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Research article

Method development and validation of Prucalopride succinate in bulk and tablet dosage form by RP-HPLC method

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Key words: RP-HPLC, Prucalopride succinate, RSD and Validation.

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Abstract

A new selective and sensitive High-performance Liquid Chromatography (HPLC) method was developed for the Quantification of Prucalopride succinate in bulk and tablet dosage form. Estimation was achieved by C_{18} column (KROMASIL 150) using Potassium dihydrogen orthophosphate and Methanol in the ratio (60:40% v/v) at ambient temperature, flow rate was 1 ml/min, injection volume 20 µl. The run time for Prucalopride succinate was 3.24 minutes and monitored at 225 nm.

The method was validated to fulfill the International Conference on Harmonization (ICH) requirements and this validation included specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision and robustness. The linearity of the proposed method was at the range of 50-150 µg/ml of Prucalopride succinate with a Correlation coefficient of 0.999 the precision (relative standard deviation – RSD) of six samples was 0.3068. The accuracy (recovery) was 100.90%, 100.05% and 100.80%.

All results were acceptable and this confirmed that the method is suitable for its intended use in routine Quality control and assay of drugs.

Introduction

Prucalopride succinate is dihydrobenzo furan carboxamide derivative acting as a selective agonist for serotonin type 4 receptors (5-HT4) and developed as an enterokinetic agent for the treatment of chronic constipation. Its chemical name is 4-amino-5-chloro-N-[1-(3-methoxypropyl)piperidin- 4-yl]-2,3-dihydro-1-benzofuran-7-carboxamide; butanedioic acid. It stimulates colonic mass movements, which provide the main propulsive force for defecation. Prucalopride has a fewer side effects than other 5-HT_4 agonists such as Tegaserod (Zelnorm) and Cisapride (Propulsid) which at therapeutic concentrations may account for the adverse cardiovascular events by interacting with other receptors, 5-HT1B/D and the cardiac HERG channel, respectively. This medication is used to treat chronic constipation; A literature review revealed has been determined and studied by several procedures; quantitative analysis of Prucalopride succinate (PRCLD) in bulk drug and pharmaceutical dosage. There is no method reported for quantification of PRCLD by HPLC. The structure of Prucalopride succinate is given below

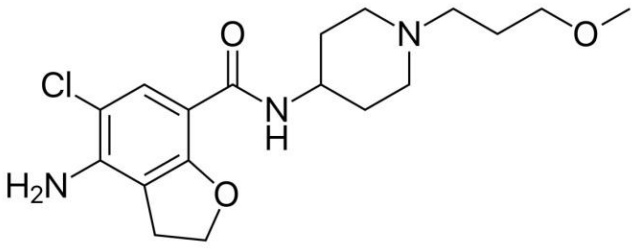


Figure 1. Structure of Prucalopride succinate.

Analytical methods keep on updating with time as per the requirements so as to develop a simple, reliable, cost effective, reproducible and above all a method bearing a high level of accuracy and precision.

A literature review revealed that PRCLD has been determined and studied by several procedures; quantitative analysis of PRCLD in bulk drug and pharmaceutical dosage is formed by Selective separation and characterization of stress degradation products and process impurities of Prucalopride Succinate by LC-QTOF-MS/MS [3] A few clinical method studies are reported [4-5]. There is no method reported for quantification of PRCLD by RP-HPLC.

Our study aimed to develop a rapid, robust, selective, sensitive, and precise HPLC method for the determination of PRCLD.

Experimental

Material and reagents

Pure PRCLD was procured as gift sample from Symed laboratories, Hyderabad, India. PRCLD tablet was purchased from local commercial sources. HPLC grade methanol was procured from Merck India, Mumbai, India. Potassium dihydrogen phosphate and ortho phosphoric acid (S.D. Fine Chemicals, Mumbai, India) were of analytical grade used for the preparation of mobile phase. HPLC grade water acquired from Millipore Milli-Q water system.

Mobile Phase

2.72 g of Potassium dihydrogen ortho phosphate was accurately weighed and dissolved in 1000 ml of water and added 1 ml of tri ethylamine, adjusted the pH 3.7 with ortho phosphoric acid and methanol in ratio of 60: 40.

Instrument Parameter

The isocratic flow rate of mobile phase was maintained at 1ml/min. The injection volume was set as 20 μl. Eluted sample was monitored at 225 nm and the run time was 5mins. The retention time of the PRCLD was about 3.24 min.

Preparation of standard stock solution

25 mg of PRCLD was accurately weighed and transferred into a 25 ml volumetric flask and dissolved in minimum quantity of methanol and made up to 25 ml with the same, to get the concentration 1000 µg/ml.

Selection of wavelength and stability studies

The standard stock solution was diluted with the mobile phase consisting of buffer: methanol (60:40) which is degassed for 15 minute by sonication to get the concentration of 10 µg/ml. The solution was scanned in UV region of 200 nm to 400 nm, which can be used for the estimation of compound by HPLC. The absorbance of same solution was measured repeatedly for stability studies; PRCLD in mobile phase was found to be stable for 3 hours.

Optimized chromatographic conditions

The following optimized parameters were used as a final method for the estimation of PRCLD tablets.

Mode of operation - Isocratic

Stationary phase - C_{18} column (KROMASIL 150)

Mobile phase - Potassium dihydrogen ortho phosphate: Methanol.

Proportion of mobile phase - 60:40 % v/v.

Detection of wave length - 225 nm

Flow rate - 1 ml/min

Temperature - Ambient

Sample Load - 20 µl

Preparation of calibration graph

In this progression, the aliquots of stock solution of PRCLD (0.5 – 1.5 mL) individually into a series of seven 10 mL volumetric flasks and made up to the mark with mobile phase, to get concentrations 50–150 µg/mL. All the solutions were injected and the chromatograms were recorded at 225 nm. The above concentration range was found to be linear and obeys Beer's law. The peak areas were plotted against concentration and the calibration curve was constructed.

Analysis of a marketed formulation

Twenty tablets of formulation (PRUWEL) were weighed accurately, powdered equivalent to 25 mg of PRCLD was transferred into a 25 mL volumetric flask and added 15 mL of methanol to dissolved the substance and made up to the volume with the same (1000 µg/mL). The solution was sonicated for 15 minutes, centrifuged at 200 rpm for 15 minutes and filtered through Whatmann filter paper No.41. From the clear solution, further dilution was made by diluting 1mL into 10mL with mobile phase, to get the expected concentration 100 µg/mL solution. A steady base line was recorded with optimized chromatographic conditions. After the stabilization of base line for 30 minutes, the test solutions were injected and recorded the chromatograms. The concentration of each test solution was determined by using slope and intercept values from the calibration graph.

Recovery studies

In order to ensure the reliability and suitability of the proposed method, recovery studies were carried out. This was done by mixing known quantities of standard drug with formulation sample and the contents were pre analysed by the proposed method. To a quantity of formulation equivalent to 25 mg of PRCLD and Standard drug PRCLD were added at 110%, 120% and 130%

levels. This was extracted, diluted and reanalyzed as per the formulation procedure. Peak areas were noted at the respective concentrations. The amount of each drug recovered from the formulation was calculated for all the drugs by HPLC method. The amount estimation was repeated in triplet in each concentration.

System suitability studies

The system suitability studies conceded as per ICH guidelines and USP. The parameters calculated was shown in table 1.

Validation of developed method

Linearity

The linearity range was checked for in the concentration range of 50 – 150 µg/mL of PRCLD. A calibration curve was plotted with concentration verses the peak area. This was linear in the concentration range.

Precision

The intermediate precision analysis of the method was confirmed by Intraday and Interday analysis. i.e., the analysis of formulation was repeated three times in the same day and on the three successive days respectively. The amount of drug present in the formulation was calculated. The percentage RSD value was calculated.

Accuracy

Accuracy of the method was confirmed by recovery studies. To the preanalysed formulation a known quantity of the standard drug solution was added and the amount of drug recovered was calculated. The percentage RSD value was calculated.

Specificity

The specificity of the HPLC method was evaluated to ensure that there was no interference from the excipients present in the formulations. The specificity was studied by injecting the excipients.

LOD and LOQ

The linearity study was carried out for three times. The LOD and LOQ were calculated based up on the calibration curve method.

Robustness

Robustness of the method was determined by subjecting the method to slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP- HPLC method developed is robust. The analytical method robustness was tested by evaluating the influence of minor modifications in HPLC conditions on system suitability parameters of the proposed method.

Results and discussion

An exertion has been made to simple, precise, rapid, specific and accurate method for the estimation of PRCLD in formulation by RP – HPLC method.

The solution of 10 µg/mL of PRCLD in diluent was prepared and the solution was scanned in the range of 200 nm to 400 nm. It shows maximum absorbance at 225 nm. Hence 225 nm was selected as detection wavelength for estimation of PRCLD by RP – HPLC method with isocratic technique. Based upon the properties of the drug the initial separation was achieved by different mobile phases with different compositions. Finally the mobile phase consisting of Potassium di hydrogen ortho phosphate Buffer (pH 3.7): Methanol (60:40% v/v) was selected after calculating all system suitability parameters, fixed the flow rate 1 mL/minute. The Chromatogram of standard drug was shown in figure 2. Chromatogram of sample drug was shown in figure 3. System suitability parameters was shown in the table 1.

With the optimized chromatographic conditions, stock solution of PRCLD was prepared by using mobile phase in the concentration range of 50 - 150 µg/mL. 20 µL of each solution were injected individually. The chromatograms were recorded at 225 nm.

The calibration curve was plotted using concentration against peak area was shown in figure 4. The procedure was repeated for three times. The Correlation coefficient value was found to be 0.999. The Optical parameters was shown in the table 2.

The optical characteristics like Slope, Intercept, LOD, and LOQ were calculated. The tablet formulation (PRUWEL) was selected for the analysis; the nominal concentration (10 µg/ mL) was prepared in the mobile phase. 20 µL was injected, the chromatogram was recorded.

The precision of the method was confirmed by repeatability of the formulation was shown in the table 3. The percentage purity of PRCLD was found to be 100.65%.

The accuracy of the method was determined by using recovery analysis. To the pre analysed formulations a known quantity of PRCLD solution was added at three different concentrations (110%, 120% and 130%) and the results was shown in the table 4. The percentage recovery of PRUWEL was found to be in the range of 99.93% to 99.76%.

The precision analysis was carried out by Inter- day and Intra-d ay and the results were shown in table 5. The standard deviation of PRUWEL was 0.0763.

The results of Robustness testing showed that a minor change of method conditions, such as the composition of the mobile phase, flow rate, and wavelength, is robust within the acceptable limits. In all modifications, good separation PRCLD was achieved, and it was observed that the percent of recovery was within acceptable limits

and the % RSD is within limit of not more than 2.0%. The tailing factors and number of theoretical plates were found within acceptable limits as well. The out come of the robustness study was shown in table 6.

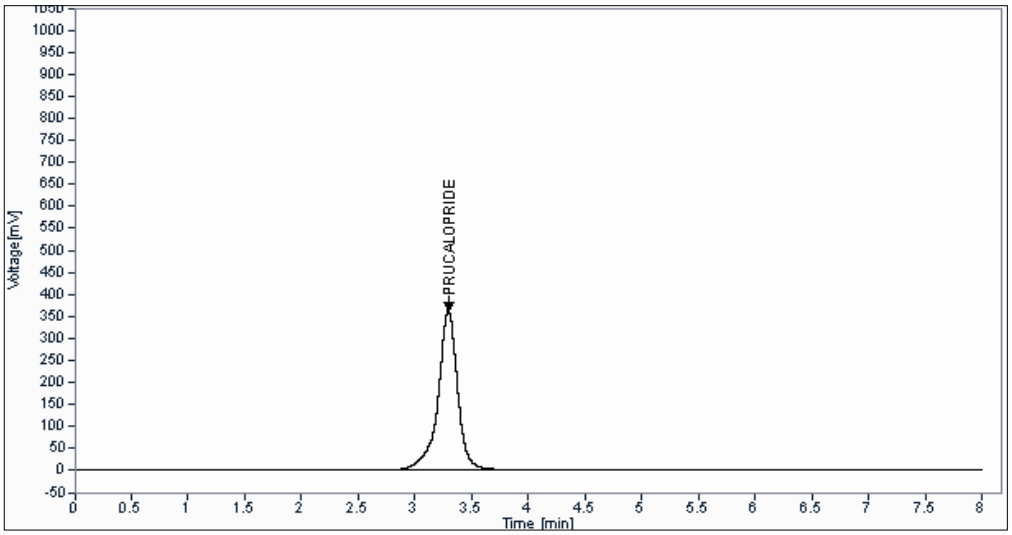


Figure 2. Chromatogram for standard drug.

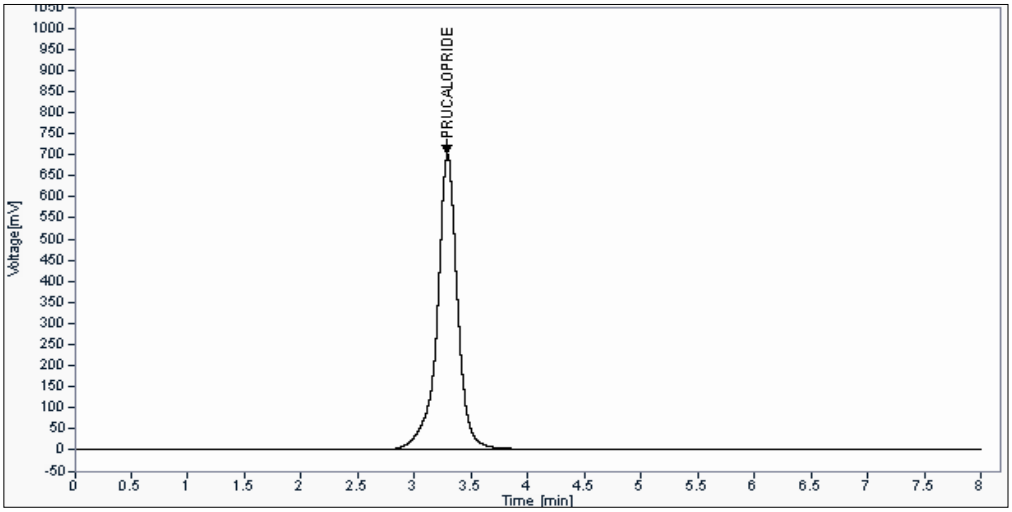


Figure 3. Chromatogram for Sample.

Table 1. System suitability parameter.

Parameters	Prucalopride succinate	Standard limit
Retention time	3.24	--
Tailing Factor	0.91	< 2
Asymmetrical factor	0.75	< 2
Theoretical plates	1963	< 2000

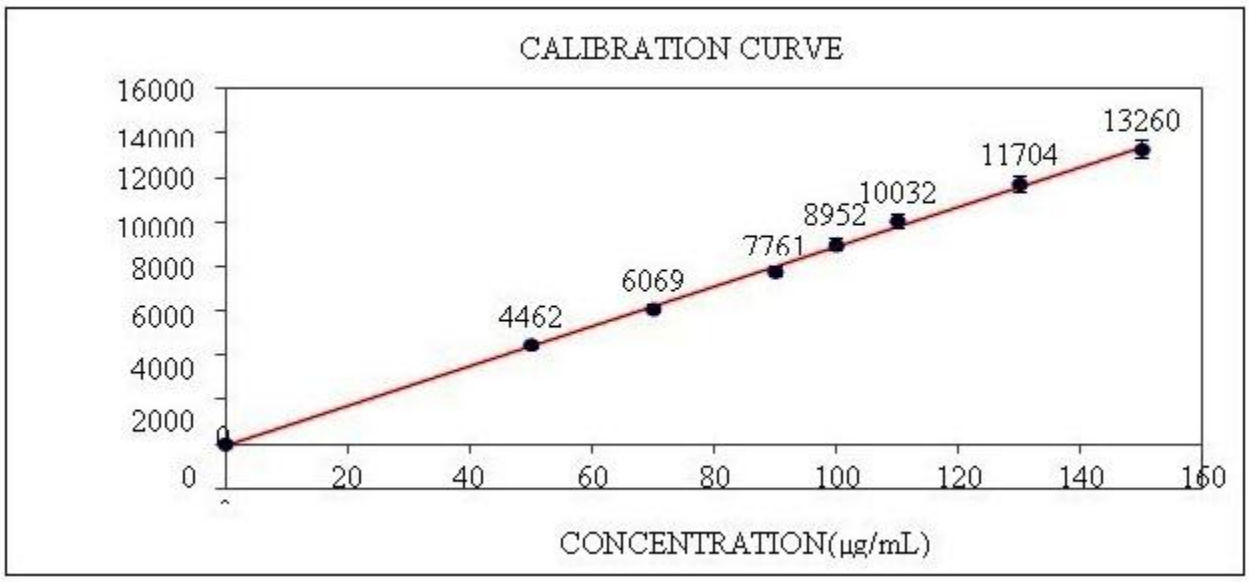


Figure 4. Calibration curve.

Table 2. Optical Characteristics.

S. No	Parameters	RP- HPLC method
1.	λ max (nm)	225 nm
2.	Beer's law limit (µg/ mL)	50 – 150
3.	Correlation co-efficient	0.999
4.	Regression equation (Y= mx + c)	Y = 89.502x -51.44
5.	Slope (m)	89.502
6.	Intercept (c)	-51.44
7.	LOD (µg/ mL)	4.6461
8.	LOQ (µg/ mL)	14.0792
9.	Standard error of mean	168.80
10.	Molar absorbtivity	47126472.56
11.	Sandell's sensitivity (µg/cm^2/0.001 A.U.)	1.11799

Table 3. Quantification of formulation.

S. NO	Labelled amount (mg/tab)	Amount found (mg/tab)	Percentage obtained (%)	Average percentage found (%)	S. D. (+/-)	% R. S. D	S. E.
1.	2	2.098	100.90				
2.	2	2.001	100.05				
3.	2	2.016	100.80	100.65 %	0.3088	0.3068	0.0085
4.	2	2.013	100.65				
5.	2	2.016	100.80				
6.	2	2.015	100.75				

Table 4. Recovery analysis.

Drug	%	Amount present (µg/mL)	Amount added (µg/mL)	Amount estimated (µg/mL)*	Amount recovered (µg/mL)*	% Recovery	Average (%) ± S.D.	% R.S.D.	S.E.
PRCLD	110	10	11	20.97	10.97	99.85	99.93	0.4609	0.0511
	120	10	12	21.90	11.90	99.54	99.73	0.1725	0.0191
	130	10	13	22.99	12.99	99.97	99.76	0.3998	0.0443

Table 5. Intraday and Interday analysis.

S.NO.	Labelled amount (mg\tab)	Percentage obtained (%)		S.D.		% R.S.D.	
		Intraday	Interday	Intraday	Interday	Intraday	Interday
1.	2	98.68	99.71	1.7069	0.9126	1.7061	**0.9160**
2.	2	101.96	100.5				
3.	2	99.5	98.68				

Table 6. Robustness study.

S.NO.	Parameter		% obtained	R.S.D
1	Change in Flow rate	0.5 ml	99.98	0.197
		1 ml (normal)	100.01	0.237
		1.5 ml	97.99	1.442
2.	Change in Wave length	245 nm	98.00	0.581
		225 nm (normal)	99.98	0.578
		300 nm	97.95	0.575
3.	Change in Mobile phase	50:50	96.90	1.20
		60:40 (normal)	99.97	1.09
		70:30	98.45	1.112

Summary and conclusion

Many studies reviewed the use of C_{18} for separation of the drug using methanol as the main solvent. The method was validated and shows satisfactory data for all the method validation parameters tested. The developed method was capable of giving faster elution, maintaining good separation more than that achieved with other available HPLC methods. The shorter run time allows the analysis of a large number of samples in a short period of time and is therefore more cost-effective for routine analysis in the pharmaceutical industries. It is suitable for rapid and accurate quality control method. Therefore, the developed analytical method can be reliably employed as an assay method for pharmaceutical study of any dosage form.

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