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Microdetermination of propofol in plasma by a rapid and sensitive liquid chromatographic method

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Abstract

A direct and sensitive liquid chromatographic method for the determination of propofol in 50 μl of plasma is described. The separation of the drug and internal standard (methyldopa) was achieved using a 4 μm particle size C_{18} cartridge (10 cm × 8 mm i.d.) in conjunction with a radial compression system and a C_{18} precolumn module. The mobile phase consisted of 0.01 M sodium acetate solution (adjusted to pH 3 with acetic acid)–acetonitrile–methanol (37:47.25:15.75, v/v/v) at a flow rate of 2 ml min^{-1}. The compounds were detected in the effluent spectrofluorimetrically with excitation and emission wavelengths of 276 and 310 nm, respectively. After the internal standard had been added, the sample was diluted with 50 μl of hydrochloric acid and centrifuged prior to injection into the chromatograph. The peaks of both propofol and internal standard under these conditions were sharp and symmetrical, and the retention times were 8.2 and 5.15 min, respectively. The peak-height ratio (drug/internal standard) varied linearly ($r > 0.9959$) with concentration in the ranges 0.002–0.1 and 0.1–10 μg ml^{-1} and the relative standard deviation was consistently $< 5.6\%$. There was no interference in the assay from the endogenous substance or other concomitantly used drug. This method is currently being used for monitoring propofol in intensive care patients and investigating its pharmacokinetics.

Keywords: Intensive care patients; Sedation; Propofol; HPLC; Plasma; Drug monitoring

1. Introduction

Propofol (2,6-diisopropylphenol) is an intravenous anaesthetic agent that is highly suitable for both induction and maintenance of anaesthesia. This drug has also become increasingly popular as a sedative in intensive care unit (ICU) patients requiring prolonged mechanical ventilation. After rapid distribution into the tissues, propofol undergoes a rapid metabolic clearance followed by a slow elimination. Because of a close relationship between the blood concentration of this drug and its anaesthetic or sedative action in patients, measurement of its concentration in plasma or whole blood is highly desirable. This is particularly important in patients with acute liver dysfunction.

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Propofol has been determined by gas chromatography [1,2] and high-performance liquid chromatography (HPLC) using electrochemical [3,4], fluorimetric [5–7] or ultraviolet (UV) detection [8]. However, these methods either involve tedious liquid–liquid or solid–liquid extraction or require 0.5 ml of biological fluid, which can only be obtained by venipuncture, which is undesirable, particularly in sick children. Further, when a smaller volume is employed, the detection limit of these assays becomes inapplicable for quantifying low concentrations frequently observed in pharmacokinetic investigations of this drug, i.e. 0.002–0.02 μg ml^{-1}. More recently, a method based on a precolumn extraction was described [9]; however, it requires closed system instrumentation, which may not be available.

This paper describes a sensitive (lowest measurable concentration = 0.002 μg ml^{-1}), rapid (total analysis time = 15–20 min) and accurate liquid chromatographic method for the direct determination of propofol in microsamples (50 μl) of unextracted plasma.

2. Experimental

2.1. Chemical and reagents

Propofol was obtained from the Pharmaceuticals Division, ICI (Macclesfield, UK) and the internal standard (IS) methyldopa from MSD (West Point, PA). Hydrochloric acid, glacial acetic acid, methanol, acetonitrile (all from Fisher Scientific, Fair Lawn, NJ) and anhydrous sodium acetate (Fluka, Buchs, Switzerland) were of HPLC grade. Reverse-osmosis water passed through a 0.45 μm pore size membrane filter (Millipore, Milford, MA) was employed to prepare water for HPLC.

2.2. Instrumentation

The liquid chromatographic system consisted of a programmable solvent-delivery pump (System Gold Module 126), an analogue interface module (Model 406) (both from Beckman Instruments, Altex Division, San Ramon, CA), an automatic sampling–injection module (Waters 715 Ultra Wisp), a radial compression separation module (Z-Module) equipped with 4 μm particle size NovaPak C_{18} Radial Pak cartridge (10 cm × 8 mm i.d.) preceded by a Guard-Pak precolumn module with a C_{18} insert and a fluorescence detector (Model 470) set at excitation and emission wavelengths of 276 and 310 nm, respectively (all from Waters, Milford, MA). A Tandon PCA-40 computer with System Gold software connected to a Citizen 180E printer were used for system monitoring and data collection.

2.3. Chromatography

The mobile phase was prepared by mixing 370 ml of 0.01 M sodium acetate solution (adjusted to pH 3 with acetic acid), 472.5 ml of acetonitrile and 157.5 ml of methanol and filtering the mixture through a 0.45 μm pore size membrane. After degassing, the mobile phase was pumped at a flow rate of 2 ml min^{-1} (pressure < 1700 psi).

2.4. Preparation of calibration curves

Since the therapeutic concentration of propofol in plasma spans a wide range, calibration curves in two ranges, 0.002–0.1 μg ml^{-1} (low range) and 0.1–10 μg ml^{-1} (high range), had to be constructed. This approach, using a different amount of IS for each range, markedly improves the quantitation of low and high concentrations of propofol observed during therapy. To construct such a curve, 50 μl aliquots of blank human plasma were supplemented with appropriate amounts of propofol to produce serial concentrations in the above ranges. To each of these samples 30 μl of 0.01 μg ml^{-1} (low range) or 70 μl of 0.1 μg ml^{-1} (high range) IS solution and 50 μl of 2 M hydrochloric acid were added. After adjusting the volume to 170 μl with HPLC-grade water, the sample was centrifuged for 5 min at 1400 rpm, and a suitable aliquot was injected by the autosampler into the chromatograph. An intact blank plasma sample containing no propofol was prepared and analyzed similarly.

2.5. Precision and accuracy

The intra-run (within-day) precision and accuracy of the assay were investigated by supplementing blank plasma with different amounts of propofol to produce concentrations of 0.01, 0.4 and 3 $\mu g\ ml^{-1}$. Each sample was divided into 10 replicates which were analysed individually, and the relative standard deviations (RSDs) of the concentration, which reflect the intra-run precision at each level, were calculated. The deviation from perfect accuracy was calculated as 100 (concentration found − concentration present)/concentration present.

2.6. Analysis of patients' samples

The applicability of the assay to quantify propofol in plasma was examined by analysing plasma samples collected at different time intervals from an ICU male patient (age = 47 years, weight = 90 kg) who received propofol by intravenous infusion at a rate of 100 mg h^{-1}. The concentration of propofol was calculated using a calibration curve constructed on the same day and obtained using identical conditions.

3. Results and discussion

To purify plasma prior to injection into the chromatograph, several approaches were attempted. While extraction with cyclohexane was a tedious and lengthy procedure, and yielded a poor chromatogram with several interfering peaks, deproteinization with a mixture of acetonitrile and perchloric acid at different ratios produced a low recovery of propofol. Similarly, the use of a micropartition filter system (Centrifree-MS from Amicon Division, W.R. Grace, Danvers, MA) gave a very low recovery, presumably because propofol was retained on the filter owing to its high protein binding. On the other hand, addition of hydrochloric acid (2 M) in an equal proportion (by volume) to the plasma produced a clean chromatogram with a recovery of 88.2%, and hence this method was adopted. It should be noted that the Guard-Pak precolumn C_{18} insert was changed daily to maintain a relatively low back-pressure and prevent interferences from plasma endogeneous substances.

Fig. 1 depicts representative chromatograms of a blank plasma sample, a plasma sample spiked with propofol and methyldopa (IS) and a plasma sample collected from a patient who had received propofol by intravenous infusion at a rate of 100 mg h^{-1}. A similar chromatogram demonstrating the sensitivity of the assay is presented in Fig. 2. As can be seen, the retention times of propofol and the IS were 8.2 and 5.15 min, respectively, and the peaks were sharp and symmetrical, typical of radial compression liquid chromatography (i.e. with a 10 cm × 8 mm i.d. Radial Pak cartridge). This system, while maintaining a large retention capacity, also allows the use of a higher flow rate, which shortens the analysis time. It is noteworthy that under these conditions the sharp and symmetrical characteristics of the peaks were maintained in spite of the high acid content of the plasma samples injected, which often causes bleeding of the column.

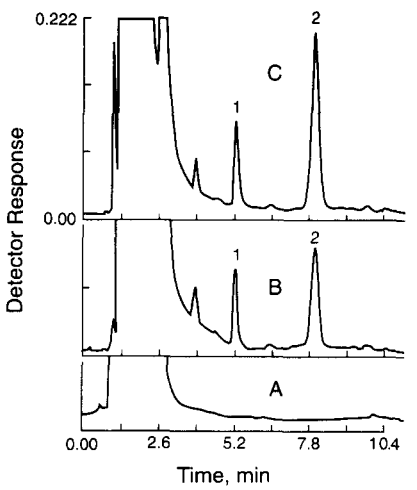

Fig. 1. Typical chromatograms of (A) a blank plasma sample, (B) a blank plasma sample supplemented with 0.4 $\mu g\ ml^{-1}$ of propofol (peak 2) and 140 $\mu g\ ml^{-1}$ of IS (peak 1); and (C) a plasma sample collected from a patient immediately after the termination of intravenous infusion of propofol at a rate of 100 mg h^{-1} for 40 h, and to which 140 $\mu g\ ml^{-1}$ of IS was added. The measured concentration of propofol in the patient's sample was 0.633 $\mu g\ ml^{-1}$.

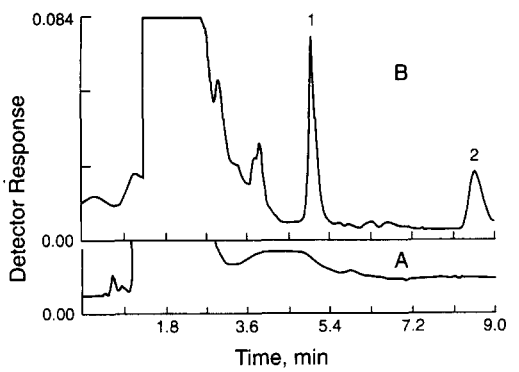

Fig. 2. Representative chromatograms of (A) a blank plasma sample (B) a blank plasma sample supplemented with 140 μg ml^{-1} of IS (peak 1) and 0.002 μg ml^{-1} of propofol (peak 2), the sensitivity limit (i.e. minimum determinable concentration) of the assay.

The chromatographic conditions employed were selected by examining the influence of the mobile phase organic–inorganic composition. As demonstrated in Fig. 3, the retention volume of propofol increased almost linearly as a function of the proportion of sodium acetate solution (0.01 M) in the mobile phase with the range 30–42%, beyond which the increase became non-linear. However, a proportion of 43% was selected to keep the chromatographic time < 10 min. It should be noted that with this mobile phase composition methyldopa (retention time = 5.15 min), which has some chemical similarity to propofol, was completely separated from it, and was therefore selected as an internal standard. With a C_{18} stationary phase, one expects that on increasing the proportion of the inorganic component (or decreasing that of the organic component) in the mobile phase, the retention volume of the drug will increase. This technique is often employed to control the retention time and optimize the chromatography.

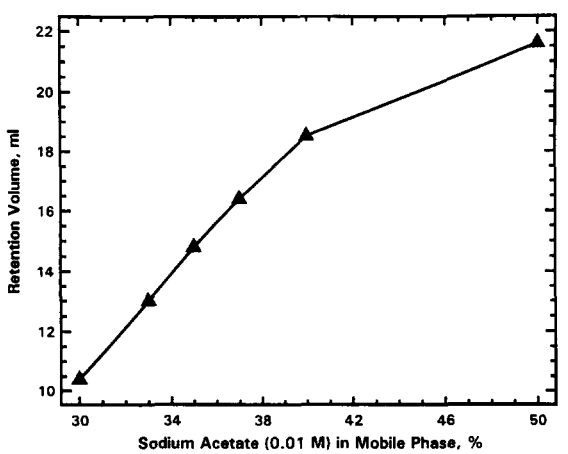

Fig. 3. Effect of the proportion of sodium acetate (0.01 M) in the mobile phase on the retention time of propofol under the described conditions.

The assay was highly linear in the concentration ranges examined for propofol; the correlation coefficient (r) for the curves of peak-height ratio (propofol/IS) versus concentration was consistently > 0.997 (mean = 0.9982, range = 0.9970–0.9999, $n = 8$) for the lower range (0.002–0.1 μg ml^{-1}) and > 0.9959 (mean = 0.9971, range = 0.9959–0.9995, $n = 8$) for the high range (0.1–10 μg ml^{-1}). The intercepts on different days were relatively small and ranged between -0.313 to 0.394 for the low range and between -0.737 and 0.987 for the high range. The accuracy and precision of the assay were equally good. As shown in Table 1, the deviation from perfect accuracy at concentrations of 0.01, 0.1 and 1 μg ml^{-1} was -2.6, 1.7 and 1.97%, respectively, and the within-run RSD, at these concentrations were 5.85, 2.5 and 2.43%, respectively.

The specificity of the assay was investigated by determining the retention times of drugs which may be used concurrently with propofol in ICU patients under the same chromatographic conditions as described above. As can be seen in Table 2, none of these drugs interferes in the assay. Further, no interference from endogenous plasma substance was noticed. This was ensured by changing the precolumn insert at the end of the day. An average of 32 deproteinized plasma samples were injected per day.

The plasma concentrations of propofol at 0, 10, 20 and 60 min after the termination of intravenous infusion of this drug at a rate of 100 mg h^{-1} for 40 h in an ICU patient were 0.633, 0.224, 0.205 and 0.193 μg ml^{-1}, respectively. Thus, following a rapid initial drop, the concentration of propofol in this patient declined at a much slower rate, typical of the pharmacokinetic characteristics of this drug.

Table 1
Within-run precision and accuracy of the described assay for propofol in plasma

Concentration present ($\mu g\ ml^{-1}$)	Concentration found[a] ($\mu g\ ml^{-1}$)	RSD (%)	Deviation[b] from perfect accuracy (%)
0.010	0.0974	5.85	−2.6
0.100	0.1017	2.50	1.7
1.000	1.0197	2.43	1.97

[a] Mean of 10 determinations.
[b] Calculated as 100(concentration found − concentration present)/concentration present.

Table 2
Retention times of drugs that may be used concurrently with propofol in ICU patients

Drug	Retention time (min)	Drug	Retention time (min)
Propofol	8.20	Ceftazidime	ND
Methyldopa	5.15	Tetracycline	2.49
Epinephrine	ND[a]	Amphotericin B	ND
Norepinephrine	ND	Miconazole	9.71
Dopamine	ND	Fluconazole	ND
Dobutamine	ND	Bleomycin	ND
Ampicillin	14.48	Taxol	2.4
Amoxicillin	ND	Metoclopromide	ND
Penicillin G, benzathine	ND	Albuterol	ND
Cefoxitin	ND	Labetalol	10.78
Cephalexin	ND	Esmolol	ND
Ceprofluoxacin	2.4	Nitrogycerin	ND
Gentamicin	ND	Nitroprusside, sodium	ND
Erythromycin	13.66	Ranitidine	ND
Streptomycin	ND		

[a] Not detected during a 15 min run.

In summary, the method presented in this paper is a sensitive, expedient and accurate method for the determination of propofol in plasma. The small sample size required, which can easily be obtained by fingertip puncture, coupled with the ease and rapidity with which the sample is processed prior to chromatography, make this assay highly suitable for pharmacokinetic monitoring of this drug in high-risk patients.

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