Journal of Pharmaceutical Research Vol. 10, No. 1, January 2011 : 21-24.

SIMULTANEOUS SPECTROPHOTOMETRIC ESTIMATION OF GATIFLOXACIN AND KETOROLAC TROMETHAMINE IN BULK DRUG AND IN OPTHALMIC DOSAGE FORM

Pawar Poonam N[1], Chintawar Pavan P[1], Harde Minal*[1], Ingale Pramod L[2], ChaudhariPraveen D[1].
[1]Modern College of Pharmacy, Yamunanagar, Nigdi, Pune 411044, Maharashtra, India.
[2]Marathwada Mitra Mandal's College of Pharmacy, Thergaon, Pune-411 033, Maharashtra, India.

Received on : 05.04.2010 Revised : 20.01.2011 Accepted : 29.01.2011

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 (λ_{max} of Gatifloxacin). The second method is Simultaneous equation method based on measurement of absorbance at two wavelength 292 (λ_{max} of Gatifloxacin) and 319 (λ_{max} of Ketorolac Tromethamine) and the third method is Area under curve method and wavelength ranges selected were 290-294 nm (λ_1-λ_2) for Gatifloxacin and 317-321 nm (λ_3-λ_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[1] (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[1]. Literature survey reveals that very few analytical methods have been reported for the determination of Gatifloxacin alone or in combination with another drugs by spectrophotometric[2,3], HPLC[4,5], HPTLC[6].

Ketorolac Tromethamine (KTA) 1*H*-pyrrolizine-1-carboxylic acid, 5-Benzoyl-2,3-dihydro-2-amino-2-(hydroxymethyl)-1,3-propanediol, has anti-inflammatory and analgesic activity. It is indicated in pain management, allergic conjunctivitis. Its liquid chromatographic method is official in U.S.P.[7] Literature survey showed that very few analytical methods have been reported for the determination of KTA alone or in combination with another drugs such as spectrophotometric[8], HPLC[9], HPTLC[10]. 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

***Correspondence :** minaltharde@yahoo.com

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 transfer into two separate 100 ml volumetric flask, 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_{max} of GTX) and 319 (λ_{max} 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)
Absorption ratio method uses the ratio of absorbances at two selected wavelengths, one at isoabsorptive point and other being the λ_{max} 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 303nm. The second wavelength used is the have λ_{max} 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_{max}) and isoabsorptive point. The concentrations of two drugs in mixture can be calculated by using following equations.

$$C_X = \frac{Q_m - Q_y}{Q_x - Q_y} \times \frac{A_1}{a_1} \quad \ldots\ldots\ldots (1)$$

$$C_Y = \frac{Q_m - Q_x}{Q_y - Q_x} \times \frac{A_1}{a_2} \quad \ldots\ldots\ldots (2)$$

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

$Q_m = A_2/A_1$,
$Q_y = ay_2/ay_1$,
$Q_x = ax_2/ax_1$.

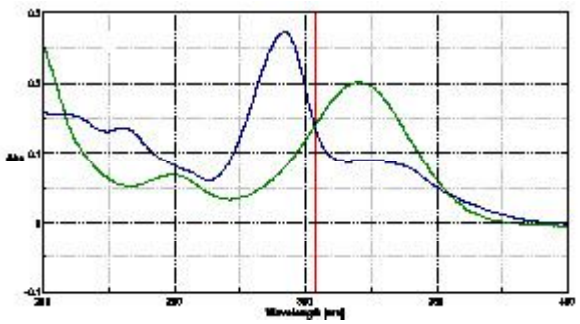

Fig 1: *Overlain spectra of GTX and KTA at 303 nm*

Method- II: Simultaneous equation method
Standard stock solutions as prepared for method I having 100µg/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_{max}. GTX has λ_{max} 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.

$$A_1 = 903.8\ C_x + 297.7\ C_Y \quad \ldots\ldots\ldots\ldots\ldots (3)$$

$$A_2 = 224.8\ Cx + 571.8\ C_y \quad \ldots\ldots\ldots\ldots\ldots (4)$$

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

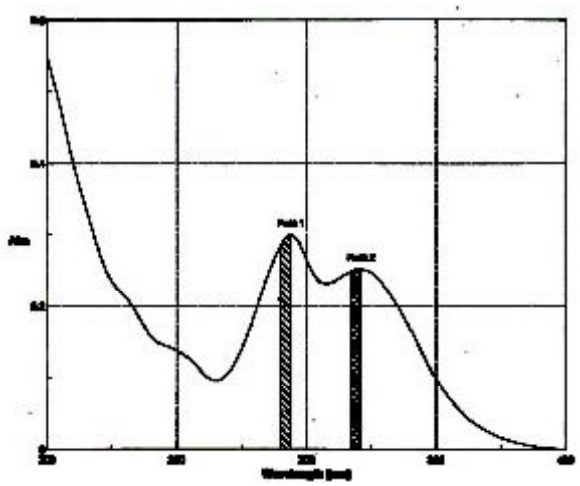

Fig 2: *AUC Spectra of mixture (GTX-KTA)*

prepared for method I having 100µg/ml of GTX and KTA were used for appropriate dilutions of two drugs separately. For area under curve method the sampling wavelengths selected for analysis of GTX and KTA were 290-294 nm (λ_1- λ_2) and 317-321 nm (λ_3 –λ_4). Both drugs showed linearity in the concentration range of 1–12 µg/ml and 1–12 µg/ml GTX and KTA respectively. Mixed standards of GTX and KTA were prepared in the ratio of 3:4 µg/ml and their area under the curve measured at selected wavelength ranges. The X value =Area under the curve of the component /concentration of the component in gm /100 ml Were determined for the two drugs at the selected wavelength ranges. A set of two equations was formed using these X values as follows,

$$A_1 = 3718.66\ C_1 + 774.12\ C_2 \quad \text{..... (5)}$$

$$A_2 = 1256.66\ C_1 + 2015.2\ C_2 \quad \text{..... (6)}$$

Where, C_1 and C_2 are the concentrations of GTX and KTA, respectively in gm/100ml in the sample solution. A_1 and A_2 are the areas for mixed standard solutions at (λ_1- λ_2) and (λ_3 –λ_4) respectively.

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 λ_{max} of GTX and at 319 nm λ_{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 (λ_1- λ_2) for Gatifloxacin and 317-321 nm (λ_3 –λ_4) 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[11]

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 80, 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.

Table 1: *Statistical data of regression equations and validation parameters for Gatifloxacin and Ketorolac Tromethamine*

Parameters	Gatifloxacin	Ketorolac Tromethamine
Intercept	0.1108	0.0532
Regression coefficient	0.9993	0.9995
Beer's lamberts range in µg/ml	1 - 16	1 - 14
Linearity in µg/ml	1- 12	1 - 12
Limit of detection in µg/ml	0.2668	0.0769
Limit of quantification in µg/ml	0.8086	0.2330

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.
2. Venugopal K, et al. Elesvier II Farmaco. 2005; 60:906-912.
3. Lopes CCGO, et al. Nota tecnica, 2008; 31(7):1831-1835.
4. Patel A B, et al. International J of Chem Tech Research. 2009; 1(3):587-590.
5. Paramane S, et al. Indian J of Pharm Science. 2007; 69(4):525-528.
6. Shah S A. Indian J of Pharmaceutical Science. 2004; 66(3):306-308.
7. United State Pharmacopoeia 30**,** NF 25, Vol.-2, Asian Edition, United States Pharmacopoeial Convention, Inc. Webcom Ltd., 2007; 2440-2441.
8. Fegade JD, et al. International J of Chem Tech Research. 2009; 1(2):189-194.
9. Chaudhary R S, et al. J of Chromatography. 1993; 614:180-184.
10. Devrajan PV, et al. J of Pharm and Biomed Ana. 2000; 22:679-683.
11. ICH, Q2A, Text on validation of analytical procedures, ICH, Geneva, 1994.