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RP-HPLC Method Development and Validation on Betahistine Dihydrochloride Tablets

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Abstract:
To provide a high-performance liquid chromatography method for the accurate, precise, and specific quantification of Betahistine Dihydrochloride in dosage forms. Mobile phase comprising Monobasic Phosphate Buffer: Methanol (80:20) v/v ratio, was utilized with a C18 column (250mm×4.6mm) in isocratic mode. The UV detector was run at 250 nm with a flow rate of 1.0 ml/min. Betahistine Dihydrochloride had a retention time of 4.46 minutes and high linearity in the concentration range of 2.5 to 15 g/ml with a correlation coefficient of 0.99. The average recovery rate was discovered to be 99.98%. According to ICH recommendations Q2(R1), the developed technique complies with validation criteria including system appropriateness, assay, system suitability, linearity, precision, accuracy and robustness. The proposed method has been validated in accordance with guidelines set by the International Council for Harmonization and has been successfully applied to bulk and pharmaceutical dosage forms. It was found to offer faster retention time with sharp resolution and linearity at a lowest concentration than previous methods.

Keywords: RP-HPLC, Betahistine Dihydrochloride, Method development, Validation, Tablet.

INTRODUCTION:

An anti-histaminic medication that is taken orally is Betahistine Dihydrochloride. Betahistine is also known chemically as N-methyl-2-(pyridin-2-yl)-ethanamide; dihydrochloride for histamine H3 receptors, betahistine has a relatively strong affinity, whereas for histamine H1 receptors, it has a weaker affinity. It has been used to treat vertigo in Meniere's disease patients; it may work by widening blood vessels in the

inner ear. [1,2] Betahistine Dihydrochloride can be estimated using a variety of ways. The liquid chromatography method is described for the analysis of Betahistine Dihydrochloride in the Indian Pharmacopoeia and British Pharmacopoeia. [3,4] One HPLC and one liquid chromatographic method are available for determining betahistine dihydrochloride in pharmaceutical preparations, according to a review of the literature. To the best of our knowledge, there has not been a reported spectrophotometric method for determining betahistine dihydrochloride in pharmaceutical formulations before the completion of our investigation. As a result, efforts are being made to create an analytical approach that is quick, focused, and sensitive for estimating betahistine dihydrochloride in tablet formulations. [5,6]

MATERIALS AND METHODS:

Betahistine Dihydrochloride was Gifted as a sample from Flamingo Pharmaceuticals, Krushnur MIDC, Nanded. HPLC grade water, Monobasic Phosphate Buffer, HPLC grade Methanol and KOH was provided by Research Lab Fine Chemical Industries, Mumbai 400 002(India).

Instruments:

During the analysis, a RP-HPLC system with a PDA detector was used. Open Lab CDS version 2.7 software was utilized to collect the data. The TC-C18 250mm×4.6mm, utilized as a stationary phase. Weighing the contents was done using a Contech (CAH - 223) electro-weighing balance.

Preparation of Mobile Phase:

The mobile phase for the chromatographic elution was composed of Monobasic Phosphate Buffer: Methanol (80:20) v/v ratio, and the column temperature was held constant at room temperature. With a 7-minute runtime, the analysis was conducted at a flow rate of 1.0 ml/min..

Chromatographic Parameters:

Diluent	: HPLC grade water
HPLC System	: Agilent Infinity II 1260
Analytical Column	: TC-C18 RP (250 x 4.6 mm, 5µ)
Mobile Phase	: Monobasic Phosphate Buffer: Methanol (80:20)
Flow Rate	: 1.0 ml/min
Pump Mode	: Isocratic
Injection Volume	: 50 µl
Detection Wavelength	: 250 nm
Detector	: PDA Detector
Column Temperature	: Room Temperature
Run time	: 7 min

Preparation of Standard Stock Solution:

- Accurately weigh 50 mg of Betahistine Dihydrochloride standard and transfer it into a 100 ml volumetric flask. Add 50 ml of diluent to the volumetric flask containing the Betahistine Dihydrochloride standard. Filter the solution through filter paper No.41. Make up the final volume up to the mark on the volumetric flask using the diluent.
- Pipette out 10 ml of the above-prepared solution and transfer it into another 100 ml volumetric flask. Make up the volume to the mark on the volumetric flask with diluent.

Method development and Validation:

Assay Preparation of Commercial preparation:

- Weigh ten tablets and calculate the average weight of the tablets. Crush the tablets into a fine powder. The amount of tablet powder equivalent to 50 mg of Betahistine Dihydrochloride is weighed and transferred into a 100 ml volumetric flask. Filter the solution containing the tablet powder through filter paper No.41 to remove any solid particles or impurities.
- Pipette out 10 ml of the filtered solution and transfer it into another 100 ml volumetric flask. Make up the volume to the mark on the volumetric flask with diluent. Inject the prepared solution once into the HPLC system for analysis.

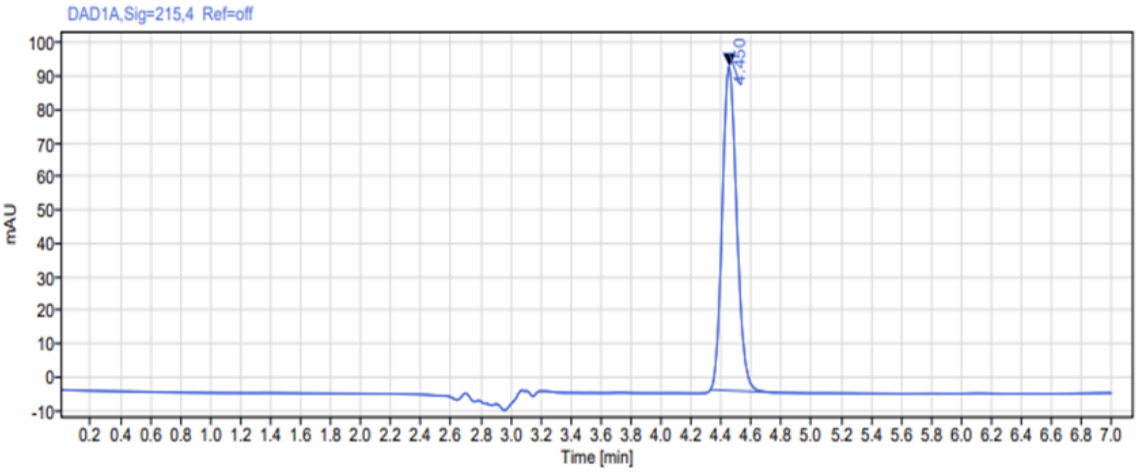

Fig.1: Chromatogram of Standard sample

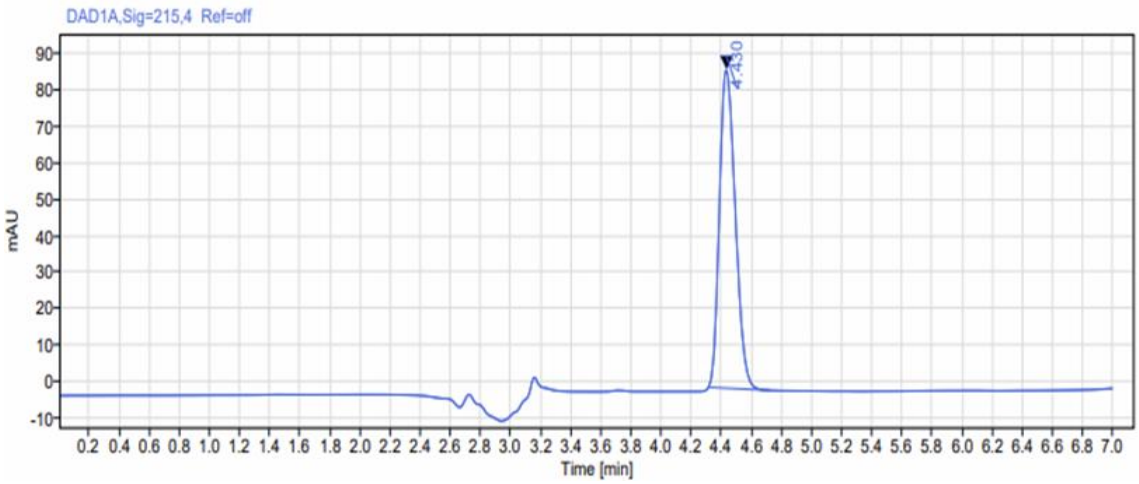

Fig.2: Chromatogram of Marketed formulation

Validation:

System Suitability:

A working standard solution of Betahistine Dihydrochloride was produced according to protocol and administered five times into the HPLC system. By determining the % RSD of retention time, tailing factor, theoretical plates, and peak areas from five replicate injections, standard Chromatograms were used to evaluate the system suitability characteristics. The results are displayed in Table 1.

Specificity:

The capacity to distinguish between the target analyte and other components in the sample is known as specificity. The method's specificity was covered by the connection of the standard, sample, blank, and placebo chromatograms.

Linearity and Range:

Inject 6 standard solutions containing at Betahistine Dihydrochloride doses ranging from 25% to 150% to show the assay method's linearity. Create a graph that shows peak area versus concentration. The acquired Y-intercept was 158.51, the obtained correlation coefficient was 0.99, the produced linearity plot was given in Fig. 5, and the obtained findings were in Table3.

Accuracy:

Three injections of 50%,80%, 100%,120% and 150% of the concentrations were made in triplicate, and the percentage of recovery came to 101.43. Table 4 presented the findings.

Precision:

Six working sample solutions containing 50 ppm were injected, and after calculating the amount obtained, the %RSD was discovered to be 0.75%. The results were displayed in Table 5.

Robustness:

Small intentional changes are introduced to the procedure, such as Flow minus, Flow plus. The above conditions' %RSD are calculated and displayed in the table 6 and 7.

Solution stability:

Standard and sample solutions were analysed for 24 hours at room temperature to show the stability of the solutions during analysis.

RESULTS AND DISCUSSION:

Table No.1: System suitability of Betahistine Dihydrochloride

Sr. No.	Surface Area	Retention Time (min)
1.	625.071	**4.433**
2.	637.981	**4.430**
3.	643.583	**4.430**
4.	653.443	**4.426**
5.	656.249	**4.431**
STDEV	**12.56**	
% RSD	**1.953%**	
Tailing Factor	1.22	

The System suitability parameters meet the acceptance criteria. When conducting an hplc analysis or any analytical testing, ensuring that the system is suitable.

Table No.2: Specificity Data for Betahistine Dihydrochloride

Sr. No.	Standard Area	Standard R.T.	Sample Area	Sample R.T.	% Assay
1	**738.985**	**4.464 min**	**738.684**	**4.464**	**100.66**

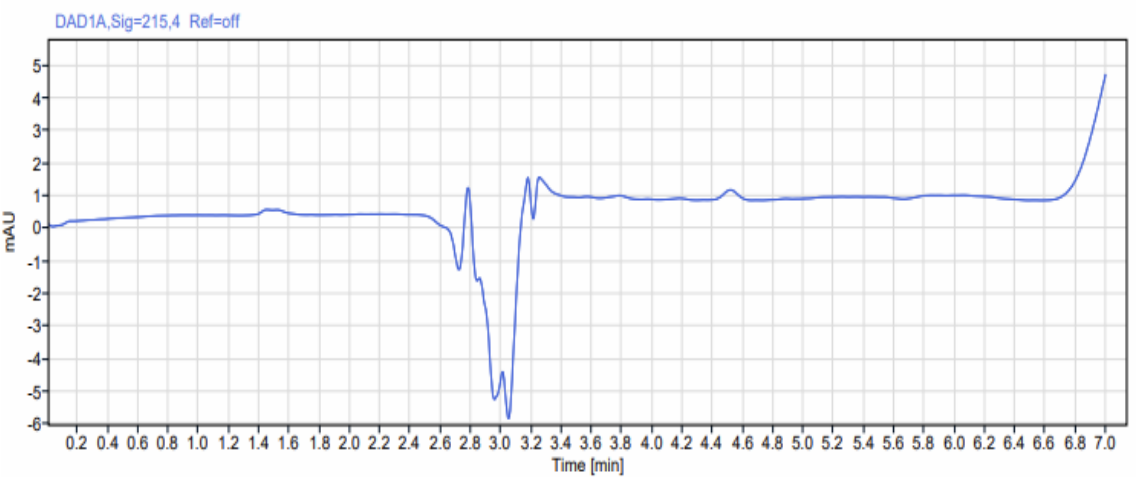

Fig.3: Specificity Blank Chromatogram

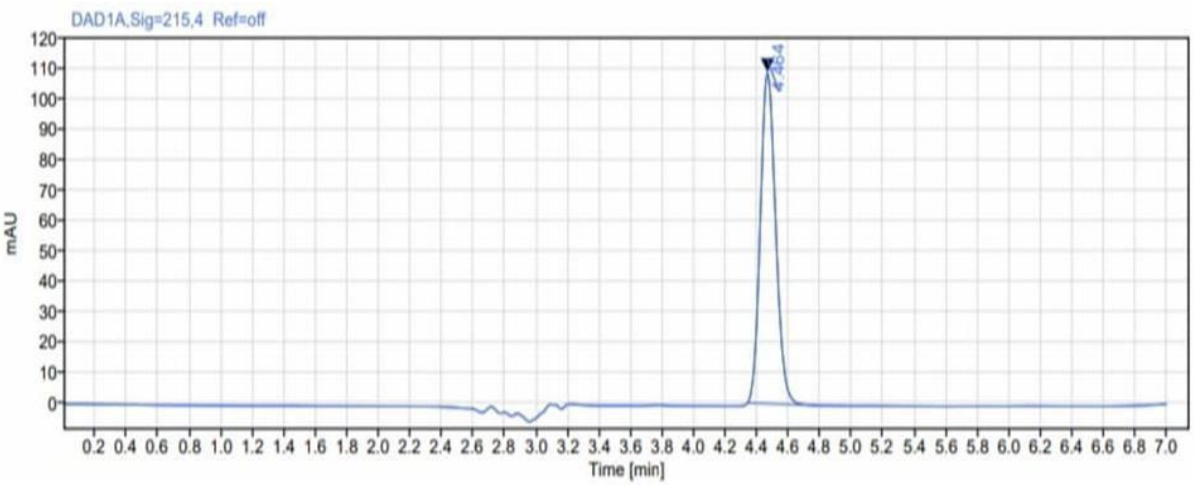

Fig.4: Specificity Sample Chromatogram

The chromatogram obtained from the sample solution of Betahistine Dihydrochloride shows similarity to the Betahistine Dihydrochloride standard solution. There is no interference of blank at the Retention time of main peak.

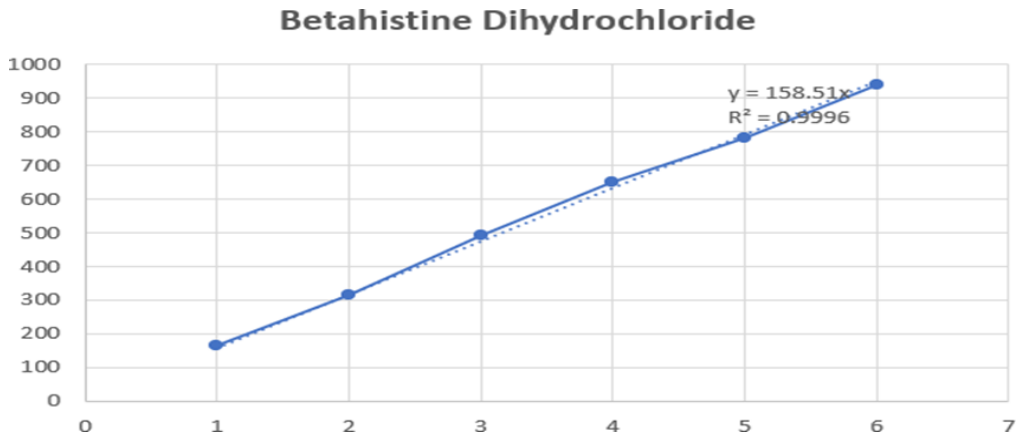

Fig.5: Calibration Curve Graph of Betahistine Dihydrochloride

Table No.3: Data sheet for Linearity

Linearity Level	Standard Conc.(ppm)	Mean Peak Area	Statistical Analysis
Level-1	12.5 ppm	164.375	**Correlation Coefficient (r^2) = 0.99**
Level-2	25 ppm	315.718	
Level-3	40 ppm	492.764	
Level-4	50 ppm	650.712	
Level-5	60 ppm	781.095	
Level-6	**75 ppm**	**940.306**	

Table No.4: Data for Accuracy of Betahistine Dihydrochloride

% Level	Set	Amount Added mg	Amount Recovery	% Recovery	Mean	SD	% RSD
50 %	1	23.5	23.90	101.73	101.40	0.427	0.43
	2	21	21.32	101.56			
	3	21	21.16	100.92			
80 %	1	32.8	32.30	98.50	99.13	0.842	0.85
	2	33.20	33.23	100.09			
	3	34.4	33.99	98.81			
100 %	1	49	49.49	101.00	102.14	0.99	0.96
	2	50	51.36	102.72			
	3	49.80	49.82	102.71			
120 %	1	60	60.26	100.44	101.73	1.12	1.11
	2	60	61.36	102.28			
	3	59.4	60.87	102.48			
150 %	1	75	76.26	101.69	102.06	0.54	0.54
	2	74.25	75.59	101.81			
	3	75	77.01	102.69			

Mean recovery is 101.43 % and %RSD is 0.929 % therefore, the HPLC method for the determination of Assay of Betahistine Dihydrochloride Injection is accurate.

Table No.5: Method Precision of Betahistine Dihydrochloride

Sample No.	Sample Area	%Assay
1.	682.652	**100.01**
2.	687.118	**100.67**
3.	692.734	**100.85**
4.	696.891	**101.62**
5.	698.868	**101.95**
6.	701.160	**101.79**
	Average	101.14
	STDEV	0.760
	%RSD	0.75

The %RSD of the method precision is 0.75 % therefore, the HPLC method for the determination of Betahistine Dihydrochloride is precise.

Table No.6: Robustness data for Betahistine dihydrochloride (For flow rate 0.8ml/min)

Flow Rate	Sr. No.	Surface Area	Retention Time
0.8ml/min	1.	918.873	**5.629**
	2.	923.198	**5.618**
	3.	943.988	**5.612**
	4.	913.567	**5.610**
	5.	942.121	**5.615**
	RSD	1.494%	

Table No.7: Robustness data for Betahistine dihydrochloride (For flow rate 1.2ml/min)

Flow Rate	Sr. No.	Surface Area	Retention Time
1.2ml/min	1.	608.149	**3.713**
	2.	624.280	**3.710**
	3.	629.449	**3.718**
	4.	616.954	**3.704**
	5.	633.053	**3.711**
	%RSD	1.067%	

The difference for each modified condition and original condition is within the limit. Hence, the method is robust.

Table No.8: Solution stability data for Betahistine Dihydrochloride

Sr. No.	Name	Area	Assay	%Relative Change	%Absolute Value
1.	Standard Solution-0 hours	745.27	NA	2.47	
2.	Standard Solution-24 hours	747.89	NA		
3.	Sample Solution-0 hours	726.85	97.52		**1.39**
4.	Sample Solution-24 hours	739.76	98.91		

Standard and sample solutions are stable for 24 hours at room temperature.

CONCLUSION:

It could be concluded that the validated RP HPLC method has been developed and is precise, easy to use, accurate, affordable, sensitive, and reproducible for the quantitative estimation of betahistine dihydrochloride in bulk and its formulation. We also came to the conclusion that this RP HPLC method is practical and efficient for research studies, quality control, and routine analysis of betahistine dihydrochloride.

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