Estimation of Cyclopentolate Hydrochloride from Ophthalmic Solutions

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One visible spectrophotometric and another fluorimetric method have been developed for the determination of cyclopentolate hydrochloride from bulk and ophthalmic solutions. Spectrophotometric method is based on the formation of greenish blue colored species on treatment with Folin-Ciocalteu (FC) reagent in alkaline medium, showing maximum absorbance at 733 nm that obeyed Beer's law in the concentration range of 20-240 µg/ml. Fluorimetric method is based on the hydrolysate solution of cyclopentolate hydrochloride in 0.5 N sodium hydroxide on excitation at 366 nm emits yellowish blue fluorescence measured at 475 nm, which showed linearity in the concentration range of 20-600 µg/ml.

Cyclopentolate hydrochloride (CY) is a tertiary amine antimuscaranic agent with actions similar to those of atropine[1]. It is used as eye drops to produce mydriasis and cycloplegia. Chemically cyclopentolate hydrochloride is 2-dimethylamino ethyl-1-hydroxy-α-phenyl cyclopentane acetate hydrochloride[2], which is official in BP[3], USP[4] and NF[5]. Literature survey reveals very few analytical methods such as a non-aqueous titration[3] and HPLC[4-5].

In the present investigation, hydrolyzed CY produced a greenish blue colored chromogen with FC reagent in alkaline medium that could be determined at 733 nm. The hydrolysate solution of CY also produced a yellowish blue fluorescence in 0.5 N sodium hydroxide. The primary and secondary filters used were 366 and 475 nm respectively.

All the reagents used were of analytical grade. Aqueous solutions of sodium hydroxide (Loba Chemie), hydrochloric acid (Qualigens) and 1N FC reagent (Loba Chemie) were prepared in double distilled water. Spectral and absorbance measurements were made on an Elico SL 159 UV/Vis spectrophotometer by using 1 cm quartz cells and fluorimetric measurements were made on an Elico flurometer model CL 53. An Elico pH analyzer, model LI-6I2 was used for the measurement of pH.

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For the visible spectrophotometric method, 100 mg of pure drug or eye drops (weight of CY equivalent to 100 mg) was treated with 40 ml of 5 M HCl and refluxed for 90 min. The remaining HCl was removed under vacuum. The crystalline residue obtained was dissolved in 10 ml of methanol and diluted with double distilled water and filtered. The final volume was made upto 100 ml with double distilled water to get 1 mg/ml and used directly. For the fluorimetric method, after getting the crystalline residue as described above, it was dissolved in 0.5 N NaOH and filtered. Then the solution was diluted up to 100 ml with 0.5 N NaOH to get a concentration of 1000 µg/ml.

For the visible spectrophotometric method, aliquots of standard hydrolyzed solution of CY ranging from (0.4-2.4 ml; 1 ml≡1000 µg/ml) were transferred in to a 10 ml volumetric flasks. Then 0.8 ml of 1 N NaOH and 0.8 ml of 1N FC reagent were added successively to each flask. The contents of each flask were mixed well, kept aside for 15 min at room temperature and diluted up to 10 ml with double distilled water. The absorbance of each solution was measured at 733 nm against a reagent blank.

For the fluorimetric method, aliquots of standard hydrolyzed CY in 0.5 N NaOH (0.2 6 ml; I ml≡1000 µg/ml) solutions were transferred into a series of 10 ml graduated test tubes and the volume was made upto 10 ml with 0.5 N

TABLE 1: COMPARATIVE ESTIMATION OF CYCLOPENTOLATE HYDROCHLORIDE.

Formulations	Labeled amount (mg/5ml)	Amount obtained (mg) Proposed methods Method A	Method B	Reported Method[3]	Percent recovery Method A[a]	Method B[a]
Eye drops						
Brand I	50	49.29	49.92	49.24	99.2±0.8	99.6±1.2
Brand II	50	49.52	49.89	49.32	99.7±0.3	100.1±0.4

Each value is average of four determinations and expressed as (mean±SD). A denotes that the amount added is 10 mg.

NaOH. Each solution was excited at 366 nm and the resulting fluorescence was measured at 475 nm filter by taking 0.5 N NaOH as a blank. The amounts of CY present in formulations were computed from their respective calibration curves.

The proposed visible spectrophotometric method shows molar absorptivity-1.001×10^3 l/mole/cm, Sandell's sensitivity-0.3278 µg/cm²/0.001 absorbance unit and linear regression of absorbance on concentration gave the equation $y=0.00305x+4.56\times10^{-4}$ with a correlation coefficient of r-0.9947. % Relative standard deviation (RSD) of 0.745 was observed for analysis of eight replicate samples and % range of error at 0.05 confidence limits was found to be ± 0.5238. The proposed fluorimetric method gave the regression equation y=0.166x-0.11 with a correlation coefficient of r-0.9999, % RSD of 0.886 and % range of error ± 0.741 at 0.05 confidence limits.

In order to determine the precision and accuracy of the proposed methods, solutions containing known amount of CY were prepared and analyzed in four replicates. The results of the analysis of pharmaceutical formulations by the proposed methods and reported method were given in Table 1. The results showed that the proposed methods have comparable precision and accuracy. These results indicate that the proposed methods are sensitive, accurate, precise and reproducible and can be successfully applied for the routine estimation of CY in bulk and in pharmaceutical formulations such as eye drops. When pharmaceutical preparations containing CY were analyzed, the results obtained by the proposed methods were in good agreement with the labeled amount and are comparable with the reference method. The recovery in both the methods was found to be 99-101%.

The spectrophotometric method is based on the reduction of FC reagent by CY in alkaline medium to form a greenish blue colored chromogen that exhibits λ_{max} 733 nm and stable for 70 min. In the case of the fluorimetric method, hydrolyzed CY in 0.5 N NaOH upon excitation at 366 nm emits yellowish blue fluorescence measured at 475 nm.

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