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Short communication

Determination of propofol in rat whole blood and plasma by high-performance liquid chromatography

M.H Yeganeh, I. Ramzan*

Department of Pharmacy, The University of Sydney, Sydney, NSW 2006, Australia

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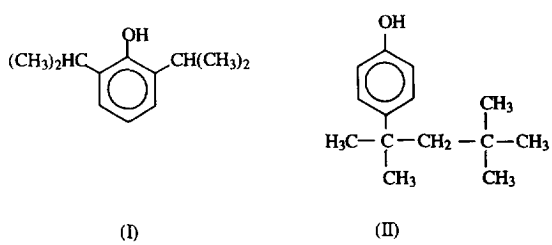
Abstract

A simple, accurate and sensitive high-performance liquid chromatographic method was developed for the determination of propofol, an intravenous anaesthetic agent, in rat whole blood or plasma samples. The method is based on precipitation of the protein in the biological fluid sample and direct injection of the supernatant into an HPLC system involving a C_{18} reversed-phase column using a methanol–water (70:30) mobile phase delivered at 1 ml/min. Propofol and the internal standard (4-*tert.*-octylphenol) were quantified using a fluorescence detector set at 276 nm (excitation) and 310 nm (emission). The analyte and internal standard had retention times of 6.3 and 10.5 min, respectively. The limit of quantification for propofol was 50 ng/ml using 100 μl of whole blood or plasma sample. Calibration curves were linear (r^2=0.99) over a 1–10 μg/ml concentration range and intra- and inter-day precision were between 4–11%. The assay was applied to the determination of propofol whole blood pharmacokinetics and propofol whole blood to plasma distribution ratios in rats.

Keywords: Propofol

1. Introduction

Propofol (2,6-diisopropylphenol) is an intravenous (i.v.) anaesthetic with a phenolic structure (I, Fig. 1). It is suitable for both induction and maintenance of anaesthesia in human and veterinary patients as well as in laboratory animals. In humans, bolus doses of 2.5 mg/kg [1] followed by constant rate infusions at 0.05–0.15 mg/min per kg for 2–4 h have been used [2]. Propofol anaesthetic concentrations in patients are reported to be between 2 and 3 μg/ml in whole blood [3] while concentrations at awakening approximate 1 μg/ml [4]. In animals propofol i.v. bolus doses have varied between 5 and 30 mg/kg [5]. Propofol whole blood concentrations at awakening in



(I) (II)

Fig. 1. Structure of (I) propofol illustrating the phenolic moiety with the two isopropyl substituents and (II) the internal standard, 4-*tert.*-octylphenol.

*Corresponding author.

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rats have been reported to be 2.8 μg/ml while in rabbits, cats, dogs and pigs these concentrations were 7.2, 4.3, 2.0 and 1.1 μg/ml, respectively [6].

Previously reported methods for assaying propofol have included high-performance liquid chromatographic (HPLC) procedures using ultraviolet detection following derivatization [7,8] or fluorescence detection [9]. Use of fluorescence detection improves the sensitivity to about 2 ng/ml [9]. Two HPLC assays, which both use direct UV detection without derivatization have also been published recently [10,11]. However, these assays for propofol have generally involved time consuming extraction procedures or have used relatively large (0.5–1 ml) volumes of blood. In this study, a HPLC method for propofol determination has been developed using fluorescence detection following precipitation of protein in the biological fluid without a separate extraction step. This direct method is simple, rapid and is suitable for analysis of propofol in biological fluids from small animals such as rats where only a limited sample volume is available.

Propofol is a lipophilic drug and despite being highly (98%) bound to serum/plasma proteins [12], it is appreciably associated with or enters erythrocytes. As a result, there is legitimate discussion in the literature as to whether propofol concentrations should be determined in whole blood or serum/plasma samples. For this reason, this newly developed assay for propofol was used to determine whole blood concentrations of propofol for pharmacokinetic purposes and to quantify propofol distribution ratios between rat whole blood and plasma.

2. Experimental

2.1. Drugs, chemicals and reagents

Propofol, pure drug, as a liquid, was a gift from ICI Australia. The internal standard, 4-*tert*.-octylphenol, [4-(1,1,3,3-tetramethylbutyl)phenol] (II, Fig. 1) was reagent grade (97% purity) obtained from Aldrich (St. Louis, MO, USA). All other chemicals and solvents including acetonitrile and methanol were HPLC grade.

2.2. Sample preparation and processing

For assay in whole blood, samples (200 μl) were mixed with aqueous EDTA solution (5 μl of 1.5 mg/ml) and the red blood cells were lysed by three freeze/thaw cycles. This did not affect the stability of propofol. Rat plasma was obtained also using EDTA as the anti-coagulant, followed by centrifugation. 4-*tert*.-Octylphenol, as internal standard (10 μl, 0.1 mg/ml in methanol), was then added to 100 μl of lysed whole blood preparation or plasma. Following precipitation of protein in the biological fluid with 250 μl of acetonitrile, and centrifugation for 3 min at 12 000 *g*, the supernatant (50 μl) was injected directly into the column.

2.3. Instrumentation and chromatographic conditions

The HPLC system consisted of a LC-10AS solvent delivery pump, SIL-6A auto-injector driven via a SCL-6A systems controller, a RF-551 fluorescence detector set at 276 nm (excitation) and 310 nm (emission), connected to a C-R3A chromatopac integrator, all from Shimadzu (Shimadzu Oceania, Sydney, Australia). The analytical column was a Versapack (Alltech Associates, Sydney, Australia) C_{18} reversed-phase column (300 mm length×4.1 mm I.D. and particle size of 10 μm) which was protected with a guard column (50 mm length×4.1 mm I.D., packed with 30–50 μm Bondapak (Waters Associates, Milford, MA, USA). The mobile phase consisted of methanol–water (70:30) delivered at 1 ml/min with an operating pressure of 200 kPa.

2.4. Calibration curves and assay validation

For routine analyses, calibration curves for propofol using five concentrations between 1 and 10 μg/ml were constructed using either spiked samples of lysed whole blood or plasma. Peak-area ratios of propofol to the internal standard were regressed against the nominal spiked concentration of propofol to generate the calibration curve data. This standard curve was extended down to 0.1 μg/ml for determination of low concentrations of propofol.

In order to determine the accuracy and precision

of the assay, whole blood or plasma samples containing different concentrations of propofol in the range 1–10 μg/ml were injected four times into the column, in a single day or on four separate days in a week to generate intra- and inter-day variability data, respectively. The recovery of propofol was determined by comparing acetonitrile treated spiked samples containing 1 and 10 μg/ml of propofol with those of equivalent propofol standards prepared in the mobile phase and treated with the same volume of acetonitrile and injected directly into the column.

2.5. Determination of propofol pharmacokinetics and whole blood to plasma ratios

Decline in propofol whole blood concentrations with time was determined after Institutional Animal Care and Ethics Committee approval in one male Sprague-Dawley rat that received a single 10 mg/kg i.v. bolus dose of propofol. Arterial blood samples (0.25 ml) were collected via a carotid artery cannula at 2, 4, 6, 8, 10, 15, 20, 30, 45, 60 min after dosing. Pharmacokinetic analysis was carried out using an iterative nonlinear regression procedure (PCNONLIN, Statistical Consultants, Apex, NC, USA). Propofol whole blood to plasma distribution ratios were quantified in a second rat that received the same dose of propofol with sampling at 2, 10 and 15 min after dosing. Each sample was divided into two aliquots; one was prepared for the analysis of propofol in lysed whole blood and the other portion was centrifuged, the plasma separated and assayed for propofol. Propofol concentrations in lysed whole blood or plasma were estimated based on either the peak-area ratio of propofol to the internal standard or on the peak area of propofol alone, to remove or minimise any artefacts related to unusual distribution of the internal standard itself into erythrocytes.

3. Results and discussion

Typical chromatograms of acetonitrile treated blank lysed rat whole blood and lysed rat whole blood containing propofol and internal standard (4-*tert.*-octylphenol) are presented in Fig. 2. The retention times for propofol and internal standard were 6.3 and 10.5 min, respectively. There were no interfering peaks from endogenous blood constituents. The major metabolite of propofol (1,4 quinol) was expected to elute prior to propofol, as it is more polar than propofol. The estimated retention time for 1,4 quinol, based on a similar HPLC assay from the literature [8] was expected to be less than 5 min. This, however, could not be verified experimentally as no authentic sample of this propofol metabolite was available.

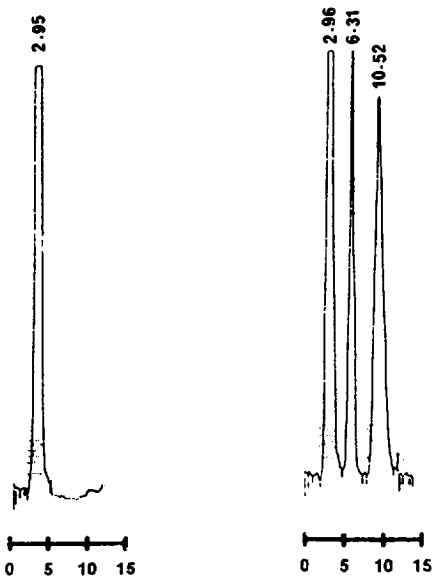

Fig. 2. Typical chromatogram of acetonitrile treated blank lysed rat whole blood (left panel) and lysed rat whole blood containing 6 μg/ml of propofol and internal standard, 4-*tert.*-octylphenol (right panel). Retention times for propofol and internal standard are 6.3 and 10.5 min, respectively.

Linear relationships were obtained between propofol to internal standard peak-area ratios and the concentrations of propofol with correlation coefficients in excess of 0.99 from both lysed whole blood and plasma (Table 1). The detection limit of propofol using this assay was 50 ng/ml using a 100 μl whole blood or plasma sample. At this concentration, the signal-to-noise ratio was 3. The intra- and inter-day precision and coefficient of variation (%C.V.) of the assay for two (1 and 10) different concentrations of propofol were generally less than 7% (Table 2).

Table 1
Calibration curve data for propofol assay from lysed rat whole blood and plasma

Concentration (μg/ml)	Whole blood			Plasma		
	Mean PAR	S.E.M.	C.V.[a] (%)	Mean PAR	S.E.M.	C.V.[a] (%)
10	1.07	0.04	4.5	0.94	0.03	4.3
6	0.72	0.03	5.6	0.60	0.02	3.4
4	0.40	0.01	5.4	0.39	0.01	2.9
2	0.21	0.01	7.2	0.20	0.02	10.0
1	0.12	0.01	10.7	0.11	0.01	10.8
Slope		0.109			0.094	
Intercept		−0.002			0.018	
r^2		0.993			0.997	

[a] C.V.% (coefficient of variation; S.D./Mean×100%).
PAR: Peak-area ratio of drug to internal standard.
Four determinations for each concentration.

Table 2
Accuracy and precision of propofol determination from lysed rat whole blood and plasma

	Intra-day			Inter-day	
	Spiked	Assayed	%C.V.[a]	Assayed	%C.V.[a]
Whole blood (μg/ml)	10	10.1±0.2	2.0	10.6±0.2	2.1
	1	0.99±0.06	7.1	1.1±0.07	7.3
Plasma (μg/ml)	10	10.4±0.4	4.3	10.2±0.5	5.2
	1	1.0±0.05	5.1	0.97±0.04	5.4

[a] C.V.% (coefficient of variation; S.D./Mean×100%).
Three determinations for each concentration.

The intra- and inter-day validation data, summarised in Table 2, indicates that this method is accurate and sensitive for quantification of unchanged propofol in lysed rat whole blood or plasma. The recovery of propofol from spiked whole blood or plasma samples from the lowest to the highest concentration ranged from 98–100% ($n=5$).

The pharmacokinetic profile of propofol in one rat receiving a 10 mg/kg i.v. bolus dose is shown in Fig. 3. In this rat the whole blood concentrations declined in a bi-exponential manner with distribution and elimination half-lives of 2 and 16 min, respectively. In the second rat where lysed whole blood and plasma concentrations of propofol were determined simultaneously, it was found that concentrations of propofol in lysed whole blood were

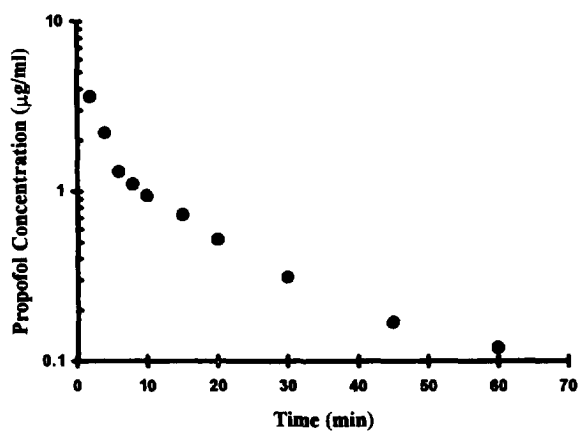

Fig. 3. Decline in propofol whole blood concentrations as a function of time after a single 10 mg/kg i.v. bolus dose.

Table 3
Lysed whole blood to plasma concentration ratios of propofol in rats determined following a 10 mg/kg i.v. bolus dose

Method[a]	Sample time (min)	Propofol concentration (μg/ml)		Whole blood/Plasma ratio
		Whole blood	Plasma	
(I)	2[b]	4.9	2.8	1.8
		4.8	3.1	1.5
	10	1.6	0.9	1.8
		1.5	0.8	1.8
	15	1.0	0.6	1.7
		1.0	0.6	1.7
	Mean±S.E.M.			1.7±0.1
(II)	2[b]	5.4	3.0	1.8
		5.1	3.2	1.6
	10	1.8	1.0	1.8
		1.7	0.9	1.8
	15	1.2	0.7	1.7
		1.1	0.6	1.7
	Mean±S.E.M.			1.7±0.1

[a] Propofol concentrations were determined using the peak-area ratio of propofol to internal standard (I) or using the area of the propofol peak alone (II).
[b] Each sample was assayed in duplicate.

consistently higher than those in plasma with the mean distribution ratio being 1.7 (Table 3). Similar distribution ratios were obtained regardless of whether the concentrations were calculated based on the area ratio of propofol to internal standard peak or on the area of the propofol peak alone (Table 3). These whole blood to plasma distribution ratios are in good agreement with the propofol whole blood to plasma concentration ratios of 1.9:1 obtained in dogs after i.v. infusion [13]. A substantial fraction of propofol is thus either associated with or enters erythrocytes at clinically relevant concentrations of 1–5 μg/ml. These results justify the measurement of propofol concentrations in whole blood in addition to that in plasma or serum.

The currently developed assay is simple and does not need prior extraction of propofol from the biological fluids. It is sensitive enough for determination of propofol concentrations for either pharmacokinetic or pharmacodynamic purposes using small volumes of biological fluids. This new propofol assay is therefore suitable for routine studies in small laboratory animals.

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