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Quality-By-Design (QBY) Approach RP-HPLC Method for the Estimation of Nilotinib Hydrochloride and Impurities in Drug Substance

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ABSTRACT

The RP-HPLC method development is to define a quality of the product and its process impurities to consistently deliver the intended quality of the drug substance. All the methods which are published are developed through knowledge base, traditional systematic scientific approach. The established method development for Nilotinib hydrochloride was through the vision of "Quality-by-Design" (QbD) approach to estimate the analytical target profile of the target analyte. The robustness of the method has been achieved with an emphasis on establishing the 'design space' of the method through the statistical model to ensure the establishment and use of knowledge on the subject. The proposed chromatographic method as Zorbax SB Phenyl (150mmX4.6mmX3.5µm) column thermo stated 45°C using 15 mM potassium dihydrogen phosphate (KH_2PO_4) with pH-3.5 and Acetonitrile for the gradient elution with the flow rate of 1.0 mL/min. The detection was carried out with the VWD/PDA detector at 240nm. The established design space has been validated against the critical quality attributes (CQA) of the method within the regulatory framework.

Keywords: Quality-by-Design, critical Quality Attributes, DoE, Design Space.

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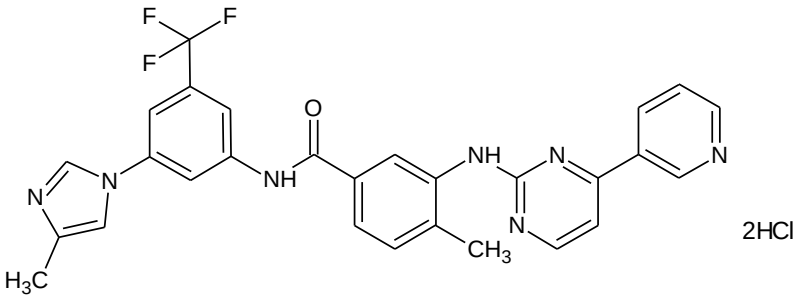
INTRODUCTION

Nilotinib hydrochloride, is 4-methyl-N-[3-(4-methyl-1H-imidazol-1-yl)-5(trifluoromethyl)-phenyl]-3-[[4-(3-pyridinyl)-2-pyrimidinyl] amino]-benzamide, dihydro-chloride, chemical composition of C28H22F3N7O. 2HCl with the molecular mass of 601.5 amu. The reported analytical method for the estimation of impurities in Nilotinib was Nilotinib monohydrochloride monohydrate[1, 2], this article designates more over the Nilotinib dihydrochloride and its related impurities. The proposed highly precise and highly robust method development has been carried through "Quality-by-Design" (QbD) approach. The concept of "Quality-by-Design" is 'Quality cannot be tested into products; quality should be built in by design"[3-7]. It was further defined as an approach which covers a better scientific understanding of the "Critical Quality Attributes" (CQAs) as well as the "Critical Method Attributes" (CMAs) of the drug substances and drug products[8]. It is the scientific, risk-based, holistic and proactive approach to pharmaceutical development. The establishment of new RP-HPLC method development was succeeded through the subject knowledge in an independent and integrated way, guidelines and mathematical models. In this method development the author used "Design Expert" (DE) as the mathematical model.



4-methyl-N-[3-(4-methyl-1H-imidazol-1-yl)-5(trifluoromethyl) phenyl]-3-[[4-(3-pyridinyl)-2-pyrimidinyl] amino] benzamide, hydrochloride,

Figure 1: Structure of Nilotinib Hydrochloride

Recent regulatory initiatives such as "Quality-by-Design" provided unprecedented opportunities to go beyond what was done in the past. The excellent scientific knowledge on the subject along with the statistical model together in a holistic manner established the "Design Space" for the RP-HPLC method within the industrial and current regulatory frame work. The "Design Space" is the multidimensional combination and interaction of input variables (e.g. material / method attributes) and process parameters that have been demonstrated to provide assurance of quality" (ICH Q8). It define the robustness of the method by increasing the flexibility of the input variables. The movement out of the design space is considered to be a change and would impact more on the regulatory perspective. The design space can illustrate understanding of parameter, interactions

and provides robustness with respect to flexibility on variables[9]. The design space can include critical and non-critical attributes on the process as well as the method. It should be verified and validated at the operational scale.

MATERIALS AND METHOD

The initial experimental design should have the important details on Nilotinib hydrochoride process and fate of impurities to identify the "Critical Method Attributes" (CMA's) and identifying the method variables through risk assessment, scientific knowledge.

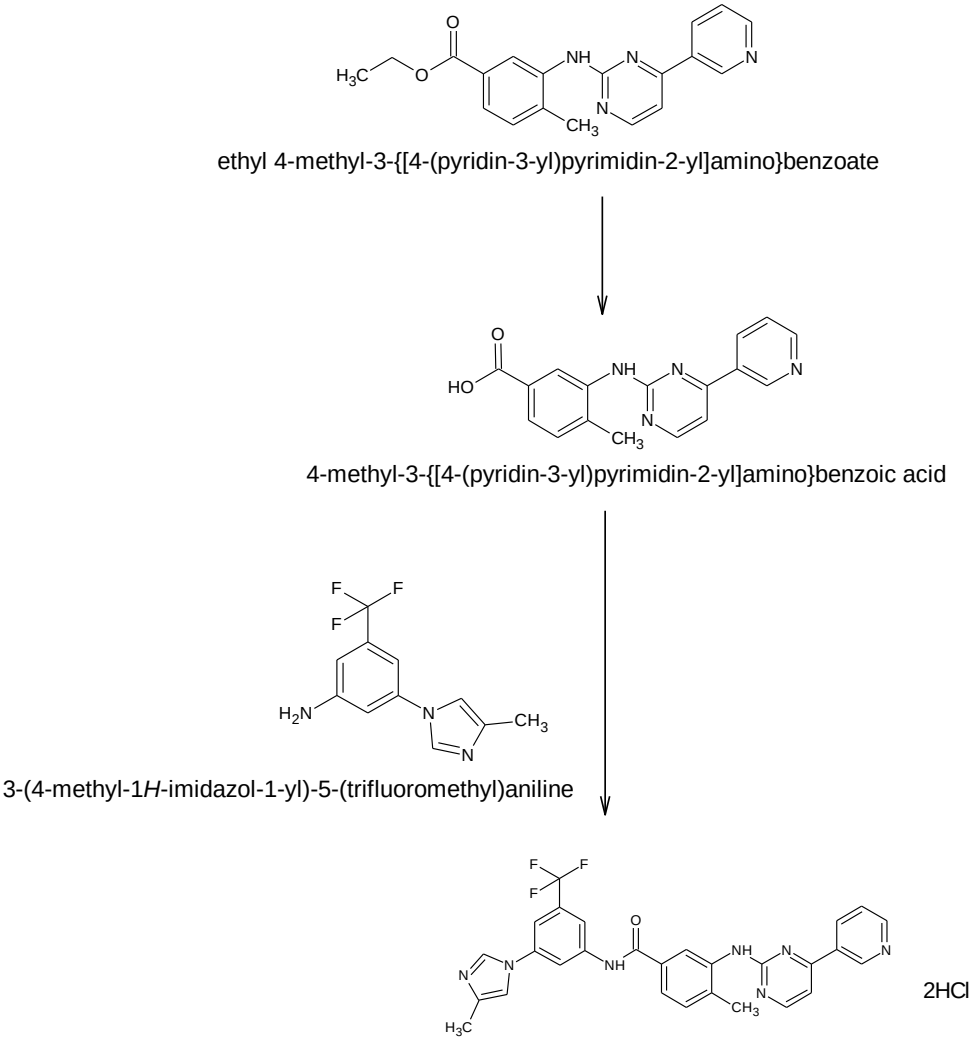


Figure 2: Schematic Diagram of Nilotinib Hydrochloride

Chemicals and Reagents

In this QbD approach RP-HPLC development, the drug substance and its related impurities synthesized and characterized by Cipla Limited, R&D centre (Bangalore, India), anhydrous potassium dihydrogen phosphate were purchased from Sigma-Aldrich (St.Louis, MO, USA). Acetonitrile 99% HPLC grade was bought from Fischer Scientifics (India).

Instrumentation and Analytical Conditions

An Agilent 1100/1200 series (Agilent Technologies, Santa Clara, USA), Ultimate 3000 HPLC (Thermo Scientific, USA) separation module equipped with UV/PDA detectors were used and the system control, the data management was through regulated compliance "CHROMELEON 6.8 SR14" (Thermo Scientific, USA) software. The multifactor designing and the screening of the experiments through factor screening and method optimization by Response Surface Methodology. The chromatographic condition of RP-HPLC method by QbD approach for the estimation of Nilotinib hydrichloride and its related impurities were Zorbax SB Phenyl (150mmX4.6mmX3.5µm) column, Mobile Phase A Buffer, Mobile phase B Acetonitrile as Organic modifier and gradient condition as follows, Time (min) / Mobile Phase A (v/v):Mobile Phase B (v/v); $T_{0.01}$/70:30, $T_{5.00}$/70:30, $T_{15.00}$/55:45, $T_{28.00}$/55:45, $T_{33.00}$/25:75, $T_{38.00}$/25:75, $T_{40.00}$/70:30 and $T_{45.00}$/70:30. The detection was carried out with the VWD/PDA detector at 240 nm.

Fate of Impurities

The purpose of fate of impurities studies were to obtained information about how the impurities were derived and how it was removed from a pharmaceutical manufacturing process. Potential impurities can be derived from starting materials or can be formed in side reaction during the process. The studies are designed to monitor and assist in the control of impurities throughout the process[10]. In our process the Impurity A as Ethyl 3-carbaminoimido-4-methylbenzoate, Impurity B as 3-(dimethyl amino)-1-(3pyridinyl)-2-propen-1-one are the precursors of starting material, Impurity C as 5-(4-Methyl-1H-Imidazole-1-yl)-3-(trifluoromethyl)aniline and Impurity D as 4-Methyl-3-[[4-(3-Pyridineyl)-2-Pyrimidinyl]amino]-benzoic acid ethyl ester are the key starting materials, Impurity E as 4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]-amino]benzoic acid is the process impurity.

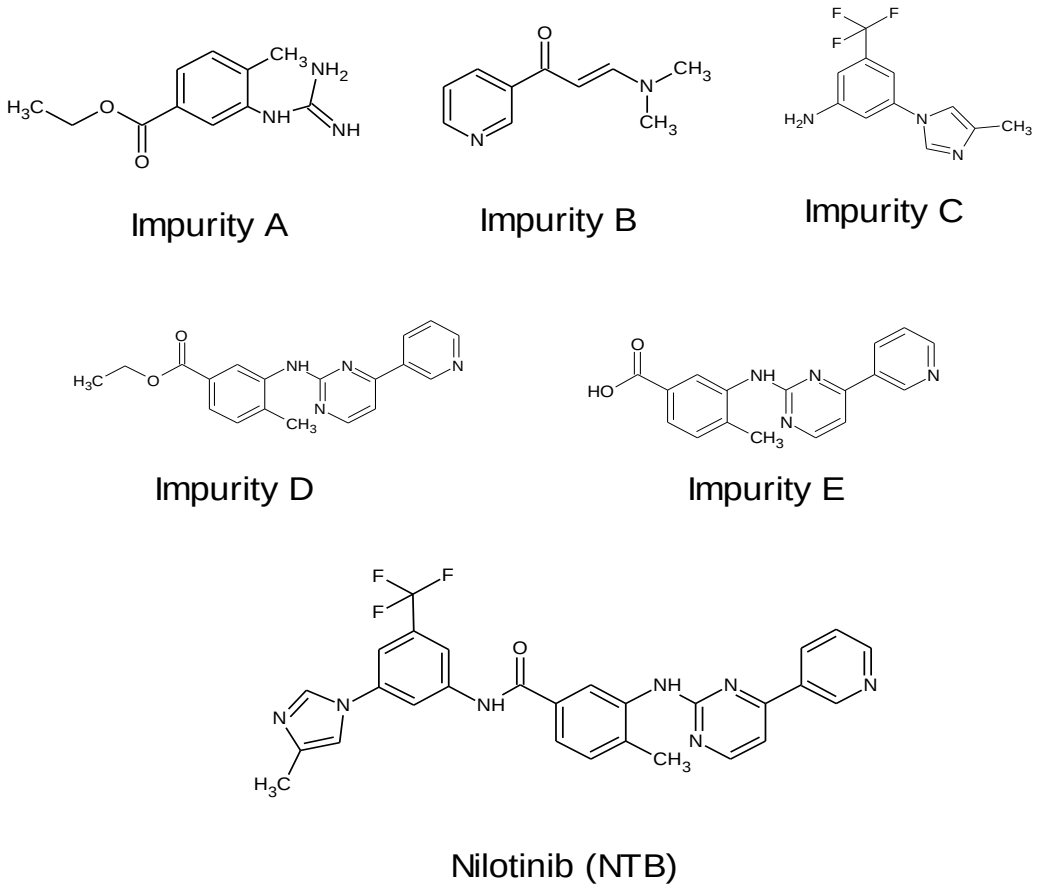


Figure 3: Fate of Impurities in Nilotinib Hydrochloride

Risk Assessment to Identify the Critical Method Attributes

Risk Assessment Identification

According to the QbD concept, analytical method development starts based on scientific knowledge and risk based matrix evaluation. In this proposed RP-HPLC method developments starts with the "Analytical Target Profile" (ATP) of System suitability as well as the critical method validation parameters as per ICH guideline as "Critical Quality Attributes" (CQAs) of the methods as Specificity, Resolution, Asymmetry, Capacity factor, Theoretical plates, etc. To achieve this ATP and CQA of this method should be empowered through the risk assessment matrix as fish bone diagram[9]. This is the tool to identify the variables affecting the CQA for this present studies.

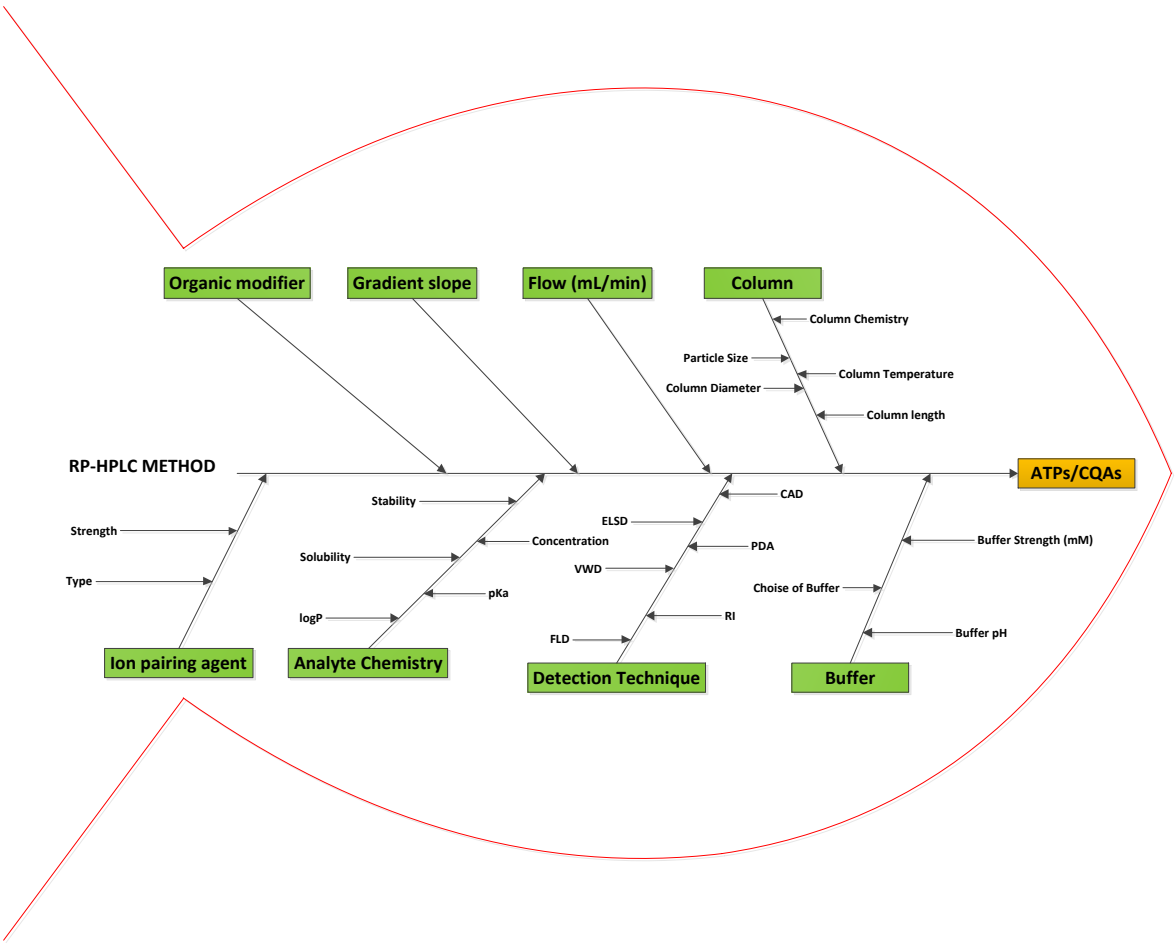


Figure 4: Fishbone Diagram for the HPLC Method Parameters which can have an Impact on the Critical Method Attributes.

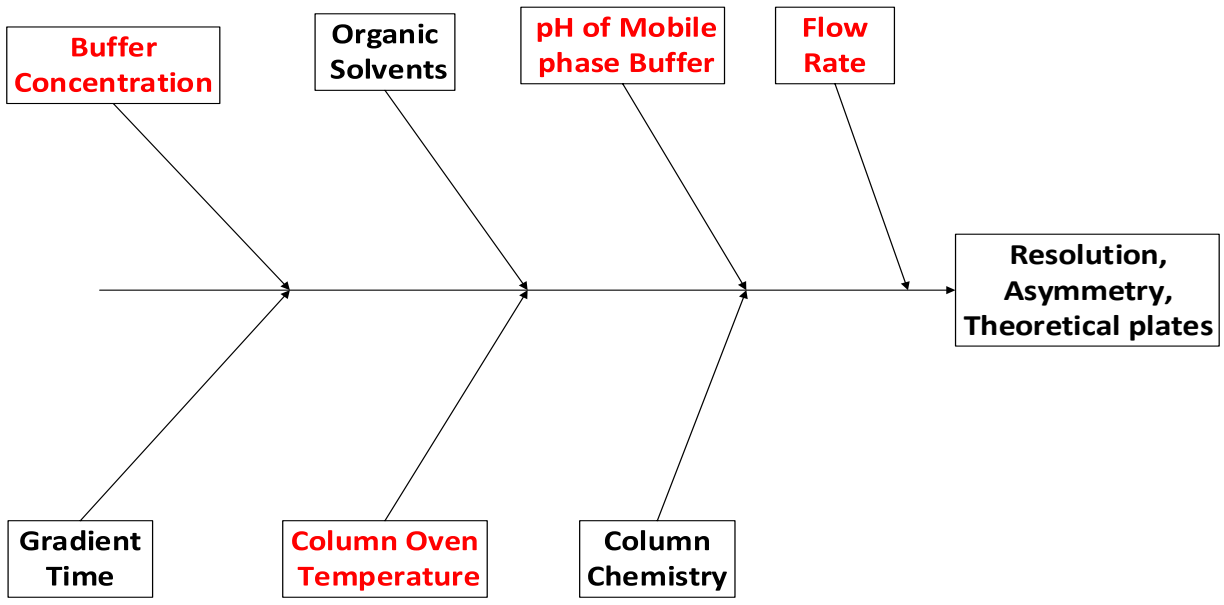


Figure 5: Fishbone Diagram for the ATP which can have an Impact on the Critical Method Attributes.

In this risk assessment matrix[5], column chemistry, gradient elution and Organic solvents are critical for the RP-HPLC method and it was fixed by using OFAT (One Factor At a Time) approach as Zorbax SB Phenyl (150mmX4.6mmX3.5µm) column, Mobile Phase A Buffer, Mobile phase B Acetonitrile as Organic modifier and gradient condition as follows, Time (min) / Mobile Phase A (v/v):Mobile Phase B (v/v); $T_{0.01}$/70:30, $T_{5.00}$/70:30, $T_{15.00}$/55:45, $T_{28.00}$/55:45, $T_{33.00}$/25:75, $T_{38.00}$/25:75, $T_{40.00}$/70:30 and $T_{45.00}$/70:30. The detection was carried out with the VWD/PDA detector at 240 nm. This all are considered as low level risk variables and the remaining parameters like buffer concentration, buffer pH, column flow and column temperature are considered as high level risk variables. The effect of critical method attributes and analytical target profiles against the CQA to be studied through the statistical tool as Design of Experiments (DoE) by Design-Expert® 9.0.5 software (Stat-Ease Inc., Minneapolis, MN, USA).

Screening of Critical Method Variables through DoE

To establish the effect of high risk variables for this method has been studied between the small ranges of variables as Mobile phase concentrations between 5mM to 15mM, pH between 3.2 to 4.2, column thermostat between 30°C to 50°C and the flow rate between 0.8 mL/min. to 1.2 mL/min. along with this another center point of all these variables also been taken for the design of experiment as multi factors at a time approach. Based on this 19 DoE has been executed and the obtained responses as analytical target profile from these DoE were augmented.

Design Type: 4 Factors 2 Levels Full Factorial with Center Point

Runs: 2^4 + 3 (centre points) = 19 runs

Since the full factorial design is a linear model, the curvature can't be studied using linear model. So the design further augmented to Central Composite Design (CCD) (Response Surface Methodology - RSM) to study the curvature observed in the responses, by which quadratic / polynomial equation to explain the curvature in the model.

RESULTS AND DISCUSSION

The experimental variables and software generated test plans as 19 unique combinations with respect to ATPs and CQAs were tabulated in Table 1. All that responses were analysed and empowered in Design-Expert software and explore to ANOVA to check the adequacy of the obtained model[8] and the significant models were tabulated as Table 1.

Table 1: Multi Factors DoE and Experimentally Obtained Responses against ATP

Response	Model equation	R-Squared	Model p-value
PLATES IMP-A	44995.5 + -20691.8 * pH + 20.5299 * Temperature + -1207.78 * Flow + -649.189 * Buffer conc + 2638.61 * pH^2 + 41.6093 * Buffer conc^2	0.9633	< 0.0001
PLATES IMP-B	-89209.3 + 56167.7 * pH + 105.769 * Temperature + -21187.7 * Flow + 1565.48 * Buffer conc + 4920 * pH * Flow + -8753.87 * pH^2 + -62.8487 * Buffer conc^2	0.9458	< 0.0001
PLATES IMP-C	-10.8862 + 7.29345 * pH + 5.91796 * Temperature + 1.63284 * Buffer conc + -1.45443 * pH * Temperature	0.9273	< 0.0001
RESOLUTION IMP-A & IMP-B	54.208 + 26.2106 * pH + 0.57111 * Temperature + -0.887132 * Flow + -0.359939 * Buffer conc + -0.05625 * pH * Temperature + 0.1475 * pH * Buffer conc + -3.35 * pH^2 + -0.004125 * Temperature^2	0.9328	< 0.0001
RESOLUTION IMP-B & IMP-C	-28.0571 + 16.612 * pH + -0.186976 * Temperature + -0.344895 * Buffer conc + 0.05875 * pH * Temperature + 0.2425 * pH * Buffer conc + -2.43 * pH^2 + -0.0163 * Buffer conc^2	0.9457	< 0.0001
RESOLUTION IMP-D & NTB	10.4268 + 7.80762 * pH + 2.73125 * pH * Temperature + 2.33125 * pH * Buffer conc + 4.69018 * pH^2	0.8957	< 0.0001
ASYMMETRY IMP-A	1.674 + -0.129142 * Flow + -0.147426 * Buffer conc + -0.06875 * pH * Buffer conc + 0.0675 * pH^2 + -0.05625 * pH^2 * Temperature + 0.19375 * pH * Temperature^2	0.9394	< 0.0001
ASYMMETRY IMP-C	0.652481 + -0.451799 * pH + 0.0701248 * Buffer conc + 0.0836828 * pH * Temperature + -0.103817 * Flow * Buffer conc + 0.328721 * pH^2 + 0.103817 * pH * Flow * Buffer conc	0.9569	< 0.0001
ASYMMETRY API	1.0125 + 0.05625 * pH * Temperature + 0.16875 * pH * Buffer conc + 0.11875 * pH^2 + -0.05625 * pH^2 * Buffer conc + -0.25625 * pH * Temperature^2	0.896	< 0.0001

The significance of the selected ATPs and CQAs are once again confirmed with the R-Squared value and the probability of the selected CQAs within the pre-defined acceptance levels as p-values <0.0001 has been computed and plotted as 3D contour plots of significant response models.

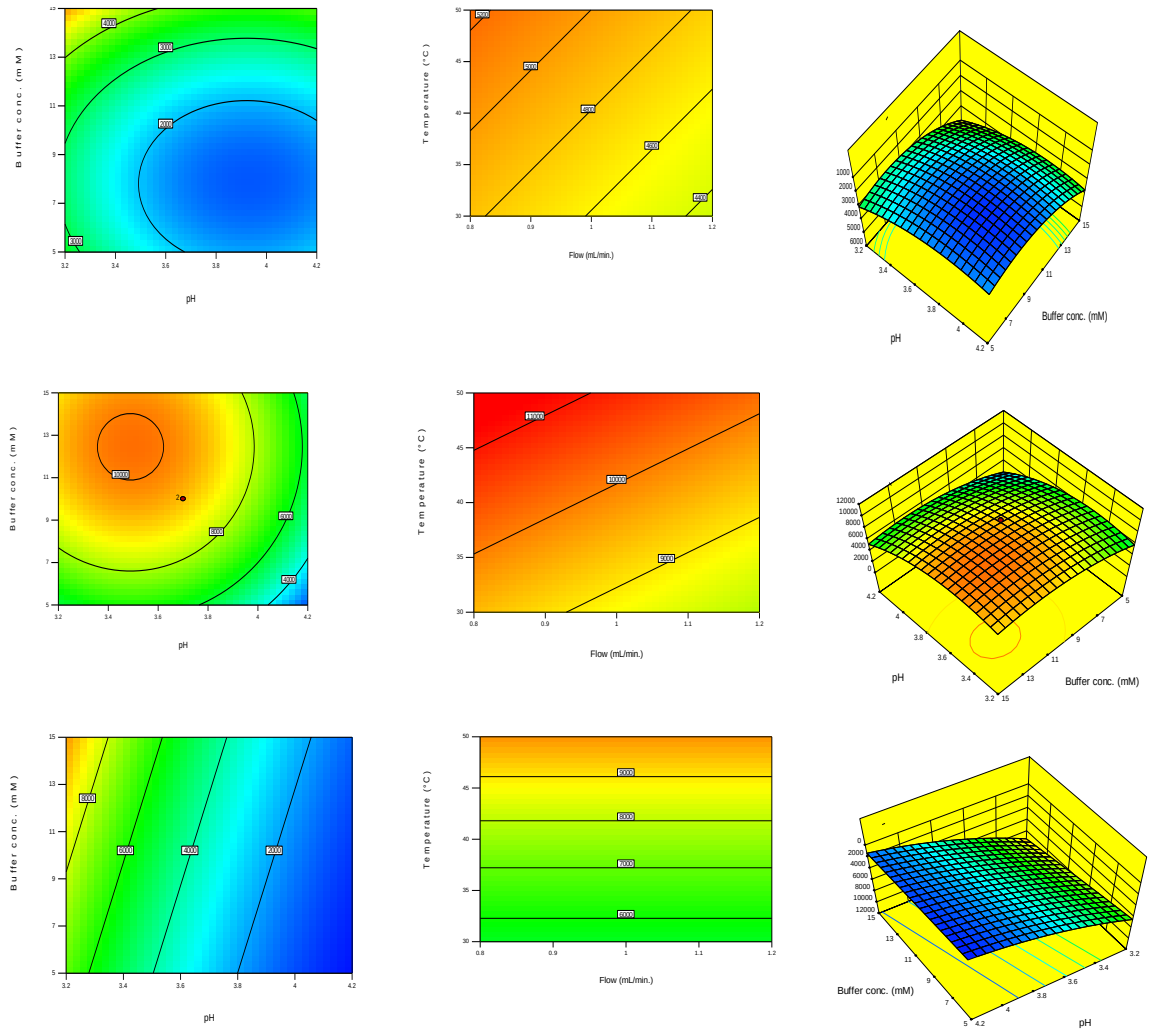


Figure 6: Contour & 3D plot for the Theoretical plate's response of Impurity A, B and C.

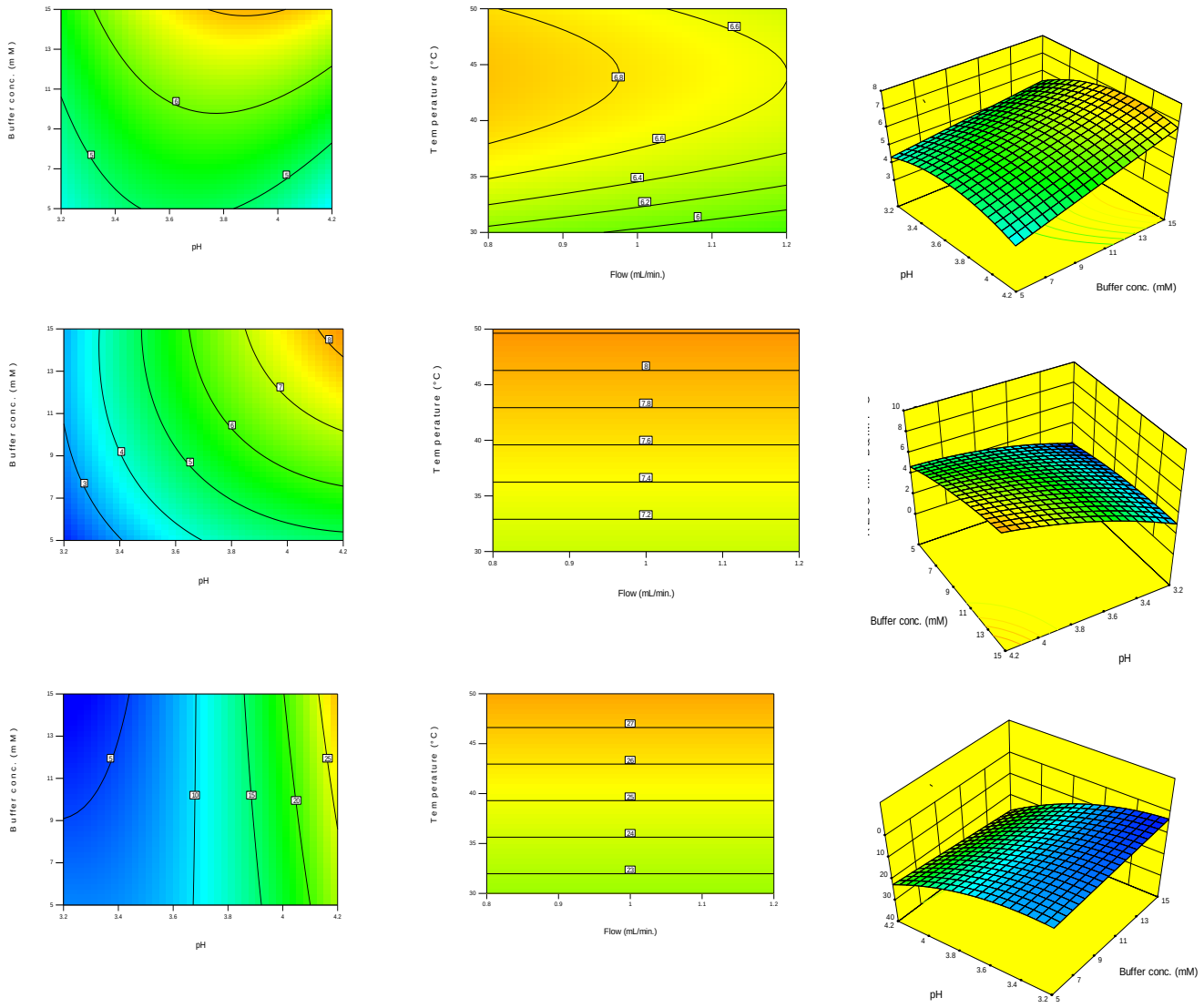


Figure 7: Contour and 3D Plot for the Resolution Response between Impurity A, B and C

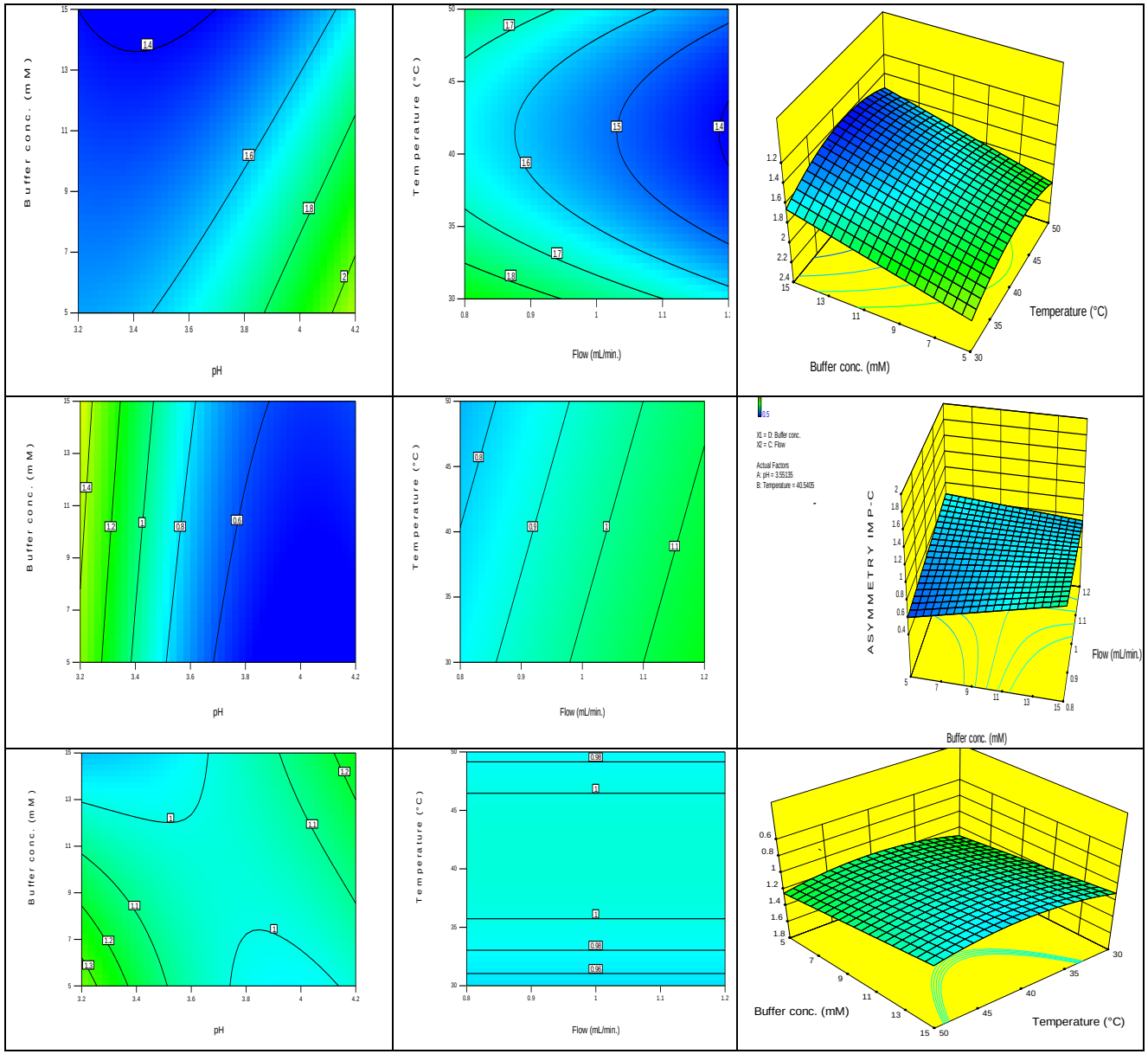


Figure 8: Contour and 3D Plot for the Asymmetry Response of Impurity A, B and C.

The contour plots for each CQA are overlapped to create the design space. Design space which the zone the method parameters can be run not only the predefined ATPs are obtained, but also the assurance of robustness of the method to achieve all CQAs.

Limits of the CQAs considered for this studies:

- Theoretical plates for Impurity-A not less than -3000
- Theoretical plates for Impurity-B not less than -3000
- Theoretical plates for Impurity-C not less than -3000
- Resolution between Impurity-A & Impurity-B not less than 4
- Resolution between Impurity-B & Impurity-C not less than 4
- Resolution between Impurity-C & Impurity-D not less than 4
- Asymmetry for the peak of Impurity-A between 0.8-1.5
- Asymmetry for the peak of Impurity-C between 0.8-1.5
- Asymmetry for the peak of API between 0.9-1.5

Design Space

The multidimensional combination and interaction of input variables (e.g., ATPs/CQAs) and process apameters that have been demonstrated to provide assurance of quality[4].

Design space shows the yellow region where all the CQAs achieved.

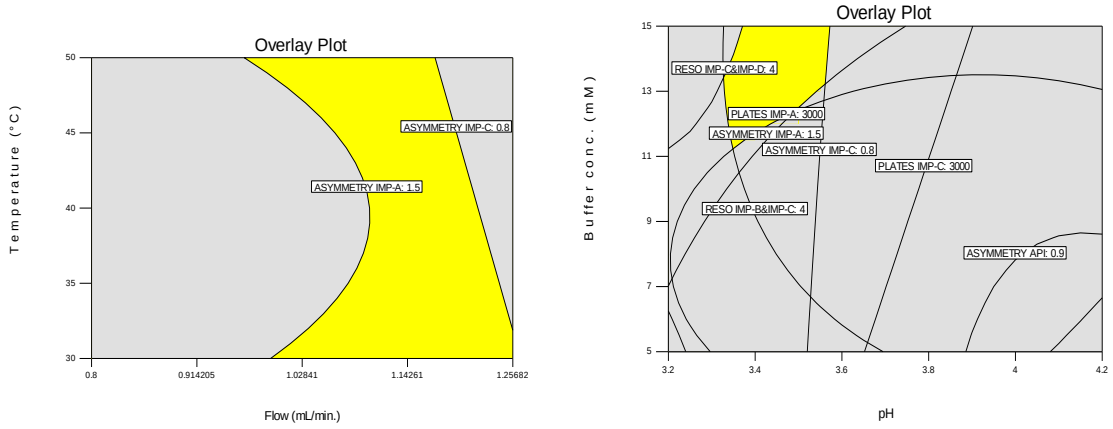


Figure 9: Contour Plot for the Design Space

All the above attributes can be achievable if the variables are set within the below given ranges;

- Temperature, Range= 32.95°C to 46.85°C.
- Flow rate, Range = 1.09 mL/min. to 1.23 mL/min.
- pH (Buffer), = 3.49
- Buffer concentration: 15 mM.

The obtained Design-Space is validated against the ICH Q2 (R1) elements and optimized the method for the reliability and reproducibility as the proposed chromatographic method for the estimation of impurities and Nilotinib hydrochloride in drug substances. During this Design-Space validation obtained values of all the responses of ATPs / CQAs are very much closer (>95%) to predicted responses. It defined the reliability and robustness of the method.

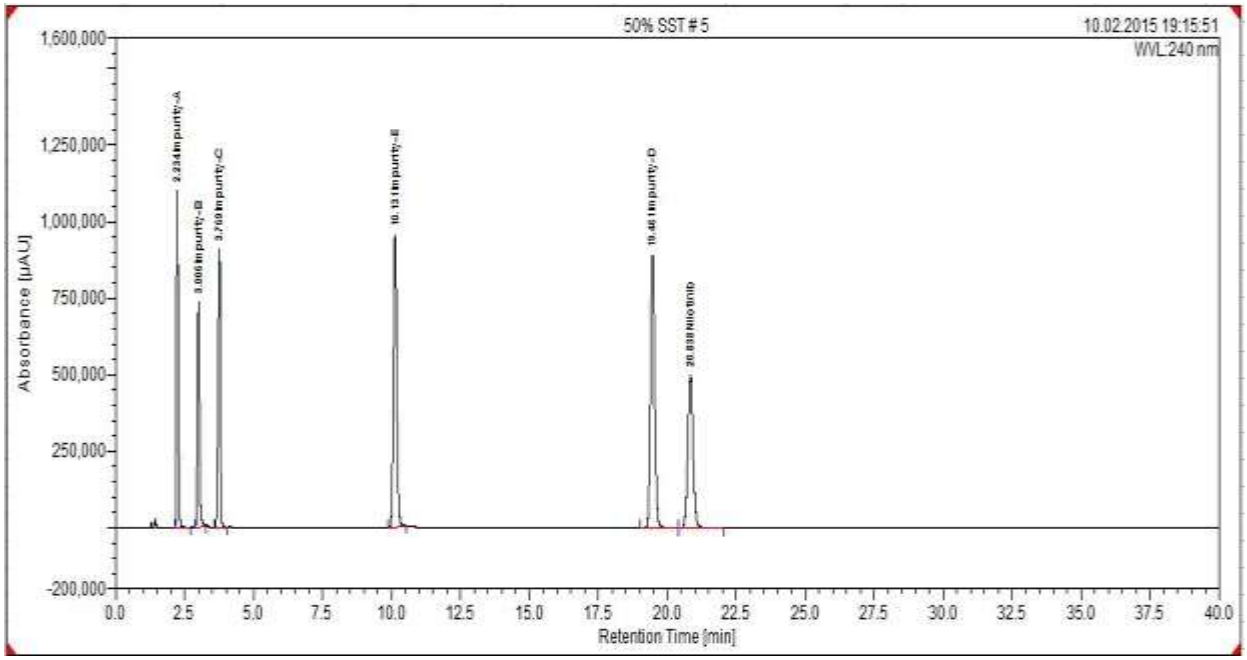


Figure 10: Typical Chromatogram of the QbD Optimized RP-HPLC Method at the Optimum.

CONCLUSION

RP-HPLC method was essentially established with Quality-by-Design approach for the estimation of related compounds in Nilotinib hydrochloride. The obtained "Design Space" was intentionally evaluated and validated against the elements as per ICH to ensure the Robustness and Ruggedness of the RP-HPLC method. This method was found to be simple, more sensitive, specific, precise, accurate and robust. This method can be used for the routine quality control analysis and stability analysis too.

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