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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF BEMPEDOIC ACID AND ROSUVASTATIN CALCIUM IN ITS TABLET DOSAGE FORM.

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ABSTRACT

For the simultaneous estimation of Bempedoic acid and Rosuvastatin calcium in tablet dosage form, a practical, sensitive RP-HPLC technique has been developed and validated by choosing chromatographic parameters. On an Inertsil ODS C18 (100 x 4.6 ID, 3 µm) with mobile phases containing a mixture of 0.1%TFA in water: ACN (40:60, %v/v), isocratic elution mode was used to separate the drugs Bempedoic acid and Rosuvastatin calcium from their process- and degradation- related substances. With a detection wavelength of 220 nm, the flow rate was set to 0.6 mL/minute, and the injection volume was set at 70 µL with a 10-minute run time. The developed method's appropriateness was examined and confirmed in accordance with the ICH (Q2 R2) guidelines for studies on specificity, Linearity, Accuracy, Precision, Detection limit, Quantification limit and Robustness. Drug compounds such as Bempedoic acid and Rosuvastatin calcium were treated to heat, hydrolysis, humidity, peroxide, and photolytic stress conditions to observe the degradation products. Bempedoic acid and Rosuvastatin calcium had retention times of 7.24 minutes and 4.11 minutes, respectively. For Bempedoic acid and Rosuvastatin calcium, linearity was found in the concentration ranges of 180-540 µg/mL and 40-120

µg/mL, respectively. For Bempedoic acid and Rosuvastatin calcium, the percentage recoveries ranged from 99.08-101.5% and 99.37-101.58%, respectively. The method was proven to be useful for determining and validating the presence of Bempedoic acid and Rosuvastatin calcium in tablet dosage form.

KEYWORDS: RP-HPLC, Bempedoic acid, Rosuvastatin calcium.

1. INTRODUCTION

The anti-hyperlipedemic medication bempedoic acid (BMP) [8-hydroxy-2,2,14,14-tetramethylpentadecanedioic acid] comes in 180 mg doses for oral use.[1,5] It is prescribed to treat familial atherosclerosis and hypercholesterolemia symptoms and indicators.[6] Bempedoic acid's molecular weight is 344.49 g/mol and its empirical formula is C19H36O5.[7,8] (Figure 1) Rosuvastatin calcium (RSV) Calcium bis((3R,5S,6E)7-[4-(4-fluorophenyl)2-(N-methylmethanesulfonamido)-6-(propan-2-yl) pyrimidin-5-yl]3,5-dihydroxyhept-6-enoate is a 40 mg oral. HMG-CoA Reductase Inhibitor that has antihyperlipidemic properties.[9-14] It is used for the treatment of Certain bodily manifestations are caused by various forms of hypercholesterolemia.[15] For instance, arcusenilis (white or grey discoloration of the peripheral cornea), xanthomta (deposition of yellowish cholesterol-rich material) of the tendons. The empirical formula for Rosuvastatin calcium is C44H54CaF2N6O12S2 and its molcular weight is 1001.1 gm/mol.[16-21] (Figure 2) According to the literature survey, the analytical methods like UV-visible spectrophotometry, HPLC, HPTLC, UPLC, LC-MS and stabilityindicating have been reported for bempedoic acid (Figure 1) and rosuvastatin calcium (Figure 2) individually.[22-27] Whereas Two RP-HPLC method has been published till date for simultaneous stimation of Bempedoic acid and Rosuvastatin calcium in combineddosage orm.[28,29] Therefore, it was thought of interest to develop and validate stability indicating RP-HPLC method for simultaneous estimation of Bempedoic acid and Rosuvastatin calcium in pharmaceutical dosage form.

Rosuvastatin calcium is official in Indian Pharmacopoeia and United States Pharmacopoeia. The present study involves development and validation of liquid chromatographic method for the estimation of BMP and RSV in combined dosage form.[30]

2. EXPERIMENTAL METHODS

2.1 Chemicals and reagents used

Potassium Dihydrogen Phosphate (Merck Life Science Pvt. Ltd.), Orthophosphoric acid (Spectrochem), Trifluoroacetic acid (Merck Life Science Pvt. Ltd.), HPLC grade methanol (Rankem), HPLC grade acetonitrile (Rankem), HPLC grade water (Merck Life Science Pvt. Ltd.), rosuvastatin calcium (gift sample, Zydus Lifesciences Ltd., Ankleshwar), bempedoic acid (gift sample, ZCL Chemicals Ltd., Ankleshwar).

HPLC INSTRUMENT

Make: Shimadzu

Model: LC 2010 CHT

Injector: 100µL fixed loop.

Detector: UV Detector

Software: LC Solution

2.2 Preparation of mobile phase

0.1% TFA in water: To prepare a 0.1% TFA solution in water, carefully pipette 1 mL of trifluoroacetic acid into 1000 mL of HPLC-grade water in a clean flask. Sonicate the solution for 10–15 minutes to ensure uniform mixing and remove air bubbles. Next, set up a vacuum filtration apparatus with a 0.45-micron filter paper and filter the solution to remove any particulates. Once filtered, transfer the solution into a clean, airtight container, label it, and store it properly for use in applications like HPLC analysis.

2.3 Preparation of standard solution

A. Rosuvastatin stock solution (400 ppm)

About 42 mg of Rosuvastatin calcium, equivalent to 40 mg of rosuvastatin, was dissolved in methanol and diluted to 100 mL to prepare a stock solution.

B. Bempedoic acid stock solution (1800 ppm)

About 180 mg of Bempedoic acid was dissolved in methanol and diluted to 100 mL to prepare a stock solution.

C. Diluent preparation

The Mobile phase was used as Diluent.

2.4 Preparation of sample solution

To assess the concentration of Rosuvastatin and Bempedoic acid present in Rozucor B 40 tablets, a sample of the powdered tablets was dissolved in methanol, sonicated, and diluted to a specific volume. The solution was then filtered, and a portion of the filtrate was further diluted to achieve final concentrations of 80 ppm Rosuvastatin and 360 ppm Bempedoic acid.

2.5 Degradation Studies

The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance.[31] The aim of work was to perform the stress degradation studies on the BMP and RSV using the proposed method. Drug products were subjected to stress conditions like acid, alkali, oxidative, thermal and photolytic degradation. (table 1 & 2).

2.5.1 Acid Degradation

A mixture was prepared by combining 2 mL of a stock solution of Rosuvastatin and 2 mL of a stock solution of Bempedoic acid in a 10 mL of flask. To this mixture, 1 mL of 1 N HCl was added, and solution was allowed to react for 6 hrs. after the reaction, acidic mixture was neutralized by adding 1 mL of 1 N NaOH. Finally, the volume was adjusted with diluent.

2.5.2 Alkali Degradation

A mixture was prepared by combining 2 mL of a stock solution of Rosuvastatin and 2 mL of a stock solution of Bempedoic acid in a 10 mL flask. To this mixture, 1 mL of 1 N NaOH was added, and the solution was allowed to react for 6 hours. Following the reaction, the basic solution was neutralized by adding 1 mL of 1 N HCl. Finally, the volume was adjusted with diluent.

2.5.3 Oxidative Degradation

A solution was prepared by combining 2 mL of a stock solution of Rosuvastatin and 2 mL of a stock solution of Bempedoic acid in a 10 mL flask. To this mixture, 2 mL of 3% hydrogen peroxide was added, and the solution was allowed to react for 6 hours.

2.5.4 Thermal Degradation

API and tablet powders were kept in hot air oven at 65°C for 6 hours. Solutions were then made according to test preparation and then injected into HPLC.

2.5.5 Photolytic Degradation

API and tablet powders were kept in UV chamber for two days. Solutions were then made according to test preparation and injected into HPLC.

2.6 VALIDATIN OF RP-HPLC METHOD

2.6.1 Linearity

The peak area was measured after various concentrations of solution were produced and injected into the chromatographic apparatus. Plot the peak area vs concentration on a graph with the concentration on the X-axis and the peak area on the Y-axis, then find the correlation coefficient.

2.6.2 Precision

a.) Repeatability: Six injections of the standard solution were made, and each time the area was measured in an HPLC. It was discovered that the %RSD for the region of six replicate injections fell between the required bounds.

b.) Intraday precision: By examining samples from the same batch in nine determinations using three Standard solutions and three replicates (n=3) each on the same day, the accuracy of the devised method was evaluated.

c.) Interday precision: Samples from the same batch were analysed in nine determinations using three Standard solutions and three duplicates (n=3) each on different days in order to evaluate the accuracy of the established method.

2.6.3 Accuracy

In this, standard solutions of Bempedoic acid and Rosuvastatin calcium were prepared at concentration levels of 50%, 100%, and 150% of the target value. These solutions were then injected into the chromatographic system for analysis. The quantities of each compound were measured, and recovery values were calculated for each concentration level. Finally, both individual and mean recovery percentages were determined to assess the accuracy of the method.

2.6.4 Robustness

To assess robustness, deliberate changes were made to the wavelength, flow rate, and column temp. Stdard and sample injections of Bempedoic acid and Rosuvastatin calcium were

performed under these altered chromatographic conditions. The results showed no significant changes in resolution, tailing factor, asymmetry, or plate count.

2.6.5 System suitability

In the standard solution, the tailing factor for the peaks caused by bempedoic acid and rosuvastatin calcium shouldn't be greater than 1.5. The Bempedoic acid and Rosuvastatin calcium peaks in standard solution should have at least 2000 theoretical plates. By injecting five distinct preparations of the Bempedoic acid and Rosuvastatin calcium standard, the method's appropriateness for the system was verified. The system suitability parameters were examined.

2.6.6 DL and QL

The DL (Detection Limit) was estimated from the set of linearity of the procedure is confirmed using calibration curves.

The DL may be calculated as

DL = 3.3 × (S.D/Slope)

Where, SD = Std deviation of the Y-intercept of Six calibration curve

Slope = Mean slope of 6 calibrationcurves

The QL (Quantitation Limit) was estimated from the set of Six calibration curves used to determine method linearity.

The QL may be calculated as,

QL = 10 × (S.D/Slope)

Where, SD = Standard deviation of the Y-intercept of Six calibration curve

Slope = Mean slope of Six calibration curves

3. RESULTS AND DISCUSSION

3.1 Forced degradation studies

To assess the stability of Bempedoic acid and Rosuvastatin calcium and the method's ability to separate them from their degradation products, the compounds were exposed to various stress conditions. These included acidic conditions (1N HCl and 1N NaOH), alkaline conditions (1N NaOH and 1N HCl), [The degradation results, showing 17.7% under acidic conditions and 23.7% under basic conditions, indicate that Bempedoic acid and Rosuvastatin calcium are more prone to breakdown in alkaline environments. In acidic conditions, the compounds exhibit moderate stability, likely due to reduced susceptibility to protonation or acid-catalysed hydrolysis. However, the higher degradation in basic conditions suggests that

alkaline hydrolysis plays a significant role in their degradation, possibly targeting functional groups like esters or amides. This higher reactivity in alkaline media reflects the compounds' greater vulnerability to nucleophilic attack in basic environments.] peroxide degradation (3% H2O2) [the peak which is obtained at 2-3 min is due to the hydrogen peroxide used, that peak is of peroxide], thermal degradation (65°C for 6 hours), and photolytic degradation (UV chamber for 48 hours). The results are summarized in (Tables 1 and 2). The chromatograms illustrating the degradation studies of Bempedoic acid and Rosuvastatin calcium are shown in (Figures 3, 4, 5, 6, and 7.)

3.2 Validation

The RP-HPLC method was validated according to the Q2 (R2) guidelines of the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH). The results are presented below.

3.2.1 **Linearity**

To assess the method's linearity, a study was conducted for both drugs at five different concentration levels. The linearity ranges for Bempedoic acid and Rosuvastatin calcium were 180-540 µg/ml and 40-120 µg/ml, respectively, as shown in (Tables 3 and 4.) Each dilution was injected into the column, and the linearity curves were illustrated in (Figures 8 and 9.) The correlation coefficient (R^2) should be at least 0.999, and the obtained value of 0.999 met this criterion.

3.2.2 **Precision**

a.) Repeatability: The data of repeatability for BMP and RSV are shown in (Table 5).

b.) Intraday precision: The data for intraday precision for BMP and RSV are depicted in the (Table 6)

c.) Interday precision: The data for interday precision for BMP and RSV are depicted in the (Table 7)

3.2.3 **Accuracy**

The % Recovery study was conducted using the Standard Addition Method. Known quantities of BMP and RSV standard solutions were added at 50%, 100%, and 150% levels to pre-measured sample solutions containing BMP (180 µg/ml) and RSV (40 µg/ml). The amounts of BMP and RSV were determined using the regression equation from the

calibration curve. The low standard deviation values indicate the accuracy of the proposed method. The recovery study results are presented in (Table 8).

3.2.4 Robustness

Some deliberate modifications were made to the test parameters to carry out the robustness investigation. typical variations are Wavelength: ±1 nm, Flow rate: ± 0.1 mL/min, Column temperature: ±2 °C (Table 9 and 10).

3.2.5 DL and QL

The DL and QL for BMP was found to be 0.0661µg/mL and 0.2003µg/mL respectively.
The DL and QL RSV was found to be 0.1464µg/mL and 0.4439µg/mL respectively.

4. CONCLUSION

An isocratic novel RP-HPLC method was developed for simultaneous determination of Bempedoic acid and Rosuvastatin calcium by using column Inertsil ODS C18 (100 x 4.6 id, 3 µm) as a stationary phase, along with a mixture of 0.1%TFA in water: ACN (40:60, %v/v) as a mobile phase at a flow rate of 0.6 mL/min. Temperature was maintained at 25°C and the detection wavelength at 220 nm. Bempedoic acid and Rosuvastatin calcium respective retention times were found to be 7.24 mins and 4.11 mins. The results of a forced degradation experiment demonstrated that there was no interference between the peaks of the degradation impurity and the analyte, which indicates that the developed method is stable.

The method yielded linear results in the range of 180-540 µg/ml for Bempedoic acid and 40-120 µg/ml for Rosuvastatin calcium, according to method validation carried out in accordance with ICH Q2 (R2) recommendations. For Bempedoic acid and Rosuvastatin calcium, the percentage recoveries ranged from 99.08-101.5% and 99.37-101.58% respectively.

Low levels of %RSD during the repeatability, intraday precision, and interday precision studies demonstrate the precision of the developed method. Since the %RSD remained below 2 even after small, intentional changes to the method, the developed method is robust. The assay value of Bempedoic acid and Rosuvastatin calcium was 101.1% and 101.5% respectively.

After the experiment was finished, it was determined that the suggested method is accurate, new, simple, precise, linear, sensitive, robust, and stable for simultaneous estimation of Bempedoic acid and Rosuvastatin calcium in raw material as well as in tablet dosage form.

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Declarations

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Conflict of interest

All authors associated with this research work declared that there is no conflict of interest for publication of work.

All authors disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work.

Data availability statement:

The datasets generated and/or analyzed during the current study are available openly in google scholar, sci hub at reference number 9f1cefc4-d254-451e-8101.

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