Semiautomated System for High-Pressure Liquid Chromatographic Determination of Dissolution Rate of Fludrocortisone Acetate Tablets

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Abstract □ A new semiautomated high-pressure liquid chromatographic (HPLC) system is described to determine the dissolution rate of fludrocortisone acetate tablets. The system uses a miniaturized dissolution basket and shaft assembly having the same geometry as that given in USP XIX. This reduced size permits use of smaller volumes of dissolution medium, allowing most very low dose oral solid dosage forms to be handled. The USP dissolution kettle was also replaced with a new miniaturized vessel that continuously filters the sample solution before it enters the flow system. Volumes of dissolution medium as small as 15 ml can be accommodated, depending on the sensitivity of the assay employed and the solubility of the drug substance under study. The concentration of fludrocortisone acetate in solution was monitored by a new HPLC system employing a reversed-phase column compatible with the aqueous dissolution medium used. A comparative dissolution study of different lots was made using different basket rotation speeds.

Keyphrases □ Fludrocortisone acetate—tablets, dissolution, high-pressure liquid chromatographic analysis □ Dissolution, *in vitro*—fludrocortisone acetate tablets, high-pressure liquid chromatographic analysis □ High-pressure liquid chromatography—analysis, dissolution of fludrocortisone acetate tablets □ Adrenocortical steroids—fludrocortisone acetate tablets, dissolution, high-pressure liquid chromatographic analysis

Fludrocortisone acetate (9α-fluoro-11β,17α,21-trihydroxy-4-pregnane-3,20-dione), a potent adrenocortical steroid (1), is usually administered as 0.1-mg tablets. The USP XIX dissolution apparatus could not be used to investigate its dissolution characteristics because of the resulting very low concentration of the active ingredient in the 900 ml of solvent specified. Under these conditions, none of the available methods, UV (1), blue tetrazolium (2), nydrazid (2), and 4-aminoantipyrine (3), has the sensitivity required for reliable quantitative analysis. The same difficulty exists in dissolution studies of other pharmaceutical formulations administered in very low doses, *e.g.*, estrogens, thyroxine fractions, digoxin, and digitoxin tablets. Since such a large volume of dissolution fluid is specified by the compendia mainly to minimize the effect of the concentration gradient on the dissolution rate, it seemed logical that the volume could be reduced significantly while still maintaining sink conditions[1].

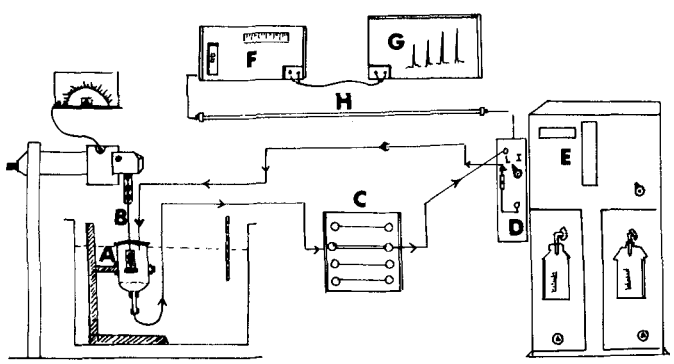

Figure 1—*Flow diagram for semiautomated system for the HPLC determination of the dissolution rate of fludrocortisone acetate tablets.*

Table I—Comparison between USP XIX and Miniaturized Basket and Drive Shaft Assembly [a]

Component	USP XIX	Miniaturized Version
Material	40-mesh, type 316 stainless steel	40-mesh, type 316 stainless steel
Shaft	26 mm × 30 cm	Upper part, 6.4 mm × 7.5 cm; lower part, 4 mm × 7.5 cm
Basket	25 × 36.6 mm	10 × 15.6 mm

[a] Details of the new design are shown in Fig. 2.

To accomplish this volume reduction, some basic modifications had to be made in the USP XIX dissolution apparatus. These modifications included the design of a miniaturized basket, drive shaft assembly, and dissolution vessel. A semiautomated high-pressure liquid chromatographic (HPLC) system also was developed to monitor the fludrocortisone acetate in solution based on the HPLC assay of fludrocortisone acetate recently developed.

EXPERIMENTAL

Apparatus—The system is modular, with the components arranged as shown in Fig. 1. The dissolution vessel (A) consists of a 30-ml glass filter funnel[2] with 2–2.5-μm diameter pores. This filter funnel is connected to a glass U tube *via* a vinyl polymer[3] sleeve and is fitted with a polytef[4] disk cover (3.8 cm i.d.) that has two circular openings. The first opening (6.7 mm i.d.), in the center, is for the basket drive shaft; the second opening (2 mm i.d.), to one side of the cover, is for the return line polyethylene probe from the HPLC injector.

The basket drive shaft assembly (B) consists of a miniaturized version[5] with the same geometry as the USP XIX assembly (Table I and Fig. 2). A proportioning pump[6] (C) was used to circulate the dissolution medium through the injector valve (D), the HPLC instrument, and back to the dissolution vessel. The HPLC instrument[7] (E) consisted of a fixed volume mobile phase reservoir (200 ml), a nitrogen cylinder to maintain system pressure, and a UV monitor operated at 254 nm. The detector (F) output was displayed on a 10-mv recorder[8] (G).

The HPLC column (H) was packed with reversed-phase packing material[9]; the mobile phase consisted of 50 ± 2% acetonitrile[10] in water, pumped at a pressure of 1000 psi, which resulted in a flow rate of 1.2–1.4 ml/min. The HPLC instrument was equipped with a 20-μl loop injector[11], the inlet of which was connected to a 0.065-mm i.d. proportioning pump tube. The outlet was connected to a polyethylene tube, positioned about 2 cm below the surface of the dissolution medium *via* a plastic nipple, which replaced the injector syringe.

[1] Defined as not allowing the concentration of the active ingredients in solution to exceed 10% of saturation level.
[2] Thomas Catalog No. 5221-B30, type 20VF.
[3] Tygon.
[4] Teflon.
[5] Made for Squibb by the Hanson Research Corp., Northridge, Calif.
[6] Technicon pump II, Technicon Instruments, Tarrytown, N.Y.
[7] Chromatronix 3100, Spectra-Physics, Santa Clara, Calif.
[8] Model 410, Pharmacia Fine Chemicals, Piscataway, N.J.
[9] C_{18}-μBondapak, Waters Associates, Milford, Mass.
[10] UV grade distilled in glass, Burdick and Jackson.
[11] Chromatronix and HPSV-20, Spectra-Physics, Santa Clara, Calif.

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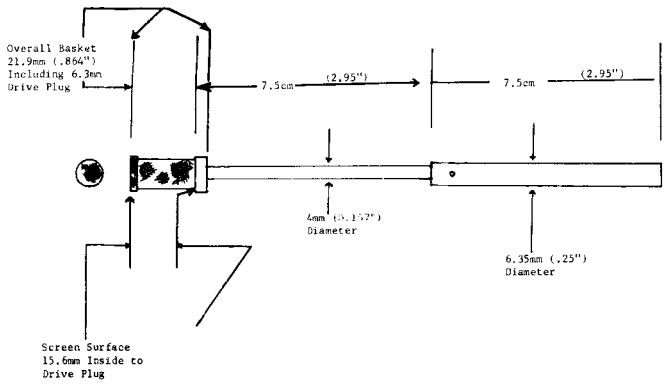

Figure 2—*Design and measurements of the miniaturized dissolution basket and shaft assembly.*

Solutions—The standard stock solution was 0.08 mg of fludrocortisone acetate/ml in acetonitrile. The working standard solutions were three aqueous solutions of standard stock solution accurately prepared to contain 3.5, 1.5, and 0.8 μg/ml.

Procedure—A system suitability test was performed by injecting the working standard solution (3.5-μg/ml level) at least three times. The relative standard deviation did not exceed 2.5%. The dissolution medium was 30 ml of distilled water, which was pipetted into the filter funnel, kept at 37 ± 0.1°, and circulated through the loop injector valve at 1.5 ml/min. One tablet was introduced into the dissolution basket, which was then immersed into the dissolution medium to a depth of 0.6 ± 0.2 cm from the bottom of the dissolution vessel.

The basket was rotated at 125 rpm; after each 15-min interval, a 20-μl aliquot of the sample solution was injected into the chromatograph by turning the injection valve from the "load" position to the "inject" position. After complete dissolution was reached, the polyethylene sampling probe was transferred from the dissolution vessel consecutively to the three working standard solutions. Each standard solution was chromatographed twice.

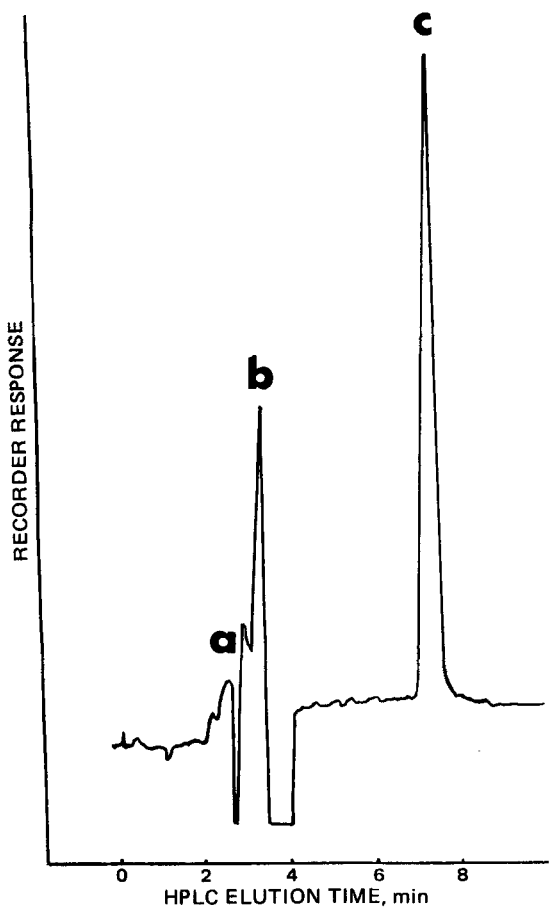

Figure 3—*Chromatogram obtained during the determination of the dissolution rate of fludrocortisone acetate tablets. Key: a, solvent; b, tablet excipients; and c, fludrocortisone acetate (~3.3 μg/ml).*

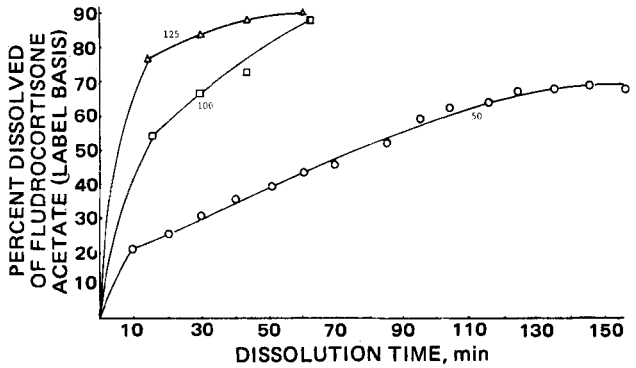

Figure 4—*Comparison between the dissolution rate of fludrocortisone acetate at different rotation speeds. Each point is an average of six tablets of the same lot. Key: ○, 50 rpm; □, 100 rpm; and △, 125 rpm.*

RESULTS AND DISCUSSION

The major difficulty in determining the dissolution rate of fludrocortisone acetate tablets was its very low concentration of active ingredient (0.1 mg/tablet). Therefore, to obtain a measurable concentration in solution, it was necessary to reduce drastically the volume of the dissolution medium recommended by the compendia. A 30-ml volume was used, which was enough to maintain sink conditions and still provide a reasonable concentration for HPLC analysis. Furthermore, the use of such a small volume of dissolution medium alleviated the homogeneity problem usually encountered with the 900-ml compendial system, especially with such low dosage forms.

A reduced version of the USP XIX dissolution system had to be designed to use such a small volume of dissolution medium. The size of the dissolution vessel can be changed on an individual basis to accommodate larger volumes of dissolution medium, depending on the solubility of the active ingredient, to ensure the maintenance of sink conditions. The use of downward direction solvent flow was necessitated by the need for simultaneous sample filtration. The prescribed slow flow rate also helped to maintain a hydrodynamic condition as close as possible to that encountered in the compendial system.

The described miniaturized dissolution assembly is generally applicable to all solid dosage forms containing very low concentrations of active ingredients, *e.g.*, estrogens, thyroxine fractions, digoxin, and digitoxin tablets. Neither the blue tetrazolium nor the nydrazid assay has enough sensitivity to measure fludrocortisone acetate in the very low concentration range encountered in this study (30% dissolution = 1 μg/ml). The 4-aminoantipyrine method described in the USP XIX for content uniformity analysis also lacks the sensitivity required for these low concentrations. The HPLC assay used in this study has a sensitivity of about 0.5 μg/ml, which is more than adequate.

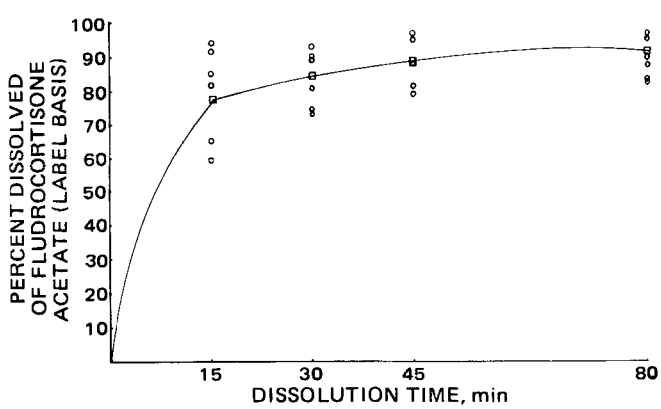

Figure 5—*Dissolution rate of six individual tablets of Lot 1 in distilled water at 125 rpm. Key: ○, individual tablet; and □, average of six tablets.*

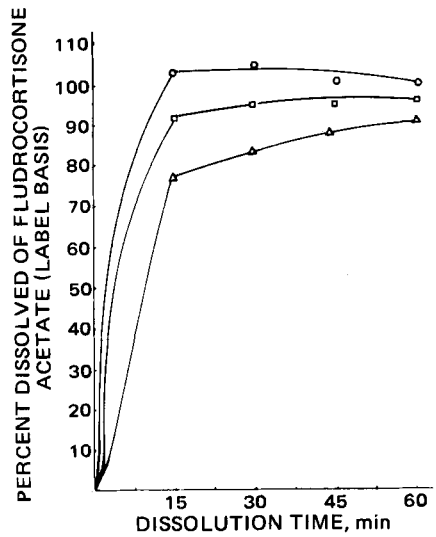

Figure 6—*Comparison between the dissolution rate of different lots of commercial fludrocortisone acetate tablets at 125 rpm in distilled water. Each point is an average of six tablets. Key: △, Lot 1; □, Lot 2; and ○, Lot 3.*

The linearity of the fludrocortisone acetate chromatographic response was checked within a concentration range of 0.8–4.0 μg/ml, equivalent to 25–120% of tablet potency. The response was linear (r = 0.999). The average relative standard deviation for triplicate injections of each standard level varied from 1 to 3%. Figure 3 shows a sample chromatogram from an actual tablet dissolution run. In studying the dissolution rate of fludrocortisone acetate tablets, three different basket rotation speeds were investigated: 50, 100, and 125 rpm. Figure 4 shows that for the same lot the dissolution rate significantly increased with an increase in basket rotation speed.

To compare the dissolution characteristics of different lots, six tablets of each of three different lots were tested in water at 125 rpm. Figure 5 shows the spread in dissolution behavior between different tablets of the same lot, and Fig. 6 compares the dissolution profiles of different lots. In all cases, DT_{50}[12] was less than 15 min while DT_{85}[13] varied between 12 and 37 min. Although the assay values of the three lots were above 95% of the label value, Lot 3 did not reach 90% dissolution even after 2 hr. These tablet-to-tablet and lot-to-lot variations in dissolution characteristics are thought to be due to differences in tablet uniformity.

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[12] DT_{50} is the time required for 50% of the label value of the drug to go into solution.

[13] DT_{85} is the time required for 85% of the label value of the drug to go into solution.

Bioavailability of Sulfonamide Suspensions I: Dissolution Profiles of Sulfamethizole Using Paddle Method

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Received September 19, 1977, from the **Department of Pharmaceutics and the* ‡*Department of Medicinal Chemistry, University of Pittsburgh, Pittsburgh, PA 15261,* and the §*Division of Biopharmaceutics, Food and Drug Administration, Washington, DC 20204.* Accepted for publication February 2, 1978.

Abstract □ A comparative bioavailability study was performed using two commercially available, chemically equivalent brands of sulfamethizole suspension. One gram of each suspension was administered to 12 different subjects following a completely randomized crossover design. Serum levels and derived pharmacokinetic parameters were compared statistically. There were no significant differences in the extent of sulfamethizole absorption from the two suspensions as evidenced by the area under the serum level–time curves. Significant differences ($p < 0.05$) in the mean serum levels at 0.5 and 0.75 hr and differences in C_{max} and t_{max} indicated that the absorption rate differed for the two products. *In vitro* tests including particle-size analysis and dissolution studies were performed. The size-frequency distribution of particles in the suspensions was studied using a resistance particle counter. The dissolution characteristics of the two products were studied using the Food and Drug Administration's paddle method and the spin-filter apparatus. Suspension A had a significantly greater amount of drug dissolved at 15 and 30 min using either method. It also had a greater percentage of particles at the smaller size range, indicating that the greater dissolution rate may be related directly to the decreased particle size. A comparison of the *in vivo* and *in vitro* results demonstrated a definite rank-order correlation between the dissolution performance of the two suspensions and the *in vivo* parameters reflecting the absorption rate. Suspension A had a greater amount of drug dissolved at 15 and 30 min and resulted in higher serum levels at 0.5 and 0.75 hr, a higher C_{max}, and a shorter t_{max}.

Keyphrases □ Sulfamethizole—bioavailability in humans and *in vitro* dissolution, two suspensions compared □ Bioavailability—sulfamethizole, two suspensions compared □ Dissolution, *in vitro*—sulfamethizole, two suspensions compared □ Antibacterials—sulfamethizole, bioavailability in humans and *in vitro* dissolution, two suspensions compared

In vitro dissolution testing can be a valuable tool for predicting or assuring the *in vivo* performance of a dosage form if appropriate correlation has been established. Several methods are employed for measuring the dissolution of the common solid dosage forms, capsules and tablets. For a drug to be absorbed, the solid dosage form must first undergo disintegration, deaggregation, and then dissolution of drug particles. The size of drug particles in the postdisintegration state affects the dissolution rate.

Suspension dosage forms correspond to the postdisin-

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