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Determination of propofol in low-volume samples by high-performance liquid chromatography with fluorescence detection

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Abstract

In order to determine propofol in rat whole-blood samples of 50 μl, we developed a rapid, simple and reliable method which is characterized by precipitation of blood elements with acetonitrile and submission of the supernatant to HPLC analysis with fluorescence detection. The method described is linear from 0.4 to 40 mg/l and the relative standard deviations in this concentration range are less than 10%. The limit of quantification proved to be 0.4 mg/l. Blood constituents do not interfere with the assay. © 1998 Elsevier Science B.V.

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1. Introduction

Propofol (2,6-diisopropylphenol) is a highly lipid-soluble anaesthetic agent, which, formulated in an intravenous fat emulsion, induces anaesthesia that is rapid both in onset and in recovery. These pharmacological properties make propofol very suitable for induction and maintenance of anaesthesia as well as for sedation in the intensive care unit. The pharmacokinetics of propofol have been widely investigated, usually by determination of propofol in whole blood by high-performance liquid chromatography (HPLC) with fluorescence detection, as previously described [1]. This method is based on extraction of propofol from whole blood with cyclohexane, evaporation of the cyclohexane layer to dryness, redissolution of the residue in the HPLC mobile phase and submission to HPLC analysis. Despite the limit of quantification of circa 2 μg/l, this method cannot be applied to samples from experiments on small animals, where the amount of blood sampled is usually restricted to 50 or 100 μl, whereas the extraction technique would require 1 ml. Furthermore, the extraction method is time-consuming and therefore unsuitable for large series.

In order to quantify propofol in low-volume samples, we developed a rapid, simple and reliable method which is characterized by precipitation of blood elements with acetonitrile and submission of the supernatant to HPLC analysis with fluorescence detection. The method described is applied to a series of whole-blood samples which were collected in a pharmacokinetic/pharmacodynamic study of propofol in rats.

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2. Experimental

2.1. Drugs and chemicals

Acetonitrile (HPLC grade) was purchased from Mallinckrodt Baker (Deventer, The Netherlands), methanol (analytical grade) and trifluoroacetic acid (spectroscopy grade) from Merck (Darmstadt, Germany) and thymol (purity>99%) from Fluka (Buchs, Switzerland). Propofol (2,6-diisopropylphenol), 97% purity, was obtained from Aldrich (Axel, Belgium) and purified in our laboratory [2]. Distilled water was obtained from NPBI (Emmer-Compascuum, The Netherlands).

2.2. Preparation of blood samples

Whole-blood samples of 50 μl were, immediately after collection, hemolysed in 250 μl of deionized water, vortexed and stored at approximately 4°C until analysis (300-μl samples). Hemolysed-blood samples were found to be stable for at least a month when kept at approximately 4°C.

2.3. Preparation of standard solutions and blood standards

Standard solutions were prepared immediately prior to running. Fifty, 100, 200, 350 and 500 μl of a stock solution of propofol in methanol (1 mg/ml) were diluted to 50.0 ml with methanol for the low-range calibration curve. For the high-range calibration curve 100, 200, 400, 700 and 1000 μl of a stock solution of propofol in methanol (5 mg/ml) were diluted to 50.0 ml with methanol. Standard solutions were found to be stable for at least a week when kept at approximately 4°C.

Blood standards were prepared by spiking 300 μl of a mixture of control, whole blood and distilled water (50 μl+250 μl) with 20 μl of each standard solution. The concentrations of the blood standards, calculated before hemolysis in distilled water, correspond to 0.4, 0.8, 1.6, 2.8 and 4.0 mg propofol per liter for the low-range calibration curve and to 4.0, 8.0, 16.0, 28.0 and 40.0 mg propofol per liter for the high-range calibration curve.

2.4. Preparation of internal standard solutions (precipitation solution)

A solution of 0.1 mg/l thymol in acetonitrile was prepared for the low-range calibration curve, and of 10 mg/l for the high-range calibration curve.

2.5. Chromatographic instrumentation and conditions

The HPLC system consisted of a Waters 600 MS system controller, a Waters in-line degasser, a Waters 717 plus autosampler, a Merck-Hitachi F 1000 fluorescence spectrophotometer and a 125×4.0 mm I.D. LiChrospher 100 RP-18 (5-μm) column (Merck, Darznstadt, Germany). The mobile phase, a mixture of acetonitrile, distilled water and trifluoroacetic acid (60:40:0.1, v/v/v), was eluted at 1.5 ml/min. It was degassed prior to use by ultrasonication for 10 min. The excitation and emission wavelengths were 276 and 310 nm, respectively. The run time was 5 min, and for the low-range calibration curve, data collection started after 1.5 min. The signals were recorded and processed by Millennium Session Manager version 2.10 (Waters, Etten-Leur, The Netherlands), which plotted the peak-height ratio of propofol to thymol against the spiked concentration of propofol. Using the regression parameters obtained from the calibration curve the concentrations of propofol in the samples were calculated.

3. Procedure

Twenty microliters of methanol were added to the 300-μl samples and were vortexed for 30 s on a Labconco vortex evaporator 432–2100 Buchler. To these samples and the blood standards of both the low- and high-range calibration curves, 500 μl of the low-range internal standard solution (0.1 mg/l) were added. The mixtures were vortexed for 2 min on a Labconco vortex evaporator 432-2100 Buchler, and centrifuged for 5 min at 2680 *g* on a Rotina 48 R-Hettich. One hundred microliters of the supernatant were transferred into autoinjector vials for HPLC analysis and 50 μl were injected. The propofol concentrations in the samples were calculated on the low-range calibration curve. During the determi-

nation of the samples on the low-range calibration curve, all tubes were stored at approximately 4°C.

The tubes of the samples with a concentration, calculated on the low-range calibration curve, greater than 4 mg/l and the tubes of the standards of the high-range calibration curve, which had been treated in exactly the same way as the samples, were centrifuged for 5 min at 2680 *g*. After adding 40 μl of the high-range internal standard solution (10 mg/l), the tubes were vortexed for 1 min and centrifuged at 2680 *g* for 5 min. One hundred microliters of the clear supernatant were transferred to autoinjector vials for HPLC analysis and 25 μl were injected. The propofol concentrations of these samples were calculated on the high-range calibration curve.

3.1. Whole-blood concentration monitoring of propofol

Blood of a rat to which an intravenous infusion of 30 mg/kg propofol (as an emulsion) in 5 min was administered, was sampled at intervals until 90 min after the start of the infusion. Samples were prepared and analysed as described in this paper.

4. Results

4.1. Chromatograms

Fig. 1 shows a chromatogram of a blood standard with a propofol concentration of 2.8 mg/l, a chromatogram of a sample obtained from a rat after an intravenous infusion of propofol and a chromatogram of a blank rat-blood extract. The retention times of thymol and propofol are 2.6 and 4.5 min, respectively. The calculated concentration in the rat-blood sample is 8.5 mg/l. The chromatogram of a blank rat-blood extract shows that blood constituents do not interfere.

4.2. Regression parameters

A linear relationship was obtained between the peak-height ratio of propofol to thymol against the spiked concentration of propofol in rat blood over the range of 0.4–4 mg/l and 4–40 mg/l. Mean

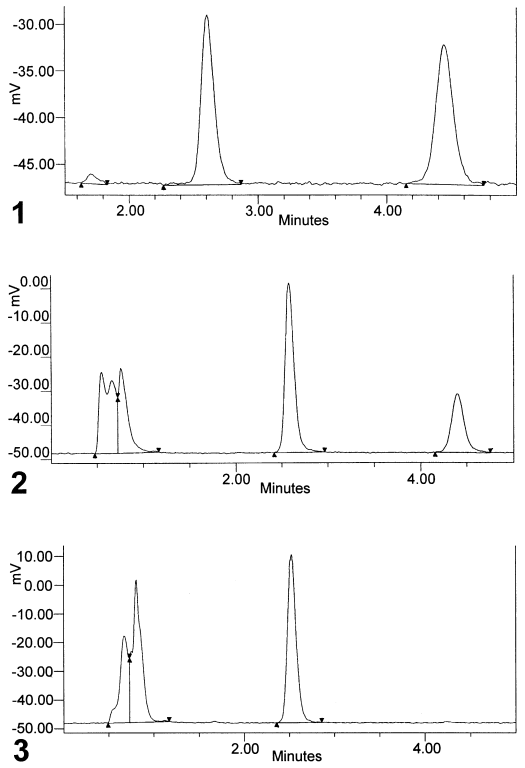

Fig. 1. Chromatograms of a rat-blood standard with a propofol concentration of 2.8 mg/l (1) (low-range calibration curve), of a sample from a rat which had been given an intravenous infusion of propofol (2) (high-range calibration curve), and of a blank rat blood sample (3). The retention times of thymol and propofol are 2.6 and 4.5 min respectively. The propofol peak in (2) corresponds to a concentration of 8.5 mg/l.

correlation coefficients (S.D.), slopes (S.D.) and intercepts (S.D.) are 0.9993 (0.0006), 0.36 (0.01) and 0.014 (0.022) respectively for the low-range calibration curve (0.4–4 mg/1) ($n=6$) and 0.9995 (0.0004), 0.026 (0.001) and 0.012 (0.011) respectively for the high-range calibration curve (4–40 mg/1) ($n=3$).

4.3. Intra-assay precision

Intra-assay variations were determined by examining a mixture of control, whole blood and distilled water (50+250 μl) spiked with propofol in four

Table 1
Intra-assay precision and recovery of low- and high-range calibration curve

Spiked concentration (mg/l)	Mean measured concentration (mg/l) and recovery (%)	Relative standard deviation
Low-range calibration curve (0.4–4 mg/l)		
0.418 (n=7)	0.460 (110%)	4.2%
0.836 (n=8)	0.859 (103%)	3.2%
2.926 (n=8)	2.915 (100%)	1.9%
4.180 (n=8)	4.249 (102%)	3.2%
High-range calibration curve (4–40 mg/l)		
4.006 (n=8)	3.586 (90%)	9.3%
16.022 (n=7)	15.185 (95%)	2.3%
40.056 (n=8)	40.549 (101%)	3.1%

concentrations of the low- and in three concentrations of the high-range calibration curve. Each concentration was prepared and analysed seven or eight times as described in this paper. Results are shown in Table 1.

4.4. Inter-assay precision

Inter-assay variations were determined by examining a mixture of control, whole blood and distilled water (50+250 μl) spiked with propofol in five concentrations of both the low- and high-range calibration curve in different runs, as described in this paper. Results are shown in Table 2.

5. Recovery

Tables 1 and 2 show recovery percentages calculated after determination of intra- and inter-assay precision. Recovery percentages vary between 90 and 111%.

5.1. Limit of quantifcation and limit of detection

The limit of quantification (LOQ) and limit of detection (LOD) of the assay were determined by measuring the noise in a control sample. The LOQ is defined as the amount of material yielding a signal-to-noise (S/N) ratio of 5. As noise corresponded to a maximum of 0.08 mg propofol/l, the LOQ was

Table 2
Inter-assay precision and recovery of low- and high-range calibration curve

Nominal concentration (mg/l)	Mean measured concentration (mg/l) and recovery (%)	Relative standard deviation
Low-range calibration curve (0.4–4 mg/l)		
0.40 (n=8)	0.445 (111%)	8.1%
0.80 (n=8)	0.815 (102%)	6.9%
1.6 (n=8)	1.526 (95%)	5.3%
2.8 (n=7)	2.800 (100%)	2.5%
4.0 (n=8)	4.043 (101%)	2.6%
High-range calibration curve (4–40 mg/l)		
4.0 (n=7)	4.395 (110%)	5.1%
8.0 (n=8)	8.117 (101%)	7.6%
16.0 (n=7)	16.010 (100%)	6.1%
28.0 (n=6)	29.060 (104%)	5.4%
40.0 (n=8)	39.846 (100%)	3.9%

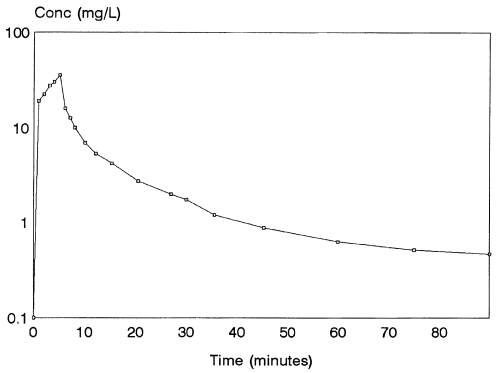

Fig. 2. Whole blood concentration–time profile of propofol in a rat (P41) to which an intravenous infusion of 30 mg/kg propofol in 5 min was administered.

determined to be 0.4 mg/l. The LOD, defined as the amount of material yielding a S/N ratio of 2.5, was determined to be 0.2 mg/l.

5.2. Whole-blood concentration monitoring of propofol

The results of the application of the described method to a series of whole-blood samples of a rat are shown in Fig. 2. The rat had been given an intravenous infusion of 30 mg/kg propofol (as an emulsion) in 5 min.

6. Discussion

The assay procedure described in this paper allows propofol determinations over a wide concentration range in rat-blood samples as small as 50 μl. No solvent evaporation under a stream of nitrogen and no transfer of propofol in extraction solvent to other tubes are necessary. By limiting the number of steps, the risk of loss of propofol or thymol are minimized. Linearity is observed from 0.4–40 mg/l and relative standard deviations are less than 10% over the described range (intra- and inter-assay). The relative standard deviation appears to be larger at low concentrations, which is probably due to the fact that the results are close to the limit of quantification. If desired, the limit of quantification can be lowered to circa 0.05 mg/l, by increasing the volume of sampled blood if available, as well as by reducing the volume of distilled water for blood hemolysis and the volume of precipitation solution.

The results of the determination in blood samples of a rat which had been given an intravenous propofol infusion confirm that the described HPLC method is suitable for routine propofol concentration monitoring and for pharmacokinetic studies. Whole-blood concentrations after (sub)anaesthetic doses are within the studied range.

A comparable sample preparation procedure has previously been described by Vree et al. [3]. The method was applied to plasma instead of whole blood, and was used to assay propofol and its metabolites by HPLC with UV detection. Fluorescence detection, in our view, is a more specific and more sensitive detection method for propofol.

Yu and Liau [4] have described an assay for propofol in plasma using gas chromatography. Since their method requires an extra centrifugation step (for the preparation of plasma from whole blood) and a slightly longer run time, we consider our method to be superior over theirs with regard to these aspects. Their lower limit of quantification, however, will allow slightly lower concentrations to be determined.

Dawidowicz [5] has argued that a precipitation method, such as the one described by Pavan et al. [6], is less precise than an extraction method, e.g. as descibed by Plummer [1], the main reason for this phenomenon being coprecipitation of propofol with denatured proteins leading to lower detector responses. In our method this disadvantage is largely overcome by using whole blood instead of serum. As propofol is associated with the formed elements of blood, the risk of loss of propofol is avoided by omitting the preliminary centrifugation step to obtain plasma or serum. A further contributing factor may be the slightly higher acetonitrile concentration we employed in the precipitation procedure, which probably led to a more complete desorption of propofol from the precipitate.

The use of whole blood instead of plasma [4] or serum [6] carries a risk of extracting interfering substances. This, in our hands, when using blank whole-blood samples, did not appear to limit the applicability of whole blood (without preparation of

plasma or serum) to propofol determination in this matrix.

In conclusion, our results demonstrate that the method we describe in this paper is a rapid, simple and reliable way to quantify propofol in low-volume rat blood samples over a wide concentration range and is suitable for routine application.

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