



# Assessment of pH-shift drug interactions of palbociclib by *in vitro* micro-dissolution in bio relevant media: An analytical QbD-driven RP-HPLC method optimization

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## ABSTRACT

In the present study, pH-dependent solubility and dissolution of Palbociclib (PB), a weakly basic cyclin-dependent kinase 4/6 inhibitor was investigated by application of analytical quality by design (QbD) approach to an reverse phase high performance liquid chromatography (RP-HPLC) method. An integrated analytical QbD approach for quantification of PB using RP-HPLC was designed by three-level three factorial, Box–Behnken design with numerical and graphical optimization. *In vitro* micro-dissolution, pH-shift experiments in bio-relevant media were carried out to predict PB's pH-dependent drug-drug interaction (DDI) behavior. RP-HPLC method developed utilizing a Box–Behnken three-stage three factorial design was shown to be specific towards PB. The optimized method leads to effective and faster chromatographic separation of PB with lower retention time value together with a satisfactory peak symmetry and low peak tailing. Based on *in vitro* micro-dissolution studies, it was observed that PB has a typical weak base pH-dependent solubility characteristic and dissolution behavior with its release ranging from 98.96% to 102.66% in simulated gastric fluid pH 1.2 before dropping to 43.43% by addition of fasted state simulated intestinal fluid pH 6.5. Overall, our findings demonstrated that *in vitro* micro-dissolution approaches may accurately predict the intensity of pH-dependent DDI and that the use of these techniques prior to clinical DDIs studies might allow for adequate prediction of pH-dependent drug absorption *in vivo*.

## INTRODUCTION

Palbociclib (PB) is an orally administrated, highly selective inhibitor of cyclin-dependent kinase 4/6 (CDK). PB is authorized for the use in conjunction with letrozole as a first line endocrine-based treatment for postmenopausal women with estrogen receptor positive, human epidermal growth factor receptor 2 (HER2)-negative advanced breast cancer (Ehab and Elbaz, 2016). The drug is available in the

market is formulated as hard capsules containing 75, 100, or 125 mg of PB as active ingredient under the brand name IBRANCE. As mentioned by the innovator, PB belongs to Biopharmaceutics Classification System Class II molecule (low solubility and high permeability) (Parylo *et al.*, 2019).

Oral cancer chemotherapy with protein kinases such as CDK inhibitors involves administration of a variety of supportive medications such as analgesics, anti-helminthics, and acid-reducing agents (ARA), such as proton pump inhibitors (PPIs) (Cazzaniga *et al.*, 2019). Supportive medicines are prescribed to alleviate the adverse effects of anticancer treatments and to address comorbidities associated with chemotherapy. PPIs are presently utilized in the top 20% of drugs worldwide to decrease gastroesophageal reflux and toxicity-related comorbidities

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associated with cancer therapy (Sharma *et al.*, 2019). In clinical practice, PPIs significantly elevate stomach pH, which may impair the absorption of other therapeutic agents, particularly those that are weakly basic and show low solubility at higher pH. This type of pH-dependent drug-drug interaction (DDI) with PPIs decreases systemic drug exposure and has a significant impact on these medicines' therapeutic outcomes (Patel *et al.*, 2020; Zhang *et al.*, 2014). After 1–3 hours, a single oral dose of esomeprazole or lansoprazole achieves effective serum concentration and raises the stomach pH in the majority of patients from 2.0 to above 6.0. Increased stomach pH produced by ARAs has been found to decrease the bioavailability and exposure of weakly basic drugs with pH-dependent solubility (Gay *et al.*, 2017). Early identification of such physicochemical factors is critical for predicting the drug's pharmacodynamic behavior (*in vivo*), and the establishment of *in vitro in vivo* correlation. In preliminary solubility analysis, 125 mg of PB was found to be completely soluble at equilibrium in 250 ml of dissolution media at pH < 4.3 with solubility surpassing 0.5 mg/ml, as observed in new drug application report submitted by innovator to U.S. Food and Drug Administration. However, the solubility fell substantially below 0.5 mg/ml when pH was raised over 4.5, which is the stomach pH commonly reached after administration of ARA (Sun *et al.*, 2017).

Experimental and computational techniques, such as *in vitro* dissolution studies in various biorelevant media, solubility profiling in gastrointestinal pH, and pharmacokinetic data, have been developed to either anticipate or eliminate the danger of pH impact. *In silico* techniques that frequently incorporate dissolution/absorption simulation and modelling with inputs from *in vitro* biorelevant dissolution have been used in drug discovery and development to simulate the effects of solubility and dissolution on drug absorption (Hamed *et al.*, 2016; Mitra *et al.*, 2020; Varma *et al.*, 2012; Zhu *et al.*, 2016). The ideal experimental model would include physiologically acceptable volumes of relevant dissolving media, as well as fluid transfer and pH changes in a dynamic process. To the authors' knowledge, there are relatively few published researches that consider the pH-effect risk across a variety of drug category, with a focus on predicting the magnitude of the risk and understanding the essential pharmacological features that drive it. The pH-modifying drug's complex effects, fluctuating pH, and transit time make clinical pH effects challenging. Due to the constraints of clinical studies, the DDI cannot be assessed with all ARA categories. Dissolution properties of chemically diverse compounds show a wide range of clinical pH-effects. To accomplish this, *in vitro* micro-dissolution assay as described by Mathias *et al.* (2013) was used to investigate dissolution and precipitation kinetics of PB by mimicking the effects of a high dosage of PPI (Mathias *et al.*, 2013). The purpose of this work was to determine the pH-dependent solubility and absorption of PB in biorelevant simulated media using a micro-dissolution technique. The use of biorelevant media with a pH shift effect to replicate the circumstances shown in the GI tract has been proposed for determining the dissolution and solubility of drugs *in vivo* and predicting their absorption. Built with suitable simulated media and hydrodynamics are beneficial in formulating strategies and establishing *in vitro in vivo* correlation, which will result in a decrease in animal experiments, bioavailability, and bioequivalence studies (Klein, 2010). The clinical pH impact

was also seen to be linked to *in vitro* dissolution experiment's pH effect. The ratio of human  $C_{max}/AUC$  with and without ARA will be calculated from literature and experimental *in vitro* solubility data was compared. This ratio helps to determine the impact of pH on drug solubility and can be correlated between *in vitro* dissolution profile and *in vivo*  $AUC_{inf}$  or  $C_{max}$  data.

Since drug solubility is the most important aspect of this experiment, the developed analytical method should be sensitive enough to detect relatively minor changes in drug solubility accurately. Design and development of analytical methods are critical in order to quantify minor solubility changes in drug solution. Various analytical techniques based on different formulations and biological fluids are available in the literature for PB quantification. However, the described analytical techniques' sensitivity, reliability, and use of a high-cost organic solvent, as well as high-end instrumental analysis utilizing mass spectrometry, among other factors, limited their applicability (Al-Shehri *et al.*, 2020; Dange *et al.*, 2018; Posocco *et al.*, 2020). Furthermore, PB is a hydrophobic and nonpolar aromatic hetero polycyclic molecule, so minor changes in critical method parameters (CMPs) such as pH or mobile phase organic content have a significant influence on the analytical method. As a result, using the analytical quality by design (QbD) approach, a highly specific, accurate, efficient, and robust reverse phase high performance liquid chromatography (RP-HPLC) technique for quantification of PB was established in the present investigation. In the development of analytical methods, analytical QbD was used to ensure that the desired development approach was adequate. It aids in identifying important quality features that have a major influence on the final outcome, in addition to knowing particular variables (Fukuda *et al.*, 2018; Peraman *et al.*, 2015a). To the best of authors' knowledge, this is the first study to disclose full development as well as optimization of PB by RP-HPLC method using a QbD approach, which will provide a wide range of analytical applications as well as a method operable region for overcoming out-of-specification findings.

## MATERIALS AND METHODS

### Chemicals and reagents

PB was kindly gifted by MSN laboratories private limited, Hyderabad. Simulated intestinal/gastric fluid powder used to make fasted state simulated intestinal fluid and fasted state simulated gastric fluid was acquired from Biorelevant.com (Croydon, Surrey, United Kingdom). Buffers and salts like potassium dihydrogen phosphate, sodium phosphate dibasic, hydrochloric acid, sodium chloride, and orthophosphoric acid were supplied by Merck. Water was purified by Milli-Q UV plus systems (Millipore Co., Bedford, MA). All remaining chemicals, if used, were of analytical grade.

### Instrumentation

#### HPLC system

The optimized chromatographic method was implemented utilizing a Shimadzu Prominence HPLC system with a 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. The data analysis was performed using LC solution software.

### Software

Design-Expert software (Version 12.0; Stat-Ease, Minneapolis, MN) was used for statistical analysis.

### Chromatographic conditions

The chromatographic method optimization was performed with integrated analytical QbD technique. The chromatographic separation was achieved using a Unison RP C8 (150 × 4.6 mm, 5 μ) column as the stationary phase and an optimized mobile phase consisting of mixture Acetonitrile, methanol and 10 mM ammonium acetate (pH 4.5) in the ratio 50:10:40. The column temperature was maintained at 25°C. The injection volume was 20 μl, with a 10-minute run time with an optimized 0.8 ml/minute flow rate at detection wavelength of 264 nm.

### Analytical design of experiment (DoE) method optimization

#### Defining analytical target profile (ATP) and critical analytical attributes (CAAs)

The first stage in developing a QbD-based RP-HPLC technique is to design an ATP (Karmarkar *et al.*, 2011; Krishna *et al.*, 2016; Peraman *et al.*, 2015b). The analytical method's quality, safety, and applicability will be assured by defining ATP. Different CAAs might be chosen to fulfil the ATP criteria. Out of these CAAs, the ATPs selected for this technique are (i) drug retention time (*Rt*) and (ii) tailing factor (*Tf*), both of which have a significant impact on the method's performance.

#### Risk assessment for screening of factors affecting the method development

A risk assessment study was initially conducted to determine the possibility of risks or failures. The Ishikawa fish-bone model was used to create a cause-effect link between critical material attributes (CMAs) and critical process parameters. It is utilized to discover the probable parameters that might have tremendous potential to impact the detected CAAs and are classified as CMPs.

#### Method development and optimization using Box-Behnken design (BBD)

BBD with three variables was designed to optimize CMA of analytical method (Table 1). Based on the screening results, three independent variables were chosen for further optimization: mobile phase pH (X1), % organic within mobile phase (X2), and flow rate (X3). The design matrix provided 15 trial runs utilizing a combination of CAA. All tests were carried out at a drug concentration of 10 μg/ml of PB. Analysis of variance (ANOVA)

statistical analysis and response surface analysis were used to further evaluate the model created by design using DoE software.

### METHOD VALIDATION

The developed analytical method was validated as per ICH (International Conference of Harmonization) Q2 (R1) guidelines (Harron, 2013) over the parameters of specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, and precision.

### Micro-dissolution pH shift studies of PB

The *in vitro* micro-dissolution test is divided into two stages—gastric and intestinal test. Considering clinical dose of PB as 125 mg, an equivalent dose of PB was added to 14 ml of pH 1.2 FaSSGF buffer taken in a glass beaker, and samples were withdrawn for 20 minutes at various time intervals. To mimic the pH change from stomach to small intestine, 21 ml of pH 6.5 fasted state simulated intestinal fluid (FaSSIF) buffer was added to the same beaker, and sampling was performed for another 180 minutes. A similar arrangement was created in another beaker with drug being added to 14 ml of pH 6.5 FaSSIF buffer, and sampled for 20 minutes at various time intervals before adding 21 ml of pH 6.8 FaSSIF buffer to the same assembly and sampled at intervals till 180 minutes. To maintain sink conditions, the withdrawn samples were replaced with equal quantity of fresh buffer. The two setups were kept stirring continuously at 350 rpm throughout the experiment with the aid of magnetic stirrers. Sampling was performed at time intervals of 0, 2, 5, 10, 15, 20, 21, 25, 30, 60, 90, 120, 150, and 180 minutes. The withdrawn samples were centrifuged at 10,000 rpm for 15 minutes to separate the precipitated drug and the supernatant was analyzed using a novel and optimized RP-HPLC technique supplemented by analytical QbD approach.

## RESULTS AND DISCUSSION

### Analytical DoE method optimization

To design an analytical technique that is both effective and sensitive, experiments with various combinations of mobile phase solvents, buffers, and stationary phases were undertaken. The buffer system is evaluated at several pH values ranging from 3 to 6 combining phosphate and acetate buffers. These combinations indicated that acetonitrile with 10% methanol as a mobile phase modifier and 10 mM ammonium acetate as the aqueous phase suited the mobile phase the best. It provided enhanced selectivity with peak symmetry and a low tailing factor, as well as increased sensitivity and theoretical plate count.

### Risk assessment

Using a three level three factorial BBD, the impact of three independent method factors, mobile phase pH (X1), %

Table 1. Independent variables and their levels.

Factor	Name	Minimum	Maximum	Coded low	Coded high	Mean	Std. Dev.
A	pH	3	6	-1 ↔ 3.00	+1 ↔ 6.00	4.50	1.13
B	% Organic	60	80	-1 ↔ 40.00	+1 ↔ 60.00	70.00	7.56
C	Flow rate	0.8	1.2	-1 ↔ 0.80	+1 ↔ 1.20	1.00	0.15

organic within the mobile phase (X2), and flow rate (X3), on two responses variables drug  $R_t$  (R1) and  $T_f$  (R2) of PB was assessed. The results were assessed by statistical analysis using Design Expert 12.0 software, and the proposed chromatographic experimental conditions by DoE were carried out using HPLC. The Ishikawa fish-bone diagram was used to do initial risk assessments for CMA of techniques like column chemistry, temperature, and HPLC system. The Ishikawa fish-bone diagram summarizes the possible risk variables that have a significant impact on the ATP of methods. The model's riskiest CMA was identified using a Pareto chart. Figure 1A shows that all three independent factors have a greater impact on PB retention, with factor X1: mobile phase pH and X2: % organic being the most relevant factors for PB tailing factor (Fig. 1B). The  $T_f$  has been impacted by the pH of the aqueous mobile phase, since acidic pH has no effect on the ionization of the silanol groups of the C18 stationary phase and increases the retention of basic drugs.

### Statistical analysis

The DoE design was validated using ANOVA, which revealed that the model was statistically significant with a  $p$ -value of  $<0.001$ . The responses R1 and R2 have model  $F$ -values of 432.28 and 13.51, respectively, indicating that the model is significant. Design Expert software recommended a quadratic model with process order for major effects and a factorial design for both responses as the best fitting model. Individual model variables

such as mobile phase pH and % organic content had  $p$ -values  $>0.005$ , indicating that these factors had a substantial influence on response R1. Similarly, the  $p$ -value for each individual model for response R2 is more than 0.005 for all independent factors, showing that these CMA have a substantial influence on method CQA (Table 2). Table 3 shows that the predicted  $R$ -squared for all responses is reasonably close to the adjusted  $R$ -squared values, with a difference of less than 0.2 in each case. The ratio of 69.62 to 11.33 indicates that the signal is acceptable (ratio  $>4.0$ ). The model's desirability was estimated to be 1.

### Perturbation plot analysis and 2D response surface plot analysis

Perturbation plots and contour plots were used to examine the visual impact of independent factors and their interactions on the response variables. Due to the existence of quadratic components in the regression model, the pH of the mobile phase has a considerable impact on tailing factor, and the output response seen in the contour plot is a curvature response surface. However, the pH of the mobile phase has less effect on retention time; therefore the model is expanded to include both quadratic and linear factors. The % of organic moiety in the mobile phase has a significant impact on the tailing factor but only a little impact on the drug's retention time. The tailing factor was unaffected by the mobile phase flow rate (Fig. 2). According to perturbation plots with smallest variation from reference point, the independent factor pH has a negative

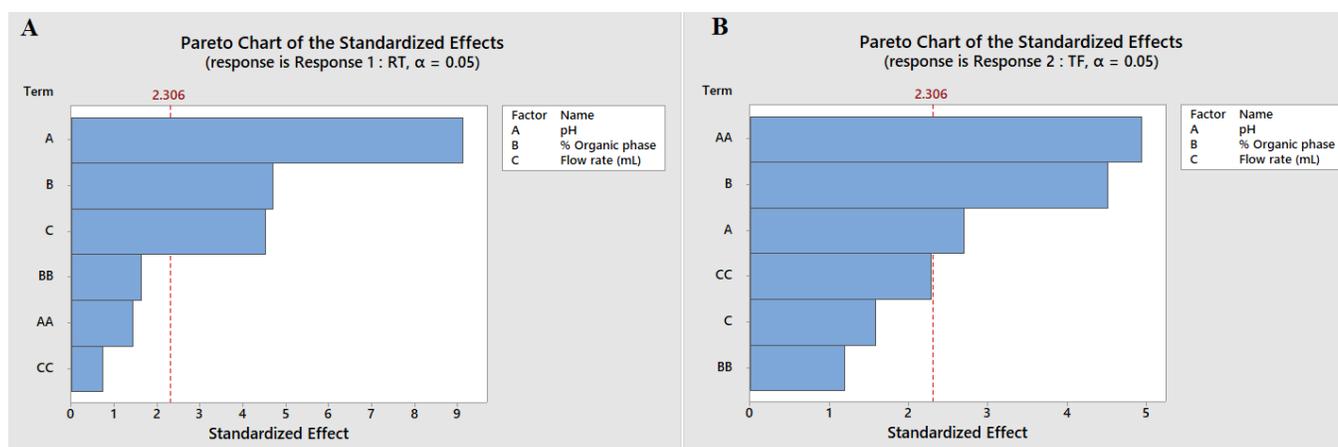


Figure 1. Pareto plot of analysis for responses R1 and R2.

Table 2. ANOVA results for responses R1: Retention time and R2: Tailing factor.

Source	Sum of squares		F-value		p-value	
	R1 ( $R_t$ )	R2 ( $T_f$ )	R1 ( $R_t$ )	R2 ( $T_f$ )	R1 ( $R_t$ )	R2 ( $T_f$ )
Model	56.800	2.9100	432.28	13.51	$<0.0001$	0.0052
A-pH	0.264	0.0592	18.08	2.47	0.0081	0.1767
B-% Organic	0.279	0.1633	19.17	6.82	0.0072	0.0476
C-Flow rate	0.320	0.0006	21.94	0.02	0.0054	0.8792
AB	2.320	0.2098	158.87	8.76	$<0.0001$	0.0315
AC	0.702	6.25E-06	48.10	0.00	0.0010	0.9877
BC	0.179	0.0206	12.310	0.85	0.0171	0.3964
Residual	0.073	0.1198	-	-	-	-
Lack of fit	0.073	0.1088	-	6.64	-	0.1336

**Table 3.** Model Fit summary for the responses R1: Retention time and R2: Tailing factor.

Component	R1 (Rt)	R2 (Tf)
Std. Dev.	0.12	0.15
Mean	5.07	2.17
% CV	2.38	7.15
$R^2$	0.99	0.96
Adjusted $R^2$	0.99	0.88
Predicted $R^2$	0.97	0.69
Adequate precision	69.62	11.33

correlation, but % organic phase and flow rate have a positive correlation towards tailing factor and PB retention time (Fig. 3).

**Optimized chromatographic condition**

After examining these interactions using contour plot analysis, a method operable design region (MODR) was established. Statistical analysis verification was used to determine the optimal experimental settings and model's desirability was found within the acceptance limit. The desirability function of Derringer was utilized to optimize two parameters to distinct goals. Desirability function, which generates  $D$  values between 0 and 1. In Figure 4, the response surface plot corresponding to the

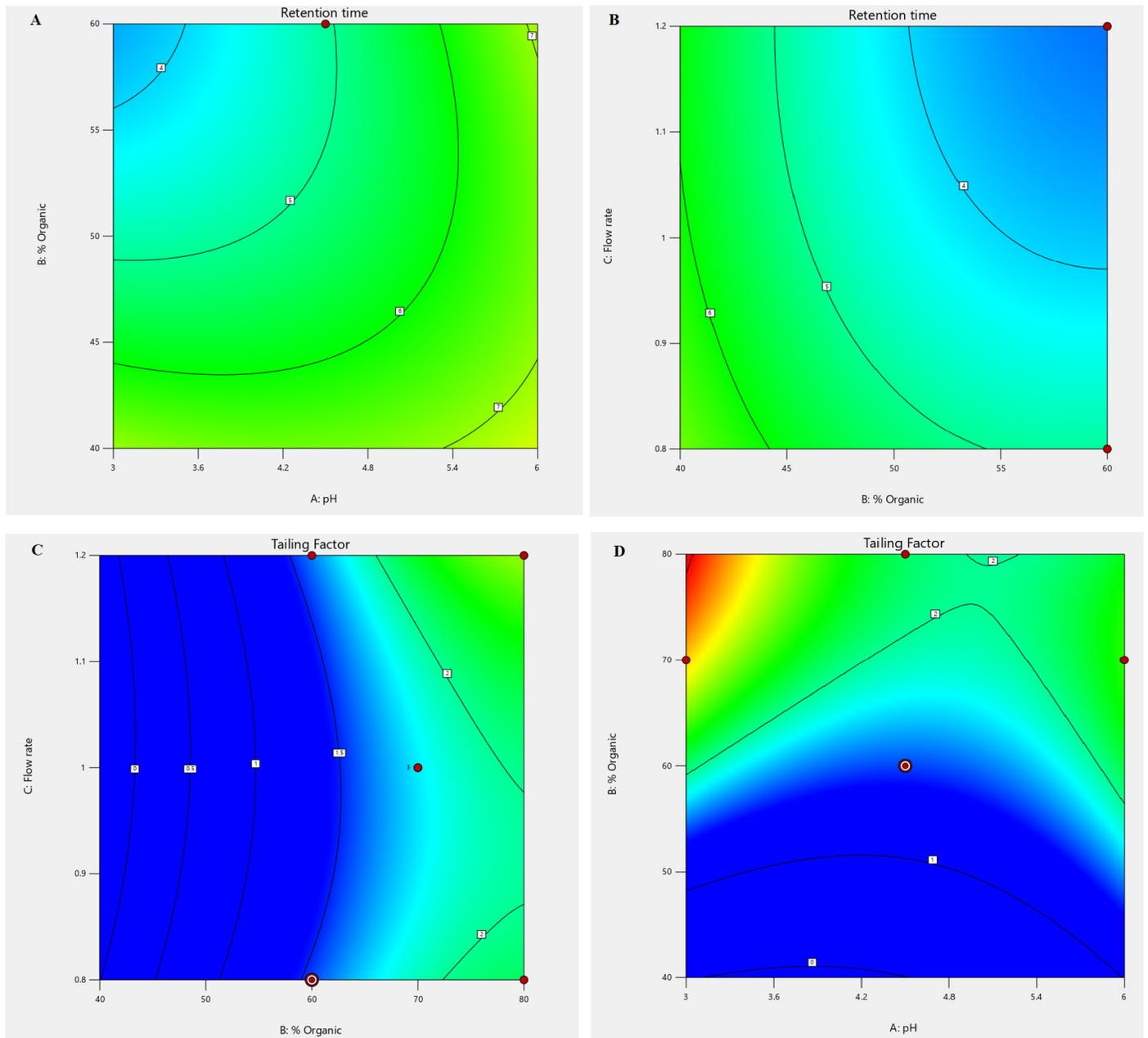


Figure 2. Contour plot of interaction effect of factor X1 and X2 on responses.

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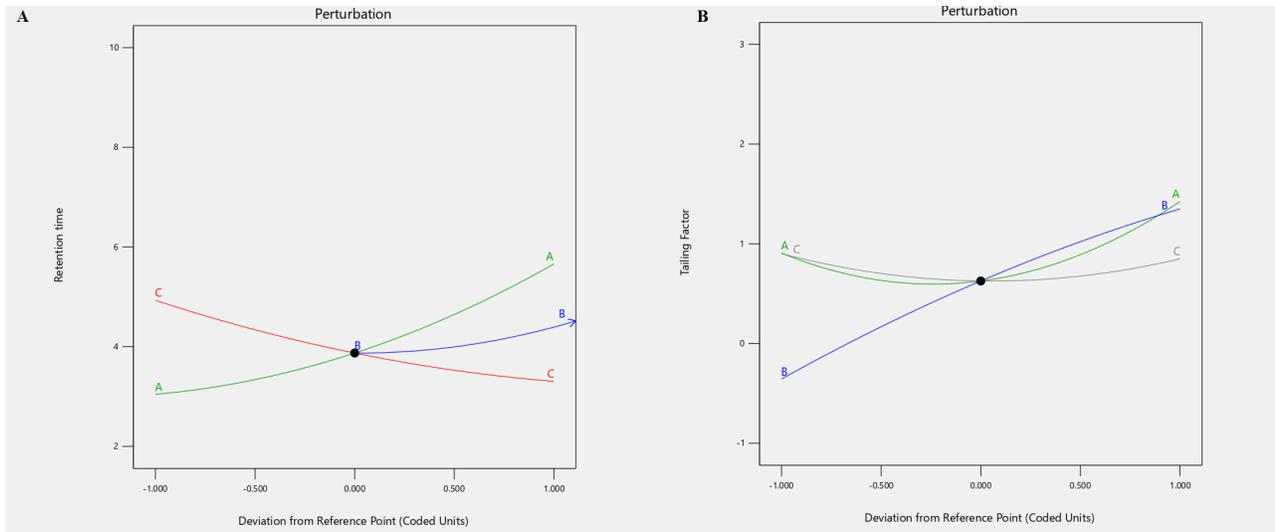


Figure 3. Perturbation plot of analysis for responses R1 and R2.

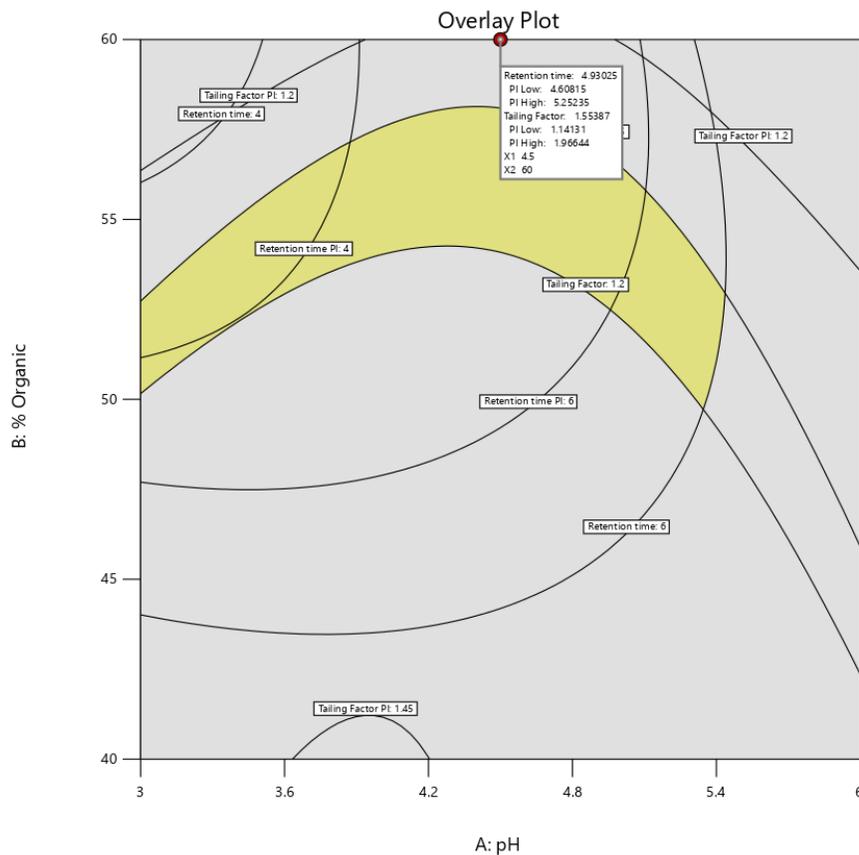


Figure 4. Design space and operable region for the independent factors.

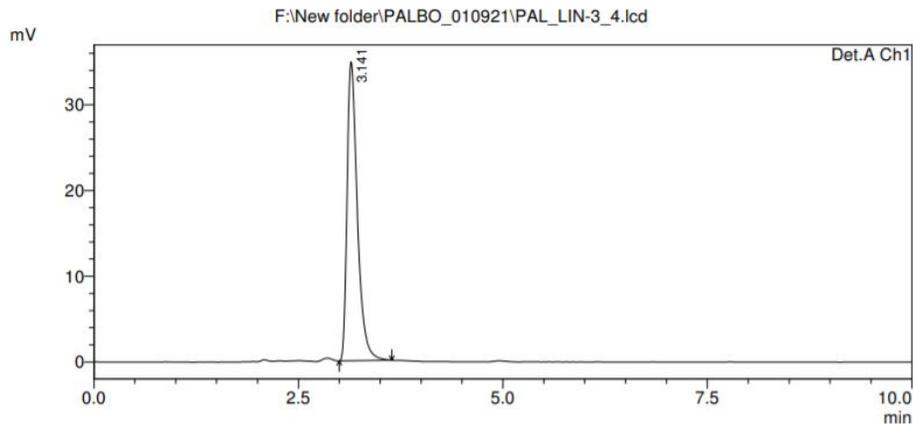
*d*-value with optimized area is indicated in yellow. Desirability coordinates denoted optimal circumstances. Working inside the MODR observed region will yield satisfactory results for our targeted criteria. In Table 4, the anticipated and actual results for responses R1 and R2 are summarized. Figure 5 represents an optimized chromatogram of PB by RP-HPLC method.

## METHOD VALIDATION

System suitability was performed to assess the reproducibility of the chromatographic method. relative standard deviation (%RSD) for peak area of six repeated injections of PB was found to be 0.59%. The developed method was found to be highly specific as there was no interfering peaks around the retention time of PB, as shown in Figure 5. The peak purity

**Table 4.** Predicted and actual point prediction at two-sided 95% confidence interval.

Response	Predicted mean	Observed	Std. Dev.	SE mean	95% CI low for mean	95% CI high for mean
R1 (Rt)	4.9302	3.14	0.1208	0.1046	4.6612	5.1992
R2 (Tf)	1.5538	1.42	0.1547	0.1340	1.2093	1.8984

**Figure 5.** Representative chromatogram of PB.

was found to be greater than the peak purity threshold, indicating that PB in both standards as well as micro dissolution processed samples was pure, and there was no interference of impurities, biorelevant dissolution components or degradants. The linearity for PB was established, and the concentration versus peak area calibration curve was plotted as linear regression analysis. The method was found to be linear for PB over the concentration range of 0.1–150 µg/ml. The correlation coefficient ( $R^2$ ) was found to be 0.999. The LOD and LOQ for PB were found to be 0.9 and 2.71 µg/ml, respectively. The mean percent recovery for PB was found to be within the limit of 98%–105% and the recovery data is shown in Table 5. The precision of the given method was determined by intermediate precision and repeatability. The results were reported in terms of %RSD as shown in Table 6. The method was found to be robust while deliberately modifying mobile phase, wavelength, column temperature, flow rate. %RSD was found to be less than 2%.

#### Micro-dissolution pH shift studies of PB

The effect of pH change on PB solubility and dissolution profile in biorelevant medium is shown in Figure 6 (FaSSGF and FaSSIF). FaSSGF was used to dissolve the initial drug concentration, where solubility of PB reached 0.5 mg/ml. As the pH of the experimental setup was increased from 1.2 to 6.5, the solubility of the drug declined drastically from 0.7 to 0.07 mg/ml. The concentration of PB in the micro-dissolution assembly steadied at 0.068 mg/ml after 180 minutes. The solubility of PB in the second set up, FaSSIF pH 6.5 to 6.8 was observed to be 0.04 and 0.02 mg/ml, respectively, while equilibrating at 0.022 mg/ml at the end of 180 minutes. The substantial drop in drug solubility after the pH shift from FaSSGF buffer highlights the influence of pH on drug solubility and validates the drug's pH sensitivity. With initial poor solubility in FaSSIF, the solubility dips even

further as the pH rises, indicating a change in solubility as the pH increases. Because the change from stomach to intestinal pH is rapid in this test, intestinal supersaturation occurs quickly upon transfer from FaSSGF pH 1.2 to FaSSIF pH 6.8. Supersaturation causes immediate precipitation and a reduction in its inherent solubility, which may be accurately determined by this *in vitro* technique at the maximum dosage. The entire research is done utilizing fasting simulation fluid to connect the dosage regime with PPIs. FaSSGF is a dissolving medium that properly mimics the contents of the stomach in a fasting state and contains physiologically relevant amounts of pepsin, bile salts, and lecithin to create a surface tension comparable to that observed *in vivo*. As PB has no dietary impact on medication absorption (Fotaki and Vertzoni, 2010), FaSSIF 6.5 has been effectively used to predict exact pH shift effects on PB absorption towards basic pH. The solubility of PB in several buffer pH ranges has been provided by the inventor, but because PB is a weakly soluble drug, it would not have been feasible to predict *in vivo* performance of PB in an increased stomach pH without biorelevant dissolving media. The ratio of the dissolution AUC profiles (AUC of FaSSIF pH 6.5 to FaSSIF pH 6.8/ AUC of FaSSGF pH 1.2 to FaSSIF pH 6.8) was used to calculate the risk of *in vitro* pH impact. This ratio aids in determining the effect of pH on drug solubility and may be linked between *in vitro* dissolution profiles and *in vivo* data. The estimated clinical pH effect ratio of PB in a human clinical study with and without ARA ( $AUC_{\text{treated}} / AUC_{\text{untreated}}$ ) is reported as 0.205 and 0.367 for  $C_{\text{max}}$  and  $AUC_{\text{inP}}$  respectively. The micro-dissolution experiment yields a value of 0.532 for the *in vitro* pH-effect risk that illustrates a parallel link between *in vitro* dissolution and clinical pH impact. The pH impact, *in vitro* and the clinical  $C_{\text{max}}$  pH effect are linearly related. In practice, the smaller the ratio, the more significant is the pH impact. A ratio of 1 indicates that there is no clinical impact of pH. Thus, PB

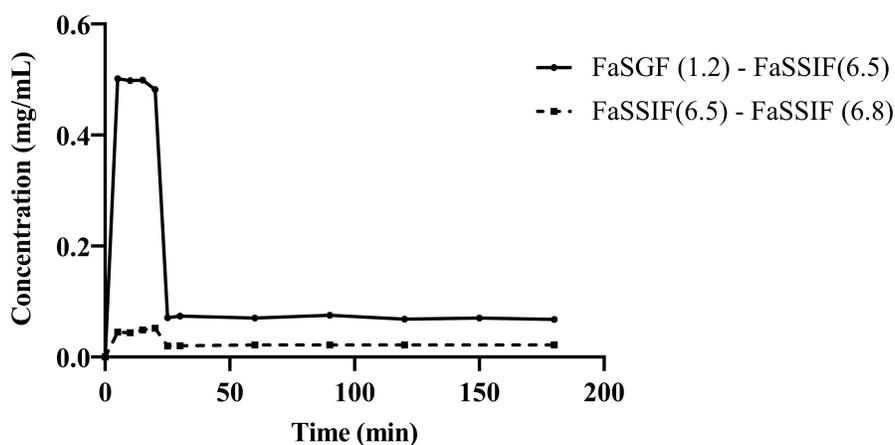
**Table 5.** Accuracy and % recovery of PB.

Accuracy level, %	Sample	% Recovery	Mean recovery	Std. Dev.	%RSD
50	1	98.61	97.80	1.0555	1.0792
	2	98.19			
	3	96.61			
100	1	105.01	104.62	0.3837	0.3668
	2	104.24			
	3	104.60			
150	1	100.27	100.07	0.6180	0.6176
	2	100.57			
	3	99.38			

**Table 6.** Interday and intraday precision of PB at quality control levels.

Sample ID	Day 1			Day 2		
	LQC	MQC	HQC	LQC	MQC	HQC
1	4.9303	10.5010	150.4054	5.0002	10.9264	150.5759
2	4.9096	10.4243	150.8500	4.8772	11.0615	148.6454
3	4.8303	10.4597	149.0689	4.9369	10.8885	148.3085
4	5.0157	10.7387	150.4054	4.8635	10.9250	151.5513
5	5.0570	10.7659	150.8500	4.9110	11.0951	149.4049
6	4.9372	10.5368	145.8121	4.9143	10.7229	151.1309
Mean	4.9467	10.5711	149.5650	4.9172	10.9366	149.9360
Std. Dev.	0.0803	0.1457	1.9517	0.0486	0.1334	1.3449
%RSD	1.6230	1.3780	1.3049	0.9886	1.2196	0.8970

LQC: Lower quantity control, MQC: Middle quantity control, HQC: Higher quantity control.

**Figure 6.** Kinetics of pH dependant dissolution from *in vitro* micro-dissolution test for PB

exhibits a significant pH-dependent absorption and DDI in both *in vitro* and clinical  $C_{max}$  pH-effects. Thus, the pH shift effect causes alterations in solubility which in turn has considerable influence on PB absorption, resulting in clinically significant interaction and poor bioavailability.

## CONCLUSION

The impact of pH on the solubility of the drug is investigated in this experiment, as solubility is a crucial factor in the

early stage of pharmacokinetic behavior, i.e., absorption. Despite the fact that computational approaches are used to predict potential interactions, this *in vitro* procedure is simple, accurate, and relevant to real-world circumstances, as well as produces accurate results. When the drug's solubility value across the physiological pH range (1.2–6.8) is less than dose/250 ml, FDA requires an *in vivo* clinical investigation (Patel *et al.*, 2020; Zhang *et al.*, 2014). Early understanding of pH-effect risk assessment is critical to influencing clinical trial design to include a pH-modifier treatment arm and

obtaining a clinical report on the pH impact of high-risk medications. PPIs generally have a longer duration of acid secretion suppression effect than H2 blockers and local acting antacids, and interfere with the intestinal absorption of weakly basic drugs to a greater extent. Use of PPIs may be considered a worst-case scenario in the *in vivo* evaluation of the clinical pH effect on novel investigational drugs. The present study comprehensively characterizes pH-dependent DDIs using an *in vitro* micro-dissolution test, which is a new, efficient, practical, and active pharmaceutical ingredient sparing technique for predicting dissolution and precipitation kinetics. This study highlights the critical need of conducting early research and interaction profiling prior to initiating clinical trials for new molecular entities and medications that are weakly basic in nature and exhibit pH-dependent solubility. While it was applied retrospectively to this compound after the effect was observed in a clinical setting, the same experimental sequences and analytical reasoning could be used to assess the risk of a pH-effect prior to clinical evaluation, thereby providing direction for physical form development and formulation strategies early in clinical development.

The implement of QbD paradigms has been entering the industrial, academic, and government sectors at an alarming rate with the objective to gain full process and product knowledge. As a consequence, for sample analysis of PB randomized order integrated QbD trials for estimate were carried out to decrease the influence of uncontrolled factors that could bias the outcome. Implementation of QbD technique has led to more robust method which can generate consistent, dependable, and quality data throughout the process and also saves time & expense.

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#### CONFLICT OF INTEREST

The authors declare no conflict of interest.

#### AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

#### DATA AVAILABILITY

All data generated and analyzed are included within this research article.

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#### ETHICAL APPROVAL

The study does not have involvement of experiments on humans or animals.

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