IJBPAS, September, 2022, 11(9): 4184-4191

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

UV VISIBLE SPECTROPHOTOMETRIC ESTIMATION OF PRUCALOPRIDE IN BULK AND ITS PHARMACEUTICAL FORMULATION BY DIAZOTIZATION METHOD USING 2-NAPHTHOL

D. VENKATESWARLU[1*], P. MALLESWARAREDDY[2] AND I.E. CHAKRAVARTHY[1]

1: Department of Chemistry, Rayalaseema University (Erstwhile, SKU PG Centre, Affiliated to Sri Krishnadevaraya University, Ananthapuramu.), Kurnool, AP-518004

2: Department of Chemistry, Sri Krishnadevaraya University, Anantapuramu, AP-515003, India

***Corresponding Author: D. Venkateswarlu: E Mail: pmeshvarreddy@gmail.com**

Received 16th Oct. 2021; Revised 20th Nov. 2021; Accepted 20th Jan. 2022; Available online 1st Sept. 2022

https://doi.org/10.31032/IJBPAS/2022/11.9.6367

ABSTRACT

A simple, sensitive, rapid and accurate spectrophotometric method has been developed for the estimation of prucalopride in pharmaceutical formulations. The proposed method was based on the formation of azo dye with 2-naphthol which form brown red color complex of prucalopride with 2-naphthol. The absorbance of the extractable azodye is measured at the wavelength of maximum absorbance 500 nm against the reagent blank. Results obtained are statistically validated and found to be reproducible.

Keywords: Spectrophotometry; prucalopride: 2-naphthol; Azo dye

INTRODUCTION

Prucalopride is 4-amino-5-chloro-N-[1-(3-methoxypropyl) piperidin-4-yl]-2,3-dihydro-1-benzofuran-7-carboxamide,

Prucalopride is a serotonin type 4 (5-HT4) receptor agonist that has potent prokinetic activity and is used as therapy for chronic idiopathic constipation. Prucalopride has been associated with a minimal rate of transient serum enzyme elevations during therapy and has not been implicated in cases of clinically apparent liver injury with jaundice.

Several analytical methods have been reported for assay of prucalopride which includes UV Spectrophotometric method **[1]**, RP-HPLC method **[2]**, UHPLC method **[3]**,

Pharmacokinetic method **[4]**, LC QTOF MS method **[5]**.

Materials and method

Instrument: All measurement were done on Milton Roy 1001spectrophotometer by using 10 mm matched quartz cuvettes.

Materials and reagents:

Preparation of reagents and solutions:

Sodium nitrite (2.0 N):13.78 g of Sodium nitrite (Merck) is dissolved in distilled water and the resulting solution is made up to the mark in 100 ml standard flask with distilled water.

Sodium Hydroxide Solution (10%): 10 g of Sodium Hydroxide (Merck) dissolved in 100 ml of distilled water.

2- Naphthol (3.0 M): 43.23 f of 2- Naphthol (Merck) dissolved in 100 ml of 10% Sodium Hydroxide (Merck) Solution.

Concentrated Hydrochloric Acid: 36 % (Merck) is directly used at necessary analytical region.

Method

The amino group in prucalopride is diazotized with sodium nitrite and hydrochloric acid at 0°C temperature. After diazotization, the diazonium salt is coupled with 2-naphthol. The orange red colored chromogen formed in the method is stable for more than 24 hours. The orange red colored chromogen is used to determine the prucalopride spectrophotometrically.

Prucalopride could be readily diazotized in acid medium and the resultant diazonium cation would then react with coupling reagent 2-naphthol by electrophilic substitution at the position ortho to the phenolic hydroxyl group of 2-naphthol results in the formation of the colored product. The reaction sequence can be shown in **Figure 1**.

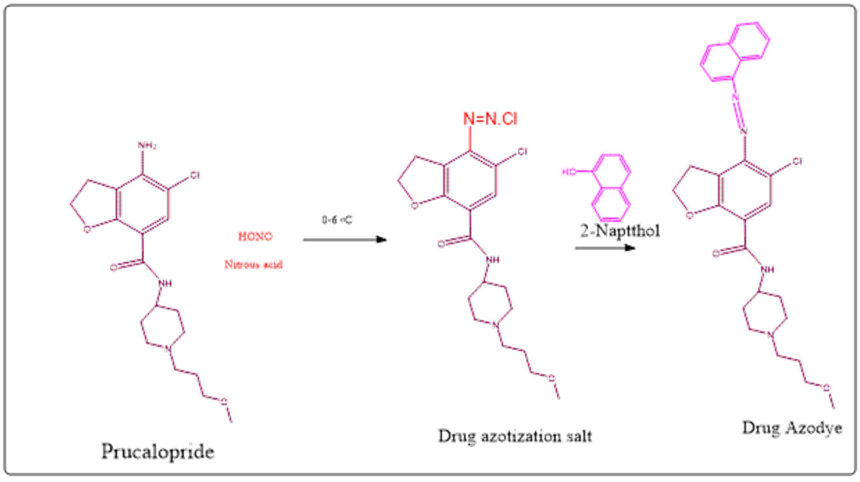

Figure 1: Schematic diagram of azotisation reaction of prucalopride

Spectrum of diazotized prucalopride:

The wavelength of maximum absorbance of the diazotised drug treated with 2-naphthol solution is ascertained by the following procedure.1 ml of prucalopride solution is transferred into a 10 ml volumetric flask. To this, 1.0 ml of 0.1N hydrochloric acid and 1.5 ml of cold 0.1N sodium nitrite solution are added. The resultant solution is well mixed, and then allowed to stand for five minutes at 0-5^{o}C temperature for diazotization. To this solution 1.0 ml of 1% urea solution is added and shaken frequently for nitrogen gas to escape. Then 1.0 ml of 0.5N sodium hydroxide and 1.0 ml of 2-naphthol solutions are added and the volume is made up to 10 ml with methanol. The absorbance of the orange red colour formed is measured in the wavelength range of 400 to 650 nm, against the reagent blank. The spectrum is given in **Figure 2**.

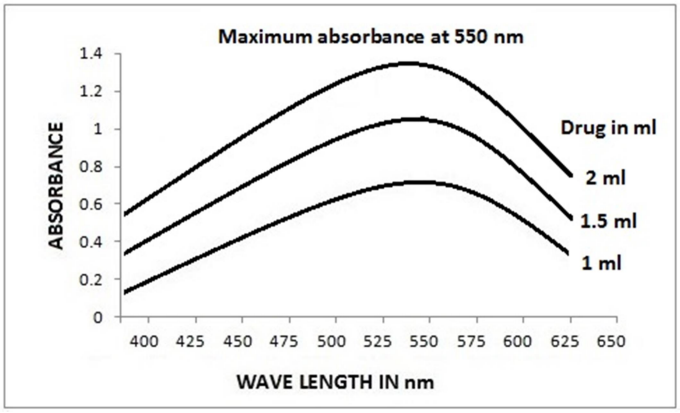

Figure 2: Absorption spectrum of prucalopride-2-naphthol azo dye

From **Figure 2** it is clear that the diazotised drug treated with 2-naphthol solution has maximum absorbance at 500 nm. Hence, all further studies are made at 500 nm. The optimal conditions for the determination of prucalopride are arrived at by the following steps.

(i). Effect of concentration of hydrochloric acid on the diazotization and coupling reaction

The stability of the colour species depends on the concentration of hydrochloric acid. The effect of hydrochloric acid on the absorbance is studied by varying the volume of hydrochloric acid (0.1N) and measuring the absorbance at 500 nm. The data is presented in **Table 1**.

Table 1: Effect of concentration of hydrochloric acid solution on absorbance

Volume of HCl (ml)	Absorbance at 500 nm.
0.5	0.283
1.0	0.651
1.5	0.648
2.0	0.645

The data in **Table 1** show that 1.0 ml of hydrochloric produces maximum absorbance and hence the same concentration is maintained throughout the experimental work.

(ii**). Effect of concentration of sodium nitrite on the absorbance of coupling reaction is studied by the following procedure**

In a series of 10 ml volumetric flasks containing 1.0 ml of prucalopride, 1.0 ml of 0.1N hydrochloric acid, 1.0 ml of 2-naphthol solution, 1.0 ml of 1% urea solution, 1.0 ml of 0.1N sodium hydroxide solution are taken and varying amounts of sodium nitrite are added. The contents are made upto the mark and set aside for 5 minutes for completion of the reaction. The absorbance of the resultant solutions is measured at 500 nm and the data are presented in **Table 2**.

Table 2: Effect of concentration of sodium nitrite

Volume of Sodium nitrite (ml)	Absorbance at 500 nm
0.5	0.247
1.0	0.485
1.5	0.660
2.0	0.641
2.5	0.659

The data in **Table 2** indicate that 1.5 ml of sodium nitrite is necessary for achieving maximum absorbance and hence maintained throughout the experimental studies.

(iii**). Effect of concentration of 2-naphthol on the coupling reaction is studied by the following procedure.**

In a series of 10 ml volumetric flasks containing 1.0 ml of prucalopride, 1.0 ml of 0.1N hydrochloric acid, 1.5 ml of 0.1N sodium nitrite solution, 1.0 ml of 1% urea solution, 1.0 ml of 0.1N sodium hydroxide solution are taken and varying amounts of 2-naphthol are added. The contents are made upto the mark and set aside for 5 minutes for completion of the reaction. The absorbance of the resultant solutions is measured at 500nm and the data are presented in **Table 3**.

Table 3: Effect of concentration of 2-naphthol

Volume of 2-naphthol (ml)	Absorbance at 500 nm.
0.5	0.387
1.0	0.657
1.5	0.682
2.0	0.678

.

The data in **Table 3** indicate that 1.0 ml of 2- naphthol is necessary for achieving maximum absorbance and hence maintained throughout the experimental studies

(b) Construction of Calibration Curve:

To study the effect of drug concentration on the absorbance of the coupling reaction under optimal conditions now arrived is studied by the following method to know the suitability of the method for the assay of prucalopride.

Various aliquots of the standard prucalopride solution (40 µg/ml) ranging from 0.5, 1.0, 1.5, 2.0, 2.5 ml are transferred into a series of 10 ml volumetric flasks. To each flask, 1.0 ml of 0.1N hydrochloric acid solution and 1.5 ml of cold 0.1N sodium nitrite solution are added. The resultant solution in each flask is well shaken and allowed to stand for five minutes at 0-5^{0}C temperature for diazotization to complete. 1.0 ml of 1% urea solution is added to each flask and the solution is shaken frequently to allow nitrogen gas to escape. Then 1.0 ml of 0.1N sodium hydroxide solution and 1.0 ml of 2-naphthol solution are added and the volume in each flask is made upto 10 ml with methanol. The absorbance of the orange red colour solution is measured at 500 nm against the reagent blank. Calibration graph is obtained by plotting absorbance values against the concentration of prucalopride solution. The calibration curve is found to be linear over a concentration range of 20 to 100 µg of prucalopride. The amount of prucalopride present in the sample is estimated from the calibration graph. The results are presented in **Figure 3**.

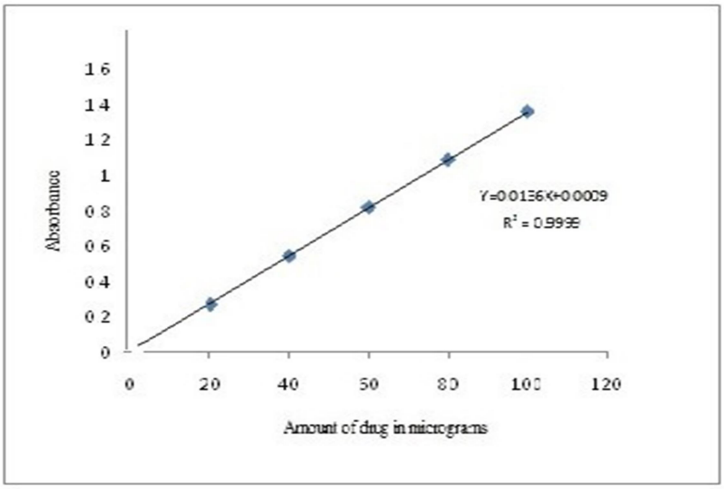

Figure 3: Calibration curve of prucalopride

(c) Assay of prucalopride in pharmaceutical formulations:

The proposed procedure for the assay of prucalopride is applied for its determination in commercial tablets.

Preparation of the sample solution:

Powdered tablet equivalent to 50 mg of the drug is weighed accurately and transferred into a 50 ml beaker and mixed well with 30 ml of methanol. The solution is filtered and transferred into a 50 ml volumetric flask and the volume is made up to 50 ml with methanol. The concentration of the drug solutions is now 1mg/ml. This stock solution is further diluted to obtain the working concentration. The pharmaceutical preparation as prepared above is analysed by the following procedure.

Assay Procedure: Known volumes of the drug formulation prepared as above are transferred into a series of 10 ml volumetric flasks and 1 ml of 0.1N hydrochloric acid solution, 1.5 ml of 0.1N sodium nitrite solution are added. The resultant solution in each flask is shaken well and allowed to stand for five minutes at 0-5^{0}C temperature for diazotization. Then 1.0 ml of 1% urea solution, 1 ml of 0.5N sodium hydroxide and 1.0 ml of 2-naphthol solution are added. The absorbance of the resultant solution is measured at 500 nm. The amount of prucalopride in the pharmaceutical formulation is evaluated from the predetermined calibration plot. The results are present in **Table 5**.

(d) RESULTS AND DISCUSSION:

Prucalopride undergoes diazotization when treated with sodium nitrite and hydrochloric acid. The excess nitrous acid during the diazotization is removed by the addition of urea solution. The solution is shaken frequently to allow the nitrogen gas to escape. The diazonium cation reacts with the coupling reagent, 2-naphthol by electrophilic substitution at the o-position of the coupling agent to produce an orange red azo product. This orange red color product shows maximum absorbance at 500 nm . The color of the product is stable for more than 24 hours. The calibration curve (concentration vs. absorbance) is linear over the range of 20-100µg of prucalopride. The optical characteristics of the proposed method such as absorption maximum, Beer´s law limits, molar absorptivity and Sandall's sensitivity are presented in **Table 4**. The molar absorptivity and Sandall's sensitivity values show sensitivity of the method. The regression analysis using method of least squares is made for the slope (b), intercept (a) and correlation coefficient(r) obtained from different concentrations and results are summarized in the **Table 4**. The value of correlation coefficient is 0.9999, which indicates the good linearity of calibration lines. The values of standard deviation are low, indicate high accuracy and

reproducibility of the method. The 't' calculated values compare well with the theoretical values of 2.78 there by indicating that the precision of the method is good. There is no effect of additives and excipients such as starch, calcium lactose and glucose in the concentrations those present in general pharmaceutical preparations.

The proposed method is found to be simple, precise, accurate and time saving, reproducible and can be conveniently adopted for routine analysis of estimation of prucalopride in bulk drugs samples and pharmaceutical formulations.

Table 4: Optical characteristics of proposed method

Parameters	Proposed method
λmax (nm)	382
Beer's law limit (µg/ml)	20-100
Molar absorptivity (l mole-^{1} cm-^{1})	2.7593X10^{4}
Sandell's sensitivity (µg cm-^{2} / 0.001 absorbance unit)	0.0362
Regression equation (Y = bx+a)	Y=0.0136x+0.0009
Slope (b)	0.00136
Intercept (a)	0.0009
Correlation coefficient (r)	0.9999

***Y = bx+a, where Y is the absorbance and X concentration in µg / ml**

Table 5: Assay of prucalopride in tablets

S. No.	Tablets (mg)	*Amount Found(mg) ±S.D*	% Label claim	*t_{cal}
1	7.5	7.54±0.04	100.53	0.7009
2	7.5	7.51±0.05	100.13	0.7659

***Average of five determination based on the label claim**

REFERENCES

[1] Vaibhavi N. Akhani *et al*, UV Spectrophotometric method for estimation of prucalopride succinate in pharmaceutical dosage form, WJPR, 9(4), 1568-1576, 2020.

[2] Vaibhavi N. Akhani *et al*, UV Spectrophotometric method for estimation of prucalopride succinate in pharmaceutical dosage form, WJPR, 9(4), 1568-1576, 2020.

[3] Virag Gophane and Ravi A Thakur, Development, Validation and Stability Indicating RP-HPLC Method for Estimation of Prucalopride in Pharmaceutical Formulation, Inventi Rapid: Pharm Analysis & Quality Assurance, 2016(3),1-8, 2016.

[4] Zhi Sun, Lihua Zuo, Jian Kang, Lin Zhou Mengmeng, Jia Zeyun Li Zhiheng, Yang Xiaojian, Zhang Zhenfeng Zhu, Development and

validation of a sensitive UHPLC–MS/MS method for quantitation of prucalopride in rat plasma and its application to pharmacokinetics study, Journal of Chromatography B, 1033-1034(1), 328-333, 2016.

[5] Vera Van de Velde, Lieve Vandeplassche, Mieke Hoppenbrouwers, Mark Boterman & Jannie Ausma , Effect of Prucalopride on the Pharmacokinetics of Oral Contraceptives in Healthy Women, , Drugs in R&D , 13(1), 43–51,2013.