GLC Analysis of Methapyrilene in Plasma and Urine Using a Sulfur-Specific Flame-Photometric Detector

R. E. SCHIRMER▲ and R. J. PIERSON

Abstract □ A GLC procedure was developed for analysis of methapyrilene in blood and urine. Samples are extracted with chloroform, dried, dissolved in methanol, and injected on a 1.22-m. (4-ft.) glass column packed with 1% cyclohexanedimethanol succinate and 10% of a 5%-substituted phenyl methyl silicone on acid-washed, silylated diatomaceous earth and operated at 220°. A sulfur-specific flame-photometric detector is used, which allows concentrations as low as 50 ng./ml. to be measured. Methapyrilene was not detected in plasma or urine of human subjects receiving normal clinical doses of methapyrilene hydrochloride, but a methapyrilene metabolite was found in the urine samples. The absence of detectable levels of methapyrilene is probably due to rapid conversion of this compound to a metabolite that is not detected by the GLC procedure.

Keyphrases □ Methapyrilene—GLC analysis in plasma and urine using sulfur-specific flame-photometric detector □ GLC—analysis of methapyrilene in plasma and urine, sulfur-specific flame-photometric detector □ Sulfur-specific flame-photometric detector—GLC analysis of methapyrilene in plasma and urine

Many analytical methods for methapyrilene have been reported in the literature, including spectrophotometric (1–6), fluorometric (7, 8), and GLC (9–14) procedures. Three reported methods are concerned with methapyrilene in blood and urine (3, 5, 7), but the methods are neither specific enough nor sensitive enough for measuring methapyrilene blood levels in human subjects following normal doses of drug (25–50 mg.). This paper reports an attempt to develop a GLC assay for methapyrilene in plasma and urine utilizing a sulfur-specific flame-photometric detector. Although the method allows detection of as little as 10 ng. of methapyrilene on-column, no methapyrilene was found in plasma or urine samples from subjects receiving the drug, presumably due to rapid metabolism of this drug substance. Some special features of the flame photometric detector also are discussed.

EXPERIMENTAL

A gas chromatograph[1] equipped with a sulfur-specific flame-photometric detector[2] was used for the analysis. The column was a 1.22-m. (4-ft.) glass U-tube (3 mm. i.d.) packed with 1% Hi-Eff 8BP and 10% SE-52 on 80–100-mesh Gas Chrom Q. The column was operated isothermally at 220° with the injection port at 250°, the detector at 225°, and helium as the carrier gas. The hydrogen–oxygen–air ratio was 100:60:20. Under these conditions, the retention times of methapyrilene and phenothiazine (internal standard) were approximately 3.5 and 6.8 min., respectively.

Several column packings were screened before selecting the mixture of 8BP and SE-52. These packings included: 0.5 and 2% OV-17 on 80–100-mesh Chromosorb G, 5% SE-52 on 80–100-mesh Chromosorb G, 1.5% Versamid 900 on 60–80-mesh acid-washed Chromosorb W, and 11% QF-1 on 80–100-mesh Gas Chrom Q. These columns were rejected because of poor peak shape when 10–50 ng. of methapyrilene was injected onto the column or because methapyrilene was not sufficiently separated from the compounds considered for use as internal standards.

[1] F&M model 402.
[2] Melpar.

Table I—Efficiency of Extraction of Methapyrilene by Chloroform and Ethyl Acetate as a Function of pH

pH	Buffer Composition	Percent Methapyrilene Extracted by[a]: Chloroform	Ethyl Acetate
1.0	0.1 *M* HCl	0.2	0.4
3.0	0.2 *M* acetate buffer	2.7	0.3
5.0	0.2 *M* acetate buffer	89.7	37.0
7.0	0.2 *M* phosphate buffer	100.0	100.0
10.0	0.2 *M* borate buffer	100.0	100.0

[a] Percent methapyrilene in the organic phase after partitioning against an equal volume of the aqueous buffer solution.

Table II—Calibration Curve for the Flame-Photometric Sulfur Detector[a]

Concentration, mcg./ml.	Response Ratio[b]
50	1.58
100	8.16
150	15.3
200	33.5
250	51.1

[a] See text for operating conditions of the detector. [b] The response ratio is calculated as the height of the methapyrilene peak divided by the height of the phenothiazine peak. All samples contained 30 mcg./ml. phenothiazine.

Plasma samples were analyzed by extracting 1 ml. of the sample three times with 5-ml. portions of chloroform. Fifty microliters of a 30-mcg./ml. solution of phenothiazine in chloroform was added to the combined chloroform extracts, and the sample was then evaporated to dryness under a stream of nitrogen. The residue was dissolved in 10.0 μl. of methanol, and 2 μl. of the methanol solution was injected into the chromatograph for analysis. A calibration curve was prepared by injecting 1.0 μl. each of solutions containing 30 mcg./ml. phenothiazine and 25, 50, or 100 mcg./ml. methapyrilene in methanol. A typical chromatogram is presented in Fig. 1. The sample is injected in a methanol solution rather than in chloroform, because chloroform causes an undesirable background response in the flame-photometric detector.

Urine samples were analyzed by adjusting the pH of a 10-ml. aliquot to 10.0 with sodium hydroxide and extracting it three times with 10-ml. aliquots of chloroform. One milliliter of a 250-mcg./ml. solution of phenothiazine in methanol was added to the combined chloroform extracts, and the sample was then evaporated to dryness under a stream of nitrogen. The residue was dissolved in 100 μl. of methanol, and 2 μl. of this solution was injected into the

Table III—Precision and Accuracy of the Analytical Method

Concentration, mcg./ml.	Number of Replicates	Mean Result	Relative Error, %	Standard Deviation of the Mean
0.250	4	0.254	+1.6	0.008
0.500	7	0.524	+4.8	0.094
0.750	11	0.702	−6.3	0.118
1.000	5	1.020	+2.0	0.120
1.250	7	1.250	0.0	0.127

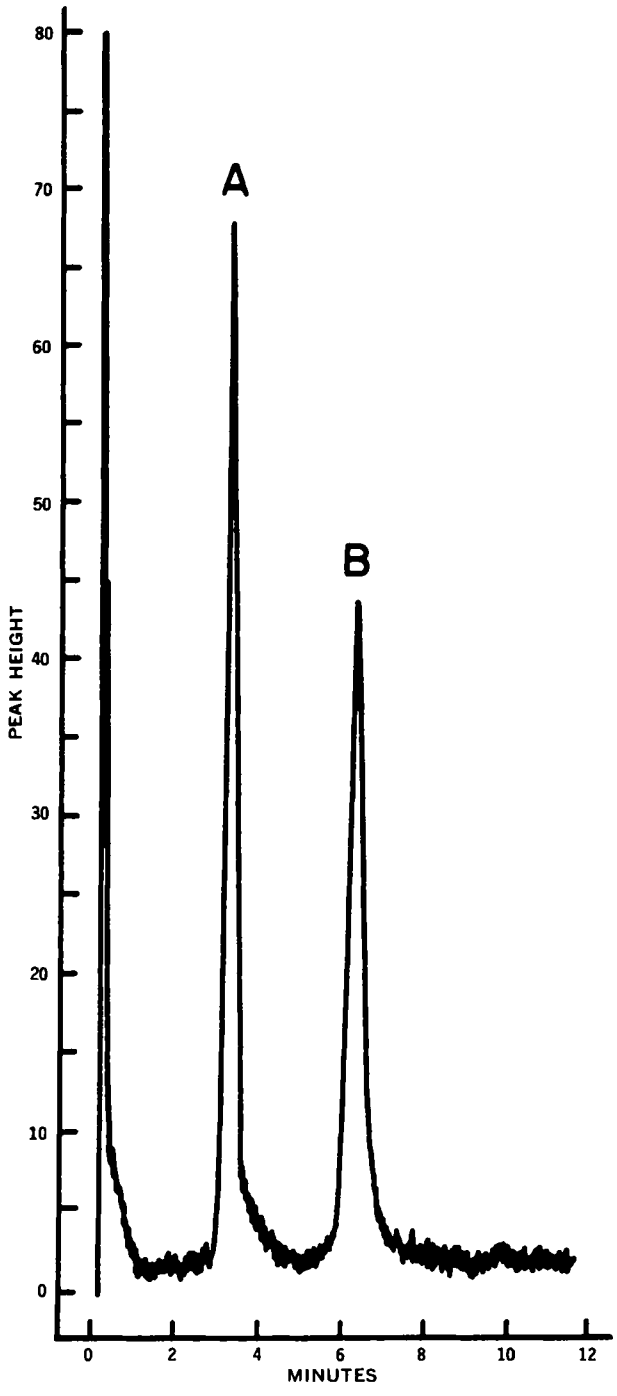

Figure 1—*Gas-liquid chromatogram of methapyrilene (A) and phenothiazine (B). See text for conditions.*

chromatograph. The calibration curve was prepared using the procedure described for plasma samples.

The efficiency of extraction of methapyrilene by chloroform and ethyl acetate was studied as a function of pH to establish conditions for the extraction in the assay. The extraction was studied by partitioning 10-ml. aliquots of 0.5 mg./ml. methapyrilene in either chloroform or ethyl acetate against 10-ml. aliquots of aqueous buffers of pH 1.0, 3.0, 5.0, 7.0, and 10.0. The percent methapyrilene remaining in the organic phase was determined by measuring the absorbance of the organic phase at 310 nm. and comparing it with the absorbance of the original 0.5-mg./ml. solution in the same solvent. The results are summarized in Table I. The extraction efficiencies from plasma and urine under the conditions outlined previously were checked and found to be identical to the corresponding values in Table I.

Studies of the response characteristics of the flame-photometric detector were reported in the literature (15, 16). In the sulfur-specific mode, the response varies linearly with the mass of the sample injected on-column under some gas flow conditions and with the square of the mass under other conditions (16). Therefore, it was considered desirable to establish the response characteristics of the detector under the flow conditions used in this procedure before using the method quantitatively. The response, R, can be written as:

$$R = k(mv)^n \qquad \text{(Eq. 1)}$$

where k is the response factor, m is the concentration of the methapyrilene solution, and v is the volume injected. By using primes to denote the analogous quantities for phenothiazine, the ratio of the response from methapyrilene to that from phenothiazine may be written as:

$$r = \frac{R}{R'} = \frac{k}{k'}\left(\frac{m}{m'}\right)^n \qquad \text{(Eq. 2)}$$

Thus, n may be evaluated by regressing log r against log m for a series of standard solutions containing a fixed concentration of phenothiazine. Methanol solutions containing 30 mcg./ml. of phenothiazine and from 50 to 250 mcg./ml. of methapyrilene in 50-mcg./ml. increments were prepared, and injections of 1.0-μl. portions of each of these solutions were made. The results are recorded in Table II. Analysis of these data gave $n = 2.10$, with 95% confidence limits of ± 0.08 and a correlation coefficient of 0.9916. A value of $n = 2.0$ was used throughout the remainder of this work.

The precision and accuracy of the method were determined by analyzing replicate samples of plasma spiked at several different methapyrilene levels. These samples were analyzed against the calibration curve presented in Table II, and the results are presented in Table III.

DISCUSSION

The standard deviations presented in Table III are quite large and are essentially constant over the upper end of the concentration range. The primary contribution to this variability is within the detector itself under the conditions of operation used in this study. It is possible that this variability could be reduced by better control of the gas flow rates or of the detector temperature. The flame is generally extinguished by each shot and must be relit before the sample peaks elute, so the effective detector temperature is probably quite variable.

In spite of the poor reproducibility, the sulfur-specific flame-photometric detector offers several significant advantages over a flame-ionization detector in the GLC assay for methapyrilene. The first advantage is greater sensitivity; the reported method can be used to measure concentrations as low as 50 ng./ml. in plasma or urine, and this limit could be reduced even further by using larger samples and injecting larger aliquots onto the GLC column. Another advantage is the very low background observed with extracts of plasma and urine. Under similar conditions, a flame-ionization detector would register many other peaks and the extraneous peaks would make it very difficult to measure methapyrilene at low levels.

Following development of the method on spiked plasma and urine samples, plasma and urine samples from human subjects who had received methapyrilene hydrochloride were analyzed. The subjects received 20 mg. by intramuscular or subcutaneous injection or 50 mg. orally. Plasma samples were obtained at 15 and 30 min. and at 1, 2, and 4 hr. An 8-hr. urine sample was also collected. The analysis revealed no methapyrilene in any plasma samples. The urine samples contained small amounts of a component whose retention time was identical to that of methapyrilene. Preliminary structural data indicate, however, that the component measured in urine is a hydroxylated metabolite of methapyrilene rather than methapyrilene itself. Work on identification of this metabolite is in progress. The presence of this metabolite in the urine, the drowsiness reported by the subjects, and the absence of detectable levels of parent compound in plasma together suggest that the absence of detectable levels of parent compound in plasma is not due to poor absorption of the drug from the gut or from the site of injection but rather to rapid conversion of methapyrilene to metabolites that are not detected by the GLC procedure.

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N-Dimethylation of β-Phenylethylamine Derivatives

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Abstract □ Direct *N*-methylation of 3,4-dimethoxyphenethylamine, 4-methoxyphenethylamine, tyramine, and 3-methoxytryamine, by refluxing each compound with a mixture of formaldehyde and formic acid in the presence of dimethylformamide, to the corresponding *N*-dimethyl derivative in 45–86% yield is reported. The synthesis of macromerine from normetanephrine by a two-step methylation, using diazomethane and formaldehyde–formic acid mixture, is also reported.

Keyphrases □ *N*-Dimethylation—aromatic primary amines in dimethylformamide using formaldehyde–formic acid □ β-Phenylethylamines and β-phenylethanolamines, *N,N*-dimethyl derivatives—as potential metabolites of levodopa □ Macromerine—synthesis, two-step methylation using diazomethane and formaldehyde–formic acid

Levodopa (L-3,4-dihydroxyphenylalanine) is used extensively in the treatment of Parkinson's disease. In spite of marked improvements in many patients, some adverse reactions, including psychotic symptoms such as confusion, hallucination, and agitation, have been noted (1, 2). *N,N*-Dimethyl-3,4-dimethoxy-β-hydroxy-β-phenethylamine (macromerine), a possible metabolite of levodopa, was reported to be hallucinogenic when tested in squirrel monkeys (3). A number of alkaloids, including *N,N*-dimethyl-3,4-dimethoxyphenethylamine, *N,N* - dimethyl - 3 - methoxytyramine, *N,N* - dimethyltyramine (hordenine), *N*-methyl-3,4-dimethoxy-β-hydroxy-β-phenethylamine (normacromerine), and macromerine have been isolated from cactus plants (4–7).

The possibility that such compounds may be formed in the human or animal body as metabolites of levodopa or 3,4-dihydroxyphenethylamine (dopamine) prompted the search for a relatively simple method of *N*-methylation of β-phenylethylamine and β-phenylethanolamine derivatives. The preparation of *N*-methylated derivatives of β-phenylethylamine and the ring-substituted compounds was reported previously (8). This method involves several steps, starting with the corresponding aldehyde, and is very time consuming. Methods for methylating aliphatic amino alcohols and amino mercaptans using formaldehyde and formic acid were described (9, 10), and a modified method for preparing *N,N*-dimethyl derivatives of other amino alcohols or amino mercaptans in 90–100% yield was published (11). This method has been modified to include a reaction medium, dimethylformamide, and extended to the *N*-methylation of β-phenylethylamine derivatives.

An attempt to identify these compounds as metabolites of levodopa in Parkinson's disease is in progress.

EXPERIMENTAL[1]

Materials—3,4-Dimethoxyphenethylamine (I) and its hydrochloride (I*a*), 4-methoxyphenethylamine (II), tyramine (III), 3-methoxytyramine (IV), normetanephrine hydrochloride (V), octopamine hydrochloride (VI), dimethylformamide[2], formaldehyde (35%)[3], formic acid (88%), ethyl acetate, ether, and methanol[4] were used.

Methylations—N,N-*Dimethyl-3,4-dimethoxyphenethylamine* (*VII*) —To 220 mg. (1 mmole) of I*a* taken in a 10-ml. round-bottom flask,

[1] All melting points were taken on a Fisher-Johns hot-stage melting-point apparatus and are uncorrected. Microanalyses were performed by PCR, Inc., Gainesville, Fla. TLC was done on silica gel G (E. Merck, Darmstadt, Germany) in two solvent systems: Solvent A, *n*-butanol–acetic acid–water (4:1:1); and Solvent B, isopropanol–10% ammonia–water (8:1:1) (12). GLC was performed in a Barber-Colman model 15 gas chromatograph provided with an ionization detector system (radium source). A glass U-tube (1.83 m. × 4 cm.) containing 12% C_6-diethylene glycol succinate on Anakrom ABS 60–70 mesh (Analabs, Inc., North Haven, Conn.), preconditioned at 200° for 72 hr. with nitrogen flowing at the rate of 100 ml./min. and maintained at 200° with an external argon pressure of 20 p.s.i. (flow rate at exit 98 ml./min.), was used as a polar column. A nonpolar column of 15% Apiezon L on 100–120-mesh Gas Chrom Q (Applied Science Laboratories, State College, Pa.), preconditioned at 255° for 144 hr. with nitrogen flowing at the rate of 66 ml./min. and maintained at 255° with an external argon pressure of 30 p.s.i. (flow rate 50 ml./min.) was also used for analyses.

[2] Sigma Chemical Co., St. Louis, Mo.

[3] E. Merck & Co., Rahway, N. J.

[4] Mallinckrodt Chemical Works, St. Louis, Mo.