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Quantitative determination of sufentanil in human plasma by liquid chromatography–tandem mass spectrometry

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Abstract

A sensitive and specific method for the quantification of sufentanil in human plasma by liquid chromatography coupled with tandem mass spectrometry has been developed. Fentanyl was used as the internal standard. Rapid sample preparation involved purification on a C_{18} solid-phase extraction column. Chromatographic separation of the analytes was obtained using an RP-C_{18} μ-HPLC column. LC–MS–MS detection was performed by atmospheric pressure ionisation (API) source equipped with an ionspray (IS) interface operating in the positive ion mode. For unambiguous substance confirmation, three analyte precursor–product ion combinations were monitored during multiple reaction monitoring (MRM) LC–MS–MS analysis. The method's performance characteristics were evaluated in blank and spiked control plasma samples. Overall accuracy (relative error, R.E., %), repeatability (relative standard deviations, R.S.D., %) and within-laboratory reproducibility (R.S.D., %) ranged from −9.28 to −2.71%, from 6.42 to 2.82% and from 13.52 to 6.06%, respectively, for sufentanil. The limit of quantification for sufentanil in human plasma samples was 0.3 ng ml^{-1}. Due to its high sensitivity and specificity, the method was successfully employed for sufentanil determination in maternal plasma samples collected immediately before epidural administration of a single sufentanil dose to women in labour, 20 min after drug administration, and at birth in arterial and venous umbilical cord plasma samples from the newborn babies. Research is in progress to adopt the method for performance of complete pharmacokinetic studies of sufentanil in human plasma.

Keywords: Sufentanil; Opioids; Plasma; Liquid chromatography–mass spectrometry

1. Introduction

Opioids are a large class of drugs that have long been used for control of labour pain. They provide analgesia during labour after intravenous, epidural

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or subarachnoid administration. However, they have many dangerous side effects.

Intravenous administration is not particularly effective for analgesia during labour and causes marked maternal sedation and muscular rigidity [1–3]. Opioids can also cause neonatal respiratory depression. The infant's central nervous system is more sensitive than the adult's to their actions due to its lower brain myelin content, higher cerebral blood flow, altered protein binding and different respiratory control mechanism [4].

More effective and with fewer side effects than the prototype analgesic morphine are the lipid-soluble opioids sufentanil and fentanyl, which have shorter action duration and are less likely to produce complicating adverse reactions when used at low doses [5]. Analgesia is in fact obtained in nulliparous women in labour following administration of the minimum analgesic dose of epidural sufentanil during the first stage of labour [6].

Sufentanil, *N*-[4-(methoxymethyl)-1-[2-(2-thienyl)ethyl]-4-piperidinyl]-*N*-phenylpropanamide and fentanyl, *N*-phenyl-*N*-[1-(2-phenyl-ethyl)-4-piperidinyl]propanamide, are members of the 4-propananilido-piperidine class of synthetic narcotic analgesics [5]. They differ structurally in the nature of the 1-substituent and the degree of substitution at the 4-position, thus affecting their relative potency and duration of action [5].

Due to their efficacy at low dosages, blood concentration of sufentanil and fentanyl from single doses falls rapidly below the limit of detection of most assay procedures. Analytical methods for quantification in biological fluids at therapeutic concentrations need to be very sensitive. Radioimmunoassay (RIA) is the most commonly employed technique for pharmacokinetic studies and detection of sufentanil [7–9] and fentanyl [10,11] in human plasma samples.

Sensitive and simple enzyme-linked immunosorbent assay (ELISA) methods have been also developed for screening of fentanyl in human urine [12,13] and fentanyl and sufentanil in equine plasma and urine [14–17]. These techniques allow the rapid and sensitive determination of sufentanil and fentanyl levels in biological fluids, but suffer from a lack of selectivity, or have low precision and show little cross reactivity with structurally similar narcotic analgesics.

High performance liquid chromatography with ultraviolet detection (HPLC-UV) methods have also been developed for the determination of fentanyl [18–20] levels in human plasma samples.

Specific determination of this has been reported by using gas chromatography (GC) methods with nitrogen-selective [21,22], nitrogen–phosphorus [23–25] and mass spectrometry detection [26,27]. GC methods have good sensitivity and specificity but require either derivative formation, glassware deactivation or intensive sample preparation.

The combination of liquid chromatography with mass spectrometry (LC–MS) and tandem mass spectrometry (LC–MS–MS) offers a rapid, simplified, specific and sensitive alternative to GC–MS methods, involving simple extraction procedures and removing the need for derivatisation reactions. A recent paper described its use for sufentanyl analysis in human serum, employing LC–MS–MS with an ion trap spectrometer [28]. This technique offers high specificity and sensitivity but is less useful for quantitative analysis.

The present study investigated the possibility of using LC–MS–MS triple-quadrupole via ionspray (IS) interface for the detection and quantification of sufentanil in arterial and venous umbilical cord plasma samples from newborns and in maternal plasma samples. The field of application of the method was quantitative analysis under real-life conditions in women in labour. Fentanyl was used as the internal standard.

2. Experimental

2.1. Chemicals and reagents

All solvents were HPLC grade and purchased from Farmitalia Carlo erba (Milan, Italy). Water was purified using MILLI-Q System (Millipore Corp, Bedford, MA, USA).

Dosage-form sufentanil citrate (FENTATIENIL®, 50 μg ml^{-1} sufentanil) was purchased from Angelini ACRAF (Rome, Italy). Fentanyl citrate (FENTANEST®, 50 μg ml^{-1} fentanyl) from Janssen Pharmaceutica NV (Beer-

ese, Belgium), was used as the internal standard (IS). FENTATIENIL® (50 μg ml^{-1}) and FENTANEST® (50 μg ml^{-1}) ampoules were used as standard stock solutions of sufentanil and fentanyl.

Individual and composite working standard solutions were prepared daily by appropriate dilution with methanol of the standard stock solutions. Stability of the analytes in solution was observed for 4 weeks in the dark at −20 °C and no degradation phenomena were observable during identification and/or quantification.

2.2. Samples

Maternal plasma samples were collected immediately before and 20 min after epidural administration of a single dose of sufentanil (10–20 μg) to women in labour and at birth in arterial and venous umbilical cord plasma samples from newborn babies.

Blank control samples of human EDTA K3 plasma were provided by Biochemed Pharmacologicals Inc. (Winchester, VA, USA) and were assayed by multiple reaction monitoring (MRM) LC–MS–MS. The absence of the analytes under investigation was verified.

All plasma samples were frozen at −70 °C until assayed.

2.3. Sample preparation procedure

Sample preparation was performed as for the procedure of Rossi et al. [25], with minor modifications. Plasma samples were shaken on a vortex mixer for 1 min and centrifuged for 10 min at $3000 \times g$. An aliquot (1.0 ml) of human plasma was spiked with 1 ng of fentanyl (IS) and purified by C_{18} solid phase extraction (SPE) (Baker, C18, 200 mg) cartridge previously conditioned with 3.0 ml of methanol, 1.0 ml of KOH (1 M), 1.0 ml of water and 0.2 ml ethanol–water (5:95, v/v). After sample loading, the cartridge was washed with 0.7 ml of K_2HPO_4 (10 mM), and 1.4 ml of water and was then dried. Finally, the analytes were eluted with 1.4 ml of methanol, the solvent was evaporated to dryness under nitrogen using an evaporation block at 37 °C and the sample was resuspended in 50 μl of methanol. 1 μl of this solution was injected into the LC–MS–MS system.

2.4. LC–MS and LC–MS–MS

Analyses were performed on a Phoenix 20 CU LC pump (Fisons, Milan, Italy) liquid chromatograph. A Valco (Houston, TX, USA) injection valve equipped with a 1 μl internal loop was used for injection by flow injection analysis (FIA)–MS, FIA–MS–MS and LC–MS–MS. Chromatographic separations were obtained under isocratic conditions using a reverse phase μ-HPLC column (300×1 mm, I.D. 5 μm) Supelcosil LC-C_{18}-DB (Supelco, Bellafonte, PA, USA) at room temperature with an acetonitrile–water (80:20, v/v) mobile phase containing 0.2% trifluoroacetic acid at a flow rate of 50 μl min^{-1}.

Mass spectrum analyses were performed on a PE-SCIEX API III plus triple-quadrupole (PE-Sciex, Thornhill, Ont., Canada) equipped with an atmospheric pressure ionisation (API) source and an IS interface operating in positive ion mode. Ultra high purity nitrogen was used as the curtain gas (0.6 l min^{-1}) and air as the nebuliser gas (400 kPa). The discharge current was set at 4 μA and the orifice potential voltage (OR) at 40 V for sufentanil and 50 V for fentanyl (IS).

Full scan mass spectra were acquired in positive ion mode in the range m/z 200–500.

For MS–MS experiments, product ion mass spectra were acquired in positive ion mode by colliding the quadrupole 1 (Q1) selected precursor ion, with argon (gas thickness 300×10^{13} molecules cm^{-2}) in quadrupole 2 (Q2) operated in radio frequency (RF)-only mode, scanning the quadrupole 3 (Q3) mass spectrometer from m/z 50 to 400.

MS and MS/MS experiments were conducted with a resolution of 0.8 amu measured at half peak height for both mass resolving quadrupoles. A collision energy of 25 eV was chosen for the collision-induced dissociation (CID) experiments. The protonated molecule $[M+H]^+$, at m/z 387 for sufentanil and 337 for fentanyl, was the precursor ion for CID. Three product ions for sufentanil and two for fentanyl were identified for MRM LC–

MS–MS analyses. Precursor–product ion combinations of m/z 387 → 140, 387 → 238 and 387 → 355 for sufentanil and m/z 337 → 105 and 337 → 188 for fentanyl (IS) were used. The dwell time for each monitored transition was 150 ms. Peak area ratios of analyte to IS were calculated using MACQUAN version 1.3 software from PE-Sciex.

2.5. Calibration and quantification

Blank control plasma samples (1 ml) were spiked with sufentanil and IS (1 ng), resulting in three analytical series each with four sufentanil concentrations (i.e. 0.3, 0.5, 1.0 and 2.0 ng ml^{-1}) and six samples per concentration. The series were analysed on each of 3 different days (total number = 18 per concentration) to evaluate the analytical method's repeatability (within-day), within-laboratory reproducibility (different operators and environmental conditions) and accuracy.

Matrix calibration curves were prepared daily by spiking control blank plasma samples with 1 ng ml^{-1} IS and sufentanil to obtain concentrations of 0.3, 0.5, 0.7, 1.0, 1.5 and 2.0 ng ml^{-1}. Estimates of sufentanil concentration in spiked, newborn and maternal plasma samples were interpolated from these calibration graphs, constructed by plotting peak area ratios of sufentanil to IS against sufentanil concentrations using a least-squares linear regression model.

3. Results and discussion

Positive ion FIA–MS was performed on individual solutions of each analyte using acetonitrile–water (80:20, v/v) containing 0.2% trifluoracetic acid at a flow rate of 50 μl min^{-1}. The protonated molecule $[M+H]^+$ was formed at m/z 387 for sufentanil and 337 for the IS fentanyl. Representative IS full scan mass spectra (mass range m/z 200–500) obtained by FIA in the MS positive ion mode for sufentanil and fentanyl are shown in Fig. 1.

The effect on ion intensity of varying the orifice potential voltage (OR) was investigated for each analyte between 30 and 100 V. ORs of 40 V for sufentanil and 50 V for fentanyl were found to offer the best compromise in terms of signal-to-noise ratio.

The simplicity of the FIA–MS spectrum is useful for identification of analytes by their molecularly related ions, although it does not provide further structural information. Tandem mass was, therefore, used to obtain additional structural information by detecting diagnostic product ions obtained by CID of the precursor ion. The protonated molecule $[M+H]^+$ served as the precursor ion for CID in MS–MS experiments, performed using FIA–MS–MS on standard solutions of each analyte. Fig. 2 shows the positive product ion mass spectra (mass range m/z 50–400) of $[M+H]^+$ of sufentanil and the IS fentanyl. Spectra comparison reveals production of the most abundant product ions at m/z 140, 238 and 355 for sufentanil and m/z 105 and 188 for fentanyl. Transitions of the respective protonated molecules to these product ions were, therefore, selected according to the MRM technique.

To achieve maximum sensitivity and a quantitative analysis MRM LC–MS–MS was performed using a reversed phase Supelcosil LC-C_{18}-DB column at room temperature with an acetonitrile–water (80:20, v/v) mobile phase containing 0.2% trifluoroacetic acid at a flow rate of 50 μl min^{-1}. Under these conditions, good separation of sufentanil (T_r = 7.4 min) and fentanyl (T_r = 6.6 min) was obtained in MRM LC–MS–MS profiles of a standard mixture (data not shown).

Specificity of the MRM LC–MS–MS method was also demonstrated by the preparation and analysis of 20 blank control plasma samples with (Fig. 3a) and without internal standard. No interference was observed around the analyte retention times in plasma samples.

Matrix calibration curves were prepared daily by spiking blank plasma control samples with the IS (1 ng ml^{-1}) and sufentanil to obtain concentrations of 0.3, 0.5, 0.7, 1.0, 1.5 and 2.0 ng ml^{-1}. Linearity was good for sufentanil throughout the tested range, with slope of 0.387 ($\pm$0.005) intercept of 0.055 ($\pm$0.006) and correlation coefficient of (r^2) > 0.9998.

Blank control plasma samples (1 ml) were spiked with sufentanil and IS (1 ng), resulting in three analytical series each with four sufentanil

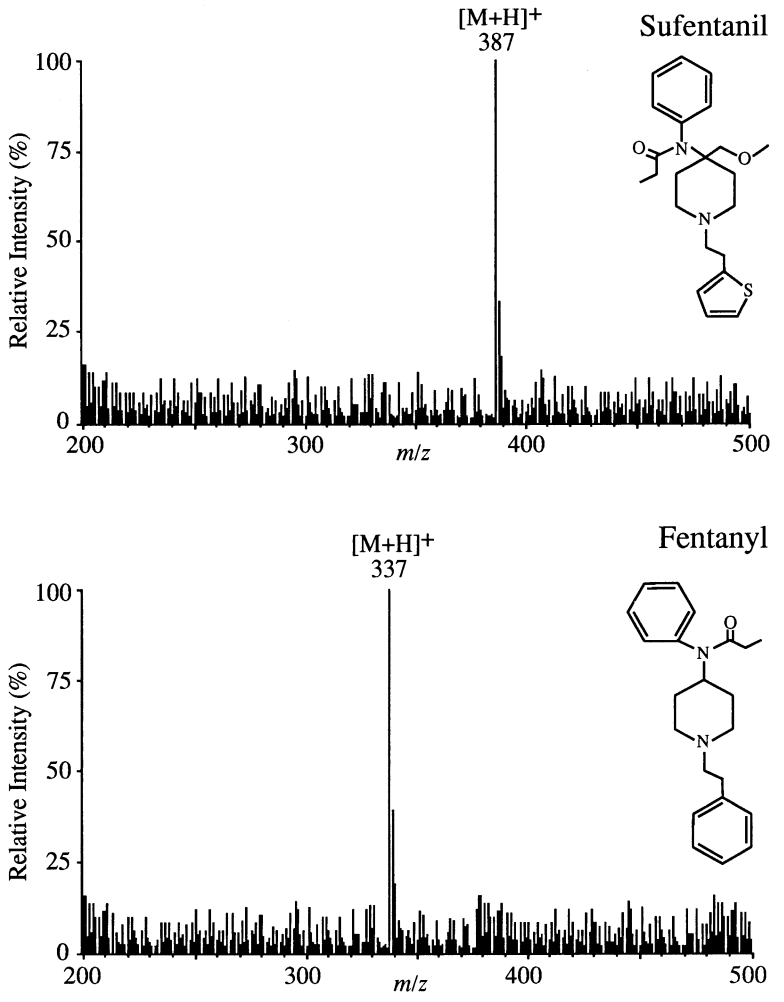

Fig. 1. Positive ion mass spectra of sufentanil and fentanyl. Conditions: FIA; mobile phase: acetonitrile–water (80:20, v/v) containing 0.2% trifluoroacetic acid; flow rate 50 μl min^{-1}; OR was set at 40 V for sufentanil and 50 V for fentanyl (IS).

concentrations (i.e. 0.3, 0.5, 1.0 and 2.0 ng ml^{-1}) and six samples per concentration ($n = 18$). Representative chromatograms of samples spiked with 0.5 ng ml^{-1} sufentanil and 1.0 ng ml^{-1} IS are shown in Fig. 3b. The relative retention time for each analyte was within 0.5% of that for the standard.

The sufentanil extraction efficiency was determined by comparing the peak areas of extracts from spiked serum and blank serum subsequently fortified with sufentanil. In the range 0.3–2.0 ng ml^{-1} extraction efficiency was found to be an average of 98.9%, similar to that achieved by Rossi et al. [25] as both were conducted using a C18 SPE cartridge. This efficiency is considerably higher than was seen by Martens-Lobenhoffer, who used conventional liquid–liquid techniques. The series were analysed on each of 3 different days to evaluate the analytical method's repeatability (within-day), within-laboratory reproducibility (different operators and environmental conditions) and accuracy.

Accuracy (relative error (R.E.), %) was calculated from the agreement between measured and nominal concentrations of the spiked samples. Precision (repeatability and within-laboratory re-

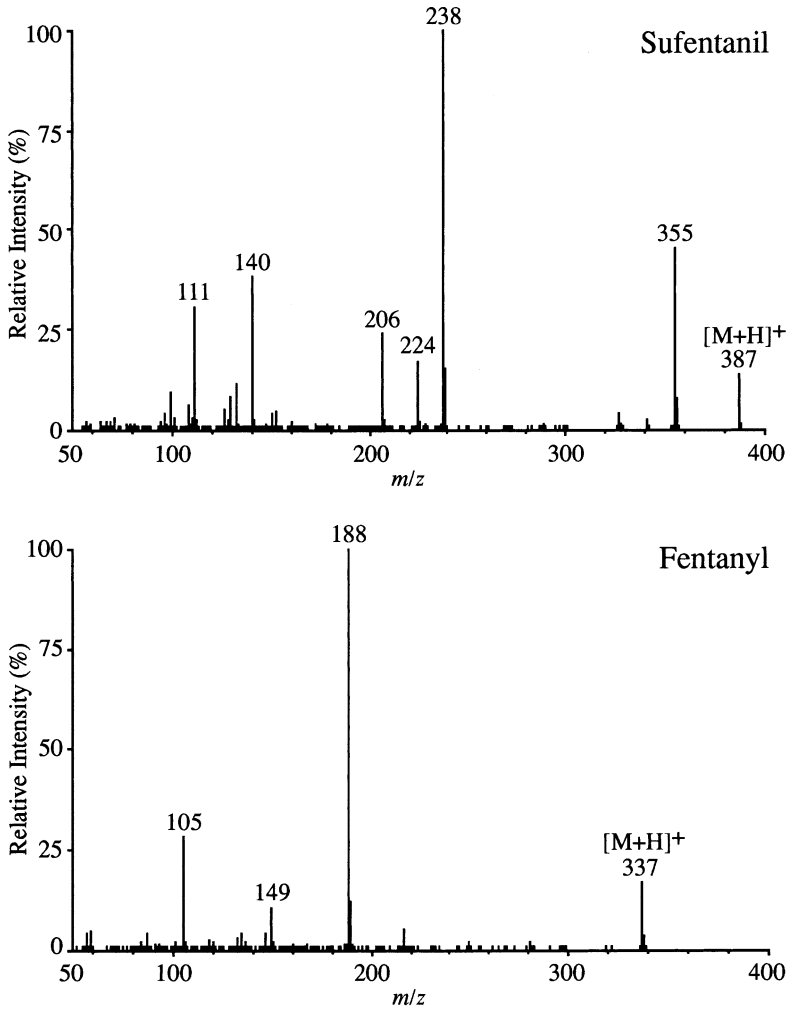

Fig. 2. Positive product ion spectra of sufentanil and fentanyl with the $[M+H]^+$ ion as precursor at m/z 387 for sufentanil and at m/z 337 for fentanyl. Conditions: FIA; mobile phase: acetonitrile–water (80:20, v/v) containing 0.2% trifluoroacetic acid; flow rate 50 μl min^{-1}; OR was set at 40 V for sufentanil and 50 V for fentanyl (IS). Argon was used as the collision gas. CID was carried out with a collision energy of 25 eV.

producibility) was calculated from the relative standard deviations (R.S.D., %) for the repeated measurements. Overall accuracy (R.E., %), repeatability (R.S.D., %) and within-laboratory reproducibility (R.S.D., %) ranged from −9.28 to −2.71%, from 6.42 to 2.82% and from 13.52 to 6.06%, respectively, for sufentanil (Table 1). The limit of quantification in human plasma for sufentanil was 0.3 ng ml^{-1}. These values are thought to be satisfactory, considering the complexity of the biological matrix.

The stability assessment of analytes in matrix under storage conditions was performed by comparing their concentration in the stored samples with their original concentrations in the fresh materials. Their stability under storage conditions

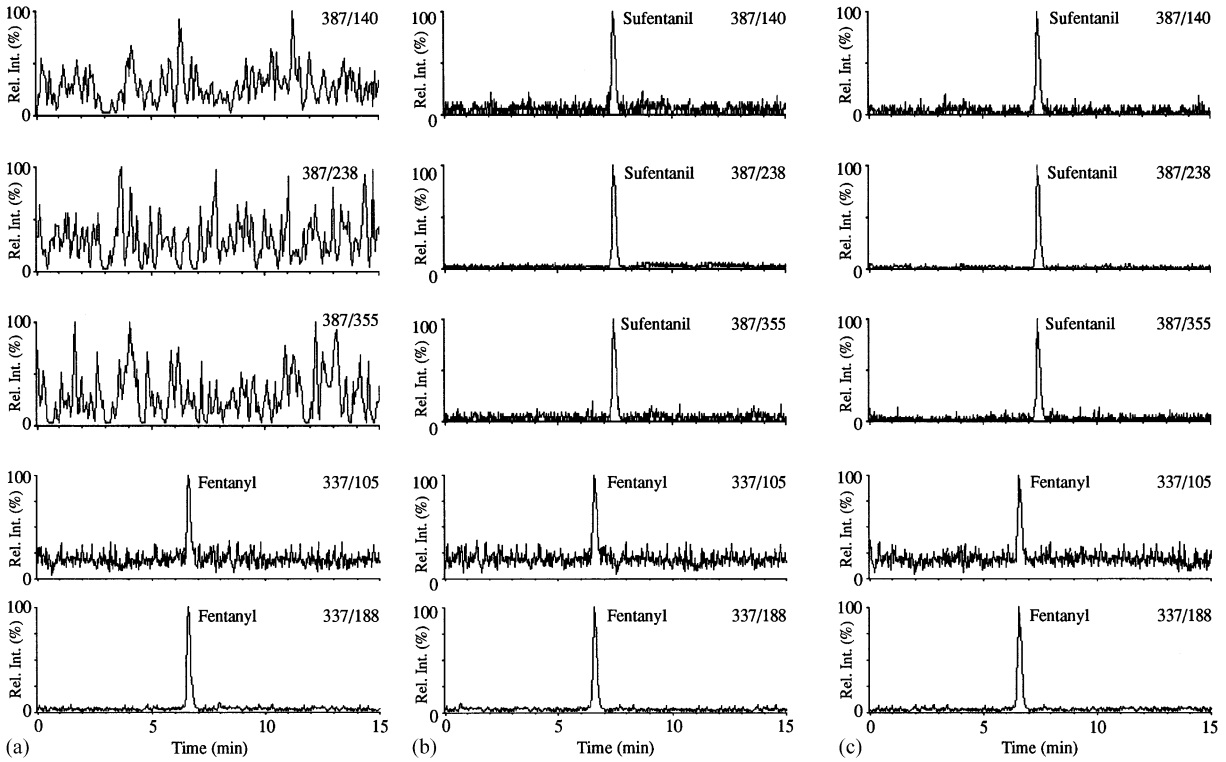

Fig. 3. MRM LC–MS–MS chromatograms of: (a) extract of blank control plasma samples spiked with 1 ng ml^{-1} of fentanyl as IS; (b) extract of blank control plasma samples spiked with 0.5 ng ml^{-1} sufentanil and 1.0 ng ml^{-1} of fentanyl as IS; (c) extract of maternal plasma sample containing 1.2 ng ml^{-1} sufentanil, which was taken 20 min after epidural administration. Precursor–product ion combinations used in MRM detection are shown. Conditions: isocratic HPLC analysis; μ-HPLC column reversed phase (300 × 1 mm I.D., 5 μm) Supelcosil LC-C_{18}-DB; mobile phase: acetonitrile–water (80:20, v/v) containing 0.2% trifluoroacetic acid; flow rate 50 μl min^{-1}; OR was set at 40 V for sufentanil and 50 V for fentanyl (IS). Argon was used as the collision gas. CID was carried out with a collision energy of 25 eV.

was verified for 4 weeks in the dark at −20 °C. No degradation phenomena were observable during identification and/or quantification.

The MRM LC–MS–MS method was finally used to analyse plasma samples taken from women in labour immediately before and 20 min after epidural administration of a single dose of sufentanil and from arterial and venous umbilical cord plasma samples from newborn babies. Fig. 3c shows representative chromatographic profiles of

Table 1
LOQ, repeatability, within-laboratory reproducibility and accuracy for sufentanil in human plasma samples

Analyte	LOQ (ng ml^{-1})	Spike level (ng ml^{-1})	Repeatability (R.S.D.,%) ($n = 6$)	Reproducibility (R.S.D.,%) ($n = 18$)	Accuracy (R.E., %) ($n = 18$)
Sufentanil	0.30	0.30	6.42	13.52	−9.28
		0.50	5.36	10.61	−7.23
		1.0	4.72	7.27	−4.34
		2.0	2.84	6.06	−2.71

a maternal plasma sample containing 1.2 ng ml^{-1} sufentanil, which was taken 20 min after epidural administration.

4. Conclusions

The suitability of MRM LC–MS–MS to identify and quantify sufentanil in human plasma has been successfully demonstrated. Sufentanil has a short therapeutic range and relevant side effects. Research is in progress to adopt the method for the complete pharmacokinetic study of sufentanil in human plasma. Using this assay we were able to quantify the drug with precision and accuracy even when administered at very low doses. The high sensitivity of this method makes it suitable for measurement of sufentanil concentration at the low levels found in women and newborns during and after epidural labour analgesia.

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