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High-performance thin-layer chromatographic method to determine sorption of propofol to infusion containers

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Abstract

A stability-indicating HPTLC method developed for the determination of propofol was used to monitor the effect of different infusion containers on the stability of propofol in 5% glucose. According to the results, the new polypropylene-lined bags and glass bottles can safely be used for administration of propofol by i.v. infusion, but polyvinyl chloride bags are not recommended.

1. Introduction

Propofol (2,6-diisopropylphenol) is an intravenous anesthetic agent used for the induction and maintenance of anesthesia during surgical procedures. The drug is only slightly soluble in water and is used as an oil in water emulsion. In addition to propofol, the formulation contains soybean oil, glycerol and egg lecithin, with sodium hydroxide added to adjust the pH. Anesthesia can be maintained by intermittent administration of propofol or by continuous infusion of the drug after it has been diluted, with 5% glucose for example, to a concentration of not less than 2 mg/ml. The sorptive loss of propofol to poly(vinyl chloride) (PVC) plastic materials during i.v. administration has been demonstrated earlier [1].

Existing methods to assay propofol include high-performance liquid chromatographic (HPLC) determination using direct UV [2–4], fluorimetric [5] or electrometric detection [6], or UV detection after precolumn derivatization with Gibb's reagent [7]. Second-derivative UV spectroscopy [4] and gas chromatography [8] have also been used to assay propofol.

The object of the study was to evaluate the stability of propofol in new polypropylene-lined infusion bags (Softbags) and, in comparison, in glass bottles and PVC bags. For the purpose, a simple, rapid and stability-indicating high-performance thin-layer chromatographic (HPTLC) method was developed to determine the concentrations of propofol in samples. To test the reliability of the method, measurements were also made with a modification of the HPLC method described by Bailey et al. [1,4].

2. Experimental

2.1. Materials

Propofol was kindly supplied by ICI Pharma (Helsinki, Finland). The identity and purity of

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the substance were verified by TLC and HPLC, and by UV and IR spectrometry. Diprivan (ICI Pharma, lots C1065A and C1049A), Glucosteril 50 mg/ml, 100-ml glass bottle (Medipolar, Orion-Farmos Pharmaceuticals, lot SHL20AA), Glucos 50 mg/ml, 100-ml PVC bag (Baxter, lots 92D22G52 and 9229G50) and Glucosteril 50 mg/ml, 100-ml Softbag (Medipolar) were kindly supplied by Orion-Farmos Pharmaceuticals, Medipolar. All reagents were of analytical grade and methanol for HPLC was of HPLC grade.

2.2. Chromatographic instrumentation and conditions

The HPTLC assay was developed using glass plates (10 × 20 cm HPTLC plates 60 F_{254}; Merck, Darmstadt, Germany) and a horizontal chamber (Camag, Muttenz, Switzerland). Before use, the plates were washed with methanol to ensure a sufficiently low background for the photometric measurements. Plates were air-dried at room temperature and used immediately. Solutions were applied at opposite ends of the plates by the Linomat spray-on technique (Linomat IV, Camag). Toluene was used for plate development and the development distance was 40 mm.

Photometric measurements were performed in the absorbance/reflectance mode with a Camag TLC scanner connected to a computer running CATS version 3.14 software. The reflection absorption spectrum of propofol was scanned in situ and wavelength 276 nm was chosen for quantitation.

Stock solution containing 25 mg/ml of propofol was prepared in methanol. For calibration, either different volumes (1–5 μl) of the standard solution (2 mg/ml), or identical volumes (4 μl) of different concentrations (0.25–2.5 mg/ml) diluted from the stock solution, were applied as bands (length 4 mm, distance apart 6 mm) to the plates. The chromatography was performed as described above. The calibration curve was constructed by plotting peak height against the concentration of propofol, using Michaelis–Menten regression 1.

2.3. Validation of the HPTLC method

To validate our HPTLC method, we determined the retention factor (R_F) of propofol, the asymmetry factor (A_s) of the peak and the resolution (R_s) in the Diprivan sample solutions (propofol versus excipients and propofol versus eluent front). The specificity of the method for quantifying propofol in the presence of excipients in Diprivan was studied by comparing the in situ spectra of propofol in standard solution and in Diprivan sample solution, in the wavelength range 190–440 nm.

Repeatability of the sample application was studied by applying the standard solution (2 mg/ml) in amounts of 1, 3 and 5 μl six times each, as 5 mm bands. Diprivan (10 mg/ml propofol) was diluted with 5% glucose solution to 1.0, 1.5 and 2.0 mg/ml and each solution was applied six times in the amount of 4 μl, as 4 mm bands.

Repeatability of the total method was studied by diluting six samples of 1.0 ml, six samples of 1.5 ml, and six samples of 2.0 ml Diprivan to 10.0 ml with 5% glucose solution, and applying to HPTLC plates 4 μl of each sample, in duplicate, as 4 mm bands.

The accuracy of the method was studied by using duplicate samples with theoretical contents of 1.0, 1.5 and 2.0 mg/ml of propofol. Two bands were applied from each solution to HPTLC plate.

Stability of propofol on the sorbent was studied before and after development. Duplicate application of 3 μl of standard solution (2 mg/ml) was made to the same plate 120, 60, 45, 30, 15 and 2 min before development. During this time the plate was stored on a laboratory bench without protection from light. The plate was then developed and the heights of the chromatographic peaks of propofol were immediately compared. In studies on the stability after development of the plate, the heights of two standards, applied 2 min before development were measured immediately after drying the plate, and after 0.5, 2, 4 and 24 h. Between measurements the plate was stored, protected from light, at room temperature.

The stability-indicating nature of the HPTLC

assay was tested with solutions containing intact propofol and propofol that had been degraded in four different ways: in 2 *M* hydrochloric acid or 2 *M* sodium hydroxide at 85°C for 48 h, in 5% glucose while exposed to a high-pressure mercury lamp (original Hanau TQ 150) for 1 h, or in 5% glucose while exposed to daylight on a sunny window sill for two weeks. Samples (4 μl) were studied on the plate.

2.4. HPLC method

The HPLC analyses were performed with an instrument consisting of an LKB 2150 pump, LKB 2151 variable-wavelength monitor (LKB, Bromma, Sweden), D-2000 chromato-integrator (Hitachi, Tokyo, Japan) and a 20-μl loop injector. Propofol was chromatographed on a phenyl column (Novapak, 15 × 0.39 cm, 5 μm). The mobile phase was an isocratic mixture of methanol–30 m*M* ammonium dihydrogenphosphate (30:70, v/v) and the flow-rate was 0.95 ml/min. Propofol was monitored at a wavelength of 276 nm.

A five-point calibration curve was prepared for the range 25–125 μg/ml of propofol, and under the conditions described above propofol eluted from the column at 3.5 min. The other components of the emulsion eluted with the solvent. The calibration curve was linear with a correlation coefficient of 0.9997 ($n = 6$).

2.5. Preparation of admixtures and samples

A 20-ml volume of fluid was withdrawn from each of three 100-ml glass bottles, PVC bags and Softbag bags containing 5% glucose infusion, and replaced by 20 ml of Diprivan solution, producing a nominal concentration of 2 mg propofol/ml. After mixing by gentle agitation, solutions were stored at room temperature (21 ± 2°C), one bottle or bag of each triplicate without protection from light, and two with protection. During the study, the glass containers were kept upright to minimize contact between drugs and the rubber stoppers. The plastic containers were hung from their support ring.

At 0, 1, 2, 4, 6 and 24 h, the admixtures of propofol were visually inspected for clarity and color change; accurately measured samples of 2 ml were then transferred from the infusion bottles and bags. The pH of the solution was measured (at 0 and 24 h) in samples of 5.0 ml (PHM 83 Autocal pH meter; Radiometer, Copenhagen, Denmark). The amount of propofol was determined by HPTLC and, for comparison, by a previously described and validated HPLC method with UV detection [1,4]. Sample pretreatment was unnecessary in the case of HPTLC whereas samples for HPLC had to be filtered before injection.

3. Results and discussion

Among the many mobile phases tested, toluene proved to be the eluent of choice for the HPTLC assay. The R_F value of propofol was 0.7 and propofol could be separated both from its degradation products and from the excipients in Diprivan (Fig. 1). The application of standards in methanol and samples in aqueous emulsions caused some difficulty: the bands of standards were narrow, whereas those of samples dried slowly and were broadened in all directions. Different solutions to the problem were investigated, among others solid-phase extraction, but the best results were obtained when the sample solutions were applied as 4 mm bands and the standards as 5 mm bands.

Baseline resolution ($R_s = 2.44$) was achieved between the peaks of propofol and excipients, and the asymmetric factor of the propofol peak

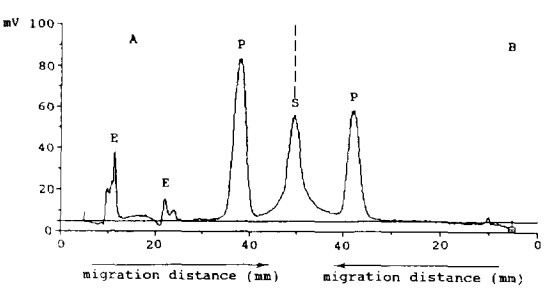

Fig. 1. A scan from the plate developed horizontally from the opposite ends. (A) Scan of diprivan, (B) scan of propofol standard. E = Excipients; P = propofol; S = solvent front.

Table 1
Repeatability of the application according to the heights of the chromatographic peaks of propofol ($n = 6$)

Solution studied	Amount applied (μl)	R.S.D. ($\pm$ %)
Standard 2.0 mg/ml	2	1.68
Standard 2.0 mg/ml	6	1.77
Standard 2.0 mg/ml	10	1.20
Sample 1.0 mg/ml	4	1.11
Sample 1.5 mg/ml	4	1.52
Sample 2.0 mg/ml	4	0.82

was 1.33. When the in situ spectra of propofol in standard solution and in Diprivan sample solution were superimposed, the spectra were seen to be identical, with a correlation factor of 0.9980. It was concluded that the excipients in Diprivan do not interfere with the measurement of propofol. The resolution between propofol and the solvent front was 0.96.

The calibration curve used in the quantitation of propofol was Michaelis–Menten regression 1 [$y = 129.04x/(6.068 + x)$]. Because of the better standard deviation of the calibration curve, different volumes of the standard solution were applied to the plate rather than identical volumes of different concentrations diluted from the stock solution (standard deviation $< \pm 2$ and $< \pm 4$, respectively). The repeatability of the application is presented in Table 1, which shows the relative standard deviation (R.S.D.) values to be less than 3%, the limit value recommended in the literature [9]. R.S.D.s measuring the repeatability of the whole method were 1.5, 1.8 and 1.8% for samples of concentration 1.0, 1.5 and 2.0 mg/ml, respectively. The accuracy of the method was good enough to monitor the sorption of propofol to different infusion containers. The mean value was 99.56% of the true value of propofol, and the R.S.D. was 2.9%. Propofol proved to be stable after application to the HPTLC plate, for at least 120 min before development, and for at least 24 h after development and before densitometric measurement.

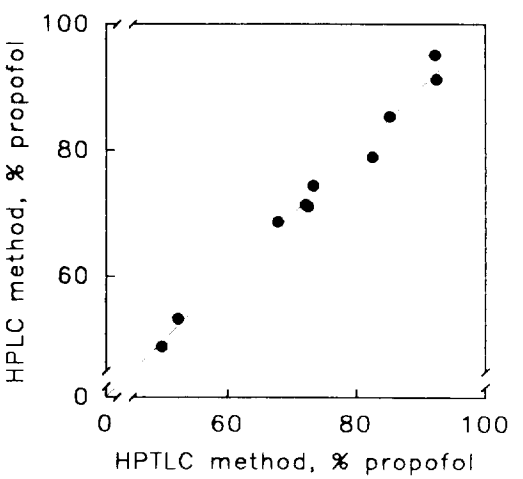

Fig. 2. Results for propofol assayed by HPTLC and by HPLC (slope 0.9962).

The reliability of our results obtained by HPTLC assay was checked by comparison with a published HPLC method. No significant difference was found in the results (Fig. 2). A paired t-test was carried out on the results obtained by the two methods, and the accuracy of the HPTLC method was found acceptable at a confidence level of 95%.

There was no decrease in the concentration of propofol in 5% glucose infusion when stored in glass or new polypropylene-lined Softbag containers for 24 h (Fig. 3). In a recent sorption study, Softbag containers also were shown to be safe for diazepam, nitroglycerine and warfarin sodium [10]. The concentration of propofol rapidly decreased in the PVC containers, however, being about 53% at 24 h (Fig. 3). This value was consistent with the results of a recent report on a static study which at 120 min a 31.54–34.74% loss of propofol had occurred to the plasticized PVC i.v. administration set [1].

The pH values of infusion solutions at 0 and 24 h were 7.2 in glass bottles and Softbag containers, whereas in PVC bags pH 5.1 was measured at 0 and pH 4.9 at 24 h. At no time was there notable discoloration or visual precipitation of sample solution. There was no sign of extra peaks in the chromatograms, confirming that the loss of propofol was caused by an absorptive process rather than degradation.

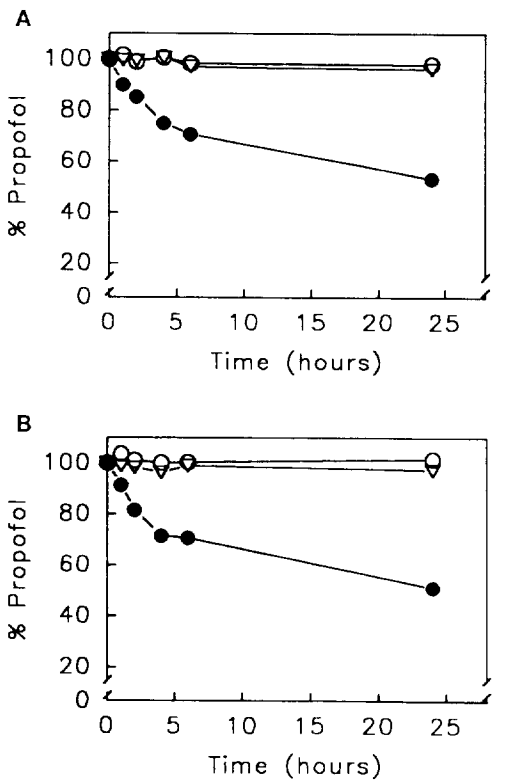

Fig. 3. Concentration of propofol, as a function of time, in glass bottles (○) and in Softbag (▽) and PVC (●) infusion bags. Values expressed as means of six measurements. (A) HPTLC; (B) HPLC.

4. Conclusions

The HPTLC method proved to be reliable and suitable for determining propofol and monitoring its sorption to infusion containers. The advantages of the developed assay over the HPLC method are: sample pretreatment is unnecessary, toluene alone forms the mobile phase which is needed only few milliliters, and the method is rapid because numerous samples can be measured on the same plate simultaneously. According to the results, the new polypropylene-lined containers (Softbags) can safely be used clinically for the administration of propofol in 0.5% glucose by intravenous infusion. Admixtures do not loose their potency when stored at room temperature with or without protection from light for 24 h.

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