Fluorimetric Quantification of Brimonidine Tartrate in Eye Drops

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A simple and sensitive spectrofluorimetric method has been developed for the estimation of brimonidine tartrate in pure and eye drops. Linearity was obeyed in the range of 0.2-3.0 µg/ml in dimethyl formamide as solvent at an emission wavelength (λ_{em}) of 530 nm after excitation wavelength (λ_{ex}) of 389 nm with good correlation coefficient of 0.998. The limit of detection and limit of quantification for this method were 22.0 and 72.0 ng/ml, respectively. The developed method was statistically validated as per International Conference on Harmonisation guidelines. The percentage relative standard deviation values were found to be less than 2 for accuracy and precision studies. The results obtained were in good agreement with the labelled amounts of the marketed formulations. The proposed method was effectively applied to routine quality control analysis of brimonidine tartrate in their eye drops.

Key words: Brimonidine, spectrofluorimetry, validation

Brimonidine tartrate (BRT) chemically 5-bromo-6-(2-imidazolidinylideneamino) quinoxaline L-tartrate is a selective alpha-2 adrenergic agonist, used as ocular hypotensive agent[1,2]. Detailed literature survey revealed that few analytical methods are reported for quantification of BRT in eye drops and ophthalmic fluids by using spectrophotometry[3], high performance liquid chromatography (HPLC)[4-6], high performance thin layer chromatography (HPTLC)[7], UPLC[8] and liquid chromatography-mass spectroscopy (LC/MS/MS) methods[9-11]. To the best of our knowledge, no method reported on the use of spectrofluorimetry for the quantification of BRT in eye drops. Spectrofluorimetry has assumed a major role in drug analysis because of its greater sensitivity and selectivity than absorption spectrophotometry[12-17]. Hence, we aimed to develop and validate a simple, precise, accurate, selective and high sensitive spectrofluorimetric method for the estimation of BRT in eye drops.

BRT pure drug was obtained as gift samples from Dr. Reddy's Laboratories Ltd, Hyderabad, India. Eye drops (Alphagan-P and Alphagan) were procured from local pharmacies and dimethyl formamide (DMF) was purchased from Hi-Media, Mumbai, India. The fluorescence spectra and measurements were recorded using Shimadzu RF-5301 PC Spectrofluorophotometer, Shimadzu, Japan, equipped with 150 W Xenon arc lamp, 1 cm non-florescence quartz cell, connected to RFPC software.

BRT molecule contains polycyclic aromatic systems like imidazole ring and quinoxaline ring, in which more π electrons are available to exhibit florescence. For spectrofluorimetric method purpose, various solvents were investigated such as acetonitrile, methanol, dimethyl sulphoxide (DMSO), ethanol, DMF and different buffer systems. The fluorescence intensity of BRT was found only in DMF solvent. Hence, we selected DMF as solvent for quantification of BRT in eye drops at emission wavelength 530 nm after excitation wave length 389 nm as shown in fig. 1.

The standard stock solution (1 mg/ml) BRT was prepared by transferring 10 mg of BRT in 10 ml volumetric flask and volume was made up to the mark with DMF. Aliquots of BRT stock solutions were taken into 10 ml volumetric flasks and diluted up to the mark with the solvent such that the final concentration of BRT was in the range of 0.2-3.0 µg/ml and analysed them by spectrofluorimeter with the proposed method. The calibration curve was constructed by plotting the analyte fluorescence intensity against the concentration (µg/ml). Calibration curve was evaluated by its correlation coefficient.

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Optimum conditions of proposed method are mentioned in Table 1.

For the assay of marketed formulations, 1 ml (Alphagan-p 0.15% and Alphagan 0.2%) of each containing 1.5 and 2 mg/ml of BRT were transferred into 10 ml volumetric flask and volume was made up to mark with DMF solvent to get the concentration 150 and 200 µg/ml of BRT, respectively. The solutions finally diluted with DMF to get concentrations within linearity range and intensity was measured by proposed method. Results were in good agreement with the label claim of the drug and are reported in Table 2. The proposed method validated according to International Conference on Harmonisation (ICH) guidelines[18]. The percentage recoveries were found to be in the range of 98.8-102.4%. This indicates that the method is accurate. Results from precision expressed in terms of %RSD, found to be less than 2. No significant differences between intraday and interday precision, which indicated that the method is reproducible and reliable. The results of limit of detection (LOD) and limit of quantification (LOQ) revealed that proposed method was highly sensitive than previous methods.

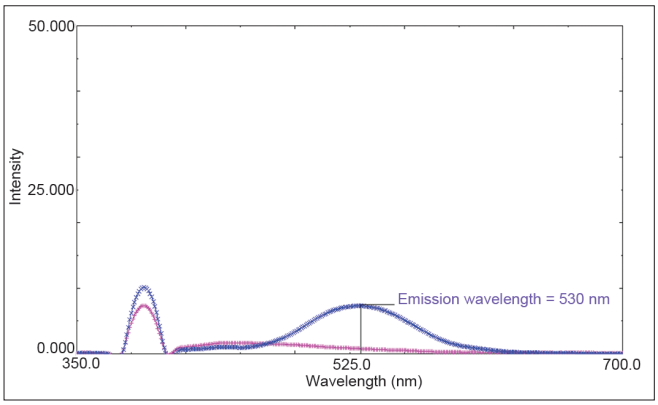

Fig. 1: Excitaion and emission spectra of brimonidine tartrate.
Excitaion and emission spectra of brimonidine tartrate (1 µg/ml) in DMF and blank DMF. The fluorescence intensity of BRT was found only in DMF solvent. It shows florescence intensity without spectral interference. The excitation wavelength (λ_{ex}) and emission wavelength (λ_{em}) of brimonidine tartrate in DMF was found to be 389 and 530 nm, respectively.
DMF blank, Brimonidine tartrate

TABLE 1: OPTIMUM CONDITIONS OF PROPOSED METHOD

Parameter	Value
Excitation wavelength (nm)	389
Emission wavelength (nm)	530
Range (µg/ml)	0.2-3.0
Limit of detection (ng/ml)	22.0
Limit of quantification (ng/ml)	72.0
Correlation co-efficient (r^2)	0.998
Slope (m)	4.389
Intercept (c)	0.341
Regression equation	Y=4.389x+0.341

The equation of the regression line was found to be Y=4.389x+0.341 with a correlation co-efficient of 0.998, revealed that BRT was showing linear relationship between concentration (µg/ml) and fluorescence intensity. BRT was linear in the range of 0.2-3 µg/ml. The LOD and LOQ of the method was found to be 22 and 72 ng/ml, respectively, which indicates the sensitivity of the method

TABLE 2: ANALYSIS OF FORMULATION

Formulation	BRT		
	Label claim (mg/ml)	Amount found (mg, mean±SD, *n*=3)	% RSD
Alphagan	2.0	2.20±0.025	1.13
Alphagan-p	1.5	1.54±0.026	1.68

The amount of BRT found in formulation-I (Alphagan 2%) was 2.2 mg and formulation-II (Alphagan-P 1.5%) was 1.54 mg. These amounts were within the limits. The % RSD for both formulations was less than 2, which indicates the accuracy of the proposed method, BRT= brimonidine tartrate, RSD= relative standard deviation

It is concluded that the proposed method was found to be simple, accurate, precise, specific and sensitive for the quantification of BRT in pure form and eye drops. The assay values were in good agreement with their respective labelled claim, which suggested no interference of formulation excipients in the estimation. The results obtained from validation proved that; the proposed method was scientifically sound. These advantages encourage that the proposed method can be routinely employed in quality control for analysis of BRT in eye drops.

REFERENCES

1. Sweetman SC, Martindale. The Complete Drug Reference. London: Pharmaceutical Press; 1999. p. 876.
2. Maryadele JO. Neil. The Merck index, an encyclopedia of chemicals, drugs and biologicals. 14th ed. White House Station: Merck and Co. Inc; 2006. p. 1375.
3. Prakash B, Pandurang D, Sarubh P, Sajeev C. Development and validation of stability indicating UV spectrophotometric method for the estimation of brimonidine tartrate in pure form, formulation and preformulation studies. Der Pharm Lett 2010;2:106-22.
4. Mohan M, Ravi T. Method development and validation of brimonidine tartrate by high performance liquid chromatography. Asian J Res Chem 2011;4:1591-4.
5. Arun P, Senthil Kumar M, Mahadevan N. Simultaneous estimation of brimonidine tartrate and timolol maleate in nanoparticles formulation by RP-HPLC. Int J Recent Adv Pharm Res 2011;3:31-6.
6. Narendra A, Deepika D, Mathrusri AM. Liquid chromatographic method for the analysis of brimonidine in ophthalmic formulations. E J Chem 2012;9:1327-31.
7. Mahajan A, Athensia F, Gandhi SV, Deshpande PB. Development and validation of high performance thin layer chromatographic method for estimation of brimonidine tartrate as bulk drug and in ophthalmic solutions. Int J PharmTech Res 2010;2:1376-9.

8. Manohar CS, Rajput AP. Development and validation of a new stability indicating analytical method for the determination of related compounds of brimonidine tartrate in drug- substance and drug product using UPLC. Int J Pharm Sci 2011;3:145-50.
9. Madhavi A, Naidu A, Subbarao DV, Srinivas. Development and validation of a new LC-method for analysis of brimonidine tartrate and related compounds. Chromatographia 2009;69:1413-9.
10. Andrew A, Diane D, Tang-liu S. Measurement of brimonidine concentration in human plasma by a highly sensitive gas chromatography/mass spectrometric assay. J Pharm Biomed Anal 1995;13:995-1002.
11. Jiang S, Chappa SK, Prokseh JW. A rapid and sensitive LC/MS/MS assay for the quantitation of brimonidine in ocular fluids and tissues. J Chromatogr B 2009;877:107-14.
12. Fawzia I, Mohie KS, Manal I. Validated stability- indicating spectrofluorimetric methods for the determination of ebastine in pharmaceutical preparations. Chem Cent J 2011;5:11.
13. Mesut A, Emrah K. Validated spectrofluorimetric method for the determination of sunitinab malate, dye complexation approach for a novel anticancer drug. Acta Pharm Sci 2010;52:469-85.
14. Mohamed W, Fathallah FB, Nahed ME. Synchronous fluorescence spectrofluorimetric method for the simultaneous determination of metaprolol and felodipine in combined pharmaceutical preparation. Chem Cent J 2011;5:70.
15. El-Wasseef DR, El-Sherbiny DT, Abu-El-Enein MA. Simultaneous determination of labetalol and furosemide by first-derivative synchronous spectrofluorimetry. J Floresec 2009;19:817-28.
16. Belal F, El-Brashy A, El-Enany N, Tolba M. Conventional and first derivative synchronous fluorometric determination of ethamsylate in pharmaceutical preparations and biological fluids, application to stability studies. J Fluoresc 2011;21:1371-84.
17. Panikumar AD, Sirisha N, Haripriya A, Sathesh Babu R, Subrahmanyam CV. First derivative synchronous spectrofluorimetric quantification of telmisartan/amlodipine besylate combination in tablets. Dhaka Univ J Pharm Sci 2013;12:35-40.
18. International Conference on Harmonization. Harmonized Tripartite Guideline, Validation of Analytical Procedures, Text and Methodology, Q2 (R1); 2005.

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Antibacterial Activity of Rhizome of *Curcuma aromatica* and Partial Purification of Active Compounds

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The hexane extract of *Curcuma aromatica*, a plant belonging to the family Zingiberaceae was tested on 10 bacterial strains (clinical isolates and standard strains). Agar diffusion method was adopted for determining the antibacterial activity of the extract. The hexane extract was found to be active against all Gram-positive strains tested, but inactive against Gram-negative strains. The minimum inhibitory concentration and minimum bactericidal concentration were determined and found to be 539 µg/ml. The phytochemical analysis of hexane extract by gas chromatography mass spectrometry revealed the presence of 13 compounds. The crude hexane extract was partially purified by thin layer chromatography. The zone showing good antibacterial activity was analysed further by gas chromatography mass spectrometry, UV/Vis spectrophotometry and Fourier transform infrared spectroscopy, which indicated the probable presence of germacrone.

Key words: *Curcuma aromatica*, antibacterial activity, crude extract, phytochemical analysis, germacrone

Resistance to antibiotics is a serious problem worldwide. In particular the multiple drug resistance among *Staphylococcus aureus* is of great concern. The increasing worldwide prevalence of infectious diseases has created an urge to look for new drugs from plants. The plant chosen in this study is *Curcuma aromatica* Salisb., used in cosmetic formulations and traditional medicinal applications[1,2], as an antiinflammatory agent, to promote blood circulation, to remove blood stasis and for the treatment of cancer[3]. Rhizomes are used in combination with astringents and aromatics for bruises, sprain, hiccough, bronchitis, cough, leucoderma and skin eruptions[4]. The paste made of benzoin and rhizome of *C. aromatica* is commonly used as a domestic remedy in headache. The monoterpenoids[5], sesquiterpenoids[5-7] and curcuminoids[8,9] of *C. aromatica* have been reported to possess antimicrobial[10,11], antifungal, antioxidant and antitumour activities[12,13].

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