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Development and Validation of an RP-HPLC Method for the Simultaneous Estimation of Rosuvastatin and Bempedoic acid in Pharmaceutical Dosage Forms

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Abstract:
This study outlines the successful development and validation of a robust reverse-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of Rosuvastatin and Bempedoic acid in pharmaceutical dosage forms. Multiple chromatographic trials were conducted to optimize key parameters including column type, mobile phase composition, and flow rate. The finalized method utilized a Thermostat ODS column (4.6×250 mm, 5 µm) with a mobile phase of 0.1% orthophosphoric acid (OPA) buffer (pH 3.5) and acetonitrile in a 30:70 v/v ratio, with detection at 240 nm. The method exhibited excellent system suitability, with acceptable retention times, resolution, peak symmetry, and theoretical plate counts, in accordance with ICH guidelines. Validation studies confirmed the method's precision, accuracy, linearity, sensitivity, and robustness. Assay results showed drug content within acceptable limits 98.3% for Rosuvastatin and 100.3% for Bempedoic acid. Linearity was established over the ranges of 8–40 µg/ml and 36–180 µg/ml for Rosuvastatin and Bempedoic acid, respectively, with correlation coefficients exceeding 0.999. The method demonstrated high repeatability and intermediate precision (%RSD < 2%), while robustness testing revealed no significant impact from minor variations in flow rate and mobile phase composition. These findings affirm the method's reliability and suitability for routine quality control of combined formulations containing Rosuvastatin and Bempedoic acid.
Keywords: RP-HPLC, Rosuvastatin, Bempedoic acid, method validation, system suitability, linearity, precision, accuracy, robustness, ICH guidelines.

Introduction

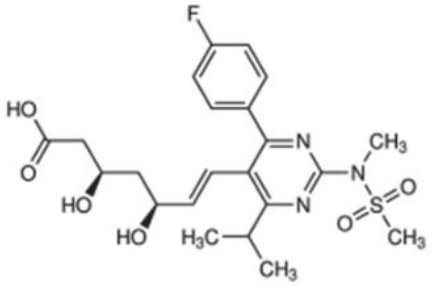

Fig.1: Rosuvastatin

Basic Information
IUPAC Name: (3R,5S,6E)-7-[4-(4-fluorophenyl)-2-(N-methyl methanesulfonamido)-6-(propan-2-yl)pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoic acid calcium
Molecular Formula: C({22})H({28})FN(3)O({6})S
Molecular Weight: 481.54 g/mol
Melting Point: Approximately 122-123°C
pKa: 4.6
Category: Drug Class: Statins (HMG-CoA reductase inhibitors)
Solubility
Solubility: Slightly soluble in water, soluble in methanol and ethanol.
Description: Rosuvastatin is a statin medication used to prevent cardiovascular disease and treat abnormal lipid levels. It is taken orally.
Mechanism of Action
Rosuvastatin works by inhibiting HMG-CoA reductase, an enzyme involved in the synthesis of cholesterol in the liver. This leads to a decrease in cholesterol levels, particularly low-density lipoprotein (LDL) cholesterol, and an increase in high-density lipoprotein (HDL) cholesterol.

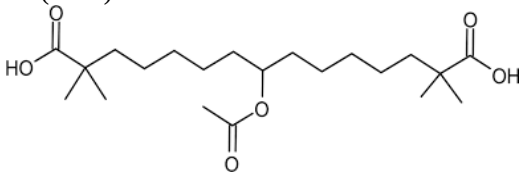

Fig.2: Bempedoic Acid

Basic Information
IUPAC Name: 8-Hydroxy-2,2,14,14-tetramethylpentadecane-dioic acid
Molecular Formula: C({19})H({36})O(_{5})
Molecular Weight: 344.49 g/mol
Melting Point: Approximately 158-160°C
pKa: 3.5
Category: Adenosine triphosphate-citrate lyase(ACL) inhibitors
Solubility
Solubility: Slightly soluble in water, soluble in methanol and ethanol.
Description
Bempedoic acid is a non-statin medication used to lower LDL cholesterol levels. It is often prescribed for patients who cannot tolerate statins or need additional LDL cholesterol reduction.
Mechanism of Action
Bempedoic acid works by inhibiting ATP citrate lyase (ACL), an enzyme involved in the synthesis of cholesterol in the liver. This inhibition reduces the production of cholesterol, particularly LDL cholesterol.

Materials and Methods

Table.1: Equipments used in the study

S. No.	Equipments	Company
1	Electronic Balance	Scaletec
2	Ultra-Sonicator	Enertech
4	Thermal oven	Yamto
5	pH Meter	Smis
6	Filter Paper 0.45 microns	Milli Pore
7	HPLC system	Agilent 1220 Infinity

HPLC Method Development:

Wave length selection:

UV spectrum of 10µg/ml Rosuvastatin and Bempedoic acid in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm and the isobestic λmax of both the drugs obtained at 240 nm.

Optimization of Column:

Thermostat ODS (4.6*250mm, 5µ) was found to be ideal as it gave good peak shape and resolution at 1.0 ml/min flow.

Optimized Chromatographic Conditions

Equipment: High performance liquid chromatography equipped with Auto Sampler and PDA detector
Column : Thermostat ODS (4.6*250mm, 5µ)
Buffer : 0.1% OPA
pH : 3.5
Mobile phase : 30% buffer : 70% Acetonitrile
Flow rate : 1.0 ml per min
Wavelength : 240 nm
Injection volume : 20 µl
Run time : 10 min.

Preparation of buffer and mobile phase:

Preparation of 0.1% OPA buffer PH-3.5: Take 1ml of OPA in 1000ml of HPLC water and adjust the PH with NAOH upto PH 3.5.

Preparation of mobile phase:

Mix a mixture of above OPA buffer 300ml (30%), 700ml Acetonitrile (70%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

Use the Mobile phase as Diluents.

System Suitability:

Tailing factor for the peaks due to Rosuvastatin and Bempedoic acid in Standard solution should not be more than 2.0.

Theoretical plates for the Rosuvastatin and Bempedoic acid peaks in Standard solution should not be less than 2000

Calculation: (For Rosiglitazone and Pioglitazone)

$$\%\ Assay = \frac{AT}{AS} * \frac{WS}{DS} * \frac{DT}{WT} * \frac{Average\ weight}{Label\ Claim} * \frac{P}{100} * 100$$

Where:
AT = average area counts of sample preparation.
AS = average area counts of standard preparation.
WS = Weight of working standard taken in mg.
P = Percentage purity of working standard
LC= Label Claim mg/ml.

Acceptance criteria of System Suitability:

- Tailing factor should be less than 2
- Theoretical Plates should be above 2000

Results and Discussion

Optimization of Column:

Thermostat ODS (4.6*250mm, 5µ) was found to be ideal as it gave good peak shape and resolution at 1.0 ml/min flow.

Optimized Chromatographic Conditions

Equipment : High performance liquid chromatography equipped with Auto Sampler and PDA detector
Column : Thermostat ODS (4.6*250mm, 5µ)
Buffer : 0.1% OPA
pH : 3.5
Mobile phase : 30% buffer : 70% Acetonotrile
Flow rate : 1.0 ml per min
Wavelength : 240 nm
Injection volume : 20 µl
Run time : 10 min.

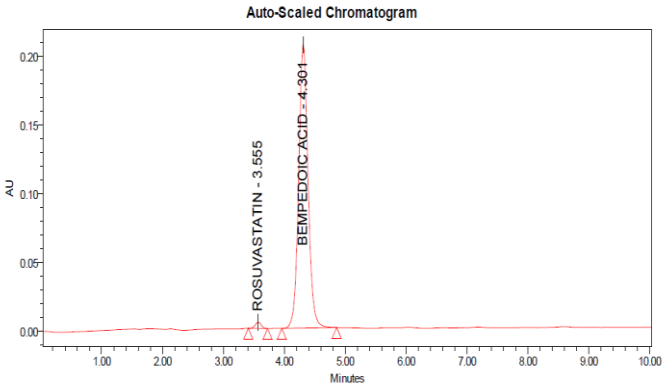

Fig.3: Chromatogram for system suitability

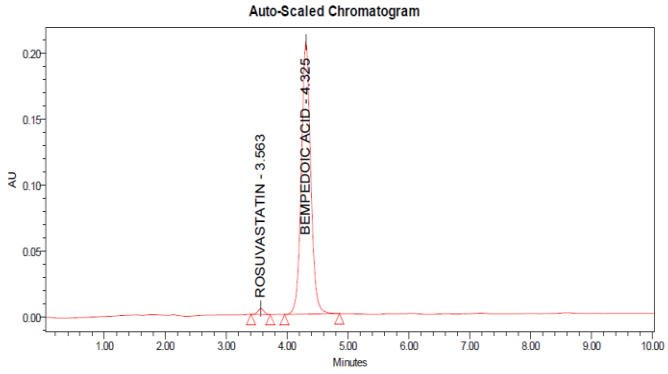

Fig.4: Chromatogram for Standard

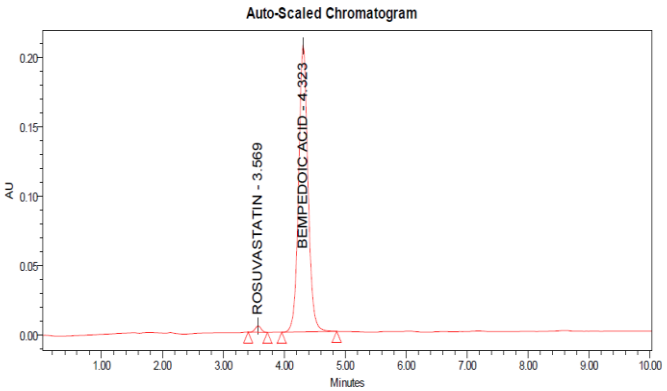

Fig.5: Chromatogram for Sample

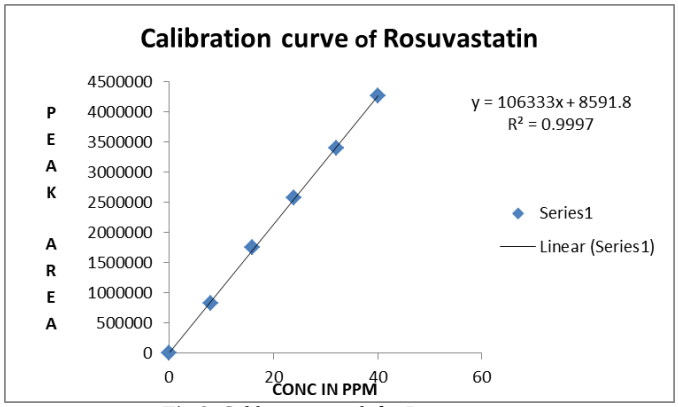

Fig.6: Calibration graph for Rosuvastatin

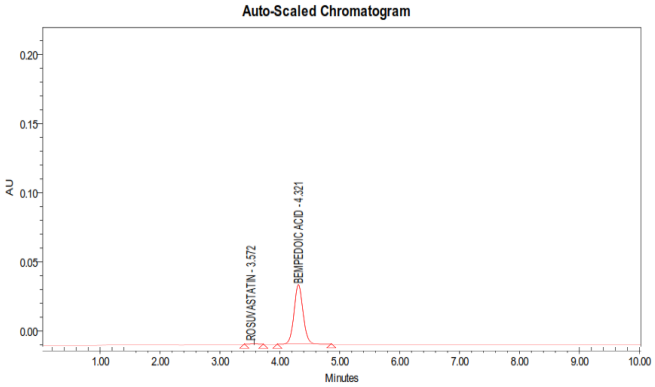

Fig.8: Rosuvastatin and Bempedoic acid showing LOD

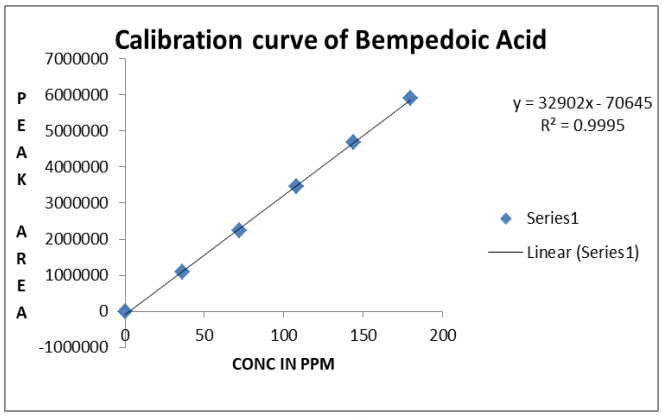

Fig.7: Calibration graph for Bempedoic acid

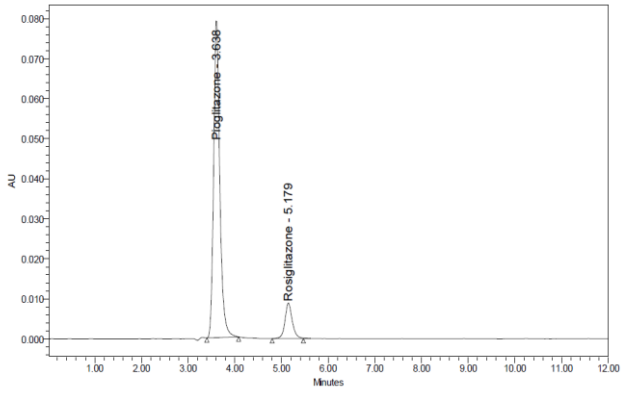

Fig.9: Rosuvastatin and Bempedoic acid showing LOQ

Table.2: Results of system suitability parameters

S.No	Name	RT(min)	Area (µVsec)	Height(µV)	USP tailing	USP platecount
1	Rosuvastatin	3.555	2578412	23934	1.25	7051
	Bempedoic acid	4.301	3818514	167538	1.15	8471

Table.3: Results of Assay for Rosuvastatin and Bempedoic acid

	Label Claim (mg)	% Assay
Rosuvastatin	40 mg	98.3%
Bempedoic acid	180mg	100.3%

Table 4: Analytical performance parameters of Rosuvastatin and Bempedoic acid

Parameters	Rosuvastatin	Bempedoic acid
Slope (m)	106333	32902
Intercept (c)	8591.8	70645
Correlation coefficient (R^2)	0.9997	0.9995

Table 5: Results of LOD

Drug name	Baseline noise(µV)	Signal obtained(µV)	S/N ratio	Conc.
Rosuvastatin	60	179	2.98	0.18 µg/ml
Bempedoic acid	60	173	2.88	0.11µg/ml

Table 6: Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained(µV)	S/N ratio	Conc.
Rosuvastatin	60	595	9.92	0.60µg/ml
Bempedoic acid	60	587	9.78	0.38µg/ml

Conclusion

In the present study, a robust, precise, and accurate RP-HPLC method was successfully developed and validated for the simultaneous estimation of Rosuvastatin and Bempedoic acid in pharmaceutical dosage forms. Several trials were conducted with varying chromatographic conditions involving different columns, mobile phases, and flow rates to optimize the method. The final optimized method employed a Thermostat ODS column (4.6×250mm, 5µ) with a mobile phase consisting of 0.1% OPA buffer (pH 3.5) and acetonitrile in the ratio of 30:70 v/v, with detection at 240 nm. The method demonstrated good system suitability, with acceptable retention times, resolution, peak symmetry, and theoretical plate counts as per ICH guidelines.

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