Journal of Chromatography, 374 (1986) 204—208
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2850

Note

Determination of debrisoquine and its 4-hydroxy metabolite in urine by high-performance liquid chromatography

P.M. HARRISON*, A.M. TONKIN, S.T. DIXON and A.J. McLEAN

Department of Clinical Pharmacology, Alfred Hospital, Commercial Road, Prahran, 3181 Melbourne (Australia)

(First received June 19th, 1985; revised manuscript received August 27th, 1985)

Debrisoquine (DEB), an antihypertensive drug, undergoes oxidative metabolism to form 4-hydroxydebrisoquine (DEB-OH), the major metabolite [1, 2]. This extensive hydroxylation has been shown to be genetically controlled with two phenotypes present, extensive metabolisers (EM) and poor metabolisers (PM), the latter group representing less than 10% of the population [3]. The drug is used to phenotype subjects with respect to aromatic hydroxylation generally. The hydroxylation status of a subject is determined by measuring the concentrations of DEB and its hydroxy metabolite in urine collected over an 8-h period after an oral dose of DEB.

Methods presently available to measure DEB and/or its hydroxy metabolite use either gas chromatography (GC) [4] or gas chromatography—mass spectrometry (GC—MS) [2, 5—7]. Three of these methods are suitable to measure the hydroxy metabolite [2, 4, 7] and they all involve lengthy preparation of an internal standard and time-consuming derivatization procedures.

We report a new extraction method, based on the use of short columns of silica modified with carboxylic acid ion-exchange functional groups (CBA Bond-Elut^{TM}), which quantitatively extracts DEB and the 4-hydroxy metabolite from urine followed by analysis using high-performance liquid chromatography (HPLC). This method is simple, rapid and highly reproducible, eliminating the need for an internal standard. Assay of the extracts using HPLC with detection by UV absorption eliminates any need for long derivatization procedures and expensive detectors (mass spectrometer), while maintaining a high degree of selectivity.

The use of short columns of modified silica (solid-phase extraction) has

0378-4347/86/$03.50

recently been applied successfully to the assay of atenolol and metoprolol [8], baclofen [9] and propranolol with its metabolites [10].

EXPERIMENTAL

Materials and reagents

Debrisoquine sulphate and 4-hydroxydebrisoquine sulphate were kindly supplied by Roche Products (Australia). Acetonitrile (HPLC grade) was supplied by Waters Assoc. (Sydney, Australia) and all other reagents were of analytical grade. CBA Bond-Elut^{TM} columns (3 ml size) and a Vac-Elut^{TM} manifold (Analytichem International, Harbor City, CA, U.S.A.) were purchased from FSE Scientific (Melbourne, Australia).

The HPLC system consisted of a Constametric III pump and a SpectroMonitor III variable-wavelength UV detector (wavelength set at 220 nm) (LDC, Riviera Beach, FL, U.S.A.), a Model 7125 injection valve with a 20-μl loop (Rheodyne, Berkeley, CA, U.S.A.) and an Omniscribe chart recorder (Houston Instruments, Austin, TX, U.S.A.). The chromatography was carried out on a 25 × 0.4 cm I.D., 10-μm C_{18} μBondapak^{TM} column (Waters Assoc.,) at room temperature.

Stock solutions containing 1 mg/ml DEB and DEB—OH were prepared in distilled water and stored at 4°C. Urine standards containing known concentrations of DEB and DEB-OH were prepared by appropriately diluting the stock solutions with drug-free urine. The urine standards were extracted in the same way as the samples and the concentration of DEB and DEB-OH in samples determined from calibration plots of HPLC peak heights versus concentration, determined with urine containing known concentrations.

Extraction procedure

DEB and DEB-OH were extracted from urine using Bond-Elut columns (3 ml size) containing silica modified with carboxylic acid ion-exchange functional groups (CBA columns). The columns were placed in luer fittings in the top of the Vac-Elut chamber which has the capacity for ten columns. A vacuum of 25—50 cmHg was applied to the manifold to effect the various stages of the extraction. Prior to use the columns were activated by washing with 1 ml of acetonitrile—0.1 *M* hydrochloric acid (40:60) followed by 1 ml of distilled water. This washing procedure enables a single Bond-Elut column to be used several times.

To the activated CBA column, 1 ml of urine (sample or standard) was added, followed by 1 ml of distilled water, 1 ml of acetonitrile—distilled water (50:50), 1 ml of distilled water and 0.5 ml of 0.1 *M* hydrochloric acid. The vacuum was then released and the stainless-steel needles of the Vac-Elut chamber wiped. Appropriately labelled polypropylene tubes were placed under the columns which were then eluted with 1 ml of acetonitrile—0.1 *M* hydrochloric acid (40:60) and the vacuum was re-applied. An aliquot (20 μl) of this extract was then injected onto the HPLC column.

Chromatography

DEB and DEB-OH could be separated from other endogenous compounds of

urine with a mobile phase of acetonitrile—0.1 *M* sodium dihydrogen orthophosphate (10:90) at a flow-rate of 2 ml/min.

Extraction recoveries

The recovery of DEB and DEB-OH was estimated by comparison with a non-extracted standard at the same concentration in the acetonitrile—0.1 *M* hydrochloric acid (40:60) (i.e. buffer used to elute CBA columns). The recoveries were determined from the mean of six replicates.

Debrisoquine and 4-hydroxydebrisoquine urine levels

DEB and DEB-OH levels were measured in an 8-h collection of urine following a 10-mg oral dose of Declinax® (debrisoquine sulphate) in nine normal volunteers.

RESULTS AND DISCUSSION

Fig. 1 shows chromatograms obtained after extraction of (A) drug-free urine and (B) urine spiked with a known amount of DEB and DEB-OH. Chromatograms obtained after extraction of urine from subject 4 and subject 9 after a 10-mg oral dose of Declinax are shown in Fig. 2A and B, respectively. Under the HPLC conditions used DEB-OH and DEB had retention times of 3.6 and 11.5 min, respectively, with no interference from any endogenous urine compounds.

The mean (± S.D., n = 6) recovery from urine was found to be 86.9 ± 1.7% for DEB and 76.4 ± 1.7% for DEB-OH at 5 μg/ml.

The intra-assay precision of the method was determined from replicate assays (n = 6) of drug-free urine spiked with known concentrations of DEB and DEB-OH. Coefficients of variation of DEB and DEB-OH were 2.0 and 2.3% with an accuracy of 4.82 ± 0.10 and 4.80 ± 0.11, respectively, at 5 μg/ml.

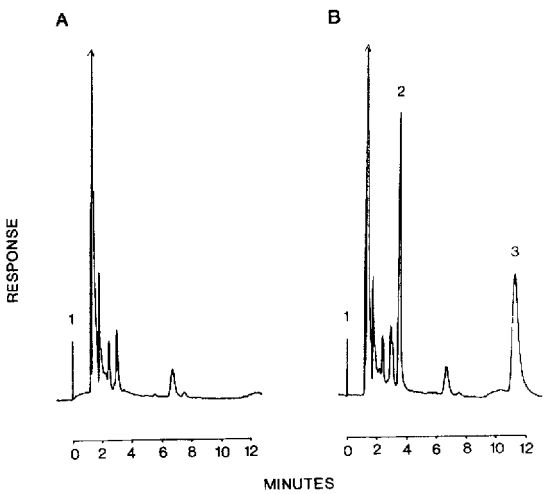

Fig. 1. HPLC profiles of (A) drug-free urine and (B) urine spiked with 5 μg/ml debrisoquine (DEB) and 5 μg/ml 4-hydroxydebrisoquine (DEB-OH). Peaks: 1 = injection site; 2 = DEB-OH; 3 = DEB.

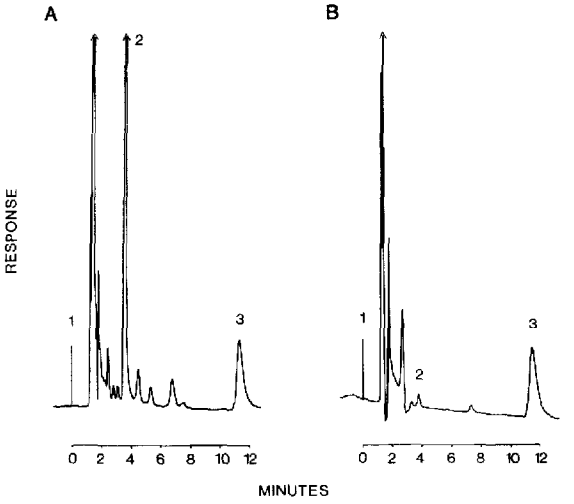

Fig. 2. HPLC profiles of urine collected (0—8 h) from (A) subject 4 (where 11.67 μg/ml DEB-OH and 2.90 μg/ml DEB were found) and (B) subject 9 (where 0.26 μg/ml DEB-OH and 4.45 μg/ml DEB were found) after a 10-mg oral dose of debrisoquine (Declinax). Peaks: 1 = injection site; 2 = DEB-OH, 3 = DEB.

The assay was linear over the urine concentration range 1—20 μg/ml (y = 10.60x, r^2 = 0.998) for DEB and 1—10 μg/ml (y = 22.14x, r^2 = 0.997) for DEB-OH.

As an example of the use of this method Table I shows the proportion of the 10-mg oral dose of Declinax excreted as DEB relative to the proportion excreted as DEB-OH in normal volunteers. According to this ratio subjects 1—8 are classified as extensive metabolisers (ratio 0—8) [3]. While the ratio for subject 9 is slightly less than the reported ratio for poor metabolisers (> 20) [3], it did give a ratio of greater than 20 when originally measured by another method based on HPLC [11] prior to twelve months storage.

In summary the method described here offers quick and straightforward sample preparation giving a total assay time of 13 min compared to much

TABLE I

URINARY LEVELS OF DEB, DEB-OH AND THE DEB/DEB-OH RATIO FOR NINE NORMAL SUBJECTS

Subject	Urinary DEB (μg/ml)	Urinary DEB-OH (μg/ml)	Percentage dose as DEB / Percentage dose as DEB-OH
1	1.17	7.82	0.16
2	0.47	3.00	0.17
3	1.31	6.96	0.20
4	2.90	11.67	0.27
5	1.22	3.51	0.37
6	2.85	1.51	2.02
7	2.62	1.33	2.11
8	14.58	3.58	4.36
9	4.45	0.26	18.34

longer times (up to 2 h) with GC methods [2, 4]. This method can accurately measure 200 ng/ml DEB and 100 ng/ml DEB-OH which is adequate for the urinary levels obtained when phenotyping for hydroxylation status. The speed and convenience of the method will substantially ease the difficulty of phenotyping large groups of subjects with DEB in population studies.

ACKNOWLEDGEMENTS

This work was supported in part by the Alfred Hospital Whole-Time Medical Specialists Private Practice Trust and the National Health and Medical Research Council of Australia. We would like to thank Dr. M.L. Mashford, University of Melbourne, Department of Medicine, St. Vincents Hospital, for supplying the urine sample of subject 9.

REFERENCES

1 M.A. Medina, A. Giachetti and P.A. Shore, Biochem. Pharm., 18 (1969) 891.
2 S.L. Malcolm and T.R. Marten, Anal. Chem., 48 (1976) 807.
3 A. Mahgoub, J.R. Idle, L.G. Dring, R. Lancaster and R L. Smith, Lancet, ii (1977) 584.
4 M.S. Lennard, J.H. Silas, A.J. Smith and G.T. Tucker, J. Chromatogr., 133 (1977) 161.
5 J.H. Hengstmann, F.C. Falkner, J.T. Watson and J. Oates, Anal. Chem., 46 (1974) 34.
6 P. Erdtmansky and T.J. Goehl, Anal. Chem., 47 (1975) 750.
7 S. Murray and K.A. Waddell, Biomed. Mass Spectrom., 9 (1982) 466.
8 P.M. Harrison, A.M. Tonkin and A.J. McLean, J. Chromatogr., 339 (1985) 429.
9 P.M. Harrison, A.M. Tonkin and A.J. McLean, J. Chromatogr., 339 (1985) 424.
10 P.M. Harrison, A.M. Tonkin, C.M. Cahill and A.J. McLean, J. Chromatogr., 343 (1985) 349.
11 B.E. Westwood, P.J. Harman and M.L. Mashford, J. Chromatogr., in press.