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Development and Validation of a RP - HPLC Method for the Simultaneous Determination of Bempedoic Acid & Ezetimibe in Pure and Pharmaceutical Dosage Form

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Abstract

A straightforward, Accurate, specific approach was created for the concurrent assessment of the Ezetimibe and Bempedoic Acid in pharmaceutical dimension structure. Chromatogram was experienced Inertial ODS C185 m (4.6 x 250mm). Portable phase having Phosphate support and also Acetonitrile in the percentage of 30:70 was siphoned through segment at a stream pace of 1ml/min. Cradle made use of at pH 4.6. Temperature was stayed on top of at Ambient. Boosted regularity for Ezetimibe as well as Bempedoic Acid was 260nm. Maintenance period of Ezetimibe and Bempedoic Acid were considered as 2.395 minutes and also 3.906 min. The percentage merit of Ezetimibe and Bempedoic Acid was considered as 100.6 percentage and also 101.3 percentage independently. The framework appropriateness boundaries for Ezetimibe and also Bempedoic Acid, for example, theoretical plates and complying with element were deemed 1.3, 1012.4 and also 1.2, 1848.2 the objective was viewed as 9.0. The linearity research for Ezetimibe and also Bempedoic Acid was discovered in focus scope of 1µg-5µg as well as 100µg-500µg and also link coefficient (r2) was considered as 0.999 and also 0.999, percentage mean recuperation was considered as 100.1 percentage and 100.4 percentage, percentage RSD for repeatability was0.31 and 0.38, percentage RSD for middle of the road accuracy was 0.12 and 0.15 separately the accuracy study was precise, powerful and repeatable. LOD esteem was 2.94 as well as 3.03, and also LOQ esteem was 9.87 and 10.1 individually.

Keywords: Ezetimibe, Bempedoic Acid, *RP*-HPLC

INTRODUCTION:

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.The combination of bempedoic and ezetimibe is also indicated with diet management and maximally tolerated statin therapy to treat elevated LDL-C levels in adults with heterozygous familial hypercholesterolemia or existing atherosclerotic cardiovascular disease who require further lowering of LDL-C [1-2]. IUPAC name is 8-hydroxy-2,2,14,14-tetramethylpentadecanedioic acid. Molecular Formula is $C_{19}H_{36}O_5$. Molecular weight is 344.4.

Bempedoic acid is sparingly soluble in aqueous buffers. For maximum solubility in aqueous buffers, Bempedoic acid should first be dissolved in DMSO and then diluted with the aqueous buffer of choice. Bempedoic acid has a solubility of approximately 0.25 mg/ml in a 1:3 solution of DMSO: PBS (pH 7.2) using this method.

Ezetimibe is indicated to reduce elevated total-C, LDL-C, Apo B, and non-HDL-C in patients with primary hyperlipidemia, alone or in combination with an HMG-CoA reductase inhibitor (statin). It is also indicated to reduce elevated total-C, LDL-C, Apo B, and non-HDL-C in patients with mixed hyperlipidemia in combination with fenofibrate, and to reduce elevated total-C and LDL-C in patients with homozygous familial hypercholesterolemia (HoFH), in combination with atorvastatin or simvastatin [3]. IUPAC name is (3R,4S)-1-(4-fluorophenyl)-3-[(3S)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4 hydroxyphenyl)azetidin-2-one. Molecular Formula is $C_{24}H_{21}F_2NO_3$. Molecular weight is 409.4. It dissolves very well in all kinds of organic solvents, e.g., ethanol, DMSO, DMF, but it is practically insoluble in water.

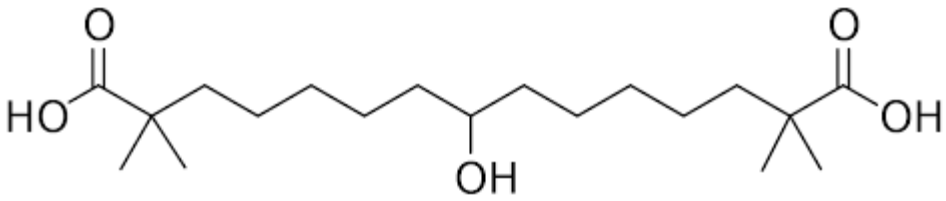

Figure 1: Structure of Bempedoic acid

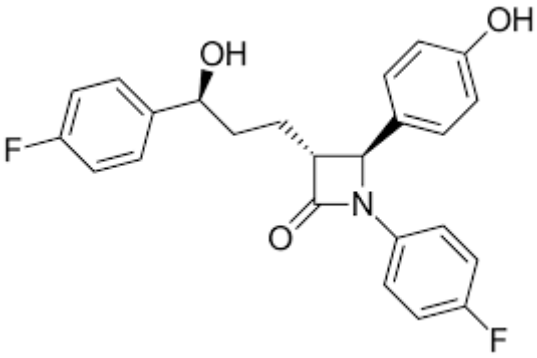

Figure 2: Structure of Ezetimibe

A literature survey conveyed that, limited methods are available for simultaneous estimation of Bempedoic acid and Ezetimibe. A few articles reported spectrophotometric techniques for estimation of Ezetimibe alone and with other drugs [4,5,6,7,8,9,10,11,12,13,14,15]. Few HPLC methods were reported for the determination of Ezetimibe alone and in combination with other drugs [16,17,18,19,20]. One RP-HPLC method was reported for simultaneous estimation of Bempedoic acid Ezetimibe [21]. Few LC–MS methods were reported for determination of Ezetimibe alone and in combination with other drugs [22,23,24,25]. One LC–MS method was reported for estimation of Bempedoic acid in human plasma and urine [26]. In view of the demand for an appropriate, cost-effective RP-HPLC method for routine analysis of Bempedoic acid and Ezetimibe synchronized evaluation of in pharmaceutical dose

type. Attempts were made to establish easy, precise, accurate as well as cost-efficient logical method for the estimate of Bempedoic acid and Ezetimibe. The recommended approach will be validated according to ICH guidelines. The objective of the recommended work is to establish a brand-new, simple, delicate, exact and economical logical method as well as recognition for the Synchronized evaluation of Bempedoic acid and Ezetimibe in pharmaceutical dose kind by utilizing RP-HPLC. To verify the established method based on ICH standards for the desired analytical application.

MATERIALS AND METHODS:

Chemicals and Reagents: Bempedoic Acid and Ezetimibe were Purchased from market. NaH_2PO_4 was analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol for HPLC (Lichrosolv (Merck).

Equipment and Chromatographic Conditions: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software. Analysis was carried out at 235 nm with column Inertsil ODS C185µm (4.6 x 250mm), dimensions at 25^0C temperature. The optimized mobile phase consists of Phosphate buffer and Acetonitril in the ratio of 30:70. Flow rate was maintained at 1 ml/min.

Preparation of solutions:

Preparation of buffer:

Weighed 6.8 grams of KH_2PO_4 was taken into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water, adjusted the pH to 4.6 with ortho phosphoric acid.

Preparation of mobile phase:

A mixture of pH 4.6 Phosphate buffer 300 mL (30%), 700 mL of ACN (70%) are taken and degassed in ultrasonic water bath for 5 minutes. Then this solution is filtered through 0.45 µ filter under vacuum filtration.

The diluents:

The Mobile phase was used as the diluent.

Preparation of the individual Ezetimibe standard preparation:

100 mg of working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask and about 2 ml of DMF is added. Then it is sonicated to dissolve it completely and made volume upto the mark with the diluant. (Stock solution). Further 10.0 ml from the above stock solution is pipette into a 100 ml volumetric flask and was diluted upto the mark with diluant.

Preparation of the individual Bempedoic Acid standard preparation:

15 mg of Bempedoic Acid working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2ml of DMF is added. Then it is sonicated to dissolve it completely and made volume upto the mark with the diluant. (Stock solution). Further 10.0 ml from the above stock solution is pipette into a 100 ml volumetric flask and was diluted upto the mark with diluant.

Preparation of Sample Solution:
Accurately 10 tablets are weighed and crushed in mortar and pestle and weight equivalent to 100 mg of Ezetimibe and 15 mg of Bempedoic Acid (marketed formulation) sample into a 10mL clean dry volumetric flask and about 7mL of Diluents is added and sonicated to dissolve it completely and made volume upto the mark with the same solvent. (Stock solution) Further 3 ml of above stock solution was pipetted into a 10 ml volumetric flask and diluted upto the mark with diluant.

Procedure:
20µL of the standard, sample are injected into the chromatographic system and the areas for Ezetimibe and Bempedoic Acid peaks are measured and the %Assay are calculated by using the formulae.

METHOD:
The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.0 ml/min for 12 minutes to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 20 µL of standard into Inertsil ODS C185µm (4.6 x 250mm), the mobile phase of composition Sodium Phospahte buffer 3.5 pH and Acetonitrile (30:70) was allowed to flow through the column at a flow rate of 1.0 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Bempedoic Acid and Ezetimibe in their tablet dosage form. The result obtained for was comparable with the corresponding labeled amounts and they were shown in Table-2.

Validation of Analytical method:

Linearity: The linearity study was performed for the concentration of 100ppm to 500ppm and1ppm to 5ppm level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. 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. The resulte are shown in table 3.

Accuracy studies: The accuracy was determined by help of recovery study. The recovery method carried out at three level 50%, 100%, 150%. Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for Bempedoic Acid and Ezetimibe and calculate the individual recovery and mean recovery values. The results are shown in table 4,5.

Precision Studies: precision was caliculated from Coefficient of variance for six replicate injections of the standard. 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. The resulte are shown in table 6,7.

Ruggedness: To evaluate the intermediate precision of the method, Precision was performed on different day. 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. The resulte are shown in table 8,9.

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The flow rate was varied at 0.8 ml/min to 1.2 ml/min. The resulte are shown in table 10,11,12,13

LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The resulte are shown in table 14.

LOD = 3.3σ/S and
LOQ = 10 σ/S, where
σ= Standard deviation of y intercept of regression line,
S = Slope of the calibration curve

RESULTS AND DISCUSSION

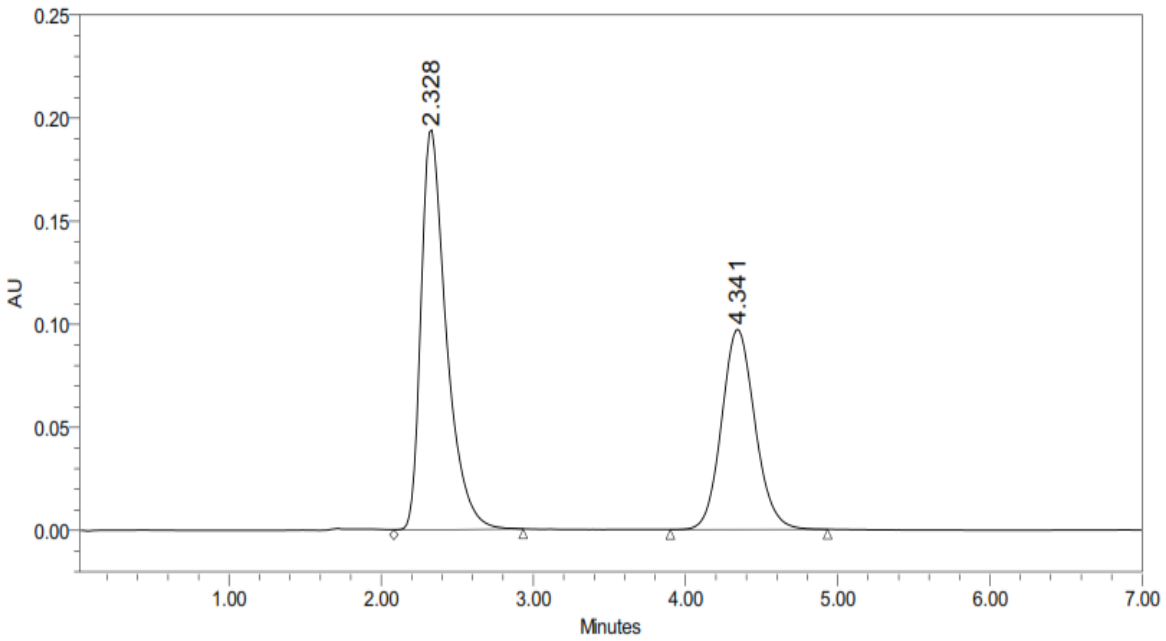

Figure 3: Standard chromatogram

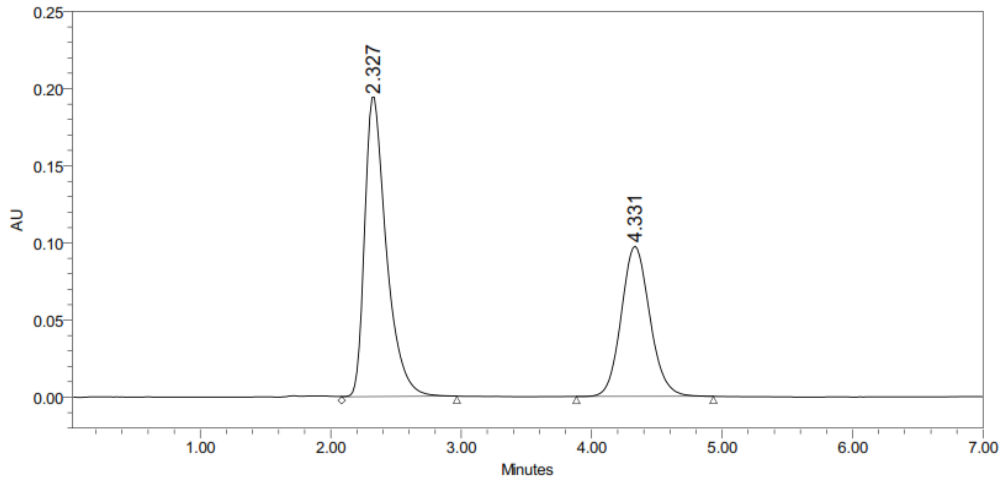

Figure 4: Sample chromatogram

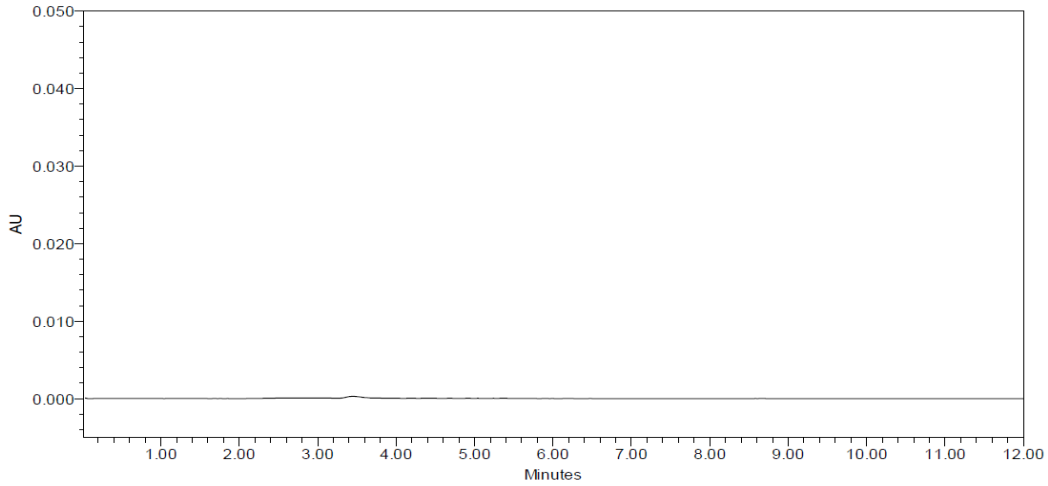

Figure 5: Blank chromatogram

Table 1: System suitability parameters

Parameters	**Bempedoic Acid**	**Ezetimibe**
Retention time	4.34	2.23
USP Plate count	2614	2632
USP Tailing	1.6	1.8

Table 2: Assay results for Bempedoic Acid and Ezetimibe

	Label Claim (mg)	% Assay
Bempedoic Acid	15	101.3
Ezetimibe	100	100.6

Table 3: Linearity results of Ezetimibe and Bempedoic Acid

S.NO	SAMPLE NAME	RT	AREA	HEIGHT	SAMPLE NAME	RT	AREA	HEIGHT
1	Linearty 1	2.309	1812101	145867	Linearty 1	4.304	1163273	74586
2	Linearty 2	2.322	2044373	176895	Linearty 2	4.323	1345955	87689
3	Linearty 3	2.324	2366122	206674	Linearty 3	4.214	1556574	101999
4	Linearty 4	2.336	2611248	228475	Linearty 4	4.524	1776565	117084
5	Linearty 5	2.340	2869662	259345	Linearty 5	4.218	1957821	129409

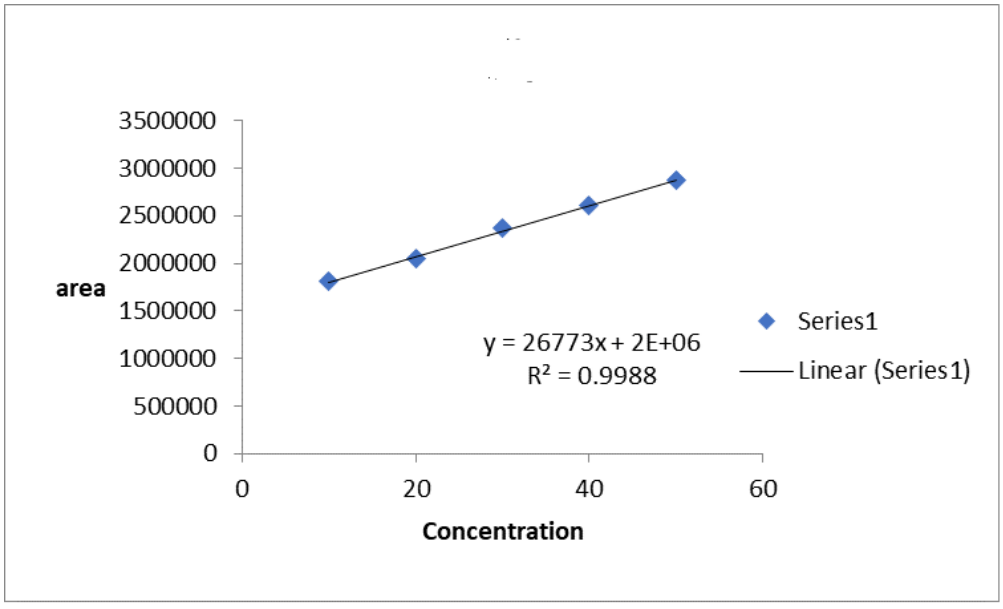

Figure 6: Linearity graph for Ezetimibe

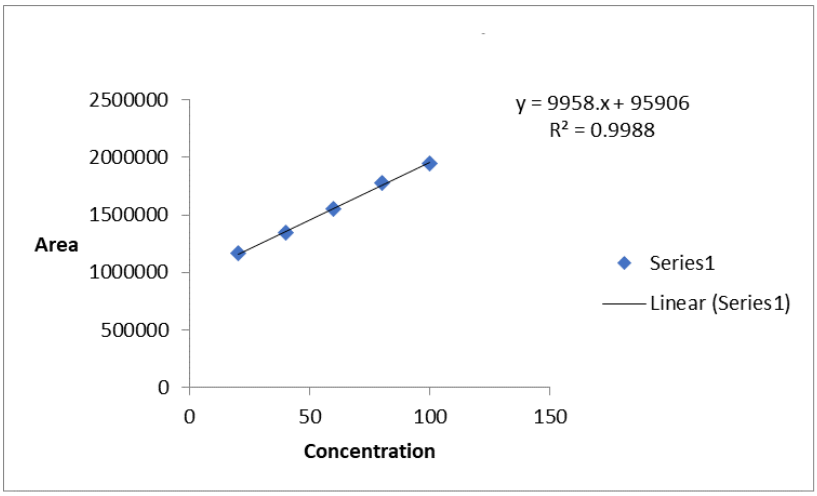

Figure 7: Linearity graph for Bempedoic Acid

Table 4: Showing accuracy results for Bempedoic Acid

%Concentration (at specification Level)	Area	Amount added(mg)	Amount found(mg)	% Recovey	Mean Recovery
50%	2331544	7.5	7.60	101.8%	100.4%
100%	3134597	15	14.8	99.9%	
150%	3917897	20	19.4	99.1%	

Table 5: Showing accuracy results for Ezetimibe

%Concentration (at specification level)	Area Area	Amount Added(mg)	Amount Found(mg)	% Recovey % Recovery	Mean Recovery
50%	353757	50	50.8	101.3%	100.1%
100%	4734988	100	99.4	99.4%	
150%	5911698	150	148.9	99.2%	

Table 6: Precision results for Bempedoic Acid

S.NO	Name	RT	Area	Height
1	Bempedoic Acid	4.302	1401375	100174
2	Bempedoic Acid	4.305	1401445	100068
3	Bempedoic Acid	4.325	1402315	98415
4	Bempedoic Acid	4.315	1404575	98155
5	Bempedoic Acid	4.312	1408514	98144
Mean			1491354	
Std.dev			5882.5	
%RSD			0.38	

Table 7: Precision results for Ezetimibe

S.NO	Name	RT	Area	Height
1	Ezetimibe	2.320	2267519	196958
2	Ezetimibe	2.341	2208588	197584
3	Ezetimibe	2.356	2275569	195874
4	Ezetimibe	2.344	2258841	194583
5	Ezetimibe	2.325	2257967	194587
Mean			2254401	
Std.dev			6535.5	
%RSD			0.31	

Table 8. Ruggedness results of Bempedoic Acid

S.NO	Name	RT	Area	Height
1	Bempedoic Acid	4.302	1401375	95613
2	Bempedoic Acid	4.305	1401442	95142
3	Bempedoic Acid	4.325	1402312	95158
4	Bempedoic Acid	4.315	1404673	95153
5	Bempedoic Acid	4.312	1408512	95143
Mean			1455158	
Std.dev			2344.5	
%RSD			0.15	

Table 9. Ruggedness results of Ezetimibe

S.NO	Name	RT	Area	Height
1	Ezetimibe	2.325	2165319	186958
2	Ezetimibe	2.315	2104788	187584
3	Ezetimibe	2.356	2147469	185874
4	Ezetimibe	2.325	2158641	184583
5	Ezetimibe	2.331	218957	184587
Mean			219556	
Std.dev			2559	
%RSD			0.12	

Robustness results

Table 10: Flow variation results for Bempedoic Acid

S.No	Flow Rate(ml/min)	System suitability results	
		USP Plate count	USP Tailing
1	0.8	1778.5	1.23
2	1.0	1547.2	1.2
3	1.2	1938.0	1.2

Table 11: Flow variation results for Ezetimibe

S.No	Flow Rate(ml/min)	System suitability results	
		USP Plate count	USP Tailing
1	0.8	882.3	1.56
2	1.0	1244.0	1.1
3	1.2	968.2	1.6

Table 12: System suitability results for Bempedoic Acid (Mobile phase)

S.No	Changein Organic Composition in the Mobile Phase	System suitability results	
		USP Plate count	USP Tailing
1	10% Less	1748.5	1.22
2	Actual	1548.2	1.2
3	10% More	1948.0	1.2

Table 13: System suitability results for Ezetimibe (Mobile phase)

S.No	Changein Organic Composition in the Mobile Phase	System suitability results	
		USP Plate count	USP Tailing
1	10% Less	878.3	1.56
2	Actual	1234.0	1.1
3	10% More	969.2	1.6

Table 14: LOD, LOQ of Bempedoic Acid and Ezetimibe

Drug	LOD	LOQ
Bempedoic Acid	3.03	10.1
Ezetimibe	2.94	9.87

CONCLUSION:

The Developed HPLC method was validated and it was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Bempedoic Acid and Ezetimibe in its pure form and in its pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Ezetimibe and Bempedoic Acid in pure and its pharmaceutical dosage forms.

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