

# A Novel Validated UV Spectroscopy Method for the Determination of Prucalopride Succinate in Solid Dosage Form

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**Abstract:-** Three simple, accurate, novel, safe and precise methods were developed by the selective UV method for the estimation of water-soluble drug Prucalopride Succinate in pharmaceutical Dosage Form. Estimation was achieved by Shimadzu -1700 Double Beam UV Visible Spectrophotometer with a pair of 10mm matched quartz cells and ELICO SL -210 Double Beam UV Spectrophotometer with a pair of 10mm matched quartz cells. By using UV Spectrophotometer, standard solution was absorbed. From the UV spectra obtained, Prucalopride Succinate showed maximum absorbance at 226nm. By using Visible Spectrophotometer, standard solution was absorbed with 2% Ferric chloride and MBTH reagent. From the Visible spectrum obtained, 483nm was selected as maximum absorbance for the analysis of Prucalopride succinate. The method was validated for specificity, linearity, precision, accuracy, robustness. The linearity of the proposed methods of UV Spectrophotometer, first order derivative spectrophotometer, Visible Spectrophotometer was at the range of 3-18µg/ml, 10-60µg/ml, 100µg/ml of Prucalopride succinate with a correlation coefficient of 0.9999. The precision (relative standard deviation – RSD) of six samples was 0.5066%. The accuracy (recovery) was 100.07 % and 100.19 %.and 99.93%.

**Keywords:-** UV Spectrophotometer, Visible Spectrophotometer, Prucalopride succinate, RSD and Validation.

## I. INTRODUCTION

Absorption Spectrophotometry in the UV and Visible region is considered to be one of the physical methods used for quantitative analysis and serves as a useful auxiliary tool for structural elucidation. UV absorption Spectroscopy is one of the best methods for detecting impurities in organic compounds. This method is much less susceptible than colorimetry to operator error and to interferences from compounds encountered in the sample itself. Spectrophotometry is currently used extensively in all

routine analytical labs and undoubtedly will be for many years to come. In Recent years, the spectrophotometer is generally inexpensive and most laboratories are easily to handle this type of equipment. UV Spectrophotometer quickly to analyze the unusual sample that cannot be conveniently determined by more rapid instrumental methods of analysis because of lack of calibration curves. In twenty first century pharmaceutical industries are focusing for new ways to in economy and shorten time for development of drugs. Prucalopride succinate (PRCS) is a "first in class" agent in a dihydrobenzo furan carboxamide derivative acting as a selective agonist for serotonin type 4 receptors (5-HT<sub>4</sub>) and developed as an enterokinetic agent for the treatment of chronic constipation [1-3]. Its chemical name is 4-amino-5-chloro-N-[1-(3-methoxypropyl) piperidin-4-yl]-2,3-dihydro-1-benzofuran-carboxamide; butanedioic acid.

The drug has also been tested for the treatment of chronic intestinal pseudo-obstruction. The primary measure of efficacy in the clinical trials is three or more spontaneous complete bowel movements per week; a secondary measure is an increase of at least one complete spontaneous bowel movement per week. This medication is used to treat chronic constipation, Prucalopride succinate is contraindicated where there is hypersensitivity to the active substance or to any of the excipients, renal impairment requiring dialysis, intestinal perforation or obstruction due to structural or functional disorder of the gut wall, obstructive ileus, severe inflammatory conditions of the intestinal tract, such as Crohn's disease, and ulcerative colitis and megarectum. In vitro data indicates that Prucalopride succinate has a low interaction potential, and therapeutic concentrations of Prucalopride succinate are not expected to affect the CYP-mediated metabolism of co-medicated medicinal products.

Prucalopride succinate stimulates motility by interacting specifically with 5-HT<sub>4</sub> receptors in the GI tract which cause a release of acetylcholine and further contraction of the muscle layer of the colon and relaxation

of the circular muscle layer leading to the propulsion of luminal content.

The survey of literature has not revealed any simple UV-Spectrometric method for the estimation of prucalopride succinate so that the objective of the present work was to develop simple, precise UV- Spectrophotometric method for estimation of prucalopride succinate in tablet dosage form.

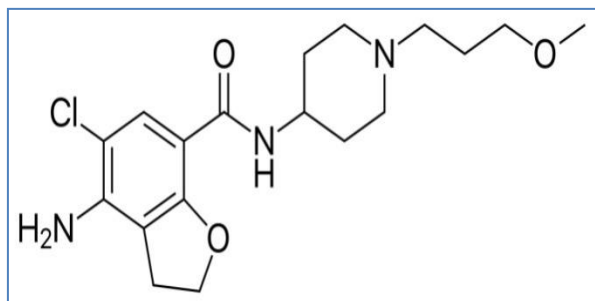


Fig. 1: Structure of Prucalopride succinate

- **Keywords and Abbreviation:** Prucalopride succinate (PRCS), UV spectrophotometer.

## II. EXPERIMENTAL

### A. Material and Reagents.

Pure PRCLD (Purity > 99.5) was procured as gift sample from Symed laboratories, Hyderabad, India. PRCLD tablet was purchased from local commercial sources. Methanol (HPLC grade) was purchased from Finar Pvt. Limited, Gujarat, India. Ethanol (AR grade) was purchased from Changzhou Hongshey fine chemicals.co. Ltd, Changzhou city, Jiangshu province. MBTH reagent (AR grade) was purchased from Loba cheme pvt Limited, Mumbai, India.

Distilled water was obtained from Double distillation unit in our Laboratory.

The high purity Milli-Q water was obtained through a Milli-Q Integral Water Purification System. All other reagents were of analytical grade and used without further purification.

## III. METHODS

- UV Spectrophotometric method
- First order derivative spectrophotometric method
- Visible Spectrophotometric method

### A. Uv spectrophotometric method

#### ➤ Selection of solvent

The solubility of PRCS was determined by using variety of solvents. Solubility study of PRCS was carried out in polar to non-polar solvents. The PRCS was found to be soluble in Distilled water, Methanol and Ethanol. Considering the economic factor, Distilled water was selected as the solvent for further analysis.

#### ➤ Preparation of standard stock solution

25 mg of PRCS working standard was accurately weighed and transferred into a 25 ml volumetric flask and dissolved in minimum quantity of distilled water and made

upto the mark, to get the concentration 1000 µg/ml of PRCS.

#### ➤ Selection of wavelength

The standard solution was further diluted with distilled water to get a concentration of 10µg/ml of PRCS. The solution was scanned between 200-400 nm ranges using distilled water as blank. From the UV spectra obtained, PRCS showed maximum absorbance at 226 nm and found to be stable for 3 hours and 30 minutes.

#### ➤ Preparation of calibration graph

The working standard solution of PRCS containing 1000µg/ml (0.3-1.8 ml) were transferred into a series of six 100 ml volumetric flasks and made up to the volume with water to get the concentration range of 3-18µg/ml. The absorbance of different concentration solutions was measured at their selected wavelength. The calibration curve was constructed by plotting concentration against absorbance.

#### ➤ Preparation of Tablet Extract

Twenty tablets were weighed and triturated to fine powder. The tablet powder equivalent to 25mg of PRCS was weighed and transferred into a 50 ml volumetric flask, added a minimum quantity of water to dissolve the substance by using ultra sonication for 15 minutes and made up to the volume with the same. The content was filtered through whatmann filter paper NO.41. Filtrate was suitably diluted to get the final concentration 9µg/ml. The absorbance was measured at 226 nm against distilled water as a blank. The absorbance of sample solution was measured with the wavelength, the repetitions were made six times for formulation. The amount of PRCS present in formulation was calculated from the slope and intercept of respective calibration curve.

#### ➤ Recovery procedure

In order to ensure the reliability and suitability of the proposed method recovery studies were carried out. It 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 PRCS and Standard drug PRCS were added at 80%, 100% and 120% levels. This was extracted, diluted and reanalyzed as per the formulation procedure. Absorbances were noted at the respective wavelength. The amount of each drug recovered from the formulation was calculated for all the drugs by UV method. The amount estimation was repeated in triplet in each concentration.

### B. First order derivative spectrophotometric method

In this First order derivative spectrometric method, selection of solvent and preparation of standard stock solution procedure to be same as the above method (METHOD A).

#### ➤ Selection of wavelength

Then by converting the normal spectrum into first order derivative spectra, From the UV spectra obtained, PRCS showed minimum absorbance at 235nm and found to be stable for 3 hours and 30 minutes.

➤ *Preparation of calibration graph*

The absorbance of different concentration of 10-60µg/ml was measured at their selected wavelength. The calibration curve was constructed by plotting concentration against absorbance.

➤ *Preparation of Tablet Extract*

Procedures were followed the same to be the above method (METHOD A). Then by converting the first order derivative spectra, the absorbance was measured at 235nm against distilled water as a blank. The absorbance measurements were made six times for formulation. The amount of PRCS present in formulation was calculated from the slope and intercept of respective calibration curve.

➤ *Recovery procedure*

Procedures were followed the same to be the above method (METHOD A). Then Absorbances were noted at the respective wavelength. The amount of each drug recovered from the formulation was calculated for all the drugs by UV method. The amount estimation was repeated in triplet in each concentration.

*C. Visible spectrophotometric method*

➤ *Principle*

PRCS has one free amino group, hence it was planned to treat with 2% Ferric chloride solution and the resulting solution was tested for various colour reactions with different chromogenic reagents like MBTH, PDAB, FC, NED. Where MBTH reagent gave a stable colour (Wavelength of 400-800 nm) with PRCS in presence of oxidizing agent (2% Ferric chloride solution) and have good linearity and obeys Beer's and Lambert law.

**Prucalopride succinate**



**0.2% MBTH  
2% FeCl<sub>3</sub>**

**Reddish orange colour**

➤ *Selection of Wavelength*

To the 1 ml of standard stock solution, added 2 ml of 2% Ferric chloride and 2 ml of MBTH reagent in a 10ml standard flask and the volume was made up to the mark with distilled water, mixed well and allowed to stand for 30 minutes. The solution was scanned between 400-800nm using reagent as blank. From the visible spectrum obtained, 483nm was selected as wavelength maximum for the analysis of PRCS. The colour was found to be stable for 2 hours.

➤ *Preparation of calibration graph*

In this method, the aliquots of working standard solutions containing 100µg/ml of PRCS (0.9-5.4 ml) were transferred into six 10 ml standard flasks, added 2 ml of 2% FeCl<sub>3</sub> and 2 ml of MBTH reagent, mixed well and made up to the volume with distilled water to get the concentration 9-

54µg/ml and allowed to stand for 30 minutes. The absorbance measurements were made at 483 nm against reagent blank. The solutions were found to be linear from 9-54 µg/ml and the calibration curve was plotted between concentration and absorbance.

➤ *Preparation of tablet extract*

Twenty tablets were weighed and triturated to fine powder. The tablet powder equivalent to 25 mg of PRCS was weighed and transferred into a 25 mL volumetric flask, added a minimum quantity of water to dissolve the substance by using ultra sonication for 15 minutes and made up to the volume with water (1000 µg/mL). The content was filtered through Whatmann filter paper No.41. Filtrate was suitably diluted to get the final concentration 100 µg/mL. From that 2.7mL was transferred into a 10mL standard flask then added 2mL of Ferric chloride solution and 2mL of MBTH reagent mixed well and made up to the mark with distilled water to get the concentration 27 µg/ml and allowed to stand for 30 minutes. The absorbance was measured at 483 nm using reagent as blank. The amount of PRCS present in the formulation was calculated from the slope and intercept value of the calibration curve.

➤ *Recovery procedure*

To determine the accuracy of the method, recovery study was performed by standard addition method. The recovery experiment was done by adding known concentrations of PRCS working standard to the pre analysed formulations. To the 50% of pre analysed formulation solution, Known quantities of standard drug that is 80,100,120% of Quantification concentration (2.16mL, 2.7mL, 3.24mL of PRCS stock solution) were added into a series of 10 ml volumetric flasks, diluted with water and added 2mL of Ferric chloride solution and 2 mL of MBTH reagent, mixed well and allowed to stand for 30 minutes. The solution were made up to the mark with distilled water and filtered through whatmann filter paper No 41. The absorbance of the resulting solutions were measured at 483 nm against reagents as blank and the amount of drug recovered from the formulation was calculated by using slope and intercept values. The procedure was repeated for three times at each level.

#### IV. VALIDATION

##### A. METHOD A

➤ *Accuracy*

Accuracy of the method was confirmed by recovery studies. To the pre-analysed 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.

➤ *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 on the three successive days respectively. The amount of drug present in the formulation was calculated. The percentage RSD value was calculated.

➤ *Limit of Detection (LOD) and Quantification (LOQ).*

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

➤ *Linearity*

The linearity range was checked for in the concentration range of 3-18 µg/ml of PRCS. A calibration curve was plotted with concentration versus the absorbance. This was linear in the concentration range.

➤ *Ruggedness*

Ruggedness of the method was performed by precision analysis.

- By using different analysts.
- By using different instruments.

The Amount, SD, %RSD and standard Error was calculated.

**B. METHOD B**

Validation of developed method procedure to be same as the above method (Method A).

**C. METHOD C**

Validation of developed method procedure to be same as the above method (Method A).

## V. RESULTS AND DISCUSSION

The UV and Visible Spectrophotometer is its quick analysis ability and easy to use. It is also one of the physical methods to analyse the sample very easily.

In UV spectrophotometric method, the calibration curve was plotted by using concentration against absorbance. The optical characteristics like correlation coefficient, Intercept, Molar absorptivity, Sandell's sensitivity, LOD and LOQ were calculated.

The average percentages of tablet formulations were found to be 100.14%, 100.62%, 101.25%, 101.01%, and 100.95% respectively. The accuracy of the method was determined by using recovery analysis. The precision analysis was carried out by Inter- day and Intra- day analysis. The standard deviation of PRCS was 0.9375 and 0.1962.

The correlation coefficient value from the calibration graph for First order derivative was found to be 0.997. This indicates the drug obeys Beer's law. The average percentages of tablet formulations for First order derivative spectroscopy were found to be 100.41%, 100.24%, 100.61%, 99.36% and 99.86% respectively. The ruggedness analysis was carried out by different analysts and different instruments. The % RSD values of PRUWEL was 0.0708 and 0.1344, PRUVICT was 0.1483 and 0.2113.

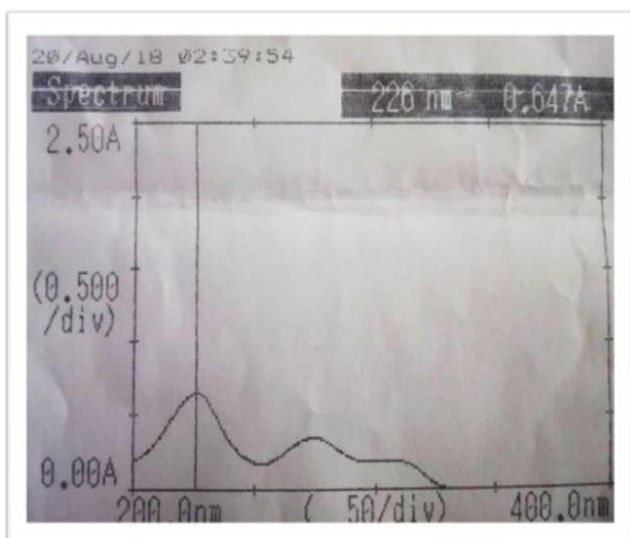
In UV Visible spectrophotometric method, the different aliquots of PRCS were prepared in the concentration ranges from 9-54 µg/ml. The absorbance of the solution was measured at 483 nm. From the linearity analysis, the calibration curve was plotted by using concentration against absorbance. The correlation coefficient value from the calibration graph was found to be 0.990. The average percentages of tablet formulations were found to be 100.44%, 100.98%, 101.03%, 99.91%, and 99.97% respectively. The ruggedness analysis was carried out by different analysts and different instruments. The %RSD values of PRUWEL was 0.148 and 0.6539.

## VI. SUMMARY AND CONCLUSION

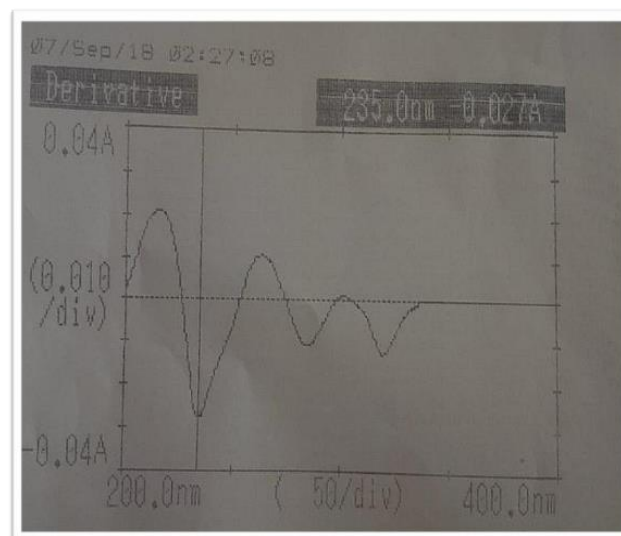
A new UV spectrophotometry, visible spectrophotometry, methods were developed for PRCS in Bulk and Tablet dosage forms. These developed methods were continuous and interrelated process that measures a parameter was intended and establishes the performance limit of the measurements. The selection of solvent, Detector, Wavelength and other condition plays a dramatic role in the method development and validation of PRCS in Tablet dosage form. These developed methods were validated according to ICH guidelines with various parameters like precision, Accuracy, LOD, LOQ etc. The advantage of these methods was high selectivity, sensitivity, Economic, Precise and accurate method. Hence these developed methods can be used in the routine analysis of PRCS in Quality control Laboratory. Hence, the method is recommended for routine quality control analysis and also sample analysis.

## ACKNOWLEDGMENT

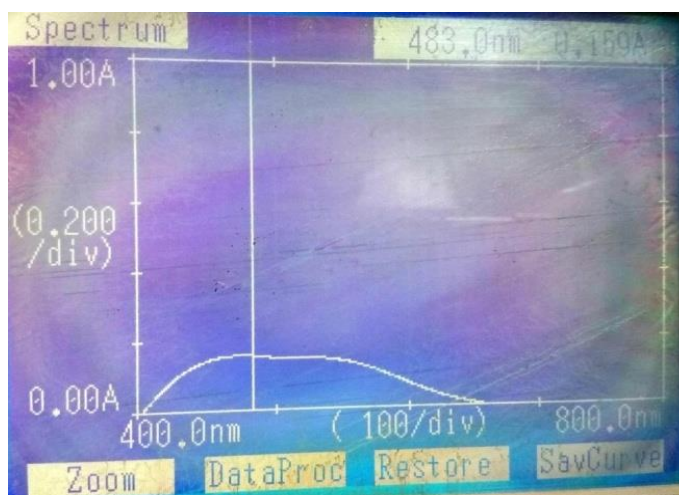
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METHOD A

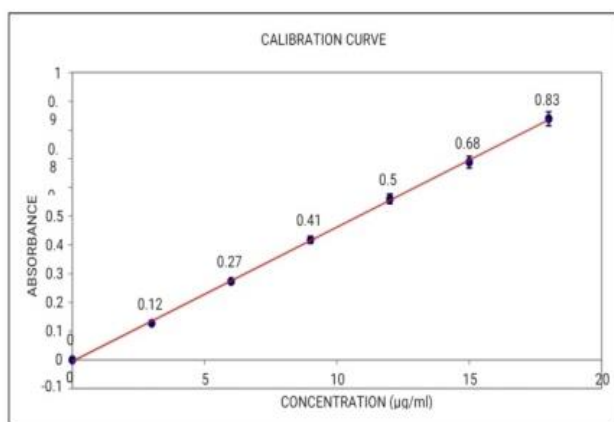


METHOD B

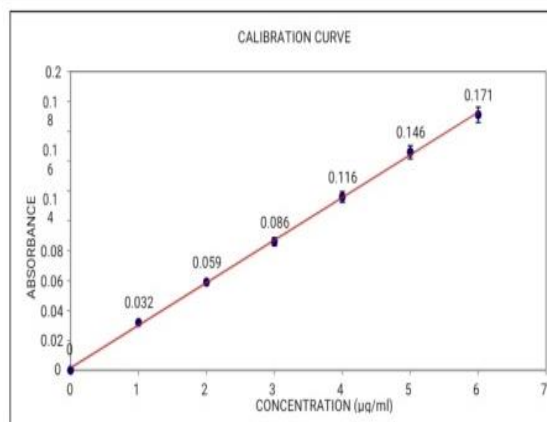


METHOD C

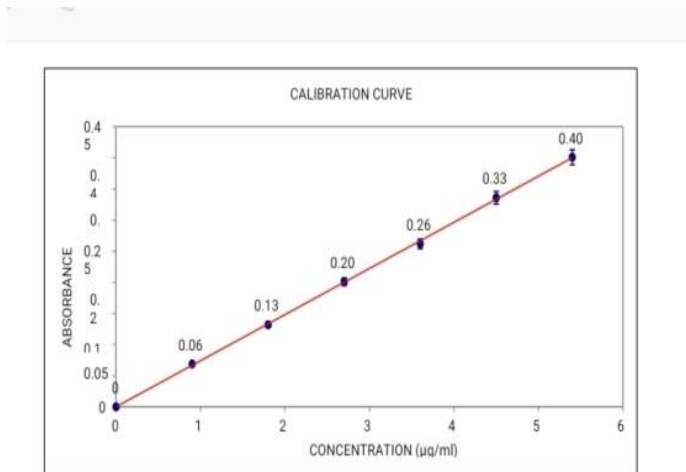
Fig. 1: UV SPECTRUM OF PRUCALOPRIDE SUCCINATE (METHOD A, METHOD B, METHOD C)



METHOD A



METHOD B



METHOD C

Fig. 2: CALIBRATION CURVE OF PRUCALOPRIDE SUCCINATE (METHOD A, METHOD B, METHOD C)

S.NO	Parameters	Method A	Method B	Method C
1	λmax (nm)	226nm	235nm	483nm
2	Beer's law limit(µg/ml)	3-18	10-160	9-54
3	Correlation co-efficient	0.999	0.9971	0.9900
4	Regression equation(y=mx+c)	Y = 0.0465x + 0.004	Y= 0.002805x + 0.00375	Y= 0.007477x + 0.03036
5	Slope(m)	0.0465	0.002805	0.007477
6	Intercept(c)	-0.004	0.00375	0.03036
7	LOD(µg/ml)	0.9759	2.387430	1.55717
8	LOQ(µg/ml)	2.9573	7.23463	6.2457
9	Standard error of mean	0.00490	0.0009380	18.9265
10	Molar absorptivity	25737.69	1777.0390	5873.8796
11	Sandell's sensitivity (µg/cm <sup>2</sup> /0.001 A.U.)	0.0216	0.3568	0.13453

Table 1: OPTICAL CHARACTERISTICS (METHOD A, METHOD B, METHOD C)

Methods	Labelled amount (mg/tab)	Amount found (mg/tab)	Percentage obtained (%)	Average percentage found (%)	S.D (+/-)	%RSD	S. E
Method A	2	2.06	100.34	100.14%	0.6093	0.6084	0.0169
	2	2.07	100.38	100.62%	0.3011	0.2993	0.0083
	2	2.04	102	101.25%	0.8215	0.8114	0.0228
Method B	2	1.996	99.8	100.41%	0.5092	0.5071	0.0141
	2	1.999	99.99	100.24%	0.3577	0.3569	0.0099
	2	2.02	101	100.61%	0.3188	0.3168	0.0088
Method C	2	2.01	100.5	100.44%	1.1938	1.18853	0.0331
	2	2.02	101.1	100.98%	0.522670	0.51756	0.0145
	2	2.04	102.21	101.03%	9990	0.9888	0.0277

Table 2: QUANTIFICATION OF FORMULATION (METHOD A, METHOD B, METHOD C)

Methods	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
METHOD A	PRCS	80	9	7.2	16.13	7.13	99.02	99.8 ± 0.7131	0.7143	0.0792
		100	9	9	18.16	9.16	101.11	0.7131	0.5266	0.0588
		120	9	10.8	19.78	10.78	99.81	100.51 ± 0.5293 99.56 ± 0.6842	0.6872	0.0760
METHOD B	PRCS	80	30	24	54.33	24.33	100.12	99.37 ± 0.7710	0.7759	0.0856
		100	30	30	59.9	29.9	99.6	0.7710	0.2533	0.0279
		120	30	36	66.01	66.01	100.02	99.33 ± 0.2516 100.14 ± 0.5021	0.5013	0.0557
METHOD C	PRCS	80	27	21.6	48.33	21.33	99.44	99.99 ± 0.4842	0.4843	0.0538
		100	27	27	53.93	26.93	99.87	0.4842	0.5716	0.0633
		120	27	32.4	59.37	32.37	99.94	99.72 ± 0.57 99.71 ± 0.5428	0.5444	0.0603

Table 3: RECOVERY ANALYSIS (METHOD A, METHOD B, METHOD C)

METHODS	LABELLED AMOUNT (mg/ tab)	PERCENTAGE OBTAINED (%)		S. D		%RSD	
		INTRADAY	INTERDAY	INTRADAY	INTERDAY	INTRADAY	INTER DAY
METHOD A	2	101.3	101.03	1.5814	0.6295	1.5811	0.6244
METHOD B	2	101.19	100.8	0.6453	0.3507	0.6403	0.3467
METHOD C	2	102.21	100.08	1.0651	1.1225	1.0530	1.1122

Table 4: INTRADAY AND INTERDAY PRECISION ANALYSIS (METHOD A, METHOD B, METHOD C)

Methods	Condition	Average percentage obtained (%)	S.D (±)	% R.S. D	S. E
METHOD A	Analyst	100.34	0.8414	0.8436	0.2103
	Instrument	100.34	0.1131	0.1126	0.0282
METHOD B	Analyst	99.8	0.0707	0.0708	0.0176
	Instrument	99.8	0.1343	0.1344	0.0335
METHOD C	Analyst	100.3	0.1414	0.1408	0.0353
	Instrument	101.03	0.6576	0.6539	0.1644

Table 5: RUGGEDNESS ANALYSIS OF FORMULATION (METHOD A, METHOD B, METHOD C)

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