(38) Morrison, A. B., Chapman, D. G., and Campbell, J. A., *J. Am. Pharm. Assoc., Sci. Ed.*, **48**, 634(1959).
(39) Campbell, J. A., and Morrison, A. B., *Practitioner*, **183**, 758(1959).
(40) Morrison, A. B., Perusse, C. B., and Campbell, J. A., *New Engl. J. Med.*, **263**, 115(1960).
(41) Morrison, A. B., and Campbell, J. A., *J. Am. Pharm. Assoc., Sci. Ed.*, **49**, 473(1960).
(42) Morrison, A. B., and Campbell, J. A., *Am. J. Clin. Nutr.*, **10**, 212(1962).
(43) Morrison, A. B., Perusse, C. B., and Campbell, J. A., *J. Pharm. Sci.*, **51**, 623(1962).
(44) Morrison, A. B., and Campbell, J. A., *Indian J. Pharm.*, **25**, 209(1963).
(45) Middleton, E. J., Davies, J. M., and Morrison, A. B., *J. Pharm. Sci.*, **53**, 1378(1964).
(46) Middleton, E. J., Nagy, E., and Morrison, A. B., *New Engl. J. Med.*, **274**, 136(1966).
(47) Levy, G., and Hayes, B. A., *ibid.*, **262**, 1053(1960).
(48) Levy, G., *J. Am. Med. Assoc.*, **177**, 689(1961).
(49) Levy, G., Antkowiak, J. M., Procknal, J. A., and White, D. C., *J. Pharm. Sci.*, **52**, 1047(1963).
(50) Levy, G., and Gumtow, R. H., *ibid.*, **52**, 1139(1963).
(51) Levy, G., *ibid.*, **52**, 1039(1963).
(52) Singh, P., Guillory, J. K., Sokoloski, T. D., Benet, L. Z., and Bhatia, V. N., *ibid.*, **55**, 63(1966).
(53) "Guide for Completing Pre-Clinical Submissions on Investigational Drugs," Food and Drug Directorate, Department of National Health and Welfare, Ottawa, Ontario, Canada, 1965.
(54) Levi, L., Walker, G. C., and Pugsley, L. I., *Can. Med. Assoc. J.*, **91**, 781(1964).
(55) World Health Organization, WHO/Pharm/66.432, unpublished working document (cited with permission of Secretariat).
(56) Cook, D., Chang, H. S., and Mainville, C. A., *Can. J. Pharm. Sci.*, **1**, 69(1966).
(57) Cook, D., *Med. Serv. J., Canada*, **23**, 323(1967).
(58) Morrison, A. B., *ibid.*, **23**, 349(1967).
(59) Wood, J. H., *Pharm. Acta Helv.*, **42**, 129(1967).
(60) World Health Assembly Document WHO 1947, World Health Organization, Geneva, Switzerland, issued May 20, 1966.
(61) World Health Organization Document A19/P&B/5, Geneva, Switzerland, issued April 4, 1966.

Determination of Antimony in Talc

By HARVEY D. SPITZ and ALEXANDER J. GOUDIE

A method has been developed to determine trace amounts of antimony present in talc. The spectrophotometric method is based upon the reaction of antimony (V) with rhodamine B in isopropyl ether after extraction of the antimony from 1.5 *M* hydrochloric acid.

RECENTLY, the Food and Drug Administration set limits for antimony in certain foodstuffs and dyes at 2 p.p.m. Because of the possible extension of this regulation to talc, a method has been developed for the semiquantitative determination of antimony in talc in the concentration range of 2 p.p.m.

Several methods have been developed to determine micro amounts of antimony. Iodide ion in acid solution (1, 2) or with iodide and pyridine (3) have been used, but not with the sensitivity of some other chromophoric reagents. Busev (4, 5) and his co-workers have developed a sensitive method using antipyrine dyes. However, the unavailability of these noncommercial dyes negated any work with them. Matulis and Guyon (6) have recently developed a sensitive system of analysis based on the enhancement by antimony of a blue hue due to the reduction of the molybdate aggregate near pH 1.4.

The most common technique has employed rhodamine B (7–14). A relatively simple and moderately accurate method for the determination of antimony in talc has been developed with rhodamine B as the chromophoric reagent.

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EXPERIMENTAL

Apparatus and Reagents—A Zeiss PMQ II spectrophotometer employing 1-cm. silica cells was used.

Hydrochloric Acid, 6 M—Dilute 500 ml. of concentrated hydrochloric acid with sufficient purified water to make 1000 ml. of solution.

Hydrochloric Acid, 1 M—Dilute 20.8 ml. of concentrated hydrochloric acid with sufficient purified water to make 250 ml. of solution.

Sulfuric Acid, 0.5 M—Dilute 2.8 ml. of concentrated sulfuric acid with sufficient purified water to make 100 ml. of solution.

Ceric Sulfate—Dissolve 3.3 Gm. of anhydrous ceric sulfate in 100 ml. of 0.5 *M* sulfuric acid.

Hydroxylamine Hydrochloride—Dissolve 1 Gm. of hydroxylamine hydrochloride in 100 ml. of purified water.

Hydroxylamine Hydrochloride, Acidic—Dissolve 1 Gm. of hydroxylamine hydrochloride in 100 ml. of 1 *M* hydrochloric acid.

Isopropyl Ether (Peroxide Free)—Saturate the isopropyl ether with 1 *M* hydrochloric acid.

Rhodamine B Reagent—Dissolve 0.02 Gm. of rhodamine B in 100 ml. of 1 *M* hydrochloric acid.

Sodium Sulfite—Dissolve 1 Gm. of reagent grade sodium sulfite in 100 ml. of purified water.

Antimony Postassium Tartrate, Reagent Grade—$[K(SbO)C_4H_4O_6 \cdot {}^1/_2H_2O]$.

Preparation of Standard—Weigh to the nearest tenth of a milligram a 274-mg. sample of antimony potassium tartrate into a 100-ml. volumetric flask. Dissolve the sample in and make up to the mark with 6 *M* hydrochloric acid. Pipet 10.00 ml. of this solution into a 100-ml. volumetric flask and dilute

to the mark with 6 *M* hydrochloric acid (solution *A*). Pipet 10.00 ml. of solution *A* into a 500-ml. volumetric flask and dilute to the mark with 6 *M* hydrochloric acid (solution *B*). One milliliter of solution *B* is equivalent to 2 mcg. of antimony.

Add to two 125-ml. separators 27.0 and 30.0 ml. of 6 *M* hydrochloric acid, respectively. Buret or pipet into the first separator 3.00 ml. of solution *B*. The second separator serves as the blank. Add 1 ml. of the sodium sulfite solution to each separator, swirl, add 15 ml. of purified water, and shake each separator gently to mix the contents.

Cool the solution in each separator to 25° or less and add 3 ml. of ceric sulfate solution. Mix thoroughly and allow the funnel contents to stand for 5 min. Add 1 ml. of aqueous hydroxylamine hydrochloride to each separator and mix the contents by gently shaking the funnels. Allow the funnel contents to stand for 1 min. Add 71 ml. of purified water to each separator and cool the contents to 25° or less. Add 5.00 ml. of isopropyl ether to each separator and shake the funnel with moderate vigor for 1 min. Allow the solution to stand for approximately 5 min. and drain off all but 0.5 ml. of the aqueous phase. Add 2 ml. of the acidic hydroxylamine hydrochloride solution to each separator and shake the funnels for approximately 5 sec. Allow the phases to separate and drain off all but 0.5 ml. of the aqueous phase. Add 2 ml. of 1 *M* hydrochloric acid to each separator and shake for approximately 3 sec. Allow the phases to separate and drain off the aqueous phase. Add 2 ml. of the rhodamine B solution to the separators and shake vigorously for 15 sec. Allow the phases to separate, drain off the lower layer, and pour the ether extract (from the top of the separator) into a cell. If so desired, one may pipet the isopropyl ether solution into the cell with the aid of a 3-ml. pipet.

Measure the absorbance of the solution at 550 mμ with the blank in the reference cell.

Procedure—Weigh to the nearest milligram a 3-Gm. sample of talc into a 100-ml. beaker. Add 25 ml. of 6 *M* hydrochloric acid to the beaker and disperse the sample thoroughly with the aid of a stirring rod. Cover the beaker with a watchglass.

Place the beaker on a steam bath for 0.5 hr. with occasional gentle stirring (approximately every 10 min.).

Add 1 ml. of the sodium sulfite solution to the beaker and stir; filter the contents through white ribbon filter paper and collect the filtrate in a 125-ml. separator. Rinse the beaker and residue in the funnel with 5 ml. of 6 *M* hydrochloric acid, followed by three 5-ml. washings with hot water.

Continue the procedure as indicated under *Preparation of the Standard*, starting with the paragraph, "Cool the solution . . . ."

The absorbance reading of the sample should not exceed that of the standard which represents 2 p.p.m.

RESULTS

Recovery studies were performed in duplicate by two operators at 4 different levels of antimony (1.0, 1.5, 2.0, and 2.5 p.p.m.). Antimony standards (ranging from 3.0 to 7.6 mcg.) were first added to the talc and the recovery was calculated (from a standard curve) after subtracting the antimony found in the talc samples. The accuracy was found to be 91.1%. The results of this study are given in Table I.

Table I—Recovery of Antimony From Spiked Talc

Antimony Added, mcg.	Antimony Found,[a] mcg.
3.0	2.7
4.6	4.2
6.0	5.5
7.6	6.9

[a] Average of 8 determinations at each level of antimony.

Analyses for the determination of antimony in two types of talc were performed by two operators in duplicate. The range of antimony found in the talcs was 0.04 to 0.15 p.p.m.

DISCUSSION

Originally, antimony trichloride was used to prepare a standard curve for the recovery study. However, antimony potassium tartrate was substituted for antimony trichloride since the latter compound required a titration for standardization, while the former could be used as a standard by simply weighing the salt. Similar results were obtained with both compounds, indicating either salt could be used as an antimony standard with equal reliability.

Ward (13) indicates that the addition of acidic hydroxylamine hydrochloride to the isopropyl ether solution decreases the concentration of iron (III) by reducing it to iron (II) which isopropyl ether does not extract. He also mentions that 1 and 2 *M* hydrochloric acid fails to extract quantitatively some other elements, including iron (III), which also reacts with rhodamine B. It has been found that a very small percentage of iron (III) is extracted from a 1.5 *M* hydrochloric acid solution (8). Hence, with this system, antimony can be detected satisfactorily in the presence of 30,000 mcg. of iron (III). Since talc contains a significant amount of iron, it was felt that 1.5 *M* hydrochloric acid would be a desirable system to reduce any possible error contributed by the iron or any other interfering elements present in the talc. This choice of acid concentration was further reinforced as higher concentrations of acid were found to be unsuitable because of the apparent loss of isopropyl ether during the extraction process. It was found that shaking 8.0, 6.0, 5.0, 3.0, and 1.5 *M* hydrochloric acid solutions with 5.00 ml. of isopropyl ether gave significantly lower recovery volumes at the 8.0, 6.0, and 5.0 *M* hydrochloric acid concentration. This decrease in volume occurred even though the ether was previously saturated with its respective acid concentration.

Van Aman and co-workers (14) have shown that dilution of concentrated hydrochloric acid before the antimony is extracted into the ether phase results in low absorbance values. The ratio of ether to acid phase (e/a = 2.1) and concentration of antimony employed by Van Aman is considerably higher as compared to that used by the authors (e/a = 0.1). However, it was not feasible to employ their system for our samples. Although lower absorbance values would be obtained with this system, it still represents a highly sensitive one.

In order to substantiate the method of preparing the standard curve for the recovery study, the standards were determined according to the exact procedure employed for the talc samples (heating, filtering). There was no significant change in the least square line as compared to a standard curve prepared without the heating and filtering steps. This indicated that any loss of antimony in the spiked samples probably involves the presence of talc.

An experiment, similar in design to the foregoing procedure, was performed with a final concentration of 3 *M* hydrochloric acid instead of the 1.5 *M* hydrochloric acid as in the preceding method. In this procedure, the concentration of acid was kept constant throughout the method. The major advantage of the modified method is the elimination of adding 71 ml. of water before extraction with the isopropyl ether. This particular step is not desirable because of the possible increase in rate of hydrolysis of antimony (V) to a nonreactive form, antimony (IV). In the original procedure, the volume of aqueous phase was 121 ml. up to the ether extraction step as compared to 65 ml. in the modified method. Thus, the latter procedure should create a more favorable environment for a higher partition coefficient.

A recovery study was performed at the 4 levels of antimony previously mentioned. The recoveries were similar to those obtained in the original method. These results seem to indicate that the partition coefficient is the same for both methods even though the ratio of organic to aqueous phase is different.

Several authors have indicated with their systems that the rhodamine B–antimony complex is fairly stable. However, the stability of the complex in isopropyl ether has been found to vary between authors. Ward and Lakin (13) state that the color is stable up to 6.5 hr., while Van Aman (14) indicates the color fades 1.2% in 1.5 hr. or 30% in 18 hr. However, Maren (8) mentions that the color is not stable, and the absorbance readings must be made within 30 min. of the extraction. We have found that there was a decrease in the absorbance readings of the color complex with time when water was used in the reference cell. A study of absorbance *versus* time for three levels of antimony and a blank was performed at 5-min. intervals for 35 min. The results obtained show that the initial absorbance readings decreased continuously for 20 min. The difference between the initial reading and those taken at 20 min. ranged from 0.017–0.027 absorbance units with 1–4 mcg. of antimony, respectively, and in most cases much less for the blank. However, from 25–35 min. the absorbance readings for the antimony complex remained almost constant (0.004 maximum difference). Subtraction of the blank absorbance reading from the antimony complex absorbance reading at the same respective time yielded a nearly constant absorbance for 35 min. In effect, absorbance readings can be made any time during the first 35 min. after the extraction step for the samples as long as the blank is prepared and read at the same time as the sample.

In conclusion, the methods show that the antimony content found in the talcs tested is considerably lower than the 2 p.p.m. level which may be required by the Food and Drug Administration.

REFERENCES

(1) Dym, A., *Analyst*, **88**, 232(1963).
(2) Elkind, A., Gayer, K. H., and Boltz, D. F., *Anal. Chem.*, **25**, 1744(1953).
(3) Clarke, S. G., *Analyst*, **53**, 373(1928).
(4) Busev, A. I., Tiptsova, V. G., Bogdanova, E. S., and Andreichuk, A. M., *J. Anal. Chem. USSR*, **20**, 887(1965).
(5) Busev, A. I., Bogdanova, E. S., and Tiptsova, V. G., *ibid.*, **20**, 542(1965).
(6) Matulis, R. M., and Guyon, J. C., *Anal. Chem.*, **37**, 1391(1965).
(7) Fredrick, W. G., *ibid.*, **13**, 922(1941).
(8) Maren, T. H., *ibid.*, **19**, 487(1947).
(9) Edwards, F. C., and Voigt, A. F., *ibid.*, **21**, 1204 (1949).
(10) Luke, C. L., *ibid.*, **25**, 674(1953).
(11) Luke, C. L., and Campbell, M. E., *ibid.*, **25**, 1592 (1953).
(12) White, C. E., and Rose, H. J., *ibid.*, **25**, 351(1953).
(13) Ward, F. N., and Lakin, H. W., *ibid.*, **26**, 1168(1954).
(14) Van Aman, R. E., Hollibaugh, F. D., and Kanzelmeyer, J. H., *ibid.*, **31**, 1783(1959).

Determination of Meprobamate in Tablets by NMR

By JOHN W. TURCZAN and THEODORE C. KRAM

A rapid NMR procedure was developed for the analysis of meprobamate tablets with malonic acid as internal standard. An average error of 0.9 percent was obtained on seven synthetic mixtures. The NMR spectrum, in addition, provided a very specific means of identification for meprobamate.

THE CURRENT development of more complex drugs and drug mixtures, and the necessity for the analysis of many more samples has led to the need for rapid and simple methods of analysis. We have successfully analyzed many high-dosage-form pharmaceuticals by extracting them with a suitable solvent and obtaining their NMR spectra with the aid of an internal standard. The recorded spectrum from the NMR spectrometer provides an identification of the active ingredient, which contributes to the specificity of the procedure. Meprobamate has been determined by this technique, using acetone as solvent and

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