Rapid, Sensitive GLC Determination of Pentobarbital and Other Barbiturates in Serum Using Nitrogen-Specific Detector

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Abstract [] A GLC method for the analysis of pentobarbital in serum was developed. After extraction from serum, a methyl derivative was prepared and quantitated by nitrogen-specific detection. The method has a sensitivity of 0.08 μg/ml for pentobarbital with only 0.1 ml of serum. Secobarbital was the internal standard. Derivatives of other barbiturates also were prepared.

Keyphrases [] Pentobarbital—GLC analysis in serum [] Barbiturates, various—GLC analysis in serum [] GLC—analysis, pentobarbital and various barbiturates in serum [] Sedatives—pentobarbital and various barbiturates, GLC analysis in serum

The GLC determination of pentobarbital and other barbiturates in biological fluids using flame-ionization detection has been reported extensively (1–11). The compounds are not readily gas chromatographed without derivatization and require carefully prepared columns, especially when nanogram amounts are injected. Recently, Sun and Chun (12) reported a sensitive GLC procedure, using electron-capture detection on the pentafluorobenzyl derivative of pentobarbital after serum extraction. However, it required numerous cleanup steps before GLC analysis. Brochmann-Hanssen and Oke (13) methylated the barbiturates by the flash-heater methylation technique with trimethylanilinium hydroxide.

Dunges and Bergheim-Irps (14) used methyl iodide for derivative formation with some barbiturates in pure drug form (the unformulated barbiturates) with subsequent flame-ionization detection. This paper reports a modification of their procedure (14) for the determination of pentobarbital in serum, using a nitrogen-specific detector to achieve selectivity and sensitivity.

EXPERIMENTAL

Reagents and Materials—Pentobarbital[1], secobarbital[2] (the internal standard), and methyl iodide[3] were used as supplied. Ether[4] and other chemicals were analytical reagent grade.

Apparatus—A mixer[5] and refrigerated centrifuge[6] at 10° were used. Methyl derivative formation at 56° was carried out on a heating block[7].

GLC—A reporting gas chromatograph[8] equipped with a dual nitrogen–phosphorus flame-ionization detector[9], a computing integrator[10], and an automatic sampler[11] were used. The glass column, 1.22 m × 2 mm (i.d.), was packed with 2% OV-101[12] on 100–120-mesh Chromosorb WHP.

[1] Abbott Laboratories, North Chicago, Ill.
[2] Eli Lilly Co., Indianapolis, Ind.
[3] J. T. Baker Chemical Co., Phillipsburg, N.J.
[4] Mallinckrodt Chemicals, St. Louis, Mo.
[5] Vortex Genie model K-550-GT, Scientific Industries, Springfield, Mass.
[6] Model RC-3, Sorvall, Newtown, Conn.
[7] Dri-block DB-3, Techne, Princeton, N.J.
[8] Model 5830A, Hewlett-Packard, Avondale, Pa.
[9] Model 18789, Hewlett-Packard, Avondale, Pa.
[10] Model 18850A, Hewlett-Packard, Avondale, Pa.
[11] Model 7671A, Hewlett-Packard, Avondale, Pa.
[12] Hewlett-Packard, Avondale, Pa.

The operating conditions were: column oven temperature, 140°; detector temperature, 250°; injection port temperature, 160°; helium carrier gas flow rate, 40 ml/min; hydrogen flow rate, 3 ml/min; air flow rate, 50 ml/min; and recorder attenuation, 16.

Preparation of Standards—The pentobarbital serum standard of 15 μg/ml was prepared from a standard solution of 500 μg/ml in methanol. Other serum standards at 0.1, 0.25, 0.5, 1.0, 2.5, 5.0, 7.0, and 10.0 μg/ml were prepared from serial dilutions of this standard with serum. For human studies, serum standards up to 2.5 μg/ml were used; this range covered the human serum pentobarbital levels expected after oral administration of a 100-mg dose of pentobarbital sodium (12).

The internal standard of 8 μg/ml in distilled water was prepared from a standard solution of 200 μg/ml in methanol.

Extraction and Derivatization Procedure—To a 15-ml conical glass centrifuge tube were added 0.1 ml of the internal standard solution, 0.1 ml of serum sample, 0.1 ml of 0.2 N HCl, and 5 ml of ether. After mixing for 15 sec twice and centrifuging at 2500 rpm for 3 min at 10°, 4 ml of organic layer was transferred into a conical tube and evaporated to dryness at 45° with filtered air. To the dry residue were added 0.5 ml of acetone, 0.1 ml of methyl iodide, and about 5 mg of sodium carbonate.

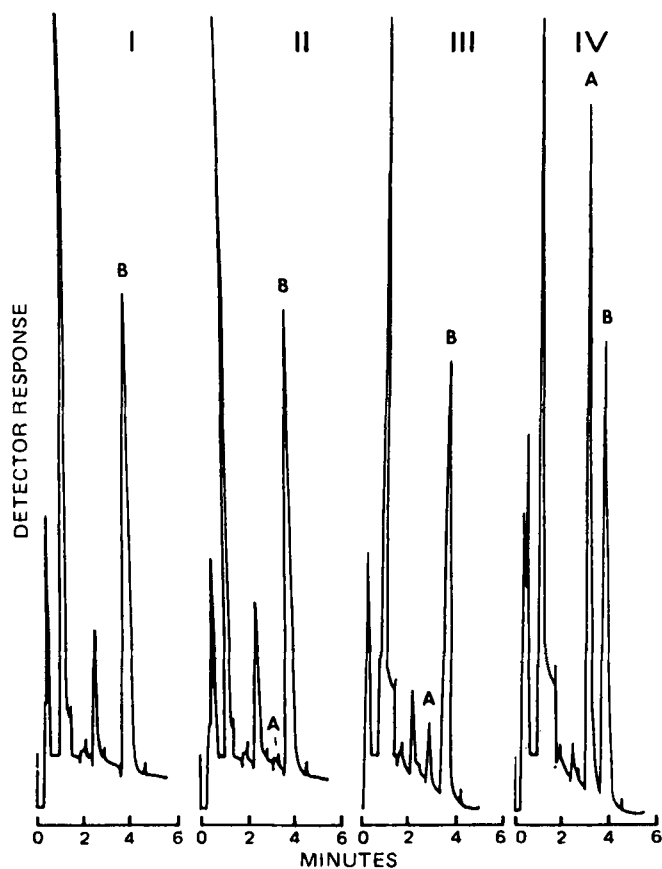The mixture was capped, agitated, heated at 56° for 30 min, and then

Figure 1—*GLC tracings of extracted serum samples containing various amounts of pentobarbital. Key: I, serum blank; II, serum pentobarbital standard at 0.1 μg/ml; III, serum pentobarbital standard at 1.0 μg/ml; IV, serum pentobarbital standard at 10.0 μg/ml; A, pentobarbital; and B, internal standard (secobarbital).*

Figure 2—*GLC tracings of N,N'-dimethyl derivatives of barbiturate mixtures. Key: I, reagent blank (composed of 0.5 ml of acetone, 0.1 ml of methyl iodide, and 5 mg of sodium carbonate); II, pure drug reacted (as N,N'-dimethyl derivatives of barbiturates injected as 5 µl of the completed reaction solution); III, serum blank extract; IV, serum extract containing different barbiturate derivatives; a, barbital and metharbital; b, allobarbital; c, aprobarbital; d, butabarbital, butethal, and butalbital; e, amobarbital; f, pentobarbital and thiopental; g, secobarbital; h, hexethal; i, hexobarbital; and j, mephobarbital and phenobarbital.*
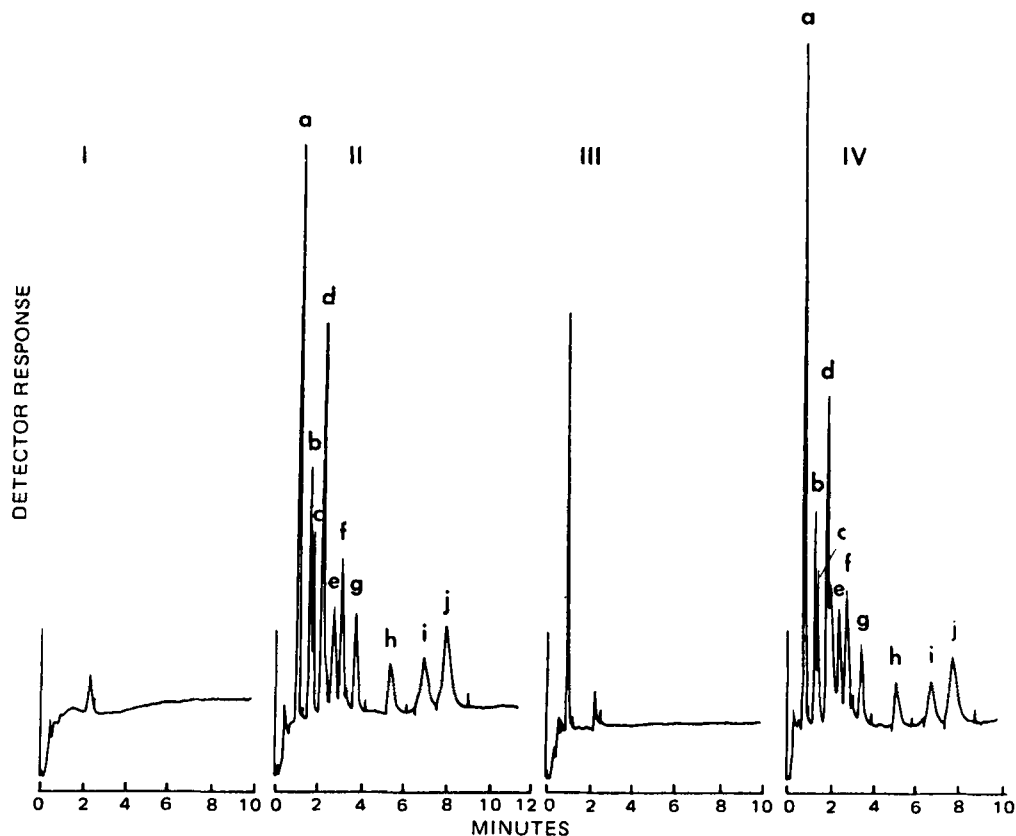
Table I—Assay Reproducibility: Serum Pentobarbital Concentration (Micrograms per Milliliter)

Theoretical	Observed Mean[a] ± SD	RSD, %
0	0	—
0.10	0.100 ± 0.0100	10.0
0.25	0.227 ± 0.0153	6.7
0.50	0.487 ± 0.0379	7.8
1.00	1.033 ± 0.0493	4.8
2.50	2.490 ± 0.0173	0.7
5.00	4.987 ± 0.2021	4.0
7.50	7.590 ± 0.0529	0.7
10.00	9.957 ± 0.0907	0.9
15.00	14.973 ± 0.4822	3.2
Average		4.3

[a] Mean of three determinations at each concentration.

cooled to room temperature. The supernate was transferred into a GLC microvial[12], and 5 µl was injected into the GLC column with the automatic sampler[11].

RESULTS AND DISCUSSION

The peak area ratio of pentobarbital to the internal standard was plotted against known standards of pentobarbital, expressed as micrograms per milliliter in serum. Values for unknown concentrations of pentobarbital in serum were calculated from this standard curve using a least-squares regression method.

Under the assay conditions, pentobarbital and the internal standard had retention times of 3.11 and 3.80 min, respectively (Fig. 1).

The precision of the assay is reported in Table I. Relative standard deviations ranged from 0.7 to 10.0%, with an average relative standard deviation of 4.3%. Statistical evaluation of the peak area ratio at 0.1 µg/ml, using a one-sided tolerance limit test, showed that the sensitivity was less than 0.1 µg/ml. The calculated sensitivity of the assay was about 0.08 µg/ml, calculated by least-squares linear regression for concentrations from 0 to 0.5 µg/ml (Table II). Sensitivity was defined as the concentration calculated by linear regression analysis to give any response greater than zero in 95% of the samples.

The accuracy of this assay was tested by analyzing 18 unknown serum samples spiked with pentobarbital sodium under blind conditions (Table

Table II—Sensitivity of Serum Pentobarbital Assay [a]

Serum Pentobarbital Concentration, µg/ml	Peak Area Ratio[b]
0	0
	0
	0
0.10	0.005
	0.007
	0.006
0.25	0.021
	0.018
	0.022
0.5	0.055
	0.047
	0.048

[a] Least-squares linear regression calculation: number of points, 12; slope, 0.1019 ± 0.0089; y-intercept ± 95% confidence limit, −0.00258 ± 0.00637; correlation coefficient, 0.988; and calculated sensitivity, 0.080 (µg/ml). [b] Pentobarbital to the internal standard.

Table III—Accuracy of Serum Pentobarbital Assay

Unknown	Serum Pentobarbital Concentration, µg/ml Theoretical	Observed	Difference	Difference, %
1	0	0	0	0
2	0.10	0.11	0.01	10.0
3	0.18	0.16	−0.02	11.1
4	0.25	0.23	−0.02	8.0
5	0.38	0.36	−0.02	5.3
6	0.50	0.53	0.03	6.0
7	0.75	0.72	−0.03	4.0
8	1.00	1.01	0.01	1.0
9	1.75	1.72	−0.03	1.7
10	2.50	2.44	−0.06	2.4
11	3.75	3.62	−0.13	3.5
12	5.00	5.22	0.22	4.4
13	6.25	6.17	−0.08	1.3
14	7.50	7.53	0.03	0.4
15	8.75	8.46	−0.29	3.3
16	10.00	9.95	−0.05	0.5
17	12.50	12.28	−0.22	1.8
18	15.00	15.14	0.14	0.9

III). The assay error exceeded 5% for all samples containing 0.50 µg/ml or less of pentobarbital. The assay error was below 5% for all samples containing pentobarbital between 0.75 and 15.0 µg/ml of serum.

The method is specific for pentobarbital. Except for thiopental, it was resolved from 13 other barbiturates (Fig. 2). A similar quantitative determination for these barbiturates should be applicable.

This rapid assay can be used to conduct single oral dose pentobarbital bioavailability studies in adult and pediatric patients using only 0.1 ml of serum. This procedure also is applicable to the general screening of some other barbiturates in biological fluids.

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Separation, Identification, and Quantitation of Anthralin and Its Decomposition Products

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Abstract □ Anthralin and its decomposition products were separated by both column chromatographic and TLC techniques. Two decomposition products were characterized by TLC, melting-point data, and UV and IR spectroscopy. Pure anthralin and its decomposition products also were determined quantitatively.

Keyphrases □ Anthralin—column chromatographic and TLC analysis and separation from decomposition products □ Chromatography, column—analysis and separation of anthralin from decomposition products □ TLC—analysis and separation of anthralin from decomposition products □ Decomposition products, anthralin—column chromatographic and TLC analysis and separation □ Antipsoriatic agents—anthralin, column chromatographic and TLC analysis and separation from decomposition products

The first use of anthralin (1,8,9-anthracenetriol) for topical therapy of psoriasis was noted in a report on chrysarobin, a related compound, in 1878 (1). Psoriasis is a skin disease characterized in part by epidermal hyperplasia and, therefore, should be controllable with antimetabolic drugs. It has been assumed that anthralin acts by blocking cell division (2). Although local treatment of psoriasis vulgaris with corticosteroid creams under plastic occlusion is accepted widely, psoriasis is still treated with anthralin pastes (USP XIX) (3–6).

In treating psoriatic lesions with commercial anthralin, two side effects were documented: staining of skin and clothing and irritation of surrounding normal skin. Anthralin is relatively unstable in the presence of air, light, and heat (7). Since commercial anthralin samples contain degradation products, it was not clear what relationship existed between anthralin and its degradation products with regard to therapeutic effectiveness and side effects. It was reported (2, 8, 9) that any therapeutic effect in

Table I—R_f Values for Anthralin and Its Decomposition Products

Solvent System	Plates	R_f (×100) Anthra-lin	R_f (×100) Dan-thron	R_f (×100) Dian-throne
Benzene–cyclohex-ane–methanol (15:15:0.25)	Silica	42	35	21
Benzene–cyclohex-ane–acetic acid (15:15:0.5)	Silica	31	20	11
Benzene–cyclohex-ane–methanol (15:15:0.25)	Compound I-treated silica	54	41	27
Benzene–cyclohex-ane–acetic acid (15:15:0.5)	Compound I-treated silica	32	20	7

psoriasis results from anthralin and not its decomposition products. These findings supported that of Comaish et al. (10), who noted that discolored anthralin pastes (possibly containing large amounts of the quinone) are ineffective in treating psoriasis.

The aims of the present research were to investigate the purity of a commercial anthralin sample and to separate, identify, and determine any decomposition products in order to judge the stability and the potency of preparations of the drug as an antipsoriatic agent.

EXPERIMENTAL

Materials—Commercial anthralin NF and reference samples of anthralin, danthron, and dianthrone were used as received[1]. All solvents

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[1] Pfaltz and Bauer, Stanford, Conn.