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Review Article

Analytical Method (HPLC) for Determination of Digitalis as a Cardiotonic Drug

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ABSTRACT

Digitalis, a cardiotonic used for the treatment of heart conditions like atrial fibrillation and heart failure is one of this well-known drugs. My favourite Cinchona80 is mixed with plant Digitalis purpurea. When the pump of Na+/K+-ATPase is inhibited, digoxin and digitoxin both augment myocardial contractility. Given the narrow therapeutic index of digitalis glycosides, their accurate quantification in pharmaceutical formulations and bio-fluids is necessary to determine drug efficacy as well as safety. The accurate and reliable quantitation of digitalis glycosides using High Performance Liquid Chromatography (HPLC) has been established. Due to the high sensitivity, selectivity and accuracy this method is well-fitting for quantitation of digitalis in plasma or urine samples. For implementation, reversed phase HPLC using UV or fluorescence detection is generally applied with C18 columns being the most popular. Adding these buffers to an acetonitrile-water mixture can ensure that the mobile phases have their pH in a right place for both analyte stability and retention.

INTRODUCTION

One of the best recognized class of life-saving medications used to treat cardiovascular disease are the digitalis glycosides. The purpurea glycosides A, B and the digitalinum glucose transferees glucogitaloxine are transformed by enzyme into secondary glycoside such as digitoxide, gitoxidet gitaloxinet in to strospesid [(1)]. As far as we know, stohs and Staba did the initial studies on biotransformation of digitoxigenin using plant cell suspension cultures [(2)]. It is 25 years that cardenolides in Digitalis leaves have been intensively investigated and many publications appeared. As separating cardenolides by chromatographic methods is not conducted very well, analysis and detection are simpler [(3)]. Therefore, these components should be identified and determined using suitable analytical methods

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in digoxin tablets and pharmaceutical materials. Those compounds were tested before by GC, HPLC and TLC [4].

1.1 Chemistry of Digitalis Glycosides

Digitalis glycosides are vital cardiac agents used in the treatment of heart failure and this comprise of an important class that is extracted from plants found within the Digitalis species such as Digitalis lanata or also known as woolly foxglove, and common purple Foxglow (digitas purpureas). A glycoside is specified by their steroid nucleus (aglycone) and also major sugar element(glycose). Solubility, melting temperature and the structural composition of a molecule play essential roles in its pharmacological activities. Aglycone (genin): attached to the C17 position is a lactone ring, and at its core, saikosaponins typically have a steroid nucleus consisting of four fused rings arranged in cis-trans-cis. Examples for common aglycones are digoxigenin, gitoxigenine and digitgenin. Glycone: The molecular sugar is at C3 of the Aglycone. Common sugars are e.g. glucose, rhamnose and digitoxose. The sugars change glycoside PKs and make them slightly water-soluble [5].

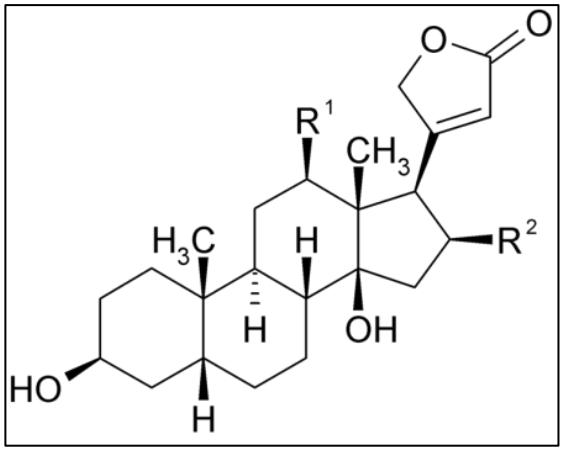


Figure 1: Chemical structure of Digitalis glycosides

Digitalis glycosides water solubility varies according on the quantity and kind of sugar units they contain. In contrast to digoxin, which has hydroxyl groups that promote its water solubility, digitoxin is more lipophilic and less water soluble. Organic Solvents: Because of their steroidal composition, particularly the aglycone portion, Alcohol and Methyl trichloride are examples of carbon-based solvents in which these glycosides are soluble. Because digitalis glycosides are crystalline in nature, they typically have high melting points. Digoxin, for instance, melts at about 235°C, while digoxin has a melting point of about 245°C [6]. The biological activity of these glycosides depends on the lactone ring at position C17, allowing them to be linked to the Na+/K+-ATPase pump; it is the primary site of action for their effects on the heart. The sugar component alters the molecule's polarity, absorption, and duration of activity [7].

2. Pharmacological Profile

2.1 Mode of Action

The blocking of the Na+/K+-ATPase pump in cardiac cells by digitalis glycosides results in a rise in the sodium level inside cells levels. Calcium within cells rises as a result of this and a decrease in the sodium-calcium exchanger's function. Elevated calcium improves cardiac output in heart failure by strengthening the heart's contractility (positive inotropic effect) [6].

2.2 Pharmacokinetics

Whereas digitoxin is nearly completely absorbed (90–100%), digoxin has a moderate oral bioavailability (60–80%). Digoxin has a vast volume of distribution and is widely dispersed throughout bodily tissues, especially the heart. It has a somewhat lower (~25%) affinity to plasma proteins than digitoxin, which has a significantly higher binding (~95%). Digoxin is mostly eliminated unaltered by the kidneys and experiences very little metabolic processing. In contrast, the liver undergoes substantial metabolism of digoxin. Digoxin is mostly eliminated by the kidneys; in patients with renal impairment, its half-life is extended to around 36–48 hours. Hepatic metabolism eliminates digoxin. Its half-life is prolonged (five to seven days) [7].

2.3 Adverse Effect

The limited therapeutic index of digitalis glycosides indicates a narrow range between therapeutic and harmful dosages. A toxic environment may cause severe effects on the heart: arrhythmias include ventricular fibrillation, tachycardia, or heart block. Effects not related to the heart include dizziness, vomiting, nausea, diarrhea, and visual abnormalities like yellow or blurred vision. Contributing elements: The risk of digitalis toxicity is increased by hypokalemia, renal insufficiency, and medication interactions (such as those with diuretics or calcium channel blockers) [7].

3. HPLC Method for Digitalis Determination

3.1 Sample Preparation

Extraction: Techniques like liquid-liquid extraction or solid-phase extraction (SPE) are used frequently to extract digitalis glycosides from biological matrices. such as plasma, serum, or urine.

Pre-treatment: To bring the sample concentration within the ideal range for HPLC analysis, samples may be diluted and filtered to eliminate particles after extraction [8].

3.2 Chromatographic condition

High-performance liquid chromatography (HPLC) is an analytical technique that is widely used for the identification, quantification, and separation of components within a compound. On the basis of HPLC is a notion for differential partitioning, wherein substances are separated according to their affinities into two phases: a liquid solvent-containing mobile phase and a stationary phase (solid or liquid on a solid support) [9].

3.3 Detection method

Analytes are transported through the stationary phase by the mobile phase as a liquid solvent or a combination of liquid solvents. Stationary Phase: Usually a column full of microscopic atoms, usually covered in various functional groups and formed of silica. The detector picks up the separated compounds as they elute from the column. Typically, mass spectrometry, fluorescence, or UV-Vis detectors are used for this [10].

3.4 Validation of HPLC method

Digitalis glycosides and other cardiovascular glycosides can be separated and quantified utilizing the potent technology for high-performance liquid chromatography (HPLC). An HPLC method's validation entails determining its accuracy, repeatability, and dependability. It usually focuses on a few important factors, including linearity, flow rate, and detection wavelength, as well as column selection and chromatographic conditions.

Column Selection

Because of their polarity and chemical makeup, Digitalis glycosides must be separated using a specific HPLC column. Due to their capacity to hold non-polar to moderately polar chemicals, C18 columns are frequently used by researchers when choosing reverse-phase columns. Particle sizes of 3 µm or 5 µm in high-efficiency columns offer superior resolution, particularly for glycosides that are structurally identical, such as digoxin and digitoxin. Optimized a C18 column for high peak resolution and validated it for the separation of digoxin and its breakdown products [11]. Utilized a C8 column in a related work to show that with small modifications in the mobile phase, several reverse-phase columns can produce satisfactory results [12].

Chromatographic Conditions

Mobile Phase: Typically, an aqueous phase that has been buffered to a particular pH is combined with organic solvents like methanol or acetonitrile. To increase glycoside separation, acetonitrile-water systems are typically improved with pH modifications (with formic or phosphoric acid). Digoxin's peak sharpness and resolution were improved by the ACN and H_2O with 0.1% methanoic acid mobile phase [13], tested different

compositions of the mobile phase, demonstrating that small pH changes can greatly extend the retention periods of particular glycosides [14].

Flow Rate: For glycoside separation, a flow rate that typically ranges from 0.8 to 1.5 mL/min is advised in order to balance resolution and run duration [15]. Detection Wavelength: Since digitalis glycosides absorb UV light strongly, UV detectors are a regular option. Since these glycosides absorb most around 220-230 nm, this is typically the wavelength used for detection. Optimized the detection sensitivity by using a 220 nm detection wavelength for digitoxin and similar chemicals [16].

Linearity

The capacity of a procedure to yield findings that are exactly proportionate to the analyte concentration within a given range is referred to as linearity. In order to evaluate linearity for method validation, standards are often analyzed at several concentrations and the peak area or height is plotted versus the concentration. To verify proper linearity, the correlation coefficient (R2) should be close to 1.0, usually over 0.99. The digoxin method has a linear range of 0.1 to 100 µg/mL and an R2 value of 0.9994, indicating great precision and accuracy [17]. Confirmed linearity for various Digitalis glycosides in a similar range, emphasizing the importance of high correlation coefficients in establishing the reliability of the method [18].

HPLC Table

Sr. No.	Drugs	Pharmaceutical or Biological Matrix	Column	Chromatographic Conditions	Linearity	Ref.
1.	DIG	Bulk Material	Symmetry C18 column (75 mm × 4.6 mm I.D., 3.5 µm)	**M.P**– H_2O: ACN (80:20,v/v) **Flow rate**– 1.0 mL/min **Mode of analysis** – Gradient **Detection** – 220 nm	5.20-1.04 µg/ml	19
2.	DIG	Bulk Material	Stainless-steel column (250 × 4.6 mm I.D.)	**M.P**– ACN: H_2O (96:4,v/v) **Flow rate** – 0.8 mL/min **Mode of analysis** – Isocratic **Detection** – 220 nm	-	20
3.	DIG	Bulk Material	Unison UK-C18 column (150 mm × 2.0 mm I.D.; 3 µm)	**M.P**– 10% ACN (solvent A) and 60% ACN (solvent B) **Flow rate** – 0.8 mL/min **Mode of analysis** – Isocratic **Detection** – 220 nm	0.8-25 µg/ml	21
4.	DIG	Bulk Material	Column (250-mm length of 4 mm I.D.)	**M.P**– Cyclohexane: Absolute ethanol: Glacial acetic acid (60:9:1) **Flow rate** – 2 mL/min **Mode of analysis** – Isocratic **Detection** – 265 or 234 nm	0-12 mg/ml	22

5.	DIG	Bulk Material	Li Chrosorb RP C-18 Column (250 × 4.6 mm, 10µm)	**M.P**– H_2O: ACN (2:1) **Flow rate** – 0.86 mL/min **Mode of analysis** – Isocratic **Detection** – 230 nm	-	23
6.	DIG	Bulk Material	LiChrosorb Si 100, particle diameter 10 pm; (length 25 cm; I.D.3 mm)	**M.P**– Methylene chloride: Methanol: H_2O (920:80:12) **Flow rate** – 1.2 mL/min **Mode of analysis** – Isocratic **Detection** – 230 nm	-	24
7.	DIG	Bulk Material	Octylsilyl bonded silica column, (150 mm × 4.6 mm, I.D. 5µm)	**M.P**– ACN: Methanol: H_2O (4:4:5) **Flow rate** – 0.5 mL/min **Mode of analysis** – Isocratic **Detection** – 220 nm	-	25
8.	DIG	Bulk Material	Cosmosil 5C18 (5 Urn, 150 × 4.6 mm I.D.)	**M.P**– Methanol: H_2O (2:1, v/v) **Flow rate** – 0.6 mL/min **Mode of analysis** – Isocratic **Detection** – 220 nm	10~80 µg	26
			Cosmosil 5Ph (5 urn, 300 ×4.6 mm I.D.)	**M.P**– ACN: H_2O (5:8, v/v) **Flow rate** – 0.4 mL/min **Mode of analysis** – Isocratic **Detection** – 220 nm		
9.	DIG	Bulk Material	Jasco SC-01 column (162 × 0.5 mm I.D.,5µm)	**M.P**– ACN: Methanol: H_2O (15:15:19) **Flow rate** – 4 µL/min **Mode of analysis** – Isocratic **Detection** – 220 nm	0.01-0.15C	27
10.	DIG	Bulk Material	PTFE column (165 × 0.5 mm I.D.)	**M.P**– ACN: Methanol: H_2O (1:1:1) **Flow rate** – 4 mL/min **Mode of analysis** – Isocratic **Detection** – 220 nm	5-25 mg/ml	28
11.	DIG	Bulk Material	Jasco SC-01 column (103 × 0.5 mm I.D.)	**M.P**– ACN: Ethanol: H_2O (21 :20:45) **Flow rate** – 8 µL/ min **Mode of analysis** – Isocratic **Detection** – 220 nm	20 µg to 100 µg	29
12.	DIG	Bulk Material	LiChrosorb Si 60	**M.P**– 8 % Methanol: Methylene chloride saturated with water	30µg/ml	30

			(15 cm; I.D. 3 mm, 5 µm)	**Flow rate** – 2.0 mL/min **Mode of analysis** – Isocratic **Detection** – 230 nm		
13.	DIG	Bulk Material	Column Dynamax, Rainin(45 mm × 11 cm)	**M.P**– Ethanol: 0.01% trifluoroacetic acid **Flow rate** – 20 mL/min **Mode of analysis** – Gradient **Detection** – 220 nm	-	31
14.	DIG	Bulk Material	stainless-steel column (250 mm × 4.6 mm I.D,5 µm)	**M.P**– ACN: H_2O (96:4, v/v) **Flow rate** – 1.0 mL/min **Mode of analysis** – Isocratic **Detection** – 220 nm	1–250 µg	32
15.	DIG	Bulk Material	Stainless - steel column (150 × 4.6 mm I.D .)	**M.P**– ACN: Methanol: H_2O (100:11:188, v/v) **Flow rate** – 0.5 mL/min **Mode of analysis** – Isocratic **Detection** – 220 nm	10 µl	33
16.	DIG	Bulk Material	Column (12.5 × 4 I.D, 5 µm)	**M.P**– ACN: Dioxane: H_2O(40:11:30) **Flow rate** – 0.4 mL/min **Mode of analysis** – Gradient **Detection** – 220 nm	2-20mg	34
17.	DIG	Bulk Material	Chemcosorb 5 C8-U column (4.6 mm × 150 mm,5 µm)	**M.P**– MeCN: MeOH: H_2O (20:1:50) **Flow rate** – 0.5 mL/min **Mode of analysis** – Isocratic **Detection** – 220 nm	10 µl	35
18.	DIG	Bulk Material	Ultrasphere reversed phase Cl8 (25 × 0.46 cm, 5 µm)	**M.P**– H_2O: Methanol: Isopropanol: Dichloromethane (51:42:5:2) **Flow rate** – 1.2 mL/min **Mode of analysis** – Isocratic **Detection** – 220 nm	50 µl	36
19.	DIG	Bulk Material	YMC-C18 (50 × 500 mm, 70 µm)	**M.P** – ACN: H_2O (20:40) **Flow rate** – 10 mL/min **Mode of analysis** – Gradient **Detection** – 220 nm	18 µg	37

20.	DIG	Bulk Material	Lichrospher RP, column (0.4 cm × 25 cm, 5-µm)	**M.P**– ACN: Trifluoroacetic acid (80:1 v/v) **Flow rate** – 0.2 mL/min **Mode of analysis** – Gradient **Detection** – 254 nm	10 µg/ml	38
21.	DIG	Bulk Material	Sep-Pak C l8 column (Shim-pack PREP-S1L, 20 mm X 25 cm)	**M.P**– ACN: Methanol (80:50 v/v) **Flow rate** – 3 mL/min **Mode of analysis** – Gradient **Detection** – 220 nm	-	39
22.	DIG	Bulk Material	Agilent Zorbax Eclipse XDB-C18 column (4.6 × 150 mm, 5 m)	**M.P**–ACN in H_2O: Acetic acid in H_2O (80:2) **Flow rate** – 1 mL/min **Mode of analysis** – Gradient **Detection** – 280-315 nm	50 µl	40
23.	DIG	Bulk Material	Poroshell 120 EC-C18 column (100 mm × 3 mm, 2.7 µm)	**M.P**– Formic acid in H_2O: ACN in H_2O (80:2) **Flow rate** – 0.4 mL/min **Mode of analysis** – Gradient **Detection** – 280-315 nm	0.5 µl	41
24.	DIG	Bulk Material	JascoSC-01 column (165 × 0.5 mm I.D)	**M.P**– Methanol: H_2O (5:2) **Flow rate** – 4 mL/min **Mode of analysis** – Gradient **Detection** – 220 nm	0.1 µl	42
25.	DIG	Bulk Material	stainless-steel column (150 mm×4.6 mm)	**M.P**– CH_3CN: Methanol: H_2O (10:15:18) **Flow rate** – 0.5 mL/min **Mode of analysis** – Gradient **Detection** – 220 nm	10-300 µg	43
26.	DIG	Bulk Material	stainless-steel column (250 × 4.6 mm I.D)	**M.P**– ACN: H_2O (9:l) **Flow rate** – 0.8 mL/min **Mode of analysis** – Gradient **Detection** – 220 nm	10 µl	44
27.	DIG	Bulk Material	Symmetry C18 (75 mm × 4.6 mm, I.D. 3.5 µm)	**M.P**– ACN: H_2O (80:20v/v) **Flow rate** – 1.0 mL/min **Mode of analysis** – Gradient **Detection** – 220 nm	10 µl	45

28.	DIG	Bulk Material	Li Chrosorb Si 100, (particle diameter 5 pm; length 15 cm; I.D. 3 mm.)	**M.P**– 40% solution of ACN: Dioxan (1.1) in H_2O **Flow rate** – 1.2 mL/min **Mode of analysis** – Gradient **Detection** – 230 nm	100 ng	46
29.	DIG	Bulk Material	stainless-steel column(150 × 4.6 mm I.D. 5µm)	**M.P**– ACN: Methanol: H_2O (100:11:188 v/v) **Flow rate** – 0.5 mL/min **Mode of analysis** – Gradient **Detection** – 220 nm	10 µl	47

Application of HPLC in Digitalis Analysis

1. Detection of Cardiac Glycosides:

Constituents, among which are Digoxin, Digitoxin, Gitoxin and other cardiac glycosides that potently increase force of contraction in the failing heart; hence HPLC identification. The cardiotonic chemicals essentially for pharmaceuticals are vital used in these studies, and the sensitivity of HPLC enables measurement with a good degree of accuracy [49].

2. Quantitative Analysis:

Quantitative analyses of digitalis extracts are performed via HPLC. Trained with 110 commercial and clinical glycoprotein samples, the algorithm offers a tuned range of high-resolution separations specific to each component. For instance, he showed how to accurately quantify glycoside in Digitalis lanata (one of the species predominantly used for digoxin production) using HPLC [50].

3. Quality Control of Pharmaceutical Preparations

Digitalis based pharmaceutical compositions likewise are evaluated by HPLC [87]. The homogeniety and safety of therapeutic usage can be gaurented by standardizing the active glycoside components in pharmaceutical formulations [51].

4. Quantitative Identification of Minor Components:

Together with the main glycosides, HPLC was also used to search for minor components in Digitalis extracts which might add to this plant´s pharmacological action. Introduction of advanced HPLC techniques like reverse-phase (RP) HPLC was followed by separation and characterization in a very accurate way for these peptides as mentioned earlier [52].

5. Detection of Impurities:

HPLC is used to determine the impurities and degradation products in Digitalis extracts. This is essential to ensuring the safety and efficacy of medicinal products. Furthermore, other studies have also found that HPLC can be utilized to investigate contaminants which accumulate during extraction and storage [53].

6.Analysis of Metabolites:

These studies also include the pharmacokinetics of cardiac glycosides metabolites using HPLC method. This approach is important as growing the therapeutic dosage of digoxin without increasing toxicity requires plasma monitoring for digoxin levels [54].

7. Adjunct Techniques:

LC-MS provides a surge in sensitivity and specificity, when combined with HPLC for Digitalis glycoside analysis. Glycoside Hunters and re-ATP, which are combined to enable a more comprehensive molecular profiling of glycones or

their metabolic derivatives including all combinations in the explanations [55].

CONCLUSION:

Cardiac glycoside is one kind of important compound used in the treatment for heart diseases, and high-performance liquid chromatography (HPLC) has been proved to be an effective analytical method which can separate identify and quantify these chemical compounds. HPLC is a highly sensitive, selective and able to cope with complex mixtures so it can be used for the analysis of cardio glycosides. Thus, studies on pharmacokinetics and quality control of heart glycosides as well as the monitoring therapeutic levels in patients and assuring proper dose content per pharmaceutical form were only to a large extent possible due high performance liquid chromatography. Due to advances in chromatographic technology and development of more specific detectors i.e., MS/MS, HPLC procedures have increased accuracy as well as precision making them applicable for both clinical use or research applications. This study is important to understand and assess cardiac glycosides which can be used as valuable chemicals for medical/biological research, (elaborating) their identification/evaluation relies hot only on proper methods but also HPLC method analysis since it could present quick, accurate results with this kind of material.

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