Novel Analytical Method Development and Validation for Simultaneous Estimation of Rosuvastatin and Bempedoic acid in Marketed Formulation using RP-HPLC

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Abstract- **The aim of the study is to develop an straight forward, precise, accurate, reliable and optimised method for the simultaneous estimation of Rosuvastatin and Bempedoic acid in the pharmaceutical formulation and the method was subsequently validated in accordance with the ICH guidelines. The suggested technique is based on the reversed phase mode separation of the two drugs using Shimadzu's C18 (250x4.6 mm, 5µm) column. The drugs were separated using the isocratic elution method with a mobile phase composed of phosphate buffer(pH 4):acetonitrile (40:60v/v) at a flow rate of 1.5 mL/min and 20 µL injection volume. UV detection was carried out at 215 nm while maintaining a column temperature at 40 °C. The procedure was approved in accordance with the ICH standards. Using the optimised technique, peak retention times of Rosuvastatin and Bempedoic acid was found to be 2.6 min and 4.1 min respectively. Both Rosuvastatin and Bempedoic acid had linearities and ranges of 20–60 µg/mL (R^2=0.9965) and 90–270 µg/mL (R^2=0.9947), respectively. When known amount of a reference drug were added to the pre-analyzed test solution for recovery test, the accuracy of the method was found to be 100.15% for Rosuvastatin and 99.85% for Bempedoic acid. Both precision and robustness study found to have % RSD values of less than 2. Thus, the suggested method can be successfully used in routine analysis for the simultaneous determination of Rosuvastatin and Bempedoic acid in dosage form.**

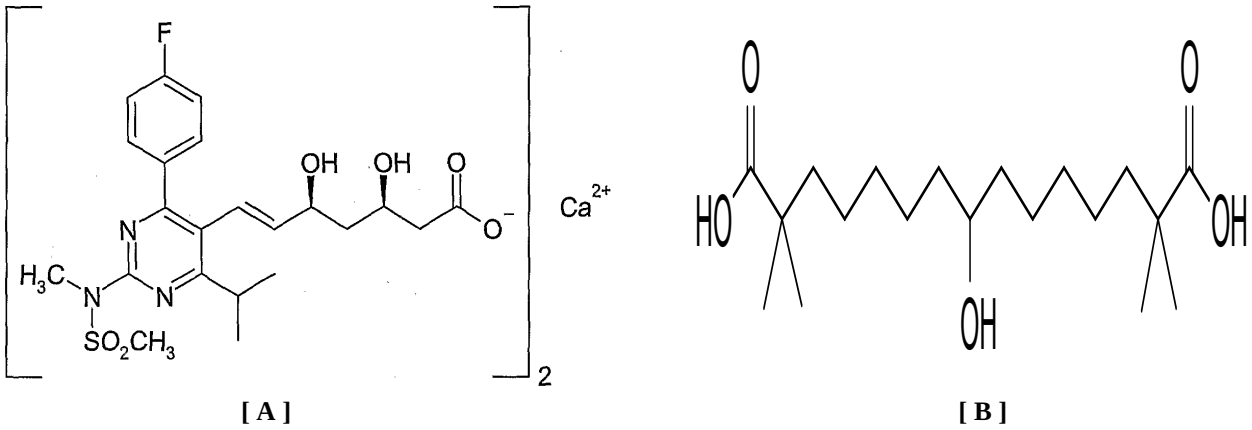
Keywords: **Rosuvastatin; Bempedoic acid; UV detection; Simultaneous estimation; Method development; RP-HPLC; Novel.**

INTRODUCTION

High level of cholesterol in the blood are referred to as hypercholesterolemia or high cholesterol. It is a kind of dyslipidemia (any abnormalities of lipid and lipoprotein levels in the blood), hyperlipoproteinemia (high levels of lipoproteins in the blood), and hyperlipidemia (high levels of lipids in the blood). Diet, obesity, inherited (genetic) disorders (such LDL receptor mutations in familial hypercholesterolemia), or the presence of additional conditions like type 2 diabetes and an underactive thyroid can all contribute to elevated levels of non-HDL cholesterol and LDL in the blood[1].

Rosuvastatin calcium(Fig. 1A), an new member of "statins" family competitively inhibits an enzyme called as HMG-CoA reductase which is an rate limiting enzyme that converts 3-hydroxy-3-methylglutaryl coenzyme A into mevalonate, a precursor to cholesterol. . Chemically, rosuvastatin calcium is (3R, 5S, 6E)-7-[4-(4-fluorophenyl)-2-(N-methyl methane sulfonamido)[[6-(propan-2-yl) pyrimidin-5-yl]3,5 dihydroxyheptenoic acid calcium salt[2-5]. Bempedoic acid(Fig. 1B) is the primary adenosine triphosphate citrate lyase (ACL) inhibitor for reducing LDL cholesterol. The chemical name for Bempedoic acid is 8-hydroxy-2,2,14,14-tetramethylpentadecanedioic acid. Bempedoic acid is a prodrug that needs to be activated in the liver. It is activated to ETC-1002-CoA, the pharmacologically active product, by the very-long-chain acyl-CoA synthetase-1 (ACSVL1) enzyme. The enzyme ATP lyase, also referred to as ATP synthase, is crucial for the production of cholesterol. Following coenzyme A (CoA) activation of the parent medication in the liver, ETC-1002-CoA blocks this enzyme directly[6-7].

A summary of the literatures showed that HPLC methods have been reported for the analysis of these drugs alone or in combination with other drugs. However, so far there is no reported HPLC method for simultaneous estimation of these two selected API. As a result, an effort has been made to create a novel isocratic reverse-phase HPLC technique for the simultaneous estimation of Rosuvastatin and Bempedoic acid in commercial formulation.



[A] **[B]**

Fig 1: Chemical structure of **[A]** Rosuvastatin [**B]** Bempedoic acid

MATERIALS AND METHODS

Instruments

HPLC of Shimadzu (LC-2030 Prominence-i) with lab solution software for data processing, UV Spectrometer of Shimadzu UV-2300 for wavelength selection, HPLC Column of Shimadzu C18 (4.6 × 250mm, 5µm) for separation and quantitation, pH meter of PCi Analytics, weighing balance of Accuratio, and Sonicator of Kromtech India were used for the analysis.

Reagents and Chemicals

Methanol and Acetonitrile of HPLC grade was procured from Thermo FisherScientific India Pvt. Ltd., Powai, Mumbai. Potassium dihydrogen phosphate and ortho phosphoric acid was purchased from High Purity Laboratory Chemicals and Milli-Q-Water(HPLC grade).

Marketed Formulation

Tablets of Rosuvastatin and Bempedoic acid under brand name ROSLAREN B™ with the strength 40 mg and 180 mg respectively marketed by La Renon Healthcare Private Ltd were obtained from local pharmacy.

Active Pharmaceutical Ingredients

Reference standard of Rosuvastatin and Bempedoic acid with 99.72%, and 99.80% potency respectively was received from Alkem Laboratories Ltd.

Selection of wavelength

The correct wavelength selection affects the method's sensitivity that employs a UV detector. For the purpose of detecting drugs, an optimum wavelength is one that provides maximum absorption and a good response. In the UV Spectrophotometer, the λ max at 215 nm was selected for the study from the overlapped spectra of Rosuvastatin and Bempedoic acid when scanned in the range of 200-400nm. Overlay Spectra of Rosuvastatin and Bempedoic acid shown in Fig 2.

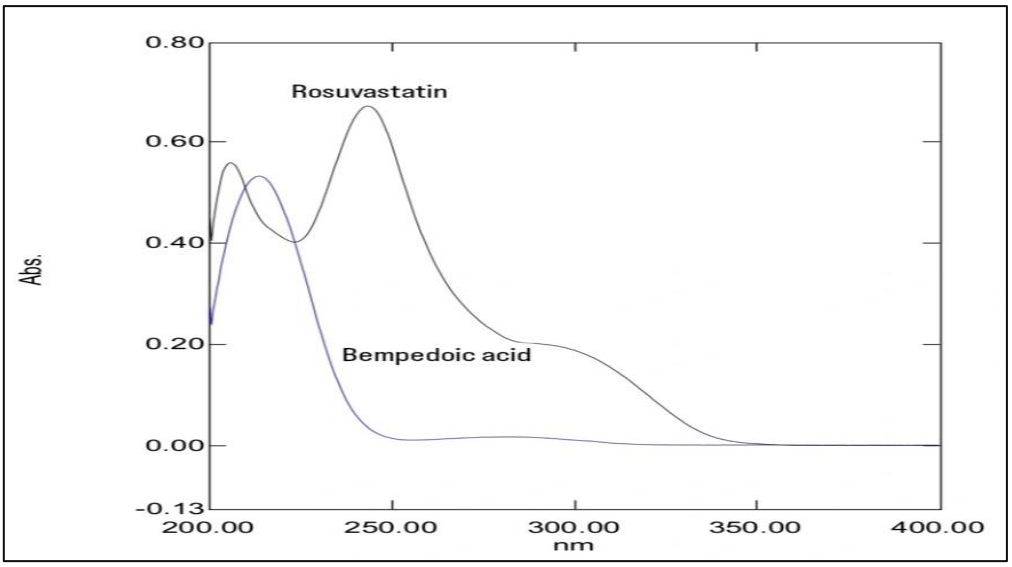


Fig 2. Overlay UV Spectra of Rosuvastatin and Bempedoic acid

Chromatographic conditions

Chromatographic method was developed on Shimadzu C 18 (4.6 x 250 mm, 5 µm) column. The mobile phase consists of phosphate buffer (pH 4):acetonitrile(40:60 v/v). The detection wavelength 215 nm was chosen by scanning a reference drug solution with a spectrophotometer throughout a wavelength range of 200-400 nm. Pump flow rate was set to 1.5 mL/min with 20 µL injection volume. The column temperature was set at 40° C.

Preparation of mobile phase
Mixed phosphate buffer and acetonitrile in the ratio of 40:60 v/v respectively and degassed.
Preparation of phosphate buffer pH 4
Dissolved 27.2g of potassium dihydrogen phosphate in 900 mL of milli Q water. Adjusted the pH 4.0±0.05 with diluted ortho phosphoric acid and make up the volume upto 1000 mL with water. The solution was sonicated and filtered through 0.45 µm membrane filter.
Preparation of diluent
Based on the solubility of the drugs, 100 % methanol was used as a diluent.
Preparation of standard stock solution
Weighed accurately 100 mg of Bempedoic acid and 10 mg of Rosuvastatin and transferred it into two different 100 mL volumetric flask. Add 70% diluent(100% methanol) in both the flask. Dissolved and sonicated with intermediate shaking. The resultant solution was then diluted upto the mark to get 1000 µL/ml and 100 µL/mL solution. Further dilutions were made using this stock solution.
Preparation of standard solution
1.8 mL was pipetted from Bempedoic acid stock solution and 4 mL from Rosuvastatin stock solution into 10 mL of volumetric flask and make up the volume with the diluent to get the final concentration of 180 µg/mL and 40 µg/mL of Bempedoic acid and Rosuvastatin respectively.
Preparation of sample solution
10 Roslaren B™ tablets were weighed and average weight calculated. All tablets were triturated with the help of motar and pestle to make fine powder. Powder equivalent to 180 mg of Bempedoic acid and 40 mg of Rosuvastatin was weighed and transferred into 100 mL volumetric flask. 70 mL of diluent added and sonicated for 1 Hr with intermediate shaking then resultant solution made up to the mark with diluent then filtered through whatmann filter and the final solution was injected into the HPLC system.
Method development
Chromatographic separation was carried out using an Shimadzu's C 18 (4.6 x 250 mm, 5 µm) column with phosphate buffer (pH 4) and acetonitrile in a 40:60 ratio as the mobile phase at a flow rate of 1.5 mL/min and a column temperature of 40° C. The detection was done at 215 nm. Bempedoic acid and Rosuvastatin was eluted by the developed, improved process in 4.1 min and 2.6 min respectively. Total run duration kept for 8 min. Chromatographic conditions are given in Table 1.

Table 1: Optimized chromatographic conditions

Parameters	Optimized Conditions
Column	Shimadzu C18 (4.6 x 250mm, 5µm)
Mobile phase	Phosphate buffer pH 4: Acetonitrile (40:60 v/v)
Flow rate	1.5 mL/min
Run time	8 min
Column temperature	40° C
Injection volume	20 µL
Detection wavelength	215 nm
Retention time of Bempedoic acid	2.6 min
Retention time of Rosuvastatin	4.1 min
Pump mode	Isocratic

Method validation
The optimised method was validated for linearity, accuracy, precision, robustness, specificity, system suitability as per ICH Q2(R1) guidelines[8].
Specificity
Specificity is the ability to assess unequivocally the analyte in the presence of components that may be assumed to be present. Typically, these might include impurities, degradants, matrices, etc. The specificity of the developed RP-HPLC method was validated by injecting blank, working standard, and sample solutions.
System Suitability
The system suitability parameter was used to confirm the ideal conditions. A system suitability test was carried out on the chromatograms in accordance with the USP standards. The theoretical plate, resolution, retention period, and tailing factor were all carried out. For the system suitability test, six replicate injections of the sample were given.
Linearity
The linearity of an analytical procedure is its capacity to produce test results that are directly proportional to the analyte in the sample. Linearity of the method was determined by preparing aliquots at five different levels of calibration curve over the concentration range of 90-270 µg/mL for Bempedoic acid and 20-60 µg/mL for Rosuvastatin. The solutions were analysed in

triplicate. The calibration curve was plotted using concentration on x-axis and peak response on y-axis. From the calibration curve, the correlation coefficient and regression equation were determined. The regression coefficient (R^2) should not be less than 0.99.

Precision

The closeness of agreement (degree of scattering) between a set of measurements taken under the specified conditions using multiple sampling of the same homogenous sample can be expressed as the precision of an analytical procedure.

I. System precision: This consists of six replicate injections of either a combination of standard solutions or just one standard solution at working concentrations. As per ICH recommendations, the percentage RSD was determined with regard to the separate areas of each marker's peak and should be less than 2%.

II. Method precision: To verify the consistency of the established method which includes six replicate injections of the sample solution at working concentrations and %RSD should be less than 2.

Accuracy

The accuracy of the procedure was determined by using a technique known as external standard addition method. By adding a known amount of standards to the test samples at three different concentration levels of 80%, 100%, and 120% of the measured amount, to determine the recovery of Rosuvastatin and Bempedoic acid from the formulation. The recovery study was carried out in triplicate. The %recovery should NLT 98% and NMT 102%.

Robustness

The chromatographic conditions were intentionally changed, and repeatability was examined in order to assess the method's robustness. All other conditions were kept fixed at their optimal values while each condition was changed independently. The proposed method's robustness was evaluated in terms of minute changes in flow rate (1.5 ± 0.1 mL/min), wavelength (215 ± 1 nm), and temperature (40 ± 1 °C).

RESULT AND DISCUSSION

Specificity

The proposed method was found to be specific because Rosuvastatin and Bempedoic acid's chromatograms showed complete separation and the retention time of the drugs in the sample solution was found to be exactly same as that of the drugs present in the standard solution and there is no interference of excipients, etc. Fig. 3A-C shown below are the chromatograms of blank, mixed standard and sample solution.

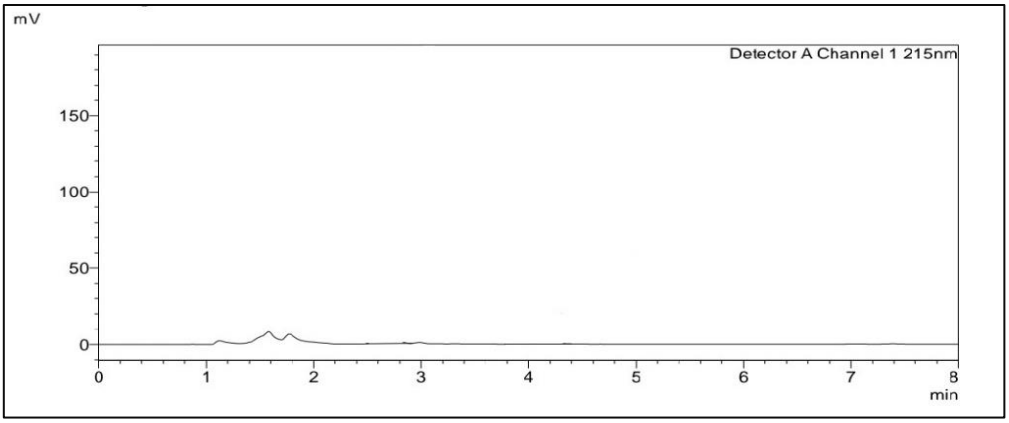


Fig 3A. Chromatogram of blank

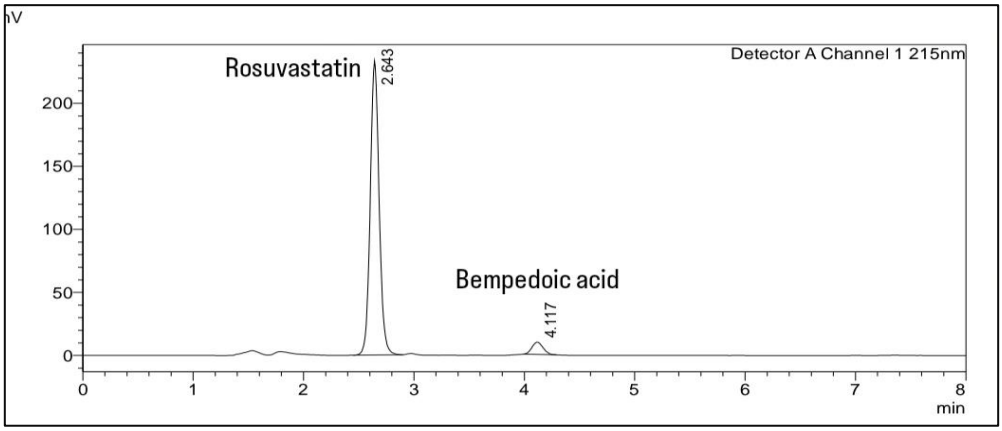


Fig 3B. Chromatogram of mixed standard solution

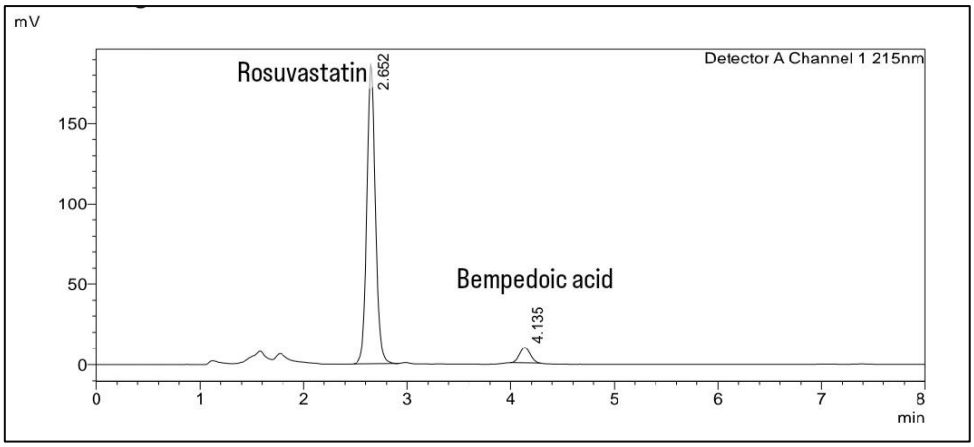


Fig 3C. Chromatogram of sample solution

System Suitability

The %RSD was found to be within the acceptable bounds (<2.0). The theoretical plates were more than 2000 and the tailing factor was less than 2. Thus, it affirms that the system was suitable for the proposed method and it delivered good performance. Results of system suitability study are given in Table 2.

Table 2. System suitability parameter results

Parameters	Observed values	
	Bempedoic acid (180 µg/mL)	Rosuvastatin (40 µg/mL)
Retention time (min)	2.643	4.117
Tailing Factor(NMT 2)	1.080	1.050
Theoretical Plates (More than 2000)	4404	6961
Resolution Factor(More than 2)	-	8.375

Linearity

From the result, it was discovered that the calibration graph(Fig. 4A and 4B), which was drawn with the values of peak response on the y-axis and concentration on the x-axis(Table 3), was linear and regression coefficient for both the drugs were more than 0.99.

Table 3. Linearity data of Rosuvastatin and Bempedoic acid

Bempedoic acid			Rosuvastatin		
Sr No.	Concentration (µg/mL)	Peak area	Sr No.	Concentration (µg/mL)	Peak area
1	90	32226	1	20	581617
2	135	46007	2	30	913238
3	180	68068	3	40	1300803
4	225	81037	4	50	1555153
5	270	99576	5	60	1883776
R^2	0.9947		R^2	0.9965	

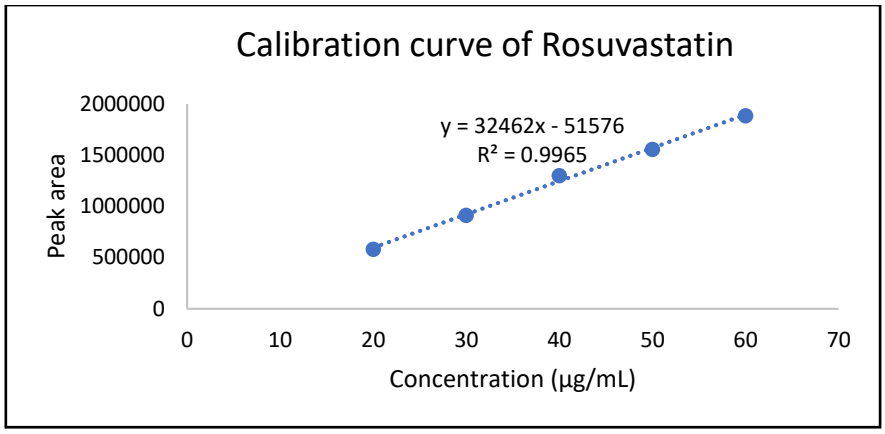


Fig 4A. Calibration curve of Rosuvastatin

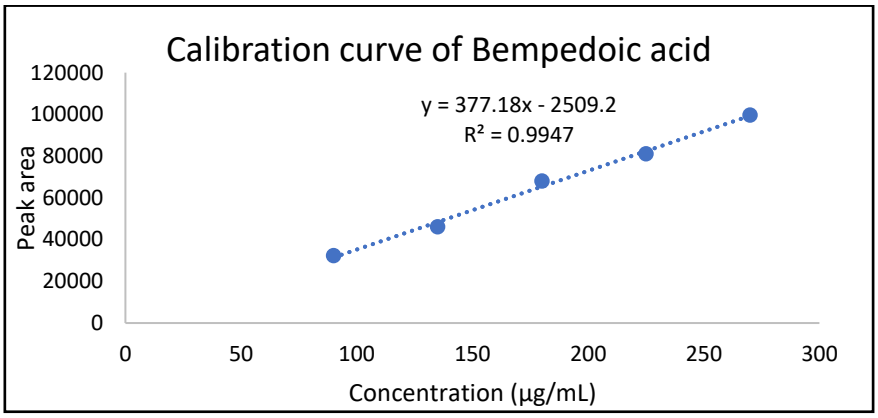


Fig 4B. Calibration curve of Bempedoic acid

Precision

Based on the result, it can be observed that the %RSD of both the system and method precision are within the acceptable limits ie, (<2.0). Hence it proved that the proposed method produce precise result. Table 4 summarises the result of precision.

Table 4. Results of method and system precision

Injection	System Precision Area of Standard		Method Precision % Assay	
	Rosuvastatin	Bempedoic acid	Rosuvastatin	Bempedoic acid
1	1312967	69146	99.96	99.33
2	1310846	69596	100.19	100.58
3	1314567	69287	99.96	101.15
4	1309246	69325	101.23	101.18
5	1310246	70015	100.96	99.04
6	1311611	69025	100.96	100.50
Mean	1311611	69399	100.54	100.30
SD	1931.453926	357.8183897	0.569936	0.910355
%RSD	**0.15**	**0.52**	**0.56**	**0.90**

Accuracy

Accuracy was performed in three different levels and from the result it can be determined that the % recovery of Bempedoic acid and Rosuvastatin was ranged within the acceptable bounds i.e, (98-102%). Thus, it confirmed that the optimized method is accurate. Accuracy data is listed in Table 5.

Table 5. Accuracy data

Pre-Analyzed Sample	Level	Amount Added (µg/mL)	Amount Recovered (µg/mL)	% Recovery	Mean of % recovery
Rosuvastatin	80%	16	16.22	101.3	100.15
	100%	20	19.61	98.07	
	120%	24	24.28	101.1	

Bempedoic acid	80%	72	72.36	100.5	99.85
	100%	90	89.69	99.65	
	120%	108	107.36	99.41	

Robustness

According to the result obtained from different variable conditions such as flow rate, wavelength and column temperature, it can be concluded that the proposed method was found to be robust i.e, system suitability criteria were within the permissible limits for all the variable conditions. Results of robustness are summarised in Table 6.

Table 6. Results of robustness study

Parameter	Level	Rosuvastatin			Bempedoic acid		
		%RSD (peak area)	Theoretical Plates	Peak Tailing	%RSD (peak area)	Theoretical Plates	Peak Tailing
Flow Rate	1.4 mL/min	1.02	4488	1.02	0.40	6302	0.99
	1.6 mL/min	1.03	4397	1.01	0.15	6257	1.00
Temperature	39 °C	0.31	4245	1.02	0.29	6001	0.99
	41 °C	1.02	4226	1.39	0.23	6298	0.98
Wavelength	214 nm	0.61	4218	1.04	0.16	5923	1.00
	216 nm	0.86	4234	1.04	0.61	6083	0.97

CONCLUSION

The pharmaceutical formulation containing Rosuvastatin and Bempedoic acid was estimated simultaneously and validated using a straightforward, unique, and reproducible RP-HPLC method. All validation parameters were determined to be within the permissible limit in accordance with the ICH standards. Simple mobile phase, increased chromatographic efficiency, less solvent consumption, shorter run time (8 min) and shorter retention period (2.6 min for rosuvastatin and 4.1 min for Bempedoic acid) are the advantages of the proposed method. The advantage of the established approach is that it is simple, quick, and affordable due to the binary solvent mixture that makes up the simple mobile phase which leads to the nature friendly chromatographic procedure and this method can be adopted for routine analysis of the dosage form.

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