Quantitative Determination of Nalbuphine in Plasma Using Electron-Capture Detection

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Abstract □ A procedure for the determination of nalbuphine in plasma is presented. The compound and an internal standard are extracted into benzene–2-propanol from plasma at pH 10.4, followed by back-extraction into 0.1 *N* HCl. After the acid phase is washed with benzene and the compound is reextracted into benzene–2-propanol, the heptafluorobutyryl derivatives are formed and determined using electron-capture GLC. The lower limit of sensitivity is approximately 0.5 ng/ml, and the upper limit of the linear dynamic range is greater than 50 ng/ml. Peak plasma levels of 50 and 150 ng were observed in two dogs 10 and 20 min, respectively, after subcutaneous administration of 1 mg of nalbuphine/kg. Nalbuphine was detectable in plasma 5 hr after administration.

Keyphrases □ Nalbuphine—electron-capture GLC analysis in plasma □ GLC, electron capture—analysis, nalbuphine in plasma □ Analgesics—nalbuphine, electron-capture GLC analysis in plasma

Nalbuphine, (−)-17-(cyclobutylmethyl)-4,5α-epoxymorphinan-3,6α,14-triol[1], is a potent analgesic with narcotic antagonist activity in rodents (1) and humans (2). Clinical studies showed that the compound has low abuse potential (2), has analgesic potency similar to morphine, and may have a longer duration of action than morphine (3).

There have been no reports on the disposition and plasma levels of nalbuphine in animals or humans because of the insufficient sensitivity of currently used analytical methods. However, sensitive electron-capture GLC assays for the structurally similar compounds naltrexone (4, 5) and naloxone (4, 6) were reported.

The development of a sensitive electron-capture GLC method for nalbuphine in plasma is the subject of this report.

EXPERIMENTAL

Analytical Methodology—Up to 3 ml of plasma, containing 200 ng of naloxone as the internal standard, was placed in a 25-ml screw-capped centrifuge tube[2] (if less was used, the volume was made up to 3 ml with water), and the pH was adjusted to 10 with 2.5% NaOH. Then 1.5 ml of pH 10.4 phosphate buffer[3] was added, followed by 1.5 g of sodium chloride. This mixture was extracted with 12 ml of benzene[4] containing 1% 2-propanol by shaking on a wrist-action shaker for 30 min.

After centrifuging for 30 min at 2200×*g*, 10 ml of the upper organic layer was removed and placed in a 15-ml glass-stoppered centrifuge tube containing 1 ml of 0.1 *N* HCl. The tube was agitated on a mixer[5] for 1 min and then centrifuged for 10 min at 1240×*g*. The upper organic phase was discarded, and the remaining aqueous layer was washed with 6 ml of benzene. After discarding the benzene, the aqueous phase was adjusted to pH 10 with 0.01 *N* NaOH, and 1.5 ml of pH 10.4 phosphate buffer was added.

The alkaline aqueous phase was extracted into 6 ml of benzene containing 1% 2-propanol by shaking for 30 min on a wrist-action shaker. After centrifuging at 1240×*g* for 10 min, 5 ml of the upper benzene phase was evaporated to dryness in a 5-ml glass-stoppered centrifuge tube[6]. The samples were then dried overnight in a desiccator.

Anhydrous ether, 50 μl, was added to the dried sample by allowing it to wash down the sides of the tube; then 25 μl of heptafluorobutyric anhydride[7] was added. The tube was stoppered, and the mixture was agi-

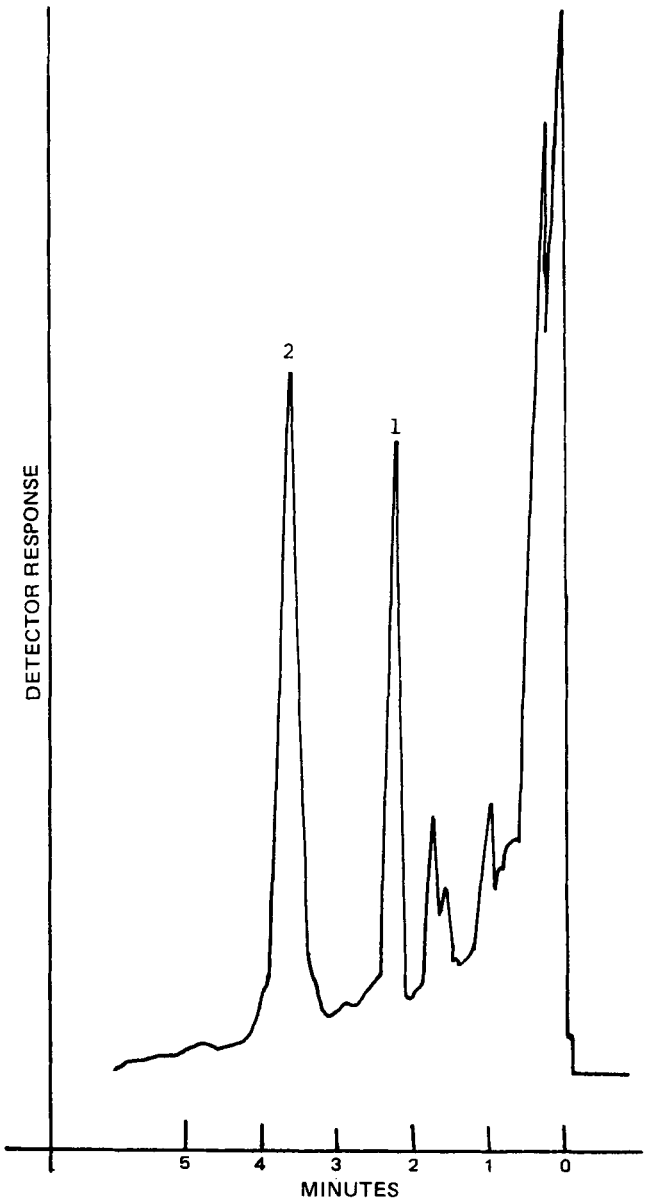

Figure 1—*Gas chromatogram of nalbuphine and internal standard (naloxone) extracted from dog plasma. Key: 1, naloxone heptafluorobutyrate; and 2, nalbuphine heptafluorobutyrate. Other peaks were due to the solvent and endogenous substances.*

[1] Nubain, Endo Laboratories.
[2] Corex.
[3] Potassium phosphate buffer was prepared by adding 5% (w/v) aqueous $K_3PO_4 \cdot x H_2O$ to 35% (w/v) aqueous K_2HPO_4 until pH 10.4 was attained.
[4] All organic solvents were Burdick and Jackson distilled in glass grade.
[5] Vortex.
[6] Evaporation was performed with a Buchler Evapo-Mix attached to a water aspirator.
[7] Pierce Chemicals, Rockford, Ill.

Table I—Plasma Nalbuphine Levels in Dogs after Subcutaneous Administration of 1 mg/kg

Hours	Plasma Nalbuphine Level, ng/ml	
	Dog 1	Dog 2
0.083	99	14
0.17	123	48
0.33	72	50
0.5	65	42
0.75	42	38
1.0	38	40
1.25	33	27
1.5	57	32
2.0	46	28
2.5	36	33
3.0	49	28
5.0	25	21

tated on a mixer for 5–10 sec, followed by incubation in a heating block[8] at 60° for 10 min. The incubated reaction mixture was then evaporated to dryness for 2 hr[9].

The dried product was dissolved in 25 μl of hexane, and 2–4-μl aliquots were injected into the gas chromatograph[10] equipped with a 2-mCi ^{63}Ni-electron-capture detector. The column was 3.8% methyl vinyl silicone gum rubber[11] (1.2 m long and 4 mm i.d.). Helium carrier gas, 75 ml/min, and 10% methane in argon (purge gas), 88 ml/min, were used. The oven, detector, and injection port temperatures were 235, 250, and 250°, respectively. The pulse interval setting was 50.

The peak heights of nalbuphine and naloxone heptafluorobutyrates were measured, and the ratio was calculated.

Dog Study—Nalbuphine hydrochloride in normal saline solution (5 mg/ml) was administered subcutaneously to two 10-kg beagle dogs, one male and one female, at a dose of 1 mg/kg. Blood was collected from the cephalic vein[12] in heparinized tubes at specified times after dosing. Plasma obtained after centrifugation was extracted and analyzed for nalbuphine by the described electron-capture GLC method.

[8] Temp-Blok, Lab-Line Instruments, Melrose Park, Ill.

[9] The Buchler Evapo-Mix was attached to a vacuum pump for this procedure. The derivatization procedure requires dry conditions and works well only when the relative humidity is 50% or less.

[10] F & M 402, Hewlett-Packard.

[11] UCC-W-982 on High Performance Chromosorb W, Hewlett-Packard.

[12] Butterfly-19 (Abbott) with a siliconized needle was placed in the vein.

RESULTS

The retention times of the heptafluorobutyryl derivatives of nalbuphine and naloxone were 3.7 and 2.2 min, respectively. Figure 1 is a typical chromatogram of nalbuphine extracted from dog plasma. Extracts of human plasma gave identical results. Human and dog plasma blanks extracted and treated with heptafluorobutyric acid showed no interfering peaks at the retention times of nalbuphine or naloxone heptafluorobutyryl derivatives.

The reproducibility of peak height ratios for quadruplicate samples of plasma made 10.0 and 1.0 ng/ml in nalbuphine hydrochloride was 5 and 14% (percent standard deviation), respectively. The recovery of nalbuphine added to plasma was 70% after correcting for aliquot losses during extraction. There was a linear relationship between the peak height ratio and the plasma concentration of naltrexone. The lower limit of sensitivity was approximately 0.5 ng/ml, and the upper limit of the linear dynamic range was greater than 50 ng/ml.

Table I presents the concentrations of nalbuphine found in the plasma of two dogs after subcutaneous administration of 1 mg of nalbuphine hydrochloride/kg. The peak concentrations were 123 and 50 ng/ml in Dogs 1 and 2, respectively. The peak times in the two dogs were 10 and 20 min (0.17 and 0.33 hr), respectively. Nalbuphine was still detectable in plasma 5 hr after administration.

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Anti-Inflammatory 1-Substituted 2-Imidazolidinones

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Received February 7, 1977, from the *Research and Development Department, Norwich-Eaton Pharmaceuticals Division of Morton-Norwich Products, Inc., Norwich, NY 13815.* Accepted for publication June 22, 1977.

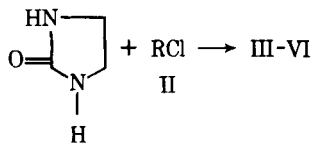
Abstract □ The synthesis and anti-inflammatory activity of a series of 1-substituted 2-imidazolidinones are reported. These compounds exerted marked anti-inflammatory activity in the carrageenan-induced hindpaw edema assay in the rat in doses that failed to elicit side effects. Their ED_{50} values were near the value of aspirin but markedly higher than the value of indomethacin.

Keyphrases □ 2-Imidazolidinones, 1-substituted—synthesized, anti-inflammatory activity evaluated □ Anti-inflammatory activity—various 1-substituted 2-imidazolidinones evaluated □ Structure-activity relationships—various 1-substituted 2-imidazolidinones evaluated for anti-inflammatory activity

A series of 1-(arylmethyl)-2-imidazolidinones was evaluated as anti-inflammatory agents. The target compounds, III–VI (Table I), were prepared by reaction of 2-imidazolidinone (I) with the appropriate arylmethyl chloride (II) (Scheme I).

DISCUSSION

The compounds were tested for anti-inflammatory activity according to a reported method (1). Each compound was suspended in 0.5% methylcellulose, and 300 mg/kg po was administered to three male Wistar



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Scheme I