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Note

Plasma and urine concentrations of methapyrilene by nitrogen—phosphorus gas—liquid chromatography

R.C. BASELT*

Department of Pathology, University of California, School of Medicine, Davis, CA 95616 (U.S.A.)

and

S. FRANCH

Office of the Medical Examiner, State of Connecticut, Farmington, CT 06032 (U.S.A.)

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Methapyrilene is an antihistamine with sedative effects which is used in numerous over-the-counter sleeping aids and cold preparations. Since methapyrilene is widely available, it is commonly encountered during the analysis of human body fluids for toxicological purposes.

Several previously reported analytical methods for methapyrilene levels in blood and urine dealt with overdose situations [1—3]. These methods lack the sensitivity and specificity needed for detection of therapeutic levels of the drug.

Two other methods were reported with sensitivity for methapyrilene in the ng/ml range. One involved gas chromatography using a sulfur-specific flame photometric detector [4], sensitive enough to detect as little as 10 ng of methapyrilene on-column. However, it was claimed that a metabolite of methapyrilene, rather than methapyrilene itself, was detected in the plasma and urine samples. The other technique was designed as a general procedure for basic drugs in postmortem blood using a nitrogen—phosphorus detector [5]. Its sensitivity limit for methapyrilene, however, was not tested beyond 100 ng/ml since the method was not specifically concerned with any one drug.

The purpose of this communication is to present a rapid, gas—liquid chromatographic (GLC) procedure for determination of therapeutic concentra-

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tions of methapyrilene, to describe in quantitative terms the human urinary excretion of the drug, and to qualitatively follow its plasma profile over several hours.

EXPERIMENTAL

Chemicals and reagents

Methapyrilene HCl and brompheniramine maleate were supplied by Schering Corporation (Kenilworth, NJ, U.S.A.). A stock solution of methapyrilene was prepared by dissolving an appropriate amount of the salt in methanol to make a 1.0 mg/ml solution as the free base. A plasma standard was prepared by adding 5 μl of the stock solution to 50 ml of drug-free human plasma, resulting in a 100 ng/ml standard. Serial dilutions of this standard were made to prepare the 50 and 10 ng/ml standards. Urine standards of 50, 100 and 300 ng/ml concentration were prepared in a similar manner. The internal standard consisted of a 40 μg/ml aqueous solution of the maleate salt of brompheniramine.

Sample preparation

A 2-ml aliquot of either plasma or urine was transferred to a 15-ml glass-stoppered centrifuge tube. Exactly 25 μl of internal standard was added and the mixture vortexed. Four drops of concentrated ammonium hydroxide were added to the plasma while three drops were added to the urine to ensure that a pH 9 medium resulted. After vortexing, 4 ml of heptane—isoamyl alcohol (98.3:1.7) were added and the tube was capped and shaken by hand for 1 min. Following centrifugation for 5 min at 2000 rpm, 3 ml of the upper solvent layer were removed by aspiration, with care taken so as not to disturb the interface area, and transferred to a clean 15-ml graduated centrifuge tube. The solvent was evaporated to dryness under a stream of dry air while the tube was in a 40°C water-bath. Methanol (50 μl) was used to reconstitute the residue immediately after drying and a 2 μl aliquot injected into the gas chromatograph.

Instrumental conditions

A Hewlett-Packard Model 5711A gas chromatograph with dual nitrogen—phosphorus detectors was used. The instrument was fitted with a 0.6 m × 2 mm I.D. glass column containing 2% OV-1 on 100—120 mesh Chromosorb G HP (liquid phase and solid support purchased separately from Applied Science Labs., State College, PA, U.S.A.). The carrier gas, extra dry nitrogen, was set to a flow-rate of 60 ml/min; hydrogen flow-rate was 3 ml/min and air flow-rate was 50 ml/min to the nitrogen—phosphorus detectors. Instrument temperatures were maintained at injector, 250°C; oven, 230°C; and detector, 300°C. Retention times under these conditions for methapyrilene and brompheniramine were 2.5 and 3.3 min, respectively.

Calculation

Once linearity was established for methapyrilene using the plasma and urine standards, the quantitation of unknown samples was estimated from a

standard curve by means of the drug/internal standard peak height ratio.

Experimental subject

A non-fasting adult male volunteer, 73 kg, received a 25-mg oral dose of methapyrilene HCl (21.9 mg of free base) at approximately 9:00 a.m. Blood was collected in heparinized tubes over the ensuing 6 h and urine was collected over a 24-h period. After the plasma was obtained by centrifuging the blood and after the urine pH was determined with a Beckman Model 3500 pH meter, the samples were refrigerated and analyzed within 4 days.

Thin-layer chromatography

Thin-layer chromatographic separation of the drugs was accomplished following extraction of 8—10-ml volumes of urine using a procedure similar to that described above, excluding the addition of internal standard. The extracts were applied to thin-layer plates coated with a 250-μm thick layer of silica gel G (J.T. Baker, Phillipsburg, NJ, U.S.A.). The plates were developed first to a height of 7.5 cm in ethyl acetate—methanol—ammonia (82:13:5), removed and air-dried, and then redeveloped to a height of 15 cm in ethyl acetate—methanol (98:2). After final drying each plate was sprayed with acidified iodoplatinate reagent. The R_F values under these conditions were 0.52 for methapyrilene and 0.33 for an apparent metabolite.

RESULTS AND DISCUSSION

Methapyrilene and brompheniramine were well separated from each other and the solvent front under the described GLC conditions (Fig. 1). There was relatively little background from the plasma sample, although an interfering substance found to be persistent in drug-free plasma eluted just after methapyrilene and was twice the height of the drug peak of the 10 ng/ml standard. One possible source of this interference was the plastic bag in which the drug-free plasma was stored. No significant background interference was found in the urine extracts.

The extracts were reasonably stable when stored overnight in the refrigerator in 1 ml of methanol. Results of the stability and reproducibility studies of a 10 ng/ml plasma standard were found to be acceptable. The coefficient of variation based on 5 determinations over a 15-day period was 13.8% (Table I).

TABLE I

DAY-TO-DAY REPRODUCIBILITY AND STABILITY OF PLASMA METHAPYRILENE MEASUREMENTS BASED ON 5 SEPARATE DETERMINATIONS OVER A 15-DAY PERIOD

Concentration (ng/ml)*		
Mean	S.D.	C.V. (%)
9.74	1.34	13.8

*A 10 ng/ml methapyrilene plasma standard was prepared with each run and used as the reference.

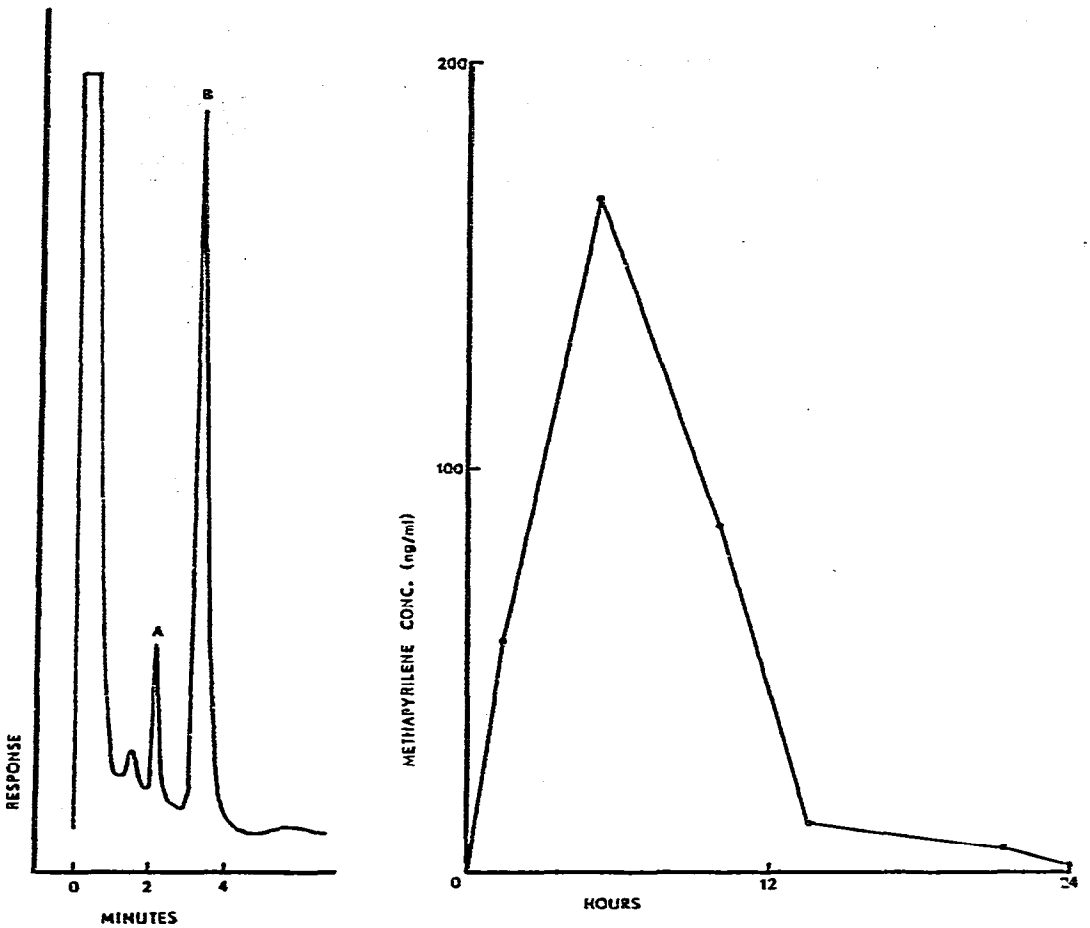

Fig. 1. Gas chromatogram of an extract of a urine sample from a subject who ingested 25 mg of methapyrilene HCl. A, Methapyrilene; B, internal standard (brompheniramine).

Fig. 2. Urine methapyrilene concentration profile showing range of concentrations for adult subject following ingestion of 25 mg of methapyrilene HCl.

Results obtained from a graph of the plasma drug/internal standard peak height ratio vs. concentration showed the volunteer's plasma concentration to be within a range lower than the 10 ng/ml plasma standard. Although the method is inaccurate at this range, we estimate that methapyrilene plasma levels were between 2 and 7 ng/ml over the 5 h following drug ingestion, with the peak concentration occurring 3 h after ingestion. The volunteer experienced no subjective feelings of drowsiness at this dosage.

The procedure was found to be linear for the urine methapyrilene concentrations over a range of 50—300 ng/ml. The urine methapyrilene concentration for the volunteer was at its maximum of 172 ng/ml at 5.3 h after oral administration of 25 mg of the drug (Fig. 2). The concentrations then fell rapidly and averaged less than 2 ng/ml at 24 h after administration.

The 24-h urinary excretion of unchanged methapyrilene amounted to

TABLE II

URINARY EXCRETION OF METHAPYRILENE*

Urine pH	Time elapsed (h)	Volume (ml)	Drug concentration (μg/ml)	Drug excreted (μg)	Urine flow-rate (ml/min)	Excretion rate (μg base/min)
6.4	1.6	90	0.054	4.9	0.9	0.051
5.8	5.3	145	0.172	25.0	0.7	0.110
5.6	10.0	175	0.080	14.0	0.6	0.049
6.1	13.8	136	0.009	1.2	0.6	0.005
6.2	21.7	335	0.005	1.7	0.7	0.004
5.8	23.2	87	0.002	0.2	1.0	0.002
5.8	24.0	53	0.002	0.1	1.1	0.002
	Total 1021 ml			Total 47.1 μg		

*In 24-h urine specimens following a single ingestion of 21.9 mg of methapyrilene base.

only 0.2% of the administered dose (Table II). The rate of excretion of methapyrilene, urinary pH, and urine flow over the 24-h period are also shown in Table II. The maximum rate of excretion was observed approximately 6 h after ingestion of the drug, while the urinary pH was steadily dropping.

In reviewing the amount of methapyrilene excreted, it is evident that either a large portion of the administered dose had not been absorbed or that the drug was extensively metabolized. Thin-layer chromatography performed on the urine sample taken 5 h after dosing showed an unknown spot of lesser intensity than the unchanged methapyrilene. It was found that this spot had the same R_F as acid-hydrolyzed methapyrilene. Thus, a portion of the methapyrilene that is absorbed may be hydrolyzed or oxidized in vivo. This is further supported by the thin-layer chromatographic properties of the apparent metabolite, which suggest that it is more polar than methapyrilene. This metabolite may be the hydroxylated compound that Schirmer and Pierson [4] detected with their GLC technique for methapyrilene. This compound was not observable during our GLC analysis of plasma or urine, however.

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