NOTE

DETERMINATION AND VALIDATION OF MEBHYDROLINE NAPADISYLATE IN TABLETS BY HPLC

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Received 4 February 2008; Accepted 15 May 2008

ABSTRACT

An accurate 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, 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 15 min, based on peak area. 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*

INTRODUCTION

Some tablet pharmaceutical preparations containing mebhydroline napadisylate as sole active ingredient, are marketed now in Indonesia [1]. Mebhydroline napadisylate in pharmaceutical preparation is not official in any pharmacopoeia and has not been published yet. An analytical reference book, Pharmaceutical Press [2,3] described column liquid chromatography (LC) methods for the determination of mebhydroline napadisylate and other drugs in the antihistamine preparations. In the present study we suggest a simple selective and validated HPLC method for the determination of mebhydroline napadisylate in tablets.

The aim of this work was to develop a simple, rapid, and validated HPLC method for routine analysis of mebhydroline napadisylate in tablet.

EXPERIMENTAL SECTION

Material

Mebhydroline napadisylate (CrossChem, Lugano, Switzerland; Batch No. 20060806; Assay 99%) was pharmaceutical grade. The substance fulfills the requirement of Pharmacopoeia specifications and was used as received for preparing laboratory-made pharmaceutical preparation and standard solutions. Acetonitrile (E. Merck, Germany) was LC grade and ammonia 25% (E. Merck, Germany) was analytical grade reagents. The solvents were used without further purification. The HPLC instrument was Shimadzu Prominence equipped with reversed phase C-18 column (Shim-pack VP-ODS) and UV-Vis detector (SPD-20A).

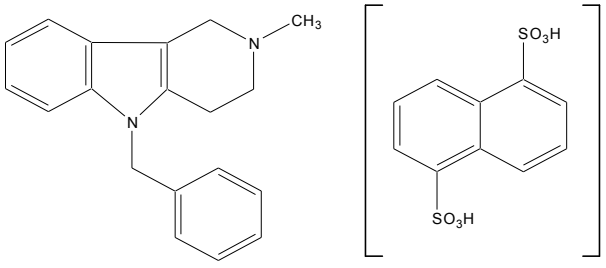

Fig 1. Chemical structure of mebhydroline napadisylate

Interhistin tablet containing 76 mg mebhydroline napadisylate were provided by Interbat Pharmaceutical Company (Buduran, Sidoarjo, Indonesia). Two commercial tablets (T-1 an T-2) containing mebhydroline napadisylate tablet were purchased in August 2006 from a local Pharmacy in Jember, East Java. T-1 and T-2 were produced in Indonesia.

Stock standard solutions were prepared daily by dissolving accurately weighed mebhydroline napadisylate in acetonitrile : ammonia 25% (80 : 20 v/v) 140, 200, 240, 350, 400 µg.mL^{-1}. For linearity studies, solution were prepared containing 80.0, 140.0, 200.0, 240.0, 350.0, and 400.0 µg.mL^{-1}.

Procedure

Sample Extraction

Ten tablets were accurately weighed and finely powdered. An amount equivalent to 10.0 mg mebhydroline napadisylate was transferred into 10.0 mL volumetric flask, and dissolved in 5.0 mL mobile phase, ultrasonicated for 5 min and then diluted to 10.0 mL with mobile phase. The solution was filtered using 0.45 µm nylon membrane filter, then 2.0 mL of filtrate

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was transferred into 10.0 mL volumetric flask and diluted with mobile phase.

Chromatographie Procedure

Chromatography was performed on a reversed phase C-18 column, using a mobile phase of acetonitrile : ammonia 25% (80 : 20 v/v). 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.

Methods Validation.

The method was validated for Linearity**,** detection limit (DL), and accuracy by the method of Funk *et al*, [4] Hahn-Dienstrop, [5] and Huber [6]. A four point accuracy study (added with 0%, 20%, 40% and 60% of the label claim) was performed on the Interhistin® tablet. For commercial preparations, accuracy studies were performed using single-point standard addition methods (40% of label claim). The precision was evaluated by analyzing six different extract aliquots from interhistin tablet according to a modified method of Renger *et al* [7].

RESULT AND DISCUSSION

The developed HPLC method was applied to the determination of mebhydroline napadisylate in tablet. To optimize HPLC assay parameters, the mobile phase composition was studied (Table 1). A satisfactory separation was obtained with a mobile phase of acetonitrile : ammonia 25% (80 : 20 v/v) using C-18 column at ambient temperature. The analysis was carried out by isocratic elution with flow rate 1.0 ml/min and detection at 320 nm. Under this condition, the chromatograms (Fig. 2) showed only two peak of mebhydrolin napadisylate (peak A at R_t 3 min and peak B at R_t 10.7 min), while other components were not detected or developed in this proposed method.

The basic calibration plot of peak area against amount of analyte was constructed within the range 20-200% of the expected values in the pharmaceutical preparations. Linearity of mebhydroline napadisylatee was achieved from 80 to 400 ppm with line equation Y=-49122.1+1870.8X, The relative process standard deviation (Vxo) and Xp values [8] of mebhydroline napadisylatee were 1.7% and 23.6 ppm, respectively (n=6; sdv=7574.4; r=0.9996). ANOVA regression-test for testing linearity of the regression line showed a significant calculated F-value (4837.6 for p<0.0001). The plots of the basic calibration graphs are not shown. The residuals were distributed at random around the regression line; neither trend nor uni-directional tendency was found.

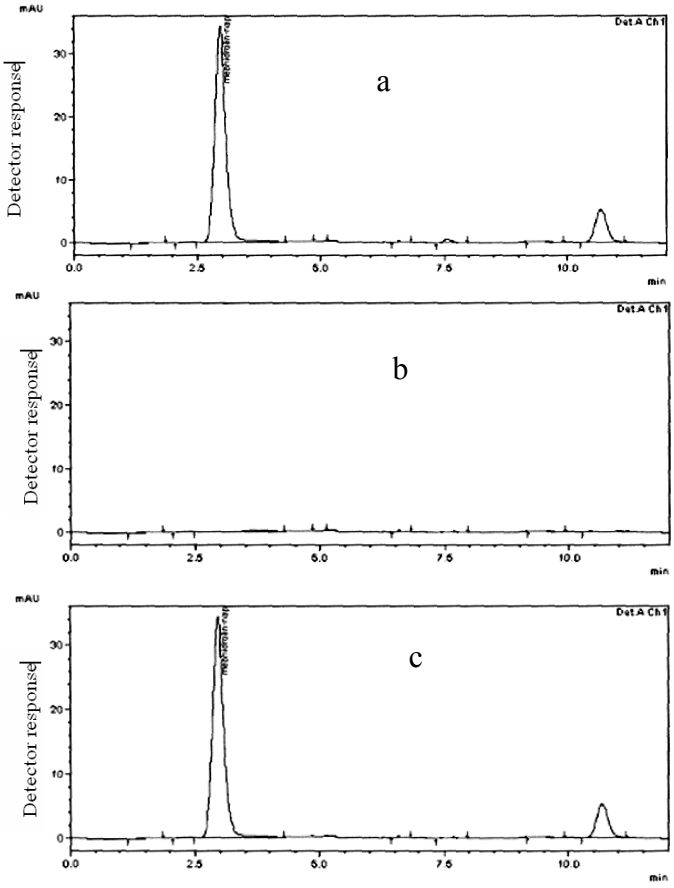

Fig 2. Chromatograms (using mobile phase of acetonitrile : ammonia 25% 80 : 20 v/v, C-18 column, isocratic elution with flow rate 1.0 ml/min and λ=320 nm) obtained from solution of standard mebhydrolin napadisylate (a), extract of excipients of laboratory-made tablet (b) and extract of laboratory-made tablet (c).

Table 1. Column efficiency parameters in various mobile phase

Mobile phase	Composition	Theoretical plate (N)	HETP	K'	Rt	
					Peak A	Peak B
Asetonitril LC Grade	100 %	449.876	555.709	311.472	3.95	6.0
Asetonitril LC Grade : ammonia 25% p.a.	95:5	735.701	339.812	335.233	3.4	10.5
Asetonitril LC Grade : ammonia 25% p.a.	90:10	2168.661	115.279	310.682	3.2	10.5
Asetonitril LC Grade : ammonia 25% p.a.	80:20	3919.832	63.778	297.095	3.0	10.7

Table 2. Accuracy results of pharmaceutical preparations

Pharmaceutical preparation	Ingredient	Label Claim (%)				
		Original[a]	Added (%)	Theory (%)	Found (%)	Recovery (%)
T-1	Mebhydroline napadisylate	98.4±0.96%	40.0[b]	240.4	246.2	102.4[c]
T-2	Mebhydroline napadisylate	100.5±0.42%	40.0[b]	239.8	244.3	101.8[c]
Interhistin tablet	Mebhydroline napadisylate	105.2±0.84%	20.0	231.0	233.1	100.9
			40.0	252.1	257.8	102.3
			60.0	273.1	276.6	101.3

[a]Mean + RSD; [b] n=3; [c]Average of two measurements

Table 3. Result from evaluation of precision of Interhistin tablets

Measurement[a]	RSD value (%, n=6)
1	0.76
2	0.98
3	1.28

[a]Each measurement was performed by a one analyst and on different days.

Detection limit was determined by making a linear regression of relatively low concentrations [4] of mebhydroline napadisylate (20 to 140 ppm n=6 Vxo=3.3%; sdv=4052.0; r=0.9981; line equation Y=34424.2+1346.0X). The ANOVA regression-test showed a significant F-value (1054.1 for p<0.0001). By this method, the calculated Xp[4] value was 17.6 ppm; in this case, DL=Xp.[4] According to Carr and Wahlich, [7] the value of quantitative limit (QL) could be estimated as three times the DL-value (52.8ppm)

Table 2 demonstrates the high accuracy of the new method as revealed by the percentage of mean recovery data (99.5-102.3%). To prove whether systematic error occurred, a linear regression of the recovery curve of Xf (percentage of label claim of the analyte found by the proposed method) against Xc (nominal percentage of label claim of the analyte after addition with the standard) of the Interhistin TR tablets was constructed.[4] In this case, the recovery curve equation was Xf=4.043 + 0.996Xc. The confidence range data (p=0.05) of the intercept VBaf (4.043 ±54.6) and slope VBbf (0.996±0.218) from the recovery curves did not reveal the accurence of constant or proportional-systematic errors.[4]

All of the values of the repeatability and intermediate precision valuations were less than 2% (Table 3). These values were also less than the required values as described by Ermer[9] and Indrayanto [10] (2.3%; specification range of 95-105%; basic lower limit 99.%; n=6). The three measurements were performed within one laboratory by different days.

CONCLUSION

The presented work describes validated HPLC methods for the assay of mebhydrolin napadisylate in tablets. The suggested method is simple, selective, and accurate. Therefore, the proposed method is suitable for the routine analysis of products of similar composition in pharmaceutical industrial quality control laboratories.

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