ABSTRACT

Three simple, accurate, economical and reproducible spectrophotometric methods have been developed for the simultaneous estimation of Gatifloxacin (GTX) and Ketorolac Tromethamine (KTA) in ophthalmic dosage form. The first method is Q analysis method based on absorbance ratio at two selected wavelengths 303 nm (iso-absorptive point) and 292 nm ($\lambda_{\text{max}}$ of Gatifloxacin). The second method is Simultaneous equation method based on measurement of absorbance at two wavelength 292 ($\lambda_{\text{max}}$ of Gatifloxacin) and 319 ($\lambda_{\text{max}}$ of Ketorolac Tromethamine) and the third method is Area under curve method and wavelength ranges selected were 290-294 nm ($\lambda_1-\lambda_2$) for Gatifloxacin and 317-321 nm ($\lambda_3-\lambda_4$) for Ketorolac Tromethamine. The linearity lies between 1-12 µg/ml for (GTX) and 1-12 µg/ml for KTA for all the three methods. The results of the analysis were validated statistically and recovery studies were carried out as per ICH guideline. As the % RSD was found less than 2, all the methods were proved to be precise and accurate and can be successfully applied for the simultaneous determination of both the drugs in bulk and ophthalmic dosage form.

Keywords: Gatifloxacin; Ketorolac Tromethamine; Q-absorbance ratio method; Simultaneous equation method; Area under curve method.

INTRODUCTION

Gatifloxacin (GTX) 1-Cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxo-3-quinolinecarboxylic acid sesquihydrate is a synthetic broad spectrum antimicrobial fluoroquinolone with extended spectrum and improved activity against Gram positive and Gram negative organisms and used in the treatment of a wide range of infections. The bactericidal activity of GTX is mediated by binding of the DNA-DNA gyrase complex to the DNA topoisomerase complex. It is indicated in acute pyelonephritis, acute bacterial exacerbation of chronic bronchitis, complicated UTI, conjunctivitis. Its liquid chromatographic method is official in I.P. Literature survey reveals that very few analytical methods have been reported for the determination of Gatifloxacin alone or in combination with another drugs such as spectrophotometric, HPLC, HPTLC. Fixed dose combination containing GTX and KTA as combined ophthalmic dosage formulation in the ratio of 3:4 mg is recently available in the market. Extensive literature survey reveled that not a single method is reported for simultaneous analysis of GTX and KTA in their combined dosage form. So a need was felt to develop new methods to analyze these drugs simultaneously. The aim of present study was to develop simple, accurate, economical and reproducible methods for GTX and KTA in bulk drug and in ophthalmic dosage forms.

EXPERIMENTAL

Instrumentation

The instrument used in the present study was UV-Visible double beam spectrophotometer, Make: Jasco, Model No. V 530 with spectral band width of 2 nm, data pitch 0.5 nm and pair of 10mm matched quartz cells. All the weighing was done on electronic balance Shimadzu, Model No. AUX 220.

Material and methods

Pure drug sample of Gatifloxacin and Ketorolac Tromethamine were obtained as gift sample from Micro labs Ltd, Bangalore-560068. Combined ophthalmic
formulation was procured from local market. All the chemicals used in spectrophotometric analysis were analytical grade. Acidic methanol was used as solvent.

**Method for preparation of solvent**
18 ml of glacial acetic acid and 900 ml methanol and diluted to 1000 ml with water.

**Preparation of Standard stock solutions:**
Accurately weighed quantity of GTX (10 mg) and KTA (10 mg) was transferred into two separate 100 ml volumetric flasks, dissolved in 25 ml acidic methanol and diluted to the mark with distilled water to get standard stock solution of concentration 100 µg/ml for each drug.

**Preparation of calibration curves**
Appropriate dilutions of the standard stock solutions were done to get 1 to 12 µg/ml of GTX and 1 to 12 µg/ml of KTA respectively. The absorption spectra of all solutions were recorded between 400 nm to 200 nm. The absorbances were measured at 292 (λ<sub>max</sub> of GTX) and 319 (λ<sub>max</sub> of KTA). Beer's lamberts range for GTX and KTA were selected and working calibration curves of both drugs were plotted separately.

**Method I: Absorbance ratio method (Q -Analysis)**
Absorbance ratio method uses the ratio of absorbances at two selected wavelengths, one at isoabsorptive point and other being the λ<sub>max</sub> of one of the two components. From the overlay spectra of two drugs, it is evident that GTX and KTA show an isoabsorptive point at 303 nm. The second wavelength used is the have λ<sub>max</sub> of GTX 292 nm (Fig 1). From stock solutions, working standard solution of GTX (3 µg/ml) and KTA (4 µg/ml) were prepared by appropriate dilution and were scanned in the entire UV range to determine the maximum absorbance (λ<sub>max</sub>) and isoabsorptive point. The concentrations of two drugs in mixture can be calculated by using following equations.

\[
C_x = \frac{Q_m - Q_y}{Q_x - Q_y} \times \frac{A_1}{a_1} \quad \text{......... (1)}
\]

\[
C_y = \frac{Q_m - Q_x}{Q_y - Q_x} \times \frac{A_1}{a_2} \quad \text{......... (2)}
\]

Where, \( C_x \) & \( C_y \) are the concentrations of GTX and KTA respectively, \( A_1 \) is the absorbance of sample solution at isoabsorptive point 303 nm, \( a_1 \) and \( a_2 \) are the absorptivities of GTX and KTA at isoabsorptive point respectively.

\[
Q_m = A_2/A_1 \\
Q_y = a_y/a_x \\
Q_x = a_x/a_x
\]

**Method- II: Simultaneous equation method**
Standard stock solutions as prepared for method I having 100 µg/ml of GTX and KTA were used for appropriate dilutions of two drugs separately. The solutions were scanned in the range of 400 nm to 200 nm to obtain λ<sub>max</sub> of GTX has λ<sub>max</sub> of 292 nm while KTA has 319 nm. The absorptivity coefficients of both these drugs were determined using calibration curve equation. Two simultaneous equations (in two variables \( C_x \) and \( C_y \)) were formed using these absorptivity coefficient values.

\[
A_1 = 903.8 C_x + 297.7 C_y \quad \text{............. (3)}
\]

\[
A_2 = 224.8 C_x + 571.8 C_y \quad \text{............. (4)}
\]

Where \( C_x \) and \( C_y \) are concentration of GTX and KTA measured in gm/100ml, in sample solutions. \( A_1 \) and \( A_2 \) are absorbance of mixture at selected wavelength 292 nm and 319 nm respectively.

**Method – III: Area Under curve method**
The area under curve method involves the calculation of integrated values of absorbance with respect to the wavelength between two selected wavelengths. This wavelength range is selected on the basis of repeated observations (Fig 2). Standard stock solutions as
Preparation and analysis of ophthalmic formulation by method I, II and III

1 ml of ophthalmic preparation was pipette out and transferred to 50 ml volumetric flask added 25 ml of acidic methanol and sonicated for 10 min. The volume was then made up to the mark with distilled water. This solution was appropriately diluted to get approximate concentration of 3 µg/ml of GTX and 4 µg/ml of KTA and analyzed by three methods as mentioned above. The analysis procedure was repeated six times. Absorbance of sample solutions were recorded at 303 nm and 292 nm and concentration of two drugs in sample were determined by using equations 1 and 2 (method I).

The ophthalmic solution sample was also subjected to analysis by simultaneous equation method. Absorbance of sample solution were recorded at 292 nm \(\lambda_{max}\) of GTX and at 319 nm \(\lambda_{max}\) of KTA and concentration of two drugs in the sample were determined by using equation 3 and 4 (method II).

The ophthalmic solution sample was also subjected to analysis in area under curve method. Areas of sample solution were recorded at 290-294 nm \((\lambda_1 - \lambda_2)\) for Gatifloxacin and 317-321 nm \((\lambda_1 - \lambda_2)\) for Ketorolac Tromethamine. Concentration of two drugs in the sample were determined by using equation 5 and 6 (method III).

RESULT AND DISCUSSION

Linearity

For all three methods linearity was observed in the concentration range of 1-12 µg/ml for GTX and 1-12 µg/ml for KTA. Marketed brand was analyzed and mean % content of GTX and KTA determined by proposed methods was 99.48 % and 99.74 % respectively with low % R.S.D. (less than 2%).

Accuracy

To determine the accuracy of proposed method different levels of drug concentration, lower concentration (LC, 80%), intermediate concentration (IC 100%), higher concentration (HC 120%) were prepared from independent stock solutions & then these solutions were added to preanalyzed solutions and percentage recoveries were calculated. Also to provide additional support to the accuracy of developed assay method, standard addition method was employed, which involves the addition of different concentration of pure drugs to preanalyzed formulation samples and total concentration was determined using proposed method. The accuracy of method was determined by calculating mean % recovery at 90, 100 and 120 % level & the % recovery ranges from 99.64 to 101.00 for GTX and KTA for all three methods.

Precision

Intra day and Inter day precision were performed by analyzing sample solutions on the same day and on the different days at specific time intervals. The precision of method was determined by % R.S.D & the % R.S.D ranges from 0.6771 to 1.1080 for GTX and KTA for all three methods.

CONCLUSION

The proposed methods were found to be simple, accurate and rapid for the routine determination of GTX and KTA in bulk and ophthalmic formulation. To study validity and reproducibility of proposed method, recovery studies were carried out. The methods were validated in terms of linearity, accuracy, precision, specificity and reproducibility (Table 1). The three methods can be easily and conveniently adopted for routine quality analysis.

ACKNOWLEDGEMENT

Authors are thankful to Micro Labs Ltd, Bangalore-560068 for providing gift sample of Gatifloxacin and Ketorolac Tromethamine.
REFERENCES
1. Indian Pharmacopoeia-Vol.2, Ghaziabad :The Indian Pharmacopoeia Commission, Central Indian Pharmacopoeia Laboratory, Govt. of India, Ministry of Health & Family Welfare, 2007 p 1158.