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Short communication

Gas chromatography–mass spectrometric assay for propofol in cerebrospinal fluid of traumatic brain patients

Mariska Y.M. Peeters [a,*], Hiltjo Kuiper [b], Ben Greijdanus [b], Joukje van der Naalt [c], Catherijne A.J. Knibbe [a,d], Donald R.A. Uges [b]

[a] *Department of Clinical Pharmacy, St. Antonius Hospital, P.O. Box 2500, 3430 EM Nieuwegein, The Netherlands*
[b] *Laboratory for Clinical and Forensic Toxicology and Drug Analysis, Department of Pharmacy, University Medical Center Groningen, The Netherlands*
[c] *Department of Neurology, University Medical Center Groningen, The Netherlands*
[d] *Division of Pharmacology, Leiden/Amsterdam Centre for Drug Research, Leiden University, The Netherlands*

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Abstract

A sensitive gas chromatography–mass spectrometry method for measuring propofol in cerebrospinal fluid is described, validated and applied to four patients after traumatic brain injury. The limit of quantitation was 2 μg/L using a volume of 0.5 mL. The inter- and intra-assay coefficients of variation were 5.9 and 5.1%, respectively. The assay covers the linear concentration range of 2–50 μg/L, which is relevant in clinical pharmacokinetic studies when propofol is used in the Intensive Care Unit as a sedative agent or to lower the intracranial pressure.

Keywords: Propofol; CSF; GC–MS

1. Introduction

Propofol (2,6-diisopropylphenol) is a short-acting highly lipophilic intravenous anesthetic that is widely used for anesthesia and sedation in the Intensive Care Unit. The mechanism of action is not completely known, but γ-aminobutyric acid (GABA) receptors within the central nervous system appear to be involved. The distribution of propofol from blood into cerebrospinal fluid (CSF) during neurosurgical procedures in patients who suffered hemorrhages, ruptured intracranial aneurysms or tumor removals, using high doses of propofol (6–12 mg kg^{-1} h^{-1}) has been investigated using high-performance liquid chromatography (LC), resulting in concentrations of 35–87 μg/L, which is approximately 40–80-fold lower than in blood, depending on the type of the procedure [1,2]. To date, the CSF–blood ratio has not been studied in the Intensive Care Unit, when propofol is given for sedation or reduction of elevated intracranial pressure, using doses up to a maximum of 5 mg kg^{-1} h^{-1}. Since especially neurological patients and prolonged use (>48 h) of propofol at doses ≥5 mg kg^{-1} h^{-1} are associated with the propofol-infusion syndrome [3,4], information of propofol concentrations in CSF of patients with different intracranial pathology will be important to optimize dose regimens. However, the administration of lower doses requires a more sensitive method for quantitation. For the bio-analysis of relative small molecules (propofol 178.3 Dalton), gas chromatography with mass spectrometry (GC–MS) may be preferred above LC–MS. In addition, our laboratory is more experienced with GC–MS than with LC–MS/MS.

In this paper, a highly sensitive and simple method is presented for the determination of propofol in CSF by GC–MS, which can be used to measure propofol concentrations during low doses of propofol and in a small volume of CSF. In addition, the influence of sample storage temperature on the propofol concentration in CSF is described.

2. Experimental

2.1. Drugs and chemicals

Pure propofol was obtained from Bufa BV (Uitgeest, The Netherlands). Thymol was purchased from Brocacef (Maarssen,

* Corresponding author. Tel.: +31 306092612; fax: +31 306093080.
E-mail address: r.peeters@antoniusmesosgroep.nl (M.Y.M. Peeters).

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The Netherlands), di-sodiumtetraborate decahydrate, *n*-heptane and methanol LichroSolv were purchased from Merck (Darmstadt, Germany).

2.2. Standard calibration curve

A stock solution of propofol (0.5 mg/mL) was prepared in methanol and stored for a maximum of 1 week at 4 °C under nitrogen gas to protect it from oxidation. The stock solution of the internal standard thymol (1 mg/L) was prepared in distilled water. Immediately prior to running the propofol stock solution was further diluted with water to 0.5 mg/L. Blank CSF samples (500 μL) were spiked with 2, 5, 10, 15, 20, 30, 40 and 50 μL of the propofol solution (0.5 mg/L), resulting in calibration samples containing 2, 5, 10, 15, 20, 30, 40 and 50 μg/L of propofol.

2.3. Extraction procedure

Thymol stock solution (1 mg/L) of 50 μL was added to 500 μL of CSF samples. Borate buffer (pH 9) of 1 mL and 75 μL *n*-heptane were added and mixed thoroughly for 20 min on a Heidolph Reax 2 mixer (Scientific Ltd., New Brunswick). After centrifugation for 5 min at 1500 × *g* the glass tube was placed for 5 min at −40 °C. The organic layer was then transferred to an injection vial and 0.3 μL was injected into the column of the GC–MS.

2.4. Apparatus and chromatographic conditions

An Autosystem XL Gas Chromatograph (Perkin-Elmer, Groningen, The Netherlands) was used combined with a Turbomass Gold Mass Selective detector (Perkin-Elmer). Separation was performed by injection in spitless mode (valve time 50 s) of 0.3 μL of the extract in a Varian Chrompack WCOT Fused Silica capillary column (25 m × 0.25 mm i.d.) coated with CP-Sil 8 CB Low Bleed/MS (0.25 μm film thickness) (Bergen op Zoom, the Netherlands). The injector temperature was 250 °C and the oven temperature was held at 80 °C at 1 min, increased at 10 °C/min up to 190 °C. The transfer line temperature was set to 225 °C. The helium gas flow rate was 1.3 mL/min. The electron energy was set at 70 eV and the electron multiplier voltage was 480 V. The propofol and thymol molecules were detected by using molecular ions at *m*/*z* 178 and 150, respectively, and for the quantitation the major ions due to loss of methylgroups at *m*/*z* 163 and 135, respectively. Under these conditions, the retention times of propofol and thymol were 7.35 and 6.47 min, respectively.

2.5. Limit of quantitation

The limit of quantitation (LOQ) was defined as the concentration where the accuracy (%) and the inter-day coefficients of variations (%CV) lie within 20% limits and as the lowest expected concentration that produces ion ratios within 20% of the averaged calibrator ratios.

2.6. Precision, accuracy and recovery

Intra- and inter-day coefficients of variations (%CV) were obtained using CSF samples spiked with four known quality control (QC) concentrations of propofol (2, 8, 25 and 45 μg/L). For the intra- and inter-assay each concentration was prepared and analyzed six times on 3 consecutive days stored at 4–8 °C. Accuracy was measured as the percent deviation from the nominal concentration. The absolute recovery was calculated by comparing the amounts of the QC sample 25 μg/L after the extraction procedure with the corresponding *n*-heptane solution six times a day, on 3 consecutive days.

2.7. Stability

Aliquots of a mediate prepared QC sample with initial concentration 23.9 μg/L were stored at room temperature (21 °C), at 4–8 °C and at −20 °C until analysis on the first 3 days and maximal 1.5 months later. The influence of one and two freezing–thawing cycles were determined by storage at −20 °C for 24 h, followed by 24 h frozen storage after thawing of the QC aliquot. To determine the extract stability in the autosampler, the organic layers of the QC samples (10 and 70 μg/L) after being extracted were transferred into the injection vials and assayed immediately and 28 h after storage at 21 °C.

2.8. Patients' samples

After approval by the Ethical Committee of the University Medical Centre Groningen and informed consent, an arterial sample and a CSF sample from the intraventricular drainage system were collected simultaneously from four different patients with traumatic brain injury admitted to the Intensive Care Unit who required continuous infusion of propofol (Propofol®-Lipuro 20 mg/mL, B Braun, Melsungen, Germany) for sedation and lowering of the intracranial pressure. The CSF samples were collected in glass tubes and stored at −20 °C until analysis. Blood samples were collected in oxalate tubes and stored at 4 °C until analysis (within 2 weeks).

2.9. Assay propofol in whole blood

Propofol concentrations in whole blood were measured using high-performance liquid chromatography with fluorescence detection [5]. The limit of quantification was 0.035 mg/L. Inter- and intra-assay coefficients of variation were less than

Table 1
Intra- and inter-day coefficient of variation (%CV) of propofol in cerebrospinal fluid

Concentration (μg/L)	Precision	
	Intra-day (%CV), $n = 18$	Inter-day (%CV), $n = 3$
2.13	5.1	2.5
8.51	4.5	0.0
26.6	4.6	4.6
47.9	3.4	5.9

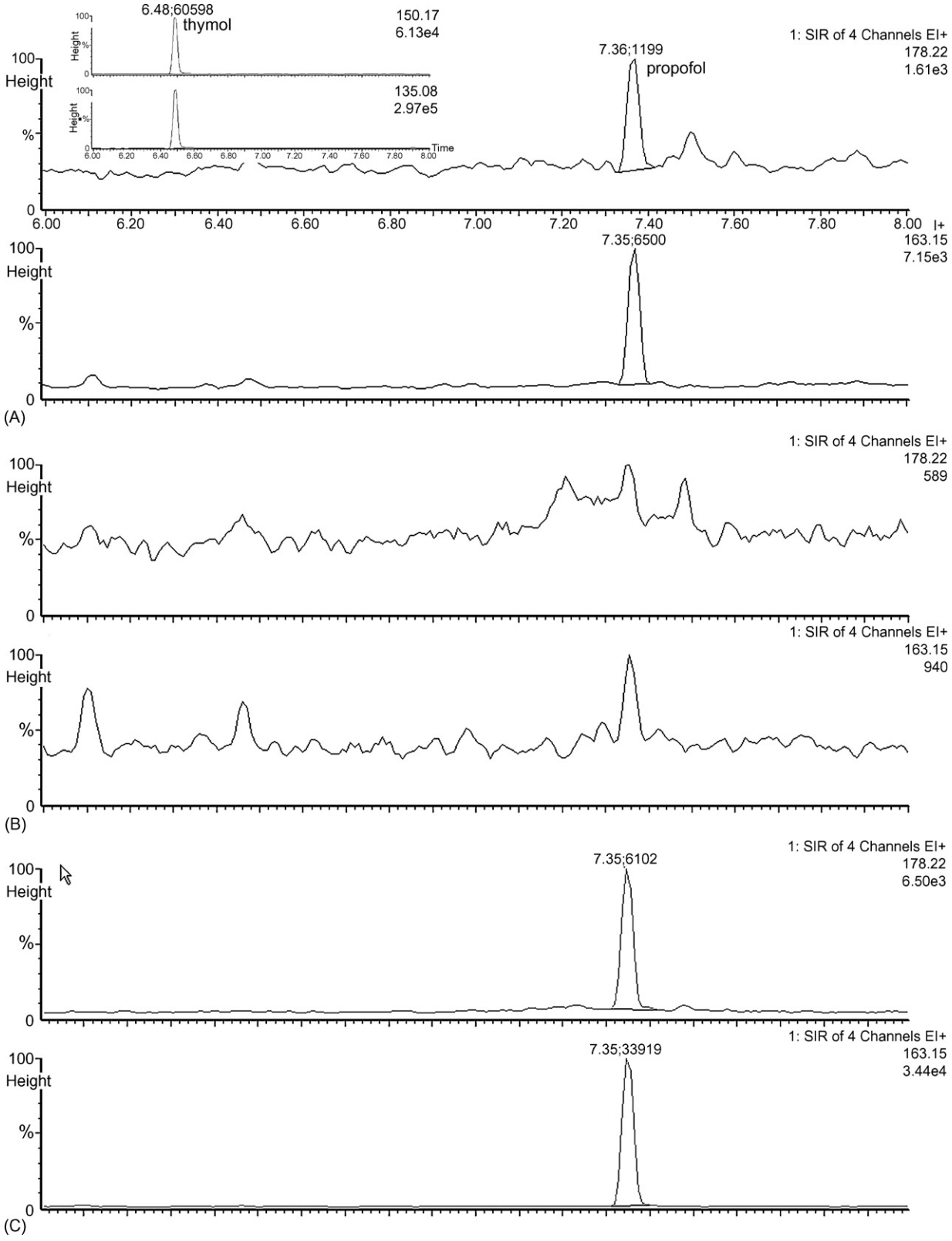

Fig. 1. Total ion chromatograms from retention time 6.00 to 8.00 min of 2 μg/L propofol (LOQ) (A) and internal standard thymol (inset A), blank human cerebrospinal fluid (B), and a patient cerebrospinal fluid sample with a concentration of 21.6 μg/L propofol (C).

9.6 and 2.6%, respectively, over the concentration range 0.5–5 mg/L.

2.10. Statistical analysis

The data are expressed as mean value with standard deviation (S.D.) where applicable.

3. Results

3.1. Limit of quantitation and linearity

The limit of quantitation was determined to be 2 μg/L. The calibration curve was linear in the range 2–50 μg/L. The linear regression equation was (mean ± S.D., $n = 3$): $y = 0.015\,x$ (±0.0011) − 0.001 (±0.000095) and the correlation coefficient 0.9997 (mean).

3.2. Precision, accuracy and recovery

The results from the validation of the method are shown in Table 1. The quality control samples were found to be −12.9, −18.1, −8.7 and −6.5% of their respective nominal value. The recovery from CSF was found to be 112.0 (S.D. 5.3) on day 1, 112.9 (S.D. 11.9) on day 2 and 117.1 (S.D. 5.2) on day 4, which resulted in a recovery of 114.0% (S.D. 2.7) for propofol and 68% for thymol.

3.3. Chromatograms

Fig. 1 shows the total ion chromatograms obtained from a LOQ spiked CSF sample, blank human CSF and a CSF sample from a patient during intravenous infusion with propofol from retention time 6.00 to 8.00 min.

3.4. Stability

The rate of propofol loss was greater in higher temperatures. The concentration of propofol assayed before and after one freezing cycle and two freezing cycles were 23.9, 24.4 and 23.0 μg/L, respectively. A storage time of 1.5 months at −20 °C did not result in any loss of propofol. When kept at 4–8 °C, propofol CSF samples were found to be stable during the first 3 days (23.9, 24.4 and 23.5 μg/L, respectively), followed by a decrease to 15.5 μg/L on day 10. Sample storage at 21 °C for 1 day resulted in a decrease of concentration from 23.9 to 21.1 μg/L. After 6 days at 21 °C the concentration decreased to 5.1 μg/L.

Twenty-eight hours storage of the organic layer of the concentrations 10 and 70 μg/L in the injection vial at 21 °C resulted in a decrease of 7.3 and 7.7%, respectively.

3.5. Patients' samples

The concentration of propofol was found to be 21.6 μg/L in CSF and 2.7 mg/L in whole blood in patient 1 (man, 18 years, 88 kg), who received 380 mg/h propofol during 32 h, 7.1 μg/L in CSF in patient 2 (man, 43 years, 85 kg) who received a continuous infusion of 400 mg/h (no whole blood available) and 15.9 μg/L in CSF and 2.1 mg/L in whole blood in patient 3 (man, 32 years, 85 kg) receiving an infusion rate of 300 mg/h during 36 h. The propofol CSF and blood concentration in patient 4 (man, 21 years, 90 kg) were 48.8 μg/L and 4.98 mg/L, respectively, taken 3 h after the infusion rate was temporary increased to 700 mg/h.

4. Discussion and conclusion

The GC–MS assay described is sensitive, precise and accurate for determination of propofol in a low volume of CSF (0.5 mL), when low doses of propofol ($\leq 5\,mg\,kg^{-1}\,h^{-1}$) are administrated.

For whole blood, using a comparable method [6], a limit of quantitation was found that was five times higher. The improved LOQ of the current method can be explained by the lower volume of the organic layer and the use of the relatively clean medium CSF, although the injected sample volume was three times lower and comparable volumes of the medium (whole blood or CSF) were used. Stetson et al. [7] also described a GC–MS method for propofol determination in plasma, but the sample pretreatment consisted of additional steps of evaporation to dryness and derivatization, which increases in our view the risks of propofol loss. The method of high pressure liquid chromatography with fluorescence detection, which has the advantage of low costs and wide availability, is not sensitive enough to cover the concentration range of interest in CSF at propofol infusion rates of $\leq 5\,mg\,kg^{-1}\,h^{-1}$. The LOQ for a precipitation method was as high as 30 μg/L for whole blood [5,8]. Only after evaporation to dryness beyond extraction, a limit of detection of 1.1 μg/L in CSF has been achieved [9–11]. Using an electrochemical detector, the level was reported to be 20 μg/L in whole blood [12], a value too high to use in intensive care patients. A similar LOQ was obtained with the method of LC–ESI/MS/MS (electrospray ionization) using a derivatization step to enhance the signal intensity [13]. Better limits in plasma (5 μg/L) were observed with solid phase extraction and APCI/MS/MS detection (atmospheric pressure chemical ionization) [14]. Analysis by LC–MS was described before for identification of metabolites of propofol, but the LOQ of the method was not reported [15]. The current described assay had a relatively high recovery, which may be a result of loss of the organic layer, but interference from substances did not occur (Fig. 1). The applicability was demonstrated by the analysis of the patients' CSF samples during continuous infusion of propofol in the neuro-intensive care, resulting in concentrations varying from 7 to 50 μg/L. The propofol concentration in CSF found was 100–130-fold lower than in whole blood. Although in only three patients the ratio between CSF and blood was measured (equal to 0.80, 0.76 and 0.98%), it seems that penetration of propofol to CSF in patients with traumatic brain injury is less than in patients surgically treated for ruptured intracranial aneurysm, tumors or hemorrhages, in which the ratio was 2.6, 1.2 and 1.6%, respectively [1,2]. This may indicate that higher doses of propofol are required in these patients' population. Furthermore, the study

shows that propofol CSF samples after collection can be stored for 3 days at 4–8 °C and for a maximum period of 24 h at room temperature. At −20 °C, CSF samples are stable for at least 1.5 month. Comparison to reported losses of propofol in blood and plasma at 4 °C indicates that speed of loss is not diminished in CSF [16]. However, evaporation may be more evident with use of a low-volume sample.

This aforementioned sensitive GC–MS assay can be used for pharmacokinetic analysis of propofol in CSF when propofol is used in the Intensive Care Unit as a sedative agent or to lower the intracranial pressure.

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