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Rp-HPLC Method Development and Validation: Strategy to Minimize Run Time and Retention Time Using Chlorpheniramine in Tablet Form.

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Abstract:
Background*: A simple, specific, linear, precise and accurate reverse phase liquid chromatographic method was developed to minimize run time and retention time of Chlorpheniramine in tablet dosage forms. The chromatographic separation was performed using Atlantis R dC18 Column (4.6 mm X 100 mm, 3 µm particle size). Mobile phase composed of buffer and acetonitrile (50:50 v/v) was selected and a flow rate of 1.2 ml/minute is monitored with injection volume of 20 µl. Detection was carried out at 225 nm. The method was validated as per ICH guidelines. The retention time for Chlorpheniramine is observed as 1.36 minutes. Linearity range was observed in concentration of 5 - 15 µg/ml for Chlorpheniramine. The percentage recovery of Chlorpheniramine is 100%. The correlation coefficients for both the components are close to 1. The proposed method was validated and successfully applied to the estimation of Chlorpheniramine in tablet dosage forms.*
Key Word*: Chlorpheniramine, method development, Validation.*

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I. INTRODUCTION

3-(4-chlorophenyl)-N,N-dimethyl-3-pyridin-2-ylpropan-1-amine is the IUPAC name of the Chlorpheniramine maleate (CPM). The empirical formula for CPM is $C_{20}H_{23}ClN_2O_4$ (Figure 1: chemical structure of CPM salt). CPM is an H-1 receptor blocker. CPM is an antihistamine used to relieve symptoms of allergy, hay fever, and the common cold. These symptoms include rash, watery eyes, itchy eyes/nose/throat/skin, cough, runny nose, and sneezing.

Various analytical techniques have been reported for the quantification of Chlorpheniramine in different matrices. High-pressure liquid chromatography detection (HPLC) is the most common used method for the determination of CPM in biological sample or dosage forms.

Analytical methods keep on updating with time as per the requirements so as to develop a simple, reliable, cost effective, reproducible and above all a method bearing a high level of accuracy and precision.

Our study aimed to develop a rapid, robust, selective, sensitive, and precise HPLC method for the determination of CPM.

Literature reveals different Assay methods like liquid chromatography-tandem mass spectrometry[3,6],high performance liquid chromatography-mass spectrometry[4], RP-HPLC[5], Phosphorimetry[7], TLC-Densitometric[8], HPLC[8,10], RP-UPLC[9] for estimation of Chlorpheniramine alone and with other drug substances. A successful attempt was made for quantitative determination of Chlorpheniramineby simple, rapid and easy to operate HPLC method which is very beneficial to the pharmaceutical industry.

The aim of this study is to develop a simple, fast, precise and accurate reverse-phase HPLC (RP-HPLC) method for the estimation of Chlorpheniraminein pharmaceutical dosage forms as per ICH guidelines [11].

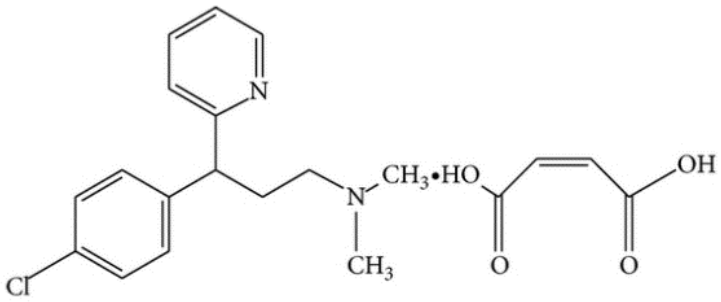

Figure 1: Chlorpheniramine maleate[1]

II. MATERIAL AND METHOD DEVELOPMENT

Instrumental

The HPLC analysis was carried with Waters 2695 with software version Empower 2- PDA detector and Shimadzu LC-2010C HT HPLC system with UV detector and auto sampler integrated with software LCsolution Version 1.25. The column used is Atlantis R d C18 Column (4.6 mm X 100 mm, 3 µm particle size) and detection was performed at 225 nm. The injection volume of sample was 20 µl and the run time was 4minutes. An isocratic mobile phase consisted of buffer and acetonitrile (50:50 v/v). The mobile phase was filtered through 0.45 µm nylon membrane filter and degassed before use.

Reagents and chemicals

Chlorpheniramine was taken from commercial source and tablets were obtained from Medley Pharmaceutical Limited. HPLC grade Acetonitrile was obtained from Finar Ltd. All other chemicals used were AR grade.

Preparation of Buffer solution

Take 8.57 g/L solution of ammonium Dihydrogen phosphate previously adjusted to pH 3 with phosphoric acid.

Preparation of mobile phase

Mix buffer and acetonitrile in the ratio of (50:50 v/v). Mobile phase is degassed before use.

Diluent 1

Acetonitrile : Water (20:80)

Diluent 2

Mobile Phase

Preparation of standard stock solution

Weigh & transfer accurately about 20 mg of Chlorpheniramine working standard in to 200 ml Volumetric flask. Add to it 150 ml of diluent 1 and sonicate until it dissolve completely. Dilute up to the mark with diluent 1& mix well.

Preparation of final stock solution

Take 5 ml solution from standard stock solution and transfer it to 50 ml volumetric flask and dilute it with diluent 2 i.e., mobile phase up to the mark and sonicate it for 5 minutes.

Preparation of sample solution

Take 20 tablets and crush. Mix uniformly and take equivalent to 20mg of Chlorpheniramine maleate of powder and transfer it into 200 ml volumetric flask. Add to it diluent 1, sonicate for 15 minutes, cool to room temperature. Further dilute 5ml of above solution to 50 ml with mobile phase. Mix well and inject.

METHOD DEVELOPMENT

Various mobile phase combination were tried to develop new method of Chlorpheniramine on C18 column. In order to achieve acceptable peak shapes and suitable run time various buffer systems are also tried systematically. Mobile phase composed of buffer and acetonitrile (50:50 v/v) indicated that peak shape was proper with lesser run time. Therefore buffer and ACN (50:50)at a flow rate of 1.2 ml/min was selected as optimized mobile phase. Atlantis dC 18 column (100mm x 4.6 mm, 3µm particle size) was used as the stationary phase to reduce the run time. To analyze drugs, detection was tried at various wavelengths but 225 nm was selected as the detection wavelength as drug showed maximum absorption. The retention time was found to be 1.36 minutes. The chromatogram obtained was shown in figure (2). The system suitability parameters were shown in Table (1)

Table I: System Suitability Parameters

Parameter	Chlorphenamine
Retention Time	1.36
Tailing factor	1.85
% RSD	0.2

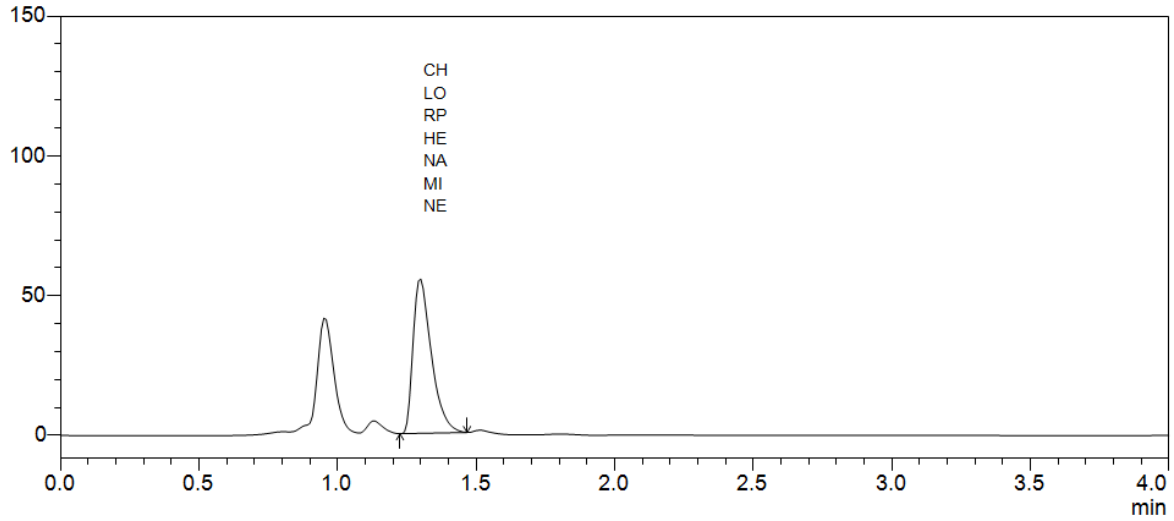

Figure 2: Representive chromatogram of test solution

III.MATERIAL VALIDATION

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The above method was validated according to ICH guidelines to establish the performance characteristic of a method (expressed in terms of analytical parameters) to meet the requirement for the intended application of the method.

They were tested using the optimize chromatographic conditions and instruments.

Specificity

Spectral purities of Chlorpheniramine peakwere evaluated for the interference of the tablet excipients, degradation components or due to the presence of impurities as per the methodology. In the work, a solution containing a mixture of the tablet excipients were prepared using the sample preparation procedure to evaluate possible interfering peaks.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration of analyte in the sample. Linearity of for Chlorpheniraminewas established by analyzing serial dilutions of a stock solution of the working standard.Five concentrations such as 5.01, 7.51, 10.02, 12.52& 15.02µg/ml for Chlorpheniraminewere prepared as per table (1) and analyzed. Correlation coefficient & %Y-axis should be within the limit.

Table 2: Linearity Concentration Levels of Chlorphenaramine

% level	Volume of stock solution	Diluted to (ml)	Final concentration in ppm
50%	1.0 ml	20	5.01
75%	1.5 ml	20	7.51
100%	2.0 ml	20	10.02
125%	2.5 ml	20	12.52
150%	3.0 ml	20	15.02

Accuracy

The accuracy of the method was determined by recovery experiments known concentrations of working standard was added to the fixed concentration of the pre-analyzed Tablet sample. Percent recovery was calculated by comparing the area with pre-analyzed sample. Three different solutions of chlorpheniraminewere prepared in triplicate at level of 50%, 100% and 150% of its predefined concentration (5, 120, 15 µg/mL). and the percentage mean and individual recovery was calculated. Data from the linearity was considered for accuracy.

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurement obtained from multiple sampling of the same homogenous sample under prescribed conditions. Repeatability of the method was checked by carrying out six independent assays of Chlorpheniramineat10µg/ml concentration. The mean area and % relative standard deviation (RSD) was calculated. % RSD should be ≤ 2 %.

Intermediate precision

The intermediate precision of the assay method was established by comparison of two independent repeatability experiments on 2 different days. The data of the 1st day was taken from the analysis of "Repeatability". The second set of experiments was performed by a different analyst or on different instrument. The standard deviation, relative standard deviation and mean value difference was calculated from the results obtained on each day.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. It was observed that the variations like sonication time&change in wavelength etc.

IV. RESULT AND DISCUSSION

The objective of the method validation was to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines.The above method was validated to establish the performance characteristics of a method (expressed in terms of analytical parameters) to meet the requirements for the intended application of the method.Chlorpheniramineshowed maximum absorbance at 225 nm.

Specificity

By comparing the chromatogramsof blank solution, placebo solution, reference solution & test solution itis observed that there is no interference of any peaks at the retention time of Chlorpheniramine. The retention time of the main peak in the chromatogram obtained with the reference solution & test solution are matching. This confirmed the specificity of the method.

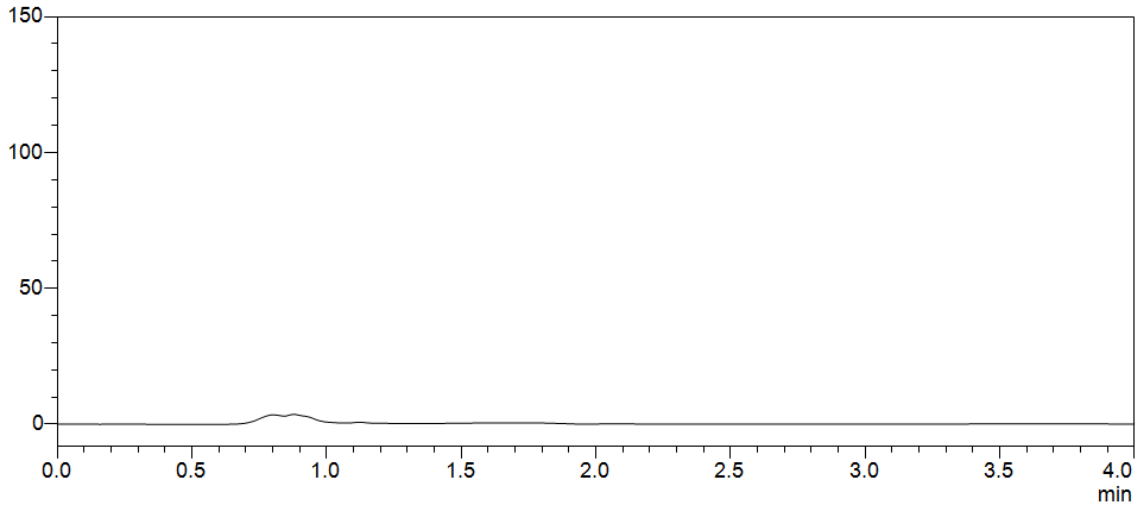

Figure 3: Chromatogram of blank solution

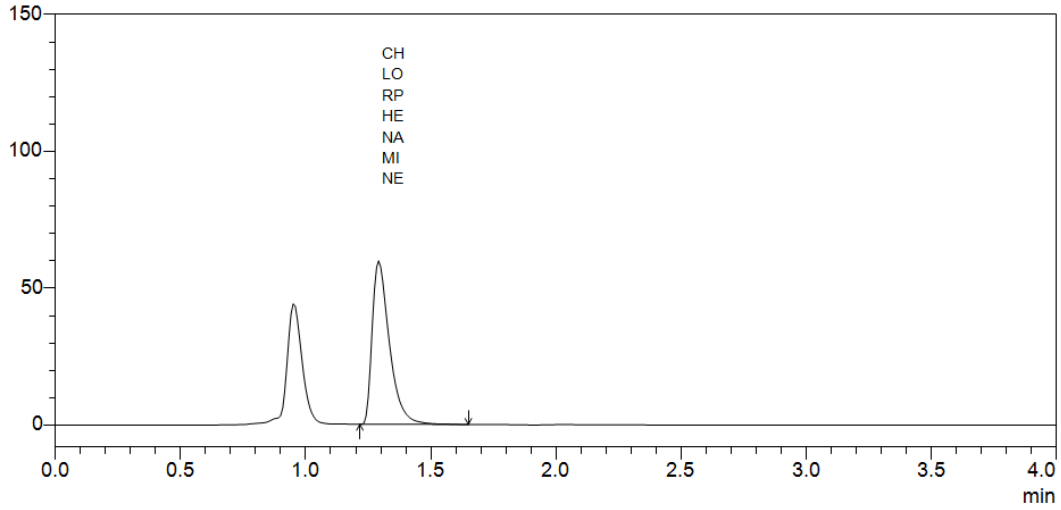

Figure 4: Chromatogram of standard solution

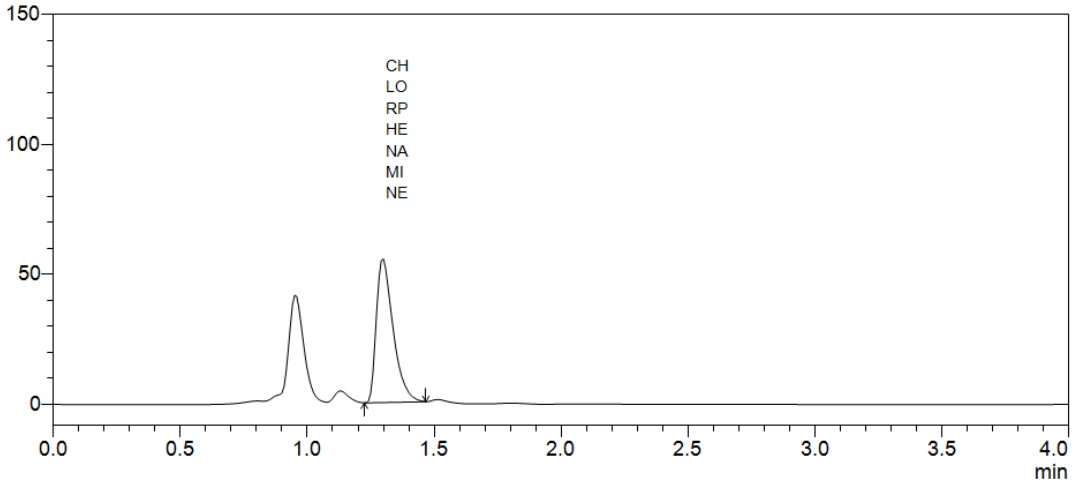

Figure 5: Chromatogram of sample solution

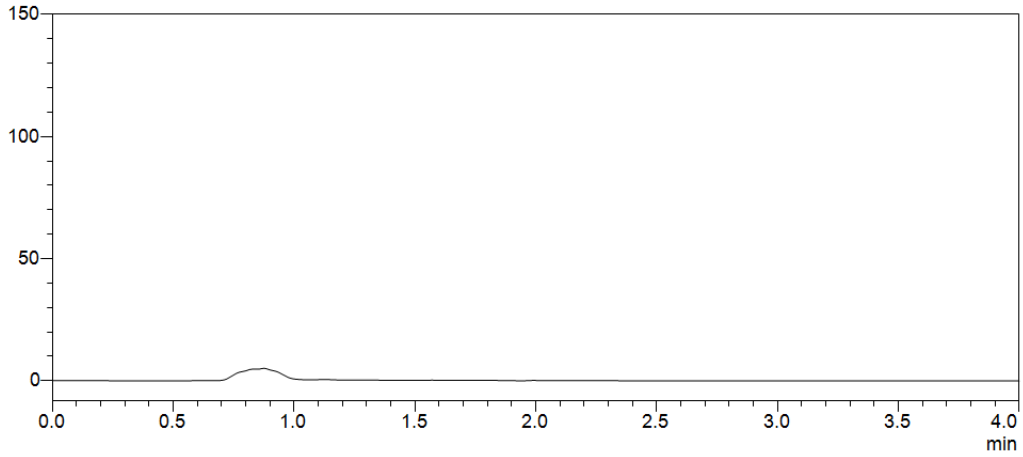

Figure 6: Chromatogram of placebo solution

Linearity

Five concentrations such as 5.01, 7.51, 10.02, 12.52& 15.02µg/ml for Chlorpheniraminewere prepared and the linearity graph was plotted using concentration verses peak areaas shown in Figure (6).Graph of Residuals against concentration was also plotted as per shown in Figure (7).A linear relationship was obtained between peak areas and quantity analyzed in the range of 50% to 150% (5.01-15.02µg/ml)

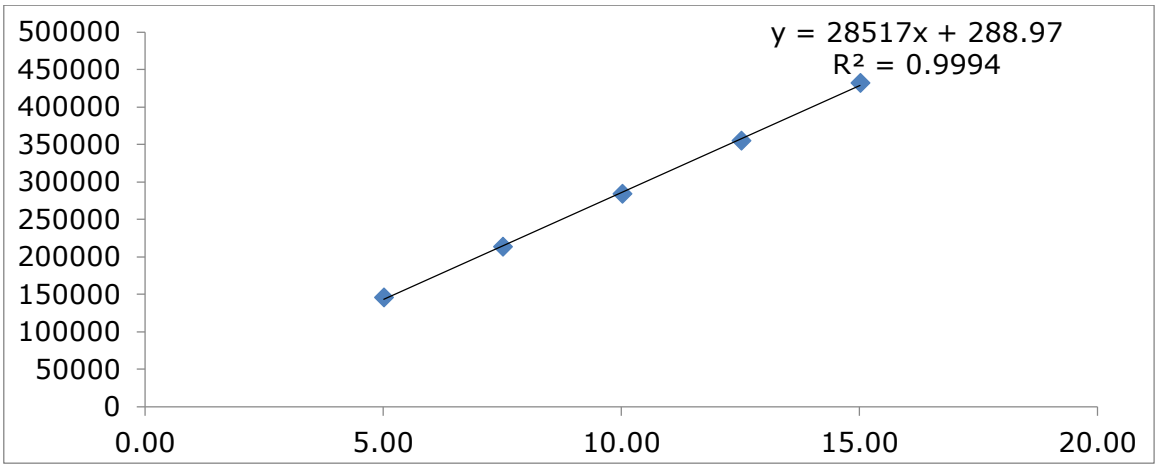

Figure 7: Linearity plot for Chlorpheniramine

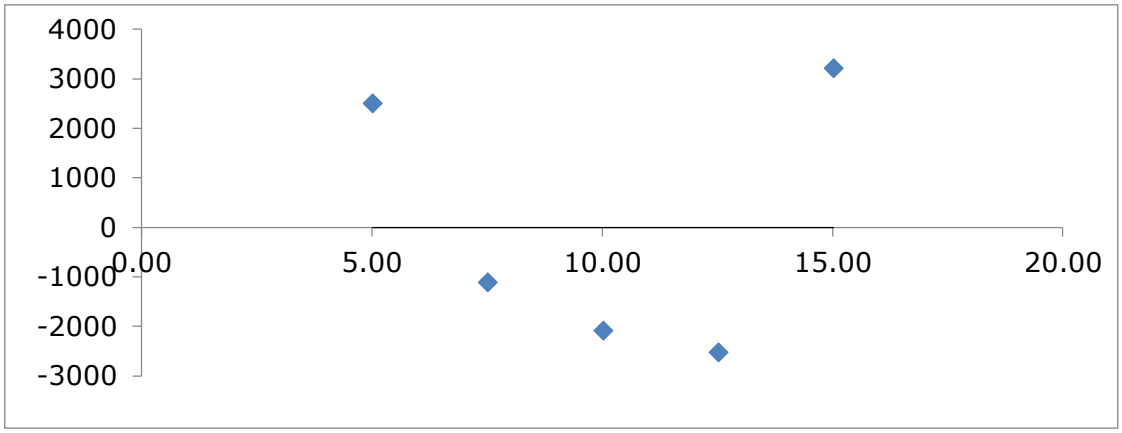

Figure 8: Plot of Residuals against concentration for Chlorpheniramine

Table 3: Observation table for linearity of Chlorpheniramine

Parameter for Linearity	Values	Acceptance Criteria
Correlation coefficient R	0.99972	$\geq$ 0.999
%Y – axis intercept	0.1	$\leq \pm$ 5 %
Slope of regression line	28516	To be reported
Residual sum of squares	28580862	To be reported

The method was considered to be linear in the range on 5.01 – 15.02µg/ml for Chlorphenaramine as Correlation coefficient & %Y-axis intercept should be within the limit.

Accuracy

The percentage recovery of Chlorpheniraminewas tabulated in table (3). The method was considered to be accurate as the % individual recovery was within the acceptance criteria of 97-103 % and the % mean recovery was within the acceptance criteria of 98 – 102 %.

Table 4: Recovery at Different Concentration Levels

Accuracy level	% recovery of Chlorpheniramine
50%	101.0
	101.6
	101.6
100%	98.4

	99.5
	100.2
150%	99.4
	100.7
	98.8
Means recovery	100.1
Minimum recovery	98.4
Maximum recovery	101.6

Precision

The exactness of the method as defined by precision and method was considered to be precised as since the relative standard deviation from 6 determinations was well within the acceptance limit of ≤ 2 %. Refer table (4).

Table 5: Method Precision

Sample No.	% Assay of Chlorpheniramine
Sample 01	99.4
Sample 02	99.5
Sample 03	99.5
Sample 04	99.6
Sample 05	99.1
Sample 06	99.3
Mean	**99.4**
STD Dev	**0.18**
% RSD	**0.18**

Intermediate Precision

The intermediate precision of the assay method was established by comparison of two independent repeatability experiments on 2 different days. Refer table (5) for % Assay of Chlorpheniramineand table (6) for comparison of two independent repeatability

Table 6: Intermediate Precision

Sample No.	% Assay of Chlorphenaramine
Sample 01	100.5
Sample 02	99.1
Sample 03	100.1
Sample 04	102.0
Sample 05	102.0
Sample 05	101.4
Mean	**100.9**
STD Dev	**1.16**
% RSD	**1.15**

Table 7: Comparison of two independent repeatability

Parameter	1st day Repeatability	2nd day Repeatability
Number of determinations	6	6
Mean (%) assay	99.4	100.9
RSD (%)	0.18	1.15
Mean value difference (%) **Acceptance Criteria:** < 2.0 % absolute	**1.5**	

Robustness

Method was found to be robust as system suitability criteria was achieved for all the robustness parameters tested. Deliberate change in parameter does not have any significant effect on the method performance, which demonstrated that the developed HPLC method was robust. The results were shown in Table (7).

Table 7: Robustness Result forChlorpheniramine

Parameter	System suitability	% Assay
	% RSD	
As per method		
--	0.05	99.4
Wavelength		
223nm	0.21	98.8
227nm	0.31	99.3
Flow rate		
1.0 mL/Minutes	0.03	98.7
1.4 mL/minutes	0.39	97.2

V. CONCLUSION

In this present work a new simple, selective, linear, precise, accurate and robust HPLCmethod was developed and validated for the estimation of Chlorphenaraminein pharmaceutical tablet dosage form in accordance with the ICH guidelines.

The minimum run time reported up till now was approximately 2.5min, here by using the buffer:ACN and column it was possible to reduce run time to 1.36min.

The current work is worthwhile as developed HPLC spectroscopic method is selective, simple and rapid whichcan be very beneficial for the routine analysis of Chlorpheniramine in pharmaceutical tablet dosage form.

REFERENCES

[1] Available at: https://en.wikipedia.org/wiki/Chlorphenaramine

[2] Valavala S, Seelam N, Tondepu S, Jagarlapudi VSK, Sundarmurthy V. Analytical Method Development and Validation for the Quantification of Acetone and Isopropyl Alcohol in the Tartaric Acid Base Pellets of Chlorphenaramine Modified Release Capsules by Using Headspace Gas Chromatographic Technique. J Anal Methods Chem. 2018;2018:8240932.

[3] Chen M, Granvil C, Ji QC, Zhang ZY, Padval MV, Kansra VV. Development and validation of a liquid chromatography-tandem mass spectrometry assay for the simultaneous quantitation of prednisolone and Chlorphenaramine in human plasma and its application in a pharmacokinetic study. J Pharm Biomed Anal. 2009;49(5):1241-1249.

[4] Wang N, Xu F, Zhang Z, Yang C, Sun X, Li J. Simultaneous determination of Chlorphenaramine and salicylic acid in human plasma by high performance liquid chromatography-mass spectrometry. Biomed Chromatography. 2008;22(2):149-156.

[5] Mallavarapu, Ravindra et al. A validated stability-indicating RP-HPLC method for Chlorphenaramine in the presence of degradation products and its process-related impurities in Pharmaceutical dosage forms. Biomedical chromatography : BMC, e5247. 2021.

[6] Qin T, Qin F, Li N, Lu S, Liu W, Li F. Quantitative determination of Chlorphenaramine in human plasma by high-performance liquid chromatography-tandem mass spectrometry and its application to a pharmacokinetic study. Biomed Chromatogr. 2010;24(3):268-273.

[7] Salinas-Castillo A, Carretero AS, Fernández-Gutiérrez A. Sensitive and simple determination of the vasodilator agent Chlorphenaramine in pharmaceutical preparations by phosphorimetry. Anal Bioanal Chem. 2003;376(7):1111-1114.

[8] El-Ragehy, Nariman A et al. "Simultaneous Determination of Aspirin, Chlorphenaramine and Two of Their Related Impurities in Capsules by Validated TLC-Densitometric and HPLC Methods." Journal of chromatographic science vol. 54,7 (2016): 1120-1128.

[9] A.P Rajput, M. C. Sonanis. Development And Validation of A Rapid RP-UPLC Method for the Determination of Aspirin and Chlorphenaramine in Combined Capsule Formulation, Int J Pharm Pharm Sci, 2011; 3(2): 156-160.

[10] M.Nuvvula, M.Jyothsna, D.Jeevan Mani Babu. Analytical method development and validation for the estimation of Chlorphenaramine in pharmaceutical dosage form by HPLC. Indian Journal of Research in Pharmacy and Biotechnology, 2017; 5(5): 356-359.

[11] ICH. Q2B Validation of Analytical Procedures: Methodology. International Conference on Harmonisation. Geneva: IFPMA; 1996.

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