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Short Communication

Rapid and sensitive pre-column extraction high-performance liquid chromatographic assay for propofol in biological fluids

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ABSTRACT

A completely automated high-performance liquid chromatographic system is described for the determination of the phenolic anaesthetic propofol. The method is based on pre-column extraction in a closed system allowing direct injection of biological samples without any sample pretreatment. The assay is sensitive (limit of quantification is 5 ng/ml serum), reliable (the variability within a series is 2%) and rapid (results are available after 6 min).

INTRODUCTION

Propofol (P), a phenolic intravenous anaesthetic, is becoming increasingly popular in clinical anaesthesia. Because of its rapid onset and short duration of action it is suitable for both induction of anaesthesia by bolus injection and maintenance of anaesthesia by constant-rate infusion. Many studies have been performed to establish pharmacokinetic parameters from which a rationale may be developed for the adjustment of the dosage regimen under different conditions. The analytical methods used so far for the determination of P are based on time-consuming extraction procedures [1–3]. We have developed a pre-column extraction high-performance liquid chromatographic (HPLC) method [4] allowing direct, rapid and sensitive analysis of P in biological fluids.

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EXPERIMENTAL

Materials

Acetonitrile and dipotassium hydrogenphosphate were obtained from Merck (Darmstadt, Germany). Pure compound of P, obtained as a generous gift from ICI England, was used for the *in vitro* experiments.

Apparatus

A Waters (Milford, MA, USA) high-performance liquid chromatograph was used. The set-up was equipped with two constant-flow pumps (Model 510), an autosampler (Model WISP 712), a fluorescence detector (Model 470), and a six-way valve (Model 7010, Rheodyne, Bischoff Analysentechnik, Leonberg, Germany). The analytical column was a LiChrosorb RP-18 (Merck) 125 mm × 4 mm I.D. column, particle size 5 μm. The pre-column was 20 mm × 4 mm I.D. (Waters, No. 84550) self-packed with Bondapak C_{18}/Corasil, particle size 37–50 μm (Waters).

The entire system was completely automatic, controlled by a personal computer (NEC Powermate 386, Software Maxima, Waters).

HPLC conditions

Dipotassium hydrogenphosphate solution (0.01 *M*, pH 7.88) was used as solvent for the extraction, and the analytical mobile phase consisted of a mixture of this phosphate buffer and acetonitrile (30:70). The flow-rate of 1.3 ml/min (pump A) produced a back-pressure of 75.8 bar at room temperature on the C_{18} analytical column, whereas at a flow-rate of 1.5 ml/min (pump B) the back-pressure of the pre-column was below 20 bar. The excitation of the fluorescence detector was set to 276 nm; the emission was recorded at 310 nm.

Procedure

An aliquot of the biological fluid (serum, protein-containing solutions, urine, etc.) was injected without any sample preparation directly onto the pre-column (extraction column) in a polar mobile phase (pump B). The polar constituents of the biological material, such as proteins, amino acids, electrolytes, etc., passed through and were eluted to waste. The lipophilic P was retained on the extraction column.

After 2 min the valve was switched automatically into position A, whereby the analytical mobile phase (pump A) back-flushed the loaded content (P and other apolar constituents of the biological sample) in the closed system from the extraction column onto the analytical column, where P was separated and detected by fluorescence (Fig. 1).

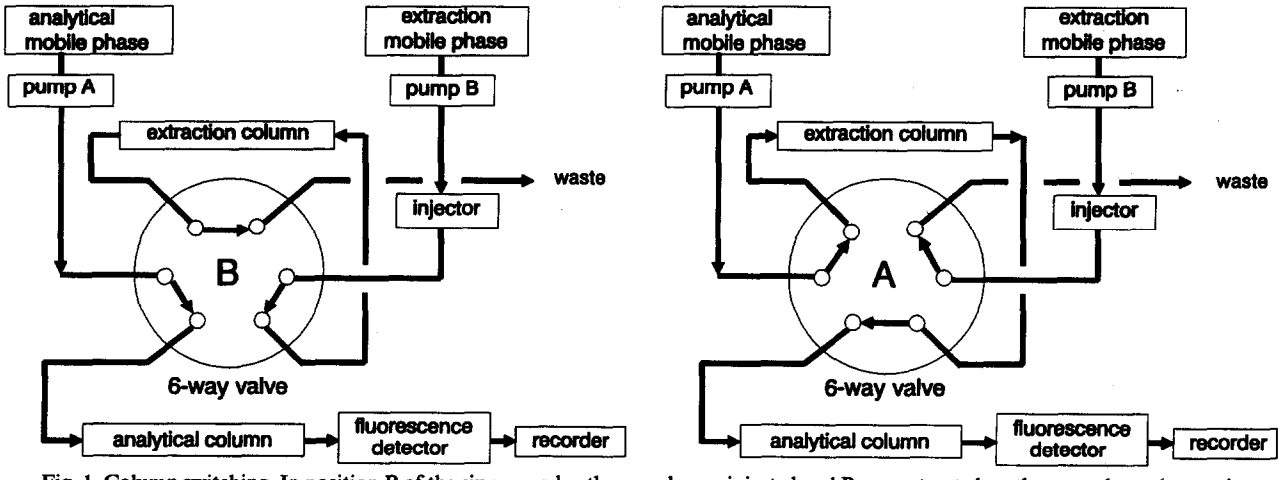

Fig. 1. Column switching. In position B of the six-way valve the sample was injected and P was extracted on the pre-column (extraction column); simultaneously the analytical column was equilibrated with the analytical solvent (pump A). Two minutes after injection of the sample the six-way valve was switched and the analytical solvent used to back-flush the pre-column, then P was brought for separation to the analytical column and detected by fluorescence.

RESULTS

Recovery rate

The efficacy of extraction or the recovery rate of P after the pre-column extraction was determined by inserting an additional valve, that allowed direct injection onto the analytical column as well as pre-column extraction. After injection of P-containing samples, increasing volumes of phosphate buffer solution were pumped through the extraction column, before the valve was switched and P was brought to the analytical column with the analytical solvent system.

With up to 5 ml of phosphate buffer solution, pumped through the pre-column, the recovery of P was nearly 100%, and even up to 15 ml it was still above 90% of the value obtained for the direct injection (Fig. 2).

Washout of polar constituents by the pre-column

The washout of serum proteins from the pre-column was determined as the function of the volume of extraction solvent *versus* analysis of the eluate of the pre-column with a photometer in the UV light range (220 nm). Increasing volumes of fresh human serum were injected and the volume of phosphate buffer necessary for washout from the pre-column was measured.

A linear relationship could be seen between the amounts of protein loaded on the pre-column and the volume of phosphate buffer required to pass the proteins through the pre-column. After injection of 1 ml of native serum all polar constituents were removed with 4.6 ml of phosphate buffer (Fig. 3).

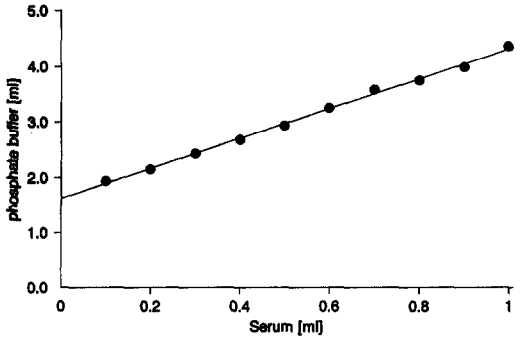

Fig. 3. Washout of the polar constituents of serum as function of the phosphate buffer volume.

Protein binding

The effect of protein binding was studied by comparing the recovery of P after injections of spiked phosphate buffer and spiked serum. The recovery was identical, indicating that P has a higher affinity for the packing material of the pre-column than for the serum proteins.

Calibration

In order to establish the conditions for standardization, two sets of calibration curves were analysed, one set with identical sample concentration of P and increasing injection volumes and a second one with increasing sample concentrations and identical injection volume (Fig. 4).

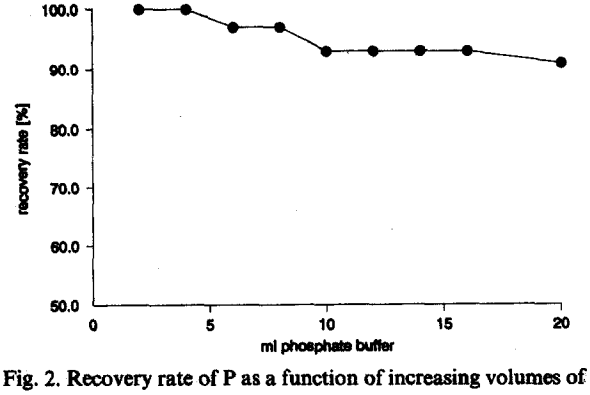

Fig. 2. Recovery rate of P as a function of increasing volumes of extraction solvent (phosphate buffer).

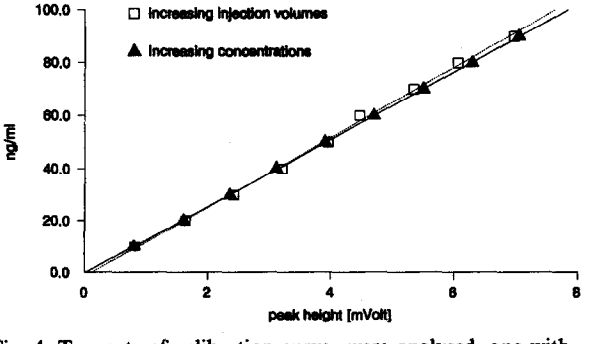

Fig. 4. Two sets of calibration curves were analysed, one with identical injection volumes and increasing sample concentrations of P and a second one with identical sample concentration and increasing injection volumes.

TABLE I

TEN SAMPLES CONTAINING 50 ng/ml P ANALYSED ON FIVE CONSECUTIVE DAYS

The variability (coefficient of variation, C.V.) within the series and from day to day was calculated.

Sample	Concentration (ng/ml)							C.V. (%)
	Day 1	Day 2	Day 3	Day 4	Day 5	Mean	S.D.	
1	50.36	48.80	53.51	53.15	53.90	51.94	2.24	4.3
2	50.08	48.14	52.18	52.24	53.64	51.26	2.15	4.2
3	50.64	50.04	53.92	52.74	53.90	52.25	1.82	3.5
4	51.13	49.24	52.84	52.57	55.02	52.16	2.14	4.1
5	50.00	49.31	53.34	55.63	54.07	52.47	2.71	5.2
6	49.72	49.97	52.68	55.87	53.81	52.36	2.61	5.0
7	49.79	50.11	53.17	56.12	53.90	52.62	2.67	5.1
8	49.93	49.97	54.08	55.29	53.90	52.63	2.51	4.8
9	50.64	48.87	53.26	54.64	51.30	51.74	2.26	4.4
10	50.29	50.63	53.26	55.21	51.04	52.09	2.10	4.0
Mean	50.26	49.51	53.22	54.35	53.45			
S.D.	0.44	0.76	0.56	1.51	1.26			
C.V. (%)	0.9	1.5	1.1	2.8	2.3			

Both calibration curves were linear and almost identical. This indicated that the calibration is independent of the injected sample volume. Because of the high reproducibility an external calibration method was used. The calibration curve for P in human serum (200 μl of serum injected) in the range 5–48 ng/ml was described by the regression line: c (ng/ml) = 68.4001 × peak height (mV) + 0.61 (ng/ml), with a correlation coefficient of r = 0.9997. The limit of quantitation of

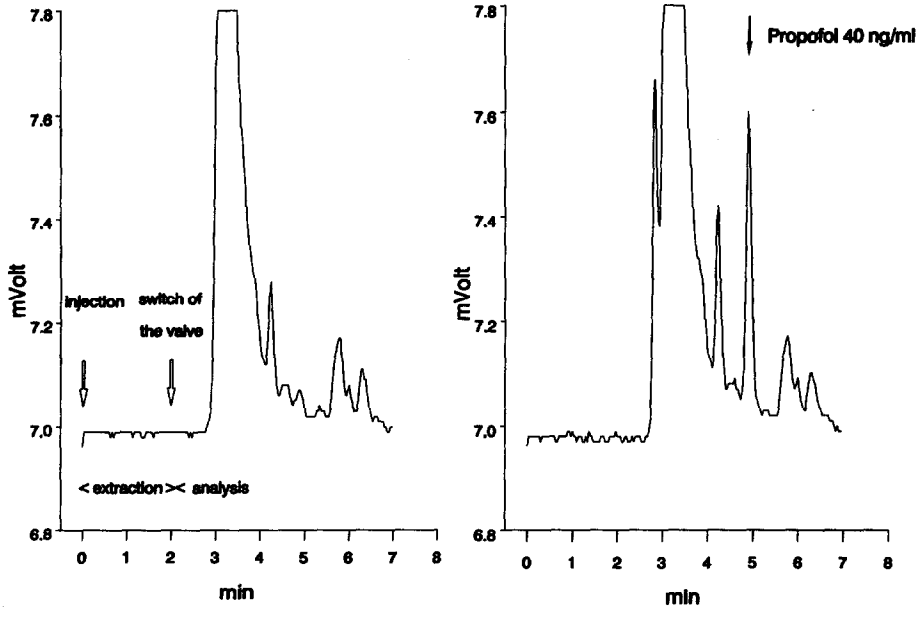

Fig. 5. Chromatograms of human serum: left = blank; right = serum of a patient after intravenous bolus of P.

the method is at least 5 ng/ml with a signal-to-noise ratio for the 5 ng/ml spiked serum of more than 5.

Accuracy and variability

Ten samples of an identical P concentration of 50 ng/ml were analysed on five consecutive days. The coefficient of variation within the series of ten samples (within-day) ranged from 0.9 to 2.8%. The day-to-day variability for these samples was between 3.5 and 5.2% (Table I).

Chromatograms

Fig. 5 shows the chromatogram of 200 μl of serum from a patient prior to general anaesthesia with P (blank). After induction of anaesthesia with an intravenous bolus of 2 mg/kg P a blood sample was taken and 200 μl of serum were injected. The propofol peak was clearly separated from serum background.

CONCLUSION

Owing to its high lipophilicity, P is well retained by the C_{18} packing material of the pre-column. At the same time the more polar constituents of more than 1 ml of native human serum can pass through the pre-column without causing a reduction in the recovery rate of P. The pre-column extraction method presented provides simple, rapid and sensitive determination of this phenolic anaesthetic in biological fluids. With one set of columns about 600 samples with different injection volumes (native human serum 10–400 μl, 4% human serum albumin solutions 10–400 μl and 4% human haemoglobin 10–400 μl) could be analysed.

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REFERENCES

1 H. K. Adam, E. J. Douglas, G. F. Plummer and M. B. Cosgrove, *J. Chromatogr.*, 223 (1981) 231.
2 G. F. Plummer, *J. Chromatogr.*, 421 (1987) 171.
3 G. Mazzi and M. Schinella, *J. Chromatogr.*, 528 (1990) 537.
4 W. Roth, K. Beschke, R. Jauch, A. Zimmer and F. W. Koss, *J. Chromatogr.*, 222 (1981) 13.