Validation of liquid and gaseous calibration techniques for quantification of propofol in breath with sorbent tube Thermal Desorption System GC–MS

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\textbf{A R T I C L E   I N F O}

\textbf{Article history:}
Received 26 April 2017
Accepted 25 May 2017
Available online 31 May 2017

\textbf{Keywords:}
Tenax tubes
Gas chromatography–mass selective detector
Volatile drugs
Propofol
Breath analysis
Calibration gas generator

\textbf{A B S T R A C T}

Plasma concentrations of intravenous drugs cannot currently be evaluated in real time to guide clinical dosing. However, a system for estimating plasma concentration of the anesthetic propofol from exhaled breath may soon be available. Developing reliable calibration and analytical validation techniques is thus necessary. We therefore compared the established sorbent tube liquid injection technique with a gas injection procedure using a reference gas generator. We then quantified propofol with Tenax sorbent tubes in combination with gas-chromatography coupled mass spectrometry in the breath of 15 patients (101 measurements). Over the clinically relevant concentration range from 10 to 50 ppb, coefficient of determination was 0.995 for gas calibration; and over the range from 10 to 100 ng, coefficient of determination was 0.996 for liquid calibration. A regression comparing gas to liquid calibration had a coefficient of determination of 0.89; slope 1.05 ± 0.01 (standard deviation). The limit of detection was 0.74 ng and the lower limit of quantification was 1.12 ng for liquid; the limit of detection was 0.90 ppb, and the lower limit of quantification was 1.36 ppb, for gas. Loaded sorbent tubes were stable for at least 14 days without significant propofol loss as determined with either method. Measurements from liquid or gas samples were comparably suitable for evaluation of patient breath samples.

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1. Introduction

Blood- and effect-site concentrations of drugs vary widely, and are hard to predict from dosing and patient characteristics. Over- and under-dosing of intravenous drug is thus common in clinical practice because blood-concentrations cannot currently be determined in real time. For long-acting drugs that are titrated over periods of weeks or months, kinetic issues are relatively easy to surmount. But anesthetic drugs are short-acting and must be precisely titrated to rapidly changing conditions over a matter of a few minutes.

Volatile anesthetic blood partial pressure can be determined in real time from end-tidal expired concentrations. It may also be possible to estimate blood concentration of the intravenous anesthetics from expired gas using ion mobility spectrometry (IMS) technologies [1]. Only a few drugs have been considered [2], and most studies evaluated the intravenous anesthetic propofol [3–8]. Propofol is an attractive drug for real-time breath analysis since: 1) it is given in large doses and thus has a high plasma concentration; and, 2) it is volatile and thus expired from the lungs. Perhaps the most promising method for real-time analysis of expired organic compounds is gas chromatography coupled to mass spectrometry (GC–MS) which is more sensitive than multicapillary column (MCC) IMS; furthermore, the national institute of standards and technology reference database for the identification of molecules from GC–MS analysis is extensive.

For gas chromatographic measurements, exhaled air is aspirated through sorbent tubes in which volatile organic compounds (VOCs) are retained by an adsorbent [9]. Tenax brand tubes are widely used for breath analysis by GC–MS [10] and even low concentrations can be evaluated by sufficiently extending sampling time and gas volume. Thermal desorption systems (TDS) are then used for transfer absorbed samples into the GC–MS system. However, data
of exhaled drugs measured with the combination of Tenax tubes and gas chromatography are limited [11].

In this study we aimed to evaluate two Tenax calibration procedures as method for propofol quantification in breath. One method is based on a liquid injection of propofol into Tenax tubes used for calibration. Then we validated this method and tested it with real patient samples. Subsequently we used the newly validated method to cross-validate a gas injection calibration. Finally a difference comparison was performed to test the accordance of both methods. As the gas injection calibration is less tedious, it would be preferable if equally suitable for propofol quantification in breath.

2. Materials and methods

2.1. GC–MS

A 7890 B gas chromatograph (Agilent, Santa Clara, United States) with a 5977 B quadrupole mass spectrometer (Agilent, Santa Clara, United States) and an XTr EI 350 ion extraction lens were used as the ion source. The system was coupled to TDS2 auto sampler (Gerstel, Mülheim, Germany) with a TDS3 thermal desorption system (Gerstel, Mülheim, Germany) and a cold injection system with a glass wool liner. Samples were injected in Tenax GR thermal desorption tubes (Gerstel, Mülheim, Germany). Helium (99.9999% pure) was used as carrier gas. Separation was performed with a HP-5MS UI (30 m x 0.25 mm, film thickness 0.25 μm) capillary column (Agilent Technologies, Santa Clara, United States).

The GC conditions were an initial column temperature of 50 °C, temperature ramp 20 K/min, final temperature 260 °C for 1 min, and a column flow 1.2 ml/min. The thermal desorption conditions were an initial temperature ramp rate of 20 K/min, final temperature 240 °C for 4 min, unsplint sample, and a desorption flow 40 ml/min. The cold injection conditions were an initial temperature of –50 °C, temperature ramp of 12 K/s, final temperature 250 °C for 3 min, 20:1 injection split. The conditions for the MSD were full scan m/z between 50 and 300, transfer tube temperature 250 °C, quadrupole temperature 150 °C, ion source temperature 230 °C, and solvent delay 4 min. Data were analyzed with the Masshunter Qualitative Analysis and the Masshunter Quantitative Analysis software (Agilent, Santa Clara, United States). With this setup propofol has a retention time (RT) from 7489 to 7501 min identified by the NIST Mass Spectral Library (version 14).

2.1.1. Liquid calibration

Propofol with a purity of 97% (Sigma Aldrich, Munich, Germany) was dissolved in HPLC-grade water (VWR, Darmstadt, Germany) to a 50 μg/ml propofol stock solution for liquid calibration. The stock solution was gravimetrically diluted in 50 ml flasks to 0, 5, 10, 15, 20, 25, 30, and 50 μg/ml calibration standard solutions. 2 μl from each standard or HPLC-grade water for blanks was pipetted onto a Tenax tube and the weight of the injection was determined with a Cubis analytical scale (Sartorius, Goettingen, Germany) to determine the exact mass of injected propofol. The resulting calibrators contained final propofol amounts of 10, 20, 30, 40, 50, 60 and 100 ng. Every tube was flushed with 1 bar synthetic air (Liquide, Düsseldorf, Germany) (20.5% O2 purity [4.5], 79.5% N2 purity [5.0]) with a purity of 99.999% for 30s to distribute the propofol evenly.

2.1.2. Gas calibration

A 90 μg/ml propofol stock solution was prepared as described in 2.1.1, with addition of 1% v/v HPLC grade ethanol (Sigma-Aldrich GmbH, Steinheim, Germany). We used a NovaCAL 4836-VOC (IAS GmbH, Oberursel, Germany) [12] gas generator. The NovaCAL generates reference gas mixtures at designated concentrations by diluting an injected analyte-of-interest with nitrogen carrier gas to the desired concentration. In our case, propofol stock solution was injected via two syringes (1702.5TLLX, Hamilton Co., Reno, USA), each with a volume of 50 μL. The propofol solution was vaporized at a temperature of 100 °C and the output of the calibration gas was 850 ml/min⁻¹. Each Tenax tube was loaded by aspirating 0.3 L reference gas with a flow of 0.4 L/min⁻¹ through a Bivoc2 gas sampling pump (Hohlbach GmbH, Wadern, Germany). The pump was connected to the reference gas generator via a 1.2 m long perfluoralkoxy transfer tubing (Rohler GmbH, Grünsfeld, Germany) with a stainless steel eight-inch t-piece (Swagelok, Frankfurt, Germany) open at one end to prevent internal pressure accumulation (Fig. 1). Tenax tubes with 10, 20, 30, 40, 50 ppb of vaporized propofol were prepared in triplicate for calibration. A blank measurement without propofol was made before each calibration to ensure that the tubing and the devices were propofol-free. For each new calibration, the reference gas generator was run for 5 min before Tenax sampling to assure equilibration at the designated concentration.

2.1.3. Calculation of ppb values

The propofol mass of the liquid calibrators was translated to ppb, so the values from each calibration method would be directly comparable. We assumed an ideal gas, and inserted \( n = m/M \) in the ideal gas formula \( \text{ppb}_m = V/(\text{Sample}) \times 10^9 \).

\[
\text{ppb}_v = m/V(\text{sample}) \times RT/pM \times 10^9 \tag{1}
\]

\[
\text{ppb}_s = m/V(\text{sample}) \times 0.137 m^2/kg \times 10^9 \tag{2}
\]

2.1.4. Validation

Validation included linearity, limit of detection, limit of quantification, precision, carry-over, stability, and robustness.

2.1.4.1. Linearity. The linearity of the calibration range was proved by the analysis of the 8 liquid calibration standards described in Section 2.1.1 as duplicates and the 5 gas calibration standards described in Section 2.1.2 as triplicates. The measured peak area of propofol was plotted vs. the known injected masses of propofol respectively vs. known ppb of propofol. Slope, intercept and linearity were determined from a least-squares linear regression.

2.1.4.2. Limit of detection quantification. The lower limit of quantification (LOQ) and the lower limit of detection (LOD) were determined according to the DIN 32645/ISO-11843-2 blank procedure with the following equations:

\[
\text{LOQ} = (9.0SD)/S
\]
and

\[ \text{LOD} = (6.\text{SD})/S, \]

where SD is the standard deviation of the mean value from 10 blank samples prepared with either the liquid or gas methods. S is the slope of the calibration curve.

2.1.4.3. Precision. The precision was determined by the analysis of 10 identical 50 ng liquid samples and 10 identical 25–ppbv, gas samples.

2.1.4.4. Carry over. Carry-over was determined by the analysis of 10 blank samples after the injection of a 100 ng liquid sample and a 50 ppbv, gas sample.

2.1.4.5. Stability.

2.1.4.5.1. Calibration standard stability. The standard solutions were prepared as in 2.1.1 described and compared with a fresh 50 ng reference solution after 0, 1, 2, 3, 4, 5, 6, 7, and 8 days incubation at 5°C.

2.1.4.5.2. Tenax tube stability. To assess short-term stability 10, 50, and 100 ng of liquid propofol were injected into Tenax tubes in duplicate; similarly, 10, 50, and 50 ppbv, propofol was vaporized into Tenax tubes in duplicate. For this purpose the calibration solutions from Section 2.1.1 were used. The tubes were maintained in a TDSA 2 auto sampler holder and stored for 24, 48, and 72 h at room temperature before measurement.

For the long-term, stability 10, 20, 30, 50, 60, and 100 ng propofol liquid injected into Tenax tubes in duplicate; similarly, 10, 20, 30, 40, and 50 ppbv, propofol was vaporized into Tenax tubes. The tubes were stored in Tenax tube containers (Gerstel, Mülheim, Germany) for 0, 7, and 14 days at room temperature before measurement.

2.1.4.6. Robustness. Column flow was adjusted (±0.2 mL/min) and the temperature ramp was adjusted (±5 K/min). 2 µL standard solution was injected into 6 thermal desorption tubes to a total amount of 10, 50, and 100 ng and analyzed in duplicate. As expected, both flow and temperature ramp have impact on the retention time of the analyte whereas an increasing column flow also led to systematic decreasing overall signal intensity. Nevertheless the signal is still definable and analyzable.

2.2. Statistical analysis.

Statistical analysis was performed with SigmaPlot (version 12.5, Systat Software, Erkrath, Germany) using repeated measures one-way ANOVA for normally distributed data, otherwise a repeated measures one-way ANOVA on ranks. \( P < 0.05 \) was considered significant. Data are expressed as means ± standard deviations (SD). All data were subjected to Grubbs test for outliers.

Correlation between patients propofol breath concentrations calibrated with gas or liquid was analyzed by linear regression. Acceptable values are defined \( R^2 > 0.85 \). A Bland and Altman analysis for repeated measures was performed to evaluate the agreement between both methods [13].

2.3. Clinical measurements.

With approval of the local Ethics Committee (Ärztekammer des Saarlandes, Saarbrücken, Germany) and written informed consent, we enrolled 15 patients scheduled for surgery with propofol and remifentanil target controlled infusions (TCI). The propofol TCI target concentrations were chosen by an anesthesiologist according to clinical need, and all were between 2 and 3 µg/mL.

![Fig. 2. Ventilation tubing with sampling setup. Endotracheal tube (1) with straight connector (2), breath sampling line with Luer lock (3), ventilation hose (4).](image)

<table>
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<th>Table 1 Validation data.</th>
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<td>LOD ng resp. ppbv</td>
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LOD lower limit of detection, LOQ lower limit of quantification, RSD relative standard deviation.

Amount tested for liquid calibration [ng]: *50, †100; Concentration tested for gas calibration [ppbv]: *25, †50.

Expired gas was sampled at 30-min intervals with a Bivoc 2 pump during anesthetic maintenance with mechanical ventilation through an endotracheal tube with a sealed cuff. The pump and Tenax tube was connected to the endotracheal tube by a 1.2-m-long perfluoralkoxy transfer tube (Bohlender GmbH, Grünfeld, Germany) with a Luer lock (Fig. 2). A volume of 0.3 L breath with a flow of 0.4 L min⁻¹ was sampled.

3. Results

3.1. Method validation.

The linearity of gas calibration was verified over the clinically relevant concentration range from 10 to 50 ppbv, with an \( R^2 \) of 0.995; for liquid calibration, \( R^2 \) was 0.996 over the range from 10 to 100 ng (Table 1). The limit of detection was 0.74 ng and the lower limit of quantification was 1.12 ng for liquid; the limit of detection was 0.90 ppbv and the lower limit of quantification was 1.36 ppbv for gas. For both methods precision and trueness were below the acceptable 15% RSD and bias. A carry-over of 0.32% for the liquid calibration and 1.38% for the gas calibration was detected. Short-term stability of the loaded Tenax tubes is presented in Figs. 3 A and 4 A for 24 h, 48 h and 72 h.

No statistically significant differences have been detected. The 10-ng calibrators show the highest variation. In comparison to the 24 h sample 16% less propofol was detected after 48 h and 11% less after 72 h. The 50 ng and 100 ng samples show only slight variations (Fig. 3A).

There were no statistically significant differences between the long-term stability tests up to 14 days for either calibration method (Figs. 3B and 4B).
3.2. Patient measurement

Propofol gas was successfully measured in all 101 breath samples of 15 different patients with propofol remifentanil anesthesia. Specificity and selectivity of the clinical measurements were tested in all samples. No interfering signals were observed at the retention time range of the analyte (Fig. 5). The mass spectra of patient samples have a reproducible pattern with a molecular ion peak at 178 m/z and a base peak at 163 m/z for propofol.

The peak areas were converted into ppb values with both calibration methods (Fig. 6). For liquid calibration, the equation in Section 2.1.3 was used to convert ng to ppb, The measured range during anesthesia of 4.07–16.49 ppb (mean 8.82 ppb ± 0.24 SE, n = 101) for the liquid calibration was reflected by a range of 4.42–18.31 ppb (mean 9.19 ppb ± 0.27 SE) for the gas calibration. Correlation between the two methods yielded a linear regression coefficient of 0.89 with a slope of 1.05 ± 0.01 (SD) for the regression line (dashed blue line).

According to the two methods was tested by a Bland & Altman plot (Fig. 7) [14]. The values are nearly equally distributed with 43% of all data points in the upper area. The plot shows three outliers, below the lower red line. The mean of the differences is 0.34 (green line) and SD is 0.92 with 95% limits of agreement ± 1.81 (red lines).

In view of the obtained propofol concentration mean values in breath of 8.8 ± 2.3SD ppb for the liquid and 9.2 ± 2.7SD ppb for the gas calibration, the limits of agreement are acceptable with a percentage of 20% of the mean breath concentration. If compared with the clinically relevant range of 1 to 5 μg/ml which creates a breath concentration of maximal 50 ppb, the limits of agreement are in a 4% range of the maximal breath concentration.

4. Discussion

We established and validated the quantitative measurement of propofol with Tenax sorbent tubes and TDS–GC–MS. The liquid calibration showed good linearity, precision, trueness, and calibrator stability. LOD and LOQ match the clinical relevant concentrations. The liquid calibration was successfully used to quantify propofol concentration in the exhalation air of 15 patients. The concentration of propofol exhaled by our patients was comparable to the values under continuous propofol measured also via TDS–GC–MS [11] and measured by a gas chromatography coupled surface acoustic wave (GC-SAW) sensor [15]. When headspace solid-phase microextraction (HS-SPME) was used for sampling, the concentrations reported by Chen and co-authors were in the ppt range [16]. How this difference can be explained is unclear. One possibility could be the limited stability of only 4 h for HS-SPME gaseous propofol samples [17], which is a significant drawback for trials that involve enough patients that samples cannot all be measured immediately. Additionally Tenax sampling can be performed at bedside without
Fig. 4. Short-term (A) and long-term (B) stability of propofol gas loaded Tenax tubes. Short-term stability was tested with 10, 30, and 50 ppb, propofol after 24, 48, and 72 h incubation ($n = 2$). Long time stability was tested with 10, 20, 30, 40 and 50 ppb, propofol after 0, 7, and 14 days incubation ($n = 2$).

Fig. 5. Extracted ion chromatograms ($m/z$ 163) with detailed mass spectrum of exhaled air sample from a mechanically ventilated patient during propofol-remifentanil anesthesia.
intermediate storage of breath samples as conducted by Miekisch and others [16,17]. Storage steps may also cause propofol loss due to interactions with the container surfaces.

The gas calibration presented comparable values for linearity, precision, trueness, calibrator stability, LOD and LOQ as the established liquid calibration method, and therefore also meets clinical demands. Previous studies used gas calibration procedures with sealed glass vials with a methanolic propofol solution heated to 40°C for evaporation instead of a gas generator [6,17,18]. At this temperature, it is doubtful whether propofol was completely vaporized and led to correct calibrations. Gong and co-authors used an advanced approach and heated their container with the propofol solution to 250°C and transferred the gas at 25°C into a Tedlar bag (Dalian Delin Gas Packing Co. Ltd, Dalian, China). Different concentrated calibrators where then generated by dilution of that gas sample with nitrogen in new Tedlar bags [16]. However, the potential loss of propofol by adsorption into Tedlar bag surfaces was not investigated.

Both liquid and gas calibrations proved reliable, with satisfactory limits of agreement. The methods show a good accordance and both are thus equally suitable for the calibration of patient breath samples. The difference analysis (Fig. 7) seems to display a slight shift in the value distribution, which can possibly be explained by the fact that the ppb, values of the liquid calibration are calculated with the assumption of an ideal gas. Although this translation is an established procedure [11,19] it may contribute to the shift. Exhaled drugs concentration are normally expressed in ppb, therefore it is useful to make the calibration also in ppb, with evaporated drugs.

The data presented here proved the accuracy and precision of a method for quantitative drug measurements in breath especially with regard to an improved gas calibration. The sampling technique is easily feasible in a clinical setting and can be used for the detection and quantification of exhaled drugs. Even drugs which are exhaled in considerably lower concentrations could be detected by increasing sampling duration. The principle question about the detectability of a drug can be answered with this method relatively fast in the laboratory setup prior to the detection tests in breath. Therefore the TDS-GC–MS with Tenax sampling is a promising method for drug detection and quantification in breath. As an online-drug monitoring this method is not suitable due to tedious analysis time and bulky equipment. For such application techniques like MCC-IMS are more appropriate [4].

At least one monitor for automatic online measurement of exhaled propofol under clinical conditions will soon be introduced commercially. Presumably other devices for propofol and other drugs will fellow. For this new class of medical devices calibration and validation technics are necessary. In this study we were able to validate a gas generator for propofol by comparative measurements. Our results indicate that the HovaCAL can be used for calibration and analytical validation of new devices for measuring volatile propofol. Limitations of the calibration with a gas generator are the space requirement, the price of the unit, and the operating costs related to the need for a carrier gas.

5. Conclusions

TDS-GC–MS with Tenax sampling is a reliable method for quantifying propofol in human breath. Calibration and validation parameters for propofol are comparable using liquid injection or propofol gas made by a gas generator. However, the gas calibration is less time-consuming and thus probably generally preferable.

Acknowledgements

Larissa Walter (Biomedical Science, Reutlingen University, Germany) was partly involved in the experimental execution of the experiments. This work was financially supported by B&S Analytik (Dortmund, Germany). All devices were loaned by B. Braun Melsungen (Melsungen; Germany).

References


