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A NEW RPHPLC METHOD FOR ANALYSIS OF MEBEVERINE HYDROCHLORIDE IN RAW MATERIALS AND TABLETS

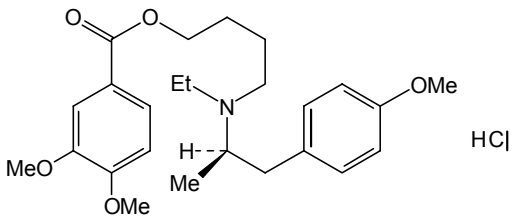
M. SAEED ARAYNE, NAJMA SULTANA* AND FARHAN AHMED SIDDIQUI
Department of Chemistry, University of Karachi, Karachi-75270, Pakistan, Email: arayne@gawab.com
**Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Karachi, Karachi*

A simple, sensitive, selective, reliable, least time consuming and rapid high-performance liquid chromatographic method for the determination and quantification of mebeverine hydrochloride using hyoscine butylbromide as internal standard has been developed. The chromatographic system consisted of a Shimadzu LC-10 AT VP pump, SPD-10 AV VP UV visible detector, and a CBM-102 Bus Module integrator. Separation was achieved on the µ Bondapak 125 a C18 10µm column at room temperature. The samples were introduced through an injector valve with a 10 µl sample loop. Acetonitrile-water (1:1 v/v) was used as mobile phase, with flow rate 1.7 ml/minutes. pH was adjusted to 2.9 with phosphoric acid. U.V detection was performed at 205 nm. The results obtained showed a good agreement with the declared content. Recovery values of mebeverine hydrochloride were from 99.80% to 100.13%. The proposed method is rapid, accurate, and selective and may be used for the quantitative analysis of mebeverine hydrochloride. The method was found to be specific, accurate, precise and reliable for the determination and quantification of mebeverine hydrochloride in form of raw materials, in bulk drugs and formulation. It was possible to determine all of them in the concentration range of 5-30 nano grams. The detection limit of mebeverine hydrochloride was 0.4-nano gram.

Keywords: Mebeverine, hyoscine, HPLC determination.

INTRODUCTION

Mebeverine hydrochloride is 4-[ethyl(4-methoxy-α-methyl-phenethyl)amino]butylveratrate hydrochloride having molecular formula $C_{25}H_{35}NO_5HCl$, molecular weight 466 and melting point 105 -107 ºC. It is white or almost white, crystalline powder, freely soluble in water and ethanol (96 %), while practically insoluble in diethyl ether.



Mebeverine hydrochloride

It is a musculotropic antispasmodic with a direct action on the smooth muscle of the gastrointestinal tract, relieving spasm without affecting normal gut motility since this action is not mediated by the autonomic nervous system. Mebeverine is rapidly and completely absorbed, and is not excreted as such but metabolized completely. Its hydrochloride is an antispasmodic with a direct action on smooth muscle of the gastrointestinal tract. It is used in conditions such as the irritable bowel syndrome [British Pharmacopoeia 2003; The Merck index. 2001; Martindale, The extra pharmacopoeia 1996].

EXPERIMENTAL

Material and reagents

Hyoscine butylbromide, and mebeverine hydrochloride reference standards were a kind gift from AGP (Private) Limited. Spasler neo tablets were purchased from the market. HPLC grade acetonitrile and phosphoric acid were obtained from Merck. The mobile phase and solution were prepared in double distilled deionized water. Stock solutions of the compounds were prepared in deionized water stored at room temperature. Fresh working solutions were prepared daily. All solutions were filtered (0.45 µm) and degassed by sonicator.

Apparatus

The HPLC system was LC-10 AT VP Shimadzu pump, SPD-10AV VP Shimadzu UV visible detector and a µ Bondapak 125 a C18 column (particle size 10 µm) was used for separation. The chromatographic and integrated data were recorded using a CBM-102 communication Bus Module Shimadzu on an IBM PC.

Chromatographic conditions

The mobile phase was acetonitrile-water (1:1); the pH of this mobile phase was adjusted to 2.9 with phosphoric acid (85 %). Before delivering into the system it was filtered through 0.45 µm filter and degassed using a vacuum. The analysis was carried out under isocratic conditions using a flow rate 1.7 ml/minutes at room temperature. Chromatograms were recorded at 205 nm using SPD-10AV VP Shimadzu UV visible detector; Injector with a 10 µ litre sample loop introduced the samples.

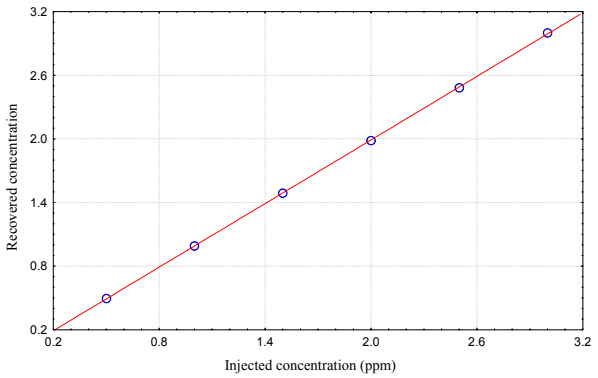


Fig. 1: Recovery of mebeverine hydrochloride.

Analytical procedure

10 mg internal standard (hyoscine butylbromide) and 10 mg of mebeverine hydrochloride were dissolved in water in 100 ml volumetric flask separately and made up volume with its solvent (Stock solution 100 µg/ml). With the help of stock solution aliquots of desired concentration of mebeverine hydrochloride were prepared by dilution. Twenty tablets of Spasler neo tablets were weighed to obtain the average tablets weight (400 mg) and were then powered; 29.63 mg of the powdered tablets (equivalent to 10 mg of active substance) was mixed with 100 ml water. This mixture was allowed to stand for 1 hour with intermittent sonication to ensure complete solubility of the drug. This stock solution was filtered to obtain clear filtrate; from this clear filtrate working solutions were prepared of desire concentration. 10 µ L volume of each sample was injected and chromatographed under above conditions.

RESULTS AND DISCUSSION

The determination and quantification by HPLC in the quality control of drugs and drug products has received considerable attention and is advantageous due number of reasons. The goal of this study was to develop a rapid, more accurate, precise reliable, less expensive and least time consuming HPLC method for the analysis of mebeverine hydrochloride using hyoscine butylbromide as internal standard. The drug can be estimated efficiently in the form of raw materials, bulk drug samples and its tablets formulations using the most commonly employed C-18 column with UV detection.

There are number of methods reported in literature for the determination of mebeverine hydrochloride. Fluorescence polarization immunoassay (FPIA) and gas chromatographic-mass spectrophotometric studies on the toxicological analysis of mebeverine after injection of a single 405 mg oral dose of mebeverine were reported by Kraemer *et al.,* (Kraemer 2001). Stockis *et al.* (2002) reported a method of identification of mebeverine acid as the main circulating metabolite of mebeverine in man while the metabolism of mebeverine in man and identification of urinary metabolites by gas chromatography/mass spectrometry were reported (Kristinsson *et al.,* 1994). The HPLC method for the determination of plasma concentration of the mebeverine was reported (Dickinson *et al.,* 1991).

Present HPLC method was developed on the basis of chemical structure and other physical properties which are most important facts that predict chromatographic behavior.

Table 1

Recovery of mebeverine hydrochloride in reference drug

Conc. injected (ppm)	<——— Reference drug ———>			<——— Dosage form ———>		
	Found	Recovered (%)	Mean (AUC)	Found	Recovered (%)	Mean (AUC)
0.5	0.4957	99.1359	17184	0.4998	99.956	17610
1	0.9903	99.0291	34332	1.0014	100.14	35284
1.5	1.4890	99.2652	51620	1.4975	99.832	52764
2	1.9845	99.2264	68800	1.9979	99.893	70395
2.5	2.4814	99.2573	86027	2.4950	99.801	87913
3	3	100	104005	3	100	105706

Table 2

Inter day accuracy and precision of proposed method

Conc injected	<—— Day 1 ——>		<—— Day 2 ——>		<—— Day 3 ——>		<—— Day 4 ——>	
	RSD	(%) Recovery	RSD	(%) Recovery	RSD	(%) Recovery	RSD	Recovery (%)
0.5	0.0039	99.134	0.0062	100.18	0.0021	100.37	0.0039	99.956
1	0.0023	99.03	0.0016	99.82	0.0026	99.98	0.0023	100.138
1.5	0.0017	99.27	0.0024	100.11	0.0015	99.86	0.0009	99.8321
2	0.0024	99.23	0.0046	99.55	0.0033	99.86	0.0012	99.8932
2.5	0.0025	99.26	0.0015	100.07	0.0018	99.83	0.0011	99.8011
3	0.0063	100	0.0037	100	0.0006	100	0.0026	100

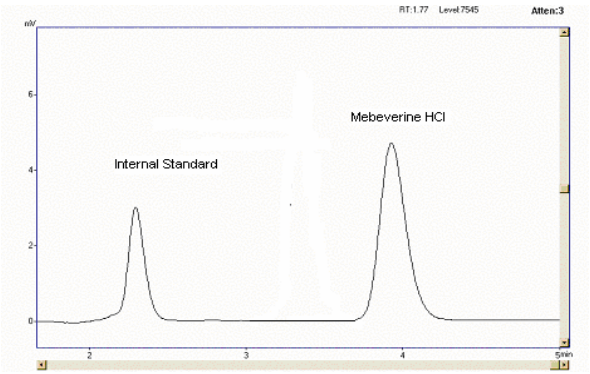


Fig. 2: *A typical chromatogram showing mebeverine hydrochloride in presence of hyoscine butylbromide.*
Retention time = Hyoscine butylbromide (Internal standard) = 2.3 minutes
Mebeverine hydrochloride = 3.9 minutes

In the present investigation the best separation was achieved using a μ Bondapak 125 a C18 (10 μm) columns. Using other types of column under the same experimental condition, peak tailing and peak broadening was observed. For the separation of mebeverine hydrochloride with internal standard (hyoscine butyl-bromide) and quantification of mebeverine hydrochloride the best results were obtained using mobile phase acetonitrile-water (1:1 v/v). The lower percentage of acetonitrile in mobile phase resulted also in peak tailing of both components and long analysis duration, while higher percentage of acetonitrile in mobile phase resulted in very little analysis duration. Optimal retention times (hyoscine butylbromide 2.3, and mebeverine hydrochloride 3.9 minutes) were achieved when the pH of mobile phase was adjusted to 2.9 with 85 % phosphoric acid. Small changes in pH of the mobile phase had a great influence to the chromatographic behavior of these substances. At the higher pH of the mobile phase, peak tailing was observed while at lower pH values, retention times of hyoscine butylbromide and mebeverine hydrochloride were extremely long. A typical chromatogram showing separation of mebeverine hydrochloride in presence of hyoscine butylbromide as internal standard is shown in figure 2.

Accuracy and precision

The accuracy of the method was evaluated by analyzing independently prepared solutions of mebeverine hydrochloride. The recovery data is expressed in tables 1-4. These tables show that the method is accurate for determination of mebeverine hydrochloride. Data of regression characteristic is expressed in table 3. All calibration curves have a correlation coefficient value of at least 0.9999. The accuracy was calculated as a percentage of the nominal concentration: Accuracy = (concentration observed/nominal concentration) x 100.

The precision of the method was investigated with respect to repeatability. For intra-day precision, six concentrations of each compound were analyzed on the same day. Each concentration of sample was injected 4 times. Table 2 summarizes the relative standard deviation (RSD). Generally acceptable repeatability of the results with in one

Table 3
Recovery and regression characteristics of proposed method

Concentration injected (ppm)	<——— Recovered concentration ———>			
	Day 1	Day 2	Day 3	Day 4
0.5	0.4957	0.5009	0.4955	0.4998
1	0.9903	0.9982	1.0037	1.0014
1.5	1.489	1.5017	1.4997	1.4975
2	1.9845	1.991	1.9972	1.9979
2.5	2.4814	2.5017	2.4958	2.495
3	3	3	3	3
Correlation Coefficient (R)	0.9999	0.9999	0.9999	0.9999
Standard Error of estimate	0.0077	0.0046	0.0035	0.0023
Standard Error	0.0071	0.0043	0.0032	0.0021
p value	0	0	0	0
Intercept	0.009	0.0006	0.001	0.0004
Slope	1	1	1	1

Table 4
Recovery of mebeverine hydrochloride from tablets (Spasler neo) by proposed method

Recovered (mg)	Error (%)	Recovery (%)	Recovered (mg)	Error (%)	Recovery (%)
134.94	0.05	99.95	135	0	100
135.18	0.13	100.13	134.73	0.2	99.8
134.77	0.17	99.83	134.85	0.11	99.89
Label claim of Spasler neo tablets = 135 mg					

day and day-to-day were observed. Data of the relative retention times obtained in a series of four consecutive injections also showed acceptable repeatability when analyzed not only on the same day but also on three consecutive days.

System suitability and specificity

System suitability of the method was evaluated by analyzing the symmetry of the internal standard (hyoscine butylbromide) and mebeverine hydrochloride peaks, theoretical plates of the column and the resolution between the peaks of internal standard and mebeverine hydrochloride. The specificity of the method was evaluated to ensure separation of internal standard and mebeverine hydrochloride. The specificity of the method was demonstrated by assaying a sample of mebeverine hydrochloride. The method demonstrated resolution between internal standard and mebeverine hydrochloride.

Calibration, quantification and detection limit

Graph (fig. 1) was constructed of the injected concentration of mebeverine hydrochloride against recovered concentration. The limit of quantification of the developed method was found 500ng/ml while detection limit was found to be120ng/ml.

Ruggedness

Ruggedness of this method was evaluated in two different labs with two different instruments. Lab No.1 was in the Department of Chemistry, University of Karachi while Lab No.2 was the Brookes Lab-1 at Research Institute of Pharmaceutical Sciences, University of Karachi.

CONCLUSION

The paper describes a new method for determination of mebeverine hydrochloride using hyoscine butylbromide as internal standard by RP-HPLC. The proposed RP-HPLC method enables determination and quantification of mebeverine hydrochloride in raw materials as well as dosage formulations (tablets & injections) because of good separation and resolution of the chromatographic peaks. The proposed method is rapid, precise and the obtained results are in a good agreement with the declared contents. The accuracy and precision of the method has been confirmed by the statistical parameters.

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THE EFFECTS OF MEFENAMIC ACID ON THE OSMOTIC FRAGILITY OF LACERTILIAN ERYTHROCYTES

MAHMOOD AHMAD, RUQAIYA HASAN, ANILA QURESHI*, MANSOOR AHMAD* AND ZULFIQAR AHMED

Department of Physiology, University of Karachi, Karachi-75270, Pakistan
**Department of Physiology, New York Medical College, Westchester Hospital, Valhalla, New York, USA*

Osmotic fragility of red cells is increased by the use of mefenamic acid. The use of this analgesic induces hemolytic anemia. Study of osmotic fragility of RBCs of control and test was observed following administration of 7.1 mg, 10.5 mg and 14 mg/day mefenamic acid to each lizard. Increased osmotic fragility was observed with increase in the amount of dose on day 6 and day 12.

Keywords: Osmotic fragility, mefenamic acid, drug induced hemolysis.