Automated Fluorometric Procedure for Unit Dose Analysis of Digitoxin and Digoxin in Tablet Formulations

L. F. CULLEN, D. L. PACKMAN, and G. J. PAPARIELLO

Abstract □ A sensitive and specific automated procedure is described for the unit dose analysis of digitoxin and digoxin in tablet formulations. The technique is based on the fluorometric measurement of the dehydration products of the cardiotonic steroids, resulting from their reaction with hydrogen peroxide in concentrated hydrochloric acid. The automated system is capable of analyzing 12 tablets per hour with a relative standard deviation of ±1.4% at the 0.05-mg. digitoxin level. Accuracy of the procedure was determined by collecting comparative data on digitoxin tablets by both the automated method and the manual USP procedure. Specificity of the method, with respect to the analysis of intact digitoxin in the presence of the products of photochemical, alkaline hydrolytic, and thermal degradation, was demonstrated by comparison to quantitative TLC values. This method has also been shown to be applicable to the unit dose analysis of digoxin in the 0.02 to 1.0 mg./tablet range.

Keyphrases □ Digitoxin—automated fluorometric method, tablets □ Digoxin—automated fluorometric method, tablets □ Automated methods—digitoxin, digoxin, unit dose analysis in tablets, diagram □ Automatic analyzer—diagram, digitoxin, digoxin determination □ TLC—analysis

Determining the precise amount of digitoxin in tablets is of the utmost interest to the pharmaceutical industry. These tablets are of low dosage, *i.e.*, 0.05–0.20 mg. per tablet, and, consequently, require unit dose analysis as a control on the homogeneity of the product. An attempt was made to automate these unit dose assays to gain the advantages of reproducibility, rapidity, and sensitivity offered by an automatic analyzer system.

Khoury (1) described an automated sulfuric acid-induced fluorescence method for the single-tablet analysis of cardiac glycosides. However, it was found that this procedure was not applicable to the analysis of digitoxin tablets which contain sugars and dyes. Since these interfering inactive components are common formulating materials, the present investigation was undertaken to develop a technique which would overcome these shortcomings.

The majority of the analytical methods reported for the quantitative determination of digitoxin are either colorimetric (2–9) or fluorometric (10–12). The colorimetric procedures did not exhibit the necessary sensitivity or specificity for single-tablet analysis at the normal digitoxin dosage levels. Thus, fluorometric procedures were investigated.

Wells *et al.* (12) presented a sensitive and specific fluorometric method for the analysis of digitoxin and digoxin in biological fluids and tissues. The technique is based on the fluorometric measurement of the dehydration products of the cardiotonic steroids resulting from the reaction of the glycosides with hydrogen peroxide in concentrated hydrochloric acid. Detailed discussions of the influence of time, temperature, and reagent concentrations on the excitation and emission properties of the glycosides and the mechanism of fluorescence formation were described by Wells *et al.* (12) and also Jelliffe (13). This procedure has been adapted and modified for continuous analysis by use of an automatic analyzer system.[1] Applicability of this method to the unit dose analysis of digoxin in the 0.02–1.0 mg. per tablet range requires only a simple change in the fluorometer filter system.

EXPERIMENTAL

Apparatus—A standard Technicon automatic analyzer system consisting of the following modules: (*a*) solid-prep sampler, programmed at 12 samples/hr.; (*b*) proportioning pumps[2] (two required); (*c*) continuous filter, speed 2; (*d*) fluorometer,[3] equipped with fluorescent lamp (Turner 110–853), quartz flowcell,[4] and filters: digitoxin analysis, primary—Corning[5] No. 7-59 and secondary—Wratten[6] No. 8; digoxin analysis, primary—Corning No. 7-60 and secondary—Wratten No. 3; and (*e*) linear recorder.

Reagents and Solutions—(*a*) Seventy percent SDA No. 30 alcohol (ethanol–methanol, 10:1) in water (v/v); (*b*) concentrated hydrochloric acid; (*c*) 0.1% ascorbic acid in methanol; (*d*) 0.065 *M* hydrogen peroxide in methanol (prepared by adding 1.0 ml. of reagent grade 30% hydrogen peroxide to 200 ml. of absolute methanol)—this reagent should be prepared fresh daily and maintained in an ice bath on the automated manifold; and (*e*) standard digitoxin and digoxin solutions—prepare a 0.5 mg./ml. solution of analytical reference standard[7] digitoxin or digoxin in 70% denatured alcohol were used. The standard calibration curve is derived from a series of diluted solutions ranging in concentration from 0.05–0.25 mg./ml.

Automated Methodology—A diagram of the flow system indicating the automated equipment arrangement for the analytical procedure is shown in Fig. 1. In performing the analyses, three standards of the appropriate levels of the cardiotonic steroid are placed on the sample plate, followed by samples of the intact or powdered tablets. At the end of a series of 12 samples, two standards are inserted to minimize the effects of reagent changes and instrumental variations. Samples are introduced into the solid-prep unit, programmed at 12 samples/hr. with its diluent volume precalibrated to deliver 150 ml., and homogenized in the 70% denatured alcohol. During the period of homogenization, the sample is diluted and dissolution of the drug occurs. A portion of the mixture is aspirated into the flow system and automatically filtered to remove insolubles. To prevent mechanical obstruction from the insoluble excipient materials at the tubing connections, a decantation trap is placed between the solid-prep unit and the proportioning pump to remove the majority of the solid matter, thus extending the operational time of the automated system before a breakdown period is required. The residual insolubles are removed by the continuous filter module. After passage through the filter module, the filtrate is segmented with air and then combined with streams of the ascorbic acid reagent, concentrated hydrochloric acid, and the hydrogen peroxide reagent. The sample stream is passed through time-delay coils to permit complete fluorometric development. Since

[1] AutoAnalyzer, Technicon Corp., Tarrytown, N. Y.
[2] Technicon model I.
[3] Technicon model I.
[4] Technicon catalog No. 126-0125.
[5] Corning Glass Works, Corning, N. Y.
[6] Eastman Kodak Co., Rochester, N. Y.
[7] USP Reference Standards, Bethesda, Md.

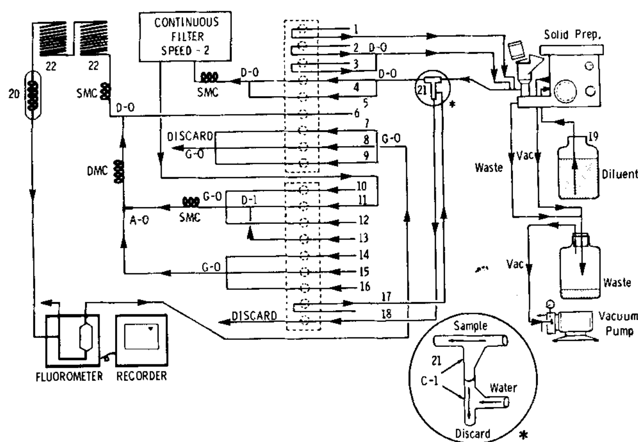

Figure 1—*Automated flow diagram. Key: 1, 0.8 ml./min. air (Tygon); 2,3, 2.76 ml./min. 70% SDA No. 30 alcohol (Acidflex); 4,5, 2.03 ml./min. sample (Acidflex); 6, 0.56 ml./min.* H_2O_2 *reagent (Solvaflex); 7,8,9, 2.03 ml./min. flowcell (Acidflex); 10, 2.42 ml./min. ascorbic acid reagent (Solvaflex); 11, 2.76 ml./min. sample (Acidflex); 12, 2.42 ml./min. ascorbic acid reagent (Solvaflex); 13, 1.60 ml./min. air (Tygon); 14,15,16, 2.76 ml./min. HCl (Acidflex); 17, 0.42 ml./min. water (Tygon); 18, 0.60 ml./min. (Tygon); 19, reservoir-70% SDA No. 30 alcohol; 20, water-cooled mixing coil (No. 114-209-1); 21, decantation trap; and 22, 12.19-m. (40-ft.) time-delay coils, 2.0 mm. i.d. (No. 105-1173-01).*

a slight fluctuation in the temperature of the final solution at the time of sample measurement in the flowcell has a significant effect on the noise level in the sample response, it is necessary to introduce a water-cooled mixing coil into the system prior to the entrance of the sample stream into the flowcell. The constant and controlled temperature of the final solution results in a significant increase in the precision of the procedure by eliminating the noise in the sample response. Calculations are made using corresponding fluorescent intensities of standards and solid dosage formulation samples.

Under the exact analytical conditions described, digitoxin exhibits excitation and emission wavelength maxima at 395 and 570 mμ, respectively. Excitation and emission wavelength maxima for digoxin are 350 and 490 mμ, respectively. These wavelength maxima are centered well within the optimum spectral transmission characteristics of the selected filters. Spectral measurements were made on a Farrand spectrofluorometer,[8] following preparation of the samples with the automatic analyzer flow system. The spectral data are in exact agreement with those reported by Wells *et al.* (12).

TLC System—Silica gel GF precoated glass plates (10 × 20 cm. with a 250-μ adsorbent layer)[9] were activated by heating at 105° for 20 min. prior to use. The chromatogram was developed in a saturated chamber with a benzene–95% ethanol (7:3, v/v) solvent system. When the solvent front ascended approximately 15 cm. from the origin, the plate was removed and air-dried. Visualization was effected by spraying the plate with chloramine–trichloroacetic acid reagent (14) and heating at approximately 105° for 10 min. Following this heating period, the plates were inspected under shortwave (253.7 mμ) and longwave (366.0 mμ) UV light.

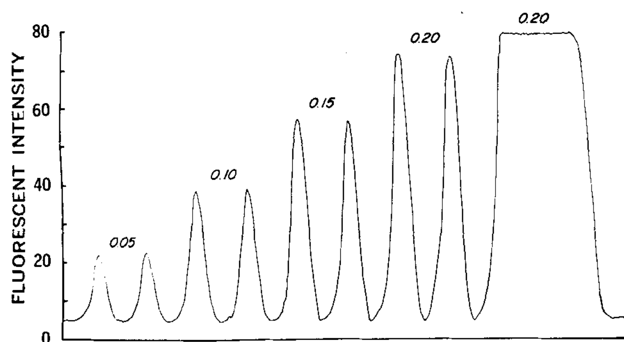

Figure 2—*Reproduction of recording curve on digitoxin standards (mg.) at the rate of 12 samples/hr.*

[8] Farrand catalog No. 104242, Farrand Optical Co., Inc., Mount Vernon, N. Y.
[9] Analtech, Inc., Wilmington, Del.

Table I—Comparison of Fluorometric and Quantitative TLC Analyses of Intentionally Degraded Digitoxin Samples

Sample Treatment	% Initial: Fluorometric Method	% Initial: TLC Method
Digitoxin powder stored at 100° for 1 month	85	87
Digitoxin powder stored at 185° for 16 hr.	76	77
Digitoxin powder exposed to shortwave UV light for 1 month at room temperature	65	63
Suspension of the digitoxin heated in 1 *N* NaOH for 1.5 hr. at 75°	46	43
Digitoxin tablet formulation stored at 100° for 1 month	87	88
Digitoxin tablet formulation exposed to shortwave UV light for 1 month at room temperature	89	91

RESULTS AND DISCUSSION

Suitability as Stability Method—Specificity of the method for analysis of intact digitoxin in the presence of its photochemical, alkaline hydrolytic, and thermal degradation products was demonstrated by comparing analytical values of intentionally degraded samples to those obtained by quantitative TLC. In the TLC procedure, the digitoxin was separated from its degradation products on the chromatographic plate. A zone of the silica gel encompassing the intact digitoxin spot was scraped from the plate. The digitoxin was eluted from the adsorbent with 70% denatured alcohol and then assayed by the automated procedure to obtain a

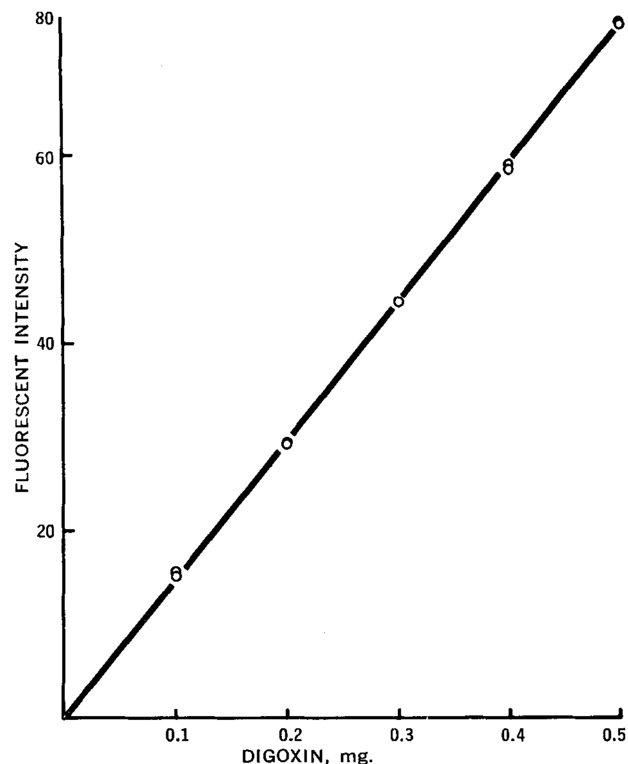

Figure 3—*Relationship of fluorescent intensity to digoxin level.*

Table II—Comparative Data Obtained on Digitoxin Tablets by Automated Procedure and Manual USP XVII Procedure

Product[a]	Labeled Amount, mg./tablet	% of Claim[b] Automated	USP
A	0.10	102	101
	0.20	100	100
B	0.10	97	98
C	0.15	100	100
	0.20	103	102
D	0.10	103	102
	0.20	101	101
E	0.10	102	102
	0.20	103	102
F	0.10	103	103
G	0.05	101	100
	0.10	100	99
	0.15	100	100
	0.20	99	100

[a] Tablets analyzed were prepared by the following manufacturers: Wyeth Labs., Inc.; The Upjohn Co.; E. R. Squibb & Sons; Parke, Davis & Co.; Eli Lilly & Co.; Davies, Rose-Hoyt; and Abbott Labs. Companies are not necessarily listed in order as given above. [b] Data from a single assay on a 10-tablet composite sample.

quantitative value. The data obtained are summarized in Table I. Since there is good agreement between the values by the two techniques, it is concluded that the fluorometric procedure is stability indicating.

Linearity and Sensitivity—Figure 2 is a reproduction of an actual recording curve obtained by analyzing standards of digitoxin in the 0.05–0.20-mg. range. The uniformity of replicate analyses at the various concentrations and the return to the base line between samples, indicating no sample overlap, can be noted. When the values for the standards of Fig. 2 are plotted, a linear relationship exists between fluorescent intensity and digitoxin concentration in the range studied. This curve also contains a recording of the steady state, during which time a 0.20-mg. standard was continuously sampled, which further defines the excellent flow characteristics of this automated system.

In the application of the method to the analysis of digoxin samples, it was also observed that a linear relationship exists between fluorescent intensity and digoxin concentration (Fig. 3).

The flow diagram of Fig. 1 is applicable to the analysis of digitoxin and digoxin samples which initially contain 0.02–1.0 mg. of the cardiac glycosides. At the 0.02-mg. level, the final concentration of the drug in the flowcell is approximately 0.07 mcg./ml.

Precision—Repeatability of the automated technique was demonstrated by performing 30 replicate assays on aliquots of both digitoxin and digoxin standard solutions. Relative standard deviations of ±1.4 and ±1.0% were determined at the 0.05- and 0.20-mg. digitoxin levels, respectively, and ±1.2% at the 0.25-mg. digoxin level. In addition, a series of 20 replicate assays run on a composite sample of a tablet formulation at the 0.10-mg. digitoxin per tablet level produced a relative standard deviation of ±1.0%.

Accuracy—The accuracy of the proposed procedure was evaluated by comparing the results obtained by the automated system with the manual USP XVII colorimetric procedure (9) on several different marketed digitoxin tablet formulations. Comparative results are shown in Table II. The agreement between the two techniques demonstrates that the automated method provides an accurate means of evaluating digitoxin content.

The effect of common inert tablet components on this fluorometric procedure for digoxin and digitoxin was investigated to uncover any possible interfering material. The inactive components evaluated were lactose, sucrose, calcium sulfate, magnesium stearate, stearic acid, alginic acid, microcrystalline cellulose (Avicel), ion-exchange resin (Amberlite IRP-88), methylcellulose (Methocel), and starch. It was found that none of these materials interfered.

REFERENCES

(1) A. J. Khoury, "Automation in Analytical Chemistry, Technicon Symposia, 1966," vol. I, Mediad, Inc., New York, N. Y., 1967.

(2) A. E. James, F. O. Laquer, and J. D. McIntyre, *J. Amer. Pharm. Ass., Sci. Ed.*, **36,** 1(1947).

(3) F. K. Bell and J. C. Krantz, *ibid.*, **37,** 297(1948).

(4) E. Soos, *Sci. Pharm.*, **16,** 29(1948).

(5) E. E. Kennedy, *J. Amer. Pharm. Ass., Sci. Ed.*, **39,** 25(1950).

(6) J. M. Rowson, *J. Pharm. Pharmacol.*, **4,** 814(1952).

(7) D. H. Tattje, *Ann. Pharm. Fr.*, **12,** 267(1954).

(8) D. H. Tattje, *J. Pharm. Pharmacol.*, **9,** 29(1957).

(9) "United States Pharmacopeia," 17th rev., Mack Publishing Co., Easton, Pa., 1965.

(10) L. J. Sciarini and W. T. Salter, *J. Pharmacol. Exp. Ther.*, **101,** 167(1951).

(11) I. M. Jakovljevic, *Anal. Chem.*, **35,** 1513(1963).

(12) D. Wells, B. Katzung, and F. H. Meyers, *J. Pharm. Pharmacol.*, **13,** 389(1961).

(13) R. W. Jelliffe, *J. Chromatogr.*, **27,** 172(1967).

(14) "Thin-Layer Chromatography," E. Stahl, ed., Academic, New York, N. Y., 1965.

ACKNOWLEDGMENTS AND ADDRESSES

Received June 16, 1969, from *Wyeth Laboratories, Pharmacy Research and Development Division, Radnor, PA 19101*

Accepted for publication October 31, 1969.

Presented to the Technicon International Congress on Automated Analysis, Chicago meeting, June 1969.