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Short Communication

Measurement of debrisoquine and 4-hydroxydebrisoquine in urine by liquid chromatography

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Introduction

Individuals vary in their ability to metabolise a number of drugs by oxidation [1], and, a defect in this ability has been shown to be genetically linked [2]. The proportion of individuals with this defective capability varies with different populations, being apparently higher in the Chinese [3]. Debrisoquine (DEB), an antihypertensive agent, has been used to estimate this oxidative capacity in individuals.

Commonly, debrisoquine and its main metabolite, 4-hydroxydebrisoquine (DEB–OH), are measured in urine by gas chromatography [4] or gas chromatography–mass spectrometry [5]. The main difficulties in the measurement of these compounds by liquid chromatography relate to their poor extractability by organic solvents and their inadequate UV absorption at higher wavelengths. Three liquid chromatographic methods have been reported. Harrison *et al.* [6] extracted DEB and DEB–OH from urine with ion-exchange Bond-Elut™ columns, and obtained recoveries for DEB and DEB–OH of 87 and 76%, respectively. Westwood *et al.* [7] injected filtered urine directly onto a reversed-phase column. Although the lower limit of detectability was sufficiently low, the DEB–OH peak was inadequately resolved from the unretained peaks. Consequently, the method suffers from occasional interference due to dietary factors. This report documents the feasibility of using liquid chromatographic methods for the pyrimidine derivatives of DEB and DEB–OH used in gas chromatography. A recent method [8] utilising a similar derivatisation procedure required extensive post-derivatisation clean-up procedures before sample injection. The method reported here is sensitive, economical and is easily automated. Although derivatisation is required, reagents are inexpensive and the method is simple.

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Experimental

Reagents

Acetonitrile and methanol were HPLC grade solvents (J. T. Baker). All other reagents were analytical grade. DEB, DEB–OH (Roche) and the internal standard, guanoxan (Pfizer), were received as pure compounds. Acetylacetone (Merck) was used as received.

Derivatisation

Aliquots (1 ml) or urine were incubated in a water bath at 50°C for 16 h (overnight) with guanoxan (80 μl; 100 μg ml^{-1}), saturated sodium bicarbonate (0.5 ml) and acetylacetone (0.5 ml). After the addition of diethyl ether, the reaction mixture was vortexed for 15 s before centrifugation (1200 **g** for 10 min). The organic phase was transferred to another test tube and back extracted with hydrochloric acid (4 N; 250 μl). The organic phase was discarded. The aqueous phase (50 μl) was transferred to a conical tube and dried under a stream of nitrogen at 25°C. The residue was reconstituted with water (50 μl) of which 10 μl was injected onto the HPLC column.

Chromatography

The HPLC system (Varian 5560) consisted of a pump with a ternary proportioning valve, a UV detector (Varian UV 200) set at 245 nm, a systems controller and data handler (Varian Vista 402). A 2-μm column inlet filter and a guard column (7.5 mm × 2.1 mm, Chrompak) filled with C_{18} reverse-phase material (10 μm) was attached before the reversed-phase C_8 column. The mobile phase was pumped at a flow rate of 1.2 ml min^{-1}. The initial mobile phase composition was acetonitrile–potassium phosphate buffer (0.002 M, pH 4.75)–methanol (26:48:26, v/v/v). The pump was then programmed to deliver the set composition for 4 min before linearly changing to a composition of (40:30:30, v/v/v) at 6 min. This final composition was then maintained to the end of the run (14 min). A 10-min equilibration time between runs was programmed.

Verification of assay

Standard urine solutions of DEB and DEB–OH were prepared for the concentration range of 0.1–20 μg ml^{-1}. Chromatography was performed after derivatisation and extraction of these standards. The ratios of the peak areas of the DEB and DEB–OH derivatives to that of the internal standard were calibrated with the concentrations of DEB and DEB–OH in the urine standards.

Ten aliquots (1 ml) of urine containing 3 μg each of DEB and DEB–OH were prepared. Five were assayed on successive days while the remaining five were assayed on the same day so as to estimate the inter-day and intra-day variability of the assay.

Human study

Six healthy volunteers were each administered 12.8 mg of debrisoquine sulphate in tablet formulation (Declinax®), equivalent to 10 mg of the free base. Urine was collected between the onset of the study and 8 h after administration of the drug. Aliquots (50 ml) of the urine were stored at −20°C until analysis.

Results

The pyrimidine derivatives of DEB–OH, guanoxan and DEB elute at 8.2, 10.1 and 13.2 min respectively. Typical chromatograms (baseline subtracted) of control urine, urine spiked with DEB, DEB–OH and guanoxan, and urine from a volunteer after consuming 10 mg of DEB are shown in Fig. 1.

Calibration curves were linear for DEB ($y = 0.19x + 0.01$; $r^2 = 0.998$) and DEB–OH ($y = 0.21x + 0.01$; $r^2 = 0.998$) over the concentration range of 0.1–20 μg ml^{-1}. The lowest limit of detection for both compounds was 0.1 μg ml^{-1}. The intra-day and inter-day precision and accuracy in the performance of the assay are presented in Table 1.

Concentrations of DEB and DEB–OH in the urine of 6 volunteers were measured without encountering any difficulty (Table 2). Using an antimode of the metabolite ratio (% of dose as DEB/% of dose as DEB–OH) of 12.6 [9], all were typed to be extensive metabolisers.

Discussion

The assay described here for DEB and DEB–OH is sensitive, specific and reproducible. It utilises the superior spectral characteristics of the pyrimidine derivatives

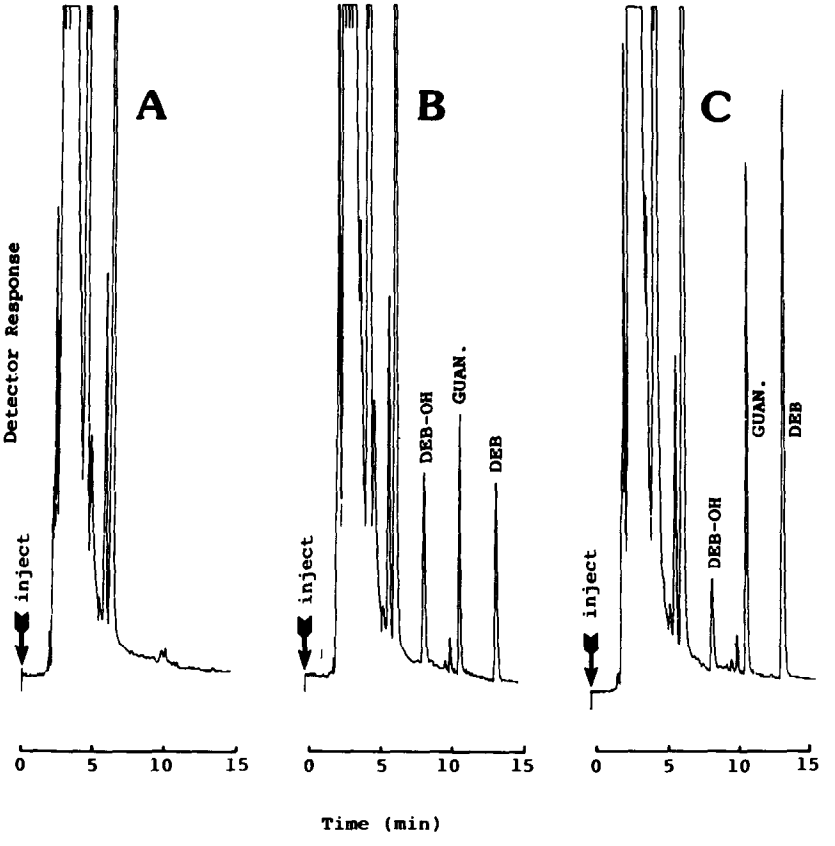

Figure 1
Typical chromatograms (baseline subtracted) of (A) control urine; (B) urine spiked with 2 μg ml^{-1} each of DEB, DEB–OH and guanoxan, and (C) urine sample from Subject 3 after consuming 10 mg DEB.

Table 1
Accuracy and precision of the assay ($n = 5$)

	DEB	DEB–OH
Prepared concentration ($\mu g\ ml^{-1}$)	3	3
Intra-day		
Measured concentration ($\mu g\ ml^{-1}$)	2.96	2.72
Relative Standard Deviation (%)	1.5	1.2
Inter-day		
Measured concentration ($\mu g\ ml^{-1}$)	2.86	2.92
Relative Standard Deviation (%)	3.6	3.1

Table 2
Concentrations of DEB and DEB–OH in the urine of 6 normal volunteers

Subject	DEB ($\mu g\ ml^{-1}$)	DEB–OH ($\mu g\ ml^{-1}$)	DEB*/DEB–OH*
1	3.7	3.3	1.23
2	4.0	8.3	0.53
3	5.7	0.9	7.01
4	4.7	0.8	6.09
5	1.4	1.5	1.08
6	5.7	1.7	3.61

* The metabolic ratio is obtained by dividing the concentration of debrisoquine in the urine by that of 4-hydroxydebrisoquine, as debrisoquine equivalents.

of DEB, DEB–OH and guanoxan. The derivatisation method has been documented elsewhere [4] and has been routinely used in the gas chromatographic analysis of these compounds. Although the derivatisation takes 16 h to complete, it is simple to perform and may be left unattended overnight. A recently reported method [8] using a similar precolumn derivatisation procedure required extensive and cumbersome clean-up before the sample could be injected onto the column. Although the method was operated isocratically, UV wavelength programming was necessary to achieve adequate sensitivity.

The method reported here is relatively simple and economical to operate. Unlike previous GC methods [4] the final carbon disulphide extraction step has been found to be unnecessary. The authors have found that the isocratic elution of the pyrimidine compounds produces broad and asymmetric peak shapes which result in markedly reduced assay sensitivity and reproducibility. In particular, a methanol–acetonitrile mixture in the mobile phase was necessary to maintain good DEB peak shape.

The method is therefore suited for the routine metabolic screening of populations, and provides a HPLC alternative to current GC methods.

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