APPLICATION OF AN ION PAIR ASSOCIATION COMPLEX FORMATION REACTION FOR THE ESTIMATION OF ACE INHIBITOR IN FORMULATIONS

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ABSTRACT

Novel and sensitive analytical technique was proposed for estimating perindopril (angiotensin-converting enzyme (ACE) inhibitor) in formulations using acidic dye as chromogenic reagent. The study was established on the development of colour species formation between perindopril erbumine and chromogenic reagent such as azocarmine G (ACG). Proposed method consists formation of an ion-pair association complex with azocarmine-G. The optical density was measured at λ_{max} of 540 nm. Regression analysis of proposed method showed that the linearity lies within the concentration limits of 20-100 µg mL^{-1}. Molar absorptivity (ϵ_{max}) values of developed method are found to be as 3.22 × 10^3 L mol^{-1} cm^{-1}. Investigated method was found to have linear correlation coefficient (r) value of 0.9999. Proposed method obeyed Lambert-Beer' law with reproducible results. Percentage recovery was found to be within the limits of 99.7 - 100.1 (± 0.41 SD, ± 0.57 SD). All the parameters were validated statistically.

Keywords: spectrophotometry, perindopril, azocaramine-G, angiotensin-converting enzyme, formulations.

INTRODUCTION

Bioactive compound affects a living organism, tissue, or cell and shows biological activity. The analysis of bioactive compounds is a vital part of life today. Drugs are an important class of bioactive compounds that need to develop more specific and sensitive procedures for their estimation based on functional group reactions with suitable chromogenic reagents. Pharmacodynamic agents are generally known as depressants, blockers, anticoagulants, antihypertensive agents, anti-acne, and ACE inhibitors. Perindopril erbumine (PPE) is a potent angiotensin-converting enzyme (ACE) inhibitor. ACE inhibitor works on hypertension, cardiovascular failure, diabetes mellitus, and kidney-related problems issues in patients. Literature survey found that HPLC [1 - 3], RP-HPLC [4, 5], spectrofluorimetric [6 - 9], UV and Visible spectrophotometric [10 - 15], kinetic spectrophotometric [16] methods were used for the determination of PPE. The literature reports also showed that analytical techniques for quantitative determination of perindopril was found in combination with other drug related substances [17]. Very few colorimetric procedures have been found in the literature. Because of its vital significance, there is a need to develop simple and fast analytical methods. As a result, the authors tried to create and validate sensitive spectrophotometric technique for formulations that used the chromogenic reagent azocarmine G (ACG). The structure of ACE inhibitor (Perindopril) is shown in the Fig. 1.

EXPERIMENTAL

Instruments Used

Shimadzu UV double beam spectrophotometer has been chosen for obtaining precise and accurate optical density measurements. In addition, to measure pH of the samples, a digital pH meter (Equiptronics, India) and an electrical balance (Dhona 200 D, India) to weigh all the materials were used.

Chemicals and reagents

All analytical grade chemicals and solvents are used for the analysis. All stock and reagent solutions were prepared using deionized water. The $5.5x10^{-4}$ mol Azocarmine G (ACG) solution was made by dissolving 50 mg of Azocarmine G in 100 mL of deionized water with the bare minimum of NaOH. To create the buffer solution for the PPE - ACG system, 711 mL of 0.1 mol L^{-1} hydrochloric acid were added to 289 mL of 0.1 mol glycine solution (14.62 g of NaCl and 18.76 g of glycine were allowed to mix in 250 mL of double deionized water). The pH of the resulting solution was then adjusted to 1.5. AR grade solvent such as chloroform (Qualigens) was used for the extraction of ion-pair association complex.

Bulk sample solution

The stock solution was generated by dissolving 100 mg of perindopril erbumine in a minimum of 0.1 mol L^{-1} sodium hydroxide. This stock solution was then diluted to 100 mL with deionized water. Chloroform was used to extract the liberated free erbumine (10 mL). The resulting erbumine-free aqueous solution was utilised as a stock solution. These stock solutions were used to generate standard working solutions with concentrations of 200 µg mL^{-1} for proposed method (PPE - ACG system).

Formulations

Different strengths of perindopril formulations were purchased from the local market. 100 mg of tablet powder was used for the extraction with chloroform (3 × 25.0 mL) and filtration. In a separating funnel, the filtrate was collected and extracted three times with 0.1 mol L^{-1} NaOH (3 × 5.0 mL). A stock solution (mg mL^{-1}) was made by diluting the aqueous alkali extract with deionized water to a volume of 100 mL. This stock solution was used to generate standard working solutions with concentrations of 200 µg mL^{-1} for proposed method (PPE-ACG system)

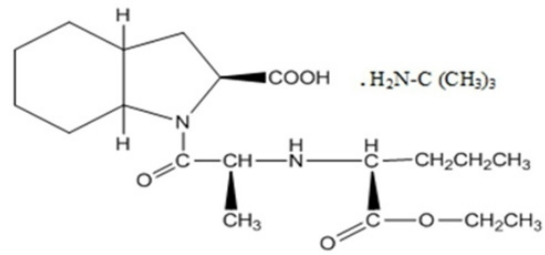


Fig. 1. Structure of ACE inhibitor perindopril erbumine.

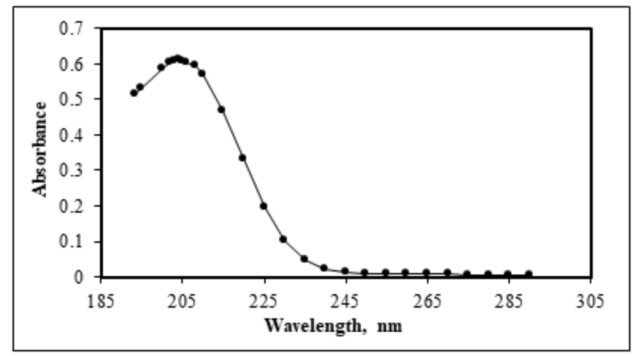


Fig. 2. Perindopril absorption spectrum (PPE = $4.53x10^{-6}$ mol L^{-1}).

Calibration curve - UV method (Reference method)

The stock solution (mg mL^{-1}) was created by dissolving 100 mg of the bulk drug sample in 100 mL of double-distilled water. Using the same solvent, a standard working solution with a concentration of 100 µg $mL^{-1}$was prepared using the same solvent. The absorption spectrum of the bulk drug sample was recorded against a reagent blank within the UV region using a Shimadzu double-beam spectrophotometer (Fig. 2).

A series of solutions were prepared by pouring 1.0 to 3.0 mL of a standard drug solution (100 g mL^{-1}) into 20.0 mL calibrated tubes. These are diluted with double distilled water to make 10.0 mL. At 204 nm, the optical densities of each sample solution were measured against reagent blank (double distilled water). The calibration curve between optical density and perindopril concentration was used to determine the drug's concentration (Fig. 3).

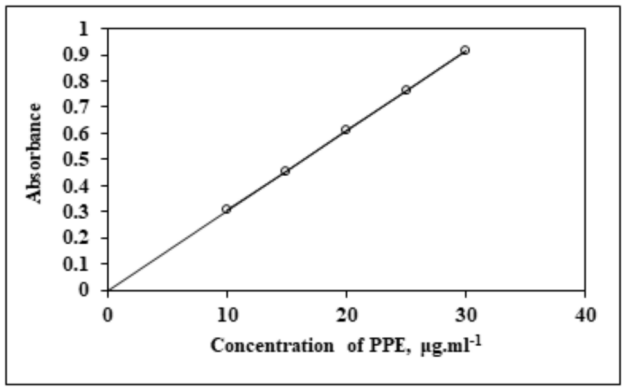


Fig. 3. Perindopril Calibration curve (PPE = $4.53x10^{-6}$ mol L^{-1}).

Protocol of proposed methods

Analytical Procedure for proposed method (PPE - ACG system)

Aliquots of the standard drug solution (1.0 - 5.0 mL, 200 µg mL^{-1}), 2.0 mL of the $5.5x10^{-4}$ mol L^{-1} azocarmine G solution, and 6.0 mL of the buffer solution (pH 1.5) were placed into 50.0 mL separating funnels to prepare a series of solutions. Each sample was diluted with distilled water to a volume of 15.0 mL, then 10.0 mL of the solvent chloroform was added. The separation funnel's contents were shaken for two minutes. After separating the two layers, the organic layer's absorbance was evaluated at 540 nm against a blank reagent. After 60 min, it was seen that the optical density of the colour species had decreased, which suggests that the coloured complex was breaking down. The calibration curve between optical density and perindopril concentration was used to determine the drug's concentration (Fig. 5).

RESULTS AND DISCUSSION

Identification of wavelength for analytical procedures

The visible area (350 - 750 nm) of the sample solution was scanned in comparison to the reagent blank. The sample solution contained a fixed amount of perindopril, azocarmine-G (an acidic dye), and other supplied variables, as indicated in the method. The absorption spectrum of the coloured species formed based on the ion-pair association complex shows maximum absorbance at 540 nm, and this wavelength has been selected for the analysis of method A (PPE - ACG system). The spectrum of the azocarmine - G (acidic dye), shows maximum absorbance whereas blank solution against chloroform shows negligible absorbance at this wavelength (Fig. 4).

Optimum conditions

The effects of several variables, including azocarmine-G (ACG) concentration (0.5 to $1.4x10^{-4}$ mol L^{-1}), the volume of extracting solvent, stability of ion-pair association complex formation time (1 to 60 min), intensity of coloured species produced, and the ratio of aqueous to $CHCl_3$ solvent, were investigated [18]. For the suggested technique A (PPE - ACG system), the following optimum conditions were established: 2.0

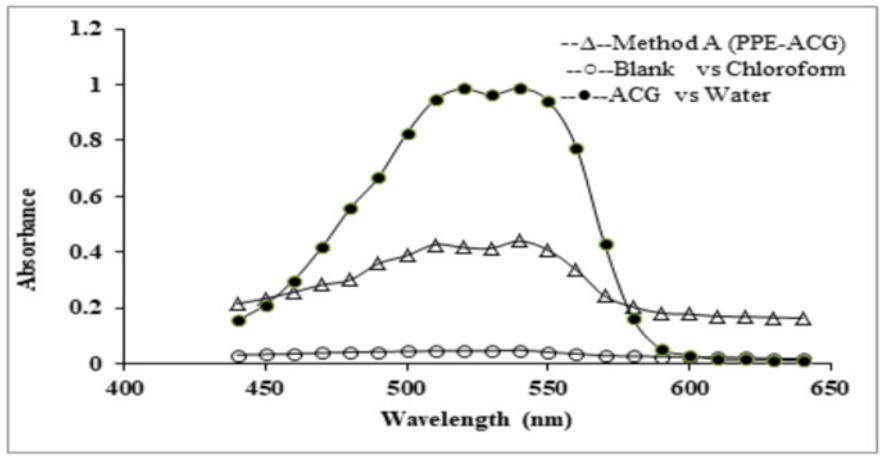


Fig. 4. Absorption spectrum of method A (PPE - ACG), blank vs chloroform, and azocarmine-G vs. water.-•- Color spectrum of (ACG = $5.5x10^{-4}$ mol L^{-1}); -Δ- ion pair association complex of the PPE - ACG system's spectrum, (PPE = $1.3587x10^{-4}$ mol L^{-1} and ACG = 1.1×10^{-4} mol L^{-1}); -○- absorption spectrum of reagent blank vs. chloroform.

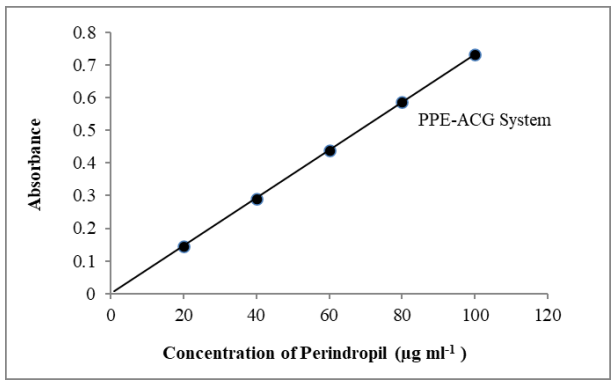


Fig. 5. Calibration curve of proposed method (PPE - ACG System).

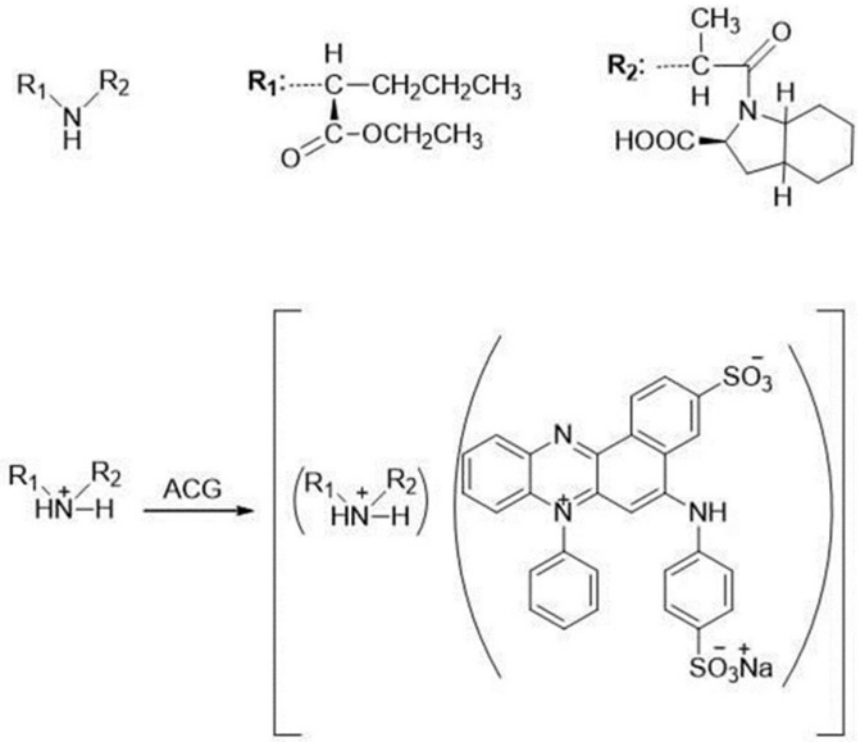
mL (0.57×10^{-4} mol L^{-1}) azocarmine-G, 1.0 mL glycine buffer (pH = 1.5) with 2 min agitation time at 28 ± 2^0C. The ion pair association complex is stable up to 60 min afterward; the absorbance slowly decreases, indicating the decomposition of the complex.

Chemistry of colored Complex

The probable sequence of ion-pair association complex formation reaction for method A (PPE -ACG system) in the presence of acid medium using buffer solution (pH = 1.5) was studied. Based on the analogy studies, the ion-pair association complex formation mechanism is explained [12]. The negative charge of the dye (azocarmine-G) and the positive charge that was seen on the nitrogen of the secondary amino group in the side chain of perindopril were held together by the electrostatic force of attraction. The result functions as a single molecule. The probable sequence of the mechanism of the reaction is given in Scheme 1.

Validations of analytical data

In accordance with the recommendations of the International Conference on Harmonization (ICH), the developed method was statistically validated [19]. For the proposed method (PPE - ACG system) the limit of linearity range was found to have 20 - 100 µg mL^{-1}.



Scheme 1: Mechanism of ion-pair association complex formation for (PPE-ACG System).

ε_{max} (L mol^{-1} cm^{-1}) and λ_{max} (nm) values were found to be $2.704x10^{-3}$ and 540. The suggested method had calibrated curve that was drawn at certain concentration levels and had linearity with a r^2 value of 0.9999. Molar absorptivity value was used to explain how sensitive the researched approach was. The suggested system, whose accuracy was justified by six (n = 6) assessments of the sample solution made under ideal circumstances. Relative standard deviation (% RSD) was used to explain them. Sandell's sensitivity, the limit of detection (LoD), and the limit of quantification (LoQ) were calculated for the developed technique. Tables 1 and 2 present the findings.

Accuracy was checked in terms of % recovery of the drug for the proposed analytical procedure. Recovery experiments were carried out by introducing a calculated quantity of drug to the pre-analysed formulations at different levels and determining the accuracy of the technique proposed. Values of % recovery ± SD was found between 99.7 - 100.1 (± 0.41, ± 0.57) (n = 3), for developed method (PPE - ACG system). Through student t- and student F- tests, the outcomes of the suggested approach (formulations) and UV reference method were compared. It was observed that no significant difference was noticed in between developed and UV reference method and found within the acceptable limits (Based on 95 % confidence limit values for student t- test (2.57) and student F-test (5.05) respectively. The results are given in Table 3.

The proposed methods were compared with literature methods and found to be more sensitive with reference to molar absorptivity, linear correlation coefficient (r), LoD, LoQ values [12, 13]. Precision and accuracy were calculated in terms of relative standard deviation (% RSD) and % recovery values. The results of RSD, and % recovery values of developed methods were found to be similar with the literature methods. The results are summarized in Table 4.

Table 1. Optical condition for proposed method (PPE - ACG system).

Wavelength (λ_{max})	540 nm
Molar absorptivity (ϵ_{max})	3.22×10^3 L mol^{-1} cm^{-1}
Limits of linearity range	20 -100 µg mL^{-1}
Limit of detection (LoD)	3.75×10^{-1} µg mL^{-1}
Limit of quantification (LoQ)	1.136 µg mL^{-1}
Standard error of estimation (Se)	7.958×10^{-4}
Sandell's Sensitivity	1.37×10^{-2}

Table 2. Results of validation parameters.

Slope (b)	7.345×10^{-3}
Standard deviation on slope (S_b)	1.258×10^{-5}
Intercept (a)	- 0.0007
Standard deviation on intercept (S_a)	8.347×10^{-4}
Linear correlation coefficient (r)	0.9999
Intra - day precision (% RSD)	0.4257
Inter - day precision (% RSD)	0.5654
0.01 Level of confidence limits	0.7007
0.05 Level of confidence limits	0.4468

Table 3. Application of proposed method (PPE -ACG System) for formulations.

Formulation batches	Quantity taken, mg	The amount detected by the reference (UV), mg	The amount detected by the present method, mg•	95 % Confidence limit values F -test@	95 % confidence limit values t- test$	% Recovery developed method*
I	2	2.00 ± 0.02	2.00 ± 0.01	3.5	0.43	99.7 ± 0.4
II	4	3.99 ± 0.02	3.99 ± 0.01	2.08	0.2	99.7 ± 0.3
III	4	3.99 ± 0.02	3.99 ± 0.01	2.07	0.22	99.9 ± 0.3
IV	8	8.04 ± 0.03	8.01 ± 0.05	1.92	2.5	100.1 ± 0.6

** Average value of three measurements (n=3).* *• Average value of six observations.*
@Tabulated value of 5.05 for student F - test. *$Tabulated value of 2.57 for student t - test.*

Table 4. Comparison of proposed method with literature methods.

Reagent used	Linearity range, μg mL^{-1}	Correlation coefficient, r	RSD, %	Recovery, %	LoD, μg mL^{-1}	LoQ, μg mL^{-1}	Ref.
BPB	5-125	0.9992	0.893	99.60	0.256	0.775	Sridevi et.al [12]
SFN-O	5-25	0.9993	0.57	100.1	0.23	0.70	Rani et.al [13]
BTB	2-20	0.9963	0.42	100.4	0.114	0.345	Rani et.al [13]
$KMnO_4$	2-20	0.9961	0.88	99.3	0.176	0.533	Rani et.al [13]
MTC	2-20	0.9999	0.60	99.7	0.188	0.569	Rani et.al [13]
ACG	20-100	0.9999	0.43	100.1	0.375	0.114	Proposed method

SFN-O: Safrranine-O, BTB: Bromothymol blue, MTC: Molybdenum(V) thiocyanate. BPB: Bromo phenol blue

CONCLUSIONS

Two accurate, precise and selective, analytical procedures were developed and validated for the assay of perindopril in formulations. The developed techniques were found to be the best among the literature methods in terms of stability of colour species formed, sensitivity and cost. The excipients commonly found in formulations or dosage forms did not intervene in the assay. Therefore, the discovered procedures can be employed in place of sophisticated tools in quality control laboratories for routine analysis of perindopril bulk and formulations.

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