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Research Article

Validated method for the simultaneous estimation of bempedoic acid and ezetimibein bulk and tablet formulation by RP-HPLC method

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Bempedoic Acid and Ezetimibe in Tablet dosage form. Chromatogram was run through Kromosil C18 150 x 4.6 mm, 5µ. Mobile phase containing kh2po4: Acetonitrile taken in the ratio 55:45 was pumped through column at a flow rate of 0.9 ml/min. Temperature was maintained at 30°C. Optimized wavelength selected was 246nm. The separation of Bempedoic Acid and Ezetimibe was done at a Retention time of 2.240 and 2.956 min Respectively. %RSD of the Bempedoic Acid and Ezetimibe were found to be 0.7and 0.4 respectively. %Recovery was obtained as 100.55% and 99.85% for Bempedoic Acid and Ezetimibe respectively. LOD, LOQ values obtained from regression equations of Bempedoic Acid and Ezetimibe were 0.25, 0.75 and 0.01, 0.05 respectively. Regression equation of Bempedoic Acid is y = 17525x + 5630.7, y = 11636x + 418.8 of Ezetimibe. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

Keywords: Method development, Bempedoic Acid, Ezetimibe, RP-HPLC

INTRODUCTION

Bempedoic Acid is first-in-class adenosine triphosphate citrate lyase (ACL) inhibitor used once a day for reducing LDL Cholesterol level in statin-Refractory patients[1,2]. Bempedoic acid was developed by Espersion therapeutics inc.[3] and approved by the FDA on February 21, 2020[4]. A combination product of Bempedoic acid and Ezetimibe was approved on February 26,2020 under the brand name NEXLIZET. The combination product used in the treatment of Hypercholesterolemia. Structurally Bempedoic acid is known as8-hydroxy-2,2,14,14-tetramethylpentadecanedioic acid, Bempedoic acid is a prodrug that requires activation in the liver[5]. The very-long-chain acyl-CoA synthetase-1 (ACSVL1) enzyme is responsible for its activation to ETC-1002-CoA, the pharmacologically active metabolite. ATP lyase (also known as ATP synthase) plays an important part of cholesterol synthesis. ETC-1002-CoA directly inhibits this enzyme after the parent drug is activated in the liver by coenzyme A (CoA)[6,7].

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Ezetimibe is a lipid lowering compound that inhibits intestinal cholesterol and phytosterol absorption[10,11].The discovery and research of this drug began early 1990s, Ezetimibe structure consists of (3R,4S)-1-(4-fluorophenyl)-3-[(3S)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl) azetidin-2-one[12], Ezetimibe is used as an adjunctive therapy to a healthy diet to lower cholesterol levels in primary Hyperlipidemia, mixed Hyperlipidemia, Homozygousfamilial hypercholesterolemia and phytosterolemia. Ezetimibe mediates its blood cholesterol-lowering effect via selectively inhibiting the absorption of cholesterol and phytosterol by the small intestine with out altering the absorption of fat-soluble vitamins and nutrients[13,14,15].

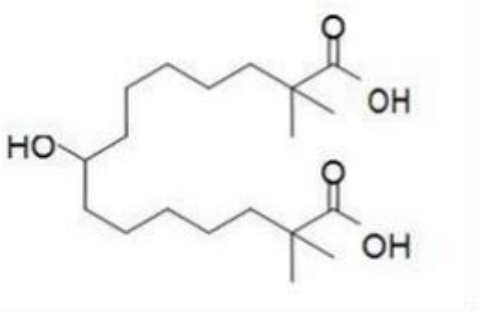

Figure-1: structure of Bempedoic acid

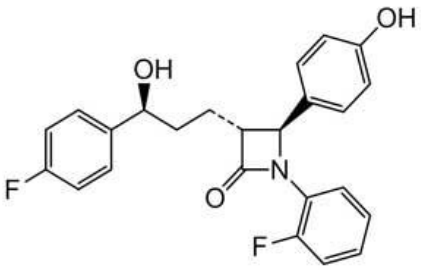

Figure-2: structure of Ezetimibe

Literature survey revealed that there are some methods reported for the simultaneous estimation of Bempedoic acid and ezetimibe, some methods for estimation of individual drugs or with other drugs are UV-Spectrophotometric methods, UPLC and RP-HPLC. The main aim of this study is to develop a simple, precise, accurate relatively sensitive and rapid RP-HPLC technique for estimation of Bempedoic acid and ezetimibe in bulk and tablet formulation.

MATERIALS AND REAGENTS

Chemicals and reagents:Bempedoic acid and Ezetimibe pure drugs (API), combination Bempedoic acid and Ezetimibe NEXLIZET (Ezetimibe 10mg, Bempedoic acid 180mg), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen, ortho phosphate buffer, ortho-phosphoric acid. All the above chemicals and solvents provided by Rankem.

Instruments and chromatographic conditions

Electronics Balance-Denver, p^H meter-BVK enterprises, India, Ultrasonicator-BVK enterprises, WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, photo diode array detector and auto sampler integrated with Empower 2 software, UV-VIS spectrophotometer PG instruments T60 with special bandwidth of 2mm and 10mm and matched quartz cells integrated with UV win 6 software was used for measuring absorbances of Bempedoic acid and Ezetimibe solutions.

Preparation of solutions:

Diluent: Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50

Preparation of Standard stock solutions: Accurately weighed 45 mg of Bempedoic Acid, 2.5 mg of Ezetimibe and transferred to 50ml volumetric flasks and 3/4th of diluents was added to these Flask and sonicated for 10 minutes. Flask Were made up with diluents and labelled as Standard stock solution. 900µg/ml of Bempedoic Acid and 50µg/ml Ezetimibe)

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (90µg/ml of Bempedoic Acid and 5µg/ml of Ezetimibe)

Preparation of Sample stock solutions: 1 vial equivalent to 180 mg Bempedoic Acid &10mg Ezetimibe was transferred into a 100ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (1800µg/ml of Bempedoic Acid and 1000µg/ml of Ezetimibe)

Preparation of Sample working solutions (100% solution): 0.5ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent.(90µg/ml of Bempedoic Acid and 5µg/ml of Ezetimibe).

Preparation of buffer:

Buffer:0.1N Potassium Dihydrogen Ortho phosphate

Accurately weighed 1.36gm of Potassium dihydrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then added 1ml of Triethylamine then PH adjusted to 3.8 with dil. Orthophosphoric acid solution.

METHOD VALIDATION

As per ICH Guidelines the method was validated and the parameters like Linearity, Specificity,

Accuracy, Precision, Limit of detection (LOD) and limit of quantification (LOQ) were assessed.

Specificity: Checking of the interferences in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method.Sothis method was said to be specific.

Linearity: stock solutions of Bempedoic acid and ezetimibe is taken in to 6 different volumetric flasks and diluted to 10ml with diluents. Linearity solutions are prepared such that 0.25, 0.5, 0.75, 1, 1.25, 1.5 m.

Accuracy: Accurately weighed 45 mg of Bempedoic Acid, 2.5 mg of Ezetimibe and transferred to 50ml volumetric flasks and 3/4th of diluents was added to these Flask and sonicated for 10 minutes. Flask Were made up with diluents and labelled as Standard stock solution. 900µg/ml of Bempedoic Acid and 50µg/ml Ezetimibe).

Preparation of 50% Spiked Solution: 0.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution: 1.0ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 150% Spiked Solution: 1.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Robustness: Small deliberatechanges in method like Flow rate, mobile phase ratio, and temperature are made but there Were no recognized change in the result and are within range as per ICH Guide lines. Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus(35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

LOD sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solutions 0.1ml each of Bempedoic Acid, Ezetimibe, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents.

LOQ sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flask and made up with diluent. From the above solutions 0.3ml each of Bempedoic Acid, Ezetimibe, and solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

System suitability parameters: The system suitability parameters were determined by preparing standard solutions of Bempedoic Acid (90ppm) and Ezetimibe (5ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

Assay: NEXLIZET, bearing the label claim Ezetimibe 10mg, Bempedoic Acid 180mg. Assay was performed with the above formulation. Average % Assay for Bempedoic Acid and Ezetimibe obtained was 100.55% and 99.85% respectively.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions:
To develop and establish a suitable RP-HPLC method for estimation Bempedoic acid and Ezetimibe in bulk and tablet dosage forms, different preliminary tests were performed and different chromatographic conditions were tested and optimized chromatographic conditions were developed which are given in Table-1. The final analysis was performed by using 55% Acetonitrile: 45% of 0.01N KH2PO4 at a flow rate of 1ml/min. samples were analysed at 246nm detector wavelength and at an injection volume of 10µl kromosil C18 (4.6 x 150mm, 5µm) with run time of 10min. the proposed method was optimized to give sharp peak with good resolution and the optimized chromatogram was obtained as shown in figure-3

Validation: linearity was established at six linear concentrations of Bempedoic acid (22.5-135µg/ml) and Ezetimibe (1.25-7.5µg/ml) were injected in a duplicate manner. Average Areas were maintained and linearity equations obtained for Bempedoic acid was y =17525x+5630.7 and of Ezetimibe was y=11636x+418.8. correlation coefficient obtained was 0.999 for the both drugs. the linearity calibration curves were plotted as shown in (figure-4&5). Retention time of Bempedoic acid was 2.240min and Ezetimibe was 2.956min, no interfering peaks in blank and placebo were found in this method .so this method holds its specificity. three levels of accuracy samples 50%, 100%, 150%

were prepared by standard addition method. triplicates injections were given %Recovery was obtained as 100.55% and 99.85% of Bempedoic acid and Ezetimibe respectively (Table-3), %RSD for system precision for Bempedoic acid was 0.7% and for Ezetimibe was 0.7%, %RSD for repeatability for Bempedoic acid was 0.7% and for ezetimibe was 0.4%. %RSD for intermediate precision for Bempedoic acid was 0.6% and ezetimibe was 0.7%, since %RSD was less then "2" the system precision was passed in this method shown in (Table-4) the LOD and LOQ values were evaluated based on relative standard deviation. The Detection limit value for Bempedoic acid was 0.25 and for Ezetimibe was 0.01, the quantification limit value for Bempedoic acid was 0.75 and Ezetimibe was 0.05 as given in (Table- 7), Robustness conditions like flow minus (0.8ml/min), flow plus (1ml/min), mobile phase minus (50B:40A), mobile phase plus (50B:50A), temperature minus (25oC) and temperature plus (35^{o}C) was maintained and samples were injected in duplicate manner (Table-6) system suitability parameters were not much affected and all the parameters were passed %RSD was with in limit, Bempedoic acid and Ezetimibe pure drug (API) was obtained from spectrum pharma research solutions ,bearing the label claim Bempedoic acid 180mg Ezetimibe 10mg . assay was performed with above formulations, average %assay for Bempedoic acid and Ezetimibe obtained was 99.98% and 99.59% respectively, the results was shown in (Table- 8) degradation studies were performedwith the formulation and all the samples passed the limits of degradation shown in the (Table-9).

Table-1: Optimized method chromatographic conditions

parameter	**Condition**
RP-HPLC	WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, photo diode array detector and auto sampler integrated with Empower 2 software
Mobile phase	55%Acetonitrile: 45%0.01N kh2po4
Flow rate	1ml/min
column	Kromosil C18 (4.6 x 150mm, 5µm)
Detector wavelength	246nm
Column temperature	30^{o}c
Injection volume	10µL
Run time	10min
Diluents	Water and acetonitrile in the ratio 50:50
Results	Both peaks have good resolution, tailing factor, theoretical plate count and resolution

Table-2: Linearity table for Bempedoic Acid and Ezetimibe.

Bempedoic Acid		**Ezetimibe.**	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	0	0
22.5	393419	1.25	16149
45	805565	2.5	29144
67.5	1188865	3.75	44183
90	1592021	5	57737
112.5	1979110	6.25	72550
135	2360949	7.5	88627

Figure-3: Optimized chromatogram

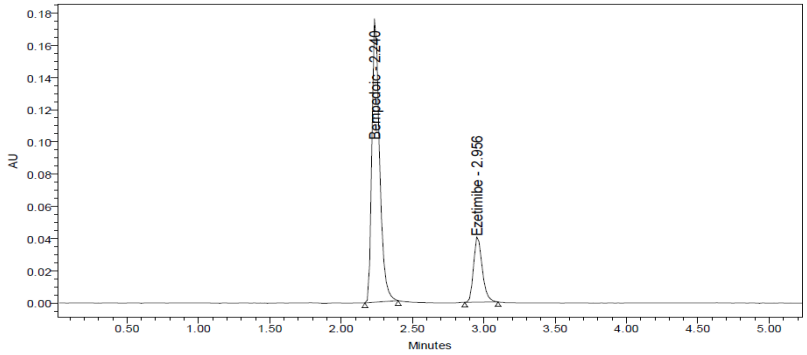

Figure-4: Calibration cure of Bempedoic acid

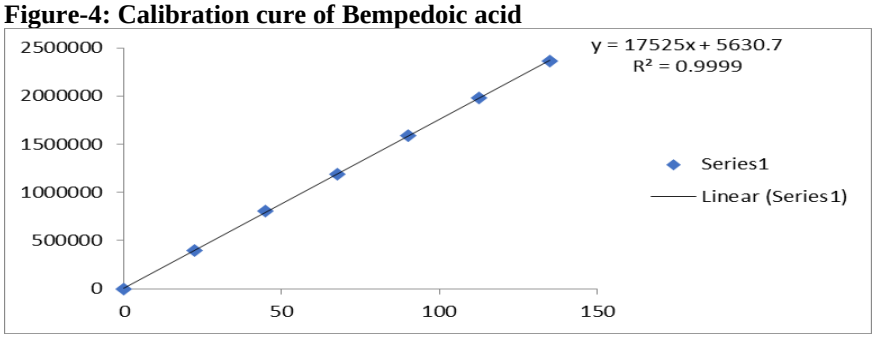

Figure -5: Calibration curve of Ezetimibe

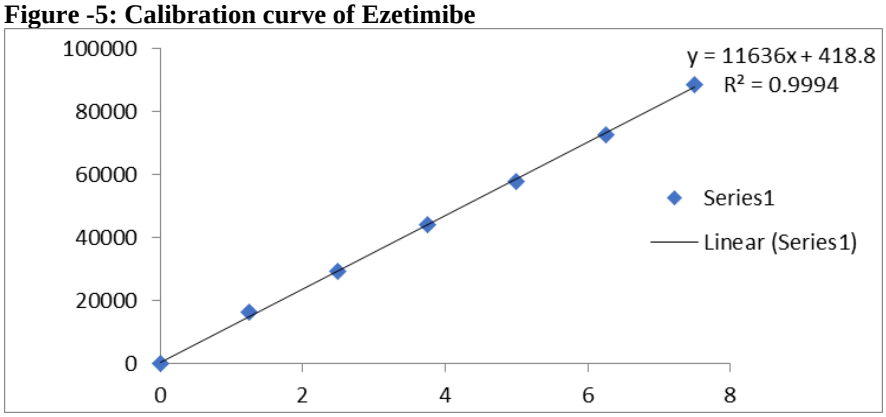

Table-3 Accuracy table of Bempedoic acid (Drug1) and Ezetimibe (Drug2)

%Level	Amount Spiked(µg/ml)		Amount Recovered(µg/ml)		% Recovery		Mean % Recovery	
	Drug 1	Drug 2	Drug 1	Drug 2	Drug 1	Drug 2	Drug 1	Drug 2
50%	45	2.5	45.3	2.53	100.8	101.26	100.55%	99.85%
	45	2.5	45.1	2.46	100.2	98.52		
	45	2.5	45.5	2.48	101.1	99.07		
100%	90	5	90.0	5.05	100.0	101.00		
	90	5	90.8	4.97	100.8	99.39		
	90	5	91.7	5.00	101.9	99.97		
150%	135	7.5	135.1	7.49	100.0	99.88		
	135	7.5	134.9	7.45	99.9	99.34		
	135	7.5	135.2	7.51	100.1	100.20		

Table-4: System precision table of Bempedoic Acid and Ezetimibe

S. No	Area of Bempedoic Acid	Area of Ezetimibe
1.	1597390	58995
2.	1576291	59053
3.	1578947	58515
4.	1590840	59349
5.	1567675	59814
6.	1590354	59121
Mean	1583583	59141
S.D	11107.4	428.4
%RSD	0.7	0.7

Table-5: System suitability parameters for Bempedoic Acid and Ezetimibe

S no	Bempedoic Acid			Ezetimibe			
Inj	RT (min)	USP Plate Count	Tailing	RT (min)	USP Plate Count	Tailing	Resolution
1	2.385	3875	1.17	2.937	6329	1.31	3.3
2	2.386	3873	1.17	2.942	6339	1.30	3.3
3	2.388	3976	1.15	2.945	6379	1.37	3.3
4	2.389	3971	1.15	2.945	6393	1.36	3.3
5	2.389	3971	1.12	2.946	6357	1.38	3.3
6	2.390	3973	1.13	2.946	6378	1.31	3.4

Figure-6: System suitability chromatogram

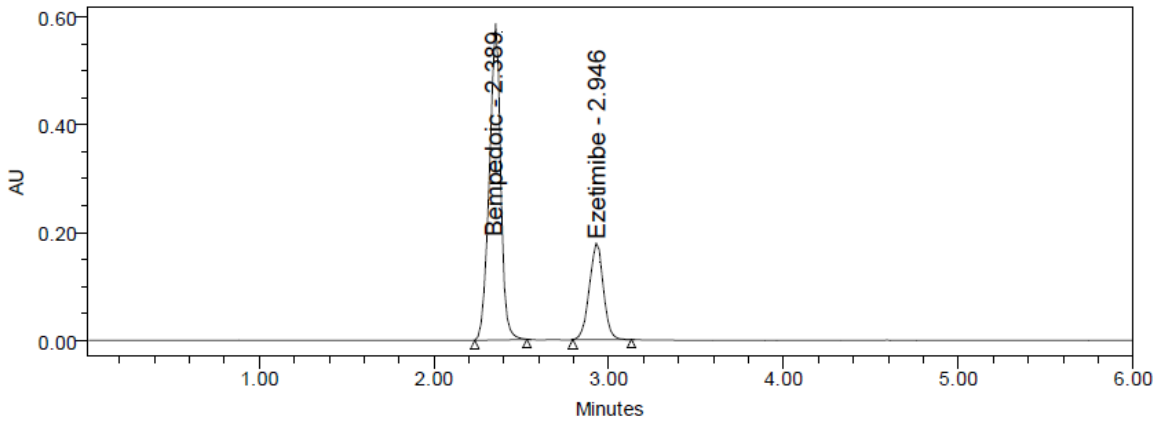

Table-6: Robustness data for Bempedoic Acid and Ezetimibe

S.no	Condition	%RSD of Bempedoic Acid	%RSD of Ezetimibe
1	Flow rate (-) 0.80ml/min	0.4	0.6
2	Flow rate (+) 1ml/min	0.6	0.6
3	Mobile phase (-) 50B:40A	0.4	0.4
4	Mobile phase (+) 50B:50A	0.3	0.6
5	Temperature (-) 25°C	0.6	0.5
6	Temperature (+) 35°C	0.7	1.0

Table-7: Sensitivity table of Bempedoic Acid and Ezetimibe

Molecule	LOD	LOQ
Bempedoic Acid	0.25	0.75
Ezetimibe	0.01	0.05

Figure-7: LOD Chromatogram of standard

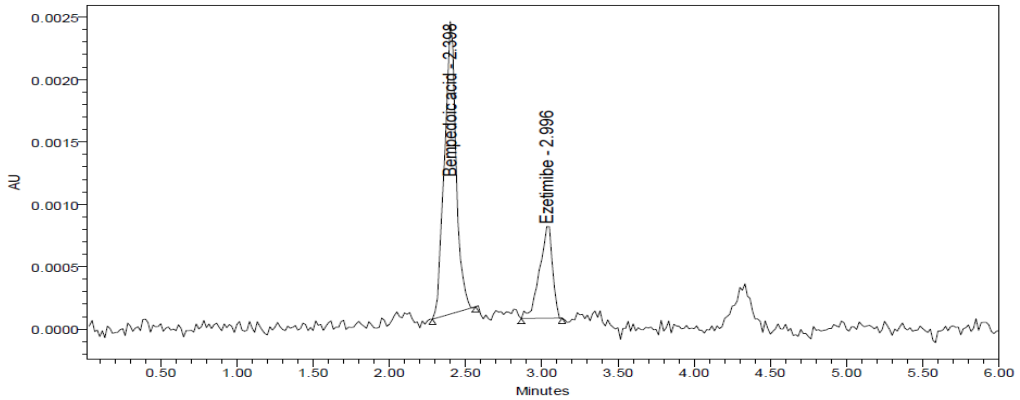

Figure-8 LOQ Chromatogram of standard

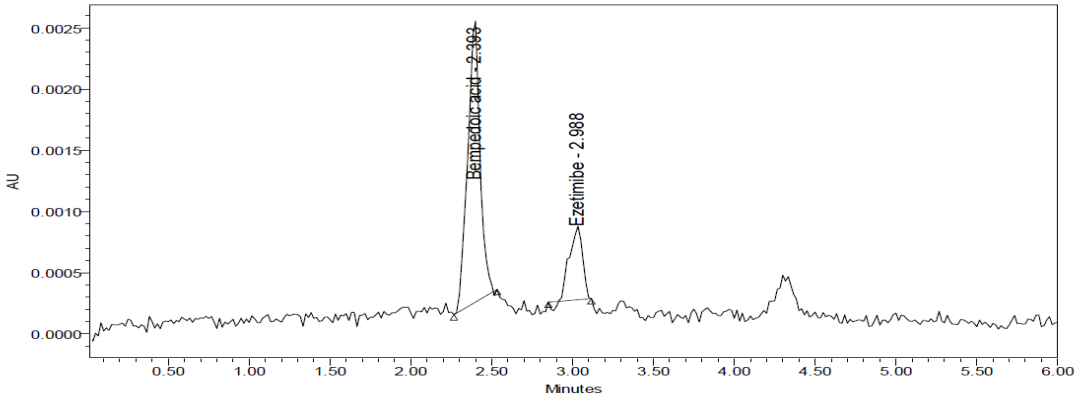

Table-8 Assay Data of Bempedoic Acid and ezetimibe

s.no	% Assay Bempedoic acid	%Assay Ezetimibe
1	99.15	99.48
2	100.81	99.28
3	99.69	99.64
4	99.29	99.11
5	100.24	99.77
6	100.69	100.25
Avg	99.98	99.59
Stdev	0.71	0.40
%RSD	0.7	0.4

Figure-9: Chromatogram of working standard solution

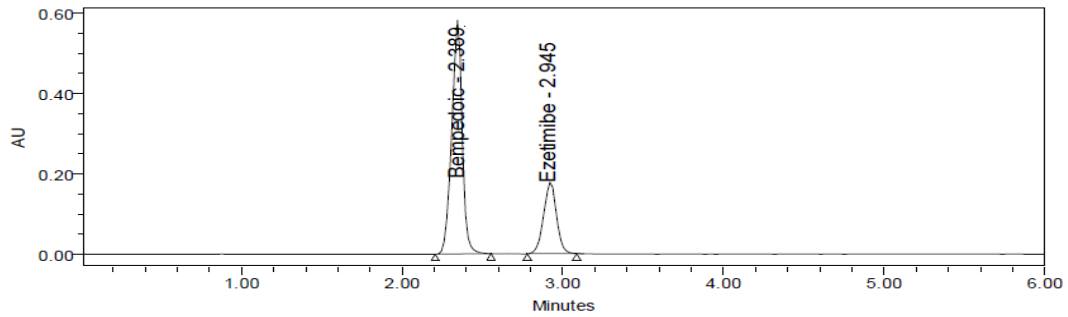

Figure-10: Chromatogram of working sample solution

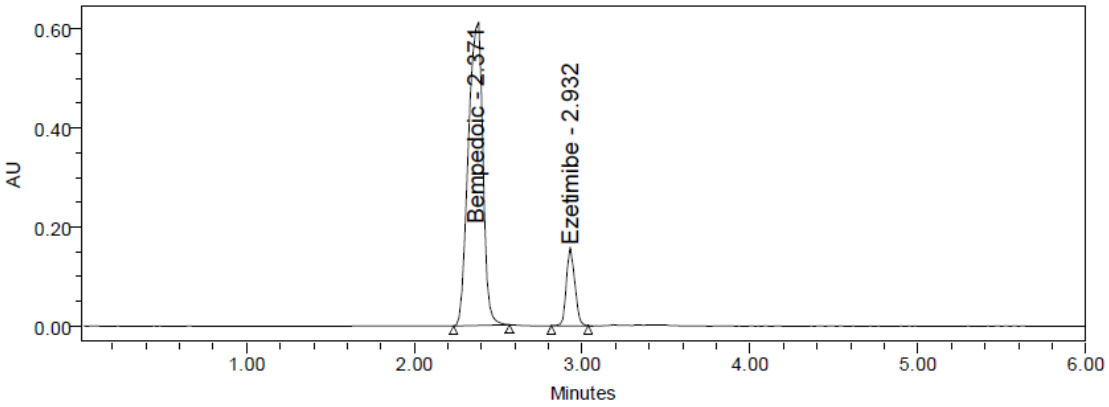

Table-9: Degradation data for Bempedoicacid and Ezetimibe

Type of degradation	Bempedoic acid		Ezetimibe	
	%RECOVERED	% DEGRADED	%RECOVERED	% DEGRADED
Acid	94.30	5.70	93.98	6.02
Base	95.12	4.88	95.60	4.40
Peroxide	95.47	4.53	95.68	4.32
Thermal	97.38	2.62	97.69	2.31
Uv	99.00	1.00	98.27	1.73
Water	99.57	0.43	99.58	0.42

CONCLUSION

A new stability indicating RP-HPLC technique was developed and validated for the simultaneous estimation of Bempedoic acid and Ezetimibe in bulk and tablet dosage form. The developed method was said to be simple, precise, accurate, with high resolution, shorter retention times with separated degradants, and economical. Hence, this method can be used for the in-process evaluation in pharmaceutical manufacturing firms and routine quality control of these drugs in drug testing laboratories.

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