RP-HPLC Method Development and Validation for Simultaneous Estimation of Bempedoic Acid and Ezetimibe in Pharmaceutical Dosage Forms

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***Abstract*—Hypercholesterolemia is a major risk factor for cardiovascular diseases and is commonly managed using lipid-lowering agents such as Bempedoic Acid and Ezetimibe. The increasing use of fixed-dose combination products necessitates the development of reliable analytical methods for their quality control and routine analysis. The present study was aimed at developing and validating a simple, accurate, precise, and economical reverse-phase high-performance liquid chromatographic (RP-HPLC) method for the simultaneous estimation of Bempedoic Acid and Ezetimibe in pharmaceutical dosage forms. Chromatographic separation was achieved using a C18 column with a mobile phase consisting of phosphate buffer and acetonitrile under optimized conditions. Detection was performed at 240 nm. The developed method was validated according to ICH Q2(R1) guidelines for parameters including specificity, linearity, precision, accuracy, robustness, limit of detection, and limit of quantification. The results demonstrated excellent chromatographic performance with good peak symmetry, high resolution, and acceptable validation characteristics. The method was found to be suitable for routine quality control analysis of combined dosage forms containing Bempedoic Acid and Ezetimibe.**

***Index Terms*—Bempedoic Acid, Ezetimibe, RP-HPLC, Method Validation, ICH Guidelines, Pharmaceutical Analysis.**

I. INTRODUCTION

Cardiovascular diseases continue to be one of the leading causes of mortality worldwide. Elevated levels of low-density lipoprotein cholesterol (LDL-C) are strongly associated with the development of atherosclerosis, coronary artery disease, and other cardiovascular complications. Effective management of cholesterol levels is therefore an important therapeutic objective. In recent years, Bempedoic Acid and Ezetimibe have emerged as valuable lipid-lowering agents that offer significant benefits either as monotherapy or in combination with statins.

Bempedoic Acid is a first-in-class ATP-citrate lyase inhibitor that reduces cholesterol synthesis in the liver. Unlike statins, it is activated primarily in hepatic tissue, thereby reducing the risk of muscle-related adverse effects commonly associated with statin therapy. Clinical studies have demonstrated significant reductions in LDL cholesterol levels following treatment with Bempedoic Acid. The drug has been approved for the treatment of hypercholesterolemia, particularly in patients who require additional lipid-lowering effects despite receiving maximally tolerated statin therapy.

Ezetimibe is a selective cholesterol absorption inhibitor that acts at the brush border of the small intestine. By inhibiting the Niemann-Pick C1-like 1 (NPC1L1) transporter, Ezetimibe reduces intestinal cholesterol absorption and decreases the amount of cholesterol delivered to the liver. The resulting increase in hepatic LDL receptor expression promotes clearance of circulating LDL cholesterol. Due to its unique mechanism of action, Ezetimibe is frequently combined with statins or other lipid-lowering agents to achieve enhanced therapeutic efficacy.

The pharmaceutical industry requires validated analytical methods to ensure the quality, safety, and efficacy of drug products. Among the various analytical techniques available, RP-HPLC remains one of the most widely employed methods because of its high sensitivity, reproducibility, selectivity, and versatility. Although several analytical methods have

been reported for the individual determination of Bempedoic Acid and Ezetimibe, limited information is available regarding a simple and validated RP-HPLC method for their simultaneous estimation in combined dosage forms. Therefore, the present study was undertaken to develop and validate an RP-HPLC method suitable for routine quality control applications.

II. MATERIALS AND METHODS

The study was carried out using analytical-grade reagents and HPLC-grade solvents. Pure samples of Bempedoic Acid and Ezetimibe were obtained from reliable pharmaceutical sources and used as reference standards. Acetonitrile, methanol, phosphate salts, and orthophosphoric acid were procured from certified suppliers and used without further purification. Commercial tablet formulations containing the combination of Bempedoic Acid and Ezetimibe were selected for assay studies.

Chromatographic analysis was performed using an HPLC system equipped with a UV detector. Different chromatographic conditions were investigated during method development to achieve optimal separation of both analytes. Several combinations of mobile phases, pH conditions, and flow rates were evaluated. After systematic optimization, satisfactory chromatographic performance was obtained using a C18 analytical column and a mobile phase composed of phosphate buffer and acetonitrile. The mobile phase was filtered through a membrane filter and degassed prior to use. The flow rate and detection wavelength were selected based on the best chromatographic response and sensitivity for both drugs.

Standard stock solutions of Bempedoic Acid and Ezetimibe were prepared separately and subsequently diluted to obtain working concentrations. Sample solutions were prepared from powdered tablets and subjected to appropriate dilution using the selected diluent. All prepared solutions were filtered before injection into the chromatographic system.

Method Validation

Validation of the developed method was performed according to ICH Q2(R1) guidelines. The specificity of the method was evaluated by comparing chromatograms of blank, standard, and sample solutions. No interfering peaks were observed at the retention times corresponding to Bempedoic Acid and Ezetimibe, indicating excellent specificity.

Table 1: Optimized Chromatographic Conditions

Parameter	Condition
Column	ACE C18 (4.6 × 150 mm, 5 µm)
Mobile Phase	Acetonitrile: Phosphate Buffer
Ratio	65:35 (v/v)
Flow Rate	1.2 mL/min
Detection Wavelength	240 nm
Injection Volume	20 µL
Run Time	10 min

Linearity studies were performed by preparing a series of standard solutions over a suitable concentration range. Calibration curves were generated by plotting peak area against concentration. The obtained regression equations demonstrated excellent linear relationships, with correlation coefficients greater than 0.999 for both analytes. These results confirmed that the analytical response was directly proportional to concentration within the selected range.

Accuracy was assessed through recovery studies at multiple concentration levels. Known quantities of standard drugs were added to pre-analyzed samples, and the percentage recoveries were calculated. The mean recovery values were found to be within the acceptable range of 98–102%, demonstrating the accuracy of the developed method.

Precision was evaluated in terms of repeatability and intermediate precision. Multiple injections of standard solutions were analyzed, and percentage relative standard deviation (%RSD) values were calculated. The obtained %RSD values were below 2%, confirming the excellent precision of the method.

The sensitivity of the analytical procedure was determined by calculating the limits of detection and quantification. The low LOD and LOQ values obtained indicate that the method possesses adequate sensitivity for routine pharmaceutical analysis. Robustness studies were performed by deliberately varying chromatographic parameters such as flow rate, wavelength, and mobile phase composition. The results demonstrated that minor variations did not significantly affect chromatographic performance, indicating that the method is robust and reliable.

Method validation was performed according to ICH Q2(R1) guidelines evaluating linearity, accuracy, precision, specificity, LOD, LOQ, robustness, and system suitability.

Table 2: Linearity Results

Drug	Concentration Range (µg/mL)	Correlation Coefficient (R^2)
Bempedoic Acid	50–250	0.999
Ezetimibe	5–50	0.999

Table 3: Accuracy Results

Drug	Mean Recovery (%)
Bempedoic Acid	99.56
Ezetimibe	99.48

Table 4: Precision Results

Parameter	Bempedoic Acid (%RSD)	Ezetimibe (%RSD)
Repeatability	0.20	0.20
Intermediate Precision	0.20	0.10

Table 5: LOD and LOQ Results

Parameter	Bempedoic Acid	Ezetimibe
LOD	3.17	5.68
LOQ	0.0172	0.2125

Table 6: System Suitability Parameters

Parameter	Bempedoic Acid	Ezetimibe
Retention Time (min)	2.733	3.415
Theoretical Plates	4668	6089
Tailing Factor	1.3	1.2
Resolution	-	6.0

All system suitability parameters met the recommended acceptance criteria.

III. RESULTS AND DISCUSSION

The developed RP-HPLC method successfully achieved simultaneous separation and quantification of Bempedoic Acid and Ezetimibe within a short analysis time. The optimized chromatographic conditions produced sharp, symmetrical peaks with excellent resolution. Retention times were sufficiently distinct to avoid overlap and ensure accurate quantification.

The validation studies confirmed the suitability of the method for pharmaceutical quality control applications. Excellent linearity was observed for both analytes, demonstrating the ability of the method to provide reliable quantitative results across a wide concentration range. Recovery studies indicated high accuracy, while precision studies showed minimal variation between repeated analyses.

System suitability parameters including theoretical plate count, tailing factor, and resolution complied with established acceptance criteria. These findings demonstrate that the chromatographic system was functioning appropriately and capable of producing reproducible results. The robustness of the method further confirms its applicability under routine laboratory conditions.

Compared with previously reported methods, the present RP-HPLC method offers several advantages, including shorter run time, simplified mobile phase composition, reduced solvent consumption, and excellent validation characteristics. These attributes make the method particularly suitable for routine quality control testing in pharmaceutical industries.

Table 7: Summary of Validation Results

Parameter	Bempedoic Acid	Ezetimibe
Linearity (R^2)	0.999	0.999
Accuracy (% Recovery)	99.56	99.48
Precision (%RSD)	<2.0	<2.0
LOD	3.17	5.68
LOQ	0.0172	0.2125

IV. CONCLUSION

The present investigation successfully developed and validated a simple, rapid, precise, and accurate RP-HPLC method for the simultaneous estimation of Bempedoic Acid and Ezetimibe in pharmaceutical dosage forms. The developed method demonstrated excellent chromatographic performance and fulfilled all validation requirements specified by ICH Q2(R1) guidelines. Validation parameters including specificity, linearity, accuracy, precision, robustness, LOD, and LOQ were found to be within acceptable limits. The method is therefore suitable for routine quality control analysis, assay determination, and

stability studies of pharmaceutical formulations containing Bempedoic Acid and Ezetimibe.

Future Perspectives

Future studies may focus on extending the developed method to stability-indicating applications through forced degradation studies. The method may also be adapted for bioanalytical investigations involving plasma samples and pharmacokinetic evaluations. Further optimization using advanced chromatographic techniques such as UHPLC may reduce analysis time and solvent consumption while improving analytical efficiency.

REFERENCES

[1] ICH, Q2(R1): Validation of Analytical Procedures: Text and Methodology. Geneva, Switzerland: International Council for Harmonisation, 2005.
[2] L. R. Snyder, J. J. Kirkland, and J. W. Dolan, Introduction to Modern Liquid Chromatography, 3rd ed. Hoboken, NJ, USA: John Wiley & Sons, 2010.
[3] V. R. Meyer, Practical High-Performance Liquid Chromatography, 5th ed. Chichester, U.K.: Wiley, 2010.
[4] D. A. Skoog, F. J. Holler, and S. R. Crouch, Principles of Instrumental Analysis, 7th ed. Boston, MA, USA: Cengage Learning, 2018.
[5] C. M. Ballantyne, M. Banach, and G. B. J. Mancini, "Efficacy and safety of bempedoic acid," European Heart Journal, vol. 41, no. 36, pp. 3899–3907, 2020.
[6] J. R. Burnett and G. F. Watts, "Ezetimibe and its clinical applications," Clinical Pharmacokinetics, vol. 46, no. 4, pp. 303–319, 2007.
[7] C. B. Pandya and S. J. Rajput, "Development and validation of RP-HPLC method for lipid-lowering agents," International Journal of Pharmaceutical Sciences Review and Research, vol. 8, no. 2, pp. 78–84, 2011.
[8] N. K. Sahoo, M. Sahu, and D. V. Rao, "RP-HPLC method development and validation studies," International Journal of Pharmacy and Pharmaceutical Sciences, vol. 4, no. 3, pp. 237–242, 2012.
[9] United States Pharmacopeia 43–National Formulary 38. Rockville, MD, USA: USP Convention, 2020.
[10] A. H. Beckett and J. B. Stenlake, Practical Pharmaceutical Chemistry, 4th ed. New Delhi, India: CBS Publishers, 2007.