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GLC Determination of Meperidine in Blood Plasma

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Abstract □ A GLC technique utilizing a flame-ionization detector is described for the analysis of meperidine in blood plasma. The meperidine is extracted with benzene from plasma that has been made basic with sodium carbonate. A linear calibration curve is found in the range 0.1–1.25 mcg./ml., with the precision of the assay estimated to be ±9.9% (*RSD*). The method has been used in the determination of the half-life of meperidine in dog plasma.

Keyphrases □ Meperidine—GLC-flame-ionization analysis in dog plasma, determination of half-life □ Plasma levels, meperidine—determination of half-life in dogs, GLC-flame ionization □ GLC-flame-ionization detection—analysis, meperidine in dog plasma, determination of half-life

Until recently, the colorimetric technique of Burns *et al.* (1), with a sensitivity of 0.3 mcg./ml., was the best method available for analysis of meperidine[1]. This method, which depends upon the formation of a methyl orange–base complex, is applicable for most amines. Thus, its adaptation for analysis of meperidine raises the question of specificity. Beckett *et al.* (2) showed that, even with modifications such as buffer washes, nicotine interferes with the assay. This should be true of many other bases as well. Dal Cortivo *et al.* (3) developed a fluorometric assay for meperidine, but a lower limit again of only 0.3 mcg./ml. was established. Jenkins *et al.* (4) used a GLC method for the estimation of meperidine in a study of its placental transfer in pregnant ewes. A sensitivity of 0.025 mcg./ml. was reported; however, in our hands this sensitivity was not obtainable. Moreover, there is a limitation of only two analyses per hour and a relatively large volume of plasma is needed (3–4 ml.).

Although other papers have been published concerning GLC techniques, either complete details of the methodology were not reported (5) or no attempt was made to adapt the method for the analysis of biological fluids (6–11). Other approaches used for the determination of meperidine in biological fluids include another colorimetric approach utilizing bromthymol blue (12, 13), a UV absorption spectrophotometric technique (14), and two methods using column chromatography, to separate meperidine from contaminating material, coupled with either paper chromatography (15) or colorimetry (16) for quantitation. Most of the therapeutic serum concentrations have been estimated using the method of Burns *et al.* (1) with widely varying values (1, 17–21). Consequently, a method having greater sensitivity and greater specificity was desired. A GLC method was developed which has a sensitivity slightly better than 0.1 mcg./ml. and the required specificity.

[1] Demerol, Sterling-Winthrop Research Institute.

EXPERIMENTAL

Apparatus—A gas chromatograph[2] equipped with a flame-ionization detector was used with gas flows of 45, 60, and 600 ml./min. for nitrogen, hydrogen, and air, respectively. A column oven temperature of 180° was used; injection port and detector temperatures were maintained at 220 and 210°, respectively.

Column—A 2-mm. i.d. × 180-cm. glass U tube column was packed with 3% OV-17 on 100–120-mesh Gas-Chrom Q[3]. Before it was packed, the empty glass column was thoroughly rinsed with methanol and acetone, dried, and conditioned 1 hr. with a 5% solution of dimethyldichlorosilane[3] in toluene to silylate reactive sites. The column was again rinsed with acetone and dried.

Analytical Procedure—Two milliliters of blood plasma[4] was transferred to a 50-ml. glass-stoppered centrifuge tube made basic with 1.5 ml. 10% sodium carbonate and extracted with 15 ml. analytical reagent grade benzene by shaking for 2 min. The tubes were centrifuged for 3 min., and 12.5 ml. of the benzene phase was then transferred to a screw-top test tube. One drop of methanolic 1 *N* HCl (prepared from 12 *N* HCl) was added to the benzene phase. The benzene was evaporated at no more than 40° under a flow of nitrogen. The residue was taken up in 30 μl. of analytical reagent grade dimethylformamide and 20 μl. of hexamethyldisilazane[3], taking care to rinse the sides of the tube well. GLC determinations were made by injecting 4 μl. of the dimethylformamide solution onto the column with a 1-μl. solvent wash. Quantitation was accomplished by the measurement of peak heights.

Standards for a calibration curve were prepared by adding aliquots of an aqueous solution of meperidine hydrochloride to 50-ml. glass-stoppered centrifuge tubes containing 2 ml. of blood plasma[5] to give final plasma concentrations of 0.1–1.25 mcg./ml. The tubes were shaken to ensure mixing and then analyzed as mentioned. To determine percent recoveries, it was necessary to compare the calibration curve data with a detector response curve. This was obtained by taking aliquots of an aqueous solution of meperidine hydrochloride to give standards of 0.2–2.5 mcg. The aliquots were evaporated to dryness and taken up in 30 μl. of dimethylformamide and 20 μl. hexamethyldisilazane, and 4-μl. samples were injected onto the column.

[2] Packard model 7301.
[3] Applied Science Laboratories, State College, Pa.
[4] Citrate and fluoride have been used successfully as anticoagulants.
[5] Red Cross citrated plasma.

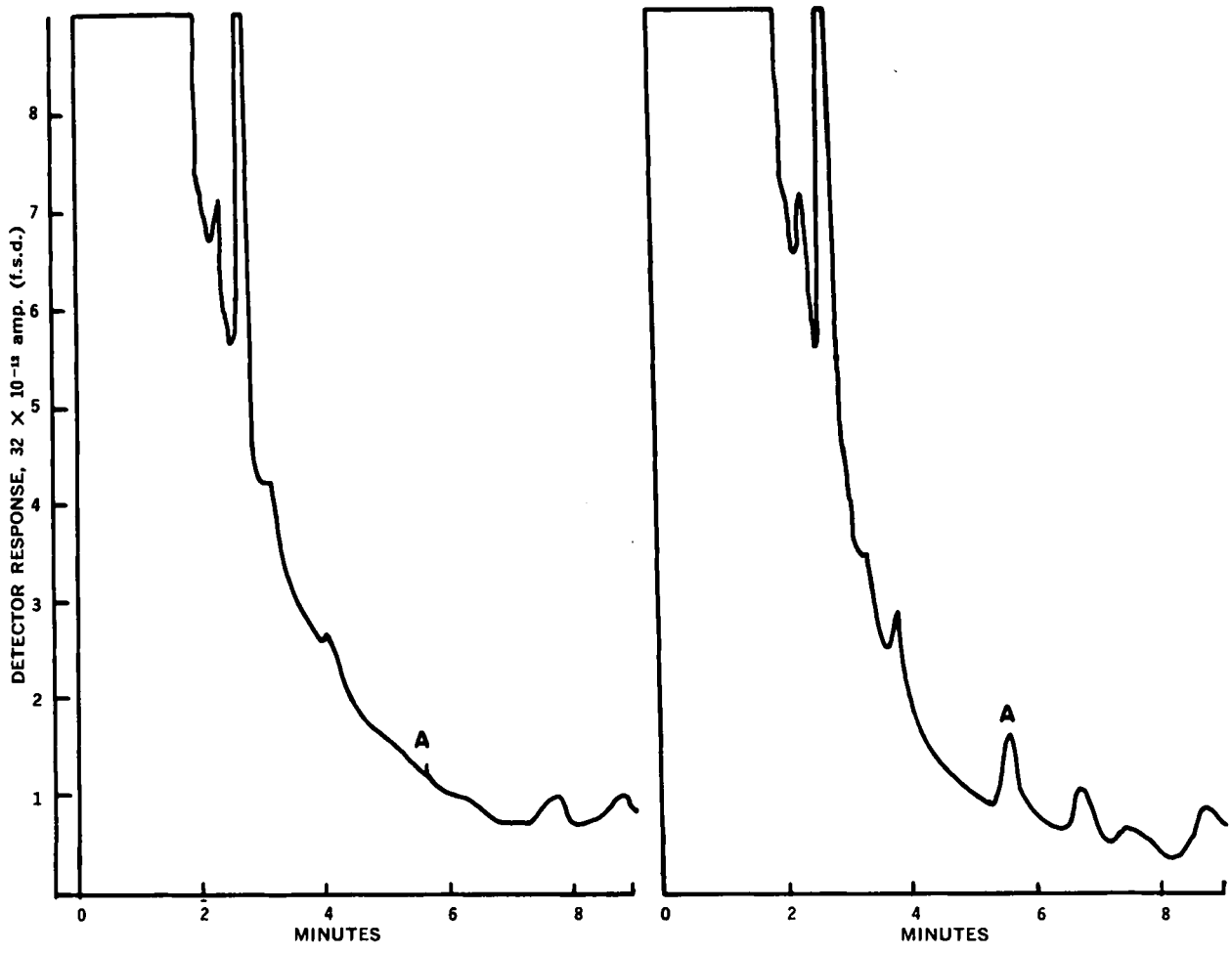

Figure 1—*Gas chromatogram of human plasma. Left: control plasma. Right: plasma containing 0.2 mcg./ml. meperidine (A).*

Precision and accuracy were determined by analyzing replicate plasma samples to which known amounts of meperidine had been added.

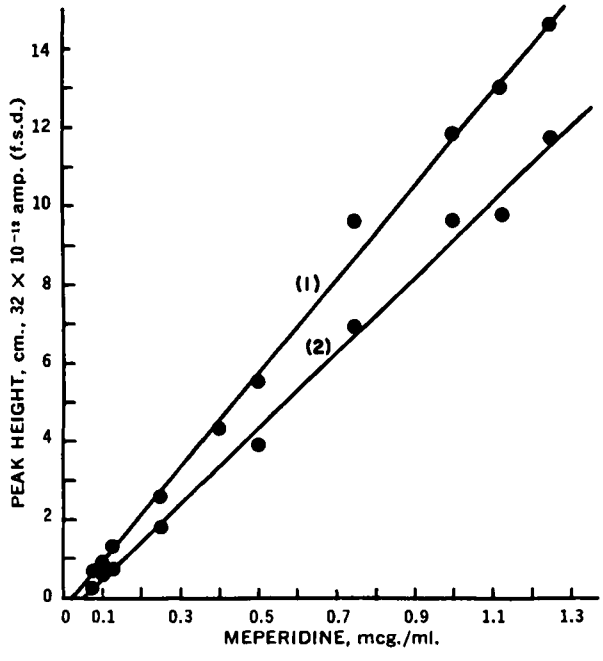

Figure 2—*Detector response curve (1) and calibration curve (2) for meperidine in plasma. Peak height was not corrected for aliquot of extraction solvent used in the analyses.*

To illustrate the utility of the method, four mongrel dogs were medicated intravenously with meperidine; the doses ranged between 15 and 25 mg. drug/kg. body weight. Multiple blood samples were taken through 2 hr. postmedication. The samples were then analyzed for meperidine, and the half-life of distribution and the half-life of elimination from blood were determined.

RESULTS AND DISCUSSION

The retention time for meperidine was found to be 5.8 min., with no interfering peaks in this area (Fig. 1). The detector response curve and the calibration curve were linear over the 0.1–1.25-mcg./ml. range (Fig. 2). No advantage was found by plotting peak areas instead of peak heights. The peak heights for the calibration curve are plotted without correcting for the volume of extraction solvent used; *i.e.*, only 12.5 ml. of benzene out of 15 ml. was used. After taking this into consideration, the percent recoveries from spiked blood plasma averaged 96%.

The precision was determined to be ±9.9% (*RSD*) for the standard curve shown (Fig. 2). For data obtained from running replicate

Table I—Precision in Measurement of Meperidine Added to Human Plasma

Added, mcg./ml.	Measured[a], mcg./ml.	*RSD*[b], %
0.2	0.22 (0.20–0.24)	±8.8
0.5	0.53 (0.52–0.55)	±2.9
1.0	1.03 (0.93–1.06)	±5.8

[a] Mean of five replicate samples (range). [b] Relative standard deviation [$(s/\bar{y})$].

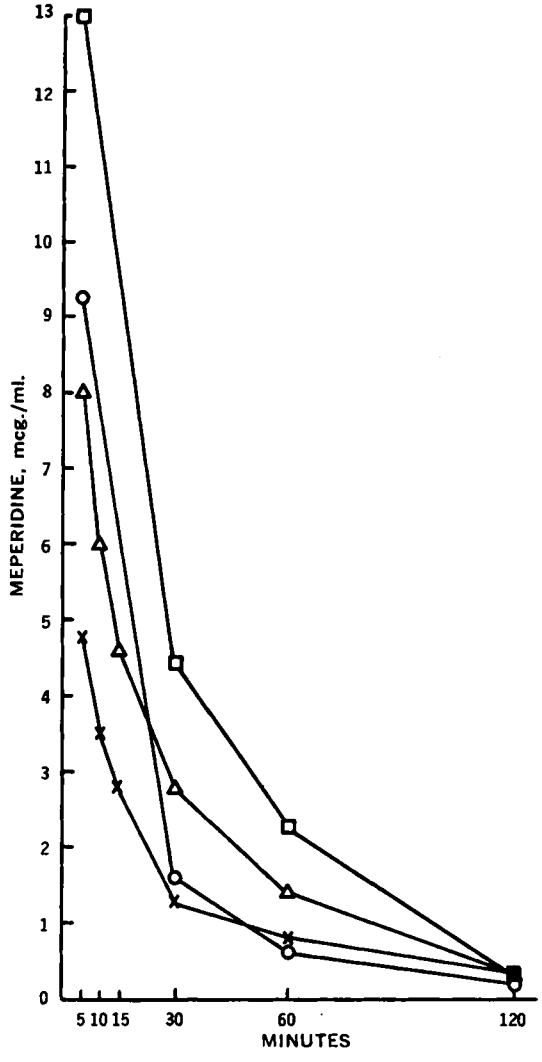

Figure 3—*Meperidine plasma concentrations in the dog after a single intravenous medication. Key: □, 25 mg./kg.; ○, 20 mg./kg.; △, 15 mg./kg.; and ×, 15 mg./kg.*

samples in the range of 0.2–1.0 mcg./ml., the precision varied from ±2.9 to 8.8% (*RSD*) (Table I).

The use of the hexamethyldisilazane along with dimethylformamide for taking up the meperidine for injection proved advantageous in two respects: (*a*) the rinsing of the walls of the tube was facilitated since the solution wet the glass very well, which was not the case with dimethylformamide alone; and (*b*) it inhibited the binding of drug to the glass, which occasionally was noticed when only dimethylformamide was used.

The facts that some tailing was noted in the chromatogram and that the standard curve, determined by the least-squares method, did not pass through zero at the 95% confidence level pointed to an interaction between the compound and the column packing. Attempts were made to overcome this problem by trying 3% OV-1 on Gas Chrom Q as the packing as well as silylating the reactive sites in the packing by hexamethyldisilazane injections; neither variation was successful. This interaction problem was not serious at the concentrations used.

Another problem was seen when on-column amounts of meperidine five times the normal range were used. A fraction of these larger amounts remained on the column and was eluted by the next dimethylformamide injection. Incomplete volatilization at the injection port seems to be a plausible explanation. However, this problem can be avoided by using on-column amounts of meperidine of 0.2 mcg. or less.

That protein binding might be a minor consideration at the lower concentrations was indicated by comparing data for extractions of meperidine from water and from plasma. Whereas the average percent recovery was greater than 90% through the 0.1–1.0-mcg./ml. range, the percent recoveries at the lower concentrations of spiked plasma dipped into the lower 80's.

It is recommended that four spiked plasma standards be run with each set of plasma samples as a routine procedure. By using the standard curve and the peak height of an unknown sample, the concentration of meperidine in a sample can be determined.

Under the conditions of the column, other peaks were noted at approximately 14 and 90 min. These could pose a problem for subsequent analyses; however, by running a sample every 10 min., it was possible to avoid interference by each 14-min. peak and thus nine samples were run before the first very large broad 90-min. peak interfered. It was then necessary to wait 90 min. until all of these latter peaks were eluted.

For this half-life study in dogs, it was shown that the metabolites of meperidine do not interfere. For future clinical studies, it was established that lidocaine, mepivacaine, bupivacaine, and nicotine do not interfere in the assay.

The analyses of the plasma samples from the dogs showed a rapid decline in the concentration of meperidine (Fig. 3). It was reported (1) previously that meperidine was metabolized at a rate of about 70%/hr., which is equivalent to a half-life of about 30 min. In this present study, the arithmetic mean and standard deviation for the half-life of distribution and the half-life of elimination from blood were calculated to be 6.5 ± 0.8 and 31.2 ± 6.4 min., respectively.

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