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IJCPA, 2014; 2(1): 18-22

International Journal of CHEMICAL AND PHARMACEUTICAL ANALYSIS

ISSN: 2348-0726

Research Article

RP-HPLC Method Development and Validation for Betahistine Hydrochloride Controlled Release Tablets

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Received: 2 August 2014 / Revised: 3 September 2014 / Accepted: 6 November 2011 / Online publication: 30 December 2014

ABSTRACT

A new simple isocratic RP-HPLC method was developed for the determination of Betahistine Hydrochloride in controlled release tablet formulation. The separation was achieved on Kromasil C18 column (250 mm x 4.6 mm, 5µm) in an isocratic elution mode. The mobile phase consisted of Buffer: Acetonitrile in the ratio of 60: 40. The column temperature was maintained at 25°C. The detection of eluent from the column was detected using UV detector at 260nm and the flow rate was maintained at 1.5 ml/min. The method was validated in terms of system suitability, specificity, accuracy, precision, linearity, filter compatibility and solution stability.

Keywords: Betahistine Hydrochloride, Controlled release tablets, Isocratic elution, RP-HPLC method, Method validation.

1. INTRODUCTION

Betahistine hydrochloride is an orally administered anti-histaminic drug. The chemical name of Betahistine is *N*-methyl-2-(pyridin-2-yl)-ethanaminehydrochloride. It was first registered in Europe in 1970 for the treatment of Meniere's disease (disorder of the inner ear that can affect hearing and balance to a varying degree.). This drug is also used in the treatment of vertigo and balance disorders. Betahistine has a very strong affinity for histamine H_3 receptors and a weak affinity for histamine H_1 receptors and it increases endogenous histamine production. It possibly acts by causing vasodilation in the internal ear. The daily dose is 24-48mg. The drug is hygroscopic and is affected on exposure to sunlight.

Betahistine hydrochloride is available in both tablet form and as an oral suspension. It is rapidly and completely absorbed. The plasma half-life of the drug is 3-4 hours and it shows very low plasma protein binding. The excretion of Betahistine hydrochloride is virtually complete in the urine within 24 hours. The release rate of Betahistine was found to be 80% in 12 hours.[1-4]

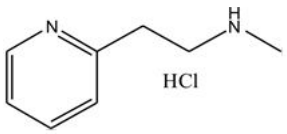

Fig.1: Chemical Structure of Betahistine Hydrochloride

The analytical methods used previously for the estimation of Betahistine are reported in literature[5, 6]. The objective of the present study was:

1) To develop and validate an HPLC Assay method for the estimation of Betahistine Hydrochloride in controlled release tablet dosage form.
2) To develop a simple, sensitive, rapid, accurate, linear and precise analytical method for estimation of Betahistine Hydrochloride in control release tablet dosage forms.

2. MATERIALS AND METHODS

2.1 Chemicals

Pure standard and controlled release tablets of Betahistine (BHT) hydrochloride was obtained from CIPLA Ltd (Mumbai,

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India.). HPLC grade acetonitrile, Sodium Dihydrogen Orthophosphate monohydrate, Sodium Dodecylsulphate and triethanolamine were obtained from E. Merck New Delhi, India. High purity deionised water was obtained from a Millipore, Milli-Q purification system.

2.2 Equipment

Waters HPLC system equipped with UV detector was used throughout the analysis. The data was acquired using Empower software. The analytical column Kromasil C18 (250mm x 4.6mm; 5µ) was used as a stationary phase. Sartorius electronic balance (Model no. 2842) was used for weighing the contents.

2.3 HPLC Method development

The chromatographic conditions were optimized by different means. (Using different column, different buffer and different mode of HPLC run).

1) The Zorbax SB phenyl, 4.6mm x 15cm, 3.5µm column was used for the study based on literature survey. The retention time of Betahistine Hydrochloride using Zorbax SB phenyl, 4.6mm x 15cm, 3.5µm column was long.
2) The Kromasil C18 column (250 mm x 4.6 mm, 5µm) used later used which yielded shorter retention times. Hence, the Kromasil C18 column (250 mm x 4.6 mm, 5µm) was used for further analysis.
3) A wavelength of 260 nm was selected after scanning the solution of the drug in the UV visible region of 200-400nm. The wavelength of maximum absorbance (260nm) was selected for further analysis.
4) Buffer: ACN was selected as the mobile phase as the drug as it showed efficient separation and of the drug. Initially, a ratio of 70:30 was used, but the retention time with this ratio was long. When the ratio was changed to 60:40, efficient separation and shorter retention times were noted.
5) The injection volume was maintained at 50µl throughout the study. A flow rate of 1.5ml/min was employed to give better separation and resolution.

Various HPLC method development trials taken are summarized in table-1.

Table 1: Method development trials

Trials taken	Observation	Inference	Present method	Observation
Column: Zorbax SB phenyl, 4.6mm x 15cm, 3.5µm, Flow rate: 1.50ml/min, Injection Volume: 50µl, Concentration: 25ppm, Mobile phase: Buffer: ACN (70:30).	The main peak was eluting at about 7 minutes. Peak shape was not good.	Different column was required in order to improve the peak shape.	Column: Kromasil C18 column (250 mm x 4.6 mm, 5µm) Flow rate:1.50ml/min Injection Volume:50µl Mobile phase: Buffer: ACN (60:40)	The main peak was eluting at 2.9 minutes and good peak shape was observed.
Column: C18 column, Flow rate: 0.50ml/min, Injection Volume: 50µl, Concentration: 25ppm, Mobile phase: Buffer: ACN (70:30).	The main peak eluted at 5 minutes. Peak shape was not good.	A different column was required to improve the peak shape. Also, the mobile phase concentration had to be changed.	Kromasil C18 column (250 mm x 4.6 mm, 5µm) Flow rate:1.50ml/min Injection Volume:50µl Mobile phase: Buffer: ACN (60:40)	The main peak was eluting at 2.9 minutes and good peak shape was observed.

2.4 Chromatographic conditions

The chromatographic elution was carried out in isocratic mode using a mobile phase consisting of Buffer: Acetonitrile in the ratio of 60:40 v/v and the column temperature was maintained at 25°C. The analysis was performed at a flow rate of 1.5 ml/min with a run time of 8 min. The eluent was monitored at wavelength of 260 nm. 50µl volume of the sample was injected by auto sampler.The optimized chromatographic parameters are summarized in table-2.

Table 2: Optimized chromatographic conditions

Column	Kromasil C18 column (250 mm x 4.6 mm, 5µm)
Flow rate	1.50ml/min
Injection Volume	50µl
Detection wavelength	260nm
Column oven temperature	25^0C
Run time	8 mins
Retention time	2.9 mins
Mobile phase	**Buffer: ACN (60:40)**

2.5 Preparation of the buffer

The buffer was prepared by dissolving 4.6 gm of Sodium Dihydrogen Orthophosphate monohydrate and 2.7 gm of Sodium Dodecylsulphate in 1000 ml of water.

2.6 Preparation of mobile phase

A mixture of Buffer: Acetonitrilewas used for the preparation of the mobile phase in the ratio of 60:40 v/v. The pH was adjusted to 7.5 using triethanolamine. The contents of the mobile phase were filtered before use through a 0.45µm membrane filter, sonicated and pumped from the solvent reservoir to the column at a flow rate of 1.5 ml/min.

2.7 Preparation of standard solutions

For the preparation of the standard solution, 25 mg of the active drug Betahistine Hydrochloride was accurately weighed and transferred to a 50 ml volumetric flask. About 10 ml of methanol was added to the flask and the mixture was sonicated for about 15 minutes. The volume was made up to 50 ml with methanol. Further, 5 ml of this solution was transferred to a 100 ml volumetric flask and the volume was made up to 100 ml with mobile phase. Thus, a 25 ppm standard solution was prepared.

2.8 Preparation of sample solutions

About 20 tablets consisting of Betahistine Hydrochloride as the active constituent were weighed and powdered. 50 mg of this powder was addedto a 500 ml volumetric flask followed by 200 ml of methanol. The solution was sonicated for about 30 minutes and allowed to attain room temperature. Further250 ml of the diluent (mobile phase) was added and the solution was stirred magnetically for 30 minutes. The volume was made up to the mark with the diluent. The solution was then filtered through a 0.22µ PVDF Whatman syringe filter. Further 5 ml of the filtered solution was taken and added to a 20 ml volumetric flask. The volume was made up to the mark with the diluent.

2.9 Method validation

The developed method was validated as per USP and ICH guide lines. [7]

2.9.1 System suitability

To know reproducibility of the method system suitability test was employed to establish the parameters such as tailing factor, theoretical plates, retention time and area.

2.9.2 Specificity

The results indicated the method was highly specific for Betahistine hydrochloride.

2.9.3 Accuracy

Accuracy test was done by adding a known amount of the drug under determination (3 levels 50%, 100% and 150%) to a weighed amount of placebo. Two standards were also prepared. Two replicates of each of the three levels were done preceded by the standards.

2.9.4 Linearity

The calibration curve was obtained at 3 concentration levels (1-3) of Betahistine Hydrochloride standard solution in the range of 12.76-38.28 ppm. The solutions (50µl) were injected in triplicate into chromatographic systems. For calculation of linearity, peak area and concentrations were subjected to least square regression analysis to calibrate equation and correlation coefficient.

2.9.5 Precision

Precision was determined as repeatability in accordance with ICH guidelines. The precision were determined by analysing the samples of Betahistine controlled release tablets at a concentration of 25µg/ml. Determination was performed with six replicates during the same day.

2.9.6 Stability of solutions

To demonstrate the stability of standard solutions during analysis, they were analysed over a period of 24h at room temperature.

2.9.7 Filter Compatibility Study

The filter compatibility study was done to check the compatibility of the filters used for analysis. The test was performed by recording the difference between the filtered solution and the unfiltered solution of the standard as well as the dosage form.

3. RESULTS AND DISCUSSION

The system suitability test parameters for Betahistine Hydrochloride controlled release tablets are shown in Table 3.

Table 3: System suitability parameters

Parameters	System suitability
Retention times (RT)	2.915 min
Placebo	NA (no interference)
HPLC Plate count (USP)	2753
Tailing factor	1.8
Area	604737

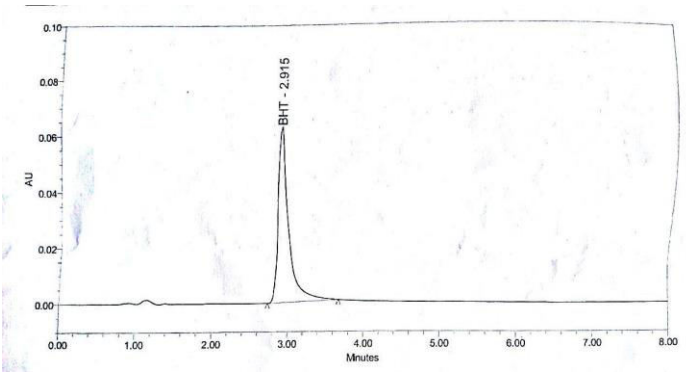

Fig.2: Betahistine Hydrochloride final developed method chromatogram

No interference peaks were found in chromatogram, indicating that the estimation of drug free from interference of blank and placebo. The results of specificity are shown in Table 4.

Table 4: Specificity results

Sample name	Retention time(RT)	Area (µAU)	% Interference
Blank	-	-	No Interference
Sample	2.915	604737	No Interference

The blank solution does not show interference at the retention time of Betahistine Hydrochloride. The values of accuracy study are shown in Table 5.

Table 5: Accuracy

Sample name	Concentration (ppm)	Area (µAU)	Amount
12.5 ppm 1	12.5	340361	99.81
12.5 ppm 2	12.5	341461	99.97
25 ppm 1	25	594911	98.39
25 ppm 2	25	611229	100.18
37.5 ppm 1	37.5	1048381	102.70
37.5 ppm 2	37.5	1064726	101.53

The summary of Linearity parameters for Betahistine Hydrochloride standard in the proposed HPLC Method is depicted in Table No. 6 and the calibration graph is shown in Fig. 3.

It was found to be linear with a correlation coefficient (r^2) of 1, the corresponding linear regression equation being y = 24107x - 47687.

Table 6: Linearity

Sr. No	Concentration (ppm)	Mean area
1	12.76	263264
2	25.52	560818
3	38.28	878467
	Slope Intercept	24107 47687

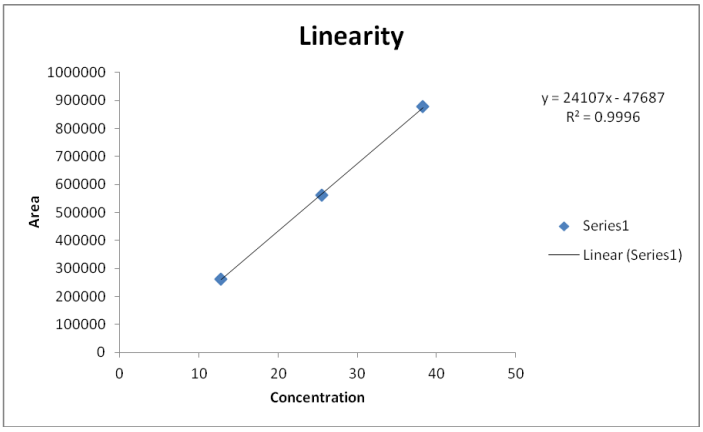

Fig.3: Linearity Curve for Betahistine hydrochloride

The data obtained for the precision study is shown in Table 7. The RSD for the content of Betahistine Hydrochloride for the samples of precision study should be less than 2.00%.

Table 7: Precision

Sample name	Area (µAU)	Amount
Sample 1	588000	102.02
Sample 2	589121	102.21
Sample 3	589378	102.16
Sample 4	590360	102.48
Sample 5	590874	102.53
Sample 6	591407	102.61
Average SD % RSD		102.34 0.24 0.23

Stability data of Betahistine Hydrochloride controlled release tablets are summarized in Table No. 8. The results showed that for both the standard as well as the dosage formulation, the retention time and peak area of Betahistine Hydrochloride remained almost unchanged and no significant degradation was observed during this period, suggesting that both solutions were stable for at least 24 h, which was sufficient for the whole

analytical process. The % relative difference for the assay of Betahistine Hydrochloride in solution stability obtained between initial and 24 hours was found to be less than 3.00%.

Table 8: Solution stability

Time in min	Area found	% Relative difference
0	1110258	0.0
2	1109318	0.08
4	1109458	0.07
6	1109246	0.09
8	1108639	0.15
24	1136127	2.28

The results of Filter compatibility studies of Betahistine Hydrochloride controlled release tablets are shown in Table 9. The difference was not more than two percent.

Table 9: Filter compatibility

Sr. No.	Sample name	Area	% difference
1	Sample unfiltered	575141	0.6845%
2	Sample filtered	579078	

4. CONCLUSION

An isocratic RP-HPLC stability indicating method developed for determination of Betahistine Hydrochloridewas simple, economic, rapid, precise, linear, accurate and specific. The method was validated as per ICH guidelines, and validation acceptance criteria were met in all cases. The method can be used for routine analysis and for assessing the stability of Betahistine Hydrochloride.

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