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Rapid UPLC–MS/MS method for the determination of sufentanil in human plasma and its application in target-controlled infusion system

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ABSTRACT

A rapid, selective and highly sensitive ultra-performance liquid-chromatography mass-spectrometry (UPLC–MS/MS) method has been developed and validated for the determination of sufentanil in human plasma. Sufentanil was separated on an ACQUITY^{TM} UPLC BEH C_{18} column (50 mm × 2.1 mm, ID 1.7 μm) and analyzed in positive-ion (PI) electrospray-ionization (ESI) mode. The mobile phase (MP) consisted of acetonitrile:water (45:55, v/v) under isocratic conditions at a flow rate of 0.2 ml/min. Sufentanil and internal-standard (IS) fentanyl were eluted at 1.47 and 1.16 min, respectively, and their responses were optimized at the transitions *m/z* 387.2 > 238.0 and *m/z* 337.2 > 188.0, respectively. The calibration curve was linear over the range 0.071–4.56 ng/ml, with coefficients of determination >0.999. The accuracy and precision of the method were between 96.49% and 100.37% (RSD < 9%), and the mean recovery of sufentanil was 84.08 ± 7.29%. The method was successfully applied to evaluate the predictive accuracy of Gepts pharmacokinetic sets in a target-controlled infusion (TCI) model, and the Gepts parameters were capable of predicting sufentanil plasma concentrations when multi-level target concentrations were acquired during surgery.

1. Introduction

Target-controlled infusion (TCI) has been investigated for its ability to achieve desired plasma or effect-site drug concentrations using a computer-controlled infusion pump driven by the published pharmacokinetics of the drug [1]. The majority of TCI studies focus on the predictive accuracy of a pharmacokinetic model. The statistical analysis involved in the accuracy evaluation is usually performed on the pre-determination of plasma concentrations of infused drugs [2]. Sufentanil is a high-efficacy opioid analgesic frequently used in clinical anesthesia and analgesia [3]. According to Derrode et al. [4], sufentanil TCI provides stable analgesia, better hemodynamic control than a bolus injection of intravenous anesthetics, anticipated recovery and improved quality of anesthesia during the perioperative period. In one of our studies, we tried to evaluate the predictive accuracy and feasibility of the sufentanil TCI system during selective surgery. However, accurate measurement of the concentrations of this narcotic drug is the key to successful evaluation of the sufentanil TCI system. Thus, there is considerable demand for a specific, sensitive and rapid method for quantitative determination of sufentanil in human plasma involving in-clinical studies for pain management and surgical applications.

Quantification of sufentanil was achieved using several methods based on HPLC [5–7], coupled with mass spectrometric methods, such as liquid chromatography–tandem mass spectrometry (LC–MS/MS) [8–10], gas chromatography–mass spectrometry (GC–MS) and radioimmunoassay (RIA) [11–13].

The quantification of this opioid drug in biological fluids is associated with numerous analytical difficulties. Historically, researchers have been successful in developing novel radioimmunoassays. This technique is now barely used because routine immunological procedures tend to suffer from cross-interference [14]. During the last decade, instrumental analyses, including both gas and liquid chromatographic separations with diverse detection systems, have been developed and validated. GC/MS analysis, which can measure concentrations at picogram levels, is well developed for the determination of opioid narcotics in human urine and hair [11,12]. Chromatographic assays using mass-selective detection procedures are specific and sensitive with limits of quantification from 0.1 to 0.3 ng/ml [6–8,10].

Recently, gradient elution has been applied to the chromatographic separation of analytes in LC–MS/MS methods for determination of sufentanil in human plasma. Martens-Lobenhoffer [15] and Schmidt et al. [9] successfully achieved the limits of quantification of 10 pg/ml and 0.25 pg/ml, respectively. However, the linear-gradient procedure results in a total run time of 7 min for each sample, which is too long for numerous clinical cases in our study.

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Based on the published literature, a method for the rapid and sensitive determination of sufentanil in human plasma is not yet well developed. In this context, the aims of this work are (1) to build a UPLC–MS/MS method to determine the plasma levels of sufentanil in volunteer patients undergoing abdominal surgery and (2) to verify whether those levels can justify the accuracy of the TCI system based on pharmaceutical parameters of sufentanil. The bioanalytical method features fentanyl as an internal standard, a small injection volume (10 μl), minimal sample preparation and very fast chromatography using the UPLC system. The detection and quantification are carried out by tandem mass spectrometry, leading to specific and reliable results.

2. Experimental

2.1. Chemicals and reagents

Sufentanil and fentanyl were obtained from YiChang Humanwell Pharmaceutical Company (Hubei, China) as pure substances in the form of citrate salts, and fentanyl citrate was used as the internal standard (IS). Acetonitrile and methanol were obtained from Merck KGaA (Darmstadt, Germany). Ammonium hydroxide was obtained from Guangzhou Chemical Reagent Factory (Guangzhou, China). All solvents were of analytical grade. Ultra-pure water was produced by a Milli Q (Milli PAKTM 4.0, Milli PORE) system. Agela Cleanert PEP-SPE (30 mg, 1 ml, Agela Technologies, China) was used in sample preparation. Eppendorf tubes (1 ml, and 5 ml) were used to stock solution during the preparation of the standard solution and plasma samples.

2.2. Apparatus and operation conditions

2.2.1. Liquid chromatography

Analyses were performed on a Waters ACQUITY^{TM} UPLC system (Waters Corp., Milford, MA, USA). Chromatographic separations were achieved on an ACQUITY UPLC BEH C_{18} analytical column (50 mm × 2.1 mm, ID 1.7 μm) based on bridged ethyl hybrid (BEH) particles. The mobile phase consisted of acetonitrile:water (45:55, v/v) at a flow rate of 0.2 ml/min. The injection volume was 10 μl in partial-loop mode.

2.2.2. Mass spectrometry

Double-tandem-mass-spectrometric detection was carried out on a Waters Micromass® Quattro Premier XE ES mass spectrometer (Waters Corp., Milford, MA, USA) with an electrospray-ionization (ESI) interface. The ESI source was used in positive ionization mode. Quantification was performed using multiple-reaction monitoring (MRM) of the transitions of m/z 387.2 → 238.0 for sufentanil and m/z 337.20 → 188.0 for IS, with scan time of 3.0 min per transition. The optimal MS parameters are as follows: capillary, 3.00 kV; cone, 30 V; extractor, 3.00 V; source temperature, 105 °C; desolvation temperature, 350 °C; cone gas flow, 50 l/h; desolvation gas flow, 500 l/h; LM 1 resolution, 15; HM 1 resolution, 15; ion energy, 1 and 0.5; entrance, −2; collision, 20; exit, 1.0; LM 2 resolution, 15; HM 2 resolution, 15; ion energy, 2 and 0.5; and multiplier, 650 V. Nitrogen was used as the desolvation and cone gas. Argon was used as the collision gas at a pressure of approximately $6.13\,e^{-3}$ mbar and a collision gas flow of 0.2 ml/min. All data collected in centroid mode were acquired and processed using MassLynx^{TM} NT 4.1 software with the QuanLynx^{TM} program (Waters Corp.).

2.3. Preparation of standard solutions

A standard solution of sufentanil was first prepared with purified water at a concentration of 4.56 ng/ml. Next, the solution was added to blank plasma, giving concentrations of 0.071, 0.142, 0.235, 0.570, 1.140, 2.280 and 4.560 ng/ml, and these new solutions were used as a serial concentration calibration standard for sufentanil. Internal standard (IS) samples were prepared with distilled water at a concentration of 2.5 ng/ml, and all solutions were stored at −4 °C after preparation. The chemical structures of sufentanil and fentanyl are shown in Fig. 1.

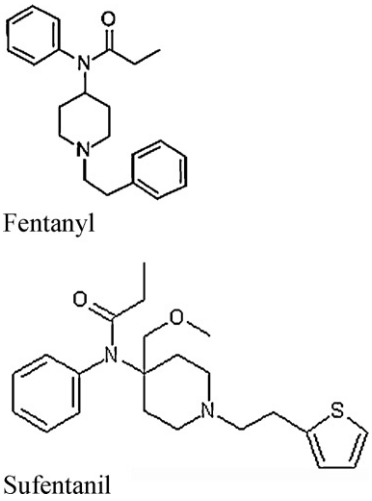

Fig. 1. Chemical structures of sufentanil and fentanyl (IS).

2.4. Plasma sample preparation

Solid-phase extraction (SPE) was used in sample preparation. A plasma aliquot of 880 μl of plasma combined with 20 μl of fentanyl (2.5 ng/ml) as an internal standard and 100 μl of 1% ammonium hydroxide was deproteinized by SPE. Having been pre-conditioned with 1 ml of pure methanol and 1 ml of purified water, each SPE cartridge was filled with a single prepared plasma sample and was then washed with 1 ml of 1% methanol (v/v) and 1 ml pure methanol. The sample was evaporated to dryness under vacuum condition for 24 h. The residue was carefully dissolved with 1 ml of the mobile-phase solution. After intense shaking for 1 min and phase separation by centrifugation for 15 min at 3000 rpm, 10 μl of centrifuged supernatant was injected into the UPLC system.

2.5. Method validation

The method was evaluated for specificity, linearity, precision and accuracy, extraction recovery, matrix effects and stability using the FDA guidelines for the validation of bioanalytical methods [16].

2.5.1. Specificity

The specificity was evaluated by comparing the sufentanil chromatograms of blank plasma samples with the corresponding standard plasma samples spiked with fentanyl (IS).

2.5.2. Linearity

Calibration curves were constructed by assaying standard plasma samples at seven concentrations of sufentanil ranging from 0.071 to 4.56 ng/ml using a weighted ($1/x$) least-squares linear regression.

2.5.3. Precision and accuracy

The intra-day accuracy was assessed by conducting a replicate analysis of quality-control (QC) sufentanil samples on the same day. The run consisted of a calibration curve and five replicates of low-, mid-, and high-concentration QC samples. To determine the inter-day precision, an analysis of three batches of QC samples was

performed on different days. The precision and the accuracy were expressed as a relative standard deviation (RSD).

2.5.4. Extraction recovery and matrix effect

According to the guidance of the FDA [16], the extraction efficiency of sufentanil was determined by analyzing five replicates of plasma samples at three QC concentrations: 0.071, 0.57 and 4.56 ng/ml. The extraction recovery was calculated by comparing the peak areas obtained from extracted spiked samples with those of the unextracted spiked samples at corresponding concentrations. The matrix effect was measured by comparing the peak response of sample spiked post-extraction with that of pure standard solution dried directly and reconstituted with the same mobile phase. The extraction recovery and matrix effects of the IS were also evaluated using the same procedure.

2.5.5. Stability

The stability of sufentanil in human plasma was assessed by analyzing replicates ($n = 5$) of low-, mid- and high-QC samples during the sample storage and processing procedure. QC samples were stored at −20 °C for 30 days and at ambient temperature (25 °C) for 4 h, to determine long-term and short-term stability, respectively. The freeze/thaw stability was determined after five freeze/thaw cycles. The post-preparation stability was estimated by analyzing the QC samples at 0 and 4 h in the autosampler at 4 °C.

2.6. Pharmacokinetic study

The pharmacokinetic study was approved by the local ethics committee. Informed consent was obtained from 17 non-obese adult patients aged 20–59 yr. All patients underwent abdominal surgery for extended gynecologic or gastrointestinal tumors or multiple lymph-node resections. Patients received sufentanil TCI from the beginning of anesthesia using the pharmacokinetic sets reported by Gepts et al. [17]. They also received other non-opioid anesthetics necessary for surgical procedures. For stable hemodynamic conditions throughout the entire surgery, target effect-site concentrations varied in multi-level (0.06–0.8 ng/ml) before some main steps of the surgery. Thus, eight surgical steps were identified as the main steps of the surgery: awake, loss of consciousness, no response to laryngoscope, tracheal intubation, scalpel incision, peritoneal traction, skin closure and spontaneous breathing. Arterial blood samples were drawn 5 min after the predicted blood concentrations reached the target effect-site concentrations. These samples were collected in separate sodium-heparin-containing tubes and centrifuged at 3 °C with a rotation speed of 3000 rpm for 15 min. The supernatant was transferred into a 1.0 ml Eppendorf tube and preserved at −80 °C. After thawing, plasma samples were similarly centrifuged as mentioned above before further sample preparation.

3. Results and discussion

3.1. Sample preparation

Using solid-phase extraction (SPE) on samples of human plasma promised high recoveries [7,9]. In our study, we found that SPE gave good results for the separation of low amounts of sufentanil from human plasma. Vacuum evaporation after the SPE procedure limited the loss of analytes before the chromatography run [18].

3.2. Chromatography and mass spectrometry

UPLC–MS/MS is used to provide quantitative and qualitative information about drug and metabolite concentrations in bioanalysis. In general, this chromatographic method features a fast and

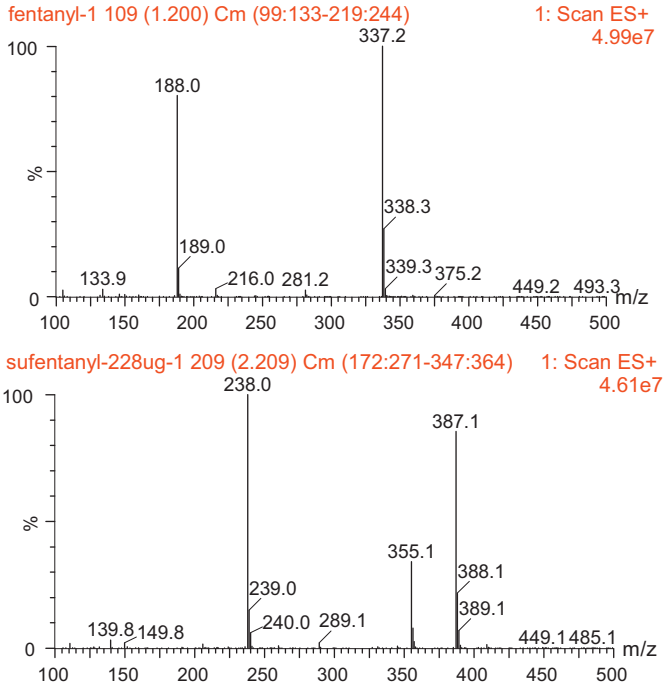

Fig. 2. Positive product ion mass spectra of sufentanil and IS with the $[M+H]^+$ ion as precursor at m/z 387 for sufentanil and at m/z 337 for fentanyl.

efficient separation and is recommended as a sensitive method for biochemical microanalysis. Churchwell et al. [19] explored the differences in LC–MS performance by comparing the analytical performances of HPLC-based mass-spectrometric methods and UPLC. Their results suggest that the narrow peaks, sensitivity, speed, and resolution of UPLC are better than those of HPLC. Pedraglio et al. [20] reported that UPLC analysis allowed bioanalytical quantitation for 24 h after administration, which is not possible using HPLC. This difference significantly affects the evaluation of pharmacokinetic parameters and oral bioavailability. In the present study, UPLC analysis produces lower quantitation of sufentanil in human plasma than previous studies [6–8,10]. However, this study has important limitations. The principal limitation is that the study applied isocratic elution for chromatographic separation, which is less sensitive than gradient elution [9,15]. The reason for this behavior might be the limitations of the mobile phase in chromatographic systems, which are set by the requirements of the ESI source of the mass spectrometer. Martens-Lobenhoffer [15] solved this limitation by extending the overall cycle time of the chromatographic separation and changing the flow rates of the mobile phase. This technique needs more than 7 min for a single run. With appropriate adjustments, we hope to be able to apply gradient elution to UPLC and spend less time on chromatographic separation in future studies.

The MS/MS detection gave exact selectivity for resemblance materials based on differences in their molecular masses. In this assay, the signals obtained in positive ionization mode with ESI for both sufentanil and IS were much greater than those in negative ionization mode. Analytical conditions such as desolvation temperature, ESI-source temperature, capillary and cone voltage, flow rate of desolvation gas and cone gas were tested to obtain a high intensity of protonated molecules, which was $[M+H]^+$ m/z 387 and 337 for sufentanil and IS, respectively. The product-ion scan spectra (Fig. 2) show high abundances of fragment ions. The collision gas pressure and collision energy of the collision-induced decomposition (CID) were optimized for the maximum response of substance fragmentation. According to Martens-Lobenhoffer [15], m/z 238

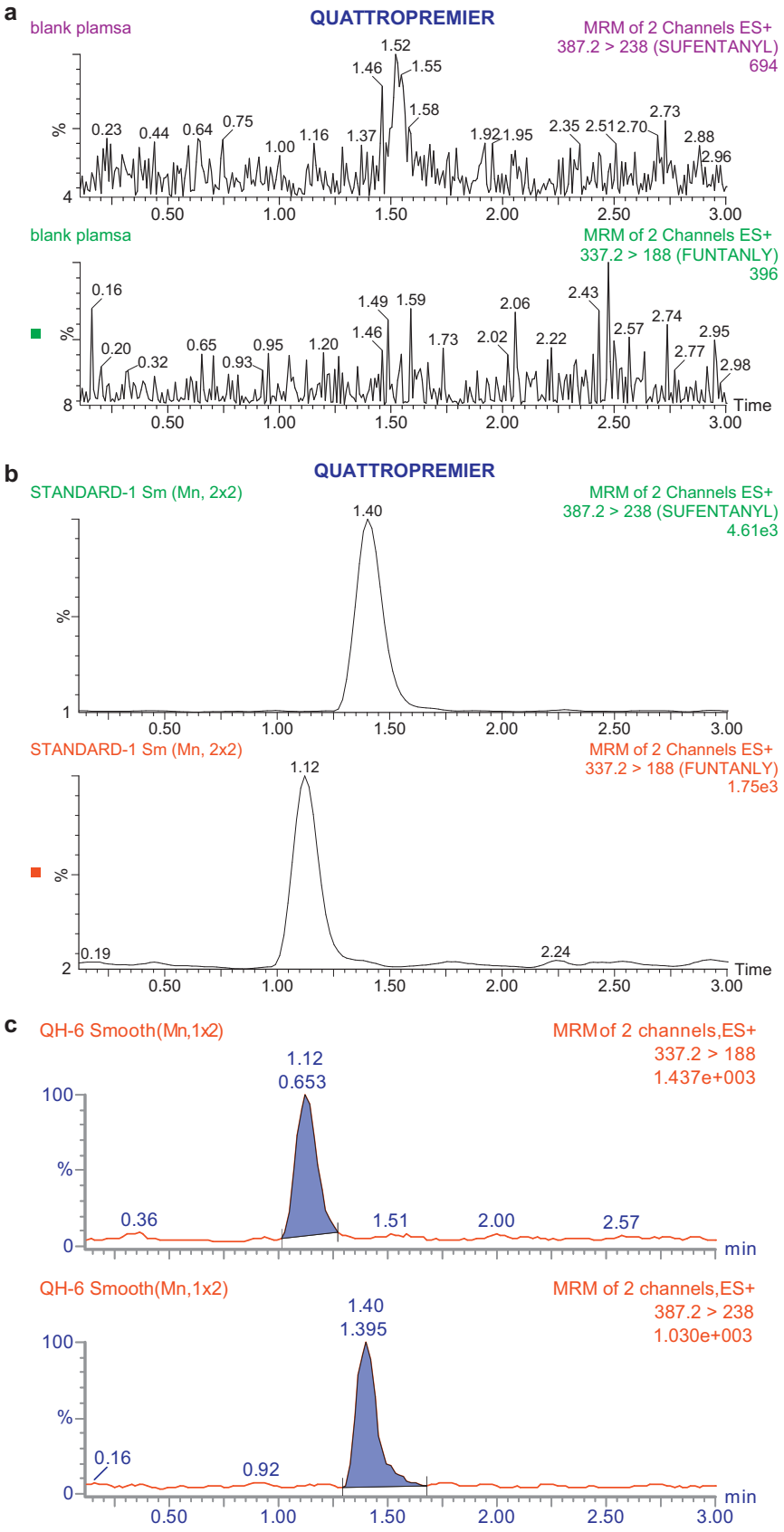

Fig. 3. MRM UPLC–MS/MS chromatograms of: (a) a blank plasma sample; (b) a blank plasma sample spiked with sufentanil and IS; and (c) a patient's plasma sample containing sufentanil with a measured concentration of 1.395 ng/ml. The retention times for sufentanil and IS were 1.40 and 1.12 min.

Table 1
Accuracy studies of sufentanil samples.

Sufentanil samples	Actual conc. (ng/ml)	Calculated conc. (ng/ml)	Accuracy (%)	Intra-day RSD (%)
Low	0.071	0.071 ± 0.006	100.14	8.21
Medium	0.570	0.550 ± 0.039	96.49	7.08
High	4.560	4.577 ± 0.146	100.37	3.19

and 188 were the most prominent product ions in the decomposition of these two analytes. Thus, we used m/z 387 → 238 and m/z 337 → 188 as the precursor → product-ion transition for MRMs of sufentanil and IS, respectively. No cross-talk was observed between the MRMs of the analytes. Compared with the LC–MS method (SIM), the LC–MS/MS method (MRM) provides higher selectivity.

3.3. Method validation

Method validation was performed according to FDA recommendations. The assay was evaluated to assess the following characteristics: specificity, linearity, precision and accuracy, extraction recovery and stability and pharmacokinetic applications.

3.3.1. Specificity

Specificity was evaluated by comparing the chromatograms of sufentanil of blank plasma with the corresponding standard plasma sample spiked with fentanyl (IS). As shown in Fig. 3, no interference from endogenous substances or other metabolites was observed in the retention time of sufentanil.

3.3.2. Linearity

The calibration curve of sufentanil (peak area ratios of analytes to IS *versus* the concentration of analytes in human plasma) was linear over the concentration range of 0.07–4.56 ng/ml. A typical equation for the calibration curve is $y = 65.33 \times 10^{-2} x + 88.46 \times 10^{-3}$, $r^2 > 0.999$.

3.3.3. Precision and accuracy

The data of the intra-day accuracy and the inter-day precision for sufentanil from QC samples are shown in Tables 1 and 2, respectively. The precision and accuracy of the present method conform to the criteria for the analysis of biological samples according to the guidance of the FDA, where the RSD determined at each concentration level is required to not exceed 15% [16].

3.3.4. Extraction recovery and matrix effect

The extraction recoveries of sufentanil from human plasma were 88.53 ± 11.90%, 76.64 ± 7.24% and 84.06 ± 10.94% at concentration levels of 0.071, 0.57 and 4.56 ng/ml, respectively, and the mean extraction recovery of IS was 83.08 ± 9.40%. The consistency of the recovery of sufentanil and IS supports the application of this procedure to routine analysis.

Matrix effects result from the co-elution of some components present in biological samples. These co-eluted components could dramatically decrease or increase the analyte response and consequently affect the sensitivity, accuracy and precision of the analytical method. Thus, the evaluation of the matrix effect of the co-eluting components is necessary for a UPLC–MS/MS method. In this study, the matrix effects of sufentanil and the IS were between 85% and 115%, indicating that no co-eluting substance influenced the ionization of the analytes and IS.

Table 3
Stability of sufentanil in human plasma at three QC ($n = 5$).

Stability	Accuracy		
	Low	Medium	High
	Mean (RSD %)	Mean (RSD %)	Mean (RSD %)
Post-preparation stability	99.15 (7.22)	98.57 (6.87)	100.67 (3.34)
Short-term stability	102.23 (8.66)	100.70 (6.83)	100.19 (3.75)
Long-term stability	99.57 (9.21)	95.58 (5.58)	95.07 (2.80)
Freeze–thaw stability	105.41 (8.87)	92.39 (7.20)	100.34 (3.99)

3.3.5. Stability

The stock solutions of sufentanil in plasma were found to be stable at room temperature for 4 h, at 4 °C for 4 h, at −20 °C for 30 days, demonstrating freeze and thaw stability (Table 3). The IS stock solutions were stable for at least 1 month, after which there was a less-than-5% difference in the measured concentrations of the stored and freshly prepared solutions. The results from all stability tests presented in Table 3 demonstrate the good stability of sufentanil over all determination steps.

3.4. Pharmacokinetic application

A modified evaluation analysis for the predictive accuracy of a pharmacokinetic model was reported as follows [21] (formulae are available in Appendix A). The prediction error (PE) is an indicator of the bias of the concentration achieved. The median prediction error (MDPE) and median absolute prediction error (MDAPE) are reflections of the bias of the TCI model. Finally, the wobble is a measurement of the intrasubject variability of PE, and the divergence is an indicator of the TCI inaccuracy during infusion and for 24 h after the infusion stops.

Predicted concentrations (C_p) of sufentanil were significantly correlated with measured concentrations (C_m) ($r^2 > 0.55$, $p < 0.05$) (Fig. 4). However, a slope of 0.5467 was found in this curve, which suggests that the measured concentrations might be twice as much as the predictive concentrations. To investigate this question from another aspect, we drew a figure of C_m/C_p *versus* surgical steps (Fig. 5). Many curves from Fig. 5 were observed above the base line, indicating that a certain group of sufentanil concentrations were slightly underpredicted during TCI. Considering a similar situation reported by Slepchenko et al. [22] and Pandin et al. [1], we confirmed that the Gepts parameters of the sufentanil TCI model were not systematically underpredicted in clinical practice.

Although several models have been proposed and validated for their ability to predict sufentanil concentrations in human plasma [1,23,24], few of them were performed when volunteers were programmed to diverse effect-site sufentanil concentrations. Except for the work of Slepchenko et al. [22], they were successful in building and analyzing a nonlinear mixed model for obese people with target concentrations ranging from 0.3 to 0.65 ng/ml. Moreover, target concentrations of anesthetic drugs might not always be fixed

Table 2
Precision studies of sufentanil samples.

Sufentanil samples	Day 1 [mean conc. (ng/ml) ± SD (RSD %), ($n = 5$)]	Day 2 [mean conc. (ng/ml) ± SD (RSD %), ($n = 5$)]	Day 3 [mean conc. (ng/ml) ± SD (RSD %), ($n = 5$)]	Inter-day [mean conc. (ng/ml) ± SD (RSD %)]
Low	0.068 ± 0.006 (8.69)	0.076 ± 0.003 (4.00)	0.067 ± 0.004 (5.22)	0.070 ± 0.006 (8.22)
Medium	0.558 ± 0.046 (8.17)	0.566 ± 0.056 (9.95)	0.534 ± 0.024 (4.44)	0.553 ± 0.041 (8.49)
High	4.526 ± 0.204 (4.52)	4.683 ± 0.118 (2.52)	4.510 ± 0.111 (2.44)	4.573 ± 0.154 (3.38)

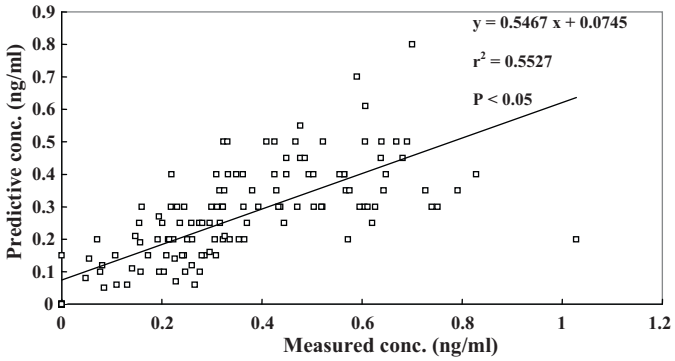

Fig. 4. Correlation between predicted and measured sufentanil concentrations using the Gepts model ($n = 136$).

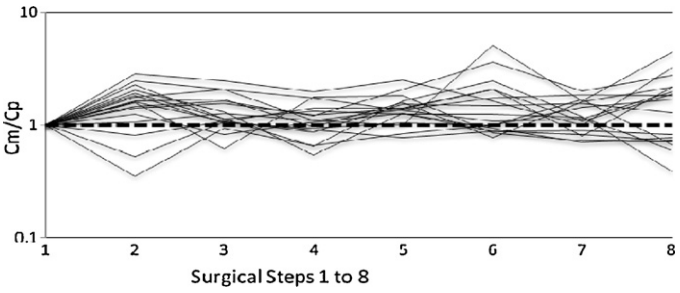

Fig. 5. Measured to predicted sufentanil concentration (C_m/C_p) ratios on a semi-logarithmic scale as a function of surgical steps. A C_m/C_p of 1 represents 100% accuracy.

Table 4
Predictive accuracy analysis of the Slepchenko et al.'s study [22] and the present study (Gepts model) for sufentanil target-controlled infusion.

Descriptive statistic	Slepchenko study	The present study
MDPE (%)	−13	12.4
MDAPE (%)	26	28.13
Wobble (%)	19.8	30.6
Divergence (%/h)	−3.4	N/A[a]

MDPE = median prediction error and MDAPE = median absolute prediction error.
[a] The divergence of the present study was unavailable for clinical reasons.

in clinical cases. Therefore, in this study, the predictive accuracy of Gepts sets was examined when the effect–site concentrations of sufentanil changed at multi-levels, from 0.06 to 0.8 ng/ml, during surgery. Table 4 shows the results of the four calculated indicators (MDPE, MDAPE, divergence, and wobble) of the predictive performance for the pharmacokinetic sets. The present results have moderate variations in the measured concentrations above the targeted concentrations. A mean 20–30% variation can be considered clinically acceptable [25]. Therefore, these results suggest that the Gepts pharmacokinetic sets for the sufentanil TCI model are accurate for predicting plasma concentrations during surgery, with a variation of target effect–site concentrations from 0.06 to 0.8 ng/ml.

4. Conclusion

In this work, we present a novel, automated and highly sensitive UPLC–MS/MS method for the analysis of sufentanil and evaluate the predictive accuracy of a Gepts set on the sufentanil TCI model. To the best of our knowledge, this work is the first report of a UPLC–MS/MS-based approach for measurement of sufentanil in human fluids. This approach enables us to separate and qualify sufentanil in human plasma in a 3 min chromatographic run. We believe that the many merits of this method will lead to its wide application for the biomedical analysis of fentanyl-like substances in the discovery and development of new pain-killing drugs. We also envision the wide application of this strategy to quantitatively measure concentrations of numerous medications and their metabolites in clinic samples for pharmacokinetics studies, regardless of how these medications are obtained [26].

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Appendix A. Appendix A

The following formulas were used for predictive accuracy analysis:

1. Median prediction error: $\mathrm{MDPE}_i = \text{median}\ \{\mathrm{PE}_{ij},\ j = 1, \ldots, N_i\}$, where N_i is the number of PE values obtained for the ith subject.
2. Median absolute prediction error: $\mathrm{MDAPE}_i = \text{median}\ \{\mathrm{PE}_{ij},\ j = 1, \ldots, N_i\}$, where N_i is the number of PE values obtained for the ith subject.
3. The divergence is defined as the slope of the linear regression line of [PE] plotted against time and is expressed in percent per hour. A positive value indicates the progressive widening of the gap between predicted and measured concentrations, whereas a negative value reveals that the measured concentrations converge on the predicted values.
4. In the ith subject, the percent wobble is calculated as follows: $\text{wobble}_i = \text{median}\ \{\mathrm{PE}_{ij} - \mathrm{MDPE}_i\ j = 1, \ldots, N_i\}$.

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