International Journal of Allied Medical Sciences and Clinical Research (IJAMSCR)

ISSN:2347-6567

IJAMSCR |Volume 11 | Issue 2 | Apr - Jun - 2023
www.ijamscr.com

Research article ***Medical research***

A stability indicating rp-hplc method developement for the simultaneous determination of ketorolac and olopatadine in bulk and in its pharmaceutical dosage forms

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Published on: 21.04.2023

ABSTRACT

A simple, rapid and precise reverse phase liquid chromatographic (RP-HPLC) method was developed and validated for the estimation of Ketorolac and Olopatadine. In this method an Symmetry C18, 250×4.6mm, (innertsil ODS). 5µ or equivalent in an isocratic mode utilizing Phosphate Buffer (4.6 pH): Acetonitrile (50:50%v/v) at a flow rate of 1.0 ml/ min and effluent was monitored at 219 nm. Olopatadine hydrochloride& Ketorolac tromethamine was 2.724, 3.849 minutes. The linearity range was found to be 25 to 150µl. The validated method was very linear and precise.

Keywords: ketorolac, olopatadine

INTRODUCTION

General Information And Importance Of Drug Analysis And Quality Control In Pharma Industry

A drug may be defined as a substance meant for diagnosis, cure, mitigation, prevention or treatment of diseases in human beings or animals or for alternating any structure orfunction of the body of human being or animals. Pharmaceutical chemistry is a science that makes use of general laws of chemistry to study drugs i.e. their preparation, chemical natures, composition, structure, influence on an organism and studies the physical and chemical properties of drugs, the methods of quality control and the conditions of their storage etc. the family of drugs may be broadly classified as.

1. Pharmacodynamic agents.
2. Chemotherapeutic agents.

It is necessary to find the content of each drug either in pure or single, combined dosage forms for purity testing. It is also essential to know the concentration of the drug and it's metabolites in biological fluids after taking the dosage form for treatment. The scope of developing and validating analytical methods is to ensure a suitable method for a particular analyte more specific, accurate and precise. The main objective for that is to improve the conditions and parameters, which should be followed in the development and validation.

Quality control is integral to all modern industrial process and the pharmaceutical industry is no exception. Testing a pharmaceutical product involves chemical, physical and sometimes microbiological analyses. Quality Control (QC) is an important task in the pharmaceutical industry. It not only protects the manufacturer against compensation claims, but also guarantees the patient a safe and effective product. QC measurements include stability testing of the drug formulation, dissolution testing1 and analysis of raw materials and synthesis products.

METHOD DEVELOPMENT AND OPTIMIZATION

Trail method 1: Initial conditions were selected from Ketorolac tromethamine & Olopatadine hydrochloride.
Preparation of mobile phase: Filtered and degassed mixture of Water and Acetonitrile in the ratio of 50:50
Buffer: Degassed mixture of HPLC grade Water.
Chromatographic conditions:
Column : C_{18}, 150×4.6mm, 5µ (thermo hypersil ODS) S Column
temperature: 25^0c
Detector : UV-Vis
Wave length : 260

Preparation of standard Stock solution

Weigh accurately about 12.5mg of Olopatadine hydrochloride and 12.5mg of Ketorolac tromethamine working standards transfer to the 25ml 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 1ml of stock solution into the 25ml of clean and dry volumetric flask andmake up the volume with diluents. Inject 20µl of Standard solution into the chromatograph. Record the chromatogram and measure the response for Ketorolac tromethamine and Olopatadine hydrochloride. Calculate the content of ketorolac tromethamine and Olopatadine hydrochloride in the solutions. The peak was not well separated so changes required for the mobile phase composition. Therefore this method was not suitable.

S.No	Peak Name	RT	Area	Height	%Area	Resolution	SymmetryFactor	USP PlateCount
1	Olopatadine	0.897	320979	45593	37.61		1.23	398
2	Ketorolac	1.647	532411	32952	62.39	2.64	1.71	267

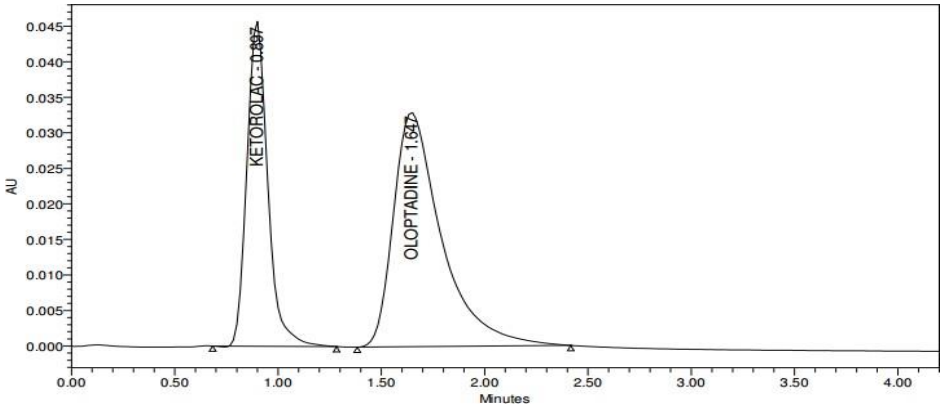

Fig 1: Typical chromatogram of the sample

OPTIMIZED CHROMATOGRAPHIC CONDITION

Mode of separation : Reverse phase isocratic
Wave length : 260
Column : innertsil ODS C_{18}(4.6×250mm) 5µ
System : HPLC
Make : Waters (alliance)
Model : waters 2695
Flow rate : 1ml/min
Temperature : 30^0 C
Injection volume: 20µl
Mobile phase : Sodium di hydrogen ortho phosphate: ACN (50:50)
Buffer used : 0.1N Sodium di hydrogen ortho phosphate with p^H 4.6
Run time : 6 mins.

METHOD DEVELOPMENT

SAMPLE : OLOPAT-KT eye drop 5ml, Ajanta Pharma Limited
LABEL CLAIM : KETORORLAC TROMETHAMINE 4mg
OLOPATADINE HYDROCHLORIDE 1mg

Method description: Preparation of mobile phase: Filtered and degassed mixture of buffer and Acetonitrile in the ratio of 50:50
Buffer: Dissolve 13.6 of 0.1N Sodium di hydrogen ortho phosphate in 1000ml water. The pH is 4.6.
Chromatographic conditions:
Column : C_{18}, 250×4.6mm, (innertsil ODS). 5µColumn temperature : 40^0c
Detector : UV-Vis detector
Wave length : 260nm
Injection volume : 20µl
Flow rate : 1ml/min
Standard stock solution preparation: Accurately Weigh and transfer the 100 mg of Olopatadine Working standard and 400 mg of Ketorolac Working standard into 100 mL of clean and dry volumetric flask, add 60 mL of diluents and sonicate to dissolve and dilute to 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 Olopatadine and 400mg 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 1ml of filtrate to 100 ml with mobile phase.

Procedure: Inject 20 µL of blank solution, placebo solution, Standard solution, Disregard peaks due to blankand placebo if any.

System suitability

The %RSD of standard area of concentration of Olopatadine should be NMT 2.0% The %RSD of standard area of concentration of Ketorolac should be NMT 2.0% Theoretical plates should be NLT 2500.

Specification limit

98% to 103% of the lable claim

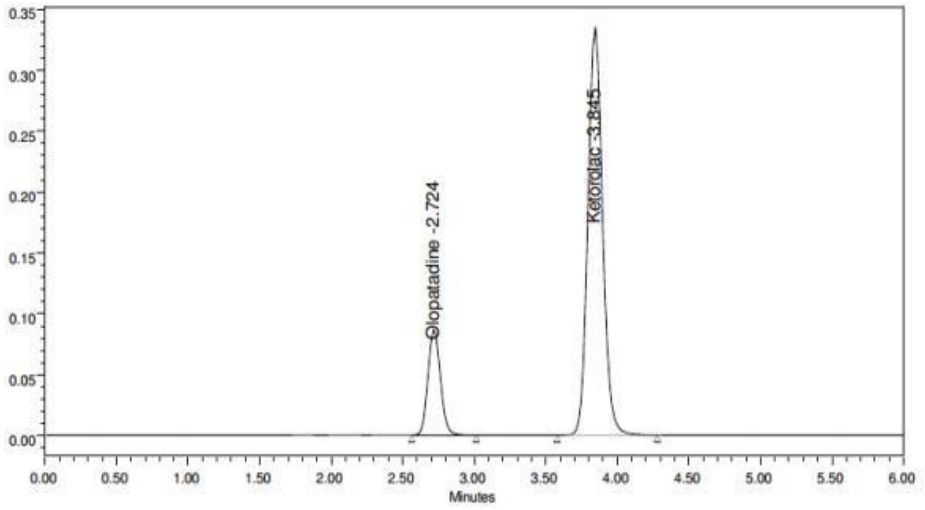

Fig 2: Typical chromatogram of the sample

S.No	Peak Name	RT	Area	Height	%Area	Resolution	Symmetry Factor	USP Plate Count
1	Olopatadine	2.724	536436	86387	18.18		1.12	4542
2	Ketorolac	3.849	2365365	333002	81.82	6.54	1.10	6936

The peak is well separated and gives satisfactory resolution. Therefore this method was suitablefor Analysis

ASSAY

Preparation of standard solution

Transfer 1 ml of standard stock solution in to 100 mL volumetric flask and make up to volume withdiluent to obtain the 50% concentration of solution.

Preparation sample solution

Transfer Sample quantitatively equivalent to 1ml Olopatadine hydrochloride and Ketorolac tromethamine eye drop in to10 mL volumetric flask add 6 mL of diluents, shake to dissolve for few minutes and dilute to volume with diluent. Further filter the solution through 0.45µ filter paper. Dilute 1 ml of filtrate to 10 ml with mobile phase.

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{\text{Avg. Wt}}{\text{Label Claim}} \times 100$$

Where:

AT = average area counts of sample preparation.As= average area counts of standard preparation.WS = Weight of working standard taken in mg.

P = Percentage purity of working standard.

LC = Label claim of ciprolet-A mg/ml.
DT= Dilution of sample preparatio
DS= Dilution of standard preparation.

Calculation of Olopatadine

$$\frac{531480}{1} \times \frac{100}{1} \times \frac{1}{10} \times \frac{10}{100} \times \frac{100}{1} \times \frac{99.4}{100} \times \frac{1}{1} \times 100 \times \frac{526541}{100} \times \frac{100}{100}$$

Calculation of Ketorolac

$$\frac{2364400}{100} \times \frac{400}{100} \times \frac{1}{1} \times \frac{10}{10} \times \frac{100}{100} \times \frac{99.5}{100} \times \frac{1}{4} \times 100 \times \frac{2364656}{100}$$

Result: =100.94%
=99.99%

S.NO	DRUG	%PURITY
1	Olopatadine	100.94%
2	Ketorolac	99.99%

Blank

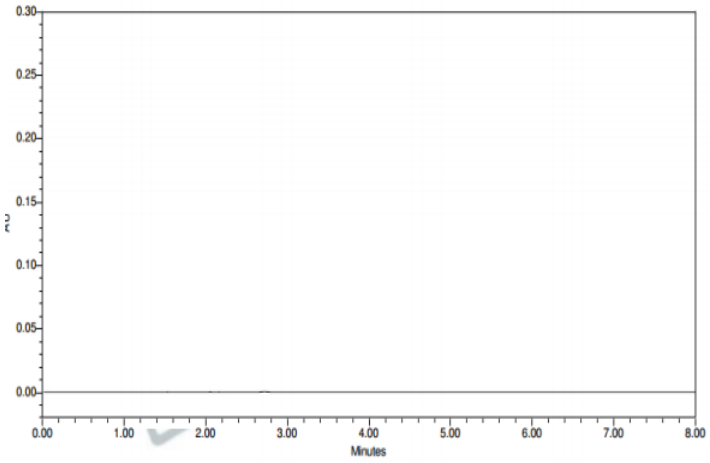

Fig 3: Typical chromatogram of the blank

Placebo

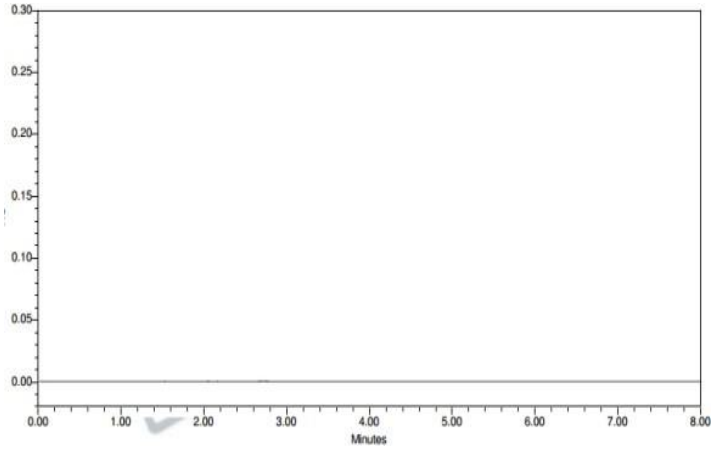

Fig 4: Typical chromatogram of the Placebo

Standard 1

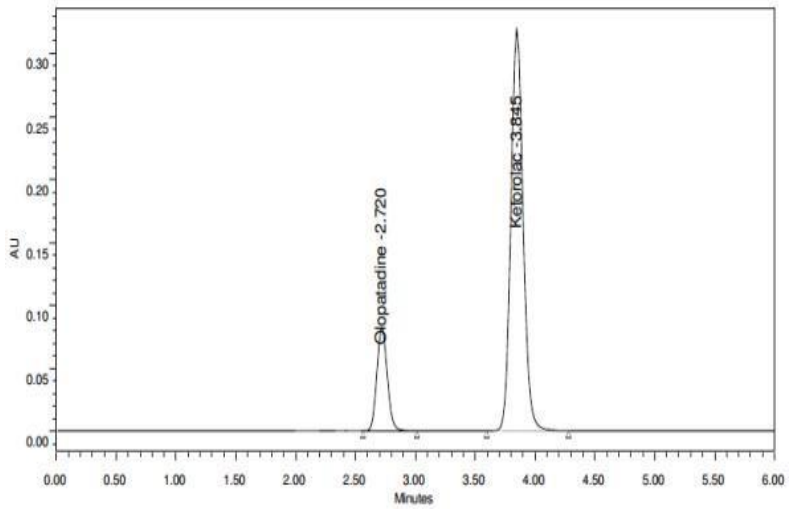

Fig 5: Typical chromatogram of the Standard 1 Injection.

Standard 2

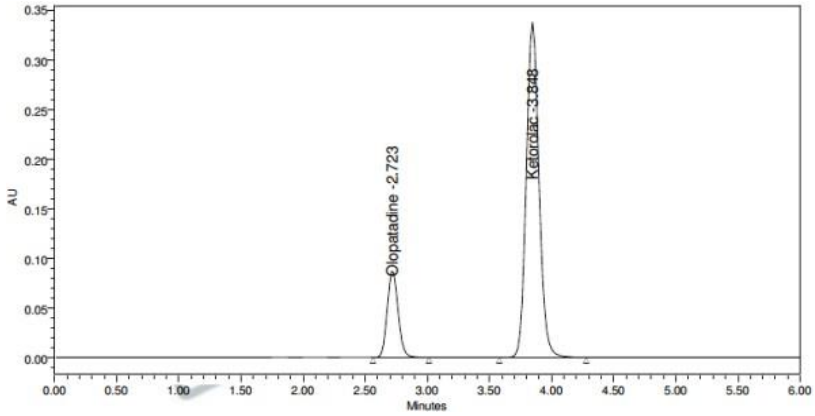

Fig 6: Typical chromatogram of the Standard 2 Injection

S.No	Peak Name	RT	Area	Height	%Area	Resolution	Symmetry Factor	USP Plate Count
1	Olopatadine	2.723	526650	87325	18.16		1.12	4355
2	Ketorolac	3.848	2364763	336754	81.84	6.14	1.12	6781

Table 8: Average calculation of Standard

	Name	RT	Area
Olopatadine	Standard-1	2.720	526431
	Standard-2	2.723	526650
Average		2.722	526541
Ketorolac	Standard-1	3.845	2364548
	Standard-2	3.848	2364763
Average		3.847	2364656

Sample 1:

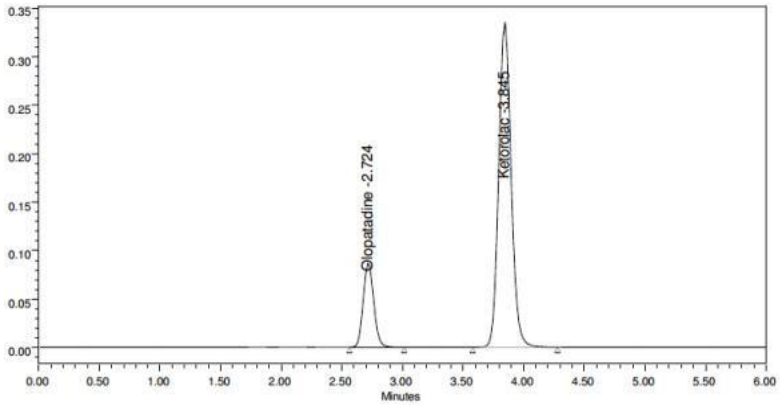

Fig 7: Typical chromatogram of the Sample 1 Injection

S.No	Peak Name	RT	Area	Height	%Area	Resolution	Symmetry Factor	USP Plate Count
1	Olopatadine	2.724	536436	86387	18.18		1.12	4542
2	Ketorolac	3.849	2365365	333002	81.82	6.54	1.10	6936

Sample 2:

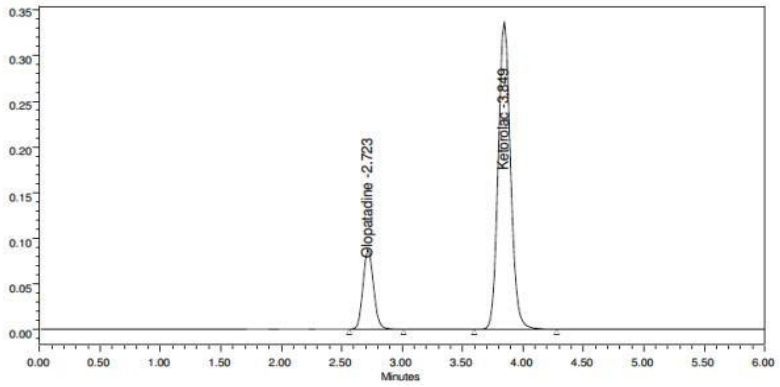

Fig 8: Typical chromatogram of the Sample 2 Injection

S.No	Peak Name	RT	Area	Height	%Area	Resolution	Symmetry Factor	USP Plate Count
1	Olopatadine	2.725	526523	87295	18.16		1.12	4666
2	Ketorolac	3.849	2364434	335412	81.84	6.61	1.08	6926

S.No	Peak Name	RT	Area	Height	%Area	Resolution	Symmetry Factor	USP Plate Count
1	Olopatadine	2.720	526431	82154	18.02		1.13	4562
2	Ketorolac	3.845	2364548	323652	81.98	6.58	1.10	6974

Table 9: Average calculation of Sample

	Name	RT	Area
Olopatadine	Sample-1	2.724	536436
	Sample-2	2.725	526523
Average		2.7245	531480

Ketorolac	Sample-1	3.849	2365365
	Sample-2	3.849	2364434
Average		3.849	2364900

SUMMARY AND CONCLUSION

Quality assurance and control of pharmaceuticals chemicals and formulations is essential for ensuring the availability of safe and effective drug formulation of consumers. Hence, pharmaceutical analysis occupies a pivotal role to statutory certification of drugs and their formulation either by the industry or by the regulatory authorities. The complexity of problems encountered in pharmaceuticals analysis coupling with the importance of achieving the selectivity ,speed, cost, simplicity, sensitivity ,precision and accuracy results in new methods of analysis being quickly adopted by pharmaceutical industry and chemical laboratories depending upon the facility available. Among the several instrumental techniques [HPLC, GC, fluorimetry, NMR, mass spectroscopy, spectrometry covering IR, UV and visible (colorimetry) regions] available for the assay of the drugs, usually UV spectrophotometric technique is simple and less expensive. HPLCis a versatile tool for the qualitative and quantitative analysis of drugs and pharmaceuticals, chemicals and biological materials and has become indispensable in pharmacokinetics studies. The development of highly efficient micro particulate bonded phase has increased the versatility of the technique and has greatly improved the analysis of multi-component mixtures. The systems used are often described as belonging to one or more among four mechanistic type, adsorption, partition, ion exchange and size exclusion. Adsorption and partition systems can be normal phase (stationary phase more polar than eluent) or reversed phase (stationary phase less polar than eluent).

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