

ISX was found to react with $KMnO_4$ in alkaline medium producing a bluish–green colour due to the production of manganate ion which absorbs maximally at 610 nm (Fig. 1). The absorbance of the reaction product remains stable for at least 60 min. As the intensity of the colour increases with time, this was used as a basis for a useful kinetic method for the determination of isoxsuprine in pharmaceuticals. The spectrophotometric properties of the coloured product as well as the different experimental parameters affecting the colour development and its stability were carefully studied and optimized. Such factors were changed individually while the others were kept constant. These factors include, effect of different oxidants, effect of different solvents, concentration of the reagents ($KMnO_4$ and NaOH), temperature, time, sensitizers, surfactants and type of alkalies. At room temperature, the reaction rate increased substantially as the colour development increased. Therefore, room temperature was selected as the optimum temperature. Heating the solution was found to increase the rate of the reaction but MnO_2 was precipitated.

The reaction rate and maximum absorbance increased with time, and upon increasing $KMnO_4$ concentration. It was found that 2.7 ± 0.2 ml of 5×10^{-3}

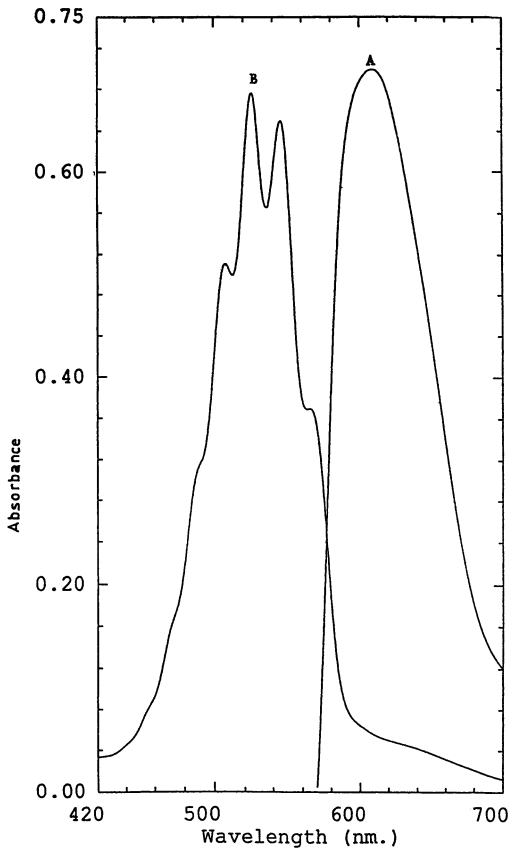


Fig. 1. Absorption spectrum of the reaction product of ISX·HCl after reaction with $KMnO_4$/NaOH system. (A) Reaction product (5 μg ml^{-1}); (B) $KMnO_4$ (5×10^{-5} M).

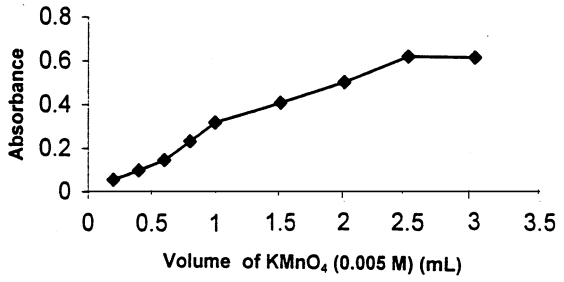


Fig. 2. Effect of volume of $KMnO_4$ (5×10^{-3} M) on the absorbance of ISX·HCl (4 μg ml^{-1}) at 610 nm.

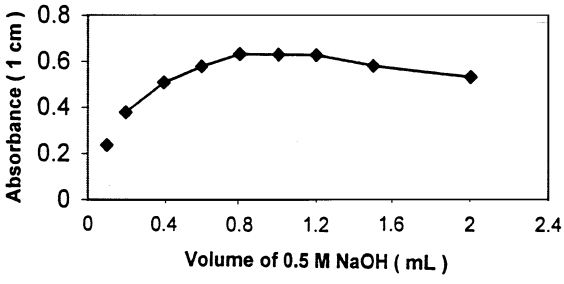


Fig. 3. Effect of volume of NaOH (0.5 M) on the absorbance of ISX·HCl (4 μg ml^{-1}) at 610 nm.

M $KMnO_4$ was adequate for the maximum absorbance (Fig. 2).

Oxidation of isoxsuprine with $KMnO_4$ was carried out in presence of NaOH. Trials were made to determine the drug through its oxidation with $KMnO_4$ in neutral and acidic media, but very little oxidation of isoxsuprine was observed.

The influence of NaOH concentration on the reaction rate was also studied using 0.1–3 ml of 0.5 M NaOH. It was found that increasing the volume of 0.5 M NaOH, would increase the absorbance of the reaction product up to 1.2 ml, further increase in volume resulted in a very slight decrease in the absorbance of the reaction product, thus, 1 ± 0.2 ml of 0.5 M NaOH was found to be the most suitable concentration for maximum absorbance (Fig. 3). Other alkalies, such as KOH and NH_4OH with the same concentration were also tested to identify the best alkaline medium. However, their effect on the colour development was less than that of NaOH, therefore the latter was used throughout the study.

Different oxidants have been used to determine ISX, such as H_2O_2, potassium persulfate in alkaline medium and cerric ammonium sulfate or potassium dichromate in strong acid medium. In case of each of H_2O_2 and persulfate, oxidation of the drug resulted in hypsochromic shift and hypochromic effect. In case of cerric ammonium sulfate, very little oxidation was attained as revealed by the very weak absorbance peaking at 492 nm ($A^{1\%}_{1\,cm} = 50$). Also, in case of dichromate very little oxidation of the drug was observed $A^{1\%}_{1\,cm}$ at 580 nm was 20.

The effect of diluting solvent was also studied. Different solvents such as water, ethanol, acetonitrile and dimethyl sulfoxide were used. It was found that water was the best solvent as it gave the highest absorbance reading, moreover its choice adds to the advantages of the method.

Different sensitizers (quinine, fluorescein and rhodamine-B), at concentrations of 5 μg ml^{-1} were tested by adding to the reactants mixture before allowing to stand for 30 min. Outstanding inhibitory effects were observed as these sensitizers reacted strongly with $KMnO_4$/NaOH system. In the same manner, the effect of surfactants on the colour development was studied. Different surfactants (cetrimide, gelatin and sodium lauryl sulfate) at three concentrations, 2.5, 7.5 and 15 μg ml^{-1}, were tested by adding to the reaction mixture prior to allowing to stand for 30 min. All tested surfactants reacted strongly with the $KMnO_4$/NaOH system with inhibitory effect, as evident from the low absorbance readings. Potassium permanganate is consumed by the surfactants, being reduced to reduction products other than the measured species (Table 1).

Table 1
Effect of surfactants on the performance of the proposed method

Surfactant	Concentration (μg ml^{-1})	Absorbance
No surfactant		0.485
Cetrimide	2.5	0.400
Sodium lauryl sulfate	2.5	0.461
Gelatin	2.5	0.454
Cetrimide	7.5	0.371
Sodium lauryl sulfate	7.5	0.420
Gelatin	7.5	0.433
Cetrimide	15	0.333
Sodium lauryl sulfate	15	0.387
Gelatin	15	0.300

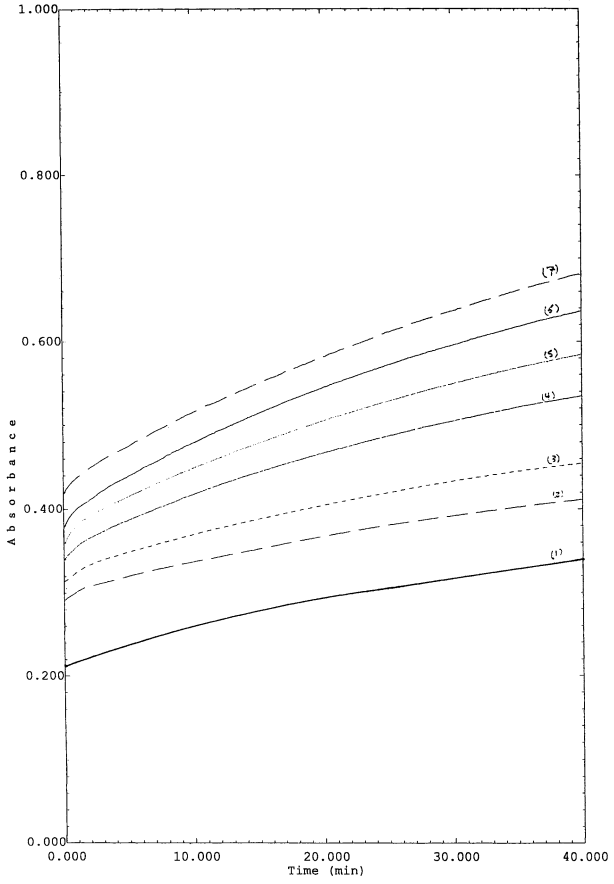


Fig. 4. Absorbance vs. time graphs for the reaction between isoxsuprine and MnO_4^-/OH^- system showing the dependence of the reaction on isoxsuprine concentrations. Concentrations of isoxsuprine are: (1) 5.92×10^{-6}; (2) 7.401×10^{-6}; (3) 8.88×10^{-6}; (4) 9.473×10^{-6}; (5) 1.036×10^{-7}; (6) 1.125×10^{-7}; (7) 1.184×10^{-7} M.

3.2. Analytical performance

The rate of the reaction was followed at room temperature with various concentrations of the drug in the range of 0.5–4 ug ml^{-1}, keeping $KMnO_4$ and NaOH concentrations constant (Fig. 4).

An alternative spectrophotometric method for the determination of ISX based upon measuring the decrease in the absorbance of $KMnO_4$ at 525 nm (Fig. 5) was developed. The difference in the absorbance was plotted against the concentration of the drug; furthermore, logarithmic analysis of the reaction rate (R) was plotted against log concentration of the drug. The rate of reaction was also found to be dependent on isoxsuprine concentrations. The rates were followed at room temperature with various concentrations of isoxsuprine in the range of 0.5–4 μg ml^{-1} keeping $KMnO_4$ and NaOH concentrations constant. The reaction rate was found to obey the following equation:

$$\text{Rate} = K^-[\text{ISX·HCl}]^n \quad (1)$$

where K^- is the pseudo-order rate constant and n is the order of the reaction.

The rate of the reaction may be estimated by the variable-time method [10] as $\Delta A/\Delta t$, where A is the absorbance and t is the time in seconds. Taking logarithms of rates and concentrations (Table 2), Eq. (1) is transformed into:

$$\text{Log(rate)} = \log \Delta A/\Delta t = \log K^- + n \log[\text{ISX·HCl}] \quad (2)$$

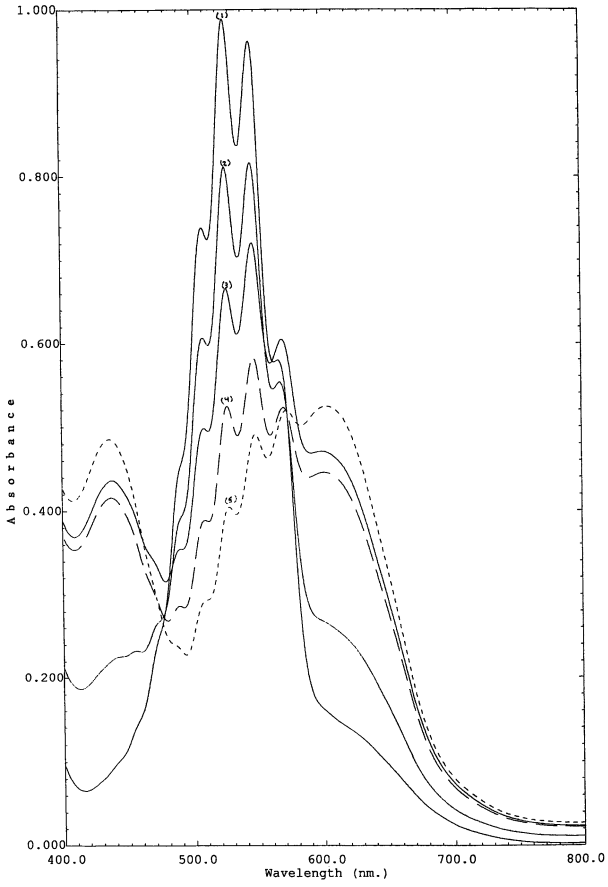


Fig. 5. Absorption spectrum of isoxsuprine after reaction with $KMnO_4$ at different concentrations (μg ml^{-1}): (1) $KMnO_4$; (2) 1 μg ml^{-1}; (3) 2 μg ml^{-1}; (4) 3 μg ml^{-1}; (5) 4 μg ml^{-1}.

Table 2
Logarithms of rates for different concentrations of ISX·HCl at room temperature at 610 nm

Wavelength (nm)	Log $\Delta A/\Delta t$	Log[ISX·HCl]
610	−4.25	−5.228
	−4.160	−5.131
	−4.050	−5.052
	−4.027	−5.024
	−3.961	−4.985
	−3.907	−4.948
	−3.890	−4.927
525	−4.103	−5.529
	−3.800	−5.228
	−3.626	−5.052
	−3.514	−5.927

Table 3
Values of K^- calculated from slopes of log A versus t graphs at 610 nm

$K^- \times 10^{-4}$ (s^{-1})	[ISX·HCl] (M)
−2.764	7.401×10^{-6}
−2.250	9.473×10^{-6}
−2.047	1.036×10^{-5}
−1.945	1.125×10^{-5}
−1.727	1.184×10^{-5}

Table 4
Values of reciprocal of time taken at fixed absorbance for different rates of variable concentrations of ISX·HCl at constant concentrations of NaOH and $KMnO_4$

$(1/t) \times 10^{-4}$ (s^{-1})	[ISX·HCl] $\times 10^{-5}$ (M)
8.333	1.036
9.259	1.065
1.165	1.125
1.389	1.154
1.684	1.184

Regression of log(rate) versus log[ISX·HCl] gave the regression equation;

$$\text{Log(rate)} = 2.195 + 1.235 \log C \quad (r = 0.9961)$$

Hence $K^- = 157\ s^{-1}$ and the reaction is first order ($n = 1.235$) with respect to isoxsuprine concentration.

$$\text{Log(rate)} = 1.336 + 0.983 \log C \quad (r = 0.9998)$$

Hence, $K^- = 22\ s^{-1}$ and the reaction is first order ($n = 0.983$).

3.3. Evaluation of the kinetic methods

The quantitation of ISX·HCl under the optimized experimental conditions outlined above, would result in a pseudo-first order with respect to its concentrations where $KMnO_4$ concentration was at least 60 times the concentration of ISX·HCl and NaOH concentration was at least 600 times the initial concentration of ISX·HCl. However, the rate was directly proportional to isoxsuprine concentration in a pseudo-first order rate equation as follows:

$$\text{Rate} = K^-[\text{ISX·HCl}] \quad (3)$$

where K^- is the pseudo-first order rate constant. Several experiments were then carried out to obtain isoxsuprine concentration from the rate data according to Eq. (3). Initial rate, rate constant, fixed concentration and fixed time methods [11,12] were tried and the most suitable analytical method was selected taking into account the applicability, the sensitivity, the intercept and the correlation coefficient (r).

3.4. Rate constant method

Graphs of log absorbance versus time for ISX·HCl concentration in the range of 7.4×10^{-6}–1.184×10^{-5} M were plotted and all appeared to be rectilinear. Pseudo-first order rate constant (K^-) corresponding to different ISX·HCl concentrations (C) were calculated from the slopes multiplied by −2.303 and are presented in Table 3. Regression of (C) versus K^- gave the following equation:

$$K^- = -4.41 \times 10^{-4} + 22.5C \quad (r = 0.9944)$$

3.5. Fixed concentration method

Reaction rates were recorded for different ISX·HCl concentrations in the range of 1.036×10^{-5}–1.184×10^{-5} M. A preselected value of the absorbance (0.5) was fixed and the time was measured in seconds. The reciprocal of time ($1/t$) versus the initial concentration of ISX·HCl (Table 4) was plotted and the following equation of the calibration graph was obtained:

$$1/t = -5.0 \times 10^{-3} + 558.6C \quad (r = 0.9706)$$

3.6. Fixed time method

Reaction rates were determined for different concentrations of ISX·HCl. At a preselected fixed time, which was accurately determined, the absorbance was measured. Calibration graphs of absorbance versus initial concentration of ISX·HCl were established at fixed times of 5, 10, 15, 20, 25, 30, 35 and 40 min with regression equations assembled in Table 5.

It is clear that the slope increases with time and the most acceptable values of the correlation coefficient (r) and the intercept were obtained for a fixed time of 30 min, which was therefore chosen as the most suitable time interval for measurement (Table 6).

After optimizing the reaction conditions, the fixed time method was applied to the determination of isoxsuprine in pure form over the range 0.5–4 μg ml^{-1}. Analysis of the data gave the following equations:

$$A = -1.557 \times 10^{-3} + 0.158C \quad (r = 0.9998)$$

where A is the absorbance at 610 nm and C is the concentration in μg ml^{-1}.

$$A = 9.0 \times 10^{-3} + 0.136C \quad (r = 0.9995)$$

Table 5
Regression equations for ISX·HCl at different fixed time over the range 1.48×10^{-6}–1.184×10^{-5} M at room temperature at 610 nm

Time (min)	Regression equation	Correlation coefficient (r)
5	$A = 0.013 + 0.117C$	0.9941
10	$A = 0.011 + 0.127C$	0.9969
15	$A = 6.495 \times 10^{-3} + 0.136C$	0.9987
20	$A = 4.352 \times 10^{-3} + 0.144C$	0.9996
25	$A = 2.000 \times 10^{-3} + 0.151C$	0.9998
30	$A = -1.557 \times 10^{-3} + 0.158C$	0.9998
35	$A = -2.539 \times 10^{-3} + 0.163C$	0.9996
40	$A = -4.831 \times 10^{-3} + 0.169C$	0.9993

Table 6
Analytical parameters for the determination of ISX·HCl in pure form using fixed time method

Parameter	Proposed method at	
	610 nm	525 nm
Concentration range (μg ml^{-1})	0.5–4.0	0.5–4.0
Minimum detection limit (M)	(1.48×10^{-7} M)	
Correlation coefficient (r)	0.9998	0.995
Slope	0.158	0.137
Intercept	-1.55×10^{-3}	9×10^{-3}
$S_{y/x}$	3.42×10^{-3}	6.74×10^{-3}
S_a	5.02×10^{-3}	0.010
S_b	9.99×10^{-4}	3.01×10^{-3}
% Error	0.34	0.54

Table 7
Application of the proposed method for the determination of ISX·HCl in pure form using fixed time method

Parameters	Proposed method at		Reference method [14]
	610 nm	525 nm	
Mean found (%) ± SD	100.40 ± 0.97	100.50 ± 1.07	99.89 ± 0.78
Variance	0.94	1.14	0.61
Student's t-value	0.81 (1.48)	0.83 (1.31)	
Variance ratio F-test	1.55 (4.74)	1.88 (9.55)	

Values in parentheses are the tabulated values of t and F at $P = 0.05$.

where A is the absorbance at 525 nm and C is the concentration in μg ml^{-1}.

The limit of detection (LOD) was found to be 0.05 μg ml^{-1} (1.48×10^{-7} M) and the limit of quantification (LOQ) was found to be 0.5 μg ml^{-1}.

The precision of the method was evaluated by analysing standard solutions of ISX·HCl. The results for pure sample were in accordance with those obtained by the reference method [14]. The latter is based on nitrosation of ISX with nitrous acid and subsequent chelation with copper acetate followed by measuring the absorbance of the copper-chelate at 525 nm.

Statistical analysis [13] of the results obtained by the proposed and reference methods, using Student's t-test and variance ratio F-test revealed no significant difference between the performance of the two methods regarding the accuracy and precision (Table 7). The validity of the method was evaluated by statistical analysis of the regression data and the results are represented in Table 7.

3.7. Pharmaceutical applications

It was found that, when the proposed method was applied to the determination of ISX·HCl in pharmaceutical preparations, the % recovery was around 150%; this might be due to the interaction of the excipients in the formulations (especially lactose and hydroxymethylcellulose which contain hydroxyl alcohol groups), with $KMnO_4/OH^-$ system. Therefore, the tablet had to be extracted with another solvent, such as acetone and the latter was thus evaporated using a rotatory evaporator till dryness. Analyses of tablets extracts spiked with known concentrations of ISX·HCl are shown in Table 8. From the results, it is evident that no interference is encountered from the tablet matrix after applying the

Table 8
Analysis of commercial tablets spiked with known concentrations of ISX·HCl at 610 nm

Preparation	Added ISX·HCl (μg ml^{-1})	% Recovery
Vascular tablets (ISX·HCl, 20 mg per tablet)	2.0	99.06
	3.0	99.53
	4.0	101.44
$\bar{X}$		100.01
± SD		0.95
Duvadilan tablets (ISX·HCl, 20 mg per tablet)	2.0	100.59
	3.0	101.19
	4.0	98.81
$\bar{X}$		100.19
± SD		0.92

Table 9
Application of the proposed method to the ISX·HCl in dosage forms

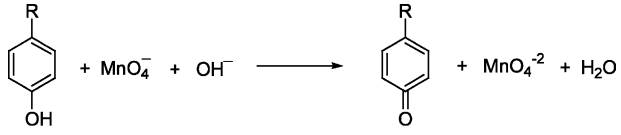
Preparations	Amount taken (μg ml^{-1})	% Recovery at 610 nm	% Recovery at 525 nm	Reference method [14]
Vascular tablets [a] (ISX·HCl, 20 mg per tablet)	2.0	101.00	99.65	100.25
	3.0	99.63	101.27	99.75
	4.0	99.08	100.70	100.08
$\bar{X} \pm$ SD		99.90 ± 0.81	100.54 ± 0.67	100.03 ± 0.21
Duvadilan tablets [b] (ISX·HCl, 20 mg per tablet)	2.0	99.30	99.31	100.05
	3.0	101.00	100.09	99.79
	4.0	98.95	99.37	99.96
$\bar{X} \pm$ SD		99.75 ± 0.89	99.59 ± 0.35	99.93 ± 0.11

[a] Product of South Egypt Drug Industries Co., 6 October City, Egypt.
[b] Product of Pharco Pharmaceuticals, Alexandria, Egypt.

extraction method. Satisfactory percentage recoveries were then obtained (Table 9).

3.8. Mechanism of the reaction

The stoichiometry of the reaction was studied adopting the limiting logarithmic method [15]. The absorbance of the reaction product was alternatively measured in the presence of excess of either $KMnO_4$ or ISX·HCl. A plot of log absorbance versus log[$KMnO_4$] and log[ISX·HCl] gave straight lines; the values of the slopes are 0.996 and 1.00, respectively (Fig. 6). Hence, it is concluded that, the molar reactivity of the reaction is 0.996/1, i.e. the reaction proceeds in the ratio of 1:1. Based on the obtained molar reactivity, and depending on the phenolic nature of isoxsuprine, the reaction pathway is proposed to proceed as follows:



The proposed mechanism of reaction between ISX·HCl and potassium permanganate inalkaline medium.

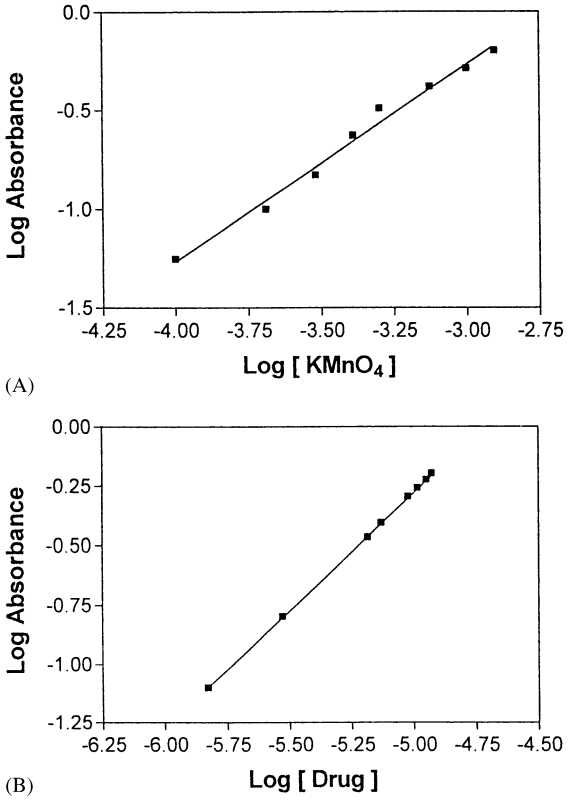


Fig. 6. Limiting logarithmic plots for the molar ratio: (A) log A vs. log[$KMnO_4$]; (B) log A vs. log[Drug].

3.9. Stability indication of the method

The proposed method is based mainly on the presence of phenolic group which is oxidized by MnO_4^-/OH^- system; hence the absence of the phenolic group by oxidation for instance—will stop the reaction pathway. Previous report; reveal that oxidation is the major pathway of degradation of isoxsuprine, especially in solutions, being oxidized to the corresponding quinine [16]. The latter would not be further oxidized. Hence, it is concluded that, the proposed method can be considered as a stability-indicating assay of isoxsuprine.

4. Conclusion

Different methods were established to determine isoxsuprine concentration kinetically, the reaction rate method, rate constant and fixed time methods were applied. Applying the fixed time method, it is clear that the slope increased with time and the most acceptable values of correlation coefficients (r) and intercepts were obtained for a fixed time of 30 min, which was therefore chosen as the most suitable time interval for measurements.

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