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MINISTRY OF AGRICULTURE, FISHERIES AND FOOD

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# The Analysis of Agricultural Materials



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LONDON: HER MAJESTY'S STATIONERY OFFICE

MINISTRY OF  
AGRICULTURE, FISHERIES AND FOOD

*Technical Bulletin 27*

THE ANALYSIS OF  
AGRICULTURAL MATERIALS

A MANUAL OF THE ANALYTICAL METHODS  
USED BY THE  
AGRICULTURAL DEVELOPMENT AND ADVISORY  
SERVICE

LONDON  
HER MAJESTY'S STATIONERY OFFICE

1973

## FOREWORD

Since the early days of the National Agricultural Advisory Service, Analysts working in Soil Science and Nutrition Chemistry Departments have been modifying, developing and improving analytical techniques for the examination of soils, plants and feedingstuffs. At first, regions tended to work independently, but over the years there has been more and more national collaboration on techniques and methods, fostered by the Analysts' Technical Committee, gradually leading to greater standardization of methods over the whole country. The publication of these methods should meet a long-felt want as there have been many enquiries from organizations, both at home and abroad, for copies of individual techniques used in our laboratories. It is highly desirable that all organizations providing advice for farmers in this country should, eventually, use the same methods and the same interpretations.

Analytical work never stands still and improvements are always being made. As new methods are produced or modified and collaboratively tested, so new sheets will be prepared and made available on demand through HMSO channels for inclusion in this cover. This first issue contains a selection of fifty-eight methods from those currently in use.

This manual has been prepared by the Analysts' Publications Committee which consists of a number of senior analysts of the Agricultural Development and Advisory Service.

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## DEFINITION OF TERMS

'Water' means water of distilled or demineralized quality.

Weights are in grammes, unless otherwise stated.

Solutions are aqueous, unless otherwise stated.

Dilutions of solutions are made with water, unless otherwise stated.

Abbreviations are in accordance with the International System of Units.

Reference may be made to *Symbols, Signs and Abbreviations recommended for scientific publications* (1969), Symbols Committee of the Royal Society, London, and to *Changing to the Metric System* (1969), P. Anderton and P. H. Bigg, HMSO.

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**FEEDINGSTUFFS AND**  
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# **THE PREPARATION OF SAMPLES**

## **INTRODUCTION**

The experienced analyst knows that, however good a method may be for the determination of a specific component, however competent the laboratory work and however precise and sophisticated the instrumentation, the result will be of little value and all the effort involved will have been wasted, if the sample actually analysed does not represent the original specimen. With certain exceptions therefore, an analysis of the plant material alone is required; not the plant material plus soil or any other contaminating substance. Equally important is the need to determine a particular component at the level at which it was in the specimen or growing plant when first collected. Many changes, however, occur in freshly cut living plant material which cause degradation of certain constituents. Some of these changes can be arrested or delayed by taking special precautions at the time of sampling. Although some analyses must be made on the fresh material, the presence of so much water often makes the use of fresh plant material difficult. Fortunately many analyses may be made after the fresh specimen has been sub-sampled, dried and ground. As every sample must be treated according to the analysis required, the following paragraphs are not intended to be a comprehensive set of instructions covering the preparation of all samples prior to analysis but should be regarded as a guide applicable to most samples.

## **WASHING**

Plants contaminated with soil may be washed under running tap water or in a weak solution of non-ionic detergent. Such washing should be carried out very quickly to minimize loss of soluble constituents and should be followed by rinsing with distilled water and drying with a cloth or paper tissue. These procedures are suitable for small whole plants and for plants to be analysed for trace elements with high soil/plant concentration ratios. Roots can simply be washed under running tap water and dried with a cloth. Silage, brewers' grains and sugar beet pulp must not be washed because of the rapid loss of sugars and other soluble constituents, nor should samples of material intended for conservation, or of conserved fodder, since such samples represent what the animal actually eats.

## **SUB-SAMPLING**

With small samples, sub-sampling is often unnecessary; large samples, however, may be sub-sampled as indicated in the following paragraphs. To avoid loss of moisture, this should be carried out as quickly as possible.

### **COMPOUND FEEDS, GRAINS AND PULSES**

The sample should be mixed on a clean non-absorbent surface and sub-sampled by quartering. To do this, form a flattened heap, divide the heap into

### (1) *Sample preparation*

quarters and reject two diagonally opposite portions. Mix the remaining material and repeat the quartering process until a sample of 200–300 g is obtained. The whole of samples weighing only a few hundred grammes should be prepared for analysis.

#### FRESH HERBAGE

Chaff into short lengths, thoroughly mix and quarter down to approx. 200 g. If a laboratory chaff cutter is not available, mix the whole sample on a clean non-absorbent surface, sub-sample several hundred grammes and quarter this down to approx. 200 g. With small samples take the whole of the sample for analysis.

#### ROUGHAGES

Roughage in long loose form cannot be satisfactorily sub-sampled. In the absence of a suitable laboratory corer, adopt the same sub-sampling procedure as for fresh herbage. Coring may be achieved in the laboratory by compressing the sample into a suitable box having a movable partition and using a corer, preferably of stainless steel, designed so that it can be operated with a variable-speed electric hand drill. Such a corer can usually be made by a local light engineering firm. When the roughage is received in the laboratory in the cored state, mix it thoroughly before sub-sampling. The whole of samples weighing less than 300 g should be prepared for analysis.

#### KALE AND OTHER GREEN BRASSICAS

Three procedures can be used to sub-sample these materials:

- a. Chop the whole plants, with leaves attached, into short lengths and sub-sample the chopped material.
- b. Split large whole plants, with leaves attached, longitudinally into two, four or six sections depending on stem thickness. Chop the split sections and sub-sample the chopped material.
- c. Remove the leaves from the stems of the plants. Weigh, chop and sub-sample the leaves and stems separately.

Take a sub-sample weighing approx. 200 g for analysis.

#### ROOTS (swedes, beet, potatoes, etc.)

Sub-sample by taking a 10 mm core or a diagonal slice approx. 5 mm thick through the root across the shoulder. Chop the slices or cores into 1 cm lengths and mix the pieces. Take a sub-sample of 200–400 g for analysis.

#### SILAGE

If the sample is cored, break up the cores and mix thoroughly. If the sample is in long form, treat as for fresh herbage. Alternatively take cores as described for roughages. It is particularly important to sub-sample without delay.

#### FOLIAGE

Remove the petiole of top and soft fruit leaves at the base of the lamina and discard. There is no standard procedure for the removal or retention of petioles in other cases, but where the petioles form a considerable proportion of the



### (I) Sample preparation

sample (e.g., tomato, sugar beet, etc.), they should be cut off at the base of the lamina.

#### APPLE FRUITS

Wash 20 apples in distilled water and dry with paper tissue. Clean each apple of strig (stalk and calyx) and make three vertical cuts at a small angle to each other through the centre of the core, giving two pairs of opposite segments. Remove the pips and collect the slices into duplicate samples, each containing one pair of opposite segments from each apple, and having a total weight of 200 g or more. Weigh each sample and homogenize for 3 min at 12,000 rpm with 1.5 times the sample weight of distilled water. Commence the analyses immediately after homogenization.

### DRYING

The sub-samples having been taken, it will frequently be necessary to dry them and in agricultural analysis this operation alone can profoundly affect the results of subsequent determinations, especially those involving sugars, vitamins, and certain trace elements. It is not sufficient to simply place material in a hot oven until it feels dry—different samples require different drying temperatures and drying times. Various ovens are used for such purposes and one with a forced-draught system is essential for fresh/plant material. The Unitherm Drying Oven, which is obtainable from S. and T. Engineering Co., Melchett Road South, Kings Norton, Birmingham 30, is an example of such equipment.

As a general procedure, weigh 200 g of sample into a suitable clean metal tray, preferably lined with greaseproof paper (particularly important for roots) and dry as indicated in the following table.

Material	Drying time (hours)	
	At 60°C	At 100 ± 2°C
Herbage, silage, hay		18
Brassicas		18
Sugar beet, carrots, mangels, swedes, parsnips, turnips	48	
Potatoes, artichokes	24 followed by	18
Cereal grains		40
Milled cereals and compound feeds		24

For the determination of *in vitro* digestibility the drying time at 100°C should not exceed 6 hr.

To avoid possible losses from samples for the determination of fluoride and selenium, the material must be dried at 40–50°C and, for the determination of boron, at 60°C (in an unlined tray). Drying at low temperature (60°C) is also desirable for materials such as onions and fruits, to prevent loss of tissue fluids and caramelization. Ideally, samples for the determination of sugars and vitamin E should not be dried at all but the use of fresh material may introduce technical difficulties in the analytical procedure. Air-drying at 30°C in a forced-draught oven is often a reasonable compromise for these analyses; there may sometimes be

### (1) *Sample preparation*

a small loss of dry matter due to respiration, but this is insignificant for most purposes.

## GRINDING

It is often necessary to analyse powdered or finely ground samples and a variety of equipment is available for satisfactorily producing homogeneous powders from the dried samples. The most frequently used grinders are hammer mills of which the 8 in. Christy and Norris laboratory mill is a typical example. Other smaller grinders, such as the Casella grain mill, can be used and for samples weighing only a few grammes these are more suitable. Brushes, sieves and trays made of brass or copper must not be used in the preparation of ground samples to be analysed for trace elements nor must these metals form parts of the grinding apparatus. The feeding chute to the mill should be made of a plastic. It is especially important to clean out the mill thoroughly between samples in order to avoid cross contamination. When a bag is used as the receiver of ground material it must be turned inside out, shaken and brushed with a nylon brush to remove adhering plant particles between each sample to be ground. Small mills, such as the Casella grain mill, may become corroded if the knives are not cleaned and dried after use.

The fineness of grinding can be important but, for general routine analyses, samples milled to pass a 1 mm mesh sieve are usually satisfactory. For certain determinations, i.e., available carbohydrates in cereal mixes, the sample should be ground to pass a 0.5 mm mesh sieve. The dried sample must be ground and the whole collected from the mill and thoroughly mixed.

The dried and ground samples should be stored away from direct sunlight in glass jars with bakelite screw caps or in sealed polythene bags.

## BIOCHEMICAL ANALYSES

Extra care and special precautions need to be exercised when preparing samples for certain biochemical analyses. The following examples give an indication of when special procedures may be required.

Analyses for sugars, amino acids, enzymes, etc., are usually carried out employing aqueous or ethanolic extracts of fresh tissue. The concentrations of these compounds change very rapidly after sampling and removal to the laboratory without delay is essential. When practicable it is advisable to inactivate the tissue immediately after sampling. For many purposes, but not for enzyme assays, the tissue can be treated with boiling 80% v/v ethanol, which prevents biochemical changes and acts as an extractant. For some enzyme investigations it may even be necessary to plunge the tissue into liquid nitrogen before transit to the laboratory.

# THE DETERMINATION OF AFLATOXIN B<sub>1</sub> IN GROUNDNUTS AND GROUNDNUT MATERIALS

## 1.0 PRINCIPLE

Aflatoxin B<sub>1</sub> is removed from defatted groundnuts, cake and meal by solvent extraction (see Note). The extract is serially diluted and the aflatoxin B<sub>1</sub> is separated by thin layer chromatography. The amount present in the sample is calculated from the minimum dilution necessary to prevent the fluorescent spot given by aflatoxin B<sub>1</sub> from being visible in controlled ultra-violet light.<sup>1</sup>

## 2.0 REMOVAL OF FAT FROM GROUNDNUTS AND GROUNDNUT CAKE

### 2.1 APPARATUS

*Coil condenser*—Quickfit Cat. No. CX3/05.

*Short necked, round bottomed flask*—250 ml, Quickfit Cat. No. FR/250/3S.

*Soxhlet extractor*—100 ml, Quickfit Cat. No. EX5/55/100.

### 2.2 REAGENT

*Petroleum spirit, free from aromatic hydrocarbons, boiling range 40–60°C.*

### 2.3 PROCEDURE

Transfer 20 g of groundnuts, grated to pass a 10 mesh sieve, or 20 g of groundnut cake, ground to pass a 1 mm mesh sieve, into a Whatman extraction thimble. Extract the fat with petroleum spirit in the Soxhlet extraction apparatus for 4 hr at a syphon rate of 10–12 changes per hr. Remove the thimble and place in a forced-draught oven at 65°C for 30 min. Retain the defatted material for the preparation of extract.

## 3.0 PREPARATION OF EXTRACT

### 3.1 APPARATUS

*Conical flask*—250 ml, with glass stopper.

### 3.2 REAGENT

*Chloroform.*

### 3.3 PROCEDURE

Transfer 20 g of groundnut meal, ground to pass a 1 mm mesh sieve, or the material defatted as described in 2.3, into a flask. Add exactly 100 ml of chloroform and swirl to disperse the solid material. Add 10 ml of water, stopper the flask, shake for 30 min and filter through an 11 cm Whatman No. 1 filter paper (solution A). Dilute 10 ml of solution A to 25 ml with chloroform (solution B).

(1) *Aflatoxin*

Concentrate 10 ml of solution A to 5 ml (solution C). Concentrate 10 ml of solution A to 0.5 ml in a graduated tapered tube (solution D).

Retain the solutions for the determination of aflatoxin B<sub>1</sub>.

#### 4.0 DETERMINATION OF AFLATOXIN B<sub>1</sub>

##### 4.1 APPARATUS

*Coating apparatus*—For thin layer chromatography.

*Chromatography tank*—For 10 × 20 cm plates.

*Drummond microcaps*—5  $\mu$ l, 10  $\mu$ l, and 20  $\mu$ l. Obtainable from Shandon Southern Instruments Ltd., Frimley Road, Camberley, Surrey.

*Goggles*—Fitted with ultra-violet absorbing glass. Obtainable from Safety Products Ltd., Holmethorpe Avenue, Redhill, Surrey. Cat. No. PCF-2.

*Plates*—10 × 20 cm. For thin layer chromatography.

*Ultra-violet lamp*—Gallenkamp Cat. No. LH-530, complete with Phillips MBW/U 125 watt bulb, wavelength 365 nm.

##### 4.2 REAGENTS

*Diethyl ether, dried by sodium.*

*Kieselgel 'G' (Merck)*—Obtainable from Anderman & Co., Ltd., Central Avenue, East Molesey, Surrey, KY8 0Q2.

*Methanol in chloroform, 5% v/v.*

##### 4.3 PREPARATION OF THIN LAYERS OF KIESELGEL 'G'

Stir 100 g of Kieselgel 'G' with 220 ml of water until a uniform slurry is obtained. This is sufficient to coat 18–20 glass plates. Coat the plates with a layer  $500 \pm 10$   $\mu$ m thick. Leave the coated plates in a dust-free atmosphere for 1 hr at room temperature. Place the plates in a forced-draught oven at 100°C for 1 hr, cool in a dust-free atmosphere for 30 min and store in a desiccator over silica gel.

##### 4.4 SEPARATION OF AFLATOXIN B<sub>1</sub>

Spot 5  $\mu$ l and 20  $\mu$ l of solution B and 20  $\mu$ l of solutions C and D on to a prepared plate 1 cm from the edge.

With extracts from samples from which the fat has not been removed (see 2.3), develop the plate with diethyl ether in subdued light until the solvent front reaches 12 cm from the base line.

Allow the plate to dry in subdued light.

After or without the treatment with ether, develop the plate with 5% methanol in chloroform until the solvent front reaches 10 cm from the base line.

TABLE I

Volume of solution taken	Concentration of aflatoxin $\mu$ g/kg of sample	
	No fluorescence	Fluorescence
5 $\mu$ l B	< 1000	> 1000
20 $\mu$ l B	< 250	> 250
20 $\mu$ l C	< 50	> 50
20 $\mu$ l D	< 5	> 5

## 4.5 ASSESSMENT OF TOXICITY LEVELS

Examine the dry developed plate, at a distance of 30 cm from the ultra-violet lamp, for purple-blue fluorescent spots at  $R_F$  0.50–0.55. From the presence or absence of spots, assess the amount of aflatoxin  $B_1$  present in the sample using Table 1.

4.6 DETERMINATION OF AFLATOXIN  $B_1$  BY SERIAL DILUTION

Under the conditions described in 4.5, the smallest weight of aflatoxin  $B_1$  giving an observable fluorescence is 0.4 ng.

Prepare a diluted extract such that when 10, 15 and 20  $\mu$ l are developed and examined as described in 4.4 and 4.5 either the 15 or the 20  $\mu$ l spot gives the minimum observable fluorescence.

## 4.7 CALCULATION OF RESULTS

Obtain the concentration of aflatoxin  $B_1$  in  $\mu$ g/kg of the sample from the expression:

$$\frac{20T}{V}$$

where  $V$  = volume in  $\mu$ l, giving minimum observable fluorescence,

$T$  = total volume in ml, to which the whole of the extract from 20 g of sample must be diluted so that the minimum observable fluorescence is given by  $V$   $\mu$ l.

## 5.0 NOTE

The method described above for groundnut materials is applicable with slight modification to a wide variety of other materials.

In certain cases it may be necessary to include a 'clean-up' procedure prior to the thin layer chromatography,<sup>2</sup> and for highly pigmented commodities such as cottonseed, to remove the pigments by precipitation with lead acetate<sup>3</sup> or copper carbonate.<sup>4</sup>

It has been found that metabolites of the mould *Macrophomina phaseoli* (Maubl) Ashby, can interfere with the evaluation of the thin layer chromatographic plate under ultra-violet light. The interference is eliminated by re-developing the plate in diethyl ether and this procedure may also be useful in dealing with other 'clean-up' problems.<sup>5</sup>

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# THE DETERMINATION OF TOTAL AND ACID-INSOLUBLE ASH IN PLANT MATERIAL

## 1.0 PRINCIPLE

The residue remaining after the destruction of the organic matter of plant material is weighed as total ash. The acid-insoluble ash is the residue remaining after the silica in the total ash has been dehydrated and the soluble mineral constituents have been removed by extraction with hydrochloric acid.

## 2.0 DETERMINATION OF TOTAL ASH

### 2.1 APPARATUS

*Evaporating basins*—45 ml, translucent vitreosil, shallow form, with round bottoms and spouts.

### 2.2 PROCEDURE

Heat a basin at approx. 600°C, cool in a desiccator and weigh. Transfer 4 g of dried plant material, ground to pass a 1 mm mesh sieve, into the basin and place the basin in a cool muffle furnace. Increase the temperature of the muffle furnace to 450°C and maintain this temperature (usually overnight) until a whitish-grey ash remains (see Note a). Place the basin in a desiccator, allow to cool and weigh. Retain the basin and contents for the determination of acid-insoluble ash.

### 2.3 CALCULATION OF RESULTS

Multiply the weight of the residue by 25. The result gives the percentage of total ash in the sample.

## 3.0 DETERMINATION OF ACID-INSOLUBLE ASH

### 3.1 REAGENTS

*Hydrochloric acid, approx. 36% w/w HCl.*

*Hydrochloric acid, approx. 6 M*—Mix equal volumes of hydrochloric acid, approx. 36% w/w HCl, and water.

### 3.2 PROCEDURE

Cover the basin and contents retained in 2.2 with a watch glass. Add 20 ml of approx. 6 M hydrochloric acid, taking care that losses due to effervescence do not occur. Remove and rinse the watch glass, collecting the washings in the basin. Place the basin on a water bath and evaporate the solution to dryness. Heat the basin over a small flame until the residue is visibly dry. Moisten the residue with 4 ml of hydrochloric acid, approx. 36% w/w HCl, cover the basin with a watch glass and gently boil for 2 min. Add approx. 20 ml of water and again boil. Remove and rinse the watch glass, collecting the washings in the basin. Filter

(1) *Ash*

through an 11 cm Whatman No. 541 filter paper and collect the filtrate and washings in a 100 ml graduated flask (see Note b). Carefully return the filter paper containing the insoluble residue to the evaporating basin and dry in an oven at 105°C. Place the basin in a cool muffle furnace. Slowly increase the temperature to 600°C and maintain this temperature until a carbon-free ash remains. Place the basin in a desiccator, allow to cool and weigh.

3.3 CALCULATION OF RESULTS

Multiply the weight of the residue by 25. The result gives the percentage of acid-insoluble ash in the sample.

4.0 NOTES

- a. When only an approximate ash content is required this residue may be weighed without removal of the residual carbon. To remove the residual carbon, moisten the cold ash with water, dry thoroughly at 105°C and reheat at 450°C.
- b. Dilution of the filtrate and washings to 100 ml provides a sample solution suitable for the determination of calcium, magnesium, phosphorus, potassium, zinc; and of sodium, if the determination is carried out immediately or the sample solution is stored in a polythene bottle.

# THE DETERMINATION OF BORON IN PLANT MATERIAL

## 1.0 PRINCIPLE

Boron in the residue remaining after the destruction of the organic matter of plant material is dissolved in phosphoric acid. The addition of calcium hydroxide to the plant material prevents loss of boron during the ashing.

Borate in the extract is converted to fluoroborate by the action of phosphoric acid and sodium fluoride. The concentration of fluoroborate is measured spectrophotometrically as the blue complex formed with methylene blue and which is extracted into 1,2-dichloroethane.<sup>1,2</sup> Nitrates and nitrites interfere: they are removed by reduction with zinc powder and phosphoric acid.

## 2.0 DETERMINATION OF BORON

### 2.1 APPARATUS

*Beakers*—Approx. 15 ml, silica. Obtainable from T. W. Wingent, Scientific Glassware, 115 Cambridge Road, Milton, Cambridge.

or

*Evaporating basins*—20 ml, translucent vitreosil, shallow form, with round bottoms and spouts.

*Dropping bottles*—125 ml, polythene, with central tapered jet on the caps.

*Phase separating paper*—7 cm Whatman No. 1 PS.

*Tubes*—15 ml, polythene (see Note a).

### 2.2 REAGENTS (see Note b)

*Boron stock solution, 100 µg/ml of boron*—Dissolve 0.572 g of boric acid ( $\text{H}_3\text{BO}_3$ ) in water and dilute to 1 litre.

*Boron solution, 10 µg/ml of boron*—Dilute 25 ml of boron stock solution to 250 ml.

*Boron working standard solutions, 0–2 µg/ml of boron*—Measure 0, 2.5, 5, 10, 15 and 20 ml of boron solution, 10 µg/ml, into six 100 ml graduated flasks and dilute to 100 ml.

*Calcium hydroxide solution, satd.*

*1,2-Dichloroethane*—A high degree of purity is required. The solvent may be purified by shaking with sodium hydroxide solution and distilling. The fraction boiling at 82–84°C should be collected for use.

*Methylene blue solution, 0.2% w/v*—This reagent is stable for one month.

*Methylene blue-hydrogen peroxide reagent*—Add 2 vol. of methylene blue solution to 1 vol. of water and add 1 vol. of hydrogen peroxide solution (100 volumes). Prepare just before use.

*Phosphoric acid, approx. 2 M*—Dilute 120 ml of orthophosphoric acid, approx. 88% w/w  $\text{H}_3\text{PO}_4$ , to 1 litre.

*Sodium fluoride solution, 2% w/v.*

*Zinc powder.*



### 2.3 PREPARATION OF STANDARD GRAPH

Pipette 5 ml of each boron working standard solution into a beaker or evaporating basin. Add 2 ml of satd. calcium hydroxide solution and evaporate the mixture to dryness on the water bath. Place the container in a cold muffle furnace and slowly increase the temperature to 450°C. Maintain this temperature for 2 hr. Allow to cool, carefully add exactly 10 ml of approx. 2 M phosphoric acid and dissolve all the soluble material. Add approx. 0.02 g of zinc powder, mix thoroughly and allow to stand for approx. 2 hr. Transfer to a polythene centrifuge tube and centrifuge at 2500 rev/min for 5 min. Pipette 4 ml of the clear supernatant solution into a polythene dropping bottle. Add 3 ml of sodium fluoride solution and either stand for 1 hr at 60°C and allow to cool to room temperature or allow to stand overnight at room temperature. Add 2 ml of methylene blue-hydrogen peroxide reagent, mix, and add 10 ml of dichloroethane. Cap the bottle and shake on a reciprocating shaking machine for 5 min. Allow the two phases to separate and run approx. 5 ml of the dichloroethane extract through a 7 cm Whatman phase separating paper into a dry tube (see Note c). Transfer a portion of the solution by means of a dropping pipette with rubber teat to a 2 mm optical cell. Measure the absorbance at 660 nm.

Construct a graph relating absorbance to  $\mu\text{g}$  of boron present. The absorbances corresponding to 0 and 4  $\mu\text{g}$  of boron are approx. 0.15 and 0.74 respectively.

### 2.4 PROCEDURE

Transfer 0.25 g of finely ground dried plant material into a beaker or basin. Continue as in 2.3 commencing at 'Add 2 ml of satd. calcium hydroxide solution . . .' and ending at 'Measure the absorbance at 660 nm.'

Carry out a blank determination.

### 2.5 CALCULATION OF RESULTS

Read from the standard graph the number of  $\mu\text{g}$  of boron equivalent to the absorbance of the sample and the blank determinations. Multiply the difference by 10. The result gives mg/kg of boron in the sample.

## 3.0 NOTES

- Borosilicate glassware must not be used at any stage of this method.
- Store all reagents in polythene bottles.
- Compress the bottle slightly between the fingers and, whilst inverting the bottle, release the compression, allowing air to be drawn into the bottle. Compress gently, transferring the organic solvent into the phase separating paper.

## 4.0 REFERENCES

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