



## DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF PRUCALOPRIDE SUCCINATE IN PHARMACEUTICAL DOSAGE FORM

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### ABSTRACT

A Simple, Precise, and Accurate RP-HPLC method was developed and validated for the estimation of Prucalopride Succinate in tablet dosage form. The method was achieved on waters C18 (150 × 4.6 mm, 5μ). The method showed a linear response in the concentration range of 10-50 μg/mL using methanol: water as mobile phase in the ratio of 70: 30 v/v with detection at 226 nm with flowrate of 1 mL/min and retention time was 2.9 min. the method was validated as per ICH guideline. The method was successfully applied for routine analysis of Prucalopride Succinate in tablet dosage form.

**KEYWORDS:** Prucalopride Succinate, RP-HPLC, validation, Pharmaceutical dosage form.

### 1. INTRODUCTION

Prucalopride succinate was selected for development in chronic constipation. Because it has a potential to address the motility problems and to provide to relief beyond increase in stool frequency.

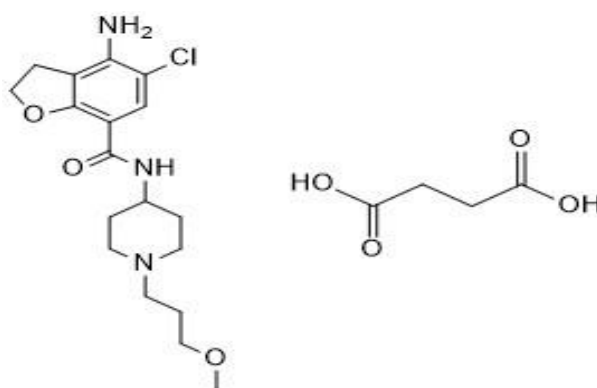
Prucalopride succinate is indicated for the treatment of chronic idiopathic constipation in adult female patients in whom laxatives failed to provide adequate relief. Prucalopride succinate is approved in Europe in women laxatives fail to provide adequate relief. Subsequently, it has been approved for use in adult that is including male patient for the same indication.

Prucalopride is a class of dihydrobenzofurancarboxamide-derivatives with potent enterokinetic activity. Prucalopride succinate is chemically designated as 4-amino-5-chloro-2,3-dihydro-N-[1-(3-methoxypropyl)-4-piperidiny]-7-benzofurancarboxamide butanedioate (1:1). It is a selective, high affinity serotonin (5-HT<sub>4</sub>) receptor agonist. This drug is not official in any Pharmacopoeia.

Prucalopride succinate is in biopharmaceutics classification system (BCS) class-I, high permeability, high solubility. Prucalopride succinate is a dihydrobenzofurancarboxamide with enterokinetic activities. It is a selective high affinity serotonin (5-HT<sub>4</sub>) receptor agonist.

Prucalopride stimulates motility by interacting specifically with 5-HT<sub>4</sub> receptors in the GI tract which causes a release of acetylcholine and further contraction of the muscle layer of the colon and relaxation of the circular muscle layer leading to the propulsion of luminal content.

A Survey of literature has not revealed any simple RP-HPLC method for the estimation of Prucalopride Succinate so that the objective of the present work was to develop Simple, Precise RP-HPLC method for estimation of Prucalopride Succinate in tablet dosage form.



**Figure 1: Chemical Structure of Prucalopride succinate.**

## 2. MATERIALS AND METHODS

### Apparatus and Instruments

- A Shimadzu HPLC Instrument (LC\_2010 CHT) [ software LC Solution, equipped with U.V. detector, Auto-sampler]
- An Analytical balance (Acculab ALC-210.4, Huntingdon Valley, PA)
- Sonicator (EN 30 US, Enertech Fast Clean, Mumbai, India)
- Volumetric Flask- 10, 50, 100 mL
- Pipettes – 1,2,5,10 mL

**Reagents and Materials**

- Prucalopride succinate standard drug
- HPLC grade Methanol (Merck Life Science Private Limited, Mumbai, India)
- HPLC grade Water (Merck Life Science Private Limited, Mumbai, India)

**Selection of detection wavelength**

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is the one that gives good response for the selected drug that is to be detected not interference of solvent effect. It gives maximum absorbance at 226 nm in distilled water. At 226 nm drug gives good height peak and not interference of solvent effect. So, this wavelength was selected for estimation of prucalopride succinate.

**Selection of Chromatographic Condition**

Proper selection of the HPLC method depends upon the nature of the sample, its molecular weight, pKa and solubility. Selection of the proper column is the first step and selection of Mobile phase is the next step. To optimize the Chromatographic conditions the effect of chromatographic variables such as mobile phase, pH, flow rate and solvent ratio were studied. And the Chromatographic parameters such as Capacity factor, asymmetric factor, and resolution and column efficiency were calculated. Finally the Condition was chosen that give the best resolution, symmetry and capacity factor was selected for estimation of the drug.

**Preparation of standard stock solution of prucalopride succinate**

Accurately weighted quantity of prucalopride succinate 10 mg was transferred into 10 ml volumetric flask, dissolved & diluted up to the mark with water. This was given a stock solution having strength of 1000 µg/ml.

**Preparation of working standard solution**

100 µg/ml of prucalopride working standard solution was prepared by diluting 1 ml of std. stock solution with water in 10 ml volumetric flask up to the mark.

**Preparation of sample Solution**

Twenty tablets were weighed and average weight was calculated. The tablets were powdered, a quantity of powder equivalent to 10 mg PRU was weighed and transferred to a 10 mL of volumetric flask containing 5 mL distilled water and sonicated for 20 minutes. The flask was

allowed to stand at room temperature for 5 min and the volume was made up to the mark with distilled water to obtain the sample stock solution (1000 µg/mL). The solution was filtered through Whatman filter paper. From this solution pipette out 1 mL and volume adjusted to mark with 10 mL volumetric flask (100 µg/mL). From this solution pipette out 1 mL and volume adjusted to mark with 10 mL volumetric flask. This was working sample solution having solution strength 10 µg/mL of PRU.

## METHOD DEVELOPMENT

### Specificity

Specificity of analytical method is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Specificity of the method was evaluated by comparison between chromatogram of standard and test solutions. There should be absence of any interfering peak with peak of analyte.

### Linearity and range

Linearity is expressed in terms of correlation coefficient of linear regression analysis.

➤ Calibration curve for PRU.

Aliquots of working solution (100 µg/mL) of PRU (1, 2, 3, 4 and 5 mL) were transferred into 10 mL volumetric flask. The volume was adjusted to the mark with the distilled water to get concentrations (10, 20, 30, 40 and 50 µg/mL). An aliquot (20 µL) of each solution was injected under the operating chromatographic conditions as described earlier. Calibration curve was prepared by plotting peak areas versus concentration, and the regression equation was calculated. Each response was average of three determinations.

### Precision

#### (a) Repeatability

The solution was prepared by pipetting out 1 mL of the working standard solution (100 µg/mL) into 10 mL volumetric flask & the volume was adjusted to mark with distilled water. This solution (10 µg/mL) was determined for 6 times. The results were reported in terms of RSD.

#### (b) Intra-day Precision

Intra-day precision was determined by analysing solution (10, 20 and 30 µg/mL) for three times on the same day. The results were reported in terms of % RSD.

**(c) Inter-day precision**

Inter-day Precision was determined by analysing the solution (10, 20 and 30 µg/ml) for three times on the different day. The results were reported in terms of % RSD.

**Accuracy**

Accuracy was determined by calculating recovery of PRU by the standard addition method. Known amounts of standard solutions PRU (16, 20, 24 µg/mL) were added to a prequantified test solutions of PRU (20 µg/mL). Each solution was injected in triplicate, and the recovery was calculated by measuring peak areas and fitting these value into the regression equation of the calibration curve by peak areas.

**Limit of Detection and Limit of Quantitation**

The LOD and LOQ of the drug were calculated using following formula according to ICH guideline.

$$\text{LOD} = 3.3 \times \sigma / s$$

$$\text{LOQ} = 10 \times \sigma / s$$

Where,  $\sigma$  = the standard deviation of response

S = the slope of the calibration curve

**Robustness**

The robustness study was performed to evaluate the influence of small but deliberate variation in the chromatographic condition. The robustness was checked by changing three small changes.

1. Flow rate ( $1.0 \pm 0.2$  mL/min)
2. Organic phase ( $70 \pm 5$  mL)
3. Injection Volume ( $20 \pm 5$  µL)

After each sample solution was injected and peak area, tailing factor and retention time were checked.

**Analysis of marketed formulation**

An aliquot of 20 µL from sample solution was injected under a chromatographic condition and peak area was measured and % assay was calculated from regression equation. Response was an average of six determinations.

### 3. RESULT AND DISCUSSION

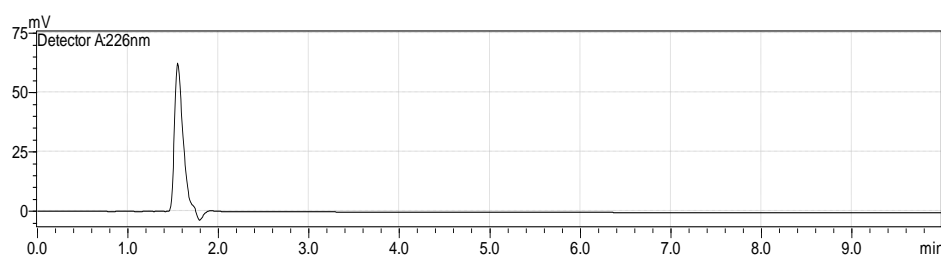
#### Optimization of Chromatographic Conditions

Stationary phase: C18 water (150 × 4.6 mm, 5μ)

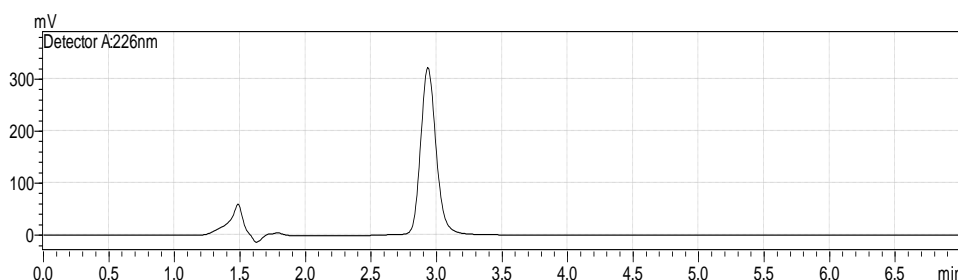
Mobile phase: Methanol: Water (70:30)

Flow rate : 1 mL/min

Run Time : 10 min



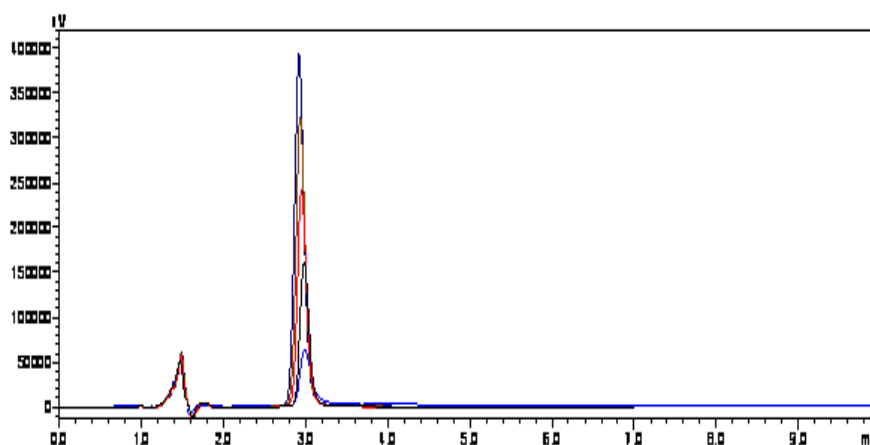
**Figure 2 Chromatogram of Diluent.**



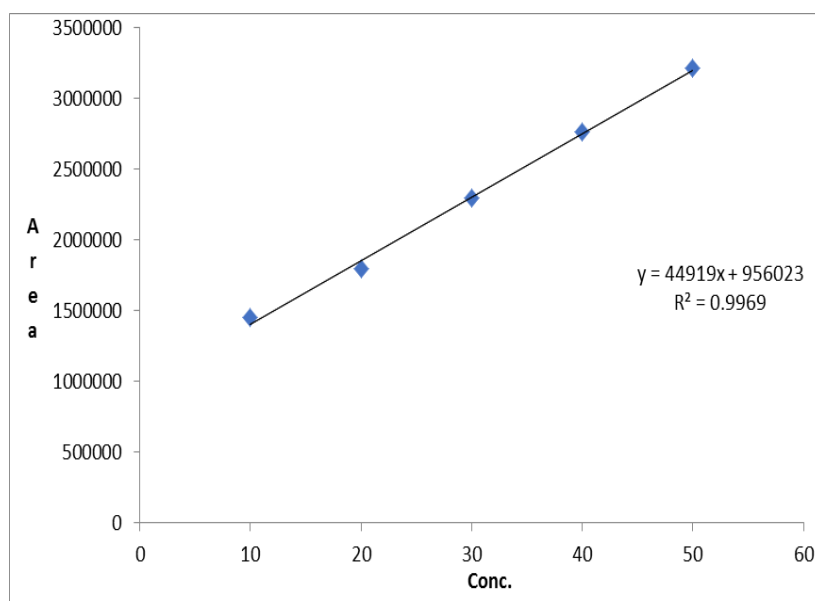
**Figure 3: Chromatogram of PRU standard.**

#### Linearity and Range

The linearity range was found between 10 – 50 μg/mL. Linearity Chromatogram is shown in the Figure 4, while Calibration Curve is shown in Figure 5 and Linearity data are shown in the table 1.



**Figure 4 Overlain Chromatogram of PRU.**



**Figure 5: Calibration Curve of Prucalopride Succinate.**

**Table 1: Calibration data of PRU.**

Sr. No.	PRU (μg/mL)	Peak Area* ± SD (n=3)	% RSD
1	10	1453553 ± 2223.953	0.15
2	20	1797275 ± 2522.746	0.14
3	30	2295265 ± 4613.361	0.20
4	40	2745640 ± 20450.9	0.7
5	50	3223106 ± 19541.83	0.6

\*Average of three determination

### Precision

The data of Repeatability, Intra-day, Inter-day is shown in Table.

**Table 2: Repeatability data for PRU.**

Conc.	Peak Area Mean* ± S.D.	% RSD
20	17944001 ± 1806.44	0.10

\*Average of six determination

**Table 3: Intra-day data for PRU.**

Conc.	Peak Area Mean* ± SD	% RSD
20	1795706 ± 2221.581	0.12
30	2294101 ± 3258.104	0.14
40	2756479 ± 6520.623	0.23

\*Average of three determination

**Table 4: Inter-day data for PRU.**

Conc.	Peak Area Mean* $\pm$ SD	% RSD
20	1792464 $\pm$ 5906.88	0.3
30	2301722 $\pm$ 9401.94	0.4
40	2799689 $\pm$ 4001.56	0.1

\*Average of three determination

**Accuracy**

Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition. Percentage recovery for PRU found to be range (98.8 – 101.09) of % shown in Table 5.

**Table 5: Accuracy Data for PRU.**

% Added	Amount of sample taken ( $\mu\text{g/mL}$ )	Amount of Std. added ( $\mu\text{g/mL}$ )	Amount Found Mean* $\pm$ S.D ( $\mu\text{g/mL}$ )	% Recovery
80 %	20	16	35.4 $\pm$ 0.3	98.82 %
100 %	20	20	39.8 $\pm$ 0.1	100.14 %
120 %	20	24	44.1 $\pm$ 0.2	101.09 %

\*Average of three determination

**Table 6: Limit of Detection and Limit of Quantitation.**

LOD	0.16 ( $\mu\text{g/mL}$ )
LOQ	0.49 ( $\mu\text{g/mL}$ )

**Robustness**

By introduction small Changes in the mobile phase composition, flow rate and injection volume on chromatographic condition on the results were examined. There were very slightly changes in peak area, tailing factor and retention time. The result was shown in table 7.

**Table 7: Robustness Data for PRU.**

Parameter	Value	Retention Time	% RSD	Area	% RSD
Flow rate (± 0.2 mL/min)	0.8	3.0	1.1 %	1452564	0.3 %
	1.2	2.95		1445123	
Mobile Phase (Methanol: Water) (± 5 mL)	65:35	2.92	1.4 %	1451412	0.11 %
	75: 25	2.86		1453854	
Injection volume (± 5 µL)	15 µL	2.84	1.7 %	1450256	0.18 %
	25 µL	2.91		1454123	
Overall % RSD	0.79 %				

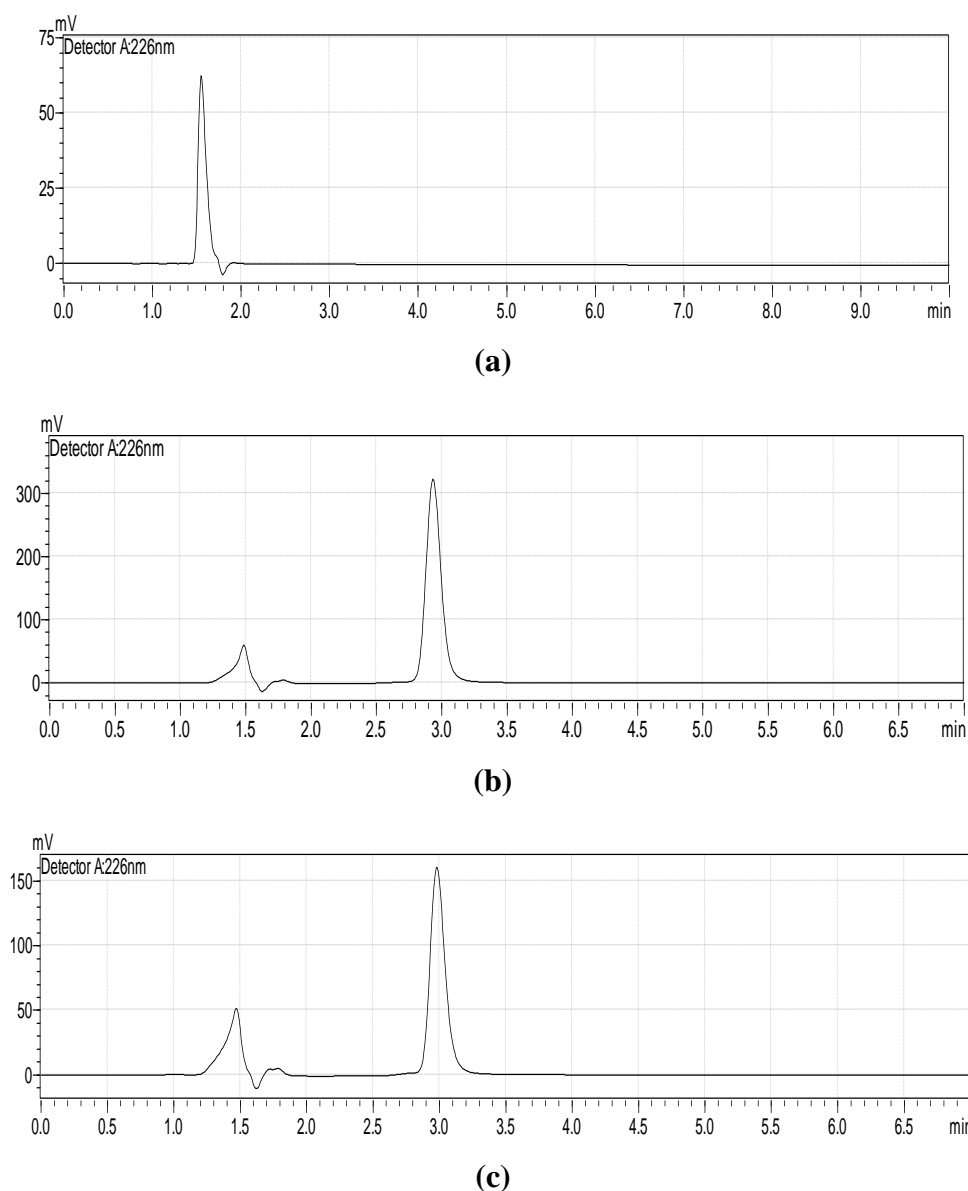
**Analysis of marketed formulation**

The Proposed RP-HPLC method was successfully applied for determination of PRU from tablet dosage form. The percentage of PRU was found to be satisfactory, which is comparable with the corresponding label claim. The result was shown in table 8.

**Table: 8 % Assay of Marketed formulation.**

Formulation	% Assay $\pm$ SD
PRUVAC (1 mg)	99.6 $\pm$ 0.7

\*Average of three determination

**Figure 6: Chromatogram of (a) Diluent, PRU (b) Standard and (c) test solution.**

## SUMMARY

The results for validation and system suitability parameters are summarized in Table 12.

**Table 9: Summary of Validation Parameters for RP-HPLC Method.**

Parameters		Prucalopride succinate
Specificity		Specific
Linearity Range		10-50 µg/mL
Correlation coefficient ( $R^2$ )		0.996
Precision (% RSD)	Repeatability	0.10 %
	Intraday	0.1-0.2 %
	Interday	0.1-0.4 %
Accuracy (% Recovery)		98.8 -101.09 %
Limit of Detection (LOD)		0.16 µg/mL
Limit of Quantitation(LOQ)		0.49 µg/mL
Robustness (% RSD)		0.79 %
% Assay		99.6 %

## 4. CONCLUSION

The Validated RP-HPLC method for the estimation of Prucalopride Succinate in tablet dosage form has been found to be Simple, Accurate & Precise. So it can be used for the routine analysis of Prucalopride Succinate in tablet formulation.

## 5. ACKNOWLEDGEMENTS

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