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Application of Quality by Design Approach for Development and Validation of Analytical RP-HPLC Method for Prasugrel HCL in Bulk and Tablet Dosage Form

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Abstract: ***QbD is a systematic risk based, proactive approach to pharmaceutical development that begins with predefined objectives and emphasizes product and process understanding. The present study describes Application of Quality by Design approach to the development and validation of analytical RP-HPLC method for Prasugrel HCl. Optimization was done by response surface methodology, applying a three level Box-Behnken design. Three factors selected were flow rate, pH, and concentration of methanol and water in mobile phase. The optimized chromatographic method was validated according to the International Conference on Harmonization (ICH) Q2 (R1) guidelines for linearity, precision, range, accuracy and robustness. Detection was done using UV detector at 254 nm. The developed method employed mobile phase methanol: water (pH 3.5) (94.7: 5.3), and flow rate 1.1 ml/min, which was optimized with the help of design expert software. High linearity of the developed method was confirmed over concentration range of 100-400 µg/ml for Prasugrel HCl with correlation coefficient of 0.999. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The proposed method can be successfully used to determine the drug contents of marketed formulation.***

Keywords: Quality by Design, Prasugrel HCL, Box-Benhken Design, Process Analytical Technology

1. Introduction

The pharmaceutical industry has used the modern concept of Quality by Design (QbD) to apply science based manufacturing principles for new and existing products to assure quality of the formulation and to increase efficiencies, provide regulatory relief and flexibility, and offer important business benefits throughout the product life cycle. It gives direction to move towards a more scientific, risk based, holistic and proactive approach to pharmaceutical development. It is also a major challenge to the Pharmaceutical industry whose processes are fixed in time, despite inherent process and material variability, In case of designing and development of a product, it is essential to define desire product performance profile Target product Profile (TPP), Target Product Quality Profile (TPQP) and identify critical quality attributed (CQA). On that basis we used to design the product formulation and process to meet the requirements for product attributes which pretend to ascertain and recognize the impact of raw materials such as critical material attributes (CMA), critical process parameters (CPP) on the CQAs and identification and control sources of variability.

What is Quality by Design:

Quality by Design (QbD) is a modern, scientific approach that formalizes product design, automates manual testing, and streamlines troubleshooting, It uses a systematic approach to ensure quality by developing a thorough understanding of the compatibility of a finished product to all of the components and processes involved in manufacturing that product. Instead of relying on finished product testing alone, QbD provides insights upstream throughout the development process. As a result, a quality issue can be efficiently analyzed and its root cause quickly identified. The design space is defined as a manufacturing area of the product including Equipment, Material, and Operators and Manufacturing conditions.

The method of optimization was earlier based on One Factor at a Time (OFAT) approach where a single component was varied with time and its effect studied. This approach was not much helpful and neglected the effect caused due to interaction of more than one factor. The approach followed is Quality by Design (QbD) which employs Design of Experiments (DoE) as important concept. DoE approach is a systematic, scientifically analyzed better understandable approach.

Regulatory Aspects of QbD:

The QbD approach which is based on scientific and methodical product development was include in the quality guidelines of International Conference on Harmonization (ICH) from 2005 onwards. This approach includes, ICH Q8 (Pharmaceutical Development), Q9 (Quality Risk Management), and Q10 (Pharmaceutical Quality System) guidelines. The Pharmaceutical products quality was also emphasized in Process Analytical Technology (PAT)

guidelines for new pharmaceutical product development and quality. In 2004, USFDA agreed to include QbD in "Pharmaceutical cGMP 21st century- A risk based approach".

Elements of QbD:

1. Define the Quality Target Product Profile
2. Identify the Quality Attributes
3. Perform a Risk (Assessment) Analysis. i.e. Determine the Critical Quality Attributes and Critical Process Parameters.
4. Determine the Design Space
5. Identify a Control Strategy
6. Lifecycle management and continual improvement

1) Analytical Target Profile (ATP):

The Analytical Target Profile (ATP) is a set of criteria what will be measured and the performance criteria to be achieved by the measurements (e.g. accuracy, precision and range). Investigation of HPLC methods can involve selection of basic conditions, For example type of column (size and composition), PH, and organic modifier.

QbD flow can start after these preliminary experiments, the QbD work flow can start, first by defining the quality target product profile (QTPP) and the critical quality attributes (CQAs). QTPP is defined as a prospective summary of the quality characteristics of a drug product that ideally will be achieved to ensure the desired quality, taking into account safety and efficacy of the drug product.

2) Critical Quality Attributes (CQA's):

CQA is a physical, chemical, biological or microbiological property or characteristic that should be within an appropriate limit, range or distribution to ensure the desired product quality.

Some examples of CQAs are the critical resolution, run time, efficiency and robustness.

3) Method Design:

Method Design is prepared to ensure the appropriate availability of material and setting various experimental conditions. In this the reagents are made available, regional and geographical conditions are taken into consideration. Feasibility of instrument is checked and experimental design is prepared.

Method Design should be done according to standardized approach. This approach helps in method transfer step from research to quality control department. Method Development Strategy (MDS) should include Design of Experiments (DoE). It is helpful in risk assessment by gaining knowledge about existing method and allows for effective control strategies for critical parameters.

4) Critical Process Parameter:

Critical Process Parameter (CPPs) are defined as those parameters whose variability have an impact on a CQA and therefore should be monitored or controlled to ensure the process produces the desired quality. Process parameters are classified into three categories: unclassified, critical or non-critical. A parameter is critical when a change in that parameter can cause the product to fail to meet the ATP.

Development studies should be able to move unclassified parameters to either noncritical or critical.

5) Risk Assessment:

According to ICH Q9 Risk Assessment can be done in three steps- Risk identification, risk analysis and risk evaluation.

There are two risk assessment tools:

a) Failure Mode Effect Analysis (FMEA)
b) Ishikawa Diagrams (Fishbone Diagrams)

6) Design Space:

Design Space is defined as the multidimensional combination and interaction of input variables (e.g. material attributes) and process parameters that have been demonstrated to provide assurance of quality. Working within design space is not considered as a change.

The working point is in the middle of the cube and represents a result of the best critical resolution.

7) Method Operable Design Region (MODR):

MODR of any analytical method, also termed as Analytical Design Space (ADS), or Proven Acceptable Range (PAR), is the multidimensional combination and interaction of input variables that have been demonstrated to provide assurance of quality. Before validating a MODR, confirmatory validation runs should be perform to rectify the empirical model resulting from a DoE exercise.

Working within MODR should not be considered as a change, as a method can be considered robust enough to work within this range.

8) Design of Experiment (DoE):

With the help of DoE, We can define many factors, create designs, construct model, define response, evaluate the models, interpret results and hence reach at decision.

Traditionally we are used to single variant study DoE help to do multivariate analysis e.g. wavelength, flow rate, concentration in case of HPLC and its impact on retention time, resolution, total run time, column performance etc.

2. Materials and Method

Chemicals and reagents

The API was obtained as gift sample from Amneal Pharmaceuticals Pvt Ltd, for this project used HPLC grade methanol and Ammonium acetate buffer were purchase from Finar Mumbai India. Prasugrel HCL tablet (PRAX 5) was purchase for analytical purpose; all the other chemicals and reagents used were of AR grade and purchase from Finechem Mumbai India.

Instrumentation and Chromatographic Condition

A HPLC system were (Waters Model Code 25P) consisting of binary LC pump (Waters 1525) the vacuum degasser UV-Visible Detector (Waters 2489), C18 reverse phase coloumn (Cosmosil, size 4.6 x 250 mm length, particle size 5um), and the colomn was kept at an ambient temerature and sample injector system (Rheodyne) with a 10ul sample size., the mobile phase was composed of Methanol: Ammonium Acetate buffer (ph 3.5) (94.7: 5.3), with flow rate 1.1 ml/min. Spectrophotometric detection were carried out on SHIMADZU double beam UV-Visible Spectrophotometer (Model: UV Probe) 1 cm quartz cell.

Software

Design Expert® 11.0 software (Design Expert trial version 11.0; State- Ease Inc., Minneapolis, MN, USA).

Preparation of Standard Stock solution

Accurately weighed quantity of Prasugrel HCl 10 mg was transferred to 10 ml volumetric flask, shaken vigorously for five minutes and volume was made up to mark with diluent. From 10 ml pipette out 2.5 ml and diluted upto 10 ml with diluent, which is equal to 250ug/ml of Prasugrel HCl.

Preparation of Marketed formulation

Standard stock solution

For Prasugrel HCl Standard stock solution prepared by weighing 10 mg of API in 10 ml, and from that 10 ml, accurately pipette out 2.5 ml which is equals to 250 ppm of stock solution.

Tablet solution preparation

Weigh accurately 20 tablets of marketed formulation (Prax 5) were taken and weight of average content was determined. Weight equivalent to 10mg Prasugrel HCL was transferred to 10 ml volumetric flask and make up the volume with methanol (1000 ug/ml) solution was sonicated and filtered through whatmann filter paper. Average weight of tablet was calculated 0.2488gms.

UV calibration curve in methanol (Selection of analytical wavelength)

Standard stock solution of Standard Prasugrel HCl was diluted with diluent to obtain final concentration of 50 µg/ml. Each solution was scanned using UV-Visible Spectrophotometer in the spectrum mode between the wavelength range of 400 nm to 200 nm. The wavelength selected was 254nm.

(Stock Solution: 50 µg/ml)

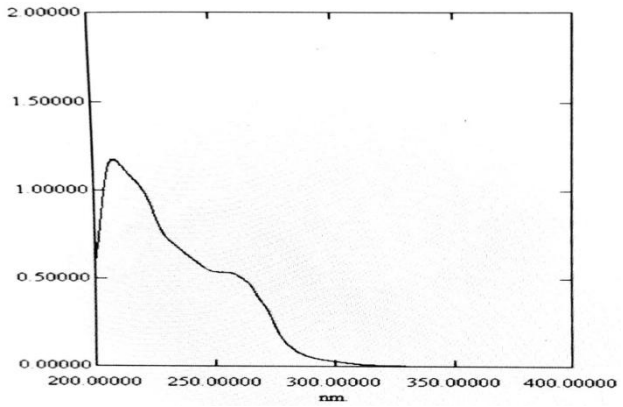

Figure 1: UV spectrum of Prasugrel HCl in Methanol

Method validation

The analytical method validation was performed on the basis of linearity, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), and system suitability in according to ICH guidelines.

Linearity

Linearity of an analytical method is its ability to elicit test results that are directly or by a well defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. It is determined by mathematical treatment of test results obtained by analysis of samples with analyte concentrations across the claimed range. Area is plotted graphically as a function of analyte concentration. Percentage curve fittings are calculated. The acceptance Criteria is to the plot should be linear passing through the origin and Correlation Coefficient should not be less than 0.999.

Accuracy (by Recovery method)

The accuracy of an analytical method is determined by applying the method to analyzed samples, to which known amounts of analyte have been added. The accuracy is calculated from the test results as the percentage of analyte recovered by the assay. Acceptance Criteria is to Mean recovery should be in the range of 98-102% and the Relative Standard Deviation should not be more than 2.0%. Prepare the standard solution by taking stock solution equivalent to 50%, 100%, and 150%, each in triplicate. Each concentration injected into the HPLC system.

Precision

Method precision: Precision of an analytical method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple Samplings of a homogenous sample. Precision of an analytical method is usually expressed as standard deviation or relative standard deviation. Prepare six different test solution of the 100% test concentration from the same sample matrix. Inject duplicate injections of each test solution. The % RSD NMT- 2% for test results. Precision should based on three level (repeatability, intermediate precision, and reproducibility).

Robustness

The robustness of an analytical method is determined by analysis of aliquots from homogenous lots by differing physical parameters that may differ but are still within the specified parameters of the assay. For example change in physical parameters like pH of mobile phase, ratio of mobile phase and wavelength.

Changes in flow rate (± 0.10 mobile phase)
Changes in wavelength (± 01 wavelength)
Changes in ratio of mobile phase (± 01 of mobile phase)

Limit of Detection and limit of Quantitation

For the calculation of LOD and LOQ of the drug used the equation according to ICH guideline. It may be calculated based on standard deviation (SD) of the response and slope of the curve (S).

LOD= 3.3 (SD)/ S and LOQ= 10 (SD)/S

Where,

SD = Standard deviation,
S = Slope of the curve.

System suitability

System suitability test is a pharmacopoeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The tests were performed by collecting data from five replicate injections of standard drug solution. The % RSD of the area of analyte peaks in standard chromatograms should NMT 2.0 %, Theoretical plates of analyte peak in Std. chromatograms should NLT 2000, and Tailing Factor (Asymmetry) of analyte peaks in Standard Chromatograms should be less than 1.75.

3. Results, Discussion

Calibration curve

The calibration studies of prasugrel HCL were performed on the basis of beers-lamberts law, for the calibration of prasugrel HCL have to prepared the different concentration of the drug sample in the range of (20, 40, 60, 80, and 100ug/ml) with methanol, the calibration curve of drug should be in linear response and this method showing its regression coefficient within in limit 0.999. the result of calibration curve shown in figure 02.

(Stock Solution: 100 ppm)

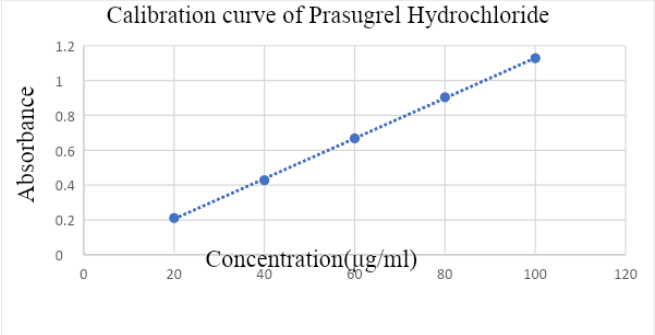

Figure 02: Beer-Lambert's plot for Prasugrel HCl in Methanol

Statistical data analysis (DOE)

Summary of factor and there level selected for 3^3 Box Behnken full factorial design as follows,

Table 01: Summary of factor and there level

Factor	Factor	Unit	Type	Minimum	Maximum	Mean	Std.Dev.
A	M P COMP	%	Numeric	-1 ↔ 89.00	+1 ↔ 95.00	92	2.121
B	pH	UNIT	Numeric	-1 ↔ 3.50	+1 ↔ 4.10	3.8	0.212
C	FLOW RATE	ml/min	Numeric	-1 ↔ 1.10	+1 ↔ 1.50	1.3	0.141

Results of Experimental Runs as per DoE

The layout of actual design of DOE with the subsequent response results are shown in Table no.02 as given below:

Table 02: Layout of Actual Design of DOE

Run	Factor 1 A: MP (ACN)	Factor 2 B: pH	Factor 3 C: flow rate	Response 1 Retention Time (min)	Response 2 TPN	Response 3 Peak Tailing
1	95	3.8	1.1	3.9	3864	1.16
2	92	3.8	1.3	3.76	1615	1.35
3	89	4.1	1.3	4.17	2132	1.03
4	92	4.1	1.5	3.4	1897	1.14
5	95	3.5	1.3	3.41	6840	1.07
6	89	3.8	1.1	5.04	2082	1.26
7	92	3.8	1.3	3.72	1663	1.25
8	95	3.8	1.5	2.88	2156	1.18
9	92	4.1	1.1	4.33	4930	1.13
10	95	4.1	1.3	3.41	4271	1.04
11	92	3.8	1.3	3.69	1837	1.31
12	92	3.8	1.3	3.65	1619	1.33
13	89	3.8	1.5	3.73	1346	1.56
14	92	3.5	1.1	4.28	8609	1.1
15	92	3.8	1.3	3.65	1791	1.36
16	92	3.5	1.5	3.22	6173	1.07
17	89	3.5	1.3	3.91	1537	1.51

2 Analysis of variance for the retention time response as dependent variable:

A) Results for the retention time of DOE:

ANOVA for response surface linear model

The analysis of variance (ANOVA) was performed to identify the significant and insignificant factors. The results of ANOVA for the retention time of DOE are as following Table no.03

Table 03: ANOVA table for retention time

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum Of Squares	Df	Mean Square	F-Value	P-Value	
Model	3.82	9	0.4248	33.00	< 0.0001	
A-M P COMP	1.32	1	1.32	102.58	< 0.0001	
B-Ph	0.0300	1	0.0300	2.33	0.1706	
C-FLOW RATE	2.33	1	2.33	181.25	< 0.0001	
AB	0.0169	1	0.0169	1.31	0.2895	
AC	0.0210	1	0.0210	1.63	0.2420	significant
BC	0.0042	1	0.0042	0.3283	0.5846	
A^2	0.0130	1	0.0130	1.01	0.3489	
B^2	0.0025	1	0.0025	0.1964	0.6710	
C^2	0.0802	1	0.0802	6.23	0.0412	
Residual	0.0901	7	0.0129			
Lack of Fit	0.0812	3	0.0271	12.13	0.0178	significant
Pure Error	0.0089	4	0.0022			
Cor Total	3.91	16				

The Model F-value of 33.00 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case A, C, C^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 21.750 indicates an adequate signal. This model can be used to navigate the design space.

B) Model assessment for the retention time response as dependent variable:

After entering the data in Design Expert software, fit summary applied to data after which "quadratic model" was suggested by the software. According to this model following polynomial equation was obtained. Polynomial equation in coded terms.

Final Equation in Terms of Coded Factors:	
RT=	3.694-0.40625*A +0.06125*B -0.54*C -0.065000000000001*AB +0.0725*AC +0.0325*BC +0.0555*A^2 -0.0245*B^2 +0.138*C^2

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels of the factors are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients

Graphical Presentation: Retention Time:

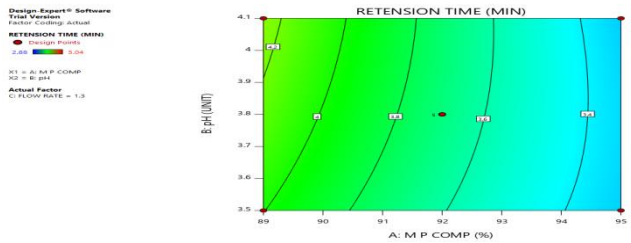

Figure 03: Contour plot for retention time (min) against mobile phase and pH (AB)

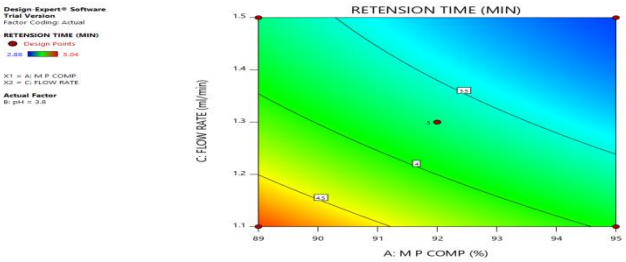

Figure 04: Contour plot for retention time (min) against mobile phase and flow rate (AC)

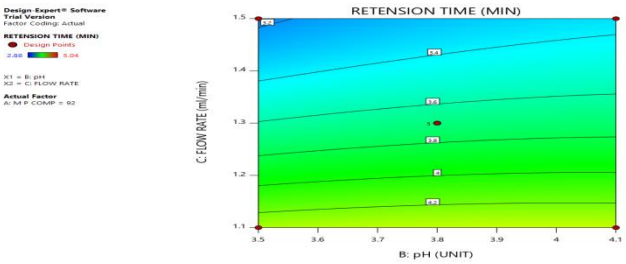

Figure 05: Contour plot for retention time (min) against pH and flow rate (BC)

Analysis of variance for the TPN response as dependent variable:

A) Results for the TPN of DOE:

Table 04: ANOVA table for TPN

ANOVA for response surface linear model

Source	Sum of squares	Df	Mean square	F-value	p-value	
Model	6.993E+07	9	7.770E+06	6.33	0.0119	
A-M P COMP	1.259E+07	1	1.259E+07	10.25	0.0150	
B-pH	1.232E+07	1	1.232E+07	10.03	0.0158	
C-FLOW RATE	7.827E+06	1	7.827E+06	6.37	0.0396	
AB	2.503E+06	1	2.503E+06	2.04	0.1965	
AC	2.362E+05	1	2.362E+05	0.1923	0.6742	significant
BC	89102.25	1	89102.25	0.0725	0.7954	
A^2	1.161E+06	1	1.161E+06	0.9453	0.3633	
B^2	2.664E+07	1	2.664E+07	21.69	0.0023	
C^2	5.884E+06	1	5.884E+06	4.79	0.0648	
Residual	8.598E+06	7	1.228E+06			
Lack of Fit	8.556E+06	3	2.852E+06	271.09	< 0.0001	significant
Pure Error	42080.00	4	10520.00			
Cor Total	7.852E+07	16				

The Model F-value of 6.33 implies the model is significant. There is only a 1.19% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case A, B, C, B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

B) Model assessment for the retention time response as dependent variable:

After entering the data in Design Expert software, fit summary applied to data after which "quadratic model" was suggested by the software. According to this model following polynomial equation was obtained. Polynomial equation in coded terms.

Final Equation in Terms of Coded Factors:	
PLATE COUNT =	1705+1254.25 *A -1241.125*B - 989.125*C -791*AB -243*AC -149.25 *BC -525.125*A^2 +2515.125*B^2 +1182.125*C^2

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels of the factors are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

C) Graphical Presentation: (TPN)

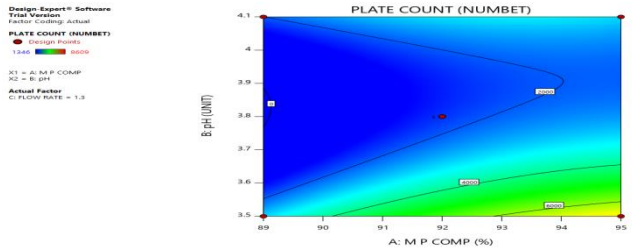

Figure 06: Contour plot for TPN against mobile phase and pH (AB)

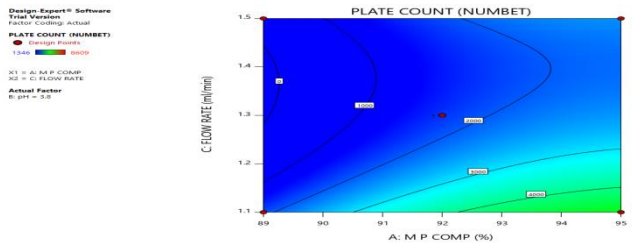

Figure 07: Contour plot of TPN against mobile phase and Flow rate (AC)

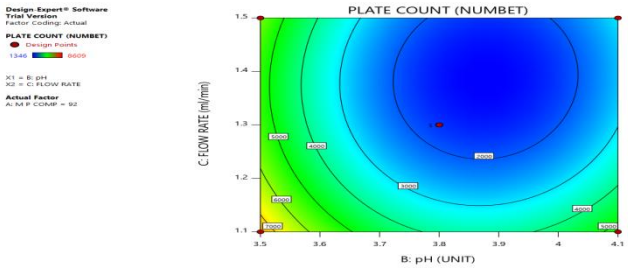

Figure 08: Contour plot for TPN against pH and flow rate (AC)

Analysis of variance for the Tailing factor:

A) Response as dependent variable:

ANOVA for response surface linear model

Table 05: ANOVA table for Tailing factor

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of squares	Df	Mean square	F-value	p-value	
Model	0.3369	9	0.0374	3.80	0.0460	
A-M P COMP	0.1035	1	0.1035	10.52	0.0142	
B-pH	0.0210	1	0.0210	2.14	0.1873	
C-FLOW RATE	0.0112	1	0.0112	1.14	0.3204	
AB	0.0506	1	0.0506	5.15	0.0576	
AC	0.0196	1	0.0196	1.99	0.2010	significant
BC	0.0004	1	0.0004	0.0407	0.8459	
A^2	0.0005	1	0.0005	0.0542	0.8226	
B^2	0.1199	1	0.1199	12.19	0.0101	
C^2	0.0072	1	0.0072	0.7281	0.4217	
Residual	0.0689	7	0.0098			
Lack of Fit	0.0613	3	0.0204	10.75	0.0220	significant
Pure Error	0.0076	4	0.0019			
Cor Total	0.4058	16				

The Model F-value of 3.80 implies the model is significant. There is only a 4.60% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case A, B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

B) Model assessment for the retention time response as dependent variable:

After entering the data in Design Expert software, fit summary applied to data after which "quadratic model" was suggested by the software. According to this model following polynomial equation was obtained. Polynomial equation in coded terms.

Final Equation in Terms of Coded Factors:	
PEAK TAILING	1.32-0.11375*A -0.05125*B +0.0375*C +0.1125*AB -0.07*AC + 0.01*BC + 0.01125*A^2

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels of the factors are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

C) Graphical Presentation: A Peak Tailing

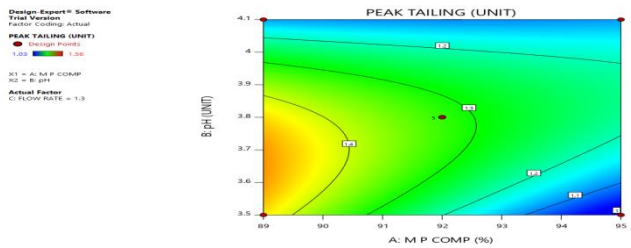

Figure 09: Contour plot for Peak Tailing against mobile phase and pH (AB)

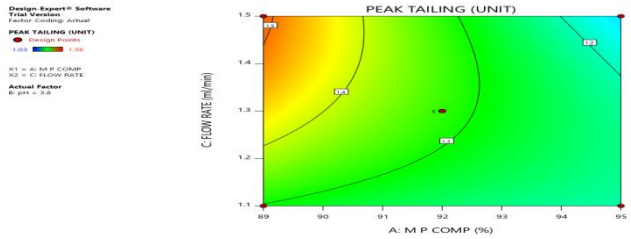

Figure 10: Contour plot for Peak Tailing against mobile phase and Flow rate (AC)

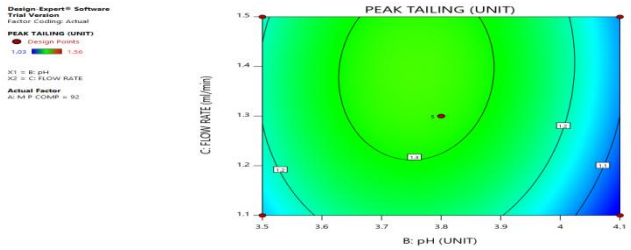

Figure 11: Contour plot for Peak Tailing against pH and Flow rate (BC)

Method Validation

A) Linearity

The calibration curve for prasugrel HCL was linear over the concentration range of 100-400 µg/mL^{-1} . the data for the peak area of the drug in corresponds to the concentration was treated by linear regression analysis (table) and the regression equation for the curve was found to be y = 8069.453571X + 38983.46429 with correlation coefficient of 0.999.

Table 05: Linearity for Prasugrel HCL

Conc	Mean peak Area ± SD	%RSD
100	825921.33 ± 1808.08	0.218
150	1267727 ± 25634.01	1.022
200	1654927.6 ± 20777.03	1.255
250	2062312.3 ± 37897.82	1.837
300	2462312.3 ± 37897.82	1.539
350	2860613.6 ± 40665.04	1.421
400	3260613.6 ± 40665.04	1.247

*mean of three replicates

B) Precision:

The % RSD range obtained was 0.905 and 1.203 for intra-day and inter-day precision studies respectively (table) and the %RSD shall NMT 2.0.

Table 06: Precision of Prasugrel HCL

Conc. (µg/ml)	Intra-day precision Mean*± SD (%RSD)	Inter-day precision Mean*± SD (%RSD)
250	2029646.33 ± 18370.83 (0.905)	2099019 ± 25260.916 (1.203)

*mean of six replicates

A) Accuracy

The method accuracy was proven by the percent recovery method was found to be 99.09-100.56, and the RSD shall be NMT 2.0.

Table 07 Accuracy of prasugrel HCL

Sr. No.	Conc. Level	Conc. (µg/mL) TAB solution	Conc. (µg/mL) for stock solution	MEAN AREA ± SD	%RSD	% Recovery
1	**80%**	**125**	**-**	**1038235 ± 19318.047**	**1.860**	**99.099**
2	**100%**	**125**	**125**	**2019692.333 ± 30338.161**	**1.502**	**98.217**
3	**120%**	**125**	**250**	**3082368.667 ± 27691.834**	**0.898**	**100.565**

*****mean of three replicates

Limit of detection and limit of quantitation

It may be calculated based on standard deviation (SD) of the response and slope of the curve (S). The LOD was found to be 8.4075 and the LOQ was found to be 25.4772.

E. Robustness:

The robustness of the analytical procedure refers to as ability to remain unchanged by small and delibrate changes in method parameters and provides the method

reliability for routine analysis. That studies performed on the basis effect of variation in Change in wavelength maxima (±) of Prasugrel HCl. Carry out the following procedure individually by changing wavelength maxima (±2nm) in chromatographic conditions.

The result obtained from the assay of the test solution were not affected by varying the condition and were in accordance with the results for standard condition. The % RSD value for assay determined was less than 2.0% and it indicates that the method was robust.

Analysis of commercial formulation (Tablets)

The chromatogram for prasugrel HCL obtained from the marketed formulation was shown in fig 6. The following method was applied for the determination of prasugrel hcl in tablets (PRAX) and 100.6 % recovery was observed indicating that the method is selective for the assay of prasugrel hcl without interference from the excipients.

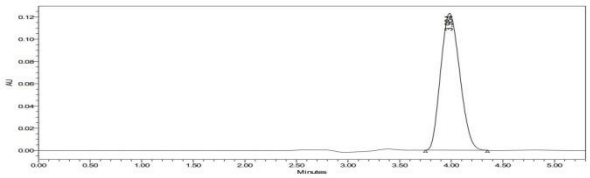

Figure 09: Representative chromatogram of Prasugrel hcl (PRAX 5)

Table 08: Analysis of commercial formulation (Tablets)

Brand name	Conc.ug/ml	Amount found	% Recovery
PRAX 5	250	251.6	100.67 %

4. Conclusion

Quality by Design approach has been successfully used for Development of RP-HPLC Method for estimation of Prasugrel HCl. The developed method employed mobile phase methanol: water (pH 3.5) (94.7: 5.3), and flow rate 1.1 ml/min, which was optimized with the help of design expert software, and utilised for method development using QbD methodology. Systematic approach was utilized to develop an efficient and robust method which includes beginning with determination of target profile characteristics, risk assessment, design of experiment and validation.

The study was done by using 3^3 Box Behnken response surface designs. In this study interaction of 3 factors; flow rate, pH and mobile phase composition vary at 3 levels. Effect of such critical process parameter on critical quality attribute of the method is studied. Responses in terms of retention times and resolution were evaluated throughout all the runs in design.

The RP-HPLC method developed for estimation of Prasugrel HCl was validated as per ICH Q2 (R1) guidelines using various parameters. High linearity of the developed method was confirmed over concentration range of 100-400µg/ml with correlation coefficient of 0.999. The % RSD for precision and accuracy of the method was found to be less than 2%. System suitability test ensures that the analytical system is working properly and can give accurate and precise results. System suitability tests includes tailing factor, number of theoretical plates, area etc.

The proposed high-performance liquid chromatographic method has also been evaluated for accuracy, precision and robustness and proved to be convenient and effective for the quality control of Prasugrel HCl. Moreover, the lower solvent consumption along with the short analytical run time of 10 min leads to a cost effective and environmentally friendly chromatographic procedure. Thus, the proposed methodology is rapid, selective, requires a simple sample preparation procedure, and represents a good procedure for Prasugrel HCl.

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References

[1] ICH Harmonized tripartite guidelines, pharmaceutical development Q8 (R2) current step 4 version dated August 2009, P: 1-24.
[2] ICH Harmonized tripartite guidelines, quality risk management Q9, Current step 4 version dated 9 November 2005 P: 1-19.
[3] ICH Harmonized tripartite guidelines, quality risk management Q10, current step 4 version dated 4 June 2008, P: 1-19.
[4] ICH Harmonized tripartite guidelines, validation of analytical procedures: Text and methodology Q2 (R1), International Conference on Harmonization Geneva; 2005, 1-13.
[5] Patwardhan. D, Amrutkar. S, Kotwal.T, Wagh. M. (2017) 'Application of Quality By Design To Different Aspects of Pharmaceutical Technologies', 8 (9), pp. 3649–3662. doi: 10.13040/IJPSR.0975-8232.8 (9).3649-62.
[6] Mastanamma Shaik, Gujjarlapudi Keerthi, Deepthi K S, Haritha K, S. P. (2013) 'Development and Validation of Colorimetric Method for the

Determination of Prasugrel HCL in Bulk and Dosage Form', International Journal of Pharmacy and Industrial Research, 03 (04), pp. 323–328.
[7] R Kishore Reddy Seerapu, Venkateswara Rao A, Lavanya.P, Pani Kumar A.D, and Rama Krishna K, P. V. S. R. (2011) 'Development of Validated RP-HPLC Method For The Estimation of Prasugrel HCl in Pure and Pharmaceutical Formulations', journal of pharmacy research, 4 (9), pp. 3105–3107.
[8] Sonia, K., Hamunyare, N. and Manikandan, K. (2017) 'Method development and validation of Prasugrel tablets by RP- HPLC', Journal of Pharmaceutical Sciences and Research, 9 (7), pp. 1133–1138.
[9] Sonia, K., Hamunyare, N. and Manikandan, K. (2017) 'Method development and validation of Prasugrel tablets by RP- HPLC', Journal of Pharmaceutical Sciences and Research, 9 (7), pp. 1133–1138.
[10] Alifiya S. Rajkotwala*, Shaikh Sirajuddin S., Dr. Zarna R. Dedania, D. R. R. D. and D. S. M. V. (2016) "QbD approach to analytical method development and validation of Piracetam by HPLC*."*, world journal of pharmacy and pharmaceutical sciences, 5 (5), pp. 1171–1784. doi: 10.20959/wjpps20165-6847.
[11] Bhatt, D. A. and Rane, S. I. (2011) 'QbD approach to analytical RP-HPLC method development and its validation', *International Journal of Pharmacy and Pharmaceutical Sciences*, 3 (1), pp. 179–187. doi: 10.20959/wjpps20165-6847.
[12] Thammana M. (2016) 'A review on high performance liquid chromatography', *journal of pharmaceutical analysis*, 5 (2), pp. 22–28. doi: 10.20959/wjpps20165-6847.
[13] Gupta, V. *et al.* (2012) 'Development and validation of HPLC method -a review.', *International Research Journal of Pharmaceutical and Applied Sciences*, 2 (4), pp. 17–25. Available at: http: //www.irjpas.com/File_Folder/Vibha Gupta et al 17-25.pdf.
[14] Indian Pharmacopoeia, Government of India Ministry of Health & Family Welfare, The Indian Pharmacopoeia Commission, Ghaziabad Vol. II, 2014 /1947-1948.
[15] Sethi P., "High Performance Liquid Chromatography", Quantitative Analysis of Pharmaceutical Formulations, 1st ed., CBS Publishers and Distributors, New Delhi, 2001, pp. 3-11, 116-120.
[16] Mendham J.; Denney, R. C.; Barnes, J. D.; Thomas, M. Vogel's Textbook of Quantitative Analysis. Pearson Education, Singapore, 2003, 8-9.
[17] Sharma B. K., Instrumental Methods of Chemical Analysis, 25thedition, Goel Publication Co. Meerut, 1983, 3- 6.
[18] Skoog D. A., Holler, F. J.; Crouch, S. R. Principle of Instrumental Analysis, 6th edition, Thomson Publications, India, 2007, 1-3, 145-147, 180.
[19] Chatwal G. R., Sharma; A. Instrumental Methods of Chemical Analysis, 5th edition, Himalaya Publishing House, Delhi, 2004; 1.1-1.5.
[20] https: \\pubchem.ncbi.nlm.nih.gov/compound /prasugrel#section =Drug –and-Medication information.
[21] Eli Lilly Nederland B. V. DOC. Ref: EMEA/ 117561/2009.

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