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Research Article

Development and Validation of Stability Indicating HPTLC Method for Determination of Ofloxacin and Ketorolac Tromethamine in Combination

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ABSTRACT

Ofloxacin is synthetic fluoroquinolone and broad spectrum antimicrobial agent for oral administration. Ketorolac is an non selective cyclooxygenase(COX) inhibitor and has anti-inflammatory, antipyretic and analgesic effects. The present study describes degradation of Ofloxacin and Ketorolac under ICH [Q1A (R_2)] prescribed stress conditions (hydrolysis, oxidation, dry heat, wet heat and photolysis) and establishment of a stability-indicating HPTLC assay method. Different degradation peaks were observed for both Ofloxacin and Ketorolac when each was exposed to different stress condition. For HPTLC, aluminium plate precoated with Silica Gel 60 F_{254} and mobile phase consisting of n-butanol : ethanol : ammonia 6: 1: 3 v/v/v was used to achieve separation. Quantitation was done at 310 nm. The method was validated as per ICH Q2 R1 guidelines and results were in limit.The method was found to be simple, specific, precise, and stability indicating.

Keywords: Ofloxacin, Ketorolac tromethamine, HPTLC, Stability indicating method.

1. INTRODUCTION

Ofloxacin±9-Fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxy-lic acid is synthetic fluoroquinolone. It is broad-spectrum antimicrobial agent for oral administration which is active against both gram positive and gram negative bacteria. It functions by inhibiting DNA gyrase a type II topoisomerase and topoisomerase IV, which is an enzyme necessary to separate replicated DNA, thereby inhibiting cell division. Ketorolac tromethamine ±-5-Benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid with 2-amino-2-(hydroxymethyl)-1,3-propanediol is an non selective cyclooxygenase (COX) inhibitor. Its anti-inflammatory, antipyretic and analgesic effects are due to inhibition of prostaglandin synthesis by competitive blocking of the enzyme (COX). It is 800 times more potent than aspirin.

Literature survey reveals some methods reported viz. High Performance Liquid Chromatographic (HPLC) methods for determination of Ofloxacin in human plasma [1], human plasma and urine [2], HPLC fluorescence [3] amd stability indicating HPTLC method [4]. For Ketorolac tromethamine some methods are also reported viz. HPLC method for determination of Ketorolac [5], determination of enantiomers of Ketorolac in plasma [6], determination in human eye samples [7], HPTLC method [8] and fluorophotometric determination [9]. Only one combination paper of Ofloxacin and Ketorolac is reported as simultaneous UV spectrophotometric methods in ophthalmic dosage form [10].

No reports were found for the stability indicating assay method as per ICH guidelines, for Ofloxacin and Ketorolac in combination by HPTLC method. This paper describes a simple, accurate, sensitive and validated stability indicating HPTLC method for combination as the bulk drug and in ophthalmic dosage forms.

The drug stability test guidelines Q1A (R2) issued by International Conference on Harmonization (ICH) requires that analytical test procedures for stability samples should be fully validated and the assay should be stability indicating. The aim of the present study accordingly was to establish inherent stability of the combination through stress studies under a variety of ICH recommended test conditions [11] and its validation [12].

2. MATERIALS AND METHOD

2.1 Material

For stability indicating HPTLC method development, Camag HPTLC system consisting of Linomat-5 applicator, Camag TLC Scanner 3 and WinCATS software V 1.4.3 were used. For photo-degradation studies, Photostability Chamber was used. (Make - Newtronic) All the weighing was done on Shimadzu balance (Model AY-120).

Working standard of Ofloxacin was provided by Arbro Pharmaceuticals Ltd. New Delhi and Ketorolac tromethamine by Cipla Ltd, Mumbai and was used as such without further purification. n- butanol, ammonia, ethanol, Conc. HCl, NaOH and H_2O_2 used were of analytical reagent grade. Marketed formulation of combination (Ketoflox containing 3mg of Ofloxacin and 5mg of Ketorolac per ml) was purchased from local market.

2.2 Method

2.2.1 Preparation of standard solution:

Standard stock solution of Ofloxacin and Ketorolac was prepared separately by dissolving 25 mg of drug in 25 ml of methanol to get concentration of 1000 µg/mL. From the standard stock solution mixed working standard solution was prepared to contain 100µg/ml of Ofloxacin and 100µg/ml of Ketorolac. Also working stock solution (100µg/ml) was prepared for both Ofloxacin and Ketorolac separately.

2.2.2 Preparation of sample solution

Ketoflox drop (Allegan India Private Limited, Madhya Pradesh) was purchased from local market with label claim as each ml contains 3mg of Ofloxacin and 5mg of Ketorolac. 1ml was withdrawn and diluted with methanol to make up the volume to 10ml in 10ml volumetric flask such that it contains 300µg/ml of Ofloxacin and 500µg/ml of Ketorolac (working mixture sample solution). Further dilutions were made to obtain solution containing Ofloxacin as 30µg/ml and Ketorolac as 50µg/ml.

2.2.3 Stress degradation studies

Stress degradation studies were carried under condition of acid/ base/ neutral hydrolysis, oxidation, dry heat and photolysis. For each study, two samples were prepared: the blank subjected to stress in the same manner as the drug solution and mixed working standard solution subjected to stress conditions. Dry heat and photolytic degradation were carried out in solid state. Then the study was extended to formulation.

2.2.4 Degradation under alkali catalysed hydrolytic condition.

2.5ml of mixed working standard solution was mixed with 2.5ml of 1N NaOH. The solution was diluted to 25 ml with methanol and kept for overnight. 10µl and 50µl of the solution was then spotted.

2.2.5 Degradation under acid catalysed hydrolytic condition.

5ml of working standard solution was mixed with 5ml of 1N HCL. The solution was diluted to 50 ml with methanol and refluxed for 1 hr at 70^0C. The solution was cooled to room tempt. and volume was made to 50ml if required. 10µl and 50µl of solution was spotted.

2.2.6 Degradation under neutral hydrolytic condition

5ml of working standard solution were mixed with 5ml water. The solution was diluted to 50 ml with methanol and refluxed for 1 hour at 70^0c. The solution was cooled to room tempt. and volume was made to 50ml if required. 10µl and 50µl of solution was spotted.

2.2.7 Degradation under oxidative condition

2.5ml of working standard solution was mixed with 2.5ml 30% solution of H_2O_2. The solution was diluted to 25 ml with methanol and was kept overnight. 10µl and 50µl of the solution was then spotted

2.2.8 Degradation under dry heat

Dry heat studies were performed by keeping drug sample in oven (100^0 C) for a period of 24 hours. Samples were withdrawn after appropriate time, dissolved in methanol and diluted to get 10µg/ml as final conc. 10µl and 50µl of the solution was spotted.

2.2.8 Photo-degradation studies

Photolytic studies were also carried out by exposure of drug to UV light up to 200 watt hours/square meter and subsequently to cool fluorescent light to achieve an illumination of 1.2 million Lux.Hr. Sample was weighed, dissolved and diluted get 10µg/ml. 10µl and 50µl of the solution was spotted.

2.2.9 Validation

2.2.9.1 Linearity and Range

The linearity of an analytical procedure is its ability (within a given range) to obtain test result which are directly proportional to the concentration (amount) of analyte in the sample. It was studied by analyzing five concentrations of the drug and process was repeated five times.

2.2.9.2 Precision

Precision of the system was evaluated by analyzing six independent standard preparations and % RSD value obtained was calculated to determine any intra-day variation. These studies were also repeated on different days to determine inter-day variation.

2.2.9.3 Accuracy

To check accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 80, 100 and 120 %. Mean percentage recovery was determined.

2.2.9.4 Limit of detection

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantitated as an exact value. Based on the Standard Deviation of the Response and the Slope, detection limit (DL) may be expressed as:

$$DL = \frac{3.3\,\sigma}{S}$$

2.2.9.5 Limit of quantitation

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy. Based on the Standard Deviation of the Response and the Slope, The quantitation limit (QL) may be expressed as:

$$QL = \frac{10\,\sigma}{S}$$

Where,

σ = *the standard deviation of the response for the lowest conc. in the range*

S = *the slope of the calibration curve.*

2.2.9.6 Specificity

The specificity of the method was ascertained by peak purity profiling studies. Purity of the drug peak was ascertained by analyzing the spectrum at peak start, middle and at peak end. The peak purity was determined on WinCATS software V 1.4.3.

2.2.9.7 Robustness

Robustness of the method was determined by carrying out the analysis under conditions during which mobile phase ratio, chamber saturation time was altered and the effects on the Rf values and area were noted.

3. RESULTS AND DISSCUSION

3.1 Optimization of mobile phase

Chromatographic separation studies were carried out on the working standard solution of Ofloxacin and Ketorolac (100µg/ml). Initially, trials were carried out using various solvents. After several trials, n-butanol: ethanol: ammonia 6: 3: 1 v/v/v was chosen as the mobile phase, for HPTLC, which resulted in good resolution and acceptable peak parameters.The retention factor was found to be: (Figure 1)

Ofloxacin = 0.32 ± 0.03; Ketorolac = 0.68 ± 0.02

3.2 Stress degradation study: (Table 1, 2)

After alkaline condition, 86.96% Ofloxacin was recovered with 2 peaks of degradation (D1, D2) and 85.92%Ketorolac was recovered with no peak of degradation (Figure 2)

After acidic condition, 82.99% Ofloxacin was recovered with 4 degraded products (D1, D2, D3, D4) and 2 peaks of degradation (d-1, d-2) was observed for Ketorolac with 7.36% as percent recovery. (Figure 3)

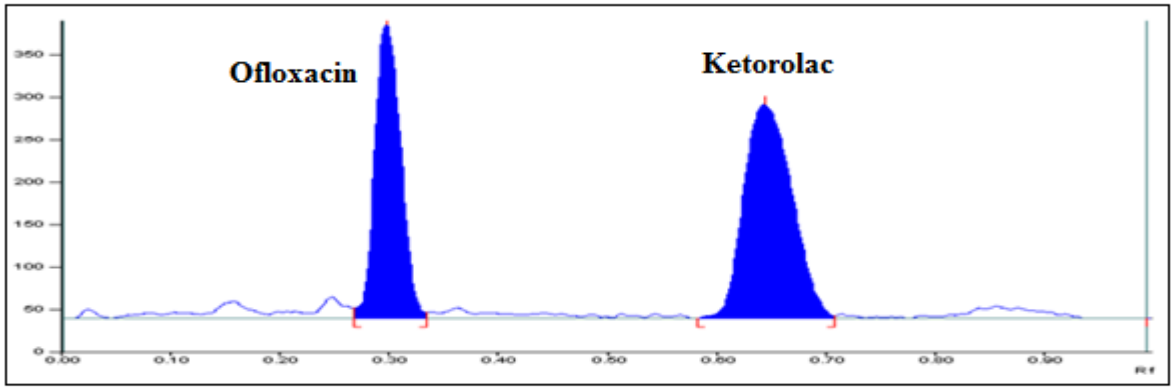

Figure 1: Densitogram of Mixture (100ng/band of each)

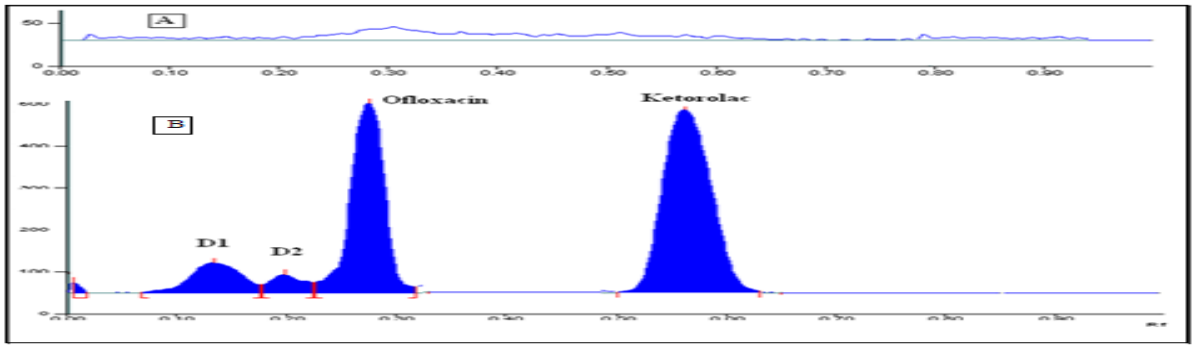

Figure 2: A- Blank NaOH, B-Mixture (500ng/band) treated with NaOH

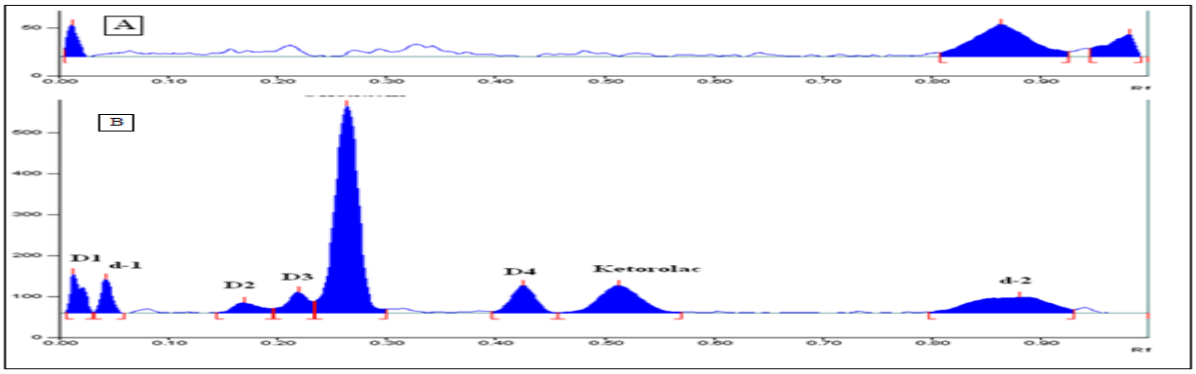

Figure 3: A- Blank HCl, B- Mixture (500ng/band) treated with HCl

Under neutral hydrolysis, no peak of degradation was observed for Ofloxacin with 88.03% recovery and Ketorolac showed single peak of degraded product (d-1) with 77.39% recovery. (Figure 4)

After oxidative condition, 85.31% Ofloxacin was recovered 3 peak of degradation product (D1, D2, D3) and no peak of degradation peak for Ketorolac was observed with 83.89% recovery (Figure 5)

After the dry heat or exposing to heat condition, single peak for degradation product was observed for Ofloxacin with 73.93% recovery and Ketorolac with 76.83% recovery. (Figure 6)

After the photo degradation study for UV light and Fluorescence light, no peaks for the degradation products were obtained but the area recovered was 88.30% for Ofloxacin and 67.31% for Ketorolac. (Figure 7)

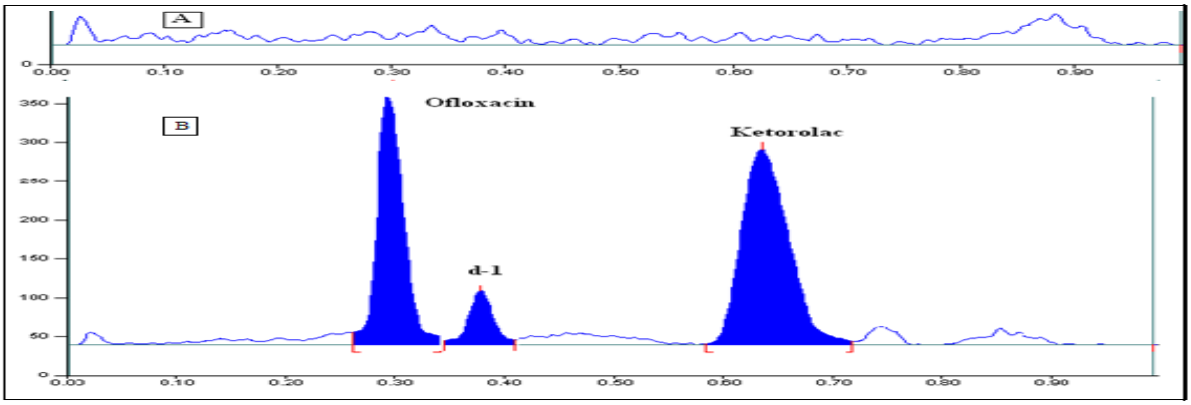

Figure 4: A- Blank H_2O, B- Mixture (500ng/band) after neutral hydrolysis

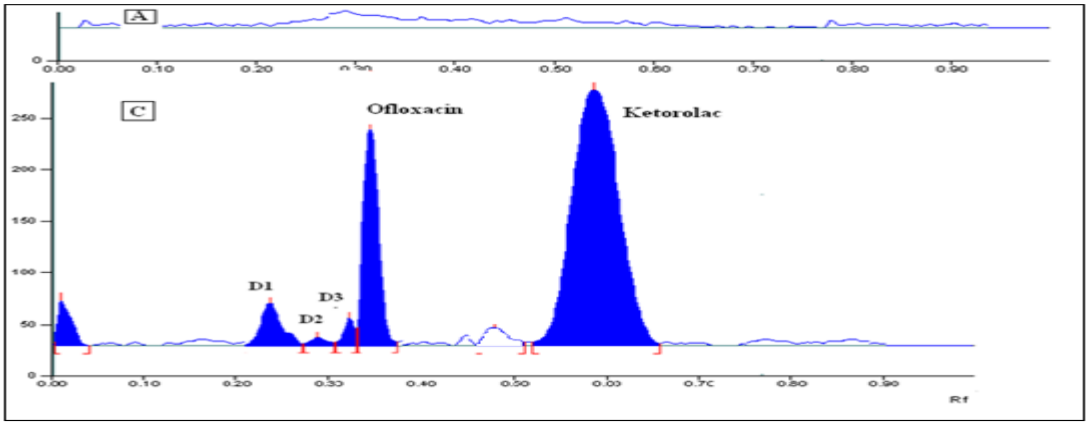

Figure 5: A- Blank H_2O_2, B- Mixture (500ng/band) after oxidation

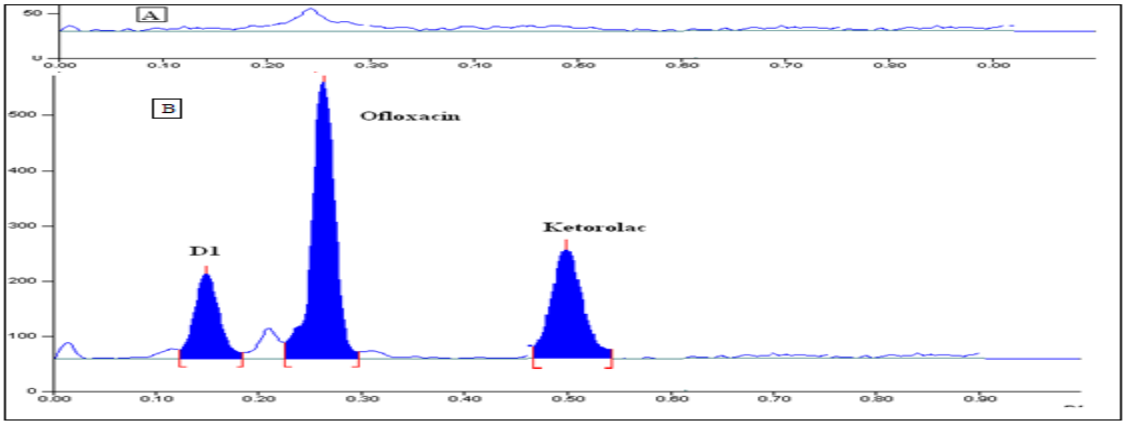

Figure 6: A- Blank MeOH, B- Mixture (500ng/band) after exposing to heat

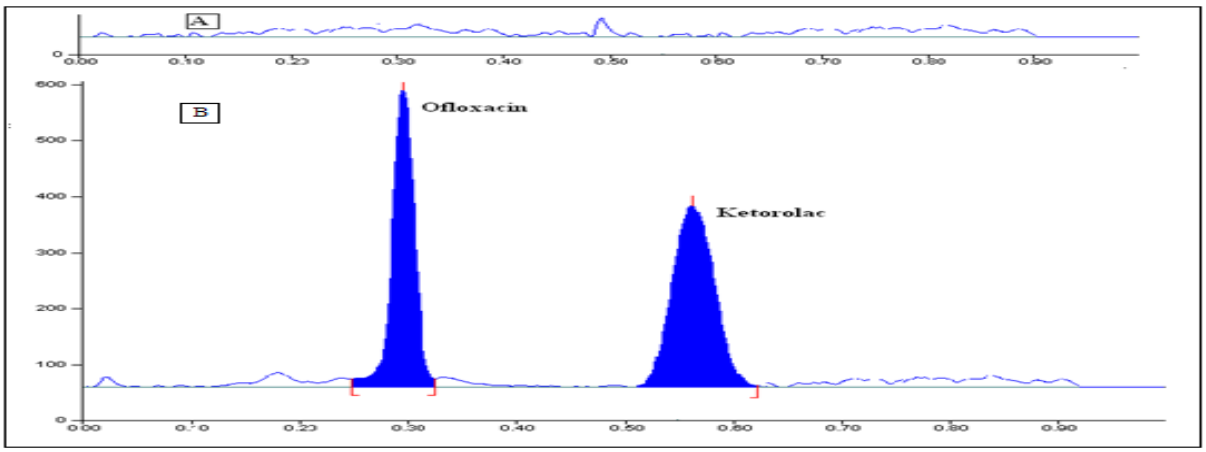

Figure 7: A- Blank MeOH, B- (500ng/band) after exposing to light

Table 1. Summary of stress degradation of Ofloxacin

Stress Degradation Condition	Peak Area	Percent Recovered %	R_f of degradation product
Initial	5961	------	-------
Base (1 N NaOH, kept overnight)	5183	86.96	D1 = 0.13 D2 = 0. 20
Acid (1 N HCl, Reflux for 1 hr at 70°c)	4947	82.99	D1 = 0.03 D2 = 0.14 D3 = 0.20 D4 = 0.40
Water (Reflux for 1 hr at 70°c)	5265	88.03	Not Found
H_2O_2 30% (kept overnight)	5085	85.31	D1 = 0.16 D2 = 0.22 D3 = 0.27
Heat dry (100°C,24 hrs.)	4406	73.93	D1 = 0.15
Photo stability [UV, 200 watt hrs/square meter Florescence , 1.2 million Lux. Hrs]	5263	88.30	Not Found

3.3 Validation study: (Table 3, 4)

The data obtained in the linearity experiment was subjected to linear-regression analysis. A linear relationship between peak areas and concentrations was obtained in the range of 30-150ng/band for Ofloxacin and 50-250ng/band for Ketorolac. The developed method was found to be precise as the % RSD value for intraday precision and interday precision were less than 2%. Excellent recoveries were obtained at each level of added concentration. The result obtained (n = 3 for each level) indicated the mean recovery between 98% to 102% for both the drugs.

The limit of detection as calculated by standard formula as given in ICH guidelines was found to be 0.6304 ng/band for Ofloxacin and 2.7155 ng/band for Ketorolac.

The limit of Quantitation as calculated by standard formula as given in ICH guidelines was found to be 1.9105 ng/band for Ofloxacin and 8.2289 ng/band for Ketorolac. The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to >0.9950, indicating the non interference of any other peak of degradation product or impurity and the method was found to be robust.

Table 2. Summary of stress degradation of Ketorolac

Stress Degradation Condition	Peak Area	Percent Recovered %	R_f of degradation product
Initial	5564	--------	-------
Base (1 N NaOH, kept overnight)	4781	85.92	Not Found
Acid (1 N HCl, Reflux for 1 hr at 70°c)	410	7.36	d-1 = 0.04 d-2 = 0.83
Water (Reflux for 1 hr at 70°c)	4306	77.39	d-1 = 0.38
H_2O_2 30% (kept overnight)	4668	83.89	Not Found
Heat dry (100^0C,24 hrs.)	4278	76.83	Not Found
Photo stability [UV, 200 watt hrs/square meter Florescence , 1.2 million Lux. Hrs]	3745	67.31	Not Found

Table.3 Robustness study

Prarmeters	Variation	% RSD	
		Ofloxacin	Ketorolac
Mobile phase composition	± 0.2 ml n-butanol	1.002	1.031
Chamber saturation period	± 3 mins	0.872	0.901

The degradation conditions mentioned above were arrived at, after number of initial trials for optimization of extent of degradation. Overall study comprised of stability indicating method development for Ofloxacin alone and in combination with Ketorolac tromethamine to study effect of Ketorolac on Ofloxacin stability study. But no considerable difference was observed. This method can be used for stability testing of these drugs in combination dosage forms.

Table.4 Summary of Validation study

Validation Parameter	Results	
	Ofloxacin	Ketorolac
Linearity	y = 48.34x + 922.8 R^2 = 0.997	y = 40.56 + 1255 R^2 = 0.998
Range	30-150 ng/band	50-250ng/band
Precision	%RSD	%RSD
A) Intraday precision	0.4695	0.9752
B) Interday precision	0.7166	0.8036
Accuracy	% recovery	% recovery
80%	101.57	99.73
100%	99.40	99.01
120%	101.69	100.79
LOD	0.6304 ng/band	2.7155 ng/band
LOQ	1.9105 ng/band	8.2289 ng/band
Specificity	Specific	Specific
Robustness	Robust	Robust

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