



Simultaneous Estimation of Drug Digoxin in Tablet Dosage Form by UV Spectrophotometric Method

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Abstract: Digoxin has a cardiac glycoside property. Aim of the present study was to develop UV - Spectrophotometric method using simultaneous equation for the drug Digoxin in tablet formulation. Objective of the study is to develop a simple and precise analytical method for the drug Digoxin with statistical data. In validation, our study includes accuracy, precision, specificity, limit of detection, limit of quantitation, linearity and range parameters. Digoxin has antihypertensive activity and various new tablet formulations were already introduced in the market, so we compared the results of marketed tablet formulation with standard drug Digoxin. Literature review helped us in planning of work that includes selection of solvent, wavelength identification, sample preparation, analyzing test & standard solutions and validation study. The detection of the drug was carried out in 220 nm. The method was linear (Correlation Coefficient= 0.99) over the range of 25 to 125 μ g/ml, precise (Standard Deviation = 0.1, Relative Standard Deviation < 2%), accurate (mean recoveries from 97.5% and 104.3%), and had a LOD and LOQ equal to 0.12 and 0.38 μ g/ml, respectively. For specific stability, the forced degradation study (alkali, acid, oxide and heat) was performed on marketed formulations to show the stability indicating ability of the developed method. The method showed robustness, remaining unaffected by deliberated variations in spectrophotometric conditions. Due to correlative results the validated analytical method was successfully applied for the quantification of Digoxin and demonstrated the uniform distribution of the drug into the systems. Finally, we developed an accurate, less time consuming and cheaper UV-Spectrophotometric method for the determination of the Digoxin in tablet formulation.

Keywords: Digoxin; UV-Spectrophotometric method; Simultaneous estimation method; Validation

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I. INTRODUCTION

Digoxin, a digitalis derivative also known as cardiac glycoside is a natural compound sharing the ability to operate as potent inhibitors of the plasma membrane Na^+ / K^+ ATPase, hence promoting via an indirect mechanism the intracellular accumulation of Ca^{2+} ions. Chemical name of Digoxin is 3β -[(O-2,6-dideoxy- β -D-ribo-hexopyranosyl-O-2,6-dideoxy- β -D-ribo-hexopyranosyl-2,6-dideoxy- β -D-ribo-exopyranosyl)oxy]-12 β ,14-dihydroxy-5 β -card-20(22)-enolide.^{1, 2} The project study designed by PCI to improve student's skills in practical and documentation knowledge related to industry as well as academics. To focus on that, present study was designed to use recent dosage forms for analytical method development. To understand and improve skilled hands on instrumental techniques, we select UV – Spectrophotometer for method development. In recent times, most percent of the population is suffering from hypertensive conditions. Many drugs were introduced in the market which was worked as antihypertensive agents. So, we select one of the drug Digoxin in standard and tablet formulation for simultaneous estimation by UV – Spectrophotometric method development. Ultraviolet/visible spectroscopy is used more extensively in assaying than in identification. It can be used to calculate very small concentrations (of the order $0.0001 \text{ mol dm}^{-3}$) with extreme accuracy. It measures a spectrum very rapidly. Organic compounds can be identified and qualitatively analyzed by UV-Visible spectrophotometer. Quantitative analysis can be performed in the presence of turbidity. These advantageous features of UV Spectrophotometer were taken into consideration for choosing them in present study.³ Aim and objective of the present study was to gain knowledge of one of the novel instrumental technique like UV – Spectrophotometer and develop simultaneous estimation method, which was related to understand the principles, instrumentation & applications of instrument, project documentation and statistical data presentation. First done literature survey related to new Digoxin tablet formulation, solvent selection, method validation and result analysis. Several reports have described the methods for quantifying Digoxin in different samples and applying various modes of detections such as liquid chromatography-tandem mass spectrometry⁴⁻⁷, LC-MS / MS analysis of Digoxin⁸, RP-HPLC method for β -acetyldigoxin⁹, pulsed amperometric detection¹⁰ and liquid chromatography with ultraviolet detection¹¹. The official Pharmacopoeia present monographs of Digoxin raw material, tablets, oral solution and injection^{12, 13}. This compendium described an isocratic high performance liquid chromatography method with ultraviolet detection for assaying Digoxin in these products. Although these methods provide the selective measurement of the Digoxin, these techniques do not represent a viable analytical method for quantifying this drug using hydro alcoholic solvent. In this study, a spectrophotometric method with ultraviolet detection was developed and validated for the determination of Digoxin in tablets. The proposed analytical method offers the advantage of being extremely simple and rapid; and requires an ordinary detection device for quantification of the Digoxin.

2. MATERIALS AND METHODS

2.1 Reagents and materials

Standard API Digoxin was gifted by Zim Laboratory, Kalmeshwar, Nagpur. Lanoxin tablet 0.25 mg (GlaxoSmith

Kline Pharmaceutical Limited) was purchased from the local market. Other chemicals used in this study were Ethanol (AR Grade) and ultra pure water.

2.2 Instrumentation and analytical conditions

Systronics 2201 double beam UV-Visible Spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all solutions. Spectra were automatically obtained by UV Probe system software. The detection of the drug was carried out at 220 nm. Hydro alcoholic solvent was used as blank. A Shimadzu analytical balance model AUW220D (Japan) and an ultrasonic bath Quimis[®] (Brazil) were also used.

2.3 Preparation of solutions

2.3.1 Digoxin standard solution:-

Weigh accurately about 25 mg of Digoxin API was transferred in a 25 ml volumetric flask. Approximately 50% of the hydro alcoholic solvent (ethanol:water:80:20) was added. The solution was sonicated for 10 minutes and the volume was adjusted to 25 ml with the same solvent (Conc. 1000 $\mu\text{g/ml}$).^{1, 12}

2.3.2 Digoxin working sample solution

An aliquot 2.5 ml standard solution was transferred to a 100 ml volumetric flask and the volume was completed with a hydro alcoholic solvent, to obtain a concentration was 25 $\mu\text{g/ml}$.^{1, 12}

2.4 Method validation¹⁶

2.4.1 Linearity

The linearity was performed according to the procedure reported in the reference.¹⁴ The calibration curve was constructed using five Digoxin standard concentrations (25; 50; 75; 100 and 125 $\mu\text{g/ml}$) in 3 independent replicates run in random order. The linear regression analysis was done by the ordinal least squares method (OLSM).

2.4.2 Range

Accurately weighed quantities of Lanoxin tablet powder equivalent to about 0.25 mg of Digoxin were transferred to five different 25.0 ml volumetric flasks and 2.5 mg, 5.0 mg, 7.5 mg and 10.0 mg of standard Digoxin were added to 2nd, 3rd, 4th and 5th flasks, respectively (representing 80 - 120% of labeled claim). This was followed by addition of hydro alcoholic solvent to about 20.0 ml in each flask then mixtures were shaken for 10 min and sonicated for 15 min. Then sufficient hydro alcoholic solvent was added to each flask to adjust the volume up to 25.0 ml mark and filtered using Whatman grade I filter paper. Then pipette out 1.0 ml of each of the filtrate from each flask and was diluted up to 100.0 ml with hydro alcoholic solvent and absorbances were recorded at 220.0 nm.

2.4.3 Precision

The intra-assay precision (repeatability) was evaluated by determining a solution at concentration 10 $\mu\text{g/ml}$ on the same day. The solution was prepared as 1.0 ml of standard

solution was transferred to a 100 ml volumetric flask and the volume was makeupsing a hydro alcoholic solvent, to obtain a concentration of 10 $\mu\text{g}/\text{ml}$ ($n = 3$ for each concentration). Similarly, the inter-assay (intermediate precision) was evaluated in two consecutive days ($n = 9$ for each concentration). The precision was expressed as standard deviation (SD), relative standard deviation (RSD) amongst responses.

2.4.4 Accuracy

Digoxin standard solution was prepared. At each level, solutions were prepared and the recovery percentage was calculated ($n = 3$). The accuracy was evaluated on three different days ($n = 9$). The percent recovery of added Digoxin was calculated by comparing absorbances of the resultant solutions with Digoxin standard solutions at the same concentration. The standard deviation (SD), relative standard deviation (RSD) was also calculated. Accurately weighed quantities of pre analyzed tablet powder equivalent to about 0.25 mg of Digoxin were transferred to five different 25 ml volumetric flasks and accurately weighed 5.0 mg of standard Digoxin were added in each flask. This was followed by addition of about 20.0 ml hydro alcoholic

solvent, shaken the mixture for 10 min and sonicated for 15 min. Sufficient hydro alcoholic solvent was added to each flask to adjust the volume up to 25.0 ml mark and filtered through Whatman grade I filter paper. Pipette out 1.0 ml of each of the filtrate and diluted up to 100.0 ml with hydro alcoholic solvent and absorbances of sample solutions were measured at 220.0 nm.

2.4.5 Robustness

Robustness was determined by analyzing the same Lanoxin tablet solution at the pre established operating condition (wavelength – 220 nm) and also by changing this operating analytical condition (wavelengths - 218 and 222 nm). The digoxin content was determined for each condition, using the hydro alcoholic solvent as blank, and the obtained data were submitted to statistical analysis (analysis of variance test).

2.4.6 Quantitation limit⁹

The limit of quantitation value (LOQ) is defined as the lowest concentration that can be quantitatively determined with suitable precision and accuracy. The LOQ was calculated directly from the calibration curve and can be expressed as –

$$LOQ = \frac{10 \sigma}{b}$$

Where, σ is the standard deviation of the response and b is the slope of the calibration curve.

2.4.7 Detection limit⁹

Detection limit or LOD (limit of detection), is the lowest quantity of a substance that can be distinguished from the absence of that substance (a blank value) with a stated confidence level (generally 99%). The detection limit is

$$LOD = \frac{3.3 \sigma}{b}$$

Where, σ is the standard deviation of response and b is the slope of the calibration curve.

2.4.8 Specific stability

The specific stability studies were carried out by attempting deliberate degradation of the Lanoxin tablet sample with exposure to stress conditions like acidic, alkaline, oxidation and heat. Accurately weighed quantities of Lanoxin tablet powder equivalent to 25.0 mg of Digoxin were transferred to four different 25 ml volumetric flasks. The samples were exposed to stress conditions for 24 h as follows-

- First flask at 50°C sample with 0.1 M NaOH (1.0 ml).
- Second flask at 50°C sample with 0.1 M HCl (1.0 ml).
- Third flask at 50°C sample with 3% H_2O_2 (1.0 ml).
- Fourth flask at 50°C only sample.

After 24 hr the flasks were cooled at room temperature and neutralize the first and second sample solutions. Diluted the resulting solutions with a hydro alcoholic solvent up to the mark, shaken the mixture for 10 min. and sonicated for 15 min. Filtered the resulting solutions through Whatman grade I filter paper. Then pipette out 10.0 ml of the resulting solutions and diluted up to 100.0 ml with a hydro alcoholic solvent.

3. STATISTICAL ANALYSIS¹⁵

Statistical analysis is the science of collecting data and analyzing it for data conformation, making future predictions based on past values and testing an experiment's hypothesis. In this study statistical analysis was done using mean, standard deviation and relative standard deviation methods.

3.1 Mean

The mean is the average of the numbers. Formula is

$$X = (X_1 + X_2 + X_3 + \dots + X_n)/n$$

Where,

X = Mean

n = Number of values

3.2 Standard Deviation

The standard deviation is a measure of how spread out numbers are. It is the square root of the variance means the average of the squared differences from the mean. Its symbol is σ (sigma). Formula is –

$$\sigma = \sqrt{\frac{1}{N} \left[\sum_{i=1}^N (ix_i - \mu)^2 \right]}$$

Where,

σ = Standard deviation

ix_i = Value of discrete observation indexed by i

N = Number of discrete observations

μ = Mean value of discrete observation

3.3 Relative Standard Deviation

Relative standard deviation is also called as percentage relative standard deviation. Relative standard deviation is the deviation measurement that tells us how the different numbers in a particular data set are scattered around the mean. This formula shows the spread of data in percentage. Formula is –

$$RSD = \frac{\sigma \times 100}{X}$$

Where,

RSD = Relative standard deviation

σ = Standard deviation

X = Mean

4. RESULTS AND DISCUSSION

4.1 Development of the spectrophotometric method

In this study, an ultraviolet spectrophotometric method was developed and validated for the determination of Digoxin in tablet formulation. It was developed as a simple, rapid and eco-friendly method. An environmental concern was in mind for the use of organic solvents in the sample preparation. So for this reason, a hydro-alcoholic solvent was selected as diluting solvent. However, the developed ultraviolet method overcomes this type of problem by replacing more toxic solvents with hydro-alcoholic solvent. So we use (ethanol: water::80:20) hydro-alcoholic mixture.¹ Initially, an ultraviolet spectroscopic scanning run for the Digoxin standard and sample solutions provided an intense absorption band with maximum wavelength at 220 nm. The ultraviolet spectrum of

the hydro alcoholic solvent was also recorded and it was verified the existence of interferences or overlaps with the Digoxin response at 220 nm, indicating that all samples should be analyzed using hydro alcoholic solvent, as blank.

4.2 Method validation¹⁶

Several solvents were tried, based on sufficient absorbance and symmetry of the peak, the most suitable solvent was found to be a hydro alcoholic solvent for the estimation of Digoxin. Digoxin working sample solution (concentration 25 μ g/ml) was scanned in the UV range of (200 - 400 nm) in 1.0 cm cell against blank to obtain the spectrum of the drug Digoxin. Digoxin showed well-defined λ_{max} at 220.0 nm and this wavelength was selected for further study. The UV absorbance spectrum of Digoxin is shown in Figure 1.^{1,3}

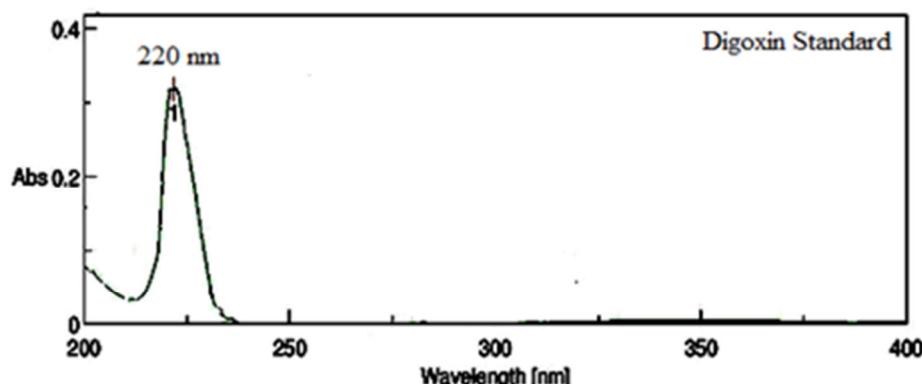


Fig 1: UV Spectra of Drug Digoxin at λ_{max} 220.0 nm

4.2.1 Study of Beer-Lambert's Law

Weigh accurately about 25 mg of Digoxin API and was dissolved in a 50% of a hydro alcoholic solvent (ethanol:water::80:20) in the volumetric flask. The solution was sonicated for 10 minutes and the volume was adjusted up to 25 ml with the same solvent. Aliquot portions of stock standard solution for Digoxin were appropriately diluted

with hydro alcoholic solvent to get a series of concentrations between 25.0 - 125.0 $\mu\text{g}/\text{ml}$. The absorbance of each solution was measured at 220.0 nm in 1.0 cm cell against solvent blank. The graph was plotted as absorbance Vs concentration at selected wavelength and found to be linear. The graph was shown in Figure 2 along with the related data given in Table I.

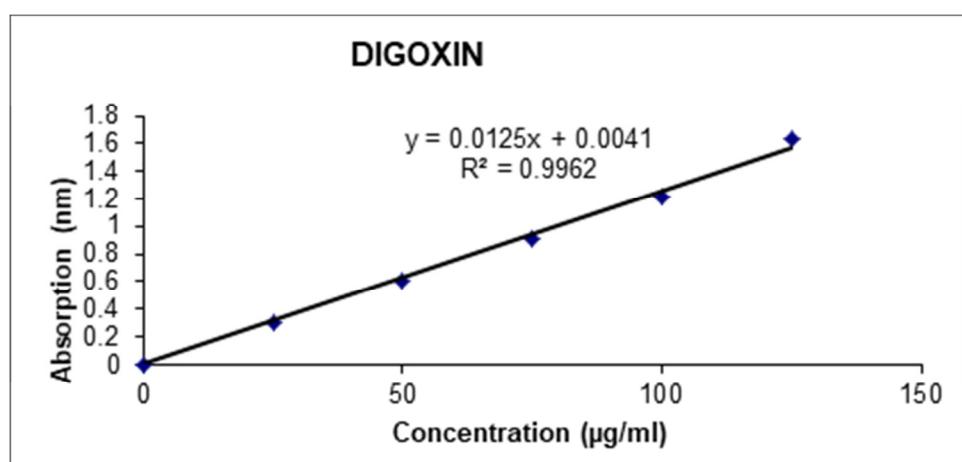


Fig 2: Plot of Beer-Lambert Study for Drug Digoxin

Table I: Characteristics of regression line

Sr. No.	Parameters	Values
1	Linear range ($\mu\text{g}/\text{ml}$)	25.0 – 125.0
2	Equation	$y = 0.0125x + 0.0041$
3	Slope	0.013
4	y intercept	0.0041
5	Correlation coefficient	0.9962
6	Standard deviation	0.5165
7	LOD ($\mu\text{g}/\text{ml}$)	0.12
8	LOQ ($\mu\text{g}/\text{ml}$)	0.38

4.2.2 Absorptivity value

Weigh accurately about 25 mg of Digoxin API and was dissolved in a 50% of a hydro alcoholic solvent (ethanol:water::80:20) in the volumetric flask. The solution was sonicated for 10 minutes and the volume was adjusted

up to 25 ml with the same solvent. Aliquot portions of Digoxin stock standard solution were diluted with hydro alcoholic solvent and obtained the concentrations as 10, 30 and 50 $\mu\text{g}/\text{ml}$. The absorbance of each solution was measured at 220.0 nm and $A (1\%, 1 \text{ cm})$ value was calculated using following formula-

$$A (1\%, 1 \text{ cm}) = \frac{\text{Absorbance}}{\text{Conc.}(\text{g}/100\text{ml})}$$

$A (1\%, 1 \text{ cm})$ value is tabulated in the following Table 2.

Table 2: Absorptivity values, A (1 %, 1 cm) of Digoxin		
Absorptivity Value	Standard Deviation	Relative Standard Deviation
134.9	0.1298	0.7628

*Average of five sample solutions

4.2.3 Analysis of marketed formulation

Twenty tablets were weighed and the average weight of the tablet was calculated. The tablets were crushed to fine powder and mixed thoroughly. Accurately weighed quantity of tablet powder equivalent to about 25 mg of Digoxin was transferred to 25.0 ml volumetric flask. Add 50% of a hydro alcoholic solvent (ethanol: water::80:20) in the volumetric flask and shaken for 10 min. Then the solution was sonicated

for 15 min. and the volume was made up to the mark using same solvent. Filtered the solution mixture using Whatman grade I filter paper.¹ The aliquot portion of filtrate (1.0 ml) was further diluted with hydro alcoholic solvent up to 100.0 ml and get a final concentration of about 10 µg/ml (on labeled claim basis). The absorbance of the sample solution was measured at 220.0 nm in 1.0 cm cell against blank. The content of Digoxin in tablet was calculated using the following formula -

$$C = \frac{A}{a}$$

Where, C is the concentration of drug in g/100 ml, A is the absorbance of drug at 220.0 nm, a is the absorptivity of drug at 220.0 nm.

$$\% \text{ Label claim} = \frac{C \times D \times W}{W_m \times L} \times 100$$

Where, C is the concentration of drug in g/100 ml, D is the dilution factor, W is the average weight of tablet, W_m is the weight of sample taken, L is the label claim of sample taken.

Tabulated data shown in Table 3. Brand name was Lanoxin tablet (0.25 mg) and average weight was 0.1403 g.

Table 3: Results of % label claim of Digoxin for marketed formulation

% Label Claim	Standard Deviation	Relative Standard Deviation
99.54	0.5370	0.5394

*Average of five sample solutions

4.2.4 Accuracy

Accuracy of the proposed method was ascertained on the basis of recovery studies performed by standard addition method. Accurately weighed five quantities of tablet powder equivalent to about 25 mg of Digoxin were transferred to 25.0 ml volumetric flasks. Add 50% of a hydro alcoholic solvent (ethanol:water::80:20) in each volumetric flask and shaken for 10 min. Add five different 5 mg API Digoxin in five

volumetric flasks. Then the solution was sonicated for 15 min. and the volume was made up to the mark using same solvent. The solution mixtures were filtered using Whatman grade I filter paper. Then pipette out 1.0 ml of the filtrate from each flask and diluted up to 100.0 ml with hydro alcoholic solvent. Then measured the absorbances of each the sample solution at 220.0 nm and the content of the drug was calculated using the following formula. The results were given in Table 4.

$$\% \text{ Recovery} = \frac{A}{B + C} \times 100$$

Where, A is the total amount of drug estimated, B is the amount of drug found on a pre analyzed basis, C is the amount of pure drug added.

Table 4: Results and statistical data for recovery study of Lanoxin tablet

Wt. of tablet powder (mg) +Amount of pure drug added (mg)	Amount of pure drug recovered (mg)	% Recovery	Standard Deviation	Relative Standard Deviation
280.04 + 5.04	5.02	99.75	0.1106	0.1108

*Average of five sample solutions

4.2.5 Precision

4.2.5.1 Repeatability

Precision of the proposed method was ascertained by replicate analysis of tablet powder sample. The tablets were crushed to fine powder and mixed thoroughly. Accurately

weighed five quantities of tablet powder equivalent to about 25 mg of Digoxin were transferred to five 25.0 ml volumetric flasks and shaken with hydro alcoholic solvent for 10 min. and the volume was made up to the mark. The solutions were sonicated for 15 min. and then filtered through Whatman grade I filter paper. The aliquot portion of filtrate (1.0 ml) from each volumetric flask was further diluted with

hydro alcoholic solvent up to 100.0 ml and get a final concentration of about 10 $\mu\text{g}/\text{ml}$ (on labeled claim basis). The absorbance of the sample solutions were measured at 220.0 nm in 1.0 cm cell against blank. The results were shown in Table 3. The repeatability result related to percent labeled claim of Lanoxin tablet was complied with United State Pharmacopeia (Range 98-102%).¹³

4.2.5.2 Intermediate precision

Intraday study

Using the same procedure as under marketed formulation analysis and the absorbance recorded at 3 hr interval within a day. The % label claim was calculated using the same formula as for marketed formulation analysis. The tablets were crushed to fine powder and mixed thoroughly. Accurately weighed three quantities of tablet powder equivalent to about 25 mg of Digoxin were transferred to five 25.0 ml volumetric flasks and shaken with hydro alcoholic solvent for 10 min. and the volume was made up to the mark. The solutions were sonicated for 15 min. and then filtered through Whatman grade I filter paper. The aliquot portion of filtrate (1.0 ml) from each volumetric flask was further diluted with hydro alcoholic solvent up to 100.0 ml and get a final concentration of about 10 $\mu\text{g}/\text{ml}$ (on labeled claim basis). The absorbance of the sample solutions were measured at 220.0 nm in 1.0 cm cell against blank. The results were given in Table 5.

Interday study

Same procedure was performed as under marketed formulation analysis and absorbances of the same sample were recorded on different days. The % label claim was calculated using the same formula as for marketed formulation analysis. The tablets were crushed to fine powder and mixed thoroughly. Accurately weighed three quantities of tablet powder equivalent to about 25 mg of Digoxin were transferred to five 25.0 ml volumetric flasks and shaken with hydro alcoholic solvent for 10 min. and the volume was made up to the mark. The solutions were sonicated for 15 min. and then filtered through Whatman grade I filter paper. The aliquot portion of filtrate (1.0 ml)

from each volumetric flask was further diluted with hydro alcoholic solvent up to 100.0 ml and get a final concentration of about 10 $\mu\text{g}/\text{ml}$ (on labeled claim basis). The absorbance of the sample solutions were measured at 220.0 nm in 1.0 cm cell against blank. The results were given in Table 5.

Different analyst

Two different analysts prepared the sample solutions. Same procedure was followed as described earlier. The tablets were crushed to fine powder and mixed thoroughly. Accurately weighed quantity of tablet powder equivalent to about 25 mg of Digoxin was transferred to 25.0 ml volumetric flask and shaken with hydro alcoholic solvent for 10 min. and the volume was made up to the mark. The solution was sonicated for 15 min. and then filtered through Whatman grade I filter paper¹. The aliquot portion of the filtrate (1.0 ml) was further diluted with hydro alcoholic solvent up to 100.0 ml and gets a final concentration of about 10 $\mu\text{g}/\text{ml}$ (on labeled claim basis). The absorbance of the sample solution was measured at 220.0 nm in 1.0 cm cell against blank. The % label claim was calculated in marketed formulation estimation and the results were given in Table 5.

Robustness study

Same procedure was performed as under marketed formulation analysis and the absorbances of sample were recorded on two different wavelengths at 218 and 222 nm. The % label claim was calculated using the same formula as for marketed formulation analysis. The tablets were crushed to fine powder and mixed thoroughly. Accurately weighed five quantities of tablet powder equivalent to about 25 mg of Digoxin were transferred to five 25.0 ml volumetric flasks and shaken with hydro alcoholic solvent for 10 min. The solutions were sonicated for 15 min. and the volume was made up to the mark. Then the solution mixtures were filtered through Whatman grade I filter paper. The aliquot portion of filtrate (1.0 ml) from each volumetric flask was further diluted with hydro alcoholic solvent up to 100.0 ml and get a final concentration of about 10 $\mu\text{g}/\text{ml}$ (on labeled claim basis). The absorbance of the sample solutions were measured at 218.0 and 220.0 nm in 1.0 cm cell against blank. The results were given in Table 5.

Table 5: Results and statistical data for precision study

Particulars	% Label Claim	Standard Deviation	Relative Standard Deviation
Intraday study	99.02	0.0750	0.0757
Interday study	99.03	0.0750	0.0750
Different analyst	98.94	0.0070	0.0070
Robustness study	100.70	0.5232	0.5195

*Average of five sample solutions

In intraday study, interday study, different analyst study (at 220.0 nm) and robustness study (at 218.0 & 222.0 nm) applied procedure shows percent labeled claim values within range with RSD values found less than 2%.

4.2.5.3 Linearity and Range

Linearity of response

Weigh accurately about 25 mg of Digoxin API and transferred in a 25 ml volumetric flask. Approximately 50% of hydro alcoholic solvent was added. The solution was sonicated for

10 minutes and the volume was adjusted up to 25 ml with the same solvent. The stock standard solution of Digoxin was diluted with a hydro alcoholic solvent to get a series of concentrations ranging from 25.0-125.0 $\mu\text{g}/\text{ml}$. Absorbances of these solutions were measured at 220.0 nm in 1.0 cm cell using solvent blank. The graph plotted as concentration Vs absorbance and depicted in Figure 2 was found to be linear.

Range of the method

Accurately weighed five quantities of tablet powder equivalent

to about 25 mg of Digoxin were transferred to five 25.0 ml volumetric flasks and shaken with hydro alcoholic solvent for 10 min. and the volume was made up to the mark. The solutions were sonicated for 15 min. and then filtered through Whatman grade I filter paper. The aliquot portions of filtrate (8.0, 9.0, 10.0, 11.0 and 12.0 ml) from each

volumetric flask were further diluted with hydro alcoholic solvent up to 100.0 ml and get a final concentration of about 80 - 120% of labeled claim. The absorbance of the sample solutions were measured at 220.0 nm in 1.0 cm cell against blank. The graph was depicted in Figure 3 and the results were tabulated in Table 6.

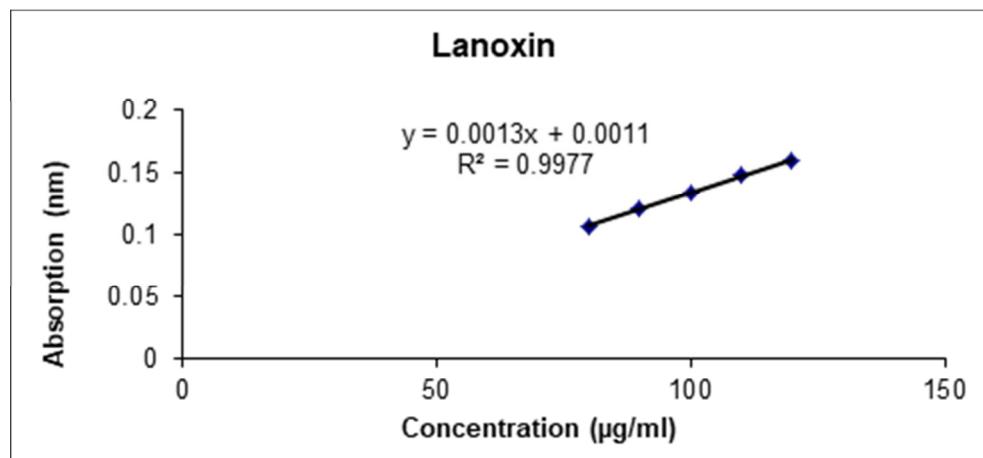


Fig 3: Plot of range study for Lanoxin tablet

Table 6: Results of range studies for Lanoxin

% Recovery	Standard Deviation	Relative Standard Deviation
99.04	0.5430	0.5482

*Average of five sample solutions

4.2.5.4 Limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ were determined by the method based on standard deviation of the response and the slope of the calibration curve was found out as per ICH guidelines.¹⁶ Weigh accurately about 25 mg of Digoxin API and was dissolved in a 50% of a hydro alcoholic solvent in the volumetric flask. The solution was sonicated for 10 minutes and the volume was adjusted up to 25 ml with the same solvent. Aliquot portions of stock standard solution for Digoxin were appropriately diluted with hydro alcoholic solvent to get a series of concentrations between 25.0 - 125.0 μg/ml. Taking the absorbances of solutions at 220.0 nm, find out the standard deviation value and using that value calculate the LOD & LOQ values of solutions. Observation was limit of detection and limit of quantitation helps in determining Digoxin molecule concentration with an acceptable level. The method of analysis was already mentioned in study of Beer's Lambert's Law and the results were shown in Table 1.

4.2.5.5 Specific stability

Samples of Digoxin tablet formulation were prepared according to procedure and absorbances were recorded at 220.0 nm. 20 tablets were taken. The tablets were crushed to fine powder and mixed thoroughly. Accurately weighed quantity of tablet powder equivalent to about 25 mg of Digoxin was transferred to four different 25 ml volumetric flasks. First flask tablet powder & 1.0 ml 0.1 M NaOH, second flask tablet powder & 1.0 ml 0.1 M HCl, third flask tablet powder & 1.0 ml 3% H₂O₂ and fourth flask only tablet powder; stored all four flasks at 50°C temperature for 24 h. Diluted the resulting solutions with a hydro alcoholic solvent up to the mark, shaken the mixtures for 10 min. and sonicated for 15 min. Filtered the resulting solutions through Whatman grade I filter paper. Then pipette out 10.0 ml of the resulting solutions and diluted up to 100.0 ml with a hydro alcoholic solvent. It was observed that the concentration of Digoxin was not declined by the stability process. There was no any interference of degraded products on Digoxin. The results for standard drug and tablet formulations were tabulated in Table 7.⁵

Table 7: Results of specific stability

Sr. No.	Name of Condition	Standard Drug (Digoxin) (%)	Tablet Formulation (%)
1	Alkali	95.64	94.53
2	Acidic	97.35	96.24
3	Oxide	96.00	95.89
4	Heat	94.59	97.58

In the analysis of Lanoxin tablet marketed formulation results were found to be % label claimed (99.54 %) lies in between 98 – 102 %, standard deviation (0.5370) less than 1 and relative standard deviation (0.5394) less than 2 %. % label claimed value of Lanoxin tablet for all above mentioned method validation parameters found in the range which was

mentioned in United State Pharmacopoeia.¹² In this method use of hydro alcoholic solvent for method development was environment friendly, less toxic and easily available. In recovery study of Digoxin result was found to be 99.75 % recovery for marketed formulation, obtained drug was 5.02 mg and RSD value less than 2% as compared to other

research paper¹. LOD and LOQ values (0.12 & 0.38) were found within range when compared with other research papers^{1,9}. In specific stability study there was no interference of any of the degradation products from the stress conditions tested. Thus, the developed method for analytical determination of Digoxin was established to be specific and stability-indicating⁹.

5. CONCLUSION

In conclusion, a spectrophotometric method for determination of Digoxin into tablets was developed and validated in terms of linearity, range, limit of quantitation, limit of detection, precision, accuracy, robustness and specific stability study. This simple and rapid method was capable of quantifying the digoxin in tablets. Using this study author understands the working procedure and handling SOP of UV Spectrophotometer with calculating the statistical parameters and knowledge about stability indicating method. Author gaining idea about how project work was completed and author ready in future for PG or Ph.D. level research work with positive approach. Therefore, this validated

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spectrophotometric method could be applied to demonstrate the ability of the different instrumental systems for control and sustained release tablets.

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7. AUTHORS CONTRIBUTION STATEMENT

Ms. Omita Paulzagade performed all experimental study related to Digoxin standard and marketed formulation with taking the assistance from laboratory technician. Dr. Anshuman Borkar analyzed the experimental study data and arrange the statistical data for presentation in journal.

8. CONFLICT OF INTEREST

Conflict of interest declared none.