Roopa K.Papanna *et al*. / *International Journal of Biological & Pharmaceutical Research.* 2015; 6(1): 56-62.

e- ISSN 0976 - 3651
Print ISSN 2229 - 7480

International Journal of Biological & Pharmaceutical Research
Journal homepage: www.ijbpr.com

IJBPR

SENSITIVE AND VALIDATED SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF PHENYLEPHRINE HYDROCHLORIDE AND PYRIDOXINE HYDROCHLORIDE IN PHARMACEUTICALS USING Ce (IV) AMMONIUM SULPHATE AND N-BROMOSUCCINIMIDE BASED ON REDOX REACTION

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ABSTRACT

A simple, low-cost, sensitive and diversely applicable UV-Visible spectrophotometric methods have been developed for the estimation of drugs i.e, Phenylephrine Hydrochloride (PEH) and Pyridoxine Hydrochloride (PRC). Method A is based on the oxidation of drugs by cerium (IV) (excess) in slightly acidic medium at room temperature and estimating the amount of unreacted Ce (IV) by amaranth dye at 520 nm. Method B is based on the oxidation of drugs by N-bromo succinimide (excess) at room temperature and estimating the amount of unreacted NBS by amaranth dye at 520 nm. Beer's law is obeyed in the concentration range of (0.5 – 4.5 µg/mL) and (1.5 – 7.0 µg/mL) for PEH and PRC [Method A] and (1.0 – 5.0 µg/mL) and (1.5 – 5.0 µg/mL) for PEH and PRC [Method B]. The limit of detection (LOD) and limit of quantification (LOQ) were reported for both the methods. Both the methods were applied for the determination of PEH and PRC in dosage forms and the results were satisfactory and were comparable with those obtained by the reference methods. The accuracy and reliability of the proposed methods were further ascertained by recovery studies.

Key Words: Phenylephrine Hydrochloride, Pyridoxine Hydrochloride, Amaranth, Spectrophotometric determination, Pharmaceutical formulations, Drug analysis.

INTRODUCTION

Phenylephrine Hydrochloride (PEH) **(**Figure 1), is a white crystalline powder, and belongs to the group of medicines called sympathomimetrics. It acts stimulating the alpha receptors in certain areas of the body. It is used locally, as decongestant, for non - specific and allergic conjunctivitis, sinusitis and nasopharyngitis. PEH syrup is also used in the treatment of congestion, cough, sneezing and watery eyes. Various analytical methods have been reported in the literature for analysis of Phenylephrine Hydrochloride. The methods include colorimetry (Hiskey C F *et al*., 1996), RP-HPLC (Mahesh P *et al*., 2013), High performance liquid chromatography (Senyuva H *et al*., 2002), Sequential injection spectrophotometry(Beyne N W *et al*., 2004), anodic stripping voltametry (Mohammad Bagher Gholivand *et al*., 2013) and Spectrophotometry (Theia'a Al-Sabha N, 2010; Shama S A, 2002; Amer MM *et al*., 1982; Ibrahim AS *et al*., 2007).

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Pyridoxine Hydrochloride (PRC), (5-hydroxy-6-methylpyridine-3,4-dimethyl)dimethanol hydrochloride (VB_6) **(**Figure 2) is a water soluble vitamin and is involved primarily in the metabolism of amino acid, carbohydrate and fat. It is also used in the treatment of sideroblastic anemia's and depression. Many techniques have been employed for the determination of Pyridoxine Hydrochloride and other water soluble vitamins. Most of these methods include Differential derivative spectrophotometry(Nevik Erk, 2001),HPLC(Pengfei Jin *et al*., 2012), HPTLC (Agreka A P *et al*., 1999), Chemiluminiscent(Abdulrahman A *et al*., 1998), Voltametry (Solange M *et al*., 2009) and spectrophotometry (Smitha Nayak V *et al*., 2013; Pons L M *et al*., 1999; Sayeed Arayne M *et al*., 2007). Nagaraja P *et al*., 2012 used the reagents cerium (IV) and amaranth dye for the assay of few antioxidants in the application of food and medicinal plants.

The present paper reports a rapid, simple and sensitive method for the colorimetric estimation by amaranth dye. The reaction is highly sensitive and more simple than most of the spectrophotometric methods reported in literature and the methods avoids heating or extraction. The structure of studied drugs is as shown in (Fig 1 and 2).

EXPERIMENTAL

Apparatus

A BL 198 Bio spectrophotometer (UV – VIS) with 1.0 cm matched quartz cuvettes cells was used for all absorbance measurements.

Reagents and chemicals

Ce(IV) ammonium sulphate (CAS) (S.D. Fine-Chem Ltd., Biosar, India), Sulfuric acid (Ranbaxy Fine Chemicals, India) , N-Bromosuccinimide (NBS) (Merck, Germany) and analytical grade amaranth dye (S.D. Fine-Chem Pvt. Ltd, Mumbai, India) were used.

Phenylephrine hydrochloride (Amrut Drug Research lab Pvt. Ltd, Surat company) and Pyridoxine hydrochloride(Mylon, Brazil) were purchased and used as received. Analytical reagent grade chemicals and double distilled water were used throughout the experiment.

Standardization of Ce (IV) solution (Method A)

Ce(IV) ammonium sulphate solution was prepared by dissolving 0.08 g of Ce(IV) ammonium sulphate in 0.2 mL concentrated H_2SO_4, which was then diluted to 25 mL; the solution was made homogeneous with a magnetic stirrer at room temperature until total dissolution, then the solution was transferred into 100 mL standard flask and diluted to the mark with distilled water. The Ce(IV) stock solution was standardized with arsenic (III) oxide by using N-Phenyl anthranilic acid indicator(Jeffer G H *et al*., 1978).

Standardization of N-Bromosuccinimide

NBS was prepared by dissolving 0.02 g of chemical in water with the aid of heat and diluting to 100 mL water and standardized (Berka A *et al*., 1965). The NBS solution was kept in a refrigerator when not in use.

Preparation of Amaranth dye **(0.1%)**

The dye was prepared by dissolving 0.1 g of dye in 100 mL water.

Procedure for pharmaceutical formulations

For the analysis of an injection, the requisite amount was transferred to a 100 mL volumetric flask and diluted with distilled water. The drug content in the diluted solution was determined as described under the general procedure.

For the analysis of a tablet, twenty tablets was weighed, powdered and mixed thoroughly. A quantity equivalent to 10 mg of each drug was transferred to 100 mL volumetric flask, dissolved in water, shaken well, sonicated and made up to the volume with water. The resultant solution was filtered and analyzed as described under general procedure.

General procedure

Accurately measured quantity of each drug containing 100 µg/mL were prepared by dissolving 10 mg of the respective drugs in 100 mL water. The solutions were further quantitatively diluted stepwise to get working concentrations of 10 µg/mL PEH and PRC for method A and method B respectively.

Method A

Different aliquots of standard 10 µg/mL PEH and PRC were transferred from stock solution to 10 mL volumetric flasks, Which could be diluted quantitatively to obtain (0.5 – 4.5 µg/mL) and (1.5 – 7.0 µg/mL) respectively. To each flask containing drugs in the order mentioned above 1.0 and 1.0 mL of Ce (IV) ammonium sulphate (0.08%). After 5 mins, 0.3 and 0.3 mL of Amaranth dye (0.1%) were added. The pink color hence developed was further diluted to the volume with water and absorbance of each solution was measured at 520 nm against corresponding reagent blank.

Method B

Various aliquots of standard 10 µg/mL PEH and PRC were transferred from stock solution to 10 mL volumetric flasks, Which could be diluted quantitatively to obtain (1.0 – 5.0 µg/mL) and (1.5 – 5.0 µg/mL) respectively. To each flask containing drugs in the order mentioned above 1.0 and 1.0 mL of N-Bromosuccinimide (0.02 %). After 10 mins, fixed volume of 0.3 and 0.3 mL of Amaranth dye (0.1%) were added. The pink color hence developed was further diluted to the volume with water

and absorbance of each solution was measured at 520 nm against corresponding reagent blank.

RESULTS AND DISCUSSION

Spectral Characteristic

The absorption spectra of the reaction product with drugs show maximum absorption (λ_{max}) at 520 and 520 nm for Phenylephrine hydrochloride and Pyridoxine hydrochloride (method A and B)respectively. The blank solution was colorless that had negligible absorbance at the λ_{max} in which the drugs were analyzed. The thus formed color was stable for more than 24 hours. The absorption spectra of PEH and PRC for Method A and Method B is as shown in the (Fig 3) and (Fig 4).

Reaction Sequence

The developed spectrophotometric methods were based on the redox reaction between the drug, dye and Ce(IV) in acidic medium (Method A) , For (Method B) drug, dye and NBS at room temperature respectively. In both the methods , Ce(IV) and NBS acts as oxidizing agents. In the Method A , drugs were reacted with excess of Ce(IV) in acidic medium and the unreacted oxidant Ce(IV) was determined by reacting with amaranth dye followed by absorption measurement at 520 nm. The absorbance increased linearly with increasing concentration of the drug, When increasing amounts of each drug were added to a fixed amount of 0.08% CAS , Consumed the latter and there occurred a concomitant decrease in the concentration of CAS. When fixed amount of dye was added to decreasing concentration of CAS, an concomitant increase in the concentration of dye was obtained , Which in turn is directly proportional to the concentration of each drug. Similarly NBS reacts with the drugs in the same way as CAS in the absence of acidic medium.

The suggested reaction sequence is as shown in scheme 1.

Optimum Reaction condition

Ce(IV) and NBS has the ability to oxidize the drug and amaranth. The order of addition of reagents plays a major role in the formulation of drugs. Drug solution was added before the addition of amaranth dye showed the maximum absorbance and this order of addition is selected for all further determinations. Reaction is carried out at room temperature (25 ± 30^0c). Maximum color development is obtained at room temperature.

VALIDATION OF THE PROPOSED METHOD

Linearity, Detection and Quantification limit

Drugs were quantified directly through their ability to reduce a fixed initial concentration of Ce(IV) in presence of dye. Calibration graphs were constructed using standard solutions under optimum condition. A linear relationship was found within the range (0.5 – 4.5 µg/mL) and (1.5 – 7.0 µg/mL) for PEH and PRC [Method A] and (1.0 – 5.0 µg/mL) and (1.5 – 5.0 µg/mL) for PEH and PRC [Method B]. The system obeyed Beer's law and the calibration graphs exhibited a straight line. The Beer's law plots of PEH and PRC were shown in the (Fig 5).

Sensitivity

Sensitivity parameters such as apparent molar absorptivity, sandell's sensitivity values and the limit of detection and quantification are calculated as per the current ICH guidelines(ICH, 1996) which are compiled in (Table 1), that speaks of the excellent sensitivity of the proposed method. The limit of detection(LOD) and limit of quantification(LOQ) were calculated according to the guidelines using the formulae

$$LOD = 3.3\sigma/S, \qquad LOQ = 10\sigma/S$$

Where σ is the standard deviation of reagent blank determination, and S is the slope of the calibration curve.

Interference studies

The effect of common excipients used in the pharmaceutical preparation were studied by analyzing synthetic sample solutions containing the quantity of drugs as mentioned in (Table 2) in presence of 100 fold more concentration of each excipients. The tolerance limit was defined as the concentration which gave an error of ± 3.0% in the determination of drugs. The common excipients such as starch, dextrose, lactose, talc, magnesium stearate, had no effect in the analysis.

Precision studies

The short term precision (intraday precision) of the drugs were evaluated by measuring 5 independent samples at 3 different concentration levels (1, 3, 4 µg/mL for PEH and 2, 4, 6 µg/mL for PRC)(Method A) and (2, 3, 5 µg/mL for PEH and 2, 3, 5 µg/mL for PRC) (Method B). Similarly the assay for daily precision (inter-day precision) at the same concentration level was repeated for 5 consecutive days (Table 3). The available pharmaceutical dosage forms of the investigated drugs were analyzed by the proposed method. The precision of the method was checked by taking five replicate measurements. The results obtained by the proposed and the Official method for the dosage forms were compared statistically by means of F- and t- test and were found not to differ significantly at 95% confidence level. The reliability and accuracy of the proposed method were further ascertained through recovery studies using the standard addition method by adding different amount of standard drugs to the preanalyzed dosage forms such that the cumulative amount after adding the drugs did not exceed their linearity range (Table 4).

Table 1. **Optical and regression characteristics of PEH and PRC for the proposed methods**

Parameters	Optical characteristics			
	(PEH)		(PRC)	
	Method A	**Method B**	**Method A**	**Method B**
Color	Pink	Pink	Pink	Pink
$\lambda_{max\ (nm)}$	520	520	520	520
Beer's law limit (µg mL^{-1})	0.5 - 4.5	1 - 5	1.5 – 7.0	1.5 – 5
Molar absorptivity (L mol^{-1} cm^{-2})	0.55728 X 10^5	0.31777 X 10^5	0.28361 X 10^5	0.34944X10^5
Sandell's sensitivity (µg cm^{-2})	0.00365	0.00640	0.00725	0.00588
Limit of Detection [LOD] (µg mL^{-1})	0.00875	0.007138	0.00996	0.000553
Limit of Quantitation [LOQ] (µg mL^{-1})	0.0265	0.021626	0.03029	0.004676
Regression equation[Y*]				
Slope [B]	0.18786	0.2490	0.12352	0.1829
Intercept[A]	0.14155	-0.20482	0.04993	-0.02358
Correlation coefficient [r]	0.9963	0.9950	0.9940	0.9909
Relative standard deviation[b]	0.826	0.373	0.383	1.369

*Y= BX+A, where X is the concentration of the measured solution in µg mL^{-1} and Y is the unit for absorbance.[b]Average of five determinations (concentrations of 1, 3 and 4 µg mL^{-1} for PEH and 2, 4, and 6 µg mL^{-1} for PRC respectively (method A), 1.5, 2.5 and 3.5 µg mL^{-1} for PEH and 2, 3, and 5µg mL^{-1} for PRC respectively (method B).

Table 2. **Recovery of drugs from solution with a 100 fold concentration of various additives used as excipients in formulation**

Excipients	%Recovery ± % RSD[a]			
	(PEH)[b]		(PRC)[c]	
	Method A	**Method B**	**Method A**	**Method B**
Dextrose	99.9 ±0.1	99.8 ± 0.2	98.4 ± 0.2	99.8 ± 0.5
lactose	100.0 ±0.1	99.9 ± 0.2	99.7 ± 0.2	99.9 ± 0.1
sucrose	99.9 ± 0.1	99.8 ± 0.2	99.8 ± 0.3	99.9 ± 0.1
starch	99.9 ± 0.2	98.7 ± 0.3	99.7 ± 0.2	99.8 ± 0.2
Talc	99.8 ± 0.3	98.6 ± 0.1	98.7 ± 0.1	99.7 ± 0.2
Magnesium stearate	99.8 ± 0.4	98.9 ± 0.2	99.6 ± 0.2	99.7 ± 0.5

[a] Mean ± R.S.D, n = 3, [a]mean of three determinations
[b]concentration of PEH used – 3 µg/mL(Method A) and 3.5 µg/mL(Method B)
[c]concentration of PRC used – 5 µg/mL(Method A) and 3 µg/mL(Method B)
% Recovery ± % RSD[a] (make it in centre in Intra-day)

Table 3. Intra day and Inter day precision data of PEH and PRC

Formulation	Amount taken µg/mL	Intra-Day %Recovery±%RSD[a]	Inter-Day %Recovery±%RSD[b]
PEH (Method A)	1.0	1.05 ± 1.1	1.03 ± 1. 0
	3.0	2.98 ± 0.74	2.96 ± 0.74
	4.0	3.99 ± 0.64	3.98 ± 0.63
PRC (Method A)	2.0	1.99 ± 0.58	1.98 ± 0.58
	4.0	3.98 ± 0.31	3.99 ± 0.30
	6.0	6.01 ± 0.26	6.05 ± 0.30
PEH (Method B)	1.5	1.49 ± 0.35	1.48 ± 0.36
	2.5	2.5 ± 0.48	2.49 ± 0.48
	3.5	3.48 ± 0.29	3.49 ± 0.30
PRC (Method B)	2.0	2.05 ± 2.4	2.0 ± 2.3
	3.0	3.01 ± 0. 007	3.02 ± 0. 007
	5.0	4.99 ± 1.7	5.01 ± 1.8

[a] Mean value of five determinations, [b]Mean of five determinations performed over a period of five days.

Table 4. Analysis of drugs in pharmaceutical formulations

Formulation studied	Label claimed mg[a]	Amount found by the proposed method ± SD, mg[a]		Standard method (BP)±SD[a]*	%Recovery by the proposed method [b] ± % RSD	
		Method A	Method B		Method A	Method B
PEH[c]	Frenin (10mg/mL)	9.98 ± 0.92	10.0 ± 1.01	9.96 ± 0.81	99.8 ± 1.07	100 ± 0.28
		t = 0.04	t = 0.07			
		F = 1.29	F = 1.55			
PRC[d]	Benadon (40 mg/tab)	40.1 ± 0.70	40.0 ± 0.64	39.93 ±0.65	100.2 ±0.41	100 ± 0.13
		t = 0.43	t = 0.18			
		F = 1.15	F=1.03			

[a]Mean of five determinations ± Standard deviation. n=5; the t- and F-values obtained after comparison to the reference methods, which have the following theoretical values at 95% confidence limit t=2.44 and F=5.05. After adding two different amounts of pure drugs to the fixed concentration of pre analyzed pharmaceutical formulations, [c]PEH equivalent to 10 mg/mL (Samarth life sciences Pvt. Ltd (unit II), India), [d]PRC equivalent to 40 mg/tab (piramal laboratories Ltd, India).

Scheme 1. Proposed reaction pathway for the pink coloration of reactants

Drug + Ce $(SO_4)_2$ excess ⟶ Oxidized Drug + Ce(III) + Ce $(SO_4)_2$ (unreacted)

Unreacted Ce $(SO_4)_2$ + Amaranth Dye ⟶ Ce(III) + Unreacted Amaranth Dye Pink color, 520 nm

Drug + NBS (excess) ⟶ oxidized drug + Unreacted NBS

Unreacted NBS + Amaranth dye ⟶ reaction product of the Drug + unreacted Amaranth Dye

Figure 1. Phenylephrine hydrochloride

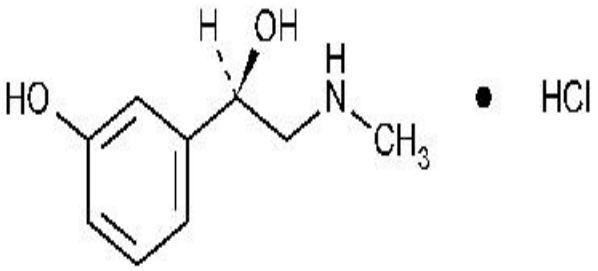


Figure 2. Pyridoxine hydrochloride

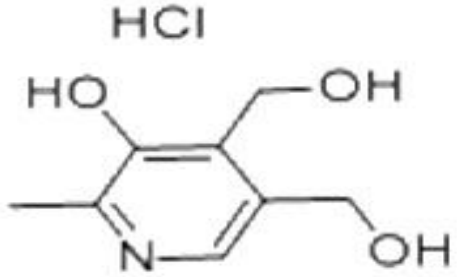


Figure 3. Absorption spectra of PEH(3ppm) and PRC(6ppm) and blank against distilled water (Method A)

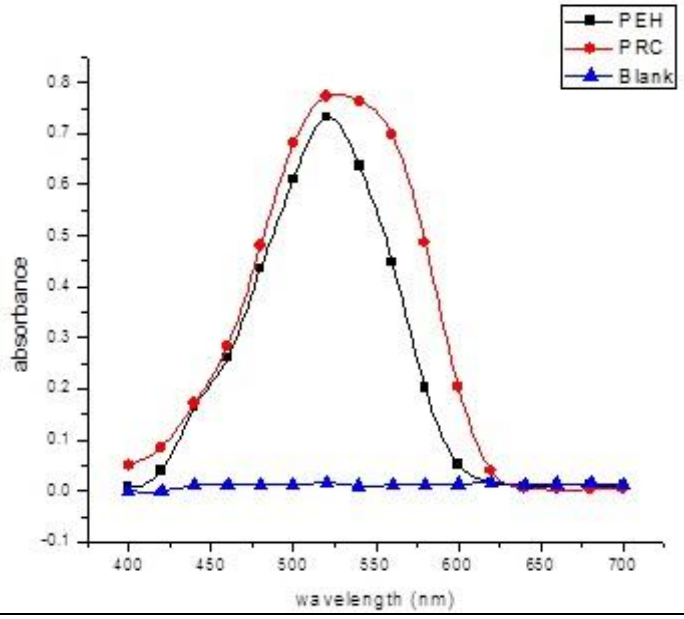


Figure 4. Absorption spectra of PEH(3ppm) and PRC(2.5ppm) and blank against distilled water (Method B)

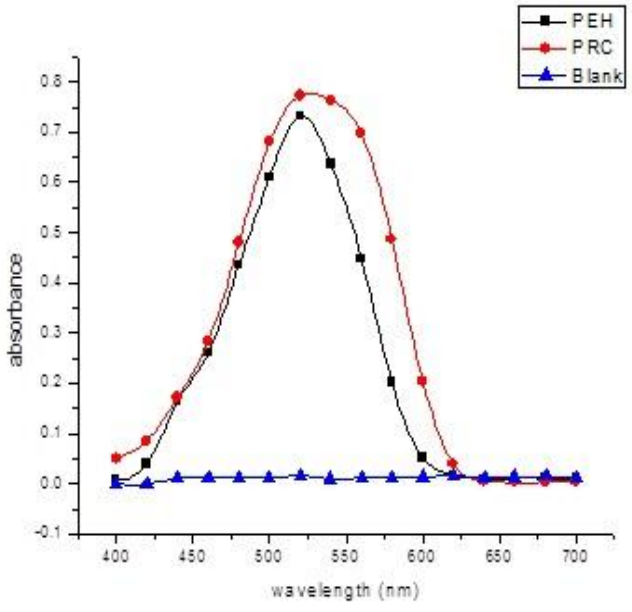
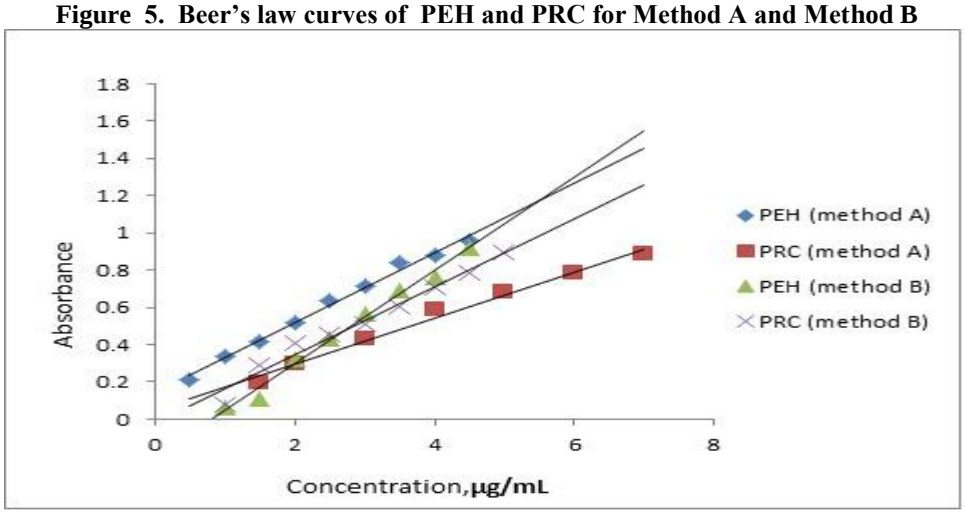


Figure 5. Beer's law curves of PEH and PRC for Method A and Method B



CONCLUSION

The proposed spectrophotometric methods for the determination of drugs are fairly sensitive, simple, and economical with reasonable precision and accuracy. Also, the procedures do not involve any critical reaction conditions or tedious sample preparation steps. So, this recommended methods are well suited for the assay and evaluation of drugs in pharmaceutical preparations.

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