Pure & Appl. Chem., Vol.51, pp.2527-2536. 0033-4545/79/1201-2527 $02.00/0
Pergamon Press Ltd. 1979. Printed in Great Britain.

INTERNATIONAL UNION OF PURE
AND APPLIED CHEMISTRY

APPLIED CHEMISTRY DIVISION

COMMISSION ON FOOD CONTAMINANTS*

DETERMINATION OF MERCURY IN FOODSTUFFS

Prepared for publication by
L. E. COLES
Institute of Preventive Medicine, Cardiff, U.K.
H. GUTHENBERG
Kungl. Generaltullstyrelsen, Stockholm, Sweden

Chairman: J. KOJIMA (Japan); *Secretary:* L. E. COLES (UK); *Titular Members:* A. D. CAMPBELL (USA); M. JEMMALI (France); P. KROGH (Denmark); M. V. TRACEY (Australia); *Associate Members:* G. BILLEK (FRG); F. BRO-RASMUSSEN (Denmark); H. GUTHENBERG (Sweden); W. KRÖNERT (FRG); J. D. LITTLEHAILES (UK); K. OHNO (Japan); P. L. SCHULLER (Netherlands); P. S. STEYN (S. Africa); *National Representatives:* H. LANGE (FRG); T. JUSZKIEWICZ (Poland).

THE DETERMINATION OF MERCURY IN FOODSTUFFS

1. SCOPE:

This method specifies a reference method for the determination of total mercury in foodstuffs.

2. FIELD OF APPLICATION:

The method described is applicable to the determination of the mercury content of all foodstuffs and biological materials down to 0.01 mg/kg in order to determine lower concentrations a concentration step should be applied as described below under 11. Special cases.

3. DEFINITION:

Total mercury contents of foodstuffs: the mercury contents determined according to the procedure described in this recommendation and expressed in milligrammes per kilogramme.

4. PRINCIPLE:

Wet combustion by means of nitric and sulphuric acids of the test portion in an all-glass apparatus according to Thiele-Pape. An oxidizing medium is maintained throughout the digestion.

The digest is diluted with water, excess oxidizing substances are removed by adding hydroxylamine and mercury (II) is reduced to metallic mercury by tin (II). Mercury is evaporated from the solution by a nitrogen current at room temperature and determined by cold vapour atomic-absorption measurement at 253.7 nm.

5. REAGENTS:

All reagents should be of analytical-reagent quality. Water must be redistilled from an all-glass apparatus of Pyrex+ or other resistant glass.

5.1 Nitric acid, 65% (m/m) d = 1.40
Should be checked for the absence of heavy metals.

5.2 Sulphuric acid, 98% (m/m) = 1.84
Should be checked for the absence of heavy metals.

5.3 Magnesium perchlorate.

5.4 Reducing solution.

Mix 2.5 ml sulphuric acid (5.2) with 30 ml redistilled water. Cool mixture to room temperature and dissolve 1.5 g hydroxylamine hydrochloride and 2.5 g of tin (II) chloride ($SnCl_2.2H_2O$). Dilute to 50 ml.

This solution will be effective as a reducing agent for several days.

5.5 Nitrogen from a cylinder.

5.6 Hydroxylamine hydrochloride solution 20% (m/v).

Dissolve 20 g hydroxylamine hydrochloride in 100 ml redistilled water. Shake the solution well for two minutes with 10 ml of dithizone solution (0.05% m/v) in chloroform. Allow the layers to separate and discard the green dithizone solution. Finally extract the aqueous solution with successive small portions of chloroform until a colourless extract is obtained.

+ Mention of a company or brand name does not constitute a guarantee or warranty of the company's product and does not imply its approval to the exclusion of other products that may be also suitable.

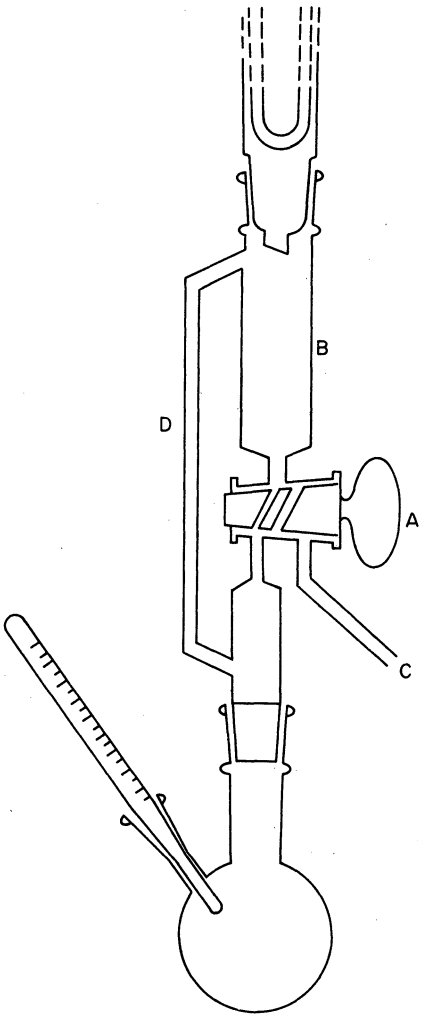
5.7 Hydroxylamine hydrochloride solution 5% (m/v).

Dilute 10 ml hydroxylamine hydrochlorine 20% to 40 ml with redistilled water.

5.8 Stannous chloride solution 20% (m/v).

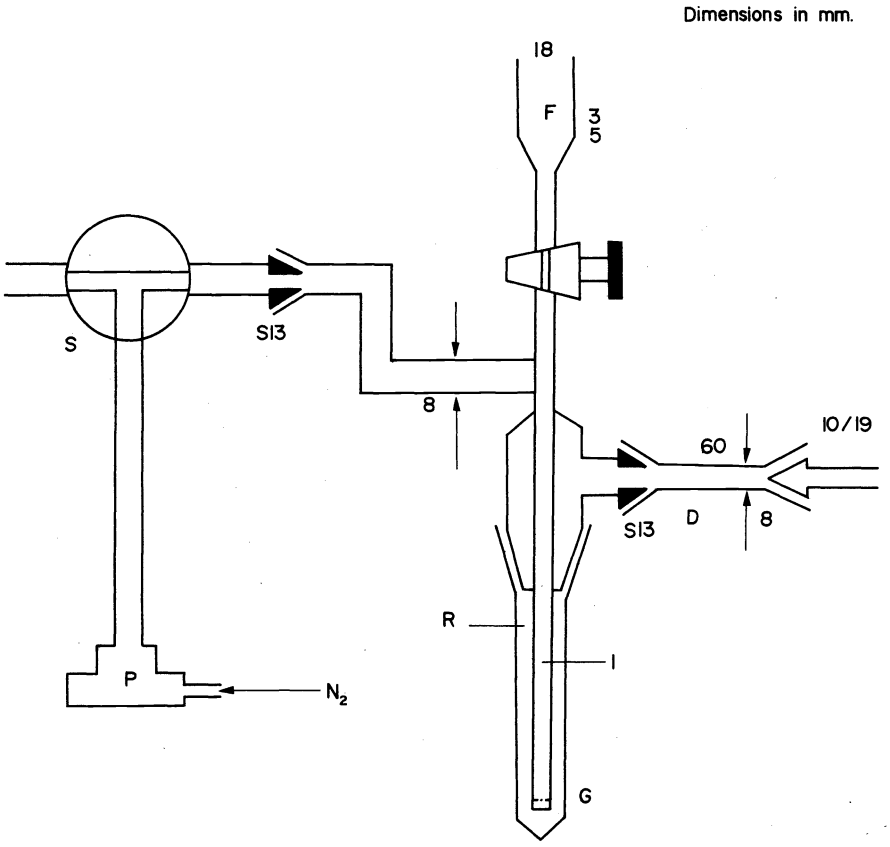
Dissolve 12 g tin (II) chloride ($SnCl_2 \cdot 2 H_2O$) in 50 ml sulphuric acid 10% (m/v).

FIGURE 1



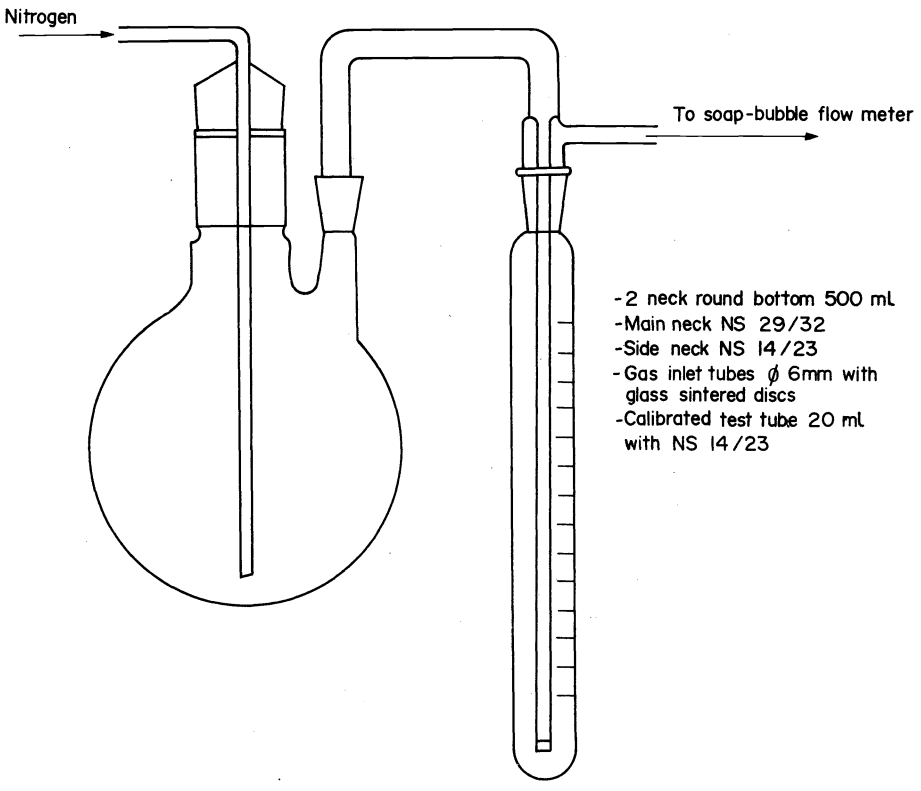
Apparatus for controlled decomposition of organic material

FIGURE 2



R = Sample tube 100 x 18
I = Inlet tube ø 6
G = Sintered glass disc
F = Funnel
P = Constant flow controller
S = Threeway tap
D = Drying tube

FIGURE 3



Apparatus for concentrating mercury

5.9 Potassium permanganate solution 5% (m/v).

Dissolve 2.5 g potassium permanganate in 50 ml redistilled water. The potassium permanganate should be checked for the absence of mercury by a blank determination.

5.10 Potassium permanganate solution 0.625% (m/v).

Dilute 12.5 ml potassium permanganate solution 5% with redistilled water to 100 ml.

5.11 Sulphuric acid solution 18N.

Dilute 245 ml sulphuric acid 98% (5.2) with redistilled water to 500 ml.

5.12 Potassium permanganate-sulphuric acid solution 0.5% (m/v).

Dilute 20 ml potassium permanganate 0.625% (m/v) with sulphuric acid 18N to 25 ml.

This solution must be prepared daily.

5.13 Concentrated mercury stock-standard solution (1.000 mg Hg/ml).

Dissolve 135.4 mg mercury (II)-chloride in 100 ml sulphuric acid (0.1N).

5.14 Mercury stock-standard solution (0.1000 mg Hg/ml).

Dilute concentrated mercury stock-standard solution (5.13) ten times by pipetting 10 ml of 5.13 into a graduated flask of 100 ml and make up to the mark with sulphuric acid (0.1N).

5.15 Diluted mercury stock-standard solution (0.0010 mg/ml).

Dilute mercury stock-standard solution (5.14) 1/100 by pipetting 5 ml of 5.14 into a graduated flask of 500 ml and make up to the mark with sulphuric acid (0.1N).

5.16 Working mercury standard solutions.

Prepare working mercury standard solutions in the range between 0.005 and 0.1 µg/ml in sulphuric acid (1 N) by serially diluting the diluted mercury stock-standard solution (5.15) pending upon the concentration expected.

WARNING:

Fresh working mercury standard solutions must be prepared just before use from the diluted mercury stock-standard solutions (5.15).

5.17 Palladium (II) chloride on glass wool: Dip a piece of glasswool into a solution of palladium chloride (1% m/v). The glass wool should be dried at room temperature.

6. APPARATUS AND GLASSWARE:

Glassware, including reagent bottles, must be of chemically resistant glass preferably Pyrex or equivalent. It should be reserved for the estimation of mercury and before its first use it must be cleaned with warm nitric acid and water. Before each use the glassware should be cleaned with dilute nitric acid (4N) and water. Before each use the digestion apparatus is cleaned by boiling with nitric acid (4N), without water circulating through the condenser.

6.1 Digestion apparatus:

Consisting of a 100 ml or 250 ml two-neck round-bottom flask equipped with a Thielepape extractor with a two-way-stopcock for withdrawing samples (Normachliff Glasgerate K.G. Wertheim (cat. no. 6.224) with a reservoir capacity of 70 ml), a Dimroth condenser length of jacket 40 cm and a thermometer up to 200°C (fig. 1).

6.2 Measuring cylinders.

For delivering liquid quantities of 250 ml, 25 ml, 10 ml.

6.3 Sampling tubes.

10 x 1.6 cm with ground joint NS 14/23.

6.4 Measuring pipettes.

For delivering quantities of 0.5 ml.

6.5 Calibrated one-mark pipettes.

For delivering quantities of 2 ml.

6.6 Stopwatch.

6.7 Atomic-absorption spectrophotometer.

Techtron AA 4 or equivalent with digital read out or 1 mv stripchart recorder, hollow cathode mercury lamp equipped with power supply or equivalent.

6.8 Vapour cell.

Standard cylindrical silica spectrophotometer cell (100 or 200 mm pathlength and 20 mm internal diameter with quartz windows).

6.9 Vapour cell holder.

Replace burner and secure cell in a spevial vapour cell holder.

6.10 Precision bore flowrator.

Fisher and Porter 08F-1/16-4/36 or equivalent. The flowrator is calibrated for 0-15 litres/h., the flowmeter is connected at the outlet of the vapour cell.

6.11 Reduction-aeration apparatus.

Assembled as shown in figure 2 consisting of a constant flow controller (Brooks 8943 B) or equivalent (P) inlet pressure 1.5 atm, a three way T-bore stopcock (S) connected to the flow controller with PVC-tubing, a gas inlet adapter with a fused in sintered glass disc diameter (G) 5 mm, maximum pore size 90-150 micron and a funnel (F) with a capacity of 3 ml equipped with stopcock for introducing the reagent, a drying tube with magnesium perchlorate.

6.12 Gas washing bottle.

Containing a potassium permanganate (0.5%)-sulphuric acid solution to prevent escape of mercury vapour into the atmosphere connected to the outlet of the reduction-aeration apparatus with the help of PVC-tubing.

6.13 Glass-beads.

Washed with warm nitric-acid (4 N) and redistilled water.

7. SAMPLE:

Proceed from a representative primary sample of at least 200 grammes.

8. PROCEDURE:

8.1 Preparation of the sample.

Make the sample homogeneous, avoid contact with metals by using porcelain where ever possible (spoons etc.). If metal food grinders are used, check them for possible mercury contamination.

8.2 Test portion.

Weigh into the digestion flask to the nearest 10 mg about 2 to 5 grammes of the homogenized sample.

8.3 Digestion.

Add 12.5 ml concentrated nitric acid, 4 ml concentrated sulphuric acid and some glass beads. +Mix well and assemble the Thielepape apparatus as shown in Fig. 1. Allow mixture to stand at room temperature overnight in order to prevent foaming during the first stage of digestion. Start refluxing and heat, first by means of a soft flame, for example, of an Argand burner, remove flame from digestion apparatus as necessary to minimize escape of nitrogen oxides from the top of condenser. Maintaining full heat, turn the tap A through 90° so that liquid distils into the reservoir B.

+Note 1: In the case of dry material such as cereals etc. 10 ml of redistilled water is first added to sample before adding nitric acid.

Temperature of the vapour in the digestion flask at this stage must not exceed 120°C. Turn the tap through a further 90° so that the distillate (mainly water) runs out through C into a 250 ml measuring cylinder. Turn the tap in such a way that liquid distils into the reservoir B.

Increase the heating in such a way that nitric acid distils into the reservoir. If the solution begins to darken stop heating until temperature of the vapour in the digestion flask is lower than 100°C. Add a few millilitres of nitric acid from the reservoir with the help of the threeway stopcock and continue heating.

Repeat this procedure till the solution remains yellow when heated at 140-145°C.+ Digestion has been now completed.

The digest is cooled and the nitric acid in the Thielepape extractor is combined with the distilled water already drained off. Rinse condenser and extractor and round-bottom flask carefully with 100 ml redistilled water. Combine the washings with the water-nitric acid portions and note the total volume. The mercury should be determined on the digestion solution.

8.4 Determination:

Switch on the atomic absorption spectrophotometer and allow the mercury lamp to warm up for at least 30 minutes. Adjust the monochromator to wavelength 253.7 nm and slitwidth to 100 micron (0.33 nm). Adjust the nitrogen current with the constant flow controller at 100 ml/minute. Set three-way stopcock in such a way that no gas passes through the aeration unit (see fig. 2).

Pipette into the sample tube 2 ml of the digestion solution (8.3) and connect the tube to the gas inlet adapter. Introduce 0.5 ml of the reduction solution into the small funnel (F) (see fig. 2) over the gas inlet adapter (stopcock closed). Introduce the reducing solution into the sample solution by opening the stopcock for a few seconds, then turn the three-way stopcock in such a way that two or three bubbles pass through the sample solution. Turn the stopcock back to its original position. Wait exactly 15 seconds and turn the stopcock in such a way so as to allow the nitrogen to bubble through the sample tube, removing the mercury that has just been formed by reduction. Note the maximum pen deflection or the maximum of absorbance (optical density). Remove the sample tube when the absorbance has diminished to half of its maximum value, insert a dry clean sample tube and wait until the digital read out has reached zero again or until the recorder pen has reached the baseline again. Start the next analysis.

Carry out two determinations, starting from the same digested solution. Read the mercury concentration of the sample solution from a calibration curve, obtained with working mercury standard solutions (8.5).

8.5 Calibration curve.

Repeat the determination procedure (8.4) with 2 ml of the working mercury standard solution instead of the sample solution.

Plot the absorption values or peak heights measured against the concentration of the working mercury standard solutions and construct the best fitting straightline through the plotted points and the origin. The determinations should be performed in duplicate starting from the same working mercury standard solutions.

9. EXPRESSION OF RESULTS AND CALCULATION:

9.1 Calculate the mercury contents (Hg) of the sample in µg/g from the formula

$$Hg = \frac{V}{W} \times C$$

where

V= total volume of the digestion solution (8.3) in millilitres.

W= mass of the test portion in gramme (8.2).

C= the mercury contents in the digestion solution expressed in microgrammes per millilitre.

+ Note 2: In some cases addition of a new portion of 10 ml of nitric acid is necessary. Drain off the nitric acid already distilled in the reservoir and combine with the water in the measuring cylinder.

Take as the results the arithmetic mean of two determinations if the requirement of repeatability is satisfied. Report the results to the nearest 0.005 p.p.m.

9.2 Repeatability.

The difference between the results of a determination in duplicate (obtained simultaneously or in rapid succession by the same analyst) shall not exceed 5% of the mercury contents.

10. CONTROL OF NON-ATOMIC ABSORPTION AND MOLECULAR EFFECTS:

10.1 Introduction.

When digestion is not complete, volatile organic compounds can also give an analytical signal resulting in too high values for the mercury content. It is necessary to control every digest for this phenomenon.

This control is carried out with palladium (II) chloride on glasswool (5.17). The mercury is then amalganized and when the digestion is complete no peak is observed. Otherwise the digestion must be repeated.

10.2 Procedure.

Remove the tube with magnesium perchlorate (D in the scheme of fig. 2). Insert tube with magnesium perchlorate and palladiumchloride on glasswool.

Take care that the nitrogen gasflow first pass the magnesium-perchlorate. Repeat the procedure as described under 8.4 for the determination of the standard and sample solution.

11. SPECIAL CASES:

If the mercury contents of the foodstuffs is lower than 0.01 ppm, transfer a measured quantity, but not more than 150 ml of the digestion solution into a two neck 500 ml round bottom flask (see fig. 3). Add one-tenth of this volume of hydroxylamine hydrochloride 20% (5.6) and wait for 15 minutes.

Pipette into a calibrated 20 ml test tube with a ground glass joint 5 ml freshly prepared potassium permanganate-sulphuric acid solution (5.12).

Connect receiver adaptor and test tube as indicated in fig. 3. Add 10 ml tin (II)-chloride 20% (5.8) to the roundbottom flask and assemble immediately as indicated in fig. 3. Adjust the nitrogen current to 750 ml/minute+, allow the nitrogen to bubble through the solution for 15 minutes.

Disconnect the test tube. Pipette 2 ml hydroxylamine hydrochloride 5% (5.7) into the test tube, allow several nitrogen bubbles to pass in order to mix. Discolouration must take place.

Pipette 2 ml of this solution into a sample tube and proceed as described under 8.4.

Calculate the mercury contents (Hg) of the sample in µg/g (ppm) from the formula

$$Hg = \frac{V \times 7}{V_1} \times \frac{C}{W}$$

where:

V= total volume of the digestion solution (8.3) in millilitres.

W= mass of the test portion in gramme (8.2).

V_1= volume of the digestion solution taken for concentration step (11).

C= mercury contents in the sample solution expressed in microgrammes per millilitres.

+ With the help of a soap-bubble flowmeter.