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Note

Improved method for the determination of propofol in blood by high-performance liquid chromatography with fluorescence detection

G.F. PLUMMER

Safety of Medicines Department, ICI Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire SK10 4TG (U.K.)

Propofol (2,6-diisopropylphenol, I.C.I. 35868), the active ingredient of Diprivan*, is currently under study as a new anaesthetic agent.

The previously reported method for propofol using high-performance liquid chromatography (HPLC) and ultraviolet detection of the indophenol derivative [1] was not considered sufficiently sensitive (limit of quantification ca. 15 ng/ml) for the monitoring of all clinical studies. The combination of HPLC and fluorescence detection has improved the sensitivity of propofol measurements. The method described is more rapid and simpler to use than the indophenol procedure and has an improved limit of quantification of ca. 2 ng/ml.

EXPERIMENTAL

Reagents and solutions

All chemicals used were of analytical grade unless otherwise stated. Double-distilled water was used. Cyclohexane (spectroscopic grade), 2-propanol, trifluoroacetic acid and sodium dihydrogen orthophosphate were obtained from BDH (Poole, U.K.). Acetonitrile (HPLC grade) was obtained from Fisons (Loughborough, U.K.). Tetramethylammonium hydroxide (TMAH) (25% in methanol) and thymol were obtained from Fluka (Buchs, Switzerland).

Internal standard solution. A solution of thymol was prepared in methanol (1 mg/ml) and further diluted with methanol to an appropriate working concentration.

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*Diprivan is a trademark, the property of Imperial Chemical Industries plc.

Phosphate buffer solution (0.1 M). Sodium dihydrogen orthophosphate (13.6 g) was dissolved in 1 l of distilled water.

Dilute TMAH solution. TMAH (25% in methanol) (1.5 ml) was added to 2-propanol (18.5 ml).

HPLC mobile phase. The HPLC mobile phase consisted of 600 ml of acetonitrile, 400 ml of distilled water and 1 ml of trifluoroacetic acid. The mobile phase was degassed by the passage of helium prior to use.

Apparatus

The high-performance liquid chromatograph used consisted of a Constametric III solvent delivery system (Laboratory Data Control, Stone, U.K.) set to deliver a solvent flow of 1.5 ml/min, a WISP 710B automatic sample injector (Waters Assoc.) and an SFM 23 fluorescence detector (Kontron, St. Albans, U.K.). The excitation and emission wavelengths were 276 and 310 nm, respectively, and both monochromator slit widths were 10 nm. The signals were recorded using a Philips PM 8252 dual-pen chart recorder (Pye Unicam, Cambridge, U.K.) set at 0.1 and 0.2 V. A Hypersil C_{18} reversed-phase column (3 μm particle size, 100×5.0 mm I.D.; Shandon Southern, Runcorn, U.K.) was used at ambient temperature.

Procedure

Propofol is significantly associated with the formed elements of blood and therefore whole blood is the preferred sample for pharmacokinetic analysis.

Blood samples were collected in oxalated blood tubes and stored at 4°C until use.

To a sample of blood (1 ml), internal standard solution (20 μl), phosphate buffer (1 ml) and cyclohexane (5 ml) were added. The mixture was then placed on an inversion mixer (Luckham, U.K.) for 15 min at 60 rpm. Following centrifugation (1150 g for 5 min) an aliquot of the cyclohexane layer (4.5 ml) was transferred to a tube containing dilute TMAH solution (50 μl). The solvent was evaporated to dryness at ambient temperature under a stream of nitrogen. The dry residue was then redissolved in HPLC mobile phase (200 μl) and an aliquot (100 μl) of the solute was submitted to HPLC analysis. Typical chromatograms of extracts containing propofol are shown in Fig. 1.

A calibration graph was prepared by the addition of known quantities of propofol to aliquots of control blood and extracted according to the above procedure. The peak-height ratio of propofol to thymol was plotted against the concentration of propofol added. The concentration of propofol in test samples was calculated using the regression parameters obtained from the calibration graph.

RESULTS AND DISCUSSION

A linear relationship was obtained between the ratio of the peak height of propofol to that of thymol and the amount of propofol added to blood over the range 0.002–10.0 μg/ml. No change in the slope was observed with different samples of blood used. These results indicate that the method permits the determination of propofol in blood over wide ranges of concentration. The regression parameters

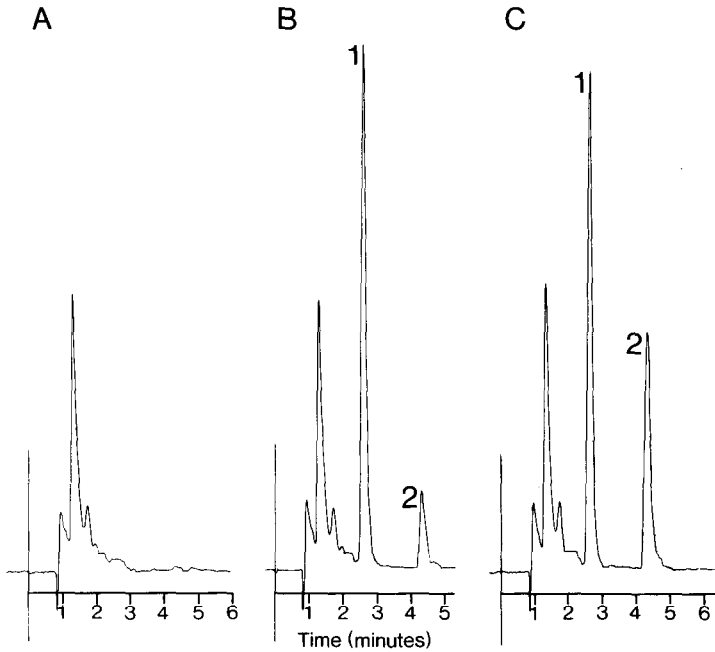A   B   C

Time (minutes)

Fig. 1. Chromatograms of extracts from (A) control human blood, (B) blood containing propofol (10 ng/ml) and (C) blood obtained from a patient having received propofol intravenously. Thymol (40 ng/ml) was added as the internal standard. Peaks 1 and 2 are thymol and propofol, respectively.

for the procedure over the most common calibration ranges used are listed in Table I.

The recovery of propofol added to control blood (incubated overnight at 4°C) was determined by comparison of the slopes for extracted calibration curves with those for non-extracted curves. Extraction efficiencies of 97.9 and 90.0% were obtained over the ranges 0–0.16 μg/ml and 0–8.0 μg/ml, respectively.

Within-batch variations were examined by performing replicate ($n=3$) analyses on blood samples to which known quantities of propofol were added. The

TABLE I

REGRESSION PARAMETERS OF COMMONLY USED CALIBRATION RANGES

Calibration range (μg/ml)	Mean correlation coefficient (S.E.)	Mean slope (S.E.)	Mean intercept (S.E.)	Internal standard (μg)
0–0.4 ($n=8$)	0.9995 (0.0002)	15.5 (0.40)	0.044 (0.021)	0.16
0–6.0 ($n=10$)	0.9993 (0.0005)	0.511 (0.009)	0.013 (0.004)	2.40
0–10.0 ($n=6$)	0.9997 (0.0003)	0.259 (0.005)	0.009 (0.004)	4.00

TABLE II

WITHIN-BATCH VARIATION

Amount spiked (ng)	Mean value found (ng)	S.D. (ng)	C.V. (%)
14.3	14.6	0.81	5.5
47.7	45.9	0.60	1.3
143	139	2.50	1.8

mean recovery was 98% with a coefficient of variation (C.V.) of between 1.3 and 5.5% over the concentration range examined (Table II). Between-batch variations were determined by performing replicate ($n = 3$) analyses on blood samples obtained from patients having received bolus doses of propofol at 2.5 mg/kg. C.V. values ranged from 2.9 to 12.3% (Table III).

The addition of dilute TMAH solution to propofol extracts prior to the evaporation of the extraction solvent is required to eliminate losses of propofol and thymol during this process. Losses of up to 75% for propofol and 45% for thymol have been recorded, for extracts reduced to dryness without the addition of dilute TMAH solution.

Propofol and thymol were found to be stable in alkaline cyclohexane (50 μl TMAH solution in 5 ml cyclohexane) for up to 6 h prior to reduction to dryness and in HPLC mobile phase for at least 18 h.

The storage of biological samples containing propofol is recommended at 4°C; under these conditions propofol has been found to be stable for up to twelve weeks. Storage at sub-zero temperatures should be avoided as significant losses of propofol have been observed when blood samples from patients were stored at $-20°C$.

A study was carried out to compare results from the fluorescence method with those from the previously described indophenol procedure. Samples obtained from patients having received propofol at 2.5 mg/kg were assayed by both procedures simultaneously. Excellent correlations were obtained between the data generated

TABLE III

BETWEEN-BATCH VARIATION

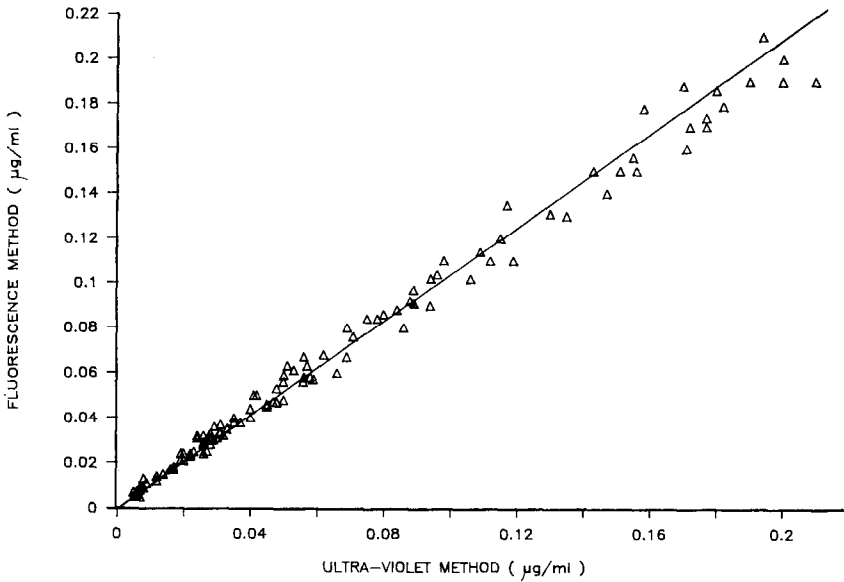| Mean (μg/ml)	SD. (μg/ml)	C.V. (%)
5.92	0.26	4.4
4.11	0.14	3.4
1.71	0.05	2.9
0.057	0.007	12.3
0.034	0.003	8.8
0.050	0.004	8.0

Fig. 2. Correlation between ultraviolet and fluorescence methods, 0.005-0.2 μg/ml of blood ($n=87$).
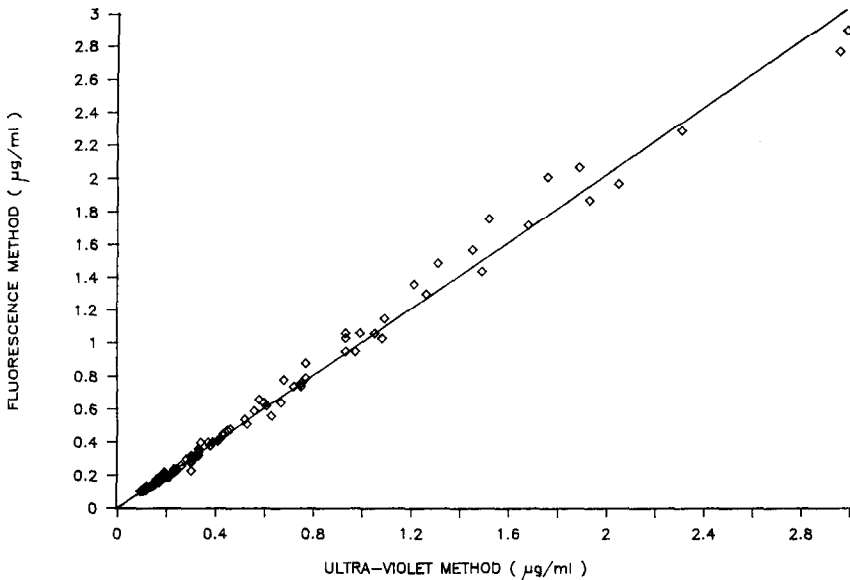

Fig. 3. Correlation between ultraviolet and fluorescence methods, 0.1-3.0 μg/ml of blood ($n=107$).

by the fluorescence and ultraviolet methods, over the ranges 0-0.20 μg/ml ($n=87$) and 0.1-3.0 μg/ml ($n=107$). These data are shown graphically in Figs. 2 and 3, and the derived regression parameters are listed in Table IV.

The known human metabolites of propofol, viz. the 1- and 4-glucuronide and 4-sulphate conjugates of 2,6-diisopropyl-1,4-quinol and propofol glucuronide, do not interfere with determinations of propofol. It has been shown by Simons et al.

TABLE IV

COMPARISON OF RESULTS OBTAINED FROM THE ANALYSIS OF BLOOD SAMPLES BY THE FLUORESCENCE AND ULTRAVIOLET PROCEDURES

Calibration range (μg/ml)	Correlation coefficient	Slope	n
0.1 –3.0	0.9946	0.984	87
0.005–0.2	0.9953	0.970	107

[2] that less than 0.3% of the radioactivity eliminated in urine following sub-anaesthetic doses of ^{14}C-labelled propofol is extractable with cyclohexane, using this procedure.

The method described is precise, sensitive and selective for propofol and is also rapid and simple to perform, it therefore has considerable advantages over the indophenol method.

REFERENCES

1 H.K. Adam, E.J. Douglas, G.F. Plummer and M.B. Cosgrove, J. Chromatogr., 223 (1981) 232.
2 P.J. Simons, I.D. Cockshott, E.J. Douglas, E.A. Gordon, K. Hopkins and M. Rowland, Postgrad. Med. J., 61 (Suppl. 3) (1985) 64.