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# ANALYSIS OF MEPERIDINE AND NORMEPERIDINE IN SERUM AND URINE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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## SUMMARY

A high-performance liquid chromatographic method is described for the simultaneous analysis of meperidine and normeperidine in serum and urine. A 1-ml sample aliquot is extracted into hexane, then back-extracted into a small volume of dilute acid which is injected onto a cyanopropyl analytical column. Absorbance of the column effluent is monitored at 205 nm. Two internal standards are employed, diphenhydramine for meperidine and nordiphenhydramine for normeperidine. Chromatography of the four compounds takes 4 min. Serum concentration—time curves of meperidine and normeperidine are presented for eight healthy subjects following single 70-mg bolus injections of meperidine.

## INTRODUCTION

Meperidine (Demerol®, Winthrop) is a narcotic analgesic commonly used for pre-operative sedation and the control of post-operative pain. It is widely used in obstetrics and bone fracture clinics. As with other opioids, the drug has a history of abuse by drug addicts due to its euphoric effect.

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The analysis of meperidine (M) and its primary N-demethylated metabolite, normeperidine (NM) in serum, blood and urine has been accomplished mainly using gas chromatography (GC). Flame-ionization [1, 2], nitrogen—phosphorus [3], electron-capture [4] and mass spectrometric [5] detection have been used. Meperidine itself has presented few analytical difficulties. However, normeperidine has always been derivatized to ensure reproducible recoveries from serum and blood extracts. The above methods have used trifluoroacetic anhydride [1], heptafluorobutyric anhydride [2, 3], trichloroethyl chloroformate [4] and N-methyl-bis-trifluoroacetamide [5] as derivatizing agents. All procedures are labour-intensive, requiring at least three extractions prior to derivatization followed by an evaporation or wash step prior to the chromatography. Radioimmunoassays are described [6] in which the two antibodies raised in rabbits were specific for either M or NM. High-performance liquid chromatography (HPLC) has been described for the analysis of M in pharmaceutical preparations using a reversed-phase $C_{18}$ column [7]. An HPLC procedure has not previously been described for the analysis of M and/or NM in biological materials.

The following is a rapid but precise method for the simultaneous analysis of both compounds from 1 ml of serum or 100 $\mu$l of urine. No derivatization is required; chromatography is complete within 4 min. M and NM are quantified in the same chromatogram by using diphenhydramine (D) and nordiphenhydramine (ND) as their respective internal standards.

## EXPERIMENTAL

### *Equipment*

HPLC was performed isocratically on a Series 2/2 pumping system coupled to an LC-85 variable-wavelength dual-beam detector which was fitted with a 1.4 $\mu$l flow cell (Perkin-Elmer, Norwalk, CT, U.S.A.). Samples were introduced via a Rheodyne 7125 injection valve fitted with a 50-$\mu$l sample loop (Rheodyne, Cotati, CA, U.S.A.). Peak area ratios were automatically converted to concentration units with a Sigma 15 integrator (Perkin-Elmer).

A Supelcosil® LC-PCN, 150 mm × 4.6 mm I.D. analytical column protected by a Supelguard® LC-CN guard column, 20 mm × 4.6 mm I.D. (Supelco, Bellefonte, PA, U.S.A.) were used. The analytical and guard columns both contained 5-$\mu$m spherical silica particles coated with cyanopropyl stationary phase. Narrow bore, 0.07 mm I.D., stainless-steel connection tubing was used from the injector to detector in order to reduce peak broadening.

### *Mobile phase*

All solvents were HPLC grade and were used as received (Fisher, Pittsburgh, PA, U.S.A.). A 15 mmol/l phosphate buffer was prepared by dissolving 2.6 g of dipotassium hydrogen phosphate in 1 l of double-distilled water. This solution was brought to pH 7.0 with 0.9 mol/l orthophosphoric acid and then was passed through a 0.22-$\mu$m pore filter under suction (Millipore, Bedford, MA, U.S.A.). The mobile phase was acetonitrile—phosphate buffer—methanol (55:25:20, v/v/v). The solution was degassed by mixing for 20 min. The mobile phase was pumped at 2.5 ml/min and the column effluent was monitored at 205 nm at a sensitivity of 0.04 a.u.f.s.

*Serum and urine standards*

Meperidine · HCl and normeperidine · HCl were gifts from Winthrop Labs. (Aurora, Ontario, Canada). The internal standard diphenhydramine · HCl was purchased from Sigma (St. Louis, MO, U.S.A.). The second internal standard, nordiphenhydramine · HCl was a gift from Warner-Lambert (Ann Arbor, MI, U.S.A.).

Stock solutions of M, NM, D and ND were prepared in 1 mmol/l orthophosphoric acid at concentrations of 100 μg/ml, expressed as the free bases. The working internal standard solution was prepared from the stock solutions to contain both D and ND concentrations of 1000 ng/ml in 1 mmol/l orthophosphoric acid. Appropriate volumes of stock M and NM solutions were combined in drug-free serum and urine pools to give 50 ml of each standard at 1000, 500, 200, 100, 50 and 10 ng/ml. Aliquots of 1.5 ml were stored in 3-ml polypropylene vials at −20°C. Sera containing M and NM have been reported to be stable for at least eighteen months when frozen at −20°C [2]. The serum- and urine-based standards were used for precision, linearity and recovery studies.

All glassware including extraction tubes, conical tubes and storage containers for stock solutions were silylated with dimethyldichlorosilane (Pierce, Rockford, IL, U.S.A.) in toluene (1:5) for 2 h. Glassware was consecutively rinsed in methanol, chloroform and hexane, then dried in an oven at 100°C.

*Sample analysis*

To 1 ml of serum in a 20-ml (150 mm × 16 mm) extraction tube were added 100 μl of the combined D + ND internal standard solution, 100 μl of 1 mol/l sodium hydroxide and 5.0 ml hexane. The tube was closed with a PTFE-lined cap and mixed by rotation (60 rpm) for 15 min. After a brief centrifugation, 4.0 ml of the hexane layer were transferred to a 13-ml conical centrifuge tube. The drugs were back-extracted into 80 μl of 1 mmol/l orthophosphoric acid by vortexing vigorously for 30 sec. The two phases were allowed to separate and 50 μl of the aqueous phase were injected. The analysis of urine specimens proceeded in the same way except that 1.0 ml saturated sodium borate buffer, pH 10.2 was used as an alkalinizing agent. The higher concentration of M and NM in urine of clinical specimens necessitated an initial 1:10 dilution into drug-free urine before analysis.

Results were calculated from peak area ratios by the Sigma 15 integrator. It was calibrated daily from extracted 100 ng/ml serum or urine standards. D was used as the internal standard for M while ND was used as the internal standard for NM.

Within-day precision was calculated from ten replicates of each serum and urine standard. Since all volumes were handled quantitatively, the peak area data from the within-day precision study were also used in the calculation of drug recoveries. Drug solutions were prepared in 1 mmol/l orthophosphoric acid at ten times the corresponding serum standard concentrations. A 50-μl injection of these solutions gave peak areas representing 100% recovery for each drug. Recovery was calculated from the ratio of corresponding peak areas in the extracted and unextracted standards. Linearity was also assessed from the within-day precision data by comparing the observed to the expected concentrations for the standards. The average values from the ten injections of

each concentration were used in regression analyses. Day-to-day precision data were obtained from single analyses of all standards on 22 days. The data were gathered over a two-month period during which time over 800 measurements on clinical specimens were also made.

*Single-dose response*

Eight healthy male volunteers who were receiving no medication each received a 70-mg intravenous dose of M · HCl over 2 min. Blood was drawn via an indwelling heparin lock in a forearm vein contralateral to the injection site. Blood samples (5 ml) were collected into plain Vacutainer® (Becton Dickenson, Rutherford, NJ, U.S.A.) tubes at 5, 10, 15, 20, 30, 45, 60, 75, 90 min and at 2, 3, 4, 5, 6, 8 and 11 h post dose. Venipunctures were used to collect 5-ml blood samples at 24, 48, 72, and 96 h post dose. The tubes were centrifuged, the serum transferred to polypropylene vials and stored frozen at −20°C until analysis. Aliquots of 24-h urine collections were also frozen. All analyses were performed in duplicate.

## RESULTS AND DISCUSSION

Typical chromatograms from serum and urine extracts are displayed in Fig. 1. M, D, NM and ND elute at 1.5, 1.9, 2.6 and 2.9 min, respectively. A valley in the baseline is evident at about 4 min. Its exact elution time varied with each batch of mobile phase but did not interfere with drug peak area quantitations. The valley appears following the injection of aqueous or organic solvents other than the mobile phase. Although each batch of phosphate buffer was brought

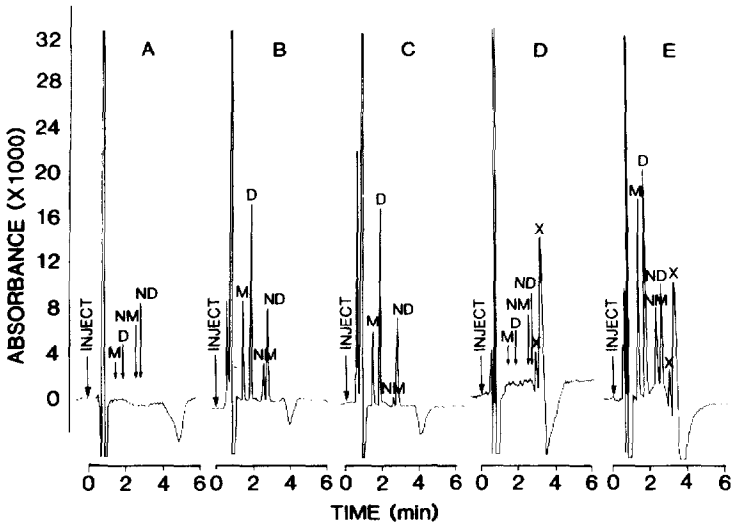


Fig. 1. Chromatograms from serum and urine extracts. (A) Serum blank; (B) serum standard containing meperidine and normeperidine each at 100 ng/ml; (C) serum from volunteer 8 h post 70-mg meperidine · HCl bolus injection, meperidine = 84 ng/ml, normeperidine = 27 ng/ml; (D) urine blank; (E) 24-h urine collection from volunteer post 70-mg meperidine · HCl bolus injection, meperidine = 150 × 10 = 1500 ng/ml, normeperidine = 170 × 10 = 1700 ng/ml. Peaks: M = meperidine; D = diphenhydramine; NM = normeperidine; ND = nordiphenhydramine; X = unknown.

to pH 7.0, it was necessary to optimize the pH of the mobile phase with a few drops of 1 mol/l sodium hydroxide or 0.9 mol/l orthophosphoric acid. The addition of alkali moved all four peaks to earlier elution times and improved the separation between the NM and ND peaks. It was sometimes necessary to add acid to lengthen all retention times so that baseline could be established before the elution of M. The addition of acid or base has little influence upon the measured pH of the phosphate buffer (pH = 6.95—7.05) or the apparent pH of the moble phase (pH = 8.9—9.0). Once the pH of the mobile phase was optimized, it remained stable and required no further adjustment. Each batch of mobile phase could be recycled three times after which the baseline became too noisy.

All four compounds displayed absorption maxima below 200 nm but the baseline was too unsteady to be of practical value. Above 210 nm there was insufficient response from NM to accurately quantify low serum concentrations. Optimal signal-to-noise was observed at 205 nm. Chromatograms from drug-free serum and urine extracts are shown in Fig. 1a and d. The baseline is clean at the elution times for M, D, NM and ND. Two additional unknown peaks appeared in urine extracts just prior to the baseline valley. Their presence did not compromise the analysis of urine specimens. M and NM are largely excreted in urine in their free and conjugated forms. The other two major metabolites are meperidinic acid and normeperidinic acid which account for 9.0—27.8% and 4.4—13.0% of the administered dose as measured by 24-h cumulative urinary excretion, respectively [8]. Based on the GC method for analysis of meperidinic acid and normeperidinic acid in urine [8], neither acid metabolite would be expected to extract into hexane at alkaline pH, and hence would not interfere with the analysis of M and NM. Attempts to procure the two acid metabolites were unsuccessful.

Results from precision studies are shown in Table I. For M, within-day coefficients of variation (C.V.) on serum standards ranged from 2.3% to 10.1%. The corresponding C.V. values for NM were slightly higher, 2.8—12.4%. During the developmental phase of the method, D was used as an internal standard for both M and NM. The precision for NM was somewhat poorer at all concentrations and most noticeable below 100 ng/ml. For comparison, serum within-day precision data have been included for NM using both D and ND as the internal standard. The secondary amine ND compensated more fully for adsorption losses of NM onto glassware than did the tertiary amine, D. The corresponding concentrations of M and NM in serum and urine standards show similar within-day C.V. values. Day-to-day and within-day C.V. values from urine standards are alike for both M and NM at low concentrations. However, the day-to-day C.V. values at 1000 ng/ml are three times as large as the corresponding values for the within-day precision. This can be attributed to the daily one-point recalibration at 100 ng/ml. All data were taken from one calibration when the within-day precision was evaluated.

Good linearity was observed for both M and NM from serum and urine standards. Calculated correlation coefficients were all 1.000; the slopes ranged from 1.02 to 1.09. The limits of detection, taken as three times maximum baseline noise, were 2 ng/ml for M and 5 ng/ml for NM.

Results from recovery experiments are summarized in Table II. Mean

TABLE I

PRECISION STUDIES

| Drug concentration (ng/ml) | Within-day ($n$ = 10) | | | | | | Day-to-day ($n$ = 22) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Meperidine | | Normeperidine* | | Normeperidine** | | Meperidine | | Normeperidine* | |
| | $\bar{X}$ (ng/ml) | C.V. (%) | $\bar{X}$ (ng/ml) | C.V. (%) | $\bar{X}$ (ng/ml) | C.V. (%) | $\bar{X}$ (ng/ml) | C.V. (%) | $\bar{X}$ (ng/ml) | C.V. (%) |
| *Serum* | | | | | | | | | | |
| 1000 | 1014 | 2.3 | 1090 | 2.8 | 1124 | 4.0 | 972 | 7.9 | 1018 | 10.6 |
| 500 | 522 | 2.2 | 520 | 5.4 | 526 | 6.1 | 486 | 5.3 | 505 | 7.8 |
| 200 | 211 | 5.1 | 192 | 8.6 | 183 | 11.4 | 201 | 7.6 | 201 | 8.8 |
| 100 | 104 | 6.6 | 93 | 8.1 | 85 | 11.1 | 102 | 6.4 | 100 | 6.8 |
| 50 | 52 | 5.5 | 50 | 7.9 | 46 | 14.8 | 53 | 8.1 | 51 | 9.6 |
| 10 | 10 | 10.1 | 11 | 12.4 | 13 | 30.8 | 11 | 11.0 | 11 | 13.4 |
| *Urine* | | | | | | | | | | |
| 1000 | 1043 | 3.7 | 1034 | 2.6 | | | 962 | 7.7 | 980 | 9.8 |
| 500 | 510 | 6.9 | 508 | 6.2 | | | 508 | 6.8 | 485 | 6.8 |
| 200 | 213 | 3.2 | 205 | 5.1 | | | 198 | 7.7 | 194 | 7.0 |
| 100 | 101 | 3.9 | 95 | 9.2 | | | 101 | 5.1 | 101 | 7.4 |
| 50 | 53 | 5.2 | 50 | 5.9 | | | 52 | 8.8 | 54 | 10.2 |

*Using nordiphenhydramine as internal standard.
**Using diphenhydramine as internal standard.

TABLE II

RECOVERY STUDIES (PERCENT ± S.D.)

In all cases $n$ = 10.

| Drug concentration (ng/ml) | Meperidine | Normeperidine | Diphenhydramine | Nordiphenhydramine |
|---|---|---|---|---|
| *Serum* | | | | |
| 1000 | 86 ± 2 | 75 ± 2 | | |
| 500 | 87 ± 3 | 68 ± 5 | | |
| 200 | 80 ± 5 | 54 ± 8 | | |
| 100 | 86 ± 5 | 55 ± 8 | 78 ± 5 | 75 ± 9 |
| 50 | 81 ± 10 | 55 ± 12 | | |
| 10 | 91 ± 13 | 73 ± 16 | | |
| *Urine* | | | | |
| 1000 | 90 ± 5 | 63 ± 5 | | |
| 500 | 93 ± 5 | 55 ± 5 | | |
| 200 | 89 ± 5 | 58 ± 5 | | |
| 100 | 82 ± 6 | 53 ± 5 | 78 ± 4 | 73 ± 6 |
| 50 | 77 ± 7 | 44 ± 7 | | |

recoveries from either serum or urine ranged between 77% and 93% for M and between 44% and 75% for NM. Mean D and ND recoveries were 78% and 75% respectively in serum. There was no difference in recovery from urine. M has been shown to extract from urine equally well at pH 7—11. However, NM was extracted optimally at pH 10 [1]. These observations were confirmed by ourselves. M, D and ND all extracted equally well at pH 8—12.5. NM was recovered maximally between pH 9.5—12.5. In the proportions used in the

method, sodium hydroxide as an alkalinizing agent raised serum to pH 10.4—10.8. Borate buffer raised urine to pH 9.8—10.0.

During the developmental phase of this work, it was observed that less than 10% of all four compounds were recovered from a pooled urine-based standard when 100 $\mu$l of 1 mol/l sodium hydroxide was used in the extraction procedure. However, saturated borate buffer gave recoveries similar to those observed in serum using sodium hydroxide. This anomaly was pursued by examining recoveries from standards prepared in drug-free urines obtained from eight normal volunteers. The urine from one of the eight consistently resulted in standards giving low drug recovery when sodium hydroxide was used but expected recovery when borate buffer was used. All other urine-based standards gave the same recovery with sodium hydroxide or borate buffer. The person in question has no health problems. An explanation for the disparity is not apparent.

The Supelcosil LC-PCN column provided reliable chromatography over a three-month period. Approximately 1000 injections were made before the column was replaced. Stability and longevity of cyanopropyl columns have been a concern to others [9, 10]. In our experience other brands of cyanopropyl columns have not provided the same extended performance. Subtle differences in peak resolution and retention times are also inherent to the source of bulk packing material. Back-extraction into a small volume of 1 mmol/l orthophosphoric acid adequately recovered all four drugs from the initial hexane extract. This step served to simultaneously clean up

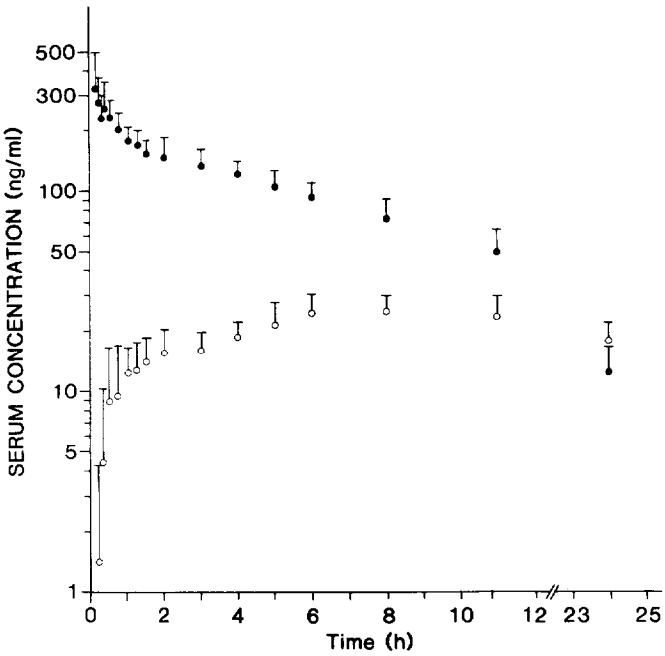


Fig. 2. Mean (+S.D.) serum concentration versus time curves for meperidine (•) and normeperidine (○) in eight healthy male volunteers following a 70-mg intravenous bolus dose of meperidine · HCl.

and concentrate the extract suitable for analysis by HPLC. No deleterious effect resulted from the injection of dilute acid onto the column. As opposed to GC procedures, it was unnecessary to perform a derivatization step in order to recover secondary amines adsorbed onto glassware.

The mean (+S.D.) serum M and NM concentration versus time curves for eight normal volunteers are displayed in Fig. 2. Serum concentrations for M and NM beyond the 24-h sample were all less than 5 ng/ml. Similar plasma concentrations have been reported for M and NM in healthy persons following a single dose [3, 5, 6, 11, 12]. The observed half-life, volume of distribution at steady state, total body clearance and 24-h urinary excretion ranges for M were 4.8—8.1 h, 3.6—5.8 l/kg, 0.40—0.73 l/kg/h, and 1.5—9.9 mg, respectively. The 24-h urinary excretion of NM ranged from 2.6 to 6.1 mg. A discussion of the effect of cimetidine on the pharmacokinetics of M and NM is forthcoming [13].

After single doses of M, the peak serum concentrations of NM ranged from 10 to 40 ng/ml. However, multiple doses of M as used clinically result in accumulation of NM owing to the long elimination half-life of the metabolite. Patients with cancer and patients with significant renal impairment are especially predisposed to this accumulation. NM concentrations of 50—1800 ng/ml have been documented in these patients [14]. We have noted M and NM concentrations of 208 and 511 ng/ml, respectively, in a renal failure patient developing seizures thought to be secondary to NM toxicity. This NM concentration is close to those reported by Szeto et al. [14] in patients developing NM-induced seizures (670 and 1800 ng/ml).

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