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Rapid Identification and Estimation of Gitoxin in Digitoxin and Digoxin Tablets by TLC

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Abstract □ A rapid TLC procedure was developed for the identification and estimation of gitoxin in digitoxin and digoxin tablets. A solution of tablet material is spotted on a TLC plate directly, and the gitoxin is estimated by visual comparison of fluorescence with that of standards after spraying with acid–ferric chloride T.S. USP XVII. The method can detect 0.5% of gitoxin in the presence of 1 mcg. of cardiac glycosides.

Keyphrases □ Gitoxin—identification, estimation □ Digitoxin, digoxin tablets—gitoxin identification □ TLC—identification, estimation □ Fluorescence, estimation—UV light

The USP XVII monograph for digitoxin tablets (1) includes separate procedures for the identification of digitoxin and the determination of other digitoxosides. Identification Test A is a color reaction using a version of the Keller–Kiliani reagent (2). The test is neither sensitive nor convenient to use for large numbers of samples. The "Other Digitoxosides" determination is also a colorimetric method based on the Keller–Kiliani reagent. Gitoxin is the principal digitoxoside other than digitoxin occurring in digitoxin tablets (3), and separation on a diatomaceous earth[1] column used in the USP XVII method is based on this premise (4). Gitoxin is also the principal fluorescing substance mentioned in the monograph for digoxin tablets in USP XVII (5). Lanatoside C may also contain gitoxin as an impurity (3), although the NF XII monograph (6) does not include a test for it.

The aglycone of gitoxin with hydroxyl groups in the 14- and 16-positions is readily dehydrated (7, 8) at room temperature to dianhydrogitoxin, which exhibits visible fluorescence when activated by UV light. The anhydro derivative of digitoxin is not visible under the same conditions. The usual dehydrating reagent for this reaction has been either glycerin–HCl or propylene glycol–HCl. Acid–ferric chloride T.S. USP XVII was found to give the same reaction and has the advantage of being volatile. The nonvolatile residues from the acidic glycerin or glycol reagents result in diffusion of spots and lowered sensitivity.

A TLC system using methylene chloride–methanol–formamide on a silica gel plate (9) was found to separate the compounds sufficiently to carry out the tests. The gitoxin content of the sample on the sprayed plate is estimated by visual comparison of its fluorescence with that of standard spots under longwave UV light (3650 Å). The digitoxin chromatogram becomes visible after the plate is heated in a 100° oven for 10 min.

The same technique can be used for the identification of digitoxin, digoxin, acetyldigitoxin, and lanatoside C and for the determination of any gitoxin present in their drug preparations. Digoxin and gitoxin are not completely separated by this chromatographic procedure. Nevertheless, digoxin is not activated to visible fluorescence at room temperature by the acid–ferric chloride. Consequently, any fluorescence present immediately after spraying is due to gitoxin alone. Heating the plate at 100° destroys the gitoxin fluorescence and converts the digoxin to a fluorescent anhydro derivative which may be seen under both UV and visible light.

EXPERIMENTAL

Chromatographic Chamber—A Mitchell tank was used (10).

TLC Plates—Coat a 200–250-μ layer on a clean glass plate, using a 2:1 slurry of water–silica gel G.[2] Air dry; then activate 30 min. at 100°. Store in a desiccator. When several samples are to be run simultaneously, 0.5–1.0-cm. channels may be scored on the plate for convenience. Precoated silica gel GF plates[3] enhance the fluorescence of the spots obtained. A source for long wavelength (3650 Å) UV light was used.

Reagents—*Mobile Solvent*—Methylene chloride, distilled in glass, plus anhydrous acetone-free methanol (Mallinckrodt or

[1] Celite 545, Johns-Manville Products Corp., New York, NY 10016

[2] Catalog No. 7731, Brinkmann Instruments Co., Westbury, NY 11590

[3] Analtech, Inc., Wilmington, DE 19801

Table I—Summary of Chromatograms of Digitoxosides Obtained by Proposed Method[a]

Drug	R_f	Heat Treatment of Chromatoplate: Room Temp. UV	Room Temp. Vis	100°, 1 min. UV	100°, 1 min. Vis	100°, 10 min. UV	100°, 10 min. Vis
Acetyldigitoxin[b]	0.90	—	—	—	BG	OF	BG
Digitoxigenin	0.83	—	—	—	—	OF	G
Digitoxin	0.83	—	—	—	BG	OF	BG
Gitoxin	0.72	BF	—	—	—	OF	BG
Digoxin	0.70	—	—	BF	BG	BF	BG
Digoxigenin	0.65	—	—	BF	—	BF	Y
Lanatoside C	0.58	—	—	BF	BG	BF	BG

[a] BF = blue fluorescence; OF = orange fluorescence; BG = blue-green; Y = yellow; G = green; UV = visualization under longwave UV light; and Vis = visualization in visible light. [b] The spot obtained from aged acetyldigitoxin standard solution exhibits a blue fluorescent fringe on the lower edge after heating 1 min.

equivalent) plus stabilized formamide (80:19:1) was used. The solvent is stable for 2 days in a glass-stoppered flask.

Spray Reagent—Acid-ferric chloride T.S. USP XVII was used. Mix 60 ml. of glacial acetic acid with 5 ml. of sulfuric acid, add 1 ml. of 9% ferric chloride, mix, and cool. Allow the reagent to age 1 day before use. It is stable at least 1 month.

Reference Standard Solutions—Prepare 1.0 mg./ml. solutions of digitoxin, digoxin, acetyldigitoxin, and lanatoside C, each containing 0.05 mg./ml. of gitoxin in ethanol–formamide (4:1). Spot 1-, 2-, 4-, 5-, and 10-μl. portions.[4]

Assay Procedures—Determine the average weight of not less than 20 tablets and reduce the sample to a fine powder. Weigh a portion of the powdered sample equivalent to 1.0 mg. of drug, and transfer to a suitable covered container. Add 1 ml. of a solution of ethanol–formamide (4:1) to the container. The formamide is necessary to produce compact spots and to reduce tailing. Swirl or stir the container occasionally during a 20-min. period; then allow it to settle for an additional 10 min. Spot 5 μl. of the supernatant liquid on a TLC plate. Spot a series of gitoxin standards containing the cardiac glycoside being identified. Place the plate in an equilibrated chromatographic chamber and develop approximately 100 mm. [Use an unequilibrated chamber without trough for plates that are 5.08 cm. (2 in.) or less in width.] Air dry the plate and spray with the acid-ferric chloride T.S. USP XVII. Ventilate 2–3 min. in a current of air and view under longwave UV light. Estimate gitoxin in the sample by visual comparison of the fluorescence with that of the gitoxin standards. Allow a short period of time for acclimation of the eyes before reading. Place the plate in a 100° oven for 10 min. All of the compounds appear as blue-green spots in visible light and may be identified by comparison of their R_f values with those of concomitant standards. The sequence of observed color changes is summarized in Table I.

RESULTS AND DISCUSSION

The gitoxin content of a series of digitoxin tablet samples was estimated by this procedure and subsequently by the USP XVII method for Other Digitoxosides (1). The results are listed in Table II. Samples that showed no gitoxin spot by TLC were checked periodically by the official procedure; in no case was any found.

A similar comparison made on a series of samples of 10 digoxin tablets each showed no gitoxin present by the TLC procedure, and the samples assayed less than 0.1% "related fluorescent substances" by the USP XVII monograph for Digoxin Tablets (5). A sample prepared to contain 0.5% gitoxin (based on digoxin declaration) assayed 0.5% by the TLC estimate and 0.45% by the USP XVII procedure.

[4] Combined standards were used so that a single spotting would produce a reference for qualitative comparison of the glycoside and quantitative estimation of the gitoxin.

Table II—Comparison of TLC Estimate with USP XVII Determination of Other Digitoxosides

Sample	Other Digitoxosides,[a] %	TLC Estimate,[b] %	Sample	Other Digitoxosides,[a] %	TLC Estimate,[b] %
1	0.5	<1	14	2.0	2.5
2	4.5	5	15	2.0	2.5
3	1.6	1	16	1.6	2
4	0.7	<1	17	5.1	5
5	0.7	<1	18	2.5	3
6	0.7	1	19	5.1	5
7	4.1	2.5	20	6.4	7
8	3.0	2.5	21	4.9	6
9	2.2	2.5	22	4.5	5
10	0.7	1	23	3.4	4
11	2.0	2	24	3.8	5
12	9.0	>5 <10	25	1.0	<1
13	4.0	5	26	0.8	<1
			27	5.7	5

[a] USP XVII monograph for digitoxin tablets; calculated from ratio of the absorbances of sample to standard as digitoxin. [b] As gitoxin.

By this procedure, 15 samples may be analyzed in less than 90 min. The official test for Other Digitoxosides requires 3–4 hr. to complete after digitoxin is eluted from the column.

Solutions of USP Reference Standard Digoxin and USP Reference Standard Digitoxin in ethanol have never given more than one spot when chromatographed. Nevertheless, it is recommended that all reference solutions be prepared at least weekly.

The procedure described in this paper was successfully applied to approximately 850 commercial samples of digitoxin tablets, 600 of digoxin, 100 of acetyldigitoxin, and 100 of lanatoside C, representing most of the formulators in the United States. The correlation between the results by the official determination of Other Digitoxosides and those by the method described in this paper is a good indication that gitoxin is the principal other digitoxoside present in current digitoxin products on the market and confirms the 1948 work of McChesney *et al.* (11).

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