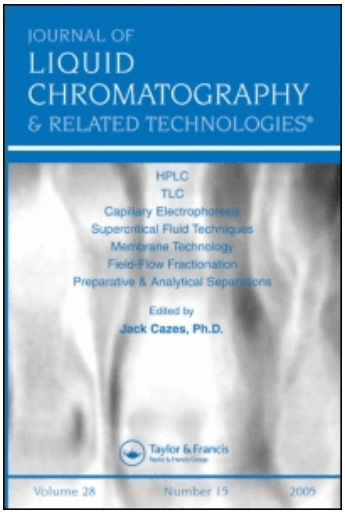

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Assay of Serum Thiopental Concentrations by High-Performance Liquid Chromatography

M. T. Parviainen[ab]; A. Harmoinen[a]; G. Baer[a]; H. Jokela[a]
[a] Department of Clinical Chemistry, Tampere University Central Hospital, Finland [b] Kuopio University Central Hospital, Finland

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ASSAY OF SERUM THIOPENTAL CONCENTRATIONS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

M.T. Parviainen*, A. Harmoinen, G. Baer and H. Jokela

Tampere University Central Hospital
Department of Clinical Chemistry
SF-33520 Tampere 52, Finland

*Present address: Kuopio University Central Hospital
SF-70210 Kuopio 21, Finland

ABSTRACT

A description is given of a specific and simple liquid chromatographic method for the determination of thiopental in protein-free serum. The analysis time per sample is only 4 min, the retention time of thiopental being 2.32 min. No drug interferences were found and the detection limit is about 0.3 mg/l. The day-to-day coefficient of variation is less than 4.3 %, and the within-day variation is less than 4.0 %. The method recovered 98.6 % of thiopental supplemented to a blank serum.

INTRODUCTION

Thiopental (5-ethyl-5(1-methylbutyl)-2-thiobarbiturate) is a short acting i.v. anaesthetic drug, which has also been used for cerebral resuscitation (1). Its use is well established as a single dose

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induction agent, but its pharmacokinetic (2) has not yet been widely studied because of the lack of a simple and sufficiently sensitive assay. Sensitivity is essential when the drug is studied as a single intravenous infusion anaesthetic (3).

Several methods exist for the determination of thiopental. The spectrophotometric method (4) involves three different wavelengths and has a sensitivity of 1.0 mg/l. The method is of limited use because of its non-specificity. Gas-chromatographic procedures (5) require a large sample volume as well as tedious sample preparation steps prior to assay. High-performance liquid chromatography has recently been utilized (6, 7, 8, 9) for the assay of thiopental in serum. The main limitations of these methods are the large sample volumes required (6, 7, 8), the tedious sample preparation (6) needed and the lack of an internal standard (7). Another drawback also is the various and rather complicated solvent systems used as a mobile phase (7, 8, 9).

The purpose of this study was to develop a sensitive and reliable assay for thiopental for use in therapeutic drug monitoring and for pharmacokinetic studies.

MATERIALS AND METHODS

Materials and reagents

The thiopental used in this study was manufactured by Lääketehdas Leiras (Turku, Finland) and the phenolphtalein internal standard came from E. Merck AG (Darmstadt, F.R.G.). The HPLC grade methanol and acetonitrile were purchased from Orion Corp. (Espoo,

Finland). An internal standard was prepared by dissolving 7.5 mg of phenolphtalein in 100 ml of acetonitrile.

Sample preparation

A serum (150 µl) was vortex-mixed with 450 µl of the internal standard solution in a 1 ml Eppendorf polypropylene tube and then centrifuged for 2 min at 12000 rpm using an Eppendorf 5411 centrifuge (Hamburg, F.R.G.) to remove the precipitated serum proteins. After centrifuging, 20 µl of the supernate was used for the analysis.

High-performance liquid chromatography

A Varian 5000 Liquid Chromatograph with variable wavelength UV-100 detector (Walnut Creek, CA, U.S.A.) was used. Sample injection was carried out via a Kontron MSI660 autosampler (Kontron AG, Zürich, Switzerland). A Shimadzu C-R1B Chromatopac computing integrator was used for automatic calculation of the final results.

A MPLC cartridge column system from Brownlee Labs. (Santa Clara, CA, U.S.A.) with a guard column (4.6 mm ID x 3 cm) and an analytical columns (4.6 mm ID x 10 cm) were used for the analysis. Both had Spheri-5 C-18 reverse-phase packing. The mobile phase consisted of 60 % methanol in distilled water.

The flow rate was 2 ml/min. The detector was set to a wavelength of 280 nm.

Calibration

The thiopental standard (20 mg/l, 75.7 µmol/l) was made up in a drug-free serum. The linearity of the

detector response to thiopental was established by using this calibrator from 0 to 250 mg/l. The standard was handled in the same way as the samples.

RESULTS AND DISCUSSION

The separation of phenolphtalein (internal standard, 1.34 min) and thiopental (2.32 min) in a patient serum is shown in Figure 1a. A drug-free serum showed no peaks in the retention times above 1 min (Figure 1b). The phenolphtalein internal standard gave a major peak at 1.34 min and minor impurity peak at 1.75 min, as illustrated in Figure 1c.

Protein in the samples is precipitated with three volumes of acetonitrile, which gives a clear protein-free supernatant (10). This assures a longer life-time of the column than when adding only two volumes of acetonitrile to the serum (9).

The absolute recovery of thiopental from serum was measured by analysing blank serum supplemented with 40 mg/l of the drug. The recovery (n=6) was 98.6 $\pm$ 2.7 % (mean $\pm$ SD), compared with the standard made up in water.

Serum samples containing potentially toxic levels of carbamazepine, clonazepam, digitoxin, disopyramide, ethosuximide, lidocaine, phenobarbitone, phenytoin, primidone, procainamide, propranolol, quinidine, theophylline or valproic acid did not interfere with the assay. Carbamazepine, if present, will elute between phenolphtalein and thiopental with a retention time of 1.94 min, and carbamazepine was the only drug peak seen in this system.

Precision was evaluated in the series by repeated analysis of serum samples containing 20.1, 14.3 and

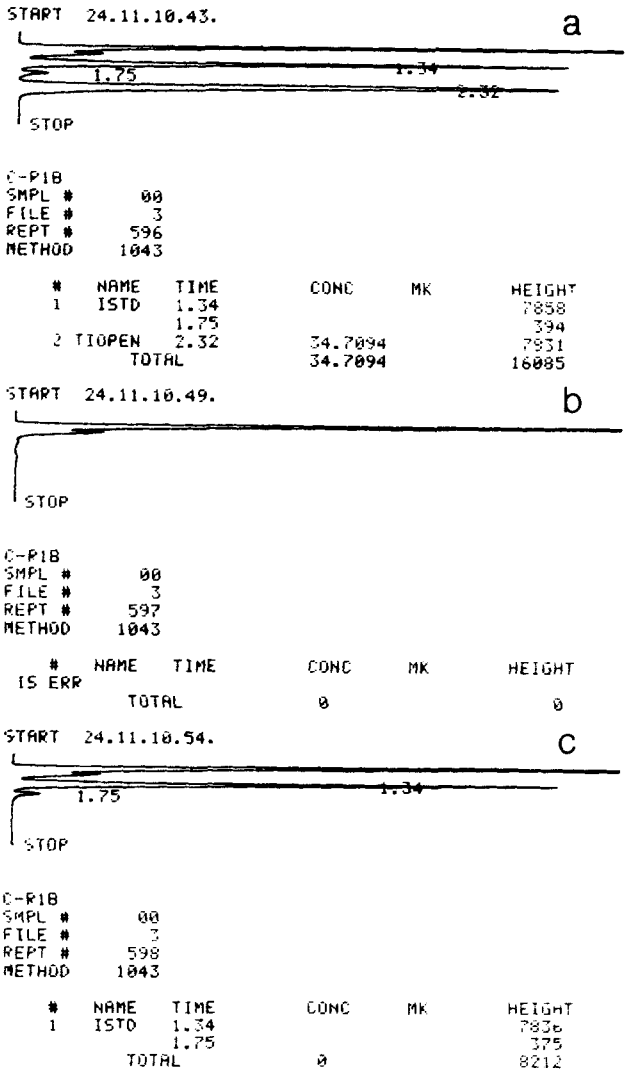

Figure 1. Separation of thiopental in serum. A) A patient serum with 34.7 mg/l of thiopental (retention time 2.32 min) and internal standard phenolphtalein (1.34 min); B) Blank serum; C) Blank serum with internal standard phenolphtalein (1.34 min). Minor peak at 1.75 min is a phenolphtalein impurity.

4.6 mg/l of thiopental. The coefficient of variation was 1.25 % (n=11), 2.40 % (n=15) and 4.0 % (n=15) respectively. The precision achieved from day to day with samples containing 14.1 and 4.5 mg/l thiopental was 2 % (n=10) 4.3 % (n=10) respectively.

We also measured serum thiopental concentrations during and after Fentanyl-complemented (0.01 mg i.v.) thiopental infusion anaesthesia (11). The induction dose was 5 mg/kg and maintenance 12.5 mg/min thiopental. The patients: 10 male and 11 female, aged 50 years (SD 10.4, range 30-64), weight 72.5 kg (SD 16, range 48-120).

The concentrations were measured 10 min, 20 min, 60 min and 24 hours after the induction dose. The concentrations were respectively 12.8 $\pm$ 4, 9.9 $\pm$ 3, 4.4 $\pm$ 2, and 0.4 $\pm$ 0.2 (mean $\pm$ 1 SD).

A detection limit of 0.3 mg/l was achieved using the routine attenuation of the detector (about 0.016 absorbance units of the full scale). This seems to be sufficient for phamacokinetic studies and for the therapeutic monitoring of the drug.

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