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*Short Communication*

# Recovery of digoxin and related glycosides from an injection dosage form by liquid–liquid and solid-phase extraction

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**Keywords**: *Digoxin; gitoxin; digoxigenin-bis-digitoxoside; liquid–liquid extraction, solid-phase extraction.*

## Introduction

Digoxin, an extremely potent cardiotonic glycoside, is a natural product extracted from the foxglove plant [1]. Digoxin medications are strictly monitored for both production impurities as well as degradation products such as gitoxin and digoxigenin-bis-digotoxoside-(DBD; see Fig. 1) [2]. In efforts to develop an HPTLC assay for the quantitation of digoxin, gitoxin and DBD in an aqueous injectable dosage form, it was discovered in these laboratories that existing sample preparation methodologies were insufficient for thorough sample clean-up.

The purpose of this study was to develop a sample preparation procedure to be used in conjunction with an HPTLC analysis of digoxin, gitoxin and DBD in an injectable dosage form. Commercial injections contain a minimum of 10% (v/v) levels of propylene glycol that is added to enhance the solubility of digoxin [3]. Propylene glycol has been shown in these laboratories to interfere with the recovery and quantitation of digoxin and the two impurities gitoxin and DBD on a HPTLC plate. It is highly viscous thus making analysis difficult since it tends to coat the HPTLC plate and remove most chromatographic interactions between the analytes and adsorbent. Propylene glycol has UV absorption characteristics that also tended to distort quantitative results for our compounds of interest by HPTLC analysis.

In this paper, both liquid–liquid and solid-phase extractions were investigated to determine the optimal procedure for the recovery of the three compounds from an injectable dosage form. A solid-phase extraction was developed on a cyclohexyl column that provided quantitative recoveries of digoxin and DBD and a near quantitative recovery for gitoxin. The method should be directly applicable to the HPTLC assay of a digoxin injection.

## Experimental

### *Materials*

Digoxin and DBD reference standards were obtained from Burroughs Wellcome Co. (Greenville, NC, USA). Gitoxin reference standard was obtained from Sigma (St Louis, MO, USA).

Propylene glycol, methanol, chloroform, iso-amyl alcohol, n-propanol, sulphuric acid, sodium dibasic phosphate, and anhydrous citric acid were all obtained from J.T. Baker (Phillipsburg, NJ, USA). Ethanol 95% was purchased from the Central Research Stores of the University of Georgia (Athens, GA, USA). Adsorbent cotton was obtained from a local pharmacy.

The 1 $cm^3$ size of C18, C8, C2, cyclohexyl, phenyl and cyanopropyl and 3 $cm^3$ size of cyclohexyl solid-phase extraction columns were obtained from Varian Sample Preparation Products (Harbor City, CA, USA).

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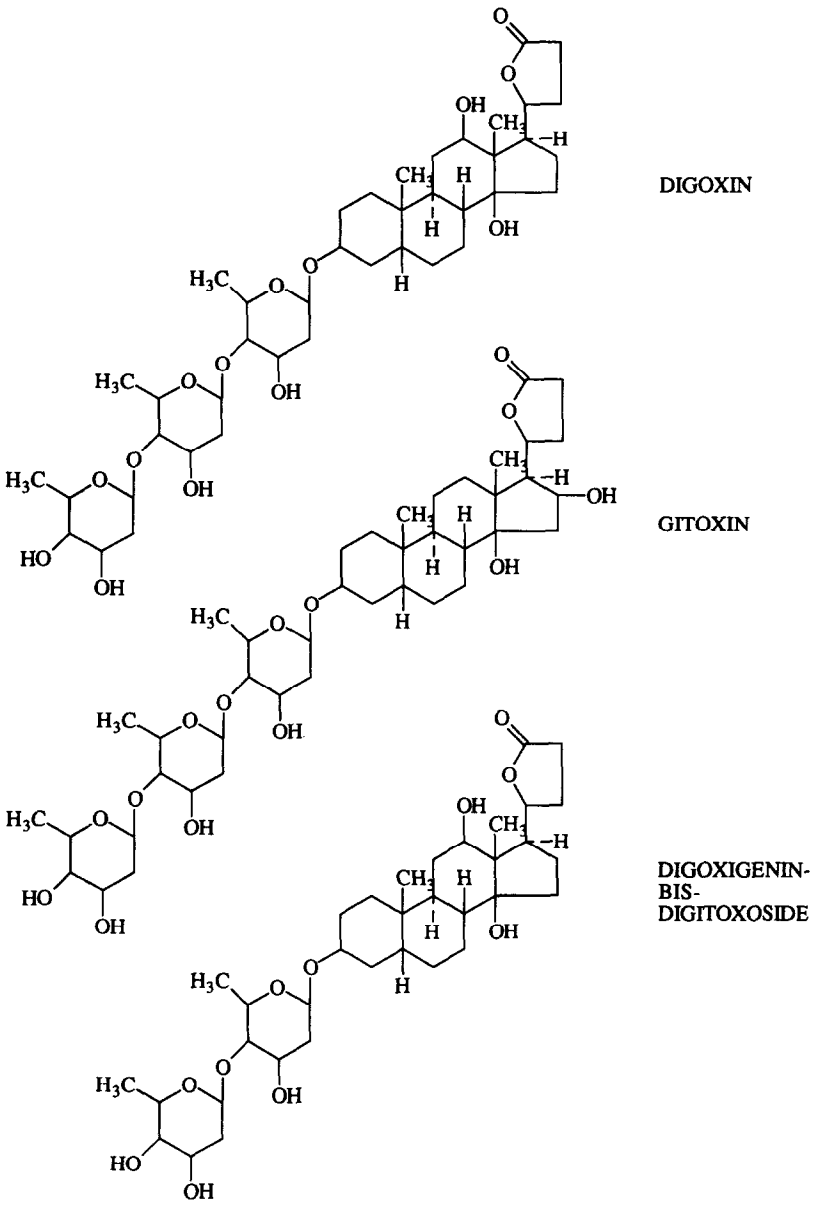


**Figure 1**
Chemical structures of analytes.

The C18 100% wettable HPTLC plates (10 × 10 cm, EM Science, Gibbstown, NJ, Cat. No. 13124-1) were scanned for digoxin, gitoxin and DBD levels using a Camag II densitometer (Camag Scientific, Wilmington, NC, USA) controlled by the Hewlett Packard Series 9000 micro-computer and Camag software system HPL 2.1 revision number 7.01 using a method developed by this laboratory.

*Preparation of injection standard solutions*

Digoxin drug substance (25 mg) was accurately weighed and placed in a low actinic 100 ml volumetric flask. Next, 0.75 mg DBD was weighed and added to the same flask. Then, 1 mg of gitoxin powder was weighed and placed in a separate 10 ml low actinic volumetric flask and ethanol–water (50:50, v/v) was added to volume.

For preparation of a spiked simulated digoxin injection, 1 ml of the gitoxin stock solution was transferred to the low actinic 100 ml volumetric flask containing the digoxin and DBD powders. To this mixture were added 10 ml of ethanol and 10 ml of water. The mixture was stirred for a minimum of 1 h or until dissolution; 40 ml of propylene glycol was added and water was added to volume.

This gave a spiked solution with concentrations of 250, 7.5 and 1 μg $ml^{-1}$ for digoxin, DBD and gitoxin, respectively. A standard solution containing these same concentrations was also prepared in 50:50 (v/v) chloroform–methanol. The gitoxin and DBD levels were 0.4 and 3.0% (w/w), respectively, of the digoxin amount. The simulated digoxin injection also contained 40% (v/v) propylene glycol and 10% (v/v) ethanol.

*Liquid–liquid extraction*

A 1 ml volume of digoxin injection, equivalent to about 2.5 mg digoxin, was diluted to 50 ml with water, placed in a separator, and 1 ml of diluted sulphuric acid was added. The following extraction methods for digoxin were performed:

*USP XIX method* [4]. The solution was extracted with 35 ml of 5:1 chloroform–n-propanol. After separation of the organic layer (bottom), the extract was washed in a second separator with 5 ml of water, and then filtered through cotton previously washed with two 30 ml portions of the 5:1 chloroform–*n*-propanol into a 100 ml volumetric flask. The extraction of the digoxin injection was repeated twice using 30 ml of 5:1 chloroform–*n*-propanol. The extracts and washed extracts were combined into the 100 ml volumetric flask and methanol was added to volume.

*Method A.* The digoxin solution was extracted with four 60 ml portions of 5:1 chloroform–*n*-propanol. The extracts were collected in a 250 ml volumetric flask and extracting solvent was added to volume.

*Method B.* The digoxin solution was extracted with five 35 ml portions of 1:1 chloroform–*n*-propanol. The extracts were collected in a 250 ml volumetric flask and extracting solvent was added to volume.

*Method C.* The digoxin solution was extracted with five 35 ml portions of 5:1 chloroform–*n*-propanol. The extracts were collected in a 250 ml volumetric flask and extracting solution was added to volume.

*Method D.* A 1 ml volume of digoxin injection, equivalent to about 2.5 mg digoxin, was diluted to 5 ml with a saturated aqueous solution of sodium chloride, placed in a separator, and 1 ml of diluted sulphuric acid was added. The solution was extracted with four 25 ml aliquots of methylene chloride. The extracts were collected in a 100 ml volumetric flask and methylene chloride was added to volume.

*Method E.* Method D was followed exactly except digoxin injection was diluted to 5 ml with water prior to extraction with methylene chloride.

*Solid-phase extraction*

The folloiwng method was used for all solid-phase extraction columns. A column was washed with two column volumes of absolute methanol (note: do not allow column to dry) followed by two column volumes of water. Then 1 ml of the simulated digoxin injection was applied to the column. The column was then washed with two column volumes of water and was allowed to air dry under vacuum for 30 min to ensure complete removal of water.

The analytes were eluted from the column using eight 250 μl aliquots of chloroform–methanol (50:50, v/v) into a 2 ml collection vial. The contents of the vial were then quantitatively transferred to a 5 ml volumetric flask and chloroform–methanol added to volume. Aliquots (40 μl) of the eluent and standard solution were spotted onto the C18 HPTLC plate and the plate was developed using a mobile phase consisting of water–methanol–ethyl acetate (50:48:2, v/v/v).

Digoxin was scanned in the absorbance mode at 218 nm. The developed plate was then exposed to concentrated hydrochloric acid vapour for 1 h and gitoxin and DBD were scanned in the fluorescence mode using an excitation wavelength of 365 nm with a K400 cut off filter. Typical chromatograms of digoxin scanned in the absorbance mode and the three analytes scanned in the fluorescence mode are shown in Figs 2 and 3. Per cent recovery of each analyte was calculated by a comparison of peak heights of extracted (eluent) to unextracted analyte (standard solution of all three analytes prepared in chloroform–methanol (50:50, v/v).

## Results and Discussion

Table 1 gives extraction recoveries for the three compounds using liquid–liquid extraction with the various extracting solvents.

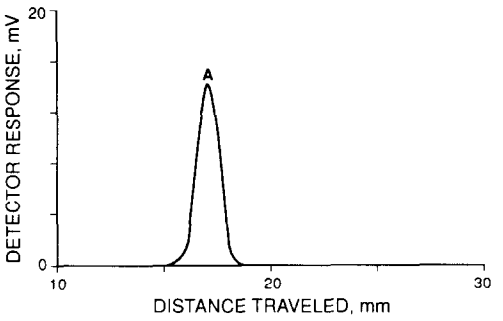


**Figure 2**
A typical chromatogram of 400 ng/5 mm band digoxin (A) scanned in the absorbance mode at 218 nm.

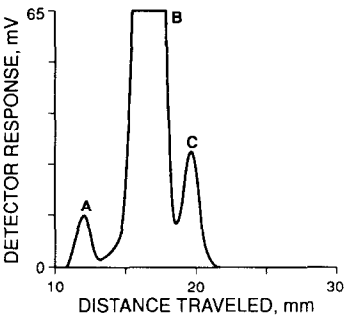


**Figure 3**
A typical chromatogram of 1.6 ng/5 mm band of gitoxin (A), 400 ng/5 mm band digoxin (B), and 12 ng/5 mm band DBD (C), after induced fluorescence and scanned in the fluorescence mode at 365 nm with a K400 filter.

**Table 1**
Recovery of digoxin, gitoxin and DBD from liquid–liquid extraction of simulated digoxin injection

| Extraction procedure | Recovery (%) | | |
|---|---|---|---|
| | Digoxin | Gitoxin | DBD |
| 1. USP XIX | 91.32 ± 3.66 | 56.65 ± 3.82 | 101.09 ± 7.69 |
| 2. USP XIX (repeat) | 91.38 ± 10.99 | —* | —* |
| 3. USP XIX (repeat) | 91.62 ± 7.88 | —* | —* |
| 4. Method A | 93.99 ± 6.03 | —* | —* |
| 5. Method B | 67.69 ± 1.03 | 64.77 ± 5.39 | 94.81 ± 4.29 |
| 6. Method C | 94.27 ± 1.73 | —* | —* |
| 7. Method D | 112.95 ± 10.99 | 92.81 ± 10.81 | 208.64 ± 26.05 |
| 8. Method E | 159.94 ± 41.92 | —* | —* |

*No data were calculated due to interfering peaks.

Repeated attempts to achieve quantitative recoveries for the three compounds using the USP XIX and other modified extraction methods were unsuccessful. Digoxin recoveries were obtained ranging from 68 to 160% and the latter value obviously showed that interferences were being co-extracted. Gitoxin recoveries ranged from 57 to 93% and there were instances in which quantifiable results were not obtainable. DBD recoveries ranged from 95 to 209% and, again, several extractions proved to have non-quantifiable results due to interferences.

It was decided to study the applicability of using solid-phase extraction to determine if recoveries would be improved over that obtained with the liquid–liquid extractions. Initial data obtained on a 1 $cm^3$ C18 solid-phase extraction column indicated that near quantifiable and reproducible data were possible for all three analytes. Other solid-phase extraction columns (C8, C2, cyclohexyl, phenyl and cyanopropyl) were investigated to optimize extraction recoveries. Extraction of an aqueous ethanolic solution (90:10, v/v) containing no propylene glycol gave recoveries on a 1 $cm^3$ cyclohexyl column of 96.0 ± 0.7, 99.5 ± 0.7 and 98.0 ± 0.1% ($n = 3$) for digoxin, gitoxin and DBD, respectively. As shown in Table 2 for a simulated digoxin injection containing propylene glycol, recoveries for digoxin and DBD were maximized on a 1 $cm^3$ cyclohexyl column, whereas gitoxin recoveries peaked with the C8 and C2 columns. Since a 1 $cm^3$ cyclohexyl SPE column had given an 85% recovery of gitoxin, it was decided to investigate a 3 $cm^3$ cyclohexyl column to determine if an increase in solid phase material would improve gitoxin's recovery without decreasing the excellent recoveries obtained for digoxin and DBD. Results from the study showed recoveries of 99.2 ± 1.1, 94.2 ± 1.62 and 104.1 ± 5.1% for digoxin, gitoxin and DBD, respectively.

The data indicated that a quantitative and reproducible extraction technique was developed for the recovery for digoxin, gitoxin and DBD from a simulated digoxin injection dos-

**Table 2**
Recovery of digoxin, gitoxin and DBD from solid phase extraction of simulated digoxin injection

| SPE column | Recovery (%) | | |
|---|---|---|---|
| | Digoxin | Gitoxin | DBD |
| 1 $cm^3$ C18 | 86.22 ± 0.68 | 87.36 ± 2.56 | 70.92 ± 2.96 |
| 1 $cm^3$ C8 | 88.07 ± 2.61 | 89.09 ± 4.32 | 73.92 ± 3.85 |
| 1 $cm^3$ C2 | 96.69 ± 4.31 | 89.07 ± 1.12 | 80.85 ± 1.29 |
| 1 $cm^3$ cyclohexyl | 99.07 ± 3.26 | 84.56 ± 7.33 | 95.72 ± 3.86 |
| 3 $cm^3$ cyclohexyl | 99.23 ± 1.09 | 94.21 ± 1.62 | 104.09 ± 5.10 |
| 1 $cm^3$ phenyl | 83.74 ± 7.29 | 85.31 ± 3.57 | 69.15 ± 1.44 |
| 1 $cm^3$ cyanopropyl | —* | —* | —* |

* Less than 50% recovery of analytes was obtained.

age form. Using a 3 $cm^3$ cyclohexyl solid-phase extraction column, quantitative recoveries were obtained for digoxin and DBD and a near quantitative recovery for gitoxin. The extraction procedure is easy to perform and it provides a usable environemt for the analysis of digoxin and its related impurities in a digoxin injection dosage form.

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