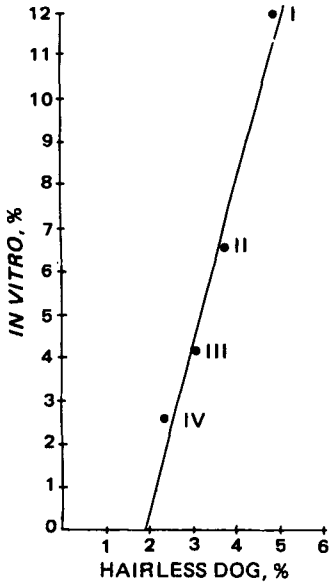

Figure 8—*Mean 12-hr percutaneous penetration (percent of applied radioactive dose)* in vitro versus *hairless dog.*

a horizontal line near its minimum effective evaporation rate. In this instance, the repellent failed almost immediately when challenged with mosquitoes (6). However, if the minimum effective evaporation rate were lower, the repellent could last a long time. The low volatility of V probably accounts for its sporadic performance[18].

In Fig. 8, 12-hr *in vitro* percutaneous penetration at the 0.3-mg/cm^2 dose is compared to 12-hr percutaneous penetration in the hairless dog at the same dose (2, 9) for four of the repellents studied (data for *in vivo–in vitro* comparison of IV were not available). Although *in vitro* percutaneous penetration was always greater than *in vivo* penetration, a good correlation exists between them ($r^2 = 0.96$).

The disposition of radioactivity 1 hr after topical application of radiolabeled II at its minimum effective dose (0.025 mg/cm^2) has been previously reported (5), both *in vitro* and *in vivo*. For the *in vitro* studies, 9.7 ± 5.9% of the applied radioactive dose evaporated, 19.7 ± 3.1% remained on the skin surface, and 50.8 ± 15.0% remained in the skin. For the *in vivo* studies, 9.6 ± 3.6% of the applied radioactive dose evaporated and 27.1 ± 11.6% remained on the skin surface. In this study, a lower percentage (4.3 ± 1.2%) of the radioactive dose of II evaporated 1 hr after *in vitro* application at the minimum effective dose. This difference may result from a closer proximity of the vapor entraining tube to the skin surface (1.5 mm *versus* 6.5 mm) in the previous report (5). A larger portion (52.8 ± 11.4%) of the applied radioactive dose was recovered from the skin surface and a correspondingly smaller portion (29.6 ± 12.9%) of the applied radioactive dose was recovered by skin oxidation, compared to the percentages cited in the previous study (5). This difference may result from the thoroughness of the skin surface rinse procedure, as the sum of the percentages of applied radioactive dose recovered by skin rinse and skin oxidation in the two studies are similar in magnitude.

The *in vitro* apparatus described here can be a useful tool for the screening of mosquito repellent formulations that incorporate a repellent whose evaporation and penetration characteristics and minimum effective evaporation rate are known. Formulations can be selected that reduce excessive evaporation, maintain evaporation rates above the minimum effective evaporation rate for longer periods of time, and reduce percutaneous penetration as compared with the unformulated repellent.

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High-Performance Liquid Chromatographic Analysis of Digitoxin Formulations

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Abstract □ A rapid, selective, and simple high-performance liquid chromatographic assay for digitoxin formulations is described. The method utilizes a conventional octadecyl-bonded phase column with detection at 220 nm. The isocratic solvent system resolves digitoxin from its potential degradation products and provides an accurate assay for tablet and injectable formulations with a relative standard deviation of 1.4 and 3.3%, respectively. The method is sufficiently sensitive to monitor content uniformity of tablets and the minimum quantifiable amount of digitoxin was determined to be 20 ng. The total chromatograph time was ~15 min.

Keyphrases □ Digitoxin—high-performance liquid chromatographic analysis of formulations, content uniformity □ Formulations—digitoxin, high-performance liquid chromatographic analysis, content uniformity □ High-performance liquid chromatography—content uniformity, analysis of digitoxin formulations

Digitoxin is a cardiac glycoside obtained from the leaves of *Digitalis purpurea* and is used in the treatment of congestive heart failure. Due to its long biological half-life the unit dose is generally low (0.1 mg). Assurance of potency and content uniformity of tablets is, therefore, a necessity for proper dosage. The determination of such a potent drug in the dosage form requires a method that is accurate, selective, and sensitive.

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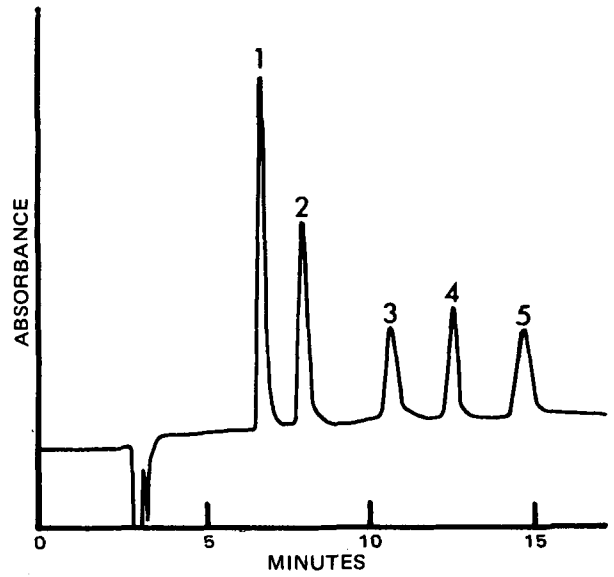

Figure 1—*Isocratic HPLC separation of digitoxin and its potential degradation products. Sequence of elution: digitoxigenin (1), digitoxigenin monodigitoxoside (2), digitoxigenin bisdigitoxoside (3), 17α-methyltestosterone (4), and digitoxin (5). Mobile phase: water–methanol–isopropanol–methylene chloride (45:38:11:6); flow rate: 1.1 ml/min.*

Quantitative colorimetric methods (1–8) lack selectivity, since the chromogenic agent reacts with either the lactone ring or the digitoxose sugar residue of the digitoxin molecule and would not determine the quantity of intact drug in the presence of any degradation products. In addition, these techniques are relatively insensitive. Fluorometric procedures (9–13) are based on the dehydration of the aglycone moiety, and although sensitive, are largely nonspecific. TLC techniques (14–17) although selective, are semiquantitative at best. A GC assay method (18) provided a highly sensitive method utilizing a heptafluorobutyrate derivative with electron-capture detection. However, the procedure requires conversion of the drug and potential impurities to the digitoxigenin portion of the glycoside and thus is nonselective. High-performance liquid chromatographic (HPLC) techniques have been reported (19–23) for the resolution of digitoxin and its potential metabolites, degradation products, and impurities; however, quantitative procedures for digitoxin in its dosage forms have not been reported.

This paper describes an HPLC method that is fast, selective, and sufficiently sensitive to allow accurate quantitation of digitoxin in single-tablet assays.

EXPERIMENTAL

Materials and Methods—A high-performance liquid chromatograph[1] equipped with dual pumps[2], a microprocessor system controller[3], a dynamically stirred mixing chamber[4], and a 20-μl loop injector[5] were used throughout. A variable wavelength detector[6] was operated at 220 nm. Data acquisition was accomplished with an electronic printer/plotter integrator[7]. The HPLC column[8] had a dimension of 4.6 mm × 250-mm i.d. and was packed with octadecyl (C-18) 5-μm bonded phase.

[1] Model 322, Beckman Instruments Inc., Toronto, Ontario, Canada.
[2] Model 100A and 110A, Beckman Instruments Inc.
[3] Model 420, Beckman Instruments Inc.
[4] Model 400, Beckman Instruments Inc.
[5] Model 210, Beckman Instruments Inc.
[6] Hitachi Ltd., Tokyo, Japan.
[7] Model C-R1A, Shimadzu Corp. Kyoto, Japan.
[8] Ultrasphere, Beckman Instruments Inc.

Table I—Recovery Data for the HPLC Analysis of Digitoxin Tablets[a]

Number	Theoretical Digitoxin Concentration, mg	Amount Added, mg	Recovery, %
1	1.0	0.5	98.6
2	1.0	0.5	102.6
3	1.0	0.5	95.8
4	1.0	0.5	101.9
Mean			99.7
RSD (%)[b]			3.1

[a] 0.1-mg tablet. [b] Relative standard deviation.

Water, methanol, isopropanol, and methylene chloride were of HPLC grade[9]. Digitoxin[10], digitoxigenin[10], digitoxigenin monodigitoxoside[10], digitoxigenin bisdigitoxoside[10], and 17α-methyltestosterone[11] were used without further purification.

The mobile phase used for the isocratic assay consisted of water–methanol–isopropanol–methylene chloride (45:38:11:6); flow rate was 1.1 ml/min and the UV detector was set at 220 nm.

An internal standard solution of 17α-methyltestosterone was prepared by dissolving 100 mg in 100 ml of methanol.

A standard solution of digitoxin was prepared by dissolving 10 mg in 60 ml of boiling methanol in a 100-ml volumetric flask. After cooling the resulting solution to room temperature, the flask was brought to volume with methanol and thoroughly mixed.

Calibration Curve—Aliquots of 1, 2, 5, 10, 20, and 30 ml of the digitoxin reference standard solution were added into 100-ml volumetric flasks along with 1 ml of internal standard solution. The solutions were diluted with methanol to produce a volume of 35 ml in each case. The resulting solutions were then brought to volume with water and thoroughly mixed. Three 20-μl aliquots of each of the calibration standards were used to determine the response ratio and linearity.

Tablet Formulation Assay—Forty tablets (0.1 mg/tablet) were weighed and triturated to a fine powder. An accurately weighed aliquot equivalent to ~1 mg of digitoxin was transferred to a 100-ml volumetric flask. Distilled water (10 ml) was added and the flask was swirled for 2–3 min, followed by the addition of 34 ml of methanol. The flask was shaken for 15 min, and the resulting suspension was filtered through filter paper[12] and the residue was washed with three, 5-ml portions of distilled water. The filtrate and washings were collected in a 100-ml volumetric flask containing 1 ml of the internal standard solution. The flask was brought to volume with water and a 20-μl aliquot was injected into the liquid chromatograph.

A single tablet assay was performed by placing one tablet in a 50-ml

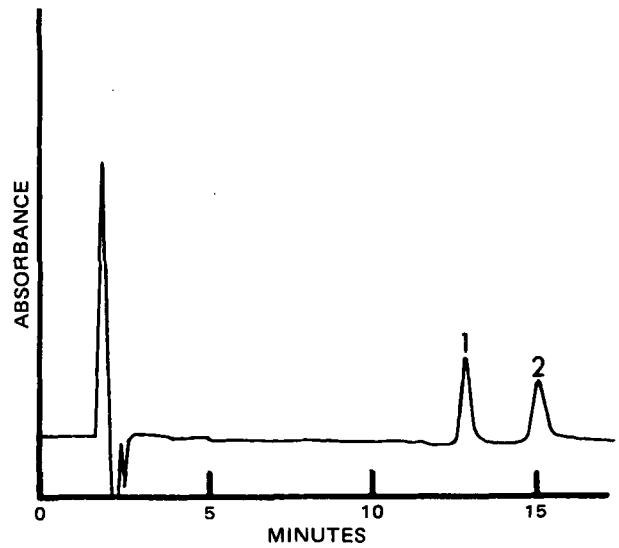

Figure 2—*High-performance liquid chromatogram of a digitoxin tablet formulation. Sequence of elution: 17α-methyltestosterone (1), digitoxin (2). Refer to Fig. 1 for HPLC conditions.*

[9] Fisher Scientific Co., Vancouver, British Columbia, Canada.
[10] Boehringer Mannheim Corp., Mannheim, G.F.R.
[11] Sigma Chemical Co., St. Louis, Mo.
[12] Whatman paper No. 1, W and R Balston Ltd., Eng.

Table II—Results of the HPLC Analysis of Digitoxin Tablet and Injectable Formulations

Number	Percent of Label Claim		
	Tablet Composite Assay[a]	Single Tablet Assay[a]	Injectable[b]
1	95.1	94.8	95.9
2	97.1	104.6	94.3
3	96.8	92.8	100.5
4	99.0	98.4	—
5	98.4	96.5	—
6	96.8	94.8	—
7	—	97.9	—
8	—	107.0	—
9	—	98.2	—
10	—	103.8	—
Mean	97.2	98.8	96.9
RSD (%)[c]	1.4	4.8	3.3

[a] 0.1-mg tablet. [b] 0.2 mg/ml. [c] Relative standard deviation.

volumetric flask and following the same procedure as for the tablet formulation assay.

Injectable Formulation Assay—Exactly 1 ml of digitoxin injection was quantitatively transferred to a 10-ml volumetric flask. A 0.1-ml aliquot of the internal standard solution along with 3.3 ml of methanol were added, and the flask was brought to volume with water.

To determine assay precision, 70 tablets were weighed and triturated to a fine powder. Six aliquots equivalent to ~1 mg of digitoxin were individually treated as described under tablet formulation assay. Three 20-μl injections were made from each of the six aliquots.

Recovery of Digitoxin from Tablets—Eighty tablets (0.1 mg/tablet) were weighed and ground to a fine powder. Three aliquots were treated and assayed in the same manner as for the tablet formulation to determine a mean assay value. Four aliquots of the ground digitoxin tablets, equivalent to an assay value of 1 mg, were prepared, and an accurately weighed quantity of digitoxin reference standard[9] equivalent to 0.5 mg was added to each sample. The samples were further treated as described under tablet assay. Three injections were made for each sample.

RESULTS AND DISCUSSION

A representative chromatogram for a standard mixture of digitoxin, its mono- and bisdigitoxosides, digitoxigenin, and the internal standard, 17α-methyltestosterone, is given in Fig. 1. From the chromatogram it can be determined that the potential impurities or degradation products are well resolved and are separated from the peak due to digitoxin. The overall chromatographic time of 15 min provides efficient assay capabilities.

The chromatogram obtained for a tablet composite assay is given in Fig. 2. The chromatogram is essentially devoid of any interfering peaks due to other glycosides or tablet excipients. As a further check, common tablet excipients such as lactose, starch, methylcellulose, and stearate lubricants were subjected to the extraction and assay procedures and were found not to interfere with the peaks due to the digitalis glycosides or the internal standard.

The composite sample used for the recovery study was initially assayed in triplicate and a mean value of 99.8% was obtained. The recovery of digitoxin from tablet formulations was thus determined by adding an amount equivalent to 50% of the tablet potency adjusted for the 99.8% potency observed. The data given in Table I indicate that the mean recovery was 99.7% of the expected total potency of the theoretical composite potency plus the amount of digitoxin added. The precision of the assay was determined by independent analysis of six tablet aliquots of a 0.1-mg tablet formulation and was found to yield a mean assay value of 97.2% with a 1.4% *RSD* (Table II).

USP assay (8) for digitoxin tablets is a relatively time-consuming procedure requiring column chromatographic isolation of the digitalis glycoside followed by a chromogenic reaction and colorimetric measurement. As such, the method requires several hours of analytical time. The HPLC method described in this paper, however, requires <1 hr to complete each assay determination. The USP limits for digitoxin tablets specify a content of 90–110% of label claim. From the data for a composite tablet assay given in Table II, the formulation examined falls within the pharmacopeial requirements. Because of the need to ensure content uniformity of digitoxin tablets, the USP specifies a sensitive but lengthy fluorometric procedure. The HPLC method described herein is sufficiently sensitive to determine content uniformity of 0.1-mg tablets. The resulting data obtained from a random sample of 10 tablets is given in Table II. The general pharmacopeial requirements for tablets specify that each dosage unit must contain 85–115% of the labeled potency. The data given in Table II indicate that the tablets examined have a mean potency of 98.8% with a 4.8% *RSD*. The range of 92.8 to 107.0% observed falls within the required limits.

Table III—Results of the Analysis of Digitoxin Tablets Using the USP XX Method[a]

Number	Label Claim, %
1	103.1
2	101.1
3	97.4
Mean	100.5
RSD (%)[b]	2.9

[a] 0.1-mg tablet. [b] Relative standard deviation.

As a final comparison with the USP assay for tablet potency, three aliquots of the 0.1-mg tablet composite used to determine the potencies given in Table II were assayed according to the pharmacopeial method. The results given in Table III for this analysis indicate that the formulation had a mean potency of 100.5% with a 2.9% *RSD*. It can be seen that the assay results obtained with the HPLC method compare favorably with those obtained with the USP method.

The HPLC analysis of a digitoxin injectable formulation is given in Table II. The assay mean (96.9%) indicates that the formulation is within the limits (90–110%) specified in the USP (8). The HPLC analytical procedure requires that the formulation only be diluted, whereas the USP method requires a prior column chromatographic procedure followed by timed chromogenic reaction and serial spectrophotometric readings. A significant time advantage is obtained with the HPLC procedure described.

The HPLC assay method described herein for tablet and injectable formulations is simple, rapid, and precise, and provides a sufficiently sensitive method for the determination of individual tablet content. The potency determinations compare favorably with those obtained with the USP assay method.

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