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NEW UV-SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION OF MEBEVERINE HCL IN PURE AND FORMULATION

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ABSTRACT

Objective: The main aim of this study was to develop spectrophotometric method for the determination of Mebeverine hydrochloride in bulk.

Method: Standard and working solutions of Mebeverine hydrochloride were prepared then aliquots of working solutions of different concentrations were prepared then linearity, Precision, Accuracy, Robustness, Ruggedness, Quantitation limit and Detection limit were determined.

Result: The linearity for this method was found to be within the range of 2-14µg/ml. Correlation coefficient (R^2) was found to be 0.999. Regression equation was found to be y = 0.066x + 0.0207.

Conclusion: A simple, specific and sensitive UV spectroscopy method was developed for the evaluation of Mebeverine hydrochloride in bulk. The projected method may be duly applied for the analysis of mebeverine hydrochloride in bulk for routine analysis.

Keywords: Ultraviolet spectroscopy, Validation, Mebeverine HCl, Method development, Assay

INTRODUCTION

Mebeverine hydrochloride (MB. HCl) is "4-[ethyl (4-methoxy-α-methyl phenethyl) amino] butylveratrate hydrochloride" which is shown in **Figure 1**. Its molecular formula (MF) is $C_{25}H_{35}NO_5$.HCl, its molecular weight (MW) is 466, and its

melting point is 105 -107 ºC. It is physically white or almost white; form of powder is crystalline, freely soluble in ethanol (96%) and water, though almost insoluble in diethyl ether. MB.HCl is an antispasmodic compound and its derivative "Methoxybenzamine" with a direct action on smooth muscle of the gastrointestinal (GI) tract. It is commonly prescribed in the treatment of abdominal ache and spasm related with gastrointestinal (GI) disorders such as mucous colitis. It has been clinically prescribe for the treatment of (IBS) irritable bowel syndrome for several years **[1-5]**. Mebeverine is indicated for apparent antispasmodic action. MB main metabolites such as "mebeverine alcohol" and "veratric acid", do not appear to acquire any action on smooth muscles **[6]**.

MATERIALS AND METHODS:

Instruments and methods: A gift sample of MEBEVERINE HCL with purity of 100was obtained. LAB INDIA (T60) double beam UV/Visible spectro-photometer and ELITE analytical balance were the instruments used. Chemicals and reagents are of analytical grade. MEBEVERINE HCL of 33mg with a brand name MEBASAP was purchased from the local market.

Preparation of standard stock solution(1000µg/ml): A standard drug solution of MEBEVERINE was prepared by adding 100mg of the drug into a 100 mL volumetric flask and made up to the mark with Acetonitrile to get a concentration of 1000µg/ml.

Preparation of working standard solution (100µg/ml): From the above standard stock solution 10 ml of the sample was transferred to a 100mL volumetric flask and made up to mark with acetonitrile to get a concentration of 100µg/mL. It was then scanned by a UV Spectrophotometer in the range of 200-400nm using acetonitrile as a blank. The absorbance was found to be maximum at 222nm.

CONSTRUCTION OF CALIBRATION CURVE: Aliquots ranging from 2-14µg/mL solutions were prepared by using acetonitrile as solvent. The samples were then analyzed at a λmax of 222nm to get respective absorbance. The values are then plotted to get a calibration curve.

PREPARATION OF THE ASSAY SOLUTION: The proposed method was applied to analyze the commercially available Mebeverine Hcl capsules.

A. Preparation of standard stock solution (1000µg/ml): A standard drug solution of Mebeverine HCl was prepared by adding 100mg of the drug into a 100 mL volumetric flask and made up to the mark with methanol to get a concentration of 1000µg/ml.

B. Preparation of working standard solution (100µg/ml): From the above

standard stock solution, 10 ml of the sample was transferred to a 100mL volumetric flask and made up to mark with acetonitrile to get a concentration of 100µg/mL. It was then scanned by a UV Spectrophotometer in the range of 200-400nm using methanol as a blank. The absorbance was found to be maximum at 222nm.

CONSTRUCTION OF CALIBRATION CURVE: Aliquots ranging from 10-100µg/mL solutions were prepared by using acetonitrile as solvent. The samples were then analyzed at a λmax of 222nm to get respective absorbance. The values are then plotted to get a calibration curve.

PREPARATION OF THE ASSAY SOLUTION: The proposed method was applied to analyze the commercially available Mebeverine HCl capsules.

% RSD= (Standard deviation)/Mean x 100

Standard deviation (SD):

$$SD=\sqrt{[\sum(x-x)^2/(n-1)]}$$

Where n=no of entries

Ruggedness: The ruggedness of an analytical procedure is the degree of reproducibility of results by analyzing the same sample under a variety of conditions like laboratories, instruments, analysis, reagents etc.

Robustness: Robustness of an analytical procedure is the capacity to remain unchanged by small but deliberate changes in parameters.

Sensitivity: Limit of detection (LOD) and Limit of quantification (LOQ) of the drug was calculated by using equations according to ICH guidelines.

Limit of Detection: It is the lowest amount of the drug in a sample that can be detected, but not necessarily quantitated.

$LOD = (3.3x\sigma)/S$

Where S=standard deviation

Limit of Quantification: It is an amount of analyte that can be quantified with a specified limit of accuracy and precision,

$$LOQ = (10x\sigma)/S$$

Linearity: Different aliquots of Mebeverine HCl were prepared from the working standard solution (100µg/mL) in the range of 2-14µg/mL. The solutions were scanned on a Double beam UV-VIS spectrophotometer in the range of 200-400nm using acetonitrile as the blank. The spectrum was recorded at 222 nm. The calibration plot was constructed as concentration Vs absorbance and can be shown in **(Table 1)**.

Precision: The precision of the method was demonstrated by intra-day and inter-day variation studies. In the inter-day variation study, the solutions of the same concentration 6µg/mL were prepared and analyzed six times, for three consecutive days and the absorbance was recorded **(Table 4)**. In the intra-day variation study,

six different solutions of the same concentration 6µg/mL were prepared and analyzed thrice a day (Morning, Afternoon and Evening) and the % RSD was calculated and reported **(Table 3)**.

Accuracy: The accuracy of the method was determined by preparing solutions of different concentrations i.e.,80,100, and 120%, in which the amount of marketed formulation Mebeverine HCl was kept constant(6µg/ml)and the amount drug was varied, that is 48µg, 60µg, 72µg for 80, 100, and 120% respectively. The solutions were prepared in triplicate and the accuracy was indicated by % recovery was calculated and reported in the **(Table 5)**.

Robustness: The Robustness of the method was carried out by analyzing the sample using three different wavelengths (±1 of lambda max) that were and respective absorbance were recorded. The results are indicated in **(Table 6)**.

Ruggedness: The ruggedness of the method was carried out by analyzing the sample using two different analysts and two different cuvettes and respective absorbance were recorded. The results are indicated in **(Table 7, 8)**.

Sensitivity: Limit of detection (LOD) and limit of quantification (LOQ) of the drug was calculated by using equations according to ICH guidelines **[6-11]**. They are calculated by checking absorbance using solvent and calculate using formulae and the results are shown in **(Table 9)**.

RESULTS AND DISCUSSION

The method was developed and validated as per ICH guidelines. The method was validated in terms of linearity, precision, accuracy, robustness, ruggedness, LOD and LOQ. Beers law obeyed over the concentration range of 10-100µg/mL, using regression analysis the linear equation y=0.066x-0.0021 with a correlation coefficient if R^2 0.999. The precision results show % RSD less than 2 at each level which indicates clearly that the method is precise enough for the analysis of Mebeverine. The accuracy of the method was checked by recovery studies. The high recovery with values indicates the accuracy of the developed method. The robustness and ruggedness studies reveal that the method is more sensitive. There was no interference observed from the excipients present in the formulation, indicated that the method is specific. Determination of Mebeverine HCl in capsules formulation showed the content of Mebeverine was very close to the label amount. The percentage RSD values in all the parameters were within the acceptable limit (<2%) all the characteristics of the method are represented in the **(Table 9)**.

Table 1: Linearity of working standard solutions

Concentration (µg/mL)	Absorbance
2	0.1767
4	0.2803
6	0.4061
8	0.5462
10	0.6864
12	0.8209
14	0.9502

Table 2: Repeatability data

Concentration (µg/mL)	Absorbance	Statistics analysis
6	0.4148	
6	0.4012	
6	0.4143	Mean:0.4118
6	0.4076	%RSD:1.94
6	0.4259	
6	0.4071	

Table 3: Intra-day study

Concentration (µg/mL)	% RSD			Average % RSD
	1	2	3	
6	1.83%	1.04%	0.55%	1.14%

Table 4:Inter-day study

Concentration (µg/mL)	%RSD			Average %RSD
	Day 1	Day 2	Day 3	
6	1.98%	1.85%	1.31%	1.71%

Table 5: Accuracy data

Levels of addition (%)	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery	% Mean recovery
80	48	47	99.1	99.93%
100	60	60	100.82	
120	72	71	99.87	

Table 6: Robustness data

Concentration (µg/ml)	Absorbance		
	221nm	222nm	223nm
6	0.4482	0.4121	0.3391
6	0.4340	0.4126	0.4060
6	0.4242	0.4132	0.4138
6	0.4259	0.4141	0.4121
6	0.4251	0.4160	0.4005
6	0.4280	0.4190	0.4141

Table 7: Ruggedness data

Concentration (µg/ml)	Absorbance	
	Analyst 1	Analyst 2
6	0.3999	0.3965
6	0.4094	0.4095
6	0.4097	0.4091
6	0.4119	0.4127
6	0.4172	0.4119
6	0.4207	0.4142

Table 8: Ruggedness data

Concentration(µg/ml)	Absorbance	
	Cuvette 1	Cuvette 2
6	0.4084	0.4142
6	0.4199	0.4130
6	0.4156	0.4134
6	0.4145	0.4141
6	0.4281	0.4148
6	0.4276	0.4053

Table 9: LOD & LOQ

Limit of Detection	Limit of Qualification
0.3457µg/Ml	1.047µg/mL

Table 10: Results of validation parameters

Parameters	Results
Absorption maxima(nm)	222nm
Linearity range(µg/mL)	2-14
Regression equation	Y=0.066x±0.0207
Correlation coefficient(R^2)	0.999
Molar extinction coefficient	0.0328
LOD(µg/ml)	0.3457
LOQ(µg/ml)	1.047
Accuracy	
(%Recovery ± SD)	99.93
Precision	
Intradayprecision(%RSD)	1.14
Inter-dayprecision(%RSD)	1.71
Sand ell's sensitivity	0.0150µg/cm^2

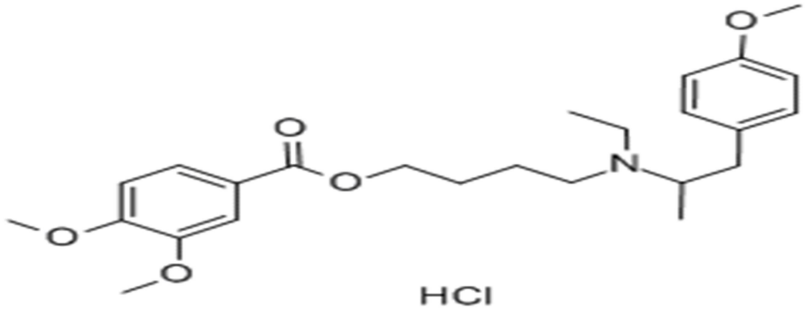

Figure 1: Structure of Mebeverine Hydrocholride

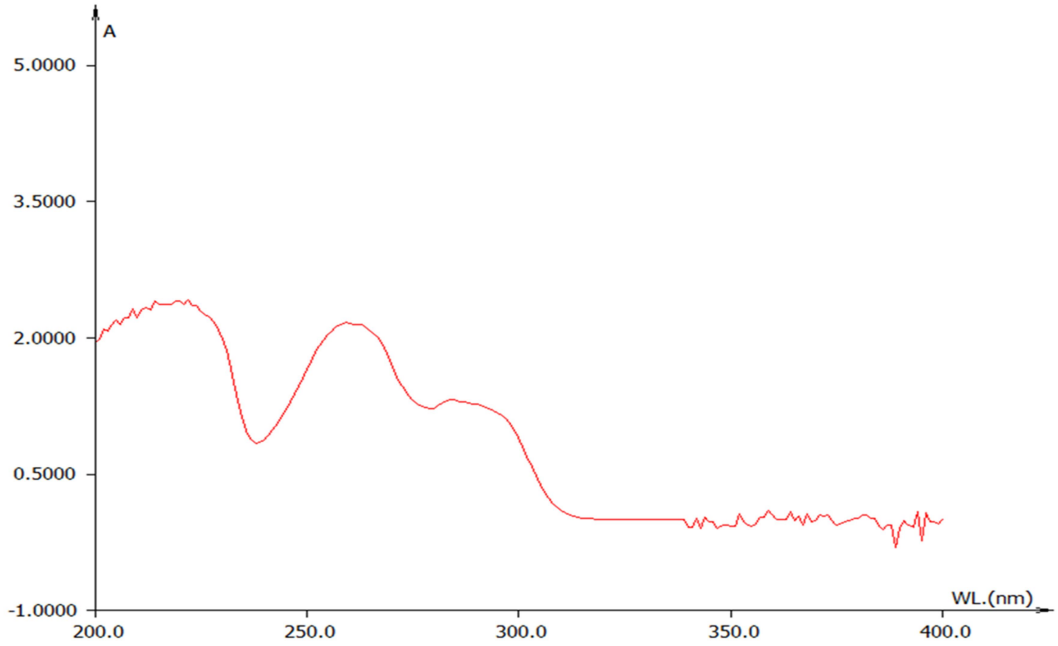

Figure 2: Absorbance of Mebeverine HCL at 222n

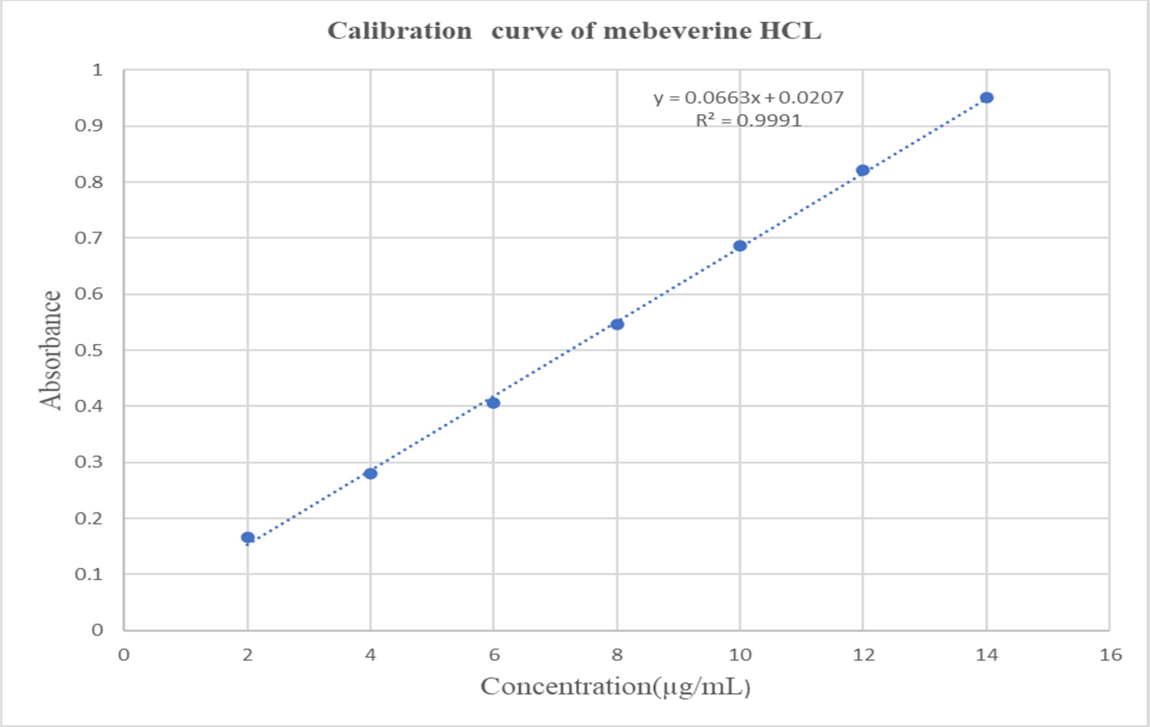

Figure 3: Calibration curve of Mebeverine Hydrochloride

CONCLUSION

A UV spectrophotometric method has been validated for the estimation of Mebeverine in bulk as well as the pharmaceutical dosage form. The developed method was found to be simple, accurate, precise, specific, reproducible and linear over the concentration range studied. The proposed method can be used for the routine analysis of Mebeverine in bulk as well as pharmaceutical formulations.

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