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Quantitation of propofol in plasma by capillary gas chromatography

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ABSTRACT

A rapid, accurate and sensitive gas chromatographic method is described for measuring plasma concentrations of propofol. The technique required only 200 μl of plasma and a single extraction process, using chloroform containing pentadecane (500 ng/ml) as an internal standard. Quantitation was achieved on an SGE BP-1 fused-silica capillary column (25 m × 0.33 mm I.D., 0.5 μm film thickness) with flame ionization detector. The peak response was linear over a wide concentration range (10–10 000 ng/ml) and the limit of quantitation was 10 ng/ml. The absolute recoveries were over 96% (n = 3). The method is applicable for both research and routine plasma level monitoring.

INTRODUCTION

Propofol (2,6-diisopropylphenol, Fig. 1) is a new intravenous anaesthetic that is chemically unrelated to the barbiturates or other anaesthetics. The onset of and the recovery from propofol anaesthesia are rapid [1]. It can be used in maintenance anaesthesia by continuous infusion or intermittent bolus injection with nitrous oxide [1]. Blood level monitoring of propofol by high-performance liquid chromatography (HPLC) has been reported [2–8]. Most of the methods are tedious, time-consuming and require more than 1 ml of blood for each analysis. Some of the methods require solvent evaporation [3,6]. Others need pre-column chemical reaction [2] or percolation of the sample solution [7]. Propofol is a colourless liquid at room temperature, and very slightly soluble in water at 20°C but soluble in most organic solvents. Its boiling temperature is not high (136°C) [9]. These physical properties suggest that assay of propofol by gas chromatography (GC) should be possible. Nevertheless, to our knowledge, analysis of propofol by GC has never before been reported.

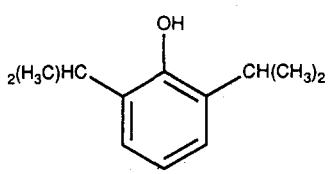

Fig. 1. Structure of propofol.

In the present study, a reliable, sensitive and simple method for quantitation of propofol in human plasma, by GC, was developed. A patient's plasma level–time profile of propofol determined by this method, as a pilot study on the clinical pharmacokinetics of propofol, is also presented.

EXPERIMENTAL

Drugs and chemicals

Pure propofol (diisopropylphenol) was supplied by ICI (Macclesfield, UK) through the local

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agents (Taipei, Taiwan). Chloroform (E. Merck, Darmstadt, Germany) and methanol (BDH, Poole, UK) were chromatography grade. Pentadecane (Sigma, St. Louis, MO, USA) was reagent grade. Blank plasma was obtained from the blood bank, and samples from patients from the Department of Anesthesiology, National Taiwan University Hospital (Taipei, Taiwan).

Preparation of standard solutions and plasma standards

Stock solutions of propofol in methanol (1 mg/ml) were prepared. The solution was placed in an airtight amber glass vial and stored in a refrigerator. The vial was filled with nitrogen gas, prior to cap closure and after each opening, to protect the drug from oxidation. The working standards, 10–10 000 ng/ml, were freshly prepared each time just before use by diluting an aliquot of stock solution with water or blank plasma.

Instrumentation

The GC apparatus (Model GC-14A, Shimadzu, Kyoto, Japan) consisted of a split–splitless capillary inlet system, a flame ionization detector and an integrator (C-R4A). A SEG BP-1 fused-silica column (25 m × 0.33 mm I.D., 0.5 μm film thickness) was used to separate the compound.

Chromatographic conditions

The operating conditions for analysis were: injection port temperature, 240°C; oven (column) temperature, 150°C; detector temperature, 280°C; nitrogen (carrier gas) flow-rate at a pressure of 98 kPa, 2.5 ml/min (split carrier gas), 60 ml/min (make up gas) and 100 ml/min (septum purge). The split ratio was 1:12. The hydrogen and the air pressure for the detector were 70 and 88 kPa, respectively. The attenuation was set at 3.

Extraction procedure

In screw-capped glass tubes, 200 μl of spiked plasma standards or aqueous standards were made slightly alkaline with 50 μl of 1 *M* sodium hydroxide. By shaking on a vortex mixer (Thermolyne Type 37600, Dubuque, IA, USA) for 5 min with 200 μl of chloroform containing pentadecane (500 ng/ml) as an internal standard, the drug was extracted. After centrifugation (Sigma 202MK, Osterode, Germany) at 4600 *g* for 10 min at 10°C, a 5-μl portion of the chloroform layer was analysed by GC.

The extraction recovery was assessed as follows. Chloroform (containing the internal standard) and plasma were spiked with propofol to the same concentration. The chloroform solution was analysed directly by GC without extraction. The response factor thus obtained was used to calibrate the analysis of the spiked plasma.

Plasma level monitoring

Plasma of a patient who had been given a 2.5 mg/kg intravenous bolus dose of propofol (emulsion type) was sampled on a time schedule up to 24 h, and analysed by the GC method immediately after blood sampling.

RESULTS

Fig. 2 shows chromatograms obtained from the extracts of a human blank plasma (A), plasma spiked with propofol (B) and a patient's plasma sampled 10 min after intravenous infusion of propofol (C). The peaks of propofol and the internal standard were well resolved and free from interference by plasma constituents.

Calibration and extraction recovery

The propofol calibration standard (500 ng/ml) was analysed, and a response factor of calibration was obtained. Using this response factor, the working standard preparations containing propofol (10, 50, 100, 500, 1000, 5000 and 10 000 ng/ml) were assayed. The spiked *versus* measured concentrations showed a linear relationship, indicating that the calibration curve should be linear over the concentration range.

The regression curves of spiked *versus* measured concentration for propofol in distilled water or in human plasma were both linear in the concentration range 10–10 000 ng/ml. The coefficient of variation (C.V.) for the inter-assay precision was less than 4%, except for the 10 ng/ml standard, which was 9.6%. The correlation coef-

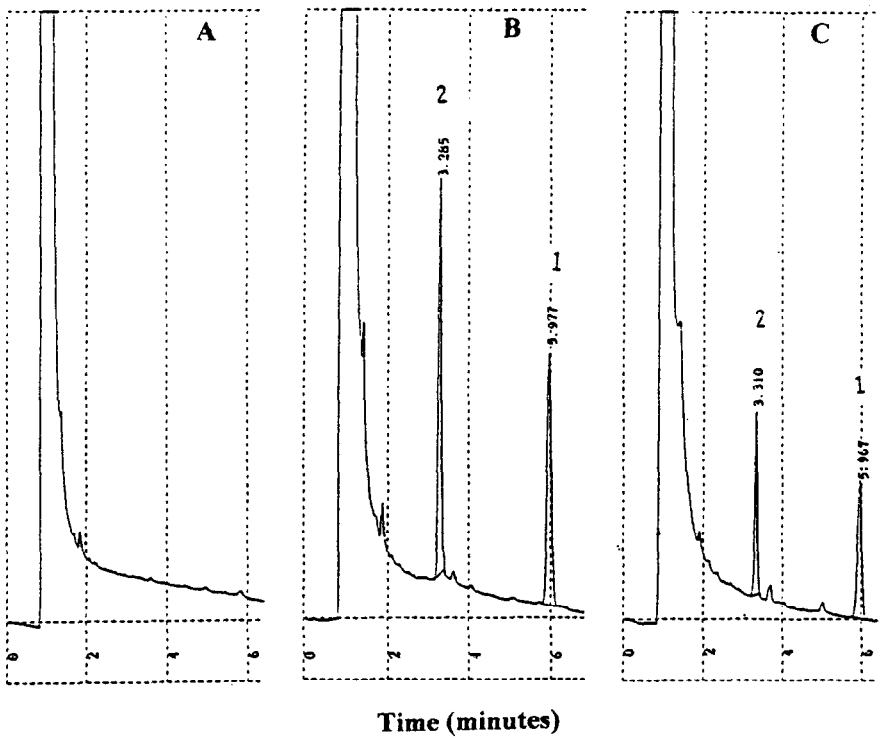

Fig. 2. Gas chromatograms of (A) plasma blank, (B) plasma spiked with propofol (500 ng/ml) and the internal standard, pentadecane (500 ng/ml) and (C) patient's plasma containing the internal standard and propofol (348.9 ng/ml), sampled 10 min after a single dose of propofol (2.5 mg/kg). Peaks: 1 = internal standard; 2 = propofol.

ficients of standard curves exceeded 0.9999. The limit of quantitation was 10 ng/ml in plasma when 0.2 ml of plasma was analysed. The extraction recovery of propofol from 0.2 ml of plasma was more than 96% (Table I). The results obtained from the spiked plasma and the spiked aqueous solution were not statistically different, indicating that plasma constituents did not hinder the extraction or interfere with the GC analysis of propofol.

Intra-day precision

Plasma samples spiked with propofol in the concentration range 10–10 000 ng/ml were analysed thrice on the same day: in the morning, at noon and in the afternoon. The C.V. for the intra-day precision ranged from 1.1 to 4.3%, except for the lowest concentration, 10 ng/ml, which showed 9.6%.

Inter-day precision

Plasma samples spiked with propofol in the concentration range 10–10 000 ng/ml were pre-

TABLE I

ABSOLUTE RECOVERY OF PROPOFOL FROM SPIKED PLASMA.

The linear regression equation was $y = 1.0289x$ (± 0.0045) $-$ 26.5968 (± 20.5916); $r = 0.9999$ (± 0.0001). The average absolute recovery was 101.4%.

Spiked, x (ng/ml)	Measured, y (ng/ml)[a]	C.V. (%)	Absolute recovery (%)
10 000	10 315.6 ± 48.4	0.47	103.16
5000	5009.6 ± 121.2	2.42	100.19
1000	977.4 ± 4.0	0.41	97.74
500	547.3 ± 6.1	1.11	109.46
100	96.5 ± 3.2	3.32	96.47

[a] Data are mean ± S.D. of three determinations.

TABLE II

PRECISION OF INTER-DAY PROPOFOL DETERMINATION

Mean ± S.D. slope: 0.9894 ± (0.0362); mean ± S.D. intercept: −0.6656 ± (12.6757); mean ± S.D. correlation coefficient: 0.9999 ± 0.00003.

Spiked (ng/ml)	Measured (ng/ml)				C.V. (%)
	Day 1	Day 2	Day 3	Mean ± S.D.	
10 000	10 065.9	10 235.7	9375.0	9892.2 ± 373.2	3.76
5000	5007.1	5032.2	4784.7	4941.3 ± 112.2	2.25
1000	1011.7	1022.8	1031.5	1022.0 ± 8.1	0.79
500	452.9	449.8	452.2	451.6 ± 1.3	0.29
100	106.4	109.4	105.6	107.1 ± 1.6	1.52
50	43.2	42.2	39.5	41.6 ± 1.6	3.76
10	11.1	10.7	13.4	11.7 ± 1.2	10.10

pared and analysed. The results obtained from a similar preparation, but analysed on different days, are presented in Table II. The C.V. for the inter-day precision ranged from 0.3 to 10%.

TABLE III

PROPOFOL DETERMINATION IN EXTENDED CONCENTRATION RANGE

Linear regression equations: Dilution factor 1: $y = 1.0049x$ (±0.0040) + 17.3816 (± 13.3999); $r = 0.9999$ (±0.00001). Dilution factor 2: $y = 0.9972x$ (±0.0096) − 3.6967 (±22.5919); $r = 0.9997$ (±0.0026). Dilution factor 0.5: $y = 1.0083x$ (±0.0078) − 10.9193 (±22.2343); $r = 0.9999$ (±0.00001).

Spiked, x (ng/ml)	D.F.[a]	Measured, y (ng/ml)[b]	C.V. (%)
20 000	1	20 114.0 ± 82.1	0.41
	2	10 047.0 ± 110.0	1.10
10 000	1	10 071.1 ± 124.4	1.24
	2	4818.2 ± 53.6	1.11
	0.5	20 156.9 ± 139.3	0.69
1000	1	1009.8 ± 8.6	0.85
	2	542.5 ± 10.5	1.94
	0.5	1986.0 ± 80.3	4.04
500	1	547.9 ± 4.6	0.85
	2	283.3 ± 3.3	1.18
	0.5	992.2 ± 9.1	0.92
10	1	9.8 ± 0.4	4.41
	0.5	19.9 ± 1.3	6.64
5	0.5	11.6 ± 0.5	4.70

[a] Dilution factor.

[b] Data are mean ± S.D. of three determinations.

Accuracy in extended concentration range

The accuracy in the quantitation of propofol outside the calibration concentration range was assessed. The results were satisfactory (Table III). Concentrations as low as 5 ng/ml can be measured by reducing the chloroform volume ratio in the extraction, and concentrations as high as 20 000 ng/ml showed recoveries proportional to the increased chloroform/plasma volume ratio in the extraction.

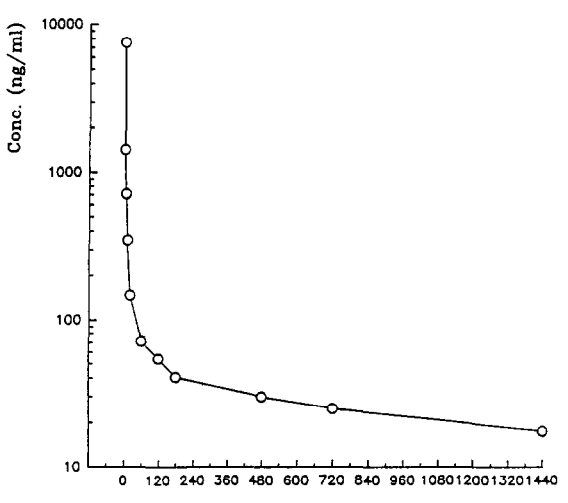

Fig. 3. Plasma level–time profile of propofol in a patient given an intravenous bolus dose of propofol (2.5 mg/kg).

Clinical application.

The plasma concentration of propofol in a patient under propofol anaesthesia was analysed by the GC method. The results are shown in Fig. 3. The plasma concentration of propofol dropped very rapidly during the first 20 min, then decreased slowly up to 60 min, and very slowly later. The plasma concentration–time profile of propofol up to 24 h after a usual dose is within the studied concentration range of the calibration.

DISCUSSION

The concentration range of propofol standards was chosen to cover the usual range of concentrations in patients under anaesthesia [8]. Interference from endogenous compounds was overcome by making the plasma sample slightly alkaline before extraction: otherwise a very small peak was noted close to the peak of propofol. Excess alkali should be avoided because of emulsion formation, which could preclude the separation of chloroform from plasma in the extraction procedure. Experience showed that acidifying the plasma sample increased the interference peak height, which suggests that the peak may belong to an endogenous organic acid.

The GC conditions were adjusted to maximize sensitivity and resolution of peaks. The temperature, the carrier gas pressure, the split and the purge flow were optimized to resolve propofol from the solvent and endogenous peaks in an acceptable time.

The limit of determination of the assay has been evaluated at 10 ng/ml. However, when half the volume of chloroform was used in the extraction, the limit of determination was improved to 5 ng/ml. One promising approach to improve the sensitivity would be to extract the plasma sample with a smaller volume fraction of chloroform. Based on the same theory, propofol concentrations beyond the concentration range of the present study could be quantitated accurately by taking a smaller fraction of plasma sample.

Analysis of a patient's plasma confirmed that the proposed GC method is suitable for routine clinical plasma level monitoring and for pharmacokinetic studies. Plasma levels are within the studied concentration range after the usual dose.

The chromatograph was operated under isothermal conditions. It took less than 7 min to complete a GC analysis. Only 0.2 ml of plasma sample and one-step extraction with a small amount of commonly available solvent were required. It was unnecessary to concentrate the organic extract, and loss of propofol through evaporation was thus avoided. The sensitivity, the reproducibility and the precision as demonstrated in the results indicated that this is a reliable, rapid and economical method for the assay of propofol in routine clinical plasma level monitoring and in laboratory research work.

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