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Fluorimetric determination of isoxsuprine hydrochloride in pharmaceuticals and biological fluids

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Abstract

Two simple and highly sensitive fluorimetric methods have been developed for the determination of isoxsuprine hydrochloride in bulk, in dosage forms and in biological fluids. The first method involves the direct measurement of the native fluorescence of the drug in the concentration range 0.4–4.0 μg ml^{-1}, the second method is based on the oxidation of isoxsuprine HCl with cerium(IV) followed by fluorimetric measurement in the concentration range 0.02–0.2 μg ml^{-1}. The average % found were 99.9 ± 0.78 and 100.0 ± 0.62 for the two methods, respectively. The minimum detectability (3 S_B) were 0.11 and 0.007 μg ml^{-1} for the two methods, respectively. The methods results showed insignificant difference with those of the official method.

Keywords: Cerium; Isoxsuprine hydrochloride; Pharmaceuticals; Biological samples; Spectrofluorimetry

1. Introduction

Isoxsuprine hydrochloride is a vasodilator which stimulates beta-adrenergic receptors. It causes direct relaxation of vascular and uterine smooth muscle. It also produces positive inotropic and chronotropic effects [1]. It is widely used in the treatment of premature labor and as a peripheral vasodilator [1].

Numerous analytical procedures have been reported for the determination of isoxsuprine hydrochloride either in pure form or in pharmaceutical preparations. The USP 24 [2] recommends a different spectrophotometric method for the analysis of isoxsuprine hydrochloride in pure form and a column partition chromatography with spectrophotometric detection for the dosage forms, while the British Pharmacopoeia [3] recommends a potentiometric titration method using sodium hydroxide for the determination of the pure form of the drug. Other reported methods include spectrophotometric methods using several color reagents [4–14], flow injection spectrophometric [15], flow injection kinetic potentiometric [16], flow injection chemiluminometric [17], polarographic [18], voltammetric [19], TLC [20], GLC [21] and HPLC methods [22,23]. Reviewing the literature revealed that, up to the present time nothing has been published concerning the fluori-

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metric determination of isoxsuprine hydrochloride. The presence of phenolic group initiated the present study.

Cerium(IV) is a well-known oxidizing agent, it is used for the determination of some drugs such as paracetamol, phenothiazines, penicillamine and triptophan [24–29].

In this paper the native fluorescence of isoxsuprine has been investigated and employed as a selective means of detection and determination in its dosage forms and biological fluids. Also, isoxsuprine has been determined via its oxidation with Ce(IV) with subsequent fluorimetric measurement.

2. Experimental

2.1. Apparatus

The fluorimetric measurements were performed on a Perkin–Elmer Luminescence Spectrometer LS 50B with the excitation and emission slit controls set at 10 mm.

2.2. Reagents and materials

The following reagents were used: sulfuric acid (Riedel-de Haen, Germany), 0.1 and 3 M solutions; aqueous Cerium(IV) sulphate (BDH, Polle, UK), 1×10^{-4} M was prepared in 3 M H_2SO_4; 1.0 M carbonate buffer, pH 9.48 was made up from 26.5 g sodium carbonate (BDH) and 21.0 g sodium bicarbonate (Koch-Light Laboratories, Colubrook Bucks, UK) in 500 ml distilled water; ethyl acetate (BDH); serum samples (Multi-Serum-Normal, Randox Laboratories, UK); urine samples were obtained from healthy volunteers.

Isoxsuprine HCl authentic was supplied by Solvay Dophar (The Netherlands); dosage forms containing isoxsuprine HCl were purchased from commercial sources.

2.3. General procedure

2.3.1. For the direct fluorimetric method

A 1.0 mg ml^{-1} stock standard solution of isoxsuprine HCl was prepared in distilled water. This solution was further diluted with water to give a 50.0 μg ml^{-1} isoxsuprine HCl working standard solution. Portions (0.2–2.0 ml) of this solution, accurately measured, were transferred into separate 25 ml volumetric flasks and completed to the mark with water. The relative fluorescence intensity was measured at $\lambda_{em} = 305$ nm with $\lambda_{ex} = 270$ nm and plotted against the concentration of isoxsuprine HCl to obtain the standard calibration graph.

2.3.2. For the oxidation with cerium(IV)

The stock standard solution (1.0 mg ml^{-1}) was further diluted with water to give a 1.0 μg ml^{-1} isoxsuprine HCl working standard solution. Portions (0.2–2.0 ml) of this solution, accurately measured, were transferred into separate 10 ml volumetric flasks, 1.0 ml Ce(IV) solution was added and diluted to the mark with distilled water. A blank experiment was performed. The relative fluorescence intensity was measured at $\lambda_{em} = 355$ nm with $\lambda_{ex} = 253$ nm and plotted against the concentration of isoxsuprine HCl to obtain the standard calibration graph.

2.4. Procedure for tablets

Ten tablets were weighed and powdered. An accurately weighed amount of powder equivalent to 10 mg of drug was transferred into a 100 ml volumetric flask. A 10 ml of 0.1 M H_2SO_4 was added and diluted to volume with water. The flask with its contents was sonicated for 30 min followed by filtration. The above procedures were then performed and the nominal content of the tablets was calculated either from a previously plotted calibration graph or using the regression equation.

2.5. Procedure for spiked biological fluids

An aliquot of serum or urine (1.0 ml) in a centrifuge tube was spiked with 1.0 ml aqueous solution containing 400 μg isoxsuprine HCl; 1.0 ml carbonate buffer (pH 9.48) and 5.0 ml ethyl acetate were added. The mixture was vortexed at high speed for 2 min, and then centrifuged at 2500 rpm for 10 min. The resulting supernatant was

transferred into a small conical flask. The extraction was repeated two times with 5 ml ethyl acetate. The combined extracts were evaporated to dryness under a stream of nitrogen at room temperature. The dry residue was dissolved in 25 ml 0.1 M H_2SO_4 and the above general procedures were then followed. A blank experiment was performed. Absolute recovery was determined by comparing relative fluorescence intensities of extracted plasma or urine with those of the standard drug at the same concentrations.

3. Results and discussion

3.1. Spectral characteristics

Aqueous solutions of isoxsuprine HCl were reported to be stable for 323 days [20]. These solutions fluoresce at $\lambda_{em} = 305$ nm with $\lambda_{ex} = 270$ nm. The fluorescence is destroyed by alkalinization, remained unchanged by acidification.

The oxidation of isoxsuprine HCl by Ce(IV) in acidic medium was found to give fluorescence due to the reduction of Ce(IV) into Ce(III) which fluoresce at $\lambda_{em} = 355$ nm with $\lambda_{ex} = 253$ nm.

3.2. Effect of experimental variables

Investigations were carried out to establish the most favorable conditions for the oxidation. The reaction was optimized using 1.0 ml of 1×10^{-4} M Ce(IV) solution in 3 M H_2SO_4 (Figs. 1 and 2). Larger volume of Ce(IV) or higher concentration of H_2SO_4 caused a marked decrease in the fluorescence. The fluorescence intensity was measured immediately since a negligible increase in the fluorescence intensity was occurred by time.

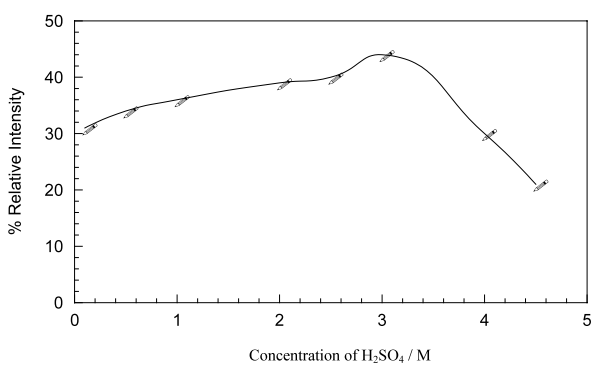

Fig. 1. Effect of sulfuric acid concentration as a diluent for cerium(IV) on the oxidation of isoxsuprine HCl (0.08 μg ml^{-1}).

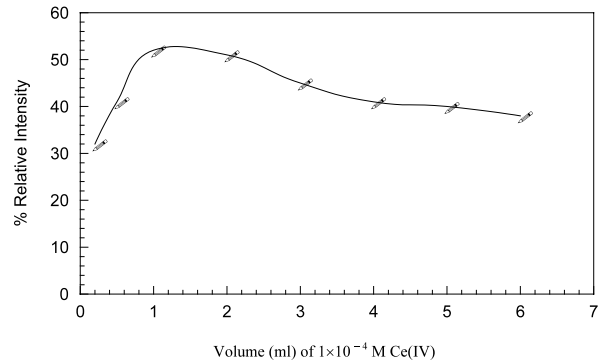

Fig. 2. Effect of volume of 1×10^{-4} M cerium(IV) on the oxidation of isoxsuprine HCl (0.1 μg ml^{-1}).

3.3. Determination of isoxsuprine hydrochloride

Under the described experimental conditions, standard calibration curves for isoxsuprine HCl by the two methods were constructed. The fluorescence intensity was linearly related to the drug concentration over the range 0.4–4.0 μg ml^{-1} by the direct method with a limit of detection (LOD) of 0.11 μg ml^{-1} and a limit of quantitation (LOQ) of 0.37 μg ml^{-1}. Linear regression analysis of the results gave the following equation:

$$\%\text{R.I.} = 5.1886 + 23.22C \quad (r = 0.9996, n = 8)$$

Also the fluorescence intensity was linearly related to the drug concentration over the range 0.02–0.2 μg ml^{-1} by the oxidation method according to the equation:

$$\%\text{R.I.} = 9.5436 + 423.11C \quad (r = 0.9994, n = 9)$$

The LOD and LOQ were 0.007 and 0.02 μg ml^{-1}, respectively. The standard deviations (S.D.s) of slopes for the direct and oxidation methods were 0.2660 and 5.625, respectively. The

S.D. of intercepts were 0.7187 and 0.6539, respectively. The repeatability of the two proposed methods were checked with 10 samples of 2.0 μg ml^{-1} by the direct method and 0.1 μg ml^{-1} of the drug for the oxidation method. The relative standard deviations (R.S.D.) were 0.53 and 1.29% for the two methods, respectively.

The precision of the two methods was evaluated by analyzing standard solutions of isoxsuprine HCl. The results in Table 1 were in accord with those obtained by the official method [2].

3.4. Analysis of tablets

The proposed methods were successfully applied to the analysis of tablets containing isoxsuprine HCl. The results in Table 1 agreed with those obtained by the official method [2].

Statistical analysis [30] of these results using Student's *t*-test and the variance ratio *F*-test showed no significant difference between the performances of the methods as regards to accuracy and precision.

3.5. Analysis of spiked urine and plasma samples

The high sensitivity attained by the proposed methods allows the determination of isoxsuprine hydrochloride in biological fluids. The extraction process was carried out using ethyl acetate at pH 9.48 [31]. Table 2 shows the results of the recovery studies of isoxsuprine hydrochloride from spiked urine and plasma.

3.6. Comparison of the proposed methods with other reported methods

Comparing the proposed methods with other reported methods shows them all to be equally accurate and precise. The proposed methods are more sensitive and rapid than most of the reported methods [4–8,10–16,18,19,22]. Also, the proposed methods are characterized by simplicity and time efficiency. On the other hand the reported chromatographic methods [21–23] are sensitive but these methods require considerable skill to obtain reliable results.

Table 1
Fluorimetric determination of isoxsuprine hydrochloride and its tablets

Drug form	Found (%)		
	Direct method	Oxidation method	Official method [2]
Isoxsuprine hydrochloride (pure sample)	99.2	100.3	
	101.0	100.4	
	100.7	100.4	
	99.8	99.0	
	99.0	99.6	
Mean ± S.D.	99.9 ± 0.78	100.0 ± 0.62	100.2 ± 0.31
Student's *t*-test[c]	0.21 (2.447)	0.51 (2.447)	
Variance ratio *F*-test[d]	6.33 (19.20)	4.00 (19.20)	
Duvadilan tablets (20 mg per tablet)[a]	100.8	100.3	
	100.0	101.4	
	99.0	101.8	
	100.0	100.2	
	100.5	101.4	
Mean ± S.D.	100.1 ± 0.68	101.0 ± 0.72	100.3 ± 0.42[b]
Student's *t*-test	0.45 (2.447)	1.51 (2.447)	
Variance ratio *F*-test	2.60 (19.20)	2.90 (19.20)	

[a] Solvay Duphar B.V., Weesp, Holland.
[b] Analyzed by difference spectrophotometric method described for pure form ($n = 3$) [2].
[c] Tabulated *t*-values at ($P = 0.05$) [30].
[d] Tabulated *F*-values at ($P = 0.05$) [30].

Table 2
Fluorimetric determination of isoxsuprine hydrochloride in spiked urine and plasma

Concentration taken (μg ml^{-1})	Found (%)		Concentration taken (μg ml^{-1})	Found (%)	
	Native method			Oxidation method	
	Urine	Plasma		Urine	Plasma
0.8	99.3	98.9	0.04	102.3	100.1
1.6	98.1	98.5	0.08	98.9	100.8
2.4	98.6	98.9	0.12	100.8	100.0
3.2	101.0	97.6	0.16	100.2	99.4
Mean ± S.D.	100.0 ± 0.94	98.5 ± 0.61	Mean ± S.D.	100.2 ± 0.89	100.1 ± 0.82

4. Conclusion

The proposed methods are accurate, simple, rapid, sensitive and suitable for quality-control analysis of pharmaceuticals formulations containing the drug. In addition, they can be considered as stability indicating since isoxsuprine like other phenolic compounds is prone to oxidation of phenolic group to give the corresponding quinone.

The direct fluorimetric procedure is more selective, while the oxidation fluorimetric method is more sensitive. Both methods are described for determination of isoxsuprine hydrochloride in dosage forms and biological fluids after extraction of both plasma and urine to avoid possible interferences.

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