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Short communication

Quantitation of propofol in whole blood by gas chromatography–mass spectrometry

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Abstract

A gas chromatographic–mass spectrometric assay, using selected-ion monitoring (GC–MS–SIM) with thymol as internal standard, was developed for quantitating propofol, an intravenous anaesthetic. The method described is rapid and sensitive for the determination of propofol in whole blood. The sensitivity of the present method is 10 ng/ml. The recovery of propofol added to human whole blood in the concentration range 10–10 000 ng/ml ranged between 95 and 100%. A single extraction procedure was used with chloroform–ethyl acetate. The assay allowed the detection of two metabolites formed during propofol metabolism: 2,6-diisopropyl-1,4-quinone and 2,6-diisopropyl-1,4-quinol.

1. Introduction

Propofol (2,6-diisopropylphenol) is an intravenous anaesthetic, active agent of Diprivan (Zeneca Pharma, Cergy, France). Propofol is employed in anaesthesiology for induction of anaesthesia by bolus injection and maintenance of anaesthesia by repeated bolus doses or by continuous infusion. Propofol is also used in intensive care for sedation.

Most methods for propofol quantitation use high-performance liquid chromatography (HPLC) with UV [1], fluorescence [2], or electrochemical [3] detection. Recently, a method was described, measuring propofol concentration in human serum by capillary gas chromatography with flame ionization detector [4].

In humans, propofol is metabolized by the liver and other organs [5]. For several species, including humans, metabolism is by conjugation of the parent compound or its quinol derivative obtained by hydroxylation [6].

This paper presents a simple, rapid, and sensitive method for quantitation of propofol by gas chromatography–mass spectrometry (GC–MS) in human whole blood. Moreover, chromato-

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graphic experimental conditions are described for the detection of two metabolites of propofol.

2. Experimental

2.1. Drugs and chemicals

Pure propofol, generously supplied by Zeneca Pharma (Cergy, France), was used for calibration standard. Thymol (5-methyl-2-isopropylphenol), sodium tetraborate, N,O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA), and pyridine were obtained from Sigma (St. Quentin Fallavier, France). Chloroform and ethyl acetate were chromatography grade and were purchased from Merck (Darmstadt, Germany). Distilled water was used.

Blank whole blood was obtained from apparently healthy human volunteers using Vacutainer collecting tubes containing EDTA (K_3) anticoagulant (Becton Dickinson, Pont de Claix, France). Samples from patients were obtained from the Department of Intensive Care and Anaesthesiology, Centre Hospitalier Universitaire Lyon Sud (Pierre Bénite, France). Blood samples were stored at 4°C until analyzed for propofol content.

2.2. Preparation of standard solutions

Stock solutions of propofol and thymol (respectively at 10 mg/ml and 1 mg/ml) were prepared in methanol and stored at 4°C. The vials were filled with nitrogen gas after each opening to protect the drug from oxidation. These solutions were further diluted with methanol to produce spiking standard solution. All spiking solutions were prepared fresh weekly, and whole blood calibration standards were prepared fresh daily at 10 000, 5000, 1000, 500, 100, 50 and 10 ng/ml, by spiking 500 μl of blank whole blood with 10 μl of the appropriate solution.

2.3. Apparatus and chromatographic conditions

The GC–MS apparatus (Model GCD, Hewlett Packard, Palo Alto, CA, USA) containing a HP-1 (25 m × 0.20 mm × 0.33 μm film thickness) capillary column was used. Injector inlet (splitless) and detector temperatures were 240°C and 280°C, respectively. The oven temperature for propofol quantitation was held at 55°C for 1 min, increased at 20°C/min to 150°C and maintained at 150°C for 3 min. For metabolite determination, the temperature gradient was similar, but after 3 min at 150°C, the oven temperature was increased at 30°C/min to 250°C. The helium gas flow-rate was 1 ml/min and the column head pressure was 8 p.s.i. at 55°C (1 p.s.i. = 6.9 kPa). The electron energy was 70 eV, the trap current 100 μA. An automatic injector (Model 7673, Hewlett Packard, Palo Alto, CA, USA) was used. Monitoring ions for the quantitation of propofol and thymol were used as follows: m/z

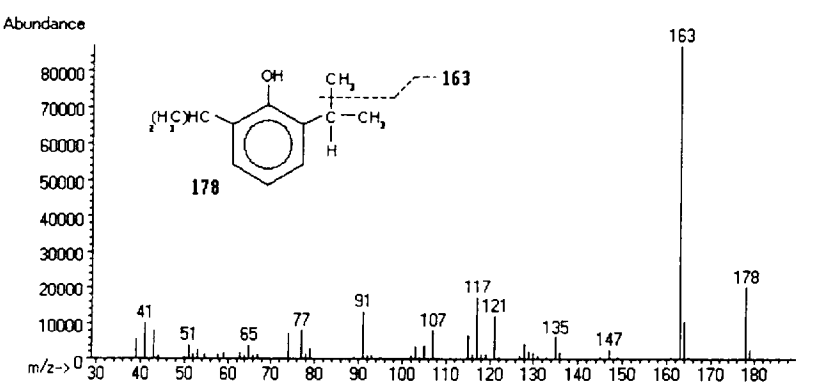

Fig. 1. Structure and mass spectrum of propofol (retention time 9.10 min). Molecular ion at m/z 178, and major fragment at m/z 163 (due to the loss of a methyl group). Propofol was detected and quantitated using selected-ion monitoring at m/z 163.

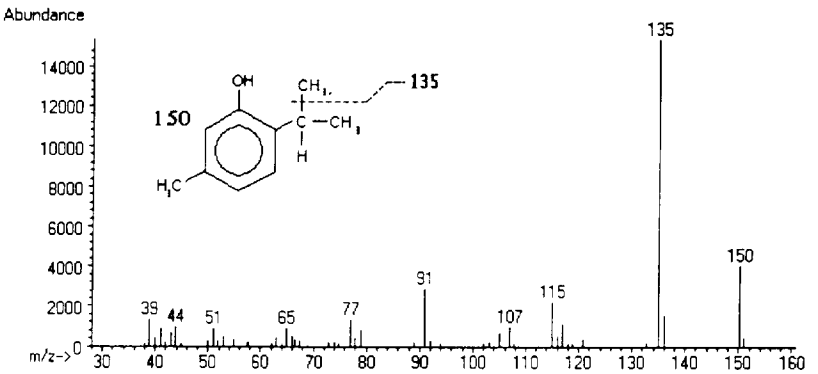

Fig. 2. Structure and mass spectrum of thymol (retention time 8.00 min). Molecular ion at m/z 150, and major fragment at m/z 135 (due to the loss of a methyl group). Thymol was detected and quantitated using selected-ion monitoring at m/z 135.

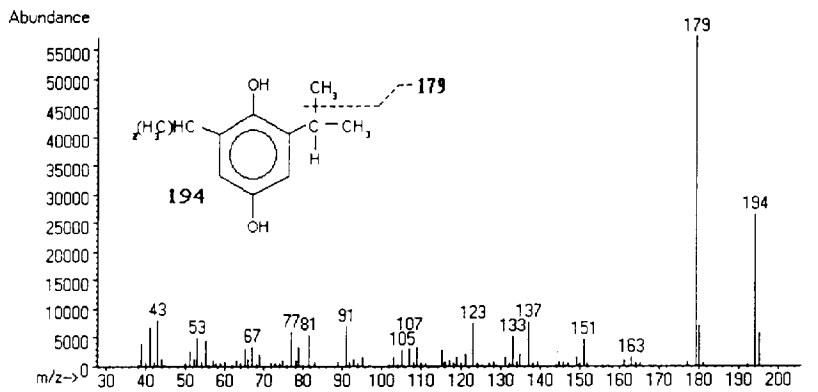

Fig. 3. Structure and mass spectrum of 2,6-diisopropyl-1,4-quinol (retention time 12.04 min). Molecular ion at m/z 194, and major fragment at m/z 179 (due to the loss of a methyl group). 2,6-Diisopropyl-1,4-quinol was detected using selected-ion monitoring at m/z 179.

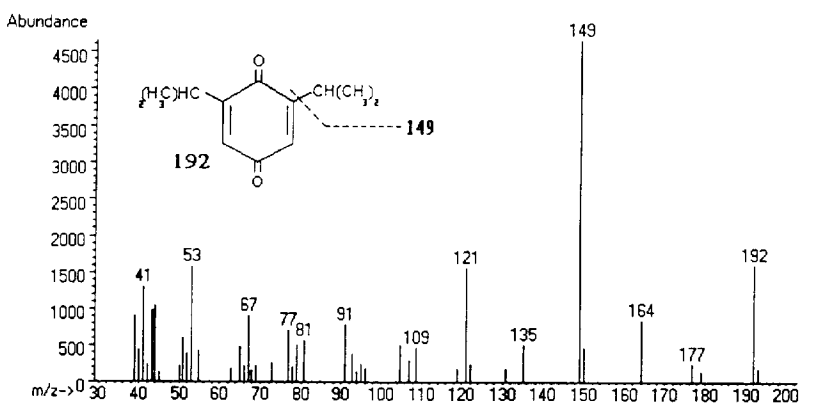

Fig. 4. Structure and mass spectrum of 2,6-diisopropyl-1,4-quinone (retention time 9.62 min). Molecular ion at m/z 192, and major fragment at m/z 149 (due to the loss of a propyl group). 2,6-Diisopropyl-1,4-quinone was detected using selected-ion monitoring at m/z 149.

163 for propofol, and m/z 135 for thymol (Figs. 1 and 2). Monitoring ions for the determination of 2,6-diisopropyl-1,4-quinone and 2,6-diisopropyl-1,4-quinol in plasma were: m/z 149 and m/z 179, respectively (Figs. 3 and 4).

2.4. Extraction procedure

Propofol is significantly associated with the formed elements of blood and therefore whole blood is the preferred sample for pharmacokinetic analysis [2]. Thus, an extraction procedure was developed in this medium.

A 0.5-ml aliquot of the blood samples or propofol calibration standard was pipetted into a 10-ml glass tube, and 50 μl of thymol (1000 ng/ml) as the internal standard working solution were added and mixed. A 1.0-ml distilled water aliquot was added and mixed thoroughly on a vortex mixer. A 0.5-ml aliquot of borate buffer (pH 9) was added and mixed on an inversion mixer (Bioblock, France) for 5 min at 20 rpm. Then 300 μl chloroform–ethyl acetate (70:30, v/v) was added, and the mixture was placed on an inversion mixture for 20 min at 50 rpm. After centrifugation (3900 g for 10 min at 10°C) an aliquot of the organic layer, without concentration, was transferred to an airtight amber glass vial and 1 μl was injected into the capillary column of the GC–MS system.

2.5. Study of metabolites

The study of 2,6-diisopropyl-1,4-quinone and 2,6-diisopropyl-1,4-quinol formation was based on rat liver microsomes which were prepared as described previously [7]. Briefly, metabolite formation was determined in 1 ml incubation mixtures (37°C, 15 min) containing 0.1 M potassium phosphate buffer (pH 7.4), propofol, rat liver microsomes and an NADPH-regenerating system (0.5 mM NADP, 5 mM glucose-6-phosphate, 1 IU/ml glucose-6-phosphate dehydrogenase). Reactions were initiated by the addition of the NADPH-regenerating system, and stopped by putting 50 μl of 1 M NaOH in the vials placed in an ice bath. The extraction procedure was similar to that previously described for propofol. Derivatization of metabolites was performed as follows. Trimethylsilyl (TMS) derivatives were prepared by adding 80 μl pyridine and 50 μl BSTFA to 100 μl of organic layer (obtained after extraction), and heating at 50°C for 30 min. Samples were transferred to auto sampler vials and analyzed by GC–MS without removing the excess derivatizing reagent.

Propofol metabolites are sulfo and glucuro conjugated in plasma [8]; consequently, chemical hydrolysis of human plasma was performed. In a glass tube, 1.0 ml of 5 M hydrochloric acid was added to 300 μl of plasma and mixed thoroughly with a vortex mixer. The vials were tightly closed and put in a thermobath at 100°C for 1.5 h. Then, the vials were cooled at 4°C, and 1.0 ml of 5 M sodium hydroxide and 1.0 ml of borate buffer were added to produce a pH 9. The extraction procedure was similar for metabolites and propofol determinations. A 1-μl aliquot of the organic layer was analysed by GC–MS.

3. Results and discussion

A linear relationship was obtained between the ratio of the peak of propofol to that of thymol and the amount of propofol added to blood in a range of 10–10 000 ng/ml. No change in the slope was observed with various samples of blood used.

The GC conditions were adjusted to maximise selectivity and sensitivity in a manner similar to that described by Yu and Liau [4].

The chromatographic specificity was tested by injecting blank plasma samples from healthy and sick human subjects. Plasma samples from subjects receiving fentanyl and midazolam before blood sampling were analysed. No peak at the expected retention time for propofol was detectable.

For the extraction procedure, borate buffer (pH 9) was preferred to sodium hydroxide because excess alkali induces emulsion formation with chloroform making the separation of organic layer and whole blood critical.

Table 1
Propofol determination precision study—within-run

Spiked (ng/ml)	n	Measured concentration (mean ± S.D.) (ng/ml)	C.V. (%)
10 000	5	9800.8 ± 50.6	0.5
500	5	509.3 ± 8.9	1.7
10	5	11.9 ± 0.3	2.9

The sensitivity of the present method is 10 ng/ml. The sensitivity can be increased, if necessary, by using a lower volume of chloroform–ethyl acetate in the extraction step, and increasing the injected sample volume and the detector amplification.

3.1. Intra-day and inter-day precision

The within-run precision was evaluated by repeated measurements of whole blood samples spiked with propofol at three concentrations: 10, 500, and 10 000 ng/ml (Table 1). The C.V. for the intra-day precision ranged from 0.5 to 2.9%. The between-run precision was evaluated using whole blood samples spiked with propofol in the range 10–10 000 ng/ml. The results obtained from a similar preparation and analysed on three different days are listed in Table 2. The C.V. for the inter-day precision ranged from 0.3 to 7.1%.

3.2. Extraction recovery

The absolute recovery of propofol added to a control blood was determined by comparison of the slopes for extracted calibration curves with those obtained by injecting chloroform–ethyl acetate solutions at the same concentrations. Mean absolute recovery for propofol was more than 95%.

3.3. Ruggedness and reproducibility

The ruggedness and reproducibility of the method were also evaluated. The results obtained from four days different preparations and analysed on different days, are presented in Table 3. The linear regression equation was (mean ± S.D.): $y = 0.934 \pm 0.046x - 0.009 \pm 0.010$, and the correlation coefficient was 0.9999 (mean).

3.4. Chromatograms

Fig. 5 shows chromatograms of the extract of a human whole blood (A) sampled 15 min after induction of anaesthesia with an intravenous bolus of propofol (2.5 mg/kg), and the extract of a same human serum (B) after chemical hydrolysis. The selected-ion monitoring (SIM) mode was used. Four peaks were clearly separated in (B): internal standard; propofol; 2,6-

Table 2
Propofol determination precision study—between-run

Spiked (ng/ml)	Measured concentration (ng/ml)				C.V. (%)
	Day 1	Day 2	Day 3	Mean ± S.D.	
10 000	9981.1	9993.3	9944.1	9972.9 ± 25.6	0.3
5000	5046.8	5017.5	5125.2	5063.2 ± 55.7	1.1
1000	965.8	985.1	950.2	967.0 ± 17.4	1.8
500	474.4	485.9	461.6	474.0 ± 12.1	2.5
100	108.0	99.6	101.9	103.2 ± 4.3	4.2
50	52.8	47.1	53.9	51.3 ± 3.6	7.1
10	13.2	13.6	12.8	13.2 ± 0.4	3.0

Table 3
Ruggedness and reproducibility of the method

Spiked (ng/ml)	Measured concentration (ng/ml)					C.V. (%)
	Extraction 1	Extraction 2	Extraction 3	Extraction 4	Mean ± S.D.	
10 000	10 043.6	10 046.0	10 020.3	9974.2	10 021.0 ± 33.2	0.3
5000	4910.4	4907.6	4960.5	5072.0	4962.6 ± 76.8	1.5
1000	1013.5	998.8	990.6	904.7	976.9 ± 49.0	5.0
500	493.7	500.2	503.8	478.5	494.1 ± 11.1	2.2
100	109.8	110.8	118.4	118.2	114.3 ± 4.6	4.0
50	57.9	61.0	55.1	50.2	56.1 ± 4.6	8.2
10	12.9	13.8	11.6	10.9	12.3 ± 1.2	10.3

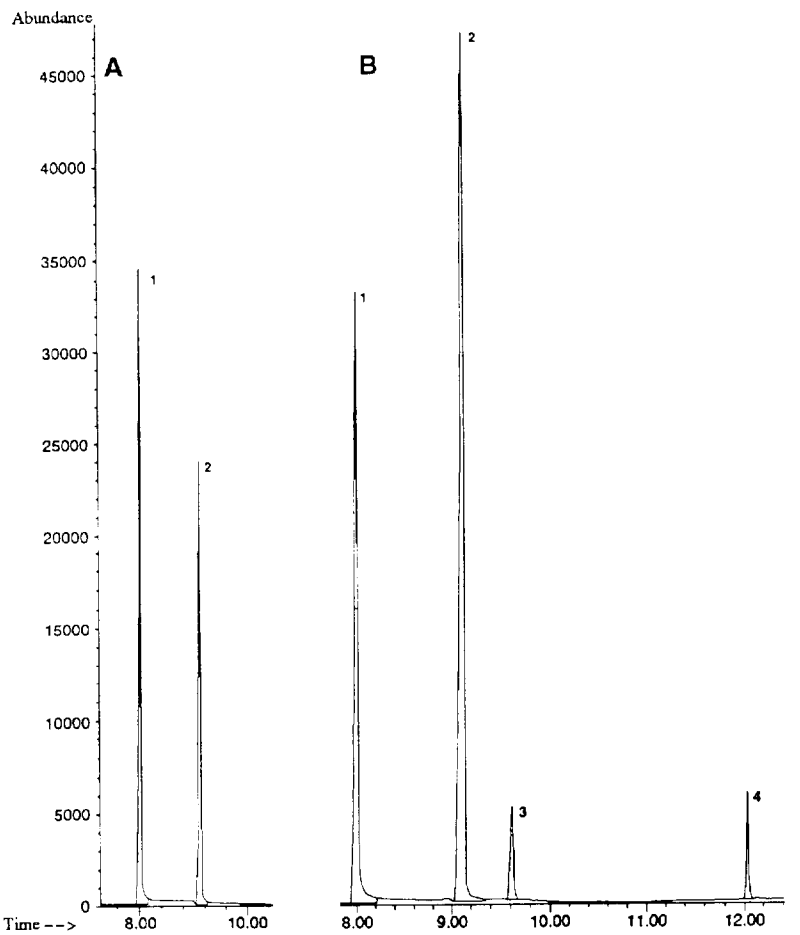

Fig. 5. Gas chromatogram profiles of propofol (748 ng/ml) in whole blood (A), and in serum (B) after chemical hydrolysis. Blood containing propofol and metabolites was obtained from the same patient, sampled 15 min after a single dose of propofol. Thymol (1000 ng/ml) was added as the internal standard. Peaks 1, 2, 3 and 4 are thymol, propofol, 2,6-diisopropyl-1,4-quinone and 2,6-diisopropyl-1,4-quinol, respectively.

diisopropyl-1,4-quinone and 2,6-diisopropyl-1,4-quinol.

3.5. Clinical application

Whole blood concentrations of propofol in patients under propofol anaesthesia or sedation were analysed by the GC–MS–SIM method. After injection, propofol blood concentrations decayed in a curvilinear manner with time. The pharmacokinetic profile was best-fitted by a three-compartment open model with central elimination. The drug is extensively distributed and rapidly cleared from the body [9]. The whole blood concentration–time profile in a patient under propofol anaesthesia is shown in Fig. 6.

3.6. Study of metabolites

Propofol glucuronide and the 1- and 4-glucuronide and 4-sulphate conjugates of 2,6-diisopropyl-1,4-quinol are described as the major metabolites in humans [8]. Propofol is extremely rapidly metabolised into its conjugates which are liberated after hydrolysis [6]. Vree et al. did not detect the 1,4-quinol derivative in human plasma after hydrolysis by 5 *M* hydrochloric acid, whereas the 1,4-quinone derivative appeared [1]. In contrast, we found both metabolites in human serum after chemical hydrolysis (Fig. 5). One explanation of this difference may be that metabolite 1,4-quinol may undergo chemical conversion in 1,4-quinone by tautomeric equilibrium. This transformation may be due to the difference in pH mixture (alkaline in our study and acid in Vree's study) used during extraction. In our study, the identification of the two metabolites in human serum was established by comparison of the retention time and the mass spectrum of the peak in the samples with those obtained from microsome incubation. Chemical derivatization of propofol metabolites was required to confirm the phenolic and ketonic structural forms of the two derivatives. For 2,6-diisopropyl-1,4-quinone, no derivatization was observed, whereas 2,6-diisopropyl-1,4-quinol was achieved by silylation by BSTFA. Two prominent ions at *m/z* 338 (diTMS derivative) and at *m/z* 323 (due to the loss of methyl group) were observed.

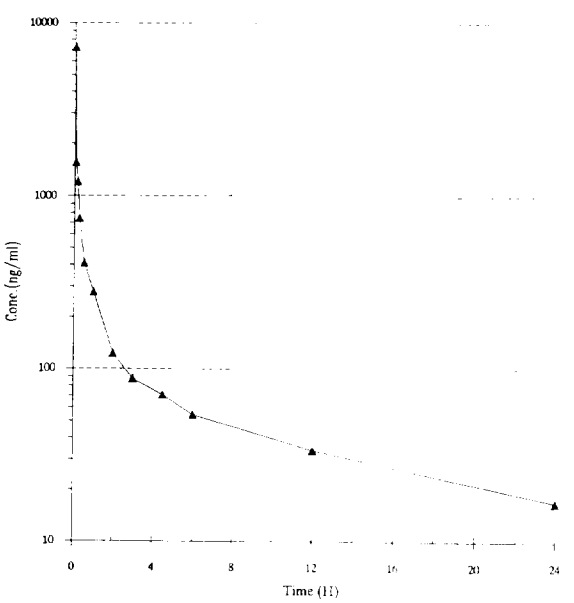

Fig. 6. Whole blood concentration of propofol as a function of time in a patient, after an intravenous bolus dose of propofol (2.5 mg/kg).

Quantitation of the metabolites was not realised due to the absence of authentic metabolite standards. Therefore, we did not try to modify the extraction procedure and optimal conditions were used for chemical hydrolysis as described above [1].

4. Conclusion

The method described here is sensitive and selective for quantitation of propofol in whole blood. It is also rapid and simple because it does not need any organic layer concentration. The method is reproducible and exhibits adequate precision and accuracy to cover the range of propofol concentrations in patients under anaesthesia. Furthermore, the assay is performed in whole blood, which is the preferred sample for pharmacokinetic analysis. Chromatographic conditions coupled to mass spectrometry detection permitted the measurement of propofol with a good specificity and to identify two metabolites

in human blood. Nevertheless, it is necessary to study the quality of extraction for derivatives.

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