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Research Article

DEVELOPMENT AND VALIDATION OF A ROBUST RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF BEMPEDOIC ACID AND EZETIMIBE IN PHARMACEUTICAL DOSAGE FORMS

Kategar Chandana Priya *, S. Shahidha Bee, C Rupasi Pratyusha, Dr. MD Sultan Ali Basha

Department of Pharmaceutical Analysis, SAFA College of Pharmacy, B.Thandrapadu, Kurnool, Andhra Pradesh- 518007, Email: priyachandana963@gmail.com.

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ABSTRACT:
A robust, accurate, and reproducible reversed-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous quantification of Bempedoic Acid and Ezetimibe in tablet dosage forms. Chromatographic separation was achieved using an Inertsil ODS C18 column (150 × 4.6 mm, 5 µm particle size) with a mobile phase composed of phosphate buffer (pH 4.7) and acetonitrile in the ratio of 70:30 v/v. The mobile phase was delivered at a flow rate of 1.0 mL/min and detection was carried out at 234 nm. The retention times were 2.3 min and 3.7 min for Bempedoic Acid and Ezetimibe, respectively. The method was validated in accordance with ICH guidelines, demonstrating excellent linearity (R^2 = 0.999), precision (RSD < 2%), accuracy (98.88% and 99.08% recovery), and robustness. The LOD and LOQ were 1.75 µg/mL, 5.3 µg/mL for Bempedoic Acid and 0.09 µg/mL, 0.28 µg/mL for Ezetimibe. The developed method is simple, rapid, and suitable for routine quality control analysis.
Keywords: Bempedoic Acid, Ezetimibe, RP-HPLC, Validation, Simultaneous estimation, Quality control.

INTRODUCTION

Bempedoic acid is a novel lipid-lowering agent that inhibits adenosine triphosphate-citrate lyase (ACL), an enzyme involved in the early stages of cholesterol synthesis in the liver. It is a prodrug, activated mainly in hepatic tissue, which helps reduce the risk of muscle-related side effects commonly observed with statins. This makes bempedoic acid a promising option, especially for patients who are unable to tolerate statins.

Ezetimibe is another well-established lipid-lowering medication that works through a different mechanism. It blocks the absorption of cholesterol in the small intestine by inhibiting the Niemann-Pick C1-like 1 (NPC1L1) transporter, thereby reducing the amount of cholesterol delivered to the liver. Ezetimibe is often used alone or in combination with other lipid-lowering agents to achieve target LDL-C levels more effectively.

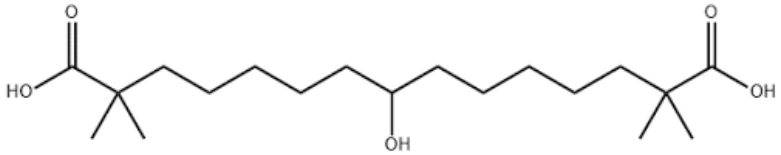

Fig:1. Chemical Structure of Bempedoic Acid

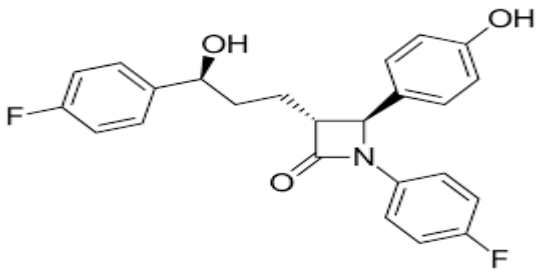

Fig:2. Chemical Structure of Ezetimibe

Address for Correspondence: Kategar Chandana Priya, Department of Pharmaceutical Analysis, SAFA College of Pharmacy, B.Thandrapadu, Kurnool, Andhra Pradesh- 518007, Email: priyachandana963@gmail.com..

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The combination of bempedoic acid and ezetimibe in fixed-dose formulations has shown significant potential in lowering LDL-C levels through dual inhibition—reducing both cholesterol synthesis and absorption. With the increasing clinical use of such combinations, there is a strong need for accurate and reliable analytical methods to monitor the quality of these formulations during manufacturing and storage.

Among the available analytical techniques, reverse phase high-performance liquid chromatography (RP-HPLC) is preferred for its efficiency, sensitivity, and reproducibility in pharmaceutical analysis. However, there is a limited number of published methods that provide a validated RP-HPLC approach for the simultaneous determination of bempedoic acid and ezetimibe in combined dosage forms. Some existing methods may lack sufficient sensitivity, involve long analysis times, or may not fully adhere to internationally accepted validation standards.

Considering these limitations, the present research focuses on the development and validation of a simple, precise, accurate, and robust RP-HPLC method suitable for the routine analysis of bempedoic acid and ezetimibe in tablet dosage forms. The method is designed and validated following the guidelines established by the International Council for Harmonisation (ICH), specifically ICH Q2(R1), covering key validation parameters such as linearity, precision, accuracy, specificity, robustness, and system suitability.

This study aims to develop and validate an RP-HPLC method for the concurrent determination of Bempedoic Acid and Ezetimibe in tablet dosage form, aligning with ICH guidelines for method validation.

Materials and Methods

Chemicals and Reagents:

The analysis involved the use of Bempedoic Acid and Ezetimibe reference standards to ensure the accuracy and reliability of the method. High-performance liquid chromatography (HPLC) grade acetonitrile was used as the organic solvent component of the mobile phase. Potassium dihydrogen phosphate and orthophosphoric acid were employed for the preparation and pH adjustment of the phosphate buffer, ensuring stable chromatographic conditions. Methanol was used as a diluent and for sample preparation where required. All solutions were prepared using Milli-Q water, which provides ultra-pure water free from contaminants, thereby maintaining the integrity of the analytical results.

Instrumentation:

The chromatographic analysis was performed using a WATERS 2965 HPLC system equipped with a photodiode array (PDA) detector, which enabled precise detection and monitoring of the analytes across a range of wavelengths. Data acquisition and processing were conducted using Empower 2 software, facilitating accurate integration and interpretation of chromatographic peaks. Additionally, a PG Instruments T60 UV-Visible Spectrophotometer was used for preliminary analysis and to support method development activities. The combination of high-quality reagents and advanced instrumentation contributed to the robustness and reliability of the developed HPLC method.

Preparation of Standard Solution

Accurately weighed 180 mg of Bempedoic Acid and 10 mg of Ezetimibe were dissolved in methanol and diluted to 100 mL with diluent (methanol:water 50:50). Further dilutions were made to obtain working standard solutions.

Sample Preparation

Five tablets were weighed, powdered, and an amount equivalent to one tablet was extracted with 30 mL diluent, sonicated for 25 min, diluted to 50 mL, filtered, and diluted further to obtain the final test solution.

Results

The HPLC method was developed using an Inertsil ODS column (150 × 4.6 mm, 5 µm) to achieve effective separation and quantification. The mobile phase comprised a phosphate buffer (pH 4.7) and acetonitrile in a 70:30 v/v ratio, which provided a suitable balance between resolution and run time. The flow rate was maintained at 1.0 mL/min, ensuring optimal elution of analytes within a total run time of 6 minutes. Detection was carried out at a wavelength of 234 nm, with an injection volume of 10 µL for each sample. The column temperature was kept constant at 30°C to enhance reproducibility and maintain peak shape. These chromatographic conditions were optimized to deliver accurate, precise, and robust results for the intended analysis.

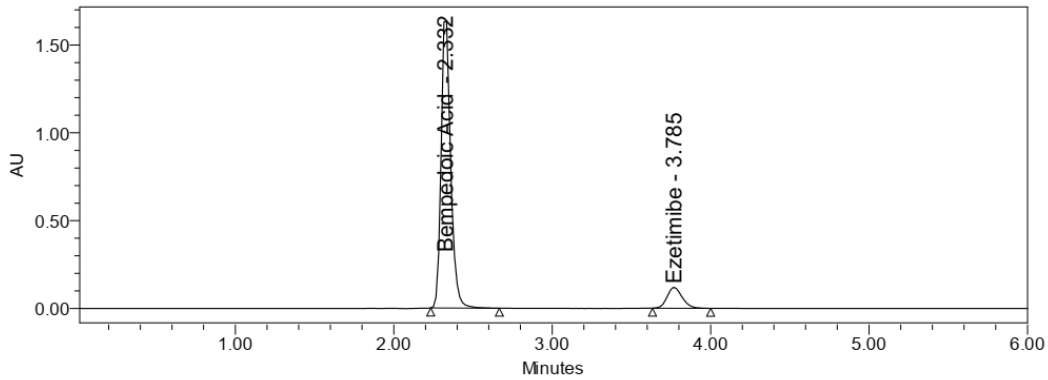

Fig 3. Optimized Chromatogram

Method Validation

System Suitability

The developed RP-HPLC method fulfilled all required system suitability criteria, confirming the efficiency and reliability of the chromatographic system. The retention times were found to be 2.3 minutes for Bempedoic Acid and 3.7 minutes for Ezetimibe, ensuring adequate separation within a short run time. Theoretical plate counts were 12,009 for Bempedoic Acid and 2,568 for Ezetimibe, indicating satisfactory column performance. The tailing factors were 1.28 and 1.35 respectively, signifying well-shaped, symmetrical peaks. The resolution between the two analytes was 2.33, which demonstrates good separation and absence of co-elution.

Table: 1 System suitability studies of Bempedoic Acid and Ezetimibe

Property	Bempedoic Acid	Ezetimibe
Retention time (Rt)	2.3min	3.7min
Theoretical plates (N)	12009	2568
Tailing factor (T)	1.28	1.35
Resoultion		2.33

Linearity

Linearity was evaluated by analyzing standard solutions of Bempedoic Acid and Ezetimibe over a range of concentrations. Bempedoic Acid exhibited linearity within the range of 45–270 µg/mL, while Ezetimibe showed linearity in the range of 2.5–15 µg/mL. The calibration curves yielded regression equations of y = 26423x + 17151 for Bempedoic Acid and y = 58509x + 13897 for Ezetimibe. The correlation coefficients (r^2) for both drugs were 0.999, confirming excellent linearity of the method across the specified ranges.

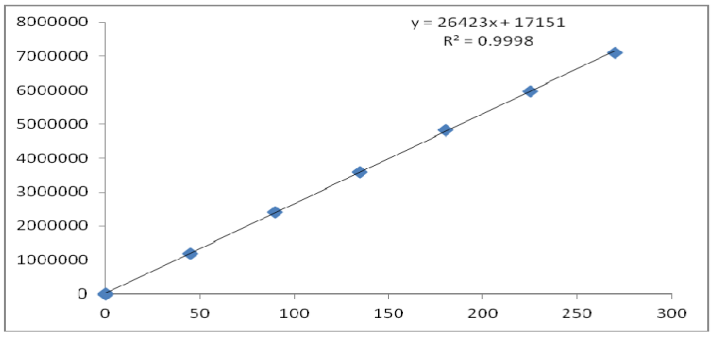

Fig : 4. Calibration curve of Bempedoic Acid

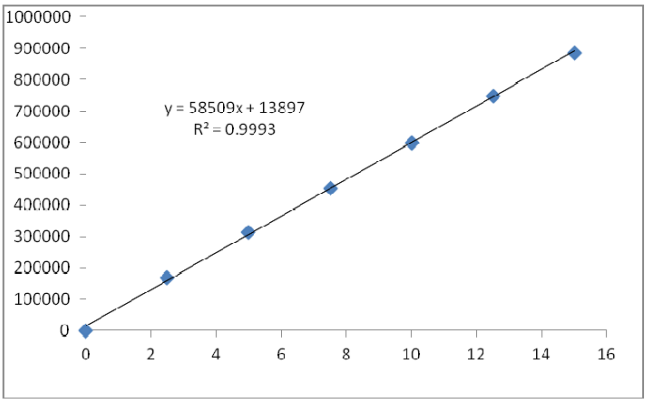

Fig : 5. Calibration curve of Ezetimibe.

Precision

Intra-day Precision (Repeatability)

Repeatability was assessed by analyzing six replicates of the same concentration on the same day. The %RSD was found to be 1.2% for Bempedoic Acid and 1.3% for Ezetimibe, indicating high consistency within the same day.

Inter-day Precision

The method's reproducibility on different days was tested by performing the analysis over three consecutive days. The %RSD values were 1.8% for Bempedoic Acid and 1.4% for Ezetimibe, demonstrating that the method maintains its precision over time. All values were below 2%, indicating good precision as per ICH guidelines.

Accuracy

Accuracy was confirmed by recovery studies conducted at three levels: 50%, 100%, and 150% of the target concentrations. The percentage recovery for Bempedoic Acid ranged from 98.30% to 99.79%, while Ezetimibe showed recoveries in the range of 95.26% to 103.4%. These results indicate that the method is accurate and is not affected by the presence of excipients in the formulation.

Table: 2 Accuracy data of Bempedoic Acid and Ezetimibe

Sample	Amount Taken (µg/ml)	Amount Recovered (µg/ml)	Recovery (%)	AVG
Bempedoic Acid	90	88.69	98.54	98.88
	180	176.95	98.30	
	270	269.44	99.79	
Ezetimibe	5	5.17	103.4	99.08
	10	9.86	98.6	
	15	14.29	95.26	

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The sensitivity of the method was determined by calculating the LOD and LOQ for both analytes. For Bempedoic Acid, the LOD was 1.75 µg/mL and the LOQ was 5.3 µg/mL. Ezetimibe demonstrated greater sensitivity with an LOD of 0.09 µg/mL and an LOQ of 0.28 µg/mL. These values confirm that the method is capable of detecting and quantifying even low concentrations of the analytes reliably.

Robustness

Robustness was assessed by deliberately introducing minor changes in chromatographic conditions, such as flow rate, mobile phase composition, and temperature. The results remained consistent under these altered conditions, with %RSD values for both analytes remaining below 2%. This indicates that the method is robust and can tolerate small, deliberate changes without compromising accuracy or precision.

Assay of Marketed Formulation

The validated method was applied to the assay of a marketed tablet formulation containing Bempedoic Acid and Ezetimibe. The mean assay result for Bempedoic Acid was found to be 100.12%, and for Ezetimibe, it was

98.71%. These results confirm that the method is suitable for routine quality control and content uniformity testing of the combined dosage form.

Results and Discussion

The proposed RP-HPLC method provides a reliable and consistent approach for simultaneous quantification of Bempedoic Acid and Ezetimibe. Excellent resolution and minimal interference were observed. Validation results confirmed the method's specificity, linearity, precision, accuracy, and robustness. This makes it suitable for routine quality control in pharmaceutical industries.

Conclusion

A simple, precise, and validated RP-HPLC method was successfully developed for simultaneous estimation of Bempedoic Acid and Ezetimibe. The method complies with ICH guidelines and can be effectively used for routine analysis of these drugs in combined tablet formulations.

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