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ANALYTICAL QUALITY BY DESIGN APPROACH IN RP-HPLC METHOD DEVELOPMENT FOR THE ASSAY OF PITAVASTATIN IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

Chromatographic method was developed according to Quality by Design (QbD) approach as per ICH Q8 (R2) guidelines for estimation of pitavastatin in pharmaceutical dosage form. By considering the current regulatory requirement for an analytical method development, a reversed phase high performance liquid chromatographic method for routine analysis of Pitavastatin has been optimized using analytical quality by design approach. Unlike routine approach, the present study was initiated with understanding of quality target product profile, analytical target profile and risk assessment for method variables that affect the method response. A liquid chromatography system equipped with a C_{18} column (250×4.6 mm, 5 µ), a binary pump and photodiode array detector were used in this work. The experiments were conducted based on plan by central composite design, which could save time, reagents and other resources. Sigma Tech software was used to plan and analyses the experimental observations and obtain quadratic process model. The process model was used for predictive solution for retention time. The predicted data from contour diagram for retention time were verified actually and it satisfied with actual experimental data. The optimized method was achieved at 1.2 ml/min flow rate of using mobile phase composition of methanol and OPA in water at 80:20 % v/v, pH adjusted to 6.5 adjusted with 10%ammonia. The method was validated and verified at flexible input variable level for high degree of robustness and system suitability during method transfer.

KEYWORDS: Analytical quality by design, Pitavastatin, analytical target profile, HPLC, Method development, Validation.

INTRODUCTION

Pitavastatin is a competitive inhibitor of HMG CoA reductase and was developed for the treatment of hypercholesterolaemia.[1-3] It can reduce plasma level of LDL cholesterol by 40% in hypercholesterolaemic patients.[4] Pitavastatin is chemically 3-hydroxy-3-methyl glutaryl coenzyme A reductase inhibitor, it is chemically (3R, 5S)-7-(2- cyclopropyl-4-(4-fluorophenyl) quinolin-3-yl) - 3,5-dihydroxy 6(E) -heptenoic acid calcium salt. Several analytical methods have been reported for quantitative determination of pitavastatin alone and in combination with other drugs in formulations as well as in biological fluids including high–performance liquid chromatographic methods[5-7], chromatography–mass spectrometric methods (LC–MS)[8-12], HPTLC[13-14], UV Spectroscopy method [15-16] and Ultra-fast performance chromatography.[17] Extensive literature survey revealed that there is no RP HPLC method available for estimation of pitavastatin in bulk and pharmaceutical dosage forms using QbD approach. Hence, it was planned to develop simple, economical, and less time consuming methods including High Performance liquid Chromatographic method for estimation of pitavastatin using QbD approach. Applying the principles of QbD to analytical method could result in more robust method which produce consistent, reliable and quality data throughout the life cycle and in turn will lead to less method incidents when used in the routine environment. This would mean less time spent on investigations and ultimately save time and money.

Quality by Design approach suggests looking into the quality of analytical process during the development stage itself. It says that quality should be built into the process design rather than testing into final results of analytical process.[18] QbD is defined as "systematic approach to development that begins with predefined objectives and emphasizes product and process understanding based on sound science and quality risk management".[19] In alignment with the approach proposed in the draft FDA guidance for process validation, a Three-stage approach[20] can be applied to method validation.

Stage 1. Method Design. Define method requirements and conditions and identify critical controls.
Stage 2. Method Qualification. Confirm that the method is capable of meeting its design intent.
Stage 3. Continued Method Verification. Gain ongoing assurance to ensure that the method remains in a state of control during routine use.

A critical function of Stage 1 is the design of an Analytical Target Profile (ATP) for the method. To design the ATP, it is necessary to determine the characteristics that will be indicators of method performance for its intended use. These are selected from the performance characteristics described in ICH Q2 as per the traditional approach.[21] Instead of being applied in a tick box manner, they are investigated by a risk assessment exercise as described in ICH Q9[22] in combination with carefully designed development studies to identify the critical method and sources of variation.[23] Variables are then investigated by robustness and ruggedness experiments to understand the functional relationship between method input variables and each of the method performance characteristics and the results are compared to the desired outcome defined in the ATP. From this, one can identify a set of operational method controls. Also, having evaluated the critical method parameters and gained a better understanding of the method through structured experimentation, a control strategy can be built into the method to ensure a consistent performance throughout its life cycle.[24] A key advantage of the QbD approach for all of the above situations is the flexibility to perform a qualification against the specific ATP defined for the intended use of the method.[25]

Implementation of QbD Approach

According to ICH Q8 (R2) guidelines, an experimental work was planned and QbD approach was implemented as follows.

Method Design: The method design stage includes establishing the method performance requirements, developing a method that will meet these requirements and then performing appropriate studies to understand the critical method variables that must be controlled to assure the method is robust.

Method Performance Requirements: Utilizing AQbD approach, it is essential at this stage that sufficient thought be given to the intended use of the method and that the objectives or performance requirements of the method be fully documented. This represents the Analytical Target Profile (ATP) [26] for the method. ATP is the estimation of pitavastatin in tablet dosage form using HPLC method.

Method Development: Once the ATP has been defined, an appropriate technique and method conditions must be selected in order to meet the requirements of the ATP.

Method Understanding: Based on an assessment of risk (i.e., the method complexity and the potential for robustness and ruggedness issues) one can perform an exercise focused on understanding the method to better understand what impact key input variables might have on the method's performance characteristics as per regulatory requirement thus HPLC is must for specificity. From this, one can identify a set of operational method controls.

Risk Assessment: Experiments can be run to understand the functional relationship between method input variables and each of the method performance characteristics. Knowledge accumulated during the development and initial use of the method provides input into a risk assessment which may be used to determine which variables need studying and which require controls.

Design of Experiment: Robustness experiments are typically performed on parametric variables using Design of Experiments (DoE) to ensure that maximum understanding is gained while minimizing the total number of experiments. Depending on the type of method, surrogate measures of characteristics such as accuracy or precision may be evaluated.

Method Design Output: A set of method conditions will have been developed and defined which are expected to meet the ATP. Those conditions will have been optimized based on understanding of their impact on method performance like accuracy and precision. Here method responses are taken as output as they are the indicator of accuracy and precision.

QbD-based treatment of the robustness of an analytical method requires the assessment of all parameters (factors) which most strongly influence selectivity (results) alone and in combination. The experimental verification of many factors simultaneously is impractical and associated with extreme technical difficulties and expense. Some authors, have employed statistical studies, such as Plackett-Burman or fractional factorial designs and risk-based approaches [27–31] to overcome the challenge and reduce the experimental workload. Other procedures include running automated robustness experiments.[32–35] The present paper, however, employs statistical analysis that is principal component analysis which exhibits factor extraction of variable parameters to evaluate robustness.

MATERIALS AND METHODS

All reagents used in the experimental work were of

HPLC grade. HPLC grade methanol and water were purchased from Merck, Mumbai, India. Pitavastatin of 97.80% purity was supplied by Hetero drugs limited, Hyderabad as gift sample.

Chromatography

Chromatographic separations were carried out using Agilent LC system (LC-1200 series), consisting of a binary pump, a Rheodyne injector with a 20 µl loop and a photodiode array detector (DAD). A chromatographic column used was Qualisil Gold C18 (150×4.6 mm, i.d., 5 µ particle size). The output signal was monitored and processed using Ezchrome Elite software resident in a Pentium computer (Digital Equipment). Peak identify was confirmed by retention time comparison. Peak purity was assessed by purity plot. The mobile phase was composed of methanol and OPA in water at variable on experimental design as stated in Table 1. The mobile phases were prepared daily, filtered through a 0.45 µ membrane filter and degassed using sonicator prior to use. The DAD detection was carried out at 245 nm wavelength, and the injection volume was 20 µl. The optimized chromatogram is shown in Fig. 1.

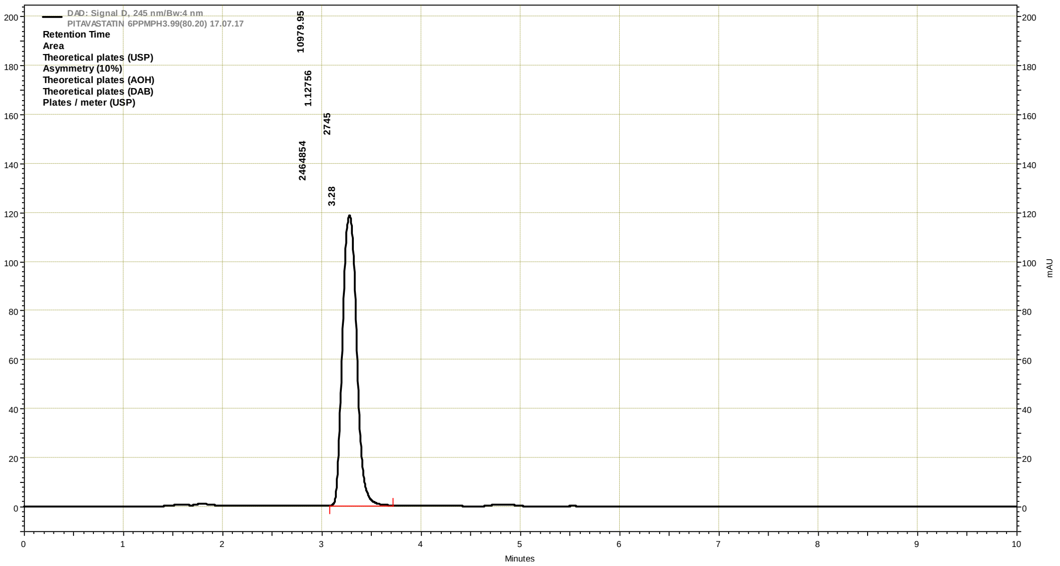


Fig. 1: Optimized RP-HPLC chromatogram of pitavastatin. Optimized RP -HPLC chromatogram of pitavastatin (t_R: 3.42 min) on C_{18} column using MODR Concept (pitavastatin eluted at 3.42 min, and the method was selected from MODR, and verified for robustness and validated).

Standard stock solution preparation

10 mg of pitavastatin was accurately weighed and transferred into a clean dry 10 ml volumetric flask, 5 ml of diluent (mobile phase) was added, sonicated for 5 minutes and made up to the final volume with diluent. . Further 1 ml from the above stock solution was taken into a 10 ml volumetric flask and made up to 10 ml with diluent.

Sample solution Preparation

20 tablets were weighed and crushed. A powder equivalent to 10 mg of pitavastatin was transferred into a clean dry 10 ml volumetric flask, 5 ml of diluent was added, sonicated for 5 minutes and made up to the final volume with diluent. Further 1 ml from the above stock solution was taken into a 10 ml volumetric flask and made up to 10 ml with diluent.

Experimental design

The present AQbD work was carried out as per the cited literatures[36–38] to investigate the impact of different variables on retention time (as method response) and to verify method performances. The levels of these variables are as follows: proportion of the aqueous (X_1) in the mobile phase (5% and 25%), pH (X_2) of aqueous phase used in the mobile phase (5.0 and 7.0), and the flow rate (X_3) of mobile phase (0.8 and 1.2 ml/min), which are given in Table 1. The retention time (Y1) and number of theoretical plates (Y2) were used as response in experimental design as controlling response, which is expected to affect and control method responses. A 2^3 factorial design consisting of 3 factors at 2 levels was considered for experimental plan initially and after confirming that the process is a nonlinear central composite design (CCD) was used. The experimental observations along with Factorial Design (DOE) plan are shown in Table 2 and the statistical analysis is given in Table 3 and 4. The MODR was defined using all three variables. From MODR suitable method conditions were selected and subjected to verification for method performance like accuracy and precision (less than 2% RSD) and robustness as targeted responses.

Table 1: Levels in design of experiments.

X	Units	-2	-1	0	+1	+2
% Aqueous (X1)	%	25	30	35	40	45
pH (X2)	NIL	4.5	5	6	6.5	7
Flow rate (X3)	Ml/min	0.8	0.9	1	1.1	1.2

Table 2: Central composite design plan and observed data (Y1) and (Y2).

S. No	Combination	% of aqueous phase (X1)	Ph of aqueous phase (X2)	Flow rate (X3)	Retention time (Y1)	No of theoritical plates(Y2)
1	I	30	5.5	0.9	3.39	1024
2	X_1	40	5.5	0.9	5.843	2413
3	X_2	30	6.5	0.9	3.84	1039
4	$X_1 x_2$	40	6.5	0.9	6.58	1190
5	X_3	30	5.5	1.1	2.92	1494
6	$X_1 x_3$	40	5.5	1.1	5.043	828
7	$X_2 x_3$	30	6.5	1.1	2.30	823
8	$X_1 x_2 x_3$	40	6.5	1.1	4.94	1183
9	Mid points	35	6	1	4.81	1295
10	Mid points	35	6	1	4.86	1332
11	Mid points	35	6	1	4.88	1339
12	Mid points	35	6	1	4.76	1245
13	CCD	35	5	1	6.90	1793
14	CCD	35	7	1	4.030	1474
15	CCD	25	6	1	2.843	2778
16	CCD	45	6	1	13.02	1390
17	CCD	35	6	0.8	5.760	1101
18	CCD	35	6	1.2	3.523	1114

CCD: Central composite design

Table 3: Central composite analysis for response (Y1; Retention time).

Coefficient	Name of variable and interaction	Value of coefficient	SS %	F-test	P-value
B0	-	4.362	-	-	-
B1	P^H	1.2395	80.358	529.7794	<0.1
B2	% aqueous phase	0.063	0.2076	1.3686	<0.01
B3	P^H and % aqueous phase	0.0955	0.477	3.1449	<0.01
B12	Flow rate and mobile phase	-0.5512	15.8911	104.766	<0.01
B13	P^H and flow rate	-0.0587	0.1802	1.1882	<0.01
B23	% aqueous phase and flow rate	-0.2337	2.8566	18.833	<0.01
B123	P^H, % aqueous phase and flow rate	0.0237	0.0294	0.1937	<0.01

P value <0.01indicates that interaction levels among variables are significant

Table 4: Central composite analysis for response (Y2; Plate count).

Combination	Name of variable and interaction	Coefficient	% SS ratio	F-Value	P-value
B0	-	1249.25	-	0.0	
B1	P^H	154.25	10.1452	12.827	<0.1
B2	% aqueous phase	-190.5	15.4739	19.5644	<0.01
B3	P^H and % aqueous phase	-26.5	0.2993	0.3786	<0.01
B12	Flow rate and mobile phase	-167.25	11.9273	15.0802	<0.01
B13	P^H and flow rate	-230.75	22.7036	28.7051	<0.01
B23	% aqueous phase and flow rate	111.5	5.301	6.7023	<0.01
B123	P^H, % aqueous phase and flow rate	283.0	34.1495	43.1766	<0.01

RESULTS

Statistical analysis

The behavior of the system was explained by the following polynomial equation. $Y1=b_0+b_1X_1+b_2X_2+b_3X_3+b_{12}X_1X_2+b_{23}X_2X_3+b_{13}X_1X_3+b_{123}X_1X_2X_3$….Eqn. 1.and

$Y2= b_0+b_1X_1+b_2X_2+b_3X_3+b_{12}X_1X_2+b_{23}X_2X_3+b_{13}X_1X_3+b_{123}X_1X_2X_3$….Eqn. 2

Where, Y1 and Y2 are the responses. B_0 is the intercept, b_1, b_2, b_3 are the regression coefficients of variables for X_1, X_2, and X_3 respectively.b_{12}, b_{23}, b_{13} are the regression coefficients for two factor interactions between $X_1X_2X_3$.

Sigma Tech software was used for the statistical analysis of the experimental observations and the analysis is given in Table 3 and **4.** The optimized method was found to be at 20% aqueous phase with a pH 5. The understanding are as follows so that co efficient of % aqueous phase was found to be negative (-190.5) with % ss ratio of 15 which was a maximum of effect was found to be among all variables. So, hence the attempt was made to reduce the % aqueous phase as low as possible. The effect of pH was found to be (coeffient) +154 with a % ratio of 10.144 indicating the positive effect on theoretical plate.

The interaction effect among variables as follows
Two variable interactions
Flow rate and mobile phase it was found to be 11.9%, pH and flow rate it was found to be 22.7% and both above interactions were found to be negative co efficient value with significant effect.

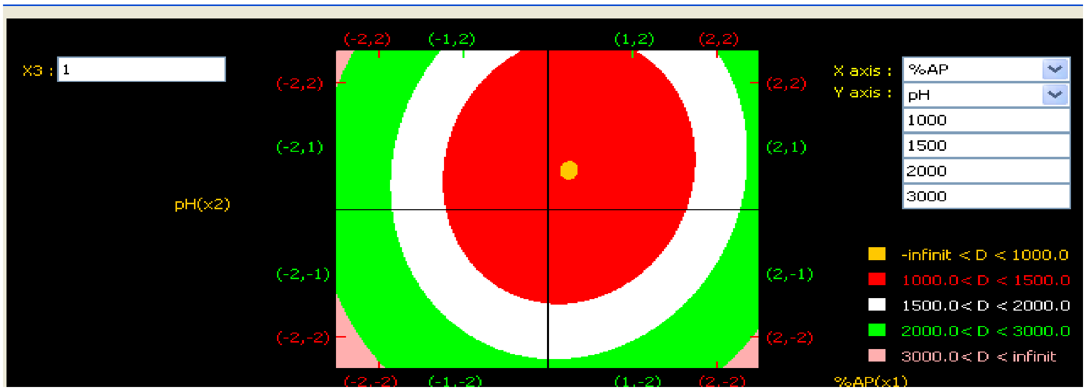
The three variable interactions also found to be positive coefficient with %SS of 34% and F value was found to be 43 indicated the interactions of three variables effect in method performance is highly significant. ANOVA indicated that the process model with X1, X2, X3 along with interactions is highly significant at 99% Confidence level (*P* <0.1) . Since the Curvature effect is significant and says it has nonlinear relationship between Y and Xs, it requires to go for CCD i.e. central composite design and accordingly the CCD plan and observed data are given below in Table 6. The following Quadratic model was obtained on application of Sigma Tech software,

$Y1=b_0+b_1X_1+b_2X_2+b_3X_3+b_{12}X_1X_2+b_{23}X_2X_3+b_{13}X_1X_3+b_{123}X_1X_2X_3$….Eqn. 1.and

$Y2= b_0+b_1X_1+b_2X_2+b_3X_3+b_{12}X_1X_2+b_{23}X_2X_3+b_{13}X_1X_3+b_{123}X_1X_2X_3$….Eqn. 2

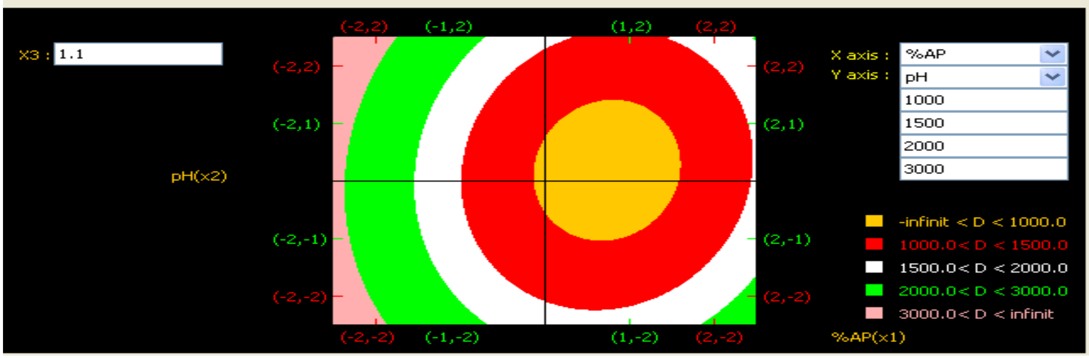
The coefficient of determination (r^2) for the above process model was 0.9812 and 0.995. Hence the Process model is well valid to predict the behavior of the process and can be used for simulation of the process model. The design space or MODR region for robustness was achieved from contours (Fig. 3**).** These regions offer robust processes parameters.

Contours
There could be different combinations, which may give a number of feasible solutions for robust process. X1 vs X2 with X3 as kept constant, X2 vs X3 with X1 as kept constant, X1 vs X3 with X2 as kept constant. Out of these combinations, whichever is the most desirable from the point of retention time that can be selected as a robust process. This contour space is called as design space in products and method operable design region (MODR) in analytical works. The MODR that control the variation in response is obtained from contours a two dimensional plot and it resembles same as Fig. 2 for other variable combinations.


X3 : 1
X axis : %AP
Y axis : pH
1000
1500
2000
3000

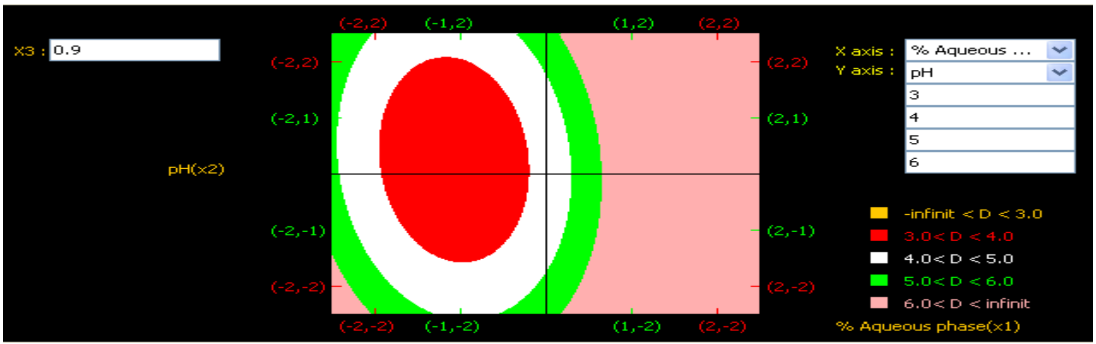
🟨 -infinit < D < 1000.0
🟥 1000.0< D < 1500.0
⬜ 1500.0< D < 2000.0
🟩 2000.0< D < 3000.0
▨ 3000.0< D < infinit

Fig. 2: P^H of aqueous phase versus % of aqueous phase contour at 1 ml/min flow rate of mobile phase.


X3 : 1.1
X axis : %AP
Y axis : pH
1000
1500
2000
3000

🟨 -infinit < D < 1000.0
🟥 1000.0< D < 1500.0
⬜ 1500.0< D < 2000.0
🟩 2000.0< D < 3000.0
▨ 3000.0< D < infinit

Fig. 3: Contour of method optimization.
P^H of aqueous phase versus % of aqueous phase contour at 1.1 ml/min flow rate of mobile phase (analyte shows large design space pitavastatin only when theoretical plates are more than 2000).


X3 : 0.9

X axis : % Aqueous ...

Y axis : pH

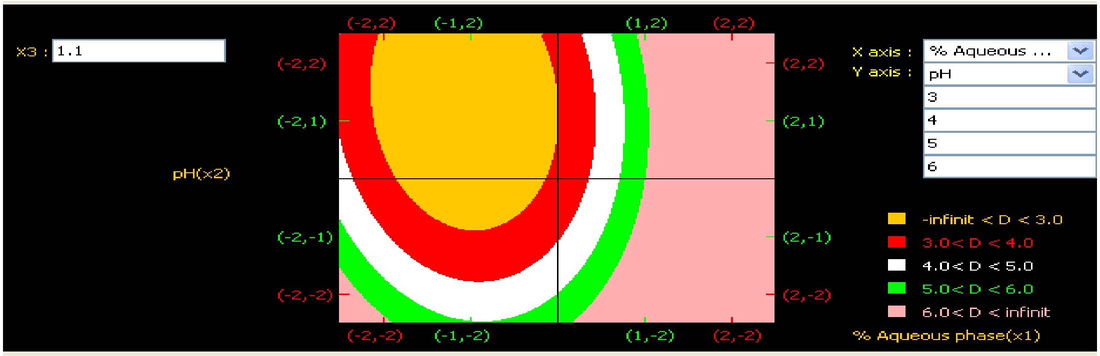
3

4

5

6

Fig. 4: P^{H} of aqueous phase versus % of aqueous phase contour at 0.9 ml/min flow rate of mobile phase.


X3 : 1.1

X axis : % Aqueous ...

Y axis : pH

3

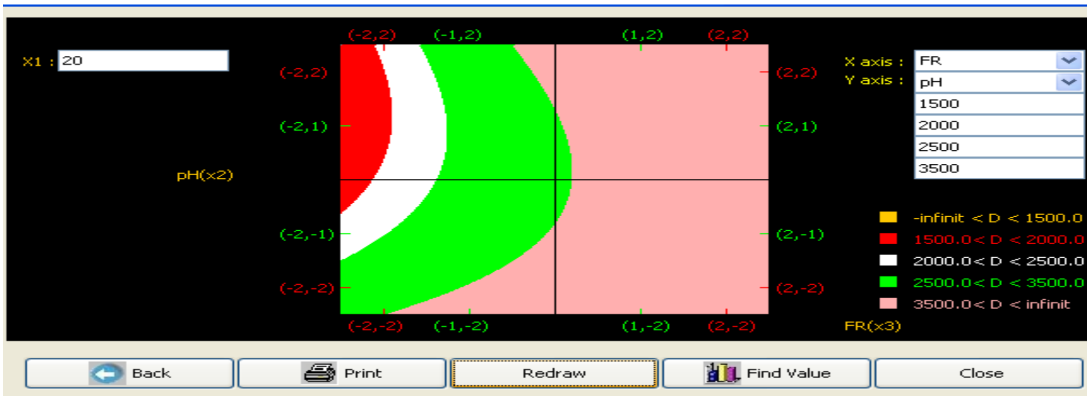
4

5

6

Fig. 5: Contour of method optimization.

P^{H} of aqueous phase versus % of aqueous phase contour at 1.1 ml/min flow rate of mobile phase (analyte shows large design space pitavastatin only when retention time is more than 3).


X1 : 20

X axis : FR

Y axis : pH

1500

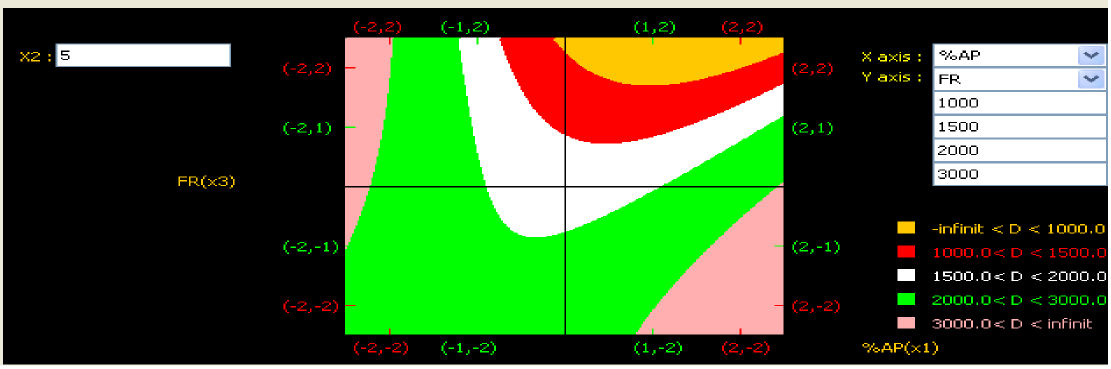
2000

2500

3500

Fig.6: Contour of method optimization.

P^{H} of aqueous phase versus flow rate contour at 20% of aqueous phase (analyte shows large design space pitavastatin only when theoretical plates is more than 3000).


X2 : 5

X axis : %AP

Y axis : FR

1000

1500

2000

3000

Fig. 7: Contour of method optimization.

% of aqueous phase versus flow rate contour at ph 5 of aqueous phase (analyte shows large design space pitavastatin only when theoretical plates is more than 2000).

Contour indicated that (at flow rate1, 1.1, 1.2, 0.9) the highest flow rate at 1.1 to 1.2 shows the acceptable plate count from 3000 – 4000 and it was also compared with suitable % aqueous phase, so indicated that the lowest % aqueous phase with highest flow rate i.e % aqueous phase < -2 level, pH > 0 level and flow rate > 0 level. Could be the most preferred levels to obtain the acceptable method performance hence the method was optimized at % aqueous phase 20, pH 5 and flow rate 1.1 ml/min. These three combinations are shown at Table 7. The three combinations, the contour Fig. 6 gave the best design space covering entire range of variables and retention time of 3 to 4 and were taken for verification purpose. It was also noted that the optimized % aqueous of 20% (X1) at flow rate 1.1 ml/min (X3), gives significant results, which are not affected by the pH from 4.5 to 5. Hence, the pH was kept at pH 5, which offered several method advantages like column long life, mobile phase stability of analyte and symmetric elution. The mathematical model the proposed contours were validated by experimental verification of predicted retention time (t_R) and the results are shown in Table 5.

TABLE 5: Summary of all contours for different method operable design region.

Range of coded values of variables X_2	Range of absolute values of variables X_1	Constant absolute value of variables	Y=retention time	Y2= no.of theoretical plates
$X_{1=}$ -0.8 to 0.2	X1= 11 to 16	X3=1.1(flow rate)	3-5	2000-3000
$X_{2=}$ -2 to 0.2	X2=5 to 7			
X1= -2 to 0. 2	X1= 5 to 25	X1=20%(aqueous)	3-4	2500-3500
X3= 0 to 2	X3= 1 to 1.2			
X2= -2 to 2	X2= 5 to 7	X2=5 (pH)	5-6	2000-5000
X3= -1.2 to 2	X3= 0.88 to 1.2			

*Constant absolute value are used as optimized method conditions

Chromatographic conditions after optimization:

After robust process was obtained as at **Fig. 1**, HPLC analyses were carried out using methanol and 0.1% OPA in water (80:20, %v/v) as mobile phase, pH adjusted to 5 and flow rate at 1.1 ml/min on C18 analytical column, UV-PDA detection wavelength at 245 nm and 20 µl of injection volume, which gave a retention time (R_t) of 3.39 min. These parameters are within MODR and hence this design space has been validated also.

Table 6: Optimized method variable and conditions

Parameter	Range	Condition adopted	Robust verified
% Aqueosu	20-30%	20%	± 3%
pH	4.5-5.0	5.0	± 0.2%
Flow rate	0.9-1.1	1.1	± 0.1
Column	C18 (250 x 4.6 mm, 5µ) Qualisil Gold		
Organic phase	Methanol		
Buffer	0.3 % (pH 5.0)		
Column temp.	Ambient		
Detection	PDA @245 nm		
Injection volume	20 µL (manual injection)		
Elution mode	Isocratic		

Verification of method by method transfer

The robust method was verified on two instruments in different laboratory and the robustness and other system suitability parameters were compared. The % assay result and its %RSD value were calculated. Accuracy and precision were compared.

Validation of the robust method

Method parameters for robust process were obtained from MODR of contour, and verified experimentally. The verified method was validated as per ICH Q2 (R2) guidelines for assay method. Method performance like assay, precision and robustness was considered as target response. The results are given in Table 7.

Table 7: Validation of the model by method verification.

S. NO	X			Y1		Y2	
	X1	X2	X3	Predicted	Experimental	Predicted	Experimental
1	30	5	0.8	4.6	4.8	2127	2245
2	35	6	0.8	5.5	5.7	2318	2345
3	45	5	0.8	9.03	10.3	1781	1888
4	25	6.5	1.2	5.7	5.8	3015	3009
5	25	5.5	1.2	4.1	4.3	2016	2001
6	20	4	1.0	3.2	3.4	2885	2996

Method validation

Method validation was performed following ICH Q2 guidelines specifications [18] for specificity, selectivity, linearity and range, accuracy, precisions, robustness, detection limit and quantitation limit.

System suitability parameters (SST)

Chromatographic conditions were tested for SST in two different laboratories. 50 µg/ml of pitavastatin was injected in replicates through manual rheodyne injector it can be detected at retention time 5.3 min with theoretical plates more than 8000 and tailing factor of 1.12. SST parameters are within the limit in both laboratories I and II,data shown in Table 8.

TABLE 8: System suitability parameters and validation results on method transfer.

Parameters	Laboratory I	Laboratory II
Chromatographic column	C18 (250 x 4.6 mm i.d, 5µm)	C18 (250 x 4.6 mm i.d, 5µm)
Mobile phase	85 % Methanol :15 % Water (0.2 % TEA) pH: 6.5	85 % Methanol :15 % Water (0.2 % TEA) pH: 6.5
Flow rate	1.1mL /min	1.1mL/ min
Detection wavelength	286 nm	286 nm
Retention time(t_R)	5.3 ± 0.1 min	5.2 ± 0.1 min
Tailing factor	1.12	1.14
Theoretical plates	> 8000	> 8000
Repeatability (% RSD)	0.45	0.52
Assay (%)	100.26 ± 0.89 (% RSD : 0.88)	99.89 ± 1.02 (% RSD : 1.02)
Robustness		
Flow rate (0.2 ml)	% RSD : 0.98	% RSD : 1.08
pH (± 0. 2 %)	% RSD : 1.08	% RSD : 1.26

Linearity

The linearity of peak area responses versus concentrations was studied from 2 to10 µg/ml for pitavastatin. A linear response was observed over the examined concentration range and the regression equation was Y=36344x+33134 (R^2=0.945) and it was good against the targeted value.

Repeatability

The system repeatability was calculated from five replicate injections of pitavastatin at the analytical concentration about 10 µg/ml and the %RSD was found to be 0.56.

Accuracy

Accuracy was studied using three different solutions, containing 90, 100 and 110 µg/ml of pitavastatin. Recovery data are reported in Table 8. The obtained values were within the range of 99.6 and 101.3%, mean %RSD was 0.19, satisfying the acceptance criteria for the study.

Precision

Both intraday and interday precisions were studied at different levels in linearity levels are reported in Table 10. Its %RSD was within the limit (below 2%). The precision was tested for the optimized method in two different laboratories, the %RSD was below 2%.

Robustness verification

Method critical parameters such as pH, wavelength and mobile phase are considered as robustness parameters and tested on as a part of validation in laboratory I and compared with the results obtained from laboratory II. The deliberate changes in variables (Xs) were made within MODR region in order to assess the robustness of the method in same and different laboratory. % Change of organic phase was tested up to 3%. The % RSD was below 1.75% for 3% change organic phase. The results for all variables are below 2% (RSD), indicated the robustness of the method. In the same way the method was robust for all test parameters. Results are shown in Table 8.

Limit of detection and limit of quantification

LOD and LOQ were determined based on signal to noise ratio. The S/N ratio of 3:1 was taken as LOD and S/N of 10:1 was taken as LOQ. LOD was found to be 1.9 µg/ml, while LOQ was 5.7 µg/ml.

DISCUSSION

There were few works reported on implementation of quality by design in analytical method development. But the sequence of implementation has to be considered as per FDA. Some papers have reported a method based on stability assay by considering resolution, as a method response to support specificity in robustness [39-40]. However method verification in design space, method performance has to be added. The knowledge based QTPP for the product of pitavastatin was constructed with the assessment of criticality for its critical attribute. Analytical target profile (ATP) was derived based on QTPP profile and then objective of this analytical QbD work was considered as assay component of QTPP of product specifications. To initiate the QbD work, the nature of chemical structure, PKa and solubility profile of pitavastatin were considered in the selection of input variables (X1, X2, and X3) for factorial design (2^3). Mid points were added to find the curvature effect. Once the curvature effect was significant, CCD was adopted to get response surface to optimize design. C18 column was chosen as stationary phase due to wide acceptability pharmaceuticals and high reproducibility. In order to achieve complete scientific understanding between method results (Y1; such as t_R and Y2; such as t_N) and input variables, a central composite design was designed and performed. The various variables and their levels were shown in Tables 1 and 2. The obtained experimental results was subjected various statistical parameters for better understanding and was found to be a nonlinear relationship between input variable and response. The statistical data and ANOVA analysis are shown in Table 3 and 4. The curvature effect was significant, so the Quadratic model (Eqn. 2) was obtained using of Sigma Tech software.

The above model was validated by coefficient of determination (R^2). The value was 1.00 indicated the process model is valid for predicting the behavior of the process and it was used for simulation of the process model and contours were obtained. The design space or MODR region for robustness was achieved from contours. These regions offer robust processes parameters and shown in Table 8. The obtained method conditions and chromatograms are shown in Fig. 1.

The AQbD approach on development of reversed phase high -performance liquid chromatographic method for pitavastatin in pharmaceutical dosage forms. The prediction form MODR has been verified by actual experimental results indicating its robustness. Thus the method developed based on AQbD is more precise, accurate, and robust during method transfer and also cost effective. This method satisfy the design space concept for analytical method (MODR) and suitable for regulatory submission under regulatory flexibility.

CONCLUSION

Chromatographic method was found to be more accurate, precise, robust, and more sensitive. Statistical analysis proves that the developed method can be used for routine analysis of pitavastatin in the pharmaceutical dosage form. Implementation of QbD approach resulted in more robust method which can produce consistent, reliable, and quality data throughout the process and also save time and money.

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CONFLICTS OF INTEREST

There are no conflicts of interest.

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