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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING METHOD FOR DETERMINATION OF PRUCALOPRIDE IN TABLET DOSAGE FORM BY RP-HPLC

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Abstract

Prucalopride (5-HT_4 receptor agonist) is used to treat chronic constipation. A simple and sensitive RP-HPLC method was developed for the quantification of prucalopride in tablets. The separation was carried on Hypersil ODS Column (125×4 mm, 5µ) column with using acetonitrile and 0.01% formic acid (80: 20% v/v) as mobile phase with 0.6 mL/min flow rate. The detention was carried at 238 nm with a runtime of 4 min. The elution of prucalopride occurred at 2.844 ± 0.07. The method was linearity over a range of 0-110 µg/mL (r^2 = 0.999). The % recovery of prucalopride was found to be 99.00% with good precision. The method was found appropriate for routine quantification of prucalopride in the solid dosage form and bulk drug. From forced degradation studies, prucalopride was susceptible to oxidative degradation.

Keywords: Prucalopride, RP-HPLC, Method validation, Stability studies, ICH guidelines

INTRODUCTION

Prucalopride is a dihydrobenzo furan carboxamide derivative used in the therapy of chronic constipation and it has selective 5-HT_4 receptor agonistic activity [1-3]. Prucalopride is an approved drug to relief the symptoms of chronic constipation in females at a normal dose of 2 mg per day. The adverse events (AEs) of prucalopride are generally well tolerated, mild to moderate, and transient in nature [4-5].

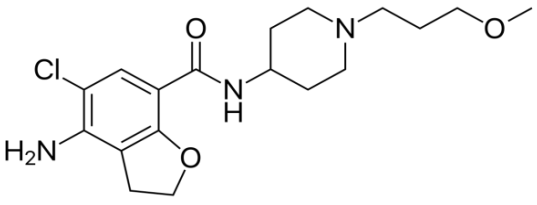

Fig 1: Structure of prucalopride

According to the literature review, some analytical techniques such as LC-MS (Liquid Chromatography-Mass Spectroscopy) [6-7], HPLC (High Performance-Liquid Chromatography) [8] methods have been reported, but these methods are not well established and not much reliable. In the present study, an effort was put together to

develop a simple and accurate method to estimate prucalopride in tablet dosage form and bulk in accordance with ICH (International Council for Harmonization) guidelines [9].

MATERIALS AND METHODS

Instrumentation

Agilent Technologies 1260 Infinity Binary HPLC equipped with photodiode array detector (PAD) was used for chromatographic studies. Absorbances were conducted using a UV-Visible spectrophotometer (Shimadzu UV1800). The materials were weighed using a Shimadzu BL220H digital scale.

Chemicals and Reagents

The pure drug of prucalopride was gifted from Shree Icon Pharmaceutical Laboratories, Vijayawada, India. The commercial formulation (PRUVICT, 2 mg) of prucalopride was purchased from the market. Grade HPLC grade methanol and acetonitrile (Merck (India) Ltd., Mumbai) and analytical grade orthophosphoric acid, formic acid, and triethyl amine were procured and used without further purification. Throughout the experiment, freshly prepared buffer solution was used.

Experimental Conditions

The elution was performed using a mobile phase consisting of acetonitrile (ACN): 0.1% formic acid (80:20 v/v) at a flow rate of 0.6 mL/min on a Hypersil, ODS (octadecylsilyl) column (125×4 mm; 5 µm). The wavelength of 238 nm was used to measure the detect the elution of the drug.

Preparation of Standards

Standard stock solution Preparation

Precisely weighed 10 mg of prucalopride was transferred into a 10 mL clean and dry volumetric flask and ACN was used as a solvent. The drug was dissolved completely under sonication for 5 minutes. Further the solution was diluted up to the mark using ACN, to obtain a stock concentration of 1000 µg/mL of prucalopride. Further dilutions were made using ACN: water (80:20) as diluent.

Preparation of Working Standards

The above standard stock solution (1000 µg/mL) was used to prepare the plucalopride standard working solutions. Different aliquots from the standard stock solution were taken and diluted with diluents separately to prepare solutions of different concentrations of 30, 50, 70, 90, and 110 µg/mL.

Preparation of sample solution

Commercial tablets available in the market were taken and made into fine powder by crushing in a motor and pestle. About 10 mg equivalent of powder prucalopride was added to 10 mL volumetric flask and the powder was dissolved using ACN. The obtained solution was sonicated for 5 minutes and filtered. The solution was further diluted with the mobile phase to produce a stock concentration of 1000 µg/mL. One mL of the aforementioned stock solution was pipette out into a 10 mL volumetric flask and the remaining volume was made up with diluents to obtain a concentration of 10 µg/mL.

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Forced Degradation Studies

The degradation studies on prucalopride was performed using three parameters, viz. oxidative degradation, acidic hydrolysis and alkali degradation. The acidic hydrolysis was carried using 2M HCl and alkali degradation was carried using 2M NaOH. About 20% H_2O_2 was used as oxidizing agent for oxidative degradation to 1 mL of prucalopride sample. The samples were analyzed by injecting to HPLC column [10].

RESULTS AND DISCUSSION

Method Development

For the selection of mobile phase, a wide variety of solvent combinations based on solubility data were tried. Various combinations of methanol, acetonitrile with water and buffers such as orthophosphate, phosphate buffer, ammonia buffer and formate buffer were used. A good separation with better sensitivity was achieved with the combination of acetonitrile and 0.01% formic acid (80:20% v/v) as mobile phase, with a flow rate of 0.6 mL/min. Using Hypersil ODS column (125×4 mm, 5µ) the drug retention time was observed at 2.926 ± 0.020 min. Based on the UV spectral data obtained, the detection wavelength was selected as 238 nm.

Validation of Method

System suitability: The prepared standard solution was injected to chromatographic system as discussed in the above section. The system suitability parameters, such as theoretical plates, resolution and asymmetric factor were also studied [11-14].

Specificity: According to its structure, prucalopride showed the wave length of 238 nm in UV spectroscopic study. In a study to ascertain the developed method's specificity, blank and placebo injections were studied under the chromatographic conditions. The excipients utilized in the tablet formulation were determined to have no interference, and the absorbance of the standard and sample showed excellent correlation. The chromatograms of the sample and the standard are presented in Figure 2 and 3.

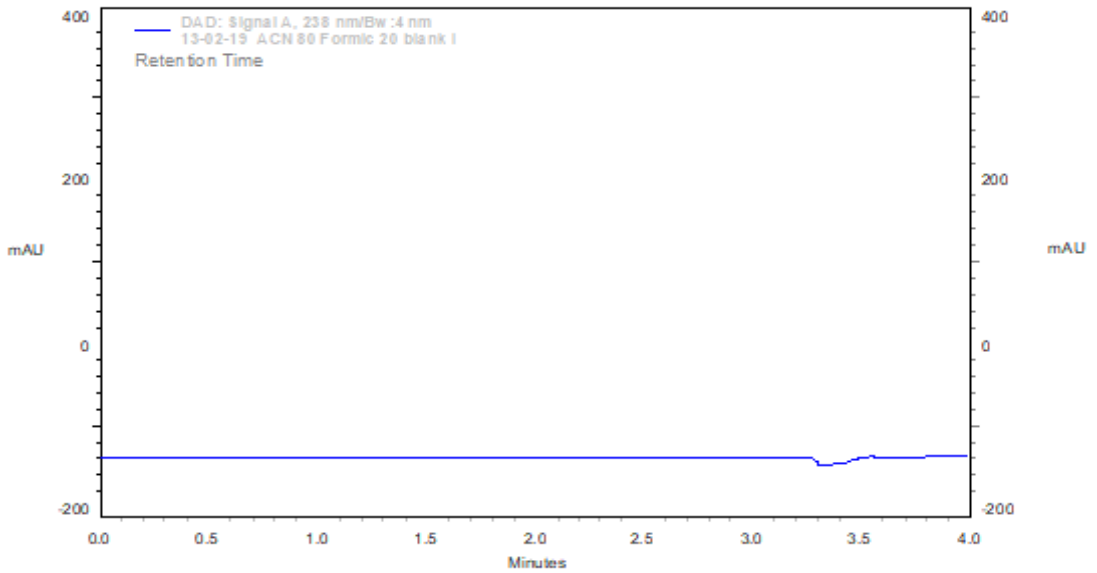

Fig 2: Blank chromatogram

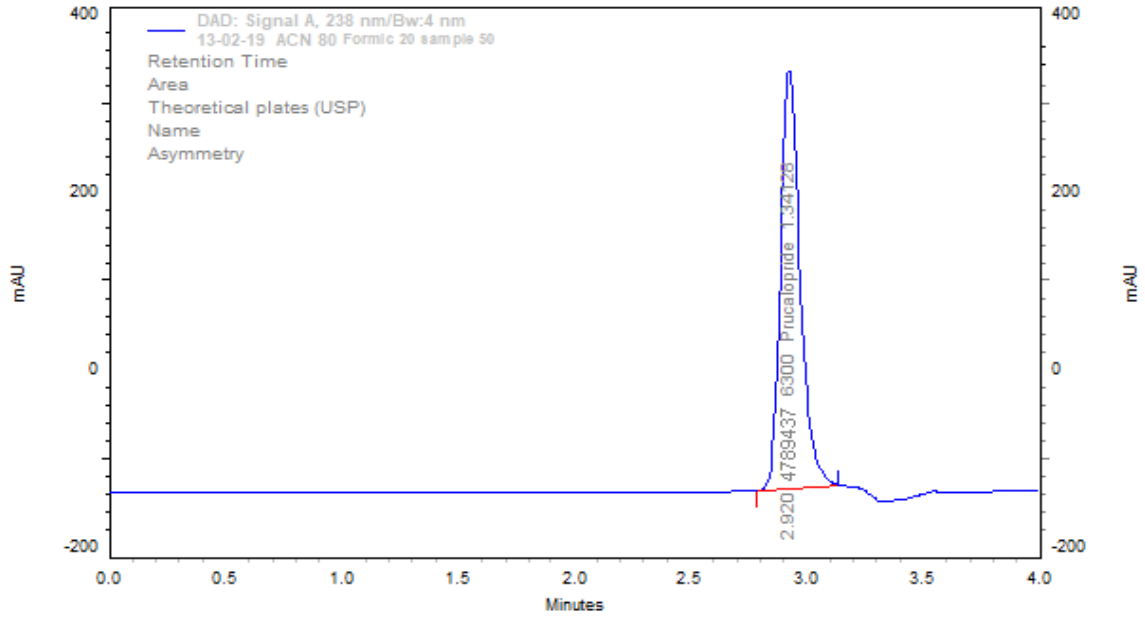

Fig 3: Standard chromatogram of prucalopride

Linearity: Linearity was established by assessing prucalopride with varying concentrations i.e., 30-11 0 µg/mL in triplicate. Regression analysis determined by using the least squares method was used to prove linearity. The linear regression equation was found to be y=93614x + 1623.7 with 0.999 regression coefficient, shows a prefect peak area and the prucalopride concentrations were correlated. The calibrating curve is presented in Figure 4.

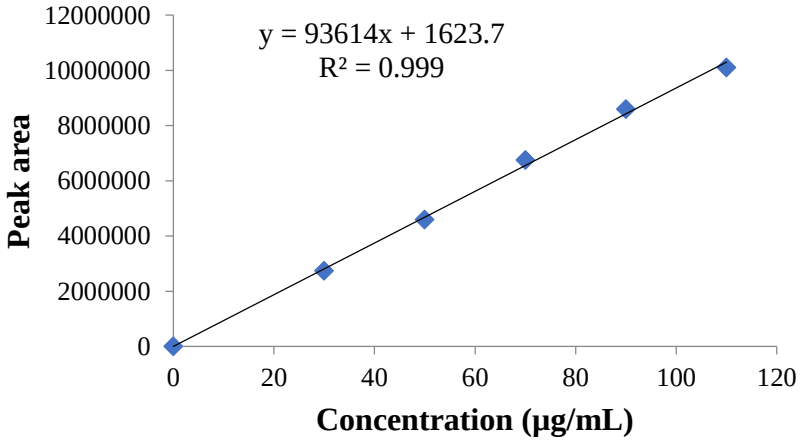

Fig 4: Calibration graph of prucalopride.

Precision: Precision is the degree to which an analytical technique can be repeated under typical operational conditions. To evaluate intra-day and inter-day precision, the analyses of drug were conducted six times on the same day and six additional times on different days. With a six-replicate mixed standard solution at the same concentration, prucalopride was tested, and the %RSD was calculated. For intra-day and inter-day precision, the %RSD was found to be 0.071% and 0.151%, respectively. These values fall within the permissible range of RSD with not more than 2.0. Results of intraday and interday precision are given in Table 1.

Table 1: Results of intra-day and inter-day precision for prucalopride.

Sl. No.	Intra-day Time (Hours)	Peak area	Inter-day Time (Days)	Peak area (n=3)
1	0	6603489	Day 1	6719398
2	3	6611279		
3	6	6599687	Day 2	6725585
4	9	6599234		
5	12	6598743	Day 3	6705743
6	15	6602648		
Mean		6602513	Mean	6716909
SD		40707	SD	10153
%RSD		0.071	%RSD	0.151

Accuracy: The accuracy of the developed method was assessed by standard addition approach. To the pre-analyzed standard, a known volume of the sample drug solution was added to the mixture. Thus, prepared solutions at a three concentration levels, 80%, 100% and 120% were evaluated in triplicate. The computed mean % recovery was determined to be 100.19% with 0.94 %RSD. It indicates that the proposed method was accurate.

Robustness

By making solutions according to the method and injecting them under various variable conditions, the robustness of the method was determined by considering parameters such as flow rate, mobile phase composition, and wavelength. The flow rate was increased by 0.1 mL, wavelength by ± 2 nm, mobile phase composition by 5% i.e., acetonitrile:buffer from (80:20% v/v) to (75:25%v/v). Parameters for system appropriateness and procedure, precision were compared. The robustness data of the developed method are given in Table 2.

Table 2: Robustness data of prucalopride.

Sl. No.	Parameter	Optimized Conditions	Used	Rt (min)	Peak area
1	Flow rate	0.6 mL/min	0.5 mL/min	2.90	6704369
			0.7 mL/min	2.89	6710468
2	Wavelength	238 nm	236 nm	2.91	6713460
			240 nm	2.90	6705634
3	Mobile Phase Composition	ACN:Buffer (80:20% v/v)	75:25	2.85	6706314
			85:15	2.90	6690341

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD is the lowest concentration of an analyte at which a quantifiable response occurs. The LOQ is the lowest concentration that can be measured consistently with a given level of accuracy and precision. For this study, the analytes were taken in six replicates which were tested and quantified at their lowest concentration. The LOD and LOQ were determined to be 0.005 µg/mL and 0.016 µg/mL, respectively.

Estimation of Prucalopride

PRUVICT, a commercial tablet dosage form, was used to evaluate the effectiveness of the method developed to identify prucalopride in formulations. Accurately weighed small quantity of powder sample of 20 tablets, which is equivalent to 10 µg of prucalopride was transferred into a volumetric flask of 10 mL capacity. The contents of the flaskwere sonicated for approximately 5 minutes and ensured that the drug was completely soluble and could be diluted with mobile phase. About 0.7 mL of the

aforementioned stock solution was pipetteted out into a 10 mL volumetric flask before adding the mobile phase.

Amounts of the drug included in the tablet dosage form were calculated using the standard solutions and sample solutions. The outcomes (Table 3) were compared with the claim on the label of pucalopride in tablet dosage forms. The assay yielded 99.00%. The assay value (% yield) indicates the developed is suitable for estimation of analyte in tablet dosage form.

Table 3: Assay of prucalopride formulation

Formulation		Label claim	Amount found	%Assay
PRUVICT	Prucalopride	2 mg	1.98 mg	99.00%

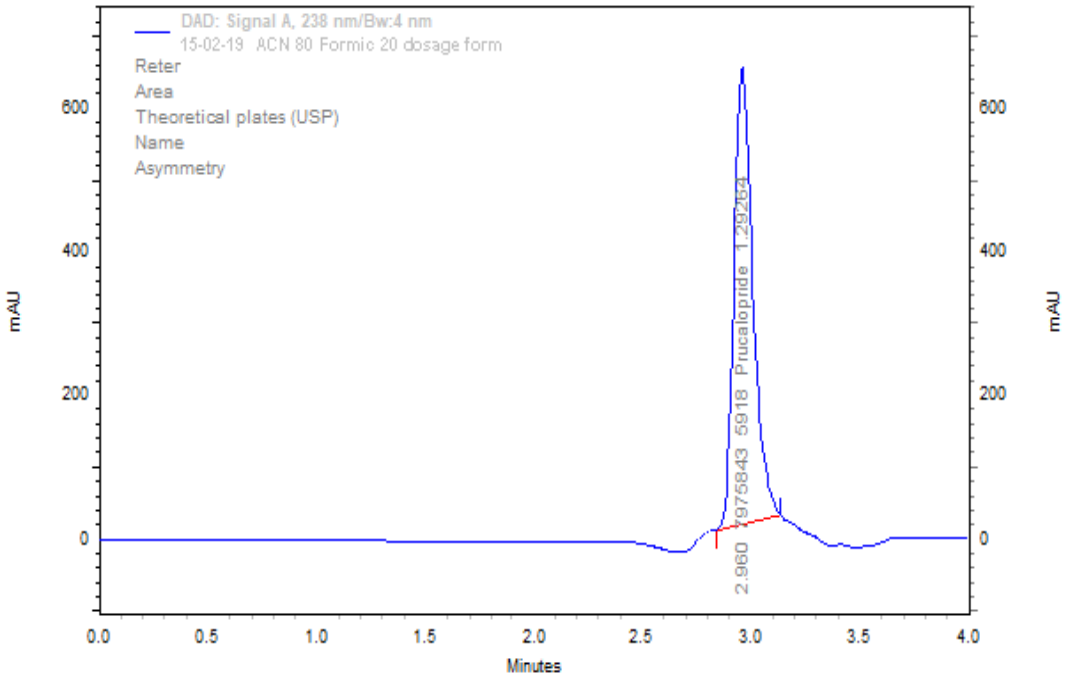

Fig 5: Sample chromatogram of prucalopride.

Stability Indicating Studies

Drug degradation studies carried out for the samples were prone to various stress conditions. Data are compiled in the Table 4 and the chromatograms are given in Figure 6. From the data obtained, the forced degradation study proved that the method was highly specific and the drug prucalopride was sensitive to oxidative degradation.

Table 4: Forced degradation data of prucalopride.

Type of Degradation	Retention Time (min)	Area (mAU)	% Assay	% Degradation
Standard (100 µg/mL)	2.813	16255711	-	-
Acid Hydrolysis	2.813	14547208	89.49	10.51
Alkaline Hydrolysis	2.813	14389923	88.52	11.48
Oxidative Hydrolysis	2.793	12383413	76.18	23.82
Neutral Degradation	2.813	15983472	98.33	1.67

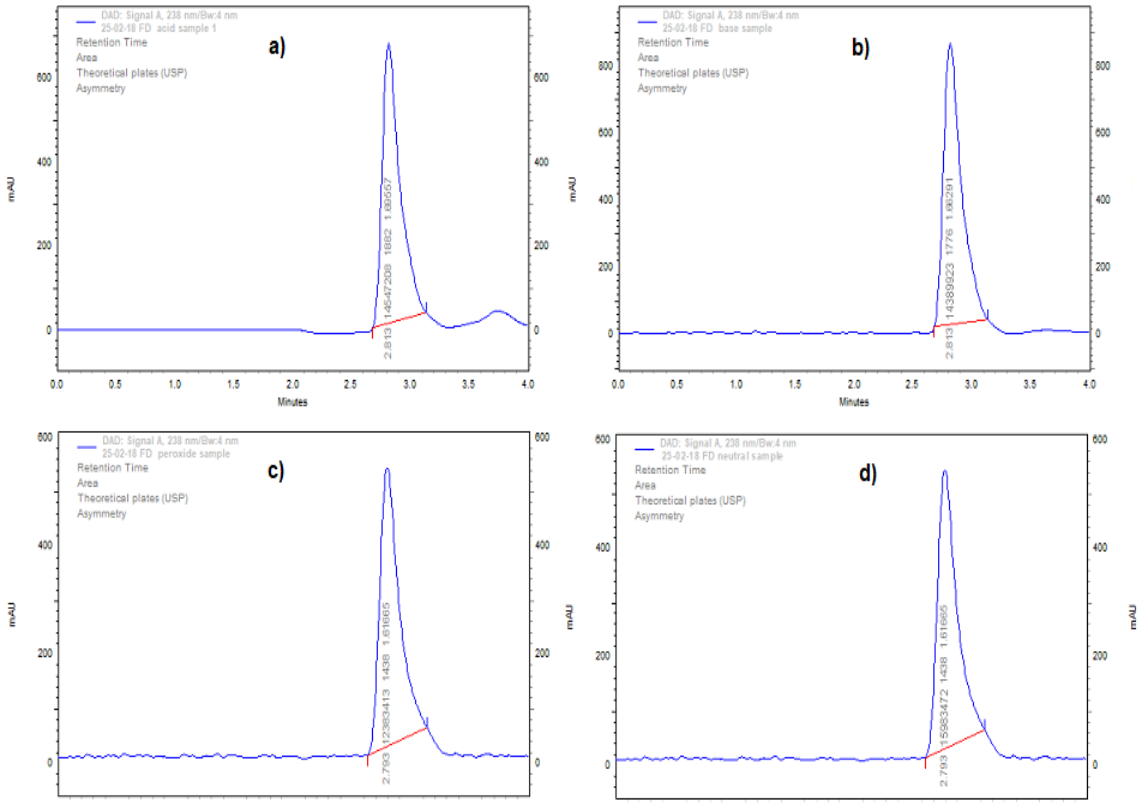

Figure 6: Chromatograms of stress studies a) acid, b) alkaline, c) peroxide and d) neutral degradative studies.

CONCLUSION

A simple, sensitive, and accurate HPLC method was developed for the estimation of prucalopride in tablet formulations and bulk drugs. The results were satisfied with the good recoveries supporting the applicability of the developed method for the regular quality control study of prucalopride in pharmaceutical dosage forms. The developed method was found valid with the ICH guidelines, and the outcomes of validation were acceptable. Prucalopride is susceptible to oxidative degradation, according to investigations on forced degradation studies. To conclude, the HPLC method is more cost-effective and accurate for analyzing prucalopride in pharmaceutical formulations and bulk drugs.

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