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Application of Oxidative Coupling Reaction using Brucine and Sodium Periodate as Chromogenic Reagent for the Assay of Perindopril Erbumine in Formulations

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Abstract: The drug Perindopril Erbumine (PE), an ACE inhibitor, and can be used to treat the patients with hypertension and cardiac failure problems. A sensitive, inexpensive, and precise analytical technique has been developed for the estimation of Perindopril in bulk and formulations. The procedure involves the development of color by forming an oxidative coupling reaction between drug (PE) and Brucine/IO_4^-). The formed colored species were measured at λ_{max}=520 nm. The developed method showed linearity within the concentration limits of 8-24 µg mL^{-1}. The linear correlation coefficient (r) and molar absorptivity were found to be 0.9999 and 9.16 x 10^3 $mol^{-1}.cm^{-1}$. % Recovery ± SD values were in the range of 99.16 - 100.7 (± 0.41 - ± 0.8) (n=3) which indicates the accuracy of the developed method. The interference of other excipients that are commonly present in formulations is found to be negligible. Precision and accuracy of the proposed method were confirmed by Student's t-test and F-tests at 95% confidence limits with (n-1) degrees of freedom. The validity parameters of the proposed method were calculated by ICH guidelines.

Keywords: Spectrophotometry, perindopril erbumine , brucine , coversyl and perigard-DF.

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INTRODUCTION

Pharmacodynamic agents refer to group of drugs which activate or reduce various functions of the body so as to bring some relief to the body without healing the disease. These are generally used as depressants or stimulants, blocking agents, antianginal, anticoagulants, antihypertensive agents, anti-acne and ACE inhibiting agents, etc. In the present investigation, the drug used namely perindopril erbumine (PDE) is referred to as one of the categories of the aforesaid agents called as angiotensin converting enzyme inhibitor (ACE inhibitor). It is used as a medicine for patients having the problems like hypertension and cardiac failure. ACE inhibitör (1) inhibits the transfer of angiotensin (AT-I) into angiotensin (AT-II).

The molecular formula of Perindopril Erbumine (PPE) is $C_{23}H_{43}N_3O_5$. Its IUPAC name is"(2S, 3aS, 7aS)-1-[(S)-N-[(S)-1-carboxy-butyl]-alanyl] hexahydro-2-indolincarboxylic acid, 1-ethylester (2), compound with tertiary-butylamine (1:1)" (Figure 1). The drug (PDE) is listed in the 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), UV-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. The authors made an attempt to develop and validate spectrophotometric method for PPE in bulk form and formulations using Brucine - IO_4^- as chromogenic reagent.

MATERIALS AND METHODS

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, Elico India). Digital pH meter (Elico LI 120) was used for measuring PH of the samples. All materials were weighed using Dhona 200D analytical balance with an accuracy of ± 0.1 mg.

Preparation of Brucine (BCN) Solution, $NaIO_4$ Solution

Reagents belonging to investigative grade, bulk, and formulation samples were made using deionized water. Brucine (BCN) (Loba; 0.2%, $5.06x10^{-3}$ M) was prepared by dissolving 200 mg of brucine initially in a minimum amount of 0.16 M H_2SO_4 and then made up to 100 mL with distilled water. Sodium metaperiodate (AR grade, BDH; 0.2%, 9.35 x 10^{-3} M) solution was prepared by dissolving sodium metaperiodate (200 mg) in 100 mL deionized water and standardized by iodometric method. Sulfuric acid (AR grade, Qualigens; 2.3N) was prepared by mixing 6.4 mL of 18 M conc. H_2SO_4 to 50 mL of deionized water initially, followed by diminishing to 100 mL with the same solvent (deionized water).

Preparation of Standard Perindopril Erbumine Solution (ppe)

We dissolved 100 mg of perindopril erbumine in a minimum quantity of 0.1 M sodium hydroxide solution followed by dilution to 100 mL with distilled water to prepare the standard stock solution (mg mL^{-1}). The released free erbumine was extracted with 10.0 mL of chloroform. The aqueous solution free from erbumine was used as the stock solution. It is further diluted stepwise with distilled water to obtain working standard solutions of concentration of 200 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 x 25.0 mL portions) and filtered. The filtrate was taken and extracted three times with 0.1 M NaOH using a separating funnel. Stock solution (mg mL^{-1}) was prepared diluting the aqueous alkaline extract to 100 mL with deionized water. The working standard of 200 µg mL^{-1} solutions was made by diluting a portion of the above stock solution and analyzed as per the developed analytical method.

Calibration Curve of Perindopril Erbumine by UV Method

100 mg of bulk drug sample was dissolved in 100 mL of distilled water to prepare the stock solution (mg mL^{-1}). The working standard solution concentration of 100 µg mL^{-1} was prepared from an aliquot portion of 10.0 mL of the above stock solution. The absorption spectrum was recorded on a spectrophotometer within the UV region against a reagent blank (Figure 3). 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 doubly distilled water. The absorbance was measured at 204 nm against deionized water as blank. The concentration of the drug sample was calculated using its calibration curve (Fig.4). The UV absorption method was chosen as a reference method.

Protocol of Proposed Method

Aliquots of standard drug solution [1.0 – 3.0 mL, 200 µg mL^{-1}], 3.0 mL of 5.067 x 10^{-3} M brucine, 1.5 mL of 9.35 x 10^{-3} M $NaIO_4$ solution and 2.0 mL of 2.3 N sulfuric acid were added successively into a series of calibrated tubes. The volume was brought up to 10.0 mL with distilled water and kept in boiling water bath for 20 min. The solutions were cooled to room temperature and the volume was made up to 25 mL with distilled water. The absorbances were measured at 520 nm against a similar reagent blank within 30 min. The stability of colored species was found as 40 minutes, afterwards the absorbance was found to decrease which may be due to the decomposition of the oxidative coupling product. The amount of PPE was computed from its calibration graph (Figure 5).

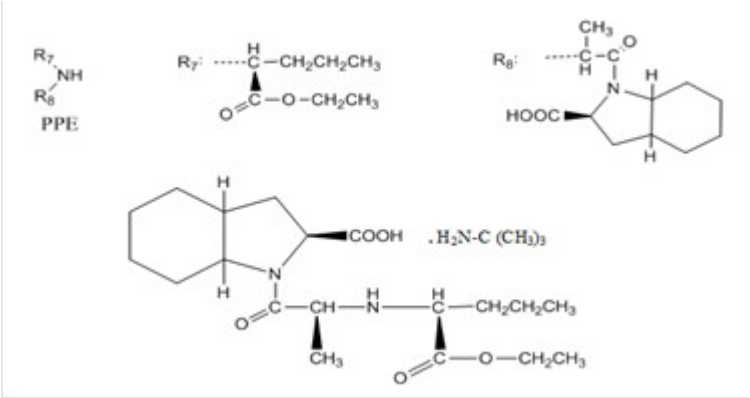


Figure 1: Structure of Perindopril Erbumine (PPE).

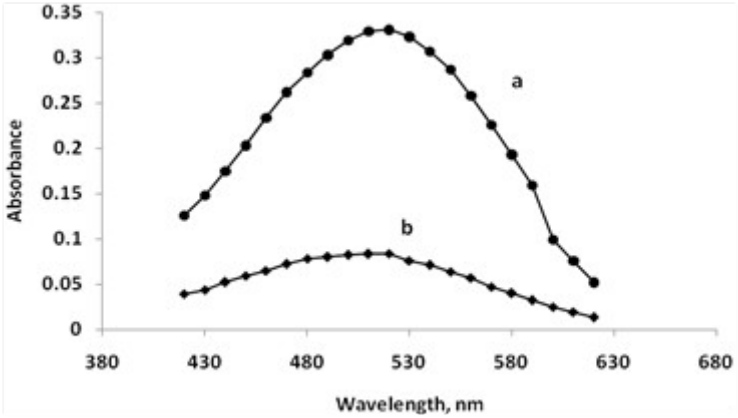


Figure 2: Absorption spectrum of PPE - Brucine – $NaIO_4$ method. a) PPE-Brucine -$NaIO_4$ method([PPE]=3.62×10^{-5} M [BCN]=6.08×10^{-4} M; [$NaIO_4$]= 5.61×10^{-4} M;[H_2SO_4]= 1.84×10^{-4} M) b) Blank Vs deionized water.

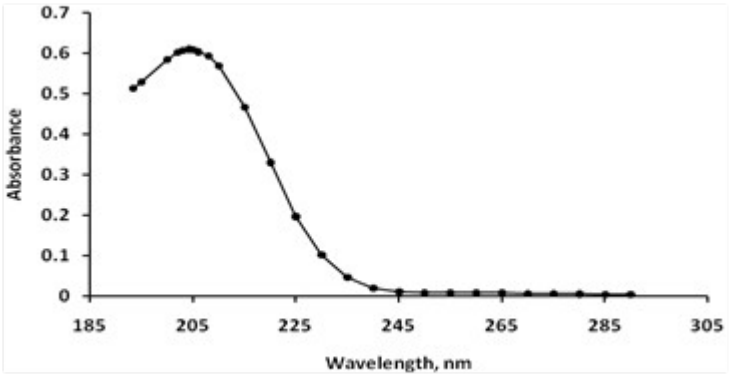


Figure 3: Absorption Spectrum of Perindopril ([PPE] = 4.53×10^{-6} M).

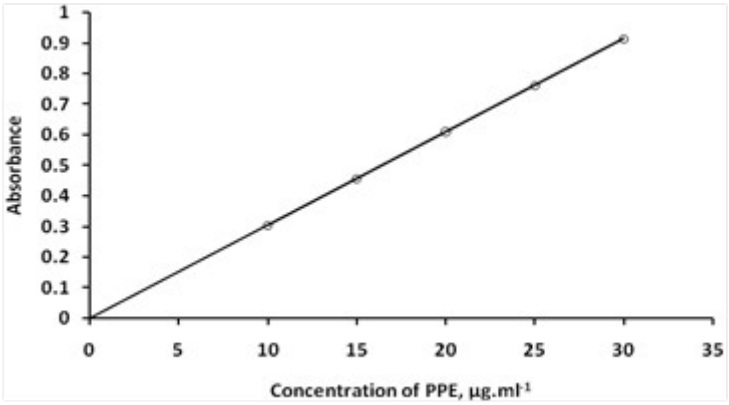


Figure 4: Calibrated Curve of Perindopril ([PPE] = [4.53×10^{-6} M]).

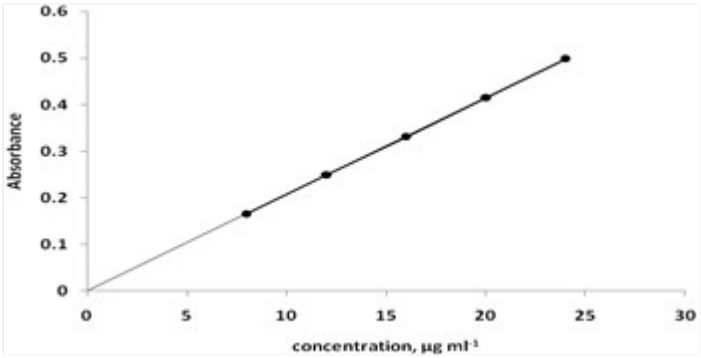
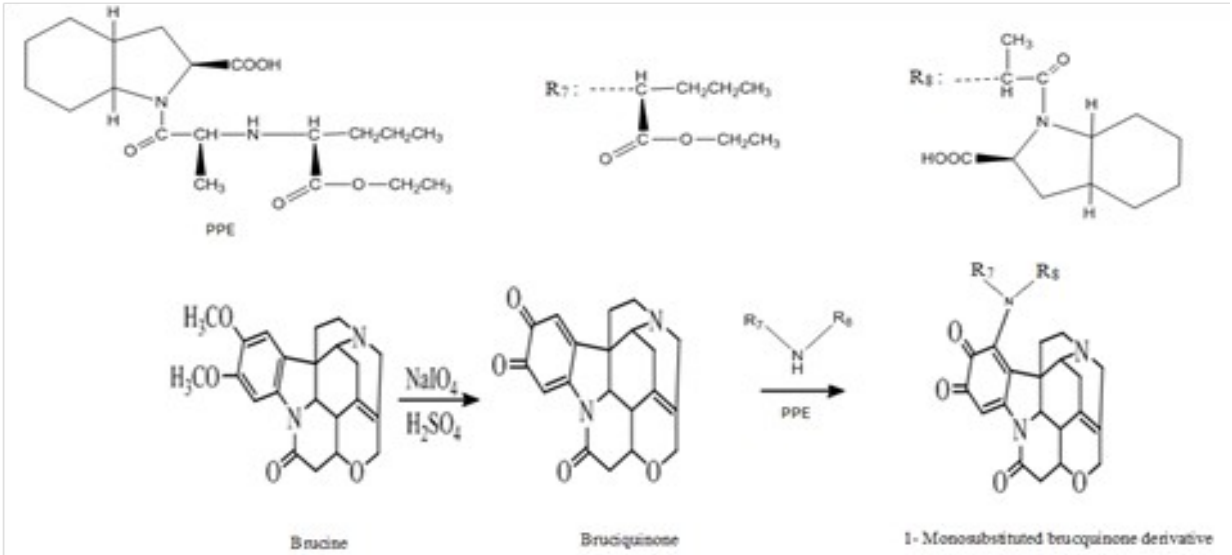


Figure 5: Beer's law plot of PPE-Brucine (BCN)-$NaIO_4$ method ([BCN] =6.08×10^{-4} M; [$NaIO_4$] = 5.61×10^{-4} M; [H_2SO_4] = 1.84×10^{-4} M).



Scheme 1: Oxidative coupling reaction of perindopril erbumine with brucine-periodate.

Table 1: Validation of PPE-BCN the method.

Wavelength	520 nm
Molar absorptivity	9.16×10^3 L $mol^{-1}cm^{-1}$
Beer's Law limits	8 – 24 µg mL^{-1}
Limit of detection	1.0×10^{-2} µg mL^{-1}
Correlation coefficient	0.9999
Limit of quantification	1.1×10^{-1} µg mL^{-1}
Relative Standard Deviation*	0.56
% of error	
0.01 Confidence Limits	0.59
0.05 Confidence Limits	0.93

***Estimation of six observations.

RESULTS AND DISCUSSION

Absorption Spectrum of Perindopril-Brucine System

For the selection of analytical wavelength, the sample solution containing fixed quantity of drug (PPE), brucine solution, and other furnished variables as outlined in the analytical procedure was scanned in the visible wavelength region 350 – 800 nm against the reagent blank. The spectrum of the oxidative coupling product observed to have maximum wavelength at 520 nm which was selected for the analysis. The spectrum of reagent blank against isopropanol solvent was also measured (Figure 2).

Mechanism for Oxidative Coupling Product Formation Reaction

In the present investigation, the chemistry of colored species was studied. Sastry et al reported brucine-periodate reagent for the spectrophotometric determinations of sulfur-containing compounds and tryptophan (30). In the present investigation, the bruciquinone formed from brucine and periodate undergoes nucleophilic attack on the most electron-rich portion of the coupler (-NH-) in PPE (free from erbumine) to give 1-monosubstituted bruciquinone derivative which is presented in Scheme 1.

Validation of Analytical Data

Following (ICH) guidelines (31), the developed method was validated for various optical and regressive characteristics such as slope, intercept, correlation coefficient, LOD, LOQ sensitivity, RSD, and percentage of error.

Linear Relationship

The developed analytical procedure showed the linear relationship within the Beer's law range (8 – 24 µg mL^{-1}). Beer's law plot (n = 6) was measured under optimum conditions and found consisting of linearity with a high correlation coefficient (r) value 0.9999. The standard calibration curve drawn at five concentration levels. Results are given in Table 1.

Limits of LOD and LOQ

Limit of detection (LOD) and Limit of quantification (LOQ) were calculated using the below given expressions.

$$(LOD) = 3.3 \times Sa / b \quad (1)$$

$$(LOQ) = 10 \times Sa / b \quad (2)$$

Where b is the slope of the calibrated curve and Sa is the standard deviation of the intercept.

Sensitivity

The sensitivity of the developed method was measured in terms of molar absorptivity (εmax), limit of detection, and limit of quantification. Results of molar absorptivity, LOD, and LOQ are 9.16×10^3, 1.0×10^{-2} µg.mL^{-1}, and 1.1×10^{-1} µg.mL^{-1}, respectively (Table 1).

Sandell's Sensitivity

It is measured as "smallest weight of substance that can be detected in column of unit cross section". The Sandell's sensitivity is the concentration of the analyte (in µg mL^{-1}) which will give an absorbance of 0.001 in a cell of path length 1 cm. Units of Sandell's sensitivity (S) is given as µg cm^{-2} , and its value was found as 4.82×10^{-2} µg.cm^{-2}

Selectivity of Method

Selectivity for the assay analytical procedure was calculated by analyzing standard drug sample solution in the presence of excipients that are commonly present in formulations. The excipients namely microcrystalline cellulose, magnesium stearate, lactose, and titanium dioxide. The results of developed method indicated that no interference from the excipients present in formulations.

Precision

Precision of the analytical procedure expresses "the closeness of agreement between a series of measurement obtained from six determinations of sample solution under prescribed conditions". The intra-day precision was calculated by measuring "absorbance of sample solution of particular concentration within the linearity range at regular intervals on the same day". The inter-day precision

was calculated by measuring "absorbance of sample solution of same concentration at a fixed time in three consecutive days". The precision of developed method expressed in terms of relative standard deviation (RSD) for the smallest concentration indicating good precision. Results are presented in Table 1.

Accuracy

Accuracy of analytical procedure was calculated as "percentage of error between the measured mean concentrations and taken concentrations". The accuracy and precision was checked by comparing the result of developed and UV reference method statistically through Student's 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. % Recovery ± SD values were in the range of 99.16-100.7 (+ 0.41 - ± 0.8) (n=3) which indicates the accuracy of developed method. Results of accuracy are given in Table-2. The interference of other excipients that are commonly present in dosage forms is found to be negligible. The proposed method is found to be sensitive and more accurate within the Beer's law range with reference to correlation coefficient value compared to literature methods (Table 3).

Table 2: Estimation of Perindopril-Erbumine (PPE) in formulations.

Formulation Batches	Quantity (mg)	Quantity found by PPE-BCN method (mg)*	95% confidence limit values F - Test	95% confidence limit values t-Test	UV reference Value
I	2	2.01 ± 0.021	1.86	0.87	2.00 ± 0.005
II	4	3.99 ± 0.028	2.69	0.18	4.00 ± 0.003
III	4	3.96 ± 0.16	1.15	1.70	4.00 ± 0.017
IV	8	8.05 ± 0.06	3.86	0.61	7.99 ± 0.021

***Average value of six observations.

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 recommended 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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