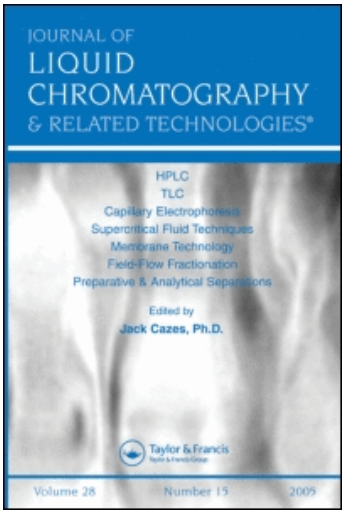

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Possibilities of Propofol Analysis in Various Blood Components by Means of HPLC

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POSSIBILITIES OF PROPOFOL ANALYSIS IN VARIOUS BLOOD COMPONENTS BY MEANS OF HPLC

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ABSTRACT

Propofol (2,6 disopropylphenol) is a short-acting hypnotic agent, effective for the maintenance of anaesthesia when given intravenously as repeated bolus injections or as a continuous infusion. The present paper deals with the analysis of this drug in different parts of the blood. The results obtained by means of HPLC show that propofol not only variously bonds with plasma or solid blood elements, but also penetrates into the interior of blood cells.

The investigations carried out with whole blood, plasma, non-washed or washed cells and washed and lysed cells suggest a different behaviour of thymol (used in chromatographic analysis as internal standard) in relation to propofol.

INTRODUCTION

Drugs in the organism are transported by blood. The leading role in this transport is played by plasma proteins, mainly albumins.[1] It is essential for drugs to cause the least possible change in the organism. Therefore, drugs transported by blood should not bond irreversibly with plasma proteins and should not damage or block morphotic blood elements.

Among numerous hypnotic agents applied in anaesthesiology, physicians are more and more interested in intravenous, short-acting pharmaceutical specifics, allowing for easy sleep guidance.[2] One of the drugs of this kind is Propofol (Diprivan, 2,6-diisopropylphenol).[3,4] It is used not only as a sleeping-draught, but also as a drug to maintain anaesthesia and as a sedative.[3,4,5]

Due to differences in metabolism and renal elimination, individual people show various sensitivities to medicines.[6] Pharmacokinetic developments supported by modern analysis make it possible to optimize the individual medicine dose by measuring the therapeutic agent concentration in blood.[7,8] In the case of propofol, the estimation of its concentration in blood helps to find its minimal dose for individual patients which will be sufficient for sleep maintenance.[9]

So far, two chromatographic procedures have been described in the literature which allow to analyse propofol concetration in blood: extraction[10] and precipitation.[11] The comparison of the data obtained from the application these two methods[12] shows that the calculated values of propofol concentration depend on the amount of the solid blood element - hematocrite.

Questions appear at this point as to whether propofol bonds with morphotic blood elements and whether it penetrates into their interior. Due to the acidic character of the propofol molecule, the problem is very important because the penetration of this substance into blood cells could damage them and handicap their functions.

EXPERIMENTAL

Equipment

A Gilson solvent delivery system, composed of two high pressure pumps Model 305 and 306, manometric module Model 805 and dynamic mixer Model 811C was used. The UV-visible variable wavelength detector was a Model 308 from MIM (Budapest, Hungary).

Chromatographic separations were carried out with a 250 x 4 mm column packed with laboratory-made reversed-phase octadecyl silane sorbent.[12]

The samples were injected into the column by an injection valve Model 7125 from Rheodyne (Cotati, CA, USA).

Reagents

The mixture composed of 67% acetonitrile (gradient grade for chromatography, LiChrosolv series from Merck) and 33% double-distilled water (pH 4.0) was used as a mobile phase. The mentioned pH was achieved by the addition of a proper amount of acetic acid (ca. 0.3-0.5 mL, depending on the water's pH).

Propofol was obtained from ICI-Pharma (Goteborg, Sweden).

Except chemicals mentioned above, the others came from the Polish Factory of Chemical Reagents - POCh (Gliwice. Poland) and were of analytical grade.

The blood for preparation of standards and the blood for analysis was collected in tubes containing sodium citrate and stored at 4 °C for not longer than 5 days.

A stock solution of thymol (used as an internal standard) in methanol (1 mg/mL) was diluted with methanol to the appropriate concentration.

Tetraethylammonium hydroxide (TEAM, 25 % in ethanol) was diluted with ethanol in the volume ratio 3 : 37.

The sorbent for chromatographic separation was obtained by the chemical modification[13,14] of a laboratory-made controlled porosity glass.[15,16] The properties of this material have been described elsewhere.

Methods

Sample preparation

To 2 mL of blood containing propofol (15 μg/mL) and internal standard solution (thymol dissolved in methanol, 20 μL) (samples A - see Table 1 in the Discussion), phosphate buffer (1 mL 0.1 M NaH_2PO_4) and cyclohexane (5 mL) were added. A vessel containing all the components was vigorously shaken for 15 min at 200 rpm. After centrifugation (1200 x g for 5 min) to separate the phases, an aliquot of the cyclohexane layer (ca. 5 mL) was transferred to a clean tube to which TEAH solution (50 μL) was added. The solvent was evaporated to dryness by means of a stream of nitrogen. The residue was redissolved in mobile phase and injected into the chromatographic column.

The same procedure was followed when analysing propofol in plasma obtained from 2 mL blood samples containing the same amounts of propofol and thymol (samples B - see Table 1 in the Discussion). The only difference was the separation of plasma from solid blood elements (by centrifugation) at the beginning of the described procedure.

In order to establish the presence of propofol bonded with solid blood elements, four types of samples were investigated:

i - solid blood elements obtained only by means of blood centrifugation (samples C - see Table 1 in the Discussion);

ii - solid blood elements (obtained after centrifugation of blood) which were next washed four times with 0.9 % NaCl solution (isotonic, isoosmotic solution) to remove the residue of plasma remaining among the solid elements (samples D - see Table 1 in the Discussion);

iii - solid blood elements in the lysed form prepared by their four-time washing with 0.9 % NaCl solution and mixing with three volumes of doubly-distilled water to cause the lysis (samples E - see Table 1 in the Discussion), and

iv - the contents of morphotic blood elements (samples F - see Table 1 in the Discussion) prepared in the following way: solid blood elements obtained as above were first carefully washed four times with 0.9 % NaCl solution, mixed with three volumes of doubly - distilled water and left for 30 min to lyse the cells. Next the suspension was centrifuged to separate the cell wall and the obtained supernatant was subjected to further stages of sample preparation.

In all these cases, the samples of solid blood elements (i-iv) (or C,D,E,F - see Table 1 in the Discussion) were obtained from 2 mL blood samples containing the same amounts of propofol and thymol. Subsequent steps of the preparation procedure were the same as described for whole blood and plasma samples.

Calibration standards

The samples for the calibration curves were prepared from the solution containing 20 mg of propofol in 100 mL of acetonitrile. The solution was appropriately diluted with blood in order to obtain the concentrations of 0.5; 1.0; 2.0; 4.0; 7.0; 10.0; 15.0 and 20.0 μg/mL. Employing these solutions and following the described procedures, six calibration curves (for blood, plasma and four types of the described solid blood element samples) were plotted. To construct these curves, the ratios of the peak heights for propofol (h_P) and internal standard - thymol - (h_T) were used

RESULTS AND DISCUSSION

As already mentioned in the introduction, there are two sample preparation procedures that can be used in the analysis of propofol in blood by means of liquid chromatography.[10-12] The first one[11] requires the precipitation of a part of the proteins contained in the examined blood before injecting the sample into the chromatographic column. According to the second procedure[10]
propofol is extracted from blood into the cyclohexane phase and, after the evaporation of the volatile solvent, the dried residue is disolved in mobile phase and chromatographed. The comparison of both methods[12] suggests the bonding of propofol with morphotic elements of blood, and it also shows that the extraction method gives more precise concentration values.

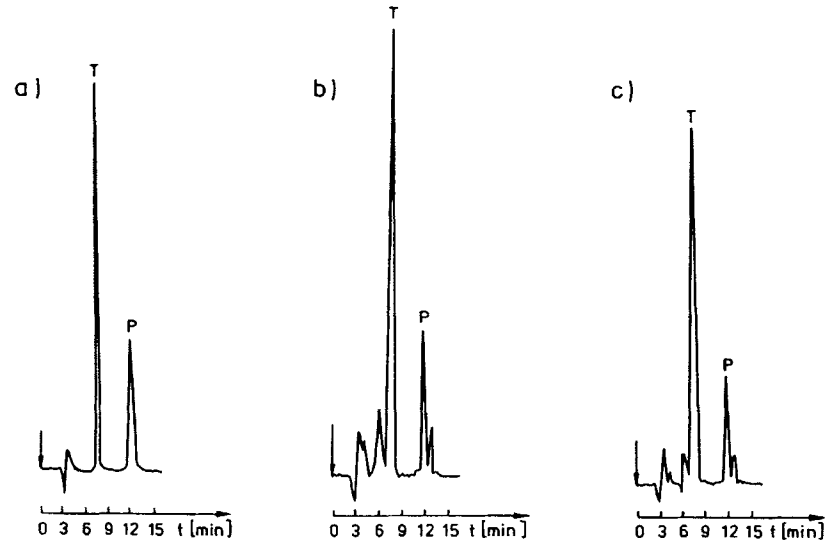

Figure 1. Chromatograms of: a) propofol-thymol mixture (peaks P and T, respectively) dissolved in acetonitrile; b) extract from blood containing propofol (15 μg/mL); c) extract from plasma separated from blood containing propofol (15 μg/ml); Conditions and marks - see Experimental and Fig. 1a.

As the observed data can result from different bonding of propofol with various blood elements, the presence of this anaesthetic agent in two parts of blood (plasma and solid blood elements) was established at the beginning.

In order to find the retention time of propofol and thymol (used as an internal standard) and to show the resolution of the applied chromatographic system, the separation of both substances diluted in acetonitrile was carried out (see Fig. 1a). As appears from the figure, the separation is complete.

In Fig. 1b, the chromatogram of the extract from blood containing 15 μg of propofol per 1 mL is shown. However, there are some substances in the prepared sample which were extracted from blood together with propofol and thymol, but their peaks are sufficiently separated from peaks representing the analyte and internal standard. This chromatogram confirms the results reported by Plummer.[10]

Fig. 1c illustrates the chromatogram of the extracts from plasma, whereas Fig. 2a shows the chromatogram of the extract from non-washed solid blood elements. These separations are similar to that presented in Fig. 1b, but

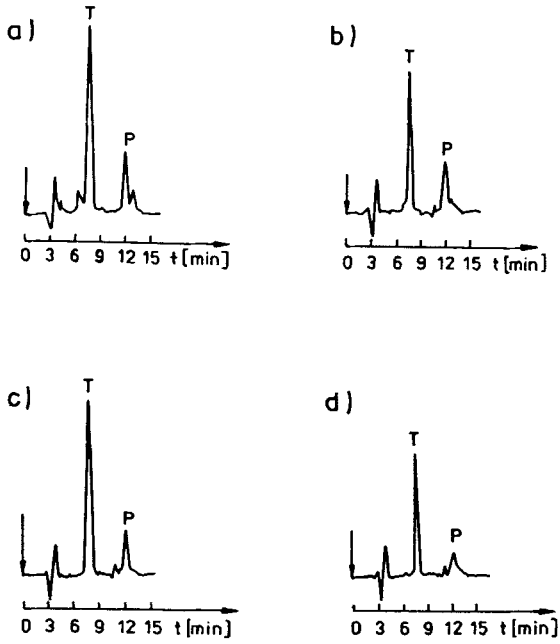

Figure 2. Chromatograms of: a) extract from non-washed solid blood element phase separated from blood containing propofol (15 μg/mL); b) extract from washed (4 times) solid blood element phase obtained by blood centrifugation. Concentration of propofol in initial blood, the same as before; c) extract from washed and lysed solid blood elements separated from blood containing propofol (15 μg/mL); d) extract from the content of solid blood elements separated from blood containing propofol (15 μg/mL); Conditions and marks - see Experimental and Fig. 1a.

distinctly lower heights of propofol and thymol peaks (peaks P and T, respectively) confirm lower amounts of both compounds in these two parts of centrifugally separated blood in relation to the whole blood (see Table 1). Besides, amounts of propofol and thymol contained in the solid blood element phase (before washing) are lower in comparison to those bonded with plasma.

The chromatograms of the extracts from washed or washed and lysed morphotic blood elements are presented in Figs. 2b and 2c, respectively. They look clearer and are very similar to those presented above. On the one hand, they suggest that the peaks unidentified before are probably connected with plasma components; on the other hand, they prove the bonding of propofol and

Table 1

Heights of Thymol and/or Propofol Peaks and their Ratios Corresponding to Investigated Samples (Average Values from 7 Separate Measurements)

Symbol of Investigated Sample	Type of Investigated Sample	Thymol Peak Height (h_T) (mm)	Propofol (h_P) (mm)	h_P/h_T
A	Blood containing propofol (15μg/mL)	199.0	64.0	0.3216
B	Plasma obtained by centrifugation of sample A	152.0	46.0	0.3026
C	(i) Solid elements of blood obtained by centrifugation of sample A	68.0	23.0	0.3382
D	(ii) Solid elements of blood (Sample C) washed to remove plasma residues	56.0	19.0	0.3392
E	(iii) Washed solid elements of blood (see D) after lysis	69.0	17.0	0.2463
F	(iv) The content of solid blood elements	47.0	0.9	0.1915

(I), (ii), (iii) and (iv) - see Experimental section.

thymol with solid blood elements. The heights of both propofol peaks in these chromatograms are similar (see Table 1), but they are lower in relation to P peak heights for initial blood, plasma or non-washed solid elements of blood.

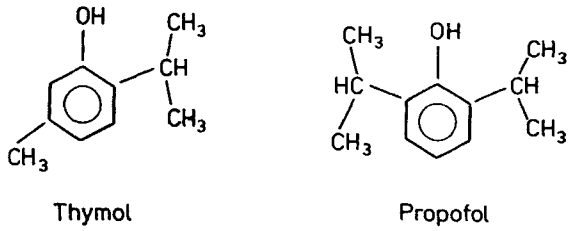

Figure 3. Chemical structures of thymol and propofol.

The chromatogram in Fig. 2d shows the penetration of some amount of propofol into the interior of blood cells. This is a very small amount, nevertheless, disadvantageous from a medical point of view. It cannot be excluded that only old or ailing blood cells (whose cell walls are not compact) are penetrated by propofol.

According to the presented results, propofol variously bonds with different components of blood. This conclusion explains the differences discussed in Ref. 12, between the propofol concentration values estimated by means of the precipitation and extraction methods.

In a typical procedure of inducing anaestesia intravenously with propofol, the drug is administrated in the amount of about 2.5 mg/kg of patient body weight. Assuming the average patient body weight of 70 kg and his blood volume of 5 liters, the concentration of propofol in blood should be about 35 μg/ml. As results from radioanalytical and chromatographic data,[17,18] its maximum concentration (about 2 - 3 min after administration) reaches the level of 5-10 μg/mL. The difference between the theoretically calculated and analytically determined values is known from literature[19] and results from propofol absorption in the adiposal tissue and other organs. Because the amount of propofol penetrating blood cells is very small and the applied uv-vis detector is not sensitive enough, blood containing 15 μg/mL of propofol was used for these experiments. The presented experiments were carried out outside the human organism. It is probable that, in natural conditions, the behaviour of propofol is a little different, but such investigation is much more difficult.

Table 1 collects the average values of peak heights and peak height ratios (n = 7) for propofol and thymol extracted from the examined samples. As appears from these data, the ratio of propofol peak height to thymol peak height is similar for first four extracts from blood preparations (blood, plasma, non-washed solid blood elements and washed solid blood elements).

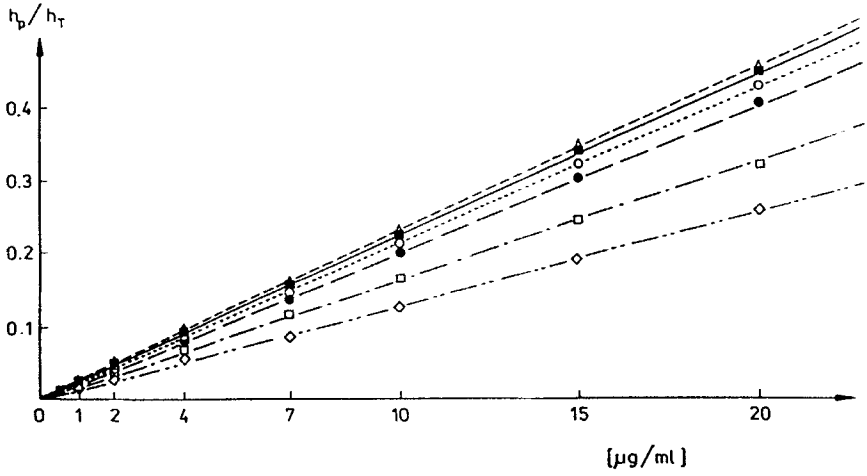

Figure 4. Calibration curves corresponding to investigated blood preparations. Height ratio of propofol peak (h_P) to thymol peak (h_T) vs. propofol conc'n in initial blood. Dotted line with open rings - sample A (see Table 1). Dashed line with black rings - sample B (see Table 1). Solid line with triangles - sample C (see Table 1). Dashed line with black squares - sample D (see Table 1). Dash-single dotted line with white squares - sample E (see Table 1). Dash-double dotted line with white rhombs - sample F (see Table 1).

For the samples E and F (washed and lysed solid blood elements (iii) and/or content of solid blood elements (iv)) the discussed value is significantly lower and decreases respectively. Considering the values presented in can be concluded that:

1. In relation to thymol, propofol is better bonded with solid blood elements then with plasma components - compare h_P/h_T ratio for:

a) plasma (samples B from Table 1) and non-washed solid blood element (samples C), or

b) plasma (samples B) and washed solid blood elements (samples D), or

c) non-washed solid blood elements containing the residual amount of plasma among them (samples C) and washed solid blood elements (samples D).

The h_P/h_T for blood (samples A which are the mixture of plasma and morphotic blood elements) reaches intermediate value.

2. Thymol penetrates better into the interior of blood cells - compare the h_P/h_T ratio for samples D (washed solid blood elements) and for samples F (the content of solid bood elements). The comparison of this value for samples D with E or E with F confirms the same.

The last conclusion can easily be explained. The molecules of thymol was proposed by Plummer as an internal stardard in this analysis due to its similar structure and character with propofol (see Fig. 3). But thymol has a little smaller diameter and is more polar than propofol molecules. In consequence, thymol molecules penerate more easily into the cell interior (over the cell wall) than propofol molecules do. Taking into account the similarity of the h_P/h_T ratio for blood plasma and solid blood elements, it can be assumed that the concentration of propofol in these parts of blood can be estimated approximately on the basis of one calibration curve constructed using one of the mentioned mediums as solvent for calibration solutions. A precise propofol analysis in these parts of blood, and especially its analysis in lysed cells or in the contents of cells require separate calibration curves in all cases. This conclusion is confirmed by the calibration plots presented in Fig. 4.

CONCLUSIONS

1) Propofol is bonded both with plasma proteins and solid blood elements. It penetrates also into the blood cell interior.

2) A precise analysis of propofol in various blood components (and especially in the content of blood cells) requires separate calibration curves constructed for the component in which this drug is investigated.

3) The concentration ratio of propofol and thymol (used as the internal standard) is similar for whole blood, plasma and solid blood elements, but different in the case of the content of blood cells.

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