Method Development and Validation for the Estimation of Prucalopride Succinate in Bulk and its Formulation by Using UV Spectroscopy

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Abstract:

The study's primary objectives were to create as well verify a simple, precise UV-Visible spectrophotometric technique to determining Prucalopride Succinate on bulk as well as pharmaceutical dosage forms. The approach is founded on Zero order Spectroscopy. The Method was developed utilizing a Shimadzu 1700 UV Visible Spectrophotometer and a set of quartz cells with matching 10mm path lengths. The responses are examined at a medium scanning speed in the 200-400 nm range. All of the parameters, including linearity, accuracy, precision and LOD and LOQ are selected and statistically confirmed by ICH criteria. The method was carried out with 50% v/v Ethanol. The maximal amount of absorption of Prucalopride Succinate came determined resulting at 226 nm. The Drug complied with Beer-Lambert's law at 10-60 µg/ml concentration range. The plot used for calibration were observed that it was simple, as well as a coefficient correlation of 0.9991 was identified. Its accuracy obtained from recovery experiments was 98.0%- 102%. A proportion of the recovered value indicated absence of interference with the formulation's excipients. Precision was carried out as described in method. The values obtained in the repeatability (precision) demonstrated it has no discernible variance in precision values; hence the developed method can be used to analyse the Prucalopride Succinate in its granular and dosage-specific form formulation. An assay for the marketed formulation was performed and was found to be 98.97%. The LOD had identified that 1.70 µg/mL & the LOQ appeared to 5.17 µg/mL in formulation.

Keywords: *UV-Spectroscopy, Prucalopride Succinate, Validation, zero order spectroscopy, ICH guidelines.*

1. Introduction

The analysis of the separation, identification, & quantification of organic substances of natural as well as manufactured substances is known as analysis-based chemistry. The chemical-based groups in the substance are identified by the qualitative assessment, which also establishes the number of a substance's constituents. Component separation is frequently conducted before the study. The number of new pharmaceuticals introduced onto the market each year is growing due to advances in analytical chemistry. There is a time lag between the date medicine is introduced to the market and its date in pharmacopeia's due to potential ambiguities in the continued and expanded use of these treatments, reports of novel toxicities, the increasing person an inability to respond, as well as the launch superior medicine from. rivals. In those situations, analytic techniques and protocols for these substances perhaps not accessible in pharmacopeia. As a result, it is critical to create, enhance, and verify newer analytical methods for such medications so that they may be explored collaboratively and implemented effectively for the study of various new molecules [1]. Prucalopride succinate is a prokinetic agent. It is chemically 4-amino-5-chloro-N-[1-(3-methoxy propyl) piperidin-4-yl]-2,3-dihydro-1-benzofuran-7-carboxamide is a selective 5-Hydroxytryptamine receptor 4 agonists with no interactions with hERG channels or 5-HT-1, considerably lowering the cardiovascular risk associated with other comparable medications. 5-HT 4 receptors are located mainly in smooth muscles, enterochromaffin cells, and the myenteric plexus of the gastrointestinal system. Its activation results in a discharge of acetylcholine, the primary excitatory neurotransmitter in the GI tract [2-3]. It was first launched in 2009 and has been available for purchase in Europe since 2010. Prucalopride's significant impact is to enhance colonic motility, which is effectively treats constipated individuals who have not responded to other regimens. Prucalopride succinate is a prokinetic medication that has just been commercially accessible to treat chronic constipation sufferers. Thus, the primary project aimed to develop as well as verify a UV spectroscopic method over figuring out Prucalopride succinate in bulk and medicinal dose form [4-6].

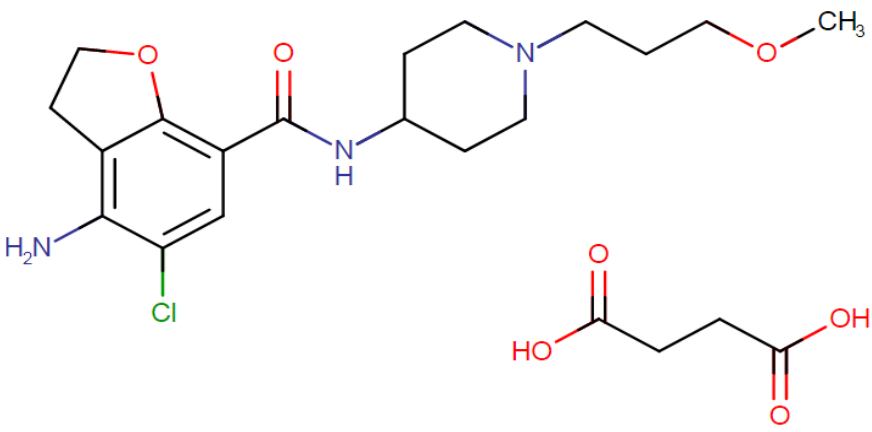

Figure 1. Structure Of Prucalopride Succinate [7-8]

2. Materials and Methods

The working standard drug (Prucalopride Succinate) was procured from Mylan Laboratories,Bangalore, Karnataka,India. The obtained Prucalopride Succinate formulation (Tablet) was manufactured by Torrent Pharmaceutical Limited, Gangtok, Sikkim, India. Ethanol was obtained from SD-Fine Chem Limited, Mumbai, India. The Milli-Q-Water utilised for the analysis was prepared on-site.

3. Instrumentation:

A Shimadzu UV 1700 twin-beam UV visible spectrometer, Quartz cells that have been compatible to which has a 10mm length of the path were employed in the investigation. Utilising UV Probe programme. A VIBRA Shinko Denshi Co.ltd. Electronic balance model NO – HT 220 was utilized for weighing purposes. The Ultra Sonicator model 2200 MH Soltech (SPINCOTECH Pvt. Ltd) was used for sonication.

4. Method Development:

The Current Analytical Method (UV Spectroscopy) was developed to determine the quantity of Prucalopride Succinate in bulk and its formulations.

4.1 Preparation of Solutions For The Study:

4.2 Preparation of Standard Stock Solution:

The standard solution had made through assessing about 10mg Prucalopride Succinate, forwarding it to 10ml volumetric flask (previously calibrated), dissolving it in 5 ml of 50 percent v/v ethanol, and sonicating it for 5 minutes with an ultrasonicator. The volume was then made up to the mark using 50 percent v/v ethanol to achieve 1mg/ml (solution A). Various aliquots were made from this using distilled water [9-10].

4.3 Preparation of Sample Solution:

The analysis for the formulation was carried out by weighing an equivalent of 50 mg drug into a 10ml volumetric flask. The flask contents were thoroughly mixed and filtered by Whatman's filter paper. Further sonication was carried out using Sonicator for 5 min to enhance solubility, and finally, that capacity seemed to produce of the mark utilizing 50% v/v ethanol. From the above-prepared solution, various aliquots were prepared using distilled water [1-13].

5. Method Validation

Determining documentation information, thereby provides a significant stage of certainty in knowing to this particular procedure shall persistently result in a product attaining its predefined requirements as well as the standard characteristics. In accordance with ICH Q2 (R1) specifications, the developed method was validated. [9-17].

5.1 Specificity:

An analytical procedure's selectivity has determined by the capacity for determining the target of the compound precisely along with some existence of any portions that might be anticipated that are contained within the samples matrix.

Intrusion with the compound's placebos effect: Conduct an assessment with duplicates using a quantity of placebo that is equal with its quantity of tests preparation along with the substance used in it. Its outcomes of specificity analysis was shown in the table No.1

From the result we have found that no interference of blank, placebo and excepients with that of drug. The spectra's of standard drug depicted by the diagram 2 and specificity info shown in Table 1. The purity was calculated and founds as greater than 0.999.

TABLE NO 1: SPECIFICITY DATA

S.NO	Name	Prucalopride Succinate UV spectra and lambda max.
I	Blank	NIL
II	Placebo	NIL
III	Standard	226 nm

UV Spectroscopic Method:

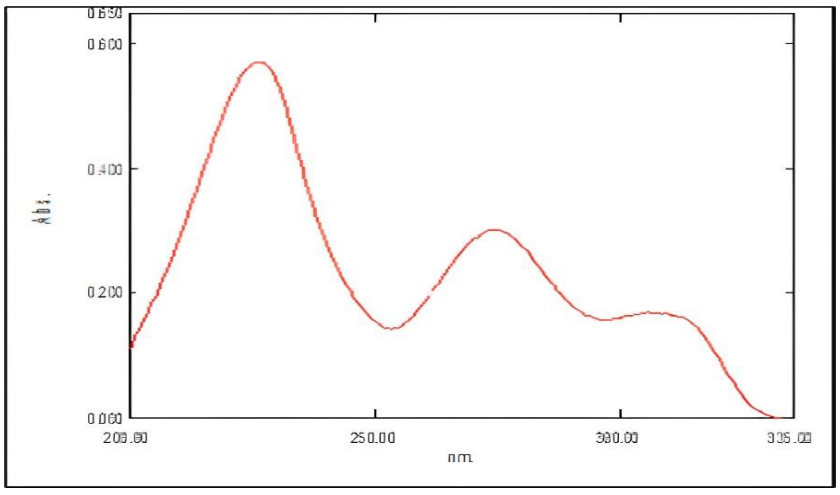

Figure 2. UV Spectra Of Standard Drug (API)

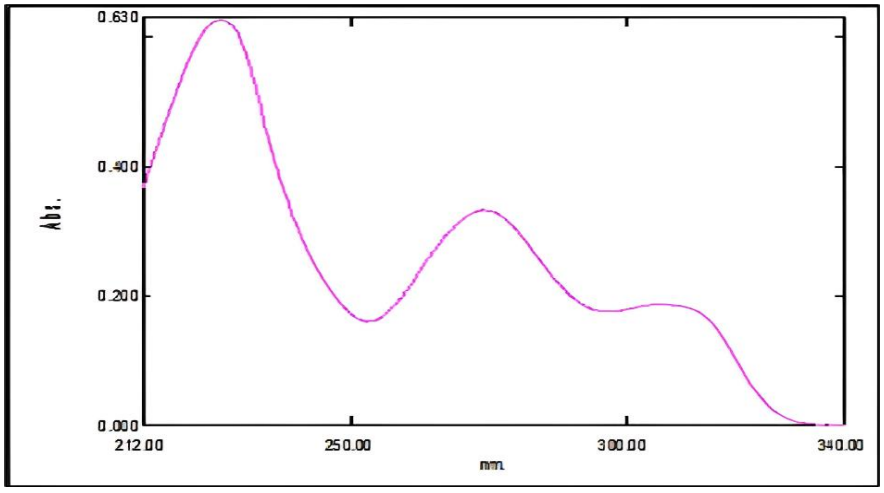

Figure 3. Uv Spectra of Formulation

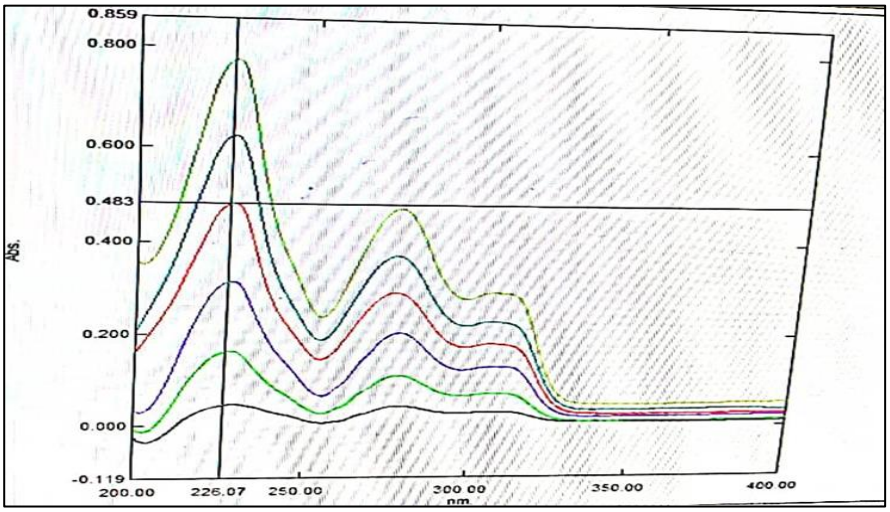

Figure 4. Overlay Spectra Of Prucalopride Succinate

5.2 Precision:

The precision of an analytical method indicates level of concordance (the level of scattering) among a number of assessments acquired through various portions associated with an exact uniform sample within the specified circumstances.

The precision had determined using intra-day and inter-day deviation. This intra-day and inter-day variance within substance mixture absorbence results is determined using the coefficient of variation. The %RSD was calculated both for intra and interday studies and was found to be less than 2. The findings of both intraday and inter day data shown in table no**.** 2

Table No 2: Intraday and Inter day precision Data

Concentration	Intraday Precision (Absorbance)	Statistical Analysis
40 ug/ml	0.483	**Mean** = 0.4805 **Std Dev**=0.006677075 **%RSD**=1.38
40 ug/ml	0.472	
40 ug/ml	0.485	
40 ug/ml	0.491	
40 ug/ml	0.473	
40 ug/ml	0.479	

Concentration	Interday Precision (Absorbance)	Statistical Analysis
10 ug/ml	0.147	**Mean**=0.1446 **Std Dev**=0.004933 **%RSD**= 3.364%
10 ug/ml	0.139	
10 ug/ml	0.148	
40 ug/ml	0.486	**Mean**=0.482 **Std Dev**= 0.003606 **%RSD**=0.7481%
40 ug/ml	0.481	
40 ug/ml	0.479	
60 ug/ml	0.758	**Mean**=0.7603 **Std Dev**=0.003215 **%RSD**=0.4228%
60 ug/ml	0.759	
60 ug/ml	0.764	

5.3 Linearity & Range:

The tendency regarding this analytical method (inside of a specified interval) to produce test outcomes that has been directly proportional to the level of substance in a sample is referred to as linearity.

The linearity Setout was determined as 10 – 60 µg/ml for Prucalopride Succinate, calibration graph along with the correlation factor were plotted. for Prucalopride Succinate determined as 0.9991 which is shown in Figure 5. Hence the results obtained within the specifications. The outcomes are reported in table no .5

TABLE NO 3: LINEARITY VALUES OF PRUCALOPRIDE SUCCINATE

S.NO	Concentration (µg/ml)	Absorbance ± SD
1	10	0.090±0.0061
2	20	0.236±0.046

3	30	0.360±0.0027
4	40	0.491±0.038
5	50	0.618±0.0085
6	60	0.735±0.0054

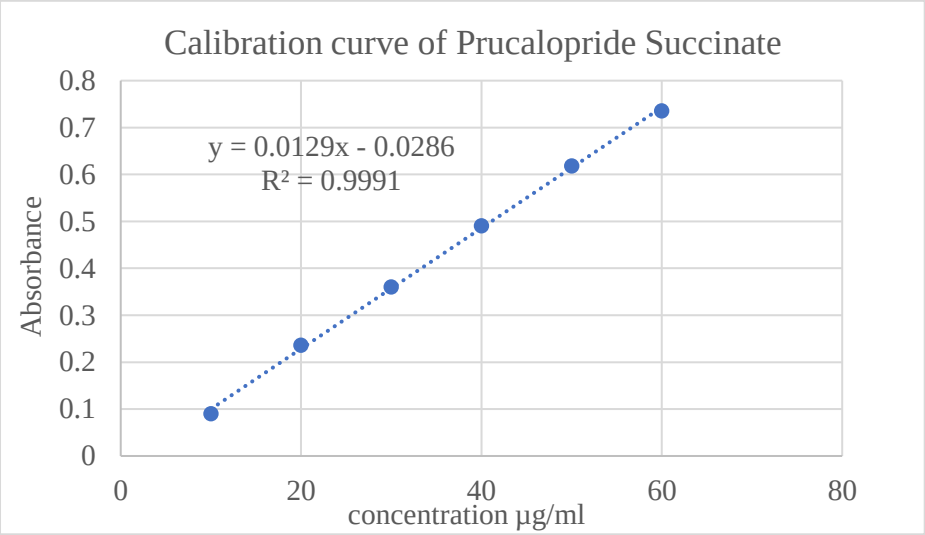

Figure 5. Calibration Curve for Prucalopride Succinate

5.4 Accuracy:

In order to determine the accuracy associated with the suggested approach, 9 test results have been executed across three concentration abilities (50%, 100%, and 150%), encompassing the desired range among repetitions. Results are shown in table No.4

50% Level:
The following standard solution of 0.2 ml (20 g/ml) is utilized and then transmitted thru ten millilitres volumetric containers before being mixed up with solvent.

100% level:
The following standard solution of 0.4 ml (40µg/ml) is utilized and then transmitted thru ten millilitres volumetric containers before being mixed up with solvent.

150% level:
The following standard solution of 0.6 ml (60µg/ml) is utilized and then transmitted thru ten millilitres volumetric containers before being mixed up with solvent.

The percentage of recovery for every stage has to be among 98.0 and 102.0% to be accepted.

S.NO	Spike level (%)	Absorbance	Amount added (µg/ml)	Amount Found (µg/ml)	% Recovery	%Mean Recovery
1	50%	0.224	19.79	20.08	101.43	100.34
2		0.241		19.83	100.20	
3		0.239		19.67	99.39	
4	100%	0.485	39.59	39.91	100.82	99.94
5		0.481		39.58	99.99	
6		0.475		39.09	98.99	
7	150%	0.735	59.38	60.49	101.86	101.02
8		0.724		59.58	100.33	
9		0.78		59.91	100.89	

TABLE NO 4: DATA OF PRESENT RECOVERY VALUES OF PRUCALOPRIDE SUCCINATE

5.5 Limit of Detection (LOD) & Limit of Quantitation (LOQ):

Limit Of Detection:

The lowest amount of substance in a sample that is able to be identified but not always quantified as specific values is the detection limit of a specific analytical method.

Utilizing the calibration graph

Samples with analytes within their LOD range must be used to examine a particular calibration graph. The variance that is measured can be the residual deviation from the mean of a regression line or the average difference from the y-intercepts of regression patterns. Results are reported in table no. 6

Limit Of Quantitation

The lowest amount of substance in a sample that can be quantitatively defined using appropriate accurateness and precision is known as the quantitation limit of a specific analytical method.

Utilizing the calibration graph

Samples with analytes within their LOQ range must be used to examine a particular calibration graph. The variance that is measured can be the residual deviation from the mean of a

regression line or the average difference from the y-intercepts of regression patterns. Results are reported in table no. 6

Table No 6: Results Showing LOD and LOQ values

S.No	Parameter	LOD & LOQ (µg/ml)
I	Limit of Detection	1.70
II	Limit of Quantification	5.17

5.6 Robustness:

It examines subtle however intentional changes to process factors like absorption maxima, pH levels, along with liquid solvent ratios in the mobile phase. Absorbance peak values were shifted by 2 nm within a current work, which involved repeating this method six instances with a 40 g/ml solution. Calculating the% RSD.

Concentration 40 ug/ml	Absorbance At 224nm (-2nm)	Absorbance At 228nm (+2 nm)	%Mean RSD
Mean	Mean 0.421	Mean 0.445	0.5970
Standard Deviation	SD 0.002646	SD 0.002517	
% RSD	%RSD 0.6285	%RSD 0.5656	

Table No 7: Summary of Optical Characteristics and Other Parameters

S. No	Validation Parameter	Results	Acceptance Criteria
I.	Absorption Maxima (nm)	226nm	-
II.	Linearity (µg/ml)	10 – 60	-
III.	Regression equation (Y)	0.0129x – 0.0286	-
IV.	Slope (b)	0.0129	-
V.	Intercept (a)	0.0286	-
VI.	Correlation Coefficient	0.9991	NLT 0.999
VII.	Intraday Precision (%RSD)	1.38	Less than 2%
VIII.	Inter Day Precision (%RSD)	1.03	Less than 2%
IX.	Accuracy (%mean recovery)	99.94-101.02	98-102 %
X.	Limit of Detection	1.70	-
XI.	Limit of Quantification	5.17	-
XII.	Assay (% Purity)	98.97	98-102 %

6. Assay:

It was accomplished by following the sample solution preparation technique.

Table8: displays the percent assay data.

Standard Absorbance	0.486
Sample Absorbance	0.483
Formulation average weight	0.05 g or 50 mg
Standard Weight	10 mg
Sample Weight	50 mg
Label amount	1 mg
Assay (% Purity)	98.97

7. Result and Discussion:

The Prucalopride Succinate was found to be soluble in Ethanol. The drug's λmax was discovered to be 226 nm. Prucalopride Succinate exhibits linearity its varying concentrations 10g/ml to 60g/ml, with a coefficient correlation of 0.9991. By combining the pure drug with the before studied sample, recovery tests had been conducted at three distinct stages, namely 50%, 100%, and 150%. By using the standard addition procedure, the percentage recovery for prucalopride succinate was assessed. It was discovered that it fulfilled the ICH-recommended acceptable level of 98.0% to 102.0%. As shown in Fig.5, the regression of the curve was y = 0.0129x − 0.0286. The detection and quantitation limits were computed as LOD (k = 3.3) and LOQ (k = 10) and were 1.70g/ml and 5.17g/ml, respectively. The precision (intra-day and inter-day measurements) findings revealed (Table no.2) significant repeatability with percent relative standard deviation (percent RSD) less than 2.0 demonstrating that the procedure is quite exact. The percent recovery number (Table no.4), greater than 100 percent, confirms the method's correctness.

8. Conclusion:

The proposed research proposes a new UV spectrophotometric technique for estimating Prucalopride Succinate in bulk and pharmaceutical dosage form utilizing appropriate diluent. Compared to other approaches, the method was validated and found to be simple, selective, accurate, exact, as well as resilient. The recovery percentage indicates that the procedure is not influenced by the excipients included in the formulation. In terms of solvent use, the approach is also cost-effective. As a result, the suggested method may be utilized regularly to analyse Prucalopride Succinate in its dose form. Developing and validating a UV-Spectroscopy method for estimation of Prucalopride succinate satisfied the acceptance criteria.

Furthermore, such established approaches might be helpful in the evaluation of medicinal products and their degradation products. This approach serves as the foundation for selecting an appropriate analytical wavelength, which aids in method development employing RP-

HPLC. As a result, we propose that such a method be used in quality control analysis for its formulation in the future.

9. Conflict of Interest: The Authors have no conflict of interest.

10. Abbreviations:

μm - Micro Meter

λ - Wave length

% - Percentage

λ max - Maximum Wavelength

nm - Nano Meter

ICH - International Council for Harmonisation

API - Active Pharmaceutical Ingredient

%RSD - Percentage Relative Standard Deviation

AUC - Area Under Curve; mg -Milligram; ml-Millilitre

Ml - Microlitre

μg /ml - Microgram/Millilitre

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