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Research Article

SPECTROPHOTOMETRIC DETERMINATION OF RAMIPRIL IN PURE AND PHARMACEUTICAL FORMS USING TRIPHENYL METHANE DYES

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ABSTRACT

Four new simple and sensitive extractive spectrophotometric methods have been developed and described for the determination of Ramipril in pure and pharmaceutical formulations. The developed methods involve formation of coloured chloroform extractable ion-pair complexes of Ramipril with triphenyl methane dyes *viz.,* bromocresol green (BCG), bromophenol blue (BPB), bromothymol blue (BTB) and bromocresol purple (BCP) in acidic medium. The extracted ion-pair complexes absorb maximally at 412, 416, 415 and 407nm with use of the BCG, BPB, BTB and BCP respectively. The stoichiometry of the complex in four cases is found to be 1:1. The Beer's law is obeyed in the concentration ranges 2.5-25µg/mL with BCG, BPB, BTB and BCP. The effect of concentration of dye, pH and interference of excipients have been studied and optimized. The limits of detection and quantification have been determined for all the four methods. These methods have been validated as per the guidelines of International Conference on Harmonization (ICH) and have been applied to the determination of Ramipril in commercial tablets and results of analysis were validated statistically through recovery studies. Thus the newly developed methods are accurate, efficient and stable.

Keywords: Spectrophotometry, Ramipril, Triphenylmethane dyes, Ion-pair complex, ICH Validation.

INTRODUCTION

The aim of the present study is to develop simple and sensitive extractive spectrophotometric methods for the determination of Ramipril in pure and pharmaceutical forms.

Chemically, Ramipril (Fig. 1) is (2*S*,3a*S*,6a*S*)-1-[(*S*)-2-[[(*S*)-1-(ethoxycarbonyl)-3-phenylpropyl]amino] propanoyl] octahydrocyclopenta[*b*]pyrrole-2- carboxylic acid (I). Ramipril is a cardiac related drug and used as an angio-tensin converting enzyme (ACE) inhibitor for the treatment of hypertension and congestive heart failure. Liver esterase enzymes convert Ramipril into its active metabolite, called ramiprilate[1]. Hypertension is a common cardiovascular disease identified in most of the adults[2,3] and is a risk factor in patients suffering from myocardial infarction (heart attack). The role of ACE inhibitors is to lower the formation of angiotension II, thus enlarging the arteries and facilitating the heart to pump blood more easily causing increase in the flow of blood through enlarged arteries[4,5].

The literature survey revealed that a few analytical methods for the determination of Ramipril based on derivatization and CT complex formation were reported. These include colorimetric and kinetic spectrophotometric methods[6-9] which were conveniently applied for its determination in pure and pharmaceutical forms. A liquid chromatographic method for the determination of Ramipril in pure and dosage forms[10] and a spectrophotometric method for its determination in solid dosage forms[11] are available in the literature.

UV Spctrophotometric method for estimation of ramipril in pharmaceutical dosage form by absorption maxima and area under curve[12], by absorbance correlation method[13] were reported. Determination of Ramipril in human plasma[14], oxidative spectrophotometric determination of Ramipril using $KMnO_4$ and methyl orange as dye[15] are available in the literature. Colorimetric determination of Ramipril from pharmaceutical formulations[16] and method development and validation of Ramipril and hydrochlorothiazide by chemometric assisted UV-Spectrophotometry in bulk and pharmaceutical form[17] were also reported.

Simultaneous estimation of Ramipril and other drugs by first derivative UV Spectrophotometric method[18], by RP-HPLC method[19] and by chemometric assisted UV-Spectrophotometric method in capsules[20] were also reported. Development of validation of stability indicating RP-HPLC method for simultaneous estimation of ramipril, aspirin and simvastatin in bulk and pharmaceutical dosage form[21] and a stability indicating HPLC method for the simultaneous determination of valsartan and ramipril in binary combination[22] are also available in the literature.

The literature survey also revealed that very few reports are available on spectrophotometric methods for the quantification of Ramipril in pure and dosage forms using dyes as analytical reagents. This encouraged to develop new spectrophotometric methods for the determination of Ramipril in bulk and pharmaceutical formulations using triphenyl methane dyes viz., bromocresol green (BCG), bromophenol blue (BPB), bromothymol blue (BTB) and bromocresol purple (BCP). In these

methods, the drug forms coloured chloroform extractable ion-pair complexes with dyes in acidic medium.

MATERIALS AND METHODS

Instruments

For recording UV-Vis spectra of the study, SHIMADZU 140 double beam spectrophotometer and ELICO SL 210 UV-Visible double beam spectrophotometer with quartz cells of 10 mm path length have been used. For *p*H measurements, an Elico model Li-120 *p*H meter was employed.

Materials

The dyes *viz*., Bromothymol blue, Bromophenol blue, Bromocresol green and Bromocresol purple of analytical grade supplied by SD Fine Chemicals Ltd. Mumbai, were used without any further purification. The solvent Chloroform (HPLC grade) and AR grade HCl and Sodium acetate supplied by SD Fine Chemicals, Mumbai were used in the study. The drug, Ramipril analysed in the present study was procured as gift sample from Hetero Drugs Pvt. Ltd, Hydeabad, Telangana.

Methods

Method A

Method A involves the interaction of the drug with Bromocresol green to form ion-pair complex, extractable into chloroform. This ion-pair complex absorbs around 415 nm in the UV region. The absorbance of this band increases with increasing the concentration of the drug from which the calibration curve is constructed for quantification of drug. 0.025% aqueous solution of BCG was used. CH_3COONa-HCl buffer of required *p*H was used and the desired *p*H was maintained with the help of a *p*H meter.

Method B

Method B involves the interaction of the drug with Bromophenol blue to form ion-pair complex, extractable into chloroform. This ion-pair complex absorbs around 415 nm in the UV region. The absorbance of this band increases with increasing the concentration of the drug from which the calibration curve is constructed for quantification of drug. 0.025% aqueous solution of BPB was used. The required pH of reaction mixture was maintained at 2.5 using CH_3COONa-HCl buffer and other experimental conditions are similar as mentioned in Method A.

Method C

Method C involves the interaction of the drug with Bromothymol blue to form ion-pair complex, extractable into chloroform. This ion-pair complex absorbs around 415 nm in the UV region. The absorbance of this band increases with increasing the concentration of the drug from which the calibration curve is constructed for quantification of drug. 0.025% aqueous solution of BTB was used. The required pH of reaction mixture was maintained at 3.5 using CH_3COONa-HCl buffer and other experimental conditions are similar as mentioned in Method A.

Method D

Method D involves the interaction of the drug with Bromocresol purple to form ion-pair complex, extractable into chloroform. This ion-pair complex absorbs around 407 nm in the UV region. The absorbance of this band increases with increasing the concentration of the drug from which the calibration curve is constructed for quantification of drug. 0.025% aqueous solution of BCP was used. The required pH of reaction mixture was maintained at 2.5 using CH_3COONa-HCl buffer and other experimental conditions are similar as mentioned in Method A.

RESULTS AND DISCUSSION

The developed methods are based on the interaction of Ramipril to form ion-pair complexes with dyes viz., BCG, BPB, BTB and BCP. The ion-pair complexes of Ramipril, quantitatively extracted into chloroform, absorbed maximally at 412, 416, 415 and 407nm with use of the cited dyes respectively (Fig. 2a, 2b, 2c and 2d), where the reagent blank under similar experimental conditions showed no absorption. 0.025% aqueous solution of dye stuffs and CH_3COONa-HCl acid buffers of *p*H 3.5, 2.5, 2.8 and 2.5 were used to get stable ion-pair complexes of Ramipril with the mentioned dyes. Appropriate pH values are maintained in all the experiments with the help of a pH meter. The developed methods can be applied for the determination of Ramipril in pharmaceutical formulations.

Formation of Ion-pair complexes

The structure of Ramipril reveals that it is more susceptible for the protonation at secondary amino group and can form ion-pair complexes with dye stuffs. The sulphonic group present in dye undergoes dissociation in acidic medium, where the lactoid ring is opened with the formation of quinoid group which is responsible for the colour of ion-pair complex. The formation of ion-pair complexes of Ramipril with the mentioned dyes is shown in Scheme 1.

Calibration curves for the methods

Into separating funnels, different aliquots of solution of Ramipril were taken, 5 ml of CH_3COONa-HCl buffer (of *p*H 3.5, 2.5, 2.8 and 2.5) and 5 ml of 0.025% aqueous solution of dye were added. The total volume of the contents in the flask was made up to 20 ml with distilled water. 10 ml of chloroform was added to this, and the contents were thoroughly shaken for 5 min in order to form a stable ion-pair complex. The flask was kept aside for 5 min to allow the organic and aqueous layers to separate. The absorbance of stable colored solution was recorded around 417 nm against blank similarly prepared. The determinations of pure Ramipril and its pharmaceutical forms were carried out using the same procedure. The calibration curves (Fig. 4) are constructed which are linear over the concentration ranges and are in permissible range.

The statistical data for the regression equations for the developed methods and optical characteristics of ion-pair complexes of Ramipril with dyes are presented in Table 1.

Procedure for the assay of pure drug

Five different solutions of pure Ramipril in the range of calibration curve were chosen for conducting recovery experiments, the results of which are presented in Table 2 along with relative standard deviations for the methods developed.

Procedure for the assay of dosage forms

Ten tablets of Cardiopril 30mg were taken and grounded to powder and dissolved in doubly distilled water. The solution was stirred thoroughly, filtered through a Whatman No. 42 filter paper, and taken into a 100 ml standard flask and diluted with required doubly distilled water. The recovery experiments were

carried out by selecting different aliquots of this solution which come in the range of calibration curve for the determination of drug in its dosage form. Table 3 represents the results of the recovery experiments for the assay of dosage forms.

Stoichiometry

The determination of molar ratio between Ramipril and dye stuffs was based on the Job's continuous variation method[23]. The solutions of Ramipril and dye stuffs (BCG, BPB, BTB and BCP) with same concentrations of 8 x $10^{-5}M$ each were mixed in varying the volume ratios such that the total volume of each mixture was maintained constant. The absorbance of each mixture solution was measured and plotted against the mole fraction of the drug. From the Figure 4, it is confirmed that 1:1 drug & dye molar ratio exists in all the complexes formed between Ramipril and each BCG, BPB, BTB and BCP. The formation constants[24] were also determined and found to be 1.0 x 10^6, 1.45 x 10^6, 1.78 x 10^6 and 2.01 x 10^6 K M^{-1} for complexes with BCG, BPB, BTB and BCP respectively.

Optimization of pH and volume of dye

The effect of pH on the absorbance of ion-pair complexes of Ramipril with BCG, BPB, BTB and BCP was studied using CH_3COONa-HCl buffer. It is evident from the Fig.5 that the absorbance of complexes with BCG, BPB, BTB and BCP was found to be constant within the *p*H ranges 2.2-3.8, 2.0-3.0, 2.0-3.0 and 2.0-3.0 respectively. Thus, all the absorbance measurements were made at *p*H 3.5, 2.5, 2.8 and 2.5 with BCG, BPB, BTB and BCP respectively.

Different volumes of BCG, BPB, BTB and BCP were added separately to a constant volume (8 µg ml^{-1}) of Ramipril for studying the effect of concentration of dye on the absorbance of ion-pair complex. It is evident from Figure 6 that the absorbance gradually increases with the volume of dye upto 3.0 ml, beyond which no change in the absorbance was observed. Hence, in all the experiments carried out with Ramipril for its determination, 5 ml of dye was used.

Effect of presence of excipients

The effect of the presence of foreign substances (excipients) along with Ramipril has been studied choosing the concentration level at 8 µg ml^{-1}. Experiments on systems with 10 ml of sample and known amount of foreign substance were carried out adopting the procedures of proposed methods. The results of these experiments and tolerance limits were tabulated in Table 4. It is appropriate to mention that any interference by the common excipients found in tablets is completely ignored as the drug content from the powdered tablets was extracted into chloroform.

Validation of the proposed method

The methods developed for the quantification of Ramipril using dye stuffs *viz.,* BCG, BPB, BTB and BCP have been validated in terms of guidelines prescribed by ICH[25] for method validation. The terms mentioned in ICH *viz*. selectivity, specificity, accuracy, precision, limits of calibration curve, LOD, LOQ, robustness, ruggedness and regression equation for the proposed methods were studied. For comparison with a reference method, the student t-test and variance F-test were performed. The results of Beer's law limits, molar absorptivity, regression equation, correlation coefficients, relative standard deviation and recoveries are presented in Table 1. Six replicate determinations were carried out to test the precision of the proposed methods. It is found that the coefficient of variation was less than 1.2% for all the procedures. The performance order of the developed methods is found to be BCP > BTB > BPB > BCG. The results of the developed methods presented in Table 2 and Table 3 were compared to those achieved by reference method in terms of *t*-test at 95% confidence level. It is observed, in all the cases, that the results achieved by developed methods and those by reference methods were identical in terms of statistical data.

Table 1: Optical characteristics and statistical analysis for the regression equation of the proposed methods for the estimation of Ramipril

Parameters	Extraction methods with[b]			
	BCG	**BPB**	**BTB**	**BCP**
λ_{max} (nm)	412	416	415	407
Beer's law limit (µg ml^{-1})	2.5-25	2.5-25	2.5-25	2.5-25
Molar absorptivity (L mol^{-1} cm^{-1})	20824	22267	21911	21925
Formation constant, K, M^{-1}	$1.0x10^6$	$1.45x10^6$	$1.78x10^6$	$2.01x10^6$
Sandell sensitivity (µg cm^{-2})	0.0222	0.0204	0.0208	0.0208
Slope (specific absorptivity), b	0.045	0.049	0.048	0.048
Intercept (a)	0.064	0.048	0.065	0.108
Correlation coefficient (r)	0.995	0.992	0.993	0.991
Standard deviation of intercepts (% n=6)	0.0101	0.0086	0.0086	0.0095
Limit of detection, µgml^{-1}	0.724	0.0581	0.592	0.656
Limit of quantification, µgml^{-1}	2.225	1.759	1.796	1.987
Regression equation[a]	Y=0.045C±0.064	Y=0.049C±0.048	Y=0.048C±0.065	Y=.0.048C±0.108

[a]With respect to $Y=bc+a$, where C is the concentration (µg ml^{-1}) and Y is absorbance
[b]Six replicate samples

Table 2: Application of proposed methods for the analysis of Ramipril in pure form

Taken (µg ml^{-1})	Proposed methods								Reference method Recovery (%)
	Found (µg ml^{-1})				Recovery (%)				
	BCG	BPB	BTB	BCP	BCG	BPB	BTB	BCP	
5	4.97	5.03	5.069	5.01	99.40	100.60	101.39	100.20	99.75
8	8.06	7.95	8.025	7.94	100.75	99.375	100.31	99.25	100.04
11	10.94	11.12	11.072	11.07	99.45	101.09	100.66	100.66	101.24
14	13.92	14.05	13.95	14.06	99.43	100.35	99.645	100.43	99.04
									100.52
									99.98
									101.58
									101.22
RSD (%)					0.665	0.7172	0.7188	0.6174	0.8713
Mean±SD					99.75 ±0.661	100.35 ±0.721	100.56 ±0.7288	100.13 ±0.619	100.42 ±0.8749
t-test					0.421	0.302	0.288	0.507	
F-test					2.603	3.097	3.160	2.276	

Table 3: Application of proposed methods for the analysis of Ramipril in pharmaceutical form

Taken (µg ml^{-1}) Cardiopril 30mg	Proposed methods								Reference method Recovery (%)
	Found (µg ml^{-1})				Recovery (%)				
	BCG	BPB	BTB	BCP	BCG	BPB	BTB	BCP	
5	5.08	5.03	5.06	5.01	100.60	100.60	101.39	100.20	98.94
8	8.05	7.95	8.02	7.94	100.63	99.37	100.31	99.25	99.14
11	10.96	11.12	11.07	11.07	99.64	101.09	100.66	100.66	101.56
14	14.11	14.05	13.95	14.06	100.78	100.35	99.64	100.43	101.28
									99.08
									100.22
									99.98
									101.10
RSD (%)					0.793	0.7173	0.7188	0.6174	0.9898
Mean±SD					100.66 ±0.806	100.35 ±0.722	100.50 ±0.729	100.13 ±0.618	100.162 ±0.9915
t-test					0.424	0.574	0.561	0.759	
F-test					1.636	1.312	1.338	0.964	

Table 4: Interference study in the estimation of Ramipril

Sl. No	Excipients	Tolerance limit (µg ml^{-1})
1	Microcrystalline cellulose	98
2	Starch	145
3	Lactose	121
4	Povidone	65
5	Silicon dioxide	68
6	Titanium dioxide	48

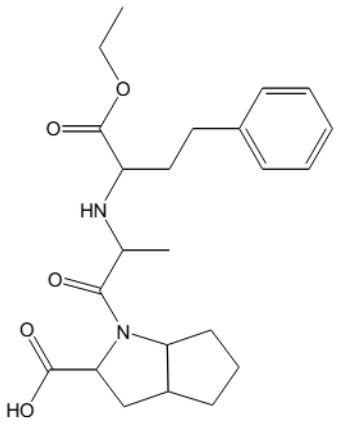


Figure 1: Structure of Ramipril

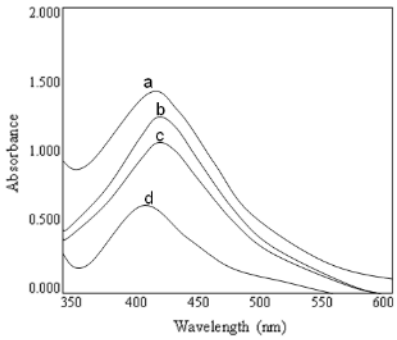
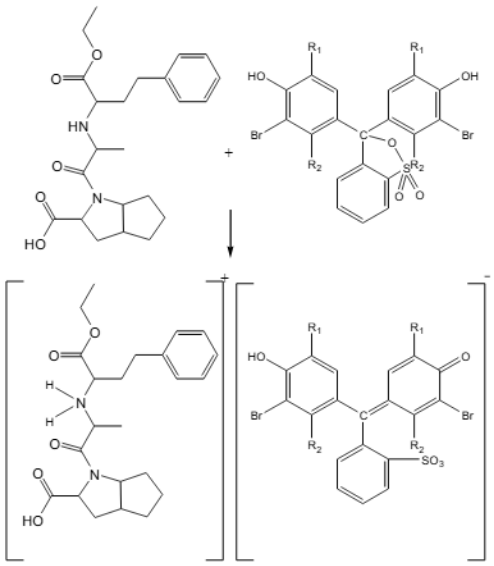


Figure 2: Absorption spectra of Ramipril-dye complex extracted into 10 ml chloroform

a. drug = 25.0 µg ml^{-1} + 5 ml of 0.025% BCG + 5 ml of pH 3.5 buffer
b. drug = 22.5 µg ml^{-1} + 5 ml of 0.025% BPB + 5 ml of pH 2.5 buffer
c. drug = 20.0 µg ml^{-1} + 5 ml of 0.025% BTB + 5 ml of pH 2.8 buffer
d. drug = 17.5 µg ml^{-1} + 5 ml of 0.025% BCP + 5 ml of pH 2.5 buffer



Scheme 1: Ramipril-dye ion-pair complex
Bromothymol blue : R_1 = isoporopyl, R_2 = $-CH_3$, Bromophenol blue : R_1 = -Br, R_2 = -H
Bromocresol green : R_1 = -Br, R_2 = $-CH_3$, Bromocresol purple: R_1= $-CH_3$, R_2= -H

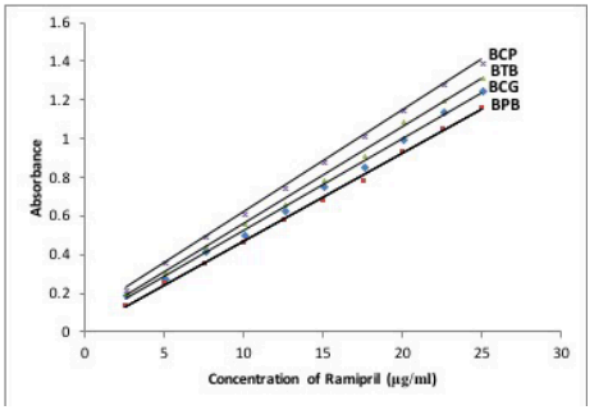


Figure 3: Calibration graphs for Ramipril-BCG, BPB, BTB & BCP ion-pair complexes

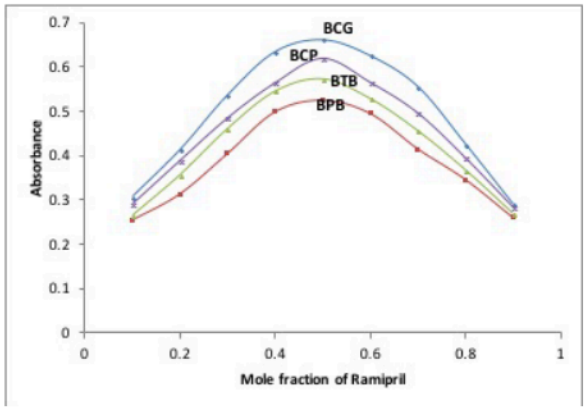


Figure 4: Continuous-variations study of drug-dye systems [Ramipril] = [Dye] = $8x10^{-5}$M

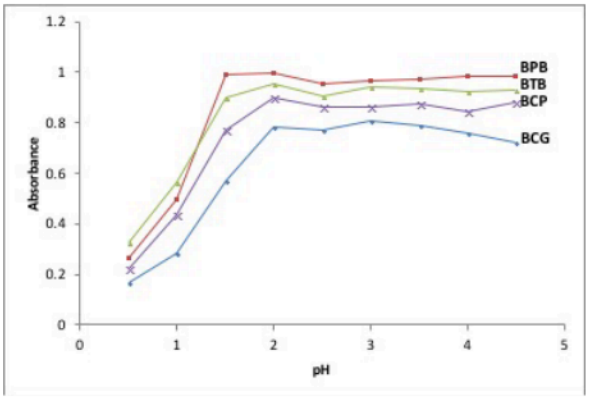


Figure 5: Effect of pH [Ramipril] = 8µg ml^{-1}, [Dye] = 5ml of 0.025%

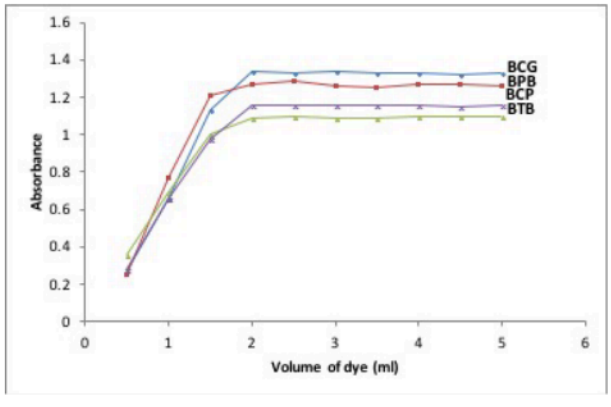


Figure 6: Influence of the volume of 0.025% dye [Ramipril] = [8µg ml^{-1}]

CONCLUSION

The formation of chloroform extractable ion-pair complexes between Ramipril and acidic triphenlmethane dyes *viz.*, bromocresol green, bromophenol blue, bromothymol blue and bromocresol purple formed the basis for the determination of Ramipril. The methods developed are simple, sensitive and can be considered as standard methods for the determination of Ramipril in pure and pharmaceutical forms. Comparative t- and F-tests grow confidence on the applicability of the methods in pharmaceutical formulations. The results obtained are satisfactorily accurate and precise as indicated by the excellent percent recovery.

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