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Simultaneous Estimation of Ofloxacin and Ketorolac Tromethamine in Ophthalmic Dosage Form by Reverse Phase High Performance Liquid Chromatography

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A simple, accurate, rapid and precise reverse phase high performance liquid chromatographic method was developed for simultaneous estimation of ofloxacin and ketorolac tromethamine in bulk and ophthalmic dosage form. Eurosphere-100 C_{18}, 250 mm × 4.6 mm, 5 µm particle size column, in isocratic mode with mobile phase methanol: 0.05 M potassium dihydrogen phosphate buffer (50:50 v/v) and pH adjusted to 3.5 ± 0.1 with ortho-phosphoric acid was used. The flow rate was 1.0 mL/min and absorbance of individual component was measured at 298 nm. The retention times of ketorolac tromethamine and ofloxacin were found to be 5.98 and 11.54 min, respectively. Linearity for ofloxacin and ketorolac tromethamine was in the range of 3-15 and 5-25 µg/mL with correlation coefficient values 0.9999 for both. The percentage recovery obtained was 100.25 and 99.67 %, respectively.

Key Words: Ofloxacin, Ketorolac tromethamine, RP-HPLC, Method validation.

INTRODUCTION

Multidrug administration is often associated with clinically significant interaction, especially of narrow therapeutic index drugs, either at pre-absorption or post-absorption stage[1]. This can limit the desired therapeutic effect of either of the drug molecules. The present study was aimed to develop simple, rapid and precise analytical method for simultaneous estimation of ofloxacin (OFLOX) and ketorolac tromethamine (KETO).

Ofloxacin[2], is an antimicrobial drug and chemically it is 9-fluro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperizinyl)-7-oxo-7*H*-pyrido[1,2,3-de]-1,4-benzoxaine-6-carboxylic acid. Various analytical methods have been reported in literature for estimation of ofloxacin in single and in combination form such as spectrophotometric[3-9], potentiometry and conductometry[10], HPLC[11-20], electrophoresis[21,22] and LC/MS/MS[23,24].

Ketorolac tromethamine[2], has antiinflammatory and analgesic activity. Chemically it is 5-benzoyl-2,3-dihydro-1*H*-pyrrolizine-1-carboxylic acid,2-(hydroxy-methyl)-

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1,3-propanediol. It is official only in USP. In literature, few analytical methods have been reported for the estimation of ketorolac tromethamine in single or combination such as spectrophotometric[25,26] , HPLC[27-32] and HPTLC[33].

Fixed dose combination containing OFLOX and KETO is available only in ophthalmic dosage form in the market. This combination was introduced recently and no method is reported for the simultaneous estimation of both these drugs. The aim of present work is to develop a simple, rapid, precise and selective RP-HPLC method for the estimation of OFLOX and KETO from ophthalmic dosage form.

EXPERIMENTAL

High performance liquid chromatography system Chemitto LC 6600 equipped with universal injector with injection volume 20 µL, Ultra-Visible (UV-Vis) detector.

A Eurosphere-100 C_{18} (KNAUER, Berlin, Germany) column (250 mm × 4.6 mm) 5 µm particle size forms the stationary phase.

The gift sample of ofloxacin (OFLOX) was obtained from Medico Pharma, Palghar and ketorolac tromethamine (KETO) was obtained from Nicholas Piramal, Pithampur. Eye drops of brand (KETOFLOX, Allergan) containing ofloxacin (3 mg) and ketorolac tromethamine (5 mg), respectively per mL was procured from a local pharmacy. Potassium dihydrogen phosphate and ortho-phosphoric acid were of analytical grade. HPLC grade methanol and HPLC grade water was obtained from qualigens.

Mobile phase: Methanol: 0.05 M potassium dihydrogen phosphate buffer (50:50 v/v), pH was adjusted to 3.5 ± 0.1 with ortho-phosphoric acid.

Potassium dihydrogen phosphate (6.8045 g) was dissolved in HPLC grade water (500 mL), to this solution HPLC grade water (500 mL) was added to get 0.05 M solution and then filtered through 0.45 µ membrane filter.

Standard stock solutions: Standard OFLOX (25 mg) was accurately weighed and transferred to a 25 mL volumetric flask and dissolved in mobile phase. The flask was shaken for 0.5 h and the volume was made up to the mark with mobile phase to get a solution of OFLOX (1000 µg/mL). Standard KETO (25 mg) was accurately weighed and transferred to a 25 mL volumetric flask and dissolved in mobile phase. The flask was shaken for 0.5 h and the volume was made up to the mark with mobile phase to get a solution of KETO (1000 µg/mL).

Working standard solution: The combined working standard solution containing OFLOX (3 µg/mL) and KETO (5 µg/mL) was prepared in mobile phase.

Sample solution: An accurately measured volume of eye drops equivalent to 3 mg of OFLOX and 5 mg of KETO were transferred to 100 mL volumetric flask containing 50 mL mobile phase, sonicated for 0.5 h and volume was made up to the mark with mobile phase. The above solution was filtered through 0.45 µ membrane filter. One mL of this solution was diluted to 10 mL with mobile phase to get the OFLOX (3 µg/mL) and KETO (5 µg/mL) solution (theoretical values).

Chromatographic conditions: The optimum composition of mobile phase containing methanol: 0.05 M potassium dihydrogen phosphate buffer (50:50 v/v), pH was adjusted to 3.5 ± 0.1 with ortho-phosphoric acid was selected as it was found to ideally resolve the peaks of OFLOX and KETO. The flow rate was set to 1 mL/min and UV detection was carried out at 298 nm. All determination were performed at ambient column temperature.

Assay: Twenty µL of the test and standard solutions (n = 3) were injected separately to an injector of HPLC and chromatograms were recorded. From the area, the amounts of both the drugs were calculated.

Linearity and calibration: From OFLOX standard stock solution, 0.3, 0.6, 0.9, 1.2, 1.5 mL of OFLOX was transferred to six 10 mL volumetric flask. Volume was made up to the mark with the mobile phase to obtain concentration of 3-15 µg/mL of OFLOX. In the same way 0.5, 1.0, 1.5, 2.0, 2.5 mL of KETO was transferred to six 10 mL volumetric flasks from the KETO standard stock solution and volume was made up to the mark with the mobile phase to obtain concentration of 5-25 µg/mL of KETO. The solution (20 µL) was injected into column with the help of Hamilton Syringe. All measurements were repeated three times for each concentration. The calibration curves of the area under curve *vs.* concentration were recorded for both the drugs.

Method validation: The analytical method was validated as per recommendations of USP[34] and ICH[35] guidelines for the parameters like recovery, precision, ruggedness and repeatability.

Recovery study: The accuracy of an analytical method is closeness of test results obtained by that method to the true value. The accuracy of an analytical method should be established across its range. A known amount of standard solution of pure drugs (OFLOX and KETO) was added to preanalyzed sample solution (OFLOX 3 µg/mL and KETO 5 µg/mL). These solutions were subjected for analysis. The lower the values of relative standard deviation (RSD) indicate the method is accurate. The mean recoveries of OFLOX and KETO were 100.25 and 99.67 % respectively and RSD values and within the limits.

Precision: The precision of an analytical method is the degree of agreement among the individual test results when the method is applied repeatedly to multiple samplings of a homogeneous sample.

Variation of results within the same day (intra day), variation of results between days (inter day) were analyzed. Intra day precision was determined by analyzing, the 3, 6 and 9 µg/mL of OFLOX and 5, 10 and 15 µg/mL of KETO concentrations, for 3 times in the same day. Inter day precision was determined by analyzing, the same concentrations of drugs daily for 3 d.

Ruggedness: The ruggedness of analytical method is the degree of reproducibility of test results obtained by the analysis of the same sample under a variety of conditions, such as different laboratories, different analysts, different instruments and different lots of reagent.

RESULTS AND DISCUSSION

Ofloxacin (OFLOX) is a synthetic fluoroquinolone antibacterial agent. It acts by inhibiting bacterial DNA gyrase enzyme which is required for DNA replication and thus causes bacterial lysis. Ketorolac tromethamine is an antiinflammatory agent and also has analgesic activity. It acts by inhibiting cyclooxygenase enzyme and prostaglandin synthesis.

The market survey revealed that the above combination is recently introduced in the market and literature survey also revealed that no methods are reported for the simultaneous estimation of OFLOX and KETO in their combined dosage form. Hence, an attempt has been made to develop the chromatographic method for simultaneous estimation of ofloxacin and ketorolac tromethamine in their pharmaceutical preparation.

Reverse phase HPLC method was developed for the simultaneous estimation of ofloxacin and ketorolac tromethamine in ophthalmic dosage form. The separation was achieved by a Eurosphere-100 C_{18} column and methanol: 0.05 M potassium dihydrogen phosphate buffer (50:50 v/v) and pH adjusted to 3.5 ± 0.1 with orthophosphoric acid as mobile phase at the flow rate of 1.0 mL/min the detection was carried out at 298 nm.

Assay result: In replicate analysis (n = 3) of two drugs by proposed method showed the content of OFLOX and KETO as 99.63 and 100.72 %, respectively (Table-1). The retention times of ketorolac tromethamine and ofloxacin were found to be 5.98 and 11.54 min, respectively (Fig. 1). Linearity was assessed by a plot of concentration *versus* area, the graphs were found to be linear in the range of 3-15 µg/mL for ofloxacin and 5-25 µg/mL of ketorolac tromethamine with correlation coefficient values 0.9999 for both the drugs (Table-2).

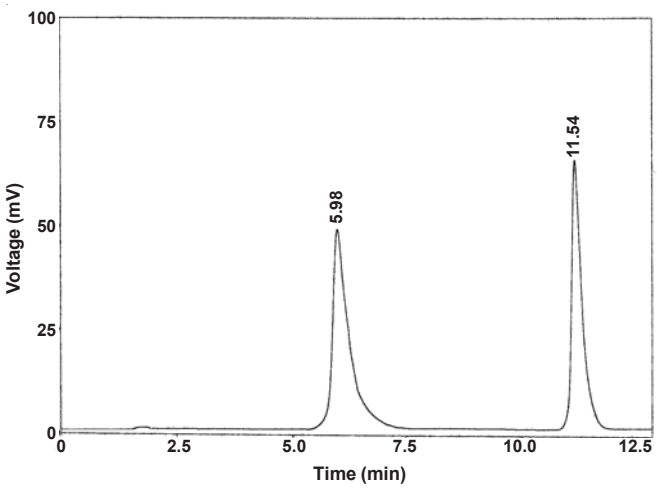

Fig. 1. Typical chromatogram of the sample solution containing ofloxacin and ketorolac tromethamine at retention time of 5.94 and 11.54 min, respectively.

TABLE-1
RESULTS OF RP-HPLC ASSAY

Formulation (Eye drops)	Actual concentration (mg)		% OFLOX* ± SD	% KETO* ± SD
	OFLOX	KETO		
KETOFLOX (Allergan)	3.0	5.0	99.63 ± 0.60	100.72 ± 0.76

*Average of three determination; SD = Standard deviation.

TABLE-2
STATISTICAL DATA FOR LINEARITY AND CALIBRATION RANGE

Parameters	OFLOX	KETO
Linear range (µg/mL)	3-15	5-25
Slope	18.144	6.830
Coefficient of variation	0.9999	0.9999

On the basis of parameters fixed, the method of estimation was validated, for the following parameters:

Recovery studies: Recovery studies were carried out by adding a known amount of standard solution of pure drugs (OFLOX and KETO) to a preanalyzed sample solution (OFLOX 3 µg/mL and KETO 5 µg/mL). These solutions were subjected to analysis. The study showed the result within acceptable limit of above 99 % and below 101 % (Table-3).

TABLE-3
RESULT FOR RECOVERY STUDIES

Sample solution (µg/mL)	Amount of standard drug added (µg/mL)	% Recovery* ± SD	% RSD
OFLOX 3	3.00	100.25 ± 0.34	0.14
KETO 5	5.00	99.67 ± 0.59	0.36

*Average of five determination; SD = Standard deviation; RSD = Relative standard deviation.

Precision: Precision studies were carried out using parameters like intra-day and inter-day analysis precision. The study showed the results within acceptable limit, *i.e.* % RSD below 2.0, indicating that the method is reproducible (Table-4).

Ruggedness: Ruggedness studies were carried out using only one parameter, *i.e.* different analyst. Results showed that the % RSD was in the range of 0.1-1.4 *i.e.* less than 2, for different analysts. This study signifies the ruggedness of the method under varying conditions of its performance (Table-5).

System suitability test: As per USP-24 system suitablity test was carried out on freshly prepared standard stock solutions of OFLOX and KETO. Twenty µL of the both drugs were injected under optimized chromatographic condition and following parameters were studied to evaluate the suitability of system. The values of system suitability test were shown in Table-6.

TABLE-4
RESULTS OF PRECISION STUDIES

Component	Intra-day precision*		Inter-day precision *	
	Area under curve	%RSD	Area under curve	%RSD
OFLOX (µg/mL)				
3	516.10	1.31	515.81	2.28
6	938.01	1.34	937.00	1.91
9	1400.79	1.40	1401.22	1.73
KETO (µg/mL)				
5	331.24	1.77	332.08	1.84
10	686.41	1.55	686.05	1.79
15	1013.27	1.59	1012.94	1.14

The inter-day and intra-day precision study data for the simultaneous estimation of ofloxacin and ketorolac tromethamine in ophthalmic dosage form.
*Average of three determination; RSD = Relative standard deviation.

TABLE-5
RUGGEDNESS STUDIES

Drug	Label claim (mg/mL)	Amount found (%)	
		Analyst I	Analyst II
OFLOX	3	99.99	100.08
KETO	5	100.23	100.09

Table shows reproducibility of proposed method.

TABLE-6
SYSTEM SUITABILITY TEST PARAMETERS

System suitability parameters	Proposed method	
	KETO	OFLOX
Retention time (t_R)	5.96 min	11.5 min
Capacity factor (k')	1.50	4.16
Theoretical plate number (N)	10732	94044
Tailing factor (T)	–	–
Resolution factor (R)	–	2.73

Table describes various validation parameters.

The proposed RP-HPLC method is simple, accurate, rapid and selective. High percentage of recovery showed that the method is free from interferences of the excepients used in the formulations. Therefore method can be useful in routine quality control analysis of these drugs.

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