trum of the ethotoin–II system was the summation of the spectra of the two components. Again there was no evidence of complexation between the two compounds. The formation of an insoluble complex between nitrofurantoin and II may explain the decrease in the dissolution rates from both the nitrofurantoin–II physical mixture and the coprecipitate.

REFERENCES

(1) K. Sekiguchi and N. Obi, *Chem. Pharm. Bull.*, **9**, 866 (1961).

(2) K. Sekiguchi, N. Obi, and Y. Ueda, *ibid.*, **12**, 134 (1964).

(3) A. H. Goldberg, M. Gibaldi, and J. L. Kanig, *J. Pharm. Sci.*, **54**, 1145 (1965).

(4) *Ibid.*, **55**, 581 (1966).

(5) *Ibid.*, **55**, 487 (1966).

(6) A. H. Goldberg, M. Gibaldi, J. L. Kanig, and M. Mayersohn, *J. Pharm. Sci.*, **55**, 581 (1966).

(7) M. Mayersohn and M. Gibaldi, *ibid.*, **55**, 1323 (1966).

(8) R. G. Stoll, T. R. Bates, K. A. Nieforth, and J. Swarbrick, *ibid.*, **58**, 1457 (1969).

(9) M. Gibaldi, S. Feldman, and T. R. Bates, *ibid.*, **57**, 708 (1968).

(10) W. L. Chiou and S. Riegelman, *ibid.*, **58**, 1505 (1969).

(11) *Ibid.*, **59**, 937 (1970).

(12) *Ibid.*, **60**, 1376, 1569 (1971).

(13) A. P. Simonelli, S. C. Mehta, and W. I. Higuchi, *J. Pharm. Sci.*, **58**, 538 (1969).

(14) T. R. Bates, *J. Pharm. Pharmacol.*, **21**, 710 (1969).

(15) G. H. Svoboda, M. J. Sweeney, and W. D. Walkling, *J. Pharm. Sci.*, **60**, 333 (1971).

(16) S. A. Said, H. M. El Fatatry, and A. S. Geneidi, *Aust. J. Pharm. Sci.*, **3**(2), 42 (1974).

(17) E. I. Stupak and T. R. Bates, *J. Pharm. Sci.*, **61**, 400 (1972).

(18) A. A. Kassen, S. A. Said, and M. El-Samaligy, *Aust. J. Pharm. Sci.*, **4**, 123 (1974).

(19) W. I. Higuchi, N. A. Mir, and S. J. Desai, *J. Pharm. Sci.*, **54**, 1405 (1965).

(20) M. H. Malone, H. I. Hochman, and K. A. Nieforth, *ibid.*, **55**, 972 (1966).

(21) D. J. Allen and K. C. Kwan, *ibid.*, **58**, 1190 (1969).

(22) T. Tachibana and A. Nakamura, *Kolloid-Polym.*, **203**, 130 (1965).

(23) T. Tsuji, K. Foukik, and T. Takaibo, *Bull. Chem. Soc. Jpn.*, **42**, 2193 (1969).

(24) R. Rastogi and P. Bassi, *J. Phys. Chem.*, **68**, 2398 (1964).

(25) S. Said, H. El Fatatry, and M. El Samaligy, *Aust. J. Pharm. Sci.*, **3** (2), (1974).

(26) D. C. Monkhouse and J. L. Lach, *J. Pharm. Sci.*, **61**, 1430 (1972).

(27) T. R. D. Shaw, K. Raymond, M. R. Howard, and J. Hammer, *Br. Med. J.*, **4**, 763 (1973).

(28) J. Linenbaum, V. P. Butler, J. E. Murphy, and R. M. Crosswell, *Lancet*, **1**, 1215 (1973).

(29) G. Levy and B. A. Hayes, *N. Engl. J. Med.*, **262**, 1053 (1960).

(30) T. R. Bates, M. Gibaldi, and J. L. Kanig, *Nature*, **210**, 1331 (1966).

ACKNOWLEDGMENTS

The authors acknowledge the technical help of Mr. M. H. Ibrahim.

GLC Determination of Meperidinic and Normeperidinic Acids in Urine

I. W. WAINER ˣ and J. E. STAMBAUGH

Received December 9, 1976, from the *Department of Pharmacology, Section of Clinical Pharmacology, Thomas Jefferson University, Philadelphia, PA 19107.* Accepted for publication February 10, 1977.

Abstract □ A GLC procedure for the determination of meperidinic and normeperidinic acids in human urine is described. After the extraction of any interfering meperidine or normeperidine, the urine samples are dried and the acids are reesterified using ethanol–sulfuric acid. The resulting meperidine and normeperidine are then extracted and quantified. With this method, the urinary excretion of these metabolites was followed in five subjects who received a single meperidine dose of 36 mg/m^2 im. This method represents an improvement over the previously described methods for meperidinic and normeperidinic acids and can be applied to clinical situations.

Keyphrases □ Meperidine metabolites—meperidinic and normeperidinic acids, GLC analyses, human urine □ GLC—analyses, meperidinic and normeperidinic acids in human urine □ Analgesics, narcotic—meperidine metabolites, GLC analyses in human urine

The pharmacokinetics of meperidine (I) were described previously (1), and its metabolites were identified (2). Unchanged meperidine was identified in the urine along with the *N*-demethylated metabolite normeperidine (II), meperidinic acid (III), normeperidinic acid (IV), and conjugated esters of these acids (Scheme I) (2). These initial studies were limited to high drug doses due to the limitations of the analytical colorimetric method.

The recent development of more sensitive GLC assays for meperidine in serum (3) and for meperidine and normeperidine in urine (4) permitted the study of the pharmacokinetics and metabolism of meperidine in humans using usual clinical doses (5). A sensitive method for the determination of urinary levels of meperidinic and normeperidinic acids has not been reported previously. This report describes a GLC technique for the analysis of these metabolites using small aliquots of urine samples.

EXPERIMENTAL

Ten milliliters of urine, 1 ml of 5 *N* NaOH, and 5 ml of chloroform were shaken for 10 min in a 50-ml glass-stoppered centrifuge tube. Then the tube was centrifuged, and 9 ml of the aqueous phase was transferred to a 50-ml round-bottom flask and evaporated to dryness by freeze drying. Absolute ethanol, 9 ml, and 1 ml of sulfuric acid were added to the residue, and the resulting mixture was refluxed for 3 hr.

After cooling, 1 ml of the reaction mixture was added to a 15-ml screw-capped centrifuge tube containing 2 ml of 2.5 *N* NaOH. Chloroform, 100 μl, containing an internal standard (lidocaine, 20 μg/ml), was added to the solution, and the resulting mixture was vortexed for 30 sec and centrifuged for 5 min. A 5-μl aliquot of the chloroform layer was then injected into the chromatograph[1].

The analysis was carried out using a hydrogen flame-ionization detector

[1] Perkin-Elmer model 3920.

Table I—Recovery of Meperidine following the Esterification of Urine Samples Containing Meperidinic Acid[a]

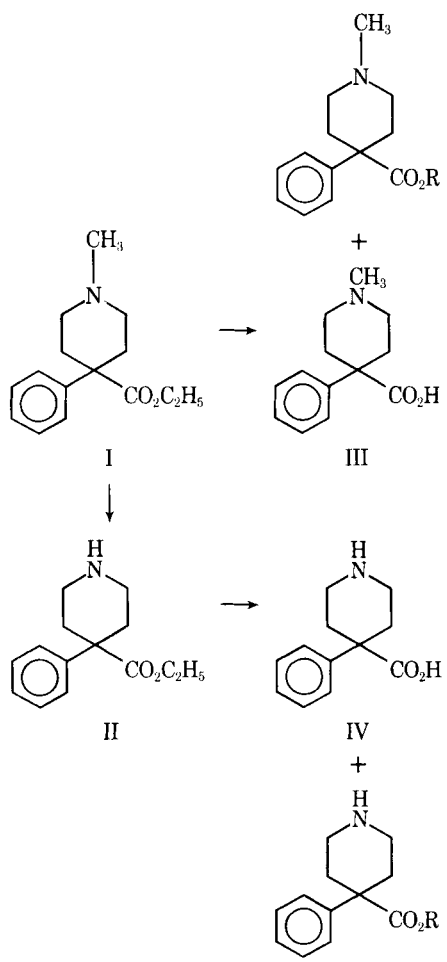
Meperidinic Acid Added, μg/ml	Meperidine Recovered, μg/ml	Conversion and Recovery, %
10	7.73 ± 0.15	77.3 ± 1.3
7	5.46 ± 0.15	78.1 ± 2.2
5	3.76 ± 0.32	75.3 ± 6.4
2	1.48 ± 0.08	74.2 ± 4.3

[a] Figures represent the mean ± *SD* for three analyses.

and a 2-mm i.d. × 1.8-m coiled glass column packed with 3% OV-17 on 100–120-mesh Gas Chrom Q[2]. A column temperature of 175° and an injector and detector temperature of 250° were used. The gas flows were: hydrogen, 60 ml/min; air, 500 ml/min; and nitrogen carrier gas, 55 ml/min. Under these conditions, the retention times of meperidine, normeperidine, and lidocaine were approximately 4.4, 5.2, and 6.8 min, respectively (Fig. 1).

Following chromatography, a baseline was drawn and the peak heights of meperidine, normeperidine, and lidocaine were measured. The ratios of meperidine and lidocaine peak heights and normeperidine and lidocaine peak heights were calculated, and the concentrations were obtained from the standard curves.

Standard curves for meperidinic and normeperidinic acids were constructed by the analysis of urine samples to which known amounts of the meperidinic and normeperidinic acids[3] had been added. The concentrations ranged from 20 to 1 μg/ml. The standard curves were described by the equations $y = 19.6x + 0.9$ with a correlation coefficient (r) = 0.99 for normeperidinic acid and $y = 3.9x - 0.4$ (r = 0.99) for meperidinic acid. The percent conversion and the percent recovery were calculated by



Scheme I

[2] Applied Science Laboratories, State College, Pa.

[3] Standards supplied by Sterling-Winthrop Research Institute, Renssalaer, N.Y.

Table II—Recovery of Normeperidine following the Esterification of Urine Samples Containing Normeperidinic Acid[a]

Normeperidinic Acid Added, μg/ml	Normeperidine Recovered, μg/ml	Conversion and Recovery, %
10	7.85 ± 0.25	78.5 ± 2.5
7	5.30 ± 0.18	75.8 ± 2.6
5	3.72 ± 0.15	74.5 ± 3.1
2	1.45 ± 0.03	72.5 ± 1.5

[a] Figures represent the mean ± *SD* for three analyses.

comparing the final meperidine and normeperidine concentrations with known standards.

For the *in vivo* study, five healthy volunteers were given a single 36-mg/m[2] im injection of meperidine hydrochloride[4]. Venous blood samples were collected at 0.5, 1, 2, 4, 6, 8, 12, and 24 hr and were analyzed for meperidine by previously described methods (5). The cumulative urinary output in each subject was collected 1, 2, 4, 6, 8, 12, and 24 hr after dosing. The volume and pH of these samples were measured, and then the samples were frozen for analysis at a later date. The 24-hr cumulative excretion of meperidinic and normeperidinic acids was determined using 10% aliquots of each sample and analyzing the pooled samples.

RESULTS AND DISCUSSION

The percent conversion of meperidinic and normeperidinic acids to the corresponding ester and the recovery of meperidine and normeperidine are presented in Tables I and II. The mean percent recovery of meperidine from urine samples with initial meperidinic acid concentrations of 2–10 μg/ml was 76.0%. The mean percent recovery of normeperidine from urine samples with initial normeperidinic acid concentrations of 2–10 mg/ml was 74.8%. The low recoveries were probably due to incomplete esterification and degradation of the molecule during the esterification process. Due to the difference in retention time and the similarity of the molecules, meperidinic and normeperidinic acids can be assayed at the same time without interference. The limit of detection was approximately 10 ng/ml of urine.

All subjects displayed normal serum meperidine concentration-time curves, with a maximum at 1 hr and with biological half-lives of 2.8–3.4 hr. The amount of meperidinic acid excreted varied considerably from

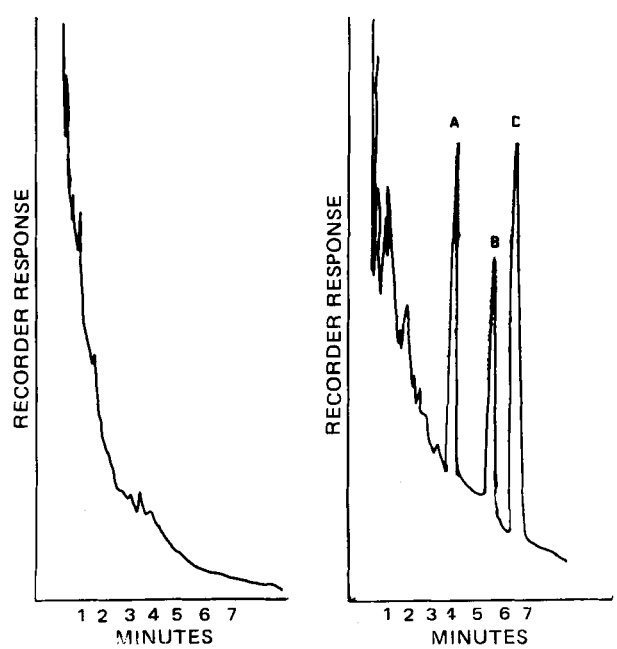


Figure 1—*Gas chromatogram of an extract after the esterification of 10 ml of control human urine containing no drugs (left) and 10 ml of human urine containing 10 μg of meperidinic acid/ml and 10 μg of normeperidinic acid/ml (right). Key: A, normeperidine; B, meperidine; and C, internal standard.*

[4] Elkins-Sinn, Inc., Cherry Hill, N.J.

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

REFERENCES

(1) J. J. Burns, B. L. Berger, P. A. Lief, A. Walack, E. M. Papper, and B. B. Brodie, *J. Pharmacol. Exp. Ther.*, **114**, 289 (1955).

(2) N. P. Plotnikoff, E. L. Way, and H. W. Elliot, *ibid.*, **117**, 414 (1956).

(3) J. E. Stambaugh and I. W. Wainer, *J. Clin. Pharmacol.*, **14**, 552 (1974).

(4) *Ibid.*, **15**, 269 (1975).

(5) J. E. Stambaugh, I. W. Wainer, J. K. Sanstead, and D. M. Hemphill, *J. Clin. Pharmacol.*, **16**, 245 (1976).

(6) A. M. Asatoor, D. R. London, M. D. Milne, and M. L. Simenhoff, *Br. J. Pharmacol.*, **20**, 285 (1963).

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

H. KINSUN *, M. A. MOULIN *x, and E. C. SAVINI ‡

Received February 8, 1977, from the **Laboratory of Functional Explorations B and Pharmacology, Centre Hospitalier Regional et Universitaire de Caen, 14033 Caen, France,* and the ‡*Laboratory of Toxicology and Pharmacology, Nice Medical School, 06000 Nice, France.* Accepted for publication March 29, 1977.

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.