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# Development and Validation of a Stability Indicating HPTLC Method for the Determination of Prucalopride succinate in Bulk and Tablet dosage form

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**ABSTRACT:**

A simple, precise, and accurate stability-indicating high-performance thin layer chromatographic method for analysis of Prucalopride succinate It has been demonstrated and proven that in the presence of its degradation products. Optimum separation among Prucalopride succinate and the results of its degradation was achieved by use of silica gel 60F254 as stationary phase with Toluene: Methanol: Glacial acetic acid (7:2.5:0.5 v/v/v) as a mobile phase. Densitometric quantification was performed at 240 nm. The retention factor was revealed to be 0.25 ± 0.10for parent drug. The drug was subjected to different stress conditions like acid, base hydrolysis, oxidation, thermal degradation and photolysis. In accordance with ICH Q2 (R1) guidelines, the approach was successfully validated. The method exhibited an adequate linearity (r =0.995), selectivity, precision and accuracy. Prucalopride succinate in bulk and pharmaceutical dose form can be routinely analysed using the developed method.

**Keywords:** Prucalopride succinate, Stability indicating method, ICH, HPTLC, Validation.

## INTRODUCTION

Prucalopride (PRU) (Fig. 1) chemically is 4-amino-5-chloro-2, 3-dihydro-N-[1-(3-methoxy propyl)-4-piperidinyl]-7-benzofurancarboxamide butanedioate). It is a dihydro benzofuran carboxamide derivative from the benzofuran family, and it possesses enterokinetic activity by specifically stimulating 5-HT4 receptors[1]. It performs a specific action on the stomach muscle· Wall thus, helping to reinstate the regular working of the human bowel[2]. There was an enhancement in the bowel motion frequency but no considerable consequence on the transit time of the colon[3]. The literature review reveals that few analytical methods have been reported for its quantitative estimation in pharmaceutical formulations, which include UV-spectrophotometry, HPLC, Stability indicating HPLC and UHPLC-MS-MS[4-15] .Stability indicating HPTLC method is not reported. Consequently, the objective of the current work was to develop and validate a novel HPTLC method for the measurement of

prucalopride succinate in pharmaceutical formulation that was simple, quick, selective, economical, and stability suggesting.

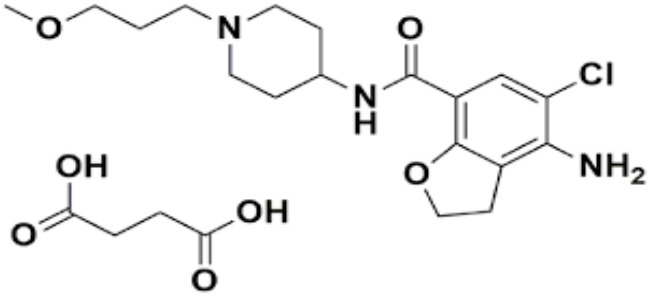


Figure 1 Structure of Prucalopride succinate.

## MATERIALS AND METHODS

### Chemicals and Reagent

Pure Prucalopride succinate was generously gifted by Alkem laboratories (Mumbai, India).The pharmaceutical dosage form used in this study was Prudac 1 (Zydus Cadila, India) Procured from the local market and labeled to contain1mg of prucalopride succinate per tablet.The solvents and chemicals used in the study were of AR grade.(Merck specialties Pvt. Mumbai, India-based Ltd.).

### Chromatographic and Instrumentation Conditions

Chromatographic separation of the drug was performed on Merck TLC plates precoated with silica gel $60F_{254}$ (10×10) from E. Merck, applying with a CAMAG Linomat 5 sample applicator (Switzerland).Weighing of the chemicals was done using Shimadzu Corporation Japan, BL-220H balance. Transonic Digital S sonicator (Athena Technology, ATS-6-LCD) was utilized for the sonication. At 240 nm, the CAMAG thin layer chromatography scanner was used for densitometric scanning. operated by win CATS software version 1.4.2. The photostability chamber (Thermolab Scientific Equipment, TS0000200G) was used for the forced degradation study.

### Selection of solvent

After trial for checking solubility in different solvents, the drug solubility found in methanol so, analytical grade methanol was used.

### Selection of detection wavelength

Further dilutions from the standard stock solution (100 ng/l) were produced using methanol and scanned between 200 and 400 nm to get spectra. It was found that the drug displayed a sizable amount of absorbance at 240 nm. This is a representation of the Prucalopride UV spectrum.

Figure: 2 UV Spectrum of prucalopride succinate

**Preparation of Standard Solution**

Standard stock solution was prepared by dissolving 1 mg of prucalopride succinate drug in 10 mL of methanol to get working standard solution of concentration 100 ng/µl from which 5 ml was further diluted to 10 ml to get solution of 50ng/µl.

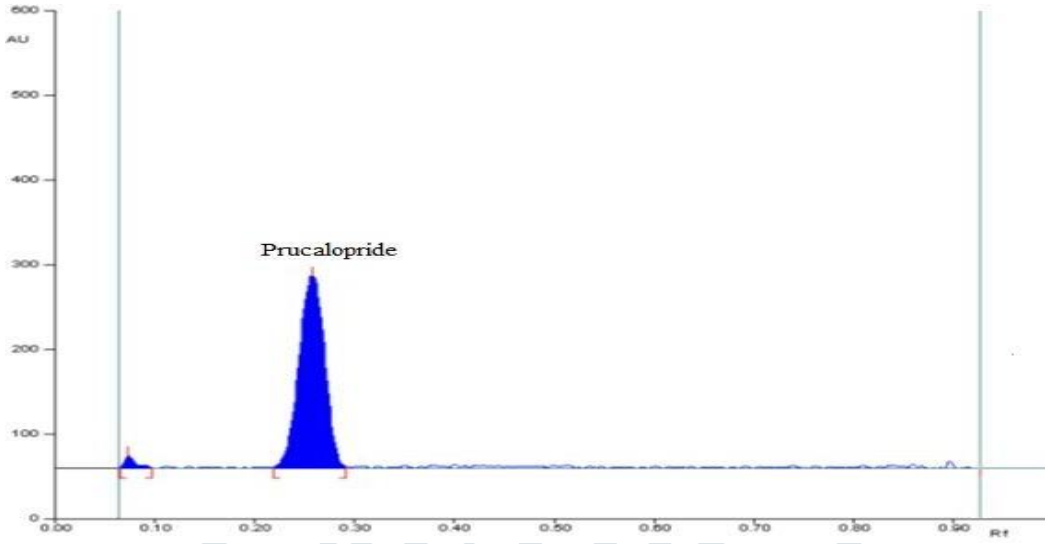


Figure 2 Densitogram of standard solution of Prucalopride succinate (150ngband-1,Rf= 0.25±0.10)

**Preparation of Sample Solution**

Tablet powder equivalent to 1 mg was transferred to 100 mL volumetric flask containing 5 mL of methanol and the contents were sonicated for 15 min and the resulting sample stock solution was filtered the volume was made up to the mark with methanol to obtain the final concentration of 50 ng/band.

**Chromatographic conditions**: The mobile phase optimization trials were performed using Toluene: Methanol, Benzene: Methanol, Ethyl acetate: Methanol in the various ratios. Hence the optimized mobile phase was Toluene: Methanol: Glacial acetic acid (7: 2.5: 0.5 v/v/v). The stationary phase used as TLC plates precoated with silica gel 60 F254 having dimension of 10 cm ×10 cm with 250 µm layer thickness. The standard solution of Prucalopride succinate was spotted on the dried, pre-coated TLC plate having band with 6 mm width. Densitometric scanning was performed on Camag thin layer chromatography scanner at 240 nm. The retention factor of prucalopride succinate was 0.25±0.10.

**Stress degradation study:**

To evaluate the stability indicating studies of the developed HPTLC method, forced degradation studies were carried out in accordance to the ICH recommendations. The standard drugs were exposed to an acid, a base, oxidation, wet heat, dry heat and photo-degradation studies. It was done to conduct the study at concentration of 100µg/ $\mu l^{-1}$.

**Acid hydrolysis**:

5 ml of the above standard drug solution was mixed 1 ml of 0.1 N hydrochloric acid and the volume was made with solvent. Solution was refluxed at 60℃ for 30 min. The densitogram of the acid degraded sample showed additional peak at Rf value 0.62 with 19.04 % degradation.

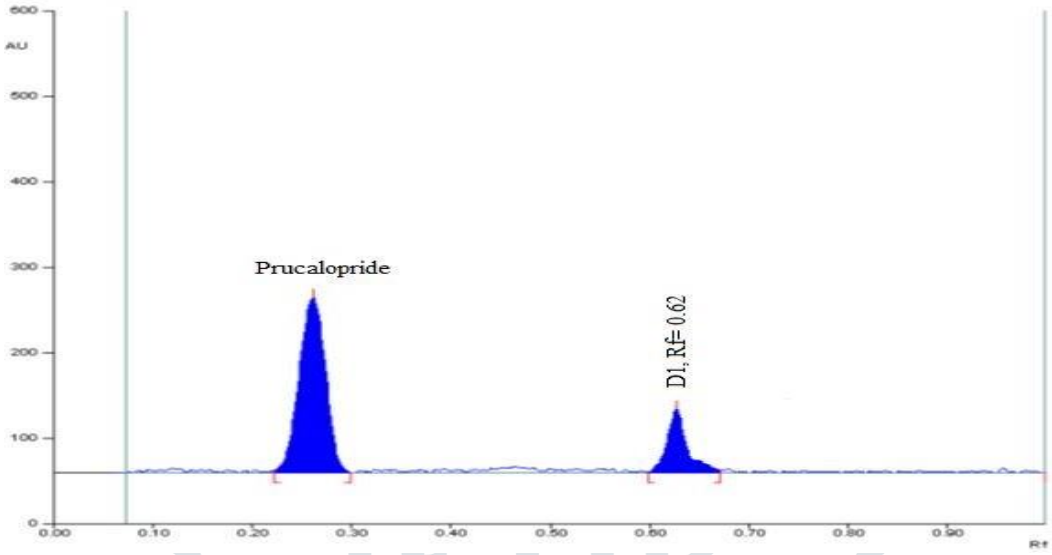


Figure 3 Densitogram after acid hydrolysis with degradation product (D1,Rf=0.62)

**Alkaline hydrolysis**:

5 ml of the above standard drug solution was mixed 1 ml of 0.1 N sodium hydroxide and the volume was made with solvent. Solution was refluxed at 60℃ for 30 min. The drug was revealed to be liable to base hydrolysis with 17.18% degradation. The densitogram had degradation product peak at Rf value of 0.40.

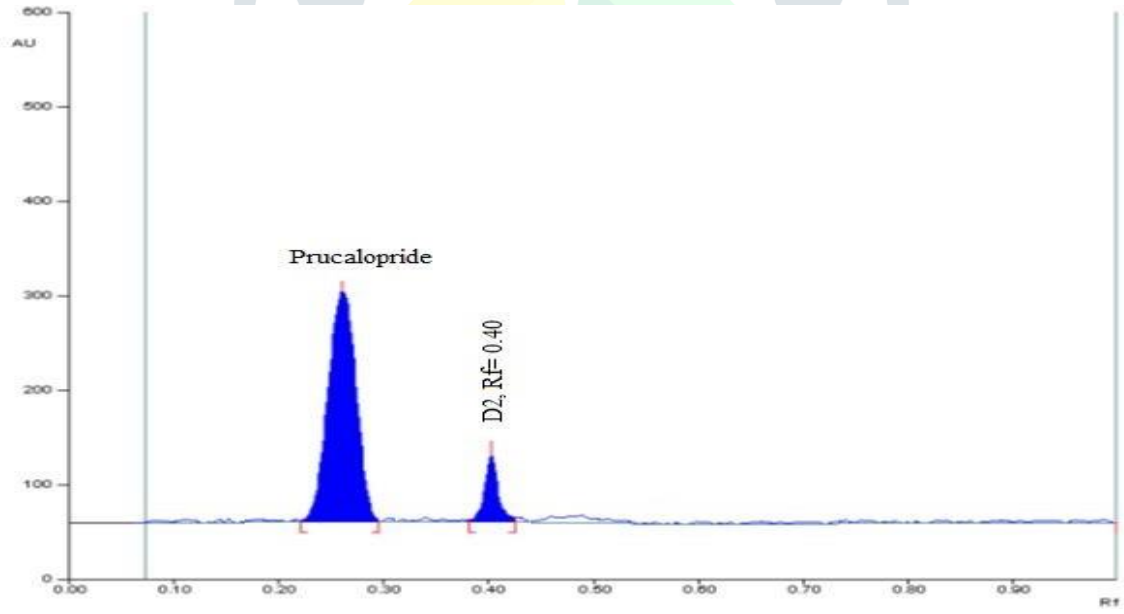


Figure 4 Densitogram after alkali hydrolysis with degradation peak (D2,Rf=0.40)

**Neutral degradation**:

5 ml of the above methanolic drug solution was mixed 1 ml of water was added and refluxed at 60ºC for 30 min. About 12.10%degradation was observed for the drug under neutral hydrolytic degradation. There was decrease in peak region of drug as compared to initial area and no additional peak for degradation was seen.

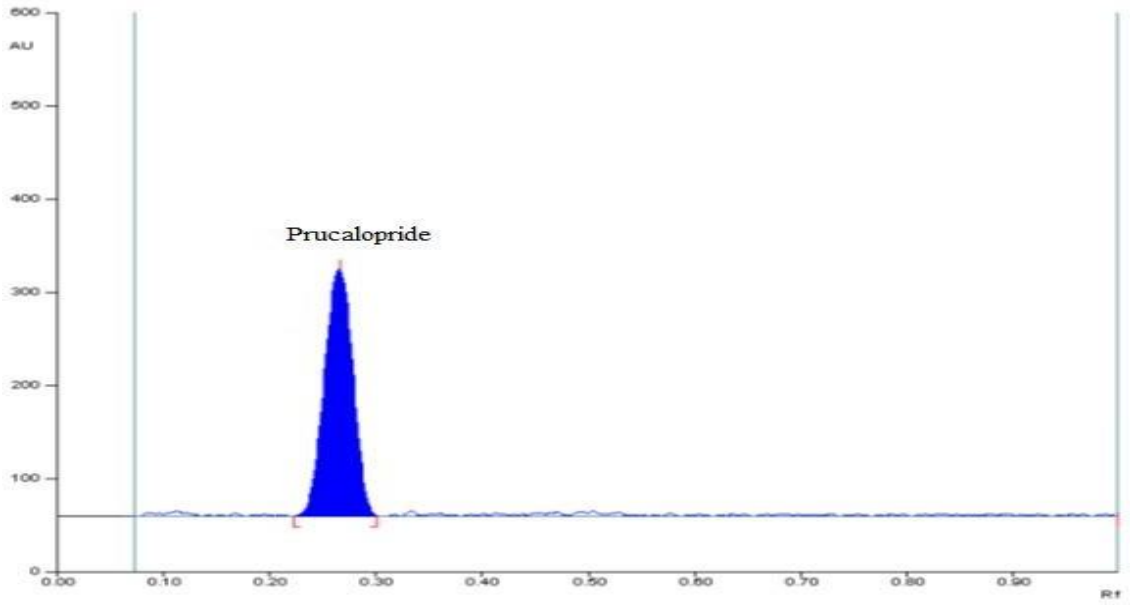


Figure 5 Representative Densitogram of prucalopride after neutral hydrolysis

**Oxidative degradation**:

Oxidative degradation was performed by mixing 5 ml of the above methanolic drug solution with 1 ml of 6 % hydrogen peroxide and refluxed at 60ºC for 30 min.The peak area of drug was reduced as compared to the initial area indicating that the drug undergoes degradation in oxidative stress condition.20.65 % degradation was observed with single degradation product at Rf value 0.48.

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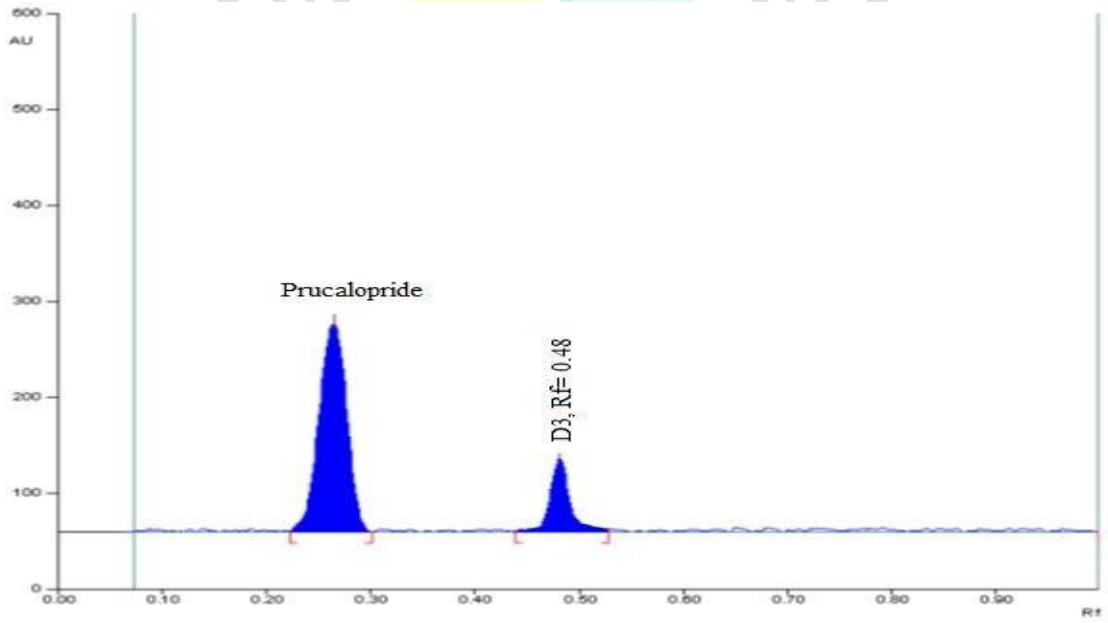



Figure 6 Densitogram with oxidative degradation product after (D3,Rf=0.48)

**Thermal degradation**

The reduction in the peak area of drug as compared to the initial area indicated 7.90% degradation but no additional degradation peak was observed. For a sample heated by dry heat, a representative densitogram was developed.

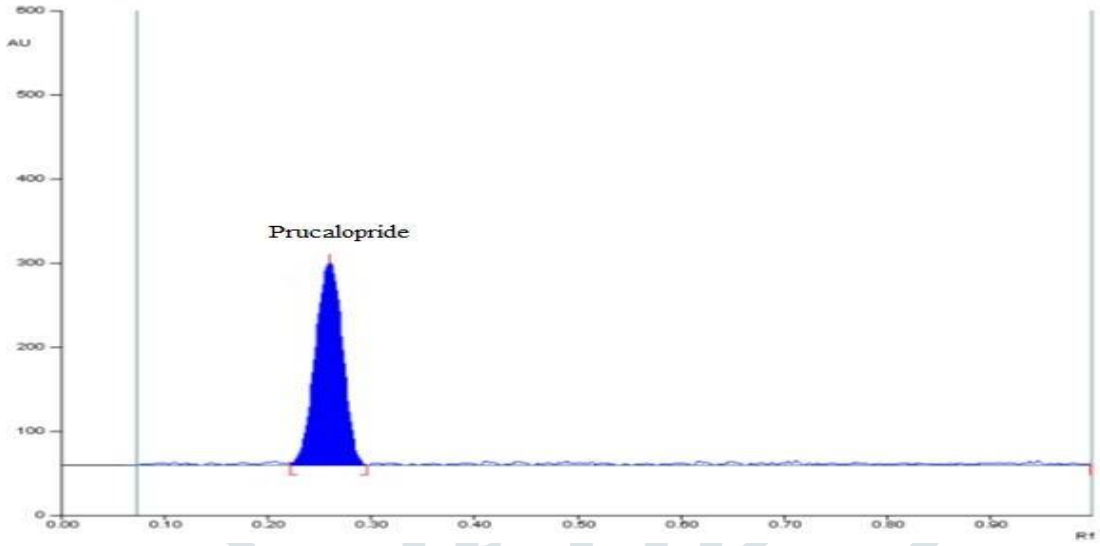



Figure 7 Densitogram obtained after dry heat degradation at70ºC for 6 h

**Degradation under dry heat:**

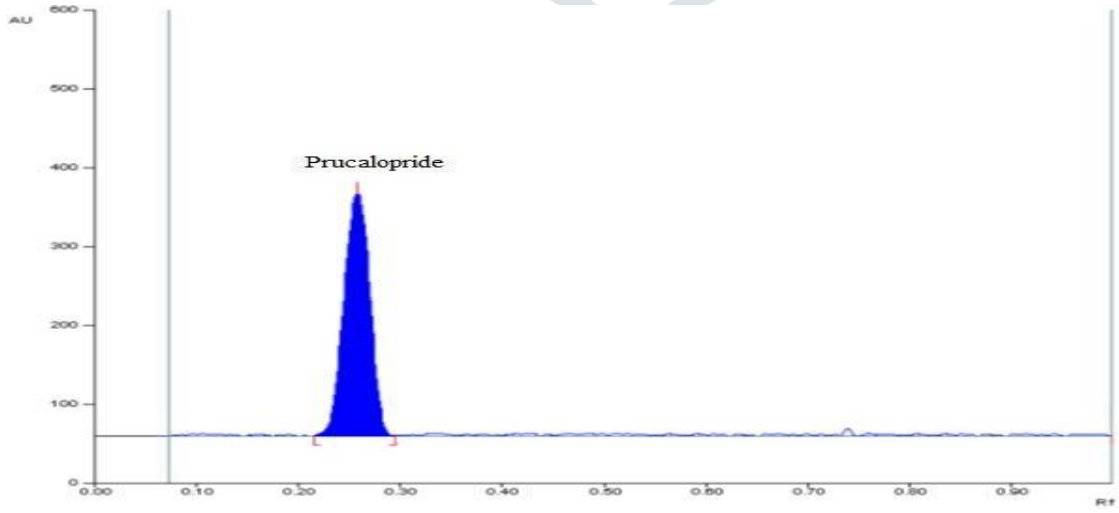
The study was undertaken by placing drug powder in oven at 70ºC for 6 h. A sample was removed from oven at suitable times and dissolved in methanol to obtain solution of 50 ng $\mu l^{-1}$.

**Photo-degradation**:

The Prucalopride standard, 10 mg was exposed to UV light in a UV chamber and sunlight for 24 hours and appropriate dilutions were made in methanol to obtain final concentration of 50ng $\mu l^{-1}$followed by 4µL

Figure 8 Densitogram obtained after exposure of drug to UV light

application, development and scanning under ideal chromatographic circumstances




Marked degradation in the densitogram was observed but the deteriorated products were well resolved from the drug indicating specificity of the method.

**Results of stress degradation study of Prucalopride succinate**

| Stress conditions /duration | %Recovered | %Degradation |
|---|---|---|
| Acidic/0.1NHCl/Refluxedat60°Cfor 30min | 80.96 | 19.04 |
| Alkaline/0.1NNaOH/Refluxedat60°C for 30min | 82.81 | 17.18 |
| Oxidative/6%$H_2O_2$/Refluxedat60°Cfor 30min | 79.35 | 20.65 |
| Neutral/$H_2O$/Refluxat60°for30min | 87.89 | 12.10 |
| Photolysis: UV light 200 watthsquaremeter$^{-1}$7days | 99.09 | ---- |
| Dry heat /70°C/6h | 92.09 | 07.90 |

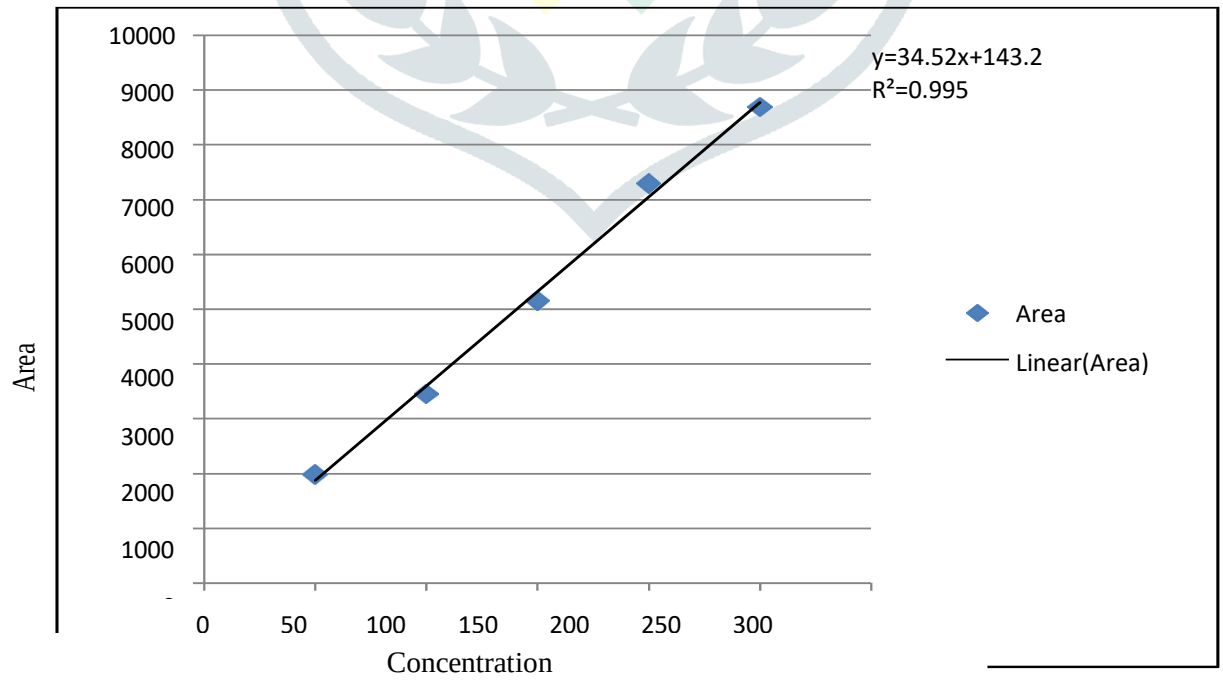
Table:1 Summary of forced degradation studies

**Validation of Method**

The method was validated in compliance with ICH guidelines.

**Linearity and Range:**

Volumes 1, 2, 3, 4 and 5 µl from standard drug solution (50 ng/µL$^{-1}$) were applied on the TLC plates with sample applicator in nitrogen stream. After spotting, plates were dried. Regression data obtained from calibration curve demonstrated excellent linear relationship over 50-250 ng band-1 concentration range.




Fig–3 Linearity curve of prucalopride succinate

Straight-line calibration curves were achieved in the 50-250 ng band$^{-1}$ concentration range through high correlation coefficient. Excellent correlation exists between peak area and concentration of drug within the

concentration range indicated above. Correlation coefficient closer to 1 for the drug also proved the linearity.

| Concentration (ng/band$^{-1}$) | Area 1 | Area 2 | Area 3 | Area 4 | Area 5 | Area 6 | Avg. Area* |
|---|---|---|---|---|---|---|---|
| 50 | 1968 | 1924 | 2088 | 1994 | 2015 | 1932 | 1987 |
| 100 | 3441 | 3417 | 3554 | 3459 | 3513 | 3369 | 3459 |
| 150 | 5193 | 5104 | 5339 | 5113 | 5219 | 5027 | 5165 |
| 200 | 7336 | 7254 | 7459 | 7245 | 7369 | 7178 | 7306 |
| 250 | 8748 | 8662 | 8759 | 8703 | 8727 | 8573 | 8695 |

Table: 2 Linearity of Prucalopride Succinate.

**Precision**

Intra and inter day deviations in results were examined by recording the values of peak area after application, three distinct concentrations in linearity range. The lesser %RSD values (<2) obtained indicate the precision of the developed method.

**1. Intra-day precision**

It was determined by analyzing prucalopride standard solutions at three different concentrations in linearity range for thrice on the corresponding day. Each concentration was spotted in triplicate and % R.S.D. was determined. The %RSD value for Intra-day precision were between 0.82 to 1.03

| Concentration applied (ng/band$^{-1}$) | Average area | %RSD |
|---|---|---|
| 100 | 3580 | 0.82 |
| 150 | 5295 | 1.03 |
| 200 | 7080 | 0.84 |

Table: 3 Intra-day precision

**2. Inter-day precision**

Standard drug solutions at three different concentrations on different three days during the course of a week were analyzed and % R.S.D. was calculated. The %RSD value for Inter-day precision were in the range of 0.79 to1.09

| Concentration applied (ng band$^{-1}$) | Average Area | %R.S.D.* |
|---|---|---|
| 100 | 3586 | 1.09 |
| 150 | 5305 | 0.79 |
| 200 | 7061 | 0.84 |

Table: 4 Inter-day precision

**Detection and Quantitation limit**

From the linearity data the Limit of quantification and detection was calculated, using the formula LOD= 3.3 σ/S and LOQ= 10 σ/S where σ represents standard deviation of response i.e., y-intercept while S denotes the calibration's slope graph. The LOD & LOQ revealed to be 6.85 ng/band$^{-1}$ and 20.77ng/band$^{-1}$ respectively.

**Specificity**

The excipients from formulation did not show any interference with analyte peak and obtained peak purity value was in the limit representing method specificity. The non-interference of degradation product peaks with the drug peak also proved that method is specific.

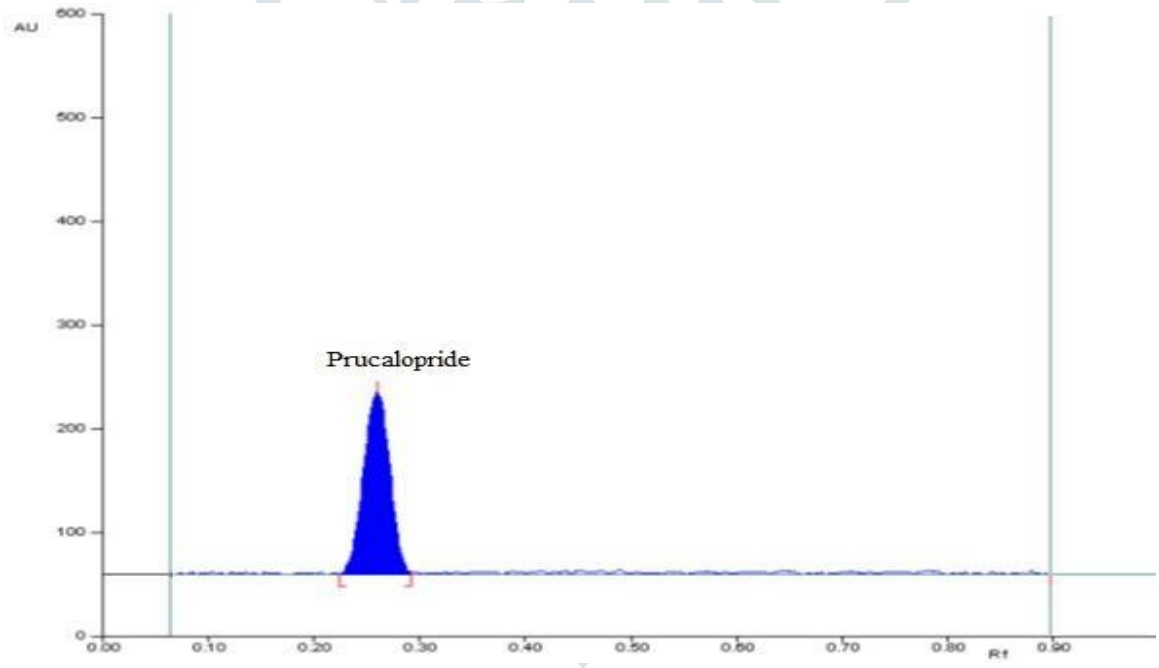



Figure 9 Densitogram of marketed formulation with concentration 100ng/band-1 (Rf= 0.25± 0.08)

**Assay**

Under the ideal chromatographic conditions, 2 ml of the drug from the sample stock solution was applied to HPTLC plates and examined. 99.99% of the medication was found to be present.

| Spotted concentration (ng/band$^{-1}$) | %Recovery | Std. Dev. | %R.S.D.* |
|---|---|---|---|
| 100 | 99.99 | 0.91 | 0.91 |

Table: 5 assay of prucalopride succinate

**Accuracy**

Recovery studies were undertaken to check method accuracy through addition of standard drug to pre-analyzed sample at three different levels (80, 100 and 120 %). The sample concentration selected was 100 ng band$^{-1}$ from tablet solution. The % average recovery was 100.28 ± 0.73 which indicated accurateness of developed method for determining drug dosage in tablet form.

| Drug | Amount taken (ng/band$^{-1}$) | Amount added (ng/band$^{-1}$) | Amount found (ng/band$^{-1}$) | %Recovery ± RSD |
|---|---|---|---|---|
| Prucalopride succinate | 100 | 80 | 180.29 | 100.15±0.82 |
| | 100 | 100 | 199.82 | 99.90±0.67 |
| | 100 | 120 | 221.79 | 100.81±0.70 |

Table: 6 Results of recovery studies

**Robustness**

By performing the analysis, the robustness of the method was determined under conditions during which mobile phase composition (± 2 % methanol), wavelength (± 1 nm) was altered and the effect on the area of drug was noted. The study was performed at concentration level of 250 ng/band [1].

| Parameters | %R.S.D.* |
|---|---|
| Composition of mobile phase (±2%methanol) | 1.01 |
| Wavelength (±1 nm) | 1.30 |

Table: 7 Robustness date

The overall review of validation parameters is given in below table:

Table: 8 Summary of validation parameters

| Sr. No. | Parameters | Results |
|---|---|---|
| 1. | Linearity(ngband$^{-1}$) | 50-250 |
| 2. | Correlation coefficient(r) | 0.995 |
| 3. | Detection limit(ngband$^{-1}$) | 6.85 |
| 4. | Quantitation limit (ngband$^{-1}$) | 20.77 |
| 5. | Accuracy | 100.28±0.73 |
| 6 | Precision(%R.S.D.) | |
| | Intraday precision | 0.82-1.03 |
| | Interday precision | 0.79-1.09 |
| 7. | Robustness | Robust |

## DISCUSSION

The development and validation of the prucalopride succinate medication in bulk and medicinal dose form is the purpose of the HPTLC approach. The absorption spectra for prucalopride succinate were recorded in the wavelength region of 200-400 nm and it determined to be 240nm. The accuracy of the method was confirmed by adding known amount of the pure drug to the formulation previously analyzed by this method and the analytical data was reported. The development and validation method and the stability studies of drug by HPTLC is important for selecting formulation and storage conditions for drug and drug products. Retention time prucalopride succinate 0.25 ± 0.10 was found shown in figure 6. The proposed method's intraday and interday precision was validated. The %RSD found less than 2.

## CONCLUSION

For the estimation of prucalopride succinate in tablet dosage form, a stable HPTLC technique has been developed and validated that does not depend on excipients or degradant products under many circumstances, including photolysis, thermal oxidation, hydrolysis, and oxidation. The outcomes showed that the technique is very specific and that the drug and its degradation compounds were successfully separated.

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