Table I—Precision and Accuracy of the Assay Applied to Spiked Human Plasma Samples

Spiked Concentration, ng/ml	Determined Concentration, ng/ml ± *SEM*[a]	Range
200	200 ± 1.0	194–205
500	500 ± 0.8	488–512
1000	998 ± 1.0	970–1026
3000	3087 ± 2.5	2870–3303

[a] $n = 6$.

The extraction procedure suggested here results in a noise-free blank for the plasma and urine. The spiked samples (prepared from a stock solution of 1 mg/ml in ethanol) showed excellent resolution. The retention time for I was 4 min, whereas the internal standard (II) appeared at 3.0 min. These short retention times are highly desirable, allowing large numbers of samples to be analyzed in a short time. The extraction efficiency was calculated to range from 75 to 80% when compared with the standard solutions prepared in carbon disulfide.

The sensitivity of the column under these conditions allowed detection of quantities as low as 10 ng injected onto the column; however, the concentration of I that could easily be detected in plasma and urine was below 100 ng/ml. The precision and accuracy of the analytical method were discerned by spiking the plasma samples with concentrations ranging from 200 to 3000 ng/ml. Table I reports the determined concentrations of the spiked samples.

The plasma I levels following administration of a 100-mg oral dose are shown in Fig. 1. Each data point represents the average of six determinations. A large degree of intersubject variation was noted in terms of the time and magnitude of the peak concentration. After a rapid rise in the plasma concentration, a sharp decline resulted in the lowering of the concentration to below 100 ng/ml within 2 hr for Subject 1 and 6 hr for Subject 2. Therefore, the bioavailability of I and its rate of absorption may differ greatly between subjects.

Less than 1% of the administered dose of I was eliminated in the urine as intact drug (Table II), which is in concurrence with a previous study (1).

Table II—Recovery of Free Butylated Hydroxyanisole in Urine[a]

Hours	Subject 1	Subject 2
0–8	0.080 ± 0.012	0.050 ± 0.011
8–16	0.570 ± 0.009	0.431 ± 0.009
16–24	0.033 ± 0.0017	0.027 ± 0.025
24–36	0.00	0.00

[a] Percent of administered dose ± *SEM*.

Detailed pharmacokinetic studies are in progress.

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Bioavailability and Related Heart Function Index of Digoxin Capsules and Tablets in Cardiac Patients

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Received August 9, 1977, from the **Istituto di Clinica Medica Ia, Cattedra di Malattie Cardiovascolari, University of Parma, Via Gramsci, 14-I-43100 Parma, Italy*, and the ‡*Istituto Simes di Cardiologia Sperimentale, Via C. Colombi, 18-I-20161 Milan, Italy.* Accepted for publication May 23, 1978.

Abstract □ A loading dose of digoxin (750 μg) in two commercial formulations was administered to 14 patients with heart disease according to a crossover design. One formulation consisted of soft gelatin capsules containing a solution of digoxin; the other formulation was compressed tablets. All parameters investigated, *i.e.*, serum peak height, time of the peak, area under the serum level–time curve (*AUC*), and area above the Q–S_2I (electromechanical systole) decrease (obtained from polycardiographic evaluation), showed better bioavailability of digoxin capsules than tablets, averaging 36.3%. The better bioavailability of digoxin capsules than tablets seems to be more evident in heart disease patients than that encountered previously in healthy subjects. The *AUC* and the area above the Q–S_2I decrease were linearly correlated only with digoxin capsules.

Keyphrases □ Bioavailability—digoxin capsules and tablets compared in cardiac patients □ Heart function index—digoxin capsules and tablets compared in cardiac patients □ Digoxin—bioavailability and heart function index, capsules and tablets compared in cardiac patients □ Cardiotonic agents—digoxin, bioavailability and heart function index, capsules and tablets compared in cardiac patients

A marked lack of uniformity in content and bioavailability of digoxin tablets was reported (1–5) for different formulations as well as for different batches of the same marketed product. In the last few years, a fairly satisfactory bioavailability level has been achieved in most commercial preparations of digoxin tablets that meet the Food and Drug Administration's recent specific requirements (6). The most important development in this field is the

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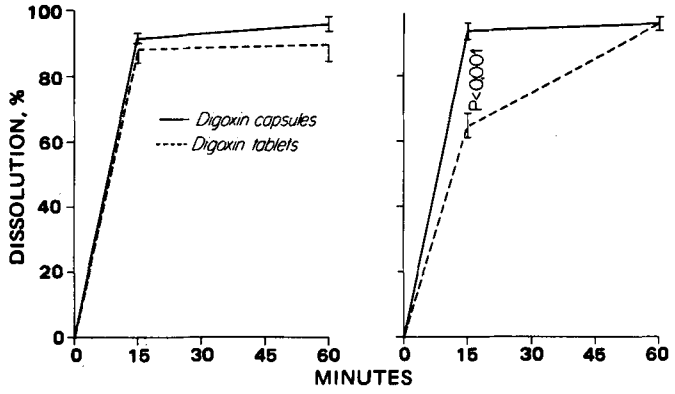

Figure 1—*Dissolution of digoxin capsules and tablets using both the paddle-water (left) and paddle-acid (right) methods (mean values of six experiments ± SE). Values of* p *were computed using the Student* t *test for independent samples.*

soft gelatin liquid-filled capsule, a new pharmaceutical formulation, which contains the glycoside in a dissolved form and allows a more satisfactory bioavailability range than tablets.

This paper reports a comparative study of the bioavailability of digoxin in capsules and tablets in volunteer patients who suffered from heart disease and used digoxin. A polycardiographic investigation enabled a useful statistical comparison between bioavailability and the related index of heart function for both formulations.

EXPERIMENTAL

Drug—The digoxin capsules[1] contained 0.25 mg of digoxin dissolved in 1.5 mg of *N,N*-dimethylacetamide and 139 mg of polyethylene glycol 400. The digoxin tablets[2] also contained 0.25 mg of the cardiac glycoside. The dissolution rate of both formulations was evaluated according to the *Federal Register* (6), using both the paddle-acid and paddle-water methods.

Treatment—The 14 patients were of both sexes and were 40–64 years old; they had an average body weight of 75 kg. They were in normal sinusal rhythm and were affected by atherosclerotic and/or hypertensive heart disease. A series of laboratory tests and clinical observations showed that the patients had no other serious diseases and that inorganic ions, serum proteins, creatinine clearance, and thyroid, GI, and renal functions were normal.

All subjects received three digoxin capsules and three digoxin tablets (750 μg of digoxin) in a crossover design at an interval of 10 days. A blood sample (5 ml) was taken from each subject before treatment and at 15, 30, and 45 min and 1, 2, 3, and 4 hr after treatment. Serum digoxin concentrations were measured with the radioimmunoassay[3] method of Smith *et al.* (7). At the same times, polycardiographic measurements were recorded[4].

Variations in systolic intervals were measured and adjusted for heart rate and sex according to Weissler *et al.* (8). Of the parameters evaluated, electromechanical systole ($Q–S_2I$) was selected as the most representative of the action of digitalis on the heart. In effect, $Q–S_2I$ is a useful index in terms of the decrease in both the preejective period and the left ventricular ejection time (9).

RESULTS

Dissolution with the paddle-acid method was complete in 15 min with capsules but was markedly slower with tablets ($p < 0.001$). Both formulations were completely dissolved in 60 min. With the paddle-water method, digoxin tablets gave a dissolution rate slightly lower than capsules, but this difference was not statistically significant (Fig. 1).

[1] Eudigox, lot A9-74, Simes.
[2] Lanoxin, lot 4 B16, Wellcome.
[3] Kits were supplied by Sorin Biomedica, Saluggia (Vercelli) Italy.
[4] Elema Schonander Mongograf 61.

Table I—Peak Height, Peak Time, Area under the Serum Level–Time Curve (*AUC*), and Area above the $Q–S_2I$ Decreases in 14 Heart Patients after Administration of a Loading Dose of Digoxin (750 μg) in Capsules and Tablets[a]

Parameter	Digoxin Capsules	Digoxin Tablets	Δ%[b]	t[c]	p[c]
Peak height, ng/ml	5.3 ± 0.5	3.6 ± 0.4	32	3.7	<0.01
Peak time, min	107 ± 19	153 ± 19	−43	−3.3	<0.01
AUC, ng/ml × hr	12.3 ± 1.6	7.6 ± 0.6	38	3.4	<0.01
Area above $Q–S_2I$ decrease, msec hr	7.0 ± 1.0	4.7 ± 0.6	32	3.0	<0.02

[a] Mean values ± *SE*. [b] Δ% = [(capsules − tablets)/capsules] × 100. [c] Evaluated with the Student *t* test for paired samples.

The peak height was significantly higher and was attained earlier with the capsules than with the tablets. The areas under the serum level–time curve (*AUC*) and above the decrement of electromechanical systole were significantly higher with the capsules than with the tablets (Table I). When a regression straight-line relationship between the *AUC* and the area above the $Q–S_2I$ decrease was calculated, a linear regression coefficient of 0.49 ($p < 0.05$) was obtained with the capsules, in contrast to 0.15 (p without any statistical significance) with the tablets.

DISCUSSION

The better bioavailability of digoxin capsules than tablets was previously encountered in dogs (10) and in healthy subjects after a single dose (11–15) and in a steady-state condition (16). The originality of this investigation lies in the experimental design, which consists of administration of a loading dose of digoxin (750 μg) in capsules and tablets to heart patients in a crossover pattern and the evaluation of bioavailability and related heart function index. The need to measure evident variations in cardiomechanical parameters indicated a loading dose of 750 μg of digoxin. The bioavailability of digoxin capsules was 36% higher than that of tablets (mean of the four Δ% in Table I) in heart patients. This difference in healthy human subjects was around 20 (12) and 27% (13) after a single dose and 26% (16) in a steady-state condition. The difference in bioavailability between digoxin capsules and tablets thus seems to be more evident in heart patients than in healthy subjects. In addition, the *AUC* and the area above the $Q–S_2I$ decrease were linearly correlated with capsules ($p < 0.05$) but not with tablets. In no case was there any evidence of GI or cardiac toxicity.

Considerable individual variability in plasma digoxin levels was observed in healthy subjects using tablets with a high dissolution rate, capsules, and, in particular, an oral solution of digoxin (12, 13, 15–17). Data from the cardiac patients in this investigation were similar to the previous healthy subject data regarding individual variability. In addition, the objective situation of the cardiopathic patients involves a specific lack of individual variability due to inadequate circulation, the liver status, and, in many cases, confinement to bed. The variability of response in digoxin absorption seems to be related mainly to the daily situation of each patient, whereas the entity of enteral absorption is closely related to the pharmaceutical formulation used.

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Quantitative Determination of Chlorthalidone in Pharmaceutical Dosage Forms by High-Pressure Liquid Chromatography

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Received October 25, 1977, from the *Department of Analytical Research and Development, USV Pharmaceutical Corporation, Tuckahoe, NY 10707.* Accepted for publication May 29, 1978.

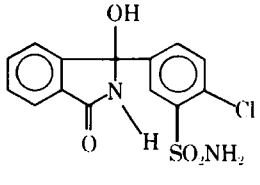
Abstract □ A reliable and selective high-pressure liquid chromatographic procedure for the quantitative determination of chlorthalidone in pharmaceutical dosage forms is described. A comparison of this stability-indicating procedure with the USP spectrophotometric assay is presented for chlorthalidone tablets and chlorthalidone tablets containing reserpine.

Keyphrases □ Chlorthalidone—high-pressure liquid chromatographic analysis in pharmaceutical dosage forms □ High-pressure liquid chromatography—analysis, chlorthalidone in pharmaceutical dosage forms □ Diuretics—chlorthalidone, high-pressure liquid chromatographic analysis in pharmaceutical dosage forms

Chlorthalidone (I), 2-chloro-5-(1-hydroxy-3-oxo-1-isoindolinyl)benzenesulfonamide, is an oral antihypertensive–diuretic administered alone and in combination with reserpine in tablets[1]. Several methods have been reported for the determination of chlorthalidone in biological media (1–4) and for its chromatographic separation and detection in the presence of other substances (5, 6). At present, there is no simple direct method for the quantitative and stability determinations of chlorthalidone in pharmaceutical dosage forms.

The USP method (7) consists of solvent extraction of the powdered tablet material and subsequent quantitative comparison of the UV absorption of a solution of the extracted chlorthalidone with the absorption of a solution of USP reference standard chlorthalidone run concomitantly. The compendial method lacks analytical specificity and selectivity for the active drug substance and is, therefore, not a reliable stability-indicating assay.

This report describes a high-pressure liquid chromatographic (HPLC) procedure that is simple, direct, and specific for chlorthalidone in tablets as the single active drug substance and in combination with reserpine.



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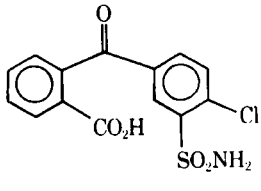

II

[1] Marketed by the USV Pharmaceutical Corp. under the trade name of Hygroton and in combination with reserpine under the trade name of Regroton.

EXPERIMENTAL

Reagents—Commercial distilled-in-glass solvents[2] and analytical reagent grade acetic acid[3] were used without additional purification.

Apparatus—A high-pressure liquid chromatograph[4], interfaced to an electronic integrator[5], was equipped with a fixed wavelength (254 nm) UV absorption detector and a constant volume injection valve[6].

Column—The column consisted of a stainless steel tube (1m × 2.2 mm i.d.) prepacked with a polyamide-coated stationary phase[7].

External Standard Solution—The external standard solution was prepared by accurately weighing approximately 50 mg of chlorthalidone reference standard[8], transferring the sample to a 25.0-ml volumetric flask, and dissolving and diluting it to volume with acetonitrile–water (9:1 v/v).

Sample Preparation—Twenty tablets were weighed and ground to a fine powder. An accurately weighed sample of the ground powder, equivalent to approximately 50 mg of chlorthalidone, was extracted with 25.0 ml of acetonitrile–water (9:1 v/v) in a 50-ml screw-capped centrifuge tube by vigorous agitation on a mixer[9] for 15 min. Then the sample was centrifuged, and the supernate was saved for chromatographic analysis.

Assay—The mobile phase, 2-propanol–acetic acid–water–*n*-hexane (30:1.5:0.5:68 v/v) was pumped through the column at a flow rate of 2 ml/min with a column head pressure of approximately 35 kg/cm². The fixed wavelength detector was operated at an attenuation of 0.32 aufs, and the column oven was maintained at 35° throughout the analysis.

Aliquots (10 μl) of the standard and sample solutions were injected in

[2] Burdick and Jackson Laboratories, Muskegon, Mich.
[3] Mallinckrodt, St. Louis, Mo.
[4] Model 830, E. I. DuPont de Nemours, Wilmington, Del.
[5] Autolab, System IV B, Spectra-Physics, Santa Clara, Calif.
[6] Six port equipped with 10-μl loop, Valco Instruments Co., Houston, Tex.
[7] Pellamidon, Whatman, Clifton, N.J.
[8] USP reference standard.
[9] Vortex Genie, Scientific Industries, Bohemia, N.Y.

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