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(RESEARCH ARTICLE)

Development and validation of simple, precise UV spectrophotometric methods for the quantification of Mebeverine HCl in API and Marketed Products

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Abstract

Simple, rapid, economic and sensitive UV spectrophotometric methods using three solvent mediums viz., 0.1N HCl, Phosphate buffer pH 6.8 and Phosphate buffer pH 7.4 were developed and validated for the estimation of Mebeverine HCl in active pharmaceutical form, Marketed tablets and capsules. The developed methods were validated in terms of linearity, accuracy, precision, and specificity, limit of detection and limit of quantification as per ICH guidelines. The purity of Mebeverine HCl was characterized by melting point and FTIR. At determined absorption maxima of 263 nm for all solvents proved to be linear in the range of 1-50 µg/ml and exhibited good correlation coefficient (R2 = 0.9999, 0.9992, 0.9999) and recovery of (99.00 – 104.55%). This method is applied for two marketed Mebeverine HCl brands and results were in good agreement with label claim. The methods were validated statistically and by recovery studies for linearity, precision, repeatability, and reproducibility. The obtained results proved that the method can be employed for the routine analysis of Mebeverine HCl in active pharmaceutical form as well as in the commercial marketed formulations viz., tablets and capsules.

Keywords: Mebeverine HCl; UV spectrophotometer; Validation; Accuracy; Precision

1. 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. Mebeverine HCl is white crystalline powder, freely soluble in water, methanol and acetonitrile[2-4]. Literature survey revealed several analytical methods for the determination of mebeverine in bulk and formulations viz., HPLC[5-7], RP-HPLC[8-10], UPLC[11], Spectrophotometric[12-14], Conductometric[15], Colorimetric[16,17]. In present study simple, rapid, cost effective and reproducible UV spectroscopic methods using solvents viz., 0.1N HCl (Solvent A), Phosphate buffer pH 6.8(Solvent B) and Phosphate buffer pH 7.4 (Solvent C) for the quantification of Mebeverine HCl in API and marketed tablets and capsules. The developed methods were optimized and validated as per the guidelines of International Conference on Harmonization (ICH)[18] and demonstrated excellent specificity, linearity, precision and accuracy for Mebeverine HCl.

2. Material and methods

Mebeverine hydrochloride (MEB) obtained as gift sample (Magnus Pharma Ltd, Birgunj, Nepal). Morease tablets (Dr. Reddy's Laboratories, Hyderabad, Telangana, India) and Normaxin MB 200 capsules (Orbit Life Science Ltd Capsules Thane, Maharashtra, India) were procured from community pharmacy. All reagents, solvents used were of analytical grade (SD Fine Chemicals, Bengaluru, India). A Shimadzu UV-VIS Spectrophotometer (UV-1900 Shimadzu Corporation, Kyoto, Japan) was used for all absorbance measurements with matched quartz cells.

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2.1. Preparation of MEB standard and working standard solutions

Transfer accurately weighed 25mg of MEB into a 25 ml volumetric flask to this add 15ml of Solvent A, sonicate the mixture for 10 min to dissolve the drug completely, then make up the volume to 25 ml to obtain 1000 µg/ml solution. Similarly prepare the standard stock solution in Solvent B and Solvent C. Transfer accurately measured volume about 2.5 ml of standard stock solutions into a series of three 25 ml volumetric flask and dilute with respective solvents to get 100 µg/ml solution considered as working standard solution.

2.2. Determination of absorption maxima (λ max)

Appropriately dilute the working standard solutions into a series of three 10 ml volumetric flasks with respective solvents to obtain 10 µg/ml solutions. All three solutions were subjected for determination of absorption maxima by scanning in the range of 200 to 400 nm UV-VIS Spectrophotometer, and observe the characteristic peak at standard wavelength (nm) for all three solvents.

2.3. Validation

The mediums viz., Solvent A, B and C were subjected for various validation parameters as per ICH guideline viz., linearity, range, accuracy, precision, robustness, ruggedness, LOD, LOQ. After validation the solvents under the study were subjected for quantification of MEB in API, marketed tablets and capsules with statistical justification.

2.3.1. Linearity and Range

For range study a series of solutions at different concentrations viz., 1, 3, 5, 7, 9, 12, 15, 20, 24, 30, 35, 40, and 50 µg/ml were prepared in all working standard solvents under the study. Measure the absorbance at 263 nm, keeping respective solvents as blank and find the range as per Beers lamberts law. For linearity study series of solutions at different concentrations viz., 1, 3, 5, 7, 9, 12, 15 and 20 µg/ml were chosen as per Beer's range, measure the absorbance of three sets of solutions at 263 nm, keeping respective solvents as blank. Plot the concentration vs absorbance curve and regression equation and statistical data was computed.

2.3.2. Precision

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

2.3.3. Robustness

Robustness studies were performed to check the influence of method parameters varied intentionally on the proposed methods. Dilute the MEB working standard solution separately with solvents under the study in a series of 10 ml volumetric flask to obtain 7 µg/ml and 12 µg/ml (n=3) concentrations and measure the absorbance at actual wavelength i.e., 263 nm and small variated wavelength i.e., ± 5 nm, interpret the results in terms of % RSD.

2.3.4. Ruggedness

Ruggedness studies were performed to check the influence of parameters varied intentionally on the proposed methods. Dilute the MEB working standard solution with solvents under the study in a series of 10 ml volumetric flask to obtain 7 µg/ml and 12 µg/ml, (n=3) concentrations and measure the absorbance at actual wavelength i.e., 263 by two different analyst. Interpret the results in terms of % RSD.

2.3.5. 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.

$$(LoD=3.3xSD/S)$$

$$(LoQ=10xSD/S)$$

2.3.6. Quantification of MEB in marketed products

Two marketed brands viz., MOREASE™135 (film coated MEB tablets) and NORMAXIN™200 (sustained release MEB capsules) were selected for the study. In case of MOREASE™ 135 tablets, 10 tablets were accurately weighed and triturate get fine powder, powder equivalent to 100 mg of MEB was used for quantification. In case of NORMAXIN™ 200 capsules, 10 capsules were weighed and empty the capsules, collect the contents and triturate to get fine powder, powder equivalent to 100 mg of MEB was used for quantification. In each case extract the MEB content in 100 ml of each solvents under the study for 2 hr followed by sonication for 15 min. Filter the contents and dilute appropriately the filtrate with solvents under the study. Determine the drug content from the calibration curve.

2.4. 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 MEB (40%, 80% and 120%) added to the test working standard solvents under the study, and the sample assayed as percent recovered in terms of RSD.

2.4.1. Solution stability

The stability of standard stock solutions of MEB in solvents under the study at room temperature (25°C), refrigerated temperature (2-8°C) and hot air oven condition (45°C) were determined. The samples were stored in tightly sealed glass containers protected from light. Appropriately dilute the standard stock solutions of proposed solvents in a series of 10 ml volumetric flask and the absorbance measured at 0 hr and 48 hr time interval.

3. Results and discussion

The optimum wavelength of maximum absorption of the proposed solvents viz., Solvent A, Solvent B and Solvent C were found to be 263 nm with characteristic peak as shown in figure 1. The calibration curve data was presented in table 1 and respective statistical data, Beer's law range, molar absorptivity, for three proposed solvents were given in table 2, respective calibration curves were shown in figures 2-4.

Table 1 Calibration curve data

Concentration (µg/ml)	**Absorbance mean ± SD (n=6)**		
	Solvent A	**Solvent B**	**Solvent C**
1	0.021±0.001528	0.019±0.003000	0.027±0.003000
3	0.068±0.001528	0.063±0.002646	0.086±0.002517
5	0.113±0.001155	0.110±0.005568	0.136±0.003055
7	0.159±0.002000	0.160±0.007572	0.197±0.002646
9	0.201±0.006245	0.195±0.007550	0.247±0.003786
12	0.270±0.001155	0.236±0.003606	0.326±0.002082
15	0.336±0.002309	0.298±0.004163	0.409±0.003055
20	0.444±0.004041	0.396±0.007024	0.546±0.0005774

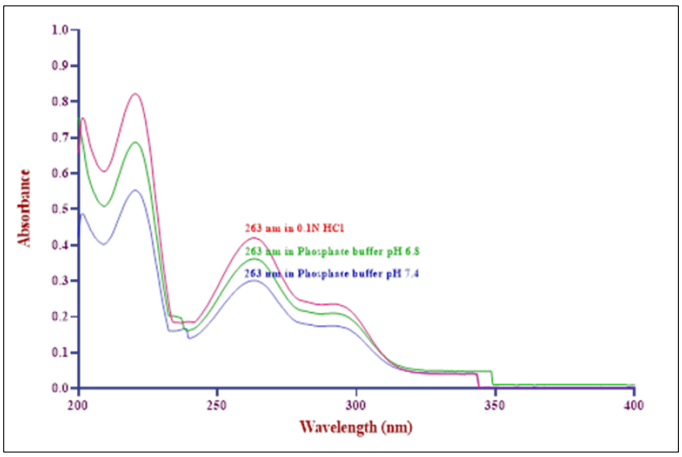

Figure 1 Absorption maxima of MEB in 0.1N HCl (Solvent A), Phosphate buffer pH 6.8 (Solvent B) and Phosphate buffer pH 7.4 (Solvent C)

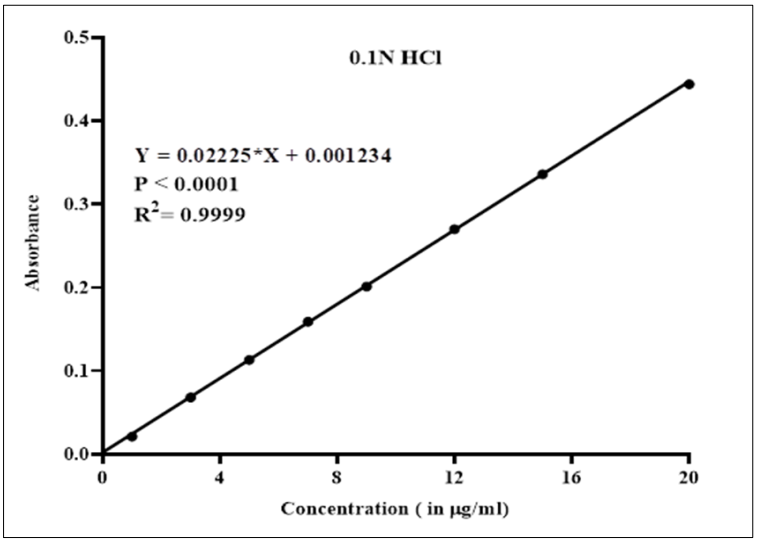

Figure 2 Calibration curve of MEB in 0.1N HCl (Solvent A)

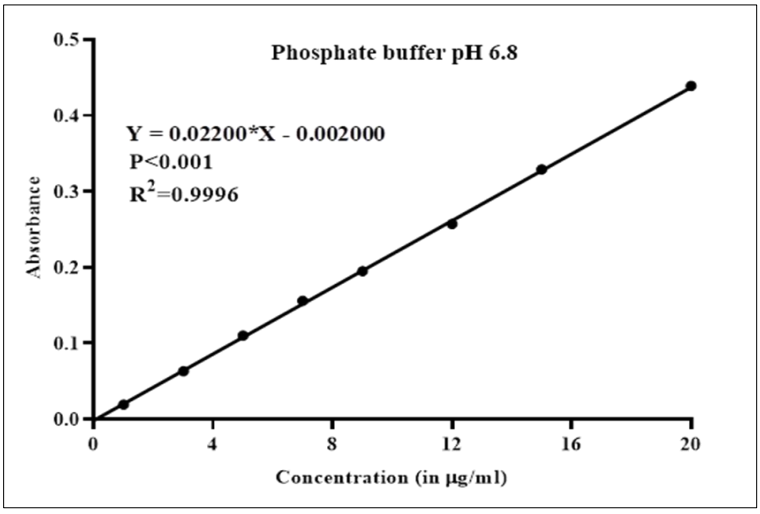

Figure 3 Calibration curve of MEB in Phosphate buffer pH 6.8 (Solvent B)

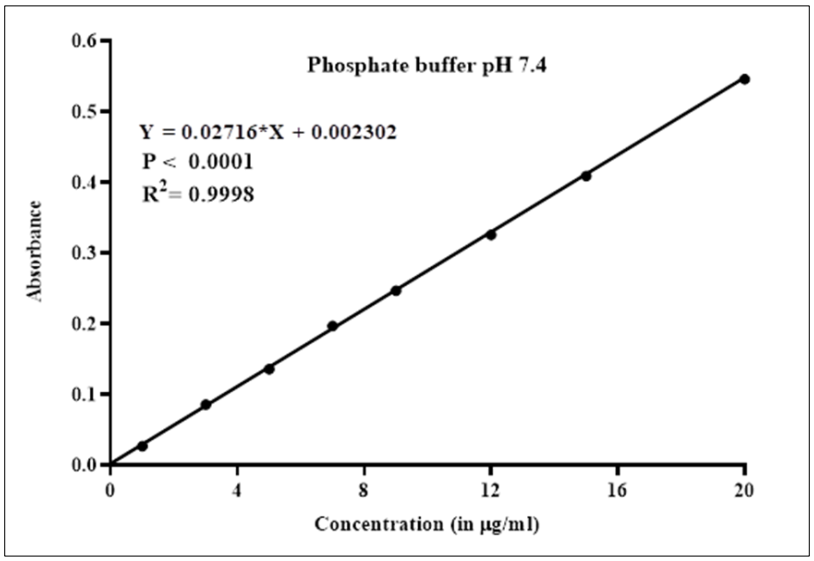

Figure 4 Calibration curve of MEB in Phosphate buffer pH 7.4 (Solvent C)

Table 2 Statistical data for calibration curves

Parameters	**Solvent A**	**Solvent B**	**Solvent C**
λ max	263 nm	263 nm	263 nm
Beer's range (µg/ml)	1-20	1-20	1-20
Molar absorptivity(ε)	$2.1\times10^{2}1/(m^{-cm})$	$1.9\times10^{2}1/(m^{-cm})$	$2.7\times10^{2}1/(m^{-cm})$
95% Confidence Intervals			
Slope	0.02199 to 0.02252	0.02158 to 0.02242	0.02678 to 0.02754
Y-intercept	-0.001611 to 0.004080	-0.006513 to 0.002513	-0.001839 to 0.006444
X-intercept	-0.1852 to 0.07170	-0.1161 to 0.2915	-0.2401 to 0.06692
Goodness of Fit			
R square	0.9999	0.9996	0.9998
P value	<0.0001	<0.0001	<0.0001
Regression Equation	Y = 0.02225*X + 0.001234	Y = 0.02200*X - 0.002000	Y = 0.02716*X + 0.002302

A linear relationship found in the concentration range of 1-20 µg/ml for all solvents under the study. The goodness of fit study suggest good correlation coefficient (R square - 0.9999, 0.9996 and 0.9998 for proposed solvent mediums) 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 MEB for the proposed solvents were found to be 0.063±0.0104 µg/ml, 0.165±0.0075, and 0.039±0.0068 µg/ml µg/ml and limit of quantitation values found to be 0.192±0.0104, 0.5±0.0075 and 0.119±0.0068 µg/ml with % RSD values less than 2 for solvent A, B and C respectively.

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 solvents were justified from the amount of MEB recovered for in fixed amount of MEB through repeatability, intra and inter day studies. The precision data was shown in table 3 and 4, the percentage RSD values were found to be less than 2 % indicate proposed solvents were precise and reproducible.

The solvents under the study were analyzed for drug content in marketed formulations viz., MOREASE™135 (film coated MEB tablets) and NORMAXIN™200 (sustained release MEB capsules) and the MEB content in two marketed

products and data were given in table 5. The results were in good agreement with the label claim with % RSD values less than 2, further accuracy was performed for the solvents under the study by standard addition method and the data was shown in table 6. The percentage recovery found to be within the permissible limits with RSD values less than 2% indicate non-interference of the excipients in the formulations.

Table 3 Repeatability data

Labelled claim (µg/ml)	**Solvent A**	**Solvent B**	**Solvent C**
	Amount recovered (µg/ml)		
7	7.12	6.98	7.07
7	7.09	7.02	7.07
7	7.02	7.09	7.01
7	7.07	7.07	7
7	7.02	6.89	7.03
7	7.10	7.10	7
Mean amount recovered	7.07	7.02	7.03
% Recovery Mean ± SD	101±0.5993	100.4±1.146	100.6±0.760
% RSD	0.5933	1.141	0.756

Table 4 Precision data

Labelled claim (µg/ml)	**Solvent A**			**Solvent B**			**Solvent C**		
	Amount recovered (µg/ml)	**% Recovery Mean ± SD (n=3)**	**% RSD**	**Amount recovered (µg/ml)**	**% Recovery Mean ± SD (n=3)**	**% RSD**	**Amount recovered (µg/ml)**	**% Recovery Mean ± SD (n=3)**	**% RSD**
	Intraday precision (n=6)								
7	7.02	100.3±0.1400	0.139	7.81	100.3±0.4157	0.145	7.18	102.6±0.409	0.398
12	12.05	100.7±0.1607	0.159	12.58	104.8±4.600	0.389	12.08	100.7±0.3143	0.312
	Interday precision (n=6)								
7	7.013	100.2±0.2972	0.296	7.59	101.9±0.1966	0.192	7.24	103.5±1.665	0.978
12	11.81	98.49±0.4689	0.476	12.46	103.9±1.537	1.479	12.01	100.1±1.081	1.079

Table 5 Drug content data in marketed formulations

Brand name	**Labelled claim (in µg/mL)**	**Amount recovered µg/mL)**	**% Recovery Mean ± SD (n=3)**	**% RSD**
Solvent A				
NORMAXIN™200	7	7.23	103.4 ± 0.4099	0.396
	12	12.01	100.1 ± 0.1762	0.176
MOREASE™135	7	7.203	101.9 ± 0.1744	0.171
	12	11.82	98.58 ± 0.4881	0.495
Solvent B				

NORMAXIN™200	7	7.05	100.8 ± 1.081	1.072
	12	12.08	100.7 ± 0.1721	0.1709
MOREASE™135	7	6.90	98.68 ± 0.3707	0.375
	12	12.18	101.5 ± 1.213	1.19
Solvent C				
NORMAXIN™200	7	7.16	102.4 ± 1.053	1.028
	12	11.87	98.92 ± 0.144	0.1415
MOREASE™135	7	7.16	102.4 ± 1.053	1.028
	12	11.87	98.92 ± 0.144	0.1415

Table 6 Accuracy data for two marketed formulations

Brand name	**Amount added (µg)**	**% addition**	**Amount recovered (µg)**	**% Recovery Mean ± SD (n=3)**	**% RSD**
Solvent A					
NORMAXIN™200	2.8	40	2.83	101.2 ± 0.5445	0.538
	5.6	80	5.42	96.90 ± 0.3747	0.3866
	8.4	120	8.56	101.9 ± 0.3020	0.2963
MOREASE™135	2.8	40	2.7	99.19 ± 0.6726	0.6780
	5.6	80	5.7	101.9 ± 0.1908	0.1872
	8.4	120	8.48	101.0 ± 0.2359	0.2335
Solvent B					
NORMAXIN™200	2.8	40	2.77	99.12 ± 0.8033	0.810
	5.6	80	5.58	99.64 ± 1.249	1.253
	8.4	120	8.61	102.6 ± 0.1328	0.1294
MOREASE™135	2.8	40	2.87	102.8 ± 0.4466	0.4344
	5.6	80	5.56	99.46 ± 0.6842	0.6879
	8.4	120	8.45	100.6 ± 0.3272	0.325
Solvent C					
NORMAXIN™200	2.8	40	2.88	103.1 ± 0.8256	0.800
	5.6	80	5.64	100.9 ± 0.6753	0.669
	8.4	120	8.4	99.99 ± 0.2021	0.2021
MOREASE™135	2.8	40	2.88	103.1±0.8256	0.800
	5.6	80	5.64	100.9 ± 0.6753	0.669
	8.4	120	8.4	99.99 ± 0.2021	0.2021

Change in λ max of ± 5 nm to the actual λ max 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. 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 7 Robustness data for proposed methods

λ max	Concentration (µg/ml)	Absorbance Mean ± SD (n=3)	% RSD
Solvent A			
Actual 263 nm	7	0.1590 ± 0.002000	0.257
	12	0.2703 ± 0.001155	0.4069
268 nm (+5nm)	7	0.1470 ± 0.0010	0.6802
	12	0.2531 ± 0.0002	0.079
258nm (-5nm)	7	0.1473 ± 0.00057	0.386
	12	0.2497 ± 0.00115	0.4605
Solvent B			
Actual 263 nm	7	0.1391 ± 0.0012	0.8713
	12	0.2346 ± 0.00034	0.1449
268 nm (+5nm)	7	0.1317 ± 0.00057	0.4328
	12	0.2251 ± 0.00017	0.0755
258nm (-5nm)	7	0.1320 ± 0.00052	0.4007
	12	0.2298 ± 0.00069	0.3002
Solvent C			
Actual 263 nm	7	0.1970 ± 0.00264	1.340
	12	0.3263 ± 0.00208	0.6374
268 nm (+5nm)	7	0.1925 ± 0.00073	0.3828
	12	0.3280 ± 0.0020	0.609
258nm (-5nm)	7	0.1923 ± 0.00152	0.7945
	12	0.3267 ± 0.00321	0.9840

Table 8 Ruggedness data for proposed methods

Parameter	Concentration (µg/ml)	Absorbance Mean ± SD (n=3)	% RSD
Solvent A			
Analyst-1	7	0.1521 ± 0.00090	0.5923
	12	0.2505 ± 0.0012	0.5109
Analyst-2	7	0.1453 ± 0.00057	0.3971
	12	0.2556 ± 0.00098	0.3849
Solvent B			
Analyst-1	7	0.1361 ± 0.00064	0.4702
	12	0.2343 ± 0.0015	0.6487
Analyst-2	7	0.1323 ± 0.00057	0.4308
	12	0.2347 ± 0.00064	0.2726

Solvent C			
Analyst-1	7	0.1920 ± 0.0034	1.77
	12	0.3273 ± 0.00057	0.1743
Analyst-2	7	0.1903 ± 0.0015	0.7987
	12	0.3280 ± 0.0050	0.6097

4. 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 use for the quantification of MEB in bulk and marketed formulations without interference with commonly used excipients and related substances.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to disclosed.

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