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METHOD DEVELOPMENT AND VALIDATION OF MEBEVERINE HCL IN BULK DRUGS BY RP-HPLC METHOD

Jagadeesh C. S., Senthilkumar G. P.* and Chandan R.

Department of Pharmaceutical Analysis, Bharathi College of Pharmacy, Bharathinagara, Mandya-Dist, Karnataka, India-571422.

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***Corresponding Author**
Dr. Senthilkumar G. P.
Department of Pharmaceutical Analysis, Bharathi College of Pharmacy, Bharathinagara, Mandya-Dist, Karnataka, India-571422.

ABSTRACT

A simple, specific, quick, isocratic Reversed Phase High Performance Liquid Chromatographic method was developed and validated for the analysis of Mebeverine HCl. RP-HPLC method was developed on a X-Terra, C-8 (4.6 × 100 mm), 5 µm particle, reversed-phase column. The mobile phase was triethanolamine (pH- 3): acetonitrile, 40:60 (v/v) at a flow rate of 0.8 ml/min to get better reproducibility and repeatability. Quantification was achieved with UV detection at 271 nm based on peak area. The retention time was found to be 4.334 min. The method was linear over the range of 1-5 µg/ml with a regression coefficient of 0.999 and validated with respect to accuracy, precision, linearity and specificity, limit of detection and limit of quantization as per the guidelines of International Conference for Harmonization (ICH). This method can be used in the industries for determination of Mebeverine HCl to analyze the quality of formulation without interference of the excipients.

KEYWORDS: Mebeverine HCl, RP-HPLC, validation, ICH.

INTRODUCTION

Mebeverine is chemically Benzoic acid, 3, 4-dimethoxy-, 4-[ethyl [2-(4-methoxyphenyl)-1-methylethyl] amino] butyl ester, with molecular formula $C_{25}H_{35}NO_5$. Mebeverine is an antimuscarinic and belongs to a group of compounds called musculotropic antispasmodics. These compounds act directly on the gut muscles at the cellular level to relax them which relieves muscle spasm pains of gut. It is used for the treatment of irritable bowel syndrome as

well as other conditions including chronic irritable colon, spastic constipation and mucous colitis.

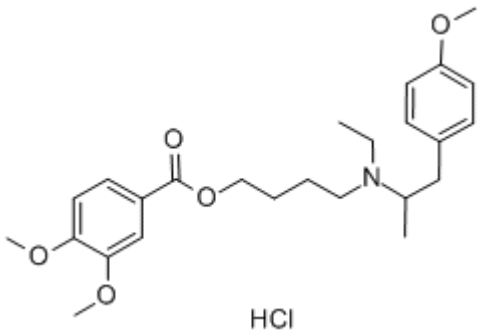

Fig.1. Structure of Mebeverine hydrochloride

A literature survey regarding Mebeverine HCl revealed that attempts were made to developed analytical methods for few HPLC and HPTLC methods for the estimation of individual and combined with other drugs.[1-7] Stability study and quantitative determination of mebeeverine hydrochloride.[8] UV spectrophotometric method was developed for Mebeverine HCl.[9] There is no HPLC method available for the analysis of Mebeverine HCl only. So, there is need for the development of a HPLC method for the analysis of Mebeverine HCl. Hence, an attempt has been made to develop a simple, quick, specific, accurate efficient and selective method for the analysis of Mebeverine HCl in bulk and pharmaceutical formulations.

MATERIALS AND METHODS

Instruments

Chromatographic separation was performed, under ambient conditions, with Waters alliance 2695 module (Waters Corporation, Milford, USA) equipment comprising a variable wavelength Waters 2487 dual λ absorbance UV detector with empower-2 software was used for the analysis. Samples (20 µl) were injected by means of a Rheodyne injector fitted with a 20µl loop. Compounds were separated on a X-Terra, C-8 (4.6×100mm), 5 µm particle, reversed-phase column. The mobile phase was triethanolamine buffer (pH- 3): acetonitrile, 40:60 (v/v) at a flow rate of 0.8 ml/min.

Chemicals

An analytically pure sample of Mebeverine HCl was procured as gift sample from Teva parm India Ltd, Goa, India. HPLC-grade Acetonitrile and Triethanolamine were purchased from

Qualigens fine chemicals, India. High-purity water was prepared using Millipore purification system. Other chemicals and reagents were of AR-grade.

Selection of mobile phase

Based on sample solubility, stability and suitability various mobile phase compositions were tried to get a good resolution and sharp peaks. The standard solution was run in different mobile phases. From the various mobile phases, triethanolamine buffer p^H-3: acetonitrile [40:60 v/v] was chosen with detection wavelength 271 nm, since it gave sharp peak with good symmetry within limits.

Preparation of diluents

Triethanolamine

To prepare 4 ml triethanolamine in 1000 ml of HPLC water, the p^H of the solution was adjust to 3.0 with 2% ortho phosphoric acid Solution.

Chromatographic conditions

The optimized parameters which were used as a final method for the estimation of represented in the Table 1.

Table 1: Optimized chromatographic conditions

Mobile phase	**Triethanolamine buffer p^H-3: acetonitrile [40:60 v/v]**
Stationary phase	X-Terra, C_{18} (4.6 x 100mm, particle size 5µm).
Wavelength	271 nm
Run time	10 min
pH mobile phase	3
Flow rate	0.8 ml per min
Injection volume	10 µl
Temperature	Ambient
Mode of operation	Isocratic elution

Preparation of working standard stock solution

Accurately weigh and transfer 10 mg of mebeverine HCl working standard into a 10 ml clean dry volumetric flask add diluent and sonicate to dissolve it completely and make volume up to the mark with the diluents (triethanolamine) to obtain standard stock solution of 1000 µg/ml (stock solution-1). From the above solution pipette out 1.0 ml into 10 ml volumetric flask and made up to the mark with diluents to obtain 100 µg/ml (stock solution-2).

Preparation of standard solution preparation

Aliquots of 0.1, 0.2, 0.3, 0.4, 0.5 ml standard stock solution (100 µg/ml) was transferred to 10 ml of volumetric flasks and made up to the mark with diluent triethylineamine to get concentration of 1, 2, 3, 4, 5 µg/ml. An aliquot (10µl) of each solution was injected under the operating chromatographic conditions and responses were recorded. Calibration curve was plotted by the peak areas versus the concentration and the regression equation was calculated.

Sample Solution Preparation

Accurately weigh and transfer equivalent to 10 mg of mebeverine HCl sample into a 10 ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with dilute up to the mark with diluent. From above stock solution pipette out 1ml solution taken in 10 ml clean dry volumetric flask make the volume by using diluents.

Procedure: Inject 2 µL of the standard, sample into the chromatographic system and measure the areas for the mebeverine HCl peaks and calculate the % Assay by using the formula.

Method Validation[10,11]

The optimized chromatographic method was completely validated according to the procedures described in ICH guidelines Q2 (R1) and Q1 A (R2) for the validation of analytical methods and Stability Testing of New Drug, respectively.

Linearity

Appropriate aliquots of standard mebeverine HCl stock solutions (100 µg/ml) were taken in different 10 ml volumetric flasks and resultant solution was diluted up to mark with diluents to obtain final concentration of drug solution. Calibration curve was plotted by peak area *vs.* applied concentration of mebeverine HCl. The slope, intercept and correlation coefficient were also determined.

Accuracy

Accuracy is the closeness of the test results obtained by the method to the true value. To study the accuracy, twenty tablets of formulation were weighed and powdered and analysis of the same was carried out. Recovery studies were carried out by standard addition method by

adding known amount of standard drug solution (50, 100 and 150%) to the sample solution and % Recovery was calculated.

Precision

The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intra-day studies, six repeated injections of standard solution were made and the response factor of drug peak and % RSD were calculated. In the inter-day variation studies, six repeated injections of standard solution were made for six consecutive days and response factor of drug peaks was observed. From the data obtained, the developed method was found to be precise.

Limit of detection (LOD) and Limit of quantification (LOQ)

The limit of detection (LOD) and limit of quantitation (LOQ) of PRG was determined by calculating the signal to noise (S/N) ratio of 3:1 and 10:1, respectively according to International Conference on Harmonization guidelines.

Robustness

The samples were analyzed separately by slightly changes in the analytical methods such as by changing flow rate of mobile phase ±0.1 ml and by changing ratio of organic composition of the mobile phase *i.e.* acetonitrile: buffer from ± 10%, the chromatograms were recorded. The retention time values were observed.

System-Suitability

System-suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. 10 µl of the standard solution was injected under optimized chromatographic conditions to evaluate the suitability of system. The values of system suitability parameters were shown in Table-2.

Table 2: Results of System suitability studies

System suitability parameters	Results
Retention time	4.334
Area	223383
Theoretical plate number	2248.22
Tailing factor	1.55

RESULT AND DISCUSSION

To develop simple and economical RP-HPLC method, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry was obtained with X-terra, C_{18} (4.6 x 100mm, 5µm particle size) column and mobile phase comprising of triethanolamine buffer p^H-3: acetonitrile [40:60 v/v] at a flow rate of 0.8 ml/min to get better reproducibility and repeatability. Quantification was achieved with UV detection at 271 nm based on peak area. The retention time was found to be 4.334 min. The optimized method was validated as per ICH guidelines.

Specificity

Specificity of the HPLC method was demonstrated by the separation of the analysts from other potential components such as impurities, degradants or excipients. A volume of 10µl of working sample solution was injected and the chromatogram was recorded. Peak was found at retention time of 4.334 min. Hence, the proposed method was specific for mebeverine HCl (Figure 2).

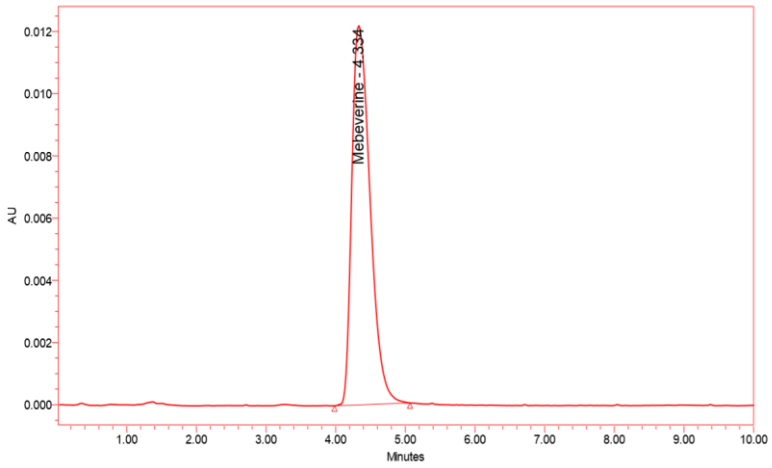

Figure 2: Chromatogram of Mebeverine HCl at 271 nm

Linearity

Under the optimum experimental conditions, the concentration vs peak area plot for the proposed method was found to be linear over the range of 1-5 µg/ml. The parameters for the regression analysis are given in Table 3. Linearity graph of different concentration of Noscapine HCl shown in Figure. 3.

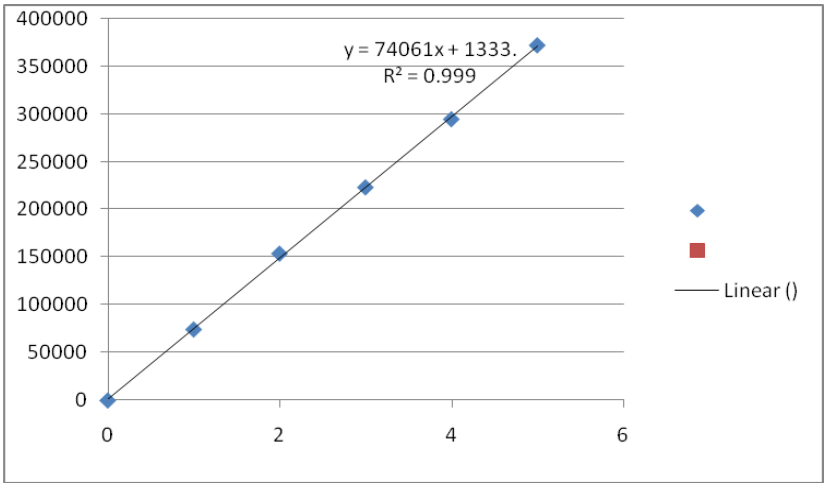

Figure 3: Calibration curve of Mebeverine HCl by RP-HPLC

Precision

The precision of the proposed method was carried in terms of the inter-day and intra-day time periods. The low % RSD values of inter-day (0.52-0.69%) and intra-day (1.0%) variations reveal that the proposed method is precise. The results of precision were tabulated in Table-4.

Table 4: Results of Precision studies

Concentration (3 µg/ml)				
Precision	**Intraday**	**Interday**		
		Day 1	**Day 2**	**Day 3**
Mean area*	219012.7	218162.4	218648.2	217822
Standard deviation	2103.5	1208.805	1529.998	1142.552
%RSD	1.0	0.55	0.69	0.52

*indicates average of six determinations, RSD indicates relative standard deviation.

Accuracy

For the accuracy of proposed method, recovery studies were performed by standard addition method at three different levels (50%, 100% and 150% of final concentration). A known amount of standard pure drug was added to preanalyzed tablet powder and the sample was then analyzed by proposed method. Results of recovery studies were found to be satisfactory and reported in Table-5.

Table 5: Results of Accuracy studies

Drug name	Levels	Amount added (µg/ml)	Amount recovered (µg/ml)	% Recovery	Mean	Standard deviation	% RSD
Mebeverine HCl	50%	1.5	1.50	100.2	111656.5	212.6	0.19
	100%	3	3.0	100.0	222771.6	1677.1	0.75
	150%	4.5	4.51	100.4	335556.3	593.4	0.17

Robustness

The robustness of the HPLC method was evaluated by analyzing the system suitability parameters after varying the pH of the mobile phase (± 0.2), organic solvent content (± 5%), wave length (±5nm). None of these alterations caused a significant change in peak area RSD, tailing factor and theoretical plates. Although the changes in the retention time were significant, yet quantitation was possible. The results were represented in Table-6.

Table 6: Results of Robustness studies

Parameters	Flow rate		Mobile phase	
	0.7 ml/min	0.9 ml/min	50:50	70:30
Mean area	234911.7	279467.3	271899.3	222644.7
Standard deviation	3883.488	2675.604	4030.405	2375.971
%RSD	1.65	0.95	1.48	1.06

Limit of detection and Limit of quantification

Limit of detection (LOD) and Limit of Quantification (LOQ) were determined by using the formula based on the standard deviation of the response and the slope. LOD and LOQ were found to be 0.105 and 0.324 µg/ml, respectively. The results were represented in Table-7.

Table7: Results of LOD and LOQ

Parameters	Results
LOD (µg/ml)	0.105
LOQ (µg/ml)	0.324

CONCLUSION

A specific, precise, accurate, rapid and reliable RP-HPLC method has been developed and validated. It has short runtime 10 min and retention time 4.334 min allows analysis of large number of samples in a short period of time. The proposed method could be useful and suitable for determination of Mebeverine HCl in bulk drugs.

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