APPLICATION OF ICH Q14 CONCEPT FOR CHEMOMETRICS-ASSISTED DEVELOPMENT OF SPECTROPHOTOMETRIC METHODS FOR ESTIMATING PIMAVANSERIN IN TABLETS

Sagar S. Panda[a]*, Ravi Kumar V. V. Bera[a] and Biswajit Sahu[a]

ABSTRACT

In the present study, a novel analytical procedure development concept is utilized along with a chemometrics approach. Two sensitive UV spectrophotometric methods were developed for quantifying a new antipsychotic agent, pimavanserin tartarate, in tablets. The scanning speed and sampling interval of the UV spectrophotometric methods were the critical method variables investigated and optimized by a face-centred cubic design during the development phase. A zero-order (222 nm) and a first derivative (maxima: 256 nm and minima: 283 nm) method displayed linear response over 0.5-100 µg mL^{-1}, with impressive validation results. Further, the procedure robustness capability index was measured by using Monte Carlo simulation. In a nutshell, the present spectrophotometry based studies reveal aptness of the novel concept for developing robust and rapid analytical methods availing regulatory flexibilities for estimating pimavanserin tartarate in tablets.

INTRODUCTION

Analytical method development has always been a critical aspect of controlling the quality of drugs and drug products[1]. When combined with a scientifically sound and systematic workflow that relies on powerful tools such as risk-based approach, design of experiments (DoE) and Monte-Carlo simulation (MCS), the result is the development of a robust and reliable analytical method that has superior method performance as well as avails regulatory flexibilities for post-approval change control purpose[2-4]. ICH has taken an initiative in this respect and instigated the recent Q14 concept on Analytical Procedure Development (APD) for promoting case studies in this field so that in the near future a sound guideline can be established[5]. The novel APD approach collectively consists of risk identification using cause-effect diagram followed by risk score estimation by Control-Noise-Experimental (C-N-X) approach, risk minimization by robustness-cum-optimization studies using DoE approach, evaluation of procedure performance capability index (C_{pk}) by using MCS study, demarcation of flexible design space (DS) and establishment of control strategy for possible post-approval change control situation.

Selection of a suitable analytical technique is a critical aspect of developing an effective and quality analytical method. Being a widely used, versatile and affordable analytical alternate, UV spectrophotometry was explored by the authors using the current novel approach. This not only produced two scientifically sound analytical methods availing regulatory flexibilities for PVN but also helped to demonstrate its aptness in analytical method development. Along with zero-order (D^0) studies, the authors attempted to utilize derivative spectroscopy (D^1), which efficiently eliminates possible variation in λ_{max} values and less deviation in absorbance measurement. The transformed D^0 spectra are generated considering a change in absorbance values with wavelengths vs. wavelength ($dA/d\lambda$)[6]. Moreover, derivatization of D^0 spectra lead to correction of a systematic error by suppression of background matrix interference and it also improves finer spectral features[7].

In the current study, a novel antipsychotic agent pimavanserin tartarate(PVN), chemically known as (2R, 3R)-2,2-dihydroxybutanedioic acid;1-[(4-fluorophenyl)methyl]-1-(1-methylpiperidin-4-yl)-3-[[4-

[a] Department of Pharmaceutical Analysis & Quality Assurance, Roland Institute of Pharmaceutical Sciences (Affiliated to Biju Patnaik University of Technology, Odisha), Khodasingi, Brahmapur-760 010, Odisha, India
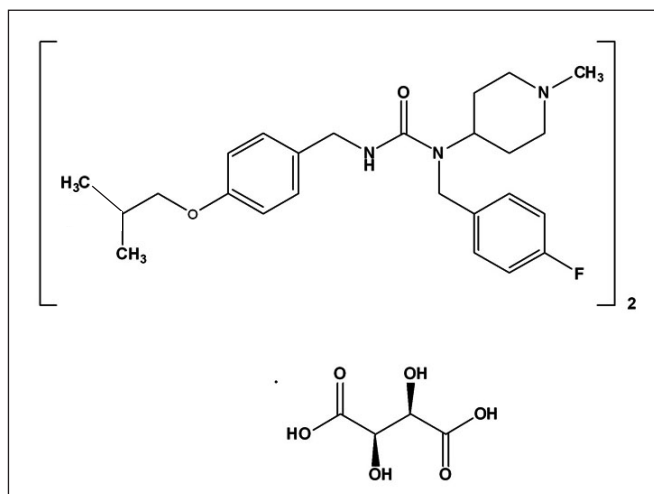*For Correspondence: E-mail: sagarguddu2002@gmail.com

Fig. 1: Chemical structure of PVN

(2-methylpropoxy) phenyl]methyl]urea (1:2) (Fig. 1), was investigated using UV spectrophotometry[8]. The target analyte has only one HPLC method reported so far and has no UV spectrophotometric methods for its quantification from dosage forms. Hence the authors took on the opportunity to develop and optimize two rapid and scientifically robust UV spectrophotometric methods for reliable quantification of PVN in dosage forms. Analytical method validation was performed within ICH guidance to ascertain method suitability for the purpose[9].

MATERIALS AND METHODS

Chemicals

Standard PVN (purity > 98%) was kindly provided by Roland Institute of Pharmaceutical Sciences, Brahmapur. Spectroscopy grade (Merck, Mumbai) methanol was used as a solvent during the spectrophotometric studies. In-house tablets containing 20 mg of PVN were prepared using excipients such as starch, magnesium stearate, microcrystalline cellulose, hypromellose, talc, polyethylene glycol and saccharin and were analyzed spectrophotometrically.

Instrumentation

A Shimadzu UV-1800 double beam spectrophotometer (Shimadzu, Kyoto, Japan) with matched 10 mm quartz cuvettes was used for spectrophotometric measurements. The instrument was controlled by UV probe 2.21 software.

Table I: Analytical target profile for estimation of PVN

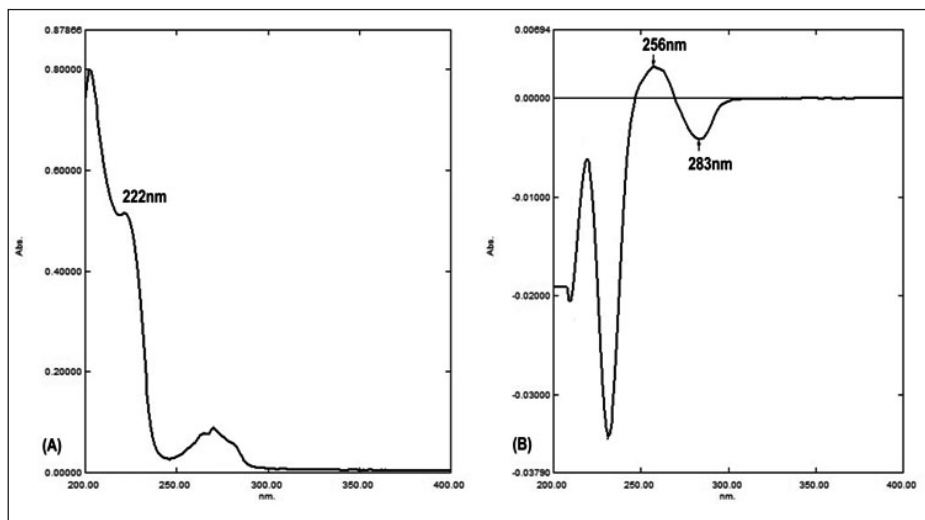| ATP	Target	Rationale
Analyte	Pimavanserin tartarate(PVN)	Lack of simple and rapid analytical methods.
Sample	API/Tablets	As per the need of the patient community.
Methodology	UV spectrophotometry (D^0 and D^1)	Suitable for analyte like PVN for routine use.
Instrumentation	Double beam UV Spectrophotometer using 10 mm matched quartz cuvettes.	Maximal detection sensitivity than compared to single beam instruments.
Method Intent	Assay with acceptable accuracy and precision	Simple and reliable quantification of PVN within product life-cycle.
Standard Preparation	Weighing, dilution of standards	An accurate calibration curve can be established by using accurately prepared standard dilutions.
Sample Preparation	Preliminary processing, weighing, ultrasonication, volumetric dilution, membrane filtration	Ensures suitable dilution of sample solution as well as the absence of extraneous matter.
CAAs	Target	Rationale
Absorbance	Maximum	Maximum absorbance values indicate that highest levels of light energy are absorbed by an analyte molecule. Hence, more accurate quantification in sample solutions.

Fig. 2: The UV absorption spectrum of PVN by D^0 method (A) and D^1 method (B)

Fig. 3: Three dimensional (A) and two dimensional (B) plots obtained for the DoE based studies

An ultrasonicator (Professional Ultrasonicator, GT Sonic) was employed for extraction of PVN from tablet samples. Design-Expert software (Stat-Ease, Inc., Minneapolis, MN, USA) and Companion by Minitab were used for performing DoE and Monte Carlo simulations (Minitab Inc, Pennsylvania, USA), respectively.

The analytical procedure development (APD) workflow

Setting up an analytical target profile (ATP) and critical analytical attribute (CAA)

The ATP for the present study was to develop and validate rapid, robust and reliable spectrophotometric methods for estimation (assay between 95 - 105 %, accuracy > 99 % and precision < 2 %) of PVN in tablets. Based on the ATP, a UV spectrophotometer was selected for the purpose (Table I). Absorbance was selected as the CAA.
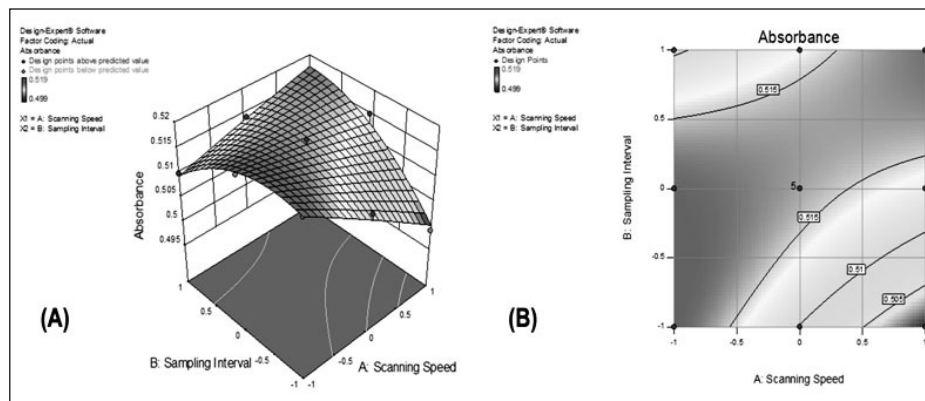
Risk-based studies

Risk assessment tools such as fish-bone diagrams were employed

Table II: Typical C-N-X based risk estimation matrix for various spectrophotometric method variables

Method variable	Risk Level on CAA	Score	C-N-X	Action taken
Scanning speed	10	100	X	DoE
Sampling interval	10	100	X	DoE
Solvent grade	3	30	N	Quality assured
Sample purity	3	30	N	Quality assessed
UV spectrophotometer	3	30	C	Intact
Calibration error	2	20	C	Calibrated
Calculation error	2	20	N	Careful measurements
Detector equilibration	2	20	C	Equilibrated

Risk Level: 1-Negligible risk, 5-Low risk, 10-High risk; Score=Risk Level of CAA×10
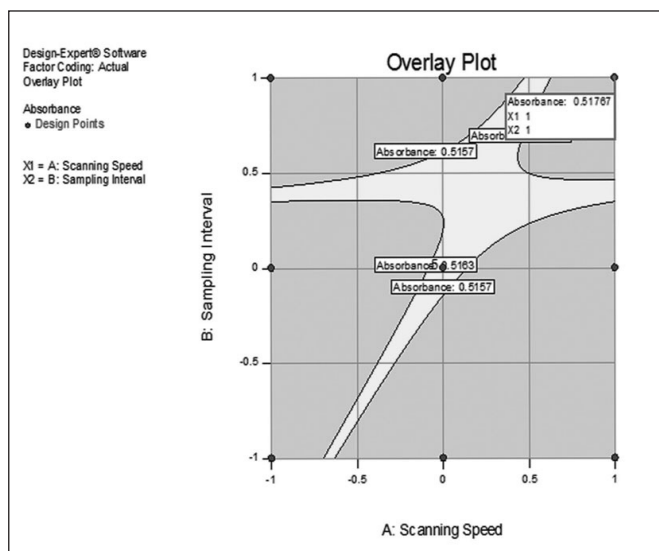
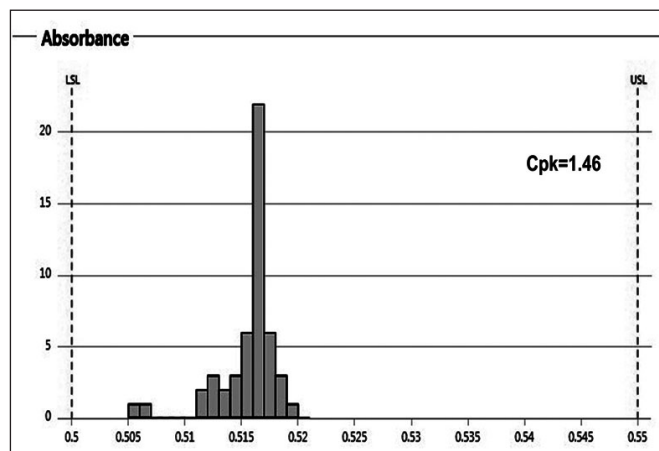**Fig. 4: Analytical DS obtained for the present method using APD approach**

Fig. 5: Typical MCS based histogram depicting method robust performance capability

Table III: Experimental design matrix and obtained observations

Run	Scanning speed	Sampling interval	Absorbance
1	-1	0	0.518
2	+1	+1	0.517
3	-1	-1	0.519
4	0	-1	0.511
5	-1	+1	0.51
6	+1	-1	0.499
7	0	0	0.516
8	+1	0	0.515
9	0	0	0.516
10	0	0	0.516
11	0	0	0.517
12	0	0	0.514
13	0	+1	0.514
Coded Levels	**Actual Level**		
Low (-1)	Slow	0.2	
Mid (0)	Medium	0.5	
High (+1)	Fast	1.0	

to sort the CMVs. Using C-N-X analysis method, variables with scores higher than 100 were selected as the critical few and were further subjected to DoE investigation. The identified variables as CMVs for DoE based investigation were scanning speed and sampling interval.

Design of experiments (DoE) avenues for method optimization

Based on the number of variables to be studied, a face-centred cubic design (FCCD) was considered as a befitting for evaluation of robustness as well as spectrophotometric method optimization. Unbiased experiments were performed with 13 runs. The basic FCCD experiments provide an enhanced understanding of the method with minimal experimentation compared

to factorial designs (FD) when there are only two CMVs. Mathematical analysis of unbiased experimental data served the purpose of identifying interaction among CMVs. In conjunction with mathematical analysis, the statistical analysis further evaluated the investigation model suitability. Model suitability assessment was established through a detailed crosschecking of polynomial equations, 2-dimensional and 3-dimensional plots. Based on the results of the above assessment, analytical DS was demarcated for further investigations. The overall DoE approach was run on the Design-Expert software.

Monte-Carlo simulation (MCS)

MCS was applied to establish the analytical process performance for the studied CMVs using Companion

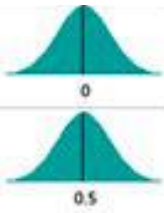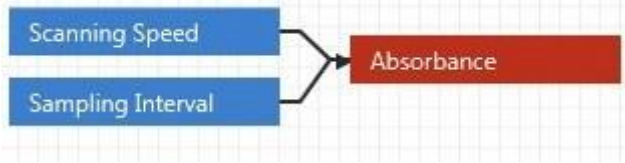**Table IV: CMVs investigated using Monte-Carlo simulation and model summary**

Sl. No.	Input CMV (X)	Distribution	CMV Range		Output CAA (Y)	Specification Limits	
		Normal	Low	High		Low	High
1	Scanning Speed		Slow	Fast	Absorbance	0.5	0.55
2	Sampling Interval		0.2	1.0			
Spectrophotometric Simulation Model Summary:							

Table V: Summary of analytical validation data obtained by UV spectrophotometric methods

Parameter	Method	
	D^0	D^1
Beer's range ($\mu g\ mL^{-1}$)	0.5 - 100	0.5 - 100
Regression equation	Y=0.023x + 0.004	Y=0.00038 + 0.00005
Correlation coefficient (R^2)	0.999	0.999
Accuracy (% recovery, % RSD)		
80 %	99.61, 0.39	99.59, 0.22
100 % (10 $\mu g\ mL^{-1}$)	99.55,0.45	99.4, 0.26
120 %	100.1, 0.30	100.93, 0.11
Precision (% RSD)		
Repeatability	0.18	0.11
Intermediate	0.24	0.12
Instrument	0.18	0.08
LOD ($\mu g\ mL^{-1}$)	0.1	0.1
LOQ ($\mu g\ mL^{-1}$)	0.5	0.5
Sandell's sensitivity ($\mu g\ cm^{-2}\ 0.001AU^{-1}$)	0.045	---
Molar extinction co-efficient(ε)(L.mol.cm^{-1})	2.23×10^5	---
Analysis of in-house tablets		
(Mean ± S.D.)	100.31 ± 0.5795	100.45 ± 0.2003

% recovery= Average of three determinations, Mean= Average of three determinations

by Minitab. Companion by Minitab is a user-friendly platform whereby the analyst can determine the process performance index (C_{pk}) using CMVs with their experimental study levels. In this study, the analytical procedure performance in terms of C_{pk} value was set as indicative of method robustness considering the variability of CMVs as input. C_{pk} values of 1.33 or higher were considered as vital for assuring method robustness performance using the upper and lower specification limits of CMVs.

Control strategy

Control strategies for the current methods were established considering DS obtained from DoE studies and MCS based investigation to obtain robust method performance throughout the product life-cycle.

Method validation

Specificity

Non-interference of placebo at measurements of analytes λ_{max} is an indication for method specificity. The placebo solution contained excipients used for preparing the in-house tablets of PVN.

Preparation of calibration curves

Exactly 25 mg accurately weighed amount of PVN was dissolved in methanol placed in the 25 mL volumetric flasks. Further, this solution was used to prepare working standard solutions of PVN within 0.5 - 100 µg mL^{-1}. The average responses (n = 3) for each concentration were used to prepare the calibration curves.

Accuracy and precision

A 10 µg mL^{-1} concentration solution of PVN present in tablets was chosen and was spiked with 80 %, 100 % and, 120 % of standard PVN. Method trueness was evaluated by the % recovery of standard PVN.

Method precision was assessed in terms of repeatability and intermediate precision at 20 µg mL^{-1} of PVN. A value below 2.0 for % relative standard deviation (% RSD) was considered an acceptable limit.

Sensitivity

Limit of detection (signal-to-noise ratio = 3) and quantitation (signal-to-noise ratio = 10) values were based on the comparison of signal to noise ratio of lowest concentrations of drug to that of blank samples. Further, Sandell's sensitivity was calculated for establishing the sensitivity of UV spectrophotometric methods.

Assay of the in-house tablets

Tablets of PVN with 20 mg of analyte were triturated to a finer state and 25 mg of it was subjected to dissolution using methanol kept in a 25 mL volumetric flask. This mixture was ultrasonicated for 30 min. Post volume make up (final concentration approx. 1000 µg mL^{-1}) was vortexed for 2 min for enhancing the homogeneity of the solution. Membrane filtration (0.45 µm) removed excipient particulates present in the solution. Thereafter, it was utilized to prepare sample solutions for spectrophotometric analysis.

RESULTS AND DISCUSSION

Spectrophotometric method development

PVN in spectroscopy grade methanol portrayed an absorption maximum of 222 nm and another secondary peak was also obtained with lesser absorbance at 270 nm (Fig. 2(A)). Further, the obtained spectra were derivatized to D^1. PVN shows maxima at 256 nm and minima at 283 nm. However, to obtain the best signal-to-noise response as well as reproducible results, the total amplitudes at 256 nm and 283 nm (Fig. 2(B)) were used. The measurements for D^1 spectra were carried out considering total amplitudes at both the wavelengths. Afterwards, the APD workflow was employed for establishing a rapid and robust analytical method for PVN availing regulatory flexibility.

With the intent of accomplishing ATP, preliminary risk assessment and its minimization carried out. The typical fish-bone based causal-effect relationship diagrams (not shown here) depicted numerous experimental variables with a possible threat to method performance quality. Certain method variables were found to possess a possible risk to the method performance i.e. CAA. For a detailed evaluation of the critical to quality nature of these variables, a C-N-X approach was initiated and the results are displayed in Table II. This approach concluded that the method variables such as scanning speed and sampling interval have the highest risk potential based on their C-N-X score towards the CAA. Further, these variables were termed as CMVs and subjected to DoE study.

Optimization studies

The FCCD was quite advantageous for the purpose and revealed maximum information on the CMVs. Experimental runs were performed and responses obtained are as shown in Table III. A second-order quadratic model investigated the interaction among the two CMVs and presented a polynomial equation in the form of equation 1:

$$Y = +0.51607 - 0.00266667A + 0.002B + 0.00675AB - 0.000241379A^2 - 0.00424138B^2 \dots (Eqn. 1)$$

where Y=Absorbance, A= Scanning speed, B=Sampling interval (nm)

Assessment of ANOVA (P < 0.0001) along with values of adequate precision (20.951; signal-to-noise ratio > 4.0 is optimum) and model F-value (35.38) favoured the aptness of the mathematical model used for response surface optimization studies.

Mapping of 3-dimensional response surface along with the 2-dimensional top contour plot revealed a complex interaction among scanning speed and sampling interval with contour lines approaching each other. A "static maximum" was obtained at around low-to-mid levels of both the CMVs (Fig. 3(A,B)). However, decreasing values were obtained over all the other levels.

The desirability, as well as overlay plot (Fig. 4), represented spectrophotometric conditions for obtaining optimum values of absorbance. At higher values of the CMVs i.e. fast scanning speed and a sampling interval of 1 nm, the method was optimized and found to be robust to changes. Experimental runs were performed for model verification within the DS region which produced results corroborating to ATP. Further, the D^0 spectra were derivatized to its D^1 form for finer spectral measurement purposes. The method validation studies were conducted according to the above-optimized conditions for D^0 and D^1 methods.

Simulation-based robustness evaluation

Method robustness performance was evaluated by utilizing a time-efficient and cost-effective simulation approach known as Monte-Carlo simulation. The Monte-Carlo simulation using Companion by Minitab software was performed using the low and high levels of the two CMVs along with lower and higher specification limits of absorbance for obtaining the optimum C_{pk} values (Table IV). Fig. 5 depicts the normally distributed simulation (n = 50,000) results obtained for absorbance. The simulation was found worthy as it produced C_{pk} values (C_{pk} for absorbance = 1.46) much greater than the ideal value of 1.33. This revealed optimum method performance and robustness for the purpose.

Control strategy

In view of the DS generated with desirability 1 and conclusions drawn from MCS study, the method was optimized at faster scanning speed and higher sampling interval. Considering the above-experimental conditions,

the method validation was performed. Further, the method was cautioned to be operated within the flexible operating range i.e. mid-to-high levels of both the CMVs.

Method validation studies

The method was found linear over the concentration range of 0.5 - 100 µg mL^{-1} (R^2=0.999). Satisfactory recoveries of PVN above 99 % advocated for optimum method accuracy and reliability. The values of % RSD were acceptable and were < 0.5 %, suggesting method preciseness. The LOD and LOQ values were quite sensitive for spectrophotometric measurements. Sandell's sensitivity along with Molar Extinction coefficients was also calculated to establish the optical aptness of the newly developed spectrophotometric methods (Table V). The results obtained for analytical validation studies are depicted in Table V.

Assay of dosage form

The visual evaluation of UV absorption spectra obtained for tablets established the capability of the present method to remain selective in presence of formulation components. The mean (n = 3) content of PVN was found to satisfactory (Table V) as per ATP, indicating method selectivity.

CONCLUSION

Two rapid and reliable spectrophotometric methods were developed and validated as per ICH guidance for PVN. The use of the novel approach of APD ensured the development of robust and reliable spectrophotometric methods for estimating PVN in bulk and tablets. Employing APD approach using chemometric tools such as DoE and MCS was found worthy as they disclosed that scanning speed and sampling interval are the two CMVs that can adversely affect the absorbance of a spectrophotometric method. Systematized risk investigation and its management provided the much-desired understanding of the CMVs and helped to optimize them for robust performance. Further, the results of validation studies revealed optimum method performance with values within federally regulated limits. Overall, this analytical method is suitable, reliable and avails regulatory flexibility for routine estimation of PVN in pharmaceuticals.

REFERENCES

1. Siddiqui M. R., Alothman Z. A. and Rahman N.: Analytical techniques in pharmaceutical analysis: a review, **Arab. J. Chem.**, 2017, 10 (S1), S1409-1421.
2. Deidda R., Orlandini S., Hubert P. and Hubert C.: Risk-based approach for method development in pharmaceutical

quality control context: a critical review, **J. Pharm. Biomed. Anal.**, 2018, 161, 110-121.

3. Manraranjan V. C., Yadav D. S., Jogia H. A. and Chauhan P. L.: Design of experiment (DoE) utilization to develop a simple and robust reversed-phase HPLC technique for related substances estimation of omeprazole formulations, **Sci. Pharm.**, 2013, 81(4), 1043-1056.

4. Beg S., Panda S. S., Katare O. P. and Singh B.: Applications of monte-carlo simulation and chemometric techniques for development of bioanalytical liquid chromatography method for estimation of rosuvastatin calcium, **J. Liq. Chromatogr. Relat. Technol.**, 2017, 40(18), 907-920.

5. Analytical Procedure Development and Revision of Q2 (R1) Analytical Validation: Final Concept paper Q14 International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use., 2018, 1-3.

6. Beckett A. H. and Stenlake J. B.: Ultraviolet-visible absorption spectrophotometry, in: Practical Pharmaceutical Chemistry: Part II, 4th (Ed.), C.B.S. Publications, New Delhi 2007, pp. 275-337.

7. Bridge T. P., Fell A. F. and Wardman R. H.: Perspectives in derivative spectroscopy Part 1-Theoretical principles, **Color Technol.**, 1987, 103, 17-27.

8. Pimavanserin tartarate: Available at https://pubchem.ncbi.nlm.nih.gov/compound/Pimavanserin-tartrate.(Accessed on June 20 2019)

9. Validation of Analytical Procedures Text and Methodology Q2 (R1) International Conference for Harmonization of Technical Requirements for Pharmaceuticals for Human Use., 2005, 1-17.