DETERMINATION OF BARBITURIC ACID DERIVATIVES AS MERCURY COMPLEXES

BY C. O. BJÖRLING, A. BERGGREN AND B. WILLMAN-JOHNSON

From the Research Laboratories, Pharmacia, Ltd., Uppsala

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Barbituric acid derivatives give precipitates with mercuric ions, which are soluble in certain organic solvents, for example, chloroform, and can be extracted. By the addition of dithizone to the extract the mercury can be determined and hence the equivalent amount of the barbiturate. As little as 0·1 μg./ml. in the final solution can be determined. Interferences seem to be few.

EXISTING methods for the photometric determination of barbituric acid derivatives are based either upon their ultra-violet absorption or upon their ability to form metal complexes. Amounts as small as 10 μg./ml. in the final solution can be determined by ultra-violet absorption[1]. The cobaltamine complexes have been commonly used[1] but require greater amounts.

Several authors[2] have used the fact that barbiturates give a precipitate with mercuric ions as a means of assay. Only Pfeil and Goldbach[3] seem to have tried this principle for micro work. In their method the precipitate is formed on a filter paper and after washing, the mercury is dissolved in hydrochloric acid and finally determined with dithizone.

Björling, Berggren, Willman-Johnson, Grönwall and Zaar[4] have shown that the mercuric complex can be extracted with an organic solvent. On the addition of dithizone the mercuric-barbiturate complex is destroyed and gives the usual red colour of the mercuric-dithizone complex. This principle has now been developed to an extremely sensitive method.

STANDARD METHOD

Reagents

Dithizone solution. Dissolve 15 mg. of dithizone in 1000 ml. of chloroform. Store the solution in a refrigerator and protect it from light. *Mercuric nitrate solution.* Dissolve 300 mg. of mercuric nitrate in 1 ml. of 0·1N nitric acid and dilute to 100 ml. with distilled water. *Buffer solutions.* M/3 phosphate, borate, and carbonate buffer solutions. *Organic solvents.* Chloroform, chloroform containing 20 per cent benzyl alcohol or 20 per cent dioxane.

Procedure

To a separating funnel add 1 ml. of the mercuric nitrate solution and 1 ml. of a suitable buffer solution and up to 10 ml. of an aqueous solution containing 30 to 300 μg. of barbiturate. Extract four times with 10 ml. of the appropriate organic solvent. Filter the extracts successively through a plug of glass wool previously moistened with the solvent to trap any entrainments. Add 5 to 40 ml. of dithizone solution and dilute to 100 ml. with chloroform.

Prepare a dilution with the same amount of dithizone solution, 40 ml. of the organic solvent and add chloroform to 100 ml. Measure the extinction of this solution against the sample solution at 605 mμ. The amount of barbiturate present may be calculated from a standard graph which can be used for any barbiturate if the values are calculated on a mol. basis. A standard curve may also be constructed from results from mercuric chloride directly dissolved in chloroform.

100 μg. of 5-ethyl-5-(3-methylbutyl)barbituric acid (amylobarbitone) equivalent to 88·66 μg. of mercury assayed by this method gives an extinction difference of about 0·35 in a 1 cm. cell.

Micro modification. The following method has been applied to amylobarbitone and there is no reason why it should not be applicable to other barbiturates.

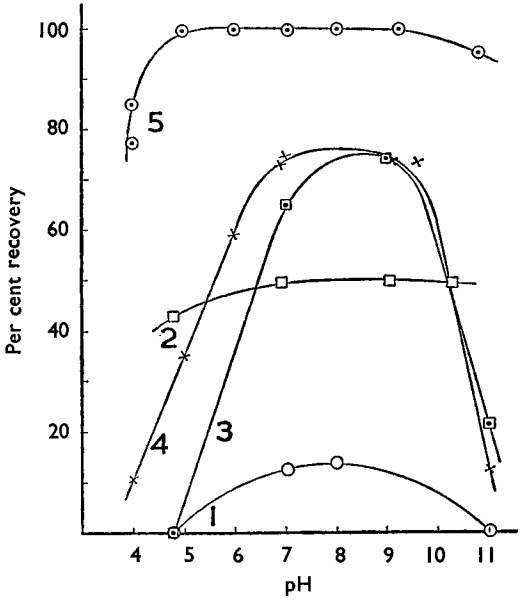


FIG. 1. Barbituric complexes extracted with chloroform at different pH values.

1. 5:5-diethylbarbituric acid. 2. *N*-methylated barbituric acids. 3. 5:5-diallylbarbituric acid. 4. 5-ethyl-5-phenyl barbituric acid. 5. 5-*iso*-amyl-5-ethyl barbituric acid.

To a separating funnel add 40 μl. of mercuric nitrate solution and 40 μl. of a buffer solution pH 7·5 and up to 400 μl. of an aqueous solution containing up to 20 μg. of barbiturate. Extract with 4 + 3 + 3 ml. of chloroform, filter through glass-wool and add 4 ml. of the dithizone solution. Dilute to 15 ml. with chloroform and continue analogously as above.

2 μg. of amylobarbitone, equivalent to 1·77 μg. of mercury, assayed by the micro modification gives an extinction difference of about 0·20 in a 4 cm. cell.

DISCUSSION

From Figure 1 it may be seen that barbiturates have an optimum pH range for their extraction from the water phase. Some barbiturates, as for instance amylobarbitone (Table I, 6), have a wide range while others such as allobarbitone (Table I, 3) are extracted only within fairly narrow pH limits. Generally a pH of 8 to 9 is suitable for the barbituric acids investigated.

From Figure 1 it is evident that the recovery of some barbiturates is not quantitative when chloroform is chosen as a solvent. These compounds can be extracted with 4 parts of chloroform and 1 part of dioxane

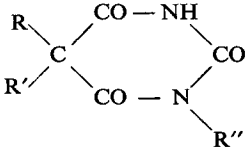
or 4 parts of chloroform and 1 part of benzyl alcohol. The compositions of the solvent mixtures were established by extracting 100 μg. of a barbiturate at pH 8 with different mixtures. For barbitone the following figures were obtained.

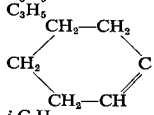
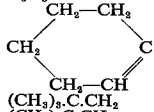
Per cent benzyl alcohol ..	5	10	20	30
Extinction of sample ..	0·310	0·369	0·420	0·379
of blank	0·004	0·002	0·021	0·056
Net extinction	0·306	0·367	0·399	0·323

The barbituric acids investigated are shown in Table I. From the values it may be concluded that a long alkyl chain promotes the extraction. Thus barbituric acid itself cannot be extracted by any of the suggested solvents, barbitone (2) can only be extracted to 95 per cent even with

TABLE I

BARBITURIC ACIDS INVESTIGATED



	R	R′	R″	pH optimum	Solvents
1.	H	H	H		80 chloroform + 20 benzyl alcohol; plain chloroform*
2.	C_2H_5	C_2H_5	H	9·0	80 chloroform + 20 benzyl alcohol
3.	C_3H_5	C_3H_5	H	9·0	80 chloroform + 20 benzyl alcohol
4.	C_3H_5	i-C_3H_7	H	9·0	80 chloroform + 20 benzyl alcohol
5.		C_2H_5	H	9·0	80 chloroform + 20 benzyl alcohol
6.	i-C_5H_{11}	C_2H_5	H	7–9	chloroform
7.	C_6H_5	C_2H_5	H	7–9	80 chloroform + 20 benzyl alcohol or dioxane
8.	C_6H_5	C_3H_5	H	9	chloroform
9.	$(CH_3)_2.CH.CH_2$	C_3H_5	H	7–9	chloroform
10.	$(CH_3)_3C.(CH_2)_6$	C_2H_5	H	4·7–10·4	chloroform
11.	C_6H_5	C_2H_5	CH_3	7–9	chloroform
12.		CH_3	CH_3	7–10·4	chloroform
13.	$(CH_3)_2.CH.CH_2$	C_2H_5	n-C_3H_7	7–9	chloroform
14.	$(CH_3)_3C.CH_2$	CH_2= $CCl.CH_2$	CH_3	7–9	chloroform
15.	$(CH_3)_2.CH.CH_2$	C_3H_5	CH_3	7–9	chloroform
16.	$(CH_3)_2.CH.CH_2$	CH_2= $CBr.CH_2$	CH_3	7–9	chloroform
17.	$(CH_3)_2.CH.CH_2$	C_3H_5†	H	7–9	chloroform
18.	$CH_3.(CH_2)_2.C(CH_3)$	C_2H_5†	H	7	chloroform

* Recovery nil.
† Thiobarbituric acid.

chloroform-benzyl alcohol. Further, for phenobarbitone (7) chloroform-dioxane is necessary while the allyl-phenyl derivative can be extracted with chloroform. The *N*-substituted barbiturates are all very easily

extracted with chloroform. Several other solvents have been tried with little or no success. Thus 1:2-dichloroethane, 1:2:2-trichloroethane were similar to but not better than chloroform while, for example, diethylether, hydrocarbons, and esters were unsatisfactory. Tetrahydrofuran 20 per cent in chloroform was unsuitable. Carbon tetrachloride was much inferior to chloroform.

The different suggested solvent mixtures slightly modify the nature of the final dithizone colour. Therefore, for accurate work, a standard curve must be prepared for each solvent. With the dioxane solvent the extinction was 5 per cent higher than with chloroform alone, and with the benzyl alcohol mixture the extinction was 2·5 per cent lower. Further, there is always a small blank with the mixed solvents. Typical extinction values for the blank in the general method are 0·01 for dioxane-chloroform and 0·02 for benzyl alcohol-chloroform. With chloroform alone the blank is zero.

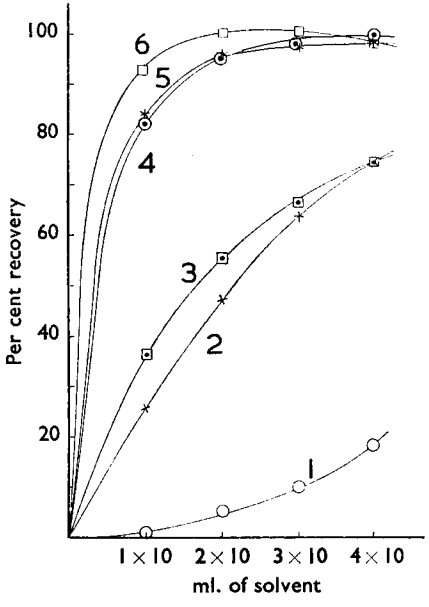


FIG. 2. Recoveries at multiple extractions of barbituric complexes.
1. 5:5-diethylbarbituric acid (chloroform). 2. 5-ethyl-5-phenylbarbituric acid (chloroform). 3. 5:5-diallylbarbituric acid (chloroform). 4. 5-*iso*amyl-5-ethylbarbituric acid (chloroform). 5. 5-ethyl-5-phenylbarbituric acid (dioxane + chloroform). 6. 5-ethyl-5-phenyl-2-methylbarbituric acid (chloroform).

With the correct choice of solvent four extractions are sufficient for quantitative recoveries. Typical successive extractions are shown in Figure 2. The figure also clearly demonstrates the effect of mixed solvents on the extraction. For biological work the method may be considerably simplified if only one standardised extraction is made.

As the method determines the barbiturates indirectly through estimation of the mercury extracted as a complex it is of the utmost importance that no entrainments are formed from the water phase. In the beginning of this investigation the organic layer was filtered through a paper moistened with the solvent. With this method good results and excellent standard curves were obtained. During experiments on the micro scale and work on the theory for the complex formation it was found that paper adsorbs small amounts of the complex. Therefore, the paper was replaced by glasswool. The adsorption of the complex by the paper seems to be proportional to the amount of the complex. Thus the slopes of the standard curves changed by 12 per cent when replacing paper with glasswool.

The amount of mercury is not critical. A 10 to 100-fold excess is provided in the general procedure and at least a 4-fold excess in the micro modification.

Other mercury salts than the nitrate can be used. Thus, mercuric acetate gave 99·1 and 98·6 per cent recovery, mercuric chloride 100·2

TABLE II

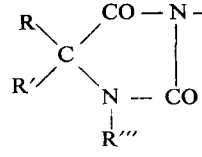
REPRODUCIBILITY OF THE PROPOSED METHODS

Barbituric acid	Taken μg.	Found μg.	Difference μg.	Error per cent
5-ethyl-5-(3-methylbutyl)	295·5	295·6	−0·1	0·3
	177·3	173·5	−4·0	2·2
	88·7	89·3	+0·6	0·7
	29·6	31·6	+2·0	6·8
	4·10	3·97	−0·13	3·1
	4·10	4·01	−0·09	2·2
	4·10	3·93	−0·17	4·1
5:5-diallyl-	229·0	233·1	+4·0	1·7
	183·2	180·9	−2·3	1·3
	91·6	93·0	+1·4	1·5
	45·8	44·1	−1·7	3·7
5-ethyl-5-phenyl-	253·5	263·7	+10·2	4·0
	202·8	204·9	+2·1	1·0
	101·4	97·1	−4·3	4·2
1-methyl-5-ethyl-5-phenyl	302·0	308·6	+6·6	2·2
	181·2	180·9	−0·3	0·2
	120·6	117·1	−3·5	2·9
	90·6	88·0	−2·6	2·9

per cent and mercuric perchlorate 96·7 and 100·9 per cent recovery when assaying about 100 μg. of amylobarbitone. Phosphate, carbonate, and borate which are present in the buffer solutions do not interfere but

TABLE III

EXTRACTION OF HYDANTOINS



R	R′	R″	R‴	Recovery per cent
CH_3	$(CH_3)_2.CH.CH_2$	H	H	0
CH_3	i-C_3H_7	H	H	0
$(CH_2)_4$		H	H	0
$(CH_2)_5$		H	H	3
CH_3	n-C_5H_{11}	H	H	14
CH_3	n-C_6H_{13}	H	H	50·6
CH_3	n-C_7H_{15}	H	H	68·6
CH_3	n-C_9H_{19}	H	H	81·0
CH_3	C_6H_5	H	H	2
C_2H_5	n-C_4H_9	H	H	10
C_6H_5	C_6H_5	H	H	41·9
C_2H_5	C_6H_5	CH_3	H	0
C_6H_5	C_6H_5	CH_3	H	61·2
C_6H_5	C_6H_5	H	CH_3	6

bromide, iodide, and cyanide are not permissible. Bromide and iodide form compounds with mercury which are extracted by the chloroform. The mercuric cyanide is so little ionized that no barbituric acid-mercury complex can be formed. In the same way EDTA (edetic acid) masks the mercuric ions and makes the determination impossible.

Generally when working with dithizone all reagents must be carefully purified to minimize blanks from heavy metals. Here this is quite unneccessary as no reagents except the solvent ever come in contact with the dithizone.

Only a slight excess of dithizone is necessary for complete reaction with the mercury in the barbituric complex. This indicates the mercury-dithizone complex to be by far the stronger of the two.

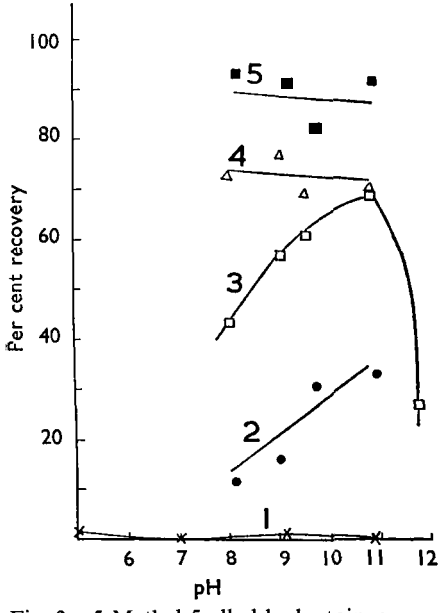


Fig. 3. 5-Methyl-5-alkyl-hydantoin complexes extracted with chloroform at different pH.
1. R = *iso*-butyl. 2. R = *n*-pentyl. 3. R = *n*-hexyl. 4. R = *n*-heptyl. 5. R = *n*-nonyl.

The complexes have been prepared by methods analogous to the analytical procedure suggested in this paper. These results will be published separately but some of them should be reported here. Barbituric acids substituted at both the nitrogen atoms give no extractable mercuric compounds. Barbituric acids substituted at one nitrogen atom form a complex consisting of 2 mols of barbiturate and 1 atom of mercury. Barbituric acids without any substitution at the nitrogen atoms form a complex consisting of 1 mol of barbiturate and 1 atom of mercury. One further condition is that the carbon atom 5 should have substituents.

The good reproducibility of the method is shown in Table II.

100 μg. of the following substances did not interfere when tested by the standard method using chloroform as the solvent at pH 7·0: theophylline, caffeine, theobromine, dihydroxypropyltheophylline, pentamethylenetetrazole, hexamethylenetetramine, sulphanilamide, sulphathiazole, nitrofuracin, acetanilide, phenacetin, amiphenazole, papaverine.

100 μg. of the following substances gave interferences which have been expressed as 5-ethyl-5-(3-methylbutyl)barbituric acid: stearic acid 2 μg., methylthiouracil 2 μg., propylthiouracil 2 μg., and phenazone 7 μg. Greater amounts sometimes gave more interference. Thus 5000 μg. of propylthiouracil corresponds to 120 μg., 5000 μg. of phenazone to 75 μg. and 5000 μg. of phenacetin to 30 μg. of 5-ethyl-5-(3-methylbutyl)barbituric acid. Greater amounts of stearic acid cause gross interference.

The hydantoins which are structurally closely related to the barbituric acid derivatives usually interfere strongly and some of them might be determined with the method. The nature of the substituents has a pronounced effect on the recoveries, this is evident from Figure 3 and

Table III. The extractions were made at several hydrogen concentrations with 100 μg. of the substances in question. In the Table only the values at pH 9 are shown. All extractions were made with chloroform. The recoveries are calculated on the assumption that 1 mol. of mercury combines with 2 mols of *N*-substituted hydantoins and with 1 mol. of the others.

Without any speculation on the theory of the extraction it must be mentioned that divalerylimide is not extracted at all while, e.g., neopentylsuccinimide is completely extracted at pH 7 to 11 and 4-ethyl-4-methyl-2:6-dioxopiperidine, a compound found useful as an antidote against barbiturate poisoning, is also completely extracted at pH 9.

References

1. e.g., Snell and Snell, *Colorimetric Methods of Analysis*, 3rd Ed., 1954, Vol. IV. p. 97–103.
2. Pedley, *J. Pharm. Pharmacol.*, 1950, **2**, 39.
3. Pfeil and Goldbach, *Z. physiolog. Chem. Hoppe-Seyler's*, 1955, **302**, 263.
4. Björling, Berggren, Willman-Johnson, Grönwall and Zaar, *Acta. chem. scand.*, 1958, **12**, 1149.