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Note

Detection of nalbuphine in plasma: an improved high-performance liquid chromatographic assay

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Nalbuphine is a partial agonist opiate derived from oxymorphone. Although approximately 0.8 times the potency of morphine at a dose of 8 mg [1], a "ceiling" effect on respiratory depression is observed at a dose of approximately 0.5 mg/kg, and very much larger doses have been employed in clinical practice [2]. Thus in a 70-kg man doses ranging from 5 to 200 mg may be used, resulting in widely variable plasma concentrations even at peak effect.

A method for the high-performance liquid chromatographic (HPLC) analysis of nalbuphine concentrations in plasma has been described using an electrochemical detector [3]. However, the method did not prove to be sufficiently sensitive or consistent for our clinical use; emulsification is likely to occur during extraction and interference peaks are common. In addition, the use of sodium hydroxide for control of pH makes uniform recovery of nalbuphine difficult. The method was therefore modified as described.

METHODS AND MATERIALS

Reagents

Ethyl acetate, toluene, isopropanol (glass-distilled) and methanol (HPLC grade) were all obtained from Rathburn (Walkerburn, U.K.). Potassium dihydrogen orthophosphate, boric acid, borax and hydrochloric acid were all AnalaR grade from BDH (Poole, U.K.). Nalbuphine hydrochloride powder was obtained from the Pharmaceutical Development Section of Endo Labs. (Garden City, NY, U.S.A.) and the internal standard, naloxone hydrochloride powder, from Sigma.

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Extraction procedure

To 2 ml of plasma in a 10-ml screw-topped test-tube were added 50 μl of an aqueous solution of naloxone, the internal standard (4 μg/ml) and 1 ml of 0.5 *M* borate buffer (pH 8.0). The plasma was then extracted twice with 5 ml of ethyl acetate—toluene—isopropanol (79:20:1, v/v/v) by mixing on a rotary tumbler at 40 rpm for 15 min and centrifuging for 5 min at 750 *g*. The organic layers were transferred to a clean tube by Pasteur pipette; 1 ml of 0.1 *M* hydrochloric acid was added, mixed on a rotary tumbler and the tube centrifuged. The organic layer was discarded and the acid layer brought to pH 8.0 with 2 ml of 0.5 *M* borate buffer. The aqueous layer was extracted twice with 5 ml of the ethyl acetate—toluene—isopropanol mixture. After centrifugation the organic top layer was collected and evaporated to dryness in tapered reaction vials under a gentle stream of nitrogen. The sample residue was then redissolved in 500 μl of methanol and injected onto the column using a 100-μl sample loop.

Nalbuphine calibration curves were prepared by spiking blank human plasma with nalbuphine at concentrations ranging from 1 to 100 ng/ml and with naloxone at a fixed concentration of 100 ng/ml. These samples were extracted and chromatographed as described below and the peak height ratios of nalbuphine relative to internal standard were plotted against the nalbuphine concentration.

High-performance liquid chromatography

A ConstaMetric III pump (Laboratory Data Control) was used in conjunction with an automatic injection system (Magnus Scientific M7110 automatic injector) and a precolumn (5 × 0.5 cm) and column (15 × 0.5 cm) both packed with Spherisorb 5-μm C_8 (HPLC Technology). The eluent was 55% potassium dihydrogen orthophosphate (0.01 *M*) and 45% methanol (HPLC grade) with a flow-rate of 0.8 ml/min. A BioAnalytical Systems LC4A electrochemical detector with a glassy carbon electrode (TL5) operating in the oxidative mode at 0.75 V was used. The current sensitivity was set at 5 nA and chromatograms were recorded on a Kipp & Zonen BD9 flat bed recorder.

RESULTS

The recovery of nalbuphine from extracted plasma at various concentrations was determined by comparing the peak height ratios of extracted nalbuphine samples with naloxone as an external standard, to those obtained with unextracted primary standard. The actual recovery determined was 76 ± 3% (mean ± S.E.M., $n = 7$).

As can be seen from the chromatograms of extracts from spiked plasma samples (Fig. 1), the retention times of the naloxone and nalbuphine were 7.5 and 10 min, respectively. A small tail peak was present on the nalbuphine peak of the extracted and unextracted standards, but this did not appear to affect the linearity of the calibration curve, the regression equation of which was $Y = 0.0177X - 0.0092$ from five separate determinations, with a correlation coefficient of 0.9999.

The reproducibility was checked by analysing plasma samples spiked with several concentrations of nalbuphine. The results are shown in Table I.

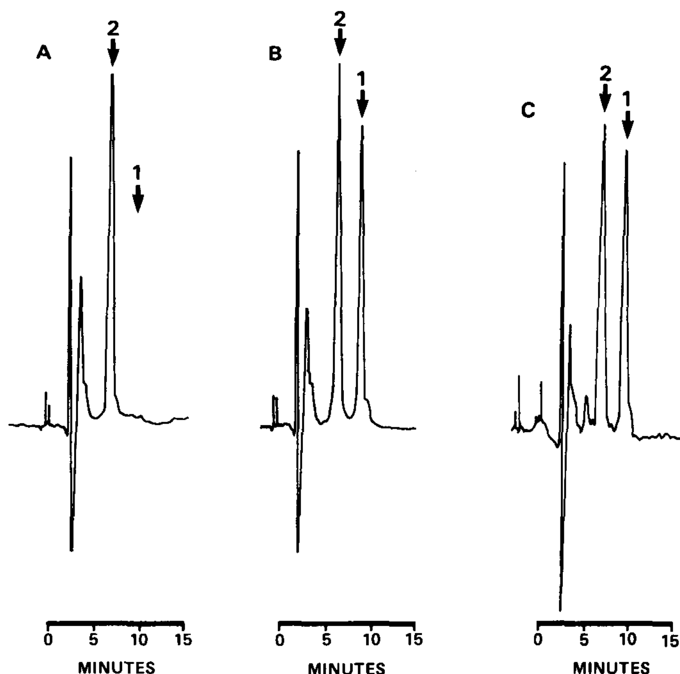

Fig. 1. Typical chromatograms of (A) control plasma to which internal standard (100 ng/ml) has been added, (B) control plasma to which have been added nalbuphine (50 ng/ml) and internal standard (100 ng/ml), and (C) patient plasma taken 1 h after administration of 0.24 mg/kg nalbuphine to which naloxone (100 ng/ml) has been added. Arrows 1 and 2 indicate the retention times of nalbuphine and naloxone, respectively.

TABLE I

REPRODUCIBILITY OF ASSAY

Added (ng/ml)	Found (ng/ml, mean ± S.E.M., n = 4)	S.E.M. (%)
10	10.26 ± 0.05	0.5
25	23.72 ± 0.40	1.7
50	47.91 ± 0.30	0.6
100	110.75 ± 0.49	0.4

DISCUSSION

This method was based on a modification of that presented by Lake et al. [3]. It was found that the extraction mixture ethyl acetate—isopropanol (9:1) with plasma was prone to the formation of emulsions which obstructed adequate removal of the organic phase. Addition of toluene to the extraction mixture resulted in an almost emulsion-free separation. In addition, there was great difficulty measuring chromatograms of plasma extracts due to interference peaks. The inclusion of a back-extraction in the extraction procedure produced a chromatogram free of interference from endogenous plasma sub-

stances. It was also found that the extraction pH was an important factor in nalbuphine recovery. Lake et al. [3] employed 0.1 *M* sodium hydroxide to adjust the plasma to pH 8.0; however, we obtained better pH control with 0.5 *M* borate buffer, with a correspondingly more consistent recovery.

The recovery was only 76% compared with a reported 94% or greater by Lake et al. [3]. However, the actual improvement in clarity of the chromatograms obtained by a more thorough extraction procedure would seem to compensate for this.

Using the method described in this paper, levels of nalbuphine down to 0.1 ng/ml of plasma were measured. The level of detection could have been increased by injection a more concentrated sample into the chromatogram, i.e. redissolving the final dried-down sample in 200 μl instead of 500 μl of methanol. This was not possible with our automated injection system as about 300 μl of final sample are required to inject 100 μl onto the column.

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