

RESEARCH

Open Access



# Stability indicating RP-UPLC method for simultaneous quantification of bempedoic acid and ezetimibe in bulk and pharmaceutical formulations

Uma Sai Teja Yarra and Sowjanya Gummadi\*

## Abstract

**Background:** Bempedoic acid and Ezetimibe acid are used in combination for treatment of hypercholesterolemia. The current work was undertaken to develop a simple and rapid stability-indicating RP-UPLC method for the simultaneous estimation of Bempedoic acid and Ezetimibe in tablets as no such method was available. The chromatographic separation was performed with Waters Acquity C<sub>18</sub> [50 × 2.1 mm, 1.7 μ] column using methanol: acetonitrile: water [50: 30: 20, by volume] as mobile phase pumped at a flow rate 0.5 mL/min. The separated analytes were detected at 260 nm using UV detector.

**Results:** The separation of Bempedoic acid (BA) and Ezetimibe (EZ) was done at a retention time of 1.827 min. and 3.577 min. respectively. The validation and stability studies of the present method were carried out according to the ICH guidelines. The linearity of the proposed method was in the range of 30–130 μg/mL and 5–50 μg/mL for Bempedoic acid and Ezetimibe respectively. Limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.1216 μg/mL and 0.3685 μg/mL for Bempedoic acid and 0.1189 μg/mL and 0.3602 μg/mL for Ezetimibe respectively. The recovery of the method was found to be in the range of 99.89–100.31% for Bempedoic acid and 98.14–99.94% for Ezetimibe while the % RSD for both drugs in the precision and robustness study was less than 2.0. The drugs did not show any major degradants in the exposed conditions.

**Conclusion:** The developed method was found to be simple, sensitive, accurate, precise, robust, rapid and yet stability indicating. The method can be adopted for simultaneous estimation of Bempedoic acid and Ezetimibe in the pharmaceutical formulation.

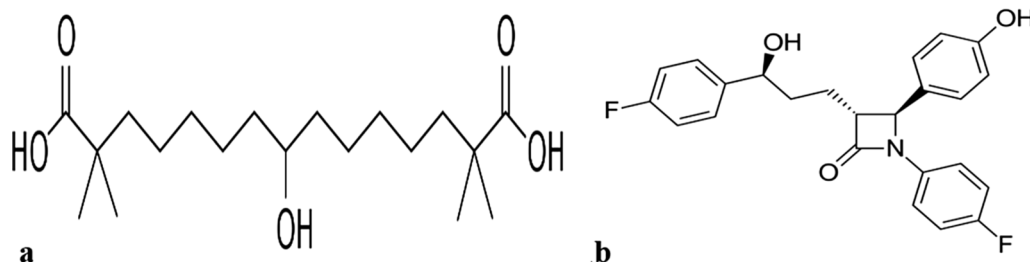
**Keywords:** UPLC, Bempedoic acid, Ezetimibe, ICH guidelines, Validation, Stability

## Background

High LDL cholesterol (LDL-C) levels represent an important risk factor for cardiovascular diseases, which can be due to genetic mutations or lifestyle factors, hypercholesterolemia can substantially reduce life quality and increase the risk of cardiovascular disease mortality.

Bempedoic acid (Fig. 1a) and Ezetimibe (Fig. 1b) acid are used in combination for treatment of hypercholesterolemia and ASCVD by reducing lipid parameters and attenuating hsCRP levels [1]. Ezetimibe is an azetidinone derivative, it prevents absorption of cholesterol by blocking the Niemann-Pick C1-like 1 (NPC1L1) protein on epithelial cells of gastrointestinal tract, and in hepatocytes. It also blocks aminopeptidase N and interfere with caveolin 1-annexin A2 complex which is in cholesterol trafficking. This decreases hepatic cholesterol and cuts the total cholesterol level in the blood [2]. Bempedoic

\*Correspondence: tatinenijishnu@gmail.com  
Department of Pharmaceutical Analysis, GITAM Institute of Pharmacy,  
GITAM (Deemed To Be University), Visakhapatnam, Andhra Pradesh  
530045, India



**Fig. 1** **a** Chemical structure of Bempedoic acid. **b** Chemical structure of Ezetimibe

acid is a prodrug, the activated form inhibits adenosine triphosphate citrate lyase (ACL) inhibitor which is responsible for biosynthesis of cholesterol in liver [3].

A literature survey conveyed that, limited methods are available for simultaneous estimation of Bempedoic acid and Ezetimibe. A few articles reported spectrophotometric techniques for estimation of Ezetimibe alone and with other drugs [4–15]. Few HPLC methods were reported for the determination of Ezetimibe alone and in combination with other drugs [16–20]. One RP-HPLC method was reported for simultaneous estimation of Bempedoic acid Ezetimibe [21]. Few LC-MS methods were reported for determination of Ezetimibe alone and in combination with other drugs [22–25]. One LC-MS method was reported for estimation of Bempedoic acid in human plasma and urine [26]. To our knowledge, this is the first report of a stability indicating RP-UPLC method for simultaneous estimation of Bempedoic acid and Ezetimibe in bulk and pharmaceutical formulations. The present work aims to develop stability indicating reverse-phase ultra-performance liquid chromatography (RP-UPLC) method for simultaneous estimation of Bempedoic acid and Ezetimibe in bulk and pharmaceutical formulations as per ICH guidelines.

## Methods

### Instrument

Acquity UPLC system equipped with quaternary pumps, Acquity TUV detector, and autosampler integrated.

### Pure samples

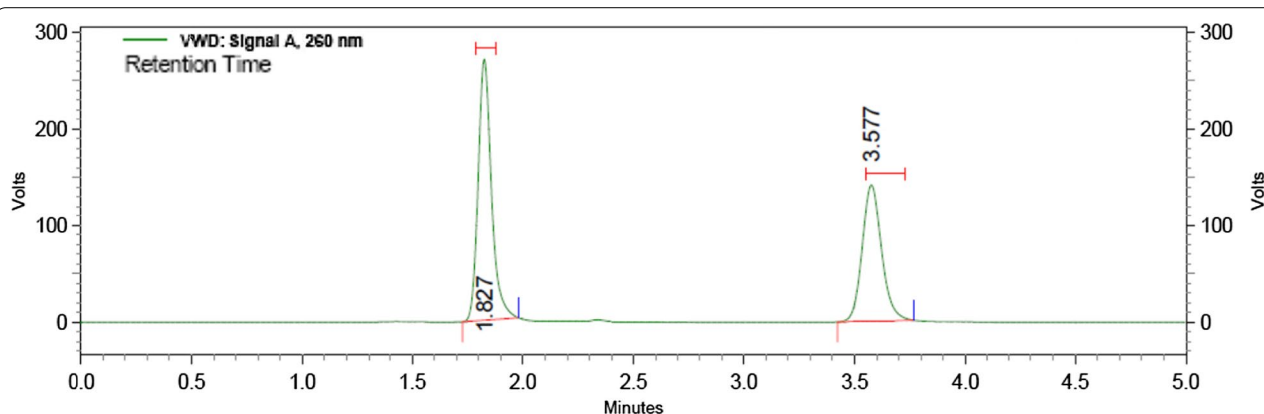
Standard samples of Bempedoic acid (99.5% w/w) and Ezetimibe (98% w/w) were procured from Madras Pharmaceuticals, Chennai.

### Formulation

Marketed formulation (tablets) of Bempedoic acid and Ezetimibe, NEXLIZET (Bempedoic acid 180 mg and Ezetimibe 10 mg) was purchased online.

### Chemicals and reagents

Acetonitrile, methanol, and water (HPLC grade) were procured from Merck. Sodium hydroxide, hydrochloric acid, hydrogen peroxide (AR grade) obtained from Rankem were utilised in the study.



**Fig. 2** Chromatogram of Bempedoic acid and Ezetimibe (Standard)

### Preparation of mobile phase/Diluent

The mobile phase consists of methanol, acetonitrile and water in the ratio 50: 30: 20, by volume. The solvent is filtered through 0.45  $\mu$  membrane filter and sonicated before use.

### Preparation of stock and standard solutions

Stock solutions of Bempedoic acid and Ezetimibe were prepared by dissolving 180 mg of Bempedoic acid and 10 mg of Ezetimibe in about 70 mL diluent, sonicated and further made up to volume in a 200 mL volumetric flask with the diluent. Pipetted 1 mL of the above solution and transferred into a 10 mL volumetric flask and made up to volume with diluent (standard solution). The stock solution was diluted as per requirement.

### Preparation of sample solution

Twenty tablets (NEXLIZET) were weighed and crushed. Quantity of powder equivalent to 180 mg of Bempedoic acid and 10 mg of Ezetimibe was taken in a 200 mL volumetric flask, 70 mL of diluent was added, sonicated it for 30 min with intermittent shaking and the volume was made up with diluent. This solution was filtered through a 0.45  $\mu$ m membrane filter and further diluted.

### Determination of working wavelength

10  $\mu$ g/mL solutions of both the components were prepared separately and scanned in the UV wavelength region of 200 nm to 400 nm. An overlaying spectrum obtained was used to determine the working wavelength (isoabsorptive point).

### Method development

Various system suitability parameters were analysed to optimize chromatographic conditions to achieve symmetric peak shape and better resolution of drugs. Optimization of mobile phase was done by combining

**Table 1** Optimized chromatographic conditions

Parameter	Optimized condition
Mobile phase	Methanol: acetonitrile: water (50: 30: 20, by volume)
Column	Waters Acquity C <sub>18</sub> (50 mm $\times$ 2.1 mm ID, 1.8 $\mu$ m)
Flow rate	0.5 mL/min
Column temperature	25 $\pm$ 2° C
Wavelength	260.00 nm (UV Detector)
Injection volume	10 $\mu$ L
Elution	Isocratic mode
Run time	5 min
Retention time	
Bempedoic acid	1.827 min
Ezetimibe	3.577 min

various suitable solvents in different ratios and finalized that methanol: acetonitrile: water (50:30:20%, v/v) as mobile phase at a flow rate of 0.5 mL/min. gave the most symmetric and resolved peaks detected at 260 nm. Optimized chromatogram is shown in Fig. 2 and chromatographic conditions are given in Table 1.

### Method validation

The validation of the proposed method was carried out as per ICH Q2 (R1) guidelines [27] and the validation parameters include system suitability, linearity, precision, accuracy, robustness, and specificity.

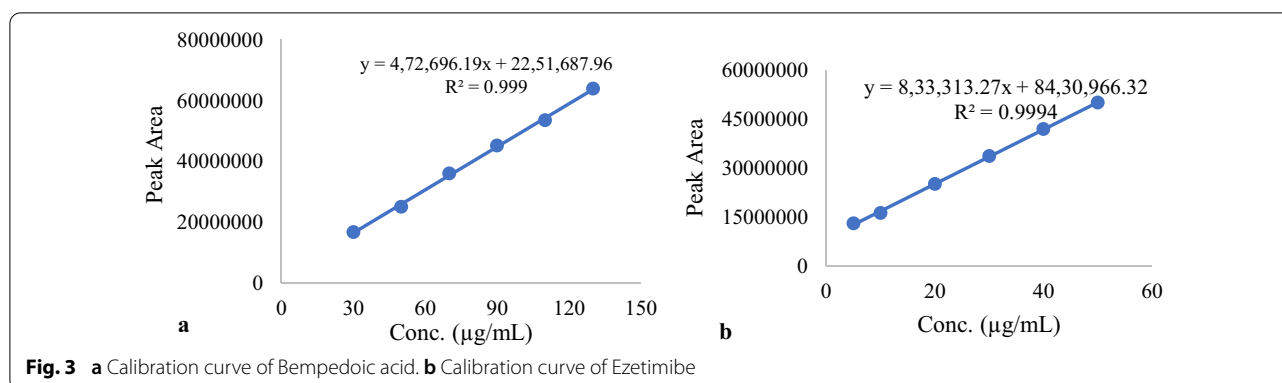
### Linearity

Linearity was determined by preparing aliquots at six different levels of calibration curve over the concentration range of 30–130  $\mu$ g/mL for Bempedoic acid and 5–50  $\mu$ g/mL for Ezetimibe. Solutions for calibration curve were prepared by serial dilutions of stock solution to obtain concentrations of 30, 50, 70, 90, 110, 130  $\mu$ g/mL of Bempedoic acid and 5, 10, 20, 30, 40, 50  $\mu$ g/mL of Ezetimibe. The solutions were analysed in triplicate. The correlation coefficient with the regression equation was determined from the calibration curve.

**Table 2** Linearity data

S. No	Bempedoic acid		Ezetimibe	
	Concentration ( $\mu$ g/mL)	*Area $\pm$ SD	Concentration ( $\mu$ g/mL)	*Area $\pm$ SD
1	30	16,674,907 $\pm$ 12,562	5	13,022,190 $\pm$ 7369
2	50	25,055,621 $\pm$ 18,075	10	16,194,209 $\pm$ 10,207
3	70	36,009,142 $\pm$ 20,674	20	25,035,716 $\pm$ 18,128
4	90	45,211,483 $\pm$ 44,895	30	33,633,340 $\pm$ 24,323
5	110	53,519,670 $\pm$ 34,322	40	41,910,697 $\pm$ 37,208
6	130	63,933,476 $\pm$ 49,587	50	49,953,204 $\pm$ 48,521

\*Mean of three determinations; SD = Standard Deviation

**Table 3** Results for intraday precision

Bempedoic acid			Ezetimibe		
Conc.	*Assay (% taken w/w) ± SD	% RSD	Concentration taken (µg/mL)	*Assay (% w/w) ± SD	% RSD
90	100.59 ± 0.1172	0.12	5	99.64 ± 0.3758	0.38

\* Mean of six determinations; SD = Standard Deviation; RSD = Relative Standard Deviation

#### Precision

The precision of the method was studied in terms of intra-day precision and inter-day precision. Each phase of precision was investigated by six replicates of injections of sample solutions of Bempedoic acid (90 µg/mL) and Ezetimibe (5 µg/mL). Precision was expressed in terms of relative standard deviation.

#### Accuracy

Accuracy was expressed in terms of recovery. It was determined by spiking a known amount of standard Bempedoic acid and Ezetimibe to pre-analysed samples at three different levels such as 50%, 100%, and 150%, and the percentage recovery was determined.

#### Robustness

The robustness of the method was determined by bringing deliberate variations in the method parameters such as flow rate, temperature and detection wavelength.

#### Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) were based on the standard deviation of the response and slope of the constructed calibration curve

**Table 4** Results for interday precision

Day	Bempedoic acid			Ezetimibe		
	Conc. taken (µg/mL)	*Assay (% w/w) ± SD	% RSD	Conc. taken (µg/mL)	*Assay (% w/w) ± SD	% RSD
1	90	100.52 ± 0.1518	0.15	5	99.58 ± 0.2725	0.27
2		100.78 ± 0.1734	0.17		99.31 ± 0.1988	0.20
3		100.72 ± 0.1353	0.13		99.40 ± 0.3483	0.35

\*Mean of six determinations; SD = Standard Deviation; RSD = Relative Standard Deviation

**Table 5** Results for accuracy

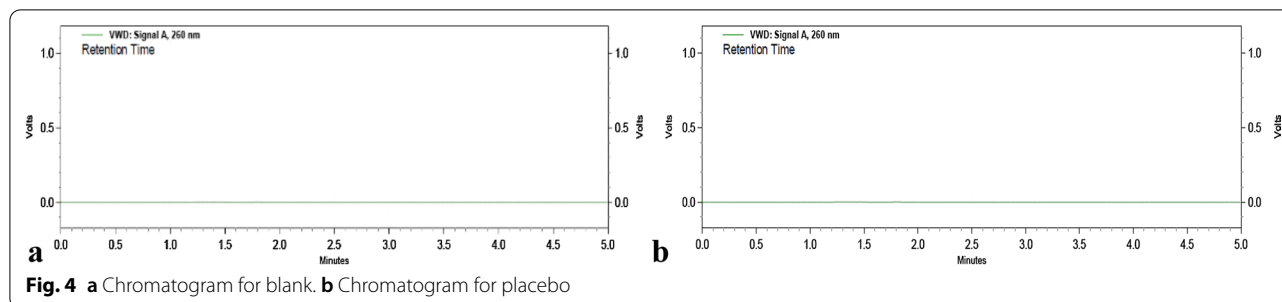
Level (%)	Tablet (µg/mL)		Standard (µg/mL)		*Conc. Found (µg/mL)		*Recovery (%) ± SD	
	BA	EZ	BA	EZ	BA	EZ	BA	EZ
50	50	10	25	5	75.07	14.99	100.31 ± 0.2902	99.94 ± 0.3938
100			50	10	100.09	19.98	100.17 ± 0.2419	99.81 ± 0.2114
150			75	15	124.58	24.87	99.89 ± 0.1993	98.14 ± 0.1116

\*Mean of three determinations; SD = Standard Deviation

**Table 6** Results for robustness study

Chromatographic changes	*Peak area			Tailing Factor		Theoretical Plates		*Assay (% w/w) ± SD, % RSD	
	BA	EZ		BA	EZ	BA	EZ	BA	EZ
Flow rate ± 0.1 (mL/min.)	0.4	45,118,072	12,608,841	1.184	1.407	3588	9450	100.59 ± 0.1799, 0.18	99.91 ± 0.3205, 0.32
	0.5	44,965,324	12,588,406	1.172	1.166	3041	7911		
	0.6	45,051,231	12,583,731	1.144	1.123	2455	7299		
Temperature ± 5 (°C)	20	45,036,493	12,584,203	1.155	1.163	3481	9827	100.51 ± 0.2184, 0.22	99.88 ± 0.4116, 0.41
	25	44,906,494	12,580,784	1.181	1.173	3112	8067		
	30	45,086,495	12,612,046	1.108	1.122	2484	6857		
Detectionwavelength ± 2 (nm)	258	44,934,914	12,597,848	1.153	1.141	3585	9708	100.53 ± 0.1820, 0.18	99.60 ± 0.3573, 0.34
	260	45,040,389	12,569,963	1.158	1.174	3088	8102		
	262	45,085,863	12,574,861	1.134	1.128	2548	6989		

\*Mean of three determinations; SD = Standard Deviation; RSD = Relative Standard Deviation

**Table 7** Results for forced degradation studies

Degradation study	*Assay (%w/w)		% Degradation		Tailing factor		Theoretical plates	
	BA	EZ	BA	EZ	BA	EZ	BA	EZ
Control	99.90	99.90	-	-	1.156	1.137	3679	7935
Acidic	99.17	99.31	0.73	0.59	1.183	1.277	4129	8367
Alkaline	99.26	99.42	0.64	0.48	1.294	1.248	3934	8753
Peroxide	99.48	99.30	0.46	0.59	1.150	1.342	4352	8243
Thermal	99.05	99.20	0.85	0.69	1.184	1.069	4253	7961
Photolytic	99.62	99.67	0.28	0.23	1.215	1.283	4472	8452

\*Mean of three determinations; BA = Bempedoic acid; EZ = Ezetimibe

( $n=3$ ). Sensitivity of the method was established with respect to LOD and LOQ for Ezetimibe and Bempedoic acid by slope method. Limit of detection and limit of quantitation was calculated using  $LOD=3.3\sigma/S$  and  $LOQ=10\sigma/S$ .

Where,  $\sigma$  is the standard deviation of the response and  $S$  is the slope of the standard curve.

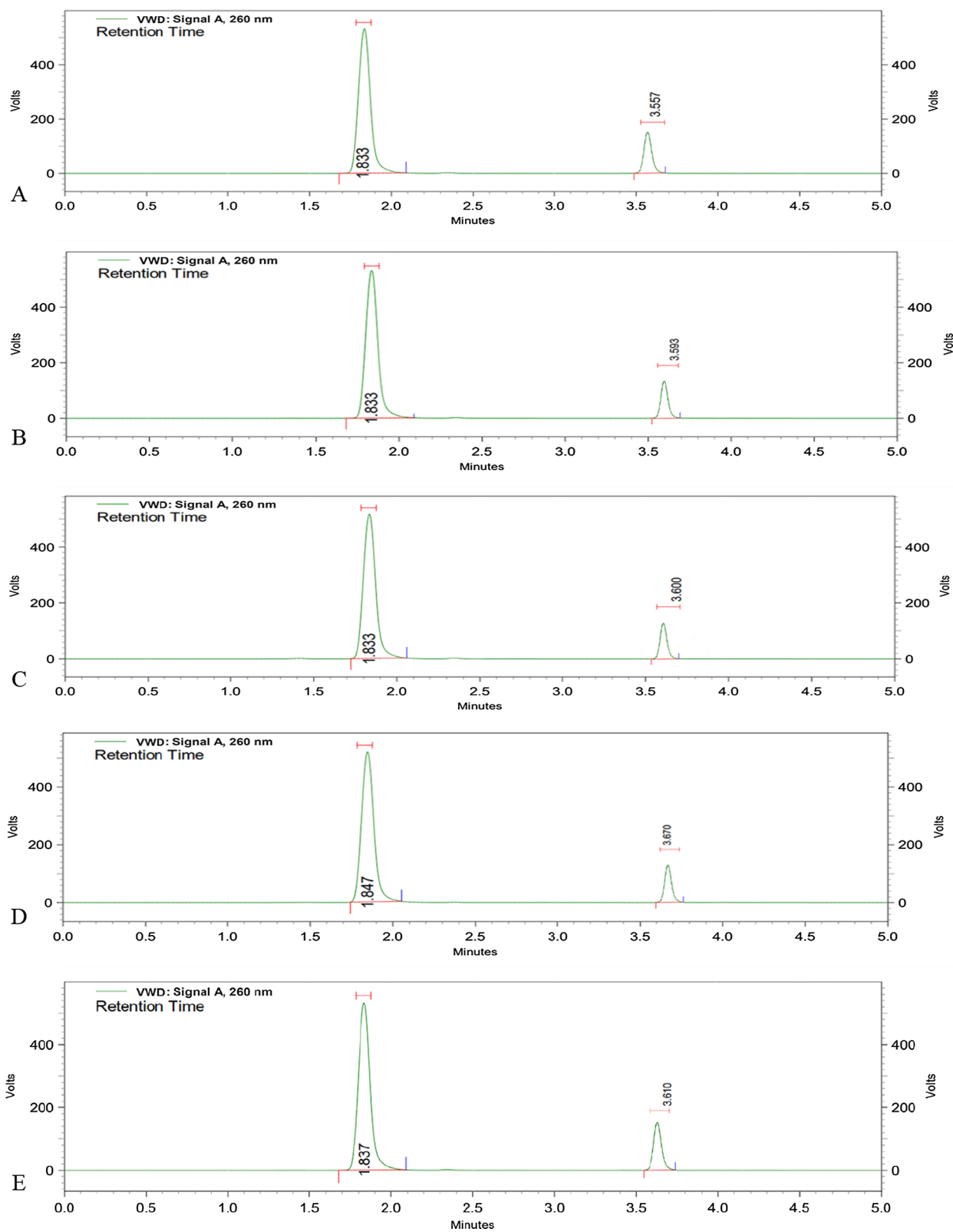
### Specificity

Specificity of the method was studied in the presence of excipients and degradants. Blank and placebo solutions

were injected into the UPLC system and observed for any interfering peaks.

### Forced degradation studies

Forced degradation studies were conducted to assess the stability-indicating property of the proposed method [28, 29]. The standard was exposed to various degradation conditions like acidic (0.1 N HCl, 60  $^{\circ}$ C, 1 h), alkaline (0.1 N NaOH, 60  $^{\circ}$ C, 1 h), peroxide (20%  $H_2O_2$ ), thermal (105  $^{\circ}$ C, 6 h) and photolytic (254 nm, 1 day) stress. The exposed solutions were injected in replicates and the system suitability parameters were studied along the percentage degradations.



**Fig. 5** Chromatograms of Bempedoic acid (90 µg/mL) and Ezetimibe (5 µg/mL) stressed samples under. **a** Acidic, **b** Basic, **c** Peroxide, **d** Thermal and **e** Photolytic degradations

**Table 8** Results for assay

Marketed formulation (tablet)	Drug	Label claim (mg)	Amount found (mg)	*Assay (% w/w) $\pm$ SD
NEXLIZET™	Bempedoic acid	180	180.92	100.51
	Ezetimibe	10	9.91	99.11

### Assay

Assay of the marketed formulation (NEXLIZET, Bempedoic acid 180 mg and Ezetimibe 10 mg) was performed by using the standard and sample solutions. 1 mL of the clear and filtered sample solution as discussed above was pipetted in to a 10 mL volumetric flask and made up to volume with diluent. The resulting solution was used to record the chromatogram.

### Results

Several trials were performed using a combination of mobile phases some of which include phosphate buffer: acetonitrile (60:40), phosphate buffer, pH 4.0: methanol: triethylamine (45:35:15), methanol: water (70:30), acetonitrile: water (60:40) and methanol: acetonitrile: water (50:30:20). A variety of stationary phases such as Phenomenex, Zorbax and Waters Acquity columns of varying dimensions were used. All these combinations resulted in chromatographic peaks with different system suitability parameters from which the optimized condition has been chosen as methanol: acetonitrile: water (50:30:20) and a Waters Acquity C<sub>18</sub> column (50  $\times$  2.1 mm ID, 1.8  $\mu$ m) at a flow rate of 0.5 mL/min.

### Linearity

Bempedoic acid and Ezetimibe obeyed linearity over the concentration range of 30–130  $\mu$ g/mL and 5–50  $\mu$ g/mL respectively. The regression equation and correlation coefficient was found to be

$y = 472,696.19x + 2,251,687.96$  (0.999) for Bempedoic acid and  $y = 833,313.27x + 8,430,966.32$  (0.9994) for Ezetimibe. Linearity data of Bempedoic acid and Ezetimibe is listed in Table 2. Calibration curves for Bempedoic acid and Ezetimibe are provided in Fig. 3a and b.

### Precision

For intraday precision % RSD was calculated and it was 0.12% for Bempedoic acid and 0.38% for Ezetimibe, while % RSD value for interday precision was obtained in the range of 0.13–0.17% for Bempedoic acid and 0.20%–0.35% for ezetimibe. All the values of % RSD were within the limits indicating a precise method. Results of intraday and interday precision are given in Tables 3 and 4, respectively.

### Accuracy

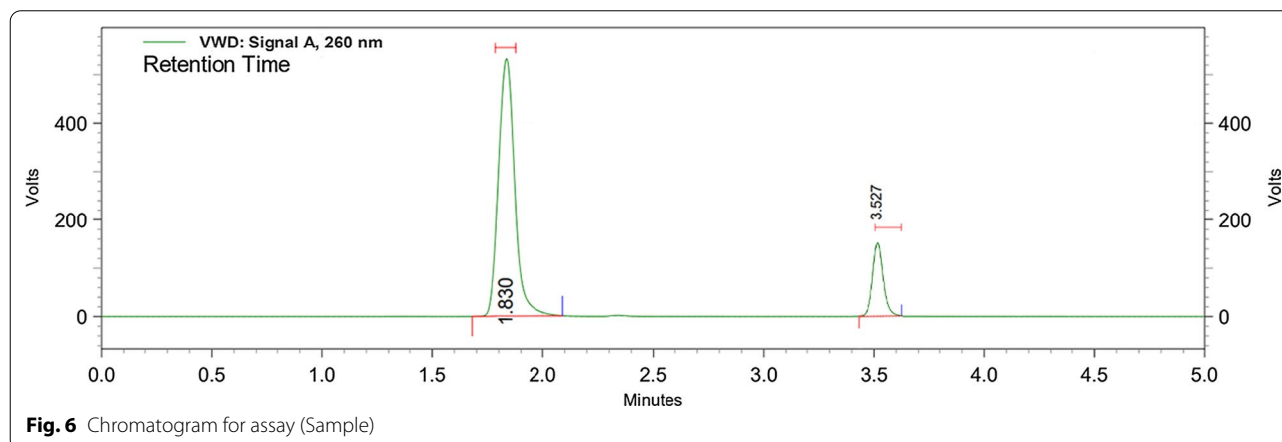
The accuracy of the proposed method was assessed at three levels. The % recovery of Bempedoic acid was found to be in the range of 99.89%–100.31% and the % recovery of Ezetimibe was found to be in the range of 98.14%–99.94% indicating the method is accurate. The accuracy results are listed in Table 5 and the data for robustness is given in Table 6.

### Limit of detection (LOD) and limit of quantification (LOQ)

Limit of detection for Bempedoic acid and Ezetimibe was found to be 0.1216  $\mu$ g/mL and 0.1189  $\mu$ g/mL respectively. Limit of quantification for Bempedoic acid and Ezetimibe was found to be 0.3685  $\mu$ g/mL and 0.3602  $\mu$ g/mL respectively.

### Specificity

No peaks were found in the diluent or placebo chromatograms at the retention times of Bempedoic acid and Ezetimibe indicating the specificity of the method in the presence of excipients as given in Fig. 4a and 4b.

**Fig. 6** Chromatogram for assay (Sample)



### Forced degradation studies

Degradation studies has revealed that no major degradation of both Bempedoic acid and Ezetimibe occurred in the exposed acidic, alkaline, peroxide, thermal and photolytic conditions. The results regarding the forced degradation studies are provided in Table 7 and chromatograms are given in Fig. 5A–E.

### Assay

The percentage assay of the sample was calculated by comparing the areas of standard and sample peaks and the results for assay of Bempedoic acid and Ezetimibe are listed in the Table 8. A representative chromatogram for assay is presented in Fig. 6.

### Discussion

An attempt has been made to develop a simple UPLC method for the simultaneous quantification of Bempedoic acid and Ezetimibe. The method optimization was started considering the solubility of the drugs in various aqueous and organic phases. A methanol: acetonitrile: water (50:30:20%, v/v) system on a Waters Acquity C<sub>18</sub> (50 mm × 2.1 mm ID, 1.8 μm) column gave the best possible chromatographic peaks. Short and small particle size columns enable faster retention and well resolved peaks within a short run time (<5 min.). The retention of both the drugs was found to be less than 4 min with a good resolution. The method validation and stability studies were carried out according to ICH guidelines. Validation parameters like precision and accuracy were within acceptable limits, indicating that the method is precise and accurate. LOD and LOQ results were less than 0.4 μg/mL and proves the sensitivity of the method. Robustness study of the proposed method illustrated that small and deliberate changes did not affect the results of system suitability parameters. Degradation studies confirmed that there was very less percentage (<1%) of degradation in all the conditions and did not affect the retention time of both the drugs. All the system suitability parameters remained unaffected in the forced degradation study.

### Conclusion

The current UPLC method was developed and validated for the simultaneous estimation of Bempedoic acid and Ezetimibe in tablets. The stability indicating nature of the UPLC method was established from the degradation studies as all the peaks were resolved within the limits of system suitability. The proposed method is simple, rapid, economical, accurate, precise and yet stability indicating. This UPLC method be successfully employed for routine

simultaneous analysis of Bempedoic acid and Ezetimibe in pharmaceutical formulations.

### Abbreviations

LDL-C: Low density lipoprotein cholesterol; ASCVD: Atherosclerotic cardiovascular disease; hs-CRP: High-sensitivity C-reactive protein; NPC1L1: Niemann-Pick C1-like 1; ACL: Adenosine triphosphate citrate lyase; RP-UPLC: Reverse phase ultra-performance liquid chromatography; TUV: Tunable ultraviolet; ICH: International council for harmonization; LOD: Limit of detection; LOQ: Limit of quantification; BA: Bempedoic acid; EZ: Ezetimibe; HPLC: High-performance liquid chromatography; LC-MS: Liquid chromatography-mass spectrometry.

### Acknowledgements

The authors are grateful to M/S GITAM Institute of Pharmacy, GITAM Deemed to be University, Visakhapatnam, India for providing the research facilities.

### Authors' contributions

UY was experimental in performing the study and generating the required data. SG was involved in the preparation of manuscript. The authors read and approved the final manuscript.

### Funding

Not applicable.

### Availability of data and material

All the required data and material are available on request.

### Declarations

#### Ethics approval and consent to participate

Not applicable.

#### Consent for publication

Not applicable.

#### Competing interests

The authors have no conflict of interest regarding the publication of this paper.

Received: 26 July 2021 Accepted: 7 October 2021

Published online: 16 October 2021

### References

1. Akshaya SB, Nadya OAM, Cain CTC, Jamal R (2021) Bempedoic acid and ezetimibe for the treatment of hypercholesterolemia: a systematic review and meta-analysis of randomized phase II/III trials. *Clin Drug Investig* 41:19–28. <https://doi.org/10.1007/s40261-020-00989-1>
2. Binh APP, Thomas DD, Peter PT (2012) Ezetimibe therapy: mechanism of action and clinical update. *Vasc Health Risk Manag* 8:415–427. <https://doi.org/10.2147/VHRM.S33664>
3. Ozlem B, Christie MB (2016) Bempedoic acid (ETC-1002): an investigational inhibitor of ATP citrate lyase. *Curr Atheroscler Rep* 18:61. <https://doi.org/10.1007/s11883-016-0611-4>
4. Khemchand G, Indrajeet, (2015) Development of UV spectrophotometric method for the estimation of ezetimibe from tablet formulation. *Int J Chem Sci* 13:1051–1056
5. Metreyi S, Deepali VM, Mahadik M, Kadam SS, Dhaneshwar SR (2008) UV and three derivative spectrophotometric methods for determination of ezetimibe in tablet formulation. *Indian J Pharm Sci* 70:258–260. <https://doi.org/10.4103/0250-474X.41471>
6. Narasimharaju BC, Devalarao G, Ramanjaneyulu S (2008) Spectrophotometric method for the determination of ezetimibe in pharmaceutical formulations. *Biomed Pharmacol J* 1:413–416
7. Kabra RP, Kadam SC, Mane VB, Kadam SS, Mamde CG (2014) Simple novel UV-spectroscopic method for estimation of ezetimibe in tablet dosage form. *Am. J Pharm Health Res* 2(9).



8. Rajput SJ, Raj HA (2007) Simultaneous spectroscopic estimation of ezetimibe and simvastatin in tablet dosage forms. *Indian J Pharm Sci* 69:759–762. <https://doi.org/10.4103/0250-474X.39429>
9. Namratha S, Uma Rajeswari B, Swathi B, Arunk S, Ratnakar N (2017) UV spectrophotometric method development and validation of ezetimibe and simvastatin in bulk and pharmaceutical dosage form. *Int J Pharma Chem Res* 3:581–585
10. Varsha BM, Surekha B, Nita K (2011) Development of UV spectrophotometric method for the simultaneous estimation of simvastatin and ezetimibe in tablet dosage form by simultaneous equation and absorbance ratio method. *Int J Pharm Tech Res* 3:1459–1466
11. Seema MD, Manjusha PY (2015) First derivative UV-spectrophotometric method for simultaneous determination of simvastatin and ezetimibe in tablet dosage form. *Pharm Lett* 7:124–128
12. Anuradha G, Vishal SD (2010) Simultaneous UV-spectrophotometric estimation of rosuvastatin and ezetimibe in their combined dosage forms. *Int J Pharm Pharm Sci* 2:131–138
13. Rajput SJ, Raj HA (2009) Simultaneous estimation of ezetimibe and rosuvastatin in drug mixture by first derivative spectroscopic method. *Int J Chem Sci* 7:2354–2362
14. Sonawane SS, Shirkhedkar AA, Fursule RA, Surana SJ (2007) Simultaneous spectrophotometric estimation of atorvastatin calcium and ezetimibe in tablets. *Indian J Pharm Sci* 69:683–684. <https://doi.org/10.4103/0250-474X.38477>
15. Manish K, Khandare MM, Kamble KG, Kamble KG (2011) UV Spectrophotometric estimation of ezetimibe and fenofibrate in bulk drug and dosage form using simultaneous equation method. *Int J Chem Technol Res* 3:749–754
16. Sistla R, Tata VSSK, Kashyap YV, Chandrasekar D, Diwan PV (2005) Development and validation of a reversed-phase HPLC method for the determination of ezetimibe in pharmaceutical dosage forms. *J Pharm Biomed Anal* 39:517–522
17. Akmar SK, Lata K, Asha T, Sumitra J, Deshpande AD (2007) Reverse phase high performance liquid chromatography method for estimation of ezetimibe in bulk and pharmaceutical formulations. *Indian J Pharm Sci* 69:695–697. <https://doi.org/10.4103/0250-474X.38482>
18. Saroj KR, Atrna Bhaskar A, Jhansi D (2015) RP-HPLC method development and validation for the simultaneous estimation of atorvastatin and ezetimibe in pharmaceutical dosage form. *Asian J Pharm Clin Res* 8:178–181
19. Mohammed IB, Vanitha PK, Krishna MG (2013) RP-HPLC method for simultaneous estimation of rosuvastatin and ezetimibe from their combination tablet dosage form. *Int J Chem Anal Sci* 4:205–209
20. Siva Kumar R, Santhanakrishnan MR, Kumar PN, Venkatanarayanan R (2008) Simultaneous RP-HPLC method for estimation of ezetimibe and eimvastatin in bulk and dosage forms. *Res J Pharm and Tech* 1:211–214
21. Fatima HB, Ayesha BK (2020) A new validated RP-HPLC method for the analysis of bempedoic acid and ezetimibe in bulk drug samples. *Int J of Pharmacy Anal Res* 9:248–252
22. Hossein D, Mehrdad H (2013) A rapid and sensitive LC–MS method for determination of ezetimibe concentration in human plasma: application to a bioequivalence study. *Chromatographia* 76:1667–1675
23. Jung-Woo B, Chang-Ik C, Sang-Hun P, Choon-Gon J, Seok-Yong L (2012) Analytical LC-MS/MS method for ezetimibe and its application for pharmacokinetic study. *J Liq Chromatogr Relat Technol* 35:141–152. <https://doi.org/10.1080/10826076.2011.597065>
24. El-Bagary RI, Ehab FE, Zeinab AES, Ahmed MK (2014) LC-MS-MS simultaneous determination of atorvastatin and ezetimibe in human plasma. *J Chromatogr Sci* 52:773–780. <https://doi.org/10.1093/chromsci/bmt109>
25. Abhaysingh B, Mallika S, Priyanka AS, Pranav SS (2018) Simultaneous quantitation of rosuvastatin and ezetimibe in human plasma by LC-MS/MS: pharmacokinetic study of fixed-dose formulation and separate tablets. *Biomed Chromatogr* 32:e4291. <https://doi.org/10.1002/bmc.4291>
26. Brian JE, Karen P, Cameron B, Clay TC, Ronald S (2020) Measurement of bempedoic acid and its keto metabolite in human plasma and urine using solid phase extraction and electrospray LC-MS/MS. *J Chromatogr B Analyt Technol Biomed Life Sci* 1154:122291. <https://doi.org/10.1016/j.jchromb.2020.122291>
27. Guidelines ICH (2005) Validation of analytical procedures: text and methodology. International Council for Harmonisation, Geneva
28. ICH Harmonized Tripartite Guideline (2003) Stability testing of new drug substances and new drug products Q1A (R2); International Council for Harmonization, IFPMA, Geneva, Switzerland.
29. ICH Harmonized Tripartite Guideline (2003) Stability testing: photo stability testing of new drug substances and new drug products Q1B; International Council for Harmonization, IFPMA, Geneva, Switzerland.

## Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**Submit your manuscript to a SpringerOpen<sup>®</sup> journal and benefit from:**

- Convenient online submission
- Rigorous peer review
- Open access: articles freely available online
- High visibility within the field
- Retaining the copyright to your article

---

Submit your next manuscript at ► [springeropen.com](https://www.springeropen.com)