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Short communication

A rapid and simple HPLC method for the analysis of propofol in biological fluids

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Abstract

A selective and sensitive high-performance liquid chromatographic method for the analysis of propofol in biological samples was developed. Propofol and thymol (internal standard) were analysed on a Purospher RP-18 endcapped (75 mm × 4 mm, 3 μm) stationary phase using acetonitrile and water (65:35, v/v) as eluents at a flow rate of 0.6 mL/min. The excitation and emission wavelengths were 276 and 310 nm, respectively. Sample treatment consisted of deproteinization by acetonitrile containing the internal standard and direct injection of the supernatant. Mean analytical recovery were 105% (CV 2.0%) at concentrations ranging from 0.05 to 10 mg/L. The quantification limit was 3 ng/mL for a 500 μL sample plasma volume and 5 ng/mL for a 500 μL blood sample. The intra-day and inter-day precisions were lower than 5.5% for three concentrations assessed (0.05, 1.0 and 10.0 mg/L). Considering the column size and the flow rate, the separation was achieved with an analysis time less than 6 min with a reduced consumption of solvent.

This rapid HPLC method using a simple treatment procedure is sensitive enough for monitoring propofol in human biological samples.

Keywords: Propofol; HPLC; Blood samples

1. Introduction

Propofol (2,6-diisopropyl-phenol), is a widely used intravenous agent for induction and maintenance of anaesthesia and for sedation in intensive care patients [1]. Propofol is metabolised into glucuronides and sulphates which are detected in plasma by HPLC [2]. However, the metabolites of propofol do not contribute to the sedating effect of propofol. Several chromatographic methods [2–12] have been reported for the dosage of propofol in biological fluids. GC–MS methods [6,7,11] require derivatization of the compounds. HPLC methods [2–5,8–10,12] reported previously, use ultraviolet [3,10] or fluorescence detection [4,5,8,9]. The use of flurorescence detection improves the sensitivity with quantification limit ranging from 2 to 50 ng/mL. Methods using LC–MS detection has been reported [12,13]. However, this equipment is not available in all clinical laboratories due to its expensive cost. Considering the binding of propofol to blood components [14], the quantification of propofol concentration in whole blood has been proposed [4,5].

To investigate the distribution ratio of propofol between whole blood and plasma in human samples, we report a simple HPLC method using a high purity reversed phase sorbent for the determination of propofol concentration in biological fluids.

2. Experimental

2.1. Chemicals

Propofol was generously supplied by Astra Zeneca. The internal standard, thymol was from Sigma (Isle d'Abeau, France).

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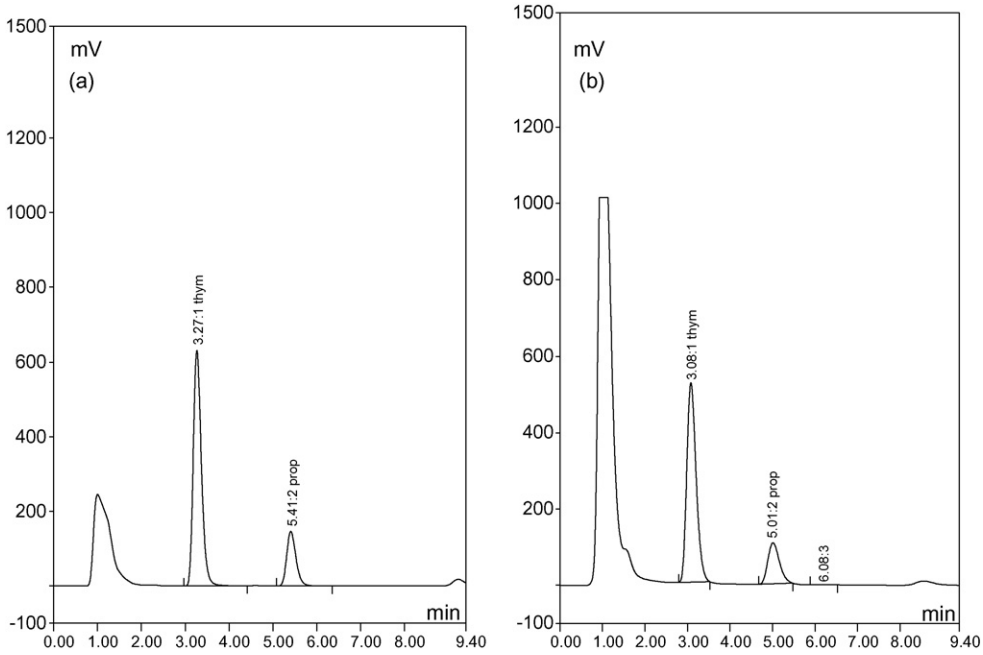

Fig. 1. Chromatograms of plasma (a) and whole blood (b) spiked with propofol at a concentration of 1.0 mg/L.

Biorad drug-free serum was used for calibration curve (Biorad, Ivry-sur-Seine, France). Acetonitrile (LiChrosolv) were purchased from Merck (Nogent-sur-Marne, France).

2.2. Apparatus and chromatographic conditions

The HPLC analysis was performed using a Kontron chromatographic system consisting of a HPLC pump 420 with an automatic injector, HPLC autosampler 460 and a fluorescence detector Jasco FP920 with a computer Targa using Kroma System 2000 software. Separation was achieved with Purospher® RP-18 endcapped (75 mm × 4 mm, 3 μm) stationary phase (Merck). The mobile phase consisted of acetonitrile–water (65:35, v/v). The flow rate was 0.6 mL/min and the excitation- and emission wavelength were 276 and 310 nm, respectively. The analyses were performed at ambient temperature (20 °C).

2.3. Standards

Stock solutions of propofol and thymol were prepared at a concentration of 1 g/L and 600 mg/L, respectively, in acetonitrile. Calibration curves from Biorad drug-free serum and from whole blood from healthy subjects were constructed in the concentration range to cover the usual concentrations expected in intensive care patients. One standard curve ranged from 0.01 to 1.0 mg/L and the other from 1.0 to 10.0 mg/L. In-house control samples at a final propofol concentration of 0.25 and 8 mg/L were incorporated into each run.

2.4. Sample collection and storage

Blood samples (5 mL) were collected into heparinized tubes. For plasma analysis, blood was centrifuged without delay at 2000 × *g* at 20 °C for 10 min. Plasma was decanted and stored at −20 °C until analysis. For whole blood analysis, blood was immediately lysed by freezing at −20 °C. As reported by Dawidowicz and Fornal [15], the lysis seems to be necessary to estimate the total amount of propofol in whole blood.

2.5. Sample treatment procedure

Five hundred microlitres of acetonitrile containing thymol (IS) were added to 500 μL of plasma or lysed whole blood. After mixing for 1 min, the mixture was centrifuged at 2000 × *g* for 10 min at 15 °C. Then, 100 μL of the supernatant was injected into the column.

3. Results and discussion

Chromatograms of a plasma and a whole blood sample spiked with propofol at a concentration of 1.0 mg/L, respectively, are shown in Fig. 1. The separation was achieved with an analysis time less than 6 min.

Table 1
Analytical recoveries of propofol and internal standard

Compound/biological fluid	Concentration (mg/L)	Recovery (%)	CV (%), $n=5$
Propofol/plasma	0.05	107.1	2.1
Propofol/whole blood	0.05	98.5	5.4
Propofol/plasma	1.0	103.0	1.7
Propofol/whole blood	1.0	104.6	1.4
Propofol/plasma	10.0	107.3	2.7
Propofol/whole blood	10.0	105.6	1.6
Thymol/plasma	1.0	96.3	2.2
Thymol/whole blood	1.0	97.7	2.4

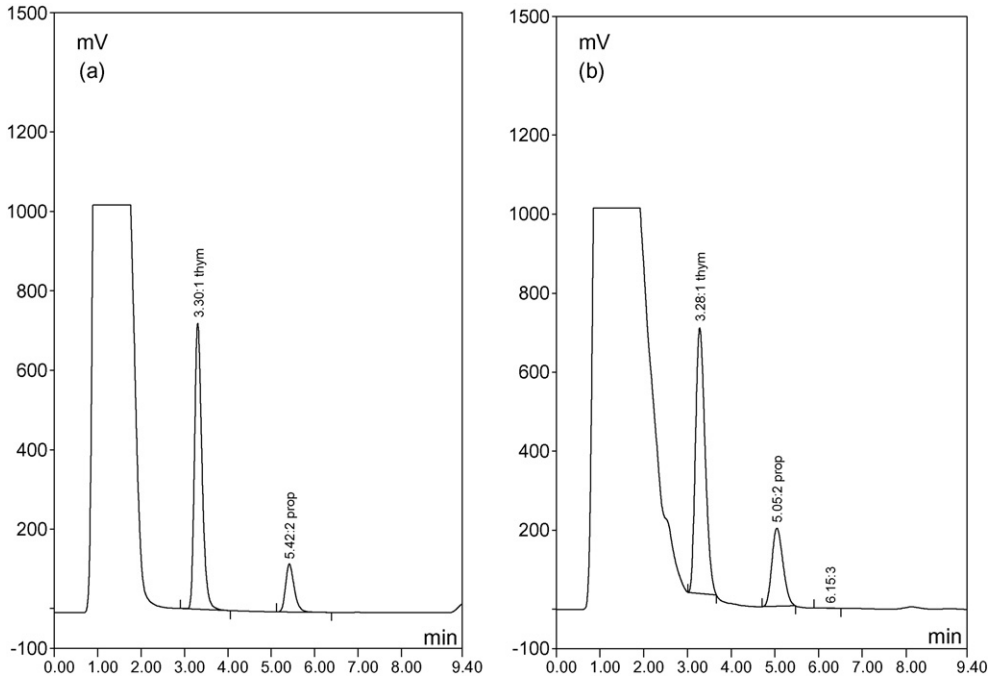

Fig. 2. Typical chromatograms of a plasma (a) and whole blood (b) from an intensive care patient.

Analytical recoveries of propofol from spiked plasma and whole blood were given in Table 1. Calibration curves fitted by plotting the peak area ratio (compound of interest/internal standard) versus the concentration were linear up to 10 mg/L with a correlation coefficient greater than 0.999 for the low as for the high calibration curve. The quantification limit was 3 ng/mL for plasma sample, and 5 ng/mL for whole blood with a coefficient of variation inferior to 13% for 500 μL sample volume. Intra-day coefficient of variation ranged from 1.2 to 1.7% and 0.3 to 5.5% for propofol at concentration of 0.05, 1.0 and 10.0 mg/L in plasma sample and whole blood, respectively. Inter-day coefficient of variation ranged from 2.4 to 4.9% and 2.7 to 3.7% for propofol at concentration of 0.05, 1.0 and 10.0 mg/L in plasma sample and whole blood, respectively.

The Purospher column has demonstrated a long lifetime with about 800 biological samples injected without any deterioration. No interference with propofol for drugs commonly used in intensive care unit such as furosemide, omeprazole, ceftriaxone, noradrenaline, erythromycine, methylprednisolone, ganciclovir, clopidogrel and amiodarone was observed.

The method was used for monitoring propofol in biological fluids from three intensive care patients. Typical chromatograms of plasma and blood samples from an intensive care patient are shown in Fig. 2. Whole blood and plasma propofol concentrations recovered in the three patients were 2.12, 0.71 and 1.40 mg/L and 1.24, 0.35 and 0.85 mg/L, respectively. The whole blood to plasma distribution ratio ranged from 1.64 to 2.02. This result is in accordance with the data reported by Dawidowicz and Fornal [15] in human biological samples.

The HPLC method described allows the simultaneous analysis of plasma and whole blood with regard to the sample treatment procedure. Considering the column size and the flow rate, the separation was performed with a short analysis time with a reduced consumption of solvent. The method is selective and sensitive enough to monitor propofol from low plasma and/or whole blood volume during pharmacokinetic studies.

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