Rapid Quantitative GLC Analysis of Digoxin

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Abstract ☐ A quantitative method of assaying digoxin by GLC was developed. The pure drug in both tablet and powder form can be assayed rapidly and accurately by this procedure.

Keyphrases ☐ Digoxin tablets and powder—GLC analysis ☐ GLC—analysis, digoxin tablets and powder

The advantages of GLC involve its speed of operation, its high degree of resolution, and its potential to yield quantitative results.

Jelliffe and Blankenborn (1) were the first to use GLC to separate cardiac glycosides. Several investigators have since used GLC qualitatively as a means of identifying these glycosides (2–4).

Various quantitative colorimetric assays for digoxin have been reported, and subsequent attempts at automating these methods have been undertaken (5–9). The GLC analysis of digoxin on nonselective phases has been studied with various degrees of success.

Watson and Kalman (10) assayed plasma levels of digoxin by GLC. Their procedure took 5 hr./assay and involved both TLC and GLC steps. Watson and Kalman (10) used electron-capture detection, while Wilson *et al.* (2) used the trimethylsilyl ethers of cardenolides which required high temperature and high flow rates of the carrier gas. Tan (3) questioned the formation of the silyl ethers reported by Wilson *et al.* and others.

It is apparent that the quantitative GLC methods of analysis reported rely on the production of derivatives and require much time as well as laborious manipulative steps. The method proposed in this investigation eliminates all of these drawbacks.

EXPERIMENTAL

Equipment—A chromatographic system[1] with a flame-ionization detector was used. The gas flow rates were: hydrogen, 35 ml./min.; air, 300 ml./min.; and carrier gas (nitrogen), 50–100 ml./min. Operating conditions were: oven temperature, 285°; and injection port and detector temperature, 330°. All injections were made with a 5-μl. syringe[2].

Columns—Three types of columns were used. Column A was a 2-m. glass U-tube (2 mm. i.d.) packed with 2.5% OV-1 on 80–100-mesh Chromosorb A[3]. Columns B and C were 0.5-m. copper U-tubes (4 mm. i.d.) packed with 2.5% OV-1 on 80–100-mesh Chromosorb A and 3% OV-17 on 80–100-mesh Chromosorb A, respectively.

Reagents—The silylating reagent was prepared just prior to use by mixing 1 ml. of trimethylchlorosilane[4] and 10 ml. of hexamethyldisilazane[4] with 10 ml. of dry pyridine as a solvent. The pyridine was stored over potassium hydroxide to ensure dryness. The hexamethyldisilazane and trimethylchlorosilane were stored under nitrogen and extracted from sealed vials with a syringe. The volume was then replaced with dry nitrogen gas. The sample to be silylated

[1] Varian model 2100.
[2] Hamilton Co., Whittier, Calif.
[3] Applied Science Laboratories, Inc., State College, Pa.
[4] Pierce Chemical Co.

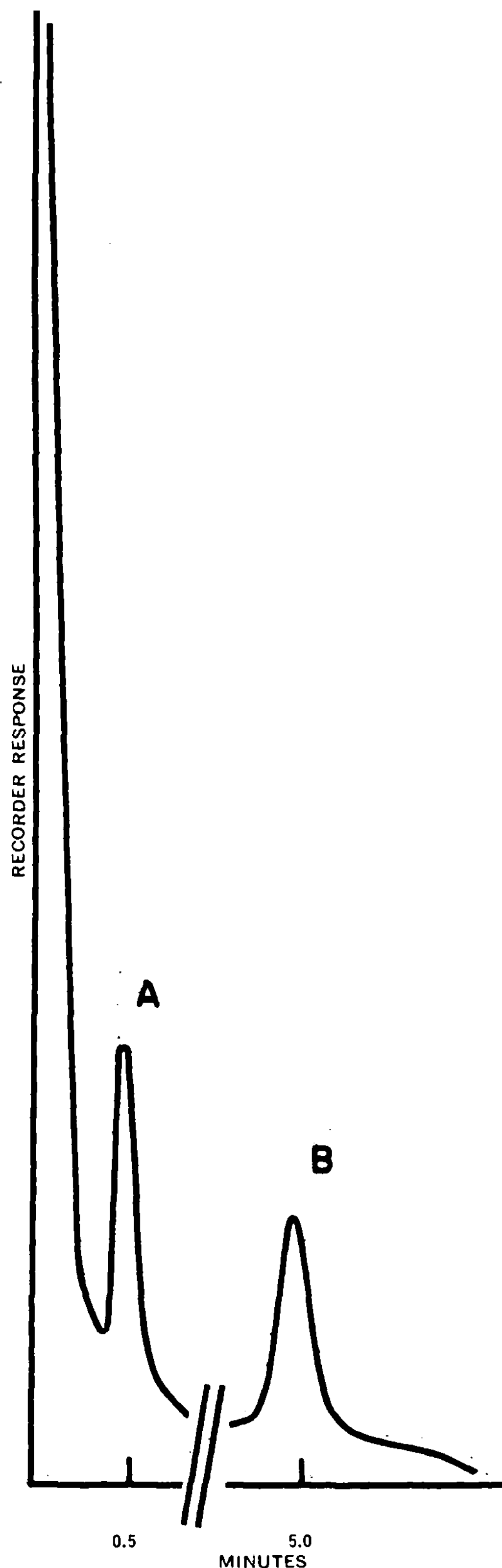

Figure 1—*Representative chromatogram of a mixture of: (A) cholesterol and (B) digoxigenin.*

Table I—Retention Time (Minutes)

	Column A	Column B	Column C
Cholesterol (unsilylated)	2.0	0.67	0.5
Cholesterol (silylated)	2.0	0.67	0.5
Digoxigenin (unsilylated)	15.0	5.33	3.67
Digoxigenin (silylated)	15.0	5.0	3.67

was made to react with 1 ml. of the reagent at room temperature before being injected into the gas chromatograph.

TLC—The purity of the digoxin[5] and digoxigenin[6] used was verified by TLC using two solvent systems: cyclohexane–acetone–acetic acid (49:49:2) and ethyl acetate–water–methanol (80:5:5). All solvents were analytical grade.

Glass plates precoated with 0.2 mm. of silica gel G[3] were used. The spots were visualized by spraying with 50% aqueous sulfuric acid and heating at 110° for 2–3 min. A second more reliable visualization method was also employed by preparing a spray consisting of a mixture of a 3% aqueous solution of chloramine-T and a 25% alcoholic solution of trichloroacetic acid. The two solutions, in a ratio of 1:4, were mixed just prior to use. After spraying, the plate was heated in an oven at 110° for 7 min. The blue spot then became visible under UV light at a wavelength of 385 nm.

Internal Standard—Cholesterol was used as an internal standard in the amount of 0.02 mg.

Reference Standard—Digoxin and digoxigenin were both employed in amounts varying from 0.05 to 0.2 mg.

Sample Preparation—A sample of either 0.1 mg. of powdered digoxin or the equivalent amount in tablet form was dissolved in 2 ml. of a pyridine-water (50%, v/v) solution. A volume of 0.1 ml. of 0.1 *N* sodium hydroxide was added, and the mixture was heated on a steam bath for 30 min. to convert completely the digoxin to digoxigenin. The solution was then evaporated to drynesss and redissolved in 100 µl. of dry pyridine. An aliquot of 10 µl. of a stock solution containing 2 mcg./µl. of cholesterol in pyridine was added. From this solution, a 1-µl. sample was injected into the gas chromatograph and the areas under the curves were calculated by means of a disk integrator.

RESULTS AND DISCUSSION

A typical chromatogram for the unsilylated compounds is shown in Fig. 1. The results indicated that both silylated and nonsilylated compounds exhibit similar retention times using all three columns (Table I). From this standpoint, there appeared to be no advantage in silylating the compound. Due to the high temperature of operation, difficulty was experienced in maintaining a firm seal between Column A and the metal connections. The Teflon ferrel, which normally would be used for this purpose, began to melt at the temperatures at which the injector and detector were operated. In addition, the retention times were much longer for Column A than for Columns B and C.

The standard curve was generated by plotting A_D/A_C against W_D/W_C, where A_D and A_C are the areas under the curve for digoxigenin and cholesterol, respectively, and W_D and W_C are the respective weights for digoxigenin and cholesterol. The results along with the analysis of variance are shown in Table II.

[5] K & K Laboratories, Plainview, N. Y.

Table II—Standard Curve Data For Digoxigenin–Cholesterol

A_D/A_C[a]	W_D/W_C[b]
3.48	16.47
2.08	10.83
2.50	8.95
1.51	7.58
0.89	6.55

Analysis of Variance

Source	df	Sum of Squares	Mean Square	F Ratio
Regression	1	22.68	22.67	29.66
Residual	4	3.06	0.76	
Total	5	25.72		

Variables in Equation

Coefficient	0.20
Standard error	0.0013
Intercept	0.057

[a] Area ratio (digoxin–cholesterol). [b] Weight ratio (digoxin–cholesterol).

As indicated under sample preparation, all the digoxin was hydrolyzed to digoxigenin. The latter can then be used indirectly to calculate the amount of digoxin in the sample by using a ratio of molecular weights. From the data in Table II, an unknown quantity of digoxigenin can be obtained by calculating W_D from Eq. 1:

$$\frac{A_D}{A_C} = 0.20\frac{W_D}{W_C} + 0.057 \qquad \text{(Eq. 1)}$$

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