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METHOD DEVELOPMENT AND VALIDATION OF DISSOLUTION OF OBETICHOLIC ACID TABLETS BY RP-HPLC

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ABSTRACT

Obeticholic acid is a potent Farnesoid X receptor agonist. The main objective of the study is to develop a sensitive and reliable method for the estimation of percentage drug release in obeticholic acid tablets. This method is accurate and sensitive. A specific and precise RP-HPLC method has been developed and validated for measuring the drug release in Obeticholic acid tablet formulation by using refractive index detector. An isocratic separation was achieved in RI detector using an Accucore C18, (50*4.6mm, 2.6µm) column with pH 3.0 KH_2PO_4 buffer and ACN (50:50v/v) respectively at a flow rate of 0.8ml/min with refractive detector sensitivity of 256. The column temperature was maintained at 45°C. The injection volume was 100µl and the run time was maintained for 10 minutes. The method was validated for specificity, linearity, precision, accuracy and robustness. The method was linear over the range from 0.6ppm to 18.0ppm. The accuracy was between 96.1 and 100.2%.The method was found to be precise, accurate, and linear which can be used for the determination of drug release in Obeticholic acid tablets.

KEYWORDS: *Obeticholic acid, refractive index detector, RP-HPLC, Isocratic separation, dissolution.*

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INTRODUCTION

Dissolution is a process in which a solid substance is solubilised in a given solvent i.e. mass transfer from the solid surface to the liquid phase.[1] Obeticholic acid is a novel semi synthetic bile acid analogue and is the most potent Farnesoid X nuclear receptor (FXR) agonist used in the treatment of primary biliary cholangitis [2-4]. Chemically it is 6-ethyl-3, 7-dihydroxy cholan-24-oic acid 5-6 (Fig.1).

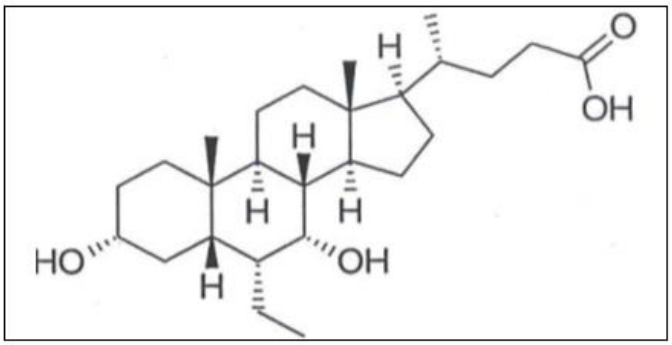

Figure 1
Obeticholic acid

Obeticholic acid, when given orally, binds to Farnesoid X receptor (FXR) which is found in the nucleus of the cells in the liver and intestine. FXR is a key regulator of bile acid metabolic pathways. Obeticholic acid increases bile flow from the liver and suppresses bile acid production in the liver, thus reducing the exposure of the liver to toxic levels of bile acids[5-6]. Content determination of obeticholic acid tablets by HPLC was reported[7]. HPLC/UV/MS method application for the separation of Obeticholic acid and its related compounds in the development process and quality control was also reported[8].

MATERIALS AND METHOD

Chemicals

Standard Obeticholic acid was obtained from Virupaksha organics limited.The chemicals used are ortho-phosphoric acid, Hydrochloric acid, Acetonitrile, Methanol, Potassium dihydrogen phosphate, Disodium hydrogen phosphate, Polysorbate 80 and water. All the chemicals used were of analytical grade. They were procured from the local market.

Instruments

Instruments used ad dissolution parameters were tabulated as follows (Table 1 and 2)

Table1
Instruments used

S.No	Instrument	Make/Model
01	HPLC	Waters Alliance Separation Module 2695 Detector: RI 2410
02	Dissolution apparatus	Lab India Model: DS 8000+
03	pH meter	LabIndia-PICO+
04	Milli-Q-Water system	Millipore, Elix-Gradient
05	Semi Micro Balance	Radwag-XA 82/220/2X
06	Pan Balance	Radwag-PS600.R2
07	Sonicator	PCI analytics

Preparation of dissolution media

70.6g of disodium hydrogen ortho-phosphate is dissolved in 9000ml of purified water and the pH is adjusted to 6.8 with 1N HCl and made up to 10,000ml with purified water and mixed well. To the above solution, 8ml of polysorbate 80 is added and mixed well.

Table 2
Dissolution parameters

Apparatus	USP II (Paddle)
RPM	75
Volume	900ml
Temperature	37.0±0.5°C
Procedure	900ml of dissolution media (pH 6.8 buffer + 0.08% polysorbate 80)
Time points	5,10,15,30 and 45minutes
Method source	USFDA drug dissolution database [9]
Standard concentration	6ppm

Instrumentation and chromatography

The HPLC system was water with a model no 2695, a refractive index detector of waters with model no 2410 with Empower 2 software for integration. Separation was achieved through Accucore C_{18}, 50 x 4.6mm, 2.6µm. The isocratic mobile phase pumped at a flow rate of 0.8ml/min consisted of pH 3.0 potassium dihydrogen phosphate buffer and Acetonitrile (50:50v/v) respectively. The prepared mobile phase was filtered through 0.45µm filter and degassed by sonication. The injection volume was 100µL and all separations were performed at a column temperature of 45°C and at a detector internal temperature of 45°C with sensitivity 256.

Standard and Sample Preparation

Preparation of standard stock (240ppm)

60mg of obeticholic acid was weighed and transferred into 250ml volumetric flask and 50ml of methanol was added and sonicated to dissolve and make up the volume to 250ml with methanol.

Preparation of standard (6ppm)

Pipette out 5ml of standard stock in 200ml volumetric flask and make up to the volume with dissolution media.

Preparation of sample

Transfer 900ml of dissolution media into each vessel and transfer tablet into each vessel. After specified time intervals sample was withdrawn and filtered through 0.45µm nylon syringe filter, discarding initial 2ml of filtrate. This is used for precision. The recoveries were determined by adding known amount of obeticholic acid reference standard.

Method validation

The method was validated according to ICH guidelines. The parameters which were used to validate the method were specificity, linearity, precision and recovery.

RESULTS AND DISCUSSION

The chromatographic conditions were optimized and separation was performed on Accucore C_{18}, 50 x 4.6mm, 2.6µm column using pH 3.0 potassium dihydrogen phosphate buffer and Acetonitrile (50:50v/v) respectively as mobile phase. The proposed mobile phase allowed suitable retention time and achieved good specificity. The retention time of obeticholic acid was found to be 5.6 (fig.2). Calibration curve was constructed using obeticholic acid standard solutions in the range of 0.6ppm to 18.0ppm. The correlation coefficient was found to be 0.998 (fig.3).

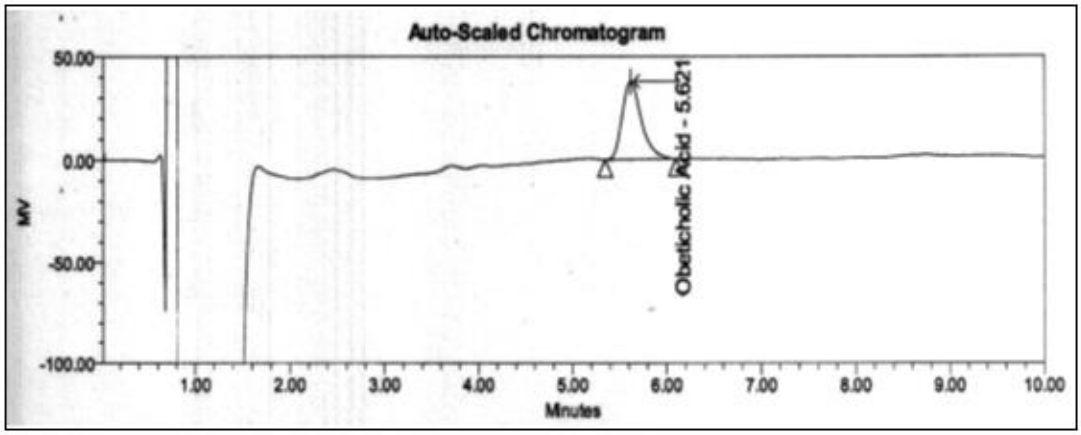

Figure 2
Standard chromatogram of obeticholic acid

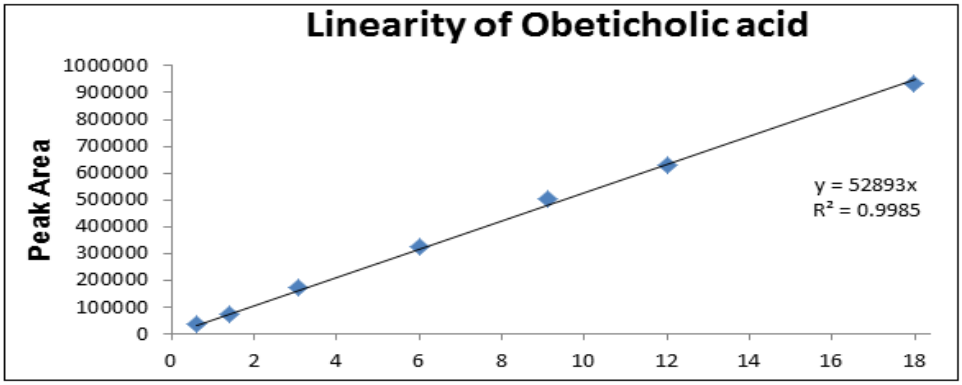

Figure 3
Linearity curve of obeticholic acid

The precision was determined by analyzing 6 injections of 30minutes sample. The %RSD was found to be less than 2.0 as shown in table 3. Recovery studies were found to be within limits (95%-105%) as shown in table 4.

Table3
Precision

S.No	Sample name	Precision
		%Drug release
01	Sample 1	95.5
02	Sample 2	91.1
03	Sample 3	92.3
04	Sample 4	91.6
05	Sample 5	92.4
06	Sample 6	90.8
	Average	92.3
	Minimum	90.8
	Maximum	95.5
	%RSD*	1.8

**** Relative standard deviation***

Table4
Recovery studies

S.No	Recovery level	Concentration (ppm)	Ppm added	Ppm found	%recovery	%average recovery
01	50	6.13	6.128	6.007	98.0	98.4
	50		6.128	6.056	98.8	
02	75	9.19	9.192	9.208	100.2	99.5
	75		9.192	9.071	98.7	
03	100	12.26	12.256	12.141	99.1	98.5
	100		12.256	11.991	97.8	
04	150	18.39	18.385	18.343	99.8	99.5
	150		18.382	18.238	99.2	

CONCLUSION

A specific, accurate and reproducible isocratic reverse phase HPLC method was developed for the estimation of %drug release in obeticholic acid tablets by RI detector. The developed method was validated. The method was found to be specific, linear, accurate and précised.

AUTHOR'S CONTRIBUTION STATEMENT

Naga Malleswari .B conceived of the presented idea. Mounica developed the theory and performed the computations. Naga Malleswari.B and Dr.S.Shobha Rani verified the analytical methods. All authors discussed the results and contributed towards the final manuscript.

CONFLICT OF INTEREST

Conflict of interest declared none.

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