Int. J. Chem. Sci.: 9(2), 2011, 601-614
ISSN 0972-768X
www.sadgurupublications.com

APPLICATION OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC TECHNIQUE FOR OLOPATADINE HYDROCHLORIDE AND ITS IMPURITY IN OPHTHALMIC SOLUTION

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ABSTRACT

The present work describes the ion pair RP-HPLC method for determination of olopatadine hydrochloride and its related substances. The separation of olopatadine hydrochloride and impurities were achieved on Kromasil 100 C18 (150 x 4.6 mm i.d.), 3.5 µ column. The mobile phase consisted of buffer and acetonitrile (80 : 20 v/v). The flow rate was maintained at 1.5 mL/min with a detection wavelength at 220 nm and the column temperature was maintained at 25°C. The method was validated for performance characteristics like specificity, response factor, limit of detection and quantitation, linearity, accuracy, precision, robustness and stability of sample solution. The detection limit for e-isomer and olopatadine hydrochloride was found to be 0.0891 µg/mL and 0.08924 µg/mL respectively. The quantitation limit was found to be 0.2701 µg/mL and 0.2704 µg/ml respectively. The linear range for olopatadine hydrochloride was found to be 0.27 – 7.5 µg/mL whereas for e-isomer it was found to be 0.27 – 3.75 µg/mL. The method has been successfully adopted for determining the quality of olopatadine hydrochloride ophthalmic solution with good recoveries of the impurities at trace levels.

Key words: Olopatadine hydrochloride, e-Isomer of olopatadine hydrochloride, Reverse phase HPLC.

INTRODUCTION

The present article proposed a novel reverse phase ion pair high performance liquid chromatographic method for determining olopatadine hydrochloride and impurities generated in ophthalmic solution over the period of time on storage. Olopatadine hydrochloride is a relatively selective H1-receptor antagonist and inhibitor of histamine release from the mast cell for topical administration to the eyes. It is 11-[(Z)-3-(dimethylamino) propylidene]-6-11-dihydrodibenz [b,e] oxepin-2-acetic acid. The known impurity e-isomer which is a geometric isomer of olopatadine hydrochloride and other

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unknown impurities in the drug product were successfully resolved and quantitated by the proposed HPLC method. The integrity of the principal peak of olopatadine hydrochloride and the e-isomer was evaluated instrumentally by determining peak purity of both the peaks using diode array detector. The stability testing[2] of the drug product using the proposed method provided insight on the quality of the drug product which varied with time under the influence of various environmental factors such as temperature, humidity and light. It is a novel, rapid and reliable HPLC method for determining olopatadine hydrochloride and impurities generated in ophthalmic solution. This method can be used for the routine and stability analysis of the drug product in quality control laboratories. In the proposed work, optimization and validation of this method are reported.

EXPERIMENTAL

Materials

Reference standards of olopatadine hydrochloride and impurity i.e. e-isomer were obtained from reputed firms with certificate of analysis. Anhydrous monobasic potassium phosphate, 1-pentane sulphonic acid sodium salt monohydrate, hydrochloric acid, sodium hydroxide and hydrogen peroxide were used of analytical grade from Merck and the HPLC grade water was obtained using Millipore water system. Standard solutions were prepared in diluent i.e. water : acetonitrile (1 : 1).

Instrumentation

The HPLC system, Water's Alliance (2695) HPLC system equipped with separation module and DAD detector (2996), was used. The chromatogram was recorded and peaks quantified by means of PC based Empower 2 software.

Preparation of standard solution

Standard solution

About 2.5 mg of e-isomer and 6 mg of olopatadine hydrochloride was weighed and transferred to 100 mL volumetric flask. About 70 mL of diluent was added to it and sonicated for 15 minutes. The volume was adjusted up to the mark with diluent to give concentration of 25 µg/mL for e-isomer and 60 µg/mL of olopatadine hydrochloride. Further 5 mL of this solution was diluted to 50 mL with diluent to give 2.5 µg/mL of e-isomer and 6 µg/mL of olopatadine hydrochloride.

Resolution solution

About 2.5 mg of e-isomer was weighed in 100 mL volumetric flask, about 70 mL of

diluent was added to it and sonicated for 15 minutes. The volume was further adjusted upto the mark with diluent to give a concentration of 25 µg/mL. Further 1 mL of this solution was taken in 10 mL of volumetric flask containing 5.6 mg of olopatadine hydrochloride. About 5 mL of diluted was added to it and sonicated for 15 minutes. Further the volume was made up to the mark with diluent to give a concentration of 2.5 µg/mL and 560 µg/mL of e-isomer and olopatadine hydrochloride respectively.

Sample preparation

About 10 mL of sample i.e. equivalent to 10 mg of olopatadine hydrochloride was transferred in 20 mL volumetric flask, about 7 mL of diluent was added to it and sonicated for 15 minutes. Further the volume was made upto the mark with diluent to give 500 µg/mL of olopatadine.

Chromatographic conditions

Chromatographic separation was achieved at 25^{o}C on Kromasil 100 C 18 (150 x 4.6 mm i.d.), 3.5 µ column. The mobile phase consisted of buffer and acetonitrile in proportion of 80 : 20 (v/v) respectively. The buffer was made up of 6.8 gm of monobasic potassium phosphate and 1.28 gm of 1-pentane sulphonic acid sodium salt monohydrate in 1000 mL water. About 3 mL of triethyl amine was added to it and the pH was adjusted to 3.0 with 85 % v/v orthophosphoric acid. The mobile phase was filtered and degassed before use. The flow rate of the mobile phase was adjusted to 1.5 mL/min. The wavelength was adjusted at 220 nm. The injection volume of the standard and sample solution was set at 10 µL.

Method development

UV spectra of olopatadine hydrochloride shows absorbance maxima at 207 nm (Fig. 1). It exhibits isomerism with e-isomer which is known geometric isomer which is controlled in active ingredient and finished product. The UV spectra of e-isomer also showed absorbance maxima at 207 nm (Fig. 2). The limit of this known impurity in ophthalmic solution is 0.50% based on the published toxicity data. The unknown impurities[3,4] were controlled at 1.0% level. The method development activity was initiated by adopting a reverse phase liquid chromatographic technique with diode array detector. Different C18 columns like Hypersil BDS C18 (150 x 4.6 mm, 5 µ), Inertsil ODS (150 x 4.6 mm, 5 µ) were evaluated for achieving satisfactory chromatographic pattern. Finally Kromasil 100, C18 (150 mm x 4.6 mm x 3.5 µ) column offered favourable chromatographic pattern with all the known and unknown impurities well resolved. The mobile phase consisted of buffer and acetonitrile in proportion of 80 : 20 (v/v) respectively. The flow rate of 1.5 mL/min offered favourable chromatographic pattern. It was found that a good

symmetrical peak of olopatadine hydrochloride was obtained at about 33 minutes and that of e-isomer at 20 minutes with all other unknown impurities well separated from the principal peak and e-isomer. The typical chromatograms of the resolution solution is given in Fig. 3 respectively. The relative chromatographic Figs. of merit are reported in Table 1.

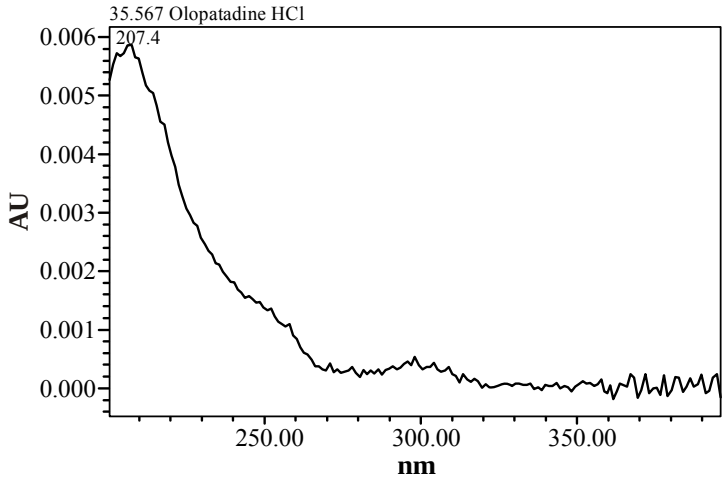

Fig. 1: UV spectra of olopatadine hydrochloride

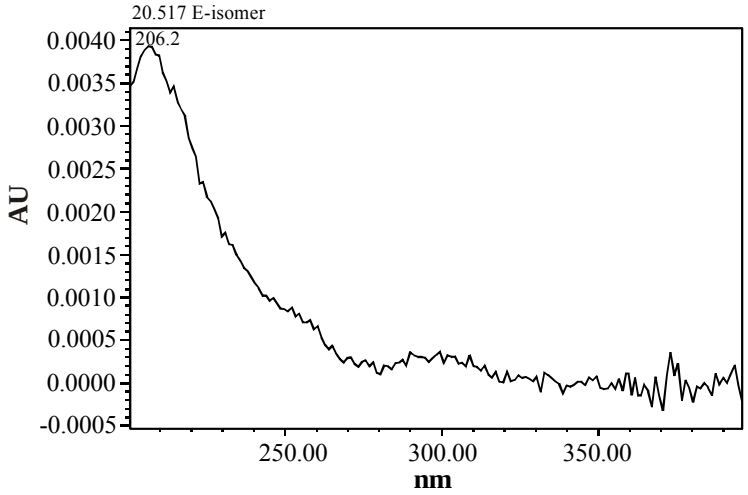

Fig. 2: UV spectra of e-isomer

RESULTS AND DISCUSSION

The results of analysis shows that the proposed analytical method is capable of resolving and quantifying olopatadine hydrochloride, e-isomer and unknown impurities in

the drug product even at trace level thereby assuring the quality of the drug product. The results of analysis showed that the degradation impurities generated during stability studies were well resolved from the principal peak of olopatadine. The developed RP-HPLC method was validated for parameters like system suitability, specificity, limit of detection, limit of quantitation, response factor, linearity, accuracy, precision, robustness and stability of the sample solution.

Method validation

The suitability of the proposed analytical procedure was demonstrated by statistically evaluating different validation characteristics like system suitability, specificity, limit of detection, limit of quantitation, linearity, accuracy, precision, stability of solution and robustness.

System suitability

System performance parameters of developed liquid chromatography method were determined by injecting standard solution of olopatadine hydrochloride. Parameters such as number of theoretical plates (N), tailing factor, resolution (R), capacity factor (K') and relative standard deviation were determined. The results are shown in Table 1 which indicates good performance of the system.

Table 1: System suitability parameters evaluated on standard solution

Component	Retention time	Area	% Area	USP plate count	USP tailing	Capacity factor
e-isomer	18.97	71065	0.43	20516	1.09	188.7
Olopatadine hydrochloride	31.64	16408101	99.57	8712	2.49	315.4

Specificity

Specificity is the ability of the method to resolve the principal peak from the known and unknown impurities. Hence to prove specificity blank (diluent), placebo, e-isomer, olopatadine hydrochloride and olopatadine hydrochloride spiked with e-isomer (resolution) were injected individually in to the chromatograph. The e-isomer peak was found to be well resolved from the principal peak of olopatadine hydrochloride. The typical chromatograms of the resolution, e-isomer and olopatadine hydrochloride are given in Figs. 3, 4 and 5, respectively.

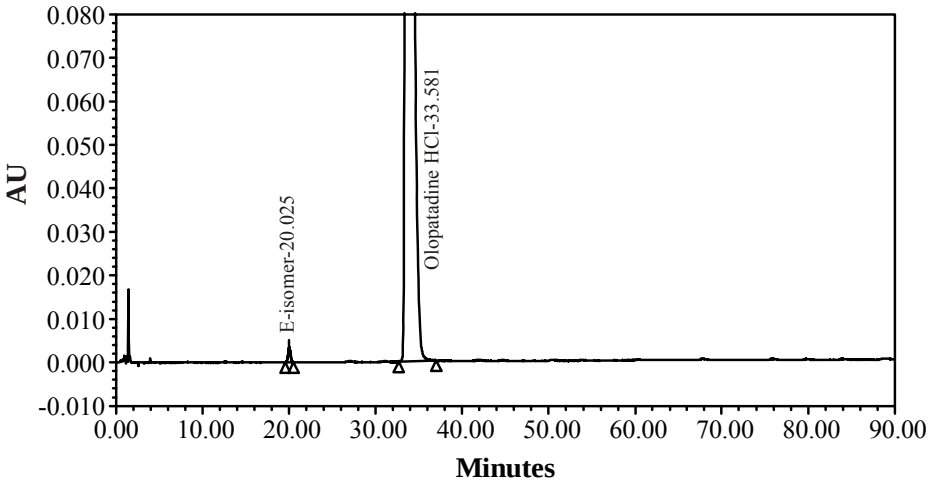

Fig. 3: Typical chromatogram of olopatadine hydrochloride spiked with e-isomer

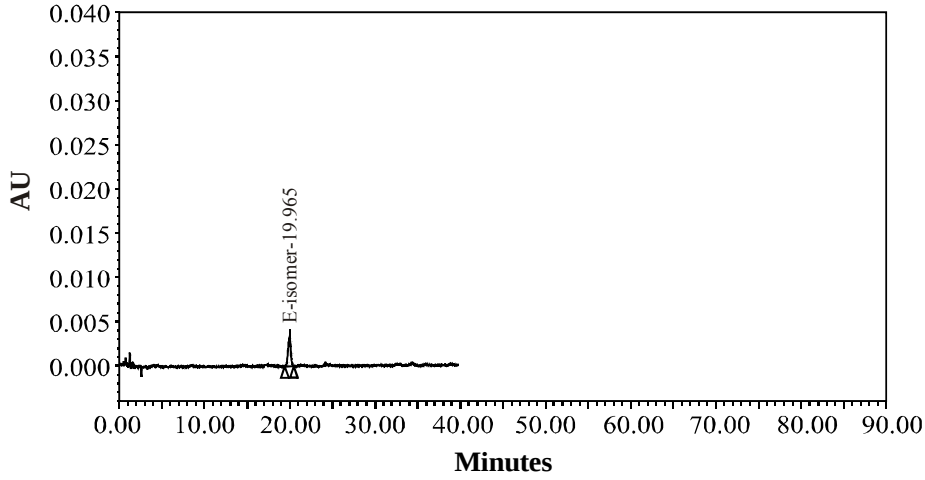

Fig 4: Typical chromatogram of e-isomer of olopatadine hydrochloride

Response factor (Molar absorptivity)

The difference in molar absorptivities of olopatadine hydrochloride and e-isomer was determined by regression analysis over a known concentration range. The difference in molar absorptivities of both olopatadine hydrochloride and e-isomer is expressed in terms of response factor. The response factor is expressed by the formula:

$$\text{Response factor} = \frac{\text{Slope of e-isomer}}{\text{Slope of olopatadine hydrochloride}}$$

The response factor is utilized in calculating percentage e-isomer in the drug product during analysis of the drug product. The response factor for e-isomer was found to be 0.84. The results of response factor determination is given in Table 2.

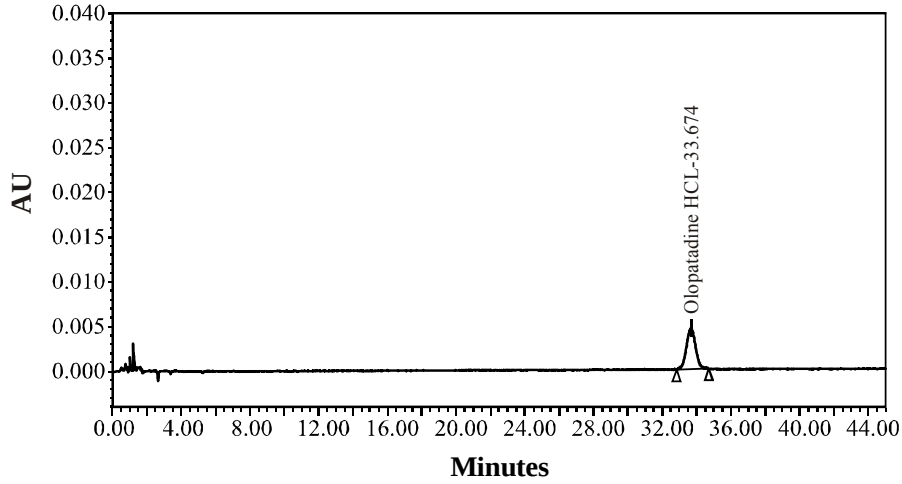

Fig. 5: Typical chromatogram of olopatadine hydrochloride

Table 2: Response factor determination of e-isomer by slope method

e-Isomer		Olopatadine hydrochloride	
Conc. (ppm)	**Mean area**	**Conc. (ppm)**	**Mean area**
0.20	7167.5	0.20	7452
0.50	14398	0.50	16897
1.00	32023.5	1.00	32226
2.00	61368	2.00	71483.5
2.50	72588.5	2.50	89289
4.00	114499	4.00	132852
Slope (e-isomer)		**28370.9**	
Slope (Olopatadine hydrochloride)		**33767.0**	
Response factor		**0.84**	

Limit of detection (LOD)

The detection limit of the analytical procedure was established by determining the lowest amount of the analytes i.e. e-isomer, olopatadine hydrochloride which can be detected

but not necessarily quantitated. The detection limit for both e-isomer and olopatadine hydrochloride was expressed as standard deviation of response and slope. The formula for the detection limit (LOD) was expressed as:

$$LOD = \frac{3.3\ \sigma}{S}$$

where σ = the standard deviation of the response

S = the slope of the calibration curve

The detection limit for e-isomer and olopatadine hydrochloride was estimated to be 0.0891 µg/mL and 0.08924 µg/mL respectively. The results of the same are tabulated in Table 3.

Table 3: Statistical evaluation of the test data subjected to detection and quantitation limit for e-isomer and Olopatadine hydrochloride

e-isomer						
Component	**Conc. (ppm)**	**Mean height**	**Slope**	**Standard error**	**LOD (ppm)**	**LOQ (ppm)**
e-isomer	0.1267	218	1320	36	0.08913	0.2701
	0.253	405				
	0.507	656				
	1.014	1372				
	5.068	6722				
Olopatadine hydrochloride	0.141	220	807	22	0.08924	0.2704
	0.281	309				
	0.562	562				
	1.124	1043				
	5.621	4640				

Limit of quantitation (LOQ)

The quantitation limit of the analytical procedure was established by determining the lowest amount of the analytes i.e. e-isomer, olopatadine hydrochloride which can be quantitated with accuracy and precision. The quantitation limit for both e-isomer and

olopatadine hydrochloride was expressed as standard deviation of response and slope. The formula for the quantitation limit (LOQ) was expressed as -

$$LOQ = \frac{10\ \sigma}{S}$$

where σ = the standard deviation of the response

S = the slope of the calibration curve

The quantitation limit for e-isomer and olopatadine hydrochloride was estimated to be 0.2701 µg/mL and 0.2704 µg/mL, respectively. The results of the same are tabulated in Table 3.

Linearity

Under the experimental conditions described above, linear calibration curves were obtained for both olopatadine hydrochloride and e-isomer. Regression analysis was done on the peak area (y) v/s concentration (x). The linear range for olopatadine hydrochloride was found to be 0.27-7.5 µg/mL whereas for e-isomer it was found to be 0.27-3.75 µg/mL. The results of the same are tabulated in Table 4.

Table 4: Statistical evaluation of the test data subjected to regression analysis

Parameters	Olopatadine hydrochloride	e-isomer
Correlation coefficient (r)	0.9998	0.9999
% Intercept (y)	-0.88	-1.03
Slope (m)	36456.38	29133.96

Accuracy

The accuracy of the proposed method was determined by carrying out recovery studies for both, active ingredient i.e. olopatadine hydrochloride and known impurity i.e. e-isomer. The recovery of the e-isomer was demonstrated by doping the known amount of the impurity i.e. e-isomer in the drug product at LOQ, 50%, 100% and 150% of the limit level. The accuracy was then calculated as the percentage of e-isomer recovered from the drug product. The recovery of the unknown impurity was represented by determining the % recovery of the active ingredient from the placebo. The results of the recovery analysis are enclosed under Table 5.

Table 5: Statistical evaluation of the test data subjected to accuracy

Component	Level (%)	Amount of component added (mg)	Amount of component recovered (mg)	% Recovery	% Error	% RSD
e-isomer	LOQ	0.2534	0.25201	99.5	0.55	2.05
	50	1.2670	1.27812	100.9	-0.88	
	100	2.5340	2.48411	98.0	1.97	
	150	3.8010	3.82253	100.6	-0.57	
Olopatadine hydrochloride	LOQ	0.2757	0.27870	101.1	-1.08	2.8
	50	2.7571	2.65571	96.3	3.68	
	100	5.5141	5.33481	96.7	3.25	
	150	8.2712	8.15525	98.6	1.40	

Precision

System precision

The integrity of system precision was established by determining the relative standard deviation of six injections of standard solution. The value of the relative standard deviation for olopatadine hydrochloride was found to be 0.29%. The results of the same are tabulated in Table 6.

Table 6: Statistical evaluation of the test data subjected to system precision

Injection number	Olopatadine hydrochloride
1	428551
2	425408
3	427713
4	427358
5	426504
6	425499
Mean	426839
Standard deviation	1258.55
% RSD	0.29

Method precision

The degree of scatter between a series of measurements obtained from multiple sampling of same homogeneous sample under prescribed conditions was expressed as percentage relative standard deviation. The results of the same are tabulated in Table 7.

Table 7: Statistical evaluation of the test data subjected to method precision

Sample number	e-isomer (%)	Unknown maximum impurity (%)
1	0.549	0.355
2	0.549	0.357
3	0.550	0.354
4	0.550	0.354
5	0.549	0.357
6	0.551	0.355
Mean	0.549667	0.355333
Standard deviation	0.000816	0.001366
% RSD	0.148544	0.384501

LOQ Precision

The precision at LOQ level was established by determining relative standard deviation (%) for olopatadine hydrochloride and e-isomer on six replicate samples. The value of the relative standard deviation for olopatadine hydrochloride and e-isomer was found to be 1.03% and 4.04% respectively. The results of the same are tabulated in Table 8.

Table 8: Statistical evaluation of the test data subjected to loq precision

Injection No.	e-isomer		Olopatadine	
	Peak area	Retention time	Peak area	Retention time
1	7752	17.499	182061	30.485
2	8684	17.510	183073	30.160
3	8445	17.631	184485	29.958
4	8154	17.549	184557	29.969

Cont…

Injection No.	e-isomer		Olopatadine	
	Peak area	**Retention time**	**Peak area**	**Retention time**
5	8474	17.555	180277	29.951
6	8036	17.588	185358	30.025
Average	8247	17.631	183302	30.091
SD	333.4	0.122	1894	0.208
% RSD	4.04	0.69	1.03	0.69

Robustness

The robustness of the method was determined to check the reliability of an analysis with respect to deliberate variations in method parameters.

The typical variations are given below -

Variation in the flow rate by + 0.2 mL/min

Variation in wavelength by + 2 nm

Variation of temperature by + $2^{o}C$

The results of the analysis of the samples under the conditions of the above variation indicated the nature of robustness of the method.

Stability of solution

The stability of the solutions under study was established by keeping the solutions at room temperature for 24 hours. The results indicated no significant change in the percentage total impurity of the sample solutions. It confirmed the stability of the drug in the solvents used for the analysis.

Method application

About 5 mL i.e. equivalent to 5.5 mg/mL of olopatadine hydrochloride of sample i.e. ophthalmic solution was pipetted in 10 mL volumetric flask, about 2 mL of diluent was added to it and sonicated for 15 minutes. Further the volume was made up to the mark with diluent to give 560 µg/mL of olopatadine hydrochloride. From this solution 10 µL was injected into chromatograph under specified conditions. The e-isomer peak was identified by

comparison with that of respective standard. The percentage impurities are expressed in Table 7 which indicates the purity of the drug product meeting the specification. The typical chromatogram of the sample preparation is given in Fig. 6.

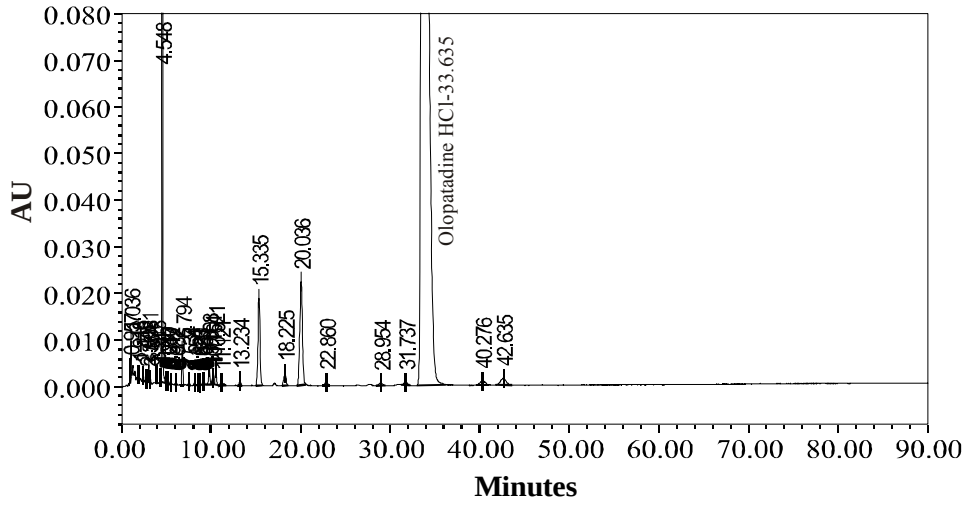

Fig. 6: Typical chromatogram of the olopatadine hydrochloride ophthalmic solution

CONCLUSION

A novel ion pair reverse phase HPLC method was developed for the analysis of olopatadine hydrochloride ophthalmic solution. The limit of detection and quantitation were established for both olopatadine hydrochloride and e-isomer. The repeatability of the proposed method was found to be satisfactory which is evident by the low values of standard deviation and percent relative standard deviation respectively. The accuracy of the proposed method was confirmed by recovery experiments, performed by adding known amount of the impurity i.e. e-isomer to the pre-analyzed formulation and reanalyzing the mixture by proposed method. The percent recovery indicated non-interference from the placebo used in the formulations. Hence the proposed RP-HPLC method can be effectively transferred to the quality control laboratories for establishing the quality of the drug substance and the drug product.

ACKNOWLEDGMENT

Authors express sincere thanks to the Principal and Head of Chemistry Department of D. G. Ruparel College, Mumbai for guidance, encouragement and providing laboratory facilities.

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Accepted : 28.01.2011