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

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ABSTRACT

A simple, rapid and accurate ultra performance liquid chromatography method is described for determination of olopatadine hydrochloride from active pharmaceutical ingredients. The separation of drug was achieved on Acquity BEH C18 (50 X 2.1 mm) 1.7µ column. The mobile phase consisted of a mixture of buffer and mobile phase B (70:30 % v/v acetonitrile and methanol). The buffer was mixtures of 0.01 M ammonium acetate adjusted the pH 4.5 with dilute acetic acid and the mobile phase B consisted a mixture of acetonitrile and methanol (70:30% v/v). The detection was carried out at wavelength 299 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, RPUPLC, Ammonium acetate, Acetic acid, Acetonitrile, Methanol

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INTRODUCTION

In this communication the present work proposes a noble UPLC method for assay of olopatadine hydrochloride from active pharmaceutical ingredients. Its chemical name is {(11Z)-11-[3-(dimethylamino) 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. Olopatadine hydrochloride was patented on 1st December 2007 by Kyowa Hakko Kogyo Company Ltd, Japan. These drugs selectively bind 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 UPLC 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. Ammonium acetate, acetic acid were used of analytical grade and the HPLC grade water was used from Merck. Standard and sample solutions were prepared in diluent [mixture of water and acetonitrile (50:50 % v/v)].

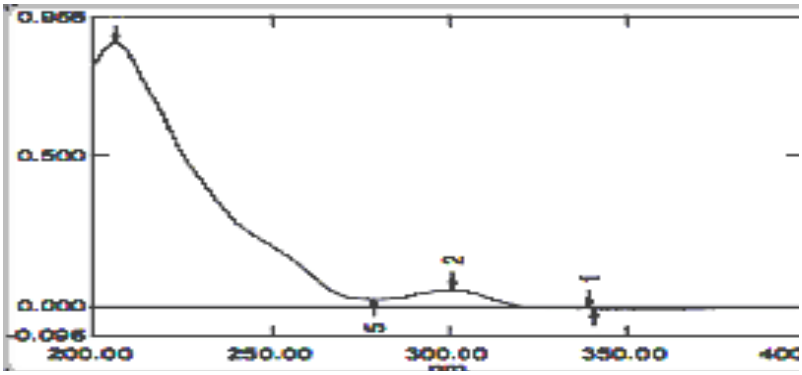
Instrumentation
The UPLC system, UPLC H-CLASS system equipped with separation module and Aquity detector, was used. The chromatogram was recorded and peak quantified by mean of PC based Empower software. A SHIMADZU analytical balance was used.

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 5 ml of 1000 µg /ml solution to 25 ml with diluent to get concentration 200 µg /ml.

Sample preparation
About 25 mg of olopatadine hydrochloride sample 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 sample solution was prepared by diluting 5 ml of 1000 µg/ml solution to 25 ml with diluent to get concentration 200 µg /ml.

Chromatographic condition
Chromatographic separation was performed at 30°C temperature on a reverse phase Acquity BEH C18 (50 X 2.1 mm) 1.7µ column. The mobile phase was a mixture of buffer and mobile phase B (70:30 % v/v). The buffer was mixtures of 0.01 M ammonium acetate adjusted the pH 4.5 with dilute acetic acid and the mobile phase B consisted a mixture of acetonitrile and methanol (70:30% v/v). The flow rate of the mobile phase was adjusted to 0.3 ml /min. The detection was carried out at wavelength 299 nm. (Fig. no.1) The injection volume of the standard and sample solution was set at 1.5 µl.

Figure 1: UV spectra of olopatadine hydrochloride



Method validation
System suitability
System performances of developed UPLC method were determined by injecting standard solutions. Parameter such as theoretical plates (N), tailing factor, 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 of Olopatadine hydrochloride

Retention Time	Area	Area %	USP Plate Count	USP Tailing
1.489 minutes	417620	100.0	2458	1.5

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.

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.

Figure 2: Typical chromatogram of olopatadine hydrochloride (standard)

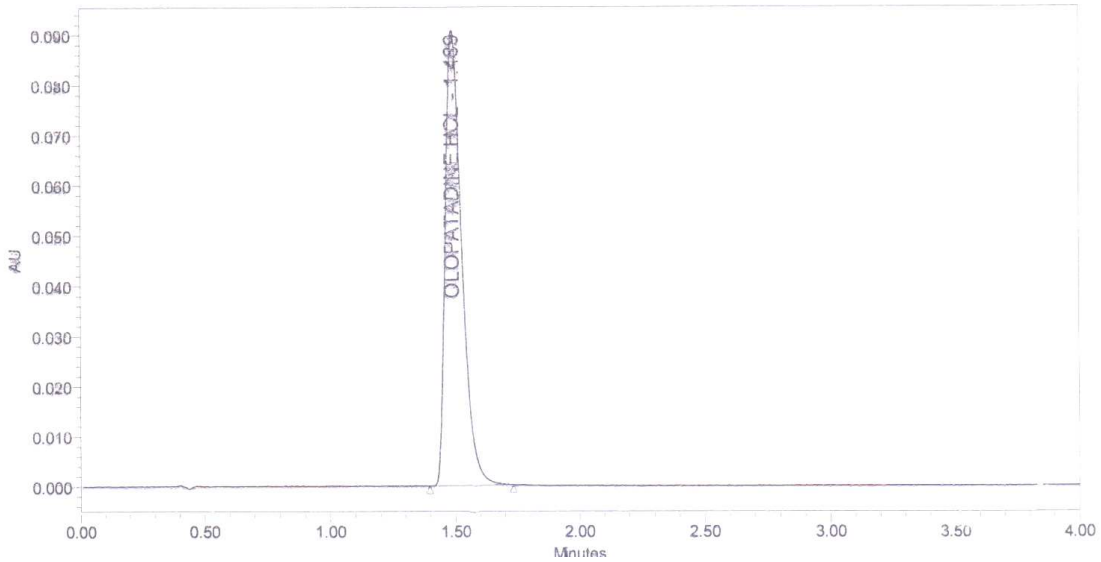


Figure 3: Typical chromatogram of olopatadine hydrochloride (sample)

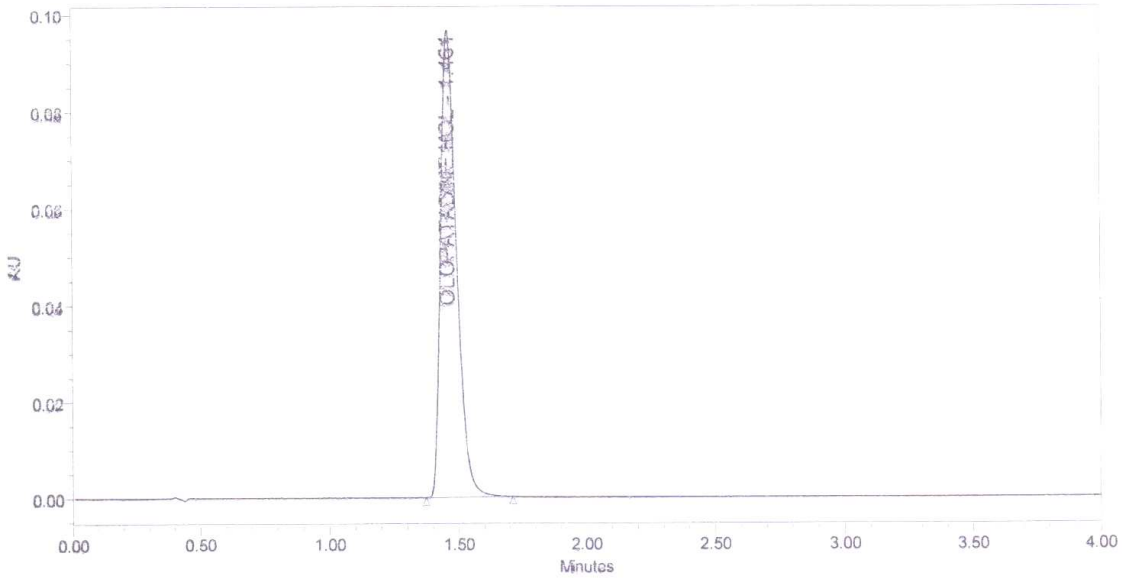


Table 2: Statistical evaluation of the data subjected to regression analysis

Parameters	Olopatadine hydrochloride
Correlation Coefficient (r)	0.9992
% Intercept (y)	-12028.56
Slope (m)	4314.85

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 recove red by the assay. The results of the recovery analysis are enclosed under table no.3.

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

Injection	Level (%)	Conc. of spiked olopatadine hydrochloride (ppm)	Area (minus from area of 10ppm)	Amount found in ppm	% Recovery
01	50 %	**104.00**	222389	50.91	101.82
02			214525	49.11	98.22
03			212792	48.71	99.70
01	100 %	**204.00**	427961	99.89	99.89
02			430573	100.50	100.50
03			431478	100.72	100.72
01	150 %	**307.20**	652826	151.79	101.19
02			655056	152.31	101.54
03			642309	149.34	99.56
				Mean	**100.35**
				Standard Deviation	**0.4204**
				% RSD	**0.42**

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

Sr. No.	Sample name	Assay (%)
01	Test solution – 1	99.79
02	Test solution – 2	99.50
03	Test solution – 3	100.17
04	Test solution – 4	99.46
05	Test solution – 5	99.88
06	Test solution – 6	99.94
	Mean	**99.79**
	Standard Deviation	**0.2712**
	% RSD	**0.27**

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 $\pm$ 0.02 ml /min

Variation in concentration of mobile phase by $\pm$ 2 %

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

Twenty tablets were weighed accurately and average weight of each tablet was determined. Powder equivalent to 25 mg of olopatadine hydrochloride sample was weighted accurately and transferred in 25 ml volumetric flask. About 15 ml diluent was added and sonicated for 2 min to dissolve it. Further volume was made up to the mark with the diluent to give 1000 µg /ml. Further the 5 ml of this solution was diluted to 25 ml with diluent to give 200 µg /ml of olopatadine hydrochloride. From this solution 1.5 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-UPLC 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-UPLC method is strongly recommended for the quality control of the raw material, active pharmaceutical ingredient and pharmaceutical formulation.

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REFERENCES

[1] http://en.*wikipedia*.org/wiki/olopatadine
[2]Suddhasattya D, Vikram reddy Y, Swetha B, Sandeepkumar D, Marthy PN, Sudhir kumar, Subhasis patro S., Subhasis Mohapatra. *Int J pharm pharm Sci*. **2010**, 2(4), 212-218.
[3]Wen-Fang H, Bao-Ping H., Li-Fen W. *Food and Drug*.,**2010**, 12.
[4]Wei-Sheng X and Chang-Qun N.*Chinese J Pharm.* **2007**,12.
[5]Rele R.V. and Warker C.B *International Journal of chemical Sciences*, **2011**, 9(2), 601-614,
[6]Liang W, Zhou H, Liu D, Hu P, and Jiang J. *J.Chinese Mass Spectrum*. **2006**, 27,193-197.
[7]Koichiro F, Xiao-Pen L, Takeshi K, Junichi S and Keizo S. *Forensic Toxicol*. **2006**, 24:8-16
[8]Fujita K, Magara H., Kobayashi H. *J Chromatogr B*. **1999**, 731(2), 345-352.
[9]Anand M, Gandhi PS, Pandita N, Gandhi SV and Deshpande PB. ,*Int J Chem Tech Res*. **2010**, 2(3), 1372-1375.