Characterization of novel stress degradation products of Bempedoic acid and Ezetimibe using UPLC–MS/MS: development and validation of stability-indicating UPLC method

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Abstract

Background: A receptive and easily comprehended technique was evolved for simultaneous assessment of Bempedoic acid and Ezetimibe and its impurities characterized by UPLC–MS/MS.

Results: This technique involves chromatographic separation with a C18 column of water symmetry (150 mm × 4.6 mm, 3.5 µm). A mobile phase of 0.1% OPA (orthophosphoric acid) and acetonitrile in 50:50 v/v with 1 mL/min flow rate and ambient temperature was used. UV observation was taken at 230 nm. The recoveries, linearity, and quantification limits were found to be within the acceptable limit.

Conclusions: This technique was successfully tested with UPLC–MS to confirm the chemical structures of newly formed degradation products of Bempedoic acid and Ezetimibe and stress studies as per ICH Q2 (R1) guidelines.

Keywords: Bempedoic acid, Ezetimibe, Validation, Characterization, UPLC, UPLC–MS

Background

Bempedoic acid is a pharmaceutical medicine utilized for the therapy of high cholesterol (high blood cholesterol levels) [1–3]. Bempedoic acid is approved for the treatment of hypercholesterolemia and therefore the highest tolerated statin therapy in adults with heterozygous [4], with hypercholesterolemia [5, 6], or with established atherosclerotic cardiovascular disorder [7, 8], who need additional lowering of LDL cholesterol [9, 10]. The most common adverse effects in clinical trials are muscle spasms, pain in the rear or within the limb, gout [11, 12], and gastrointestinal problems [13] like diarrhea [14, 15].

A less common but more serious effect was tendon rupture [16] within the structure of the shoulder, the biceps tendon, or the Achilles tendon [17].

Ezetimibe is a pharmaceutical drug unused and treats high blood cholesterol and certain other lipid abnormalities. Generally, it is used alongside dietary changes and a statin [18, 19]. It is preferred low in statin. It is taken orally. It is also available within the fixed combinations of Ezetimibe/Simvastatin, Ezetimibe/Atorvastatin, and Ezetimibe/Rosuvastatin. Usual consequences include upper respiratory infections, joint pain, diarrhea, and body exhaustion. Serious side effects include anaphylaxis [20, 21], liver problems, depression, and muscle breakdown. Its usage in pregnancy and breastfeeding [22, 23] is unsafe. Ezetimibe lowering the cholesterol involvement the intestines (Fig. 1). The experiments provided details on the conditions under which the drug was unstable to
prevent possible instability, and suitable steps were taken during formulation.

Methods
Reagents and chemicals
Acetonitrile (HPLC mark), orthophosphoric acid (HPLC mark), and water (HPLC mark) were obtained from Merck India Ltd., Worli, Mumbai, India. APIs of Bempedoic acid (purity 99.8%) and Ezetimibe (purity 99.9%) were obtained from Cipla Pharmaceutical Company, Mumbai.

Instrumentation
UPLC
A chromatographic software of empower version 2 was used. Waters Acquity UPLC with a quaternary pump and PDA detector with empower 2.0 software was employed.

UPLC and MS/MS conditions
The chromatographic process involved the column of symmetry C_{18} (150×4.6 mm, 3.5µ) with ambient temperature. An isocratic elution containing 50% of 0.1% OPA and 50% of acetonitrile was used as mobile phase, and the flow rate of 1 mL/min with a dose volume of 20 µL was employed in UPLC.

In the forced degradation study, UPLC was connected to a mass spectrophotometer with the conditions and the splitter placed before the ESI source, allowing entry of only 35% of an eluent. The standard operating source conditions for MS scan of Bempedoic acid and Ezetimibe on positive ESI mode were optimized as follows: The fragmented voltage was set at 80 V, the capillary was set at 3000 V, the skimmer was set at 60 V, nitrogen was used as drying and nebulizing gas (45psi), and highly filtered nitrogen gas was used as collision gas.

Preparation of standard solution
Accurately weighed 180 mg of Bempedoic acid and 10 mg of Ezetimibe were transferred into a 100-mL volumetric flask, and 70 mL of diluent was added and sonicated to dissolve it. Then, the volume was made up to the mark with diluent. Further, diluted 5 mL of the above solution was transferred into a 50-mL volumetric flask with diluent. And concentration of Bempedoic acid is 180 µg/mL and Ezetimibe is 10 µg/mL.

Preparation of sample solution
The samples were prepared by dissolving the finely ground tablets powder equivalent to 180 mg of Bempedoic acid and 10 mg of Ezetimibe sample, and they were transferred into a 100-mL volumetric flask, and 70 mL...
of diluents was added, ultrasonicated for 15 min, and diluted up to 100 mL mark with diluents. Further, diluted 5 mL of the sample stock solution was transferred into a 50-mL volumetric flask with diluents. Finally, the solution was filtered by utilizing a 0.45-µm syringe before injecting into the LC column.

Method validation
The systematic technique UPLC was confirmed by evaluating the parameters such as system suitability, linearity, accuracy, the limit of detection, the limit of quantification, and robustness, and therefore, the results were found to be within the suitable range of ICH requirements.

System suitability
To check the system performance, we used the parameters such as USP tailing, USP plate count, and percentage of relative variance.

Linearity and accuracy
Linearity was studied by using standard solutions of Bempedoic acid and Ezetimibe at several dilution levels (10%, 25%, 50%, 75%, 100%, 125%, 150%, and 200%). Accuracy was studied in three different dilution levels of 50%, 100%, and 150%. Finally, % of recovery and % of RSD were calculated.

Precision
Precision is of three types, namely

  System Precision  Reference standard solution of Bempedoic acid and Ezetimibe was injected six times and % RSD was calculated .

  Method Precision Three levels of sample solutions of Bempedoic acid and Ezetimibe with concentrations of 90, 5 µg/mL (50%), 180, 10 µg/mL (100%), and 270, 15 µg/mL (150%) were injected and % recovery and % RSD were calculated.

  Intermediate Precision Three levels of sample solutions of Bempedoic acid and Ezetimibe with concentrations of 90, 5 µg/mL (50%), 180, 10 µg/mL (100%), and 270, 15 µg/mL (150%) were injected in different days by using different columns. Then, % recovery and % RSD were calculated.

Robustness
This technique was studied by changing the flow of±0.02%, organic phase of±10%, and wavelength of±5 nm.

LOD and LOQ
LOD means little quantity of analyte during a sample which will be observed with tolerable precision accuracy. The limit of detection and limit of quantification for Bempedoic acid and Ezetimibe were determined by injecting progressively low concentrations of ordinary solutions using the developed UPLC method. The limit of detection and limit of quantification were calculated as 3 s/n and 10 s/n, respectively, as per ICH guidelines where s/n indicates the signal-to-noise.

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\text{LOD} = 3.3 \times \text{Standard deviation}/\text{Slope} \\
\text{LOQ} = 10 \times \text{Standard deviation}/\text{Slope}.
\]

Stress degradation
Stress degradation will not interfere between the peaks obtained for the chromatograms of forced degradation preparations. Stress degradation learnings were performed as reported by ICH guidelines Q1 (A) R2. The degradation peaks should be separated from one another, and therefore, the resolution between the peaks should be a minimum of 1.0. Therefore, the peak purity of the principle peak shape was passed. The forced degradation work was performed by different kinds of stresses to get the degradation of about 20%.

Acid degradation
In acid degradation, the sample having 5 mL of 1 N HCl was transferred into a 100 mL volumetric flask and the flask was heated in a water bath at 60 °C for 30 min, allowed to cool to room temperature, and neutralized with 5 mL of 1 N NaOH. Then, it was made up to the mark with diluent. Further, diluted 5 mL of the above solution was transferred into a 50-mL volumetric flask with diluent and then filtered and injected into UPLC–MS system.

Alkali degradation
In alkali degradation, the sample having 5 mL of 1 N NaOH was transferred into a 100-mL volumetric flask and the flask was heated in a water bath at 60 °C for 30 min, allowed to cool to room temperature, and neutralized with 5 mL of 1 N NaOH. Then, it was made up to the mark with diluent. Further, diluted 5 mL of the above solution was transferred into a 50-mL volumetric flask with diluent and then filtered and injected into UPLC–MS system.

Peroxide degradation
In peroxide degradation, sample having 5 mL of 30% hydrogen peroxide was transferred into a 100-mL volumetric flask. After that, the flask was heated in a water bath at 60 °C for 30 min. and allowed to cool to room
temperature. Then, it was made up to the mark with diluent, and further, diluted 5 mL of the above solution was transferred into a 50-mL volumetric flask with diluent and then filtered and injected into UPLC–MS system.

**Reduction degradation**
In reduction degradation, sample having 5 mL of 10% sodium bisulfate solution was transferred into a 100-mL volumetric flask. The flask was heated in a water bath at 60 °C for 30 min and allowed to cool to room temperature. Then, it was made up to the mark with diluent, and further, diluted 5 mL of the above solution was transferred into a 50-mL volumetric flask with diluent and then filtered and injected into UPLC–MS system.

**Thermal degradation**
In thermal degradation, 1gm sample powder was weighed in a Petri dish and exposed to dry heat at 105 °C for 6 h. After that, equivalent weight of 180 µg/mL of Bempedoic acid and 10 µg/mL of Ezetimibe sample was weighed, transferred into a 100-mL volumetric flask, and dissolved in a diluent. Then, it was made up to the mark with diluent. Further, diluted 5 mL of the above solution was transferred to a 50-mL volumetric flask with diluent.

**Photolytic degradation**
In photolytic degradation, tablets were ground finely into powder form and 1gm sample was exposed to photolight UV 200 W-hrs and fluorescence light 1.2 million lux-hours. After that, equivalent weight of 180 µg/mL of Bempedoic acid and 10 µg/mL of Ezetimibe sample was weighed, transferred into a 100-mL volumetric flask, and dissolved in a diluent. Then, it was made up to the mark with diluent. Further, diluted 5 mL of the above solution was transferred into a 50-mL volumetric flask with diluent.

**Results**
An isocratic elution of Bempedoic acid and Ezetimibe involved symmetry C18 column with a flow rate of 1 mL/min, and ambient temperature was maintained within the column. A mobile phase of 0.1% OPA and acetonitrile in 50:50 v/v was used. UV observation was taken at 230 nm.

**System suitability**
The standard solution of Bempedoic acid (180 µg/mL) and Ezetimibe (10 µg/mL) was injected into the UPLC system, and the chromatogram of UPLC is shown in Fig. 2. %RSD was calculated by using the peak areas, and the results were found to be within the acceptable limit. Results of system suitability are shown in Table 1.

**Specificity**
Specificity was not used to test the power of the assay of the method but to eliminate the consequences of all interfering substances in Bempedoic acid and Ezetimibe peak results, specifically by comparing the chromatograms

| S. no. | System suitability parameter | Acceptance criteria | Drug name        | Bempedoic acid | Ezetimibe |
|--------|-----------------------------|---------------------|------------------|----------------|-----------|
| 1      | % RSD                       | NMT 2.0             |                  | 0.11           | 0.27      |
| 2      | USP Tailing                 | NMT 2.0             |                  | 1.03           | 1.01      |
| 3      | USP plate count             | NLT 2000            |                  | 3111           | 6605      |

![Standard chromatogram of UPLC](image-url)
of the blank samples presented in Fig. 3. The justified technique exhibited that the selected drugs were eluted without the involvement of peaks that occurred by the excipients in the market products.

**Linearity**

Linearity of the developed test method was proven by preparing a series of linearity of solutions containing Bempedoic acid and Ezetimibe at eight different concentrations ranging from Bempedoic acid 18–360 µg/mL (18, 45, 90, 135, 180, 225, 270, and 360 µg/mL) and Ezetimibe 1–20 µg/mL (1, 2.5, 5, 7.5, 10, 12.5, 15, and 20 µg/mL). The calibration curves were linear throughout the concentration series of Bempedoic acid and Ezetimibe. The values of linearity are listed in Table 2 and Fig. 4. The coefficient of correlation values of both analytes Bempedoic acid and Ezetimibe were 0.9997 and 0.99964 in the calibration curve, respectively.

**Accuracy**

Accuracy of Bempedoic acid and Ezetimibe depends on recovery studies, which were administered at three different dilution levels (50%, 100%, and 150%). APIs with concentrations of 90, 180, 270 µg/mL of Bempedoic acid and 5, 10, 15 µg/mL of Ezetimibe were prepared. According to the test procedure, the test solutions were injected as three preparations of each spike level and therefore the assay was performed. The shared recovery values were observed to be within the range of 98%–102%, and the results are shown in Table 3.

**Precision**

The precision of this analysis was assessed in terms of method and intermediate variations. The intraday studies were calculated by executing three levels of sample solutions of Bempedoic acid and Ezetimibe with concentrations of 90, 5 µg/mL (50%), 180, 10 µg/mL (100%), and 270, 15 µg/mL (150%) in an equivalent day under the equivalent experimental conditions. Intermediate precision of the tactic was administered within the same laboratory by studying the analysis with different days and different columns. The tactic was very precise, and RSD values were found to be <2%. Good recoveries (98 to 102%) of the selected drugs were obtained at each attached concentration and showed that the tactic was accurate. The results are given in Table 4.

**LOD and LOQ**

LOD and LOQ were separately determined by the calibration curve method; LOD and LOQ of the compounds were calculated by injecting continuous lower

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**Table 2** UPLC results of linearity

| Linearity  | Bempedoic acid | Ezetimibe |
|------------|----------------|-----------|
| Conc. (µg/ml) | Area           | Conc. (µg/ml) | Area           |
| Linearity-10% | 18             | 382,238   | 1               | 219,908       |
| Linearity-25% | 45             | 818,812   | 2.5             | 541,877       |
| Linearity-50% | 90             | 1,655,675 | 5               | 1,089,663     |
| Linearity-75% | 135            | 2,463,729 | 7.5             | 1,533,475     |
| Linearity-100% | 180            | 3,255,329 | 10              | 2,188,257     |
| Linearity-125% | 225            | 4,003,213 | 12.5            | 2,607,096     |
| Linearity-150% | 270            | 4,869,046 | 15              | 3,134,284     |
| Linearity-200% | 360            | 6,486,358 | 20              | 4,233,526     |
| Slope      | 17,903.72     | 210,294.97 |
| Intercept  | 27,530.84     | 10,156.17  |
| CC         | 0.99992       | 0.99964    |
accumulation of standard solutions using the developed UPLC method. The LOD values for Bempedoic acid and Ezetimibe were observed as 0.225 µg/mL and 0.013 µg/mL and s/n values were 7 and 4, respectively. LOQ values were 0.743 µg/mL and 0.043 µg/mL and 27 and 21 were the s/n values, respectively.

### Robustness

As per ICH norms, deliberate variations were made within the method parameters such as change in flow (±0.02%), organic content in the mobile phase (±10%), and wavelength of detection (±5 nm). So there is no tactic capacity to stay unaffected by system suitability. Table 5 shows the robustness of the tactic evaluated by observing the result of the modified parameters on retention time, tailing factor, and content percentage using UPLC. The degree of reliability of the consequences which were obtained by small deliberate variations showed that the tactic was strong.

### Stability

To assess the steadiness of the sample, a solution was analyzed initially for 24 h at different intervals of time. No significant degradation was observed during this era, and therefore, the mean deviation and mean were not quite 5.0%, suggesting that the solutions were stable for a minimum period of 24 h, which was sufficient for the entire analytical procedure for UPLC.
According to ICH stability guidelines, there are various types of forced conditions, i.e., thermal, basic, acidic, oxidative, photolytic, and reductive forced degradation studies were conducted by using the sample brand name Nexlizet (containing 180 mg of Bempedoic acid and 10 mg of Ezetimibe) (Fig. 5). Seven numbers of DPs, DP1–DP7, were observed and characterized by UPLC–MS. The studies provided information about the conditions in which the drug is unstable to avoid potential instabilities; proper measures were often taken during formulation. Tables 6 and 7 represent the degradation results and validation parameters of Bempedoic acid and Ezetimibe.

### Acid degradation

In acid degradation, the selected samples were hydrolyzed with 1 N HCl for 3 h at 60 °C, 16.1% of Bempedoic acid and 12.4% Ezetimibe degradation was observed using HPLC, and 16.4% of Bempedoic acid and 11.6% of Ezetimibe degradation was observed using UPLC, and three degradation products, namely DP1, DP2, and DP5, were formed.

### Alkali degradation

Alkali degradation of selected samples was initiated with 1 N NaOH, 15.2% of Bempedoic acid and 13.5% Ezetimibe degradation was observed using HPLC, and 17.7% of Bempedoic acid, 13.6% of Ezetimibe was observed using UPLC, and three degradation products, namely DP1, DP2, and DP5, were formed.

### Peroxide degradation

Peroxide decomposition of selected drug sample was studied in 30% hydrogen peroxide, 18.7% of Bempedoic acid and 15.8% of Ezetimibe degradation was observed using UPLC, and four degradation products, namely DP1, DP2, DP5, and DP7, were formed.

### Reduction degradation

Reduction degradation of selected drugs was studied in 30% sodium bisulfate solution, 18.5% of Bempedoic acid and 16.4% of Ezetimibe degradation was observed using UPLC, and one DP1 degradation product was formed.
Fig. 5 UPLC degradation chromatograms
Fig. 5 continued
Thermal degradation

The thermal degradation sample was exposed at 105 °C for 6 h, 16.3% of Bempedoic acid and 16.6% of Ezetimibe degradation was observed in UPLC, and two degradation products, namely DP6 and DP2, were formed.

Photolytic degradation

The sample was exposed to sunlight for 12 h, 16.2% of Bempedoic acid and 16.8% of Ezetimibe degradation was observed using UPLC, and three degradation products, namely DP1, DP3, and DP4, were formed.

Collision-induced dissociation of Bempedoic acid and Ezetimibe

DP1: Scheme 1 shows the fragmentation mechanism of DP1, and the ESI spectrum showed the most intense [M+H]+ ion of m/z-449, which was observed under acid, alkalai, peroxide, and photolytic degeneration conditions.

The MS/MS spectrum of DP1 displayed abundant product ions at m/z-361 (loss of C4H8O2), m/z-273 (loss of C4H8O2 from m/z 361), and m/z-157 (loss of C6H12O6 from m/z 273). The MS/MS experiments combined with accurate mass measurements have confirmed the proposed scheme. Figures S6 and S7 represent collision induced dissociation of Bempedoic acid and Ezetimibe and MS spectral data.

DP2: Scheme 2 shows the fragmentation mechanism of Ezetimibe DP2, and the MS/MS spectrum showed more intense [M+H] ion of m/z-373, which was noticed under acid, alkalai, thermal, and peroxide conditions. The spectrum displayed abundant product ions at m/z-295 (loss of benzene), m/z-217 (loss of benzene from m/z 292), m/z-123 (loss of phenol from m/z 217), and m/z-63 (loss of C3H8O from m/z 123). The MS/MS experiments combined with correct mass evaluations have confirmed the proposed scheme.

### Table 6 Degradation results of Bempedoic acid and Ezetimibe

| Deg condition | Time/Temp | Bempedoic acid | Ezetimibe | Number of DPs formed |
|---------------|-----------|----------------|-----------|---------------------|
|               |           | % Deg | % Assay | % MB | % Deg | % Assay | % MB |                  |
| Acid deg      | 3 h, 60 °C | 16.4  | 86.1   | 102.5 | 11.6  | 89.6   | 101.2 | DP1, DP2 and DP5 |
| Alkali deg    | 3 h, 60 °C | 17.7  | 81.8   | 99.5  | 13.6  | 87.2   | 100.8 | DP1, DP2 and DP5 |
| Peroxide deg  | –         | 18.7  | 81.2   | 99.9  | 15.8  | 84.1   | 99.9  | DP1, DP2, DP5 and DP7 |
| Reduction deg | 3 h, 60 °C | 18.5  | 83.4   | 101.9 | 16.4  | 84.7   | 101.1 | DP1 |
| Thermal deg   | 24 h, 105 °C | 16.3  | 85.3   | 101.6 | 16.6  | 84.8   | 101.4 | DP1 and DP2 |
| Photolytic deg | UV–Vis light | 16.2  | 83.5   | 99.7  | 16.8  | 83.9   | 100.7 | DP1, DP3 and DP4 |

### Table 7 Method validation results of Bempedoic acid and Ezetimibe by UPLC

| Parameter                  | Bempedoic acid | Ezetimibe |
|----------------------------|----------------|-----------|
|                            | Concentration (µg/ml) | Result | Concentration (µg/ml) | Result |
| Linearity                  | 18–360         | CC: 0.999 | 1–20          | CC: 0.999 |
| Accuracy                   | 90             | % Rec: 100.1 | 5 | %Rec: 99.5 |
|                           | 180            | %Rec: 99.9  | 10 | % Rec: 99.3 |
|                           | 270            | % Rec: 99.6 | 15 | % Rec: 99.9 |
| Intraday precision         | 180            | %RSD: 0.87  | 10 | %RSD: 0.65 |
| Interday precision         | 180            | %RSD: 0.36  | 10 | %RSD: 0.61 |
| Robustness                 | Flow Plus      | 0.41       | 10  | 0.33       |
|                            | Flow Minus     | 0.37       | 10  | 0.82       |
|                            | Organic Plus   | 0.78       | 10  | 0.51       |
|                            | Organic Minus  | 0.52       | 10  | 0.78       |
|                            | Wavelength Plus| 0.42       | 10  | 0.84       |
|                            | Wavelength Minus| 0.39      | 10  | 0.92       |

CC correlation coefficient  
% REC-% Recovery  
%RSD: Relative standard deviation
DP3: Scheme 3 shows the fragmentation mechanism for DP3 of m/z 427 with molecular formula C_{24}H_{23}F_{2}NO_{4}, which was noticed under photolytic conditions. The MS spectrum displays abundant product ions at m/z-274 (loss of C_{6}H_{12}OF), m/z-179 (loss of m/z C_{6}H_{12}F from m/z 274), m/z-93 (loss of C_{3}H_{6}O from m/z 153), and m/z-85 (loss of phenol from m/z 179). The MS/MS measurements combined with correct mass evaluations have confirmed the proposed scheme.

DP4: Scheme 4 shows the fragmentation mechanism for DP4 of m/z-499, which was noticed under photolytic degradation condition. The spectrum displays abundant product ions at m/z-346 (loss of C_{6}H_{12}OF), m/z-173 (loss of m/z C_{6}H_{12}F from m/z 346), m/z-93 (loss of m/z C_{3}H_{6}O from m/z 153), and m/z-93 (loss of C_{3}H_{6}O from m/z 173). The MS/MS experiments combined with correct mass evaluations have confirmed the proposed scheme.

DP5: Scheme 5 shows the fragmentation mechanism for DP5 of m/z-393.4, which was noticed under acid, alkali, and peroxide degradation conditions. The spectrum displays abundant product ions at m/z-137 (loss of C_{6}H_{12}F), m/z-95 (loss of C_{6}H_{12}F from m/z 256), and m/z-94 (loss of C_{3}H_{6}OH from m/z 161). The MS/MS experiments combined with correct mass evaluations have confirmed the proposed scheme.

DP6: Scheme 6 shows the fragmentation mechanism for DP6 of m/z-154, which was noticed under thermal degradation condition. The spectrum displays abundant product ions at m/z-72 (loss of C_{6}H_{12}O) and m/z-84 (loss of C_{3}H_{12}). The MS/MS experiments combined with correct mass evaluations have confirmed the proposed scheme.

DP7: Scheme 7 shows the fragmentation mechanism of degradation product 7 of m/z-493, which was noticed under peroxide degradation condition. The spectrum displays abundant product ions at m/z-399 (loss of C_{6}H_{12}F), m/z-359 (loss of C_{6}H_{12}O_{2} from m/z-493), m/z-265 (loss of C_{3}H_{12}F from m/z-359), m/z-205 (loss of C_{11}H_{14}FO_{2} from m/z-399), and m/z-71 (loss of C_{11}H_{14}FO_{2} from m/z-265). The MS/MS experiments combined with correct mass evaluations have confirmed the proposed scheme.

Discussion
We have developed a responsive, robust, and fast UPLC process. The factors influencing the efficiency of the system were optimized, and the resulting method displayed high sensitivity and selectivity. A literature survey found that little attention was paid to the structural elucidation of the degradation products (DPs) of Bempedoic acid and Ezetimibe. A few attempts have been made for major impurities. According to the ICH stability guidelines [24–28], there are different forms of forced conditions, i.e., thermal, basic, acidic, oxidative, photolytic, and reductive forced degradation studies have been conducted [29–34]. Thus, in continuation of our previous efforts [35, 36], seven DPs (DP1–DP7) were observed and characterized by UPLC–MS, and few articles were mentioned in the last few years for quantification and analysis of Bempedoic acid and Ezetimibe in various chemical and biological matrices by using HPLC, UPLC, and characterization of its degradation products [37–44]. In the present study, we intended to explore a specific, sensitive, and new UPLC method toward the analysis of Bempedoic acid, Ezetimibe, and characterization of its new degradation products by UPLC–MS.

Conclusions
In this study, a unique, simple, rapid, economical, sensitive, and simply available UPLC technique was developed for the coincident determination of Bempedoic acid and Ezetimibe in bulk and tablet dosage form. The advantages of this method are shorter run time, low price, accessibility, reliability, sensitivity, and reproducibility. The degradation actions of the drugs were examined under hydrolysis (acid, base, and neutral), oxidation, and photolytic and thermal stress conditions. The drugs were found to be stable in thermal hydrolysis and unstable in acid, alkali, and oxidative conditions. The degradation products were identified [M + H]^+ ion, and the proposed structures were supported by UPLC–MS/MS experiments combined with correct mass evaluations. The UPLC method was supported as per ICH guidelines and finally applied to the marketed formulations.

Abbreviations
UPLC: Ultra-performance liquid chromatography; LOD: Limit of detection; LOQ: Limit of quantization; ICH: International Council for Harmonization; UPLC–MS: Ultra-performance liquid chromatography–mass spectrometry.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s43094-021-00381-6.

Additional file 1. Collision induced dissociation of Bempedoic acid and Ezetimibe and Mass spectral data.

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Authors’ contributions
SMT and AV designed the study, performed the method development and validation, wrote the protocol, and wrote the first draft of the manuscript. CHR helped in the analyses of the study and literature searches. All authors read and approved the final manuscript.
References
1. Bhatnagar D, Soran H, Durrington PN (2008) Hypercholesterolemia and its management. BMJ 337:a993
2. Awad K, Mikhailidis DP, Katsiki N, Muntner P, Banach M (2018) Effect of ezetimibe monotherapy on plasma lipoprotein concentrations in patients with primary hypercholesterolemia. A systematic review and meta-analysis of randomized controlled trials. Drugs 78(4):453–462
3. Zech LA Jr, Hoeg JM (2008) Correlating corneal arcus with atherosclerosis in familial hypercholesterolemia. Lipids Health Dis 7(1):7
4. Martin C, Samuel C, David WR, Andrew P, Smith H, Fowler K (2006) Assigning sex to pre-adult stalk-eyed flies using general disc morphology and X chromosome homogeneity. BMC Dev Biol 6(1):29
5. Scientific steering committee on behalf of the Simon Broome register (2009). Atherosclerosis 142(1):105–112
6. Marais AD, Blom DJ, Firth JC (2002) Statins in homozygous familial hypercholesterolaemia. Curr Atheroscler Rep 4(1):19–25
7. Faxon DP, Creager MA, Smith SC, Pasternak RC, Olin JW, Bettmann MA, Cirqui MH, Milani RV, Loscalzo J, Kaufman JA, Jones DW, Pearce WH (2004) Atherosclerotic vascular disease summary. Executive disease summary. Atherosclerotic vascular disease conference proceedings for health professionals from a special writing group of the American heart association. Circulation 109(2):2595–604
8. Zhao DF, Edelman JJ, Seco M, Bannon PG, Wilson MK, Byrom MJ, Thourani V, Lamy A, Taggart DP, Psukas JD, Valley MP (2017) Coronary artery bypass grafting with and without manipulation of the ascending aorta. A network meta-analysis. J Am Coll Cardiol 69(8):924–936
9. Dashty M, Motsazacker MM, Levels J, de Vries M, Mahmoudi M, Peppelenbosch MP, Razaee F (2014) Proteome of human plasma very low density lipoprotein and low density lipoprotein exhibits a link with coagulation and lipid metabolism. Thromb Haemost 111(3):518–530
10. Ahotupa M (2017) Oxidized lipoprotein lipids and atherosclerosis. Free Radical Res 51(4):439–447
11. Robinson PC, Stamp LC (2016) Management of gout: Must has changed. Aust Family Phys 45(5):299–302
12. Choi HK (2018) A prescription for lifestyle changes in patients with hyperuricemia and gout. Curropinheumatol 22(2):165–172
13. Helander HF, Fandirinks L (2014) Surface area of the digestive tract-revisited. Scand J Gastroenterol 49(6):681–689
14. Dupont HL (2014) Acute infectious diarrhea in immune competent adults. New Engl J Med 370(16):1532–1540
15. Sweeters S (2012) Evaluating the patients with diarrhea. A case based approach. Mayo Clin Proc 87(6):596–602
16. Thomas JR, Lawtron Jr. (2017) Biceps and triceps ruputures in athletes. Hand Clin 33(1):35–46
17. Wu Y, Lin L, Li H, Zhao Y, Liu L, Jia Z, Wang D, He Q, Ruan D (2016) Is surgical intervention more effective than non surgical treatment for acute Achilles tendon rupture. A systematic review of overlapping Meta-analyses. Int J Surg 36(Pt A):305–311
18. Taylor FC, Huffman M, Ebrahim S (2013) Statin therapy for primary prevention of cardiovascular disease. JAMA 310(22):2451–2452
19. Abd TT, Jacobson TA (2011) Statin induced myopathy. A review and update. Expert Opin Drug Saf 10(3):373–387
20. Sampson HA, Munoz Furlong A, Campbell RL, Adkinson Jr NF, Bock S, A, Braman A, Camargo Jr CA, Cydulka R, Galli SJ, Giddu J, Gruchalla RS, Harlor Jr AD, Hepner DL, Lewis LM, Lieberman PL, Metcalfe DD, O'Connor R, Mutaro A, Rudman CS, Schwerer D, Simons FE, Thomas S, Wood JP, Decker WW (2006) Second symposium on the definition and management of anaphylaxis. Summary report-Second national institute of allergy and infectious disease/food allergy and anaphylaxis network symposium. J Allergy Clin Immunol 117(2):391–7
21. Tejedor-Alonso MA, Moro-Moro M, Mugica-garcia MV (2015) Epidemiology of anaphylaxis, Contributions from the last 10years. J Invest Allergol Clin Immunol 25(3):163–175
22. Kremer KP, Kremer TR (2018) Breastfeeding is associated with decreased childhood maltreatment. Breastfeed Med 13(1):18–22
23. Spencer B, Wambach K, Domain EW (2015) African American women’s breastfeeding experiences, cultural, personal and political voices. Qual Health Res 25(7):974–987
24. ICH validation of analytical procedures methodology
25. Validation of compendia methods. United states pharmacopoeia, 2003, 21st edition, 2440.
26. International Conference on Harmonization. Validation of analytical procedures: methodology ICH Q2 (R1)2005. http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/ Q2R1Guideline.pdf
27. 22ICH (2003) Q1A (R2) Stability testing of new drug substances and products. https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q1A_R2/Step4/Q1A_R2_Guideline.pdf
28. ICH (1997) Q1R Stability testing. Photostability testing of New drug substances and products.https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q1B/Step4/Q1B_Guideline.pdf
29. Narasimha SL, Chandrasekhar K, Srinivas KS, Ravi teja Y (2020) Separation and characterization of new forced degradation products of dasatinib in tablet dosage formulation using LC-MS and stability-indicating HPLC methods. Chromatographia 83(6):947–962
30. Zhuang T, Wang G, Cui X, Chen Y, Chen L, Zhang G (2016) Isolation and structure characterization of two novel degradation products in Filipristine maleate formulation by prep-HPLC, LC-MS/Q-TOF and 2D-NMR Chromatographia 79(5):1041–1047
31. Zaman B, Hassan W (2018) Development of stability indicating HPLC-UV method for determination of daclatasvir and characterization of forced degradation products. Chromatographia 81(3):785–797
32. Santa Z, Kota J, Szoke K, Vukics K, Szanta J (2002) Structure of the major degradant of Ezetimibe. J Pharm Biomed Anal 111(11):1587–1664
33. Prasad K, Venkatappaiah V, Pallavi A, Saeed SA, Mukant K, Parthasaradhi D (2016) LC-MS/MS characterization of the forced degradation products of Ezetimibe: development and validation of a stability-indicating UPLC method. J Taibah Univ Sci 10(1):148–160
34. Saranjit S, Baljinder S, Rakesh B, Lalit W, Rahul S (2006) Stress degradation studies on Ezetimibe and development of a validated stability-indicating HPLC assay. J Pharm Biomed Anal 41(3):1037–1040
35. Subrahmanyam T, Anuradha V, Prathyusha KA (2021) New validated RP-HPLC method for cisplatin and topotecan in API and vaccine form and its stress studies. Int J Res Pharm Sci 12(1):808–814
36. Subrahmanyam T, Anuradha V, Murthy SNB, Prathyusha KA (2021) A newly developed reverse phase-high performance liquid chromatography method for the assay of dexmedetomidine and sedemxemiphene-date with PDA. J Pharm Res Int 33(318):203–211
37. Elawady T, Ibrahim F, Khedr A, Belal F (2021) Simultaneous determination of Ezetimibe, atorvastatin and Simvastatin using quadrupole LC-MS: application to combined tablets and plasma after SPE. Acta Chromatogr 33(3):245–252
38. Li Y, Tang L, Wang Y (2021) Simultaneous quantification of Ezetimibe and Ezetimibe glucuronide in human plasma: a pharmacokinetic study in healthy Chinese volunteers. Lat Am J Pharm 40(4):735–741
39. Devi DPV, Narayanaaro KVM, Shyamala P, Krishna RM, Prasad KS (2020) HPLC estimation of new impurity methyl Ezetimibe in Ezetimibe drug. Asian J Chem 32(6):1309–1313
40. Ramadevi P, Rambabu K (2020) Bio analytical method development and validation for Ezetimibe and pitavastain and its applications to pharmacokinetic studies in rabbit plasma by using LC-MS/MS. Int J Res Pharm Sci 11(4):7854–7862
41. Kurbanoglu S, Esem O, Ozkan CK, Savaser A, Ozkan Y, Uslu B, Ozkan SA (2020) Stability-indicating liquid chromatographic method for the simultaneous determination of Rosuvastatin and Ezetimibe from pharmaceuticals and biological samples. J Turk Chem Soc Sect A Chem 7(3):865–874
42. Li Y, Tang L, Wang Y (2020) A sensitive and reliable method for the determination of Ezetimibe by lc-ms/ms and its application to a pharmacokinetic study in healthy Chinese volunteers. Latin Am J Pharm 39(10):1921–1926
43. Shah U, Shah K, Patel R (2019) Stability-indicating analytical method development using quality by design approach for simultaneous estimation of Ezetimibe and glimepiride. Indian J Pharm Sci 81(2):273–281
44. Kurbanoglu S, Esem O, Ozkan CK, Savaser A, Ozkan Y, Ozkan SA (2019) Development and validation of RP-LC method for the simultaneous determination of simvastatin and ezetimibe in fixed-dose combination tablets and in rabbit serum. Chromatographia 82(1):279–285

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