

# Stability Indicating RP-HPLC Method Development and Validation for the Quantification of Obeticholic Acid in Bulk and its Pharmaceutical Dosage Form

R. Kiran Jyothi<sup>1</sup>, K.B. Chandrasekhar<sup>2</sup>, M. Balakrishnan<sup>3</sup>, B. Siva Sai Kiran<sup>4</sup>

<sup>1</sup>Research Scholar, Jawaharlal Nehru Technological University-Anantapur, Ananthapuramu, Andhra Pradesh, INDIA.

<sup>2</sup>Vice Chancellor, Krishna University, Machilipatnam, Andhra Pradesh, INDIA.

<sup>3</sup>Principal, Sri Seshachala College of Pharmacy, Puttur, Andhra Pradesh, INDIA.

<sup>4</sup>Assistant Professor, Jawaharlal Nehru Technological University-Anantapur, Ananthapuramu, Andhra Pradesh, INDIA.

## ABSTRACT

**Aim and Objectives:** The aim of present research work is to develop stability indicating validated RP-HPLC method for the quantification of Obeticholic acid in bulk and its pharmaceutical dosage form. **Materials and Methods:** Chromatographic method was carried on C<sub>18</sub> column (Ascentis 150mm x 4.6 mm, 5m). Mobile phase was prepared by mixing 0.1% OPA: Acetonitrile in the ratio of 60:40. The flow rate was 1.0 mL/min and the injection volume was 10  $\mu$ L. The absorbance maxima of obeticholic acid was measured at 210 nm. The retention time was found to be 2.90 min. **Results:** The method was proved to be specific and linear in the range of 2.5 - 15  $\mu$ g/mL with correlation coefficient of 0.999. The percentage RSD for precision was found to be less than 2 % and the mean percentage recovery was 100.08 %. All the validation parameters were statistically validated according to ICH guidelines and were found to be within acceptance criteria. **Conclusion:** The developed method was simple, specific, precise, accurate and robust. The described HPLC method can be successfully employed for the analysis of Obeticholic acid.

**Key words:** Obeticholic acid, RP-HPLC method, 0.1 % OPA, Acetonitrile, Validation, ICH guidelines.

## INTRODUCTION

Obeticholic acid belongs to hepatoprotective category.<sup>1</sup> It is a semi-synthetic bile acid, which acts as a farnesoid X receptor agonist and is used for treating primary biliary cholangitis.<sup>2</sup> Chemically obeticholic acid is a dihydroxy-5 beta-cholanic acid, 3 alpha-hydroxy steroid and 7 alpha-hydroxy steroid. It was derived from a chenodeoxycholic acid.<sup>3-5</sup> The key role of the farnesoid X receptor (FXR) as a regulator of bile and cholesterol metabolism in the liver, with preclinical data from numerous studies providing strong rationale for the advancement of FXR agonists as hepatoprotective therapeutics in chronic liver disease.<sup>6-8</sup> The chemical structure of Obeticholic acid was presented in Figure 1. Literature survey revealed that only a few analytical methods were reported for the

estimation obeticholic acid. Bio analytical methods (LC-MS/MS) have been reported for the quantification of obeticholic acid in biological fluids.<sup>9</sup> There is no RP-HPLC method for the analysis of obeticholic acid.

## MATERIALS AND METHODS

### Materials

Obeticholic acid pure drug was obtained as gift sample from Biophore pharma, Hyderabad. Obeticholic acid tablets (OCALIVA) were purchased from local market. HPLC grade acetonitrile, methanol and distilled water were purchased from Merck, Mumbai. Analytical grade phosphate buffer, potassium dihydrogen,

Submission Date: 12-09-2019;

Revision Date: 11-11-2019;

Accepted Date: 26-02-2020

DOI: 10.5530/ijper.54.2s.92

Correspondence:

Mrs. Kiran Jyothi

Research Scholar, Jawaharlal Nehru Technological University Anantapur, Ananthapuramu-+515002, Andhra Pradesh, INDIA.

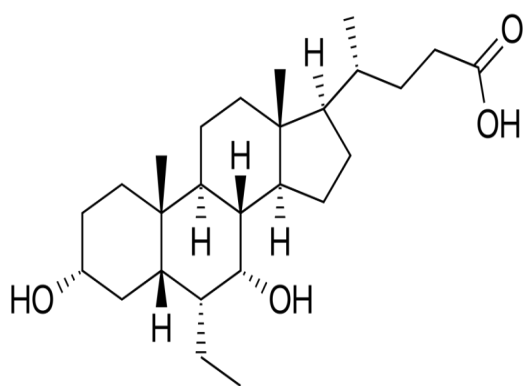
Phone: +91 8309839388

E-mail: jyothikiran460@

gmail.com



www.ijper.org



**Figure 1: Chemical Structure of Obeticholic acid.**

ortho-phosphoric acid were obtained from Rankem laboratories, Hyderabad.

### Instrument

The liquid chromatographic system was performed on WATERS HPLC 2965 system equipped with auto injector and PDA Detector. Empower software was used for data acquisition, processing and reporting.

### Chromatographic conditions

The method development was achieved on Ascentis C<sub>18</sub> column (150 x 4.6 mm, 5µm). Mobile phase was freshly prepared by mixing 0.1% OPA and acetonitrile in the ratio of 60:40 v/v. The flow rate was maintained at 1.0 mL/min. Detector wavelength was monitored at 210 nm and the injection volume was 10 µL and run time was kept 5 min.

### Preparation of standard stock solution

Accurately weighed 2.5 mg of Obeticholic acid was dissolved in 25 ml mobile phase (100µg/ml of Obeticholic acid standard stock solution). From the above standard stock solution 1 ml was transferred into a 10 ml volumetric flask and made up to the mark with mobile phase (10µg/ml of Obeticholic acid).

### Preparation of Sample stock solutions

Average weighed amount equivalent to of each tablet was calculated. Weight equivalent to 10 mg was transferred into a 100 ml volumetric flask and made up to the mark with mobile phase (100 µg/ml of Obeticholic acid). The solution was sonicated for 25 min and filtered by HPLC filters. Further pipette out 1 ml of filtered sample stock solution was transferred to 10 ml volumetric flask and made up with mobile phase (10 µg/ml of Obeticholic acid).

### Method validation

#### System suitability parameters

System suitability was evaluated in terms peak tailing and USP plate count by injecting 6 replicates of 10 µg/

mL Obeticholic acid standard drug concentration. The calculated % RSD for the area of six standard injections results should not be more than 2% (Table 1).

### Specificity

The specificity of developed method was evaluated by injecting blank sample (mobile phase) to demonstrate the absence of interference with elution of Obeticholic acid standard solution (10 µg/mL).

### Linearity

Six different concentrations ranging from 2.5- 15 µg/mL obeticholic acid standard stock solution were prepared and injected. Calibration curve was constructed by plotting mean response factor against the respective concentration. The method was evaluated by determination of the correlation coefficient and intercept value. (Table 2)

### Accuracy

Recovery assessment was obtained by using standard addition technique which was performed by adding known quantities of pure standards at three different levels in 50%, 100% and 150% to the pre-analysed sample formulation. From the amount of drug found and amount of drug recovered, percentage recovery was calculated. (Table 3)

### Precision

Obeticholic acid standard concentrations (10 µg/mL) were analyzed for 6 times in consecutive days (inter day precision) and 6 times during the same day (intra-day precision). The precision of proposed method was obtained by calculating the relative standard deviation (RSD) values for intra-day and inter-day analysis with acceptance criteria of ≤ 2% RSD. (Table 4)

### Robustness

Robustness of the method was evaluated by small but deliberate changes in method like Flow rate at ± 0.1 ml/min and temperature by ± 5°C. (Table 5)

### Assay

Assay of the marketed formulation was carried out by injecting sample corresponding to equivalent weight into HPLC system to calculate the percentage purity. (Table 6)

## RESULTS AND DISCUSSION

### System Suitability

Standard solutions of Obeticholic acid working standard was prepared as per procedure and were injected six times into the HPLC system. The system suitability parameters were evaluated from standard

Chromatograms obtained by calculating the % RSD of retention time, tailing factor, theoretical plates and peak areas from five replicate injections are within range and results were shown in Table 1 and Figure 2.

### Linearity

To demonstrate the linearity of assay method, 6 standard solutions with concentrations of about 2.5 µg/mL to 15 10 µg/mL of Obeticholic acid were injected. Calibration curve plotted between peak areas versus respective concentration which was shown in Figure 3. Slope obtained was 37816 Y-Intercept was 1506.8 and Correlation Co-efficient was found to be 0.999.

### Accuracy

Obeticholic acid spiked standard concentrations (5 µg/mL, 10 µg/mL, 15 µg/mL) at all the three levels were analyzed for percentage recoveries and the results were presented in Table 3. The mean percentage recoveries

of three levels (3 samples from each concentration were injected) was found to be 100.08%. The accuracy results were within the accepted limits from 98.0% to 102.0% which proves that the method was found to be accurate. Good recovery results obtained for the developed method indicates that this method can be used for regular quality control assay test for Obeticholic acid.

### Precision

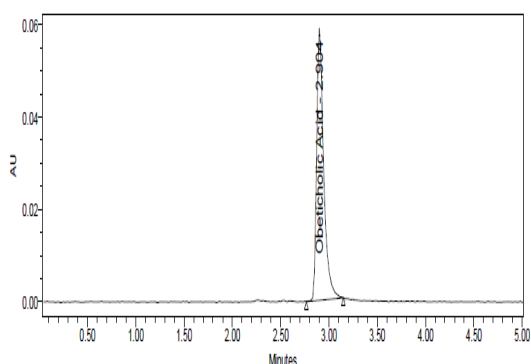
The precision of a method determines the closeness of agreement between a series of measurements of the same sample. The intraday and interday precisions were carried out 6 times at concentration of 10 µg/mL and the %RSD were found to be 0.7 and 0.7, respectively. The precision result (Table 4) was within the accepted limits of ≤ 2 % RSD which proves that the method was precise.

### LOD and LOQ

LOD is a limit test parameter and it is a test to determine whether the analyte concentration was present within the specification limit or not. LOQ is a parameter for quantitative assay used particularly for determination of impurities or degradation products as it used for minimum concentrations of analytes in sample. The LOD and LOQ were found to be 0.03 and 0.08

**Table 1: System suitability parameters.**

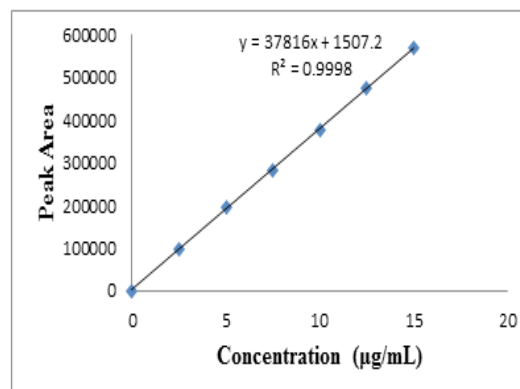
S.no	Peak name	Rt	Area	USP Plate count	USP tailing
1	Obeticholic Acid	2.898	380975	8258	1.46
2	Obeticholic Acid	2.900	370962	8198	1.46
3	Obeticholic Acid	2.904	377054	7955	1.46
4	Obeticholic Acid	2.905	372797	8087	1.45
5	Obeticholic Acid	2.905	377132	8045	1.48
6	Obeticholic Acid	2.906	373011	8129	1.47
Mean			375322		
SD			3713.7		
%RSD			1.0		



**Figure 2: System suitability Chromatogram.**

**Table 2: Results of Linearity.**

Linearity Level (%)	Concentration (µg/mL)	Peak Area
0	0	0
25	2.5	95519
50	5	196685
75	7.5	283363
100	10	376309
125	12.5	473485
150	15	570515



**Figure 3: Calibration curve of Obeticholic acid.**

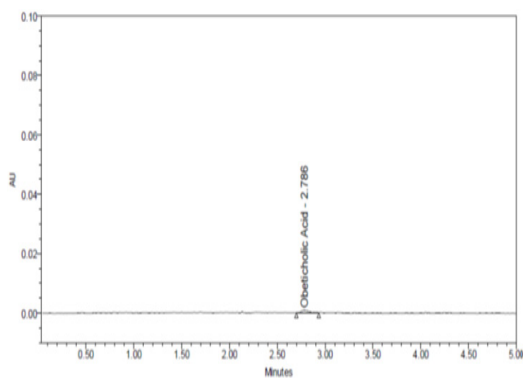


Figure 4: LOD Chromatogram of Obeticholic acid.

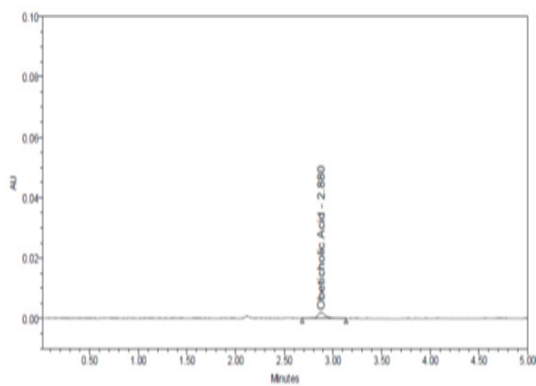


Figure 5: LOQ Chromatogram of Obeticholic acid.

Table 3: Accuracy data.

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	50	4.98	99.55	100.08%
	50	5.01	100.12	
	50	5.00	99.96	
100%	100	10.10	100.97	
	100	10.02	100.19	
	100	10.08	100.76	
150%	150	14.97	99.82	
	150	15.04	100.26	
	150	14.87	99.11	

respectively which proves the method was sensitive. The results were presented in Figure 4 and 5.

### Robustness

Robustness of the method was performed by changing flow rate ( $\pm 0.1$  mL/min) and change in temperature ( $\pm 5^\circ$  C). The results were summarized in Table 5. It

Table 4: Precision data.

Si No	Peak Area	
	Intraday	Interday
1	379391	331698
2	378339	336270
3	375437	333793
4	373357	336099
5	372826	330602
6	376644	333808
AVG	375999	333712
Std Dev	2636.7	2279.7
%RSD	0.7	0.7

Table 5: Robustness Data.

Parameter	%RSD
Flow Minus	0.5
Flow Plus	0.6
Mobile phase Minus	0.7
Mobile phase Plus	0.5
Temperature Minus	0.6
Temperature Plus	0.8

Table 6: Assay of Formulation.

Sample No	%Assay
1	100.68
2	100.40
3.	99.63
4.	99.08
5.	98.94
6.	99.95
AVG	99.78
SD	0.70
%RSD	0.7

was observed that even in minor changes of method conditions there was no marked changes in the results demonstrate that the HPLC method developed was robust. The robustness results were within the accepted limits of  $\leq 2\%$  RSD.

### Assay of Marketed Formulation

Sample solution was injected separately 6 times from the same sample individually into the system and chromatograms were recorded and %RSD was reported from the calculated percentage purity values. The assay and degradation study results were presented in Table 6 and 7, respectively.

Table 7: Degradation Data of Obeticholic acid.

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	5.53	0.154	0.298
2	Alkali	4.68	0.160	0.304
3	Oxidation	3.79	0.245	0.312
4	Thermal	2.41	0.151	0.311
5	UV	1.02	0.156	0.309
6	Water	1.02	0.145	0.310

## CONCLUSION

The developed method was validated as per ICH guidelines. All the validation parameters were found to be well within the acceptance criteria. We concluded that the method is accurate, precise, linear and robust. The developed method can be successfully applied for the analysis of Obeticholic acid bulk and pharmaceutical dosage form in quality control laboratories.

## ACKNOWLEDGEMENT

The authors are thankful to Biophore Pharma, Hyderabad, India, for providing the gift sample of Obeticholic acid.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ABBREVIATIONS

**RP-HPLC:** Reverse Phase High Pressure Liquid Chromatography; **OPA:** Ortho Phosphoric Acid; **ICH:** International Council for Harmonization; **FXR:** farnesoid X receptor; **PDA:** Photo Diode Array; **Rt:** Retention time; **USP:** United States Pharmacopeia; **SD:** Standard Deviation; **RSD:** Relative Standard Deviation; **LOD:** Limit of Detection; **LOQ:** Limit of Quantification; **UV:** Ultra Violet.

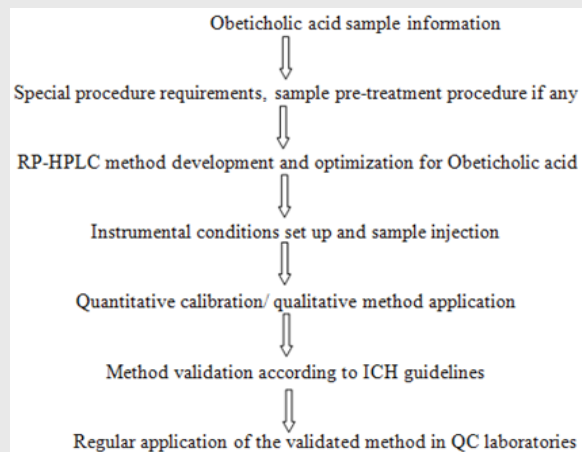
## REFERENCES

1. Fiorucci S, Cipriani S, Mencarelli A, Baldelli F, Bifulco G, Zampella A. Farnesoid X receptor agonist for the treatment of liver and metabolic disorders: focus on 6-ethyl-CDCA. *Mini Rev Med Chem.* 2011;11(9):753-62.
2. Verbeke L, Farre R, Trebicka J, Komuta M, Roskams T, Klein S. Obeticholic acid, a farnesoid-X receptor agonist, improves portal hypertension by two distinct pathways in cirrhotic rats. *Hepatology.* 2013;59(6):2286-98.
3. Walters JR, Johnston IM, Nolan JD, Vassie C, Pruzanski ME, Shapiro DA. The response of patients with bile acid diarrhoea to the farnesoid X receptor agonist obeticholic acid. *Aliment. Pharmacol Ther.* 2015;41(1):54-64.
4. Akare S, Jean-Louis S, Chen W, Wood DJ, Powell AA, Martinez JD. Ursodeoxycholic acid modulates histone acetylation and induces differentiation and senescence. *Int J of Cancer.* 2006;119(12):2958-69.
5. Poupon RE, Balkau B, Eschwege E, Poupon R. UDCA-PBC Study Group. A multicenter, controlled trial of ursodiol for the treatment of primary biliary cirrhosis. *N Engl J Med.* 1991;324:1548-54.
6. Hirschfield GM, Mason A, Luketic V, Lindor K, Gordon SC, Mayo M. Efficacy of obeticholic acid in patients with primary biliary cirrhosis and inadequate response to ursodeoxycholic acid. *Gastroenterology.* 2015;148(4):751-61.
7. Eipel C, Abshagen K, Vollmar B. Regulation of hepatic blood flow: The hepatic arterialbuffer response revisited. *World J Gastroenterol.* 2010;16(48):6046-57.
8. Pellicciari, R. et al. 6 alpha-ethyl-chenodeoxycholic acid (6-ECDCA), a potent and selective FXR agonist endowed with anticholestatic activity. *J Med Chem.* 2002;45(17):3569-72.
9. Dousa M, Slavikova M, Kubelka T, Cerny J, Gibala P, Zezula J. HPLC/UV/MS method application for the separation of obeticholic acid and its related compounds in development process and quality control. *J Pharm Biomed Anal.* 2018;5:214-24.

## SUMMARY

- In this present research work a novel RP-HPLC validated method for the quantification of obeticholic acid in bulk and its pharmaceutical dosage form was developed. After a series of trials 0.1% OPA and Acetonitrile in a ratio of 60:40 v/v was confirmed as mobile phase. System suitability parameters were studied by injecting the standard six times and results were well under the acceptance criteria. Linearity study was carried out between 25% to 150% levels,  $R_2$  value was found to be as 0.999. Precision was found to be 0.7 for repeatability and 0.7 for intermediate precision. LOD and LOQ were 0.03 µg/ml and 0.08 µg/ml respectively. By using above method assay of marketed formulation was carried out 100.36% was present. Degradation studies of Obeticholic acid were done, in all conditions purity threshold was more than purity angle and within the acceptable range and the method can be used for routine analysis of Obeticholic acid in quality control laboratories.

### PICTORIAL ABSTRACT



**Cite this article:** Jyothi RK, Chandrasekhar KB, Balakrishnan M, Kiran BSS. Stability Indicating RP-HPLC Method Development and Validation for the Quantification of Obeticholic Acid in Bulk and its Pharmaceutical Dosage Form. *Indian J of Pharmaceutical Education and Research*. 2020;54(2s):s344-s349.