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Note

Gas—liquid chromatographic determination of methohexital in plasma or whole blood with electron-capture detection of the pentafluorobenzyl derivative

SVEN BJÖRKMAN*, JAN IDVALL and PÅL STENBERG

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

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Methohexital (methohexitone, Brietal®) is an ultra-short-acting barbiturate used for induction of anaesthesia or as an intravenous sedative [1]. Quantitative analysis of methohexital in biological samples has been done by means of ultraviolet spectrophotometry [2], gas—liquid chromatography (GLC) with flame ionization detection [3] and GLC with nitrogen-selective detection [4, 5]. Only the last method has sufficient sensitivity and selectivity for pharmacokinetic studies.

Analysis of barbiturates as pentafluorobenzyl derivatives, with electron-capture detection (ECD), has not become a standard method, perhaps because of the tedious work-up and derivatization procedures reported [6]. We therefore wish to describe a simple and rapid GLC—ECD method for methohexital in biological samples.

EXPERIMENTAL

Reagents and chemicals

Methohexital was supplied by Eli Lilly Sweden (Stockholm, Sweden). Hexobarbital was of European Pharmacopoeia quality. The barbiturates were dissolved in 6.7 m*M* phosphate buffer (pH 7.4) and the appropriate stock solutions were then prepared by dilution with distilled water. Pentafluorobenzyl (PFB) bromide was purchased from Aldrich-Europe (Beerse, Belgium) and triethylamine was Eastman synthetic grade (Rochester, NY, U.S.A.). Toluene (8325; Merck, Darmstadt, F.R.G.), methanol (6009; Merck), cyclohexane (9666; Merck) and absolute ethanol (Svensk Sprit, Stockholm, Sweden) were used without further purification.

*To whom correspondence should be addressed at Sjukhusapoteket, Allmänna Sjukhuset, S-214 01 Malmö, Sweden.

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Instrumentation

A Varian (Palo Alto, CA, U.S.A.) 3700 gas chromatograph equipped with a ^{63}Ni electron-capture detector and a Model 8000 autosampler was used. The glass column (150 × 0.2 cm I.D.) was packed with 3% OV-17 on 100—120 Supelcoport. The oven temperature was 188°C and the injector and detector temperatures were 240°C and 250°C, respectively. Nitrogen (30 ml/min) was used as carrier gas. Mass spectra were obtained, under similar gas chromatographic conditions, on a Finnigan (Sunnyvale, CA, U.S.A.) 4510 quadrupole instrument operated in the chemical ionization mode.

Method

To 1.0 ml samples of plasma or haemolysed (frozen and thawed) whole blood containing methohexital (0—16.0 μg/ml = 0—61.2 μ*M*) were added 0.5 ml of internal standard solution (hexobarbital 10.0 μg/ml = 42.3 μ*M* in water), 0.5 ml of 0.25 *M* hydrochloric acid and some 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 extracted with 2 ml of 0.01 *M* sodium hydroxide. The aqueous phase was separated, acidified with 1 ml of 0.25 *M* hydrochloric acid and extracted with 3 ml of toluene. The solvent was evaporated on a sand bath (50 ± 5°C) under a stream of dry air.

The residue was taken up in 0.1 ml of 0.5 *M* triethylamine in methanol and 5 μl of pentafluorobenzyl bromide were added. The mixture was heated for 10 min on a 56 ± 1°C water bath. The volatiles were evaporated on the sand bath under a stream of dry air. The residue was taken up in a few drops of absolute ethanol and diluted to 4 ml with cyclohexane. By means of the autosampler, 1 μl was injected into the gas chromatograph.

The extraction recovery was determined by adding methohexital to 1 ml of plasma or whole blood (final concentration 2.0 μg/ml), freezing and thawing the whole blood and extracting by the standard procedure with 3.0 ml of toluene. To 2.0 ml of the toluene phase 0.5 ml of hexobarbital in methanol (10 μg/ml) was added as external standard. The solvent was evaporated and the residue derivatized and assayed. A mixture of methohexital—hexobarbital (2:5) served as reference. Four samples of each, plasma, blood and reference, were used.

RESULTS AND DISCUSSION

Work-up

For plasma samples containing >1 μg/ml methohexital the re-extraction step can be omitted. The recovery of methohexital from plasma was 93 ± 1% and from haemolysed whole blood 88 ± 5%.

Derivatization procedure

The derivatization conditions are those given by Walle [7], who did not, however, work with biological samples. The reaction was tried out by running the recovery experiment as described but adding hexobarbital pentafluorobenzyl derivative at the end as an external standard. With reaction times

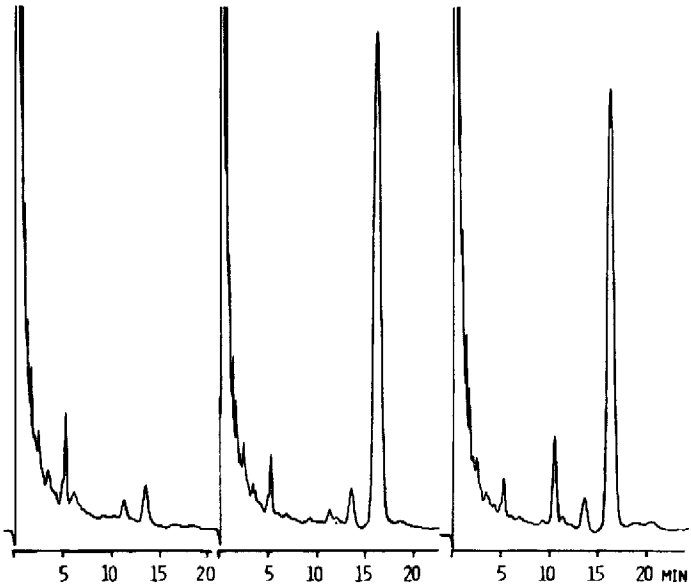

Fig. 1. Chromatograms of plasma samples from a patient undergoing minor surgery. From left to right: a blank sample taken before anaesthesia; a blank sample with internal standard added; and a sample taken 10 min after induction of anaesthesia with intravenous methohexital sodium 1.25 mg/kg. The methohexital concentration of the last sample is 0.75 μg/ml.

exceeding 5 min the relative height of the methohexital peak vs. the standard peak did not change. Instead, with protracted reaction times (> 30 min) extraneous peaks interfering with the desired ones began to appear.

The derivatives are stable in solution for at least a month at room temperature. Typical chromatograms are shown in Fig. 1.

On-column methylation with 0.2 *M* trimethylanilinium hydroxide in methanol [8] was also tried. The methyl derivative of hexobarbital was not stable, however, but decomposed in a way previously described for N,N-dimethylphenobarbital [9].

Mass spectra

The methane chemical ionization mass spectra confirmed the putative structures of the methohexital and hexobarbital PFB derivatives. The pseudomolecular ions $[M+H]^+$ appeared as expected at *m/z* 443 and 417, respectively. Fragmentation, in the form of cleavage of the PFB group from the barbiturate [10], was observed for the hexobarbital derivative only.

Standard curve, precision and sensitivity

Standard curves drawn on analysis of duplicate samples containing 0.125, 0.250, 0.500, 1.00, 2.00 and 4.00 μg/ml methohexital were linear (generally r = 0.977—0.999 on a four-point standard curve). With high sample concentrations, 8.00 or 16.00 μg/ml, the final solution of derivative had to be diluted four- to five-fold before injection. Otherwise these points would drop below the straight line. "Unknown" samples showing methohexital concentrations above 4 μg/ml should be re-run after similar dilution.

Analysis of eight samples spiked with 1.00 μg/ml methohexital (3.83 μM) gave a mean value of 1.005 μg/ml (3.84 μM) with a standard deviation of 0.065 μg/ml (0.25 μM), which gives a relative S.D. of 6.5%.

The lowest plasma methohexital concentration which can be satisfactorily quantitated is around 0.1 μg/ml (0.4 μM).

CONCLUSIONS

Several attempts have been made to determine barbiturates in biological samples by pentafluorobenzylation and ECD [6]. The derivatization of pentobarbital with pentafluorobenzyl bromide in aqueous sodium carbonate solution required prolonged heating of the reaction mixture [10]. Extractive alkylation of phenobarbital from saliva worked well but removal of excess reagent required the use of a pre-column venting system [11]. Pentobarbital and phenobarbital form di-pentafluorobenzyl derivatives. The N-methylated barbiturates methohexital and hexobarbital form mono derivatives and, in

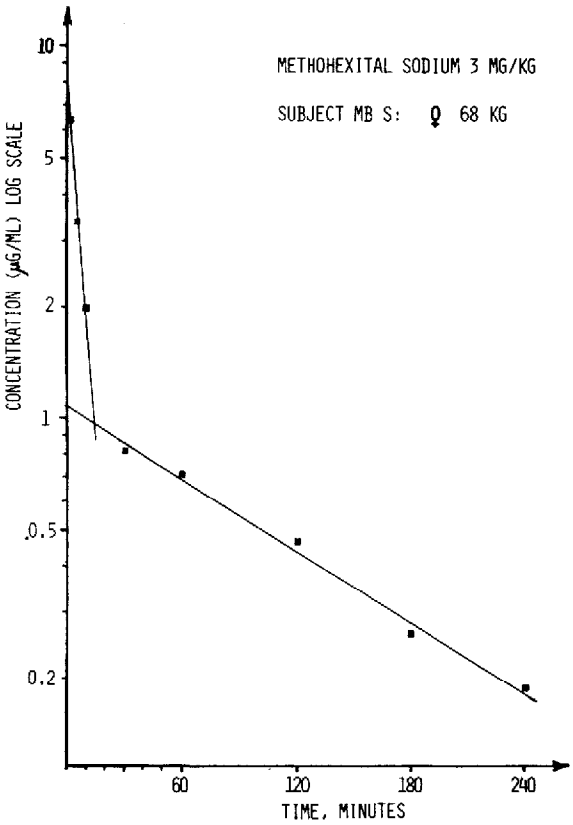

Fig. 2. Plasma methohexital concentration curve from a patient under methohexital—halothane—nitrous oxide anaesthesia. Three mg/kg methohexital sodium (Brietal), corresponding to 2.76 mg/kg free acid, was given as an intravenous bolus dose. The apparent volume of distribution (V_d = 2.6 l/kg) and the terminal half-life (β = 95 min) were calculated from the five-point regression line.

the triethylamine—methanol system, the reaction is easily driven to completion with a moderate excess of reagent. This makes our method feasible for multi-sample analysis and its usefulness in pharmacokinetic work is exemplified in Fig. 2.

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