Original Article

Evaluation of pH Dependent Solubility and Examination of Variation in Pharmacokinetic Properties of Alectinib: A Quantitative Study by Implementing Integrated Quality by Design Approach for RP-HPLC Method Development and Optimization

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ABSTRACT

Introduction: Alectinib, an anaplastic lymphoma kinase inhibitor of BCS class IV, is said to have pH-dependent solubility and is susceptible to interactions with co-prescribed acid-reducing agents. **Objectives:** A micro-dissolution study was performed to determine the effect of modulations in gastrointestinal pH using biorelevant media and a sensitive RP-HPLC technique was developed for quantification of alectinib in the same using quality by design approach. **Materials and Methods:** Analytical method was developed and optimized in accordance with box-behnken design followed by micro-dissolution experiment mimicking physiological pH shift. **Results:** The solubility of alectinib in FaSSGF decreased from 0.648 μg/ml to 0.270 μg/ml whereas in FaSSIF it dropped down from 0.574 μg/ml to 0.108 μg/ml at the end of the micro-dissolution experiment. This reveals that elevation of pH from 1.2 to 6.8 has no significant impact on its solubility and hence will not influence drug absorption. **Conclusion:** Nonetheless, the study would be useful for therapeutic medication monitoring, dose adjustment of co-administered drugs, proactively driving clinical research design, and obtaining a readout on pH liability for high-risk anticancer medications.

Keywords: Alectinib, QbD, pH, ALK kinase, Micro dissolution.

INTRODUCTION

Alectinib (ALK) is a second-generation anaplastic lymphoma kinase inhibitor that is used to treat malignancies with anaplastic lymphoma kinase mutated genes, non-small cell lung cancer, lymphomas, and CNS disorders. ALK is administered in capsules with a daily dose of 600 mg (3 capsules of 150mg) and has a low systemic clearance and moderate bioavailability.[1-3] Due to the pH gradient in the GI fluid during the passage from the stomach to the intestine, it is shown to have a complicated solubility pattern as a weakly basic medication belonging to BCS class IV. Several anticancer medications that are weakly basic have pH-dependent solubility, and co-administration of acid reduction agents, such as proton pump inhibitors, which contribute to an elevation in gastric pH, would cause modulation in drug absorption and modify the drug's pharmacokinetic pathway.[4-5] Macros *et al.* conducted a human investigation in healthy volunteers who were administered with ALK and esomeprazole in fasted and fed settings to study the clinical impact of acid-reducing agents (ARA) and the possibility of interaction and modifications in the pharmacokinetic pathway.[6]

Although numerous *in-silico* modeling strategies, as well as human studies, are designed for drug-interaction prediction based on physiochemical data that have been

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documented, a simple, sensitive, and reliable experimental method will be favored for a better understanding and identification of the prospective risk of interaction.[7-8] In this study, the effect of pH on the target drug due to pH modulation because of administration of proton pump inhibitors in the stomach and intestine was investigated using an *in vitro* micro dissolution experiment. To create data acceptable for interpretation and *in vitro* – *in vivo* correlation, the experiment assembly simulated the pH shift and clinically relevant conditions of changes in the stomach and intestine.[9]

Along with the importance of understanding the drug's pH dependence on solubility, designing an accurate analytical process is also a key focus in such sensitive investigations. Since solubility is the most significant aspect to evaluate in this experiment, the established analytical method must be sensitive enough to detect even the smallest possible amount of drug present. For this, a broadly adopted approach in analytical process designing - quality by design approach, was employed to instill quality in the final output. The application of the AQbD approach creates a roadmap for developing and validating methodological approaches for components in a short period of time.[10-11] It not only aids in the determination of factors impacting the interaction in the experiment, but it also assists in the monitoring of the performance and minor alterations of the designed approach, as well as shortens the process. It also assists in identifying the important qualitative attributes that have a substantial impact on the result, in addition to comprehending specific aspects and variables.[12]

However, various aspects of biomedical research would influence ALK's clinical activity in different ways. This experiment focuses on the absorption step in the pharmacokinetics of ALK and is meant to determine the influence of pH changes attributable to modulations in absorption.[11-20] This would be the sole study in our reference to design, develop, and optimize an RP-HPLC method for quantification of ALK by implementing a QbD technique, which would enable a broad spectrum of qualitative and quantitative application as well as a method operational region for resolving non-specified discoveries. An analytical quality by design (AQbD) methodology - for building a new simple, accurate, precise, and robust RP-HPLC method for quantification of ALK in the samples of the micro-dissolution experiment would be carried out for assessment of pH-dependent interaction and to study the variation in bio-performance.

MATERIALS AND METHODS

Reagents and chemicals: MSN (Hyderabad) provided alectinib as a complimentary sample. Biorelevant (Croydon, Surrey, United Kingdom) simulated intestinal fluid powder was acquired for the preparation of FaSSGF and FaSSIF media. All the chemicals, solvents, and reagents utilized in this experiment were HPLC grade. Finar and Spectro chem (Mumbai) delivered the methanol and acetonitrile, respectively. Potassium dihydrogen phosphate and orthophosphoric acid were purchased from Merck (Mumbai).

Analytical Target Profiling and Risk Assessment Parameters

Analytical target profile: The QbD-based RP-HPLC approach begins with the identification of an ATP. ALK, chemically denoted as (9-ethyl-6,6-dimethyl-8-[4-(morpholin-4-yl) piperidin-1-yl]-11-oxo-6,11-dihydro-5*H*-benzo[*b*]carbazole-3-carbonitrile hydrochloride) is a heterocyclic chemical entity with sensitive physiochemical profile. The accuracy and applicability of the developed method will all be influenced by the same.

Critical Quality Attributes (CQA) and Categorisation of Critical Process Parameters (CPP): To achieve the intended output i.e., an accurate, robust, and a specific analytical method for quantification and meet the ATP criteria, critical process parameters (CPPs), and critical quality attributes (CQAs) were selected. These factors were chosen based on the Ishikawa fishbone diagram which included several factors that are found to influence analytical method development.[20-21] As essential quality features, drug retention time (RT) and tailing factor (TF) were chosen, whereas pH of the aqueous mobile phase, percentage of organic mobile phase, and flow rate were chosen as critical process factors that could have a substantial impact on the method's performance (Table 1).

Instrumentation, Chromatographic conditions, and Software

Instrument and chromatographic conditions: The chromatographic method was established utilizing a Shimadzu Prominence HPLC system with configuration of LC-20AD quaternary pumps, DGU-20A5 degasser unit, SPD-M 10A Photo Diode Array detector, SIL 20AC HT autosampler, and CTO -10AS column oven. As a stationary phase, a phenomenex C8 (150 mm* 4.6 mm, 5 µ) column was used to achieve chromatographic separation. A 10µg/ml concentration of ALK was selected for the experimental runs. The buffers used as the mobile phase were sonicated, filtered, and degassed before the experiment.

Table 1: Independent variables and their levels.							
Factor	Name	Units	Type	Minimum	Maximum	Coded low	Coded high
A	pH	1	Numeric	3	6.8	-1 ↔	+1 ↔
B	% Aqueous	%	Numeric	60	80	-1 ↔	+1 ↔
C	Flow rate	ml	Numeric	0.8	1.2	-1 ↔	+1 ↔

Software: LC Solution software was used for post-run analysis in RP-HPLC and Design Expert® software version 11 for designing the experimental runs, incorporation of obtained data, and carrying out statistical analysis based on the responses obtained for identification of the extent of impact and interaction of critical process parameters selected on ATP.

Design Selection, assessment factors, and response analysis

Design selection: A DoE based three-level three factorial box-behnken design was selected for designing the experimental runs and mathematical model fitting.[12] The relevance of the design was established by using the ANOVA approach to assess statistical parameters and for response surface analysis.

Risk parameters: Amongst the several method parameters of mobile phase, stationary phase, temperature, etc., the impact of three independent process factors, namely mobile phase pH (A), percent organic within the mobile phase (B), and flow rate (C) was examined on defined analytical target profile.

Assessment factors: Based on two response variables, retention time (RT) and tailing factor (TF), the most critical process parameter was investigated. Main effect and statistical analysis were studied based on a 3D counter plot and perturbation plot denoting the interaction between various process variables affecting the Analytical target profile.

Validation of Optimized Method

Specificity, linearity, the limit of detection (LOD), the limit of quantification (LOQ), accuracy, precision, and robustness of the established methodical technique were all validated according to the ICH Q2 (R1) guideline.

In-vitro micro dissolution for quantification ALK in a pH shift experiment

The *in vitro* micro dissolution test was categorized into two sections: gastric (FaSSGF buffer) and intestinal (FaSSIF buffer), with pH levels variations of 1.2, 6.5, and 6.8.[15-16] Since ALK is administered as a 150 mg capsule, the strength was calculated per 250 ml glass of water consumed with it. The calculated quantity of ALK was added to 14ml of pH 1.2 FaSSGF buffer, and dissolution was monitored up to 20 min followed by pH shift with the addition of 28mL of pH 6.5 FaSSIF buffer into the same container. The micro-dissolution assembly was tracked for 180 min for sampling. In a different container, a similar arrangement was made with 14 ml of pH 6.5 FaSSIF buffer, and dissolution was carried out for 20 min in pH 6.5 FaSSIF buffer followed by the addition of 28 ml of pH 6.8 FaSSIF buffer was added to the same assembly. The sink conditions were maintained by the replacement of the same volume of fresh buffer. The assembly thoroughly mixed the experimental fluids with the help of magnetic stirrers revolving at 350rpm. The withdrawn samples were centrifuged at 10,000 rpm and the supernatants were analyzed by the optimized RP-HPLC method.

RESULTS AND DISCUSSION

Risk assessment and Statistical analysis

Effect of three independent variables i.e., pH of aqueous mobile phase (A), % organic mobile phase (B), and flow rate (C) on the responses RT and TF i.e., retention time and tailing factor of ALK was assessed statistically after carrying out the experimental runs suggested in Box-Behnken design. The effect of the independent variables was significantly visible on the response factors which signified the importance of the selection of critical process parameters and quality attributes.

Impact of independent process variables: The independent variable A, i.e., the pH of the aqueous mobile phase, had an impact on the tailing factor, implying that the ATP of weakly basic medication is sensitive to acidic pH and produces the desired output. The retention time of ALK, on the other hand, was heavily influenced by the flow rate of the mobile phase. As a result, the crucial process parameters for the intended reaction were the flow rate and pH of the aqueous mobile phase.

Statistical Analysis

Table 2 summarizes the results of statistical analysis by ANOVA for both the response factors. Design expert suggested linear and quadratic models as best-fitting models for both the responses. The model was found statistically significant with a p-value of < 0.0001 and

Table 2: ANOVA results for retention time and tailing factor.

Response	Retention time	Tailing factor
Std. Dev.	1.06	0.0858
Mean	11.84	1.732
P-value	0.0015	< 0.0001
F-value	11.27	26.33
R^2	0.7717	0.8876
Adjusted R^2	0.7032	0.8539
Predicted R^2	0.5170	0.7508
Adequate Precision	9.9351	16.6844

f-values of 11.27 and 26.33 for RT and TF respectively. Adequate precision i.e., signal to noise ratio for both the response factors was found to be above 4 as per the desirability, whereas predicted R^2 and adjusted R^2 were found to be in reasonable agreement with each other having a difference of less than 0.2 between them displaying the significant interactions between process variables and its correlation with the response factor in selected design with the desirability of 1.

Perturbation plot analysis and 3D response surface plot analysis

The visual/graphical impact of the independent variables and their interactions concerning responses were studied with help of perturbation plots and 3D contour plots and the results were correlated with the statistical data. Amongst the various critical process parameters including column chemistry, column length, choice of organic solvent, etc. pH of the aqueous mobile phase, flow rate, and percent organic phase was found to be most relevant for the selected analyte profile. The interaction diagram displayed the strong impact of flow rate on the retention time of ALK. Whereas the pH of the mobile phase considerably affected the tailing factor of the peak. However, the % organic mobile phase and pH of the aqueous phase did not show a significant impact on the retention time of ALK (Figure 1). According to the representation in perturbation plots (Figure 2)., flow rate exhibits both, positive and negative co-relation concerning RT and TF, whereas pH of the aqueous mobile phase shows significant positive deviation for tailing factor followed by % organic phase. This embarks the impact of acidic pH on ionization of a weakly basic drug and the role of flow rate in elution of analyte.

Optimized Chromatographic Condition

Method validation: Method validation was initiated with system suitability i.e., six repeatable injections to

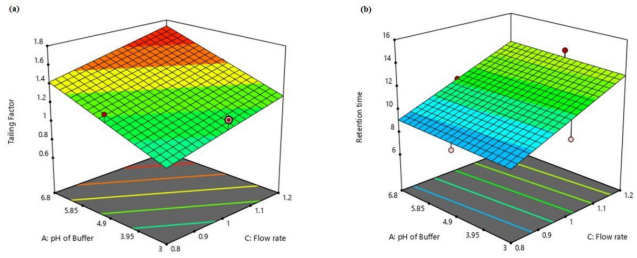

Figure 1: 3D counterplot analysis for variable factors F1 and F3 for (a) Tailing Factor and (b) Retention time.

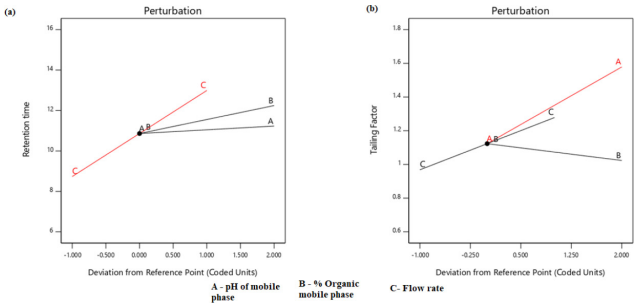

Figure 2: Perturbation plot analysis for (a)Retention time and (b) Tailing factor

Table 3: Summary of validation parameters.

Validation parameter	Acceptance criteria	ALK
System suitability	% RSD NMT 2.0	1.566
Linearity	Correlation coefficient (r^2) NLT 0.9990	0.999
Accuracy	% Recovery should be in between 98.0- 102.0%	
	Recovery levels	
	80%	100.09
	100%	99.61
	120%	100.29
Intra-day precision	% RSD NMT 2.0	0.359
Inter-day precision	% RSD NMT 2.0	0.807

calculate percent relative standard deviation. Linearity was established by analyzing the samples of various concentrations in linear range and the graph was plotted of concentration versus peak area to calculate the correlation coefficient (R^2) which was found to be 0.999. LOD-LOQ, repeatability, intra- day, inter-day precision, and robustness are tabulated in (Table 3) which complied with the acceptable limits. The optimized method was found to be sensitive and robust with no interference of extra peaks around the peak of an analyte represented in Figure 3. Also, the method was favorable for analyzing the samples of micro-dissolution as the mobile phase composition and other

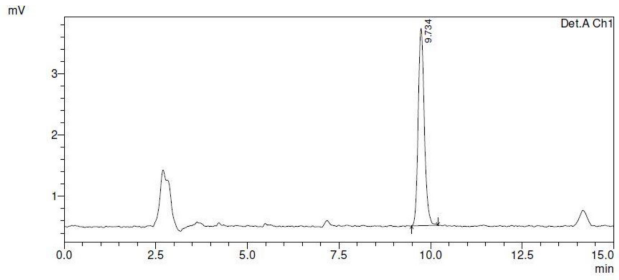

Figure 3: Representative chromatogram of ALK.

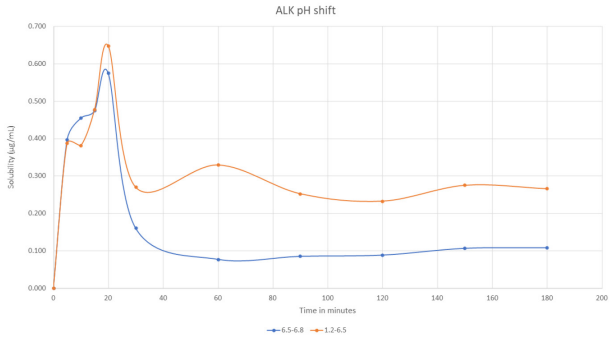

Figure 4: Effect of pH on solubility of ALK for FaSSGF pH 1.2→FaSSIF pH 6.5 transition and for FaSSIF pH 6.5→FaSSIF pH6.8 transition from *in vitro* micro dissolution test

conditions did not show interaction with biorelevant media ingredients used in the experiment.

In vitro micro-dissolution Test Analysis

Even though computational approaches are used for the determination of probable interactions, this *in-vitro* method is simple, accurate, and is relevant to the actual conditions, and gives results that are clinically co-relatable. Considering cancer as a critical disease and prescription of oral anticancer therapy a specific approach towards it, even a minimal variance in dose or pharmacokinetics would affect the therapeutic efficacy and clinical significance of the drug. In the current study, an attempt was made to determine the severity of possible pH-dependent absorption interaction of ALK in biorelevant conditions and pH shift. The experiment attempted to study the solubility of the drug which is a key factor with respect to the primary stage of the pharmacokinetic behavior of the drug i.e., absorption. The solubility was determined by the optimized RP-HPLC method developed using the QbD approach in fasted gastric and intestinal pH, which concluded that the ALK is not susceptible to changes in the pharmacokinetic pathway because of shift and elevation in gastric pH.[17-20]

The impact of pH shift in FaSSGF and FaSSIF mimicking the biorelevant conditions in stomach and intestine in fasting state with pH 1.2, 6.5 and 6.8 is displayed in Figure 4. The concentration of ALK (equivalent human dose) in FaSSGF had initial concentration of 0.387 µg/ml to 0.648 µg/ml in acidic pH conditions. After 20 min (considering gastric emptying period) on the transfer of FaSSIF the concentration declined drastically to 0.270 µg/ml and reached 0.266 µg/ml at the end of 180 min. In the case of FaSSIF pH 6.5→6.8 ALK, the concentration of ALK reduced from 0.397 µg/ml to 0.108 µg/ml at the end of 180 min. As a BCS class IV drug, ALK is known to have poor solubility which was found to be extremely decreased with elevation in pH from 1.2 → 6.5. The dissolution profiles of both the setups were plotted and compared to study the pH shift interaction. As compared with FaSSGF and FaSSIF it was observed that even though there is an increase in pH from 1.2 to 6.5, there is no significant impact on the solubility of ALK revealing that the difference in solubility might not show a significant effect on the absorption of ALK. The FDA studies report the solubility of ALK in various buffers individually in fasted and fed states but do not include a pH shift experiment demonstrating physiological pH transition in the human body. Since this shift in the body, as well as the elevation in gastric pH due to the administration of acid-reducing agents, are vital factors in this study, the results of this experiment should be considered for their significance in the determination of pH-dependent interaction.

CONCLUSION

The pharmacokinetic behavior of a drug is influenced by its physiochemical features. In the presence of acid-reducing agents and pH shift, weakly basic drugs of BCS class IV are observed to have pH-dependent solubility and varied pharmacokinetic characteristics. As a result, the study's findings suggest that concomitant usage of pharmaceuticals with oral anti-cancer therapies should be carefully prescribed or adjusted after assessing the effect and severity of any potential interaction. Being specific about the class of PPI, modification of pH and their prolonged duration of action are the prime factors that should be taken into consideration while the dosage of these drugs is suggested with oral anticancer agents or any high-risk medications. This study demonstrates the need for preliminary investigation and interaction profiling before clinical trials for new medical entities and medications that are weakly basic and have pH-dependent solubility. The study signifies the importance of drug-drug interaction studies and development of sensitive experimental procedures which would yield real-time results for the same. By modifying the experimental

sequences and analytical parameters to assess the risk of a pH-effect before clinical evaluation, this preliminary study can be taken to the next level to investigate the clinical implications, thereby providing direction for physical form development and formulation strategies in the primary stage of clinical development.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ALK: Alectinib; **AQbD:** Analytical Quality by design; **ANOVA:** Analysis of variance; **ATP:** Analytical target profile; **CQA:** Critical quality attributes; **CMA:** Critical method attributes; **DoE:** Design of experiments; **RT:** Retention time; **TF:** Tailing factor; **R1:** Response variable for Retention time; **R2:** Response variable for Tailing factor.

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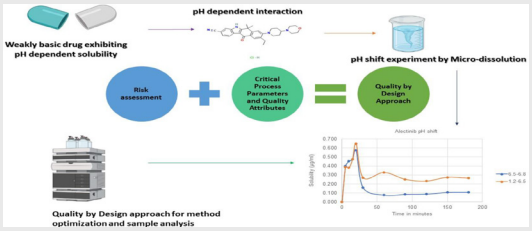
PICTORIAL ABSTRACT



SUMMARY

To assess the pH mediated interaction for alectinib, approach of micro-dissolution in biorelevant media was selected and to analyze the samples of the experiment, a sensitive and robust analytical method was developed by implementation of quality by design approach. Micro-dissolution assembly mimicked pH conditions of gastric (FaSSGF) and intestinal (FaSSIF) compartments where in solubility of alectinib in FaSSGF decreased from 0.648 μg/ml to 0.270 μg/ml whereas in FaSSIF it dropped down from 0.574 μg/ml to 0.108 μg/ml at the end of the micro-dissolution experiment with pH shift. Although there was no significant difference observed in solubility profile of alectinib because of pH shift conditions, the experiment lays a foundation of a technique to analyze the risk of pH dependent interaction by *in-vitro* method and establish the correlation of the same with absorption and pharmacokinetic properties of the drug.

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