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Analytical Method Development and Validation of Simultaneous Estimation of Bempadoic Acid & Ezetimibe by RP-HPLC in Bulk and Pharmaceutical Dosage Form

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Abstract: ***A simple, rapid, precise, sensitive and reproducible reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the quantitative analysis of Bempedoic acid and Ezetimibe in pharmaceutical dosage form. Chromatographic separation of Bempedoic acid and Ezetimibe was achieved on Waters Alliance-e2695 by using Waters X Terra RP-18 (250x4.6mm, 5µ) column and the mobile phase containing Methanol: 0.1% TEA pH-2.5/OPA in the ratio of 20:80% v/v. The flow rate was 1.0 ml/min; detection was carried out by absorption at 232nm using a photodiode array detector at ambient temperature. The number of theoretical plates and tailing factor for Bempedoic acid and Ezetimibe were NLT 2000 and should not more than 2 respectively. % Relative standard deviation of peak areas of all measurements always less than 2.0. The proposed method was validated according to ICH guidelines. The method was found to be simple, economical, suitable, precise, accurate & robust method for quantitative analysis of Bempedoic acid and Ezetimibe study of its stability.***

Keywords: RP-HPLC, Bempadic acid, Ezetimibe, Analytical Method development, TEA & ICH

1. Introduction

Bempedoic acid is a prodrug that requires activation in the liver. The very-long-chain acyl-CoA synthetase-1 (ACSVL1) enzyme is responsible for its activation to ETC-1002-CoA, the pharmacologically active metabolite. ATP lyase (also known as ATP synthase) plays an important part of cholesterol synthesis. BETC-1002-CoA directly inhibits this enzyme after the parent drug is activated in the liver by coenzyme A (CoA). This inhibition leads to upregulation of the LDL cholesterol receptor, reducing serum LDL-C via increased uptake and LDL clearance in the liver. Chemical name of Bempadoic acid is 8- hydroxy-2, 2, 14, 14-tetramethyl pentadecane dioic acid and it belongs to the adenosine triphosphate citrate lyase (ACL) inhibitors 1,2.

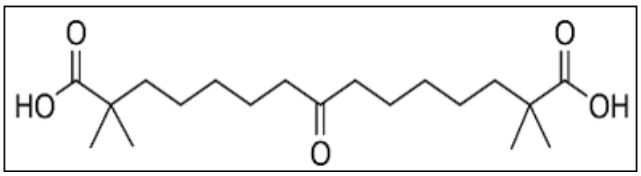

Figure 1: Molecular structure of Bempedoic acid

Ezetimibe is an azetidine derivative, it is Inhibits absorption of cholesterol at the brush border of the small intestine via the sterol transporter, Niemann-Pick C1-Like1 (NPC1L1). This leads to a decreased delivery of cholesterol to the liver, reduction of hepatic cholesterol stores and an increased clearance of cholesterol from the blood, decreases total C, LDL-cholesterol (LDL-C), ApoB, and triglycerides (TG) while increasing HDL-cholesterol (HDL-C). Ezetimibe is chemically (3R, 4S)-1-(4-fluorophenyl)-3-[(3S)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl) azetidin-2 one. and it belongs to the class cholesterol-lowering medications 1, 2.

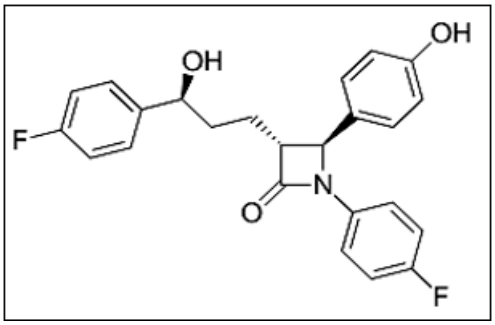

Figure 2: Molecular structure of Ezetimibe

Only few Methods have been reported for estimation of Bempadoic acid and Ezetimibe Bulk and Pharmaceutical dosage form. The aim of this study to develop & validate RP-HPLC Method for estimation of Bempadoic acid Ezetimibe Bulk and Pharmaceutical dosage form. It was low cost, accurate, precise and high resolution & short run time method developed for simultaneous estimation of bempadoic acid and ezetimibe by RP-HPLC.

2. Materials and Methods

Instrumentation: The chromatograpy method was done by using Waters Alliance-e2695 System equipped with Photo diode arry detector and data processing was done by using Empower software 2. 0versions.The separation technique was conducted Waters X Terra RP-18 (250x4.6mm, 5µ). Weights was taken by using an Sartouris BSA224S-CW analytical balance & and pH was adjusted by using Eutech

7000 pH meter. In this study Pipettes, beakers and Burettes was used Class-A Borosil glass ware.

Chemicals and reagents: Methanol is used HPLC grade manufactured by Merck & Ortho Phosphoric acid, 0.1% Triethyl amine was used AR grade manufactured by Merck and Milli-Q Water were used. The API Bempadoic acid and ezetimibe was obtained from manufactured by Honours labs.

Determination of Working Wavelength (λ_{max})

The wavelength of maximum absorption of the solution of the drugs in mixture of Methanol and 0.1% TEA pH-2.5/OPA (20:80) were scanned using PDA Detector within the wavelength region of 200–400 nm against Methanol and 0.1% TEA pH-2.5/OPA (20:80) as blank. The absorption curve shows isobestic point at 232nm. Thus 232 nm was selected as detector wavelength for the HPLC chromatographic method.

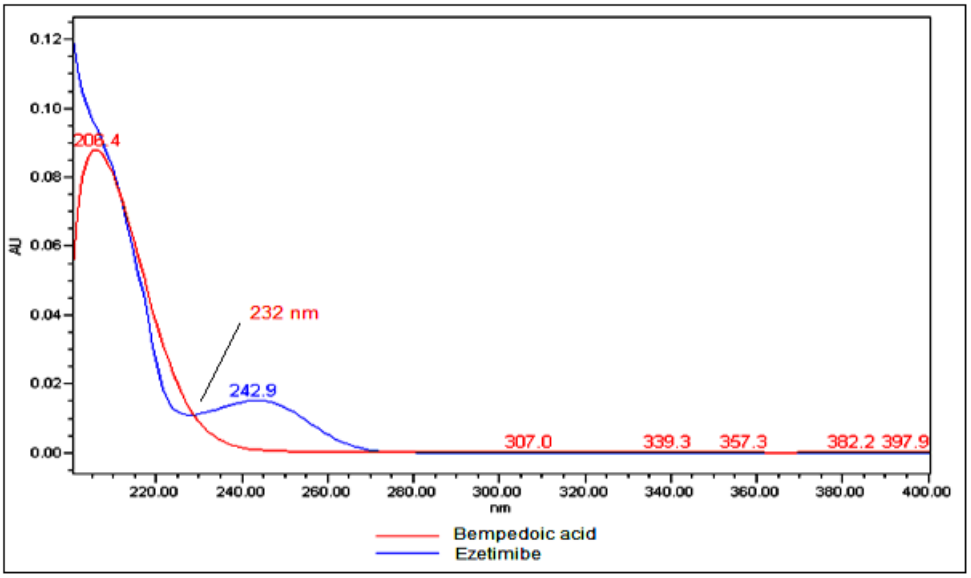

Figure 3: PDA - Spectrum of Bempedoic acid & Ezetimibe

Chromatographic conditions:

Table 1: Chromatographic conditions

Column	Waters X Terra RP-18 (250x4.6mm, 5µ)
Mobile phase ratio	Methanol: 0.1% TEA pH-2.5/OPA (20:80)
Detection wavelength	232nm
Flow rate	1ml/min
Injection volume	10µl
Run time	6min

Preparation of 0.1% TEA: 1ml of Triethyl amine is dissolved in 1 litre of HPLC water, adjust its pH-2.5 with OPA and filter through 0.45µ membrane filter paper.

Preparation of Mobile Phase: Mobile phase was prepared by mixing Methanol and 0.1% TEA pH-2.5/OPA taken in the ratio 20:80. It was filtered through 0.45µ membrane filter to remove the impurities which may interfere in the final chromatogram.

Preparation of standard solution: Accurately weigh and transfer 180 mg of Bempedoic acid and 10 mg of Ezetimibe working standard into 100 ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 5 ml of the above stock solutions into a 50 ml volumetric flask and dilute up to the mark with diluent. (180ppm of Bempedoic acid, 10ppm of Ezetimibe).

Preparation of sample solution: Accurately weighed and transfer 30.2mg of Bempedoic acid and Ezetimibe sample into a 10mL clean dry volumetric flask add Diluent and sonicate it up to 30 mins to dissolve, and centrifuge for 30min. to dissolve it completely and make volume up to the mark with the same solvent. Then it is filtered through 0.45 micron Injection filter (Stock solution).

Further pipette 1 ml of the above stock solutions into 10 ml volumetric flask and dilute up to the mark with diluents. (180ppm of Bempedoic acid, 10ppm a of Ezetimibe).

Method development: Chromatographic separations was performed by Waters X Terra RP-18 (250x4.6mm, 5µ) column with the Methanol and % TEA (20:80) as mobile phase at the flow rate 1ml/min and column temperature was 25° C where detection was carried 232nm by using PDA Detector. the developed, optimized results in the separation of bempadoic acid at 2.345 min and Ezetimibe 3.532 min and the total run time was 6 min respectively.

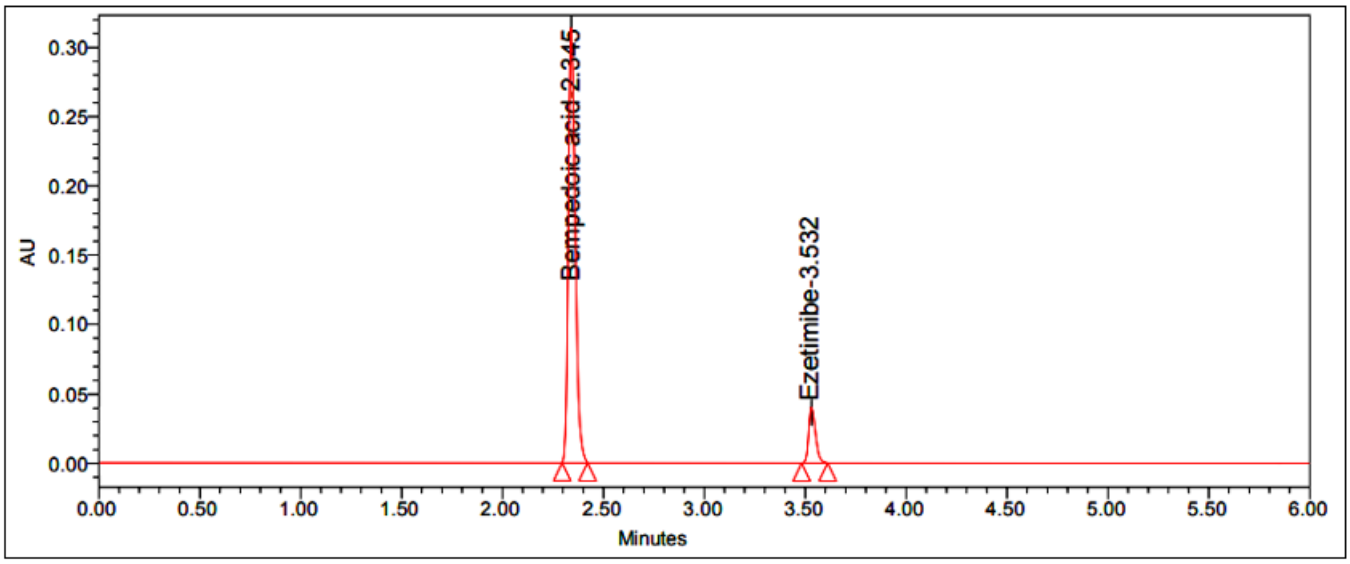

Figure 4: Optimized chromatogram

Table 2: Results for (Optimized trail)

S. No	Name	RT	Area	USP Plate Count	USP Tailing	USP Resolution
1	Bempedoic acid	2.345	3125468	9846	1.12	-
2	Ezetimibe	3.532	176001	5431	1.03	8.42

Table 3: Optimized chromatographic conditions

Parameters	Observation
Instrument used	Waters HPLC with auto sampler and PDA detector.
Injection volume	10µl
Mobile Phase	Methanol: 0.1% TEA pH-2.5/OPA (20:80)
Column	Waters X Terra RP-18 (250x4.6mm, 5µ)
Detection Wave Length	232nm
Flow Rate	1 mL/min
Runtime	6min
Temperature	Ambient(25° C)
Mode of separation	Isocratic mode

Optimized chromatogram of Bempadoic acid and Ezetimibe are shown in **Fig-4** and Optimized chromatographic conditions are tabulated in **Table -3**

System suitability parameters:
System suitability parameters was conducted to verify the optimized coditions and the system suitability tests was performed as ICH guidelines. various parameters evaluated, such as Retention time, Plate count, Tailing factor, Resolution, %RSD. they all system suitability parameters are were within the range and found satisfactory as per ICH guidelines. the obtained system suitability results was summarized **Table -4**

Table 4: System suitability parameters for Bempedoic acid & Ezetimibe

S.no	Parameter	Bempedoic acid	Ezetimibe
1	Retention time	2.345	3.532
2	Plate count	9846	5431
3	Tailing factor	1.12	1.03
4	Resolution	----	8.42
5	%RSD	0.22	0.52

3. Results and Discussion

The developed RP-HPLC method for ezetimibe and bempedoic acid was validated as per ICH guidelines.

Analytical method validation: The method was validated for its linearity range, accuracy, precision and specificity. Method validation was carried out as per ICH guidelines

Specificity: Specificity of an analytical method is ability to measure specifically the analyte of interest without interference from blank and known impurities. For this purpose blank chromatogram, standard chromatogram and sample chromatogram were recorded. The chromatogram of blank shows no response at the retention times of drugs which confirms the response of drugs was specific.

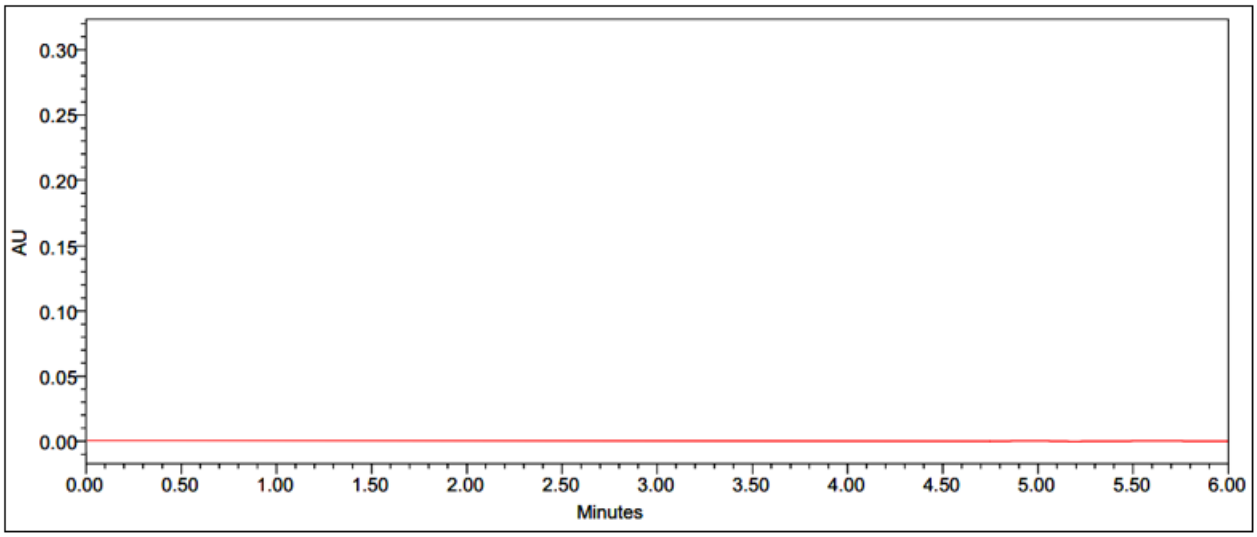

Figure 5: Chromatogram of blank

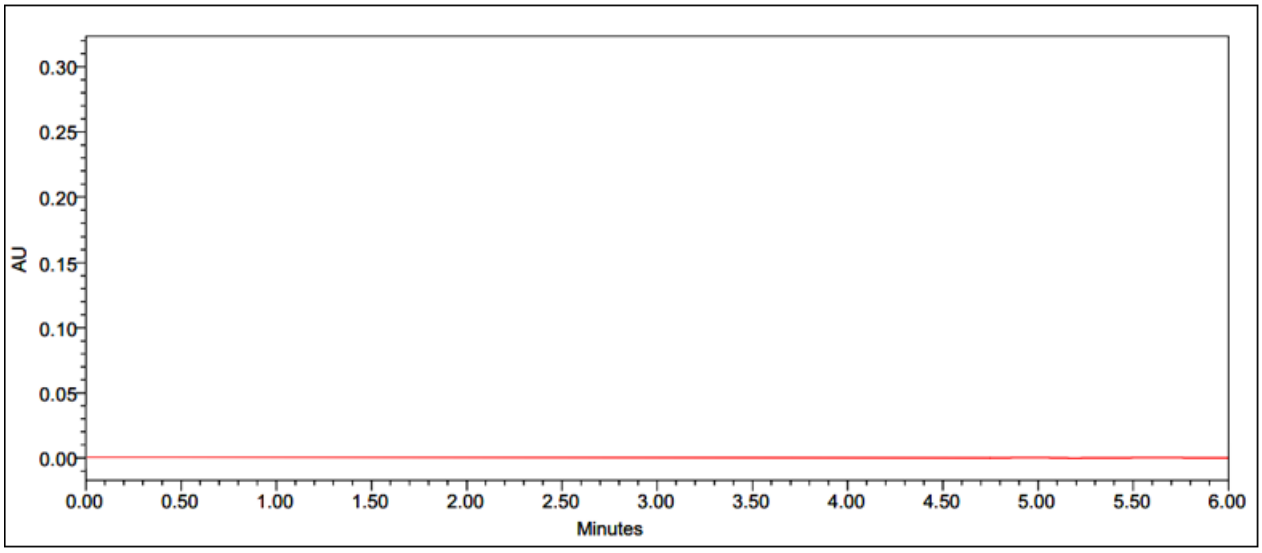

Figure 6: Chromatogram of placebo

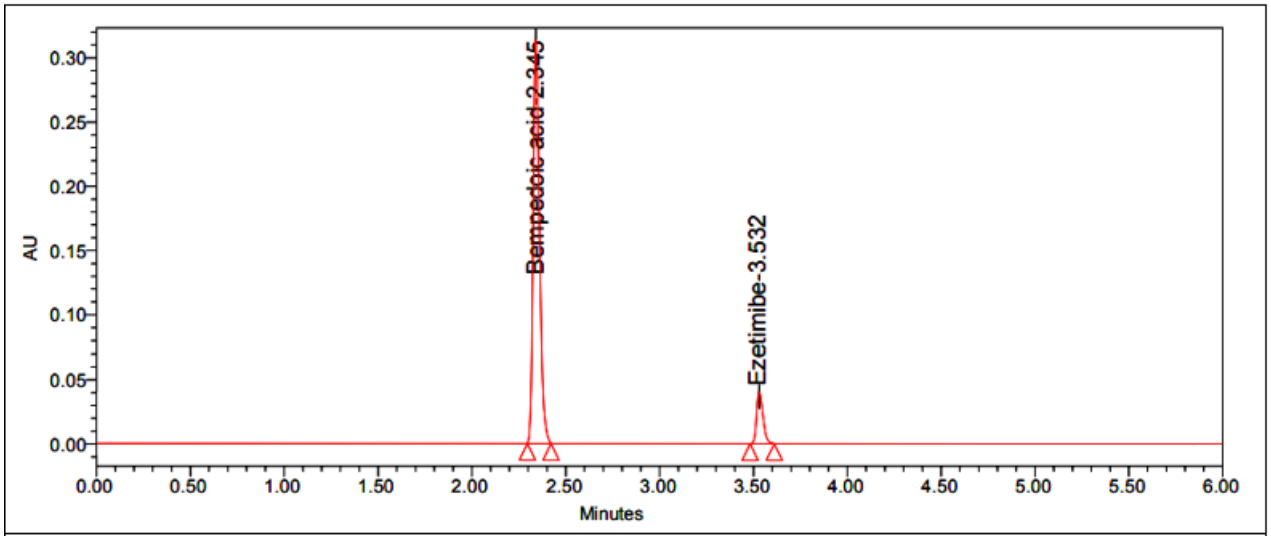

Figure 7: Chromatogram of standard

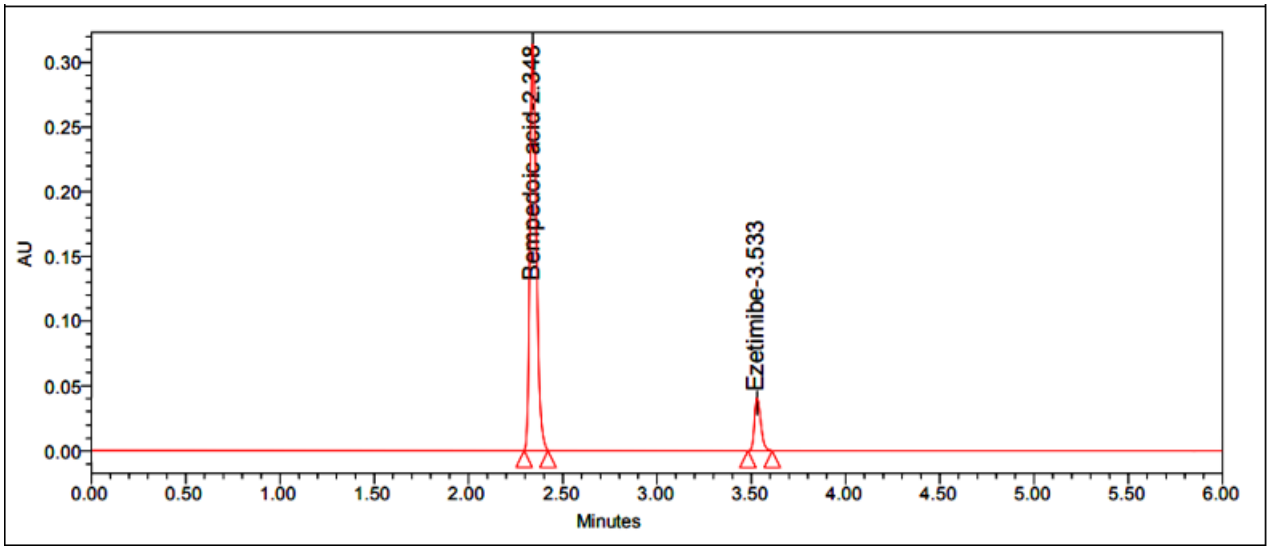

Figure 7: Chromatogram of sample

Retention times of Bempedoic acid and Ezetimibe were 2.348 min and 3.533 min respectively. The blank and placebo chromatograms was shown in **Fig-5& Fig-6** and Standard and sample chromatograms shown in **Fig-7 & Fig-8**. We did not found and interfering peaks in blank and placebo at retention times of these drugs in this method. So, this method was said to be specific.

Linearity data of Bempadoic acid Ezetimibe:

Table 5: Results of linearity for Bempedoic acid & Ezetimibe

S.NO	Bempedoic acid		Ezetimibe	
	Conc.(µg/ml)	Peak area	Conc.(µg/ml)	Peak area
1	45.00	783546	2.50	44289
2	90.00	1589630	5.00	88547
3	135.00	2301647	7.50	132648
4	180.00	3145268	10.00	176953
5	225.00	3824775	12.50	221454
6	270.00	4732154	15.00	255242
Regression equation	y = 17329.01x +157.36		y =17263.74x + 1826.64	
Slope	17329.01		17263.74	
Intercept	157.36		1826.64	
R^2	0.99967		0.99945	

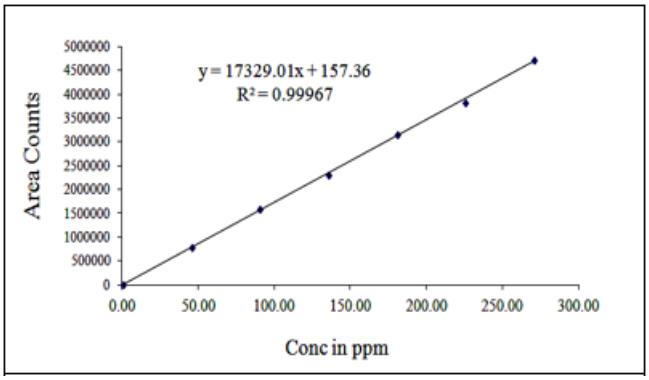

Figure 8: Calibration curve for Bempedoic acid

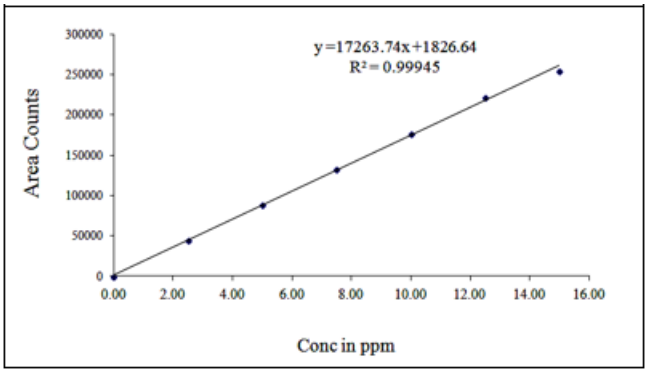

Figure 9: Calibration curve for Ezetimibe

Accuracy:

Table 6: Accuracy results of Bempedoic acid by RP-HPLC method

% Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1563967	9.00	9.00	100.0	99.4
	1548796	9.00	8.91	99.0	
	1550342	9.00	8.92	99.1	
100%	3139587	18.00	18.07	100.4	99.9
	3114072	18.00	17.92	99.6	
	3122304	18.00	17.97	99.8	
150%	4678968	27.00	26.93	99.7	99.8

	4688942	27.00	26.99	100.0	
	4673163	27.00	26.89	99.6	

Table 7: The Accuracy results for Ezetimibe by RP-HPLC method

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	87277	0.50	0.496	99.2	99.4
	87542	0.50	0.497	99.4	
	87614	0.50	0.498	99.6	
100%	175426	1.00	0.997	99.7	99.7
	176324	1.00	1.002	100.2	
	174648	1.00	0.993	99.3	
150%	262542	1.50	1.492	99.5	99.8
	263145	1.50	1.495	99.7	
	264847	1.50	1.505	100.3	

Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 99.7% and 99.6% for Bempedoic acid and Ezetimibe respectively. The summarized results was tabulated in Table No-6 &Table No -7

Precision: Precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling if same sample under the prescribed conditions .7

System Precision:

Table 8: System precision table of Bempedoic acid & Ezetimibe

	Concentration Bempedoic acid (µg/ml)	Area of Bempedoic acid	Concentration of Ezetimibe (µg/ml)	Area of Ezetimibe
1.	180	3125468	10	176001
2.	180	3132605	10	177513
3.	180	3121475	10	175170
4.	180	3132549	10	175128
5.	180	3118103	10	175546
6.	180	3135639	10	176434
Mean	3127640		175965	
S.D	7020.150		908.370	
%RSD	0.22		0.52	

From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated for two drugs. % RSD obtained as 0.22% and 0.52% respectively for Bempedoic acid and Ezetimibe. As the limit of Precision was less than “2” the system precision was passed in this method. The results was described in **Table-8.**

Robustness: Robustness is a measure of its capacity to remain unaffected by small deliberate in the chromatographic method parameters and provides an indication of its reliability.7

Table 9: Robustness results of Bempedoic acid by RP-HPLC

Parameter	Bempedoic acid					
	Condition	Retention time(min)	Peak area	Tailing	Plate count	% RSD
Flow rate Change (mL/min)	Less flow (0.9ml)	2.486	2982134	1.18	9725	0.36
	Actual (1.0ml)	2.345	3125468	1.12	9846	0.22
	More flow (1.1ml)	2.154	3263595	1.06	9958	0.25
Organic Phase change	Less Org (18:82)	2.503	2854969	1.19	9633	0.36
	Actual (20:80)	2.348	3132605	1.16	9830	0.22
	More Org (22:78)	2.088	3426398	1.10	10199	0.21

Table 10: Robustness results of Ezetimibe by RP-HPLC

Parameter	Ezetimibe						
	Condition	Retention time(min)	Peak area	Resolution	Tailing	Plate count	% RSD
Flow rate Change (mL/min)	Less flow (0.9ml)	3.716	153564	8.98	1.11	5312	0.20
	Actual (1.0ml)	3.532	176001	8.42	1.03	5431	0.52
	More flow (1.1ml)	3.491	184817	9.45	1.01	5563	0.65
Organic Phase change	Less Org (18:82)	3.853	142256	9.70	1.08	5254	0.57
	Actual (20:80)	3.533	177513	8.48	1.04	5433	0.52
	More Org (22:78)	3.222	197248	8.07	1.00	5784	0.46

This was done by small, deliberate changes in chromatographic conditions at 2 different levels and retention time of ezetimibe and bempedoic acid. The factors selected were flow rate & Organic phase changes. It was observed that there were no changes in the chromatogram which is performed that the RP-HPLC method development robust.the results is tabulated in **Table-9 & Table-10.**

4. Conclusion

Based on the results chromatographic separation method was successfully developed as per ICH guidelines for simultaneous estimation of bempadoic acid and ezetimibe .the method was costffective, accurate, precise, high resolution & short run time (6min).Method was validated s per ICH guideline and this method is suitable for estimation of bempadoic acid and ezetimibe bulk and pharmaceutical dosage form.the method was used for routine analysis in laboratories & pharmaceutical quality control.

References

[1] Drug Dictionary.com Unbridge Vol.1.1, Random house 20 September 2007.
[2] Journals Ranked by Impact: Toxicology 2014. Journal Citation Reports. Web Sciences (Sciences ed.). Thomson Reuters 2015.
[3] Mula. Anusha Reddy1, C. Parthiban, M. Sudhakar, RP-HPLC Method Development and Validation for the Simultaneous Estimation of Bempedoic Acid and Ezetimibe in Pharmaceutical Dosage Form: Ijppr. Human, 2022; Vol. 26 (1): 106-121.
[4] A Sai Datri1*, KS Nataraj2 and A Lakshmana Rao, Development and Validation of Novel Analytical Method for the Simultaneous Estimation of Bempadoic Acid and Ezetimibe in Bulk and Pharmaceutical Dosage Form by RP-UPLC: Journal of Drug and Alcohol Research, (2022) Volume 11, Issue 7.
[5] Kasa Maheshwari, Satla Shobha Rani, Validated method for the simultaneous estimation of bempedoic acid andezetimibe in bulk and tablet formulation by RP-HPLC method: WORLD J PHARM SCI, 2022; 10(09): 20-69.
[6] S krishna bhuvanagiri1, Jayendra Kumar, Development and validation of a RP - HPLC method for the simultaneous determination of bempedoic acid & ezetimibe in pure and pharmaceutical dosage form: Dogo Rangsang Research Journal, Vol-12, Issue-01, No. 03 January 2022.
[7] ICH Harmonized Tripartite Guidelines. Validation of analytical procedures: Text and methodology Q2 (R1). Geneva 2005: 13.