World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Available online at: https://wjpsonline.com/
Research Article

A Novel Rp-Hplc Method Development and Validation for Determination and Estimation of Bempedoic Acid and Ezetimibe drug with its Bulk form and Tablet Formulation.

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Received: 24-08-2025 / Revised Accepted: 27-08-2025 / Published: 29-08-2025

ABSTRACT:
The assessment of Ezetimibe and Bempedoic Acid at the same time. Through Sunfire (250mm 4.6mm, 5µ), the chromatogram was conducted. At a flow rate of 1.0 ml/min, a mobile phase comprising KH_2PO_4, acetonitrile in a 65:35 v/v ratio was injected across the column. A constant temperature of 30°C was maintained. Bempedoic Acid and Ezetimibe had an ideal wavelength of 245 nm. %RSD of Bempedoic Acid and Ezetimibe was found 1.5 and 0.6 to be Bempedoic Acid and Ezetimibe were shown to have retention times of 2.132 and 2.595 minutes, respectively. Bempedoic Acid and Ezetimibe regression equations yielded LOD and LOQ values of 0.58, 1.75, and 0.01, 0.03 correspondingly. Bempedoic Acid's regression equation is y = 7380.3x + 1190.5, while Ezetimibe is y = 12372x + 121.34.

Key Words: Bempedoic Acid, Ezetimibe, Rp Hplc, Validation.

INTRODUCTION

The fat known as low-density lipoprotein cholesterol, or LDL cholesterol, travels throughout the body to the locations where it is required for cell repair and deposits itself inside artery walls. Triglycerides and cholesterol must bind to proteins in order to pass through the hydrophilic bloodstream because they are insoluble in water.1 Recent developments and approvals of several new cholesterol-lowering medications have expanded the pharmacological arsenal beyond statins. Both bempedoic acid, a novel medication that inhibits the same biosynthetic pathway as statins target but at an early stage, and ezetimibe, which has been on the market for 20 years, are effective treatments for hypercholesterolaemia, especially in certain patient populations. LDL-C levels appear to be considerably lowered by bempedoic acid, either by itself or in conjunction with ezetimibe; this effect has also been noted in patients who are statin intolerant.2 Bempedoic acid chemically written as 8-hydroxy-2,2,14,14-tetramethylpentadecanedioic acid is first-in-class adenosine triphosphate-citrate lyase (ACL) inhibitor used once a day for reducing LDL cholesterol levels in statin-refractory patients.3 Ezetimibe chemically written as (3R,4S)-1-(4-fluorophenyl)-3-[(3S)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl)azetidin-2-one. 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 4,5

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How to Cite this Article: Nune Deepthi. A Novel Rp-Hplc Method Development and Validation for Determination and Estimation of Budesonide and Albuterol drug with its Bulk form and Tablet Formulation. World J Pharm Sci 2025; 13(03): 96-106; https://doi.org/10.54037/WJPS.2022.100905

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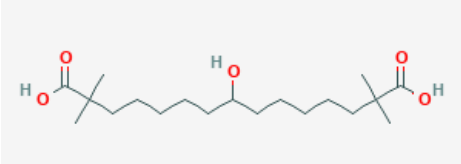

Figure 1: structure of Bempedoic Acid

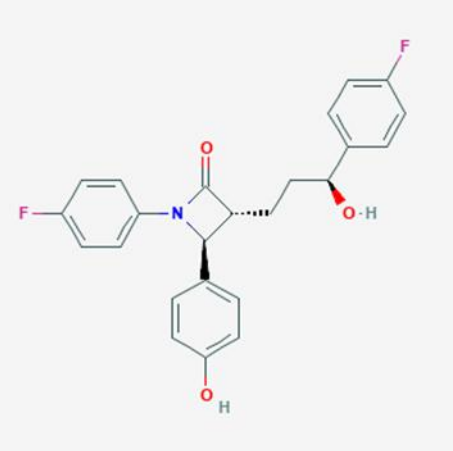

Figure 2: Structure of Ezetimibe

Extensive literature research has unearthed a multitude of recorded analytical procedures, including the discovery of more economically efficient ways. Nevertheless, there is currently no documented approach for calculating stability studies. Hence, a reliable and cost-effective approach is suggested for assessing the stability of Bempedoic Acid, Ezetimibe, and their medicinal dose form using RP-HPLC [6- 12] must be validated and developed as per ICH guidelines

Materials and Methods: Spectrum pharma Research Solution with Bempedoic Acid and Ezetimibe pure drugs (API) gift samples and Combination Bempedoic Acid and Ezetimibe tablets (Eztarica BM). The chemicals and buffers utilized in this estimation were obtained from Rankem, an Indian supplier.

Instrumentation: The development and method validation were conducted using a WATERS HPLC, specifically the model 2695 SYSTEM, equipped with a Photo diode array detector. The system also included an automated sample injector and the Empower 2 software.

Objective: In order to fulfill ICH standards, we need to design and test an HPLC technique that can detect Ezetimibe and Bempedoic Acid in pharmaceutical formulations at the same time.

Table 1: Chromatographic Conditions

Mobile phase	Acetonitrile and KH2PO4(65:35 v/v)
Flow rate	1 ml/min
Column	Sunfire C18 (4.6 x 150mm, 5µm)
Detector wave length	245 nm
Column temperature	30°C
Injection volume	10mL
Run time	5.0 min
Buffer	KH_2PO_4

Buffer Preparation: 0.01N KH2PO4 Buffer: Accurately weighed 1.36gm of Potassium dihyrogen 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 PH adjusted to 5.0 with dil. Orthophosphoric acid solution.

Preparation of Standard stock solutions: Accurately weighed 36mg of Bempedoic Acid, 2mg of Ezetimibe and transferred to 50ml individual volumetric flasks and 3/4 th of diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (720µg/ml of Bempedoic Acid and 40µg/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. (72µg/ml of Bempedoic Acid and 4µg/ml of Ezetimibe).

Preparation of Sample stock solutions: Average weigh 5 tablet is taken and transferred into a 100 ml volumetric flask, 50 ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (720µg/ml of Bempedoic Acid and 40µg/ml of Ezetimibe).

Preparation of Sample working solutions (100% solution): 0.4ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (72µg/ml of Bempedoic Acid and 4µg/ml of Ezetimibe).

System suitability parameters: Bempedoic Acid (72 ppm) and Ezetimibe (4 ppm) standard solutions were prepared, injected six times, and metrics such as peak tailing, resolution, and USP plate count were measured in order to evaluate the system suitability parameters. The region of six standard injection results should have an RSD of no more than 2%.

Specificity: 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.

Table 2: System suitability results

S.no	Bempedoic Acid			Ezetimibe			
Inj	RT (min)	USP Plate Count	Tailing	RT (min)	USP Plate Count	Tailing	Resolution
1	2.131	6355	1.23	2.591	7158	1.17	3.9
2	2.132	6241	1.27	2.592	6630	1.12	3.7
3	2.132	6071	1.23	2.593	8604	1.15	3.9
4	2.132	5977	1.22	2.595	7298	1.18	3.7
5	2.133	5794	1.26	2.596	7013	1.24	3.9
6	2.135	6280	1.26	2.597	7517	1.14	4.0

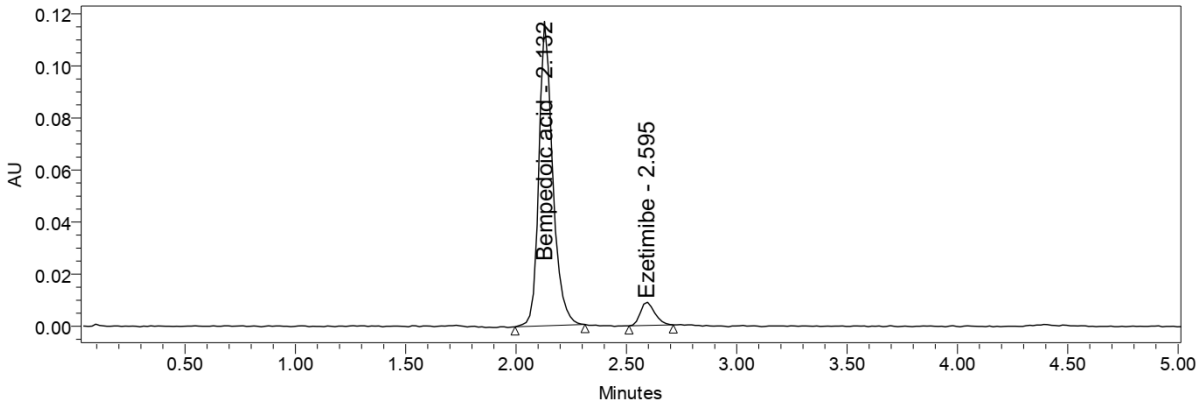

Figure 3: system suitability Chromatogram

Table 3: Specificity data

Sample name	Retention time	Area	Plate count	Tailing	Resolution
Bempedoic Acid	2.132	537749	6280.1	1.3	
Ezetimibe	2.595	44233	7517.3	1.1	4.0

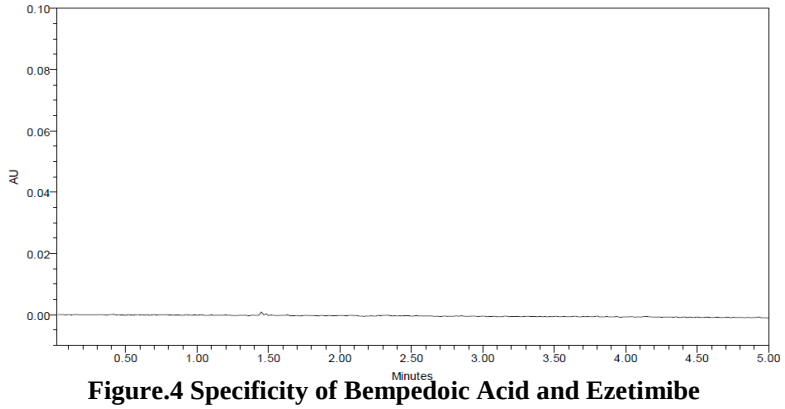

Figure.4 Specificity of Bempedoic Acid and Ezetimibe

Linearity:

Calibration data is given in table 4 and regression data in table 5 and calibration curve in figure 6, 7

Table 4: Calibration data of Bempedoic Acid and Ezetimibe

Bempedoic Acid		Ezetimibe	
Conc (µg/mL)	**Peak area**	**Conc(µg/mL)**	**Peak area**
0	0	0	0
18	131415	1	12440
36	263773	2	24738
54	409187	3	37611
72	535373	4	49530
90	664120	5	62515
108	794221	6	73821

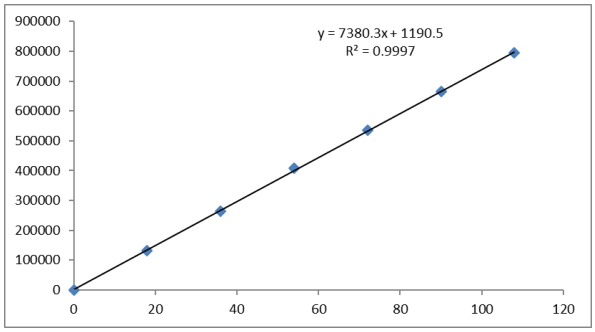

Figure.5 Calibration curve of Bempedoic Acid

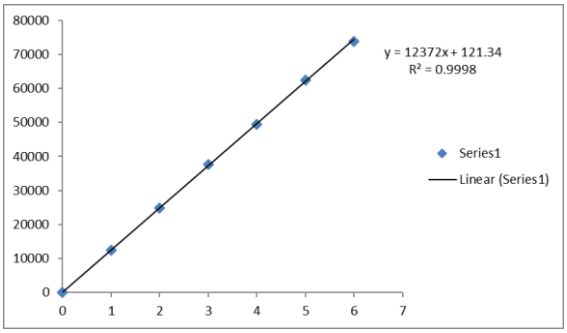

Figure.6 Calibration curve of Ezetimibe

Table 5: regression data

Parameter	Bempedoic Acid	Ezetimibe
Conc range (µg/mL)	18 – 108 µg/ml	1 – 6 µg/ml
Regression Equation	y = 7380.3x + 1190.5	y = 12372x + 121.34
Co-relation	0.999	0.999

Accuracy:
Recovery data shown in table 6

Table 6: recovery data of Bempedoic Acid and Ezetimibe

	Bempedoic Acid			Ezetimibe		
% Level	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**
50%	36	35.82	99.49	2	1.97	98.38
	36	35.76	99.32	2	1.98	99.09
	36	35.75	99.31	2	1.98	99.08
100%	72	72.31	100.43	4	4.01	100.18
	72	71.60	99.44	4	3.99	99.86
	72	71.70	99.59	4	3.99	99.87
150%	108	108.08	100.08	6	5.96	99.33
	108	107.70	99.72	6	5.95	99.17
	108	107.85	99.86	6	5.93	98.89
% recovery	**99.69**			**99.32**		

System precision was performed and the data was shown in table 8

Table 7: System precision of Bempedoic Acid and Ezetimibe

S. No	Area of Bempedoic Acid	Area of Ezetimibe
1.	549613	49545
2.	529640	50253
3.	530703	50149
4.	533494	49836
5.	528612	49620
6.	535713	49756
Mean	534629	49860
S.D	7790.9	285.0
%RSD	1.5	0.6

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

Method Precision: The precision of the method was determined by analyzing a sample of Bempedoic Acid and Ezetimibe and shown in table 8.

Table 8: method Precision

S. No	Area of Bempedoic Acid	Area of Ezetimibe
1.	536471	50112
2.	528221	50016
3.	530059	50023
4.	530851	49627
5.	537884	49951
6.	537140	49510
Mean	533438	49873
S.D	4195.2	244.3
%RSD	0.8	0.5

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

Robustness: Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.0ml/min), mobile phase minus (40B:60A), mobile phase plus (50B:50A), temperature minus (27°C) and temperature plus(33°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.

Table 9: Robustness data for Bempedoic Acid and Ezetimibe.

Condition	%RSD of Bempedoic Acid	%RSD of Ezetimibe
Flow rate (-) 0.9ml/min	0.8	0.6
Flow rate (+) 1.1ml/min	0.7	0.7
Mobile phase (-) 60B:40A	0.8	0.3
Mobile phase (+) 70B:30A	0.9	0.4
Temperature (-) 27°C	0.9	0.4
Temperature (+) 33°C	0.6	0.5

Sensitivity:

Table 10: sensitivity of Bempedoic Acid and Ezetimibe

Molecule	LOD	LOQ
Bempedoic Acid	0.058 µg/ml	1.75 µg/ml
Ezetimibe	0.01 µg/ml	0.3 µg/ml

Force Degradation Studies: table 11 shows degradation conditions and table 10 shows the obtained degraded data and purity plot chromatogram in figure 8, 9.

Table 11: degradation conditions

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

Table 12: degradation data

Type of degradation	Bempedoic Acid			Ezetimibe		
	area	%recovered	% degraded	area	%recovered	% degraded
Acid	498355	93.03	6.97	46587	93.25	6.75
Base	501229	93.57	6.43	46438	92.95	7.05
Peroxide	503229	93.94	6.06	47174	94.42	5.58
Thermal	514843	96.11	3.89	48392	96.86	3.14
Uv	523299	97.68	2.32	48902	97.88	2.12
Water	531551	99.23	0.77	49518	99.12	0.88

Acid degradation chromatogram

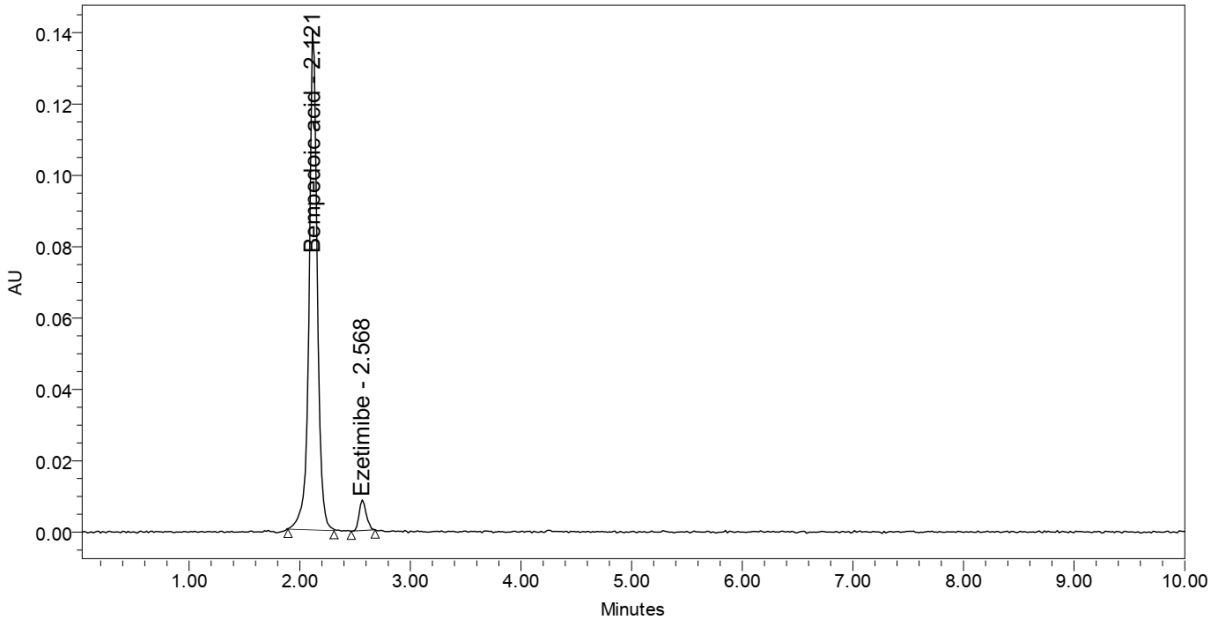

Figure.7 acid

Base degradation chromatogram

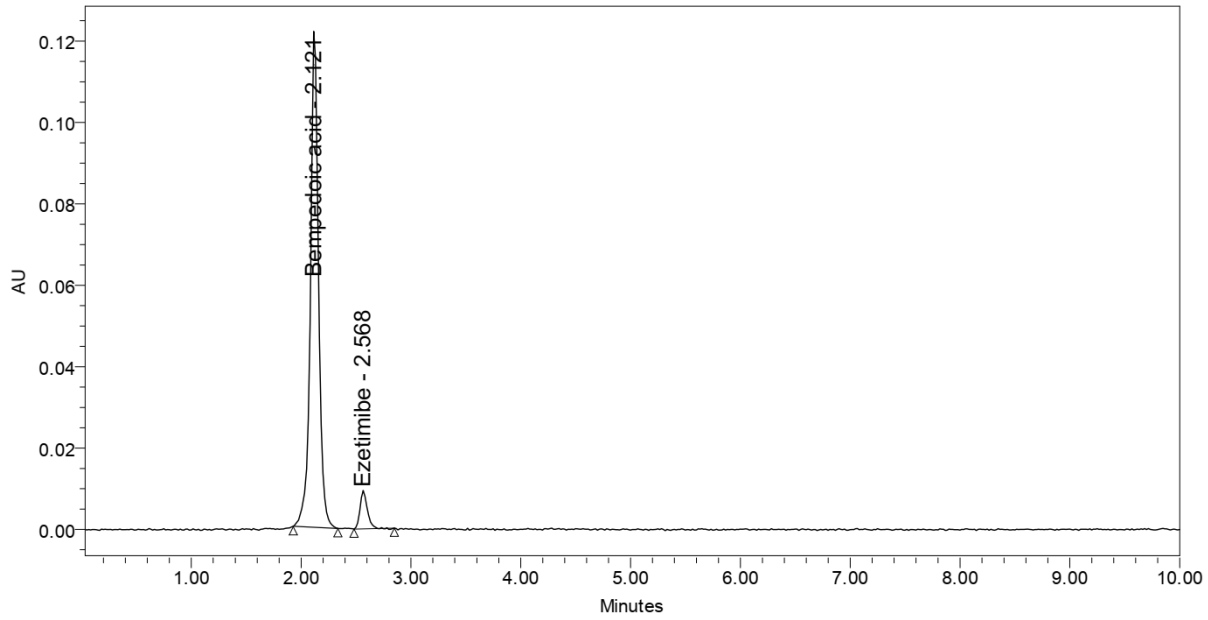

Figure.8 base

Peroxide degradation chromatogram

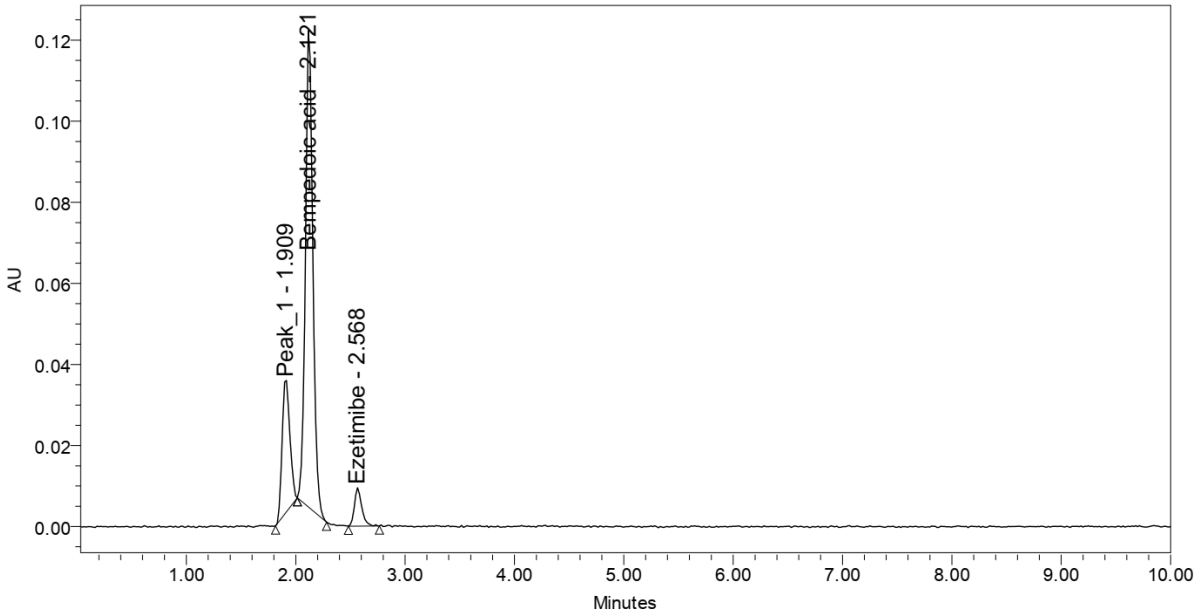

Figure.9 peroxide

Thermal degradation chromatogram

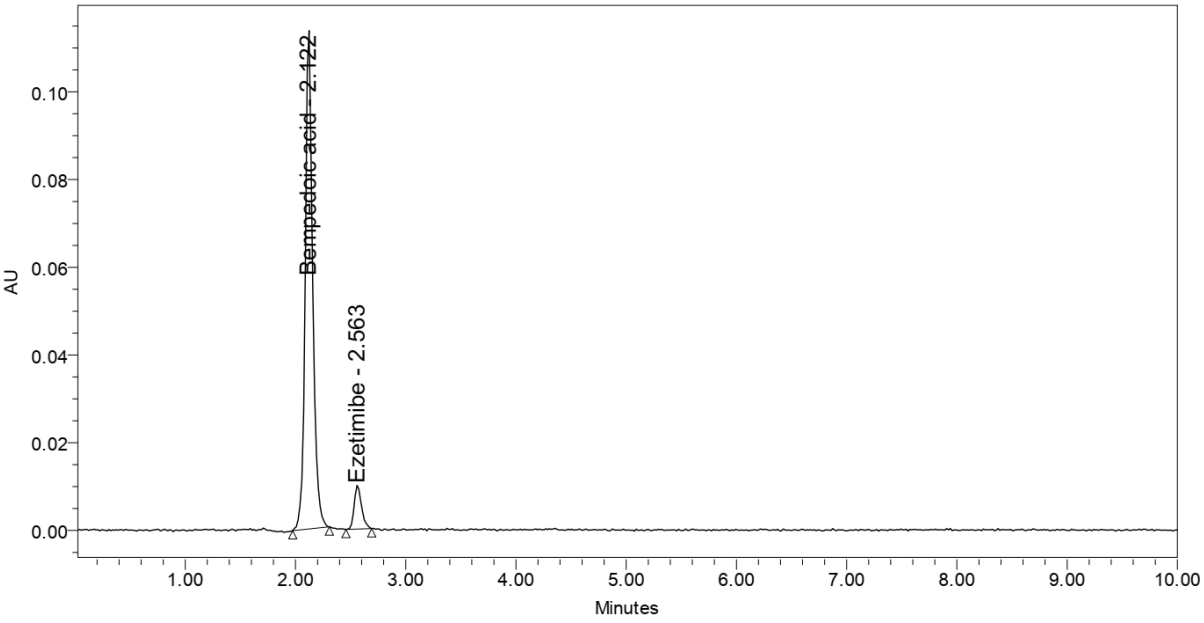

Figure.10 thermal

UV degradation chromatogram

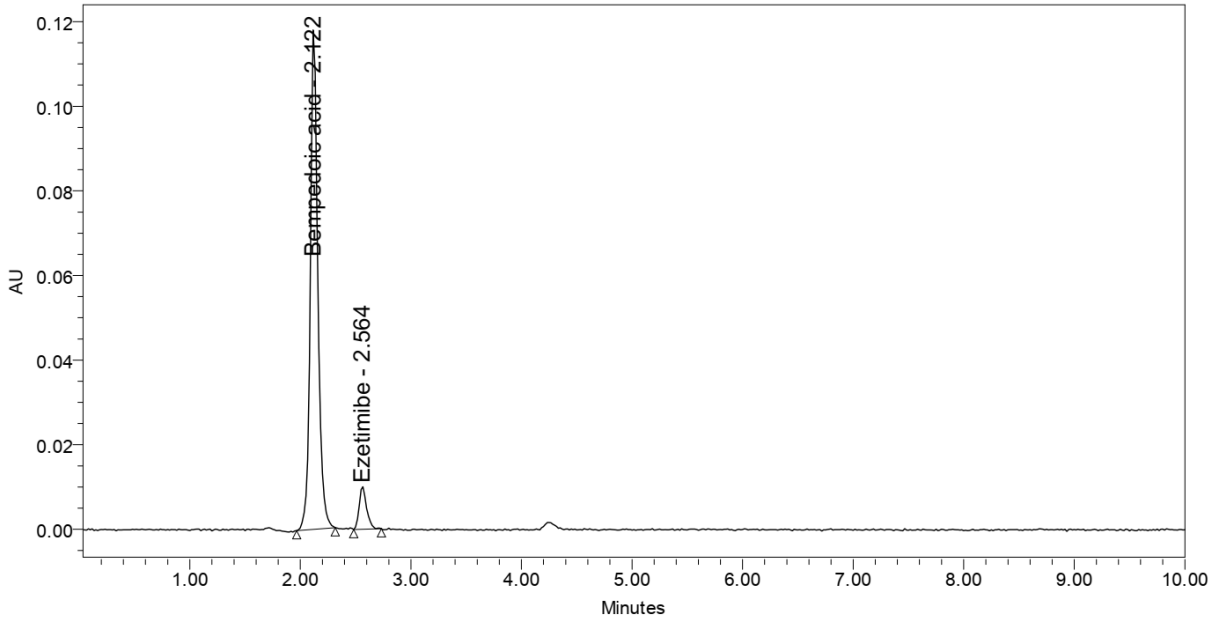

Figure.11 UV

Water degradation chromatogram

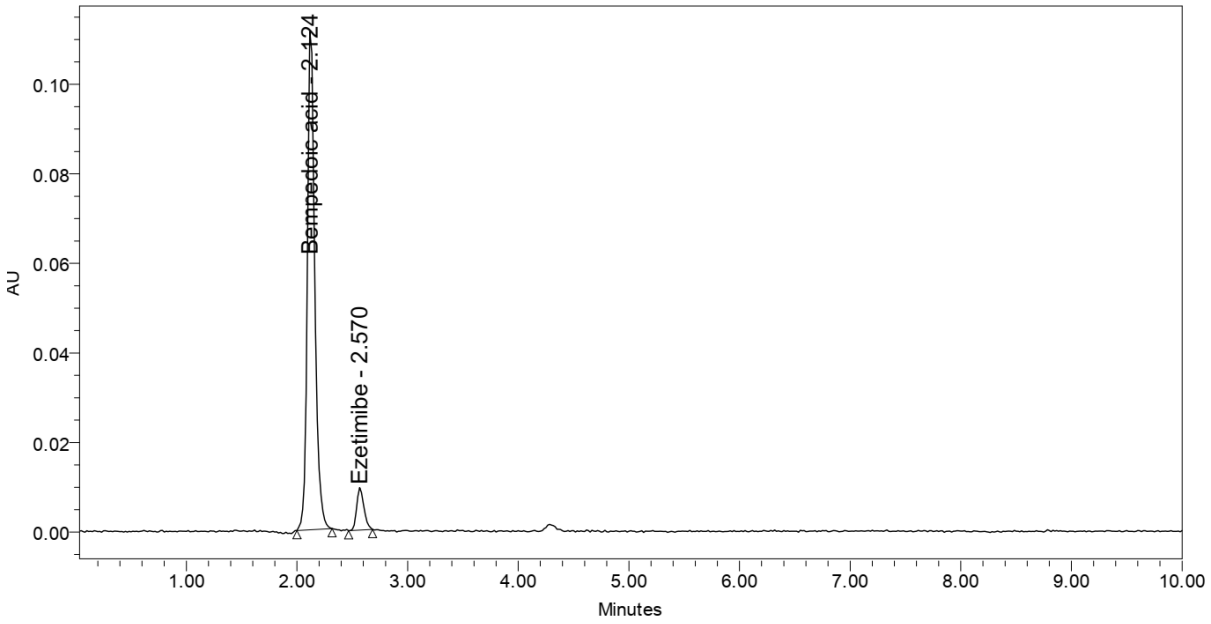

Figure.12 water

Assay: Eztarica BM, bearing the label claim Bempedoic Acid 180mg, Ezetimibe 10mg. Assay was performed with the above formulation. Average % Assay for Bempedoic Acid and Ezetimibe obtained was 99.58% and 99.83% respectively.

Table 13: assay data

	Bempedoic Acid			Ezetimibe		
S.no	**Std Area**	**Sample area**	**% Assay**	**Std Area**	**Sample area**	**% Assay**
1	549613	536471	***100.14***	49545	50112	***100.30***
2	529640	528221	***98.60***	50253	50016	***100.11***
3	530703	530059	***98.95***	50149	50023	***100.13***
4	533494	530851	***99.09***	49836	49627	***99.33***
5	528612	537884	***100.41***	49620	49951	***99.98***
6	535713	537140	***100.27***	49756	49510	***99.10***
Avg	534629	533438	***99.58***	49860	49873	***99.83***
Stdev	7790.9	4195.2	***0.8***	285.0	244.3	***0.5***
%RSD	1.5	0.8	***0.8***	0.6	0.5	***0.6***

Assay was calculated by the formula:

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{WS}{100} \times \frac{1}{10} \times \frac{100}{1} \times \frac{10}{1} \times \frac{P}{100} \times \frac{FV}{L.C} \times 100$$

AT	Average Peak area of sample in test solution
AS	Mean peak area of sample in standard solution
WS	Weight of drug working standard taken in mg
P	Assay of drug working standard in % on dried basis
L.C	Label Claim

Figure.13 Formula

Conclusion:

The study's results will help a lot with checking the quality of affordable medications that contain Bempedoic Acid and Ezetimibe. This might be because the study used a simple way to prepare the samples, which only needed a short analysis time and a small amount of mobile phase. After testing two medicines together in a single dose, the data showed that the newly developed analysis method was very close to being 100% effective.

ACKNOWLEDGEMENT:
The authors are thankful to, Department of Pharmaceutical Analysis, Pulla reddy college of Pharmacy, Affiliated to JNTUH India and Spectrum Pharma Research Solutions, Hyderabad, Telangana, India.

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