DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF OBETICHOLIC ACID

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Abstract

The goal of this work was to create a new, fast, and accurate UV spectrophotometric method for quantifying obeticholic acid in pure and capsule dose forms. Water & ethanol were used in the ratio of 50:50 as a diluent. The highest absorbance of obeticholic acid was measured at 210 nm, and the linearity ranged from 2.5 to 15 g/ml. The regression equation for obeticholic acid was y= y=0.0504x + 0.0026, with a correlation value of 0.9995. The percentage of recovery ranged from 100.7 to 101.6 percent. The relative standard deviation for method precision and intraday precision was determined to be less than two. Obeticholic acid LOD and LOQ were determined to be 0.01 µg/ml and 0.04 µg/ml, respectively. The spectrometric technique was validated in accordance with ICH criteria and was found to be suitable for routine quantitative measurement of obeticholic acid in pure and tablet dose forms.

Keywords*: Method development, Spectrometric technique, Obeticholic acid, Regression equation*

1. Introduction

In a quantitative analysis, the quantity or concentration of an analyte is ascertained (estimated) and expressed as a numerical value in the proper units. The primary methods that have been used for the quantitative analysis of pharmaceutical compounds include a number of techniques like ultraviolet/visible spectrophotometry, fluorimetry, titrimetry, electro analytical techniques, chromatographic methods (thin-layer chromatography, gas chromatography, and high-performance liquid chromatography), capillary electrophoresis, and vibrational spectroscopies[1]. Obeticholic acid is a hepatoprotective substance. It is a semi-synthetic bile acid that treats primary biliary cholangitis by acting as a farnesoid X receptor agonist. Obeticholic acid is a dihydroxy-5 beta-cholinic acid, a 3- and a 7-hydroxysteroid chemically speaking. It came from an acid called chenodeoxycholic. Preclinical results from various research and the farnesoid X receptor's (FXR) crucial role as a regulator of bile and cholesterol metabolism in the liver serve as a compelling justification for the development of FXR agonists as hepatoprotective therapies in chronic liver disease[2]. The chemical structure of Obeticholic acid was shown in fig 1.

There were RP-HPLC methods that have been reported in the literature for the determination of obeticholic acid in pharmaceutical dosage form [2,3,4,5]. Bio analytical (LC-MS/MS) in biological fluids, and HPLC/UV method have been reported for the quantification of obeticholic acid [6,7]. There was no method that describes the quantitative determination of obeticholic acid by UV- Visible spectrometry. An attempt has been made to develop a UV-visible spectrometric method that was simple, specific, rapid, precise, and economical for the quantitative determination of obeticholic acid in a pharmaceutical dosage form. This method was validated as per the guidelines of international conference on harmonization (ICH) Q2 (R1).

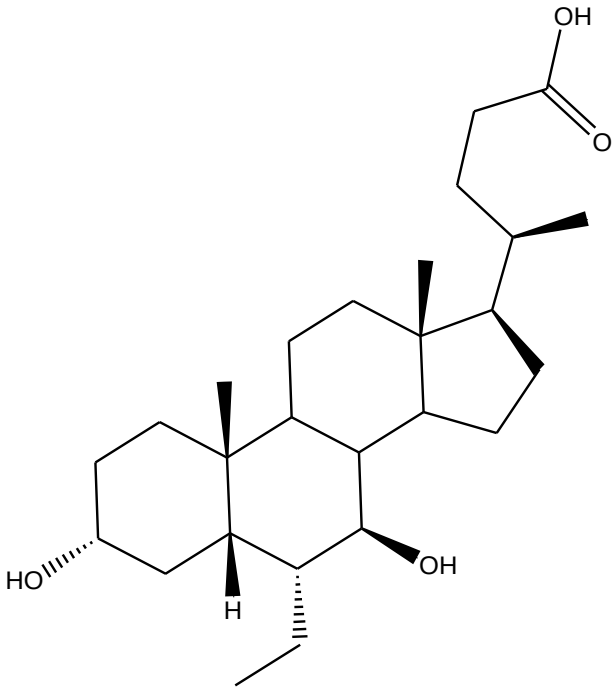

Figure 1. Structure of obeticholic acid

2. Materials and Methods

Instrumentation

The absorbance of obeticholic acid solution was measured using a UV 1800 double beam UV visible spectrophotometer with unique bandwidth of 10mm. The above instrument consists of matched quartz cells combined with UV solutions 2.42 software. For measuring purposes, an electronic balance was employed. Pipettes and volumetric flasks made of borosilicate glass were used in the experiment.

Chemicals and reagents

Spectrum pharma research solutions (Hyderabad, India) provided a free sample of obeticholic acid. The chemicals utilised were all of analytical grade.

Preparation of obeticholic acid standard solution

Ten milligrams of obeticholic acid was weighed accurately and transferred to a hundred millilitres volumetric flask. 30 ml of diluent was added and sonicated to dissolve. Then, add diluent until the desired level was reached. 1 ml of this solution was added to a 100 ml volumetric flask and diluted with diluent to the desired strength. Then, filtered through 0.45µm nylon membrane filter.

Preparation of test solution

The average weight of 10 tablets was calculated and made into powder. 25 mg of obeticholic acid tablet sample powder (equivalent to 10 mg of obeticholic acid) was weighed and transferred in to a hundred millilitres volumetric flask. 30 ml of diluent was added and sonicated to dissolve. Then, add diluent until the desired level was reached. 1 ml of this solution was added to a 100 ml volumetric flask and diluted with diluent to the desired strength. Then, the solution was filtered through 0.45µm nylon membrane filter.

Preparation of diluent

The solvent was prepared by using 50 ml of water and 50 ml of ethanol. Both solutions were mixed in a hundred millilitres volumetric flask.

Preparation of calibration curve

Six volumetric flasks of volume 10 ml were used in this experiment. From working standard solution, 0.25 - 1.50 ml samples were taken into volumetric flasks and made up with water: ethanol (50:50 v/v) to get 2.5-15 µg/ml solutions. The solutions were scanned with a UV-visible spectrophotometer in the 200-400 nm UV range.

Method validation

Linearity

By graphing concentration vs corresponding absorbance, the linearity was discovered. Solutions ranging from 2.5 µg/ml to 15 µg/ml were prepared from a standard stock solution (100 µg/ml). By plotting absorbance vs concentration, the calibration curves were created, and the regression equations were derived.

Accuracy

Recovery studies were carried out at three distinct levels, i.e., 80%, 100%, and 120%, to evaluate the suggested method's accuracy. The pre-analyzed sample solution received three additions of a known quantity of the reference drug solution, and the absorbance was measured after each addition. Next, the recovery percentage was determined.

Precision

Precision was calculated by inter day and intraday variation. In intraday study, standard stock solutions were taken in a ten millilitres volumetric flasks and final volume was made up to the mark with diluent. Three times in one day, the absorbances of each of these solutions were independently tested and recorded. In the inter-day precision investigation, standard stock solutions were taken in 10 ml volumetric flasks and volume were made up to the mark with diluent. These solutions' specific absorbances were measured three times over the course of three days and recorded.

Sensitivity

The lower limit of quantification and the limit of detection were derived by means of the subsequent equations based on the slope of the calibration curve and the SD of responses.

LOD = 3.3 × Standard deviation (SD) / slope
LOQ = 10 × Standard deviation (SD) / slope

Robustness

Two samples of same concentration (10 µg/ml) were prepared and analysed by small adjustments in the UV wavelength settings (±2 nm).

Assay

Obeticholic acid (Brand name: OCALIVA) was used for assay. Ten tablets were weighed, and weight equivalent to 10 mg was transferred to a volumetric flask and dissolved in diluent. The flask was sonicated for 10 minutes. The solution was filtered and diluted with water. Diluent was used to modify the volume. The absorbance was measured at 210 nm.

3. Results and Discussion

Linearity

The ability to obtain test findings that were proportional to analyte concentration in samples within an appropriate range was termed as linearity. The developed approach showed linearity in the 2.5-15 µg/ml range. For obeticholic acid, the linearity equation was found to be y = 0.0504x + 0.0026, with a correlation coefficient of 0.9995. From the obtained linearity data, the coefficient of correlation was found to be less than 1. Hence, the result was within the acceptable limits. The linearity result was illustrated in table 1 and the graph was plotted in fig 2.

Table 1. Linearity data of obeticholic acid

Conc (µg/ml)	Absorbance
2.5	0.126
5	0.252
7.5	0.392
10	0.505
12.5	0.606
15	0.758

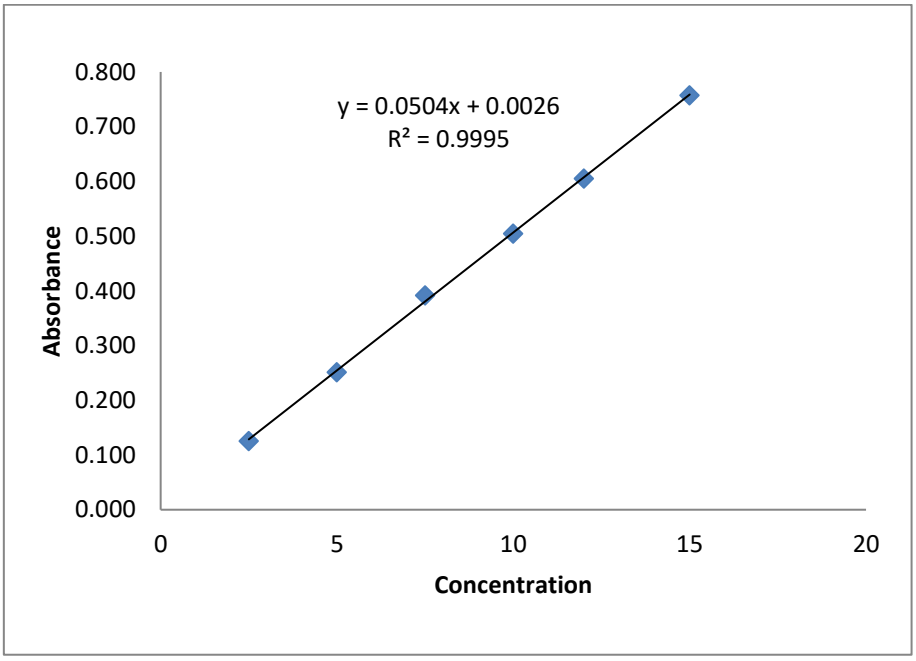

Figure 2. Calibration curve of obeticholic acid

Accuracy

Accuracy was the degree to which the measured value agrees with the accurate value. Mean percentage recovery of obeticholic acid was found between 100.7-101.6% and can be concluded that the results were within the limits. The observed data was within the range, indicating that the suggested analytical method had good recovery and accuracy. Accuracy results were shown in table 2.

Table 2. Accuracy data of obeticholic acid

% Recovery level	Pure drug added (µg/ml)	Drug recovered (µg/ml)	Average Recovery	Recovery (%)	SD	RSD (%)
80%	8	8.06	8.13	101.6	0.45	0.44
	8	8.08				
	8	8.26				
100%	10	10.14	10.07	100.7		

	10	10.06				
	10	10.02				
120%	12	12.13	12.16	101.3		
	12	12.2				
	12	12.15				

Intraday Precision

Precision was defined as the degree of agreement between individual test findings when the procedure was tested on several uniform samples. Percentage relative standard deviation (RSD) for intraday precision was found between 0.2%. The percentage RSD of precision studies was < 2 and within the acceptable range. The intraday precision results were shown in table 3.

Table 3. Intraday precision

Concentration taken (µg/ml)	Absorbance	Mean	SD	%RSD
10	0.518	0.517	0.0011	0.2
10	0.519			
10	0.517			
10	0.516			
10	0.518			

Inter day precision

Percentage relative standard deviation for interday precision was found to be 0.2%. The percentage relative standard deviation of precision studies was less than 2 and within the acceptable range. The intraday precision was summarised in table 4.

Table 4. Inter day precision

Concentration taken (µg/ml)	Absorbance	Mean	SD	%RSD
10	0.502	0.503	0.0013	0.2
10	0.505			
10	0.504			
10	0.505			
10	0.503			

Robustness

Robustness was an estimation of its capacity to remain unchanged by little but planned changes in analytical process settings and provided a hint of its consistency throughout usage. % RSD was found to be 0.17 and can be reported that it was within the limit. The robustness data also represented that the values were within the limits. The results of robustness were illustrated in table 5.

Table 5. Robustness data of obeticholic acid

Wavelength (nm)	Concentration (µg/ml)	Absorbance	Wavelength (nm)	Concentration ((µg/ml)	Absorbance
208	10	0.503	212	10	0.504
	10	0.502		10	0.503
	Average	0.502		Average	0.503
	SD (±)	0.0007		SD (±)	0.0007
	(%) RSD	0.14		(%) RSD	0.14

Assay

Percentage purity was found to be 98.7 %. According to the label claim, the drug content obtained from the values of sample solutions was found to be in the permissible range of 90–110 %. The assay data was illustrated in table 6. The developed technique was validated according to the ICH guidelines. The summary of the results was tabulated in table 7.

Table 6. Assay data of obeticholic acid

Brand name	Available form	Label Claim	Concentration found	% Assay
OCALIVA	Tablet	10mg	9.87	98.7

Table 7. Summary of validation parameters

Parameters	Obtained values
Maximum absorbance (λ max)	294nm
Linearity (µg/ml)	2.5-15
Intercept (c)	0.0026
Slope (m)	0.0504
Intra-day precision (%RSD)	0.2
Inter-day precision (%RSD)	0.2
Recovery (%)	100.7 - 101.6
Robustness (%RSD)	0.14
Assay	98.7
LOD	0.01 µg/ml
LOQ	0.04 µg/ml

4. Conclusion

The present analytical method was validated as per ICH Q2(R1) guideline and it meets to specific acceptance criteria. It is concluded that the analytical method was precise, linear, accurate, and robust. The present analytical method can be used for its intended purpose.

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Conflict of interest

The authors declare that there was no conflict of competing financial interests.

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