RESEARCH PAPER

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

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ABSTRACT

Validated analytical methods are the backbone of pharmaceutical quality assurance, ensuring that drug products meet safety and efficacy standards. The present study aimed to develop and validate a simple, accurate, precise, and cost-effective reverse-phase high-performance liquid chromatographic (RP-HPLC) method for the simultaneous quantification of Bempedoic acid and Ezetimibe in bulk powder and tablet dosage form, suitable for routine pharmaceutical quality control.

Chromatographic separation was accomplished using a Kromosil C18 column (150 × 4.6 mm, 3.0 µm particle size) with a mobile phase of 0.01 N potassium dihydrogen phosphate (KH2PO4) buffer and methanol (70:30 v/v), pumped at a flow rate of 0.9 mL/min. The column temperature was maintained at 30°C, with UV detection at 260 nm and an injection volume of 10 µL. The method was validated in accordance with ICH Q2(R1) guidelines for system suitability, linearity, precision, accuracy, robustness, limit of detection (LOD), limit of quantification (LOQ), specificity, and drug assay.

Retention times were 2.780 min for Bempedoic acid and 2.123 min for Ezetimibe. Excellent linearity was observed with regression equations y = 28262x + 4418.6 (R^2 = 0.9999) for Bempedoic acid and y = 28796x + 190.13 (R^2 = 0.9999) for Ezetimibe. System precision %RSD values were 0.6% and 1.3% for Bempedoic acid and Ezetimibe, respectively. Mean percentage recoveries were 99.87% and 100.12%, respectively. LOD values were 0.10 and 0.01 µg/mL, and LOQ values were 0.35 and 0.03 µg/mL for Bempedoic acid and Ezetimibe, respectively.

The developed RP-HPLC method was found to be simple, rapid, specific, and reliable for the simultaneous estimation of Bempedoic acid and Ezetimibe in bulk and tablet dosage forms, with compliance to ICH guidelines confirming its suitability for industrial quality control applications.

Keywords: *Bempedoic acid; Ezetimibe; RP-HPLC; Simultaneous estimation; ICH validation; Tablet dosage form; LOD; LOQ; Precision; Accuracy*

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INTRODUCTION

Ensuring pharmaceutical quality is a fundamental requirement for patient safety and therapeutic efficacy. Quality assurance and quality control programs are indispensable components of drug manufacturing, demanding robust, validated, and reproducible analytical methods. The physicochemical diversity of active pharmaceutical ingredients (APIs) makes method development a technically demanding process, requiring careful optimization of selectivity, sensitivity, precision, and accuracy. High-performance liquid chromatography (HPLC), particularly the reverse-phase mode, is the most widely adopted technique in pharmaceutical analysis due to its exceptional resolving power, broad applicability, and compatibility with various detection systems.

Bempedoic acid is a first-in-class ATP-citrate lyase (ACL) inhibitor approved by the US Food and Drug Administration (FDA) on February 21, 2020, under the brand name Nexletol (Nilemdo). It is indicated as an adjunct to diet and maximally tolerated statin therapy for adults with heterozygous familial hypercholesterolemia or established atherosclerotic cardiovascular disease who require additional LDL-cholesterol (LDL-C) lowering. Its molecular formula is $C_{19}H_{36}O_5$ with a molecular weight of 344.492 g/mol. As a prodrug, it undergoes hepatic activation via very-long-chain acyl-CoA synthetase-1 (ACSVL1) to form the active metabolite ETC-1002-CoA, which inhibits ACL and ultimately upregulates LDL-C receptor expression, enhancing LDL clearance.

Ezetimibe is a selective cholesterol absorption inhibitor that targets the Niemann-Pick C1-Like 1 (NPC1L1) transporter in the small intestine, reducing intestinal cholesterol delivery to the liver without interfering with the absorption of fat-soluble vitamins or nutrients. Ezetimibe is indicated for primary hyperlipidemia, mixed

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hyperlipidemia, and homozygous familial hypercholesterolemia. The fixed-dose combination of Bempedoic acid (180 mg) and Ezetimibe (10 mg), marketed as Bempetol, was approved on February 26, 2020, offering enhanced LDL-C reduction in statin-intolerant patients.

A literature survey revealed that while some analytical methods exist for the individual determination of these drugs, limited validated RP-HPLC methods have been reported for their simultaneous estimation with short run times. The present study describes the development and ICH Q2(R1)-compliant validation of a rapid, simple, and economical RP-HPLC method for the simultaneous quantification of Bempedoic acid and Ezetimibe in bulk and tablet dosage form.

MATERIALS

Drug Samples and Reagents

Bempedoic acid and Ezetimibe were obtained as working standards. The commercial tablet formulation used was Bempetol, containing Bempedoic acid (180 mg) and Ezetimibe (10 mg) per tablet. HPLC-grade methanol, potassium dihydrogen orthophosphate (KH2PO4), orthophosphoric acid, and Milli-Q water were used for mobile phase preparation and sample dissolution.

Instrumentation

Chromatographic analysis was performed on a WATERS HPLC 2695 system equipped with quaternary pumps, a photodiode array (PDA) detector, and an autosampler integrated with Empower-2 data processing software. UV spectral scanning was carried out using a PG Instruments T60 UV-VIS spectrophotometer (2 nm bandwidth, 10 mm matched quartz cells) with UV Win 6 software. An electronic analytical balance (Denver Instruments) and a pH meter (BVK Enterprises, India) were used for weighing and pH adjustment, respectively. An ultrasonicator (BVK, India) was used for dissolution and degassing.

RP-HPLC Method Development

UV Spectral Analysis

Solutions of Bempedoic acid and Ezetimibe were individually scanned over the UV wavelength range of 200–400 nm. Both drugs displayed UV absorption maxima at 260 nm. Accordingly, 260 nm was chosen as the common detection wavelength for simultaneous RP-HPLC analysis.

Selection of Chromatographic Conditions

Reverse-phase HPLC was selected based on the polar nature and aqueous solubility profiles of the analytes. Multiple mobile phase combinations were evaluated to achieve adequate resolution, acceptable retention times, and satisfactory peak symmetry. A Kromosil C18 column (150 × 4.6 mm, 3.0 µm) was selected as the stationary phase. The optimized mobile phase consisted of 0.01 N KH2PO4 buffer and methanol (70:30 v/v) with the buffer pH adjusted to 4.0 using dilute orthophosphoric acid. Under these conditions, both analytes were resolved with acceptable peak shapes, tailing factors, and plate counts within a total run time of 5 minutes.

Table 1. Optimized chromatographic conditions

Parameter	Condition
Column	Kromosil C18 (150 × 4.6 mm, 3.0 µm)
Mobile phase	0.01 N KH2PO4 buffer : Methanol (70:30 v/v)
Flow rate	0.9 mL/min
Detection wavelength	260 nm
Column temperature	30°C
Injection volume	10 µL
Run time	5 min
Diluent	Milli-Q water

ANALYTICAL METHOD — SOLUTION PREPARATION

Preparation of Buffer Solutions

0.01 N KH2PO4 Buffer: Exactly 1.36 g of potassium dihydrogen orthophosphate was accurately weighed and dissolved in approximately 900 mL of Milli-Q water in a 1000 mL volumetric flask, followed by degassing and sonication. The volume was adjusted to 1000 mL with Milli-Q water, and the pH was set to 4.0 using dilute orthophosphoric acid.

0.1% Orthophosphoric Acid Solution: Exactly 1 mL of concentrated orthophosphoric acid was transferred to a 1000 mL volumetric flask, diluted with approximately 100 mL of Milli-Q water, and made up to the final volume.

Preparation of Standard Stock Solutions

Exactly 2.5 mg of Ezetimibe and 45 mg of Bempedoic acid were accurately weighed and transferred separately into 50 mL volumetric flasks. Three-quarters of the diluent volume was added to each flask, followed by sonication for 10 minutes. The volumes were made up to the mark with diluent to obtain Standard Stock 1 (50 µg/mL

Ezetimibe) and Standard Stock 2 (900 µg/mL Bempedoic acid).

Preparation of Standard Working Solutions (100%)
One milliliter each from Standard Stock 1 and Standard Stock 2 was pipetted into a 10 mL volumetric flask and diluted to the mark with diluent to obtain final concentrations of 5 µg/mL Ezetimibe and 90 µg/mL Bempedoic acid.

Preparation of Sample Stock Solutions
Five tablets of Bempetol were accurately weighed and the mean tablet weight was calculated. A quantity equivalent to one tablet was transferred to a 10 mL volumetric flask, and 5 mL of diluent was added. The mixture was sonicated for 25 minutes, and the volume was made up to the mark. The solution was filtered through HPLC-grade syringe filters to yield concentrations of 100 µg/mL Ezetimibe and 1800 µg/mL Bempedoic acid.

Preparation of Sample Working Solutions (100%)
A volume of 0.5 mL of the filtered sample stock solution was transferred to a 10 mL volumetric flask and diluted to the mark with diluent to obtain a final concentration of 5 µg/mL Ezetimibe and the corresponding concentration of Bempedoic acid.

Analytical Method Validation
The developed RP-HPLC method was validated as per ICH Q2(R1) guidelines. Validation parameters assessed included system suitability, linearity, precision (system precision and repeatability), accuracy, robustness, LOD, LOQ, specificity, and drug assay.

System Suitability
System suitability was assessed by injecting six replicate injections of the standard working solution. Parameters including retention time, USP plate count, tailing factor, and resolution were recorded and compared against acceptance criteria (tailing factor ≤ 2.0, plate count > 2000, resolution> 2.0).

Linearity
Calibration standards were prepared at six concentration levels ranging from 25% to 150% of the working concentration for both analytes. Peak areas were plotted against concentration, and regression analysis was performed to obtain the slope, intercept, and coefficient of determination (R^2).

Precision
System precision was evaluated by analyzing six replicate injections from a single working standard solution. Method precision (repeatability) was assessed by preparing six independent sample working solutions of the same concentration and injecting each once. %RSD was computed for both drugs.

Accuracy
Accuracy was determined by the standard addition method at three concentration levels (80%, 100%, and 120% of the working concentration), with triplicate injections at each level. The mean percentage recovery was calculated for both analytes.

Robustness
Robustness was studied by deliberately introducing small changes to the optimized method parameters: flow rate minus (0.8 mL/min) and flow rate plus (1.0 mL/min), mobile phase ratio minus (65B:35A) and plus (75B:25A), and column temperature minus (24°C) and plus (34°C). Samples were injected in triplicate under each condition, and %RSD values were calculated.

Limit of Detection and Limit of Quantification
LOD and LOQ were determined from the calibration curve regression data using the following equations:

$$LOD = 3.3 \times \sigma / S \quad (Eq.\ 1)$$

$$LOQ = 10 \times \sigma / S \quad (Eq.\ 2)$$

where σ is the standard deviation of the y-intercept and S is the mean slope of the calibration curve.

Specificity
Specificity was assessed by examining chromatograms of blank solution, standard solution, and sample solution to confirm that no interfering peaks eluted at the retention times of Bempedoic acid and Ezetimibe.

Degradation Studies
Forced degradation studies were conducted under acid (HCl), alkali (NaOH), oxidative (H2O2), thermal, and UV stress conditions. Degraded samples were injected and the percentage drug remaining was calculated, confirming that degradation products did not interfere with the analyte peaks.

RESULTS AND DISCUSSION

UV Spectral Analysis
UV scanning of Bempedoic acid and Ezetimibe solutions over the range 220–400 nm revealed a common absorption maximum at 260 nm for both drugs. This wavelength was selected for HPLC detection to ensure maximum sensitivity for simultaneous quantification.

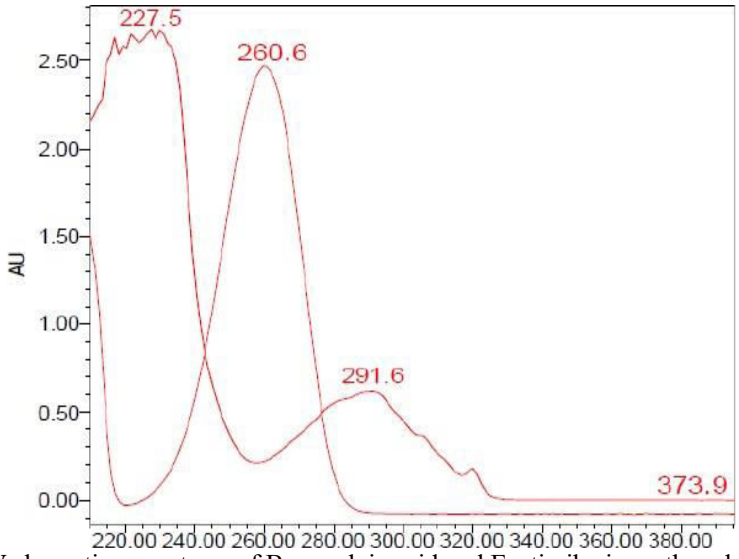

Fig. 1. UV absorption spectrum of Bempedoic acid and Ezetimibe in methanol (260 nm).

System Suitability

The results of system suitability evaluation from six replicate injections are presented in Table 2. Retention times were reproducible at 2.119–2.123 min for Ezetimibe and 2.773–2.780 min for Bempedoic acid. USP plate counts, tailing factors, and resolution values all met the prescribed acceptance criteria, confirming that the chromatographic system was suitable for the intended analysis.

Table 2. System suitability parameters

Inj.	Ezetimibe			Bempedoic Acid			
No.	RT (min)	Plate Count	Tailing	RT (min)	Plate Count	Tailing	Res.
1	2.119	7255	1.46	2.773	9342	1.32	6.0
2	2.120	7071	1.45	2.774	9111	1.34	5.9
3	2.121	7101	1.43	2.774	9133	1.34	5.9
4	2.122	7068	1.39	2.776	9291	1.33	5.9
5	2.122	7058	1.39	2.779	9331	1.29	5.9
6	2.123	6825	1.38	2.780	9708	1.28	6.0

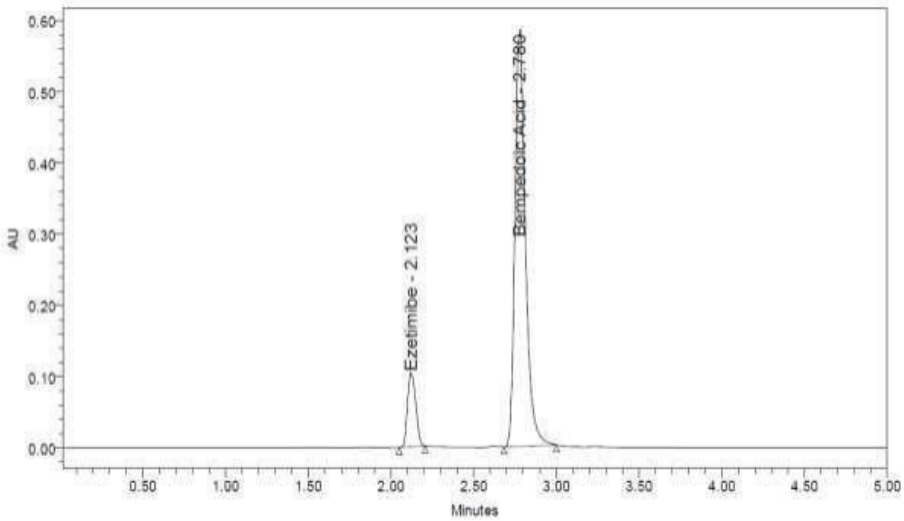

Fig. 2. Opimized chromatogram of Bempedoic acid and Ezetimibe Linearity

The calibration curves for both analytes demonstrated excellent linearity over the studied concentration ranges. For Bempedoic acid, linearity was established over 22.5–135 µg/mL with regression equation y = 28262x + 4418.6 (R^2 = 0.9999). For Ezetimibe, the range was 1.25–7.5 µg/mL with regression equation y = 28796x + 190.13 (R^2 = 0.9999), confirming strong linear relationships between analyte concentration and peak area response.

Table 3. Linearity parameters for Bempedoic acid and Ezetimibe

Parameter	Bempedoic Acid	Ezetimibe
Linearity range (µg/mL)	22.5 – 135	1.25 – 7.5
Regression equation	y = 28262x + 4418.6	y = 28796x + 190.13
Slope (m)	28262	28796
Intercept (c)	4418.6	190.13
R^2	0.9999	0.9999

Precision

System precision was evaluated through six replicate injections of the standard working solution. The %RSD values were 0.6% for Bempedoic acid and 1.3% for Ezetimibe, both well within the ICH acceptance limit of ≤2.0%, confirming satisfactory system repeatability. Method precision (repeatability) was assessed by analyzing six independently prepared sample working solutions. %RSD values of 0.4% for Bempedoic acid and 0.6% for Ezetimibe were obtained, demonstrating excellent intra-day reproducibility of the method.

Table 4. Precision data for Bempedoic acid and Ezetimibe

Parameter	Bempedoic Acid	Ezetimibe
System precision (%RSD)	0.6	1.3
Method precision (%RSD)	0.4	0.6
Acceptance limit	≤ 2.0%	≤ 2.0%

Accuracy

Accuracy was determined by the standard addition method at 80%, 100%, and 120% concentration levels in triplicate. The mean percentage recovery values were 99.87% for Bempedoic acid and 100.12% for Ezetimibe, both within the ICH-prescribed range of 98–102%, confirming the trueness and reliability of the method.

Table 5. Accuracy data — Mean % Recovery

Parameter	Bempedoic Acid	Ezetimibe
Mean % Recovery	99.87%	100.12%
Acceptance criteria	98 – 102%	98 – 102%

Robustness

Robustness testing was conducted under six varied conditions: flow rate minus (0.8 mL/min), flow rate plus (1.0 mL/min), mobile phase ratio minus (65B:35A), mobile phase plus (75B:25A), column temperature minus (24°C), and column temperature plus (34°C). Under all conditions, system suitability parameters remained within acceptable limits and %RSD values were within 2.0%, demonstrating that the method is robust to minor deliberate variations in operating conditions.

Table 6. Robustness data (%RSD values)

Condition	Abbreviation	Bempedoic acid %RSD	Ezetimibe %RSD
Flow rate minus (0.8 mL/min)	FM	0.4	1.6
Flow rate plus (1.0 mL/min)	FP	0.3	1.0
Mobile phase minus (65B:35A)	MM	0.2	1.8
Mobile phase plus (75B:25A)	MP	0.1	0.5
Temperature minus (24°C)	TM	0.2	1.9
Temperature plus (34°C)	TP	0.2	0.8

Limit of Detection and Limit of Quantification

The LOD and LOQ values were calculated from the regression data of the calibration curves. For Bempedoic

acid, LOD = 0.10 µg/mL and LOQ = 0.35 µg/mL. For Ezetimibe, LOD = 0.01 µg/mL and LOQ = 0.03 µg/mL. These low sensitivity limits confirm that the method is capable of detecting and quantifying trace levels of both analytes with adequate precision and accuracy.

Table 7. LOD and LOQ values

Drug	LOD (µg/mL)	LOQ (µg/mL)
Bempedoic acid	0.10	0.35
Ezetimibe	0.01	0.03

Drug Assay

The assay of the commercial tablet formulation (Bempetol) was performed using the validated method. The mean percentage assay values were 100.27% for Bempedoic acid and 100.13% for Ezetimibe, both within the acceptable range of 90–110%, confirming the suitability of the method for drug content determination in pharmaceutical dosage forms.

Degradation Studies

Forced degradation studies were performed under acid, alkali, oxidative, thermal, and UV conditions. The results, summarized in Table 8, indicate that both drugs underwent measurable degradation under the stress conditions applied. Chromatographic analysis of the degraded samples confirmed that the degradation products were adequately resolved from the main analyte peaks, demonstrating the specificity and stability-indicating capability of the method.

Table 8. Degradation study results

S.No.	Degradation Condition	% Drug Degraded	% Drug Undegraded
1	Acid (HCl)	97.49	2.51
2	Alkali (NaOH)	93.40	6.60
3	Oxidation (H_2O_2)	93.51	6.49
4	Thermal	97.70	2.30
5	UV Radiation	98.38	1.62

Summary of Validation Parameters

Table 9. Summary of validation parameters for Bempedoic acid and Ezetimibe

Parameter	Bempedoic Acid	Ezetimibe	Limit
Linearity range (µg/mL)	22.5 – 135	1.25 – 7.5	–
Regression coefficient (R^2)	0.9999	0.9999	–
Regression equation	y = 28262x + 4418.6	y = 28796x + 190.13	–
Assay (% mean)	100.27%	100.13%	90 – 110%
Specificity	Specific	Specific	No interference
System precision %RSD	0.6	1.3	NMT 2.0%
Method precision %RSD	0.4	0.6	NMT 2.0%
Accuracy % Recovery	99.87%	100.12%	98 – 102%
LOD (µg/mL)	0.10	0.01	–
LOQ (µg/mL)	0.35	0.03	–
Robustness FM	0.4	1.6	%RSD NMT 2.0
Robustness FP	0.3	1.0	%RSD NMT 2.0
Robustness MM	0.2	1.8	%RSD NMT 2.0
Robustness MP	0.1	0.5	%RSD NMT 2.0
Robustness TM	0.2	1.9	%RSD NMT 2.0
Robustness TP	0.2	0.8	%RSD NMT 2.0

CONCLUSION

A validated, simple, accurate, and precise RP-HPLC method was successfully developed for the simultaneous estimation of Bempedoic acid and Ezetimibe in bulk and tablet dosage form. The method employed a Kromosil C18 column with a mobile phase of 0.01 N KH_2PO_4 buffer and methanol (70:30 v/v), at a flow rate of 0.9 mL/min, with UV detection at 260 nm. Chromatographic separation was achieved within a total run time of 5 minutes, with retention times of 2.780 min for Bempedoic acid and 2.123 min for Ezetimibe.

All validation parameters including linearity (R^2 = 0.9999), system precision (%RSD 0.6% and 1.3%), method precision (%RSD 0.4% and 0.6%), accuracy (99.87% and 100.12% recovery), robustness, LOD, LOQ, specificity, and drug assay complied with ICH Q2(R1) acceptance criteria. The method demonstrated stability-indicating capability through forced degradation studies. The short run time, straightforward sample preparation, and low solvent consumption make this method economical and environmentally favorable, rendering it well-suited for routine quality control testing of Bempedoic acid and Ezetimibe in pharmaceutical industries.

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