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Validated Method for the Simultaneous Estimation of Bempedoic Acid and Ezetimibe in Bulk and Tablet Formulation by RP-HPLC Method

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Abstract

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Bempedoic Acid and Ezetimibe, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Phenomenex Gemini C18 (4.6 x 150mm, 5µm) column using a mixture of Methanol: Water (25:75% v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 240 nm. The retention time of the Bempedoic Acid and Ezetimibe was 2.256, 5.427 ±0.02min respectively. The method produce linear responses in the concentration range of 5-25mg/ml of Bempedoic Acid and 25-125mg/ml of Ezetimibe. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: Bempedoic Acid and Ezetimibe, RP-HPLC, validation

A R T I C L E I N F O

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Contents:

1. Introduction

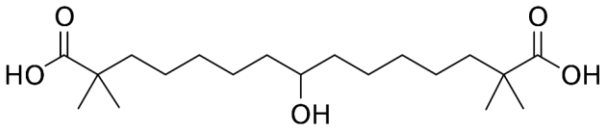

Fig.1. Bempedoic Acid

IUPAC Name:
8-Hydroxy-2,2,14,14-tetramethylpentadecanedioic acid
Molecular Formula : $C_{19}H_{36}O_5$
Molecular Weight : 344.492 g·mol^{-1}

Physicochemical properties:
Description(Physical State): Solid
Solubility: highly soluble in water: 0.0211 mg/mL
Melting point : 87-92°C
pKa (strongest acidic) : 4.44
Log P: 3.65

Pharmacokinetic properties:
Bioavailability : Bempedoic acid is rapidly absorbed in the small intestine.
Half-life : 21±11 hrs
Absorption : Bempedoic acid is rapidly absorbed in the small intestine.1,2 The Tmax of the 180mg tablet is estimated at 3.5 hours.
Volume of Distribution : 18L
Protein binding : 99.3%
Metabolism : Glucuronidation
Excretion : 70% urine, 30% feces
Adverse effects/Side effects: muscle spasms

Pharmacodynamics:

Mechanism of action: Normally, LDL cholesterol is produced in the liver and circulates in the blood. When the blood becomes saturated, excess LDL deposits in blood vessels including the coronary arteries, increasing the risk of cardiovascular events. Therapeutic efficacy/ Indications: Bempedoic acid is indicated as an adjunct to diet and maximally tolerated statin therapy for adults with heterozygous familial hypercholesterolemia or existing atherosclerotic cardiovascular disease that warrants additional lowering of LDL-C.

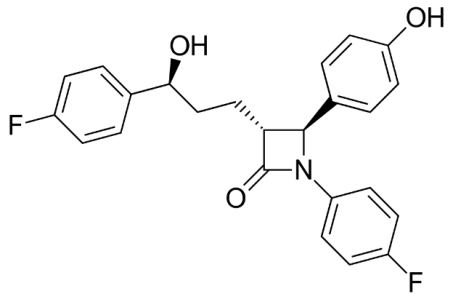

Fig.2: Ezetimibe

IUPAC Name : (3R,4S)-1-(4-fluorophenyl)-3-[(3S)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl)azetidin-2-one

Molecular Formula : $C_{24}H_{21}F_2NO_3$

Molecular Weight : 409.433 g·mol^{-1}

Physicochemical properties:

Description (Physical State): Solid

Solubility: Practically insoluble

Melting point: 163°C

pKa(strongest acidic): 9.48

Log P: 4.14

Pharmacokinetic properties:

Bioavailability : 35% to 65%

Half-life : 19 h to 30 h

Absorption : 3.4-5.5 ng/mL within 4-12 hours

Volume of Distribution : 0.16 L/kg

Protein binding : >90%

Metabolism : Intestinal wall, liver

Excretion : Kidney 11%, fecal 78%

Adverse effects/Side effects: headache and/or diarrhea.

2. Materials and Methods

Table.1: Instruments used

S.N	Instruments	Model
1	HPLC	WATERS Alliance 2695 separation module, software: Empower 2, 996 PDA Detector.
2	pH meter	Lab India
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Labman

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Bempedoic Acid and Ezetimibe working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.1ml of the above Bempedoic Acid and 0.3ml of the Ezetimibe stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization:

Initially the mobile phase tried was Methanol: Water and Water: Acetonitrile and Methanol: Phosphate Buffer: ACN with varying proportions. Finally, the mobile phase was optimized to Acetonitrile: Phosphate Buffer in proportion 45:55 v/v respectively.

Optimization of Column:

The method was performed with various columns like C18 column, Symmetry and Zodiac column. Phenomenex Luna C18 (4.6×250mm, 5µm) particle size was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Optimized chromatographic conditions:

Instrument used : Waters HPLC with auto sampler and PDA Detector 996 model.

Temperature : 35ºC

Column : Phenomenex Luna C18 (4.6×250mm,5µm) particle size

Buffer : Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 4.6 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultra-sonication.

pH : 4.6

Mobile phase : Acetonitrile: Phosphate Buffer (45:55 v/v)

Flow rate : 1ml/min

Wavelength : 245 nm

Injection volume : 10 µl

Run time : 7 min

Validation

Preparation of buffer and mobile phase:

Preparation of potassium dihydrogen phosphate (KH2PO4) Buffer (Ph-4.6): Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 4.6 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultra-sonication.

Preparation of mobile phase:

Accurately measured 450 ml (45%) of Methanol, 550 ml of Phosphate buffer (55%) were mixed and degassed in digital ultrasonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation: The Mobile phase was used as the diluent.

Validation Parameters

System Suitability: Accurately weigh and transfer 10 mg of Bempedoic Acid and 10mg of Ezetimibe working standard into a 10ml of clean dry volumetric flasks add

about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.1ml of the above Bempedoic Acid and 0.3ml of the Ezetimibe stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Procedure: The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Specificity study of drug:

Preparation of Standard Solution:

Accurately weigh and transfer 10mg of Bempedoic Acid and 10mg of Ezetimibe working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)**.** Further pipette 0.1ml of the above Bempedoic Acid and 0.3ml of the Ezetimibe stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of Sample Solution:

Take average weight of one Tablet and crush in a mortor by using pestle and weight 10 mg equivalent weight of Bempedoic Acid and Ezetimibe sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 0.1ml of the above Bempedoic Acid and 0.3ml of the Ezetimibe stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Linearity:

Accurately weigh and transfer 10 mg of Bempedoic Acid and 10mg of Ezetimibe working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

6 ppm of Bempedoic Acid & 18ppm of Ezetimibe:

Pipette out 0.06ml of Bempedoic Acid and 0.18ml of Ezetimibe stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

8 ppm of Bempedoic Acid & 24ppm of Ezetimibe:

Pipette out 0.08ml of Bempedoic Acid and 0.24ml of Ezetimibe stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

10 ppm of Bempedoic Acid & 30ppm of Ezetimibe:

Pipette out 0.1 ml of Bempedoic Acid and 0.3ml of Ezetimibe stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

12 ppm of Bempedoic Acid & 36ppm of Ezetimibe:

Pipette out 0.12 ml of Bempedoic Acid and 0.36ml of Ezetimibe stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

14 ppm of Bempedoic Acid & 42ppm of Ezetimibe:

Pipette out 0.14ml of Bempedoic Acid and 0.42ml of Ezetimibe stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Procedure: Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Precision

Bempedoic Acid and Ezetimibe Product Solution for Precision: Accurately weigh and transfer 10mg of Bempedoic Acid and 10mg of Ezetimibe working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.1ml of the above Bempedoic Acid and 0.3ml of the Ezetimibe stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent. The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Intermediate precision:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure:

DAY 1: The standard solution was injected for Six times and measured the area for all Six injections in HPLC. The %RSD for the area of Six replicate injections was found to be within the specified limits.

DAY 2: The standard solution was injected for Six times and measured the area for all Six injections in HPLC. The %RSD for the area of Six replicate injections was found to be within the specified limits.

Accuracy:

For preparation of 50% Standard stock solution:

Accurately weigh and transfer 10 mg of Bempedoic Acid and 10mg of Ezetimibe working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.05ml of the above Bempedoic Acid and 0.15ml of the Ezetimibe stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

For preparation of 100% Standard stock solution:

Accurately weigh and transfer 10 mg of Bempedoic Acid and 10mg of Ezetimibe working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Bempedoic Acid and 0.3ml of the Ezetimibe stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

For preparation of 150% Standard stock solution:

Accurately weigh and transfer 10 mg of Bempedoic Acid and 10mg of Ezetimibe working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)**.** Further pipette 0.15ml of Bempedoic Acid and 0.45ml of Ezetimibe from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:

Inject the Three replicate injections of individual concentrations (50%,100%,150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Bempedoic Acid and Ezetimibe and calculate the individual recovery and mean recovery values.

Robustness:

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results. .

For preparation of Standard solution:

Accurately weigh and transfer 10 mg of Bempedoic Acid and 10mg of Ezetimibe working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)**.** Further pipette 0.1ml of the above Bempedoic Acid and 0.3ml of the Ezetimibe stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Variation of flow conditions:

The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

Variation of mobile phase organic composition: The sample was analyzed by variation of mobile phase i.e. Acetonitrile: Phosphate Buffer was taken in the ratio and 50:50, 40:60 instead (45:55), remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

3. Results and Discussions

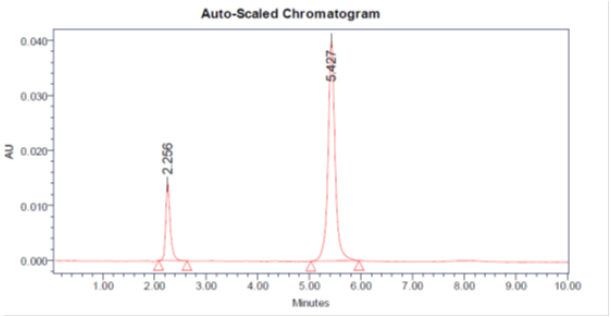

Fig.3: Optimized chromatogram (Standard)

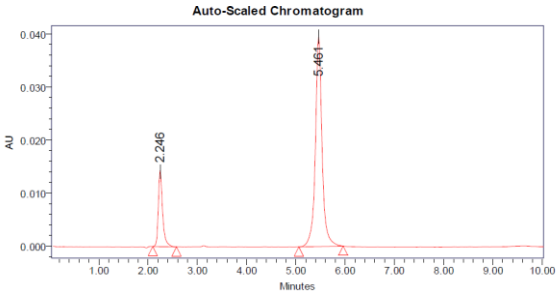

Fig.4: Optimized chromatogram (Sample)

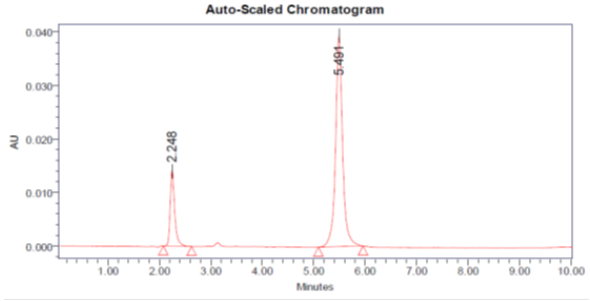

Fig.5: System suitability

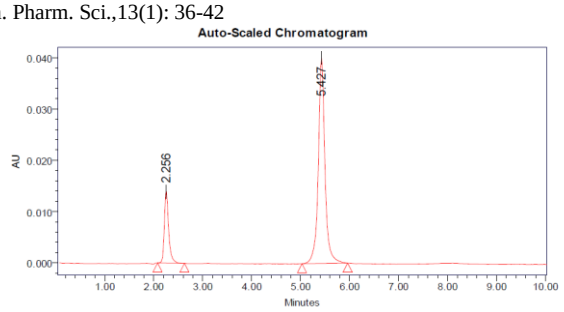

Fig.6: Chromatogram for assay Standard

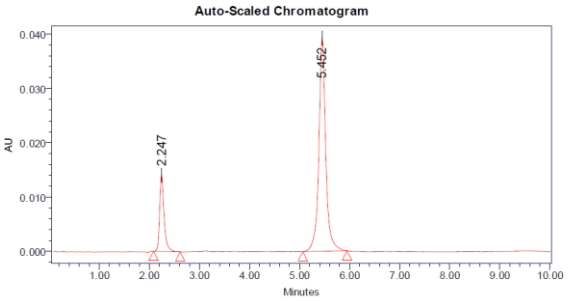

Fig.7: Chromatogram for assay Sample

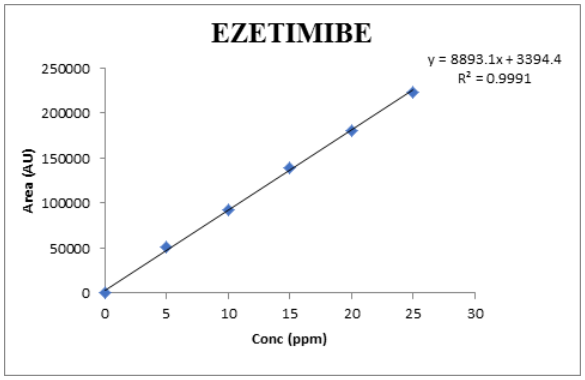

Fig.8: Calibration graph for Ezetimibe

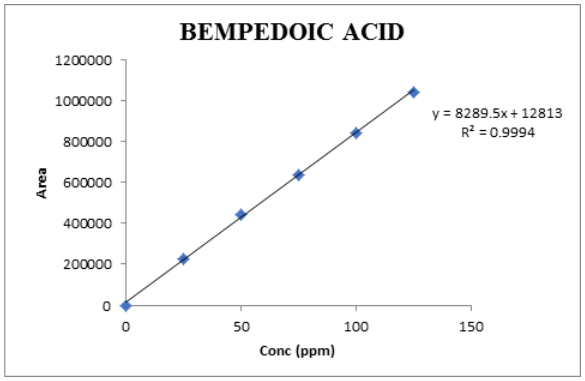

Fig.9: Calibration graph for Bempedoic Acid

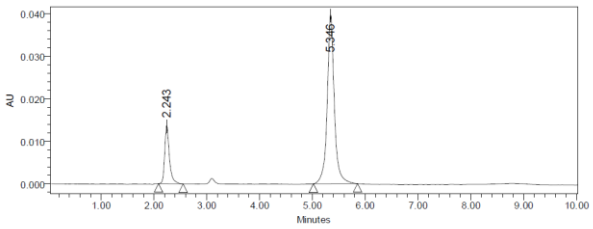

Fig.10: Intermediate precision (Day-1)

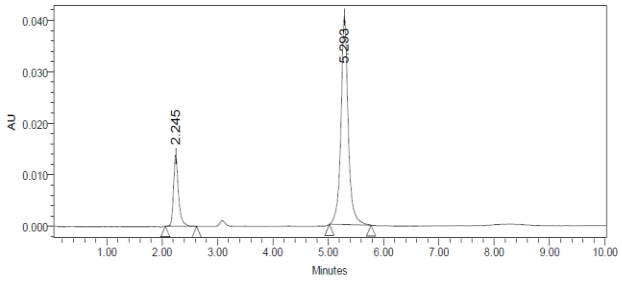

Fig.11: Intermediate precision (Day-2)

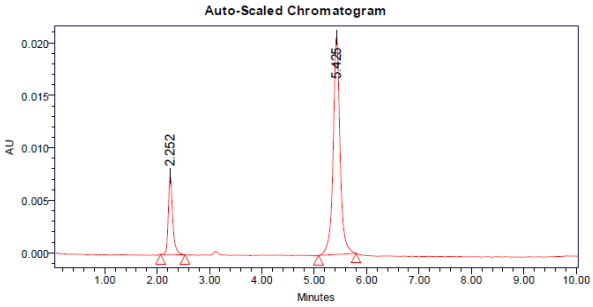

Fig.12: Accuracy-50%

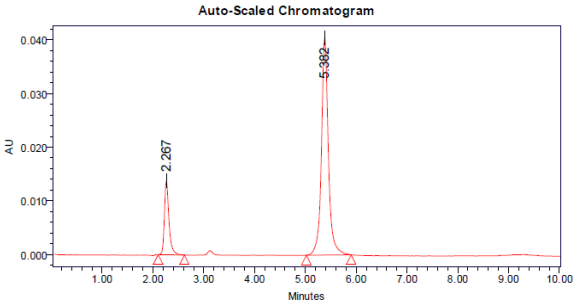

Fig.13: Accuracy-100%

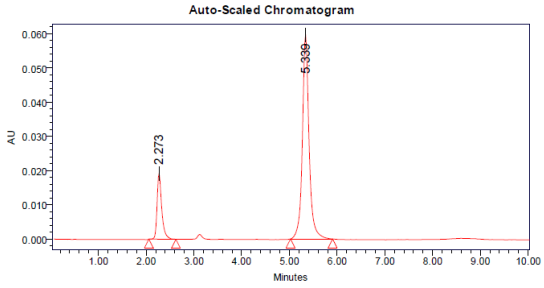

Fig.14: Accuracy-150%

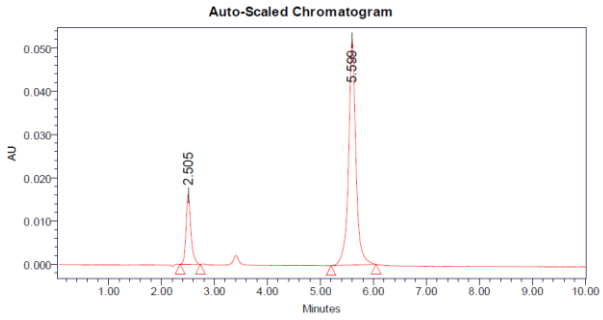

Fig.15: Robustness (less organic composition)

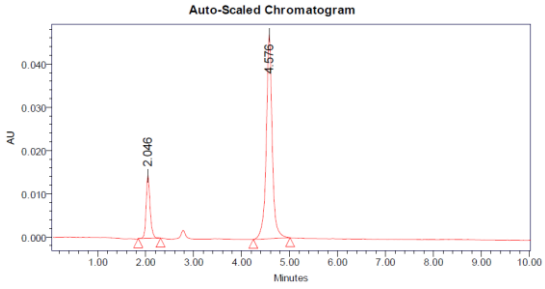

Fig.16: Robustness (more organic composition)

Table.2: Results of system suitability for Ezetimibe

S.n	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Ezetimibe	2.247	86093	14052	5507	1.36
2	Ezetimibe	2.246	85627	14026	5675	1.2
3	Ezetimibe	2.248	85558	14133	5299	1.2
4	Ezetimibe	2.252	86142	14307	5033	1.0
5	Ezetimibe	2.248	86558	14153	5811	1.33
Mean			85995.6			
Std. Dev			410.662			
% RSD			0.477538			

Table.3: Results of system suitability for Bempedoic Acid

S n	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Bempedoic Acid	5.452	376066	39374	9147	1.04	15.0
2	Bempedoic Acid	5.484	373326	39428	9025	1.5	15.5
3	Bempedoic Acid	5.491	373434	39404	9166	1.2	15.3
4	Bempedoic Acid	5.482	375114	39746	9077	1.1	15.1
5	Bempedoic Acid	5.491	373436	39404	9328	1.2	15.2
Mean			374275.2				
Std. Dev			1247.338				
% RSD			0.333268				

Table.4: Results of Intermediate precision for Ezetimibe

S.N	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Ezetimibe	2.248	84028	13604	3518.3	1.4
2	Ezetimibe	2.245	84203	13521	3373.9	1.4
3	Ezetimibe	2.242	84746	13637	3412.8	1.4
4	Ezetimibe	2.239	85443	13776	3324.5	1.3
5	Ezetimibe	2.243	85536	13769	3434.4	1.4
6	Ezetimibe	2.246	85698	13738	3337.9	1.3
Mean			84942			
Std. Dev			720.3716			
% RSD			0.8			

Table.5: Results of Intermediate precision for Bempedoic Acid

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Bempedoic Acid	5.284	366832	40103	9181.2	1.1	15.8
2	Bempedoic Acid	5.293	368857	40465	9156.6	1.1	15.5
3	Bempedoic Acid	5.306	370175	39978	9038.6	1.0	15.5
4	Bempedoic Acid	5.319	370604	40749	9118.3	1.1	15.8
5	Bempedoic Acid	5.346	372579	39773	9184.9	1.1	15.6
6	Bempedoic Acid	5.352	376551	40084	9008.1	1.1	15.9
Mean			370933				
Std. Dev			3349.08				
% RSD			0.9				

Table.6: The accuracy results for Ezetimibe

%Concentration	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	69863.33	7.6	7.48	99.7	98.9%
100%	135468.7	16	14.9	98.7	
150%	199977	22.6	22.2	98.3	

Table.7: The accuracy results for Bempedoic Acid

%Concentration	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	322955	37.6	38.4	98.7	99.8%
100%	632156	76	75.7	99.7	
150%	945871.3	113.5	113.5	101	

4. Conclusion

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Bempedoic Acid and Ezetimibe in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Bempedoic Acid and Ezetimibe was freely soluble in ethanol, methanol and sparingly soluble in water. Methanol: Water (25:75% v/v) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the spectrophotometric methods. This method can be used for the routine determination of Bempedoic Acid and Ezetimibe in bulk drug and in Pharmaceutical dosage forms.

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