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Original Article

VALIDATED SPECTROPHOTOMETRIC METHODS FOR DETERMINATION OF ENALAPRIL MALEATE IN PURE AND DOSAGE FORMS

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ABSTRACT

Objective: Simple, sensitive, precise, reproducible and validated visible spectrophotometric methods have been developed for the determination of an angiotensin converting enzyme inhibitor (ACE) drug, namely enalapril maleate (ENP) in pure and pharmaceutical dosage forms.

Methods: The methods are based on the formation of yellow colored ion-pair complexes between enalapril with two sulphonphthalein acid dyes, bromocresol purple (BCP) and bromophenol blue (BPB) at pH 2.8 and 3.0 using BCP and BPB, respectively followed by their extraction with chloroform. Several parameters such as pH, buffer type, reagent volume, sequence of addition and effect of extracting solvent were optimized to achieve high sensitivity, stability, low blank reading and reproducible results.

Results: The absorbance is measured at 408 and 414 nm using BCP and BPB reagents, respectively. The stoichiometric ratio of the formed ion-pair complexes was found to be 1:1 (drug: reagent) for both methods as deduced by Job's method of continuous variation. Under the optimum reaction conditions, linear relationships with good correlation coefficients (0.9993-0.9996) were found between the absorbance's and the concentrations of enalapril over the concentration ranges of 2.0–24 µg ml^{-1} and 2.0–28 µg ml^{-1} with limits of detection (LOD) of 0.39 and 0.45 µg ml^{-1}, using BCP and BPB methods, respectively. Various analytical parameters have been evaluated and the results have been validated by statistical data.

Conclusion: The proposed methods were validated in accordance with ICH guidelines and successfully applied to the determination of enalapril in pure and Dosage forms. Statistical comparison of the results obtained by applying the proposed methods with those of the official method revealed good agreement and proved that there were no significant difference in the accuracy and precision between the results.

Keywords: Enalapril maleate, Ion-pair complex, Bromocresol purple, Bromophenol blue, Spectrophotometry, Dosage forms.

INTRODUCTION

Enalapril maleate (ENP); (S)-1-[N-[1-(ethoxycarbonyl)-3-phenyl propyl]-L-alanyl]-L-proline hydrogen maleate belong to the class of a dicarboxylate containing group of angiotensin converting enzyme inhibitors (ACEIs) (fig. 1). Enalapril malate is widely used in the treatment of hypertension and some types of chronic heart failures [1, 2]. The official methods of analysis of ENP in pharmaceuticals are high-performance liquid chromatography (HPLC) in USP [1] and British Pharmacopoeia (BP) [2]. The analytical profiles of the drug have been reviewed [3].

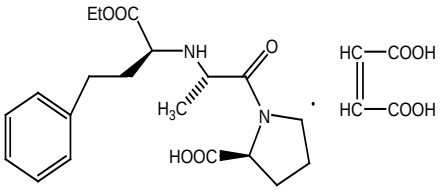


Fig. 1: Chemical structure of enalapril maleate (ENP)

A review of the literature revealed that several methods have been reported for the quantitative determination of ENP both in pharmaceutical preparations and in biological fluids, including high-performance liquid chromatography (HPLC) methods [4-14], potentiometric methods [15, 16], chemiluminescence method [17] and nuclear magnetic resonance spectroscopy (^{1}H-NMR) [18].

Most of the reporting procedures are not simple for routine analysis and require expensive or sophisticated instruments. Hence, it is always required to develop simple, fast, inexpensive analytical methods that can be readily adopted for routine analysis at a relatively low-cost to the different requirements of analytical problems.

Visible spectrophotometry, because of its simplicity and cost effectiveness, sensitivity and selectivity, fair accuracy, precision and easy access in most quality control laboratories, has remained competitive in an area of chromatography techniques for pharmaceutical analysis. Ultraviolet [19-21] and visible [22-37] spectrophotometric methods have been reported for the determination of ENP in pharmaceuticals. However, most of the reported visible spectrophotometric methods [22-37] suffer from one or the other disadvantage like the narrow range of determination, poor sensitivity, the use of heating, strict pH control, indirect method etc. as shown in table 1.

Ion pair extractive spectrophotometric procedures are popular for their sensitivity in the assay of drugs[38 46]. In the present work, two simple, accurate, sensitive, low-cost and validated extractive spectrophotometric methods based on ion-pair formation reaction between ENP and an anionic dyes such as bromocresol purple (BCP) and bromophenol blue (BPB) are proposed for the determination of ENP in pure form and pharmaceutical preparations.

MATERIALS AND METHODS

Apparatus

All absorption spectra were made using Kontron Unikon 930 (UV-Visible) spectrophotometer (German) with a scanning speed of 200 nm/min and a band width of 2.0 nm, equipped with 10 mm matched quartz cells. The pH values of different buffer solutions were checked using a Hanna pH-meter instrument (pH 211) (Romania) equipped with a combined glass-calomel electrode.

Materials and reagents

All chemicals and reagents were of analytical grade and used without further purification and all solutions were prepared fresh daily. Water has been always doubly distilled.

Table 1: Comparison between the reported methods for spectrophotometric determination of ENP

Method	Wavelength (nm)	Beer's law (µg ml^{-1})	Molar absorptivity (l mol^{-1} cm^{-1})	Detection limit (µg ml^{-1})	References
Potassium permanganate in neutral medium	340	2.0-12	1.8 x 10^4	0.028	[23]
Potassium permanganate in H_2SO_4 medium	550	7.0-70	3.8 x 10^3	0.115	
Potassium permanganate in acidic medium/					[24]
Acid red 73 (AR)	510	0.7-12,	4.96 x 10^4	0.197	
Amaranth dye [acid red 27] (AM)	521	0.5-7.6	6.49 x 10^4	0.138	
Acid orange 7 (AO)	484	0.6-9.5	5.09 x 10^4	0.147	
Bromothymol blue (BTB)	404	10-48		0.40	[25]
Molybdenum (V)-thiocyanate	468	10-80		0.18	
Molybdenum(V) thiocyanate	517	4.0-36	3.4×10^4		[26]
Molybdenum(V) thiocyanate/benzalkonium chloride	545	3.0-27	5.0×10^4		
Potassium iodate (KIO3)/(KI)	352	2.5-50		1.13	[27]
p-chloranilic acid (pCA) in 1, 4-dioxan-methanol medium,	510	20-560		3.07	
2, 3-dichloro 5, 6-dicyano 1, 4-benzoquinone (DDQ) in acetonitrile-1,4 dioxane medium	565	5.0-75		0.39	
Iodine	365	10-200		3.20	
Methyl orange	425	4.0-20			[28].
Palladium(II) chloride	362.8	50-300	1.3×10^3		[30]
Palladium(II), eosin/methylcellulose	550.6	8.0-56	2.7×10^4		
Copper(II), eosin	533.4	56-112			[31]
Copper(II), eosin/methylcellulose	558.8	19-32			
Iron(III), thiocyanate	436.6	60-132			
Bromothymol blue	410	10–160	3.414x10^3		[32]
FeCl3/Potassium Ferricyanide	778	10–60	3.656x10^4		
FeCl3/2, 2'–Bipyridyl	520	10-60	2.811x10^3		
Tropeolin 00/HCl	415	10-100	5.206 x 10^3	2.03	[33]
Potassium dichromate (0.1N)/50 % H2SO4	574	5–60		0.99	[34]
Potassium dichromate	610	25-900		7.59	[35]
Potassium permanganate	520	10-200		2.4	
Cu(II)/Bromothymol blue	426	200-500	-	9.907	[36]
Bromocresol purple (BCP)	408	2.0-24	7.714 x 10^3	0.39	Proposed
Bromophenol blue (BPB)	414	2.0-28	7.325 x 10^3	0.45	work

Materials

Standard ENP was obtained from Egyptian International of Pharmaceutical Industries Company (EIPICO), 10th of Ramadan City, Egypt.

Pharmaceutical formulations

All pharmaceutical preparations were obtained from commercial sources in the local markets.

- Acapril tablets labeled to contain (20 mg ENP/tablet) were purchased from Alpha Chem Advanced Pharmaceutical Industries Company (ACAPI), Bader Industrial City, Cairo, Egypt.

- Ezapril tablets labeled to contain (10 mg ENP/tablet) were purchased from El-Kahira Pharmaceutical and Chemical Industries Company, Cairo, Egypt.

- Enalapril tablets labeled to contain (20 mg ENP/tablet) were purchased from Egyptian International of Pharmaceutical Industries Company (EIPICO), 10th of Ramadan City, Egypt.

Preparation of stock standard solution

A stock standard ENP solution (100 µg ml^{-1}) and (1.0 × 10^{-3} mol l^{-1}) were prepared by dissolving an exact weight 10 and 49 mg of pure drug in bidistilled water and adjusted to 100 ml with bidistilled water in 100 ml measuring flask. The standard solutions were found stable for at least one week without alteration when kept in an amber colored bottle and stored in a refrigerator when not in use. Serial dilution with the same solvent was performed to obtain the appropriate concentration range.

Reagents

All reagents and solvents used were of analytical-reagent grade. Bromocresol purple (BCP) and bromophenol blue (BPB) (BDH Chemicals LTD, Poole, England) and used without further purification. Stock solutions (1.0×10^{-3} mol l^{-1}) of reagents were prepared by dissolving the appropriate weight of each reagent in 5.0 ml of 96% ethanol and diluted to 100 ml in a calibrated flask with bid stilled water. These solutions were stable for at least one week if kept in the refrigerator.

Series of buffer solutions of NaOAc–HCl (pH=1.99-4.92), NaOAc–AcOH (pH=3.4-5.6) and potassium hydrogen phthalate–HCl (pH=2.0-7.0) were prepared by following the standard methods [47]. The pH of each solution was adjusted to an appropriate value by the addition of 0.2 mol l^{-1} hydrochloric acid or sodium hydroxide with the help of the pH meter. Freshly prepared solutions were always employed. Chloroform and dichloromethane (BDH), anhydrous sodium sulfate (Prolabo), ethanol (BDH).

General procedures

Accurately measured aliquots (0.2–2.8 ml) of the standard ENP solution (100 µg ml^{-1}) were transferred to 10 ml measuring flasks. A volume 2.0 ml acetate buffer of pH 2.8 and 3.0 using BCP and BPB, respectively was added. Then add 2.5 ml of 1.0 × 10^{-3} mol l^{-1} BCP or BPB reagent solution. The total volume of each solution was completed to 10 ml with bidistilled water. The formed ion associate complexes were extracted with 10 ml chloroform by shaking for 2.0 min, then allowed to stand for clear separation of the two phases and the chloroform layer was passed through anhydrous sodium sulfate. The absorbance of the yellow colored ion-pair complexes were measured at 408 and 414 nm using BCP and BPB, respectively against corresponding reagent blank similarly prepared. All measurements were made at room temperature (25±2 °C). In both methods, a standard curve was prepared by plotting the absorbance values versus concentrations of drug. A linear equation for the standard curve was calculated by linear regression.

Applications to pharmaceutical formulations

The contents of twenty tablets were crushed, finely powdered, weight out and the average weight of one tablet was determined for each drug. An accurate weight equivalent to 10 mg ENP was transferred into a 100 ml calibrated flask, dissolved in bidistilled

water with shaking for 5.0 min and filtered through a sintered glass crucible (G_4). The filtrate was diluted to 100 ml with bidistilled water in a 100 ml measuring flask to give 100 µg ml^{-1}stock solutions. Aliquot of the cited solutions was taken and analyzed as described under the above recommended procedures for construction of calibration curves. For the proposed methods, the content of tablets was calculated using the corresponding regression equation of the appropriate calibration graph. The method of standard addition was used for the accurate determination of ENP content.

Stoichiometric relationship

The stoichiometric ratios of the ion-associates formed between ENP and the reagents were determined by applying the continuous variation [48] and the molar ratio [49] methods at the optimum wavelengths of maximum absorbance. In continuous variation method, an equimolar solution was employed: a 1.0 x 10^{-3} mol l^{-1} standard solution of ENP and 1.0 x 10^{-3} mol l^{-1} solution of dye was used. A series of solutions was prepared in which the total volume of ENP and the dye was kept at 2.0 ml. The drug and reagent were mixed in various complementary proportions (0.2:1.8, 0.4:1.6, 0.6:1.4, 0.8:1.2, 1.0:1.0, 1.2:0.8, 1.4:0.6, 1.6:0.4, 1.8:0.2) and completed to volume in a 10 ml calibrated flask with the appropriate solvent for extraction following the above mentioned procedure. In the molar ratio method, the concentrations of ENP are kept constant to 1.0 ml of (1.0 x10^{-3} mol l^{-1}) while that of dye (1.0 x10^{-3} mol l^{-1}) is regularly varied (0.2–2.4 ml). The absorbance of the prepared solutions was measured at the optimum wavelength for each complex.

RESULTS

Absorption spectra

The nitrogenous drugs are present in positively charged protonated forms and anionic dyes of sulphonpthalein group present mainly in anionic form at a pH ≥ 2.5. So when treated with an acid dye at a pH range (2.5-5.0) of acidic buffer solutions, a yellow ion-pair complex which is extracted with chloroform is formed. The absorption spectra of the ion-pair complexes, which were formed between ENP and (BCP or BPB) reagents were measured in the range 350–550 nm against the blank solution. The maximum absorbance's of ion-pair complexes show at 408 and 414 nm using BCP and BPB, respectively (fig. 2).

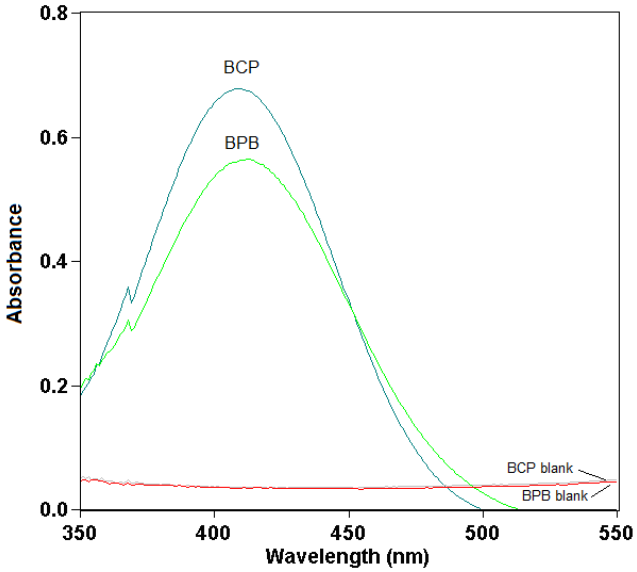


Fig. 2: Absorption spectrum of ion-pair complexes of ENP and (1.0 x 10^{-3} mol l^{-1}) BCP and BPB reagents against reagent blank

Optimum reaction conditions for complex formation

The optimization of the methods was carefully studied to achieve the complete reaction formation, highest sensitivity and maximum absorbance. Reaction conditions of the ion-pair complex were found by studying with preliminary experiments such as pH of buffer, the type of organic solvent, volumes of the dye, reaction time and temperature for the extraction of ion-pair complexes.

Effects of pH on ion-pair formation

The effect of pH on the drug–reagent complex formation was studied by extracting the colored complexes in the presence of various buffers such as NaOAc–HCl (pH 1.99-4.92) and NaOAc–AcOH (pH 3.6-5.6). It was noticed that the maximum color intensity and highest absorbance value were observed in NaOAc-HCl buffer of pH 2.8 and 3.0 for BCP and BPB method, respectively (fig. 3). Buffer volume was determined by applying the same experiment and variation the volume regularly (0.5-4.0 ml). The higher absorbance value and reproducible results were obtained by using 2.0 ml of buffer solutions.

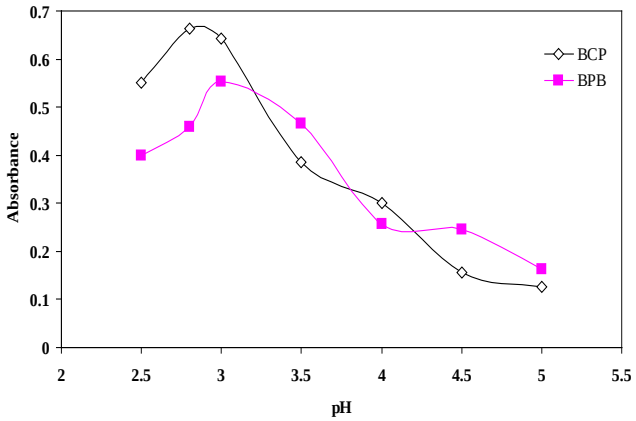


Fig. 3: Effect of pH of buffer solution on ion pair complex formation between (24 µg ml^{-1}) ENP and (1.0 x 10^{-3} mol l^{-1}) BCP and BPB reagents

Effects of reagent concentration

The effect of the reagent was studied by measuring the absorbance's of solutions containing a fixed concentration of ENP and varied amounts of the respective reagents 1.0×10^{-3} mol l^{-1} (BCP or BPB) from 0.5–4.0 ml. The maximum color intensity of the complex was achieved with 2.5 ml of (1.0 × 10^{-3} M) (BCP or BPB) reagent solutions. After this volume, the absorbance remains constant by increasing the volume of the reagents (fig. 4). So an excess of reagents has no effect on the determination of the drug.

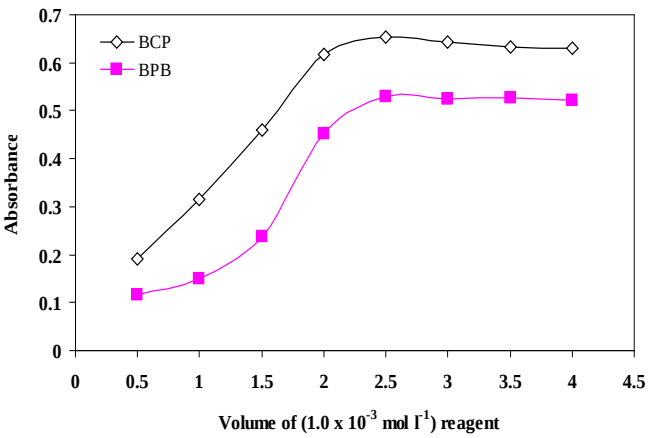


Fig. 4: Effect of volume of (1.0 ×10^{-3} mol l^{-1}) BCP and BPB reagent on the ion pair complex formation with (24 µg ml^{-1}) ENP

Effect of extracting solvent

Different organic solvents as dichloromethane, carbon tetrachloride, chloroform and ether were tested as extractive solvents for the proposed methods. Chloroform was preferred to other solvents for its selective and obtained the highest absorbance with chloroform. It was also observed that only one extraction with total volume 10 ml solvent was adequate to achieve a quantitative recovery of the complexes, maximum absorbance intensity and considerably lower extraction ability for the reagent blank and the shortest time to reach the equilibrium between both phases.

Effect of shaking time and temperature

The optimum shaking time was investigated by shaking from 0.5-5.0 min. Maximum and constant absorbance value was obtained when extracted after 1.5 min shaking. Therefore, shaking time of 2.0 min was maintained throughout the experiment. The effect of temperature on colored complexes was studied by measuring the absorbance values over the temperature range 20-35 °C. It was found that the absorbance of the colored ion pair complex was constantly up to 30 °C. At higher temperatures, the drug concentration was found to increase due to the volatile nature of the chloroform. Therefore, the temperature chosen was room temperature (25±2 °C) as the best temperature for micro-determination of ENP in pure and dosage forms. The absorbance of both complexes remains stable for at least 12 h at room temperature.

Stoichiometric relationship

The molar ratio between ENP and BCP or BPB in the ion-pair complexes was determined by Job's method of continuous variation [48]. Continuous variation method of equimolar solutions was employed: a 1.0×10^{-3} mol l^{-1} standard solution of drug base and 1.0×10^{-3} mol l^{-1} solution of BCP or BPB were used. A series solution was prepared in which the total volume of drug and reagent was kept at 2.0 ml in the total volume of 10 ml of the aqueous layer. The absorbance of extracting ion-pair in each instance was measured at the optimum wavelength and plotted against the mole fraction of the drug. The results indicate that the molar ratio of (drug: dye) is (1:1) complex was formed through the electrostatic attraction between the positive charged ENP^{+}ions and negatively charged BCP^{-} and BPB^{-} dye, (D^{-}) ions (fig. 5).

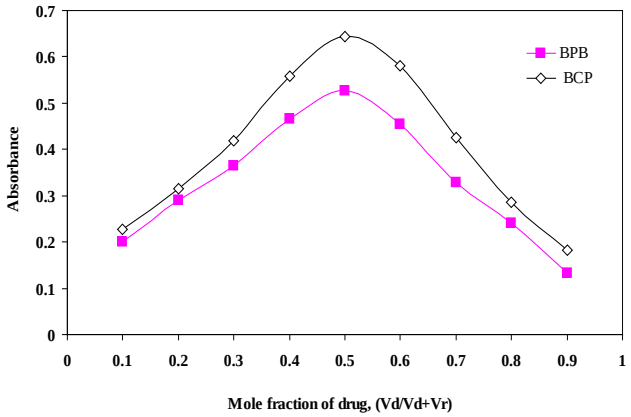
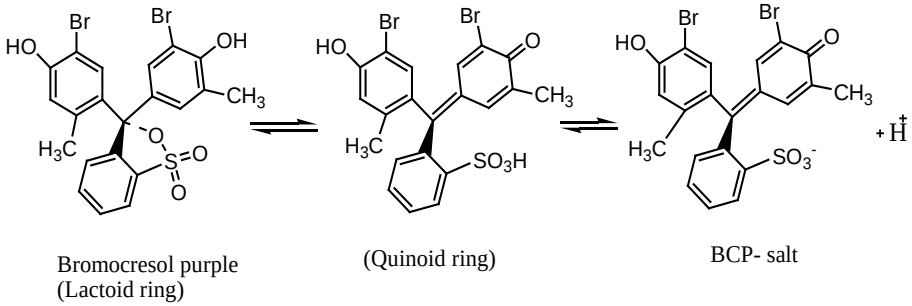
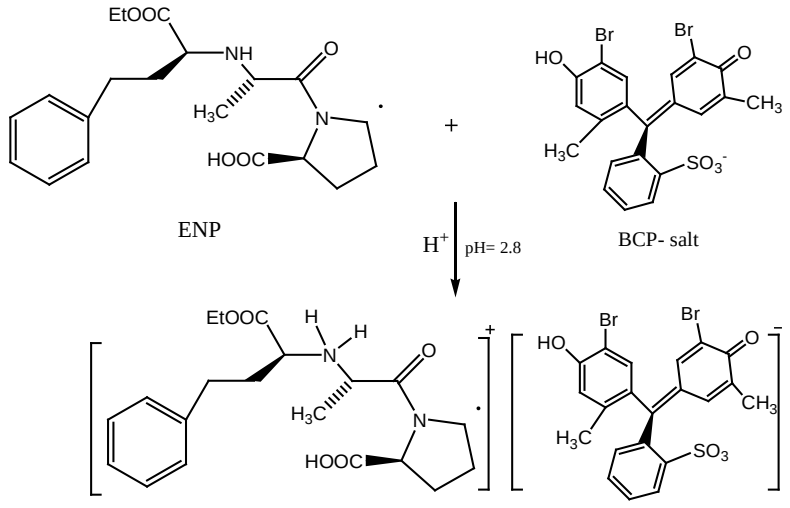


Fig. 5: Job's method of continuous variation graph for the reaction of ENP with the studied dyes, [drug] = [dye] = (1.0×10^{-3} mol l^{-1}





ENP- BCP ion-pair complex

Scheme 1: Proposed reaction mechanism for the ion pair complex formation between ENP and BCP

Method of validation

Linearity

At described experimental conditions for ENP determination, standard calibration curves with reagents were constructed by plotting absorbance vs. concentration of ENP. The statistical parameters were given in the regression equation calculated from the calibration graphs A = aC+b, where A is the absorbance and C is concentration in µg ml^{-1}. The linearity of calibration graphs was proved by the high values of the correlation coefficient (*r*) and the small values of the *y*-intercepts of the regression equations. The apparent molar absorptivity of the resulting colored ion-pair complexes and relative standard deviation of response factors for each proposed spectrophotometric method were also calculated and recorded in table 2. The molar absorptivity of BCP>BPB ion-pair complexes with ENP.

Sensitivity

The limits of detection (LOD) and quantitation (LOQ) for the proposed methods were calculated using the following equation [50, 51]:

$$LOD = 3s/k \text{ and } LOQ = 10\ s/k$$

Where *s* is the standard deviation of the response of the blank or the standard deviation of intercepts of regression lines and *k* is the sensitivity, namely the slope of the calibration graph. In accordance with the formula, the limit of detection was found to be 0.39 and 0.45 µg ml^{-1} for BCP and BPB methods, respectively. The limit of quantitations was found to be 1.30 and 1.50 µg ml^{-1} using BCP and BPB methods, respectively (table 2).

Accuracy and precision

In order to evaluate the accuracy and precision of the proposed methods, solutions containing three different concentrations of ENP were prepared and The assay procedure was analyzed in six replicates, and percentage relative standard deviation (% R. S. D) values were obtained within the same day to evaluate repeatability (intra-day precision) and over five different days to evaluate intermediate precision (inter-day precision). The analytical results of intra-day and inter-day precision and accuracy were summarized in table 3. The low values of percentage relative standard deviation (R. S. D %) as precision and percentage relative error (RE %) as accuracy of the proposed methods were calculated. The percentage relative error was calculated using the following equation:

$$RE\ \% = [(founded–added)/added] \times 100$$

These results of accuracy and precision show that the proposed methods have good repeatability and reproducibility

Table 2: Statistical analysis of calibration graphs and analytical data in the determination of ENP using the proposed methods

Parameters	BCP	BPB
Wavelengths λ_{max} (nm)	408	414
Beer's law limits (µg ml^{-1})	2.0-24	2.0-28
Molar absorptivity ε, (l mol^{-1} cm^{-1}) x 10^3	7.714	7.325
Sandell's sensitivity (ng cm^{-2})	50.89	53.59
Regression equation [a]		
Intercept (*a*)	0.0002	-0.0006
Slope (b)	0.0194	0.0189
Correlation coefficient (*r*)	0.9996	0.9993
LOD (µg ml^{-1})[b]	0.39	0.45
LOQ (µg ml^{-1})[b]	1.30	1.50
mean±SD	99.79±1.87	99.90±1.18
RSD%	1.87	1.18
RE%	1.96	1.24
t-test [c]	0.66	0.75
F-test[c]	4.31	1.72

[a] A = a+bC, where C is the concentration in µg mL^{-1}, A is the absorbance units.

[b] LOD, limit of detection; LOQ, limit of quantification; ε,molar absorptivity.

[c] The theoretical values of t and F at P= 0.05 are 2.571 and 5.05, respectively.

Table 3: Intra-day and Inter-day precision and accuracy data for ENP obtained by the proposed methods

Method	Added (µg ml^{-1})	Intra-day				Inter-day			
		Recovery %	Precision RSD % [a]	Accuracy RE %	Confidence Limit [b]	Recovery %	Precision RSD % [a]	Accuracy RE %	Confidence limit [b]
BCP	6.0	99.30	0.51	-0.70	5.958± 0.032	100.20	0.45	0.20	6.012± 0.028
	12	99.50	0.85	-0.50	11.94± 0.107	99.10	0.64	-0.90	11.892± 0.08
	24	100.20	1.40	0.20		100.50	1.20	0.50	
BPB	6.0	99.00	0.60	-1.00		99.50	0.36	-0.50	5.97± 0.023
	12	100.60	0.70	0.60		99.80	0.58	-0.20	11.976±0.073

24	99.40	1.10	0.60		100.70	0.95	0.70	24.168± 0.241

[a] Mean of six determination, RSD%, percentage relative standard deviation; RE%, percentage relative error.

[b] Confidence limit at 95% confidence level and five degrees of freedom (t = 2.571).

Robustness and ruggedness

The robustness of the method was evaluated by making small incremental changes in volume of dye, pH and shaking time, and the effect of these changes on the absorbance of the colored systems was studied. The changes had negligible influence on the results as revealed by small intermediate precision values expressed as RSD (≤ 3.0 %). Method ruggedness was demonstrated by having the analysis done by two analysts, and also by a single analyst performing analysis on two different instruments in the same laboratory. The results showed no statistical differences between different analysts and instruments, suggesting that the developed methods were robust and rugged. Intermediate precision values (RSD) of this study were ≤ 3.0 % indicating acceptable ruggedness.

Effects of interference

To assess the usefulness of the method, the effect of diluents, excipients and additives which often accompany ENP in its dosage forms (tablets) (starch, lactose, glucose, saccarose, talc, sodium chloride, titanium dioxide, and magnesium stearate) were studied. The results indicated that there is no interference from excipients and additives, indicating a high selectivity for determining the studied ENP in its dosage forms.

Applications of pharmaceutical formulations

The proposed methods have been successfully applied to the determination of ENP in dosage forms (Acapril tablets; Ezapril tablets and Enalapril tablets). Six replicates determinations were made. Moreover, to check the validity of the proposed methods, dosage forms were tested for possible interference with standard addition method (table 4). There was no significant difference between the slopes of calibration curves and standard addition method. Therefore, it is concluded that the excipients in pharmaceutical preparations of ENP did not cause any interference in the analysis of ENP. The results were compared with those obtained using the reported method for ENP [24]. Statistical analysis of the results did not detect any significant difference between the proposed methods and the reported method [24] in pharmaceutical formulations with respect to accuracy and precision as revealed by the Student's t-value and variance ratio F-value at 95% confidence level [51]. The results show that the Student's t-and F-values at 95 % confidence level did not exceed the theoretical values which confirmed that there is a good agreement between the results obtained by the proposed methods and the reported method [24] with respect to accuracy and precision (table 4).

Table 4: Application of the standard addition technique for the determination of ENP in dosage forms using the proposed methods

Sample	Taken	BCP		BPB		reported method [24]
	(µg ml^{-1})	Added (µg ml^{-1})	Recovery[a] (%)	Added (µg ml^{-1})	Recovery[a] (%)	
Acapril tablets	4.0	-	99.70	-	99.30	
		4.0	99.30	8.0	100.70	
		8.0	100.40	12	99.20	
		12	99.00	16	99.80	
		16	98.80	20	99.10	
mean±SD			99.44±0.635		99.62±0.661	99.30±0.60
R. S. D%			0.631		0.658	0.596
V			0.403		0.437	0.355
S. E			0.284		0.296	0.267
t-value[b]			0.36		0.80	
F-value[b]			1.12		1.21	
Ezapril tablets	4.0	-	99.60	-	100.05	
		4.0	100.30	8.0	99.70	
		8.0	99.80	12	100.00	
		12	99.10	16	99.50	
		16	100.10	20	99.00	
mean±SD			99.78±0.466		99.65±0.427	99.70±0.52
R. S. D%			0.465		0.468	0.518

V			0.217		0.183	0.270
S. E			0.208		0.191	0.233
t-value[b]			0.16		0.17	
F-value[b]			1.25		1.48	
Enalapril tablets	4.0	-	99.80	-	99.20	
		4.0	99.50	8.0	98.70	
		8.0	99.40	12	100.40	
		12	98.90	16	99.60	
		16	100.10	20	99.50	
mean±SD			99.54±0.451		99.48±0.622	99.40±0.70
R. S. D%			0.449		0.619	0.696
V			0.203		0.387	0.49
S. E			0.201		0.278	0.313
t-value[b]			0.38		0.19	
F-value[b]			1.09		1.27	

[a]Average of six determinations.

[b] The theoretical values of *t* and *F* are 2.571 and 5.05, respectively at confidence limit at 95% confidence level and five degrees of freedom (p= 0.05).

DISCUSSION

The proposed methods are based on the reactivity of tertiary amine group of ENP with two acid dyes (BCP and BPB). ENP forms an ion-association complex with acid dyes which is extractable into chloroform from the aqueous phase. The protonated nitrogen (positive charge) of ENP as hydrochloride is expected to attract the oppositely charged part (negative charge) of the dye in acidic buffer solution at pH ≥ 2.5 and behave as a single unit being held together by electrostatic attraction and a yellow ion-pair complex which is extracted with organic solvent is formed. The absorption spectra of the yellow ion-pair complexes, which were formed between ENP and BCP or BPB reagents and show maximum absorbance's at 409 and 415 nm, respectively against the blank solution.

The results indicate that the molar ratio of (drug: dye) is (1:1) complex was formed through the electrostatic attraction between the positive charged ENP^{+}ions and negatively charged dye, D^{-} ions. The extraction equilibrium can be represented as follows:

$$ENP^{+}_{(aq)} + D^{-}_{(aq)} \longleftrightarrow ENP^{+}D^{-}_{(aq)} \longleftrightarrow ENP^{+}D^{-}_{(org)}$$

Where, ENP^{+} and D^{-} represent the protonated drug and the anion of the dye (BCP^{-} or BPB^{-}), respectively, and the subcript (aq) and (org) refer to the aqueous and organic phases, respectively.

CONCLUSION

This paper describes the application of extractive ion–pair complexation reaction with acid dyes for the quantification of ENP in pure and dosage forms. Compared with the existing spectrophotometric methods, the proposed methods are relatively simple, rapid, cost-effective, free from auxiliary reagents and more sensitive for determination of ENP in pure and dosage forms. Moreover, the proposed methods are free from tedious experimental steps such as heating unlike the previously reported methods. The most attractive feature of these methods is its relative freedom from interference by the usual diluents and excipients in amounts far in excess of their normal occurrence in pharmaceutical formulations. The statistical parameters and the recovery data reveal good accuracy and precision of the methods. Therefore, the validated methods could be useful for routine quality control assay of ENP in pure and dosage forms.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests with the company name used in the paper.

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