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A NOVEL CHROMATOGRAPHIC ESTIMATION OF BEMPEDOIC ACID AND EZETIMIBE IN BEMPEDOIC ACID AND EZETIMIBE PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT
A simple, accurate, precise method was developed for the estimation of Bempedoic acid and Ezetimibe in the tablet dosage form of Bempedoic acid and Ezetimibe. Optimized separation was achieved on an Ascentis C18 150x 4.6mm, 5µ using mobile phase composition of phosphate buffer pH 4.0 and acetonitrile in the ratio of 700 mL:300 mL (v/v), at a flow rate of 1.0 mL/min, the injection volume is 10 µL and run time 6 minutes in isocratic elution. UV detection was carried out at a wavelength of 230 nm. The temperature was maintained at 30°C. Well-resolved peaks were observed with high numbers of theoretical plates, lower tailing factor, and reproducible relative retention time. The method was validated and all the validation parameters were found to be within the acceptable limits.

KEYWORDS: *Bempedoic acid: Ezetimibe: stress degradation: RP-HPLC method development:Validation*.

INTRODUCTION
Bempedoic acid and Ezetimibe Tablet (trade name is NEXLIZET company: ESPERION THERAPS INC), Bempedoic acid is an inhibitor of adenosine triphosphate-citrate lyase (ACL), an enzyme in the cholesterol and fatty acid biosynthesis pathways. Ezetimibe is an inhibitor of intestinal cholesterol absorption[I,II].Low density lipoprotein cholesterol (LDL-C) is a primary therapeutic target for atherosclerotic cardiovascular disease (ASCVD) risk reduction. Meta-analyses of clinical trials and Mendelian studies have reaffirmed the principle of "lower is better" for LDL-C reduction for ASCVD prevention[III,IV,V,VI].

A literature search confirms that there is no method reported for the simultaneous estimation of Bempedoic and Ezetimibe quantitatively in tablet dosage forms. Hence the present work aimed to develop simple stability indicating RP-HPLC method for the separation and quantification of Bempedoic acid and Ezetimibe.The main aim of this method

was to determine and validate the Bempedoic acid and Ezetimibe based on International Conference on Harmonization guidelines[7]. This method was made for a reproducible procedure for the quantitative analysis of drug samples as the bulk drug and in tablet dosage forms. The designed method was considered advisable to develop a precise, accurate, simple RP-HPLC method. The chemical name for bempedoic acid is 8-hydroxy-2,2,14,14-tetramethyl-pentadecanedioic acid. The molecular formula is $C_{19}H_{36}O_5$, and the molecular weight is 344.5 grams per mole.

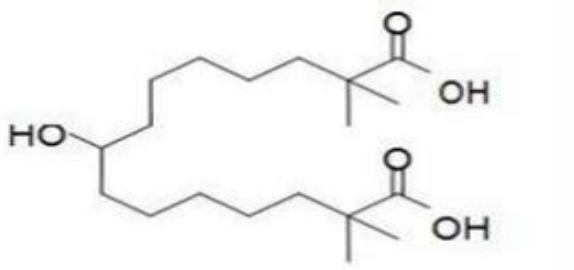

Fig:1.Chemical structures of Bempedoic acid

The chemical name for ezetimibe is 1-(4-fluorophenyl)-3(R)-[3-(4-fluorophenyl)-3(S)hydroxypropyl]-4(S)-(4-hydroxyphenyl)-2-azetidinone. The molecular formula is $C_{24}H_{21}F_2NO_3$ and the molecular weight is 409.4 grams per mole.

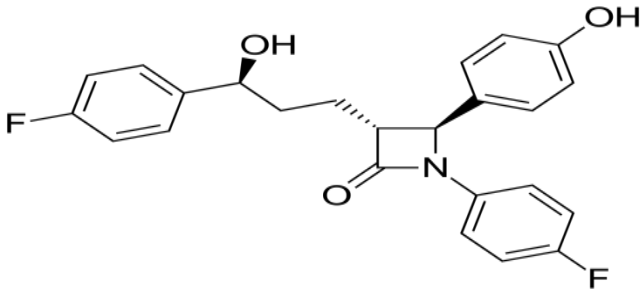

Fig:2.Chemical structures of Ezetimibe

MATERIAL AND METHODS

Chemicals, reagents and instruments:

Bempedoic acid, Ezetimibe, Orthophosphoric acid (H3PO4), potassium dihydrogen phosphate (KH2PO4), Acetonitrile, and Milli-Q water. Ascentis C18 150x 4.6mm, 5µ column, HPLC instrument equipped with UV-VIS spectrophotometer & PDA detector.

Mobile phase and solutions preparation:

Preparation of buffer:

Accuratly Weighted 1.36 gms of potassium dihydrogen phosphate dissolve in 1000 mL of water, degasified by sonication, and adjusted the pH of the solution to 4.0 with diluted ortho phaspharic acid.

Preparation of Mobile phase:

Mixed 700 mL of buffer and 300 mL of acetonitrile. Degasified this solution through sonication.

Preparation of Diluent:

Mixed 500 mL of water and 500 mL of acetonitrile and Degasified this diluent through sonication

Chromatographic conditions:

Flow rate: 1.0 mL, Injection volume: 10 µL, Detector: 230 nm, column temperature: 30°C, Column:Ascentis C18 150x 4.6mm, 5µ, Run time: 6 minutes.

Standard Preparation:

Accurately Weighted and transferred 45 mg of Bempedoic acid and 2.5 mg of Ezetimibe working Standards into a 50 ml clean dry volumetric flasks, added 10 mL of diluent, sonicated for 10 minutes, and make up to the final volume with diluent. (900 µg/mL Bempedoic acid and 50 µg/mL of Ezetimibe),1mL from the above two stock solutions was taken into a 10mL volumetric flask and made up to 10mL. (90µg/ml Bempedoic acid and 5µg/ml of Eztimibe).

Sample Preparation:

Accurately weighted equivalent weight of one tablet combination powder sample transfer into a 100 mL volumetric flask, 50 mL of diluents was added and sonicated for 25 minutes, further, the volume was made up with diluent and filtered the solution through 0.45µm membrane filter (1800 µg/mL Bempedoic acid and 100 µg/mL of Ezetimibe).

0.5 mL of filtered sample stock solution was transferred to a 10 mL volumetric flask and made up with diluent. (90 µg/mL Bempedoic acid and 5 µg/mL of Ezetimibe).

Degradation studies:

Oxidation:

Taken 1 ml of stock solution of 1800 µg/mL Bempedoic acid and 100 µg/mL of Ezetimibe& 1 ml of 20% hydrogen peroxide (H_2O_2) was added separately. The resultant solution was kept for 60 min at 30°C. For the HPLC study, the resultant solution was diluted to obtain 90 µg/mL Bempedoic acid and 5 µg/mL of Ezetimibe, then 10 µL injection volume was injected into the system and the chromatogram was recorded to assess the stability of the sample.

Acid Degradation Studies:

Taken 1 ml of stock solution of 1800 µg/mL Bempedoic acid and 100 µg/mL of Ezetimibe& 1 ml of 2N hydrochloric acid was added separately. The resultant solution was refluxed for 30 min at 60°C and neutralized acid with an equivalent volume of sodium hydroxide solution. For the HPLC study, the resultant solution was diluted to obtain 90 µg/mL Bempedoic acid and 5 µg/mL of Ezetimibe, then 10 µL injection volume was injected into the system and the chromatogram was recorded to assess the stability of the sample.

Alkali Degradation Studies:

Taken 1 ml of stock solution of 1800 µg/mL Bempedoic acid and 100 µg/mL of Ezetimibe& 1 ml of 2N sodium hydroxide solution was added separately. The resultant solution was refluxed for 30 min at 60°C and neutralized the base with an equivalent volume of hydrochloric acid solution. For the HPLC study, the resultant solution was diluted to obtain 90 µg/mL Bempedoic acid and 5 µg/mL of Ezetimibe, then 10 µL injection volume was injected into the system and the chromatogram was recorded to assess the stability of the sample.

Thermal Degradation Studies:

Taken 1 ml of stock solution of 1800 µg/mL Bempedoic acid and 100 µg/mL of Ezetimibe& exposed the solution to heat at 105°C for 6 hours. For the HPLC study, the resultant solution was diluted to obtain 90 µg/mL Bempedoic acid and 5 µg/mL of Ezetimibe, then 10 µL injection volume was injected into the system and the chromatogram was recorded to assess the stability of the sample.

UV light studies:

Taken 1 ml of stock solution of 1800 µg/mL Bempedoic acid and 100 µg/mL of Ezetimibe& exposed the solution to UV light by keeping into the chamber. For the HPLC study, the resultant solution was diluted to obtain 90 µg/mL Bempedoic acid and 5 µg/mL of Ezetimibe, then 10 µL injection volume was injected into the system and the chromatogram was recorded to assess the stability of the sample.

Neutral Degradation Studies:

Taken 1 ml of stock solution of 1800 µg/mL Bempedoic acid and 100 µg/mL of Ezetimibe& 1 ml of water was added separately. The solution was refluxed for 6 hours at 60°C. For the

HPLC study, the resultant solution was diluted to obtain 90 µg/mL Bempedoic acid and 5 µg/mL of Ezetimibe, then 10 µL injection volume was injected into the system and the chromatogram was recorded to assess the stability of the sample.

RESULTS AND DISCUSSION:

With the progress of International Conference on Harmonization (ICH) guidelines, the determination of a stability-indicating Assay method has developed more clearly and obligatory. The guidelines are necessary the handling of forced degradation studies under different conditions, like acid, base, oxidation, UV, heat and water. Hence the necessity of separation of several components through the study of stability samples, HPLC has gained reputation instability studies due to its specificity, sensitivity and high-resolution capacity. The work planned in this research was subjected to the study of the chromatographic actions of the samples of stress degradation of Bempedoic acid and Ezetimibe in the tablet dosage formulation. The stability-indicating study of the present combination drug has not been reported so far in the literature as per our knowledge and motivated us to develop an RP-HPLC-PDA stability-indicating test where the degradation products were resolved from the integral drugs.

Method development

The standard drug solution containing 90 µg/mL of Bempedoic acid and 5 µg/mL of Ezetimibe was used initially for method development studies, the resultant chromatogram revealed in Figures 3& 4.

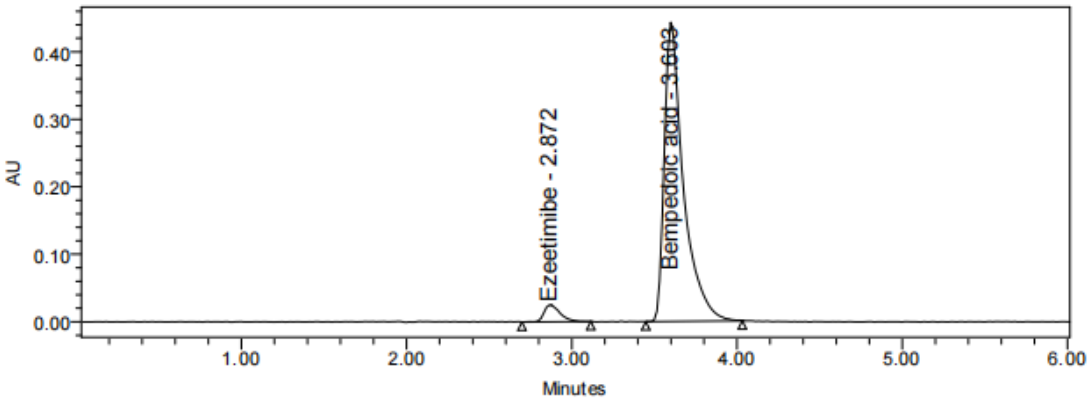

Figure 3: Standard chromatogram of Bempedoic acid and Ezetimibe

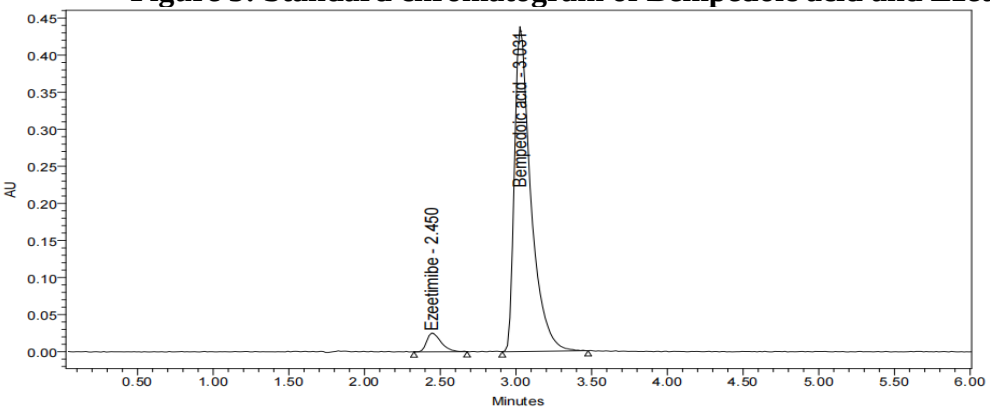

Figure 4: Sample chromatogram of Bempedoic acid and Ezetimibe

Method validation

The method was validated as per ICH guidelines. The different validation parameters were performed as follows: linearity, method precision, accuracy, specificity, and the limit of

detection, limit of quantification, robustness, degradation studies, and the stability-indicating capability.

System suitability test:

System suitability was evaluated with freshly prepared standard solutions. Five replicate standard solution was performed and calculated the % RSD for peak areas. Other parameters theoretical plates and tailing factors were measured. System suitability results were tabulated in tables 1& 2. % RSD values were within the limit not more than 2%.

Table 1: System suitability results for Bempedoic acid

Injections	Retention Time(min)	Area	USP Plate Count	USP Tailing factor
1	2.674	2959336	4401	1.74
2	2.720	2928213	4379	1.73
3	2.729	2959687	4514	1.75
4	2.729	2947298	4521	1.74
5	2.763	2943603	4380	1.75
Mean		2947627		
Std. Dev.		12997.6		
% RSD		0.4		

Table 2: System suitability results for Ezetimibe

Injections	Retention Time(min)	Area	USP Plate Count	USP Tailing factor
1	2.167	128176	3338	1.47
2	2.186	127721	3395	1.46
3	2.209	125518	3389	1.46
4	2.209	127156	3359	1.46
5	2.225	128591	3371	1.47
Mean		127432		
Std. Dev.		1195.8		
% RSD		0.9		

Specificity

Specificity tests were carried out on a freshly prepared blank and placebo of Bempedoic acid and Ezetimibe Tablets and resultant chromatograms indicate that no interference was observed from blank and placebo at retention times of Bempedoic acid and Ezetimibe in the optimize method conditions. The resultant chromatograms are attached in Figures-5&6.

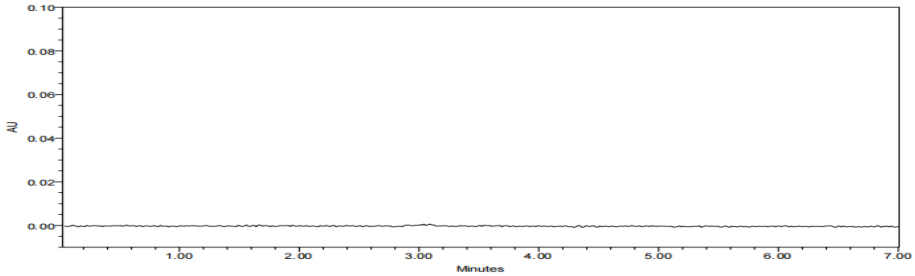

Figure 5: Blank chromatograms

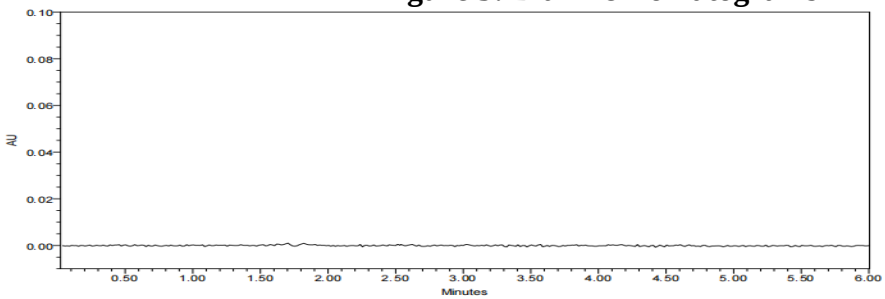

Figure 6: Placebo chromatogram

Linearity:

Linearity parameter was evaluated with standard drug solutions by preparing six different concentrations. Linearity levels are25%, 50%, 75%, 100%, 125%, 150% concentrations and all six linearity solutions were injected into the HPLC system, calculated the correlation coefficient values. The correlation coefficient was calculated for concentration versus peak area. Results were obtained in table3. Results were satisfactory, correlation coefficient values were above 0.999.

Table 3: Linearity concentration table

Linearity level	**Bempedoic acid**		**Ezetimibe**	
	Concentration (µg/mL)	**Peak area**	**Concentration (µg/mL)**	**Peak area**
25 %	22.5	717005	1.3	33620
50 %	45.0	1471191	2.5	70693
75 %	67.5	2227987	3.8	109222
100 %	90.0	2967170	5.0	139303
125%	112.5	3625113	6.3	172872
150 %	136.5	4320206	7.5	207140
Correlation coefficient	0.999		0.999	

Method Precision:

Method Precision was performed by preparing the six replicate sample preparations from a homogeneous sample. Six replicate solutions were carried out as per the test procedure mentioned in the materials and method section. method precision % of RSD results were calculated and tabulated in table 4. Precision results were found satisfactory and % RSD values were below 5 %.

Table 4:Method precision results

Method precision		
S. No	**Bempedoic acid**	**Ezetimibe**
1.	100.34	98.96
2.	99.61	101.36
3.	100.72	100.09
4.	101.29	101.30
5.	99.17	101.14
6.	100.74	100.15
Mean	100.31	100.50
S.D	0.79	0.95
%RSD	0.8	0.94

Accuracy:

The Accuracy of the method was determined on three concentration levels by performing recovery experiments. The recovery studies were carried out by different concentrations of both drugs added to the placebo from 50 %, 100 % and 150 % were evaluated. Accuracy recovery and % RSD were calculated and tabulated in table 5. % of recovery results were between 97 % to 103 %.

Table 5:Accuracy results

Accuracy (%Recovery)			
S. No	**Recovery level**	**Bempedoic acid**	**Ezetimibe**
1.	50%-1	99.0	99.36
2.	50%-2	99.2	99.12
3.	50%-3	100.7	99.76
4.	100%-1	100.5	98.22
5.	100%-2	99.4	100.31
6.	100%-3	99.7	99.13
7	150%-1	99.3	99.69
8	150%-2	100.9	101.42
9	150%-3	100.3	100.99

Robustness:

Robustness of the method was performed by changing flow rate, buffer pH, Organic, temperature. System suitability was conducted to check the variations and results were found satisfactory.

Limit of detection and limit of quantification:

Limit of detection (LOD) is the least concentration of analyte in a sample that can be identified but not quantified. Limit of quantification (LOQ) is defined the least concentration of analyte

in a sample that can be estimated with tolerable precision, accuracy, and reliability by a specified method under affirmed experimental conditions. The LOD were found to be 0.96 and 0.02 for Bempedoic acid and Ezetimibe respectively.The LOQ were found to be 2.91 and 0.07 for for Bempedoic acid and Ezetimibe respectively.

Degradation Studies: Degradation studies are acid, base, peroxide, thermal, UV and neutral conditions were evaluated. Further all stress results and force degradation results were tabulated in the table 6 and the resultant chromatograms were revealed in the Figure 7 to 12.

Table 6: Degradation results for bempedoic acid

Stress condition	**% Amount remaining**	**% Amount degraded**	**Peak Purity**	
			Purity Angle	**Purity Threshold**
Acid	95.00	5.00	0.260	0.478
Base	95.54	4.46	0.285	0.454
oxidation	94.04	5.96	1.230	1.399
Thermal	96.87	3.13	0.266	0.474
UV	98.11	1.89	0.221	0.441
Neutral	99.28	0.72	0.224	0.448

Table 7: Degradation results for Ezetimibe

Stress condition	**% Amount remaining**	**% Amount degraded**	**Peak Purity**	
			Purity Angle	**Purity Threshold**
Acid	94.72	5.28	1.414	1.743
Base	94.98	5.02	1.192	1.445
oxidation	94.43	5.57	4.076	4.171
Thermal	96.48	3.52	0.626	0.704
UV	97.64	2.36	1.078	1.390
Neutral	98.83	1.17	1.211	1.472

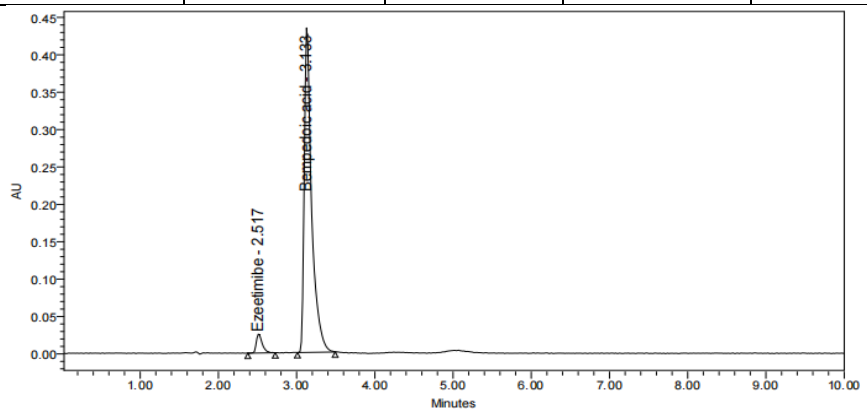

Figure 7 : Acid degradation chromatogram.

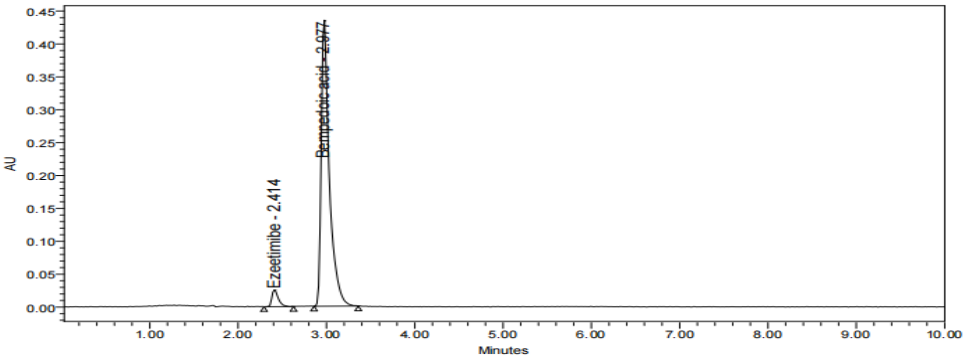

Figure 8: Base degradation chromatogram

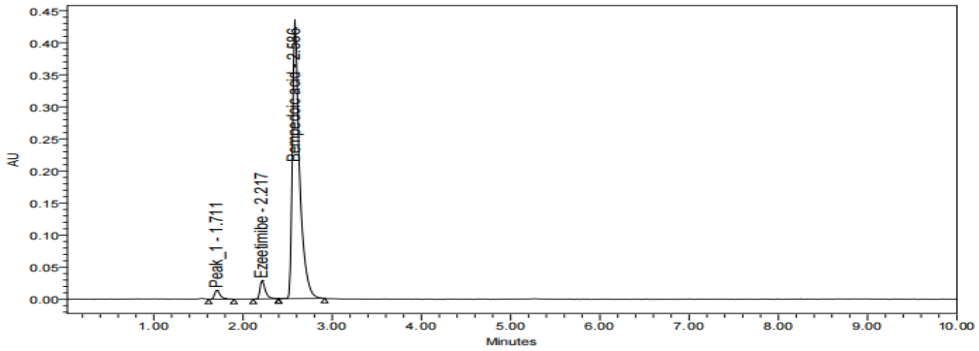

Figure 9: Oxidation degradation chromatogram

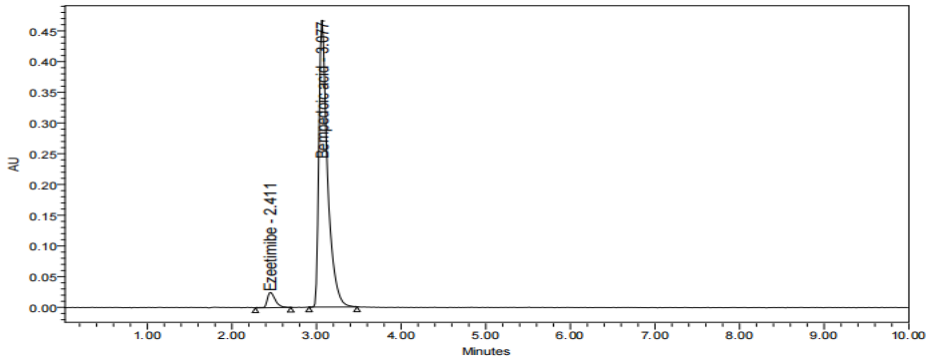

Figure 10 : Thermal degradation chromatogram

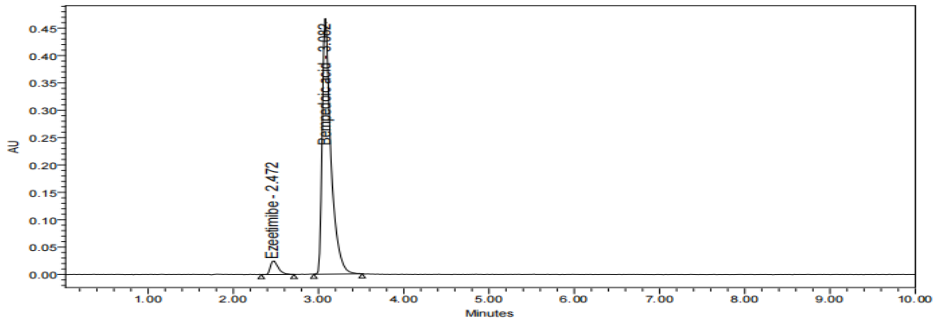

Figure 11 : UV degradation chromatogram

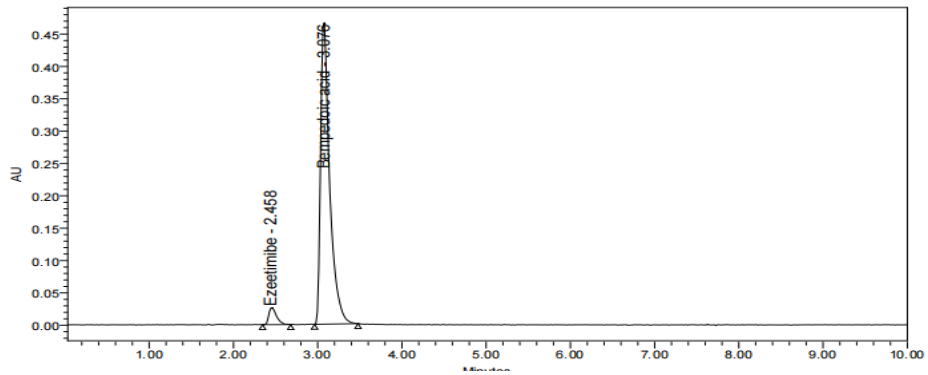

Figure 12: Water degradation chromatogram

RESULTS AND DISCUSSIONS:

The current study describes a new and simple, reliable, economic elution RP-HPLC-PDA method for the estimation of Bempedoic acid and Ezetimibe tablets dosage form. The forced degradation studies were conducted for the by using several degradation conditions like acidic, alkali, oxidation, thermal, UV, neutral conditions and the proposed method was effectively employed from the resolution of employed sample peaks. To our present knowledge, no such detailed and stability indicating method has been presented for this tablet dosage form. The developed method finished use of PDA as a tool for peak integrity and purity confirmation. Therefore the proposed study method can be used for the quantification of Bempedoic acid and Ezetimibe in the pharmaceutical dosage form. Finally, this method was carefully validated; as a result, it can be suggested for routine analysis and for testing quality through stability studies of the drugs.

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