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Simultanious Spectrophotometric Estimation of Ofloxacin and Ketorolac Tromethamine in Ophthalmic Dosage Form.

Jitendra D. Fegade, * Harshal P. Mehta, Rajesh Y. Chaudhari, Vijay R.Patil.

Department of Pharmaceutical Chemistry, TVES's Hon. Madhukarrao Chaudhari College of Pharmacy, Nehru Vidyanagar, Savda Road, Faizpur, (MS), India-425 503.

E-mail-jitufegade@gmail.com

Abstract: A spectrophotometric method for the simultaneous estimation of ofloxacin and ketorolac tromethamine in their combined dosage form have been developed and validated for linearity, accuracy, precision, ruggedness and repeatability. The wavelength selected to develop the equation were 300.0 nm and 319.2 nm in acidic methanol. Both drugs shows linearity in the concentration range of 1-11 µg/mL for ofloxacin and 3-13 µg/mL for ketorolac tromethamine. The results have been statistically validated and were found to be simple, rapid, accurate, precise and reproducible.
Keywords: Ofloxacin, Ketorolac tromethamine, Validation.

Introduction and Experimental

Multi-drug 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 spectrophotometric 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-7H-pyrido[1,2,3-de]-1,4-benzoxaine-6-carboxylicacid. Extensive literature survey revels that various analytical methods have been reported for the estimation of OFLOX in single and in combination dosage 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 anti- inflammatory and analgesic activity. Chemically it is 5-benzoly-2,3-dihydro-1H-pyrrolizine-1-carboxylicacid,2-(hydroxymethyl)-1,3-propanediol and official only in USP. Literature survey showed that very few analytical methods have been reported for the estimation of KETO in single or in combination such as, Spectrophotometric,[25,26] HPLC[27-32] ,and HPTLC[33].

Fixed dose combination containing OFLOX and KETO in ophthalmic dosage form is recently available in the market and no single method is yet reported for the simultaneous estimation of both these drugs. The aim of present work is to develop a simple, rapid, precise and selective spectrophotometric method for the estimation of OFLOX and KETO in ophthalmic dosage form.

Instrument:

PC based UV-Vis. double beam spectrophotometer, model Systronic 2202 with 1 cm quartz cells was used.

Chemicals and reagents:

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. Methanol and hydrochloric acid were of analytical grade.

Preparation of solutions:

Standard stock solution of OFLOX and KETO were prepared separately to the concentration of 1mg/mL in acidic methanol. Suitable aliquots were pipeted out from standard stock solution to obtained working standard solution of OFLOX and KETO, 3 µg/mL and 5 µg/mL, respectively. Both solutions were scanned over a range of 380 nm to 210 nm in the spectrum mode and the overlain spectra of two were recorded. From the overlain spectra (Fig.1), wavelengths 300 and 319 nm were selected for the formation of simultaneous wave equations. For construction of calibration curves, two series of different concentrations in range of 1-11 µg/mL for OFLOX and 3-13 µg/mL for KETO were prepared from stock solution. The calibration curves were plotted at 300 and

319 nm. The absorptivities (A1%, 1 cm) of the drugs at selected wavelengths were determined. These calculated values were the mean of five independent determinations.

Analysis of laboratory mixture by proposed method:

In order to see the feasibility of proposed method for simultaneous estimation of OFLOX and KETO in marketed pharmaceutical formulations, the method was first tried for estimation of drugs in standard laboratory mixture.

Accurately weighed quantities OFLOX (3mg) and KETO (5mg) were taken in a volumetric flask (100mL) and dissolved in acidic methanol. The volume was made up to the mark by acidic methanol filter through whatman filter paper. The aliquot portions of this stock solution were further diluted with solvent to get final concentrations OFLOX (3µg/mL) and KETO (5µg/mL) and the absorbance were measured at 300.0 nm and 319.2 nm against acidic methanol as blank. The quantitative estimation of the drugs were carried out by solving simultaneous equation,

$Cx = (A_2ay_1 - A_1ay_2)/ (ax_2ay_1 - ax_1ay_2)$ (1)

$Cy = (A_1ax_2 - A_2ax_1)/ (ax_2ay_1 - ax_1ay_2)$ (2)

Where A_1 and A_2 are absorbance's of mixture at 300 and 319nm, respectively ax_1 and ax_2 are absorptivities of x at 300 and 319 nm, respectively, ay_1 and ay_2 are absorptivities of y at 300 and 319 nm, respectively and Cx is the concentration of OFLOX and Cy is the concentration of KETO. Results as shown in **Table-1.**

Table-1 Analysis of OFLOX and KETO in laboratory mixture:

Sr .No.	Amount Taken	Amount found* (µg/mL)	(%)Amount found ± SD
1	OFLOX (3 µg/mL)	2.98	99.40± 0.52
2	KETO (5 µg/mL)	4.99	99.84± 0.32

*Mean of five estimations

Application of proposed method for analysis of marketed formulation:

An accurately weighed sample equivalent to of OFLOX (3mg) and KETO (5mg) was taken in a volumetric flask (100mL), acidic methanol (40mL) was added and sonicated for 10 min. Volume was made up to the mark with the acidic methanol and filter through whatman filter paper. The aliquot portions of above solutions were further diluted with solvent to get final concentrations of OFLOX (3µg/mL) and KETO (5µg/mL), respectively. The above solution was analyzed for the content of OFLOX and KETO using the method described above. Results are reported in **Table-2**.

Table-2 Application of Proposed Method for Analysis of marketed prep

Sample	Label claimed	% amount found * ± SD
KETOFLOX	KETO 5mg/mL	99.32 ± 0.18
	OFLOX 3mg/mL	100.79 ± 0.09

***Mean of five estimations**

Validation of Proposed Method:

The proposed 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 were added to preanalysed sample solution OFLOX 3µg/mL and KETO 5µg/mL. These solutions were subjected for analysis. The lower the values of relative standard deviation (RSD) indicate the method is accurate.

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 three times in the same day. Inter day precision was determined by analyzing, the same concentrations of drugs daily for three days.

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.

Ruggedness of the proposed method is determined by analysis of aliquots from homogenous slot by two analyst using same operational and environmental conditions and the results are reported in **Table-3**.

Repeatability:

Repeatability was determined by analyzing ofloxacin (3µg/mL) and ketorolac tromethamine (5µg/mL) concentration of drug solutions for six times and results are reported in **Table-6**.

Table-3: Recovery Study Data

Sr.No	Sample solution	Amount of standard drug added (µg/mL)	%Recovery* ± SD	%RSD
1	OFLOX (3 µg/mL)	3.00	100.34 ± 0.19	0.19
2	KETO (5 µg/mL)	5.00	99.59 ± 0.24	0.24

*Mean of five observations

Table-4: Precision Data

SR.NO	Drug	Conc.	Intraday	%RSD	Inter-day	%RSD
1	OFLOX	3 µg/mL	2.96	0.543	2.96	0.6548
		6 µg/mL	6.01	1.09	5.99	0.9957
		9 µg/mL	8.95	0.7586	8.91	1.0325
2	KETO	5 µg/mL	4.99	0.8964	4.97	0.3658
		10 µg/mL	9.96	1.799	9.93	1.536
		15 µg/mL	14.94	0.6217	15.07	0.7359

Table-5: Ruggedness Data

Drug	Amount taken (µg/mL)	Analyst I*	%RSD	Analyst II*	%RSD
OFLOX	3	2.99	0.10	3.00	1.42
KETO	5	4.96	0.11	4.95	0.70

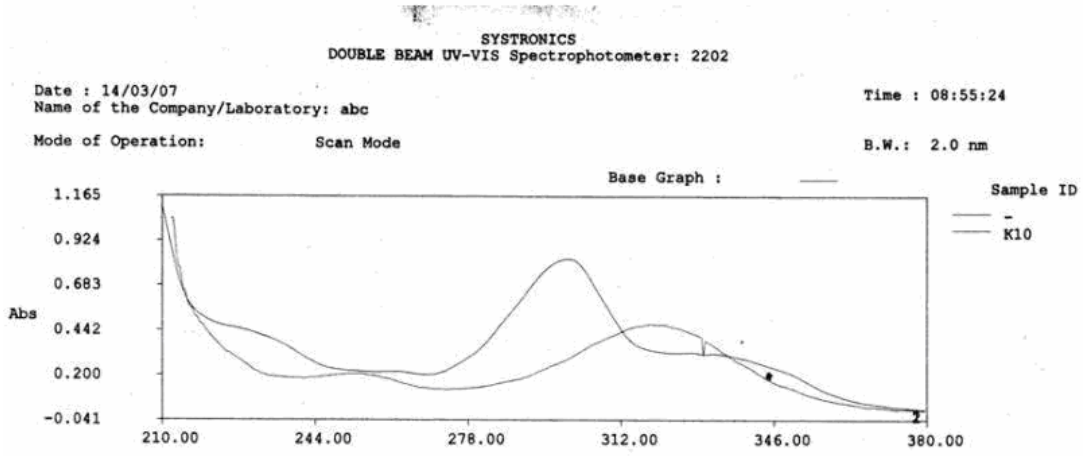
*Mean of five estimations

Table-6: Repeatability Data

Sr.No	Drug	Amount taken* (µg/mL)	Amount found (µg/mL)	%RSD
1	**OFLOX**	3	3.94	0.81
2	**KETO**	5	4.96	0.57

*Mean of six observations

Figure No. 1 Overlain UV Spectra of Ofloxacin and Ketorolac tromethamine



Results and Discussion:

Ofloxacin (OFLOX) is a synthetic fluoroquinolone antibacterial agent., acts by inhibiting bacterial DNA gyrase enzyme which is required for DNA replication and thus causes bacterial lysis. Ketorolac Tromethamine is an anti-inflammatory 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 spectrophotometric method for simultaneous estimation of Ofloxacin and Ketorolac Tromethamine in ophthalmic dosage form.

The overlain spectra of both drugs showed good absorbances at 300 and 319 nm, hence these wavelengths were selected for estimation of OLOX and KETO. Linearity of both OFLOX and KETO were obeyed in the concentration range of 1-11 µg/mL and 3-13 µg/mL with the correlation coefficient 0.9946 and 0.9923, respectively. The absorptivities was then calculated and substituted in equation 1 and 2 to obtained concentration of both drugs.

Assay result:

In replicate analysis (n= 5) of two drugs by proposed method showed the content of OFLOX and KETO as 99.32% and 100.72% respectively **(TABLE 1).**

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 preanalysed sample solution (OFLOX 3µg/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% and lower values of RSD indicates the proposed method is accurate **(TABLE 3).**

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 less than 2, for different analysts. This study signifies the ruggedness of the method under varying conditions of its performance **(TABLE 5).**

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