CHROMBIO. 1168

Note

Micromethod for automated identification and quantitation of fifteen barbiturates in plasma by gas—liquid chromatography

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(First received July 1st, 1981; revised manuscript received November 9th, 1981)

A great number of methods for the determination of barbiturates in blood have been published the last years, essentially by gas—liquid chromatography (GLC) [1—8]. We ourselves have described the measurement of fifteen barbiturates in biological fluids using two columns of different polarity [10, 11].

Taking advantage of some new improvements in GLC such as specific detection by nitrogen—phosphorus detector, fused-silica capillary column, automation and data computer, we present an optimized technique for the identification and quantitation of fifteen barbiturates whose structures are given in Table I.

EXPERIMENTAL

Reagents and chemicals

The following reagents were used: methanol—water solution (90:10, v/v) saturated with potassium carbonate; dimethylsulfate (Normapur; Prolabo, Paris, France); hexane (Normapur; Prolabo); sodium acetate buffer, 0.2 M, pH 6.0.

Methohexital (Lilly, Basingstoke, Great Britain), was used as internal standard, 0.1 mg/ml in water. Stock barbiturate solutions are 1 mg/ml in methanol. The working standard is a drug-free plasma spiked with the most commonly encountered barbiturates (amobarbital, pentobarbital and seco-barbital at 12.5 mg/l, phenobarbital at 25 mg/l).

TABLE I

BARBITURATE FORMULA, RETENTION TIME AND RELATIVE RETENTION INDEX
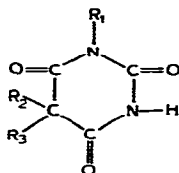
No.	Name	R_1	R_2	R_3	Retention time (min)	Relative retention index
1	Barbital	H	Ethyl	Ethyl	4.72	0.385
2	Butalbital	H	Allyl	1-Methylpropyl	7.28	0.594
3	Butobarbital	H	Ethyl	Butyl	7.47	0.610
4	Amobarbital	H	Ethyl	3-Methylbutyl	8.70	0.710
5	Pentobarbital	H	Ethyl	1-Methylbutyl	9.54	0.779
6	Vinylbital	H	Vinyl	1-Methylbutyl	9.71	0.793
7	Vinbarbital	H	Ethyl	1-Methyl-1-butenyl	10.38	0.847
8	Secobarbital	H	Allyl	1-Methylbutyl	10.80	0.882
9	Brallobarbital	H	Allyl	2-Bromoallyl	11.79	0.962
10	Methohexital	CH_3	Allyl	1-Methyl-2-pentynyl	12.25	1.000
11	Hexobarbital	CH_3	Methyl	1-Cyclohexen-1-yl	14.14	1.154
12	Phenobarbital	H	Ethyl	Phenyl	14.84	1.211
13	Cyclobarbital	H	Ethyl	1-Cyclohexene-1-yl	15.14	1.236
14	Heptabarbital	H	Ethyl	1-Cyclohepten-1-yl	16.78	1.370
15	Reposal	H	Ethyl	Bicyclo[3.2.1.]oct-2-en-3-yl	18.07	1.475

Apparatus

An HP 5880 gas chromatograph (Hewlett-Packard, Les Ulis, France) equipped with an HP 7671 automatic sampler and nitrogen—phosphorus detector was used. Qualitative and quantitative data were given by an HP 5880 GC terminal (level four).

The column was a flexible fused-silica capillary column (12 m × 0.2 mm I.D.) coated with dimethylsilicone fluid (SP 2100, Hewlett-Packard).

Operating conditions were: injection port temperature in splitless conformation, 300°C; detector temperature, 300°C; oven temperature programmed from 50°C (initial time 0.5 min) to 140°C (final time 5 min) at 30°C/min and then from 140°C to 190°C (final time 5 min) at 5°C/min; carrier gas (helium), 1.5 ml/min.

Procedure

To 100 μl of plasma in polypropylene Eppendorf microtubes (Roucaire, Paris, France) were added 25 μl of the internal standard solution, 250 μl of the methanolic solution and 10 μl of dimethylsulfate. The stoppered tubes were shaken and placed in a water-bath at 50°C for 5 min. Then, 250 μl of sodium acetate buffer and 600 μl of hexane were added and mixed for 30 sec. After brief centrifugation, the hexane layer was transferred to automatic sampler vials and 1 μl was injected.

Quantitation

Barbiturate determination was carried out using methohexital as internal standard. Calibration curves were obtained by spiking plasma with variable

amounts of stock solutions (5, 10, 20, 30, 60 mg/l), and a constant amount of internal standard (25 mg/l). Two working standards were run with each set of analyses. Quantitation was done by the peak area ratio method.

Within-run variation was determined for two concentrations with ten spiked plasma samples. Day-to-day precision was studied using working standards.

RESULTS AND DISCUSSION

The retention times of the fifteen barbiturates and typical chromatograms of standard, blank and patient samples are shown, respectively, in Table I and Figs. 1—3. The linearity of the method is very good within the concentration range encountered in plasma and up to 100 mg/l, with correlation coefficients of 0.9995 or more. Within-run variation and day-to-day reproducibility are presented in Table II. For levels as low as 1.25 mg/l for amobarbital, pentobarbital, secobarbital and 2.5 mg/l for phenobarbital, the coefficient of variation is below 5%. Concentrations of 0.5 mg/l can be easily detected, but minor concentrations could be measured after solvent concentration.

The specificity of the method has been widely tested in this laboratory. More than 350 plasma samples from patients suspected of intoxication have been analyzed simultaneously by this method and by another method which permits identification and quantitation of about 100 drugs acting on the central nervous system [9]. No interference has been encountered.

This method presents many advantages compared to the technique previously described [10, 11], which required the use of two packed columns of different polarity (SE-30 and NPGA) and 3 ml of blood. Here, the separation of
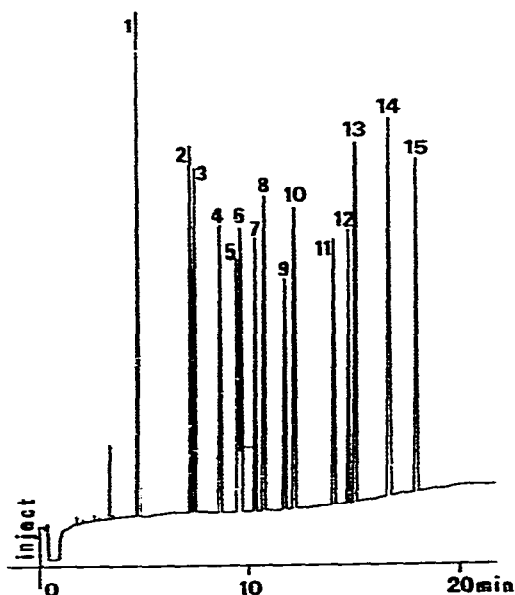

Fig. 1. Separation of a mixture of pure barbiturates. Peak numbers correspond to those of the compounds in Table I.
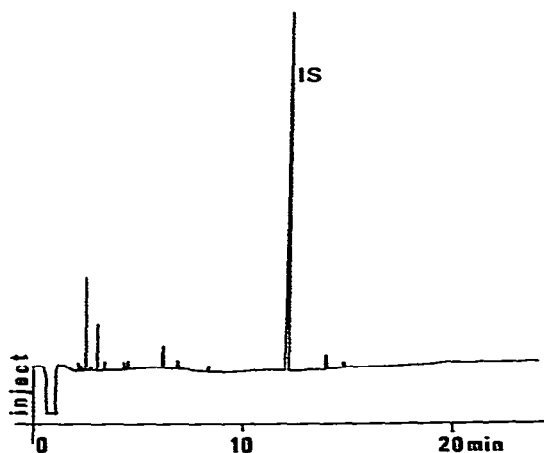
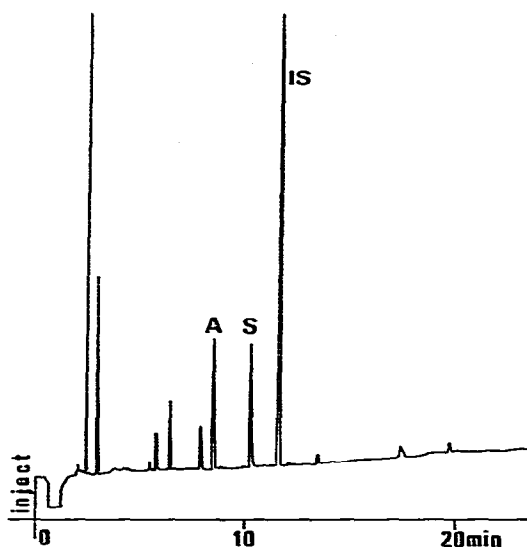Fig. 2. Chromatogram of blank plasma. IS = internal standard.

Fig. 3. Chromatogram of patient plasma containing amobarbital (A) (5.1 mg/l) and seco-barbital (S) (4.6 mg/l).

TABLE II

WITHIN-RUN AND DAY-TO-DAY VARIABILITY OF THE METHOD

	Within-run			Day-to-day		
	Amount added (mg/l)	Amount found ($\bar{x} \pm$ S.D., $n = 10$)	C.V.* (%)	Amount added (mg/l)	Amount found ($\bar{x} \pm$ S.D., $n = 34$)	C.V.* (%)
Amobarbital	1.25	1.29 ± 0.04	3.1	12.50	12.55 ± 0.62	4.9
	10.00	9.81 ± 0.17	1.7			
Pentobarbital	1.25	1.29 ± 0.04	3.1	12.50	12.47 ± 0.62	4.9
	10.00	9.69 ± 0.19	2.0			
Secobarbital	1.25	1.29 ± 0.04	3.1	12.50	12.48 ± 0.38	3.0
	10.00	9.70 ± 0.15	1.6			
Phenobarbital	2.50	2.69 ± 0.12	4.5	25.00	25.10 ± 0.91	3.6
	20.00	20.73 ± 0.40	2.0			

*C.V. = coefficient of variation = (S.D./$\bar{x}$) × 100.

the fifteen barbiturates studied is performed on one flexible fused-silica capillary column which also presents the advantage of being less fragile and easier to connect to the injector and detector fittings than conventional glass capillary columns. This point is not negligible when routine analysis is concerned. More than 1000 analyses have been performed with only minor loss of resolution. The nitrogen—phosphorus detector is more specific and sensitive than a flame ionization detector, so a more simple purification procedure with only 100 μl of plasma is possible. As a matter of fact, methyla-tion can occur directly in plasma or in other biological fluids without any prior purification, and hexane extraction is performed in the same tube without

further evaporation. The analysis time for one sample including preparation of plasma and chromatographic run is only 30 min, which is very interesting especially in the case of intoxication. Moreover, the rapidity and simplicity of sample preparation permit the rapid preparation of large series of samples during therapeutic control and to perform the chromatographic analysis overnight using the automatic sampler. The data system gives, after each run, a report including the name and concentration of any of the fifteen barbiturates present in the plasma and also the name of the patient.

In conclusion, this technique, involving a small quantity of plasma, minimal sample pretreatment and automated gas chromatography, is highly suitable for routine analysis.

REFERENCES

1   J. MacGee, Clin. Chem., 17 (1971) 587.
2   E.A. Fiereck and N.W. Tietz, Clin. Chem., 17 (1971) 1024.
3   H.V. Street, Clin. Chim. Acta, 34 (1971) 357.
4   R.G. Cooper, M.S. Greaves and G. Owen, Clin. Chem., 18 (1972) 1343.
5   A. Brachet-Liermain, L. Ferrus, Y. Clerc and D. Michon, Ann. Biol. Clin., 30 (1972) 243.
6   D.J. Berry, J. Chromatogr., 86 (1973) 89.
7   T. Walle, J. Chromatogr., 114 (1975) 345.
8   J.F. Menez, F. Berthou, D. Picart, L. Bardou and H.H. Floch, J. Chromatogr., 129 (1976) 155.
9   A. Cailleux, A. Turcant, A. Premel-Cabic and P. Allain, J. Chromatogr. Sci., 19 (1981) 163.
10 A. Premel-Cabic and P. Allain, Therapie, 28 (1973) 951.
11 A. Premel-Cabic and P. Allain, Therapie, 28 (1973) 969.