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Utility of Redox Reaction for Spectrophotometric Determination of Propranolol and Isoxsuprine Hydrochlorides in Pure and Dosage Forms

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Two simple, accurate and sensitive spectrophotometric procedures for the assay of propranolol hydrochloride (PPH) and isoxsuprine hydrochloride (ISH) in pure and in dosage forms have been proposed. The methods are based on the oxidation of the drug by a known excess of standard N-bromosuccinimide (NBS) (**method A**) or by cerium (IV) sulfate (**method B**), in an acidic medium followed by the reaction of excess oxidant with amaranth dye (A17) or rhodamin 6G (Rh6G), respectively. The absorbance values described linearly with increasing drug concentration regression analysis of Beer's plots showed good correlation in the concentration range of 0.2–6.4 μg mL^{-1}. The apparent molar absorptivity, Sandell sensitivity, detection and quantification limits were calculated. From more accurate analysis, Ringbom optimum concentration ranges were employed successfully in the range 0.4–6.0 μg mL^{-1}. Analyzing pure and dosage forms containing (PPH) and (ISH) tested the validity of the proposed procedures. The relative standard deviations were ≤ 1.70 with recoveries 98.75–101.0%.

Key Words: Redox reaction, Spectrophotometry, N-Bromosuccinimide, Cerium(IV) sulphate, Propranolol, Isoxsuprine HCl, Dosage forms.

INTRODUCTION

Propranolol hydrochloride (PPH), the prototype of a pure beta to adrenergic, blocking compound without intrinsic activity, represents an outstanding advance in the treatment of certain cardiovascular disorders and hypertension. It is one of the best drugs of choice for sustained action dosage forms, because its therapeutic index is very high[1]. Isoxsuprine hydrochloride (ISH) 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[2]. It is widely used in the treatment of premature labour and as a peripheral vasodilalor.

Both PPH and ISH are official in BP[3] and USP[4]. Expanding indications and a more widespread use of PPH and ISH require simple, sensitive and easily available analytical methods for their assay in pure and dosage forms. The

analytical techniques used to determine PPH and ISH, such as ^{1}H NMR spectrometry[5], high-performance liquid chromatography[6–8], voltammetry[9, 10] are expensive. Spectrophotometric techniques continue to be the most preferred method for routine analytical work because of its simplicity and reasonable sensitivity with significant economical advantages. Thus many spectrophotometric methods have been described for the determination of PPH[11–15] and ISH[16–20]. Most of these methods suffer from limitations for instance, these involve extraction[11, 19], heating[12, 17], long standing[13, 16] for colour development or have low sensitivty[11–20].

In the present work, two simple and sensitive spectrophotometric methods for the determination of PPH and ISH in bulk samples and pharmaceutical formulations are given. The methods are based on the oxidation of drugs by N-bromosuccinimide (NBS) or cerium(IV) in acidic medium followed by the reaction of unconsumed NBS or Ce(IV) with amaranth and rhodamine 6G, respectively, to yield uncoloured radical cations.

EXPERIMENTAL

A Hitachi UV-Vis spectrophotometer (Model U-2001) with 10 mm matched quartz cells was used for all absorbance measurements. All chemicals used were of either pharmaceutical or analytical reagent grade and high-purity distilled water was used throughout. Stock solutions 1×10^{-3} of pure PPH and ISH were prepared by dissolving the requisite amount in distilled water; the diluted solutions were prepared daily by accurate dilution with deionized water just before use. Solution of 0.02% NBS (Aldrich) was freshly prepared by dissolving an accurate weight in least amount of warm water in a 100 mL measuring flask, then diluted with water to the mark. A 5.0 M HCl solution was prepared and standardized as recommended previously[21] prior to use. A solution of cerium sulfate (May and Baker, 1×10^{-3} M) was prepared by dissolving an accurate weight of $Ce(SO_4)_2$ in least amount of warm 1.0 M H_2SO_4 in a 250 mL measuring flask, then diluted with the same acid to the mark.

Aqueous solutions of amaranth (E. Merk, 2.0×10^{-3} M) and rhodamine 6G (Rh6G) (BDH, 1×10^{-3} M) were prepared by dissolving an appropiate weight of dye in water and completed to the mark in a 100 mL measuring flask.

Procedure A: To each 10 mL measuring flask containing 2.0–6.4 μg mL^{-1} PPH or ISH solution, 0.5 mL of 5.0 M HCl, 1.0 mL of 0.02% NBS and 1.0 mL of 1.0% KBr were added and the solutions were diluted to 7.0 mL. After 3.0 min of mixing, 1.0 mL of 2×10^{-3} AM dye was added, mixed well and completed to the mark with water. The absorbance was measured at 521 nm against a reagent blank prepared in the same way without the drug. The concentrations of PPH or ISH were computed from the calibration curves.

Procedure B: Aliquot containing 2.0–52 μg of the examined drug was added to an excess volume (2.0 mL) of 1×10^{-3} M Ce(IV) containing 2.0 mL of 1.0 M H_2SO_4. The mixture was boiled in a water bath for 3.0 min, cooled and 0.5 mL of 1×10^{-3} M Rh6G was added to the hot solution. The volume was completed to 10 mL with water; a decrease in colour intensity of Rh6G was measured

spectophotometrically at λ_{max} 526nm. The concentration range was determined by plotting the concentration of PPH or ISH against absorbance at the corresponding λ_{max}.

Procedure for dosage forms: Twenty tablets of the selected drug were finely powdered. An accurately weighed amount of powder equivalent to 10 mg was transferred to a 100 mL measuring flask. Using a mechanical stirrer, the powder was completely disintegrated in distilled water. The solution was filtered through a Whatmann filter paper No. 42 and the filtrate was made up to 100 mL with distilled water. The above procedures (A and B) were then performed and the nominal content of the tablets was calculated either from a previously plotted calibration graph or using the regression equation.

RESULTS AND DISCUSSION

It is known that NBS oxidizes PPH[13], while Ce(IV) oxidizes ISH[20]. Preliminary investigations have also shown that amaranth (AM) and Rh6G undergo reversible one-electron oxidation by various oxidants in an acidic medium to yield uncoloured radical cations. These analytical aspects have been successfully utilized to develop a simple and sensitive spectorphotmeric procedure for the assay of PPH and ISH in pure and dosage forms. The procedures are based on the oxidation of PPH and ISH by NBS and Ce(IV) in acidic medium and subsequent measurement of unconsumed AM or RH6G respectively spectrophotometrically. Investigations were carried out to establish the most favourable conditions for the completion of the redox reaction. The influence of some variables on the reaction has been tested as follows:

Results of procedure A: It involves two steps of oxidation of PPH or ISH with an excess of freshly prepared NBS and estimation of the unreacted oxidant using known excess of AM dye. The excess of AM dye is then measured spectrophotometrically.

Effect of acid concentration: A series of mineral acids (sulfuric, nitric, phosphoric and hydrochloric acids) were examined at various concentrations. Moreover, 0.5 mL of 5.0 M HCl was chosen as the best volume, in a 10 mL measuring flask.

Effect of shaking time and temperature: The reaction takes place completely in the presence of KBr after 3.0 min of mixing. Raising the temperature does not affect the oxidation process and does not give reproducible results; thus the optimum temperature is the ambient (25 ± 1°C). The effect of time after addition of AM indicates that shaking for 1.0 min is sufficient to give reliable results.

Influence of KBr: 1.0 mL of 1.0% KBr, chosen as the best volume, is taken in 10 mL of total volume to accelerate the oxidation process.

Effect of sequence of addition: The optimum sequence is drug—acid—oxidant—KBr—AM. The sequences gave lower absorbance values under the same experimental conditions.

Effect of amaranth concentration: Investigation of the optimum AM concentration was done by taking different volumes (0.2–1.4 mL) of

2×10^{-3} M AM. The results revealed that the optimum volume used for the production of maximum and reproducible colour intensity is 0.8 mL (Fig. 1).

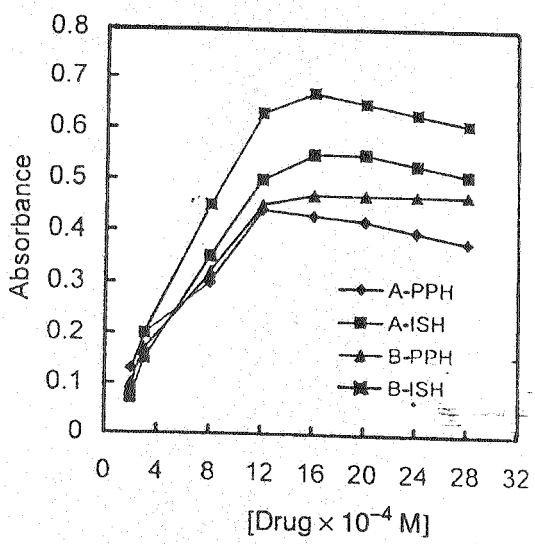

Fig. 1. Effect of [AM] and [Rh6G] on the absorbance of 3.0 μg mL^{-1} drug and 1.0 mL of 0.02% NBS

Results of procedure B: It involves two stages of oxidation of PPH and ISH with an excess of Ce(IV) in acidic medium under the effect of heating and determination of the unreacted oxidant by measurement of the decrease of Rh6G at λ_{max} = 526 nm.

Effect of acidity: Of the examined acids (H_2SO_4, HNO_3, HCl,and H_3PO_4), the most favourable acid to be used with Ce(IV) was found to be H_2SO_4 (1.0 M) if present as 1.0 mL in total volume of the reaction mixture (10 mL).

Effect of temperature and time: Sample solutions containing PPH or ISH, Ce(IV) and H_2SO_4 were heated at different temperatures ranging from 25–100°C. The time required to complex the redox reaction is 3.0 min. Addition of Rh6G to the hot solution gives maximum absorbance, so there is no need to cool the solution before addition of Rh6G.

Effect of Rh6G concentration: Different volumes of 1×10^{-3} M Rh6G solution were examined to select the optimum concentration (Fig. 1). The optimum volume used for the production of maximum and reproducible colour intensity is 0.5 mL of Rh6G.

Analytical features

The Beer's law limits, the molar absorptivity and the Sandell sensitivity values were evaluated. Regression analysis of Beer's law plots revealed a good correlation. Graphs of the absorbance *vs.* concentration showed a zero intercept and are described by the regression equation $Y = a + bX$ (where Y is the absorbance of a 10 mm layer, b is the slope, a is the intercept and X is the concentration of each of PPH or ISH in μg mL^{-1}) obtained by the least-squares method[22]. The results are summarized in Table-1. The low relative standard deviation values and the range of error at 95% confidence level for the analysis

of six replicates of PPH and ISH indicated good precision and accuracy of the proposed procedures. The detection and quantification limits were calculated from the standard deviation of the absorbance measurements obtained from a series of 13 blank solutions for each procedure.

TABLE-1
OPTICAL CHARACTERISTICS, PRECISION AND ACCURACY DATA

Parameters	NBS-AM		Ce(IV)-Rh6G	
	PPH	ISH	PPH	ISH
λ_{max} (nm)	521	521	525	527
Beer's law limit ($\mu g\ mL^{-1}$)	0.2–6.4	0.2–4.5	0.2–5.2	0.2–3.8
Ringbom range ($\mu g\ mL^{-1}$)	0.5–6.0	0.5–4.2	0.4–4.9	0.5–3.5
Detection limit ($ng\ mL^{-1}$)	58	50	60	55
Quantitation limit ($ng\ mL^{-1}$)	195	170	205	180
Molar absoptivity ($L\ mol^{-1}\ cm^{-1}$)	4.03×10^4	7.2×10^4	4.31×10^4	7.56×10^4
Sandell's sensitivity ($ng\ cm^{-2}$)	5.88	4.69	6.42	4.47
Regression equation, y[a]				
Slope, b	0.145	0.185	0.156	0.224
Intercept, c	–0.003	0.005	0.007	–0.006
Correlation coefficients, r	0.9992	0.9988	0.9996	0.9990
Relative standard deviation (%)[b]	1.34	1.70	1.52	1.63
% range of error[b] (95% confidence limit)	0.85	0.73	0.92	0.64

a: Y = a + bx, where x is the concentration in $\mu g\ mL^{-1}$.
b: For six replicate analyses within Beer's law limits.

The limits of detection (K = 3) and of quantification (k = 10) were established according to IUPAC definitions[23]. In order to determine the accuracy and precision of the procedures, solutions containing three different concentrations of PPH and ISH were prepared and analyzed in six replicates. The analytical results are summarized in Table-2 The percentage SD (≤ 1.45) and the percentage range of error at 95% confidence level (≤ ± 1.60) can be considered to be satisfactory.

Interference studies

In order to assess the possible analytical applications of the proposed procedures, the effects of excipients that often accompany PPH and ISH in various dosage forms were studied by adding different concentrations of each excipient to know the concentrations of PPH and ISH. It was observed that talc, glucose, dextrose, magnesium stearate, lactose, starch and gelatin did not interfere in the determination of PPH and ISH at the following levels (mg):

1. PPH (4.0 μg), talc (500), glucose (420), dextrose (550), magnesium stearate (400), lactose (350,) starch (275) and gelatin (300).
2. ISH (4.0 μg), talc (660), glucose (525), dextrose (475), magnesium stearate (450), lactose (400), starch (300) and gelatin (270).

TABLE-2
EVALUATION OF THE ACCURACY AND PRECISION OF THE PROPOSED PROCEDURE

Method	Drug	Taken ($\mu g\ mL^{-1}$)	Recovery (%)	RSD[a] (%)	RE (%)	Confidence limits[b]
A	PPH	2.0	98.5	1.38	1.45	1.97 ± 0.0210
		4.0	100.75	1.20	1.27	4.03 ± 0.0315
		6.0	99.17	0.94	1.00	5.95 ± 0.0085
	ISH	1.2	99.17	0.85	0.89	1.19 ± 0.0096
		2.4	101.25	0.67	0.70	2.43 ± 0.0106
		3.6	101.39	1.19	1.25	3.65 ± 0.0165
B	PPH	1.5	99.67	0.54	0.56	1.48 ± 0.0084
		3.0	99.00	0.80	0.84	2.97 ± 0.0049
		4.5	101.11	1.17	1.23	4.55 ± 00204
	ISH	1.0	99.00	0.75	0.78	0.99 ± 0.0092
		2.0	100.50	0.48	0.50	2.01 ± 0.0069
		3.0	99.33	0.68	0.72	2.98 ± 0.0088

a: RSD for six determinations.
b: 95% confidence limits and five degrees of freedom.

A suitable concentration of each mixure was analyzed using the procedure described for (dosage forms) the percentages of PPH and ISH found by the proposed procedures in the ranges 98.75–1008 and 99.2–101.0 receptivity, with RSD values less than 1.70 for six replicates (Table-3).

TABLE-3
DETERMINATION OF PPH AND ISH IN DOSAGE FORMS USING THE PROPOSED AND OFFICIAL METHODS

Dosage forms	Supplier	Nominal value	Recovery ± SD (%)[b]		
			Procedure A	Procedure B	Official
Indeval tablets	Kahiro	40 mg PPH	98.8 ± 0.83 t = 1.15 F = 2:68	99.5 ± 0.96 t = 0.87 F = 2.09	98.1 ± 1.56
Duvadilan Tablets	Solvay B.V., Weesp, Holland	20 mg ISH	100.7 ± 0.56 t = 0.69 F = 1.83	100.45 ± 0.67 t = 0.96 F = 2.22	98.8 ± 1.25

a: Average of six determinations ± SD%.
b: Theoretical values for t and F-values for five degrees of freedom and 95% confidence levels are 2.57 and 5.05, respectively[24].

The results of the analysis were compared statistically by the Student's *t*-test and by the variance ratio *F*-test with those obtained by the official method[3, 4]. The Student's *t*-values at 95% confidence level did not exceed the theoretical values, indicating that there was no significant difference between the proposed

and official procedures. It was also noticed that the variance ratio *F*-values calculated for p = 0.05 did not exceed the theoretical values, indicating that there was no significant difference between the precisions of the proposed procedures and official methods. The results are given in Table-3.

Conclusion

The proposed procedures are simple and sensitive compared to the reported methods[11, 20]. The utility of the proposed procedures for the determination of PPH and ISH in dosage forms has been well demonstrated. The assay procedures did not involve any stringent experimental conditions and were also free from interference by common excipients. Hence, the proposed procedures could be used for routine quality control.

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