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Simultaneous determination of propofol and its glucuronide in whole blood by liquid chromatography–electrospray tandem mass spectrometry and the influence of sample storage conditions on the reliability of the test results

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ABSTRACT

Propofol (2,6-diisopropylphenol) is commonly used as an anaesthetic agent but is also abused for recreational purposes. Several cases of fatalities involving self-administered propofol have been reported. For rapid quantification of propofol and propofol β-D-glucuronide (propofol G) in clinical and forensic cases, an ultra-performance liquid chromatography–tandem mass spectrometry method using pneumatically assisted electrospray ionisation has been developed. The technique has been validated on both ante-mortem and post-mortem human whole blood. The proteins in the blood samples were removed by the addition of a mixture of methanol and acetonitrile, and the extract was cleaned up by solid phase extraction. The extract was concentrated in dimethyl sulphoxide. The system was calibrated using matrix-matched calibrants combined with isotope dilution. The lower limits of quantification were 0.01 and 0.02 mg/L for propofol and 0.02 and 0.04 mg/L for propofol G in ante-mortem and post-mortem whole blood, respectively. The relative intra-laboratory reproducibility standard deviation was less than 10% at concentrations of 0.2 mg/L or higher. The mean true extraction recovery was 85% for propofol and 81% for propofol G. The trueness of the propofol determination expressed as the relative bias of the test results was within ±6% at concentration levels of 0.01–8.5 mg/L. Propofol was less stable in blood stabilised with a citrate–EDTA–fluoride mixture than in blood stabilised with an oxalate–fluoride mixture. The stability was lower at −20 °C than at 5 °C and −80 °C. Propofol G did not show instability under the storage conditions tested.

1. Introduction

Propofol (2,6-diisopropylphenol), an intravenous anaesthetic agent formulated in an oil–water emulsion, is used for the induction and maintenance of anaesthesia. Induction normally requires a propofol concentration of 2–10 mg/L blood, while 2–4 mg/L is sufficient for the maintenance of narcosis [1,2]. Propofol is rapidly redistributed and metabolised to inactive water-soluble substances by the glucuronidation of the parent drug and through *p*-hydroxylation and further conjugation with glucuronic acid and sulphate [1].

Several adverse reactions are associated with the use of propofol, including bradycardia, hypotension, cardiac arrhythmia, somnolence and seizures [1]. Propofol is also used recreationally, especially among health care professionals [3,4], due to its sedative and relaxing properties and potential sexual illusions and euphoric feelings [5]. Several cases of fatal propofol abuse or suicide due to single or combined drug intake have been reported [4–10]. In the fatal cases, the measured propofol concentrations in the blood were in the range of 0.08–8.7 mg/L.

Quantification of propofol in blood must take into consideration the highly lipophilic and volatile properties of the substance and its significant association with proteins and erythrocytes. Several methods based on high-performance liquid chromatography combined with fluorescence or UV detection and gas chromatography with mass spectrometric detection have been published and reviewed [11]. Many of these methods are based on the liquid–liquid extraction of propofol with non-polar solvents. In few cases liquid chromatography tandem mass spectrometry (LC–MS/MS) has been applied for the determination of propofol in plasma samples cleaned up by solid phase extraction (SPE) using either atmospheric pressure chemical ionisation [12] or

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electrospray ionisation (ESI) [13]. However, propofol is difficult to ionise, and the fragmentation efficiency is very weak. To improve the sensitivity, derivatisation of propofol prior to LC–MS/MS has been applied using dansyl chloride [14], 2-fluoro-1-methyl-pyridinum-*p*-toluene sulphonate [15] and a diazonium salt from aniline [16]. However, the specificity of the method is not necessarily improved by the derivatisation.

The present ultra-performance liquid chromatography tandem mass spectrometry (UPLC–MS/MS) method was developed and validated as a simple and quantitative mass spectrometric technique that is sufficiently sensitive for the direct determination of propofol in both ante-mortem and post-mortem whole blood samples from abusers and persons who have received anaesthetic or sedative treatments. The method simultaneously determines the glucuronic acid conjugate of propofol, which may be useful in the interpretation of the results.

2. Materials and methods

2.1. Standards and reagents

Propofol, propofol β-D-glucuronide (propofol G), propofol-D_{17} and propofol-D_{17} β-D-glucuronide (propofol-D_{17} G) were purchased from Toronto Research Chemicals, Inc. (North York, Canada). Acetic acid (HAc) was purchased from Merck (Darmstadt, Germany). Methanol (MeOH), acetonitrile (MeCN) and dimethyl sulphoxide (DMSO) were purchased from Sigma–Aldrich (Schnelldorf, Germany). Water was purified using a Direct-Q 3 apparatus (Millipore, Bedford, MA).

Separate stock solutions (1 mg/mL) of the substances were prepared in MeOH. Combined standard solutions to spike the samples and prepare the calibrants were prepared by diluting the stock solutions with MeOH. An internal standard solution of stable isotope-labelled substances (SIL-IS) containing 5 μg/mL each of propofol-D_{17} and propofol-D_{17} G was also prepared in MeOH. The mobile phases A and B consisted of 0.02% HAc in 10% MeOH (v/v) and 0.02% HAc in MeOH/MeCN (1:1, v/v), respectively.

2.2. Materials

Blank ante-mortem whole blood samples for calibration and validation were obtained from the blood bank at Aarhus University Hospital (Skejby, Denmark). The ante-mortem whole blood with authentic analyte content and post-mortem whole blood used for the method validation were obtained from the Institute of Forensic Medicine, University of Aarhus. Ante-mortem blood was collected and preserved in Venosafe VF-054SFX tubes containing 9 mg of sodium fluoride (NaF) and 9 mg of potassium oxalate (FO mixture) for a 4 mL draw volume of blood (Terumo Europe, Leuven, Belgium). Venosafe VF-053SFC32 tubes containing 6.8 mg of NaF and 15.7 mg of citrate-EDTA buffer ingredients (FC mixture) for a 3 mL draw volume of blood and Venosafe VF-052SDK tubes containing 3.9 mg of K_2EDTA for a 2 mL draw volume were used in a stability study on the analytes in whole blood. Post-mortem blood samples were preserved with 200 mg of NaF per 30 mL of blood.

2.3. Equipment

The liquid chromatography system was a Waters Acquity UPLC instrument consisting of a binary pump, an autosampler with a 10 μL sample loop set at 7 ± 2 °C and a column oven set at 40 ± 2 °C (Waters, Milford, MA). The mass spectrometer was a Waters Xevo TQMS triple-quadrupole instrument with an electrospray ionisation (ESI) source. The separation was performed using an Acquity UPLC HSS T3 (1.8 μm, 2.1 mm I.D. × 100 mm) column (Waters). Disposable 2 mL polypropylene Safe-Lock tubes (Eppendorf, Hamburg, Germany) were used for the extractions. Strata-X solid phase extraction (SPE) cartridges containing 60 mg of polymeric reversed phase sorbent (Phenomenex, Torrance, CA) were used for the clean-up of extracts. A VacMaster-20 vacuum manifold (Biotage, Uppsala, Sweden) was used during the SPE procedure. A TurboVap LV (Caliper Life Sciences, Hopkinton, MA) was used for solvent evaporation by a nitrogen stream. Tubes (15 mL), and autosampler vials made of glass were used for the handling and storage of extracts. The other equipment used included pipettes (Biohit, Helsinki, Finland) and a Heraeus Biofuge Pico (Thermo Scientific, Langenselbold, Germany).

2.4. Extraction

A 200 μL volume of sample was transferred to a disposable 2 mL centrifuge tube. Next, 50 μL of SIL-IS solution and 50 μL of MeOH were added, and the tube contents were gently mixed. Shortly thereafter, a 500 μL volume of MeCN was added, and the tube was immediately closed and vigorously vortexed for few seconds. After a standing time of approximately 10 min, the mixture was centrifuged at 10,000 × *g* for 5 min. A 500 μL volume of clear supernatant was mixed with 3 mL of water and applied onto an SPE cartridge previously conditioned with 1 mL of MeOH followed by 1 mL of water. The cartridge was rinsed with 1 mL of water, sucked dry by the application of a full vacuum for several seconds and eluted with 2 mL of MeCN. The eluate was mixed with 100 μL of DMSO and evaporated to a residual volume of approximately 100 μL at 30 °C. The extract was diluted with 100 μL of water and transferred to an autosampler vial.

2.5. Calibration

Calibrants based on blank donor blood were used for the construction of 6-point calibration curves. The calibrants were treated according to the above procedure, except that 50 μL of MeOH was replaced by 50 μL of the mixed standards containing propofol and its glucuronide. Calibrants were prepared with concentrations of 0.01, 2, 4, 6, 8, and 10 mg/L of propofol and twice the propofol concentrations for propofol G. The calibration curves were created by weighted ($1/x$) linear regression analysis of the SIL-IS normalised peak areas (analyte area/IS area) and were forced through the origin.

2.6. LC–MS/MS conditions

All sample extracts were stored at 5 ± 2 °C until analysis. A 10 μL volume was injected onto an Acquity UPLC HSS T3 column running 55% mobile phase A and 45% mobile phase B. The mobile phase was changed to 100% B using a linear gradient over 3 min. Seven minutes after injection, the gradient was returned to 55% A over 0.2 min, and the column was equilibrated for 2.8 min before the next injection, resulting in a total run time of 10 min. The eluent was diverted to waste during the intervals of 0–1 min and 4–10 min after the injection using a post-column switch. The column flow rate was 400 μL/min, and the column temperature was kept at 40 ± 1 °C. The source and desolvation temperatures were set at 150 °C and 600 °C, respectively, and the cone and desolvation gas flows were set at 50 L/h and 800 L/h, respectively. The mass spectrometer was operated in negative ion mode with capillary and cone voltages of 2.2 kV and 35 V, respectively. The dwell time was 0.1 s for all ion transitions. Selected reaction monitoring (SRM) was applied under the conditions shown in Table 1. Argon was used for the collision-induced dissociation. Data acquisition and processing were performed using MassLynx 4.1 (Waters).