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Note

Determination of methyprylon and its dehydro metabolite, 5-methylpyrithyldione, in plasma by high-performance liquid chromatography

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Since the introduction in 1956 of methyprylon (Noludar®) (Fig. 1, I) into clinical practice as a non-barbituarate, sedative hypnotic, reports describing its pharmacokinetics [1—8] have varied considerably as to the half-life of elimination of this drug. One reason for these inconsistencies was the lack of an assay with suitable specificity to quantitate the parent drug in the presence of its metabolites (Fig. 1, II—V) [9—11]. These methods included colorimetry [9], thin-layer chromatography with UV spectrometry [11—13], and gas chromatography with flame ionization detection (GC—FID) [14]. More recently, GC—FID assays with conventional packed columns [7—15], and capillary columns [16], have been reported for the simultaneous assay of methyprylon and its metabolites in overdose cases. However, these assays are not sufficiently sensitive ($\geqslant$ 1—2 μg/ml) to determine the drug and its major plasma metabolite, 5-methylpyrithyldione (II), following single oral doses of methyprylon.

The present work describes the development of a normal-phase, high-performance liquid chromatographic (HPLC) assay with UV detection (214 nm) for the determination of methyprylon and its principle plasma metabolite (II). The assay was applied to the determination of methyprylon elimination in beagle dogs [17], and is herein described for the measurement of methyprylon (I) and 5-methylpyrithyldione (II) in man following a single oral 300-mg dose of methyprylon (Noludar).

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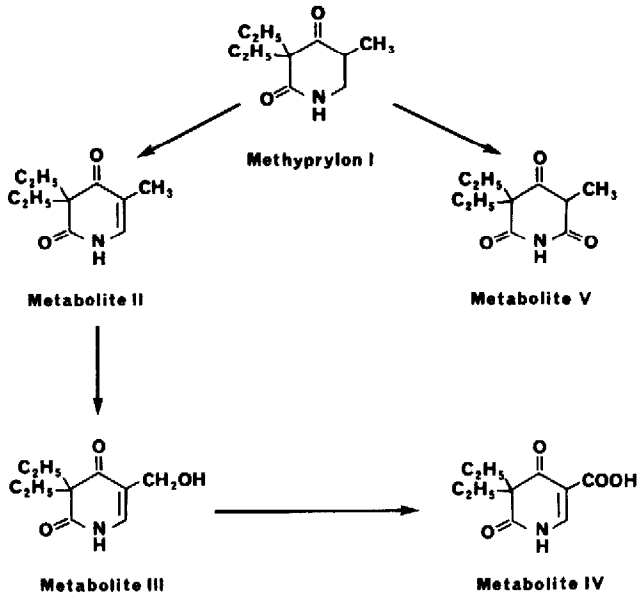

Fig. 1. Biotransformation of methyprylon in dog and man [10, 11].

EXPERIMENTAL

Reagents

All reagents were of analytical (ACS) grade purity, including concentrated hydrochloric acid, diethyl ether, and methanol. HPLC solvents were obtained from Burdick & Jackson Labs., Muskegon, MI, U.S.A. All aqueous solutions were prepared in double-distilled water.

Methyprylon (I) (3,3-diethyl-2,4-dioxo-5-methylpiperidine), and metabolites III (3,3-diethyl-2,4-dioxo-5-hydroxymethyl-1,2,3,4-tetrahydropyridine) and IV (3,3-diethyl-2,4-dioxo-5-carboxy-1,2,3,4-tetrahydropyridine) were obtained from Hoffmann-La Roche, Nutley, NJ, U.S.A. Metabolites II (3,3-diethyl-2,4-dioxo-5-methyl-1,2,3,4-tetrahydropyridine, 5-methylpyrithyldione) and V (3,3-diethyl-2,4,6-trioxo-5-methylpiperidine) were synthesized according to the methods of Dickson [14]. The internal standard, VI (3,3-diethyl-2,4-dioxo-1,2,3,4-tetrahydropyridine, pyrithyldione), was obtained from Aldrich, St. Louis, MO, U.S.A.

Standard solutions

Drug-free plasma, obtained from the Michigan Red Cross, was supplemented with varying concentrations of methyprylon and II according to the following procedure. Individual stock solutions were prepared to contain 200 μg of I and 20 μg of II per ml of distilled water. Appropriate aliquots of each solution were then added to 1.0 ml of plasma to obtain concentrations of 0.1, 1.0, 2.5, 5.0, and 10.0 μg/ml of methyprylon and 0.01, 0.1, 0.5, and 1.0 μg/ml of II. After vortexing, these standards were processed using the scheme described under Sample preparation, the results of which were used to construct calibration curves for both I and II.

Sample preparation

Blood samples were collected in heparinized vacutainers and immediately centrifuged and the plasma was stored at −20°C until processed. Prior to analysis, the samples were thawed at room temperature and centrifuged again to remove precipitated proteins. To 1.0 ml of sample or standard were added 100 μl of 0.1 *N* hydrochloric acid, 50 μl of internal standard (12.0 μg of VI per ml of distilled water), and 10 ml of diethyl ether. The mixture was vortexed for 20 sec and the layers separated by centrifugation at 1500 *g* for 5 min. The ether layer was transferred to a 15-ml conical centrifuge tube and evaporated under a stream of nitrogen at 40°C; all tubes were then stored at −20°C until analyzed. At such time, the sample extract was dissolved in 50 μl of methanol and 25—40 μl aliquots were injected into the chromatograph, as described below.

Instrumental parameters

HPLC analyses was performed on a system consisting of a Model 6000A pumping module, a Model U6K injector, and a Model 450 variable-wavelength UV detector (Waters Assoc.). Separations were obtained on a μPorasil column (10 μm, 3.9 mm I.D. × 30 cm, Waters Assoc.) using a mobile phase composed of hexane—tetrahydrofuran—methanol (72:6:2) at a flow-rate of 2.0 ml/min. Chromatographic peaks were detected at 214 nm and recorded on a single-channel strip chart recorder (Houston Instruments). The injection of 2.0 μg of methyprylon, 0.2 μg of II, and 0.3 μg of internal standard per 25 μl resulted in peaks of nearly 50% full-scale response at a sensitivity of 0.04

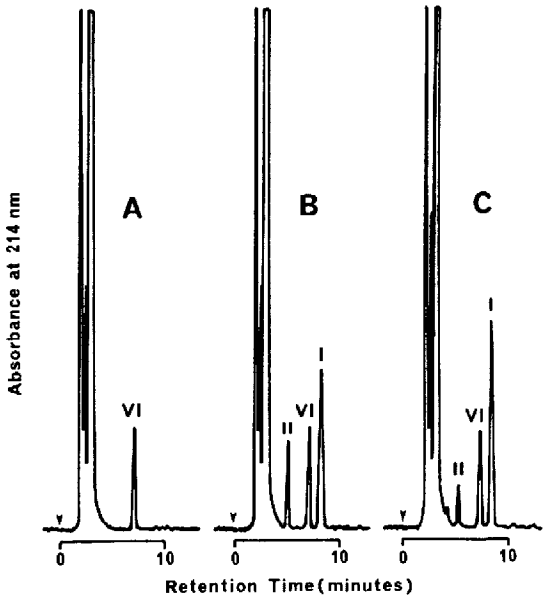

Fig. 2. (A) Chromatogram of drug-free plasma processed as described under Sample preparation. (B) Chromatogram of plasma supplemented with I (1.0 μg/ml) and II (0.1 μg/ml). (C) Chromatogram of plasma taken 1 h after oral administration of 300 mg of methyprylon (Noludar).

a.u.f.s. The chart speed was 0.067 inch/min. Fig. 2 shows representative chromatograms obtained using these conditions.

RESULTS

Statistical validation

The intra-assay linearity and precision of the method was determined over the plasma concentration ranges of 0.1—10.0 μg/ml and 0.01—1.0 μg/ml for I and II, respectively (Table I). Triplicate samples of each concentration were analyzed and peak height ratios calculated (peak height of I or II to peak height of VI). Calibration curves were then constructed by weighted linear ($1/y$) regression analysis of peak height ratio vs. concentration data.

For methyprylon, a correlation coefficient of 0.998 was obtained with an average coefficient of variation (C.V.) of 9.4% over the concentration range studied. Recovery, determined by comparing peak heights of processed vs. unprocessed standards, averaged 84—86%, with a lower limit of sensitivity of 0.1 μg/ml using 1.0-ml samples. For metabolite II, a correlation coefficient of 0.999 was obtained with an average C.V. of 8.4%. Recovery averaged somewhat higher than that of the parent compound, ranging between 88 and 90%, with a sensitivity limit of 0.01 μg/ml.

TABLE I

INTRA-ASSAY STATISTICAL EVALUATION

Compound	Concentration added (μg/ml)	Mean concentration found ± S.D. (n = 3)	Coefficient of variation (%)
Methyprylon*	0.1	0.095 ± 0.021	22.1
	1.0	1.11 ± 0.08	7.2
	2.5	2.40 ± 0.13	5.4
	5.0	5.23 ± 0.35	6.7
	10.0	9.83 ± 0.53	5.4
		Mean =	9.4
Metabolite II**	0.01	0.010 ± 0.002	20.2
	0.10	0.11 ± 0.01	5.6
	0.50	0.48 ± 0.02	3.8
	1.00	1.01 ± 0.04	4.2
		Mean =	8.5

*$Y = 0.481X + 0.004$ ($r = 0.998$).

**$Y = 2.718X + 0.010$ ($r = 0.999$), where X = concentration of I or II and Y = peak height ratio.

Selectivity

The selectivity of the assay for I and II was demonstrated by their resolution from the other three metabolites of methyprylon, III—V (Fig. 3). While the extraction of compounds III—V using the described procedure was not evaluated in this study, earlier reports have described the extraction of methyprylon and its metabolites from urine [14].

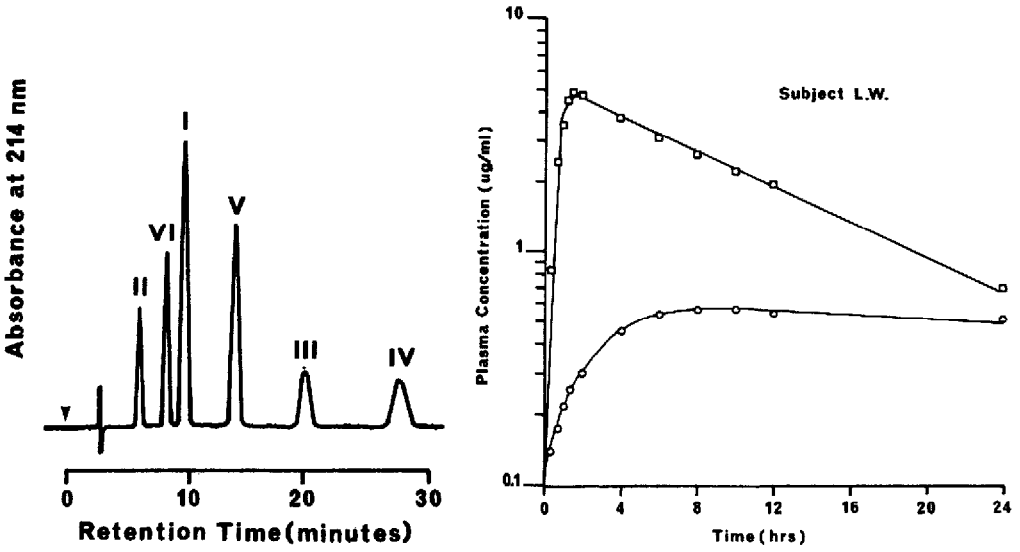

Fig. 3. Chromatogram of authentic standards I—VI obtained as described under Instrumental parameters.

Fig. 4. Plasma concentration vs. time profile for methyprylon (□) and its major plasma metabolite II (○) in a single, healthy, human volunteer (L.W.), following a single 300-mg dose of methyprylon (Noludar).

Preliminary biological applications

The assay was applied to the determination of concentrations of I and II following a single 300-mg dose of methyprylon (Noludar) in a single healthy subject (Fig. 4). A peak concentration of 4—5 μg of I per ml of plasma was determined 2—3 h post dosing, declining to 0.8 μg/ml at 24 h. Concentrations of II demonstrated a maximum plateau of approximately 0.5 μg/ml from 7 to 24 h. The pharmacokinetic profile of I in ten subjects will be the subject of a future publication [18].

DISCUSSION

During the initial development of this assay, several reversed-phase HPLC systems were investigated, including that developed by Kabra et al. [19] for the screening of sedative hypnotics in serum [19]. However, due to the similarity in lipophilic character between methyprylon and II, acceptable separation of these two compounds could not be achieved, even when ion pairing reagents (e.g. heptane sulfonic acid) were added to the mobile phase(s). The normal-phase system described herein results in baseline separation of both I and II in plasma, with retention times of 8.5 and 5.0 min, respectively.

Methyprylon displays two UV absorption maxima, at 298 and 214 nm, while metabolite II displays maxima at 307 and 218 nm. Since the molar absorptivity of methyprylon at 214 nm is approximately ten-fold greater than that at 298 nm (ϵ = 550 at 214 nm), the lower wavelength was chosen for quantitation. Although its absorptivity is low, compared to metabolite II (ϵ = 5100 at 214 nm), the lower limit of detection of this method was never-

theless sufficient to be acceptable for clinical pharmacokinetic studies.

The internal standard used in this assay, namely pyrithyldione or persedon, was chosen from several candidates based on its retention time relative to the other two compounds (7.0 min) and because of its ready availability. The dihydro derivative of pyrithyldione, with a retention time of 13.7 min, could also be used, the only disadvantages being its relatively low UV absorbancy and commercial unavailability.

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REFERENCES

1 N. Burstein and H. Strauss, J. Amer. Med. Ass., 194 (1965) 237.
2 M. Yudis, C. Swartz, G. Onesti, O. Ramirez, D. Snyder and A. Brest, Ann. Intern. Med., 68 (1968) 1301.
3 G. Xanthaky, A. Freireich, W. Matusiak and L. Lukash, J. Amer. Med. Ass., 198 (1966) 1212.
4 J.M. Mandlebaum and N. Simon, J. Amer. Med. Ass., 216 (1971) 139.
5 A. Pancorbo, P. Palagi, J. Piecoro and H. Wilson, J. Amer. Med. Ass., 237 (1977) 470.
6 R.A. Polin, D. Henry and C.E. Pippinger, J. Pediatr., 90 (1977) 831.
7 J.-L. Benedetti, L.A. Ferron, A.J. Nantel, J.Y. Savoie and T.H. Schwartz, 2'eme Congres International Franco-Americo-Canadien de Toxicologie Clinique et Analytique, Sainte-Adele, Quebec, Canada, September 1—5, 1977.
8 Y.S. Brownstone, D.Y. Brownstone, M.J. Diamond and E. Foreman, Clin. Chem. (Winston-Salem, N.C.), 24 (1978) 1060.
9 L.O. Randall, V. Ilier and O. Brandman, Arch. Int. Pharmacodyn., 106 (1956) 388.
10 O. Pribilla, Arch. Toxicol., 18 (1959) 39.
11 J. Bosche and G. Schmidt, Arzneim.-Forsch., 16 (1966) 548.
12 J. Bosche, Arzneim.-Forsch., 19 (1969) 123.
13 J. Bosche, F. Reibel and Gg. Schmidt, Arch. Toxikol., 25 (1969) 65.
14 S. Dickson, Forensic Sci., 4 (1974) 177.
15 R.R. Bridges and M.A. Peat, J. Anal. Toxicol., 3 (1979) 21.
16 M. VanBoven and I. Sunshine, J. Anal. Toxicol., 3 (1979) 174.
17 P. Gwilt, M. Pankaskie and J. Mitala, Can. Anaesth. Soc. J., 29 (1982) 381.
18 P. Gwilt, M. Pankaskie, R. Zustiak and J. Thornburg, in preparation.
19 P. Kabra, H. Koo and L. Marton, Clin. Chem. (Winston-Salem, N.C.), 24 (1978) 657.