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Simultaneous detection of ketamine, lorazepam, midazolam and sufentanil in human serum with liquid chromatography–tandem mass spectrometry for monitoring of analgosedation in critically ill patients

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ABSTRACT

A liquid chromatography–tandem mass spectrometry (LC–MS/MS) method has been developed and validated for the determination and quantification of four predominantly used analgosedatives in the intensive care unit: ketamine, lorazepam, midazolam and sufentanil in human serum. The extraction procedure consisted of protein precipitation of serum samples with acetonitrile and subsequent centrifugation. D_5-fentanyl and D_4-midazolam served as internal standards (ISTD). Separation of analytes was performed with a Hypersil C18 column and a mobile phase with acetonitrile and 0.1% formic acid (60/40, v/v) under isocratic conditions at a flow rate of 280 μl/min. Analytes were simultaneously detected with a triple-stage quadrupole mass spectrometer (LC–MS/MS) in a selected reaction monitoring (SRM) mode with positive heated electrospray ionization (HESI) within a single 2-min run. Calibration curves were linear over a range of 50–2000 for ketamine, 10–1000 for lorazepam, 5–500 for midazolam and 1–100 for sufentanil (ng/ml). The limit of detection and the lower limit of quantification were 0.01 and 10.00 for ketamine, 0.005 and 10.00 for lorazepam, 0.018 and 5.00 for midazolam and 0.068 and 0.25 for sufentanil (ng/ml). Intra- and inter-day accuracies and precisions of all analytes were less than 15%. Bench stability with spiked serum samples was ensured after 12, 24 and 48 h at room temperature, freeze- and thaw-stability after 3 cycles of thawing and freezing. The method was successfully established according to International Conference on Harmonization (ICH) guideline Q2 (R1) "Validation of Analytical Procedures" and applied in critically ill adult patients in the intensive care unit. We suggest its suitability for parallel quantification of the sedative analgesics ketamine, lorazepam, midazolam and sufentanil. The method serves as an instrumental tool for therapeutic drug monitoring (TDM) and pharmacokinetic studies [1].

1. Introduction

One of the most important issues in intensive care medicine is the establishment of an individual analgosedation profile for each patient with respect to the clinical situation.

Generally, the intensive care unit (ICU) presents two different patient collectives – Elective ICU-patients: postoperatively monitored patients, and non-elective ICU-patients: emergency patients. Normally, optimization of the sedation status of adult ICU patients consists of subjective monitoring of several scores and scales like the "ramsay" sedation scale (RAMSAY), the Glasgow coma scale, sedation-agitation-score (SAS), nurse judgements, Richmond agitation sedation score (RASS), Minnesota sedation assessment tool, comfort scale or Brussels sedation scale without measurements of analgosedatives [2]. Objective tools are electroencephalography or current bispectral-EEG (BIS) measurements, although these approaches are laborious and not well established yet [3].

In general, the analgesic and sedation dose regime will be adjusted to the clinical situation and the constitution of each single patient to shorten duration of therapy and to reduce morbidity. The latest guidelines of sedation recommend a light target level of sedation in ICU patients, which is associated with a shorter duration of mechanical ventilation and a shorter ICU length of stay [3]. In addition, over- and underdosages should be avoided [4,5]. For instance, it is recognized that over dosage of sedatives may increase the

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incidence of pneumonia, hypotension, delirium, insomnia, psychic post trauma and the costs of hospitalization [6]. Besides a limited presence of attending physicians, a varying number of breathing machines and complex disease patterns of older multimorbid medical patients, it is indispensable to ensure a high level of treatment quality [7].

In summary, therapeutic drug monitoring is necessary to ensure drug effects, dosage regimes, monitor physiological changes and, when appropriate, adapt the medical health care of each patient in the ICU. Currently, anticonvulsants and psychotropic drugs are mainly determined in the daily routine of analytical laboratories [8]. Nevertheless, recommendations in the recent years have tended to expand generally the assortment of analytical methods in the ICU (such as antibiotics) and increase the improvement of drug safety and individual patient treatment [9–11].

Ketamine, an anaesthesia- and analgesic agent, is characterized by poor sedative and strong analgesic effects. It is applied as an adjuvant if standard sedation with midazolam and sufentanil is insufficient [12,13]. The benzodiazepines lorazepam and midazolam are prescribed for their sedative, anxiolytic, anticonvulsant, and amnestic properties which are especially useful in anaesthesia in the ICU. Midazolam represents currently the sedative choice of procedural sedation and is used in long-term ventilated patients because of its short acting and context sensitive half-life time [13]. In comparison, lorazepam is being applied as an additional therapeutic option because of its long-acting effects and difficult controllability. Both agents are characterized by a rapid onset of action and a low acute toxicity; while the elimination half-life is 13.0–16.0 h for lorazepam and 1.5–2.5 h for midazolam [14–16]. To introduce an anaesthetic intervention, midazolam is normally combined with sufentanil, a semisynthetic opioid [13,15,17]. Sufentanil, which is especially marked with strong analgesic effects (1000 times as potent as morphine), a short context sensitive half-life time, fast distribution and reversibility, constitutes a possible monoanaesthesic drug but is widely applied to supplement general combined analgesic sedation [13].

Up to now information about correlation of analgosedative concentrations and pharmacodynamic parameters is still lacking.

Thus, there is a considerable demand for a robust, specific, highly sensitive and rapid liquid chromatography–tandem mass spectrometry method for quantification of analgosedatives. A combination of analgosedative concentrations and pharmacodynamic parameters may better predict the sedation status of critically ill patients. To the best of our knowledge, the simultaneous measurement of ketamine, lorazepam, midazolam and sufentanil in long term ventilated adult patients has not been described before.

The present work was developed to determine a representative group of analgosedatives in serum samples of critically ill adults in the ICU and to establish a robust and highly sensitive liquid chromatography–tandem mass spectrometry (LC–MS/MS) method for assessment of the sedation status.

2. Experimental

2.1. Chemicals and reagents

Ketamine (KET) and sufentanil (SUF) were purchased from Lipomed (Weil, Germany), lorazepam (LOR) from Sigma–Aldrich (Steinheim, Germany) and midazolam (MID) from Alsachim (Ilkirch Graffenstaden, France). ISTD midazolam-D_4 (MID-D_4) for LOR and MID was obtained from Alsachim (Ilkirch Graffenstaden, France). ISTD Fentanyl-D_5 (FEN-D_5) for KET and SUF was acquired from Lipomed (Weil, Germany).

All reagents were of analytical or high-performance liquid chromatography (HPLC) grade. Acetonitrile and methanol were obtained from Roth (Karlsruhe, Germany), formic acid from Merck (Darmstadt, Germany). Deionized water was applied and produced by a Milli-Q water purifying system (Millipore Corporation, Bedford, MA).

2.2. Materials

Sample-analysis was performed by liquid chromatography–tandem mass spectrometry (LC–MS/MS) carried out with a TSQ Vantage triple-stage quadrupole mass spectrometer using a selected reaction monitoring (SRM) mode with heated electrospray ionization (HESI). The system was provided with an Accela 1250 pump and an Accela auto sampler, assembled with a temperated tray and column oven. The Thermo XCalibur software (version 2.1.0.1139) was applied for the instrumental control and registration of data. In order to optimize the MS/MS conditions, Thermo TSC Tune Master-software (version 2.3.01206 SP1) was used. The chromatographic separation was achieved on a Hypersil Gold C18 column (50 mm × 2.1 mm, 1.9 μm; Thermo Fisher Scientific).

2.3. Stock solutions, calibration standards and quality control samples

Stock solutions of all analytes and ISTDs were prepared in 50% methanol and 50% distilled water (50/50, v/v). The concentrations of stock solutions were 218, 334, 298 and 50 mg/l for KET, LOR, MID and SUF, respectively. For preparation of calibration standards (CS) all stock solutions were diluted in distilled water in predefined concentrations and next added to blank serum, giving concentrations in 5 aliquot mixtures (Suppl. Table 1). Blank serum was purchased from the Department of Transfusion Medicine of the Department of the University of Cologne. Serum internal quality controls (IQC) with low, medium, and high concentrations were prepared in an analogous way. (Suppl. Table 2). The ISTDs were assembled into one aliquot with concentrations of 50 ng/ml for D_5-FEN and 20 ng/ml for D_4-MID. All CS and IQC samples were stored at $\vartheta = -80\,^{\circ}C$ until analysis.

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jchromb.2014.10.006.

2.4. Sample preparation

Using a precipitation method, ISTD (50 μl) was added with serum (100 μl) and acetonitrile (300 μl). The samples were vortexed (Bender and Hobein, Zurich, Switzerland) for 30 s and centrifuged for 10 min at 14,000 rpm ($g = 15{,}800$) and $\vartheta = 20\,^{\circ}C$ (Eppendorf, Hamburg, Germany). The clear supernatant was transferred into an auto sampler LC–MS vial (2 ml glass vial with 200 μl glass insert; Macherey-Nagel, Düren, Germany), capped and analyzed subsequently. The calibration standards were treated in an analogous way.

2.5. Liquid chromatography

The injection volume was $V = 2$ μl with a solvent flow rate of 280 μl/min, a column pressure of 145–155 (2103–2248 psi) and a single run time of 2 min. A mobile phase consisting of acetonitrile and 0.1% formic acid (60/40, v/v) was applied under isocratic conditions. The column temperature was kept at $\vartheta = 25\,^{\circ}C$ and the tray temperature in the auto sampler at $\vartheta = 20\,^{\circ}C$. The system was equilibrated until stability of total ion current (TIC) was reached for a minimum of 1.5 h. All analytical peaks were integrated with the Thermo Scientific processing software LCquan (version 2.6) in