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Research Article

DISSOLUTION METHOD DEVELOPMENT FOLLOWED BY SPECTROPHOTOMETRIC ANALYSIS AND VALIDATIONOFBETAHISTINE HYDROCHLORIDE CONTROLLED RELEASE TABLETS

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ABSTRACT

The aim of this work was to develop and validate a dissolution test for Betahistine Hydrochloride in controlled release tablet dosage form by using UV visible spectrophotometric method. The dissolution established conditions were: 900 mL of phosphate buffer pH 6.8, using a paddle apparatus at a stirring rate of 100 rpm. The drug release was evaluated by UV visible spectrophotometry method at 260 nm. The method was validated as per the ICH guidelines. The validation included accuracy, precision and linearity. In addition, filter compatibility and drug solution stability in medium were demonstrated.

Keywords: Dissolution, Validation, Betahistine Hydrochloride tablets.

1. INTRODUCTION

Betahistine hydrochloride is an orally administered anti-histaminic drug. The chemical name of Betahistine is *N*-methyl-2-(pyridin-2-yl)-ethanamine hydrochloride. 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. Betahistine hydrochloride has a very strong activity as an antagonist for histamine H_3 receptors and a weak affinity as an agonist for histamine H_1 receptors.[1-4]

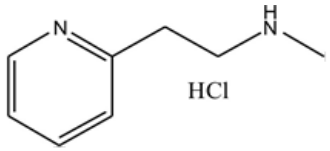

Fig.1. Chemical structure of Betahistine hydrochloride

2. EXPERIMENTAL WORK

2.1 Materials

Pure standard and controlled release tablets of Betahistine (BHT) hydrochloride was obtained from CIPLA Ltd (Mumbai, India.). Analytical grade methanol was obtained from E. Merck New Delhi, India. High purity deionised water was obtained from a Millipore, Milli-Q purification system. The buffer solution was prepared according to the USP Pharmacopoeia.

2.2 Equipment

Dissolution test was performed in a Lab India dissolution test system (n=6 and n=12), in accordance to USP Pharmacopoeia general method. The mediums were vacuum degassed under house vacuum and were maintained at 37.0 ± 0.5°C by using a thermostatic bath. Shimadzu UV 1700 double beam spectrophotometer was used throughout the analysis. The spectral bandwidth was 1 nm and the path length was 1 cm. Electronic balance was used for weighing the contents.

2.3.1 Dissolution Method Development

Dissolution studies were performed on Betahistine Hydrochloride controlled release tablets and the aliquots were analysed by UV and HPLC. The following steps were involved in the dissolution testing of Betahistine Hydrochloride tablets:

a. Selection and optimization of the dissolution medium.

The most crucial step in carrying out dissolution studies is the selection of the appropriate dissolution medium. The following trials were performed for the selection of the dissolution medium:

Table No. 1: Dissolution Medium Optimization

Trial number	Dissolution medium used	Percentage of drug released.
1	900 ml of distilled water.	55%
2	500 ml of distilled water.	59%
3	900 ml of citrate buffer pH 6.8.	70%
4	900 ml of phosphate buffer pH 6.8.	80%

b. Selection of the dissolution method:

Phosphate buffer with pH 6.8 was selected as the appropriate medium for the dissolution testing. The dissolution method was optimized as follows:

Table No. 2: Dissolution Method Optimization

Sr. No.	Time period for which the testing was done	Rotations per minute	Percentage of drug released
1	2 hours	100	59
2	4 hours	100	75
3	6 hours	100	84
4	8 hours	100	88

After 8 hours of dissolution testing, more than 80% of the drug (Betahistine Hydrochloride) was released from the Betahistine Hydrochloride controlled release tablet dosage form, which was optimum for performing further analysis.

The final optimized parameters for dissolution testing are as mentioned in table no.3.

Table No. 3: Final optimized method for dissolution

Medium selected	Volume of the medium	pH of the medium	Time period for which the dissolution testing was performed	Rotations per minute
Phosphate buffer	900 ml	6.8	8 hours	100

2.3.2 Development of dissolution test conditions

Dissolution is an official test used by pharmacopoeias for drug evaluation release of solid and semisolid dosage forms, and it is routinely used in Quality Control (QC) and Research & Development (R&D). The purpose of in vitro dissolution studies in QC is batch to batch consistency and detection of manufacturing deviation while in R&D the focus is to provide some predictive estimate of the drug release in respect to the in vivo performance of a drug product. For QC, an over-discriminatory test might be suitable to detect even small production deviations. However, for prediction of the in vivo performance of drug product a dissolution test should be sensitive and reliable.

2.3.3 Dissolution test conditions

Drug release tests were carried out according to conventional dissolution procedures recommended for single-entity products, using paddle (USP Apparatus 2) at 100 rpm. Sampling aliquots of 10.0 mL were withdrawn at 2, 4, 6 and 8 hours. After the end of each test time, samples aliquots were filtered, diluted in dissolution medium, when necessary, and quantified. The cumulative percentage of drug released was plotted against time, in order to obtain the release profile and to calculate the in vitro dissolution data. Betahistine Hydrochloride stability in dissolution medium was evaluated at 37.0 ± 0.5ºC for 0 hr, 2 hr, 4 hr, 6 hr, 8 hr and 24 hrs. The filtration procedure of Betahistine Hydrochloride tablets was carried out using a 0.22 µm PDVF membrane filter. The absorbances of filtered and unfiltered (centrifuged) solutions were evaluated.

2.3.4 Preparation of Solutions

Preparation of the dissolution medium:

The dissolution medium (6 litres) was prepared by adding 1500 ml of Anhydrous Sodium Hydrogen Orthophosphate (0.2M) and 700 ml of Sodium hydroxide solution to 3800 ml of distilled water. The pH was adjusted to 6.8 using Sodium Hydroxide.

Preparation of the standard solution:

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 the diluent (dissolution medium). Further, 5 ml of this solution was transferred to a 100 ml volumetric flask and the volume was made up to 100 ml with the diluent. Thus, a 25 ppm standard solution was prepared.

Preparation of the sample solution

The sample solution was obtained by performing dissolution studies. Six units of the dosage formulation were added to six different bowls of the dissolution tester. A weighed amount of placebo was also added to a separate bowl. Dissolution studies were performed using phosphate buffer pH 6.8 for a period of 8 hours and a speed of 100 rpm was maintained throughout the study. After 8 hours, the aliquots obtained were filtered using PVDF syringe filter. This solution was used for further analysis.

Preparation of the placebo

A weighed amount of placebo was also added to a separate bowl in the dissolution tester. Dissolution studies were performed using phosphate buffer pH 6.8 for a period of 8 hours and a speed of 100 rpm was maintained throughout the study. After 8 hours, the aliquot obtained was filtered using PVDF syringe filter. This placebo solution was used for further analysis.

2.3.5 Method validation

UV/VIS spectrophotometry and high performance liquid chromatography are the most commonly used analytical methods used for quantifying drug release in dissolution tests. The UV spectrophotometric method may be used if drug has a UV chromophore and no UV interferences due excipients used in the formulation are observed. This method has the advantage of very rapid time of analysis and the relative low cost for the routine quality control. In all cases, the absorbance was measured at 260 nm.The UV visible spectrophotometric method used to analyze the Betahistine Hydrochloride controlled release tablet samples in phosphate buffer pH 6.8 dissolution medium was validated for specificity, linearity, precision and accuracy, according to USP and ICH guidelines. [5]

a) Accuracy

Accuracy test was done by adding a known amount of the drug under determination (4 levels 10%, 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. The percent recovery was calculated. The percent recovery for dissolution studies should be between 95% and 105%.

Preparation of the stock and solutions for accuracy:

About 225 mg of Betahistine Hydrochloride standard was weighed and transferred into a 100 ml volumetric flask. About 30 ml of diluent (dissolution medium) was added and the flask was sonicated for about 10 minutes to dissolve the drug and the volume was made up with the diluent (2250 ppm). The Accuracy was determined by preparing different concentrations of standard stock solution ranging from 10%, 50%, 100% & 150%. Further dissolution studies were performed using the prepared solutions.

Procedure for dissolution studies

Dissolution was carried out in 900 ml of phosphate buffer pH 6.8 for a period of 8 hours and a speed of 100 rpm was maintained throughout. A 12 bowl Lab India Dissolution tester was used. Each of the four levels, prepared in duplicated were tested. The solutions were added to the specified volume of the dissolution medium (900 ml) and a weighed amount of placebo was added to each bowl. Also, the placebo was added in a separate bowl to 900 ml of the dissolution medium. After 8 hours, the aliquots were taken and filtered with PVDF syringe filter and analysed using Ultra Violet Visible Spectroscopy.

b) Linearity

The calibration curve was obtained at 4 concentration levels (1-4) of Betahistine Hydrochloride standard solution in the range of 2.52-38 ppm. For calculation of linearity, peak area and concentrations were subjected to least square regression analysis to calibrate equation and correlation coefficient.

Preparation of Linearity Stock:

Accurately about 25mg of Betahistine Hydrochloride standard was weighed and transferred into a 50 ml volumetric flask. About 30 ml of diluent (dissolution medium) was added and the flask was sonicated to dissolve the drug and the volume was made up with diluent (500 ppm). The prepared 500 ppm stock was used for further dilutions.

Procedure

Level 1 was analysed in triplicate, level 2 and level 3 in duplicate and level 4 in triplicate. The absorbance was recorded. The mean absorbance of each level was considered for the determination of linearity. A graph of Concentration (ppm) v/s Absorbance was plotted. The linear regression coefficient, slope of regression and the % Y-intercept was determined.

c) Precision

Precision studies were performed with six solutions of the sample of equal strength. Six units of the dosage formulation were added to six different bowls of the dissolution tester. Dissolution studies were performed using phosphate buffer pH 6.8 for a period of 8 hours and a speed of 100 rpm was maintained throughout the study. After 8 hours, the aliquots obtained were filtered using PVDF syringe filter. For precision studies, six readings of the solution from the first bowl were recorded. The absorbance of the aliquots from the first bowl and the remaining five bowls was also recorded. The relative standard deviation of the absorbance of the first solution (n=6) as well as all the six solutions was calculated.

d) Stability of solutions

To demonstrate the stability of standard solutions during analysis, they were analysed over a period of 24h at room temperature. The results showed that for both the standard as well as the dosage formulation, the absorbance 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 percent difference between the initial reading and the reading at 24 h should not be more than 3%.

To demonstrate the stability of dissolved sample solutions during analysis, they were analysed over a period of 24 hours at room temperature. The aliquots obtained by dissolution testing at the end of 2 hours, 4 hours, 6 hours, 8 hours and 24 hours were analysed. The results showed that for both the standard as well as the dosage formulation, the absorbance 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 hours, which was sufficient for the whole analytical process.

e) Filter Compatibility Study

The filter compatibility study was performed to check the compatibility of the filters used for the analysis of the solutions. The unfiltered and filtered solutions of the standard as well as the samples were analysed. The percentage difference between the unfiltered and the filtered solution was recorded.

3. RESULTS AND DISCUSSIONS

3.1 Accuracy of Betahistine Hydrochloride controlled release tablets:

The values are shown in Table-4.

Table No. 4: Results for Accuracy

Sample name	Concentration (ppm)	Absorbance	Amount
2.5 ppm 1	2.5	0.041	102.24
2.5 ppm 2	2.5	0.041	102.24
12.5 ppm 1	12.5	0.206	102.72
12.5 ppm 2	12.5	0.207	102.24
25 ppm 1	25	0.406	101.24
25 ppm 2	25	0.407	101.49
37.5 ppm 1	37.5	0.595	98.91
37.5 ppm 2	37.5	0.595	98.91

3.2 Summary of Linearity parameters for Betahistine Hydrochloride standard in the proposed HPLC Method

It was found to be linear with a correlation coefficient (r^2) of 1, the corresponding linear regression equation being y = 0.016x + 0.0025. The data of linearity was shown in Table-5 and the calibration graph is shown in Fig. 2.

Table No. 5: Results for Linearity

Sr. No	Concentration (ppm)	Mean absorbance
1	2.52	0.0416
2	12.61	0.205
3	25.21	0.41
4	38	0.61
	Slope Intercept	0.016 0.0025

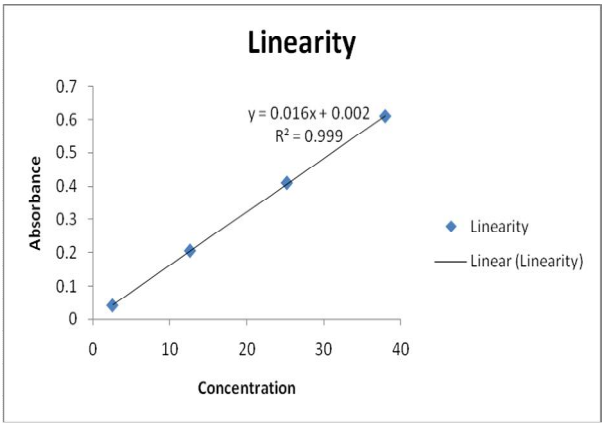

Fig.2. Linearity Curve for Betahistine hydrochloride

3.3 Precision of Betahistine Hydrochloride controlled release tablets

The RSD for the content of Betahistine Hydrochloride for the samples of precision study should be less than 10.00%. The data is shown in Table-6.

Table No. 6: Results for Precision

Sample name	Absorbance	Amount (%)
Sample 1	0.386	87.65
Sample 2	0.388	88.10
Sample 3	0.386	87.65
Sample 4	0.388	88.10
Sample 5	0.393	89.24
Sample 6	0.391	88.78
Average		**88.25**
SD		**0.64**
% RSD		**0.72**

3.4 Stability of Betahistine Hydrochloride controlled release tablets

The % relative difference for the assay of Betahistine Hydrochloride in solution stability obtained between initial and 24 hours should be less than 3.00%.Results are shown in Table-7.

Table No. 7: Results for Solution Stability

Time in hours	Absorbance	% Relative difference
0	0.373	-
2	0.374	0.27
4	0.370	0.81
6	0.371	0.54
8	0.373	0.00
24	0.376	0.80

3.5 Filter compatibility studies of Betahistine Hydrochloride controlled release tablets

The results are shown in Table-8.

Table No.8: Results for Filter Compatibility

Sr. No.	Sample name	Absorbance	% difference
1	Sample unfiltered	0.398	1.005%
2	Sample filtered	0.394	

4. CONCLUSION

A spectroscopic dissolution method was developed for determination of Betahistine Hydrochloride in controlled release tablet dosage form. The conditions that allowed the dissolution determination were 900 mL of sodium phosphate buffer pH 6.8 at 37.0 ± 0.5 ºC, paddle apparatus, 100 rpm stirring speed and filtration with quantitative filter. The methodwas simple, economic, rapid, precise, accurate and specific. The method was validated as per ICH guidelines, and validation acceptance criteria were met in all cases.

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