IJPSR (2018), Volume 9, Issue 11 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 13 March, 2018; received in revised form, 22 May, 2018; accepted, 21 October, 2018; published 01 November, 2018

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

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Keywords:

Analytical quality by design, Pitavastatin, analytical target profile, HPLC

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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.

INTRODUCTION: Pitavastatin is a competitive inhibitor of HMG CoA reductase and was developed for the treatment of hypercholestero-laemia [1-3]. It can reduce plasma level of LDL cholesterol by 40% in hypercholesterolaemic patients [4].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.9(11).4992-01

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.9(11).4992-01

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. 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**.

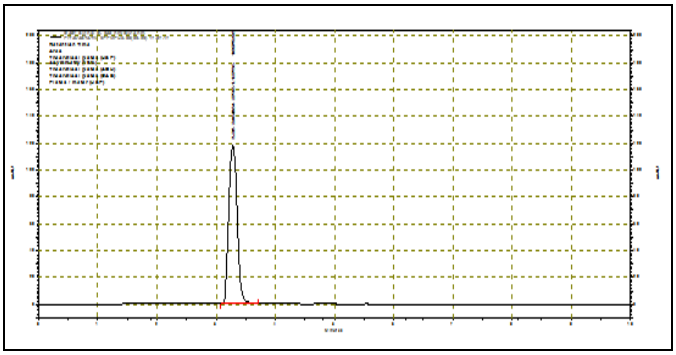

FIG. 1: OPTIMIZED RP-HPLC CHROMATOGRAM OF PITAVASTATIN. OPTIMIZED RP -HPLC CHROMATOGRAM OF PITAVASTATIN (T_R: 3.42 min) ON C_{18} COLUMN USING MODR CONCEPT (PITAVASTATIN ELUTED AT 3.42 min, AND THE METHOD WAS SELECTED FROM MODR, AND VERIFIED FOR ROBUSTNESS AND VALIDATED)

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 min 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 response.

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 theoretical 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_2x_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	pH	1.2395	80.358	529.7794	<0.1
B2	% aqueous phase	0.063	0.2076	1.3686	<0.01
B3	pH 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	pH 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	pH, % aqueous phase and flow rate	0.0237	0.0294	0.1937	<0.01

P value <0.01 indicates 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	pH	154.25	10.1452	12.827	<0.1
B2	% aqueous phase	-190.5	15.4739	19.5644	<0.01
B3	pH 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	pH 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	pH, % 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.

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

$Y1=b_0+b_1X_1+b_2X_2+b_3\ X_3+b_{12}X_1X_2+b_{23}X_2X_3+b_{13}X_1X_3+b_{123}X_1X_2X_3$….Eqn. 1.

$Y2=b_0+b_1X_1+b_2X_2+b_3\ X_3+b_{12}X_1X_2+b_{23}X_2X_3+b_{13}X_1X_3+b_{123}X_1X_2X_3$….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.

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.

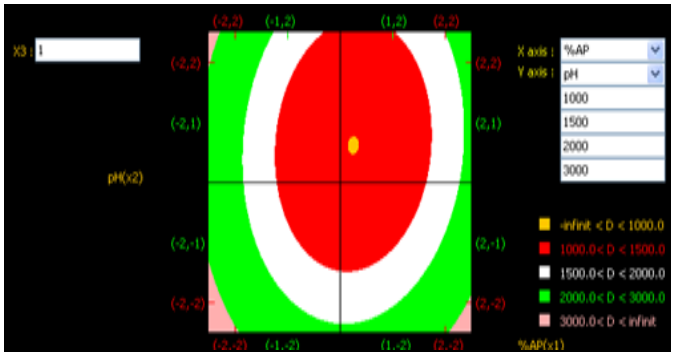

FIG. 2: pH OF AQUEOUS PHASE VERSUS % OF AQUEOUS PHASE CONTOUR AT 1 ml/min FLOW RATE OF MOBILE PHASE

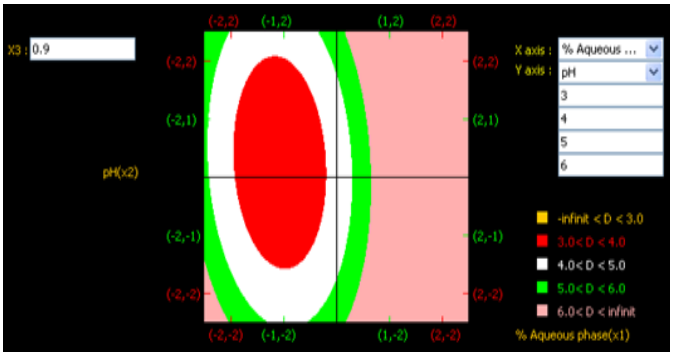

FIG. 4: pH OF AQUEOUS PHASE VERSUS % OF AQUEOUS PHASE CONTOUR AT 0.9 ml/min FLOW RATE OF MOBILE PHASE

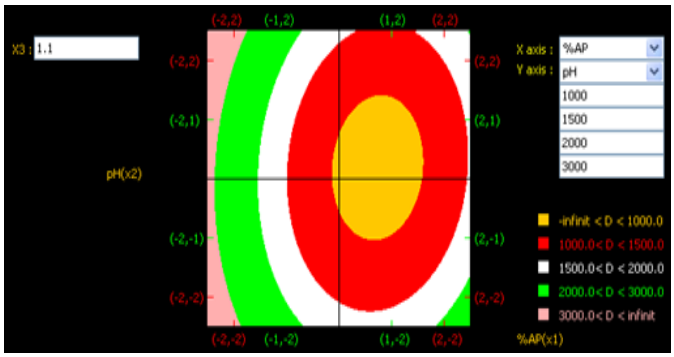

FIG. 3: CONTOUR OF METHOD OPTIMIZATION. pH OF AQUEOUS PHASE VERSUS % OF AQUEOUS PHASE CONTOUR AT 1.1 ml/min FLOW RATE OF MOBILE PHASE (ANALYTE SHOWS LARGE DESIGN SPACE PITAVASTATIN ONLY WHEN THEORETICAL PLATES IS MORE THAN 2000)

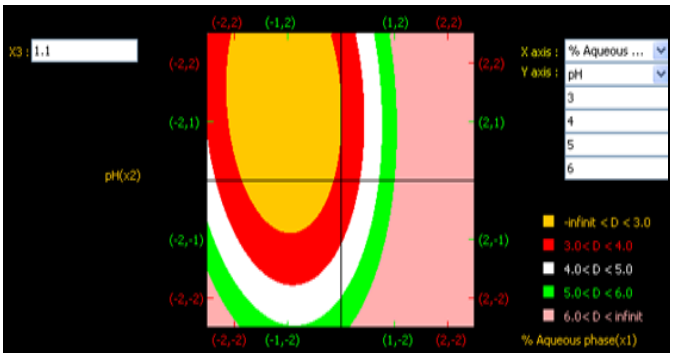

FIG. 5: CONTOUR OF METHOD OPTIMIZATION. pH OF AQUEOUS PHASE VERSUS % OF AQUEOUS PHASE CONTOUR AT 1.1 ml/min FLOW RATE OF MOBILE PHASE (ANALYTE SHOWS LARGE DESIGN SPACE PITAVASTATIN ONLY WHEN RETENTION TIME IS MORE THAN 3)

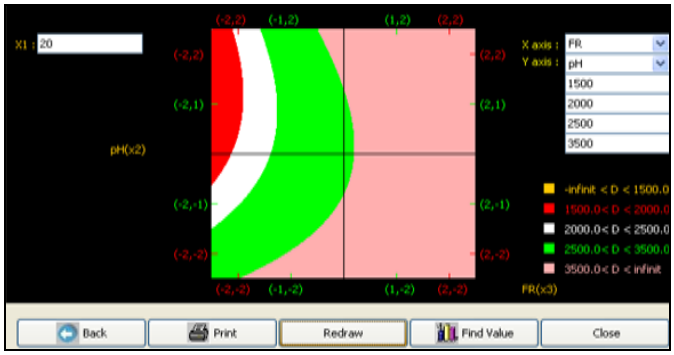

FIG. 6: CONTOUR OF METHOD OPTIMIZATION. pH OF AQUEOUS PHASE VERSUS FLOW RATE CONTOUR AT 20% OF AQUEOUS PHASE (ANALYTE SHOWS LARGE DESIGN SPACE PITAVASTATIN ONLY WHEN THEORETICAL PLATES IS MORE THAN 3000)

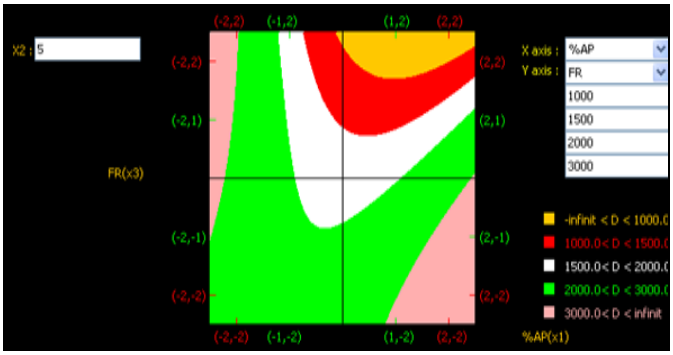

FIG. 7: CONTOUR OF METHOD OPTIMIZATION. % OF AQUEOUS PHASE VERSUS FLOW RATE CONTOUR AT PH 5 OF AQUEOUS PHASE (ANALYTE SHOWS LARGE DESIGN SPACE PITAVASTATIN ONLY WHEN THEORETICAL PLATES IS MORE THAN 2000)

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 $X_{2 =}$ -2 to 0.2	X1 = 11 to 16 X2 = 5 to 7	X3=1.1 (flow rate)	3-5	2000-3000
X1 = -2 to 0. 2 X3 = 0 to 2	X1 = 5 to 25 X3 = 1 to 1.2	X1=20% (aqueous)	3-4	2500-3500
X2 = -2 to 2 X3 = -1.2 to 2	X2 = 5 to 7 X3 = 0.88 to 1.2	X2=5 (pH)	5-6	2000-5000

*Constant absolute value are used as optimized method conditions

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 × 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		

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.

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**.

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.

TABLE 8: SYSTEM SUITABILITY PARAMETERS AND VALIDATION RESULTS ON METHOD TRANSFER

Parameters	Laboratory I	Laboratory II
Chromatographic column	C18 (250 × 4.6 mm i.d, 5µm)	C18 (250 × 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	1.1 mL /min	1.1 mL/ min
Detection wavelength	286 nm	286 nm
Retention time (*t*R)b	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

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 8**. 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 **Table 1** and **2**.

The obtained experimental results was subjected various statistical parameter 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.

ACKNOWLEDGEMENT: The authors wish to thank the Spain and Sigma Tech Services private limited, Hyderabad, India for supporting this work. This work was conducted at Research laboratory of Raghavendra Institute of Pharmaceutical Education and Research, Andhra Pradesh, India in collaboration with Sigma Tech. Consultancy, Hyderabad, India.

CONFLICT OF INTEREST: There are no conflicts of interest.

REFERENCES:

1. Aoki T, Nishimura H, Nakagawa S, Kojima J, Suzuki H, Tamaki T, Wada Y, Yokoo N, Sato F, Kimata H, Kitahara M, Toyoda K, Sakashita M and Saito Y: Pharmacological profile of a novel synthetic inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A reductase. Arzneimittel forschung 1997; 47(8): 904-909.
2. Wensel TM, Waldrop BA and Wensel B: Pitavastatin: a new HMG-CoA reductase inhibitor. Ann Pharmacother 2010; 44(3): 507-514.
3. Mukhtar RY, Reid J and Reckless JP: Pitavastatin. Int J Clin Pract 2005; 59(2): 239-252.
4. Saito Y, Yamada N, Teramoto T, Itakura H, Hata Y and Nakaya N: A randomized, double-blind trial comparing the efficacy and safety of pitavastatin versus pravastatin in patients with primary hypercholesterolaemia. Atherosclerosis 2002; 162(2): 373-379.
5. Ojha A, Guttikar S, Vayeda C and Padh H: Determination of pitavastatin from human plasma using high performance liquid chromatography with fluorescence detection. Se Pu 2007; 25(5): 715-728.
6. Kojima J, Fujino H, Yosimura M, Morikawa H and Kimata H: Simultaneous determination of NK–104 and its lactone in biological samples by column-switching high–performance liquid chromatography with ultraviolet detection. J Chromatogr B Biomed Sci Appl 1999; 724(1): 173-180.
7. Nanjappan SK, Narayanan N, Jayabalan N, Narayanan S and Bagyalakshmi J: HPLC determination of Pitavastatin calcium in pharmaceutical dosage form. Pharma Ana Acta 2011: 2(2): 1-4.
8. Di B, Su MX, Yu F, Qu LJ, Zhao LP, Cheng MC and He LP: Solid–phase extraction and liquid chromatography / tandem mass spectrometry assay for the determination of pitavastatin in human plasma and urine for application to phase I clinical pharmacokinetic studies. J Chromatogr B Analyt Technol Biomed Life Sci 2008; 868(1-2): 95-101.
9. Tian L, Huang Y, Jia Y, Hua L and Li Y: Development and validation of a liquid chromatography-tandem mass spectrometric assay for pitavastatin and its lactone in human plasma and urine. J Chromatogr B Analyt Technol Biomed Life Sci 2008; 865(1-2): 127-132.
10. Lv H, Sun JG, Wang GJ, Zhu XY, Zhang Y, Gu SH, Liang Y and Sun J: Determination of pitavastatin in human plasma via HPLC-ESI-MS/MS and subsequent application to a clinical study in healthy Chinese volunteers. Clin Chim Act 2007; 386(1-2): 25-30.
11. Deng JW, Kim KB, Song IS, Shon JH, Zhou HH, Liu KH and Shin JG: Determination of two HMG-CoA reductase inhibitors, pravastatin and pitavastatin, in plasma samples using liquid chromatography-tandem mass spectrometry for pharmaceutical study. Biomed Chromatogr 2008; 22(2): 131-135.
12. Shen-Tu J, Xu X, Liu J, Hu X, Chen J, Wu L, Huang M and Zhou H: Determination of Pitavastatin in human plasma by LC-MS-MS. Chromatogr 2009; 69(10): 1041-1047.
13. Kumar SN and Baghyalakshmi J: Determination and quantification of pitavastatin calcium in tablet dosage formulation by HPTLC method. Analytical letters 2007; 40(14): 2625-2632.
14. Hiral JP, Bhanubhai NS, Natvarlal JP and Bhavesh HP: A simple and sensitive HPTLC method for estimation of pitavastatin calcium in tablet dosage form. J. Planar Chrom 2008; 21: 267-270.
15. Krishna MV and Sankar DG: Adaptation of color reactions for spectrophotometric determination of pitavastatin calcium in bulk drugs and in pharmaceutical formulations. E-J.Chem 2007; 4(2): 272-278.
16. Vadia NH, Vandana P and Bhalara HN: Spectrophotometric determination of cefetamet pivoxil

hydrochloride and pitavastatin calcium in tablet dosage form. Indian J Pharm Sci 2008; 70(5): 649-651.
17. Antony RG. Pannala RR. Nimmakayala S and Jadi S: Degradation pathway for pitavastatin calcium by validated stability indicating UPLC method. American J. Ana. Chem 2010; 1(2): 83-90.
18. ICH Topic Q8 (R2), "ICH harmonised tripartite guideline," in Proceedings of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH '09), Pharmaceutical Development 2009.
19. Godfrey AB, Kenett RS and Joseph MJ: A perspective on past contributions and future impact. Quality and Reliability Engineering International 2007; 23(6): 653-663.
20. Nethercote P, Borman P and Bennett T: QbD for Better Method Validation & Transfer, Pharmaceutical Manufacturing 2010.
21. ICH Topic Q2 (R1), "ICH harmonised tripartite guideline," in Proceedings of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH '94), Validation of Analytical Procedures 1994.
22. ICH Topic Q9, "ICH harmonised tripartite guideline," in Proceedings of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH '94), Quality Risk Management, 2005.
23. Borman P, Nethercote P and Chatfield M: The Application of Quality by Design to Analytical Methods. PharmTech 2007; 31(10).
24. Yu LX: Pharmaceutical quality by design: product and process development, understanding, and control. Pharm Res 2008; 25(4): 781-791.
25. Pohl M, Schweitzer M and Hansen G: Implications and opportunities of applying the principles of QbD to analytical measurements, Pharmaceutical Technology Europe 2010; 22(2): 29-36.
26. Schweitzer M, Pohl M, and Hanna BM: Implications and opportunities of applying QbD principles to analytical measurements. Pharmaceutical Technology 2010; 34(2): 52-59.
27. Bianchini RM, Castellano PM and Kaufman TS: Development and validation of an HPLC method for the determination of process-related impurities in pridinol mesylate, employing experimental designs. Analytica Chimica Acta 2009; 654(2): 141-147.
28. Monks K, Molnar I, Rieger HJ, Bogati B and Szabo E: Quality by design: multidimensional exploration of the design space in high performance liquid chromatography method development for better robustness before validation. J. Chromatogr. A 2012; 1232: 218-230.
29. Srinubabu G, Raju CAI, Sarath N, Kumar PK and Rao JS: Development and validation of a HPLC method for the determination of voriconazole in pharmaceutical formulation using an experimental design. Talanta 2007; 71(3): 1424-1429.
30. Khamanga SM and Walker RB: The use of experimental design in the development of an HPLC-ECD method for the analysis of captopril. Talanta 2011; 83(3): 1037-1049.
31. Sheldon EM and Downar JB: Development and validation of a single robust HPLC method for the characterization of a pharmaceutical starting material and impurities from three suppliers using three separate synthetic routes. J Pharm Biomed Anal 2000; 23(2-3): 561-572.
32. Gavin PF and Olsen BA: A quality by design approach to impurity method development for atomoxetine hydrochloride (LY139603). J Pharm Biomed Anal 2008; 46(3): 431-441.
33. Dejaegher B and Vander HY: Experimental designs and their recent advances in set-up, data interpretation, and analytical applications. J Pharm Biomed Anal 2011; 56(2): 141-158.
34. Awotwe OD, Agarabia C and Faustinoa PJ: Application of quality by design elements for the development and optimization of an analytical method for protamine sulfate. J Pharm Biomed Anal 2012; 62: 61-67.
35. Vasselle B, Gousset G and Bounine JP: Development and validation of a high performance liquid chromatographic stability-indicating method for the analysis of Synercid in quality control, stability and compatibility studies. J Pharm Biomed Anal 1999; 19(5): 641-657.
36. Lewis GA, Mathieu D and Phan Tan L: Pharmaceutical Experimental Design. New York: Marcel Dekker 1999.
37. Wen YH, Pei CL, Ling KH, Li PL and Wayne CL: Stability studies of ascorbic acid 2-glucoside in cosmetic lotion using surface response methodology. Bioorg Med Chem Lett 2013; 23: 1583-1587.
38. Srinubabu G, Raju CH, Sarath N, Kiran Kumar P and Rao JVS: Development and validation of a HPLC method for the determination of voriconazole in pharmaceutical formulation using an experimental design. Talanta 2007; 71(3): 1424-1429.
39. Beg S, Sharma G, Katare OP, Lohan S and Singh B: Development and validation of stability-indicating liquid chromatographic method for estimating olmesartan medoxomil using quality by design. J Chromatogr Sci 2015: 53(7): 1048-1059.
40. Kurmi M, Kumar S, Singh B and Singh S: Implementation of design of experiments for optimization of forced degradation conditions and development of a stability-indicating method for furosemide. J Pharm Biomed Anal 2014; 96(5): 135-143.

How to cite this article:
Gundala A, Bharathi K and Prasad KVSRG: Analytical quality by design approach in RP-HPLC method development for the assay of pitavastatin in tablet dosage form. Int J Pharm Sci & Res 2018; 9(11): 4992-01. doi: 10.13040/IJPSR.0975-8232.9(11).4992-01.