Table III—Cumulative 24-hr Excretion of Meperidinic and Normeperidinic Acids

Subject	Meperidinic Acid, mg	Percent Dose	Normeperidinic Acid, mg	Percent Dose
1	8.89	17.8	3.56	7.1
2	6.49	12.8	4.73	9.5
3	13.91	27.8	4.17	8.3
4	4.49	9.0	2.22	4.4
5	12.98	26.0	6.51	13.0

subject to subject (Table III), and the 24-hr cumulative excretion of meperidinic acid accounted for between 9.0 and 27.8% of the administered dose. These results are consistent with the previously reported findings for these metabolites of 10.3–40.9% of the administered dose when 200–1180 mg (over 20 hr) (2) and 100 mg (6) of meperidine were administered.

The 24-hr cumulative excretion of normeperidinic acid ranged between 4.4 and 13.0% of the administered dose (Table III); these results are also consistent with previous studies of normeperidinic acid, which accounted for 2.7–28.3% of the administered dose (2, 6). No other attempt was made to quantitate the conjugate esters of meperidinic and normeperidinic acids, which accounted for 0–16.1 and 3.8–22.3% of the administered dose, respectively.

The hourly excretion of meperidine, normeperidine, and meperidinic and normeperidinic acids was followed in one subject (Table IV). The meperidine excretion rate reached a maximum of 3.50 μg/ml hr at 2 hr. Meperidinic acid also reached its maximum at 2 hr with a rate of 10.24 μg/ml hr. The maxima for normeperidine and normeperidinic acid were reached at 6 hr with rates of 0.60 and 2.64 μg/ml hr, respectively.

The described GLC procedure is reliable and easily performed, and it can be applied to the study of the pharmacokinetic and metabolic parameters of meperidine in humans using average clinical doses.

Table IV—Hourly Excretion of Meperidine, Normeperidine, and Meperidinic and Normeperidinic Acids by Subject 3

Hour	Meperidine, mg	Normeperidine, mg	Meperidinic Acid, mg	Normeperidinic Acid, mg
1	0.01	0.00	0.61	0.00
2	0.44	0.08	1.28	0.36
4	0.46	0.06	1.12	0.34
6	0.56	0.28	3.37	0.81
8	0.08	0.08	1.49	0.58
12	0.04	0.24	2.68	0.89
24	0.19	1.07	3.36	1.19
Total	1.71	1.81	13.91	4.17

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Simultaneous GLC Determination of Phenylpropanolamine and Chlorpheniramine in Urine Using a Nitrogen Selective Detector

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Abstract □ A simple, rapid, and sensitive simultaneous quantitative determination of phenylpropanolamine and chlorpheniramine in human urine by GLC, using a nitrogen specific detector, is described. After alkaline extraction from urine, phenylpropanolamine and chlorpheniramine are analyzed directly by GLC, without a derivatization step. Promethazine was used as the internal standard. The total assay time is less than 30 min. The method is useful in studies of pharmacokinetic and pharmacological interactions of drug combinations.

Keyphrases □ Phenylpropanolamine—GLC analysis in presence of chlorpheniramine, human urine □ Chlorpheniramine—GLC analysis in presence of phenylpropanolamine, human urine □ GLC—analyses, phenylpropanolamine and chlorpheniramine simultaneously, human urine □ Adrenergic agents—phenylpropanolamine, GLC analysis in presence of chlorpheniramine, human urine □ Antihistaminics—chlorpheniramine, GLC analysis in presence of phenylpropanolamine, human urine

Phenylpropanolamine has been identified and quantitated by a spectrophotometric method after periodate oxidation (1) according to the original method of Shinn and Nicolet (2). This method was time consuming in comparison to GLC for phenylpropanolamine and chlorpheniramine analysis (3–7).

GLC, using a nitrogen selective detector, is sensitive and allows specific detection (8–11).

The purpose of this work was to develop a simultaneous quantitative method for the determination of phenylpropanolamine and chlorpheniramine in human urine by GLC with a nitrogen specific detector.

EXPERIMENTAL

Instrumentation—The gas chromatograph[1] was equipped with flame-ionization and nitrogen detectors[2] connected to a recorder[3] with a scale range of 1 mv. The stainless steel column (2.17 mm × 2 m) was packed with 3% OV-1[4] on 100–120-mesh Gas Chrom Q[5] and conditioned at 260° for 34 hr with 35 ml of nitrogen (U quality)/min.

The chromatographic conditions were as follows: injected quantity of sample, 1–2 μl; injector temperature, 240°; detector temperature, 280°; column temperature, 230° in isotherm; carrier gas (nitrogen U) flow rate, 35 ml/min; hydrogen (U) flow rate, 30 ml/min; air (medical quality) flow

[1] Girdel model 3000 1 ERPT, Paris, France.
[2] Girdel model 15 489, Paris, France.
[3] Servotrace PU Sefram, Paris, France.
[4] Pierce Chemical, Rockford, IL 61105.
[5] Applied Sciences, Inglewood, CA 90304.

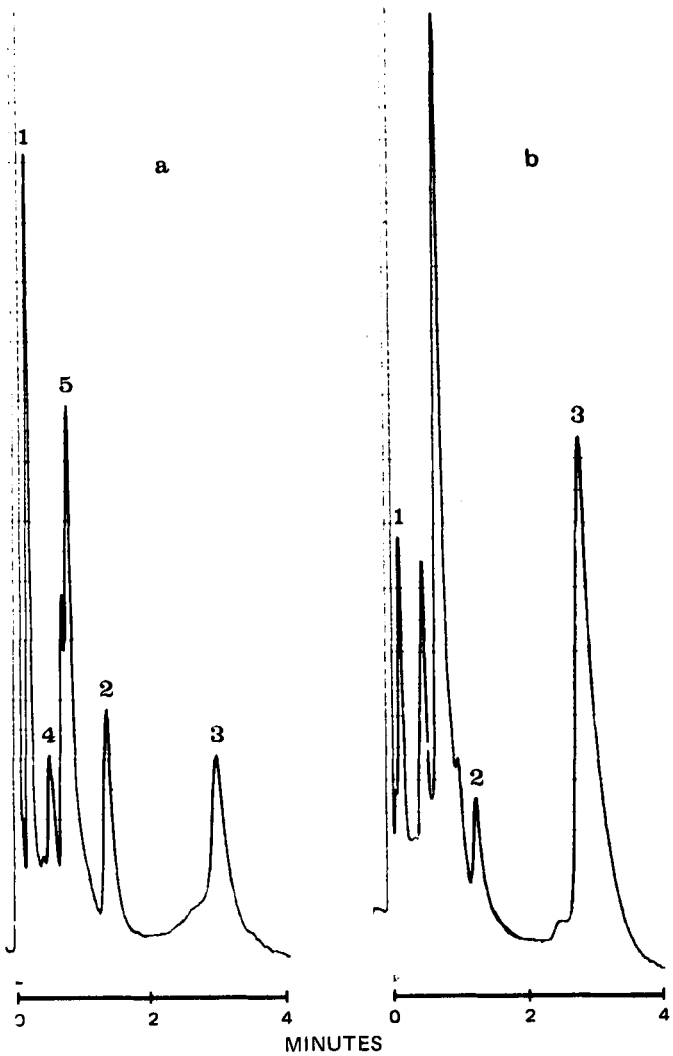

Figure 1—(a) *Gas chromatogram obtained during the peak height ratio standardization in the standard curve, Phenylpropanolamine (1), chlorpheniramine (2), promethazine (3), a constituent of the solvent (4), and a urinary constituent (5) were present.* (b) *Representative chromatogram from a patient receiving an oral dose of 8-mg of chlorpheniramine and 50 mg of phenylpropanolamine in a combination dosage form.*

rate, 350 ml/min; sensitivity and attenuation, 1 × 64; and chart speed, 10 mm/min.

Standard Solutions—The stock standard solutions (1 mg/ml) of promethazine chlorhydrate[6], phenylpropanolamine chlorhydrate[7], and chlorpheniramine maleate[7] were prepared by directly dissolving them in methanol. After some decomposition in the first 48 hr, the promethazine chlorhydrate standard solution was stable for several months (12). The standard solutions were stored at 4° in a cold chamber.

Extraction Procedure—To 1 ml of normal human urine in a glass test tube[8] (polytef-lined screw cap) were added successively 15 μl of promethazine (15 μg), 50–150 μl of phenylpropanolamine (50–150 μg), 2.5–10 μl of chlorpheniramine (2.5–10 μg) from stock standard solutions, and 0.5 ml of 1 *N* NaOH. The mixture was shaken and extracted with 2 × 3 ml of ether with vigorous stirring. The organic fractions were mixed and dried on anhydrous sodium sulfate and then evaporated under an air flow in a water bath at 30°.

The residue was dissolved in methanol (30 μl) by ultrasonic shaking for 30 sec. Then 1–2 μl of the mixture was injected into the gas chromatograph.

[6] Specia, Paris, France.
[7] Smith Kline and French, Paris, France.
[8] Sovirel, Paris, France.

RESULTS AND DISCUSSION

Figure 1*a* represents the gas chromatogram obtained during the peak height ratio standardization of phenylpropanolamine on promethazine and of chlorpheniramine on promethazine in a standard curve. In spite of the nitrogen specific detection, five peaks were observed. Two came from the impurity of the solvent and a urinary constituent. The relative retention times (to the solvent) were 0.3 min for phenylpropanolamine, 1.55 min for chlorpheniramine, and 3.35 min for the internal standard. The total analysis time was 30 min or less, suitable for several analyses.

The chromatographic yield of phenylpropanolamine ($C_{19}H_{13}NO$) with one nitrogen atom was less than that of chlorpheniramine ($C_{16}H_{19}ClN_2$), but phenylpropanolamine had better permeability through the 3% OV-1 packed column.

The selectivity and sensitivity of the nitrogen selective detector (8–10) contribute to the suitable separation of the tailing peak of methanol (solvent) from that of phenylpropanolamine on the weak polar phase OV-1. This separation was not possible with flame-ionization detection because of the short retention time of phenylpropanolamine and the unfavorable ratio of the amount of phenylpropanolamine to the solvent.

Figure 1*b* shows the representative chromatogram of a biological specimen from a patient receiving an oral dose of phenylpropanolamine chlorhydrate (50 mg) and chlorpheniramine maleate (8 mg) in a combination dosage form. One hundred samples were assayed in this laboratory.

The standard curves were prepared from normal human urine samples spiked with 0–150 μg of phenylpropanolamine, 0–10 μg of chlorpheniramine, and a constant quantity of the internal standard. The curves of the peak height ratio *versus* the added amount of phenylpropanolamine and chlorpheniramine were linear over these ranges.

The concentration in urine was within the range of the concentrations found following daily doses of 8–16 mg of chlorpheniramine and 50–100 mg of phenylpropanolamine.

The errors on the peak height ratio in standard curves for any of the three points were 7.5% for phenylpropanolamine (50, 100, and 150 μg) and 6% for chlorpheniramine (2.5, 5, and 10 μg). The recovery for both products was 98.5 ± 3.5%, and the coefficients of variation within and between assays were 2.5 and 7.5%, respectively.

The limit of sensitivity was not reached and might be improved.

After alkaline extraction, phenylpropanolamine, chlorpheniramine, and promethazine were in a relatively volatile basic form. Evaporation at room temperature under nitrogen may be desirable.

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