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Short Communication

Determination of dextromethorphan and dextrorphan in urine by high-performance liquid chromatography after solid-phase extraction*

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Introduction

Recent studies have indicated that *O*-demethylation of dextromethorphan (DMO) in man shows a complete co-segregation with genetically controlled debrisoquine–sparteine hydroxylation polymorphism [1–3]. In contrast to debrisoquine, DMO, an antitussive drug, has a high therapeutic index and is widely available. Thus, DMO is preferable for the determination of this clinically important hydroxylation capacity. The phenotype is characterized by a bimodal frequency distribution with about 8–10% of the Caucasian population belonging to the "poor" metabolizers, whilst the remainder are "extensive" metabolizers [2, 3]. The isoenzyme of cytochrome P-450, P450IID6, which is responsible for this metabolism controls more than 20 therapeutically important drugs [4].

For phenotyping in clinical routine, DMO and its main *O*-demethylated metabolite dextrorphan (DOR) are measured in urine collected 8 h after an oral dose of 20–30 mg DMO and the ratio between these two compounds is calculated.

Several analytical methods have been used for the measurement of DMO and DOR including gas–liquid chromatography (GLC) [5, 6] and high-performance liquid chromatography (HPLC) [7–11]. The chromatographic methods require an extensive sample clean-up of the urine samples for which time-consuming liquid–liquid extraction procedures are used in most cases. In the present study a HPLC method has been developed based upon the use of a solid-phase extraction technique suitable for the routine determination of DMO hydroxylation polymorphism.

Experimental

Reagents and materials

DMO, DOR and levallorphan tartrate were kindly supplied by Roche (Basel, Switzerland). Beta-glucuronidase arylsulphatase solution (from *Helix pomatia*, approx. 100,000 Fisherman units/ml) was obtained from Boehringer (Mannheim, Germany) and 1-octanesulphonic acid sodium salt from Fluka (Buchs, Switzerland). All other reagents were of analytical grade and purchased from E. Merck (Darmstadt, Germany). De-ionized water was used throughout. For the solid-phase extraction 3 ml (200 mg) C-18 Bond Elut extraction columns, Analytichem (Harbor City, CA, USA) were utilized.

Apparatus

The solvent delivery system was a Constametric II pump, Milton Roy (Philadelphia, PA, USA). Samples were injected via a WISP 710 B autosampler, Waters Assoc. (Milford, MA, USA). For the protection of the analytical column a RP-2, 15 × 3.2 mm i.d. precolumn, Brownlee Labs Inc. (Santa Clara, CA, USA) was connected to a Zorbax Phenyl, 5 μm, 250 × 4.6 mm i.d. analytical column, DuPont (Wilmington, DE, USA). The eluent was monitored by a fluorescence spectro-

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photometer, Model 650-10 LC, Perkin Elmer (Norwalk, CT, USA), using excitation and emission wavelengths of 270 and 312 nm, respectively, with slits set at 15 nm. The column temperature was maintained at 30°C by a column heater. A SP 4270 integrator, Spectra Physics (San Jose, CA, USA) was used.

Patient protocol

For characterization of the oxidative *O*-demethylation phenotype, 74 patients received an oral dose of 25 mg dextromethorphan hydrobromide, Bexin, Spirig Corp. (Egerkingen, Switzerland) at 22.00 h, and urine was collected overnight for 8 h. The total volume of urine was measured and an aliquot was kept frozen at −20°C until required for analysis. Under these conditions it has been shown [12] that the analytes are stable for at least 16 weeks. Subsequently after HPLC analysis of the urine samples the metabolic ratio DMO–DOR was calculated.

Analytical procedures

Enzymatic hydrolysis of DOR conjugates in urine. A 0.75-ml aliquot of urine was added to 0.75 ml of a mixture of 20 μl β-glucuronidase–arylsulphatase in 0.1 M sodium acetate buffer, pH 5.0, and 0.05 ml of 0.6 M sodium azide. This mixture was incubated for 18 h in a water bath at 37°C.

Solid-phase extraction. To a 0.25-ml aliquot of hydrolysed urine 0.1 ml of internal standard solution containing 1 μg levallorphan tartrate in water and 2 ml of 0.1 M sodium carbonate buffer, pH 9.2, were added and applied to a preconditioned 3 ml C18 Bond Elut extraction column. The preconditioning was carried out by sequentially washing the column with 6 ml methanol, 6 ml water, and 4 ml 0.1 M sodium carbonate buffer (pH 9.2). After the urine samples had been applied, the columns were washed with 2 ml water and 1 ml acetonitrile. The components of interest were eluted with 3 ml of methanol–acetonitrile–2% phosphoric acid (5:3:2, v/v/v). The extracts were evaporated to dryness at 70°C under a stream of nitrogen and the residues redissolved with 0.5 ml mobile phase; 20 μl were injected into the HPLC system. For the exact determination of very low DMO concentrations in extensive metabolizers the assay was slightly modified as follows: a 0.5-ml aliquot hydrolysed urine was used for the extraction. The internal standard solution contained 0.1 μg levallorphan tartrate and, finally, the evaporated extract was redissolved in 0.25 ml mobile phase of which 120 μl were injected into the HPLC system. All analysis were performed in duplicate.

Chromatography

The chromatographic conditions were similar to the method of Zysset *et al.* [11] using a phenyl reversed-phase column and ion pair separation. The mobile phase consisted of acetonitrile–methanol–2.5 mM 1-octanesulphonic acid in 10 mM phosphate buffer pH 2.5 (27:13:60, v/v/v). The flow rate was 1 ml min^{-1} and the mobile phase was recycled for 3 days. For quantification peak height ratios between the components of interest and internal standard were used. The standard curves were calculated by means of an unweighted linear regression analysis of five standard concentrations.

For the preparation of the standard samples a stock solution, containing 50 mg l^{-1} DMO and 500 mg l^{-1} DOR in water, was diluted with blank urine to give final concentrations of 0.05, 0.1, 0.5, 1 and 5 mg l^{-1} DMO, and 0.5, 1, 5, 10 and 50 mg l^{-1} DOR.

Results

With the present mobile phase the retention times of DOR, levallorphan and DMO were 7.0, 9.5 and 15.0 min, respectively. The chromatograms of drug-free urine samples contained no peaks that might interfere with any of the compounds of interest. The analytical column remained stable for more than 1000 injections.

Examples of the chromatographic separation of urine from poor and extensive metabolizers are shown in Fig. 1. Chromatograms were completed within 20 min. Extraction recoveries (mean values ±1 SD, $n = 6$) for DMO and DOR were 90.6 ± 1.1 and 92.8 ± 1.7%, respectively. Taking a signal-to-noise ratio of 3 as a criterion, the detection limits of the proposed standard method for DMO and DOR were found to be 12 and 8 ng ml^{-1}, respectively. Using the modified method with increased sensitivity, the detection limit could be improved by a factor of 8. Within-assay and between-assay relative standard deviations are listed in Table 1. The standard curves showed excellent linearity for the whole range of

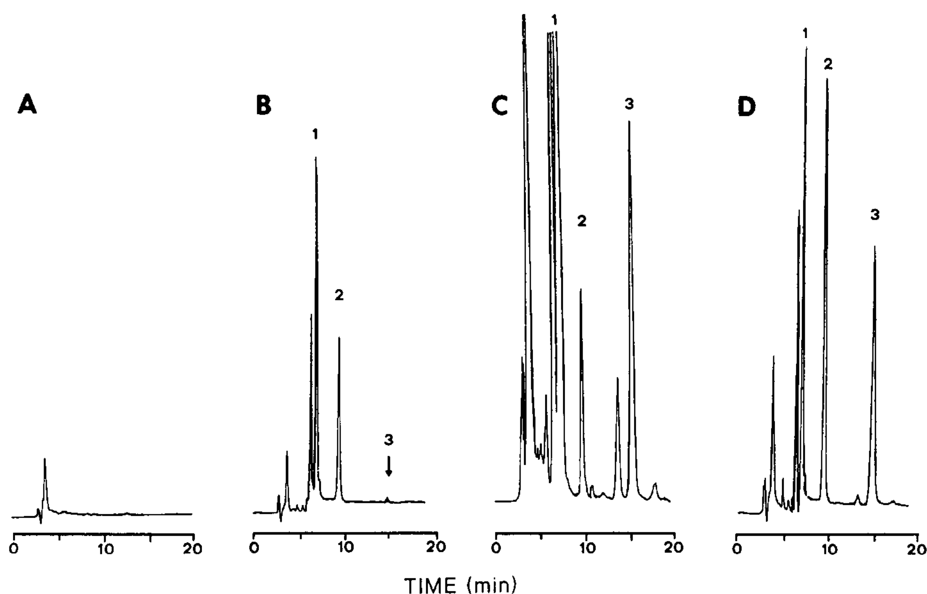

Figure 1
HPLC chromatograms of extracted urine samples from patients after an oral dose of 25 mg DMO: peak 1 = DOR, peak 2 = levallorphan (IS), peak 3 = DMO. (A) Drug-free urine, (B) extensive metabolizer (DOR = 13 mg l^{-1}, DMO = <0.1 mg l^{-1}), (C) extensive metabolizer after modified sample preparation (DMO = 0.7 mg l^{-1}) and (D) poor metabolizer (DOR = 5.9 mg l^{-1}, DMO = 4.6 mg l^{-1}).

Table 1
Accuracy and precision of analysis for DMO and DOR in urine

Compound	Spiked concentration (mg l^{-1})	*n*	Mean (mg l^{-1})	RSD (%)
Within-day				
DMO	0.15	15	0.14	3.9
	2.5	15	2.57	2.6
DOR	1.5	15	1.59	0.94
	30.0	15	29.0	1.5
Between-day				
DMO	0.15	11	0.13	8.0
	2.5	11	2.51	6.1
DOR	1.25	11	1.25	11.9
	25.0	11	24.7	2.8

standard concentrations (coefficients of correlation >0.999).

Discussion

Although most published HPLC methods for the determination of DMO and DOR in urine utilize a specific fluorimetric detection, an effective sample clean-up procedure is necessary to eliminate interfering peaks and to obtain a sufficiently low signal-to-noise ratio. In order to avoid time-consuming liquid–liquid extraction [7, 8, 11] or complex column switching procedures [10] a reliable HPLC method incorporating an efficient solid-phase extraction procedure, using reversed-phase extraction columns, has been developed.

The metabolic ratios DMO–DOR in the urine of the patients examined were between 0.001 and 4.7. The separation point for poor and extensive metabolizers described in the literature is given as 0.3 [2]. However, the absolute concentration of DMO and DOR in urine depends not only on the very large inter-individual variations of the metabolism, even within a genetically defined population, but also on the urine produced within the collec-

tion period. For the 74 patients examined the concentrations of DMO and DOR were between <0.05 and 10 $\mu g\ ml^{-1}$ and between 0.16 and 69 $\mu g\ ml^{-1}$, respectively, that is an almost 1000-fold difference between the lowest and the highest concentrations. Therefore, for the precise determination of very low DMO concentrations (<0.08 $\mu g\ ml^{-1}$) in some of the extensive metabolizers, it might be necessary to employ the slightly modified sample preparation as described in the methodological section. If the determination of DMO is used only for the distinction between the two genetically different groups this modification can be avoided.

In conclusion, the fast and reliable method for the measurement of DMO and DOR in urine proposed in the present study should support a more frequent application of the safe and non-invasive method for the determination of the debrisoquine–sparteine type genetic polymorphism in man.

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