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DEVELOPMENT AND VALIDATION OF A RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF BEMPEDOIC ACID AND EZETIMIBE IN PURE AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A novel and advanced high-performance liquid chromatography (HPLC) technique has been successfully developed for the simultaneous determination of Bempedoic Acid and Ezetimibe in both their pure form and pharmaceutical dosage forms. The separation was achieved using an Kromasil C18 column with a UV detector set at a wavelength of 224 nm. A mobile phase consisting of Acetonitrile and water (pH 2.1 adjusted with orthophosphoric acid) in a ratio of 80:20 was utilized for the chromatographic separation. The retention times for Bempedoic Acid and Ezetimibe were determined to be 7.1 min and 8 min respectively.

The developed method was fully validated using various validation parameters. Ezetimibe exhibited a linear response over the concentration range of 5-40 µg/mL, while Bempedoic Acid showed linearity in the range of 90-720 µg/mL. The correlation coefficients (r^2 values) obtained for Ezetimibe and Bempedoic Acid were 0.9973 and 0.996, respectively, indicating a strong correlation between the concentration and the response.

The precision of the method was also assessed, with the percentage relative standard deviations (% RSD) for Ezetimibe and Bempedoic Acid determined to be 0.944 and 1.326, respectively. The newly developed HPLC method is characterized by simplicity, linearity, precision, accuracy, suitability, and specificity. This validated method can be effectively utilized for the

routine analysis of Bempedoic Acid and Ezetimibe in pharmaceutical formulations, ensuring reliable and consistent results.

Keywords: Bempedoic Acid, Ezetimibe, Correlation Coefficient, Assay, Precision

INTRODUCTION:

Hyperlipidemia is a common metabolic disorder characterized by elevated levels of lipids in the bloodstream, particularly cholesterol and triglycerides. It is a major risk factor for cardiovascular diseases, which are the leading cause of morbidity and mortality worldwide **[1-3]**. Bempedoic acid and ezetimibe are two commonly prescribed medications for the management of hyperlipidemia **[4-6]**. Bempedoic acid is a novel ATP-citrate lyase inhibitor that reduces cholesterol biosynthesis, while Ezetimibe inhibits the absorption of cholesterol from the gastrointestinal tract **[7]**.

The simultaneous determination of Bempedoic acid and Ezetimibe in their pharmaceutical dosage forms is crucial for quality control and monitoring of their concentrations in biological samples **[8-10]**. High-performance liquid chromatography (HPLC) is a widely used analytical technique for the quantitative analysis of drugs due to its high sensitivity, selectivity, and precision **[5]**.

In this study, we aimed to develop and validate a reversed-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous determination of Bempedoic Acid and Ezetimibe in pure form and pharmaceutical dosage forms. The developed method will be validated as per ICH guidelines to ensure its reliability, accuracy, and reproducibility **[11-13]**.

MATERIALS AND METHODS:

Instrumentation:

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

Chemicals and Reagents:

Bempedoic Acid (99.8) reference standard was a gift sample from Optum Healthcare, Gujarat and Ezetimibe was purchased from Shobha Lifesciences Gujarat . Acetonitrile (Merck) and Milli-Q water (HPLC Grade) were used for preparing the mobile phase.

Selection of Wavelength:

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

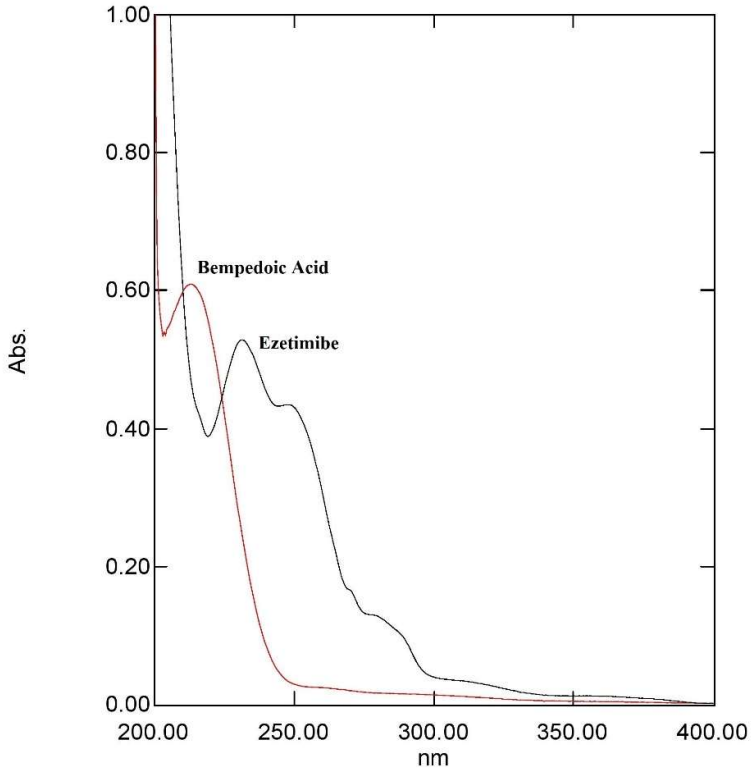

Figure 1: UV Spectrum of Bmpedoic Acid and Ezetimibe

Chromatographic Condition:

The method was developed using a Kromasil C_{18} column (250 x 4.6 mm, 5 µm). The mobile phase consisted of acetonitrile and water (pH 2.1) in a ratio of 80:20. Detection was performed at a wavelength of 224 nm by scanning a standard drug solution across a range of wavelengths from 400-200 nm using a spectrophotometer. The flow rate of the pump was set at 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:

Precisely weighed 180 mg Bempedoic Acid and 10 mg Ezetimibe and separately

transfer into 100 ml volumetric flask then add 50 ml methanol and sonicate for 5 min . Finally make up the volume. The solution contain 1800 µg/ml Bempedoic Acid, 100 µg/ml Ezetimibe .

Standard Final Solution:

Dilute 5 ml of the stock solution into a 50 ml volumetric flask and adjust the volume with diluent to obtain a final concentration of 180 µg/ml Bempedoic Acid and 10 µg/ml Ezetimibe.

Method Development:

A Kromasil C_{18} column with dimensions of 250 x 4.6 mm and particle size of 5 µm was used for chromatographic separation. The mobile phase consisted of acetonitrile and water in an 80:20 ratio, flowing at a rate of 0.5 mL/min. Detection was carried out at 224 nm. Following development and optimization of the procedure, Bempedoic Acid eluted at 8.1 minutes and Ezetimibe eluted at 7 minutes. The total run time for the analysis was 10 minutes.

The development of the analytical method took into account a number of factors, including pH, flow rate and mobile phase ratio. Several mobile phase ratios and combinations were tested in order to determine the maximal response.

Analytical method validation:

The developed method was validated in accordance with ICH principles to ensure its suitability for its intended use. Validation was conducted using the following parameters

Linearity and range:

The linearity of Ezeimibe and Bempedoic Acid was determined by preparing solutions with concentrations ranging from 5-45 µg/ml and 90-720 µg/ml, respectively. The solutions were injected into the HPLC system, and the peak areas were recorded. A calibration curve was then constructed by plotting the concentration versus peak area values.

The linear regression equation, slope, and correlation coefficient of the calibration curve were calculated to assess the linearity of the method. The linearity study helps to establish the reliability and accuracy of the HPLC method for the quantification of Ezeimibe and Bempedoic Acid in pharmaceutical formulations or biological samples.

Assay:

Twenty tablets containing a combination of Bempedoic Acid and Ezetimibe were pulverized into a fine powder in a mortar. From the powdered sample, 180 mg of Bempedoic Acid and 10 mg of Ezetimibe were measured and transferred to a 100 ml volumetric flask. The flask was then filled up to the mark with a suitable diluent and the mixture was sonicated for 20 minutes.

After filtration through a 0.45 µm membrane filter, the solution was further diluted to achieve a concentration of 10 µg/ml of

Ezetimibe and 180 µg/ml of Bempedoic Acid. This diluted solution was then analyzed using an HPLC system to determine the percentage of medication present in the tablets.

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

The limit of detection (LOD) is the lowest concentration of an analyte that can be reliably detected. It is determined using the formula LOD = 3.3 × σ/S, where σ is the standard deviation of the y-intercept and S is the slope of the calibration curve.

The limit of quantification (LOQ) is the lowest concentration of an analyte that can be accurately measured. It is calculated as LOQ = 10 × σ/S, where σ is the standard deviation of the y-intercept and S is the slope of the calibration curve **[15-18]**.

By establishing the LOD and LOQ, researchers can determine the sensitivity and accuracy of their analytical methods.

Precision:

Six replicate standard solutions were prepared and injected into an HPLC to evaluate the precision within the same day. Additionally, the same six replicate standard solutions were injected into the HPLC on three consecutive days to assess the precision between days. The peak area and percentage relative standard deviation (RSD) were calculated for each set of injections **[19]**.

Accuracy:

For the recovery test, known amounts of Ezetimibe and Bempedoic Acid were spiked into samples at three different levels (80%, 100%, and 120% of the expected concentration) and analyzed using the same method as before. The recovery percentage was calculated by comparing the measured concentration to the expected concentration **[20-22]**.

RESULT AND DISCUSSION:

Method development and optimization:

Multiple trials were conducted on an Kromasil C18 column using various mobile phase compositions to optimize separation. The best results were achieved with an 80:20 acetonitrile:water (pH 2.1) mobile phase adjusted with orthophosphoric acid. Ezetimibe and Bempedoic Acid were successfully separated with retention times of 7 and 8.1 minutes, respectively **(Figure 2)**.

Method validation:

The regression equation for Ezetimibe and Bempedoic Acid was obtained by plotting the calibration curve of peak area versus concentration of analyte in the range of 5-45 µg/ml and 90-720 µg/ml respectively. The linear regression equation for calibration curve of Ezetimibe was determined and the correlation coefficient (r^2) was found as 0.9982. The linear regression equation for calibration curve of Bempedoic Acid was determined and the correlation coefficient (r^2) was found as 0.9992 **(Figure 3, 4)**.

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 mentioned in the **Table 1**.

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

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 and respectively. All the calculated values are mentioned in the **Table 3**.

Accuracy:

Accuracy was determined as % Recovery at three different levels i.e., 80 %, 100 % and 120 %. All the calculated values are mentioned below in the **Table 4**.

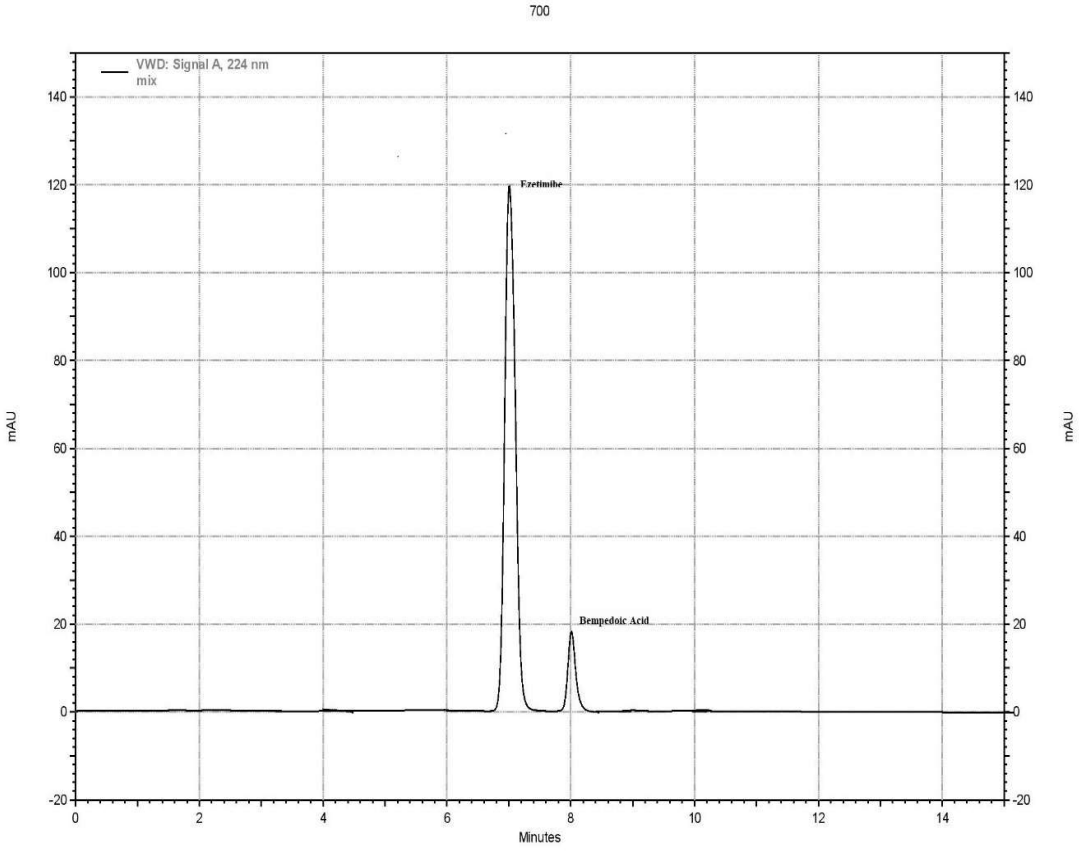

Figure 2: HPLC Chromatogram of Bempedoic Acid and Ezetimibe

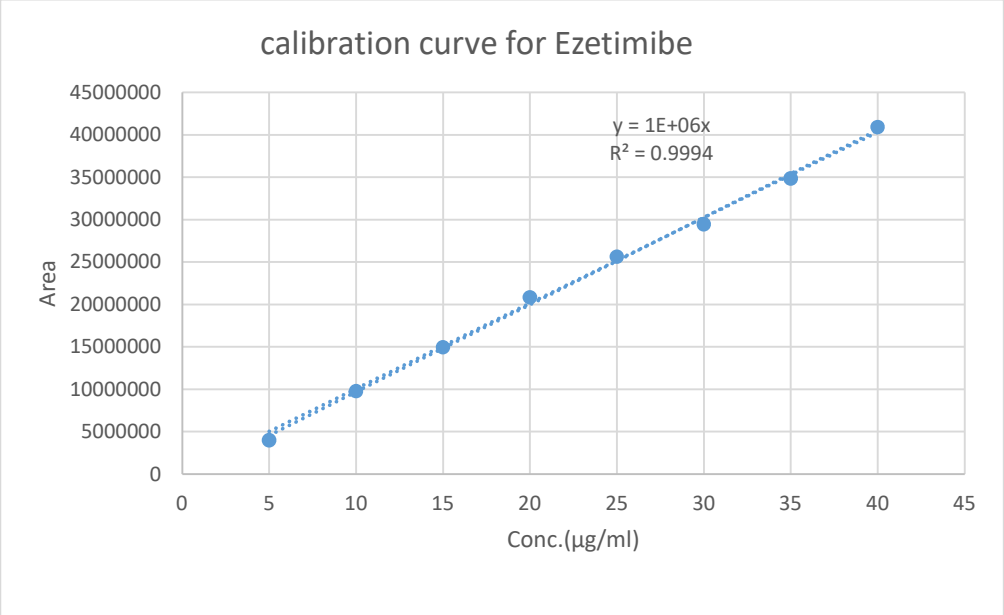

Figure 3

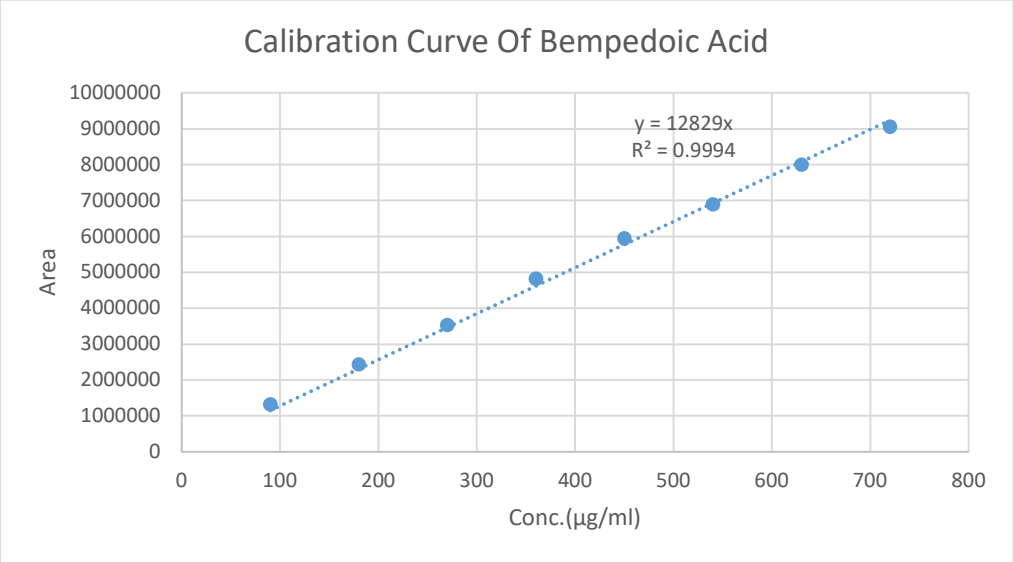

Figure 4

Table 1: Summary of Assay

Drug	Label Claim (mg/ tab)	Amount Found* [n=6] (mg/tab)	% Assay	SD	%RSD
Ezetimibe	10	9.95	99.51	116320.4	1.168
Bempedoic Acid	180	179.60	99.77	18291.26	0.793

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

Table 3: Summary of precision

Drug	Level	Conc. Added *[*n = 6]	Conc. Found by graph	SD	%RSD
Ezetimbe	Intra- Day	10	9.78	92361.57	0.944
Bempedoic Acid	Intra – Day	180	178.90	30447.30	1.32

Drug	Level	Conc. Added *[*n = 6]	Conc. Found by graph	SD	%RSD
Ezetimibe	Inter –Day 1	10	9.75	49216.14	0.504
	Inter –Day 2	10	9.73	82254.9	0.846
	Inter –Day 3	10	9.72	78948.8	0.812
Bempedoic Acid	Inter –Day 1	180	179.81	7353.9	0.319
	Inter –Day 2	180	179.05	7817.8	0.340
	Inter –Day 3	180	179.48	14969	0.650

Table 4: Summary of Accuracy

Drug	Level	Amount Added *[*n = 6]	Amount Recovered *[*n=6]	%Recovery
Ezetimibe	80%	8	7.89	98.62
	100%	10	9.96	99.30
	120%	12	11.98	98.91
Bempedoic Acid	80%	8	7.94	99.25
	100%	10	9.93	99.30
	120%	12	11.98	98.91

CONCLUSION:

In conclusion, the developed RP-HPLC method for simultaneous determination of Bempedoic Acid and Ezetimibe in pure and pharmaceutical dosage form has been successfully validated. The method demonstrated good linearity, accuracy, precision, and specificity, making it suitable for routine analysis of these two drugs. Therefore, this method can be effectively used for quality control analysis of Bempedoic Acid and Ezetimibe in pharmaceutical formulations.

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