Improved Delivery of Methoxsalen

JÖRG KREUTER * **and TAKERU HIGUCHI** *

Received May 2, 1978, from the *Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS 66044.* Accepted for publication September 11, 1978. *Present address: School of Pharmacy, Federal Institute of Technology, Clausiusstr. 25, CH-8092 Zürich, Switzerland.

Abstract □ Significant improvement in the effective bioavailability of methoxsalen was achieved when it was administered to rats and dogs in a solution as compared to a suspension. Much earlier and higher peak levels were observed for the solution in both animals. The possible impact of these observations on current use of this agent for psoriasis treatment is discussed.

Keyphrases □ Methoxsalen—solution formulated for improved delivery, bioavailability characteristics, rats and dogs □ Bioavailability—methoxsalen, improved delivery, rats and dogs □ Formulations—improved delivery of methoxsalen using solution form □ Dosage forms—methoxsalen solution compared to suspension, bioavailability characteristics evaluated, rats and dogs

Methoxsalen (I) is commercially available as hard gelatin powder capsules[1]. Because of the poor water solubility of this drug (1), its bioavailability was suspected to be rather poor. This study aimed at developing both a simple, yet sensitive, method for its analysis in blood samples and a drug formulation that would permit more rapid and effective drug absorption. Such a dosage form would permit more optimal exposure to a UV source and significantly reduce the required amount of the rather toxic drug substance.

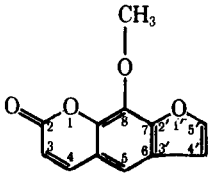
BACKGROUND

Plants containing furocoumarins (psoralens) have been used to produce skin pigmentation for at least 3000 years (2). Modern usage of psoralens for repigmenting vitiligo began in the early 1950's (3–5). Methoxsalen (xanthotoxin) proved to be one of the most potent photosensitizers of this group of compounds (6, 7). Over the past few years, this drug has been shown to be effective in the treatment of psoriasis (8–10), a chronic, intractable skin disease common in 1–3% of the world's population.

Methoxsalen, together with longwave UV irradiation, leads to the formation of a thicker and denser stratum corneum and, therefore, to enhanced pigmentation (2, 11). Its mechanism of action seems to be a photoreaction with the thymine bases of the body DNA (7, 8, 12). UV light (365 nm) induces formation of C_4-cycloadducts between the 5,6-double bond of thymine and the 4′,5′- or 3,4-double bond of methoxsalen. Acting as a bifunctional reagent, it forms interstrand cross-linkages between pyrimidine bases in opposite strands of the DNA double helix.

Only limited information is available regarding the pharmacokinetics of methoxsalen and related compounds. Two studies investigated the elimination of radioactivity after oral (13, 14) and topical (14) application of psoralen (13), trimethylpsoralen (13), and methoxsalen (14). Approximately 80% of the radioactivity was excreted within 8 hr. After application of methoxsalen, 80% of the radioactivity appeared in the urine and 20% in the feces. Urine analysis showed that methoxsalen was totally metabolized (14). Gazith and Schaefer (15) followed plasma levels of unchanged methoxsalen in two humans for 5 hr after oral application of 50 mg (15).

The reason for the very limited information on methoxsalen biopharmaceutics was the lack of sufficiently sensitive analytical methods. Lerman and Borkman (16) recently detected methoxsalen concentrations of 10^{-5} *M* (2.2 μg/ml) in intact rat lenses. Gazith and Schaefer (15) detected 0.1 μg/ml in serum using a combined TLC–GLC method. Since their procedure is rather complicated, it is not suitable for extensive investigations. Moreover, these two methods are not sensitive enough for analysis of low plasma or blood levels.



I

In a recent study with nine patients, Steiner *et al.* (17) found enormous variations of peak plasma levels, ranging from 0.15 to 4.58 μg/ml after administration of commercial methoxsalen powder capsules. The time required to reach maximal plasma levels also differed significantly from patient to patient. These variations suggest great differences in the absorption rate, probably largely caused by poor solubility.

The present article reports results of a biopharmaceutical study on the furocoumarin in two oral dosage forms. Data are presented for experiments conducted on rats and dogs based on the high-pressure liquid chromatographic (HPLC) determination of the parent drug.

EXPERIMENTAL

Materials—Methylene chloride[2], methanol[2], 2-propanol[2], ethylene chloride[3], and heptane[3] were all HPLC grade. All other materials were reagent grade.

Calibration Curve—Methoxsalen[4], 20 mg, was dissolved in 100 ml of methylene chloride. This solution was twice diluted 1:10 with methylene chloride to give a solution containing 2.0×10^{-3} mg/ml (Solution I). Blood sample blanks were spiked with different amounts (2.5–100.0 μl) of a solution containing 20.0 mg of drug in 1000 ml of distilled water (Solution II); after mixing with a vortex mixer for 1 min and standing for 4 hr at room temperature, the spiked blood samples were assayed as described later.

Methoxsalen standards prepared by further dilution of Solution I with methylene chloride served as a comparison. All standard solutions were freshly prepared before usage.

Assay—The blood samples of rats were extracted with 2 ml of a mixture of 20% methylene chloride and 80% heptane by agitation with a vortex mixer for 1 min. The blood samples of dogs were weighed and then extracted with 5 ml of the same mixture (20% methylene chloride and 80% heptane) by the same procedure. After separation by centrifugation with a laboratory centrifuge for 15 min, the organic layer was transferred into a stoppered tube with a disposable pipet[2].

The organic solvents were then evaporated under nitrogen by heating in a water bath (60°), and the residue was stored in a vacuum desiccator for 12 hr. The residue was then redissolved in 1 ml of methylene chloride, containing 3.0 μg of griseofulvin/ml as an internal standard, by agitation with a vortex mixer for 30 sec. Replicate 10-μl injections were made for each sample using the HPLC method described later. Control blanks for each biological fluid were also included.

Chromatographic Procedure—A high-pressure liquid chromatograph[5] equipped with a fixed wavelength (254 nm) detector and recorder[6] was used. The mobile phase was 4% methanol, 20% ethylene chloride, and 76% heptane. The degassed mobile phase was pumped through the column[7] at a flow rate of 2 ml/min at room temperature until a stable baseline was reached. Replicate 10-μl injections of samples or standard solutions were made with a 10-μl syringe. The column had to be flushed with 2-propanol for 45 min after 15–20 injections.

[1] Oxsoralen, Paul B. Elder Co., Hamilton, Ind.

[2] Fisher Scientific Co., Fair Lawn, N.J.
[3] Burdick & Jackson Laboratories, Muskegon, Mich.
[4] Sigma Chemical Co., St. Louis, Mo.
[5] Waters ALC 202 equipped with a U6K universal injector.
[6] Omniscribe, Houston Instruments, Austin, Tex.
[7] μBondapak CN, Waters Associates, Milford, Mass.

0022-3549/79/0400-0451$01.00/0

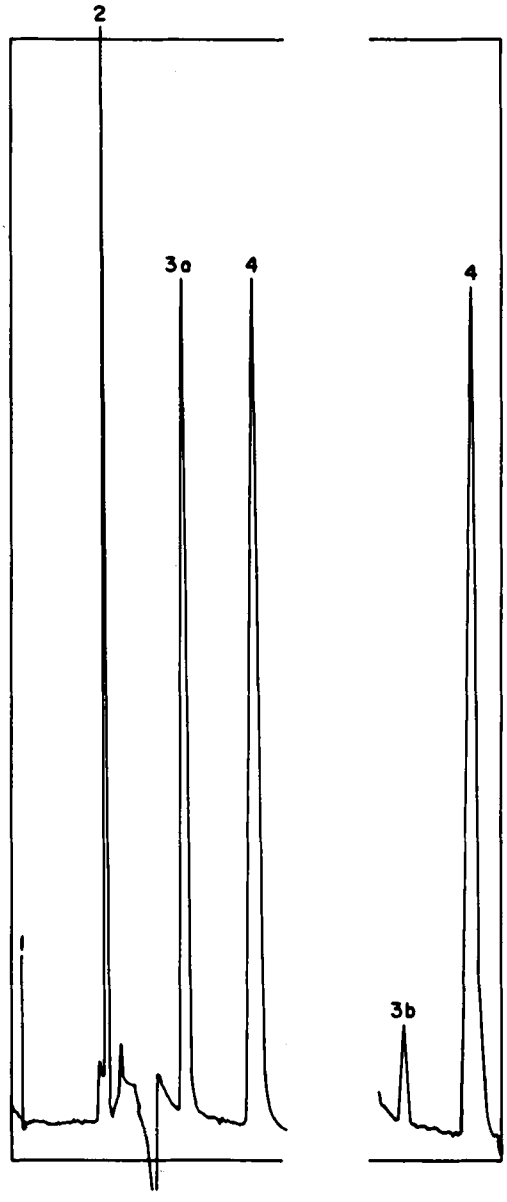


Figure 1—*Liquid chromatogram of rat blood extracts. Key: 1, injection marker; 2, solvent front; 3a, 800 ng of methoxsalen/ml of blood; 3b, 100 ng of methoxsalen/ml of blood; 4, internal standard, 3.0 μg of griseofulvin/ml.*

During the first run of both the rat and dog experiments, samples with the solution resembling the methoxsalen zone (Fig. 1, peak 3) were collected after HPLC separation and investigated with TLC using silica gel 60 F-254 plates[8] (0.2 mm thick). Benzene[2] was the mobile phase. After drying, the plates were examined with UV light.

Rat Experiments—Male rats[9], 250–300 g, were deprived of food, but not water, 12 hr prior to dosing. Five groups of three rats were formed, and three rats per day received one of three formulations. For Formulation A, 10.0 mg of methoxsalen[4] was dissolved in 2 ml of ethanol[2]; 100 μl of this solution (0.5 mg of drug) was administered into the penal dorsal vein.

For Formulation B, 100.0 mg of methoxsalen[4] was dissolved in 1 ml of 2,2,2-trichloroethanol[10]. This solution was mixed with 4 ml of peanut oil[11]. For Formulation C, one capsule was suspended in 5 ml of distilled water by agitation with a vortex mixer. One hundred microliters of Formulation B and 1 ml of Formulation C, both containing 2.0 mg of drug, were administered with an oral syringe.

A 200-μl blood sample was taken by tail clipping with a graded pipet[2] after 5, 10, and 30 min and 1, 1.5, 3, 4, 6, and 8 hr. The blood samples were assayed as already described.

Dog Experiments—The dog experiments were designed as a 3 × 3 crossover study with a latent time of 2 weeks between each run. Three beagle dogs were used: Dog A, 9 kg; Dog B, 10 kg; and Dog C, 7 kg. The dogs were deprived of food, but not water, 24 hr prior to and 12 hr after dosing.

For Formulation D, 5.0 mg of methoxsalen[4] was dissolved in 1 ml of ethanol[2] and mixed with 1 ml of 5% dextrose solution in water[12]. For Formulation E, 100.0 mg of methoxsalen[4] was dissolved in 1 ml of 2,2,2-trichloroethanol[10] and mixed with 4 ml of peanut oil[11]. A hard gelatin capsule[13], size 000, was filled with 1 ml of this solution (20.0 mg of drug). Formulation F was two capsules[1] containing a total of 20 mg of drug.

Formulation D was administered intravenously. Formulations E and F were administered orally with 100 ml of water. Blood samples of 1.0–1.5 ml were drawn before dosing and after 10, 20, 40, and 90 min and 3, 6, 12, and 25 hr and assayed as already described.

RESULTS AND DISCUSSION

Analysis of trace substances in a biological system is often limited by method sensitivity. In previous studies on methoxsalen in biological systems, a fluorometric (15) or a combined TLC–GLC (14) method was used. Neither method was sensitive enough for analysis of low plasma or blood levels.

In the present work, HPLC was used. Figure 1 shows the chromatogram of spiked rat blood samples. The method allows the detection of 10 ng of methoxsalen/ml of blood. Thus, it is 10 times more sensitive than the method of Gazith and Schaefer (15) and 100 times more sensitive than the method of Lerman and Borkman (16). The sensitivity of this HPLC method can be extended by increasing the volume injected into the liquid chromatograph and the amount of serum taken for extraction.

The specificity of the HPLC separation was tested by further investigation of the methoxsalen fraction (peak 3, Fig. 1) with TLC. TLC using

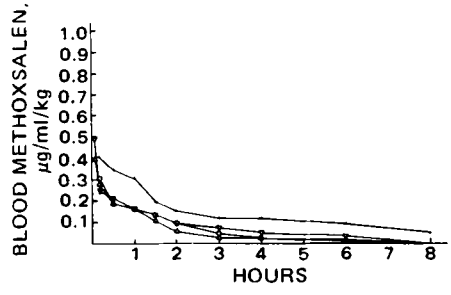


Figure 2—*Blood levels in rats after intravenous administration of 0.5 mg of methoxsalen (Formulation A). Key: ●, Rat 1A; ○, Rat 2A; △, Rat 3A; and ▽, Rat 4A.*

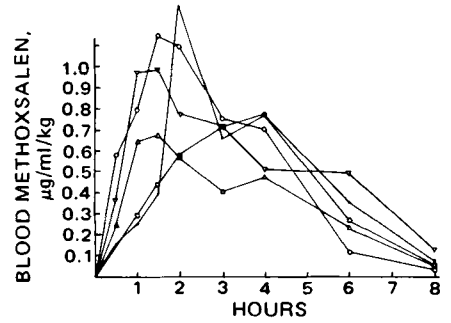


Figure 3—*Blood levels in rats after oral administration of 2.0 mg of methoxsalen in a trichloroethanol–peanut oil formulation (Formulation B). Key: ●, Rat 1B; ○, Rat 2B; △, Rat 3B; ▽, Rat 4B; and □, Rat 5B.*

[8] E. Merck, Darmstadt, West Germany.
[9] Sprague-Dawley, Madison, Wis.
[10] Aldrich Chemical Co., Milwaukee, Wis.
[11] Planters Manufacturing Co., New York, N.Y.
[12] Abbott Laboratories, North Chicago, Ill.
[13] Eli Lilly and Co., Indianapolis, Ind.

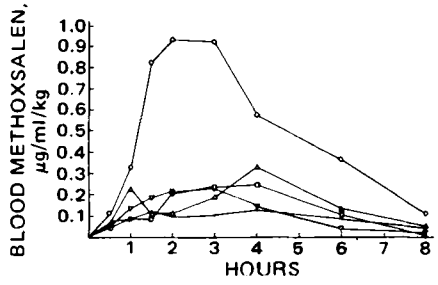


Figure 4—*Blood levels in rats after oral administration of 2.0 mg of methoxsalen as a suspension of a commercial capsule formulation (Formulation C). Key: ●, Rat 1C; ○, Rat 2C; □, Rat 3C; △, Rat 4C; and ▽, Rat 5C.*

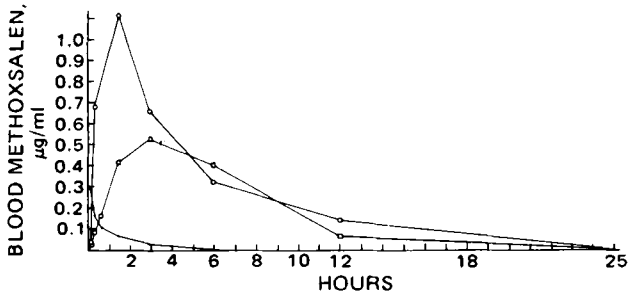


Figure 5—*Blood levels of methoxsalen in Dog A. Key: ●, intravenous administration (5.0 mg), Formulation D; ○, oral administration (20.0 mg), Formulation E; and □, oral administration (20.0 mg), Formulation F.*

the mobile phase from HPLC resulted in only one spot with the same R_f value as methoxsalen.

The assay presented is uncomplicated. A single extraction led to a reproducible 96% recovery of drug from blood samples. A second extraction yielded an additional 4%.

Because of the good reproducibility, only one extraction is required for routine analysis. The chromatogram is characterized by short retention times. With a flow rate of 2 ml/min, the methoxsalen peak appeared after 3 min; griseofulvin, the internal standard, appeared after 4 min 15 sec. Thus, total assay time is less than 5 min for each sample.

The blood level data (Figs. 2–7) are characterized by broad individual variations. Nevertheless, the oral liquid formulations (Formulations B and E) generally gave higher blood levels (Figs. 3 and 5–7) and better bioavailability (area under the concentration–time curve) (Tables I and II) than the powder formulations (Formulations C and F) (Figs. 4–7), which were administered as suspensions to the rats and as intact capsules to the dogs. Exceptions to these findings were Rat 2C and Dog C (Tables I and II) in whom the powder formulations were equivalent in bioavailability to the oral liquid formulations.

The intravenous data suggest that methoxsalen pharmacokinetics follow a two-compartment model with a rapid α-phase, significant for only 10 min in rats and for about 30 min in dogs. The intravenous bioavailability data (Tables I and II) calculated from the concentration–time curves were, however, unexpectedly low in comparison to the oral formulations. Since the intravenous dose was one-fourth of the oral doses, the expected bioavailability should be at least one-fourth of the oral bioavailability.

With two exceptions (Rat 1A and Dog B), the bioavailability of several oral formulations was significantly higher than four times the intravenous bioavailability. There may be two reasons for this finding. Either

Table I—Bioavailability [(µg min/ml)/kg] of Methoxsalen in Rats

Rat	Formulation A	Rat	Formulation B	Rat	Formulation C
1A	75	1B	238	1C	42
2A	34	2B	249	2C	238
3A	28	3B	171	3C	66
4A	38	4B	260	4C	78
		5B	201	5C	54

Table II—Bioavailability [(µg min)/ml] of Methoxsalen in Dogs

Dog	Formulation D	Formulation E	Formulation F
A	38	382	252
B	64	183	93
C	51	257	239

methoxsalen does not show linear pharmacokinetics or the drug precipitates after intravenous administration due to its poor water solubility and then is redissolved very slowly over a long period with blood levels below the detection limit. Occasional inflammation at the injection site of the rats may support the latter hypothesis. The smaller dose was used to limit the injection volume because of separate effects of the vehicle.

In the blood level curves of rats, a second peak, or at least a shoulder, was observed about 4 hr after application of most oral formulations (Figs. 3 and 4). It may possibly be caused by analytical errors, by variations in the absorption rate, or by enterohepatic recycling. Analytical errors are unlikely, however, because the rat experiments and the assay procedures were carried out on different days. The occurrence of enterohepatic recycling seems questionable since no second peaks are observable after intravenous administration. Enterohepatic recycling would have to be determined by analysis of unchanged drug and metabolites after bile cannulation.

Comparison of the bioavailabilities, measured by the area under the concentration–time curves, is of only limited value if nonlinear pharmacokinetics exist because the area under the curve is then not only dependent on the drug formulation but to a great extent also dosage dependent. For the therapeutic use of methoxsalen, bioavailability is of limited relevance because it is used as a "hit-and-run" (18) drug. The desired photoreaction of this drug occurs only if the patient is exposed to UV irradiation. Therefore, the effectiveness of the drug is dependent on the drug concentration in the epidermis. For this reason, blood levels shortly before or at the time of irradiation are of main interest. To avoid a prolonged photosensitivity of the patient (8), prolonged absorption is not desirable.

The UV treatment (irradiation time of about 10 min) is normally carried out 2 hr after drug administration. Gazith and Schaefer (15) reported maximal serum methoxsalen levels 1.5 hr after administration (drug formulation not specified). In the present study, maximal blood levels were observed in rats 2–4 hr and in dogs 3 hr after administration of the oral powder formulations (C and F). After administration of the oral liquid formulations (B and E), the time for the appearance of the maximal blood levels was reduced in both rats (1.5–2 hr) and dogs (20–90 min). In addition, the maximal blood levels in both species were increased significantly ($2p < 0.05$ according to the Student t-test) relative to the powder formulations. Therefore, the oral liquid formulations considerably enhanced the absorption rate and the maximal blood levels.

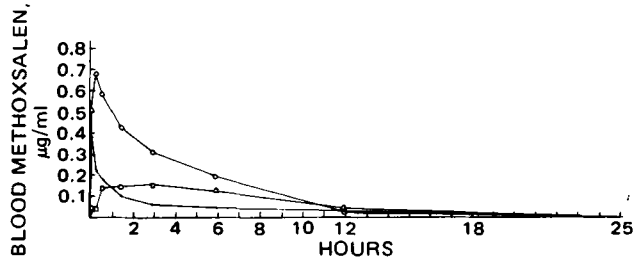


Figure 6—*Blood levels of methoxsalen in Dog B. Key: see Fig. 5.*

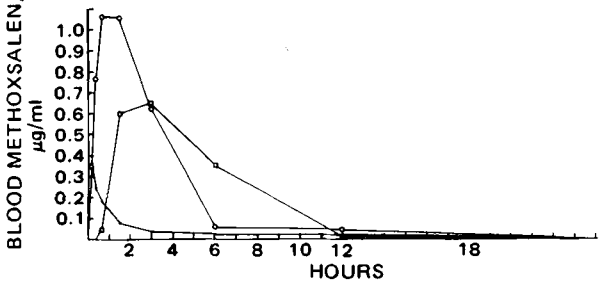


Figure 7—*Blood levels of methoxsalen in Dog C. Key: see Fig. 5.*

REFERENCES

(1) *Drug Stand.*, **25**, 89 (1957).
(2) S. W. Becker, Jr., *J. Am. Med. Assoc.*, **173**, 1483 (1960).
(3) E. Sidi and J. Bourgeous-Gavardin, *J. Invest. Dermatol.*, **18**, 391 (1952).
(4) A. B. Lerner, C. R. Denton, and T. B. Fitzpatrick, *ibid.*, **20**, 299 (1953).
(5) E. W. Kelly, Jr., and H. Pinkus, *ibid.*, **25**, 453 (1955).
(6) M. A. Pathak and T. B. Fitzpatrick, *ibid.*, **32**, 255 (1959).
(7) G. Rodighiero, L. Musajo, F. Dall'Acqua, S. Marciani, G. Carporale, and L. Ciavatta, *Biochim. Biophys. Acta*, **217**, 40 (1970).
(8) J. F. Walter, J. J. Voorhees, W. H. Kelsey, and E. A. Duell, *Arch. Dermatol.*, **107**, 861 (1973).
(9) J. A. Parrish, T. B. Fitzpatrick, L. Tanenbaum, and M. A. Pathak, *N. Engl. J. Med.*, **291**, 1207 (1974).
(10) J. W. Melski, L. Tanenbaum, J. A. Parrish, T. B. Fitzpatrick, and H. L. Bleich, *J. Invest. Dermatol.*, **68**, 328 (1977).
(11) S. W. Becker, Jr., *Science*, **127**, 878 (1958).
(12) F. Dall'Acqua, S. Marciani, L. Ciavatta, and G. Rodighiero, *Z. Naturforsch.*, **26b**, 561 (1971).
(13) M. A. Pathak, F. Dall'Acqua, G. Rodighiero, and J. A. Parrish, *J. Invest. Dermatol.*, **62**, 347 (1974).
(14) W. Schalla, H. Schaefer, B. Kammerau, and A. Zesch, *ibid.*, **66**, 258 (1976).
(15) J. Gazith and H. Schaefer, *Biochem. Med.*, **18**, 102 (1977).
(16) S. Lerman and R. F. Borkman, *Science*, **197**, 1287 (1977).
(17) I. Steiner, T. Prey, F. Gschnait, J. Washüttl, and I. Greiter, *Arch. Derm. Res.*, **259**, 299 (1977).
(18) J. G. Wagner, "Fundamentals of Clinical Pharmacokinetics," 1st ed., Drug Intelligence, Hamilton, Ill., p. 45.

ACKNOWLEDGMENTS

Supported in part by funds provided by INTERx Research Corp.

High-Pressure Liquid Chromatographic Determination of Amoxicillin in Urine

TEH LO LEE ˣ, LUCIUS D'ARCONTE †, and MARVIN A. BROOKS

Received June 23, 1978, from the *Department of Biochemistry and Drug Metabolism, Hoffmann-La Roche Inc., Nutley, NJ 07110.* Accepted for publication September 12, 1978. †Deceased.

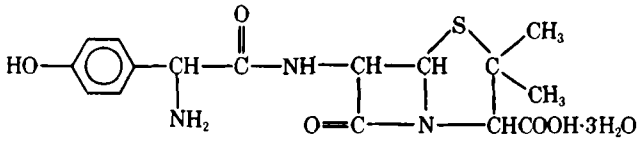
Abstract □ A rapid and specific high-pressure liquid chromatographic (HPLC) assay was developed for the simultaneous determination of amoxicillin and its penicilloic acid metabolite in urine. The two compounds, assayed directly in urine or after dilution with water–methanol (85:15), are separated by reversed-phase chromatography and quantitated spectrofluorometrically following postcolumn derivatization with fluorescamine. Linear calibration curves were measured in the ranges of 25–250 and 50–400 ng injected for amoxicillin and the penicilloic acid metabolite, respectively. The sensitivity limit of the assay is 2.5–5.0 μg/ml of urine for amoxicillin and the penicilloic acid metabolite. Urine samples (0–8 hr) taken from six subjects following single 250-mg po doses and assayed by HPLC showed ranges of cumulative percent of the dose excreted as amoxicillin and the penicilloic acid metabolite (reported as amoxicillin equivalents) of 50.2–68.0 and 21.6–30.0%, respectively. An excellent correlation (r = 0.985) was demonstrated for the measurement of amoxicillin concentrations by the HPLC and microbiological assays.

Keyphrases □ Amoxicillin—high-pressure liquid chromatographic determination in urine □ Antibacterials—amoxicillin and penicilloic acid metabolite, high-pressure liquid chromatographic determination in urine □ High-pressure liquid chromatography—analysis, amoxicillin and penicilloic acid metabolite in urine

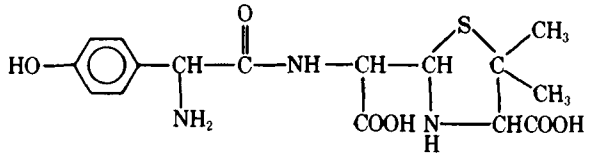
Amoxicillin[1] (I) [D-(−)-α-amino-*p*-hydroxybenzylpenicillin trihydrate], synthesized from 6-aminopenicillanic acid, is an orally absorbed, acid-stable, semisynthetic, broad-spectrum antimicrobial agent (1–3). Studies on its biotransformation demonstrate that it is excreted in urine intact and as the penicilloic acid of amoxicillin (II) [6-D-(−)-α-amino-*p*-hydroxyphenylpenicilloic acid] (4, 5).

BACKGROUND

Routine determinations of I in biological fluids are usually performed by microbiological assay using one of a variety of sensitive bacterial strains



I



II

(6). Since the metabolic cleavage of the β-lactam ring to II inactivates this compound toward microbiological activity, the microbiological assays are specific for I in the presence of II. The analysis of II has been reported using a separate aliquot of the sample by iodometric titration (7) and by TLC, followed by formation of an ammonium–molybdate complex and spectrophotometry (5).

Spectrofluorometric methods are capable of measuring ampicillin (8–10) and amoxicillin (11–14) in serum, plasma, or urine following therapeutic doses. The spectrofluorometric assays are based on the formation of strongly fluorescent products by heating either in the presence of formaldehyde (8, 11–13) or uranylacetate in acid solution (10) or at room temperature in the presence of mercuric chloride in neutral solution (9, 14). Jusko (8) postulated that the fluorescent product formed is a 3,6-disubstituted diketopiperazine and demonstrated that ampicillin levels measured by the spectrofluorometric assay were appreciably higher than those measured in identical samples by a microbiological assay. This result was attributed to the formation of the identical fluorophore by both the penicilloic acid and the penicillin.

Thus, the spectrofluorometric methods described using formaldehyde (8, 11–13) or uranylacetate (10) are "total" assays, which measure the sum of the penicillin and penicilloic acid. The spectrofluorometric methods using mercuric chloride described for ampicillin (9) and amoxicillin (14) differ from the other methods employing formaldehyde (8, 11–13) in that

[1] Larotid, Hoffmann-La Roche Inc., Nutley, NJ 07110.

0022-3549/79/0400-0454$01.00/0