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Note

Determination of the diuretic agent metolazone in plasma by high-performance liquid chromatography

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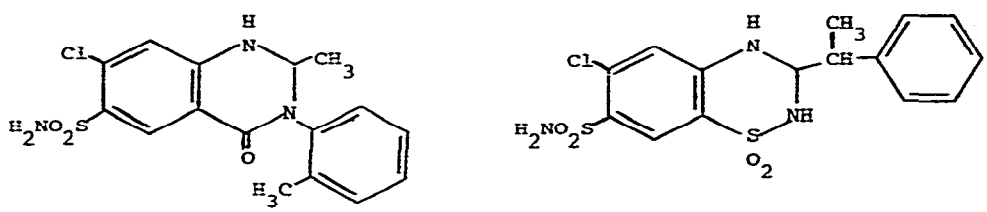
Metolazone (Fig. 1) is a recently introduced diuretic drug structurally and pharmacologically related to quinethazone [1]. It causes increased excretion of sodium and chloride, and, to a lesser extent, of potassium. On a weight basis the drug is about ten times more potent in the rat for promoting the excretion of sodium than is quinethazone or hydrochlorothiazide, but its effect on the excretion of potassium in humans is less than that produced by other diuretics [2].



Metolazone Bemetizide

Fig. 1. Chemical structures of metolazone and internal standard (bemetizide).

Metolazone has been measured in urine by high-performance liquid chromatography (HPLC) over the concentration range of 1—10 μg/ml [3, 4] and more recently by fluorimetry over the concentration range 0.1—10 μg/ml [5]. In order to obtain reliable pharmacokinetic data, accurate, precise, sensitive (< 10 ng/ml) and specific methods of measurement of drug concentrations in plasma are usually required. This paper describes an HPLC method for the measurement of metolazone in plasma over the concentration range

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2—200 ng/ml. It involves a simple liquid—liquid partition extraction stage followed by chromatography in a reversed-phase mode and detection by ultraviolet (UV) absorption at 236 nm. The thiazide diuretic bemetizide is used as the internal standard and the method has been applied to the measurement of unchanged drug in the plasma of rhesus monkeys receiving oral doses of 2.5 mg of metolazone administered in solution.

Since the work described in this paper was performed, another HPLC method for the measurement of metolazone in plasma and urine has been published [6]. This method requires the use of a fluorimetric detector and although this appears to be a precise and perhaps more sensitive (1 ng/ml) method than the one described here (2 ng/ml), it requires the extraction of a larger plasma volume (2 ml instead of 0.5 ml), which is not always practical, particularly in the case of animal studies as are described here.

EXPERIMENTAL

Materials

All reagents were of analytical grade and all inorganic reagents were prepared in freshly glass-distilled water. Acetonitrile was HPLC grade (Fisons Scientific Apparatus, Loughborough, Great Britain) and diethyl ether was freshly redistilled prior to use. Metolazone (7-chloro-1,2,3,4-tetrahydro-2-methyl-4-oxo-3-*o*-tolyl-6-quinazolinesulfonamide) was provided by Sandoz, Basle, Switzerland, and bemetizide [3-(α-methylbenzyl)-6-chloro-7-sulphamoyl-3,4-dihydro-1,2,4-benzothiadiazine-1,1-dioxide], used as internal standard, was from Sanol Schwarz-Monheim, Monheim, G.F.R. Standard solutions of metolazone were prepared in methanol at concentrations of 1 μg/ml and 10 μg/ml and bemetizide at 11 μg/ml, and stored at —20°C throughout the study.

Extraction procedure

Plasma samples (0.5 ml) were transferred into 10-ml pointed centrifuge tubes and spiked with internal standard (5 μl, containing 55 ng of bemetizide). Distilled water (0.5 ml) was added together with sodium hydrogen carbonate (about 0.2 g) and the samples were extracted by mixing them with diethyl ether (5 ml) for 30 sec using a vortex mixer. After centrifugation of the extract at 2000 *g* for 10 min, the organic layer was transferred to another pointed centrifuge tube and the solvent was evaporated to dryness at 37°C under a stream of nitrogen. The residue was washed to the bottom of the tube with a small amount of ether which was again removed by evaporation. The residue was taken up in mobile phase (40 μl, see later), centrifuged at 2000 *g* for 10 min, and the resulting clear solution transferred to autosampling vials. Aliquots (15 μl) of this solution were injected into the chromatograph.

Liquid chromatography

The liquid chromatograph consisted of a Waters M6000A pump (Waters Assoc., Northwich, Great Britain) coupled to a Pye LC3 variable-wavelength UV-absorption detector (Pye-Unicam, Cambridge, Great Britain) operated at 236 nm. Injection was via an automatic injector (Wisp™, Waters Assoc.). Chromatograms were recorded on a Hewlett-Packard 3380A recording inte-

grator (Hewlett-Packard, Slough, Great Britain) using a 1-V input and an attenuation setting of 16; peak height measurements were preferred to peak area measurements. The column was constructed of stainless steel (30 cm × 0.4 cm I.D.), pre-packed with μBondapak C_{18} (mean particle diameter 10 μm) (Waters Assoc.). A pre-column (7 cm × 0.2 cm I.D.) constructed of stainless steel and dry-packed with pellicular Co:Pell® ODS (particle diameter 25—37 μm) (Whatman, Maidstone, Great Britain) was installed in front of the analytical column to protect it from contamination and was changed routinely if the back-pressure in the system increased.

Chromatography was performed in a reversed-phase mode using a mobile phase of 40% (v/v) acetonitrile in aqueous potassium dihydrogen orthophosphate (0.1%, w/v) with a flow-rate of 2 ml/min. The retention times of metolazone and bemetizide (internal standard) were 3.6 min and 5.5 min, respectively (Fig. 2).

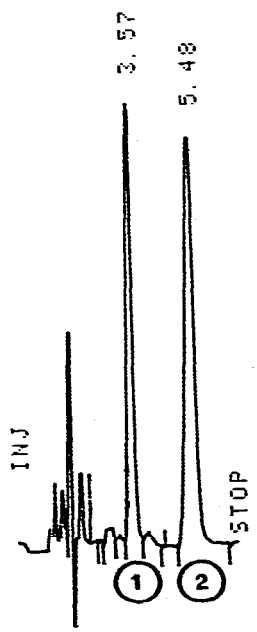

Fig. 2. Chromatogram of reference standards; peak 1 = metolazone, peak 2 = internal standard. Column, 30 × 0.4 cm I.D., pre-packed with μBondapak C_{18}; flow-rate, 2 ml/min; solvent system, 40% (v/v) acetonitrile—aqueous potassium dihydrogen orthophosphate (0.1%, w/v); detector, UV at 236 nm.

Calibration procedure

Calibration lines of peak height ratio measurements of metolazone to internal standard were constructed over the concentration range up to 200 ng/ml. Samples of blank (drug-free) plasma (0.5 ml) were spiked with metolazone to give concentrations of 2, 10, 40, 100, 140 and 200 ng/ml and with internal standard at a fixed concentration of 110 ng/ml. The samples were taken through the extraction procedure described previously.

Studies in monkeys

Plasma samples were obtained from eight adult female rhesus monkeys (*Macaca mulatta*) (bodyweights about 5—7 kg) after administration of 2.5 mg of metolazone, dissolved in a mixture of propylene glycol—water (5 ml, 2 : 3, v/v), given by oral intubation and washed in with distilled water (5 ml). During 16 h before dosing and for 6 h thereafter, each monkey was fasted, although water was allowed during this period.

Blood samples (3 ml) were withdrawn from the femoral veins of the animals into heparinised tubes before dosing and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 24, 32, 48, and 72 h after dosing. Blood cells were separated by centrifugation and discarded. The separated plasma was stored at —20°C until analysed. Metolazone was found to be stable in plasma stored at —20°C for several weeks.

RESULTS AND DISCUSSION

Precision

Extraction and measurement at each concentration was repeated on five occasions. The precision of the method for the measurement of metolazone in plasma as indicated by the coefficient of variation of peak height ratio measurements of drug to internal standard (Table I) was ± 12% at 10 ng/ml, ± 4% at 100 ng/ml and ± 6% at 200 ng/ml; the precision at the limit of detection of the method, 2 ng/ml, was ± 50%.

TABLE I

RECOVERY AND PRECISION MEASUREMENTS OF METOLAZONE FROM PLASMA

Concentration of metolazone added to plasma (ng/ml)	Recovery* (%)	Coefficient of variation (%)
10	72	12
40	73	8
100	72	4
140	72	4
200	74	6
Mean	73 ± 1 S.D.	

*Mean of five determinations at each concentration.

Accuracy

The calibration line for the measurement of metolazone in plasma was constructed from five replicate measurements at six concentrations over the range up to 200 ng/ml; plots of peak height ratio against concentration of metolazone (ng/ml) were linear ($y = 0.0110x - 0.0315$) and the value of the intercept was not significantly different from zero ($p > 0.05$).

The variance about the calibration was found to be concentration depen-

dent (inhomogeneous) and Bartlett's statistic [7] was significant at the 1% level.

The variance was homogenised by logarithmic transformation of the data, and a plot of $\log_e$ of peak height ratio against $\log_e$ concentration was linear ($\log_e y = a + b \log_e x$) where $a = -5.0429$ and $b = 1.1037$. The accuracy of the method as indicated by the coefficient of variation about the fitted line was ± 16% over the concentration range 2—200 ng/ml.

Recovery

The recovery of internal standard (110 ng/ml) from plasma (0.5 ml) was 80% ± 3 S.D. ($n = 5$). The mean absolute recovery of metolazone from plasma over the concentration range 10—200 ng/ml was determined by comparison of peak height ratio measurements of non-extracted standards to those of extracted standards corrected for losses of internal standard, and was 73% ± 1 S.D. (Table I).

Limit of detection

No interfering peaks were present in pre-dose (control) plasma with the same retention times as metolazone or internal standard (Fig. 3). The limit of detection of metolazone under the experimental conditions used (signal-to-noise ratio = 2 : 1) was 2 ng/ml when a 0.5-ml plasma sample was analysed.

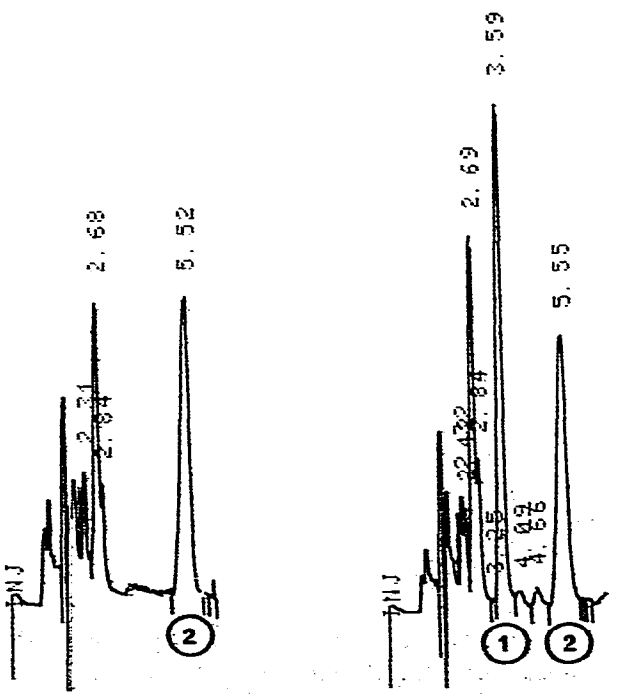

Fig. 3. Chromatograms of (left) pre-dose (control) plasma containing internal standard, and (right) 3-h post-dose plasma sample containing metolazone at a concentration of 166 ng/ml. Peak 1 = metolazone, peak 2 = internal standard. Chromatographic conditions as for Fig. 2.

Concentrations of metolazone in plasma

After single oral doses of 2.5 mg of metolazone to eight rhesus monkeys, a peak of mean concentrations of 202 ng/ml was reached at 1 h after dosing (Table II). Mean concentrations declined to 23 ng/ml at 8 h and were below the limit of detection at 24 h. The mean half-life of metolazone in plasma was 2.20 h ± 1.04 S.D. (Table III).

TABLE II

CONCENTRATION OF METOLAZONE IN THE PLASMA OF EIGHT RHESUS MONKEYS AFTER A SINGLE ORAL DOSE OF 2.5 mg

Results are expressed in ng/ml. For concentrations > 200 ng/ml, smaller aliquots of plasma (0.1—0.25 ml) were analysed.

Time (h)	Animal No.								Mean ± S.D.
	1	2	3	4	5	6	7	8	
0.5	9	58	82	274	24	395	425	270	192 ± 169
1	13	202	192	296	52	315	355	192	202 ± 122
1.5	26	206	214	222	62	305	275	182	187 ± 97
2	29	208	214	184	68	181	167	143	149 ± 67
3	166	156	184	114	127	102	105	103	132 ± 32
4	191	125	121	84	127	70	64	61	105 ± 44
6	100	58	52	43	94	37	25	36	56 ± 27
8	36	25	26	21	28	18	10	18	23 ± 8
24	< 2	< 2	< 2	9	4	< 2	< 2	< 2	< 2
32	< 2	< 2	< 2	3	< 2	< 2	< 2	< 2	< 2
48	< 2	< 2	< 2	3	< 2	< 2	< 2	< 2	< 2
72	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2

TABLE III

HALF-LIVES OF THE TERMINAL LINEAR SECTIONS OF THE PLASMA METOLAZONE CONCENTRATION—TIME RELATIONSHIPS

Animal No.	Half-life (h)	r^2
1	1.66	0.98
2	1.72	1.00
3	1.80	1.00
4	—	<0.90
5	4.48	0.92
6	2.02	1.00
7	1.47	1.00
8	2.27	0.99
Mean ± S.D.	2.20 ± 1.04	

ACKNOWLEDGEMENT

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