Journal of Chromatography, 495 (1989) 361–363
Biomedical Applications
Elsevier Science Publishers B V , Amsterdam — Printed in The Netherlands

CHROMBIO 4884

Letter to the Editor

Dextromethorphan phenotypes determined by high-performance liquid chromatography and fluorescence detection

Sir,

Dextromethorphan is a non-narcotic antitussive agent, which is metabolized to a major O-demethylated metabolite, dextrorphan, and various minor metabolites Dextromethorphan demethylation co-segregates with the polymorphic debrisoquine hydroxylation in humans [1] This reaction is genetically controlled. By measuring dextromethorphan metabolic ratios in urine, two phenotypes can be identified: a major group of extensive metabolizers and a minor group of poor metabolizers (3% of the French caucasian population) [2] Several high-performance liquid chromatographic (HPLC) methods, using UV detection, are available to measure dextromethorphan and dextrorphan in urine [3,4] We report here a simple extraction method for dextromethorphan analysis using HPLC system coupled with a fluorescence detector We also compared the metabolic ratios obtained using UV or fluorescence detection

Dextromethorphan hydrobromide (DEM), dextrorphan tartrate (DOR), hydroxymorphinan hydrochloride (HYD) and methoxymorphinan hydrobromide (MET) were kindly supplied by Hoffman La Roche Labs (Basle, Switzerland) *Helix pomatia* β-glucuronidase/arylsulphatase was purchased from Boehringer (Mannheim, F.R.G) Bond-Elut columns containing silica modified with carboxylic acid ion-exchange functional groups (CBA columns) were purchased from Analytichem International (Harbor City, CA, U.S.A) All other chemicals and solvents were analytical grade

Stock solutions of DEM, DOR, HYD and MET base were prepared in distilled water and stored at 4°C. Standards were prepared by appropriate dilutions of stock solutions with drug-free urine. Urine samples (1 ml) and standards were adjusted to pH 5–5.5, and the β-glucuronidase/arylsulphatase mixture was added The samples were incubated at 37°C for 18 h Using a Vac-

0378-4347/89/$03 50

Elut chamber, CBA columns were activated by washing with 1 ml of acetonitrile–0 1 *M* hydrochloric acid (40 60, v/v) and 1 ml of distilled water A 1-ml volume of the sample was added and the column was washed with 1 ml of distilled water and 0 5 ml of 0 1 *M* hydrochloric acid The column was then eluted with 1 ml of acetonitrile–0 1 *M* hydrochloric acid (40 60, v/v). An aliquot was injected into the HPLC system This consisted of a Spectra Physics (SP) Model 8800 pump, an SP 8780 autosampler, a JASCO Model FP820 fluorescence detector (excitation 280 nm, émission 310 nm) and an SP 4290 integrator connected to a Winner system (Epson PCE) Chromatography was carried out on a 5 μm particle size Zorbax Phenyl column (250 mm) The mobile phase was acetonitrile–0 1 *M* potassium dihydrogenphosphate (45 55, v/v), adjusted to pH 4, pumped at a flow-rate of 1 5 ml min^{-1}

Under the chromatographic conditions described, the retention times of HYD, DOR, MET and DEM were 4 1, 4 8, 6 25 and 8 6 min, respectively Following extraction, the respective recoveries were determined at 0 5 and 5 μg ml^{-1} ($n=5$) and were 90 and 96% for HYD, 85 and 90% for DOR, 70 and 76% for MET and 65 and 66% for DEM. The concentrations of DEM and DOR in urine are used to determine individual metabolic ratios The detection limit was 0 03 μg ml^{-1} for DEM and 0 02 μg ml^{-1} for DOR for an injection volume of 20 μl, at a signal-to-noise ratio of 2 1 The calibration plots of peak areas versus concentrations were linear over the range 0 1–10 μg ml^{-1} for DEM and DOR The inter-assay coefficient of variation (C V) for the slopes of the calibration curves was 1 65% for DEM and 2 2% for DOR The intra- and inter-assay C V s were studied at 0 5 and 5 μg ml^{-1} for both compounds ($n=5$) and did not exceed 6%

The determination of individual metabolic status was then undertaken in 33 healthy volunteers They received 20 mg of DEM orally and collected urine for 8 h overnight (The selection of subjects was not random, because 5 were already known to be poor metabolizers and 28 were tested for the first time)

TABLE I

URINARY ANALYSIS OF DEXTROMETHORPHAN POLYMORPHISM

Dextromethorphan (56 8 μmol) was administered orally to 33 subjects, and results (mean ± S D) obtained with UV and fluorescence (FLUO) detection are compared

Subjects	*n*	DEM (μmol per 8 h)		DOR (μmol per 8 h)		DEM/DOR ratio	
		UV	FLUO	UV	FLUO	UV	FLUO
Extensive metabolizers	28	0 306 ±0 08	0 228 ±0 07	11 960 ±1 04	11 550 ±1 00	0 658 ±0 24	0 520 ±0 22
Poor metabolizers	5	1 211 ±1 01	1 366 ±1 05	0 229 ±0 07	0 333 ±0 17	4 198 ±3 54	3 280 ±2 07

Following the same sample extraction, chromatography was carried out as described except that an SP 8450 variable-wavelength UV detector (operating at 280 nm) was added in series. With fluorescence, the chromatographic profiles were free of any interfering peaks The results of individual metabolic ratios are presented in Table I and show a high correlation between the two methods ($r = 0\ 98$) It is known that the DEM concentration in urine is not detectable in very extensive metabolizers In such cases, the minimum quantifiable concentration is used to calculate the metabolic ratio DEM/DOR The minimum detectable level of DEM is lower in fluorescence than in UV (0 03 versus 0 1 $\mu g\ ml^{-1}$) and the number of overestimated ratios is reduced (two with fluorescence and five with UV in our study)

The single-step solvent extraction reported here is rapid and simple for routine preparation of urine samples As previously reported [5], the sensitivity is increased by using a fluorescence detector

Hôpital Robert-Debre, E JACQZ-AIGRAIN*
Unité de Pharmacologie Clinique, Y MENARD
48 Boulevard Sérurier, M POPON
75935 Paris Cedex 19 (France) H MATHIEU

1 B Schmid, J Bircher, R Preisig and A Kupfer, Clin Pharmacol Ther , 38 (1985) 618
2 E Jacqz-Aigrain, H Dulac and H Mathieu, Eur J Clin Pharmacol , 35 (1988) 167
3 D Larrey, G Amouyal, M Tinel, P Letteron, A Berson, G Labbe and D Pessayre, Br J Clin Pharmacol , 24 (1987) 676
4 N Motassim, D Decolin, A Nicolas and G Siest, J Chromatogr , 423 (1987) 340
5 T Kronbach, D Mathys, J Gut, T Catin and U A Meyer, Anal Biochem , 162 (1987) 24

(First received January 3rd, 1989, revised manuscript received May 30th, 1989)