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 \( \lambda_{em} \) of 530 nm after excitation wavelength \( \lambda_{ex} \) 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\cite{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\cite{3}, high performance liquid chromatography (HPLC)\cite{4-6}, high performance thin layer chromatography (HPTLC)\cite{7}, UPLC\cite{8} and liquid chromatography-mass spectroscopy (LC/MS/MS) methods\cite{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\cite{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 \( \pi \) 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.
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.

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


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

S. REVATHI* AND N. S. MALATHY
Departments of Plant Biology and Plant Biotechnology, PSGR. Krishnammal College for Women, Coimbatore-641004, India

Revathi and Malathy: Antibacterial Activity Curcuma aromatica

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 monoterpeneoids[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].

*Address for correspondence
E-mail: revashi02@gmail.com