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Short communication

Design and validation of an automated solid phase extraction liquid chromatography coupled mass spectrometry method for the quantification of propofol in plasma

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ABSTRACT

Propofol concentration in human plasma can be quantified by liquid chromatography coupled mass spectrometry. Sample preparation usually requires solid phase extraction to remove matrix components and enrich the analyte. To facilitate user-independent measurements and speed extraction, we developed and validated a fully automated high throughput in-line sample preparation system with direct injection into liquid chromatography coupled mass spectrometry. We assessed linearity of each method over the clinically relevant concentration range from 0.5 μg/mL to 8 μg/mL plasma concentration. R^2 values were 0.99 for the automated process and 0.98 for manual sample preparation. The limit of detection was 6 ng/mL and the lower limit of quantification was 18 ng/mL for the automated method; for the manual process, the limit of detection was 1.58 ng/mL and the lower limit of quantification was 4.79 ng/mL. Intra-day precision for low, medium and high concentration range of the automated method was validated 4.14%, 9.68% and 3.04% relative standard deviation and 0.29%, 0.12% and 0.52% for the manual process. Carry over was 0.4% with the automated method, whereas there was no carry over with the manual method. Stability of plasma samples was tested with the manual method at concentrations of 1, 4, and 6 μg/mL propofol and found to be stable over 150 days at −20 °C. The manual sample preparation method has successfully been transferred to a fully automated process with appropriate sensitivity and precision but the automatization failed with regard to trueness and working time due to lengthy sample preparation runtime. Therefore it is not suitable for daily use in a hospital laboratory e.g. for brain death diagnosis in the intensive care unit.

1. Introduction

Propofol is used for induction of most general anesthetics, for maintenance and sedation. Liquid chromatography in combination with mass spectrometry (LC–MS) is commonly used to quantify propofol in plasma because the method is fast, accurate, and robust [1].

In most cases, samples are purified before LC–MS quantification, often using solid-phase extraction (SPE). SPE uses solid chromatographic packing material to separate sample components. This method requires many manual steps which are tedious, time consuming, and use many disposable items. Furthermore, the quality of the assay depends on the dexterity of the executor which may add variability to the process.

An automated solid-phase extraction process may eliminate variability consequence to human performance. An automated process may also reduce working time. We thus developed and validated an automatic Multi-Purpose-Sampler for in-line injection into an LC–MS system. Our primary outcomes were analytical accuracy and investigator time required for analysis.

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2. Material and methods

2.1. Materials

2.1.1. Standards for calibration and validation

The certified propofol reference standard (1 μg/μL) was purchased from Sigma-Aldrich (Steinheim, Germany). As reference plasma a lyophilized drug-free serum (Bio-Rad, Munich, Germany) was used.

2.1.2. Solid phase extraction (SPE)

The manual SPE was performed on Oasis Prime HLB 96-well plate (Waters, Eschborn, Germany) with phosphoric acid (Sigma-Aldrich), LC–MS grade methanol (VWR BDH Prolabo, Darmstadt, Germany), LC–MS grade water (Merck, Darmstadt, Germany) and LC–MS grade acetonitrile (VWR BDH Prolabo). For the automated SPE Oasis HLB cartridges with shortened top, an attachment for the MPS gripper, and a cannula at the bottom for elution into septum sealed vials was used.

2.1.3. LC–MS

The eluent was composed of LC–MS grade acetonitrile (VWR BDH Prolabo) and LC–MS grade water (Merck) with the eluent additive ammonium hydroxide (Sigma-Aldrich).

2.1.4. Instrumentation

The manual method was performed with a Positive Pressure 96-Processor (Waters). The fully automated sample preparation was performed on a MultiPurpose Sampler (MPS, Gerstel, Mülheim, Germany) with a centrifuge model 4-16KL (Sigma, Osterode am Harz, Germany) and two mobile arms. The arms were equipped with one glass syringe each. One syringe had a total volume of 2.5 mL for preparation and the other had a volume of 10 μL for sample injection into the LC valve with a 5 μL sample loop. The mobile arm with the 2.5 mL syringe had additionally a vial gripper for sample vial handling.

Separation was carried out on an Agilent 1260 Infinity series LC system consistent of a binary pump model G1312B, a degasser model G4225A, a thermostat model G1330 B and a temperature controlled column compartment model G1316A. Detection was performed on an atmospheric pressure ionization-electrospray coupled mass selective detector (API-ES MSD) model G6130BA (Agilent, Waldbronn, Germany).

2.2. Methods

The general difference between both methods is that in the manual procedure the steps of the SPE are performed for all samples in parallel whereas the automated method processes the SPE successively.

2.2.1. Manual solid phase extraction

Plasma was mixed 1:1 with 600 μL 4% phosphoric acid. Subsequently, 1 mL of the plasma supernatant was transferred to the 96-well plate and 3–4 bar N_2 pressure was applied over several seconds to enhance the sample flow through the filter material. Then, the wells were washed two times with 500 μL 5% methanol. The elution was performed twice with 125 μL acetonitrile (ACN). Both elution steps were pooled.

2.2.2. Automated solid phase extraction

An automated method was designed based on the steps of the manual method. Subsequently, optimization steps had to be made for the following processes to adjust the automated method: 1) draw up and eject speed of the syringe 2) needle penetration depth 3) LC injection volume 4) syringe washing steps (Fig. 1). The resulting method is as follows: Plasma samples are kept on a 10 °C cooling tray prior to the SPE process. First 600 μL plasma is mixed with 600 μL 4% phosphoric acid. Then a centrifugation over 10 min at 6000 rpm with a subsequent syringe washing step is carried out. In the meantime the SPE cartridge is conditioned with 1 mL methanol and 1 mL H_2O and dries with a N_2 gas stream. Afterwards, 1 mL of the plasma is transferred to the cartridge and flushed through with N_2. The cartridge is washing two times with 1.5 mL 5% methanol and flushed with N_2. The elution is performed with 250 μL ACN which is flushed trough the cartridge with 2.5 mL air. After nine washing steps with water and methanol, 8 μL of the flow is injected into the LC–MS valve.

2.2.3. LC–MS measurements

The chromatographic separation was the same for both solid phase extraction methods. Separation was performed on a Waters Xselect CSH C18 (100 mm × 2.1 mm i.d., 3.5 μm) column with a Zorbax SB-C18 (2.1 × 5 mm; 3.5 μm) guard column under isocratic conditions at 40 °C. As mobile phase a mixture of 0.05% ammonium hydroxide in water and acetonitrile (30:70 v/v) with a flow rate of 0.4 mL/min was used. The spectra were generated in full scan mode once for development of the method. Then the most abundant propofol ion m/z 177 was monitored in selected ion monitoring mode (SIM) with negative polarity (heat 400 °C, nebulizer 35psi, dry gas nitrogen at 12 L/min, dry gas temperature 350 °C). The retention time for propofol in this setup was 1.8 min.

2.2.4. Method validation

The validation was assessed for specificity and selectivity, trueness, linearity, limit of detection/quantification, inter-day precision, accuracy, robustness and free-thaw stability of plasma samples. All validation steps were performed for both methods except robustness and stability which were determined with the manual method. The plasma was spiked by hand for the manual method and by the MPS for the automated method using drug free plasma. The validation was performed with the optimized automated method described in Section 3.

2.2.4.1. Calibration. Manual and automated calibration curves were prepared using nine blank plasma aliquots spiked with propofol (1 μg/μL) to the final concentrations of 8, 6, 5, 4, 3, 2, 1, 0.5, and 0.25 μg/mL. Subsequent an automated (Section 2.2.2) or manual (Section 2.2.1) SPE was performed. Each plasma concentration was measured three times by LC–MS and the measured mean peak area was transferred to the current concentrations using linear fit.

2.2.4.2. Quality control samples. For each LC–MS run 2 quality control samples (QC) at concentrations of 1, 4 and 6 μg/mL where made separately and then measured as triplicate.

2.2.4.3. Specificity and selectivity. With approval of the local Ethics Committee (Ärztekammer des Saarlandes, Saarbrücken, Germany) and written informed consent, 145 plasma samples of 15 different patients with propofol administration were checked to test the specificity and selectivity in the clinical setting.

2.2.4.4. Trueness. For the determination of the trueness the mean from 2 QC samples (2.2.4.2) at each low, medium and high concentration was calculated. Each sample was measured as triplicate. The calculation was performed in the following way: Bias [%] = [(mean – nominal value)/nominal value]*100