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RP-HPLC analytical method development and validation of obeticholic acid in bulk and marketed formulation

Pawan S. Avhad
Research Scholar Dr. APJ Abdul Kalam University, Indore
*Corresponding author email: pawanavhad@gmail.com

Dr. Revathi Gupta
Faculty of Pharmacy, Dr. APJ Abdul Kalam University, Indore

***Abstract*---**The purpose of this study is to develop a new analytical RP-HPLC method for quantifying Obeticholic Acid in bulk and commercial formulations. Liquid chromatography is performed on a C18 column and the mobile phase is prepared using 0.1% orthophosphoric acid and acetonitrile in a ratio of 65:35. The flow rate was 1.0 ml / min and the injection volume was 10 ml. The maximum absorption of the drug was 210 nm. The retention time was found to be 2.80 minutes. This method was found to be accurate and linear with a correlation coefficient of 0.999 in the range 2.4-14 mg / mL. The percentage RSD for accuracy was less than 1.8%, averaging 100.05%. All validation parameters were statistically validated according to ICH guidelines and were within acceptable criteria. This method turned out to be simpler, more accurate, more specific, more accurate and more robust. This HPLC method is used for the analysis of obeticholic acid.

***Keywords*---**acetonitrile, obeticholic acid, RP-HPLC, validation.

Introduction

Obeticholic acid is bile acid activator drug also act as farnoside X receptor agonist use to treat Primary Biliary Cholangitis. Chemical name for Obeticholic acid is dihydroxy-5 beta colanic acid 3 alpha hydroxyl steroid it is obtained from Chenodeoxycholic acid. The farnoside X receptor regulate bile and cholesterol metabolism in liver. As this molecule has been recently approved by US FDA very few methods are available for its development. Some bioanalytical methods were developed but very few RP-HPLC methods for analysis of this drug. Goal of this work is to develop newer method for analysis of drug in new solvent combination

using RP- HPLC method. This method is unique, newer, accurate and precise also.

Structure

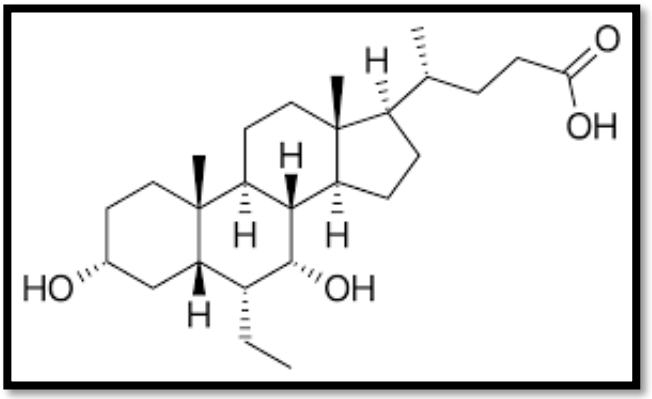


Figure 1. Obeticholic Acid

Mechanism of action

Obeticholic Acid is a semi-synthetic hydrophobic BA analogue. This one is a highly selective agonist of FXR, Revitalization power similar to that of the endogenous one But BA chenodeoxycholic acid is 100 times higher Powerful 5OCA also induces intestinal expression Hormones, especially FGF19. Beneficial effects of OCA on the resulting glucose Lipid metabolism, especially the liver Inflammation makes it a potential candidate Pharmacological treatment of various diseases Including PBC and non-alcoholic steatohepatitis (NASH).

Primary biliary cholangitis

Formerly known as primary biliary cirrhosis, PBC6 is a rare chronic autoimmune progressive disease. Cholestasis liver disease, Inflammation and selective destruction of small things And the intermediate intrahepatic bile duct. 7 Usually It progresses over the years, causing cholestasis and the liver. Fibrosis, and finally cirrhosis and liver failure. Presentation, natural history and clinical course it is variable. Causes of PBC shared with others autoimmune disorders are complex and multifactorial. It's a series of events, including complicated ones. Immunopathological changes leading to injury Small and medium bile ducts immortalized by Cholestasis and toxic effects of hydrophobic BA Causes bile duct leakage, fibrosis and cirrhosis.

Material and Method

Obeticholic acid pure drug received as gift sample from Amneal Pharmaceutical, Gujrat, and OVALIVA tablet was purchased from market, HPLC grade acetonitrile, methanol, Analytical grade phosphate buffer, potassium dihydrogen orthophosphoric acid were purchased from Vishal Chemical Supplier, New Mumbai.

Instrument

Water's HPLC equipment is used to perform this work in college laboratory.

Chromatographic condition

Mobile phase used is 0.1% OPA and Acetonitrile in ratio 65:35. Rate of liquid flow in column kept 1.0 mL/minutes, volume of injection keeps at 10ml. Absorbance maxima of drug was 210 nm. The time for retention was found to be 2.80 minutes. Column used is Grace C18.

Stock solution preparation

Weigh 2.5 gm of Obeticholic Acid dissolved in 25 ml of solution which gives 100 µg/ml of Obeticholic Acid Stock Solution. Dilute 1 ml above in 10 ml to prepare 10 µg/ml of stock solution for injecting into machine.

Sample stock solution preparation

Average weight of tablet was calculated and 10 mg drug transfer in 100 ml solution of volumetric flask, (100 µg/ml of Obeticholic Acid). Prepared solution was sonicate for 30 min in sonicator and then filter to obtained clear liquid. Further 1 ml removed and transfer to volumetric flask to make 100 ml final stock solution with mobile phase it gives 10 µg/ml of Obeticholic Acid.

Specificity

Method Specificity was evaluated by using blank sample mobile phase to demonstrate absorbance of interference with elution of Obeticholic acid standard solution.

Linearity

Different six concentrations ranging from 2 to 12 µg/mL Obeticholic Acid standard stock solution was prepared and injected, calibration curve was constructed and plot a graph of response factor against concentration.

Accuracy

Drug recovery is gained by adding quantity of pure standard at three different levels of 50%, 100%, and 150% to reanalysed sample sample formulation.

Precision

Drug stock solution was analysed for 6 times in constructive day i.e. Interday precision, 6 times during same day i.e. Intraday precision. Precision was calculated by Relative Standard Deviation.

Roubstness

This method was evaluated by small changes in method like flow rate ± 0.1 ml/ min and temperature by ± 5°C.

Assay

Assay is carried out by injecting sample corresponding to equivalent weight into HPLC machine to calculate percentage purity.

Result and Discussion

System Suitability

Obeticholic acid working standard was prepared according to the procedure and injected into the machine 6 times. This parameter was obtained by calculating the retention time, tailing factor, number of theoretical plates, and% RSD of peak area from 5 repeated injections within range, shows in table 1 and Figure 1.

Table 1
System Suitability Parameter

Sr. No.	Peak Name	Rt	Area	USP Plate Count	USP tailing
1	Obeticholic Acid	2.829	391135	8126	1.45
2	Obeticholic Acid	2.904	381051	8096	1.45
3	Obeticholic Acid	2.908	378029	8011	1.45
4	Obeticholic Acid	2.902	376905	7945	1.47
5	Obeticholic Acid	2.906	374819	8008	1.48
6	Obeticholic Acid	2.902	373128	8002	1.45
Mean			379177	8059	
SD			3715.6		
% RSD			1.0		

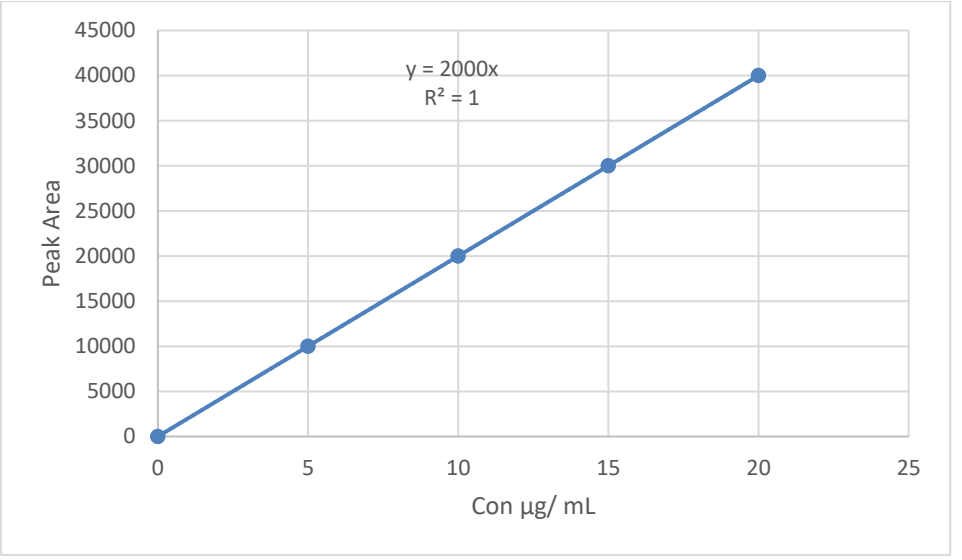
Linearity

To find out the linearity of assay method 6 standard solutions with concentration about 2.4 µg/ mL to 14, 10 µg/mL of Obeticholic acid was injected. Calibration curve is plotted by peak areas against respective concentration. Slope obtained was 386971, correlation coefficient was 0.999.

Table 2
Linearity Result

Linearity Level %	Concentration µg/mL	Peak Area
0	0	0
24	2.4	94138
50	5	185841
80	8	271257

100	10	386971
120	12	462158
140	14	584212



Accuracy

Obeticholic acid standard solution was (5µg/mL, 10µg/mL, and 15 µg/mL) analysed to get percentage recovery and result presented in Table. Mean of 5 recovery was about 100.11%. Accuracy result is within accepted limit i.e. 98.0% to 102% this proves that method was accurate. Excellent recovery result is obtained from above developed method, which indicates that this method can be used regularly for quality control as well as assay of the drug.

Table 3
Accuracy Result

% Level	Amount Spiked µg/ mL	Amount Recovered µg/ mL	% Recovery	Mean % Recovery
50%	50	4.88	99.85	
	50	4.96	99.98	
	50	5.01	100.04	100.06 %
100%	100	10.6	100.96	
	100	10.04	100.40	
	100	10.08	100.80	
150%	150	14.92	99.04	
	150	15.10	100.30	
	150	14.86	99.80	

Precision

Intraday and Interday reading was obtained done at 6 times of 10 µg/mL and % RSD were found 0.6. Precision result was found in given limit ≤ 2 % RSD it shows that method was precise.

Table 4
Precision Data

Sr. No.	Peak Area	
	Intraday	Interday
1	389518	341279
2	388641	341055
3	387426	340960
4	386312	340752
5	385122	340631
6	384059	340589
AVG	386846	340760
Std Dev	2735	2358
%RSD	0.8	0.8

Roubstness

This can be done by change in flow rate (± 0.1mL/min) also temperature (± 5o C). It was observed that little change in condition does not effect on HPLC method. The Roubstness effect was within range ≤ 2 % RSD.

Table 5
Roubstness

Name of Parameter	% RSD
Flow -	0.4
Flow +	0.5
Mobile Phase -	0.6
Mobile Phase +	0.7
Temperature -	0.7
Temperature +	0.8

Assay of Formulation

Prepared sample solution is introduce 6 times into the machine and chromatogram were recorded and %RSD were reported from calculated % purity values. Assay and Degradation result was shown in given table.

Table 6
Assay of Tablet

Sample No	% Assay
1	100.48
2	100.35

3	99.72
4	98.26
5	98.15
6	97.83
Average	99.13
SD	0.80
%SD	0.8

Conclusion

The newly developed method has been validated according to ICH guidelines. It turns out that all validation parameters are within acceptable limits. It was concluded that the procedure was accurate, accurate, linear and robust. This method can be used to analyse bulk and finished obeticholic acid in quality control labs.

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 forthe 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, Slavíkova 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.Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). Get vaccinated when it is your turn and follow the local guidelines. *International Journal of Health Sciences*, *5*(3), x-xv. https://doi.org/10.53730/ijhs.v5n3.2938

10. Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). Health and treatment of diabetes mellitus. *International Journal of Health Sciences*, *5*(1), i-v. https://doi.org/10.53730/ijhs.v5n1.2864
11. Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). The COVID-19 pandemic. *International Journal of Health Sciences*, *5*(2), vi-ix. https://doi.org/10.53730/ijhs.v5n2.2937