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DEVELOPMENT AND VALIDATION OF VISIBLE SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF PHENYLEPHRINE HYDROCHLORIDE IN PHARMACEUTICAL DOSAGE FORM

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Abstract:
A simple, sensitive, precise, accurate, economic and rapid visible spectrophotometric method has been developed and validated for estimation of Phenylephrine Hydrochloride using Folin-Ciocalteu (F.C.) reagent in presence of 10% Sodium Carbonate solution. The blue colour chromogen formed is measured at wavelength of maximum absorption 749 nm against reagent blank. The chromogen showed linearity in the concentration range of 2-16 µg/ml for Phenylephrine Hydrochloride. The optimum parameters for maximum absorbance were found to be 2.0 ml, 3.5 ml and 15 minutes for F.C. reagent, 10% Sodium Carbonate solution and time, respectively. The method was validated for linearity, precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ) as per ICH guidelines. The percentage RSD values obtained were 0.96 - 1.44 and 0.82 – 1.39 for interday and intraday precision, respectively. The average percent recovery was found to be 101.05 ± 0.67 for proposed method. The assay results obtained was 101.10 ± 0.59 for Phenylephrine Hydrochloride using proposed method. The proposed method was found to be simple economic and sensitive, hence can be used for the routine analysis and quality control checking of Phenylephrine Hydrochloride in pharmaceutical dosage form.

Keywords: Phenylephrine Hydrochloride, Folin-Ciocalteu, Chromogen, Visible Spectrophotometric, Validation.

Introduction

Phenylephrine Hydrochloride (PEH) is chemically (R)-1-(3- hydroxy phenyl) -2-methyl amino ethanol hydrochloride. [1] PEH is α1-adrenoreceptor agonist which stimulates postsynaptic alpha receptor cause vasoconstriction, systolic and diastolic pressure. It is indicated for nasal congestion, minor eye irritations and open angle glaucoma. [2, 3] It is official in IP [4], BP [5] and USP. [6] The pure drug is estimated by titration but the formulations are assayed by UV Spectrophotometry and HPLC according to these official books. The estimation of PEH using simple UV Spectroscopy, HPLC and HPTLC has been reported in combination with other drugs. [7-11] Colorimetry were developed on PEH present in formulations as single component. [12-13] The present communication describes simple, sensitive, accurate, rapid and economical spectrophotometric method for the estimation of PEH in pharmaceutical dosage form using Folin-Ciocalteu (F.C.) [14-16] reagent in presence of 10% Sodium Carbonate solution.

MATERIALS & METHODS

Apparatus

- A double beam UV-visible spectrophotometer (Shimadzu, UV-1800, Japan) attached to a computer software UV prob 2.0, with a spectral width of 2 nm, wavelength accuracy of 0.5 nm and pair of 1 cm matched quartz cells.
- Analytical balance (CP224S,Sartorius, Germany)
- Ultrasonic cleaner (Frontline FS 4, Mumbai, India)
- Corning volumetric flasks and pipettes of borosilicate glass were used in the study.

Reagents & Materials

- PEH pure powder and Benzalkonium Chloride (BKC) were gifted from Medwin Pharmaceutical, Naroda GIDC, Ahmedabad.
- F.C. reagent (S.D. Fine Chemical Ltd., Mumbai, India)
- Sodium Carbonate (S.D. Fine Chemical Ltd., Mumbai, India)
- Distilled water
- The marketed formulation (Eyedrop) included in the study was DROSYN® 10% eyedrops (FDC, M.I.D.C., Waluj, Aurangabad) for PEH.

Preparation of F.C. reagent and Sodium Carbonate (10%) solution

F.C. reagent was prepared by diluting 1 part of reagent with 2 part of distilled water. Sodium Carbonate solution (10%) was prepared by dissolving 10 gram Sodium Carbonate to 100 ml with distilled water.

Preparation of Standard Drug Solution

Standard stock solution of PEH was prepared by dissolving 20 mg of PEH in 100 ml volumetric flask using distilled water to obtain final concentration 200 µg/ml.

Methodology

Standard stock solution of PEH (1.0 ml) was transferred to a 10 ml corning volumetric flask. 10% Sodium Carbonate solution (3.5 ml) and F.C. reagent (2 ml) was added. After a thoroughly shaking the flask was set aside for 15 minutes for the reaction to complete. The volume of flask was adjusted to 10 ml with distilled water. The solution was scanned in the range of 400 to 800 nm against reagent blank, prepared similarly in which volume of standard drug solution was replaced by an equal volume of distilled water. Maximum absorbance was obtained at 749 nm.

Optimization of Reaction Condition

Optimization of Volume of F.C. Reagent

Standard stock solution of PEH (1 ml) was transferred to a series of ten different 10 ml volumetric flasks. To each flask, 10% Sodium Carbonate solution (3 ml) was added. Then different volume of F.C reagent (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 ml) was added in each flask. For each flask, volume was adjusted up to mark with distilled water. The absorbance of resulting colored solutions was measured at 749 nm against reagent blank.

Optimization of Volume of 10% Sodium Carbonate Solution

Standard stock solution of PEH (1 ml) was transferred to a series of ten different 10 ml volumetric flasks. To each flask, different volume of 10 % Sodium Carbonate solution (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 ml) was added. Then F.C reagent (2 ml) was added in each flask and mixed. For each flask, volume was adjusted up to mark with distilled water. The absorbance of resulting colored solutions was measured at 749 nm against reagent blank.

Optimization of Reaction Time

Standard stock solution of PEH (1 ml) was transferred to a series of seven different 10 ml volumetric flasks. To each flask, 10 % Sodium Carbonate solution (3.5 ml) was added in each flask. Then, F.C reagent (2 ml) was added and mixed. All flasks were kept aside for different time interval in minute (5, 10, 15, 20, 30, 45 and 60) for reaction. Then to each flask, volume was adjusted up to mark with distilled water. The absorbance of resulting colored solutions was measured at 749 nm against reagent blank.

Preparation of Calibration Curve

Aliquot of 0.1 to 0.8 ml portion of standard drug solutions was transferred to a series of 10 ml corning volumetric flasks. To each flask, 10% Sodium Carbonate solution (3.5 ml) and F.C. reagent (2 ml) was added. After a thoroughly shaking, the flasks were kept aside for 15 minutes for the reaction to complete. The volume of each flask was adjusted to 10 ml with distilled water. The absorbance of solution in each flask was measured at 749 nm against reagent blank and calibration curve was plotted. Similarly the absorbance of sample solution was measured and the amount of PEH was determined by referring to the calibration curve.

VALIDATION

The proposed method was validated as per ICH guidelines. [17]

Linearity

Calibration curve was plotted over a concentration range of 2-16 µg/ml for PEH. An accurately measured standard stock solution of PEH (0.1, 0.2, 0.3, 0.4, 0.6 and 0.8 ml) was transferred to a series of 10 ml corning volumetric flasks. To each flask, 10% Sodium Carbonate solution (3.5 ml) and F.C reagent (2 ml) was added and mixed. The flasks were kept at room temperature for 15 minutes and the volume in each flask was adjusted to 10 ml with distilled water. The absorbance of the resulting solutions was measured at 749 nm against reagent blank. Calibration curve was constructed for PEH by plotting graph of concentration versus absorbance at 749 nm. Each reading was an average of five determinations.

Method Precision (Repeatability)

The precision of the instrument was checked by repeated scanning and measuring the absorbance of solutions (n=6) of PEH (8 µg/ml) without changing the parameters of the proposed visible spectrophotometric method. The results are reported in terms of percentage relative standard deviation (%RSD).

Intermediate Precision (Reproducibility)

The interday and intraday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of PEH (4, 8 and 12 µg/ml). The results are reported in terms of percentage relative standard deviation (%RSD).

Limit of Detection (LOD) & Limit of Quantification (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) of the method were calculated by using the following equations as per ICH guidelines.

$$LOD = 3.3*\sigma/S$$

$$LOQ = 10*\sigma/S$$

Where, σ = standard deviation of the absorbance

S = slope of the calibration curve

Accuracy (Recovery Study)

The accuracy of the method was determined by calculating recovery of PEH by the standard addition method. Known amounts of standard solution of PEH was added at 50%, 100% and 150% levels to pre-quantified sample solutions of PEH (6 µg/ml).

Analysis of Pharmaceutical Preparation (DROSYN® 10% Eyedrops, 5 ml)

An accurately measured 0.2 ml of eyedrops of PEH was transferred in 100 ml volumetric flask and volume was made up to 100 ml with distilled water to obtain final concentration equivalent to 200 µg/ml. The solution was then suitably diluted with distilled water to get final concentration of 6 µg/ml of PEH. This solution was taken for analysis and the solution was then analyzed as described under calibration curve procedure against reagent blank in presence of 0.01% BKC standard. The amount of PEH was determined by referring to the calibration curve. The analysis procedure was repeated five times with pharmaceutical formulation.

RESULTS & DISCUSSION

As per Lewis acid-base theory, PEH contains nitrogen having unshared pair of electrons. Due to electron transfer mechanism, PEH reduces F.C. reagent in alkaline condition forming blue colored chromogen molybdenum blue. Therefore the proposed work is based on the similar reaction principle. In the present work, the quantitative reaction of PEH with F.C. reagent is proposed. The reaction is based on the reduction of phosphomolybdotungstic acid in F.C. reagent by PEH in presence of 10% Sodium Carbonate solution, thereby producing reduced species molybdenum blue having characteristic blue color with maximum absorption at 749 nm. In pharmaceutical preparation, BKC is present as a preservative and it contains quaternary nitrogen having no electron pair. So there is no color generation due to presence of BKC and is not interfere during analysis.

The linearity was found in the concentration range of 2 to 16 µg/ml ($r^2 = 0.9980$) (Figure 2). The reproducibility, repeatability and precision of method are very good as shown by the low values of standard deviation and relative standard deviation (%RSD). The % recovery value in the range of 98.66 to 102.33 for pharmaceutical formulation indicates non-interferences from the formulation excipients. The data of recovery studies and assay result are given in Table 1 and Table 2, respectively. Optical characteristics of method and summary of validation parameters for PEH was given in Table 3.

Table 1 Recovery data for the proposed method

Drug	Level	Amount of sample taken (µg/ml)	Amount of standard spiked (%)	Mean % Recovery ± % RSD (n=3)
PEH	I	6	50 %	98.66 ± 0.43
	II	6	100 %	102.33 ± 0. 85
	III	6	150 %	102.17 ± 0.72

Table 2 Analysis of Pharmaceutical Formulation of PEH (n=3)

Formulation	Label claim (%)	Amount found (%)	% Label claim ± % RSD (n=3)
EYE DROPS	10	10.11	101.10 ± 0.59

Table 3 Optical Characteristic and Summary of Validation Parameter for the Proposed Method

Parameters	Results
λ max (nm)	749 nm
Concentration range (µg/ml)	2-16
Sandell's sensitivity (µg/cm^2/0.001 A.U.)	0.011942
Slope	0.0671
Intercept	0.0942
Correlation coefficient (R^2)	0.9980
LOD[a] (µg/ml)	0.54
LOQ[b] (µg/ml)	1.65
Accuracy (n[c] = 3)	101.05 ± 0.67
Repeatability (% RSD[d], n = 6)	0.99
Precision (%RSD)	
Interday (n = 3)	0.96 - 1.44
Intraday (n = 3)	0.82 – 1.39

a = Limit of Detection b = Limit of Quantification

c = Number of Determinations d = Relative Standard Deviation

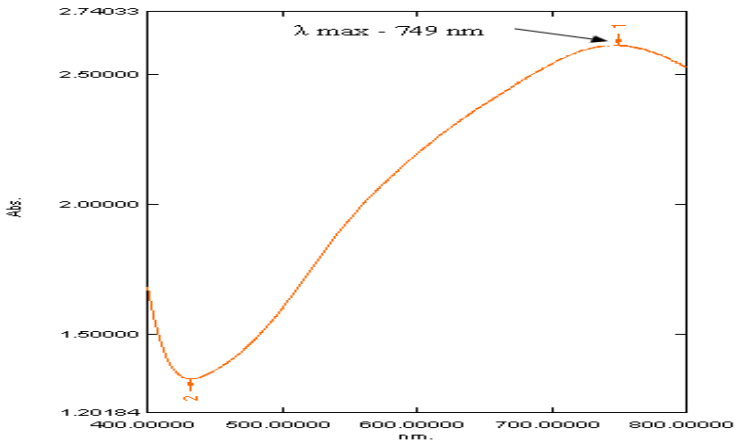

Figure 1. Representative Absorption Spectra of PEH (50 µg/ml) with F.C Reagent in Alkaline Condition Showing λmax at 749 nm.

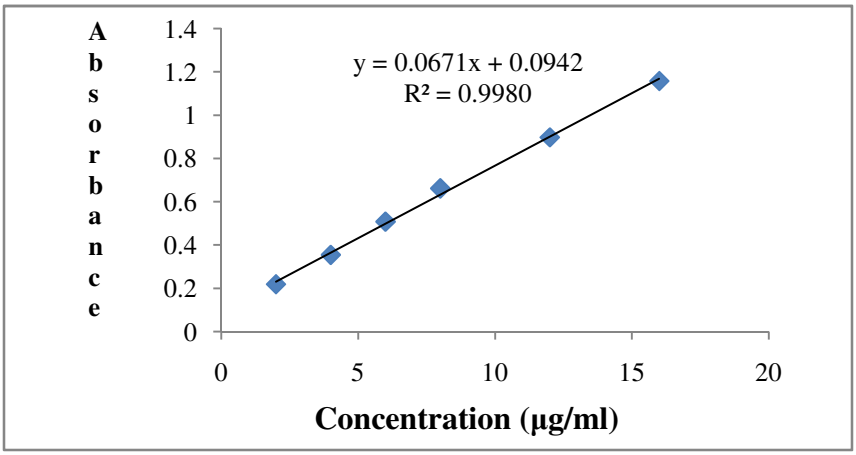

Figure 2. Linearity curve of PEH (2-16 µg/ml)

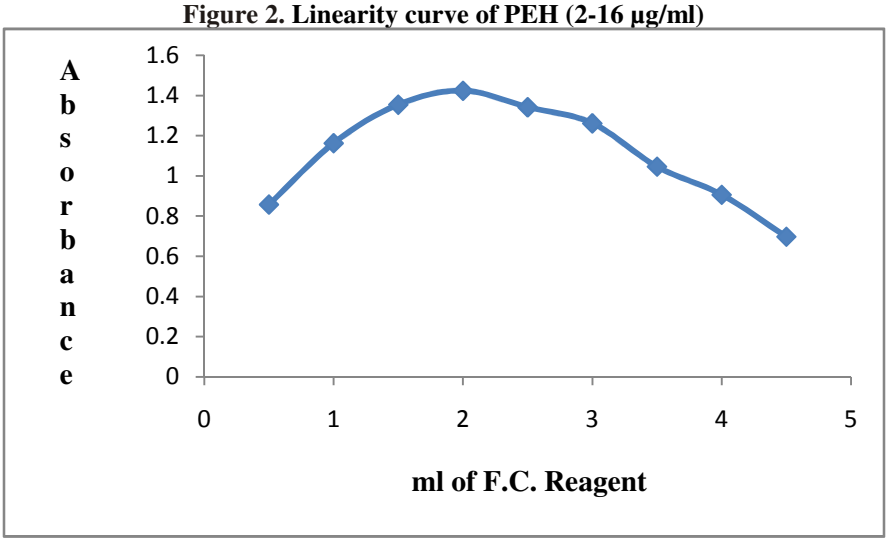

Figure 3. Optimization of Volume of F.C. Reagent (ml)

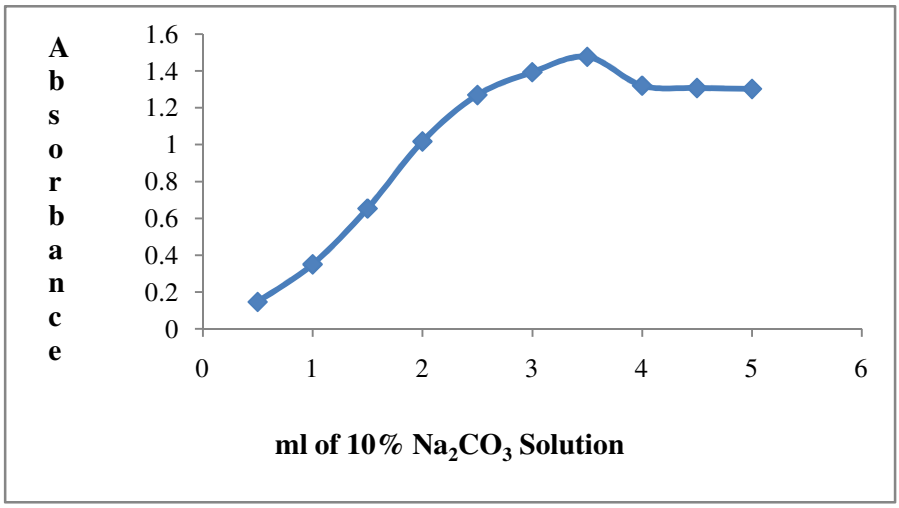

Figure 4. Optimization of Volume of 10 % Sodium Carbonate Solution (ml)

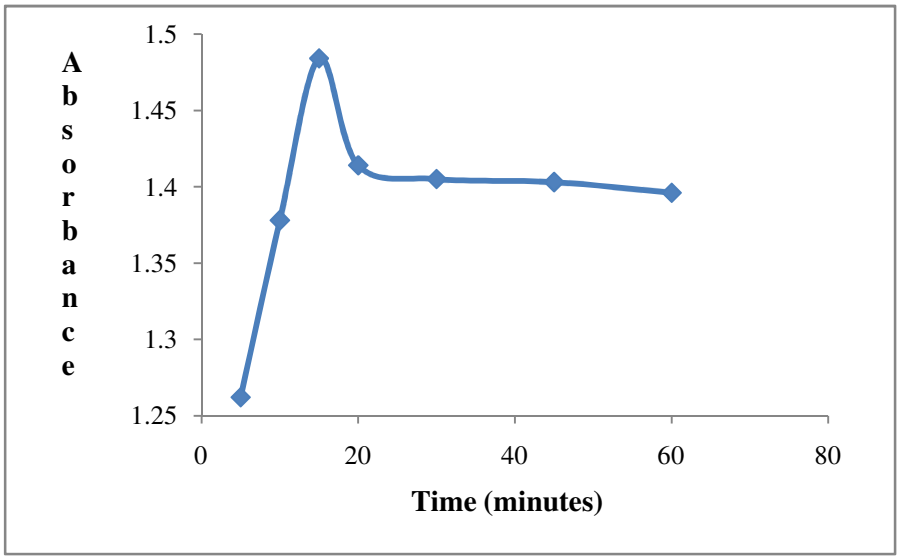

Figure 5. Optimization of Reaction Time (minutes)

CONCLUSION

A simple, sensitive, repeatable and specific visible spectrophotometric method has been developed for the estimation of PEH using Folin-Ciocalteu (F.C.) reagent in presence of 10% Sodium Carbonate solution. The method was validated for accuracy, precision, linearity, LOD and LOQ. The result of analysis of pharmaceutical formulation by the proposed method is highly reproducible and reliable and it is in good agreement with the label claim of the drug. The method can be used for the routine analysis of the PEH in pharmaceutical dosage form without any interference of excipients.

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