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Estimation of olopatadine hydrochloride by RP–HPLC and U.V spectrophotometry method in pure and pharmaceutical formulation

Bhanu Prakash Nayak.M* B.Thangabalan
Sims College of Pharmacy, Mangaldas Nagar, Guntur, A.P-522001
***Corresponding author: Bhanu Prakash Nayak.M**
E-mail id: bprakashpharma@gmail.com

ABSTRACT

A simple and sensitive Reversed Phase High Performance Liquid Chromatographic method and UV Spectrophotometry has been developed and validated for the of the Olopatadine Hcl in pure and pharmaceutical dosage form. In RP-HPLC Method the separation was carried out using mobile phase consisting of Methanol and Acetonitrile in the ratio of 60:40 (v/v). The column used was inertsil ODS 3V C_{18}, (250 mm x 4.6 mm i.d., 5µm) with flow rate 1.2ml/min using UV detection at 254 nm. The method was linear over a concentration range of 10 – 250µg/. The retention time of 2.857min. Results of analysis were validated statistically and by recovery studies. The mean recovery was 98.2 to101.5. The limit of detection (LOD) and the limit of quantification (LOQ) were found to be 0.024 and 0.075µg/ml. The %RSD for the method precision was found to be less than 2%. To develop simple and economical UV spectrophotometric method for the estimation of Olopatadine in pharmaceutical dosage form available in the market for conjunctivitis.The method was validated as per the ICH guidelines and the results were statistically validated. Linearity was observed in concentration range of 10-60µg/ml for Olopatadine. The accuracy of the method was evaluated by recovery studies and good recovery results were obtained between 98% to 100% and the relative standard deviation was found to be below 2% . A simple, accurate, sensitive and economical UV-spectrophotometric method for the estimation of Olopatadine pharmaceutical dosage form has been developed which can be employed in the industry for the routine analysis.

Keywords: Olopatadine (OLO), RP-HPLC, UV spectrophotometry

INTRODUCTION

Olopatadine is chemically {(11*Z*)-11-[3-(dimethyl amino)propylidene]-6,11-dihydro dibenzo [*b*,*e*] oxepin-2-yl} acetic acid Olopatadine hydrochloride is an antihistamine (as well as anticholinergic and mast cell stabilizer), sold as a prescription eye drop (0.2% solution, Pataday (or Patanol S in some countries), manufactured by Alcon). It is used to treat itching associated with allergic conjunctivitis (eye allergies). Olopatadine hydrochloride 0.1% is sold as Patanol (or Opatanol in some countries). A decongestant nasal spray formulation is sold as Patanase, which was approved by the FDA on April 15, 2008

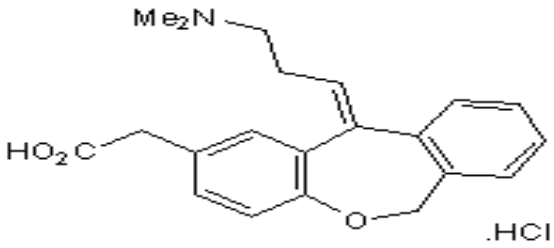


Figure 1: Chemical structure of olopatadine

Several HPLC[5,6,7], GC[8,9] and LC/MS-MS[10-14] methods have been reported for the analysis of olopatadine in plasma that suffer from either undesirably long chromatographic run times and requirement for gradient analysis or use of an internal standard.

The objective of this study was to develop reverse phase high performance liquid chromatography method for the estimation of olopatadine in pure and pharmaceutical dosage form without any derivatization and having short retention time. This method was found to be linear, precise, accurate, sensitive, specific, and robust, and therefore suitable for routine analysis.

MATERIALS AND METHOD

HPLC Instrumentation and Chromatographic conditions

The analytical separations were carried out on a waters 2487 HPLC system equipped with Photo Diode Array detector. The output of signal was monitored and integrated using LC – solutions 2000 software. The analytical column was inertsel ods C_{18} (250 × 4.6mm, 5µ). Mobile phase consisted Acetonitrile and methanol in the ratio of 40:60. Mobile phase was mixed, filtered through 0.45µmembrane filter and degassed under ultrasonication. The mobile phase was used as diluent. The flow rate was 1.0 ml/min and runtime was 5 minute. The column was maintained at ambient temperature. UV detection was measured at 254 nm and the volume of sample injected was 10 µl.

Preparation of standard stock solution

For both uv & rp-hplc method:50mg of olopatadine was weighed accurately and dissolved in 50ml of mobile phase to get the concentration of 1000 µg/ml. Resultant solution was filtered through Whatman filter paper. The standard chromatogram for olopatadine (100µg/ml) was shown in figure 2.

Preparation of working standard solution

For RP-HPLC method

Working standard solutions of olopatadine were prepared by accurately transferring the (0.1, 0.5, 1.0,1.5,2.0 and 2.5 ml) aliquots of the standard stock solution into a series of six 10 ml volumetric flasks. The volume was made up to mark with mobile phase to obtain concentration range of 10 – 250 µg/ml.

For UV method

Working standard solutions of olopatadine were prepared by accurately transferring the (0.1, 0.2 ,.0,3 ,0.4 ,0.5and 0.6ml) aliquots of the standard stock solution into a series of six 10 ml volumetric flasks. The volume was made up to mark with mobile phase to obtain concentration range of 10 –60µg/ml

Preparation of sample solutions

For both uv & rp-hplc method

25mg of tablet powder was weighed accurately and was taken into 25mL volumetric flask and then the sample was diluted to 25ml with mobile phase to get concentration of 1000µg/ml and used for analysis.

RESULTS AND DISCUSSION

HPLC method development and optimization

To optimize the chromatographic conditions, different columns, mobile phases, flow rates etc., were tested. Acetonitrile and water in the ratio of 40:60 was preferred as mobile phase because it resulted in a greater response to olopatadine after several preliminary investigatory runs compared with the different mobile phase combinations. The effect of the

flow rate was studied in the range 0.9 to 1.5ml/min and 1.2ml/min was preferred to be effective. Under these conditions, the analyte peak obtained was well-defined and free from tailing. The retention time (RT) was found to be 2.8min. The optimized chromatographic parameters were listed in table 1

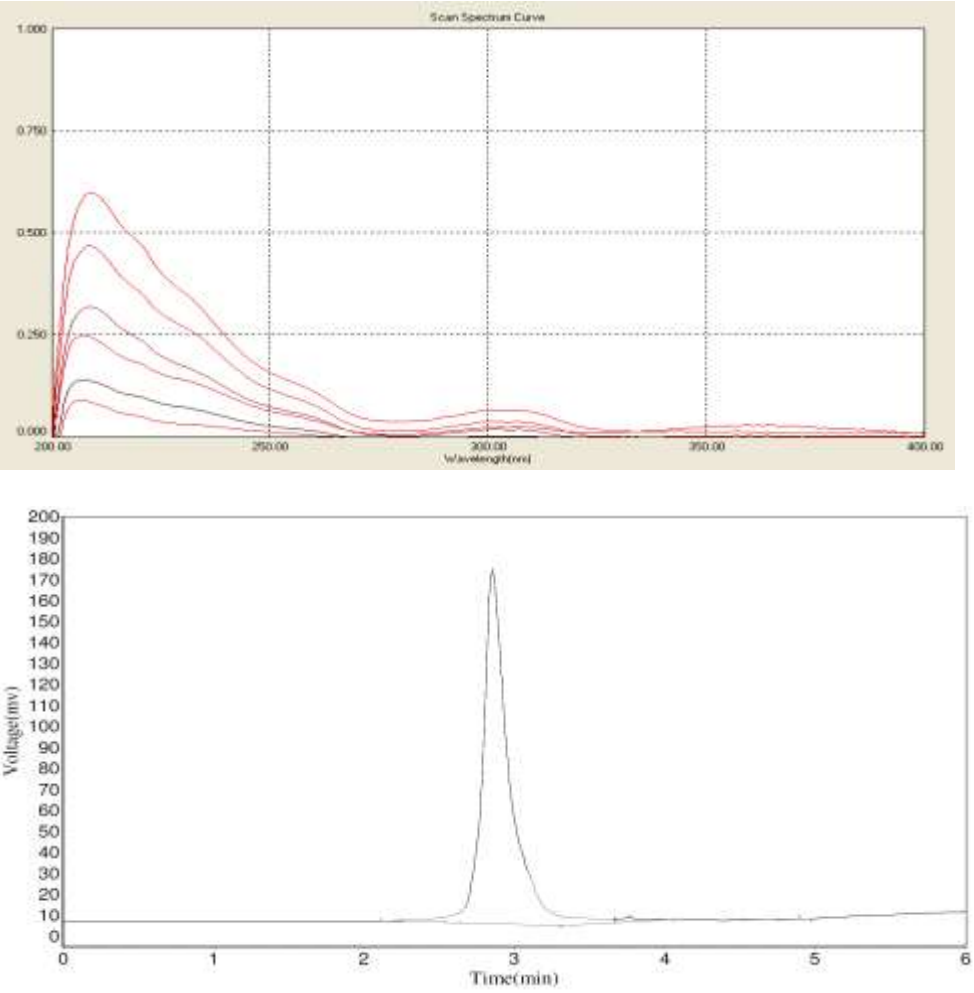


Table 1: Optimized chromatographic parameters

Optimized Chromatographic parameters	
Elution	gradient
Mobile phase	acetonitrile :methanol (40:60)
Column	inertsil c18 column
Flow rate	1.2ml/min
Detection	254nm
Injection volume	10µl
Temperature	Ambient
Retention time	2.87min
Run time	5.0 min
Concentration	10 - 250µg/ml

Validation of the method

When method development and optimization are complete, it is necessary to accomplish method validation. The validation studies include linear range (correlation coefficient), method precision (RSD, %), method accuracy (% recovery and RSD, %), sensitivity studies (LOD & LOQ), and robustness.

System suitability studies

System-suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (RT), tailing factor (T), and peak asymmetry (AS), resolution (RS) were evaluated. The system suitability test was performed using five replicate injections of standards before analysis of samples. The system suitability method acceptance criteria set in each validation run were: capacity factor > 2.0, tailing factor ≤ 2.0 and theoretical plates > 2000. In all cases, the relative standard deviation (R.S.D) for the analytic peak area for two consecutive injections was < 2.0%. System suitability parameters were shown in table 2.

Table 2: System suitability parameters

Parameters	Values
Retention time	1.862min

Linearity

The linearity of the method was evaluated by preparing six series of standard solutions of olopatadine in the range of 10 – 250 µg/ml in mobile phase and injecting the solutions into the HPLC system. Excellent correlation between olopatadine peak area and concentration was observed with R^2 = 0.998 (Figure.3). The regression equation was found to be Y =5844 x +75913. Statistical data are presented in table 3 and the calibration curve was shown in figure 3.

Table 3: Linearity results for olopatadine

s.no	concentration	Area
1	10	417079
2	50	805358
3	100	13898789
4	150	1974988
5	200	2484887
6	250	3092886

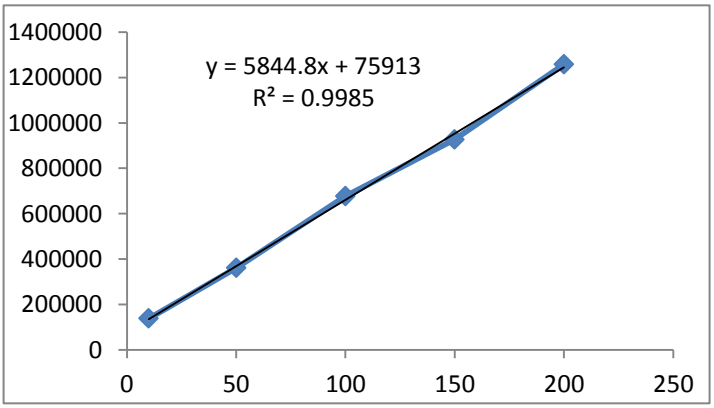
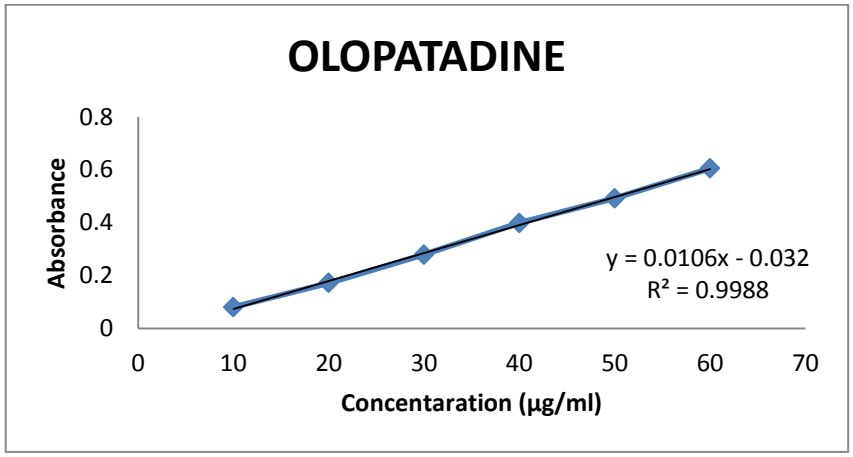


Figure 3: Calibration curve of olopatadine by RP –HPLC method

s.no	concentration	Absorbance
1	10	0.081
2	20	0.172
3	30	0.279
4	40	0.399
5	50	0.492
6	60	0.605



Calibration curve for olopatadine by uv method

Precision

System precision: (Repeatability)

To study precision, five replicate standard solutions of olopatadine (100µg/ml) were prepared and analyzed using the proposed method. The percent relative standard deviation (% RSD) for peak responses was calculated. Results of system precision studies were shown in table 4.

Table 4: Results of system precision for olopatadine

s.no	Retention time(min)	Area(Mv. sec)
1	2.89	1389879
2	2.89	1390870
3	2.94	1381709
4	2.81	1371808
5	2.82	1387968
6	2.857	1375058
mean	2.89	1382882
Standard deviation	0.047545968	8048.07964

% RSD 1.645189498 0.581978769

Method precision: (Reproducibility)

The intraday and inter-day precision of the proposed method was determined by analyzing the corresponding responses 6 times on the same day and on different days for concentration of sample solutions of 100µg/ml. The result was reported in terms of relative standard deviation (% RSD). Results of method precision studies were shown in table 5.

Table 5: Results of Method precision for olopatadine by using rp-hplc method

s.no	Peak area	%labelled claim
1	1375623	100.5276
2	1367299	101.139
3	1381142	100.125
4	1358812	101.771
5	1385642	99.8008
6	1399221	98.8322

25µg/ml	test abs	std abs	wt to be taken	wt taken	avg wt	label claim	amt found	% labele caliam
pre1	0.221	0.222	0.145	0.145	0.145	5	4.977	99.54954955
pre 2	0.218	0.222	0.145	0.145	0.145	5	4.910	98.1981982
pre3	0.215	0.222	0.145	0.145	0.145	5	4.842	96.84684685
pre4	0.219	0.222	0.145	0.145	0.145	5	4.932	98.64864865
pre5	0.212	0.222	0.145	0.145	0.145	5	4.775	95.4954955
pre 6	0.217	0.222	0.145	0.145	0.145	5	4.887	97.74774775

Table 5: Results of Method precision for olopatadine by using uvmethod

Intermediate precision

The intermediate precision of the proposed method was determined by performing the method by two analysts (Analyst 1 and Analyst 2) for concentration of sample solutions 100µg/ml. The percent relative standard deviation (% RSD) for peak responses was calculated. The results for intermediate precision were shown in table 6.

Table 6: Results of Intermediate precision for olopatadine by using rp –hplc method

	ANALYST 1		ANALYST 2	
S.NO	RT (MIN)	AREA (MV.SEC)	RT (MIN)	AREA(MV.SEC)
1	2.893	1389879	2.884	1385965
2	2.901	1397256	2.875	1377715
3	2.871	1372689	2.912	1366121
4	2.869	1377661	2.819	1345688
5	2.877	1388821	2.902	1366127
6	2.903	1401127	2.833	1399910
MEAN	2.885	1387905.5	2.8705	1373587.667
SD	0.014463748	10986.0312	0.037022966	17092.40841
%RSD	0.50134307	0.791554699	1.128774111	1.244362397

	analyst 1	analyst 2
day1	**0.229**	**0.221**
day2	**0.225**	**0.224**

Table 6: Results of Intermediate precision for olopatadine by using uv method

Acuracy

Accuracy of the method was confirmed by the standard addition method, which was carried out by performing recovery studies at 3 different concentrations 100%, 150% and 200% of these expected, in accordance with ICH guidelines, by replicate analysis (n=3). Known amount of standard drug solution (100µg/ml) was added to a pre analyzed sample solution (100, 150, 200 µg/ml) and percentage drug content was measured. The closeness of obtained value to the true value indicates that the proposed method is accurate. Recovery studies were shown in table 7.

$$\%Recovery = [(Ct - Cpa)/ Cs] \times 100.$$

Where, Ct = Total concentration of analyte

Cpa = Concentration of pre-analysed sample

Cs = Concentration of standard added to pre-analysed sample.

Table 7: Results of recovery studies for olopatadine by using rp –hplc method

s.no	level	std	Amount added	Total recovery	recovered	%recovery
1	50	100	50	150	50	100
2	50	100	50	150.057	50.057	100.114
3	50	100	50	150.78	50.78	101.56
4	100	150	100	200.421	100.421	100.421
5	100	150	100	200.602	100.036	100.326
6	100	150	100	247.117	147.117	98.078
7	100	150	150	247.271	147.271	98.18066
8	100	150	150	247.067	147.067	98.044

s.no	STND(µg/ml)	test (µg/ml)	absorbence at 217nm
1	**25**	**20**	**0.444**
2	**25**	**25**	**0.485**
3	**25**	**30**	**0.512**

Table 7: Results of recovery studies for olopatadine by using uvmethod

Robustness

The robustness study was performed to evaluate the influence of small but deliberate variation in the chromatographic condition. The robustness was checked by changing parameters like flow rate of mobile phase and detection wavelength

- Change in the detection wavelength by ± 2nm (294nm and 290nm)
- Change in flow rate by ± 0.1 ml/minute (1.6 ml/min and 1.4 ml/minute)

After each change, sample solution was injected and % assay with system suitability parameters were checked. Robustness values were given in table 8

Table 8: Results of Robustness for olopatadine by using rp – hplc method

parameter	Rt(min)	Area(mvsec)
Flow rate(ml/min)1.3	2.7	1485965
1.1	3.2	1300145
Wavelength(nm)252	2.78	1328815
256	2.76	1215141

Table 8: Results of Robustness for olopatadine by using uvmethod

WAVELENGTH	ABSORBENCE					
215	0.217	0.219	0.217	0.218	0.22	0.219
219	0.215	0.216	0.218	0.219	0.217	0.218

Limit of Detection and Quantitation

Detection and Quantitation limit were calculated by the method based on the standard deviation (σ) and slope of the calibration plot, using the formula

Limit of Detection = σ × 3.3/S

Limit of Quantitation = σ × 10/S

Where σ = the standard deviation of the response.

S = the slope of the calibration curve (of the analyte).

Results of LOD & LOQ were shown in table 9.

Table 9: Results of LOD, LOQ for olopatadine

S.No	LOD	LOQ
1	0.024982	0.075702

By using uv **2.** 0.518571 1.571429

Specificity

Specificity of an analytical method is its ability to measure the analyte accurately and specifically in the presence of component that may be expected to be present in the sample matrix. Chromatograms of standard and sample solutions were compared in order to provide an indication of specificity of the method.

Assay of pharmaceutical formulation

The proposed validated method was successfully applied to determine olopatadine in their pharmaceutical dosage form And the % Assay results were shown in table 10.

Table 10: Results of % assay by using RP – HPLC method

S.No	Amount Found	%Assay
1	5.0314	100.628
2	5.03590	100.718
3	5.0357	100.715

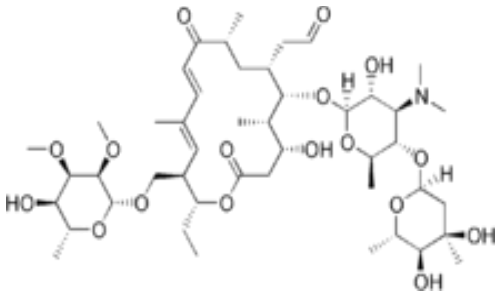
DRUG PROFILE

TYLOSIN TARTRATE

Drug substance: tylosin tartrate

Category: anti bacterial agent.

Chemical structure



IUPAC name

[(2R,3R,4E,6E,9R,11R,12S,13S,14R) -12- {[3,6-dideoxy-4-O-(2,6-dideoxy-3-C-methyl-α-L-ribo-hexopyranosyl) -3-(dimethylamino)-β-D-glucopyranosyl]oxy}-2-ethyl-14-hydroxy-5, 9,13-trimethyl-8, 16-dioxo-11- (2-oxoethyl) oxacyclohexa deca-4,6-dien-3-yl] methyl 6-deoxy-2,3-di-O-methyl-β-D-allopyranoside.

Molecular Formula: $C_{46}H_{77}NO_{17}$

Molecular Mass: 916.10g/mol

Synonyms: TYLOSIN TARTRATE SALT;TYLOSINE TARTRATE;pharmasin; Tylosin, [R-(R*,R*)] -2,3-dihydroxybutanedioate (salt);Tylosin, (2R,3R)-2,3-dihydroxybutanedioate (salt);TYLOSINTARTRATE,BP;

PHYSICAL PROPERTIES

Colour: clear, yellow-green

State / form: powder form

Solubility: freely soluble in water, dichloro methane.slightly soluble in alchol.

Boiling Point(℃): 1116.3°C at 760 mmHg

pka:7.3

MECHANISM OF ACTION

Tylosin tartate targets primarily gram positive bacteria and species of the *Mycoplasma* genus.categorised under macrolide antibiotics.Macrolide antibiotics inhibit bacterial growth by targeting the 50S ribosomal subunit preventing peptide bond formation and translocation during protein synthesis. Resistance to tylosin tartate is commonly attributed to mutations in 50S rRNA preventing tylosin tartate binding allowing the cell to synthesize proteins free of error. Tylosin is a macrolide, bacteriostatic antibiotic. It is similar in structure, mechanism of action, and spectrum as that of erythromycin. Tylosin is a macrolide antibiotic related structurally to erythromycin, tylosin is produced from Streptomyces fradiae. It is made naturally by the bacterium “Streptomyces fradiae” and acts to inhibit bacterial protein synthesis by inhibiting the 50S ribosome, a cellular structure only certain bacteria have and use to make internal proteins. It occurs as an almost white to buff-colored powder with a pKa of 7.1. It is slightly soluble in water and soluble in alcohol. Tylosin is considered to be highly lipid soluble. The tartrate salt is soluble in water. The injectable form of the drug (as the base) is in a 50% propylene glycol solution.

Tylosin is an antibiotic veterinary drug for the treatment of disease in food producing animals, including cattle, swine, and poultry, and for growth promotion in pigs. In small animals, tylosin is used for its anti-inflammatory properties in the large intestine rather than for its ability to fight infection. While few formal studies have been performed to examine this non-antibacterial property of tylosin, this has not stopped tylosin from being widely used in the treatment of colitis in pets. Tylosin is a broad spectrum antibiotic; it has good anti-bacterial activity against most pathogenic organism such as gram positive bacterium, some gram negative bacterium, vibrio, spirochete,

coccidian, etc. It is one of the first-choice drugs against infections caused by mycoplasma. Tylosin is fast acting; it can be absorbed quickly after oral administration and distributed widely in the organs (mainly concentrated in lung, liver, kidney, spleen and gallbladder). Bioavailability of Tylosin is high, which makes Tylosin's action rapid and long lasting. However, Tylosin is very safe. And residue in tissues is very rare. It is eliminated from the body upon withdrawal of the drug. Tylosin is very stable, against environmental conditions, feed processing, etc. Tylosin is a good growth promotant. It cannot only prevent diseases, but continual use also increases growth rate and can improve breeding efficiency. Because of Tylosin's broad spectrum activity it is quite effective when used as the only antibiotic for animal and poultry thus, cross-drug-resistance is avoided.

Chemical Formula: C46H80N2O13
Molecular Weight: 869.15
Cas No.: 108050-54-0
Specification: Tilmicosin consists of 82-88% cis isomer and 12-18% trans isomer, as determined by liquid chromatographic assay. (Assay: Min. 85%)

CONCLUSION

A simple, rapid, accurate, and precise RP-HPLC method for the analysis of olopatadine in pure and in pharmaceutical dosage forms had been developed and validated in accordance with ICH guidelines. The RP-HPLC method developed is cost-effective due to short retention time which enabled analysis of olopatadine samples with a small amount of mobile phase. From the % RSD values of precision and recovery studies the method was found to be precise and accurate. The low detection and quantification limits achieved indicate the method is very sensitive. The robustness data gathered during method validation showed that the method is not susceptible to small changes in chromatographic conditions. The proposed RP-HPLC method developed by the author is suitable for routine analysis and quality assessment of olopatadine in pharmaceutical products.

Table 12: Summary of validated parameters for proposed method

Parameter	Result
Linearity range	10 – 250 µg/ml
Regression equation	Y =5844 x +75913
Slope	5844
Intercept	75913
Correlation coefficient	0.998
System precision (% RSD, n=5)	0.58197
Intermediate precision (% RSD, n=5)	1.2443
LOD (µg/ml)	0.099
LOQ (µg/ml)	0.075702
% Recovery (Accuracy, n =3)	98.044%
% Assay (% Assay, n=3)	100.365%

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