Journal of Chromatography, 307 (1984) 481—487
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2058

Note

A high-performance liquid chromatographic method for methohexital and thiopental in plasma or whole blood

SVEN BJÖRKMAN* and JAN IDVALL

Hospital Pharmacy and Department of Anesthesiology, Malmö General Hospital, S-214 01 Malmö (Sweden)

(First received October 12th, 1983; revised manuscript received December 23rd, 1983)

Methohexital (methohexitone, Brietal®) is an ultra-short-acting barbiturate used for induction of anaesthesia or as an intravenous sedative [1]. Gas chromatographic methods for the determination of methohexital in biological samples have been reported, the most sensitive of which utilize nitrogen-selective detection [2, 3] or electron-capture detection of a pentafluorobenzyl derivative [4].

For the purposes of pharmacokinetic work, we have developed a rapid and sensitive liquid chromatographic analysis permitting the determination of methohexital in plasma or blood in the nanogram per millilitre range. The method is also applicable to thiopental, for which a number of high-performance liquid chromatographic (HPLC) methods have, however, already been reported [5—13]. During the preparation of this manuscript a brief outline of another HPLC method for methohexital appeared in the literature [14].

EXPERIMENTAL

Reagents and chemicals

Methohexital was supplied by Eli Lilly Sweden (Stockholm, Sweden). Hexobarbital was of European Pharmacopoeia quality. These compounds were each dissolved in 6.7 m*M* phosphate buffer (pH 7.4) and the appropriate stock solutions were then prepared by dilution with distilled water. Thiopental sodium (Pentothal®, Abbott, Campoverde, Italy), containing 86.1% free acid, was dissolved in water. The methohexital and hexobarbital solutions were kept at room temperature and the thiopental solutions in the refrigerator. Toluene

*Correspondence address: Sjukhusapoteket, Allmänna sjukhuset, S-214 01 Malmö, Sweden.

0378-4347/84/$03.00 © 1984 Elsevier Science Publishers B.V.

(8325; Merck, Darmstadt, F.R.G.), acetonitrile (30; Merck) and $NaH_2PO_4 \cdot H_2O$ (6346; Merck) were used without further purification. The water was freshly distilled and collected in a stainless-steel vessel.

Instrumentation

The liquid chromatography system consisted of an LDC/Milton Roy (Riviera Beach, FL, U.S.A.) Minimetric II pump, a Rheodyne 7125 loop injector and an LDC Spectro Monitor III variable-wavelength UV detector. A LiChroCart RP-18 7-μm column (250 × 4 mm) was used in conjunction with a Hibar precolumn (30 × 4 mm) filled with Perisorb RP-18 (all from Merck). The mobile phase was a 1:1 mixture of 0.050 *M* NaH_2PO_4 solution (pH 4.6) and acetonitrile. The flow-rate was 1.00 ml/min and the detection wavelength was 195 nm. Ultraviolet spectra were recorded on an LKB Beckman Model 25 scanning photometer (LKB, Stockholm, Sweden) with the barbiturates dissolved in aliquots of mobile phase.

Methods

To 1.00-ml (or smaller) samples of plasma or haemolysed (frozen and thawed) whole blood containing methohexital (0—16.0 μg/ml = 0–61.2 μM) or (and) thiopental (0—16.0 μg/ml = 0—66.3 μM) were added 0.500 ml of internal standard solution (hexobarbital, 2.00 μg/ml = 8.46 μM in water), 0.5 ml of 0.25 *M* hydrochloric acid and 0.04 g of sodium chloride. The samples were extracted with 3 ml of toluene on a Hook and Tucker rotamixer and the solvent layers were separated by centrifugation. The toluene layer was transferred to another tube and the solvent was evaporated on a sand bath (50 ± 5° C) under a stream of dry air. The residue was taken up in 0.20 ml of acetonitrile, and 0.20 ml of 0.050 *M* sodium dihydrogen phosphate solution was added; 20 μl of this solution were injected into the chromatograph.

Extraction recoveries were determined by adding methohexital and thiopental to 1.0 ml of plasma or whole blood (final concentration of methohexital 0.50 μg/ml and thiopental 1.0 μg/ml), freezing and thawing the blood and extracting by the standard procedure with 4.00 ml of toluene. Of the toluene phase, 2.00 ml were transferred to another tube, 0.500 ml of hexobarbital (1.00 μg/ml in methanol) was added and the solvents were evaporated. The residue was taken up in acetonitrile—0.050 *M* sodium dihydrogen phosphate solution and injected. A mixture of methohexital—thiopental—hexobarbital (1:2:2) prepared from the same stock solutions served as reference. Four samples each of plasma, blood and reference were analysed.

The quality of the stock solutions was checked by addition of internal standard and direct injection into the chromatograph. Freshly made solutions served as references for old ones.

The stability of methohexital and thiopental in blood samples was assessed by spiking 6 ml of freshly drawn, heparinized blood with 50 μl of methohexital or thiopental solution, 100 μg/ml (final concentration in the blood 0.7 μg/ml). To mimic careless sample handling, the tubes were then left on the bench for 24 h. Aliquots were withdrawn at 0, 1, 2, 4, 8, 12 and 24 h, frozen (at −20° C) and analysed on the following day.

For the pharmacokinetic verification of the method, blood samples were

drawn from consenting day patients undergoing minor (orthopaedic) surgery under nitrous oxide—halothane anaesthesia. The induction doses of intravenous barbiturate were titrated individually and ranged from 1 to 2 mg/kg for methohexital and from 3 to 5 mg/kg for thiopental. The blood samples were collected in heparinized tubes, kept in the refrigerator and centrifuged after a few hours. The plasma samples were then stored at −20°C until analysed.

RESULTS

General considerations

Typical chromatograms from methohexital and thiopental analysis are shown in Figs. 1 and 2. The retention times of six representative barbituric acids in this system are: barbital 2.9 min, phenobarbital 3.6 min, pentobarbital 4.5 min, hexobarbital 4.8 min, thiopental 6.8 min and methohexital 7.3 min. Of possible interfering compounds, salicylic acid and acetylsalicylic acid are not retained while caffeine and indomethacin appear at 2.5 and 8.6 min, respectively.

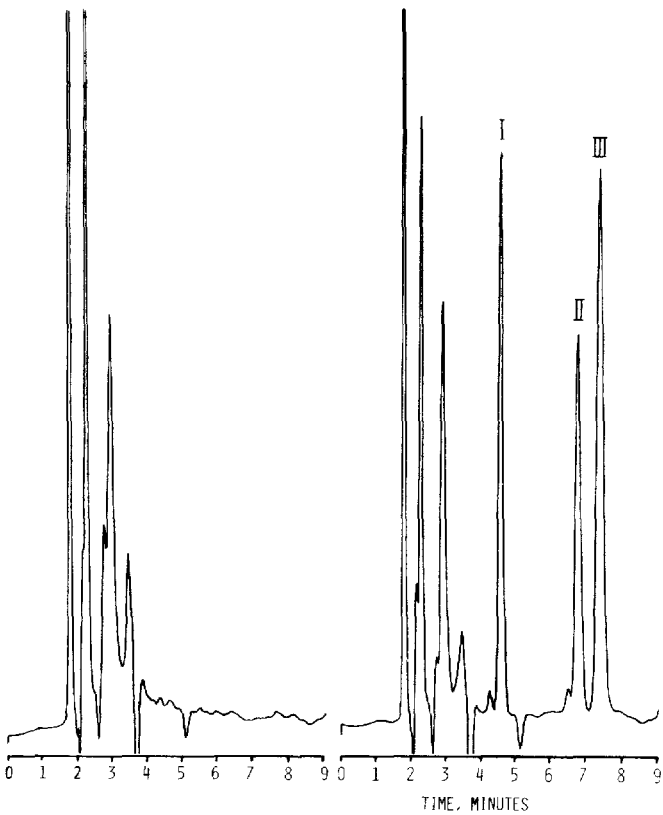

Fig. 1. Chromatograms of an extract of haemolysed blood and of a similar sample spiked with hexobarbital (I, 1.00 μg/ml), thiopental (II, 4.00 μg/ml) and methohexital (III, 2.00 μg/ml). The small peaks just in front of the hexobarbital and thiopental peaks are impurities in the thiopental (Pentothal) preparation. Extracts of plasma give similar chromatograms. Some samples also give large, unidentified peaks at 10 and 13 min, and in practice the interval between injections is around 14 min. Detector: 0.02 a.u.f.s. Recorder: 10 mV, 10 mm/min.

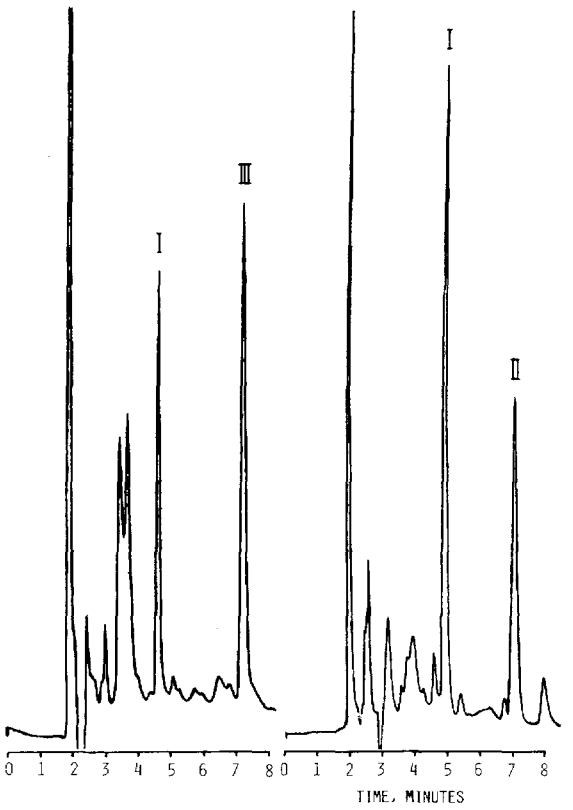

Fig. 2. Chromatograms of plasma extracts from patients. Left: a sample taken 5 min after induction of anaesthesia with methohexital sodium, 1.7 mg/kg. The methohexital concentration is 1.2 μg/ml. Right: a sample taken 30 min after induction of anaesthesia with thiopental sodium, 4.1 mg/kg. The thiopental concentration is 3.2 μg/ml. Peak identification numbers and detector and recorder settings are as in Fig. 1.

The ultraviolet spectra of hexobarbital and methohexital (as free acids) show absorption peaks at 195 nm, with shoulders at 220 nm. Also thiopental gives an absorption maximum at 195 nm, albeit a lower one than at 236 and 286 nm. The relative detector responses of the three compounds, measured as peak areas at 195 nm in a chromatogram of an equimolar mixture, are 1:0.70:0.32. The lower ultraviolet absorption of thiopental compared to methohexital is reflected in a lower sensitivity of the analysis.

The stock solutions of methohexital and hexobarbital were stable for at least six months at room temperature, and the thiopental stock solutions for at least one month at +8° C.

Methohexital

Standard curves drawn on the analysis of duplicate samples containing 0.125, 0.250, 0.500, 1.00, 2.00, 4.00, 8.00 and 16.0 μg methohexital per ml were linear (generally $r = 0.999$ on a six-point standard curve). Quantitation was by peak height. In addition, samples of very low concentration were analysed with 0.500 ml of 0.50 μg/ml hexobarbital as internal standard. Under these conditions, linear (typically $r = 0.998$) standard curves could be obtained

TABLE I

ANALYSIS OF SPIKED PLASMA AND BLOOD SAMPLES (n = 8)

Amount added (μg/ml)		Amount found (μg/ml)	Relative S.D. (%)
In plasma			
Methohexital	1.00	0.993 ± 0.026	2.6
Methohexital	0.250	0.250 ± 0.0083	3.3
Methohexital	0.0625	0.0625 ± 0.0028	4.4
Thiopental	2.00	2.04 ± 0.055	2.7
Thiopental	0.50	0.54 ± 0.032	6.1
In blood			
Methohexital	1.00	0.963 ± 0.032	3.3
Methohexital	0.250	0.246 ± 0.0049	2.0
Methohexital	0.0625	0.0641 ± 0.0018	2.8
Thiopental	2.00	1.99 ± 0.036	1.8
Thiopental	0.50	0.503 ± 0.015	3.0

on duplicate samples containing 31.3, 62.5, 125, 250, 500 and 1000 ng/ml. The precision of the analysis is given in Table I.

Shifting the detector wavelength to 220 nm lowered the sensitivity of the detection without improving the selectivity. Sample clean-up by back-extraction into 0.01 M sodium hydroxide [4] tended to decrease the peak-to-noise ratio of the chromatograms and did not improve the sensitivity of the analysis.

The recovery of methohexital was 78 ± 3% from plasma and 78 ± 3% from haemolysed whole blood, as measured at a sample concentration of 0.50 μg/ml. At 2.00 μg/ml the recoveries were 93 ± 1% and 88 ± 5% [4].

Thiopental

Standard curves drawn from the analysis of duplicate samples containing 0.250, 0.500, 1.00, 2.00, 4.00, 8.00 and 16.0 μg/ml were linear (generally r = 0.999 on a five-point standard curve). Quantitation was by peak height. The precision of the analysis is given in Table I. The recovery of thiopental (at 1.0 μg/ml sample concentration) was 91 ± 1% from plasma and 82 ± 2% from haemolysed blood.

Stability of blood samples

There was, within the limits of error of the method, no apparent degradation of methohexital or thiopental in blood samples left on the bench (at 21 ± 1°C) for 24 h.

Pharmacokinetic verification of the method

Representative plasma concentration curves from two patients given intravenous bolus doses of barbiturates are shown in Figs. 3 and 4.

DISCUSSION

Separation of barbiturates on chemically bonded octadecylsilyl phases is highly dependent on the pH of the eluent, and for good peak shapes and

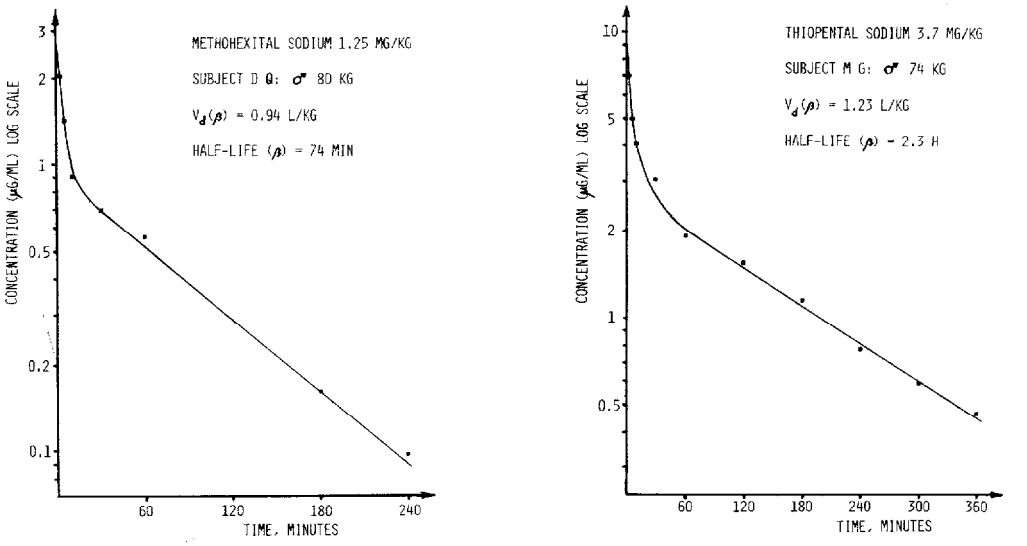

Fig. 3. Plasma concentration of methohexital in a patient under methohexital—halothane—nitrous oxide anaesthesia. The dose of methohexital sodium (Brietal) corresponds to 1.15 mg/kg free acid. The apparent volume of distribution (V_d) and the half-life (β) given in the figure were calculated from the six-point regression line.

Fig. 4. Plasma concentration of thiopental in a patient under thiopental—halothane—nitrous oxide anaesthesia. The dose of thiopental sodium (Pentothal) corresponds to 3.4 mg/kg free acid. The apparent volume of distribution (V_d) and the half-life (β) given in the figure were calculated from the six-point regression line.

efficient separations acidic systems are often preferable [15]. Determination of barbiturates as free acids requires short-wavelength ultraviolet detection, typically 195 nm, which precludes the use of alcohols in the mobile phase. Water—acetonitrile mixtures are consequently employed. In spite of the low wavelength the detection is quite selective and permits determination of the barbiturates in the ng/ml range [14, 16].

In conclusion, we have developed an HPLC method for the determination of methohexital or thiopental or, if need should arise, the simultaneous determination of both compounds. Its sensitivity, for methohexital, is at least as good as that of the gas—liquid chromatographic methods utilizing nitrogen-selective detection [2, 3]. It also compares favourably with our gas—liquid chromatographic method with electron-capture detection [4], with a roughly two-fold higher precision and sensitivity and no need for sample derivatization. The barbiturates proved sufficiently stable in blood to permit sample handling without special precautions. The feasibility of the method for pharmacokinetic work is demonstrated.

ACKNOWLEDGEMENT

We thank Miss Ulla Jönsson for skilful technical assistance.

REFERENCES

1 J.G. Whitwam, Anaesthesiol. Wiederbeleb., 57 (1972) 2.
2 D.D. Breimer, Brit. J. Anaesth., 48 (1976) 643.
3 H. Heusler, J. Epping, S. Heusler, E. Richter, N.P.E. Vermeulen and D.D. Breimer, J. Chromatogr., 226 (1981) 403.
4 S. Björkman, J. Idvall and P. Stenberg, J. Chromatogr., 278 (1983) 424.
5 G.L. Blackman and G.J. Jordan, J. Chromatogr., 145 (1978) 492.
6 A.N. Masoud, G.A. Scratchley, S.J. Stohs and D.W. Wingard, J. Liquid Chromatogr., 1 (1978) 607.
7 J.H. Christensen and F. Andreasen, Acta Pharmacol. Toxicol., 44 (1979) 260.
8 W. Toner, P.J. Howard, J.W. Dundee and P.D.A. McIlroy, Anaesthesia, 34 (1979) 657.
9 D.J. Freeman, Clin. Chem., 27 (1981) 1942.
10 C. Salvadori, R. Farinotti, P. Duvaldestin and A. Dauphin, Ther. Drug Monit., 3 (1981) 171.
11 G.K. Shiu and E.M. Nemoto, J. Chromatogr., 227 (1982) 207.
12 R. Sauerbrey and H. Reiber, Z. Anal. Chem., 311 (1982) 412.
13 J.R. Sharman and K.M. Ahern, J. Anal. Toxicol., 7 (1983) 37.
14 R.J. Hudson, D.R. Stanski and P.G. Burch, Anesthesiology, 59 (1983) 215.
15 R. Gill, A.H. Stead and A.C. Moffat, J. Chromatogr., 204 (1981) 275.
16 H.L. Levine, M.E. Cohen, P.K. Duffner, K.A. Kustas and D.D. Shen, J. Pharm. Sci., 71 (1982) 1281.