Identification and Quantitation of Impurities in Dapsone Preparations

G. R. GORDON ˣ, D. C. GHOUL, and J. H. PETERS

Abstract □ Chromatographic and fluorometric procedures were developed to isolate and quantitate small amounts of 2,4′-diaminodiphenyl sulfone and 4-aminodiphenyl sulfone in pharmaceutical preparations of the antileprosy drug 4,4′-diaminodiphenyl sulfone (dapsone). Identification was accomplished by comparison with authentic compounds employing UV absorption, fluorometry, and mass spectrometry in addition to TLC and high-pressure liquid chromatography.

Keyphrases □ Dapsone—isolation, identification, and quantitation, pharmaceutical preparations □ TLC—determination, dapsone impurities in pharmaceutical preparations □ High-pressure liquid chromatography—determination, dapsone impurities in pharmaceutical preparations

Dapsone (4,4′-diaminodiphenyl sulfone, I) has emerged as the most efficacious drug in the therapy of leprosy (1). It is also of value in the suppression of chloroquine-resistant malaria (2, 3).

During studies on the purity of I, some samples were found to be contaminated[1] with 2,4′-diaminodiphenyl sulfone (II), 4-aminodiphenyl sulfone (III), and 4-amino-4′-chlorodiphenyl sulfone (IV). The purpose of this study was to isolate, identify, and quantitate any such impurities in several pharmaceutical preparations of I. These preparations were being used at that time by various clinical collaborators for the treatment of leprosy patients.

EXPERIMENTAL

Reagents and Standards—All reagents were of analytical grade except ethylene dichloride, which was purchased as a high purity solvent[2]. Compound I was technical grade[3] purified by crystallization from 95% ethanol. Compounds II–IV were used as received[1].

TLC and Microbore Column Chromatography—One dapsone tablet of the various pharmaceutical preparations (Table I) was triturated in 100 ml of 70% ethanol, and the insoluble excipients were removed by centrifugation at 2600×*g*. An aliquot of the clarified solution (containing approximately 250 μg of I) was streaked on a 250-μm thick silica gel plate[4]. The chromatogram was developed with chloroform. After air drying, the plate was viewed with a shortwave (254 nm) UV lamp[5]. The plate was then sprayed with a solution of 1 g of *p*-dimethylaminobenzaldehyde in 100 ml of ethanol containing 1.0 ml of concentrated hydrochloric acid.

Microbore column chromatography was performed using a glass column, 300 × 2.75 mm i.d.[6]. The column was slurry packed in small increments with silica gel[7] in ethyl acetate. A mixture of chloroform–carbon tetrachloride (70:30 v/v) was then pumped[8] through at a rate of 0.65 ml/min and 35.15 kg/cm² (500 psi). The absorbance of the column effluent liquid was measured at 280 nm using a UV monitor[9].

For chromatography, aliquots of each tablet solution (equivalent to 300 μg of I) were evaporated to dryness at 50° under a stream of nitrogen[10]. The residue was dissolved in 100 μl of chloroform, and 50 μl was injected into the solvent stream of the microbore column using a pneumatically actuated valve[11]. Individual fractions (≃0.3 ml) containing II and III were collected manually every 30 sec. After 42 fractions were collected, an additional 30-ml aliquot of the effluent liquid containing I was collected in a 50-ml volumetric flask. The latter collection was subsequently adjusted to 50 ml with additional solvent.

The first 42 fractions and 50 μl of the last collection were evaporated to dryness using nitrogen as previously described. To each residue was added 3 ml of ethylene dichloride, and the relative fluorescence was determined[12]. Fractions 1–24, containing III, were measured at 295/405 nm (activation/emission wavelengths); fractions 25–42, containing II, were measured at 295/375 nm; and the aliquot containing I was measured at 285/350 nm. The quantity of each compound was calculated by comparison to reference standards prepared for each chromatographic separation. Recoveries of the reference compounds from the chromatographic column were quantitative.

Spectrometry—UV spectra[13] of II and III isolated from dapsone preparations were obtained by pooling the respective fractions from several separations, evaporating the samples to dryness, and dissolving the residue in 95% ethanol. Reference spectra of authentic II and III were obtained in the same solvent. The fluorescent activation and emission spectra of II and III isolated by column chromatography were obtained from small aliquots of the ethanolic solutions used for UV spectrometry. After evaporating the ethanol, the residue was dissolved in ethylene dichloride. Similar spectra were obtained for authentic II and III. The mass spectra of II and III (authentic samples and isolated compounds) were obtained by subjecting samples to GC and then introducing the pure sulfone into the mass spectrometer[14] as it emerged from the gas chromatograph.

RESULTS

After TLC, Compounds I, II, III, and IV exhibited R_f values of 0.00, 0.11, 0.17, and 0.20, respectively. Compounds I and II exhibited a blue fluorescence, and Compounds III and IV yielded a yellowish-blue fluorescence under UV light. All yielded a yellow-to-orange color with the spray reagent. When aliquots of the various preparations of I were chromatographed, only bands corresponding to I, II, and III were detected. The retention times of I, II, and III after column chromatography were 44, 14, and 8 min, respectively. Each compound exhibited a linear relationship between peak height and quantity injected over the range of 0.5–2.0 μg. Only three peaks were found in the pharmaceutical preparations tested; they exhibited retention times identical to those of I, II, and III.

Because the fluorescence of all sulfones was considerably greater than their UV absorption per unit weight, all quantitative estimations were based upon fluorescent measurements. Figure 1 shows a

[1] P. Lim, Pharmaceutical Analysis Department, Stanford Research Institute, Menlo Park, Calif., personal communication.
[2] Burdick and Jackson Laboratories, Muskegon, Mich.
[3] Merck and Co., Rahway, N.J.
[4] E. Merck, Darmstadt, Germany.
[5] Model UVS-11, Ultra-Violet Products, Inc., San Gabriel, Calif.
[6] Chromatronix, Inc., Berkeley, Calif.
[7] Silica gel 7, J. T. Baker Co., Phillipsburg, N.J.
[8] Milton-Roy minipump, Milton Roy Co., St. Petersburg, Fla.
[9] Model UA-2, Instrumentation Specialties Co., Lincoln, Nebr.
[10] N-Evap model 105, Organomation Associates, Shrewsbury, Mass.
[11] Model SVA-8031, Chromatronix, Inc., Berkeley, Calif.
[12] Aminco-Bowman spectrophotofluorometer, American Instrument Co., Silver Spring, Md.
[13] Model 220 spectrophotometer, Gilford Instrument Laboratories, Oberlin, Ohio.
[14] Model 9000 gas chromatograph–mass spectrometer, LKB Instruments, Rockville, Md.

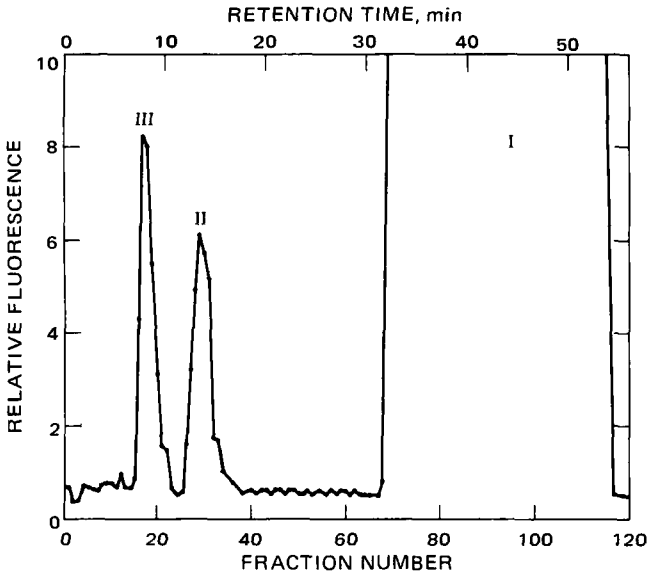

Figure 1—*Typical chromatographic separation and fluorometric quantitation of I, II, and III found in a pharmaceutical preparation of dapsone. The fluorescence of fractions 14–24 was determined at 295/405 nm (activation/emission wavelengths), of fractions 25–42 at 295/375 nm, and of fractions 67–116 at 285/350 nm.*

typical elution pattern of a dapsone preparation when the fluorescence of each fraction was measured. During each separation, I was always present in large amounts (150 μg) so the elution volume containing I (fractions 65–120) was routinely collected as a single sample beginning after fraction 42.

Figure 2 shows calibration curves obtained for I, II, and III in ethylene dichloride *via* fluorometry. The sensitivities (fluorescent intensity units per nanogram per milliliter) were 0.372, 0.236, and 0.173, respectively. Coefficients of variation for these sensitivity factors were 4.6, 3.9, and 6.3%, respectively, for 1 week of daily determinations. The lower limit of detection of II and III in 150 μg of I was about 0.05%.

Compound I exhibited fluorescence maxima of 285/350 nm (activation/emission) in ethylene dichloride (4). The recovery of I from the six pharmaceutical preparations averaged 96.9% (range 91.8–99.2%).

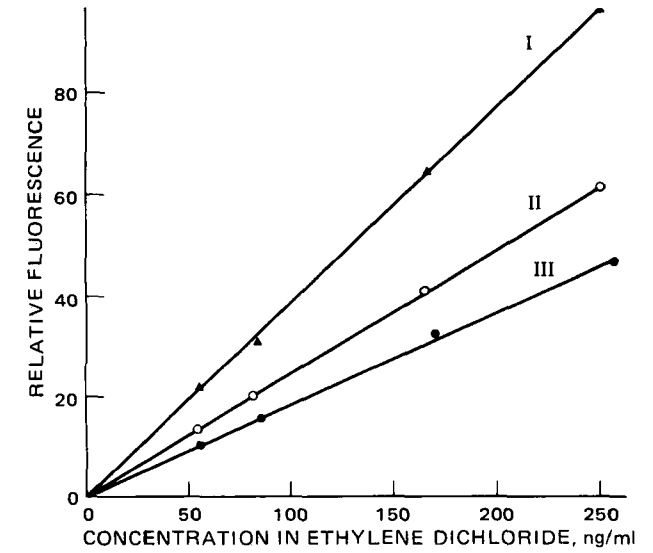

Figure 2—*Fluorescence calibration curves for I, II, and III. The activation/emission wavelengths were 285/350, 295/375, and 295/405 nm, respectively.*

Table I—Quantities of Impurities in Dapsone Preparations

Manufacturer[a]	Formulation	Percent of Impurity[b] II	III
1	25-mg tablet	0.16	0.64
1	50-mg tablet	0.31	0.76
2	25-mg tablet	0.49	0.65
2	50-mg tablet	0.80	0.99
3	100-mg tablet	0.35	0.51
4	100-mg tablet	0.20	0.27
5	Crystalline	3.3	2.7
6	Crystalline, technical grade	<0.05	0.70
6	Crystalline, technical grade, recrystallized	<0.05	0.18

[a] 1, P.C.B., Brussels, Belgium; 2, Laboratoire Lafran, Paris, France; 3, Ayerst Laboratories, New York, N.Y.; 4, Imperial Chemical Industries, Cheshire, England; 5, K&K Laboratories, Hollywood, Calif.; and 6, Merck & Co., Rahway, N.J. [b] Relative to the quantity of dapsone.

Compound II exhibited a UV_{max} at 280 nm, and the uncorrected maximum fluorescent wavelengths were 295/375 nm. The characteristics of the peak eluting at 14 min were identical to II, and its mass fragmentation spectrum was also identical to II. Since two other isomers of I, 3,4′- and 3,3′-diaminodiphenyl sulfone, yield mass spectral fragmentation patterns significantly different from I and II, possible confusion among these isomers and I and II is eliminated.

Compound III exhibited a UV_{max} at 290 nm, and its fluorescent characteristics were 295/405 nm. The peak that eluted at 8 min yielded these same characteristics in addition to exhibiting the same mass fragmentation pattern as authentic III.

The percentages of II and III found in several preparations of I are shown in Table I.

DISCUSSION

In the six tablet preparations of I, the quantity of II averaged 0.38% while the level of III averaged 0.64%. In one crystalline preparation (not pharmaceutical grade), the amounts of II and III were found to be considerably greater. The uncrystallized technical grade preparation contained 0.70% III, and the level of II was below the sensitivity of the procedure. On recrystallization, the level of III in this preparation was reduced to 0.18%.

The antimicrobial activities of II and III were compared with I. Using an *in vitro* assay method employing *Mycobacterium* sp. 607, Colwell *et al.* (5) showed that neither compound was as effective in preventing growth at concentrations at which I was very active. Likewise, the *in vivo* mouse foot pad test system showed that II and III were inactive in suppressing the multiplication of *M. leprae*[15] wherein I is very active (6).

The therapeutic regimen for the treatment of leprosy is generally 50 or 100 mg of I/day. Thus, preparations of I containing the levels of II and III found in these studies would result in a daily ingestion of less than 1.0 mg of either II or III. Although these quantities appear to be small compared with the daily dose of I, the long-term effects may be important because therapy using I in leprosy patients usually extends for many years and, in some cases, for the lifetime of the patients.

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Rapid Fluorometric Determination of Procainamide Hydrochloride Dosage Forms

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Abstract □ A fluorometric procedure for procainamide hydrochloride was developed, and it offers improvements in ease, speed, and sensitivity over the official method. The new procedure is based on the reaction with fluorescamine in aqueous medium at pH 7.5 to form a fluorophore, with activation and emission wavelengths of 400 and 485 nm, respectively. The fluorescence is linear (r = 0.999) over the 0.04–1-μg/ml concentration range and is stable for at least 2 hr. Recovery data appeared to be accurate, quantitative, and reproducible. The overall recovery was 99.8% with a standard deviation of ±1.14 (n = 5). The method was successfully applied to commercially available dosage forms.

Keyphrases □ Procainamide hydrochloride—rapid fluorometric determination, bulk and dosage forms, fluorescamine reagent □ Fluorometry—analysis, procainamide hydrochloride dosage forms □ Fluorescamine—reagent in fluorometric analysis of procainamide hydrochloride dosage forms

The USP XVIII (1) assay procedure for procainamide hydrochloride capsules is time consuming, since it is based on a prior separation of the drug from the dosage form followed by a diazotization titration of the compound with standard sodium nitrite at low temperatures and starch iodide paper as the external indicator. This method is subject to variations between individuals in determination of the end-point. The assay method (2) for procainamide hydrochloride injections is also lengthy, since the sample must be evaporated and dried before the drug can be analyzed by nonaqueous titrimetry with standard perchloric acid.

Recently, it was reported (3–5)[1] that 4-phenylspiro[furan-2(3*H*),1′-phthalan]-3,3′-dione (fluorescamine) is a reagent for the detection of primary amines in the picomole range. Its reactions with amines is almost instantaneous at room temperature in aqueous solutions. The resulting fluorophores are fluorescent, whereas the reagent and its degradation products are nonfluorescent. This report presents a sensitive, simple, and accurate method for the assay of procainamide hydrochloride and its dosage forms with fluorescamine in an aqueous medium.

EXPERIMENTAL

Instruments—The following were used: a fluorescence spectrophotometer[2] with 1-cm cells, a mixer[3], and an analytical balance[4]. The response of the fluorescence spectrophotometer was calibrated daily, using a 0.5-μg/ml quinine sulfate solution in 0.1 *N* sulfuric acid, at activation and emission wavelengths of 350 and 445 nm, respectively.

Materials and Reagents—The following were used: procainamide hydrochloride[5], acetone[6], a 0.15% (w/v) solution of fluorescamine[7] in acetone, and pH 7.5 phosphate buffer. This buffer solution was prepared by adding 40.8 ml of 0.2 *M* sodium hydroxide to 50.0 ml of 0.2 *M* monobasic potassium phosphate in a 200-ml volumetric flask and diluting to volume with water. The pH of the resulting buffer solution was checked with a pH meter. All chemicals were analytical grade.

Preparation of Standard Curve—A stock solution of procainamide hydrochloride was prepared by dissolving 50.0 mg of the compound in 1000 ml of water. Further dilutions were made to obtain procainamide hydrochloride standard solutions containing 0.25, 0.5, 1.0, 1.5, 2.0, and 2.5 μg/ml. One milliliter of each solution was utilized for fluorescence development as described under *Assay Procedure for Procainamide Hydrochloride*. The stock and standard solutions were stable for at least 3 weeks.

Assay Procedure for Procainamide Hydrochloride—Pipet 1.0 ml of an aqueous solution containing 0.25–2.5 μg of procainamide into a 25 × 200-mm test tube. To this solution, add 1.0 ml of phosphate buffer (pH 7.5), and place the test tube on a vortex mixer. While shaking the tube vigorously, add 0.5 ml of the fluo-

[1] After completion of this work, while this article was being prepared, a paper entitled "Spectrophotofluorometric Analysis of Procainamide and Sulfadiazine in Presence of Primary Aliphatic Amines Based on Reaction with Fluorescamine," by J. M. Sterling and W. G. Haney [*J. Pharm. Sci.*, **63**, 1448(1974)] was published.

[2] Model 204, Perkin-Elmer Corp., Norwalk, Conn.

[3] Vortex-Genie, Scientific Industries, Springfield, Mass.

[4] Mettler type H-18, Mettler Instrument Corp., Princeton, N.J.

[5] E. R. Squibb & Sons, Princeton, N.J.

[6] Spectranalyzed grade, Fisher Scientific Co., Pittsburgh, Pa.

[7] Roche Diagnostics, Nutley, N.J.