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Method Validation and Development of RP-HPLC Method of Mebhydroline Napadisylate in Bulk and its Tablet Dosage Form

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ABSTRACT

An accurate, simple, rapid and sensitive HPLC method has been developed for the determination of mebhydroline napadisylate in the tablet. The Chromatography was performed on a reversed phase C-18 column(150 mm × 4.6 mm id, 5µm) by isocratic elution, using a mobile phase of acetonitrile : ammonia 25% (80 : 20 v/v) at ambient temperature 25±5 °C and UV detection operates at 320 nm in an overall analysis time of about 10 min.The total retention time was 1.612 min with a flow rate of 1.0 ml/min. % 0f RSD values for precision is found to be 0.293 (< 2).The limits of detection (LOD) and quantification (LOQ) were 0.03µg/ml and 0.096133µg/ml, respectively. The correlation coefficient for Mebhydroline Napadisylate 0.9972 indicates linearity of the methods within the limits. The linear range of determination for Mebhydroline Napadisylate was 100-500µg/ml. However, the change in flow rate and column temperature also did not show any significant variance. The % recovery was found to be 99.70%-99.41%. As per ICH guidelines the proposed method is fully validated and found to be linear over a workable drug concentration, accurate, precise and robust. This HPLC method is selective, precise, and accurate and can be used for routine analysis of pharmaceutical preparation in industrial quality-control laboratories.

Keywords: HPLC, mebhydroline napadisylate, validation, Tablet.

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INTRODUCTION

Mebhydroline(Napadisylate) is Mebhydroline 1,5-naphthalenedisulfonate salt(Figure 1) which is used as anti-histamine[1]. It is also called Bexidal (BD) and Diazolin (RU) with INN name Mebhydrolin. It is chemically (C19H20N2)2•C10H8O6S2 with IUPAC name 5-Benzyl-2-methyl-2,3,4,5-tetrahydro-1H-pyrido[4,3b]indole1,5naphthalenedisulfonate. It belongs to H1-antagonist pharmacological group on the basis of mechanism of action. Mebhydroline is white or almost white crystalline and soluble in the methanol, chloroform, ether. Freely soluble in the water and ethanol[2,3]. Oral absorption of Mebhydroline is found to be 100%. Mebhydroline is available in tablet dosage form 50 mg and in suspension 50mg/5ml. It is used for symptomatic relief of allergic symptoms caused by histamine release, including nasal allergies and allergic dermatosis. Mebhydroline is primarily indicated in conditions like Allergy, Angioedema, Angiooedema, Eczema, Hay fever, Pruritus, Rhinitis, Urticaria[4]. Mebhydrolin has been shown to enhance the performance-deficit effects of alcohol[5].

Mebhydroline napadisylate in pharmaceutical preparation is not official in any pharmacopeia and has not been published yet. On detailed literature survey, it was found that Mebhydroline can be estimated by HPLC (based on peak area)[6] and HPTLC method[7]. An analytical reference book, Pharmaceutical Press[8,9] described column liquid chromatography (LC) methods for the determination of mebhydroline napadisylate and other drugs in the antihistamine preparations.Since no simple HPLC Method validation are reported for the simple, specific, less time consuming estimation of Mebhydroline Napadisylate, therefore in the present work a successful attempt has been made to estimate this drug by simple RP-HPLC Method. The proposed methods were optimized and validated as per ICH guidelines[9].

MATERIALS AND METHOD

Reagents and Chemicals:

The bulk drug of Mebhydroline was obtained as gift sample from ESKAYEF BANGLADESH LIMITED (Gazipur, Bangladesh).The commercial fixed dose combination product of Mebhydroline 50 mg Bexidal (BEXIMCO PHARMA, Tongi, Bangladesh) was procured from the local market. The solvent used was a) Ammonia **(**Merck KGaA, 64271 Darmstadt Germany, Web: www. merk- chemicals.com) b) HPLC grade Acetonitrile(Active Fine Chemicals Ltd., Dhaka.,Bangladesh, Web:www.afchem.com) c) The water for RP-HPLC filter(Globe Pharmaceutical Limited, Noakhali, Bangladesh).

Instrument used and Chromatographic conditions:

A HPLC instrument compromised of Reservior Tray (Shimadzu Corporation, Kyoto Japan), Prominance UV/ VIS Detector (Model: SPD-20A, Shimadzu Corporation, Kyoto Japan), Prominance Degrassing Unit (Model: DGU-20A 3R, Made in USA, Shimadzu Corporation, Kyoto Japan), Prominance Chromatograph (Model: LC-20AT, Made in Japan), Columnpan Oven (Model: CTO-10ASVP, Shimadzu Corporation, Made in Japan).

Other instruements were: UV Spectrophotometer (Model: UV-1800 240V, Shimadzu Corporation, Made In Japan), Triple distillation unit consisting of borosilicate glass, Analytical Balance (*II*AXIS, Model: AGN220C, Spolkazoo, Made in Poland), Ultrasonic Cleaner (Model: Power sonic 505, Made by Hwashin Technology, Seoul Korea), Oven: (Model: Binder, Made in Germany)

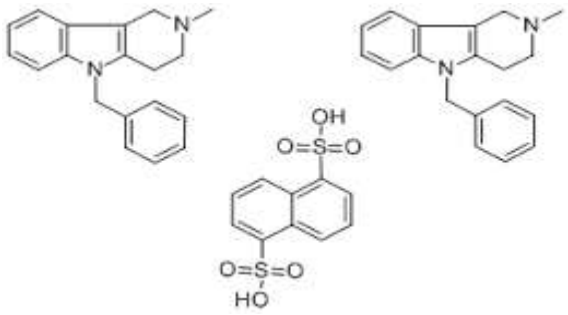

Figure 1: Chemical Structure of Mebhydroline Napadisylate. It is chemically (C19H20N2)2·C10H8O6S2

The Chromatographic separations were performed using a Thermo Hypersil reversed phase C-18 column ODS (with 150mm x 4.6 mm and 5 µm particle size), **Acetonitrile:Ammonia25% (80:20 v/v)** as a mobile phase,flow rate of 1.0 ml/min with isocratic elution, Injection volume was 20µl and retention time 5 min etc. The wavelength 320nm was detected by UV Spectrometer (Model: UV-1800, Made by Shimadzu Corporation) (Figure 2).

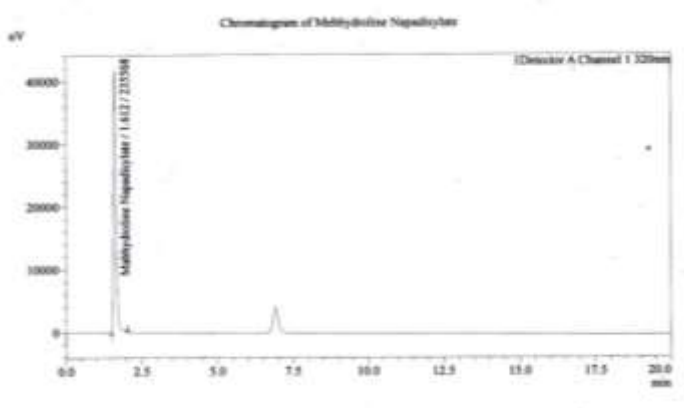

Figure 2: A typical chromatogram of a mixture of Mebhydroline. The wavelength 320nm was detected by UV Spectrometer (Model: UV-1800, Made by Shimadzu Corporation)

Preparation of standard stock solution of Mebhydroline:

Accurately weighted quantity of Mebhydroline 20 mg was transferred into 50 ml volumetric flask, dissolved and diluted up to mark with Mobile phase. Which later give a stock solution having strength of 400µg/ml.

Preparation of working standard solution of Mebhydroline:

200µg/ml of Mebhydroline solution was prepared by diluting 5ml of stock solution to 10 ml with mobile phase. This solution was diluted in mobile phase further to get the concentration range of 100, 300, 500µg/ml of Mebhydroline.

Mobile phase preparation:

Acetonitrile: Ammonia 25% (80:20v/v) was used as mobile phase. At first 25% ammonia was mixed with acetonitrile. The solvent was sonicated for 15 min and then filtered by vaccum filter. Then mobile phase was run for 60 min for stability.

Method development and Optimization

A RP-HPLC method was optimized with an intention to develop an accurate and reproducible method for simultaneous estimation of Mebhydroline. Isocratic elution is simple, requires only one pump and flat baseline separation for easy and reproducible results. The drug showed absorbance at 320 nm. Therefore the wavelength selected for the determination of Mebhydroline was 320 nm. The final chromatographic conditions were set for stationary phase giving satisfactory resolved peak and run time with reversed phase C18 Luna (150 mm × 4.6 mm id, 5µm) column. Mebhydroline was soluble in the methanol, chloroform, ether. Freely soluble in water and ethanol. So a series of mobile phases consisted of varying pH and volume fractions of methanol and water were tested and the best results were obtained using the mobile phase consisted of Acetonitrile:Ammonia25% (80:20 v/v), giving well resolved, sharp peak for Mebhydroline with a retention time of 1.612 min(Figure. 2). The flow rate of 1.0 ml/ min at 320nm. The mobile phase was filtered by using a 0.45 µm nylon membrane filter and degassed in an ultrasonic bath before used. The samples were also filtered using 0.45 µm nylon membrane filter. The flow rate was set at 1.0 mL/min and UV detection at 320 nm. The column was allowed to stand for 15 min before analysis was performed. All determinations were performed at ambient temperature 25±5 °C and the injection volume was 20 µL.

System Suitability and Method validation

All the system suitability parameters were assessed as per ICH guidelines (ICH, 2005)[10-11].

RESULT & DISCUSSION:

Method Validation

The method was validated for Linearity**,** detection limit (DL), and accuracy by the method of Funk *et al*, Hahn-Dienstrop, and Huber.

Linearity and range

For Mebhydroline the linear regression data for the calibration curves showed good linear relationship over the concentration range of 100-500µg/ml .Typically, the regression equations for the calibration curve was found to be **y=1153x + 12936 for Mebhydroline .**The calibration curve was plotted by considering the peak areas(y) versus corresponding concentration(x) in **µg/ml.** (Figure 3, table 1)

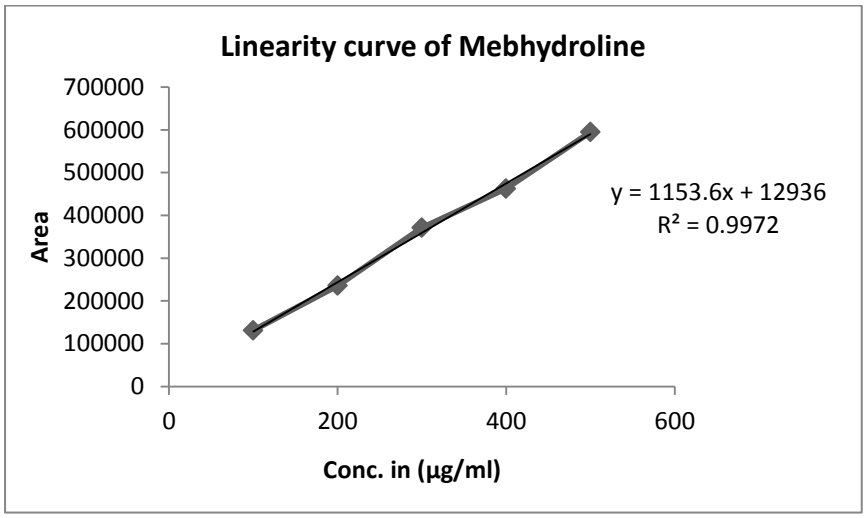

Figure 3: Linearity curve of Mebhydroline. For Mebhydroline the linear regression data for the calibration curves showed good linear relationship over the concentration range of 100-500µg/ml.

Table 1 Linearity result of Mebhydroline by RP-HPLC method

Sr. No	Concentration (µg/ml)	Area	Slope (S)	Intercept	r^2
1	100	131331			
2	200	235568			
	300	370957	1153	12936	0.9972
4	400	462610			
5	500	594608			

Limit of detection (LOD) and limit of quantitation (LOQ)

For this study, three replicates of analyte at lowest concentrations were measured and quantified. The equations are LOD=3.3×σ/S and LOQ=10×σ/S where, 'σ' is standard deviation **of the peak areas of the drug (n=3)** and 'S' is slope of linearity equation. The LOD and LOQ were 0.03µg/ml and 0.096133µg/ml respectively (table 2).

Table 2 LOD and LOQ result of Mebhydroline

Sr No	Conc.	Area	σ	S	LOD	LOQ
1	100	131331				
2	200	235568	120150.0127	1153.6	0.03	0.096133
3	300	370957				

Precision

The precision of the developed HPLC method was expressed in terms of percent relative standard deviation (% RSD). At first, standard sample (200µg/ml) was run for six times. After confirmation of system suitability parameters (number of theoretical plates, tailing factors etc.), six samples were injected. Then % RSD was calculated as 0.392 (table 3). % RSD values less than 2, revealed high precision of the method. These values were also less than the required values as described by Ermer and Indrayanto.

Table 3 Precition results of Mebhydroline

Sr. no	Sample preparation	Weight of sample	Area of sample	Calculated value	Assay mg	Average value	SD	% RSD
1	Sample 1	65.68	233756	.00021002	49.09			
2	Sample2	65.70	235146	.00021002	49.39			
3	Sample3	65.68	236147	.00021002	49.60	49.37	0.19335632	0.392
4	Sample4	65.71	234365	.00021002	49.22			
5	Sample 5	65.69	235161	.00021002	49.39			
6	Sample 6	65.70	235946	.0002002	49.55			

Robustness

There are different ways to check the robustness of methods such as variations of pH in a mobile phase, variations in mobile phase composition, different columns (different lots and/or suppliers), flow rate, detection wavelength, temperature etc. In this study, standard deviation of peak areas was calculated by changing two parameters. Temperature was increased to 30ºC and flow rate was changed to 0.8ml/min. Concentration of Mebhydroline was not changed. % RSD was found to be 0.293 (< 2) (table 4). Being less than 2, the values of % RSD indicated the robustness of the method. These values were also less than the required values as described by Ermer and Indrayanto. It was observed that there were no marked changes in chromatograms, which demonstrated that the RP-HPLC method developed is robust.

Accuracy

Accuracy was performed in triplicate after spiking pure drug equivalent to 80, 100, and 120% of the standard concentration of Mebhydroline (200µg/ml). Recovery was found in the range from 99.93-99.95%. The recovery of Mebhydroline by proposed method is satisfactory as percent of

relative standard deviation is not more than ± 2.0% and mean recovery between 98.0 - 102.0% (table 5).

Table 4 Robustness results of Mebhydroline

Sr. no	Sample preparation	Weight of sample	Area of sample	Calculaed value	Assay mg	Average value	SD	% RSD
1	Sample 1	65.70	237976	.00021002	49.98			
2	Sample 2	65.68	236986	.00021002	49.77			
3	Sample 3	65.72	236387	.00021002	49.65	49.742	0.142886902	0.302
4	Sample 4	65.70	236391	.00021002	49.65			
5	Sample 5	65.69	237153	.00021002	49.81			
6	Sample 6	65.68	236097	.00021002	49.59			

Table 5 Accuracy results of Mebhydroline

Sample	Area of sample	Theoretical value	Experimental value	% of recovary	Mean
80% S-1	235146	40.43	40.12	99.23	
80% S-2	235161	40.43	40.31	99.70	99.41
80% S-3	233756	40.43	40.15	99.31	
100% S-1	236986	50.53	50.16	99.27	
100% S-2	236076	50.53	50.32	99.58	99.56
100% S-3	236391	50.53	50.45	99.84	
120% S-1	237976	60.64	60.54	99.83	
120% S-2	237153	60.64	60.39	99.58	99.70
120% S-3	237586	60.64	60.45	99.68	

CONCLUSION:

Statistical analysis proves that the method is suitable for the analysis of Mebhydroline as bulk drug and in pharmaceutical formulation without interference from the excipients. Acceptable regression values, RSD % and standard deviations which make it versatile and valuable for simultaneous estimation of drugs in tablet formulation. The modalities adopted in experiment were successfully validated as per ICH guidelines. It is thus inferred that this newly developed method was found to be accurate, simple, precise, and reproducible.The short run (i.e. within 10 min) time of this method will significantly reduce the analysis time and cost. Therefore, this developed RP-HPLC method can be could be conveniently adopted for quality control analysis of Mebhydroline simultaneously from tablet dosage form. It may be extended to study the degradation kinetics of Mebhydroline and also for its estimation in plasma and other biological fluids.

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