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RP-HPLC Method Development and Validation for the Simultaneous Estimation of Bempedoic Acid and Ezetimibe in Pharmaceutical Dosage Form

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Mula. Anusha Reddy[1], C. Parthiban*[1], M. Sudhakar[1]

*[1]M. Pharmacy, Department of Pharmaceutical Analysis, Malla Reddy College of Pharmacy, Affiliated to Osmania University[1], Dhulapally, Hyderabad-500014, Telangana. India. *[1]Head of the department and Professor. Department of Pharmaceutical Analysis, Malla Reddy College of Pharmacy, Affiliated to Osmania University[1], Dhulapally, Hyderabad-500014, Telangana. India. [1]Principal and Professor. Department of Pharmaceutical Analysis, Malla Reddy College of Pharmacy, Affiliated to Osmania University[1], Dhulapally, Hyderabad-500014, Telangana. India.*

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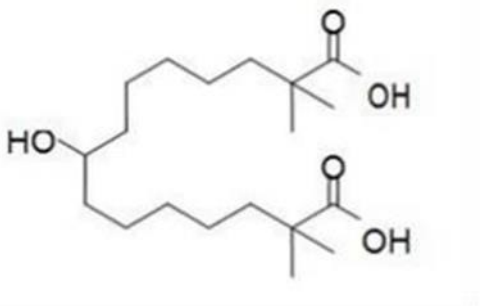
ABSTRACT

For the simultaneous estimation of the Bempedoic acid and ezetimibe in Pharmaceutical Dosage Form, a straightforward, accurate, and precise method was developed. Chromatogram was run through Agilent 150 x 4.6 mm, 5mm. Mobile phase containing Acetonitrile: KH2 (0.1% OPA is added to adjust ph 2.2) 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 230nm. Retention time of Bempedoic Acid and Ezetimibe were found to be 2.252 min and 2.987. % RSD of the Bempedoic Acid and Ezetimibe were and found to be 0.5 and 0.7 respectively. % Recovery was obtained as 100.07% and 99.96% for Bempedoic Acid and Ezetimibe respectively. LOD, LOQ values obtained from regression equations of Bempedoic Acid and Ezetimibe were 0.20, 0.60 and 0.03, 0.10 respectively. Regression equation of Bempedoic Acid is y = 6086.x + 434.6, y = 35280x + 593.0 of Ezetimibe. Because retention times and run times were reduced, the method developed was simple and cost-effective, and it can be used in regular quality control tests in industries.

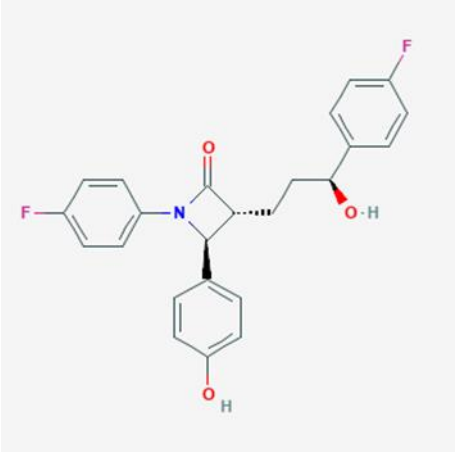
INTRODUCTION:

Bempedoic Acid is a first-in-class adenosine triphosphate citrate lyase (ACL) inhibitor that is taken once daily to lower LDL cholesterol levels in statin-resistant patients [1,2], Espersion therapeutics inc. [3] developed bempedoic acid, which was approved by the FDA on February 21, 2020 [4]. On February 26, 2020, NEXLIZET, a combination product of bempedoic acid and ezetimibe, was approved. The combination medication used to treat hypercholesterolemia. Structurally Bempedoic acid is also known as 8-hydroxy-2,2,14,14-tetramethyl penta decanedioic acid. It is a prodrug that needs to be activated in the liver {5}. The very-long-chain acyl-CoA synthetase-1 (ACSVL1) enzyme is responsible for its conversion to the pharmacologically active metabolite ETC-1002-CoA. The enzyme ATP lyase (also known as ATP synthase) is essential for cholesterol synthesis. After the parent drug is activated in the liver by coenzyme A (CoA), ETC-1002-CoA directly inhibits this enzyme [6,7], 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, Homozygous familial hypercholesterolemia and phytosterolemia. Ezetimibe mediates its blood cholesterol-lowering effect via selectively inhibiting the absorption of cholesterol and phytosterol by the small intestine without altering the absorption of fat-soluble vitamins and nutrients[13,14,15].

There are some other RP-HPLC methods published [17,18,19].



Structure of Bempedoic acid



Structure of Ezetimibe

Figure No. 1: Structures of Bempedoic acid and Ezetimibe

A review of the literature revealed that some methods for simultaneous estimation of Bempedoic acid and ezetimibe have been reported, as well as methods for estimation of individual drugs or in combination with other drugs such as UV-Spectrophotometric methods, UPLC, and RP-HPLC. The primary goal of this research is to create a simple, precise, accurate, relatively sensitive, and fast RP-HPLC technique for estimating Bempedoic acid and ezetimibe in tablet formulations.

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.

INSTRUMENTATION

WATERS HPLC, model: 2695 SYSTEM with Photo diode array detector was used for the development and method validation, with an automated sample injector with software Empower 2.

CHROMATOGRAPHIC CONDITIONS:

Flow rate:	1ml/min
Column:	Agilent 150mm x 4.6 mm, 5µm
Mobile phase:	Acetonitrile: KH_2 (0.1% OPA is added to adjust ph 2.2) taken in the ratio 55:45
Detector:	230.0 nm
Temperature:	Ambient
Injection volume:	10.0µL
Run time:	6.0 mins

PREPARATION OF SOLUTIONS

Preparation of 0.01N Potassium dihydrogen phosphate Buffer: 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 4.0 with dil. Orthophosphoric acid solution.

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

Standard Working 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 solution: 5 to 10 tablets were weighed and taken 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).

Sample working 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)

METHOD VALIDATION

The validation of the HPLC method was carried out in accordance with the ICH recommendations [25] for the simultaneous estimation of Ezetimibe and Bempedoic acid drug material to show that the method is suitable for routine analysis.

System suitability:

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. System suitability chromatogram was shown in figure 2 and values are mentioned in the table 1.

Specificity (Selectivity): Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So, this method was said to be specific. Representative chromatogram is shown in Figure 3 and experimental data is given in Table 2.

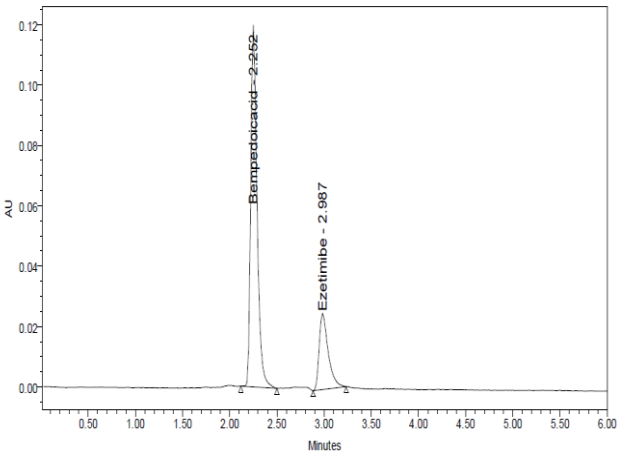


Fig. No. 2: Optimized Chromotogram

RESULT

Bempedoic Acid and Ezetimibe and were eluted at 2.252 min and 2.987 min respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated.

Table No. 1: System suitability results

S no	**Ezetimibe**			**Bempedoic acid**			
Inj	RT (min)	USP Plate Count	Tailing	RT (min)	USP Plate Count	Tailing	Resolution
1	2.252	5480	1.34	2.987	4868	1.51	4.9
2	2.252	5519	1.34	2.987	4815	1.60	4.8
3	2.252	5434	1.34	2.990	4803	1.57	4.9
4	2.257	5415	1.40	2.991	4890	1.56	4.9
5	2.258	5476	1.43	2.996	4828	1.55	4.8
6	2.268	5448	1.35	2.976	4817	1.54	4.8

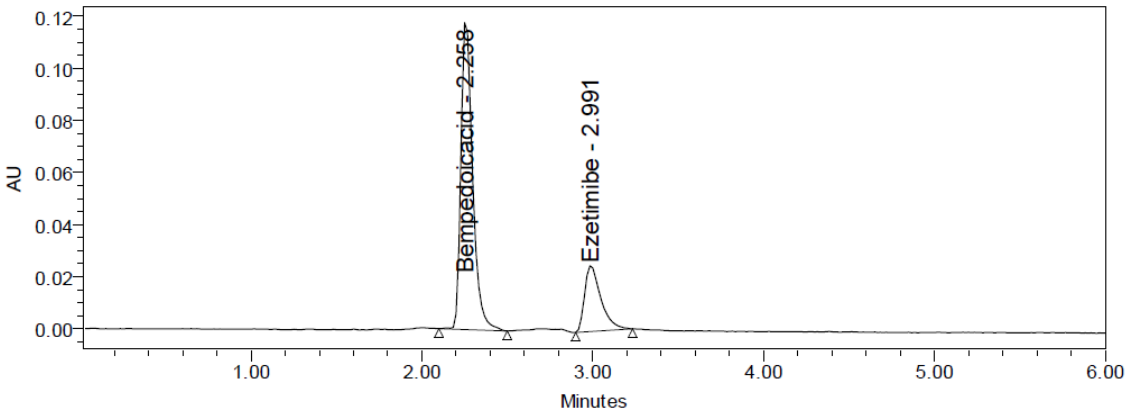
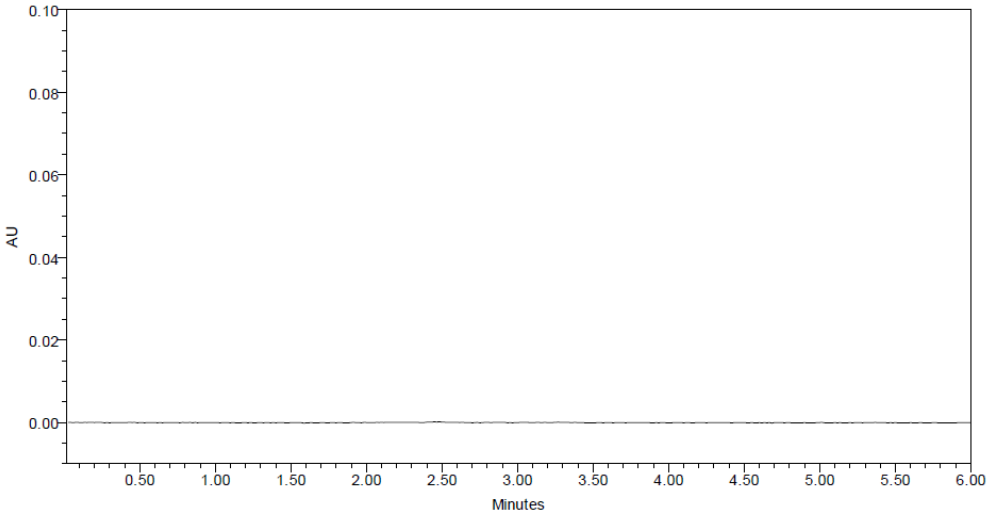


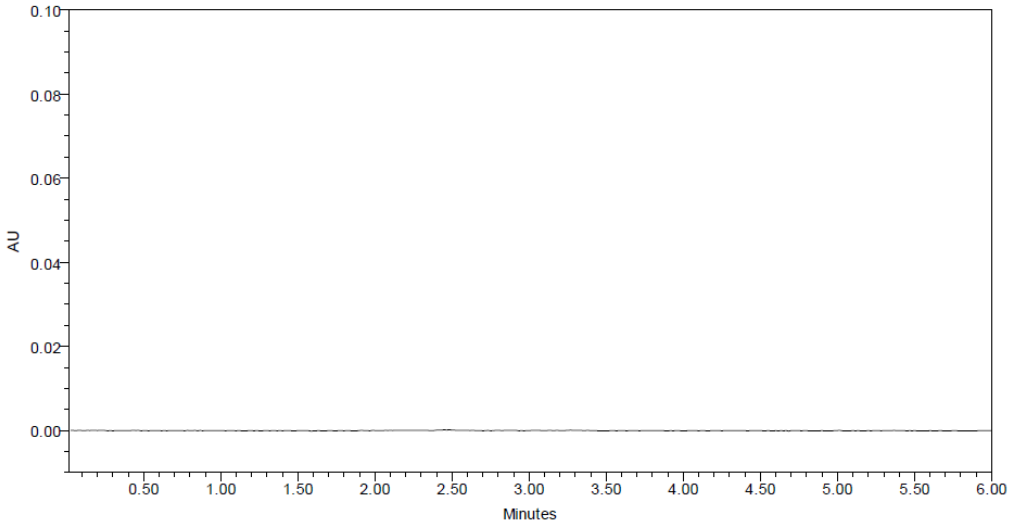
Figure No. 3: System suitability Chromatogram of Ezetimibe and Bempedoic acid.

Table No. 2: Specificity data

Sample name	Retention time(mins)
Ezetimibe	2.260
Bempedoic acid	2.986



Blank Chromatogram



Placebo Chromatogram

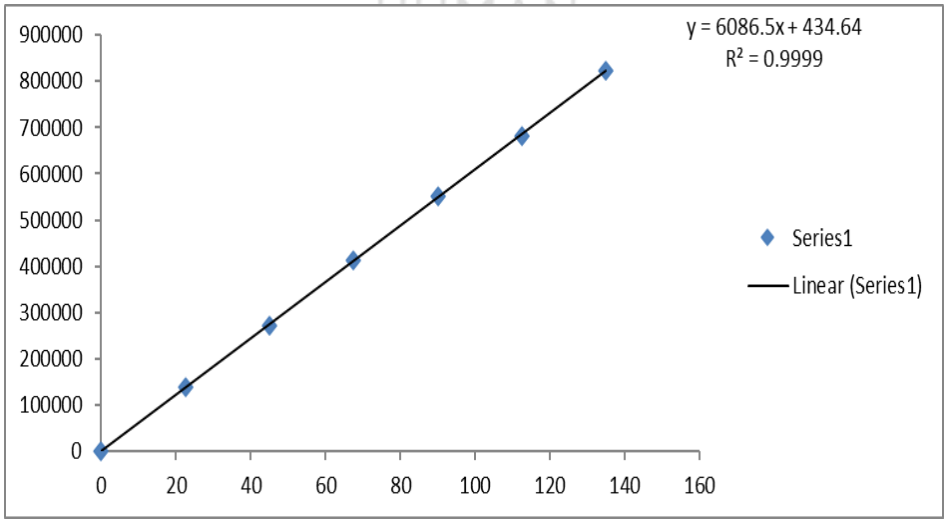
Figure No. 4: Specificity Chromatograms of Ezetimibe and Bempedoic acid.

Table No. 3: Linearity

Six linear concentrations of Bempedoic acid (0-60ml/ µg/ml)) and Ezetimibe (0-90µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Bempedoic acid was y = 38940x + 2460 and of Ezetimibe was y = 32637x + 5878.

Correlation coefficient obtained was 0.999 for the two drugs.

Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
0	0	0	0
22.5	139006	1.25	44482
45	271011	2.5	88879
67.5	413108	3.75	132445
90	550968	5	178677
112.5	681905	6.25	223330
135	822910	7.5	262432



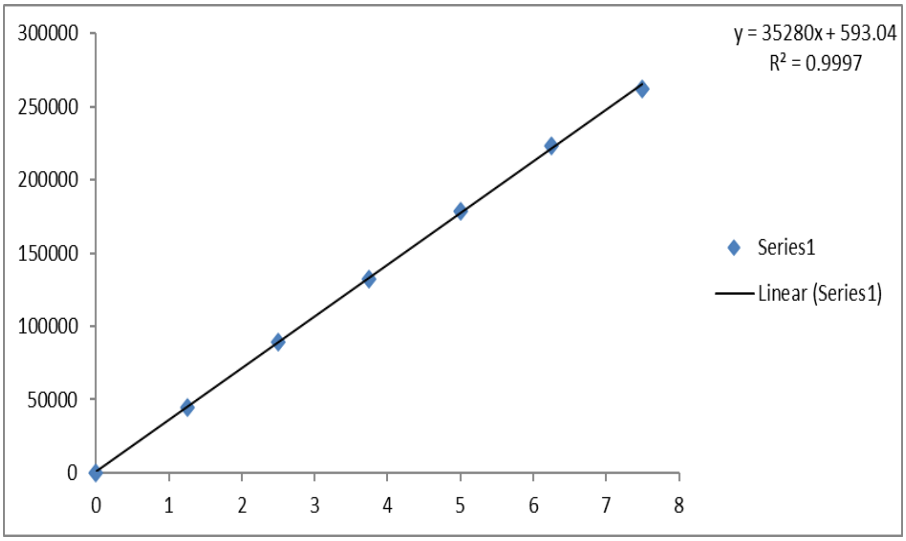


Figure No. 5: Calibration curves of Bempedoic acid and Ezetimibe

Table No. 4: Accuracy (% Recovery data)

% Level	**% Recovery**					
50% Level	**Ezetimibe**			**Bempedoic acid**		
	Amt added	Amt found	%Rec	Amt added	Amt found	%Rec
	2.5	2.50	99.97	45	44.8	99.6
	2.5	2.48	99.09	45	45.2	100.4
	2.5	2.52	100.85	45	45.4	100.9
100%Level	5	4.97	99.30	90	89.9	99.9
	5	5.04	100.81	90	89.2	99.1
	5	4.99	99.78	90	90.6	100.6
150%Level	7.5	7.50	99.97	135	135.0	100.0
	7.5	7.46	99.42	135	134.6	99.7
	7.5	7.53	100.46	135	135.3	100.2
Mean%			**99.96**			**100.07**

System Precision: From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated for two drugs. % RSD obtained as 0.5% and 0.7% respectively for Bempedoic Acid and Ezetimibe. As the limit of Precision was less than "2" the system precision was passed in this method. Results of peak area are summarized in Table 5.

Table No. 5: System precision data

Inj	Ezetimibe	Bempedoic acid
1	553042	176662
2	559223	176944
3	556800	175047
4	557693	176134
5	552832	178140
6	554267	175156
Avg	555643	176347
Std dev	2642.6	1168.3
% RSD	0.5	0.7

The % RSD for the peak areas of Ezetimibe and Bempedoic acid obtained from six replicate injections of standard solution was within the limit.

Method Precision: Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.7% and 0.7% respectively for Bempedoic Acid and Ezetimibe. As the limit of Precision was less than "2" the system precision was passed in this method. As the limit of Precision was less than "2" the system precision was passed in this method. Data obtained is summarized in Table 6.

Table No. 6: Method precision data

Injection	Ezetimibe	Bempedoic acid
1	550715	177969
2	559678	177156
3	558956	174734
4	559405	176156
5	554422	175878
6	559156	174659
Avg	557055	176092
Std dev	3675.8	1311.7
% RSD	0.7	0.7

From the above results, the % RSD of method precision study was within the limit for Ezetimibe and Bempedoic acid.

Sensitivity

Molecule	LOD	LOQ
Bempedoic acid	0.20	0.60
Ezetimibe	0.03	0.10

Table No. 7: Robustness results

Chromatographic condition	Ezetimibe (RSD)	Bempedoic acid (RSD)
Flow rate (-) 0.8ml/min	0.5	0.9
Flow rate (+) 1.0ml/min	0.4	0.7
Mobile phase (-) 45B:55A	0.7	0.5
Mobile phase (+) 60B:40A	0.6	0.9
Temperature (-) 25°C	0.6	0.6
Temperature (+) 35°C	0.5	0.6

Table No. 8: Forced degradation conditions for Ezetimibe and Bempedoic acid.

Stress condition	Solvent	Temp(^{0}C)	Exposed time
Acid	2N HCL	60^0c	30 mins
Base	2N NAOH	60^0c	30 mins
Oxidation	20% H_2O_2	60^0c	30 mins
Thermal	Diluent	105^0c	6 hours
Photolytic	Diluent	-	-
Hydrolytic	Water	60^0c	

From the results, no degradation was observed when the samples were exposed to acid, base, hydrolysis, thermal, light and water. According to the stress study, none of the degradant co-eluted with the active drug peaks formed.

Table No. 9: Degradation profile results

Type of degradation	**Ezetimibe**		**Bempedoic acid**	
	% RECOVERED	% DEGRADED	% RECOVERED	% DEGRADED
Acid	93.57	6.43	93.38	6.62
Base	93.09	6.91	92.35	7.65
Peroxide	93.15	6.85	93.22	6.78
Thermal	97.29	2.71	97.42	2.58
Uv	98.18	1.82	98.34	1.66
Water	99.03	0.97	99.84	0.16

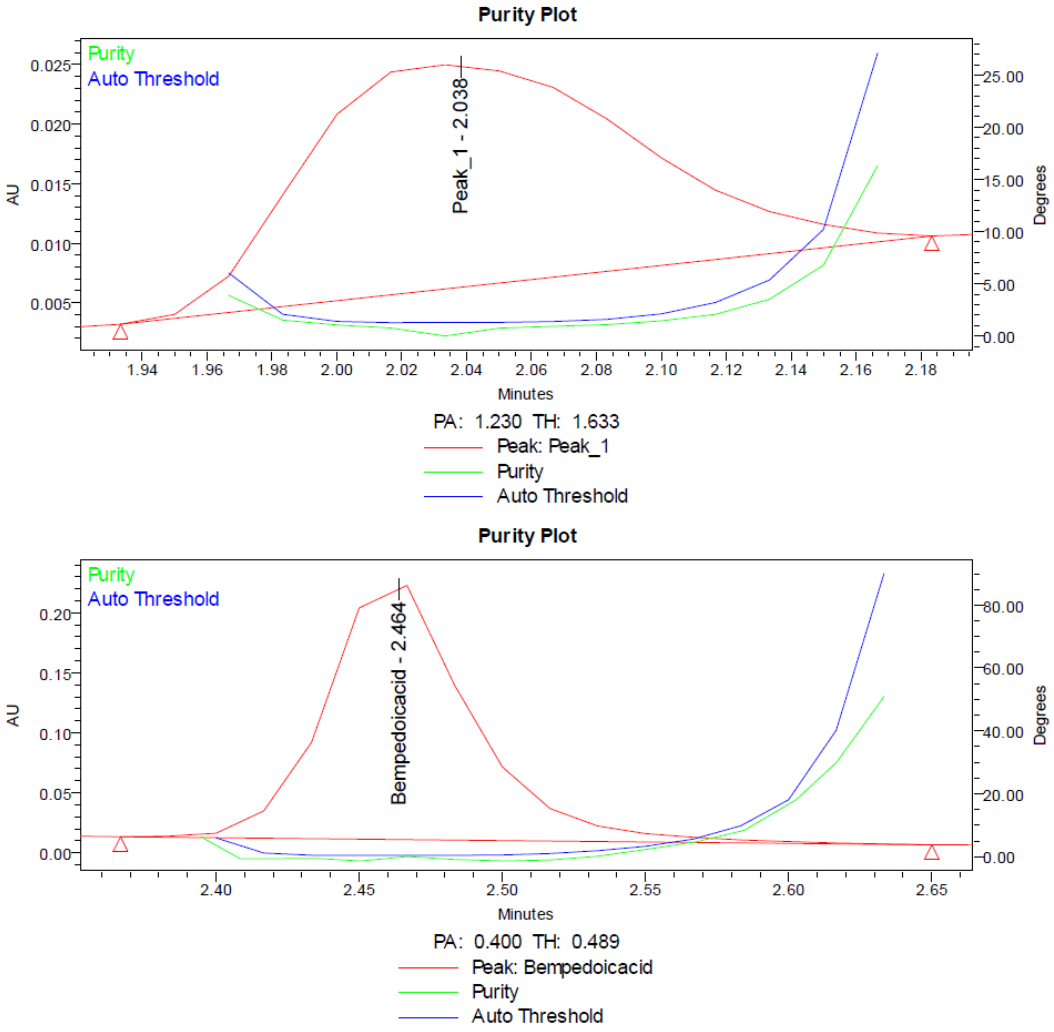


Fig. No. 6: Degradation purity plots

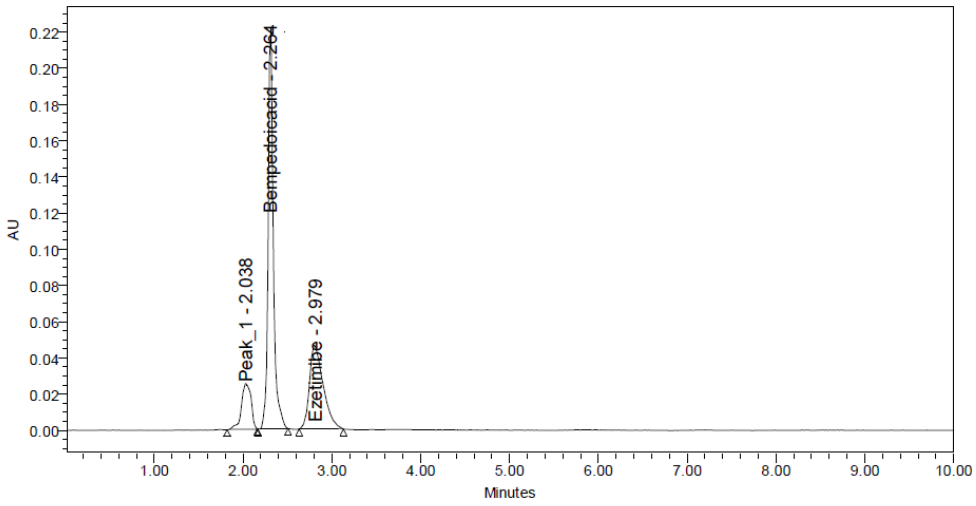


Fig. No. 7: Acid degradation chromatogram

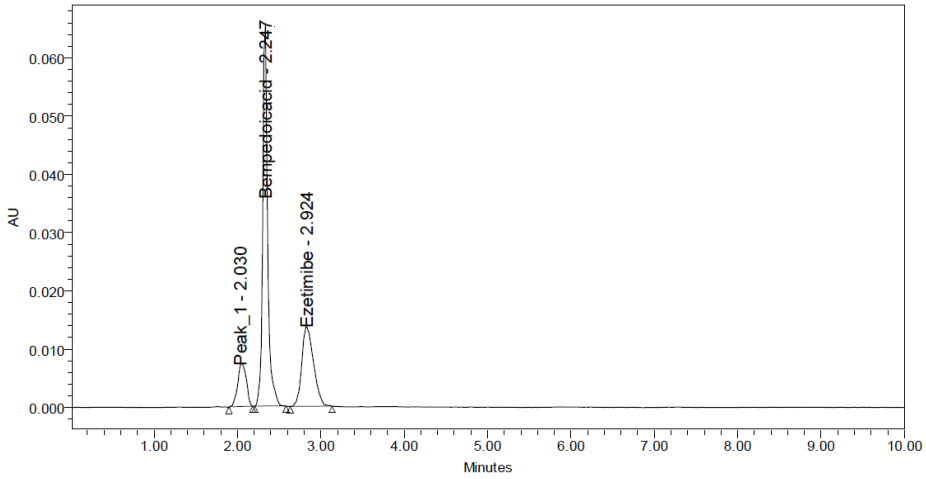


Fig. No. 8: Base degradation chromatogram

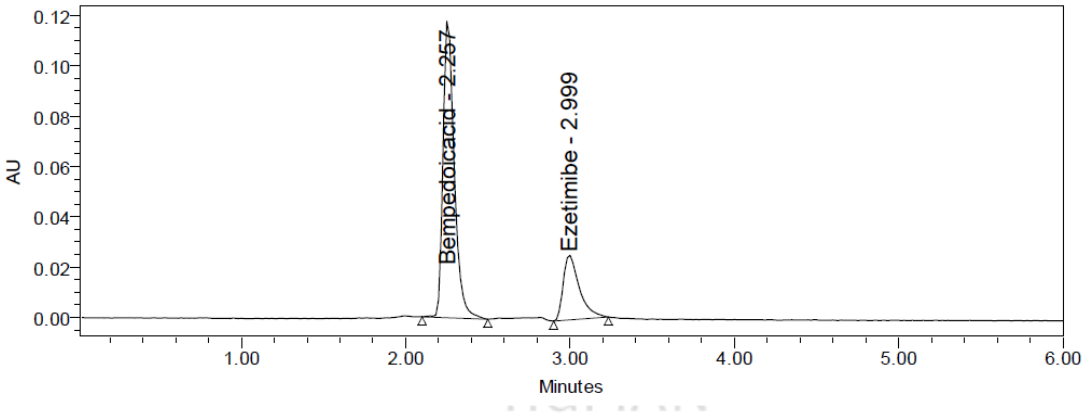


Fig no 9: Chromotogram of Ezetimibe and Bempedoic acid

Table No. 10: Assay results for Ezetimibe and Bempedoic acid

Drug name	**Label claim dose**	**% Assay**	**Brand**
Ezetimibe	10mg	99.76%	Nexlizet
Bempedoic acid	180mg	100.15%	

CONCLUSION

The RP-HPLC methodology was used to create and evaluate a new stability indicating analytical approach. The sample preparation is straightforward, uses less mobile phase, and takes very little time to analyse. The results of the study will be highly beneficial for quality

monitoring of Ezetimibe and Bempedoic acid in pharmaceutical dosage forms. The assay examination of two medications from a combination dosage form using this devised method yielded results that were nearly 100 % accurate. The results of the recovery studies were good, indicating that there was no interference from excipients.

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