Journal of Chromatography, 305 (1984) 210—213
Biomedical Applications
Elsevier Science Publishers B.V , Amsterdam — Printed in The Netherlands

CHROMBIO. 1906

Note

Rapid gas chromatographic assay for serum thiopental

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(First received May 18th, 1983; revised manuscript received August 2nd, 1983)

Several methods have been described for thiopental analysis, some of them using gas liquid chromatography (GLC) involving various solvents, extensive extraction procedures and derivatization techniques [1—5]. Also four high-performance liquid chromatographic (HPLC) methods [6—9] have been reported.

This communication describes a simple and rapid GLC method for quantitation of thiopental in serum. With minor modifications to the extraction procedure and chromatographic conditions we used for monitoring anticonvulsant drugs [10, 11], the thiopental samples can be prepared in the same way, therefore only one extraction is needed and the thiopental is analyzed in the underivatized form.

METHODS

Reagents and materials

Thiopental in free form is supplied by Abbott Labs. Methylene chloride and 85% phosphoric acid were pro analysi grade from Merck (Darmstadt, F.R.G.).

Stock solutions of thiopental (1 mg/ml) were prepared in methanol, and in methanol adding 1 ml of 85% phosphoric acid to 100 ml of methanol.

Internal standard solutions were prepared by diluting a stock solution (1 mg/ml) of 5-methylphenylhydantoin from Supelco (Bellefonte, PA, U.S.A.) to 50 μg/ml and 12.5 μg/ml.

Procedure

Internal standard (10 or 2.5 μg) was added to each extraction tube and the methanol was evaporated with a stream of nitrogen. Then 0.5 ml of serum

sample was poured into the extraction tube. After mixing, the sample was acidified with 100 μl of 85% phosphoric acid and mixed. Then the sample was extracted with 5 ml of methylene chloride for 10 min and centrifuged for 5 min at 2100 *g*. The organic layer was transferred to an 8-ml tube (Kimax 13 × 100 mm 45066) and evaporated to dryness with nitrogen. The extract was reconstituted with 100 μl of methylene chloride (when the amount of internal standard was 10 μg) or with 60 μl (when the amount of internal standard was 2.5 μg), and 1.2 or 2 μl were injected into the GLC system.

Calibration was made by analysing in duplicate serum spiked with 1—5 μg of thiopental and a constant quantity of internal standard (2.5 μg) and 5—30 μg of thiopental and 10 μg of internal standard. Standard curves were constructed plotting the peak height ratio and the peak area ratio of thiopental to the internal standard (2.5 or 10 μg) against 1—5 μg and 5—30 μg of thiopental, respectively. Thiopental concentration was calculated from the standard curves.

The recovery of thiopental from serum was determined by comparing thiopental/internal standard peak area and height ratios in serum with those obtained by direct injection of equal amounts of the thiopental in methanolic solution.

Gas chromatographic conditions

A Perkin Elmer 3920 B chromatograph (Perkin Elmer, Norwalk, CO, U.S.A.) equipped with a flame ionization detector was used. A 1.8-m glass column, 2 mm I.D., was packed with 2% SP 2110—1% SP 2510 DA on 100—120 mesh Supelcoport (Supelco).

The analysis was carried out isothermally with the oven temperature at 205°C, the injector and detector at 250°C, carrier gas (nitrogen flow-rate of 50 ml/min, hydrogen flow-rate of 35 ml/min and air flow-rate of 300 ml/min.

A minigrator M2 (Perkin Elmer) was used to measure the areas of the desired peaks.

RESULTS AND DISCUSSION

A representative chromatogram of an extract of a patient sample is shown in Fig. 1.

Two different amounts (2.5 and 10 μg) of internal standard were used. This method should prove to be widely applicable. A concentration range of 1—30 μg permits quantitation of serum concentrations after administration of a wide range of doses of thiopental.

The linearity of each range was evaluated by a least-squares regression analysis of the ratio of the peak heights and areas of thiopental to internal standard (Fig. 2).

Any conversion to pentobarbital (retention time 1.9 min) was reflected in the chromatograms of the standard curves. No difference was observed after storage at −15°C for six weeks between the concentration of thiopental in methanolic solution and in methanol with 1% of 85% phosphoric acid [8]. Pentobarbital is a minor oxidation metabolite of thiopental in man and pentobarbital serum concentrations of 10% of thiopental concentrations have been

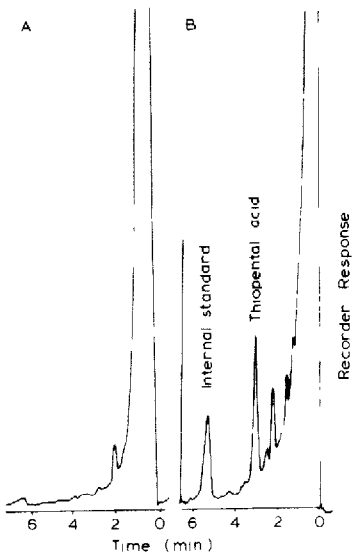

Fig 1 Gas chromatograms of 0.5-ml serum extracts: (A) serum blank; (B) serum from a patient receiving sodium thiopental, serum level = 2.5 μg.

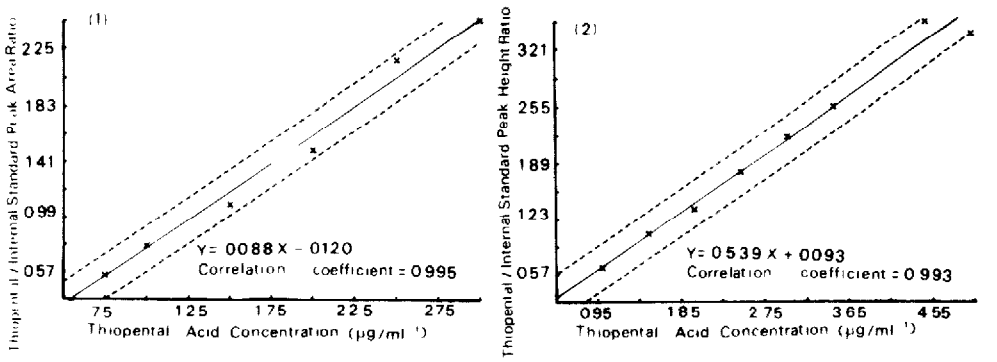

Fig. 2. Standard curves obtained from duplicate analyses of extracts of serum samples spiked with sodium thiopental. (1) high concentration range, derived from 0.5-ml serum samples with 10 μg of internal standard; (2) low concentration range, derived from 0.5-ml serum samples with 2.5 μg of internal standard.

reported during high-dose thiopental therapy [12]. A trace of pentobarbital arising from "in vivo" biotransformation was observed.

The absolute recovery of thiopental was about 95%.

The coefficient of variation obtained by extraction and analysis of six replicates of 15.5 μg was 5.2% while a similar analysis of six replicates of 7.3 μg gave a coefficient of variation of 6.1%.

Several GLC methods with different detection systems have been published for the determination of thiopental in serum. The disadvantage of most of these methods is that some of them analyse the drug in the derivatized form or they need several extraction steps. The method proposed analyses the thiopental in free form and with only one extraction step then, it is simpler than the others.

The sensitivity with the flame ionization detector (0.6 μg/ml) is satisfactory to determine the concentration of thiopental after administration of a wide range of doses in order to optimize the drug dosage selection.

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