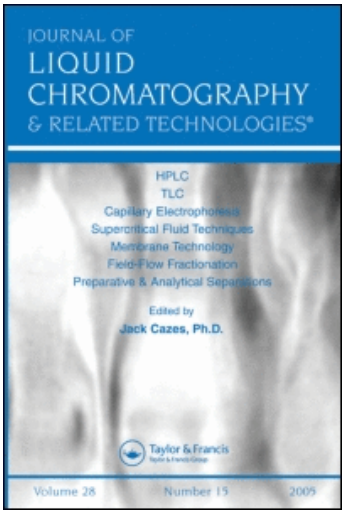

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Rapid High-Performance Liquid Chromatographic Method for the Analysis of Debrisoquine and 4-Hydroxydebrisoquine in Urine without Derivatization

S. Wanwimolruk[a]; D. G. Ferry[a]
[a] Department of Pharmacy, MRC Toxicology Research Unit University of Otago Medical School, Dunedin, New Zealand

To cite this Article Wanwimolruk, S. and Ferry, D. G.(1990) 'Rapid High-Performance Liquid Chromatographic Method for the Analysis of Debrisoquine and 4-Hydroxydebrisoquine in Urine without Derivatization', Journal of Liquid Chromatography & Related Technologies, 13: 5, 961 — 968
To link to this Article: DOI: 10.1080/01483919008049225
URL: http://dx.doi.org/10.1080/01483919008049225

JOURNAL OF LIQUID CHROMATOGRAPHY, 13(5), 961-968 (1990)

RAPID HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE ANALYSIS OF DEBRISOQUINE AND 4-HYDROXYDEBRISOQUINE IN URINE WITHOUT DERIVATIZATION

S. WANWIMOLRUK AND D. G. FERRY
Department of Pharmacy and
MRC Toxicology Research Unit
University of Otago Medical School
Dunedin, New Zealand

ABSTRACT

A simple and rapid HPLC method has been developed for the simultaneous determination of debrisoquine (D) and its main metabolite, 4-hydroxydebrisoquine (4OHD) in urine, without derivatization of the compounds. Guanoxan is used as the internal standard. The chromatographic separation was accomplished with a mobile phase comprising 15% acetonitrile and 85% 8 mM phosphate buffer (pH 5) at a flow rate of 1 ml/min. The HPLC column was packed with 5 μm Spherisorb-CN. The eluant was monitored at 214 nm. The intra- and inter-assay coefficients of variation were less than 6%. The lowest limit of detection for D was 0.1 μg/ml and for 4OHD was 0.05 μg/ml. The method is suitable for determining the oxidation phenotype of populations and is presently being used to study the oxidation polymorphism in Maori and South Pacific Polynesians.

INTRODUCTION

Debrisoquine (D), an antihypertensive drug, is metabolised essentially to 4-hydroxydebrisoquine (4OHD). This 4-hydroxylation has been shown to be genetically controlled with two phenotypes present; extensive metabolisers and poor metabolisers (1). The oxidation status of subjects is assessed from the metabolic ratio, determined as the D/4OHD ratio in urine collected, usually over an 8 hour period, after an oral dose of D.

Several methods are available to determine D and 4OHD concentrations in urine. Gas chromatography and gas chromatographic-mass spectroscopic techniques can be used after derivatization of compounds with acetylacetone (2-4). These methods involve lengthy sample preparation and time-consuming derivatization procedures which limit the number of samples assayed per day. Five high-performance liquid chromatographic (HPLC) methods for D and 4OHD have been developed. Two of these do not use an internal standard (5,6) and two require derivatization with acetylacetone before chromatographic separation (7,8). The recent HPLC method published by Duche et al (9), appears to be a simple and rapid HPLC assay for the determination of D and 4OHD, using an internal standard without derivatization of the compounds. In their method, the urine sample is extracted with hexanol and the hexanol layer is then injected onto the HPLC column. We found that there was a large negative shift in the chromatographic baseline due to the presence of hexanol which caused an interference with measurement of the 4OHD peak. We have improved the method by using an alternative column and including a back-extraction step. The new procedure provides a simple and rapid HPLC assay for the determination of D and 4OHD, using an internal standard and without derivatization of compounds.

MATERIALS AND METHODS

Reagents and Chemicals

All chemicals were analytical grade, pharmaceutical quality or better and were used without further purification. Declinax (10 mg tablets), debrisoquine

sulfate and 4-hydroxydebrisoquine sulfate were gifts from Roche (New Zealand). Guanoxan (G) hemisulfate was kindly supplied by Pfizer (New Zealand). HPLC-grade acetonitrile was purchased from Fisher Scientific Company (Fair Lawn, NJ). 1-Hexanol and diethyl ether were purchased from BDH Chemicals (Poole, UK). Water was double glass distilled and MilliQ® filtered. All glassware was cleaned and silanized with 0.5% Aquasil® (Pierce, Rockford, IL) before use.

Stock solutions containing 1 mg/ml of D, 4OHD and G separately were prepared in methanol and stored at -20°C until required. Urine standard solutions containing known concentrations of D and 4OHD were prepared by appropriate dilution of the stock drug solutions with drug-free urine. The internal standard solution of G (100 μg/ml) was prepared by dilution of the stock solution with water. The urine standards were extracted in the same way as the samples. The concentrations of D and 4OHD in samples were then determined from calibration plots of the chromatographic peak height ratios D/G and 4OHD/G versus concentration.

Extraction Procedure

To 2 ml of urine were added 100 μl of 4M NaOH, 200 μl of the internal standard solution (G, 100 μg/ml) and 6 ml of 1-hexanol/ether (60:40, %v/v). The samples were shaken for 15 minutes on a mechanical shaker, then were centrifuged at 4°C (1500 g, 10 minutes). After centrifugation, the upper organic layer was transferred to a tapered glass centrifuge tube containing 250 μl of 0.1% orthophosphoric acid. The mixture was shaken for 15 minutes and centrifuged at 4°C (1500 g) for 10 minutes. The organic layer was aspirated, the acidic layer was transferred to the autosampler vials and 70 μl were injected onto the HPLC column.

Chromatographic Procedures

The HPLC system consisted of a Perkin Elmer LC pump, Model 250 (Perkin Elmer, CT, USA) connected to a Waters 712 auto-injector (Milford, MA, USA). The detector was a Spectroflow 757 (Kratos Analytical Instrument, NJ) variable wavelength ultraviolet-detector. The

chromatographic response was recorded by a Hitachi D-2500 integrator (Hitachi, Tokyo, Japan). The HPLC column was 15 cm x 4.6 mm I.D. packed with 5 μm Spherisorb-CN (Hichrom, UK). The mobile phase consisted of acetonitrile and 8 mM potassium dihydrogenphosphate pH 5 (15:85, %v/v). The flow rate of the mobile phase was 1 ml/min (pressure 1200 psi). Detection was by UV spectrophotometry at 214 nm using a setting of 0.1 AUFS. Separations were done at room temperature.

RESULTS AND DISCUSSION

The liquid chromatographic separation of D and 4OHD from the endogenous urine peaks was accomplished using reversed-phase techniques consisting of a cyano stationary phase with an aqueous acetonitrile mobile phase. The separation and resolution of D and 4OHD were better with the cyano stationary phase column than with a C8 stationary phase column. Figure 1 shows chromatograms obtained from the analysis of the blank urine and a standard urine sample spiked with 0.5 μg/ml each of D and 4OHD using a mobile phase of acetonitrile and pH 5 phosphate buffer (15:85). Under these chromatographic conditions, no endogenous sources of interference were observed and the resolution between 4OHD, internal standard (G) and D was very satisfactory. The retention times for 4OHD, G and D were 7.2, 9.8 and 11.8 minutes, respectively. A mixture of hexanol and ether (60:40 %v/v) gave a cleaner extract and less interference than hexanol alone with no loss of extraction efficiency. The mean (n = 6) extraction recovery from urine was found to be 68.7% for 4OHD, 79.2% for G and 96.8% for D. The inclusion of a back-extraction step into dilute acid additionally provided a cleaner extract and a more concentrated sample.

Calibration curves were linear for D ($y = 0.127x + 0.014$; $r^2 = 0.993$) and 4OHD ($y = 0.204x + 0.015$; $r^2 = 0.999$) over the concentration range of 0.1 - 25 μg/ml. The day-to-day coefficients of variation of the slope of the calibration curves were 6.9% for D and 4.1% for 4OHD (n=5). The precision of the assay was evaluated for two concentrations of D and 4OHD (1 and 10 μg/ml) by analysing each one four times on the same day. The intra-assay

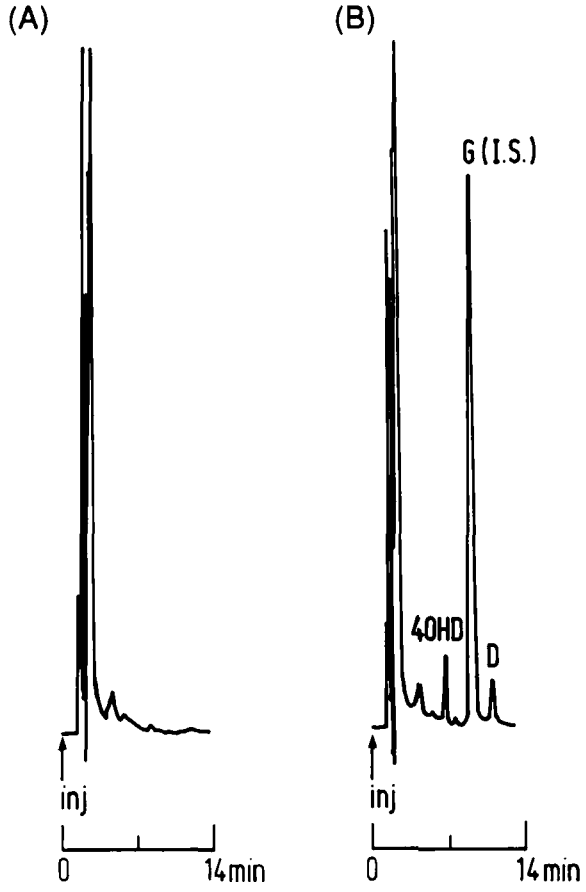

FIGURE 1 Chromatograms of human urine extracts (A) a blank urine and (B) a urine spiked with 0.5 μg/ml debrisoquine (D) and 0.5 μg/ml 4-hydroxydebrisoquine (4OHD).

Peaks: Inj = injection; 4OHD = 4-hydroxydebrisoquine; G = guanoxan (internal standard); D = debrisoquine

coefficients of variation were 3.0% for D and 1.4% for 4OHD at 1 μg/ml and 2.1% for D and 1.9% for 4OHD at 10 μg/ml. The inter-assay coefficients of variation (n = 4 days) were 2.7% for D and 2.8% for 4OHD at 1μg/ml and 4.0% for D and 2.7% for 4OHD at 10 μg/ml. Using the criterion of signal to noise ratio of 3 the sensitivity for D was 0.1 μg/ml and for 4OHD was 0.05 μg/ml. The detection limits can be increased by using UV-detection at lower wavelengths but this was not necessary. Urine samples stored at -20°C for up to 7 weeks showed no signs of decomposition and practically the same metabolic ratio value was obtained, suggesting that D and 4OHD are stable under these storage conditions for at least 7 weeks.

Possible interference by other drugs was examined. None of the following drugs interfered with the assay for D and 4OHD: acetaminophen, alprenolol, antipyrine, aspirin, caffeine, cefoxitin, ceftriaxone, cephalothin, cephradine, dapsone, diclofenac, ephredine, frusemide, gentamicin, gentisic acid, hydrocortisone, 4-hydroxyantipyrine, indapamide, indomethacin, labetolol, lorazepam, methadone, metoprolol, morphine, phenacetin, phenylbutazone, phenytoin, piroxicam, propranolol, quinidine, quinine, salicylic acid, satolol, theophylline and verapamil. The tested drugs that interfered with the assay were: codeine, diazepam, lidocaine, mepivacaine, pindolol, practolol and triazolam.

The urine samples collected from healthy volunteers taking 10 mg Declinax tablet were analysed by this method without any difficulty. Two examples of the chromatograms obtained from the subjects are illustrated in Figure 2. One is from a poor metaboliser who had a metabolic ratio of 108. The other one is from an extensive metaboliser having a metabolic ratio of 1.6.

In summary, a HPLC procedure has been described for the quantitative analysis of debrisoquine and its metabolite, 4-hydroxydebrisoquine in human urine. D, 4OHD and the internal standard (guanoxan) can be resolved from the endogenous substances in urine using a reversed-phase cyano column. Chromatographic analysis was optimized using an acetonitrile and phosphate buffer mobile phase. The mixture of hexanol and ether used for urine extraction was found to be the most appropriate solvent to minimize sources of interference from the urine co-extractives. The method described utilizes

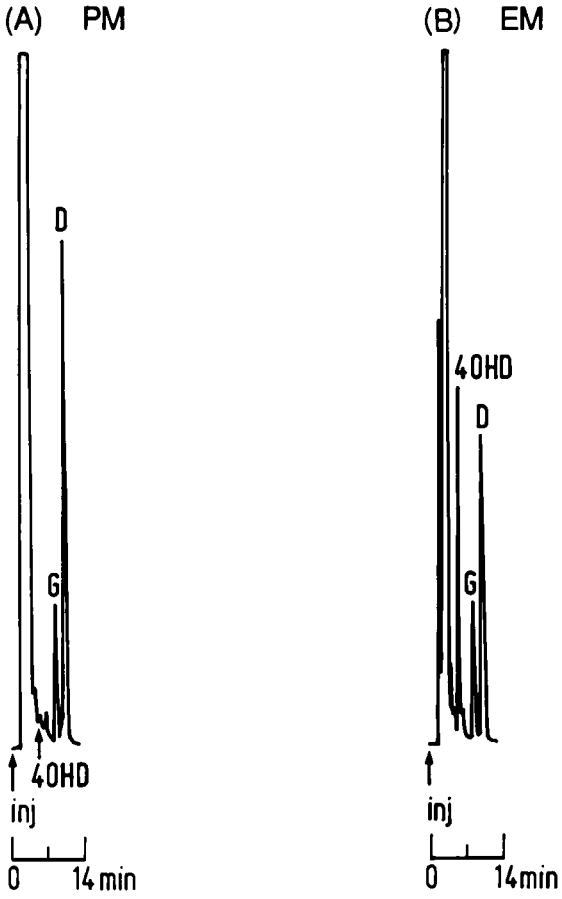

FIGURE 2 Chromatograms of human urine extracts of (A) a poor metaboliser who had a metabolic ratio of 108 and (B) an extensive metaboliser having a metabolic ratio of 1.6. Both subjects took an oral dose of 10 mg D and urine samples were collected for 4 hours after dose.
Peaks: 4OHD = 4-hydroxydebrisoquine (0.42 μg/ml for A; 16.8 μg/ml for B); G = guanoxan (internal standard); D = debrisoquine (41.8 μg/ml for A; 24.7 μg/ml for B)

widely available equipment and is less time-consuming than most other methods as derivatization of the compound is not required. The procedure is suitable for the routine analysis (30-48 urine samples per day) of specimens obtained for the metabolic screening of populations. This assay is being employed for the determination of debrisoquine oxidation phenotypes in Maori and South Pacific Polynesians.

REFERENCES

1 Mahgoub A., Idle J.R., Dring L.G., Lancaster R. and Smith R.L. Lancet 2, 584, 1977.

2 Lennard M.S., Silas J.H., Smith A.J. and Tucker G.J. J. Chromatogr., 133, 161, 1977.

3 Allen J.G., Erst P.B., Francis R.J. and Haigh J.L. Drug Metab. Dispos., 3, 332, 1975.

4 Erdtmansky P. and Goehl T.J. Anal. Chem. 47, 750, 1975.

5 Harrison P.M., Tonkin A.M., Dixon S.T. and McLean A.J. J. Chromatogr. 374, 204, 1986.

6 Westwood B.E., Harman P.J. and Mashford M.L. J. Chromatogr. 374, 200, 1986.

7 Rona K., Vereczkey L. and Gachalyi B. J. Chromatogr. 378, 509, 1986.

8 Lee E.J.D. and Ang S.B. J. Pharmaceut. Biomed. Anal. 5, 435, 1987.

9 Duche J.C., Barre J. and Tillement J.P. J. Chromatogr. 423, 340, 1987.