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A Comprehensive Review of Analytical Method – Development and Method Validation of Digoxin Tablets

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Abstract

The main objective of this review is to give an outline of the method development and validation process of digoxin, and this review covers the different types of spectroscopic and chromatographic techniques, such as HPLC, UHPLC-Tandem mass spectroscopy, TLC, and LC-MS. A novel method for the determination of digoxin in biological samples has been developed and validated, focusing on improving sensitivity, specificity, and reproducibility. The method was validated in terms of linearity, accuracy, precision, specificity, detection limit, quantification limit, and stability according to the guidelines of the international conference on harmonization (ICH). The precision and accuracy of the method met the required standards for clinical applications, demonstrating minimal intra and inter day variation digoxin a cardiac glycoside used in the treatment heart failure and arrhythmias, requires precise quantification due to its narrow therapeutic index, high performance liquid chromatography coupled with ultraviolet detection, optimized for the separation and quantification of digoxin in plasma, the method development process involved selecting an relevant mobile phase composition, stationary phase and ensuring the chromatographic condition, such as flow rate and temperature, the linearity limit of detection(LOD), limit of quantification, precision, and accurately assessed the method development provide reliable, systematic and applicable for clinical and pharmacokinetic studies of digoxin.

Keywords: LC-MS, limit of detection, digoxin, pharmacokinetic studies, ICH, method validation, thin layer chromatography, analytical method

INTRODUCTION

Analytical method validation ensuring the quality and reliability of analytical results. Synthetic materials constituted with one or more compound, chemical additives, Identification of herbal, separation, quantification are the process involved in analytical chemistry [1].

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It is mainly classified into two categories:

1. Qualitative rate that expresses the identification regarding the chemical additives subsisting in the sample.
2. Quantitative rate estimates the amount of positive detail or compound present in the sample.

Digoxin and digitoxin are cardiac glycosides obtained in purified form from the leaves of *digitalis lanata* and digitalis purpura. Congestive heart failure, atrial fibrillation, and cardiac arrhythmias are treated by using digoxin [2]. The presence of pharmaceutical excipients and equal formulation of degradation products introduce additional requirements of precision. The study of digoxin and digitoxin in

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their several dosage forms arouses for methods that are sensitive enough unit dose amount of digoxin (0.125 mg per tablet) and digitoxin (0.1 mg per tablet) with acceptable selectivity to prevent any interference from other compounds.

Digoxin dosage form prescribed a high degree of content uniformity for low unit dosages, narrow toxic to therapeutic dosage ratio accompanying with inter subject variation of sensitivity (Figure 1) [3].

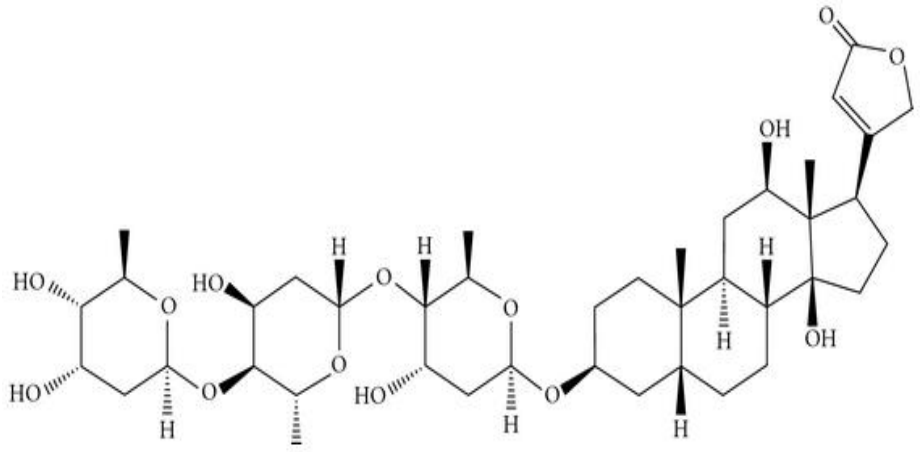

Figure 1. Structure formula of digoxin.

Name

It is known by the names cardioxil, davoxin, dicaxin, lanoxin, rougoxin, vanoxin.

Appearance

Digoxin is an odorless, white crystalline powder, and its crystals appear radially arranged.

Pharmacological Action

Digoxin is a cardiac glycoside medication that exhibits the pharmacological actions: (1) Inhibition of Na+/k+ATP as pump in cardiac cells, lead to increase in intracellular sodium and calcium levels. (2) Increased contractility (increased calcium levels enhance contractility, resulting in improved cardiac output and reduced symptoms of heart failure. (3) Decreased heart rate (digoxin slows heart rate by increasing the effective refractory period and prolonging the AV node conduction). (4) Increased ventricular filling time. (5) Prolonged AV node conduction, (6) Increased effective refractory.

ANALYTICAL METHOD DEVELOPMENT

When there are no determinative techniques that can be afforded, new methodologies proceed. To inspect the presence of both pharmacopeial and non-pharmacopeial products, new techniques are developed to bring down the values aside time for eminent precision and strength. Analytical data support to provide batch consistency during manufacturing. In combination with dosage form and organic fluids, the drug evaluation exhibits the identity characterization and resolution of drugs.

Importance requirements for the development of an analytical method for digoxin tablets

- Sensitivity (to detect and quantify digoxin at low concentrations (e.g., 0.1–0.2 μg/ml).

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- Instruments – qualified and calibrated.
- Impurities and degradation products: Evaluation of the analytical method's ability to detect and quantify.
- Assuring quality.
- Mandatory requirement purpose for accreditation as per ISO 17025 Guidelines.
- Required accuracy and required range of analysis (Figure 2) [4].

Figure 2. Process involved in analytical method development.

Chromatographic and Spectroscopic Technique for the Development of Digoxin

Cardiac glycoside and its aglycones are separated by

- Thin-layer chromatography.
- High-performance liquid chromatography.
- Liquid chromatography.
- UV Spectroscopy.
- Liquid chromatograph-mass spectrophotometer (LC-MS).
- New ultra-high performance liquid chromatography – tandem mass spectroscopy.
- IR Spectroscopy is the technique that has been utilized for the separation and quantitation of cardiac glycosides.

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High-Performance Liquid Chromatography

High-performance liquid chromatography is used to separate complex mixtures. It utilizes a stationary phase that has a very small particle size (3–20 μm). This increased the elution rate over 100-fold. HPLC is a more flexible technology than gas chromatography, and it is not suitable for volatile and thermally stable substances. It may be employed with a large variety of mobile and stationary phases. HPLC utilizes a column that holds a packing material (stationary phase), a column and detector which shows the retention time. This time varies depending upon the molecules analyzed [5]. The basic concept is that a sample solution is put into a stationary phase composed of porous material, and then the mobile phase is pumped through the column at high pressure. The normal flow rate in HPLC is 1 to 10 ml/min for analysis and for preparative analysis flow rate is up to 30 ml/min. This should generate high pressure, up to 6000 psi. Isocratic [same ratio of mobile phase composition during entire analysis], gradient means altering the mobile phase composition.

METHOD A

Sample Preparation

Sample equivalent to 1.0mg of digitoxin in 100 ml volumetric flask, add water and swirl for few minutes. Add 34 ml of MeOH and shake for 10 minutes. Filter and wash the residue with distilled water, then add the internal standard and dilute with water to volume and mix.

Preparation of Standard Solution

20mg of digoxin is transferred into the volumetric flask with 80 ml of methanol. Dissolve and cool and make up with methanol. Transfer a solution to six aliquots to this add stock solution to volumetric flask and add 2.5 ml of internal standard solution and make up 35 ml of methanol. Dilute the solution to 100 ml with distilled water and mix.

PROCEDURE

Equilibrate the column with solvent system, water /methanol /isopropanol /dichloromethane:45/38/11/6. 20 μl of the sample is injected into a liquid chromatogram, which has been adjusted to specified conditions flow rate is 1.1 ml/min: uv detection at 220 nm, range of 0.02, speed chart of 0.5 cm/min. record the chromatogram.

METHOD B

Separation of Digoxin and Its Metabolites After Fluorogenic Post Column Derivatization: Preparation of Hydrogen Peroxide Solution

Add 1 ml of hydrogen peroxide solution to 200 ml of water and mix.

Preparation of Dehydroascorbic Acid Solution

100 mg of ascorbic acid and dissolve in 200 ml of water. Add 5 ml of hydrogen peroxide solution dropwise.

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Procedure

Position solvaflex orange green and orange blue and acid flex white tube pump tubes on the technicon to deliver air, acid solution, segment the stream of acid, connect the tube delivering air acid and HPLC column effluent, pass the segmented solution through the reaction coil, cooling coil and assemble the debubbling setup for 100% fluid recovery .using acid flex tubing connect the vertical exit of the debubbler to the tapering, position the glass tube for circulating the fluid and entering the debbubler through the horizontal exit into the fluorescent detector, optimize the debubbling processes and adjust the flow rate about 1ml/min (Table 1).

Table 1. Chromatographic conditions.

Parameters	Conditions
Solvent	Water/methanol/isopropanol.
Uv detection	254 nm.
Fluorescent detection	360 nm.
Flowrate	0.4 ml/min.
Speed chart	0.5 cm/min.

HPLC Procedure

Standardize the column with solvent system. Dissolve the mixture of digoxin in the eluting solvent system. The sample solution is injected into the liquid chromatogram. Which has been adjusted to specified conditions flow rate –0.4ml/min, measure the uv detection at 254 nm. Speed chart of 0.5 cm/min. Record the chromatogram.

METHOD C

There are some methods for determination of digoxin after the dissolution test. In digoxin, water/0.1M Hcl is used as a dissolution medium after dissolution withdraw a sample analyzed by using uv or HPLC technique. Standard and sample solutions are prepared at 2ppm concentration with suitable diluents, such as methanol, acetonitrile, and water (Table 2).

Table 2. Chromatographic condition [6]:

Pump	Binary pump G1312A
Dissolution apparatus	Erweka DT700CH
UV detector	G1315D
Column	Symmetry C18 3.5
Column length	75 mm x 4.6 mm
Degasser	Vacuum degasser G1379B
Autosampler	G1329A
Column temperature	20°C
Flow rate	0.8 ml min_

UHPLC-Tandem Mass Spectroscopy

Prepared digoxin standard solution at 1mg /ml and the blood samples were centrifuged about 4000–5000 rpm then collects the plasma. All samples were previously tested with a routine immunoassay. Patient plasma samples were kept at –20°C preceding analysis within 2 weeks (Table 3).

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Table 3. Chromatographic conditions.

Parameters	Conditions
Column	C18 (2.1 × 50 mm, 1.7 µm)
Column temperature	50°C
Mobile phase A	Water, 5 mm ammonium formate, and formic acid 0.01%
Mobile phase B	Acetonitrile, 0.1 % of formic acid
Flow rate	0.4 ml per min
Injection volume	2 µl
UHPLC Instrument	Model 1 UHPLC BEH
Liquid chromatography equipment	Model 6500 triple quadrupole mass spectrometer with Ion Drive Turbo V

PROCEDURE

A simple LIQUID- LIQUID extraction procedure used for this determination plasma sample (200 µl) was transferred in a polypropylene tube is added with 1ml of methyl tert- butyl ether with 10 µl of digoxin solution to final concentration of 10 ng\ml, then the mixture was vortex mixed for 2 mins, centrifuged at 13000 rpm for 15 minutes in microfuge light centrifuged. The supernatant was collected and transferred into glass tubes, where it was evaporated under nitrogen steam. Separation was performed by using model 1 UHPLC, and the column was thermostated. Injected samples were eluted with linear gradient from 30 to 60% of solvent column was washed with 90% of mobile phase B and digoxin was eluted in minutes.

Infrared Spectrum of Digoxin

Prepare a kBr pellet and record the spectrum on the Beckman IR-10 spectrophotometer. The spectrum obtained on IR was compared with a standard reference spectrum and has the same characteristics as absorption bands.

Procedure

Weigh 10.0 mg of digoxin in a Cahn electro balance. With the help of 50 ml of boiling methanol, transfer it into a 100 ml volumetric flask. The above solution is diluted from 10 ml to 100 ml with 35% methanol, using 35% methanol as the blank. The maximum absorption of radiant energy was found to take place at 220 nm.

Thin Layer Chromatography

Thin-layer chromatography is also one of the separation techniques.

EXAMPLE

Sample Solution Preparation

Mixture of equal volume of dichloromethane and methanol containing 1.0%w/v of the substance, 1.0% w/v of digoxin RS, 0.020%w/v of digoxin RS, 0.010%w/v of digoxin, 0.010%w/v of digitoxin RS, 0.020w/v of gitoxin RS (Table 4).

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Table 4. Chromatographic conditions.

Parameter	Conditions
Coating substance	Kieselguhr G
Mobile phase mixture	50 volumes of butanone, 50 volumes of xylene, 4 volumes of formamide
UV light	365 nm
Detecting agent	15 volumes of 25%w/v of trichloroacetic acid, ethanol 95% & 1 volume 3%w/v of chloramine

PROCEDURE

Coat the plate using a coating substance. Place the dry plate in a solvent tank containing a mixture of 90 volumes of acetone and 10 volumes of formamide. Dip the plate about 5 mm into the liquid and impregnate solvent to ascend 15 cm. Remove the plate from the tank and allow it to stand for 30 minutes.

Utilize the mobile phase a mixture. Coat the plate separately with 2 µl of each of the six sample solutions in a mixture of equal volumes of dichloromethane and methanol and dip the plate into a solvent tank containing mobile phase. After removing the plate, dry it in a current of cold air till the lower edge is in a quiescent motion.

Repeat the development and dry the plate at 115° for 15 minutes, pass to cool, spray the mixture of detecting agent. Examine under the UV light at 365 nm. Detect the spots in the chromatogram [7].

Liquid Chromatography

LC is an analytical chromatographic technique that separates molecules in a liquid mobile phase and a solid stationary phase. The separation of substance depends upon the rate of absorption (Table 5).

- Identification solution [a]: 10 mg of digoxin Identification solution [b]: 1ml of test solution
- Identification solution [c]: 2.5 mg of digoxigenin and methanol as a diluent Identification solution [d]: 50 mg of lanatoside
- Identification solution [e]: 5 mg of digoxin.
- Test solution: 0.5 mg /ml test solution prepared by methanol.

Table 5. Chromatographic conditions

Column size	0.15 m Ø = 3.9 mm
Stationary phase	Octadecyl silyl silica gel for chromatography (5 µm)
Mobile phase A	Acetonitrile R water R (10:90 V/V)
Mobile phase B	Water R acetonitrile R (10:90 V/V)
Flow rate	1.5 ml /min
Detection	Spectrophotometer 220 nm
Injection	10µl of the test solution and standard solution

PROCEDURE

The test sample impurity, retention time, and relative retention time were identified by using an identification solution.

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UV-SPECTROSCOPY

Ultraviolet spectroscopy measured electromagnetic radiation in the range of 200–400 nm for the UV region and 400–800 nm for the visible region.

SIMULTANEOUS ESTIMATION OF UV SPECTROPHOTOMETRIC METHOD

Reagents and Materials

Lanoxin tablets (0.25 mg), ethanol, and ultra-pure water.

Sample Solution Preparation

In aliquot, 2.5 ml standard solution was transferred to a 100 ml volumetric flask and makeup the volume with hydroalcoholic solvent to obtain 25 µg/ml concentration

Standard Solution Preparation

Weigh about 25 mg of digoxin and transfer to a 25 ml volumetric flask. Approximately 50% of the hydroalcoholic solvent was added and sonicated for 10 minutes, and the volume was adjusted to 25 ml with the same solvent (Table 6).

Table 6. Instrumentation and analytical conditions [8].

Parameters	Conditions
UV visible instrument	Systronics 2201 double beam spectrophotometer width 2 nm
Sample cell	Pair of 10 mm matched quartz cell
Software	UV probe system software for spectra detection
Blank solution	Hydroalcoholic solvent (ethanol: water)
Wavelength	220nm with 0.5 nm accuracy
Analytical instrument	Shimadzu analytical balance

PROCEDURE

Hydro alcoholic solvent is used as a diluting solvent in an environmental concern. The organic solvent is used in sample preparation, so we use (ethanol: water) hydro alcoholic mixture. Digoxin standard and sample solution give an intense absorption band with 220 nm. The ultraviolet spectrum of the hydroalcoholic solvent was also recorded; it verified the existence of interferences.

Method for Digoxin Determination in Liquid Chromatograph-Mass Spectrophotometer (LC-MS)

A narrow therapeutic window of 0.5–2 ml in plasma increases the complexity of using digoxin, and TDM is needed to assure the suitable dose is administered. Two quick and sensitive methods used in practical digoxin monitoring are radioimmunoassay and LC/MS/MS. A novel, rapid, and sensitive approach using formate-adduct ions was developed in this study (Table 7).

Chemicals and Reagents

Digoxin, formic acid, ammonium formate, methanol, acetonitrile, deionized water

Table 7. Optimized chromatographic condition [9].

Parameter	Condition
Mobile phase	0.05% ammonium carbonate
Limit of quantitation	0.2 ng/ml
Injection volume	5 µl
Column length	100 mm × 2.1 mm
Formic acid concentration	0.01% (0.005%, 0.002%, 0.01%, 0.1%v/v)

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Total run time	3 minutes
Plasma concentration in rat	0.1 ml

PROCEDURE

A basic method involved a mobile phase consisting of ammonium carbonate degrading the silica in some columns and large injection volume with suggested limit, which allows analysis of trace amounts of sample and increased column performance in the LC/MS/MS. Some methods use gradient elution. lead to higher column performance and HPLC analysis.

METHOD VALIDATION

From the point of view of ICH Q2 guidelines, "creating a documented proof, which provides a high degree of assurance that a specific process will often produce a specified resultant its prearranged specification and quality characteristics". It is a process of producing a controlled design to assure that the product has its identity, strength, quality, and purity [10]. This concept was first introduced in the United States in 1978. It has widely expanded to cover various activities in sectors like medical research and device manufacturing. Its main work is to monitor the test for medical items, labels, control procedures, and labels, and ensure that computer systems are working efficiently (Figures 3 & 4).

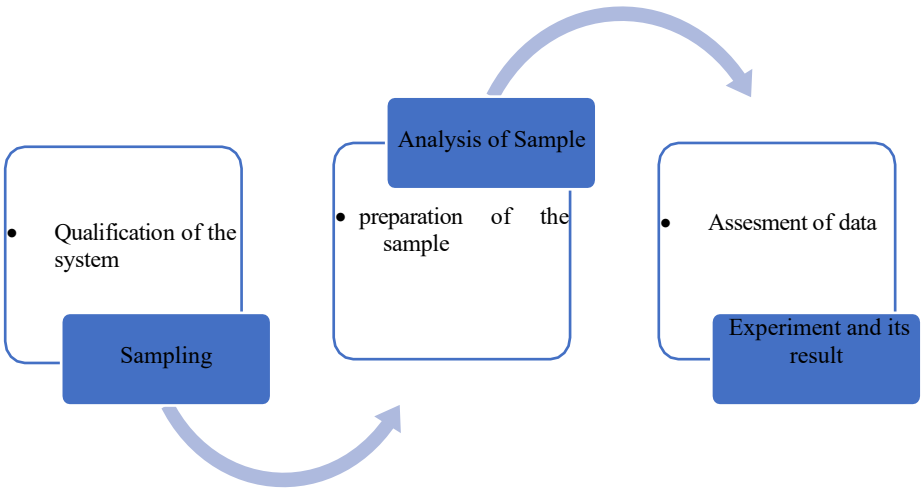

Figure 3. Different steps in method validation.

Validation Parameters

- Accuracy.
- Precision.
- Repeatability.
- Specificity/sensitivity.
- Limit of detection.
- Linearity.
- Range.
- Limit of quantitation.
- Robustness.
- Ruggedness.

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Accuracy
Defined as the closeness of results obtained by the method of true value.

Precision
Closeness of two or more measurements to each other indicating the repeatability.

Specificity
The analyte is assessed unequivocally in the presence of components that may be expected to be present, e.g., impurities

Linearity and Range
Test sample preparation 50–150% of linear concentration level the resultant of the test is directly proportional to concentration [11–13].

Limit of Detection (LOD)
In this analytical method, the lowest concentration of components that can be reliably detected is not quantified.

Limit of Quantitation (LOQ)
Lowest detection and quantifiable.

Robustness
Robustness is a deliberate change of analytical method parameters, such as temperature, flow rate, mobile phase composition, etc.

Ruggedness
It is a measure of method reproducibility under various terms within specified parameters of test method.

CONCLUSIONS

This review emphasizes the various types of chromatographic conditions and spectroscopic conditions that are used for the analysis of digoxin and provides information about the validation parameters. UV-visible spectrometry is suitable for certain applications, particularly those requiring rapid and simple analysis. The validation and method development of an analytical method for digoxin require careful consideration, including chromatographic conditions, extraction procedures, and detection techniques.

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