RESEARCH ARTICLE

Implementation of Quality by Design Approach for Analytical Method Development and Validation for Estimation of Prucalopride Succinate in the Bulk and Solid Dosage Form

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Received: 10th August, 2020; Revised: 15th September, 2020; Accepted: 19th October, 2020; Available Online: 25th Decemeber, 2020

ABSTRACT

Quality by design (QbD) based analytical method development and optimization has proved to be of immense importance in the pharmaceutical industry. It has been used in the drug development stage to identify and optimize critical parameters and evaluate its effects on the critical quality attributes. An attempt was made to develop and validate a reverse phase-high performance liquid chromatography (RP-HPLC) method for the determination of Prucalopride succinate in bulk form and tablet formulation using QbD approach. The Box-Behnken design was used for screening where the effect of buffer pH, % ACN and flow rate (critical parameters) on retention time, number of theoretical plates (NTP), and symmetry factor (critical quality attributes) was evaluated. The developed method was validated according to guidelines of the International Conference on Harmonization (ICH). An isocratic mode of elution comprising of 0.01M ammonium formate with pH 5.0 and acetonitrile (ACN) in a ratio of 77:23 v/v as mobile phase at a flow rate of 1.0 mL/min over Inertsil ODS C_{18}, 250 mm x 4.6mm x 5µmcolumn at 27°C temperature was maintained. A Photo-diode array (PDA) detector was used for detection at 226nm. The method showed a linear response with correlation coefficient value of 0.9987 for Prucalopride succinate which was within the limit (correlation coefficient ≥ 0.995). The limit of detection and limit of quantification (LoQ) was found to be 0.2 µg/ml and 0.8 µg/ml, respectively. Forced degradation studies were also carried out.

Keywords: Prucalopride succinate, QbD, RP-HPLC, forced degradation, ICH, validation.

International Journal of Pharmaceutical Quality Assurance (2020); DOI: 10.25258/ijpqa.11.4.10

How to cite this article: Chawathe AS, Hamrapurkar PD. Implementation of Quality by Design Approach for Analytical Method Development and Validation for Estimation of Prucalopride Succinate in the Bulk and Solid Dosage Form. International Journal of Pharmaceutical Quality Assurance. 2020;11(4):510-517.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Quality by design (QbD) was integrated into current good manufacturing practices (cGMPs) by the United States Food and Drug Administration (FDA) in 2004. Since then, it has become an important part of analytical method development in the pharmaceutical industry.[1] It has been extensively used for the optimization of various drug development processes, which involves use of tools like experimental design space and response surface modeling. The QbD approach often leads to a better understanding of the relationship between different variables, which helps in building an efficient and flexible system. Unlike the quality by testing (QbT) approach, QbD essentially focuses on building the quality into the process design rather than testing into the analytical method's final results. It involves identifying critical parameters and determining their interaction effects on the system's response parameters, thereby establishing the optimized design space for the method.

Prucalopride succinate is a novel enterokinetic compound and is the first representative of the benzofuran class.[2] Prucalopride succinate is a potent, selective, and specific serotonin 5-HT4 receptor (5-HT4-R) agonist. It is used to treat adults with chronic idiopathic constipation (CIC) in whom laxatives have failed to provide adequate relief.

Chemically it is 4-amino-5-chloro-N-[1-(3-methoxypropyl) piperidin-4-yl]-2,3-dihydro-1-benzofuran-7-carboxamide; butanedioic acid. The molecular formula is $C_{22}H_{32}ClN_3O_7$. It has a molecular weight of 485.96 g/mol. Chemical structure of Prucalopride succinate is shown in Figure 1. It is white to an off-white powder. It is slightly soluble in acetonitrile, soluble in methanol and freely soluble in water and dimethyl sulphoxide.

Literature survey revealed that few analytical methods like HPLC, ultra-high performance liquid chromatography (UHPLC) and bioanalytical method involving use of liquid chromatography-tandem mass spectrometry (LC-MS/MS) are

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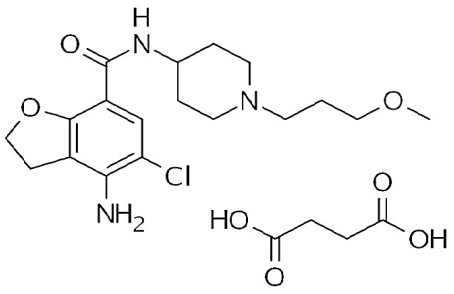

Figure 1: Chemical structure of Prucalopride succinate

available for estimation of Prucalopride succinate from bulk drugs and dosage forms.[3-5]

An attempt was made to develop and validate a sensitive, precise and stability indicating RP-HPLC method for Prucalopride succinate using QbD approach.

MATERIALS AND METHODS

Chemicals and Reagents

Prucalopride succinate was procured from Symed Labs, Hyderabad as a gift sample. The HPLC grade water (millipore), acetonitrile (finar), ammonium formate (SDFCL), and formic acid (Merck) were used.

Equipment

The analysis was carried out using Waters Alliance 2695 HPLC system with Waters 2996 PAD and Empower Pro software as an integrator. Also, analytical balance (Citizon CY 204), pH meter (Thermo Electron Company Orion 420A+), and a sonicator (Ultra sonicator) were used. The column used for the separation of Prucalopride succinate was Inertsil ODS C18 (250mm x 4.6mm x 5µm).

Stock Solution Preparation

A 25 mg of prucalopride succinate was accurately weighed and transferred to a 25 ml volumetric flask. The sample was dissolved in a sufficient amount of ammonium formate buffer (pH 5.0) and volume was made up to the mark (1000 µg/mL) (Solution A). A 2.5 mL of solution A was diluted to 25 mL with buffer (100 µg/mL) (Solution B). Further, 1 mL of solution B was diluted to 10 mL with buffer (10 µg/mL) (Solution C). Solution C was used to prepare further dilutions for carrying out validation procedures.

Wavelength Selection

UV spectrum of a solution having a concentration of 10 µg/mL of Prucalopride succinate was recorded using methanol as blank. It showed maximum absorbance at a wavelength of 226nm; therefore, it was selected as a detection wavelength.

Column Selection

C_8 and C_{18} columns were used for conducting experimental trials. Analysis using C_8 column yielded in broad peak shape because of poor retention of the analyte. C_{18} column, on the other hand, showed improved peak shape owing to better retention of the analyte.

Mobile Phase Selection

Various combinations of water and organic phase compositions were used for initial experimentation. Mixture of methanol and water showed high baseline noise in the chromatogram. Thus, interference was observed at the retention time of the analyte. A mixture of acetonitrile and water yielded showed reduced interference, but there was no precision in the analyte's retention time. This problem necessitated the use of a buffer. Therefore, 0.63 gm of ammonium formate was weighed accurately and transferred in 1,000 mL beaker. 1000 mL of water was added, sonicated and the pH was adjusted to 5.0 with formic acid. The buffer and acetonitrile were used in the ratio of 77:23 as the mobile phase.

Method Optimization by QbD Approach

Software aided method development: A QbD based RP-HPLC method was developed for the estimation of Prucalopride succinate in bulk form and dosage form. Two phases are involved in method development by QbD approach: -

Screening Phase

Screening phase involves screening of major contributors of selectivity and peak shape which include buffer pH, flow rate, and % organic modifier. The screening phase was carried out using Design Expert 12 software as shown in Table 1. In this software, Box-Behnken statistical screening design was used to optimize the critical process parameters (CPPs) or critical method parameters (CMPs) and to evaluate interaction effects of these parameters on the critical quality attributes (CQAs). This Box Behnken statistical screening design is a 3 factor-3 level design which was specifically selected since it requires fewer experimental runs than other screening design. All the parameters are varied simultaneously, unlike the conventional OFAT (one factor at a time) approach. The responses obtained after carrying out 13 experimental trials were fed back into DoE software.

Statistical Analysis and Final Optimization

Statistical analysis was used to identify significant chromatographic factors and the impact of their interaction on the three responses i.e., retention time, NTP and symmetry factor of Prucalopride succinate. Figures 2-10 represent 3D surface plots as well as actual vs predicted plots. Analysis of surface plots was used to estimate as to which method parameter gave the most acceptable responses. Additionally, with the help of statistical analysis tools like ANOVA, the p-value (probability) was used to determine the significance of each method parameter for each individual response. The significance level for the probability of a null hypothesis (H_0) was defined at $p \geq 0.05$. The null hypothesis indicates variation in all factors, which has no influence on the responses. The response variables i.e., retention time, NTP, and symmetry factor were statistically evaluated, and the results obtained were found within the acceptable criteria. Final optimized chromatographic conditions are summarized in Table 2.

Table 1: Factor screening by Box-Behnken design

Run	*Factor1 pH*	*Factor2 % ACN*	*Factor 3 Flow rate*	*Response 1 Retention time*	*Response 2 NTP*	*Response 3 Symmetry factor*
1	5	23	1	5.85	10373	1.1
2	5	26	1.5	3.1	4633	0.98
3	4.6	26	1	4.43	6688	1.28
4	5.4	23	1.5	4.26	4644	1.1
5	5.4	20	1	9.74	6010	1.48
6	5.4	26	1	5.02	6838	1.35
7	5	20	1.5	5.89	4994	0.99
8	4.6	23	1.5	3.85	5552	1.1
9	4.6	20	1	12.12	9562	1.39
10	5.4	23	0.5	13.63	9334	1.49
11	4.6	23	0.5	11.33	9132	1.37
12	5	26	0.5	9.07	9988	1.38
13	5	20	0.5	17.01	9082	1.42

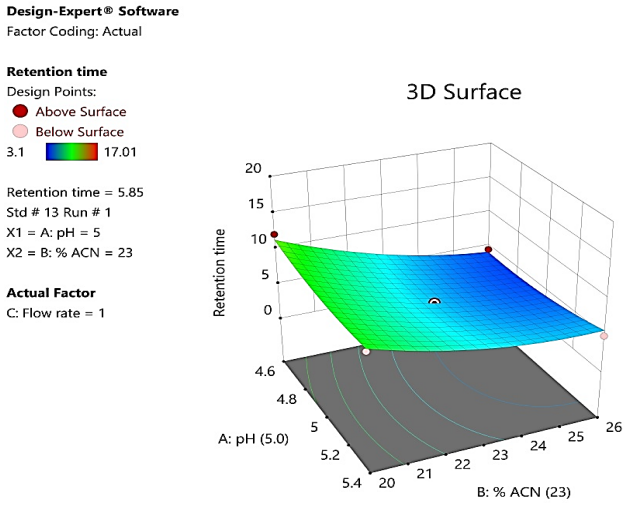

Figure 2: Effect of pH and % ACN on retention time of Prucalopride succinate

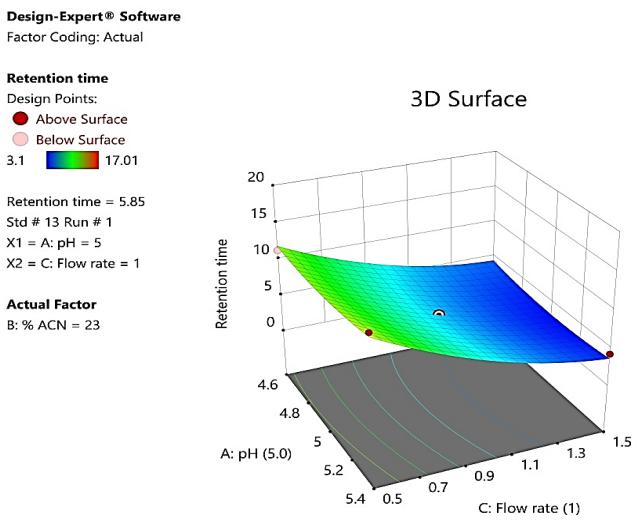

Figure 3: Effect of pH and flow rate on retention time of Prucalopride succinate

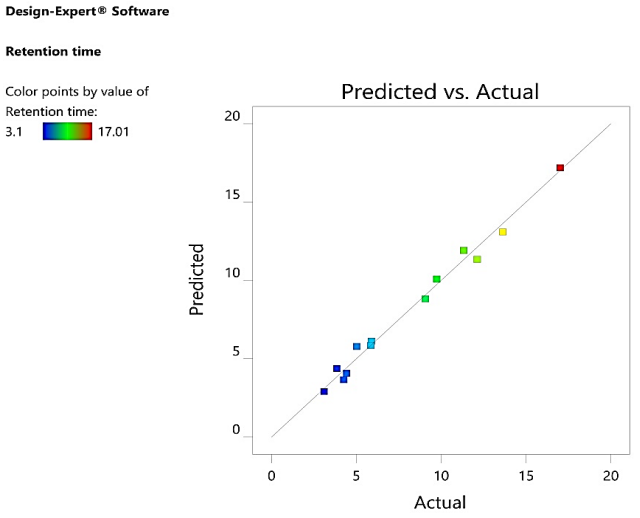

Figure 4: Actual vs Predicted plot for retention time of Prucalopride succinate

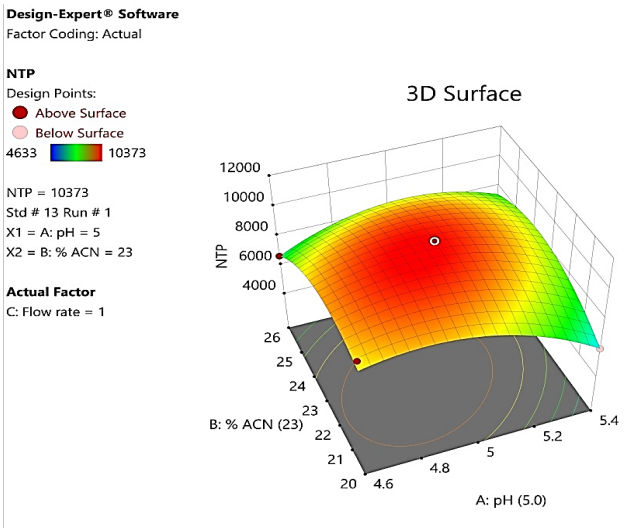

Figure 5: Effect of pH and % ACN on NTP of Prucalopride succinate

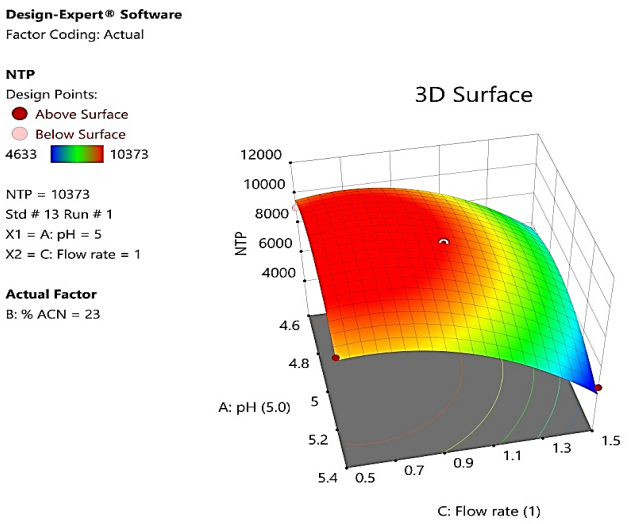

Figure 6: Effect of pH and flow rate on NTP of Prucalopride succinate

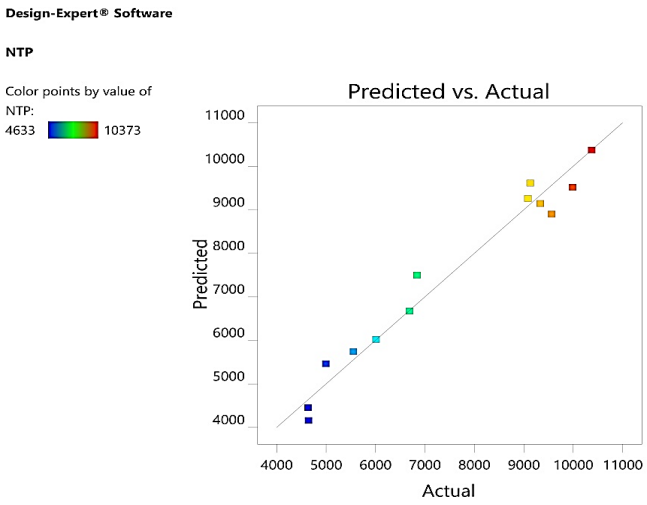

Figure 7: Actual vs Predicted plot for NTP of Prucalopride succinate

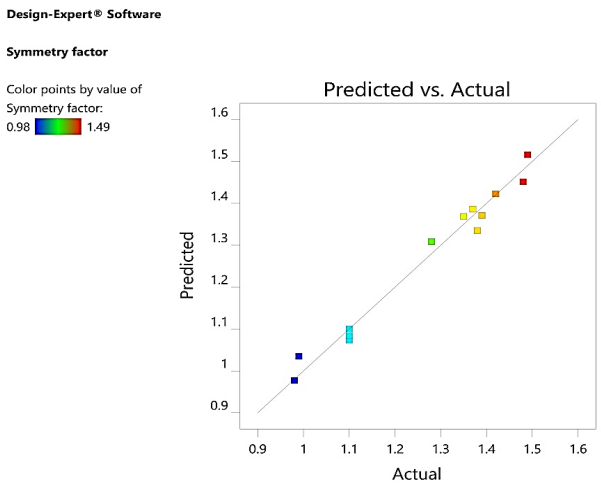

Figure 10: Actual vs Predicted plot for symmetry factor of Prucalopride succinate

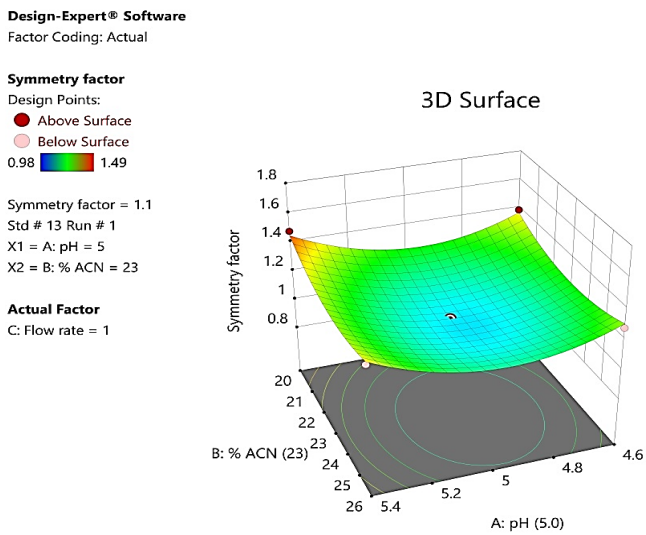

Figure 8: Effect of pH and % ACN on symmetry factor of Prucalopride succinate

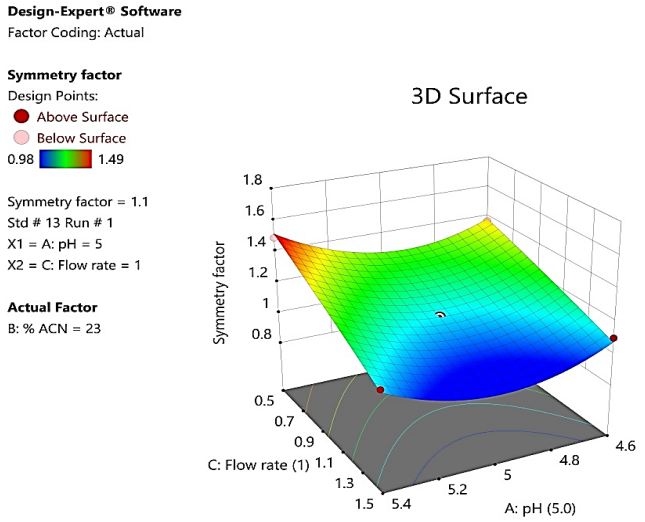

Figure 9: Effect of pH and flow rate on symmetry factor of Prucalopride succinate

1. Retention time (Rt)
2. NTP
3. Symmetry factor

Forced Degradation Studies[6,7]

Forced degradation was carried out to prove that the method is stability-indicating.

Acid Hydrolysis

A 2 mL of solution A (1000µg/mL) was pipetted out in a 10 mL volumetric flask. The solution was treated with three different concentrations of HCL, i.e., 1N, 2N, and 5N separately and then neutralized with the same molar concentrations of sodium hydroxide solution, i.e., 1N, 2N and 5N separately and the volumes were made up with the diluent to give a solution of 200µg/mL. Finally, the solution was loaded into HPLC system. No degradation was observed.

Table 2: Optimized chromatographic conditions

Parameter	*Specification*
HPLC pump	Waters 2695 Separations Module
HPLC detector	Waters 2996 PAD
Integrator	Empower Pro
Column	Inertsil ODS- C_{18}
Wavelength	226 nm
Mobile Phase	23:77 v/v (Acetonitrile: Ammonium formate buffer adjusted to pH 5.0)
Injection volume	10µL
Flow rate	1 mL/min

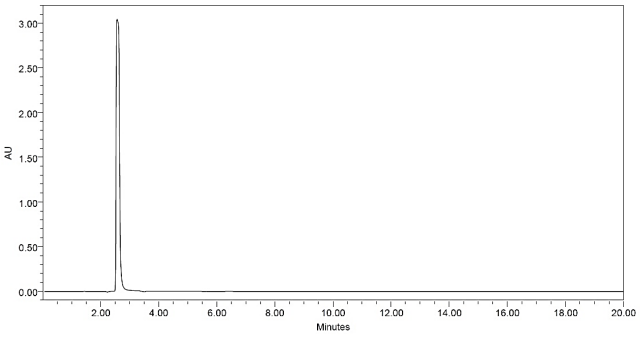

Figure 11: Chromatogram of blank for oxidative degradation

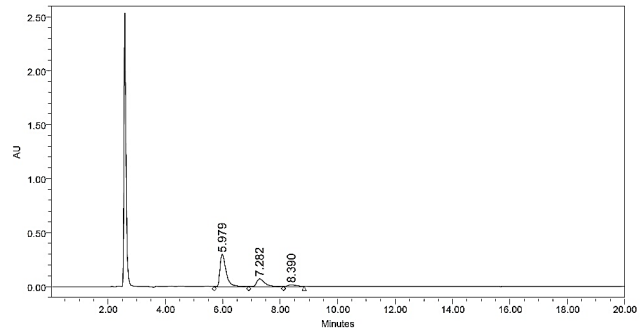

Figure 12: Chromatogram of stock solution subjected to oxidative degradation

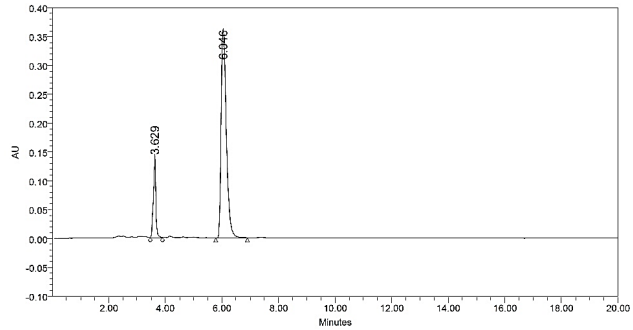

Figure 13: Chromatogram of stock solution subjected to photolytic degradation

Base hydrolysis

A 2 mL of solution A (1000µg/mL) was pipetted out in a 10 mL volumetric flask. The solution was treated with 2 mL of 2N and 5N sodium hydroxide separately and then neutralized with 2 mL of 2N and 5N hydrochloric acid. The volume was made up with the diluent to give a solution of 200 µg/mL. Finally, the solution was loaded into the HPLC system. No degradation was observed.

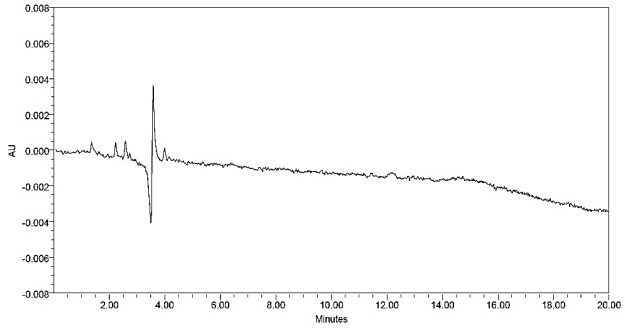

Figure 14: Chromatogram of blank

Oxidative Degradation

A 2 mL of solution A (1000µg/mL) was pipetted out in a 10 mL volumetric flask. The solution was treated with 1 mL of 6% H_2O_2 solution kept as such at room temperature for 12 hours. The volume was made up with the diluent to give a solution of 200 µg/mL. Finally, the solution was loaded into HPLC, and the corresponding chromatogram was recorded. Figures 11 and 12 represents the chromatograms for blank and stock solution subjected to oxidative degradation, respectively.

Thermal Degradation

2 mL of the solution A (1000 µg/mL) was pipetted out in a 10mL volumetric flask. The solution was kept in a water bath at 60°C for 12 hours. The volume was made up with the diluent to give a solution of 200 µg/mL. This solution was injected into the HPLC system. No degradation was observed.

Photolytic Degradation

A 2 mL of solution A (1000 µg/mL) was pipetted out in a 10 mL volumetric flask. The solution was kept in sunlight for 30 minutes. The volume was made up with the diluent to give a solution of 200 µg/mL. Finally, the solution was loaded into HPLC and the corresponding chromatogram was recorded. Figure 13 represents the chromatogram of stock solution subjected to photolytic degradation.

Forced degradation studies indicate that the drug is susceptible to oxidative degradation and photolytic degradation. Observed degradation of oxidative degradation was 8.2%, whereas it was 10.6% for photolytic degradation.

RESULT AND DISCUSSION

Method Validation[8]

Validation was performed according to ICH guidelines for the parameters such as specificity, system suitability, sensitivity, linearity, accuracy, and precision.

Specificity

No other interfering peak around prucalopride's retention time succinate (Rt–5.8 minutes); thus, the method was specific. Figure 14 represents the chromatogram for blank.

System Suitability

Prucalopride succinate standard solution of 2 µg/mL was injected in six replicates. The mean of system suitability parameters was obtained. Chromatogram for system suitability is shown in Figure 15, along with the peak information.

Peak Name	Rt (min)	Area	Symmetry factor	NTP
Prucalopride succinate	5.8	66334	1.15	10225

Sensitivity

The LoD for Prucalopride succinate was found to be 0.2 µg/mL
The LoQ was found to be 0.8µg/mL

Linearity and Range

The Linearity was confirmed in the range of 1–3µg/ml. The Correlation Coefficient (r2) was found to be 0.9982, and the equation of the line was y = 30417x + 9476.2 as evident from the calibration curve shown in Figure 16. Thus, the data shows that the response was found to be linear.

Precision

Intraday Precision (Repeatability) of Prucalopride succinate was determined by injecting six replicates of 1, 2 and 3 µg/mL concentration each at different time intervals. Inter-day precision (Intermediate precision) was determined by injecting six replicates of the same three concentrations on two consecutive days. The % RSD values for Intraday and interday precision of three concentration levels was found to be 1.32% and 1.45%, respectively. Results of intraday precision and inter-day precision are summarized in Tables 3 and 4, respectively.

Accuracy

% Recovery study was performed by in triplicates by spiking 50, 100, and 150% of working level concentration (1.0 µg/mL, 2.0 µg/mL, 3.0 µg/mL) and obtaining the percent recovery by putting the values of the areas of the peak obtained in the calibration curve to obtain the values of the concentration

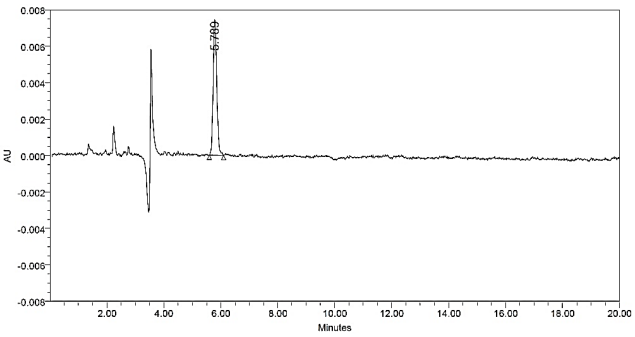

Figure 15: Chromatogram for system suitability

Table 3: Intraday precision data of Prucalopride succinate

Intraday precision						
Conc.	*1 µg/mL*	*2 µg/mL*	*3 µg/mL*	*1 µg/mL*	*2 µg/mL*	*3 µg/mL*
Replicates	*Retention time (min)*			*Peak Area*		
1	5.82	5.82	5.82	39584	65119	100025
2	5.85	5.83	5.82	39686	64573	98352
3	5.82	5.82	5.85	41074	64301	99730
4	5.83	5.83	5.82	40715	65598	97454
5	5.81	5.82	5.87	40855	66334	98291
6	5.82	5.82	5.9	40297	65801	100669
Mean	5.83	5.82	5.85	40368.5	65287.7	99086.8
SD	0.01378	0.00516	0.03327	623.03	770.369	1235.84
% RSD	0.23664	0.08868	0.56898	1.54336	1.17996	1.24723

Table 4: Inter-day precision data of Prucalopride succinate

Inter-day precision						
Conc.	*1 µg/mL*	*2 µg/mL*	*3 µg/mL*	*1 µg/mL*	*2 µg/mL*	*3 µg/mL*
Replicates	*Retention time (min)*			*Peak Area*		
1	5.81	5.82	5.85	39574	64120	99085
2	5.81	5.81	5.82	40102	63973	97352
3	5.82	5.82	5.85	39074	65501	97530
4	5.82	5.83	5.82	38715	64596	98954
5	5.81	5.82	5.86	39855	63858	100291
6	5.82	5.81	5.89	39997	66418	100669
Mean	5.82	5.82	5.85	39552.8	64744.3	98980.2
SD	0.00548	0.00753	0.02639	551.7	1016.47	1366.46
% RSD	0.09419	0.12938	0.45132	1.39484	1.56997	1.38054

Table 5: Accuracy data of prucalopride succinate

	Peak Area					
Conc. (µg/ml)	*Inj. 1*	*Inj. 2*	*Inj. 3*	*Mean*	*Recovered conc.*	*% Recovery*
1	39644	39363	38982	39329.7	0.9815	98.1473
2	69193	70285	69077	69518.3	1.9740	98.6983
3	100777	102945	102010	101911	3.0389	101.2969

Table 6: Results of validation

Sr. No.	*Validation Parameters*	*Observations*	*Acceptance criteria*
1.	System suitability	Retention time - 5.8 NTP- 10015 %RSD- 1.314% Symmetry factor-1.14	- > 2000 < 2% 0.8–2.0
2.	Specificity	No interference was found at the Rt of the analyte	-
3.	LoD	0.2µg/mL	-
4.	LoQ	0.8µg/mL	-
5.	Linearity	R^2 = 0.9982 y = 30417x + 9476.2	R^2 > 0.995 -
6.	Range	1–3 µg/mL	-
6.	Accuracy: % Recovery % RSD	 99.38% 0.96 %	 98–102% < 2%
7.	Inter-day precision (%RSD)	1.45 %	< 2%
8.	Intraday precision (%RSD)	1.32%	< 2%
9.	Solution Stability (%RSD)	1.55 %	< 2%

Table 7: Assay of Prucalopride succinate

Formulation	*Label claim (mg)*	*Amount found (mg)*	*% Amount found*
PRUVICT-1	1 mg	0.98 mg	98.05 %

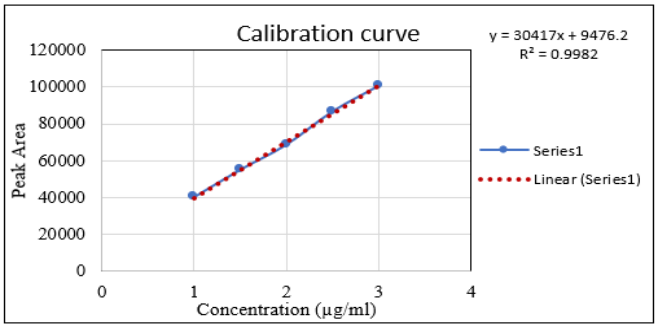

Figure 16: Calibration curve for Prucalopride succinate

injected. % Recovery was found to be 99.38 %, and % RSD of response was found to be 0.96 %. Table 5 shows the accuracy data of Prucalopride succinate.

Solution Stability

The stability of drug solution was evaluated for three different concentrations - 1, 2 and 3µg/mL. The samples were stored at a refrigerated temperature of 10ºC-15 ºC. The analysis was performed at initial time 0 and then after 6, 9, 24, 32, 48, and 72 hours. The stability solution test results indicated that the drug solutions were stable up to 72 hours.

The results of validation are summarised in Table 6.

Application of the Method to Marketed Formulation

Ten tablets of PRUVICT-1 containing Prucalopride succinate 1 mg each were accurately weighed and the average weight of a tablet was found out. The 10 tablets were finely powdered and powder equivalent to 10 mg of Prucalopride succinate was taken and transferred into a 10 mL volumetric flask. 7 mL of diluent was added and sonicated with occasional shaking for few minutes and the volume was made upto the mark with diluent (solution A). A 1-mL of this solution A was further diluted to 10 ml with diluent to give a concentration of 100 µg/mL (solution B). A 1-mL of this solution B was further diluted with 10 mL diluent to give a concentration of 10µg/mL (solution C). 2 mL of solution C was diluted with 10 mL of diluent to give a final concentration of 2 µg/mL (solution D). This solution D was injected into the HPLC system. The average area of 3 injections was taken for calculation. Assay results of Prucalopride succinate are shown in Table 7.

CONCLUSION

The developed and validated RP-HPLC method was found to be simple, sensitive and stability-indicating. It was validated according to current ICH guidelines. The use of the QbD approach led to reduced number of trials and failure. The

developed method can be successfully applied for the routine analysis of drug in bulk form and tablet form.

ACKNOWLEDGEMENT

The authors are grateful to Symed Labs for providing the gift sample of the API. The authors would also like to thank the staff members of Ultra Pure Analytics for providing the required facilities which helped in the completion of the project.

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