PHARMACOPŒIAS AND FORMULARIES

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SOME OBSERVATIONS ON THE TESTS FOR PURITY

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IN the official tests for degree of purity, and the methods of assay for chemicals, the new British Pharmacopœia shows remarkable progress when compared to the previous one (1932). Throughout the book the formulation is more precise, the tests are more comprehensive, and frequently more rational; and many new methods have been introduced.

Determination of melting-points.—The technique—the capillary method—is that generally described in modern pharmacopœias, and is completely identical with the method used in the Pharmacopœia of 1932. The melting temperatures may be regarded as corrected temperatures consistent with the technique employed. On the other hand the definition, viz.: The temperature at which liquefaction of the substance occurs; this is indicated by the formation of a definite meniscus, may be subject to criticism, because it is expressed by a single temperature and not by a range between two temperatures. In general, commercial products are rarely of such purity that their melting-points are sharply defined; usually the melting-range extends over one or two degrees. The United States Pharmacopœia uses the term melting-range or melting-temperature, which is thus defined "The temperature at which the column of the sample (in the capillary tube) is observed to collapse definitely against the side of the tube at any point is defined as the beginning of melting, and the temperature at which the sample becomes liquid throughout is defined as the end of the melting." Many other Pharmacopœias (e.g. Danish, Swiss, Swedish) give a similar definition.

Determination of boiling-points.—Both the apparatus used in the determination of the boiling-point and the procedure are described in detail, as in the Pharmacopœia of 1932. The method is the conventional one, as employed in, e.g. the test for oils, and it is well known that this gives reproducible results, although the use of a cylindrical flask as described respectively in the Swiss and the new Danish Pharmacopœias is more rational.[1,2] It is a disadvantage that the procedure demands a large quantity of the substance, 100 ml. In most cases the procedure is employed to decide the character of mixtures of compounds as, for instance, creosote and cresol. It would have been an advantage if the technique in the determination of substances with low boiling-point as e.g cyclopropane (− 34.5°C.) and ethyl chloride (about + 12·5°C.) had been described in detail.

The determination of the boiling-point in the manner described can scarcely be regarded as a practical test for identity and such a test might be useful in many cases, e.g. amphetamine. A number of methods requiring small quantities only and giving reproducible results are available for that purpose; the method described by Siwoloboff,[3] and modifications,[4] may be mentioned.

Limit test for chlorides.—In practically every relevant instance the limit test for chloride is used; although vague expressions such as "no opalescence occurs immediately" have not been completely eliminated. The limit test is carried out in Nessler glasses using 50 ml. of the solution to be tested; to this is added 1 ml. of nitric acid and a solution of silver nitrate (N/10); then the mixture is stirred with a glass rod, and the observation of the reaction

is made 5 minutes later. The opalescence produced is compared to a standard opalescence, equivalent to 0·355 mg. Cl^-/50 ml. (0·071 mg. Cl^-/ml.).

The limit test for chlorides has been carefully investigated by Thörn[5] and by Reimers and Gottlieb[6]. The results of these investigations may be summarised as follows. The opalescence produced by minute quantities of chloride is in inverse ratio to the speed at which the reagent and the test solution are mixed together. Thus rapid mixing does not lead to the maximum sensibility, but gives the most reproducible results. If the reagent is added without shaking, it will give the maximum of sensibility, but with poor reproducibility. For instance the opalescence produced when the solution of silver nitrate is added to the solution to be tested and the liquid shaken after 1 minute, is about 3 times more intense than that produced after rapid mixing. The procedure as set out in the B.P. gives good reproducible results.

Limit test for sulphates.—The test for sulphates is a simple one. Dissolve the substance in water and transfer to a Nessler glass; add hydrochloric acid: dilute to 50 ml. with water, and add 1 ml. of solution of barium chloride. Stir immediately with a glass rod and set aside for 5 minutes. The turbidity produced is then compared to a standard turbidity equivalent to 2·4 mg. of SO_4/50 ml. (0·048 mg./ml.). The conditions here are not quite so simple as in the limit test for chlorides. Thörn[7] and Reimers and Gottlieb[8] have proved that to obtain good reproducibility, it is necessary for the solution to contain a minute quantity of scarcely visible crystals of barium sulphate. Actually the solubility of barium sulphate increases with the decrease in size of the particles owing to the greater surface energy of the small particles. The precipitation of barium sulphate from very dilute sulphate solutions must commence with the formation of microcrystals, the solubility of which may be as much as 1000 times greater than that of large barium sulphate crystals. The precipitation will be markedly inhibited unless the test is carried out with a reagent containing barium sulphate to induce the precipitation in the test sample. The Danish Pharmacopœia describes the use of such a seeding reagent—the same quantities of sulphate in the test and in the standard turbidity—the concentration of which is chosen so that the precipitation is rapidly completed. The application of the seeding reagent results in both an increased sensibility and an increased reproducibility. The simple method of the B.P. cannot be said to lead to exact reproducible results.

Limit test for iron.—The limit test for iron is now carried out with thioglycollic acid in a solution containing citric acid to produce complex compounds with other cations, and an excess of ammonia to make the solution alkaline. In the presence of iron a pinkish-violet colour is produced. This is a notable improvement on the thiocyanate method formerly employed. Woods and Mellon[9] state that the thiocyanate method in general is inferior to several other methods, especially those using *o*-phenanthroline, and $\alpha\alpha$-dipyridyl or thioglycollic acid.

Thiogycollic acid as a reagent for iron was first proposed by Andreasch[10] and has subsequently been investigated by others, of whom Swank and Mellon[11] state that the thioglycollic acid method is remarkably free from the influence of other common anions, many of which must be entirely absent in other colorimetric methods. The following ions, in concentrations of 500 mg./100 ml. of solution, had no effect on the colour: — fluoride, iodide, nitrate, orthophosphate, sulphate, chlorate, tartrate, oxalate, citrate, acetate, bromide, thiocyanate, sulphite, and chloride; 250 mg. of boron trioxide, present as tetraborate ion, also has no effect. Pyrophosphate ion, when present in an amount equivalent to 500 mg. of phosphorus pentoxide decreases

the colour intensity by about 8 per cent., but 200 to 300 mg. can be present without serious error. Cyanide ion interferes seriously and must be absent. The lack of interference by nearly all anions and the reproducibility and sensitivity of the colour reaction makes the method superior to various other colorimetric determinations of iron. The procedure is just as simple as the thiocyanate method. The standard colour is equivalent to 0·04 mg. Fe/50 ml. (0·0008 mg./ml.)

Reaction.—For the determination of the reaction of a solution various indicators are used. It is not quite clear what intervals in the *p*H scale are covered by the designations, strongly acid, weakly acid, neutral, weakly alkaline, and strongly alkaline. Many pharmacopœias use similar terms to describe defined intervals of the *p*H scale. In many cases it would be more decisive to determine the *p*H value, or the interval between two *p*H values as a means of characterising the degree of purity. As the B.P. gives a full description of the colorimetric determination of *p*H values and the standard buffer solutions for preparing solutions with *p*H 1·2 to 10·0 such an indication might easily have been inserted more generally and more consistently. It must be regarded as a disadvantage that no test for the sensitivity of litmus paper is given, because the commercial grades of litmus paper vary considerably in this respect. The U.S.P. and the new Danish Pharmacopœia have specified tests, both for sensitivity and for the content of buffer substances in the paper.

Limit tests for metals.—The limit tests for lead and arsenic do not differ much from the previous Pharmacopœia. The expression of the limits in parts per million might well be introduced more generally into other Pharmacopœias. For lead the diphenylthiocarbazone method is used in some cases. It must be regarded as an advance that the B.P. specifies the tests for various individual metals (e.g. copper, zinc) and only in a very few cases uses the general term "heavy metals," which is used in some other Pharmacopœias, e.g. in the Danish, Swedish, Swiss, and U.S.P.

The test for arsenic takes the form used in the British Empire and U.S., while the modern continental Pharmacopœias use the hypophosphite test, which is simpler in technique and equally accurate.

Readily carbonisable substances.—The deletion of the test for readily carbonisable substances is comprehensible in a country where supplies are generally of great purity. One thing is certain, that, if the test is to be of any value, it is essential to have a series of matching fluids at one's disposal as given in the U.S.P. and in the new Danish Pharmacopœia. In the latter, the matching fluids are used not only in this test but generally to determine the colour of many faintly tinted solutions, also for some colorimetric determinations, for instance morphine in codeine and papaverine and for the colorimetric determination of the concentration of adrenaline in solutions.

As an exception, the B.P. includes a test for carbonisable substances in liquid paraffin and similar products. In these cases the colour which is produced in sulphuric acid, after shaking with the paraffin, is measured by means of standardised coloured glasses in accordance with the system of colour measurement adopted at the National Physical Laboratory, Teddington. Such standardised glasses might advantageously be used instead of matching fluids to measure the colour in the test for readily carbonisable substances and also for other approximate colorimetric measurements.

The tests for identification and purity must be said to meet all reasonable demands, a comment equally applicable to the assays. The tests for purity are not numerous, but are adequate for practical purposes, to ensure com-

pounds of sufficient purity. As a large number of new substances have been included a few of the tests and assays will be mentioned.

Ultraviolet absorption.—The absorption of ultraviolet light as a test covering both purity and identity has been included for the following substances: ascorbic acid, ethisterone, calciferol, dienœstrol, œstrone, progesterone, and the following drugs containing vitamin A: halibut-liver oil, concentrated solution of vitamin A, concentrated solution of vitamins A and D, and cod-liver oil. The extinction coefficient is referred to a 1 per cent. w/v solution and indicated for a given wave-length. For the practical purpose to which it is here applied this indication is more suitable than the molecular extinction coefficient. In some cases it might have been of value to give the absorption not only in the maximum but also in the minimum of the extinction curve.

The identification of substances which are so expensive that only small amounts are available are carried out by melting-point determinations on the pure substances or simple derivatives of them, these tests likewise are satisfactory. Among the more modern tests for identification the cyanogen bromide test for nicotinic acid and nicotinamide may be mentioned. For the determination of iodine ion the titration using potassium iodate in presence of potassium cyanide is used; this is an easy and reliable method, which is used also for determination of iodide in iodoxyl after hydrogenation with zinc dust and glacial acetic acid and in iodophthaleim after destruction by heating with anhydrous sodium carbonate. For the determination of iodine in thyroid the powder is heated with sodium carbonate and the iodine ion oxidised to iodate and titrated in the usual way.

For the qualitative test for organically bound chlorine reduction by sodium and amyl alcohol and subsequent titration of the chloride ion formed has been substituted; a more rational method than the old one. This test is used for benzoic acid, benzaldehyde, mandelic acid and its calcium salt, and vinyl ether.

For the determination of the content of bismuth in bismuth salts the old method of ignition is used in several cases, but in some the determination of bismuth as bismuth phosphate is used. This latter procedure is an excellent method and might have been adopted more widely, especially for the salicylate and subgallate, in both of which cases the ignition is protracted.

The assay of organic compounds, which cannot be titrated, in many cases proves difficult In some cases the determination of the content of nitrogen solves the problem. This method might also advantageously have been used for barbitone. For hexabarbitone and the sodium salt the determination of the double bond in the same way as the iodine value would have been a good assay.

The determination of alkaloids in alkaloidal salts has been discussed by van Os[12]. Here it should be sufficient to mention that the principle of weighing or titration of the base is always used in the B.P. and this is the rational way, although other pharmacopœias use only the determination of the anion.

The titrations with titanous chloride for some chemicals (menaphthone, methylene blue, crystal violet) are good.

For the determination of theobromine the excellent method of methylating and weighing the caffeine formed is used. For methylthiouracil no really satisfactory test for identification has been given, as the assay mentioned is inadequate for identification.

The Pharmacopœia contains a large number of Appendices which seem to be comprehensive and very satisfactory. Appendix I gives a list of

materials and solutions employed in tests, these describe the usual reagents, with a complete description of tests for identification and purity.

The solutions of reagents are also listed. It is regrettable that the respective concentrations are not given in simple molarity, but always in per cent. This is an unpractical and old-fashioned way. At least the concentrations of commonly used acids, bases, and some salts ought to be given in simple molarity as is the case in e.g. the Danish, Netherlands, Swedish, and Swiss Pharmacopœias, but, strangely enough, not in the U.S.P.

Appendix V, qualitative reactions and tests for substances mentioned in the Pharmacopœia, gives briefly but exhaustively most of the common identity tests.

The form of the individual monographs is clear, practical and well arranged, and all in all, the new Pharmacopœia is a great improvement on the previous one.

REFERENCES

1. Kempf and Kutter, *Schmelzpunkttabellen zur organischen Molekular-Analyse*, Braunschweig, 1928, 47.
2. Baggesgaard Rasmussen and Reimers, *Dansk. Tidsskr. Farm*, 1942, **61**, 182.
3. Siwoloboff, *Ber. dtsch, chem. Ges*, 1886, **19**, 795.
4. Cheronis, *Semimicro- and Macro-organic Chemistry*. New York, 1947, 60.
5. Thörn, *Farm. Revy*, 1941, **40**, 38.
6. Reimers and Gottlieb, *Grænseprøver for urenheder. (Limit tests for impurities)*. Contributions from the Danish Pharmacopœia Commission. Vol. I, Copenhagen 1946, 19-28, 179-180.
7. Thörn, *Farm. Revy*, 1942, **41**, 445, 453, 461, 473, 481.
8. Reimers and Gottlieb, *loc. cit.* 43-55, 181-182.
9. Woods and Mellon, *Indust. Engng. Chem., Anal. Ed.*, 1941, **13**, 551.
10. Andreasch, *Ber. dtsch. chem. Ges.*, 1879, **12**, 1391.
11. Swank and Mellon, *Indust. Engng. Chem. Anal. Ed.*, 1938, **10**, 7.
12. D. van Os, *J. Pharm. Pharmacol.*, 1949, **1**, 55.

ABSTRACTS (*continued from page 342*)

results calculated by Schild's method. Eight satisfactory assays were performed and the mean percentage error was 2·16. Fiducial limits for the estimate of potency were calculated and were found to be about half the value for the original Dale and Laidlaw method. The mean experimental time for an assay was 3¼ hours. S. L. W.

Sulphaguanidine, Absorption of. M. R. Fabre, M. T. Régnier and M. E. Grasset. (*Ann. pharm. Franc.*, 1948, **6**, 205.) The method of investigation previously applied to the absorption of sulphanilamide has been extended to sulphaguanidine. After a feed rich in fats dogs were anæsthetised with somnifen and a sample of chyle was collected by catheter. A dose of 5 g. of sulphaguanidine was then given into the stomach. The chyle and blood was examined at intervals for the presence of the drug. The results show that sulphaguanidine is absorbed more slowly than sulphanilamide. In the blood, the compound was first detected after 20 minutes, reaching a maximum after 6 hours. The maximum concentration in the blood was 2·2 mg./100 ml., which compares with the figure of 10·5 mg./100 ml. previously obtained for sulphanilamide. In the chyle, the first signs were detected after 70 minutes, reaching a maximum of 1·9 mg./100 ml. at 5 hours. On post mortem examination, no sulphaguanidine was recovered from the organs, the main quantity being in the urine (13 mg./100 ml.) and fæces (1·20 g./100 g.) G. M.