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Short communication

Determination of alfentanil and noralfentanil in human plasma by gas chromatography–mass spectrometry

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Abstract

This report describes a simple gas chromatographic–mass spectrometric (GC–MS) assay for the simultaneous analysis of alfentanil and its major metabolite, noralfentanil, in human plasma. The method facilitates the processing of numerous samples for pharmacokinetic analysis. Alfentanil and noralfentanil are extracted from plasma under basic conditions and noralfentanil is converted to the pentafluoropropionyl derivative. The extraction efficiencies for noralfentanil and alfentanil were >99% and 70%, respectively. Standard curves were linear ($r^2 = 0.99$) over the ranges of 5–500 ng/ml for alfentanil and 0.4–10 ng/ml for noralfentanil. Inter-day coefficients of variation were 3.2% for alfentanil (50 ng/ml) and 3.1% for noralfentanil (1 ng/ml). This assay represents a significant improvement over existing HPLC assays which require radiolabelled alfentanil. The simultaneous disposition of alfentanil and noralfentanil in plasma after intravenous administration in humans is described.

1. Introduction

Alfentanil (Fig. 1) is a potent short-acting opioid used intravenously as an anesthetic agent. Alfentanil is metabolized extensively *in vivo* with less than one percent excreted unchanged, and eliminated rapidly with a half-life of 1–2 h [1]. The major metabolite, noralfentanil (NALF), results from an exo-cyclic N–C cleavage at the piperidine ring of alfentanil (ALF) catalyzed by hepatic cytochrome P450-3A3/4 [2,3]. NALF appears in urine several minutes after the administration of alfentanil and accounts for approximately 30% of the dose after 24 h [1]. NALF is excreted in urine along with other primary and secondary metabolites of alfentanil, some of which may arise from further metabolism of noralfentanil. Interest in the inter-individual variability in alfentanil pharmacokinetics and metabolism led us to develop an assay for the simultaneous determination of alfentanil and noralfentanil in human plasma.

Radioimmunoassay (RIA), enzyme-linked immunosorbentassay (ELISA) [4,5], gas chromatography combined with nitrogen–phosphorus detection (NPD) [6–8], and GC–MS [9] have been reported to be sensitive assays suitable for the analysis of non-labelled alfentanil in blood, plasma, and serum. Alternatively, high-performance liquid chromatography (HPLC) with

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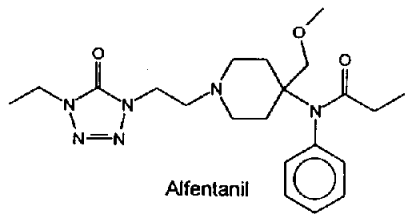

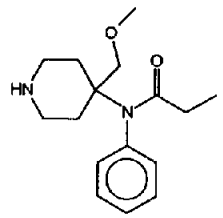

Fig. 1. Structures of alfentanil and noralfentanil. The internal standard, R38527 is a structural analog of ALF possessing an additional methylene unit between the piperidine and tetrazole rings. The internal standard, $^{2}H_{5}$-NALF was deuterated on the aromatic ring.

radiodetection [1] has also been used to monitor the fate of tritium-labelled alfentanil.

Relatively few methods are available for the analysis of noralfentanil. Tritium-labelled alfentanil metabolites, including noralfentanil, have been separated by reversed-phase HPLC and measured simultaneously by radiodetection [1]. However, the use of radiolabelled compounds is complicated by the ethical requirement for patient consent. In an abstract, Bovill *et al.* [9] reported the analysis of noralfentanil in plasma as the heptafluorobutyryl derivative using GC–MS. However, to our knowledge, non-labelled alfentanil and noralfentanil have not been analyzed simultaneously by any method.

We have employed GC–MS with selected-ion monitoring (SIM) and a derivatization scheme to provide a sensitive and selective assay for alfentanil and noralfentanil. Under our mild derivatizing conditions, alfentanil can be simultaneously assayed with noralfentanil without detriment.

2. Experimental

2.1. Chemicals and reagents

Alfentanil, N-{1-[2-(4-ethyl-4,5-dihydro-5-oxo-1-H-tetrazol-1-yl)ethyl]-4-(methoxymethyl)-4-piperidinyl}-N-phenylpropanamide and R38527, N-{1-[2-(4-ethyl-4,5-dihydro-5-oxo-1-H-tetrazol-1-yl)propyl]-4-(methoxymethyl)-4-piperidinyl}-N-phenylpropanamide were purchased from Research Diagnostics (Flanders, NJ, USA). Commercial sources provided the pentafluoropropionic anhydride (Pierce, Rockford, IL, USA), ethyl acetate (Baker, Phillipsburg, NJ, USA), heptane (Aldrich, Milwaukee, WI, USA), and methanol (Mallinckrodt, Paris, KY, USA). Noralfentanil and $^{2}H_{5}$-noralfentanil were prepared as described elsewhere [10]. Out of date plasma was purchased from the Puget Sound Blood Bank.

2.2. Instrumentation

The gas chromatograph was a Hewlett-Packard Model 5890 II equipped with a 5972A mass selective detector (MSD), 7673 liquid automatic sampler, split–splitless capillary inlet system, and electronic pressure control system. The capillary column was a 15 m × 0.25 mm I.D., 0.25 μM film thickness DB-5MS (J and W Scientific, Folsom, CA, USA)

2.3. Operating parameters

Injections of 1 μl were made in the splitless mode with a pulse pressure (104 kPa) of the helium carrier gas. After 1 min, the head pressure was decreased to 14 kPa and the inlet purged. The oven temperature was held at 40°C for 1 min then increased to 120°C at 40°C/min and then to 290°C at 30°C/min. The injector and transfer line temperatures were 290 and 300°C, respectively.

The MSD was operated in the electron-impact mode (70 eV) with selected-ion monitoring. The detector monitored the 321 and 326 m/z fragments (M-101, loss of methoxymethyl and prop-

ionyl groups) with a dwell time of 75 ms for the pentafluoropropionyl derivatives of $^2H_0$- and $^2H_5$-noralfentanil (NALF-PFP) [10] and 289 and 282 m/z for ALF (M-127) and R38527 (M-148), respectively, with a dwell time of 100 ms.

2.4. Sample preparation

Culture tubes (13 × 100 mm) received 1 ml of plasma, the internal standards $^2H_5$-NALF (6 ng) and R38527 (60 ng) in methanol (60 μl), 1 ml of 0.2 *M* aqueous sodium hydroxide, and 2 ml of ethyl acetate–heptane (1:1, v/v). The tubes were sealed tightly with PTFE lined caps and shaken horizontally for 15 min at 60 cycles/min. The emulsion was broken by centrifugation at 1400 *g* for 3 min. The samples were then frozen and the organic phase transferred to new culture tubes. The plasma samples were extracted again. The combined organic layers were dried under a nitrogen stream at 40°C. Samples were derivatized with 25 μl of pentafluoropropionyl anhydride (PFPA) in 200 μl of ethyl acetate and heated at 50°C for 30 min. The excess PFPA and ethyl acetate were evaporated by a gentle stream of nitrogen at 40°C. The samples were reconstituted with 50 μl of methanol and transferred to autosampler vials for GC–MS analysis.

2.5. Calibration and quantification

A stock plasma solution containing 500 ng/ml of alfentanil and 10 ng/ml of noralfentanil was used to produce the calibration samples. Appropriate amounts of the plasma stock solution were mixed with blank plasma to yield the 1-ml calibration samples. The concentrations of the seven calibration samples were 0/0, 5/0.1, 20/0.4, 50/1, 100/2, 200/4, and 500/10 (ng alfentanil/ng noralfentanil). The calibration samples were treated identically to the unknown samples.

2.6. Quality control (QC) samples

Culture tubes received 1 ml of plasma spiked with 50 ng of alfentanil and 1 ng of noralfentanil. The tubes were sealed by PTFE lined caps and stored at −20°C. A QC sample was analyzed at the beginning, middle, and end of each batch of clinical samples.

2.7. Extraction recovery

Calibration samples in plasma were prepared as described above. Analytical samples in methanol were reduced in volume to a residue by a gentle stream of nitrogen. The analytical samples were treated with PFPA and processed along with the plasma samples. The percent recovery was defined as follows: % recovery $= (C_p/C_{std}) \cdot 100$, where C_p is the plasma sample concentration and C_{std} is the mean analytical standard concentration.

3. Results and discussion

A typical selected-ion chromatogram for $^2H_0$-NALF-PFP, $^2H_5$-NALF-PFP, ALF, and R38527 is shown in Fig. 2. Derivatization with PFPA improved the limit of noralfentanil quantitation by several orders of magnitude. Alfentanil stability was unaffected if the excess derivatizing agent was removed prior to analysis. The majority of the excess PFPA was removed by evaporation and the remainder by reaction with methanol. Reconstitution in methanol also improved the chromatography by selectively extracting the analytes from the lipophilic residue.

Standard curves of peak-area ratios, ($^2H_0$-NALF-PFP/$^2H_5$-NALF-PFP) and (ALF/R38527), *vs.* analyte concentration were linear ($r^2 > 0.99$) for 5–500 ng/ml alfentanil and 0.4–10 ng/ml noralfentanil. Regression equations were $y = (0.57 \pm 0.08)x - (20 \pm 49)$ for alfentanil and $y = (0.12 \pm 0.05)x + (0.015 \pm 0.024)$ for noralfentanil ($n = 6$). Inter-assay precision and accuracy data are summarized in Table 1. For the QC samples, the concentrations were 46 to 54 ng/ml and 0.88 to 1.03 ng/ml for alfentanil and noralfentanil, respectively. Noralfentanil was quantifiable (%C.V. < 20) at 0.4 ng/ml in plasma as the pentafluoropropionyl adduct,

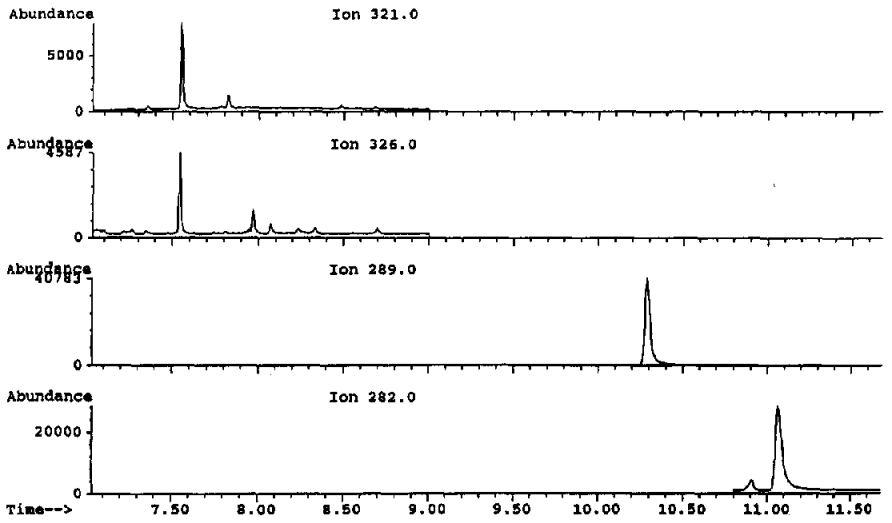

Fig. 2. Selected-ion chromatogram of $^{2}H_{0}$-NALF-PFP, $^{2}H_{5}$-NALF-PFP, alfentanil, and R38527. $^{2}H_{0}$-NALF-PFP (m/z 321), $^{2}H_{5}$-NALF-PFP (m/z 326), alfentanil (m/z 289), and R38527 (m/z 282) appear at 7.54, 7.54, 10.28, and 11.08 min, respectively. The plasma sample was drawn 1.5 h after the patient received a 100 μg/kg intravenous bolus of alfentanil.

while alfentanil was routinely measurable at 5 ng/ml. The extraction efficiency for noralfentanil (1 ng/ml) was >99% ($n = 4$), while the efficiency for alfentanil (50 ng/ml) was 70% ($n = 4$). No attempts were made to improve the detection limit for alfentanil, since the assay was optimized for noralfentanil which was present in substantially lower concentrations.

3.1. Application

The assay has been used to quantitate plasma alfentanil and noralfentanil concentrations in patients undergoing surgery. Typical plasma concentrations in a patient receiving 90 μg/kg alfentanil intravenously are shown in Fig. 3. Noralfentanil appeared in plasma within 1 min and plasma concentrations typically peaked at 15 min. Plasma alfentanil concentrations were undetectable after 24 h, while noralfentanil was usually detected at concentrations less than 2 ng/ml.

This assay represents a substantial improvement over the existing HPLC assay utilizing radiolabelled alfentanil and scintillation counting of the parent drug and metabolite peaks. The assay may be widely applied to pharmacokinetic investigations in surgical patients or normal volunteers. The capability to monitor metabolite kinetics in addition to parent drug disposition may help clarify existing variabilities in alfentanil pharmacokinetics.

Table 1
Inter-assay comparison of quality control samples

Compound	Amount added (ng/ml)	n	Amount found (mean ± S.D.)(ng/ml)	C.V. (%)
Alfentanil	50.0	10	50.0 ± 1.6	3.2
Noralfentanil	1.00	10	0.98 ± 0.03	3.1

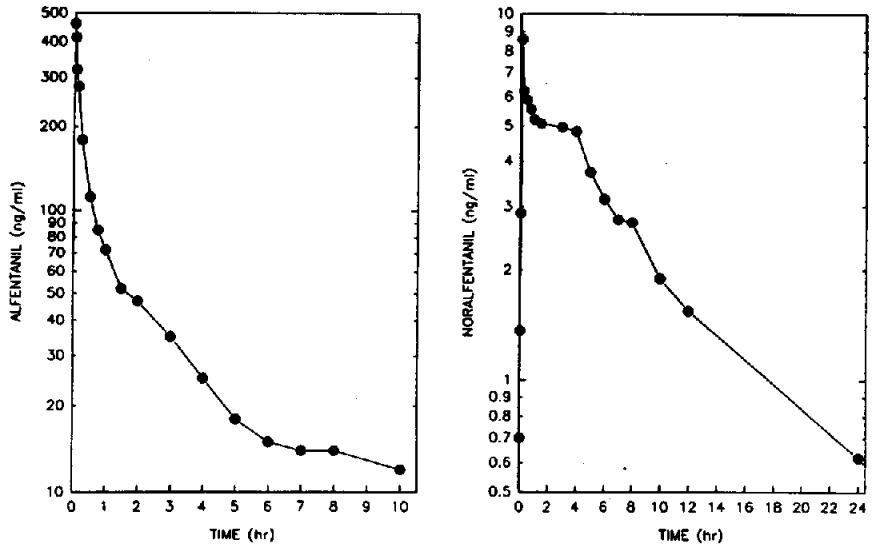

Fig. 3. Plasma concentration–time data for alfentanil and noralfentanil. (Left) Plasma alfentanil concentrations in a typical patient following a 90 μg/kg alfentanil intravenous bolus. (Right) Plasma noralfentanil concentrations in the same patient.

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