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Note

Micromethod for determination of thiopental in human plasma by high-performance liquid chromatography

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Since 1935, thiopental, an ultra-short-acting barbiturate, has been used to induce anesthesia. Lately, thiopental has been proposed for protection of the brain in comatose patients with head trauma [1—3]. In this indication, large doses of thiopental are given intravenously over a period of a few days and a control of plasma levels is necessary.

For the determination of thiopental in plasma, gas chromatographic [4] and high-performance liquid chromatographic (HPLC) [5—9] methods have been reported in the literature. The HPLC method described in this paper, requiring a minimum pretreatment of the plasma sample, presents the advantage of being rapid and simple.

EXPERIMENTAL

Chemicals

Acetonitrile (Burdick and Jackson Labs., Muskegon, MI, U.S.A.) used was of non-spectro grade. Stock thiopental (Specia, Paris, France) solution was 1 g/l in water. Flunitrazepam (Roche, Neuilly/Seine, France), used as internal standard, was 12.5 mg/l in acetonitrile.

Liquid chromatography

A liquid chromatograph Model 5040 (Varian, Orsay, France) combined with a Vista CDS 401 data system (Varian) was used throughout this work. The UV Spectromonitor III detector (LDC, Riviera Beach, FL, U.S.A.) was operated at 280 nm. The automatic injector was a WISP 710 B Model (Waters Assoc., Paris, France).

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Analysis was performed on a 5-μm Spherisorb C_6 column (20 cm × 4.6 mm) from Phase Sep (Queensferry, Great Britain), operating at 30°C. The analytical column was protected by a small precolumn (5 cm × 4.6 mm) packed with 30—40 μm Permaphase C_{18} (Dupont, Orsay, France).

The mobile phase was a mixture (70:30, v/v) of 0.01 *M* sodium acetate adjusted to pH 3.6 by concentrated acetic acid and acetonitrile. The flow-rate was kept at 1.5 ml/min.

Preparation of plasma samples

To 50 μl of plasma, in a 1.5-ml polypropylene microtube (Eppendorf, Hamburg, G.F.R.), were added 200 μl of the internal standard solution. The stoppered tube was shaken for 3 min on an Eppendorf shaker. After brief centrifugation, the supernatant was transferred to an automatic sampler vial and a 25-μl aliquot was injected.

Quantitation

Quantitation was done by the peak height ratio method with flunitrazepam as internal standard. Calibration curves were obtained by spiking control plasma with various amounts of stock thiopental solution (0, 12.5, 25, 50, 75, 100 mg/l) and a constant amount of internal standard (50 mg/l).

Within-run variation was determined by analyzing tenfold two plasma samples containing, respectively, 5 mg/l and 25 mg/l thiopental.

Stability

The stability of plasma thiopental was tested by using fresh control plasma surcharged with thiopental at the following concentrations: 12.5, 25, 50 and 100 mg/l.

Samples of 50 μl from each concentration were placed into separate 1.5-ml polypropylene microtubes and stored either at room temperature for 24 h or at −20°C for one to eight weeks.

RESULTS AND DISCUSSION

Typical chromatograms of a blank plasma and a normal plasma supplemented with thiopental are shown, respectively, in Fig. 1a and b. The retention times of thiopental and flunitrazepam are, respectively, 9.7 min and 11.1 min, which permit a chromatographic run of only 13 min. The chromatograms of patients receiving thiopental show a peak with a retention time of 3.0 min (Fig. 1c). We have not yet identified this peak which probably corresponds to a thiopental metabolite.

The linearity of the method is very good up to 100 mg/l. The detection limit for plasma samples is 0.5 mg/l. Reproducibility and accuracy of the method are illustrated in Table I. For concentrations of 25 mg/l as well as 5 mg/l, the coefficient of variation is below 2%.

Plasma thiopental is stable for at least 24 h at room temperature and no appreciable degradation was observed after storage for eight weeks at −20°C (Table II).

More than 500 patient plasma samples were analyzed and no interference

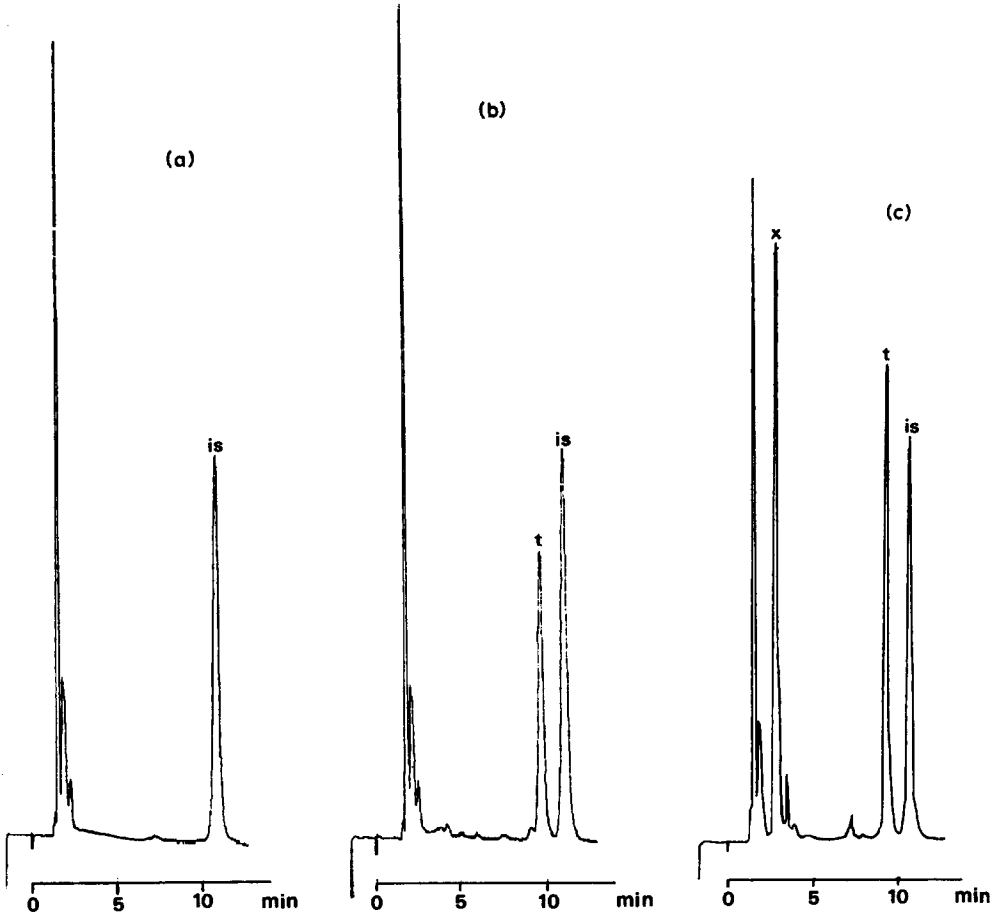

Fig. 1. Chromatograms of: (a) blank plasma, (b) normal plasma with 12.5 mg of thiopental added per liter, (c) a patient plasma (thiopental = 21 mg/l). x = unknown peak, t = thiopental, is = internal standard.

TABLE I

WITHIN-RUN VARIABILITY OF THE METHOD

Added (mg/l)	Found ($\bar{x}$ ± S.D.) (n = 10)	C.V.* (%)
5.00	5.03 ± 0.06	1.19
25.00	24.83 ± 0.34	1.37

*C.V. = coefficient of variation = (S.D./$\bar{x}$) × 100.

was encountered. Moreover, the barbiturates having a molecule without a sulphur atom, and in particular pentobarbital, a thiopental metabolite, do not absorb at 280 nm. The UV absorption spectra of thiopental and pentobarbital are shown in Fig. 2. In our laboratory, pentobarbital quantitation is carried out by a gas chromatographic method previously described [10].

TABLE II

STABILITY OF THIOPENTAL DURING PROLONGED STORAGE OF THE SAMPLES

Storage at 20°C (h)	Storage at −20°C (weeks)	Thiopental concentration found (mg/l)			
		12.5 mg/l added	25 mg/l added	50 mg/l added	100 mg/l added
0		12.5	23.5	52.0	95.0
4		13.0	26.0	53.0	99.0
24		12.5	26.0	54.0	99.5
	1	12.0	23.0	53.0	102.0
	2	12.0	23.0	52.0	109.0
	4	12.8	23.6	43.2	97.0
	8	12.0	24.5	45.5	106.0

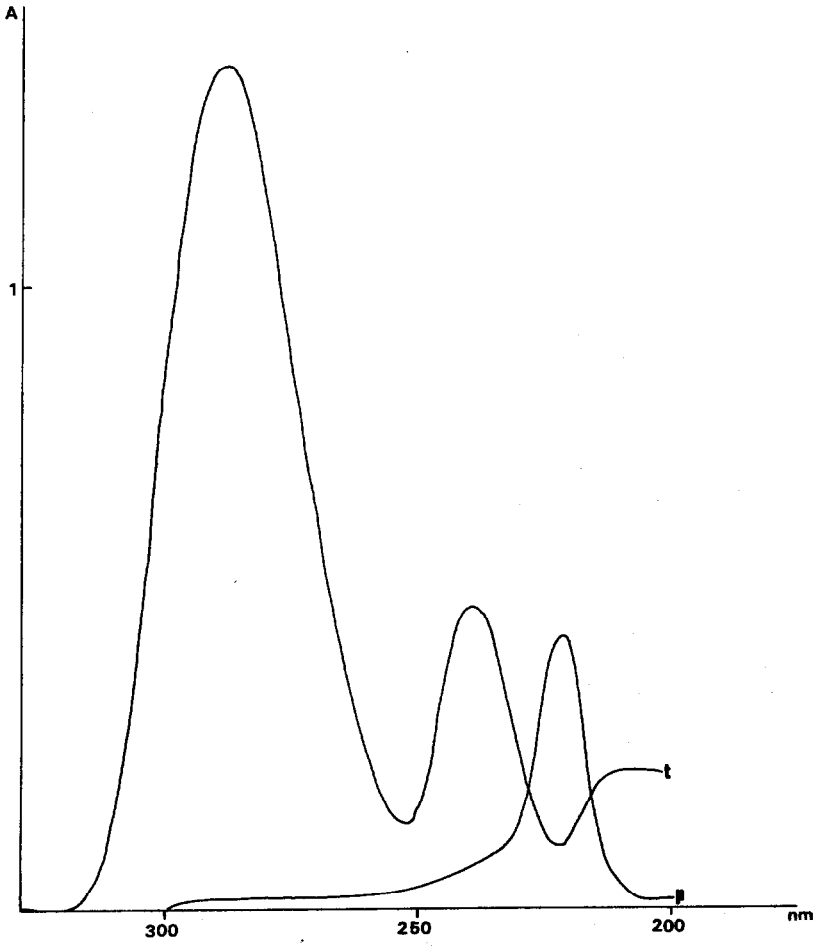

Fig. 2. UV absorption spectra of thiopental (t) and pentobarbital (p). The concentrations were 8 mg for thiopental and 16 mg for pentobarbital per liter of mobile phase.

In conclusion, this technique involving a small quantity of plasma, rapid sample preparation and automated liquid chromatography is highly suitable for routine analysis.

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