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Analytical Method Development and Validation of Obeticholic acid Along With Stability Indicating Method by Quality By Design Approach

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ABSTRACT

A chromatographic method was developed to determine the of assay of Obeticholic acid with the help of Quality by design approach. QBD is a regulated technique for the process development. In Quality by Design, The method development starts with the specific intentions. A simple method was developed focusing on two critical quality attributes for development of the sturdy and polished method. The implication of self-sufficient factors was resolved using Fisher's statistical test for Analysis of the Variance (ANOVA) model that was predicted. The aim of the present work is to develop a simple, precise, accurate method for the estimation of obeticholic acid by reverse phase chromatographic technique. Improved chromatographic conditions were observed on Thermo C18 column (150mm x 4.6mm 5μm), using the mixture of Orthophosphoric acid (0.1 % OPA) and Acetonitrile (ACN) in the ratio of 55:45% v/v as mobile phase at the flow rate of 1.0mL/minute. The column temperature was set at 25°C and wavelength for detection of moiety was 210nm. The system suitability parameters of the instrument used were found within acceptance criteria as per the ICH guidelines. Linearity study was carried out over a range from 100 ppm to 300 ppm and R^2 value was found to be 0.9995. The precision study was calculated by injecting six samples of same concentration and the %Relative standard deviation was observed to be 0.3%. The mean percentage recoveries for 50%, 100% and 150% concentration were found to be 100.5%, 99.9% and 100.8% respectively. The percentage purity of the commercial sample assayed using the proposed method was found to be 99.8%. The developed method was validated according to ICH guidelines-Q2 (R1) and all the results were present within the acceptance criteria, Hence the proposed method can be employed for regular quality control analysis of obeticholic acid in bulk and pharmaceutical dosage forms.

Keywords: Analytical method development, Antipsychotic drug, Chromatography, Design expert, HPLC, Obeticholic acid, QBD approach.

INTRODUCTION

Obeticholic acid is a natural bile acid which was identified as most effective physiological ligand for farnesoid X receptor in 1999. Obeticholic acid got the approval from U.S. Food and drug Association for the treatment of primary biliary cholangitis in 2016. It got its approval as an orphan

drug as it is rarely available and cannot be produced for profitable purpose without the government assistance. Obeticholic acid (OBE), also known as 6-ethyl-chenodeoxycholic acid, is a brand-new semi-synthetic bile acid analogue. OBE is the strongest FXR agonist, and the presence of an ethyl group in the 6-position makes it possible to increase the activity of FXR-dependent processes as therapeutic targets for human disease.[1] Obeticholic acid is a dihydroxy-5 beta-cholanic acid, 3 alpha-hydroxy steroid, and 7 alpha-hydroxy steroid in its chemical form.[2]

Analytical method development and Analytical method validation are considered as fundamentals for each and every pharmaceutical development schedule. A chromatographic analytical method is developed to determine purity of the compound to be analysed. A successful method development make sure that the resources that are available in the laboratory are amended and the methods fulfil the manifesto that are mandatory at ascending stages of the method development of the drug. An appropriate definition of method validation can be penned as "The process of demonstrating that analytical procedures are suitable for their intended use."[3,4,5]

A chromatographic analytical method development is considered to be an instinctive and tedious process. In this modern and competitive world, the companies hastens the drug development curriculum and the competent drug pass through this process and it is necessary that the method developed must be rapid and robust.Most of the analytical methods are developed manually following the OFAT (One Factor At a Time) process, where only one factor is taken in to the consideration and the further modifications are dependent on the results obtained from the variations made in the factor. This is the time consuming process and repeated till the any advancement is observed and other parameter can be used[6,7,8,9,10,11]. The improvement can be made in this process for the reduction of time by applying QBD (Quality By Design) approach. QBD is applied using the software based DOE (Design Of Experiment) operations that depends on multivariate modelling to anticipate accordingly and produce the advanced analytical chromatographic method which are then performed on HPLC system, resulting in significant growth in productivity.[12,13,14,15,16,17]

MATERIALS AND METHODS

Instrumentation and chromatographic conditions

HPLC system of Agilent technologies 1260 Infinity Quaternary LC system model attached to DAD detector, auto sampler, column oven and degasser was used during the method development, forced degradation and validation. Data were evaluated on Chromeleon software version 6.8. Thermo C-18 column (150mm*4.6mm*5μ) was used as stationary phase for separation. Ultrasonic bath, Sartorius analytical balance and Vacuum micro unit with 0.45μ membrane filter were used during the study.

Chemicals and Reagents

Obeticholic acid API was received as a gift sample from reputed pharmaceutical company. HPLC grade Acetonitrile (Rankem), Orthophosphoric Acid (Rankem), Hydrochloric acid (Merck), sodium hydroxide (Rankem), Hydrogen peroxide (Rankem) was used during development and analysis.

Chromatographic conditions

Separation was obtained in Thermo C-18 column (150mm*4.6mm*5μ) when column and sampler temperature were maintained at ambient temperature. The isocratic elution method was applied with Buffer: Acetonitrile (55:45)(%v/v) as a mobile phase. The flow rate was adjusted to 1 mL/min and the injection volume was 20μL. The maxima for Obeticholic acid was detected at 210nm. The peak purity of main peak was checked using DAD detector.

0.1% Orthophosphoric acid

1 mL of orthophosphoric acid was diluted in 1000 mL milli Q water, mixed well and sonicated for 2 minutes. The Buffer was filtered through 0.45μ membrane filter.

Mobile phase preparation

Mobile phase was prepared by mixing 550 mL buffer and 450 mL acetonitrile.

Selection of wavelength

The wavelength for detection of Obeticholic

acid was selected by scanning 200 ppm sample of Obeticholic acid in diluent in UV spectrophotometer and the maxima for the drug was observed at 210nm.

Selection of mobile phase

The trials for the different mobile phases were injected in the HPLC system for finalizing of mobile phase. Mobile phases used were different proportion of water, Acetonitrile, Methanol and Buffers. It was concluded that mixture of 0.1% Orthophosphoric acid and acetonitrile in (55:45) v/v amount provided the gratifying results which passes the criteria to be followed according to ICH guidelines. Thus the finalized mobile phase is 0.1% Orthophosphoric acid and acetonitrile (55:45 v/v).

Preparation of standard solution

Standard stock solution of 200 ppm was prepared by dissolving 20 mg Obeticholic acid API in 100 mL mobile phase. 20 ppm diluted standard solution was prepared by diluting 5 mL of standard stock solution to 50 mL in mobile phase.

All the critical parameters used in the study passed the acceptance criteria according to ICH Guidelines. The system suitability criteria for the analytical method to be performed on HPLC are RSD%, tailing factor and theoretical plates. The solvent ratio and the flow rate were studied and optimized with design of experiments. The pH was not taken into the consideration as it did not show any major variation in retention time and the area of analyte during analysis. The effect of flow rate and the solvent ratio in the mobile phase in the variation of the results were studied. Most of the development studies follows OFAT (one Factor at a Time) but with the help of DOE multiple factors can be studied at one go. Thus the Central composite method, one of the widely used methods, is followed in QBD. Totally 13 experimental trials were implied by the software for analysing the cooperation of each level on formulation characters and the peak area (R1), tailing factor (5%) (R2) and theoretical plates were taken as dependent factors. The connotation of self-reliant factors was calculated using Fisher's statistical test for Analysis of the Variance (ANOVA) model that was estimated.

Table 1: 2^2 Factorial design with high & low level factor statistical sequence optimization

Sr. No	A:Mobile Phase (0.1% OPA) (mL)	B:Flow Rate mL/min
1	53	0.9
2	57	0.9
3	53	1.1
4	57	1.1
5	52	1
6	58	1
7	55	0.86
8	55	1.14
9	55	1
10	55	1
11	55	1
12	55	1
13	55	1

RESULTS

System suitability and precision

System suitability and precision were demonstrated by injecting six replicates of standard solution, prepared as per the test method. The peak area of analyte was recorded for replicate injections of standard solution. The precision was calculated by computing the relative standard deviation for the peak areaof replicate injections.

The observations are mentioned in Table 2.

Table 2: Replicates of Standard solution

Injection no	Area of analyte
1	2908163
2	2899220
3	2908999
4	2889663
5	2898626
6	2909615
Average	2902381
% RSD	0.27

Table 3: System suitability observation

Parameters	Results
The relative standard deviation for peak area of Obeticholic Acidconcluded from six replicate injections of standard solution	0.27 %
Average tailing factor for Obeticholic Acid peak	1.17
Average theoretical plates for Obeticholic Acid peak	3950

The obtained results of the system suitability and precision are found within limit, which indicates that the system is correct and literal for analysis.

Method precision

Method precision was demonstrated by preparing six different samples of same batch as per the test method. The correctness of the method was studied by calculating the relative standard deviation for %Assay value. The results of the precision study are mentioned in Table 4.

Table 4: Method Precision Sample Replicates

Precision sample set no	% Assay
1	99.6
2	100.1
3	99.0
4	100.7
5	99.3
6	99.3
Average	100.3
%RSD	0.63 %

As the results shown are found to be within limit, thus it is concluded that method is suitable for determination of Assay in the sample.

Stability of analytical solution

Stability of analytical solutions (sample solution & standard) were established by preparing standard solution and sample as per method and by injecting at different intervals of time. The study was conducted at temperature 25°C up to 26 h for standard solution and sample solution.

Table 5: Stability of standard solution at temperature: 25°C

Time (hours)	Area	%RSD
Initial	2908163	NA
7	2899265	0.2
9	2894563	0.2
15	2896546	0.2
24	2885642	0.3
26	2889652	0.3

Table: 6 Stability of sample solution at temperature: 25°C

Time (hours)	Assay	Absolute Difference (%)
Initial	99.6	NA
7	99.4	0.1
9	99.8	0.2
15	99.6	0.2
24	100.6	0.5
26	99.0	0.5

Sample and standard solutionsare found stable up to 26 h at 25°C temperature.

Linearity

The linearity of Obeticholic acid was carried out by preparing solutions of Obeticholic acid standard over the range of 50% to 150% of standard concentration. These solutions were analyzed into the HPLC system and area response of the solutions were reported. A graph of concentration Vs. Obeticholic acid peak area was plotted for the calculation ofthe correlation co-efficient between concentration and Obeticholic acid peak. The observations are mentioned in Table 6.

The study shows that the response of Obeticholic acidis linear from 50% to 150% of standard concentration.

Table 6: Linearity of Obeticholic acid

Linearity level	Peak area
50%	1369141
80%	2254210
100%	2908135
120%	3578877
150%	4415850
Correlation coefficient (R)	0.9985
Resodual Sum Of Square (R2)	0.9970
The y-intercept (100% level)	-4.5278
Slope	14386.1700
Intercept	-61991.4000

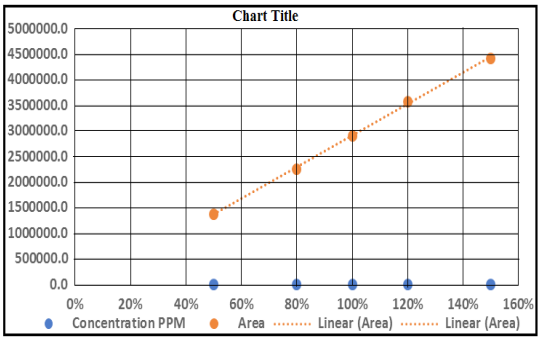

Fig. 1. A plot of concentration Vs. Obeticholic acid peak area

Accuracy

The accuracy of the anlytical method was demonstrated by preparing recovery samples at the level of 50%, 100% and 150%. The recovery samples were prepared in set of three at each level. The above samples were analysed and the amountof API recovered from sample was calculated. The

observations are tabulated in below Table 7, 8 and 9.

Table 7: Recovery at 50% level

Sample no	Amount spiked (mcg/mL)	Amount recovered (mcg/mL)	%Recovery
1	50	50.092	100.2
2	50	49.850	99.7
3	50	50.875	101.7
Average			100.5
%RSD			1.1%

Table 8: Recovery at 100% level

Sample no	Amount spiked (mcg/mL)	Amount recovered (mcg/mL)	%Recovery
1	100	100.235	100.2
2	100	99.804	99.8
3	100	99.573	99.6
Average			99.9
%RSD			0.3 %

Table 9: Recovery at 150% level

Sample no	Amount spiked (mcg/mL)	Amount recovered (mcg/mL)	% Recovery
1	150	152.146	101.4
2	150	149.961	100.0
3	150	151.595	101.1
Average			100.8
%RSD			0.8 %

As the recovery results obtained for Obeticholic acid was within the acceptable limits of recovery, it is been proved that the method is specific for quantification of Obeticholic acid in the range of 50% to 150% of target concentration.

Specificity

The specificity of the test method was demonstrated by studying the interference from diluent and forced degradation samples. The samples with the stress conditions such as Acid degradation, Base degradation, Thermal degradation, Photolytic degradation and Oxidative degradation were prepared and injected. No degradation sample showed the interference with main peak.

Robustness

The robustness was done by change in various parameters such as Flow rate of mobile phase, pH value of the buffer and the solvent ratio in the mobile phase. The samples were injected in triplicates. The results obtained are demonstrated in the following Table 11.

Table 10: Degradation Data for specificity

Stress Type	%Assay	%Degradation	Peak purity
As such	99.6	No degradation	Pass
Acid Hydrolysis	78.1	21.5%	Pass
Alkali Hydrolysis	92.2	7.4%	Pass
Peroxide Oxidation	97.7	1.9 %	Pass
Thermal Degradation	98.2	1.4 %	Pass
UV light Degradation	99.1	0.5 %	Pass

Table 11: Robustness data

Parameter	Condition	Mean Peak area	%RSD(%)
Flow rate	0.9 ml/min	2996604	0.46
	1.1 ml/min	2734830	1.16
Solvent Ratio	47%	2865965	0.44
	43%	3049977	1.38
Column Temperature	23	2789867	0.27
	27	2856393	0.12

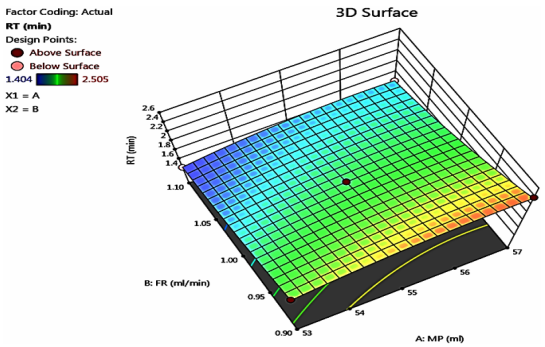

Fig. 2. 3D presentation of for impact of Flow and OPA on Retention Time

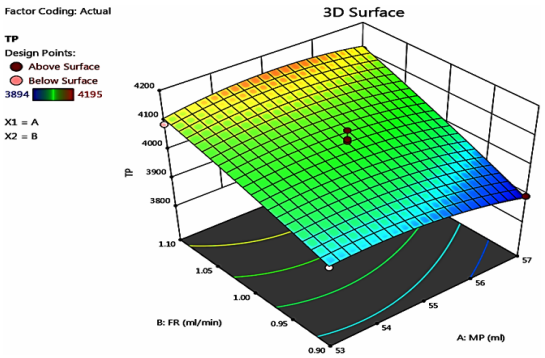

Fig. 3. 3D presentation of for impact of Flow and OPA on Theoretical Plates

CONCLUSION

A sturdy method for development of

Obeticholic acid API was developed on basis of Quality by Design approach on Design-Expert® Software, Version 12. Two self-reliant factors were taken in to consideration such as flow rate and solvent ratio. Total 13 trial were recommended by the software for studying the synergy of individual level on the peak area, tailing factor and number of theoretical plate which the major response factors (dependent factors). The method validation was carried out following ICH guidelines. Specificity of the method was performed byanalysing degradation samples. The assay method for the API was prepared with present chromatographic condition developed, moreover it is found to be more authentic and decisive. The %Assay and %RSD appeared in range 100±1.5% and <2, respectively, which meets the criteria mentioned in the ICH guideline. The stability studies were carried out and the samples are found to be stable for more than 26 hours. According ICH guidelines, the forced degradation study was performed and no obstruction were observed because of degradation products elution time of the main product. Design Expert was able to automatically predict and test, optimized analytical methods.

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