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Quantification of Bempedoic Acid and Ezetimibe with Comprehensive Stability Assessment in Drug Substance and Drug Product By UPLC

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A sensitive and robust UPLC method was developed and validated for the simultaneous analysis of bempedoic acid and ezetimibe in drug substance and drug product. Chromatographic conditions were accomplished using a UPLC (Acquity) CSH C18 column (100 × 2.1 mm, 1.7 μm) under isocratic conditions, utilizing a movable phase comprising of acetonitrile and heptane sulphonic acid buffer (pH 2.5) /OPA in a 5:95 v/v ratio. The absorption wavelength was set at 232 nm, with a flow rate of 0.5 mL/min. International Council for Harmonisation Q2(R1) standards were used for method optimization and included stress studies. The retention times for bempedoic acid and ezetimibe were 0.365 and 1.326 minutes, respectively. The method demonstrated a regression coefficient ($R^2 > 0.999$), ruggedness (% RSD < 2%), recovery range (between 98–102%) and robustness. The LOD was 0.54 μg/mL for bempedoic acid and 0.03 μg/mL for ezetimibe. Both compounds exhibited sensitivity to acidic, oxidative, and thermal degradation, confirming the stability-indicating method of the compounds. Overall, the developed UPLC method is precise, accurate, specific, and suitable for quality analysis and stability assessment of bempedoic acid and ezetimibe in both drug substances and drug products.

Keywords: Bchandru@shcptirupati.edu.inempedoic Acid; Ezetimibe; ICH; Method Development; Stability Assessment; Simultaneous Quantification; UPLC; Validation.

Bempedoic acid is a lipid-lowering agent that acts as an ATP citrate lyase (ACL) inhibitor. [1] It reduces cholesterol biosynthesis in the liver, thereby lowering low-density lipoprotein cholesterol (LDL-C). It is particularly beneficial for patient's intolerant to statins. [2,3] Figure 1: Shows the structure of Bempedoic acid.

Ezetimibe is a cholesterol absorption inhibitor that blocks the Niemann–Pick C1-like 1 (NPC1L1) transporter in the intestinal brush border. This reduces the absorption of dietary and biliary cholesterol, complementing other lipid-lowering therapies. The combination of Bempedoic acid and Ezetimibe has demonstrated synergistic effects in the management of hyperlipidemia [4] Figure. 2 Represents the structure of Ezetimibe

Few analytical methods are available that can accurately quantify both compounds

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simultaneously with high sensitivity and speed. Ultra-Performance Liquid Chromatography (UPLC) is an advanced chromatographic technique that offers superior resolution, sensitivity, and speed compared to conventional HPLC. UPLC provides high efficiency and reduces analysis time, making it an ideal tool for pharmaceutical method development and validation. [4, 5]

Thus, the present work focuses on the development and validation of a simple, precise, and robust UPLC method for the simultaneous estimation of bempedoic acid and ezetimibe in bulk and pharmaceutical dosage forms, in accordance with ICH guidelines. The ICH standards were followed for development and validation of the method and subjected to stress degradation under various stress tests to ensure its applicability for stability analysis.

MATERIALS AND METHODS

Chemicals and Reagents

Bempedoic acid and ezetimibe pure drug samples were obtained from Shree Icon Pharmaceutical Laboratories Research centre Vijayawada. Acetonitrile, (HPLC grade, Merck, India) heptane sulfonic acid and ortho-phosphoric acid (AR grade, Merck, India) were used for the analysis. All other reagents and chemicals employed were of either analytical reagent (AR) grade or HPLC grade. High-purity Milli-Q water was used throughout the method for solution preparation and mobile phase composition.

Instrumentation

The chromatographic separation was performed on an ACQUITY UPLC system (Waters) equipped with Empower software version 2.0. A digital pH meter (Eutech) was used for pH adjustments. Weighing of samples was carried out using an analytical balance (Sartorius). A UV/VIS spectrophotometer (model UV-1700) and an ultrasonicator (model UCA 701, Unichrome) were employed during the study. Additionally, an isocratic pump model was used to maintain consistent flow rates during the chromatographic runs.

Selection of Detection Wavelength (ëmax)

A mixture of ACN and heptane sulfonic acid (pH 2.5) in a 5:95 ratio was used as the solvent. The drug mixture was scanned in the 200–400 nm range using a PDA detector, and the isosbestic point was identified at 232 nm. This wavelength was thus selected for detection in the UPLC method. Figure 3 Represents PDA - Spectrum of Bempedoic acid and Ezetimibe.

Chromatographic Conditions

Numerous trials were conducted to determine the most suitable chromatographic conditions. After optimization, the best set of parameters was chosen for consistent and effective separation of the analytes.

Preparation of Standard Solution

Accurately weigh 18 mg of bempedoic acid and 10 mg of eze working standards and transfer them into a clean 10 mL volumetric flask. Add about 7 mL of diluent and sonicate the mixture to achieve complete dissolution, then make up the volume to the 10 mL mark with the same diluent. From this stock solution, pipette 1 mL into a separate 10 mL volumetric flask and dilute to the mark. Additionally, mix 1 mL each of the bempedoic acid and ezetimibe stock solutions in another 10 mL volumetric flask and dilute to the mark. The final concentrations of 180 ppm for BA and 10 ppm for Eze.

Preparation of Sample Solution

Accurately weigh 23 mg of the sample containing both bempedoic acid and ezetimibe and transfer it into a 10 mL volumetric flask. Add the required volume of diluent and sonicate the mixture for 30 minutes to facilitate dissolution. After sonication, centrifuge the solution for 30 minutes to ensure clarity. Finally, make up the volume to the 10 mL mark with diluent and filter the solution through a 0.22 µm membrane filter prior to analysis.

General Preparations

Preparation of Heptane Sulfonic Acid Buffer

Dissolve 1.8 g of heptane sulfonic acid in 1 L to bring the pH to 2.5 using (OPA). Clarify the solution to eliminate particulate matter.

Preparation of Mobile Phase

The mobile phase was prepared by mixing the ACN and heptane sulfonic acid buffer (pH 2.5, adjusted with OPA) a ratio 95:5 (v/v). The mixture was then filtered to remove any impurities that might interfere with chromatographic performance.

Method Validation Parameters

The developed UPLC method was validated as per the (ICH) standards Q2(R1), addressing the following key parameters:

System Suitability

The system suitability studies were satisfied as the theoretical plate count exceeded 2000, the tailing factor remained below 2, and the resolution between the peaks was greater than 2.v w

Specificity

The developed method proved to be highly specific, as it clearly distinguished the analytes from excipients, degradation products, and possible impurities. Chromatograms of blank and placebo samples showed no interfering peaks at the retention times of BA and Eze. [8,9]

Linearity

Calibration curves were prepared for BA (45–270 µg/mL) and Eze (2.5–15 µg/mL). Both analytes exhibited excellent linearity with correlation coefficients (R^2) of 0.99989 and 0.99981, respectively.

Precision

System precision was confirmed as six replicate injections of the standard gave %RSD values below 2% for both drugs. Method precision (repeatability) with six sample preparations showed %RSD values of 0.90% for BA and 0.55% for Eze. Intermediate precision, evaluated through day-to-day variability, also met the acceptance criteria. [1p 11]

Accuracy

Recovery studies at 50%, 100%, and 150% levels yielded mean recoveries of 100.3% for BA and 99.9% for Eze, which fall within the acceptable recovery range of 98–102%, confirming the method's accuracy. [12 13]

Robustness

Robustness testing was carried out by making small variations in the flow rate (±0.05 mL/min) and the organic phase ratio (±0.5%). These deliberate changes produced %RSD values less than 2%, establishing the robustness of the method. [1t ,1u]

LOD and LOQ

The limits of detection and quantification were calculated using the ICH formulae:

LOD = 3.3 × ó/S

LOQ = 10 × ó/S

For bempedoic acid, the LOD and LOQ were 0.54 µg/mL and 1.80 µg/mL, respectively, whereas for ezetimibe, the LOD was 0.03 µg/mL and the LOQ was 0.10 µg/mL. These values indicate the high sensitivity of the developed method. [1v ,1w]

RESULTS

System Suitability

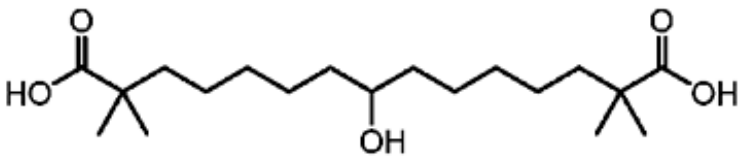
Retention time for bempedoic acid was 0.365 min, ezetimibe was 1.326 min, theoretical plates were >8000, tailing factor was <1.2 and resolution was >9. The results are summarized in Table 1. Figure 4 Displays blank and standard chromatogram for bempedoic acid and ezetimibe.

Specificity

Specificity was confirmed by the absence of interference peaks at the retention time of analytes in the blank and placebo chromatograms.

Precision

For system precision, % RSD for BA is 0.98% and Eze is 1.31%. In method precision (repeatability), % RSD for BA is 0.90%, and Eze is 0.55%. Intermediate Precision (Day-to-Day) % RSD is <1.3 for both drugs. Table 2 summarizes



(1)

Fig. 1. Structure of Bempedoic acid

system precision results of BA and Eze. Table 3 Method Precision Data for BA and Eze Table 4 Presents intermediate precision (day variation) for BA and Eze.

Linearity

Regression line of BA was 45–270 µg/mL (R^2 = 0.99989), and Eze was 2.5–15 µg/mL (R^2 = 0.99981). Table 5 Summarizes the results of linearity for BA and Eze, Figure 5 Shows the Calibration curve for Bempedoic acid and Figure 6 displays the Calibration curve for Ezetimibe.

Accuracy

Mean recovery for BA is 100.3% and Eze is 99.9%. Recovery range within 98–102% for all levels. Table 6 and Table 7 Summarizes the Accuracy results of Bempedoic acid and Ezetimibe.

Robustness

Method was robust against small variations in flow rate and organic phase. All % RSD values <2%. Table 8 and Table 9 Represents the Robustness results of BAand Robustness results of Eze.

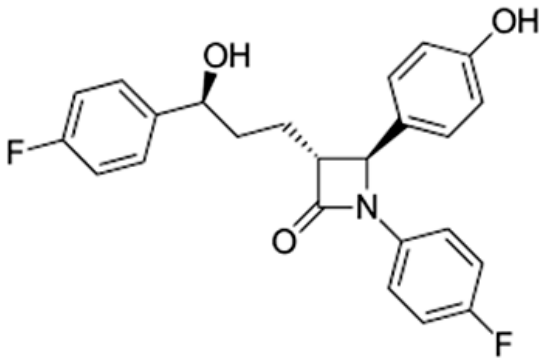


Fig. 2. Structure of Ezetimibe

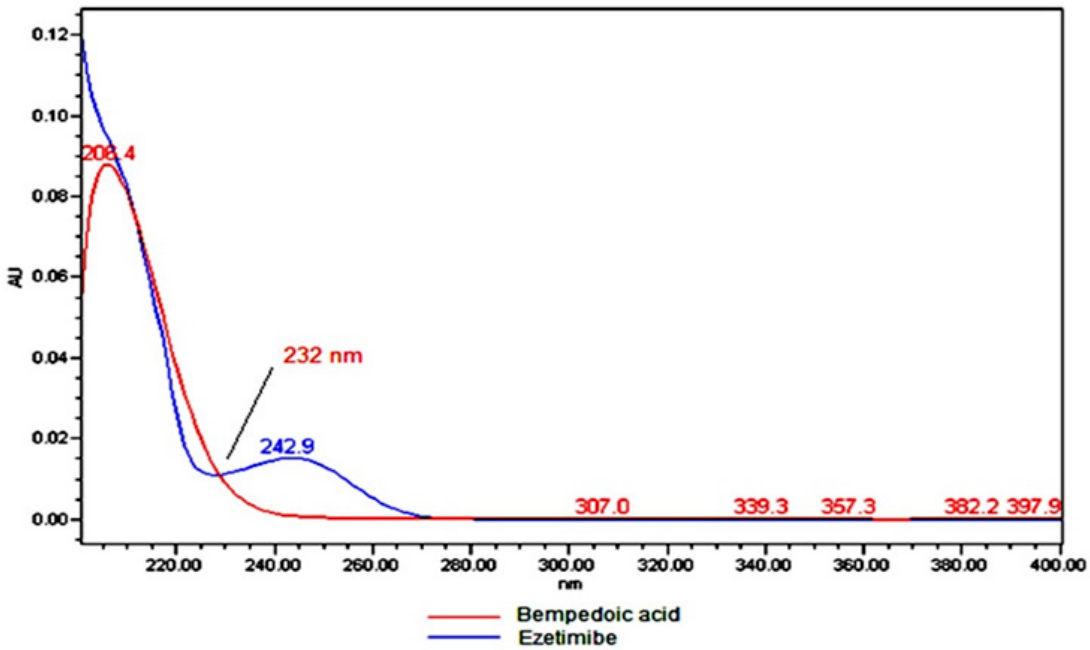


Fig. 3. PDA - Spectrum of Bempedoic acid and Ezetimibe

LOD and LOQ

The LOD was 0.54 and 1.80 for BA and the LOQ was 0.03 and 0.10 for Eze. Table 10 summarizes the sensitivity parameters (LOD & LOQ) by UPLC.

Table 1. System suitability parameters for Bempedoic acid and Ezetimibe

No	Parameter	Bempedoic acid	Ezetimibe
1	Retention time	0.365	1.326
2	Theoretical plates	14875	8320
3	Tailing factor	1.15	0.88
4	Resolution	—	9.45
5	%RSD	0.98	1.31

Stress Degradation Study

The analytes were exposed to a range of stress conditions in accordance with ICH Q1A(R2) in order to demonstrate the methods stability-indicating assessment.

Acid Hydrolysis

Stock solution of 1 mL of was mixed with 1 mL of 1N HCl and heated at 60°C for one hour. After neutralization with 1N NaOH, the solution was diluted with diluent to the required volume and filtered prior to analysis. [1]x

Alkali Hydrolysis

A mixture of 1 mL stock solution and 1 mL of 1N NaOH was heated at 60°C for one hour. The solution was then neutralized using 1N HCl, diluted, filtered, and analysed.[1]

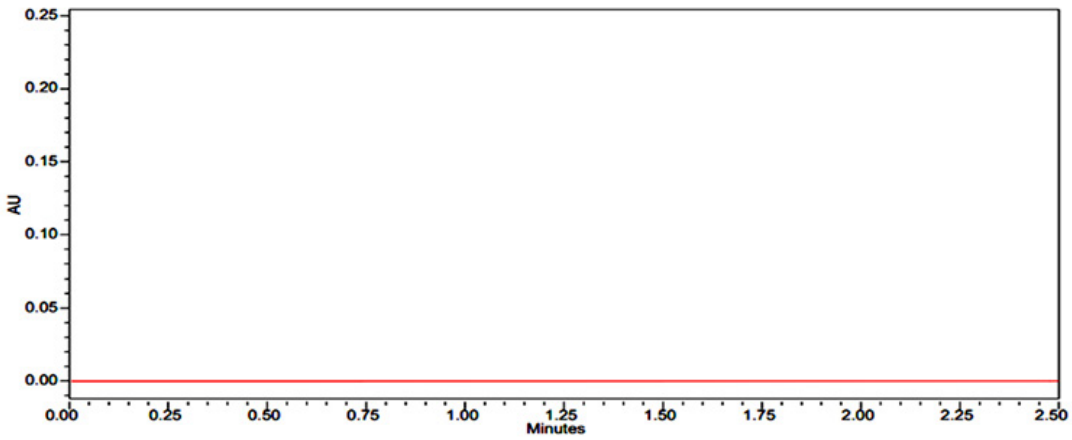


Fig. 4. Blank Chromatogram for Bempedoic acid and Ezetimibe

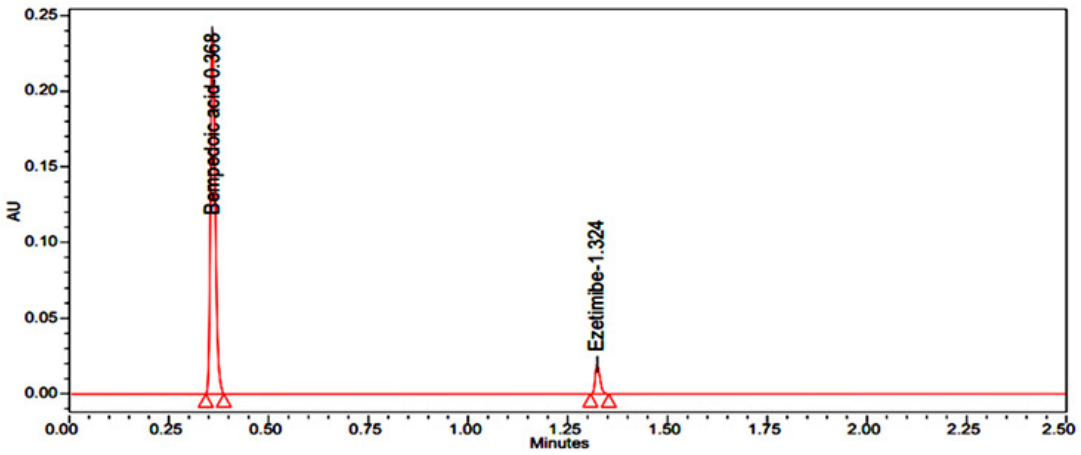


Fig. 5. Standard Chromatogram for Bempedoic acid and Ezetimibe

Oxidative Degradation

To induce oxidative stress, 1 mL of stock solution was combined with 1 mL of 10% H_2O_2 and heated at 60°C for one hour. The sample was cooled, diluted, filtered, and injected into the system.

Thermal Degradation

The solid drug substance was placed in a petri dish and exposed to dry heat at 105°C for 3 hours. The degraded material was dissolved in diluent and analysed.

Table 2. System precision table of Bempedoic acid and Ezetimibe

No	Concentration Bempedoic acid (µg/ml)	Area of Bempedoic acid	Concentration of Ezetimibe (µg/ml)	Area of Ezetimibe
1.	180	2254187	10	122840
2.	180	2236410	10	121682
3.	180	2208641	10	124874
4.	180	2217413	10	123564
5.	180	2234512	10	122462
6.	180	2267451	10	120158
Mean		2236436		122597
SD		21978.020		1611.400
%RSD		0.98		1.31

Table 3. Method Precision for Bempedoic acid and Ezetimibe

No.	Area for Bempedoic acid	Area for Ezetimibe
1	2218460	123487
2	2214587	122132
3	2256974	123652
4	2251478	123461
5	2261058	123054
6	2231476	122187
Average	2239006	122996
Standard Deviation	20198.104	677.050
%RSD	0.90	0.55

Table 4. Intermediate Precision (Day variation) for Bempedoic acid and Ezetimibe

S. No.	Area for Bempedoic acid		Area for Ezetimibe	
	Day-1	Day-2	Day-1	Day-2
1	2210369	2263140	123045	125364
2	2240178	2271036	122368	124632
3	2236204	2256418	125132	123459
4	2254123	2201436	123925	122874
5	2210632	2210547	122024	121850
6	2240165	2245178	123236	125450
Average	2231945	2241293	123288	123938
Standard Deviation	17692.491	28773.923	1123.562	1450.692
%RSD	0.79	1.28	0.91	1.17

Table 5. Results of linearity for Bempedoic acid and Ezetimibe

S. No	Bempedoic acid		Ezetimibe	
	Conc.(µg/ml)	Peak area	Conc.(µg/ml)	Peak area
1	45.00	563546	2.50	30710
2	90.00	1127093	5.00	61420
3	135.00	1690639	7.50	94130
4	180.00	2254187	10.00	122840
5	225.00	2817733	12.50	153550
6	270.00	3321280	15.00	181260
Regression equation	y =12380.40x + 10713.86		y = 12155.43x + 821.43	
Slope	12380.40		12155.43	
Intercept	10713.86		821.43	
R^2	0.99989		0.99981	

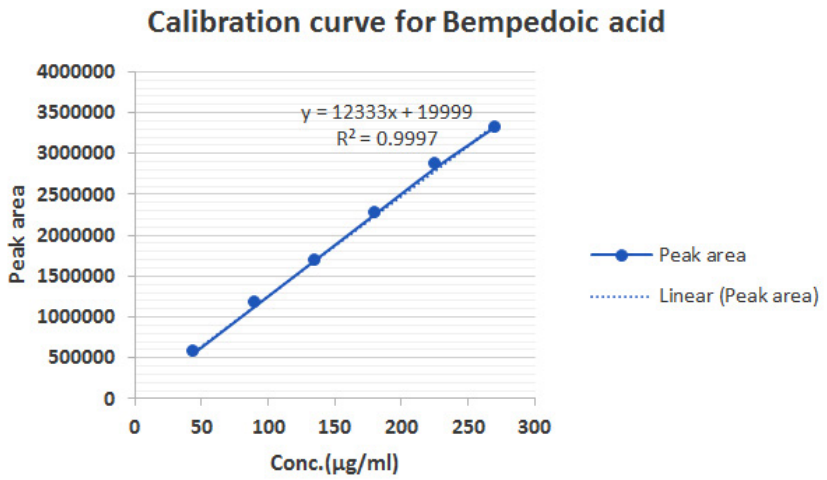


Fig. 5. Calibration curve for Bempedoic acid

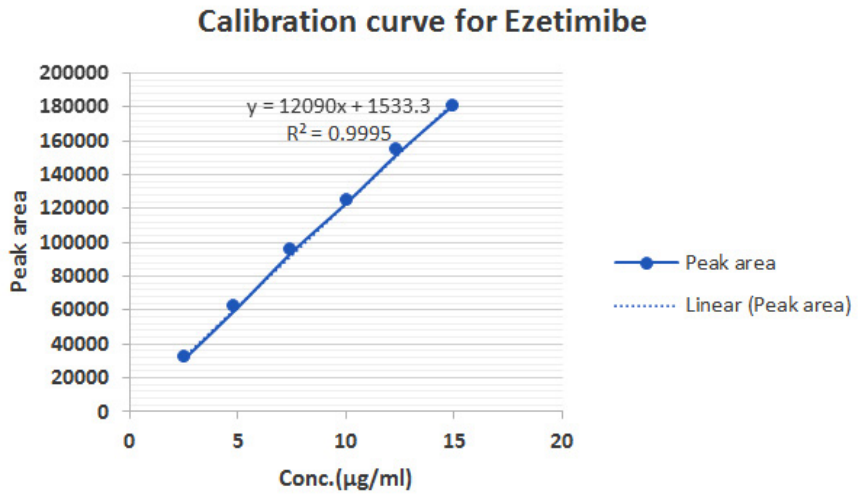


Fig. 6. Calibration curve for Ezetimibe

Photolytic Degradation

Solid samples were exposed to UV light in a photostability chamber for 3 hours. The treated material was then diluted with diluent and subjected to chromatographic analysis.

Reduction Degradation

For reduction stress, 1 mL of stock solution was mixed with 1 mL of 10% sodium bisulfite solution and heated at 60°C for one hour. After cooling, the sample was filtered and injected.

Table 6. Accuracy results of Bempedoic acid

% Concentration	Response	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1115729	9.00	8.98	99.8	100.3
	1123105	9.00	9.04	100.4	
	1126941	9.00	9.07	100.8	
100%	2231458	18.00	17.96	99.8	100.4
	2241305	18.00	18.04	100.2	
	2265841	18.00	18.24	101.3	
150%	3347187	27.00	26.94	99.8	100.0
	3352694	27.00	26.98	99.9	
	3365321	27.00	27.09	100.3	

Table 7. The Accuracy results for Ezetimibe

% Concentration	Response	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	61421	0.5	0.501	100.2	99.9
	61132	0.5	0.499	99.8	
	61230	0.5	0.499	99.8	
100%	120648	1.0	0.984	98.4	99.3
	121870	1.0	0.994	99.4	
	122541	1.0	1.000	100.0	
150%	182472	1.5	1.488	99.2	100.1
	183642	1.5	1.498	99.9	
	186403	1.5	1.520	101.3	

Table 8. Robustness results of Bempedoic acid

Parameter	Bempedoic acid						
	Condition	Retention time(min)	Peak area	Resolution	Tailing	Plate count	% RSD
Flow rate Change (mL/min)	Less flow (0.45ml)	0.468	2031732	-	1.19	14753	0.76
	Actual (0.50ml)	0.365	2254187	-	1.15	14815	0.98
	More flow (0.55ml)	0.273	2351468	-	1.11	14956	0.70
Organic Phase Change	Less Org (4.5:95.5)	0.563	1945328	-	1.21	14623	0.60
	Actual (5:95)	0.368	2236410	-	1.12	14898	0.98
	MoreOrg (5.5:94.5)	0.186	2540187	-	1.09	15021	1.11

Hydrolytic Degradation

A mixture of 1 mL stock solution and 1 mL of HPLC-grade water was heated at 60°C for one hour. The resulting solution was filtered and analysed.[20]

DISCUSSION

The developed UPLC method demonstrated excellent system suitability with retention times of 0.365 min for Bempedoic acid (BA) and 1.326 min for Ezetimibe (Eze),

Table 9. Robustness results of Ezetimibe

Parameter	Condition	RT (min)	Peak area	Ezetimibe Resolution	Tailing	Plate count	% RSD
Flow rate Change (mL/min)	Less flow (0.45ml)	1.541	114230	10.17	0.92	8121	1.26
	Actual (0.50ml)	1.326	122840	9.42	0.88	8320	1.31
	More flow (0.55ml)	1.225	131524	9.33	0.85	8475	0.62
Organic Phase Change	Less Org (4.5:95.5)	1.601	96146	9.88	0.99	8063	0.63
	Actual (5:95)	1.324	121682	9.44	0.86	8369	1.31
	MoreOrg (5.5:94.5)	1.011	143210	8.21	0.81	8566	1.00

Table 10. Sensitivity parameters (LOD & LOQ)

Drug Name	LOD(µg/ml)	s/n	LOQ(µg/ml)	s/n
Bempedoic acid	0.54	3	1.80	10
Ezetimibe	0.03	3	0.10	10

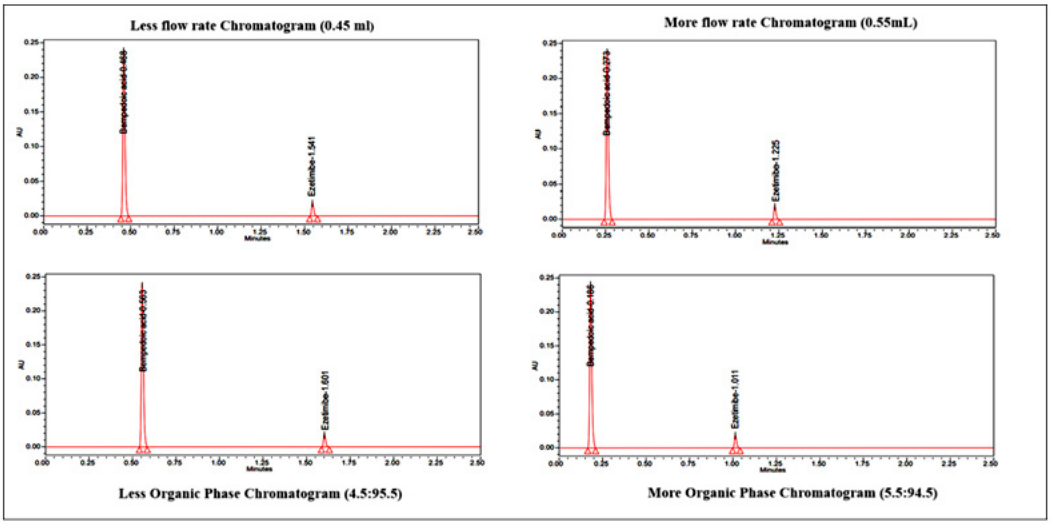


Fig. 7. Robustness chromatograms of Bempedoic acid and Ezetimibe

theoretical plates exceeding 8000, a tailing factor below 1.2, and resolution greater than 9. Linearity was observed over the ranges of 45–270 µg/mL for BA (R^2 = 0.99989) and 2.5–15 µg/mL for Eze (R^2 = 0.99981). Precision studies showed %RSD values below 2% for system, method, and intermediate precision, while accuracy studies gave mean recoveries of 100.3% for BA and 99.9% for

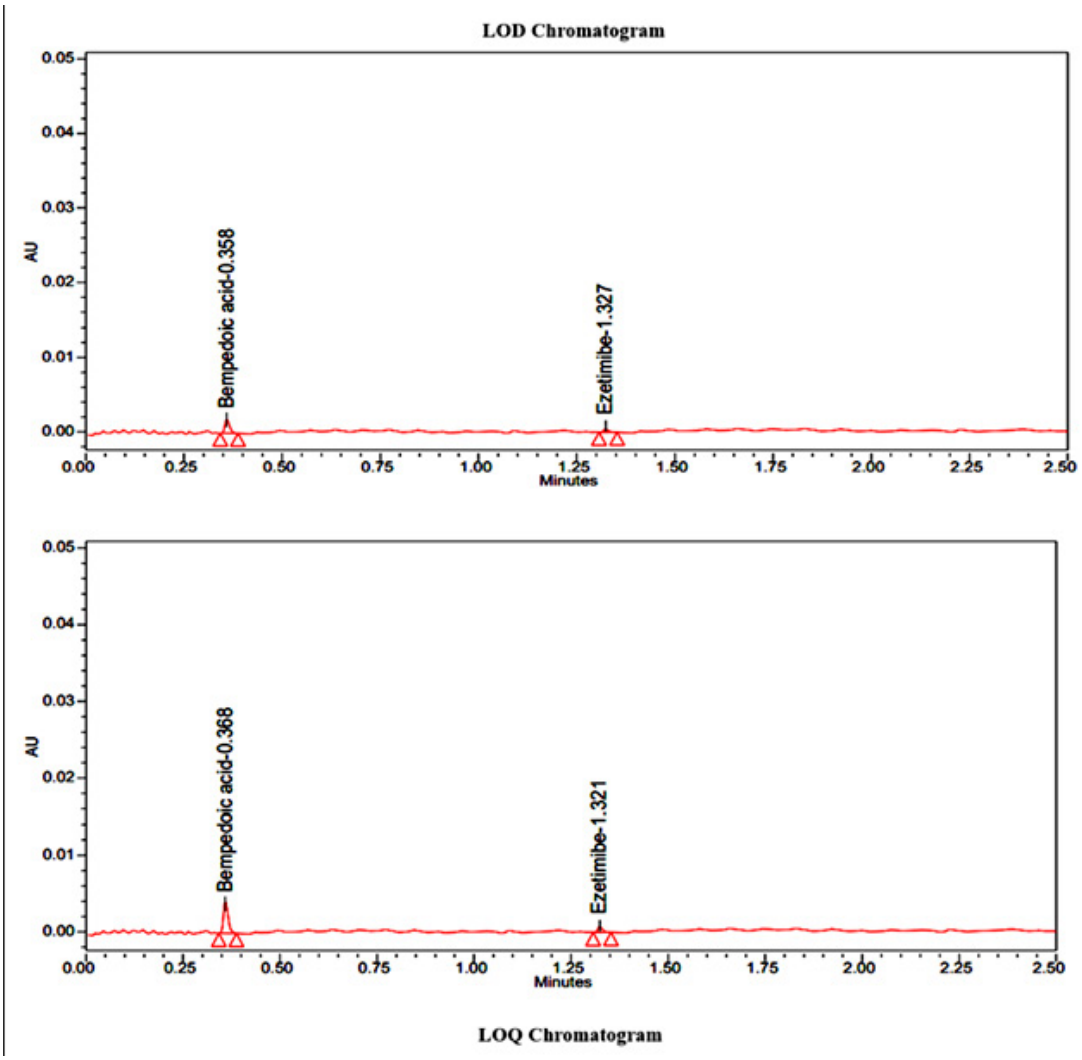


Fig. 8. LOD & LOQ chromatograms of Bempedoic acid and Ezetimibe

Table 11. Forced Degradation results for Bempedoic acid and Ezetimibe

Results:	Bempedoic acid					Ezetimibe				
% Degradation results	Area	% Assay	% Deg	Purity Angle	Purity Threshold	Area	% Assay	% Deg	Purity Angle	Purity Threshold
Control	2229638	100	0	6.325	9.959	123091	100	0	5.128	16.295
Acid	2154889	96.6	3.4	6.318	9.947	112346	91.3	8.7	5.163	16.241
Alkali	1985423	89.0	11.0	6.359	9.921	118330	96.1	3.9	5.154	16.255
Peroxide	1941404	87.0	13.0	6.336	9.963	105584	85.8	14.2	5.102	16.239
Reduction	2142098	96.0	4.0	6.344	9.989	119437	97.0	3.0	5.187	16.203
Thermal	2038517	91.4	8.6	6.302	9.914	122589	99.6	0.4	5.149	16.257
Photolytic	2210212	99.1	0.9	6.369	9.932	119028	96.7	3.3	5.183	16.298
Hydrolysis	2213965	99.3	0.7	6.387	9.991	121410	98.6	1.4	5.106	16.243

Eze, well within the acceptance range of 98–102%. The method proved robust to minor changes in chromatographic conditions, with all %RSD values remaining below 2%. Sensitivity was confirmed with LOD/LOQ values of 0.54/1.80 µg/mL for BA and 0.03/0.10 µg/mL for Eze. Forced degradation studies revealed that both drugs were relatively stable under hydrolytic and photolytic conditions, while moderate degradation occurred under acidic, basic, and oxidative stress. The highest degradation was observed for BA under alkaline conditions (11.0%) and for Eze under peroxide stress (14.2%). In all stress conditions, the purity angle values were less than the purity threshold, confirming the stability-indicating capability of the method. Overall, the results demonstrate that the proposed UPLC method is specific, precise, accurate, robust, and suitable for the stability evaluation and routine quality control of bempedoic acid and ezetimibe in fixed-dose formulations. [21,22]

Moreover, our method achieved fast runtime (2.5 min), excellent resolution (9.45), and low LOD/LOQ values. Use of an isocratic mode, which simplifies the method compared to gradient-based HPLC methods. This makes the present UPLC method one of the most efficient,

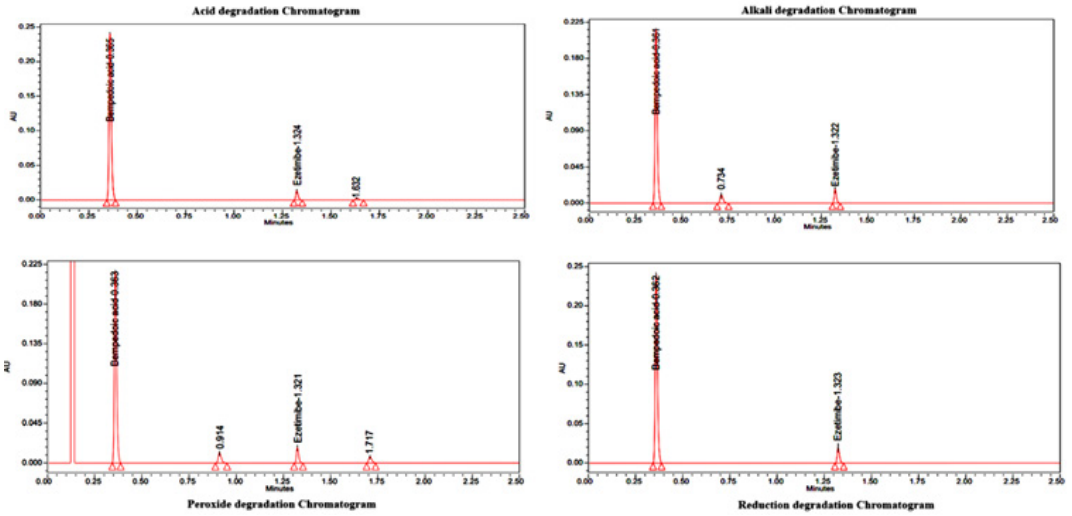


Fig. 9. Forced Degradation chromatograms Bempedoic acid and Ezetimibe

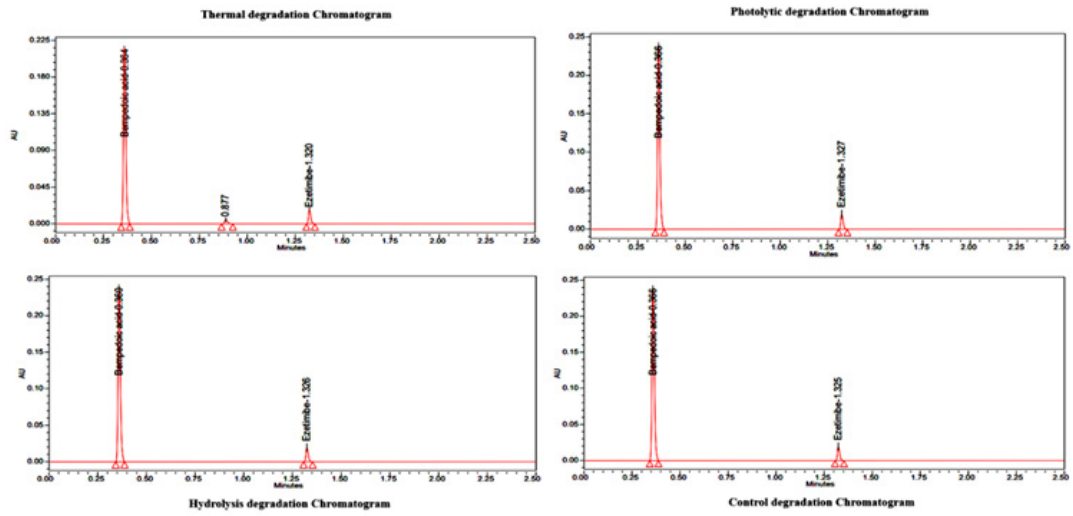


Fig. 10. Forced Degradation chromatograms Bempedoic acid and Ezetimibe

Comparative literature table

Column / System	Mobile Phase	Flow / λ	Retention (BEM/EZT)	Linearity & LOD/LOQ	Reference
Waters Acquity C18 UPLC, 50×2.1/ mm, 1.7/ µm	Methanol: ACN: Water (50:30:20), 0.5/ mL/min	UV @/ 260/ nm	1.83/ min / 3.58/ min	BEM: 30–130/ µg/mL; EZT: 5–50/ µg/mL; LOD H"0.12/ µg/mL, LOQ H"0.36/ µg/mL	14
X-Bridge Phenyl (150×4.6/ mm, 3.5/ µm) RP-UPLC	0.1% formic acid/ ACN (70:30), 1.0/ mL/min	UV @/ 230/ nm	1.16/ min / 3.21/ min	BEM: 27–337.5/ µg/mL (R^2=0.9991); EZT: 1.5–18.75/ µg/mL (R^2=0.9993); LOD/LOQ BEM: 0.27/2.7/ µg/mL; EZT: 0.015/0.15/ µg/mL	15
Waters C18 (150×4.6/ mm, 3.5/ µm) UPLC–MS/MS	0.1% OPA/ACN (50:50), 1.0/ mL/min	UV @/ 230/ nm; MS detection	BPA 2.457 EZM 3.888	LOD BPA 0.225 µg/mL and EZM 0.013 µg/mL LOQ 0.743 µg/mL and 0.043 µg/mL	16
Phenyl XBD (100×2.1/ mm, 1.7/ µm) RP-UPLC	0.1% TFA/H, O–ACN, ambient	UV @/ 230/ nm	BPA 0.422 EZM 0.87	LOD/LOQ approx. 0.225/0.743/†µg/mL (BEM); 0.013/0.043/ µg/mL (EZT)	17

robust, and cost-effective analytical tools available for quality analysis of the BA–Eze combination in drug products. [23,24]

CONCLUSION

A sensitive and robust UPLC method was developed and validated for the simultaneous analysis of Ezetimibe (Eze) and Bempedoic acid (BA). The method proved to be robust, linear across a wide concentration range, specific, accurate, and precise. Stress degradation studies confirmed that the method meets ICH requirements for stability-indicating assays by effectively distinguishing the active drug substances from their degradation products. Therefore, this method can be reliably applied for stability testing and quality evaluation of fixed-dose combination formulations containing both drugs.

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This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials.

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Not Applicable

Author Contributions

Madhu Reddemma: Conceptualization, Methodology; Chandrasekar Raju: Writing – Original Draft; Pratyusha Valligatla: Data Collection, Analysis; Chandrasekar Raju: Writing – Review & Editing; Sivagami Bojan: Visualization,

Supervision; Mounika Tummalapalli: Funding Acquisition; Charumathi Salva: Resources, Supervision; Sunil Kumar Ellampati: Project Administration.

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