Spectrofluorimetric Method for the Determination of Atenolol in Tablet Dosage Forms

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A simple and sensitive spectrofluorimetric method was developed for the determination of atenolol in tablet dosage forms. The method was based on the reaction of atenolol with 0.1 N sodium hydroxide solution in boiling water bath, which shows strong fluorescence having excitation and emission wavelengths 278 nm and 302 nm, respectively. Linear relationship for the fluorescence intensity was obtained in the concentration range of 5.0-25.0 μg/ml. The method was validated statistically and was applied successfully for the determination of atenolol in tablet dosage forms.

Atenolol is a beta-blocker, cardio selective drug having no intrinsic sympathomimetic activity and used in the treatment of hypertension. Atenolol is chemically 4-(2-hydroxy-3-isopropylaminopropoxy)-phynylacetamide[1]. Atenolol is official in IP and USP. Various spectrophotometric[2,3] and HPLC[4,5,6] methods have been described for its estimation. In the present study, atenolol gives strong fluorescence having excitation and emission wavelengths 278 nm and 302 nm, respectively in 0.1 N NaOH in boiling water bath. So it was thought of interest to develop a simple, sensitive, accurate and precise spectrofluorimetric method for the routine analysis of atenolol in bulk drug and in their formulations.

The recovery experiments were performed by adding known amount of drug to the preanalyzed formulation and reanalyzing the mixture by proposed method. The results were validated statistically and the % recovery was found in the range of 99.2 to 100.3. The proposed method is new, simple, sensitive, accurate, and precise and can be successfully employed in the routine analysis of atenolol in tablet dosage forms.

Fluorescence spectrophotometer with single quartz cell of 1 cm path length (Hitachi, F-2000) was used to measure fluorescence intensity of resulting solutions. A sonicator (Frontline FS-4) and constant temperature water bath (Labtronic) were also used in the study. Atenolol (Torrent Pharmaceuticals Ltd., Ahmedabad) was used in this study. The tablets were procured from a local pharmacy. Aqueous solutions of 0.1 N NaOH was prepared in double distilled water.

Sodium hydroxide solution (0.1 N) was prepared by dissolving 4.0 g of sodium hydroxide in double distilled water in a 1000 ml volumetric flask. A standard solution containing 1 mg/ml of atenolol was prepared by dissolving 100 mg of pure drug in 100 ml of 0.1 N NaOH. It was further diluted to 100 μg/ml with the same solvent. Twenty tablets were weighed and powdered. Powder equivalents to 100 mg of atenolol was accurately weighed and dissolved in 0.1 N NaOH, the solution was filtered through Whatman filter paper No. 41and the volume was made to 100 ml. It was further diluted to 100 μg/ml with the same solvent.

Standard stock solution (0.5-2.5 ml, 5-25 μg/ml) or sample solution (1.0 ml) were transferred to a series of 10 ml corning volumetric flasks and heated for 30 min on boiling water bath, cool to room temperature and volume was adjusted up to the mark with 0.1 N NaOH. The fluorescence intensity of resulting solution was measured at emission

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TABLE 1: ANALYSIS OF ATENOLOL IN TABLET DOSAGE FORMS

Formulation#	Label claim(mg/tab)	% of label Claim± S.D	%CV%	Recovery*± S.D
Tablet 1	25	99.1±1.03	1.040	100.3±1.14
Tablet 2	50	99.7±0.79	0.793	99.2±0.89
Tablet 3	25	101.6±0.85	0.837	99.7±0.93

*Mean of five determinations. #The commercial preparations used were; Tablet 1: Betacard, Torrent Pharmaceuticals Ltd. Tablet 2 and 3: Atelol, Themis Pharmaceuticals Ltd.

wavelength 302 nm keeping excitation wavelength 278 nm. The calibration curve was prepared by plotting concentration of atenolol Vs corrected fluorescence intensity of the respective solution. The correction in fluorescence intensity is made by subtracting the native fluorescence of blank solution from the observed fluorescence intensity of standard or sample solution.

To test the accuracy and reproducibility of the proposed methods, recovery experiments were carried out by adding known amount of the drug to the preanalyzed formulation and reanalyzing the mixture by proposed methods. The results are shown in Table 1.

Effect of sodium hydroxide concentration was evaluated by taking 2.0 ml solution of atenolol standard in a series of 10 ml volumetric flasks and treated with varying concentration of sodium hydroxide solution (0.1-1.0 N). The solution was analyzed as described under fluorimetric method. Optimum heating time required to obtain fluorophore having maximum intensity was evaluated by taking 2.0 ml solution of atenolol standard in a series of 10 ml volumetric flasks and heated in boiling water bath for different time (0-90 min) and the solution was analyzed as described under fluorimetric method. The stability of developed fluorophore was determined by measuring the fluorescence intensity of the treated sample up to 2.0 h. The proposed method was validated in terms of accuracy, precision, linearity, limit of detection and limit of quantification.

The proposed reaction mechanism was that the acetamido ($-CH_2CONH_2$) group of atenolol gets hydrolyzed to $-NH_2$ group in alkaline as well as acidic medium. $-NH_2$ group is a fluorescent group and was unionized in alkaline medium while in acidic medium, ionization takes place and gets converted in to their protonated form (NH_4^+), which gives the lower intensity than the unionized form. The literature shows that the $-NH_2$ substituted structure give the maximum fluorescence in alkaline pH while the acetamido ($-CH_2CONH_2$) group is non-fluorescent at all pH values[7]. Therefore, it was thought of interest to exploit the fluorescence of compound in basic medium (0.1 N NaOH) for its estimation in market formulations.

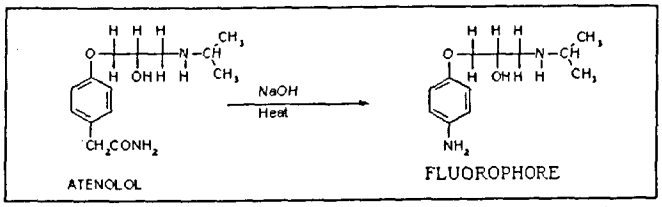

Fig. 1: Reaction scheme for development of fluorophore

The developed method was optimized using different parameters such as sodium hydroxide concentration, heating time required for development of maximum fluorescence intensity and stability of developed fluorophore. Maximum fluorescence intensity was obtained with 0.1 N sodium hydroxide solution in double distilled water. On further increase in concentration of sodium hydroxide fluorescence intensity get decreased. The minimum heating time required to get maximum fluorescence intensity was 30 min heating on boiling water bath. No change in fluorescence intensity was observed on further heating. The developed fluorophore was stable up to 90 min at room temperature (29±1.0°). After that

TABLE 2: VALIDATION PARAMETERS FOR SPECTROFLUORIMETRIC METHOD

Parameter	Range
Linearity range (µg/ml)	5.0-25.0
Detection limit (µg/ml)	0.5
Quantification limit (µg/ml)	5.0
Correlation co-efficient	0.9996
Precision (% CV) (n=5)	0.647-1.643
Accuracy (n=5)	99.2-100.3%

fluorescence intensity diminished gradually. The optimized method was validated in terms of accuracy, precision, linearity, limit of detection and limit of quantification. The results are summarized in Table 2. The proposed method was successfully applied for the determination of atenolol in pharmaceutical dosage forms.

The analysis results of marketed formulations (tablets) are in good agreement with the labeled claim. The reproducibility, repeatability and accuracy of these methods were found to be good, which is evidenced by low standard deviation. The percent recovery obtained was 99.2-100.3 indicates non-interference from the common excipients and colour used in the formulations. Thus the developed spectrofluorimetric method was simple, sensitive, accurate, precise and reproducible and can be successfully applied for the routine estimation of atenolol in bulk and pharmaceutical dosage forms.

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Inclusion Complexation of Rofecoxib with Dimethyl β-Cyclodextrin

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An attempt has been made to enhance solubility and dissolution of rofecoxib by complexation using dimethyl β-cyclodextrin. Complexes were prepared by physical mixture, kneading and spray drying methods. The prepared complexes were evaluated by Fourier transform infra-red spectroscopy, X-ray diffraction, differential scanning calorimetry and scanning electron microscopy. Release profile of the drug from the complexes were studied in pH 1.2 and pH 7.4 and it was found that the marketed preparation showed lesser release characteristics as compared to the complex prepared by kneading method.

Cyclodextrins are cyclic maltooligosaccharides, which have been extensively used to increase aqueous solubility of poorly soluble drugs[1,2]. Amongst the existing cyclodextrins, β-cyclodextrin (β-CD) has been used extensively to modify the physico-chemical properties[3-5]. Rofecoxib is a selective cox-2 inhibitor, which is used in the treatment of osteoarthritis and rheumatoid arthritis[6]. This drug is practically insoluble in water and has a longer onset of action. Since it is used in the treatment of osteoarthiritis, its prolonged use is associated with incidence of side effects that include GI perforations, ulcerations and bleeding. Therefore, an attempt has been made to improve the aqueous solubility of rofecoxib by complexing it with dimethyl β-cyclodextrin (DiMEB), thus enhancing its dissolution rate, thereby showing a faster onset of action and less GI mucosal toxicity.

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Rofecoxib and DiMEB were obtained as a gift sample