WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Method Development and Validation of Mebeverine Hydrochloride in Capsules by RP-HPLC

Puvvada Venkata Kavya*, Karavadi Thejomoorthy and Ch. Saibabu

Department of Pharmaceutical Analysis, Malineni Lakshmaiah College of Pharmacy, Kanumalla, Singarayakonda-523101

ABSTRACT:

An isocratic reverse phase liquid chromatography (RP-HPLC) method has been developed and subsequently validated for the determination of Mebeverine Hydrochloride in Bulk and its pharmaceutical formulation. Separation was achieved with a Devosil BDS C18; (250×4.6 mm;) I.D; particle size 5 µm)) Column and Ammonium acetate (pH adjusted to 5.2 ± 0.05 with diluted glacial acetic acid): Acetonitrile (62:38) v/v as eluent at flow rate 1.0 mL/min and the Column temperature was 25°C. UV detection was performed at 263 nm and sample temperature was maintained at 5°C. The method is simple, rapid, and selective. The described method of Mebeverine Hydrochloride is linear over a range of 40 µg/mL to 60 µg/mL. The method precision for the determination of assay was below 2.0% RSD. The method enables accurate, precise, and rapid analysis of Mebeverine Hydrochloride. It can be conveniently adopted for routine quality control analysis of Bulk and pharmaceutical formulations.

Keywords: Mebeverine Hydrochloride, RP-HPLC, Uv detector, Method validation.

Introduction:

Mebeverine hydrochloride is a Musculo tropic antispasmodic agent commonly employed for the management of irritable bowel syndrome. This medication is known for its favorable safety profile, as it is associated with minimal adverse effects. Mebeverine hydrochloride (HCL) exerts its therapeutic effects by directly targeting the cellular level to induce relaxation of the stomach muscles. The biological half-life of the substance is 2.5 hours. The oral administration of the drug results to the reaching the peak plasma concentration within a time frame of 1 to 3 hours. Additionally, it is observed that approximately 75% of the drug is bound to plasma proteins. Mebeverine hydrochloride (HCL) undergoes substantial first-pass metabolism in both the gut wall and liver. Plasma concentrations of Veratric acid, a primary inactive metabolite of Mebeverine HCL, were observed at high levels approximately twenty to thirty minutes following oral administration. Concurrently, minimal levels of the parent medication were also detected. Mebeverine hydrochloride (HCL) was selected as a representative medication due to its possession of the required pharmacokinetic and physicochemical characteristics suitable for controlled administration. Irritable bowel syndrome (IBS) is a commonly observed condition that is primarily characterized by symptoms such as cramping, abdominal discomfort, bloating, constipation, and diarrhea. Irritable bowel syndrome elicits significant discomfort and emotional distress, but it does not induce permanent damage to the intestines or give rise to severe ailments such as cancer[1].

Materials & Methods:

Instrument:

The chromatographic separation was carried out using HPLC Agilent model A1100 equipped with reciprocating pump waters-510, connected to Uv/visible detector. The data was acquired by EZ-Chrome. The equipments used are analytical balance (Make: Mettler Toledo), pH meter (Make: Elico), Sonicator: Ultrasonic bath sonicator.

Chemicals & Reagents:

Mebeverine hydrochloride working standard was procured from sigma Aldrich. Methanol, Acetonitrile used are of HPLC grade solvents. Chemicals Ammonium acetate, Glacial acetic acid are of AR grade.

METHOD DEVELOPMENT

Method development and optimization of chromatographic parameters for the estimation of Mebeverine Hydrochloride capsules by RP-HPLC are discussed below.

Solubility studies:

Solubility studies for Mebeverine hydrochloride revealed the solubility of drug in ethanol and methanol. Mebeverine hydrochloride was insoluble in ether.

Selection of detector wave length:

An UV spectrum of 10 µg / ml Mebeverine hydrochloride in 10 ml diluent (Ethanol) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength of 263 nm was selected. At this wavelength Mebeverine hydrochloride standard showed good absorbance. For spectrum refer fig.1.

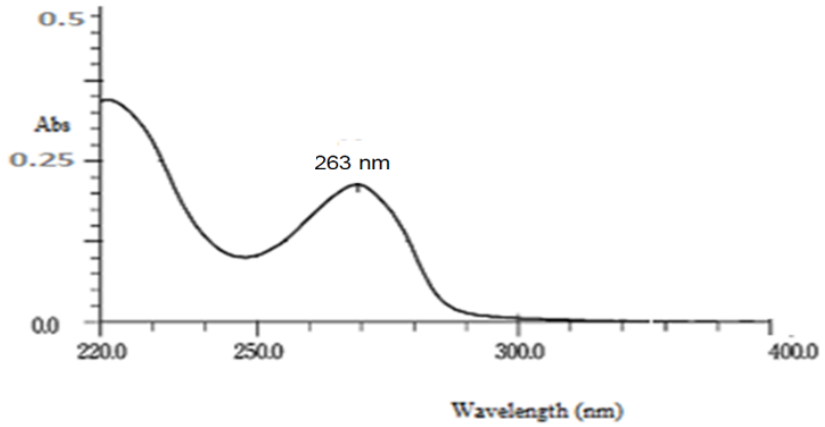

Fig.no.1 Spectra of Mebeverine HCl showing λ max of 263nm

Optimized method:

Buffer preparation: 3.8g of Ammonium acetate was dissolved in 500ml of Hplc grade water, sonicate and make up to 1000ml with water and adjust pH to 5.2 with glacial acetic acid

Mobile phase: Prepare a filtered and degassed mixture of buffer and acetonitrile in the ratio of 62:38v/v

Diluent: Mobile phase

Chromatographic conditions

Flow rate : 1ml/min

Column : Devosil C_{18} column (250×4.6 mm; 5µ)

Detector wave length : 263nm

Column temperature : 25±2ºC

Injection volume : 20µl

Run time : 20mins

Standard preparation:

25mg of Mebeverine HCl was accurately weighed into 100ml volumetric flask. 20ml of diluent is added and sonicated to dissolve. Solution was cooled to room temperature and make up volume with diluent. 5ml of above solution transfered into 25ml volumetric flask and diluted to volume with mobile phase. For results refer table -1. For chromatogram refer fig No.2.

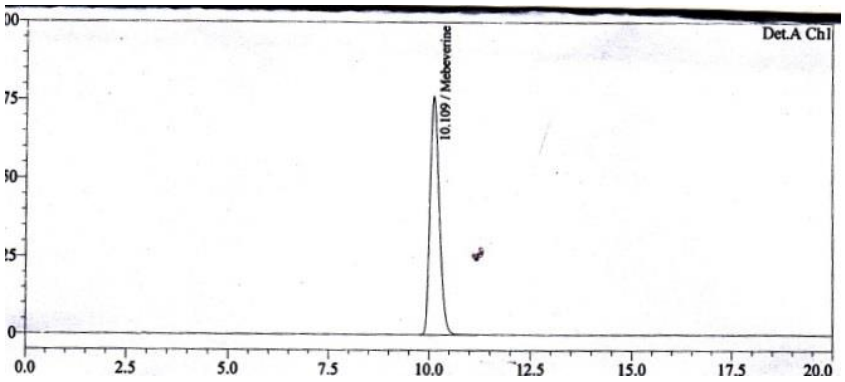

Fig.no.2 Chromatogram of Optimised method

Table 1: system suitability parameters of standard

S.no	Peak name	RT	Area	%Area	Theoritical plates	Peak purity index	Tailing factor
1	Mebeverine HCl	10.109	1589511	100.00	9168	1.00000	1.52

Observation: peak is eluted at 10.109 in this trial

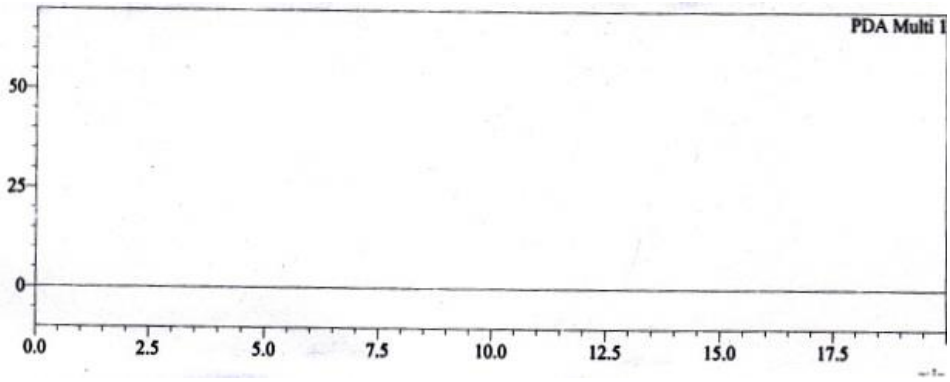

Fig.no.3 Chromatogram of blank

Placebo preparation: 6.25mg of placebo was weighed and transfered in to a 100 ml volumetric flask to this 60ml of diluent was added, sonicated and made up to the volume and then filtered (0.45µmPVDF). 5ml of the above solution was made up to 25 ml by using the same diluent.

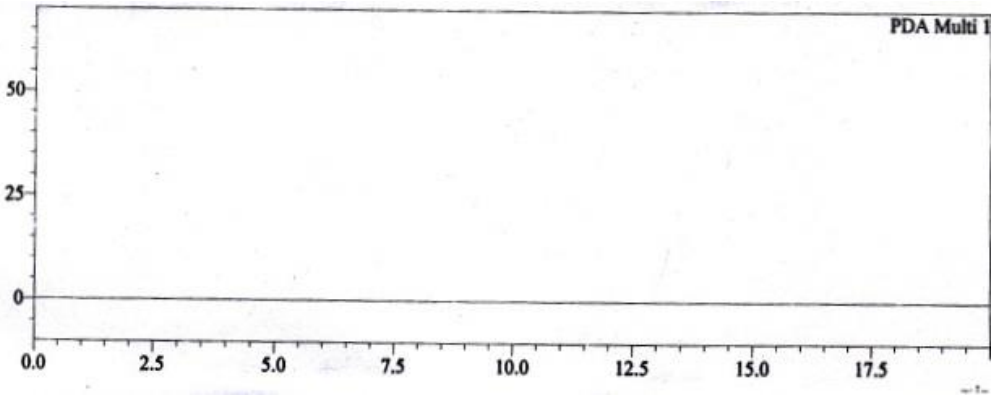

Fig.no.4 Chromatogram of placebo

Sample preparation: Portion of the powder, equivalent to 25mg of Mebeverine HCl was weighed and transferred into 100ml volumetric flask. 50ml of diluents was added and sonicated for 30mins with occasional shakings. Solution was cooled to room temperature and diluted to volume with dilluent. solution is filtered through 0.45µm nylon filter.

5ml of above solution was transferred into 25ml volumetric flask and volume diluted with mobile phase

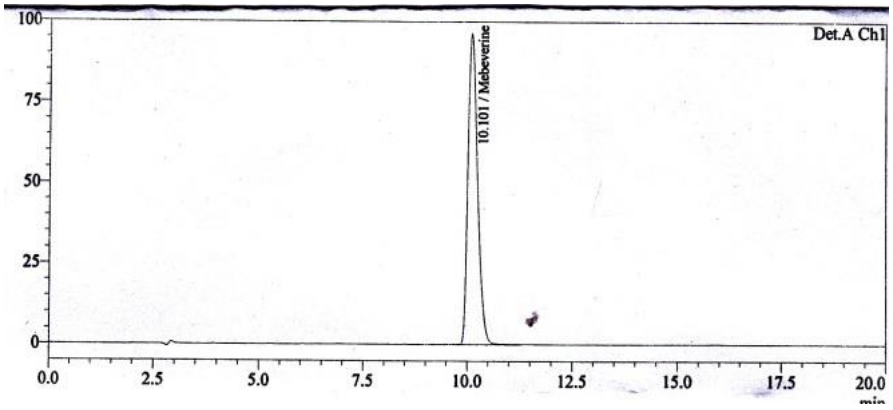

Fig.no.5 Chromatogram of sample

Table:2 system suitability parameters for sample:

S.no	Peak name	RT	Area	%Area	Theoritical plates	Peak purity index	Tailing factor
1	**Mebeverine HCl**	**10.101**	**1521446**	**100.00**	**9178**	**1.00000**	**1.56**

Calculations:

Calculate the amount of Mebeverine Hydrochloride in mg/ml of solution using the following formula

% Content of Mebeverine Hydrochloride:

$$= \frac{Ru}{Rs} \times \frac{Ws}{100} \times \frac{5}{25} \times \frac{100}{Wr} \times \frac{P}{5} \times \frac{100}{100} \times \frac{100}{Lc} \times 100$$

$$= \frac{1521446}{1589511} \times \frac{25}{100} \times \frac{5}{25} \times \frac{100}{12.5} \times \frac{25}{5} \times \frac{99}{100} \times \frac{100}{200} \times 100$$

Where

Ru = Peak area of Mebeverine Hydrochloride in sample solution

Rs = Average peak area Mebeverine Hydrochloride in standard solution

Ws = Weight of Mebeverine Hydrochloride working standard taken in mg

Wr = Weight of sample taken in mg

P = Assay Mebeverine Hydrochloride working standard used on as basis

Lc = Label claim

Result of assay: Mebeverine HCl-99.98%

METHOD VALIDATION

Definition:

Validation is a process of establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce meeting, its predetermined specifications and quality attributes.

The objective of the analytical procedure should be clearly understood since this will govern the validation characteristics which need to be evaluated. Typical validation characteristics which should be considered are listed below:

Validation parameters:

System suitability testing

Specificity

Accuracy

Precision

Linearity & Range

Ruggedness

Robustness

Solution stability

Filter compatibility

System Suitability:

The standard solution and sample solution are prepared as per the assay method of concentration 10µg/ml and filter it through Millipore filter and sonicate. Carried out the system suitability studies for Standard solution and sample solution with a minimum of six replicates of single preparation. Calculate %RSD for area and retention time of Mebeverine Hydrochloride, and record the tailing factor & theoretical plate count details were given in table-3&4.

Table 3: system suitability parameters of standard solution 5 replicate injections

Standard Solution				
S.NO.	**Retention Time**	**Area**	**Tailing Factor**	**Theoretical Plates**
1	**10.129**	**1544494**	**1.5**	**10053**
2	**10.148**	**1545311**	**1.5**	**10042**
3	**10.129**	**1549914**	**1.5**	**10050**
4	**10.125**	**1550421**	**1.5**	**10028**
5	**10.127**	**1550173**	**1.5**	**10031**
% RSD		**0.17**		

Table 4: system suitability parameters of sample solution of 5 replicate injections

Sample Solution				
S.NO.	**Retention Time**	**Area**	**Tailing Factor**	**Theoretical Plates**
1	**10.132**	**1544494**	**1.5**	**10086**
2	**10.105**	**1545311**	**1.5**	**10064**
3	**10.104**	**1549914**	**1.5**	**10040**
4	**10.101**	**1550421**	**1.5**	**10054**
5	**10.132**	**1550421**	**1.5**	**10028**
%RSD		**0.18**		

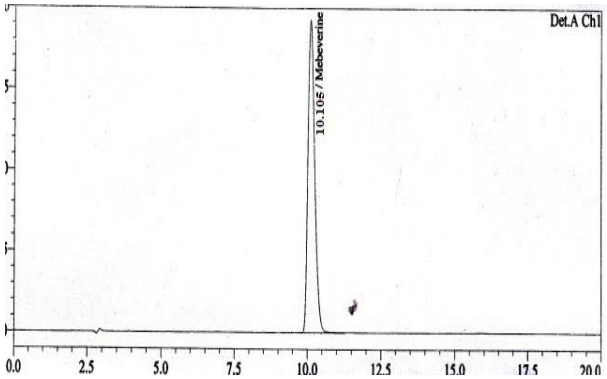

Fig.6 Standard Chromatogram

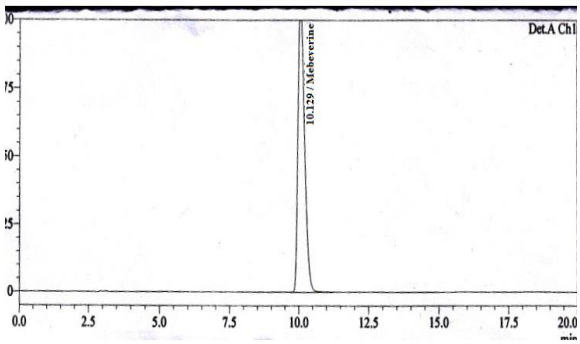

Fig.7 Sample chromatogram

Specificity:

Procedure:

Individually inject 20µl of each solution individually the prepared solutions of standard and sample and develop chromatograms check the retention time of individual injection sample in the chromatogram and observe whether the retention time is matching with that of standard. Resulted chromatograms are shown in the figures.

Placebo Interference:

A study to establish the interference of placebo was conducted. Samples were prepared in triplicate by taking the placebo equivalent to about the weight in portion of test preparation as per the test method. Chromatogram of placebo did not show any additional peaks. This indicates that the excipients used in the formulation do not interfere in the assay of Mebeverine Hydrochloride in capsules. Resulted chromatograms are shown in the figures.

Acceptance criteria: No interference at the retention times of Mebeverine HCl.

Table-5 Data of Specificity:

Injections	**Interference**	**RT**
Blank	**Nil**	**No interference at RT of Analyte Peak**
Placebo	**Nil**	**No interference at RT of Analyte Peak**
Sample	**Nil**	**Mebeverine HCl – 10.101**

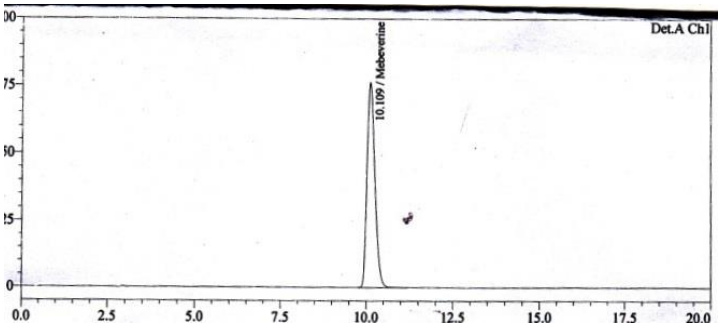

Fig.8 Standard chromatogram

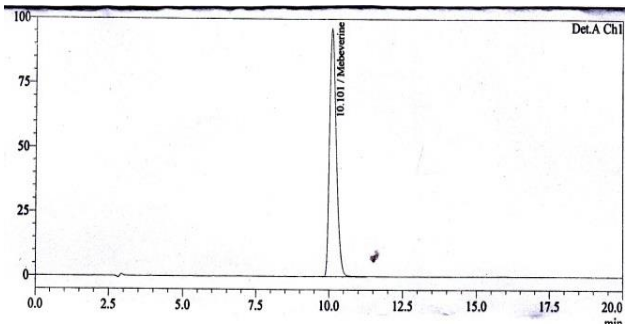

Fig.9 Sample chromatogram

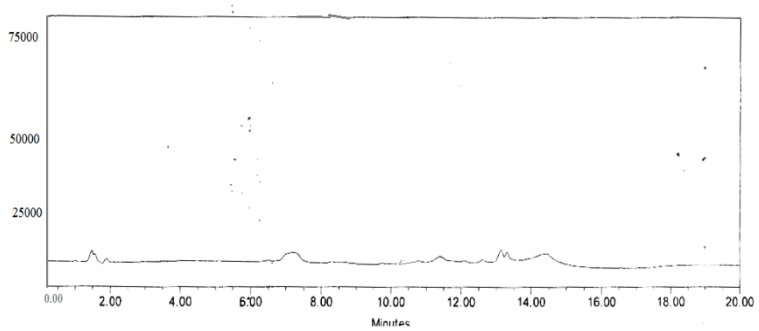

Fig. 10 Sample placebo

Data Interpretation: On the basis of these chromatograms, we can say that there is no interference of blank and placebo.

Accuracy:

Accuracy of test method was carried out by spiking known amount of drug substance to get concentration of Mebeverine HCl 80 %, 100 % and 120 % of target concentration in triplicate for each level. Each solution was injected in triplicate. The average % recovery of Mebeverine

Data Interpretation: The recovery results indicating that the test method has an acceptable level of accuracy shown in table.

Table 6: Data of Accuracy

Concentration level	**Amount added(mg/ml)**	**Amount found(mg/ml)**	**%Recovery**	**Average % recovery**
80%	**0.04124** **0.04174** **0.04090**	**0.04123** **0.04123** **0.04055**	**99.98** **99.36** **99.15**	**99.49**
100%	**0.0496** **0.04990** **0.04962**	**0.04937** **0.04946** **0.04899**	**99.49** **99.11** **99.74**	**99.44**
120%	**0.06010** **0.06030** **0.06050**	**0.06004** **0.05995** **0.06060**	**99.91** **99.42** **100.16**	**99.83**

Precision:

System Precision:

The standard solution was prepared as per the assay method of concentration 0.05mg/ml and filter it through Millipore filter and sonicate.

Procedure:

Carried out the system precision for Standard solution with a minimum of six replicates of single preparation.

Calculate %RSD for peak area and retention time of Mebeverine Hydrochloride. The data for system precision was given in table.

Data Interpretation: % RSD of the Mebeverine HCl tablet from six units was found to be 0.42, as shown in the table.

Table 7: Data for System precision:

Standard Solution				
S.NO.	Retention Time	Area	Tailing Factor	Theoretical Plates
1	10.106	1616866	1.5	9181
2	10.109	1619647	1.5	9221
3	10.107	1625056	1.5	9174
4	10.126	1631471	1.5	9179
5	10.123	1631662	1.5	9140
MEAN		1626237		
%RSD		0.42		

Method Precision:

Six sample preparations were prepared individually using single batch of Mebeverine HCl as per assay method and injected each solution. Resulted data was shown in the Table.

Procedure:

Carried out the method precision studies for Mebeverine hydrochloride at 100% concentration of the test solution with a minimum of six determinations of individual preparations. Calculated %RSD for Mebeverine hydrochloride area and RT.

Data Interpretation: % RSD of the Mebeverine HCl capsule from six units was found to be 0.20, as shown in the table

Table 8: Data for Method Precision:

Sample no	%assay of Mebeverine Hcl 200mg capsules
1	98.6
2	98.1
3	98.4
4	98.3
5	98.4
6	98.6
Average	98.4
SD	0.19
%RSD	0.20

Linearity & Range:

Method: A study to establish the linearity of detector response of Mebeverine HCl was conducted. Linearity of detector response of Mebeverine HCl was conducted from 80% level of metoprolol standard to 120% of the Mebeverine HCl standard concentration. Plotted linearity graphs of standard Mebeverine HCl concentration versus area level 80%, 90%, 100%, 110%, 120% and has been found linear in the prescribed range.

Table-9:Data for Linearity:

Linearity level (%)	**Volume of stock taken(ml)**	**Diluted to (ml)**	**Final concentration(mg/ml)**
80	4	25	0.0400
90	4.5	25	0.0450
100	5	25	0.0500
110	5.5	25	0.0550
120	6	25	0.0600

Table 10: Data of linearity:

% level	**Concentration(mg/mL)**	**Peak area(average)**
80	0.0400	1208873
90	0.0450	1397064
100	0.0500	1572211
110	0.0550	1710688
120	0.0600	1869844
Correlation coefficient(r)		0.9996
Squared correlation coefficient(r^2)		0.9993
Slope		31068
Y- intercept		421.10

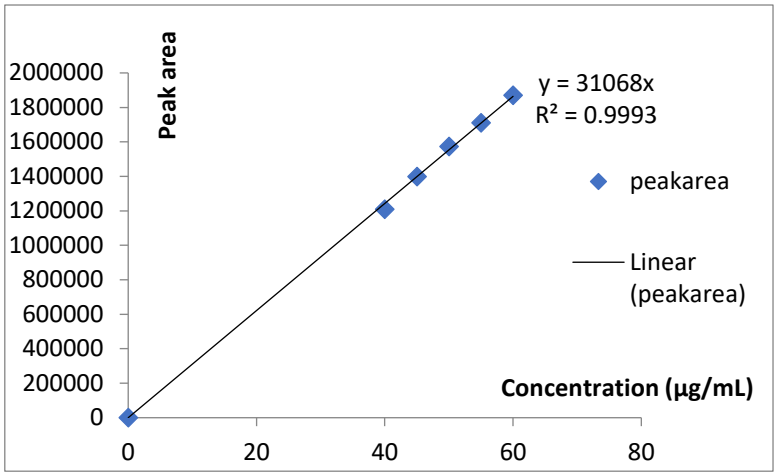

Fig 11: Linearity Plot

Data Interpretation: The correlation coefficient was found to be 0.9993 for Mebeverine HCl. From the above study it was established that the linearity of test method is from 40 µg/ml to 60 µg/ml of target concentration shown in linearity plots**.**

Ruggedness:

Intermediate precision expresses within laboratory variation as on different days or with different analysts or equipment within the same laboratory. It is not considered necessary to study these effects individually.

Table11: Comparative results:

Sample no	**Mebeverine Hcl capsules 200mg**	
	Day 1/ analyst 1/ inst.1	**Day 2/ analyst 2/ inst.2**
	% assay	**% assay**
1	**98.6**	**98.5**
2	**98.1**	**98.1**
3	**98.4**	**98.9**
4	**98.3**	**98.4**
5	**98.4**	**98.7**
6	**98.6**	**98.7**
Average	**98.4**	**98.6**
%RSD	**0.2**	**0.5**
Cumulative % RSD	**0.2**	

Data Interpretation: From the above data, it was concluded that the method is rugged.

Robustness:

Influence on flow rate variation: robustness of assay method is demonstrated by changing the flow rate for 0.9ml/min and 1.1ml/min instead of specified flow rate (1ml/min). By injecting the 6 replicate injections of standard in 0.9ml/min and 1.1ml/min flow rate and found that system suitability parameters are passed. The % RSD, tailing factor and theoretical plates of Mebeverine HCl standard are within the limits.

Table 12: Data of robustness (Influence on flow rate variation)

S.NO	**SYSTEM SUITABILITY PARAMETERS**	**RESULTS**		**ACCEPTANCE CRITERIA**
		Low flow (0.9ml)	**High flow (1.1ml)**	
1	**% RSD of peak area**	**0.17**	**0.32**	**NMT 2**
2	**Tailing factor**	**1.5**	**1.42**	**NMT 2**
3	**Theoretical plate count**	**9167**	**8715**	**NLT 2000**

Data Interpretation: Results are within the limit.

The analytical method was found to be robust with respect to change in flow rate.

Influence on variation of column temperature:

Robustness of assay method is demonstrated by changing the column temperature for 20ºC and 30ºC instead of column temperature (25ºC). By injecting the 6 replicate injections of standard in 20ºC and 30^{0}C column temperature and found that system suitability parameters were passed. The %RSD, tailing factor and theoretical plates of Mebeverine HCl standard was found to be within the limits.

Table 13: Data of robustness (Influence on Column temperature variation)

S.NO	**SYSTEM SUITABILITY PARAMETERS**	**RESULTS**		**ACCEPTANCE CRITERIA**
		Low Temp (20ºC)	**High Temp (30ºC)**	
1	% RSD of peak area	0.27	0.42	NMT 2
2	Tailing factor	1.5	1.42	NMT 2
3	Theoretical plate count	8375	9178	NLT 2000

Data Interpretation: Results are within the limits. The analytical method was found to be robust with respect to change in column temperature.

Influence on variation of buffer composition:

The robustness of assay method is demonstrated by changing the composition of buffer composition as 60% and 64% instead of 62%. standard solutions was prepared as per test procedure and injected 6 replicates into the chromatograph with variation of buffer phase composition in mobile phase as 60% and 64% and evaluated the system suitability parameters. The %RSD, tailing factor and theoretical plates of Mebeverine HCl standard was found to be within limits.

Table 14: Data of robustness (Influence on buffer composition variation)

S.NO	SYSTEM SUITABILITY PARAMETERS	RESULTS		ACCEPTANCE CRITERIA
		Low Buffer (60%)	High Buffer (64%)	
1	% RSD of peak area	0.24	0.11	NMT 2
2	Tailing factor	1.5	1.42	NMT 2
3	Theoretical plate count	8900	8577	NLT 2000

Data Interpretation : Results are within the limits

Influence on variation of buffer pH:

The robustness of assay method is demonstrated by changing the composition of buffer pH as 5.0 and 5.4 instead of 5.2.By injecting 6 replicates into the chromatograph with variation of pH in buffer phase as 5.0 and 5.4 and evaluated the system suitability parameters. The %RSD, tailing factor and theoretical plates of Mebeverine HCl standard was found to be within limits.

Table 15: Data of robustness (Influence on variation of buffer pH)

S.NO	SYSTEM SUITABILITY PARAMETERS	RESULTS		ACCEPTANCE CRITERIA
		Low pH 5.0	High pH 5.4	
1	% RSD of peak area	0.19	0.43	NMT 2
2	Tailing factor	1.5	1.42	NMT 2
3	Theoretical plate count	8914	8451	NLT 2000

Data Interpretation: Results are within the limit

The analytical method was found to be robust with respect to change in Buffer pH.

Solution Stability:

Sample and standard solutions should be tested over 24-48hrs period under normal laboratory conditions and refrigerated conditions (2^0-8^{0}C) and potency of solution should be determined by comparison to freshly prepared standards. Data should also be generated to establish the use before date and storage conditions of the standard solutions.

Method:

A study to establish the stability of standard and test preparation at controlled room temperature (25±5^{0}C) and refrigerated (2-8^{0}C) condition was conducted over a period of 48hrs. the difference in percentage assay of standard and test preparations at initial, after 24hrs and after 48hrs of room temperature and refrigerator conditions were found within the limits.

Standard solution preparation:

Table 16: Solution stability results for Mebeverine HCl standard solution at controlled room temperature

Time	**At controlled room temperature (25±5^{0}C)**	
	Assay (%)	**Difference (%)**
Initial	99.7	NA
After 24hrs	99.9	0.2
After 48hrs	100.3	0.6

Table 17 : Solution stability results for Mebeverine HCl standard solution at refrigerator

Time	**At refrigerator (2-8^{0}C)**	
	Assay (%)	**Difference (%)**
Initial	99.7	NA
After 24hrs	100.1	0.4
After 48hrs	100.3	0.6

Table 18: Sample solution preparation: at controlled room temperature (25±5^{0}C)

Time	**Sample 1**		**Sample 2**	
	Assay %	**Difference %**	**Assay %**	**Difference %**
Initial	100.8	NA	100.9	NA
After 24hrs	101.5	0.7	101.2	0.3
After 48hrs	101.3	0.5	101.5	0.6

Table 19: Sample solution preparation at refrigerator (2-8^{0}C)

Time	**Sample 1**		**Sample 2**	
	Assay %	**Difference %**	**Assay %**	**Difference %**
Initial	**100.8**	**NA**	**100.9**	**NA**
After 24hrs	**101.9**	**1.1**	**101.2**	**0.3**
After 48hrs	**101.2**	**0.4**	**101.4**	**0.5**

Data Interpretation: Results are within the limits. Standard and test preparations are stable for a period of 48hrs at room temperature (25±2^{0}C) and at refrigerator condition (2-8^{0}C)

Filter Compatibility:

A study was conducted to determine the effect of filter on the assay, dissolution and impurities. Centrifuged and filtered different portions of the test preparation separately injected and analysed.

Table 20: Filter compatibility results for Mebeverine HCl through nylon filter

Sample no	Centrifuged sample	Filtered through 0.45µm nylon filter (%)	Difference (%)
1	101.6	101.4	0.2
2	101.4	101.1	0.3

Table-21: Filter compatibility results for Mebeverine HCl through PVDF filter

Sample no	**Centrifuged sample**	**Filtered through 0.45µm PVDF filter (%)**	**Difference (%)**
1	**101.6**	**101.1**	**0.5**
2	**101.4**	**101.5**	**0.1**

CONCLUSION

High performance liquid chromatography is at present one of the most sophisticated tools of analysis. The estimation of Mebeverine hydrochloride in capsules was done by Reverse Phase HPLC. The mobile phase used consists of Buffer containing Ammonium acetate and mobile phase ratio of Ammonium acetate (p^H 5.2): Acetonitrile. A C18 column containing Octadecyl silane (ODS) chemically bonded to porous silica particles (250 × 4.6mm, 5µ particle size) was used as the stationary phase. The detection was carried out using UV detector set at 263nm. The solutions are chromatographed at a constant flow rate of 1.0 ml/ min. The retention time for Mebeverine hydrochloride was around 10.101 min. The quantitative estimation was carried

out on the capsule using RP HPLC. The quantitative results obtained are subjected to the statistical validation. The values of RSD are less than 2.0%, indicating the accuracy and precision of the method. The percentage recoveries vary from 98.0 – 102.0% for Mebeverine hydrochloride. The results obtained on the validation parameters met the ICH and USP requirements. It is inferred that the method was found to be simple, specific, precise and linear. The method was found to have suitable applications in routine laboratory analysis with high degree of accuracy and precision.

References:

1. Parchaki Pranitha & M. Sunitha Reddy (2022). Formulation and Evaluation of Mebeverine Hydrochloride Sustained Release Capsules by Pelletization Technique. Saudi J Med Pharm Sci, 8(11): 644-649.
2. Lough WJ.Wainer IW.High Performance Liquid Chromatography: fundamental principles & practice. Glasgow (UK): Blackie Academic & Professional ; 1995
3. Sweetman SC: *Martindale: The complete Drug Reference*. 35th edition. The Pharmaceutical Press, London; 2007:119-692
4. University of Alexandria, Faculty of Pharmacy, Pharmaceutical Analytical Chemistry Department, Elmessalah 21521, Alexandria, Egypt.
5. journal of AOAC International, Volume 93, Number 4, July 2010 , pp. 1192-1200
6. *Czechowicz, M. W. McCulloch, C. Raper, M. J. Rand*, Pharmacol. Res. Commun.(1969)
7. *J. D. Pinkston, G. J. Venkatramani, L. J. Tulich, D. J. Bowling, K. R. Wehmeyer*, J. Chromatogr. Biomed. Appl.133, 209 (1993). Crosreff **7**. *E. M. Hassan, A. A. Gazy, M. M. Bedair*, Drug Dev. Ind. Pharm.21, 633 (1995).
8. Drug Testing and Analysis Volume 5, Issue 5, pages 325–333, May 2013
9. Zayed SI. Simultaneous determination of mebeverine hydrochloride and sulpiride using the first derivatives of ratio spectra and chemometric methods. Anal Sci. 2005;21:985–989. doi: 10.2116/analsci.21.985.
10. The Pharmaceutical Press, London, 1989. *British Pharmacopoeia, Vols I & II*, Her Majesty's Stationery Office, London, 1988
11. Stockis A, Guelen PJ, de Vos D. Identification of mebeverine acid as the main circulating metabolite of mebeverine in man. J Pharm Biomed Anal. 2002;29:335–340. doi: 10.1016/S0731-7085(02)00023-7