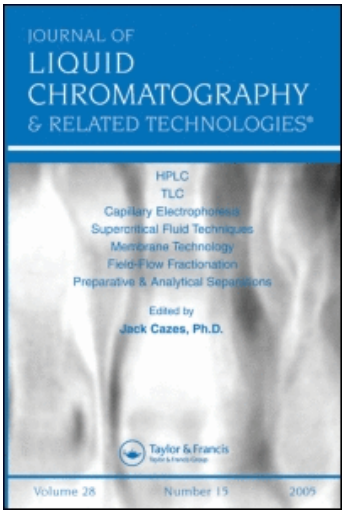

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A STABILITY-INDICATING HPLC METHOD FOR THE DETERMINATION OF ISOXSUPRINE IN DOSAGE FORMS. APPLICATION TO KINETIC STUDIES OF ISOXSUPRINE

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ABSTRACT

A simple, rapid, and stability-indicating HPLC method has been developed for the determination of isoxsuprine in the presence of its degradation products. Acetonitrile: potassium dihydrogen phosphate (0.01 M solution) adjusted to pH 2.2 ± 0.1 with phosphoric acid (18:82 v/v), was used as the mobile phase, at a flow rate of 2.5 mL/min.

A μ Bondapak™ C_{18} 10 μm column (3.9 mm i.d. × 150 mm) was utilized as a stationary phase. Detection was affected spectrophotometrically at 275 nm. Methyl p-hydroxybenzoate was used as an internal standard. The method was applied for the determination of isoxsuprine in the presence of its main degradation products, namely: 4-hydroxy-benzaldehyde, 4-hydroxybenzyl alcohol, 4-hydroxybenzoic acid, and 4-hydroxyacetophenone. Linearity range for isoxsuprine was 2-40 μg/mL with correlation coefficient (r) of 0.9998 and minimum detectability was 0.2 μg/mL (~ 6×10^{-7}M).

The proposed method was further applied to the analysis of commercial tablets containing the drug, the percentage recoveries

± SD were 99.60 ± 0.76 and 99.78 ± 0.88, and these values were in agreement with those given with the official methods. The method was also adopted to detect isoxsuprine in spiked human plasma at its therapeutic level of concentration (0.4 μg/mL). The proposed HPLC method was successfully applied to study the degradation kinetics of isoxsuprine. The photothermal degradation of isoxsuprine upon exposure to UV light was first-order with regard to its concentration.

INTRODUCTION

Isoxsuprine, 1-(4-hydroxyphenyl)-2-(1-methyl-2-phenoxy-ethylamino) propan-1-ol, 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]

Several analytical methods have been reported for the determination of isoxsuprine 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 for that drug include spectrophotometry[3,4] and HPLC.[5]

Reviewing the literature revealed that, till present, no reports on the use of HPLC for the determination of isoxsuprine in the presence of its degradation products are available. The aim of this work is to develop and validate a stability-indicating HPLC assay procedure for the quality control of isoxsuprine in pharmaceutical preparations, and its detection in spiked human plasma. The method may also be adopted to study the kinetics of degradation of isoxsuprine upon exposure to UV light.

EXPERIMENTAL

Materials and Reagents

Isoxsuprine HCl (Batch No. 191401) was purchased from Sigma, Saint Louis, MO, USA. Tablets containing the drug: Duvadilan® tablets, labeled to contain 20 mg each (Batch No. 544066) and Vascular® tablets, labeled to contain 20 mg each (Batch No. 1295127) were obtained from commercial sources.

Acetonitrile (Hipersolv®) HPLC grade, (BDH, Pool, England); Potassium dihydrogen phosphate (Riedel-de Häen, Germany); Phosphoric acid, 85% (E. Merck, Darmstadt, Germany); Methyl p-hydroxybenzoate (Sigma, Saint Louis, MO, USA); 4-Hydroxybenzaldehyde (Aldrich, Gillingham, U.K.); 4-Hy-

droxybenzyl alcohol (Aldrich, Gillingham, U.K.); 4-Hydroxybenzoic acid (Aldrich, Gillingham, U.K.); 4-Hydroxyacetophenone (Aldrich, Gillingham, U.K.).

Standard Solutions

The following stock solutions were prepared and diluted with the mobile phase to give the proper concentrations: Isoxsuprine HCl stock solutions containing 0.10 mg/mL and 1.0 mg/mL; 4-Hydroxybenzoic acid stock solution containing 0.10 mg/mL; 4-Hydroxybenzyl alcohol stock solution containing 0.10 mg/mL; 4-Hydroxybenzaldehyde stock solution containing 0.10 mg/mL; 4-Hydroxyacetophenone stock solution containing 0.10 mg/mL; Methyl p-hydroxybenzoate stock solution containing 8.0 μg/mL (Internal standard).

Apparatus

Waters liquid chromatograph 600 E, equipped with Waters-U6K injector, Waters-486 tunable absorbance detector and Waters-746 data module, was used. The column used was stainless steel, 3.9 mm i.d. × 150 mm, μ Bondapak™ C_{18} (Waters).

UV lamp model UV GL-25 Mineralight® lamp multiband UV-254/366 nm, 215-250 Volts, 50/60 Hz, 0.12 Amps®, Son Gabriel, USA was used. The lamp was fixed to a wooden cabinet in a horizontal position.

Chromatographic Conditions

Chromatographic analysis was carried out at ambient temperature. The compounds were separated isocratically with a mobile phase consisting of acetonitrile : potassium dihydrogen phosphate (0.01 M solution) with the pH adjusted to 2.2 ± 0.1 with phosphoric aicd (18:82). The flow rate was 2.5 mL/min.

The chart speed was 0.5 cm/min and the effluent was monitored spectrophotometrically at a wavelength of 275 nm with an attenuation of 4. The mobile phase was filtered by passing through a 0.22 μm membrane filter (Millipore, Bradford, MA, USA). The mobile phase was degassed by pumping pure helium gas into the solvent reservoir at a rate of 20 mL/min.

Calibration Curve

Transfer aliquots of the stock solution into separate 10 mL volumetric flasks; to each flask add 2.0 mL of the internal standard solution and complete

to the mark with the mobile phase. Make triplicate 20 μL injections for each solution and plot the peak area ratio to the internal standard against the corresponding concentrations to obtain the calibration graph. Alternatively, derive the corresponding regression equation.

The ruggedness and precision of the proposed method were checked at different days; within day (n = 4) and between days (n = 16) for three different concentrations of the drug at low, medium, and high levels of the standard curve. The relative standard deviations were calculated for each level of concentration.

Application of the Proposed Method to Tablets Containing the Drug

Weigh and finely powder ten tablets, then transfer an accurately weighed amount of the powder equivalent to 20.0 mg of the drug into a 100 mL volumetric flask. Add 50 mL of the mobile phase and heat on a boiling water bath for 30 min. Cool, dilute to the mark with the mobile phase, then filter. Transfer 1.0 mL of the filtrate into a 10 mL volumetric flask. Complete as under "Calibration Curve". Determine the nominal content of the tablets either from the calibration graph or using the corresponding regression equation.

Recovery Studies

To study the accuracy of the proposed method, and to check the interference from excipients used in the formulations, recovery experiments were carried out by the standard addition method.

2.0 mL of isoxsuprine HCl standard solution (0.1 mg/mL) and 2.0 mL of the internal standard solution (8.0 μg/mL), were transferred into a 10 mL volumetric flask (flask No. 1) to give a solution containing 20.0 μg/mL of isoxsuprine and 1.6 μg/mL of internal standard. The volume was completed to the mark with the mobile phase.

For isoxsuprine content: a stock sample solution containing isoxsuprine HCl (0.1 mg/mL) was prepared. One mL of this solution was transferred into a 10 mL volumetric flask; 2.0 mL of isoxsuprine standard solution (0.1 mg/mL) were also added, followed by 2.0 mL of internal standard solution (8.0 μg/mL). The volume was completed to the mark with the mobile phase (flask No. 2).

One mL of the stock sample solution was transferred into a 10 mL volumetric flask, followed by 2.0 mL of internal standard solution and the volume was completed to the mark with the mobile phase (flask No. 3). Triplicate injec-

tions were made for each solution (flasks No. 1, 2, and 3) and the added recoveries were calculated as follows:

$$\% \text{ Recovery} = P_{(d)} - P_{(sp)} / P_{(std)} \times 100$$

where, $P_{(d)}$ = Peak ratio for added solution.

$P_{(sp)}$ = Peak ratio for sample solution.

$P_{(std)}$ = Peak ratio for standard solution.

Alkaline Hydrolysis

0.2 g of isoxsuprine HCl was dissolved in 100 mL of 2 M NaOH solution and heated on a water-bath at 80°C. After different time intervals, (0, 30, 60, 90, 120, 150, 180, 210, and 240 min), 1.0 mL aliquots of the solution were transferred into 25 mL volumetric flasks, then neutralized with 1.0 mL of 2 M HCl; the volume was completed to the mark with the mobile phase.

From this solution, 2.0 mL were transferred into 10.0 mL volumetric flasks and 2.0 mL of internal standard solution were added, triplicate 20 µL injections were made for each sample. The isoxsuprine content was obtained either from the calibration graph or using the regression equation.

Photothermal Degradation Studies

The effect of UV radiation on the degradation of isoxsuprine was studied by exposing a solution of isoxsuprine HCl containing 1.0 mg/mL in a 1 cm quartz cell to strong UV radiation (heat was also emitted from this source) for different time intervals, (0, 30, 60, 90, 120, 150, 240, 510, and 570 min). The solution was placed at a distance of 3 cm apart from the UV light source.

After each time interval, 200 µL of this solution was transferred into separate 10 mL volumetric flasks and 2 mL of internal standard solution were added to each; triplicate 20 µL injections were made for each sample.

Detection of Isoxsuprine in Spiked Human Plasma

To 1 mL of the spiked plasma, add 3 mL of acetonitrile, mix well, then centrifuge for 10 minutes. Transfer 2 mL of the supernatant to a 10 mL volumetric flask and complete to the mark with the mobile phase. Inject 20 µL of the final solution under the above described chromatographic conditions.

RESULTS AND DISCUSSION

Chromatographic Conditions

The chromatogram of isoxsuprine solution and its major degradation products namely: 4-hydroxybenzaldehyde, p-hydroxyacetophenone, 4-hydroxybenzyl alcohol, and p-hydroxybenzoic acid,[6] is shown in Figure 1. Different chromatographic conditions (organic modifier, flow-rate, ionic-strength, pH, and type of column) have been investigated in order to optimize the separate elution of these compounds.

Mobile phases containing only acetonitrile or methanol were first tried; sodium acetate or potassium dihydrogen phosphate solutions were also tried with different concentrations of either acetonitrile or methanol as organic modifiers. The optimum conditions were found upon using a mixture of potassium dihydrogen phosphate 0.01 M solution and acetonitrile (82:18 v/v). The pH of

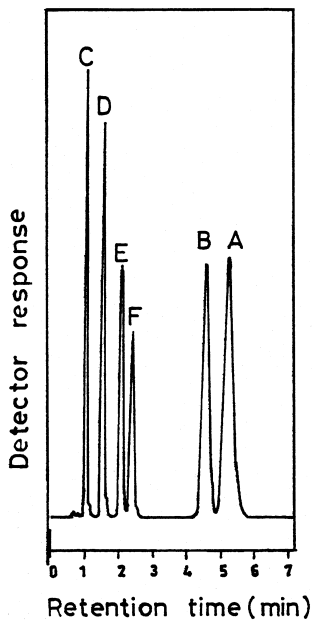

Figure 1. Typical chromatogram of isoxsuprine HCl and its degradation products under the described chromatographic conditions: A: isoxsuprine HCl., B: methyl-p-hydroxybenzoate (internal standard). C: 4-hydroxybenzoic acid. D: 4-hydroxybenzyl acid. E: 4-hydroxybenzaldehyde. F: 4-hydroxyacetophenone.

potassium dihydrogen phosphate solution was adjusted to 2.2 ± 0.1 with phosphoric acid.

Under these conditions, the eluted peaks were well defined, resolved, and free from tailing. The tailing factors were less than 1.5 for all peaks. The elution order was 4-hydroxybenzyl alcohol (t_R = 1.09 min), 4-hydroxybenzoic acid (t_R = 1.61 min), 4-hydroxybenzaldehyde (t_R = 2.19 min), 4-hydroxyacetophenone (t_R = 2.5 min), methyl p-hydroxybenzoate internal standard (t_R = 4.8 min) and isoxsuprine (t_R = 5.48 min) at a flow rate of 2.5 mL/min as shown in (Figure 1).

Several drugs were tested for selection of a suitable internal standard, however, methyl p-hydroxybenzoate was found to be a suitable internal standard for this study.

Calibration Curves

The calibration curve of isoxsuprine was found to be linear over the range of 2-40 μg/mL. Linear regression analysis of the data gave the following equation:

$$P = 0.003 + 0.086\ C \quad (r = 0.9998)$$

where C is the concentration in μg/mL and P is the peak ratio. The detection limit (S/N = 2) is 0.2 μg/mL ($\approx 6 \times 10^{-7}$ M).

Precision

Within-day and between-day variation was determined by calculating the relative standard deviation (RSD.) of the results of percentage recoveries obtained in the same day at three levels of concentration, low (2.0 μg/mL), medium (16.0 μg/mL), and high (32 μg/mL).

The results are shown in Table 1. The ruggedness and precision of the method was also evaluated by calculating the RSD of the results of percentage recovery over a period of 16 days, for three different levels of concentration.

The results are abridged in Table 2. The precision of the method is reflected by the excellent values of RSDs % as shown in Tables 1 and 2.

Stability-Indication of the Method

To prove that the method is stability-indicating, the drug was assayed in presence of its degradation products obtained either under stress conditions e.g.

Table 1

Within-Day Reproducibility and Precision of the Proposed Method as Evaluated by Peak Ratio

	Low Conc. (2 μg/mL)		Medium Conc. (16 μg/mL)		High Conc. (32 μg/mL)	
Run No.	Peak Ratio	% Recovery	Peak Ratio	% Recovery	Peak Ratio	% Recovery
1	0.180	100.00	1.39	99.29	2.750	99.638
2	0.182	101.11	1.41	100.71	2.761	100.036
3	0.179	99.44	1.42	101.43	2.753	99.746
4	0.181	100.56	1.38	98.57	2.758	99.928
Mean	0.181	100.28	1.40	100.00	2.756	99.837
S.D.	0.0013	0.72	0.018	1.30	0.005	0.179
%RSD	0.713	0.72	1.29	1.30	0.181	0.179

after alkaline hydrolysis or upon exposure to UV light at 254 nm or by injecting the drug with its degradation products.

Alkaline Hydrolysis

The chromatograms obtained from the alkaline hydrolysate of isoxsuprine revealed that there is no significant change in the peak ratios of the drug and internal standard. Hence no measurable degradation was detected upon heating the drug in 2 M NaOH solution for up to 4 hours at 80°C. The drug seems to be stable under these conditions.

Photothermal Decomposition

The chromatograms obtained from the photothermal degradate revealed that the peak ratio of isoxsuprine to the internal standard was reduced proportionally with time. Figure 2 shows the semi logarithmic plots of concentration of isoxsuprine (log A) *versus* time (t) for a degraded sample of isoxsuprine. It

Table 2

Between Days Ruggedness and Precision of the Proposed Method as Evaluated by Peak Ratio using Three Different Concentrations*

	Peak Ratio		
Day	**2 μg/mL**	**16 μg/mL**	**32 μg/mL**
Initial	0.180	1.40	2.760
After 2 days	0.170	1.41	2.750
After 4 days	0.175	1.41	2.754
After 6 days	0.172	1.39	2.761
After 8 days	0.173	1.38	2.758
After 10 days	0.181	1.41	2.740
After 12 days	0.169	1.43	2.750
After 14 days	0.180	1.39	2.759
After 16 days	0.173	1.40	2.770
Mean	0.175	1.40	2.756
S.D.	0.005	0.015	0.009
%RSD	2.587	1.056	0.309

*Each result is the average of three separate determinations.

indicates a first-order degradation behavior with a rate constant (k) of 0.001382 min^{-1} and a correlation coefficient of 0.995.

The first order disappearance rate constant was calculated using the following formula:[7]

$$\log [A] = \log [A]_o - \frac{Kt}{2.303}$$

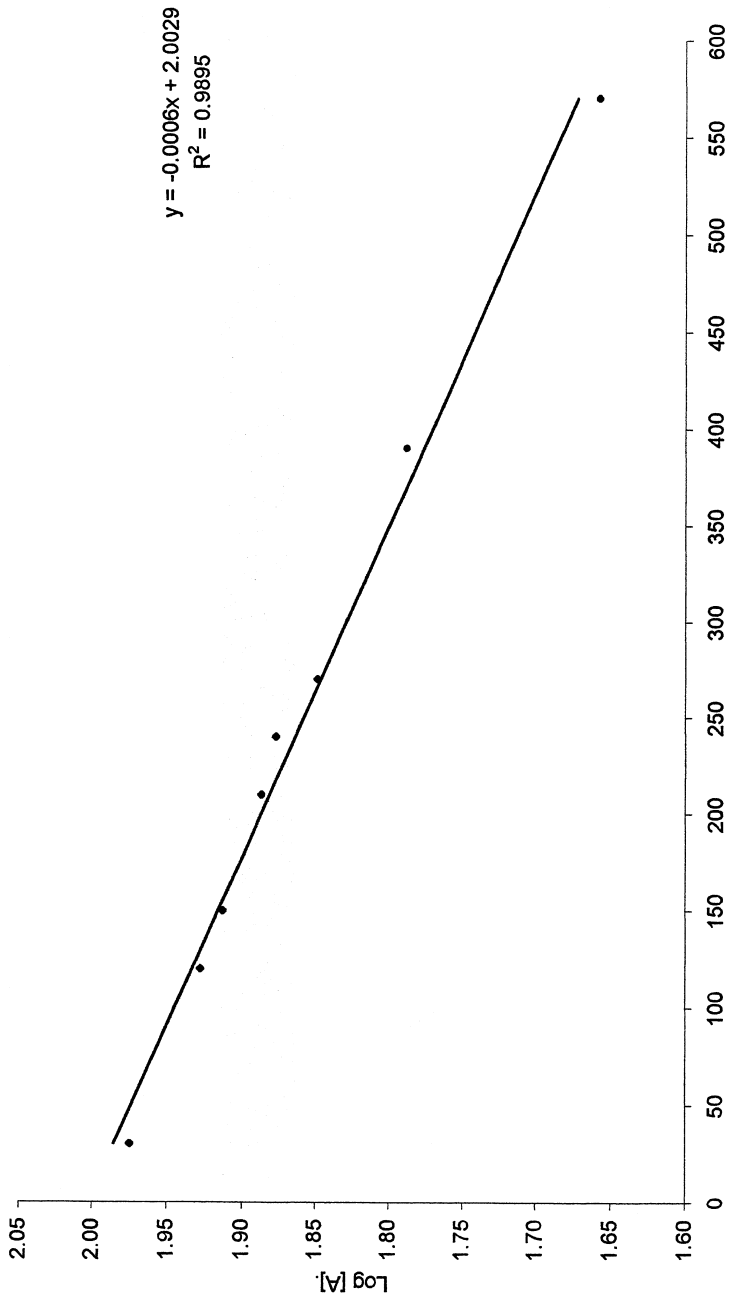

Figure 2. Semilogarithmic plot of isoxsuprine HCl (0.1 μg/mL) versus time of exposure to UV light.

where $[A]_o$ is the initial concentration of isoxsuprine at t = 0 and $[A]_t$ is its concentration at time t.

The half-life time ($t_{1/2}$) was calculated according to the following formula:[7]

$$t_{1/2} = \frac{0.693}{K}$$

and it was found to be about 500 min.

Figure 3 shows the chromatograms of isoxsuprine after zero, 120, and 570 min exposure time to UV radiation. It illustrates the reduction of the peak height of isoxsuprine and the development of the new peaks of its degradation without any interference. It also points out that the main degradation product is that which is eluted at a retention time of 2.19 min, and with measurable peaks eluted at retention times of 0.8, 1.09, 1.61, 2.5, 3, and 3.5 min.

By comparing the chromatogram obtained by injecting isoxsuprine with its degradation products postulated by Volpe et al,[6] with that obtained by exposing isoxsuprine to UV light, the compound eluted at 2.19 min, most probably is 4-hydroxybenzaldehyde, and those eluted at 1.09, 1.61, and 2.5 min are 4-hydroxybenzyl alcohol, p-hydroxybenzoic acid and p-hydroxyacetophenone, respectively. The other peaks eluted at 0.8, 3, and 3.5 min may be for other minor degradation products.

Application of the Proposed Method to the Analysis of Authentic Sample of Isoxsuprine HCl

After establishing the experimental conditions, the validity of the method was assessed by analyzing an authentic sample of isoxsuprine HCl powder. The results obtained were compared with those given adopting the British Pharmacopoeia 1993 method.[8] Statistical analysis of the results obtained by both methods using the student t-test and the variance-ratio, F-test, revealed no significant difference between the two methods regarding accuracy and precision, respectively.[9] The results are shown in Table 3.

Application of the Proposed Method to the Analysis of Isoxsuprine HCl in Its Dosage Forms

The proposed method could be successfully applied to the determination of isoxsuprine HCl in its tablets. The results obtained (Table 4) were in good agreement with those given using the official method[8] as revealed by statistical

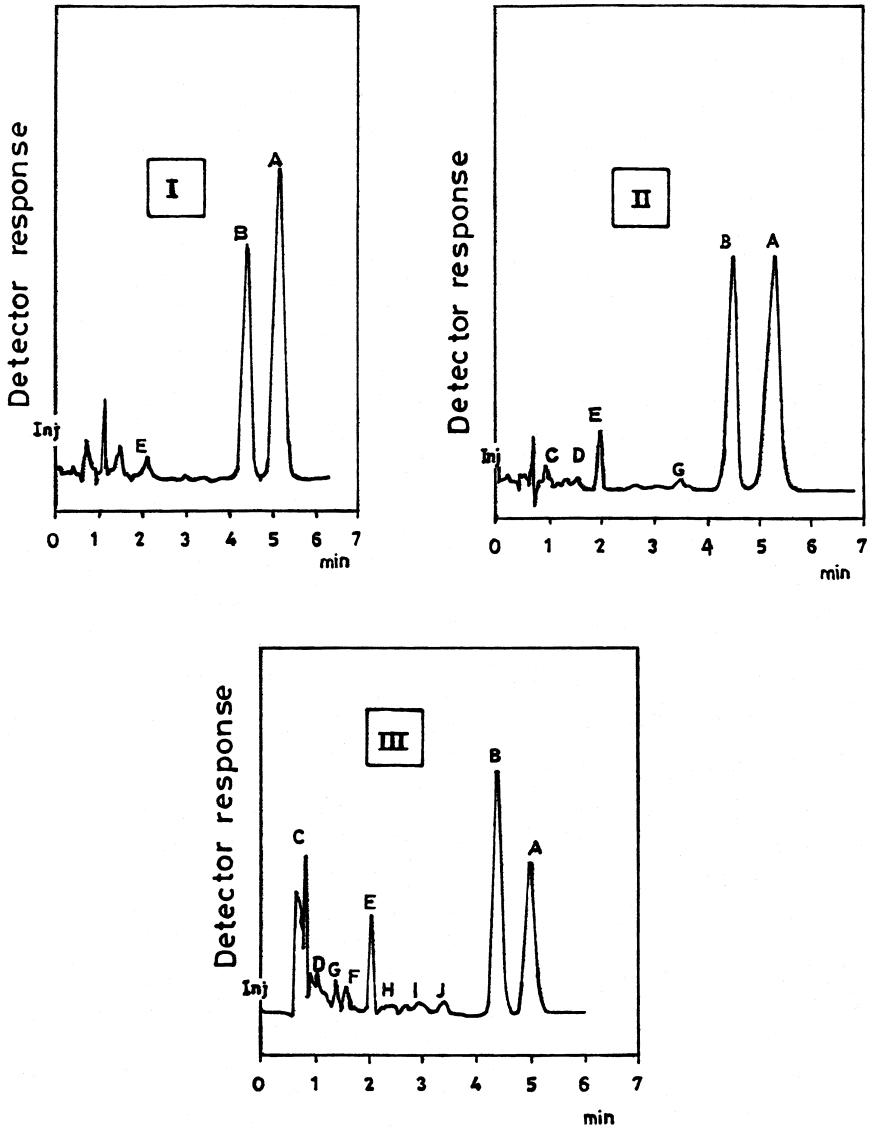

Figure 3. Chromatogram of the degradation products of isoxsuprine upon exposure to UV light obtained under the same chromatographic conditions of Figure 1. G, H, I and J are unidentified degradation products. I: zero exposure time. II: 120 min. exposure time. III: 570 min exposure time.

Table 3

Application of the Proposed Method to the Determination of Isoxsuprine in its Pure Form

	Proposed Method		Official Method[8]
Amount Taken (μg/mL)	Amount Found (μg/mL)	% Recovery	% Recovery
4.00	3.972	99.3	99.65
6.00	6.03	100.5	100.78
8.00	8.06	100.8	101.34
10.00	9.98	99.8	100.78
12.00	11.96	99.7	100.20
Mean		100.02	100.55
± S.D		0.614	0.645
t		1.33 (2.228)*	
F		1.10 (6.390)*	

* Tabulated values of t and F at 95% confidence limits.

analysis.[9] Common tablet excipients, such as talc, lactose, avisil, starch, magnesium stearate, and gelatin did not interfere with the assay.

Detection of Isoxsuprine in Spiked Human Plasma

Isoxsuprine is orally administered in a dosage of 20 mg three times daily. The anticipated plasma level concentration is around 0.4 μg/mL, that is higher than the minimum detection limit (0.2 μg/mL). The proposed method could be successfully applied to detect isoxsuprine in spiked human plasma, after precipitation of the protein using acetonitrile.

Table 4

Determination of Isoxsuprine in its Pharmaceutical Preparations by the Proposed Method.

		Proposed Method		Official Method[8]	
Preparation	Amount Taken (μg/mL)	Amount Found (μg/mL)	% Recovery	% Recovery	Added Recovery*
Duvadilan®	4	3.96	99.00	100.00	
Tablets	9	8.00	100.00	99.70	
(20 mg of	12	12.05	100.42	100.55	
isoxsuprine	16	15.80	98.75	99.70	
HCl/tablet)	20	20.15	100.75	99.70	
Mean ± S.D.			99.78 ± 0.88	99.89 ± 0.41	99.93 ± 0.41
t			0.254 (2.228)†		
F			4.565 (6.390)†		
Vascular®	4	3.95	98.75	100.2	
Tablets	8	8.02	100.25	99.8	
(20 mg of	12	11.94	99.50	99.3	
isoxsuprine	16	16.08	100.50	99.5	
HCl/tablet)	20	19.80	99.00	100.4	
Mean ± S.D.			99.60 ± 0.76	99.84 ± 0.46	99.55 ± 0.27
t			0.602 (2.228)†		
F			2.728 (6.390)†		

* Each result is the average of three separate determinations. † Tabulated values of t and F at 95% confidence limits.

CONCLUSION

A sensitive and simple method has been developed for the determination of isoxsuprine hydrochloride whether per se, or in formulations in the presence of its main degradation products. The method can measure concentrations down to 2 μg/mL with good accuracy. The minimum detectability is 0.2 μg/mL (~ 6 x 10^{-7} M) and, therefore, it can be applied for the detection of the drug in spiked human plasma.

The method is simple and rapid, and is, therefore, recommended for routine quality control of isoxsuprine in dosage forms.

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