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RESEARCH ARTICLE

RP-HPLC assay method development and validation of ketorolac and olopatidine in opthalmic liquid dosage forms

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ABSTRACT

A simple reverse phase high performance liquid chromatographic assay method was developed and validated for the simultaneous estimation of Ketorolac and olopatadine in ophthalmic liquid dosage forms. The chromatographic separation was achieved on phenomenex C18 (250 mm × 4.6 mm id, 5 µm particle size) column by using the mobile phase composition of ammonium acetate: acetonitrile: methanol buffer (50:30:20 v/v, pH 5.5), the detection of analyte was done at 212 nm for ketorolac and olopatadine respectively by PDA detector. LOD was found to be 0.78µg/ml and 0.45µg/ml and LOQ was found to be 2.36µg/ml and 1.37µg/ml for ketorolac and olopatadine respectively.

Key words: RP-HPLC, Ketorolac, olopatadine, validation.

INTRODUCTION:

Ketorolac is chemically 5-benzoyl-2, 3-dihydro-1H-pyrrolizine-1-carboxylic acid [1, 2]. It is used as a treatment of postoperative pain and for topical use for allergic conjunctivitis. 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 [3,4].

Olopatidine is chemically 2-[(2Z)-2-[3-(dimethylamino)propylidene]-9-oxatricyclo [{3,8}]pentadeca-1(11),3(8),4,6,12,14-hexaen-5-yl]acetic acid [1, 2]. It is used for anti-histamine; treat to itching and redness in the eyes due to allergic. Olopatidine is a selective histamine H_1 antagonist that binds to the histamine H_1 receptor. Thus blocks the action of endogenous histamine, which subsequently leads to temporary relief of the negative symptoms brought on by histamine. Olopatidine is devoid of effects on alpha-adrenergic, dopamine and muscarinic type 1 and 2 receptors [5,6]. Several assay methods has been developed for the determination of Ketorolac [7,8,] and Olopatadine [9,10] in formulations and biological fluids but there is a need for most economic and accurate method which is very useful for the simultaneous determination in ophthalmic liquid dosage forms.

2. MATERIALS AND METHODS:

2.1. Reagents and chemicals:

Ketorolac and Oloptidine were obtained as gift samples from Siri Pharmaceuticals Pvt. Ltd., Hyderabad. Methanol, ammonium acetate used as a solvent was purchased from Merck (Mumbai, India). All other reagents and solvents used were of analytical and HPLC grade.

2.2. Instruments:

Shimadzu HPLC instrument equipped with a PDA detector, assisted with LC- Solution software, Metler Toledo p^H meter and Model XI 5522050 of Millipore, vaccum filter

2.3. Preparation of buffer solution:

3.85 g of ammonium acetate was taken in a 1000 ml volumetric flask, 1 ml of triethyl amine was added and volume was made up to the mark with water. The pH of this buffer solution was adjusted to 5.5 with glacial acetic acid[3, 4].

2.4. Preparation of mobile phase:

Mobile phase was prepared having composition of ammonium acetate: acetonitrile: methanol buffer (50:30:20 v/v, pH 5.5)[3, 4].

2.5. Preparation of standard solution of Ketorolac & Oloptidine:

32mg of Ketorolac working standard and 40mg of Olopatadine working standard were weighed and transfered into 50ml volumetric flask. 30ml of diluents

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were added and sonicated to dissolve and diluted to volume with diluent. 10ml of standard stock solution were taken into 100ml volumetric flask and diluted to volume with diluents.[10, 12]

The solution was injected once into the present chromatographic system

1. Again the solution was injected once into the same chromatographic system.[12]

2. The peak areas were noted down and %RSD were calculated. To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions.[11, 13, 14]

2.6. Sample Preparation:

Transfer sample quantitatively equivalent to 16mg Ketorolac and 20mg of Olopatadine in to 50ml volumetric flask add 30ml of diluent, sonicate to dissolve for 10 minutes and diluted to volume with diluents. Further the solution was filtered through 0.45µ vacuum filter. Then 10ml of filtrate was further diluted to 100ml with mobile phase.[8]

Table 1: Optimized method conditions

Mode of separation	Isocratic separation
Mobile phase	p^H 5.5 KH_2PO_4:ACN
Column	Athene C18 (250mmX4.6mm,5µ)
Flow rate	1.0 ml/min
Detector wavelength	212 nm
Injection volume	20µl
Oven temperature	Ambient
Run time	6 min

2.7. Validation:

2.7.1. Linearity:

The standard solution of Ketorolac and Olopatadine was diluted with mobile phase to get concentration of 19.2-44.8 µg/ml and 24 – 56 µg/ml respectively. Then this solution was injected in HPLC and peak area was calculated, the calibration graph was plotted as concentration versus peak area[5, 6].

2.7.2. Accuracy:

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out at 80%, 100% and 120% levels by standard addition method. Standard deviation and percentage recovery was calculated[5, 7].

2.7.3. Precision:

Precision of analytical method is the degree of agreement among individual test results when the methods apply repeatedly to multiple sampling of homogenous sample[9]

2.7.4. Acceptance Criteria:

The results obtained indicate that the % RSD was found to be less than 2 % which was in the acceptance limits[5]

2.7.5. Robustness:

Small deliberate changes were made in the method parameters and the peak area of sample solution was calculated.

3. RESULTS AND DISCUSSION:

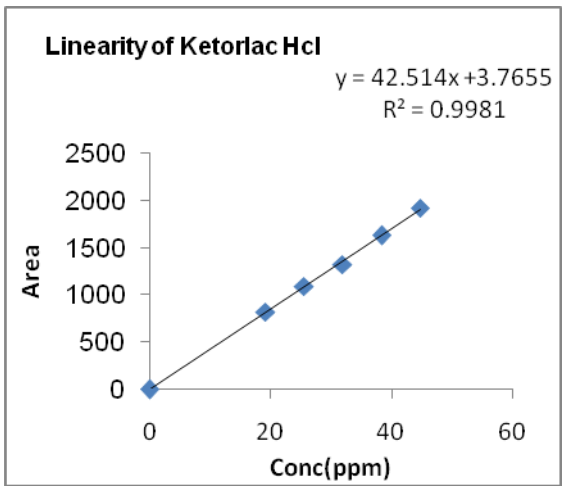
The method developed in the present study was by using phenomenex C18 (250 mm × 4.6 mm id, 5 µm particle size) column by using the mobile phase composition of ammonium acetate: acetonitrile: methanol buffer (50:30:20 v/v, pH 5.5). The detectors response to Ketorolac and Oloptidine was found to be linear in range 19.2-44.8 and 24-56 (Table 2,3). The R^2 values were found to be 0.9981 for Ketorolac and 0.999 for Olopatidine respectively, which indicates good linearity between concentration and peak area, the interday and intraday assay variance indicated the precision and reproducibility of the proposed method (Fig. 1). The mean % recovery was close to 100 which indicate the accuracy of the method. LOD was found to be 0.78µg/ml for Ketorolac and 0.45µg/ml for Oloptidine and LOQ was found to be 2.36µg/ml for Ketorolac and 1.37µg/ml for Oloptidine respectively. The results are presented in the table

Table 2: Validation results for Ketorolac and Olopatidine:

Parameters		Ketorolac	Olopatadine
Specificity	Sample	1410.5	3835.77
	standard	1378.89	3749.69
Linearity		19.2-44.8	24-56
		R^2-0.9981	R^2-0.9999
Accuracy		101.26	101.28
precision	**Average area**	1431.713	3883.960
	SD	23.416	63.620
	%RSD	1.6355	1.6380
Robustness			
Flow rate		0.8	0.8
		1.2	1.2
Retention time		3.080	4.987
		1.877	3.057
Wave length		210	210
		214	214
Asymmetry		1.409	1.405
		1.419	1.418
Assay		99.28	99.654

Table 3: Precision

S.no	Ketorolac Area	Olopatadine Area
1	1467.678	3966.918
2	1447.199	3954.345
3	1430.934	3865.243
4	1427.653	3841.342
5	1401.169	3806.319
6	1415.644	3869.59
Average area	1431.713	3883.960
SD	23.416	63.620
%RSD	1.6355	1.6380



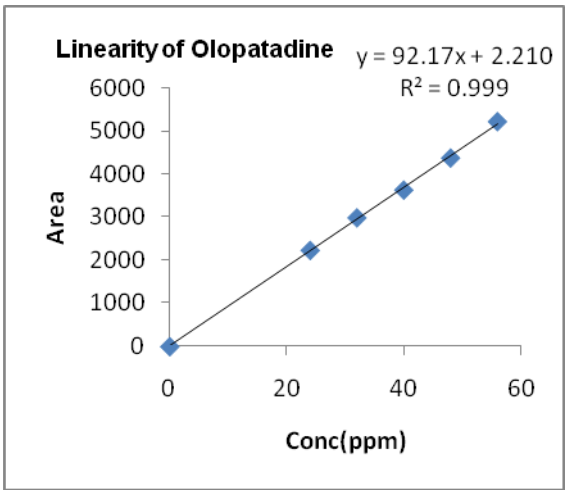


Figure 1: Calibration of drugs

4. CONCLUSION:

A suitable chromatographic method was developed through optimization by changing various parameters such as the mobile phase, injection volume, flow rate etc.In the present method Athene C_{18}, 250 X 4.6 mm, 5µ, column has been used for Ketorolac& Olopatadine drugs respectively. Potassium dihydrogen ortho phosphate (p^H 5.5): Acetonitrile mobile phase were used for the separation of Ketorolac& Olopatadine drugs, retention time was found to be more influenced by the mobile phase. The separation of the two peaks was also effected by buffer and the percentage of mobile phases. Ketorolac and Olopatadine were eluted at acceptable retention times and good resolution was obtained. This method was validated as per ICH-Q2 (R1) guidelines and met the regulatory requirements for selectivity, accuracy and stability. Considering the obtained data, it was possible to affirm that the proposed method was fast, simple and suitable for the accurate determination of drug Ketorolac & Olopatadine in ophthalmic liquid dosage forms.

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