II

(Acts whose publication is not obligatory)

COMMISSION

COMMISSION DIRECTIVE 93/1/EEC

of 21 January 1993

amending Directive 77/535/EEC on the approximation of the laws of the Member States relating to methods of sampling and analysis for fertilizers

(Analysis methods for trace elements)

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Economic Community,

Having regard to Council Directive 76/116/EEC of 18 December 1975 on the approximation of the laws of the Member States relating to fertilizers (1), as last amended by Directive 89/530/EEC (2), and in particular Article 9 (2) thereof,

Whereas Article 8a of the Treaty establishes an area without internal frontiers in which the free movement of goods, persons, services and capital is ensured;

Whereas Directive 89/530/EEC supplements and amends Directive 76/116/EEC in respect of the trace elements boron, cobalt, copper, iron, manganese, molybdenum and zinc in fertilizers;

Whereas Commission Directive 77/535/EEC (3), as last amended by Directive 89/519/EEC (4), provides for official controls for Community fertilizers for the purpose of checking compliance with the requirements imposed by Community provisions concerning the quality and composition of fertilizers; whereas Directive 77/535/EEC should be supplemented so that fertilizers to which Directive 89/530/EEC applies can also be checked;

Whereas, in view of the scope and effects of the proposed action, the Community measures provided for by this Directive are not only necessary but also indispensable for the attainment of the stated objectives, whereas these objectives cannot be achieved by Member States individually, and whereas their attainment at Community level is, in fact, already provided for by Directive 76/116/EEC;

Whereas the measures provided for in this Directive are in accordance with the opinion of the Committee on the Adaptation to Technical Progress of the Directives for the Removal of Technical Barriers to Trade in Fertilizers,

HAS ADOPTED THIS DIRECTIVE:

Article 1

The text set out in the Annex to this Directive is hereby added to Annex II to Directive 77/535/EEC.

The methods are applicable to Community fertilizers for the determination of each trace element the declared content of which is less than or equal to 10 %.

Article 2

1. Member States shall bring into force the provisions necessary to comply with this Directive by 31 December 1993. They shall immediately inform the Commission thereof.

When Member States adopt these provisions, these shall contain a reference to this Directive or shall be accompanied by such reference at the time of their official publication. The procedure for such reference shall be adopted by Member States.

(1) OJ No L 24, 30. 1. 1976, p. 21.
(2) OJ No L 281, 30. 9. 1989, p. 116.
(3) OJ No L 213, 22. 8. 1977, p. 1.
(4) OJ No L 265, 12. 9. 1989, p. 30.

2. Member States shall communicate to the Commission the texts of the provisions of national law which they adopt in the field covered by this Directive.

Article 3

This Directive is addressed to the Member States.

Done at Brussels, 21 January 1993.

For the Commission
Martin BANGEMANN
Member of the Commission

ANNEX

'Methods 9

TRACE ELEMENTS

Method 9.1

EXTRACTION OF TOTAL TRACE ELEMENTS

1. SCOPE

This method defines the procedure for extracting the following trace elements: total boron, total cobalt, total copper, total iron, total manganese, total molybdenum and total zinc. The aim is to carry out the minimum number of extractions, making use wherever possible of the same extract to determine the total level of each of the trace elements listed above.

2. FIELD OF APPLICATION

This procedure concerns EEC fertilizers covered by Council Directive 89/530/EEC (1) containing one or more of the following trace elements: boron, cobalt, copper, iron, manganese, molybdenum and zinc. It is applicable to each trace element the declared content of which is less than or equal to 10 %.

3. PRINCIPLE

Dissolution in boiling dilute hydrochloric acid.

Note: The extraction is empirical and may not be quantitative depending on the product or the other constituents of the fertilizer. In particular, in the case of certain manganese oxides, the quantity extracted may be substantially smaller than the total quantity of manganese which the product contains. It is the responsibility of the fertilizer manufacturers to ensure that the declared content actually corresponds to the quantity extracted under the conditions pertaining to the method.

4. REAGENTS

4.1. Dilute hydrochloric acid (HCl) solution, about 6 M:

Mix 1 volume of hydrochloric acid (ρ = 1,18 g/ml) with 1 volume of water.

4.2. Concentrated ammonia solution (NH_4OH, ρ = 0,9 g/ml)

5. APPARATUS

Electric hotplate with variable temperature control.

Note: Where the boron content of an extract is to be determined, do not use borosilicate glassware. As the method involves boiling, teflon or silica is preferable. Rinse the glassware thoroughly if it has been washed in detergents containing borates.

6. PREPARATION OF THE SAMPLE

See Method 1 (Directive 77/535/EEC, OJ No L 213, 22. 8. 1977, p. 1).

7. PROCEDURE

7.1. Test sample

Take a quantity of fertilizer weighing between 2 and 10 g depending on the declared content of element in the product. The following table shall be used to obtain a final solution which, after appropriate dilution, will be within the measuring range for each method. Samples should be weighed to within 1 mg.

Declared content of trace element in the fertilizer (%)	< 0,01	0,01- < 5	≥ 5-10
Mass of test sample (g)	10	5	2
Mass of element in the sample (mg)	1	0,5-250	100-200
Volume of extract V (ml)	250	500	500
Concentration of element in extract (mg/l)	4	1-500	200-400

Place the sample in a 250 ml beaker.

(1) OJ No L 281, 30. 9. 1989, p. 116.

7.2. Preparation of the solution

If necessary moisten the sample with a little water, add 10 ml of dilute hydrochloric acid (4.1) per gram of fertilizer carefully, in small amounts, then add about 50 ml of water. Cover the beaker with a watchglass and mix. Bring to the boil on the hotplate and boil for 30 minutes. Allow to cool, stirring occasionally. Transfer quantitatively to a 250 or 500 ml volumetric flask (see Table). Make up to volume with water and mix thoroughly. Filter through a dry filter into a dry container. Discard the first portion. The extract must be perfectly clear.

It is recommended that the determinations be carried out without delay on aliquot portions of the clear filtrate, if not the containers should be stoppered.

Remark: Extracts in which the boron content has to be determined: Adjust the pH to between 4 and 6 with concentrated ammonia (4.2).

8. DETERMINATION

The determination of each trace element is to be carried out on the aliquot portions indicated in the method for each individual trace element.

If necessary, remove organic chelating or complexing substances from an aliquot portion of the extract by using Method 9.3. In the case of determination by atomic absorption spectrometry, such removal may not be necessary.

Method 9.2

EXTRACTION OF WATER-SOLUBLE TRACE ELEMENTS

1. SCOPE

This method defines the procedure for extracting water-soluble forms of the following trace elements: boron, cobalt, copper, iron, manganese, molybdenum and zinc. The aim is to carry out the minimum number of extractions,making use wherever possible of the same extract to determine the level of each of the trace elements listed above.

2. FIELD OF APPLICATION

This procedure concerns EEC fertilizers covered by Directive 89/530/EEC containing one or more of the following trace elements: boron, cobalt, copper, iron, manganese, molybdenum and zinc. It is applicable to each trace element the declared content of which is less than or equal to 10 %.

3. PRINCIPLE

The trace elements are extracted by shaking the fertilizer in water at 20 °C ± 2 °C.

Note: The extraction is empirical and may or may not be quantitative.

4. REAGENTS

4.1. Dilute hydrochloric acid (HCl) solution, about 6 M:

Mix 1 volume of hydrochloric acid (ρ = 1,18 g/ml) with 1 volume of water.

5. APPARATUS

5.1. Rotary shaker set at about 35 to 40 rpm.

5.2. pH-meter.

Note: Where the boron content of the extract is to be determined, do not use borosilicate glassware. Teflon or silica is preferable for this extraction. Rinse the glassware thoroughly if it has been washed in detergents containing borates.

6. PREPARATION OF THE SAMPLE

See Method 1 (Directive 77/535/EEC, (OJ No L 213, 22. 8. 1977, P. 1)).

7. PROCEDURE

7.1. Test sample

Take a quantity of fertilizer weighing between 2 and 10 g depending on the declared content of the element in the product. The following table shall be used to obtain a final solution which, after appropriate dilution, will be within the measuring range for each method. The samples should be weighed to within 1 mg.

Declared content of trace element in the fertilizer (%)	< 0,01	0,01 - < 5	≥ 5-10
Mass of test sample (g)	10	5	2
Mass of element in the test sample (mg)	1	0,5-250	100-200
Volume of extract V (ml)	250	500	500
Concentration of element in extract (mg/l)	4	1-500	200-400

Place the sample in a 250 or 500 ml flask (according to the Table).

7.2. Preparation of the solution

Add about 200 ml of water to the 250 ml flask or 400 ml of water to the 500 ml flask.

Stopper the flask well. Shake vigorously by hand to disperse the sample, then place the flask on the shaker and shake for 30 minutes.

Make up to volume with water and mix thoroughly.

7.3. Preparation of the test solution

Filter immediately into a clean, dry flask. Stopper the flask. Carry out the determination immediately after filtering.

NB: If the filtrate gradually becomes cloudy, make another extraction following 7.1 and 7.2 in a flask of volume Ve. Filter into a calibrated flask of volume W which has previously been dried and has received 5,00 ml of dilute hydrochloric acid (4.1). Stop the filtration at the exact moment when the calibration mark is reached. Mix thoroughly.

Under these conditions the value of V in the expression of results is:

$$V = Ve \times W / (W - 5).$$

The dilutions in the expression of results depend on this value of V.

8. DETERMINATION

The determination of each trace element is carried out on the aliquot portions indicated in the method for each individual trace element.

If necessary, remove organic chelating or complexing substances from an aliquot portion by using Method 9.3. In the case of determination by atomic absorption spectrometry, such removal may not be necessary.

Method 9.3

REMOVAL OF ORGANIC COMPOUNDS FROM FERTILIZER EXTRACTS

1. SCOPE

This method defines a procedure for removing organic compounds from fertilizer extracts.

2. FIELD OF APPLICATION

This procedure is applicable to analysing samples of fertilizers extracted by Methods 9.1 and 9.2 for which a declaration of total and/or water-soluble element is required by Directive 89/530/EEC.

Note: The presence of small quantities of organic matter usually does not affect determinations by means of atomic absorption spectrometry.

3. PRINCIPLE

The organic compounds in an aliquot portion of the extract are oxidized with hydrogen peroxide.

4. REAGENTS

4.1. Dilute hydrochloric acid (HCl) solution, about 0,5 M:

Mix 1 volume of hydrochloric acid (ρ = 1,18 g/ml) with 20 volumes of water.

4.2. Hydrogen peroxide solution (30 % H_2O_2, ρ = 1,11 g/ml), free from trace elements

5. APPARATUS

Electric hotplate with variable temperature control.

6. PROCEDURE

Take 25 ml of the extract solution obtained by Method 9.1 or Method 9.2 and place in a 100 ml beaker. In the case of Method 9.2, add 5 ml of the dilute hydrochloric acid solution (4.1). Then add 5 ml of the hydrogen peroxide solution (4.2). Cover with a watchglass. Allow oxidation to occur at room temperature for about one hour, then bring gradually to boiling and boil for half an hour. If necessary, add a further 5 ml of the hydrogen peroxide to the solution once it has cooled. Then boil to remove the excess hydrogen peroxide. Allow to cool and transfer quantitatively to a 50 ml volumetric flask and make up to volume. Filter where necessary.

Account should be taken of this dilution when taking aliquot portions and calculating the percentage of trace element in the product.

Method 9.4

DETERMINATION OF TRACE ELEMENTS IN FERTILIZER EXTRACTS BY ATOMIC ABSORPTION SPECTROMETRY

(GENERAL PROCEDURE)

1. SCOPE

This document defines a general procedure for determining the levels of certain trace elements in fertilizer extracts by atomic absorption spectrometry.

2. FIELD OF APPLICATION

This procedure is applicable to analysing samples of fertilizers extracted by Methods 9.1 and 9.2 for which a declaration of total and/or water-soluble element is required by Directive 89/530/EEC.

Adaptations of this procedure for the various trace elements are detailed in the methods defined specifically for each element.

Note: In most cases the presence of small quantities of organic matter will not affect determinations by atomic absorption spectrometry.

3. PRINCIPLE

After the extract has been treated where necessary to reduce or eliminate interfering chemical species, the extract is diluted so that its concentration is in the optimum range of the spectrometer at a wavelength suitable for the trace element to be determined.

4. REAGENTS

4.1. Dilute hydrochloric acid solution (HCl), about 6 M:

Mix one volume of hydrochloric acid (ρ = 1,18 g/ml) with 1 volume of water.

4.2. Dilute hydrochloric acid solution (HCl), about 0,5 M:

Mix one volume of hydrochloric acid (ρ = 1,18 g/ml) with 20 volumes of water.

4.3. Lanthanum salt solutions (10 g of La per litre).

This reagent is used for determinations of cobalt, iron, manganese and zinc. It can be prepared either :

(a) with lanthanum oxide dissolved in hydrochloric acid (4.1). Place 11,73 g of lanthanum oxide (La_2O_3) in 150 ml of water in a 1 litre volumetric flask and add 120 ml of 6 M hydrochloric acid (4.1). Allow to dissolve and then make up to 1 litre with water and mix thoroughly. This solution is approximately 0,5 M in hydrochloric acid ; or

(b) with solutions of lanthanum chloride, sulphate or nitrate. Dissolve 26,7 g of lanthanum chloride heptahydrate ($LaCl_3$ $7H_2O$) or 31,2 g of lanthanum nitrate hexahydrate [$La(NO_3)_3$ $6H_2O$] or 26,2 g of lanthanum sulphate nonahydrate [$La_2(SO_4)_3$ $9H_2O$] in 150 ml of water, then add 85 ml of 6 M hydrochloric acid (4.1). Allow to dissolve and then make up to 1 litre with water. Mix thoroughly. This solution is approximately 0,5 M in hydrochloric acid.

4.4. Calibration solutions

For the preparation of these, see the individual determination method for each trace element.

5. APPARATUS

Atomic absorption spectrometer fitted with sources emitting radiation characteristic of the trace elements to be determined.

The analyst must follow the manufacturer's instructions and be familiar with the apparatus. The apparatus must allow background correction so that it can be used whenever necessary (Co and Zn). The gases to be used are air and acetylene.

6. PREPARATION OF THE SOLUTION TO BE ANALYSED

6.1. Preparation of extract solutions of the trace elements to be determined

See Method 9.1 and/or 9.2 and, if appropriate, 9.3.

6.2. Treatment of the test solution

Dilute an aliquot portion of the extract obtained by Method 9.1, 9.2 or 9.3 with water and/or hydrochloric acid (4.1) or (4.2) so as to obtain, in the final solution for measurement, a concentration of the element to be determined that is appropriate to the calibration range used (7.2) and a hydrochloric acid concentration of at least 0,5 M and not more than 2,5 M. This operation may require one or more successive dilutions.

Take an aliquot portion of the final solution obtained by dilution of the extract, let (a) be its volume in ml, and pour into a 100 ml volumetric flask. When determining the cobalt, iron, manganese or zinc content, add 10 ml of the lanthanum salt solution (4.3). Make up to volume with the 0,5 M hydrochloric acid solution (4.2) and mix thoroughly. This is the final solution for measurement. Let D be the dilution factor.

7. PROCEDURE

7.1. Preparation of a blank solution

Prepare a blank solution by repeating the whole procedure from the extraction stage, omitting only the test sample of fertilizer.

7.2. Preparation of calibration solutions

From the working calibration solution prepared using the method given for each individual trace element, prepare in 100 ml volumetric flasks a series of at least five calibration solutions of increasing concentration within the optimum measuring range of the spectrometer. If necessary, adjust the concentration of hydrochloric acid to bring it as close as possible to that of the diluted test solution (6.2). For determining cobalt, iron, manganese or zinc add 10 ml of the same lanthanum salt solution (4.3) as used in 6.2. Make up to volume with the 0,5 M hydrochloric acid solution (4.2) and mix thoroughly.

7.3. Determination

Prepare the spectrometer (5) for the determination and adjust to the wavelength given in the method for the individual trace element concerned.

Spray three times in succession the calibration solutions (7.2), the test solution (6.2) and the blank solution (7.1), noting each result and flushing the instrument with distilled water between individual sprayings.

Construct the calibration curve by plotting the average spectrometer reading for each calibration solution (7.2) along the ordinate and the corresponding concentration of the element, expressed in µg/ml, along the abscissa.

From this curve, determine the concentrations of the relevant trace element in the test solution x_s (6.2) and in the blank solution x_b (7.1), expressing these concentrations in µg per ml.

8. EXPRESSION OF RESULTS

The percentage of trace element (E) in the fertilizer is equal to:

$$E\ (\%) = [(x_s - x_b) \times V \times D] / (M \times 10^4).$$

If method (9.3) has been used:

$$E\ (\%) = [(x_s - x_b) \times V \times 2D] / (M \times 10^4),$$

where:

E is the amount of the trace element determind, expressed as a percentage of the fertilizer;

x_s is the concentration of the test solution (6.2), in µg/ml;

x_b is the concentration of the blank solution (7.1), in µg/ml;

V is the volume of the extract obtained by Method 9.1 or 9.2, in ml;

D is the factor corresponding to the dilution carried out in (6.2);

M is the mass of the test sample taken in accordance with Method 9.1 or 9.2, in grams.

Calculation of dilution factor D:

If (a1), (a2), (a3), ., ., ., (ai) and (a) are the aliquot portions and (v1), (v2), (v3), ., ., ., (vi) and (100) are the volumes in ml corresponding to their respective dilutions, the dilution factor D will be equal to:

$$D = (v1/a1) \times (v2/a2) \times (v3/a3) \times . \times . \times . \times (vi/ai) \times (100/a).$$

Method 9.5

DETERMINATION OF BORON IN FERTILIZER EXTRACTS BY MEANS OF SPECTROPHOTOMETRY WITH AZOMETHINE-H

1. SCOPE

This method describes a procedure for determining boron in fertilizer extracts.

2. FIELD OF APPLICATION

This procedure is applicable to analysing samples of fertilizers extracted by Methods 9.1 and 9.2 for which a declaration of total and/or water-soluble boron is required by Directive 89/530/EEC.

3. PRINCIPLE

In an azomethine-H solution, borate ions form a yellow complex the concentration of which is determined by molecular absorption spectrometry at 410 nm. Interfering ions are masked with EDTA.

4. REAGENTS

4.1. EDTA buffer solution

Place in a 500 ml volumetric flask containing 300 ml of water:

— 75 g of ammonium acetate (NH_4OOCCH_3);

— 10 g of disodium salt of ethylene diamine tetraacetic acid (Na_2EDTA);

— 40 ml of acetic acid (CH_3COOH, ρ = 1,05 g/ml).

Make up to volume with water and mix thoroughly. The pH of the solution, checked by means of a glass electrode, must be 4,8 ± 0,1.

4.2. Azomethine-H solution

Place in a 200 ml volumetric flask

— 10 ml of the buffer solution (4.1);
— 400 mg of azomethine-H ($C_{17}H_{12}NNaO_8S_2$);
— 2 g of asorbic acid ($C_6H_8O_6$).

Make up to volume and mix thoroughly. Do not prepare large quantities of this reagent as it is stable for only a few days.

4.3. Boron calibration solutions

4.3.1. Boron stock solution (100 µg/ml)

Dissolve 0,5719 g of boric acid (H_3B0_3) in water in a 1 000 ml volumetric flask. Make up to volume with water and mix thoroughly. Transfer to a plastic bottle for storage in a refrigerator.

4.3.2. Boron working solution (10 µg/ml)

Place 50 ml of stock solution (4.3.1) in a 500 ml volumetric flask. Make up to volume with water and mix thoroughly.

5. APPARATUS

Spectrometer fitted for molecular absorption with cells having a 10 mm optical path and set to a wavelength of 410 nm.

6. PREPARATION OF THE SOLUTION TO BE ANALYSED

6.1. Preparation of the boron solution

See Methods 9.1 and/or 9.2 and, if appropriate, 9.3.

6.2. Preparation of the test solution

Dilute an aliquot portion of extract (6.1) to obtain a boron concentration as specified in 7.2. Two successive dilutions may be necessary. Let D be the dilution factor.

6.3. Preparation of the correction solution

If the test solution (6.2) is coloured, prepare a corresponding correction solution by placing in a plastic flask 5 ml of test solution (6.2), 5 ml of EDTA buffer solution (4.1) and 5 ml of water and mix thoroughly.

7. PROCEDURE

7.1. Preparation of the blank solution

Prepare a blank solution by repeating the whole procedure from the extraction stage, omitting only the test sample of fertilizer.

7.2. Preparation of the calibration solutions

Transfer 0, 5, 10, 15, 20 and 25 ml of the working calibration solution (4.3.2) to a series of 100 ml volumetric flasks. Make up to 100 ml with water and mix thoroughly. These solutions contain between 0 and 2,5 µg/ml of boron.

7.3. Colour development

Transfer 5 ml of the calibration solutions (7.2), test solutions (6.2) and blank (7.1) to a series of plastic flasks. Add 5 ml of the EDTA buffer solution (4.1). Add 5 ml of the azomethine-H solution (4.2).

Mix thoroughly and allow the colour to develop in the dark for 2 $^1/_2$ to three hours.

7.4. Determination

Measure the absorbance of the solutions obtained at 7.3 and if appropriate the correction solution (6.3) against water at a wavelength of 410 nm. Rinse the cells with water before each new reading.

8. EXPRESSION OF RESULTS

Plot a calibration curve of the concentration of the calibration solutions (7.2) along the abscissa and the absorbance given by the spectrophotometer (7.4) along the ordinate.

Read off the calibration curve the concentration of boron in the blank (7.1), the concentration of boron in the test solution (6.2) and, if the test solution is coloured, the corrected concentration of the test solution. To calculate the latter, subtract the absorbance of the correction solution (6.3) from the absorbance of the test solution (6.2) and determine the corrected concentration of the test solution. Note the concentration of the test solution (6.2), with or without correction, $X(x_s)$ and of the blank (x_b).

The percentage of boron in the fertilizer is given by:

$$B\ \% = [(x_s - x_b) \times V \times D] / (M \times 10^4)$$

If Method 9.3 is used:

$$B\ \% = [(x_s - x_b) \times V \times 2D] / (M \times 10^4)$$

where:

B is the quantity of boron expressed as a percentage of the fertilizer;

x_s is the concentration (μg/ml) in the test solution (6.2), with or without correction;

x_b is the concentration (μg/ml) in the blank (7.1);

V is the volume in ml of extract obtained in accordance with Method 9.1 or 9.2;

D is the factor corresponding to the dilution carried out in 6.2;

M is the mass in grams of the test sample taken in accordance with Method 9.1 or 9.2.

Calculation of the dilution factor D: if (a1) and (a2) are succesive aliquot portions and (v1) and (v2) are the volumes corresponding to their respective dilutions, the dilution factor D is given by:

$$D = (v1/a1) \times (v2/a2).$$

Method 9.6

DETERMINATION OF COBALT IN FERTILIZER EXTRACTS BY ATOMIC ABSORPTION SPECTROMETRY

1. SCOPE

This method describes a procedure for determining cobalt in fertilizer extracts.

2. FIELD OF APPLICATION

This procedure is applicable to analysing samples of fertilizers extracted by Methods 9.1 and 9.2 for which a declaration of total and/or water-soluble cobalt is required by Directive 89/530/EEC.

3. PRINCIPLE

After suitable treatment and dilution of the extracts, the cobalt content is determined by atomic absorption spectrometry.

4. REAGENTS

4.1. Hydrochloric acid solution, about 6 M.

See Method 9.4 (4.1).

4.2. Hydrochloric acid solution, about 0,5 M.

See Method 9.4 (4.2).

4.3. Lanthanum salt solutions (10 g of La per litre)

See Method 9.4 (4.3).

4.4. Cobalt calibration solutions

4.4.1. Cobalt stock solution (1 000 µg/ml)

In a 250 ml beaker, weigh to the nearest 0,1 mg, 1 g of cobalt, add 25 ml of 6 M hydrochloric acid (4.1) and heat on a hotplate until the cobalt is completely dissolved. When cool, transfer quantitatively to a 1 000 ml volumetric flask. Make up to volume with water and mix thoroughly.

4.4.2. Cobalt working solution (100 µg/ml)

Place 10 ml of the stock solution (4.4.1) in a 100 ml volumetric flask. Make up to volume with 0,5 M hydrochloric acid solution (4.2) and mix thoroughly.

5. APPARATUS

Atomic absorption spectrometer : see Method 9.4, (5). The instrument must be equipped with a source of rays characteristic of cobalt (240,7 nm). The spectrometer must allow background correction to be made.

6. PREPARATION OF THE SOLUTION TO BE ANALYSED

6.1. Cobalt extract solution

See Methods 9.1 and/or 9.2 and, if appropriate, 9.3.

6.2. Preparation of the test solution

See Method 9.4 (6.2). The test solution must contain 10 % (v/v) of a lanthanum salt solution (4.3).

7. PROCEDURE

7.1. Preparation of blank solution

See Method 9.4 (7.1). The blank must contain 10 % (v/v) of the lanthanum salt solution used in 6.2.

7.2. Preparation of calibration solutions

See Method 9.4 (7.2).

For an optimum determination range of 0 to 5 µg/ml of cobalt, place 0, 0,5, 1, 2, 3, 4 and 5 ml respectively of working solution (4.4.2) in a series of 100 ml volumetric flasks. If necessary adjust the hydrochloric acid concentration as closely as possible to that of the test solution. Add to each flask 10 ml of the lanthanum salt solution used in 6.2. Make up to 100 ml with 0,5 M hydrochloric acid solution (4.2) and mix thoroughly. These solutions contain 0, 0,5, 1, 2, 3, 4 and 5 µg/ml respectively of cobalt.

7.3. Determination

See Method 9.4 (7.3). Prepare the spectrometer (5) for measurement at a wavelength of 240,7 nm.

8. EXPRESSION OF RESULTS

See Method 9.4 (8).

The percentage of cobalt in the fertilizer is given by :

$$Co\ \% = [(x_s - x_b) \times V \times D] / (M \times 10^4)$$

If Method 9.3 is used :

$$Co\ \% = [(x_s - x_b) \times V \times 2D] / (M \times 10^4)$$

where :

Co is the quantity of cobalt expressed as a percentage of the fertilizer ;

x_s is the concentration in µg/ml of the test solution (6.2) ;

x_b is the concentration in µg/ml of the blank solution (7.1) ;

V is the volume in ml of extract obtained in accordance with Method 9.1 or 9.2 ;

D is the factor corresponding to the dilution carried out in 6.2 ;

M is the mass in grams of the test sample taken in accordance with Method 9.1 or 9.2.

Calculation of the dilution factor D : if (a1), (a2), (a3), ., ., ., (ai) and (a) are aliquot portions and (v1), (v2), (v3), ., ., ., (vi) and (100) are the volumes in ml corresponding to their respective dilutions, the dilution factor D is given by :

$$D = (v1/a1) \times (v2/a2) \times (v3/a3) \times . \times . \times . \times . \times (vi/ai) \times (100/a).$$

Method 9.7

DETERMINATION OF COPPER IN FERTILIZER EXTRACTS BY ATOMIC ABSORPTION SPECTROMETRY

1. SCOPE

This method describes a procedure for determining copper in fertilizer extracts.

2. FIELD OF APPLICATION

This procedure is applicable to analysing samples of fertilizers extracted by Methods 9.1 and 9.2 for which a declaration of total and/or water-soluble copper is required by Directive 89/530/EEC.

3. PRINCIPLE

After suitable treatment and dilution of the extracts, the copper content is determined by atomic absorption spectrometry.

4. REAGENTS

4.1. Hydrochloric acid solution, about 6 M

See Method 9.4, (4.1).

4.2. Hydrochloric acid solution, about 0,5 M

See Method 9.4, (4.2).

4.3. Hydrogen peroxide solution (30 % H_2O_2, ρ = 1,11 g/ml), free from trace elements.

4.4. Copper calibration solutions

4.4.1. Copper stock solution (1 000 µg/ml)

In a 250 ml beaker weigh to the nearest 0,1 mg, 1g of copper, add 25 ml of 6M hydrochloric acid (4.1) add 5 ml hydrogen peroxide solution (4.3) and heat on a hotplate until the copper is completely dissolved. Transfer quantiatively to a 1 000 ml volumetric flask. Make up to volume with water and mix thoroughly.

4.4.2. Copper working solution (100 µg/ml)

Place 20 ml of the stock solution (4.4.1) in a 200 ml volumetric flask. Make up to volume with 0,5 M hydrochloric acid solution (4.2) and mix thoroughly.

5. APPARATUS

Spectrometer equipped for atomic absorption : see Method 9.4 (5). The instrument must be fitted with a source of rays characteristics of copper (324,8 nm).

6. PREPARATION OF THE SOLUTION TO BE ANALYSED

6.1. Copper extract solution

See Methods 9.1 and/or 9.2 and, if appropriate, 9.3.

6.2. Preparation of the test solution

See Method 9.4, (6.2).

7. PROCEDURE

7.1. Preparation of blank solution

See Method 9.4, (7.1).

7.2. Preparation of calibration solutions

See Method 9.4, (7.2).

For an optimum determination range of 0 to 5 µg/ml of copper, place 0, 0,5, 1, 2, 3, 4 and 5 ml respectively of working solution (4.4.2) in a series of 100 ml volumetric flasks. If necessary adjust the hydrochloric acid concentration as closely as possible to that of the test solution (6.2). Make up to 100 ml with 0,5 M hydrochloric acid solution (4.2) and mix thoroughly. These solutions contain 0, 0,5, 1, 2, 3, 4 and 5 µg/ml respectively of copper.

7.3. Determination

See Method 9.4, (7.3). Prepare the spectrometer (5) for measurement at a wavelength of 324,8 nm.

8. EXPRESSION OF RESULTS

See Method 9.4, (8).

The percentage of copper in the fertilizer is given by:

$$Cu\ \% = [(x_s - x_b) \times V \times D] / (M \times 10^4)$$

If Method 9.3 is used:

$$Cu\ \% = [(x_s - x_b) \times V \times 2D] / (M \times 10^4)$$

where:

Cu is the quantity of copper expressed as a percentage of the fertilizer;

x_s is the concentration in µg/ml of the test solution (6.2);

X_b is the concentration in µg/ml of the blank solution (7.1);

V is the volume in ml of extract obtained in accordance with Method 9.1 or 9.2;

D is the factor of the dilution carried out in 6.2;

M is the mass in grams of the test sample taken in accordance with Method 9.1 or 9.2.

Calculation of the dilution factor D: if (a1), (a2), (a3) ., ., ., (ai) and (a) are aliquot portions and (v1), (v2), (v3), ., ., ., (vi) and (100) are the volumes in ml corresponding to their respective dilutions, the dilution factor D is given by:

$$D = (v1/a1) \times (v2/a2) \times (v3/a3) \times . \times . \times . \times (vi/ai) \times (100/a)$$

Method 9.8

DETERMINATION OF IRON IN FERTILIZER EXTRACTS BY ATOMIC ABSORPTION SPECTROMETRY

1. SCOPE

This method describes a procedure for determining iron in fertilizer extracts.

2. FIELD OF APPLICATION

This procedure is applicable to analysing samples of fertilizers extracted by Methods 9.1 and 9.2 for which a declaration of total and/or water-soluble iron is required by Directive 89/530/EEC.

3. PRINCIPLE

After suitable treatment and dilution of the extract, the iron content is determined by atomic absorption spectrometry.

4. REAGENTS

4.1. Hydrochloric acid solution, about 6 M

See Method 9.4 (4.1).

4.2. Hydrochloric acid solution, about 0,5 M

See Method 9.4 (4.2).

4.3. Hydrogen peroxide solution (30 % H_2O_2, ρ = 1,11 g/ml) free from trace elements.

4.4. Lanthanum salt solutions (10 g of La per litre)

See Method 9.4, (4.3).

4.5. Iron calibration solutions

4.5.1. Iron stock solution (1 000 µg/ml)

In a 500 ml beaker, weigh to the nearest 0,1 mg, 1 g of pure iron wire, add 200 ml of 6 M hydrochloric acid (4.1) and 15 ml of hydrogen peroxide solution (4.3). Heat on a hotplate until the iron is completely dissolved. When cool, transfer quantitatively to a 1 000 ml volumetric flask. Make up to volume with water and mix thoroughly.

4.5.2. Iron working solution (100 µg/ml)

Place 20 ml of the stock solution (4.5.1) in a 200 ml volumetric flask. Make up to volume with the 0,5 M hydrochloric acid solution (4.2) and mix thoroughly.

5. APPARATUS

Atomic absorption spectrometer : see Method 9.4 (5). The instrument must be fitted with a source of rays characteristic of iron (248,3 nm).

6. PREPARATION OF THE SOLUTION TO BE ANALYSED

6.1. Iron extract solution

See Methods 9.1 and/or 9.2 and, if appropriate, 9.3.

6.2. Preparation of the test solution

See Method 9.4 (6.2). The test solution must contain 10 % (v/v) of a lanthanum salt solution.

7. PROCEDURE

7.1. Preparation of blank solution

See Method 9.4 (7.1). The test solution must contain 10 % (v/v) of the lanthanum salt solution used in 6.2.

7.2. Preparation of calibration solutions

See Method 9.4 (7.2).

For an optimum determination range of 0 to 10 µg/ml of iron, place 0, 2, 4, 6, 8 and 10 ml respectively of working solution (4.5.2) in a series of 100 ml volumetric flasks. If necessary adjust the hydrochloric acid concentration as closely as possible to that of the test solution. Add 10 ml of the lanthanum salt solution used in 6.2. Make up to volume with 0,5 M hydrochloric solution (4.2) and mix thoroughly. These solutions contain 0, 2, 4, 6, 8 and 10 µg/ml respectively of iron.

7.3. Determination

See Method 9.4 (7.3). Prepare the spectrometer (5) for measurement at a wavelength of 248,3 nm.

8. EXPRESSION OF RESULTS

See Method 9.4 (8).

The percentage of iron in the fertilizer is given by :

$$Fe\ \% = [(x_s - x_b) \times V \times D)] / (M \times 10^4)$$

If Method 9.3 is used :

$$Fe\ \% = [(x_s - x_b) \times V \times 2D)] / (M \times 10^4)$$

where :

F_e is the quantity of iron expressed as a percentage of the fertilizer ;

x_s is the concentration in µg/ml of the test solution (6.2) ;

x_b is the concentration in µg/ml of the blank solution (7.1) ;

V is the volume in ml of extract obtained in accordance with Method 9.1 or 9.2 ;

D is the factor of the dilution carried out in 6.2 ;

M is the mass in grams of the test sample taken in accordance with Method 9.1 or 9.2.

Calculation of the dilution factor D : if (a1), (a2), (a3), ., ., ., (ai) and (a) are aliquot portions and (v1), (v2), (v3), ., ., ., (vi) and (100) are the volumes in ml corresponding to their respective dilutions, the dilution factor D is given by :

$$D = (v1/a1) \times (v2/a2) \times (v3/a3) \times . \times . \times . \times (vi/ai) \times (100/a).$$

Method 9.9

DETERMINATION OF MANGANESE IN FERTILIZER EXTRACTS BY ATOMIC ABSORPTION SPECTROMETRY

1. SCOPE

This method describes a procedure for determining manganese in fertilizer extracts.

2. FIELD OF APPLICATION

This procedure is applicable to analysing samples of fertilizers extracted by Methods 9.1 and 9.2 for which a declaration of total and/or water-soluble manganese is required by Directive 89/530/EEC.

3. PRINCIPLE

After suitable treatment and dilution of the extracts, the manganese level is determined by atomic absorption spectrometry.

4. REAGENTS

4.1. Hydrochloric acid solution, about 6 M

See Method 9.4, (4.1).

4.2. Hydrochloric acid solution, about 0,5 M

See Method 9.4, (4.2).

4.3. Lanthanum salt solutions (10 g of La per litre)

See Method 9.4, (4.3).

4.4. Manganese calibration solutions

4.4.1. Manganese stock solution (1 000 µg/ml)

In a 250 ml beaker, weigh to the nearest 0,1 mg, 1 g of manganese, add 25 ml of 6 M hydrochloric acid solution (4.1). Heat on a hotplate until the manganese is completely dissolved. When cool, transfer quantitatively to a 1 000 ml volumetric flask. Make up to volume with water and mix thoroughly.

4.4.2. Manganese working solution (100 µg/ml)

Dilute 20 ml of the stock solution (4.4.1) in the 0,5 M hydrochloric acid solution (4.2) in a 200 ml volumetric flask. Make up to volume with the 0,5 M hydrochloric acid solution (4.2) and mix thoroughly.

5. APPARATUS

Atomic absorption spectrometer: see Method 9.4 (5). The apparatus must be fitted with a source of lines characteristic of manganese (279,6 nm).

6. PREPARATION OF THE SOLUTION TO BE ANALYSED

6.1. Manganese extract solution

See Methods 9.1 and/or 9.2 and, if appropriate, 9.3.

6.2. Preparation of the test solution

See Method 9.4, (6.2). The test solution must contain 10 % by volume of lanthanum salt solution (4.3).

7. PROCEDURE

7.1. Preparation of the blank solution

See Method 9.4 (7.1). The blank solution must contain 10 % by volume of the lanthanum salt solution used in 6.2.

7.2. Preparation of the calibration solutions

See Method 9.4 (7.2).

For an optimum interval of 0 to 5 µg/ml manganese, place 0, 0,5, 1, 2, 3, 4 and 5 ml, respectively, of the working solution (4.4.2) in a series of 100 ml volumetric flasks. Where necessary, adjust the hydrochloric acid concentration to bring it as close as possible to that of the test solution. To each flask add 10 ml of the lanthanum salt solution used in 6.2. Make up to 100 ml with the 0,5 M hydrochloric acid solution (4.2) and mix thoroughly. These solutions contain 0, 0,5, 1, 2, 3, 4 and 5 µg/ml manganese respectively.

7.3. Determination

See Method 9.4 (7.3). Prepare the spectrometer (5) for measurements at a wavelength of 279,6 nm.

8. EXPRESSION OF RESULTS

See Method 9.4 (8).

The percentage of manganese in the fertilizer is as follows:

$$Mn\ \% = [(x_s - x_b) \times V \times D] / (M \times 10^4)$$

If Method 9.3 has been used:

$$Mn\ \% = [(x_s - x_b) \times V \times 2D] / (M \times 10^4)$$

where:

Mn is the quantity of manganese expressed as a percentage of the fertilizer;

x_s is the concentration in µg/ml of the test solution (6.2);

x_b is the concentration in µg/ml of the blank solution (7.1);

V is the volume in ml of the extract obtained using Method 9.1 or 9.2;

D is the factor corresponding to the dilution performed in 6.2;

M is the mass in g of the test sample taken using Method 9.1 or 9.2.

Calculation of dilution factor D: where (a1), (a2), (a3), ., ., ., (ai) and (a) are aliquot portions and (v1), (v2), (v3), ., ., ., (vi) and (100) the volumes in ml corresponding to their respective dilutions, dilution factor D will be equal to:

$$D = (v1/a1) \times (v2/a2) \times (v3/a3) \times . \times . \times . \times (vi/ai) \times (100/a).$$

Method 9.10

DETERMINATION OF MOYLBDENUM IN FERTILIZER EXTRACTS BY SPECTOPHOTOMETRY OF A COMPLEX WITH AMMONIUM THIOCYANATE

1. SCOPE

This method describes a procedure for determining molybdenum in fertilizer extracts.

2. FIELD OF APPLICATION

This procedure is applicable to analysing samples of fertilizers extracted by Methods 9.1 and 9.2 for which a declaration of total and/or water-soluble molybdenum is required by Directive 89/530/EEC.

3. PRINCIPLE

Molybdenum (V) forms a complex $[MoO(SCN)_5]^{--}$ in an acid medium with SCN^- ions.

The complex is extracted with n-butyl acetate. Interfering ions such as those of iron remain in the aqueous phase. The yellow-orange colour is determined by molecular absorption spectrometry at 470 nm.

4. REAGENTS

4.1. Dilute hydrochloric acid solution (HCl), about 6 M

See method 9.4 (4.1).

4.2. Copper solution (70 mg/l) in 1,5 M hydrochloric acid

Dissolve 275 mg of copper sulphate ($CuSO_4\ 5H_2O$) weighed to within 0,1 mg in 250 ml of the 6 M hydrochloric acid solution (4.1) in a 1 000 ml volumetric flask. Make up to volume with water and mix thoroughly.

4.3. Ascorbic acid solution (50 g/l)

Dissolve 50 g of ascorbic acid ($C_6H_8O_6$) in water in a 1 000 ml volumetric flask. Make up to volume with water, mix thoroughly and keep in a refrigerator.

4.4. n-butyl acetate

4.5. Ammonium thiocyanate solution, 0,2 M

Dissolve 15,224 g of NH_4SCN in water in a 1 000 ml volumetric flask. Make up to volume with water; mix thoroughly and store in a dark-coloured bottle.

4.6. Stannous chloride solution (50 g/l) in 2 M hydrochloric acid

This solution must be perfectly clear and prepared immediately before use. Very pure stannous chloride must be used otherwise the solution will not be clear.

To prepare 100 ml of solution, dissolve 5 g of ($SnCl_22H_2O$) in 35 ml of 6 M HCl solution (4.1). Add 10 ml of the copper solution (4.2). Make up to volume with water and mix thoroughly.

4.7. Molybdenum calibration solutions

4.7.1. Molybdenum stock solution (500 µg/ml)

Dissolve 0,920 g of ammonium molybdate [$(NH_4)_6Mo_7O_{24}$ $4H_2O$] weighed to within 0,1 mg in the 6 M hydrochloric acid (4.1) in a 1 000 ml volumetric flask. Make up to volume with that solution and mix thoroughly.

4.7.2. Molybdenum intermediate solution (25 µg/ml)

Place 25 ml of the stock solution (4.7.1) in a 500 ml volumetric flask. Make up to volume with 6 M hydrochloric acid (4.1) and mix thoroughly.

4.7.3. Molybdenum working solution (2,5 µg/ml)

Place 10 ml of the intermediate solution (4.7.2) in a 100 ml volumetric flask. Make up to volume with 6 M hydrochloric acid (4.1) and mix thoroughly.

5. APPARATUS

5.1. Spectrometer fitted for molecular absorption with cuvettes having a 20 mm optical path and set to a wavelength of 470 nm.

5.2. 200 or 250 ml separating funnels.

6. PREPARATION OF THE SOLUTION TO BE ANALYSED

6.1. Molybdenum extract solution

See Methods 9.1 and/or 9.2 and, if appropriate, 9.3.

6.2. Preparation of the test solution

Dilute an aliquot portion of the extract (6.1) with 6 M hydrochloric acid solution (4.1) so as to obtain an appropriate molybdenum concentration. Let D be the dilution factor.

Take an aliquot portion (a) from the extract solution containing 1 to 12 µg molybdenum and place it in the separating funnel (5.2). Make up to 50 ml with the 6 M hydrochloric acid solution (4.1).

7. PROCEDURE

7.1. Preparation of the blank solution

Prepare a blank solution by repeating the whole procedure from the extraction stage, omitting only the test sample of fertilizer.

7.2. Preparation of the series of calibration solutions

Prepare a series of at least six calibration solutions of increasing concentration corresponding to the optimum response range of the spectrometer.

For the interval 0-12,5 µg molybdenum, place 0, 1, 2, 3, 4 and 5 ml, respectively, of the working solution (4.7.3) in the separating funnels (5.2). Make up to 50 ml with 6 M hydrochloric acid (4.1). The funnels contain, respectively, 0, 2,5, 5, 7,5, 10 and 12,5 µg molybdenum.

7.3. Development and separation of the complex

To each separating funnel (6.2, 7.1 and 7.2), add in the following order:

— 10 ml of the copper solution (4.2)

— 20 ml of the ascorbic acid solution (4.3);

mix thoroughly and wait for two or three minutes. Then add:

— 10 ml of n-butyl acetate (4.4), using a precision pipette;

— 20 ml of the thiocyanate solution (4.5).

Shake for one minute to extract the complex in the organic phase; allow to precipitate; after the separation of the two phases, draw off the entire aqueous phase and discard it; then wash the organic phase with:

—10 ml of the stannous chloride solution (4.6).

Shake for one minute. Allow to precipitate and draw off the entire aqueous phase. Collect the organic phase in a test tube; this will make it possible to collect the drops of water in suspension.

7.4. Determination

Measure the absorbencies of the solutions obtained at 7.3 at a wavelength of 470 nm using the 0 µg/ml molybdenum calibration solution (7.2) as a reference.

8. EXPRESSION OF RESULTS

Construct the calibration curve by plotting the corresponding masses of molybdenum in the calibration solutions (7.2) expressed in µg along the abscissa and the corresponding values of the absorbancies (7.4) given by the spectrometer reading along the ordinate.

From this curve determine the mass of molybdenum in the test solution (6.2) and the blank solution (7.1). These masses are designated (x_s) and (x_b) respectively.

The percentage of molybdenum in the fertilizer is:

$$Mo\ \% = [(x_s - x_b) \times V/a \times D] / (M \times 10^4)$$

If Method 9.3 has been used:

$$Mo\ \% = [(x_s - x_b) \times V/a \times 2D] / (M \times 10^4)$$

where:

Mo is the quantity of molybdenum expressed as a percentage of the fertilizer;

a is the volume in ml of the aliquot taken from the last dilute solution (6.2);

x_s is the Mo mass in µg in the test solution (6.2);

x_b is the Mo mass in µg in the blank solution (7.1) the volume of which corresponds to the volume (a) of the aliquot of the test solution (6.2);

V is the volume in ml of the extract solution obtained in accordance with Method 9.1 or 9.2;

D is the factor corresponding to the dilution performed in 6.2;

M is the mass in g of the test sample taken in accordance with Method 9.1 or 9.2.

Calculation of the dilution factor D: where (a1), (a2) are successive aliquot portions and (v1), (v2) are the volumes corresponding to their respective dilutions, the dilution factor D will be:

$$D = (v1/a1) \times (v2/a2).$$

Method 9.11

DETERMINATION OF ZINC IN FERTILIZER EXTRACTS BY ATOMIC ABSORPTION SPECTROMETRY

1. SCOPE

This method describes a procedure for determining zinc in fertilizer extracts.

2. FIELD OF APPLICATION

This procedure is applicable to analysing samples of fertilizers extracted by Methods 9.1 and 9.2 for which a declaration of total and/or water-soluble zinc is required by Directive 89/530/EEC.

3. PRINCIPLE

After suitable treatment and dilution of the extracts, the zinc level is determined by atomic absorption spectrometry.

4. REAGENTS

4.1. Hydrochloric acid solution, about 6 M

See Method 9.4 (4.1).

4.2. Hydrochloric acid solution, about 0,5 M

See Method 9.4 (4.2).

4.3. Lanthanum salt solutions (10 g of La per litre)

See Method 9.4 (4.3).

4.4. Zinc calibration solutions

4.4.1. Zinc stock solution (1 000 µg/ml)

In a 1 000 ml volumetric flask dissolve 1 g of zinc powder or flakes weighed to within 0,1 mg in 25 ml of 6 M hydrochloric acid (4.1). When completely dissolved, make up to volume with water and mix thoroughly.

4.4.2. Zinc working solution (100 µg/ml)

In a 200 ml volumetric flask, dilute 20 ml of the stock solution (4.4.1) in 0,5 M hydrochloric acid solution (4.2). Make up to volume with the 0,5 M hydrochloric acid solution and mix thoroughly.

5. APPARATUS

Atomic absorption spectrometer : See Method 9.4, (5). The apparatus must be fitted with a source of lines characteristic of zinc (213,8 nm). the spectrometer must allow background correction to be made.

6. PREPARATION OF THE SOLUTION TO BE ANALYSED

6.1. Zinc extract solution

See Methods 9.1 and/or 9.2 and, if appropriate, 9.3.

6.2. Preparation of the test solution

See Method 9.4, (6.2). The test solution must contain 10 % by volume of lanthanum salt solution.

7. PROCEDURE

7.1. Preparation of the blank solution

See Method 9.4, (7.1). The blank solution must contain 10 % by volume of the lanthanum salt solution used in 6.2.

7.2. Preparation of the calibration solutions

See Method 9.4, (7.2).

For an optimum interval of 0 to 5 µg/ml of zinc, place 0, 0,5, 1, 2, 3, 4 and 5 ml, respectively, of the working solution (4.4.2) in a series of 100 ml volumetric flasks. Where necessary, adjust the concentration of hydrochloric acid to bring it as close as possible to that of the test solution. Add 10 ml of the lanthanum salt solution used in (6.2) to each volumetric flask. Make up to 100 ml with the 0,5 M hydrochloric acid solution (4.2) and mix thoroughly. These solutions contain, respectively : 0, 0,5, 1, 2, 3, 4, and 5 µg/ml of zinc.

7.3. Determination

See Method 9.4 (7.3). Prepare the spectrometer (5) for measurements at a wavelength of 213,8 nm.

8. EXPRESSION OF RESULTS

See Method 9.4 (8).

The percentage of zinc in the fertilizer is equal to :

$$Zn\ \% = [(x_s - x_b) \times V \times D] / (M \times 10^4)$$

If method 9.3 has been used :

$$Zn\ \% = [(x_s - x_b) \times V \times 2D] / (M \times 10^4)$$

where :

Z_n is the quantity of zinc expressed as a percentage of the fertilizer ;

x_s is the concentration in µg/ml of the test solution (6.2) ;

x_b is the concentration in µg/ml of the blank solution (7.1) ;

V is the volume in ml of the extract solution obtained in accordance with Method 9.1 or 9.2 ;

D is the factor corresponding to the dilution performed in (6.2) ;

M is the mass in g of the test sample taken in accordance with Method 9.1 or 9.2.

Calculation of the dilution factor D : where (a1), (a2), (a3), ., ., ., (ai) and (a) are successive aliquot portions and (v1), (v2), (v3), ., ., ., (vi) and (100) are the volumes corresponding to their respective dilutions, the dilution factor D will be :

$$D = (v1/a1) \times (v2/a2) \times (v3/a3) \times . \times . \times . \times (vi/ai) \times (100/a).'$$