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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 C_{18} 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

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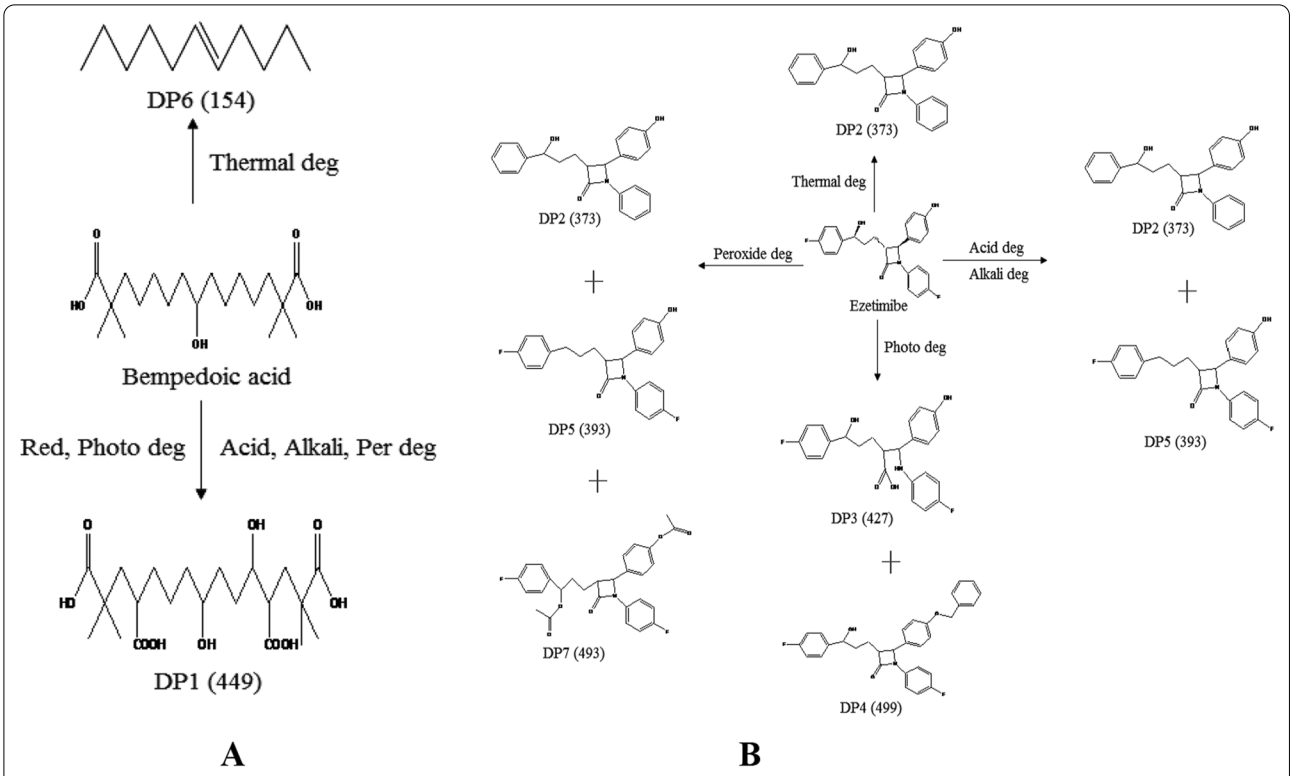


Fig. 1 Schematic representation of degradation products of **A** Bempedoic acid and **B** Ezetimibe

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 weighed180 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 detected, while LOQ explains the 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.

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

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) R_2. 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 C_{18} 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

Table 1 System suitability results

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

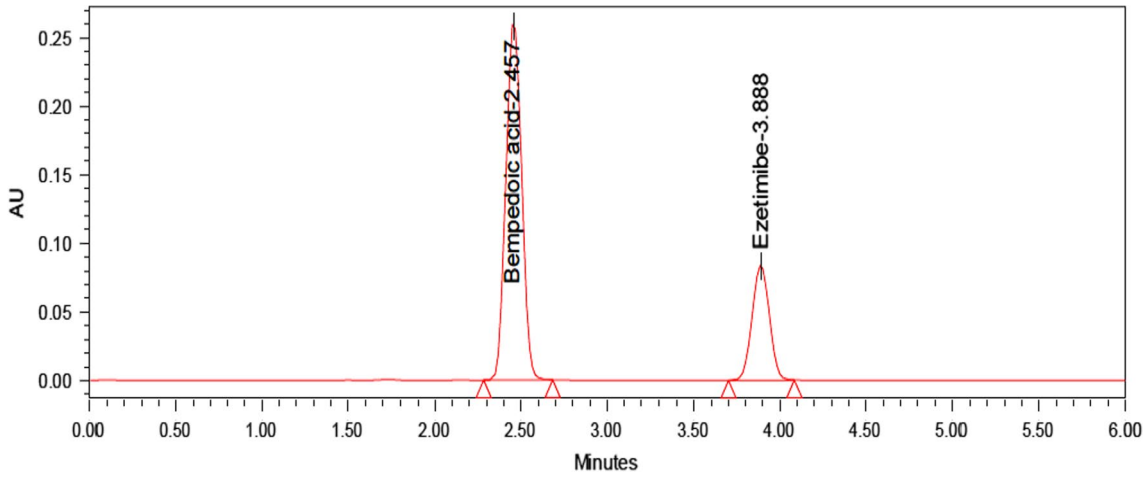


Fig. 2 Standard chromatogram of UPLC

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 .

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	

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

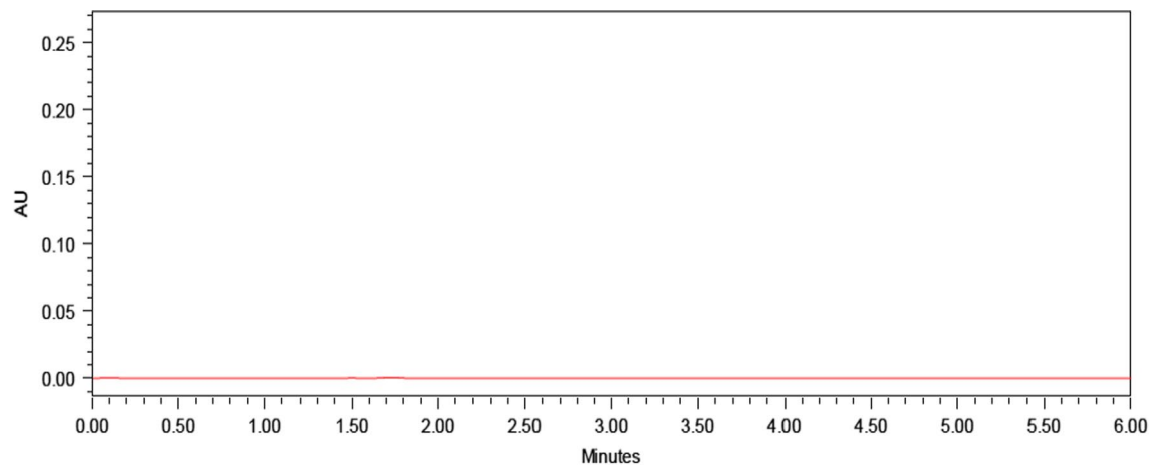


Fig. 3 Blank chromatogram of UPLC

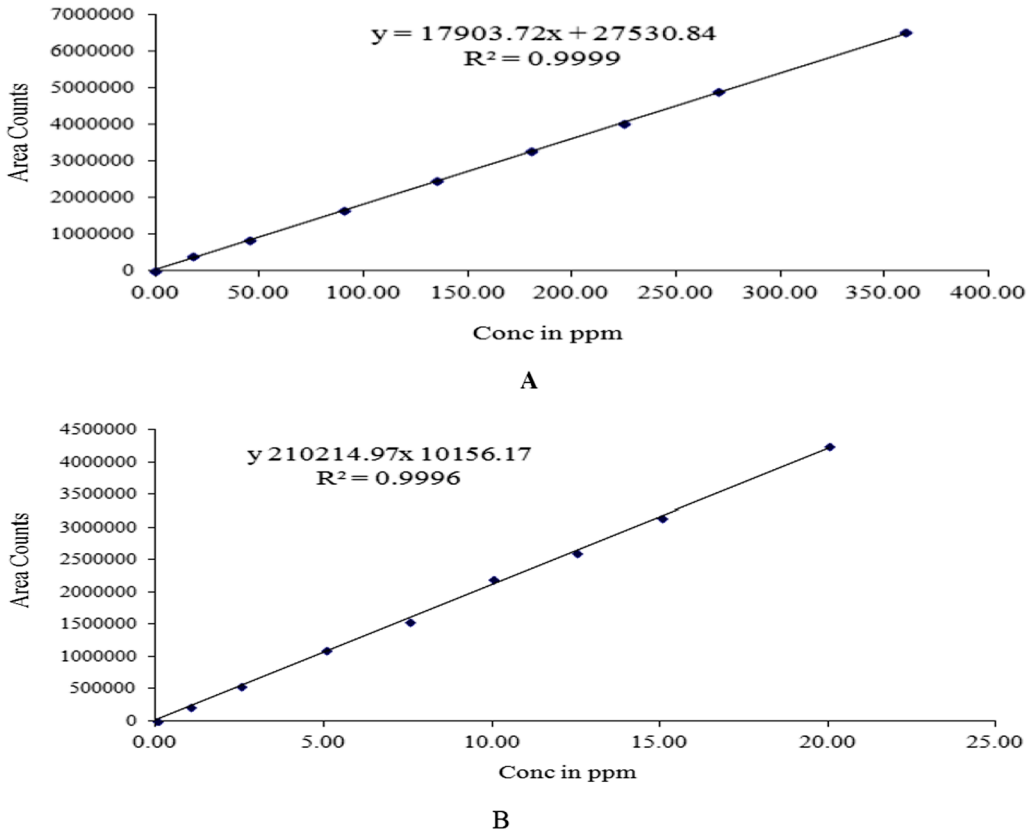


Fig. 4 Linearity plots of **A** Bempedoic acid and **B** Ezetimibe

Table 3 UPLC results of accuracy of (A) Bempedoic acid and (B) Ezetimibe

S. no	Concentration (µg/ml)	Mean ± SD, %RSD	% Recovery
A			
1	90	90.12 ± 0.062, 0.18	100.1
2	180	180.06 ± 0.024, 0.39	99.9
3	270	270 ± 0.057, 0.38	99.6
B			
1	5	5.04 ± 0.039, 0.74	99.5
2	10	10.11 ± 0.028, 0.22	99.3
3	15	15.05 ± 0.063, 0.52	99.9

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.

Table 4 UPLC precision results of (A) Bempedoic acid and (B) Ezetimibe

S. no.	Amount added (µg/ml)	Mean ± SD	% RSD
A			
Method precision results			
1	90	89.98 ± 0.011	0.36
2	180	180.14 ± 0.053	0.87
3	270	270.15 ± 0.074	0.65
Intermediate precision results of Day-1			
1	90	90.15 ± 0.046	0.88
2	180	180.01 ± 0.035	0.53
3	270	270.08 ± 0.015	0.27
Intermediate precision results of Day-2			
1	90	90.19 ± 0.024	0.74
2	180	179.99 ± 0.039	0.65
3	270	270.04 ± 0.055	0.39
Intermediate precision results of column-1			
1	90	90.14 ± 0.052	0.61
2	180	0.07 ± 0.049	0.36
3	270	270.06 ± 0.078	0.34
Intermediate precision results of column-2			
1	90	90.11 ± 0.025	0.91
2	180	180.07 ± 0.034	0.68
3	270	270.06 ± 0.048	0.42
B			
Method precision results			
1	5	5.09 ± 0.035	0.74
2	10	10.05 ± 0.024	0.85
3	15	15.03 ± 0.049	0.34
Intermediate precision results of Day-1			
1	5	4.99 ± 0.024	0.28
2	10	10.21 ± 0.047	0.42
3	15	15.14 ± 0.056	0.53
Intermediate precision results of Day-2			
1	5	5.04 ± 0.041	0.46
2	10	10.17 ± 0.027	0.62
3	15	15.14 ± 0.011	0.28
Intermediate precision results of column-1			
1	5	5.07 ± 0.024	0.465
2	10	9.97 ± 0.044	0.61
3	15	15.06 ± 0.021	0.38
Intermediate precision results of column-2			
1	5	5.10 ± 0.024	0.28
2	10	10.07 ± 0.078	0.11
3	15	15.07 ± 0.039	0.96

Table 5 Results of robustness of Bempedoic acid and Ezetimibe

Change in parameter	%RSD of Bempedoic acid	%RSD of Ezetimibe
Flow (0.8 ml/min)	1.24	1.22
Flow (1.2 ml/min)	0.84	0.79
Org Phase (45:55)	1.36	1.65
Org Phase (55:45)	0.97	0.77
Wavelength (225 nm)	0.82	0.85
Wavelength (235 nm)	0.79	0.81

Forced degradation studies of Ezetimibe and Bempedoic acid

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

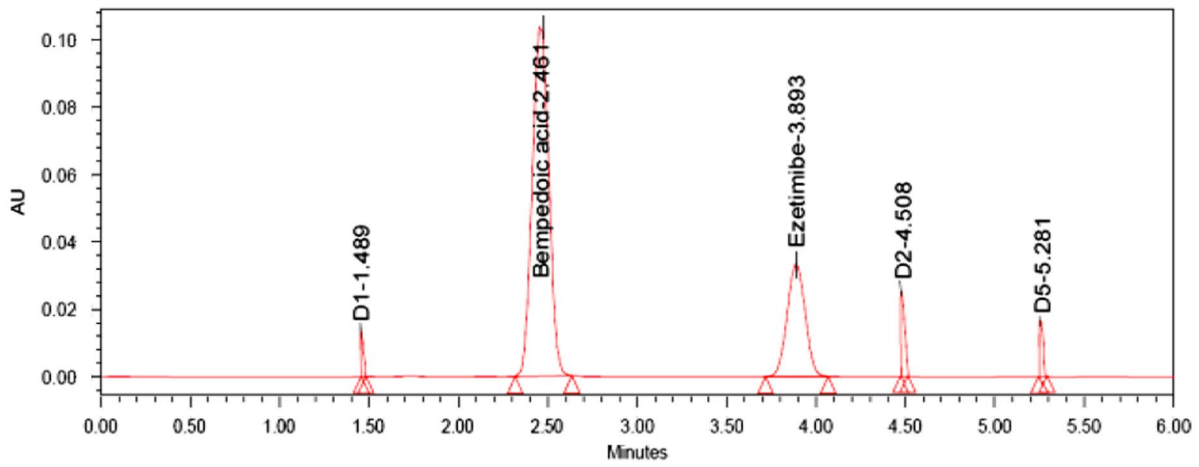
Alkali degradation of selected samples was initiated with 1 N NaOH, 15.2% of Bempedoic acid and 13.5% Ezetimibe degradation was observed using HPLC, and 17.7% of Bempedoic acid, 13.6% of Ezetimibe was observed using UPLC, and three degradation products, namely DP1, DP2, and DP5, were formed.

Peroxide degradation

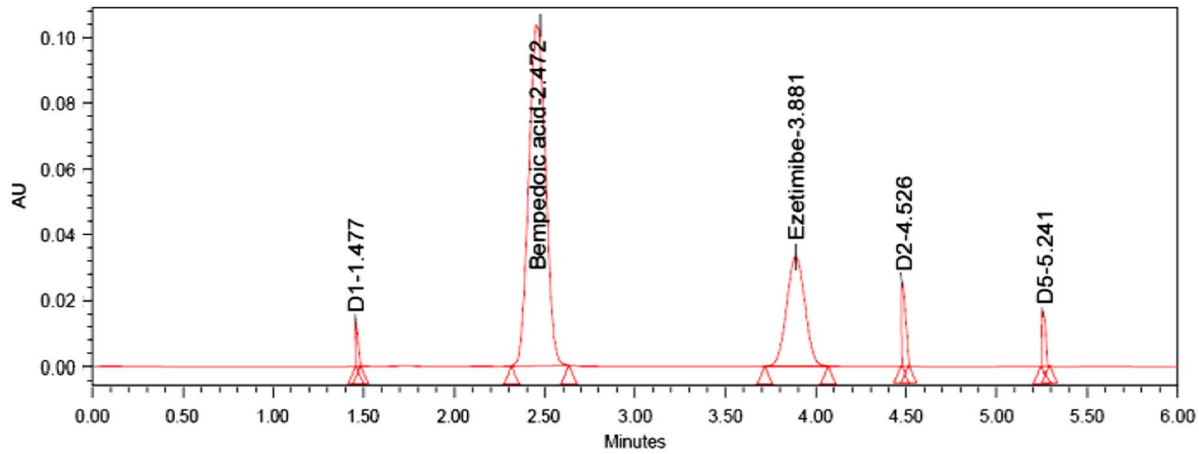
Peroxide decomposition of selected drug sample was studied in 30% hydrogen peroxide, 18.7% of Bempedoic acid and 15.8% of Ezetimibe degradation was observed using UPLC, and four degradation products, namely DP1, DP2, DP5, and DP7, were formed.

Reduction degradation

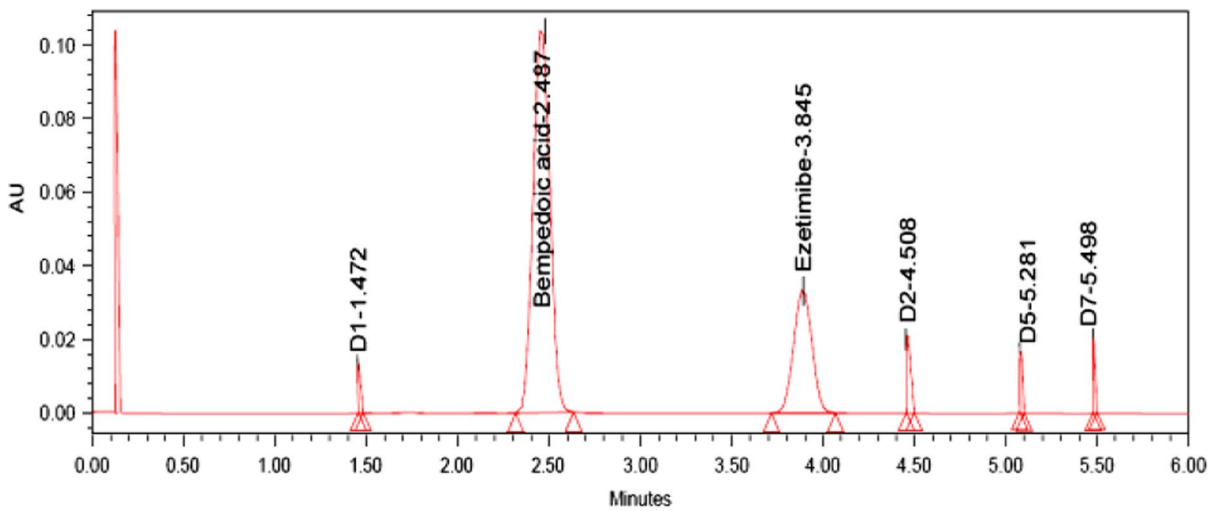
Reduction degradation of selected drugs was studied in 30% sodium bisulfate solution, 18.5% of Bempedoic acid and 16.4% of Ezetimibe degradation was observed using UPLC, and one DP1 degradation product was formed.



UPLC Acid degradation

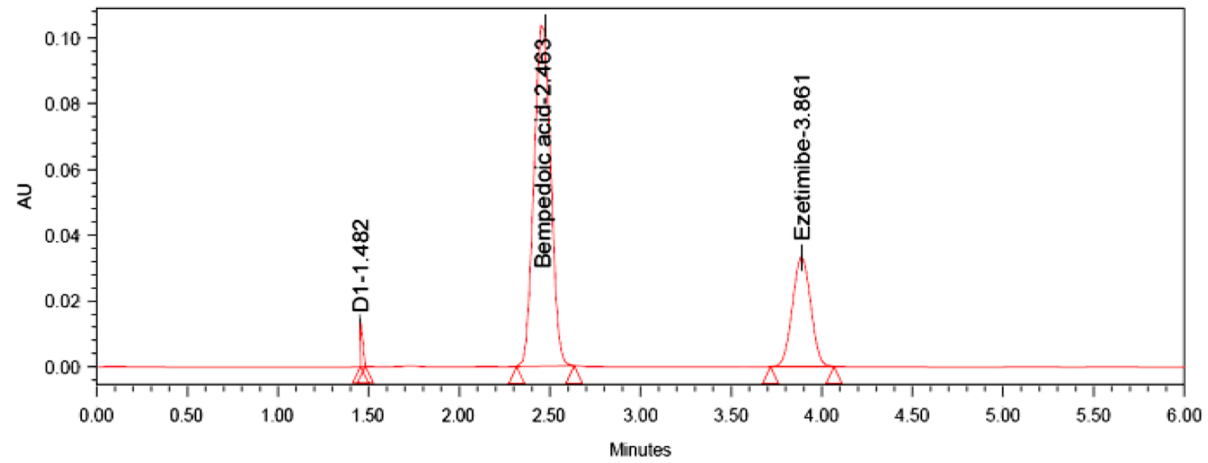


UPLC Alkali degradation

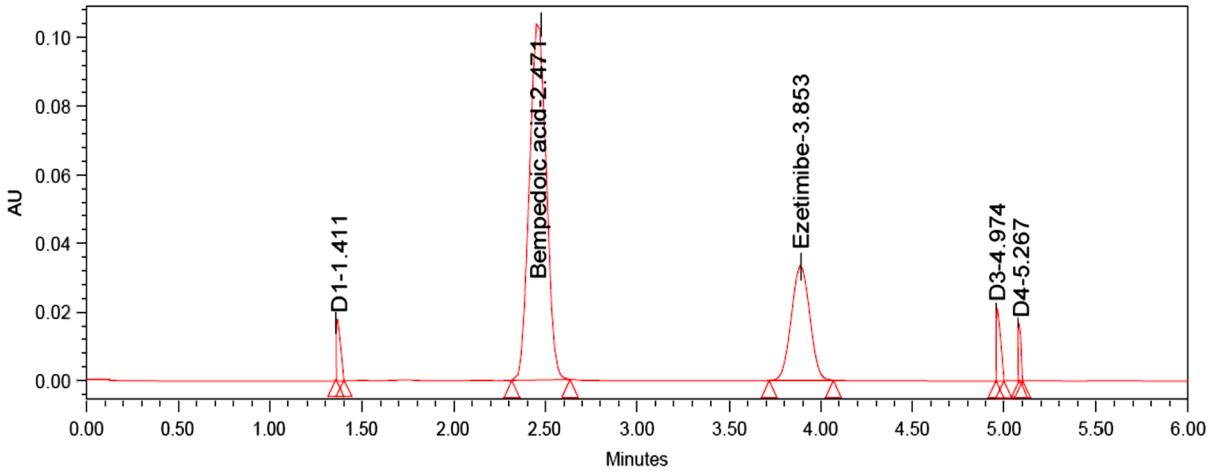


UPLC Peroxide degradation

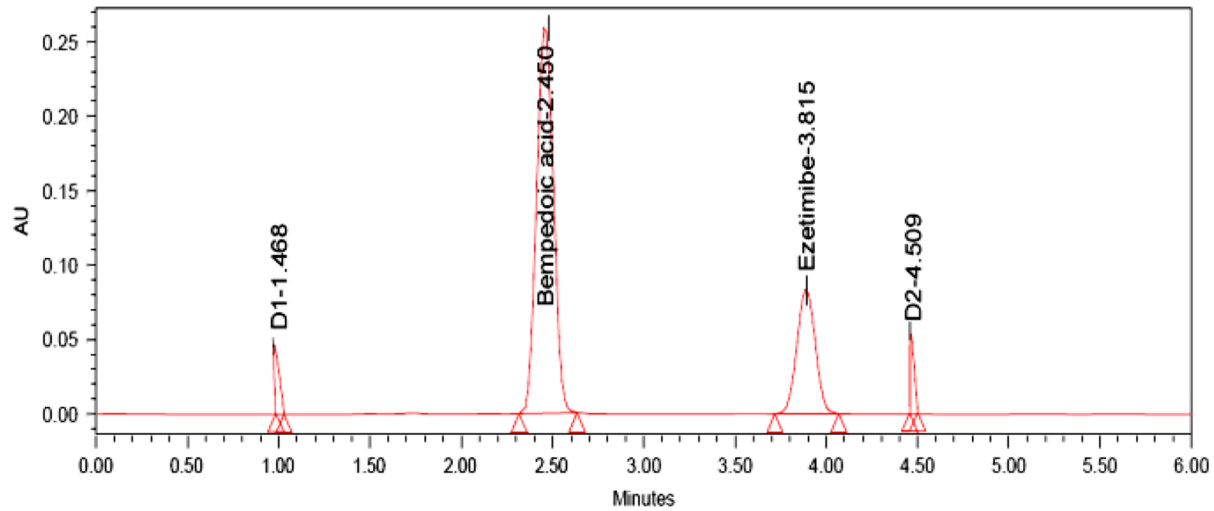
Fig. 5 UPLC degradation chromatograms



UPLC Reduction degradaiton



UPLC Photo degradation



UPLC Thermal degradation

Fig. 5 continued

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	180	0.41	10	0.33
Flow Minus	180	0.37	10	0.82
Organic Plus	180	0.78	10	0.51
Organic Minus	180	0.52	10	0.78
Wavelength Plus	180	0.42	10	0.84
Wavelength Minus	180	0.39	10	0.92

CC correlation coefficient

% REC-% Recovery

%RSD: Relative standard deviation

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, alkali, 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 represents 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, alkali, 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 C_3H_8O from m/z 123). The MS/MS experiments combined with correct mass evaluations have confirmed the proposed scheme.

DP3: Scheme 3 shows the fragmentation mechanism **DP3** of m/z 427 with molecular formula $C_{24}H_{23}F_2NO_4$, which was noticed under photolytic conditions. The MS spectrum displays abundant product ions at m/z-274 (loss of $C_9H_{11}OF$), m/z-179 (loss of m/z C_6H_5F from m/z 274), m/z-93 (loss of C_3H_8O 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 $C_9H_{11}OF$), m/z-173 (loss of m/z C_6H_5F from m/z 346), m/z-93 (loss of m/z C_3H_8O from m/z-153), and m/z-93 (loss of C_7H_8O 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 $C_9H_{11}F$), m/z-95 (loss of C_6H_5F from m/z 256), and m/z-94 (loss of C_6H_5OH 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_6H_{12}) and m/z-84 (loss of C_5H_{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_6H_5F), m/z-359 (loss of $C_8H_8O_2$ from m/z-493), m/z-265 (loss of C_6H_5F 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 (DP_1–DP_7) 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

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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.

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Declarations

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Competing interests
The authors declare that they have no competing interests.

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