

SCIENTIFIC SECTION

THE ASSAY OF SALICYLATES AND BENZOATES.*

BY A. H. CLARK.

The ideal in any quantitative analysis is 100% accuracy and the ideal and only accurate result is one which accounts for 100% of all the material present. In order to accomplish this a method must be theoretically perfect. Many times, in practice, no attempt is made to attain the ideal and this is particularly the case in the methods of the United States Pharmacopœia: Nearly all the determinations of the Pharmacopœia depend upon the isolation of only one constituent of the compound or mixture under examination. This idea is carried out in the determination of acids where the hydrogen ion only is titrated; in the titration of bases where the hydroxyl ion only is determined; in the determination of the purity of halide salts by titration with silver; in the determination of the purity of organic salts of sodium and potassium by ignition, and in other ways. Such methods have their faults but these faults are common to them all and a better and more accurate method would be the determination of all the constituents present. From the standpoint of the Pharmacopœia this is neither convenient nor necessary because the tests for identity and purity exclude foreign material which might interfere with the accuracy of the determination.

In accord with the above reasoning any method for the assay of salicylates and benzoates which determines with accuracy either the metal or the acid radical is satisfactory. The only way to prove the accuracy of a method is to try it on samples of known purity or upon samples which are prepared in such a way that they are certain to be pure. The testing of a method upon commercial samples is worthless because it is a well known fact that commercial products are not intended to be theoretically perfect in composition but may contain small quantities of foreign material. This principle is recognized by the Pharmacopœia when it sets a limit for impurities in almost every case where assay methods are prescribed. The important thing is an accurate method for the determination of one or more of the constituents present and then the Pharmacopœia may place whatever limit it decides is reasonable on the amount of foreign materials permitted.

For the work recorded here pure samples of sodium salicylate, sodium benzoate and ammonium benzoate were prepared. The sodium salicylate was prepared by recrystallizing a commercial sample from solution in hot absolute alcohol. The truth of the statement made above regarding the character of commercial samples was very clearly evidenced in the preparation of the pure sodium salicylate. One of the commercial samples used for this purpose gave solutions in alcohol that were very highly colored and showed the presence of appreciable quantities of splinters of wood, dirt and the like. In this case it was necessary to recrystallize from absolute alcohol three or four times to secure

* The work recorded here was started some time ago in the University of Illinois School of Pharmacy and concluded in the University of Michigan College of Pharmacy during the summer session of 1925.

a specimen which seemed free from foreign material. Other samples required but one or two crystallizations.

It was a much more difficult matter to prepare a pure sample of sodium benzoate. This was finally accomplished by neutralizing an alcoholic solution of benzoic acid with sodium hydroxide and carefully washing the precipitated sodium benzoate with alcohol and ether and then drying to constant weight at 110° C.

A pure ammonium benzoate was quite readily prepared by neutralizing an alcoholic solution of benzoic acid with ammonia and very carefully washing the precipitated ammonium benzoate with alcohol and finally with ether and drying in a desiccator.

The melting point of the acid extracted was determined in every case and was taken as an index of the purity of the sample.

Numerous methods have been proposed for the determination of the purity of these salts among which are colorimetric ones; methods depending upon the ignition of the salt and titration of the carbonate; methods depending upon the conversion of sodium to sulphate or chloride; methods depending upon the isolation of the acid and either weighing or titrating it; volumetric methods in which the aromatic group is determined by absorption of iodine or bromine and finally a few gravimetric methods have been proposed. A discussion of those methods which have been tested and the results reported in this paper are given below.

METHODS.

1. *Ignition and Titration of Carbonate Formed.* (a) *U. S. P. VIII and IX Modifications.*—Simple ignition of the salt and leaching out of the carbonate formed and its subsequent titration is the procedure directed in U. S. P. VIII. Since experience showed this method to be quite unsatisfactory for benzoates and salicylates it was thought that a general method of assay for organic salts of alkali metals might be included in the U. S. P. IX by a slight modification of it. The modification consists in adding to the charred mass an excess of standard acid, boiling for one-half hour, filtering and determining the acid consumed by titration with standard alkali. Even this has not proven satisfactory for benzoates and salicylates and is unnecessary for salts of other organic acids.

(b)—For a great many years the writer has used a slight modification of the simple ignition process which consists in moistening the original charred mass with a few drops of water, drying and igniting once more and if necessary repeating this moistening and drying until a residue perfectly white and free from carbon is obtained. The carbonate is then added to water and titrated directly with standard acid. This procedure is entirely satisfactory for all alkali metal salts excepting benzoates and salicylates and the U. S. P. X has adopted practically the same procedure as a general method but directs for the latter a special procedure which is as follows:

(c) *U. S. P. X Method.*—This method is a modification of the U. S. P. IX procedure in which after charring the salt, treating it with excess of standard acid, boiling, filtering, etc., the filter and carbon are again ignited, the ash thus obtained being added to the standard acid and the titration of the excess acid with alkali completed, using methyl orange as an indicator. This procedure is

exceedingly cumbersome and time-consuming, requiring by count of time actually spent as much as one hour. Aside from this it is rather difficult to avoid errors due to loss during the boiling and the subsequent filtrations and to the difficulties due to the use of methyl orange as an indicator.

The writer has been very much interested in the cause of the failure of the usual methods in the case of benzoates and salicylates when they work so well with other salts. Many efforts have been made to discover some plausible explanation for this fact but at the present time there is nothing definite to offer.

2. *Conversion of the Sodium into a Salt.* (a) *Into Sulphate.*—The usual procedure for the determination of sodium as sulphate when applied to this problem works very well. It is not nearly so simple, however, as the method outlined later under 2b. Dr. Lyons' (1) procedure for the determination of sulphate is cumbersome and rather time consuming and in the writer's opinion has no special advantages over the regular sulphate determination.

(b) *Weighing the Metal as Chloride.*—While this method is frequently mentioned in connection with sodium benzoate and salicylate, the writer has never seen any special directions for carrying out the determination. The following procedure has proven satisfactory. A quantity of the salt, about one Gm., is placed in a tared dish, preferably of platinum, dissolved in a small amount of water and an excess of strong hydrochloric acid added. The dish with its contents is then placed on a steam- or water-bath and heated to near dryness. It is then transferred to a hot plate or to an oven heated to about 120° C. until all the acid is expelled and then gently ignited and weighed as sodium chloride. In the case of ammonium benzoate it is necessary to dissolve the sample in water and transfer the solution to a separator and add a liberal quantity of ether and shake until the acid has dissolved. The solution of ammonium chloride is then drawn into a tared dish, the ether washed two or three times with water and then the combined washings and solution evaporated to dryness and dried in an oven at 100° to constant weight. Care must be taken when the dish is placed on the steam-bath to avoid loss by spattering when the dissolved ether escapes from the solution as it becomes heated. This method applied to sodium salts is very simple and exclusive of the time required for the weighings will not consume five minutes of the operator's time.

3. *Extraction of Acid.*—This in some form is about the oldest and most widely used method of all.—(a) Moerk and Ehman (2) extracted the acid and without removal of the solvent titrated it with alkali using phenolphthalein as indicator. There are some objections to the titration of benzoic and salicylic acids with sodium hydroxide and phenolphthalein as indicator. The U. S. P. directs the use of barium hydroxide in this titration. The authors do not state whether their results were obtained on purified or commercial samples. Their results range from 98.088 to 100.12 per cent on sodium salicylate and from 99.063 to 97.41 on sodium benzoate. These are not very concordant results and unless a method gives better results than this it is not very satisfactory since the Pharmacopœia requires a purity of 99.5 per cent in the case of sodium salicylate and 99 per cent for sodium benzoate. Any suitable method must surely give results agreeing within a range of 0.5 per cent. This method was not tried.

(b) *Weighing of the Acid.*—This method has been criticized by Taub (3) on the

ground that it may give results which are too high due to the retention of the solvent, or results that are too low, due to the loss of acid during the evaporation. In the writer's experience both benzoic and salicylic acid can be extracted after its liberation by means of hydrochloric acid and accurately weighed by proceeding as follows:

About 1 Gm. of the sample is dissolved in water and transferred to a separator and an excess of hydrochloric acid added. The liberated benzoic or salicylic acid is extracted with chloroform (*ether, because of its tendency to take up water, will not answer*) taking care that no moisture is carried along with the chloroform. This can be best accomplished by drawing the chloroform through a pledget of cotton placed in the stem of a small funnel and moistened with chloroform. The chloroform is collected in a tared dish and set aside to evaporate spontaneously. If the extraction is made in the late afternoon the dish may be set aside and usually the chloroform will have completely evaporated by morning. The dish with the acid is then placed in a desiccator for an hour or more and weighed. A little experience will enable one to select a location in the laboratory where this evaporation will take place over night. It has been found after numerous trials that the drying in a desiccator is not always necessary, in many cases the loss being negligible.

4.—This method is a combination of 2*b* and 3*b* and is the ideal method since it recovers all of the constituents. A weighed quantity of the sample, about 1 Gm., is dissolved in water and transferred to a separator and an excess of hydrochloric acid added. The liberated acid is extracted with chloroform as described under 3*b*. The solution of chloride remaining in the separator is drawn off into a tared dish, the separator washed with several small portions of water and the combined washings and solution evaporated and weighed according to 2*b*.

5. *Volumetric Methods.* (a) *By Absorption of Bromine.*—This method is applicable to salicylates only, since benzoates do not absorb bromine quantitatively. These methods have been severely criticized and have been the subject of much study. Fresenius and Grünhut (4) state that they give only fair results. Seidell (5) made a very thorough study of them and concluded that the results were decidedly variable while A. and H. Taub (3) found Jones' (6) modification to be the most applicable of *all* the methods of determination. This method is essentially that of the U. S. P. for the assay of phenol. A solution of the salicylate is added to *N*/10 bromine V. S. and the mixture acidulated. After 15-20 minutes potassium iodide is added and the bromine not absorbed is determined by titration with thiosulphate. In the experience of the writer this bromine absorption method is unreliable, giving results which are decidedly variable depending upon the factors of time, acidity, and concentration.

(b) *Methods Depending upon the Absorption of Iodine.*—In the author's experience the absorption of iodine by phenols and similar bodies is exceedingly uncertain, the results depending upon the relation between the iodine and the quantity of substance being determined as well as upon the alkalinity of the mixture. This was pointed out by Puckner and Clark (7) and has been a subject of very thorough study by Seidell. (8) Other analysts seem to agree that the method is too uncertain to be of very much value. It was not tried on any of the samples used in these experiments.

The results obtained by the various methods tried are given below.

SODIUM SALICYLATE.

Quite a number of assays were made by methods *1a*, (U. S. P. VIII and IX) and also Method *1b*. Results were invariably low in some cases as low as 95 per cent while the usual result was from 98 to 99 per cent.

Method *1c*. Many assays were made by this method and the results were found to be quite variable as shown in the following table. It was thought that better results might be obtained with a platinum dish but upon trial it was found that it had little if any influence on the results.

Results obtained by the other methods tried are shown in the table.

	<i>1b.</i>	<i>1c.</i>	Methods.		<i>4.</i>	<i>5a.</i>	
			<i>2b.</i>	<i>3b.</i>			
Sodium Salicylate, pure	99.43	99.52	99.86	99.79		99.45	
	99.46	98.73	100.00	99.80		97.05	
	99.24	101.00	99.65	99.95		99.63	
	99.59	99.05	99.94	99.91		99.96	
		with Pt	99.89	99.87		96.95	
		dish	100.08	99.64			
			99.32	100.03			
			98.53	100.03			
			99.91	99.55			
	Commercial samples		97.40	99.71	99.28		
			98.24	99.91	99.34		
			97.61	99.22	100.25		
			99.42	99.38	100.11		
			99.75	99.71	100.13		
		98.63					
		98.33					
		100.43					
		100.65					
		99.84					
		98.42					
Sodium Benzoate, pure		98.70	100.02	100.02	99.85 &		
		98.30	99.97	99.16	99.82		
			99.46		100.05 &		
					99.73		
					99.66 &		
Commercial samples		99.38	99.77	100.99	99.95		
		97.30	100.22	100.60	99.38 &		
			99.60		99.64		
			99.91	99.95	99.60 &		
			99.72	99.86	99.16		
Ammonium Benzoate, pure					100.30 &		
					99.90		
					100.03 &		
					100.10		

CONCLUSIONS.

These results show that in the assay of sodium salicylate, sodium benzoate and ammonium benzoate most accurate and uniform results are obtained by either weighing the metal as chloride or extracting the liberated benzoic acid with chloroform and weighing it. If a method of general application is desired, the extraction of the acid and weighing it is the only advisable one. It is quite unlikely that lithium, mercury, or strontium could be weighed as chloride. In addition to its accuracy, Method 2*b*, the weighing of the metal as chloride, is exceedingly simple and time saving. Determinations upon the sodium salts by this method require practically no time beyond that of the two weighings necessary.

Acknowledgment is made to Mrs. Frances Stogis of the University of Illinois School of Pharmacy for her careful and conscientious work in making many of the determinations, especially those on sodium salicylate.

BIBLIOGRAPHY.

- (1) A. B. Lyons, *JOUR. A. PH. A.*, 7, 603 (1918).
- (2) Moerk and Ehman, *JOUR. A. PH. A.*, 2, 156 (1913).
- (3) A. and H. Taub, *JOUR. A. PH. A.*, 11, 517 (1922).
- (4) W. Fresenius and H. Grinhut, *Z. anal. Chem.*, 38, 38 (1899).
- (5) A. Seidell, *Am. Chem. J.*, 47, 520 (1912).
- (6) H. W. Jones, *JOUR. A. PH. A.*, 9, 878 (1920).
- (7) W. A. Puckner and A. H. Clark, *PROC. A. PH. A.*, 56, 830 (1908).
- (8) A. Seidell, *J. Am. Chem. Soc.*, 31, 1168 (1909).

TOXICITY OF QUININE-ASPIRIN MIXTURE ON WARM-BLOODED ANIMALS.*

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At the meeting of the ASSOCIATION one year ago, we presented a paper to this section on the Toxicity of Quinine-Aspirin Mixture.¹ The frog was the test-animal in the work reported on at that time. It was suggested that different results might be obtained on warm-blooded animals. This paper presents the results of the work done during the past year on rats and guinea-pigs.

Referring to the report of last year (*JOUR. A. PH. A.*, November 1924, 1009) we find that the minimum fatal dose of the freshly made Quinine-Aspirin Mixture (quinine alkaloid two parts to aspirin three parts) was about 0.00017 gram per gram body-weight of the frog and that the minimum fatal dose of the old Quinine-Aspirin Mixture (after it has changed to the brown-red mass) is 0.00016 gram. There is only a difference of 0.00001 gram, which might easily be due to experimental error on the part of the operator, and the variation of action on the animal.

Consequently, we can say that the old Quinine-Aspirin Mixture is no more toxic for the frog than the freshly prepared mixture.

The animals used this year were white rats and guinea-pigs. The rats were reared in our own laboratory; were all in healthy condition; were between three and four months old and weighed from 61 to 175 grams. Sixty-one rats were

* Read before Scientific Section, *A. PH. A.*, Des Moines meeting, 1925.

¹ *JOUR. A. PH. A.*, November 1924, p. 1009.