

A METHOD FOR THE QUANTITATIVE DETERMINATION OF THEOPHYLLINE AND ETHYLENE DIAMINE THEOPHYLLINE.*

BY PHILIP W. SCHMITT.¹

Ethylene diamine theophylline or euphyllin, as it is commonly called, has been used for a long time in the treatment of certain types of cardiac cases. Our laboratory, engaged in the synthesis of this material, found it necessary to develop a method for the chemical control of the product. A survey of the literature showed no reference to a detailed procedure for the analysis of theophylline. The literature mentions the fact that theophylline forms an insoluble silver compound with silver nitrate. A silver nitrate method (1) for the analysis of theobromine, which is an isomer of theophylline, was applied to theophylline and excellent results were obtained. This method cannot be applied to the analysis of ethylene diamine theophylline because not only does the theophylline react with the silver nitrate but the ethylene diamine also reacts with the silver salt giving results that are too high.

In order to analyze ethylene diamine theophylline it was necessary to develop a reagent which would not react with ethylene diamine. Silver ammonium chloride was found to be a suitable reagent.

DETERMINATION OF THEOPHYLLINE.

Preparation of the Reagent.—Weigh out 12.0 Gm. of silver nitrate and dissolve in 300–400 cc. of water. Acidify with a little nitric acid and add sufficient ammonium chloride to precipitate all of the silver. Wash three or four times by decantation. Add 300 cc. of water and 90 cc. of ammonia water (28%). Shake until the silver chloride is dissolved. Dilute to one liter and filter. (The reagent should be kept in the dark when not in use.)

Standardization of the Reagent.—Burette out 50 cc. of the reagent and acidify with nitric acid (1 : 1). Heat to boiling to coagulate the silver chloride. Cool. Filter and wash with nitric acid (1 : 50) and finally wash with cold water. Dry at 100° C. and heat to constant weight at 130° C.

Method.—Weigh out about 0.1 Gm. of anhydrous theophylline and dissolve in 2.5 cc. of ammonia water (1 : 9), or 0.12 Gm. of ethylene diamine theophylline and dissolve in water. Make total volume about 20 cc. Add 50 cc. of the reagent. (While the reagent is being added, the contents and flask must be kept in a water-bath, at 60–65° C.) The flask is then put in a water-bath for 30 minutes at 60–65° C. Cool the flask and contents to room temperature, dilute to 100 cc. and filter. Measure out a 40 cc. sample. Acidify with nitric acid (1 : 1) and heat to boiling. Cool. Filter and wash the precipitate with nitric acid (1 : 50) and finally with cold water. Dry at 100° C. and heat to constant weight at 130° C.

Calculation.—(Weight of AgCl)(2.5)(75.26%) = Gm. of silver not used to precipitate theophylline.

$$\begin{array}{r} \text{Weight of silver in 50 cc. reagent} \\ - \text{Weight of silver found in the filtrate} \\ \hline = \text{Weight of silver used to precipitate theophylline.} \end{array}$$

(Silver used to precipitate theophylline) (1.6693) = Gm. of theophylline in the sample.

NOTES.

1. The reagent was worked out by experiment and it was found that concordant results were obtained when the ratio employed above was used in the experiment.

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2. The flask must be kept in a water-bath from the time the reagent is added to the theophyllin solution, otherwise a very gelatinous precipitate will be obtained which will not allow the solution to filter.

3. The flasks must be lightly stoppered from the time the reagent is added to the ethylene diamine theophylline until the solution is ready to filter. During the filtration the funnels should be covered with a watch glass to prevent (as much as possible) the air from coming in contact with the silver solution.

4. The percentage error in this method is not very large. It is well within the tolerances allowed by the Government for the standardization of pharmaceutical preparations.

DETERMINATION OF ETHYLENE DIAMINE.

Ethylene diamine in combination with another compound cannot be determined by ordinary chemical methods. We can calculate the ethylene diamine content from the figures obtained by subtracting from the total nitrogen content of the ethylene diamine theophylline, the calculated nitrogen content of the theophylline as determined by the silver method.

Method.—A sample of about 0.12 Gm. of ethylene diamine theophylline is placed in a 500-cc. Kjeldahl flask with 5 Gm. of anhydrous sodium sulphate, 0.25 Gm. of hydrated copper sulphate and 10 cc. of concentrated sulfuric acid. The flask is heated gently at first and finally heated vigorously until all the ethylene diamine theophylline has been digested. Cool. Add water and alkali, and distill the ammonia into 35 cc. of *N*/10 sulphuric acid. I prefer to add 50 cc. of water and 75 cc. of sodium hydroxide solution (1 : 1) to the digestion mixture and aerate for one hour, absorbing the liberated ammonia in 35 cc. of *N*/10 sulphuric acid.

A method of calculation for the ethylene diamine theophylline, based on the theophylline and total nitrogen determination follows:

METHOD OF CALCULATION OF ETHYLENE DIAMINE THEOPHYLLINE FROM THE TOTAL NITROGEN AND THEOPHYLLINE DETERMINATIONS.

NITROGEN BY KJELDAHL METHOD.

30.61 cc. of *N*/10 H_2SO_4 required to neutralize the ammonia liberated from a 0.12 Gm. sample of ethylene diamine theophylline.

TO CALCULATE THEOPHYLLINE IN TERMS OF *N*/10 H_2SO_4 .

1 cc. of *N*/10 H_2SO_4 = 0.0014 Gm. of nitrogen.

Molecular weight of theophylline ($C_7H_8N_4O_2$) = 180.09

$\frac{180.09}{40,000} = 0.00450$ Gm. of theophylline corresponds to 1 cc. of *N*/10 H_2SO_4 .

$\frac{0.0914}{0.00450} = 20.31$ cc. of *N*/10 H_2SO_4 = 0.0914 Gm. of theophylline found in 0.12-Gm. sample of

ethylene diamine theophylline by the silver method.

TO CALCULATE ETHYLENE DIAMINE FROM *N*/10 H_2SO_4 .

30.61 cc. total *N*/10 H_2SO_4 used

20.31 cc. *N*/10 H_2SO_4 corresponding to theophylline

10.30 cc. *N*/10 H_2SO_4 corresponding to ethylene diamine.

1 cc. *N*/10 H_2SO_4 = 0.0014 Gm. nitrogen

Molecular weight ethylene diamine ($C_2H_8N_2$) = 60.084

$\frac{60.084}{20,000} = 0.0030$ Gm. of ethylene diamine corresponding to 1 cc. *N*/10 H_2SO_4

10.30
0.003
 0.0309 Gm. of ethylene diamine in 0.12 Gm. of ethylene diamine theophylline
 0.0914 Gm. of theophylline by silver method
 0.0309 Gm. of ethylene diamine
 $\frac{0.1223}{0.12} = 1.019 (100) = 101.9\%$ ethylene diamine theophylline in the sample considering ethylene diamine theophylline as mono-derivative (C₂H₂(NH₂)₂)(C₇H₈N₄O₂).

Ethylene diamine theophylline may be either a mono-theophylline, a di-theophylline ethylene diamine or a mixture of the two.

The preparation made by our laboratory is almost entirely mono-theophylline ethylene diamine as is shown by the analysis given by Table II. Usual commercial samples indicate a mixture of the two compounds.

The reagent is fairly sensitive. Under favorable conditions 0.5 mg. of theophylline in 5 cc. can be detected. The following tables show the accuracy with which theophylline and ethylene diamine theophylline may be determined.

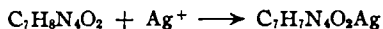
TABLE I.

Anhydrous theophylline taken	0.05	0.07	0.09	0.11	0.13	0.15	0.17	0.25	0.27	0.29	0.31
	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.
Per cent of theophylline found by several determinations	99.3	99.5	99.0	101.6	98.9	98.6	100.0	98.5	97.8	99.3	98.1
	98.2	98.3	99.6	100.3	99.2		100.2	98.1	97.8	99.0	
	98.3	97.2			99.9				98.38	98.0	
					99.2				98.00		
Average per cent found	98.6	98.3	99.3	100.9	99.4	98.6	100.1	98.3	97.99	99.0	98.1

TABLE II.

Sample of 5 cc. of a solution of ethylene diamine theophylline labeled as containing 0.12 Gm. of dry material per 5 cc.	LABORATORY PREPARATION OF ETHYLENE DIAMINE THEOPHYLLINE.		COMMERCIAL SAMPLE OF ETHYLENE DIAMINE THEOPHYLLINE.	
	Ethylene diamine.	Theophylline.	Ethylene diamine.	Theophylline.
Per cent found	0.0309 Gm.	0.0914 Gm.	0.0260 Gm.	0.0834 Gm.
Weight of ethylene diamine theophylline found	25.26	74.73	23.76	76.23
Per cent of ethylene diamine theophylline found	0.1223 Gm.		0.1094 Gm.	
	101.9%		91.1%	

The above results show that the method gives very consistent results and is relatively simple. Theophylline reacts with silver ammonium chloride to form a white gelatinous precipitate of silver theophylline which coagulates on heating.



Silver and nitrogen contents of the dried precipitate are in harmony with this formula.

TABLE III.

SILVER THEOPHYLLINE.

	C ₇ H ₇ N ₄ O ₂ Ag.	
	Calculated	Found.
Silver	37.59	37.00
Nitrogen	19.52	19.70

DISCUSSION.

As the chemical name for this product is rather long and since the only other synonym is a proprietary term of European origin, I would like to propose the word *aminophylline* as a non-proprietary name to designate these preparations. It is a short name and indicative of the chemical constitution, particularly since the ethylene diamine is used only for the purpose of keeping the theophylline in solution with a non-volatile amino base.

The method described in this paper has not been checked as to all possible interfering substances that may be met with in practice, but it has the advantage of not being influenced by ordinary salts and not being affected by the different hydrogen-ion concentrations of different samples.

Theobromine and caffeine do not give a precipitate with silver ammonium chloride. If theobromine and caffeine are used as substitutes in commercial preparations, only the theophylline will be determined by this method. However, the solubility of theobromine in the presence of ethylene diamine is very small. Whereas one mole of ethylene diamine will dissolve one mole of theophylline, it will only dissolve one fortieth of a mole of theobromine under the best conditions. Therefore the ethylene diamine content determined by this method and found to be that of pure ethylene diamine theophylline would only allow approximately two per cent, at the most, of theobromine as a substitute for theophylline. As caffeine is still less soluble than theobromine, under these conditions the method may be considered quite specific for theophylline.

Ammonia as a substitute for ethylene diamine may be determined by adding sodium hydroxide to the sample and aerating. Ten cc. of sodium hydroxide solution containing 0.8 Gm. of sodium hydroxide was added to 10 cc. of ethylene diamine theophylline solution containing 0.03 Gm. of ethylene diamine and aerated for one hour. No ethylene diamine could be found to have vaporized into the standard sulphuric acid. The same experiment was performed with a sample of an ammonia solution of theophylline containing 0.0039 Gm. of ammonia. One hundred per cent of the ammonia was recovered, showing the ease with which ammonia may be detected in the sample.

SUMMARY.

A method for the determination of ethylene diamine theophylline has been developed, based upon the use of silver ammonium chloride as a precipitant for theophylline, and the total nitrogen content by the Kjeldahl method.

It has been shown that the method gives concordant results under various experimental conditions and is sufficiently specific to exclude any substances likely to be used as substitutes.

REFERENCE.

- (1) "Food Inspection and Analysis—Leach," Fourth Edition, 1920, page 414.

ABSTRACT OF DISCUSSION.

Arno Viehoveer asked what was gained by abbreviating the name so the composition could not be recognized. He thought that the name amino theophyllin would be more satisfactory than aminophyllin.

The author replied that the thought was in mind to provide a short name for the product being placed on the market. He agreed that the name amino theophyllin would be more indicative of the constitution of the product; that they were endeavoring to get away from the proprietary name of European origin Euphyllin. He, however, agreed that the name suggested by Dr. Viehoever was preferable.

THE EFFECT OF STABILIZERS IN LARD IN RELATION TO ITS USE IN OINTMENT OF POTASSIUM IODIDE, N. F. V.***

BY WILLIAM J. HUSA.***

Using the Kreis test as a criterion of the degree of rancidity, Husa and Husa (1) found that benzoic and cinnamic acids are not effective in retarding the development of rancidity in lard. The effect of benzoin could not be determined by the method used, because Siam benzoin contains aldehydes or other compounds which give a pronounced red color in the Kreis test.

In a further study by the same authors (2), it was found that hydroquinone reduces the rate of development of rancidity in fats; this result indicated that there was at least some basis for the patent claims of Moureu and Dufraisse (3). The following compounds, in a concentration of 0.5%, were found to have no effect on the rate of development of rancidity: salicylic acid, acetylsalicylic acid, beta-naphthol, liquefied phenol, *dl*-alanine, pyrogallol, resorcinol and thymol.

As indicated in the review of the literature in a previous paper (1), the value of benzoin as a preservative of lard seems well established in the older literature. During the past 50 or 60 years, however, no further progress has been made in the study of the effect of benzoin. In fact there has been a tendency to question the value of benzoin. A chemist who has done considerable work on the rancidity of fats has stated (4) that he considers benzoin and other substances to be of no value as preservatives. According to Fiero (5), "benzoin . . . is far from a perfect preservative. A sample of benzoinated lard in sunlight at 33° C. developed rancidity in three days. Samples of benzoinated lard or ointments prepared from this compound are commonly found rancid in drug stores."

Since the Kreis test is not applicable to benzoinated lard, and since the odor of benzoin interferes more or less with the detection of rancidity by the sense of smell, it has seemed desirable to apply some other method for the measurement of rancidity. It was felt that a further study would give a better idea of the real effect of benzoin, and would make it possible to directly determine the relative value of other stabilizers as compared with benzoin.

It is well known that rancid fats liberate iodine from potassium iodide, due in all probability to the peroxide linkages in the partially oxidized fat. In Ointment of Potassium Iodide, N. F. V, 1% of sodium thiosulphate is included to prevent the appearance of free iodine for a reasonable time, it being further directed that the ointment should not be dispensed unless it has been freshly prepared.

Deschamps, in 1843, in the paper (6) in which he reported his discovery of the preservative effect of benzoin, indicated that potassium iodide could be used

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