*Journal of Chromatography*, 258 (1983) 267–270
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 15,528

## Note

# Analysis of barbiturates in plasma and urine using gas chromatography without prior derivatization

TOMAS VILLÉN* and INGA PETTERS
*Department of Clinical Pharmacology, Huddinge Hospital, S-141 86 Huddinge (Sweden)*
(First received September 6th, 1982; revised manuscript received November 17th, 1982)

The identification and quantification of barbiturates are frequently used to diagnose overdoses in patients in emergency wards. Barbiturates are also commonly abused and there is a need for a rapid qualitative method for barbiturate screening.

Commercially available immunological methods give no information about concentrations, and the detection levels differ from one barbiturate to another. Endogenous substances may also interact in these methods and a positive result demands identification and quantification by a more specific method.

Specific methods for barbiturate analysis have mostly been based on gas chromatography (GC) with flame ionization detection (FID) and packed columns or support-coated open tubular columns (SCOT)[1–3]. In these methods the barbiturates have to be derivatized, *e.g.* 1,3-diethyl or 1,3-dimethyl derivatives, to avoid tailing peaks and to achieve good separation. A review of GC methods for barbiturate analysis was recently presented[4].

The use of fused silica capillary columns makes it possible to achieve good chromatographic behaviour of the barbiturates without derivatization. By employing splitless injection and nitrogen–phosphorus sensitive detection (NPD), the sensitivity was sufficient to detect barbiturate concentrations less than 2 μmol/l, as required when analysing barbiturates in urine.

## EXPERIMENTAL

### *Apparatus*

A Hewlett-Packard 5800 gas chromatograph equipped with a splitless injection system, a multilevel temperature programming system and an NPD was used. The column was a 12-m fused silica capillary column deactivated with Carbowax 20M®, and with methyl silicone fluid as stationary phase (Part no. 19091-60010; Hewlett Packard, Palo Alto, CA, U.S.A.).

### *Chromatographic conditions*

Helium was used as carrier gas at a flow-rate of 0.8 ml/min. The injection port temperature was 200°C and the detector temperature was 300°C. The time between injection and purge activation of the injection system was 30 sec. The power applied to the detector bead was adjusted to give a baseline response of 10–20 pA. The

0021-9673/83/0000–0000/$03.00 

hydrogen and air flow-rates were 3 and 100 ml/min, respectively; that of the make-up gas (helium) was 25 ml/min. The oven temperature was held for 1 min at 100°C, programmed at a rate of 20°C/min to 155°C, isothermal for 2 min, programmed at a rate of 20°C/min from 155°C to 240°C and finally isothermal for 1 min at 240°C. Due to the smaller response of the later peaks the attenuation was increased 8.3 min after injection.

*Chemicals and reagents*

All chemicals were of analytical grade. One-ml glass tubes (5 mm I.D.) were used for plasma extraction and were purchased from Svenska AB Philips (sample vials for an autoinjector, Part no. 299493; Svenska Philips, Stockholm, Sweden).

*Analytical methods*

A 400-$\mu$l plasma sample was mixed with 100 $\mu$l of a saturated solution of $KH_2PO_4$ (pH 4.4) and 200 $\mu$l of diisopropyl ether containing 4 $\mu$g of methylphenobarbital and 8 $\mu$g of 5-allyl-5-phenylbarbituric acid (internal standards, peaks 12 and 17, Fig. 2). This mixture was shaken for 10 min and after centrifugation 0.8 $\mu$l of the organic phase were injected into the gas chromatograph. Standards were prepared by adding known amounts of barbiturates to plasma from a drug-free

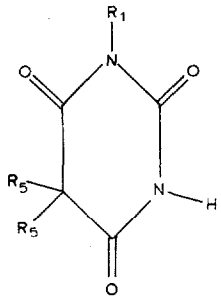


Fig. 1.

| | | $R_5$ | $R_1$ |
|---|---|---|---|
| 1 | Metharbital | diethyl | methyl |
| 2 | Barbital | diethyl | hydrogen |
| 3 | Aprobarbital | isopropyl, 2-propenyl | hydrogen |
| 4 | Allobarbital | di-2-propenyl | hydrogen |
| 5 | Butethal | butyl, ethyl | hydrogen |
| 6 | Butalbital | isobutyl, 2-propenyl | hydrogen |
| 7 | Amobarbital | ethyl, isopentyl | hydrogen |
| 8 | Pentobarbital | ethyl, isopentyl | hydrogen |
| 9 | Hexobarbital | 1-cyclohexenyl, methyl | methyl |
| 10 | Vinbarbital | ethyl, 1-methyl-1-butenyl | hydrogen |
| 11 | Secobarbital | 1-methylbutyl, 2-propenyl | hydrogen |
| 12 | Methylphenobarbital | ethyl, phenyl | methyl |
| 13 | Brallobarbital | 2-bromo-2-propenyl, 2-propenyl | hydrogen |
| 14 | Cyclobarbital | 1-cyclohexenyl, ethyl | hydrogen |
| 15 | Phenobarbital | ethyl, phenyl | hydrogen |
| 16 | Heptabarb | 1-cycloheptenyl, ethyl | hydrogen |
| 17 | 5-Allyl-5-phenyl-barbituric acid | phenyl, 2-propenyl | hydrogen |

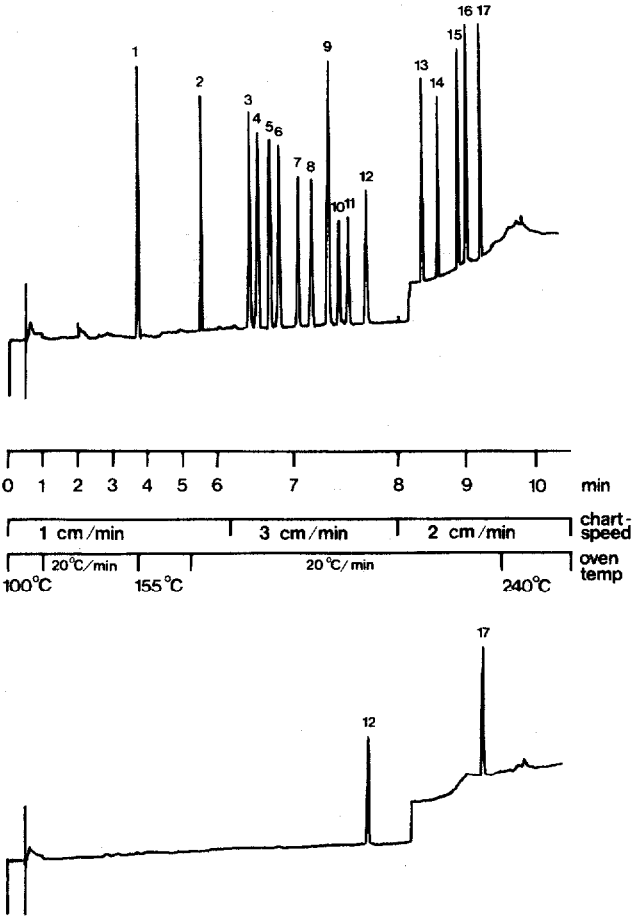


Fig. 2. Chromatogram (upper) from a human plasma sample spiked with approximately 100 μmol/l of all barbiturates, and (lower) from a human plasma sample to which only the two internal standards had been added.

healthy human subject. Concentrations were calculated by comparing the peak height ratio between the drug and one of the internal standards in the unknown sample and in the standard.

Urine (5 ml) was analysed after addition of 1 ml of a saturated solution of $KH_2PO_4$ and 500 μl of diisopropyl ether containing 10 μg and 20 μg of the internal standards respectively. This mixture was shaken for 10 min and after centrifugation 1.0 μl of the organic phase was injected into the gas chromatograph. A standard containing 10 μmol/l each of the barbiturates in water was run along with the samples. The urine analysis was used only for identification of barbiturates and a concentration higher than 2.5 μmol/l was regarded as positive.

## RESULTS AND DISCUSSION

The barbiturates were characterized by their retention times, which showed a

very small within-day variation. For positive identification the retention times were not allowed to differ by more than 0.01 min from a standard injected the same day.

Plasma samples from patients who had received carbamazepine, amitriptyline, phenytoin or theophylline were tested and found not to give any peak interfering with the barbiturates. A small peak which occurs in almost all plasma samples had a slightly longer retention time than butalbital (Fig. 2, peak 6). The retention time of this peak is similar to that of caffeine which has a high response on the NPD but is poorly extracted into diisopropyl ether.

Barbiturates are poorly extracted into non-polar solvents such as hexane. Caffeine gives a huge peak interfering with butalbital when chloroform is used. When using diisopropyl ether the extraction of barbiturates is 75–100% in the phase proportions described. Diisopropyl ether is, however, not ideal for the splitless injection technique when the initial oven temperature is as high as 100°C. This affects the shape of the earliest eluting peaks in the chromatogram. Substances with higher boiling points seem to be cold-trapped (condensed) in the column to a larger extent. Use of a lower initial oven temperature would result in a considerably longer analysis time.

Standard curves were constructed using the two internal standards methylphenobarbital and 5-allyl-5-phenylbarbituric acid for barbiturates 1–11 and 13–16, respectively. The range of the standard curve was 20–100 μmol/l and the correlation coefficient varied from 0.96 (metharbital and barbital) to 0.998 (hexobarbital and secobarbital).

We have used this method for more than 1 year for determination of barbiturates in plasma from intoxicated patients admitted to the hospital and in urines from patients in drug addiction wards. All samples analysed were first found positive with enzyme immunoassay or radioimmunoassay. The only problem we have had is that the retention times decrease by 1–2 sec from one week to another. This makes it necessary to change the oven temperature program every other month in order to separate all peaks. Usually after more than 6 months this is no longer possible and the column has to be exchanged.

## REFERENCES

1 E. Brochmann-Hanssen and T. O. Oke, *J. Pharm. Sci.*, 58 (1969) 370–371.
2 M. Garle and I. Petters, *J. Chromatogr.*, 140 (1977) 165–169.
3 B. Kinberger, A. Holmén and P. Wahrgren, *J. Chromatogr.*, 224 (1981) 449–455.
4 D. N. Pillai and S. Dilli, *J. Chromatogr.*, 220 (1981) 253–274.