Determination of Drug Stability in Aspirin Tablet Formulations by High-Pressure Liquid Chromatography

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Abstract □ Salicylic acid and aspirin were resolved from the other salicylates in thermally degraded multicomponent tablets and determined quantitatively. The analytical method involved wetting the powdered tablet with acetic acid and diluting with chloroform to extract the drug components. Automated high-pressure liquid chromatographic analyses of filtered extracts were performed on a silica column with a mobile phase of acetic acid in heptane. The method was capable of resolving the major thermally induced transformation products in tablet formulations. It was sensitive to ~0.1 mg of salicylic acid/tablet. Good agreement with the compendial method for free salicylic acid was obtained.

Keyphrases □ Salicylic acid—simultaneous high-pressure liquid chromatographic analysis with aspirin in multicomponent tablets □ Aspirin—simultaneous high-pressure liquid chromatographic analysis with salicylic acid in multicomponent tablets □ High-pressure liquid chromatography—analysis, salicylic acid and aspirin multicomponent tablets □ Analgesics—aspirin and salicylic acid, high-pressure liquid chromatographic analysis in multicomponent tablets

Of the numerous methods reported for the determination of aspirin and salicylic acid in pharmaceutical preparations, few permit a simultaneous quantitative determination of both components in thermally degraded samples or have been evaluated as stability-indicating assay procedures. Free salicylic acid has been determined by column chromatography (1–3) and direct UV spectrophotometry (4, 5). Other analytical methods include fluorometry (6–8), GLC (9), and high-pressure liquid chromatography (HPLC) (10–12). Aspirin has been determined using spectrophotometry (13–15), GLC (16), NMR spectroscopy (17), nonaqueous titrimetry (18–20), TLC (21–23), and HPLC (24, 25). Aspirin and salicylic acid have been determined simultaneously by direct spectrophotometry (26), fluorometry (27), GLC (28, 29), IR spectroscopy (30), nonaqueous titrimetry (31), and HPLC (32–35).

This paper discusses an HPLC method developed for the simultaneous determination of aspirin and salicylic acid with the demonstrated precision and sensitivity required for assessing drug degradation. It is stability indicating for tablets containing aspirin stored at 50° or less. Aspirin stability can be determined either alone or in combination with drugs frequently present in analgesic formulations, such as propoxyphene, caffeine, and codeine.

EXPERIMENTAL

Apparatus—The high-pressure liquid chromatograph[1] was equipped with flow control, a variable-wavelength detector, automatic sampling, an integrator–recorder, and a 10-μm microparticulate silica column[2]. Sample extracts were prepared using an ultrasonic bath[3] and filtered through chloroform-resistant membranes[4] prior to analysis.

Reagents—Aspirin[5], salicylic acid[6], caffeine[7], and codeine phosphate[8] were USP grade. Acetic acid[6], methanol[6], ethyl acetate[6], and acetone[6] were reagent grade. Chloroform[9], *n*-heptane[9], and trichloroethane[9] were distilled HPLC grade.

HPLC Operating Conditions—The mobile phase was 5% (v/v) acetic acid in *n*-heptane. The flow rate was 3.5 ml/min at 30°. The UV detector was operated at 300 nm. Instrument conditions were maintained for ~0.5 hr prior to the initial injection. Fifty microliters of either the standard or sample solution was injected onto the silica column. After use, the column was prepared for storage by flushing with *n*-heptane.

Preparation of Sample Solutions—For each tablet formulation, the average tablet weight was calculated by weighing 10 tablets. These tablets subsequently were pulverized to a fine powder. A quantity of powdered sample, approximately equivalent to one average tablet weight, was weighed accurately and transferred to a 100-ml volumetric flask. With the flask in an ultrasonic bath, 5 ml of acetic acid was added to wet the entire solid material. After 30 sec in the ultrasonic bath, the contents of the flask were diluted with 70 ml of chloroform, allowed to remain another 10 min in the bath, and then diluted to volume (100 ml) with chloroform.

Preparation of Standard Solutions—A stock solution of salicylic acid was prepared by weighing accurately ~50 mg, transferring the powder to a 100-ml volumetric flask, dissolving it in chloroform, and diluting the solution to volume with chloroform. A standard solution was prepared by weighing accurately an amount of aspirin, approximately equivalent to the labeled content of one tablet, and transferring this amount to a 100-ml volumetric flask. This solid was treated with 5 ml of acetic acid and diluted with 70 ml of chloroform in the same manner and preferably at the same time as the sample preparation. A 10.0-ml aliquot of salicylic acid stock solution was added to the aspirin solution and then diluted to volume with chloroform. Changes in aliquot size or the composition of the stock solution were made to match approximately the quantity of salicylic acid present in the standard with that in the sample if extensive degradation was anticipated.

Chromatographic Separation and Analysis—A standard solution was chromatographed in duplicate to check for system suitability. In a typical chromatogram, the k' values for salicylic acid and aspirin were ~2 and 9, respectively. The resolution factor for salicylic acid and aspirin was preferably not less than 7 to allow for resolution from the possible degradation products. The tailing factor was <1.5 for each component.

Prior to injection, samples were filtered into appropriate vials. A standard was prepared simultaneously with two or three samples to permit frequent calibration. The quantity of salicylic acid and aspirin was determined by comparing peak areas of the sample and the external standard. The content of salicylic acid was expressed as a percentage of the label claim for aspirin.

Identification of Degradation Products—About 100 mg of an aspirin sample stressed at 95° for 5 weeks was dissolved in ~1 ml of chloroform and applied to a silica gel TLC plate[10] predeveloped with methanol. The plate was developed three times with 30% acetic acid in heptane, dried, and examined under UV illumination. The band (R_f 0.6) between salicylic acid and the slower moving components was scraped from the plate, extracted with 5% acetic acid in chloroform, evaporated to dryness,

[1] Model 830 high-pressure liquid chromatograph with model 833 flow controller, model 834 automatic sampler, and model 837 variable-wavelength spectrophotometer, DuPont Instruments, Wilmington, Del., or model 1084 B high-pressure liquid chromatograph with variable-wavelength detector, Hewlett-Packard, Avondale, Pa.

[2] Partisil 10, 4.6 × 250 mm PXS 1025, Whatman, Clifton, N.J.

[3] Model ME 46, Mettler Electronics Corp., Anaheim, Calif.

[4] Metricel Alpha-450, Gelman Instrument Co., Ann Arbor, Mich.

[5] Dow Pharmaceuticals, Richmond Hill, Ontario, Canada.

[6] American Chemicals Ltd., Montreal, Quebec, Canada.

[7] Pfizer Co., Pointe Claire, Quebec, Canada.

[8] F. E. Cornell and Co. Ltd., Montreal, Quebec, Canada.

[9] Fisher Scientific Co., Fair Lawn, N.J.

[10] Silica gel GF, 1-mm precoated TLC plates, Analtech Inc., Newark, Del.

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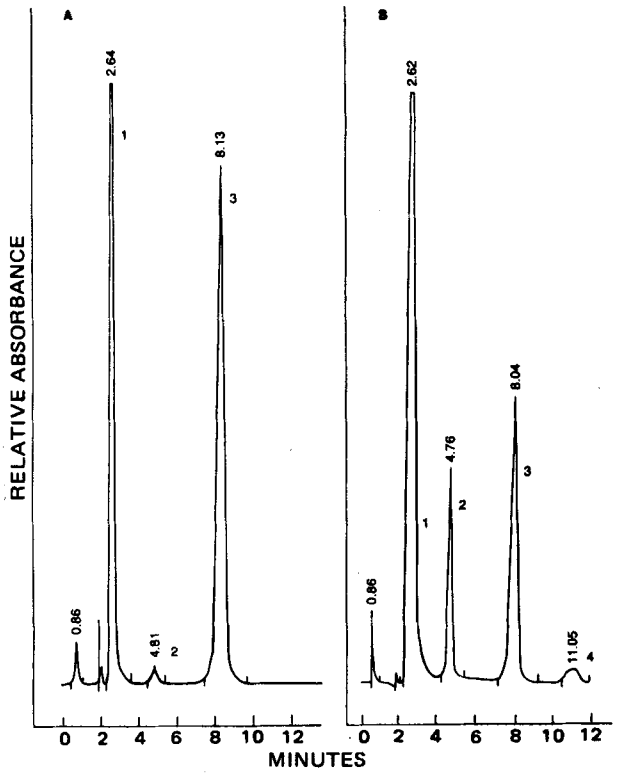

Figure 1—*High-pressure liquid chromatograms of aspirin tablet formulations stressed at 50° for 16 weeks (A) and at 80° for 2 weeks (B). Key: 1, salicylic acid (k′ = 2.2); 2, salicylsalicylic acid (k′ = 4.9); 3, aspirin (k′ = 9.0); and 4, acetylsalicylsalicylic acid (k′ = 12.6). The column was Partisil 10 silica, and the mobile phase was 5% acetic acid in heptane.*

and dried in a vacuum desiccator over sodium hydroxide pellets. About 10 mg was recovered from six plates. Under HPLC analytical conditions, this component eluted with a k' value of 4.9, which is identical to that of the unknown compound that eluted immediately after salicylic acid (Fig. 1). The structure corresponded to salicylsalicylic acid; NMR (deuterochloroform): δ 10.4 (1H), 8.4–7.9 (2H), and 7.7–6.8 (6H), no methyl protons; mass spectrum: major ions at m/z 258 ($M^{+\cdot}$), 121, and 120; UV (5% acetic acid in heptane): λ_{max} 312 nm.

The third degradation product (k' = 12.6 in Fig. 1) was not successfully isolated from TLC plates because of contamination from aspirin and other degradation products with lower R_f values. Instead, 10 fractions were collected from a concentrated solution on the chromatograph. A 200-μl sample of the stressed aspirin in methanol was injected. Fractions were collected, evaporated, and dried in a vacuum desiccator over sodium hydroxide pellets. The structure corresponded to acetylsalicylsalicylic acid; mass spectrum: major ions at m/z 180, 163, 138, 120, 92, and 43; UV (5% acetic acid in heptane): λ_{max} 282 and 312 nm.

RESULTS AND DISCUSSION

Quantitative extraction of aspirin and salicylic acid was not obtained with combinations of methanol and chloroform, probably because of adsorption on tablet excipients (36, 37). Successful drug release was achieved by initially wetting the pulverized sample with 98% formic acid, followed by dilution with chloroform (37). Aspirin hydrolysis was ~0.02%/min in the presence of the concentrated acid and 0.02%/hr after dilution with chloroform. Substitution of acetic acid in place of formic acid provided adequate drug release, similar hydrolysis rates, and reduced instrument corrosion. To compensate for aspirin hydrolysis in the sample, standards also were wetted with acetic acid and prepared at the same time as the samples.

To minimize aspirin hydrolysis, only nonaqueous mobile phases were investigated. With a mobile phase of 10% ethanol and 5% acetic acid in chloroform, a polar bonded phase column[11] resolved aspirin and salicylic acid in unstressed tablet formulations. However, inadequate resolution of the drug and its decomposition products occurred with thermally stressed formulations. Successful resolution of the latter was achieved on a silica column using acetic acid in heptane as the mobile phase (Fig. 1). Additional basic drug components commonly present in analgesic preparations, such as propoxyphene, caffeine, and codeine, were not eluted. Assays of thermally stressed samples containing various combinations of these drugs with aspirin did not indicate any interference by these additional drug components with the eluting salicylates. The HPLC column was regenerated periodically to remove the retained components by flushing with trichloroethane, ethyl acetate, acetone, methanol, and

[11] Partisil 10 PAC, 4.6 × 250 mm PXS 1025 PAC, Whatman, Clifton, N.J.

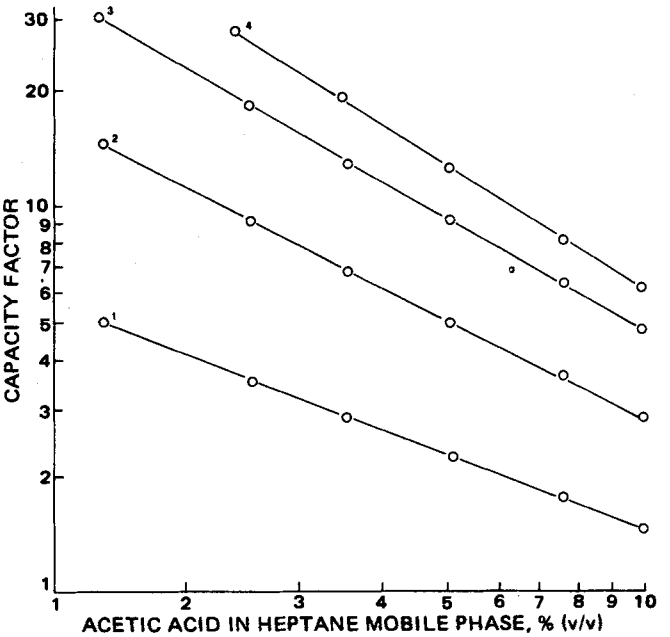

Figure 2—*Logarithmic correlations of capacity factors* versus *the percent of acetic acid in the mobile phase for the four salicylates. Key: 1, salicylic acid; 2, salicylsalicylic acid; 3, aspirin; and 4, acetylsalicylsalicylic acid.*

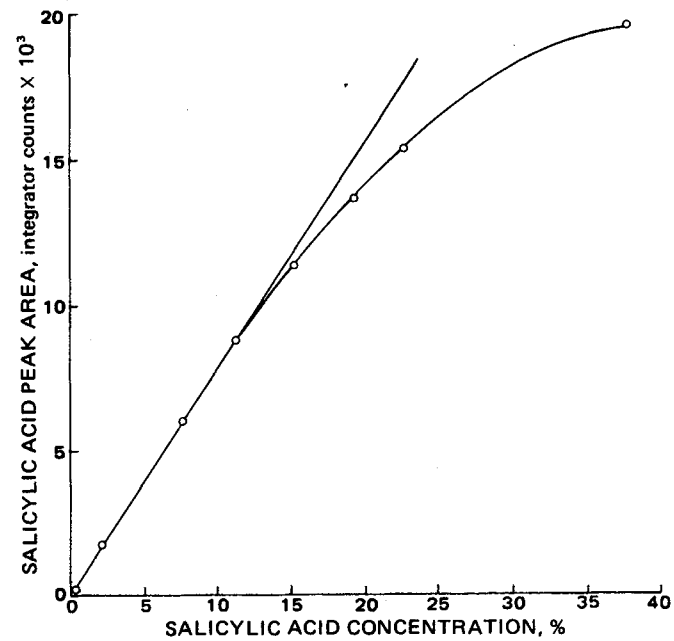

Figure 3—*Salicylic acid peak areas* versus *the percent of salicylic acid in standard solutions containing an increasing concentration of salicylic acid and a corresponding decreasing concentration of aspirin. In all cases, the percent of salicylic acid refers to the weight percent of salicylic acid expressed as a percentage of the label claim for aspirin. The aspirin label claim for all formulations was 650 mg.*

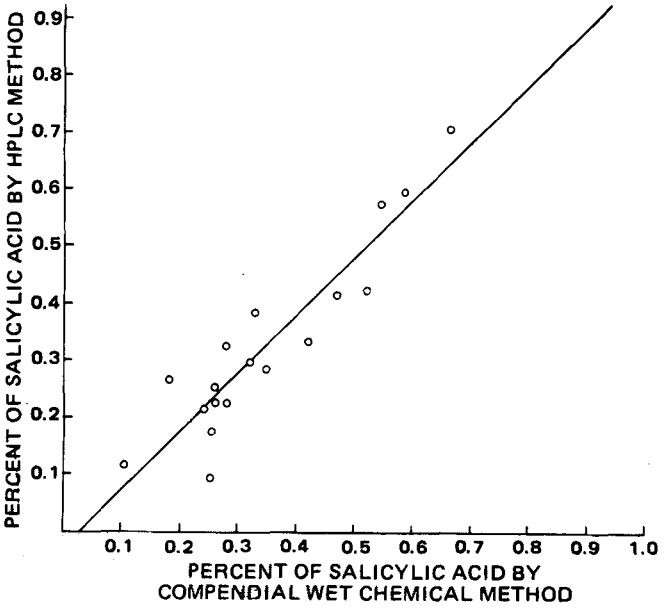

Figure 4—*Correlation of the HPLC salicylic acid results* versus *the wet chemical results. The latter method was based on the assay for free salicylic acid in USP XIX. With 18 data points, a slope of 1.0 ± 0.1, an intercept of −0.028 ± 0.039, and a correlation coefficient of 0.929 were calculated from the linear regression analysis.*

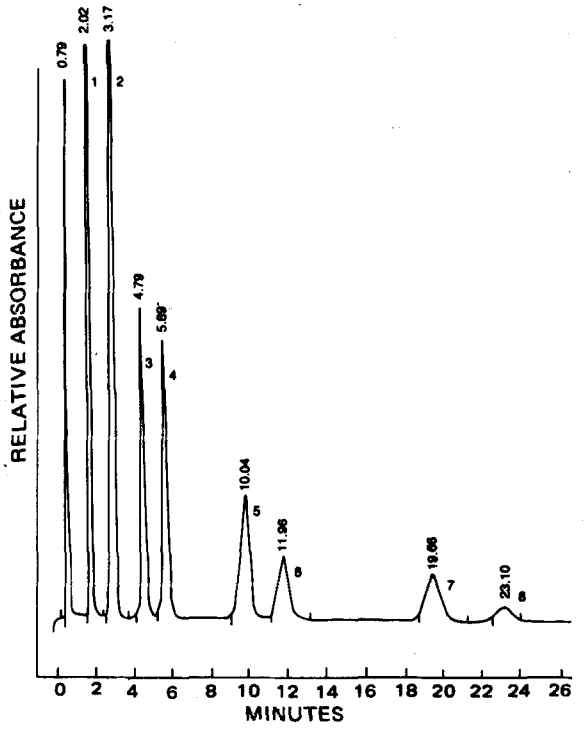

Figure 5—*High-pressure liquid chromatogram of aspirin stressed at 95° for 5 weeks showing the four salicylates in Fig. 1 (peaks 1–4) and the four additional components eluting at longer retention times (peaks 5–8). The column was Partisil 10 silica, and the mobile phase was 10% acetic acid in heptane.*

water and then reversing the sequence as recommended by the manufacturer.

Aspirin and three salicylate decomposition products were resolved with the silica column. In accelerated thermal stability trials, samples contained primarily salicylic acid with trace quantities of salicylsalicylic acid. Where salicylic acid concentrations were >3%, a third degradate, acetylsalicylsalicylic acid, was observed in trace quantities.

Although the time required to obtain a satisfactory chromatogram could be reduced by adding small amounts (1–2%) of a modifier, such as chloroform or ethyl acetate, to the mobile phase, no modifier was used because its presence caused dead volume interference with the salicylic acid. The presence of acetic acid was mandatory for the satisfactory elution of the acidic hydrogen-bonded species. Figure 2 illustrates the observed decrease in the capacity factors of the four components with increasing acetic acid concentrations in the heptane mobile phase.

A wavelength of 300 nm was selected for detecting the eluting components. It provided adequate sensitivity for salicylic acid and permitted the detection of salicylsalicylic acid and acetylsalicylsalicylic acid. Aspirin has a small absorptivity at 300 nm, but the absorbance was sufficient for satisfactory quantitative determination because of its large concentration. At 312 nm, the absorption maximum for salicylic acid, aspirin was difficult to detect.

The linearity of the detector response for aspirin and salicylic acid was investigated by preparing a series of samples containing decreasing amounts of aspirin and correspondingly increasing amounts of salicylic acid. Linear response was satisfactory for aspirin over the concentration range of 650–300 mg. Appreciable deviations from linearity were observed for salicylic acid concentrations of 15% (98 mg/tablet) (Fig. 3). Typical assay values of <5% salicylic acid (33 mg/tablet) were well within the linear range.

A small positive intercept was obtained at zero salicylic acid concentration. Based on nine data points, a linear correlation was obtained for salicylic acid peak areas *versus* percent of salicylic acid (concentration range 0–0.36%). The correlation coefficient from the regression analysis was 1.0, and the intercept was equivalent to ~0.1% salicylic acid (0.7 mg/tablet). This amount of salicylic acid probably was generated from aspirin hydrolysis during the preparation of both the sample and the standard. As a result, a significant error was introduced when employing the external standard analytical method for samples containing <0.1% salicylic acid. Samples with 0.01–0.1% salicylic acid were assayed successfully using a linear calibration technique.

The accuracy of the HPLC method was evaluated by comparing the HPLC assay results obtained from the stressed tablet formulations containing aspirin with data obtained on the same samples using the method for free salicylic acid reported by Levine (38) and later established as a compendial method (39). The linear correlation obtained (Fig. 4) approximated a theoretical slope of 45° and a zero intercept, indicating reasonable agreement between the two methods. Based on three analysts assaying a single powdered sample on 3 different days, the HPLC data in Table I indicated good precision for both aspirin and free salicylic acid.

To demonstrate that the method was capable of detecting and resolving all major degradation products in samples from accelerated thermal stability trials, a sample of pure aspirin stressed under abnormally severe conditions (95° for 5 weeks) was examined by HPLC. In addition to the four previously identified components, four other unidentified compounds eluted later in the chromatogram with 10% acetic acid in the heptane mobile phase (Fig. 5). Traces of these four unknowns were detectable in formulations stressed at 80° for 2 weeks but were undetectable in samples stressed at 50° for 16 weeks. Only the three identified components contributed significantly to total drug degradation in the accelerated stability samples stressed at or below 50°. If the samples were exposed to more severe conditions or contained abnormal amounts of salicylic acid, increasing the acetic acid concentration in the mobile phase reduced the peak broadening and enhanced the detection of these late-eluting components.

Table I—Assay Results for Aspirin and Salicylic Acid from a Study of the HPLC Analytical Precision

	Aspirin, % of label claim	Salicylic Acid, %[a]
	98.9	0.38
	97.7	0.38
	99.0	0.38
	100.3	0.34
	98.1	0.36
	99.6	0.39
	98.2	0.36
	101.1	0.37
	98.3	0.37
Average	99.0	0.37
SD	1.1	0.02

[a] The percent of salicylic acid refers to the weight percent of salicylic acid expressed as a percentage of the label claim for aspirin.

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Hypoglycemic Activity of Oral Hypoglycemics with Increased Hydrophilicity

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Abstract □ The effect of increasing the hydrophilicity of acetohexamide and tolbutamide with hydroxypropyl methylcellulose and methylcellulose on drug dissolution and hypoglycemic activity in rats was examined. The dissolution rate of both drugs was increased according to the type and concentration of the polymer. The oral absorption of both drugs was improved, as indicated by potentiation of the reduction in blood glucose in rats. The efficiency of the polymer in increasing the dissolution rates of the drugs correlated with the hydrophilicity of the polymer.

Keyphrases □ Hypoglycemics—improved absorption through increased hydrophilicity □ Tolbutamide—improved absorption through increased hydrophilicity □ Acetohexamide—improved absorption through increased hydrophilicity □ Absorption—tolbutamide and acetohexamide, improved absorption through increased hydrophilicity

Certain oral hypoglycemics, *e.g.*, acetohexamide and tolbutamide, are poorly water soluble and have irregular dissolution rates (1–3). Coprecipitation with povidone and solid dispersion of tolbutamide in polyethylene glycols have been utilized to increase the dissolution rate (4, 5) and bioavailability (5, 6). Inclusion of acetohexamide in cyclodextrin enhanced its dissolution and hypoglycemic activity (3).

The increased hydrophilicity of drugs, *i.e.*, the conversion of the hydrophobic surface of the drug to a hydrophilic one through treatment with a film-forming water-soluble polymer, increased the dissolution of hexobarbital (7) and the bioavailability of phenytoin (8) and griseofulvin (9). The polymers used were methylcellulose, hydroxyethyl cellulose (7), and hydroxypropyl cellulose (9).

The objective of the present study was to investigate the

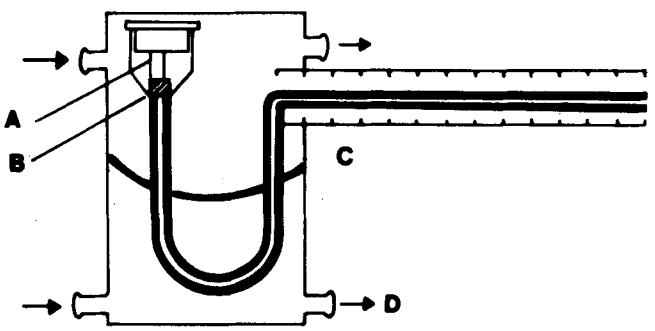

Figure 1—*Apparatus for measuring the uptake of dissolution fluid by drug disks. Key: A, disk stager; B, drug disk; C, graduated pipet; and D, water bath.*

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