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DEVELOPMENT AND VALIDATION OF NOVEL RP-HPLC METHOD FOR THESIMULTANEOUS ESTIMATION OF EZETIMIBE AND BEMPEDOIC ACID IN A TABLETDOSAGE FORM

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Keywords:

RP-HPLC, Ezetimibe, Bempedoic acid, Method Development, Validation

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ABSTRACT: A simple, novel, precise, and cost-effective reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated to simultaneously estimate ezetimibe and bempedoic acid in the marketed tablet dosage form. The chromatographic separation was carried out on a Prontosil C18 (250 x 4.6 mm, 5 µm) column using a mobile phase of Acetonitrile: water (60:40 v/v). The flow rate was 1.0 mL/min with detection at 225 nm using UV detector. The retention time for bempedoic acid was 4.7 min, and for ezetimibe 5.7 min. Ezetimibe showed a linear response in the concentration range of 20-60 µg/mL. Bempedoic acid showed a linear response in 180-540 µg/mL concentration range. The correlation coefficient ('r²' value) for ezetimibe and bempedoic acid was 0.9982 and 0.9998, respectively. The results of analysis have been validated as per different validation parameters. The percentage recoveries obtained for ezetimibe and bempedoic acid range from 98%-102%.

INTRODUCTION: Ezetimibe is an azetidine derivative, it prevents absorption of cholesterol by blocking the Niemann Pick C1 like 1(NPC1L1) protein on epithelial cell of gastrointestinal tract, and in hepatocytes [1]. Ezetimibe is chemically (3R, 4S)-1-(4-fluorophenyl)-3-[(3S)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl) azetidin-2-one and it belongs to the class cholesterol-lowering medications. Bempedoic acid is chemically 8-hydroxy-2, 2, 14, 14-tetramethyl pentadecane dioic acid and belongs to the adenosine triphosphate-citrate lyase (ACL) inhibitors [4].

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Bempedoic acid and Ezetimibe are used in combination for the treatment hypercholesterolemia and ASCVD by reducing lipid parameters and Attenuating hsCRP levels [3-4]. Several spectroscopic RP-HPLC and UPLC-MS have been reported to estimate ezetimibe and bempedoic acid individually and in combination with other drugs. Therefore, it was thought worthwhile to develop an accurate, precise, and Cost-effective rapid RP-HPLC method for simultaneous estimation of ezetimibe and bempedoic acid in the tablet dosage form.

MATERIALS AND METHOD:

Instrumentation: Chromatography was performed on Shimadzu prominence – i 2030 system with lab solution software for data processing. Separation and quantitation were made on Prontosil C 18 column (250×4.6 nm, 5µm).

Chemicals and Reagents: Ezetimibe (99.10) and bempedoic acid (99.8) reference standard was a gift sample from Alkem laboratory Mumbai. Acetonitrile (Merck) and Milli-Q water (HPLC Grade) were used for preparing the mobile phase.

Selection of Wavelength: Each solution was scanned using a double beam UV visible spectrophotometer between the range 200nm to 400nm, and overlain spectra were obtained.

The wavelength selected was 225, which is an isosbestic point. The overlaid spectra of bempedoic acid and ezetimibe are shown in **Fig. 1.**

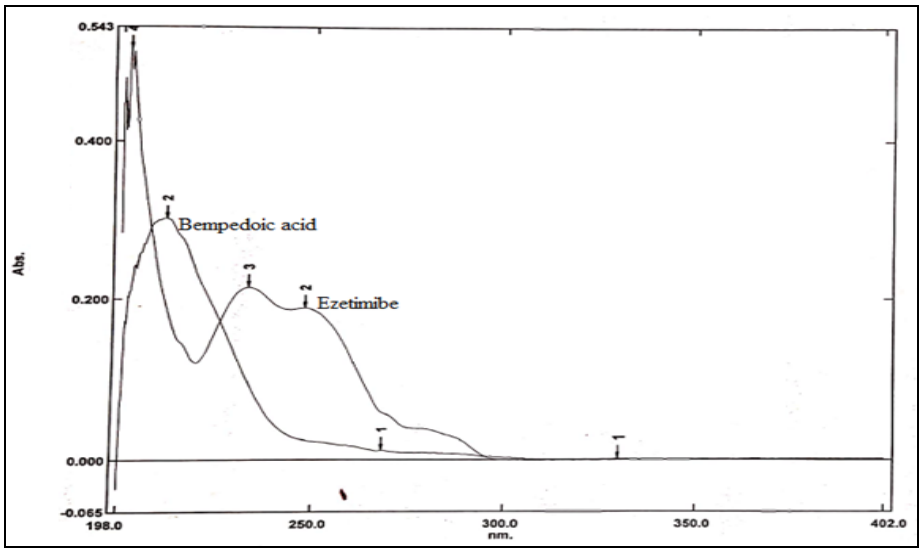

FIG. 1: UV SPECTRUM OF EZETIMIBE AND BEMPEDOIC ACID

Chromatographic Condition: Method was developed using a Prontosil C18 (250 x 4.6 mm, 5 µm) column. Mobile phase Acetonitrile: water (60:40) was used. Detection wavelength 225nm was selected by scanning standard drug solution over a wide range of wavelengths 200-400 nm using a spectrometer. The pump's flow rate was set 1.0 mL/min and the volume 10µl. The column temperature was set as 30°C.

Preparation of Mobile Phase: 60 volumes of HPLC grade acetonitrile and 40 volumes of water were used as the mobile phase.

Preparation of Diluent: Based on the Solubility of drug, diluent was selected as Water: Acetonitrile: Methanol (20:40:40).

Preparation of Standard Stock Solution: About 10 mg of each of reference standard of ezetimibe and bempedoic acid was weighed accurately and transferred to two separate 10 mL volumetric flask. Both drugs were dissolved in 60 % of diluent with shaking and volume was made up to the mark with diluent to get 1000 µg/mL of standard stock solution of each drug.

Standard Final Solution: Take 3.6 mL of standard stock solution of bempedoic acid and 0.2 mL of standard stock solution of ezetimibe transferred to 10 mL volumetric flask. Volume was made up to with diluent to get 360 µg/mL of bempedoic acid and 20µg/mL of ezetimibe standard final solutions.

Preparation of Sample Solution: Ten tablets were weighed and crushed to get a fine powder. The tablet powder equivalent to 360 mg of bempedoic acid and 20 mg of ezetimibe was transferred to a 100 mL volumetric flask and dissolved in diluent and the flask was kept in ultrasonication for 30 min. Finally, the volume was made up to the mark with the help of diluent. This solution was further diluted by taking 1ml from above solution and making up the volume up to 10 mL with diluent.

Method Development: Chromatographic separation was achieved by using Prontosil C18 (250 x 4.6 mm, 5 µm) column with acetonitrile and water in the ratio of (60:40) as the mobile phase at the flow rate of 1.0 mL/min and column temperature 30°C the detection was carried out at 225 nm. The developed, optimized method resulted in the elution of bempedoic acid at 4.7 min and

ezetimibe at 5.7 min. The total run time was 10 min.

TABLE 1: OPTIMIZED CHROMATOGRAPHIC CONDITIONS FOR EZETIMIBE AND BEMPEDOIC ACID

Parameters	Optimized conditions
Pump mode	Gradient
Column	Prontosil C18 (250 x 4.6 mm, 5 µm)
Mobile Phase	Acetonitrile: Water (60:40)
Flow rate	1.0 mL/min
Column temperature	30°C
Injection Volume	10 µL
Detection wavelength	225 nm
Retention time	5.7 min and 4.7 min respectively

Chromatograms of standard and sample of ezetimibe and bempedoic acid are shown in **Fig. 2** and **3,** respectively. The optimized chromatographic conditions are tabulated in **Table 1.**

RESULT AND DISCUSSION: The developed RP-HPLC method for ezetimibe and bempedoic acid was validated as per ICH guidelines.

Specificity: Specificity is the ability to assess the analyte unequivocally in the presence of components that may be expected to be present[6].

Method specificity was determined by observing and comparing the test result obtained for the sample solution with the standard result obtained for a pure drug. The blank, standard drug and sample chromatogram is shown in Fig. 2, 3, and **4**.

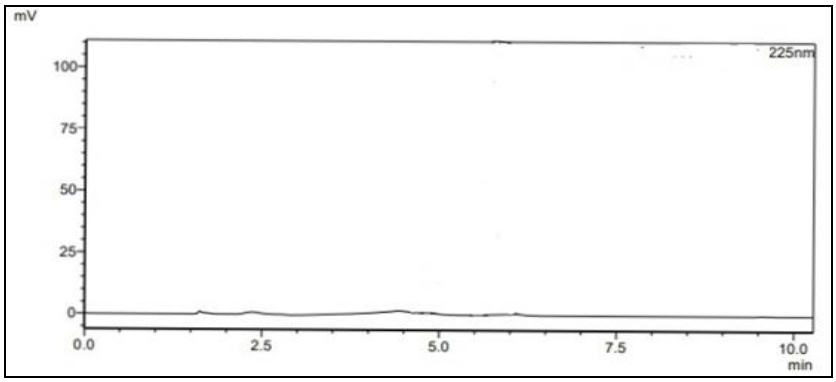

FIG. 2: CHROMATOGRAM OF BLANK SOLUTION

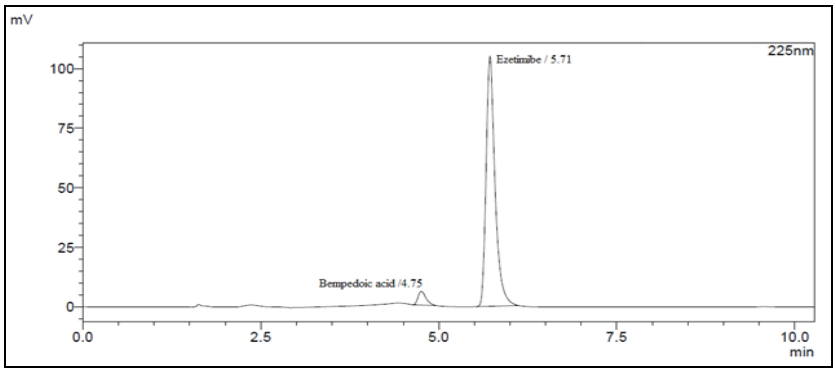

FIG. 3: CHROMATOGRAM OF STANDARD SOLUTION OF BEMPEDOIC ACID AND EZETIMIBE

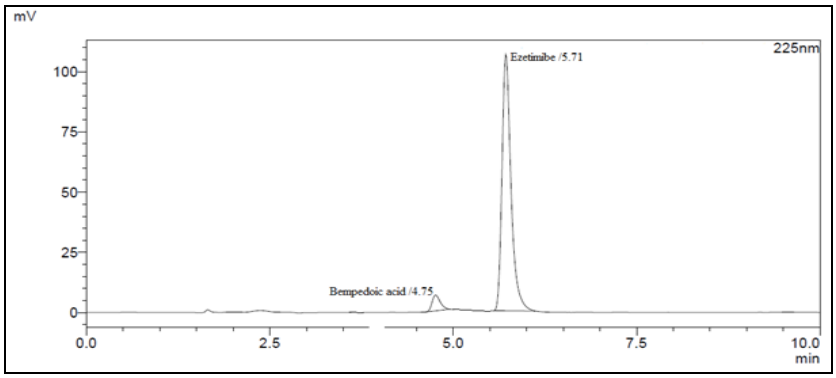

FIG. 4: CHROMATOGRAM OF SAMPLE SOLUTION OF BEMPEDOIC ACID AND EZETIMIBE

System Suitability: system suitability parameter was studied to verify the optimum conditions. System suitability test was performed as per USP guidelines on the chromatograms. The different parameter was evaluated, such as retention time, tailing factor, theoretical plate, and resolution. The obtained results are summarized in **Table 2.**

TABLE 2: SYSTEM SUITABILITY PARAMETER RESULTS

Parameter	Bempedoic acid	Ezetimibe
Retention time	4.75	5.71
Tailing factor (Less than 2)	1.27	1.41
Theoretical plate (More than 2000)	6935	9720
resolution	-	4.42

Linearity: The standard curve was obtained within the 10 -30 µg/mL concentration range for ezetimibe and 180 -540 µg/mL for Bempedoic acid. The linearity of this method was evaluated by linear regression analysis. The linearity graph was plotted by taking the concentration of the drug on the X-axis and the corresponding peak area on the Y-axis, as shown in **Fig. 5** and **Fig. 6**. The linearity data is summarized in **Table 3.**

TABLE 3: LINEARITY DATA OF EZETIMIBE AND BEMPEDOIC ACID

Concentration of Ezetimibe (PPM)	Peak Area of Ezetimibe	Concentration of Bempedoic acid (PPM)	Peak Area of bempedoic acid
30	1304739	540	70355
25	1106539	450	55758
20	893182	360	40606
15	669887	270	25844
10	422047	180	11926
Correlation Coefficient (r^2)	0.9982	Correlation Coefficient (r^2)	0.9998

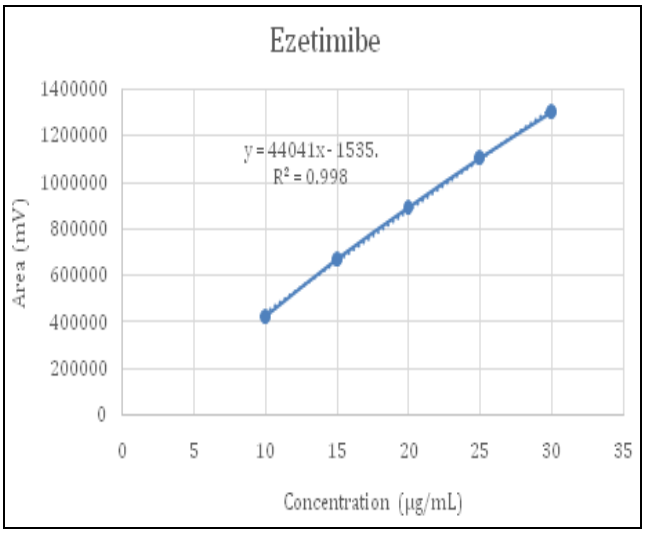

FIG. 5: CALIBRATION CURVE OF EZETIMIBE

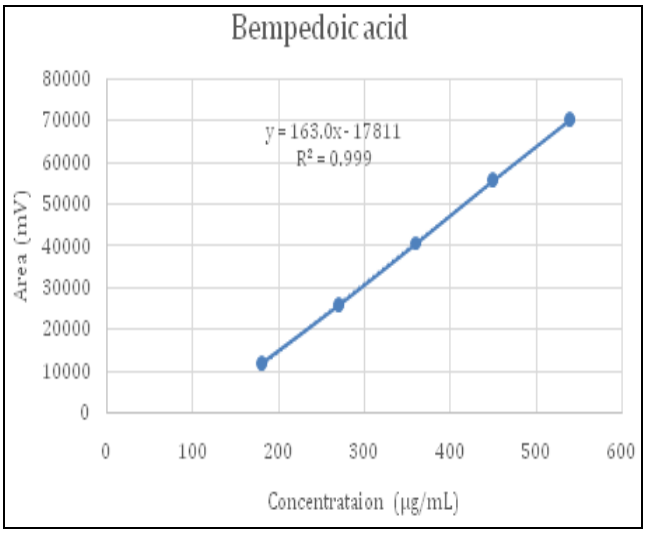

FIG. 6: CALIBRATION CURVE OF BEMPEDOIC ACID

Precision: Precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling if same sample under the prescribed conditions [6].

System precision was performed by injecting six replicate injection of the standard solution of ezetimibe (20µg/ml) and bempedoic acid (360µg/mL). The average, standard deviation (SD) and % RSD of the area in six replicate injection was calculated and reported. And method precision was performed by injecting replicate injection of sample solution of ezetimibe (20µg/mL) and bempedoic acid (360µg/mL).

Its % assay, average, standard deviation (SD), and %RSD were calculated and reported. The result of system precision and method precision are summarized in **Table 4**.

TABLE 4: SYSTEM PRECISION & METHOD PRECISION RESULTS

Injection	System Precision Area of Standard		Method Precision % Assay	
	Ezetimibe	Bempedoic acid	Ezetimibe	Bempedoic acid
1	918910	51125	99.70	99.24
2	921249	51222	99.73	99.36
3	919716	51257	99.00	98.46
4	914897	50394	99.48	99.8
5	914167	50556	99.01	97.62
6	914161	51767	99.39	100.2
Mean	917183.3	51053.5	99.38	99.11
SD	3142.786	503.2784	0.321	0.936
%RSD	0.342656	0.985786	0.323	0.944

Accuracy / Recovery Studies: To study the accuracy and reproducibility of the proposed method recovery experiment were carried out [6]. A fixed amount of preanalyzed sample was taken and the standard drug was added at 50%, 100% and 150% levels. Each level was repeated three times. The content s of ezetimibe and bempedoic acid found by the proposed method is shown in **Table 5.** The mean of recoveries of bempedoic acid and ezetimibe was 98.88% and 100.02%, respectively, which shows no interference from the excipient.

TABLE 5: ACCURACY DATA OF EZETIMIBE AND BEMPEDOIC ACID

Pre-Analyzed Sample	Level	Amount Added (mg)	Amount Recovered (mg)	% Recovery	Mean of % recovery
Ezetimibe	50%	0.1	0.099	99.74	
	100%	0.2	0.20	100.00	100.02
	150%	0.3	0.30	100.33	
Bempedoic acid	50%	1.8	1.805	100.27	
	100%	3.6	3.53	98.26	98.88
	150%	5.4	5.29	98.13	

Assay of Marketed Formulation: Ten tablets were weighed and powdered finely. tablet powder equivalent to 360 mg of bempedoic acid and 20 mg of ezetimibe was transferred into 100 mL volumetric flask add 60 ml diluent sonicate for 30 min make up the volume up to mark. This solution was further diluted by taking 1ml from the above solution in 10 mL volumetric flak and making up the volume up to 10 mL with diluent to obtain 360 µg/mL of bempedoic acid and 20µg/mL of ezetimibe.

TABLE 6: ANALYSIS OF THE FORMULATION

Tablet	Drug	% Assay
Nexlizet tab	Bempedoic acid	98.46
	Ezetimibe	99.00

Robustness: Robustness is a measure of its capacity to remain unaffected by small deliberate in the chromatographic method parameters and provides an indication of its reliability [6].

This was done by small, deliberate changes in chromatographic conditions at 3 different levels and retention time of ezetimibe and bempedoic acid. The factors selected were flow rate, column temperature, and wavelength.

It was observed that there were no deliberate changes in the chromatogram, which demonstrate that the RP-HPLC method developed is robust. The result is described in **Table 7.**

TABLE 7: RESULT OF ROBUSTNESS STUDY

Parameter	Level	Bempedoic acid			Ezetimibe		
		Retention time	Number of Theoretical Plates	Peak Tailing	Retention time	Number of Theoretical Plates	Peak Tailing
Flow Rate	0.8 mL/min	5.97	6961	1.16	6.80	9716	1.40
	1 mL/min	4.75	6935	1.24	5.71	9720	1.41
	1.2 mL/min	3.92	6881	1.16	4.80	9690	1.27
Temperature	28°C	4.78	6861	1.62	5.79	9258	1.34
	30°C	4.75	6935	1.24	5.71	9720	1.41
	32°C	4.71	6961	1.38	5.63	9675	1.32

Wavelength	223 nm	4.72	6893	1.33	5.66	9774	1.39
	225 nm	4.75	6935	1.24	5.71	9720	1.41
	227 nm	4.75	6894	1.27	5.72	9631	1.36

CONCLUSION: Based on the results, it is concluded RP-HPLC method was successfully developed for simultaneous estimations of bempedoic acid and ezetimibe pharmaceutical formulation. Both drugs have good resolution with short analysis time 10 min. The developed HPLC method was found to be simple, accurate, linear, precise and robust.

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CONFLICTS OF INTEREST: Nil

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