Notes on the Determination of Theophylline, Alone and in Combinations, by the U. S. P. Aminophylline Method*

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In adapting argentometric methods to the assay of certain nitrogenous organic compounds, the U. S. P. assay of theophylline was reviewed in detail. Increased precision of results was noted when (*a*) the precipitated mixture was cooled before filtration, and (*b*) when the ammonia presently required in the assay was omitted. The substitution of other buffers (lactates and acetates) for the ammonia was not advantageous. Physical and chemical characteristics of silver theophyllinate were reviewed, and modification of the present U. S. P. assays of theophylline and aminophylline is suggested.

THE PRESENT U. S. P. method for the assay of theophylline in aminophylline became official with the Eleventh Revision, Second Supplement (1), and has continued virtually unchanged through subsequent pharmacopeial revisions. It has since been adopted for the determination of theophylline in theophylline tablets, theophylline and sodium acetate, and theophylline and sodium acetate tablets. While other official works and standard methods (2, 3) employ gravimetric procedures, the U. S. P. assay is argentometric, and is based upon the reaction of theophylline with 0.1 *N* silver nitrate to form silver theophyllinate, $Ag(C_7H_7N_4O_2)$. Precipitation is carried out in a slightly ammoniacal solution (about 1 per cent); the mixture is digested for fifteen minutes on a water bath and filtered while hot. On cooling, excess 0.1 *N* silver nitrate is titrated with 0.1 *N* ammonium thiocyanate using ferric ammonium sulfate T. S. as an indicator (4).

Since silver nitrate forms insoluble compounds with many nitrogenous organic substances, assay methods similar to the official procedure for theophylline in its compounds have been suggested for many such substances (5–7). These methods differ from the U. S. P. in the details of temperature of filtration, quantity of ammonia used, and addition of buffer salts. In recent re-evaluations of these methods (8), it has been noted that many of the silver precipitates fail to form, or are redissolved, when a slight excess of ammonia is present. Further, some partially dissolve upon moderate heating, and precipitation has been observed in theophylline filtrates upon cooling after warm filtration. Also, the use of buffers in theophylline determinations has not been reported. These facts have made an investigation of the present U. S. P. method for the determination of theophylline in compounds seem warranted.

EXPERIMENTAL

Samples of theophylline and aminophylline were assayed by the U. S. P XIII method for theophylline in aminophylline, as shown in Table I. Samples were then analyzed by modifying this method by allowing the sample to cool to room temperature before filtration. Results are given in Table I under modification I. The addition of 2 cc. of a 10% solution of sodium acetate as a buffer prior to precipitation yielded the results given in Table I as modification II, and with 2 cc. of a 50% solution of sodium *r*-lactate as a buffer, those of modification III. Samples were then analyzed by omitting the ammonia required by the U. S. P., using no buffer, and cooling the precipitated solution to room temperature before filtration. Results are given in the table under modification IV. This last modification appears to give the most uniform results.

DISCUSSION

When the U. S. P. method was used, precipitation sometimes occurred in the hot filtrate as it was cooled to room temperature for titration. Many other organic silver complexes are quite soluble in hot water (8). Other similar argentometric methods direct cooling before filtration (5, 9). By comparison of the results in the table, it may be seen that the maximum deviation of results from the average values obtained appreciably decreases if the mixture is not filtered "while still warm" (4).

Mangouri and Milad (5) suggested the use of a sodium acetate buffer in the argentometric assay of barbituric acid derivatives. They called attention to the fact that such solutions tended to become more acidic as the silver compounds were precipitated, and that this condition sometimes caused incomplete precipitation, and could be prevented by the use of the buffer. However, when sodium acetate was used in the theophylline determinations, unusually high percentages were obtained (modification II). Due to the possibility that this error was caused by the co-precipitation of silver acetate, a salt which would yield a more soluble silver compound, sodium *r*-lactate, was employed in a further series of deter-

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minations. The high values again obtained (modification III) and the formation of bulky, gelatinous precipitates which were difficult to filter and wash, and which were relatively unstable, seemed to indicate that addition of alkaline buffers would not increase the accuracy of the U. S. P. method.

The use of ammonia in the argentometric precipitation of the xanthines was first suggested by Kunze (10) for the precipitation of theobromine. No other supporting evidence for the use of ammonia has been found in the literature. Ammonia was used in the proposed U. S. P. XI assay for theophylline in aminophylline (11) and was required in the adopted U. S. P. XI assay, although the amount was changed from 7 to 8 cc. per sample. While ammonia may aid the initial solution of the sample, no difficulty was encountered when it was omitted. The character of the precipitate is relatively unchanged as to stability and ease of filtration in nonammoniacal solution.

On the other hand, silver theophyllinate and most other silver precipitates are soluble in ammonia, and this places a quantitative aspect upon the amount of ammonia used which the assay does not closely control. Its omission, accompanied by cold filtration, yielded the best series of determinations (modification IV).

It should be noted that the results for theophylline in aminophylline when ammonia is omitted are somewhat higher than those obtained when it is used. The reason for this is not apparent, but it is not due to any reaction of the ethylene diamine, as was shown by running blank determinations with ethylene diamine alone.

It was also observed that the precipitates formed were less gelatinous when a larger excess of silver nitrate was present, and the use of 50 ml. of 0.1 *N* silver nitrate in place of 20 ml. aids in forming a less bulky precipitate.

TABLE I.—DETERMINATION OF THEOPHYLLINE ALONE AND IN AMINOPHYLLINE BY U. S. P. METHOD AND FOUR MODIFICATIONS

Method	Sample	Sample Wt.	Theophylline, %
U. S. P. XIII	Theophylline A	0.3231	99.08[a]
	Theophylline B	0.2966	98.31
	Theophylline C	0.3275	97.86
	Theophylline D	0.2794	98.57
		Av.	98.45
	Aminophylline A	0.2349	80.01[b]
	Aminophylline B	0.3470	81.48
	Aminophylline C	0.2167	81.09
		Av.	80.86
Modification I[c]	Theophylline A	0.3088	98.41[a]
	Theophylline B	0.3335	98.38
	Theophylline C	0.3126	98.12
	Theophylline D	0.2579	98.38
		Av.	98.32
	Aminophylline A	0.2026	80.67[b]
	Aminophylline B	0.2171	81.34
	Aminophylline C	0.1717	80.98
		Av.	81.00
Modification II[d]	Theophylline A	0.2597	101.77[a]
	Theophylline B	0.2525	103.02
	Theophylline C	0.2476	103.63
	Theophylline D	0.2503	99.58
		Av.	102.00
	Aminophylline A	0.3160	84.53[b]
	Aminophylline B	0.4000	83.45
	Aminophylline C	0.3720	83.69
		Av.	83.89
Modification III[e]	Theophylline A	0.2500	97.94[a]
	Theophylline B	0.2544	102.37
	Theophylline C	0.2530	100.65
	Theophylline D	0.2503	97.61
		Av.	99.14
	Aminophylline A	0.2450	87.80[b]
	Aminophylline B	0.2520	86.90
	Aminophylline C	0.2430	88.92
		Av.	87.87
Modification IV[f]	Theophylline A	0.1585	98.04[a]
	Theophylline B	0.2013	98.07
	Theophylline C	0.2490	97.93
	Theophylline D	0.3470	98.12
	Theophylline E	0.2012	97.99
	Theophylline F	0.2015	97.84
		Av.	98.00
	Aminophylline A	0.1448	83.50[b]
	Aminophylline B	0.1731	82.86
	Aminophylline C	0.2930	82.15
	Aminophylline D	0.2739	83.30
	Aminophylline E	0.3181	83.35
	Aminophylline F	0.1920	82.94
		Av.	83.02

[a] Expressed as theophylline monohydrate.
[b] Expressed as anhydrous theophylline.
[c] Precipitated mixture cooled before filtration.
[d] 2 cc. of 10% sodium acetate added before precipitation.
[e] 2 cc. of 50% sodium *r*-lactate added before precipitation.
[f] Ammonia omitted and mixture cooled.

CONCLUSIONS

1. The U. S. P. XIII method for the determination of theophylline in aminophylline has been subjected to laboratory evaluation in original and modified procedures.

2. The cooling of the precipitated mixture before filtration appeared to increase the precision of the assay.

3. The addition of sodium acetate or sodium *r*-lactate as a buffer was not advantageous.

4. The addition of 8 cc. of ammonia T. S. as directed in the present monograph appears unnecessary and may constitute a source of error.

5. Increasing the volume of 0.1 *N* silver nitrate to 50 ml. per sample aids in forming a less bulky precipitate.

6. It is suggested that the present assay may be improved by omitting the ammonia T. S., increasing the volume of 0.1 *N* silver nitrate used, and cooling the precipitated mixture before filtration.

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