Quantitative Analysis of Mebeverine in Dosage Forms by HPLC

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Key Words

Column liquid chromatography
Mebeverine in dosage forms

Summary

A simple and rapid, high performance liquid chromatographic (HPLC) procedure for determination of mebeverine in dosage forms (tablet and liquid) is described. Reversed-Phase chromatography was carried out using a mobile phase containing 0.05 M ammonium acetate buffer and acetonitrile, [(45 %, v/v) pH 5.2] with UV-detection (263 nm). Replicate regression analyses of three standard plots in the concentration range of 0.5–10 mcg mL^{-1} obtained on three different days gave a correlation coefficient > 0.9995 and the coefficient of variation of the slopes < 2.2 %. The assay was precise within day and between days as indicated by ANOVA test. The recoveries from 10 replicate tablets of two commercial mebeverine brands and liquid were in order 99.3, 100.5 and 100.1 % of the label amount and their coefficient of variations were 1.41, 0.89 and 0.69 %, respectively. The limit of quantitation of mebeverine was 5 ng mL^{-1}.

Introduction

Mebeverine, 3,4-dimethoxybenzoic acid 4-{ethyl-[2-(4-methoxyphenyl)-1-methylethyl] amino} butyl ester hydrochloride, is a spasmolytic agent with a direct nonspecific relaxant effect on gastrointestinal smooth muscle and colon [1], with more selective effect on colon [2]. The clinical significance of the drug in the treatment of irritable bowel syndrome has been confirmed [3].

Several techniques and methodology for assaying mebeverine in various biological specimens, e.g. plasma, tissues and urine, and in bulk form and tablet formulations, have been developed. The investigated methods include non-aqueous titrimetry [4], colorimetry by charge transfer complexation [5], direct [6] and derivative [7] UV-spectrophotometry, capillary supercritical fluid chromatography with mass spectrometric detection [8], high performance thin layer chromatography [9, 10] and high performance liquid chromatography [11–13]. In contrast with the work described here, the previously reported HPLC methods for the determination of mebeverine in tablets and pure form [11–13] utilized a range of mobile phase compositions with high percentage of organic solvents. These methods also entail a relatively higher detection limits than the present HPLC procedure. In addition, no studies have yet been undertaken to demonstrate the applicability of the proposed HPLC procedures for quantification of the drug in pharmaceutical preparations, i.e. tablets and liquids. The main task of the present work was to develop a rapid and sensitive method for accurate determination of mebeverine. The established HPLC procedure was to be applied for determination of the drug content in tablets and liquids.

Experimental

Chemicals and Reagents

Reference mebeverine hydrochloride [14] and methyl paraben [15] were utilized without further treatments. Water was bidistilled in an all-glass distillator for all analytical purposes. Acetonitrile [16] and methanol [16] were of HPLC grade. All other chemicals and reagents were of U.S.P. or A.C.S. quality and were used as received.

Instrumentation

Waters liquid chromatograph [17] equipped with Waters-5O1 pump, Waters-WISP 712B autosampler, Waters-480 UV-detector set at 263 nm at 0.02 AUFS and Waters-730 data module was utilized. Chromatographic separation was accomplished using C_{18} column, 3.9 × 300 mm μBondapack C_{18} column with 10 μm packing. Solvent degassing with pure helium is preferable to get the best reproducibility by adopting the following chromatographic conditions.

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Chromatographic Conditions

The mobile phase containing 0.05 M ammonium acetate buffer/acetonitrile (45:55, v/v) adjusted to pH 5.2 with glacial acetic acid, was prepared and degassed for 5 min prior to use. Column equilibrium with the eluent was established by pumping the mobile phase at a rate of 0.2 mL min^{-1} for overnight. The flow rate was set at 1.0 mL min^{-1} during analysis. The chromatogram was recorded and integrated at a speed of 0.25 cm min^{-1}. All analysis were done at room temperature.

Internal Standard

A stock solution of methyl paraben containing 10 mg in 100 mL methanol was prepared weekly and stored at 4 °C.

Standard Solution of Mebeverine Hydrochloride

A stock solution of mebeverine hydrochloride was prepared by dissolving 10 mg of the drug in 10 mL methanol. Seven aliquots equivalent to 0.5, 1, 2, 4, 6, 8, and 10 mcg of mebeverine hydrochloride were added to 1 mL volumetric flask. After the aliquot of the internal standard equivalent to 1 mcg was added, the flasks were brought to volume by acetonitrile and thoroughly mixed. Twenty five µL of the standard solutions were injected onto the column for analysis. The peak area ratio of the drug internal standard were plotted versus the standard mebeverine hydrochloride concentrations. Least square linear regression analysis was performed to determine the slope, y-intercept, and the correlation coefficients of the standard plots.

Sample Preparation

1. Tablets [14]: Individual tablets were powdered using a mortar and pestle, and completely transferred to 100 mL volumetric flask. The volume was adjusted with methanol and the flask was mechanically shaken for five min. Five mL of the solution were removed into a centrifuge tube and centrifuged at 3000 r.p.m. for 5 min. Fifty µL were transferred to a 10 mL volumetric flask containing 10 µL of methyl paraben stock solution, and the volume was completed with mobile phase. Twenty five µL were loaded into the sample loop for chromatography. Ten replicate commercial tablets of mebeverine. hydrochloride were analyzed for statistical evaluation of the assay.

2. Liquid [14]: Ten mL of the liquid (each 1 mL is equivalent to 10 mg of mebeverine hydrochloride as claimed by the manufacturers) were transferred to 100 mL volumetric flask. The volume was adjusted with methanol and the flask was mechanically shaken for five minutes. Five mL of the solution were removed into centrifuge tube and centrifuged at 3000 r.p.m. for 10 min. Fifty µL were transferred to a 10 mL volumetric flask containing 10 µL of methyl paraben stock solution, and the volume was completed with mobile phase. Twenty five µL were loaded into the sample loop for chromatography.

Quantitation

The amount of mebeverine per tablet was determined from the following equation:

$$Q = [R/A + B] \times \text{dilution factor}$$

were Q is the mg mebeverine per tablet, R is the peak area ratio (drug/internal standard), A is the slope of the calibration curve and B is the y-intercept.

Limit of Detection and Quantitation

The smallest detectable quantity of mebeverine hydrochloride, defined as at least three times the baseline noise signal was about 5 ng mL^{-1}. Lower concentrations could be quantitated by injecting larger sample volumes.

Recovery Testing

To 1 mL of the drug extract in the mobile phase claimed to contain 50 µg an equal mass of the reference drug substance was added from the stock solution in the mobile phase, followed by 100 µL of the internal standard in the mobile phase in 10 mL volumetric flask. The volume was completed with the mobile phase, mixed well to homogenize. Triplicate injections were made to calculate the average ratio response, due to the added masses i.e. the area of each added drug compared with that of the internal standard.

Statistical Analysis [18]

All the results are expressed as mean ± SD. The relative standard deviation RSD% was calculated for all values. The Student's t-test was used to examine the concentration difference at each day, and one-way analysis of variance (ANOVA) was employed to evaluate the reproductivity of the assay. The level of confidence was 95 %.

Results and Discussion

Typical HPLC-separation of the drug from the tablets extracts in the mobile phase is shown in Figure 1. Following the chromatographic conditions described above, mebeverine and methyl paraben were well separated and their retention times (T_R) were 8.8 and 4.72 min, respectively. For both compounds sharp and symmetrical peaks were obtained with good baseline resolution and minimal tailing, thus facilitating the accurate measurement of the peak area ratio. No interfering peaks were found in the chromatogram due to excipients and other formulation additives. Figure 2 shows a calibration plot for the peak area ratio of varying amounts of mebeverine (0.5–10 µg mL^{-1}) to a constant amount of methyl paraben (1 mcg mL^{-1}). The plots were linear (Correlation Coefficient r = 0.9996) and the regression analysis of the data gave the slope and intercept as:

$$Y = 0.1383\ X - 0.00573$$

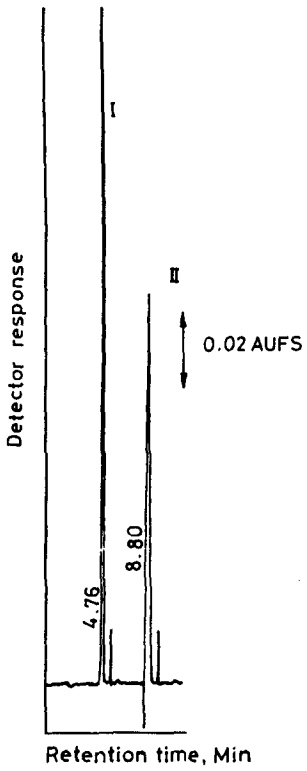

Figure 1
Chromatogram of mebeverine hydrochloride tablet. Key: I. Methyl-paraben; II. Mebeverine hydrochloride.

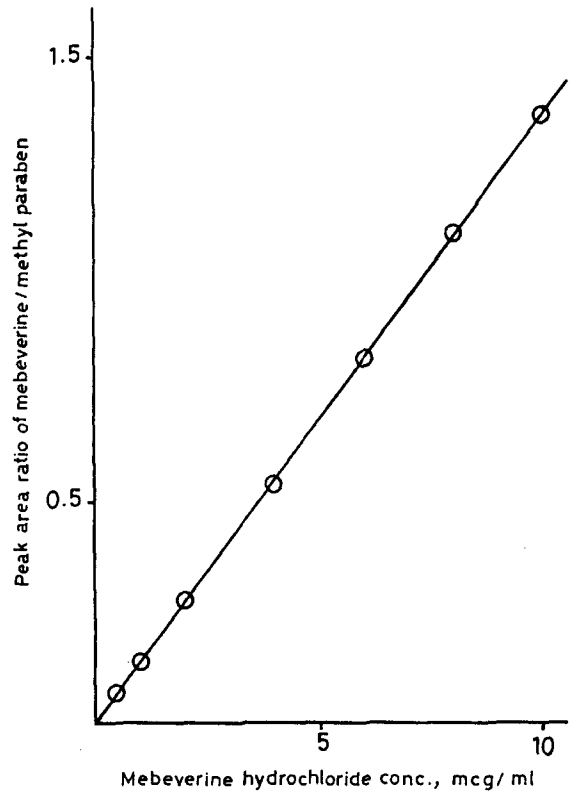

Figure 2
Standard calibration plot of mebeverine hydrochloride.

Table I. Regression analyses of the three standard plots of mebeverine.

Standard[a]	Slope[b]	Intercept[b]	Correlation Coefficient[b](r)
1	0.1383	-0.005733	0.9999
2	0.1336	-0.006200	0.99952
3	0.1315	-0.006200	0.99943

[a]obtained in 3 different days
[b]The mean of 3 determinations at each drug concentration.

Table IIa. Analysis of Variance for Intra- and Interday Precision [concentration 0.75 μg mL^{-1}].

Day/ assay	1	2	3	4	5	6	7	8
1	0.796	0.682	0.695	0.704	0.75	0.708	0.716	0.809
2	0.810	0.785	0.827	0.760	0.749	0.732	0.808	0.793
3	0.665	0.736	0.698	0.711	0.731	0.744	0.773	0.711

Mean = 0.745 mg, SD = 0.04486, RSD% = 6.02

ANOVA

Source of variation	DF	Sum of squares	Mean of squares	*F value
Total	23	0.029761		
Between	2	0.00123	0.000616	0.45
Within	21	0.02853	0.001360	

F(95 %) tabulated = 3.74, *No significant difference at $P < 0.05$.

where Y and X are the peak area ratio and mebeverine concentration, respectively. Three replicate analyses of the drug at concentration of 0.5–10 mcg mL^{-1} were performed at three different days over one week period. The results of this evaluation are summarized in Table I. The average correlation was higher than 0.9995 and the coefficient of variation of the slopes of the three lines was < 2.2 °. Analysis of variance of the data showed no detectable difference in the slopes of the three standard plots ($F = 3.2$, $P > 0.01$). The high correlation coefficients and the similarities in the slopes are good indication of the excellent reproducibility and linearity of the proposed method. The assay results also revealed its accuracy and precision within the assay day as well as between assay days.

Precision: The reproducibility of the present method was assessed by assaying replicate mebeverine samples (n = 8) spiked at three concentrations (0.75, 5 and 9 μg mL^{-1}) for three, four and five consecutive days, respectively, for intra- and interday precision studies (Tables IIa-IIc). Estimates of day to day and within day precision were calculated by ANOVA test. The calculated F values were smaller than the table values. Thus, it was

Table IIb. Analysis of variance for intra- and interday precision [concentration 5 μg mL^{-1}]

Day/ assay	1	2	3	4	5	6	7	8
1	4.968	4.993	4.874	5.317	5.095	5.002	4.857	4.806
2	5.037	5.029	4.902	4.096	5.062	4.826	4.810	5.163
3	5.010	5.076	4.955	4.990	4.873	5.003	4.887	4.710
4	4.843	5.021	5.062	5.091	5.079	4.946	4.992	4.934

Mean = 4.95 mg, SD = 0.19306, RSD% = 3.9

ANOVA

Source of variation	DF	Sum of squares	Mean of squares	*F value
Total	31	0.45920		
Between	3	0.01758	0.00586	0.37
Within	28	0.44162	0.01577	

F(95 %) tabulated = 2.95, *No significant difference at $P < 0.05$.

Table IIc. Analysis of variance for intra- and interday precision [concentration 9 μg mL^{-1}]

Day/ assay	1	2	3	4	5	6	7	8
1	9.282	9.226	8.645	9.145	9.365	8.671	8.751	8.714
2	9.258	9.291	9.409	8.660	8.643	8.727	9.098	9.426
3	8.827	8.891	9.066	9.026	8.827	9.042	8.811	8.994
4	9.227	8.975	9.368	9.025	8.988	9.165	8.42	8.620

Mean = 8.99 mg, SD = 0.2678, RSD% = 2.98.

ANOVA

Source of variation	DF	Sum of squares	Mean of squares	*F value
Total	31	1.96669		
Between	3	0.08276	0.02759	0.41
Within	28	1.88394	0.06728	

F(95 %) tabulated = 2.95, *No significant difference at $P < 0.05$.

Table III. Assay and recovery of mebeverine in dosage forms by adopting the proposed HPLC procedure.

Formulation	Assay+	Recovery+
Tablet A	98.9 ± 0.9 (0.91, 10)	99.3 ± 1.4 (1.41, 10)
Tablet B	101.0 ± 0.75 (0.74, 10)	100.5 ± 0.9 (0.89, 10)
Liquid	104.0 ± 0.58 (0.55, 10)	100.1 ± 0.7 (0.69, 10)

X ± SD (Cv, n) each run is the average of at least 10 experiments.

concluded that there was no significant difference for the assay which was tested within day and between days.

Assay and Recovery of Mebeverine Hydrochloride Tablets

Table III collects the HPLC-assay results of the drug in tablets (two commercial dosage forms), and liquid, in addition to the results of recoveries of added 50 % drug mass. The precision of the method is clearly reflected as the obtained low deviations and variations.

Conclusion

The investigated HPLC procedure indicated excellent resolution and peak symmetry of mebeverine. The applicability of the method for routine drug analysis revealed that the proposed procedure is simple, rapid and precise enough for the determination of mebeverine in dosage forms.

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