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Extraction-spectrophotometric determination of an ACE inhibitor with naphthol blue-black in formulations

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A new analytical method has been proposed for the assay and validation of ACE inhibitors in formulations. A bioactive compound, perindopril erbumine (PPE), is selected for its assay in bulk and pharmaceutical formulations. The procedure involves the development of colour by forming an ion-pair association complex between drug, perindopril erbumine (PPE), and naphthol blue-black (NBB). The absorbance of the colored species was measured at λ_{max} = 605 nm. The above-developed method showed linearity within the concentration limits of 25–125 μg mL^{-1}. The correlation coefficient (*r*) value was found as 0.9999. The molar absorptivity was found to be 1.983×10^3 mol^{-1} cm^{-1}. Percent recovery was found within the limits of 99.7–99.96 (±0.51–±0.24). The developed method was statistically validated as per ICH guidelines.

Keywords: Spectrophotometry, perindopril erbumine, naphthol blue-black (NBB) and formulation.

Introduction

Pharmacodynamic agents are generally used as depressants or stimulants, blocking agents, antianginal, anticoagulants, antihypertensive agents, anti-acne and ACE inhibiting agents, etc.[1]. The drug perindopril erbumine (PPE), an ACE inhibitor[1], can be used to treat hypertension and heart failure. The molecular formula of perindopril erbumine (PPE) is $C_{23}H_{43}N_3O_5$. Its chemical name is known as "(2*S*, 3*aS*, 7*aS*)-1-[(*S*)-*N*-[(*S*)-1-carboxy-butyl]-alanyl]hexahydro-2-indolincarboxylic acid, 1-ethyl ester[2], with tertiary-butylamine (1:1)" (Fig. 1). Angiotensin-converting enzyme (ACE) inhibitor (perindopril erbumine) is mainly used to lower hypertension, and heart failure issues in patients. It also helps in preventing kidney related problems or chronic renal failures. The significance of ACE inhibitor is to control the symptoms of blood pressure, renal failures, diabetes mellitus and hearts attacks.

The drug (PDE) is listed in British Pharmacopoeia[3], Remington[4] and Physician's desk reference[5]. A survey of the literature revealed that UV[6,7], HPLC[8–11], RP-HPLC[12–18], spectrofluorimetric[19,20], visible spectrophotometric[21–24], kinetic spectrophotometric[25,26], LC-MS[27,28] and GC-MS[29] methods were reported for the estimation of PPE. It was found that there are very few spectrophotometric methods are reported for the assay of PPE. Due to the growing importance of the perindopril erbumine, the development of a simple, sensitive and rapid method for its determination is of urgent need. The authors developed a spectrophotometric method for PPE in bulk form and formulations using naphthol blue-black (NBB) as a chromogenic reagent. The results of the proposed validated method were reported in this communication. The structure of perindopril erbumine is shown in Fig. 1.

Experimental

Instrumentation:

Precise and accurate wavelength measurements were made using UV wavelength scanning double beam spectrophotometer (UNICAM UV-500, Thermo Electron Corporation, UK) and visible scanning spectrophotometer (SL-177 of

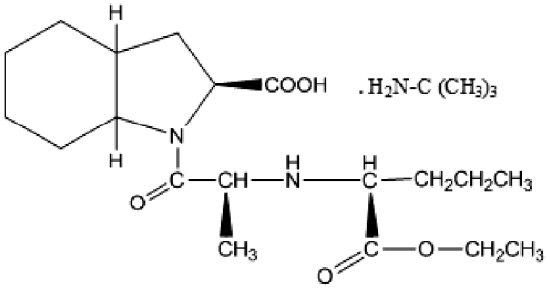


Fig. 1. Structure of perindopril erbumine (PPE).

Elico.Elico India). Elico LI 120 digital pH meter was used for measuring pH of the samples. All the reagents and samples were weighed using Dhona 200D analytical balance with an accuracy of ± 0.1 mg.

Reagents, chemicals and standards:

Reagents:

All chemicals and solvents are of analytical grade (AR). The reagent solution (Chroma; 0.2%, 3.2×10^{-3} *M*) was made by taking 200 mg of naphthol blue-black and solubilized in 100 mL of triple distilled water. The resulting solution was washed with chloroform solvent-soluble impurities

Solvent: AR grade of chloroform (Qualigens) solvent was used.

Glycine-HCl buffer solution:

Glycine solution was prepared by transferring 14.62 g of NaCl and 18.76 g of glycine into 250 mL triple distilled water. This solution was mixed with a requisite volume of 0.1 *M* hydrochloric acid and the pH of this buffer solution was regulated to a value of 1.5.

Standard drug solution:

The standard solution (mg/mL) of perindopril erbumine (bulk drug) was prepared by dissolving 100 mg of drug in 100 mL of deionized water. The working standard of 500 μg mL^{-1} was prepared by diluting a portion standard solution with deionized water.

Procedure for formulations:

The formulations of PPE procured from the local market were Coversyl (Serdia Pharmaceuticals (India) Pvt. Ltd., India), Coversyl plus (Serdia Pharmaceutical Ind. Ltd., India), Périgard-DF (Glenmark Pharmaceuticals Ltd., India) and Aceon (Solvay Pharmaceuticals Inc.). Tablets equivalent to 2 mg, 4 mg, and 8 mg per tablet respectively were selected for this study. The tablet powder equivalent to 100 mg was taken for extraction into chloroform (4×25.0 mL portions) and filtered. The filtrate was taken and extracted three times with 0.1 *M* NaOH using separating funnel. Stock solution (mg/mL) was prepared diluting the aqueous alkali extract to 100 ml with deionized water. From the stock solution, 500 μg mL^{-1} working solutions were made using double distilled water and analyzed as per the developed analytical method.

Calibration curve by UV method:

The working standard of 100 μg mL^{-1} was prepared from the stock solution (mg/mL) which was made by dissolving 100 mg of bulk drug in 100 mL distilled water. The absorption spectrum was drawn within the UV region against a blank solution (Fig. 2). A portion of the working standard drug solution (1.0–3.0 mL, conc. 100 μg mL^{-1}) was taken in a series of 25.0 mL calibrated tubes, and diluted to 10.0 ml with triple distilled water. The linearity of the curve was recorded at 204 nm against deionised water as blank. A calibration curve was drawn to calculate the concentration of the drug (Fig. 3). The UV absorption method was chosen as a reference method.

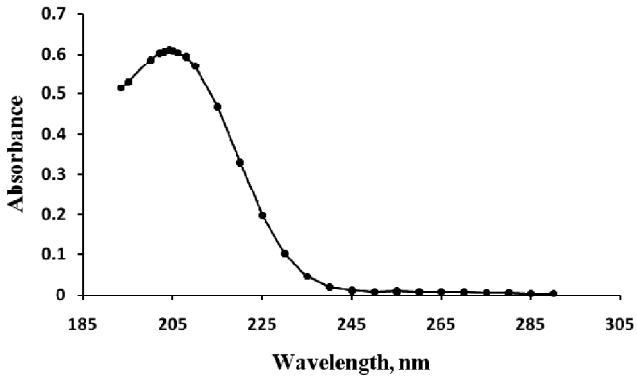


Fig. 2. UV absorption spectrum of PPE against deionised water as reagent blank ([PPE] = 4.53×10^{-6} *M*).

The protocol of the proposed method:

Into a series of 50.0 mL separating funnels, a portion of working standard bulk drug solution of concentration 500 μg mL^{-1} (0.5–2.5 mL), buffer solution (6.0 mL), and 3.2×10^{-3} *M* of dye solution (2.0 mL) were added. A final volume of 15.0

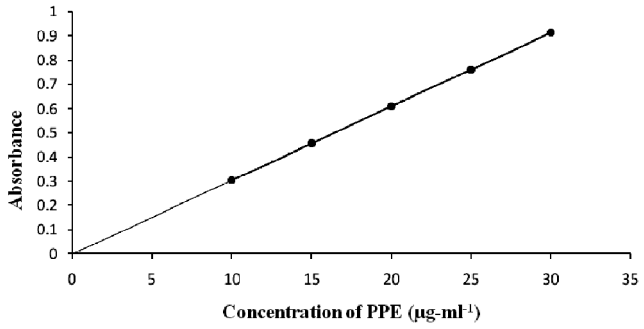


Fig. 3. Beer's law plot of perindopril (PPE) using UV method ([PPE] = [4.53×10^{-6} *M*]).

ml was maintained in each separating funnel with deionized water after the addition of 10.0 mL of the solvent (chloroform) to extract the complex. The separated chloroform layer whose absorbance was recorded against reagent blank at 605 nm. A calibration curve was drawn to calculate the amount of drug present (Fig. 4).

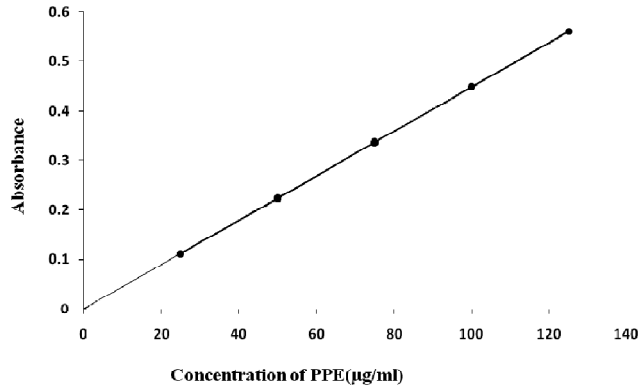


Fig. 4. Beer's law plot of PPE-NBB ion pair complex ([PPE] = [1.698×10^{-3} *M*] and [NBB] = [6.4×10^{-4} *M*]).

Results and discussion

Selection of analytical wavelength:

For the selection of analytical wavelength, the sample solution containing a fixed quantity of drug (PPE), naphthol blue-black (NBB) solution, and other furnished variables as outlined in the analytical procedure was scanned in the visible region against reagent blank. The spectrum of the ion-pair association complex is observed to have a maximum wavelength at 605 nm which was selected for the analysis. The spectrum of the naphthol blue-black (NBB) reagent (acidic dye) in the aqueous phase (at pH = 1.5) against distilled water was found to have very high absorption peak whereas blank solution showed very low absorption peak in this region. The spectrum of reagent blank against the chloroform solvent was also measured (Fig. 5).

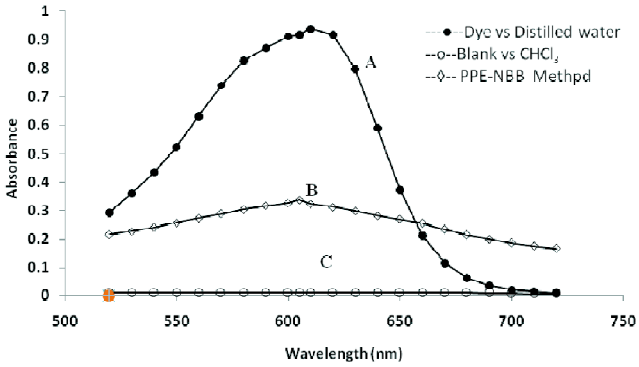


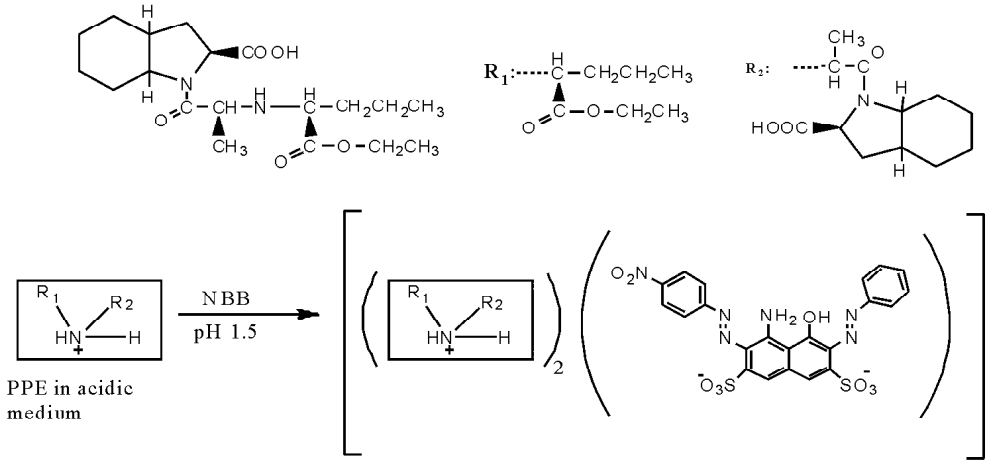
Fig. 5. (A) Absorption spectrum of [NBB] = [6.4×10^{-4} *M*]; (B) Absorption spectrum of ion pair association complex of PPE-NBB ([PPE] = [1.698×10^{-3} *M*] and [NBB] = [6.4×10^{-4} *M*]); (C) Absorption spectrum of reagent blank vs chloroform.

Optimum conditions:

The responses of several factors like the volume of primary solvent used for extraction, the concentration of the dye used, buffer solution, stability period of the complex formed, the intensity of the color of the species formed, the ratio of primary solvent phase to aqueous phase during separation, were studied[30]. The optimum conditions identified for the proposed method are 1.0–3.0 mL (0.32–0.96×10^{-3} mol L^{-1}) of the naphthol blue-black (NBB) solution, 5.0–7.0 mL of buffer solution (pH = 1.5), laboratory temperature of (28±2°C) and mixing time considered as 1–5 min. In this method, 2.0 mL of (0.64×10^{-3} mol L^{-1}) naphthol blue-black (NBB), 6.0 mL of buffer solution (pH = 1.5), and two minutes mixing time essential for highest color growth were found to be optimum conditions. The stability period of the ion-pair association complex was found as 50 min, afterward, the absorbance was found to decrease, which may be due to the decomposition of the complex. The optimum conditions are given in Table 1.

Table 1. Optimum conditions of the proposed developed established

Parameter	Optimum range	Conditions in procedure
Wavelength (λ_{max})	610–630 nm	605 nm
Influence of pH on formation of colour	1.3–1.7	1.5
Volume of glycine-HCl buffer used for obtaining maximum intensity colour	5.0–7.0 ml	6.0 ml
Volume of NBB used for producing maximum colour	1.0–3.0 ml	2.0 ml
Suitability of organic solvent for extraction of the complex	Chloroform	Chloroform
Ratio of organic to aqueous phase used for extraction	2:3	2:3
Shaking time	1–5 min	2 min
Influence of temperature for colour development	Lab. temp. (28±30°C)	Lab. temp.
Stability of the coloured species	1–50	Absorbance measured after 5 min



Scheme 1. Mechanism of ion-pair association complex of PPE-NBB.

Mechanism of ion-pair association complex:

In the present investigation, the chemistry of colored complex was studied in acid medium and found that the positive charge appeared on the nitrogen of the drug (PPE) molecule and the negative charge of the dye held together through electrostatic force of attraction and behave as a single entity. From the analogy studies, the probable mechanism of the colored complex formed between PPE with NBB is given in Scheme 1.

Validation of analytical data:

Following (ICH) guidelines[31], the analytical technique (PPE-TPOOO) developed was validated for various optical and regression characteristics. The molar absoptivity was

Table 2. Validation of PPE-NBB the method

Wavelength	605 nm
Molar absorptivity	1.987×10^{3} L mol^{-1} cm^{-1}
Beer's law limits	25–125 μg mL^{-1}
Standard error of estimation (S_e)	3.65×10^{-4}
Limit of detection (LOD)	2.81×10^{-1} μg mL^{-1}
Correlation coefficient (r)	0.9999
Limit of quantification (LOQ)	8.52×10^{-1} μg mL^{-1}
Relative standard deviation (RSD)•:	
Intra-day precision	0.90
Inter-day precision	0.87
% of error:	
0.01 Confidence limits	1.47
0.05 Confidence limits	0.94

•Estimation of six observations.

Table 3. Estimation of perindopril erbumine (PPE) in formulations

Formulation batches	Quantity taken (mg)	Quantity found by UV absorption method (mg)	Quantity found by developed method (mg)[a]	95% Confidence limit values F-test[b]	95% Confidence limit values t-test[d]	% Recovery[c]
I	2	2.00±0.005	1.99±0.01	3.64	1.3	99.7±0.51
II	4	4.00±0.005	3.99±0.01	3.04	0.36	99.96±0.24
III	4	4.01±0.017	4.001±0.04	4.28	0.8	99.94±0.9
IV	8	8.00±0.022	7.98±0.04	3.51	0.74	99.7±0.52

[a]Average value of six observations.

[b]Tabulated F-value at 95% confidence level is 5.05.

[c]Average of three determinations.

[d]Tabulated t-value at 95% confidence level is 2.57.

found to be as 1.983×10^3 mol^{-1} cm^{-1} and the wavelength was measured at 605 nm. Linearity was detected within the concentration limits (25–125 μg/mL). Beer's law plot (n = 6) was found consisting of linearity with a high correlation coefficient (*r*) value 0.9999. Results of LOD and LOQ are established as 2.81×10^{-1} μg mL^{-1} and 8.52×10^{-1} μg mL^{-1} respectively by using the formulas such as LOD = 3.3×*Sa*/*b* and LOQ = 10×*Sa*/*b* where *b* is the slope of the calibration line and *Sa* is the standard deviation of the intercept.

For the proposed method, precision was checked in two ways such as intra-day precision and inter-day precision. It was calculated by repeating the procedure six times a day and repeating the same on six consecutive days. RSD values for the proposed method were found to be as 0.90 and 0.87. Results of optical and regression parameters, RSD, and percentage of error for the developed method are given in Table 2.

The accuracy was checked by comparing the result of the developed and UV reference method statistically through student t- and student F-tests at theoretical values of 95% confidence limits with (n–1) degrees of freedom. It was observed that the values obtained for t- and F-tests for the proposed method are found to be lower than the tabulated values[29] of 2.57 and 5.05 respectively. Further, the accuracy studies were carried out by the addition of a fixed known quantity of the drug to the pre-estimated dosage forms. The results obtained by proposed and reference method are in good agreement with each other. Recovery ± SD values were in the range of 99.7–99.96 (±0.51–±0.24 (n = 3) which indicates the accuracy of the developed method. Results of accuracy are given in Table 3. The interference of other excipients that are commonly present in dosage forms is found to be negligible. The developed method is found to be more accurate within the Beer's law range with reference to correlation coefficient value compared to literature methods (Table 4).

Table 4. Comparison of visible spectrophotometric method

Sl. no.	Reagent	Wavelength λ_{max} (nm)	Beer's law range (μg mL^{-1})	Correlation coefficient (*r*)	Ref.
1.	Safranine-O	520	5–25	0.9993	24
2.	Bromothyol blue	410	2–20	0.9996	24
3.	BPB	425	5–125	0.9992	21
4.	NBB	605	25–125	0.9999	Present paper

Conclusion

The sensitivity of the technique lies only like the reaction with an appropriate chromogenic reagent selected but not on the sophistication of the instrument. For routine analysis in bulk and formulations, the method developed is specific and can be used as a substitute to GLC, HPLC, GC-MS, and LC-MS etc. in quality control laboratories.

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