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Note

Rapid liquid chromatographic determination of debrisoquine and its hydroxy metabolite in human urine to define hydroxylation phenotypes

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Debrisoquine (D), an effective antihypertensive drug, is now used as a probe for determining a particular phenotype of hydroxylation. It is mainly metabolized into 4-hydroxydebrisoquine (HD) by cytochrome P450 isozyme aliphatic 4-hydroxylation [1,2], and the urinary D/HD concentration ratio allows a classification into poor (PM) or extensive (EM) metabolizer phenotypes in the caucasian population [3]. Several methods are currently available to measure D and HD. Gas chromatography [4] and gas chromatography–mass spectrometry [2,5] can be used after reaction with acetylacetone, but derivatization procedures of the drug and its metabolite are involved.

Three column liquid chromatographic (LC) methods for D and HD have been developed, but two did not use a internal standard [6,7] and the third again used acetylacetone derivatization [8].

This paper describes a simple and rapid LC method for the simultaneous determinations of D and HD, using an internal standard without derivatization of compounds.

EXPERIMENTAL

Reagents

Debrisoquine sulphate and 4-hydroxydebrisoquine sulphate were gifts from Hoffmann-La Roche (Basle, Switzerland). Guanoxan (G) sulphate was kindly supplied by Pfizer (Orsay, France).

Acetonitrile and hexanol were purchased from Merck (Darmstadt, F.R.G.), and all other reagents were of analytical grade.

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Stock solutions containing 100 μg/ml G and 100 μg/ml D and HD were prepared in 0.001 and 0.003 *M* hydrochloric acid, respectively, and stored at 4°C. Urine standards containing known concentrations of D, HD and G were prepared by appropriate dilution of stock solutions with drug-free urine. The urine standards were extracted in the same way as the samples. The concentrations of D and HD in samples were then determined from calibration plots of the LC peak-height ratios D/G and HD/G versus concentration.

Extraction procedure

To 1 ml of urine were added 50 μl of G solution (100 μg/ml), 1 ml of 4 *M* sodium hydroxide and 2 ml of hexanol. The samples were shaken for 10 min. After centrifugation (1500 *g*, 10 min), the upper hexanol layer was removed and an aliquot (50–200 μl) of this extract was injected onto the LC column.

Chromatographic conditions

The LC system consisted of a Model 6000A pump and a WISP automatic injector (Waters Assoc., Milford, MA, U.S.A.), a variable-wavelength UV detector (Cecil Instruments, Cambridge, U.K.) and a chart recorder (Ifelec, Paris, France). Chromatography was carried out on a C_8 reversed-phase column (100×5 mm I.D., 10 μm) in a Z module system (Waters Assoc.). The mobile phase was acetonitrile–8 m*M* potassium dihydrogenphosphate pH 4.8 (45:55) at a flow-rate of 2 ml/min. D, HD and G were monitored at 200 nm.

RESULTS AND DISCUSSION

Fig. 1 shows the chromatograms obtained after extraction of two drug-free urine samples, the first supplemented with only 5 μg/ml G as internal standard (A) and the second spiked with 5 μg/ml D and HD (B). The chromatograms obtained after extraction of urine from a PM and EM subject are shown in Fig. 2. Under these LC conditions, the retention times of HD, G, D were 4.4, 5.8, 6.8 min, respectively. When added to urine, the respective recoveries (mean ± S.D., $n=10$) were 67.5 ± 3.2% for HD, 97.3 ± 2.3% for D, 94.3 ± 3.8% for G.

The linear regression curve for calibration can be described by the equations $y_D = 0.142x + 0.014$ ($r=0.999$) and $y_{HD} = 0.149x + 0.028$ ($r=0.999$) where y_D and y_{HD} are the D/G and HD/G peak-heights ratios. The coefficients of variation (C.V.) for identical samples were 5.4% for D and 2.5% for HD at 1 μg/ml and 1.3% for D and 1.5% for HD at 10 μg/ml ($n=10$ at each concentration). The day-to-day C.V. for the slopes of the calibration curves were 3.3% for D and 2.1% for HD ($n=5$). The detection limit was found to be lower than 0.2 μg/ml D and HD.

In the method of Lennard et al. [4], the three compounds were derivatized with acetylacetone before extraction, in order to increase their solubilities in organic solvents. We found these compounds may be extracted from alkaline urine by hexanol, with a good recovery, without any prior transformation. Hexanol, a polar solvent, has a lower polarity than other solvents, dimethyl ether, ethyl acetate and chloroform. This explains why it can extract both the more and the less

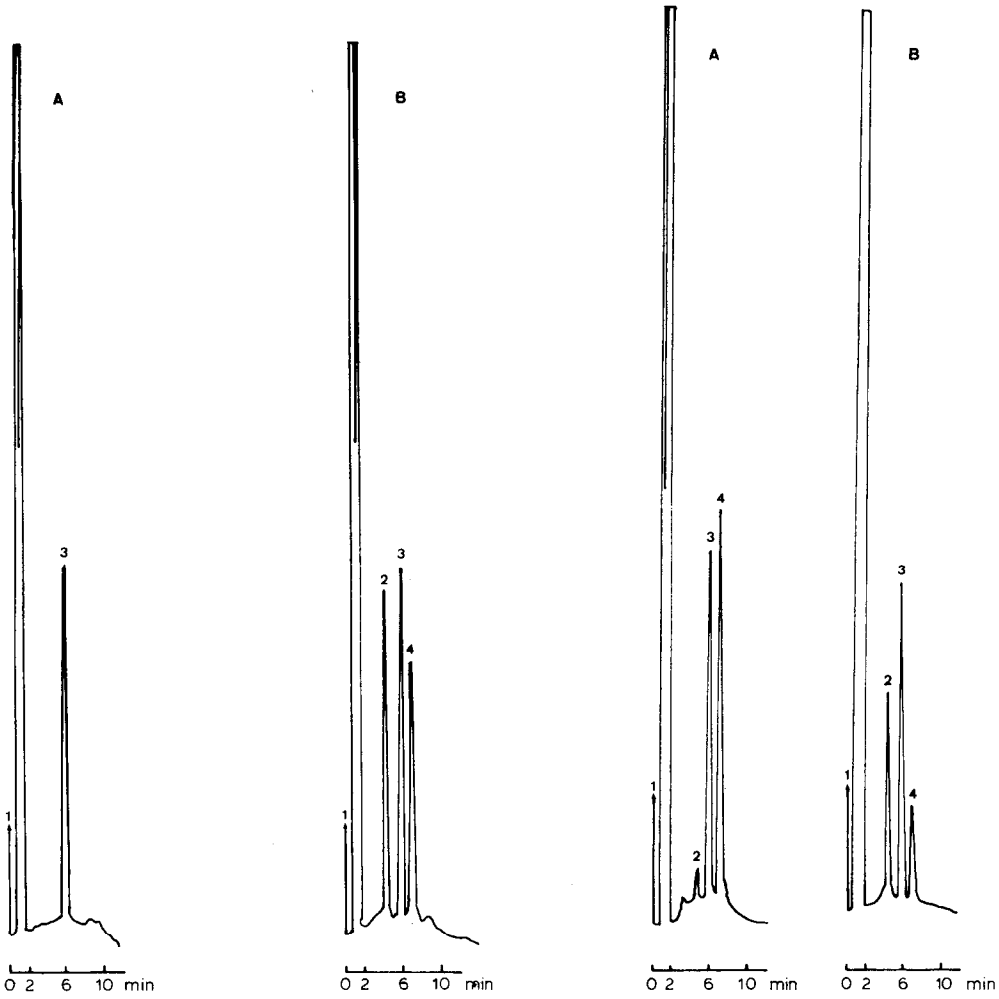

Fig. 1. Chromatograms of (A) a urine extract (100 μl) containing 5 μg/ml guanoxan as internal standard, and (B) a urine extract (100 μl) spiked with 5 μg/ml debrisoquine (D) and 5 μg/ml 4-hydroxydebrisoquine (HD), monitored at 100 nm. Peaks: 1 = injection site; 2 = 4-hydroxydebrisoquine; 3 = guanoxan; 4 = debrisoquine.

Fig. 2. Chromatograms of urine extracts (100 μl) collected (0–8 h) (A) from a PM subject (where 7.45 μg/ml D and 0.50 μg/ml HD were found), and (B) from an EM subject (where 1.85 μg/ml D and 3.38 μg/ml HD were found) after a 10-mg oral dose of debrisoquine sulphate. D and HD were monitored at 200 nm. Peaks: 1 = injection; 2 = HD; 3 = G; 4 = D.

polar compounds, HD and D (and G), respectively. The corresponding chromatograms are clear. This may seem surprising but it is due to the fact that all polar compounds of urine are not extracted and probably also to the presence of some impurities in the solvent front. However, for some samples from patients interfering peaks may occur; in these cases an additional urine washing step is carried out with chloroform or dimethyl ether. To separate the three compounds, it was

necessary to use phosphate buffer with low molarity (8 m*M*) associated with acetonitrile.

In conclusion, this method is accurate, rapid and available for routine estimation of debrisoquine hydroxylation phenotypes.

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