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DETERMINATION OF BARBITURATES AND SOME NEUTRAL DRUGS IN SERUM USING QUARTZ GLASS CAPILLARY GAS CHROMATOGRAPHY

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SUMMARY

Rapid methods for the glass capillary gas chromatographic determination of barbiturates and some neutral drugs are described. The analysis of barbiturates was performed using a nitrogen—phosphorus selective detector (NPD). The barbiturates were recovered from serum using charcoal adsorption followed by extraction with methylene chloride. The drugs were then alkylated by means of the Claisen carbonate method. Neutral drugs were extracted simultaneously with the barbiturates. The neutral drugs were determined underivatized with a flame ionization detector. In the underivatized form the barbiturates were not stable on the quartz column used. The selectivity of derivatization combined with an NPD was used to determine the barbiturates in the presence of neutral drugs with the aid of retention data.

INTRODUCTION

Barbiturate analysis by glass capillary gas chromatography (GC/GC) has been described by Garle and Petters [1]. An extractive alkylation procedure was used to recover barbiturates and glutethimide selectively from other types of drugs. A sensitive GC/GC determination of barbiturates was reported by Dünges et al. [2], who claimed that barbiturates could be identified using the Claisen carbonate method to prepare various derivatives of the same compound. A rapid procedure for the extraction of drugs from serum was studied by Meola [3]. Barbiturates and other drugs were adsorbed on activated charcoal, from which they were extracted with a solvent mixture. After evaporation of the organic phase, the residue was dissolved in a small volume of ethyl acetate and the solution was injected directly into a gas chromatograph equipped with packed columns. This method is very simple.

Preliminary studies on underivatized barbiturates using commercially available deactivated quartz glass capillary columns showed serious adsorption/

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catalysis on the glass surface, resulting in poor sensitivity. Sandra et al. [4] separated underivatized barbiturates successfully on a persilylated glass capillary column coated with OV-1. However, free barbiturates give low responses but alkylated barbiturates give high responses if a nitrogen-sensitive detector was used. Therefore, the derivatization method proposed by Dünges et al. [2] was combined with quartz GC/GC and the nitrogen—phosphorus selective detector (NPD) was applied.

Serum samples were investigated using a minor modification of the rapid extraction procedure of Meola [3] followed by the alkylation procedure reported by Dünges et al. [2].

EXPERIMENTAL

Apparatus

One Packard 427 gas chromatograph with an NPD and one Packard 427 gas chromatograph with a flame ionization detector (FID), both equipped with split injectors, were used. The gas chromatographs were connected to Sigma 10 Chromatography Data Station (Perkin-Elmer, Norwalk, CT, U.S.A.). Fused silica capillary columns (25 m × 0.20—0.21 mm I.D.), deactivated with Carbowax 20M, and with SP-2100 as the stationary phase, part number 19091-60025 (Hewlett-Packard, Avondale, PA, U.S.A.), were applied. The GC/GC conditions were as follows: injection port temperature, 230°C; oven temperature, programmed from 110 to 230°C at 10°C/min and then kept isothermally at 230°C for 5 min. Nitrogen was employed both as a carrier gas at a flow-rate of 0.8 ml/min and as the make-up gas at a flow-rate of 10 ml/min. The splitting ratio was 1:10.

Reagents

The reagents used were of analytical-reagent grade, unless specified otherwise: acetone (Merck, Darmstadt, G.F.R.); activated charcoal (Riedel-de Haën, Hannover, G.F.R.); methylene chloride (Baker, Phillipsburg, NJ, U.S.A.) (HPLC grade); ethyl iodide (iodoethane) (BDH, Poole, Great Britain); potassium carbonate, anhydrous.

Serum standards, barbiturates

To drug-free serum were added the barbiturates metharbital, barbital, aprobarbital, butalbital, amobarbital, pentobarbital, vinbarbital, secobarbital, hexobarbital, phenobarbital, cyclobarbital and heptabarbital, all in the range 5—40 μg/ml.

Serum standard, neutral drugs

To drug-free serum were added hexapropymate (30 μg/ml), methyprylon (10 μg/ml), persedon (10 μg/ml), phenazone (5 μg/ml), methaqualone (10 μg/ml) and meprobamate (10 μg/ml).

Internal standard solution for the determination of barbiturates

A 10-mg amount of allobarbital was dissolved in 10 ml of ethanol.

Extractant

Methylene chloride containing 7.5 μg/ml of eicosane ($C_{20}H_{42}$) (eicosane was used as an internal standard for the determination of underivatized neutral drugs) was employed.

Procedure

To 500 μl of serum were added approximately 10 mg of activated charcoal with the aid of a calibrated spatula, then 10 μl of the allobarbital internal standard solution were added (if underivatized neutral drugs were of prime interest, addition of this solution was omitted) and the mixture was vortexed for 30 sec. After centrifugation for 2 min at 2500 *g* the aqueous phase was aspirated off and discarded. The charcoal precipitate was evenly distributed in the bottom of the extraction tube by vortexing. The drugs were then extracted with 300 μl of methylene chloride containing eicosane by vortexing for 1 min. After centrifugation for 2 min at 2500 *g* the organic phase was transferred into a conical mini-vial (volume approximately 400 μl) and evaporated to dryness using a gentle stream of air.

Screening of neutral drugs, GC/GC—FID

The residue was dissolved in 10 μl of acetone and 1 μl of the extract was injected into a gas chromatograph equipped with an FID.

Alkylated barbiturates, GC/GC—NPD

An additional 30 μl of acetone, 10 μl of ethyl iodide and a few grains of potassium carbonate were added to the mini-vial, which was sealed, vortexed and placed in an oven at 80°C for 30 min. Then 0.5 μl of the extract was injected into the gas chromatograph equipped with an NPD.

RESULTS

Fig. 1 (left chromatogram) shows the chromatographic separation of thirteen barbiturates, including the internal standard allobarbital, using the NPD. Retention times were very stable (± 1.2 sec). No major interfering peaks of endogenic origin were found in the serum extracts. Fig. 1 (right chromatogram) shows a serum blank. The GC/GC calibration graphs (peak area ratios to that of the internal standard, allobarbital, versus barbiturate concentration) were linear in the range 5—40 μg/ml for all of the barbiturates studied. All of the correlation coefficients were greater than 0.98. The detection limits were approximately 1 μg/ml or better, except for that of phenobarbital (2 μg/ml). Table I gives the precision data obtained by replicate analyses of sera to which all of the barbiturates had been added (data for three levels are given). Retention times are given in Table II.

As pointed out, many other drugs are co-extracted with the barbiturates. Some of the drugs may be analysed by GC/GC and detected underivatized with the FID. Fig. 2 (upper chromatogram) shows results for neutral drugs analysed in this way. Eicosane was used as the internal standard for the FID procedure. Eicosane was not detected by the NPD and thus did not interfere with the determination of barbiturates. Fig. 2 (lower chromatogram)

shows a serum blank obtained with the FID procedure. The GC/GC calibration graphs were linear in the ranges shown in Table III. The precision data were comparable to those obtained in the analysis of barbiturates (see Table I).

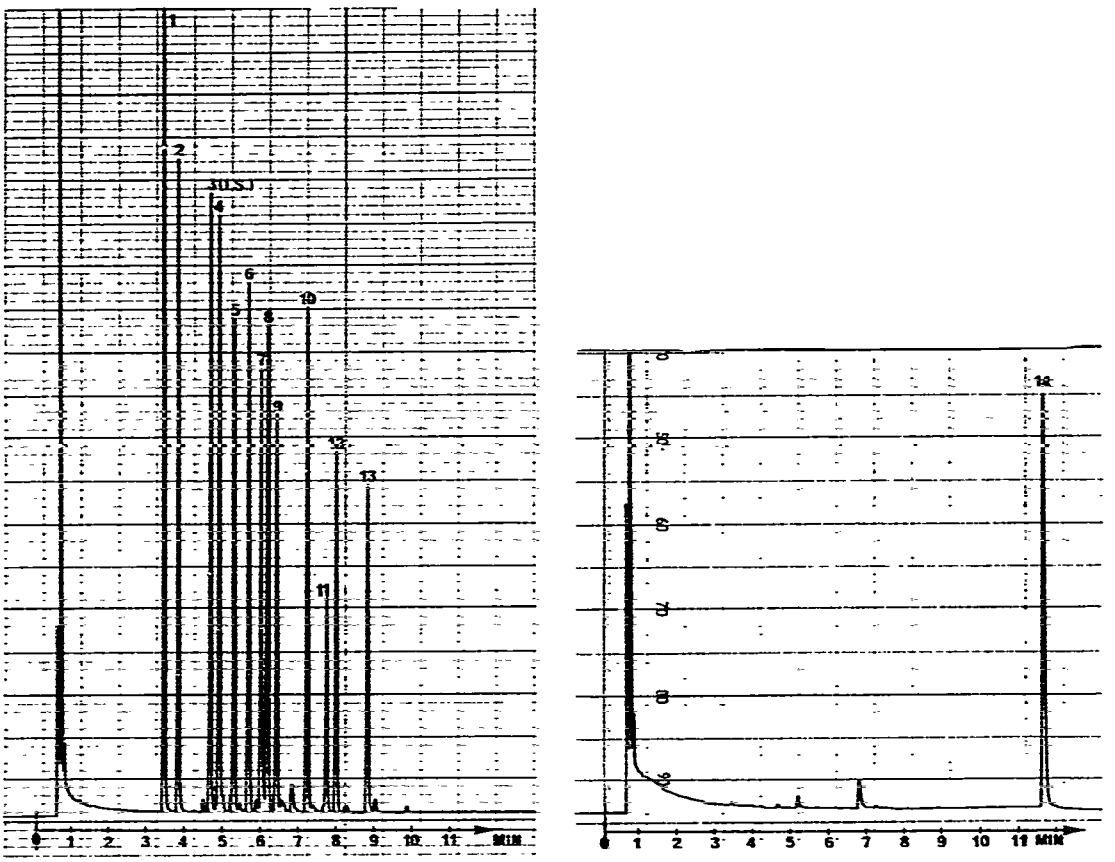

Fig. 1. The left chromatogram shows the results of an analysis of a serum barbiturate standard (concentration of each barbiturate = 20 μg/ml) obtained by the GC/GC—NPD procedure. Peaks: 1 = metharbital; 2 = barbital; 3 = allobarbital (internal standard); 4 = aprobarbital; 5 = butalbital; 6 = amobarbital; 7 = pentobarbital; 8 = vinbarbital; 9 = secobarbital; 10 = hexobarbital; 11 = phenobarbital; 12 = cyclobarbital; 13 = heptabarbital. Retention times are given in Table II. The right chromatogram shows the results for a serum blank (GC/GC—NPD procedure) without addition of internal standard (allobarbital). Peak 14 = unknown peak that is often found in the serum extracts.

DISCUSSION

The rapid extraction procedure chosen in this study is not selective for barbiturates. However, speed and simplicity of the sample preparation methods are great assets in routine clinical chemistry work. Further, when analysing serum samples from intoxicated patients valuable information may be lost if the extraction technique is selective for a group of drugs. Many methods

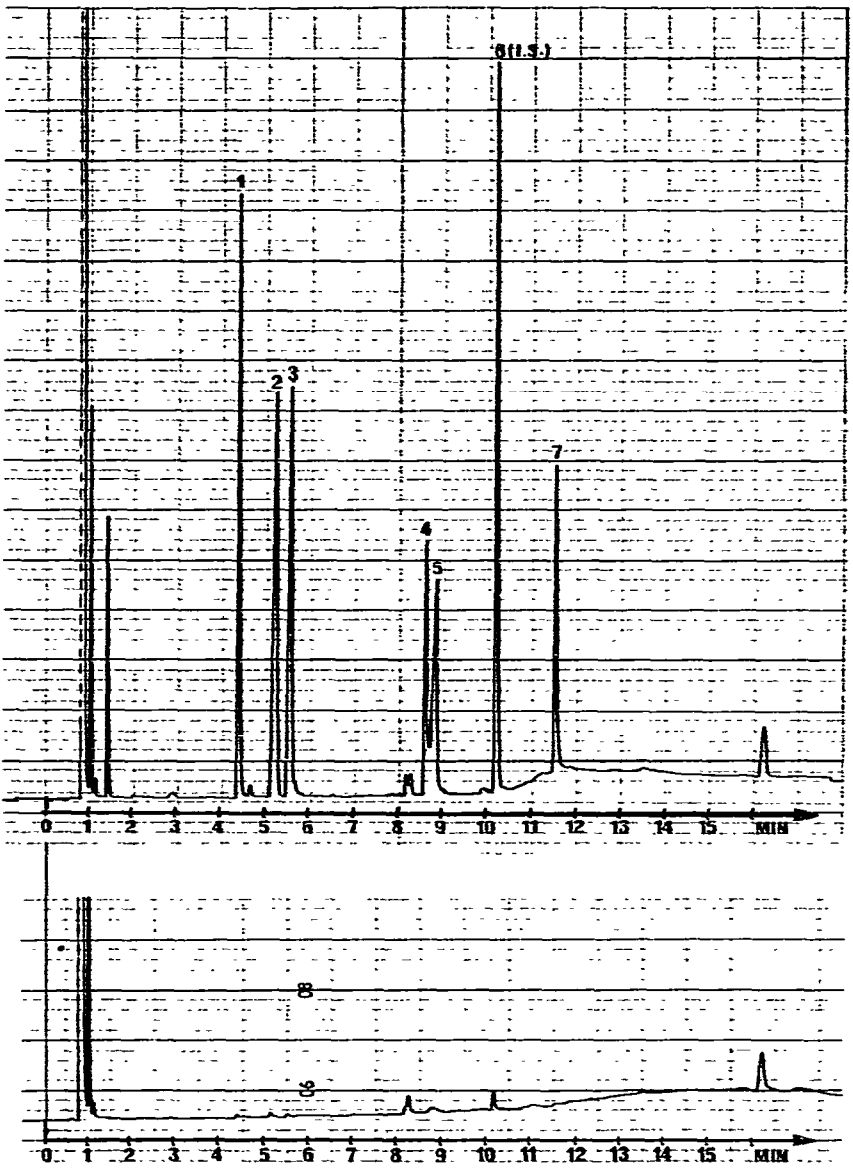

Fig. 2. The upper chromatogram shows the results of an analysis of a "serum neutral drug standard" obtained by the GC/GC—FID procedure. Peaks: 1 = hexapropymate; 2 = methyprylon; 3 = persedon; 4 = phenazone; 5 = meprobamate; 6 = eicosane (internal standard); 7 = methaqualone. Allobarbital addition was omitted (retention time of allobarbital is approximately 7.0 min and thus it does not interfere with the neutral drugs if added). Retention times are given in Table III. The lower chromatogram shows a serum blank (GC/GC—FID procedure) without addition of the internal standards eicosane and allobarbital.

TABLE I

PRECISION DATA FOR DETERMINATION OF BARBITURATES BY GC/GC—NPD PROCEDURE

Compound	Level 1 (n = 5)		Level 2 (n = 10)		Level 3 (n = 5)	
	Mean (μg/ml)	C.V. (%)*	Mean (μg/ml)	C.V. (%)*	Mean (μg/ml)	C.V. (%)*
Metharbital	9.8	11.4	19.5	7.6	39.8	5.6
Barbital	9.4	8.3	19.9	2.1	39.8	3.5
Aprobarbital	10.1	6.6	21.2	4.6	40.4	2.2
Butalbital	10.2	5.9	21.0	3.4	40.1	2.5
Amobarbital	10.2	5.3	21.1	4.4	40.4	2.9
Pentobarbital	9.8	7.5	21.4	5.5	41.3	3.4
Vinbarbital	9.9	8.2	21.5	4.6	41.6	2.5
Secobarbital	9.9	7.9	21.6	5.0	41.8	4.5
Hexobarbital	9.8	5.1	24.4	6.6	43.3	6.0
Pentobarbital	10.3	7.6	21.9	6.3	41.2	2.9
Cyclobarbital	10.1	7.4	23.7	5.8	42.1	3.6
Heptabarbital	9.8	8.9	23.8	8.7	43.9	5.9

*C.V. = coefficient of variation.

TABLE II

RETENTION DATA FOR THE GC/GC—NPD PROCEDURE (ETHYLATED DRUGS)

Peak No.	Compound	Retention time (min)	Relative retention
1	Metharbital	3.51	0.74
2	Barbital	3.88	0.82
3	Allobarbital (internal standard)	4.73	1.00
4	Aprobarbital	4.96	1.05
5	Butalbital	5.35	1.13
6	Amobarbital	5.73	1.21
7	Pentobarbital	6.07	1.28
8	Vinbarbital	6.23	1.32
9	Secobarbital	6.47	1.37
10	Hexobarbital	7.30	1.54
11	Phenobarbital	7.81	1.65
12	Cyclobarbital	8.06	1.70
13	Heptabarbital	8.90	1.88
—	Ethosuximide	1.88	0.40
—	Persedon (2 peaks)	3.66	0.77
		4.34	0.92
—	Methyprylon	4.27	0.90
—	Caffeine	6.83	1.44
—	Phenazone	7.25	1.53
—	Methaqualone	9.98	2.11

of analysis must then be applied to screen for drugs that could be the cause of intoxication.

Some of the neutral drugs may be detected underivatized with the NPD, but we chose the FID because we required a more general detection method

TABLE III

RETENTION DATA AND LINEARITY DATA FOR THE GC/GC—FID PROCEDURE (UNDERIVATIZED DRUGS)

Peak No.	Compound	Retention time (min)	Relative retention	Range of linearity of calibration graph (μg/ml)
1	Hexapropymate	4.43	0.43	2—40
2	Methyprylon	5.26	0.51	2—40
3	Persedon	5.59	0.55	2—40
4	Phenazone	8.63	0.84	1—40
5	Meprobamate	8.84	0.86	Not studied, severe peak tailing
6	Eicosane (internal standard)	10.23	1.00	—
7	Methaqualone	11.57	1.13	1—40
—	Caffeine	8.28	0.81	—
—	Glutethimide	8.63	0.84	2—40
—	Barbiturates	—	—	Severe peak tailing

instead of a sensitive method for some neutral compounds. If a drug was detected only with the GC/GC—FID procedure and not with the GC/GC—NPD procedure, the drug is not a barbiturate. Underivatized barbiturates are subject to adsorption and catalytic phenomena on the quartz capillary column, resulting in badly tailing peaks. A comparison of the retention data from the two modes of gas chromatography gives valuable information that could help in the identification of peaks. Of course, as Dünges et al. [2] pointed out, alkyl derivatives other than the ethyl derivatives could be prepared in order to assist with the identification.

The methods described here are adequate for the determination of any one of the investigated drugs, except meprobamate, in serum from patients on known medication programmes.

REFERENCES

1 M. Garle and I. Petters, J. Chromatogr., 140 (1977) 165—169.
2 W. Dünges, R. Langlais and R. Schlenkermann, J. High Resolut. Chromatogr. Chromatogr. Commun., 2 (1979) 361—365.
3 J.M. Meola, Chromatogr. Newsl., 5 (1977) 1—3.
4 P. Sandra, M. Van den Broeck and M. Verzele, J. High Resolut. Chromatogr. Chromatogr. Commun., 3 (1980) 196.