Determination of serum propofol concentrations by breath analysis using ion mobility spectrometry

T. Perl¹*, E. Carstens¹, A. Hirn¹, M. Quintel¹, W. Vautz², J. Nolte² and M. Jünger²

¹Department of Anaesthesiology, Emergency and Intensive Care Medicine, University of Göttingen, Robert-Koch-Str. 40, 37099 Göttingen, Germany. ²ISAS—Institute for Analytical Sciences, Metabolomics Department, Dortmund, Germany

*Corresponding author. E-mail: tperl@med.uni-goettingen.de

Background. We aimed to measure propofol concentrations in exhaled air with an ion mobility spectrometer coupled to a multicapillary column for pre-separation (MCC–IMS). In addition, we aimed to compare the values of these measurements with serum propofol concentrations, as determined by gas chromatography–mass spectrometry (GC–MS).

Methods. Thirteen patients, ASA I or II, undergoing elective ENT surgery were studied. Anaesthesia was induced with propofol 2.1 (0.7) mg kg⁻¹, rocuronium 0.5 (0.1) mg kg⁻¹, and remifentanil 0.5 μg kg⁻¹ min⁻¹. After tracheal intubation, anaesthesia was maintained with a continuous infusion of propofol 3.9 (1.8) mg kg⁻¹ h⁻¹ and remifentanil 0.5 μg kg⁻¹ min⁻¹. Simultaneously, a venous blood sample was obtained. Propofol concentrations in serum were determined by GC–MS and compared with the height of the respective propofol signals achieved by MCC–IMS.

Results. Twenty-four pairs of samples were obtained. The comparison of propofol concentrations in exhaled air and serum presented a bias of −10.5% and a precision of ±12.3%. With these values, the 95% limits of agreement were 14.1% and −35.1%.

Conclusions. MCC–IMS may be a suitable method to determine propofol concentrations in exhaled air, and may be used to predict propofol concentrations in serum.

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Monitoring the level of volatile anaesthetics in end-tidal breath during anaesthesia is a standard procedure to ensure safety of the patient and to adjust anaesthetic depth. Presently, the monitoring of i.v. anaesthetics is difficult, as complex and time-intensive off-line analysis of blood samples is necessary. High doses of propofol can be responsible for haemodynamic instability, and low dosage of propofol may lead to intraoperative awareness.¹ A method to determine serum concentrations of i.v. anaesthetics such as propofol is desirable to improve safety and adjust anaesthetic depth or the level of sedation in the intensive care unit.

Propofol has been measured in exhaled gas by methods such as proton transfer reaction–mass spectrometry (PTR–MS),² ³ thermal desorption gas chromatography–mass spectrometry (GC–MS),⁴ ⁵ and ion molecule reaction–mass spectrometry (IMR–MS).⁶ With these methods, a close correlation between the propofol concentration in exhaled air and that in plasma was found.³–⁶ All methods used mass spectrometry, which requires complex or demanding techniques like vacuum pumps and, due to technical and methodological complexity, is unlikely to be applicable during routine clinical use for closed-loop anaesthesia with propofol.

For the present investigation, an ion mobility spectrometer (IMS) in combination with a multicapillary column (MCC) was used to directly quantify propofol in exhaled air. IMS has successfully been used for the detection of chemical warfare agents, explosives, and illegal drugs,⁷–¹⁰ and MCC–IMS may have potential medical applications.¹¹–¹³ IMS provides high sensitivity (detection limits in the ng litre⁻¹, pg litre⁻¹, ppb, and ppt ranges)
combined with high-speed data acquisition. Owing to the relatively low technical expenditure, the equipment is transportable, less bulky, and inexpensive.

In this study, we calibrated an MCC–IMS to humidified gas mixtures with known propofol concentrations. Subsequently, we measured the propofol concentration in expired air and compared the results with the serum concentrations of propofol as measured by GC–MS.

Methods

The study was approved by the Ethics Committee of the University of Göttingen (protocol no. 01/07/08). All patients gave written consent to participate in the investigation.

Thirteen patients (nine males and four females), ASA I or II, mean (range) age 45 (18–69) yr, mean (sd) BMI 24.6 (6.2), undergoing elective ENT surgery, were enrolled. Anaesthesia was induced with propofol 2.1 (0.7) mg kg⁻¹ (Disoprivan 1%; AstraZeneca, Wedel, Germany) and remifentanil 0.5 μg kg⁻¹ min⁻¹. To facilitate tracheal intubation, rocuronium 0.5 (0.1) mg kg⁻¹ was administered. After tracheal intubation, lungs were ventilated with a standard anaesthesia respirator (Cato, Dräger, Lübeck, Germany). High air flow was used (air flow >minute ventilation volume) with an $F_{I_o}$ of 0.5. Ventilator settings were a tidal volume of 6.9 (1.6) ml kg⁻¹, a ventilatory frequency of 11.5 (1.1) bpm, and an inspiration/expiration ratio of 1:1.5. Anaesthesia was maintained without a specific protocol by continuous administration of propofol 3.9 (1.8) mg kg⁻¹ h⁻¹ and remifentanil 0.5 μg kg⁻¹ min⁻¹, adjusted to clinical requirements.

Multicapillary column–ion mobility spectrometer

A custom-designed IMS (Fig. 1) with a β-radiation source ($^{63}$Ni, 550 MBq) for ionization was used for breath analyses. As the operating principle of MCC–IMS has been described before, only a brief description will be given here. The gas of an expired air sample is pre-separated by a multicapillary chromatographic column. Owing to pre-separation, the compounds contained in expired air enter the drift tube with different retention times. The gas-phase propofol is ionized by β-radiation. It is then injected into a drift tube where it is simultaneously attracted by an electrical field to a detector and decelerated by a counter-current gas (i.e. drift gas). The operating principle is explained in more detail in the Appendix.

Measurement of serum propofol concentrations by GC–MS

The sample was injected in one bolus at 250°C into an Agilent Technologies 6890N GC-system connected to an Agilent Technologies 5973 mass selective detector (MSD; Gerstel, Mülheim, Germany). An HP-5MS capillary column (Wicom GmbH, Heppenheim, Germany) was used for compound separation with helium as carrier gas at a constant flow rate of 1.0 ml min⁻¹. Electron ionization mode was used with 70 eV, and a mass range of $m/z$ 33–450 was detected.

Fig 1 Schematic diagram of an MCC–IMS.
After induction of anaesthesia, gas sample tubing (polytetrafluoroethylene, 220 cm, 1.6 mm S1810-26, Fa. Bohlender, Grünsfeld, Germany) was attached on the patient side of the HME filter (Teleflex Medical, Humid-Vent® Filter Compact S, REF 19401, Teleflex Medical GmbH, Kernen, Germany). Gas sampling control was performed as basically described by Schubert and colleagues15 (Fig. 2). Carbon dioxide content was measured by mainstream capnography (IRMA, Fa. PhaseIn, Danderyd, Sweden). If carbon dioxide exceeded 3.33 kPa, the sample loop (8 ml) was rinsed with the sample over a T-piece connection using a pump to obtain an end-tidal sample. A sample (8 ml) was obtained from multiple breaths (20 s expiratory time) and was then injected into the MCC directly.

At the same time as gas sampling, venous blood was drawn for analysis from the arm that was not used for the infusion.

All samples were collected under steady-state conditions, which were achieved by constant administration of propofol and remifentanil for at least 15 min. Before each sample acquisition, a blank measurement using humidified synthetic air was performed to ensure a propofol-free system. Depending on the duration of surgery, one or two samples were obtained per patient.

To determine the relationship between exhaled breath propofol concentration ($c_{AP}$) and serum propofol concentration ($c_{PP}$), single linear regression analysis was performed. Statistical calculations were done with Statistica 8.0 (StatSoft Inc., Tulsa, OK, USA). The agreement between both measures was assessed according to Bland and Altman.16 The concentration of propofol in exhaled air is described in ppb, whereas the propofol serum concentration is given in μg ml$^{-1}$. As the IMS signal of propofol in exhaled air and propofol serum concentrations have different units, both signals were equalized. The maximum measured value for each method was defined as 100%.17

**Results**

The linear range of the GC–MS analysis of serum propofol concentration was verified to range from 2 to 10 μg ml$^{-1}$ ($r^2=0.93$). The intraday precision was 12.2% (8.9–14.8%).

For the observed range of 1–10 ppb, we found a linear relationship between gas concentrations supplied by HovaCal and signal intensities as determined by MCC–IMS ($r^2=0.984$). The limit of detection (LOD) was 700 ppt, and the limit of quantification (LOQ) was 1.5 ppb. Furthermore, intraday precision was calculated for six concentrations (four measurements each) with 2.5% (0.8–4.3%) (Table 1).

The correlation coefficient ($r^2$) between exhaled breath propofol concentration ($c_{AP}$) and serum propofol concentration ($c_{PP}$) was 0.73. Results of linear regression analysis are presented in Figure 3.

Serum propofol concentrations ranged from 0.3 to 4.3 μg ml$^{-1}$. The observed propofol concentrations in exhaled

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**Fig 2** Schematic diagram of the sample acquisition device mounted on the respirator circuit. The valve control toggles the two-way valve to the sample loop if carbon dioxide exceeds a level of 3.33 kPa. A/D, analogue to digital.
air ranged from 5 to 15.6 ppb. Propofol concentrations in exhaled air measured with MCC–IMS and serum propofol concentrations measured with GC–MS were normalized. The mean difference between the normalized values was 10.5% with an SD of 12.3% and a 95% confidence interval from +14.1% to 35.1% (Fig. 4).

**Discussion**

The study demonstrates that an MCC–IMS can be used to determine the propofol concentration in expired air. The propofol concentration in expired air correlates with serum propofol concentrations during steady-state total i.v. anaesthesia, and therefore, the measurement may be used as a non-invasive technique for determination of serum propofol concentration. The MCC–IMS provides the technique for point of care, online, and end-tidal propofol monitoring.

The determination of propofol in exhaled air under total i.v. anaesthesia has been studied by several other investigators. The techniques used varied from thermal desorption–gas chromatography–mass spectrometry to IMR–MS and to PTR–MS. All investigations describe the feasibility of propofol measurement in exhaled air with a close relationship to serum/blood concentrations. The current investigation strongly supports these findings by using the MCC–IMS system as a novel technique in this context.

The advantage of direct mass spectrometry systems is the possibility of real-time measurements at point of care. As the analysis focused on a single compound (propofol), the time for one measurement could be reduced to 1 min. It is therefore conceivable that in the future, propofol infusion rates may be controlled by end-tidal propofol concentrations.

However, analysis of propofol breath concentrations as a clinical application has potential limitations. Miekisch and colleagues investigated the relationship of venous and arterial blood propofol concentrations to exhaled air propofol concentration. They described a correlation between arterial blood and exhaled air propofol concentration, but were unable to find a correlation between venous and exhaled air propofol concentration. This issue contrasts to a study by Grossherr and colleagues describing an animal experiment resulting in a close correlation between arterial and mixed venous propofol concentrations. Differences between mixed venous and arterial propofol concentration might occur due to an extrahepatic metabolism or first pass effect. In the present study, we did not measure arterial serum propofol concentrations, but found, in contrast to Miekisch and colleagues, a correlation between venous serum and exhaled air propofol concentration, consistent with Grossherr and colleagues. For a discontinuous measurement of exhaled air, the dynamic changes of propofol concentrations due to bolus injections or changes in administration rate have to be considered. From injection
of the propofol bolus to the first detection of volatile propofol, a time range of 41 s was observed. Maximum volatile propofol concentration was observed 333 s after bolus injection.3

The slope of regression from the relationship of serum propofol concentration and exhaled air propofol concentration varied between different species (goats and pigs) with 10-fold higher exhaled air concentrations in pigs.4 Inter-individual variation of the slope of regression was also reported.4 Physiological parameters as an impaired ratio of ventilation and perfusion due to intrapulmonary right–left shunt might lower exhaled air propofol concentrations, as demonstrated in patients undergoing lung surgery.18

The method of sample acquisition is a recently discussed topic. Harrison and colleagues2 and Hornuss and colleagues5 measured mixed gas samples with a side-stream technique. A quantification of mixed gas samples is not appropriate because the inspiratory to expiratory ratio may vary. End-expiratory samples can be obtained by CO24 15 or temperature measurement.3 In this study, a CO2-controlled sample acquisition was used. The delay in CO2 data acquisition for mainstream sensors is more than 150 ms and may lead to invalid sample acquisition, if the ventilatory frequency or inspiratory time ratio changes. The effect is more important for multiple breath analysis4 19 than for continuous breath analysis.3 A further improvement in sample acquisition technique may allow more precise sampling and therefore more accurate breath analysis results.

The technical and methodological problems described above suggest that none of the methods published so far is ready for clinical routine application. The MCC–IMS system, in terms of technical and methodical expenditure, is one step closer to clinical use. However, further investigations regarding the described potential limitations are necessary for the development of a validated quasi-online and non-invasive tool for the determination of blood/serum propofol concentrations via breath analyses.

In conclusion, this is the first time an MCC–IMS has been used to measure exhaled gas propofol concentrations. We found a correlation between these values and venous propofol concentrations. The MCC–IMS method can give a point of care measurement of exhaled gas that may allow non-invasive assessment of blood propofol concentration. Fortunately, a pre-concentration of breath samples is not necessary. Even if a real-time measurement is not possible, the online measurement (~1 min after sample acquisition) of exhaled propofol is feasible. An infusion control might be possible as well.

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Appendix
Multicapillary column–ion mobility spectrometry and calibration procedures
The pre-separation was performed isothermally at 40°C on a 20 cm non-polar multi-capillary chromatographic column (OV-5, Multichrom, Novosibirsk, Russia). The column contains ~1000 capillaries with an inner diameter of 43 μm each and a film thickness of 0.2 μm. The sum of the single capillaries allowed a gas flow of 150 ml min−1. After pre-separation, the gas-phase analyte is ionized by a β-radiation source. A Bradbury–Nielsen grid is opened every 100 ms for 300 μs, thus introducing an ion cloud into the drift tube. Under the influence of an external electrical field, the ions move towards a detector (Faraday plate). During their drift to the detector, they collide with the drift gas molecules moving in the opposite direction. Therefore, the ions are decelerated depending on their size and shape and are in the ideal case totally separated. The ion drift time is measured and the velocity of the ions can be calculated for a known drift distance. By relating the ion velocity to the electric field, ion mobility is calculated, and then by correcting for temperature and pressure, an expression for ion mobility can be calculated that is characteristic for the ion and independent of other factors.4

Calibration
For calibration of MCC–IMS with propofol concentrations in humid gas samples, a calibration gas generator was used (HovaCal 3834SC VOC, Inspire Analytical Systems GmbH, Frankfurt am Main, Germany). This generator provides gas mixtures of up to three components in the ppb–ppt range and relative humidity up to 100%. All generated gaseous propofol concentrations (1, 3, 5, 7, 9, and 10 ppb) were analysed with the MCC–IMS and peak intensity was correlated to each expected concentration of propofol. The linearity of the method was determined by linear regression analysis. The LOD was calculated as 3×
sd+mean signal intensity in the peak area from multiple blank samples. Additionally, the LOQ was determined from multiple blank samples, which was defined as 10× sd+mean signal intensity.21 Intraday assay precision was determined by calculation of relative standard deviation (%RSD) from 24 propofol measurements of a defined gas mixture.

We used propofol-spiked serum concentrations of 2, 4, 5, 6, 8, and 10 µg ml⁻¹ to calibrate the GC–MS analysis. Calibration for internal standardization was achieved by linear regression analysis curve fitting. Integrated signals (total ion chromatogram) from the internal standard thymol (m/z 135 150) and from propofol (m/z 163 178) were evaluated by AMDIS/NIST (Automated Mass Spectral Deconvolution and Identification System; version 2.62, 2005; NIST version 2.0, 2005).

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