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Reversed phase high performance liquid chromatography method for determination of olopatadine hydrochloride from active pharmaceutical dosage form

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ABSTRACT

A simple, rapid and accurate high performance liquid chromatography method is described for determination of olopatadine hydrochloride from active pharmaceutical ingredients. The separation of drug was achieved on chromatopak peerless basic C18 (50 x 4.6 mm i.d.) with 3 µ particle size column showed most favorable chromatographic pattern over the other columns. The mobile phase consisted of a mixture of buffer and acetonitrile (70:30 % v/v). The buffer was mixtures of 0.01 % tri-ethyl amine adjusted the pH 3.3 with ortho-phosphoric acid. The detection was carried out at wavelength 220 nm. The mixture of water and acetonitrile (50:50% v/v) was used as a diluent. The method was validated for system suitability, linearity, accuracy, precision, robustness, stability of sample solution. The method has been successfully used to analyze olopatadine hydrochloride from active pharmaceutical ingredients.

Keywords: Olopatadine hydrochloride, acetonitrile, tri-ethyl amine

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INTRODUCTION

In this communication the present work proposes a noble reverse phase high pressure liquid chromatographic method for assay of olopatadine hydrochloride from active pharmaceutical ingredients. Its chemical name is {(11Z)-11-[3-(di-methyl-amino) propylideine]-6, 11-dihydrodibenzo [b, e] oxepin-2-yl} acetic acid. Olopatadine hydrochloride is a selective histamine H1 receptor-antagonist activity and inhibits the release of histamine from mast cell. It shares many of the pharmacologic effect of mast cell stabilizers. It is used to treat itching associated with allergic conjunctivitis. Its principal effects are inhibition of H1 receptors. The drug selectively binds to H1 receptors there by blocking the actions of endogenous histamine. They act on the bronchi, capillaries, and other smooth muscles [1]. Literature survey reveals that spectrophotometric [2], HPLC [3-5], LC-MS [6-8] and HPTLC [9] methods for the determination of olopatadine hydrochloride. A new, simple, rapid and reliable HPLC method is developed for the determination of olopatadine hydrochloride. This method can be used for the routine analysis. In the proposed method optimization and validation of this method are reported.

MATERIALS AND METHODS

Chemical and reagents

Reference standard of olopatadine hydrochloride was obtained from reputed firm with certificate of analysis. Tri-ethylamine, acetonitrile and ortho-phosphoric acid were used of analytical grade and HPLC grade water was used from Millipore. Standard and sample solutions were prepared in diluent [mixture of water and acetonitrile (50:50 % v/v)].

Instrumentation
The HPLC system used was MERCK Hitachi HPLC system equipped with auto sampler (D 7200 separation module) and UV detector (D- 7400). The chromatogram was recorded and peaks quantified by means of PC based EZChrom Elite software.

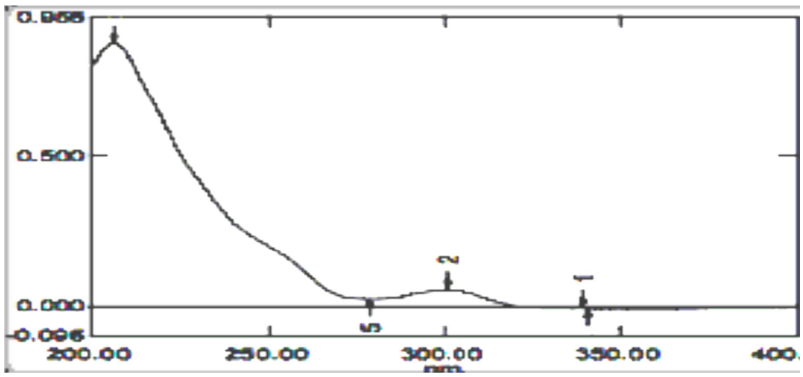
A SHIMADZU analytical balance(0.01 mg) was used.

Preparation of Standard preparation
Standard solution
A 25 mg of standard olopatadine hydrochloride was weighted accurately and transferred in 25 ml volumetric flask. About 15 ml of diluent was added and sonicated for 2 minutes. The volume was adjusted up to the mark with diluent to give concentration as 1000 µg /ml. The working standard solution was prepared by diluting 1 ml of 1000 µg /ml solution to 10 ml with diluent to get concentration 100 µg /ml.

Sample preparation
About 10 mg of olopatadine hydrochloride sample was weighted accurately and transferred in 10 ml volumetric flask. About 5 ml of diluent was added and sonicated for 10 minutes. The volume was adjusted up to the mark with diluent to give concentration as 1000 µg /ml. The sample solution was prepared by diluting 1 ml of 1000 µg/ml solution to 10 ml with diluent to get concentration 100 µg /ml.

Chromatographic condition
Chromatographic separation was performed at ambient temperature on a reverse phase chromatopak peerless basic C18 (50 x 4.6 mm i.d.) with 3 µ particle size column. The mobile phase was a mixture of buffer and acetonitrile (70:30 % v/v). The buffer was mixtures of 0.01 % tri-ethyl amine adjusted the pH 3.3 with ortho-phosphoric acid. The flow rate of the mobile phase was adjusted to 1 ml /min. The detection was carried out at wavelength 220 nm. (Fig. no.1) The injection volume of the standard and sample solution was set at 1.0 µl.

Figure 1: UV spectra of olopatadine hydrochloride



Method validation
System suitability
System performances of developed HPLC method were determined by injecting standard solutions. Parameter such as theoretical plates (N), symmetry, area and % area 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 of Olopatadine hydrochloride

Retention Time	Area	Area %	USP Plate Count	Symmetry
6.673	8825181	100	2852	1.65

Specificity
Specificity is the ability of the method to resolve the active ingredients. Hence blank, standard olopatadine hydrochloride was injected to prove specificity. The typical chromatogram of the standard and sample assayed are given in figure 2 and 3 respectively.

Figure 2: Typical chromatogram of olopatadine hydrochloride (standard)

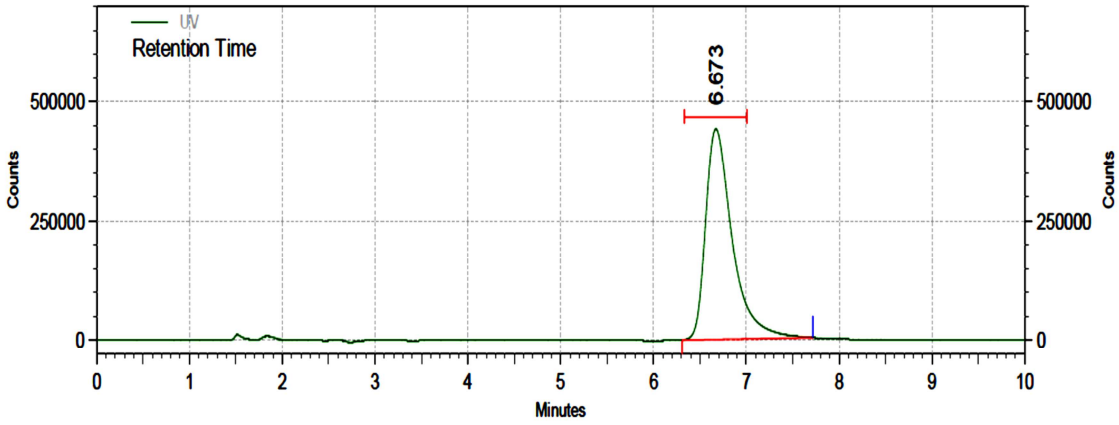
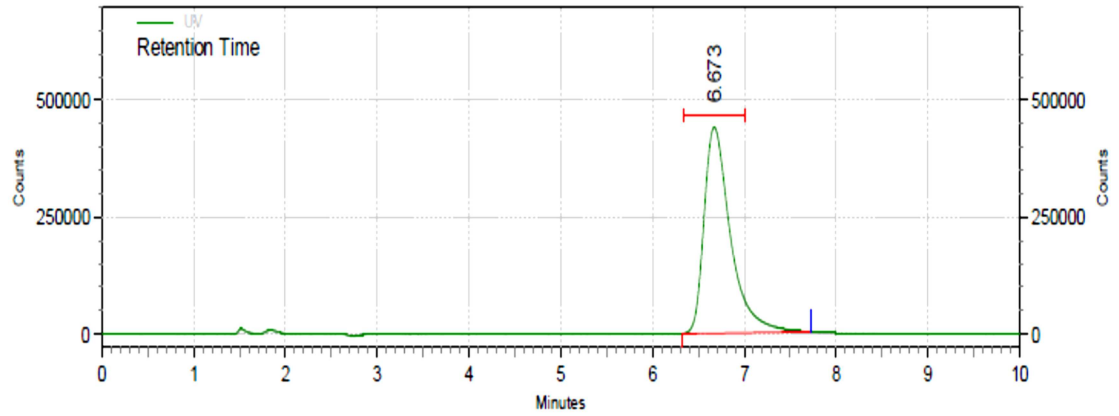


Figure 3: Typical chromatogram of olopatadine hydrochloride (sample)



Linearity

Under the experimental conditions described above, linear calibration curve were obtained throughout the concentration range studied. Regression analysis was done on the peak area (y) v/s concentration (x). The regression analysis data obtained is tabulated in table no. 2.

Table 2: Statistical evaluation of the data subjected to regression analysis

Parameters	Olopatadine hydrochloride
Correlation Coefficient (r)	**0.9997**
% Intercept (y)	**119406**
Slope (m)	174764

Table 3: Statistical evaluation of the data subjected to accuracy of olopatadine hydrochloride

level	test	wt in mg	area	quantity added in ppm	quantity recovered in ppm	% recovery	mean recovery
80%	1	10.33	7179982	41.52	42.33	101.95	**101.86**
	2	10.45	7172975	41.52	42.29	101.85	
	3	10.3	7168112	41.52	42.26	101.78	
100%	1	10.11	8876357	51.9	52.33	100.83	**100.87**
	2	10.21	8888559	51.9	52.40	100.96	
	3	10.13	8874799	51.9	52.32	100.81	
150%	1	10.26	10719195	62.28	63.19	101.47	**101.38**
	2	10.26	10703509	62.28	63.10	101.32	
	3	10.28	10708621	62.28	63.13	101.37	
					Mean		**101.37**

Accuracy
The accuracy method was determined by applying proposed method to synthetic mixture containing known amount of drug corresponding to 50 %, 100 % and 150 %. The accuracy was then calculated as the percentage of analyte recovered by the assay. The results of the recovery analysis are enclosed under table no.3.

Precision
The method precision was established by carrying out the analysis of olopatadine hydrochloride. The assay was carried out of the drug using analytical method in five replicates. The value of relative standard deviation lies well with the limits. The results of the same are tabulated in the table no. 4.

Table 4: Statistical evaluation of the data subjected to method precision of olopatadine hydrochloride

Test	wt of test sample	Area	% assay
Test solution -1	10.31	8825181	99.57
Test solution -2	10.29	8835262	99.49
Test solution -3	10.29	8775228	98.81
Test solution -4	10.32	8782021	99.18
Test solution -5	10.18	8857850	98.68
Test solution -6	10.26	8853660	99.41
	Mean Assay		**99.19**
	SD		**0.370**
	RSD		**0.373**

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 $\pm$ 0.2 ml /min
Variation in mobile phase composition by $\pm$ 2 %
Variation in wavelength ± 5 nm

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

Method application
Eye drop sample equivalent to 10 mg of olopatadine hydrochloride sample was weighted accurately and transferred in 10 ml volumetric flask. About 5 ml diluent was added and sonicated for 10 minutes to dissolve it. Further volume was made up to the mark with the diluent to give 1000 µg /ml. Further the 1 ml of this solution was diluted to 10 ml with diluent to give 100 µg /ml of olopatadine hydrochloride. From this solution 1.0 µl was injected specific conditions. The analyte peak was identified by comparison with that of respective standard. The (%) assay results were expressed in table no. 4. It indicates the amount of olopatadine hydrochloride in the product meets the requirement.

RESULTS AND CONCLUSION

The reproducibility, repeatability and accuracy of the proposed method were found to be satisfactory which is evidenced by low values of standard deviation and percent relative standard deviation. The accuracy and reproducibility of the proposed method was confirmed by recovery experiments, performed by adding known amount of the drug to the pre-analyzed active pharmaceutical ingredient and reanalyzing the mixture by proposed method. Thus the proposed RP-HPLC method is used for estimation of olopatadine hydrochloride from active pharmaceutical ingredient. It is more precise, accurate, linear, robust, simple and rapid method. Hence the proposed RP-HPLC method is strongly recommended for the quality control of the raw material, active pharmaceutical ingredient and pharmaceutical formulation.

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