



## RP-HPLC Method Development and Validation for Simultaneous Estimation of Bempedoic Acid and Ezetimibe in Bulk and Pharmaceutical Dosage Form

Dr. Gampa Vijay Kumar<sup>1</sup>, Dr. D. Naresh<sup>2</sup>, S. Prasanna Bharathi<sup>3</sup>

<sup>1</sup>Professor and Head, Dept. of Pharmacy, KGR Institute of Technology and Management, Rampally, Kesara, Rangareddy, Telangana, India.

<sup>2</sup>KGR Institute of Technology and Management, Rampally, Kesara, Rangareddy, Telangana, India.

<sup>3</sup>KGR Institute of Technology and Management, Rampally, Kesara, Rangareddy, Telangana, India.

### ABSTRACT

New method was established for simultaneous estimation of Bempedoic acid and Ezetimibe by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Bempedoic acid and Ezetimibe by using ACE C18 column (4.6×150mm) 5 $\mu$ , flow rate was 1.2 ml/min, mobile phase ratio was (70:30 v/v) methanol: Phosphate buffer pH 3 (pH was adjusted with orthophosphoric acid), detection wavelength was 240nm. The instrument used was Shimadzu, model No. SPD-20MA LC+20AD, Software- LC-20 Solution. The retention times were found to be 2.733 mins and 3.415 mins. The % purity of Bempedoic acid and Ezetimibe was found to be 101.04% and 99.24% respectively. The system suitability parameters for Bempedoic acid and Ezetimibe such as theoretical plates and tailing factor were found to be 4668, 1.3 and 6089 and 1.2, the resolution was found to be 6.0. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Bempedoic acid and Ezetimibe was found in concentration range of 50 $\mu$ g-250 $\mu$ g and 5 $\mu$ g-50 $\mu$ g and correlation coefficient ( $r^2$ ) was found to be 0.999 and 0.999, % recovery was found to be 99.56% and 99.48%, %RSD for repeatability was 0.2 and 0.2, % RSD for intermediate precision was 0.2 and 0.1 respectively. The precision study was precise, robust, and repeatable. LOD value was 3.17 and 5.68, and LOQ value was 0.0172 and 0.2125 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Bempedoic acid and Ezetimibe in API and Pharmaceutical dosage form.

**Keywords:** ACE C18 column, Bempedoic acid and Ezetimibe, RP-HPLC

### ARTICLE INFO

#### \*Corresponding Author

Dr. Gampa Vijay Kumar

Professor and Head, Dept. of Pharmacy,

KGR Institute of Technology and Management,

Rampally, Kesara, Rangareddy, Telangana, India.



**ARTICLE HISTORY:** Received 09 March 2021, Accepted 29 April 2021, Available Online 12 November 2021

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**Citation:** Gampa Vijay Kumar, et al. RP-HPLC Method Development and Validation for Simultaneous Estimation of Bempedoic Acid and Ezetimibe in Bulk and Pharmaceutical Dosage Form. *Int. J. Pharm. Natural Med.*, 2021, 9(1): 08-12.

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## 1. Introduction

Bempedoic acid, sold under the brand name Nexletol among others, is a medication for the treatment of hypercholesterolemia (high blood cholesterol levels). The most common side effects include hyperuricemia (high blood levels of uric acid), pain in arms or legs, and anemia (low red blood cell counts). Bempedoic acid blocks an enzyme in the liver called adenosine triphosphate-citrate lyase, which is involved in making cholesterol.

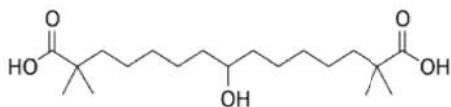


Figure 1: Bempedoic acid

Ezetimibe is a medication used to treat high blood cholesterol and certain other lipid abnormalities. Generally it is used together with dietary changes and a statin. Alone, it is less preferred than a statin. It is taken by mouth. The most commonly reported adverse events include upper respiratory tract infections, joint pain, diarrhea, and tiredness. Serious side effects may include anaphylaxis, liver problems, depression, and muscle breakdown. Use in pregnancy and breastfeeding is of unclear safety. Ezetimibe works by decreasing cholesterol absorption in the intestines.

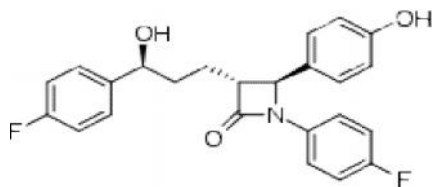


Figure 2: Ezetimibe

## 2. Materials and Methods

HPLC- Shimadzu, model no. SPD-20MA LC+20AD, Software- LC-20 Solution, U.V double beam spectrometer UV 3000+ U.V win soft ware Lab India Digital weighing balance(sensitivity 5mg) pH meter Sonicator. Bempedoic acid and Ezetimibe, Ortho phosphoric acid,  $\text{KH}_2\text{PO}_4$ ,  $\text{K}_2\text{HPO}_4$ , Acetonitrile, Methanol, Water.

### Chromatographic conditions

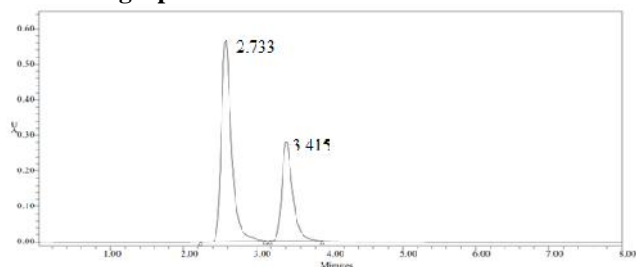


Figure 3

### Observation

The separation was good, peak shape was good, so we conclude that there is no required for reduce the retention times of peaks, so it is taken as final method.

### Trial -5 (optimized method)

#### Chromatographic conditions

Column : ACE C18 (4.6×150 mm) 5.0  $\mu\text{m}$

Column temperature : Ambient  
Wavelength : 240 nm  
Mobile phase ratio : 70:30 Methanol: Phosphate buffer  
Flow rate : 1.2 ml/min  
Auto sampler temperature : Ambient  
Injection volume : 10  $\mu\text{l}$   
Run time : 10.0 minutes

### Assay Preparation of the Bempedoic acid and Ezetimibe standard and sample solution

#### Sample solution preparation:

10 mg of Bempedoic acid and 1 mg Ezetimibe tablet powder were accurately weighed and transferred into a 10 ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent (Stock solution). Further pipette 10ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

#### Standard solution preparation

10 mg Bempedoic acid and 1 mg Ezetimibe working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

#### Method Validation

- Linearity
- Accuracy
- Precision
- Intermediate Precision
- Limit of Detection
- Limit of Quantification
- Robustness
- System suitability testing

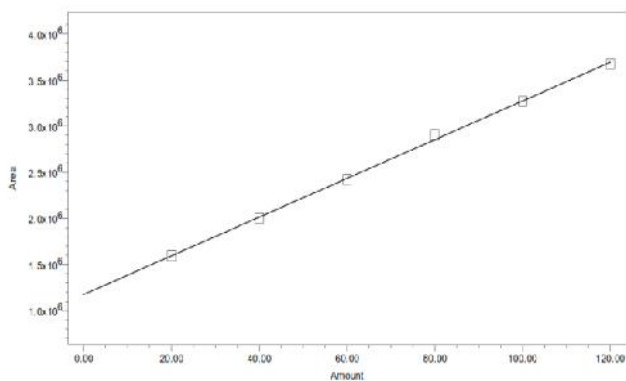
## 3. Results

Table.No.1 Linearity Results for Bempedoic acid

S.No	Linearity Level	Concentration	Area
1	I	50 ppm	471543
2	II	100 ppm	656277
3	III	150 ppm	794999
4	IV	200 ppm	946124
5	V	250 ppm	1002139
Correlation Coefficient			0.999

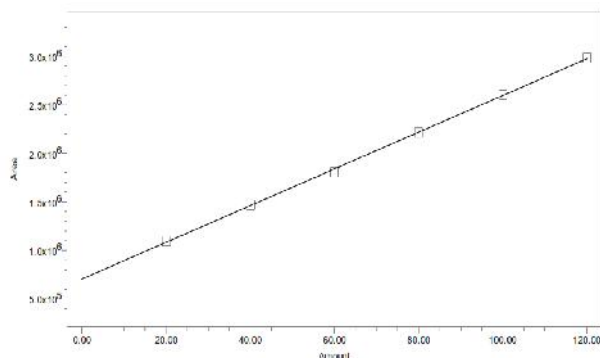
Table.No.2 Linearity Results for Ezetimibe

S.No	Linearity Level	Concentration	Area
1	I	5ppm	56472
2	II	10 ppm	73841
3	III	15ppm	92655
4	IV	20ppm	111541
5	V	25ppm	130567
Correlation Coefficient			0.999



Bempedoic acid  $r^2 = 0.999$

Fig.No.4 showing Calibration graph Bempedoic acid



Ezetimibe  $r^2 = 0.999$

Fig.No.5 Showing calibration graph for Ezetimibe

**Accuracy**

**Table.No.3 Showing accuracy results for Bempedoic acid**

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	656659	5	4.96	99.91%	99.56%
100%	1304258	10	9.98	99.18%	
150%	1854608	15	15.02	99.60%	

**Table.No.4 Showing accuracy results for Ezetimibe**

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	65312	0.5	0.99	99.53%	99.47%
100%	124509	1.0	1.05	99.38%	
150%	178517	1.5	1.495	99.52%	

**Table.No.5 Showing % RSD results for Bempedoic acid**

	Peak Name	RT	Area ( $\mu V \cdot sec$ )	Height( $\mu V$ )
1	Bempedoic acid	2.343	1302729	248455
2	Bempedoic acid	2.344	1309759	248699
3	Bempedoic acid	2.344	1302947	249526
4	Bempedoic acid	2.345	1303977	246695
5	Bempedoic acid	2.345	1303236	250012
Mean			1304529.8	
Std.Dev.			2961.1	
%RSD			0.2	

**Table.No.6. Showing %RSD results for Ezetimibe**

	Peak Name	RT	Area ( $\mu V \cdot sec$ )	Height( $\mu V$ )
1	Ezetimibe	3.285	124263	19458
2	Ezetimibe	3.287	124487	19634
3	Ezetimibe	3.287	124175	19600
4	Ezetimibe	3.288	124894	19327
5	Ezetimibe	3.288	124495	19540
Mean			124462.7	
Std.Dev.			278.6	
%RSD			0.2	

**Table.No.7 Showing results for intermediate precision of Bempedoic acid**

	Peak Name	RT	Area ( $\mu V \cdot sec$ )	Height( $\mu V$ )
1	Bempedoic acid	2.342	1305937	247870
2	Bempedoic acid	2.343	1306476	246764

3	Bempedoic acid	2.344	1304520	245696
4	Bempedoic acid	2.344	1300148	247140
5	Bempedoic acid	2.345	1308271	247280
Mean			1305070.2	
Std.Dev.			3061.8	
%RSD			0.2	

**Table.No.8 Showing results for intermediate precision of Ezetimibe**

	Peak Name	RT	Area ( $\mu\text{V}\cdot\text{sec}$ )	Height( $\mu\text{V}$ )
1	Ezetimibe	3.278	122962	19165
2	Ezetimibe	3.281	122487	19115
3	Ezetimibe	3.281	122632	19073
4	Ezetimibe	3.281	122626	19003
5	Ezetimibe	3.283	122702	19123
Mean			122681.8	
Std.Dev.			174.8	
%RSD			0.1	

**Table .No.9 Showing results for Limit of Detection**

Drug name	Standard deviation( )	Slope(s)	LOD( $\mu\text{g}$ )
Bempedoic acid	382625.50	572175863	3.17
Ezetimibe	5862.40	467579210	0.0172

**Table.No.10 Showing results for Limit of Quantitation**

Drug name	Standard deviation( )	Slope(s)	LOQ( $\mu\text{g}$ )
Bempedoic acid	381727.80	583265980	5.80
Ezetimibe	5681.30	469828490	0.212

**Table.No.11 Showing system suitability results for Bempedoic acid**

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	5339	1.4
2	<b>1</b>	<b>4668</b>	<b>1.3</b>
3	1.2	5216	1.4

**Table.No.12 Showing system suitability results for Ezetimibe**

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	7036	1.3
2	<b>1</b>	<b>6089</b>	<b>1.2</b>
3	1.2	6998	1.3

**Table.No13 Showing system suitability results for Bempedoic acid**

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	6232	1.4
2	<b>*Actual</b>	<b>4668</b>	<b>1.3</b>
3	5 % more	6387	1.4

**Table.No.14 Showing system suitability results for Ezetimibe**

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	5437	1.3
2	<b>*Actual</b>	<b>6089</b>	<b>1.2</b>
3	5 % more	4817	1.2

#### 4. Conclusion

The presented validated method is rapid, economic, simple, accurate, sensitive, robust, specific and linear. It can be used for routine analysis of bempedoic acid and ezetimibe in combination products.

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