ejpmr, 2022,9(11), 465-471

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR THE QUANTIFICATION OF MEBEVERINE HYDROCHLORIDE IN BULK AND MARKETED TABLETS

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Article Received on 12/09/2022 **Article Revised on 03/10/2022** **Article Accepted on 24/10/2022**

ABSTRACT

Two Simple and precise UV spectroscopic methods were developed and validated as per ICH guidelines. The solvent systems such as Acetonitrile: Methanol (1:1) and Distilled water: Methanol (2:1) selected for the study. The two proposed solvent systems validated for linearity, accuracy, precision, robustness, ruggedness and solution stability. The percent recovery in the marketed tablet formulation was found to be good agreement with the label claim. The proposed methods validated statistically and the results suggest these methods can employ for the routine analysis of mebeverine hydrochloride in bulk and marketed formulations.

KEYWORDS: Mebeverine hydrochloride, UV spectroscopy, Validation, Accuracy, Precision.

INTRODUCTION

Mebeverine HCl is a drug used to alleviate some of the symptoms of irritable bowel syndrome.[1] Chemically it is 4-(ethyl-(1-(4-methoxyphenyl) propan-2-yl) amino) butyl 3, 4-dimethoxybenzoate; hydrochloride (figure 1). White, crystalline powder, freely soluble in water, methanol and acetonitrile.[2-4]

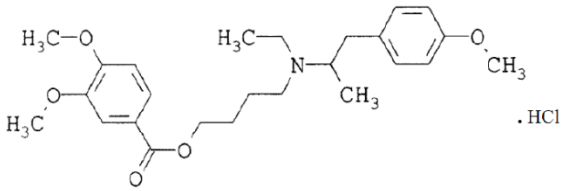

Figure 1: Chemical structure of Mebeverine hydrochloride.

Few analytical methods have been reported for determination of mebeverine in bulk and formulations viz., HPLC[5-7], RP-HPLC[8-10], UPLC[11], Spectrophotometric[12-14], Conductometric[15], Colorimetric[16] and analytical[17] method. There is a need for a simple, rapid, cost effective and reproducible method for assay of mebeverine HCl. Therefore, it was thought of interest to develop effective method for the analysis of mebeverine HCl, this paper describes development and validation of simple, specific, sensitive, accurate and precise UV spectroscopic method for the quantification of mebeverine in bulk and marketed tablets.

Materials

Mebeverine hydrochloride obtained as gift sample (Magnus Pharma Ltd, Birgunj, Nepal). Morease tablets (Dr. Reddy's Laboratories, Hyderabad, Telangana, India) Normaxin MB 200 caps (Orbit Life Science Ltd Capsules Thane, Maharashtra, India) and tablets procured from local community pharmacy. All reagents, solvents used were of analytical grade (SD Fine-Chemicals, Bengaluru, India). UV-1900 UV-VIS Spectrophotometer-Shimadzu Corp/Japan; UV-1700 PharmaSpec UV-VIS Spectrophotometer-Shimadzu Corp, KYOTO JAPAN used for the study.

Methods

Preparation of mebeverine hydrochloride standard and working standard solutions: Transfer accurately weighed 50 mg of mebeverine hydrochloride into a 50 ml volumetric flask to this add 40ml of medium under the study viz., Acetonitrile: Methanol (1:1), sonicate the mixture for 10min dissolve completely the drug, then make up the volume to 50ml to obtain 1mg/ml solution. Similarly prepare the standard stock solution in Methanol: Distilled water (1:2). Transfer accurately measured volume about 2.5ml of standard stock solution into a 25ml volumetric flask and dilute with Acetonitrile: Methanol (1:1) to get 0.1mg/ml solution considered as working standard solution. Similarly prepare the working standard solution in Methanol: Distilled water (1:2).

Determination of absorption maxima (λ max): Appropriately dilute the working standard solution with

Acetonitrile: Methanol (1:1) solution in 10ml volumetric flask to get 10µg/ml solution, similarly prepare another solution in Methanol: Distilled water (1:2). Scan both the solutions in the range of 200 to 400 nm using double beam UV spectrophotometer, and observe the characteristic peak at standard wavelength (nm).

Validation

The mediums under the study were subjected for various validation parameters as per ICH guideline viz., linearity, range, accuracy, precision, robustness, ruggedness, LOD, LOQ. The mediums were further subjected for solution stability and forced degradation studies. After validation the mediums under the study were subjected for determination of mebeverine hydrochloride in bulk and marketed tablets with statistical justification.

Range: Appropriately dilute the mebeverine hydrochloride working standard solution with Acetonitrile: Methanol (1:1) solution in a series of 10ml volumetric flask to obtain 1-40µg/ml, similarly prepare series of mebeverine hydrochloride working standard solutions at 1-40µg/ml concentrations in Methanol: Distilled water (1:2). Measure the absorbance of both set of solutions at 265 nm, keeping respective mediums as blank. Plot the concentration vs absorbance curve find the range from the curve.

Linearity: The linearity is the ability of analytical procedure to produce test results, which are proportional to the concentration (amount) of analyte in samples within a given concentration range, linearity should be determined by using a minimum of six standards. Appropriately dilute the mebeverine hydrochloride working standard solution with Acetonitrile: Methanol (1:1) solution in a series of 10ml volumetric flask to obtain 1, 2, 3, 4, 5, 6, 8, 10, 12, 16 and 20µg/ml concentrations. Similarly prepare series of mebeverine hydrochloride working standard solution i.e. 1, 2, 3, 4, 5, 6, 8, 10, 12, 16 and 20µg/ml concentrations in Methanol: Distilled water (1:2). Measure the absorbance of both set of solutions at 265 nm, keeping respective mediums as blank. Plot the concentration vs absorbance curve and regression equation and statistical data was computed.

***Precision*:** Precision of proposed analytical method were carried out at different concentrations prepared by diluting appropriately the mebeverine hydrochloride working standard solution in medium under the study and express the results in terms of % RSD, similarly inter-day and intra-day precision were performed.

Robustness: Robustness studies perform to check the influence of method parameters varied intentionally on the proposed method results. Dilute the mebeverine hydrochloride working standard solution separately with Acetonitrile: Methanol (1:1) and Methanol: Distilled water (1:2) in a series of 10ml volumetric flask to obtain 4µg/ml, 8µg/ml, 12µg/ml (n=5) concentrations and measure the absorbance at actual wavelength i.e., 265 nm and small variated wavelength i.e., ± 2-5 nm, interpret the results in terms of % RSD.

Ruggedness: Ruggedness studies perform to check the influence of parameters varied intentionally on the proposed method results. Dilute the mebeverine hydrochloride working standard solution separately with Acetonitrile: Methanol (1:1) and Methanol: Distilled water (1:2) in a series of 10ml volumetric flask to obtain 4µg/ml, 8µg/ml, 12 µg/ml (n=5) concentrations and measure the absorbance at 265nm by two different analyst and two different UV spectrophotometer. Interpret the results in terms of % RSD.

LoD and LoQ: Limit of detection (LoD) is the lowest amount of an analyte detected in a sample and Limit of quantitation (LoQ) is the lowest amount of an analyte quantified in a sample with a suitable precision and accuracy. Both are determined based on standard deviation (SD) of response and slope by using the following equations.

(LoD=3.3xSD/S)

(LoQ=10xSD/S)

Quantification of mebeverine hydrochloride in marketed tablets: For this study two marketed brands were selected viz. Morease and Normaxin. In each case of marketed tablets, triturate accurately weighed 20 tablets to get fine powder. Weigh accurately triturated powder equivalent to 50 mg of mebeverine hydrochloride and transfer into 50ml volumetric flask, add 50ml of Acetonitrile: Methanol (1:1), extract the content by shaking for 90 min and sonicated for 10min, filter the content through whatmann filter paper No.44. Appropriately dilute this working standard solution with Acetonitrile: Methanol (1:1), similarly perform the extraction and prepared working standard solution of tablets in Methanol: Distilled water (1:2). Determine the drug content for both the solutions from the linearity curve.

Accuracy: The most common technique for determining accuracy in analytical method development studies are the recovery method, recovery defined as the ratio of the observed result to the expected result expressed as a percentage. Standard addition method applied for recovery studied, in which a sample assayed with known amount of mebeverine hydrochloride (40%, 60% and 80%) added to the test working standard mediums under the study, and the sample assayed as percent recovered.

Solution stability: The stability of standard stock solutions of mebeverine hydrochloride in proposed mediums studied at room (25°C) and refrigerated temperature (2-8°C). The samples were stored in tightly sealed glass containers protected from light. Appropriately dilute the standard stock solutions of proposed mediums in a series of 10ml volumetric flask and the absorbance measured at 0hr and 24hr time interval.

RESULTS AND DISCUSSION

The optimum wavelength of maximum absorption of the proposed mediums viz., Acetonitrile: Methanol (1:1) and Methanol: Distilled water (1:2) were found to be 265 nm with characteristic peak as shown in figure 2, 3.The Beer's law range, molar absorptivity, best fit values for two proposed mediums viz., Acetonitrile: Methanol (1:1) and Methanol: Distilled water (1:2) are given in table 1, 2 and linearity curve in figures 3.

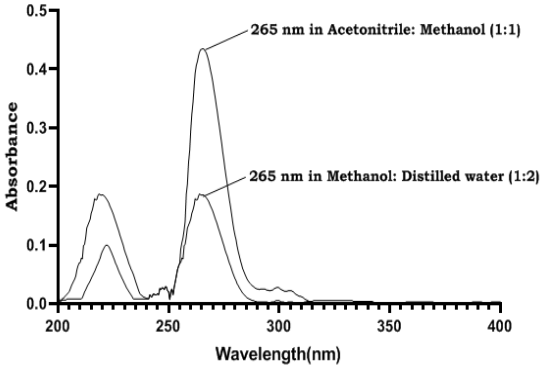

Figure 2: Absorption maxima of mebeverine hydrochloride in Acetonitrile: Methanol (1:1) and Methanol: Distilled water (1:2).

A linear relationship found in the concentration range of 1-20µg/ml for both methods. The goodness of fit study suggest good correlation coefficient (R square - 0.9999 and 0.9998 for proposed methods) shows the validity of Beer's law with intercept response < 2% calculated by the least square method indicating functional linearity between the concentration of analyte and the absorbance. Based on the standard deviation of the response and the slope the limit of detection values for mebeverine hydrochloride for the proposed methods found to be 0.0858 ± 0.0104µg/ml, 0.0616 ± 0.0075µg/ml and limit of quantitation values found to be 0.2602 ± 0.0104µg/ml, 0.186±0.0075µg/ml with % RSD values less than 2.

Table 1: Linearity curve data.

Concentration (µg/ml)	Absorbance mean ± SD (n=5)	
	Acetonitrile: Methanol(1:1)	Methanol: Distilled Water(1:2)
1	0.030 ± 0.00057	0.042 ± 0.0011
2	0.059 ± 0.00115	0.081 ± 0.001
3	0.090 ± 0.00115	0.123 ± 0.001
4	0.121 ± 0.00057	0.160 ± 0.0015
5	0.148 ± 0.001	0.202 ± 0.0005
6	0.177 ± 0.00057	0.242 ± 0.001
8	0.242 ± 0.00173	0.319 ± 0.001
10	0.293 ± 0.0036	0.399 ± 0.0005
12	0.362 ± 0.00152	0.485 ± 0.0005
16	0.493 ± 0.001	0.640 ± 0.0011
20	0.599 ± 0.00057	0.799 ± 0.001

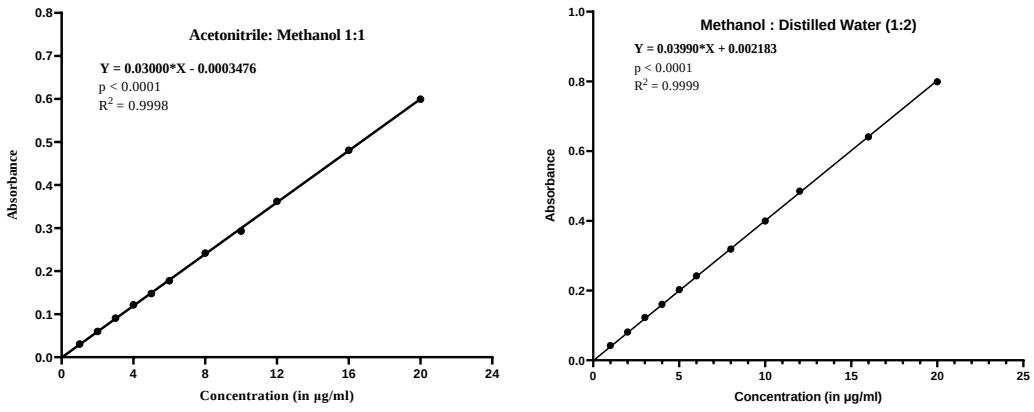

Figure: Linearity curve of mebeverine hydrochloride.

Table 2: Statistical data of linearity curve for proposed mediums.

Parameters	Acetonitrile: Methanol (1:1)	Methanol: Distilled water (1:2)
Absorption maxima (λmax)	265nm	265nm
Beer's range (µg/ml)	1-20	1-20
Molar absorptivity(ε),	$5.872x10^3$ 1/(m-cm)	$1.7227x10^4$ 1/(m-cm)
Sandell's sensitivity(л)	0.1359 µg/cm^2/0.001	0.0286 µg/cm^2/0.001
Best-fit values		
Slope	0.03990	0.03000
Y-intercept	0.002183	-0.0003476
X-intercept	-0.05471	0.01159
1/slope	25.06	33.34
95% Confidence Intervals		
Slope	0.02967 to 0.03033	0.03967 to 0.04013
Y-intercept	-0.00359 to 0.00290	-3.08e-005 to 0.0043
X-intercept	-0.09756 to 0.1188	-0.1107 to 0.0007680

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The precision of the proposed methods were justified from the absorbance values obtained viz., six replicates in repeatability studies, two concentrations and three replicates in intra and inter day studies of a fixed amount of mebeverine hydrochloride in proposed mediums. The SD and % RSD calculated for the proposed methods and are given in table 3, 4. The percentage RSD values for repeatability studies, intraday and inter day studies is less than 2 % indicate proposed methods were precise and reproducible.

Table 3: Repeatability precision data.

Concentration (µg/ml)	Absorbance (n=5)	
	Acetonitrile: Methanol (1:1)	Methanol: Distilled water (1:2)
4	4.0	3.95
4	4.1	3.97
4	4.06	3.95
4	4.03	3.92
4	3.93	3.9
4	3.96	3.95
% Recovery Mean ± SD	100.41 ± 1.559	98.54 ± 0.645
% RSD	1.5525	0.6550

Table 4: Inter day and intraday precision data.

Intra day precision*						
Amount tested (µg/ml)	Acetonitrile: Methanol(1:1)			Methanol: Distilled Water (1:2)		
	Amount recovered (µg/mL)	% Recovery Mean ± SD (n=3)	% RSD	Amount recovered (µg/mL)	% Recovery Mean ± SD (n=3)	% RSD
8	8.04	100.55±.055	0.478	8.01	100.14±0.625	0.625
12	12.01	100.09±0.623	0.160	12.00	100.0±.0.041	0.045
Inter day precision*						
Amount tested (µg/ml)	Acetonitrile: Methanol(1:1)			Methanol: Distilled Water (1:2)		
	Amount recovered (µg/mL)	% Recovery Mean ± SD (n=3)	% RSD	Amount recovered (µg/mL)	% Recovery Mean ± SD (n=3)	% RSD
Day 1						
8	8	100 ± 0.416	0.416	7.98	99.80 ± 0.477	0.4764
12	12.03	100.27± 0.277	0.277	11.9833	99.86 ± 0.318	0.317
Day 2						
8	8.01	100.13 ± 0.63	0.6364	7.95	99.44 ± 0.245	0.240
12	12	100 ± 0.277	0.277	11.95	99.58 ± 0.208	0.204
Day 3						
8	8.022	100.27±0.27	0.277	8.0666	100.92 ± 0.24	0.243
12	12.05	100.45=0.16	0.160	12.0166	100.09 ± 0.22	0.217

*n=5 in all cases

The results of stability study of mebeverine hydrochloride in proposed methods were within the acceptable limit and indicate solutions in proposed methods stable over the period of 24hr.

Table 5: Accuracy data of proposed methods for two marketed formulations.

Brand name Labelled claim	Amount Added (µg) (Pure drug)	% Added (marketed drug)	Amount recovered (µg/mL)	% Recovery Mean ± SD (n=3)	% RSD
Acetonitrile : Methanol (1:1)					
Morease 135	10	40	3.94	98.611±1.279	1.2553
	10	60	5.92	98.707±0.327	0.3167
	10	80	7.92	99.028±0.481	0.4768
Normaxin 200	10	40	3.98	99.722±0.481	0.4765
	10	60	5.98	99.811±0.327	0.3201
	10	80	7.95	99.444±0.245	0.2392
Methanol : Distilled water (1:2)					
Morease 135	10	40	3.99	99.999±0.368	0.3608
	10	60	5.98	99.916±0.481	0.5192
	10	80	7.98	99.791±0.312	0.3118
Normaxin 200	10	60	5.98	99.736±0.481	0.4798
	10	80	7.98	99.803±0.477	0.4764
	10	120	11.98	99.8686±0.240	0.2403

The proposed methods analyzed for assay in two marketed tablet formulations and data given in table 5. The percentage recovery was within the permissible limit with RSD values less than 2%. The accuracy performed for the proposed methods by standard addition method and the percentage recovery found within the permissible limits with RSD values less than 2% indicate non-interference of the excipients in the formulations. The mebeverine hydrochloride content of two marketed products determined by the proposed methods was in good agreement with the label claim with % RSD values less than 2 and data given in table6.

Table 6: Drug content data in marketed tablet formulations.

Brand name	Labelled claim (in µg/mL)	Acetonitrile: Methanol(1:1)			Methanol: distilled water (2:1)		
		Amount recovered (µg/mL)	% Recovery Mean ± SD (n=3)	% RSD	Amount recovered (µg/mL)	% Recovery Mean ± SD (n=3)	% RSD
Morease 135	4	4.02	100.72±0.481	0.479	4	100 ± 0.625	0.652
	8	7.97	99.81±0.481	0.480	7.95	99.37±0.315	0.313
	12	11.98	99.72±0.481	0.479	11.95	99.58±0.208	0.204
Normaxin 200	4	3.98	99.55±0.96	0.965	4	100±0.625	0.625
	8	7.98	99.72±0.63	0.634	7.98	99.75±0.180	0.179
	12	11.96	99.90±0.42	0.423	11.98	99.86±0.318	0.317

Change in λmax of ± 2nm to the actual λmax in robust analysis results significant different in the percentage recovery in both proposed methods indicates the methods were not robust. In ruggedness, analysis by different analyst and change of instrument indicates the proposed methods were significantly rugged. The robustness and ruggedness data given in tables 7, 8.

Table 7: Robustness data for proposed methods

λmax	Concentration (µg/mL)	Amount recovered (µg/mL)	% Recovery Mean ± SD (n=3)	% RSD
Acetonitrile : Methanol (1:1)				
Actual 265 nm	4	4.05	101.38±1.27	1.2555
	8	8.07	100.92±0.24	0.2388
	12	12.06	100.56±0.55	0.5524
263nm (-2nm)	4	3.68	92.22±0.48	0.484
	8	7.3	91.25±0.41	0.417
	12	10.96	91.39±0.27	0.278

267 nm (+2nm)	4 8 12	3.35 6.63 9.93	0.484±0.57 0.416±0.502 0.277±0.335	0.573 0.502 0.335
Methanol : Distilled water(1:2)				
Actual 265 nm	4 8 12	4 7.97 11.97	99.72±0.481 99.81±0.481 99.72±0.481	0.479 0.480 0.479
263nm (+2nm)	4 8 12	3.79 7.59 11.29	94.75±0.75 94.875±0.65 94.083±0.89	0.753 0.661 0.901
267 nm (-2nm)	4 8 12	3.76 7.54 11.26	94±0.60 94.25±0.52 93.83±0.42	0.607 0.543 0.428

Table 8: Ruggedness data for proposed methods.

Parameter	Concentration (µg/ml)	Amount Recovered (µg/ml)	% Recovery Mean ± SD (n=3)	% RSD
Acetonitrile : Methanol (1:1)				
Analyst-1	4 8 12	4.05 8.07 12.06	101.38±1.27 100.92±0.24 100.56±0.55	1.2555 0.2388 0.5524
Analyst-2	4 8 12	4.02 8.04 12.01	99.72±0.481 100.55±.055 100.09±0.623	0.476 0.546 0.618
UV-1700	4 8 12	4 8.04 12.01	100±0.83 100.55±0.55 100.092±0.160	0.833 0.552 0.1602
UV-1900	4 8 12	4.05 8.06 12.05	101.388±0.27 100.07±0.24 100.04±0.55	0.281 0.238 0.556
Methanol : Distilled water(1:2)				
Analyst-1	4 8 12	4.01 7.94 11.93	100.25±0.360 99.25±0.312 99.41±0.4166	0.362 0.313 0.42
Analyst-2	4 8 12	3.97 8.01 12.02	99.25±0.625 100.125±0.625 100.167±0.240	0.6248 0.6252 0.2392
UV-1700	4 8 12	3.98 7.92 12.02	99.5±0.360 99±0.180 100.166±0.120	0.359 0.181 0.122
UV-1900	4 8 12	4 7.97 11.97	100±0.158 99.625±0.172 99.75±0.210	0.158 0.170 0.214

CONCLUSION

The results and the statistical parameters demonstrate that the proposed UV spectrophotometric methods are simple, rapid, specific, accurate and precise. Therefore, this method can used for the quantification of mebeverine hydrochloride in bulk and marketed tablet formulations without interference with commonly used excipients and related substances.

ACKNOWLEDGMENT

We wish to thank Mr. Sarvotam Giri Technical director Magnus Pharma Ltd, Nepal for providing gift sample of the mebeverine hydrochloride and thanks to the principal and management of V.L.College of pharmacy for providing the facilities to carry out the work.

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