Micromethod for Determination of Meperidine in Plasma

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Abstract □ A GLC method utilizing a flame-ionization detector is described for the analysis of meperidine in blood plasma. Meperidine is extracted with ether from plasma that has been made basic with sodium hydroxide. The ether extract is evaporated to dryness, and the residue is dissolved in carbon disulfide for GLC analysis. An internal standard, *N',N'*-diethylaminoacetyl-2,6-xylidine, is used to quantitate meperidine. The extraction efficiency from plasma is 85%, and as little as 0.05 μg of meperidine can be quantitatively determined in 1 ml of plasma.

Keyphrases □ Meperidine—GLC analysis, human plasma □ GLC—analysis, meperidine, human plasma □ Analgesics, narcotic—meperidine, GLC analysis, human plasma

Since the development (1) of a spectrophotometric analysis of meperidine[1] (I), several investigators reported procedures with greater sensitivity and specificity for plasma meperidine (2). However, some of these newer methods require several extractions (3), lack internal standard techniques (4), and demonstrate long retention times, which limit the number of samples that can be processed (5).

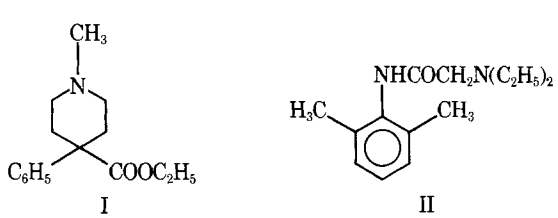
During prolonged administration of meperidine to pediatric patients, a micromethod of analysis is needed for the determination of peak plasma concentrations and the half-life of the drug. Therefore, a GLC method was developed for the rapid analysis of small amounts of meperidine in plasma using an internal standard for quantitation. The sensitivity of this method was greater than 0.05 μg/ml of plasma, and no interference was observed with peak identification. To evaluate this method, meperidine disappearance in plasma was studied in two infants following therapeutic drug administration.

EXPERIMENTAL

Apparatus—A gas chromatograph[2] equipped with a flame-ionization detector was maintained with gas flows of 20, 30, and 300 ml/min for nitrogen, hydrogen, and air, respectively. A column oven temperature of 215° was used; the injection port and detector temperatures were maintained at 240°.

Column—A glass column, 2 m × 2 mm, was packed with 3% SP 2250 on Chromosorb W, AW/DMCS[3] (80–100 mesh). The column was rinsed before packing with methanol and acetone; it was then dried and conditioned for 6 hr with a 10% solution of dimethyldichlorosilane[4] in toluene to silylate reactive sites. Following silylation, the column was again rinsed with acetone and dried.

Analytical Procedures—One milliliter of blood plasma was transferred to a 25-ml glass-stoppered centrifuge tube, mixed with the internal standard *N',N'*-diethylaminoacetyl-2,6-xylidine[5] (II) (1.0 μg/0.5 ml), made basic with 1 *N* NaOH (0.2 ml), and extracted with reagent grade ether (10 ml) by stirring on a vortex mixer (30 sec). The ether was then transferred by disposable pipet to a dry, clean, 12-ml centrifuge tube and evaporated to dryness in a water bath (45°) using nitrogen flow. The residue was redissolved in 20 μl of spectrophotometric grade carbon disulfide[6], and 1 μl was injected into the gas chromatograph.



I

II

A standard calibration curve was prepared by adding aliquots of an aqueous solution of meperidine hydrochloride to 1 ml of plasma to give a final concentration ranging from 0.05 to 0.20 μg/ml of meperidine in plasma. The internal standard (1 μg) was then added to the sample, and the analyses were made. Sixteen samples, four per concentration of meperidine, were analyzed for the determination of the standard curve. Each sample was injected in triplicate, and the determination for the entire standard was replicated once.

[^{14}C-*N*-Methyl]-meperidine hydrochloride[7] was used to estimate the extraction efficiency of meperidine from plasma by measurement of the total radioactivity in the extract[8].

RESULTS AND DISCUSSION

The GLC retention times for I and II were 1.4 and 2.5 min, respectively. A gas chromatogram from a control human plasma and plasma containing 0.1 μg of meperidine/ml with the internal standard (0.5 μg/ml) is shown in Fig. 1. Since peaks for both agents were symmetrical, quantitation was made by comparison of peak heights. Detector responses and calibration curves were linear for both compounds over the 0.05–0.20-μg/ml range. Blanks were prepared from plasma of drug-free subjects, and no peaks were observed that would interfere with the measurement of meperidine or the internal standard.

Linear regression analysis of the individual data points from the standards, plotted as the ratio of peak height of meperidine–peak height of the internal standard *versus* concentration of meperidine in plasma gave a computed slope of 1.37/μg of meperidine with a 0.01 *SE* of the

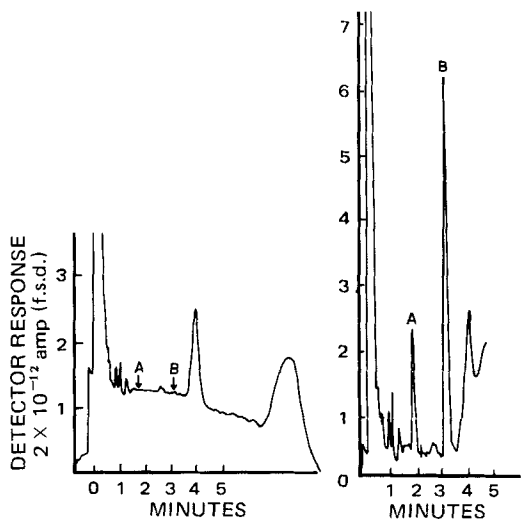


Figure 1—*Gas chromatogram of human plasma. Left: control plasma. Right: plasma containing 0.1 μg of meperidine/ml (A) and 0.5 μg of the internal standard/ml (B).*

[1] Demerol, Sterling-Winthrop Research Institute.
[2] Packard model 419.
[3] Supelco, Inc. Bellefonte, Pa.
[4] Applied Science Laboratories, State College, Pa.
[5] Astra Pharmaceutical Inc., Worcester, Mass.
[6] Mallinckrodt Chemicals, St. Louis, Mo.
[7] California Bionuclear, Sun Valley, Calif.
[8] Packard Tri-Carb model 3320.

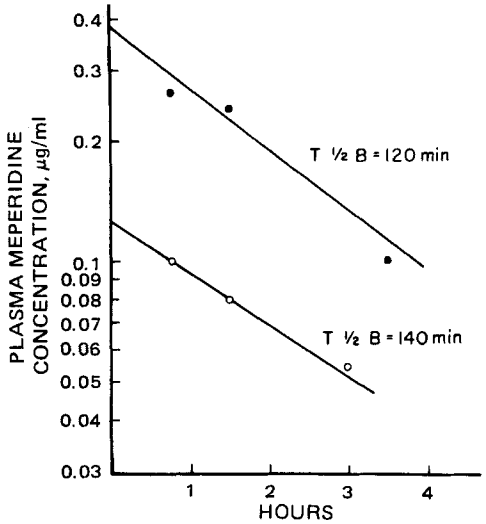


Figure 2—*Plasma concentration–time profile for meperidine in two infants following therapeutic drug administration. Sample size ranged from 0.5 to 1.5 ml of plasma.*

estimate and an intercept of zero. Extraction efficiency based on results from 10 samples using ^{14}C-meperidine hydrochloride was estimated at 85%.

Results of meperidine analysis in two infants following therapeutic intravenous administration of the drug are shown in Fig. 2. Although the calculated half-life was 1.5 hr for both infants, no clear definition of biological half-life in infants can be made since only two subjects were available for complete analysis. Burns *et al.* (1) demonstrated a biological half-life of 3–4 hr in adult humans with variations up to 100%.

This rapid method of analysis may prove useful for meperidine determinations when sample size is limited and many samples must be analyzed.

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Flavonoid Constituents from *Eupatorium altissimum* L. (Compositae)

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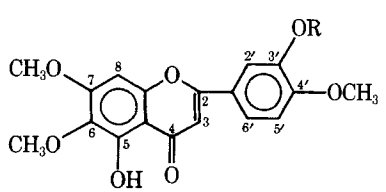
Abstract □ An aqueous ethanol extract of *Eupatorium altissimum* L. (Compositae) showed confirmed activity in the P-388 lymphocytic leukemia assay in mice, and the chloroform solubles showed both cytotoxic activity in the 9KB carcinoma of the nasopharynx cell culture assay and antitumor activity in the P-388 lymphocytic leukemia assay. Two flavones, eupatorin and 5-hydroxy-3′,4′,6,7-tetramethoxyflavone, were isolated and identified. Both were devoid of cytotoxic and antitumor activity.

Keyphrases □ *Eupatorium altissimum*—aqueous ethanol whole plant extract, two flavones isolated, cytotoxic and antitumor activity evaluated □ Flavones—isolated from *Eupatorium altissimum* aqueous ethanol whole plant extract, cytotoxic and antitumor activity evaluated □ Cytotoxic activity—evaluated in *Eupatorium altissimum* aqueous ethanol whole plant extract and isolated flavones □ Antitumor activity—evaluated in *Eupatorium altissimum* aqueous ethanol whole plant extract and isolated flavones

As part of a continuing search for antitumor compounds from plant sources, it was found that an aqueous ethanol extract of *Eupatorium altissimum* L. (Compositae) showed confirmed activity against the P-388 leukemia assay in mice and that the chloroform solubles showed both antitumor activity in the P-388 leukemia and marginal activity against the 9KB cell culture (cytotoxicity). In the only previous investigation of *E. altissimum*, a defatted ethanol extract was reported to exhibit antimicrobial activity against *Leuconostoc citrovorum* (1).

Extracts of various *Eupatorium* species have been reported to possess cytotoxic and/or antitumor activity. Cytotoxic flavonoids have been isolated from *E. semiserratum* (2–4) and *E. cuneifolium* (4); cytotoxic and antitumor sesquiterpene lactones have been isolated from *E. semiserratum* (5), *E. cuneifolium* (6, 7), *E. formosanum* (8), and *E. rotundifolium* (9–11).

In this investigation, two flavonoids were isolated from a fraction possessing cytotoxic activity. One was identified as eupatorin (I), a flavone previously isolated from *E. semiserratum* (2). The other flavonoid was verified as 5-hydroxy-3′,4′,6,7-tetramethoxyflavone (II). Although II



I: R = H

II: R = CH_3