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Research Article

Simultaneous estimation of olopatadine hydrochloride and Ketorolac tromethamine in bulk and pharmaceutical dosage form by RP-HPLC method

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ABSTRACT

A new analytical RP-HPLC chromatographic method was developed and validated for the simultaneous estimation of Olopatadine Hydrochloride (OPH) and Ketorolac Tromethamine (KLT) in bulk drug and pharmaceutical dosage forms. The method was validated as per ICH guidelines. The separation was carried out by using a mobile phase consisting of buffer: acetonitrile in the ratio of 50: 50. The column used was inertsil ODS C_{18} (4.6×250mm) 5µ with flow rate of 1.0 ml / min using UV detector at 260 nm. The described method was linear over a concentration range of 2.5-15.0 µg/ml for OPH (r^2 =0.999) and 10-60 µg/ml for KLT (r^2 =0.999). The retention times were found to be 2.7 min for OPH and 3.8 min for KLT. The limit of detection (LOD) was found to be 0.11 µg/ml for OPH and 0.94 µg/ml for KLT. The limit of quantification (LOQ) was found to be 0.36 µg/ml for OPH and 2.80 µg/ml for KLT. The study results showed that the proposed RP-HPLC method is simple, sensitive, rapid, precise, accurate and specific, which is useful for the routine simultaneous determination of OPH and KLT in its dosage form.

Keywords: Ketorolac tromethamine; Method development; Olopatadine hydrochloride; RP-HPLC; Simultaneous estimation; Validation

INTRODUCTION

Olopatadine hydrochloride (OPH) is 11-[(Z)-3-(Dimethylamino) propylidene]-6-11-dihydrodibenz [b, e] oxepin-2-acetic acid hydrochloride. The chemical structure of OPH is shown in Fig.1. (United State Pharmacopoeia; Sweetmam sc Martindale, 2009; the Merck index; Indian Pharmacopoeia, 2007; British Pharmacopoeia, 2009; British National Formulary, 2009) It is used as anti-allergic agent, H1 antagonist and antihistamine. It is a structural analogue of doxepin, non-steroidal, non-sedating, topically effective anti-allergic molecule that exerts its effects through multiple distinct mechanisms of actions (Cook EB *et al.*, 2000; Abelson MB *et al.*, 1993; Friedlander MH, 1989)

Ketorolac tromethamine (KLT) is (±)-5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid, compound with 2-amino-2-(hydroxymethyl)-1,3-propanediol (1:1). The KLT chemical structure is shown in Fig.2 (United state Pharmacopoeia; Sweetmam sc Martindale, 2009; The Merck index; Indian Pharmacopoeia, 2007; British Pharmacopoeia, 2009). It is a Non steroidal anti-inflammatory drug (NSAID) chemically related to indomethacin and tolmetin. It is a racemic mixture of [-] S and [+] R enantiomeric forms, with the S-form having analgesic activity. 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 (Wendell H Rooks, 1990; Jung D *et al.,* 1988). There are very few HPLC methods available in literature for OPH alone (Bhatt Parth D *et al.,* 2011; Nageswara Rao K *et al.,* 2012) and KLT alone & in combination with gatifloxacin, moxifloxacin and ofloxacin (Dubey SK *et al.,* 2013; Jayant B Dave *et al.,* 2011; Sunil G *et al.,* 2013; Syed Naeem Razzaq *et al., 2012*; Qandil AM *et al.,* 2008). So far, no method has been reported for estimation of OPH and KLT in combined dosage forms, hence we attempted to develop a simple, accurate, and economical analytical method.

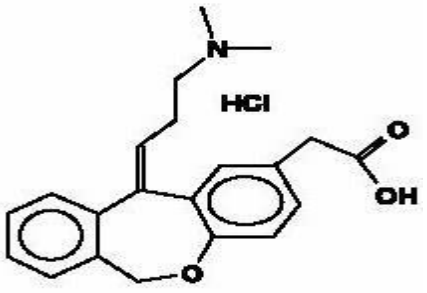

Figure 1: Chemical structure of Olopatadine Hydrochloride

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Table 1: Parameters Optimised Conditions

Parameters	Optimised Conditions
Chromatograph	HPLC Waters
Column	Inertsil ODS C18, 100 x 4.6 mm, 5µm
Mobile Phase	Buffer: ACN (50:50). (Buffer 0.1N sodium dihydrogen ortho phosphate by pH adjusted to 4.6 with ortho phosphoric acid)
Flow rate	1 ml/min
Detection wavelength	260 nm
Injection volume	20µl
Column temperature	30°C

Table 2: Results of Analysis of Formulation

Drug Name	Quantity label claim(mg)	Quantity found ± SD	% Assay ± SD	%RSD
Olopatadine Hydro-chloride	1.0	1.0 ± 0.02	100.94 ± 0.27	0.0020
Ketorolac Tro-methamine	4.0	3.99 ± 0.12	99.99 ± 0.21	0.0027

Table 3: System Suitability Parameters

Parameters	Olopatadine Hydrochloride	Ketorolac Tromethamine
Linearity range	2.5-15µg/ml	10-60µg/ml
Correlation coefficient	0.9999	0.9999
Slope	52720	59393
Retention time	2.723 min	3.848 min
Resolution factor	--	6.63
USP plate count	4657	7042
USP tailing	1.10	1.09
Limit of Detection (LOD)	0.11µg/ml	0.94µg/ml
Limit of Quantitation (LOQ)	0.36 µg/ml	2.8 µg/ml

Table 4: Accuracy (Recovery) Study Data

Parameters	Olopatadine Hydrochloride		Ketorolac Tromethamine	
	% Estimated*	% RSD	% Estimated*	% RSD
50%	99.87%	0.72	99.84%	0.42
100%	100.33%	0.29	99.07%	0.27
150%	99.56%	0.41	99.98%	0.52

*Mean of six determinations (n=6)

This paper describes validated RP-HPLC for simultaneous estimation of OPH and KLT in combination using Inertsil ODS C_{18} Column, 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 was done by using 0.1N sodium dihydrogen ortho phosphate buffer (pH adjusted to 4.6 with ortho phosphoric acid) and acetonitrile (50:50). The run time was set for 6 minutes. The retention time of OPH and KLT were found to be 2.724 min and 3.849 min respectively.

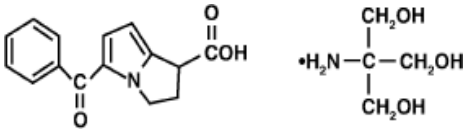

Figure 2: Chemical structure of Ketorolac Tromethamine

MATERIALS AND METHODS

Instrumentation

HPLC was performed on a High performance liquid chromatography equipped with Waters-515 pump and 2489 UV/VIS detector, and Inertsil ODS C18, 100 x 4.6 mm, 5µm column was used. A Rheodyne injector with a 20 µl loop was used for the injection of sample. The data processing was performed using EM-Power software.

Standards and Chemicals

Standard bulk drug samples of OPH and KLT were provided by Ajanta Pharma, Mumbai. The ophthalmic preparation of combined OPH and KLT was procured from the local market (OLOPAT –KT eye drops - 5 mL,

Table 5: Results for Precision Study

Drug	Intra day assay		Inter day assay	
	% Obtained*	% RSD	% Obtained*	% RSD
Olopatadine Hydro-chloride	100.2%	0.10	99.4%	0.36
Ketorolac Tro-methamine	99.8%	0.08	100.6%	0.72

*Mean of six determinations (n=6)

Table 6: Results of Robustness Study

Factor	Level	Retention time		Area ($\mu v^2 sec$)	
		OPH	KLT	OPH	KLT
Standard		2.723	3.850	528811	2378945
Flow rate	0.9	2.914	4.119	561547	2536221
	1.1	2.551	3.607	488583	2209222
pH of Buffer	4.4	2.653	3.745	471413	2134388
	4.8	2.593	3.648	471183	2128999
% of ACN	48	2.815	3.847	524212	2359870
	52	2.720	3.817	525410	2365200

Ajanta Pharma, Mumbai). All other reagents, procured from Merck, were of HPLC grade.

Chromatographic Conditions

The mobile phase used in this study was a mixture of buffer: ACN (50:50). The buffer 0.1N sodium dihydrogen ortho phosphate was adjusted to pH 4.6 with ortho phosphoric acid. The mobile phase was filtered using 0.45 micron membrane filter and ultrasonicated for 15 min. Stationary phase was Inertsil ODS C18, 100 x 4.6 mm, 5µm column with a flow rate of 1.0 mL /min at a wavelength 260 nm and Column temperature is 30°C; injection volume 20µl. The retention time of OPH and KLT were found to be 2.724 min and 3.849 min respectively. The run time was 6 minutes. The chromatographic conditions were given in Table 1.

Standard Stock Solution Preparation

Accurately weigh and transfer 100 mg of OPH working standard and 400 mg of KLT working standard into 100 mL of clean and dry volumetric flask, add 60 mL of diluents and sonicate to dissolve. Then again dilute to required volume with diluents.

Standard preparation

Transfer 1 mL of standard stock solution into 100 mL volumetric flask and dilute to volume with diluents.

Sample Preparation

Transfer sample quantitatively equivalent to 100 mg OPH and 400 mg of KLT into 100 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. The diluted solution (10µg/ml) was analyzed under optimized chromatographic conditions and chromatogram is depicted.

The results of analysis were given in Table 2 and the chromatogram was given in Figure 3.

Validation Methods

Linearity

Linearity was demonstrated by analysing five different concentrations of active compound. Peak areas were recorded for all the peaks and calibration plot was constructed by plotting peak area versus concentrations of OPH and KLT which were found to be linear in the range of 2.5 – 15 µg/ml and 10 - 60 µg/ml respectively given in Table. 8. Coefficient of correlation OPH and KLT were 0.999985 and 0.999933 respectively. The calibration curves were given in Figure 4(a,b).

Accuracy

The accuracy of the method was determined on three concentration levels by recovery experiments. The recovery studies were carried out six times by spiked placebo recovery method and the percentage recoveries with standard deviations [SD] were calculated. From the data obtained in Table 4, the method was found to be sufficiently accurate.

Precision

To demonstrate agreement among results, a series of measurements are done with OPH and KLT. Six replicate injections of the specific standard at various time intervals on the same day and on different days were done. Percentage relative standard deviation (%RSD) was found to be less than 2% for within a day and day to day variations, which proves that the method is precise. The results for precision were given in Table 5.

Limit of Detection and Limit of Quantification:

The limit of detection (LOD) and limit of quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the stan-

Table 7: Ruggedness Data for OPT and KLT

OPT		Area	KLT		Area
S.No	RT (min)	(µv2sec)	S.No.	RT (min)	(µv2sec)
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**	**524385**	Average	**3.846**	**2353324**
SD	**0.002**	**556.168**	**SD**	**0.002**	**5777.67**
%RSD	**0.08**	**0.11**	**%RSD**	**0.06**	**0.25**

Table 8: Linearity of Olopatadine Hydrochloride and Ketorolac Tromethamine

Olopatadine Hydrochloride (OPT)		Ketorolac Tromethamine (KLT)	
Concentration (µg/mL)	Area (µv²sec)	Concentration (µg/mL)	Area (µv²sec)
2.5	131875	10.0	600183
5.0	266830	20.0	1213706
7.5	397849	30.0	1801949
10.0	533804	40.0	2394248
12.5	659693	50.0	2976117
15.0	789260	60.0	3565845

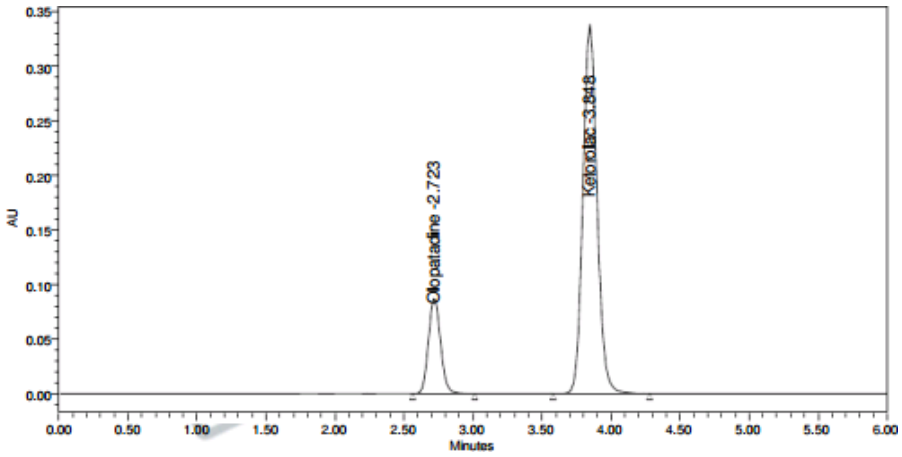

Figure 3: Chromatogram of Olopatadine Hydrochloride and Ketorolac Tromethamine

dard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The LOQ is the smallest concentration of the analyte, which gives response that can be accurately, quantified (signal to noise ratio of 10). The values of LOD and LOQ were given in Table. 2.

Ruggedness and Robustness

Ruggedness of the method was determined by making slight changes in the chromatographic conditions, such as different analysts and instruments, days, change in composition of mobile phase, flow rate and pH of mobile phase. It was observed that there were no marked changes in the chromatograms, which demonstrates that the RP-HPLC method developed is rugged and robust. The robustness limit for flow rate and buffer variation were well within the limit, which shows that the method is having good system suitability and precision under given set of conditions and were within the acceptance criteria of not more than 2%. The results for robustness and ruggedness were given in Table 6 & Table 7.

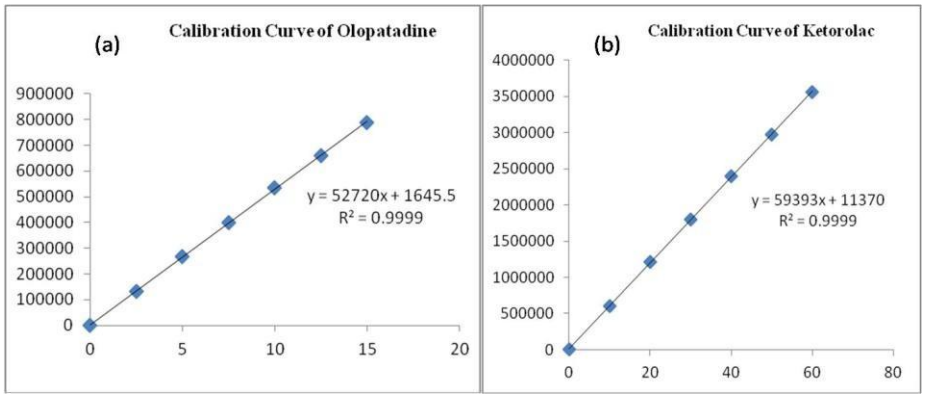

Figure 4: Calibration curve of Olopatadine Hydrochloride and Ketorolac Tromethamine

RESULTS AND DISCUSSION

In order to develop an effective method for the analysis of the drugs in pharmaceutical formulations, preliminary tests were performed in order to select adequate and optimum conditions. Parameters such as detection of wavelength, ideal mobile phase and their proportions, optimum pH and concentration of the standard solution were studied. Several binary or ternary eluents were tested using various proportions of solvents including buffer and acetonitrile with various proportions. The flow rate of 1.0 ml/min for the mobile phase was selected after the preliminary tests. The goal of this study was to develop a rapid HPLC method for the analysis of OPH and KLT in a finished ophthalmic preparation using a commonly employed reverse phase C-18 column with UV detector. The proposed method is simple, rapid and statistically validated for its accuracy. No interfering peaks were found in the chromatograms indicating that the ophthalmic excipients had not interfered in analysis of drugs. The calibration curve showed linearity over a concentration range of 2.5-15.0 µg/ml for OPH (r^2 is 0.999985) and 10-60 µg/ml for KLT (r^2 is 0.999933).

CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for simultaneous determination of OPH and KLT in bulk and ophthalmic preparations. The mobile phase is easy and simple to prepare and economical. The sample recoveries in formulation were in good agreement with their respective label claims. Hence, it can be easily and conveniently adopted for routine analysis of OPH and KLT in eye drops.

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