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Development and Validation of Stability Indicating UV Spectrophotometric Method for the Estimation of Brimonidine Tartrate in Pure Form, Formulations and Preformulation Studies

Prakash Bhagav [1], Pandurang Deshpande [1], Saurabh Pandey [1], Sajeev Chandran [1,2]

[1]Formulation Development & Pharmacokinetics Laboratory, Pharmacy Group, Birla Institute of Technology and Science, Pilani. INDIA
[2]Innovation Cell, Pharma Research, Lupin Research Park, 46/47A, Nande Village, Mulshi Taluka, Pune. INDIA

Abstract

A new simple, sensitive and selective UV- spectrophotometric method was developed and validated for the estimation of brimonidine tartrate (BRT) in bulk and in formulations. The method was also applied for the determination of drug solubility, apparent partition coefficient and pKa. BRT was estimated at 248 nm in phosphate buffer (pH 7.4). The linearity range was found to be 3-18 µg/ml with a regression equation of Absorbance = 0.0630 × (concentration in µg/ml) + 0.0050. The LOD and LOQ were found to be 0.15 µg/ml and 0.45 µg/ml respectively. The method was found to be accurate (mean percentage accuracy of 99.92) and precise with % RSD less than 1.97 (for intra-day) and less than 1.36 (for inter-day). The developed method was successfully employed for the determination of BRT in pharmaceutical dosage forms (ophthalmic solutions, ocuserts and gels). Forced degradation studies with acid, alkali and temperature based stress conditions showed that the method is stability indicating. The determined preformulation parameters (solubility, apparent partition coefficient and pKa) were found to be similar to the earlier reported results.

Keywords: Brimonidine tartrate, spectrophotometry, UV method, validation, stability indicating, preformulation studies.

INTRODUCTION

Brimonidine tartrate (BRT) [5-bromo-6-(2-imidazolidinylideneamino) quinoxaline L-tartrate] (Fig. 1) is a selective alpha-2 adrenergic agonist, used as ocular hypotensive agent.

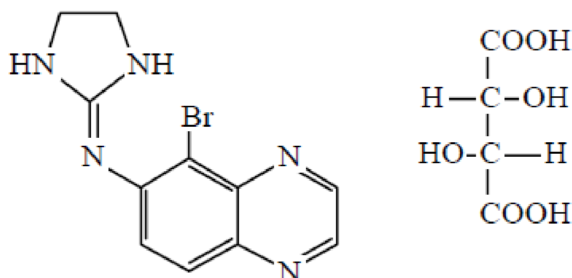


Fig. 1: Structure of brimonidine tartrate

Its ocular hypotensive effect is due its ability to decrease aqueous humor production and increase uveoscleral outflow [1,2]. Its selectivity towards alpha-2 adrenergic receptors [2,3] and its neuroprotective activity on retinal ganglionic cells [3] makes it as an important therapeutic agent for the treatment of open angle glaucoma. Its superiority in treating glaucoma in cardiopulmonary patients makes this drug as a better alternative to timolol [5,6] In few clinical trials it has been shown to have comparable ocular hypotensive activity to that of timolol [7,8]. In another clinical trial, it was found to have Intra Ocular Pressure (IOP) - lowering efficacy greater than that of betaxolol [9]. Its use in ischemic neuropathy has also been reported [4]. There is always a need for a simple, sensitive, accurate, rapid, reproducible analytical method for the estimation of BRT in pure form and in formulations with its ever increasing use as antiglaucoma agent and a good number of formulations entering into the market each year.

An extensive survey of literature did not reveal any validated UV-spectrophotometric method for the estimation of BRT in pure form and in pharmaceutical dosage forms of ophthalmic formulations. An UV- spectrophotometric method for the quantitation of the drug at 319 nm in phosphate buffer saline (pH 7.4) has been reported as part of formulation design and evaluation procedure [10]. The validation data is not presented in the report. However, few liquid chromatographic (LC) methods have been reported for the analysis of BRT in eye drops [11], pharmaceutical formulations and in biological samples [12] and a mass spectrophotometric method for the identification of synthetic impurities of BRT [13]. Few LC methods have been reported for the estimation of BRT in plasma, serum and in aqueous humor [14,15] and in ocular tissues [12,16]. An on-line H/D exchange LC-MS/MS and stable-isotope tracer methods has been reported for the estimation of metabolites of BRT [17]. The reported chromatographic and other techniques employ sophisticated instrumentation, are time consuming, require costly solvents and other chemicals, and hence cannot be used for routine laboratory analysis. A simple, sensitive, accurate and cost effective UV- spectrophotometric method is required for the routine analysis of drug in bulk, in pharmaceutical formulations and also samples obtained from in vitro dissolution studies and stability studies.

In the present study a simple, sensitive, accurate and reproducible analytical method with better detection range for estimation of BRT in pure form and in its pharmaceutical dosage forms was developed and validated. Based on forced degradation studies, the method was also tested for its

stability indicating ability. The results of the analysis were validated by applying suitable statistical methods [18, 19, 20] and by recovery studies [21]. The developed and validated method was employed in the determination of preformulation parameters such as solubility, apparent partition coefficient and pKa.

MATERIALS AND METHODS

Experimental

Instruments

A scanning UV-VIS NIR spectrophotometer (Jasco, Tokyo, Japan, Model V-570) connected to computer loaded with spectra manager software, with automatic wavelength accuracy of 0.1 nm, a 10 mm matched quartz cells was used for all the absorbance measurement.

Materials

BRT was obtained as gift sample from FDC Ltd. Mumbai, India. Potassium dihydrogen orthophosphate, sodium hydroxide and isopropyl alcohol were purchased from S.D. Fine Chemicals, Mumbai, India. Excipients used in the preparation of the formulations; hydroxypropyl methylcellulose (HPMC), ethyl cellulose (EC), benzalkonium chloride were purchased from Sigma-Aldrich India. High quality pure water was prepared using Millipore purification system (Millipore, Molsheim, France, Model Elix SA 67120). BRT ophthalmic solution (0.2% w/v) was prepared in laboratory using phosphate buffer saline (pH 7.4) under aseptic conditions. Ocuserts were composed of drug HPMC, EC, micro crystalline cellulose (MCC), magnesium stearate, poly vinyl pyrrolidone K-30, while ocugels were composed of HPMC, carbopol, benzalkonium chloride, benzyl alcohol and buffers. All other chemicals required were of pharmaceutical/analytical grade.

Method development

To develop a sensitive UV-spectrophotometric method, various solvent systems were investigated in order to optimize the method parameters. Parameters like sensitivity, interference from the matrix, ease of preparation, need for pH adjustment, tolerance for pH variation, suitability for drug content estimation and stability, analysis time and cost factors were taken into consideration while selecting the solvent.

Preparation of standard calibration curve

Three different stock solutions of BRT were prepared by dissolving 5 mg of drug in 100 ml of phosphate buffer pH 7.4 [0.05M KH_2PO_4 (79.1 parts) and 0.045M NaOH (20.9 parts)] to get a final concentration of 50 µg/ml. Suitable dilutions of the stocks were prepared in series of 10 ml calibrated volumetric flasks using the same media. Various concentrations were prepared in the range of 3-18 µg/ml. By scanning a suitable standard solution in the UV-VIS spectrophotometer in the wavelength range of 200-400 nm with the scanning speed of 400 nm per min, the λ_{max} of the drug in the above media was determined, shown in Fig. 2(a) and (b). Absorbance values for standard dilutions were measured at λ_{det} 248 nm.

Analytical method validation

Following procedure was employed for validating the developed method. [18, 19, 20, 22]

Linearity of the proposed method was determined by measuring the absorbance of the standard solutions in the concentration range of 3-18 µg/ml and performing least square regression analysis. Accuracy was determined in terms of the mean percent recovery along with standard deviation (n = 9). In addition, the accuracy of the proposed method was checked using standard addition method. To the pre-analyzed standard calibration and formulation samples, a known amount of drug samples were added and the total concentration was determined by measuring absorbance of the resultant solution by using the proposed method. The percent analytical recovery was calculated by comparing the concentration resulted with the addition of spiked samples with actual expected theoretical increase in concentration. Intra-day precision was determined by carrying out the analysis at three different time points for three concentrations in a day. Similarly inter-day precision was determined by performing analysis on three consecutive days. LOD and LOQ of the proposed methods were calculated on the basis of standard deviation of response and slope of the regression equation. Specificity of the proposed method was determined by preparing drug and placebo solutions using some commonly used excipients like HPMC, EC, lactose, starch, methyl cellulose, MCC, sodium chloride, dextrose, talc, magnesium chloride, benzalkonium chloride, benzyl alcohol separately in the solvent as per the proposed method. The samples were then checked for the absorbance at the wavelength of 200-400 nm. Robustness of the method was determined by changing the pH of the medium, phosphate buffer of pH from 6.9 to 7.9 and measuring the absorbance of standard solutions. Effect of change of analyst was also investigated on the robustness of the method.

Recovery studies

The method employed for the estimation of drug from the formulations is as follows:

Ophthalmic solutions: An aliquot of ophthalmic solution of BRT equivalent to 2 mg was taken and suitably diluted with the medium under study and analyzed by the proposed method. From the absorbance values of five replicates, the drug content/ml was calculated on average concentration basis**.**

Ocuserts: Ten ocuserts were accurately weighed and finely powdered. An aliquot of the triturate equivalent to 1 mg of BRT was accurately weighed and dissolved in phosphate buffer (pH 7.4), sonicated for 30 mins. The resulting solution was filtered through Whatman filter paper No # 40, suitably diluted and analyzed using the proposed method. From the absorbance values of five replicates, the drug content in terms of mg/l2 mg ocusert was calculated on average weight basis.

Ocugel: Aliquots of brimonidine gel equivalent to 2 mg of drug was taken and dissolved in phosphate buffer (pH 7.4) by sonication. The resultant solution was filtered using Whatman filter paper No # 40, suitably diluted and analyzed by the proposed method. The drug content/g was calculated from the absorbance values of five replicates, on average weight basis**.**

Bench top and accelerated stability studies:

The bench top stability studies of the drug solution was carried out by storing the standard solution of concentration 12 µg/ml in phosphate buffer (pH 7.4) at controlled room temperature (25°C ± 2°C). At different time intervals up to 24 hrs, samples were withdrawn and diluted suitably with the medium and analyzed for the amount of drug.

Forced degradation studies
The forced degradation studies provide an idea of specificity and stability indicating nature of the developed analytical method. This study was carried out by subjecting the drug to stress conditions like acid and alkaline treatment and temperature stress conditions. However no attempts were made to isolate the degradation products from the drug. The details of the procedure followed are as follows: For acid degradation studies, in a calibrated volumetric flask (in triplicates), a 4.5 ml of 50 µg/ml solution of drug in phosphate buffer (pH 7.4), 8.0 ml of 0.1N hydrochloric acid was added and volume was made up to 25 ml with phosphate buffer (pH 7.4). The solution was allowed to stand for 9 hrs at 50°C. At an interval of 3, 6, 9 hrs, samples were withdrawn and UV- spectrum of the samples were recorded at 200-400 nm. Similar procedure was employed for alkali degradation studies where in 0.1N hydrochloric acid was replaced with 0.1N sodium hydroxide in the procedure.

STUDIES

The developed and validated analytical method was employed in the preformulation parameters of the drug like solubility at buffered conditions, apparent volume of distribution and spectrophotometric determination of dissociation constant.

(a) Solubility studies
The solubility profile of BRT was determined using modified shake flask method in buffered solutions. An excess of BRT was added to each of the 2 ml of the buffers taken in micro centrifuge tubes maintained on a mechanical orbital shaker maintained at 37 ± 0.5° C. An excess of undissolved drug was always maintained in each of the tubes for the entire duration of the study. The tubes were shaken on mechanical orbital shaker for 24 hours. Samples from the tubes were collected, suitably diluted and analyzed by the above developed and validated UV spectrophotometric method.

Phosphate buffers of varying pH ranging from 4.0 to 8.0 (pH 4.0, 4.5, 5.0, 5.5, 6.0, 6.8, 7.0, 7.4, 8.0) was employed for solubility determination studies. The phosphate buffer of different pH was prepared as per the procedures of Indian pharmacopoeia (2007). NaCl was added in sufficient amount to adjust the ionic strength to 0.3M.

(b) Determination of apparent partition coefficient
The apparent partition coefficient (P_{App}) of BRT was determined by traditional shake flask method at 37.0 ± 0.5 ºC temperature using n-octanol or chloroform as organic phase and water or phosphate buffer (pH ranging from 5.5 to 8.0, pH adjusted with 0.1N NaOH or 0.1N HCl) as aqueous phase. The organic and aqueous phases were equilibrated with each other by shaking them together for about 12 hours and then separating at 37.0 ± 0.5 ºC. The ionic strength of the buffers was adjusted to 0.15 M with NaCl. The aqueous phase was centrifuged after equilibration in order to remove small n-octanol droplets befouling it as a result of the emulsion formation during shaking. To a known volume (2 mL) of aqueous phase containing drug dissolved in it, 2 mL of organic phase was added into a 5 mL tube, and allowed to stir for 24 hours on an orbital shaker at a temperature of 37.0 ± 0.5 ºC, in triplicates. Samples were taken 12 and 24 hours, centrifuged (4000 rpm, 15 mins), diluted suitably with phosphate buffer pH 7.4 and analyzed spectrophotometrcally at 248 nm. The concentration of drug in aqueous phase at time zero (C_0)

and the concentration at time t (C_t) were determined. The apparent partition coefficient was calculated using the equation,

$$P_{App} = [C_{0\,(Aqueous)} - C_{t\,(Aqueous)}] / C_{t\,(Aqueous)}] \qquad \text{Eq. (1)}$$

(c) Determination of dissociation constant (pKa)

If the criterion of different UV absorbance profile for ionized and unionized species is met, an accurate and precise measurement of dissociation constant is possible by spectrophotometric method.

The procedure for the determination and calculation of pKa was followed as reported earlier [23, 24]

Buffers of varying pH in the range of 1-12 were prepared using 0.2 M NaH_2PO_4, 0.2 M K_2HPO_4 and 0.2 M NaCl to give a final buffer molarity of 0.01M and ionic strength of 0.02M. The pH of these were adjusted with 0.1N NaOH or 0.1 N HCl. The primary stock solution of BRT (1000 µg/ml) was prepared in TDW and an aliquot volume was transferred into individual calibrated volumetric flasks and diluted with media of varying pH to give a concentration of 10 µg/ml. The resulting solutions were immediately scanned in the range of UV wavelength of 200-400 nm at a scanning speed of 200 nm/sec and UV absorbance spectras were recorded. The obtained spectras were then overlapped to determine the wavelength where maximum change in the absorbance amongst the absorbing species at different pH was seen. The absorbance values at the wavelength was plotted against pH. A first derivative absorbance spectrum was plotted by taking ΔAbs/ΔpH Vs pH and the pKa was directly obtained as the point of inflection in ΔAbs/ΔpH Vs pH curve (graphical method). Subsequently pKa was also be calculated using the following equation.

$$pKa = pH + \log (A_i - A / A - A_u) \qquad \text{Eq. (2)}$$

where A_i absorbance of the ionized species (absorbance of BRT in 0.1 N HCl), A_u is the absorbance of neutral species (absorbance of BRT in 0.1 N NaOH), and A is the absorbance of test solution. The pKa values obtained is an average of three determinations for each set of pH.

RESULTS AND DISCUSSION

Method development

In the present work, a UV-spectrophotometric method was developed and validated for the estimation of BRT in pure form and in formulations. The various solvents such as water, buffers such as phosphate (pH 5.6-8.0), acetate (pH 3.5-5.6) and citrate (pH 3.0-7.0) and organic solvents like methanol, acetonitrile alone or in combinations of different proportions were investigated. The selection of phosphate buffer (pH 7.4) was based on the sensitivity, pH tolerance, ease of preparation and applicability in routine analysis, stability of the drug in the solvent and cost. The λ_{det} of BRT in phosphate buffer (pH 7.4) was found to be 248 nm and the UV- absorption spectra of 12 µg/ml standard solution of BRT in phosphate buffer (pH 7.4) is shown in the Fig. 2(a). The

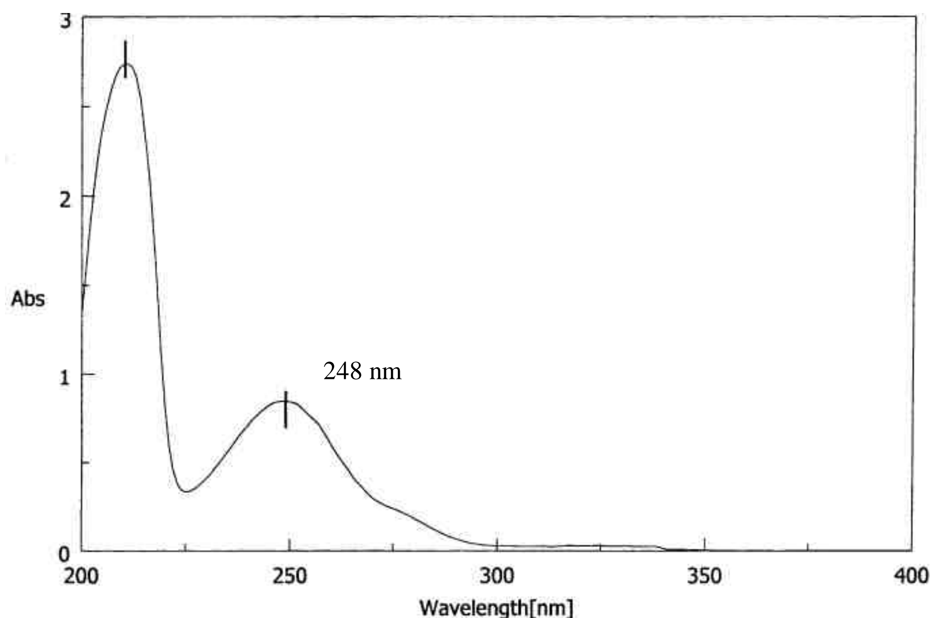
absorbance values of BRT standard solutions in the concentration range of 3-18 µg/ml is shown in the Table 1 and the overlay spectra is presented in the Fig. 2(b).

Table-1: Calibration data of the proposed UV method for the estimation of brimonidine tartrate at 248 nm in phosphate buffer (pH 7.4)

Concentration (µg/ml)	Absorbance (± S.D)[a]	% RSD
3	0.1938 ± 0.0033	1.69
6	0.3825 ± 0.0018	0.48
9	0.5807 ± 0.0010	0.18
12	0.7613 ± 0.0013	0.17
15	0.9510 ± 0.0020	0.21
18	1.1423 ± 0.0028	0.24

[a] mean ± standard deviation (SD) of nine separate determinations; RSD is Relative Standard Deviation

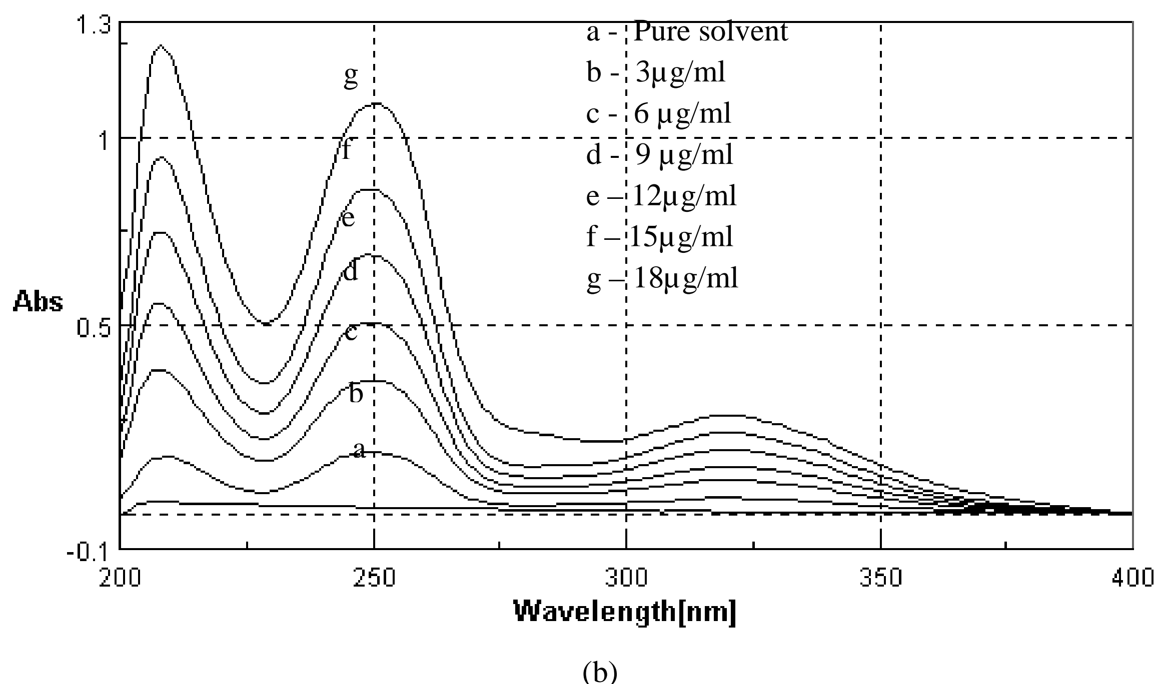
The linear regression equation of drug in phosphate buffer (pH 7.4) was found to be Y = 0.0630 X + 0.0050, where Y is the absorbance (µV) and X is the concentration (µg/ml), with a regression coefficient of 0.9999. The regression analysis data for linearity of the developed method for the estimation of BRT in phosphate buffer pH 7.4 at 248 nm is presented in the Table 2.



(a)

The linearity of the regression equation was demonstrated by excellent regression coefficient with a negligible scatter of points around the regression line. The slope value without intercept on the ordinate fell within the 95% confidence interval limits of reported slope which indicated

that the calibration line did not deviate much from the origin. The standard error of slope, intercept and estimate was found to be 3.047 x 10^{-4} and 3.560 x 10^{-3} and 3.811 x 10^{-3} respectively, further confirming the precision of the proposed method.



(b)

Fig.2 (a): UV- absorption spectrum of 12 µg/ml standard solution of brimonidine tartrate in potassium phosphate buffer (pH 7.4) at λ_{det} of 248 nm, (b) Overlay spectrum of brimonidine tartrate standard solutions (3-18 µg/ml) in phosphate buffer (pH 7.4).

Table-2: Regression analysis data for linearity of developed method for the estimation of brimonidine tartrate at 248 nm in phosphate buffer (pH 7.4)

Statistical parameters	Values
Regression equation	Y = 0.0630 X + 0.0050
Regression coefficient (R^2)	R^2 = 0.9999
Standard error of slope	3.047 x 10^{-4}
Standard error of intercept on ordinate	3.560 x 10^{-3}
Standard error of estimate	3.811 x 10^{-3}
95% confidence interval of intercept	- 0.0041 to 0.0156
95% confidence interval of slope	0.0622 to 0.0639
Slope without intercept	0.0635

Method validation

The proposed method was validated as per ICH Q2B guidelines [19,20] and statistical methods [21]. The validation summary for the proposed method is presented in the Table 3.

The linearity of the method was determined by linear regression and residual analysis of the results of calibration standard solutions of BRT. The linearity range of the method was found to be 3-18 μg/ml at λ_{det} of 248 nm. As the slope without intercept was found to be well within the 95 % confidence interval of slope, the proposed method can be considered as linear over the range of 0-18 μg/ml. A one-way ANOVA test of linearity was performed on absorbance values obtained for calibration standards [21]. The calculated F value was found to be very less compared to the tabulated value at 5% level of significance indicating that the method is linear in the proposed range.

Accuracy of the proposed method (expressed as mean % recovery) was found to be 99.92 ± 0.94 in the selected solvent. The precision of the developed method was studied by determining the repeatability and intermediate precision. The repeatability or intra assay and intermediate precision values expressed as % RSD, was found to be 0.60 ± 0.67 and 0.49 ± 0.76 respectively. The low values of % RSD indicate the excellent precision of the developed method.

Table-3: Validation report for the estimation of brimonidine tartrate in standard solutions at 248 nm in phosphate buffer pH 7.4.

Analytical parameter	Results		
Accuracy	% Recovery : 99.92 ± 0.94		
Precision (%RSD)	Conc. (μg/ml)	Intra-day	Inter-day
	3	1.391	1.366
	9	0.195	0.038
	18	0.223	0.077
	Mean % RSD ± SD	0.60 ± 0.67	0.49 ± 0.76
Linearity	3-18μg/ml		
Specificity	A 12 μg/ml solution of drug in phosphate buffer (pH 7.4) buffer pH 7.4 at λ_{det} of 248 nm will show an absorbance of 0.7613 ± 0.0013.		
LOD[a]	0.15μg/ml		
LOQ[a]	0.45μg/ml		
Robustness	A change in solvent pH by ± 0.5 and change in analyst resulted in % RSD not more than 1.23		

[a] Based on S.D. of the response and the slope of the regression curve

The LOD of analytical method is the lowest absolute concentration of the analyte which can be detected but not necessarily be quantified under the given experimental conditions. Similarly LOQ is the lowest concentration of an analyte which can be quantified with acceptable precision and accuracy. LOD of the proposed method was found to be 0.15 μg/ml and the LOQ was found to be 0.45 μg/ml respectively. Low LOD and LOQ values indicate high sensitivity of the developed method.

The proposed method was found to be specific for BRT, as UV- spectrum of the drug remained unchanged in the presence of common excipients added in the study. A drug solution of 12 µg/ml in phosphate buffer (pH 7.4) at λ_{det} 248 nm shows specifically an absorbance of 0.7613 ± 0.0013.

No significant change in the absorbance was observed with change in the pH of the media by ± 0.5. Also there is no significant change in the absorbance due to change in the instrument and analyst with % RSD value was found to be not greater than 1.23.

Drug content estimation from formulations and analytical recovery studies

The developed method was further validated by estimating the drug content from prepared formulations. Results are shown in the Table 4. The estimated drug content values for different formulations were obtained as 1.97 ± 0.02 mg/ml for ophthalmic solution; 0.98 ± 0.02 mg/12 mg of ocusert for the prepared ocuserts; and 1.97 ± 0.01 mg/g for the prepared ocugel. The % RSD in all the cases was less than 1%. A two-way ANOVA test at 5% level for drug content estimation from these formulations indicated low $F_{Calculated}$ value when compared to tabulated value suggesting non- interference from the varied formulation matrix in the estimation of drug. The percent analytical recovery values ranged from 96.49 to 99.28% in case of all the formulations further indicating specificity of the proposed method.

Table-4: Results of recovery studies of brimonidine tartrate from its formulation matrix by the proposed method

Formulation	Label claim	Recovery		% Analytical recovery (Mean ± SD)
		Mean ± SD	% RSD	
Ophthalmic solution	2.0 mg/ml	1.9674 ± 0.018 [a]	0.925	98.37 ± 0.91
Ocusert	1.0 mg/12 mg ocusert	0.9746 ± 0.016 [b]	0.828	97.30 ± 0.80
Ocugel	2.0 mg/g	1.9721 ± 0.007 [c]	0.363	98.60 ± 0.35

[a] mg/ml , [b] mg/12mg ocusert, [c] mg/g

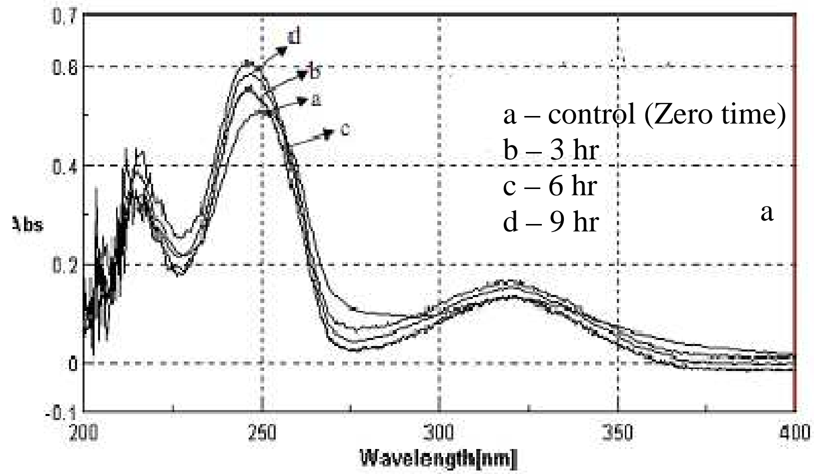
Bench top stability studies

The bench top stability at controlled room temperature (25 ± 2°C), showed that there was no significant change in drug's absorption spectra upto 24 hrs. This suggests that the method can be applied in the routine analysis of pure drug and its formulations in quality control testing without any fear of sample degradation.

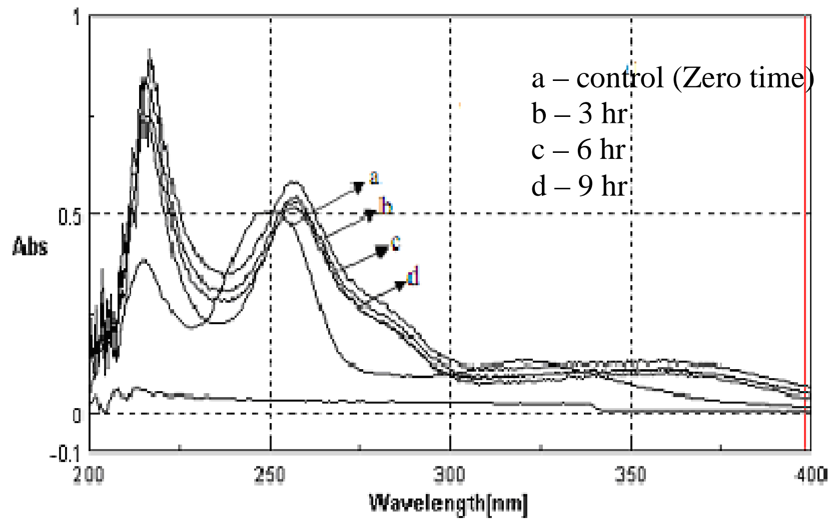
Forced degradation studies

The forced degradation studies were performed to study the stability indicating attribute of the proposed method. The ability of the method to trace out the degradation of the drug, as evident by the shift in the UV- absorption spectra of the drug is an indicator for the method's specificity for the drug in the given experimental conditions and also provide information on its stability

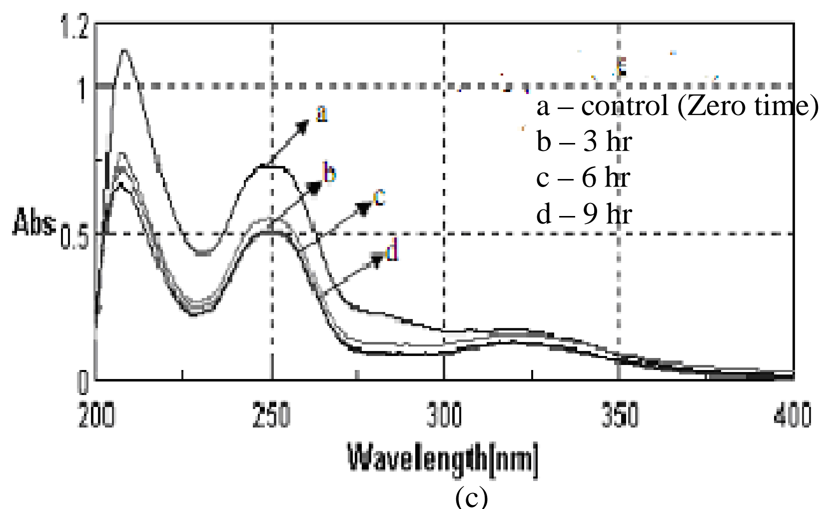
indicating property. The UV spectrum (200-400 nm) of BRT after exposure to acidic, alkaline and heat treatments showed a significant deviation from the zero time control as shown in Fig 3.



(a)



(b)



(c)

Fig 3: Overlayed UV-absorption spectrum of 9 µg/ml concentration of BRT at at 0,3, 6, 9 hrs under (a) temperature stress condition, (b) in 0.1N NaOH and (c) in 0.1N HCl

Preformulation studies

The above method was successfully employed in the determination of preformulation parameters of BRT in the preformulation studies such as drug solubility studies in buffers and in water, apparent partition coefficient.

(a) Solubility studies

The solubility results of BRT were shown in the Fig. 4. The solubility of BRT is found be pH dependent, with solubility higher on acidic side and decreasing as pH is increased. From the pKa results, it is evident that drug is weakly basic in nature. It exists in predominately in ionized form at lower pH, hence a higher solubility of free base. As the pH is increased, solubility was found to decrease. The solubility in TDW was found to be 29.85 mg/ml.

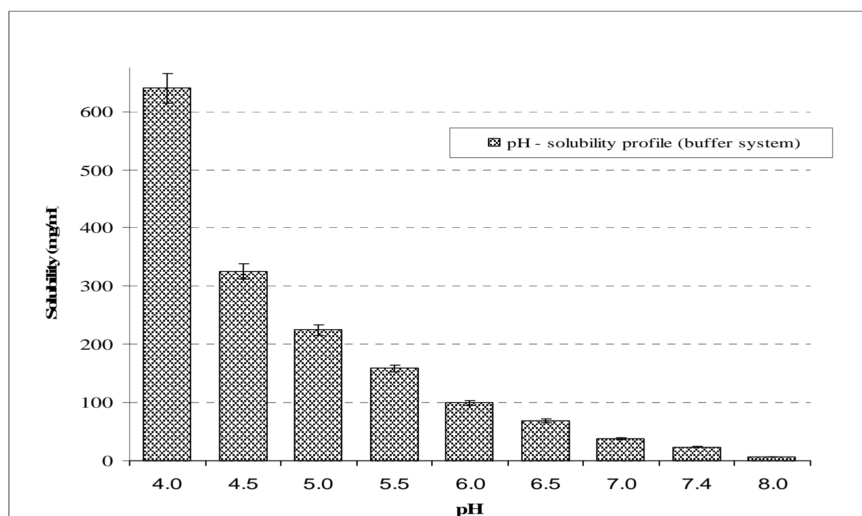


Fig. 4: pH-solubility profile of brimonidine tartrate in aqueous buffer systems.

(b) Dissociation constant

The pKa of BRT was determined using spectrophotometric method and the results are shown in the Table 5. The pKa of the drug was found to be 7.22 ± 0.18 The earlier reported value of determined by potentiometric method was 7.5 [25].

The UV absorbance spectras of various absorbing species at different pH were recorded in the range of 200-400 nm. They are overlapped in order to determine the wavelength for pKa determination. The wavelength(s) selected for the determination of absorbance of BRT in ionized and neutral forms, was the point where the maximum difference existed between various species (ionized, neutral) of the BRT at varying pH solutions. For the accurate determination of pKa, a range of wavelength from 234-250 were selected and absorbance of BRT in varying pH buffers were noted down. The representative pH absorbance of BRT at wavelength of 248 nm is presented in the Fig 5. A gradual decrease in absorbance was observed beyond pH 6.0.

A first derivative spectra of ΔAbs/ΔpH Vs pH (Fig. 6) showed the pKa to be in the range of 6-8, with a sharp change in absorbance with respect to ΔAbs/pH against pH. Graphically pKa was found be 7.3, as determined from the peak of first derivative spectra. Also the results of pKa determination using Handersson-Hasselbach equation (Eq. 2) is presented in the Table-5. The pKa by this method was found to be 7.22 ± 0.18. The pKa values were found to be almost same at the entire wavelength selected. The ionisation at lower pH was due to the protonation of amino groups of BRT, which decreases as the pH is increased. At pH = pKa, a equal amount of ionised (protonated) and unionised (non- protonated) species were formed.

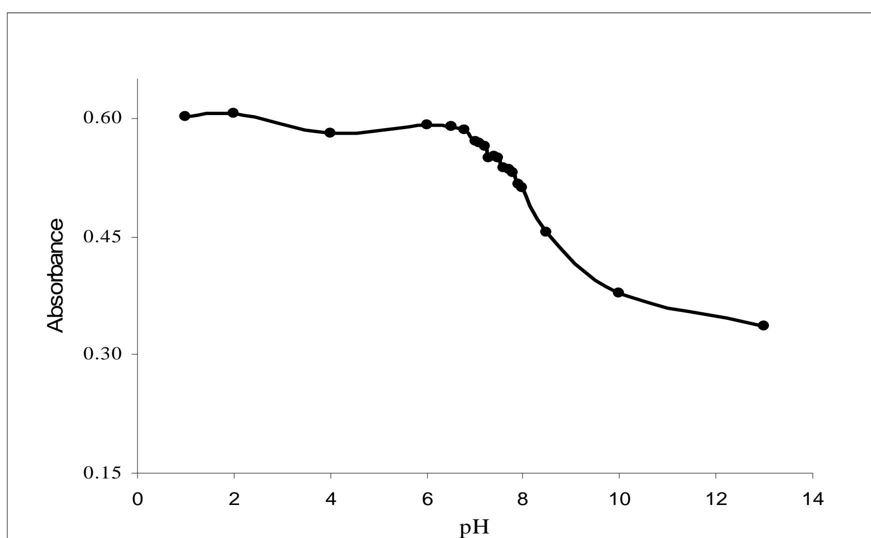


Fig. 5: pH- absorbance profile of brimonidine tartrate at 248 nm

Table-5: pKa of Brimonidine tartrate in multiple wavelengths and multiple pH range.

pH	pKa at Wavelength (nm)								
	234	**236**	**238**	**240**	**242**	**244**	**246**	**248**	**250**
6.5	7.33	7.13	7.03	6.98	7.42	7.33	7.09	7.32	7.10
6.8	7.03	6.91	6.88	6.8	7.02	6.99	6.9	7.31	7.39
7.0	7.09	6.97	7.11	7.06	7.10	7.01	7.15	7.08	7.18
7.1	7.34	7.20	7.28	7.14	7.20	7.08	7.12	7.16	7.27
7.22	7.43	7.29	7.28	7.24	7.36	7.19	7.22	7.26	7.36
7.31	7.29	7.38	7.47	7.42	7.22	7.35	7.25	7.43	7.53
7.51	7.02	7.51	7.41	7.04	7.33	7.30	7.12	7.22	7.90
7.67	6.92	7.22	7.32	7.31	7.41	7.08	7.21	7.21	7.45
7.82	6.96	6.92	7.42	7.45	7.32	7.22	7.32	7.16	7.28
7.99	7.42	7.43	7.38	7.21	7.04	7.10	7.40	7.21	7.23
Average pKa: 7.22 ± 0.18									

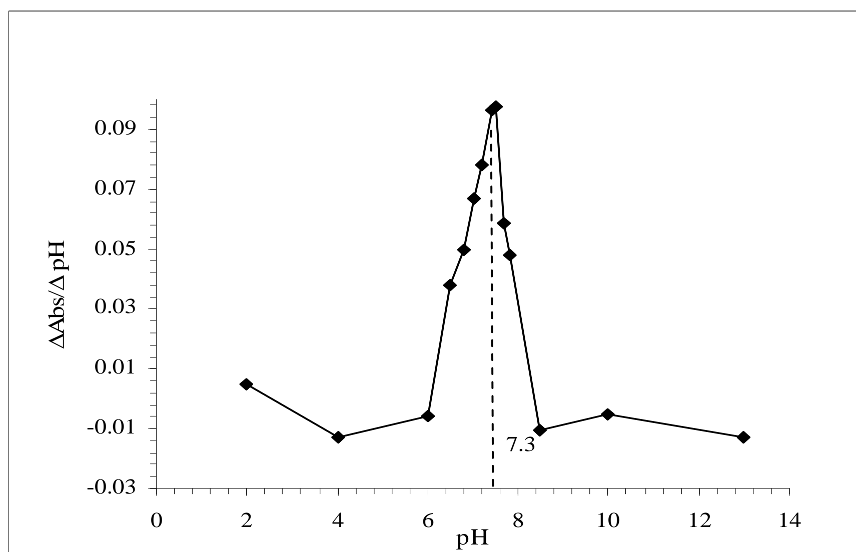


Fig. 6: First derivative absorbance spectra of brimonidine tartrate in varying pH.

(c)Apparent partition coefficient

The partition coefficient of BRT between chloroform/ aqueous media system and octanol/aquoues media system is tabulated in the Table 6. From the Table 6, it was evident that, the P_{App} values in octanol/buffer system was highly pH dependent. The P_{App} values were less at pH 5.5 and increased gradually with an increase in the pH of the buffer media. From the pKa determination studies, BRT was found to be weakly basic (pKa 7.22). Therefore at acidic pH, a

relatively low P_{App} was obtained due to the ionisation of drug below its pKa, because of presence of basic functional groups in the drug molecule whereas at neutral pH range drug exists as zwitterion form. The degree of ionisation of drug majorly governs its partition across the organic and aqueous phases. As the pH is increased, P_{App} values increased due to fact that pH was approaching pKa of the drug and the degree of ionization was relatively lesser. A very high value of P_{App} was obtained at pH 7.4 and 8.0, as drug predominately existed in unionized form. The pH-partition profile is depicted in the fig.7.

Table- 6: pH-apparent partition coefficient of BRT in chloroform/aqueous media and Octanol/aqueous media (Buffer used is phosphate buffer)

Results in Chloroform / Aq. media			Results in Octanol / Aq. media		
Composition of Aq. media	P_{App} (Mean ± SD)	Log P_{App} (Mean ± SD)	Composition of Aq. media	P_{App} (Mean ± SD)	Log P_{App} (Mean ± SD)
pH 5.5 buffer	1.80 ± 0.01	0.25 ± 0.01	pH 5.5 buffer	0.14 ± 0.01	-0.86 ± 0.02
pH 6.0 buffer	1.93 ± 0.03	0.28 ± 0.04	pH 6.0 buffer	0.16 ± 0.01	-0.78 ± 0.03
pH 6.5 buffer	2.01 ± 0.02	0.30 ± 0.02	pH 6.5 buffer	0.26 ± 0.01	-0.58 ± 0.03
pH 7.0 buffer	2.10 ± 0.03	0.32 ± 0.03	pH 7.0 buffer	0.42 ± 0.01	-0.37 ± 0.02
pH 7.4 buffer	2.19 ± 0.02	0.34 ± 0.02	pH 7.4 buffer	0.78 ± 0.01	-0.11 ± 0.01
pH 8.0 buffer	2.32 ± 0.01	0.37 ± 0.01	pH 8.0 buffer	1.80 ± 0.02	0.25 ± 0.03
pH 8.5 buffer	2.33 ± 0.01	0.37 ± 0.03	pH 8.5 buffer	1.70 ± 0.01	0.23 ± 0.02
Water	1.78 ± 0.02	0.25 ± 0.03	Water	1.78 ± 0.02	0.25 ± 0.04

In the case of chloroform/aqueous media system, a relatively higher value of P_{App} was obtained in comparison to octanol/aqueous system at all the pH conditions. This may be because of the stronger acidic nature of chloroform, thereby resulting in higher partitioning of drug into the chloroform layer compared to that of octanol layer.

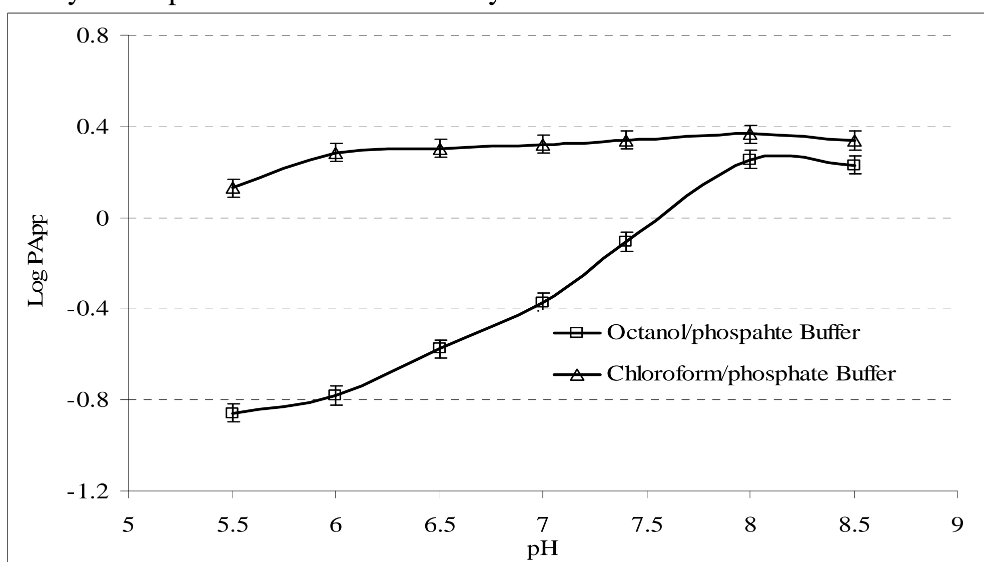


Fig. 7: pH- partition coefficient profile of brimonidine tartrate in organic-aqueous media.

CONCLUSIONS

To conclude, the developed and validated method for the estimation of BRT in pure form and its ophthalmic formulations is simple, accurate, rapid, precise and cost effective. The method is very sensitive with LOD and LOQ of 0.15 µg/ml and 0.45 µg/ml. The proposed method does not utilizes special extraction procedures in recovering the drug from the formulation excipients matrices, hence it is fast and has low probability of sample preparation error. The drug content estimation values in different formulations and analytical recovery values were in agreement with the labeled claims indicating the specificity of the proposed method and non- interference of formulation additives. Hence the method can be a good tool in estimating BRT is pure drug, in dosage form, and in the analysis of samples of in vitro dissolution studies and in stability studies. Further the method also can be used as stability indicating method due to the high stability of the analyte in the solvent system used and detection of any degradation at the selected wavelength of the analysis.

The developed method was successfully implemented for determination of solubility and apparent partition coefficient with values found to be matching with earlier reported values using different techniques.

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