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Research Article

STABILITYINDICATING RP-HPLC METHOD FOR SIMULTANEOUSDETERMINATION OF KETOROLACTROMETHAMINE AND OLOPATADINE HYDROCHLORIDE IN BULK AND ITS PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A simple and new RP – HPLC method was developed for the simultaneous determination of Ketorolactromethamine and Olopatadine Hydrochloride in combined dosage form. An Inertsil C18 column was used with mobile phase of composition Acetonitrile :Sodiumdihydrogen Orthophosphate (50:50 v/v at pH 4.6) at a flow rate of 1.0 mL/min and injection volume of 20µL with UV detection at 260 nm for separating Ketorolactromethamine and Olopatadine hydrochloride. The retention time of Olopatadine hydrochloride and Ketorolactromethamine were 2.724 min and 3.849 min respectively. The specificity, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), ruggedness and robustness of the developed method were studied to validate as per ICH guidelines. The Linearity range for Ketorolactromethamine andOlopatadine hydrochloride were 10.0 – 60.0 µg/ml and 2.5 – 15.0 µg/ml, respectively. The percentage recoveries were in the range for Ketorolactromethamine and Olopatadine hydrochloride 99.07-99.98 % and 99.56-100.33%, respectively. The developed method could be used for routine analysis of Ketorolactromethamine and Olopatadine hydrochloride in their combined dosage forms. The other formulation excipients did not influence and interrupt the developed method for simultaneous of the two drugs. Hence this method can be conveniently used for the routine analysis of Ketorolactromethamine andOlopatadine hydrochloride in quality control formulations and also in the dissolution studies.

Keywords: Ketorolac tromethamine, Olopatadine hydrochloride, Validation.

INTRODUCTION

Ketorolac tromethamine (KETO) is a white crystalline substance. Chemical name is (±)-5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid, compound with 2-amino-2-(hydroxymethyl)-1,3- propanediol (1:1). Ketorolac tromethamine is a nonsteroidal anti-inflammatory drug (NSAID) chemically related to

indomethacin and tolmetin. Ketorolac tromethamine is a racemic mixture of [-]S- and [+]R-enantiomeric forms, with the S-form having analgesic activity[1-5]. Its anti-inflammatory effects are believed to be due to inhibition of both cylooxygenase-1 (COX-1) and cylooxygenase-2 (COX-2) which leads to the inhibition of prostaglandin synthesis leading to decreased formation of precursors of prostaglandins and thromboxanes from arachidonic acid. The resultant reduction in prostaglandin synthesis and activity may be at least partially responsible for many of the adverse, as well as the therapeutic, effects of these medications. Analgesia is probably produced via a peripheral action in which blockade of pain impulse generation results from decreased prostaglandin activity. However, inhibition of the synthesis or actions of other substances that sensitize pain receptors to mechanical or chemical stimulation may also contribute to the analgesic effect. In terms of the ophthalmic applications of ketorolac - ocular administration of ketorolac reduces prostaglandin E2 levels in aqueous humor, secondary to inhibition of prostaglandin biosynthesis.

Olopatadine is a crystalline powder. Chemical name of it is11-[(Z)-3-Dimethylamino) propylidene]-6-11dihydrodibenz[b,e] oxepin-2-acetic acid hydrochloride. Olopatadine, a structural analog of doxepin, is a non-steroidal, non-sedating, topically effective anti-allergic molecule that exerts its effects through multiple distinct mechanisms of action. Olopatadine is a mast cell stabilizer and potent, selective histamine H1antagonist [6,7] that inhibits the in vivo type 1 immediate hypersensitivity reaction. Olopatadine inhibits the release of mast cell inflammatory mediators [i.e., histamine, tryptase, prostaglandin D2 and TNFα [26] as demonstrated in in-vitro studies and confirmed in patients [27]. Olopatadine is also an inhibitor of pro-inflammatory cytokine secretion from human conjunctival epithelial cells.

A number of HPLC, HPTLC, UPLC, LC/MS, UV-Vis, Fluorometric and Voltametric methods were reported for the quantification of Ketorolac tromethamine and Olopatadine hydrochloride alone and in combination with other drugs[8-25]. All these have resulted in simple and sensitive methods for separation and determination of these drugs alone and in combination with other drugs and no method was reported for the determination of Ketorolac tromethamine and Olopatadine in combination.In all these methods reported till now, no degradation studies were carried out to prove that the method is stability indicating method. The present work describes the development of a validated stability indicating analytical RP-HPLC method, which can quantify these Components simultaneously from a combined dosage form.

Aim of present work was to develop simple, eco nomical, rapid, accurate and precise RP-HPLCmethods for determination of these drugs i n fixed dose combination.

The proposed method was optimized and validat ed as per the International Conference on Harm onization (ICH) guidelines [28,29]

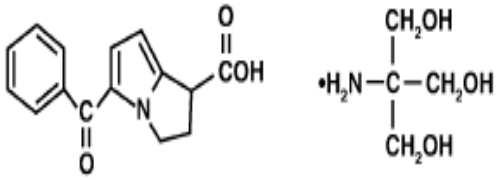

Fig. 1A: Structure of Ketorolac tromethamine

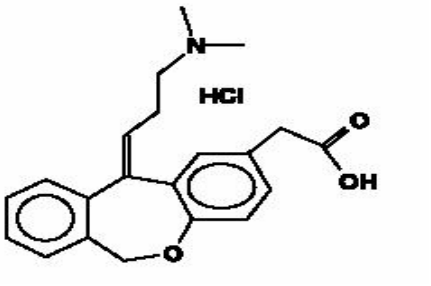

Fig. 1B: Structure of OlopatadineHCl

MATERIALS AND METHODS

Materials

HPLC grade Sodiumdihydrogenorthophosphate (NaH_2PO_4), sodium hydroxide, and acetonitrilewere procured from Merck India. All dilutions were performed in standard class-A, volumetric glassware. For the estimation of commercial formulation, Olopat KT eye drops 5 mL having ketorolac tromethamine4mg and 1.0mg Olopatadine hydrochloride were procured from the local market.

Instrumentation

Waters2695 compact LC chromatographic system, with UV-Vis detector 2996 and a fixed injector equipped with 20µL loop was used for

the chromatographic separation. The chromatogram was recorded at ambient temperature and peaks quantified by means of Empower software. Chromatographic separation was carried out on a C18 column [Inertsil, 250mm x4.5mm 5µ]. Sartorious electronic balance was used for weighing the samples. Ultra-sonic bath sonicator was used for degassing and mixing of the mobile phase.

Chromatographic conditions

Chromatographic separation of Ketorolac tromethamine and Olopatadine hydrochloride was carried on a C18 column. The mobile phase was composed of acetonitrile and sodium di hydrogen orthophosphate buffer (pH 4.6) in the ratio of 50:50 v/v. It was filtered through a 0.45 µ membrane filter and degassed for 15 minutes. The flow rate of the mobile phase was maintained at 1.0 ml/min. Detection was carried out at 260 nm at ambient temperature.

Method development

Preparation of Standard Stock Solutions

Weighed accurately about 12.5mg of Olopatadine hydrochloride and 12.5mg of Ketorolac tromethamine working standards transfer to the 25 mL of clean and dry volumetric flask, to these add the 15 mL of diluents to sonicate to dissolve the few minutes then make up the volume with diluents. Filter the solution through 0.45µm

Standard preparation

Transfer the filtrate 1 mL of stock solution into the 25 mL of clean and dry volumetric flask and make up the volume with diluents.

Preparation of Sample solutions

Transfer Sample quantitatively equivalent to 100 mg Olopatadine and 400 mg of Ketorolac in to100 mL volumetric flask add 60 mL of diluents, sonicate to dissolve for 10 minutes and dilute to volume with diluents. Further filter the solution through 0.45µ filter paper. Dilute 1 mL of filtrate to 100 mL with mobile phase.

Method validation

simultaneous determination of Ketorolac tromethamine and Olopatadine hydrochloride by RP- HPLC method was validated as per the ICHguidelines[28, 29].

System suitability and System Precision

System suitability for chromatographic separation was checked on each day of validation to evaluate the components of the analytical system in order to show that the performance of the system meet the standards required by the method. System suitability parameters established for the developed method include number of theoretical plates (efficiency), Resolution, Tailing factor. The HPLC system was equilibrated using the initial mobile phase composition, followed by 6 injections of the standard solution of 100% concentration containing 40µg/mL ketorolac tromethamineand 10 µg/ml Olopatadine hydrochloride. These 6 consecutive injections were used to evaluate the system suitability on each day of method validation. The result was given in the Table 1.

Specificity

Blank interference

A study to establish the interference of blank was conducted. Diluent was injected into the chromatograph in the above defined chromatographic conditions and the blank chromatograms were recorded. Chromatogram of Blank solution **(Fig. no.-2)** showed no peaks at the retention time of ketorolac tromethamine and Olopatadine hydrochloride peak. This indicates that the diluent solution used in sample preparation do not interfere in estimation of Ketorolac tromethamine and olopatadine hydrochloride in Olopat KT eye drops. Similarly typical representative chromatogram of standard is also shown (**Fig. No. -3**)

Table 1: System suitability parameters for Ketorolac tromethamine and Olopatadine hydrochloride by proposed method

Name of the Compound	Retention Time	Tailing factor	Theoretical plates	USP Resolution
Ketorolac tromethamine	3.849	1.10	6889	6.52
Olopatadine hydrochloride	2.724	1.11	4581	----

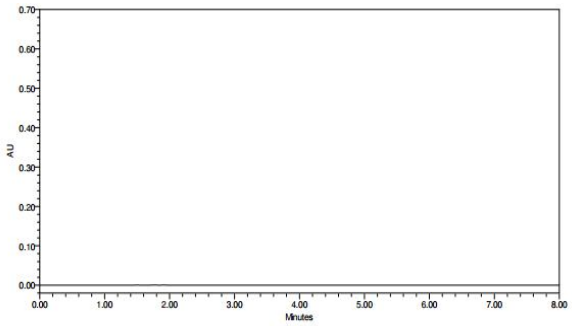

Fig. 2: A typical HPLC Chromatogram showing the no interference of diluent for Ketorolac tromethamine and Olopatadine hydrochloride

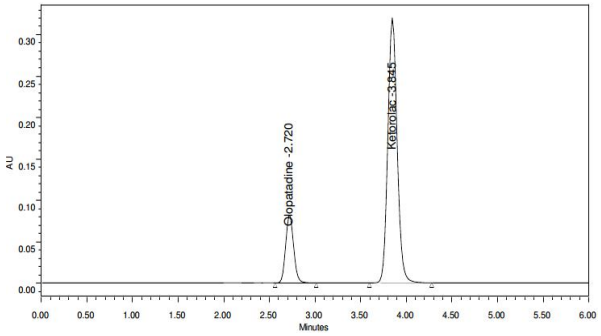

Fig. 3: A typical HPLC Chromatogram showing the peak of Ketorolac tromethamine and Olopatadine hydrochloride

Forced Degradation

In case of forced degradation studies a sample was prepared equivalent to 1.0mLof Ketorolac tromethamine and Olopatadine hydrochloride. Then they are transferred into a 10 mL volumetric flask into which 6 mL of diluent was added and then sonicated for 15 minutes with intermittent shaking at controlled temperature. The solution was then filtered through 0.45 µ filter paper. Control sample was prepared by transferring 1 mL of the above solution into a 10 mL of volumetric flask and diluted to volume with diluent. Acid and Base degradation studies were performed by adding acid and base before making up the volume to 10 mL before filtering through 0.45 µ filter paper. From this controlled sample was prepared latter.

Thermal degradation was studied by preparing sample using common procedureas mentioned above.Similarly Sunlight exposure stress sample was prepared and checked for their purity by proposed method. The figures 4A, 4B, 4C, 4D and 4E represent the typical chromatograms of control sample, acid, base, thermal, photolyticdegradation. The results of degradation studies were given in table 2.

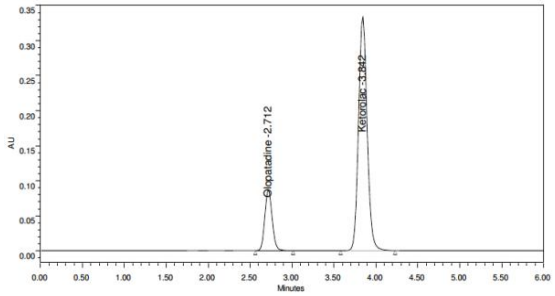

Fig. 4A: A typical HPLC Chromatogram showing the Control Sample profile of Ketorolac tromethamine and Olopatadine hydrochloride by proposed method

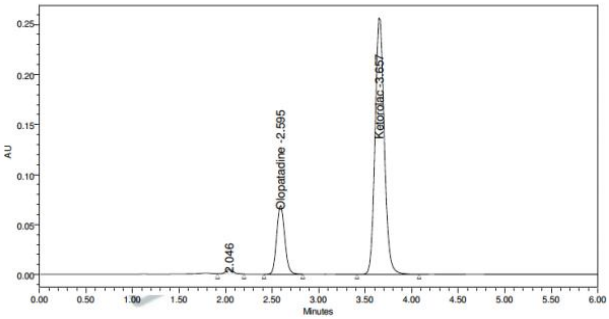

Fig. 4B: A typical HPLC Chromatogram showing the Sample profile of Ketorolac tromethamine andOlopatadine hydrochloride in Acidic hydrolysis by proposed method

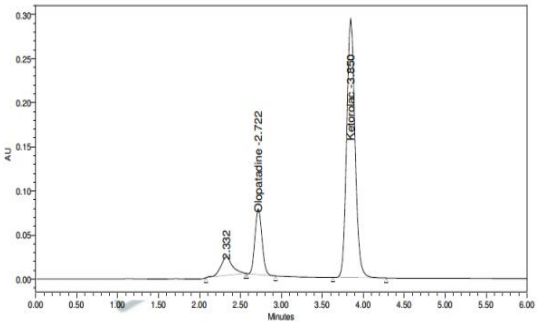

Fig. 4C: A typical HPLC Chromatogram showing the Sample profile of Ketorolac tromethamine and Olopatadine hydrochloride in Base hydrolysis by proposed method

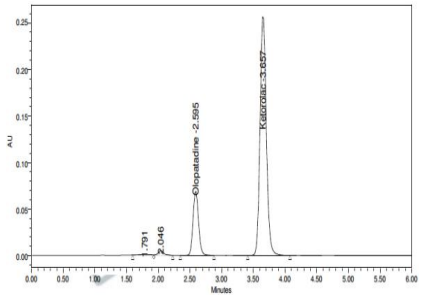

Fig. 4D: A typical HPLC Chromatogram showing the Sample profile of Ketorolac tromethamine and Olopatadinehydrochloride in Thermal hydrolysis by proposed method

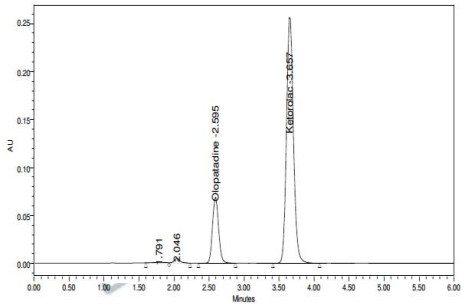

Fig. 4E: A typical HPLC Chromatogram showing the Sample profile of Ketorolac tromethamine and Olopatadine hydrochloride in photolytic degradation by proposed method

Linearity and range

Linearity and range of Ketorolac tromethamine and Olopatadine hydrochloride were determined by weighing accurately about 100gm of Olopatadine hydrochloride and 400 mg of Ketorolactromethamine in to a 100 mL of clean and dry volumetric flask, add 70 mL of diluents, shake and sonicate to dissolve the content, make up the volume with diluents. Filter the solution through 0.45µm. 0.5 mL, 0.75 mL, 1.0 mL, 1.25mL and 1.5 mL of above solution were diluted in 100 mL volumetric flask with diluent separately to get 50%, 75%, 100%, 125% and 150% concentration solutions.

Standard curves for Ketorolac tromethamine and Olopatadine hydrochloride were obtained in the range of 10.0 – 60.0 µg/ml and 2.5 – 15.0 µg/ml respectively. A statistical method known as linear regression analysis was used to evaluate the linearity of the curve. To assess the linearity of the proposed method slope, intercept and correlation coefficient [r^2] of standard curve were calculated and were given in Figure-5A(For ketorolac tromethamine) and Figure-5B(For olopatadine hydrochloride). The results of linearity were given in Table 3 and 4.From the data obtained (For KETO and OLOP) the method was found to be linear within the proposed range.

Table 2: Forced degradation specificity data for Olopatadine and Ketorolac

Conditions	Retention time(min)		Area (μv^2sec)		% Area of active drug		Asymmetry factor	
	OLP	KT	OLP	KT	OLP	KT	OLP	KT
ACID DEGRADATION	2.5	3.6	408257	176915	18.53	80.32	1.09	1.12
BASE D EGRADATION	2.7	3.8	447859	2065989	16.46	75.92	1.14	1.11
THERMALDEGRADATION	2.5	3.6	410146	1769157	18.51	79.85	1.09	1.12
PHOTOLYTIC DEGRADATION	2.7	3.8	395834	1793487	17.92	81.18	1.09	1.11

Table 3: Linearity Data for KETOROLAC

Concentration(mcg/mL)	Area (μv^2sec)
10.0	600183
20.0	1213706
30.0	1801949
40.0	2394248
50.0	2976117
60.0	3565845

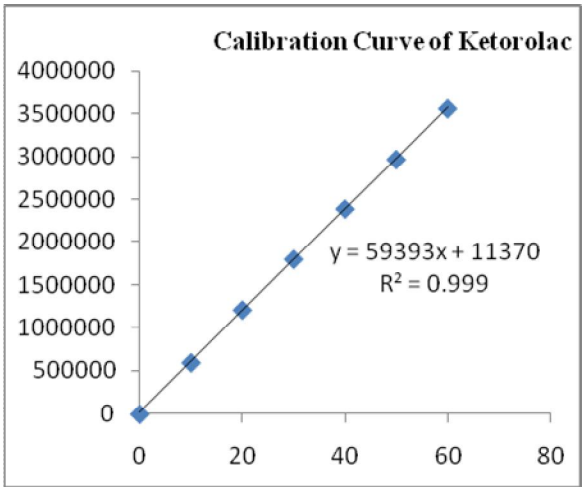

Fig. 5A: Calibration curve for Ketorolac tromethamine

Table 4: Linearity Data for OLOPATADINE

Concentration(mcg/mL)	Area (μv²sec)
2.5	131875
5.0	266830
7.5	397849
10.0	533804
12.5	659693
15.0	789260

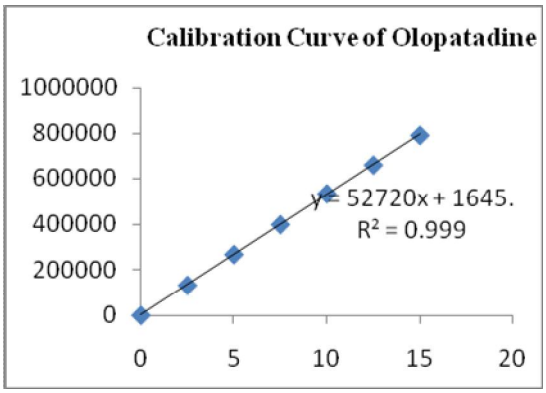

Fig. 5B: Calibration curve for Olopatadine

Accuracy

Accuracy is defined as the closeness of results obtained by that method to the true value for the sample. In general Accuracy is expressed in terms of percentage recovery. Recovery % was assessed by standard addition method. In the present investigation to understand the accuracy the recovery studies were carried out at 50%, 100% and 150% spiked levels. The results of Recovery % were given in Table 5. The chromatograms of accuracy study of 50%, 100%, 150% and including that of sample were given in figures 6A, 6B, 6C and 6D respectively.

Precision

The closeness of agreement (degree of scatter) between a series of measurements obtained from multiple samplings of the same homogeneous sample. The precision of the method was assessed by six replicate injections of 100% test concentration. The precision was expressed in terms of standard deviation and %RSD. The results were given in Table 7 and the corresponding chromatogram was given in figure 7.

Ruggedness

Degree of reproducibility of test results obtained by analyzing the same sample under variety of normal test conditions such as different analysts, instruments, days, reagents, column etc. The Ruggedness of the method was verified by analyzing the six samples of same batch for method precision as per test method by different analysts using different instrument, different days. The analyst's prepared six sample of the same batch on two different day's .Calculated %RSD for two different days in six samples for ruggedness results with the method precision. The results of ruggedness were given in table 8 and the chromatograms corresponding to ruggedness studies of day 1 and day 2 were given in figures 8A and 8B respectively.

Table 5: Accuracy data for Olopatadine and Ketorolac

S. No.	OLOPATADINE			KETOROLAC		
	Area (μv² sec)					
	50%	100%	150%	50%	100%	150%
Injection 1	257679	401264	530784	1202039	1824112	2425989
Injection 2	269865	401123	531865	1212433	1802842	2409678
Injection 3	269904	400275	530455	1204892	1802892	2426256
Average	265822	400887	531035	1206455	1809949	2420641
*Amount recovered (μg)	**+ 49.94**	**100.33**	**149.34**	**49.92**	**99.07**	**149.97**
*** % Recovery**	**99.87**	**100.33**	**99.56**	**99.84**	**99.07**	**99.98**

***Each value is a mean of three readings**

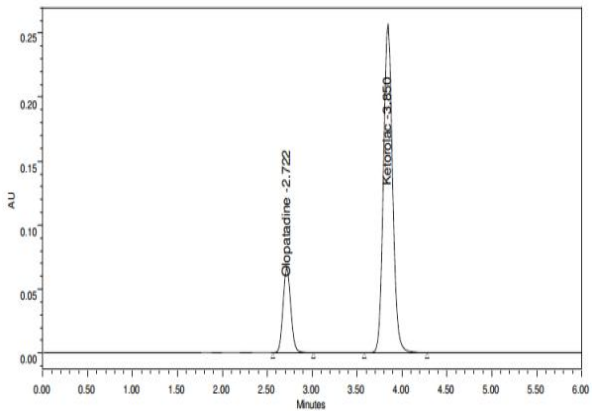

Fig. 6A: Chromatogram ofOlopatadineandKetorolac Accuracy – 50%

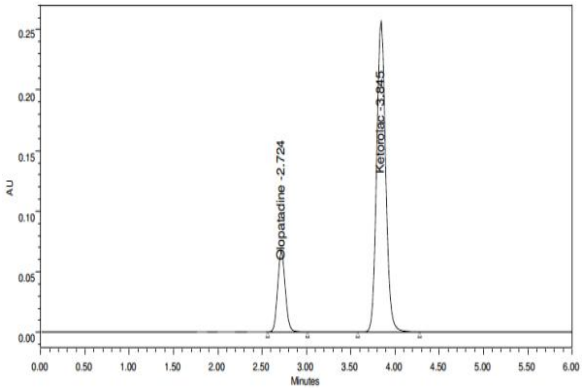

Fig. 6B: Chromatogram ofOlopatadine and Ketorolac Accuracy -100%

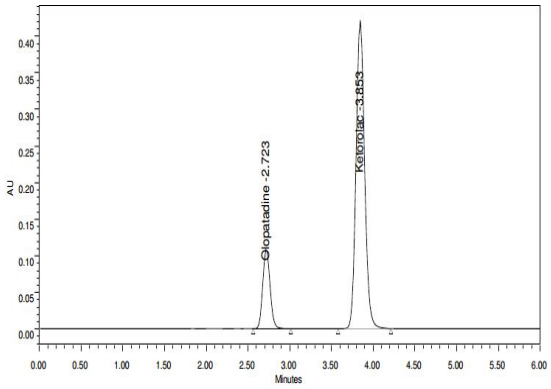

Fig. 6C: Chromatogram ofOlopatadine and Ketorolac Accuracy -150%

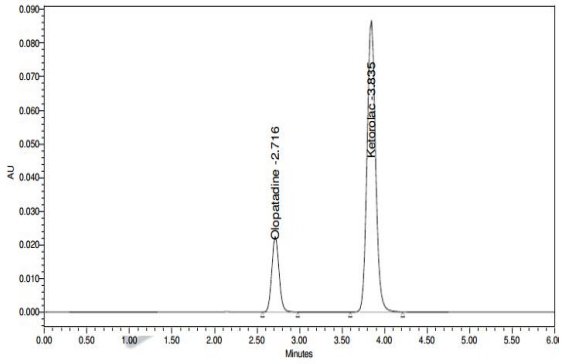

Fig. 6D: Chromatogram of Olopatadine and Ketorolac sample Accuracy

Table 7: Method Precision studies for Ketorolac tromethamine and Diclofenac by proposed method

S.No.	OLOPATADINE	KETOROLAC
	Area (μv^2sec)	
Injection 1	525232	2365043
Injection 2	525021	2365131
Injection 3	525453	2365545
Injection 4	526023	2361320
Injection 5	526432	2362313
Injection 6	526210	2361890
Average	**525729**	**2363540**
Standard Deviation	**572.1**	**1895.6**
%RSD	**0.071**	**0.080**

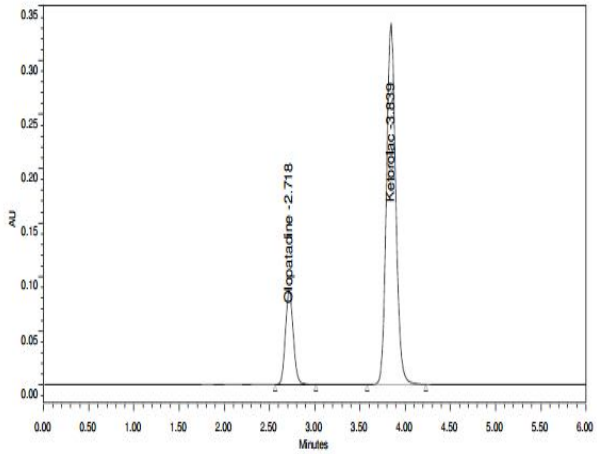

Fig. 7: Chromatogram ofOlopatadineandKetorolac Method precision

Table 8: Ruggedness Data for OLOPATADINE and KETOROLAC

Olopatadine			Ketorolac		
S.No.	RT (min)	Area (μv^2sec)	S.No.	RT (min)	Area (μv^2sec)
1	2.719	524215	1	3.842	2358786
2	2.719	524098	2	3.843	2358324
3	2.72	523987	3	3.845	2357987
4	2.721	524034	4	3.846	2357675
5	2.721	524212	5	3.847	2359870
6	2.723	524650	6	3.849	2359453
7	2.716	525675	7	3.843	2348765
8	2.717	524876	8	3.844	2348990
9	2.717	523656	9	3.845	2346745
10	2.718	523905	10	3.847	2344540
11	2.72	524434	11	3.848	2349544
12	2.722	524875	12	3.849	2349204
Average	**2.719**	**524384.750**	Average	**3.846**	**2353323.583**
Standard deviation	**0.002**	**556.168**	Standard deviation	**0.002**	**5777.666**
%RSD	**0.08**	**0.11**	**%RSD**	**0.06**	**0.25**

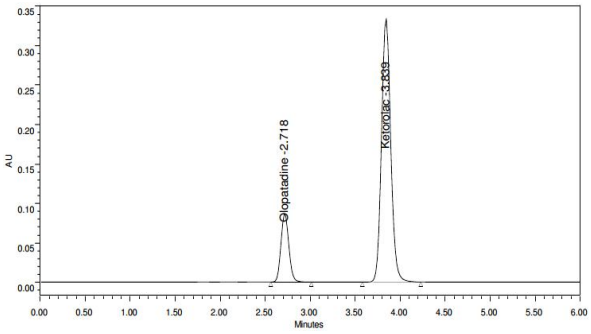

Fig. 8A: Chromatogram of Olopatadine and KetorolacRuggedness on day-1

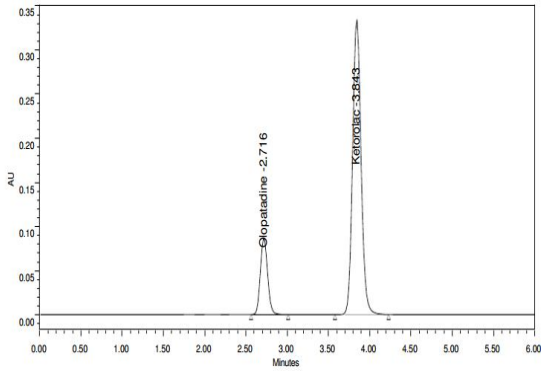

Fig. 8B: Chromatogram of Olopatadine and Ketorolac Ruggedness on -day-2

LOD and LOQ

The formulae 3.3 σ/S and 10 σ/S were used to calculate LOD and LOQ respectively. σ is the mean of standard deviation of y intercepts of the three calibration curves and S is the mean of slopes of the calibration curves. The results were given in Table 9 and 10.

Table 9: Data for limit of detection of Olopatadine and Ketorolac

S. No.	LIMIT OF DETECTION	
	SYTEM PRECISION	METHOD PRECISION
OLOPATADINE(µg/mL)	0.11	0.33
KETOROLAC(µg/mL)	0.94	0.10

Table 10: Data for limit of quantification of Olopatadine and Ketorolac

S. No.	LIMIT OF QUATITATION	
	SYTEM PRECISION	METHOD PRECISION
OLOPATADINE(µg/mL)	0.36	1.00
KETOROLAC (µg/mL)	2.80	0.31

Robustness

The ability of the developed method to remain unaffected by the small changes in the parameters is known as Robustness. Robustness was assessed by varying the parameters such as percent organic content, pH of the mobile phase, buffer concentration,

temperature, injection volume and flow rate. In the present investigation, a variation ± 0.1 mL/min in the flow rate, a variation in buffer composition were carried out andthe results were tabulated in Table 11.

Table 11: Data for Robustness study of Olopatadine and Ketorolac

S.No.	Olopatadine		Ketorolac	
	RT	Area	RT	Area
Standard				
1	2.723	528811	3.850	2378945
Robust-1 Flow -1				
2	2.551	488583	3.607	2209222
Robust-2 Flow-2				
3	2.914	561547	4.119	2536221
Robust-3 Buffer-1				
4	2.653	471413	3.745	2134388
Robust-3 Buffer-2				
5	2.593	471183	3.648	2128999

RESULTS AND DISCUSSION

In present study a new analytical reversed phase HPLC method for the simultaneous determination of Olopatadine Hydrochloride and Ketorolac Tromethamine in Ophthalmic preparation for eye drops was developed. The column used in this method is Inertsil ODS C18, 100 x 4.6 mm, 5µm with a flow rate of 1.0 mL /min at a wavelength 260 nm and Column temperature is 30°C. The mobile phase preparation done by using buffer 0.1N sodium di hydrogen ortho phosphate by PH adjusted to 4.6 with ortho phosphoric acid. The mobile phase combination was Buffer: ACN (50:50). The run time was set for 6 minutes. The retention time of Olopatadine and Ketorolac were found to be 2.724 min and 3.849 min respectively.

The new HPLC method developed and validated for simultaneous determination of Olopatadine and Ketorolac in pharmaceutical dosage forms and assured the satisfactory precision and accuracy and also determining lower concentration of each drug in its solid combined dosage form by RP-HPLC method. The linearity range for Olopatadine and Ketorolac is 0-15µg/mL the co-relation co-efficient was found to be 0.999. The percentage RSD obtained for system precision of Olopatadine and Ketorolac were 0.36 and 0.72 respectively. The percentage RSD obtained for method precision of Olopatadine and Ketorolac were 0.11 and 0.080.

The Limit of detection values for Olopatadine and Ketorolac were 0.11 and 0.94 for system precision respectively. The Limit of detection values for Olopatadine and Ketorolac for method precision was 0.33 and 0.10 respectively. The Limit of quantification values for Olopatadine and Ketorolac for system precision was 0.36 and 2.8 and for method precision were 1.00 and 0.31 respectively.

The Ruggedness of the method has been verified by analyzing the six samples of same batch for method precision as per test method by different analysts using different instrument, different days. The analyst's prepared six sample of the same batch by two different day's .calculated %RSD for two different days in six samples for ruggedness results with the method precision.

The system suitability was evaluated in each condition and compare the results with method precision results the method is robust for change in flow rate and, mobile phase buffer solution.

To conform that during stability or throughout shelf life any degradation product if found will not interfere with the main peak of Olopatadine and Ketorolac also the force degradation study will help to identify the type of degradation(with alkali,acidic,thermal and photolytic) for each of the degradents . No peaks are observed at the time of retention time of Olopatadine and Ketorolac the method was found to be specific.

The sample solution was injected and the amount of Olopatadine and Ketorolac present in the formulation was calculated from the calibration curve. The amount of Olopatadine hydrochloride and Ketorolac Tromethamine present per each mL was found to be 1.0 ± 0.02 and 3.99 ± 0.12 respectively. The results of assay of Olopatadine and Ketorolac were found to be 100.94% and 99.99% respectively.

CONCLUSION

All the proposed methods for Olopatadine hydrochloride& Ketorolac tromethamineare simple, selective, reproducible and specific with good precision and accuracy. The method was

proved to be superior to most of the reported methods. These proposed methods for estimation of selected drugs were successfully applied either in bulk or pharmaceutical formulations.

The proposed method can be used as alternative method to the reported ones for the routine determination of selected drugs under the study in bulk and pharmaceutical dosage forms.

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