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Analytical Method Development and Validation of UHPLC and UV Spectroscopy for the Determination of Obeticholic acid in Bulk and Pharmaceutical Dosage Form

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Abstract

Quantitative determination of obeticholic acid is simple, quick, sensitive, accurate, precise, and robust. Using a C18 column (4.6mm X 100mm) as the stationary phase, 0.05% OPA (pH 3.7), and Methanol: water (0.05% OPA) 50:50 as the mobile phase, chromatographic separation has been performed at a flow rate of 0.7 ml. The wavelength used for UV detection was 210 nm. Linearity, accuracy, range, and robustness were all within acceptable bounds as per the ICH criteria. The determination of Obeticholic acid performed. The Obeticholic acid to provide well retained, sharp and symmetric peak at3.859 min and 1.435 min. The mean % recovery for in accuracy study was observed to be 98-102%. LOD and LOQ values were found to be 0.0147 µg/mL and 1.3930 µg/mL, respectively. The UHPLC method linear detector response was found to be linear over the concentration range of 10-50µg /ml, with a correlation coefficient of 0.999 and a regression equation of y=14.07x+4.764 and UV method of 1-5µg /ml, with a correlation coefficient of 0.999 and a regression equation of y= 0.085x+0. 012.The intermediated precision study was determined using intra-day and inter-day data Received from the proposed method of evaluating Obeticholic acid. Repeatability studies on UHPLC and UV method for Obeticholic acid was found to be, The %RSD was less than 2%, which shows high percentage amount found in between 98% to 102% indicates the analytical method that concluded. Intraday and Inter day Precision studies on UHPLC and UV method for Obeticholic acid which shows the high precision %amount in between 98% to 100% indicates to analytical method that concluded. The robustness of Obeticholic acid changes were did flow rate (±1 ml/ min-1), PH of mobile phase composition (±1 ml/ min-1), and Wavelength (±1 ml/ min-1). %RSD for peak area was calculated which should be less than 2%. The mean % recovery for in accuracy study was observed to be 98-102%. LOD and LOQ values were found to be 0.0147 µg/mL and 1.3930 µg/mL, respectively. The ICH guideline for the validation of analytical recommended UHPLC,UV analytical method for the quantitative determination of Obeticholic acid is simple, quick, sensitive, accurate, precise, and robust. The findings of all validation parameters are well within the acceptable requirements set by the guideline.

Keywords: UHPLC, UV Spectroscopy, Obeticholic acid, validation method.

Introduction

Obeticholic acid (OCA), also referred to as 6alpha-ethyl-3alpha, 7alpha-dihydroxy-5-cholon-24-oic acid, is a main bile acid that is made in the liver from cholesterol and is quite hydrophobic. Chenodeoxycholic acid (CDCA, 3alpha, 7alpha, dihydroxy-5-cholon-24-oic acid) is a semi-synthetic derivative of this acid. OCA, a farnesoid X receptor (FXR) agonist, is essential for the flow of bile acid through the enterohepatic system. The most physiologically active ligand for the farnesoid X receptor (FXR), which is involved in many physiological and pathological processes, was identified in 1999. After being taken orally, Obeticholic acid targets and binds to FXR that is present in the liver and gut, activating FXR-mediated inflammatory, fibrotic, and metabolic pathways as well as bile acid production. By preventing bile acid synthesis in the hepatocytes and promoting bile acid transport out of the hepatocytes, this reduces hepatic exposure to bile acids [1-3].The small intestine (particularly the distal ileum) and liver are two organs where FXR is highly expressed. Chenodeoxycholic acid and other Bas are the principal naturally occurring ligands for FXR[4]. The IUPAC name of OCA is (4R)-4-[(3R,5S,6R,7R,8S,9S,10S,13R,14S,17R)-6-ethyl-3,7-dihydroxy-10,13-dimethyl 2, 3, 4, 5,6,7,8,9,11,12,14,15,16,17-tetradecahydro-1H cyclopenta phenanthren-17-yl] pentanoic acid. The molecular formula is C26H44O4, and it has a molecular weight of 420.6 g/mol. It is an atypical anti-inflammatory drug and is soluble in water soluble methanol and ethanol, aceton. Its melting point is about 108-110 °C. It is a white to off-white powder [5-7].

The goal of the current work is to provide a precise and trustworthy development of UHPLC technique and UV spectrophotometer of Obeticholic acid in solid Dose form.

Chemically, Obeticholic acid is: 6 alpha-ethyl-Chenodeoxycholic acid and has the structural Formula as shown on Fig1.

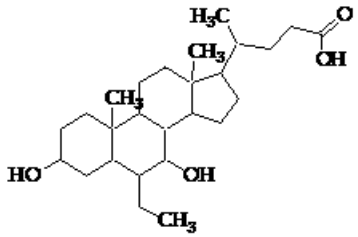

Figure No.1: Obeticholic acid

Methods and Materials

Chemicals and reagents

HPLC grade water,Acetonitrile, methanol, and OPA from Merck.ltd Were used in this study. An analytically pureObeticholic acid working standard was obtained from Swapnaroop Drug & Pharmaceutical.A local store provided Obeticholic acid 5 mg Tablet.

Instrumentations

The analysis of the drug was carried out on Agilent Tech. Gradient System with Auto injector, (DAD) & Gradient Detector. Equipped with Reverse Phase (Agilent) C18 column (4.6mm x 100mm; 2.5µm), and UV730D Absorbance detector and running chemstation 10.1 software.

Selection of wavelength

Accurately weigh and transfer 10 mg Obeticholic acid working standard into 10 ml volumetric flask as about dilute Methanol prepared in completely and make volume up to the mark with the same solvent to get 10 µg/ml standard (stock solution) and 15 min sonicate to dissolve it and from the resulting solution 0.1 ml was transferred to 10 ml volumetric flask and the volume was made up to the mark with Methanol. Shows that when the solution was scanned in the 200–400 nm range, the absorbance of Obeticholic acid was discovered at 210 nm. Show in (Fig. 2)

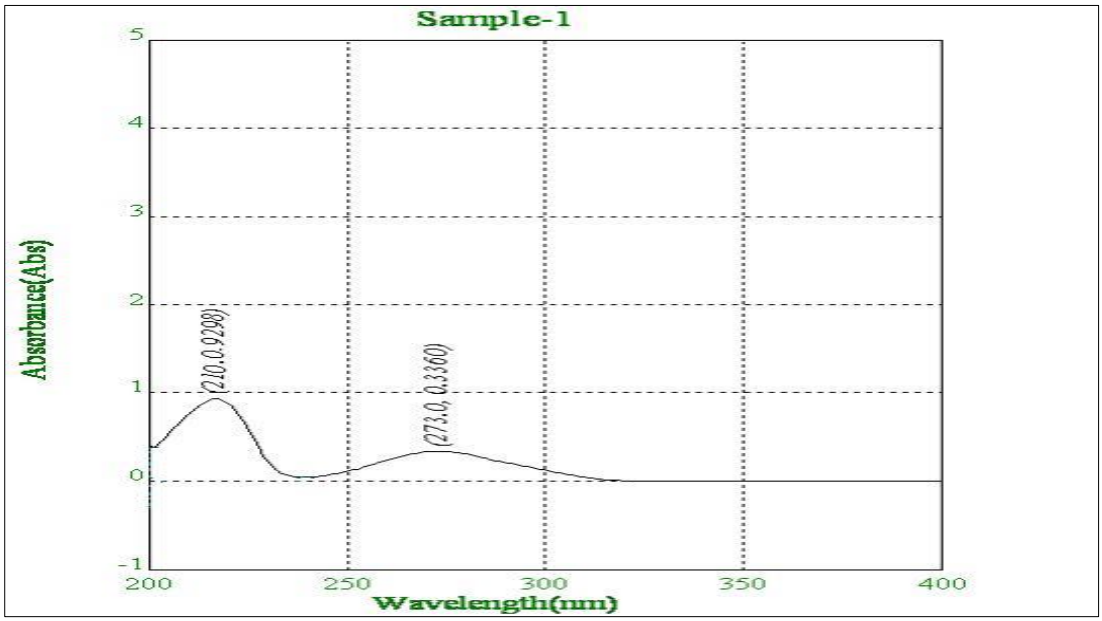

Figure No.2 : UV Spectrum of Obeticholic acid

Preparation of drug standard solution

Accurately weight and transfer 10 mg Obeticholic acid working standard into 10 ml volumetric flask as about diluent Methanol completely and make volume up to the mark with the same solvent to get 1000 µg/ml standard (stock solution) and 15 min sonicate to dissolve it and the resulting stock solution 0.1ml was transferred to 10 ml volumetric flask and the volume was made up to the mark with mobile phase Methanol: Water (0.1% OPA) Water, prepared in (50 ml MEOH: 50ml WATER v/v).

Preparation of sample solution

To quantify the amount of Obeticholic acid in commercially availabletablets, the average weight of 20 tablets was computed. Tablets were triturated,Weight of 50 mg of

Obeticholic acid powder was weighed and transfered to 10 mL volumetric flask & diluent was added to make up the volume. Sonicated for 10 min with occasional swirling. The above solution was filtered through 0.45µm membrane filter 0.1 ml of this solution diluted upto 10 ml with diluent.

Chromatogram trails

Table no. 1: Chromatographic behavior of Obeticholic acidmobile phase of various compositions

Sr.no.	Column used	Mobile phase, Flow rate and wavelength	Injection vol.	Observation	Conclusion
1.	C18 (Agilent) (4.6mm x 100 mm), 2.5 µ)	Methanol + 0.05% OPA (70+30)PH3.7, 210 nm, Flow rate 0.7mL	20µl	Sharp Peaks were not obtained (Less TF)	Hence rejected
2.	C18 (Agilent) (4.6mm x 100 mm), 2.5 µ)	Methanol+ 0.05% OPA ,Ph3.7 (80+20),210 nm 20 Mcg, Fl. 0.7ml	20 µl	Sharp Peaks were not obtained (TF is high)	Hence rejected
3.	C18 (Agilent) (4.6mm x 100 mm), 2.5 µ)	Methanol + 0.05% OPA (70+30)PH3.7, 210 nm, Flow rate 0.7mL	20 µl	Sharp Peaks were not obtained (Broad Peak shape)	Hence rejected
4.	C18 (Agilent) (4.6mm x 100 mm), 2.5 µ)	Methanol + 0.05% OPA, (60+40)PH3.7, 210 nm, Flow rate 0.7 mL	20 µl	Sharp Peaks were not obtained	Hence rejected
5.	C18 (Agilent) (4.6mm x 100 mm), 2.5 µ)	Acetonitrile + 0.05% (0.05% OPA)(50+50 %v/v)PH3.0, Flow rate 0.7 mL	20 µl	Peak was not Ressolve	Hence rejected
6.	C18 (Agilent) (4.6mm x 100 mm), 2.5 µ)	Methanol + 0.05% (0.05% OPA)(50+50 %v/v)PH3.0, Flow rate 0.7 mL	20 µl	Sharp and well resolved Peaks were obtained	Hence selected

Thus, from the above, it has been observed that, using mobile phase of meoh+0.05% OPA (50:50 % v/v),PH 3.7,210 nm, Flow rate 0.7 ml gave adequate retention at 3.859 min with good peak shape (Theoretical plates: Obeticholic acid 7075

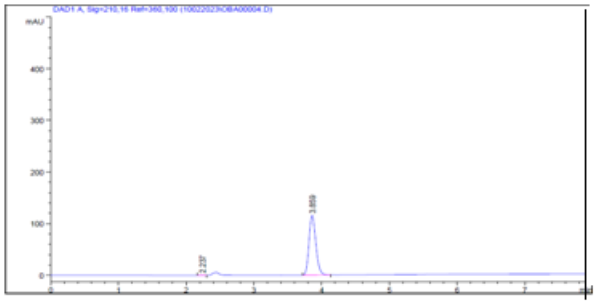

Figure No. 3: Result of chromatogram (final)

Validation method

According to ICH method validation criteria, analytical method validation was completed Q2 (R1). in relation to a number of factors, including linearity, accuracy, precision, repeatability, robustness, rugdness and resilience.

Linearity

Different working standard solutions (10-50µg/ml for HPLC and1-5 µg/mL for UV) weremade from Obeticholic acid standard stock solution in the mobile phase 20 µlof sample solution were then injected into the chromatographic system using a mixed volume Loop injector. There were chromatograms made. The area for each concentration was measured (Table no.2 and Table no.3 respectively), and the calibration curve is depicted in (Fig.4 and Fig.5 respectively).

Table no. 2 Linearity data for Obeticholic acid (UHPLC)

Method	Conc µg/ml	Peak area(µV.sec)		Average peak area (µV.sec)	S.D. of Peak Area	% RSD of Peak Area
		1	2			
RP-UHPLC Method	10	142.67	145.23	143.95	1.81	1.26
	20	286.21	285.46	285.84	0.53	0.19
	30	426.77	425.74	426.26	0.73	0.17
	40	582.4	572.93	577.67	6.70	1.16
	50	702.03	701.97	702.00	0.04	0.01
	Equation		Y= 14.07x+4.764			
	R^2		0.999			

Table no. 3 Linearity data for Obeticholic acid (UHPLC)

Method	Conc µg/ml	Peak area(µV.sec)		Average peak area (µV.sec)	S.D. of Peak Area	% RSD of Peak Area
		1	2			
UV Method	1	0.0975	0.0967	0.0971	0.0006	0.58
	2	0.1849	0.1851	0.1850	0.0001	0.08
	3	0.2711	0.2711	0.271	0.0000	0.00
	4	0.3545	0.3548	0.355	0.0002	0.06
	5	0.44	0.441	0.4405	0.0007	0.16
	Equation		y= 0.085x+0.012			
	R^2		0.999			

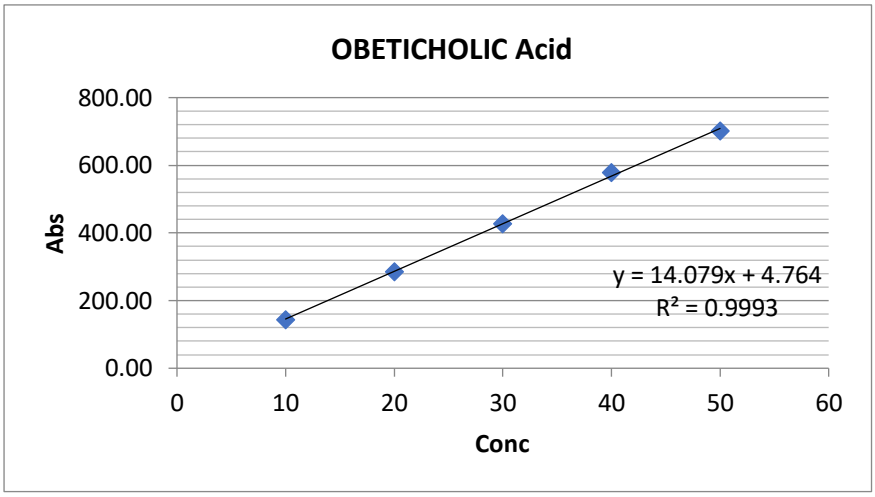

Figure No.4: Calibration curve of Obeticholic acid (HPLC)

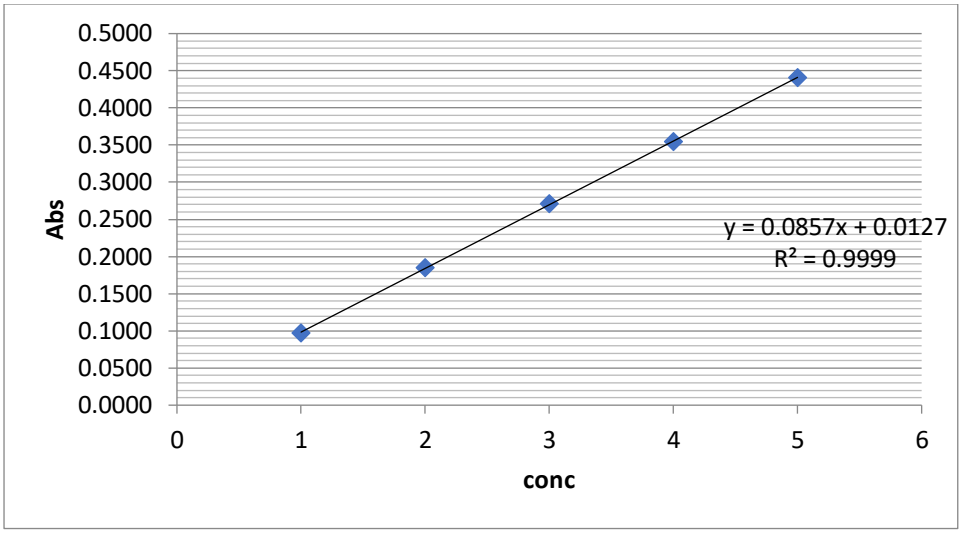

Figure No.5: Callibration curve of Obeticholic acid (UV)

Accuracy

Recovery studies were performed to validate the accuracy of developed method. To pre analyzed tablet solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed (Table No. 4). Statistical validation of recovery studies shown in (Table No. 5).

Table No.4: Result of Recovery data for Obeticholic acid

Method	Drug	Level (%)	Amt. taken (µg/ml	Amt. Added (µg/ml	Absorbance /Area Mean* ± S.D.	Amt. recovered Mean *±S.D.	%Recovery Mean *± S.D.
UHPLC Method	OA	80%	10	8	17.92±0.07	20.58±0.04	99.06±0.87
		100%	10	1	19.86±0.04	20.58±0.04	98.56±0.35
		120%	10	12	21.98±0.05	20.58±0.05	99.83±0.45
UV Method	OA	80%	1	0.8	1.80±0.001	0.80±0.001	100.22±0.10
		100%	1	1	2.00±0.002	1.00±0.002	100.24±0.17
		120%	1	1.2	2.20±0.003	1.20±0.003	99.71±0.28

***mean of each 3 reading for RP-UHPLC method and UV method**

Table 5: Statistical Validation of Recovery Studies Obeticholic acid

Method	Drug	Level (%)	Mean % Recovery	Standard Deviation*	% RSD
UHPLC Method	OA	80%	99.06	0.87	0.88
		100%	98.56	0.35	0.36
		120%	99.83	0.45	0.45
UV Method	OA	80%	100.22	0.10	0.10
		100%	100.24	0.17	0.17
		120%	99.71	0.28	0.28

***Denotes average of three determinations for RP-UHPLC and UV method**

System Suitability Parameter

(Repeatability)

Precision of the system was determined with the sample of RP-UHPLC& UV Method for. Three replicates of sample solution containing 40 mg of Obeticholic acid were injected and peak areas were measured and %RSD was calculated. Is was repeated for two times result are shown in (Table no. 6, Table no.7).

Table No.6: Repeatability studies on RP-UHPLC for Obeticholic acid

Sr. No.	Concentration of Obeticholic acid (mg/ml)	Peak area	Amount found (mg)	% Amount found
1	40	580.27	40.88	101.17
2	40	579.77		
Mean			580.02	
SD			0.96	
%RSD			0.17	

Table No.7: Repeatability studies on UV method for Obeticholic acid

Sr No.	Conc	Absorbance	Amt Found	%Amt Found
1	5	0.4412	5.06	101.12
2	5	0.4423		
Mean			0.44	101.12
SD			0.0008	0.0008
% RSD			0.18	0.18

Precision: The method was established by analyzing various replicates standards of Obeticholic acid. All the solution was analyzed thrice in order to record any intra-day & inter-day variation in the result thatconcluded. The result obtained for intraday is shown in(Table No. 8) respectivel concluded.

Table No.8: Result of Intraday and Inter day Precision studies on RP-UHPLC and UV method for Obeticholic acid

METHOD	Drug	Conc (µg/ml)	Intraday Precision		Interday Precision	
			Mean± SD	%Amt Found	Mean± SD	%Amt Found
UHPLC METHOD	OA	10	143.49±0.30	98.50	145.5±0.32	98.99
		30	423.87±0.44	101.63	433.254±0.46	101.66
		50	699.55±0.18	98.76	699.55±0.18	99.12
		2	0.18±0.0001	101.15	0.18±0.0001	101.12
UV METHOD	OA	3	0.27±0.0004	100.33	0.27±0.0004	100.10
		4	0.36±0.0006	101.03	0.36±0.0002	100.93

***Mean of each 3 reading for UHPLC and UV methods**

Robustness

The Robustness of a method is its ability to remain unaffected by small deliberate changes in parameters. To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters were done. The effect of

changes in mobile phase composition and flow rate, wavelength on retention time and tailing factor of drug peak was studied.

The mobile phase composition was changed in(±1 ml/min-1) proportion and the flow rate was varied by(±1ml/min-1), and wavelength change(±1 ml/min-1)of optimized chromatographic condition. The results of robustness studies are shown in (Table No.9).

Parameters	Conc.	Amount of detected(mean ±SD)	%RSD
Mobile phase composition-(49+51)	40	589.6±0.95	0.16
Mobile phase composition-(51+49)	40	591.45±0.72	0.12
Wavelength change 209 nm	40	591.73±0.24	0.04
Wavelength Change 211 nm	40	538.7±2.04	0.38
Flow rate change(0.6ml)	40	516.67±0.64	0.12
Flow rate change(0.8ml)	40	590.87 ± 0.35	0.06

Table No.9: Result of Robustness Study of Obeticholic acid

Limit of Detection

The LOD is the lowest limit that can be detected. Based on the S.D. deviation of the response and the slopethe limit of detection (LOD) may be expressed as:

LOD=3.3XAvd.SD/Slope

Where, SD = Standard deviation of Y intercept

S = Slope

Limit of Quantification

The LOQ is the lowest concentration that can be quantitatively measured. Based on the S.D. deviation of the response and the slope,

The quantitation limit (LOQ) may be expressed as:

LOQ = 10 (SD)/ S

Where, SD = Standard deviation Y intercept

S = Slope

ANALYSIS OF TABLET FORMULATION

Weigh 20 Obeticholic acid Tablet and calculated the average weigh 0.50 gms accurately weigh and transfer the sample equivalent to 50 mg Obeticholic acid into 10 ml volumetric flask. Add about 10 ml of diluent and sonicate to dissolve it completely and make volume up to the mark

with diluent.Mix well and filter through 0.45 µm filter. Further pipette 0.2 ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluents. (20 µg/ml). The simple chromatogram of test Obeticholic acid Shown in (Fig No:6) the amounts of Obeticholic acid per Tablet were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated five times with tablet formulation. Tablet Assay for %Lable claim for %RSD Calculated, Result was shown in (Table No. 1

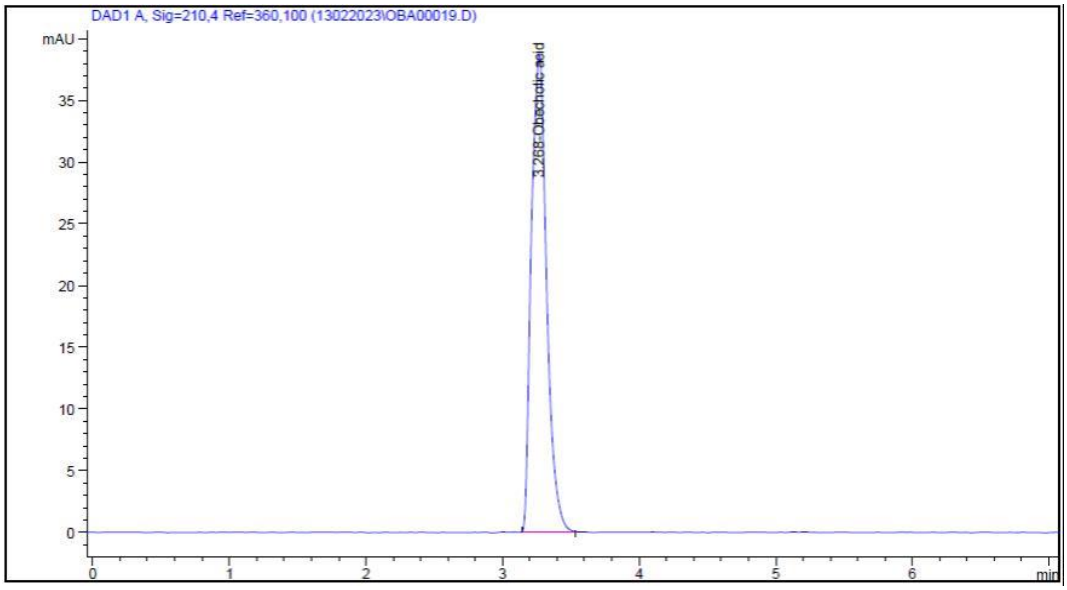

Figure No.6: Chromatogram of Marketed Formulation (20 Mcg)

Table No.10 :. Analysis of marketed formulation

Assay	Drug	Label Claimed	Amt Found	%Label Claim	SD	%RSD
RP-UHPLC Method	**OA**	20	20.33	101.65	1.29	0.44
		20	20.20	101.00	0.09	0.45
UV Method	**OA**	4	4.0376	100.94	0.0001	0.040
		4	4.0352	100.88	0.0002	0.041

Results

Research in the pharmaceutical industry is faced with the requirement to validate an analytical method on an almost daily basis, because sufficiently validated methods are required for approved regulatory submission.The unique and simple have been created for the determination of Obeticholic acid in satisfactory to provide a well retained, sharp and symmetric peak at 3.859 min, with an average number of therotical plates of 7075, indicating the column's efficient performance.

Linearity: The UHPLC method linear detector response was found to be linear over the concentration range of 10-50µg/ml, with a correlation coefficient of 0.999and a regression equation of y=14.07x+4.764 and UV method of 1-5µg /ml, with a correlation coefficient of 0.999 and a regression equation of y= 0.085x+0.012.

Accuracy: Method's accuracy was found to be 98-102 percent, indicating that there was no interference from excipients.

System suitability parameter Repeatability studies on UHPLC and UV method for Obeticholic acid was found to be ,The %RSD was less than 2%, which shows high percentage amount found in between 98% to 102% indicates the analytical method that concluded .

Precision: The intermediated precision study was determined using intra-day and inter-day data Received from the proposed method of evaluating Obeticholic acid.Intraday and Inter day Precision studies on UHPLC and UV method for Obeticholic acid which shows the high precision %amount in between 98% to 100% indicates to analytical method that concluded.

Robustness: The robustness of Obeticholic acid changes were did flow rate (±1 ml/ min-1), PH of mobile phase composition (±1 ml/ min-1), and Wavelength (±1 ml/ min-1). %RSD for peak area was calculated which should be less than 2%.the result shown in analytical method that concluded.

LOD: The LOD of Obeticholic acid was found to be 0.0147 (µg/mL) analytical methods that concluded.

LOQ: The LOQ of Obeticholic acid was found to be 1.3930 (µg/mL) analytical method that concluded.

% Lable claim: Analysis of marketed formulation were also %Lable Claim was found to be 98-102% Satisfactory are concluded.

Discussion

A rapid, accurate, and exact UHPLC and UV-Spectroscopic technique has been used to establish and confirm the process analysis of obeticholic acid in API and tablet dosage forms. These methods can be employed independently of one another to detect the presence of obeticholic acid in single-component formulations. The UHPLC and UV spectroscopy method of obeticholic acid and its validation parameters, such as linearity, precision, accuracy, robustness, repetability, LOD, and LOQ, were carried out and described in this study.

Conclusion

The process analysis of obeticholic acid in API and tablet dosage forms has been established and validated using a simple quick, accurate, and precise RP-UHPLC and

UV-Spectroscopic approach. Without interfering with one another, these techniques can be used to determine the presence of obeticholic acid in single-component formulations. For routine and quality control analysis of the pharmaceutical preparations' examined drug components, the developed procedures are advised. The amount discovered using the suggested methods was fairly consistent with the formulation's label claim. The calculated coefficient of variation and standard deviation values were also adequately low, demonstrating the viability of the suggested approaches for the routine calculation of tablet dosage forms.

Abbreviation

OCA - OBETICHOLIC ACID
Meoh - Methanol
C2H3N - Acetonitrile
CH3COOH / H3PO4 - Acetic acid (0.1 %OPA)
R2 -Correlation coefficient
µL - Microlitre
mL - Millilitre
µg - Microgram
Mg - Milligram
G–Gram
S.D. - Standard deviation
%RSD - Relative standard deviation
LOD – Limit of Detection
LOQ – Limit of Qautification
HPLC – High Performance Liquid Chromatography
UHPLC –Ultra High Performance Liquid Chromatography

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