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research article

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

A receptive and easily comprehended technique was evolved for simultaneous assessment of Bempedoic acid and Ezetimibe in tablet formulation by using UPLC. This technique involves the chromatographic separation with a C18 column of water symmetry (150mmx4.6mm, 3.5µ).A movable phase of 0.1% OPA (Ortho phosphoric acid) and acetonitrile in 50:50 v/v with 1mL/min flow rate and ambient temperature was used. UV observation was taken at 230 nm. Good results were obtained using these conditions of linearity over a range of 18-360 µg/mL of Bempedoic acid and 1-20 µg/mL of Ezetimibe using UPLC. By employing the above mentioned assay method, the results of other validation parameters of UPLC like system precision, method precision, accuracy, robustness and degradation studies were achieved within the permitted limit, according to ICH guidelines.

Keywords: Bempedoic acid; Ezetimibe; validation; Charcterization; UPLC; UPLC-MS

INTRODUCTION

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 together with diet and therefore the highest tolerated statin therapy in adults with heterozygous [4] familial hypercholesterolemia [5,6] or with established atherosclerotic cardiovascular disorder, [7,8] who needs 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 archilles tendon [17].

Ezetimibe is a pharmaceutical drug unused treats high blood cholesterol and certain other lipid abnormalities. Generally it is used alongside to 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, Ezetimibe/ Rosuvastatin. Usual consequences include upper respiratory infections, joint pain, diarrhea, and body exhaustion. Serious side effects include anaphylaxis [20, 21], liver problems, and depression and muscle breakdown. Its usage in pregnancy and breast feeding [22, 23] is unsafe. Ezetimibe effects by lowering cholesterol involvement within the intestines.

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 [29-36]. Seven DPs (DP1-DP7) were observed and characterized by UPLC-MS, since UPLC-MS is the first opinion on the quantification and characterization of drugs (Fig.1). The experiments provided

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details on the conditions under which the drug was unstable in order to prevent possible instability and suitable steps were taken during formulation.

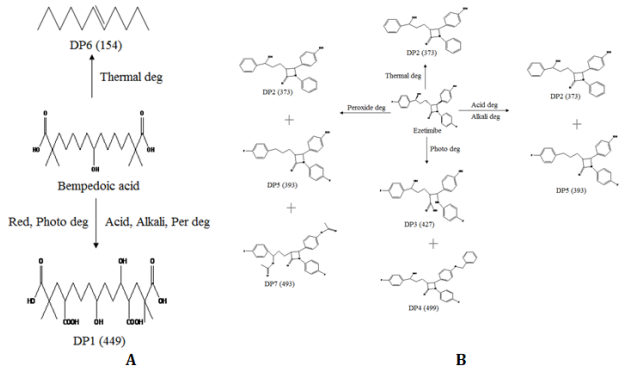

Figure 1: Schematic representation of degradation products of (A) Bempedoic acid and (B) Ezetimibe

MATERIALS AND METHODS

Reagents and Chemicals

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

Instrumentation

UPLC

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

UPLC & MS/MS Conditions

The chromatographic process involved the column of symmetry C18 (150x4.6mm, 3.5µ) with ambient temperature. An isocratic elution containing 50% of 0.1% OPA and 50% of acetonitrile was used as movable phase, flow rate of 1ml/min with dose volume of 20 µL was employed in UPLC.

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

Preparation of Standard Solution

A standard solution containing Bempedoic acid (180µg/mL) and Ezetimibe (10µg/mL) was prepared by dissolving 180mg of Bempedoic acid and 10 mg of Ezetimibe in sufficient amount of mobile phase and structure to the mark 100mL, 5mL .And further dilution was obtain up to 50 mL with diluents (50% of 0.1% Ortho phosphoric acid and 50% of Acetonitrile).

Preparation of Sample Solution

For quantitative analysis sample solutions were prepared by dissolving finely powdered drug samples, 180mg of Bempedoic acid and 10mg of Ezetimibe was transferred into 100mL volumetric flask and add 70ml of diluents and ultra sonicated to15 minutes and diluted upto100 mL mark with diluents. Further diluted to 50 mL, with 5mL of the sample stock solution with diluents finally, filter the solution by utilizing 0.45µ syringe before injecting into the LC column.

Method Validation

The systematic technique UPLC was confirmed by evaluating the parameters like system suitability, linearity, accuracy, limit of detection, limit of quantification, robustness etc., 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 like 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 concentration levels (10%, 25%, 50%, 75%, 100%, 125%, 150% and 200%).Accuracy was studied in three different levels of 50%, 100% and 150%. Finally % of recovery and % of RSD was calculated.

PRECISION

Precision is of three types namely,

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

Method Precision: Six individual sample solutions of Bempedoic acid and Ezetimibe were injected and calculated % recovery and % RSD.

Intermediate Precision: Inter-day precision study was managed for sample solutions of Bempedoic acid and Ezetimibe and calculated % recovery, % RSD.

Robustness

This technique was studied by changing the flow of ±20%, organic phase of ±10% and wavelength of ±5nm.

LOD and LOQ

LOD means little quantity of analyte during a sample which will be detected while LOQ explain about the little quantity of analyte during a sample which will be observed with tolerable precision accuracy. Limit of detection and limit of

quantification for Bempedoic acid and Ezetimibe are determined by injecting progressively low concentrations of ordinary solutions using the developed UPLC method. The limit of detection and limit of quantification are calculated as 3 s/n and 10s/n respectively as per ICH guidelines where s/n indicates signal-to-noise.

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 and therefore the peak purity of the principle peak shape shall pass. Forced degradation work was performed by different kinds of stress to get the percentage degradation of about 20%.

Acid Degradation

In acid degradation the sample having add 5ml of 1N HCl into a 100ml volumetric flask heat the flask on water bath at 60°C for 30min., allowed to cool room temperature and neutralize with 5ml of 1N NaOH. Then made up to the mark with diluent. Further dilute 5ml of above solution into 50ml volumetric flask with diluent then filtered and injected into UPLC-MS system.

Alkali Degradation

In alkali degradation the sample having add 5ml of 1N NaOH into a 100ml volumetric flask heat the flask on water bath at 60°C for 30min, allowed to cool room temperature and neutralize with 5ml of 1N NaOH. Then made upto the mark with diluent. Further dilute 5ml of above solution into 50ml volumetric flask with diluent then filtered and injected into UPLC-MS system.

Peroxide Degradation

In peroxide degradation sample having add 5ml of 30% hydrogen peroxide into a 100ml volumetric flask allowed to room temperature for 30min. after that heat on a water bath 60°C for 30min. allowed to cool room temperature. Then made upto the mark with diluent, further dilute 5ml of above solution into 50ml volumetric flask with diluent then filtered and injected into UPLC-MS system.

Reduction Degradation

In reduction degradation sample having add 5ml of 10% sodium bisulphate solution into a 100ml volumetric flask heated on a water bath 60°C for 30min. allowed to cool room temperature. Then made upto the mark with diluent, further diluted 5ml of above solution into a 50ml volumetric flask with diluent 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 6hr. After that equivalent weight of 180µg/ml of Bempedoic acid and 10µg/ml of Ezetimibe sample was weighed and transferred into a 100ml volumetric flask, dissolved in a diluent then make upto the mark with diluent. Further dilute 5ml of above solution to 50ml volumetric flask with diluent.

Photolytic Degradation

In photolytic degradation tablets are crushed finely to powder form and 1gm sample exposed to photo light UV 200W-hrs and fluorescence light 1.2 million lux-hours. After that equivalent weight of 180µg/ml of Bempedoic acid and 10µg/ml of Ezetimibe sample was weighed and transferred into a 100ml volumetric flask, dissolved in a diluent then make upto the mark with diluent. Further dilute 5ml of above solution to 50ml volumetric flask with diluent.

RESULTS AND DISCUSSION

An isocratic elution of Bempedoic acid and Ezetimibe involves symmetry C18 column with a flow of 1ml/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 brought at 230nm.

System Precision

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

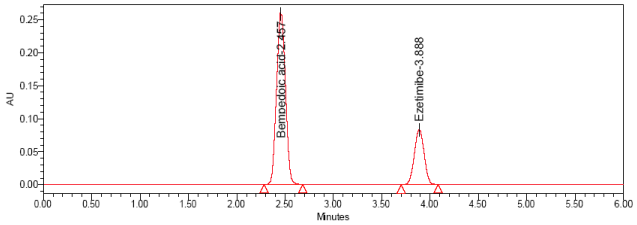

Figure 2: Standard Chromatogram of UPLC

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 of the blank samples represents in Fig. 3. The justified technique exhibited that the selected drugs were eluted without involvement of peaks occurred by the excipients in the market products.

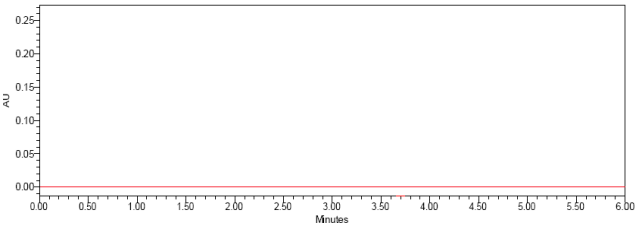

Figure 3: Blank Chromatogram of UPLC

Linearity

Linearity of the tactic was evaluated by preparing a typical solution containing 180 µg/ml of Bempedoic acid and 10 µg/ml

of Ezetimibe. Successive dilutions were carried out to the given dilutions at 10, 25, 50, 75, 100, 125, 150 and 200% of the selected concentrations was injected into UPLC (Fig.4). The calibration curves were linear throughout the concentration series of Bempedoic acid and Ezetimibe. The values of linearity were tabulated in Table. 1. The coefficient of correlation values of both analytes were 0.9997 and 0.99964 in Calibration curve for Bempedoic acid and Ezetimibe.

Accuracy

Accuracy depends on recovery studies, which were administered at three different concentration levels (50%, 100% and 150% levels). APIs with concentration 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 share recovery values were observed to be within the range of 98%-102% and the results were shown in Table .2.

Precision

Precision of this analysis was assessed in terms of method and intermediate variations. The intraday studies were calculated by executing six repeated sample solutions of Bempedoic acid and Ezetimibe to equivalent day under the equivalent experimental conditions. Intermediate precision of the tactic was administered within the same laboratory by studying the analysis with different analysts and different instruments. 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 furnished in Table 3.

LOD and LOQ

LOD and LOQ were separately determined by calibration curve method; LOD and LOQ of the compounds were calculated by injecting continuous lower accumulation of standard solutions using the developed UPLC method. The LOD values for Bempedoic acid and Ezetimibe were observed as 0.18 µg/mL, 0.01 µg/mL and s/n values were 7 and 4 respectively. LOQ values were 1.8 µg/mL and 0.1 µg/mL and 27, 21were the s/n values respectively.

Robustness

As per ICH norms, deliberate variations were made within the method parameters like change in flow (±20%), organic content in the mobile phase (±10%), and wavelength of detection (±5nm) .So there is no tactic capacity to stay unaffected of system suitability. Table. 4 show the robustness of the tactic was 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 had showed that the tactic was strong.

Stability

To assess the steadiness of the sample, a solution was analyzed initially for 24 hrs at different intervals of time. No significant degradation was observed during this era and therefore the mean deviation and mean was not quite to 5.0%. Suggesting that the solutions were stable for a minimum time period of 24hrs, which was sufficient for the entire analytical procedure for UPLC. Table .6 represents overall method validation results of Bempedoic acid and Ezetimibe.

Forced Degradation studies of Ezetimibe and Bempedoic acid

According to ICH stability guidelines various types of forced conditions i.e. Thermal, Basic, Acidic, Oxidative, photolytic and reductive Forced degradation studies were performed (Fig.5). A seven number 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. Table .5 represents degradation results of Bempedoic acid and Ezetimibe.

Acid Degradation

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

Alkali Degradation

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

Peroxide Degradation

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

Reduction Degradation

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

Thermal Degradation

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

Photolytic Degradation

Sample was exposed to sunlight for 12 hrs and 16.2% Bempedoic acid, 16.8% of Ezetimibe was observed in UPLC and three degradation products DP1, DP3 and DP4 were formed.

Collision induced dissociation of Bempedoic acid and Ezetimibe

Scheme 1: Shows the fragmentation mechanism of DP1,the ESI spectrum showed 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. Fig.6. & Fig.7 represents Mass fragmentation schemes and MS spectras degradation products.

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 C3H8O from m/z 123). The MS/MS experiments combined with correct mass evaluations confirmed the proposed scheme.

Scheme 3: Shows the fragmentation mechanism DP3 of m/z 427 with molecular formula C24H23F2NO4 which was noticed under photolytic condition. The MS spectrum displays abundant product ions at m/z-274 (loss of C9H11OF), m/z-179 (loss of m/z C6H5F from m/z 274), m/z-93 (loss of C3H8O from m/z 153), and m/z-85 (loss of phenol from m/z 179). The MS/MS measurements combined with correct mass evaluations confirmed the proposed scheme.

Scheme 4: Shows the fragmentation mechanism for DP4 of m/z-499 which was noticed at photolytic degradation condition. The spectrum displays abundant product ions at m/z-346 (loss of C9H11OF), m/z-173 (loss of m/z C6H5F from m/z 346), m/z-93 (loss of m/z C3H8O from m/z-153), and m/z-93 (loss of C7H8O from m/z 173). The MS/MS experiments combined with correct mass evaluations confirmed the proposed scheme.

Scheme 5: Shows the fragmentation mechanism for DP5 of m/z-393.4 which was noticed at acid, alkali and peroxide degradation condition. The spectrum displays abundant product ions at m/z-137 (loss of C9H11F), m/z-95 (loss of C6H5F from m/z 256), and m/z-94 (loss of C6H5OH from m/z 161). The MS/MS experiments combined with correct mass evaluations have confirmed the proposed scheme.

Scheme 6: Scheme 6 shows the fragmentation mechanism for DP6 of m/z-154 which was noticed at thermal degradation condition. The spectrum displays abundant product ions at m/z-72 (loss of C6H12) and m/z-84 (loss of C5H12). The MS/MS experiments combined with correct mass evaluations have confirmed the proposed scheme.

Scheme 7: Scheme 7 shows the fragmentation mechanism of degradation product 7 of m/z-493 which was noticed at peroxide degradation condition. The spectrum displays abundant product ions at m/z-399 (loss of C6H5F), m/z-359 (loss of C8H8O2 from m/z-493), m/z-265 (loss of C6H5F from m/z-359), m/z-205 (loss of C11H14FO2 from m/z-399), and m/z-71 (loss of C11H14FO2 from m/z-265). The MS/MS experiments combined with correct mass evaluations have confirmed the proposed scheme.

CONCLUSION

In this study, a complete 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, assessibility, 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, 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.

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