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Development and Validation of HPLC Method for Determination of Degradation Impurities for the Qualification of Mebevrine HCl

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ABSTRACT

Reverse phase high performance liquid chromatographic method for the estimation degradation impurities of Mebeverine HCl in bulk drug is illustrated. The method consists of; Mobile phase B: Acetonitrile and Mobile phase A: 0.051 M Phosphate buffer (pH-4.5) in gradient pump mode. A linear response was observed in the range of 0.8 to 48 µg/ml with a regression coefficient of 0.999 for both Mebeverine HCl and Veratric acid. The force degradation study was performed to determine the degradation pathways which also proves the specificity of the method. The method is cost effective as well as sensitive. Validation parameters were carried out as per the guidelines of International Conference for Harmonization.

Keywords: HPLC, Mebeverine HCl, Veratric acid, Validation and Method development.

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INTRODUCTION

High performance liquid chromatography which was previously termed as High pressure liquid chromatography is an analytical technique which is used to separate, Quantify and identify different components in a given mixture. The principle mainly involves the use of pumps which pass the pressured liquid solvent that contains mixture of a sample inside the column filled by adsorbent material. The components in the mixture of sample has different affinity towards adsorbent, hence all the components flow with different flow rate and hence eluted from the column at different time intervals. HPLC technique is widely useful in medical (like detection of vitamin D levels in blood serum), for legal (e.g. detection of performance enhancing drugs in urine samples), for research (e.g. separation of the components for a complex sample, in similar synthetic molecules from each other), and in manufacturing (e.g. while the production procedure of pharmaceutical as well as biological products) purposes.

The mass transfer phenomenon which includes adsorption is called as chromatography. The important part in HPLC is pumps which passes the pressurized mobile phase along with the sample solution through a column containing sorbent, in the process which carries out the separation for components in the sample. The important part of a column is a sorbent, which is granular material of different size from 2-50 micrometers. The sample components have different affinities towards the sorbent, and hence they get separated while passing through the column. The mobile phase contains different types of solvents, mainly water or methanol or acetonitrile or also tetrahydrofuran. The flow rate and temperature also have an important feature in separation of the sample components, because the affect the affinity of the sample molecules for a stationary phase. The affinity of the sample molecules for a stationary phase may be due to interactions like hydrophobic, dipole-dipole and ionic.

HPLC is different from the traditional liquid chromatography in the sense of the use of high operational pressures i.e. 50 to 350 bar, were as traditional chromatography is mainly dependent on the gravitational force to pass a mobile phase inside the stationary phase. Since very small amount of sample can be separated in the HPLC, the HPLC columns have diameter of 2.1 to 4.6 diameter, while 30-250 mm of length. The size of a stationary phase is usually 2-50 micrometer. Hence HPLC gets a superior separation over traditional chromatographic techniques.

[1]De Schutter J.A *et al*[2], have done stability study and quantitative determination of Mebeverine hydrochloride in tablets by means of reversed-phase high-performance liquid chromatography. The column used for the HPLC method was C8 while the mobile phase was mixture of methanol and water (75% as to 25%) which was containing 0.05% hexylamine. The pH of the solution was

adjusted to 5.0 with orthophosphoric acid. [2] Mohamed I.W. *et al*[6], have carried out simultaneous determination of sulpiride and Mebeverine by HPLC method using fluroscence detection: Application to real human plasma. The separation of molecules was achieved within 6 minutes. The mobile phase consists of Acetonitrile and 0.01 dihydrogenphosphate buffer (45: 55), pH-4.0. The flow rate of the method was 1.0 ml/min. The detection was carried out by fluorescence detection at 300 nm and 365 nm for emission. [3]Simon E *et al*[7], have done investigative implication of the instability and metabolism of Mebeverine. The case with over dosage of mebeverine was studied for detection of Mebeverine, Mebeverine-Alcohol and Veratric acid in the post mortem blood samples. The compounds were measured by using HPLC which consist of DAD detector. Gas chromatography was also carried out to detect veratric acid.

There are various methods available for determination of Mebeverine from pharmaceutical dosage forms as well as in the active pharmaceutical ingredient. But there are not much references for the determination of degradation impurities of Mebeverine HCl in pharmaceutical dosage form as well as in pure drug form. Hence there is a scope for the development of analytical method for the determination of degradation impurities from Mebeverine HCl. HPLC is the most sensitive and accurate analytical technique used for quantification and qualification of pharmaceutical components. Hence it was decided to develop the HPLC method for determination of degradation impurities in the Mebeverine HCL by HPLC in active pharmaceutical ingredient.

MATERIALS AND METHOD

Separation was performed on a Waters Sunfire, C18, 250 x 4.6 mm, 5 µ particle size column using mobile phase consisting of buffer pH-2.5 and Acetonitrile. 2.72 g of the Potassium dihydrogen phosphate was accurately weighed and then transferred to a 1000 ml of the Milli-Q water followed by mixing. 2.0 ml of the Triethylamine was then added and the pH of solution is adjusted to exactly 2.5 with the use of orthophosporic acid. Gradient pump program flow mode is used were mobile phase A contains Buffer pH-2.5 and mobile phase B is 98% Acetonitrile in water. The separation process was performed at 1.5 ml/min flow rate with injection volume of 10 µL. Detection wavelength was 220 nm on PDA/UV detector. The column temperature was 45°C while sample temperature was 10°C.

Chromatographic system and conditions

The column used for the method was Waters Sunfire (C-18, 250 x 4.6 mm, 5µ). The proposed method was performed using chromatographic conditions as mentioned in Table 1.

Table 1: Chromatographic conditions of HPTLC for Mebeverine HCl

Chrmatographic mode	Chromatographic conditions
HPLC system	Waters alliance 2695
Detector	Waters 2487 dual λ absorbance UV detector
Column	Sunfire C-18, 250 x 4.6 mm, 5 µ
Software	Empower 3
Flow rate (ml/min)	0.8
Injection Volume (µl)	30
Column temperature	45°C
Run time (min)	19
Mobile phase	Mobile Phase A: Buffer pH-2.5 Mobile Phase B: Acetonitrile
Diluents	0.1 N HCl
Pump Mode	Gradient

Selection of mobile phase

The pure drug of the Mebeverine HCl along with its related substance Veratric acid is injected into HPLC system and was run in different solvent systems. Various mobile phases *viz.*, methanol and water, methanol/acetonitrile and (0.05 M) phosphate buffers having pH such as 6, 5.5 and 3.5, and (0.05M) triethylamine buffer pH-8/methanol were prepared to find a best possible conditions for separation of the Noscapine HCl and Papaverine. It was found that acetonitrile and octane sulponic acid buffer pH-3.0 gives satisfactory results as compared to other mobile phases. Finally, the optimal composition of the mobile phase was determined to be 98% acetonitrile in water and Phosphate buffer pH-2.5 in gradient mode. This mobile phase produced good resolution, reasonable retention time and acceptable peak symmetry for the drug.

Preparation of octane sulphonic acid buffer pH-2.5

Weighed and transferred accurately 2.72 g potassium dihydrogen phosphate in 1000 ml water. Add 2.0 ml of triethylamin and the pH was adjusted to 2.5 with dilute orthoposporic acid.

Gradient Programme

Time (Mins)	Buffer pH-3.0	Acetonitrile
0	80	20
4	65	35
14	55	45
15	80	20
19	80	20

Preparation of standard solution

Stock solution of MebeverineHCl and Veratric acid of concentration of 320 µg/ml were prepared separately by using 0.1 N HCl. Standard stock preparation was diluted with the 0.1 N HCl to get 32µg/ml of standard solution. Standard solution was found to be stable for 24 hours.

Preparation of the sample solution

MebeverineHCl API was dissolved in 0.1 N HCl to get 320 µg/ml of sample solution.

Stressed degradation samples

Acid degradation sample:

3.2 mg of Mebeverine hydrochloride is accurately weighed and then transferred to 10 ml of volumetric flask. 1 ml of the 1 N HCl is added in the flask and it was kept in room temperature for about 1 hour. Solution was then neutralized with adding 1 ml of the 1 N NaOH. The volume was then made up by 0.1 N HCL.

Base degradation sample:

3.2 mg of Mebeverine hydrochloride is weighed and then transferred to a 10 ml of volumetric flask. Then 1 ml of the 1 N NaOH was added in the flask and it was kept in room temperature for about 1 hour. Solution was then neutralized with adding 1 ml 1 N HCl. The volume was then made up by 0.1 N HCL.

Preparation for Peroxide degradation sample:

3.2 mg of the Mebeverine hydrochloride is weighed and then transferred in to 10 ml of volumetric flask. 1 ml of the 30% H_2O_2 was then added into flask and it was kept in the room temperature for about 1 hour. The volume was then made up with the 0.1 N HCL.

Conditions of the columns

Before the new run of HPLC, conditioning of the columns was done by passing HPLC grade acetonitrile at 1 ml/min flow rate for 30 min, so as to remove the remains of the previous run's present in the column.

Loading of mobile phase

Filtered and degassed mobile phase was filled in the channel. Priming was done for each channel by using freshly prepared mobile phase.

Validation of RP-HPLC method

Validation for the analytical test is an activity to establish by the laboratory study. Validation studies weather if the performance characteristic for a method meets requirements of intended analytical requirements. Performance characteristics are expressed by the terms for analytical parameters.

Specificity:

Specificity is performed by determining the interference for diluents and by performing forced degradation study. No blank peak was interfering at retention time for Mebeverine and also Veratric acid.

The forced degradation is carried out with stressing a sample solution by Acid hydrolysis, Base hydrolysis and Peroxide hydrolysis condition. Maximum degradation was obtained at peroxide hydrolysis stress condition for about and peak was found to be pure in all degraded solutions for sample and also mass balance is also found to matching.

Linearity

Appropriate aliquots for the standard Mebeverine HCl and Veratric acid stock solutions (320 µg/ml) were taken in various individually 10 ml of volumetric flasks. The solution was then diluted to the mark by diluent to active the final concentration for the drug solution. Calibration curve of Mebeverine HCl and Veratric acid was then constructed for plotting the peak area *vs*. applied concentration. The slope, intercept and the correlation coefficient was also calculated and tabulated in Table 2 and 3. The results reflect that excellent correlation is existing between peak area along with concentration of the drugs which are within a concentration range.

Table 2: Calibration data of Mebeverine HCl by RP-HPLC method

Sr. No	Concentration (µg/ml)	Area
1	0.8	19055
2	16	421161
3	24	638758
4	32	845793
5	40	1056316
6	48	1239102

Table 3. Calibration data of Veratric acid by RP-HPLC method

Sr. No	Concentration (µg/ml)	Area
1	0.8	42640
2	16	779890
3	24	1153596
4	32	1517737
5	40	1874774
6	48	2190959

Precision

The precision for a method is demonstrated with injecting six replicate injections of the standard solution. Response factor for the drug peak and also % R.S.D were calculated (Table 4). With the data obtained, the developed test method was observed to be precise.

Table 4. Precision results of Mebeverine HCl by RP-HPLC method

Sr. No	Concentration (µg/ml)	Precision
1	0.8	100.33
2	0.8	101.99
3	0.8	103.45
4	0.8	102.96

5	0.8	101.15
6	0.8	101.25
Avg.	-	1.18
S.D*	-	101.85
%R.S.D*	-	1.16

***is average of six determinations.

Accuracy

The Accuracy is a closeness of a test results that are obtained by method to a true value. The Recovery study was performed by using the method for standard addition by adding the known amount for standard drug solutions (LOQ, 50, 100 and 150%) to diluent. The % Recovery was then calculated and tabulated in Table 5.

Table 5. Recovery study data of Mebeverine HCl by RP-HPLC method

Recovery level (%)	Average Area	Amount of sample recovered (%)
LOQ	37392	98.19
	37212	97.71
50	775318	101.80
	774811	101.73
100	1491016	97.88
	1520023	99.79
150	2232054	97.69
	2229545	97.58
SD*		1.82
AVG*		99.04
%RSD*		1.84

*is average of 3 determinations

Limit of detection

Limit of detection for the developed test method is determined with injecting progressively very low concentrations of a standard solution by using a developed HPLC test method. The LOD is a smallest concentration for the analyte which gives the measurable response (the signal to noise ratio should be 3: 1).

Limit of quantitation (LOQ)

Based on a LOD value, the LOQ concentrations were determined by multiplication to three times. The chromatogram of the LOQ was shown in the Fig. 3.

Robustness

For determination of the method's robustness, the number of the method parameters like flow rate and the column temperature was varied within the realistic range, and its quantitative influence for variables was determined. Hence it was determined that there was no any marked changes in a chromatogram, hence it was demonstrated that a HPLC test method is robust.

Robustness for analytical test procedure is the measure of its capability to be remained unaffected with small, but by deliberate variations in the method parameters and that provides the indication of the methods reliability during its routine use. The samples were analyzed separately by slightly changes in the analytical method as given below:

By changing flow rate of mobile phase ±0.1 ml, chromatograms were obtained and the retention time values were measured. The robustness results are tabulated in the Table 6 and 7.

Table 6. Robustness studies of Mebeverine HCl by changing flow rate by RP-HPLC method

Sr. No	Flow rate (ml/min)	System suitability results Plate count	Tailing	Retention time (min)
1	1.4	27809	1.2	7.495
2	1.5*	28099	1.2	7.569
3	1.6	28217	1.2	7.484

*is actual flow rate.

Table 7 Robustness studies of Veratric acid by changing flow rate by RP-HPLC method

Sr. No	Flow rate (ml/min)	System suitability results Plate count	Tailing	Retention time (min)
1	1.4	15107	1.2	5.398
2	1.5*	14433	1.2	5.376
3	1.6	14653	1.2	5.388

System-suitability

System-suitability parameters are the integral part for method development which are used for ensuring the adequate performance from a chromatographic system. Retention time (t_R), the number for theoretical plates and the tailing factor were determined of six repeated injections for the drug of concentration 10 µg/ml. Results were found to be within the acceptable limits which are tabulated in Table 8 and 9.

Table 8. System suitability studies of Mebeverine HCl by RP-HPLC method

Parameters	Values	Required limits
Retention time (min)	7.569	R.S.D ≤ 1%
Theoretical plates (N)	28099	N > 2000
Tailing factor (T)	1.2	T ≤ 2

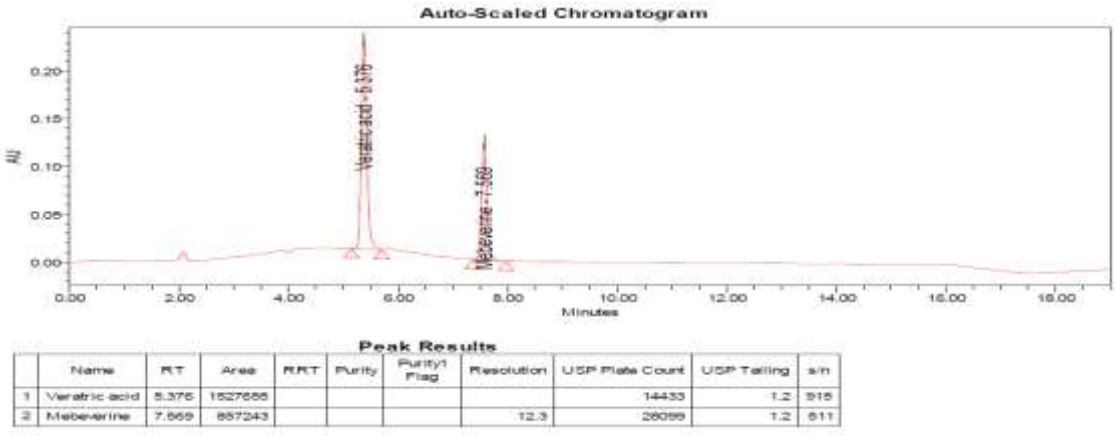
Table 9. System suitability studies of Veratric acid by RP-HPLC method

Parameters	Values	Required limits
Retention time (min)	5.376	R.S.D ≤ 1%
Theoretical plates (N)	14433	N > 2000
Tailing factor (T)	1.2	T ≤ 2

RESULTS AND DISCUSSION

In the current RP-HPLC method chromatographic conditions were optimized with a mobile phase which consists of acetonitrile and phosphate buffer pH-2.5 with gradient pump mode and Waters Sunfire (C-18, 250 x 4.6 mm, 5µ) column as stationary phase. In RP-HPLC method, different parameters were studied for system suitability and % R.S.D for retention time is observed to be less than 2 and the tailing factor is found less than 2. The number of a theoretical plates [*n*] was found more than 2000. Other parameters like the theoretical plates every meter [N] and the Height Equivalent Theoretical Plate [HETP] was observed to be in the acceptance limit. Thus, proposed RP-HPLC method shows system suitability for estimation of Mebeverine HCl.

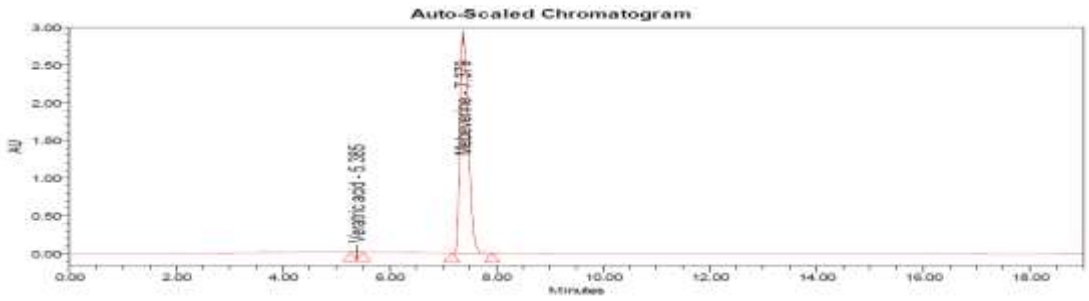
In RP-HPLC method, chromatograms recorded showed peaks separated from other impurities of the bulk drug. The percentage recovery of Mebeverine HCl was observed to be in the limit under most conditions and didn't showed any significant change when the critical parameter like flow rate was modified for robustness study in RP-HPLC method. In RP-HPLC method, tailing factor of Mebeverine HCl and veratric acid was always found to be below than 2.0 and they were well separated with acceptable system suitability parameters when all changes were performed. Considering the results of robustness studies for RP-HPLC method, it can be finally concluded that, proposed chromatographic method is robust. The results obtained from precision studies show % R.S.D less than 2.0 %. It shows that proposed methods are rugged under routine analytical variations.



Peak Results

	Name	RT	Area	RRT	Purity	Purity Flag	Resolution	USP Plate Count	USP Tailing	s/n
1	Veratric acid	5.376	1527666					14433	1.2	918
2	Mebeverine	7.569	887243				12.3	28099	1.2	611

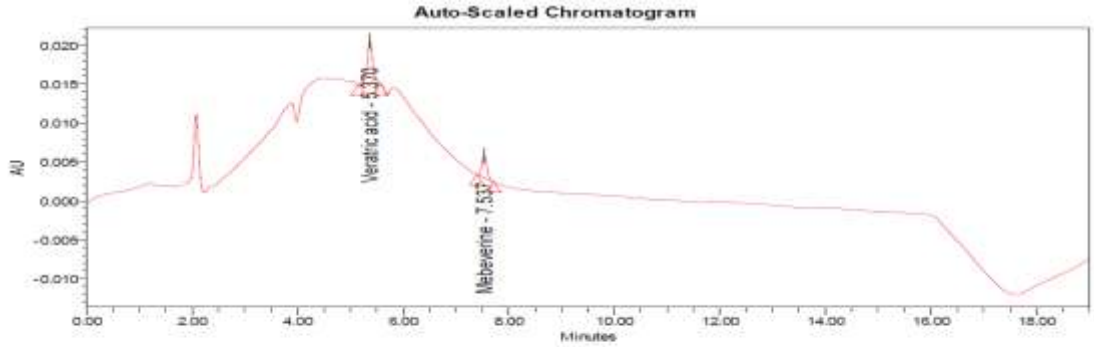
Figure 1: Cromatogram of standard solution by RP-HPLC method



Peak Results

	Name	RT	Area	RRT	Purity	Purity Flag	Resolution	USP Plate Count	USP Tailing	s/n
1	Veratric acid	5.385	39487					16363	1.0	29
2	Mebeverine	7.379	22246573				8.4	10124	1.4	13574

Figure 2: Chromatogram of Sample solution by RP-HPLC method



Peak Results

	Name	RT	Area	RRT	Purity	Purity Flag	Resolution	USP Plate Count	USP Tailing	s/n
1	Veratric acid	5.370	40667					13741	1.0	23
2	Mebeverine	7.537	18432				12.2	29346	1.3	12

Figure 3: Chromatogram of LOQ sample by RP-HPLC method

CONCLUSION

In the present investigation, we have developed a simple, sensitive, precise and accurate RP-HPLC method for the quantitative estimation of the related substances in Mebeverine HCl in Active pharmaceutical ingredient. The results expressed for RP-HPLC method are found to be promising. The current RP-HPLC method is very much sensitive, accurate and precise as compared to the various other spectrophotometric methods.

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