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Toxicology-Focused Development and Validation of an RP-HPLC Technique for Quantifying Bempedoic Acid and Rosuvastatin in a Fixed-Dose Combination

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Abstract

A straightforward, precise method was developed to simultaneously assess bempedoic acid and rosuvastatin in both bulk and tablet forms. Utilizing a DIKMA Spursil C18 column (4.6 mm × 250 mm, 3.0 µm), chromatography was conducted with a mobile phase comprising 30% phosphate buffer and 70% acetonitrile, flowing at 1 mL/min. Operating at ambient temperature, the optimized wavelength for detection was set at 240 nm, revealing retention times of 4.418 min for bempedoic acid and 3.524 min for rosuvastatin. Both compounds exhibited high purity levels, with bempedoic acid at 100.57% and rosuvastatin at 100.15%. The system's suitability parameters, such as theoretical plates and tailing factor, fell within acceptable thresholds at 4402.39 and 6959.54, respectively, with values of 1.12 and 3.60. Linearity studies demonstrated strong correlation coefficients (r^2) of 0.999 for both bempedoic acid and rosuvastatin, with mean recovery percentages of 100.33% and 100.74%, respectively. Repeatability showed low % RSD (relative standard deviation) values of 1.0 and 0.3, while intermediate precision displayed % RSD values of 0.7 and 1.2, respectively. Overall, the precision study affirmed the method's reliability, robustness, and repeatability. These findings validate the proposed RP-HPLC method as a dependable, rapid, and reproducible technique suitable for routine estimation of bempedoic acid and rosuvastatin in both bulk and tablet forms.

Keywords: Bempedoic acid, rosuvastatin, reverse-phase high-performance liquid chromatography (RP-HPLC), simultaneous estimation

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INTRODUCTION

Bempedoic acid is utilized alongside lifestyle adjustments or other medications to manage refractory hypercholesterolemia. Typically, low-density lipoprotein (LDL) cholesterol is synthesized in the liver and circulates within the bloodstream. However, when blood cholesterol levels reach saturation, excess LDL accumulates in blood vessels, including the coronary arteries, heightening the risk of cardiovascular incidents. Bempedoic acid, functioning as a prodrug, necessitates activation within the liver [1]. Activation occurs through the very-long-chain acyl-CoA synthetase-1 (ACSVL1) enzyme, yielding ETC-1002-CoA, the biologically active metabolite. Following activation, bempedoic acid directly inhibits adenosine triphosphate (ATP) lyase (also known as ATP synthase), a pivotal enzyme in cholesterol synthesis. Its IUPAC name is 8-hydroxy-2,2,14,14-

tetramethylpentadecanedioic acid, with a molecular formula of $C_{19}H_{36}O_5$ and a molecular weight of 344.4 [2]. Rosuvastatin, an HMG-CoA (hydroxymethylglutaryl coenzyme A) reductase inhibitor, is prescribed to reduce lipid levels and mitigate cardiovascular risks such as myocardial infarction and stroke. Rosuvastatin, functioning as a statin medication, competitively obstructs the enzyme HMG-CoA reductase. This enzyme facilitates the conversion of HMG-CoA to mevalonate, which is a pivotal stage in the biosynthesis of cholesterol [3]. Its primary action occurs in the liver, where diminished hepatic cholesterol levels prompt the upregulation of hepatic LDL receptors, thereby increasing the uptake of LDL cholesterol by the liver as seen in Figures 1 and 2.

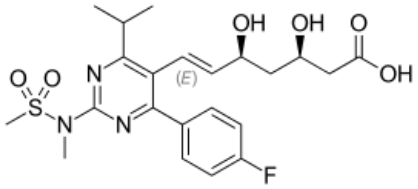

Figure 1. Structure of bempedoic acid.

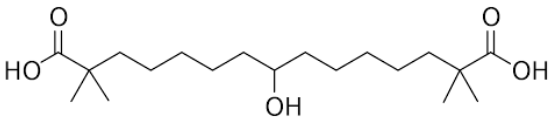

Figure 2. Structure of rosuvastatin.

The review of existing literature indicates a scarcity of methods documented for assessing bempedoic acid and rosuvastatin individually or in conjunction with other compounds, whether in their pure forms or within drug formulations, using RP-HPLC (reverse-phase high-performance liquid chromatography) techniques. Recognizing the need for a suitable, economical RP-HPLC approach to routinely analyze bempedoic acid and rosuvastatin in pharmaceutical formulations, efforts were directed towards devising a straightforward, precise, accurate, and cost-effective analytical method for quantifying these substances [4].

MATERIALS AND METHODS

Chemicals and Reagents: Bempedoic acid and rosuvastatin were purchased from Honour Lab. NaH_2PO_4 was analytical grade supplied by Finerchem Limited; orthophosphoric acid (Merck), and water and methanol for HPLC (Lichrosolv) (Merck).

Equipment and Chromatographic Conditions: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, ultraviolet (UV) detector and Empower 2 software. Analysis was carried out at 240 nm with column Spursil C18 (250 × 4.6 mm, 5 µm), dimensions at 25°C temperature. The optimized mobile phase consists of phosphate buffer 300 mL (30%) and 700 mL acetonitrile (70%). Flow rate was maintained at 1 mL/min.

Preparation of Solutions

Preparation of Phosphate Buffer pH 5.0

To prepare phosphate buffer solution, by adding 6.8 gm of phosphate buffer in a 1000 mL water. Adjust this solution to pH 5.0 by using sodium hydroxide.

Preparation of Mobile Phase

Mix a mixture of phosphate buffer 300 mL (30%) and 700 mL acetonitrile (70%) and degas in ultrasonic water bath for 5 minutes. Filter through 4.5 µm filter under vacuum filtration.

Diluents Preparation

Phosphate buffer:acetonitrile (30:70) ratio.

Standard Solution Preparation

Accurately weigh and transfer 90 mg of bempedoic acid and 20 mg of rosuvastatin working standard into a 25 mL clean dry volumetric flask and add about 7 mL of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (stock solution).

Further pipette 0.3 mL of the above stock solutions into a 10 mL volumetric flask and dilute up to the mark with diluent [5].

Sample Solution Preparation

Accurately weigh and transfer of equivalent tablet powder of 90 mg of bempedoic acid and 20 mg of rosuvastatin (330 mg) into a 25 mL clean dry volumetric flask add about 7 mL of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (stock solution).

Further pipette 0.3 mL of the above stock solutions into a 10 mL volumetric flask and dilute up to the mark with diluent [6].

Procedure

Inject 20 μL of the standard, sample into the chromatographic system and measure the areas for bempedoic acid and rosuvastatin peaks and calculate the percentage assay by using the formulae.

RESULTS AND DISCUSSION

Method

The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness, and robustness as per ICH guidelines [7].

Chromatographic Parameters

Equipment:	High performance liquid chromatography equipped with auto sampler and PDA (photo-diode array) detector
Column:	Spursil C18 (250 × 4.6 mm, 5 μm)
Flow rate:	1.0 mL/min
Wavelength:	240 nm
Injection volume:	20 μL
Column temperature:	Ambient
Run time:	10 min

Retention time, tailing factor, and USP (United States Pharmacopeia) theoretical plate count of the developed method are shown in Table 1.

Table 1. System suitability parameters.

Parameter	Bempedoic Acid	Rosuvastatin
Theoretical plates	4402.39	6959.54
Retention time	4.418	3.524
Tailing factor	1.12	3.60

Assay of Pharmaceutical Formulation: The proposed validated method was successfully applied to determine bempedoic acid and rosuvastatin in their tablet dosage form as seen in Figures 3–5. The result obtained was comparable with the corresponding labeled amounts and they were shown in Table 2.

Table 2. Assay results for bempedoic acid and rosuvastatin.

	Label Claim (mg)	% Assay
Bempedoic acid	90	100.57
Rosuvastatin	20	100.15

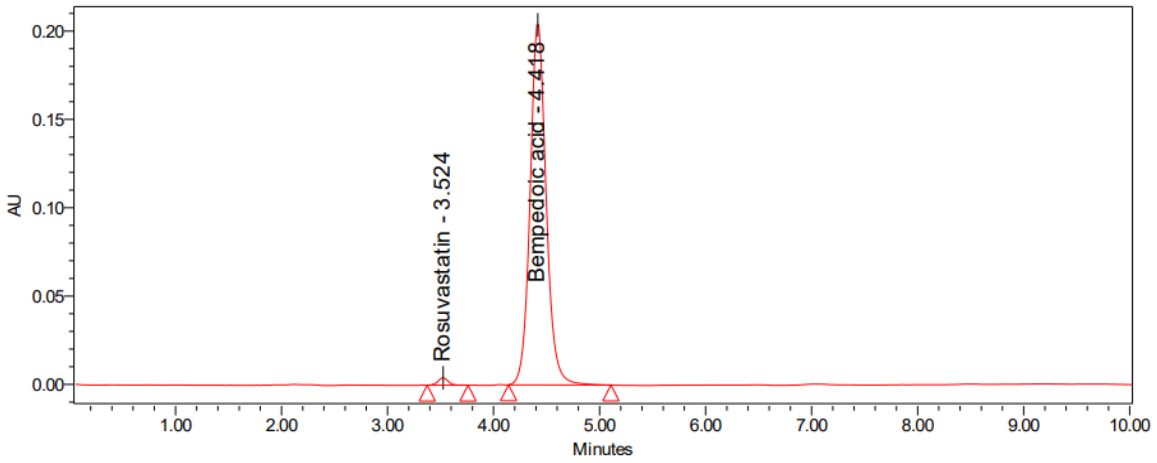

Figure 3. Standard chromatogram.

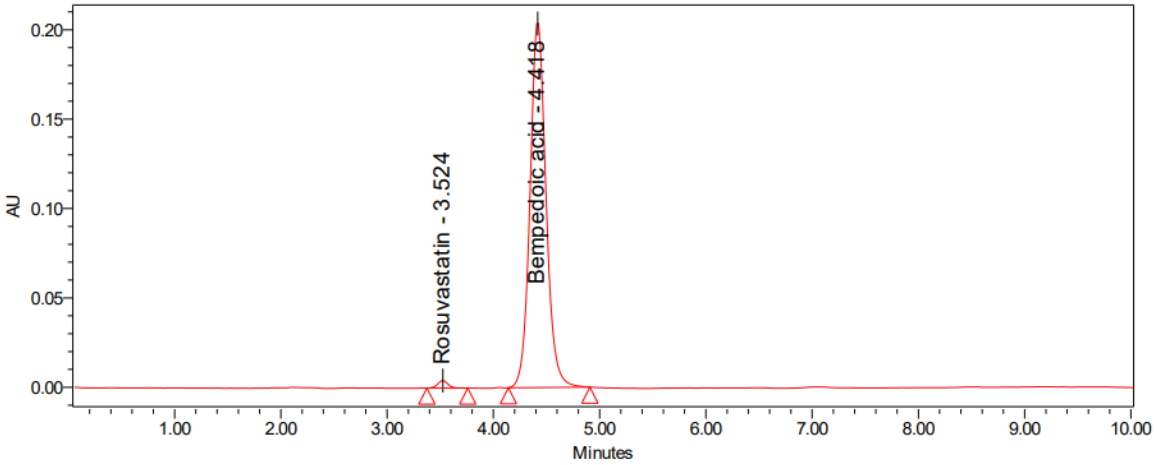

Figure 4. Sample chromatogram.

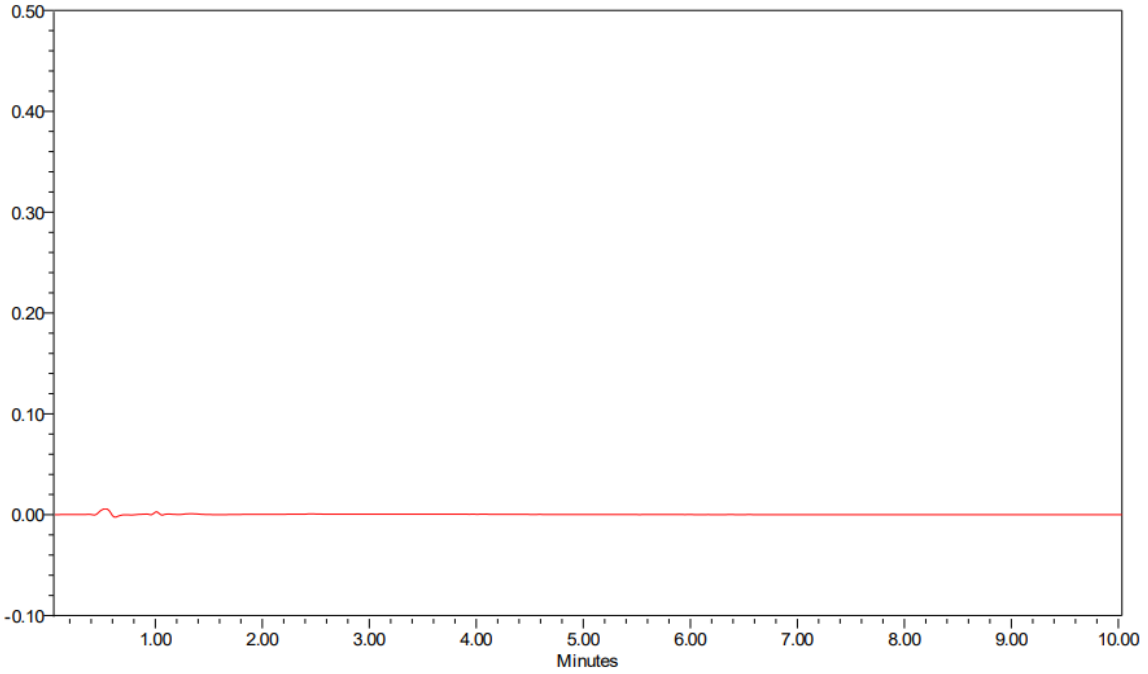

Figure 5. Blank chromatogram.

Validation of Analytical Method

Linearity: The linearity study was performed for the concentration of 36 ppm to 180 ppm and 8 ppm to 40 ppm level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis peak area) and calculate the correlation coefficient [8]. The results are shown in Tables 3 and 4.

Accuracy Studies: The accuracy was determined by help of recovery study. The recovery method carried out at three level 50%, 100%, 150% and 50%, 100%, 150% Inject the standard solutions into chromatographic system. Calculate the amount found and amount added for bempedoic acid and rosuvastatin and calculate the individual recovery and mean recovery values. The results are shown in Tables 5 and 6.

Table 3. Linearity results of bempedoic acid.

S.N.	Linearity Level	Concentration	Area
1	I	36	679688
2	II	72	1398189
3	III	108	2166875
4	IV	144	2784702
5	V	180	3525027
Correlation coefficient			0.999

Table 4. Linearity results of rosuvastatin.

S.N.	Linearity Level	Concentration	Area
1	I	8	21599
2	II	16	22895
3	III	24	24297
4	IV	32	25545
5	V	40	27085
Correlation coefficient			0.999

Table 5. Accuracy results for bempedoic acid.

% Concentration (at Specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1055764	45	45.08	100.17	100.33
100%	2132744	90	91.06	101.17	
150%	3151028	135	134.53	99.65	

Table 6. Accuracy results for rosuvastatin.

%Concentration (at Specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	13883	10	10.26	102.58	100.74
100%	27022	20	19.97	99.84	
150%	40483	30	29.91	99.71	

Precision Studies: Precision was calculated from coefficient of variance for six replicate injections of the standard. The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD (relative standard deviation) for the area of six replicate injections was found. The results are shown in Table 7.

Table 7. Precision results for bempedoic acid and rosuvastatin.

Injection	Area for Bempedoic Acid	Area for Rosuvastatin
Injection-1	2128651	27340
Injection-2	2141733	27340
Injection-3	2084645	27497
Injection-4	2125632	27287
Injection-5	2135815	27442
Injection-6	2149902	27310
Average	2127730	27369.33
Standard deviation	22867.51	81.97
%RSD	1.0	0.3

Ruggedness: To evaluate the intermediate precision of the method, Precision was performed on different day. The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found. The results are shown in Table 8.

Robustness: As part of the robustness, deliberate change in the flow rate, mobile phase composition, temperature variation was made to evaluate the impact on the method (Figure 6, 7). The flow rate was varied at 0.9 mL/min to 1.1 mL/min. The results are shown in Tables 9 to 12.

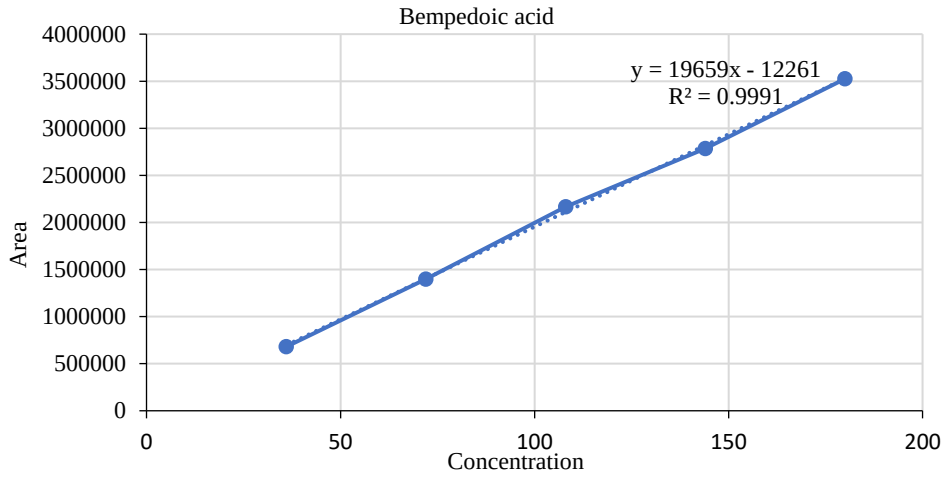

Figure 6. Linearity graph for bempedoic acid.

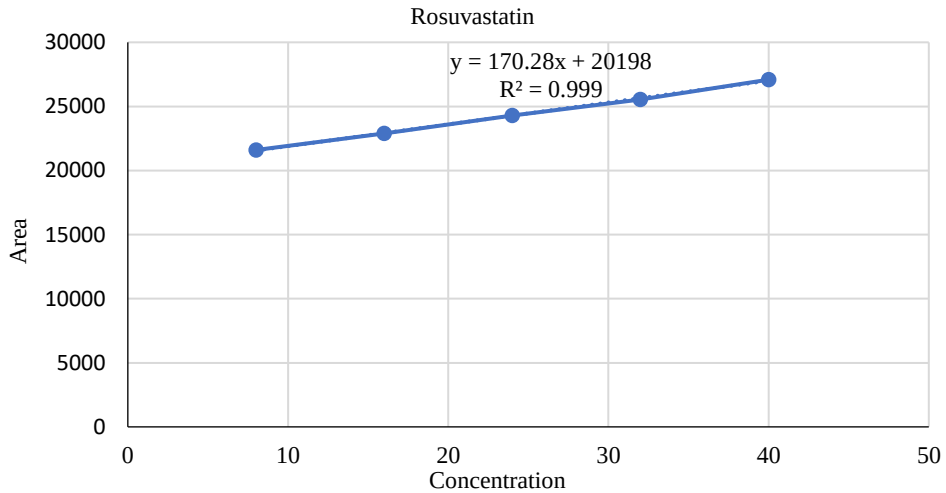

Figure 7. Linearity graph for rosuvastatin.

Table 8. Ruggedness results of bempedoic acid and rosuvastatin.

Injection	Area for Bempedoic Acid	Area for Rosuvastatin
Injection-1	2041733	27340
Injection-2	2084645	27497
Injection-3	2025632	27287
Injection-4	2008618	27287
Injection-5	2035815	27310
Injection-6	2052672	26910
Average	2041519	27271
Standard deviation	25890.91	194.0
%RSD	0.7	1.2

Table 9. Flow variation results for bempedoic acid.

S.N.	Flow Rate (mL/min)	System Suitability Results	
		USP Plate Count	*USP Tailing*
1	0.9	4565.01	1.09
2	1	4402.39	1.12
3	1.1	4525.26	1.08

Table 10. Flow variation results for rosuvastatin.

S.N.	Flow Rate (mL/min)	System Suitability Results		
		USP Plate Count	*USP Tailing*	*USP Resolution*
1	0.9	5705.32	1.01	3.59
2	1	6959.54	1.01	3.60
3	1.1	5402.20	1.08	3.66

Table 11. Change in organic composition in the mobile phase.

S.N.	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	*USP Tailing*
1	10% less	3796.37	1.06
2	*Actual	4402.39	1.12
3	10% more	4246.82	1.06

Table 12. Change in organic composition in the mobile phase on System Suitability Parameters.

S.N.	Change in Organic Composition in the Mobile Phase	System Suitability Results		
		USP Plate Count	*USP Tailing*	*USP Resolution*
1	10% less	5384.81	1.02	2.85
2	*Actual	6959.54	1.01	3.60
3	10% more	4785.72	1.01	3.55

DISCUSSION

The passage discusses the validation of an HPLC method for simultaneously analyzing bempedoic acid and rosuvastatin in bulk and tablet forms. It emphasizes the method's reliability, simplicity, precision, accuracy, and sensitivity, highlighting its suitability for routine quality control analysis in pharmaceutical manufacturing [9]. In essence, the text underscores the thorough validation process undertaken to ensure the efficacy of the HPLC method. It discusses how the method was designed to be user-friendly, with straightforward procedures to facilitate ease of use by laboratory personnel. The precision of the method was verified through repeated analyses, demonstrating consistent results within acceptable margins of error. Accuracy was confirmed by comparing the method's results with those

obtained from reference standards or established methods, establishing its reliability in quantifying the target compounds accurately. Furthermore, the method's sensitivity was demonstrated by its ability to detect and quantify bempedoic acid and rosuvastatin at low levels, which is crucial for pharmaceutical analysis where precise measurement of drug concentrations, even at trace levels, is essential for ensuring product quality and efficacy [10]. The successful validation of this method highlights its potential for routine quality control analysis in pharmaceutical manufacturing, with its adaptability to various dosage forms enhancing its utility. By providing a reliable means of assessing drug content and purity, the method contributes to ensuring the consistency and compliance of pharmaceutical products with regulatory standards. In summary, the validated HPLC method offers simplicity, precision, accuracy, and sensitivity, making it a robust tool for routine quality control analysis in pharmaceutical laboratories. Its adoption can streamline analytical processes and instill confidence in the quality of bempedoic acid and rosuvastatin formulations, ultimately benefiting patient safety and treatment efficacy.

CONCLUSION

The developed HPLC method underwent thorough validation procedures, demonstrating its efficacy in analyzing bempedoic acid and rosuvastatin simultaneously in both bulk and tablet forms. The validation process encompassed various parameters, ensuring the method's reliability and suitability for routine quality control analysis. In terms of simplicity, the method was designed to be user-friendly, with straightforward procedures that can be easily followed by laboratory personnel. Its precision was verified through repeated analyses, showing consistent results within acceptable margins of error. Accuracy, a critical aspect in analytical methods, was confirmed by comparing the method's results with those obtained from a reference standard or an established method, establishing its reliability in quantifying the target compounds accurately. Moreover, sensitivity, an essential characteristic for detecting low concentrations of analytes, was demonstrated through the method's ability to detect and quantify bempedoic acid and rosuvastatin at low levels. This attribute is particularly advantageous for pharmaceutical analysis, where precise measurement of drug concentrations, even at trace levels, is crucial for ensuring product quality and efficacy. The successful validation of this method underscores its potential for routine quality control analysis in pharmaceutical manufacturing. Its adaptability to both bulk and tablet dosage forms further enhances its utility, offering versatility in analyzing different formulations of bempedoic acid and rosuvastatin. By providing a reliable means of assessing drug content and purity, this method contributes to ensuring the consistency and compliance of pharmaceutical products with regulatory standards. In summary, the validated HPLC method for the simultaneous estimation of bempedoic acid and rosuvastatin demonstrates simplicity, precision, accuracy, and sensitivity, making it a robust tool for routine quality control analysis in pharmaceutical laboratories. Its adoption can streamline analytical processes and bolster confidence in the quality of bempedoic acid and rosuvastatin formulations, ultimately contributing to the delivery of safe and effective medications to patients.

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