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Research Article

DEVELOPMENT AND VALIDATION OF A ULTRA PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR ASSAY OF MEBEVERINE HYDROCHLORIDE

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ABSTRACT

Objective: The main objective of current study is to develop and validate UPLC method, simple, precise, accurate and specific chromatographic method for the determination of mebeverine hydrochloride in tablets.

Methods: An ultra performance liquid chromatography instrument and waters symmetry, C18, 75 x 4.6 mm, 3.5 µ were used for determination of mebeverine hydrochloride. The flow rate of 1.0 mL/min was set with isocratic, the temperature of column compartment maintained at 50°C and ultra violet detection done at 242nm wavelength. The injection volume was 3 µL. The mebeverine hydrochloride peaks eluted at 1.306 minute and then run time was set as about 2 minutes.

Results: The correlation coefficient (≥ 0.999) shows the linearity of response against concentration over the range of 50 to 400%. The observed result shows that the method was rapid, precise, accurate and simple. The method was validated as per ICH guidelines.

Conclusion: The developed and validated UPLC method was suitable for determination of mebeverine hydrochloride in pharmaceutical formulations which is more useful with respect to regular laboratory analysis. This method can be conveniently used in a quality control laboratory for routine analysis of mebeverine hydrochloride.

Keywords: Mebeverine hydrochloride, Method development, UPLC, Validation

INTRODUCTION

Mebeverine hydrochloride (Fig.1) is an anti muscarinic. The IUPAC name was (RS)-4-(ethyl [1-(4-methoxyphenyl) propan-2-yl] amino) butyl 3, 4-dimethoxybenzoate with molecular formula $C_{25}H_{35}NO_5$. It belongs to a group of compounds called musculotropic antispasmodics [1]. These compounds act directly on the gut muscles at the cellular level to relax them. This relieves painful muscle spasms of the gut, without affecting its normal motility. Mebeverine is used to relieve symptoms of irritable bowel syndrome and related intestinal disorders that are the result of spasms in the intestinal muscles [2]. These include colicky abdominal pain and cramps, diarrhoea alternating with constipation and flatulence. Mebeverine is also an inhibitor of calcium-depot replenishment. Therefore, it has dual mode of action which normalizes the small bowel motility. It was first registered in 1965 and is marketed as Colofac, Duspatal, Colotal, Colospa, Mebeverine, Rudakol, Boots IBS relieve, Fomac, Mebecon and Duspatalin by Abbott Laboratories. British Pharmacopoeia described a non aqueous titrimetric method for determination of MEB in pure form [2]. Literature survey revealed that there was so many HPLC and spectroscopy methods have been reported for the estimation of mebeverine hydrochloride. Combination of MEB and SUL is used for treatment gastrointestinal and colic spasms which are a consequence of psychosomatic manifestation of nervous tension, mental stress or anxiety. MEB and SUL were determined in their binary mixture via derivative spectroscopy [3, 4], first derivative synchronous fluorescence spectroscopy [5], HPLC [6] and chemometric techniques [7].The present work describes first UPLC isocratic method for the determination of mebeverine hydrochloride in tablets as for ICH guidelines [8-9].

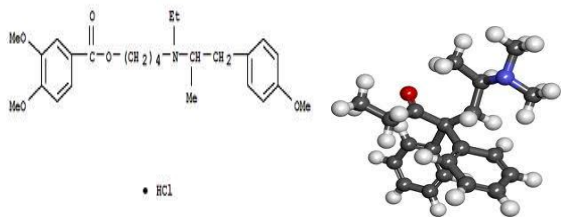

Figure 1: Structure of mebeverine hydrochloride

MATERIALS AND METHODS

Chemicals

Qualified standards and samples of mebeverine hydrochloride were obtained from local laboratories and were used without any further purification. The chemicals like diammonium hydrogen phosphate, acetonitrile and phosphoric acid were purchased from Merck, Mumbai. Millipore water generated from TK water system. The analytical column used was Waters Symmetry C18, 75x4.6 mm, 3.5µ.

Instruments

An Acquity UPLC system manufactured by Waters which consist of Photo Diode Array (PDA) detector, Quaternary solvent manager, Sample manager, column heating compartment was used for determination of mebeverine hydrochloride.

UPLC instrument was controlled by Waters Empower chromatographic software. A Waters Symmetry C18, 75x4.6 mm, column with particle size of 3.5µm was used as stationary phase for chromatographic separation and determination of mebeverine hydrochloride. Sartorius semi micro analytical balance was used for all weighing, Thermo pH meter was used for buffer pH adjustment, and Bandelin sonicator used to dissolve the standard, sample and were centrifuged by using Hermle centrifuge machine.

Standard preparation

Weighed and transferred about 81.0mg of mebeverine hydrochloride standard in to 100 mL volumetric flask, dissolve and make up the volume with mobile phase and mix well. Further dilute 5.0mL of above solution in 20mL volumetric flask, make up the volume with mobile phase and mix well.

Sample preparation

Weighed and transferred about 400.0mg of mebeverine hydrochloride sample in to 500 mL volumetric flask, dissolve and make up the volume with mobile phase and mix well. Centrifuge a portion of this preparation at 3000 RPM for 10 minutes. Pipette out 5 mL of the clear supernatant solution into a 20 mL volumetric flask, dilute to volume with the mobile phase and mix and filter through 0.45µm filter.

Chromatographic conditions

The chromatographic column used for analysis was Waters symmetry C18 with dimensions 75x4.6 mm, 3.5 µm. The isocratic method was employed with the mobile phase mixture of buffer and Acetonitrile in the ratio of 60:40 v/v. And adjust pH5.2 with phosphoric acid. The buffer was prepared as 6.6 g of Diammonium hydrogen phosphate in 1000 ml of purified water. The column temperature was maintained at 50.0ºC and detection was monitored at a wavelength of 242 nm. Injection volume was 3 µl and the mobile phase flow was set at 1.0 mL/min. The mobile phase was used as diluents for preparation of solutions.

Method validation

The developed method for determination of mebeverine hydrochloride was validated for system suitability along with method selectivity, specificity, linearity, range, precision (Repeatability and Intermediate precision),accuracy, range, ruggedness, robustness according to the ICH guidelines.

System suitability

The system suitability was conducted using standard preparation and evaluated by injecting five replicate injections.

Specificity

Specificity is the ability of analytical method to assess un equivocally the analyte in the presence of component that may be expected to be present. Performed the specificity parameter of the method by injecting Diluent, placebo into the chromatographic system and evaluated by show any peak at the retention time of analyte.

Linearity

Performed the linearity with mebeverine hydrochloride in the range of 10 to 300% of specification limit. Recorded the area response for each level and calculated slope, intercept & correlation coefficient. Also performed precision at higher level by injecting six times into the chromatographic system

Precision and Accuracy

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of homogeneous sample. The precision of analytical method is usually expressed as the standard deviation or relative standard deviation (Coefficient of variation) of series of measurements. The system precision was conducted using mebeverine hydrochloride and evaluated by making six replicate injections. The Accuracy of the method by recoveries of mebeverine hydrochloride sample solutions at different concentration levels ranging from 10 to 300%

Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

Method development includes selection of appropriate chromatographic conditions/factors like detection wavelength, selection, optimization of stationary and mobile phases. The wavelength of 242 nm was selected due to it produces less noise, which minimizes problems that may exhibit around the active ingredient when attempting to quantify mebeverine hydrochloride. Preliminary development trials were performed with various C18 columns of different types and dimensions from different manufacturers were tested for the peak shape and the number of theoretical plates for specification concentrations. Finally by switching to Waters, symmetry C18, 75x4.6 mm, 3.5µ, column there was a substantial increase in the theoretical plates (5263) with a significant improvement in the peak shapes with 1.19 tailing factor.

System suitability

The RSD from five replicate injections of standard preparation was 0.4 %. Theoretical plates for mebeverine hydrochloride peak 5263. Tailing factor for Mebeverine hydrochloride peak was 1.19.

Selectivity

Performed the specificity parameter of the method by injecting Diluent, Standard preparation, and Sample preparation, Placebo preparation into the chromatographic system and recorded the retention times. Specificity study of the method proved no peak observed at retention time of mebeverine hydrochloride. Specificity results of mebeverine hydrochloride given in the below Table-1.The selectivity chromatograms shown in the Figures-2 and 3

Table 1: Selectivity results of mebeverine hydrochloride

Solution Retention Time in (min)
Diluent -
Placebo -
Standard preparation- 1.337
Sample as such- 1.306

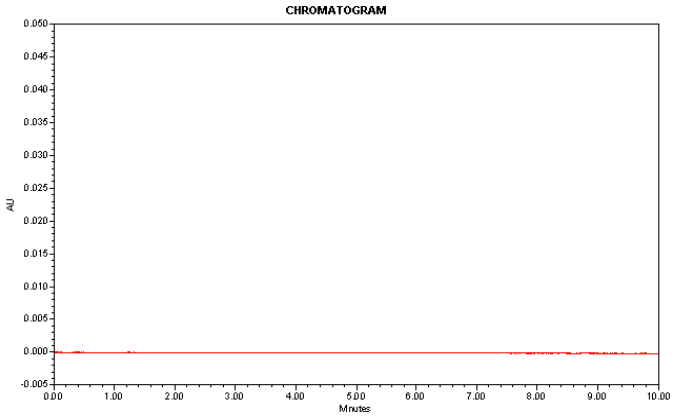

Fig: 2 Chromatogram of placebo

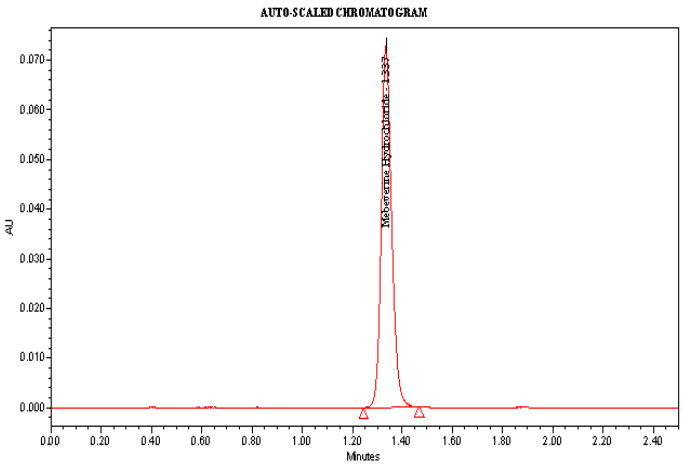

Fig: 3 Chromatogram of mebeverine hydrochloride

Linearity

To demonstrate the linearity with mebeverine hydrochloride standard in the range of 50 to 400% of specification limit. Correlation coefficient of mebeverine hydrochloride was 1.000.The linearity results shown in the below Table -2.The linearity curves of mebeverine hydrochloride shown in the Figure-4

Table 2: Linearity results of mebeverine hydrochloride

S. No.	Concentration in ppm	Area Response
1.	50	52909
2.	100	107616
3.	150	160062
4.	200	212272
5.	250	268750
6.	300	317384
7.	400	429773

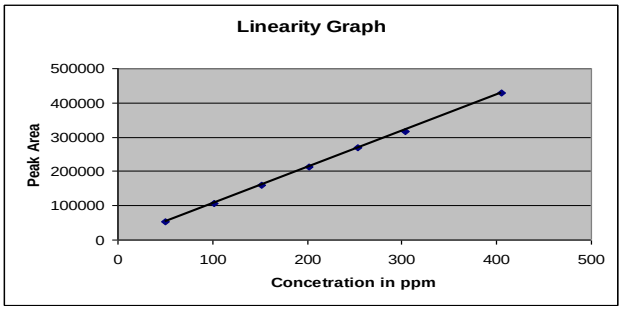

Fig. 2: Linearity curve of Mebeverine hydrochloride

Accuracy

Accuracy study found that the mean % of recovery was more than 95.0% and less than 105.0% at each level 50 to 150% of concentration levels, hence method is accurate. The accuracy results are given in the Table-3.

Table 3: Accuracy results

S.No	Level in %	% Mean Recovery
1	50	100.5
2	75	100.4
3	100	100.4
4	125	100.8
5	150	100.6

Precision

The precision of test method was validated by assaying six samples prepared on mebeverine hydrochloride and calculate relative standard deviation of Assay results. The precision results are given in the Table-4.

Table 4: Precision results

S. No.	Preparation No.	% Assay of mebeverine
1	Preparation No - 1	98.62
2	Preparation No - 2	98.35
3	Preparation No - 3	98.57
4	Preparation No - 4	98.87
5	Preparation No - 5	98.30
6	Preparation No - 6	98.79
Average % Assay of Mebeverine	98.58	
% Relative Standard Deviation	0.23	

Table 5: Robustness results

Condition	Tailing factor	Theoritical plates	% RSD
Limits	NLT 2.0	NLT 1500	NMT 2.0
Normal Condition	1.26	3689	0.15
Flow rate 1.7ml/min	1.26	3711	0.14
Flow rate 1.3/min	1.26	3844	0.16
Column Temperature 45°C	1.27	3531	0.31
Column Temperature 55°C	1.26	3985	0.13
Organic phase +10.0%	1.28	3804	0.08
Organic phase -10.0%	1.26	4037	0.09

Robustness

The method robustness was studied by injecting the system suitability solution at change in the percentage of organic modifier, flow rate, and column temperature. The results were obtained as shown in the below Table-5

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CONCLUSIONS

A simple isocratic UPLC method has been developed and validated for the determination of assay of mebeverine hydrochloride tablets. The developed method has been found to selective, sensitive, precise, robust and stability indicating. The method can be directly adopted in quality control laboratories for routine analysis with respect to determination and quantification of mebeverine hydrochloride and also for the analysis of stability samples.

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