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Note

Determination of nitrofurantoin (Furadantine®) and hydroxymethylnitrofurantoin (Urfadyn®) in plasma and urine of man by means of high-performance liquid chromatography

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Nitrofuran derivatives are highly effective chemotherapeutic drugs in the treatment of chronic pyelonephritis and other infections of the urinary tract [1]. The effectiveness of the treatment is influenced by the urinary pH, the concentration of the drug in the urine and the nature and concentration of the bacteria [1—3]. The widely used colorimetric method of Conklin and Hollifield has a limit of sensitivity of 2 μg/ml [4,5]. This does not allow accurate measurement of plasma concentrations, and also lacks specificity with regard to possible metabolites [6]. For the purpose of the study of bioavailability of nitrofuran derivatives from their pharmaceutical preparations, a high-performance liquid chromatography (HPLC) method was developed. This method enables the determination of plasma concentrations as low as 0.02 μg/ml and therefore can be used for study of the bioavailability of these drugs and their rate of dissolution as a function of their pharmaceutical formulation. Pharmacokinetic parameters of these drugs such as half-lives, renal clearance constants and other distribution parameters may be obtained in this way.

MATERIALS AND METHODS

Apparatus

A Spectra Physics 3500 B high-performance liquid chromatograph equipped with a spectrophotometric detector (Model 770) was used. The detector was

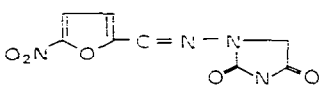
connected to a 1 mV recorder (BD7; Kipp & Zonen, Delft, The Netherlands). A stainless-steel column, 15 cm × 4.6 mm I.D. packed with LiChrosorb RP-8, particle size 5 μm, was used. An injection loop of 100 μl was used. The detection of the nitrofurantoin and its derivatives was performed at 370 nm, the detection limit being 0.02 μg/ml.

Solvent

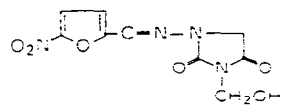
The solvent was a mixture of water with 5% ethanol. The solvent flow accounted for 1.6 ml/min at a pressure of 174 atm.

Drugs

Nitrofurantoin, nitrofurazolidone and nitrofural were from Norwich Benelux (Utrecht, The Netherlands), hydroxymethylnitrofurantoin was from Inpharzam (Amsterdam, The Netherlands) and metronidazole was obtained from Specia (Amstelveen, The Netherlands).



NITROFURANTOIN



HYDROXYMETHYLNITROFURANTOIN

Subjects

Six healthy volunteers, all employees of the Department of Clinical Pharmacy participated in this study. Nitrofurantoin was administered in a dose of 100 mg (0.42 mole) as Furadantine® tablets. Hydroxymethylnitrofurantoin was administered in a dose of 120 mg (0.45 mole) Urfadyn® tablets. The drug was taken orally in the morning, 1.5 h after a standard breakfast of two slices of bread and a cup of tea [6,7].

Blood samples of 0.2 ml were collected at scheduled time intervals by fingertip puncture (Microlance No. 433, Becton-Dickinson). Spontaneously voided urine was collected over 15 h. The pH of the urine was maintained alkaline (pH 7—8) in the volunteers by the regular intake of 10 g of sodium bicarbonate per day. An acidic urine pH was reached by the intake of 8 g of ammonium chloride per day (pH 5—6) [8,9].

Sample preparation

Plasma. A 10-μl volume of human plasma is mixed thoroughly on a Vortex mixer with 0.5 ml of perchloric acid (0.33 *N*). After centrifugation at 2600 *g* for 5 min (Heraeus Christ centrifuge), 100 μl of the supernatant is injected onto the column. A calibration curve is produced by adding known concentrations of either nitrofurantoin or hydroxymethylnitrofurantoin to blank human plasma.

Urine. A 10-μl volume of human urine is mixed on a Vortex mixer with 0.5 ml of perchloric acid (0.33 *N*). A 100-μl aliquot of the solution is injected onto the column.

Sample stability

An aqueous solution of nitrofurantoin appeared to be unstable on standing at room temperature. Fig. 1 shows the degradation of nitrofurantoin in a urine sample, diluted with water or with perchloric acid. After 4 h of standing at room temperature only 50% of the peak height related to a freshly extracted sample could be measured, also the peak shape of nitrofurantoin was altered (peak broadening). This phenomenon may be due to photochemical degradation of the compound in solution at room temperature, which leads to compounds with only a slightly altered retention time, but with a reduced molar extinction. Therefore all blood and urine samples were processed upon receipt and within 5 min of preparation of the samples for injection. Blood and urine samples remained stable when stored at - 20° [10].

Recovery

Recovery of nitrofurantoin and hydroxymethylnitrofurantoin added to human plasma in the concentration range of 0.02—10 μg/ml was found to be 92 ± 4 % S.D. The recovery of nitrofurantoin and hydroxymethylnitrofurantoin added to urine was 100 ± 2 % S.D.

RESULTS

Nitrofuran derivatives are well separated from each other and from the structurally related metronidazole (Flagyl®), as can be seen from Fig. 2 and Table I. Chromatograms of blanks did not show any interfering substances from plasma

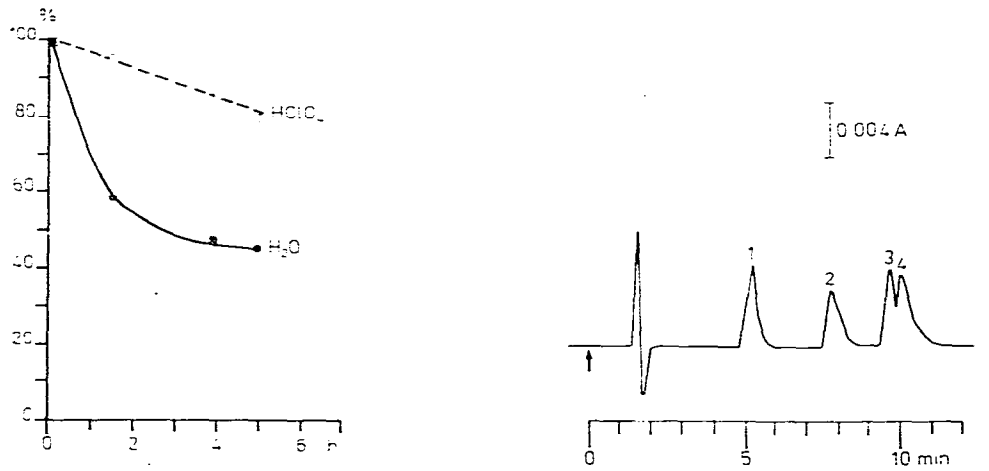


Fig. 1. Instability of nitrofurantoin solutions in water or perchloric acid upon standing at room temperature. The peak height of the nitrofurantoin in the chromatogram obtained after standing is expressed as a percentage of the peak height of a freshly extracted sample.

Fig. 2. High-performance liquid chromatogram of a mixture of nitrofuran derivatives. Column, LiChrosorb 5 RP-8; solvent, 5% ethanol in water; solvent flow, 1.6 ml/min, 1 = metronidazole, 2 = nitrofurantoin and hydroxymethylnitrofurantoin, 3 = nitrofural, and 4 = nitrofurazolidone.

and urine at the wavelength of detection, 370 nm. Within the indicated concentration ranges for plasma and for urine a linearity between peak height ratio and concentration could be established ($r = 0.99$).

The pharmacokinetic parameters of nitrofurantoin and hydroxymethyl-

TABLE I

RELATIVE RETENTION TIMES OF NITROFURANTOIN, HYDROXYMETHYLNITROFURANTOIN AND SOME RELATED COMPOUNDS

Column, LiChrosorb 5 RP-8; solvent, water + 5% ethanol; solvent flow, 1.6 ml/min.

Compound	Retention time relative to the unretained compound (K')
Metronidazole (Flagyl®)	3.21
Nitrofurantoin (Furadantine®)	4.72
Hydroxymethylnitrofurantoin (Urfadyn®)	4.72
Nitrofural (Nitrofurazone®, Furacine®)	5.88
Nitrofurazolidone (Furoxone®)	6.12

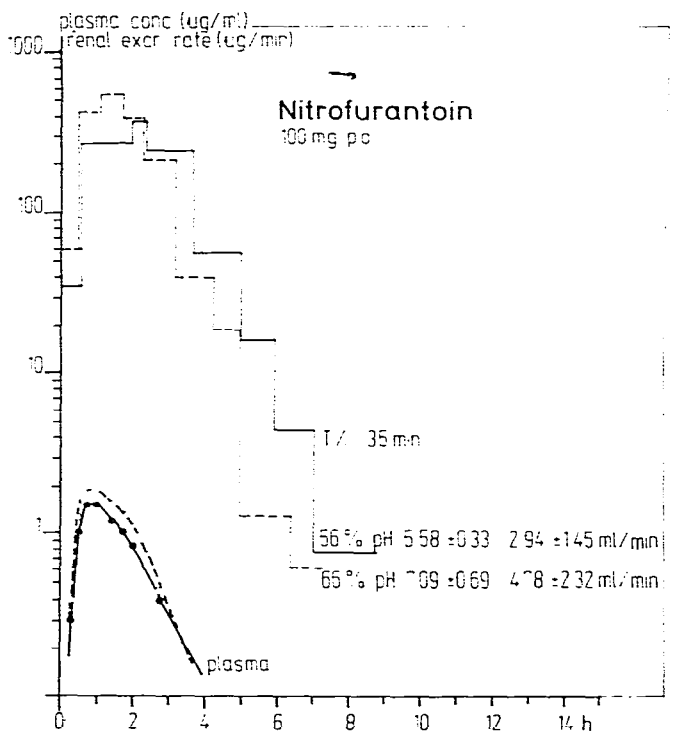


Fig. 3. Plasma concentration (μg/ml) and renal excretion rate (μg/min) of nitrofurantoin in man after administration of 100 mg (Furadantine orally. Note that in the same subject under alkaline urinary conditions the absorption and renal excretion rate are somewhat higher than under acidic urinary conditions (Table II).

TABLE II

SOME PHARMACOKINETIC PARAMETERS OF NITROFURANTOIN AND HYDROXYMETHYLNITROFURANTOIN IN MAN

Compound	Subjects (*n*)	Urine pH	Bodyweight (kg)	Dose (mg)	Amount excreted unchanged (%)	Plasma t½ (min)	Maximum plasma concentration (μg/ml)	Maximum renal excretion rate (μg/min)
Nitrofurantoin	6	5—7	63.7 ± 4.5	100	70.4 ± 20.8	45 ± 15	2.33 ± 0.42	466 ± 106
Hydroxymethyl-nitrofurantoin	6	5—7	63.7 ± 4.5	120	65.4 ± 15.6	45 ± 10	1.20 ± 0.30	360 ± 55

nitrofurantoin in man under either alkaline or acidic urinary pH conditions reveal only small differences. Fig. 3 shows that nitrofurantoin in one volunteer is eliminated with the same half-life of 35 min under alkaline as well as acidic urinary pH conditions. The total amount excreted under alkaline conditions is 66% while in the same subject this figure under acidic urinary conditions was 56%.

Hydroxymethylnitrofurantoin is excreted with almost the same half-life ($t_{1/2}$ = 30 min) as nitrofurantoin (Fig. 4). The amount of hydroxymethylnitrofurantoin excreted unchanged under alkaline conditions was somewhat less (54%) than under acidic conditions (60%). These differences are within the variation when the amounts totally excreted in the whole group of volunteers (Table II) are considered. The renal clearance (K_r) of both compounds is not dependent on the urinary pH and the urine flow, and shows a linear relationship between the renal excretion rate and plasma concentration, indicating that the excretion process is a linear one.

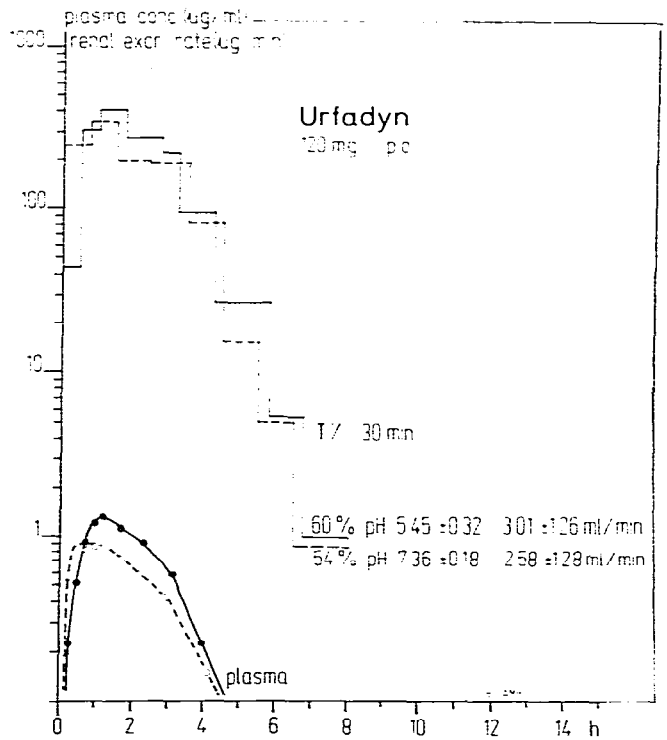


Fig. 4. Plasma concentration (μg/ml) and renal excretion rate (μg/min) of hydroxymethylnitrofurantoin in man after administration of 120 mg Urfadyn orally. Note that in the same subject under acidic urinary conditions the absorption and renal excretion are slightly higher than under alkaline urinary conditions (Table II).

DISCUSSION

The HPLC method of determining the nitrofuran derivatives has the advantages over the colorimetric method of higher sensitivity, specificity and rapidity, allowing the measurement of plasma concentration in the therapeutic range. A limit of sensitivity of 0.2 μg/ml was already reached with the improved spectrophotometric Hyamine 10 X method [11], and a recently published alternative HPLC method [10] had reached the limit of 0.02 μg/ml. However, this method [10] required 0.2 ml of biological fluid, which can only be obtained by venipuncture. The present method needs only 0.01 ml of plasma allowing fingertip puncture. The advantage of the present method over the method of Aufrère et al. [10] furthermore is its rapidity, its ease of handling during sample preparation and also the possibility of determining, with no modification, the structurally-related drug metronidazole (Flagyl).

The simplicity of the method makes it useful for studies on the bioavailability of the drug [12], for routine monitoring, e.g., for drug compliance studies, and of course for the determination of pharmacokinetic parameters. Influence of renal impairment on plasma concentration and renal excretion rate can be studied in this way. The difference in chemotherapeutic behaviour with changing pH of the urine [2,3,9] is not related to differences in pharmacokinetic behaviour as renal excretion rates and half-lives are almost identical under alkaline and acidic urinary conditions. Derivatives and pharmaceutical formulations have been produced with the prospect of reduced side-effects, especially in the stomach.

The clinically observed differences in tolerance and therapeutic efficiency of the various marketed products have stimulated the investigation of the relative bioavailabilities of hydroxymethylnitrofurantoin and nitrofurantoin from their pharmaceutical formulation. This method is appropriate to such pharmacokinetic bioavailability studies of different nitrofuran-derivatives and pharmaceutical formulations, and the results of the study will be published elsewhere, together with its clinical implications.

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