



CAPILLARY GAS CHROMATOGRAPHY OF UNDERIVATIZED BARBITURATES IN HUMAN BLOOD WITH A NITROGEN-PHOSPHORUS DETECTOR AND WITH SPLITLESS INJECTION

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CAPILLARY GAS CHROMATOGRAPHY OF UNDERIVATIZED BARBITURATES IN HUMAN BLOOD WITH A NITROGEN-PHOSPHORUS DETECTOR AND WITH SPLITLESS INJECTION

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窒素リン検出器ならびにスプリットレス注入によるヒト血中非誘導体化バルビツール酸系薬物の
キャピラリーガスクロマトグラフィー

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Summary

The most updated method has been presented for analysis of underivatized barbiturates in human blood by capillary gas chromatography (GC) with a nitrogen-phosphorus detector and with splitless injection. The drug-containing samples, after mixing with dilute acid solution, were directly applied to a Sep-Pak C₁₈ cartridge and eluted with chloroform/methanol (9:1). Separation of the eight drugs from each other and from impurities was satisfactory with use of an intermediately polar DB-17 capillary column. The detection limits of the barbiturates were 0.2-4 ng on column except for phenobarbital; that of the latter was 20 ng on column. The recovery from the whole blood was more than 88 % for 6 of 8 drugs tested.

Key words : Analytical toxicology ; Barbiturates ; Phenobarbital ; Gas chromatography (GC) ; Capillary GC ; Nitrogen-phosphorous detector ; Splitless injection

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Introduction

Barbiturates are used as hypnotics and antiepileptics, and sometimes cause death in suicide and accidents because of their narrow safety dose ranges. Barbiturates contain many functional groups in their structures and are generally not suitable for analysis by gas chromatography (GC) with packed columns in their underivatized forms; but thanks to the introduction of capillary columns, their analysis by GC has been realized, because of their low adsorption to the capillary columns [1-3]. These authors, however, used capillary columns with a flame ionization detector (FID), and with split injection which makes sensitivity lower.

In this study, we present the most updated method for analysis of underivatized barbiturates in human blood by capillary GC with a nitrogen-phosphorous detector (NPD) and with splitless injection; a simple isolation procedure with a Sep-Pak C₁₈ cartridge is also described.

Experimental

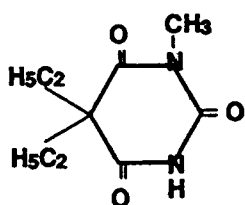
Materials

Molecular structures and weights of 8 barbiturates used in the present study are shown in Fig.1. Metharbital was obtained from Dainippon Pharmaceutical Co., Ltd., Osaka; barbital from E. Merck AG, Darmstadt, Germany; amobarbital from Nippon Shin-yaku Co., Ltd., Kyoto; pentobarbital calcium from Tanabe Seiyaku Co., Ltd., Osaka; secobarbital sodium from Yoshitomi Pharmaceutical Ind., Ltd., Osaka; hexobarbital from Teikoku Chemical Ind., Co., Ltd., Osaka; mephobarbital from Bayer AG, Leverkusen-Bayerwerk, Germany; and phenobarbital from Fujinaga Pharmaceutial Co., Ltd., Tokyo. Sep-Pak C₁₈ cartridges were purchased from Waters Associates, Milford, MA, USA. Other common chemicals used were of the highest purity commercially available. Whole blood was obtained from a healthy subject.

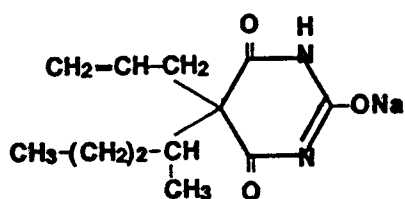
Isolation with Sep-Pak C₁₈ cartridges

The drugs were extracted on Sep-Pak C₁₈ cartridges according to our previous paper [4] with a minor modification. For pretreatment of a cartridge, 10 ml of methanol and 10 ml of distilled water were passed through it.

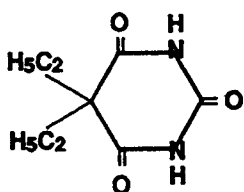
One milliliter of whole blood was spiked with 100 μ l methanolic solution of barbiturates. To the sample, 19 ml of 0.01 N HCl solution was added and mixed completely. The solution was poured into the pretreated cartridge at a flow rate not greater than 5 ml/min. It was washed with 10 ml 0.01 N HCl solution twice. Finally, 3 ml chloroform/methanol (9:1) was passed through it to elute barbiturates and it was collected in a vial. The eluate consisted of a major amount of an organic layer (lower phase) and a small amount of aqueous layer (upper phase); the latter was discarded with a Pasteur pipette. The organic layer was evaporated to dryness



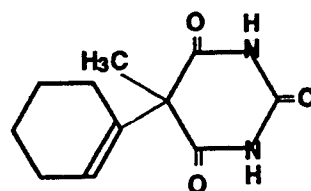
METHARBITAL
MW.198



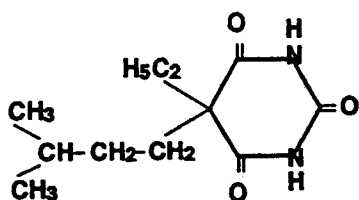
SECOBARBITAL SODIUM
MW.260



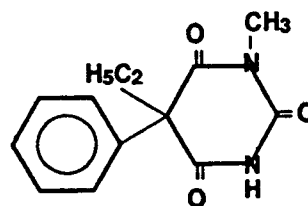
BARBITAL
MW.184



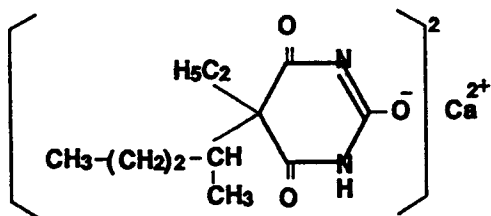
HEXOBARBITAL
MW.236



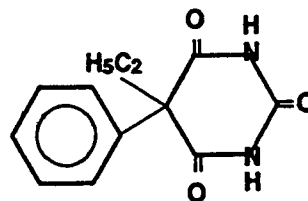
AMOBARBITAL
MW.226



MEPHOBARBITAL
MW.246



PENTOBARBITAL CALCIUM
MW.226(FREE FORM)



PHENOBARBITAL
MW.232

Fig. 1. Chemical structures of eight barbiturates used in the present study.

under the stream of nitrogen. The residue was dissolved in 100 μ l methanol and a 2 μ l aliquot of it was subjected to GC analysis.

GC conditions

GC was carried out on an HP-5890A instrument with a fused silica capillary column (DB-1, 30 m \times 0.32 mm i.d., film thickness 0.25 μ m, J & W Scientific, Folsom, CA, USA), with an NPD and with a split-splitless injector. The GC conditions were : column temperature, 100–280 $^{\circ}$ C (8 $^{\circ}$ C/min) ; injection temperature, 200 $^{\circ}$ C ; helium flow-rate, 25 cm/s. The samples were injected in the splitless mode at 100 $^{\circ}$ C, and the splitter was opened after 1 min.

Results

Figure 2 shows gas chromatograms obtained with a DB-17 capillary column with an NPD and with splitless injection. All drugs showed sharp peaks and could be satisfactorily separated from each other (Fig. 2, left panel). In the chromatogram for whole blood extract, which had been spiked with the 8 drugs, the peak of mephobarbital (peak 7) appeared very close to a big adjacent impurity peak ; but the drug peak was separated from it (Fig. 2, middle panel). Other drug peaks were not interfered with by impurities.

Table 1 shows retention times and recoveries of the eight drugs, which had been added to 1 ml whole blood. Metharbital and barbital showed somewhat low recoveries, but other 6 drugs showed very excellent values. The recovery of phenobarbital exceeded 100% (Table 1 and Fig. 2).

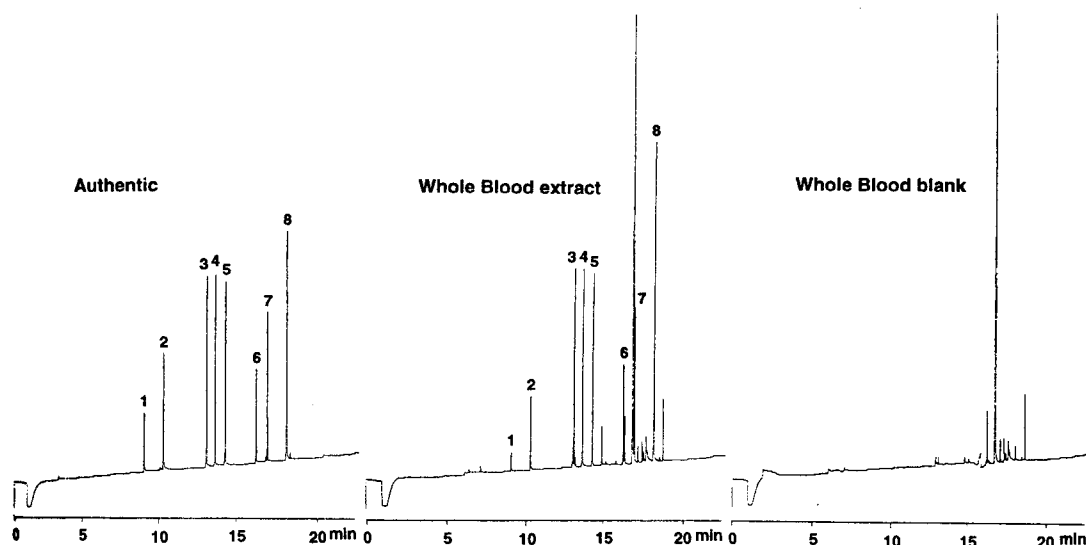


Fig. 2. Capillary GC with an NPD for barbiturates isolated from human whole blood by use of Sep-Pak C₁₈ cartridges. Peaks : 1. metharbital ; 2. barbital ; 3. amobarbital ; 4. pentobarbital ; 5. secobarbital ; 6. hexobarbital ; 7. mephobarbital ; 8. phenobarbital.

Table 1. Retention times and recoveries of eight barbiturates

Compound	Retention time (min)	Amount of drug added to 1 ml blood (μg)	Recovery (%)
Metharbital	9.22	0.2	28.4
Barbital	10.4	1.0	47.9
Amobarbital	13.2	2.0	90.1
Pentobarbital	13.7	2.0	88.1
Secobarbital	14.4	2.0	89.7
Hexobarbital	17.3	0.2	96.3
Mephobarbital	17.0	0.4	89.8
Phenobarbital	18.2	5.0	125

Table 2. The equation parameters and detection limits for calibration curves of eight barbiturates

Compound	$y = ax + b$		r value	Detection limit (ng on column)
	a	b		
Metharbital	15.70	-318.9	0.9945	0.2
Boarbital	1.835	-0.3212	0.9964	4.0
Amobarbital	2.582	-0.6617	0.9982	2.0
Pentobarbital	2.524	-0.6956	0.9985	2.0
Secobarbital	1.9757	-0.6035	0.9987	3.0
Hexobarbital	20.70	-462.2	0.9999	0.2
Mephobarbital	19.80	-574.7	0.9979	0.2
Phenobarbital	0.2730	-0.3962	0.9977	20.0

$y=ax+b$: x values are expressed as μg drug/ml blood. The values were obtained from curves of 3-5 plots.

Figure 3 shows calibration curves for mephobarbital and phenobarbital as examples. Both curves intersected the horizontal axis, showing adsorptions of the drugs to the column at low concentrations. Table 2 shows the summary of all calibration curves, with each equation parameter, r value and detection limit. The highest sensitivity was obtained with hexobarbital, mephobarbital and metharbital ; and the lowest sensitivity with phenobarbital. All drugs showed very excellent r values.

Discussion

To our knowledge, this is the most updated method for GC analysis of underivatized barbiturates among those so far reported [1-4]. In addition, the present procedure for isolation of barbiturates from blood samples, by solid phase extraction with use of Sep-Pak C_{18} cartridges, is very simple and rapid.

In the previous paper, we reported wide-bore capillary GC for underivatized barbiturates with an FID [4]. The sensitivity of the present NPD method with splitless injection is about 10-20

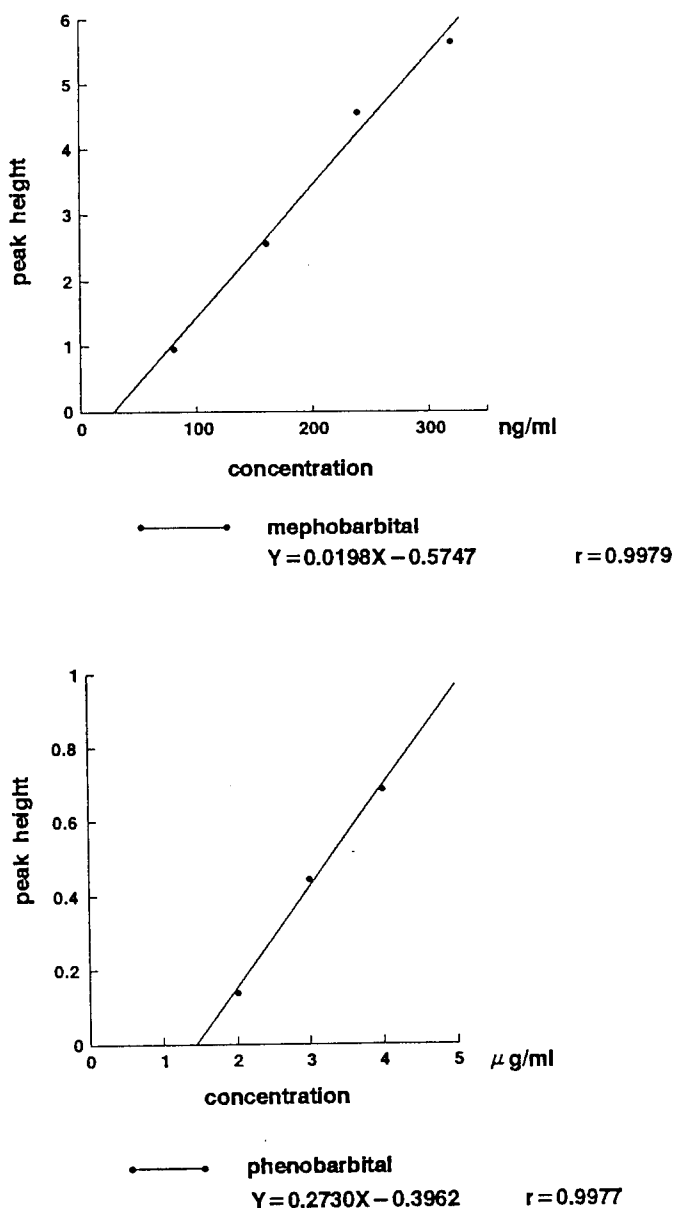


Fig. 3. Calibration curves for mephobarbital and phenobarbital.

times higher than that of the previous FID method [4]. The peaks with the present middle-bore capillary column (i.d., 0.32 mm) are much sharper (Fig.2) than those with the wide-bore capillary column (i.d., 0.52 mm), resulting in higher resolution. No decomposition of the barbiturates were found even with the present middle-bore capillary column with longer exposure to heat.

The use of middle-bore capillary GC is advantageous for its combination with a mass spectrometry (MS) instrument in that the capillary column can be directly introduced into an ion

source chamber, which results in high sensitivity for MS measurements; while a wide-bore capillary column should be connected to a separator to remove the carrier gas, which causes much loss of compounds to be analyzed and thus lower sensitivity.

The recovery of phenobarbital in the whole blood extract exceeded 100 % (Table 1 and Fig. 2). This phenomenon is not due to contamination by impurities; the gas chromatogram for the extract without any addition of drugs did not show any impurity peak where the drug would be expected to appear (Fig. 2, right panel). This phenomenon may be due to certain factors contained in the extract, which may prevent them from adsorbing to the column.

The detection limit of phenobarbital was as large as 20 ng on column (Table 2), which is equivalent to 1.0 $\mu\text{g}/\text{ml}$ blood, owing to its strong adsorption to the column especially in low concentration ranges. However, it gives no problem for actual drug monitoring in clinical pharmacology, because therapeutic concentrations of phenobarbital in blood is 2–30 $\mu\text{g}/\text{ml}$ [5].

The present capillary GC method with an NPD and a splitless injector, together with a simple isolation procedure, should become more popular for drug analysis in the field of forensic chemistry, clinical toxicology and clinical pharmacology.

Acknowledgement

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