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Development and Validation of a RP-HPLC Method for Simultaneous Determination of Bempedoic Acid and Ezetimibe in Pharmaceutical Dosage Form.

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KEYWORDS
RP-HPLC, Bempedoic Acid, Ezetimibe, Correlation Coefficient, Assay, Precision

ABSTRACT:

A new and advanced Reversed Phase High-Performance Liquid Chromatography (RP-HPLC) technique has been successfully developed for the simultaneous determination of Bempedoic Acid and Ezetimibe in pharmaceutical dosage form. The analysis utilized a Kromasil C18 column and a wavelength of 224 nm for detection. Chromatographic separation was achieved using an 80:20 ratio of Acetonitrile and water (pH 2.1 adjusted with orthophosphoric acid). The retention times for Bempedoic Acid and Ezetimibe were determined to be 7.1 min and 8 min respectively.

Extensive validation was conducted, demonstrating the linear response of Ezetimibe in the concentration range of 5-40 µg/mL and Bempedoic Acid in the range of 90-720 µg/mL. The correlation coefficients (r^2 values) for Ezetimibe and Bempedoic Acid were 0.9973 and 0.996, respectively, indicating a strong concentration-response relationship.

Precision assessment revealed percentage relative standard deviations (% RSD) of 0.944 for Ezetimibe and 1.326 for Bempedoic Acid. The newly developed HPLC method exhibits characteristics of simplicity, linearity, precision, accuracy, suitability, and specificity. This validated method can reliably be used for routine analysis of Bempedoic Acid and Ezetimibe in pharmaceutical formulations, ensuring consistent and dependable results."

Introduction:

Hyperlipidaemia, a prevalent metabolic disorder marked by heightened lipid levels in the bloodstream, significantly heightens the risk of cardiovascular diseases, the foremost cause of global morbidity and mortality.[1,2] Bempedoic Acid and Ezetimibe are frequently prescribed medications for hyperlipidemia management.[3,4] Bempedoic acid, a novel ATP-citrate lyase inhibitor, diminishes cholesterol biosynthesis, while Ezetimibe inhibits cholesterol absorption from the gastrointestinal tract.[5,6,7]

Accurate determination of these drugs in pharmaceutical formulations is vital for quality control and monitoring their concentrations in biological samples.[8] High-performance liquid chromatography (HPLC), renowned for its sensitivity, selectivity, and precision, is extensively employed for drug quantification[9] This study endeavours to establish and validate a reversed-phase HPLC method for simultaneously quantifying Bempedoic Acid and Ezetimibe in both their pure forms and pharmaceutical formulations. Validation of the method adheres to ICH guidelines, ensuring its reliability, accuracy, and reproducibility. [10, 11, 12]

Materials and methods:

Instrumentation: Chromatography was conducted

Chromatography was conducted using an Agilent system with EZ Chrome Elite software. A Kromasil C18 column (250×4.6 nm, 5µm) was used for separation and quantitation.

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Chemicals and Reagents:

The Bempedoic Acid reference standard, with a purity of 99.8%, was graciously provided as a gift sample by Optum Healthcare, Gujarat.

Selection of Wavelength:

Each solution was analysed using a UV-visible spectrophotometer, scanning within the range of 400 nm to 200 nm [Overlapping spectra were obtained and the wavelength of 224 nm was selected as an isobestic point] [14]. The overlaid spectra of Bempedoic Acid and Ezetimibe are shown in Fig. 1.

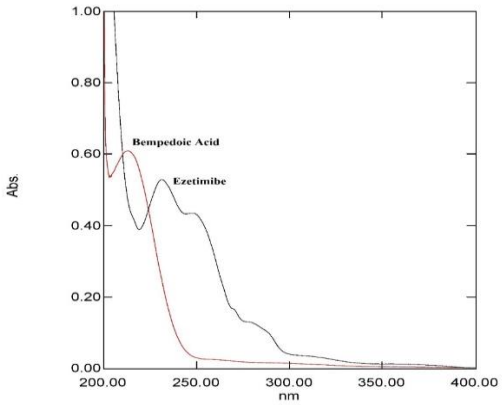

Fig 1: UV Spectrum of Bempedoic Acid and Ezetimibe

Chromatographic Condition:

The method development utilized a Kromasil C_{18} column (250 x 4.6 mm, 5 µm). The mobile phase comprised a mixture of acetonitrile and water (pH 2.1) in an 80:20 ratio. Detection was conducted at a wavelength of 224 nm by scanning a standard drug solution across wavelengths ranging from 400 to 200 nm using a spectrophotometer. The flow rate was 0.5 mL/min.

Preparation of Mobile Phase:

The mobile phase for HPLC analysis was chosen based on factors including solvent type, buffer pH, and sample properties. Various mobile phase combination was checked .Various combinations of water and acetonitrile were tested to achieve sharp peaks for Ezetimibe and Bempedoic Acid. The final mobile phase consisted of 20 volumes of pH 2.1 water adjusted with ortho-phosphoric acid and 80 volumes of HPLC-grade acetonitrile.

Preparation of Diluent: Based on the Solubility of drug, diluent was selected as Methanol.

Preparation of Stock solution:

Accurately weighed 180 mg of Bempedoic Acid and 10 mg of Ezetimibe, then individually poured them into 100 mL volumetric flasks. Subsequently, add 50 mL of methanol, the solutions were sonicated for 5 minutes. Finally, the volume was adjusted with methanol. The resulting solutions contained concentrations of 1800 µg/mL for Bempedoic Acid and 100 µg/mL for Ezetimibe. [15, 16]

Standard Final Solution:

Dilute above 5 ml stock solution with diluent and adjust the 50 ml volume, resulting in a final concentration of 180 µg/mL for Bempedoic Acid and 10 µg/mL for Ezetimibe.

Method Development:

A Kromasil C_{18} column measuring 250 x 4.6 mm with a particle size of 5 µm facilitated chromatographic separation. The mobile phase, comprising acetonitrile and water in an 80:20 ratio, flow rate of 0.5 mL/min at 224 nm. Through procedure development and optimization, Bempedoic Acid eluted at 8.1 minutes, while Ezetimibe eluted at 7 minutes. The total run time for the analysis was 10 minutes. The method development considered various factors such as pH, flow rate, and mobile phase ratio. Numerous mobile phase ratios and combinations were evaluated to determine the optimal response.

Analytical method validation:

The developed method underwent validation in accordance with ICH principles to ensure its appropriateness for its intended application. [17] Validation encompassed the assessment of the following parameters:

Linearity and range:

The linearity of Ezetimibe and Bempedoic Acid was evaluated by preparing solutions with concentrations ranging from 5 to 45 µg/mL and 90 to 720 µg/mL respectively. Then inject the sample and the resulting peak areas were recorded. Subsequently, a calibration curve was generated by plotting concentration against

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peak area values. The linear regression equation, slope, and correlation coefficient of the calibration curve were computed to assess the method's linearity. This study aids in establishing the reliability and accuracy of the HPLC method for quantifying Ezetimibe and Bempedoic Acid in pharmaceutical formulations or biological samples. [18]

Assay:

Twenty tablets [Brillo-EZ] were ground into a fine powder using a mortar. From this powdered sample, accurately weighed 180 mg of Bempedoic Acid and 10 mg of Ezetimibe and transferred into a 100 mL volumetric flask. The flask was then filled up to the mark with an appropriate diluent, then mixture was sonicated for 20 minutes. Following filtration through a membrane filter, the sample underwent further dilution to achieve concentrations of 10 µg/mL for Ezetimibe and 180 µg/mL for Bempedoic Acid. This diluted solution was subsequently analysed to determine the percentage of medication present in the tablets.[19]

Limit of detection (LOD) and Limit of quantification (LOQ):

LOD refers to the minimum concentration of analytes that can be reliably detected. It is computed using the formula LOD = 3.3 × σ/S, where σ represents the standard deviation of the y-intercept, and S indicates the slope of the calibration curve. LOQ denotes the lowest concentration of analytes that can be accurately measured and is determined as LOQ = 10 × σ/S, where σ represents the standard deviation of the y-intercept and S is the slope of the calibration curve. Establishing the LOD and LOQ enables researchers to assess the sensitivity and accuracy of their analytical methods. [19]

Precision:

To evaluate precision, six replicate standard solutions were prepared and injected into an HPLC within the same day. Furthermore, the same six replicate standard solutions underwent injection into the HPLC on three consecutive days to determine precision across days. For each set of injections, the peak area and percentage relative standard deviation (RSD) were calculated. [20]

Accuracy:

In the recovery test, predetermined quantities of Ezetimibe and Bempedoic Acid were added to samples at three distinct levels. These solutions were then analysed utilizing the employed method. The recovery percentage was determined by comparing the measured concentration with the expected concentration .[21]

Result and discussion:

Method development and optimization:

The most favourable outcomes were achieved using 80% acetonitrile and 20% water (pH 2.1), which was modified with orthophosphoric acid.

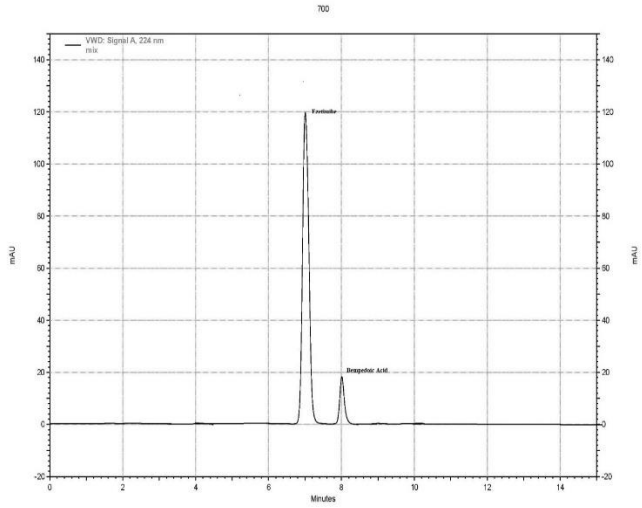

Fig 2: HPLC Chromatogram of Bempedoic Acid and Ezetimibe

Method validation:

The regression equations for Ezetimibe and Bempedoic Acid were derived by plotting the peak area versus analytes concentration within the ranges of 5-45 mg/mL and 90-720 µg/mL, respectively. The linear regression equation for the c of Ezetimibe was determined, yielding a correlation coefficient (r^2) of 0.9973. Similarly, the linear regression equation for the Bempedoic Acid was established, with a correlation coefficient (r^2) of 0.9969.

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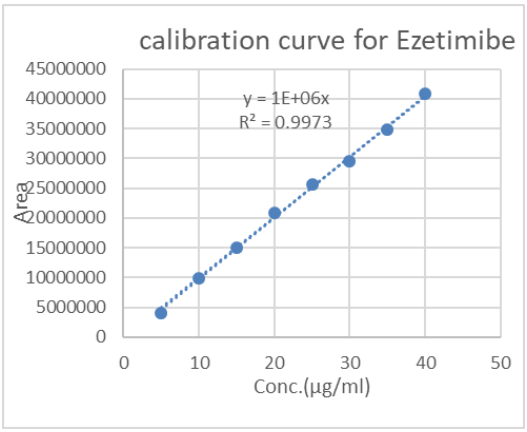

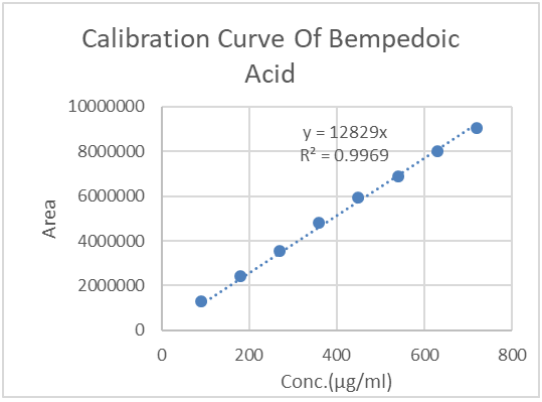

Assay:

The assay was determined as % drug content. The % drug content for Ezetimibe and Bempedoic Acid was found to be 99.51% and 99.77% respectively. All the calculated values are given in the table 1.

Table 1: Summary of Assay

Drug	**Label Claim (mg/ tab)**	**Amount Found* [n=6] (mg/tab)**	**% Assay**	**%RSD**
Ezetimibe	10	9.95	99.51	1.168
Bempedoic Acid	180	179.60	99.77	0.793

Limit of detection (LOD) and Limit of quantification (LOQ): LOD and LOQ values for Ezetimibe and Bempedoic Acid are mentioned in the table 2.

Table 2: Summary of LOD and LOQ

Drug	**LOD** (µg/ml)	**LOQ** (µg/ml)
Ezetimibe	0.06	0.19
Bempedoic Acid	4.70	14.25

Precision:

The precision was measured as intra-day and inter-day precision. For intra-day precision, the % RSD for Ezetimibe and Bempedoic Acid was found to be 0.944 and 1.32 respectively. All the calculated values are mentioned in the table 3.

Table 3: Summary of precision

Drug	**Level**	**Conc. Added *[*n = 6]**	**Conc. Found by graph**	**%RSD**
Ezetimibe	**Intra-Day**	10	9.78	0.944
Bempedoic Acid	**Intra–Day**	180	178.9	1.32
Drug	**Level**	**Conc. Added *[*n = 6]**	**Conc. Found by graph**	**%RSD**
Ezetimibe	**Inter – Day 1**	10	9.75	0.504
	Inter – Day 2	10	9.73	0.846
	Inter – Day 3	10	9.72	0.812
Bempedoic Acid	**Inter – Day 1**	180	179.81	0.319
	Inter – Day 2	180	179.05	0.340
	Inter – Day 3	180	179.48	0.650

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Accuracy:

Accuracy was determined as % Recovery at 80 %, 100 % and 120 %. All the calculated values are mentioned below in the table 4.

Drug	Level	Amount Added *[*n = 6]	Amount Recovered *[*n=6]	%Recovery
Ezetimibe	80%	8	7.89	98.62
	100 %	10	9.96	99.3
	120 %	12	11.98	98.91
Bempedoic Acid	80%	8	7.94	99.25
	100 %	10	9.93	99.3
	120 %	12	11.98	98.91

Table4: SummaryofAccuracy

Conclusion:

The RP-HPLC method developed for the simultaneous determination of Bempedoic Acid and Ezetimibe in pharmaceutical dosage forms has undergone successful validation. The method has exhibited favourable attributes in terms of linearity, accuracy, precision, and specificity, thus establishing its suitability for routine analysis of these two drugs. Consequently, this validated method can be reliably utilized for quality control analysis of Bempedoic Acid and Ezetimibe in pharmaceutical formulations."

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