spectrophotometric determinative step because of the low absorptivity of atropine and hyoscyamine, *e.g.*, *a* of atropine sulfate = 6.31 in 0.1 *N* H_2SO_4 (18).

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TECHNICAL ARTICLES

Automated Assay of Single Tablets of Digoxin

JAMES W. MYRICK

Abstract □ An automated analytical system has been used to determine the amount of digoxin in single tablets at a level of 0.25 mg. per tablet. The active ingredient in an alcoholic solution was oxidized with periodate; after removal of the excess periodate with arsenite, reaction with 2-thiobarbituric acid at 75° produced a visible color which was recorded from a colorimeter. Interference by dextrose, which may be present in the tablet, was eliminated by a simultaneous determination at a different wavelength. Relative standard deviation of the method was 0.7% for powdered tablet samples and 1.0% for an authentic tablet formulation.

Keyphrases □ Digoxin tablets—analysis □ Automated procedure—digoxin, single tablets □ Diagram–automated analysis, digoxin □ Colorimetric analysis—spectrophotometer

Digoxin, one of the active ingredients isolated from *Digitalis lanata*, is frequently used in the treatment of congestive heart conditions to increase the force of contraction and to increase cardiac tone. Patients placed under digitalis medication undergo two phases of administration—the initial course for digitalization and the second for maintenance. Both of these phases require individualized supervision to secure proper results. Since the duration of action of digoxin is one-third to one-seventh as long as digitoxin, the dosage of digoxin needed to control the patient must be accurately quantitated. Because of this need for accuracy, the USP (1) has established a content uniformity requirement in its monograph for digoxin tablets.

Various colorimetric assays for digoxin have been reported. Alkaline picrate was used as reagent by Baljet (2), xanthydrol by Pesez (3), 3,5-dinitrobenzoic acid by Tattje (4), acetone-phosphoric acid by Dequeker and Loobuyck (5), ferric chloride, acetic acid, and sulfuric acid by James *et al.* (6), 2,4-dinitrodiphenylsulfone by Tattje (7), thiobarbituric acid by Mesnard and Devaux (8), and *m*-dinitrobenzene by Houk *et al.* (9). An automated method based on acid-induced fluorescence was proposed by Khoury (10) but it was not reproducible in our laboratory because of instability of the fluorometer. Literature on applications of automated analyses to pharmaceutical formulations are now fairly extensive and several compilations exist (11). A survey of the colorimetric analytical methods indicated a lack of sensitivity or suitability for automation

of individual tablet analysis in all except the thiobarbituric acid method (8).

This report describes a successful application of automated methods to the individual tablet analysis of digoxin in which thiobarbituric acid is used as the color reagent.

EXPERIMENTAL

Apparatus—The analytical train included the following modules:[1] liquid sampler II; proportioning pump; heating bath; two colorimeters, one with a 50-mm. tubular flow cell and a 530-mμ filter, the second with a 15-mm. tubular flow cell and a 460-mμ filter; a dual pen recorder (Bristol) linear in transmission, provided with paper printed in absorbance units (No. R0487) moving at 18 in./hr.

Reagents—*Wash Solution*—95% ethyl alcohol.

Periodate Solution.—Add 3.6 g. of potassium periodate (*meta*) to 900 ml. of distilled water. Heat to 80–90° and stir to obtain solution. Cool. Add 3.0 ml. of 98% sulfuric acid and dilute to 1 l.

Arsenite solution—Add 20.0 g. of arsenious acid (As_2O_3) and 7.0 g. of sodium hydroxide pellets to 100 ml. of distilled water. Heat to boiling to dissolve all As_2O_3. Dilute to 900 ml. with distilled water. Add 60 ml. of 37% hydrochloric acid and dilute to 1 l.

Thiobarbituric Acid Solution—Place 15.0 g. of 2-thiobarbituric acid[2] and 4.5 g. of sodium hydroxide pellets in 900 ml. of distilled water. Stir to obtain solution. Add 37% hydrochloric acid slowly to bring the pH to 3.5–4.0 as tested by paper (about 1–2 ml. is required). Filter and dilute to 1 l.

Standards—*Digoxin, 5 mcg./ml.*—Prepare a stock solution of 25.0 mg. of USP reference standard digoxin dissolved in 500.0 ml. of 95% ethyl alcohol. This solution will keep for several weeks if tightly stoppered. Prepare a working solution fresh daily by adding 2 drops of distilled water to a 10.0-ml. aliquot of the stock solution and diluting to 100.0 ml. with 95% ethyl alcohol.

Dextrose, 2.4 mg./ml.—Place 12.0 g. of anhydrous dextrose (reagent grade) in a 500-ml. volumetric flask and dissolve in 100 ml. of distilled water. Dilute to volume with 95% ethyl alcohol. Prepare a working solution fresh daily by placing a 10.0-ml. aliquot of stock solution in a 100-ml. volumetric flask, adding 38 ml. of absolute ethyl alcohol, and diluting to volume with 95% ethyl alcohol.

Sample Preparation—Individual tablets containing 0.25 mg. digoxin were placed in a 60-ml. snap-cap vial;[3] tablets containing 0.50 mg. were placed in a 120-ml. snap-cap vial. The tablets were softened by placing 2 drops of distilled water directly on the tablet and allowing to stand 5 min. Then 50.0 ml. of 95% ethyl alcohol

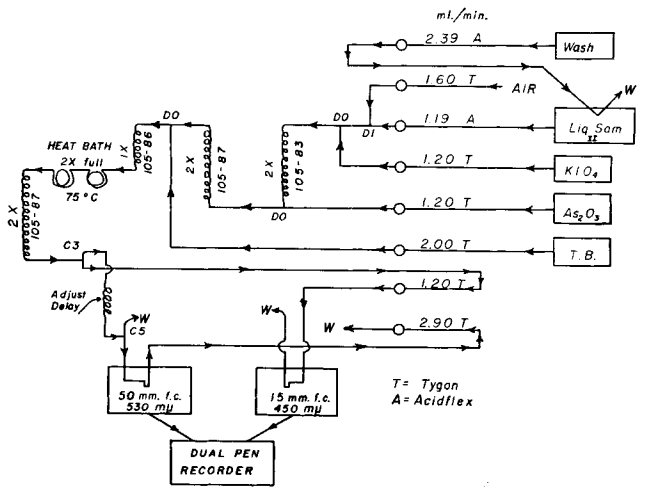

Figure 1—*Schematic diagram of the automated system for digoxin determinations.*

[1] AutoAnalyzer, Technicon Corp., Tarrytown, NY 10591.
[2] Catalog No. 660, Distillation Products Industries, Eastman Organic Chemicals Department, Rochester, NY 14063.
[3] Catalog No. 24836, Wheaton Glass Co., Millville, NJ 08332.

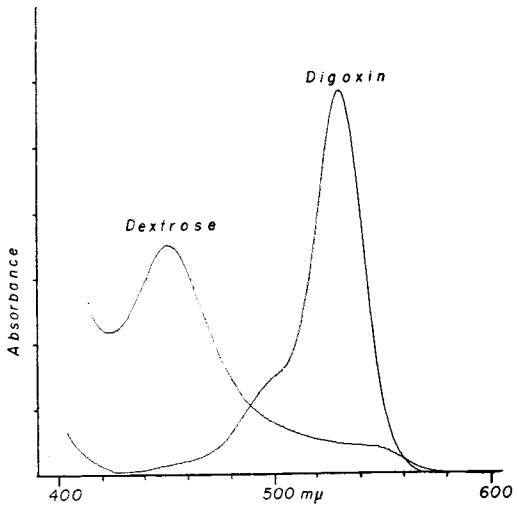

Figure 2—*Spectral characteristics of the reaction products of digoxin and dextrose with thiobarbituric acid.*

was added to the 0.25-mg. tablet (or 100.0 ml. to the 0.50-mg. tablet). The vial was capped and shaken intermittently for 1 hr., and undissolved material was allowed to settle.

Procedure—A schematic diagram of the automated analytical system used is presented in Fig. 1.

The sample solutions were decanted into 8.5-ml. polystyrene cups, loaded into the 40-place sample storage wheel of the liquid sampler, and covered with the protective cover. A pattern of five solutions of individual tablets, a standard solution, and five individual tablets was used. Occasionally, after these 11 cups, a solution prepared from one average tablet weight of a ground composite of the lot was inserted. A blank cup of 95% alcohol was used to isolate the curves of a lot on the chart paper. A sampling rate of 30/hr. with a sample-to-wash ratio of 2/1. was used for the timing program.

In operation, the sampled solution was segmented with air, periodate solution was added, and the digoxin was allowed to oxidize in a 4-mm. double mixing coil for a period of about 2 min. Arsenite solution was then added to remove the excess periodate, after which the color reagent, thiobarbituric acid, was added. Color was developed in a 2X full-coil heating bath at 75°. The solution was then split between the two colorimeters, the first with a 50-mm. tubular flow cell and a 530-mμ filter to measure the digoxin moiety, and the second with a 15-mm. tubular flow cell with a 460-mμ filter to measure the interference color due to dextrose, which is sometimes used as a filler or excipient in some tablet formulations. Sufficient delay was built into one leg of the final solutions being read so that both colorimeter peaks were synchronized on the dual pen chart recorder.

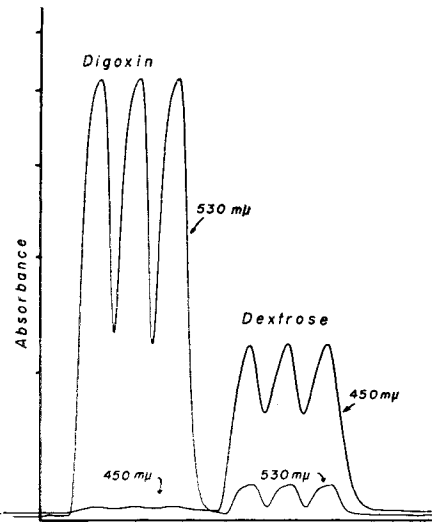

Figure 3—*Standard curves for digoxin and dextrose.*

Table I—Reproducibility of Standard and Sample Solutions

Absorbance	
Standard Digoxin[a]	Sample Digoxin[b]
0.460	0.435
0.462	0.435
0.462	0.438
0.460	0.437
0.460	0.439
0.460	0.437
0.459	0.437
0.458	0.436
0.460	0.437
0.459	0.438
Av. 0.460	0.437
Relative *SD* ±0.27%	±0.30%

[a] 5 mcg./ml. [b] Ten 0.25-mg. tablets/500 ml.

At the beginning of the day, determinations were made of the contribution of dextrose to the digoxin color curve (Fig. 2) by running three cups of pure standard digoxin (5 mcg./ml.), a blank cup of 95% alcohol, and three cups of pure standard dextrose (2.4 mg./ml.) (Fig. 3).

The equipment was cleaned weekly by pumping approximately 0.1 *N* sodium hydroxide solution through all supply lines for about 15 min. followed by a distilled water rinse of equal duration.

Calculations—A factor equivalent to the ratio of the absorbance of the dextrose peak at 530 mμ divided by the absorbance at 460 mμ was established. If a sample showed an appreciable absorption peak at 460 mμ, the absorption was multiplied by the factor to obtain the contribution of dextrose to the digoxin peak. A base line correction was applied to each set of curves for a lot by drawing a line on the chart paper from the lowest points of the 95% alcohol blank cup and assuming that there was uniform drift between samples of the lot. The base line correction was added to the dextrose correction and this total was subtracted from the total absorption of the digoxin at 530 mμ to determine the *net* absorption of each sample. Calculation of the percentage of digoxin in each tablet was made from the formula:

$$\% \text{ found} = \frac{A_u \times S \times V}{A_s \times W \times 10}$$

where A_u = net absorption of tablet solution; A_s = net absorption of standard digoxin; S = mcg./ml. of standard digoxin; V = volume in milliliters of each tablet solution; W = milligrams declared digoxin per tablet.

RESULTS

Reproducibility of Standards—The reproducibility of the method was checked by establishing steady state conditions, then analyzing 10 cups of the same standard solution as presented in Fig. 4 and Table I. At a sampling program rate of 30/hr. and a sample-to-wash ratio of 2/1., the peak height was about 97% of steady state.

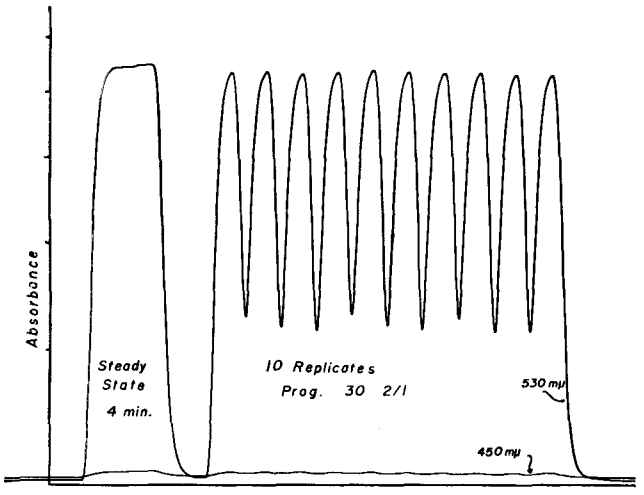

Figure 4—*Reproducibility of a standard digoxin solution.*

Table II—Analysis of Powdered Composite Sample

Sample[a]			Standard Digoxin[b], Absorbance
Weight, g.	Absorbance	Declared, %	
0.1426	0.428	96.96	0.446
0.1408	0.426	97.74	0.450
0.1429	0.436	98.56	0.449
0.1402	0.428	98.62	0.442
0.1439	0.440	98.76	—
0.1404	0.428	97.60	—
0.1396	0.430	98.62	—
0.1420	0.433	97.63	—
0.1431	0.439	98.22	—
0.1418	0.434	97.99	—
0.1424	0.432	97.35	—
0.1459	0.443	97.43	—
0.1484	0.455	98.38	—
0.1395	0.430	98.91	—
0.1421	0.431	97.33	—
0.1420	0.430	98.71	—
0.1413	0.430	99.20	—
0.1424	0.431	98.66	—
0.1398	0.422	98.40	—
0.1409	0.426	98.55	—
Av.	—	98.18	—
Relative *SD*	—	±0.70%	—

[a] Average tablet weight = 0.1407 g., equivalent to 0.25 mg. digoxin; composite assayed 97.8% by USP method (1). [b] 5.12 mcg./ml., inserted after every fifth sample cup.

Reproducibility of Samples—A total of 10 tablets containing 0.25 mg. of digoxin/tablet was dispersed in 500 ml. of alcohol, and the solution was used to fill 10 cups of the sampler. Absorption values obtained are presented in Table I.

The average tablet weight of 100 tablets was determined. They were ground to pass a 60-mesh sieve, and the powder was mixed thoroughly. Twnety samples of approximately one average tablet weight were weighed from this composite, placed in vials, and treated with 50 ml. of alcohol. Portions of each solution were placed in individual cups of the automatic analyzer in a pattern of five samples, standard, five samples, standard, *etc.* Results of the analysis are presented in Table II.

Recovery of Authentic Tablet Formulation—An excipient powder was prepared as indicated in Table III by premilling all ingredients in a laboratory ball mill for 2 hr. A portion of the excipient powder was accurately weighed and ball-milled for two hours with a known amount of pure digoxin as indicated in Table III. Twenty samples were weighed from the resulting authentic powder and analyzed with the automated system (Table IV). For comparison, nine additional portions of the authentic powder were weighed and analyzed by the USP official method (1). These results are shown in Table V.

DISCUSSION

Interferences—The possible interference of common tablet excipients, fillers, and binders was studied. Dextrose contributed to

Table III—Formulation for Authentic Tablet Powder

Ingredients	Parts
Excipient	
Dextrose, anhydrous powder	10.0
Lactose powder, USP	53.5
Dicalcium phosphate, USP	19.8
Cornstarch, USP	14.4
Talc	1.8
Magnesium stearate	0.36
Sodium benzoate	0.09
Tablet Powder[a]	
Excipient powder	15.2233 g.
Pure digoxin	0.0782 g.

[a] Equivalent to 0.514 mg. digoxin/tablet weight of 0.1000 g.

Table IV—Analysis of Authentic Tablet Formulation by Automated Method

Authentic Formulation[a]			
Weight, g.	Absorbance	Recovery, %	Standard[b], Absorbance
0.1081	0.523	100.88	0.440
0.1073	0.512	99.50	0.439
0.1030	0.495	100.21	0.440
0.1032	0.500	101.03	0.438
0.1035	0.500	100.74	—
0.1009	0.490	101.50	—
0.1026	0.491	100.02	—
0.1042	0.508	101.89	—
0.1034	0.498	100.65	—
0.1072	0.512	99.82	—
0.1078	0.512	99.04	—
0.1095	0.536	102.07	—
0.1106	0.530	99.93	—
0.1053	0.502	99.41	—
0.1022	0.491	100.18	—
0.1030	0.491	99.40	—
0.1033	0.499	100.73	—
0.1090	0.515	98.52	—
0.0994	0.468	98.18	—
Av.	—	100.19	—
Relative *SD*	—	±1.06%	—

[a] 0.1000 g. = 0.514 mg. digoxin. [b] 5.24 mcg./ml., inserted after every fifth sample cup.

the absorption at 530 mμ to a considerable extent (see Fig. 2), since it can also be oxidized by periodate (12) and reacts with thiobarbituric acid to produce a yellow color with maximum absorbance at 450 mμ. The effect of this contribution can be eliminated by measuring the final color stream at the two wavelengths and correcting for those samples which contain dextrose, as described in the procedure. Tablets that do not contain dextrose or other interfering materials have a negligible peak at 460 mμ. A very slight response was obtained at 460 mμ for starch and lactose but these were considered negligible.

Glycerol produces a strong absorbance at 460 mμ but has no contribution at the 530-mμ peak. Shellac produces a larger peak at 530 mμ than at 460 mμ. When used as a binder on punched tablets, it appears to cause a wide apparent assay variation of the individual tablets, probably because it is not uniformly applied to each tablet.

For the most accurate work, it is recommended that whenever possible a blank of all raw materials except digoxin be prepared for each formulation in order to estimate the necessary corrections properly.

Solvents—Literature references indicate that digoxin is most soluble in 80% ethanol–water (v/v); however, dextrose is also more soluble at this concentration and greater corrections were necessary when that solvent was used to treat the tablet.

Other Reactions—This color reaction depends on the oxidation of the terminal sugar of the glycoside into a malonic dialdehyde configuration which then couples with the thiobarbituric acid. A color reaction will also be obtained with digitoxin or other digitoxosides which may be present as impurities in the digoxin raw material. No reaction was observed with lanatoside C or acetyldigitoxin, where the acetate group is on the terminal sugar and prevents oxidation by the periodate to the necessary malonic dialdehyde configuration.

CONCLUSIONS

The method as presented has been used at the National Center for Drug Analysis as a screening procedure for more than 400 samples, as part of the assigned program in the investigation of identity, purity, and potency of a wide variety of drugs purveyed throughout the United States. Assay limits on 10 individual tablets of a lot were set inside official limits by twice the relative percent standard deviation of the method. This permitted us to work with a confidence level of at least 95%. In this case, the USP (1) requires a content uniformity of 85 to 115% of declared. All lots assaying outside the limits of 87 to 113% of declared by the automated procedure were subjected to the official manual analysis.

Table V—Analysis of Authentic Tablet Formulation by USP Method (1)

	Digoxin Recovered	
Weight, g.[a]	mg.	%
0.1089	0.528	94.4
0.1046	0.512	95.3
0.1102	0.549	96.9
0.1164	0.589	98.4
0.1122	0.568	98.6
0.1064	0.547	100.0
0.1182	0.582	95.9
0.1086	0.530	96.5
0.1221	0.601	95.7
Av.	—	96.9
Relative *SD*	—	±1.81%

[a] 0.1000 g. = 0.514 mg. digoxin.

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