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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OFBEMPEDOIC ACID IN BULK DRUG AND DOSAGE FORM BY QbD APPROACH

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For the simultaneous estimation of Bempedoic acid in tablet dosage form, a quick, specific, precise and accurate method has been developed for RP-HPLC. The JASCO UV spectrophotometer achieved the Spectra scan and quartz quvets were used for sample analysis. By taking too much, the detection wavelength of Bempedoic acid was found to be 212nm. Phenomenex C18, 250 mm X 4.6mm ID, 5 µm column with the mobile phase Acetonitrile: 0.05% OPA in water (60:40) was achieved in the RP-HPLC process separation. 1ml/min is the flow rate. For Bempedoic acid, the retention time was found to be 4.18 min respectively A good linear response in the range of 50-150µg/ml was obtained for each. Bempedoic acid recoveries were found in the range of 99.41% respectively. The LOD (Limit of Detection) of Bempedoic acid was found to be 8.914 µg/ml and 27.012 µg/ml respectively. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory. According to ICH guidelines, the proposed method was validated using various parameters such as linearity, precision, accuracy, LOD and LOQ, selectivity range, roughness and roughness as per ICH guidelines, and successfully applied in tablet dosage form to the estimation of Bempedoic acid.

Keywords:- Bempedoic acid, RP-HPLC, Simultaneous estimation, UV, Validation

Introduction

Bempedoic acid acts by inhibiting adenosine triphosphate-citrate lyase (ACL) and consequently cholesterol biosynthesis, leading to increased expression of LDL receptors and increasing low-density lipoproteins (LDL-C) plasma clearence. It is a prodrug for oral administration with intracellular activation.The literature review says that various analytical method reported for Dapagliflozin in UV-spectrometry,HPLC,RP-HPLC individually and common. This work shows that the Development and validation of RP-HPLC method for simultaneous estimation of Dapagliflozin in tablet dosage form.

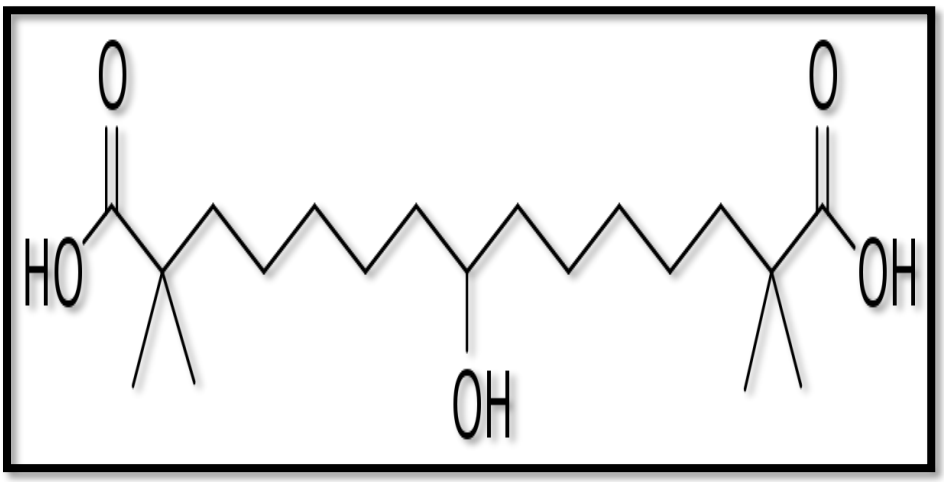

Fig.1: Structure of Bempdoic acid

MATERIALS AND METHOD

Materials: Bempdoic acid pure API, tablet of drugs(B),Distilled water, Phosphate buffer, Methanol, ortho phosphoric acid, Acetonitrile.

Instruments Electronic balance, pH meter, Ultrasonicator, HPLC system, Uv-VIS spectrophotometer.

Methods

Diluent: Based upon the solubility the diluents was selected. Acetonitrile: 0.05% OPA in water (60:40)

Preparation of Std. stock solution

Bempedoic acid Standard stock solution was prepared by dissolving 20 mg Bempedoic acid into a 20 mL clean and dried volumetric flask, added about 14 mL of methanol to dissolve it completely and made volume up to the mark with methanol (1000 PPM).

Preparation of Sample stock solutions Weighed 20 tablets transferred in mortar pestle and crushed to fine powder. Mixed the contents with butter paper uniformly. Weighed the powder material equivalent to 100 mg of Bempedoic acid and transferred to clean and dried 50 mL of volumetric flask. Added 35 mL of Metahnol, sonicated for 10 minutes with intermittent shaking. After 10 minutes allow to cool the solution to room temperature and made volume up to the mark with Methanol. Filtered the solution through suitable 0.45 µ syringe filter discarding 3-5 mL of initial filtrate. Further diluted 5.0 ml of filtered stock solution to 10 ml with methanol. (1000 mcg of Bempedoic acid), injected the resultant solution and chromatograms were recorded and results are recorded.

Determination of Analytical wavelength: Methanol as a blank and Bempedoic acid standard solution having concentration 20 PPM & 400 PPM were scanned from 400 nm to 200 nm. Absorption maxima were determined for drug. 212 nm wavelength used for further analysis.

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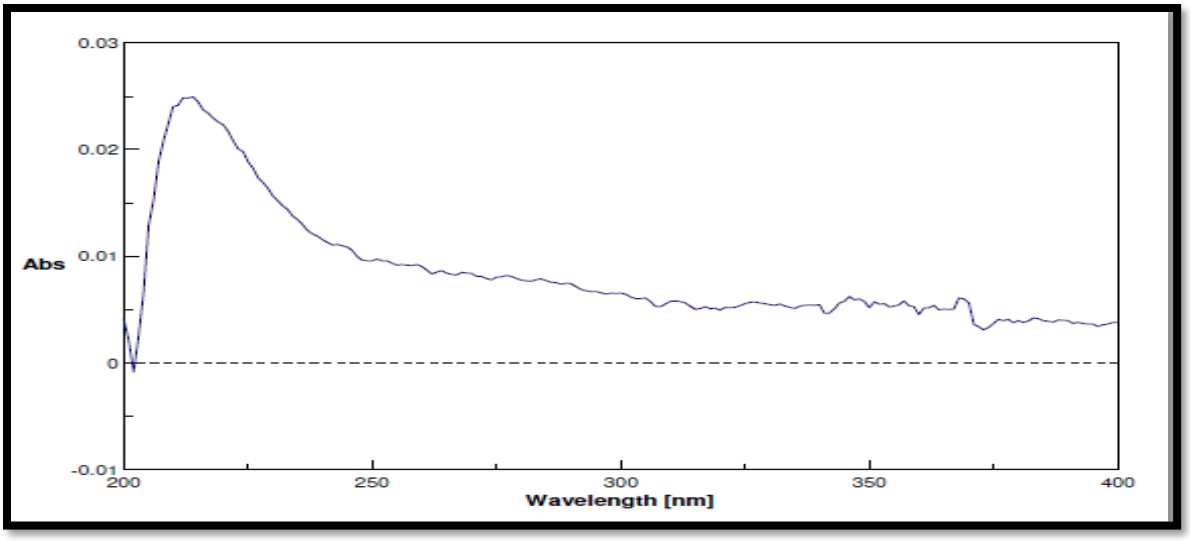


Fig. 3 Wavelength spectra of Bempdoic acid

RESULTS AND DISCUSSION

Bempdoic acid having good resolution, plate count and tailing factor, asymmetry, so that's why this method was optimized and validated. All the system suitability parameters was within the range as per ICH guidelines.

Linearity Weighed 50 mg of Dapagliflozin and dissolved in 50 mL with Methanol. Further diluted 5 ml of stock solution to 50 mL with Methanol (100µg/mL). The correlation coefficient of both drugs was 0.999 for both drugs.

Precision

Precision is called as the closeness of agreement between a series of measurements obtain from multiple samples of the same sample. The known concentrations of Dapagliflozin and Saxagliptin has been analysed into HPLC column n the same day.The precision determine by injecting the samples of same concentrations on 2 different days. The peak

area all conc were taken and Standard deviations, % Relative standard deviation was calculated.

Accuracy: The accuracy of the analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value of the value found, Accuracy will be conducted in the range from 50 % to 150 % of working concentration. Solution of each accuracy level was prepared in triplicate. Calculated % Recovery for each sample, Mean % recovery for each level and overall recovery and also calculated % RSD for each level and % RSD for overall recovery.

LOD (Limit of detection): LOD (Limit of detection) and LOQ (Limit of Quantitation) of Dapagliflozin determine by calibration curve method. Dilutions were prepared in linearity range. The average area was plotted against concentrations and they are calculated by following formula.

Robustness: The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Determination: Blank and Standard solution were injected under different chromatographic conditions as shown below.

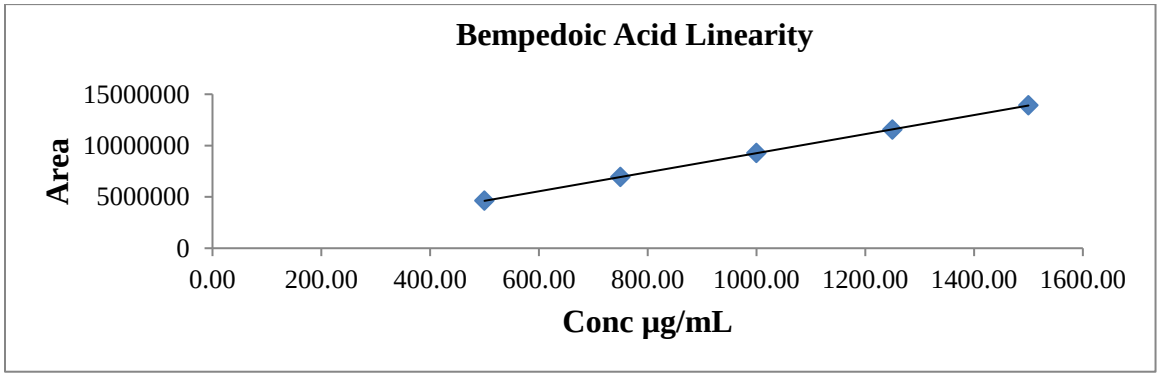
a). Changes in flow rate by ±10%. (± 0.1ml/min)
b). Change in column oven temperature. (± 2ºC)
c) Change in wavelength (± 3 nm)
the analytical robustness. The changes in flow rate and wavelength**.**

Linearity: The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. 5 levels of Linearity was performed from 50% to 150% of working concentration

Table 1: Linearity data of Bempedoic acid

Level	Conc (µg/mL)	Area	Mean	% RSD
50%	500.00	4626060	4619581	0.177
		4610368		
		4622314		
75%	750.00	6946744	6934849	0.181
		6936014		
		6921789		
100%	1000.00	9276279	9273296	0.109
		9281604		
		9262004		
125%	1250.00	11547439	11538081	0.244
		11506479		
		11560324		
150%	1500.00	13960140	13924315	0.225
		13910346		
		13902460		

Fig 4.Calibration curve for Bempedoic acid



Precisions:

The precision of the Bempedoic acid was found to be within the limits (RSD<2)

Repeatability (Intra-day)	**Sample**	**Test Sample (mg)**	**Area**	**% Assay**
	Sample 1	249.6	9240145	99.71
	Sample 2	249.2	9268491	100.18
	Sample 3	248.9	9200145	99.56
	Sample 4	249.3	9125840	98.60
	Sample 5	249.1	9236014	99.87
	Sample 6	249.5	9166581	98.96
	Mean			**99.48**
	STD DEV			**0.590965**
	% RSD			**0.594**
Intermediate precision (Inter-Day)	Sample 1	248.9	9138931	98.90
	Sample 2	249.3	9247801	99.92
	Sample 3	249.1	9141039	98.84
	Sample 4	249.5	9295813	100.36
	Sample 5	248.8	9100463	98.52
	Sample 6	249.4	9247046	99.87
	Mean			**99.40**
	STD DEV			**0.741739**
	% RSD			**0.746**
Repeatability Plus Inter-day	**Mean**			**99.441**
	STD DEV			**0.64070**
	% RSD			**0.644**

Table no: 3 Result of Intra- day and Inter- Day Precision for Bempedoic acid test sample

Accuracy: Three levels 50%, 100%, 150% for accuracy shown in below table and it done by standard addition method in which known amount of standard drug was added into blank placebo and standard solutions of Dapagliflozin respectively. The average accuracy and % RSD was within the limit.

Table No.7 Result and statistical data of Accuracy of Bempdoic acid

Level (%)	Area	Recovered conc (µg/mL)	Added conc (µg/mL)	% Recovery	Mean Recovery	% RSD
50	4602581	497.88	502.00	99.18	99.47	0.9595
	4562103	493.50	500.00	98.70		
	4656310	503.70	501.00	100.54		
100	9286014	1004.51	1001.00	100.35	99.70	0.7189
	9235608	999.06	1001.00	99.81		
	9145600	989.32	1000.00	98.93		
150	13792018	1491.95	1501.00	99.40	99.05	0.5975
	13658091	1477.46	1502.00	98.37		
	13800495	1492.87	1502.00	99.39		

Overall Recovery: 99.45 %

%RSDforOverallRecovery:0.77

Limit of Detection (LOD) and Limit of Quantitation (LOQ):

σ = 25081.21701 (Residual standard deviation of a regression line)

s = 9285.08 (Slope)

Detection limit (LOD):

LOD = 3.3 σ / S

LOD = 3.3 x 25081.21701 / 9285.08

LOD = 8.914 µg/mL

Quantitation limit (LOQ):

LOQ = 10 σ / S

LOQ = 10 x 25081.21701 / 9285.08

LOQ=27.012µg/mL

Robustness:- The parameters like change in flow rate, change in wavelength was maintained.System suitability parameters were not much affected and all parameters passed.%RSDwerewithinthelimiT

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Following changes made under Robustness:

- Change in Wavelength
- Change in flow rate
- Change in column oven temperature

Table No.9 Result of Robustness study:

Change in Parameter	**R.T.**	**Standard area**	**Asymmetry**	**Theoretical plates**
Wavelength by +3 NM (215 NM)	4.95	9254602	0.99	10824
Wavelength by -3 NM (209 NM)	4.95	8782333	0.98	10950

Flow rate by +10% (1.32 mL/min)	4.57	8562516	0.99	10473
Flow rate by -10% (1.08 mL/min)	5.39	10154938	0.97	11217
Column oven temp by +2ºC (40 ºC)	4.92	9276035	1.00	11126
Column oven temp by -2ºC (36 ºC)	4.97	9246813	0.99	10936

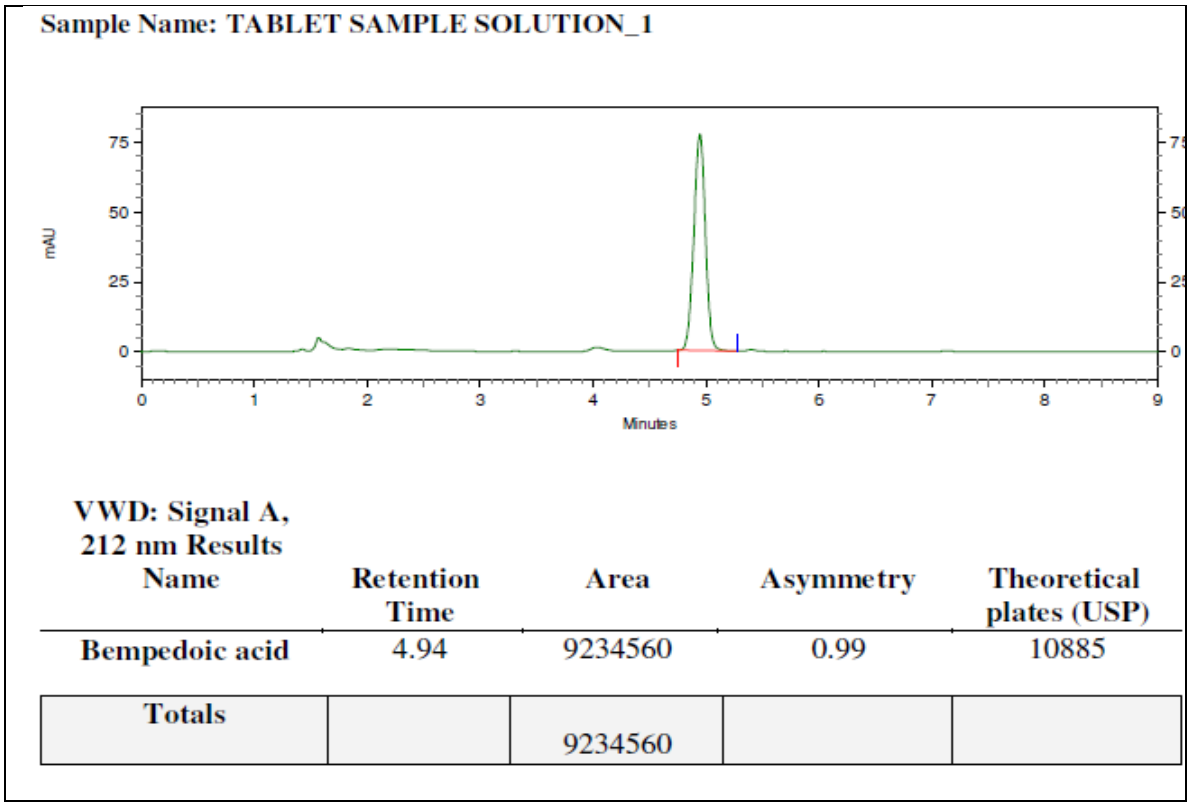
Assay a) Bempesta 180 mg Tablet:

Weight of 20 tablets = 8.9640 gm

Average weight of tablet = 8.9640 / 20 = 0.4482 gm = 448.2 mg

Table no 13:Assay results of Bempesta 180 mg Tablet:

Sample	**Area**	**% Assay**	**Mean Assay**
Sample 1	9234560	99.77	100.02
Sample 2	9276092	100.26	



VWD: Signal A, 212 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Bempedoic acid	4.94	9234560	0.99	10885
Totals		9234560		

Fig. No. 45 Typical chromatogram Bempesta 180 mg Tablet sampleFig.6 Chromatogram of

Conclusion

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 212nm and the peak purity was excellent. Injection volume was selected to be 20µl which gave a good peak area. The column used for study was (250 mm X 4.6 mm i.d.) 5µm chosen good peak shape. Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time. Different pH and ratios of mobile phase were studied, mobile phase with ratio of Acetonitrile: 0.05% OPA in water (60:40) was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study. Methanol was selected because of maximum extraction sonocation time was fixed to be 10min at which all the drug particles were completely soluble and showed good recovery. The present recovery was found to be 99.41% was linear and precise over the same range. Both system and method precision

was found to be accurate and well within range. Detection limit was found to be 8.914 µg/Ml and limit of quantitiation was found to be 27.012 µg/mL. Linearity study was, correlation coefficient and curve fitting was found to be. The analytical method was found linearity over the range of 50-150ppm of the target concentration. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

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