Calibration and validation of a MCC/IMS prototype for exhaled propofol online measurement

Felix Maurer a,*, Larissa Walter a,b, Martin Geiger a, Jörg Ingo Baumbach b, Daniel I. Sessler c, Thomas Volk a, Sascha Kreuer a

a CBR—Centre of Breach Research, Department of Anaesthesiology, Intensive Care and Pain Therapy, Saarland University Medical Centre and Saarland University Faculty of Medicine, 66424 Homburg, Saar, Germany
b Faculty Applied Chemistry, Reutlingen University, Alteburgstrasse 150, 72762 Reutlingen, Germany
c Michael Cudahy Professor & Chair, Department of OUTCOMES RESEARCH, Anesthesiology Institute, Cleveland Clinic, Cleveland, Ohio, USA The Centre of Breath Research is part of the OUTCOMES RESEARCH Consortium, Cleveland, OH, USA

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A B S T R A C T

Propofol is a commonly used intravenous general anesthetic. Multi-capillary column (MCC) coupled ion-mobility spectrometry (IMS) can be used to quantify exhaled propofol, and thus estimate plasma drug concentration. Here, we present results of the calibration and analytical validation of a MCC/IMS pre-market prototype for propofol quantification in exhaled air. Calibration with a reference gas generator yielded an $R^2 > 0.99$ with a linear array for the calibration curve from 0 to 20 ppb. The limit of quantification was 0.3 ppb, and the limit of detection was 0.1 ppb. The device is able to distinguish concentration differences > 0.5 ppb, for the concentration range between 2 and 4 ppb, and > 0.9 ppb, for the range between 28 and 30 ppb. The imprecision at 20 ppb is 11.3% whereas it is 3.5% at a concentration of 40 ppb. The carry-over duration is 3 min. The MCC/IMS we tested provided online quantification of gaseous propofol over the clinically relevant range at measurement frequencies of one measurement each minute.

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1. Introduction

The dosing of propofol during anesthesia is usually based on morphometric characteristics and clinical needs. However, the relationship between administered dose and blood concentrations varies considerably from person-to-person as a function of drug distribution and metabolism. Concentrations estimated from pharmacokinetic equations can thus differ substantially from measured values. Excessive propofol concentrations may provoke haemodynamic instability and delayed recovery, whereas inadequate concentrations increase the risk for awareness and recall [1]. Real-time monitoring of propofol blood concentration thus seems preferable to pharmacokinetic estimates.

A series of studies using various devices have shown that propofol is detectable in patients’ breath [2–6] over the clinically relevant range of 0–39 ppb, [7], and that exhaled concentrations correlate with plasma concentrations [3]. Propofol can be quantified in exhaled air with multi-capillary column ion mobility spectrometry (MCC/IMS) [5,8,9] during anesthesia at a rate of once per minute. However, signals from MCC/IMS systems are in volts so calibration is necessary for comparison with other devices or to estimate plasma concentration. Our goal was thus to calibrate a pre-market prototype MCC/IMS system designed for online measurement of exhaled propofol.

We established a propofol calibration method for MCC/IMS devices using a commercial reference gas generator. Recently, we cross-validated the reference gas generator calibration by gas-chromatography coupled mass spectrometry (GC–MS) measurements of liquid injected sorbent tubes [10]. With the reference gas generator we tend now to calibrate and analytically validate a pre-market prototype for online measurement of exhaled propofol.

2. Materials and methods

2.1. MCC/IMS parameters

The pre-market prototype MCC/IMS system (B&S Analytik, Dortmund, Germany) has an OV5 MCC (Multichrom Ltd, Novosibirsk, Russia) of 12 cm length and with a temperature of 90 °C, providing
a retention time of 23 s for propofol. After pre-separation, ionization is performed by a Na\(^{69}\)\(\beta\)-radiation source. Ions enter the 20 cm long drift region through a Bradbury–Nielsen grid which is opened every 50 ms for 30 μs.

As drift and carrier gas synthetic air (Air Liquide, Düsseldorf, Germany) (20.5% \(\pm\) 0.5% \(\mathrm{O}_2\) in \(\mathrm{N}_2\), purity \(\geq 99.999\%\)) was used. The gas flows were 100 mL min\(^{-1}\) for sample loop flushing, 150 mL min\(^{-1}\) in the MCC and 100 mL min\(^{-1}\) in the drift tube. Samples were collected for 20 s at 1-min intervals with a gas flow of 350 mL min\(^{-1}\).

2.2. Calibration gas generator parameters

A calibration gas generator model HovaCAL 4836-VO (IAS, Oberursel, Germany) \(^{[1]}\) was used with a calibration gas flow of 850 mL min\(^{-1}\). Dosing syringes with a volume of 12.5, 50, 125 and 250 μL were purchased (Hamilton, Planegg, Germany). The propofol solution was vaporized at a temperature of 100°C. Pure water (B. Braun Melsungen, Melsungen, Germany) was simultaneously evaporated to provide a relative humidity of 100% at the selected reference temperature of 37°C, representing physiologic conditions. The carrier gas was \(\mathrm{N}_2\) (purity 3.5%). ViewCAL 1.2.1 (IAS, Oberursel, Germany) software was used for HovaCAL control.

2.2.1. Stock solution

A 90 μg mL\(^{-1}\) propofol solution was used for all measurements. It was prepared by dissolving propofol > 97% (Sigma Aldrich, Steinheim, Germany) in 1% v/v absolute ethanol (Sigma-Aldrich, Steinheim, Germany) in HPLC-grade water (VWR, Darmstadt, Germany). Propofol was gravimetrically dosed with an analytical balance model MSA225P-1CE-DU (Sartorius, Gottingen, Germany) to calculate the exact mass of the propofol concentration in solvent. The stock solution was kept in a 250 mL glass bottle and freshly prepared once per week.

2.3. Experimental setup

The propofol gas was piped through a 2 m long 50°C heated PFA transfer tubing (IAS, Oberursel, Germany) to the sample-in of the MCC/IMS. The tubing contains a stainless steel 1/8” t-piece (Swagelok, Frankfurt, Germany) in the middle to avoid build-up of internal pressure. The experimental setup is illustrated in Fig. 1.

2.4. Calibration

The following propofol concentrations were produced by the reference gas generator and measured with the MCC/IMS: 60, 50, 40, 30, 20, 10, and 5 ppb\(\_v\). Each concentration was held for 30 min, corresponding to 30 measurements. Before and after each concentration, 15 blank measurements were obtained. For dosing into the HovaCAL, a syringe volume of 50 μL was used from 5 to 30 ppb, and a 125 μL syringe was used for the higher concentrations.

2.5. Validation

2.5.1. Linearity

The measured MCC/IMS signal intensity of propofol was plotted vs. the gas concentration of propofol from HovaCAL, excepting the five initial and final measurements at each concentration. Linear regression was used to determine the slope, intercept, and linear range.

2.5.2. Limit of detection/limit of quantification

The limit of quantification (LOQ) as well as the limit of detection (LOD) were determined according to the International Conference on Harmonisation of “Technical Requirements for Registration of Pharmaceuticals for Human Use,” \(^{[12]}\) and based on the signal-to-noise ratio of 3:1 for LOD and 10:1 for LOQ. Specifically, the test concentration was decreased in 0.1 ppb, decrements from an initial concentration of 1 ppb\(\_v\). Each concentration was maintained for 20 min. The noise was calculated as the mean intensity of 10 initial blank measurements.

2.5.3. Precision

Precision of the method was analyzed by holding a concentration of 20 ppb\(\_v\) respectively 40 ppb\(\_v\) for two hours with the reference generator while continuously measuring with MCC/IMS. The standard deviation was determined and imprecision was additionally estimated as difference between the largest and smallest measured value as a percentage of the mean.

2.5.4. Carry over

Concentrations of 20 ppb\(\_v\) and 40 ppb\(\_v\) were maintained for 1 h before changing the concentration to 0 ppb\(\_v\). Concentrations were assayed over the subsequent 15 min to determine the time required for the propofol signal to disappear.

2.5.5. Resolution

The resolution was tested in the concentration ranges from 2 to 4 ppb\(\_v\), and 28 to 30 ppb, in 0.2 ppb, steps. One concentration was held for 20 min with 5 blank measurements before and after each concentration. For dosing into the HovaCAL 50 μL syringes were utilized.

2.6. Data analysis

Data analysis for IMS spectra and peak intensities was performed by VisualNow 3.7 (B&K Analytik, Dortmund, Germany).

2.7. Statistical analysis

Statistical analyses were conducted with SigmaPlot (version 12.5, Systat Software, Erkrath, Germany) using repeated-measures one-way ANOVA for normally distributed data. Alternatively, a repeated-measures one-way ANOVA on ranks was performed. Normality was determined by Shapiro-Wilk test. \(P \leq 0.05\) was considered significant.

3. Results

Fig. 2 shows the MCC/IMS signal intensity in volts vs. the propofol gas concentration in ppb\(\_v\) of the reference gas generator. The middle 20 (of 30) values of each concentration were evaluated (blue dots). The exponential calibration curve (red line) shows a coefficient of determination \(\geq 0.99\). The linear range is short. A linear fitting with a coefficient of determination of 0.92 is just available between 0 and 20 ppb\(\_v\).

The calculations based on the signal-to-noise ratio, provided a LOD of 85 ppt\(\_v\), (0.004 V) and a LOQ of 258 ppt\(\_v\) (0.012 V). The corresponding peaks are shown in Fig. 3. The precision measurements at 20 ppb\(\_v\), and 40 ppb\(\_v\), had a relative standard deviation of 1.8% and 0.8 % and a statistical range of 11.3% and 3.5% of the mean. The statistical range at 40 ppb\(\_v\), is small because of the non-linear measurement range of the device at 40 ppb\(\_v\).

The carry-over was measured after changing a concentration of 20 ppb\(\_v\), and 40 ppb\(\_v\), to 0 ppb\(\_v\). The first value after the propofol evaporation stopped shows a carry-over of 3.5% respectively 6.1% of the average signal intensity at 20 ppb\(\_v\), and 40 ppb\(\_v\).

The resolution was evaluated between 2 and 4 ppb\(\_v\), and 28 – 30 ppb\(\_v\). The measurements between 2 and 4 ppb\(\_v\) (Fig. 4 upper left) can be described with a linear fit yielding an \(R^2 = 0.96\). Using the 95%
Fig. 1. Schematic experimental setup with HovaCAL gas generator and MCC/IMS connected by a heated gas transfer tube with t-piece. Both devices are supplied with carrier gas and are controlled by an external software.

Fig. 2. MCC-IMS calibration with 20 values (blue dots) at each concentration over the range from 0 to 60 ppb<sub>v</sub>. The calibration curve in red increases exponentially: f(x) = 0.5392(1-exp-0.0873) with R² = 0.99. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The measuring range between 28 and 30 ppb<sub>v</sub> shows a correlation coefficient of 0.81 for a linear fit and 0.87 for a polynomial fit. The predicted ppb<sub>v</sub> range for 0.1 V has a width of 0.5 ppb<sub>v</sub> (2.6–3.1 ppb<sub>v</sub>). Hence, the minimal distinguishable concentration difference in this measurement range is > 0.5 ppb<sub>v</sub>.

The prediction interval (red lines), upper and lower prediction boards for 0.1 V can be calculated in ppb<sub>v</sub>. The predicted ppb<sub>v</sub> range for 0.1 V has a width of 0.5 ppb<sub>v</sub> (2.6–3.1 ppb<sub>v</sub>). Hence, the minimal distinguishable concentration difference in this measurement range is > 0.5 ppb<sub>v</sub>.

(28.1–29.3 ppb<sub>v</sub>) when the polynomial fit is used. In this measurement range the differentiation between individual concentrations is possible for differences > 0.9 ppb<sub>v</sub>.

4. Discussion

The propofol calibration curve shows a short linear range and therefore the device is hardly able to differentiate signals above 0.5 V. Several publications reported an expected clinical relevant concentration range between 0 and 39 ppb<sub>v</sub> [2,3,6,9,13–15]. Based on these publications, the linear range of the device is too short for the clinical quantification of propofol in breath. However, one study based on three patients reported a propofol breath concentration of 0.0043 ppb<sub>v</sub>–0.0335 ppb<sub>v</sub> during anesthesia [16]. How this much lower range can be explained remains unclear, but the detected concentration can be influenced by diverse factors as sampling duration and volume, measurement method and many more. As the study of Gong and co-authors is based only on 9 data points, the power is limited. It is probable that the average propofol concentration during anesthesia is higher than reported in that study.

Since the linear range is optimal for quantification measurements the precision at 20 ppb<sub>v</sub> is of particular interest. The statistical range of the precision was evaluated 11.3% for 20 ppb<sub>v</sub>. It is difficult to determine if this statistical range is tolerable for clinical measurements. Although the correlation between propofol blood and breath concentration has been described by several pharmacokinetic modeling approaches in humans [17,18], further studies are needed to design models suitable for clinical practice. The impact of an 11.3% imprecision in breath on the estimation

Fig. 3. Illustration of background noise, LOD, and LOQ propofol signal intensities. Darker blue areas represent higher signal intensity. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
of blood concentration is thus unclear. From an analytical point of view a deviation up to 15% is commonly considered satisfactory.

A carry over effect could be observed in the first minute after a concentration change with a value below 7% of the average signal intensity for 40 ppb. A reason for the effect could be the adhesion of propofol to the inner surfaces of the HovaCAL and the MCC/IMS. Interactions of gaseous propofol with plastic surfaces have been investigated [19]. Plastic in form of polytetrafluoroethylene (PTFE) tubing is installed in the MCC/IMS as gas-conveying line. Further sorption sites in the reference gas generator are stainless steel elements, syringes consisting of glass, or glass bottles with plastic lock. Potentially all of these surfaces can contribute to the carry over. However, after three measurements at one-minute intervals, the carry-over was only 2.4% at 0 ppb, and thereby lower than the imprecision of our method. Furthermore, the low carry over after just one minute of washout is suitable for clinical use.

Also the limits of detection and quantification with values of 85 ppt, and 258 ppt, are lower than required for quantitative breath measurements during anesthesia as breath concentrations are in the ppb range. Furthermore the limits are considerably lower than those reported by Perl and colleagues in a similar setup with MCC/IMS [9]. The technical parts of the used prototype were redesigned and optimized for propofol measurement. This included changing the material of all used tubes and a 2 mL higher volume of the sample loop. An increased loop volume leads to a higher sample volume. Therefore, the detection and quantification limits are lower in our MCC/IMS.

The validation of the resolution obtained minimal distinguishable concentration differences of >0.5 ppb for the lower concentration range and >0.9 ppb for the upper concentration range. Therefore, the resolution over the tested concentration ranges is sufficient for clinical needs.

Our calibration and validation of MCC/IMS is based on measurements of defined propofol gas concentrations. The gas was produced with a reference gas generator, which is more elegant than headspace measurements [20] or gas dilution in Tedlar bags [16]. Nevertheless, the gas concentrations can be inaccurate for two reasons: First, the generator estimates concentrations using ideal gases laws, which is the same for all common methods, but is not exactly correct. However, it can be assumed that the related deviation is negligibly small. Second, inner surfaces can lead to carry over and falsify the concentration of the output gas at least as long as sorption and desorption are not in equilibrium. However, the calibration with the reference gas generator was cross-validated with a liquid injection to sorbent tubes for thermal desorption GC–MS [10]. When we compared the method to the liquid injection technique, the reference gas generator method showed satisfactory limits of agreement and a good accordance.

We aimed to calibrate and analytically validate a pre-market prototype for online measurement of exhaled propofol. The calibration technique proved to be uncomplicated and fast. The limit of detection and quantification were sufficiently to permit propofol quantification over the full clinical range.
5. Conclusions

The calibration and validation procedure of a pre-market prototype MCC/IMS with a reference gas generator was successfully established. The validation demonstrated that the analytical requirements except the linear measurement range are fulfilled to conduct propofol monitoring in breath.

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