Institute of Clinical Pharmacology, Medical Faculty, Otto-von-Guericke University, Magdeburg, Germany

Stability of thiopental and pentobarbital in human plasma determined with a new easy and specific gas chromatography-mass spectrometry assay

J. MARTENS-LOBENHOFFER

A gas chromatographic-mass spectrometric (GC-MS) assay for the determination of thiopental and its main metabolite pentobarbital in human plasma is presented in this study. The sample preparation consists only in the addition of the internal standard barbital and an acidic extraction with ethyl acetate. Analytical separation is accomplished on a RTX-1 15 m × 0.25 mm capillary column with a film thickness of 0.5 μm. The effluent is observed by a mass selective detector operating in the single ion monitoring mode. The limits of detection are 5 ng/ml for pentobarbital and 10 ng/ml for thiopental, the intra-day variabilities are 2.2% and 4.0% and the inter-day variabilities are 3.3% and 7.1% at concentrations of 5 μg/ml, respectively. Applying this assay, the stability of thiopental and pentobarbital in human plasma was tested at concentrations of 5 μg/ml each. Thiopental is stable in human plasma at least over 41 days stored at −20 °C and 5 °C, respectively. A decay of about 2%/day is observed under storage at ambient temperature (19–20 °C). Pentobarbital is stable under all storage conditions. Methanolic solutions of thiopental are stable for 83 days under storage at 5 °C. Aqueous solutions of thiopental-sodium are stable for at least 23 days under storage at 5 °C or ambient temperature.

1. Introduction

Thiopental is used as an ultrashort-acting intravenous anesthetic [1], for the treatment of seizures unresponsive to anticonvulsant therapy [2] and in patients with increased intracranial pressure [3]. Its main metabolite is pentobarbital, which also exhibits narcotic activity. For the diagnosis of brain death the determination of thiopental and pentobarbital plasma levels is imperative to exclude a barbiturate induced coma after thiopental administration [4]. There are several easy HPLC-methods for the determination of thiopental in human plasma [5–7], but the simultaneous determination of pentobarbital is either not very sensitive [8] or needs a post-column photochemical reactor to facilitate the sensitive detection of both compounds [9]. A simultaneous determination of thiopental and pentobarbital by GC is also described [10], but the method is not validated according to modern Good Laboratory Practice (GLP) guidelines. In this paper, we describe a GC-MS assay for thiopental and pentobarbital which is easy, selective and sensitive. Moreover, due to concerns about the stability of thiopental in solutions [5], we investigate the concentration-time curves of thiopental and pentobarbital in human plasma under different storage conditions and in methanolic and aqueous solutions.

2. Investigations, results and discussion

2.1. GC-MS assay for thiopental and pentobarbital

The assay for thiopental and pentobarbital is calibrated from 0.5 to 8 μg/ml to cover the therapeutic range of 1 to 5 μg/ml for both substances [10]. Samples from patients with increased intracranial pressure sometimes contain much higher concentrations and have therefore to be diluted with drug free plasma prior to analysis. To increase the precision of the assay, the internal standard barbital is added to the samples. The only sample preparation step is a simple acidic extraction with ethyl acetate. The extraction yield, determined by comparison of ethyl acetate solutions of the analytes according to a theoretical extraction yield of 100% with actual plasma extracts, is (n = 5) 98 ± 4.6% for pentobarbital and 78 ± 8.3% for thiopental, respectively. The extracts are injected without any further treatment into the GC-MS. The complete sample processing time is about 45 min from start to result. Typical chromatograms obtained with this procedure are depicted in Fig. 1. As it can be seen, no interferences arise from endogenous substances. The calibration curves are linear for pentobarbital (slope = 0.245, intercept = 9.73×10^{-3}, $r^2 = 0.997$) and thiopental (slope = 0.076, intercept = -9.72×10^{-3}, $r^2 = 0.996$). The intra-day relative standard deviation (n = 10) is 2.2% for pentobarbital and 4.0% for thiopental at the 5 μg/ml concentration level and 7.7% and 6.3% at the 0.5 μg/ml concentration level, respectively. The day-to-day relative standard deviation (n = 9) is 3.3% for pentobarbital and 7.1% for thiopental at the 5 μg/ml concentration level. The limits of detection, which were defined as a signal to noise ratio of 3, are 0.005 μg/ml for pentobarbital and 0.01 μg/ml for thiopental, respectively.

2.2. Stability of plasma samples containing thiopental and pentobarbital

In Fig. 2 the concentration time curves of pentobarbital and thiopental in human citrate plasma (concentration for both compounds: 5 μg/ml) under different storage conditions are depicted. Pentobarbital is stable under all conditions for at least 41 days. For thiopental, stability over this

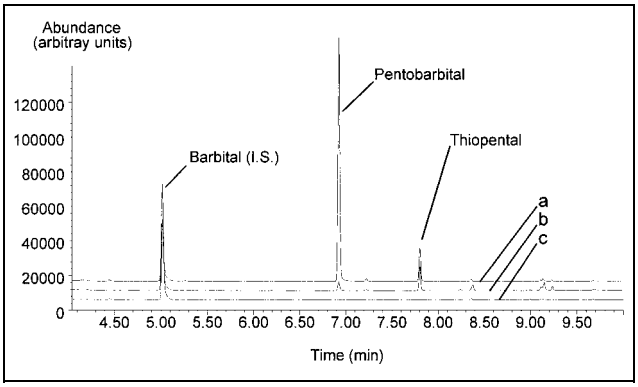

Fig. 1: Chromatograms obtained from human plasma. a) Calibration sample 8 μg/ml thiopental and pentobarbital, b) patient sample containing 5.5 μg/ml thiopental and 0.4 μg/ml pentobarbital (dose: 400 mg thiopental peripartal, 5 min post applicationem), c) blank control from drug free human plasma

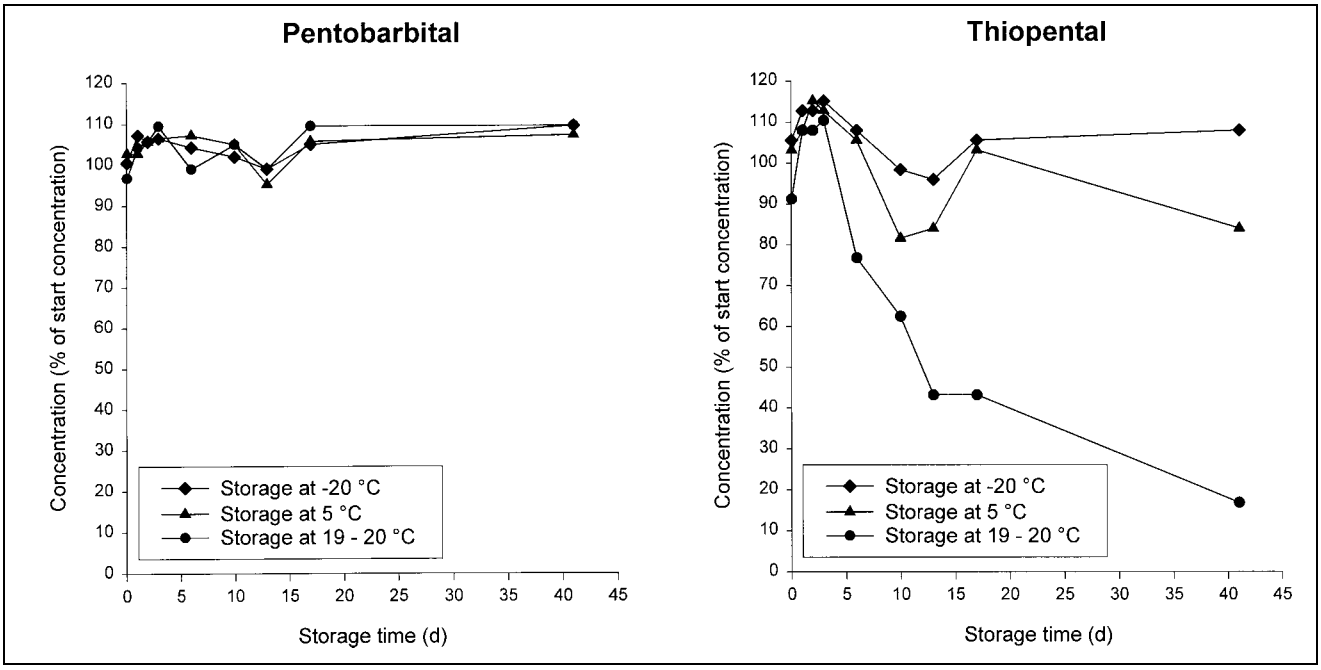

Fig. 2: Stability of pentobarbital and thiopental in human citrate plasma stored at −20 °C, at 5 °C and at ambient temperature

time is only achieved by storage at −20 °C. A trend towards loss in concentration can be seen at storage at 5 °C, but the differences are not significant. A significant concentration loss results from storage at 19–20 °C, where thiopental decomposes with a rate of about 2%/day. The larger scattering of the thiopental concentration measurements in comparison to pentobarbital is due to the higher day-to-day variability of the thiopental assay. The *in vitro* decomposition of thiopental does not lead to the formation of pentobarbital as suggested by Russo et al. [5], since the concentrations of pentobarbital in the samples remain constant. Furthermore, the decomposition rate we have found is much slower than the one reported [5], where 43% of the initial thiopental concentration is lost within 6 h at storage at 4 °C.

2.3. Stability of methanolic and aqueous solutions of thiopental

To assess the durability of the calibration solutions of thiopental used in this study, the concentrations of thiopental vs. time in methanolic solutions (50 µg/ml) under storage at 5 °C are measured. No decay of thiopental could be determined over a period of 83 days. This finding is in contrast to literature data [5], where the stability of thiopental in methanol at 4 °C is told to be no longer than 30 h. A similar test for the stability of solutions of thiopental-sodium salt in water over 23 days also showed no significant loss in concentration, whether the solutions are stored at ambient temperature or at 5 °C. These findings confirm the results published by Chernin and Stewart and Haws et al. [11, 12], where thiopental-sodium solutions in water are found to be stable for at least 10 respectively 6 days at room temperature and for 13 respectively more than 7 days at 4 °C.

2.4. Conclusion

The method for the determination of thiopental and its main metabolite pentobarbital in human plasma presented here is easy, sensitive and precise. It has been used in a number of cases to support the differential diagnosis of brain death in patients treated in the intensive care unit of our hospital and by a number of patients enroled in a pharmacokinetic study currently carried out in our institution. No interferences from endogenous substances or comedication have ever been observed. The stability study carried out for thiopental in human plasma have shown that special care for the samples during the transport and the sample preparation is not needed.

3. Experimental

3.1. Reagents

Thiopental, pentobarbital and barbital were purchased from Sigma (Deisenhofen, Germany), thiopental-sodium salt (Trapanal®) is produced by Byk Gulden (Konstanz, Germany). Sodium hydroxide, hydrochlorid acid (both quality grade p. A.) and methanol (gradient grade) were purchased from Merck (Darmstadt, Germany) and ethyl acetate (reagent grade) from Baker (Griesheim, Germany), respectively. Drug free human citrate plasma was a gift from the Institute of Transfusion Medicine and Immunehematology of the University Hospital Magdeburg, Germany.

3.2. Apparatus and chromatographic conditions

The GC-MS assay was performed on a Hewlett Packard HP5890 II with autosampler HP7673 and mass selective detector HP5972 (Waldbronn, Germany). The analytical column was a Restec RTX-1 15 m × 0.25 mm ID × 0.5 µm film thickness (Bad Soden, Germany).
From the ethyl acetate extract, 1 µl was injected in the splitless mode at an injector temperature of 300 °C, the split valve was opened after 1 min applying a split flow of 20 ml/min. Carrier gas was helium with a flow rate of 1 ml/min constant throughout the oven temperature program which was 100 °C constant for 1 min, then 15 °C/min up to 200 °C, 30 °C/min up to 290 °C and a final constant hold for 1 min. The MS-detector worked in the single ion monitoring mode observing the ion traces 156, 173, 141, 197 and 242 m/z. The first two ion traces served for the quantitation, the others as peak qualifiers.

3.3. Sample preparation

3.3.1. Calibration samples

Precisely measured volumes of a solution of thiopental and pentobarbital in methanol (5 mg/100 ml of each compound) were evaporated in 10 ml glas test tubes and reconstituted with 50 µl 1 M NaOH. Drug free human plasma (1 ml) was added to each sample.

3.3.2. Extraction procedure

In a 10 ml glas test tube, 50 µl 1 M NaOH (to achieve the same conditions as in the calibration samples) and 1 ml of a patient plasma sample were mixed. To each of the patient- and calibration samples 50 µl of the internal standard solution (10 mg/ml barbital in methanol) and 100 µl 1 M HCl were added. The mixtures were extracted with 3 ml ethyl acetate by shaking for 25 min. From the resulting extracts, about 1 ml was transferred into autosampler vials for GC-MS analysis.

3.4. Stability testing of plasma samples

Drug free human citrate plasma was spiked with thiopental and pentobarbital to achieve concentrations of 5 µg/ml for each compound and was divided into 1 ml portions. One set of these plasma samples was stored at ambient temperature (19–20 °C), one set in a refrigerator at 5 °C and one set was frozen at −20 °C. Over a range of 41 days, samples of the different sets were analyzed for their concentrations of thiopental and pentobarbital.

3.5. Stability testing of methanolic and aqueous solutions of thiopental

Calibration solutions of thiopental in methanol with concentrations of 5 mg/100 ml were stored for 0, 25, 63 and 83 days at 5 °C. With this calibration solutions plasma was spiked to generate samples containing a nominal concentrations of 5 µg/ml thiopental. Five samples from each calibration solution were measured for their concentration of thiopental. In a similar manner solutions of thiopental-sodium in water (5 mg/100 ml) were stored at 5 °C and at ambient temperature (19–20 °C) for 0, 18 and 23 days with subsequent analysis for their actual concentrations.

References

1 Yamamoto, L. G.; Yim, G. K.; Britten, A. G.: Pediatr. Emerg. Care **6**, 200 (1990)
2 Roberts, M. R.; Eng-Bourquin, J.: Emerg. Med. Clin. North Am. **13**, 489 (1995)
3 Schalen, W.; Messeter, K.; Nordstrom, C. H.: Acta Anaestesiol. Scand. **36**, 369 (1992)
4 Grattan-Smith, P. J.; Butt, W.: Arch. Dis. Child. **69**, 151 (1993)
5 Russo, H.; Allaz, J. L.; Bressolle, F.: J. Chromatogr. B Biomed. Sci. Appl. **694**, 239 (1997)
6 Altmayer, P.; Buch, U.; Buch, H. P.: Methods Find. Exp. Clin. Pharmacol. **9**, 817 (1987)
7 Gruhl, H.; Mayer, H.: J. Clin. Chem. Clin. Biochem. **22**, 385 (1984)
8 Kelner, M.; Bailey, D. N.: Clin. Chem. **29**, 1097 (1983)
9 Schmid, R. W.; Wolf, C.: J. Pharm. Biomed. Anal. **7**, 1749 (1989)
10 Meyer, F. P.: Int. J. Clin. Pharmacol. Ther. **32**, 71 (1994)
11 Chernin, E. L.; Stewart, J. T.; Smiler, B.: Am. J. Health Syst. Pharm. **53**, 1576 (1996)
12 Haws, J. L.; Herman, N.; Chlark, Y.; Bjoraker, R.; Jones, D.: Anesth. Analg. **86**, 208 (1998)

Received November 6, 1998
Accepted December 28, 1998

Dr. J. Martens-Lobenhoffer
Institut für Klinische Pharmakologie
Otto-von-Guericke Universität
Leipziger Str. 44
D-39120 Magdeburg