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DETERMINATION OF TRIMETHOPRIM AND SULFAMETHOXAZOLE (CO-TRIMOXAZOLE) IN BODY FLUIDS OF MAN BY MEANS OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A high-performance liquid chromatographic method for the determination of trimethoprim, sulfamethoxazole and its metabolite and a series of structurally related sulfonamides is described. The half-life time of elimination of sulfamethoxazole and its metabolite N_4-acetylsulfamethoxazole is 9 h for both compounds. The renal excretion rate of sulfamethoxazole depends strongly on the urinary pH. The renal excretion rate of the metabolite N_4-acetylsulfamethoxazole is not dependent on the urinary pH.

INTRODUCTION

Sulfamethoxazole in combination with trimethoprim (Co-Trimoxazole®) is nowadays accepted and used as a highly effective antibacterial formulation. Both compounds interfere with folate synthesis in bacteria and many papers have appeared devoted to the clinical applications and its chemotherapeutic activity [1—5].

However in papers dealing with the various aspects of the pharmacokinetic parameters of absorption, metabolism and tissue concentration of the two components of co-trimoxazole, the determinations were carried out with rather intricate or non-specific methods such as microbiological [6], spectrophotometric [7,8], spectrofluorimetric [9] and spectrodensitometric [10] assays or pyrolysis gas chromatographic—mass spectrometric analysis [11].

The use of high-performance liquid chromatography (HPLC) for pure sulfonamides has been described recently [12,13] and some determinations of these compounds in biological fluids have been accomplished [14,15].

The measurement of plasma concentration and renal elimination of sulfon-

amides, especially in patients with kidney insufficiency [16—18] can be of advantage in treatment, as the renal excretion of sulfamethoxazole and of its metabolite N_4-acetylsulfamethoxazole account for 50—100% of the total elimination [19,20].

This has led to the development of an HPLC determination of trimethoprim and sulfamethoxazole in the body fluids, plasma, serum and urine of man. The method described in this communication covers the therapeutic range of concentrations in serum and urine for trimethoprim from 0.1 μg/ml up to 10 μg/ml and for sulfamethoxazole from 1 μg/ml to 200 μg/ml.

The differences in pharmacokinetic behaviour of sulfamethoxazole and its metabolite N_4-acetylsulfamethoxazole under different urinary pH conditions have been investigated.

MATERIALS AND METHODS

Apparatus

A Spectra Physics 3500 B high-performance liquid chromatograph was used, equipped with a spectrophotometric detector (model 770). The detector was connected to a 1 mV recorder (BD7, Kipp & Zonen, The Netherlands). A stainless steel column, 15 cm × 4.6 mm I.D. packed with LiChrosorb RP 8, particle size 5 μm, was obtained from Chrompack (Middelburg, The Netherlands). An injection loop of 100 μl was used. Detection of sulfonamides only was effected at 260 nm, the detection limit is 0.5 μg/ml. Detection for sulfonamides and trimethoprim simultaneously was effected at 225 nm. The detection limit of trimethoprim is 0.75 μg/ml.

Solvents

Sulfonamides. The solvent is a mixture of phosphate buffer and methanol of pH 6.7. Therefore 390 ml of 0.067 *M* KH_2PO_4 were mixed with 10 ml of 0.067 *M* Na_2HPO_4 and 80 ml of methanol. The solvent flow-rate is 1.6 ml/min at a pressure of 115 atm (Fig. 1, Table I).

Sulfamethoxazole + trimethoprim. The solvent is a mixture of 390 ml 0.067 *M* KH_2PO_4, 10 ml of 0.067 *M* Na_2HPO_4 and 80 ml of ethanol. The solvent flow-rate is 1.2 ml/min at a pressure of 85 atm (Fig. 2 and Table II).

Drugs

Sulfamethoxazole, N_4-acetylsulfamethoxazole and trimethoprim were obtained from Hoffmann-La Roche (Mijdrecht, The Netherlands) by the courtesy of Dr. J. Kuitert. Sulfapyridine, sulfadiazine, sulfathiazole, sulfadoxine were obtained from the St. Radboud Hospital Pharmacy. Sulfadimidine and sulfamethoxypyridine were obtained from the Department of Pharmacology, State University of Groningen, The Netherlands by courtesy of Dr. D.K.F. Meyer. According to the HPLC chromatogram all compounds were 100% pure.

Subjects and patients

Ten subjects, all employees of the Department of Clinical Pharmacy participated in this study. Sulfamethoxazole was administered in doses of 800, 400, 200, and 100 mg (powder in a gelatine capsule). The drug was taken orally in

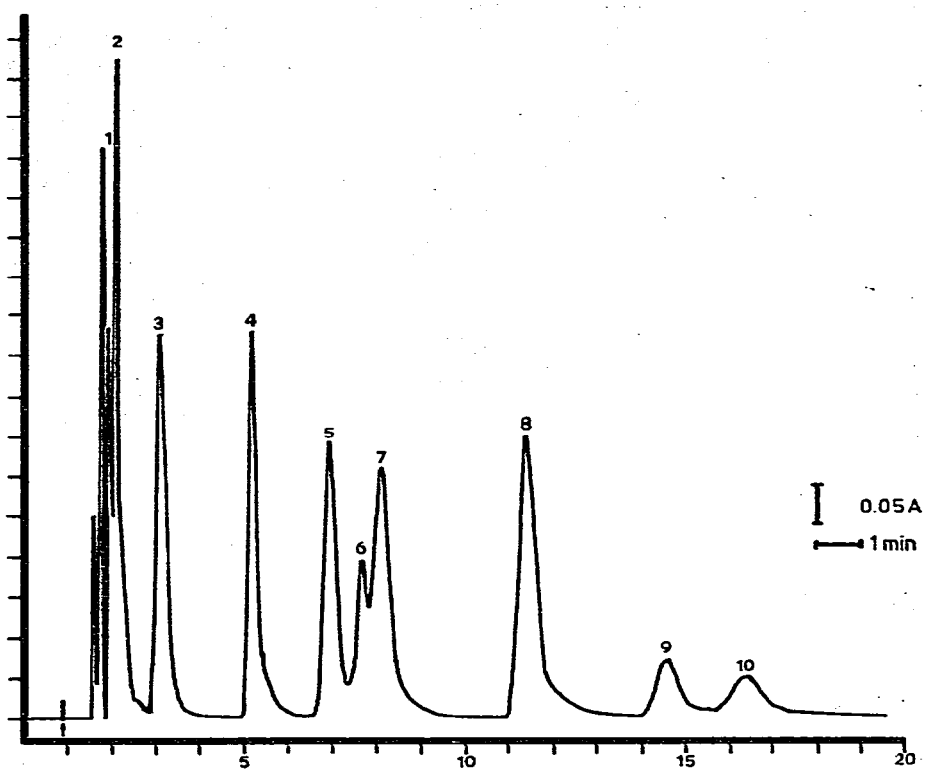

Fig. 1. HPLC chromatogram of sulfamethoxazole and related sulfonamides. The column used was LiChrosorb RP 8, and the solvent a phosphate buffer of pH 6.7 + 20% methanol. Flow-rate, 1.6 ml/min. 1 = Sulfacetamide; 2 = sulfanilamide; 3 = sulfadiazine; 4 = sulfamethoxazole; 5 = sulfathiazole; 6 = sulfadoxine; 7 = sulfapyridine; 8 = N_4-acetylsulfamethoxazole; 9 = sulfamethoxypyridine; 10 = sulfadimidine.

TABLE I

RETENTION TIMES OF SULFAMETHOXAZOLE AND RELATED SULFONAMIDES RELATIVE TO THE UNRETAINED COMPONENT (K′)

Column, LiChrosorb RP 8; solvent, phosphate buffer (pH 6.7) + 20% methanol.

Compound	Relative retention time K′
Sulfacetamide	1.53
Sulfanilamide	1.87
Sulfadiazine	3.00
Sulfamethoxazole	5.60
Sulfathiazole	8.20
Sulfadoxine	9.00
Sulfapyridine	9.73
N_4-Acetylsulfamethoxazole	14.40
Sulfamethoxypyridazine	18.40
Sulfadimidine	20.73

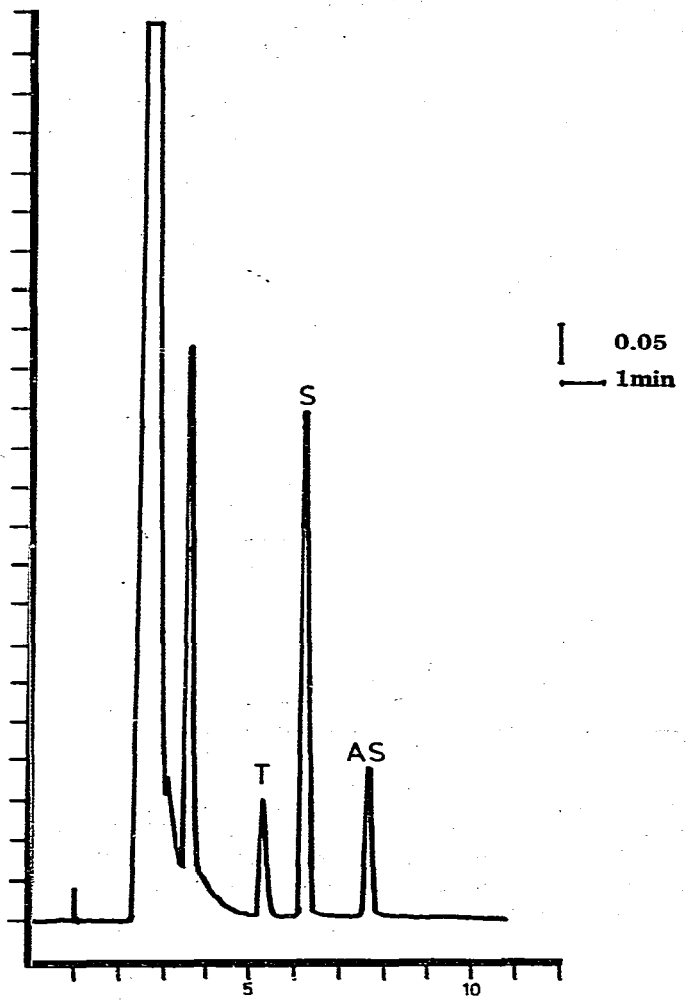

Fig. 2. HPLC chromatogram of sulfamethoxazole (S), its metabolite N_4-acetylsulfamethoxazole (AS) and trimethoprim (T) in plasma of a patient receiving co-trimoxazole as treatment. The column used was LiChrosorb RP 8 and the solvent a phosphate buffer of pH 6.7 + 20% ethanol. Flow-rate, 1.2 ml/min.

TABLE II

RETENTION TIMES OF SULFAMETHOXAZOLE, ITS METABOLITE AND TRIMETHOPRIM RELATIVE TO THE UNRETAINED COMPONENT (K′)

Column, LiChrosorb RP 8; solvent, phosphate buffer (pH 6.7) + 20% ethanol.

Compound	Relative retention time K′
Trimethoprim	3.50
Sulfamethoxazole	4.33
N_4-Acetylsulfamethoxazole	5.50

the morning, 1.5 h after a standard breakfast. Blood samples of 0.2 ml were collected at scheduled intervals by fingertip puncture (Microlance no. 433, Becton Dickinson). Spontaneously voided urine was collected during 56 h. An alkaline urine pH was reached by the daily intake of 10 g of sodium bicarbonate (pH 7—8). An acidic urine pH was reached by the daily intake of 8 g of ammonium chloride (pH 5—6).

Blood samples from patients receiving co-trimoxazole as treatment were obtained from the Intensive Care Unit (Dr. R. van Dalen) of the St. Radboud Hospital.

Sample preparation

Human serum is 10 times diluted with distilled water, and to 0.2 ml of the diluted serum a 0.8 ml volume of perchloric acid (0.33 *M*) is added. The solution is mixed thoroughly on a Vortex mixer and subsequently allowed to stand for 10 min. After centrifugation at 2600 *g* for 5 min, 100 μl of the supernatant is injected onto the column.

A calibration curve is made by adding known concentrations of sulfamethoxazole to blank human serum. Once a calibration curve has been made, it is desirable to check 3 points of this curve with each series of determinations in order to confirm whether the column is still stable.

The human urine sample is prepared by addition of 0.5 ml of perchloric acid (0.33 *M*) to 10 μl of urine. The solution is mixed and 100 μl is injected onto the column.

Recovery

Recovery of sulfamethoxazole added to human serum in the concentration range of 1—200 μg/ml was found to be 88 ± 4%. For trimethoprim the recovery was found to be 83 ± 2% in the concentration range of 0.5—16 μg/ml.

If, for the estimation of sulfamethoxazole, the serum is not diluted, the recovery after deproteinization is somewhat less, 80 ± 7%.

The recovery of sulfamethoxazole and trimethoprim added to urine is 100 ± 2%.

RESULTS

Sulfamethoxazole is well separated from its metabolite N_4-acetylsulfamethoxazole, other related sulfonamides and trimethoprim, with which it may occur frequently in chemotherapeutic combination (as can be seen in Figs. 1 and 2 and Tables I and II).

A dose of 100—800 mg of sulfamethoxazole results in plasma concentrations of sulfamethoxazole and N_4-acetylsulfamethoxazole that can easily be measured (0.5 μg/ml—200 μg/ml). The urine concentrations of both compounds after a dose of 100—800 mg range from 10—500 μg/ml.

The pharmacokinetics of sulfamethoxazole in man under acidic and alkaline urinary pH conditions reveal a difference in the excretion pattern of sulfamethoxazole which is shown in Fig. 3 and Table III. The most striking observation is that sulfamethoxazole is hardly excreted unchanged with acidic urine, while

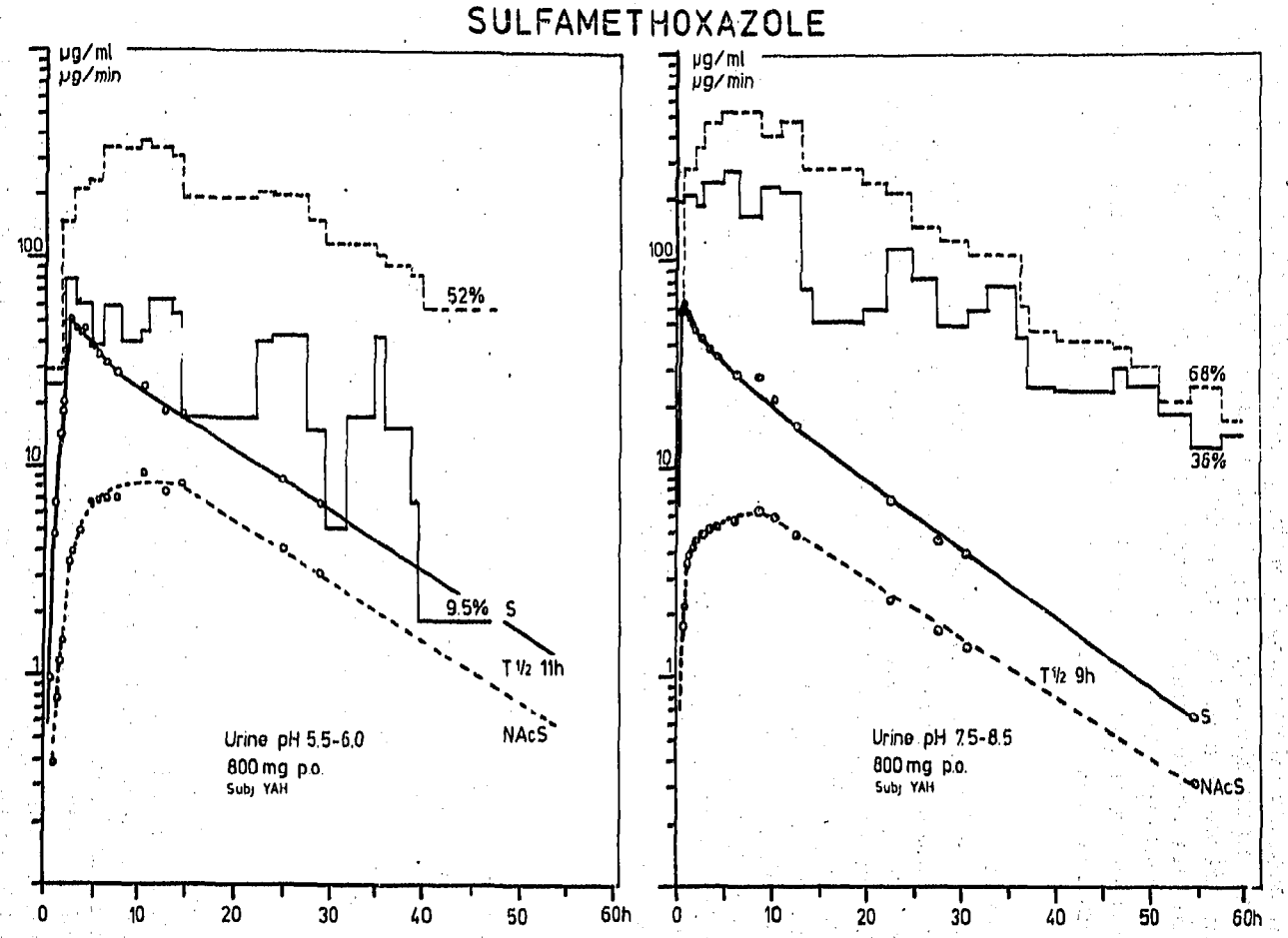

Fig. 3. Plasma concentration–time profile of sulfamethoxazole (S) and its metabolite N_4-acetylsulfamethoxazole (NAcS) and the renal excretion rate of both compounds in a volunteer who took 800 mg sulfamethoxazole orally. The experiment was performed under different urinary pH values. Note that with acidic urine (pH 5.5–6.0) only 9.5% of sulfamethoxazole is excreted unchanged, while this value is 36% when the urine is maintained alkaline (pH 7.5–8.5). Due to the enhanced excretion under alkaline urine conditions the T½ of elimination is shorter (9 h) than under acidic urine conditions (11 h).

TABLE III

PHARMACOKINETIC PARAMETERS OF SULFAMETHOXAZOLE IN MAN

Subjects	Dose	pH Urine	$T_{1/2}$ (h)		Excreted in the urine (%)	
			Sulfameth.	N-Acetylsulf.	Sulfamethoxazole	N_4-acetylsulfamethoxazole
$n = 10$	200 mg (5) 400 mg (2) 800 mg (3)	7—8	9.6 ± 2.5	11.2 ± 2.4	35.0 ± 2.0	42.8 ± 12.9
$n = 5$	400 mg (2) 800 mg (3)	5—6	9.5 ± 3.5	9.0 ± 1.7	7.1 ± 3.2	44.0 ± 6.9

the percentage of the dose of N_4-acetylsulfamethoxazole followed by excretion in all volunteers is almost constant.

The renal clearance constant, the proportionality constant between the renal excretion rate (μg/min) and the plasma concentration (μg/ml) of sulfamethoxazole are strongly dependent on the urinary pH (Fig. 4), while the renal clearance constant of the metabolite N_4-acetylsulfamethoxazole is hardly affected by the urinary pH.

Fig. 5 shows an example of the plasma concentrations of sulfamethoxazole,

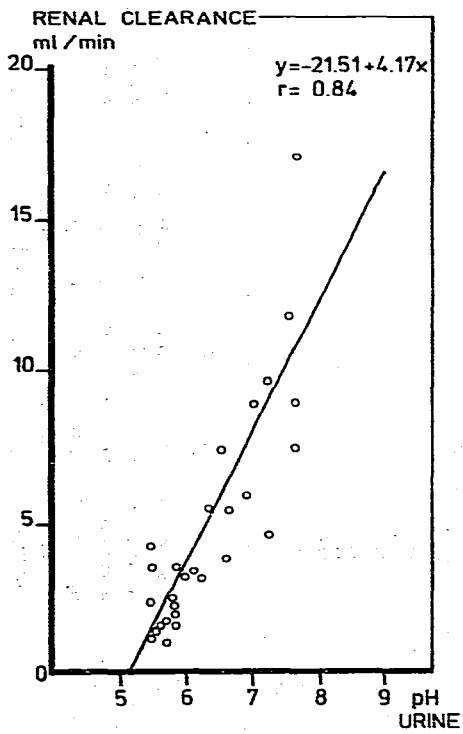

Fig. 4. Relationship between renal clearance (ml/min) of sulfamethoxazole and the urinary pH. Both variables were measured in each urine portion voided. Note that the renal clearance is increased by a factor 10 when the pH changes from pH 5 to pH 8. No obvious relationship could be observed ($r = 0.25$) for the metabolite N_4-acetylsulfamethoxazole.

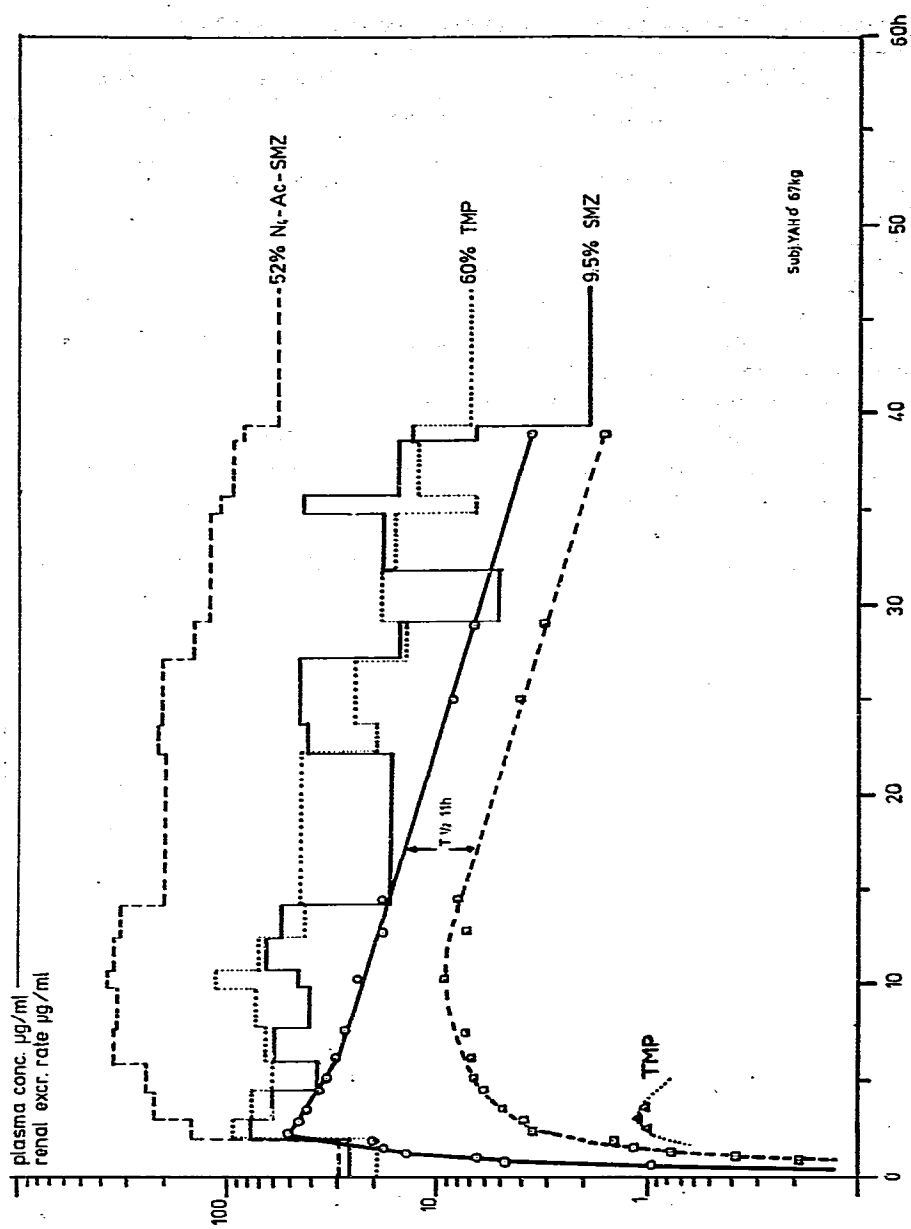

Fig. 5. Plasma concentration—time profile and renal excretion rate of sulfamethoxazole (SMZ), its metabolite N_4-acetylsulfamethoxazole (N_4 AcSMZ) and trimethoprim (TMP) in a volunteer who took 800 mg sulfamethoxazole and 160 mg of trimethoprim orally. The urinary pH is not influenced and appeared to be mainly acidic (pH 5—6). Trimethoprim concentrations could be measured in plasma only for a short period, its renal excretion accounts for 60% of the dose administered. Sulfamethoxazole is excreted 9.5% unchanged and 52% as the N_4-acetylsulfamethoxazole.

its metabolite N_4-acetylsulfamethoxazole and the co-medication trimethoprim. While the concentrations of both sulfa- compounds are relatively high and can be measured over a long period, trimethoprim can only be measured for a short time.

The renal excretion rate of trimethoprim is much higher than that of sulfamethoxazole. 60% of trimethoprim is excreted unchanged, while for sulfamethoxazole this value is 9.5% and 52% is excreted as metabolite. The pH of the urine in this experiment is not influenced.

DISCUSSION

The HPLC method for sulfonamides has the advantage over the Bratton and Marshall photometric method [7,8] that the compounds are measured specifically and selectively and that several sulfonamides ingested at the same time, can be simultaneously measured [13]. The pharmacokinetic parameters can be compared under exactly the same circumstances. As plasma and urine concentrations can be measured conveniently, the renal clearance constant can be calculated. It was shown that the renal excretion of sulfonamides is strongly dependent on the urinary pH [21—23]. The implications of this pharmacokinetic behaviour for clinical treatment are the subject of further research.

It may be deduced that in the case of an infection of the urinary tract, the urine should be kept alkaline in order to ensure that the concentration of sulfamethoxazole is as high as possible, because the metabolite is inactive. When trimethoprim is used in chemotherapy in combination with sulfamethoxazole, three compounds can be easily recognized in the HPLC chromatograms of plasma and urine samples (Fig. 1 and Table I).

The ease of the sample preparation and the avoidance of extraction procedures, the small sample volume required, the possibility of simultaneous determination of several sulfonamides and the short retention time (10—15 min) all make this method useful for the analysis of large series of samples for both research and routine applications.

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