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Research Article

Quantification of Olopatadine from Intranasal Formulation by Using Validated RP-HPLC Method

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Abstract

Olopatadine is an anti-histamine drug. Olopatadine is effective in various kinds of allergic conditions. The authors have developed a stability indicating RP-HPLC method for the quantification of Olopatadine in nasal formulation. The chromatographic separation was achieved by using Waters 2695e HPLC system connected to PDA detector and Ultracarb ODS 20 (150 x 4.6mm, 5µm) column on gradient elution mode using a mixture of 50 mM potassium dihydrogen orthophosphate (pH 4.0) and methanol (60:40) as mobile phase A and methanol as mobile phase B with flow rate 0.8 ml/min (UV detection 295 nm) and injection volume 25 µl. Olapatadine has shown linearity over the concentration range 1-300 µg/ml with linear regression equation, y = 6344.4x + 2441.4 (R^2 = 0.9998). The LOD and LOQ were found to be 0.3098 mg/ml and 0.9412 mg/ml respectively. Olapatadine was exposed to different stress conditions such as acidic, basic, oxidative, thermal, and photolytic degradations and the method was validated as per ICH guidelines.

Keywords: Olopatadine; Nasal Formulation; RP-HPLC; Validation; Stress Degradation Studies; ICH Guidelines

Introduction

Olopatadine hydrochloride (OLP) is a selective H_1 receptor antagonist activity. It is a tricyclic compound which acts against multiple allergic conditions [1]. It has a molecular weight of 337.412 g/mol [2]. It is chemically {(11*Z*)-11- [3-(dimethyl amino) propylidene]-6,11-dihydrodibenzo [*b, e*] oxepin-2-yl} acetic acid (Figure 1).

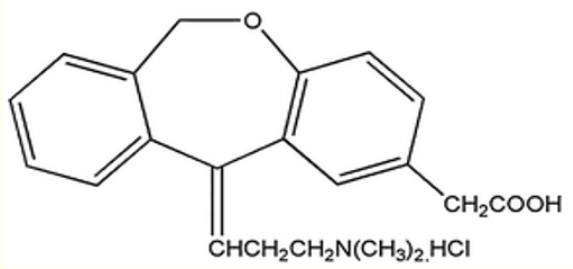


Figure 1: Chemical structure of Olopatadine hydrochloride (OLP).

Basniwal and Jain., *et al.* [3] developed RP-HPLC method for the estimation of Olopatadine using ZORBAX Eclipse Plus C18 column with a mixture of 0.1% Formic acid: Methanol (35:65) as mobile phase with flow rate 1.0 ml/min (Detection wavelength 300 nm) where Olopatadine was eluted at 3.77 min and the linearity was shown over the concentration range 1-20 µg/ml.

Basniwal and Jain proposed RP-HPLC-DAD-HRMS method [4] for the estimation of Olopatadine using ZORBAX Eclipse Plus C18 column on gradient mode with a mixture of 0.1% Formic acid: (Methanol: Acetonitrile, 50:50) as mobile phase with flow rate 1.0 ml/min (Detection wavelength 300 nm) where Olopatadine and five degradants were identified during the forced degradation studies using HRMS and the linearity was shown over the concentration range 2-10 µg/ml.

Jelena Maksić., *et al.* proposed hydrophilic interaction liquid chromatography technique [5] for the separation of Olopatadine,

its geometrical isomer, E-isomer impurity and Benzalkonium chloride using DoE methodology (Box-Behnken experimental design) with a mobile mixture Acetonitrile: 15 mM ammonium acetate (pH 4.5) (82.50: 17.50) (Isocratic mode) with flow rate 1.0 ml/min (Detection wavelength 257 nm) and the linearity was shown over the concentration range 37.5-1000 µg/ml.

Varghese., *et al.* developed HPLC and HPTLC methods [6] for the estimation of Olopatadine. Olopatadine was estimated using RP-HPLC method using RP-18 column using 0.1% orthophosphoric acid (adjusted to pH 4.5 with triethylamine)-acetonitrile (75: 25, v/v) as mobile phase with flow rate 1 ml/min in presence of internal standard, Paracetamol where Olopatadine was eluted at 11.30 min and Paracetamol was eluted at 4.70 min. For the HPTLC method, precoated silica gel 60 F254 aluminum sheets were used as stationary phase and a mixture of Methanol: Chloroform: Ammonia (8: 2: 0.1, v/v) as mobile phase where the detection of the analyte band was carried out at 301 nm. Linearity was observed over the concentration range 0.1-1.0 µg/ml and 0.1-0.9 µg/band for HPLC and HPTLC respectively.

Saroj Kumar., *et al.* developed RP-HPLC method [7] for the estimation of Olopatadine using Waters HPLC system (2695 module) equipped with 2487 dual lambda detector with auto Sampler with Waters Empower software. Kromasil C_{18} column was chosen with Methanol: Phosphate buffer (60:40, v/v) as mobile phase (Detection wavelength 246 nm) and Olopatadine has shown linearity over the concentration range 10-60 µg/ml.

Munjas Jurkic., *et al.* developed a UHPLC method [8] for the estimation of Olopatadine related compounds using Waters Acquity CSH C18 column and mobile phase consisting of 6 mM phosphate buffer (pH adjusted to 2.4 with freshly diluted ortho phosphoric acid) and Acetonitrile (Gradient mode) (Detection wavelength 220 nm) and the linearity was shown to be 0.04-3.4 µg/ml for impurity isomer E, Olopatadine related compound C, α-hydroxy olopatadine and 0.02-4 µg/ml for Olopatadine related compound B respectively.

Jelena Maksić., *et al.* developed a bioanalytical HILIC/ESI/MS/MS method [9] for the estimation of Olopatadine in human tears using UPLC Acquity BEH amide column and mobile phase mixture, 0.1% aq. formic acid and acetonitrile in presence of Mianserin hydrochloride, an internal standard. The matrix experiments were defined using Box-Behnken design created by Design-Expert and the linearity was shown over the concentration range 0.01-10 µg/ml.

In the present work a new RP-HPLC method has been developed and validated for the estimation of Olopatadine in intranasal formulations and stress degradation studies were performed.

Materials and Methods

Olopatadine hydrochloride (API) was obtained from Glenmark Pharma Ltd (India) as a gift sample. HPLC grade methanol and triethylamine were purchased from Thermo fisher (Mumbai, India). Sodium hydroxide, hydrochloric acid, hydrogen peroxide, potassium dihydrogen orthophosphate, and ortho phosphoric acid used during the study were of analytical grade only. MilliQ water obtained from inhouse facility was used throughout the study.

Instrumentation

The HPLC instrument used for separation was Waters 2695e HPLC connected to a PDA detector, temperature-controlled column oven, and autosampler unit. The column used for the study was Ultracarb ODS 20 (150 x 4.6mm, 5µm) from Phenomenex (USA) with a C18 Phenomenex guard column. For the degradation study, a precision water bath was used. The Photostability chamber was used for performing photolytic studies. All the buffer salts and active substances were weighed accurately using a sartorius weighing balance. pH was checked and adjusted using calibrated Eutech pH 510 pH meter. The prepared mobile phase was filtered through 0.45µ nylon filter paper using vacuum filtration. The filtered mobile phase was sonicated using ultra sonicator before use.

Preparation of nasal solution of Olopatadine

To develop Olopatadine nasal solution, aqueous phase was divided into two parts. In one part povidone (2% w/v) was dissolved and stirred for 30 min to get clear solution. In another part dibasic sodium phosphate (0.5% w/v), sodium chloride (0.41%w/v), edetate disodium (0.01% w/v) and benzalkonium chloride (0.0.1%) were dissolved and the resultant solution was stirred for 15 min and both the parts were mixed together and stirred for 15 min to get clear solution. Olopatadine hydrochloride (0.665% w/v) was added into resultant solution and stirred to solubilize the drug and finally, the volume was adjusted to 100 ml and the so far developed formulation was stored in air tight light resistance container.

Preparation of buffer solution

The buffer solution was prepared by dissolving 6.8 grams of potassium dihydrogen orthophosphate in 1000 ml of Milli-Q water, followed by the addition of 5 ml tri ethylamine and the pH was adjusted to 4.0 ± 0.05 with ortho phosphoric acid. A mixture of buffer and methanol (60: 40, v/v) was used as mobile phase A and methanol as mobile phase B. The mobile phase was filtered through 0.45 µ nylon filter paper and sonicated to degas prior to use. Mobile phase A is used as diluent.

Preparation of Olopatadine Hydrochloride standard and sample solutions

20 mg of OLP was accurately weighed and transferred in to a 100 ml volumetric flask and dissolved in 100 ml of mobile phase A followed by sonication for 5 min. The working solution was prepared from a stock solution of OLP where 3ml of stock solution was transferred to 100 ml of volumetric flask and made up the volume using mobile phase A. The sample solution of OLP was prepared by transferring 3 ml formulation in to a 100 ml volumetric flask and made up to volume with mobile phase A.

Optimized chromatographic conditions

A new stability indicating RP-HPLC method has been developed and validated for the estimation of Olopatadine in intranasal formulations. Waters 2695e HPLC system connected to PDA detector and Ultracarb ODS 20 (150 x 4.6 mm, 5µm) column was used for the chromatographic study. 50 mM potassium dihydrogen orthophosphate (pH 4.0) and methanol (60:40) as mobile phase A and methanol as mobile phase B (Gradient mode) with flow rate 0.8 ml/min (UV detection 295 nm) for the chromatographic study and the injection volume 25 µl (Table 1).

Table 1: Gradient program.

Time (min)	% Mobile phase A	% Mobile phase B
0.0	80	20
10.0	80	20
10.01	30	70
13.0	30	70
13.1	80	20
18.0	80	20

Method validation [10]

Linearity, precision, accuracy and robustness

A series of 1.0-300 µg/ml of Olopatadine solutions were prepared from the stock and diluented and each of these solutions were injected thrice into HPLC system and the chromatograms were observed. The peak area of each chromatogram was noted and the mean peak area was calculated. A calibration curve was drawn by plotting the concentration of Olopatadine on the x-axis and the corresponding mean peak area on y-axis. The LOD and LOQ were calculated from the signal to noise ratio (S/N).

Intra-day (n = 6) and the interday precision studies were performed for the method validation and the % RSD was calculated.

The accuracy of the method was evaluated at 50%, 100% and 150% concentration levels and the % recovery was calculated.

The robustness of the developed method was assessed by varying flow rate, change in organic content in mobile phase A and change in pH and the mean % assay of OLP was calculated to ensure the robustness of the method. The minor changes incorporated in the chromatographic conditions are flow rate (± 0.2 ml/min), change in organic phase of mobile phase A (± 6%) and change in pH of buffer (± 0.2 unit). For each parameter, 3 replicate injections of 200µg/ml were injected in HPLC and the mean value of the percentage assay of OLP was calculated.

The solution stability was performed to identify the stability of the working solution. For the study, the standard solution of OLP (200 µg/ml) was analyzed at zero time, 8 hours, 24 hours and 36 hours. The peak area observed was used for the analysis and the stability was assessed by comparing with the zero-time sample.

System suitability

System suitability is performed to ensure the working performance of the HPLC system. The system suitability test is also involved in the ICH guideline of method validation. For system suitability 5 injections of the same concentration (200 µg/ml) were injected and the RSD in the observed area, tailing factor, and number of theoretical plates were calculated.

Stress degradation studies [11]

Olopatadine was subjected to different stress conditions such as acidic hydrolysis, basic hydrolysis, oxidation, thermal and photolytic degradations.

For acidic degradation, 3 ml stock solution of OLP was diluted with 20 ml of diluent addition of 10 ml of 2N HCl and the mixture was subjected to heating in a water bath at 80°C for 30 min. The stressed sample was allowed to be cooled and then neutralized the solution with 10 ml of 2N NaOH. The sample was diluted up to 100ml mark with diluent and mixed well before injecting into HPLC.

For alkaline degradation, 3 ml stock solution of OLP was diluted with 20 ml of diluent 10 ml of 2N NaOH. in a volumetric flask and the mixture was heated at 80°C on a water bath for 30 min. The stressed sample then cooled and neutralized the solution with 10 ml of 2N HCl. The sample was diluted up to 100 ml mark with diluent and mixed well before injecting into HPLC.

Oxidative degradation was performed by mixing 3 ml of stock solution of OLP with 20 ml of diluent and 10 ml of 6% H_2O_2. The mixture was heated for 15min at 80°C in a water bath. Followed by cooling and diluting up to 100ml before injecting to HPLC.

For thermal degradation, 3ml stock solution of OLP was diluted with 20 ml diluent. The sample was heated at 60°C for 60 min on a water bath, cooled down to room temperature, and diluted to 100 ml before the HPLC run.

The photolytic degradation was performed by exposing 3 ml stock solution of OLP to UV light for 24 hours. Further, dilute it to 100 ml with diluent before injecting it to HPLC.

Results and Discussion

A new stability indicating RP-HPLC method has been developed on gradient mode and the method was validated for the estimation of Olopatadine in intranasal formulations. Waters 2695e HPLC system connected to PDA detector and Ultracarb ODS 20 column using a mixture of 50 mM potassium dihydrogen orthophosphate (pH 4.0) and methanol (60:40) as mobile phase A and methanol as mobile phase B with flow rate 0.8 ml/min (UV detection 295 nm) was used for the chromatographic study and the injection volume 25 µl. The present RP-HPLC method was compared with the previously published methods and some of the parameters were highlighted in Table 2.

Table 2: Literature survey of Olapatadine.

Mobile phase (v/v)	Method	Linearity (µg/ml)	Ref
0.1% Formic acid: Methanol (35:65)	HPLC	1-20	[3]
0.1% formic acid: (Methanol: Acetonitrile, 50:50) (Gradient mode)	HPLC-DAD-HRMS (Stability indicating)	2-10	[4]
Acetonitrile: 15 mM Ammonium acetate (pH 4.5) (82.50: 17.50)	HILIC (Impurity E-isomer) DoE	37.5-1000 (Olopatadine) 0.375-10 (Impurity E-isomer) 20-150 Benzalkonium chloride	[5]
0.1% ortho phosphoric acid (adjusted to pH 4.5 with triethylamine): Acetonitrile (75: 25) Methanol: Chloroform: Ammonia (8: 2: 0.1)	HPLC (Paracetamol internal standard) HPTLC	0.1-1.0 0.1-0.9/band	[6]
Methanol: Phosphate buffer (60:40)	HPLC	10-60	[7]
6 mM phosphate buffer (pH adjusted to 2.4 with freshly diluted ortho phosphoric acid): Acetonitrile (Gradient mode)	UHPLC Related compounds	0.04-3.4 0.02-4.0	[8]
0.1% aq. formic acid: Acetonitrile (Mianserin HCl internal standard)	UPLC/MS/MS (Human tears) DoE	0.01-10	[9]
50 mM potassium dihydrogen orthophosphate (pH 4.0) and methanol (60:40) and mobile phase B consisted of methanol	HPLC Ultracarb ODS 20 C18	1-300	Present method

Method validation

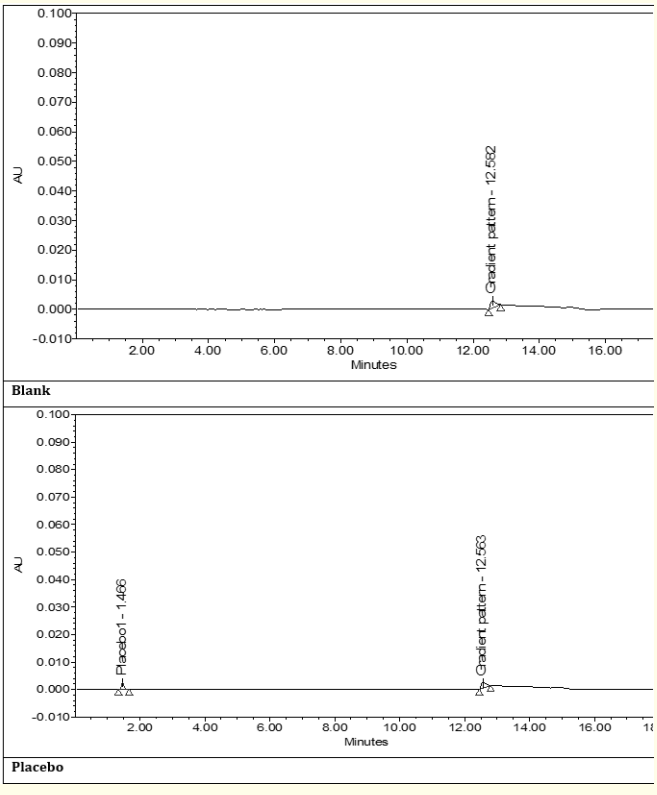
Linearity, precision, accuracy and robustness

Olopatadine obeys Beer-Lambert's law over the concentration range of 1-300 µg/ml (Table 3) and the respective chromatograms of blank, placebo, Olopatadine standard and Olopatadine nasal formulation were shown in Figure 2. The regression equation was found to be y = 6344.4x+2441.4 (Figure 3) with correlation coefficient 0.9998.

Table 3: Linearity of Olopatadine.

Conc. (µg/ml)	*Mean peak area
1	5841
2	12751
5	31253
10	64256
25	160358
50	321569
100	645326
150	964525
200	1275966
250	1566895
300	1912547

*Mean of three replicates



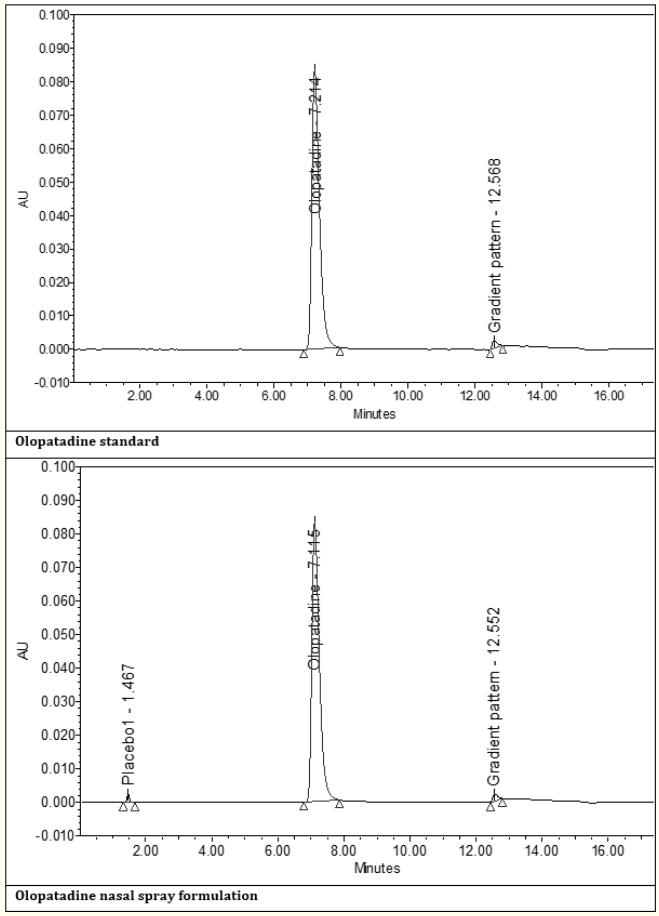


Figure 2: Representative chromatograms of Olopatadine.

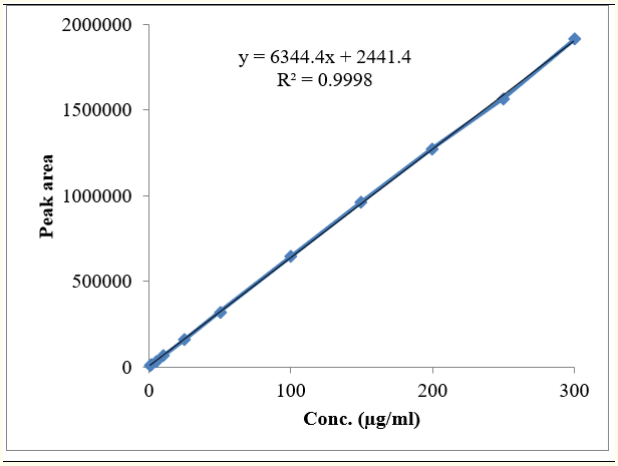


Figure 3: Calibration curve of Olopatadine hydrochloride.

The LOD and LOQ were found to be 0.3098 µg/ml and 0.9412 µg/ml respectively. The %CV for intraday and inter day precision was found to be 0.25% and 0.94% respectively (Table 4 and Table 5) which is less than 2.0 indicating that the method is precise. The accuracy of the developed method was evaluated at 50%, 100%, and 150% concentration levels and the recovery at all three-concentration levels was found to be in the RSD of ± 2% i.e., 100, 99.16, and 99.73% respectively (Table 6). The data of robustness study is summarized in table 3 where the mean % assay of OLP was calculated to ensure the robustness of the method. The robustness study was also found to be less than 2% indicating that the method is robust (Table 7). The standard solution was injected at different time and compared with the zero time. The % deviation from zero-time sample should be in the range of ± 3%. The % deviation for 8h, 24h, and 36h was found to be 1.4, 2.9, and 2.4%, respectively.

Table 4: Method precision study.

Preparation	% Assay	% Deviation from mean assay value (Limit: ± 2.0%)
1	101.93	-0.37
2	102.31	-0.01
3	102.53	0.21
4	102.15	-0.16
5	102.34	0.02
6	102.63	0.31
Mean	102.32	-
± SD	0.25	-
%RSD	0.25	-

Table 5: Intermediate precision study.

Preparation	% Assay	% Deviation From Mean Assay Value (Limit: ± 2.0%)
1	103.28	0.47
2	102.36	-0.43
3	103.90	1.08
4	102.97	0.17
5	101.11	-1.64
6	103.15	0.34
Mean	102.80	-
± SD	0.96	-
RSD	0.94	-

Table 6: Accuracy study.

Simulated dosage nominal	Preparation	Amount recovered %	% Recovery
50%	1	50.33	100.7
	2	50.15	100.3
	3	49.48	99.0
100%	1	98.67	98.7
	2	99.39	99.4
	3	99.21	99.2
150%	1	148.00	98.7
	2	149.88	99.9
	3	150.95	100.6
*Mean			99.60
± Standard Deviation			0.80
% Relative Standard Deviation			0.80

Table 7: Robustness study.

S. No	Chromatographic conditions	*Mean % assay of Olopatadine hydrochloride
1	Increase in flow rate by 0.2 ml/min i.e. at 1.2 ml/min	101.10
2	Decrease in flow rate by 0.2 ml/min i.e. at 0.8 ml/min	102.21
3	Increase in organic concentration in mobile phase A by 10% (Buffer: Methanol, 660:340)	101.28
4	Decrease in organic concentration in mobile phase A by 10% (Buffer: Methanol, 540:460)	102.12
5	Increase in pH of buffer by 0.2 unit i.e. pH 4.2	101.83
6	Decrease in pH of buffer by 0.2 unit i.e. pH 3.8	101.60

System suitability

Five replicate injections of 200 µg/ml were analyzed by HPLC using the optimised method and the % RSD observed for peak area, tailing factor and number of theoretical plates were considered to determine the system suitability and the results are shown in Table 8.

Table 8: System suitability.

Parameter	Mean ± SD (n = 5)	%RSD
Peak Area	1306390.2 ± 4095.03	0.31
Tailing Factor	1.54 ± 0.005	0.32
Number of theoretical plates	4955.5 ± 67.5	1.36

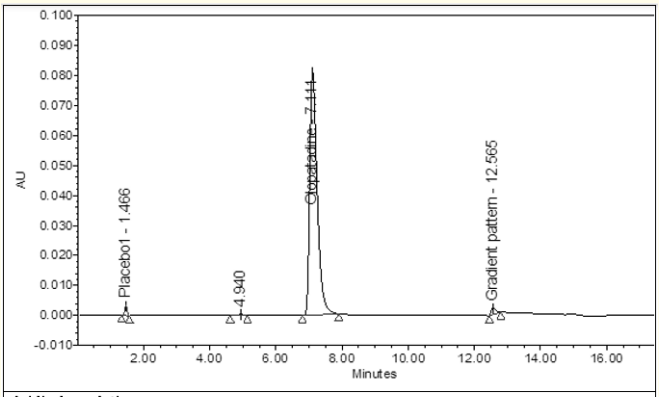
Stress degradation studies

Olopatadine was eluted at 7.111 min, 7.146 min, 7.214 min, 7.068 min and 7.021 min during the acidic, alkaline, oxidation, thermal and UV degradations and the system suitability parameters such as theoretical plates were greater than 2000 and tailing factor is less than 1.5 which are within the acceptable criteria (Table 9). The method is specific as no degradant peaks were interfering with Olopatadine drug peak and the respective chromatograms observed during the stress degradation studies were shown in Figure 4.

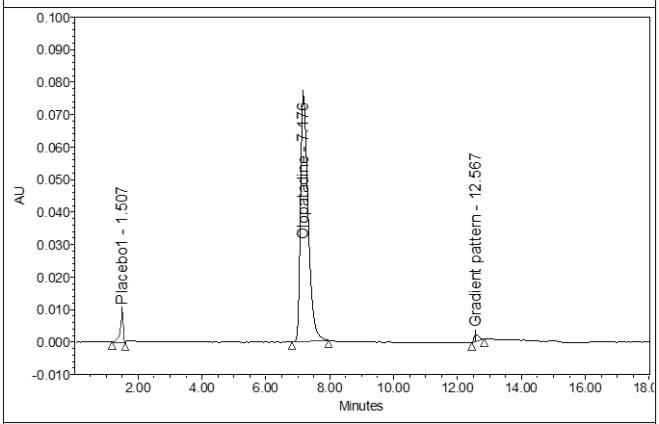
Table 9: Stress degradation studies.

Stress conditions	% Assay	Purity angle	Purity threshold
Untreated sample	103.43	0.054	0.287
Acid degradation (10 ml; 2N HCl, heated it in water bath at 80°C for 30 minutes)	101.40	0.058	0.282
Alkali degradation (10 ml 2N NaOH, heated it in water bath at 80°C for 30 minutes.)	101.04	0.058	0.273
Peroxide degradation (10 ml 6 % H_2O_2, heated it in water bath at 80°C for 15 minutes.)	98.17	0.063	0.272
UV degradation (24 Hrs at 254 nm)	94.55	0.054	0.268
Thermal degradation (Heat at 60°C, 60 mins)	101.13	0.065	0.283

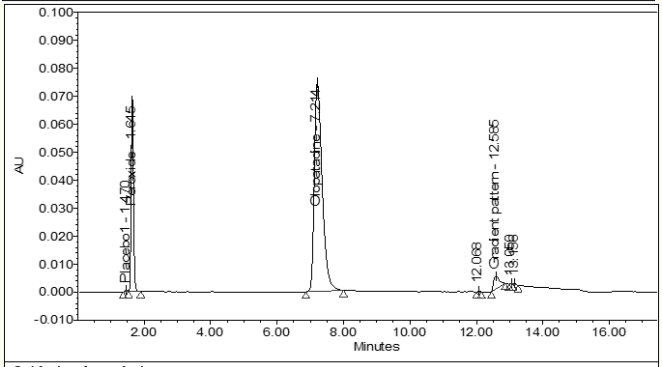
*Mean of three replicates.



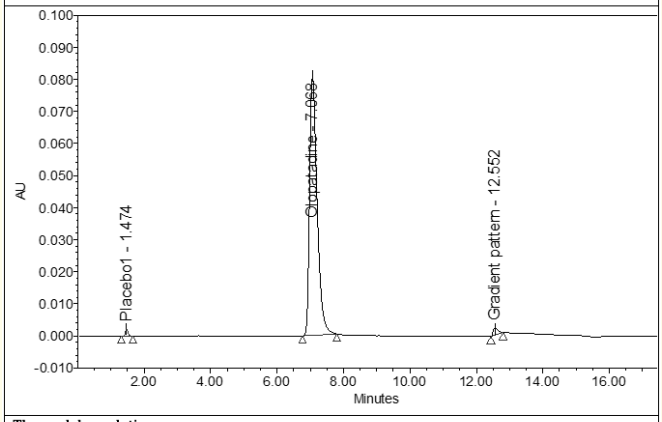
Acidic degradation



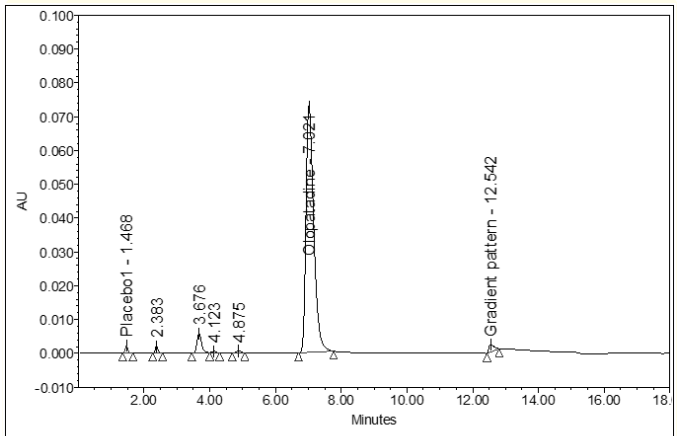
Alkaline degradation



Oxidative degradation



Thermal degradation



UV degradation

Figure 4: Representative chromatograms of Olopatadine during stress degradation studies.

Conclusions

Olopatadine is an approved as nasal delivery formulation for various types of allergic conditions. No interference was observed from any excipient at the retention time of drug. The proposed RP-HPLC method is simple, accurate, precise and robust. This method can be used for routine analysis of Olopatadine hydrochloride in nasal formulation.

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