A VOLUMETRIC DETERMINATION OF BARBITURIC ACID DERIVATIVES

By E. Pedley

From the North Western Forensic Science Laboratory, Preston.

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Several volumetric methods of determination of barbituric acid derivatives have been described in the literature, most of them depending upon the acidity of the imido hydrogen. These methods have recently been reviewed by Mangouri and Milad[1], who classified them into two classes, one class consisting of methods involving direct titration with alkali, and the other titration with standard silver nitrate solution. These authors found most of the published methods to be wanting in accuracy unless modified, and they developed a method depending upon the precipitation of the barbiturate as its silver salt with silver nitrate solution and determination of the excess of silver. Apart from gravimetric determination of tablets and sodium salts, there are no pharmacopœial methods of assay of the barbiturates.

Barbituric acid derivatives give precipitates with other heavy metals besides silver and precipitation with a strong nitric acid solution of mercuric nitrate (Millon's Reagent) is commonly used as a preliminary qualitative test for their presence. This precipitate appears to be quite insoluble in excess of the cold reagent, but is appreciably soluble on boiling. It was considered feasible that a method of assay depending upon this precipitate could be developed. The solution of mercuric nitrate, however, is necessarily strongly acid and dilute neutral volumetric solutions are therefore difficult to obtain.

It was found however, that a solution of mercuric perchlorate also gives a precipitate with neutral solutions of the barbiturates and as this may be readily obtained in a dilute, almost neutral, solution, an attempt was made to develop a method of assay using this reagent. A solution of mercuric perchlorate is readily prepared in a 0·1M concentration by dissolving mercuric oxide in dilute perchloric acid solution[2], and the method developed consists essentially of adding a known excess of this solution to an aqueous solution of the barbiturate, filtering off the precipitate of mercuric barbiturate and determining the excess of mercury volumetrically.

Precipitation with this solution is not specific for the barbiturates and a precipitate is obtained with the sulphonamides, cinchophen, phenazone and carbromal. As, however, these drugs (except perhaps the last) are not usually found associated with the barbiturates, this fact would not appear to detract from the value of the method, at least in its application to pharmaceutical preparations.

Experimental

Mercuric Perchlorate Solution.—An approximately 0·1M solution was prepared by boiling an excess (25 g.) of mercuric oxide with 28 g. of

60 per cent. perchloric acid in 200 ml. of water, adjusting to 1 l. and filtering. This solution contains approximately 36·6 g. of mercuric perchlorate and remains stable indefinitely.

Determination of the Mercury.—Earlier determinations of the excess of mercury were carried out, using the process described in the British Pharmacopœia for the assay of mercuric chloride, i.e., reduction with alkaline formaldehyde and absorption of the precipitated mercury with excess of standard iodine. This method, however, did not prove completely successful, as widely varying results were often obtained. As it was found that the perchlorate ion differs from the chloride ion in not forming complexes in the presence of the thiocyanate ion, the ammonium thiocyanate method of assay was used and found to be completely satisfactory. It was found that the final oxidation of the solution by boiling with nitric acid before titration with ammonium thiocyanate solution was not essential, and the same titration figures were obtained with boiled solutions as with the unboiled ones. This was found to be so even in the presence of starch.

Precipitation of the Barbiturate.—Analysis of the precipitate obtained with a barbiturate containing an unsubstituted imido nitrogen indicated a monomolecular compound with the mercury, e.g. barbitone gave a precipitate containing 7·26 per cent. of nitrogen and 51·43 per cent. of mercury, $(C_8H_{10}O_3N_2)Hg$ requires N, 7·33 per cent., Hg, 52·3 per cent. Hence 2 l. of 0·1M mercuric perchlorate solution is equivalent to one molecule of the barbiturate.

As the barbituric acid derivatives themselves are insoluble in water, preliminary experiments were performed by adding the mercuric perchlorate solution to a boiling solution of the barbiturate in water. Owing to the slight solubility of the precipitate in boiling water, however, low results were obtained. This tendency was emphasised when the volume of water used to dissolve the barbiturate was increased. At high concentration also, low figures were obtained, probably due to complex formation[3], but this was only manifest in concentrations over 8·0 millimoles/l. and if the concentration was maintained between 2·5 millimoles and 6·25 millimoles/l., concordant results were obtained (Table I).

In the method finally adopted, mixing of the two solutions was carried out at room temperature at a dilution of about 0·2 g. in 150 ml. The effect of buffering the barbituric acid solution was also investigated, as

TABLE I

EFFECT OF CONCENTRATION OF BARBITURATE

	Amount added	Dissolved in ml.	Found	Percentage
	g.		g.	
Barbitone	0·1005	200	0·0995	98·97
	0·2010	200	0·1989	98·95
	0·3015	200	0·2947	97·74
Phenobarbitone	0·1000	200	0·0999	99·91
	0·2000	200	0·1998	99·68
	0·3000	200	0·2966	98·87

the sodium salts of the barbiturates are alkaline in reaction, and here again it was found that a wide variation of *p*H was possible without any appreciable effect on the results obtained (Table II). The precipitate was found to be appreciably soluble in excess of perchloric acid however, and no precipitate is obtained in the presence of excess of chloride ion or mineral acids.

TABLE II

EFFECT OF *p*H OF SOLUTION OF SODIUM PHENOBARBITONE

Amount added g.	Dissolved in ml.	*p*H	Found	Percentage
0·2438	50	8·6	0·2296	94·18
0·2438	50	8·0	0·2306	94·58
0·2438	50	7·0	0·2296	94·18
0·2438	50	6·0	0·2296	94·18
0·2438	50	5·0	0·2306	94·58

No difficulty was experienced in filtering the solution after precipitation, and the presence of starch had no effect on filtration if the solution was boiled to partially hydrolyse this. In order to avoid a long washing of the precipitate, complete filtration was avoided and an "aliquot part" method used throughout.

METHOD ADOPTED

Weigh out approximately 0·2 g. of the barbiturate (or its equivalent in powdered tablets) and dissolve in 50 ml. of boiling distilled water. Boil for a few minutes after solution has been achieved and add about 80 ml. of water and allow to cool to room temperature. Transfer the solution to a 200 ml. measuring flask and wash the container several times adding the washings to the contents of the flask, until the volume is about 150 ml. Now add slowly, with rotation of the contents of the flask, 25 ml. of the solution of mercuric perchlorate and allow the mixture to stand with frequent shaking for 15 minutes. Adjust the volume of the solution to 200 ml. and then filter through a double fluted filter paper into a dry 100 ml. measuring flask. Reject the first 50 ml. of the clear solution and then collect 100 ml. Transfer this filtrate to a conical flask, washing the measuring flask with 20 ml. quantities of 10 per cent. nitric acid solution, and adjust the volume of liquid to about 250 ml. Add 1 ml. of a saturated solution of ferric alum and titrate with N/10 ammonium thiocyanate. Repeat the operation without the barbiturate. The difference in the two titrations represents the number of ml. of mercuric perchlorate solution required for the barbiturate.

Each ml. of N/10 ammonium thiocyanate is equivalent to the molecular weight of the barbiturate/20,000, e.g.:—barbitone 0·00921 g.; barbitone sodium 0·0103 g.; phenobarbitone 0·0116 g.; phenobarbitone sodium 0·0127 g.

RESULTS

This procedure was applied to the Pharmacopœial barbiturates and their preparations. As a check on the method, the B.P. assay was used in

the case of the sodium salts and a Kjeldahl assay was carried out on the barbiturates themselves.

TABLE III

BARBITURATES

Compound	Perchlorate Method			B.P. Method or Kjeldahl
	Added g.	Found g.	Percentage	Percentage
Barbitone	0·2021	0·2008	99·35	99·75
	0·2029	0·2019	99·50	
Barbitone Sodium	0·2531	0·2523	99·68	99·59
	0·2522	0·2513	99·64	
Phenobarbitone	0·1984	0·1972	99·40	99·94
	0·2030	0·2018	99·40	
	0·1796	0·1786	99·44	
Phenobarbitone Sodium	0·2273	0·2230	98·10	99·12
	0·2223	0·2198	98·87	

TABLETS

Various makers' tablets were used and as checks on the method, the B.P. gravimetric assay was used throughout. In this application of the suggested procedure, certain advantages were seen over the B.P. assay. In the latter, some makers' tablets tended to give difficulty by the formation of emulsions in the preliminary extraction. Other makers' tablets on the other hand, were quite free from this difficulty. The starch vehicle gave no difficulty in the mercuric perchlorate method but in order to ensure rapid filtration from the mercury precipitate, 5 minutes' gentle boiling was used in order to partially hydrolyse the starch. After this, filtration was as rapid as in the absence of starch.

It will be noticed that in the majority of cases the perchlorate method gave slightly higher results than the Pharmacopœial method. No explanation of this was discovered but it may be that the starch present in the tablets is a disturbing factor. This is borne out to some extent by the fact that though no such discrepancy was observed with the sodium salts of the barbiturates it was observed with a factitious " tablet," i.e., a simple mixture of starch and barbiturate. These figures are given in Table IV, " Maker B," and Table V, " Maker C."

N-*Substituted Barbiturates.*—The assay outlined above was applied to methylphenobarbitone and hexobarbitone sodium. Satisfactory results were obtained only with the former. In the case of hexobarbitone sodium the precipitated mercury compound was appreciably soluble in water and results obtained were very low.

Methylphenobarbitone on the other hand, though closely related to hexobarbitone gives a practically insoluble mercury derivative, but owing to the almost complete insolubility of the methylphenobarbitone itself the method had to be modified in the following way:—Heat 0·4 to 0·5 g. of the methylphenobarbitone with 20 ml. of N/10 sodium hydroxide and

120 ml. of water to 60°C. until dissolved. While maintaining at this temperature add 10 ml. of 10 per cent. acetic acid followed immediately by 25 ml. of mercuric perchlorate solution. Allow the mixture to cool to room temperature, transfer to a measuring flask with washing, adjust to 200 ml., stand for 15 minutes with shaking and proceed as in the original method.

TABLE IV

TABLETS OF BARBITONE AND BARBITONE SODIUM

Compound	Maker	Perchlorate Method			B.P. Method
		Added	Found	Percentage	Percentage
Barbitone 5 grains	A	0·1665	0·1423	85·46	82·52
		0·2000	0·1704	85·20	
		0·1747	0·1491	85·34	
	B	0·2102	0·1308	62·22	61·28
		0·2107	0·1308	62·07	
	C	0·2005	0·1510	75·50	73·27
		0·2015	0·1528	75·83	
Barbitone Sodium 5 grains ...	A	0·2090	0·1463	70·00	67·36
		0·2193	0·1535	70·00	
	B	0·2000	0·1669	83·45	83·35
		0·2062	0·1730	83·89	
	C	0·1995	0·1607	80·55	77·69
		0·2034	0·1627	80·00	

TABLE V

TABLETS OF PHENOBARBITONE AND PHENOBARBITONE SODIUM

Tablet	Maker	Perchlorate Method			B.P.
		Added	Found	Percentage	Percentage
Phenobarbitone 1 grain... ...	A	0·2103	0·1183	56·25	53·60
		0·2006	0·1137	56·69	
		0·1892	0·1067	56·39	
	B	0·1949	0·1298	66·64	66·00
		0·2329	0·1554	66·71	
		0·2092	0·1392	66·53	
	C	0·2226	0·1392	62·53	61·23
		0·1990	0·1253	62·96	
Phenobarbitone Sodium 1 grain	A	0·1945	0·0965	49·61	48·16
		0·2253	0·1118	49·62	
½ grain	B	0·3967	0·2083	52·51	52·53
		0·3996	0·2108	52·78	
	C	0·2538	0·1067	42·03	40·30
		0·2461	0·1041	42·30	

In this case one molecule of mercury combines with two molecules of the barbiturate so that 1 ml. of N/10 ammonium thiocyanate is equivalent to 0·02464 g. of methylphenobarbitone. Results obtained are given in Table VI.

TABLE VI

METHYLPHENOBARBITONE

Added	Found	Percentage	B.P.
0·4200	0·4139	98·57	99·51
0·3785	0·3746	98·97	
0·4820	0·4762	98·91	

SUMMARY

1. A method of assay of Pharmacopœial barbiturates and their tablets is suggested depending upon their precipitation as a mercury compound with excess of a standard solution of mercuric perchlorate followed by determination of the excess of mercury.

2. The precipitates obtained with the various barbiturates vary in solubility in water, but it is only in the case of hexobarbitone that the solubility is sufficient to interfere seriously with the assay.

REFERENCES

1. Mangouri and Milad, *Quart. J. Pharm. Pharmacol.*, 1947, **20**, 109.
2. Britton, *Conductimetric Analysis*, 1934, 117.
3. Britton, *ibid.*, 118.