METHODS FOR THE DESTRUCTION OF ORGANIC MATTER

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BUREAU OF CEYLON STANDARDS
53, DHARMAPALA MAWATHA,
COLOMBO - 3.

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BUREAU OF CEYLON STANDARDS
53, DHARMAPALA MAWATHA,
COLOMBO-3.

Telephone: 26055
26054
26051

Telegrams: "PRAMIKA"

SRI LANKA STANDARD METHODS FOR THE DESTRUCTION OF ORGANIC MATTER

FOREWORD

This Sri Lanka Standard has been prepared by the Drafting Committee on Chemical Test Methods. It was approved by the Agricultural & Chemicals Divisional Committee of the Bureau of Ceylon Standards and was authorised for adoption and publication by the Council of the Bureau on 5th December 1973.

In the preparation of this standard the assistance derived from the following publications is acknowledged:

(i) Pearson, David. Chemical analysis of food. J. A. Churchill, London. 6th ed. 1970.

(ii) Garrat. D. C. Quantitative analysis of drugs. Chapman & Hall, London. 3rd ed. 1964.

(iii) Middleton, G. & Stuckey, R. E. The destruction of organic matter. **Analyst** vol 79. p 137. 1954.

1. SCOPE

This standard prescribes methods for the destruction of organic matter for the purpose of preparing test solutions for analysis.

2. METHODS

2.1 Dry ashing — Weigh accurately a suitable quantity of the well mixed sample in a tared platinum or silica dish. Heat the dish cautiously with a low flame until the material begins to char. It is possible to regulate charring by placing the flame at one side of

the dish so that charring will spread gradually over the dish without excessive foaming. The charred residue may be heated in a temperature controlled muffle furnace at a temperature preferably not exceeding 420° C. Some products may be ashed by heating the dish directly in a place free from draughts where the material has to be stirred from time to time with a platinum wire. The temperature is critical as halides are slowly volatalised and alkali phosphates fuse to a glass mass entrapping particles of carbon thereby protecting them from complete oxidation. The ashing should be continued until a light grey or white ash is obtained. Oxidation may be hastened by wetting the ash directly with water which will also prevent loss of fluffy ashes.

In some cases the difficulty of effecting complete combustion and the danger of loss by volatalisation on ignition may be overcome by moistening the substance or the ash with strong sulphuric acid and repeating the ignition.

In order to hasten the process of ashing and to prevent fusion of the ash to a glossy mass several aids to ashing are used. These modifications are given below:-

(a) Light magnesium oxide as the ashing aid

Weigh a suitable amount of the material into a tared silica or platinum basin containing light magnesium oxide (up to 2 per cent of the mass of the sample) distributed over the base and partly up the sides of the basin. Support the basin in a hole cut in asbestos board so that at least two-thirds of the basin projects below the asbestos. Heat first by means of a soft flame, such as that of an Argand burner, to volatilise as much as possible of the organic matter, then transfer the basin to a temperature-controlled muffle furnace at a temperature preferably not exceeding 420° C, and heat until no carbon remains.

(b) Magnesium nitrate solution as the ashing aid

Magnesium nitrate solution - Adjust the pH of a 50 per cent $\frac{m}{v}$ solution of magnesium nitrate to 9.5 with ammonium hydroxide using thymol blue as indicator and extract with successive portions of dithizone solution in chloroform until the dithizone layer remains green.

Weigh a quantity of the sample equivalent to not more than 5 g of solids into a tared silica or platinum basin. Heat first by means of a soft flame, such as that of an Argand burner to drive off any moisture and to volatilise as much as possible of the organic matter, continue with increasing heating until white fuming ceases and a dry char is obtained. Break down the char with a clean glass rod and moisten it with a little magnesium nitrate solution. Transfer the basin to a temperature - controlled muffle furnace, bring the temperature to about 420° C, and maintain the furnace at this temperature until no carbon remains.

(c) Sodium carbonate as the ashing aid.

Mix intimately a suitable quantity of the well-mixed sample with anhydrous sodium carbonate (20 per cent of the mass of the sample) in a tared silica or platinum basin. Heat first by means of a soft flame, such as that of an Argand burner, until all volatile carbonaceous matter is driven off. Transfer the basin to a temperature - controlled muffle furnace at a temperature as low as possible, and in any case not exceeding 420° C, and heat until a grey powdery ash is obtained. Care must be taken not to fuse the ash, having regard to the fact that its melting-point will possibly be much lower than that of pure sodium carbonate.

If it is still difficult to burn all the carbon, cool the ash, add a slight excess of dilute hydrochloric acid or a mixture of one volume of concentrated nitric acid and two volumes of water, warm on a water bath and note whether any colour is extracted or whether

organic matter is still present. If so evaporate the mixture to dryness on a water bath and gently char the residue over a small flame until all the organic matter has been destroyed or better repeat the ignition at a higher temperature or for a longer period.

Notes (i) Dry ashing is applicable to the determination of most of the common metals in organic matter with the exception of mercury and arsenic.

(ii) The quantities recommended for use are at least 2 g of dry matter for fish products, grain and livestock feed, 3-5 g of cereal food, 5-10 g of sugar or sugar products or vegetable products, 25 g of juice of fresh fruit or canned fruit and 10 g of jellies, syrups, jams or dried fruit.

(iii) A few drops of an ashless vegetable oil when added to the material will usually prevent excessive swelling or foaming.

(iv) Material containing much water should be dried in an oven before ashing.

(v) Dry ashing in the presence of halides results in loss of certain metals (e.g. Zn, Sn and Sb). This may be minimised by ensuring that an alkaline ash remains.

(vi) Modification (a) can be used for those material with a low ash content (b) for sugars, syrups and biological material ash (c) for the determination of several metals (excluding mercury) whose salt may be volatile in dyestuffs and medicinals.

2.2 Wet methods

Wet oxidation methods are preferable to the dry ashing procedures because they obviate difficulties due to loss of more volatile constituents during ashing and slow solution of the residue after ashing. However, the necessity for constant vigilance and the possibility of high (and sometimes uncertain) blanks render the method less suitable for routine work. The following procedures are commonly used:

2.2.1. Wet ashing - The procedure is carried out in a suitable vessel of borosilicate glass or silica with a large flat area of base, and which can be covered by a clock glass. Lipless beakers, which can be closed more effectively by a clock glass, are preferable. For quantities of material equivalent to about 5 g of dry matter, 1 and 2 litre beakers are used; but the same size of vessel can be used for such larger quantities, e. g. 50 g of dried material, although, of course the procedure then takes longer.

Procedure - Place about 5 g of material in the beaker with 10 to 20 ml of water and 5 to 10 ml of nitric acid sp. gr. 1.4 containing 5 per cent of sulphuric acid, and heat gently until the sample has dispersed. Evaporate the mixture, adding if necessary a few drops of capryl alcohol to control frothing and take care to avoid loss by spurting. Leave uncovered on the hot plate (temperature 310-350° C) until there is little or no further visible change-this stage is generally reached in about 15 minutes. Allow the beaker to cool, add sufficient nitric acid, sp. gr. 1.4 to moisten the residue cover with the clock glass, and leave the beaker on the hot plate for at least 15 minutes after the residue has become dry. Allow the beaker to cool and repeat the operation as often as necessary. When the residue is whitish with dark patches, use fuming nitric acid in place of ordinary nitric acid, and continue until a white residue is left.

With some materials, such as animal tissues, in order to dissolve the residue it is necessary to add about 1 to 3 ml of concentrated sulphuric acid and 20 ml of water and to heat on the hot plate until the residue fumes strongly. Then allow the mixture to cool, and dilute it with water.

Possible modifications in the procedure

The procedure detailed above is a general one and can be applied directly to most materials, but where possible it is advisable to carry out a preliminary test with the

material in question, as this may iudicate that certain modifications are desirable or advantageous, as follows:-

(i) Dilution of the acid in the first stage is used to moderate the reaction and facilitate the dispersion of the material. When the material does not react with diluted acid, there is no point in diluting it.

(ii) Sulphuric acid is added to the first portion of nitric acid only to moderate the reactivity of the 'char' and thereby to prevent ignition. It can often be omitted as it only slows down the procedure.

(iii) For substances containing fats and fatty materials, the first heating (uncovered) is used to remove a large proportion of the volatile matter.

(iv) Any volatile matter (e.g. from fat) that condenses on the clock glass should be removed with cellulose wadding at intervals.

(v) The change to fuming acid should be made at the earliest convenient occasion at which it is possible to do so without causing incandescence; this depends on the material being destroyed. If the weaker acid does not appear to be having any appreciable effect, it is probably safe to change to the fuming acid.

(vi) As an alternative to the procedure described above for getting an insoluble final residue into solution, it may be digested for an hour on the water bath with 5 per cent sodium hydroxide, after which it should dissolve readily on the addition of hydrochloric acid. If phosphorous is absent, the final residue may dissolve directly in dilute acid.

(vii) The total amount of acid used should be noted so that allowance can be made for any trace metals in it. The amount used at each stage need only be sufficient to damp the residue, and therefore decreases continually as the bulk of residue decreases.

(viii) In general, no metallic contamination is to be anticipated from the use of borosilicate glass vessels, but silica could be used if desired. It may be necessary to distil the nitric acid in silica before use in order to remove traces of metals.

2.2.2. Wet Oxidation

2.2.2.1. **Method 1** - Into a Kjeldahl digestion flask, place a suitable quantity of the sample (usually 5-10 g), 20 ml of concentrated nitric acid and upto 20 ml water (depending on the water content of the sample). Boil so that the volume is reduced to about 20 ml, cool and add 10 ml of concentrated sulphuric acid. Boil again, and as the liquid begins to blacken add further small quantities of nitric acid. (Delay may cause loss of elements specially arsenic.) When the addition of nitric acid is no longer necessary (i e. the liquid no longer blackens), continue the heating until white fumes are well in evidence. Cool and add 10 ml of saturated ammonium oxalate solution and boil again until copious white fumes are again produced. The ammonium oxalate treatment assists in removing yellow colourates due to resinous materials, fats etc. so that the final solution is colourless. The blank should be prepared by boiling out the same volumes of reagents used (nitric acid, sulphuric acid, ammonium oxalate).

2.2.2.2. Method 2

(a) Weigh 5 g (or a suitable amount) of the well-mixed sample into a 100 ml. Kjeldahl flask and add 10 ml of a mixture of 1 volume of concentrated nitric acid and 2 volumes of water. As soon as any initial reaction subsides, heat gently until further reaction ceases, and then cool the mixture. If the initial reaction is violent as in the case of carbohydrates heating should be delayed, if necessary, even overnight. Further nitric acid may then be added as necessary. With some extremely reactive organic compounds it is necessary to carry out the preliminary treatment with dilute nitric acid in a 500-ml beaker, heating the beaker slowly on a water-bath until the initial reaction is completed. If excessive frothing is experienced in the earlier stages, a drop or two of sec.-octyl alcohol may be added or the preliminary treatment may be carried out in a 500-ml borosilicate-glass beaker with the addition of glass beads to prevent bumping. Add, gradually, up to 10 ml of concentrated sulphuric acid at such a rate as not to cause excessive frothing or heating (five to ten minutes are usually required) and then heat until the liquid darkens appreciably in colour.

Continue as given below (Clause 2.2.2.2 e)

(b) Weigh 5 g (Or a suitable amount) of the well-mixed sample into a 100 ml Kjeldahl flask and add 5 ml of concentrated nitric acid. As soon as any vigorous initial reaction subsides, heat gently until further vigorous reaction ceases, and then cool the mixture. Add, gradually, 8 ml of concentrated sulphuric acid, at such a rate as not to cause excessive frothing or heating (five to ten minutes are usually required), and then heat until the liquid darkens appreciably in colour.

Continue as given below (Clause 2.2.2.2 e).

(c) Weigh 5 g (or a suitable amount) of the well-mixed sample into a 100-ml Kjeldahl flask and add a mixture of 8 ml of concentrated sulphuric acid and 10 ml of concentrated nitric acid. Warm cautiously until the reaction subsides, and then boil rapidly until the solution begins to darken owing to incipient charring.

Continue as given below (Clause 2.2.2.2 e).

(d) Treat 5 g (or a suitable amount) of the material in a 100-ml Kjeldahl flask with 20ml of a mixture of 1 volume of concentrated nitric acid and 2 volumes of water and warm until the initial vigorous reaction is over. At this point a spongy, tarry cake is formed. Cool the mixture pour off the acid into a beaker, and wash the tarry residue with a small amount of water (three or four 1-ml portions), adding the washings to the acid liquor in the beaker. Add 8 ml of concentrated sulphuric acid to the tarry residue, agitate to disperse the cake, and introduce concentrated nitric acid, drop by drop, with warming if necessary, until vigorous oxidation ceases. Return the original acid liquor to the flask, and boil until the solution just begins to darken. Continue as given below (Clause 2.2.2.2 e).

(e) Methods 2 (a)-(d) to be continued to completion by one of the methods given below.

(i) Without addition of perchloric acid or hydrogen peroxide.

Add concentrated nitric acid slowly in small portions (1 to 2 ml), heating after each addition, until darkening again takes place, Do not heat so strongly that charring is excessive, or loss of

arsenic may occur; a small, but not excessive, amount of free nitric acid must be present throughout. Continue this treatment until the solution fails to darken on prolonged heating to fuming (five to ten minutes). The criterion of completion of oxidation is that the final solution is fuming when hot and colourless when colder but if much iron is present the solution will be pale yellow in colour frequently with a granular precipitate soluble on dilution. Allow to cool somewhat, dilute the solution with 10 ml of water (this should give a colourless solution or a faintly yellow one if iron is present), and boil gently to fuming. Allow the solution to cool again add a further 5 ml of water and boil gently to fuming. Finally cool, and dilute the solution with 5 ml of water.

(ii) With the addition of perchloric acid or hydrogen peroxide,

With addition of perchloric acid - Add concentrated nitric acid, slowly in small portions, heating after each addition, until darkening takes place. Do not heat so strongly that charring is excessive, or loss of arsenic may occur; a small, but not excessive, amount of free nitric acid must be present throughout. Continue this treatment until the solution fails to darken in colour on prolonged heating (five to ten minutes) / and is only pale yellow in colour. Run into the flask 0.5 ml of 60 per cent perchloric acid, m/m and little more nitric acid, and heat for about fifteen minutes, then add a further 0.5 ml of perchloric acid and heat for a few minutes longer. Allow to cool somewhat, and dilute the mixture with 10ml of water. The solution should be quite colourless

except when much iron is present, when it may be faintly yellow. Boil gently taking care to avoid bumping until white fumes appear, allow the solution to cool, add a further 5 ml of water and again boil gently to fuming. Finally cool and dilute the solution with 5 ml of water.

With addition of hydrogen peroxide - Proceed as given above, to the point of addition of perchloric acid. In place of this add analytical - reagent grade 100-volume hydrogen peroxide, in small quantities (1 or 2 ml), with a few drops of concentrated nitric acid. Heat to fuming after each addition of hydrogen peroxide until the residue is colourless or no further reduction of the pale yellow colour can be obtained; cool the solution, dilute it with 10 ml of water, and evaporate to fuming; again dilute the solution with 5 ml of water, and evaporate to fuming. Finally dilute the solution with 5ml of water.

2.2.2.3 Method 3.

Introduce into a 100-ml conical borosilicate glass flask an amount of sample containing not more than 2g of dry matter. Add 1ml of water, 3ml of concentrated nitric acid and 2 ml of 60 per cent $\frac{m}{m}$ perchloric acid and heat the flask on an electric hot-plate, starting the plate from cold. Use a layer of asbestos paper, if necessary, to moderate the heat, When the liquid begins to turn brown, add further nitric acid, drop by drop, to avoid further darkening. (Note: the darkening of digestion mixtures containing perchloric acid is regarded by some workers as indicating the onset of dangerous conditions. The real significance of this darkening probably depends upon the nature of the sample but in any case the condition should not be allowed to persist longer than can be avoided). A colour-

less solution and white fumes of perchloric acid indicate completion of oxidation.

2.2.2.4. Method 4 - Destruction with nitric acid and ammonium nitrate

Transfer 1 g of the dried and powdered sample to a 250-ml Kjeldahl flask, and add 10 ml of concentrated nitric acid. Gently warm the flask until solution is complete, add 10 ml of oxidising reagent (a solution of 50 g of ammonium nitrate and 25 g of concentrated nitric acid, make upto 100 ml with water) and heat gently to expel water so that oxidation (indicated by efferverscence) proceeds in a melt of ammonium nitrate. From time to time add more reagent if necessary, and continue until no browning of the solution is observed and a clear melt has been obtained. In the presence of much fat, oxidation is slower and more reagent may be required, and there may be excessive initial frothing if much carbohydrate is present. When oxidation is complete, heat the clear melt more strongly to volatilise the excess of ammonium nitrate (avoid overheating), holding the flask over a free flame to expel salt subliming on the side. Then dissolve the residue in 2 ml of concentrated hydrochloric acid, evaporate to dryness, fuse the residue in the flask, so as to remove all nitric acid, dissolve it in about 10 ml of N hydrochloric acid, and evaporate the solution to a small bulk to ensure conversion of any metaphosphate to orthophosphate. Evaporate to dryness in a stream of warm air, dissolve the residue in a few millilitres of warm water, add a few drops of N hydrochloric acid and dilute to 10 ml with water.

In all the above methods employing perchloric acid special precautions should be taken.

Notes

(i) The methods given in clauses 2.2.1 and 2.2.2.1 are of general application. The method given in clause 2.2.1 is specially effective for all animal tissues, proteins and other biological material which are in general the most difficult to deal with by other methods.

(ii) The methods given in clause 2.2.2.2. are applicable to almost all materials although unfamiliar materials must always undergo preliminary treatment on a small scale before the method to be used is selected.

Given suitable selection, methods given in clause 2.2.2.2. (a)-(d) are applicable to the destruction of most organic materials, including such materials as dyestuffs, intermediates and medicinals, before the determination of most of the common trace metals, but they are not recommended in the presence of appreciable amounts of alkaline-earth metals, since the insoluble sulphates formed absorb a considerable proportion of trace metals, particularly lead. In such instances the method given in clause 2.2.2.3. should be used. The method given in clause 2.2.2.2. (b) is suitable for less reactive substances than those that the method given in clause 2.2.2.2. (a) is suitable for and the method given in clause 2.2.2 2. (c), a more rapid method than methods given in clauses 2.2.2.2. (a) or (b) is appropriate for substances that decompose quietly. For substances that are liable to deflagrate violently during charring with risk of incurring appreciable losses of arsenic the method given in clause 2.2.2.2. (d) must be used.

The choice of the continuation method for the methods given in clause 2.2.2.2. (a) - (d) may be left to the individual operator, The use of perchloric acid or hydrogen peroxide speeds digestion and hence reduces the amount of nitric acid required and shortens the time taken to complete the removal of all the organic matter but of course perchloric acid must not be used if the

presence of chloride is detrimental in the procedure for determination of the metals.

(iii) The method given in clause 2.2.2.3 is suitable for sugar products when lead is to be determined.

(iv) The method given in clause 2.2.2.4 was orginally devised for the determination of calcium, magnesium, sodiun, potassium and sulphur all on one sample, since sodium and potassium which have volatile compounds, cannot be determined after dry ashing and methods involving the use of sulphuric acid as an oxidising agent, which leave unchanged acid in the residue preclude the determination of sulphur. The method can also be applied when iron, copper and similar elements are to be determined in samples, in which the amount of organic matter is small and is easily decomposed.

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