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Analytical method for the assay of Perindopril Erbumine in formulations by ion association complex formation using Tropaeolin OOO (TPOOO)

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A novel, inexpensive and responsive analytical technique was developed for the assay and validation of Perindopril Erbumine (PPE) in dosage forms. The procedure involves the development of colour by forming ion pair association complex between drug (PPE) and Tropaeolin OOO (TPOOO). The absorbance of the colored species were measured at λ_{max} = 480 nm. The above developed method showed linearity within the concentration limits of 5–25 μg ml^{-1}. The correlation coefficient (*r*) value was found as 0.9999. Percent recovery was found within the limits of 99.03±0.82–99.79±0.66. Developed method was statistically validated as per ICH guidelines.

Keywords: Spectrophotometry, Perindopril Erbumine, Tropaeolin OOO (TPOOO), 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] and can be used as a medicine for patients having the problems like hypertension and heart failure.

Molecular formula Perindopril Erbumine (PPE) is $C_{23}H_{43}N_3O_5$. It's chemical name (IUPAC) is "(2S, 3aS, 7aS)-1-[(S)-N-[(S)-1-carboxy-butyl]-alanyl] hexahydro-2-indolincarboxylic acid, 1-ethylester"[2] (Fig. 1). PPE 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[12–28], spectrofluorimetric[19,20], visible spectrophotometric[21–23], kinetic spectrophotometric[24,25], LC-MS[26,27] and GC-MS[28] were reported for the estimation of PPE. It was found that there are very few spectrophotmetric methods are reported for the assay of PPE. The authors made an attempt to develop and validate spectrophotometric method for PPE in bulk form and formulations using Tropaeolin OOO as chromogenic reagent.

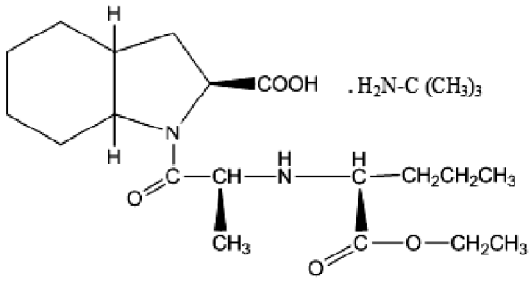


Fig. 1. Structure of Perindopril Erbumine (PPE).

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 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, solvents and bulk drug solution:

All chemicals and solvents are of analytical grade (AR). Tropaeolin OOO solution (TP OOO) (Fluka; 0.2%, 5.70×10^{-3} *M*) was prepared by dissolving 200 mg of Tropaeolin OOO in 100 ml deionised water and subsequently washed with chlroform to remove solvent soluble impurities.

The standard solution (mg/mL) of Perindopril Erbumine (bulk drug) was prepared by dissolving 100 mg of drug in 100 mL of deionized water. A portion of stock solution further diluted stepwise with deionised water to obtain standard solution of 100 μg mL^{-1}.

Procedure for formulations:

Coversyl (Serdia Pharmaceuticals (India) Pvt. Ltd., India), Coversyl plus (Serdia Pharmaceutical Ind. ltd., India), Perigard-DF (Glenmark Pharmaceuticals Ltd., India) and Aceon (Solvay Pharmaceuticals Inc.) containing Perindopril Erbumine were procured from local market. Tablets equivalent to 2 mg, 4 mg and 8 mg per tablet respectively were selected for this study. Tablet powder equivalent to 100 mg was taken for extraction with 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 deionised water. From the stock solution, 100 μg ml^{-1} working solutions were made using double distilled water and analyzed as per the developed analytical method.

Calibration curve by UV method:

Bulk drug sample (100 mg) was dissolved in 100 mL distilled water to prepare stock solution (mg/mL). Aliquot portion (10.0 mL) of this stock solution was further diluted stepwise with distilled water to achieve feasible standard solution concentration 100 μg mL^{-1}. The absorption spectrum was recorded on spectrophotometer within the UV region against a reagent blank (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 10.0 mL calibrated tubes, and diluted to 10.0 ml with double distilled water. The absorbance was measured at 204 nm against deionised water as blank. The concentration of the drug sample was calculated using its calibration curve (Fig. 3). The UV absorption method was chosen as a reference method.

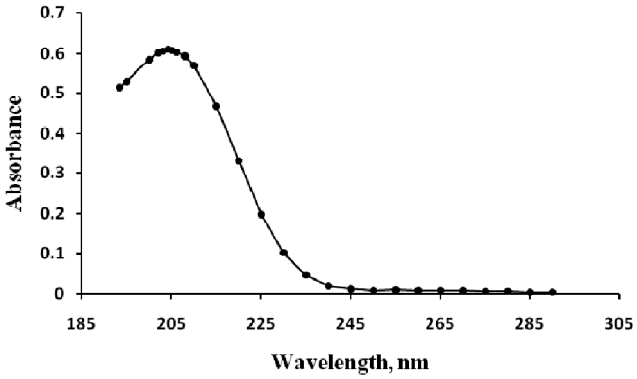


Fig. 2. UV absorption spectrum of PPE against deionised water as reagent blank ([PPE] = 4.53×10^{-6} *M*).

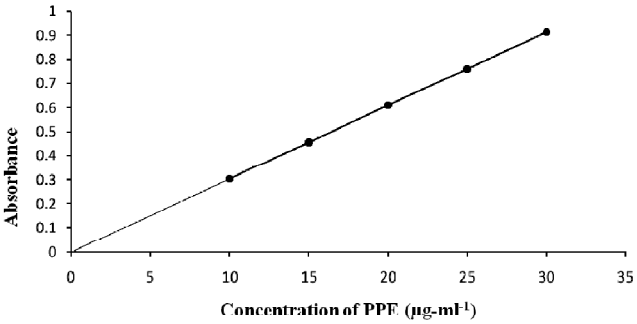


Fig. 3. Beer's law plot of Perindopril (PPE) using UV method ([PPE] = [4.53×10^{-6} *M*]).

Protocol of proposed method:

Into a series of 100.0 mL separating funnels, a portion of working standard bulk drug solution of concentration 100 μg mL^{-1} (10–5.0 mL), 0.1 *M* HCl solution (6.0 mL), 5.7×10^{-3} *M* of dye solution (2.0 mL) were added. The total volume of 15.0 ml was maintained in each separating funnel with deionised water and 10.0 mL of the solvent chloroform was added for the extraction of complex. The absorbance of the separated chloroform layer was measured at 480 nm against reagent blank. A calibration curve was drawn to calculate the amount of drug present (Fig. 4).

Results and discussion

Selection of analytical wavelength:

For the selection of analytical wavelength, the sample solution containing fixed quantity of drug (PPE), Tropaeolin OOO solution and other furnished variables as outlined in

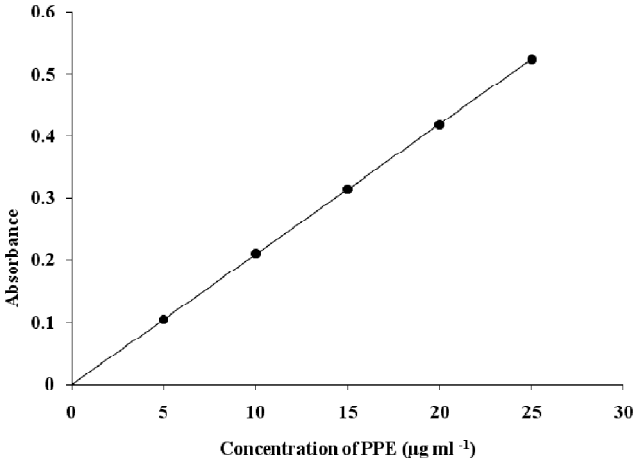


Fig. 4. Beer's law plot of PPE-TPOOO ion pair complex. ([PPE] = [3.40×10^{-5} *M*] and [TPOOO] = [1.142×10^{-3} *M*]).

the analytical procedure was scanned in the wavelength region 350–800 nm against the reagent blank. The spectrum of the ion-pair association complex is observed to have maximum wavelength at 480 nm which was selected for the analysis. The spectrum of the Tropaeolin OOO reagent (acidic dye) against distilled water was reported which has very high absorption peak whereas blank solution showed very low absorption peak in this region. The spectrum of reagent blank against chloroform solvent was also measured (Fig. 5).

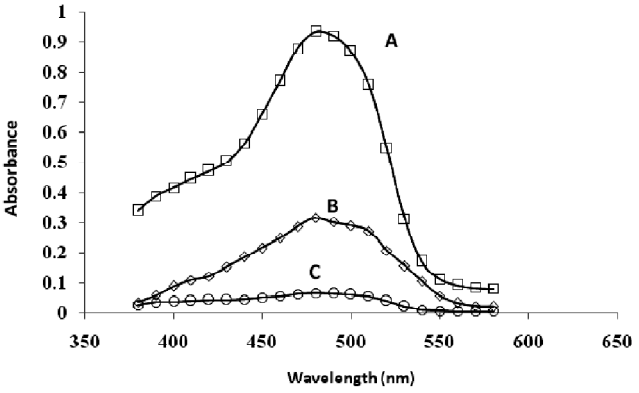


Fig. 5. (A) Absorption spectrum of TPOOO ([(TPOOO] = [1.142×10^{-3} *M*), (B) absorption spectrum of ion pair association complex of PPE-TPOOO ([PPE] = [3.40×10^{-5} *M*] and [TPOOO] = [1.142×10^{-3} *M*]) and (C) absorption spectrum of reagent blank vs chloroform.

Optimum conditions:

The responses of several factors like the volume of primary solvent used for extraction, concentration of the dye used, buffer solution, stability period of the complex formed, intensity of the colored species formed, the ratio of primary solvent phase to aqueous phase during separation, were studied[29]. The optimum conditions identified for the proposed method are: 1.5–2.5 mL (0.86–1.4×10^{-4} mol L^{-1}) of the Tropaeolin OOO solution, 5.0–7.0 mL (5.0–7.0×10^{-2} mol L^{-1}) of 0.1 *M* HCl solution, laboratory temperature of (28±2°C) and mixing time considered as 1–5 min. In this method, 2.0 mL of (1.1×10^{-3} mol L^{-1}) Tropaeolin OOO, 6.0 mL (6.0×10^{-2} mol L^{-1}) of 0.1 *M* HCl solution and two minutes mixing time essential for highest color growth were found to be optimum conditions. Solvent chloroform was used for the extraction of complex from the aqueous phase, and proportion of aqueous to primary solvent stage was taken as 3:2. Stability period of ion-pair association complex was found as 60 min, afterwards the absorbance was found to decrease which may be due to the decomposition of the complex. The optimum conditions are given in Table 1.

Mechanism of ion-pair association complex:

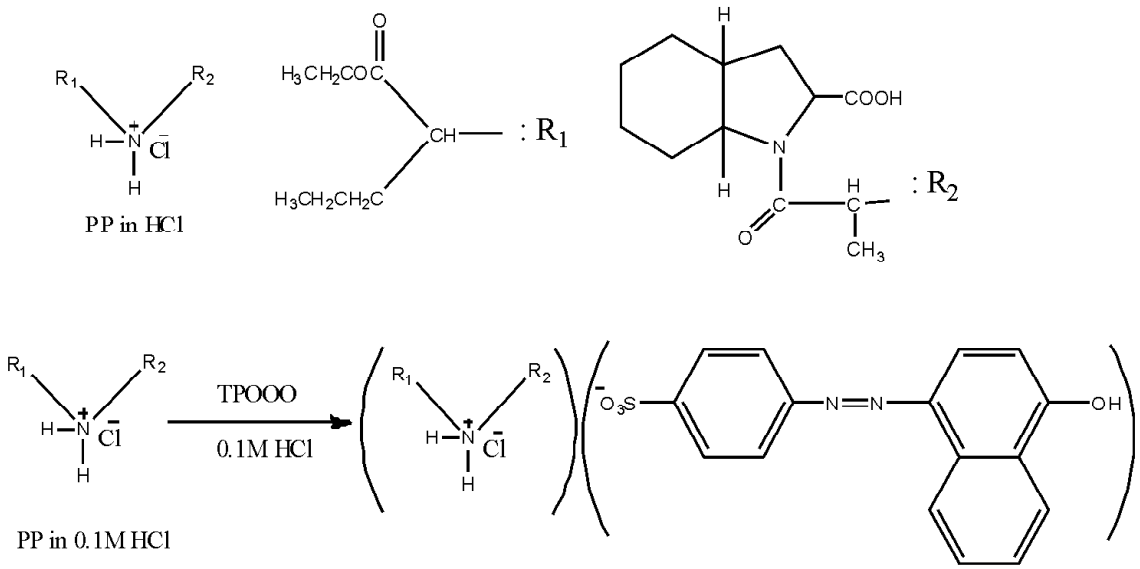
The mechanism of ion-pair association complex formation reaction was studied and found that in acid medium, the positive charge appeared on nitrogen of the drug (PPE) molecule and the negative charge of the dye held together by means of electrostatic force of attraction and behave as a single entity. The probable mechanism of ion pair association complex formation between PPE with Tropaeolin OOO is given in Scheme 1.

Validation of analytical data:

Following international conference on harmonization instructions (ICH) guidelines[31], the analytical technique (PPE-TPOOO) developed was statistically validated for various optical and regression characterstics. The ε_{max} (mol^{-1}/cm) and λ_{max} (nm) values were found to be 9.31×10^3 and 480.

Table 1. Optimum conditions of the proposed developed method

Parameter	Optimum range	Conditions in procedure	Remarks
λ_{max} (nm)	480–490	480	–
Effect of acid conc. on colour development	0.08–0.12 *M*	0.1 *M*	Beyond the optimum range resulted in low absorbance values
Volume of acid required for maximum intensity of colour	5.0–7.0 ml	6.0 ml	High absorbance was found at the specified condition and noticed low absorbance beyond the lower limit
Effect of volume of 5.079×10^{-3} *M* TPOOO	1.5–2.5 ml	2.0 ml	Specified volume in the procedure was found to be necessary for covering the broad range of Beer's law limits
Choice of organic solvent for the extraction of the coloured species	Chloroform	Chloroform	$CHCl_3$ was preferred as best solvent compared to other solvents
Effect of the ratio of aqueous to organic phase on extraction	3:2	3:2	Specified ratio was found to be the best for the extraction of the coloured species
Effect of shaking time	1–5 min	2 min	Constant absorbance values were obtained within the shaking period time
Effect of temp. on the coloured species	Laboratory temp. (28°±2°C)	Laboratory temp.	Stability of coloured species were found to be constant at laboratory temp
Stability of the coloured species	Up to 60 min	Absorbance measured after 5 min	The coloured species were stable for 60 min. Afterwards absorbance decreased which is due to dissociation of complex



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

Linearity was detected within the concentration limits (5–25 μg mL^{-1}). Beer's law plot (n = 6) was found consisting of linearity with a high correlation coefficient (r) value 0.9999. Following ICH instructions, limit of detection (LOD) and limit of quantification (LOQ) values were calculated using the following formulas. DL = $3.3\times S_a/b$ and QL = $10\times S_a/b$, where b

Table 2. Optical characteristics, precision, accuracy of the proposed developed method

λ_{max} (nm)	480
Beer's law limits (mg/ml)	5–25
LOD (mg/ml)	9.5×10^{-2}
LOQ (mg/ml)	3.172×10^{-1}
Molar absorptivity (L mol^{-1} cm^{-1})	9.31×10^{3}
Slope (b) of regression equation ($y = a + bC$)[a]	2.09×10^{-2}
Standard deviation on slope (S_b)	4.0×10^{-5}
Intercept (a)	1.0×10^{-3}
Standard deviation on intercept (S_a)	6.63×10^{-4}
Standard error of estimation (S_e)	6.32×10^{-4}
Correlation coefficient (r)	0.9999
Relative standard deviation[b]	0.73
% Range of error (confidence limit):	
0.05 level	0.77
0.01 level	1.21

[a]$y = a + bC$ where C is the concentraion of component in mg/ml and y is the absorbance unit, [b]six determinations used to calculate.

The accuracy was ascertained by comparing the result of proposed and UV (reference) method statistically through student *t*- and *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 performed by adding a fixed known amount of the drug to the pre estimated dosage forms. This comparison shows that there is no significant difference between the results obtained by proposed and reference method. RSD value for the proposed method was found to be 0.73. Recovery ±SD values were in the range of 99.03–99.79 ($\pm$0.82–±0.66) (n = 3) which indicates the accuracy of 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.

Table 3. Estimation of Perindopri 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[c]	% Recovery[d]
I	2	1.99±0.02	1.99±0.01	4.78	0.43	99.6±0.35
II	4	3.99±0.02	3.98±0.02	1.41	1.51	99.43±0.5
III	4	3.99±0.02	3.99±0.03	3.05	0.17	99.79±0.66
IV	8	8.04±0.03	7.92±0.07	3.89	2.09	99.03±0.82

[a]Average value of six observations. [b]Tabulated *F*-value at 95% confidence level is 5.05. [c]Tabulated *t*-value at 95% confidence level is 2.57. [d]Average of three determinations.

is the slope of the calibration line and S_a is the standard deviation of the intercept. Results of LOD and LOQ are established as 9.5×10^{-2} μg mL^{-1} and 3.172×10^{-1} μg mL^{-1} respectively. Precision of the proposed method was checked in terms of intra-day and inter-day precision. It was calculated by repeating six times a day and repeating on six consecutive days. Results of optical and regression parameters, RSD, and percentage of error for developed method are given in Table 2.

Conclusion

Sensitivity of the technique lies only on the nature of the reaction with an appropriate chromogenic reagent selected but not on the sophistication of the instrument. The method developed is specific to be used for routine analysis in bulk and formulations as a substitute to GLC, HPLC, GC-MS, and LC-MS etc. in quality control laboratories where the sophisticated and expensive instruments are not available.

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