Liquid Chromatography-Tandem Mass Spectrometry Method for the Quantification of Fentanyl and its Major Metabolite Norfentanyl in Critically Ill Neonates

Linnerz K1, Wiesen HJM1, Blaich C1, Junghaenel-Welzing S2, Welzing L3, Roth B2 and Müller C1*

1Division of Therapeutic Drug Monitoring, Institute of Pharmacology, University of Cologne, Cologne, Germany
2Department of Pediatrics, Children’s Hospital, University of Cologne, Cologne, Germany
3Children’s Hospital, Department of Neonatology, University of Bonn, Bonn, Germany

Abstract

Fentanyl is a widely used opioid analgesic in the intensive care unit. In critically ill neonates, continuously infused fentanyl is part of the standard treatment regimen for sedation and pain control. Little is known about fentanyl pharmacokinetics in specific clinical indications, as in asphyxiated neonates with therapeutic hypothermia treatment. In this report, we introduce a liquid chromatography-tandem mass spectrometry method (LC-MS/MS-method) to determine concentrations of fentanyl and its major metabolite norfentanyl in critically ill newborns. 100 µl serum samples were precipitated with acetonitrile containing the isotopically labeled internal standard fentanyl-D5. The supernatant was evaporated to dryness and reconstituted with mobile phase. Chromatographic separation was achieved on a C18 column with a gradient flow. LC-MS/MS detection was performed using a triple-stage quadrupole mass spectrometer working in selected reaction monitoring mode with positive electrospray ionization. Linearity was demonstrated over the concentration range of 0.1 to 40 and 39.4 ng/ml for fentanyl and norfentanyl respectively. Inter- and intraday accuracies and precisions were within the acceptance ranges. The lower limit of quantification was 0.02 and 0.09 ng/ml for fentanyl and norfentanyl. The method was successfully applied to newborns with hypoxic-ischemic encephalopathy treated with whole body hypothermia, which allowed accurate determination of fentanyl and norfentanyl concentrations as a basis for pharmacokinetic analysis.

Keywords: LC-MS/MS; Fentanyl; Low volume; Newborn; Hypothermia

Abbreviations: BE: Base Excess; CS: Calibration Standard; ESI: Positive Electrospray Ionization; HIE: Hypoxic-Ischemic Encephalopathy; LC-MS/MS: Liquid Chromatography Tandem Mass Spectrometry; LLOQ: Lower Limit of Quantification; LOD: Limit of Detection; QC: Quality Control; RE: Relative Error; RSD: Relative Standard Deviation; SD: Standard Deviation; SRM: Selected Reaction Monitoring

Introduction

Fentanyl is a synthetic µ-opioid receptor agonist that is 75 to 100-fold more potent than morphine [1]. It is well established in clinical practice as an opioid analgesic providing pain relief with favorable characteristics such as lack of active metabolites, a rapid onset, and a short duration of action [2,3]. Fentanyl is also suitable for application in trauma, cardiac, or intensive care unit patients as it was shown to have beneficial hemodynamic effects [4]. Following intravenous administration, systemic clearance occurs primarily in the liver by the cytochrome P450 isof orm CYP3A4. It is mainly degraded to norfentanyl by N-dealkylation and excreted in the urine [5,6].

Neonatal therapeutic hypothermia is a medical treatment to reduce body temperature to 33.5°C-34.5°C for 72 hr [7]. It has emerged as standard treatment in perinatal hypoxic-ischemic encephalopathy (HIE) which is usually secondary to perinatal asphyxia [8,9]. The neuroprotective effects of mild hypothermia are complex and may not only be explained by a reduction in brain metabolic rate [10]. Asphyxiated neonates should receive a sedative and opioid analgesic to reduce intensive care treatment related distress and pain as these factors can counteract the neuroprotective effects of hypothermia treatment [11].

Little is known about fentanyl blood concentrations and pharmacokinetics in asphyxiated neonates with hypothermia treatment. In clinical practice, fentanyl medication used in newborns are derived from recommended dosages in adults and adjusted to body weight [12]. However, it is well known that pharmacokinetics in newborns differ considerably when compared to adults [13]. A deeper understanding of fentanyl pharmacokinetics in critically ill neonates may guide optimization of dose regimens in this patient group. This study describes the development of a liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) method for determination of fentanyl and its major metabolite norfentanyl in serum of critically ill newborns.

Materials and Methods

Chemicals and reagents

Fentanyl, norfentanyl HCL and a deuterated fentanyl isotope (fentanyl-D5) were obtained from Lipomed (Weil am Rhein, Germany) and stored at ϑ=-18 °C. Solvents used for sample preparation and mobile phase were of analytical or high-performance liquid-chromatography grade. Acetonitrile and formic acid were obtained from Merck.
The mobile phase consisted of solvent A acetonitrile and solvent B 0.1% formic acid. Deionized water was purified by a Milli-Q Plus ultra pure water system (Millipore Corporation, Bedford, MA, USA).

**LC-MS instrumentation and equipment**

Samples were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using a TSQ Vantage triple-stage quadrupole mass spectrometer (ThermoFischer Scientific, San Jose, CA, USA), working in selected reaction monitoring (SRM) mode with positive electrospray ionization (ESI). The system was equipped with an Accela 1250 pump and an Accela autosampler, fitted with a tempered tray and column oven. Mass spectrometry conditions were optimized using the Thermo TSQ Tune Master software (version 2.3). Thermo XCalibur software served for instrument control and data acquisition. The Thermo Scientific processing software LCquan (Version 2.6) was used to integrate the peaks obtained by the Interactive Chemical Information System (ICIS) peak detection and integration algorithm. All chromatograms were reviewed and, if necessary, reintegrated manually.

**Calibration standards and quality control samples**

Primary stock solutions for fentanyl and norfentanyl were prepared in 50% methanol and distilled water (50:50, v/v) and the stock solution of the internal standard fentanyl-D5 in 100% methanol. All stock solutions were stored at -18°C. Blank pooled human serum units were supplied by the Department of Transfusion Medicine of the University Hospital of Cologne. Different blank serum units were used to prepare calibration standards (CS) and quality control (QC) samples. Blank serum samples were spiked with stock solution of fentanyl and norfentanyl to obtain 6 different calibration standards with concentrations ranging from 0.1-40 ng/ml for fentanyl and 0.1-39.4 ng/ml for norfentanyl. Similarly, quality control samples were prepared with concentrations of 0.5, 4.0, and 20 ng/ml for both fentanyl and norfentanyl.

**Sample preparation**

100 µl calibration standard or quality control sample was extracted with 200 µl acetonitrile containing 5 ng/ml internal standard fentanyl-D5. After vortexing, the mixture was centrifuged at 8°C and 15,000 g for 15 min. The clear supernatant was transferred into micro tubes (Sarstedt, Nümbrecht, Germany) and evaporated to dryness in an evaporation concentrator (RC10.22, Thermo Electron, Waltham, MA, USA) at 40°C for 60 min. The samples were reconstituted with 55 µl mobile phase (formic acid 0.1% and acetonitrile, 50:50, v/v). Subsequently, the mixture was vortexed for 25 s and centrifuged at identical conditions. The supernatant was transferred to LC-MS glass vials (Macherey-Nagel, Düren, Germany) and then subjected to LC-MS/MS analysis.

**Liquid chromatography**

The system was equilibrated for a minimum of 45 min until stability of total ion current was reached. Column and tray temperature were set to 25°C and 20°C. A volume of 5 µl of the extracted samples was injected for chromatographic separation, which was achieved by a Hypersil Gold C18 column (50 mm × 2.1 mm × 1.9 µm) from ThermoFisher Scientific (San Jose, CA, USA). Flow rate was 320 µl/min, run time 2.5 min. Different mobile phase compositions were evaluated to increase sensitivity and sharpness of the peaks. For the most suitable solvents A (acetonitrile) and B (formic acid 0.1%) were combined in a gradient: 0-0.3 min: 28% A, 0.3-0.4 min: linear from 28 to 60% A, 0.4-1.7 min: 60% A, 1.7-2 min: return to initial conditions and keep until 2.5 min.

**Mass spectrometry conditions**

Mass spectrometer parameters were: spray voltage 3.5 kV, heated capillary temperature 300°C, and vaporizer temperature 350°C. Nitrogen was used as sheath and auxiliary gas and set to 37 and 10 (arbitrary units). The argon collision gas pressure was set to 1.5 mTorr. Collision energies were set to 22 eV for fentanyl, 16 eV for norfentanyl and 33 eV for fentanyl-D5. The following SRM transitions of precursor ions to product ions were selected: Fentanyl, m/z 337.2→188.2; norfentanyl, m/z 233.2→84.1; fentanyl-D5, m/z 342.3→188.1. The scan time was set to 100 ms for all analytes.

**Method validation**

The validation of the method was based on the International Conference of Harmonization (ICH) guideline Q2 (R1) “Validation of Analytical Procedures” (ICH harmonised Tripartite Guideline, n.d.) [14].

**Linearity, accuracy, precision:** Six calibration standards were analyzed and the ratio of the peak areas for fentanyl and norfentanyl to the internal standard fentanyl-D5 plotted versus the concentration of fentanyl and norfentanyl (x-axis). Standard curves were generated by least-squares linear regression analysis with a weighing factor of 1/x². QC samples contained analytes in low, midrange, and high concentrations (QC1-QC3). Accuracy (bias, %) was calculated as the deviation between measured and the nominal concentration. Precision was expressed as percent relative standard deviation (RSD) evaluated as the standard deviation (SD) of the observed concentration divided by the mean concentration. Intraday variability was determined by repeated analyzing of six individually extracted QC1-QC3 samples at one day (n_{sample}=18). The interday variability was calculated by measuring QC1-QC3 samples on eight different days during a three week period (n_{sample}=24).

**Limit of detection and quantification:** The limits of detection (LOD) and lower limits of quantification (LLOQ) were derived from interday assay data (n_{sample}= 24). These limits were calculated using the equations LOD= (3.3σ)/S’ and LLOQ= (10σ)/S’, where σ is the standard deviation of the blank response and S’ is the slope of the calibration curve.

**Stability testing:** Stability of the analytes was evaluated by freeze-and-thaw and bench stability tests. During three freeze-and-thaw cycles, the QC samples were thawed at room temperature for 1 hr and again frozen at -18°C overnight. Bench stability was tested by thawing QC samples and keeping at room temperature for 24 and 48 hr. All samples subjected to stability testing were compared to freshly prepared QC samples (as described in 2.4). The analytes were considered stable if the relative error [RE (%)]=(measured concentration-reference concentration)/reference concentration × 100 was below ± 15% for different conditions.

**Recorvery:** Three working solutions were prepared in blank serum units resulting in concentrations of 1, 10, and 40 ng/ml for fentanyl and norfentanyl. Samples were processed and analyzed as described in sections 2.4-2.6. Blank serum was precipitated with acetonitrile (1:2), then vortexed for 25 s and centrifuged for 15 min. New working solutions were prepared with the supernatant of the precipitated serum resulting in the same concentrations. These samples were directly submitted to LC-MS/MS analysis. Recoveries were determined as quotients of the areas of extracted samples to reference samples.

---

**Citation:** Linnerz K, Wiesen HJM, Blaich C, Junghaenel-Welzing S, Welzing L et al. (2015) Liquid Chromatography-Tandem Mass Spectrometry Method for the Quantification of Fentanyl and its Major Metabolite Norfentanyl in Critically Ill Neonates. J Chromatograph Separat Techniq S6:004. doi:10.4172/2157-7064.S6-004
Ion suppression: Ion suppression experiments were investigated by means of a continuous post column infusion (flow rate 20 μl/min) of mobile phase containing the analytes with a concentration of 154 ng/ml. The solvent flow rate was 300 μl/min. During the infusion, blank serum samples were subjected to LC-MS/MS analysis using chromatographic conditions as described in section 2.5. The responses of fentanyl and norfentanyl were examined for possible matrix effects.

Interference: Drugs which may frequently be used during HIE treatment were tested for mass spectrometric or analytical interference with fentanyl and norfentanyl, cefotaxime, ampicillin, furosemide, norepinephrine, dobutamine, vecuronium, piritramide, and midazolam. Briefly, aliquots of blank serum were spiked with fentanyl and norfentanyl. Aliquots of these samples were then spiked with aforementioned drugs, ultimately achieving therapeutic concentrations [15-19]. As control, the same volume of distilled water was added to fentanyl and norfentanyl containing serum samples instead of aliquots of concomitant drugs. All samples were processed and analyzed as described in 2.3-2.5 and responses of the SRM transitions for norfentanyl and fentanyl examined.

Clinical application

The method was applied to a male newborn patient (gestational age 40+2 (week+day), birth weight 3.4 kg) with hypoxic ischemic encephalopathy due to meconium aspiration. Apgar scores were 3/4/3. The initial umbilical cord pH and base excess (BE) at birth were 7.1 and -4.5 mmol/l, the lowest pH and base excess determined within the first hour were 6.65 and -25.6 mmol/l, respectively. Initial lactate concentration was 17.8 mmol/l. Blood samples for the determination of fentanyl and norfentanyl concentrations were obtained from an indwelling arterial catheter and collected during the course of hypothermia treatment (0-72 hr) and during the elimination phase after discontinuation of fentanyl administration. The mean body temperature during hypothermia treatment was 34.7°C Approval by the local ethics committee and written informed consent by the children’s parents were obtained prior to sample collection.

Results

Liquid chromatography and mass spectrometry

Representative chromatograms of extracted blank plasma and calibration standards (CS) containing low (0.5 ng/ml) and high concentrations (10 ng/ml) are shown in Figures 1-3. Retention times were R<sub>t</sub>=1.46 min for fentanyl and R<sub>t</sub>=0.56 min for norfentanyl. The deuterated internal standard fentanyl-D5 co-eluted with fentanyl at R<sub>t</sub>=1.45 min. During the validation of the method, retention times and peak shapes did not change. SRM transitions used for quantitation were: fentanyl [M+H]<sup>+</sup>, m/z 337.2→188.2; norfentanyl [M+H]<sup>+</sup>, m/z 233.2→84.; fentanyl-D5 [M+H]<sup>+</sup>, m/z 342.3→188.1.

Linearity, accuracy, precision

Linearity could be shown over a concentration range of 0.1-40 ng/ml for fentanyl and 0.1-39.4 ng/ml for norfentanyl (Table 1). Representative equations of the calibration curves were: fentanyl, y=0.000133+0.014084x (R²=0.9992); and norfentanyl, y=0.000171+0.008817x (R²=0.9897). The intraday precision expressed as RSD was less than 5.5 and 10.3% for fentanyl and norfentanyl, respectively. The intraday accuracies varied from -1.4 to 0.5% for fentanyl and from 2.2 to 3.0% for norfentanyl, respectively (Table 2).
Limit of detection and quantification

The calculated lower limits of quantification (LLOQ) for fentanyl and norfentanyl were 0.02 and 0.09 ng/ml and the calculated limits of detection (LOD) were 0.01 and 0.03 ng/ml respectively (Table 3).

Stability

Short term stability tests showed that fentanyl and norfentanyl were stable in human plasma when compared to control. Bench tests for 24 and 48 hours at ambient temperature showed a percentage deviation of less than 8% for both fentanyl and norfentanyl. After three freeze-and-thaw cycles, percentage deviation from control was less than 15%. Long term stability of fentanyl and norfentanyl were investigated in previous studies and therefore not reexamined [20,21].

Recovery

Extraction recovery after protein precipitation with acetonitrile, evaporation and reconstitution was 44.4% for fentanyl and 82.1% for norfentanyl (Table 3).

Ion suppression and interference

The post-column infusion experiments confirmed potential ion suppression effects for fentanyl, norfentanyl, and fentanyl-D5; however with maximum effect at 0.44 min (Figure 4). Based on the fentanyl and norfentanyl peak responses of extracted concomitant drug containing and control serum samples, no mass-spectrometric interferences were observed except for dobutamine containing samples. Serum samples spiked with dobutamine exhibited 35% lower peak responses for norfentanyl compared to control serum samples. No chromatographic interferences were detected.

Clinical application

The method was successfully applied to serum samples of a newborn with hypoxic ischemic encephalopathy (HIE) with therapeutic hypothermia treatment (Figure 5). Fentanyl was continuously administered at dosages ranging from 2 to 8 µg/kg/h. Liver enzymes (ASAT 49 IU/l, ALAT 19 IU/l) and creatinine (0.79 mg/dl) remained within normal limits during hypothermia treatment. Fentanyl and norfentanyl serum concentrations ranged from 0.41 to 2.77 ng/ml and 0.41 to 0.78 ng/ml. Based on a 2-compartment model, the derived fentanyl terminal elimination half-life t1/2 was 339.6 min (MW/Pharm, version 3.50., MediWare, Groningen, the Netherlands).

Discussion and Conclusions

Previously, various approaches for the determination of fentanyl in human serum have been described including high-performance liquid chromatography (HPLC) [22], LC-MS/MS [1,23,26], gas chromatography-mass spectrometry (GC-MS) methods [27,28], and radio immunoassays [29-31]. Apart from human serum, fentanyl concentrations have been analyzed in different biological matrices including plasma, whole blood, and dried blood spots [32, urine [20], or hair [33,34]. Few articles deal with the analysis of fentanyl in blood samples of neonates [32].

In critically ill neonates, especially those with low birth weight, blood loss due to laboratory testing is the primary cause of anemia [35]. To limit blood draw, tubes with fill-lines or micro blood sampling systems have been developed for laboratory analysis [35,36]. Sample volumes between 500 and 3600 µl have been employed for determination of fentanyl concentrations in different studies [1,2,22-26]. The present work describes the development of a very sensitive LC-MS/MS method for the analysis of fentanyl and norfentanyl in serum of neonates using a sample volume of 100 µl. Using this method, fentanyl and norfentanyl in concentrations of 0.1-40 ng/ml could be determined (Table 1). These concentration ranges are also covered in other studies; however, higher sample volumes were used [2,23-25,38]. The LLOQ for fentanyl was determined at 0.02 ng/ml (Table 3) and far below the LLOQ reported in other studies [1,2,22,25,26,32]. Lennerström et al. reported the same LLOQ, but with a 10 fold higher sample volume (24). In the literature, run times vary from R1=2.5-35 min and mobile phase flow rates are up to 1000 µl/min [1,2,21,23,25,26,32]. Hence, with a run time of R1=2.5 min and a flow rate of 320 µl/min, the new method offers an economic and time advantage. The method is highly sensitive for fentanyl determination, enabling detection of minuscule serum concentrations using low sample volumes. The relatively low recovery of fentanyl did not impact its accurate quantitation. A focus was rather put on the optimization of the norfentanyl recovery to assure precise quantification of low metabolite concentrations (Table 3).

In mass spectrometry, matrix effects are considered to affect quantitative assays by suppressing or enhancing ionization potential [39]. Thus, isotope-labeled internal standards that can compensate for these effects are recognized as gold-standard in quantitative LC-MS/MS. In the present study, fentanyl-D5 was used for quantification of both fentanyl and norfentanyl. Matrix effects potentially affecting norfentanyl quantification were examined by means of an ion suppression experiment (Figure 4), and the influence of co-medication on the ionization potential was assessed. The maximum ion suppression dip occurred prior to the maximum norfentanyl peak;
however, after addition of dobutamine to the matrix, peak responses for norfentanyl were found 35% lower compared to control. In patients with co-administration of dobutamine, it is therefore advisable to employ a separate isotopically labeled norfentanyl molecule as internal standard.

Clinical applicability was shown in a newborn patient with HIE (Figure 5). Fentanyl concentrations were fitted to a 2-compartment model and the derived fentanyl terminal elimination half-life was $t_{1/2} = 339.6$ min. This is in line with previous studies where fentanyl half-lives between 87 and 407 minutes were reported [4,40-42].

In summary, the presented work provides a highly sensitive LC-MS/MS method with minimal sample volume for the analysis of fentanyl and its metabolite norfentanyl. Open circle and triangles indicate fentanyl and norfentanyl concentrations in critically ill neonates suitable for pharmacokinetic studies in these vulnerable patients.

References

1. Coopman V, Cordonnier J, Plen K, Van Varenbergh D (2007) LC-MS/MS analysis of fentanyl and norfentanyl in a fentanyl fatality due to application of multiple Durogesic transdermal therapeutic systems. Forensic Sci Int 169: 223-227.
2. Cooreman S, Deprez C, Martens F, Van Bockxlaer J, Croes K (2010) A comprehensive LC-MS-based quantitative analysis of fentanyl-like drugs in plasma and urine. J Sep Sci 33: 2654-2662.
3. Drewes AM, Jensen RD, Nielsen LM, Dronye J, Christrup LL, et al. (2013) Differences between opioids: pharmacological, experimental, clinical and economical perspectives. Br J Clin Pharmacol 75: 60-78.
4. Yaster M, Deshpande J, Deshpande J (1989) Management of pediatric pain with opioid analgesics. J Pediatr 113: 421-426.
5. Labrou RB, Paine MF, Thumbel KE, Kharasch ED (1997) Fentanyl metabolism by human hepatic and intestinal cytochrome P450 3A4. Implications for interindividual variability in disposition, efficacy, and drug interactions. Drug Metab Dispos 25: 1072-1080.
6. Saari TI, Laine K, Neuvonen M, Neuvonen PJ, Oikkola KT (2008) Effect of voriconazole and fluconazole on the pharmacokinetics of intravenous fentanyl. Eur J Clin Pharmacol 64: 25-30.
7. Perlman JM, Wylie J, Kattwinkel J, Atkins DL, Chameides L et al. (2006) Intervention strategies for neonatal hypoxic-ischemic cerebral injury. Clin Ther 9: 1353-1365.
8. Azzopardi D, Strohm B, Edwards AD, Dyet L, Halliday HL, et al. (2009) Moderate hypothermia to treat perinatal asphyxial encephalopathy. N Engl J Med 361: 1349-1358.
9. Zhou W, Cheng G, Shao X, Liu X, Shan R, et al. (2010) Selective Head Cooling with Mild Systemic Hypothermia after Neonatal Hypoxic-Ischemic Encephalopathy: A Multicenter Randomized Controlled Trial in China. J Pediatr 3: 367-372.
10. Sandestg A, Rommer B, Grände PO (2014) Therapeutic Hypothermia in Children and Adults with Severe Traumatic Brain Injury. Ther Hypothermia Temp Manag 4: 10-20.
11. Welzint L, Junghaenel S, Weiss V, Roth B, Mueller C, et al. (2013) Disposition of midazolam in asphyxiated neonates receiving therapeutic hypothermia—a pilot study. Klin Padiatr 225: 398-404.
12. Encinas E, Calvo R, Lukas JC, Vozzediano V, Rodriguez M, et al. (2013) A predictive pharmacokinetic/pharmacodynamic model of fentanyl for analgesia/seizures in neonates based on a semi-physiologic approach. Paediatr Drugs 15: 247-257.
13. Keams GL, Abdel-Rahman SM, Anderer SW, Blowey DL, Leeder JS, et al. (2003) Developmental pharmacology—drug disposition, action, and therapy in infants and children. N Engl J Med 349: 1157-1167.
14. ICH harmonised Tripartite Guideline (2005) Validation of Analytical Procedures: Text and Methodology Q2 (R1).
15. Ahsman MJ, Wildschut ED, Tibboel D, Mathot RA (2010) Pharmacokinetics of cefotaxime and desacetylcefotaxime in infants during extracorporeal membrane oxygenation. Antimicrob Agents Chemother 54: 1734-1741.
16. Müller C, Kremer W, Hartlinger S, Doroshyenko O, Jetter A, et al. (2006) Pharmacokinetics of pilramide in newborns, infants and young children in intensive care units. Eur J Pediatr 165: 229-239.
17. Nahata MC, Vashi VI, Swanson RN, Messig MA, Chung M (1999) Pharmacokinetics of ampicillin and sulbactam in pediatric patients. Antimicrob Agents Chemother 43: 1225-1229.
18. Reich DL, Hollinger I, Harrington DJ, Seiden HS, Chakravorti S, et al. (2004) Comparison of citalopram and venlafaxine by influence in neonates and small infants after congenital heart surgery. Anesthesiology 101: 1122-1127.
19. van der Vorst MJ, Hartigh J, Wildschut E, Tibboel D, Burggraaf J (2007) An exploratory study with an adaptive continuous intravenous furosemide regimen in neonates treated with extracorporeal membrane oxygenation. Crit Care 5: R111.
20. Huyyn NH, Tyrellas N, Ekman L, Johansson M (2005) Determination of fentanyl in human plasma and fentanyl and norfentanyl in human urine using LC-MS/MS. J Pharm Biomed Anal 37: 1095-1100.
21. Shou WZ, Jiang X, Beato BD, Naidong W (2001) A highly automated 96-well solid phase extraction and liquid chromatography/tandem mass spectrometry method for the determination of fentanyl in human plasma. Rapid Commun Mass Spectrom 15: 466-476.
22. Ebrahimzadeh H, Yamin Y, Ghoozadeh A, Sedighi A, Kasraee S (2008) Determination of fentanyl in biological and water samples using single-drop liquid-liquid-liquid microextraction coupled with high-performance liquid chromatography. Anal Chim Acta 626: 193-199.
23. Gregov M, Nokua P, Vuori E, Oikkola KT (2009) Simultaneous screening and quantification of 25 opioid drugs in post-mortem blood and urine by liquid chromatography-tandem mass spectrometry. Forensic Sci Int 186: 36-43.
24. Lennernäs B, Hedner T, Holmberg M, Bredenberg S, Nyström C, et al. (2005)
Pharmacokinetics and tolerability of different doses of fentanyl following sublingual administration of a rapidly dissolving tablet to cancer patients: a new approach to treatment of incident pain. Br J Clin Pharmacol 59: 249-253.

25. Musshoff F, Trafkowski J, Kuepper U, Madea B (2006) An automated and fully validated LC-MS/MS procedure for the simultaneous determination of 11 opioids used in palliative care, with 5 of their metabolites. J Mass Spectrom 41: 633-640.

26. Verplaetse R, Tytgat J (2010) Development and validation of a sensitive ultra performance liquid chromatography tandem mass spectrometry method for the analysis of fentanyl and its major metabolite norfentanyl in urine and whole blood in forensic context. J Chromatogr B Analyt Technol Biomed Life Sci 878: 1987-1996.

27. Bagheri H, Es-haghi A, Khalilian F, Rouini MR (2007) Determination of fentanyl in human plasma by head-space solid-phase microextraction and gas chromatography-mass spectrometry. J Pharm Biomed Anal 43: 1763-1768.

28. Portenoy RK, Southam MA, Gupta SK, Lapin J, Layman M, et al. (1993) Transdermal fentanyl for cancer pain. Repeated dose pharmacokinetics. Anesthesiology 78: 36-43.

29. Campistron G, Giroux M, Dumas JC, Hoff M, Desprats R, et al. (1988) Fentanyl RIA improved by a single-step extraction. Clin Chem 34: 2157.

30. Schütter J, White PF (1984) Optimization of the radioimmunoassays for measuring fentanyl and alfentanil in human serum. Anesthesiology 61: 315-320.

31. Singleton MA, Rosen Ji, Fisher DM (1987) Plasma concentrations of fentanyl in infants, children and adults. Can J Anaesth 34: 152-155.

32. Clavijo CF, Thomas JJ, Cromie M, Schniedewind B, Hoffman KL, et al. (2011) A low blood volume LC-MS/MS assay for the quantification of fentanyl and its major metabolites norfentanyl and despropionyl fentanyl in children. J Sep Sci 34: 3569-3577.

33. Lendoire E, Quintela O, de Castro A, Cruz A, López-Rivadulla M, et al. (2012) Target screening and confirmation of 35 licit and illicit drugs and metabolites in hair by LC-MS MS. Forensic Sci Int 217: 207-215.

34. Musshoff F, Lachenmeier K, Trafkowski J, Madea B, Nauck F, et al. (2007) Determination of opioid analgesics in hair samples using liquid chromatography/tandem mass spectrometry and application to patients under palliative care. Ther Drug Monit 29: 655-661.

35. Lin JC, Strauss RG, Kulhavy JC, Johnson KJ, Zimmerman MB, et al. (2000) Phlebotomy overdraft in the neonatal intensive care nursery. Pediatrics 106: E19.

36. Jung W, Ahn CH (2013) A micro blood sampling system for catheterized neonates and pediatrics in intensive care unit. Biomed Microdevices 15: 241-253.

37. Day J, Slawson M, Lugo RA, Wilkins D (2003) Analysis of fentanyl and norfentanyl in human plasma by liquid chromatography-tandem mass spectrometry using electrospray ionization. J Anal Toxicol 27: 513-516.

38. Ghassabian S, Moosavi SM, Valero YG, Shekar K, Fraser JF, et al. (2012) High-throughput assay for simultaneous quantification of the plasma concentrations of morphine, fentanyl, midazolam and their major metabolites using automated SPE coupled to LC-MS/MS. J Chromatogr B Analyt Technol Biomed Life Sci 903: 126-133.

39. Srinivas NR (2009) Dodging matrix effects in liquid chromatography tandem mass spectrometric assays—compilation of key learnings and perspectives. Biomed Chromatogr 23: 451-454.

40. Johnson KL, Erickson JP, Holley FO, Scott JC (1984) Fentanyl Pharmacokinetics In The Pediatric Population. Anesthesiology 61: A441-A441.

41. Koehntop DE, Rodman JH, Brundage DM, Hegland MG, Buckley JJ (1986) Pharmacokinetics of fentanyl in neonates. Anesth Analg 65: 227-232.

42. Poklis A (1995) Fentanyl: a review for clinical and analytical toxicologists. J Toxicol Clin Toxicol 33: 439-447.