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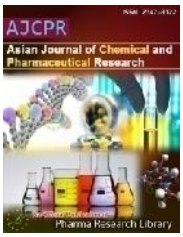

Research Article Open Access

Analytical Method Development and Validation for the Estimation of Betahistine HCL by RP-HPLC Method in Bulk and Pharmaceutical Dosage Form

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A B S T R A C T

The chromatographic conditions were successfully developed for the separation of Betahistine HCL by using Agilent C_{18} Column (150mm x 4.6mm)5µm, flow rate was 1ml/min, mobile phase ratio was Methanol: Phosphate buffer (75:25 v/v), detection wavelength was 270 nm. The Spectroscopic method was done in solvent using mobile phase and the instrument lab India 3000+ with UV win software. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector, Empower-software version 2. The retention time was found to be 2.145 min. The % purity of Betahistine HCL was found to be 98.56%. The system suitability parameters for Betahistine HCL such as theoretical plates and tailing factor were found to be 4343, 1.6. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Betahistine HCL was found in concentration range of 20µg-100µg and correlation coefficient (r^2) was found to be 0.999 respectively, % recovery was found to be 98.96% respectively. %RSD for repeatability and precision was found to be <2.LOD value was 0.439 and LOQ value was found to be 1.466 respectively for Betahistine HCL.

Keywords: Betahistine HCL, HPLC.

A R T I C L E I N F O

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1. Introduction

Betahistine has a very strong affinity as an antagonist for histamine H_3 receptors and a weak affinity as an agonist for histamine H_1 receptors. Betahistine seems to dilate the blood vessels within the inner ear which can relieve pressure from excess fluid and act on the smooth muscle. Betahistine has two modes of action. Primarily, it has a direct stimulating (agonistic) effect on H_1 receptors located on blood vessels in the inner ear. This gives rise to local vasodilation and increased permeability, which helps to reverse the underlying problem of endolymphatic hydrops. More importantly, betahistine has powerful antagonistic effects at H_3 receptors. This stimulation explains the potent vasodilatory effects of betahistine in the inner ear. It is postulated that betahistine's increase in the level of serotonin in the brain stem inhibits the activity of vestibular nuclei.

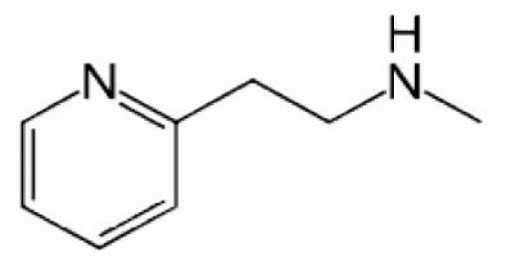


Figure 1: Betahistine HCL

Analytical methods

Analytical methods can be separated into classical and instrumental methods.

Classical methods

Classical methods are also known as wet chemistry methods, where, Separation of analytes was performed by precipitation, extraction, or distillation. Qualitative analysis by reaction of analytes with reagents that yield products that could be recognized by their colors, boiling or melting points, solubilities, optical activities, or refractive indexes. Quantitative analysis by gravimetric or titrimetric techniques. In Gravimetry, the amount of a substance is determined by the mass of product generated by a chemical reaction. In Titrimetry, concentration is determined by the volume of a reagent needed to completely react with the analyte. These methods are highly accurate and precise but require a sufficient amount of sample, and a concentration of analyte in the sample of at least 0.1 percent. Furthermore, these analyses require the constant attention of a trained scientist. Classical methods use separations such as precipitation, extraction, and distillation and qualitative analysis by color, odor, or melting point.

Instrumental methods

Instrumental methods use an apparatus to measure physical quantities of the analyte such as light absorption, fluorescence / conductivity. Highly efficient chromatographic and electrophoretic techniques began to replace distillation, extraction and precipitation for the separation of components of complex mixtures prior to their qualitative or quantitative determination. These newer methods for separating and determining chemical species are known collectively as Instrumental methods of Analysis.

2. Materials and Methods

Apparatus

The instrument used for the study was Waters HPLC Auto Sampler, Separation module 2695, photo diode array detector with Empower-software version-2.

Reagents and Materials

The solvents used were Methanol, Potassium dihydrogen ortho phosphate, Dipotassium hydrogen ortho phosphate, Tri Ethyl Amine of HPLC Grade and HPLC Water, Betahistine HCL

Selection of chromatographic condition

Proper selection of the method depends upon the nature of the sample, its molecular weight and solubility. The drug selected in the present study is polar in nature and hence reversed phase or ion-pair or ion exchange chromatography method may be used. The reversed phase HPLC was selected for the separation because of its simplicity and suitability.

Selection of detection wavelength:

The sensitivity of method that uses PDA detector depends upon the proper selection of wavelength. An ideal wavelength is that gives maximum absorbance and good response for both the drugs to be detected. Standard solution of Betahistine HCL was scanned in the UV range (200-400nm) and the spectrum was recorded. From the spectrum, 270 nm was selected as the detection wavelength for the present study.

Selection of mobile phase:

Initially the mobile phase tried was methanol and water, methanol and Methanol, buffer and water in various proportions. Finally, the mobile phase was optimized to Buffer: Methanol in proportion 25:75 v/v respectively.

Chromatographic trials for Estimation of Betahistine HCL by RP- HPLC.

Trial-1 Chromatographic conditions

Column	:	Xterra , RP18 4.6x150mm ,5µm
Mobile phase ratio	:	MeOH: H_2O (60:40%v/v)
Detection wavelength	:	270nm
Flow rate	:	1 ml/min
Injection volume	:	10 µl
Run time	:	8 min
Retention time	:	1.791min

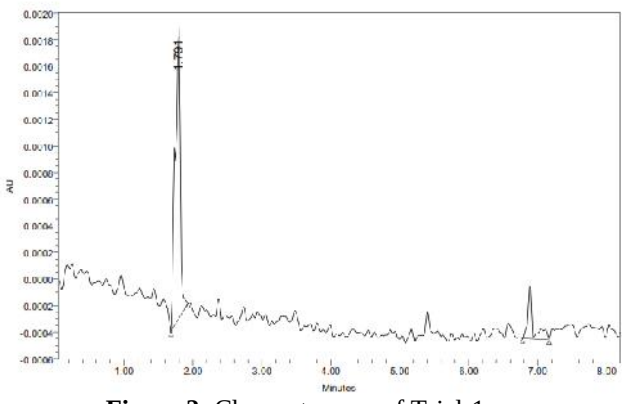


Figure 2: Chromatogram of Trial-1

Observation: The trial shows no good peak separation, so more trials were required for obtaining peaks.

Trial-2 Chromatographic condition

Column : Inertsil ODS 4.5×150mm 5.0 µm
Mobile phase ratio : ACN: H_2O (80:20%v/v)
Detection wavelength : 270nm
Flow rate : 1ml/min
Injection volume : 20µl
Column temperature : Ambient
Auto sampler temperature : Ambient
Run time : 8.0 min

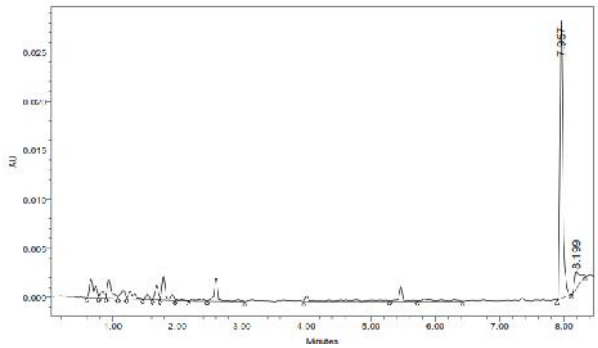


Figure 3: Chromatogram of Trial-2

Observation: In this trial no peak was observed, still more trials was required for good peaks

Trial-3 Chromatographic condition

Column : Xterra RP18 4.6x150mm 5µm
Mobile phase ratio: ACN: pH 6.8 phosphate buffer (75:25 % v/v)
Column temperature : Ambient
Auto sampler temperature : Ambient
Detection wavelength : 270 nm
Injection volume : 10 µl
Run time : 10 mins
Retention time : 4.162 mins

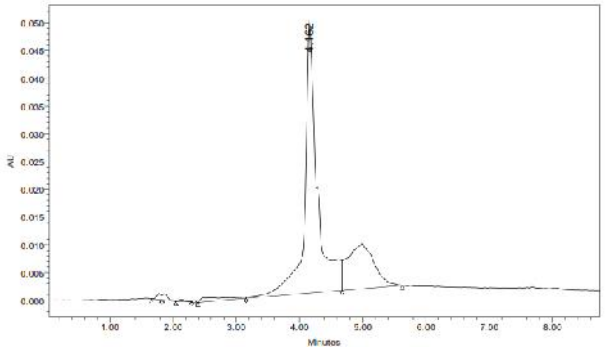


Figure 4: Chromatogram of Trial-3

Observation: In this trial Betahistine HCL was eluted but there is no proper separation. Still more trials was required for better separation in peak.

Trial -4 Chromatographic conditions

Column : Agilent C18 4.6×250mm 5µm
Mobile phase ratio: ACN: pH 3 phosphate buffer (60:40% v/v).
Detection wavelength : 270nm
Flow rate : 1.0 ml/min
Injection volume : 10 µl
Column temperature : Ambient
Auto sampler temperature : Ambient
Run time : 20 min
Retention time : 13.336 mins

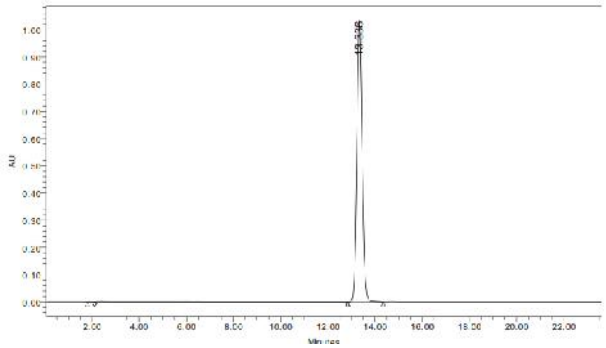


Figure 5: Chromatogram of Trial-4

Observation: The separation was good; peak shape was good, still more trials were required to decrease the retention times of peak.

Trial-5 Chromatographic condition(Optimized Method)

Column : Agilent (4.6×150mm) 5µ
Mobile phase ratio : Phosphate buffer: MeoH (25:75% v/v)
Detection wavelength : 270 nm
Flow rate : 1.0 ml/min
Injection volume : 10µl
Column temperature : Ambient
Auto sampler temperature : Ambient
Run time : 10min
Retention time : 3.668min

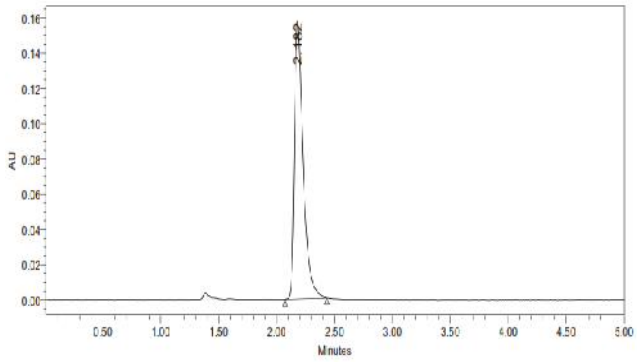


Figure 6: Chromatogram of Trial-5 (Optimized)

Observation: The separation was good, peak shape was good, and so we conclude that there is no trial required to decrease the retention time of peak, so it is taken as final method.

Procedure

Preparation of Buffer:

About 7.0g of potassium dihydrogen orthophosphate was dissolved in 1000ml of HPLC grade water and pH 4.0 was adjusted with Orthophosphoric acid. It was filtered through 0.45µm nylon membrane filter and degassed with sonicator.

It was used as a diluent for the preparation of sample and standard solution.

Preparation of mobile phase

Mix a mixture of 250 ml Phosphate buffer (25%) and 750 ml of Methanol (75%) and degassed in ultrasonic water bath for 5 minutes. Filter through 0.22 µ filter under vacuum filtration. Mobile phase was used as the diluent.

Preparation of the individual Betahistine HCL standard preparation

10 mg of Betahistine HCL working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask and add about 2 ml of diluent and sonicated to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1.0 ml from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

Sample solution preparation:

10 mg of Betahistine HCL tablet powder was accurately weighed and transferred into a 10 ml clean dry volumetric flask, add about 2ml of diluent and sonicated to dissolve it completely and making volume up to the mark with the same solvent(Stock solution). Further pipette 10ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

3. Results and discussion

Method Validation Parameters

1. Specificity The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by Injecting blank

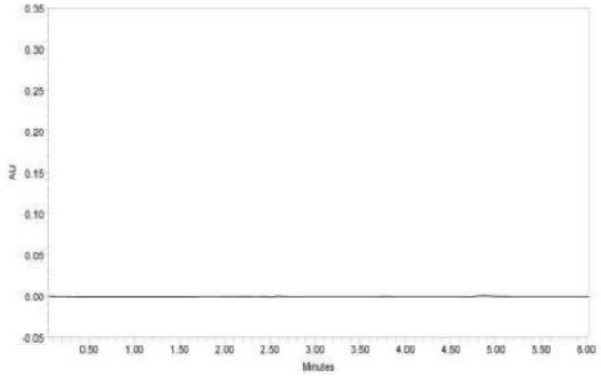


Figure 7: Chromatogram of Blank

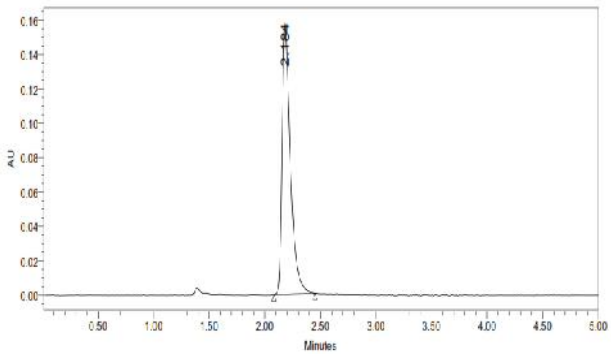


Figure 8: Chromatogram of Sample

2. Linearity

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range.Serial dilutions of Betahistine HCL (20-100 µg/ml) were injected into the column and detected at a wavelength set at 270 nm. The calibration curve was obtained by plotting the concentration vs. peak area.

Acceptance criteria: Correlation coefficient should be not less than 0.999.

3. Range

Based on precision, linearity and accuracy data it can be concluded that the assay method is precise, linear and accurate in the range of 20-100 µg/ml for Betahistine HCL respectively

4. Accuracy

Accuracy of the method was determined by recovery experiments. There are mainly 2types of recovery studies are there.

Standard addition method:

To the formulation, the reference standard of the respective drug of known concentration was added, analyzed by HPLC and compared with the standard drug concentration.

Percentage method:

For these assay method samples are prepared in three concentrations of 50%, 100%, and 150% respectively.

Acceptance criteria: The mean % recovery of the Betahistine HCL at each level should be not less than 95.0% and not more than 105.0%.

Assay procedure

10µL of the blank, standard and sample was injected into th e chromatographic system and areas for the Betahistine HCL the peak was used for calculating the % assay by using the formulae.

5. Precision

Method precision also called as repeatability/Intra-day precision indicates whether a method gives consistent results for a single batch. Method precision was demonstrated by preparing six test solutions at 100% concentration as per the test procedure & recording the chromatograms of six test solutions. The % RSD of peak areas of six samples was calculated. The method precision was performed on Betahistine HCL formulation.

Acceptance criteria: The % RSD for the area of six sample injections results should not be more than 2.

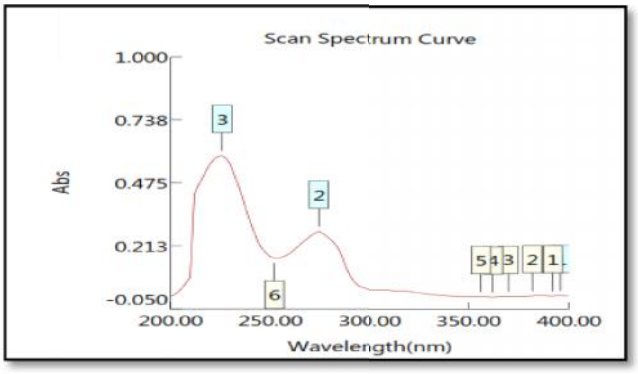


Figure 9: Overlain Spectra of Betahistine HCL

Selection of solvent

The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10 µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400 nm. The overlay spectrum of Betahistine HCL was obtained and the isobestic point of Betahistine HCL showed absorbance's maxima at 270 nm.

Validation of the method

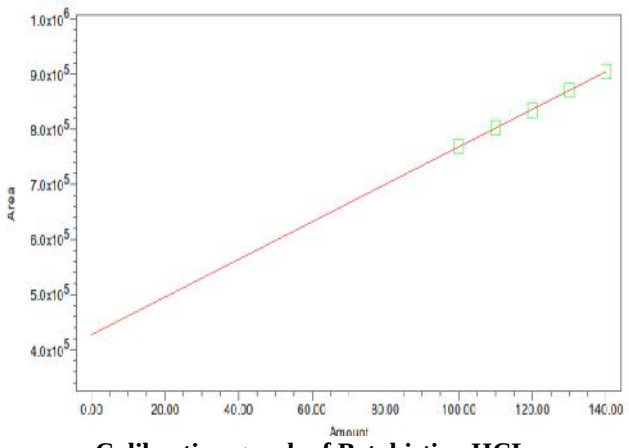
Linearity

Betahistine HCL:

Serial dilutions of Betahistine HCL (20-100 µg/ml) were injected into the column and detected at a wavelength set at 270 nm. The calibration curve was obtained by plotting the concentration vs. peak area and the correlation coefficient was found to be 0.999 respectively.

Table 1: Calibration data of Betahistine HCL

S.No	Linearity Level	Concentration	Area
1	I	20	905957
2	II	40	1033632
3	III	60	1200130
4	IV	80	1403642
5	V	100	1608820
Correlation Coefficient			0.999



Calibration graph of Betahistine HCL

Recovery studies

In order to ensure the suitability and reliability of proposed method, recovery studies were carried out. To an equivalent quantity of formulation powder a known quantity of standard Betahistine HCL were added at 50%, 100% and 150% level and the contents were re-analyzed by the proposed method.

Precision:

Table 2: Intermediate Precision

	Peak Name	RT	Area	Height
1	Betahistine Hcl	2.180	830760	160374
2	BetahistineHcl	2.184	832532	160030
3	Betahistine Hcl	2.185	823385	159662
4	Betahistine Hcl	2.188	840724	161107
5	Betahistine Hcl	2.188	829385	160286
Mean			831357.4	
Std. Dev.			6263.2	
% RSD			0.8	

Table 3: Method Precision

	Peak Name	RT	Area	Height
1	Betahistine Hcl	2.185	824170	158772
2	Betahistine Hcl	2.191	826053	157336
3	Betahistine Hcl	2.204	823442	156124
4	Betahistine Hcl	2.207	818967	155674
5	Betahistine Hcl	2.210	823476	156033
Mean			823221.9	
Std. Dev.			2604.2	
% RSD			0.3	

4. Conclusion

A new method was established for estimation of Betahistine HCL by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Betahistine HCL by using Agilent C18 column (4.6×150 mm) 5µ, flow rate was 1 ml/min, mobile phase ratio was (75:25 v/v) methanol: Buffer, detection wavelength was 270 nm. Precision and recovery studies were also found to be with the range. The proposed HPLC method was found to be simple, specific, precise, accurate, rapid and economical for simultaneous estimation of Betahistine HCL in tablet dosage form. The developed method was validated in terms of accuracy, precision, linearity, robustness and ruggedness, and results will be validated statistically according to ICH guidelines. The Sample recoveries in all formulations were in good agreement with their respective label claims. Hence the suggested RP-HPLC method can be used for routine analysis of Betahistine HCL in API and Pharmaceutical dosage form.

Table 4: Accuracy results for Betahistine HCL

% Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	1143519	5	4.86	98.81%	**98.96%**
100%	2938342	10	9.88	99.08%	
150%	4452758	15	15.0	100.0%	

Table 5: Suitability results for Betahistine HCL

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	4517	1.7

2	1.0	4343	1.6
3	1.2	4209	1.6

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	4623	1.6
2	*Actual	4543	1.6
3	5 % more	4864	1.6

LOD and LOQ

Drug name	Standard deviation(σ)	Slope(s)	LOD(µg)
Betahistine HCL	2604.5	17757	0.439

Drug name	Standard deviation(σ)	Slope(s)	LOQ(µg)
Betahistine HCL	2604	17757	1.466

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