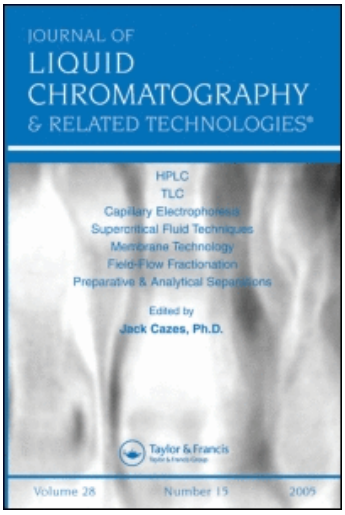

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DETERMINATION OF SUFENTANIL IN HUMAN PLASMA BY CAPILLARY ELECTROPHORESIS AND GAS CHROMATOGRAPHY–MASS SPECTROMETRY

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ABSTRACT

A fast capillary electrophoretic method and a gas chromatographic - mass spectrometric method were developed for the determination of sufentanil in human plasma. Spiked plasma samples and patient plasma samples were prepared by liquid-liquid extraction. In the capillary electrophoretic analysis, both inorganic and organic buffers were tested with and without surfactants, at various pHs. The final separation with fast analysis time was performed with organic 3-[N-morpholino]propanesulfonic acid solution at pH 7.00, with a bubble cell capillary and UV detection at 195 nm. The gas chromatographic separation was performed without sample derivatization, in a nonpolar column material with fentanyl as the internal standard. The mass spectrometric identification was done in electron impact mode. The limit of detection for sufentanil was lower in the gas chromatographic - mass spectrometric technique than in capillary electrophoresis with UV, therefore more sample was required in the capillary

electrophoretic analysis. Comparison of the techniques was done with spiked plasma samples. Both techniques were found to be excellent for the determination of sufentanil. Advantages of the capillary electrophoretic analysis over the gas chromatographic method are better efficiency and the faster elution.

INTRODUCTION

Sufentanil (N-[4-(methoxymethyl)-1-[2-(2-thienyl)ethyl]-4-piperidinyl]-N-phenylpropanamide) and fentanyl (N-phenyl-*N*-[1-(2-phenylethyl)-4-piperidinyl] propanamide) are potent short-acting opioid analgesics used as anesthetic drugs (Figure 1). Comparison shows that sufentanil is five to ten times more potent than fentanyl, although both opioids have short half-lives in blood. Because sufentanil has no seriously adverse haemodynamic effects, not even at high doses, it has been preferred in long-durational cardiac surgical procedures such as coronary artery bypass grafting (CABG). During these procedures, sufentanil (or fentanyl) is administered intravenously as citrate complex. The given dose is dependent on patient's weight and his diseases.[1]

In human plasma of patients undergoing non-cardiac surgery, about 92 per cent of sufentanil is complexed with proteins and other plasma compounds, while the rest of it, which is the pharmacologically active moiety of the drug, exists free.[2] By contrast, the free fraction of sufentanil, and therefore, also the therapeutic level of the free drug, is not reliably known for cardiac surgical patients.

In routine clinical laboratory work sufentanil is quantified with a radioimmunoassay (RIA) technique, a sensitive method with a determination limit as low as 50 pg/mL.[3] However, there are a number of disadvantages to the use of radioactive labels, including an analysis time of several hours and incapability of separating the free active drug from the inactive protein-bound one and from metabolities. In view of these drawbacks, other methods, gas chromatographic-mass spectrometric methods in particular, have been developed for the determination.[4,5,6] Capillary electrophoresis (CE) offers a further choice, as the separation efficiency of the technique is excellent.[7] Although detectability is poor with CE, especially with the UV detector, this disadvantage can be overcome by developing fast electrophoretic methods for quantitation that reduce the width of the sample zones. As well, on-line coupling with a mass spectrometer can be expected to improve the detectability at very low concentrations, as achieved in many other applications.[8]

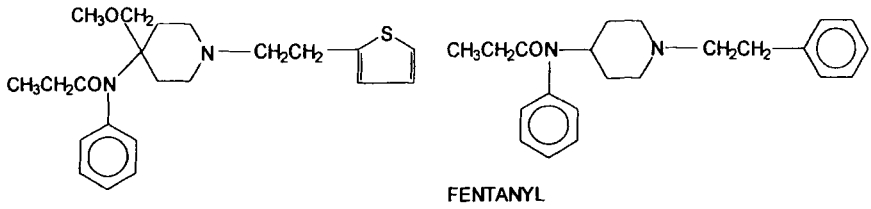


Figure 1. Chemical structures of sufentanil and fentanyl.

We have developed a fast capillary zone electrophoretic (CZE) method and a gas chromatographic mass spectrometric (GC-MS) method for determination of the free and total sufentanil in plasma samples. The capillary zone electrophoretic method is based on the separation of analytes under a strong electric field in an aqueous electrolyte solution made of organic buffer (pH 7.00), whereas the gas chromatographic - mass spectrometric method depends on the separation of components with a retention gap and nonpolar stationary phase column. In both cases the method development included sample pretreatment with liquid-liquid extraction (LLE) and thorough validation of the separation methods.

EXPERIMENTAL

Chemicals and Reagents

Sufentanil (MW 386.6 g/mol) and fentanyl (MW 336.5 g/mol) were purchased from Janssen Pharmaceutica, Beerse, Belgium (Sufenta forte 50 μg/mL and Fentanyl 50 μg/mL). Ethyl acetate (Rathburn Chemicals Ltd., Walkerburn, Scotland) and methanol (E.Merck, Darmstadt, Germany) were HPLC grade, and *n*-heptane was spectroscopy quality (Merck). 3-[N-Morpholino]propanesulfonic acid (MOPS, MW 209.3 g/mol), sodium dodecylsulfate (SDS, MW 288.4 g/mol), and N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (HEPES, MW 238.2 g/mol) were purchased from Sigma (99.5%, Sigma Ultra, St. Louis, MO, USA). Betaine was from Fluka (Buchs, Switzerland) and sodium dihydrogen phosphate, disodium hydrogen phosphate, and glycine were from Merck.

Water was distilled, ion-exchanged (Water-I, Gelman, Ann Arbor, MI, USA) and filtered with 0.45 μm membrane filters (Millipore, Molsheim, France). Helix pomatia juice was from Sepracor (France). Pooled and freeze-dried plasma was from Labquality Ltd. (Finland). All chemicals were used without further purification.

Instrumentation

Capillary electrophoretic analysis was done on an HP Chemstation 3D CE (Hewlett-Packard, Palo Alto, California, USA). The final separations were achieved by using inside uncoated bubble cell capillaries (length 58 cm, i.d. 50 μm, L_{det} 50 cm, Hewlett-Packard). Injections were made hydrostatically within 30 s (50 mbar pressure). The temperature and applied voltage were 22°C and 20 kV, respectively. Detection was accomplished with a diode array detector (DAD) at wavelength 195 nm.

GC-MS analyses were performed on a Hewlett-Packard 5890A gas chromatograph interfaced to an HP 5989A MS Engine quadrupole mass spectrometer (Hewlett-Packard). The final analytical column was HP-5 fused silica capillary column (length 11 m, internal diameter 0.32 mm, film thickness 0.17 μm, Hewlett-Packard). A DPTMDS-deactivated retention gap (3 m * 0.32 mm i.d., HNU-Nordion, Helsinki, Finland) was connected in front of the analytical column. The helium flow rate was 1.5 mL/min (calculated at 150°C). Injections of 1 μL were made with an HP 7673 autosampler (Hewlett-Packard) in splitless mode. The temperature was kept at 60°C for 1.5 min, after which it was programmed at 30°C/min to 265°C, at 15°C/min to 280°C, and finally at 20°C/min to 300°C, where it was kept for 2 min. The injector, interface, ion source and quadrupole temperatures were 290°C, 290°C, 280°C, and 120°C, respectively.

The mass spectrometer was operated in EI-mode (70 eV, multiplier 2500 V) by using selected ion monitoring (SIM) with m/z 245 for fentanyl and m/z 140 and 289 for sufentanil. Data were recorded and evaluated with an HP 9000/345 data system.

A Jenway 3030 pH meter and an electrode (Jenway, Felsted, England) containing 3 M KCL in saturated AgCl solution were used to adjust the pH of the electrolyte solutions. The pH meter was calibrated with CONVOL standard buffer solutions purchased from BDH (BDH Chemicals Ltd., Poole, England).

Preparation of Electrolyte Solution

Phosphate, MOPS, HEPES, phosphate - betaine (5:3 v/v), glycine - SDS, phosphate - SDS and MOPS buffers with concentrations varying from 10 mM to 3 M were used to validate the separation method. The pHs of the electrolyte solutions ranged from 6.50 to 10.60. The final running electrolyte solution used for the fast separations was 100 mM MOPS of pH 7.00 adjusted with 10 mL of 0.1 M NaOH.

Sample Pretreatment

Preparation of reference solutions for quantitation

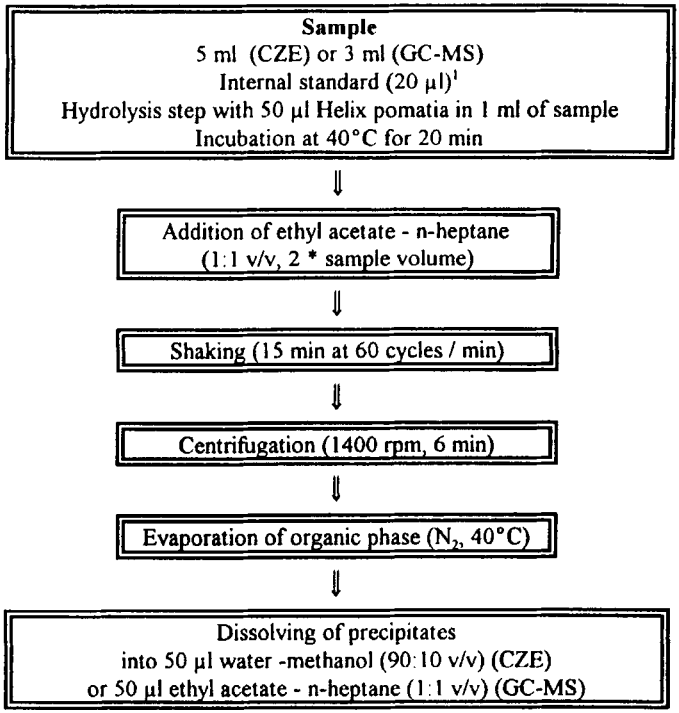
Pooled blank plasma was spiked with Sufenta forte at concentrations varying from 1 to 100 ng/mL. The concentrations of sufentanil in spiked plasma samples were 1, 2, 5, 10, 20, 50, and 100 ng/mL. In CZE, methanol was used to control the electroosmosis during the runs. For the GC-MS analyses, each standard plasma sample also contained fentanyl as internal standard.

Plasma samples

The plasma samples from coronary artery bypass grafting (CABG) patients (five patients, both sexes, unknown ages and weights) were taken three times during surgical operations: 1. before induction of anesthesia, 2. after induction of anesthesia, but before administration of heparin, and 3. after administration of heparin, before the start of cardiopulmonary bypass. Arterial blood for the determination of sufentanil concentrations was drawn into pre-chilled EDTA tubes, and the plasma was separated and stored frozen before analysis.

Liquid-Liquid Extraction

Free sufentanil was determined from test plasma samples spiked with Sufenta forte in different concentrations by isolating with liquid-liquid extraction (Figure 2). Sample volumes were 3 mL for GC-MS analysis and 5 mL for CZE analysis. Sample preparation was based on an earlier reported method.[9]



1= fentanyl in the determination sufentanil by GC-MS
2= hydrolyser excluded in analyses of free sufentanil

Figure 2. Preparation of plasma samples.

The extraction method was also tested for patient plasma samples. The final aim was to separate the free and the conjugated fractions of patient plasma samples. This was done by adding a hydrolysis step into the extraction procedure (Figure 2). The hydrolysis was done after extraction of free sufentanil with enzymatically with Helix pomatia solution.

RESULTS

Optimization of the Separation in CZE and GC-MS

The capillary electrophoretic separations were optimized with sufentanil

and fentanyl standards, by choosing a pH value and buffer concentration that would give the fastest electrophoretic separation and quantitation of sufentanil from patient plasma. The electrolyte solutions tested were neutral or basic buffers of different concentration and content. In the basic region sufentanil was neutral and could be separated from the endogenic and other exogenic compounds only with electrolyte solutions containing SDS, but at neutral pH it was in cationic form and migrated fast within five minutes. The basic electrolyte solution was excluded, since the migration times were increased in SDS solution decreasing the detectability of sufentanil at pg level in patient plasma samples (Figure 3). Buffers without additives allowed the fastest analyses and, owing to the low currents at high voltages, the organic buffers were better than inorganic buffers for fast separation since they could be used at high concentrations. MOPS solution at 100 mM concentration was accordingly chosen for further optimization in CZE. However, only 30 mM phosphate buffer - 50 mM SDS in CE could provide separation of sufentanil and fentanyl (Figure 3), and concentrations should then be above 0.1 μg/mL. Accordingly, in this study we used MOPS buffer to obtain very sharp peaks and UV-responses at low concentration levels.

In the case of GC-MS, the optimization was carried out to give good separation of sufentanil and fentanyl, and the method was optimized to give baseline separation for both.

Also, the instrumental parameters were optimised. The capillary lengths in CZE the separation of sufentanil from endogenic and exogenic compounds was optimized by using 40 to 70 cm capillaries. In GC-MS the capillary lengths of 10 to 18 metres were tested. The final capillary lengths were 50 cm and 11 metres in CZE and GC-MS, respectively.

The optimised separations of spiked test samples with both techniques are presented in Figures 4 and 5.

Interferences

In CZE the plasma proteins interfered with the separation, especially when aqueous inorganic buffers with additives were used; with MOPS, however, they migrated after electroosmosis and offered no interference in the analyses. Figures 6 and 7 show the electropherograms of the analyses of plasma samples from CABG patient. In Figure 6 sufentanil has determined by CZE. The negative peak interference was due to the high ionic strength of the sample after 200-fold concentration. Figure 7 shows that also in the MEKC method tested in our studies during the method development procedures, the

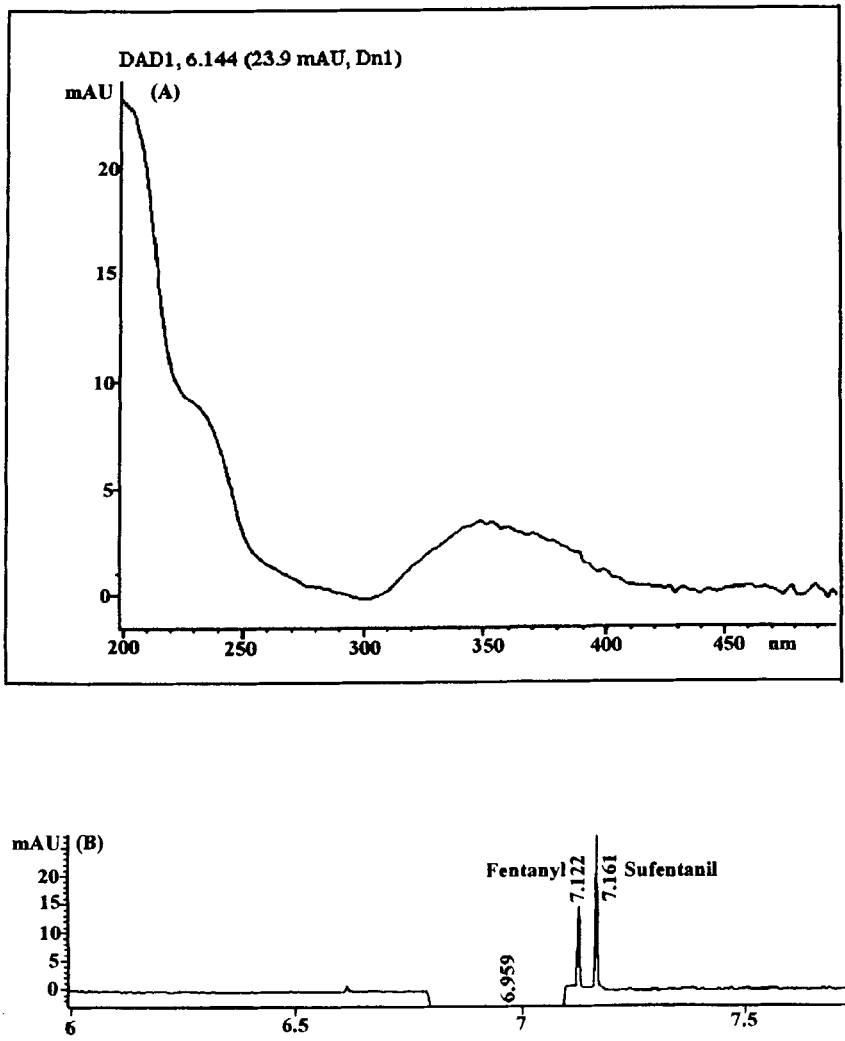
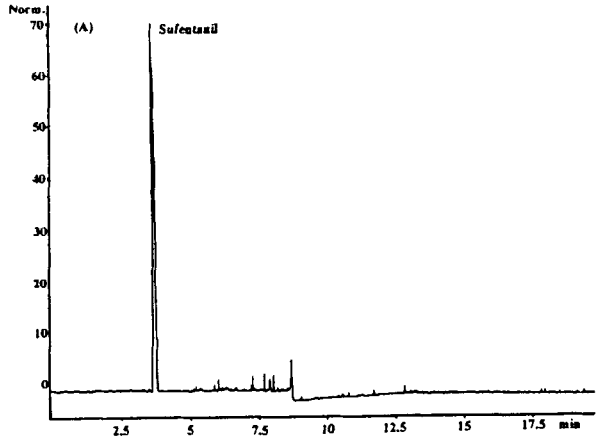
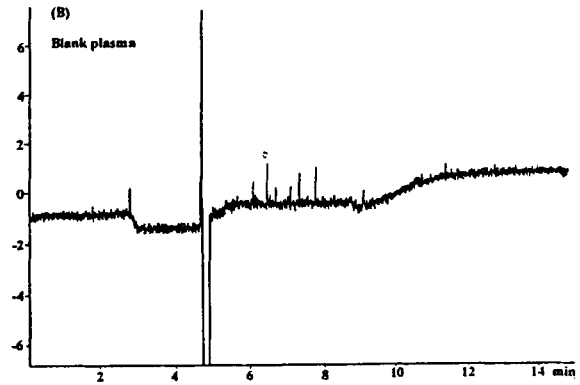
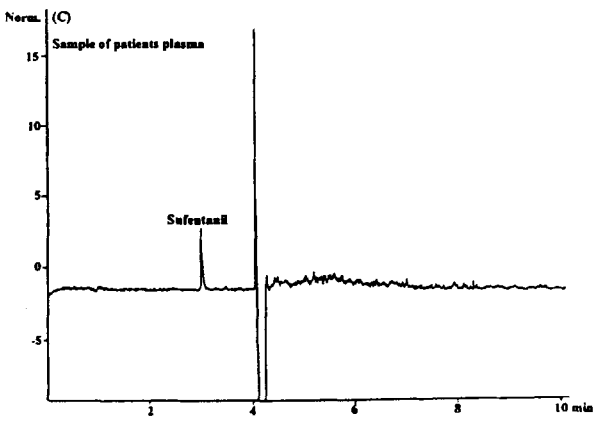


Figure 3. (A) The UV spectrum of sufentanil obtained with DAD, (B) electropherogram of fentanyl and sufentanil separation in 30 mM phosphate - 50 mM SDS electrolyte solution. Concentration of standards 20 μg/mL.

Figure 4 (right). Electropherograms obtained for the quantitation of sufentanil in patient plasma. (A) Standard sufentanil spiked to pooled blank plasma, (B) blank plasma and (C) plasma sample from CABG patient.







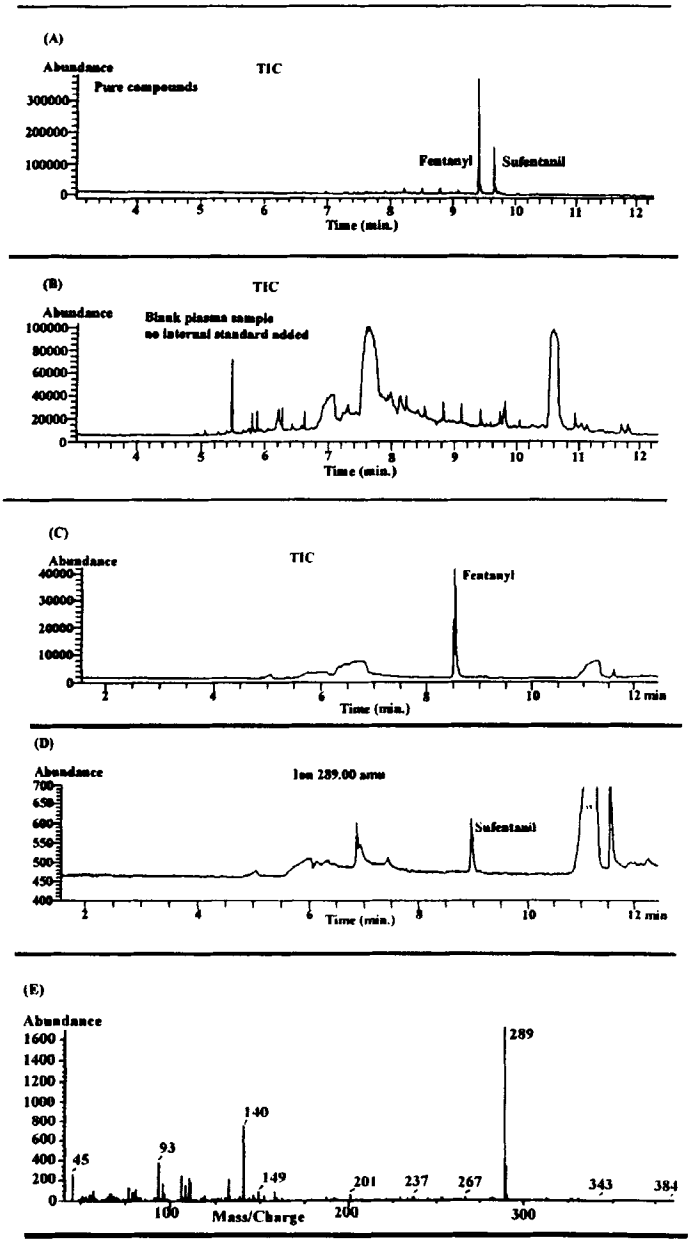


Figure 5. (A) Total ion chromatogram (TIC) of pure fentanyl and sufentanil after liquid -liquid extraction, (B) TIC of blank plasma, (C) TIC of free drug fraction of patients plasma sample, (D) ion 289 from extraction of free drug fraction of patients plasma sample and (E) mass spectrum of sufentanil.

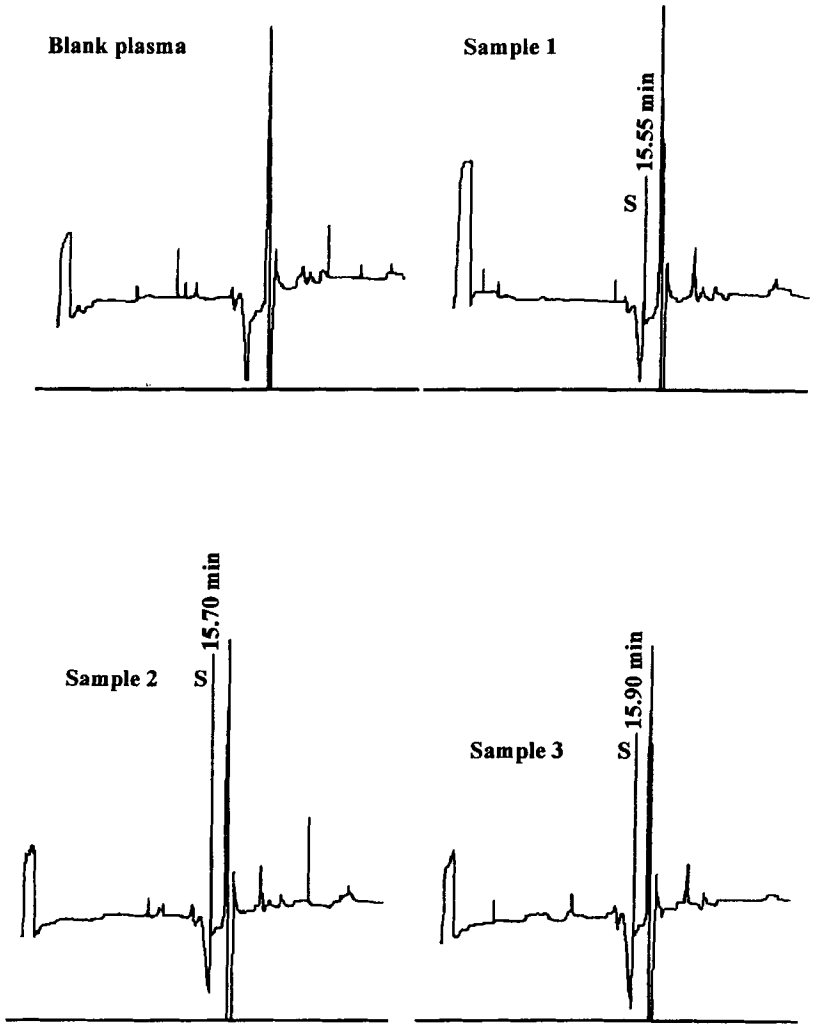


Figure 6. Electropherograms recorded from samples of CABG patient X in electrolyte solution 50 mM phosphate - 3 M betaine (5:3 v/v, pH 7.0). (S) sufentanil. Samples 1, 2 and 3 as described in the Experimental section.

quantifying of sufentanil was not repeatable, because of the broad peak due to endogenic compounds remaining in the sample after the extraction step. In the GC-MS analyses, however, endogenous compounds in plasma which may not be isolated during the pretreatment steps. did not interfere with the analysis.

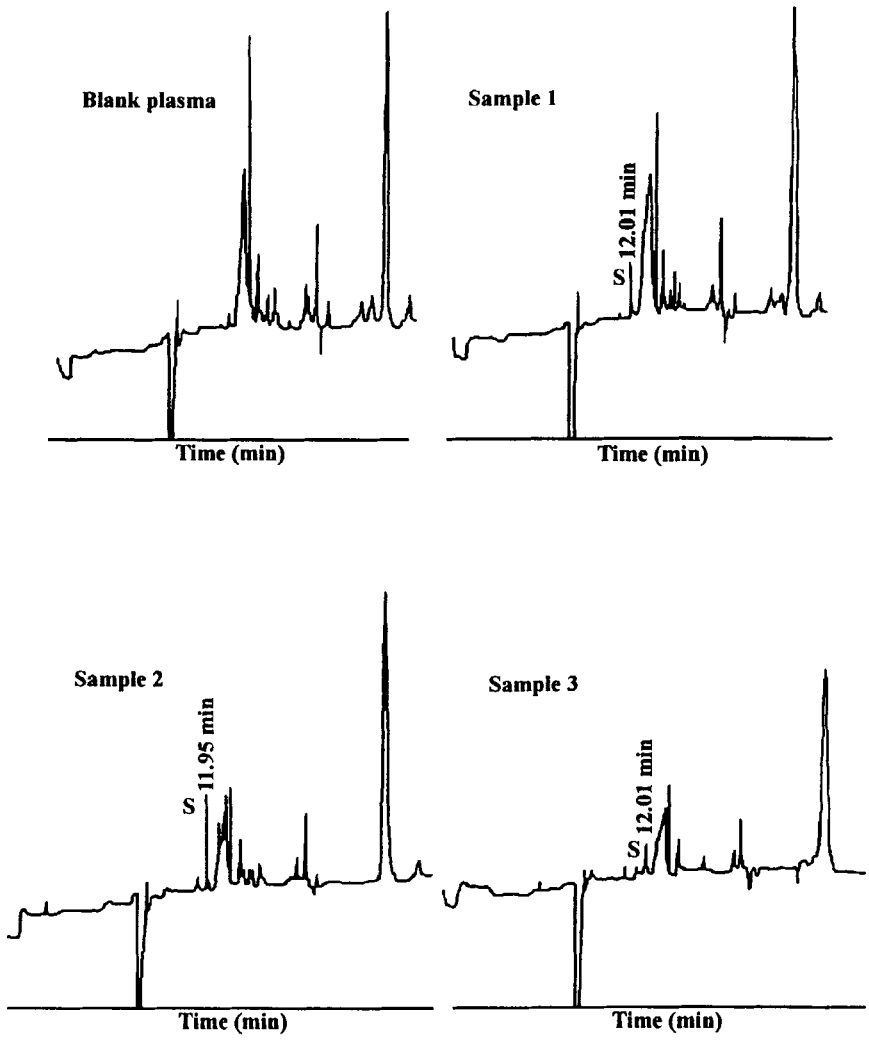


Figure 7. Electropherograms recorded from samples of CABG patient X in electrolyte solution 30 mM glycine - 50 mM SDS (pH 10.5). (S) sufentanil. Samples 1, 2 and 3 as described in the Experimental section.

Linearity

Calibration curves were determined, both in GC-MS and in CZE, on the basis of samples of pooled drug-free plasma spiked with reference compounds.

Table 1

The Concentration of Sufentanil of One Patient During CABG Operations

Sample	CZE-DAD (conc./sample amount)	GC-MS (conc./sample amount)
0 (before induction of anesthesia)	-----[1]	-----[1]
1 (after induction of anesthesia and before administration of heparin), free sufentanil	10.0 ng/3 mL	3 ng/1 mL
1, free + conjugated sufentanil	12.5 ng/3 mL	4 ng/1 mL
2 (after administration of heparin, just before start of cardiopulmonary bypass) free sufentanil	0.003 ng/3 mL	0.0009 ng/ 1mL
2, free + conjugated sufentanil	4 ng/3 mL	1.2 ng/1 mL

1 = sufentanil not added to the sample.

The results showed linear correlations (r=0.997) for peak height (CZE) and peak area (GC-MS) within the concentration range 1 ng/mL to 100 ng/mL. With CZE the detection limit was 3 ng/mL and with GC-MS 20 pg/mL (S/N=3, n=5). The limit of determination was 8 ng/mL in CZE and 50 pg/mL (n=5) in GC-MS.

The free and bound sufentanil fractions were determined from the patient samples. The amount of conjugated sufentanil varied from 3 to 24 per cent of the amount of free sufentanil, which means an amount of bound sufentanil in plasma between 1 and 24 ng/mL.

Table 2

Recoveries of the Spike Plasma Samples at Two Concentration Levels

Sample, Quality (free/conjugated sufentanil)	CZE-DAD (conc. /sample amount)	GC-MS (conc. /sample amount)
0.1 μg/mL, free sufentanil	0.167 μg/5 mL	0.179 μg/3 mL
0.1 μg/mL, conjugated sufentanil	-----[1]	0.020 μg/3 mL
1 μg/mL, free sufentanil	0.987 μg/5 mL	1.02 μg/3 mL
1 μg/mL, conjugated	0.110 μg/5 mL	0.105 μg/3 mL

1 = not quantified

The plasma concentration of sufentanil is dependent on the time of sampling: highest concentrations of both free and bound sufentanil were measured in samples taken before the administration of heparin. In addition, the doses are individual which reflects the concentrations in the patient samples.

Quantitation

The quantitation of sufentanil in patients plasma samples was made with help of a calibration curve. Table 1 shows data on the concentrations of sufentanil in one patient before and during CABG operation.

Compatibility of the Results

To check the agreement between the CZE and GC-MS results, we used plasma samples spiked at concentration levels of 1 μg/mL and 0.1 μg/mL. These samples were extracted as described in Experimental. As expected, almost all sufentanil was in the fraction containing the free form of the drug (Table 2).

DISCUSSION

The liquid-liquid extraction gave good recoveries, and no cross-contamination was observed between the free and bound fractions. Our studies also showed that the sample preparation step needs still to be less time-consuming, however, and more specific for the isolation of sufentanil especially when low concentrations of the drug is to be determined.

Both capillary zone electrophoresis and gas chromatography - mass spectrometry are excellent methods for the determination of sufentanil. Both methods are rapid and repeatability is good. More sample was needed for CZE than for GC-MS because of the low concentration levels.[1] However, the total analysis time was shorter in CZE than in GC-MS. The fast separation and overlapping with fentanyl means that sufentanil and fentanyl cannot be determined simultaneously in CZE. Our studies nevertheless suggest that the CZE method is also suitable for individual fentanyl analysis, though this was not specifically investigated. The GC-MS technique is excellent for the identification of both sufentanil and fentanyl, especially when they are present in plasma samples at picogram to femtogram level.

The best injection technique for sufentanil and the matrix in GC-MS analyses was splitless injection, since the concentrated samples were still contaminated with proteins and sugars. That was also the reason for using a retention gap, which by retaining the contaminants protects the analytical column. However, elution of compounds coextracted with the analyte can never entirely be avoided and produces noise in GC-MS analyses.

A drawback in CZE technique was the low response of the UV detector to the analyte, which could nevertheless be enhanced by concentrating the samples. The disturbance caused by the simultaneous increase in the concentration of endogenous compounds could be avoided by fast analysis under high voltage while the ionic strength was kept high. In summary, CZE is a highly promising technique for the opioid analysis from body fluids and can be used as a reference method for gas chromatographic-mass spectrometric methods.

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