ABSTRACT: A simple, fast, accurate and precise method has been developed for the determination of Betahistine Dihydrochloride from pharmaceutical formulation by Reversed-phase high performance liquid chromatography. The separation was carried out on C18 column using mobile phase consisting of acetonitrile, methanol and sodium acetate buffer in the ratio 60:10:30 at pH 3 with a flow rate of 1ml/min. The UV detection was carried out at a wavelength of 240 nm. The retention time was found to be at 2.27 min. Regression coefficient (r²) was 0.998 and method was found to be linear. The propose method was validated according to ICH guidelines and can be successfully applicable to pharmaceutical preparation.

KEYWORDS: Betahistine Dihydrochloride, validation, Hplc.

1. INTRODUCTION

Chemically Betahistine is 2-[2-(methylamino)ethyl] pyridine dihydrochloride. It is official in British Pharmacopoeia and mainly used as an antivertigo drug. Literature survey reveals UV and colorimetric methods for the determination of betahistine dihydrochloride. The present work describes simple, sensitive, rapid, accurate, precise RP-HPLC method for the determination of betahistine dihydrochloride in pharmaceutical dosage form.

MATERIALS AND METHODS

Material-

Authenticated sample of Betahistine dihydrochloride was supplied by Suraksha Pharma Pvt.Ltd, Delhi. The tablet formulation containing (ELVERT8mg) was purchased from local market. All the chemicals used are of HPLC or AR grade.

Method-

INSTRUMENTATION

WATERS HPLC

Pump : 515- HPLC
Column : Spherisorb 5µ silica (4 x 250mm i.d.)
Detector : PDA – 2998
Software : Empower 2

UV VISIBLE DOUBLE BEAM SPECTROPHOTOMETER
Shimadzu, Japan UV – 1700

CHROMATOGRAPHIC CONDITION

A mobile phase consisting of Acetonitrile:Methanol:Sodium acetate buffer in the ratio 60:10:30 was selected and are filtered through 0.45 µ membrane filter. A flow rate of 1 ml was maintained throughout the analysis. Detection was carried out at 240 nm using UV
detector.

**PREPARATION OF SOLUTIONS**

**STANDARD STOCK SOLUTION**

Weigh accurately 10mg of Betahistine Dihydrochloride in 10ml standard flask, dissolve and made up the volume with mobile phase (acetonitrile:methanol: sodium acetate buffer (60:10:30 v/v)). The solution has a concentration of 1mg/ml. From the resulting solution, 0.5 ml, 1 ml, 1.5 ml, 2 ml, 2.5 ml were pipette out into each of 10 ml standard flask, made up the volume with mobile phase. To obtain final concentrations of 5 µg/ml, 10 µg/ml, 15 µg/ml, 20 µg/ml & 25 µg/ml respectively.

**SAMPLE SOLUTION**

Ten tablets (ELVERT) each containing 8 mg of Betahistine Dihydrochloride were weighed. Their average weight was determined and finely powdered using glass mortar and pestle. The tablet powder equivalent to 10 mg of Betahistine Dihydrochloride was transferred to 10ml volumetric flask and dissolve in approximate 8ml of mobile phase, sonicated for 15 min. And filter the solution using whatmann No.1 filter paper to 10 ml standard flask with the washings make up the solution. The stock solution was further diluted with mobile phase to obtain working sample solution of 15 µg/ml.

**III. METHOD VALIDATION**

Validation was carried out as per ICH guidelines by considering the following parameters.

**LINEARITY**

For linearity determination, the standard solutions having 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml and 25µg/ml were prepared. Each five concentrations, 20µl injected to the column individually, peak area was noted.

**PRECISION**

Precision was carried out by the repeated scanning and measurement of absorbance of the solution of Betahistine without changing the parameters of the method. The percentage standard deviation (%RSD) was found to be within the limit of not more than 2%.

**INTERMEDIATE PRECISION**

The intraday and interday precision was determined by analysing the corresponding responses times on the same day and on three different days over a period of one week. The percentage standard deviation was found to be within the limit.

**ACCURACY**

The accuracy of the method was determined by recovery experiments. The recovery experiments were carried out by the standard addition method. In this method known amount of an analyte is spiked at different levels in to sample matrix that already contain some quantity of the analyte. To study the accuracy of the method and to check the interference from excipients, recovery studies were carried out by the addition of standard drug solution to sample at three different levels (80%, 100%, 120%) of the test concentration.

**LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION**

The limit of detection (LOD) and limit of quantification (LOQ) of drug was determined as per ICH guidelines using the following equation.

\[
LOD = 3.3 \times \sigma / S
\]

\[
LOQ = 10 \times \sigma / S
\]

Where \( \sigma = \) standard deviation

\( S = \) slope of the calibration curve.

**ROBUSTNESS**

Robustness of the method was determined by small change in flow rate, mobile phase ratio, and wavelength detection. Flow rate was changed to 1± 0.1ml/min. The mobile phase
ratio was changed to ±2% wavelength of detection was changed to 240±5nm.

RESULTS AND DISCUSSION

The method was found to be simple, accurate, precise and economic for routine examination of Betahistine dihydrochloride in tablet dosage form.

- In HPLC method development experiments were carried out under optimized conditions using C18 column. The wavelength selected at 240 nm was optimum. Experiments are carried out at a flow rate of 1ml/min. The mobile phase selected was Acetonitrile:methanol:sodium acetate buffer at 60:10:30 ratio. The retention time was observed at 2.27 min.

- The linearity was tested with a concentration range of 5-25µg/ml for Betahistine Dihydrochloride. The linear regression equation for Betahistine was found to be

  \[ Y = 57438 \times \text{with correlation coefficient } R^2=0.998. \] 
  \[ R^2 \text{ value of 0.998 confirms the linearity of the method.} \]

- The precision was evaluated at the repeatability and intermediate precision level. % RSD value of less than 2% indicates that the proposed method was precise.

- The accuracy of the method was determined by measuring the drug recoveries by the standard addition method. The mean % recovery of the drug were within the limit of 98-102 % which indicates that the method is accurate.

- The LOD and LOQ was found to be 0.1443 µg/ml and 0.4373 µg/ml respectively.

- Robustness determination small variation in the experiment condition was carried out. But there was no marked changes in the chromatogram. It indicates that the method was robust.

The drug content, expressed as % label claim for betahistine were 100.09 % and the %RSD value was 0.486. The low %RSD value indicate the suitability of this method for routine analysis of Betahistine dihydrochloride in pharmaceutical dosage form.

CONCLUSION

The proposed RP-HPLC method were found to be simple, precise accurate, rapid and economic. Statistical analysis indicates that the developed method was reproducible and selective for the analysis of Betahistine Dihydrochloride in solid pharmaceutical dosage form without interferences from excipients. Hence this method can be easily and conveniently adopted for the routine analysis of Betahistine dihydrochloride in its solid dosage form.

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