(37) J. H. Robertson and K. Tsuji, *J. Pharm. Sci.*, **61**, 1633 (1972).
(38) G. Kobrehel, Z. Tamburasev, and S. Djokic, *J. Chromatogr.*, **133**, 415 (1977).
(39) G. Richard, C. Radecka, D. W. Hughes, and W. L. Wilson, *ibid.*, **67**, 69 (1972).
(40) T. T. Anderson, *ibid.*, **14**, 127 (1964).
(41) A. Vilim, M. J. LeBelle, W. L. Wilson, and K. C. Graham, *ibid.*, **133**, 239 (1977).
(42) K. Tsuji and J. F. Goetz, *ibid.*, **147**, 359 (1978).
(43) K. Tsuji, *ibid.*, **158**, 337 (1978).
(44) K. Tsuji and J. F. Goetz, *J. Antibiot.*, **31**, 302 (1978).
(45) S. Omura, Y. Suzuki, A. Hakagawa, and T. Hata, *ibid.*, **26**, 794 (1973).
(46) D. C. Grove and W. H. Randall, "Assay Methods of Antibiotics, A Laboratory Manual," Medical Encyclopedia Publishers, New York, N.Y., 1955, pp. 96–103.
(47) S. C. Bell, J. W. Hamman, and W. E. Grundy, *Appl. Microbiol.*, **17**, 88 (1969).
(48) J. V. Bennett, J. L. Bodie, E. J. Benner, and W. M. Kirby, *ibid.*, **14**, 170 (1966).
(49) "Code of Federal Regulations, Title 21," U.S. Government Printing Office, Washington, D.C., 1980, Parts 436.105 and 436.106.
(50) T. A. Geissman, "Principles of Organic Chemistry," 3rd ed., F. H. Freeman, San Francisco, Calif., 1968, p. 625.

ACKNOWLEDGMENTS

Presented at the APhA Academy of Pharmaceutical Sciences, San Antonio meeting, November 1980.

The authors thank Mr. Tim Porter and staff for their assistance in the validation of this methodology and Ms. Diane Penza for her assistance in preparing the manuscript.

Gas Chromatographic Analysis of Meperidine and Normeperidine: Determination in Blood after a Single Dose of Meperidine

PEYTON JACOB III ‡x, JEAN F. RIGOD ‡, SUSAN M. POND *‡, and NEAL L. BENOWITZ *‡

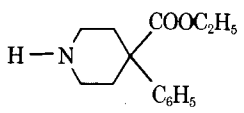
Received February 13, 1981, from the *Division of Clinical Pharmacology, Department of Medicine, School of Medicine, University of California, San Francisco, CA 94143 and the ‡Division of Clinical Pharmacology, San Francisco General Hospital, San Francisco, CA 94110. Accepted for publication June 25, 1981.

Abstract □ A method is described for the determination of meperidine and its pharmacologically active metabolite, normeperidine, in blood, plasma, and urine using gas chromatography with nitrogen–phosphorus detection. Structural analogs of both meperidine and normeperidine were used as internal standards. Unlike previously reported assays, this procedure was sensitive and convenient enough for use in pharmacokinetic studies of both meperidine and normeperidine following single doses of meperidine. The assay was sensitive to 5 ng of meperidine/ml and 2.5 ng of normeperidine/ml extracted from a 1-ml biological sample. The between-assay coefficients of variation at these concentrations were 9.4 and 10.4%, respectively.

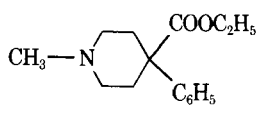
Keyphrases □ Meperidine—gas chromatographic analysis in blood □ Normeperidine—gas chromatographic analysis in blood after single dose of meperidine □ Gas chromatography—analysis of meperidine and normeperidine in blood □ Analgesics—meperidine, gas chromatographic analysis in blood

Normeperidine (I), the *N*-demethylated metabolite of the analgesic drug meperidine (II), is pharmacologically active (1) and may cause seizures in humans (2). Therefore, blood levels of I should be determined when delineating the side effects and drug interactions that occur with II. To carry out such studies after single doses of II, a sensitive and specific gas chromatographic (GC) assay for I and II in biological fluids was developed. The assay allows measurement of II up to 24 hr and I up to 48 hr after a single dose of II.

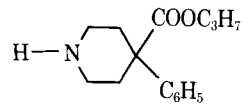
Previously published methods with adequate sensitivity for the measurement of I in plasma after a single dose have involved GC–mass spectrometry with selected ion monitoring (3, 4), radioimmunoassay (5), or GC using electron-capture detection (6). GC methods with flame-ionization detection (7–10) may be satisfactory for determining therapeutic concentrations of II, but have marginal sensitivity for the determination of plasma I concentrations (7–9) following single doses of the parent drug.



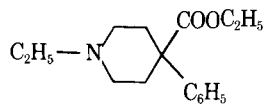
I



II



III



IV

This report describes the GC determination of I and II using a nitrogen–phosphorus detector, which is highly selective toward nitrogen-containing compounds such as II and its derivatives. The assay is significantly faster and more convenient than previously reported GC methods, and is applicable to whole blood, plasma, or urine.

EXPERIMENTAL

Materials—Meperidine hydrochloride and normeperidine hydrochloride were obtained[1]. Heptafluorobutyric anhydride and sodium cyanoborohydride were obtained commercially[2]. Normeperidinic acid *n*-propyl ester (III) was synthesized by a previous method (6). *N*-ethylnormeperidine (IV) was synthesized as will be described. All other chemicals and solvents were analytical reagent grade.

Synthesis of *N*-Ethylnormeperidine Hydrochloride—Sodium cyanoborohydride (100 mg) was added to a solution of normeperidine hydrochloride (250 mg) and acetaldehyde (0.5 ml) in 50 ml of 50% aqueous

[1] Kindly supplied by Sterling-Winthrop Research Institute, Rensselaer, N.Y.
[2] Aldrich Chemical Co., Milwaukee, Wis.

0022-3549/82/0200-0166$01.00/0

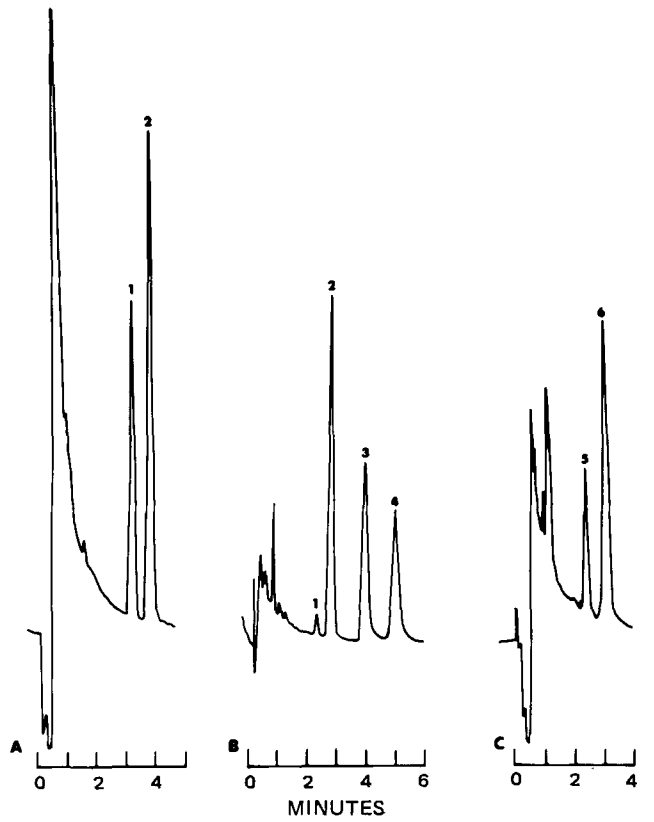


Figure 1—*Chromatograms of extracts containing normeperidine (I) and meperidine (II). Key: A, plasma containing 28 ng of II/ml; B, urine sample containing 108 and 1300 ng/ml of II and I, respectively; and C, plasma containing 10 ng of I/ml. GC conditions are described in the text; and 1 = II; 2 = IV; 3 = I; 4 = III; 5 = heptafluorobutyryl derivative of I; and 6 = heptafluorobutyryl derivative of III.*

ethanol. The solution was stirred and the pH was maintained at 6–7 by the dropwise addition of aqueous sodium acetate. After 2 hr, the pH was adjusted to 2 by the addition of concentrated hydrochloric acid. This procedure was done in a fume hood because hydrogen cyanide is evolved.

The solution was extracted twice with 20-ml portions of methylene chloride. The extracts were discarded and the aqueous phase was brought to pH 12 with aqueous sodium hydroxide. The basic aqueous phase was extracted twice with 20-ml portions of methylene chloride, and the combined extracts were dried over anhydrous potassium carbonate and then evaporated under reduced pressure. The oily residue was dissolved in 3 ml of absolute ethanol, acidified to pH 2 with concentrated hydrochloric acid, and then diluted with 25 ml of anhydrous ether, added in portions with stirring and scratching with a glass rod. The product slowly crystallized and was collected by filtration and air dried. A white crystalline powder (57 mg) was obtained, mp 170.5–172° [lit. (11) mp 171°]. GC analysis indicated a purity of >99.5%.

Instrumentation—GC analyses were performed using an instrument[3] equipped with dual nitrogen–phosphorus detectors. The nitrogen (carrier gas), air, and hydrogen flow rates were 30, 50, and 5 ml/min, respectively. Columns used for II analyses or for simultaneous II and underivatized I analyses (urine) were 1.8 m × 2-mm i.d. glass. The columns were configured for on-column injection, packed with 2% polyethylene glycol and 2% potassium hydroxide on 100–120 mesh chromatographic diatomaceous earth[4], and operated at 190° for determination of II in blood and 205° for simultaneous determination of I and II in urine. Columns used for derivatized I analyses were 1.8 × 2-mm i.d. glass, packed with 3% methyl silicone liquid phase on 100–120 mesh base-deactivated chromatographic diatomaceous earth[5] operated at 205°.

The injection port and detector temperatures were 250 and 300°, respectively. A strip-chart recorder[6] was used for recording chromatograms.

[3] Model 5711A, Hewlett-Packard, Avondale, Pa.
[4] Carbowax, 20 *M* potassium hydroxide on Gas Chrom P, Applied Science Laboratories, State College, Pa.
[5] SP2100 DB, Supelco, Bellefonte, Pa.
[6] Model 9176, Varian Co., Sunnyvale, Calif.

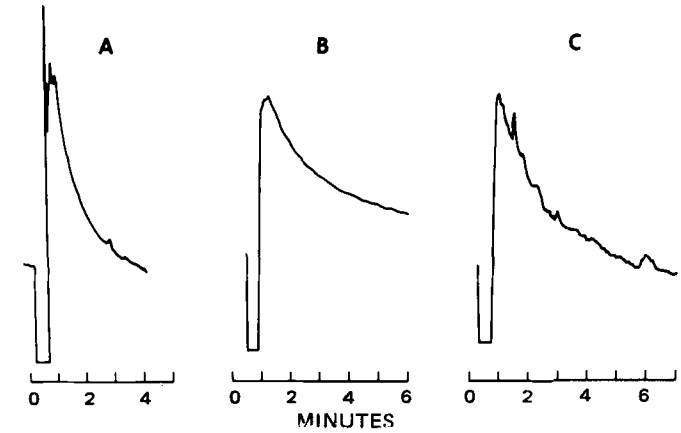


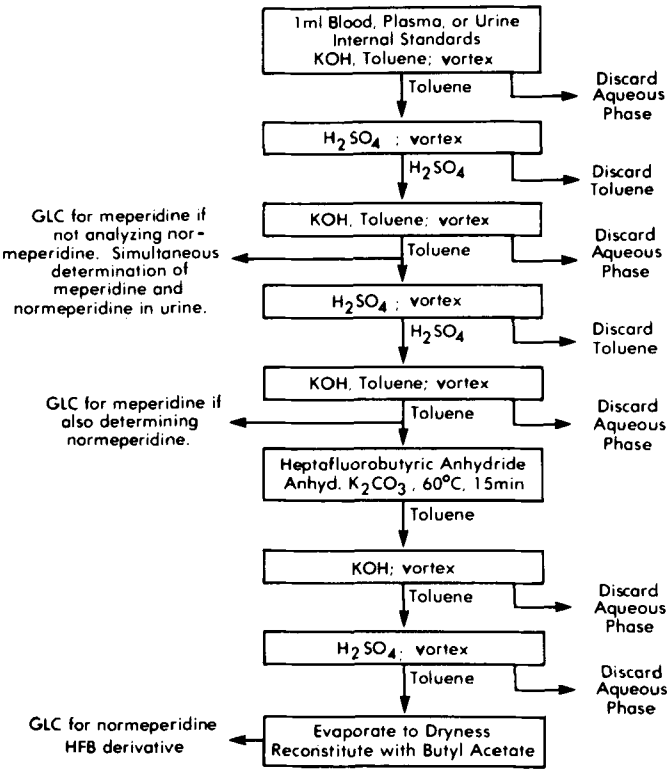
Figure 2—*Chromatograms of extracts of drug-free plasma and urine specimens. Key: A, plasma extracted for II; B, urine extracted for both I and II; and C, plasma extracted for I and derivatized with heptafluorobutyric anhydride.*

All extractions were carried out by agitating for 3 min on a multiple-tube vortex mixer[7]; an analytical evaporator[8] was used for evaporating solvent from extracts with a nitrogen stream.

Assay Procedure—A flow diagram of the extraction procedure is shown in Scheme I. To 1-ml samples of whole blood, plasma, or urine contained in 13-mm × 100-mm culture tubes were added 100-μl aliquots of an aqueous solution of the internal standards, III (for normeperidine) and IV (for meperidine). Methanolic 2 *N* potassium hydroxide (0.5 ml) was added, and the samples were extracted with 3 ml of toluene.

After centrifuging for 5 min, the aqueous layers were frozen by placing the tubes in a dry ice–acetone bath, and the toluene layers were poured into culture tubes containing 0.5 ml of 0.2 *N* sulfuric acid. The tubes were vortexed for 3 min and then centrifuged. The aqueous layers were frozen, the toluene layers discarded, and the aqueous acid layers were allowed to thaw. Aqueous 2 *N* potassium hydroxide (1 ml) was added, and the samples were extracted with 2 ml of toluene and then centrifuged.

At this stage, if the concentrations of I and II were expected to be high



Scheme I—Flow diagram of extraction procedure.

[7] Model VB-1, Kraft Apparatus, Mineola, N.Y.
[8] N-Evap model 111, Organomation Associates, Shrewsbury, Mass.

Table I—Day-to-Day Variations of Identical Plasma Samples

	Meperidine (II)						Normeperidine (I)					
Concentration given, ng/ml	5	10	20	50	100.0	200	2.5	5	10	20	50	100
Concentration found, mean ng/ml	4.9	10.2	20.1	49.6	100.0	202.4	2.3	5.0	10.4	20.8	50.0	98.8
Standard deviation, ng/ml	0.5	0.8	1.3	3.0	6.0	9.6	0.2	0.4	0.5	0.8	1.1	1.9
Coefficient of variation, %	9.4	8.2	6.4	6.1	6.0	4.7	10.4	8.4	5.2	4.0	3.9	1.9

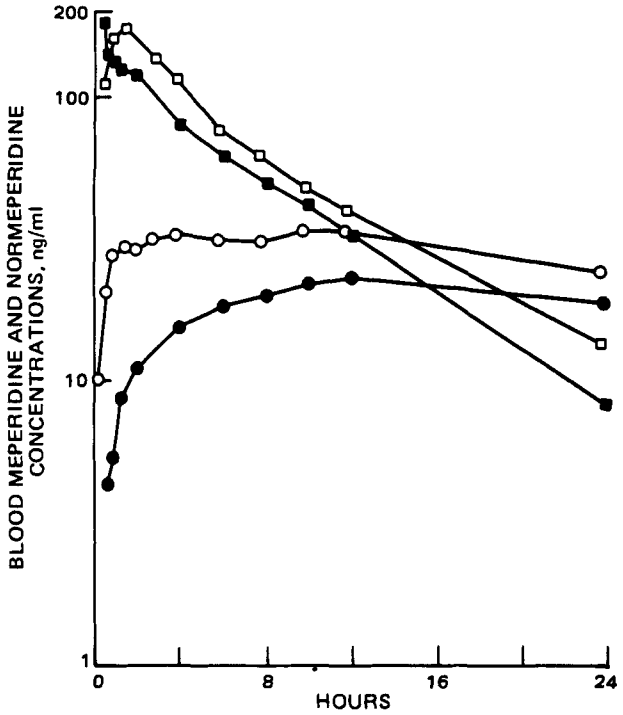


Figure 3—*Blood II (■) and I (●) concentrations following administration of 50 mg iv of II; blood II (□) and I (○) concentrations following administration of 100 mg po of II in a human subject.*

(*e.g.*, as in urine), the toluene extracts were analyzed by GC for both I and II. The retention times were 2.30, 2.75, 4.00, and 4.95 min for II, IV, I, and III, respectively. The extraction recoveries of I and II from urine averaged 70 and 73%, respectively, over the concentration range of 1–20 μg/ml.

The toluene extracts from blood or plasma were back-extracted with 0.5 ml of 0.2 *N* sulfuric acid. The sulfuric acid layers were made basic with 1 ml of 2 *N* aqueous potassium hydroxide and extracted with 0.5 ml of toluene. The toluene layers were transferred to vials containing anhydrous potassium carbonate (~40 mg) to dry the solvent. Aliquots (2–4 μl) of the toluene extracts were injected into the chromatograph for analysis of II. Examples of chromatograms from plasma extracts are presented in Fig. 1. The retention times of II and the internal standard (IV) were 3.30 and 3.85 min, respectively. Extraction recovery from blood over 5–200 ng/ml averaged 67%.

Heptafluorobutyric anhydride (10 μl) was added to the remaining toluene extracts. The tubes were capped and heated at 60° for 15 min on a heating block to derivatize I and its internal standard. After cooling to room temperature, 2 ml of 2 *N* sodium hydroxide was added and the tubes were vortexed for 5 min to destroy excess heptafluorobutyric anhydride. Following centrifugation, the toluene layers were transferred to clean tubes containing 1 ml of 0.2 *N* sulfuric acid.

After extraction for 1 min and centrifugation, the toluene layers were transferred to small vials and evaporated to dryness under a nitrogen stream. Butyl acetate (50 μl) was added to reconstitute the samples, and 2–6 μl was injected into the chromatograph for analysis of I (Fig. 1).

The retention times for the heptafluorobutyryl derivatives of I and III were 2.48 and 3.05 min, respectively. Extraction recoveries from blood averaged 66% over 2.5–100 ng/ml. Chromatograms of blank plasma and urine extracts (Fig. 2) were free from interfering peaks under the extraction conditions employed. The detection limit for each substance was ~1 ng/ml for 1-ml plasma samples.

Standard Curves—Standards were prepared by spiking drug-free blood, plasma, or urine samples with known amounts of drug and internal standard. Standards with at least four different concentrations over the expected range were run with each batch of samples and used to construct standard curves. The amounts of IV added were 2.5 μg for urine and 50 ng for blood or plasma; the amounts of III were 1 μg for urine and 25 ng for blood or plasma. Regression equations relating peak height ratios of standards to their concentrations were used to determine concentrations of I and II. The regression lines were linear over all concentrations studied (0–200 ng/ml for blood, and 0–20 μg/ml for urine) and passed through the origin.

Analytical Variables—Between-run precision was determined by analyzing identical plasma standards with six consecutive runs. Coefficients of variation for six concentrations representative of the therapeutic ranges found in plasma are presented in Table I.

Pharmacokinetic Study in a Human Subject—A healthy male volunteer, age 38 and weight 72 kg, was given 50 mg iv of meperidine hydrochloride over 1 min. One week later, the same subject was given 100 mg po of meperidine hydrochloride in solution. Twelve blood samples were collected over the 24 hr following drug administration and plasma separated for analysis of I and II (Fig. 3).

RESULTS AND DISCUSSION

Losses of basic drugs due to adsorption on glassware and/or GC columns may be variable and reduce the precision of an assay if the internal standard is not chemically similar to the drug being measured. Therefore, an internal standard that is a structural analog of II was used. Sharp peaks and baseline separation of II and IV (the internal standard) were obtained on both polyethylene glycol–potassium hydroxide (Fig. 1) and base-deactivated methyl silicone columns. Good reproducibility was obtained even at concentrations as low as 5 ng/ml (Table I). Consequently, this assay can be used to measure plasma II concentrations for as long as 24 hr after a single dose.

The described procedure is suitable for the analysis of large numbers of samples and its sensitivity should greatly facilitate studies of the kinetic behavior of normeperidine in meperidine drug interactions and in patients with disease states in which normeperidine kinetics may be altered (2).

REFERENCES

(1) J. W. Miller and H. H. Anderson, *J. Pharmacol. Exp. Ther.*, **112**, 191 (1954).

(2) H. H. Szeto, C. E. Inturrisi, R. Houde, S. Saal, J. Cehigh, and M. M. Reidenberg, *Ann. Intern. Med.*, **86**, 738 (1977).

(3) C. Lindberg, M. Berg, L. O. Boreus, P. Hartvig, K.-E. Karlsson, L. Palmer, and A.-M. Thornblack, *Biomed. Mass. Spectrom.*, **5**, 540 (1978).

(4) E. L. Todd, D. T. Stafford, and J. E. Morrison, *J. Anal. Toxicol.*, **3**, 256 (1979).

(5) D. S. Freeman, H. B. Gjika, and H. Van Vanakis, *J. Pharmacol. Exp. Ther.*, **203**, 203 (1977).

(6) P. Hartvig, K.-E. Karlsson, C. Lindberg, and L. O. Boreus, *Eur. J. Clin. Pharmacol.*, **11**, 65 (1977).

(7) U. Klotz, T. S. McHorse, G. R. Wilkinson, and S. Schenker, *Clin. Pharmacol. Ther.*, **16**, 667 (1974).

(8) K. Chan, M. J. Kendall, and M. Mitchard, *J. Chromatogr.*, **89**, 169 (1974).

(9) H. H. Szeto and C. E. Inturrisi, *ibid.*, **125**, 503 (1976).

(10) M. A. Evans and R. D. Harbison, *J. Pharm. Sci.*, **66**, 599 (1977).

(11) E. Walton and M. B. Green, *J. Chem. Soc.*, **1945**, 315.

ACKNOWLEDGMENTS

Supported by Grants DA 01696 and DA 4RG012 from the National Institute on Drug Abuse.

The clinical study was carried out in the General Clinical Research Center RR-00083 at San Francisco General Hospital Medical Center with support by the Division of Research Resources, National Institutes of Health.