



# “RP HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF BEMPEDOIC ACID, EZETIMIBE, AND ATORVASTATIN IN SYNTHETIC MIXTURE”

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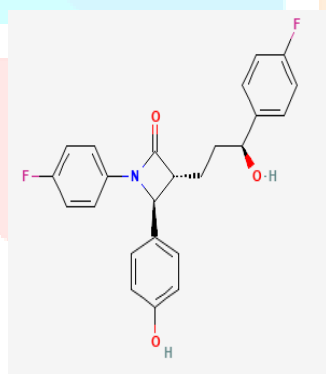
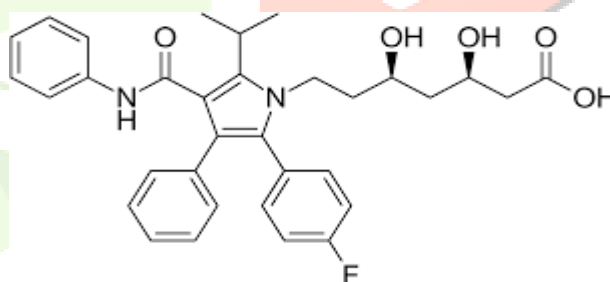
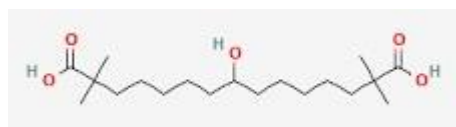
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**ABSTRACT:** A reverse phase high performance liquid chromatography (RP-HPLC) Method was developed for the determination of bempedoic acid, ezetimibe and atorvastatin in a synthetic mixture. The developed method was validated as per of ICH guidelines. the chromatographic separation was achieved isocratically on C 18 (250 mm x 4.6 mm), 5 µm at ambient temperature using Potassium dihydrogen phosphate, methanol and acetonitrile (30:60:10) – 5.8 ph of buffer, as a mobile phase at flow rate of 1 ml/min and UV detection at 262 nm. Retention time for bempedoic acid, ezetimibe and atorvastatin were 3.76, 5.49 and 6.85 min respectively. The calibration curve obtained for Bempedoic Acid (BEM) in the range of 90-540 µg/ml, Ezetimibe (EZT) 5- 30 µg/ml and Atorvastatin (ATR) 10 – 60 µg/ml. The correlation coefficient of Bempedoic Acid (BEM), Ezetimibe (EZT) and Atorvastatin (ATR) was found to be 0.9987, 0.9973 and 0.9997 respectively. Accuracy of method was carried out at three level (50 %,100 % and 150 %). % Recovery for Bempedoic Acid (BEM) was found to be in range of 99.90-103.46 %, for), Ezetimibe (EZT) it was found to be range of 97.02 -102.43 % and Atorvastatin (ATR) it was found to be range of 97.97 -102.17.

Keywords: ezetimibe (EZM) Bempedoic acid (BEM) atorvastatin (ATR) reversed-phase high-performance liquid chromatography (RP-HPLC).]

**INTRODUCTION:** Statins are the drugs most commonly used to treat hyperlipidaemias in primary and secondary prevention, in order to lower levels of cholesterol-rich plasma lipoproteins and reduce the risk of coronary disease.[1] Ezetimibe (EZM) is a selective inhibitor of the intestinal cholesterol and related phytosterols absorption used as adjunctive therapy to a healthy diet to lower cholesterol levels in cases of hyperlipidaemia [3]. Bempedoic acid is an Adenosine Triphosphate-Citrate Lyase Inhibitor. The mechanism of action of Bempedoic acid is as an Adenosine Triphosphate-Citrate Lyase Inhibitor. Bempedoic acid is first-in-class adenosine triphosphate-citrate lyase (ACL) inhibitor used once a day for reducing LDL cholesterol levels in statin-refractory patients. It was developed by Esperion Therapeutics Inc. The number of drugs introduced into the market is increasing every year. These drugs may be either new entities or partial structural modification of the existing one. Very often there is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, reports of new toxicities (resulting in their withdrawal from the market), development of patient resistance and introduction of better drugs by competitors. Under these conditions, standards and analytical procedures for these drugs may not be available in the pharmacopoeias. Thus, it becomes necessary, to develop newer analytical methods for such drugs. [4-5]

**EZETIMIBE****ATORVASTATIN**

### **BEMPEDOIC ACID**

**MATERIALS AND METHODS:** Sample of Atorvastatin (ATR), Ezetimibe (EZT) and Bempedoic Acid (BEM) procured from ZuventusHealthcare Ltd., Maharashtra.

## EXPERIMENTAL CONDITION:

**Apparatus:** HPLC manufactured by Cyber Lab having LC-100 model no. was used in this method development. Cyber-Sil, C18 column (250mm x 4.6mm, 5 µg) was used as a stationary phase. For Identification of api by using UV Visible Spectrophotometer and FT-IR UV Visible Spectrophotometer is manufactured by Shimadzu having UV 1700 model no and FT-IR is manufactured by Agilent Technologies having Cary 630 model no.

**Chemical:** HPLC Grade Water, Methanol, Acetonitrile which is manufactured by Ranchem Ltd. AR Grade Phosphate buffer, Orthophosphoric acid which is manufactured by Ranchem Ltd.

### Solubility Study

Solubility of Atorvastatin (ATR), Ezetimibe (EZT) and Bempedoic Acid (BEM) was performed using various solvents like water, methanol, acetonitrile etc.

### IR Spectra

Drug was placed in sample compartment of FT-IR instrument, where it was scanned in the range of 4000 - 650  $\text{cm}^{-1}$ . Principle IR peaks were observed for drug are shown in table and from this data it was concluded that drugs were found to be authentic.

### UV Absorption Study

Accurately weighed 10 mg of Atorvastatin (ATR), Ezetimibe (EZT) and Bempedoic Acid (BEM) were transferred separately in 10 ml volumetric flasks, dissolved in small volume of methanol and then volume was adjusted to the mark with methanol to obtain concentration of 1000 µg/ml. These solutions were further diluted to obtain concentration of 10 µg/ml. These standard solutions of Atorvastatin (ATR), Ezetimibe (EZT) and Bempedoic Acid (BEM) in methanol were scanned in UV range, 200-400 nm in 1 cm cell using methanol as blank and maximum absorbance was measured for selection of  $\lambda_{\text{max}}$  of Atorvastatin (ATR), Ezetimibe (EZT) and Bempedoic Acid (BEM).

## Method development and validation:

### Selection of Diluent

Based on solubility Atorvastatin (ATR), Ezetimibe (EZT) and Bempedoic Acid (BEM) was sparingly soluble in methanol. Hence, methanol was selected as diluent.

### Preparation of Stock solution

Accurately weighed and transferred about Bempedoic acid 180 mg, ezetimibe 10 mg, and atorvastatin 20 mg in to 100 ml of volumetric flask, 50 ml of methanol was added and sonicated to dissolve. Volume was making up to the mark with diluent. Concentration of 1800 µg/ml Bempedoic acid, 100 µg/ml ezetimibe, and 200 µg/ml for atorvastatin. Further diluted 5 ml of above solution to 50 ml volumetric flask and volume was make up to the mark with diluent. Concentration of 180 µg/ml Bempedoic acid, 10 µg/ml ezetimibe, and 20 µg/ml for atorvastatin. The optimum wavelength was

selected for the estimation was 265 nm where gives maximum absorbance, which was obtained by scanning solution in the range of 200-400 nm in UV spectrophotometer.

### **Selection of Mobile Phase**

Mobile phase selection involved selection of buffer, pH of buffer, selection of solvent and buffer to solvent ratio. Proper selection of the HPLC method depends upon the nature of the sample, its molecular weight and solubility. For pH control buffer is required. As the acidic compound retain at low pH while base retained at high pH. The mobile phase was selected on the basis of good separation, peak purity, Tailing factor, theoretical plate etc. Various mobile phases were tried in different composition and different pH to achieve sharp peak of Bempedoic acid, ezetimibe, and atorvastatin.

### **Preparation of 0.1 M Phosphate Buffer**

Weigh accurately and transfer about 1.36 g of Sodium Phosphate Dibasic Heptahydrate and 13.126 gm of Sodium Phosphate Monobasic Monohydrate in 800 ml of water, add water sufficient water to produce 1000 ml. Adjust the pH 5.8 with NaOH.

### **Preparation of Mobile Phase**

Prepare a mixture of Potassium Dihydrogen Phosphate: Methanol: Acetonitrile (30:60:10 % V/V/V)- 5.8 pH of buffer. Mix well and sonicate to degas the mixture.

**Preparation Bempedoic acid (BEM) Standard Solution:** Accurately weigh and transfer 100 mg of Bempedoic acid into a 100 mL volumetric flask. Add about 70 % diluent and sonicate to dissolve. Dilute upto mark with diluent and mix well. The solution formed will have concentration of Bempedoic acid 1000 µg/mL.

- Take 0.9, 1.8, 2.7, 3.6, 4.5, 5.4 ml above linearity solution to get series of concentration 90 – 600 ppm for BEM. Dilute the solution were filtered through 0.45 µm membrane filters.

**Preparation ezetimibe (EZE) Standard Solution:** Accurately weigh and transfer 10 mg of ezetimibe into a 100 mL volumetric flask. Add about 70 % diluent and sonicate to dissolve. Dilute upto mark with diluent and mix well. The solution formed will have concentration of ezetimibe 100 µg/mL.

- Take 0.5, 1, 1.5, 2, 2.5, 3 ml above linearity solution to get series of concentration 5 – 30 ppm for EZE. Dilute the solution were filtered through 0.45 µm membrane filters.

### **Preparation atorvastatin (ATR) Standard Solution:**

- Accurately weigh and transfer 10 mg of atorvastatin into a 100 mL volumetric flask. Add about 70 % diluent and sonicate to dissolve. Dilute upto mark with diluent and mix well. The solution formed will have concentration of Chlorthalidone 200 µg/mL.
- Take 1,2,3,4,5 and 6 ml above linearity solution to get series of concentration 10 – 60 ppm for atorvastatin. Dilute the solution were filtered through 0.45 µm membrane filters.

**SELECTION OF COLUMN:** C18 analytical column were selected for HPLC method. The column was used Cybersil C18 column (250 mm × 4.6 mm, 5 µm) was used for the development of the method.

## METHOD VALIDATION:

### System-Suitability Tests

The system-suitability tests are integral part of gas and liquid chromatography. They are used to verify that the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The tests are based on concept that the equipment, electronics, analytical operations, and sample to be analysed constitute an integral system that can be evaluated as such. The system suitability parameters like resolution, theoretical plates and asymmetric factor were calculated and compared with standard values. The system suitability test was carried out on freshly prepared working standard stock solution of BEM (180 µg/ml), EZE (10 µg/ml) and ATR (20µg/ml), respectively.

### Accuracy

- To ensure the reliability of the above method recovery studies were carried out by mixing standard quantity of standard drug with the pre analyzed sample synthetic mixture and the contents were re-analyzed by the proposed method.
- Recovery studies were carried out at 50,100 and 150 % level. The recovery study was performed three times at each level.
- Level 50 %: Take 1 ml of 90 ppm of BEM, 5 ppm of EZE and 10 ppm of ATR, spiked with 1 ml of 45 ppm of BEM, 2.5 ppm of EZE and 5 ppm of ATR, and Add 50 ml methanol and sonicate to dissolved, Dilute upto mark with diluent and mix well. Further diluted 1 ml of above solution to 10 ml volumetric flask and volume was make up to the mark with diluent. Concentration of 135 µg/ml Bempedoic acid, 7.5 µg/ml ezetimibe, and 15 µg/ml for atorvastatin
- Level 100 %: Take 1 ml of 90 ppm of BEM, 5 ppm of EZE and 10 ppm of ATR, spiked with 1 ml of 90 ppm of BEM, 5 ppm of EZE and 10 ppm of ATR, and Add 50 ml methanol and sonicate to dissolved, Dilute upto mark with diluent and mix well. Further diluted 1 ml of above solution to 10 ml volumetric flask and volume was make up to the mark with diluent. Concentration of 180 µg/ml Bempedoic acid, 10 µg/ml ezetimibe, and 20 µg/ml for atorvastatin.
- Level 150 %: Take 1 ml of 90 ppm of BEM, 5 ppm of EZE and 10 ppm of ATR, spiked with 1 ml of 135 ppm of BEM, 7.5 ppm of EZE and 15 ppm of ATR, and Add 50 ml methanol and sonicate to dissolved, Dilute upto mark with diluent and mix well. Further diluted 1 ml of above solution to 10 ml volumetric flask and volume was make up to the mark with diluent. Concentration of 225 µg/ml Bempedoic acid, 12.5 µg/ml ezetimibe, and 25 µg/ml for atorvastatin.

### Precision

- Repeatability was determined by analyzing solution containing mixture of Concentration of 180 µg/ml Bempedoic acid, 10 µg/ml ezetimibe, and 20 µg/ml for atorvastatin. Peak area of same concentration was measured six times and % RSD was calculated.

➤ Intra – Day Precision of Concentration of 90, 270, 540 µg/ml Bempedoic acid, 5, 15, 30 µg/ml ezetimibe, and 10, 30, 60 µg/ml for atorvastatin of respectively as a mixture of drugs were analyze at three different time intervals in a day and RSD was calculated.

Inter – Day Precision of Concentration of 90, 270, 540 µg/ml Bempedoic acid, 5, 15, 30 µg/ml ezetimibe, and 10, 30, 60 µg/ml for atorvastatin of respectively as a mixture of drugs were analyze at three different time intervals in a day and RSD was calculated.

### **Robustness**

➤ According to ICH, the robustness of an analytical procedure refers to its capability to remain unaffected by small and deliberated variations in method parameters here changes in different conditions were considered:

- 1.Change in Flow rate (1 mL/min ± 1)
- 2.Change in Mobile phase composition (30:60:10 % v/v ± 2ml)
- 3.Change in Wavelength (262 nm ± 3)

**Specificity:** Specificity were ensured by the use of a standard, diluent and placebo to examine the % interference of excipients. The specificity of proposed method was determined by analysing spiking of placebo to standard and cauculate the % interference.

### **Assay of synthetic mixture**

➤ The synthetic mixture of Bempedoic acid 180 mg, ezetimibe 10 mg, and atorvastatin 20 mg. Common excipients like Microcrystalline cellulose (4080 mg), Cross Povidone (300 mg), Calcium Phosphate (300 mg), Magnesium Stearate (111 mg), (HPMC) Hydroxypropyl methylcellulose K100 (30 mg), Cross Carmellose Sodium (150 mg) were weighed accurately and transfer into motor pestle along with Bempedoic acid 1800 mg, ezetimibe 100 mg, and atorvastatin 200 mg which is equivalent to 10 tablets. Weight accurately equivalent to Bempedoic acid 180 mg, ezetimibe 10 mg, and atorvastatin 20 mg and transfer it in 10 ml volumetric flask containing 5.0 ml of methanol and sonicated for 15 min. The solution was filtered using Whatman filter paper No.42 and collects the filtrate in another 10 ml volumetric flask and the residue was wash with 3.0 ml amount of methanol, the filtrate and residue was combined and volume was diluted to the mark with the methanol. Pipette out 1.0 ml aliquot from the above solution, transfer it in another 10 ml volumetric flask and volume made upto the mark with methanol, from above solution, Pipette out 1.0 ml aliquot and transfer it into another 10 ml volumetric flask and makeup volume upto the mark and from the above solution, to obtain final concentration of 180 µg/ml for BEM, 10 µg/ml for EZE and 20 µg/ml for ATR respectively. The possibility of interference from other components of the synthetic mixture in the analysis was studied. It was analysed under proposed chromatographic conditions and chromatogram recorded. The amount of BEM, EZE and ATR were computed using regression equation.

**RESULT AND DISCUSSION:****IDENTIFICATION OF DRUG****Melting Point Study**

The observed melting point of each mentioned drugs were similar to the standard melting point reported for respective drugs as evident from Table 4.3.

**Table 1: Melting Point Study**

Drugs	Reported Melting Point (°C)	Observed Melting Point (°C)
Atorvastatin (ATR)	175-177 °C <sup>[10]</sup>	175-177 °C
Ezetimibe (EZT)	161°C -163°C [12]	160°C -163°C
Bempedoic Acid (BEM)	87-92 °C <sup>[14]</sup>	88-90 °C

**N = 3, Mean of 3 replicates**

**Solubility Study**

The solubility of substance fundamentally depends on the physical and chemical properties of the solute and solvent as well as temperature, pressure and the pH of the solution. The solubility profile is used for solvent selection in method development. The solubility of each drug in different solvent is shown in Table 4.2.

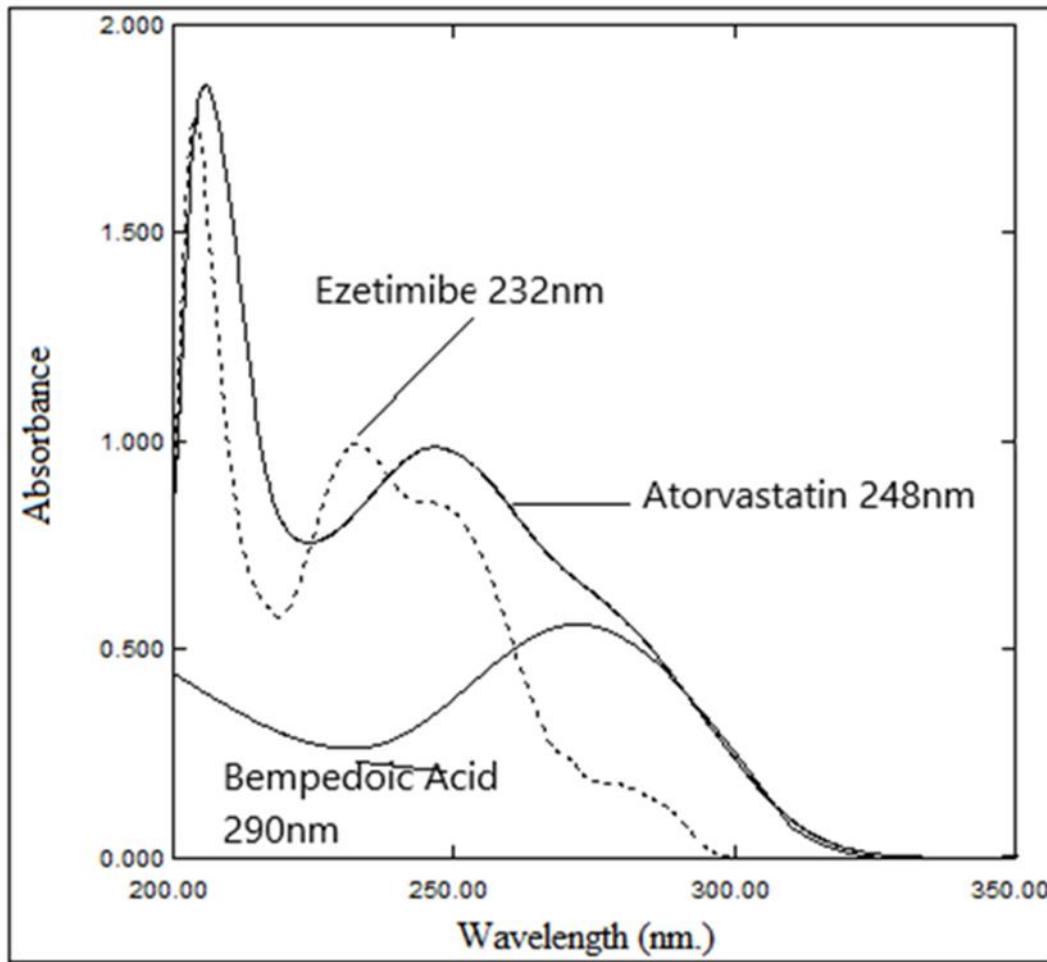
**Table 2: Solubility Study**

Drugs	Atorvastatin (ATR)	Ezetimibe (EZT)	Bempedoic Acid (BEM)
Water	Slightly soluble	Insoluble	Insoluble
Methanol	Soluble	Very Soluble	Soluble
Acetonitrile	Slightly soluble	Soluble	Slightly soluble

**UV Absorption Study**

UV spectra of drugs in methanol depicted that the wavelength maxima of Atorvastatin (ATR), Ezetimibe (EZT) and Bempedoic Acid (BEM) were at 254 nm, 248nm and 275 nm respectively as shown in Figure 6.1.

For High Performance Liquid Chromatography 262 nm was selected wavelength.



**Figure 1: Overlain UV Spectrum in methanol**

### IR Spectra

An IR spectrum of reference sample shown in figure 2, figure 3 and figure 4 observed frequency was within the standard frequency range. So, concluded that given sample content was Atorvastatin (ATR), Ezetimibe (EZT) and Bempedoic Acid (BEM) results are shown in table 3,4and 5.



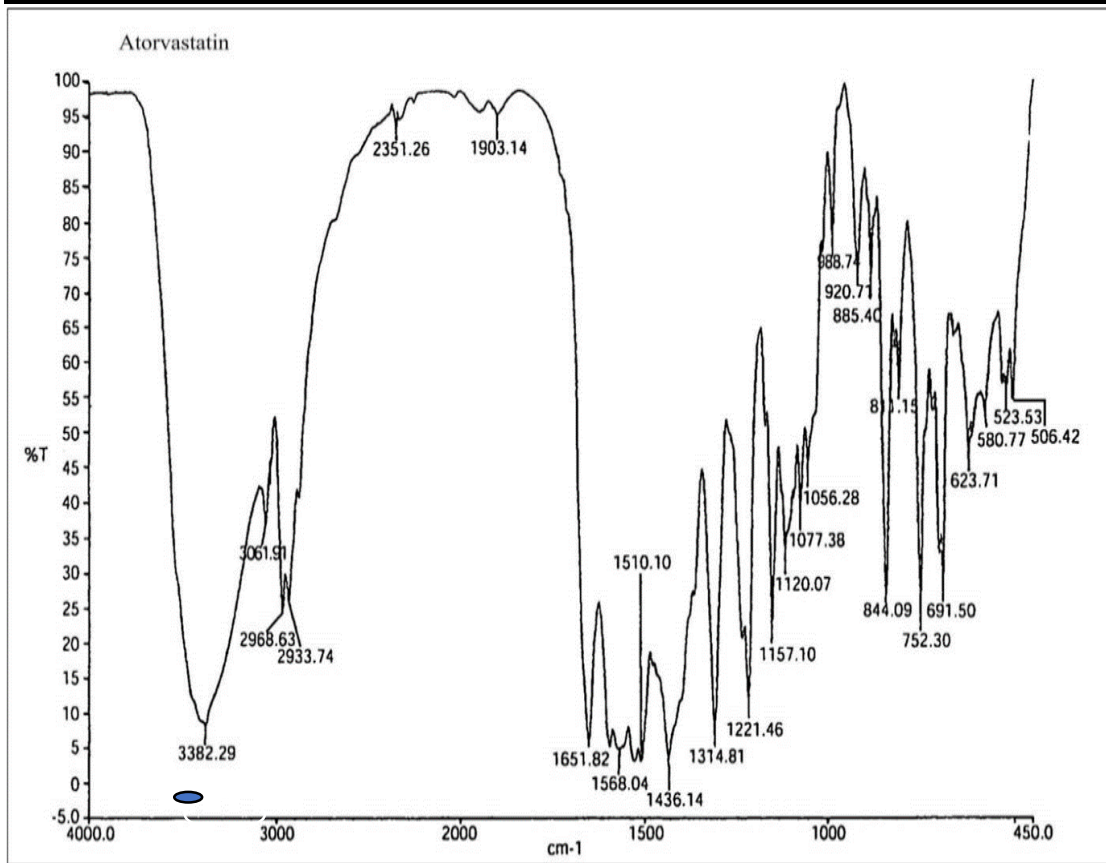


Figure 2: IR Spectrum of Atorvastatin (ATR)

Table 3: IR value for Atorvastatin (ATR)

Sr. No.	Functional Group	Reported Wavenumber (cm <sup>-1</sup> )	Observed Wavenumber (cm <sup>-1</sup> )
1.	O-H	3400-3200	3051.58
2.	N-H	3500-3100	3382.29
3.	C=O	1725-1705	1651.82
4.	C=N	1690-1640	1588.04
5.	C-H	3100-3000	2968.68

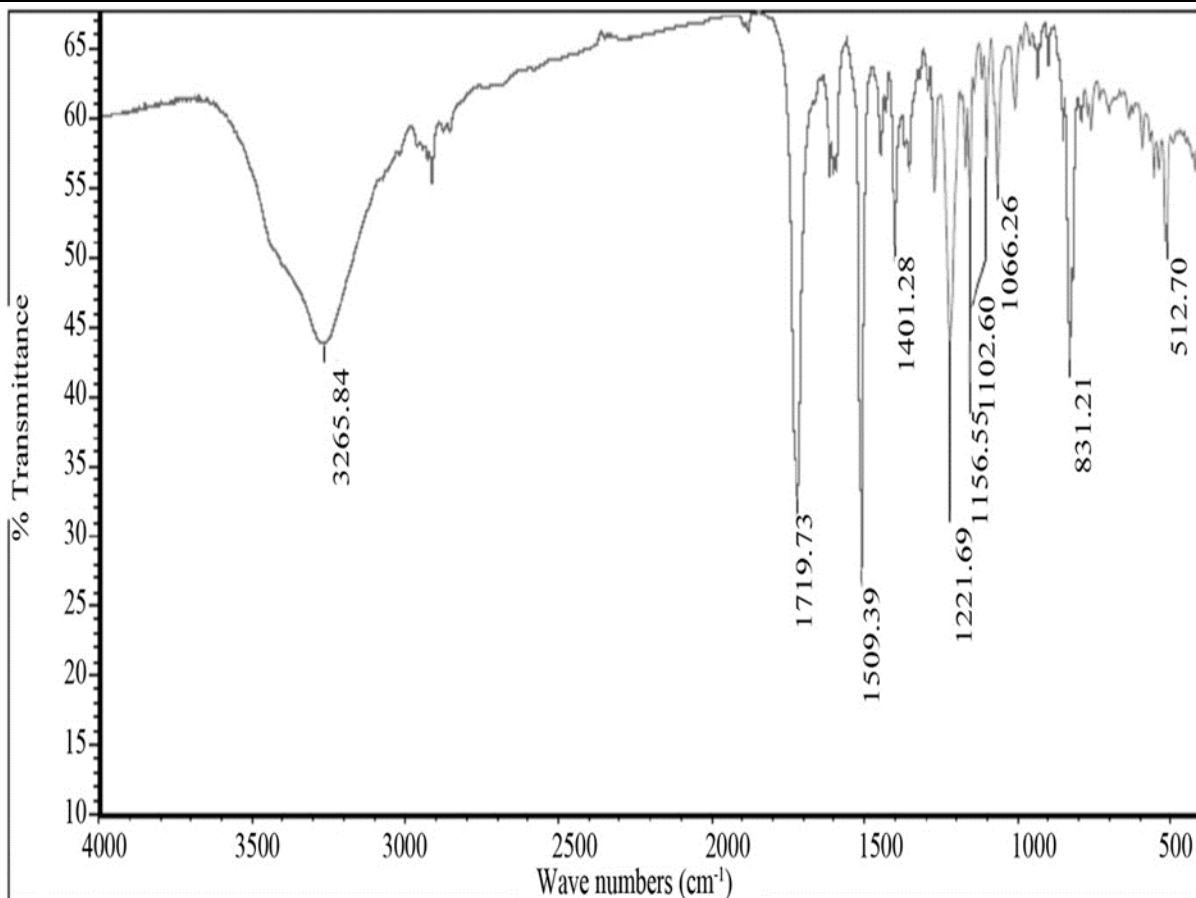
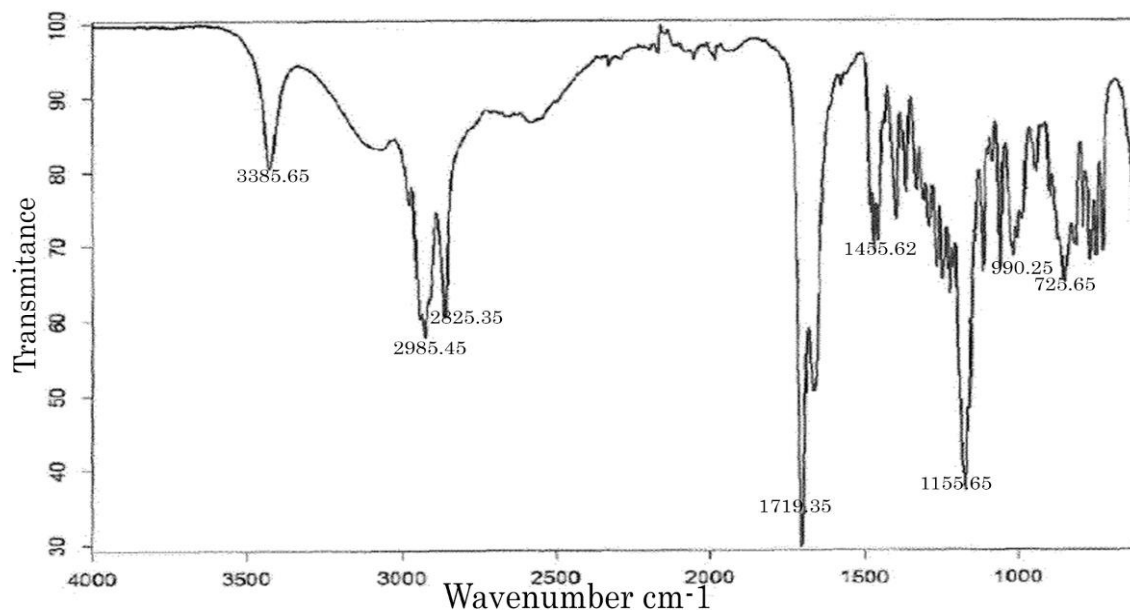


Figure 3: IR Spectrum of Ezetimibe (Ezt)

Table 4: IR Value for Ezetimibe (Ezt)

Sr. No.	Functional Group	Reported Wavenumber (cm <sup>-1</sup> )	Observed Wavenumber (cm <sup>-1</sup> )
1.	C=O	1750-1730	1719.73
2.	C-H	1300-1000	1509.39
3.	O-H	3550-3200	3265.84
4.	C-F	1400-1000	1401.28
5.	C-N	1250-1020	1221.69



**Figure 4: IR Spectrum of Bempedoic Acid (BEM)**

**Table 5: IR Value for and Bempedoic Acid (BEM)**

Sr. No.	Functional Gap	Reported Wavenumber (cm <sup>-1</sup> )	Observed Wavenumber (cm <sup>-1</sup> )
1.	O-H Carboxylic acid	3400-3200	3385.65
2.	C=O Carboxylic acid	1720-1706	1719.35
3.	C-H Bending	1465-1450	1455.62
4.	O-H Alcohol	1205-1124	1155.65
5.	C=C Stretching	995-985	990.25

**CONCLUSION**

From the obtained results of identification study, it can be interpreted that the Atorvastatin (ATR), Ezetimibe (EZT) and Bempedoic Acid (BEM) are pure and authentic.

**Table 6: Method Development Trial**

<b>Trial No.</b>	<b>Condition</b>	<b>Observation</b>
1	<b>Column:</b> C 18 (250 mm x 4.6 mm),5 µm <b>Mobile Phase:</b> Water: Methanol (30:70 % V/V) <b>Flow Rate:</b> 1 ml/min <b>Wavelength:</b> 262 nm <b>Injection Volume:</b> 20 µl	Peak shape was not proper only Ezetimibe (EZT) and Bempedoic Acid eluted and Atorvastatin Was not found.
2	<b>Column:</b> C 18 (250 mm x 4.6 mm),5 µm <b>Mobile Phase:</b> Water: Acetonitrile (30:70 V/V) <b>Flow Rate:</b> 1 ml/min <b>Wavelength:</b> 262 nm <b>Injection Volume:</b> 20 µl	Peak shape was not proper only Ezetimibe (EZT) and Atorvastatin eluted and Bempedoic Acid was not Found.
3	<b>Column:</b> C 18 (250 mm x 4.6 mm),5 µm <b>Mobile Phase:</b> Water: Methanol: Acetonitrile (20:40:40 % V/V/V) <b>Flow Rate:</b> 1 ml/min <b>Wavelength:</b> 262 nm <b>Injection Volume:</b> 20 µl	Peak shape was not proper. Peak tailing observed
4	<b>Column:</b> C 18 (250 mm x 4.6 mm),5 µm <b>Mobile Phase:</b> Water: Methanol: Acetonitrile (20:60:20 % V/V/V) <b>Flow Rate:</b> 1 ml/min <b>Wavelength:</b> 262 nm <b>Injection Volume:</b> 20 µl	Peak shape was not proper. Resolution is less and peak tailing observed
5	<b>Column:</b> C 18 (250 mm x 4.6 mm),5 µm <b>Mobile Phase:</b> Buffer: Methanol: Acetonitrile (30:50:20 % V/V/V) 3.5 pH of buffer <b>Flow Rate:</b> 1 ml/min <b>Wavelength:</b> 262 nm <b>Injection Volume:</b> 20 µl	Peak shape was not proper. Resolution is less
6	<b>Column:</b> C 18 (250 mm x 4.6 mm),5 µm	Shorter retention time of Drug and peak shape was proper, resolution is good but tailing observed

	<p><b>Mobile Phase:</b> Potassium Dihydrogen Phosphate: Methanol: Acetonitrile (30:60:10 % V/V) 4 pH of buffer</p> <p><b>Flow Rate:</b> 1 ml/min</p> <p><b>Wavelength:</b> 262 nm</p> <p><b>Injection Volume:</b> 20 µl</p>	
7	<p><b>Column:</b> C 18 (250 mm x 4.6 mm),5 µm</p> <p><b>Mobile Phase:</b> Potassium Dihydrogen Phosphate: Methanol: Acetonitrile (30:60:10 % V/V)- 5.8 pH of buffer</p> <p><b>Flow Rate:</b> 1 ml/min</p> <p><b>Wavelength:</b> 262 nm</p> <p><b>Injection Volume:</b> 20 µl</p>	<p>Peak shape was proper, resolution good and tailing factor is less than 1.5 and theoretical plate greater than 2000</p>

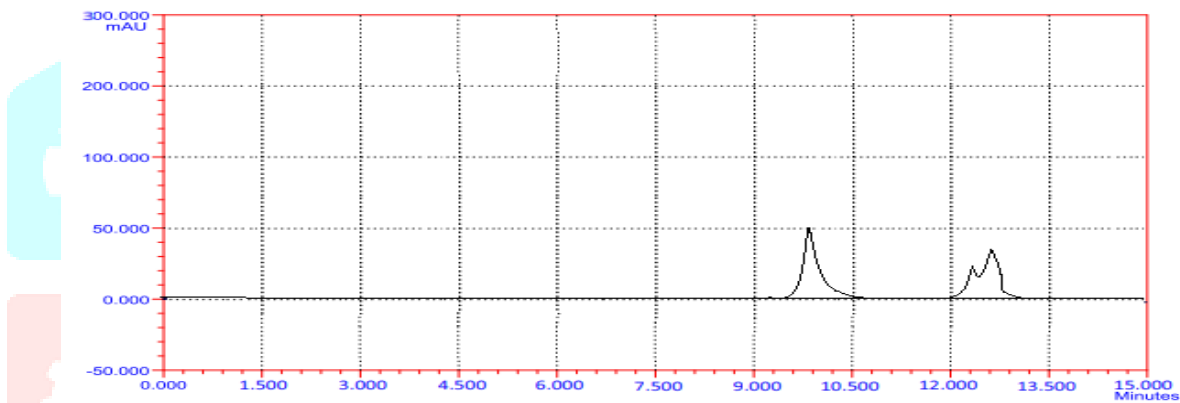


Figure1: Trail 1: Mobile Phase: Water: Methanol (30:70 % V/V), HPLC Chromatogram

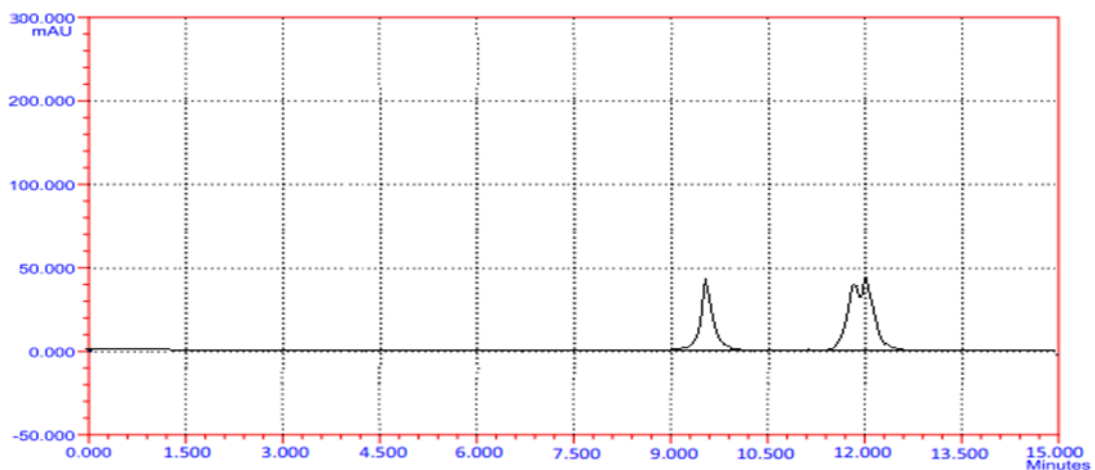
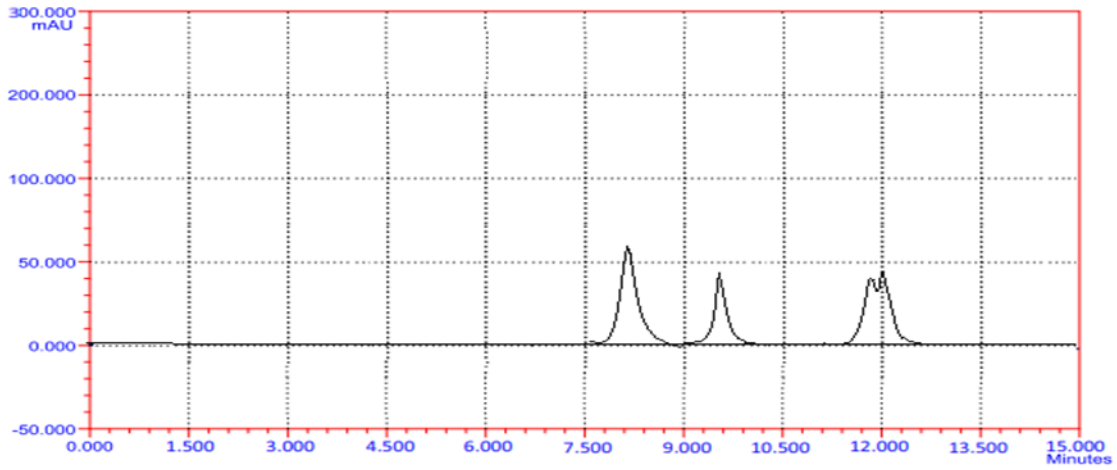
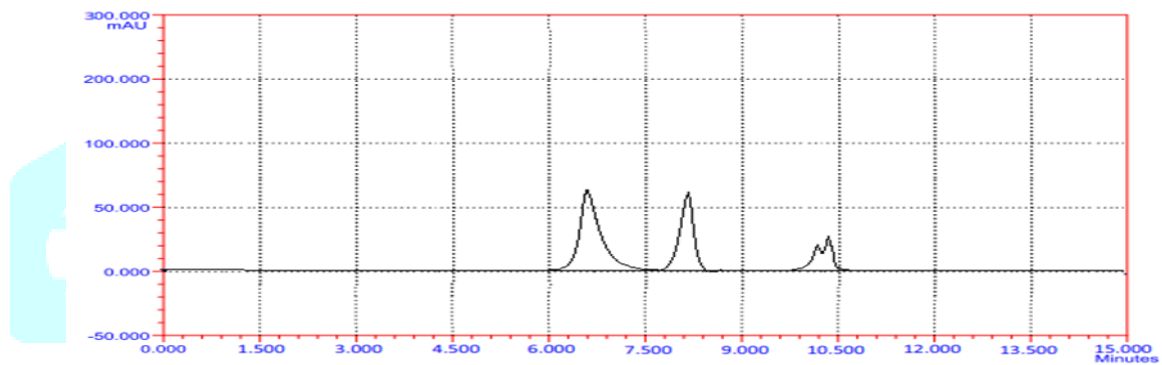


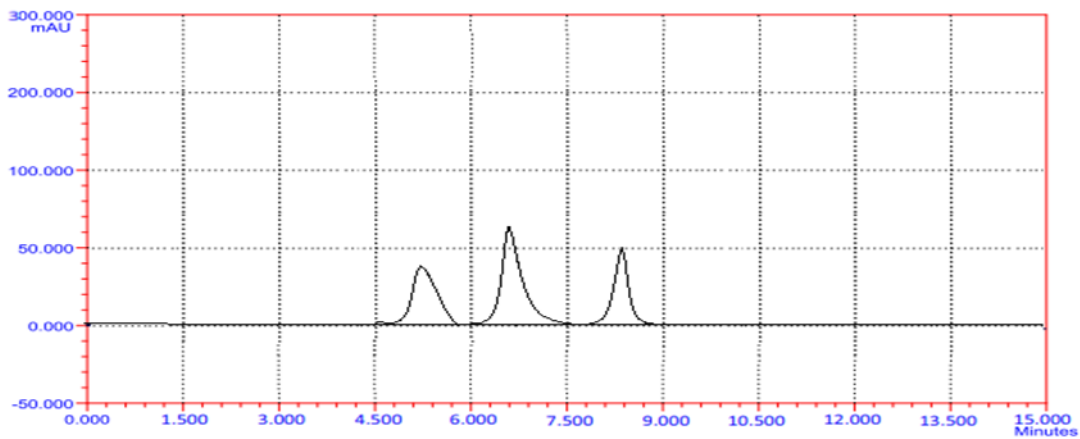
Figure 0: Trail 2: Water: Acetonitrile (30:70 V/V), HPLC Chromatogram



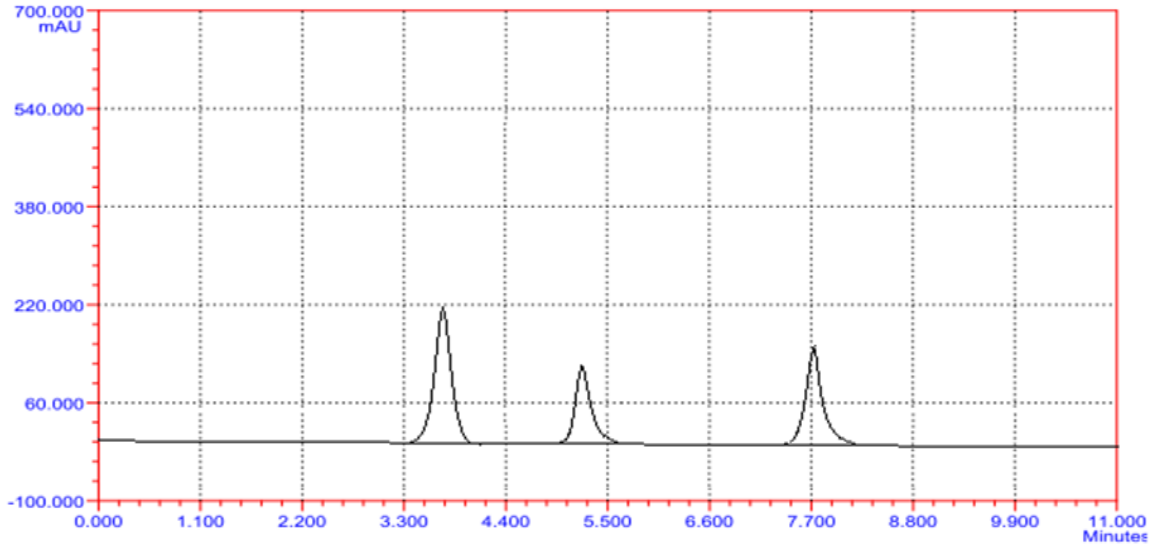
**Figure 7: Trail 3: Mobile Phase: Water: Methanol: Acetonitrile (20:40:40 %V/V/V), HPLC Chromatogram**



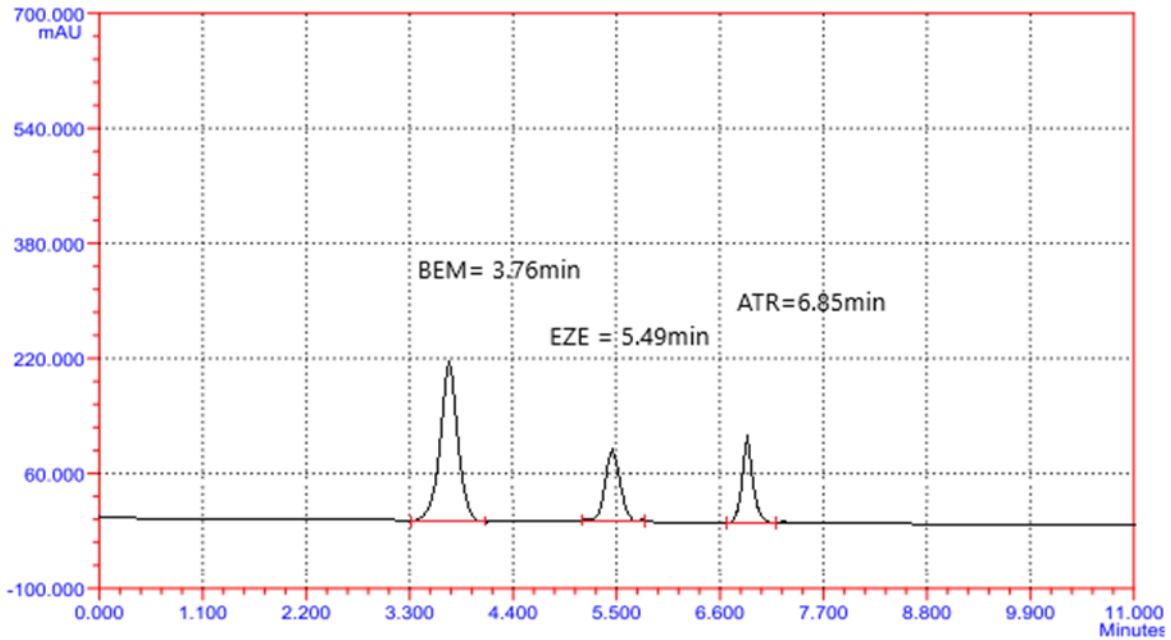
**Figure 8: Trail 4: Mobile Phase: Water: Methanol: Acetonitrile (20:60:20 %V/V/V), HPLC Chromatogram**



**Figure 9: Trail 5: Mobile Phase: Buffer: Methanol: Acetonitrile (30:50:20 %V/V/V) 3.5 pH of buffer, HPLC Chromatogram**

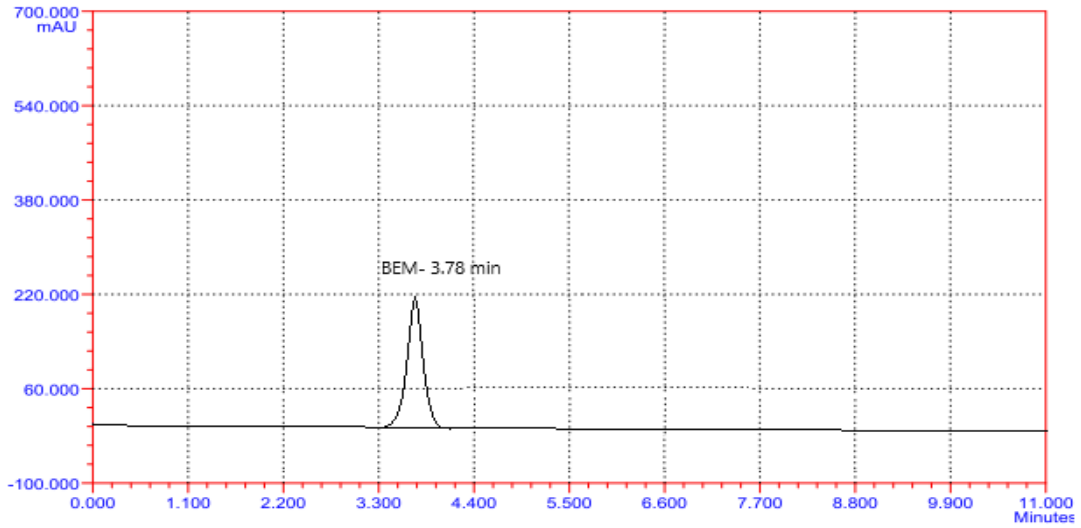


**Figure10: Trail 6: Mobile Phase: Potassium Dihydrogen Phosphate: Methanol: Acetonitrile (30:60:10 % V/V) 4 pH of buffer, HPLC Chromatogram**

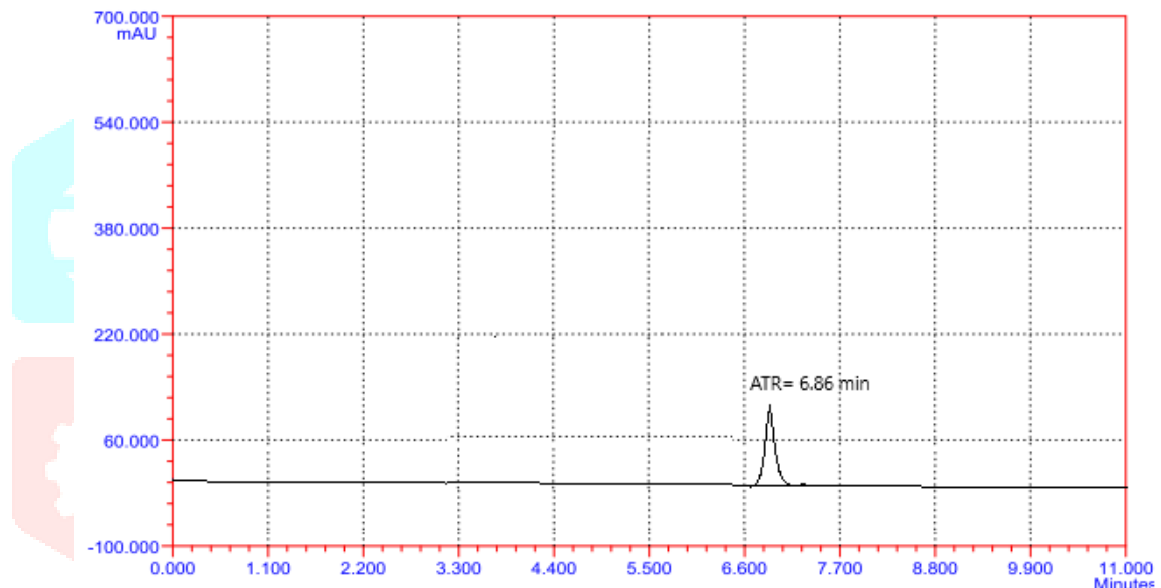


**Figure**

**11: Trail 7: Mobile Phase: Potassium Dihydrogen Phosphate: Methanol: Acetonitrile (30:60:10 % V/V)- 5.8 pH of buffer, HPLC Chromatogram**

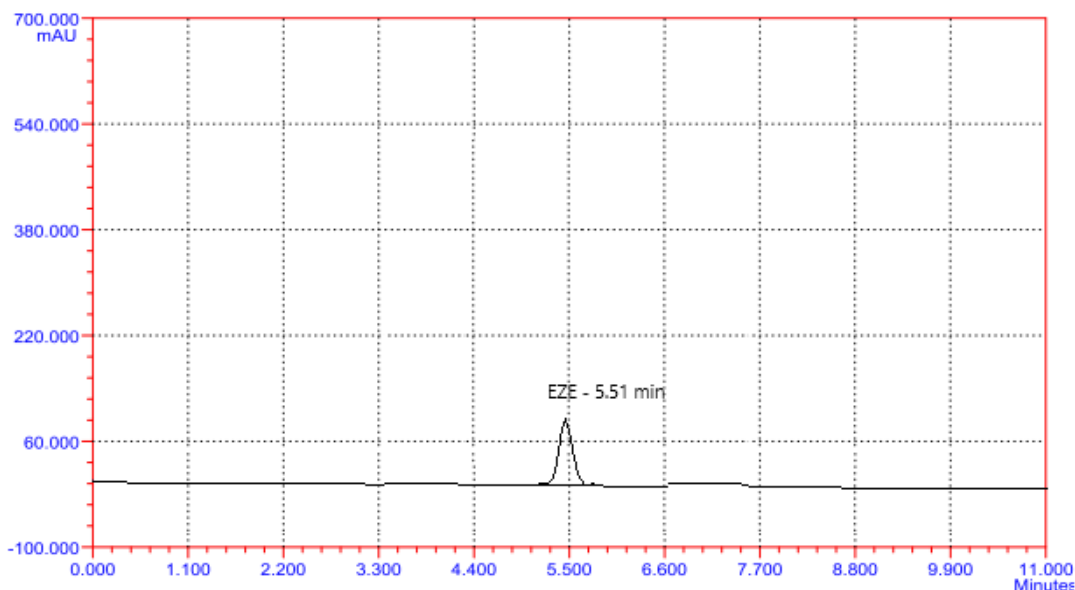


**Figure 12: Bempedoic Acid (BEM) HPLC Chromatogram**



**Figure 13: Ezetimibe (EZE) HPLC Chromatogram**





**Figure 14: Atorvastatin (ATR) HPLC Chromatogram**

**Table 7: Optimization of RP-HPLC chromatographic condition**

Sr. No.	Chromatographic parameter	Optimize Condition		
1	Flow Rate	1 ml/min		
2	Detection Wavelength	262		
3	Mobile Phase composition	Potassium Dihydrogen Phosphate: Methanol: Acetonitrile (30:60:10 % V/V)- 5.8 pH of buffer		
4	Column	C18 (250 mm×4.6 mm×5 μm)		
5	Injection Volume	20 μl		
6	pH of buffer	5.8± 0.02		
7	Retention time (min)	BEM		
		EZE		
		3.76	5.49	6.85

**System Suitability Parameter:** The system suitability parameters were calculated and all system suitability parameter are within the acceptable range.

**Table 8: system suitability parameter**

Parameter	BEM	EZE	ATR
Retention Time(min)	3.76	5.49	6.85
Resolution	0.00	7.25	5.68
Theoretical plate	2245.31	4316.74	3894.35
Symmetric Factor	1.10	1.29	1.47

Peak Area	24444.32	15399.76	11733.56
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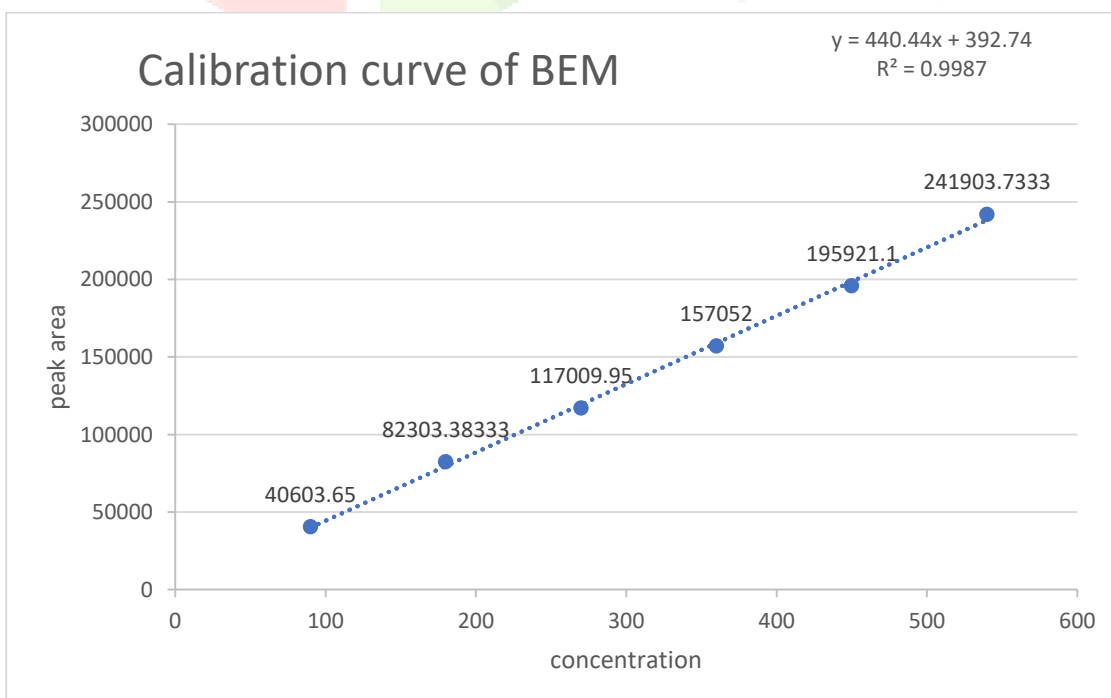
**METHOD VALIDATION:**

**Linearity**

The calibration curve obtained for Bempedoic Acid (BEM) in the range of 90-540 µg/ml, Ezetimibe (EZT) 5- 30 µg/ml and Atorvastatin (ATR) 10 – 60 µg/ml. The correlation coefficient of Bempedoic Acid (BEM), Ezetimibe (EZT) and Atorvastatin (ATR) was found to be 0.9987, 0.9973 and 0.9997 respectively. The calibration curve for Bempedoic Acid (BEM), Ezetimibe (EZT) and Atorvastatin (ATR) given in Fig. no. 15, 16 and fig. no 17. The overlay HPLC chromatogram of drugs was given in fig no. 18

**Table 9: Linearity Data of Bempedoic Acid (BEM)**

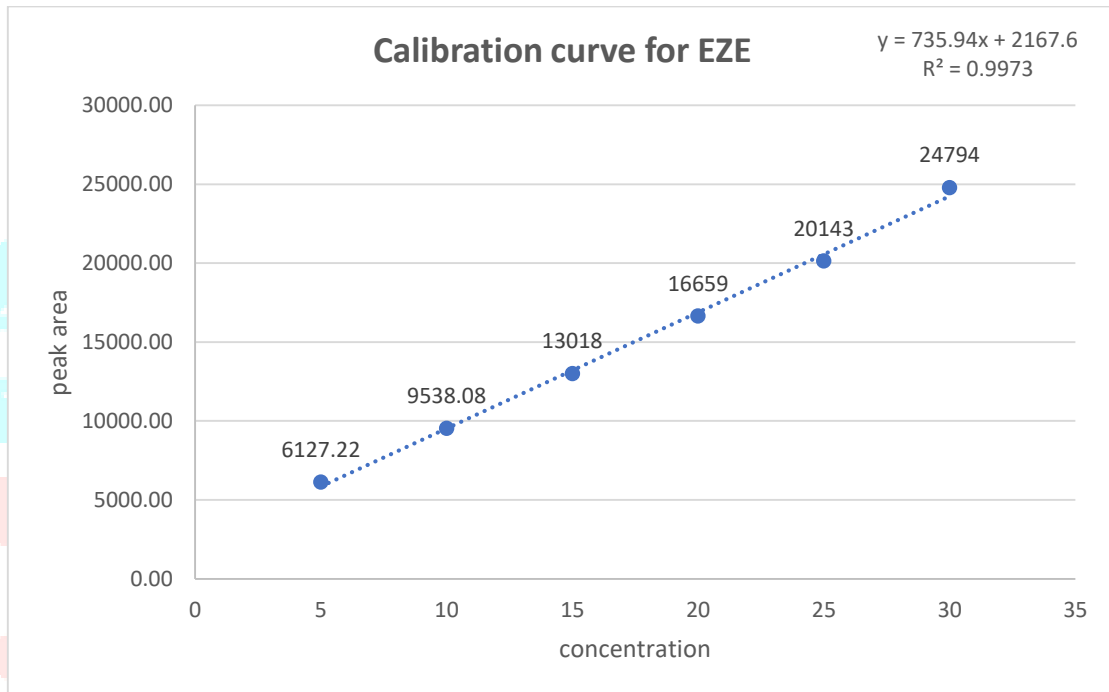
Conc.	Peak Area ± SD	RSD
90	40603.65 ± 506.66	1.25
180	82303.38 ± 861.63	1.05
270	117009.95 ± 670.78	0.57
360	157052 ± 1032.36	0.66
450	195921.1 ± 1215.58	0.62
540	241903.73 ± 4156.44	1.72



**Figure 15: Calibration curve for Bempedoic Acid (BEM)**

**Table 10: Linearity Data of Ezetimibe (EZT)**

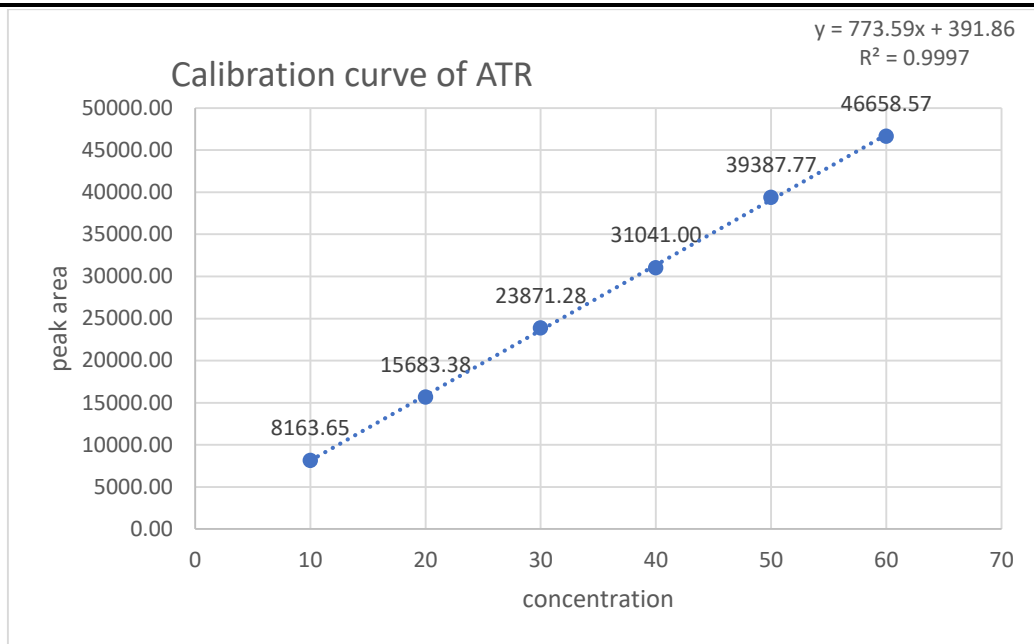
Conc.	Peak Area ± SD	RSD
5	6127.22 ± 88.34	1.44
10	9538.08 ± 102.50	1.07
15	13018 ± 253.96	1.91
20	16659 ± 312.54	1.90
25	20143 ± 363.99	1.80
30	24794 ± 327.65	1.32



**Figure16: Calibration curve for Ezetimibe (EZT)**

**Table 11: Linearity Data of Atorvastatin (ATR)**

Conc.	Peak Area ± SD	RSD
10	8163.65 ± 101.40	1.24
20	15683.38 ± 304.08	1.94
30	23871.28 ± 444.95	1.86
40	31041.00 ± 507.27	1.63
50	39387.77 ± 572.28	1.45
60	46658.57 ± 835.57	1.79



**Figure 17: Calibration curve for Atorvastatin (ATR)**

**Accuracy**

➤ Accuracy of method was carried out at three level (50 %,100 % and 150 %). % Recovery for Bempedoic Acid (BEM) was found to be in range of 99.90-103.46 %, for, Ezetimibe (EZT) it was found to be range of 97.02 -102.43 % and Atorvastatin (ATR) it was found to be range of 97.97 -102.17 % are shown in Table 12.

**Table 12: Accuracy data**

Level (%)	Target Conc. (µg/ml)	Spiked Conc. (µg/ml)	Total Conc. (µg/ml)	Area	Conc. Found (µg/ml)	% Recovery
Bempedoic Acid (BEM)						
0	90	0	90	40604.90	91.30	101.44
50	90	45	135	59793.67	134.87	99.90
100	90	90	180	82416.23	186.23	103.46
150	90	135	225	102091.74	230.90	102.62
Ezetimibe (EZT)						
0	10	0	10	9493.31	9.95	99.54
50	10	5	15	13475.15	15.36	102.43
100	10	10	20	16447.98	19.40	97.02
150	10	15	25	20418.90	24.80	99.20
Atorvastatin (ATR)						
0	20	0	20	15549.57	19.59	97.97
50	20	10	30	24102.83	30.65	102.17
100	20	20	40	31326.80	39.99	99.97

150	20	30	50	39519.67	50.58	101.16
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**Precision**

➤ Repeatability was determined by analyzing solution containing mixture of Concentration of 180 µg/ml Bempedoic acid, 10 µg/ml ezetimibe, and 20 µg/ml for atorvastatin. Peak area of same concentration was measured six times and % RSD was calculated.

**Table 13: Repeatability Data of Bempedoic acid, ezetimibe, and atorvastatin.**

Bempedoic acid			Ezetimibe			Atorvastatin		
Sr. No	Conc. (µg/ml)	Area	Sr. No	Conc. (µg/ml)	Area	Sr. No	Conc. (µg/ml)	Area
1	180	82598.7	1	10	9531.53	1	20	15298.7
2	180	82474.2	2	10	9416.85	2	20	15774.2
3	180	82975.8	3	10	9531.53	3	20	15575.8
4	180	81849.3	4	10	9721.58	4	20	15449.3
5	180	81758.6	5	10	9551.33	5	20	15564.21
6	180	81163.7	6	10	9285.55	6	20	15863.7
Average	44178.60		Average	9506.40		Average	15587.65278	
SD	663.89		SD	145.86		SD	207.04	
% RSD	0.81		RSD	1.53		RSD	1.33	

Intra – Day Precision of Concentration of 90, 270, 540 µg/ml Bempedoic acid, 5, 15, 30 µg/ml ezetimibe, and 10, 30, 60 µg/ml for atorvastatin of respectively as a mixture of drugs were analyze at three different time intervals in a day and RSD was calculated.

Inter – Day Precision of Concentration of 90, 270, 540 µg/ml Bempedoic acid, 5, 15, 30 µg/ml ezetimibe, and 10, 30, 60 µg/ml for atorvastatin of respectively as a mixture of drugs were analyze at three different time intervals in a day and RSD was calculated.

For Repeatability, Intraday and Interday precision RSD was found to be less than 2

**Table 14: Intraday and Interday precision of method**

<b>Bempedoic acid</b>				
<b>Conc</b>	<b>Intraday precision</b>		<b>Interday precision</b>	
	Peak Area (Mean ± SD) <sup>n</sup>	%RSD	Peak Area (Mean ± SD) <sup>n</sup>	%RSD
90	40235.40 ± 324.01	0.81	41271.90 ± 769.71	1.86
270	116756.17 ± 614.24	0.53	116930.40 ± 1260.24	1.08
540	245890.29 ± 1154.70	0.47	241249.84 ± 3729.43	1.55
<b>Ezetimibe</b>				
5	6217.63 ± 41.69	0.67	6066.80 ± 43.87	0.72
15	13208.48 ± 128.97	0.98	13419.48 ± 198.03	1.48
30	24412.00 ± 78.14	0.32	24801.55 ± 106.04	0.43
<b>Atorvastatin</b>				
10	8122.07 ± 59.15	0.73	8205.23 ± 130.46	1.79
30	23902.83 ± 295.71	1.24	23647.07 ± 238.58	1.78
60	46556.96 ± 1000.0	2.15	46765.17 ± 844.35	0.41

**LOD and LOQ**

LOD & LOQ of Bempedoic acid, ezetimibe, and for atorvastatin of were determined by equation according to ICH guideline calculation of these was given in Table 15.

**Table 15: LOD and LOQ of Bempedoic acid, ezetimibe, and for atorvastatin**

Drug	Bempedoic Acid	Ezetimibe	Atorvastatin
Limit of detection (LOD)	6.38 µg/ml	0.72 µg/ml	0.80 µg/ml
Limit of quantification (LOQ)	21.28 µg/ml	2.41 µg/ml	2.68 µg/ml

**Robustness**

➤ Deliberate change in parameter like flow rate, wavelength, mobile phase composition ratio and showed RSD of peak area less than 2 %, indicating that the method was robust, result is shown in table 16.

**Table 16: Robustness**

**EFFECT OF CHANGE IN VOLUME OF PHOSPHATE BUFFER**

25 ml			30 ml			35 ml			
	Peak Area	SD	%RSD	Peak Area	SD	%RSD	Peak Area	SD	%RSD
<b>BEM</b> <b>(90 µg/ml)</b>	40235.40	324.01	0.81	40971.9	360.49	0.88	40604.90	546.36	1.35
<b>EZE</b> <b>(5 µg/ml)</b>	6228.87	102.98	1.65	6070.13	38.12	0.63	6070.13	38.12	0.63
<b>ATR</b> <b>(20µg/ml)</b>	8168.23	105.23	1.29	8205.233	130.46	1.59	8171.72	120.25	1.47

**EFFECT OF CHANGE IN FLOWRATE**

0.9 ml/min			1 ml/min			1.1 ml/min			
	Peak	SD	%RSD	Peak	SD	%RSD	Peak	SD	%RSD
<b>BEM</b> <b>(90 µg/ml)</b>	40870.75	315.05	0.77	40971.9	360.49	0.88	40971.57	707.56	1.73
<b>EZE</b> <b>(5 µg/ml)</b>	6217.63	41.69	0.67	6070.13	38.12	0.63	6199.67	60.96	0.98
<b>ATR</b> <b>(20µg/ml)</b>	8172.10	99.06	1.21	8205.233	130.46	1.59	8205.23	130.46	1.59

**EFFECT OF CHANGE IN DETECTION**

251 nm			254 nm			257 nm		
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	Peak Area	SD	%RSD	Peak Area	SD	%RSD	Peak Area	SD	%RSD
<b>BEM</b> <b>(90 µg/ml)</b>	40235.40	324.01	0.81	40971.9	360.49	0.88	40971.90	360.49	0.88
<b>EZE</b> <b>(5 µg/ml)</b>	6180.87	88.65	1.43	6070.13	38.12	0.63	6106.90	86.86	1.42
<b>ATR</b> <b>(20µg/ml)</b>	8208.10	145.85	1.78	8205.233	130.46	1.59	8171.72	120.25	1.47

### Specificity

➤ Specificity were ensured by the use of a standard, diluent and placebo to examine the % interference of excipients. It was proved by comparing chromatogram of blank, standard solution and sample preparation solution, there was no any interference of excipients with peak of Bempedoic Acid (BEM), Ezetimibe (EZE) and Atorvastatin (ATR)

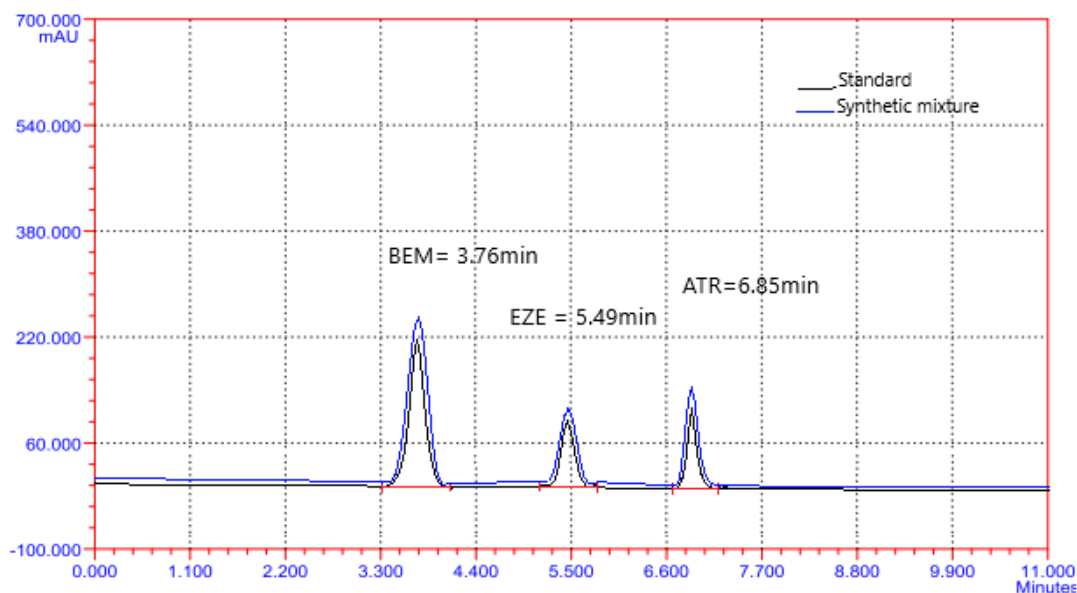
### Table 17: Summary of validation parameters for bempedoic acid ezetimibe and atorvastatin by RP-HPLC method

- Summary of validation parameters for bempedoic acid ezetimibe and atorvastatin by RP-HPLC method
- 

### Assay of synthetic mixture

➤ The synthetic mixture of Bempedoic acid 180 mg, ezetimibe 10 mg, and atorvastatin 20 mg was analysed using the developed method. Final concentration of 180 µg/ml for BEM, 10 µg/ml for EZE and 20 µg/ml for ATR respectively chromatogram of drug mixture indicating no interference of the excipients and Result are shown in table 6.17 and they were found satisfaction.





**Figure 18:** Overlay chromatogram of API and Synthetic mixture on optimized Mobile Phase

**Table 18: Analysis of Synthetic mixture**

Drugs	Conc.	Peak Area	% Assay
Bempedoic acid	180 µg/ml	82433.1 ± 564.37	103.48 ± 0.711
Ezetimibe	10 µg/ml	9556.65 ± 153.91	100.40 ± 2.09
Atorvastatin	20 µg/ml	15599.77 ± 163.77	98.29 ± 1.05

**Table 19 Summary of validation parameter for EZM BEM &ATR**

Sr.no	Parameters	Result		
		ezetimibe	Bempedoic acid	atorvastatin
1	Concentration range (µg/ml)	5-30	90-540	10-60
2	Retention time (min.)	5.49	3.67	6.85
3	Detection limit (µg/ml)	0.72	6.38	0.80
4	Quantification limit(µg/ml)	2.41	21.28	2.68
5	Accuracy (% Recovery) (n=3)	97.02-102.43	99.90-103.46	97.97-101.16
6	Precision (% RSD)			
	Repeatability (n=6)	1.57	0.81	1.33
	Intraday Precision (n=3)	0.32-0.98	0.47-0.81	0.73-2.15
	Interday Precision (n=3)	043-1.48	1.08-1.86	0.41-1.79
7	Specificity	Specific	Specific	Specific
8	Robustness	Robust	Robust	Robust

## CONCLUSION

Proposed method is developed for the identification and quantification of Bempedoic Acid, Ezetimibe, And Atorvastatin in bulk and synthetic mixture. The developed method is simple, accurate, less time consuming, economical and sensitive when compared to other reported analytical methods. According to ICH guideline the method was found to be accurate, sensitive and precise. Statistical analysis proved that the method was repeatable and selective for the analysis of Bempedoic Acid, Ezetimibe, And Atorvastatin without any interference of excipients. This method was successfully used in determination of prepared synthetic mixture.

In HPLC (High Performance Liquid Chromatography) by using Cybersil C-18 column (250 mm × 4.6 mm, 5 µm) equilibrated with mobile phase Potassium Dihydrogen Phosphate: Methanol: Acetonitrile (30:60:10 % V/V)- 5.8 pH of buffer, flow rate was maintained to 1ml/min and analysis was carried out by using UVdetector. The common detection wavelength was found to be 262 nm. The method was validated for linearity, precision, accuracy and robustness, limit of detection and limit of quantification.

The calibration curve obtained for Bempedoic Acid (BEM) in the range of 90-540 µg/ml, Ezetimibe (EZT) 5- 30 µg/ml and Atorvastatin (ATR) 10 – 60 µg/ml. The correlation coefficient of Bempedoic Acid (BEM), Ezetimibe (EZT) and Atorvastatin (ATR) was found to be 0.9987, 0.9973 and 0.9997 respectively. Accuracy of method was carried out at three level (50 %,100 % and 150 %). % Recovery for Bempedoic Acid (BEM) was found to be in range of 99.90-103.46 %, for, Ezetimibe (EZT) it was found to be range of 97.02 -102.43 % and Atorvastatin (ATR) it was found to be range of 97.97 -102.17 %

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