Research Article
AMERICAN JOURNAL OF PHARMACY AND HEALTH RESEARCH
www.ajphr.com
2014, Volume 2, Issue 4
ISSN:2321–3647(online)

Validated Spectrophotometric Methods for Quantitative Determination of Gatifloxacin and Ketorolac Tromethamine in Bulk and Marketed Formulation

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ABSTRACT

Three simple, accurate and precise UV- Spectrophotometric methods has been developed and validated for quantitative determination of Gatifloxacin (GTX) and Ketorolac Tromethamine (KTA) in bulk and marketed formulation. First method is based on simultaneous equations and the wavelengths selected for analysis were 292 nm (λmax of Gatifloxacin) and 319 nm (λmax of Ketorolac tromethamine). Second method is based on the ratio of absorbances at two selected wavelengths, one at isoabsorptive point and the other being λ_{max} of one of two components. Third method is based on area under curve and the wavelengths ranges selected for analysis were 290-294 nm for Gatifloxacin and 317-321 nm for Ketorolac tromethamine. Linearity was obtained in the concentration range of 2-12 µg / ml for both drugs. The results of analysis have been validated statistically and by recovery studies. The utility of the developed methods has been demonstrated by analysis of commercial formulation containing these drugs.

Keywords: Gatifloxacin, Ketorolac tromethamine, simultaneous equation method, absorbance ratio method, area under curve method.

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Received 23 March 2014, Accepted 05 April 2014

Please cite this article in press as: Phuge AN *et al*., Validated Spectrophotometric Methods for Quantitative Determination of Gatifloxacin and Ketorolac Tromethamine in Bulk and Marketed Formulation. American Journal of Pharmacy & Health Research 2014.

INTRODUCTION

Gatifloxacin (GTX), chemically is [1-cyclopropyl-6-fluro, 1, 4- dihydro-8 methoxy-7 (3-methyl piprazine -1-yl)-4-oxo-3-quinoline carboxylic acid]. It is a fluoroquinolone antibiotic. Its mode of action depends on inhibition of DNA synthesis via inhibition of the enzymatic activities of two members, DNA gyrase and topoisomerase. It is official in IP. [1, 3-5] Ketorolac Tromethamine (KTA), chemically is **[**5-benzoyl-2, 3-dihydro-1H-pyrrolizine-1-carboxylicacid, 2 - (hydroxymethyl) -1,3 propanediol]. It is a non-selective COX inhibitor and is used for the symptomatic relief of moderate to severe postoperative pain, including that associated with abdominal, gynecologic, ophthalmologic surgery. Also used for the relief of acute renal colic, pain associated with trauma. It is official in USP.[2] Literature survey reveals that few UV, HPLC, HPTLC and colorimetric method have been reported for the determination of both Gatifloxacin and Ketorolac Tromethamine as single component in bulk, formulation and in biological fluids. However not a single analytical method is reported so far for simultaneous analysis of GTX and KTA. Present work describes three simple, rapid, accurate and precise methods for simultaneous determination of GTX and KTA in bulk and marketed formulation. The proposed methods were validated as per ICH guidelines.

MATERIAL AND METHODS

Procurement of drug samples and formulation

Standard samples of Gatifloxacin and Ketorolac tromethamine were purchased by Aarti Drugs Ltd., Mumbai and Aligns International, Mumbai respectively. Combined dose marketed formulation containing Gatifloxacin (3 mg) and Ketorolac tromethamine (4 mg), manufactured by Sun Pharma Pvt. Ltd., were purchased from local market.

Reagents and chemicals used

Methanol-AR was used as solvent.

Instrument Used

A double-beam Shimadzu UV - visible spectrophotometer, 1700 Pharmaspec, with spectral bandwidth of 2 nm, wavelength accuracy ± 0.5 nm and a pair of 1 cm matched quartz cells was used to measure absorbance of solution.

Preparation of standard stock solution

Accurately weighed quantity of GTX (10 mg) and KTA (10 mg) was transferred 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 solutions of concentration 100 µg/ml for each drug.

Method I: Simultaneous Equation Method

For the selection of analytical wavelengths, standard solution of GTX (3 µg/ml) and KTA (4 µg/ml) were prepared separately by appropriate dilution of standard stock solution with acidic methanol and scanned in the entire UV range to determine λ_{max} of both the drugs. The λ_{max} of GTX and KTA were found to be 292 nm and 319 nm respectively. A series of standard solutions of GTX and KTA were prepared separately having concentration range of 2-12 µg/ml. The absorbance of resulting solutions was measured at 292 nm and 319 nm and calibration curves were plotted. Both the drugs obeyed linearity in the concentration range under study. Absorptivity values were then determined for both the drugs at selected wavelengths. Two simultaneous equations (in two variables C_X and C_Y) were formed using absorptivity coefficient values obtain and are as follows:

A_1 at 292 nm = 905.0 C_X + 224.0 C_Y (1)

A_2 at 319 nm = 297.6 C_X + 569.6 C_Y (2)

Where A_1 and A_2 are the absorbance of sample solution at 292 nm and 319 nm, respectively. C_1 and C_2 are the concentrations of GTX and KTA measured in mg/ml, in sample solutions. Absorptivity values 905.0 and 224.0 are of GTX at 292 nm and 319 nm, respectively. Similarly, 297.6 and 569.6 are absorptivity values of KTA at 292 nm and 319 nm, respectively. By applying the Cramer's rule to equation 1 and 2, the concentration C_{GTX} and C_{KTA} can be obtained as follows,

$C_{GTX} = A_2 (297.6) – A_1 (596.6)/- 448825.6$ (3)

$C_{KTA} = A_1 (224.0) – A_2 (905.0)/ - 448825.6$ (4)

Method II: Absorbance Ratio Method

In this method, appropriate dilutions were prepared for each drug from the standard stock solution and scanned in the spectrum mode from 400 nm to 200 nm. Absorbance ratio method uses the ratio of absorbances at two selected wavelengths, one at isoabsorptive point and the other being λ_{max} of one of two components. From the overlain spectra of two drugs, it is evident that GTX and KTA have isoabsorptive point at 303 nm and λ_{max} of GTX is at 292 nm. Fig. No – 1 represents the overlain spectra of both the drugs.

Method III: Area under Curve Method

From the overlain spectra of drugs (Figure 1), area under the curve in the range of 290-294 nm for Gatifloxacin and 317-321 nm for Ketorolac tromethamine were selected for the analysis. The calibration curves for GTX and KTA were plotted in the concentration range of 2-12 µg/ml. the 'X' values for both the drugs were determined at the selected AUC range. The 'X' value is the

ratio of area under the curve at selected wavelength ranges with the concentration of component in gm/lit. A set of two simultaneous equations obtained by using mean 'X' values are as follows:

$A_1 = 3718.66\ C_1 + 774.12\ C_2$ (at 290-294 nm) (5)

$A_2 = 1256.66\ C_1 + 2015.2\ C_2$ (at 317-321 nm) (6)

Where A_1 and A_2 are area under curve of sample solution at the wavelength ranges 290-294 nm and 317-321 nm, respectively. The 'X' values 3718.66 and 774.12 are of GTX at wavelength range 290-294 nm and 317-321 nm, respectively. Similarly, 1256.66 and 2015.2 are 'X' values of KTA at wavelength range 290-294 nm and 317-321 nm, respectively. The concentration of GTX and KTA in sample solution was determined by using the equations (5) and (6).

Analysis of Marketed Formulation by Proposed Methods

For the analysis of marketed formulation accurately about 1.5 ml ophthalmic formulation was transferred to a 50 ml volumetric flask and dissolved in about 12.5 ml of acidic methanol, then the volume was made up to the mark with distilled water. The solution was diluted further to prepare sample solutions containing GTX and KTA within their beers–Lambert's ranges.

In method I, concentration of both GTX and KTA were determined by measuring absorbance of sample solution at 292 nm and 319 nm and using equations (3) and (4). In method II, concentration of both GTX and KTA were determined by measuring their absorbances at 303 nm (isoabsorptive point) and at 292 nm (λ_{max} of GTX). In method III, concentration of both GTX and KTA was determined by measuring area under curve in the range of 290-294 nm (for Gatifloxacin) and 317-321 nm (for Ketorolac tromethamine) and values were substituted in equations (5) and (6) to obtain concentration of both the drugs. Results of analysis of marketed formulation are shown in Table 1. The analysis was carried out for the marketed formulation containing GTX and KTA in a concentration of 3: 4 mg respectively.

RESULTS AND DISCUSSION

The methods discussed in the present work provide a convenient and reliable way for quantitative determination of GTX and KTA in bulk and marketed formulation. The analytical methods were validated with respect to parameters such as linearity, precision and accuracy. Linearity for GTX and KTA was observed in the concentration range of 2 - 12 µg/ml for GTX and 2 - 12 µg/ml for KTA, for all three methods. Percent drug found for GTX and KTA in marketed formulation, by all the methods, was found in the range of 99.00 to 99.90 %. Percent recovery for GTX and KTA, by all the methods, was found in the range of 99.48 % to 100.25 % with standard deviation well below 2 indicating accuracy of the methods. Intra-day and Inter-day precision studies were carried out by analyzing marketed formulation, by all the methods, three

times on the same day and on three different days, respectively. Standard deviation and coefficient of variance for intra-day and inter-day precision studies was satisfactorily low indicating high degree of precision and reproducibility of proposed methods.

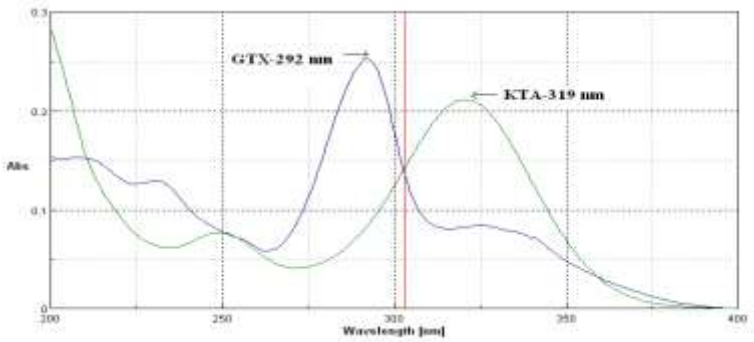

Figure. 1: Overlain spectra of Gatifloxacin and Ketorolac tromethamine

Table 1: Result of Analysis of Marketed Formulation

Method	Drug	Concentrations of Drugs taken(mg/ ml)	Concentrations of drugs found(mg/ ml)	* % Drug found
I	GTX	3	2.99	99.90
	KTA	4	3.97	99.33
II	GTX	3	2.99	99.66
	KTA	4	4.01	100.33
III	GTX	3	2.97	99.00
	KTA	4	3.99	99.75

*Mean of six determinations. GTX = Gatifloxacin, KTA = Ketorolac tromethamine

Validation

The proposed methods were validated as per ICH guidelines. [6-7]

Accuracy

To ascertain the accuracy of the proposed methods, recovery studies were carried out by standard addition method at three different levels (80%, 100% & 120%). The results of recovery studies, expressed as percent recovery, were satisfactory and are presented in Table. 2.

Table 2: Result of Recovery Studies

Level of recovery	Amount of pure Drug added (mg)		Percent Recovery* Method – I		Method – II		Method – III	
	GTX	KTA	GTX	KTA	GTX	KTA	GTX	KTA
80	5.4	7.2	100.23	99.79	99.89	99.48	100.08	99.66
100	6.0	8.0	99.96	99.89	100.25	99.88	100.12	100.19
120	6.6	8.8	100.05	100.10	99.75	100.21	99.85	99.92
Mean % Recovery			100.08	99.92	99.96	99.85	100.01	99.92
S. D.			0.1374	0.1584	0.2579	0.3656	0.1459	0.2650
C. V.			0.1372	0.1585	0.2580	0.3661	0.1458	0.2652

* Mean of three determinations, GTX = Gatifloxacin, KTA = Ketorolac tromethamine, S.D. = Standard Deviation, C.V. = Coefficient of Variance

Precision

Precision of the method was determined with marketed formulation. 1.5 ml of ophthalmic formulation was measured and the sample solutions were prepared in the same manner as described in analysis of the marketed formulation. Repeatability is given by intra-day and inter-day precision. Intra-day precision was determined by analyzing the sample solution for three times in the same day. Inter-day precision was determined by analyzing the sample solution for three different days. The results of analysis are shown in Table no. 3

Table 3: Precision Study Data

Methods	Drug	Concentration in µg/ml	Intra-day study		Inter-day study	
			*S.D.	*% RSD	*S.D.	*% RSD
I	GTX	3	0.0189	0.7562	0.0179	0.7159
	KTA	4	0.0310	0.8890	0.0208	0.8338
II	GTX	3	0.0251	0.8175	0.0351	1.1676
	KTA	4	0.0378	0.9458	0.0351	0.8740
III	GTX	3	0.0351	1.1670	0.0282	0.9306
	KTA	4	0.0351	0.8741	0.0321	0.7971

***Average of six determinations**

CONCLUSION

Based on the results obtained, it can be concluded that the proposed UV-Spectrophotometric methods (Simultaneous equation method, Absorbance ratio method and Area under curve method) for simultaneous determination of Gatifloxacin and Ketorolac tromethamine are rapid, economical, accurate and precise. The utility of the developed methods has been demonstrated by analysis of combined dose marketed formulation. Hence, the proposed methods can be used for quantitative determination of pharmaceutical formulation containing these ingredients in combination.

ACKNOWLEDGEMENT

The authors are thankful to Dr. Sanjay B. Wagh, Principal and Professor, NDMVP Samaj's College of Pharmacy, Nashik, Maharashtra for providing necessary facilities.

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