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DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS DETERMINATION OF ROSUVASTATIN AND BEMPEDOIC ACID IN PHARMACEUTICAL DOSAGE FORMS BY USING RP-HPLC METHOD

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ABSTRACT

The chromatographic conditions were successfully developed for the separation of Rosuvastatin and Bempedoic acid by using Inertsil ODSC18 column (4.6×250mm)5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) ACN : KH2PO4 pH 3, detection wavelength was 225nm. The instrument used for HPLC, WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 3.598 mins and 4.487 mins. The % purity of Rosuvastatin and Bempedoic acid was found to be 100.15% and 100.57% respectively. The system suitability parameters for Rosuvastatin and Bempedoic acid such as theoretical plates and tailing factor were found to be 4260, 1.2 and 5085 and 1.2, the resolution was found to be 3.67. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The precision study was precision, robustness and repeatabilty. LOD value was 3.72 and 0.0242 and LOQ value was 7.40 and 0.0202 respectively.

INTRODUCTION

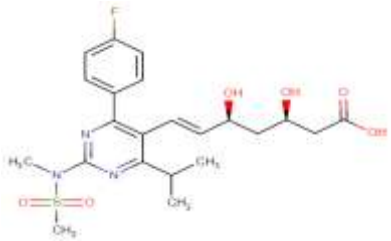
ROSUVASTATIN

Description: Rosuvastatin is an antilipemic agent that competitively inhibits hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase. HMG-CoA reducuase catalyzes the conversion of HMG-CoA to mevalonic acid, the rate-limiting step in cholesterol biosynthesis. Rosuvastatin belongs to a class of medications called statins and is used to reduce plasma cholesterol levels and prevent cardiovascular disease.

IUPAC Name: (3R, 5S, 6E)-7-[4-(4-fluorophenyl) -2- (N-methyl methane sulfo namido)-6-(propan-2-yl) pyrimidin-5-yl]-3, 5-dihydroxyhept-6-enoic acid.

Melting point:>151^{0}C

Structure:



Chemical Formula: $C_{22}H_{28}FN_3O_6S$

Molecular weight: 500.57g/mol

Solubility: DMSO (Slightly), Methanol (Slightly)

Indication: Used as an adjunct to dietary therapy to treat primary hyperlipidemia (heterozygous familial and nonfamilial), mixed dyslipidemia and hypertriglyceridemia. Also indicated for homozygous familial hypercholesterolemia as an adjunct to other lipid-lowering therapies or when other such therapies are not available. Furthermore, it is used to slow the progression of atherosclerosis and for primary prevention of cardiovascular disease.

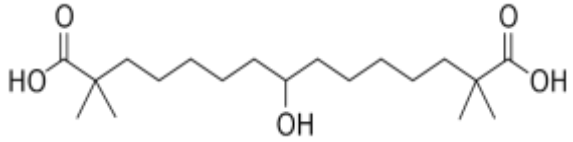
Mechanism of action: Rosuvastatin is a competitive inhibitor of HMG-CoA reductase. HMG-CoA reductase catalyzes the conversion of HMG-CoA to mevalonate, an early rate-limiting step in cholesterol biosynthesis. Rosuvastatin acts primarily in the liver. Decreased hepatic cholesterol concentrations stimulate the upregulation of hepatic low density lipoprotein (LDL) receptors which increases hepatic uptake of LDL. Rosuvastatin also inhibits hepatic synthesis of very low density lipoprotein (VLDL). The overall effect is a decrease in plasma LDL and VLDL. In vitro and in vivo animal studies also demonstrate that rosuvastatin exerts vasculoprotective effects independent of its lipid-lowering properties. Rosuvastatin exerts an anti-inflammatory effect on rat mesenteric microvascular endothelium by attenuating leukocyte rolling, adherence and transmigration (PMID: 11375257).

Affected organisms: Humans and other mammals

2. Bempedoic Acid:

Description: Bempedoic acid is a drug used in conjunction with lifestyle modification and/or other agents for the treatment of refractory hypercholesterolemia.

Structure:



Systematic (IUPAC) name: 8-hydroxy-2,2,14,14-tetramethylpentadecanedioic acid.

PHYSIOCHEMICAL DATA:

Solubility: 0.0211 mg/mL
Formula: $C_{19}H_{36}O_5$
Molecular weight: 344.492/mol
Melting point: 87-92^{0}C

3. Materials and methods

Instruments used

Sl. No	Instrument	Model
1	HPLC	Waters, software: empower, 2695 separation module,uv detector.
2	UV/VIS spectrophotometer	Labindia uv 3000$^+$
3	PH meter	Adwa – ad 1020
4	Weighing machine	Afcoset er-200a
5	Pipettes and burettes	Borosil
6	Beakers	Borosil

Chemicals used:

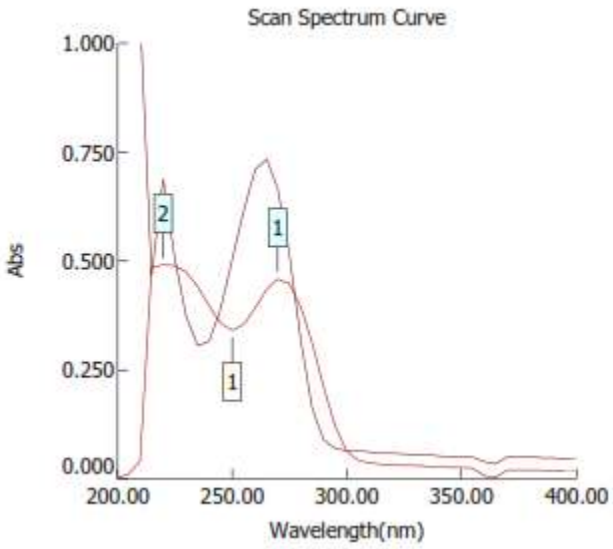
Sl. No	Chemical	Company name
1	Rosuvastatin	Glenmark
2	Bempedoic acid	Glenmark
3	KH_2PO_4	Finer chemical ltd
4	Water and methanol for HPLC	Lichrosolv (merck)
5	Acetonitrile for HPLC	Molychem
6	Ortho phosphoric acid	Merck

HPLC METHOD DEVELOPMENT:

Mobile Phase Optimization: Initially the mobile phase tried was methanol: Ortho phosphoric acid buffer and Methanol: phosphate buffer ,Acetonitrile : methanol with various combinations of pH as well as varying proportions. Finally, the mobile phase was optimized to Phosphate buffer (pH 3.0), Acetonitrile in proportion 70: 30 v/v respectively.

Wave length selection: UV spectrum of 10 µg/ml Telmisartan and 10 µg/ml Azelnidipine in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 240 nm. At this wavelength both the drugs show good absorbance.

UV Graph



Optimization of Column: The method was performed with various columns like C18 column Phenomenex column, YMC, and Inertsil ODS column. Inertsil ODS (4.6 x 250mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1.0 ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Instrument used	:	Waters UPLC with auto sampler and uv detector.
Temperature	:	Ambient (25° C)
Mode of separation	:	Isocratic mode
Column	:	Inertsil ODS (4.6*250mm, 5µ)
Buffer	:	Phosphate buffer
pH	:	3.0
Mobile phase	:	70% buffer 30% ACN
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 Phosphate buffer: 3.4g of Potassium di hydrogen ortho phosphateis taken in 1000 ml of HPLC water pH was adjusted with 0.1M NAOH up to 3.0.final solution was filtered through 0.45 µm Membrane filter and sonicate it for 10 mins.

Preparation of mobile phase: Accurately measured 700 ml (70%) of above buffer and 300 ml of Acetonitrile HPLC (30%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation: The Mobile phase was used as the diluent.

PREPARATION OF THE BEMPEDOIC ACID& ROSUVASTATIN STANDARD & SAMPLE SOLUTION:

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 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 10ml volumetric flask and dilute up to the mark with diluent.

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 10ml volumetric flask and dilute up to the mark with diluent.

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 %Assay by using the formulae.

SYSTEM SUITABILITY:

Tailing factor for the peaks due to Bempedoic acid and Rosuvastatin in Standard solution should not be more than 2.0. Theoretical plates for the Bempedoic acid and Rosuvastatin peaks in Standard solution should not be less than 2000. Resolution for the Bempedoic acid and Rosuvastatin peaks in standard solution should not be less than 2.

Calculation: (For Bempedoic acid)

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

Where:

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

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RESULTS AND DISCUSSION

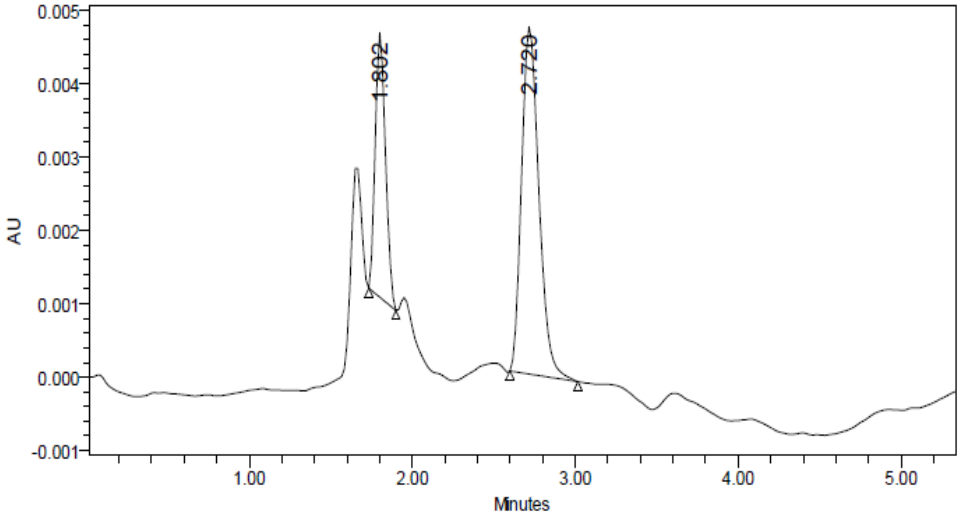


Fig.3: Chromatographic conditions Trial -1

Column	:	Inertsil C18 4.6x150mm, 5µm
Mobile phase ratio	:	MeOH: H_2O (50:50%v/v)
Detection wavelength	:	240 nm
Flow rate	:	1ml/min
Injection volume	:	10µl
Run time	:	10min

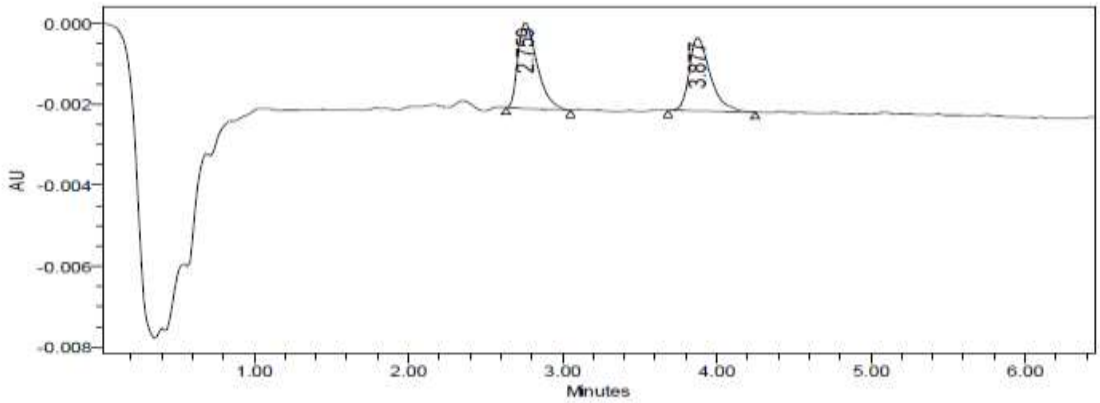


Fig.4: Chromatographic conditions Trial -2

Column	:	Zodiacsil C18 4.6x150mm 5µm
Mobile phase ratio	:	ACN: H_2O (50:50%v/v)
Detection wavelength	:	240 nm
Flow rate	:	1ml/min
Injection volume	:	20µl
Column temperature	:	Ambient
Auto sampler temperature	:	Ambient
Run time	:	8.0 min

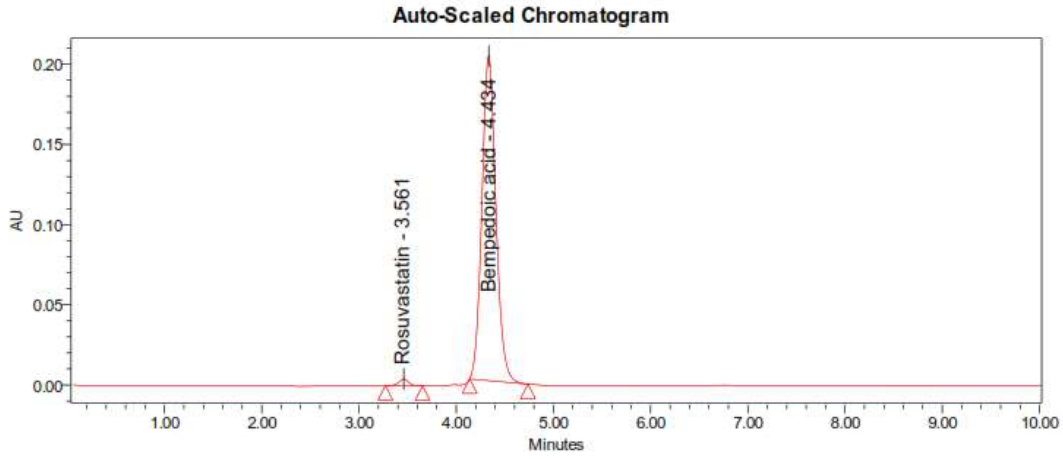


Fig.5: Optimized Chromatographic Conditions

Equipment	:	High performance liquid chromatography equipped with Auto Sampler and PDA detector
Column	:	Inertsil ODS (4.6*250mm, 5µ)
Buffer	:	Phosphate buffer
pH	:	3.0
Mobile phase	:	70% buffer 30% ACN
Flow rate	:	1.0 ml per min
Wavelength	:	240 nm
Injection volume	:	20 µl
Run time	:	10 min.

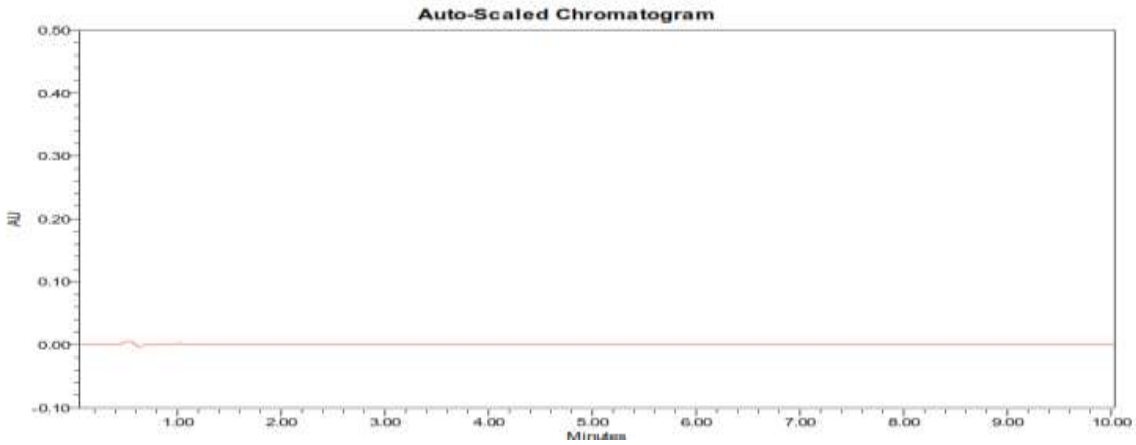


Fig 6-Chromatogram of blank

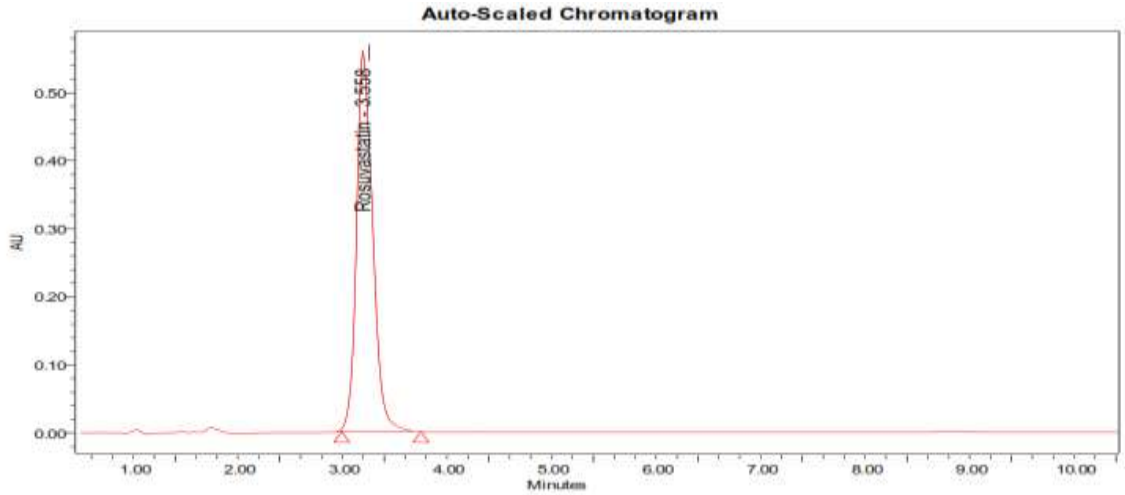


Fig 7-Chromatogram of Rosuvastatin standard

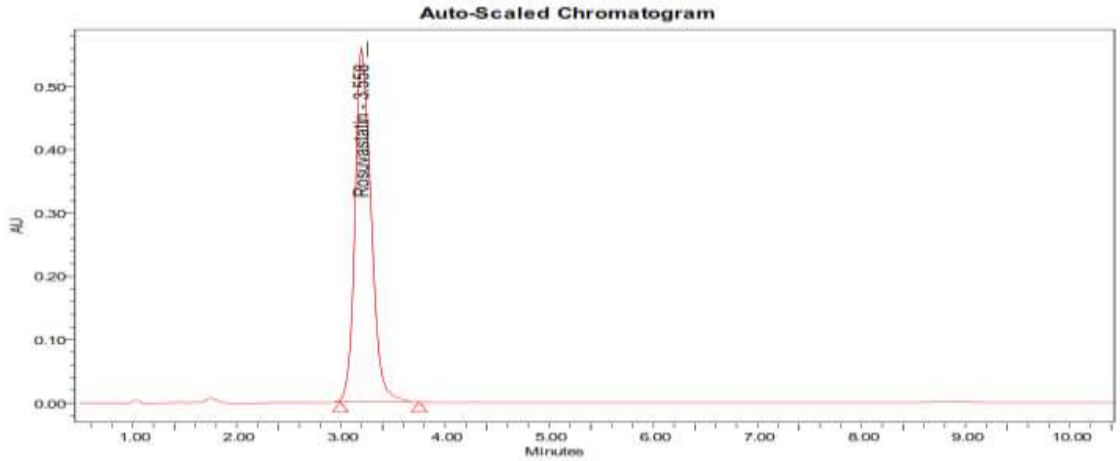


Fig 8-Chromatogram of Bembodoic acid standard

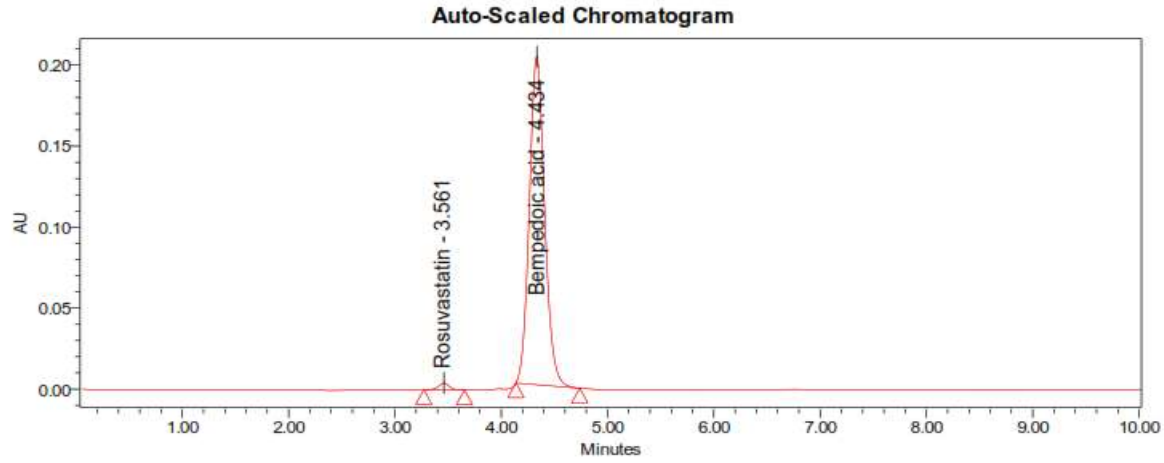


Fig 9-Chromatogram of Standard chromatogram for Rosuvastatin and Bembodoic acid:

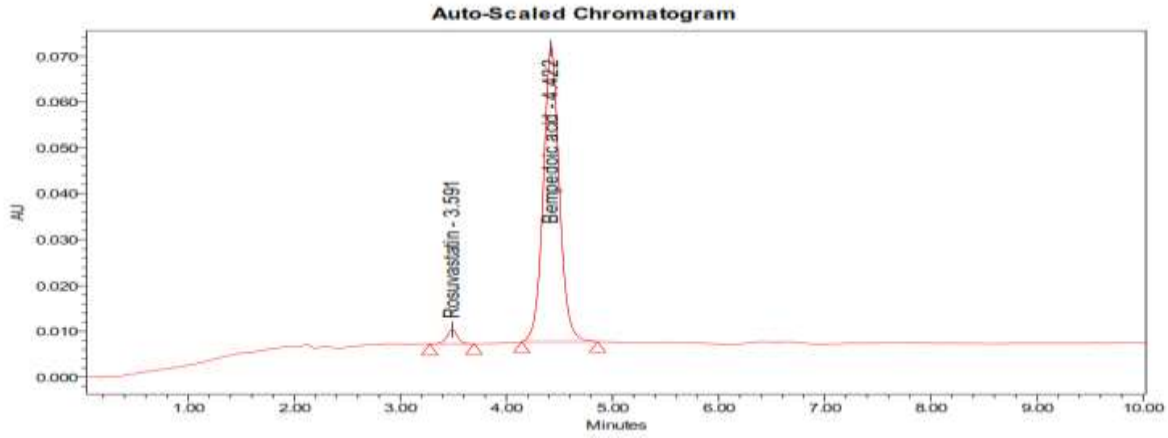


Figure10-Chromatogram of Rosuvastatin and Bembodoic acid L-1

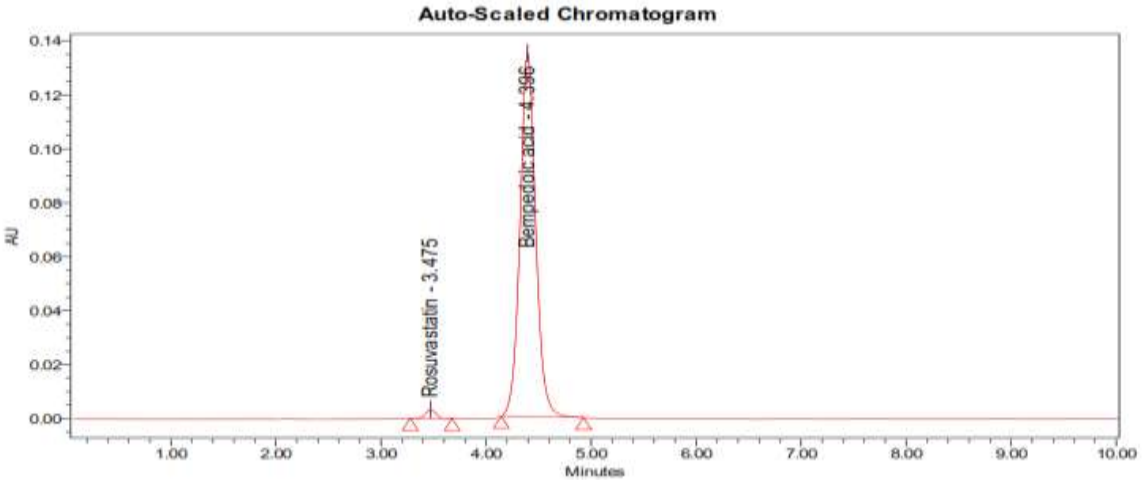


Figure 11-Chromatogram of Rosuvastatin and Bembodoic acid L-2

Table 3. Linearity results of Bembodoic acid

S. No	Linearity Level	Concentration(µg/ml)	Area
1	I	36	65787
2	II	72	131783
3	III	108	194311
4	IV	144	256245
5	V	180	317748
Correlation Coefficient			0.999

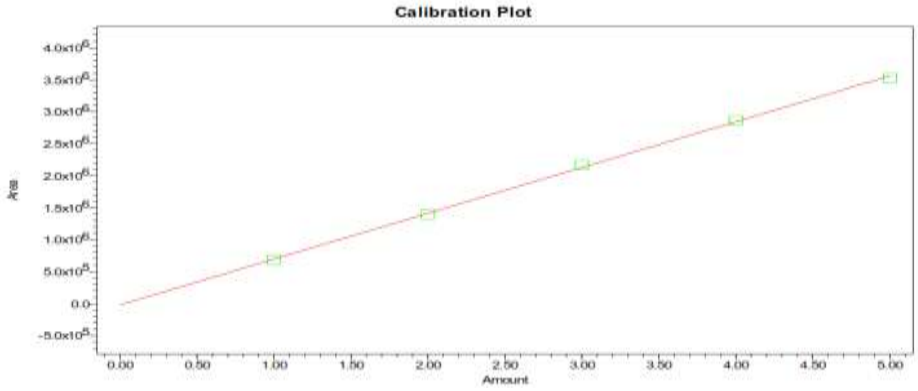
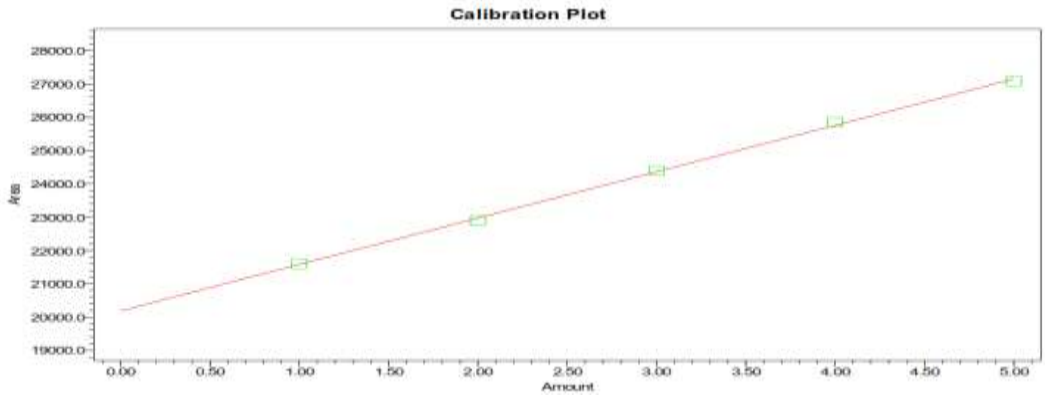


Figure 12-Linearity graph of Bembodoic acid

Table 4. Linearity results of Rosuvastatin

S.No	Linearity Level	Concentration(µg/ml)	Area
1	I	8	32441
2	II	16	67728
3	III	24	100630
4	IV	32	134448
5	V	40	172463
Correlation Coefficient			0.999



Linearity graph of Rosuvastatin

Table 5. System suitability results for Bembodoic acid

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	3726.18	1.21
2	*Actual	3417.62	1.14
3	10% more	3343.64	1.34

Table 6. System suitability results for Rosuvastatin

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results		
		USP Plate Count	USP Tailing	USP Resolution
1	10% less	3175.92	1.31	4.96
2	*Actual	2381.56	1.11	4.42
3	10% more	34445.92	1.23	4.96

CONCLUSION

A new method was established for simultaneous estimation of Rosuvastatin and Bempedoic acid by RP-HPLC methods. The chromatographic conditions were successfully developed for the separation of Rosuvastatin and Bempedoic acid by using Inertsil ODSC18 column (4.6×250mm)5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) ACN : KH2PO4 pH 3, detection wavelength was 225nm. The instrument used for HPLC , WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 3.598 mins and 4.487 mins. The % purity of Rosuvastatin and Bempedoic acid was found to be 100.15% and 100.57% respectively. The system suitability parameters for Rosuvastatin and Bempedoic acid such as theoretical plates and tailing factor were found to be 4260, 1.2 and 5085 and 1.2, the resolution was found to be 3.67. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). Hence the suggested RP-HPLC can be used for routine analysis of Rosuvastatin and Bempedoic acid in API and Pharmaceutical dosage form.

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