A Validated RP-HPLC Technique for the Determination of Obeticholic Acid in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

*The RP-HPLC methodology was used to produce a simple, precise, and accurate method for estimating obeticholic acid. Successful elution of the drug was achieved using STD BDS C18 (150mm*4.6mm 5µm) column with 0.01N KH_2PO_4: acetonitrile in a 70:30 % v/v ratio as mobile phase. The ultraviolet detection was monitored at a wavelength of 215 nm at flow rate 1.0 mL/min. The validation of proposed method was carried for linearity, precision, accuracy, limit of detection, limit of quantification and robustness were determined in accordance with ICH guidelines. The system suitability parameters were investigated by injecting the standard six times, with the results falling into the acceptance standards. Between 25 and 150 percent levels, a linearity study was done, and the R^2 value was found to be 0.999. Precision for repeatability was determined to be 0.8% and 0.7% for moderate precision. The LOD and LOQ values were 0.15 µg/ml and 0.44 µg/ml, respectively. The marketed formulation was assayed using the aforesaid method, and 99.50 % of it was present. Obeticholic acid degradation investigations were performed under all situations, the purity threshold was more than the purity angle and within the suitable range. The developed HPLC method provides short analysis time, high reproducibility and high sensitivity.*

Key Words: *Obeticholic acid, RP-HPLC, Buffer, Validation*

Introduction

An adult patient's primary biliary cholangitis may be treated with obeticholic acid, a bile acid analogue and farnesoid X receptor agonist, if they were not responding to UDCA or becoming intolerable to it. PBC, or primary biliary cirrhosis, is a chronic, progressive disease that damages the liver and frequently progresses to end-stage liver failure, necessitating liver transplantation [1,2]. Obeticholic acid belongs to hepatoprotective category. As a farnesoid-X receptor (FXR) agonist, obeticholic acid is used to treat this illness and may increase survival [3,4]. Ursodeoxycholic acid, which had previously been the standard therapy for this ailment, was approved in 2016 to be used in conjunction with other medications to treat primary biliary cholangitis [1]. Primary biliary cirrhosis is an autoimmune condition in which the liver and bile ducts gradually deteriorate, resulting in fibrosis and cirrhosis. Bile acids raise the possibility of bile duct fibrosis and injury [5]. Figure 1 shows the chemical structure of obeticholic acid.

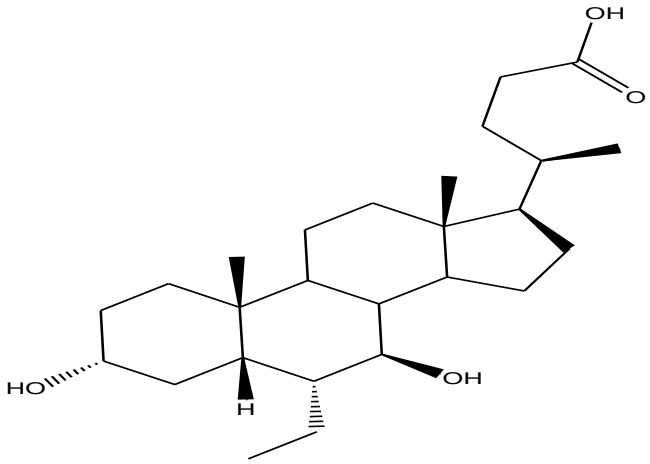

Figure 1. Structure of obeticholic acid

There were RP-HPLC methods that have been reported in the literature for the determination of obeticholic acid in pharmaceutical dosage form [4,7]. Bio analytical (LC-MS/MS) in biological fluids, and obeticholic acid measurement using an HPLC/UV method has been documented.[6]. There was no method that describes the stress studies for the determination of obeticholic acid, an attempt has been made to develop an RP-HPLC method that was simple, specific, rapid, precise, and economical method for the quantitative determination of obeticholic acid in pharmaceutical dosage form. This method was validated as per the guidelines of international conference on harmonization (ICH Q2 (R1).

Experimental

Chemicals and reagents

Obeticholic acid standard, acetonitrile (HPLC grade), HPLC grade water (Millipore), potassium dihydrogen phosphate, ortho phosphoric acid, sodium hydroxide (NaOH), hydrochloric acid (HCl) and hydrogen peroxide (H_2O_2) were purchased from Rankem, India.

Instrumentation

Waters 2695 HPLC with PDA detector 2996 was the instrument employed in the study. Empower 2 software was used to integrate and monitor it. Mettler Toledo, Model XP56, electronic balance was used. Elma S300H sonicator, brand model was utilized. Hot air oven by serve well instruments, model H02436 was used. A digital pH meter from Mettler Toledo and a UV-Visible chamber from Mack Equipment, Model: MK-2 were utilized.

Preparation of Standard Stock Solution

Obeticholic acid of 2.5 mg was accurately weighed and added to 25 ml volumetric flask. Then 75% of diluents were added and sonicated for 10 minutes. The flasks were filled with dilutants and labelled standard stock solution (100µg/ml obeticholic acid).

Preparation of Standard Working Solution

Obeticholic acid stock solution of 1ml was pipetted into a 10 ml volumetric flask and diluted with dilutant (10 µg/ml).

Preparation of Sample Stock Solution

Five tablets were weighed and the average weight of each tablet was calculated. The weight corresponding to one tablet was put into a 50 ml volumetric flask. After adding 50 ml of diluents, 25 minutes of sonication followed. Additionally, the volume was diluted and HPLC-filtered (using 200 µg/ml of obeticholic acid).

Preparation of Sample Working Solution

A sample working solution of obeticholic acid was prepared by transferring 0.5 ml of the filtered sample stock solution to a 10 ml volumetric flask and diluent was added to it (10 µg/ml of obeticholic acid).

Method Validation

The method was validated as per ICH guidelines, and the validation parameters included specificity, linearity, accuracy, precision, sensitivity (LOQ and LOD), and robustness.

System Suitability Parameters

System suitability was determined by introducing six replicate injections of the drug standard solution (10 µg/ml). Parameters like tailing factor, plate count and column efficiency were noted.

Specificity

The specificity was evaluated by comparing the placebo solution with the drug solution by injecting samples into the HPLC system. The resulting chromatograms were seen for the interference of placebo response with a drug peak response.

Linearity

Dissimilar drug standard solutions were made to evaluate the linearity by diluting the drug stock solutions with diluents in different concentrations of obeticholic acid ranging from 2.5 to 15 µg/ml. The linearity plot of the calibration curve was assessed by linear regression analysis.

Sensitivity

The lowest limit of detection and limit of quantification were determined by means of the subsequent equations based on the slope of the calibration and the SD of the responses using different concentrations of the standard stock solution.

Limit of detection= 3.3 × standard deviation of the response/ slope of calibration curve of the analyte.

Limit of quantification=10 × standard deviation of the response/ slope of calibration curve of the analyte.

Accuracy

Accuracy was determined at 50%, 100% and 150% by adding an acknowledged amount of sample stock solution of obeticholic acid to the standard stock solution. The percentages of recoveries were calculated.

Precision

Precision was studied as system precision, intraday and inter day. Intraday precision was determined by injecting six different concentrations of standard solutions in the same day. The peak area was measured, and the percent RSD was computed. Inter-day precision was measured by injecting six different concentrations of standard solutions three times a week for three days. The peak area was measured, and the percent RSD was computed.

Robustness

Robustness was estimated varying the flow rate, temperature, ratio of the mobile phase. Samples were injected into the system and the %RSD was determined.

Degradation

To conduct the forced degradation experiment, standard stock solutions of obeticholic acid was exposed to various stress conditions, including 1 ml of 20% H_2O_2 (for oxidative degradation), 1 ml of 2N HCl (for acidic degradation), and 1 ml of 2N NaOH (for basic degradation). The produced solutions were refluxed for 30 minutes at 60^{o}C. To examine the descent, the standard solutions were also subjected to UV radiation and temperature conditions. The resulting solutions were diluted to yield 10 µg/ml of obeticholic acid for degradation studies. To examine sample stability, 10 µl samples were fed into the system and chromatograms were obtained.

Chromatographic conditions

Method development was carried on Std BDS C18 (150x4.6mm, 5µm) column. Buffer (0.01N Potassium dihydrogen phosphate) and acetonitrile were mixed in a 70:30 v/v ratio. The analysis was performed in isocratic mode with a flow rate of 1.0 ml/min and an injection volume of 10

µl. The temperature of the column was 30°C, and the run time was 5 minutes. The data was collected at 215 nm detection wavelength with the Empower 2 software the data will be acquired. Obeticholic acid retention time was at 2.288mins with good resolution which was shown in Figure 2. The plate count and tailing factor were highly excellent, the technique conditions were optimized and the same conditions were used for validation.

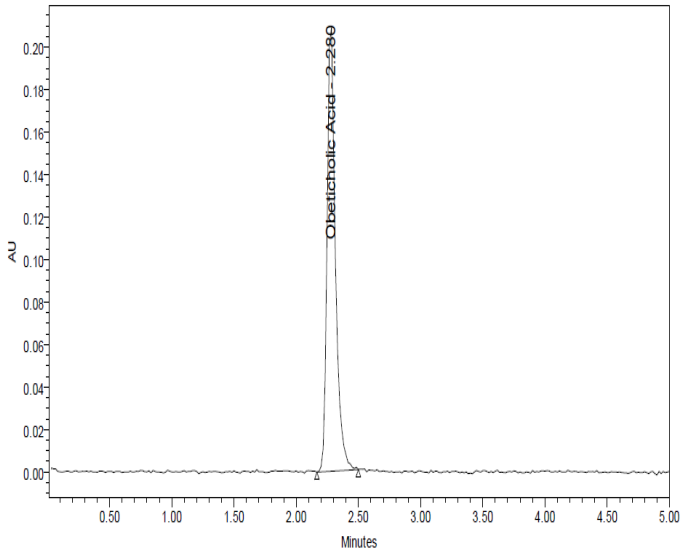

Figure 2. Optimized chromatogram of standard solution

RESULTS AND DISCUSSION

System Suitability Parameters

The system suitability parameters were assessed by making standard solutions of obeticholic acid (10 µg/ml) and injecting them six times. Peak tailing, resolution, and USP plate count were all determined. For three medications in combination, the USP Plate count exceeded 2000 and the tailing factor was less than 2. All of the system's appropriate parameters were passed and remained within the limitations. Table 1 shows the results.

Table 1. System suitability data of proposed HPLC method

	Obeticholic acid		
S. No	RT (min)	USP plate count	Tailing
1	2.266	5957	5714
2	2.272	5720	5648
3	2.273	5532	6054
4	2.274	1.31	1.34
5	2.280	1.36	1.37
6	2.282	1.34	1.33

Linearity

Analytical method linearity is defined as the ability of the method to obtain test results that are directly proportional to the analyte concentration, within a specific range. The mean peak area obtained from the HPLC was plotted against corresponding concentrations to obtain the

calibration graph. Six linear concentrations of obeticholic acid (2.5-15µg/ml) were injected in triplicate manner. Correlation coefficient obtained was 0.999 indicating a linear relationship between the concentration of analyte and area under the peak. The results were shown in Table 2 and Figure 3.

Table 2. Linearity data for obeticholic acid

Conc (µg/ml)	Peak area
2.5	245798
5	472284
7.5	672216
10	885284
12.5	1127901
15	1339175

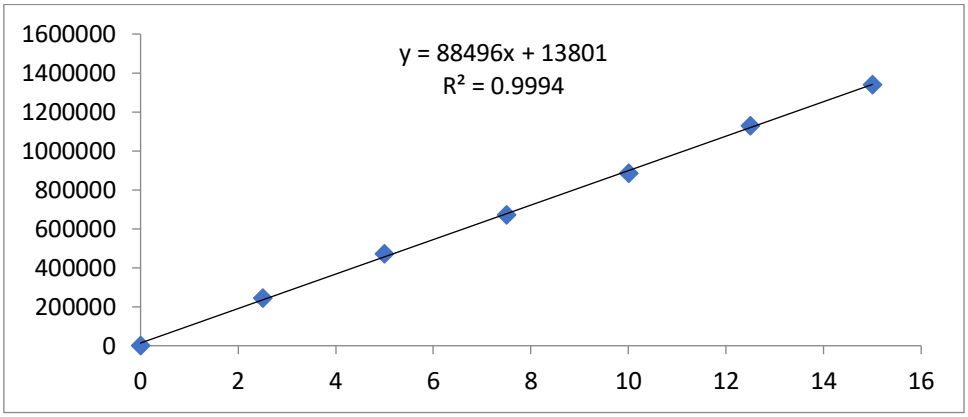

Figure 3. Standard calibration curve of obeticholic acid

Specificity

In the optimized method, the interference was checked. Obeticholic acid, had retention time of 2.269 minutes. There were no interfering peaks in the chromatograms of blank and placebo samples during the retention periods of the drug in the approach. As a result, this procedure was stated to be particular. Figures 4, 5, and 6 show the chromatograms for specificity.

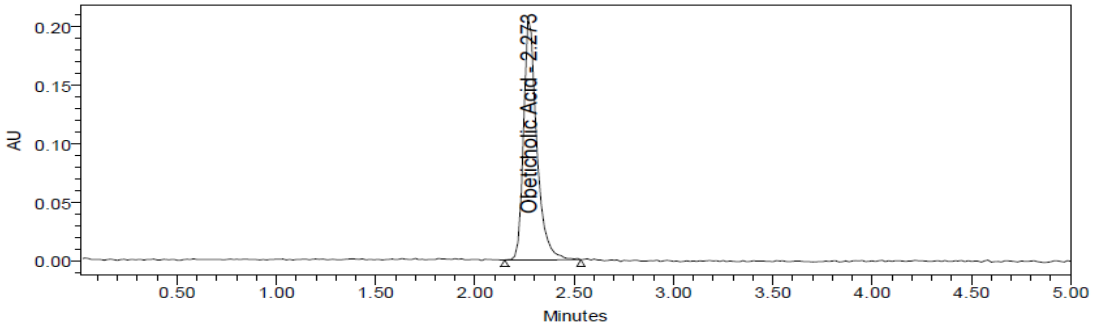

Figure 4. Chromatogram of standard solution

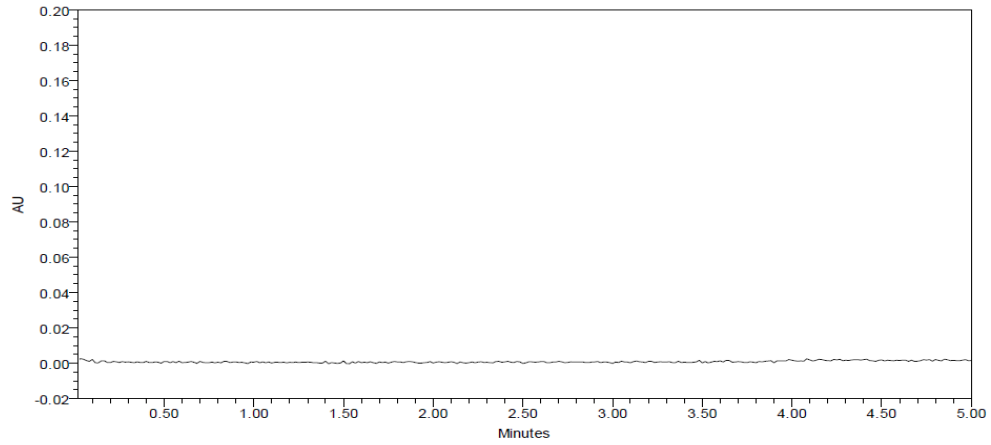

Figure 5. Chromatogram of blank solution

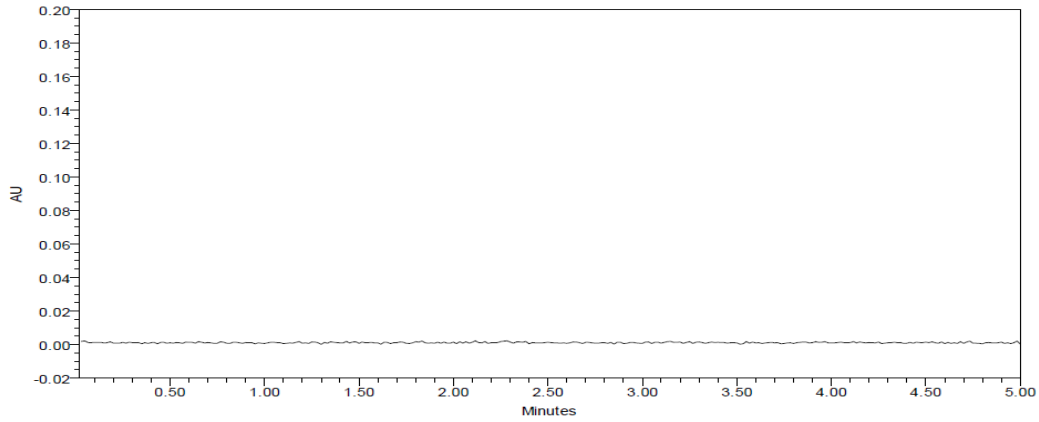

Figure 6. Chromatogram of placebo

Precision

Repeatability

From a sample stock solution, six working sample solutions of the same concentrations (10 µg/ml obeticholic acid) were created. Each injection was given from each solution. The average area, standard deviation, and % RSD for the medication were computed and found to be 0.8% for obeticholic acid. The system precision was passed for this procedure since the precision limit was less than "2 %." Table 3 shows the information results.

Table 3. Repeatability data for obeticholic acid

S. No	Obeticholic acid
1	908887
2	898461
3	906292
4	891821
5	894875
6	908636
Mean	901495
S. D	7419.7
%RSD	0.8

Intermediate Precision

From a sample stock solution, multiple samples were obtained, and six working sample solutions of the same concentrations (10 µg/ml of obeticholic acid) was prepared. The areas obtained are listed in Table 4 after each injection from each working sample solution was administered the day after sample preparation. The average area, SD, and % RSD for the medication was computed and found to be 0.7% for obeticholic acid. Because the precision limit was less than "2%" the intermediate precision was used for this procedure.

Table 4. Intermediate precision results for obeticholic acid

S. No	Obeticholic acid
1	864196
2	854899
3	856861
4	857175
5	860204
6	847446
Mean	856797
S. D	5613.3
%RSD	0.7

Accuracy

The accuracy of an analytical procedure expresses the closeness of results obtained by that method to the true value. The results of accuracy showed percentage recovery at all three levels in the range of 99.35–101.34% and the mean % recovery was found to be 100.54% (Table 5). The results of percentage recovery were within the accepted limits from 98.0% to 102.0%, which indicates the applicability of the method for routine drug analysis.

Robustness

Robustness conditions such as flow minus (0.9 ml/min), flow plus (1.1 ml/min), mobile phase minus (75:25 v/v), mobile phase plus (65:35 v/v), temperature minus (27°C), and temperature plus (33°C) were maintained, and samples (10 µg/ml obeticholic acid) was injected in duplicate. The % RSD was computed and determined to be within the acceptable range. Table 6 shows the data.

Assay

Obeticholic acid tablets had a label claim of obeticholic acid 10 mg (Brand Name: GILOTRIF) per unit formulation. The afore mentioned formulation was used for the assay. The average % assay achieved for obeticholic acid was 99.50%.

Degradation Studies

The forced degradation studies were performed to assess the stability of obeticholic acid under stressed conditions such as acid-alkali hydrolysis, thermal degradation and photo degradation by UV light. Peak purity test results obtained from the detector confirmed that the obeticholic acid peak was pure and homogeneous in all the analyzed stress samples.The results were shown in Table 7.

Table 5. Accuracy data for the proposed HPLC method

% Level	Amount spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	5	5.02	100.49	100.54%
	5	5.00	99.96	
	5	4.97	99.35	
100%	10	10.08	100.80	
	10	10.13	101.31	
	10	10.09	100.85	
150%	15	15.20	101.34	
	15	15.06	100.38	
	15	15.06	100.40	

Table 6. Robustness Data

S. No	Condition	% RSD of obeticholic acid
1	Flow rate (-) 0.9ml/min	0.8
2	Flow rate (+) 1.1ml/min	0.3
3	Mobile phase (-) 60B:40A	0.8
4	Mobile phase (+) 50B:50A	0.7

5	Temperature (-) 27°C	0.6
6	Temperature (+) 33°C	0.4

Table 7. Degradation studies results for obeticholic acid

S. No	Condition	Obeticholic acid % degraded	Obeticholic acid % obtained
1	Acid	92.44	7.56
2	Base	94.94	5.06
3	Oxidation	94.25	5.75
4	Dry heat	97.78	2.22
5	UV Light	98.56	1.44

Conclusion

The developed approach was validated in accordance with ICH recommendations. All validation parameters were found to be within acceptable limits. Hence, the approach was exact, linear, and resilient. The described approach may be successfully used for obeticholic acid bulk and pharmaceutical analyses in quality control laboratories. The method was robust enough to reproduce accurate and precise results under different chromatographic conditions.

Conflicts of Interest

Authors have no conflicts of interest to declare.

Acknowledgements

The author expresses sincere thanks to GITAM deemed to be university, Visakhapatnam for providing the necessary research facilities to carry out the research work.

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