Simultaneous Automated Determination of Free and Total Sulfisoxazole and Sulfamethoxazole in Plasma and Urine

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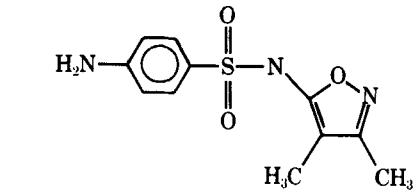
Abstract □ A fully automated method for the determination of sulfisoxazole, N_4-acetylsulfisoxazole, sulfamethoxazole, and N_4-acetylsulfamethoxazole in human plasma and urine was developed. Untreated plasma is analyzed by automation of dialysis, hydrolysis, color development, and quantitation. The method has a sensitivity limit of 2 μg/ml of plasma and has been used successfully to determine sulfonamide levels following administration of sulfisoxazole and a combination drug product containing sulfamethoxazole and trimethoprim in humans. Samples are processed at the rate of 40 per hour, with a minimum of sample handling, data reduction, and materials.

Keyphrases □ Sulfisoxazole—automated analysis, human plasma and urine, with sulfamethoxazole □ Sulfamethoxazole—automated analysis, human plasma and urine, with sulfisoxazole □ Automated analysis—sulfisoxazole and sulfamethoxazole, human plasma and urine □ Antibacterial agents—sulfisoxazole, sulfamethoxazole, automated analysis, human plasma and urine

The ability to analyze numerous biological specimens rapidly has been the goal of many laboratories responsible for the routine application of analytical procedures. Samples resulting from bioequivalency and pharmacokinetic studies have placed increased demands on laboratories in recent years, in part due to increased drug regulatory requirements and drug development programs.

The sulfonamides have been determined in biological fluids by modifications of the Bratton–Marshall manual procedure since its publication in 1939 (1). All of these manual methods involve time-consuming sample preparations and sequential additions of reagents to form the chromophore, which is then measured spectrophotometrically. Other analytical methods for sulfonamide determination in various media including biological fluids, are fluorometry (2), TLC (3), GLC (4–6), and high-pressure liquid chromatography (HPLC) (7, 8). Although these methods often provided improved sensitivity and specificity when compared to the nonspecific, although sensitive, colorimetric assay, they also involved additional sample preparatory steps, including solvent extractions, derivatization, evaporation, sample injection, and quantitation.

The need to process numerous samples in a single workday led to the development of the automated method described here. The procedure allows for the simultaneous determination of "free" [1] sulfisoxazole (I) and "total" [2] sulfisoxazole [predominantly N_4-acetylsulfisoxazole (II)] or sulfamethoxazole (III) and its major plasma metabolite, N_4-acetylsulfamethoxazole (IV), in plasma and urine by employing the principles of continuous-flow analysis, with emphasis on reliability. Although use of continuous-flow systems is not unique, this procedure permits direct plasma analysis with no prior sample manipulation and utilizes automated dialysis, sample hydrolysis, and direct concentration readout.



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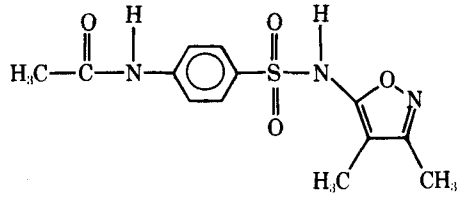

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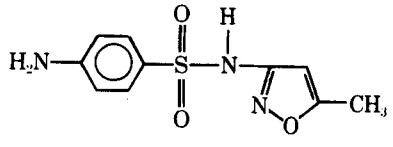

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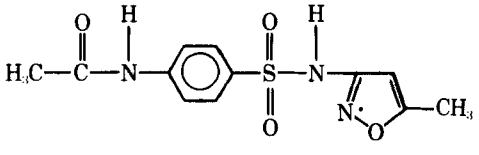

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BACKGROUND

The introduction of automated analytical equipment based on continuous-flow principles and techniques in 1957 by Skeggs (9) and subsequent application of such techniques to sulfonamides made possible the rapid processing of numerous samples. Falk and Kelly (10) described a procedure for measuring free sulfisoxazole levels in dog plasma following drug administration. The automated procedure is adequate for dog plasma sulfonamide levels since that species does not acetylate sulfonamides. It would be applicable to human plasma provided only free sulfonamide levels were required. In pharmacokinetic and bioavailability studies in humans, however, both free and total sulfonamide levels are needed. The plasma levels of both intact sulfonamide and its N_4-acetylated metabolite must be measured in renal insufficiency patients so that changes in the biotransformation and elimination of these compounds can be detected.

A previously reported semiautomated procedure (11) for determining free and total sulfisoxazole levels in human plasma required manual hydrolysis of protein-free filtrates of whole blood or plasma prior to automated colorimetry. A more recent procedure (12) for determining

[1] "Free" sulfonamide levels reflect those sulfonamides that react with the Bratton–Marshall reagents without hydrolysis. They reflect not only intact sulfonamide but any sulfonamide metabolite that still possesses a free primary aromatic amine group, *e.g.*, N_1- or ring N-glucuronides and possibly sulfanilamide.

[2] "Total" sulfonamide levels include all free components plus all metabolites that, upon acid hydrolysis, yield a substance capable of producing the Bratton–Marshall chromophore. The N_4-acetyl sulfonamide derivative is usually the major metabolite in humans. Generally, the sum of all of these components is expressed in equivalents of the intact drug.

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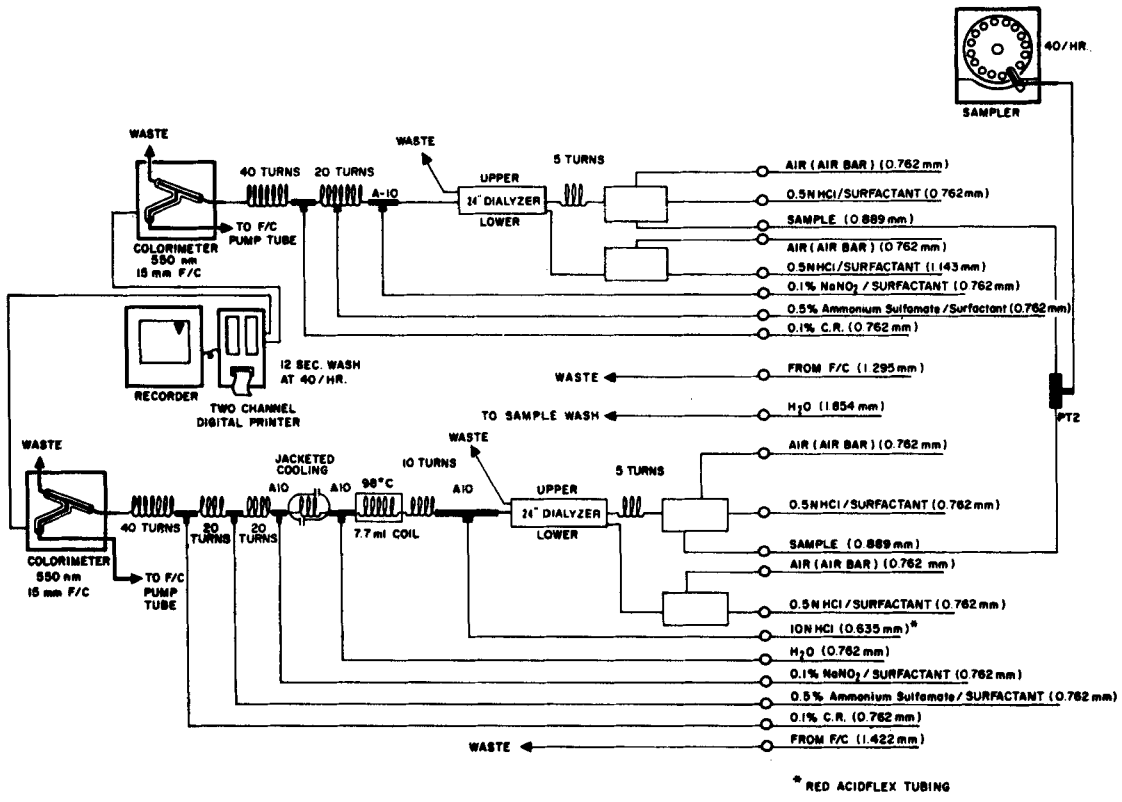


Figure 1—*Manifold for simultaneous automated determination of free and total sulfonamides in plasma and urine. Pump tubing, with size indicated, was all Tygon, except where noted.*

sulfamethoxazole still required manual sample preparation and hydrolysis prior to automation to quantitate the N_4-acetylated component.

The fully automated system described here is capable of simultaneously measuring free and total sulfisoxazole[3] or sulfamethoxazole[4] in undiluted plasma and in diluted urine. The sensitivity requirements for the determination of I and III in human plasma depend on the dose administered and typically range from 2 to 200 μg/ml of plasma during 24 hr following a single oral 2.0-g dose in normal subjects (13, 14). The automated system has a sensitivity limit of ~2 μg of I or III/ml of plasma, is precise, and can accelerate analysis greatly with a minimum of sample handling.

EXPERIMENTAL[5]

Reagents—All chemicals were reagent grade: hydrochloric acid, 0.5 *N* containing 1 ml of surfactant[6]/liter; hydrochloric acid, 10 *N*; sodium nitrite, 0.1% aqueous[7], containing 0.5 ml of surfactant[6]/liter; ammonium sulfamate, 0.5% aqueous[7], containing 0.5 ml of surfactant[6]/liter; *N*-(1-naphthyl)ethylenediamine dihydrochloride, 0.1% aqueous[7]; methanol; and concentrated ammonium hydroxide.

Standards—Compounds of pharmaceutical grade purity (>99%) were used to prepare the analytical standards: sulfisoxazole (I) [N_1-(3,4-dimethyl-5-isoxazolyl)sulfanilamide], mol. wt. 267.31; N_4-acetylsulfisoxaole (II), mol. wt. 309.35; sulfamethoxazole (III) [N_1-(5-methyl-3-isoxazolyl)sulfanilamide], mol. wt. 253.28; and N_4-acetylsulfamethoxazole (IV), mol. wt. 295.32.

Standard Solutions for Plasma Analysis—Solutions of I–IV were prepared as follows. Ten mg of I and III and 11.6 mg of II and IV were weighed into separate 10-ml volumetric flasks. The compounds were dissolved in absolute methanol to yield 1 mg of I and III/ml and solutions of II and IV equivalent to 1 mg of I and III/ml. These solutions were used to prepare internal standards of I–IV in plasma.

Standard Solutions for Urine Analysis and Aqueous Standard Curves—Aqueous stock solutions of I–IV were prepared as follows. One hundred milligrams of I and III and 116.6 mg of II and IV were weighed into four separate 100-ml volumetric flasks. About 10 ml of distilled water was added to suspend the compounds, and a sufficient amount of concentrated ammonium hydroxide was added (dropwise) to dissolve them. Solutions were brought to volume with distilled water to yield 1 mg of I and III/ml and solutions of II and IV equivalent to 1 mg of I and III/ml.

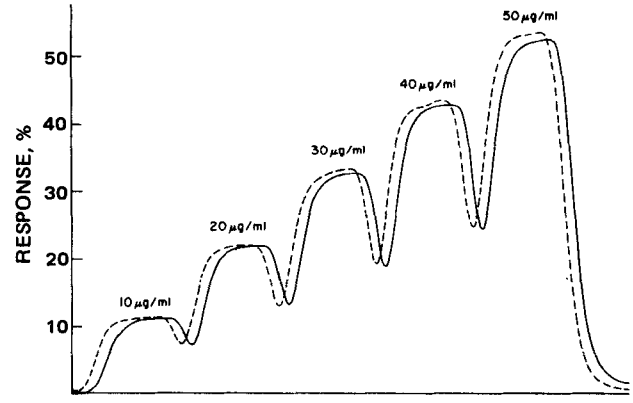


Figure 2—*Chart recordings of aqueous standard solutions of III analyzed on the automated manifold. Key: —, free channel; and - - -, total channel.*

[3] Sulfisoxazole is the active drug substance in Gantrisin, Roche Laboratories Division, Hoffmann–La Roche Inc.

[4] Sulfamethoxazole is the active drug substance in Gantanol and one active component in the combination drug Bactrim (combination of sulfamethoxazole and trimethoprim), both products of Roche Laboratories Division, Hoffmann–La Roche Inc.

[5] The equipment and instruments used were one Technicon sampler IV (Technicon Instruments Corp., Tarrytown, N.Y.); one Technicon proportioning pump, model III; two Technicon colorimeters equipped with 15-mm flowcells and 550-nm interference filters; two Technicon voltage stabilizers; two Technicon analytical cartridges containing 24-in. dialyzers with type "C" premount dialysis membranes and Auto Analyzer II glassware; and one Technicon two-pen recorder (chart speed: 60 in./hr) for use with Technicon digital printer, one Technicon modular digital printer (two channel), and one Techne heating module, modified Dri-block model DB-3 (Techne, Inc., Princeton, N.J.), machined to accept a 7.7-ml capacity Technicon coil (Fig. 1).

[6] Brij-35 (Technicon).

[7] Stored refrigerated in an amberized or light-protected container; stable for several weeks.

Table I—Standard Curve Preparation

Concentration, μg/ml	I–IV, ml
10	1.0
20	2.0
30	3.0
40	4.0
50	5.0
75	7.5
100	10.0

With the aqueous stock solutions of each standard I–IV, the aliquots shown in Table I were pipetted into four separate 100-ml volumetric flasks and diluted to volume with distilled water.

For convenience, these solutions were stored in 125-ml plastic drop-dispensing bottles[8] under refrigeration. The squeeze-type bottles provided a quick means of transferring the standards to sample cups for analysis. The dilutions were used to establish standard curves and to determine percent recovery, dialysis, and hydrolysis.

Sample Preparation—*Plasma*—An internal standard of 50 μg of sulfonamide/ml of plasma was prepared by evaporating 500 μl of the methanolic standards of I and II or III and IV to dryness in a 15-ml glass-stoppered centrifuge tube, adding 10 ml of plasma (control, drug free), and mixing well[9] to dissolve the compounds in the plasma. Unknown plasma specimens were run without dilution by transfer of ~1.5 ml into sample cups[10] and placement on the sampler.

Aqueous sulfonamide standards were run by transferring ~1.5 ml into sample cups along with the unknowns at concentrations of 10, 20, 30, 40, 50, 75, and 100 μg/ml. These standards were used to establish a curve and to determine the percent recovery of dialysis, percent hydrolysis of the 50-μg/ml internal standards, and linearity of response.

Urine—One milliliter of urine was diluted to 20 ml with distilled water, and ~1.5 ml was transferred to sample cups for analysis.

An internal standard of 50 μg of sulfonamide/ml of diluted urine was prepared by adding 1 mg (1.0 ml) of the aqueous stock standard solutions of I and II or III and IV separately to 1.0-ml urine samples (control, drug free) in a 25-ml graduated cylinder and diluting to 20 ml with distilled water.

External aqueous standards, ranging in concentration from 10 to 100 μg of sulfonamide/ml, were run along with the samples to determine percent recovery of dialysis and hydrolysis.

Unknowns that were off scale were diluted further and then were reanalyzed.

Automated Analytical Procedure—The complete automated system is diagrammed in Fig. 1. The sampler was operated at a rate of 40 samples/hr using a 12-sec wash[11]. All pump tubing lines were placed in distilled water containing 0.5 ml of surfactant[6]/liter and allowed to pump through the entire system for 5 min. Then the lines were transferred to appropriate reagent containers and pumped for at least 15 min to establish reagent baselines for each channel on the recorder.

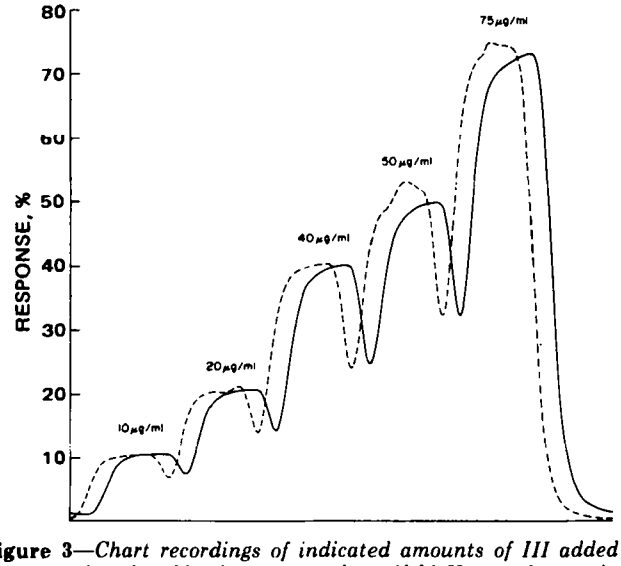


Figure 3—*Chart recordings of indicated amounts of III added to plasma and analyzed by the automated manifold. Key: —, free; and ---, total.*

Table II—Percent Dialysis, Hydrolysis, and Recovery of Compounds I–IV

Sample and Concentration Range	Percent Dialysis	Percent Hydrolysis	Percent Recovery (Overall)
10–100 μg of I/ml, aqueous	—	—	100
10–100 μg of I/ml of plasma	96.1	—	96.1
10–100 μg of II/ml, aqueous	87.3	84.8	74.0
10–100 μg of II/ml of plasma	47.2	84.8	40.0
10–75 μg of III/ml, aqueous	—	—	100
10–75 μg of III/ml of plasma	93.5	—	93.5
10–75 μg of IV/ml, aqueous	79.6	79.6	63.4
10–75 μg of IV/ml of plasma	50	79.6	39.8

Approximately 3.0 ml of the internal standard (50 μg/ml of plasma or 50 μg/ml of diluted urine) was transferred to a 3.5-ml conical base sample cup and placed on the sampler. The sampler probe was placed into the cup and allowed to sample for 2–2.5 min, introducing a continuous sample stream to permit adjustment of the "steady-state" plateau generated on the recorder for the internal standard. Day-to-day instrumental fluctuations were eliminated by adjustment of the colorimeter sensitivities for each module (free and total) to give a set recorder deflection (50% of full scale).

Once this parameter was established, the identical internal standard was placed in two or three sample cups (2.0 ml) and run with the sampler operating at a rate of 40 per hour to adjust the digital printer[11], which provided a concentration readout based on peak height of the recorder response and preset calibration of the digital printer range.

The internal standards containing 50 μg of I or 50 μg of III/ml of plasma or diluted urine served to adjust both the free and total channel (digital printer and recorder) results. In this manner, a more precise estimation of the total concentration could be attained (see *Calculations*).

The manifold was designed for the simultaneous determination of free and total sulfonamide by use of an initial sample stream splitter and parallel analytical manifolds, one for free sulfonamide (without hydrolysis) and the other for total sulfonamide (with hydrolysis) (Fig. 1). The free sample stream was adjusted so that its chart recording did not coincide with that of the total peak recording. This was accomplished by introduction of time delay coils (10 or more turns, as required) after the addition of *N*-(1-naphthyl)ethylenediamine dihydrochloride and before the colorimeter in the hydrolysis cartridge. In this manner, the free and total results associated with a particular sample were not confused. A two-pen recorder with different colored inks for each channel was used.

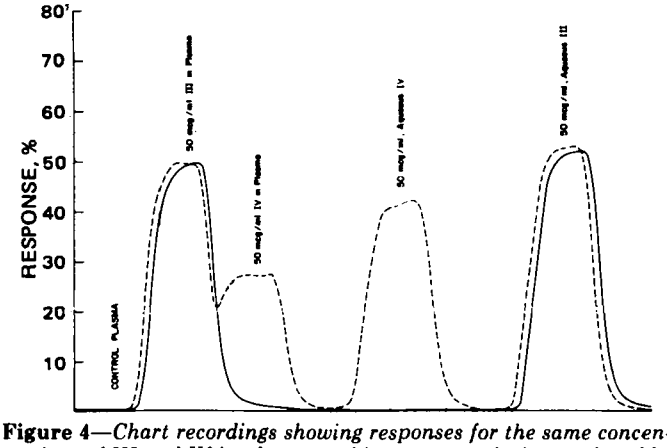


Figure 4—*Chart recordings showing responses for the same concentrations of III and IV in plasma and in aqueous solution analyzed by the automated manifold. Key: —, free; and ---, total.*

[8] Nalgene Labware, Rochester, N.Y.
[9] Vortex mixer.
[10] Technicon, polystyrene, 2-ml size, conical base or equivalent.
[11] Digital printer controlling samples and wash cycles. For digital printer operation, refer to Technicon Operations Manual TA1-170-10.

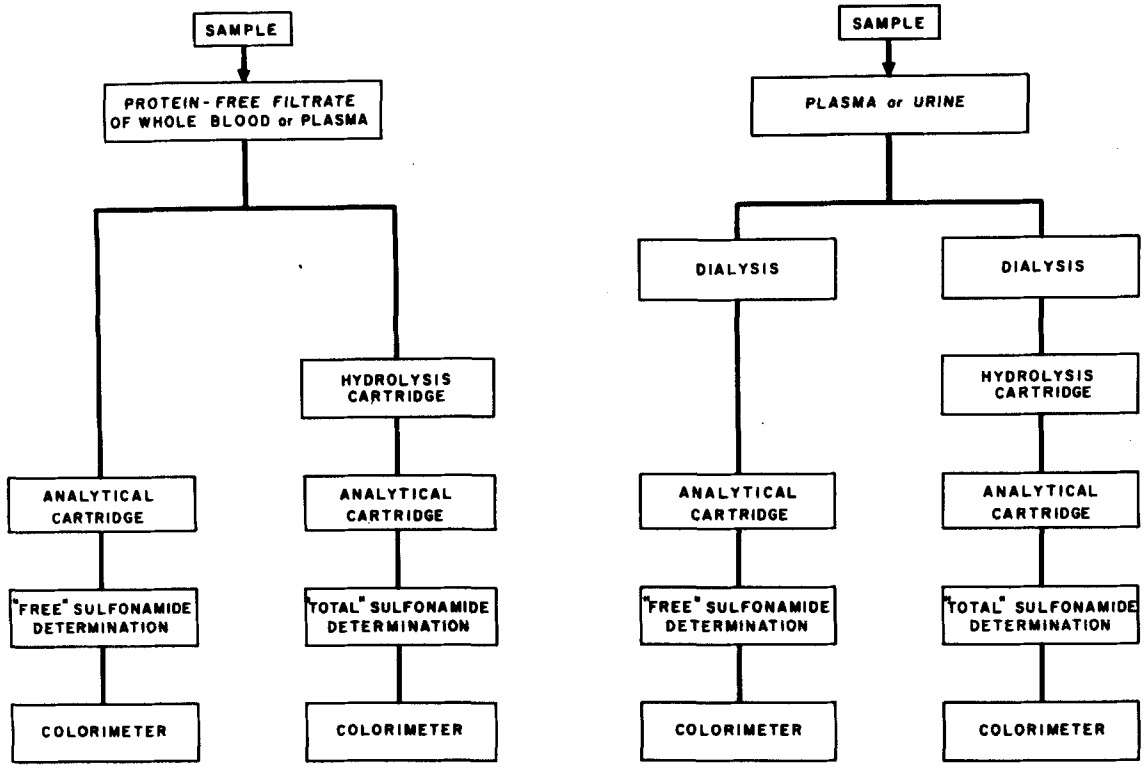


Figure 5—*Sample preparation schemes and analytical modules for automated analysis. Left: protein-free filtrates requiring a manual sample preparation step prior to automation (no dialysis). Right: untreated plasma directly analyzed (with dialysis).*

RESULTS

Standard Curves—The aqueous standards were added to individual sample cups, and their responses were used to establish a calibration curve for 10–100 μg/ml. Typical responses are illustrated in Fig. 2 for aqueous III standards in the range of 10–50 μg/ml. Typical responses of III added to plasma (10–75 μg/ml) and analyzed by the automated procedure are shown in Fig. 3. For the analysis of urine samples, the identical standards were used to establish a calibration curve.

Calculations and Determination of Percent Recovery—Calculation of plasma and urine concentrations required determination of the degree of metabolite hydrolysis to intact compound and the extent of metabolite dialysis (or recovery) from the plasma and urine matrix. These factors were determined for I–IV separately in plasma and in urine by running standards, added to plasma through the dialysis system and following them with aqueous standards of the same concentrations (Fig. 4). The percent recovery values of I–IV from plasma and the percent hydrolysis values of II to I and of IV to III are summarized in Table II.

In calculating the results from unknown specimens following dialysis and hydrolysis, it was unnecessary to introduce any correction for the free analysis since the colorimeter and digital printer were adjusted using the I or III internal standard. The total analysis represents an amount of I or III equal to the already determined free plus the amount of II or IV dialyzed, converted to free by acid hydrolysis, and measured colorimetrically following reaction. Thus, to determine the amount of N_4-acetyl metabolite in the sample, these equations are used:

$$\text{total } \mu\text{g/ml} - \text{free } \mu\text{g/ml} = \mu\text{g of II or IV/ml} \quad \text{(Eq. 1)}$$

$$(\mu\text{g of II or IV/ml})\,(\text{dialysis factor}) \times (\text{hydrolysis factor}) = \text{corrected } \mu\text{g of II or IV/ml} \quad \text{(Eq. 2)}$$

Assay Specificity—Interferences from drugs with primary aromatic amine groups other than the sulfonamides or their metabolites can produce false-positive values. In controlled studies, *e.g.*, bioequivalency or pharmacokinetic studies, this interference can be held to a minimum or eliminated entirely by screening of individuals. In such studies, the classical free and total sulfonamide measurements in plasma represent primarily intact parent drug (free) and N_4-acetyl metabolite (total − free), as demonstrated by Rieder (15, 16) using a procedure developed to quantitate the intact, active sulfonamide component in plasma.

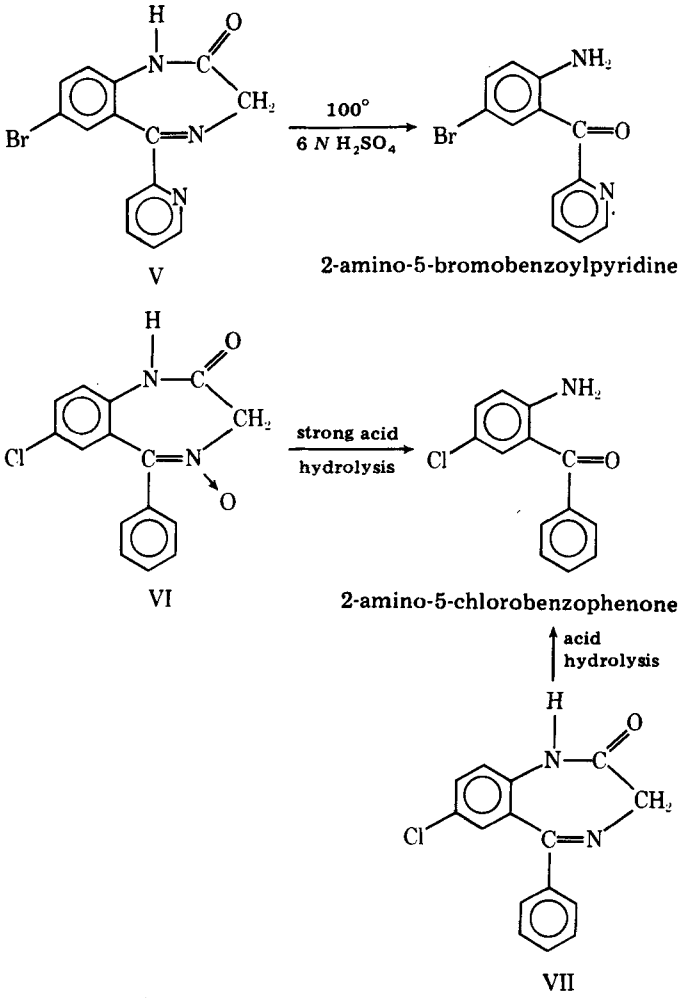
In another study, comparison of results by TLC densitometric and colorimetric assays demonstrated essentially no differences in the plasma sulfonamide levels (17). Therefore, the colorimetric assay is specific for plasma sulfonamide determinations. Interferences from other drug substances, which can occur in clinical situations, are generally not a major problem in plasma since the sulfonamide levels following the relatively high doses used therapeutically far exceed levels of other classes of compounds (*e.g.*, benzodiazepines). In addition, no interferences in the colorimetric assay were reported due to trimethoprim, chloramphenicol, tetracycline, colistin, and ampicillin, all of which are often administered in situations requiring sulfonamide therapy (12, 18, 19).

Standards of the following benzodiazepines were introduced into the automated system: bromazepam (V), demoxepam (VI), and *N*-desmethyldiazepam (VII) (Scheme I). Each of these compounds, in addition to chlordiazepoxide, oxazepam, lorazepam, and other 1,4-benzodiazepines, is capable of being acid hydrolyzed to the corresponding benzophenone, which contains a primary aromatic amine and will, therefore, give a positive Bratton–Marshall diazotization reaction under the conditions used in the automated sulfonamide assay. However, only concentrations greater than 2 μg of benzodiazepine/ml could be detected, so plasma samples containing benzodiazepines would have to be present in much higher than usual therapeutic concentrations to cause errors in the sulfonamide quantitation. In addition, the extent of dialysis of these compounds from a biological matrix has not been established and could further reduce potential interferences.

In urine, free and total sulfonamides were measured. However, metabolites other than the N_4-acetyl metabolite, *e.g.*, the N_1- and the ring *N*-glucuronides, represent a significant percentage of the urinary excretion products in humans (20). Therefore, free and total measurements in urine by the automated assay yield results only in terms of equivalents of intact drug and the N_4-acetyl derivative. The urinary data are useful, however, in determining the percent recovery of the administered dose and for monitoring purposes.

Application to Biological Specimens—Plasma samples collected following the intravenous administration of 2 g of I to one subject in a study to determine the pharmacokinetic profile of the drug in anephric patients were analyzed by the manual sulfonamide procedure (1) and by the described automated procedure. Plasma I concentrations ranged from 11 to 134 μg/ml and metabolite levels (total − free) were from 16 to 117 μg/ml in a set of 18 specimens collected over 4 days. The coefficients of correlation between the two methods were 0.999 and 0.970, respectively, for the two data sets.

Study designs that involved oral administration of the combination product containing III and trimethoprim to humans provided for whole



V

2-amino-5-bromobenzoylpyridine

VI

2-amino-5-chlorobenzophenone

VII

Scheme I—Structures of three benzodiazepines (V–VII) and the hydrolysis conditions under which a benzophenone containing a primary aromatic amine function is produced.

blood specimens rather than plasma. In these studies, a minor modification of the automated method involved preparation of protein-free filtrates of whole blood[12] (11) and used the same automated manifold (Fig. 1) with dialyzers removed from both channels (Fig. 5). There was no increase in sensitivity by using whole blood or a protein-free filtrate for analysis, and the results were analytically similar to those obtained using direct untreated plasma (Fig. 6) for standards of III added to blood. In a series of blood samples collected from subjects who had received a combination of 2 g of sulfamethoxazole plus 400 mg of trimethoprim orally, the blood levels ranged from 11 to 65 μg of free sulfamethoxazole/ml of blood and from 9 to 94 μg of total sulfamethoxazole/ml. Comparison of 25 data sets analyzed by the manual and automated methods showed a coefficient of correlation of 0.996 for the free. For 51 sets of data for the total analyses, the coefficient of correlation was 0.990.

DISCUSSION

During the development of this automated methodology, certain inconsistencies were discovered when the N_4-acetyl metabolites of I and III were analyzed. Compounds II and IV were not as extensively dialyzed from the plasma matrix as I and III (Table II); only by running standards of those compounds alone, with and without plasma, could a suitable correction factor be introduced into the calculations. The alternative was to prepare and hydrolyze manually the protein-free filtrates as previously reported (11, 12). The automated method successfully overcomes these problems; by coupling the system to a suitable peak height detection system[11], direct concentration readout was accomplished. It is still necessary to prepare internal standards for the automated system and to tool-up the system prior to sample introduction. However, once this is accomplished, the system is capable of operating an entire workday and beyond if new samples are placed in the sampler tray as the samples are processed.

[12] Attempts to dialyze whole blood directly in this automated system were unsuccessful due to clogging of tubing and dialyzers.

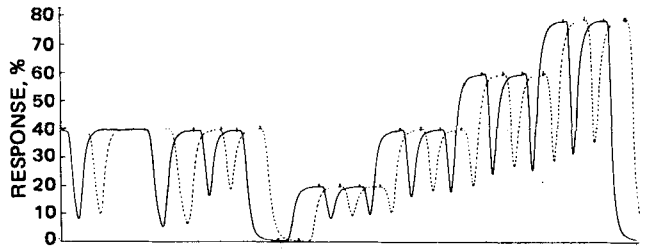


Figure 6—*Chart recording showing, from left to right: steady-state plateau of a continuously sampled 5 μg of III/ml of protein-free filtrate (equivalent to 50 μg of III added to 0.5 ml of blood) (11); duplicate determinations of the same sample analyzed at 40 per hour rate; and duplicate determinations of a series of aqueous standards of increasing concentrations (2, 4, 6, and 8 μg of III/ml). Key: —, free; and ---, total; using the automated manifolds without dialyzers (Figs. 1 and 5).*

The rate of automated analysis is 40 samples per hour, which allows for 200–240 samples per workday, leaving sufficient time for startup and washout periods after all analyses are completed. It is unlikely that any other available analytical method could provide results at the same rate and with as little sample manipulation, glassware, and time as the automated plasma method. Although the recently published HPLC procedure for sulfisoxazole in plasma (8) is fairly rapid, sample manipulation is still extensive. The procedure requires centrifugation and transfer of the supernate followed by injection into the liquid chromatograph, so the number of samples and standards that could be processed in a single day would be ~30.

In addition, the automated system is flexible in that it is easily converted to a colorimetric reaction system (without dialyzers) when sample preparation must be performed manually (Fig. 5). The reaction system also is useful for processing compounds other than sulfonamides if they yield a primary aromatic amine following acid hydrolysis (Scheme I). Although no immediate application was found for the benzodiazepines other than to demonstrate this feasibility, automated acid hydrolysis can be an interface with other automated analytical procedures when required. This application would be useful only when the reactant concentrations were in microgram quantities or where other more sensitive techniques (*e.g.*, fluorometry) were employed following hydrolysis.

In conclusion, although more sensitive instrumentation and specific methodologies have been developed in recent years, the classical Bratton–Marshall method for sulfonamide quantitation in biological fluids remains useful in certain analytical situations, such as in bioequivalency studies involving *in vivo* comparison of formulations containing sulfisoxazole and sulfamethoxazole. In such situations, the administered dose is sufficiently high to permit the use of the colorimetric assay (Bratton–Marshall) to define a plasma level–time profile for at least 24 and possibly 48 hr. By totally automating the analysis following the introduction of untreated plasma into the system, the analyst is freed of manual sample preparation steps.

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Pharmacokinetics and Relative Bioavailability of Oral Theophylline Capsules

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Abstract □ The oral bioavailability of liquid-filled theophylline capsules relative to a nonalcoholic aminophylline solution was determined in normal volunteers. In addition, theophylline absorption and elimination kinetics were reexamined. There were no statistically significant differences between the bioavailability of capsules and liquid as measured by the area under the curve (AUC) from time $0 \rightarrow \infty$ ($p > 0.05$). The bioavailability parameters of C_{max}, t_{max}, and AUC were determined from actual serum theophylline concentration–time data and from a nonlinear least-squares fit of the serum concentration–time data. Theophylline absorption from the capsules was noticeably faster than from the liquid in most subjects, although the differences in absorption rates were not significantly different ($p > 0.05$). The determined apparent volume of distribution, elimination half-life, and plasma clearance of theophylline were similar to values reported by other investigators. Marked inter- and intraindividual variations in the elimination half-life were noted.

Keyphrases □ Theophylline—oral dosage forms, pharmacokinetics, bioavailability, capsule compared to liquid □ Muscle relaxants (smooth muscle)—theophylline, oral dosage forms, pharmacokinetics, bioavailability, capsule compared to liquid □ Bioavailability—theophylline, various oral dosage forms

The clinical importance of satisfactory oral theophylline bioavailability is well recognized in the scientific and medical communities. Bioavailability problems, particularly with tablets and capsules, are thought to be related primarily to the dosage form formulation and not to physiological factors that influence absorption (1).

Although reported bioavailability studies of various theophylline oral dosage forms have demonstrated satisfactory bioavailability, all commercial oral theophylline dosage forms may not have equally satisfactory bioavailability (2, 3). It is important to evaluate the bioavailability of each theophylline formulation to confirm that formulation factors do not affect *in vivo* absorption.

One major goal of this investigation was to evaluate the relative bioavailability of liquid-filled oral theophylline capsules[1] in a random, crossover study. The rationale was to determine if any formulation factors associated with liquid-filled capsules might affect theophylline bioavailability when compared to the bioavailability from a nonalcoholic, rapidly absorbed aminophylline oral liquid[2]. Another major goal was to examine the absorption and elimination kinetics of theophylline when administered as a liquid-filled capsule or as a liquid.

EXPERIMENTAL

Subjects—Seven male and seven female subjects, 21–40 years old, were selected with the approval of the Institutional Human Subjects Review Committee. Valid written informed consent was obtained from each subject prior to entrance into the study. The body weight (mean ± *SD*, 67 ± 12 kg) of the volunteers was within 10% of normal limits for their height and build (4).

All subjects were determined to be in good physical health, with no history of alcoholism or cardiovascular disease. They were judged to be medically sound based on a medical history, physical examination, vital signs, ECG, and the usual battery of blood and urine clinical chemistry tests. All subjects were nonsmokers and had not smoked regularly at any time within the last 3 years.

All subjects were instructed to refrain from any medication for at least 7 days prior to the study and to abstain from alcohol and xanthine-containing foods or beverages for 24 hr prior to dosing. All volunteers were fasted, with the exception of water, for 12 hr prior to dosing, and the fasting was continued for 4 hr after dosing. A modest meal, low in carbohydrates and fat, was served at 4 hr, and a light dinner, likewise low in fat and carbohydrates, was served at 8 hr after dosing.

Drug Administration and Blood Sampling—On each study day, an oral dose equivalent to 300 mg of anhydrous theophylline was randomly administered either as liquid-filled capsules or as a nonalcoholic aminophylline solution. Previous analysis for potency showed the capsules to contain 98% of the label claim. The study days were separated by a washout period of 7 days, after which the subjects took the alternate formulation. On each study day, the subjects were administered the medication with 240 ml of water at approximately 8:00 am.

Predose blood samples (1 ml) were obtained immediately before dosing *via* an indwelling catheter in the forearm vein. After dosing with the capsules, blood samples were collected at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 10, 12, 16, and 24 hr. After dosing with the liquid, blood samples

[1] Somophyllin capsules, lots 02977 and 07677, Fisons Corp., Bedford, Mass.

[2] Somophyllin oral liquid, lot 02287, Fisons Corp., Bedford, Mass.

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