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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF ROSUVASTATIN CALCIUM AND BEMPEDOIC ACID IN TABLETS

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ABSTRACT

A simple, rapid, economical, precise, and accurate stability-indicating RP-HPLC method has been developed for the simultaneous estimation of Rosuvastatin Calcium and Bempedoic Acid in tablet dosage form. A reverse phase high performance liquid chromatographic method was developed for the estimation of Rosuvastatin Calcium and Bempedoic Acid in tablets. The separation was achieved using a C18 column (250 mm × 4.6 mm, 5 µm) with a mobile phase consisting of Buffer (Ammonium Acetate + TFA) and Acetonitrile in the ratio of 55:45, at a flow rate of 1.0 mL/min. Detection was carried out at 220 nm. The retention time for Rosuvastatin Calcium and Bempedoic Acid was found to be approximately 2.729 min and 6.463 min respectively. The method was validated as per ICH guidelines for parameters such as linearity, accuracy, precision, detection limit, and quantitation limit. Linearity was observed over a suitable concentration range for both drugs. The developed method was found to be accurate, precise, and reliable for the estimation of both drugs. The drugs were subjected to various stress conditions such as hydrolysis (acidic and alkaline), oxidation, photolysis, and thermal degradation under the same chromatographic conditions. The degradation products were well separated from the main drug peaks, indicating the stability-indicating nature of the method.

KEYWORDS: Rosuvastatin Calcium, Bempedoic Acid, RP-HPLC, Method Validation, Stability Indicating Method.

INTRODUCTION

Hyperlipidaemia is a chronic metabolic disorder characterized by elevated levels of lipids such as cholesterol and triglycerides in the blood. It is one of the major risk factors for cardiovascular diseases including atherosclerosis, myocardial infarction, and stroke. Under normal physiological conditions, lipids are transported through the bloodstream and utilized for various biological functions. However, an imbalance in lipid metabolism leads to their accumulation in blood vessels, resulting in serious health complications.[1,2]

Rosuvastatin Calcium

Rosuvastatin Calcium is a lipid-lowering agent belonging to the class of statins. It acts by selectively inhibiting the enzyme HMG-CoA reductase, which is responsible for the conversion of HMG-CoA to mevalonate, a key step in cholesterol biosynthesis. By inhibiting this enzyme, Rosuvastatin reduces hepatic cholesterol synthesis and increases the uptake of low-density lipoprotein (LDL) cholesterol from the bloodstream. This results in a significant reduction in total cholesterol and LDL levels, thereby lowering the risk of cardiovascular diseases.[3]

Bempedoic Acid

Bempedoic Acid is a novel lipid-lowering drug that inhibits ATP-citrate lyase, an enzyme involved in the cholesterol biosynthesis pathway upstream of HMG-CoA reductase. It reduces cholesterol synthesis in the liver and enhances LDL receptor expression, leading to increased clearance of LDL cholesterol from circulation. Bempedoic Acid is particularly useful in patients who are intolerant to statins or require additional lipid-lowering effects.[4]

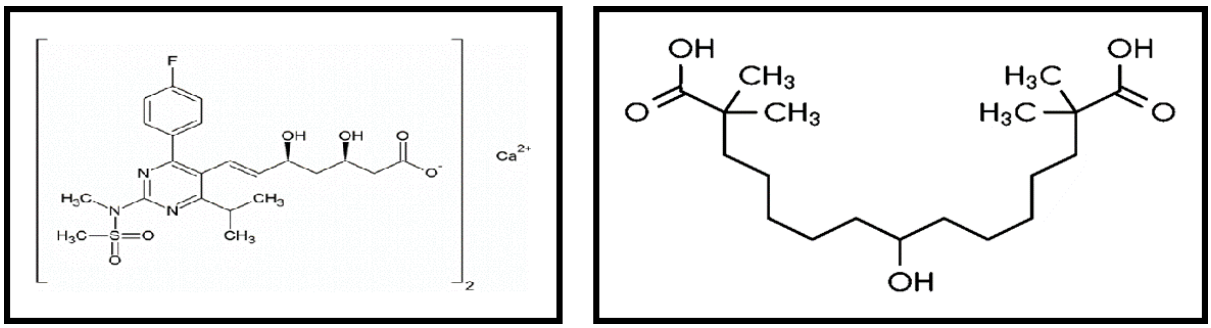


Fig. 1: Structure of Rosuvastatin Calcium and Bempedoic Acid.

The literature survey reveals that several analytical methods have been reported for the estimation of Rosuvastatin Calcium and Bempedoic Acid individually or in combination, including RP-HPLC methods[5,8] spectrophotometric methods,[9-,10] and HPTLC techniques.[11] However, very limited methods are available for stability-indicating[12-13] simultaneous estimation of this combination in pharmaceutical dosage forms. Therefore, the present study aims to develop a simple, precise, accurate, and stability-indicating RP-HPLC method for the simultaneous estimation of Rosuvastatin Calcium and Bempedoic Acid in tablet dosage form. The developed method is intended to be suitable for routine quality control analysis and stability studies as per ICH guidelines.

MATERIALS AND METHODS

Chemical and Reagents

The Rosuvastatin Calcium (ROSU) API and Bempedoic Acid (BEMP) API were generously provided as a gift sample by Ami lifesciences and West coast respectively.

All chemicals and reagents used were of analytical grade, and HPLC grade water was used throughout the experiment.

Preparation of Standard Stock Solution

Master Stock Solution of Rosuvastatin calcium

40 mg of Rosuvastatin calcium working standard and transferred into 10 mL volumetric flask. Add 10 mL solution containing Buffer: ACN (55:45) and dissolved by sonication. This Solution was used as a stock solution.

Master Stock Solution of Bempedoic acid

180 mg of Bempedoic acid working standard and transferred into 10 mL volumetric flask. Add 10 mL of solution containing Buffer: ACN (55:45) and dissolved by sonication. This solution was used as a stock solution .

Preparation of standard solution of binary mixtures of Rosuvastatin calcium and Bempedoic acid

1 mL of Rosuvastatin stock solution + 1 mL of Bempedoic stock solution into 10 mL volumetric flask. Make volume up to the mark with diluent to get mixed standard solution.

Preparation of Sample Solution

Sample solution

Weigh 10 tablets and determine the average tablet weight. Crush the tablet into fine powder using mortar and pestle. Transfer powder equivalent to 1 tablet into a 100 mL volumetric flask. Add about 70 mL of diluent and sonicate for 15 minutes to extract the drug completely. Cool to room temperature and make up the volume with diluent. Mix well and filter through 0.45 µm Nylon filter. Use the filtrate as sample solution.

Diluent: Buffer (Ammonium Acetate + TFA): ACN (55: 45)

Forced Degradation Study

1) Acid Degradation

Transfer 1 tablet equivalent powder into a 100 mL volumetric flask. Add 20 mL of methanol. Add 5 mL of 1N HCl. Sonicate for 10 minutes and keep at 60°C for 30 minutes. Cool to room temperature. Neutralize with 5 mL 1N NaOH. Make volume up to mark with methanol. Mix well and filter through 0.45 µm Nylon filter.

2) Base Degradation

Transfer 1 tablet equivalent powder into a 100 mL volumetric flask. Add 20 mL of methanol. Add 5 mL of 1N NaOH. Sonicate for 10 minutes and keep at 60°C for 30 minutes. Cool to room temperature. Neutralize with 5 mL 1N HCL. Make volume up to mark with methanol. Mix well and filter through 0.45 µm Nylon filter.

3) Oxidation Degradation

Transfer 1 tablet equivalent powder into a 100 mL volumetric flask. Add 20 mL of methanol. Add 10 mL of 3% Hydrogen Peroxide (H_2O_2). Sonicate for 10 minutes and Keep the solution at room temperature for 30 minutes. Make volume up to mark with methanol. Mix well and filter through 0.45 µm Nylon filter.

4) Photolytic Degradation

Transfer powder equivalent to one tablet into a volumetric flask. Expose the sample to UV light. Expose the sample for 24 hours. Add about 70 mL methanol and sonicate for 15 minutes. Make up the volume to 100 mL with methanol. Filter through 0.45 µm nylon filter.

5) Thermal Degradation

Transfer powder equivalent to one tablet into a volumetric flask. Place the flask in a hot air oven maintained at 100 °C. Expose the sample for 1 hour. Allow the stressed sample to cool to room temperature. Add about 70 mL methanol and sonicate for 15 minutes. Make up the volume to 100 mL with methanol. Filter through 0.45 µm nylon filter.

METHOD VALIDATION

1. Linearity and Range (n=3)

Linearity was evaluated for Rosuvastatin and Bempedoic Acid using five concentration levels ranging from 200–600 µg/mL and 900–2700 µg/mL, respectively. The calibration curves showed excellent linearity with correlation coefficients of 0.999 for Rosuvastatin and 0.999 for Bempedoic Acid.

2. Precision

Precision was assessed in terms of repeatability and intermediate precision. For repeatability, six replicate injections of sample solution were analyzed. The %RSD was found to be 0.34% for Rosuvastatin and 0.37% for Bempedoic Acid, indicating good precision.Intermediate precision also showed %RSD less than 2%, confirming method reproducibility.

3. Accuracy

The accuracy of the method was assessed using the standard addition technique, where a known amount of the working standard was spiked into a placebo at three concentration levels: 50%, 100%, and 150% of the standard concentration. Each solution was injected in triplicate and the recovery was calculated by measuring peak areas. The % recovery was found within 98–102% for both drugs, confirming the accuracy of the method.

4. Robustness

The robustness of the analytical procedure was evaluated to assess its ability to remain unaffected by minor but deliberate variations in method parameters, ensuring its reliability during routine use. Robustness testing was conducted (n=3) by altering key parameters, including:

Flow rate of the mobile phase (± 0.1 mL/min)

Buffer pH

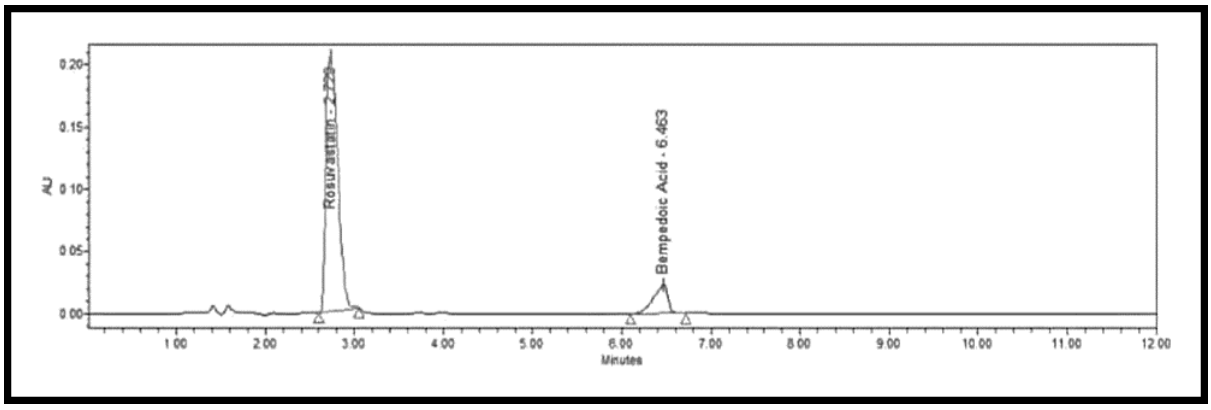
Mobile phase composition

Column temperature (± 5°C).

RESULT AND DISCUSSION

Final Optimized Conditions

Shimadzu HPLC system was used for method development, degradation studies, and validation. Data acquisition was performed using the HPLC system. The separation was achieved on a Zodiac C18 column (250 mm × 4.6 mm, 5 µm). The column temperature was maintained at 40°C, and detection was carried out at 220 nm. The mobile phase consisted of buffer (ammonium acetate with trifluoroacetic acid) and acetonitrile in the ratio of 55:45 (% v/v) at a flow rate of 1.0 mL/min. The injection volume was 10 µL. The method showed good peak separation with acceptable system suitability parameters.



Forced Degradation Study

Acid Degradation

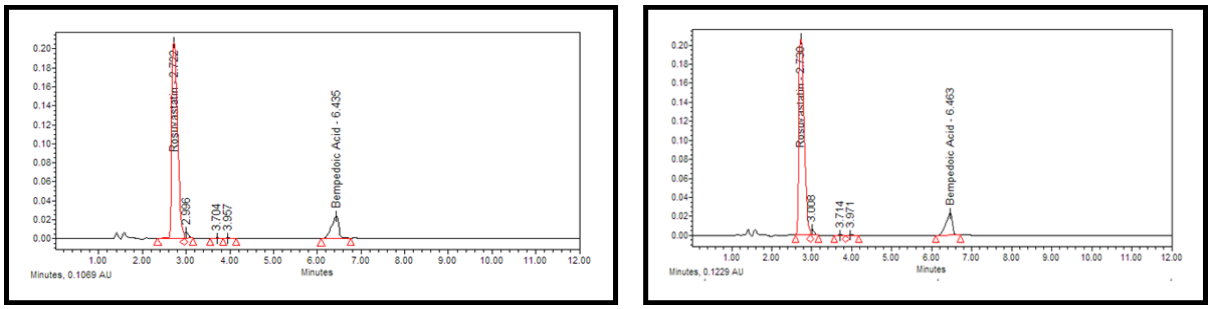


Fig. 3: Chromatogram of Acid Degradation Std & Sample.

Base Degradation

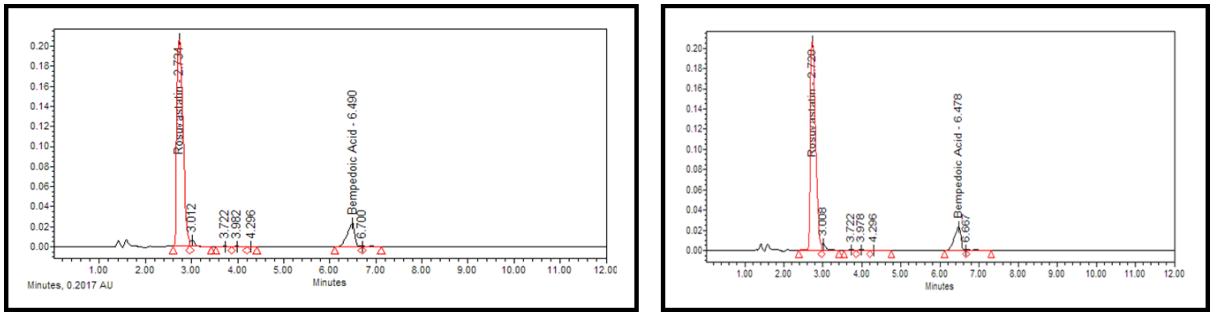


Fig. 4: Chromatogram of Base Degradation Std & Sample.

Oxidative Degradation

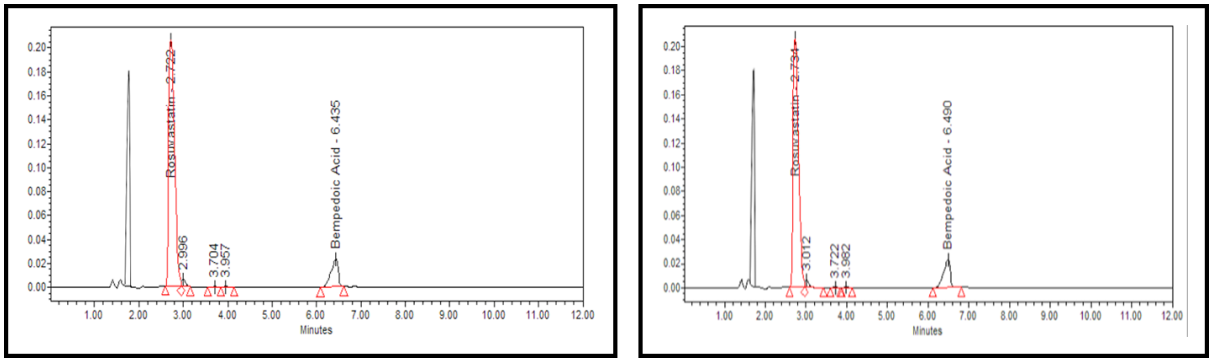


Fig. 5: Chromatogram of Oxidative Degradation Std & Sample.

Thermal Degradation

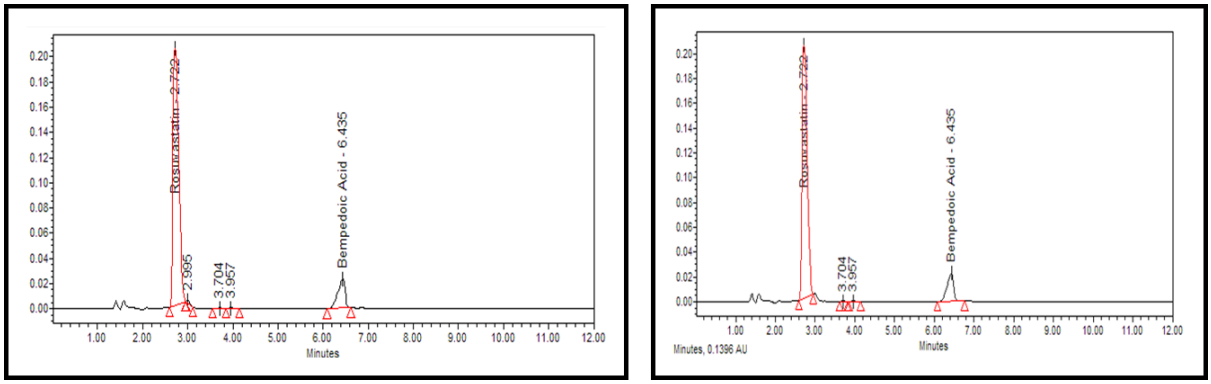


Fig. 6: Chromatogram of Thermal Degradation Std & Sample.

Photolytic Degradation

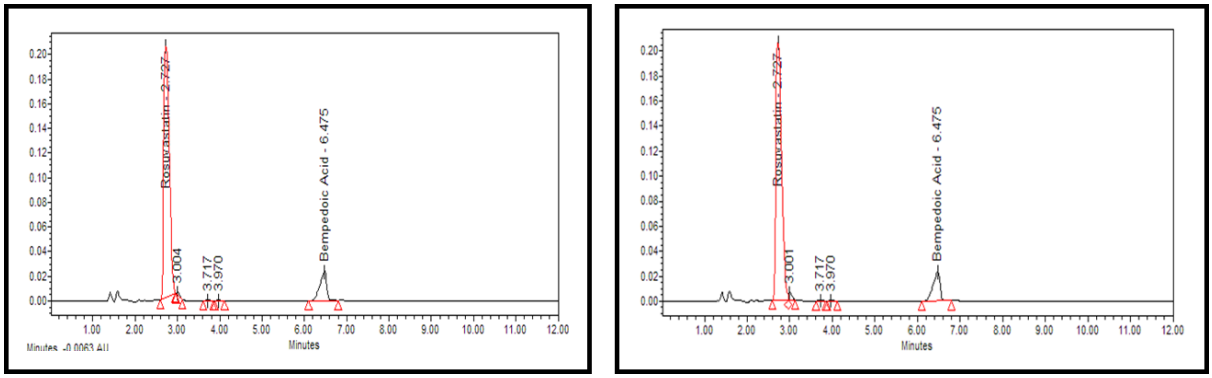


Fig 7: Chromatogram of Photolytic Degradation Std & Sample.

Table 1: Degradation of Rosuvastatin Calcium.

Condition	Sample		API	
	Area	%Degradation	Area	%Degradation
Acid	1719082	8.2	1697725	10.5
Base	1639467	12.4	1626987	14.3
Oxidative	1733654	7.4	1748853	7.8
Thermal	1840042	1.7	1858011	2.1
Photo	1865743	0.4	1878942	1.0

Table 2: Degradation of Bempedoic Acid.

Condition	Sample		API	
	Area	%Degradation	Area	%Degradation
Acid	227698	11.9	228632	11.7
Base	219936	14.9	214913	17.0
Oxidative	237018	8.3	234569	9.4
Thermal	247225	4.3	249854	3.5
Photo	256985	0.6	256169	1.1

Validation of Development RP-HPLC Method

Specificity

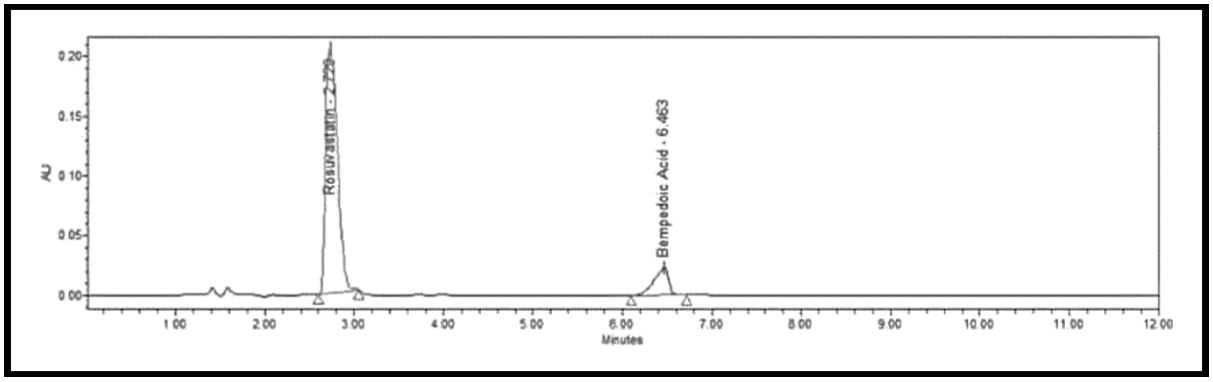


Fig. 8: Chromatogram of Sample.

Linearity and Range

Linearity was assessed by preparing five standard solutions of Rosuvastatin Calcium and Bempedoic Acid. The method demonstrated linearity over the concentration range of 200–600 µg/mL for Rosuvastatin Calcium with a correlation coefficient (R^2 = 0.999) and 900–2700 µg/mL for Bempedoic Acid with a correlation coefficient (R^2 = 0.999). The results of the linearity study for Rosuvastatin Calcium and Bempedoic Acid are presented in Table 3.

Table 3: Linearity Data of Rosuvastatin and Bempedoic.

Rosuvastatin Calcium		Bempedoic Acid	
Concentration (µg/mL)	**Area (n = 5)**	**Concentration (µg/mL)**	**Area (n = 5)**
200	933786	900	129050
320	1516002	1440	207165
400	1896437	1800	260510
480	2275520	2160	312612
600	2840203	2700	385417
Regression coefficient	R^2 = 0.999	Regression coefficient	R^2 = 0.999
Y Intercept	12976	Y Intercept	1583.7
Slope	4763.4x	Slope	142.98x

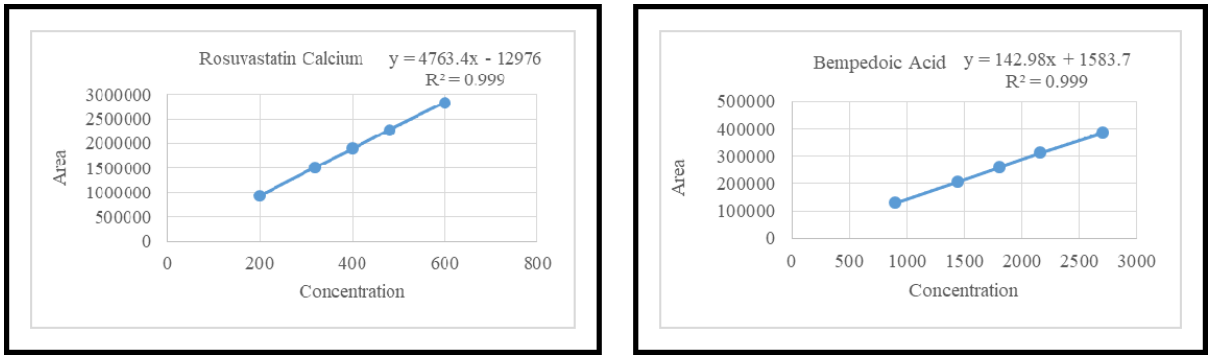


Fig 9: Calibration curve of Rosuvastatin and Bempedoic.

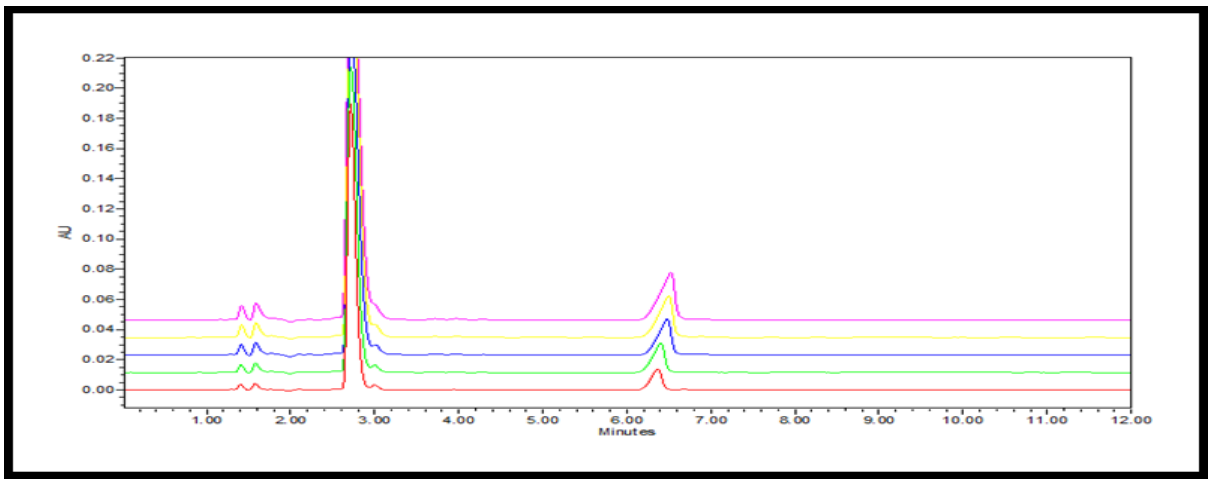


Fig. 10: Overlay Linearity Chromatogram of Rosuvastatin Calcium & Bempedoic Acid.

Precision.

Repeatability

Table 4: Repeatability data for Rosuvastatin Calcium and Bempedoic Acid.

Conc. of Rosuvastatin Calcium (µg/mL)	Peak Area	Conc. of Bempedoic Acid (µg/mL)	Peak Area
400	1896437	1800	260510
	1885025		260430
	1874466		260222
	1851433		259974
	1897821		259884
	1889132		260764
Mean (n = 6)	1882386	Mean (n = 6)	260297.3
SD	17372.15	SD	335.16
% RSD	0.92	% RSD	0.13

Table 5: Interday and Intraday data for Rosuvastatin and Bempedoic Acid.

Precision	**Intraday**		**Interday**	
Drug	Rosuvastatin	Bempedoic	Rosuvastatin	Bempedoic
Concentration	**200**	**900**	**200**	**900**

(µg/mL)	400	1800	400	1800
	600	2700	600	2700
Mean peak area ± SD (n = 3)	935842± 7010.549	130162± 634.493	935142± 7015.540	130652± 641.412
	1882589± 17376.8	260760± 334.95	1885289± 17370.10	260452± 336.65
	2850684± 7290.97	386009± 470.82	2846684± 7250.81	386462± 445.52
% RSD	0.73	0.45	0.70	0.48
	0.90	0.13	0.99	0.15
	0.23	0.14	0.28	0.11

Accuracy

Accuracy of the method was confirmed by recovery study at three levels (50%, 100% and 150%) of placebo addition.

Table 6: Recovery data of Rosuvastatin and Bempedoic Acid.

Name of drug	Conc Levels%	Amount Added (µg/mL)	Amount recovered (µg/mL)	% Recovery	% Mean Recovery ±SD	% RSD
Rosuvastatin Calcium	50%	200	199.67	99.84	99.61±0.22	0.22
		200	198.80	99.40		
		200	199.16	99.58		
	100%	400	400.17	100.04	100.02±0.05	0.05
		400	399.82	99.96		
		400	400.23	100.06		
	150%	600	598.98	99.83	98.85±0.03	0.03
		600	599.31	99.89		
		600	598.96	99.83		
Bempedoic Acid	50%	900	898.23	99.80	99.71±0.09	0.09
		900	896.63	99.63		
		900	897.32	99.70		
	100%	1800	1796.52	99.80	99.57±0.67	0.67
		1800	1798.63	99.92		
		1800	1799.69	99.98		
	150%	2700	2695.61	99.83	99.82±0.12	0.12
		2700	2698.45	99.94		
		2700	2692.12	99.70		

Robustness

Table 7: Robustness data for Rosuvastatin and Bempedoic acid.

Sr No	Area at Temp. (-5ºC)	Area at Temp. (+5ºC)	Area at Flow rate (-0.1 mL/min)	Area at Flow rate (+0.1 mL/min)	Area at Mobile Phase (-2%)	Area at Mobile Phase (+2%)
Rosuvastatin Calcium						
1.	1864265	1868565	1871466	1875032	1897241	1850218

2.	1864894	1868489	1871569	1875101	1897365	1855365
3.	1865212	1868645	1872365	1875094	1896236	1856263
Mean	1864790	1868566	1871800	1875076	1896947	1853949
%RSD	0.026	0.004	0.026	0.002	0.033	0.175
Bempedoic Acid						
1.	260526.0	259894.0	260719.0	259981.0	260805.0	259206.0
2.	260415.0	259759.0	260895.0	259899.0	260798.0	259456.0
3.	260562.0	259846.0	260789.0	259789.0	260896.0	259641.0
Mean	260501.0	259833.0	260801.0	259890.0	260833.0	259434.0
%RSD	0.029	0.026	0.034	0.037	0.021	0.084

Assay

Table 8: Assay of Rosuvastatin and Bempedoic Acid.

Drug	Label Claim	Amount Found	Assay (%) Mean ± SD (n = 3), % RSD
Rosuvastatin Calcium	40 mg	39.98 mg	99.94 ± 0.045, 0.045
Bempedoic Acid	180 mg	179.50 mg	99.72 ± 0.25, 0.25

The developed method showed good resolution with retention time of 2.7 min and 6.4 min respectively. Linearity was observed with correlation coefficient >0.999. Accuracy showed recovery in the range of 98-102%. Precision showed %RSD <2%. Forced degradation studies showed clear separation of degradation products.

CONCLUSION

Based on the experimental results, the proposed method is accurate, simple, precise, linear, sensitive, robust, and stability-indicating for the simultaneous estimation of Rosuvastatin Calcium and Bempedoic Acid in tablet dosage form. The method was successfully validated as per ICH guidelines and was found suitable for routine quality control analysis and stability studies of pharmaceutical formulations.

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