INTERNATIONAL UNION OF PURE AND APPLIED CHEMISTRY

COMMISSION FOR THE DETERMINATION OF TRACE ELEMENTS IN FOOD

DETERMINATION OF LEAD CONTENT OF FOODSTUFFS

INTRODUCTION

The Commission for Determination of Trace Elements in Food consisted of the following permanent or part-time members: R. Cultrera (Parma), A. R. Deschreider (Brussels), W. Diemair (Frankfurt), J. C. Gage (Welwyn, Herts), J. F. Reith (Utrecht; chairman), L. L. Ramsay (Washington), G. Taylor (London), L. Truffert (Paris) and H. J. Wichman (Washington).

Sessions were held at Zürich, Lisbon, Paris, Munich and London. One or more sessions were also attended by D. W. Kent-Jones (Honorary Secretary of the Food Division), Miss Olliver (Histon, England), H. G. Irving (Oxford) and R. Truhaut (Paris).

The Commission prepared methods for the determination of traces of copper,† lead and mercury in food.

1. Principle

The organic material in the sample may be destroyed by strong acids or by dry ashing.

The digest or the ash solution is diluted to an acid concentration of not more than 1 N and is extracted with a solution of diethylammonium diethyldithiocarbamate in carbon tetrachloride. The extract which contains lead, bismuth, iron and other metals but no magnesium or calcium and no phosphates, is evaporated and the residue destroyed with strong acids.

The residue is diluted and hydroxylamine is added to prevent oxidation of dithizone. Citrate and cyanide are added to mask interfering metals such as iron, copper and zinc. The pH is adjusted to 8·5–9 by the addition of ammonium hydroxide. Lead and bismuth are extracted by means of a solution of dithizone in carbon tetrachloride from which the lead and only a small part of bismuth are extracted with 0·025 per cent nitric acid.

After the addition of hydroxylamine and citrate, the aqueous layer is adjusted to pH 8·5–9 by means of potassium cyanide, and lead and bismuth are again extracted with a known volume of dithizone solution.

The lead content of this solution is determined by a reversion technique;

† The method for the determination of copper was published by Butterworths as an I.U.P.A.C. report in 1959.

one-half of the solution is shaken with 0·025 per cent nitric acid which detaches lead from its complex with dithizone but leaves any bismuth dithizonate unchanged. The difference in optical density of the two portions of the extract is measured at 620 mμ and converted into micrograms of lead by means of a factor determined by calibration.

In an alkaline solution containing cyanide, only lead, bismuth and thallium react with dithizone. The reversion technique removes the interference from bismuth and if sufficient citrate is present during the preliminary and final extraction with dithizone solution, thallium will not interfere as this metal reacts slowly with dithizone under these conditions. With materials containing much calcium it is preferable to destroy the organic matter by dry ashing rather than by oxidation with nitric–sulphuric acid, as with the latter the subsequent precipitation of calcium sulphate may entrain some lead. Dry ashing should be avoided if the sample contains much halogen.

The collaborative work of the Commission has indicated that the method is satisfactory if the lead content of the sample taken for analysis lies between 4 and 40 μg. If the lead content is higher than this range, a stronger dithizone solution than that indicated in **3.3** below should be preferred.

2. Requisites

2.1 *Reagents*

All reagents should be of Analytical Reagent quality if available.

Ammonium citrate solution 5%—Adjust the pH of a 5 per cent w/v aqueous ammonium citrate solution to about 8·5 by the addition of ammonium hydroxide solution, using an external indicator. Shake the solution with portions of a solution of dithizone in carbon tetrachloride (25 mg/litre) until two successive extracts show the unchanged green colour of the reagent. Shake the citrate solution with portions of chloroform to extract any dissolved dithizone and remove the chloroform by shaking with carbon tetrachloride.

Ammonium hydroxide 4 N—Dilute strong ammonium hydroxide solution with water to about 4 N. If no strong ammonium hydroxide with a low lead content is available, conduct aqueous ammonia into water.

Carbon tetrachloride—Test for the absence of oxidizing substances by adding a few drops of dithizone solution to obtain a faintly perceptible green colour and storing in the dark; the colour should not change within an hour. If the colour fades in this period, distil off the carbon tetrachloride, rejecting the first and last tenth; check the distillate for the absence of oxidizing substances. Store in a cool dark place and test again before use.

Carborundum grains—Wash with hot nitric acid.

Cotton-wool—Wash cotton-wool with dilute nitric acid, then with water until free from acid and dry. Wash it with dithizone solution, then with carbon tetrachloride and dry.

DDDC reagent—Dissolve 40 mg diethylammonium diethyldithiocarbamate in 100 ml carbon tetrachloride; this solution should be prepared freshly each day.

Stock dithizone solution—Dissolve 40 mg dithizone in 100 ml carbon tetrachloride in a separating funnel and extract the solution with three successive 100 ml of 1 per cent ammonia. Wash the combined ammonia extracts with carbon tetrachloride, add dilute sulphuric acid until the solution is acid to Congo Red paper and extract the dithizone with three portions of 100 ml carbon tetrachloride. Wash the combined tetrachloride extracts with 25 ml of water and filter through paper. Store in a cool dark place. Some samples of dithizone have given satisfactory results in this method for lead determination without purification; with such samples it is sufficient to dissolve 40 mg dithizone in 300 ml carbon tetrachloride.

Dithizone solution 6 mg/litre—Dilute the stock dithizone solution with carbon tetrachloride so that the optical density of the solution, when measured at 620 mμ in a 1 cm cell, is 0·65.

Hydrochloric acid concentrated (38%)—Test for the absence of lead as described for nitric acid concentrated.

Hydroxylamine solution—Prepare a 10 per cent w/v aqueous hydroxylamine hydrochloride solution and purify as described for ammonium citrate solution. Residual dissolved dithizone may be removed by washing with carbon tetrachloride.

Magnesium nitrate solution—Dissolve 500 g magnesium nitrate in water and dilute to 1 litre. Purify the solution as described for ammonium citrate solution.

Metacresol purple solution—Dissolve 1 g metacresol purple in 500 ml ethanol and dilute to 1 litre with water.

Nitric acid concentrated (65% weight, sp. gr. 1·40)—Test for the absence of lead by mixing 50 ml with 5 ml of concentrated sulphuric acid and carrying out the lead determination described below in 3·3–3·5.

The lead content so determined should not be more than 0·5 μg. If necessary, purify the sample by distillation in the presence of sulphuric acid. Store in the dark.

Nitric acid 0·025%—Dilute 0·3 ml concentrated nitric acid to about 1 litre with water. Titrate and adjust the concentration to 0·004 N. Store in the dark in an amber bottle.

Potassium cyanide solution 5%—Dissolve 50 g potassium cyanide in water and dilute to 100 ml. Purify the solution as described for ammonium citrate, using only a small excess of dithizone as this is difficult to remove. Dilute the solution to 1 litre with water.

Standard lead solution (*1 μg lead per* ml)—Dissolve 0·1598 g lead nitrate in a litre of 0·025 per cent nitric acid solution to give a stock solution containing 100 μg lead per ml. Dilute this stock solution 1/100 with 0·025 per cent nitric acid immediately before use.

Sulphuric acid concentrated (*95 to 98%*)—Determine the lead content of 5 ml of the concentrated sulphuric acid by the procedure described in **3.3–3.5**, this should be not more than 0·2 μg lead.

Water—Redistil distilled water from an apparatus constructed of resistant glass or purify by de-ionization.

2.2 *Glassware*

All glassware, including the reagent bottles, must be of chemically resistant glass, preferably Pyrex, or of polyethylene. It should be reserved for the estimation of lead 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 and water. The necks of the bottles should be protected against dust. Cork and rubber bungs must not be used.

2.3 *Laboratory*

The laboratory should be as dust-free as possible preferably entirely devoted to the determination of traces of metals. Apparatus and glassware should be covered against dust.

3. Procedure

3.1 *Destruction of organic matter*

Weigh a representative portion of the material containing preferably between 4 and 40 μg lead, into a suitable porcelain or silica dish. Dry the wet samples on a steam bath or in an oven. Add a few ml of the magnesium nitrate solution to products difficult to ash or to furnish bulk to materials with a low ash content. Mix well and dry.

Char the sample on a hot-plate or by a gas burner with a soft flame. Do not allow the material to ignite. With some samples better ashing is obtained if the magnesium nitrate solution is added to the charred sample, broken up with a clean glass rod. Place the dish in a muffle furnace and raise the temperature slowly to 500°C and maintain the furnace at a temperature between 450°C and 500°C overnight. If a clean ash is not obtained by this treatment, remove the dish from the furnace, allow it to cool, moisten the ash with magnesium nitrate solution and repeat the heating at 500°C.

When a clean ash is obtained, cool and cautiously add 15–20 ml lead-free concentrated hydrochloric acid. Evaporate to dryness on a steam bath and dissolve the residue in N hydrochloric acid, filter, wash any residue on the paper with N hydrochloric acid and make the total volume up to 50 ml with the washings. If more than a trace of insoluble residue is obtained, the procedure described in the original reference should be followed. If the organic matter is to be destroyed by digestion with strong acids, transfer a weighed amount of the sample, corresponding to not more than 5 g dry

weight, into a suitable round-bottom flask, together with a few grains of carborundum. Add water if the sample contains less than 75 per cent water and about 2 ml nitric acid per gram dry matter. Add 5 ml sulphuric acid.

Carry a blank along with the unknown through all the subsequent steps, using the same amounts of reagents throughout.

Warm the flask slightly and discontinue heating if foaming becomes excessive. When the initial reaction has subsided, heat cautiously until the contents of the flask commence to boil. Continue boiling off the solution, maintaining oxidizing conditions at all times during the digestion by adding cautiously small quantities of nitric acid whenever the mixture begins to darken. Continue digestion until the organic matter is destroyed and sulphur trioxide fumes are copiously evolved.

The final solution should be colourless or at most light straw in colour.

To remove nitrosylsulphuric acid, allow to cool, add 25 ml water and boil off to fuming point. Repeat this operation with another 25 ml water. Allow to cool, add about 180 ml water and boil if necessary to dissolve calcium sulphate.

3.2 *Extraction with diethylammonium diethyldithiocarbamate solution (DDDC)*

Transfer the ash solution or the solution of the digest, which should have not more than normal acidity, to a separating funnel, add 10 ml DDDC reagent and shake for 1 minute (200 shakes). Transfer the lower layer to a flask and repeat the extraction 4 times more. To the combined extracts add 0·5 ml concentrated sulphuric acid and evaporate off the carbon tetrachloride. Oxidize the residue by means of about 1 ml nitric acid as described in **3.1**.

Remove nitrosylsulphuric acid as described in **3.1**, using two successive 5 ml water. Dilute the residue with about 10 ml water.

3.3 *Extraction with dithizone solution*

Transfer the solution to a separating funnel and wash in with a further 5 ml water. Add 1 ml hydroxylamine solution, 1 ml citrate solution and a few drops of metacresol purple solution and adjust the pH to 8·5–9·0 (indicator changes to purple) by means of ammonium hydroxide solution. Add 1 ml potassium cyanide solution and extract the lead by shaking for one minute with successive 2 ml portions of dilute dithizone solution until two successive extracts show the unchanged green colour of the reagent. Run the extracts into a separating funnel, taking care to avoid transferring any of the aqueous phase.

3.4 *Extraction with dilute nitric acid*

Shake the combined extracts for one minute with 25 ml 0·025 per cent nitric acid. Discard the carbon tetrachloride layer and wash the aqueous layer with 1 ml carbon tetrachloride.

3.5 *Determination of lead by reversion*

The following procedure is suitable for a spectrophotometer with 1 cm cells of not more than 4 ml capacity.

To the acid extract from **3.4** add 1 ml 5 per cent ammonium citrate solution, a few drops of metacresol purple solution and potassium cyanide until the pH reaches the range 8·5–9. Add exactly 10 ml of the dilute dithizone solution and shake for one minute; if the dithizone layer is bright red add further 10 ml portions of dithizone solution, shaking for one minute after each addition, until the colour of the organic layer is intermediate between bright red and the green of unchanged dithizone.

Let the total volume of the organic layer be V.

Plug the stem of the funnel with a piece of cotton-wool, and fill a dry spectrophotometer cell with the lower layer discarding the first few drops. Cover the cell with a lid. Run the remaining carbon tetrachloride layer into a separating funnel containing 20 ml 0·025 per cent nitric acid and shake for one minute. Plug the stem of the funnel with cotton-wool and fill a second spectrophotometer cell with the lower layer. Measure the difference R between the optical densities of the two solutions at 620 mμ.

Determine the lead content of the sample by the expression RV/F, where V is the total volume of dithizone solution used and F is a factor determined as described in **4.**

4. Calibration

Measure a series of volumes of the standard lead solution in the region of the expected lead content of the sample, and dilute each to 25 ml with 0·025 per cent nitric acid. Proceed as described in **3.5.**

Calculate for each the factor F by the expression $F = R'V'/M$, where R' is optical density difference as defined in **3.5** (minus the reagent blank), V' is the volume of dithizone used and M is the weight of lead taken in micrograms. The value of F will vary slightly with the amount of lead, and that value should be taken which is nearest to the lead content of the sample; this may be estimated approximately by the amount of dithizone solution required in **3.5.** The following table is an example of the calculation of F:

M (μg *lead*)	V' (ml *dithizone solution*)	R' (*optical density difference*)	F
4·0	10	0·127	0·318
8·0	15	0·171	0·321
16·0	20	0·268	0·328

5. Bibliography

Analytical Methods Committee of the S.A.C. *Analyst* **79**, 397 (1954); **84**, 127 (1959).
H. Bode. *Z. Anal. Chem.* **172**, 1 (1960).
J. C. Gage. *Analyst* **80**, 793 (1955); **82**, 453 (1957); **83**, 672 (1958).
T. T. Gorsuch. *Analyst* **84**, 135 (1959).
H. V. Hart. *Analyst* **76**, 692 (1951).
H. M. Irving and E. J. Butler. *Analyst* **78**, 571 (1953).
H. C. Lockwood. *Analyst* **79**, 143 (1954).
A. D. Maynes and W. A. E. McBryde. *Anal. Chem.* **29**, 1259 (1957).
N. Strafford, P. F. Wyatt, and F. G. Kershaw. *Analyst* **70**, 232 (1945); **78**, 624 (1953).
S. L. Tompsett. *Biochem. J.* **33**, 1231 (1939); *Analyst* **81**, 330 (1956).
S. L. Tompsett and A. B. Anderson. *Biochem. J.* **29**, 1851 (1935).

DETERMINATION OF MERCURY CONTENT OF FOODSTUFFS

1. Principle

The organic material in the sample is digested by means of sulphuric and nitric acids in an all-glass apparatus according to A. K. Klein[1]. An oxidizing medium is maintained throughout the digestion.

The digest is diluted with water, excess oxidizing substances are removed by adding urea and hydroxylamine, and mercury is extracted from the acid solution by means of a solution of dithizone in chloroform. The mercury is transferred to an aqueous phase by destroying the organic complex with a dilute hydrochloric acid solution containing sodium nitrite. The excess of nitrite is removed by adding hydroxylamine.

The mercury content of the aqueous phase may be determined by a titrimetric method (**3.4**) or by a photometric method (**3.5**).

Gold, platinum, silver and copper, if present in a relatively large amount, form also dithizonates in an acid medium. Gold and platinum are very unlikely to occur in larger amounts, silver cannot interfere owing to the presence of chloride ions; only a very small part of copper present is extracted in the first stage if a solution of dithizone in chloroform is used[2] and this amount of copper does not inhibit the final determinations with dithizone solution in chloroform, if the sample has contained less than 100 μg of copper.

The collaborative work of the Commission has indicated that the method is satisfactory if the mercury content of the sample taken for analysis lies between 2 and 20 μg.

2. Requisites

2.1 *Reagents*

All reagents should be of Analytical Reagent quality if available.

Acetic acid solution 4 N—Shake a 4 N acetic acid solution with portions of dithizone solution until the latter remains green, wash with chloroform until the washings are colourless and filter.

Carborundum grains—Wash with hot nitric acid.

Chloroform—Chloroform should be checked for the absence of oxidizing substances by adding a few drops of dithizone solution (see below) until a faint green colour is obtained. This colour should not diminish after the chloroform has been stored for one hour in the dark. Chloroform which does not satisfy this test may be purified by allowing it to stand over calcium oxide for 24 hours and then distilling it into a receiver containing an amount of absolute ethanol equal in volume to about 1 per cent of the chloroform. The first few millilitres of the distillate are discarded and not more than

three-quarters of the chloroform is collected. Before use the chloroform should be checked as described above for the absence of oxidizing substances. Store chloroform in a cool dark place.

Cotton-wool—Wash cotton-wool with dilute nitric acid, then with water until free from acid and dry. Wash it with dithizone solution, then with chloroform and dry.

Dithizone solution—Dissolve about 30 mg dithizone in 100 ml chloroform in a separating funnel and extract the solution with three successive 100 ml of 1 per cent ammonia. Wash the combined ammonia extracts with chloroform, add dilute sulphuric acid until the solution is acid to Congo Red paper and extract the dithizone with three portions of 100 ml chloroform. Wash the combined chloroform extracts with 25 ml water and filter through paper. Dilute with chloroform to 1 litre. Store in a cool dark place.

Some samples of dithizone have given satisfactory results in this method for mercury determination without purification; with such samples it is sufficient to dissolve 25 mg dithizone in 1 litre chloroform.

Dilute dithizone solution—Dilute the dithizone solution with chloroform so that the optical density of the solution, when measured at 605 mμ in a 0·5 cm cell, is 0·28 (=4 mg dithizone per litre, corresponding to 1·8 μg Hg per ml).

Hydrochloric acid 0·1 N—Purify the solution as described for acetic acid.

Hydroxylamine solution—Prepare a 10 per cent w/v aqueous hydroxylamine hydrochloride solution and purify as described for acetic acid.

Nitric acid concentrated (65% weight, sp. gr. 1·40)—Test for the absence of mercury as follows: mix 50 ml with 5 ml of concentrated sulphuric acid. Evaporate until only sulphuric acid is left, dilute to 100 ml, add 2 ml hydroxylamine hydrochloride solution and shake vigorously with 0·2 ml of the dilute dithizone solution. The latter should remain green. If necessary, purify the sample by distillation in the presence of sulphuric acid. Store in the dark.

Sodium nitrite solution—Prepare a 0·5 per cent aqueous sodium nitrite solution and purify the solution as described for acetic acid.

Standard mercury solution (1 μg Hg per ml)—Dissolve 0·1354 g mercuric chloride in 100 ml 1 per cent nitric acid to give a stock solution containing 1 mg mercury per ml. Dilute this stock solution 1/1000 with 1 per cent nitric acid immediately before use.

Sulphuric acid concentrated (95–98%)—Test for mercury by diluting 10 ml of the acid to 100 ml with water and shaking vigorously with 0·2 ml of the dilute dithizone solution. The latter should remain green.

Urea solution—Prepare a 40 per cent w/v aqueous urea solution. Shake the solution vigorously with portions of dithizone solution until the lower layer

remains green, acidify slightly with sulphuric acid, remove excess dithizone by washing with chloroform and filter.

2.2 *Glassware*

All glassware, including the reagent bottles, must be of chemically resistant glass, preferably Pyrex or of polyethylene. 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 and water. The necks of the bottles should be protected against dust. Cork and rubber bungs must not be used. Pear-shaped separating funnels of 500, 150 and 75 ml capacity and of uniform shape, and burettes of 5 and 10 ml are used. The digestion apparatus is covered with asbestos lagging from the liquid surface to the condenser.

2.3 *Laboratory*

The laboratory should be as dust-free as possible, and preferably entirely devoted to the determination of traces of metals. Apparatus and glassware should be covered against dust.

3. Procedure

3.1 *Digestion*

Weigh a representative portion of the material containing preferably between 2 and 20 μg mercury and corresponding to not more than 5 g dry weight. Transfer the weighed part into the flask A (see *Figure 1*) together with a few grains of carborundum. Add water if the sample contains less than 75 per cent water. Add about 2 ml nitric acid per gram dry matter. Add 5 ml sulphuric acid. Carry a blank along with the unknown through all the subsequent steps, using the same amounts of reagents throughout. Warm the flask slightly, and discontinue heating if foaming becomes excessive. When the initial reaction has subsided heat gently for about 15 minutes. If much fat or nitroproducts (*e.g.* from synthetic dyestuffs) are present, make a partial digestion, allow to cool, decant the liquid into a second digestion flask, transfer the solid mass to a mortar, treat it with water, filter the extract through a wad of cotton-wool and add the filtrate to the second digestion flask.

Close the stopcock of the Klein apparatus. Distil by heating in a stream of air at 250–300°C. Towards the end of the distillation the digest will begin to evolve brown vapours. When the evolution of brown vapours almost ceases start adding nitric acid at the rate of about one drop per second, regulating the heat supply so that brown vapours keep coming off, and the digest shows no brown colour (charring). Avoid, however, undue excess of nitric acid. When the digestion is complete no more brown vapours are given off and the digest should remain practically colourless. At this point, stop the addition of nitric acid and remove the flame.

Adjust another flask to the apparatus, allow the distillate to flow into it, and add 2 ml sulphuric acid and carborundum. Distil once more. Remove any organic material in the residue by dropwise addition of nitric acid as in the first digestion. Allow to cool.

Add 175 ml water to the two combined digests, and cool down to room temperature. Transfer the solution to a 500 ml separating funnel and rinse the flask with water, so that the total volume in the funnel is about 200 ml.

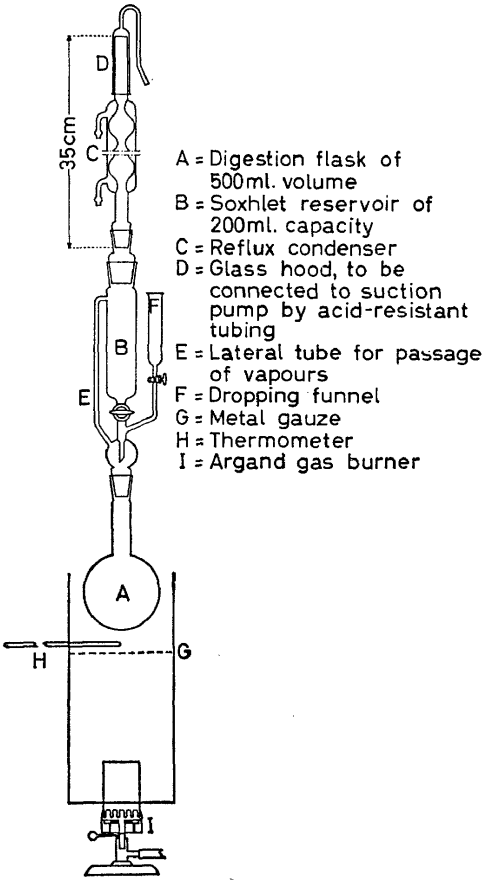

Figure 1

The carborundum is left in the flask. Add 5 ml urea solution and 5 ml hydroxylamine solution and allow to stand for 15 minutes.

3.2 *Separation of mercury from the digest*

Shake the contents of the separating funnel with 10 ml chloroform. Draw off and discard the non-aqueous layer. The separation of layers in this and all subsequent extractions should be as complete as possible; floating droplets of the chloroform are caused to sink by rocking the funnel; the bore of the stopcock must be filled with this phase.

Shake vigorously with 10 ml of dithizone solution and draw the lower layer into a 75 ml separating funnel, containing 10 ml 0·1 N hydrochloric acid. Continue this extraction with 1 ml portions of the dithizone solution

until two consecutive portions remain pure green and then, with 2 ml chloroform, add these extracts to the first.

3.3 *Purification of the dithizone extract*

Add 1 ml sodium nitrite solution. Shake the funnel vigorously for one minute, reject the lower layer, wash the aqueous layer with a few ml of chloroform and add 1 ml hydroxylamine solution. Rinse stopper and neck of the separating funnel with water and mix. Allow to stand for 30 minutes. Add 5 ml 4 N acetic acid. Proceed according to **3.4** or **3.5.**

3.4 *Final determination by titration*

Add dilute dithizone solution in small portions from a burette to the solution obtained in **3.3**, shaking vigorously between each addition until the separated lower layer shows a colour intermediate between the orange of the mercury complex and the green colour of dithizone.

Place 10 ml 0·1 N hydrochloric acid, 5 ml 4 N acetic acid and 1 ml hydroxylamine solution in another 75 ml separating funnel, add the same volume of dilute dithizone solution as is used for the unknown and titrate small portions of mercury standard solution from another burette, vigorously shaking after each addition, until the same colour is obtained as in the unknown. The number of millilitres of standard mercury solution used is equivalent to the content of mercury in the sample, expressed in μg.

3.5 *Final determination by colorimetry*

Run in 0·5 or 1 ml of dilute dithizone solution to the solution obtained in **3.3**, shake vigorously for about 5 seconds and run the lower layer quantitatively into a small separating funnel containing 5 ml 2 N acetic acid solution. Repeat this extraction as long as the colour of the chloroform layer remains clear orange; when it appears smoky orange, extend the shaking to 10 seconds and continue with additions of 0·2 ml reagent until its colour after shaking is grey or greenish. Add a known volume of chloroform to the combined extracts if necessary to obtain sufficient volume to fill a cell of the photometer and shake the funnel containing acetic acid and the extracts for about 10 seconds. Plug the stem of the funnel with a piece of cotton wool, run the lower layer into a dry 1 cm cell discarding the first few drops, cover the cell with a lid and measure the optical density at 485 mμ.

Read the number of micrograms of mercury per ml solution from a calibration graph and multiply by the volume of the solution, expressed in millilitres. This gives the number of μg of mercury in the sample taken. Express the result in mg Hg per kg of sample (p.p.m.).

In order to obtain the calibration graph place into a separating funnel 10 ml of 0·1 N hydrochloric acid and 1 ml of hydroxylamine solution, add appropriate amounts of standard mercury solution to cover the range 0 to 10 μg and proceed as described in the first two paragraphs of **3·5.** Plot μg Hg per ml of combined extracts against optical densities.

References

[1] A. K. Klein. *J. Assoc. Offic. Agr. Chemists* **35**, 537 (1952).
[2] H. Irving, G. Andrew, and E. J. Risdom. *J. Chem. Soc.* **1949, 541.**