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Quantification Analysis of Betahistine in Tablet Dosage Forms Using HPLC-ZIC-HILIC with Ultraviolet Detection

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Vertigo, Anti-inflammatory drugs, ZIC-HILIC, Betahistine, Tablet dosage forms.

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Abstract
A simple ZIC-HILIC-UV technique was developed and applied to determine Betahistine (N-methyl-2-(pyridin-2-yl) ethanamine) in tablet dosage forms. The separation took place in the ZIC1 and ZIC5 homemade stationary phases. Mix an acetate buffer and acetonitrile in the mobile gradient phase and detect at 235 nm. The Betahistine conduct with varying acetate buffer concentration, buffer pH, and acetonitrile has been tested. The results have established the mechanism of separation is based on the hydrophilic partitioning of Betahistine. For the ZIC1 and ZIC4 stationary phases, the developed ZIC-HILIC techniques had high precision (0.99%), linear ranges of 0.01-10 ppm, and detection limits of 0.0074 and 0.0060 ppm, with a coefficient of determination of 0.9999 and 0.9998, respectively. The method was a valuable alternative to Betahistine quantification in tablet dosage forms.

1. Introduction

Betahistine (BET) is a chemical compound with the general formula (N-methyl-2-(pyridin-2-yl)ethan-amine). It is also called (Betaserc). It is a drug used in the treatment of vertigo. It was registered in Europe as a treatment for Meniere's disease, a disease in which a person becomes dizzy due to the influence of the inner ear responsible for balance. Figure 1. Shows the chemical composition of Betahistine [1]. A restricted number of publications were originated in the literature regarding the evaluation of Betahistine either in dose forms or in biological-matrix [2]. Specific analytical techniques have been described for the evaluation of Betahistine which include very few publications have been described for evaluation of Betahistine such as spectro-photometric analysis and High Performance Liquid Chromatographic(HPLC) [3]. Spectro-photometric, atomic absorption spectrometric (AAS) and high-performance-liquid-chromatographic (HPLC) procedures have been described for the analysis of Betahistine in pharmaceutical preparation [4].

The methodical systems employed beforehand for the valuation of Betahistine are described in works [5]. The objective of the current study was:

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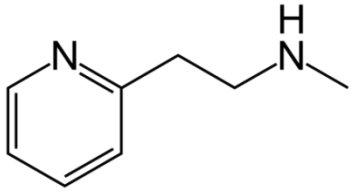
1) To validate and develop a high-performance-liquid-chromatographic (HPLC) assess technique for the evaluation of Betahistine in measured release tablet pharmaceutical form.

2) To develop a simple, rapid, sensitive, accurate, precise and linear analytical technique for estimate of Betahistine in control release tablet pharmaceutical forms.

Figure 1. Structure of Betahistine.



Numerous high-performance liquid chromatography (HPLC) procedures for the examination of Betahistinein pharmaceutical dose form and biological sample has been documented [6].

Zwitterionic-hydrophilic-interaction-liquid-chromatography (ZIC-HILIC) is a method for combining a polar stationary-phase with a high-water-content mobile-phase while increasing the proportion of a less polar solvent. Separations are typically performed using a 5–40% water concentration; the procedure is also compatible with gradient elution. In 1990, Alpert coined the term HILIC to capture the notion and its substantial applications [7]. In comparison to RP-HPLC, ZIC-HILIC favours the retention of hydrophilic and polar compounds over hydrophobic neutral substances. There are numerous studies on the quantifying of Betahistine in HPLC; there is no published literature on quantifying Betahistine using ZIC-HILIC techniques. The effect of the ZIC-HILIC columns' chain length on Betahistine retention behavior has not been investigated previously. Previous studies [8] have examined the effect of chain length on ZIC-HILIC stationary phases and their impact on the behavior of medicines. They observed that the greater chain lengths of ZIC-HILIC stationary phases, the more interaction between the pharmaceuticals and stationary phases occurred, resulting in a longer retention time. The ultimate objective was to create a straightforward method for quantifying Betahistine in bulk and commercial pharmaceutical materials[9].

2. Materials and methods

2.1. Chromatographic and ZIC-HILIC conditions

Betahistine was detected in UV areas with a wavelength of 235 nm at a flow rate of 0.5 mL/min. A 10 µL injection loop is supplied with a Merck Hitachi HPLC system equipped UV-visible L-4200 and L-6200 gradient pump. Chromatography analysis was monitored using the N2000 Data Workstation software. For Betahistine separation, ZIC1 and ZIC4 columns (100 mm x 4 mm ID) were built on polystyrene-divinylbenzene (PS/DVB) using a grafted sulfobetaine monomer [10]. Identified the systematic cycle of the grafting process. Millipore water conductivity of 18.2 MΩ (System-US Millipore) was employed [11].

2.2. Chemicals and Stock solution for Betahistine

Betahistine was purchased from Sigma-Aldrich. Acetic acid and HPLC acetonitrile grade were purchased from BDH. Sodium acetate was obtained for Fluka. A stock solution of Betahistine(25 ppm) was prepared by dissolving the appropriate quantity of Betahistine(0.6250 mg) in 25 mL of Millipore water. A 0.22 m filter was used to filter the stock solution.

2.3. Preparation of commercial pharmaceutical materials.

Nine tablets of three commercial pharmaceutical materials were weighed and ground to a fine powder to determine the content of pharmaceutical formulations. 8-24 mg APL Swift Services limited Betaserc–India Solvay Pharmaceuticals-Netherlands were transferred to a 100 mL volumetric flask containing 25 mL acetonitrile. After 15 minutes of sonication, the solutions were diluted with acetonitrile. A working solution was generated the following filtration by diluting the stock solution with acetonitrile. Next to that, the solution was filtered using Millipore filters (0.22 μm). By diluting the stock solution, other standard solutions were developed [13].

3. Results and discussion

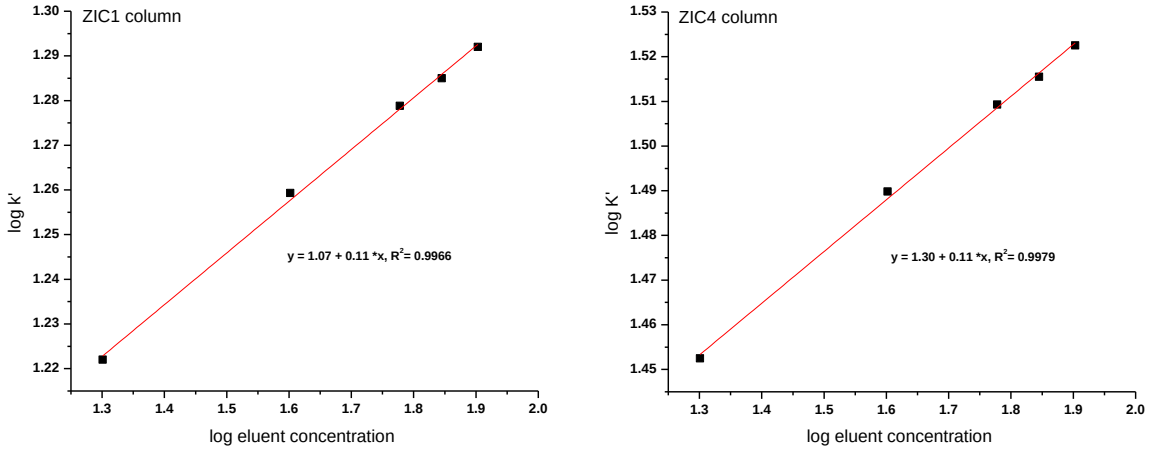
The purpose of this study was to design and validate a chromatographic test for Betahistine estimation in bulk and commercial pharmaceutical materials utilizing HPLC-ZIC-HILIC-UV. The quality frameworks were examined by varying the acetonitrile content (70%-95%), buffer acetate concentration (20-80 mM), pH buffer acetate (3.3-5.5) and estimating their values with good sensitivity and selectivity.

3.1. Method development

3.1.1. Influence of Betahistine retention on acetonitrile content

Increased Betahistine drug retention is demonstrated in Figure 2 by raising the acetonitrile content from 70% to 95% at a fixed buffer concentration and pH (40 mM, pH =4.75). And therefore, Betahistine exhibits hydrophilic (HILIC) behavior with two ZIC-HILIC stationary phases in lower mobile phase water content. This property of Betahistine is owing to its hydrophilicity. The reason for this is that Betahistine logP_{ow} value (-1.36) [14].

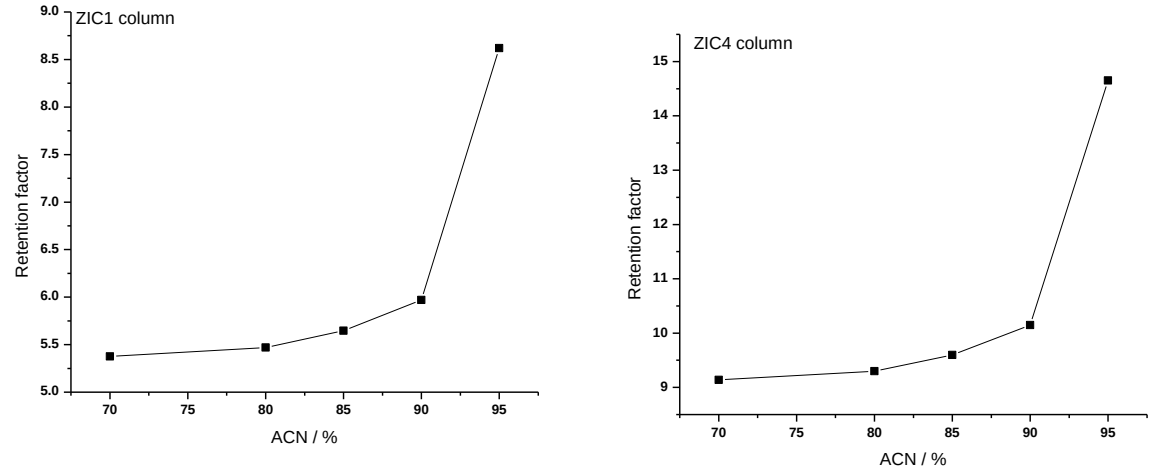
Figure 2. Acetonitrile content impact on Betahistine retention using ZIC1 and ZIC4 columns.



3.1.2. Eluent Impact on retention of Betahistine

In most cases, greater buffer concentrations retained ZIC-HILIC, which resulted in the deactivation of intra-molecular ion pairs. Thus, the presence of acetonitrile improves the linearization of functional phase groups. Increased buffer levels resulted in an increase in Betahistine retention in ZIC-HILIC columns. The influence of the acetate buffer on the Betahistine retention behavior in eluents containing 20-100 mM (pH 4.75) and 90 percent acetonitrile has been described. The results are given in (Figure 3). By increasing the buffer concentration in the acetate eluent, the Betahistine retention factor of the column is increased. Betahistine behavior is due to its hydrophilicity, which is intimately associated with the ZIC-HILIC material's stationary phase.

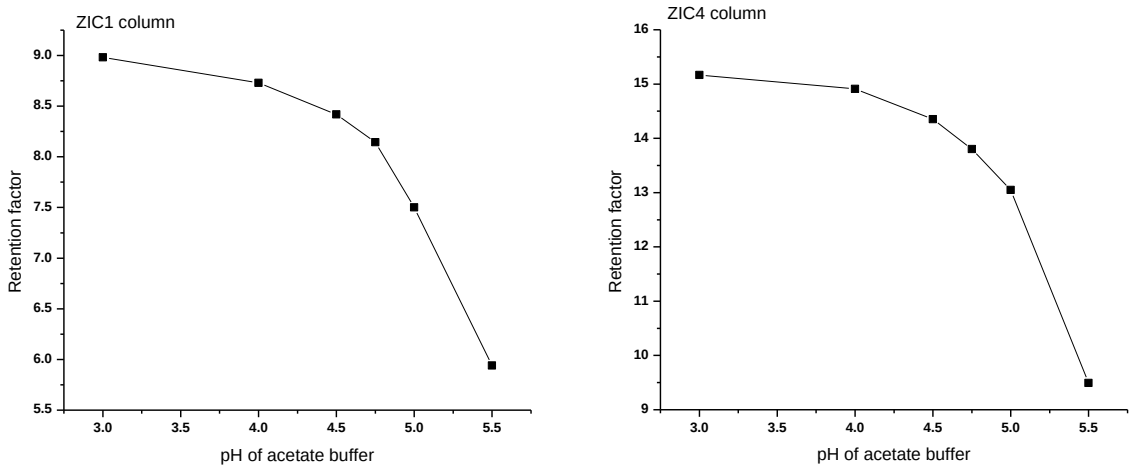
Figure 3. Eluent concentration impact on Betahistine retention using ZIC1 and ZIC4 columns.



3.1.3. Eluent pH Effect on Betahistine retention

The buffer pH should be varied to complete the separation mechanism of Betahistine. Betahistine retention was reduced when raised pH from 3 to 5.5 with sodium acetate concentration maintained at 40 mM and 95 percent of acetonitrile, as shown in Figure 4. Betahistine behavior is due to its pKa (1.63) and isoelectric point (5.36) values [14]. As a result, lowering the positive charge on the amine group of Betahistine reduces the interaction of Betahistine with the ZIC1 and ZIC4 columns.

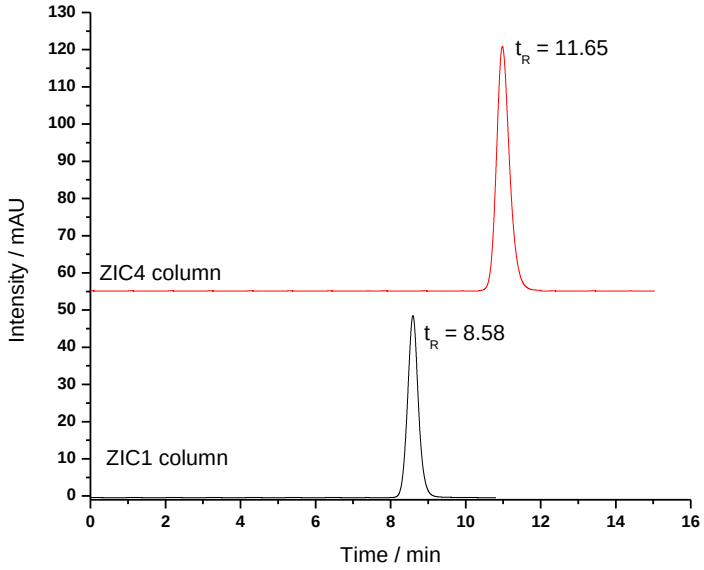
Figure 4. Buffer pH impact on Betahistine retention using ZIC1 and ZIC4 columns.



3.1.4. The optimum separation of Betahistine

Figure 5 illustrates the chromatogram. The chromatogram was prepared using sodium acetate 40 mm (pH 4.7) and acetonitrile 95 percent. As seen by the ZIC4 stationary phase in Figure 5, the interaction between Betahistine and two ZIC1 and ZIC4 stationary phases is clear; by increasing the chain length, the retention duration of Betahistine increased. The methyl group grows between charges in the ZIC stationary phases.

Figure 5. Chromatogram for the separations of Betahistine using ZIC1 and ZIC4 columns.



3.2. Method validation results

Linearity was determined for Betahistine on ZIC1 and ZIC4 stationary phases over the concentration range of 0.01-9 ppm. Calibration curves were developed between the peak area ratios and the associated Betahistine concentrations. The regression coefficients ranged between 0.9999 and 0.9998, indicating an excellent linear response for the stationary phases of ZIC1 and ZIC4, respectively. Table 1 summarizes the parameters of the regression equations for Betahistine. The limit of detection (LOD) and limit of quantitation (LOQ) values (Table 1) for Betahistine were determined with appropriate precision and accuracy for sensitivity determination, showing that the devised method was susceptible.

Table 1. Factors of the regression equations for the estimation of Betahistine using ZIC1 and ZIC4 stationary phases.

Factors	ZIC1 stationary phase	ZIC4 stationary phase
Regression equation	y= 7128.81+ 15987.9* x	y=18387.07+19380.93* x
r^2	0.9999	0.9998
Linear range (ppm)	0.01-9	0.01-9
LOD (ppm)	0.0052	0.0038
LOQ (ppm)	0.0177	0.0135
Intra-Inter day accuracy (%Recovery)	99.12-100.78	99.45-101.00
Intra-Inter day precision (% RSD)	0.25-0.66	0.48-0.77

3.3. Betahistine content determination in commercial pharmaceutical materials

The proposed methodologies were successfully applied to the analysis of Betahistine in commercial pharmaceutical materials, as shown in Table 2. The results were compared to data collected during the evaluation of the ZIC-HILIC methods' competence and performance following the United States Pharmacopeia method (USP) [15].

Table 2. The recommended methods for quantifying Betahistinein commercial pharmaceutical materials.

Commercial pharmaceutical	Present (mg)	Get it (mg)	%RSD n=5	%Rec.
APL Swift Services limited-turkey	ZIC1 stationary phase			
	8	7.9	0.71	98.75
Betaserc -India	16	15.7	0.44	98.21
	24	24.05	0.22	99.79
Solvay Pharmaceuticals-Netherlands	ZIC4 stationary phase			
	8	7.8	0.65	99.72
	16	15.9	0.53	99.64
	24	24.08	0.34	100.33

For data analysis, the t-test and F-test variance ratios with a confidence of 95% are used (Table 3). To guarantee that the accuracy of Betahistine determination in commercial pharmaceutical materials was not significantly different between the three methods, the t and F values of the measurements were kept within theoretical values.

Table 3. Comparing the suggested procedures to the USP method.

Commercial pharmaceutical	ZIC1 method	ZIC4 method	USP method [16]	t-Test (theor.)	F-Test (theor.)
APL Swift Services limited	99.98	99.82	99.22	0.5080* (2.7764)	0.1069* (19.000)
Betaserc -India	99.45	99.74	100.32		
Solvay Pharmaceuticals-Netherlands	100.04	100.08	101.11	0.5061** (2.7764)	0.0767** (19.000)

*ZIC1 method
**ZIC4 method

4. Conclusion

This article discusses the ZIC-HILIC method for determining the presence of Betahistinein commercial pharmaceutical products. This adaptability is advantageous because ZIC-HILIC traders between charged groups with one to four methylene groups under various circumstances. The ZIC4 stationary phase has longer retention duration and a lower detection limit for Betahistine than the ZIC1 stationary phase, which could be due to the geometric orientation of the ZIC4 stationary phase. The observations suggest that hydrophilic behavior is responsible for Betahistine retention. Commercial pharmaceutical compounds have been successfully analyzed using the existing procedures.

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