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Note

Gas chromatographic determination of barbiturates by extractive alkylation and support coated open tubular column separation

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The analysis of barbiturates has mostly been based on gas chromatography (GC) or mass spectrometry with packed columns[1–5]. Since the separation of related barbiturates appears to be a problem on packed columns, the use of support coated open tubular (SCOT) columns[6] might be a solution. The chromatography of barbiturates as their 1,3-dimethyl derivatives has been discussed by Brochmann-Hansen and Oke[7]. The disadvantage of using trimethylanilinium hydroxide as a flash methylating agent is the appearance of so-called "early peaks" from phenobarbital and some other barbiturates. These were identified by Osiewicz and collaborators[8] as breakdown products. These peaks interfere with barbiturates eluting at a lower column temperature than phenobarbital. The use of extractive alkylation[9,10] has the advantage that no "early peaks" appear, and different alkyl derivatives can easily be formed. For these reasons a method for the determination of barbiturates in body fluids of overdose patients has been developed.

EXPERIMENTAL

Apparatus

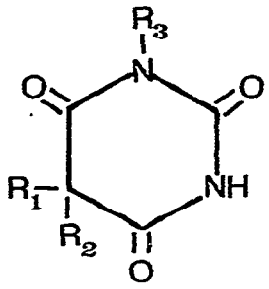
A Hewlett Packard 5720 A gas chromatograph with a temperature programming unit and flame ionization detector (FID) was used. The SCOT column and injection system were from SGE (N. Melbourne, Australia). The gas chromatograph was equipped with an inlet system for the make-up gas.

Chromatographic system

The column was made of glass, 43 m × 0.5 mm, coated with SE-30 (type GSC/SE-30/S). The GC conditions were as follows: injection port 250°, detector 300°, oven temperature isothermal at 170° for 4 min and then programmed from 170° to 260° at 4°/min. The carrier gas was helium at a flow-rate of 2 ml/min. The make-up gas for the FID was nitrogen.

Chemicals and materials

The barbiturates and glutethimide were obtained from manufacturers their respective. Tetrabutylammonium hydrogen sulphate (TBA-HSO_4) was purchased from Lab Kemi (Stockholm, Sweden), and prepared as a 1 *M* solution in



	Compound	R_1	R_2	R_3
1	Metharbital	ethyl	ethyl	methyl
2	Barbital	ethyl	ethyl	hydrogen
3	Allobarbital	allyl	allyl	hydrogen
4	Aprobarbital	allyl	isopropyl	hydrogen
5	5,5-dipropyl barbituric acid	propyl	propyl	hydrogen
6	Butethal	ethyl	butyl	hydrogen
7	Amobarbital	ethyl	3-methylbutyl	hydrogen
8	Pentobarbital	ethyl	1-methylbutyl	hydrogen
9	Vinbarbital	ethyl	1-methylbutenyl	hydrogen
10	Secobarbital	allyl	1-methylbutyl	hydrogen
11	Hexobarbital	methyl	1-cyclohexenyl	methyl
12	Glutethimide			
13	Phenobarbital	ethyl	phenyl	hydrogen
14	5-allyl-5-phenyl barbituric acid	allyl	phenyl	hydrogen
15	Heptabarb	ethyl	1-cycloheptenyl	hydrogen

Fig. 1. Structural formulae of the barbiturates.

1 *M* sodium hydroxide. The charcoal used was "Norit A", a neutral, pharmaceutical grade obtained from Amend Drug and Chemical Co., Irwington, N.J., U.S.A. It was prepared as follows: to *ca.* 500 mg of charcoal were added 50 ml of distilled water which was mixed thoroughly with a magnetic stirrer. All other chemicals were of reagent grade.

Analytical method

A 0.5 ml plasma sample was mixed thoroughly with 1 ml of the charcoal suspension and allowed to stand for a few minutes. After centrifugation as much as possible of the supernatant was aspirated off and discarded. Then 0.5 ml of 1 *M* sodium hydroxide, 50 μl of TBA-HSO_4 and 200 μl of ethyl iodide were added to the charcoal suspension and extracted with 1 ml of dichloromethane for 45 min. After centrifugation the aqueous phase was aspirated and the dichloromethane layer was transferred to a new tube and evaporated to dryness in a sandbath at 40° under a gentle stream of nitrogen. The residue was reconstituted in 50 μl of hexane. Standards

were prepared by the addition of known amounts of drugs (see Fig. 1) to human plasma. Drug concentrations were obtained by plotting the peak-height ratio of drug to internal standard.

RESULTS AND DISCUSSION

The adsorption of drugs onto charcoal from plasma and urine is well known and has been used as a clean-up procedure prior to GC[11] and liquid chromatographic analyses[12]. Extractive alkylation can be carried out directly, which simplifies the procedure to a single extraction step. The polar counter-ion, tetrahexylammonium sulphate, which gives a shorter derivatization time[10], could not be used because it contained impurities that would interfere with some barbiturates. To achieve quantitative derivatization with the less polar TBA-HSO_4 it was necessary to extract for 45 min at room temperature. This could, however, be speeded up by performing the extraction in a thermo-block at a higher temperature. The ethyl derivatives of all

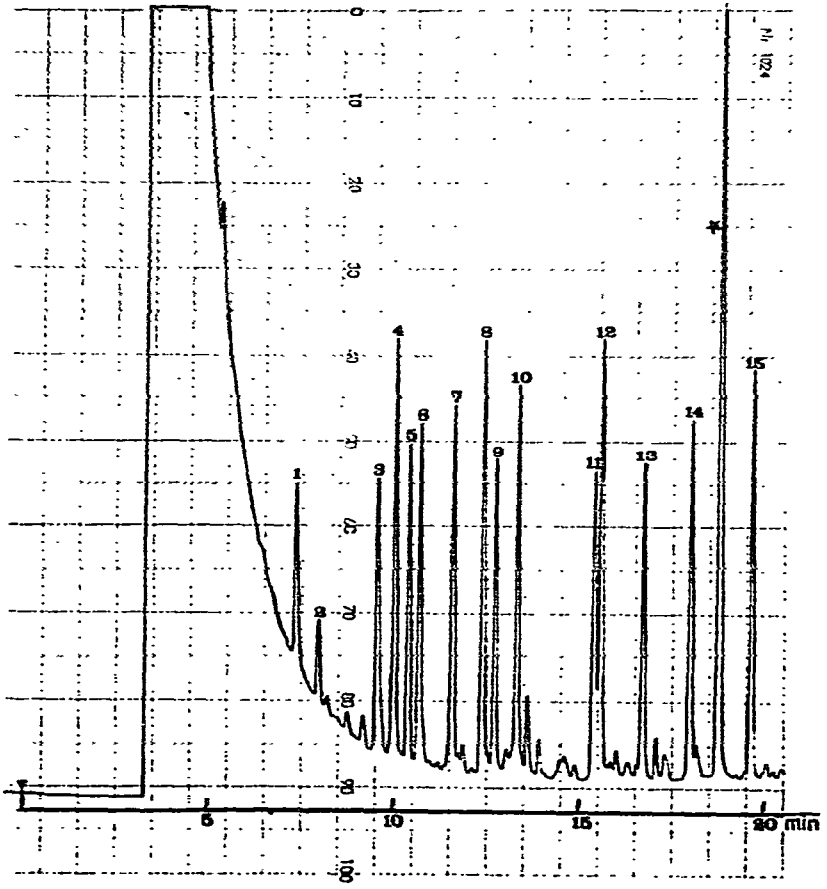


Fig. 2. Chromatogram of a spiked plasma sample: concentration of all drugs 20 μg/ml. Drugs numbered as in Fig. 1. ★ = peak from plasma.

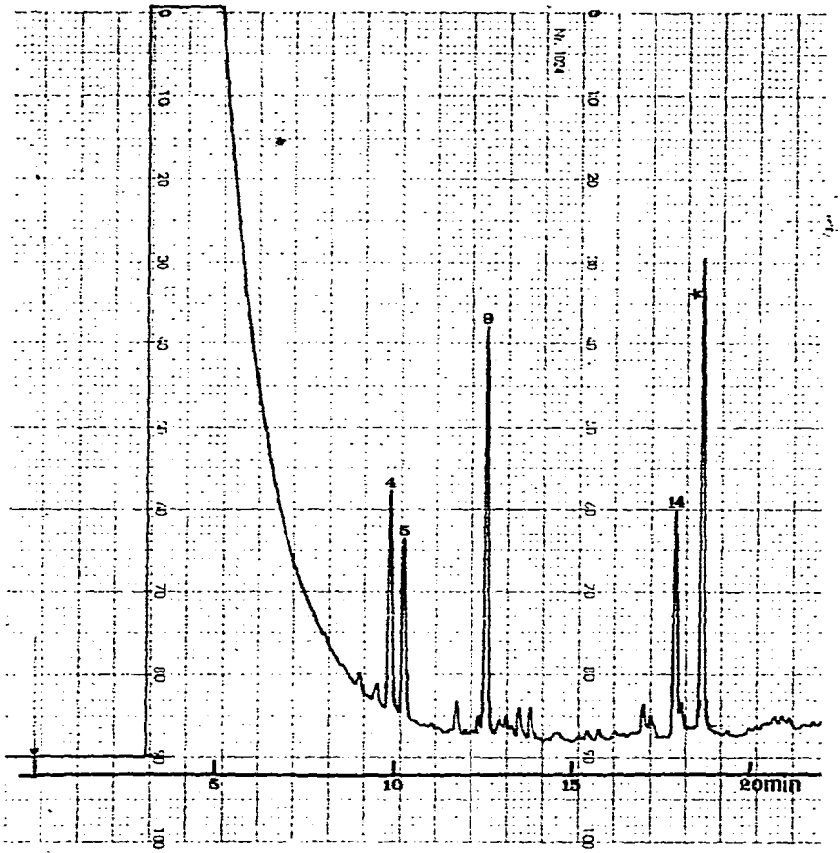


Fig. 3. Chromatogram of a plasma sample containing 19 μg/ml aprobarbital and 46 μg/ml vinbarbital. Internal standards (5,5-dipropyl barbituric acid and 5-allyl-5-phenyl barbituric acid) were added.

TABLE I

MEANS ($\bar{X}$) AND STANDARD DEVIATIONS (S.D.) FOR 21 SINGLE DETERMINATIONS ON PLASMA SAMPLES

Drug		*y (μg/ml)**	*$\bar{X}$ (μg/ml)*	*S.D.*
1	Metharbital	15.0	8.8	3.5
1**	Metharbital	15.0	15.7	2.1
2	Barbital	14.4	10.2	3.4
3	Allobarbital	17.7	14.0	1.6
4	Aprobarbital	20.8	18.5	1.1
6	Butethal	15.0	14.6	0.6
7	Amobarbital	15.0	15.5	1.5
8	Pentobarbital	15.0	16.5	2.3
9	Vinbarbital	15.0	16.8	2.7
10	Secobarbital	14.4	16.0	2.8
11	Hexobarbital	16.5	17.8	1.5
12	Glutethimide	15.8	15.5	1.3
13	Phenobarbital	15.0	14.5	0.9
15	Heptabarb	15.8	16.1	1.0

* Known plasma concentration.

** Ten determinations of metharbital with barbital as internal standard.

drugs give a better GC separation. Glutethimide and the barbiturates are ethylated in the nitrogen position. The structures of the barbiturates are shown in Fig. 1, and a chromatogram from a spiked plasma sample is shown in Fig. 2.

Standard curves were determined for all barbiturates and glutethimide using two different internal standards, dipropylbarbituric acid for barbiturates 1–10 (Fig. 1) and allylphenylbarbituric acid for the remaining barbiturates and glutethimide. The range of the standard curves was 5–40 μg/ml and they showed a good linear relationship. The correlation coefficient varied from 0.969 (barbital) to 0.999 (butethal). Means and standard deviations (S.D.) for 21 determinations at a concentration of *ca.* 15 μg/ml in plasma (all drugs added to spiked plasma) are in Table I. The quantitation of metharbital and barbital showed a higher S.D. depending on the large differences in retention time to internal standard. This was shown by running 10 determinations of metharbital with barbital as internal standard (Table I). The barbiturates are identified from their retention times. For positive identification the retention time should not differ by more than 0.5% from a standard sample containing all barbiturates. No interference was found by running acetylsalicylic acid, paracetamol, phenytoin, methaqualone or diazepam through the procedure. A chromatogram of a plasma sample from a patient is shown in Fig. 3, and the peaks can be identified as aprobarbital and vinbarbital. These barbiturates are in a multiple drug "Diminal-Duplex", one of the most common hypnotics in Sweden.

SCOT columns are not yet in common use but they appear to be preferable to packed columns when barbiturate separation is a problem. An unsplit inlet system can be used, and up to 1 μl solvent and 10 μg drug/peak could be injected without any detrimental long-term effect or overloading. In the end step of the analyses, 1 μl of hexane contains not more than 1 μg of each drug.

The method has been in use in our laboratory for more than a year, with good results. The sensitivity of the method in 500 μl samples seems sufficient for detection and quantitation in overdose patients.

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