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Short Communication

Determination of nalbuphine using high-performance liquid chromatography coupled to photodiode-array detection and gas chromatography coupled to mass spectrometry

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ABSTRACT

A procedure involving capillary column gas chromatography coupled to mass spectrometry and a method involving liquid chromatography coupled to a diode-array detector have been developed for the analysis of nalbuphine. The extraction step is the same for both techniques and involves extraction under alkaline conditions in chloroform–2-propanol–*n*-heptane (50:17:33, v/v/v) with levallorphan as the internal standard. After purification by acidic extraction and back alkaline extraction, drugs are derivatized with N,O-bis-(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane for gas chromatography–mass spectrometry and directly injected for high-performance liquid chromatography–diode-array detection. The limits of detection are 2.0 and 25.0 ng/mg, respectively.

INTRODUCTION

Nalbuphine, commercially available under the name Nubain, is a synthetic narcotic agonist–antagonist of the phenanthrene series. Chemically related to naloxone (an antagonist) and oxymorphone (an agonist), it is an agonist at kappa receptors and an antagonist at other opioid receptors [1]. With respect to analgesic efficacy and respiratory depressant effects, nalbuphine is equipotent to morphine on a weight basis and three items as potent as pentazocine [2,3]. The usual adult dose is 10 mg every 3–6 h as needed, and plasma peak concentrations of *ca.* 15 ng/ml are attained in 1 h after a single oral dose of 30 mg [4]. After intravenous use, its action begins in 2–3 min and lasts for 3–6 h. Nalbuphine produces sedation, central nervous system depression, hallucinations and physical and psychological dependence when abused, but is useful in reversing the respiratory depression after opioid administration [5,6].

The few methods that have been described to detect nalbuphine in plasma or urine include gas chromatography (GC) with electron-capture detection [7] and column liquid chromatography [8–12].

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To the best of our knowledge, no GC method coupled to mass spectrometry (MS) has been described for assaying nalbuphine. Therefore, we have developed such a technique, to improve specificity, particularly for forensic applications.

EXPERIMENTAL

Chemicals and reagents

Nalbuphine hydrochloride was a gift from DuPont Pharmacie (Paris, France). Levallorphan bitartrate, the internal standard, was purchased from the Theta Corporation (Media, USA). N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA), 1% TMCS (trimethylchlorosilane) and β-glucuronidase were supplied by Pierce (Rockford, USA) and Sigma (St. Louis, USA), respectively. Chloroform, 2-propanol, *n*-heptane, acetonitrile, tetrahydrofuran and methanol were HPLC grade and obtained from Merck (Darmstadt, Germany). All other chemicals were analytical grade.

Stock solutions (100 μg/ml, free base) were prepared in methanol and stored at 4°C. The nalbuphine standard concentrations obtained by dilution with methanol in drug-free human plasma were 5, 10, 20, 50, 100, 200 and 350 ng/ml. For the extraction, phosphate buffer was prepared with a saturated solution of K_2HPO_4, 40% diluted with deionized water, and adjusted to pH 9.2.

Extraction procedure

Enzymic hydrolysis prior to urine extraction to cleave the opiate glucuronides was done with 0.25 ml β-glucuronidase (20 000 I.U./ml) in 0.5 ml of 1 *M* sodium acetate buffer (pH 5.2) at 56°C for 12 h. The 2-ml sample was then pipetted into a 15-ml Pyrex centrifuge tube, followed by 1 ml of phosphate buffer (40%, pH 9.2), 50 μl of levallorphan (10 μg/ml) and 10 ml of chloroform–2-propanol–*n*-heptane (50:17:33, v/v). After agitation and centrifugation (10 min, 2000 *g*), the organic phase was purified by acidic extraction (5 ml of 0.2 *M* HCl). The aqueous layer was re-extracted in 5 ml of chloroform, after addition of 1 ml of phosphate buffer and 0.5 ml of concentrated ammonia solution. After agitation, the organic phase was removed and evaporated to dryness at 45°C in a Speed Vac concentrator (Savant Instruments, Farmingdale, USA).

GC–MS analysis

To the dried extract, 40 μl of BSTFA containing 1% TMCS were added. The mixture was incubated at 70°C for 20 min, and then 3 μl were directly injected into a BP 5 capillary column (SGE, Ringwood, Australia; 12 m × 0.22 mm I.D.; particle size 0.25 μm). The flow-rate of the carrier gas (helium, purity grade N 55) through the column was 1.8 ml/min. The column oven temperature was programmed to rise from an initial temperature of 60°C to 280°C at 30°C/min and was kept at 280°C for the final 4 min. Splitless injection with a split valve off-time of 1 min was employed. The injector port temperature was 230°C.

The GC system consisted of a Perking Elmer (8500) chromatograph with an ion trap detector, operated at 70 eV with an ion source temperature of 210°C; the *m/z* range from 200 to 550 was used to determine suitable selected ions. The electron multiplier voltage was set at 1400 V.

Selected-ion monitoring (*m/z* 446 and 355 for nalbuphine and levallorphan, respectively) was used to prepare calibration graphs and to calculate concentrations.

HPLC–photodiode-array analysis

The dried residue was dissolved in 100 μl of mobile phase, and 70 μl were injected into the HPLC system. The system consisted of a pump (Waters 600 E, Milford, USA) with an autoinjector (Waters 715 Ultra Wisp). A UV–VIS diode-array spectrophotometer (Waters 991) with a wavelength range from 190 to 800 nm was used. The system was monitored by a computer (Powerblate SX Plus, NEC, Boxborough, USA) with software allowing the creation of a personal library of substances, and on-line comparison of current analytical data (retention time and UV spectra) with data of the previously stored compounds.

The separation was achieved isocratically on a Waters NovaPak C_{18} column (300 × 3.9 mm

I.D.; particle size 4 μm), set at 30°C during all analyses. The eluent was methanol–tetrahydrofuran–10 m*M* KH_2PO_4 (pH 2.6) (65:5:30, v/v/v). The flow-rate was set at 0.8 ml/min, with an average pressure of 196.5 bar.

Full spectra from 200 to 400 nm were recorded during the entire analysis time. The spectral resolution and sampling interval (delay between acquisition of two consecutive spectra) were set at 1.3 nm and 1.0 s, respectively.

RESULTS AND DISCUSSION

Following extraction and silylation, plasma or urine samples spiked with nalbuphine were found to contain only one chromatographic peak. No other by-products or impurities were observed.

The electron impact mass spectrum of nalbuphine is shown in Fig. 1. The ions chosen for monitoring were the base peaks at *m/z* 446 (nalbuphine) and 355 (internal standard). To be considered positive for nalbuphine, the selected-ion monitoring analysis must show coincident peaks in the *m/z* 446, 501, and 486 ion current profiles. The retention times of the internal standard and nalbuphine were 7.42 and 10.06 min, respectively. The retention time of the internal standard varied by less than 0.11 min over a 4-month period, and it was clearly separated from nalbuphine. Moreover, the latter is also separated from other opiates: codeine (retention time 8.08 min, *m/z* 371), ethylmorphine (8.19 min, *m/z* 385), morphine 8.27 min, *m/z* 429), and 6-monoacetylmorphine (8.47, *m/z* 399). This is of particular interest since several opiates can be identified in the same sample, especially in addicted subjects. Moreover, no other by-products were identified as a potential sources of interference (Fig. 2).

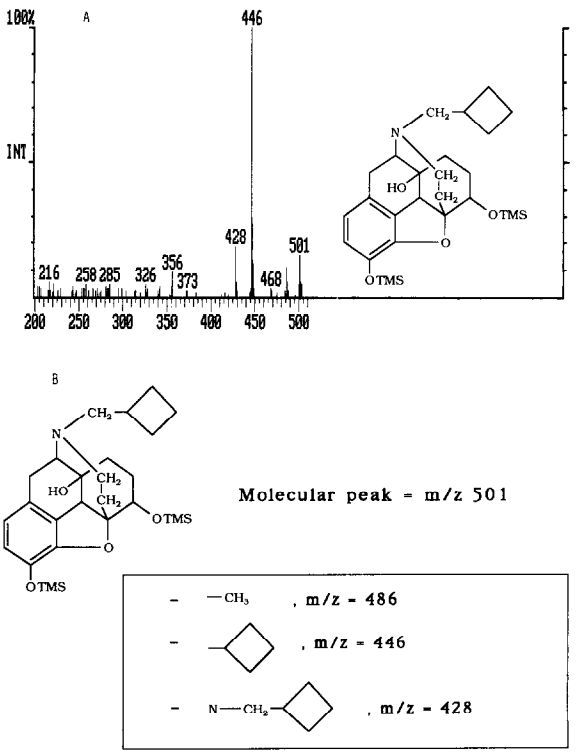

Fig. 1. (A) Electron impact mass spectrum of nalbuphine. (B) Fragmentation pattern of nalbuphine (molecular mass 501).

Concentration *versus* response curves for nalbuphine were linear from 5 ng/ml to 350 ng/ml.

TABLE I

WITHIN-RUN PRECISION AND ACCURACY OF GC–MS ASSAY FOR NALBUPHINE IN PLASMA AND URINE

Fluid	Concentration given (ng/ml)	Concentration found (mean ± S.D.) (ng/ml)	R.S.D. (%)	Error (%)
Plasma	10	9.3	7.9	7.0
	50	48.1	4.8	3.8
	100	106.1	4.3	6.1
Urine	10	10.6	7.8	6.0
	50	48.3	5.1	3.4
	100	104.2	4.7	4.2

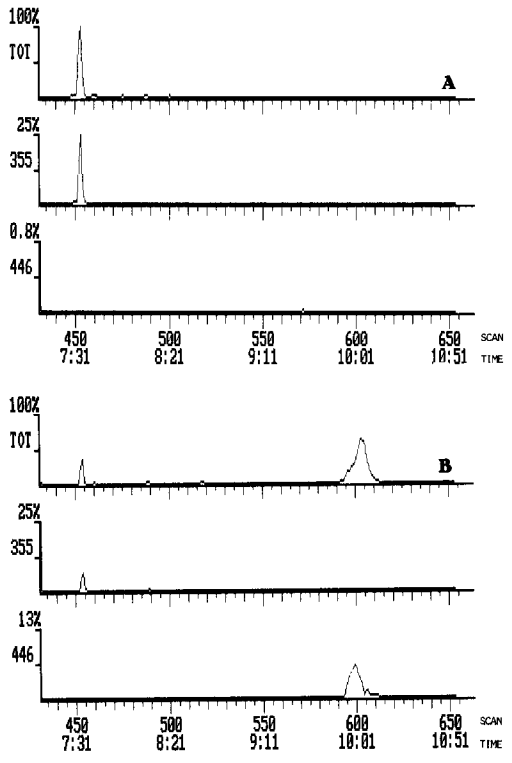

Fig. 2. (A) Selected-ion chromatogram of a blank sample. (B) Selected-ion chromatogram of an experimental sample (nalbuphine 46 μg/l). Time in min.

Coefficients of determination (r^2) ranged from 0.9978 to 0.9994 for four independently established calibration curves.

Within-run precision and accuracy data for spiked plasma and urine samples are presented in Table I. The assay was accurate to within 7% of the target concentrations at 10, 50, and 100 ng/ml nalbuphine. Coefficients of variation for within-run data were less than 8% at the three concentrations.

Chloroform–2-propanol–*n*-heptane (50:17:33, v/v/v) was chosen as the extraction solvent on the basis of its ability to minimize emulsion formation during the extraction and to result in a suitable recovery of 81.3 ± 4.6% (n = 4). The minimum detectable amounts at m/z 446, which represents a signal-to-noise ratio of 3:1, are 2.0 ng/ml and 3.0 ng/ml fdor plasma and urine, respectively. This detection limit is suitable for forensic and clinical analyses, but not for single-dose pharmacokinetic studies.

HPLC coupled to diode-array detection was also investigated for nalbuphine analysis. The high detection limit (25 ng/ml) associated with a poor informational spectrum led us to use HPLC only for quantification in urine, or as pure solution. Fig. 3 shows a chromatogram obtained from a urine extract after hydrolysis in a case involving nalbuphine therapy. The retention times of nalbuphine and levallorphan were 3.83, and 4.38 min, respectively. The calibration curve was linear over the range 50–2500 ng/ml for nalbuphine. Coefficients of determination (r^2) ranged from 0.9975 to 0.9997 for four independently established calibration curves. Within-run precision and accuracy data for spiked urine samples are presented in Table II.

HPLC with electrochemical detection, and GC with electron-capture detection, have limits of detection in the range 0.3–0.5 ng/ml [7–12], but GC–MS dramatically increases the specificity of the assay. Negative ion chemical ionization,

TABLE II

WITHIN-RUN PRECISION AND ACCURACY OF HPLC PHOTODIODE-ARRAY DETECTION ASSAY FOR NALBUPHINE IN URINE

Concentration given (ng/ml)	Concentration found (mean ± S.D.) (ng/ml)	R.S.D. (%)	Error (%)
50	52.7	6.1	5.4
100	104.6	5.1	4.6
500	487.8	4.0	2.4

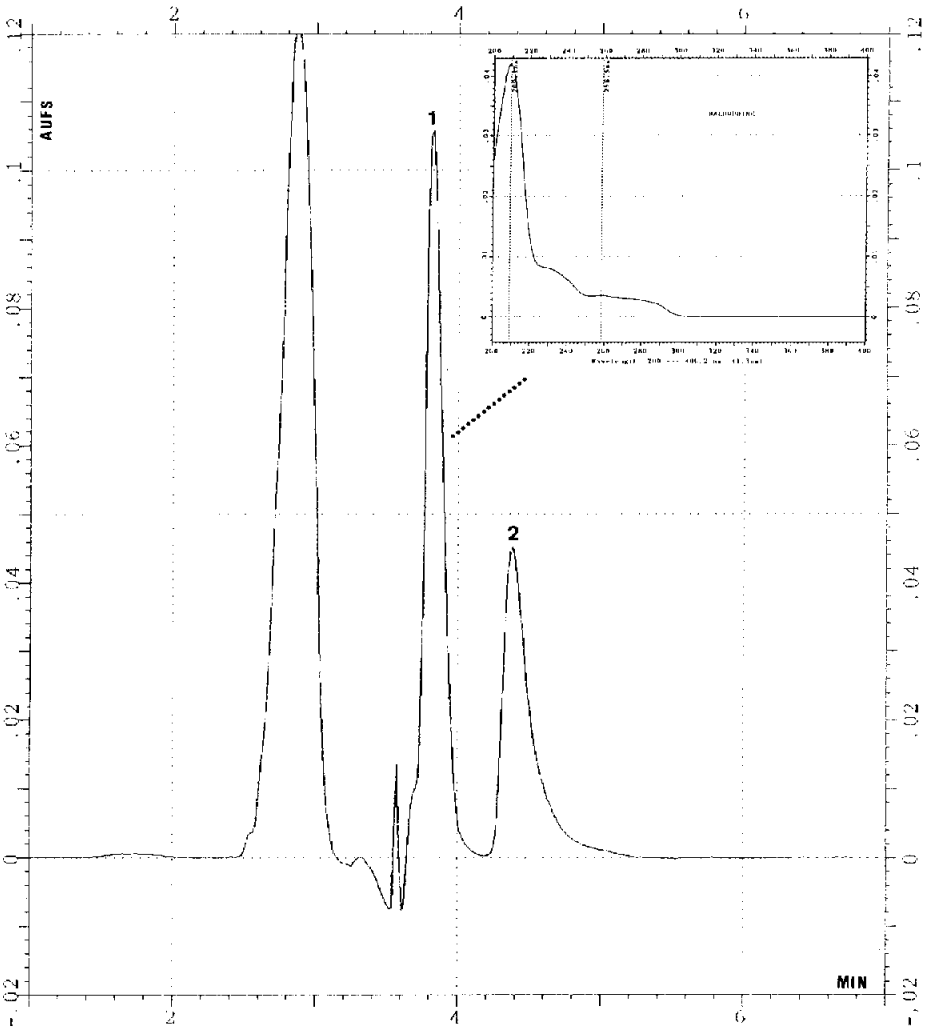

Fig. 3. Chromatogram obtained from a urine extract from a patient treated with nalbuphine. The chromatogram was obtained in the full scan mode between 215 and 238 nm. The UV spectrum of nalbuphine is also shown. Peaks: 1 = nalbuphine, 324 ng/ml, at 3.83 min; 2 = levallorphan, at 4.38 min.

which has greater sensitivity, could be a good alternative for single-dose pharmacokinetic studies, but such an application is limited by its cost.

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