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Article

Determination of perindopril erbumine by oxidative coupling reaction using 2, 6-dichloroquinone-4-chlorimide

Manikya Sastry Thuttagunta[*1], Ramakrishna Karipeddi[2]

[1]Department of Chemistry, Gayatri Vidya Parishad College of Engineering (Autonomous), Kommadi Junction, Madhurawada, India, [2]Department of Chemistry, Institute of Science, GITAM (Deemed to be University), Rushikonda, India

Perindopril erbumine (Perindopril tert-butylamine salt) is a potent angiotensin-converting enzyme (ACE) inhibitor. It is used to treat the patients with hypertension and heart 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 colour by forming an oxidative coupling reaction between drug (PPE) and reagent such as 2, 6-dichloroquinone-4-chlorimide (DCQC). The formed colored species were measured at lmax=520 nm. The developed method showed linearity within the concentration limits of 25-75 µg mL^{-1}. The linear correlation coefficient (r) and molar absorptivity were found to be 0.9999 and 3.285 x 10^3 $mol^{-1}cm^{-1}$. % Recovery ± SD values were in the range of 99.69 - 100.51 (+ 0.42 - ± 0.41) (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 t-test and F-tests at 95% confidence limits with (n-1) degrees of freedom. The validity parameters of proposed method were calculated by ICH guidelines.

Keywords: Spectrophotometry. Perindopril erbumine. Gibb's reagent (DCQC). Formulation.

INTRODUCTION

Pharmacodynamic agents are generally used as depressants, blocking agents, antianginal, anticoagulants, and antihypertensive agents, anti-acne and ACE inhibiting agents, etc (Sweetman, 2009). 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-indo-lincarboxylic acid, 1-ethyl ester with tertiary-butylamine* (1:1)*"* (Figure -1).

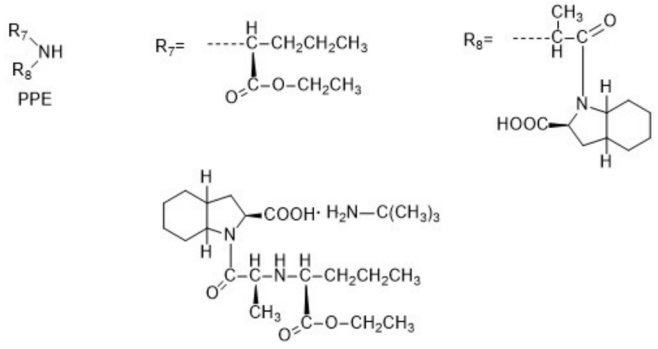


FIGURE 1 - Structure of perindopril erbumine (PPE).

The drug perindopril erbumine is listed in (British Pharmacopeia commission, 2009). Remington (Joseph, Alfonso, 2000) and physician's desk reference (Montvale, 2000)**.** A survey of the literature revealed that UV (Nayak, Pillai*,* 2011; Kale *et al.,* 2011), HPLC(El-Gizawy *et al.,* 2014; Gizawy *et al.,* 2013; Pattan, *et al.,*2013; Singh *et al*., 2011), RP-HPLC(Chaudhary,

***Correspondence:** M. S. Thuttagunta. Department of Chemistry. Gayatri Vidya Parishad College of Engineering (Autonomous). Kommadi Junction, Madhurawada, Visakhapatnam, 530047, India. E-mail: tmsastry@yahoo.com. ORCID 0000-0003-3003-0158

Patel, Chaudhary 2010; Mastannamma *et al*., 2016; Dugga, Peraman, Nayakanti, 2014; Joseph, Philip, 2011; Patel *et al.,* 2011; Prajapati *et al.,* 2011; Prameela Rani, Bala Sekaran, 2009), spectrofluorimetric (Fael Sakur 2015a.; Fael Sakur 2015b), kinetic spectrophotometric (Rahman, Rahman, Haque, 2017; Rahman, Anwar, Kashif, 2006), LC-MS(Deepak, *et al.,*2006) and GC-MS(Maurer, Kraemer, Arlt,1998) methods were reported for the estimation of PPE. There are limited spectrophotmetric methods (Sridevi,Jahnavi, Sekaran, 2012; Rahman,Rahman,Khatoon,2012; Rahman, Rahman 2011; Prameela Rani *et al.,* 2012) are reported for the assay of perindopril erbumine (PPE). In an attempt to develop and validate the spectrophotometric method for PPE in bulk form and formulations, the authors proposed a simple method using 2, 6-dichloroquinone-4-chlorimide (DCQC) as a chromogenic reagent.

MATERIAL AND METHODS

Chemicals and reagents

Perindopril erbumine (PPE) (Glenmark Pharmaceuticals Ltd., India). Formulations namely Perigard-DF (Glenmark Pharmaceuticals Ltd., India), Coversyl (Serdia Pharmaceuticals Pvt Ltd., India). Coversyl plus (Serdia Pharmaceutical Ind. Ltd., India), and Aceon (Solvay Pharmaceuticals Inc.) were procured from the local market. 2, 6-dichloroquinone-4-chlorimide (DCQC) (Loba, Mumbai, India), isopropyl alcohol (IPA), sodium hydroxide and chloroform (Qualigens Mumbai, India). All the chemicals and solvents procured for this study are of analytical grade.

Preparation of bulk drug and Gibb's reagent (DCQC) solution

Dissolved 100 mg of bulk drug perindopril erbumine in a minimum quantity of sodium hydroxide (0.1 $molL^{-1}$) solution followed by dilution to 100 ml with distilled water to prepare the standard stock solution (mg/ml). The released free erbumine was extracted with 10.0 ml chloroform. The aqueous solution free from erbumine was used as a stock solution. It is further diluted stepwise with distilled water to obtain working standard solutions of concentration (250 µg mL^{-1})

2, 6-dichloroquinone-4-chlorimide (DCQC) solution (Loba; 0.2%, 9.52 x 10^{-3} mol L^{-1}) was prepared by dissolving 20 mg of Gibb's reagent (DCQC) in 100 ml of isopropyl alcohol (IPA). AR grade of isopropyl alcohol ((IPA, Qualigens) solvent was used for the preparation of reagent and dilution of the test solution.

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

Procedure for formulations

Coversyl (Serdia Pharmaceuticals 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 the 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 sodium hydroxide (0.1 $molL^{-1}$) using separating funnel. Stock solution (mg/mL) was prepared to dilute the aqueous alkali extract to 100 mL with deionized water. Working standard solution (250 µg mL^{-1}) 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 distilled water (100 mL) to prepare a stock solution (mg/mL). Working standard solution concentration (100 µg mL^{-1}) was prepared from an aliquot portion (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.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 deionized water as blank. The concentration of the drug sample was calculated using its calibration curve (Figure.3). The UV absorption method was chosen as a reference method.

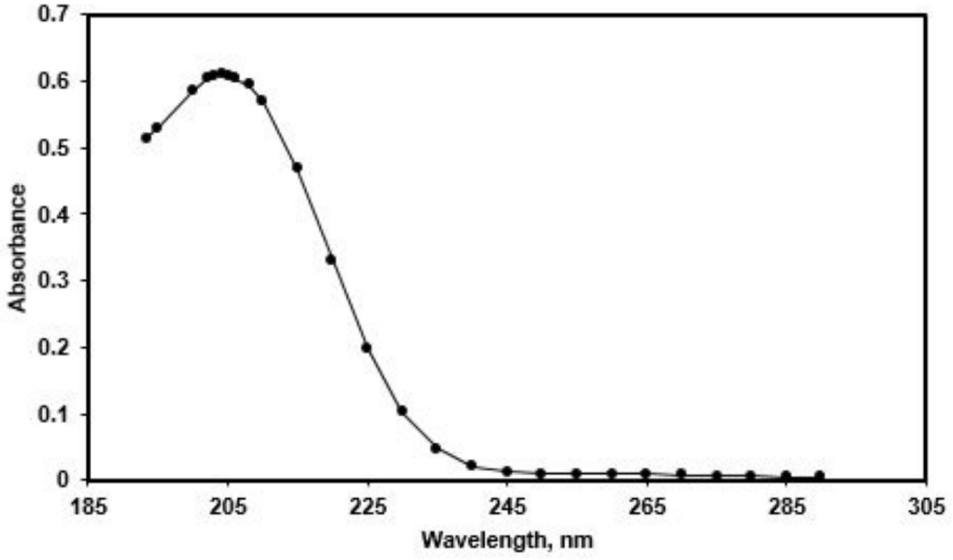


FIGURE 2 - Absorption spectrum of perindopril ([PPE] = 4.53×10^{-6} M).

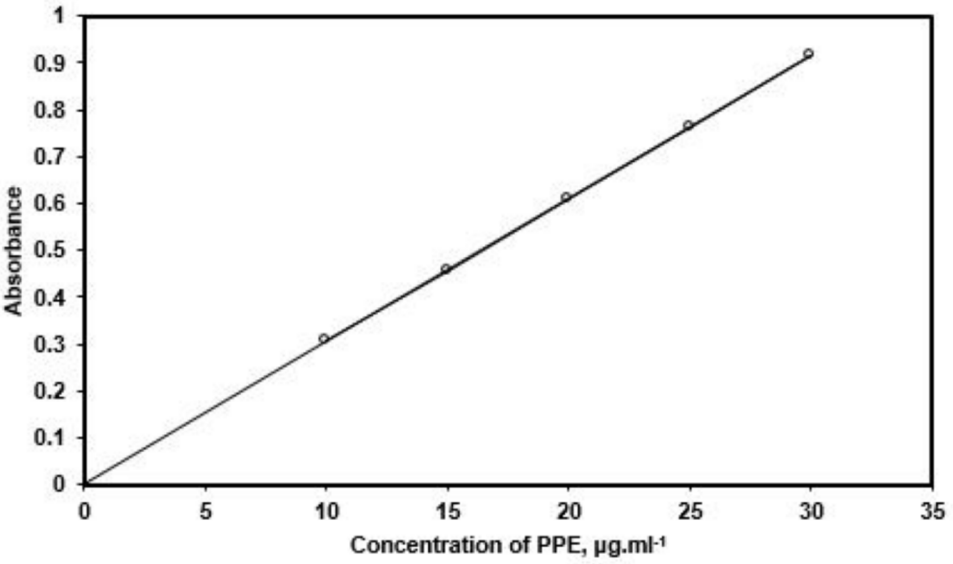


FIGURE 3 - Calibrated curve of perindopril ([PPE] = [4.53×10^{-6}M]).

Protocol of the proposed method

Into a series of separating funnels (100.0 mL), a portion of working standard bulk drug solution concentration 250 µg mL^{-1} (10-5.0 mL), 1.0 mL of 9.52 x 10^{-3} $molL^{-1}$ DCQC was added and volume made up to 10.0 mL with isopropanol and kept it on a hot water bath for 20 min, then cooled to room temperature and the volume again made up to 10.0 mL with isopropanol. The absorbances of the colored species were measured at 520 nm against a reagent blank prepared simultaneously. The constancy of color species was found to be stable for 30 min. A calibration curve was drawn to calculate the amount of drug present (Figure 4).

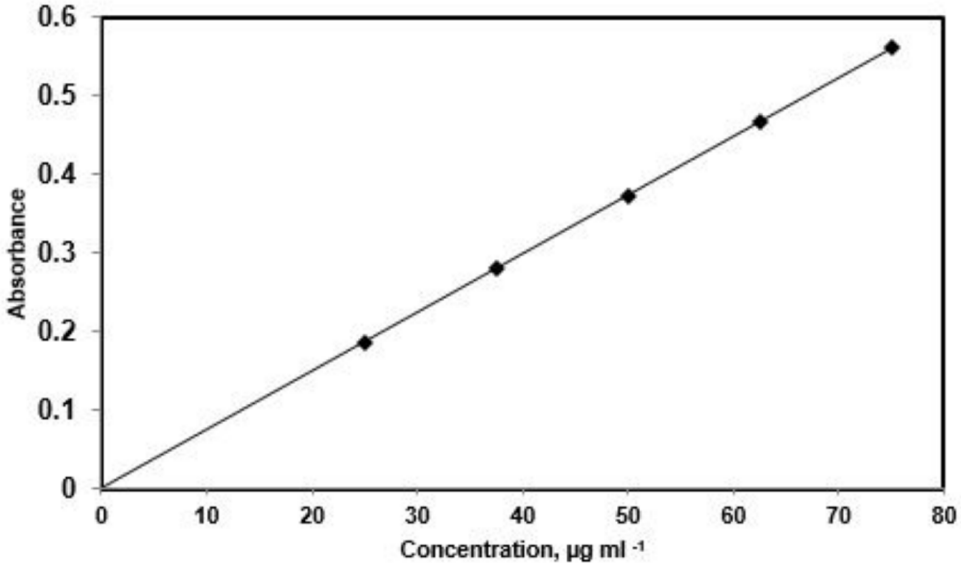


FIGURE 4 - Calibrated curve of PPE-DCQC method.

[PPE] = [1.13×10^{-4} M] and [DCQC] = [9.52×10^{-4}M].

RESULTS AND DISCUSSION

Absorption spectrum of perindopril-DCQC system:

For the selection of analytical wavelength, the sample solution containing a fixed quantity of drug, DCQC 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 a maximum wavelength at 520 nm which was selected for the analysis. The spectrum of reagent blank against isopropanol solvent was also measured (Figure 5).

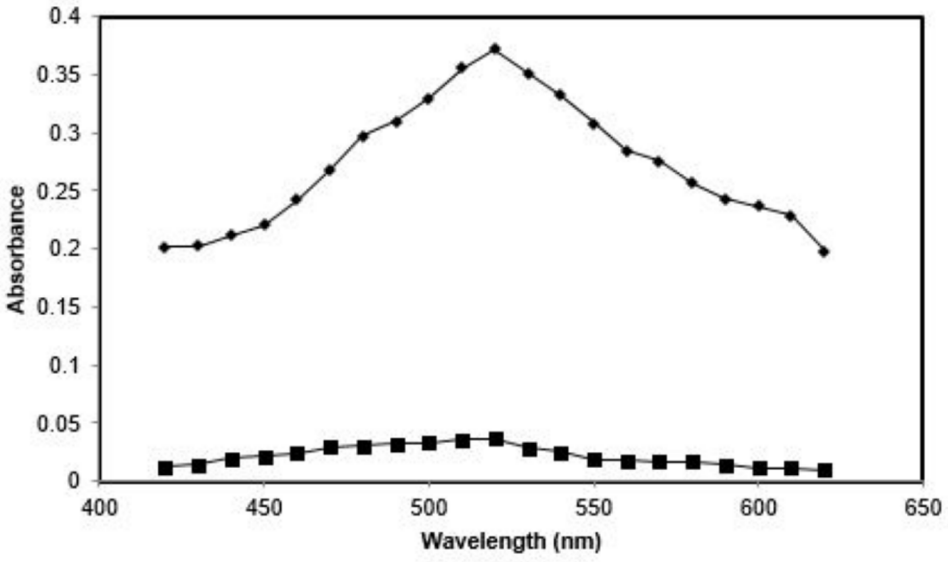


FIGURE 5 - Absorption spectra of 2, 6- dichloroquinone chloride [DCQC] and reagent blank.

Absorption spectrum oxidative coupling product of PPE-DCQC ([PPE]= [1.13×10^{-4} M] and [DCQC]= [9.52×10^{-4}M].
Absorption spectrum of reagent blank vs isopropanol

Optimum conditions

The responses of several factors like the concentration of the DCQC, stability period of the complex formed, intensity of the colored species formed, were studied (Massart *et al.,* 1988). The optimum conditions identified for the proposed method are 0.75-1.5 mL (0.0.71 –1.43×10^{-3} molL^{-1}) of the DCQC solution, laboratory temperature of (70 - 80^{0}C) and time required for colour development 15-30 minutes. In this method, 1.0 mL of (9.52×10^{-3} mol L^{-1}) DCQC, and 20 minutes essential for the highest color growth were found to be optimum conditions. The stability period of the oxidative coupling product was found as 30 min, afterward the absorbance was found to decrease which may be due to the decomposition of the oxidative coupling product.

Validations of analytical data

Following (ICH) guidelines (Tripartite, 2005) the developed method was validated for various optical and regression 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 ((25 – 75 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 I

Limits of LOD and LOQ

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

$$(LOD) = 3.3\times S_a / b \quad (1)$$
$$(LOQ) = 10\times S_a / b \quad (2)$$

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 the limit of quantification. Results of Molar absorptivity, LOD and LOQ are 3.285 x 10^3 L $mol^{-1}cm^{-1}$, 1.866 x 10^{-1} µg $mL^{-1}$and 5.653 x 10^{-1} µg mL^{-1} (Table I)

Sandell's sensitivity

Sandell's sensitivity (µg/cm^2 /0.001 Abs unit) is measured as the "smallest weight of a substance that can be detected in a column of the unit cross-section". Sandell's sensitivity is the concentration of the analyte (in µg mL^{-1}) which gives an absorbance of 0.001 in a cell of path length 1 cm. Sandell's sensitivity value is found to be 1.344 x 10^{-1} µg/cm^2

The selectivity of method:

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

Precision

The precision of the analytical procedure expresses "the closeness of agreement between a series of measurements 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 the developed method expressed in terms of relative standard deviation (RSD) for the smallest concentration indicating good precision. Results are presented in Table I.

Accuracy

Accuracy of analytical procedure was calculated as "percentage of error between the measured mean concentrations and taken concentrations". The accuracy and precision were checked by comparing the result of the developed 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 (Massart *et al.,* 1988) of 2.57 and 5.05 respectively. % Recovery ± SD values were in the range of 99.69 - 100.51 (+ 0.42 ± 0.41) (n=3) which indicates the accuracy of the developed method. Results of accuracy are given in Table II.

TABLE I - Validity parameters of perindopril-DCQC system

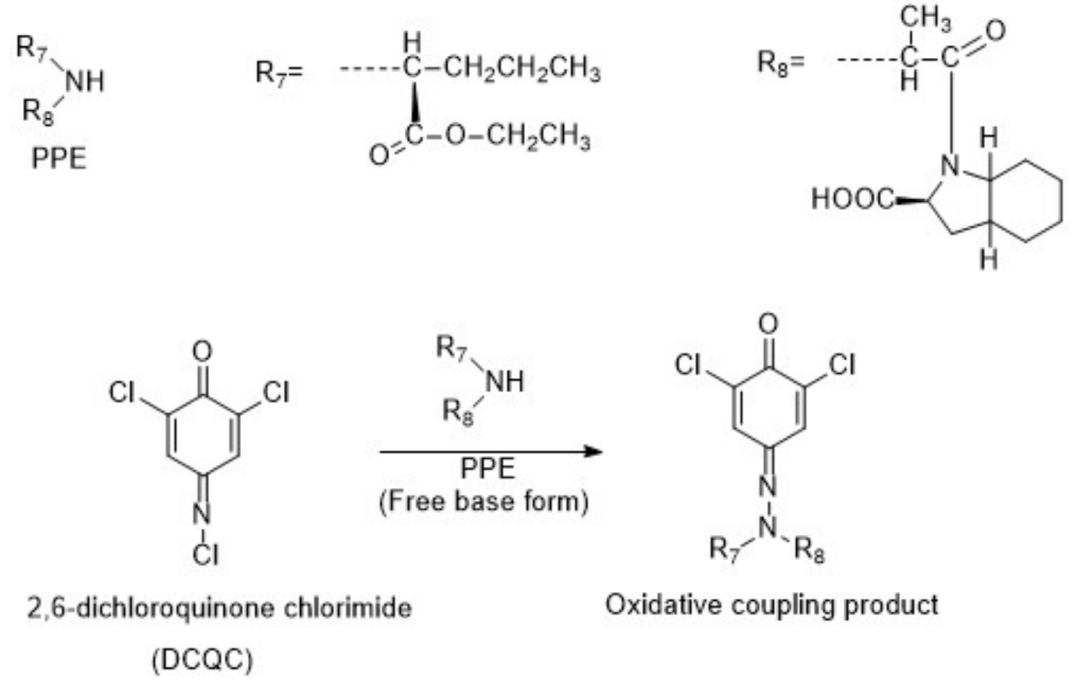
Optimum wavelength (l_{max})	**520 nm**
Beer's Law limits	25-75 µg mL^{-1}.
Molar absorptivity (ε_{max})	3.285 x 10^3 L $mol^{-1}cm^{-1}$
Limit of detection (LOD)	1.866 x 10^{-1} µg mL^{-1}.
Limit of quantification (LOQ)	5.653 x 10^{-1} µg mL^{-1}.
Sandell's sensitivity (µg. cm^{-2} /0.001 Abs unit)	1.344 x 10^{-1} µgcm^{-2}
Relative Standard Deviation(RSD)@	
Inter day precision	0.28
Intraday precision	0.50
Percentage of error (%)	
Confidence limit at 0.05 level	0.53
Confidence limit at 0.01 level	0.83

TABLE II - Assay of perindopril erbumine (PPE) in pharmaceutical formulations

Formulation Batches	Quantity taken (mg)	Quantity found by UV absorption method (mg)	Quantity found by developed method (mg)	95% Confidence limit values F -Test (Tabulated value = 5.05)	95% Confidence limit values t-Test (Tabulated value = 2.57)	% Recovery
Batch I	2	2.00 ± 0.01	2.00 ± 0.02	2.76	0.06	99.87±0.77
Batch II	4	3.99 ±0.01	3.99±0.02	1.88	0.12	99.69±0.42
Batch III	4	3.99 ±0.02	3.99±0.02	1.09	0.25	99.89.2±0.38
Batch IV	8	8.04 ±0.05	8.04±0.03	2.7	0.22	100.51± 0.41

Proposed scheme for the formation of coloured species

Earlier workers estimated the phenols or amines using 2, 6-dichloroquinone-4-chlorimide (DCQC) reagent (Rao *et al*., 1985). In the present investigation, the chemistry of color species is studied and found that the iminio group in perindopril couples directly with N-Cl in DCQC to get the colored product. The probable scheme for the formation of oxidative coupling product between drug and DCQC is presented in scheme 1.



SCHEME 1 - Formation of oxidative coupling product between drug (PPE) and DCQC

CONCLUSION

The proposed method is more sensitive than the reported methods. The sensitivity of the technique lies only with 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.

CONFLICT OF INTEREST

None

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