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Determination of diprivan in urine by a supported liquid membrane technique and liquid chromatography–electrochemical detection

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Abstract

A supported liquid membrane technique was used for the extraction and enrichment of propofol in a spiked sample of urine. An acidic solution of propofol and thymol as an internal standard was passed over the membrane and after enrichment the acceptor solution was analyzed by LC with an electrochemical detector. The acceptor and donor pH, flow-rate, and volume of donor and different membrane solvents were varied to optimize the extraction efficiency. The detection limit for 100 ml of a spiked urine sample was 10 ppt of propofol.

Keywords: Diprivan

1. Introduction

Propofol (2,6-diisopropylphenol, I.C.I. 35868), the active ingredient of diprivan or disoprivan, is currently under study as a new intravenous anaesthetic agent. In animals and man it rapidly produces anaesthesia of short duration devoid of excitory side-effects.

The previously reported methods for determination of propofol using liquid chromatography (LC) had a quantitation limit of about 100 ng/ml (100 ppb) [1], and in the case of indophenol derivative 25 ng/ml (25 ppb) [2] for ultraviolet detection, and 5 ng/ml (5 ppb) [3] or 2 ng/ml (2 ppb) [4] with fluorescence detection and they were not considered sufficiently sensitive. One report using electrochemical detection obtained a detection limit of 10 ng/ml (10 ppb) [5].

Altmayer et al. [3] proposed a method based on pre-column extraction in a closed system allowing direct injection of biological samples without any pretreatment where the polar constituents of the biological material such as proteins, amino acids, electrolytes etc. were passed through and were eluted to waste. The lipophylic propofol was retained and than extracted with the analytical mobile phase.

Another method [1] proposed isolation of propofol from serum by protein precipitation and after centrifugation the supernatant was directly injected onto an LC system. Mazzi and Schinella [5] also used an extraction procedure for the isolation of propofol which was separated on a phenyl column and detected electrochemically.

Plummer [4] and Dawidowicz and Fijalkowska [6] extracted propofol from blood using cyclohexane. After centrifugation an aliquot of the cyclohexane layer was transferred to a tube with tetramethylammonium hydroxide and evaporated to dryness, then redissolved in the mobile phase and injected.

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We have explored the application of supported liquid membranes [7] for extraction of propofol from large volumes of sample (urine, cerebrospinal fluid) which permits us to determine substances at concentrations in the ppt range using LC with electrochemical detection. This method was also used for the determination of amines in urine [8], blood plasma [9], a basic drug and its metabolite from blood plasma samples [10,11].

2. Experimental

2.1. Apparatus and chromatographic conditions

The LC apparatus consisted of a HPP 4001 syringe pump (Laboratorni Pristroje Prague, Czech Republic), Reodyne valve injector (Berkeley, CA, USA) equipped with a 20-μl loop and electrochemical detector (Coulochem Model 5100A, ESA, Bedford, MA, USA), with applied potential +0.45 V and +0.2 V for detector 1 and the guard cell, respectively. The chromatography was carried out on a LiChrosorb 5-μm RP-18 (E. Merck, Darmstadt, Germany) column (100×4 mm I.D.). A mixture of 0.05 *M* sodium phosphate buffer (pH 3.8)–methanol (1:3, v/v) was used as the mobile phase. The mobile phase was filtered (17 G5 glass filter) and degassed with a water vacuum pump for 5 min to prevent bubble formation in the detector.

2.2. Reagents and solvents

Thymol and propofol (Fig. 1) were obtained from ICI Pharmaceutical Division (Macclesfield, Cheshire, UK). A solution was prepared in methanol (Chemical Factory Oświęcim, POCh Gliwice, Poland).

Sulphuric acid was obtained from Polchem (Toruń, Poland), and sodium hydroxide, sodium dihydrogenorthophosphate and orthophosphoric acid were obtained from POCh (Gliwice, Poland).

Di-*n*-hexyl ether (Sigma, St. Louis, MO, USA) and *n*-undecane (Reachim, Russia) were organic solvents used for impregnation of the membranes.

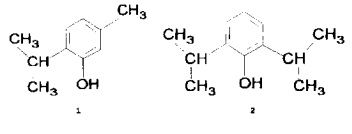

Fig. 1. Structural formula of investigated substances: (1) thymol (2-isopropyl-5-methylphenol), (2) propofol (2,6-diisopropylphenol).

2.3. Extraction procedure

Sulphuric acid and sample solutions were pumped into a mixing coil that consisted of about 1 m×0.5 mm I.D. PTFE tubing coiled with a 20 mm diameter (Fig. 2a). Mixed solutions were passed over the liquid membrane in a membrane separator which was made of two PTFE blocks (diameter 120 mm and thickness 8 mm) with machined spiral grooves facing each other (depth 0.25 mm, width 1.5 mm, length 250 cm and total volume of about 0.80 ml) (Fig. 2b). Aluminium blocks 6 mm thick were used to make the construction rigid.

A porous PTFE membrane with polyethylene backing was from Millipore (Bedford, MA, USA) (pore size 0.2 μm, total thickness 175 μm, of which 115 μm is polyethylene net, porosity 0.70). After impregnation by soaking for 15 min in *n*-undecane

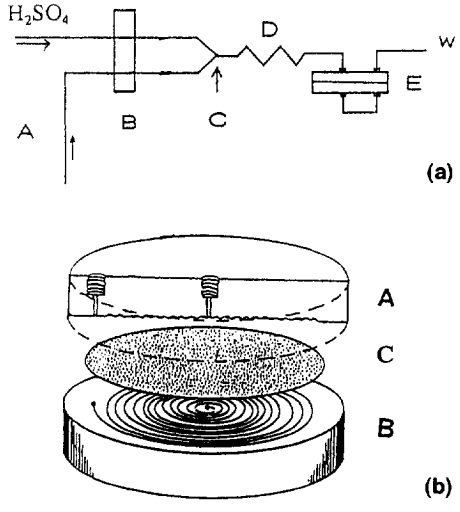

Fig. 2. (a) Set-up for membrane enrichment of propofol in urine: (A) sample; (B) peristaltic pump; (C) confluence point of sample and sulphuric acid solution; (D) mixing coil; (E) membrane separator with stagnant acceptor solution; (W) waste; and (b) The membrane separator: (A) alumunium back-up; (B) PTFE block with grooves like Archimedes' spiral; (C) impregnated liquid membrane.

or dihexyl ether the membrane was placed between two PTFE blocks and the whole separator was clamped together with eight screws. Excess of solvent on the surface of the liquid membrane was removed by pressing with water through both channels. In the extractor the membrane separated two channels: the donor for extraction of thymol and propofol from acidic solutions into membrane and the acceptor with stagnant sodium hydroxide solution for reextraction of analytes from the membrane. After extraction 10 μl or 20 μl of neutralized acceptor was injected onto the LC system.

3. Results and discussion

3.1. Optimization of membrane extraction

To optimize the membrane process the enrichment factor was plotted as a function of acceptor pH, donor pH, flow-rate of donor solution and volume of donor solution. Different organic solvents (*n*-undecane, dihexyl ether) for impregnation of the membrane were also investigated.

The enrichment factor F_e was expressed as $F_e = c_a/c_d$, where c_a is concentration of analyte in acceptor solution after extraction and c_d is concentration of analyte in donor solution.

3.2. Influence of acceptor pH

The pH of the acceptor solution was changed by adding sodium hydroxide. The influence of acceptor pH on thymol and propofol enrichment was investigated using *n*-undecane as the extraction solution in the liquid membrane. As a donor solution 0.05 *M* H_2SO_4 was chosen, containing 100 ppt of thymol and propofol which was passed over the membrane with flow-rate $F_d = 0.12$ ml/min during 80 min. After extraction, 20 μl of acceptor solution was injected onto the LC system.

Fig. 3 shows the influence of acceptor solution's pH on the enrichment factor. We observed an increase of the enrichment factor for thymol and propofol at pH ranging from 6 to 11 and a significant increase at pH 13 (up to 23 and 10, respectively).

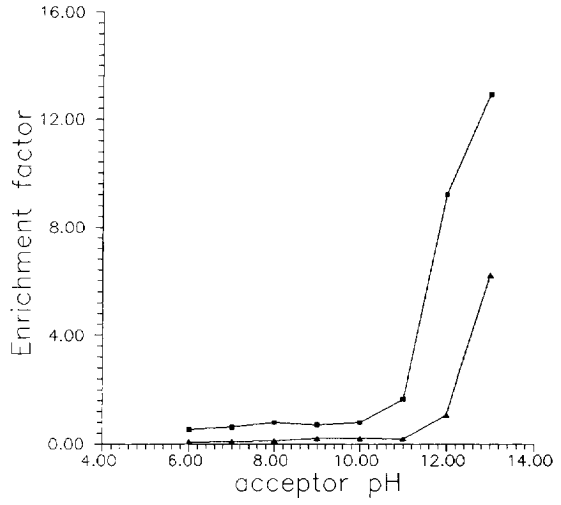

Fig. 3. Enrichment factor for thymol (■) and propofol (▲) versus acceptor pH. Acceptor: different concentrations of NaOH, extraction time of 80 min, membrane impregnated with *n*-undecane, $F_d = 0.12$ ml/min, donor: 0.1 *M* H_2SO_4 containing 100 ppt of thymol and propofol.

3.3. Influence of donor pH

Different pH values of the donor phase containing 100 ppt of thymol and propofol were prepared to investigate its influence on enrichment factor of analytes. We chose 0.05 *M* sulphuric acid as pH 1 and 0.05 *M* sodium phosphate buffer at pH values from 2 to 8 to prepare the donor solution. The acceptor solution was 0.1 *M* sodium hydroxide with a donor flow-rate 0.12 ml/min and an extraction time of 80 min. After enrichment, the acceptor solution was neutralized with 1 *M* H_2SO_4. The extraction of thymol and propofol was the highest for 0.05 *M* H_2SO_4, decreased slowly up to pH 7 and was very low at pH 8 (Fig. 4).

The investigated substances are derivates of phenol and they have acidic properties, for this reason they are easily extracted from the donor solution at low pH and not extracted at pH above 7 where they are ionic.

3.4. Influence of donor flow-rate

To examine the influence of donor flow-rate with *n*-undecane as a membrane solvent, 0.05 *M* H_2SO_4 with 10 ppb of investigated substances as the donor

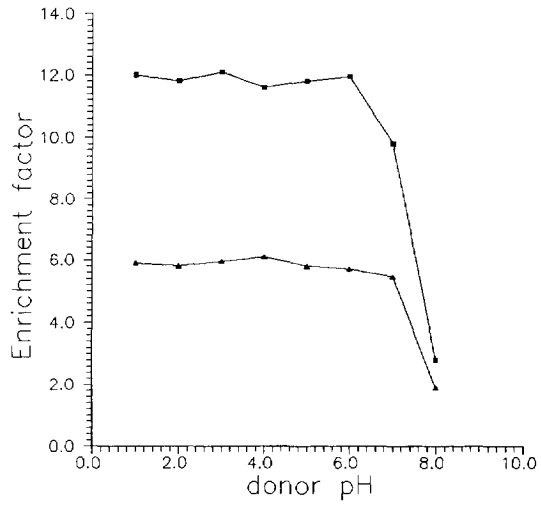

Fig. 4. Enrichment factor for thymol (■) and propofol (▲) versus donor pH (0.05 *M* sulphuric acid, sodium phosphate buffer pH 2–8). Acceptor was 0.1 *M* sodium hydroxide. Liquid membrane impregnated with *n*-undecane, extraction time of 80 min, $F_d = 0.12$ ml/min.

solution and 0.1 *M* NaOH as the acceptor solution were used. Flow-rates of 0.14, 0.28, 0.55, 1.8, 2.8 and 4.8 ml/min were applied for the 20 ml of donor. From Fig. 5 we can see that the enrichment factor decreases by about 30% for both compounds when the flow-rate increases from 0.14 to 2.8 ml/min. Using a high flow-rate we can reduce significantly the time of analysis with some loss of extracted substances.

3.5. Influence of donor volume

Volumes from 10–200 ml of donor solution were passed over the membrane with *n*-undecane at a flow-rate 0.12 ml/min, with a concentration of propofol and thymol of 10 ppb. After injection of the acceptor phase we observed a linear response of the electrochemical detector with increasing volume of the donor solution (Fig. 6).

3.6. Calculation of extraction efficiency

Extraction efficiency E is expressed as percent of analyte extracted from donor solution to the acceptor solution and was calculated from the equation

$$E = \frac{V_a \cdot h_a}{f_d \cdot t_e \cdot h_d} \cdot 100\%$$

where V_a = volume of acceptor phase (ml), h_a = peak height of analyte in acceptor after enrichment de-

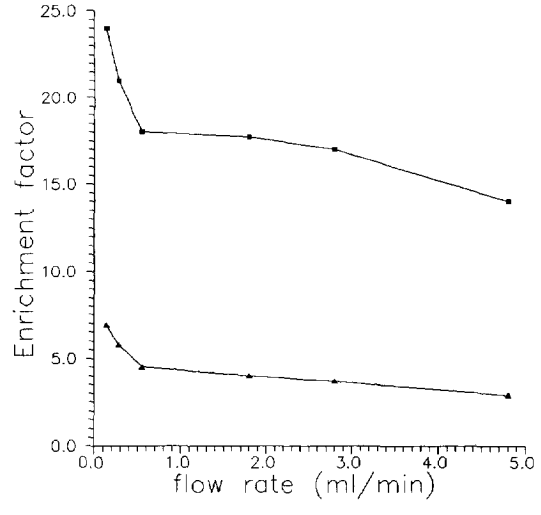

Fig. 5. Influence of donor flow-rate on enrichment factor. *n*-Undecane was used as a membrane solvent, 20 ml of 0.05 *M* H_2SO_4 with 10 ppb of thymol and propofol were pumped with different flow-rates, 0.1 *M* NaOH as acceptor solution.

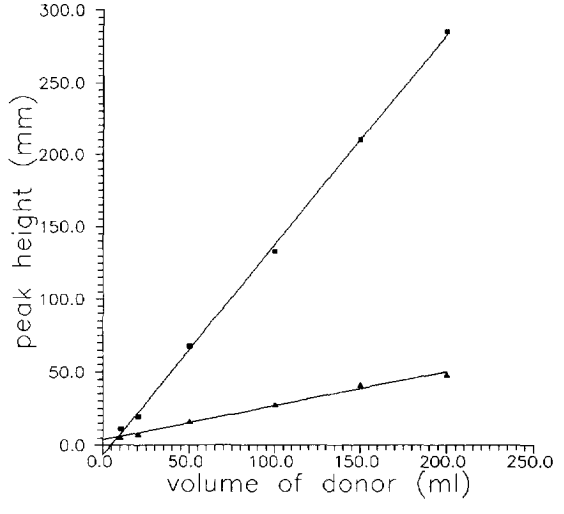

Fig. 6. Influence of donor volume on the peak height of thymol and propofol. *n*-Undecane was used as a membrane solvent, different volumes of 0.05 *M* H_2SO_4 with 10 ppb of thymol and propofol were pumped with flow-rate 0.12 ml/min, acceptor solution was 0.1 *M* NaOH.

termined by LC, f_d = flow-rate of donor phase (ml/min), t_e = time of extraction (min) and h_d = peak height of analyte in donor (determined by HPLC).

3.7. Influence of solvent in membrane

n-Undecane, dihexyl ether and a 1:1 mixture *n*-undecane–dihexyl ether were chosen as a membrane solvents to investigate the enrichment factor of thymol and propofol. In this experiment 20 ml of donor at 10 ppt of thymol and propofol were passed over each type of membrane. The highest efficiency of extraction appeared in the case of *n*-undecane (72% and 53%), lower for the solvent mixture (61% and 36%) and the lowest for dihexyl ether (50% and 29% for thymol and propofol, respectively). Because *n*-undecane is less polar then dihexyl ether we concluded that the investigated substances have somewhat nonpolar properties in the donor solution.

To increase effectiveness a solid-phase extraction procedure was also applied. For this purpose the acceptor solution (ca. 500 μl) was passed through a microcolumn containing 20 mg silicagel 40-60 μm with chemically bonded phase C_8. After washing with 1 ml water thymol and propofol were eluted with 50 μl ethyl octane or methanol, giving an 8-fold operate response.

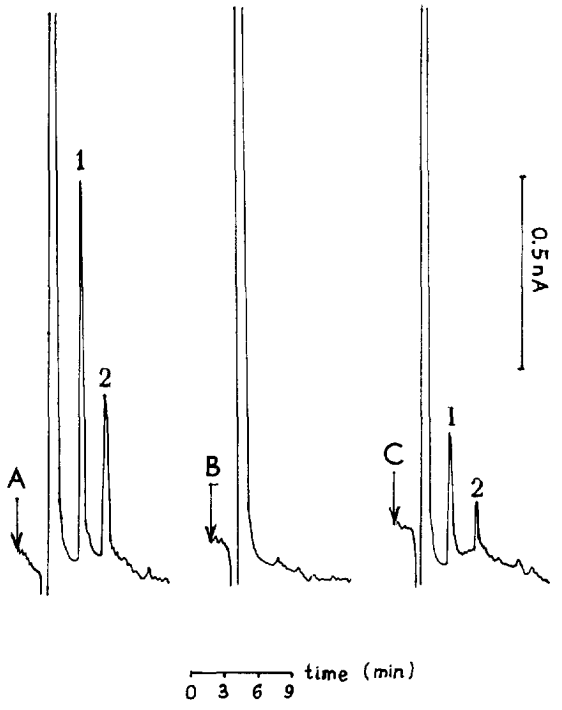

Fig. 7. Chromatograms of (A) standard solution (1 = thymol, 2 = propofol), (B) sample of 100 ml of urine after SLM enrichment, (C) sample of 100 ml of urine spiked with 100 ppt of investigated substances. SLM extraction with *n*-undecane was done at donor pH 1, flow-rate 0.12 ml/min, acceptor was 0.1 *M* NaOH. HPLC with electrochemical detector (+0.45 V), column 100×4 mm I.D. with LiChrosorb 5 μm RP-18; ammonium phosphate buffer pH 7 and methanol (80:140, v/v) was used as mobile phase.

4. Conclusions

Using the above mentioned chromatographic conditions satisfactory separation of thymol (1) and propofol (2) was achieved in 10 min. Fig. 7A shows a typical chromatogram of 1 ppb standard mixture of the investigated substances. To apply the described method 100 ml of urine spiked with thymol and propofol was adjusted with concentrated sulphuric acid to pH 1 and passed over a membrane impregnated with *n*-undecane. The acceptor was 0.1 *M* NaOH. After extraction of urine 20 μl of acceptor solution was injected into the LC (chromatogram in Fig. 7B). Fig. 7C shows the chromatogram obtained after enrichment of urine spiked with thymol and propofol.

The limit of propofol determination was 10 ppt. This range is over 100 times more sensitive then reported methods (100 ppb [1], 25 ppb [5], 10 ppb [5] and 5 ppb [3]). The use of this method based on the supported liquid membrane technique permits investigation of propofol in urine or cerebrospinal fluid from patients after anaesthesia.

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