Assay of Aminophylline-Vinbarbital Solutions*

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An assay for aminophylline-vinbarbital solutions through their silver salts has been developed. This method combines the U. S. P. XIV assay for aminophylline with a modification of the Budde assay for barbiturate. The ability of the Budde assay to eliminate interference by barbiturate decomposition products is investigated and confirmed. Discussion and quantitative evaluation of sources of error in the assay are presented. One of these errors is directly applicable to the U. S. P. XIV assay of aminophylline.

THE PROBLEM of assaying barbiturate solutions for active barbiturate, i. e., unhydrolyzed barbiturate, has been approached by many workers with several different methods. The U. S. P. XIV method, that of extraction with an immiscible solvent, evaporating the solvent, and weighing the residue as barbiturate, only partially solves the problem of decomposition. Rotondaro (1) and van Itallie and Steenhauer (2) were able to separate the barbiturate from its decomposition products by a complicated and tedious series of extractions. Bell (3) discussed several reported complexes as being unreliable, the unreliability being due probably to insufficient knowledge of these complexes.

The direct alkaline titration of barbiturates as described by von Babitsch (4) does not eliminate the acidic decomposition compounds (usually stronger acids than the barbiturate), and conductimetric titration [Bartilucci and Discher (5)] would also find quantitative differentiation between the barbiturate and its decomposition products difficult.

The formation of insoluble mercury barbiturate and assay by weighing this salt [von Babitsch (4)] is subject to the errors caused by acidic decomposition.

Budde (6) developed a method of titrating barbiturates in sodium carbonate solution with silver nitrate, the end point being the first permanent appearance of a precipitate of silver carbonate. This method has been found to be quantitative for phenobarbital in the presence of the usual decomposition products (7). The method depends on the fact that silver barbiturate is soluble in sodium carbonate solution but is undissociated, whereas the silver compounds of the decomposition products are dissociated and therefore cannot hold the silver in solution. The barbiturate actually can dissolve silver carbonate under these conditions and therefore obviously is dissociated less than is the silver carbonate in the titration medium. In the case of vinbarbital,[1] the silver barbiturate fluoresces in alkaline medium and obscures the end point under the conditions of Budde's assay as modified by Jatul (7).

The cobalt colorimetric method for barbiturates reported first by Zwikker (8), modified by Koppanyi (9, 10), and made quantitative by Cohen (11), involves the development of a blue color, using as reagents cobaltous chloride and isopropylamine in anhydrous chloroform. No conclusive investigation regarding possible interference by hydrolysis products of the barbiturate has been presented.

PROCEDURE

The silver salts of barbiturates are soluble in ammonium hydroxide solution as well as in sodium carbonate solution. Therefore, the U. S. P. XIV assay for aminophylline, involving precipitation of silver theophylline and subsequent titration of the filtrate with ammonium thiocyanate for excess silver, need only be modified by the condition that sufficient silver nitrate be added to accommodate both the barbiturate and the theophylline, plus a suitable excess. This excess is required to prevent competition for the silver between the theophylline and the barbiturate.

An aliquot of the filtrate can be titrated for the theophylline assay. Another aliquot is made slightly acidic with nitric acid and is extracted with chloroform in five 25-ml. portions. The chloroform contains the barbiturate and its decomposition products. The combined chloroform extracts are evaporated to dryness in an Erlenmeyer flask on a steam bath, the residue is taken up in 5% sodium carbonate solution, a slight excess of silver nitrate added, and the solution is brought to a definite volume. Then it is centrifuged in the presence of nitrobenzene to remove the slight amount of precipitated silver carbonate, and an aliquot is made acidic and titrated with ammonium thiocyanate. The amount of silver found in the sample is converted by calculation to its equivalent of the unhydrolyzed barbiturate.

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[1] "Delvinal" is Sharp & Dohme's trade mark for this compound.

EXAMPLE OF PROCEDURE AND RESULTS

The vinbarbital acid and theophylline, U. S. P., were dried at 105° for four hours. Four mixtures of the two, representing approximately 275 mg. of vinbarbital and 1 Gm. of anhydrous theophylline in each sample, were placed in 200-ml. volumetric flasks. Each sample was treated as follows:

There were added 0.5 ml. of 95–100% ethylenediamine, 2.86 ml. of 28% reagent ammonia water, and 100 ml. of distilled water, and solution was effected. Exactly 75 ml. of 0.1 *N* silver nitrate was added, and this was followed by sufficient distilled water to make a volume of 200 ml. The mixture was allowed to stand at room temperature for one hour. (Heat, which is used in the U. S. P. XIV assay, was not employed, since this would hydrolyze some of the barbiturate.) The flask was cooled in an ice bath with occasional agitation for twenty-five minutes.

The solution was kept cold while it was filtered through a sintered filtering crucible of medium porosity. The filtrate was allowed to return to room temperature; then a 50-ml. aliquot was titrated with 0.1 *N* ammonium thiocyanate in the presence of 6 ml. of nitric acid and 2 ml. of ferric ammonium sulfate T. S. for the theophylline assay.

An aliquot (100-ml.) of the filtrate was made slightly acidic by the addition of 7 ml. of nitric acid in a separatory funnel, after which it was extracted with five 25-ml. portions of chloroform, the chloroform extracts being pooled in an Erlenmeyer flask and evaporated to dryness on a steam bath.

The residue was taken up in 40 ml. of 5% sodium carbonate solution in two portions, and the flask was washed with four 10-ml. portions of distilled water, the solutions being collected in a 100-ml. volumetric flask. There was added 1.3 ml. of 10% silver nitrate solution, followed by sufficient distilled water to make a volume of 100 ml.; then the mixture was agitated thoroughly.

The solution was placed in a centrifuge tube, 2 ml. of nitrobenzene was added, the mixture was thoroughly agitated and then was centrifuged at 2,400 r. p. m. for twenty-five minutes. An aliquot (25-ml.) was removed carefully, made acidic with 4 ml. of nitric acid, and 2 ml. of ferric ammonium sulfate T. S. was added. This sample then was titrated with 0.01 *N* ammonium thiocyanate, and the silver found was converted by calculation to vinbarbital. The results of this assay procedure may be found in Table I.

TABLE I.—ASSAY OF AMINOPHYLLINE-VINBARBITAL SOLUTIONS

Sample	Theophylline Used, Gm.	Theophylline Found, Gm.	%
1	1.1758	1.1670	99.25
2	0.9729	0.9715	99.86
3	1.0090	1.0062	99.72
4	1.0426	1.0363	99.40
Sample	Vinbarbital Used, Gm.	Vinbarbital Found, Gm.	%
1	0.2738	0.2711	99.01
2	0.2860	0.2837	99.20
3	0.2803	0.2843	101.43
4	0.2660	0.2653	99.74

DISCUSSION

The procedure of bringing a suspension to a definite volume and then filtering it (the initial step of this method) is subject to error due to the volume of the precipitate. By a pycnometric method, the specific gravity of a prepared sample of silver theophylline was determined to be 2.11. The error can be calculated for both determinations from the specific gravity of the silver theophylline and the assay results for the theophylline and barbiturate.

For the theophylline correction:

$$\frac{-1.6\ (\text{Gm. of theophylline found})}{\text{Sp. gr. of silver theophylline}} \times {}^{1}/_{2} \times \frac{\text{mg. theophylline found}}{100}$$

or

$$-0.0000038\ (\text{mg. theophylline found})^2 = \text{correction in mg. for theophylline}$$

For the vinbarbital correction, by the same reasoning:

$$-0.0000038\ (\text{mg. theophylline found})\ (\text{mg. vinbarbital found}) = \text{correction in mg. for vinbarbital}$$

These corrections are of the order of 0.4% for the amounts here assayed.

The U. S. P. XIV procedure of washing the precipitated silver theophylline could be followed if the filtrate then were brought to some definite volume. Considerably more time would be required, however, for that procedure.

It was found that the U. S. P. XIV assay for aminophylline yields low results of approximately 2.66 mg. of theophylline in the quantities here tested, due to the solubility of silver theophylline in ammonium hydroxide solution. This was determined by following the procedure with approximately 90% of the silver nitrate necessary to combine with all the theophylline used. The experiment was done in quadruplicate on the same parent solution of theophylline; two of the precipitated solutions being heated and two being allowed to stand at room temperature. The unheated samples showed titratable silver after filtration equivalent to 2.70 mg. of theophylline, while the heated samples showed titratable silver after filtration equivalent to 2.62 mg. Ammonium thiocyanate, 0.01 *N*, was used in this titration. These results cannot be considered significantly different. The point made here is that the procedure of heating the suspension of silver theophylline is not necessary from the standpoint of accuracy; it facilitates filtration only.

The solubility of silver theophylline in the solution to be filtered, using the U. S. P. XIV method of assay (i. e., addition of ammonium hydroxide), was not compared with its solubility in the absence of the ammonium hydroxide, as suggested by Griffenhagen and Brady (12). However, these values placed the error to be found in the U. S. P. XIV method on a quantitative basis.

The question of error due to the use of nitrobenzene to facilitate removal of silver carbonate from the sample for titration of silver vinbarbital was approached as follows: the solubility of the nitrobenzene in a 2% sodium carbonate solution, essentially

the solution present in the sample, was found to be 0.16% v/v. This agrees with the published solubility in water (13). To get the complete answer immediately, a parent solution of vinbarbital in 2% sodium carbonate with sufficient silver nitrate added to react with only 90% of the barbiturate was divided into four parts. The proper amount of nitrobenzene was added to two of these portions, after which they were carried through the centrifugation and titration procedures. The portions treated with nitrobenzene showed an average value of 0.14% less than the untreated portions, thus approaching the maximal dilution attributable to solubility of nitrobenzene. Since saturation would only be approached by the nitrobenzene in the procedure used, this result is quite logical and indicates that no loss of silver barbiturate is incurred due to solubility of the compound in nitrobenzene.

Corrections for the sources of error described above should be made in the following sequence:

1. +0.16% (maximum) to the vinbarbital result due to the solubility of nitrobenzene in the sodium carbonate solution.
2. +2.66 mg. to the theophylline result because of the solubility of silver theophylline in ammonium hydroxide solution.
3. A negative correction to the vinbarbital result for the volume occupied by the silver theophylline in the original volumetric solution.
4. A negative correction to the theophylline result as applied in correction No. 3.

The latter two corrections should be made using the figures corrected by No. 1 and No. 2, but should not influence each other; i. e., No. 3 should not use the value for theophylline already corrected by No. 4.

The results of these corrections on the assays tabulated above are shown in Table II.

TABLE II.—APPLICATION OF CORRECTIONS TO ASSAYS IN TABLE I

Sample	Theophylline, Found, Uncorr. %	Total Correction, %	Theophylline Found, Corr., %
1	99.25	0.21	99.04
2	99.86	0.11	99.75
3	99.72	0.12	99.60
4	99.40	0.14	99.26
Av.	99.56		99.41
Sample	Vinbarbital, Found, Uncorr. %	Total Correction, %	Vinbarbital Found, Corr., %
1	99.01	0.29	98.72
2	99.20	0.21	98.99
3	101.43	0.22	101.21
4	99.74	0.23	99.51
Av.	99.85		99.61

TABLE III.—APPLICATION OF ASSAY TO HEAT-TREATED SAMPLES

Treatment	Vinbarbital Av. 2 Samples, Mg.	% Decompn. Compared to Initial
No heat	35.88	0
1/2 hr. 120° C.	34.11	95.07
1 hr. 120° C.	32.36	90.19
2 hr. 120° C.	29.49	82.19

DECOMPOSITION OF VINBARBITAL

In order to show that this assay method will evaluate the decomposition of the barbiturate, a parent solution of vinbarbital was made using ethylenediamine as the base. This was divided into portions, two of which were not heated, the remainder being autoclaved and assayed according to the full recommended procedure. No precipitate appeared in any sample when the silver nitrate was added. The results of this experiment are shown in Table III.

These results show a positive and significant response of the assay to the hydrolysis of the barbiturate and reaffirm the validity of the silver method as described by Budde.

SUMMARY

1. An assay method for aminophylline-vinbarbital solutions is presented. This assay determines both substances through their silver salts.

2. It is shown that the method determines active, or unhydrolyzed, barbiturate. Other methods either do not or have not been shown to eliminate errors due to decomposition products of the barbiturate.

3. Sources of error in the assay are discussed and evaluated quantitatively.

4. An error inherent in the U. S. P. XIV assay of aminophylline is placed upon a quantitative basis.

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