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Note

Simple and practical high-performance liquid chromatographic assay of propofol in human blood by phenyl column chromatography with electrochemical detection

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Propofol (2,6-diisopropylphenol) is an intravenous anaesthetic that can be administered either by repeated bolus dose or by continuous infusion. Isolated instances of bradycardia, epileptiform movements and a few cases of severe hypotension [1] have been reported in patients anaesthetized.

The previously reported high-performance liquid chromatographic (HPLC) methods for propofol determination use direct UV detection [2], fluorimetric detection [3] or UV detection after precolumn derivatization by Gibb's reagent [4]. Propofol has also been selected as a model compound for direct plasma injection using internal surface reversed-phase chromatography [5].

This paper describes a new HPLC analysis of propofol in human whole blood using an electrochemical detector.

EXPERIMENTAL

Apparatus

The HPLC apparatus consisted of a 114 or 116 solvent-delivery module, a 450 data system and an Omniscribe d-5000 recorder (Beckman Instrument International, Geneva, Switzerland), a Gilson Medical Electronics (Villiers-

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Le-Bel, France) Model 401/231 sample injector and a Bio-Rad Labs. (Milan, Italy) Model 1340 electrochemical detector. A Viosfer phenyl analytical column (250 mm×4.0 mm I.D., 5 μm particle size) and a phenyl guard column (30 mm×4.6 mm I.D., 5 μm particle size) were obtained from Violet (Rome, Italy) and Brownlee Labs. (Santa Clara, CA, U.S.A.), respectively.

Reagents and solutions

HPLC-grade acetonitrile and ethanol, and HPLC-grade methanol and 2-propanol were obtained from Farmitalia Carlo Erba (Milan, Italy) and Merck (Darmstadt, F.R.G.), respectively. Demineralized water distilled together with potassium permanganate was used. A 1-ml C_{18} Bond-Elut cartridge and a Vac-Elut manifold were obtained from Analytichem International (Harbor City, CA, U.S.A.). Thymol and propofol (I.C.I. 35 868) were obtained from I.C.I. (Macclesfield, U.K.).

The propofol calibration standard was prepared by dissolving propofol and diluting to 10.0 μg/ml with methanol. The solution was stable for at least 30 days at −20°C. A 10-μl aliquot of this solution was added to 500 μl of pooled propofol-free whole blood to achieve a 200 μg/l concentration of calibration standard. The internal standard working solution was prepared by dissolving thymol in water–methanol–2-propanol (90:5:5, v/v/v) and diluting to 5.0 μg/ml with water.

The mobile phase was methanol–25 m*M* potassium phosphate buffer (pH 6.0) (1:3, v/v).

Extraction

Whole blood specimens from anaesthetized patients were collected in EDTA tubes. A 0.5-ml aliquot of the unknown blood or propofol calibration standard was pipetted into a 5-ml polypropylene tube, and 25 μl of the internal standard working solution were added and mixed. A 1.0-ml acetonitrile aliquot was added and mixed thoroughly on a vortex mixer. Following centrifugation the entire clear supernatant was transferred to a 10-ml polypropylene tube containing 3.5 ml of water, and mixed. A 1-ml C_{18} Bond-Elut extraction cartridge with column reservoir, assembled on the Vac-Elut, was rinsed thoroughly under vacuum with 3 ml of 95% ethanol in water and 3 ml of 15% acetonitrile in water. The mixture containing propofol was poured into the cartridge and allowed to slowly percolate through under vacuum. The effluent was discarded. The cartridge was washed with 3 ml of 15% acetonitrile in water and 0.5 ml of 20% ethanol in water. Again the effluents were discarded. The last stages of the extraction were as follows:

- The column was allowed to dry out for several seconds.
- The vacuum was disconnected.
- The reservoir was removed.
- The metal tips were wiped.

– A tube was placed in the collection rack.
– The propofol was slowly eluted with 0.5 ml of 75% ethanol in water.

Chromatography

A 25-μl aliquot of ethanolic eluate was injected into the analytical column, kept at 37°C. The flow-rate was 0.9 ml/min. The oxidation potential of the electrochemical detector was 0.8 V, the sensitivity 5 nA/V f.s. and the filter was set at 5. Chromatograms were recorded at a 0.5 cm/min chart speed and at 10 mV f.s. (Fig. 1). The peaks were acquired by computer and calculated by plotting the propofol/thymol peak-height ratio against the 200 μg/l propofol calibration standard.

RESULTS AND DISCUSSION

The within-run precision was evaluated by repeated measurements of specimens at three concentrations. The samples were obtained by diluting whole blood containing an increased concentration of propofol with pooled drug-free whole blood. The between-run precision was evaluated using the same three specimens, stored in aliquots at −20°C, by duplicate measurements performed monthly. The mean concentrations and coefficients of variation (C.V.) are listed in Table I.

The absolute recovery was calculated by comparing chromatograms obtained by processing whole blood samples at 500 ng/ml propofol and 1000 ng/ml thymol concentrations, with chromatograms obtained by injecting ethanolic solutions at the same concentrations. Mean absolute recoveries in repeated determinations were 72% for propofol and 75% for thymol. After correction of the recovery on the basis of the internal standard level, the mean analytical recovery of propofol was 96%, over the whole range of linearity, thus providing the accuracy of the method.

The sensitivity of the present method is at least 20 ng/ml and the signal-to-noise ratio for the 20 ng/ml spiked whole blood is at least 4. The sensitivity

TABLE I

PROPOFOL DETERMINATION PRECISION STUDY

Within-run			Between-run		
n	Mean concentration (ng/ml)	C.V. (%)	*n*	Mean concentration (ng/ml)	C.V. (%)
9	41	5.4	6	40	5.6
10	184	2.2	9	181	4.3
10	857	1.0	10	879	3.6

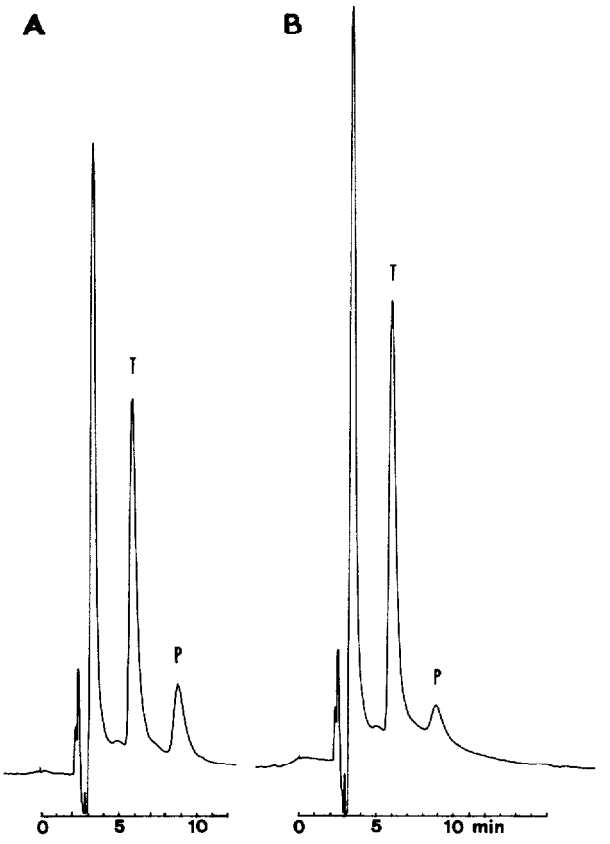

Fig. 1. Chromatograms of the whole blood of two patients: (A) 250 ng/ml thymol and 145 ng/ml propofol; (B) 250 ng/ml thymol and 55 ng/ml propofol. Peaks: T = thymol; P = propofol.

can be considerably increased by using a lower concentration of internal standard, and increasing the injection volume and the detector sensitivity.

The linearity of the method was checked up to 2000 ng/ml concentration.

The phenyl column increases the selectivity of stationary phase for propofol and thymol, which are aromatic compounds, and it also improves the quality of chromatograms. The electrochemical detector increases the sensitivity of the method, avoiding the need for both precolumn derivatization [4] and time-wasting extractions in order to concentrate the sample [3]. The use of internal surface reversed-phase HPLC [5] minimizes the problems of sample purification, but is necessary to use plasma rather than whole blood. Propofol is significantly associated with formed elements of blood, and therefore whole blood is preferred for pharmacokinetic analysis [3].

To achieve satisfactory purification of the sample, it is sufficient to precip-

itate proteins with acetonitrile, which solubilizes the more hydrophobic molecules, and to purify it by solid-phase extraction.

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