RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF CHLORPHENIRAMINE MALEATE IN LIQUID DOSAGE FORM.

KANDEKAR VIKAS*, RANE SUCHITRA

SIDDHANT COLLEGE OF PHARMACY,SUDUMBARE,MAVAL, PUNE-411041, INDIA.

***Corresponding Author**:

KANDEKAR VIKAS

Professor Quality assurance department

Siddhant College of Pharmacy,

Sudumbare,Maval, Pune-41,Maharashtra , India.

ABSTRACT

A high performance thin layer chromatographic method was developed and validated for determination for Chlorpheniramine maleate which comply with ICH Guidelines. The chromatographic development was performed using a Hypersil CN C18,(250×4.6mm, 5.0µm particle size) and a mobile phase composed of sodium dihydrogen o-phosphate buffer:Acetonitrile:Methanol (35:34:31 v/v/v), pH 6.2 adjusted with triethylamine at 1.3 ml/min flow rate.

The retention factor (R_f) was found to be 7.65 min.The linear calibration curves at concentration range 80-120% (r^2 =0.9999) for Chlorpheniramine maleate.

Keywords:

Chlorpheniramine maleate, ICH, High performance thin layer chromatography,

validation ,development.

INTRODUCTION

Chlorpheniramine maleate chemically 1-(p-Chlorophenyl)-1-(2-pyridyl)-3-dimethy-

laminopropanebimaleate (Fig.1).It is is used as antihistaminic in temporary relief of sneezing, itchy, watery eyes, itchy nose or throat, and runny nose caused by hay fever (allergic rhinitis), or other respiratory allergies.

Chlorpheniramine binds to the histamine H1 receptor. This block the action of endogenous histamine, which subsequently leads to temporary relief of the negative symptoms brought on by histamine.

.Literature survey shows that few analyticaland bioanalytical methods including UV-Vis spectoscropy[2], Spectrofluorimetry[3], HPLC[4]and some combinational methods have been published for the estimation of Chlorpheniramine maleate.

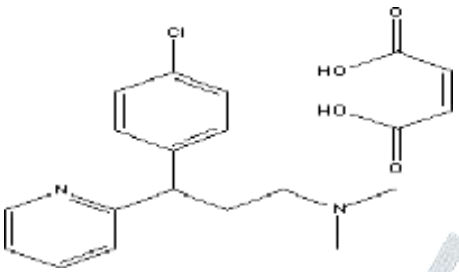

Fig 1:Structure of Chlorpheniramine Maleate

MATERIAL AND METHODS

Material and reagents

Methanol(AR grade), Acetonitrile (AR grade) and Sodium Dihydrogen phosphate NaH2PO4 Buffer were purchased from MERCK, Mumbai. Triethylamine (AR grade)was purchased from LobaChemiePvt. Ltd., Mumbai.

Preparation of standard stock solution.

Accurately weigh 40 mg of Chlorpheniramine maleate,Add into 25 ml water and ultrasonicate for 5 minutes.(Make up the volume using water upto 50 ml).Dilute 5 ml of this solution to 50 ml with water.

Further dilute 5 ml from this solution to 100 ml with water.

Preparation of sample solution.

Accurately weigh 40 mg of Chlorpheniramine maleate,Add into 30 ml water and ultrasonicate for 15 minutes.(Make up the volume using water upto 50 ml).Dilute 5 ml of this solution to 50 ml with water.

Shake and filter through 0.45 micron filter and inject.

Selection of analytical wavelength

Stock solutions 40 µg/ml of Chlorpheniramine maleate was prepared in Water and scanned in UV range. The drug shows maxima at two positions. But the maximum absorbance of Chlorpheniramine maleate was found at 228 nm (Fig.2). Spectra of Chlorpheniramine Maleate drug in UV region indicates that 228 nm was an ideal wavelength for determination by HPLC analysis .

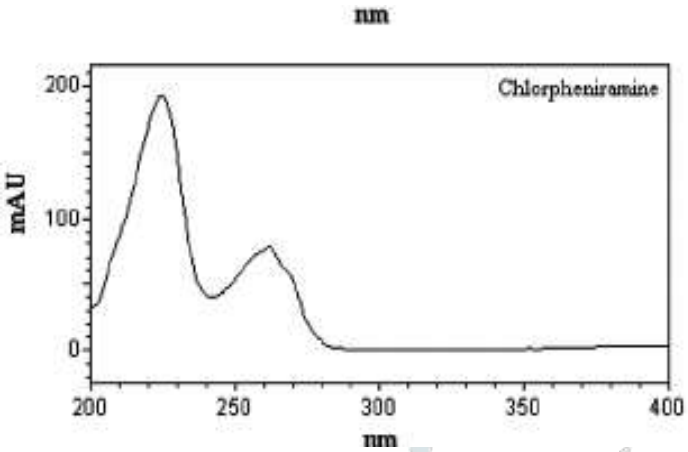

Fig.2: UV spectra of Chlorpheniramine Maleate

Solubility Studies:

These studies were carried out to reconfirm literature data of solubility. Chlorpheniramine Maleate is freely soluble in water (0.55g/100ml); soluble in alcohol and in chloroform slightly soluble in ether and in benzene

From solubility studies, it was concluded that the drug is freely soluble in water(0.55g/100ml). Therefore, water was selected as suitable solvent for further studies.

Results and discussion

Validation of Analytical Method

The method was validated as per ICH guidelines

Specificity

The method was found to be specific.

Linearity and Range

Standard solutions at various concentrations above and below the nominal concentrations were injected. A regression line of concentrations mg/ml Vs. Response (Peak area ratio) was obtained for Chlorpheniramine maleate. The linearity of calibration graphs and validated by high value of correlation coefficient and the S.D. for intercept value was less than 2%..The results obtained are shown in Table 1.The peak areas were plotted against the corresponding concentrations to obtain the calibration curve as shown in(Fig .3)

Sr.no.	Concentration (%)	Peak area
1	0.0	0.0
2	80	103364
3	90	116179
4	100	127902
5	110	142052
6	120	155835

Table 1: Linearity and range of Chlorpheniramine Maleate.

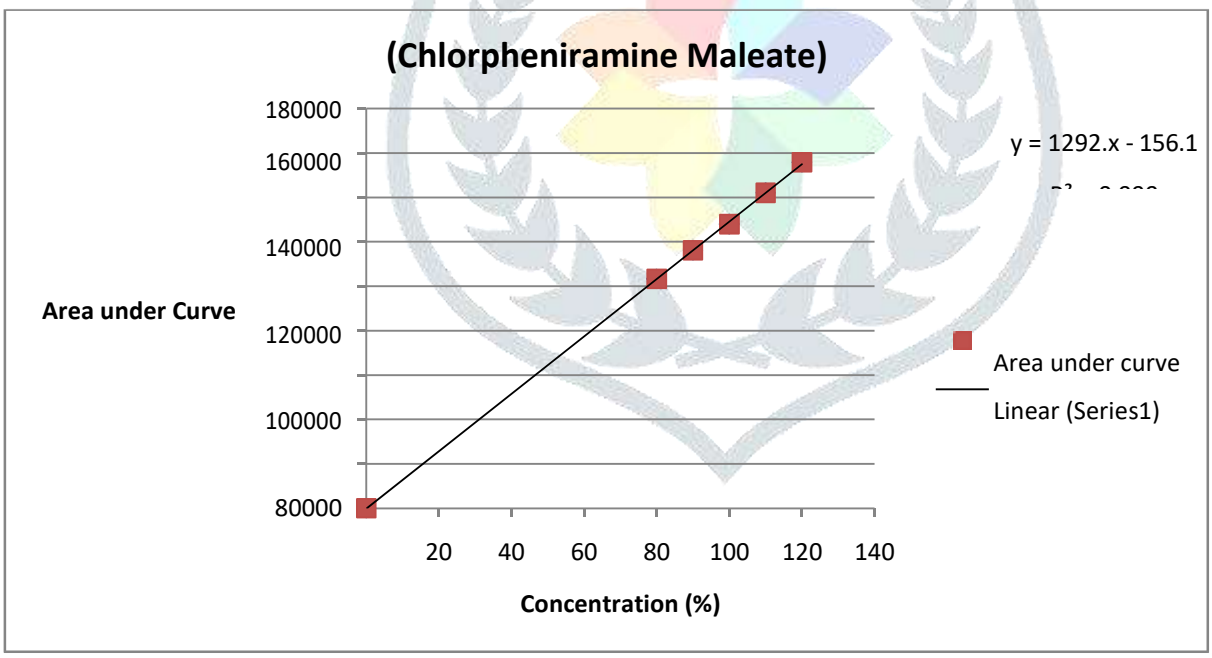

Fig.3: Linearity graph of Chlorpheniramine Maleate

Precision

The precision of the method was demonstrated by intra-day and inter-day variation studies.

1)System Precision: The % R.S.D. (retention time and peak area response) was verified by injecting concentration of both standard drugs in six replicate.

2)Method Precision: The % assay value of each sample and % RSD of average assay value calculated by injecting six replicate of sample solution.

For intraday precision and inter-day precision results obtained are shown in Table 2.

	Value	Acceptance Criteria
Method precision (Repeatability) N=6	**0.2576**	**% RSD < 2**
System precision N=6	**0.2**	
Intermediate Precision n=6		
Analyst 1 (Day 1)	**0.1447**	
Analyst 1 (Day 2)	**0.1866**	
Analyst 2(Day 1)	**0.211**	
Analyst 2(Day 2)	**0.1633**	

Table 2:Precision of Chlorpheniramine Maleate.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

From the linearity data the limit of detection and quantitation was calculated, using the formula LOD = 3.3 σ / S and LOQ = 10 σ / S where, σ = standard deviation of the response at lowest concentration in range and S = slope of the calibration curve. The LOD and LOQ were found to be 0.398 µg/ml and 1.2 µg/ml.

Accuracy

To check accuracy of the method, recovery studies were carried by spiking the standard drug to the sample solution at three different levels 80, 100 and .The results obtained are shown in Table 3.

Parameter	Value	Acceptance Criteria
Accuracy(Recovery Studies)		**98-102 % (individual)**
80%	**99.65**	
100%	**99.51**	
120%	**100.66**	

Table 3:Accuracy (Recovery Studies) of Chlorpheniramine Maleate.

Robustness

The critical parameters like, flow rate of Mobile Phase and mobile phase proportion as shown in the table were changed and carried out the analysis with each change in parameter. Change in parameters are shown in Table 4.

Parameter(%R.S.D)	Observation	Acceptance Criteria
A)Change in flow rate		
1)At 1.1ml/min	0.0369	NMT 2.00
2)At 1.5ml/min	0.1796	
B)Change in mobile phase composition Buffer:ACN:Methanol		
1)34:33:33	0.1371	NMT 2.00
2)36:35:29	0.3810	
C)Change in Injection Volume		
1)At 16ul	0.4742	N.M.T 2.00
2)At 24ul	0.9524	
D)Change in wavelength		
1)At 226 nm	0.4278	N.M.T 2.00
2)At 230nm	0.141	

Fig 4:Robustness for Chlorpheniramine Maleate

Conclusion

A simple, precise, reliable, sensitive and accurate stability indicating HPLC for Chlorpheniramine maleate has been developed according to ICH guidelines.

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REFERENCES

1.Khopkar SM. Basic concepts of Analytical Chemistry, 3rd ed., Newage International, 2008; 1-2,113-115.

2.Christian GD. Analytical Chemistry, 6th ed., John Wiley and sons (Asia) pvt. Ltd. Singapore, 2007; 1,5.

3.Skoog DA, West DM, Holler FJ, Crouch SR. Fundamental of analytical chemistry, 8th ed., Thomson Brooks/Cole. 2007;1-5.

4.History of analytical chemistry in the U.S.A., Talanta 1989, Vol 36, Iss1, p1-9.

5.Anjeneyulu Y, Chandrasekhar K, Manikam V. A textbook of Analytical Chemistry. Parma Book Syndicate, Delhi, 2005; 3-5,27-31.

6.Somenath M. Sample Preparation Techniques in Analytical Chemistry, 1st ed., Wiley Interscience New York 2003; Vol. 62:p13-16,105.

7.Sharma BK. Instrumental Methods of Chemical Analysis. Eleventh edition, Goel Publishing House, Meerut, 1991; 1-9.

8. HH, Merritt LL, Jr. Dean JA, Frank AS. Instrumental method of analysis, CBS publishers and Distributors, New Delhi, 7th ed., 1986; 1-5.

9.Londhe SV, Mulgund SV, Mallade PS, Jain KS. Ind. J. Pharm. Edu. Res. 2008; Vol.42, (4): 395-403.

10.Skoog DA, Holler FJ, Nieman TA. Principles of Instrumental Analysis. 6th ed., Thomson Brooks/Cole. 2007; 762-63,816-827.