FERTILIZERS AND ANIMAL FOODSTUFFS (ANALYSIS) RULES, 1972

[L.N. 215/1972, L.N. 292/1974.]

1. These Rules may be cited as the Fertilizers and Animal Foodstuffs (Analysis) Rules, 1972.

2. (1) Samples for analysis shall be taken from Official Samples which have been taken and packed in the manner prescribed in the Fertilizers and Animal Foodstuffs (Sampling) Rules, 1972.

(2) The following apparatus shall be used in the preparation of a sample for analysis—

(a) sieves with apertures of 1 mm square and of between 2 and 3 mm square respectively;

(b) a laboratory mill fitted with a screen with 1 mm aperture;

(c) a porcelain pestle and mortar;

(d) an oven set to operate at 100°C; and

(e) stoppered or screw-capped storage bottles 250 ml and 500 ml.

3. (1) If the Official Sample is in a fine condition and passes through a sieve having apertures about 1 mm square it shall be thoroughly mixed and a portion not less than 250 gm in weight shall be placed in a storage bottle. From this portion the quantities for analysis shall be taken.

(2) If the Official Sample does not wholly pass through the sieve having apertures about 1 mm square, and wholly passes through the sieve having apertures between 2 and 3 mm square or if a change in a moisture content is likely to occur during the preparation of the Official Sample for analysis, the Official Sample shall be thoroughly mixed and a portion for the determination of moisture shall be taken at once.

(3) If the Official Sample is in a coarse condition (but can be pulverized) as, for example, pieces of broken cake, it shall be carefully pulverized until the whole passes through the sieve having apertures between 2 and 3 mm square. It shall then be thoroughly mixed and a portion for the determination of moisture shall be taken at once.

(4) Material which does not admit of being pulverized in its natural condition so that it will pass through the sieve having apertures between 2 and 3 mm square shall be mixed as thoroughly as its condition will allow. A portion of the coarse material shall then be taken for the determination of moisture and if the dry material can be pulverized, sufficient of the coarse material to produce at least 250 g of dried material shall similarly be dried and then pulverized, so that it will pass through the aforesaid sieve. It shall then be thoroughly mixed. A portion of the material which has been prepared in the manner prescribed in rules 3(1), 3(2), 3(3) and 3(4) of this rule weighing not less than 250 g shall then be taken and if necessary further pulverized until it passes through the sieve having apertures about 1 mm square. The portion of the sample so prepared shall be placed in a storage bottle and from it the quantities for analysis shall be taken.

(5) If the Official Sample is a liquid, it shall be placed in a storage bottle and shall be well stirred immediately before each sample is taken for analysis.

(6) For a grading analysis, the sample shall be taken direct from the Official Sample.

(7) Fertilizers or animal foodstuffs which separate readily into separate fractions which are not susceptible to mixing together during preparation for analysis shall be separated into their component fractions, each fraction shall be analysed as if it was a separate fertilizer or animal foodstuffs and the proportion of the separate fractions shall be allowed for in reporting the analysis.

(8) The moisture content of material which gains or loses moisture during its preparation for analysis shall be determined on a sample of the material which has been prepared for analysis each time a sample is taken for analysis.

(9) Any change in moisture content during preparation of the sample shall be allowed for in presenting the results of analysis.

(10) The samples taken for analysis and for moisture determination shall be drawn in as equal portion as possible from several well scattered points in the material which has been prepared for analysis or whose moisture content is being determined.

4. (1) The following apparatus shall be used for the determination of moisture in a fertilizer or in an animal foodstuff where the sample tested is deemed by the analyst to be suitable for drying at 100°C—

(a) a laboratory spoon or such other sampling tool as the analyst shall consider suitable for drawing the sample;

(b) a weighing bottle or boat;

(c) a laboratory balance which is sensitive to 1 mg or less, and its weights;

(d) a laboratory oven set to operate at 100°C;

(e) a desiccator suitably charged with active desiccant.

(2) About 5 grams of the sample (or the minimum larger amount that, with regard to its coarseness, the analyst shall consider to be representative of the sample), weighed to the nearest milligram shall be heated in the oven for 2 to 3 hours. The sample shall then be placed in the desiccator to cool and shall then be re-weighed, again to the nearest milligram. The heating, cooling and weighing process shall be repeated until the difference in weight before and after heating is less than 5 mg. The percentage moisture shall be taken as the total loss in weight as a result of the heating process, in milligrams, multiplied by 100 and divided by the weight also in milligrams of the sample taken for the moisture determination.

(3) Where the sample is deemed by the analyst to be of a nature unsuitable for drying at 100°C, he may undertake the drying at a reduced pressure and at a much lower temperature as is compatible with the stability of the product and he may utilize phosphorus pentoxide or some other desiccating agent to assist the process, or he may employ any other method which is suited to the determination of the moisture content of the sample and he may use such specialized apparatus as the method may require. In these circumstances the moisture content reported shall be qualified by an indication of the method employed.

5. (1) The following apparatus and reagents shall be used for the determination of oil in an animal foodstuff—

(a) a laboratory spoon for drawing the sample;

(b) a weighing bottle or boat;

(c) a laboratory balance which is sensitive to 1 mg or less and its weights;

(d) a laboratory oven set to operate at 100°C;

(e) an extraction apparatus of 60 ml capacity fitted with a water cooled reflux condenser;

(f) a fat-free extraction thimble to fit the aforesaid extraction apparatus;

(g) a receiving flask of 150 ml capacity whose weight is known to within 1 mg and which will fit the aforesaid extraction apparatus;

(h) a second receiving flask of 150 ml capacity whose weight is known to within 1 mg, which also will fit the aforesaid extraction apparatus;

(i) a radiant heat heater unit with energy regulated heat control to accommodate the aforesaid receiving flasks;

(j) a water cooled distillation condenser to fit the aforesaid receiving flask and a distillate receiver;

(k) a 9 to 11.5 cm outside diameter porcelain mortar and pestle;

(l) a dry 10 cm diameter glass filter funnel, 15 cm diameter general purpose filter papers;

(m) a desiccator suitably charged with active desiccant, glass rods;

(n) wash bottles;

(o) petroleum spirit boiling between 40° to 60°C.

(2) Between 3 and 5 gm weighed to the nearest milligram, of the analysis sample shall be placed in the extraction thimble and this shall then be placed in the extraction apparatus. The extraction apparatus shall then be fitted with a receiving flask into which has been placed about 100 ml of the petroleum spirit. The apparatus so assembled shall be heated for a total of 16 hours in the heater unit the heat control being kept so adjusted that condensate falls from the reflux condenser into the extraction apparatus throughout that time at the rate of 5 or 6 drops per second. The bulk of the petroleum spirit in the receiving flask shall then be distilled through the distillation condenser into the distillate receiver and the flask shall then be heated in the oven for 30 minutes, placed in the desiccator to cool and weighed to the nearest milligram. The heating, cooling and weighing process shall be repeated until the difference in weight of the receiving flask and its contents before and after heating is less than 3 mg. The percentage oil found shall be taken as the weight of the residue in the receiving flask in milligrams, multiplied by 100 and divided by the weight also in milligrams of sample taken for the analysis. Where an allowance for change in moisture content under rule 2(c) of these Rules must be made, the percentage oil reported shall be the percentage found divided by (100—the percentage moisture content of the sample determined as prescribed in rule 2(a) of these Rules) and multiplied by (100—the percentage moisture content of the Official Sample). Otherwise the percentage found shall be the percentage reported.

6. (1) The following apparatus and reagents shall be used for the determination of fibre in an animal foodstuff—

(a) a laboratory spoon for drawing the sample;

(b) a weighing bottle or boat;

(c) a laboratory balance which is sensitive to 1 mg or less, and its weights;

(d) a laboratory oven set to operate at 100°C;

(e) a laboratory furnace;

(f) a 400 ml beaker on which has been marked the level to which it would be filled by 200 ml of liquid measured at room temperature;

(g) a hot plate with an energy regulator heat control;

(h) a porcelain Buchner funnel about 10.5 cm diameter, 8 and 15 cm diameter glass filter funnels, muslin cloth whose weight shall be known to the nearest milligram;

(i) 4 cm diameter porcelain or silica dishes;

(j) a suction pump;

(k) a heat resistant suction flask;

(l) washing bottles, some with necks and mouthpieces insulated for handling hot liquids;

(m) beakers;

(n) a desiccator suitably charged with active desiccant;

(o) glass rods;

(p) a time piece and optionally, the extraction apparatus, energy regulated heating unit and a receiving flask as prescribed under rule 5 of these Rules;

(q) petroleum spirit boiling between 60° to 80°C;

(r) 0.255N sulphuric acid;

(s) 0.313N sodium hydroxide which is free or nearly free of sodium carbonate;

(t) a 1 per cent hydrochloric acid solution prepared by diluting 10 ml of concentrated hydrochloric acid with water to 1 litre;

(u) 95 per cent alcohol; and

(v) Diethylether.

(2) A small weighed portion of the sample which has been prepared for analysis in the manner prescribed in rule 2 of these Rules, shall be heated with an excess of the 1 per cent hydrochloric acid.

(3) If no effervescence is observed, between 2.7 and 3.0 gm, of the sample which has been prepared for analysis in the manner prescribed in rule 2 of these Rules shall be taken and weighed to the nearest milligram.

(4) The weighed sample shall be extracted with the petroleum spirit in the manner prescribed in rule 6 of these Rules or by stirring, settling and decanting three times with the petroleum spirit in a small beaker. The sample shall in no manner be ground in the extraction process. The extracted sample shall be air-dried and transferred to a 400 ml beaker. Sufficient of the 0.255N sulphuric acid to fill the flask to its 200 ml mark shall then be heated to its boiling point. 30 to 40 ml of this solution shall then immediately be added to the extracted sample.

(5) The beaker shall be gently swirled to disperse the sample. Sufficient of the heated sulphuric acid solution shall then immediately be added to fill the beaker to the 200 ml mark. The beaker and its contents shall be heated on the hot plate so that they come to the boil within 1 minute. The boiling shall then be continued gently for exactly 30 minutes. During boiling the beaker shall be swirled every few minutes in order to mix the contents and remove particles from the sides. Meanwhile the Buchner funnel shall be prepared by placing a muslin cloth over the holes of the plate. The Buchner funnel shall then be fitted in the suction flask and boiling water shall be poured into the funnel and this shall be allowed to remain in the funnel until the funnel is hot. The hot water shall then be drawn away by the application of suction. At the end of the 30 minutes of boiling the beaker shall be removed from the hot plate and the acid mixture poured at once into a shallow layer of hot water which is under gentle suction in the prepared funnel. The suction shall then be so adjusted that the filtration of the bulk of the 200 ml of acid mixture is completed within 10 minutes. If the filtration takes longer than 10 minutes, the determination shall be discarded and a new determination shall be undertaken. The residues from the sulphuric acid extraction shall then be washed with boiling water until the washings are free from acid. Sufficient of the 0.313N solution of sodium hydroxide to fill the beaker to the 200 ml mark shall be brought to boiling point in an insulated wash bottle and some of it shall be used to wash the residue on the muslin cloth and in the funnel back into a 400 ml beaker. Sufficient of the hot sodium hydroxide solution shall then immediately be added to fill the beaker to its 200 ml mark. The beaker shall then be put on the hot plate and again heated so that its contents come to the boil within 1 minute. Boiling shall then be continued gently and continuously for exactly 30 minutes. During boiling the beaker again shall be swirled every few minutes in order to mix its contents and remove particles from the sides. At the end of this 30-minute boiling period, the beaker shall be removed from the hot plate and its contents shall be transferred to a muslin cloth in a 15 cm filter funnel. Any insoluble material remaining in the beaker shall be transferred to the muslin cloth by washing with

boiling water and the residue shall then be well washed on the muslin cloth with boiling water. The residue shall then be washed with the 1 per cent hydrochloric acid solution. The residue shall then be rewashed with boiling water until it is free from acid. The residue shall then be washed twice with the 95 per cent alcohol and three times with the diethylether. The residue on the muslin cloth shall be transferred with diethylether to a weighed dried silica dish whose weight shall have been determined to the nearest milligram. The silica dish and its contents shall then be heated in the oven for 30 minutes, placed in the desiccator to cool and weighed to the nearest milligram. The heating, cooling and weighing process shall be repeated until the difference in weight before and after heating is less than 3 mgm. The silica dish and its contents shall then be placed in the silica capsule and the capsule and its contents shall be placed in the furnace, the furnace then being cool. The furnace and its contents shall then be heated until the contents of the silica capsule are incinerated, but the temperature inside the furnace during that time shall not be allowed to exceed 600°C. The capsule and its incinerated content shall be placed in the desiccator to cool and shall then be reweighed to the nearest milligram. The amount whereby the weight of the silica dish and its contents after the incineration exceeds the known weight of the said silica dish, empty, shall be taken as the weight of ash in the residue. The apparent fibre content of the sample shall be taken as the amount whereby the weight of dry residue collected on the muslin cloth exceeds the weight of ash in the residue. The actual fibre content multiplied by the factor (0.0102t—0.02), where 't' is the observed boiling point of water in °C in the laboratory at which the determination was carried out. The percentage fibre found shall be taken as the actual fibre content so found in milligrams, multiplied by 100 and divided by the weight also in milligrams, of sample taken for analysis. Where an allowance for change in moisture content under rule 2 of these Rules must be made, the percentage fibre reported shall be the percentage found, divided (100—the percentage moisture content of the sample determined as prescribed in rule 2(b) of these Rules) and multiplied by (100—the percentage moisture content of the Official Sample). Otherwise the percentage found shall be the percentage reported.

(6) If an effervesence is observed in application of the test prescribed in section (a) of this rule, the suspension shall be stirred well and allowed to settle. The supernatant liquid shall be decanted through a 12.5 cm general purpose filter paper of known weight in an 8 cm filter funnel and the residue shall be washed twice and then transferred to the filter paper. The residue and the filter paper shall then be dried in the oven and reweighed.

(7) The quantity of the sample taken for the test in accordance with these Rules which would be sufficient to give 2.7 gm and 3.0 gm respectively of residue after treatment in the manner prescribed in rule 6(b) of this rule shall then be calculated.

(8) A quantity of the sample which has been prepared for analysis in the manner prescribed in rule 2 of these Rules which shall be between the weights calculated in rule 6(7) of this rule shall then be taken and weighed to the nearest milligram. The weighed sample shall then be extracted with the petroleum spirit in the manner prescribed in rule 6(4) of this rule and transferred to the 400 ml beaker. The extract sample shall then be treated in the beaker with an excess of the 1 per cent hydrochloric acid and the suspension shall be agitated well and allowed to settle. The supernatant liquid shall be decanted through a muslin cloth in an 8 cm filter funnel. The residue shall then be washed twice with water by decantation through muslin cloth. The residue in the beaker and on the muslin cloth shall then be allowed to drain thoroughly. Sufficient of the 0.255N sulphuric acid to fill the 400 ml beaker to its 200 ml mark shall then be heated to its boiling point and 30 to 40 ml of the hot acid shall be used to wash any particles on the muslin cloth back into the beaker. The fibre determination shall then proceed in the manner prescribed in rule 6(5) of this rule.

7. (1) The following apparatus and reagents shall be used for the determination of nitrogen in a fertilizer or in an animal foodstuff—

(a) a laboratory spoon for drawing the sample;

(b) a weighing bottle or boat;

(c) a laboratory balance which is sensitive to 1 mg or less, and its weights;

(d) pipettes 25, 50 and 100 ml minimum specification B.S. 1583, Class B;

(e) burettes 50 ml minimum specification B.S. 846, Class B;

(f) measuring cylinders 25 ml;

(g) flat bottom flasks 50 and 100 ml;

(h) glass rods;

(i) a time piece;

(j) 50 per cent sodium hydroxide solution, prepared by dissolving 500 gm of sodium hydroxide in water and diluting to 1 litre;

(k) 0.1N, 0.2N or 0.5N standard hydrochloric acid or sulphuric acid, the concentration used shall be as prescribed under this rule as the case may be. The standard acid shall be adjusted to exact normality against a corresponding standard sodium carbonate solution;

(l) 0.1N, 0.2N or 0.5N standard sodium hydroxide, the concentration used shall be as prescribed under this rule as the case may be. The standard sodium hydroxide shall be adjusted to exact normality against the corresponding standard acid;

(m) methyl red solution, prepared by adding 0.5 ml of 0.1N sodium hydroxide to 5 ml of 90 per cent industrial methylated spirits, dissolving in this solution 25 mgm of methyl red and diluting the resultant solution to 250 ml with 50 per cent industrial methylated spirits.

(2) The following apparatus and reagents in addition to those specified in paragraph (1) of this rule shall be used for determination of nitrogen in a fertilizer in which nitrogen is declared to be present only in organic and ammonium forms or protein in an animal foodstuff—

(a) Kjeldahl flasks 500 to 600 ml;

(b) ammonia distillation apparatus to fit the aforesaid Kjeldahl flasks, each comprising a Liebig condenser mounted vertically and connectable to its flask through a splash-head and vertical delivery still head connecting tube;

(c) radiant heat heater units with energy regulator heat controls to accommodate the aforesaid Kjeldahl flasks;

(d) concentrated sulphuric acid;

(e) paraffin wax;

(f) potassium sulphate or anhydrous sodium sulphate;

(g) crystalline copper sulphate or elemental selenium;

(h) litmus indicator papers; and

(i) pure sucrose.

(3) About 2 gm, weighed to the nearest milligram, of the sample which has been prepared for analysis in the manner prescribed in rule 2(a) of these Rules shall be placed in a Kjeldahl flask and to it shall be added 25 ml, measured by measuring a cylinder, of the concentrated sulphuric acid. The flask then shall be gently heated until frothing ceases. If frothing is excessive 0.5 gm of the paraffin wax shall also be added.

(4) 10 gm of potassium sulphate or anhydrous sodium sulphate and 0.5 gm of the copper sulphate or elemental selenium shall then be added and the flask strongly heated until the colour of the clear liquid ultimately obtained ceases to diminish. Heating shall then continue for a further one and a half hours. The contents of the Kjeldahl flask shall then be allowed to cool and water shall be added, at first in small quantities with further intervals of cooling of the flask as necessary, until a total volume of about 250 ml is

obtained. A piece of litmus paper shall then be placed in the diluted solution and, with swirling of the flask to ensure mixing, the 50 per cent solution of sodium hydroxide shall be added slowly until the litmus paper just turns blue. A further 10 ml, measured by measuring cylinder of the sodium hydroxide solution shall then be poured carefully down the side of the flask so that it does not mix at once with the other constituents of the flask. The flask shall then at once be mounted in a heater unit and connected to the ammonia distillation apparatus, the outlet shall dip into a measured volume of standard sulphuric or hydrochloric acid, in a 500 ml flat bottom flask. The amount and normality of standard acid into which the ammonia distillation apparatus outlet shall dip shall be determined by the amount of nitrogen which the fertilizer or the amount of crude protein which the animal foodstuff is believed to contain and shall be in accordance with the following table—

TABLE 1

VOLUME AND NORMALITY OF STANDARD ACID IN THE DETERMINATION OF NITROGEN

Where the N content is believed to be:	*or where the crude protein is believed to be:*		*the No. of ml. of standard acid taken shall be:*	*and its normality shall be:*
	(a) in pure wheat products:	*(b) in all other Animal Foodstuffs:*		
less than 3%	less than 16%	less than 17.5%	50	0.1N
at least 3% but less than 4%	at least 16% but less than 23%	at least 17.5% but less than 25.0%	75	0.1N
at least 4% but less than 6%	at least 23%	at least 25.5% but less than 37.5%	100	0.1N
at least 6% but less than 8%		at least 37.5% but less than 50.0%	125	0.1N
at least 8% but less than 12%		at least 50.0%	100	0.2N
at least 12% but less than 16%			125	0.2N
at least 16% but less than 20%			150	0.2N
at least 20% but less than 30%			100	0.5N
at least 30% but less than 40%			125	0.5N
at least 40%			150	0.5N

The volume of standard acid taken shall be measured using suitable combinations of the pipettes.

Where less than 100 ml of standard acid is taken it shall be made up to about 100 ml with distilled water.

The contents of the boiling flask shall then be mixed and the heat control on its heater unit shall be so adjusted that not less than 150 ml of distillate shall be collected in the standard acid in 30 minutes.

The excess of standard acid remaining after the distillate has been collected in it shall be titrated from a burette with standard sodium hydroxide of the same normality as that of the acid used to take up the distillate, a few drops of the methyl red solution being used as indicator.

(5) A blank determination shall be undertaken alongside the actual determination using the same amounts of the same reagents for the digestion and distillation, and for taking up the distillate and the same standard alkali for titrating the excess acid as were used in the actual determination, but with 2 gm of pure sucrose in place of the sample. In the blank determination the bulk of the standard alkali used to neutralize the excess of standard acid may be measured by a 50 or 100 ml pipette as the case may be. The titrations shall be made to the nearest 0.05 ml. If the number of millilitres and parts of a millilitre of standard sodium hydroxide required to neutralize the standard acid in the blank determination is expressed as x and the number of millilitres and parts of a millilitre similarly required in the actual determination is expressed by y, the percentage nitrogen found shall be taken as (x-y) multiplied by 1.4 times the normality of the standard acid used to take up the distillate and divided by the weight in grams and parts of a gram of sample taken for the analysis.

(6) Where the animal foodstuff is a pure wheat product, the percentage crude protein found shall be taken as percentage nitrogen found multiplied by 5.70. In all other animal foodstuffs, the percentage crude protein found shall be taken as the percentage nitrogen found multiplied by 6.25.

(7) Where an allowance for change in moisture content under rule 2(c) of these Rules must be made the percentage nitrogen or crude protein reported shall be the percentage found, divided by (100—the percentage moisture content of the sample determined as prescribed by rule 2(b) of these Rules) and multiplied by (100—the percentage moisture content of the Official Sample). Otherwise the percentage found should be the percentage reported.

(8) The following apparatus and reagents in addition to those specified in this rule shall be used for the determination of nitrogen in Sulphate of ammonia, Diammonium phosphate or in a compound fertilizer in which the nitrogen is declared to be present only as ammonium nitrogen—

(a) a Markham ammonia distillation apparatus which embodies a sample chamber, a funnel, a splash-head welded onto a Liebig Condenser and a steam inlet;

(b) volumetric flasks 250 ml minimum specification B.S. 846, Class B;

(c) flat bottom flasks 1,000 ml;

(d) 8-15 cm diameter glass filter funnel; and

(e) 15-24 cm diameter general purpose filter papers.

(9) About 2 gm, weighed to the nearest milligram, of the sample which has been prepared for analysis in the manner prescribed in rule 2(a) of these Rules, shall be transferred to a 250 ml volumetric flask. Here it shall be dissolved in about 200 ml of water with vigorous shaking to ensure complete solution if necessary. The contents of the flask shall then be diluted to volume and the solution shall be filtered if cloudy or if it contains visible insoluble matter through a dry filter paper in the filter funnel into a dry 500 ml flat bottom flask.

(10) 10 ml measured by pipette of the clear solution shall be transferred into the sample chamber of the Markham still through the funnel, and 10 ml measured by measuring cylinder of 50 per cent sodium hydroxide solution shall be introduced slowly into the sample chamber and the provided plug shall be replaced into the funnel. Steam shall be slowly introduced into the mixture by closing the washings outlet. The distillate outlet shall dip into a measured volume of 2 per cent boric acid solution containing a mixed indicator of methyl red and bromocresol green. The distillate is collected in 3 minutes. The amount of ammonia in the distillate shall then be titrated from a burette with standard hydrochloric or sulphuric acid. A blank determination shall be undertaken alongside the actual determination, using the same amount of the same 50 per cent sodium hydroxide solution as was used in distillation, the same amount of 2 per cent boric acid solution as was used to take up the distillate and the same standard acid as was

used to titrate the ammonia in the actual determination. The titrations shall be made to the nearest 0.05 ml. Express results in terms of nitrogen 1 ml 0.02N acid = 0.00014 gm nitrogen. Where an allowance for change in moisture content under these Rules must be made, the percentage nitrogen reported shall be the percentage found divided by (100— the percentage moisture content of the sample determined as prescribed under these Rules) and multiplied by (100—the percentage moisture content of the Official Sample). Otherwise the percentage found shall be the percentage reported.

(11) The following reagents in addition to the apparatus and reagents specified under the rule shall be used for the determination of nitrogen in Ammonium Sulphate Nitrate, Calcium Ammonium Nitrate, Nitrate of Soda or in a compound fertilizer in which nitrogen is declared to be present entirely as nitrate nitrogen or as ammonium nitrogen and nitrate nitrogen: Devarda's alloy, finely powdered so that not less than 80 per cent will pass through a sieve having apertures about 0.25 mm square. A solution of the fertilizer shall be prepared in the manner prescribed under this rule. 10 ml of the clear solution shall then be transferred to a Markham distillation apparatus and to it shall be added 1 gm of the Devarda's alloy and 5 ml of water. 10 ml measured by measuring cylinder of the 50 per cent sodium hydroxide solution shall be poured carefully down the side of the sample chamber so that it does not mix at once with the other constituents of the flask. The distillate outlet shall dip into a measured volume of 2 per cent boric acid solution in a 100 ml flat bottom flask. The contents of the distillation apparatus shall then be mixed and allowed to stand in the cool for 10-15 minutes. Steam shall then be introduced slowly increasing gently to a steady bubbling. The distillate is collected in the boric acid in six minutes. The nitrogen determination shall then proceed in the manner prescribed under this rule.

(12) The apparatus specified under this rule and the reagents specified under this rule shall be used for the determination of nitrogen in urea or in a urea containing compound fertilizer in which no nitrate nitrogen is declared to be present.

(13) About 2 gm, weighed to the nearest milligram, of the sample which has been prepared for analysis in the manner prescribed under these Rules shall be placed in a Kjeldahl flask and to it shall be added, first 50 ml of water and then slowly with frequent shaking and cooling, 25 ml measured by measuring cylinder, of concentrated sulphuric acid. The Kjeldahl flask which shall then be gently heated until the water is expelled. The Kjeldahl flask with contents shall then be cooled to room temperature. The digest shall then be transferred to a volumetric flask of 500 ml capacity using small quantities of distilled water and the volume shall be adjusted to 500 ml. The nitrogen determination shall then proceed in the manner prescribed in subsection (c)(iii) of this rule.

(14) The following reagent in addition to the reagents specified in this rule and the apparatus specified in this rule shall be used for the determination of nitrogen in an organic nitrogen or urea containing compound fertilizer in which nitrate nitrogen is also declared to be present—

Crystalline Sodium Thiosulphate.

(15) About 2 gm weighed to the nearest milligram, of the sample which has been prepared for analysis in the manner prescribed in these Rules shall be taken and treated with sulphuric acid in the manner prescribed in these Rules accordingly as the fertilizer is or is not believed to contain urea. It shall then be cooled, 5 gm of the crystallized sodium thiosulphate shall be added in small amounts and the whole shall be allowed to stand for one hour with occasional shaking. The nitrogen determination shall then proceed in the manner prescribed in subsection (b)(ii) and part of subsection (b)(iii) of this rule.

8. (1) The following apparatus and reagents shall be used for the determination of phosphate in a fertilizer or in an animal foodstuff—

(a) a laboratory spoon for drawing the sample;

(b) a weighing bottle or boat;

(c) a laboratory balance which is sensitive to 1 mg or less, and its weights;

(d) volumetric flasks 100 ml and 250 ml minimum specification B.S. 1792, Class B;

(e) volumetric flasks 50 and 200 ml minimum specification B.S. 1792, Class B, if prescribed in this rule;

(f) pipettes 10, 25 and 50 ml minimum specification B.S. 1583, Class B;

(g) burettes 50 ml minimum specification B.S. 846, Class B. Graduated pipettes 25 ml, minimum specification B.S. 700, Class B, Type 2;

(h) measuring cylinders 5, 10, 25 and 500 ml, minimum specification B.S. 604, Class B;

(i) wash bottles;

(j) a spectrophotometer, with a monochromator capable of being set to give a source of light with wavelength of 4200A°, or a colorimeter or absorptiometer fitted with a 425A°, violet light filter, with two cells of 1 cm optical length;

(k) a time piece;

(l) a dry flat bottom flask 1,000 ml;

(m) a dry 20 cm diameter glass funnel;

(n) 32 cm diameter medium fine filter papers;

(o) glass rods;

(p) graph paper, 1 mm rulings;

(q) sodium sulphate solution, prepared by dissolving 54 gm anhydrous sodium sulphate in 500 ml water;

(r) vanadium molybdate reagent, prepared by dissolving separately 20 gm of ammonium molybdate and 1 gm of ammonium vanadate in water, mixing, acidifying with 140 ml of concentrated nitric acid, and diluting with water to 1 litre;

(s) phosphate stock solution, prepared by dissolving in water, 1.9173 gm of potassium dihydrogen phosphate which had previously been dried at 150°C for one hour and cooled in a desiccator and diluting to 1,000 ml in a volumetric flask; and

(t) standard phosphate solution, prepared by diluting 50 ml measured by pipette, of phosphate stock solution to 250 ml, in a volumetric flask. This solution contains 0.2 mgm of phosphorus pentoxide per millilitre.

(2) The following apparatus and reagents in addition to those specified above shall be used for the determination of water soluble phosphate in a fertilizer—

(a) volumetric flasks 50 and 500 ml, minimum specification B.S. 1792, Class B;

(b) a laboratory shaking machine accommodating the aforesaid 500 ml volumetric flask;

(c) a safety pipette 1 ml;

(d) beakers 50 ml;

(e) a hot plate with an energy regulator heat control;

(f) litmus paper;

(g) concentrated nitric acid;

(h) approximately 1.0N sodium hydroxide.

(3) Between 9.9 and 10.1 gm, weighed to the nearest milligram, of the sample which has been prepared for analysis in the manner prescribed in rule 2(a) of these Rules, shall be transferred to a 500 ml volumetric flask. 400 ml of water at 20°C shall then be added and the flask shall be shaken continuously for 30 minutes on the shaking machine. The contents shall then be diluted to volume, mixed well and filtered through a dry 32 cm filter paper in the dry filter funnel into the dry flat bottom flask.

(4) 25 ml measured by pipette, of the filtrate shall be transferred to the 50 ml beaker and to it shall be added by safety pipette, 1 ml of the concentrated nitric acid. The solution shall then be heated to incipient boiling on the hot plate and maintained at this temperature for 10 minutes. It shall then be cooled, a piece of litmus paper shall be placed in the solution and approximately 1.0N sodium hydroxide solution shall be added slowly, with stirring, until the paper starts to turn blue. The solution shall then be transferred to a 50 ml volumetric flask and diluted to volume.

(5) This solution shall then be further diluted with water at 20°C to an extent which shall be determined by the percentage of phosphorus pentoxide which is believed to be present in accordance with the following table—

TABLE 2

PHOSPHATE DILUTION IN RELATION TO PHOSPHATE LEVELS IN FERTILIZERS

Where the P_2O_5 content being measured is believed to be:		*the Nos. of ml of solution which shall be taken for dilution shall be:*	*and it shall be diluted in a volumetric flask to:*
	less than 5%	24	50ml
At least 5%	but less than 5.5%	22	50ml
At least 5.5%	but less than 6%	20	50ml
At least 6%	but less than 6.5%	19	50ml
At least 6.5%	but less than 7%	17	50ml
At least 7%	but less than 7.5%	16	50ml
At least 7.5%	but less than 8%	15	50ml
At least 8%	but less than 8.5%	14	50ml
At least 8.5 %	but less than 9.5%	13	50ml
At least 9.5 %	but less than 10%	12	50ml
At least 10%	but less than 11%	22	100ml
At least 11%	but less than 12%	20	100ml
At least 12%	but less than 13%	19	100ml
At least 13%	but less than 14%	17	100ml
At least 14%	but less than 15%	16	100ml
At least 15%	but less than 16%	15	100ml
At least 16%	but less than 17%	14	100ml
At least 17%	but less than 19%	13	100ml
At least 19%	but less than 20%	12	100ml
At least 20%	but less than 22%	22	200ml
At least 22%	but less than 24.5%	20	200ml
At least 24.5%	but less than 27.5%	18	200ml
At least 27.5%	but less than 30.5%	16	200ml
At least 30.5%	but less than 33%	15	200ml
At least 33%	but less than 35%	14	200ml

TABLE 2—*continued*

Where the P_2O_5 content being measured is believed to be:		*the Nos. of ml of solution which shall be taken for dilution shall be:*	*and it shall be diluted in a volumetric flask to:*
At least 35%	but less than 38%	13	220ml
At least 38%	but less than 41%	12	200ml
At least 41%	but less than 45%	11	200ml
At least 45%	but less than 49.5%	10	200ml
At least 49.5%		9	200ml

The solution taken for dilution shall be measured by graduated pipette.

(6) 25 ml measured by pipette, of the solution obtained as a result of the dilution prescribed in subsection (b)(iv) of this rule shall then be placed in a 100 ml volumetric flask.

(7) Into a series of seven 100 ml volumetric flasks shall be measured by a burette, 25.0, 26.0, 27.0, 28.0, 29.0, 30.0 and 31.0 ml of the Standard Phosphate solution (containing respectively 5.0, 5.2, 5.4, 5.6, 5.8, 6.0 and 6.2 mgm P_2O_5).

(8) To each of these samples of the Standard Phosphate solution and to the 25 ml aliquot of diluted sample solution shall be added by pipette, 10 ml of the sodium sulphate solution and 25ml of the vanadium molybdate reagent at a temperature of 20°C. The solution in each flask shall then be diluted to volume with water at 20°C, shall be mixed and shall be allowed to stand for ten minutes while its colour develops.

(9) While these solutions are so standing, the spectrophotometer shall be set to operate at 4200A° (or the colorimeter or absorptiometer shall be brought into operation as the case may be). Then the ten minutes standing period having been completed and following the procedures which are appropriate to the operation of the instrument used, the optical densities of its cells shall be compared, using the coloured standard solution containing 5.0 mgm P_2O_5 per 100 ml if the comparison reveals that there is a small difference between the two cells being used, the cell with the lower optical density shall be used as the standard reference cell. The apparent optical densities of the coloured standard solutions and of the coloured sample solution relative to the coloured standard solution containing 5.0 mgm P_2O_5, per 100 ml shall then be determined. The apparent optical densities of the coloured standard solutions shall be plotted on a calibration graph against their phosphorus pentoxide content values. The number of milligrams and parts of a milligram of phosphorus pentoxide per 100 ml of coloured standard solution having the same optical density as the coloured sample solution shall then be determined by interpolation on the calibration graph. The determination shall be made to the nearest 0.01 mgm. A new Standard Phosphate solution, a new set of coloured standards prepared as prescribed in this rule and a new calibration graph shall be prepared for each phosphate determination.

(10) If the number of milligrams and parts of a milligram of phosphorus pentoxide so found in the 100 ml of coloured sample solution is expressed as x, if the number of millilitres of solution taken in making the dilution prescribed in this rule, is expressed as y, and if the sample is believed to contain less than 10 per cent water soluble phosphorus pentoxide, then the percentage water soluble phosphorus pentoxide found in the sample shall be taken $\frac{x}{y}$ multiplied by 200 and divided by the weight in grams and parts of a gram of sample taken for the analysis. If the sample is believed to contain at least 10 per cent but less than 20 per cent water soluble phosphorus pentoxide, then the percentage found in the sample shall be taken as $\frac{x}{y}$ multiplied by 400 and divided by the

weight in grams and parts of a gram of sample taken for the analysis. If the sample is believed to contain at least 20 per cent water soluble phosphorus pentoxide, then the percentage found shall be taken as $\frac{x}{y}$ multiplied by 800 and divided by the weight in grams and parts of a gram of sample taken for the analysis. Where an allowance for change in moisture content under these Rules must be made, the percentage water soluble phosphorus pentoxide reported shall be the percentage found, divided by (100—the percentage moisture content of the sample determined as prescribed under these Rules) and multiplied by (100—the percentage moisture content of the Official Sample). Otherwise, the percentage found shall be the percentage reported.

(11) The following apparatus and reagents in addition to those specified in this rule shall be used for the determination of citric soluble phosphorus pentoxide in a fertilizer—

(a) a stoppered shaking bottle, 1 litre;

(b) a laboratory shaking machine to accommodate the aforesaid shaking bottle;

(c) a volumetric flask 1,000 ml minimum specification B.S. 1792, Class B;

(d) a measuring cylinder 500 ml;

(e) graduated pipettes 5 and 10 ml minimum specification B.S. 700, Class B, Type 2;

(f) as required for the 2 per cent citric acid solution additions prescribed in this rule;

(g) two per cent acid, prepared by dissolving 20 gm of pure crystallized citric acid, monohydrate in water and diluting to volume at 20°C in a 100 ml volumetric flask.

(12) Between 4.9 and 5.1 gm weighed to the nearest milligram of the sample which has been prepared for analysis in the manner prescribed in these Rules shall be placed in the stoppered shaking bottle. To it shall be added 500 ml measured by measuring cylinder, of the 2 per cent citric acid solution, with shaking so as to avoid the possibility of the fertilizer caking. The flask shall then be shaken continuously for 30 minutes on the shaking machine. After shaking, the whole of the liquid shall be poured at once into a dry filter paper in the dry filter funnel and the filtrate collected in the dry flat bottom flask. If the filtrate is not clear it shall be passed again through the same filter. The solution shall be diluted in the manner prescribed in this rule.

(13) 25ml measured by pipette of the diluted solution shall then be placed in a 100 ml volumetric flask.

(14) Into a series of seven 100 ml volumetric flasks shall be measured by burette 25.0, 26.0, 27.0, 28.0, 29.0, 30.0 and 31.0 ml of the standard phosphate solution containing respectively 5.0, 5.2, 5.4, 5.6, 5.8, 6.0 and 6.2 mgm P_2O_5. If the fertilizer is believed to contain less than 10 per cent of phosphorus pentoxide which is soluble in 2 per cent citric acid, 0.5ml of the 2 per cent acid solution shall be added to each of these standard phosphorus solutions for every 1 ml of sample solution used in the dilution to 50 ml prescribed in this rule. If the fertilizer is believed to contain at least 10 per cent but less than 20 per cent of phosphorus pentoxide which is soluble in 2 per cent citric acid, 0.25 ml of the 2 per cent citric solution shall be added to each of these standard phosphorus solutions for every 1 ml of sample solution used in the dilution to 100 ml prescribed in this rule. If the fertilizer is believed to contain at least 20 per cent of phosphorus pentoxide which is soluble in 2 per cent citric acid, 0.125 ml of the 2 per cent citric acid solution shall be added to each of these standard phosphorus solutions for every 1 ml of sample solution used in the dilution to 200 ml prescribed in this rule. These citric acid solution additions shall be measured by graduated pipette and shall be to the nearest 0.1 ml. The phosphate determination shall then proceed in the manner prescribed in this rule.

(15) If the number of milligrams and parts of a milligram of phosphorus pentoxide so found in the 100 ml of coloured sample solution is expressed as x; if the number of millilitres of solution taken in the dilution in the manner prescribed in this rule is expressed as y, and if the sample is believed to contain less than 10 per cent of phosphorus pentoxide which is soluble in 2 per cent citric acid, then the percentage citric soluble phosphorus pentoxide found in the fertilizer shall be taken as $\frac{x}{y}$ multiplied by 100 and divided by the weight in grams and parts of a gram of sample taken for the analysis. If the sample is believed to contain at least 10 per cent but less than 20 per cent of phosphorus pentoxide which is soluble in 2 per cent citric acid, then the percentage found shall be taken as $\frac{x}{y}$ multiplied by 200 and divided by the weight in grams and part of a gram of sample taken for analysis. If the sample is believed to contain at least 20 per cent of phosphorus pentoxide which is soluble in 2 per cent citric acid, then the percentage found shall be taken as $\frac{x}{y}$ multiplied by 400 and divided by the weight in grams and parts of a gram of sample taken for the analysis. Where an allowance for a moisture content under these Rules must be made, the percentage citric soluble phosphorus pentoxide reported shall be the percentage found, divided by 100 minus the percentage moisture content reported of the sample determined as prescribed under these Rules and multiplied by 100 minus the percentage moisture content of the Official Sample. Otherwise, the percentage found shall be the percentage reported.

9. (1) The following apparatus and reagents in addition to those specified under rule 8(1) of these Rules shall be used for the determination of total phosphorus pentoxide in a mineral fertilizer—

(a) a beaker 400 ml;

(b) a hot plate with energy regulator heat control;

(c) a volumetric flask 500 ml minimum specification B.S. 1792, Class B;

(d) safety pipettes 10 ml;

(e) concentrated nitric acid;

(f) concentrated hydrochloric acid.

(2) Between 4.9 and 5.1 gm weighed to the nearest milligram of the sample which has been prepared for analysis in the manner prescribed in rule 2(a) of these Rules shall be placed in the 400 ml beaker. 100 ml of water shall be added and the whole shall be stirred thoroughly. The mixture shall then be brought to the boil and to the boiling liquid shall be added by safety pipette, 10 ml of the concentrated hydrochloric acid in a fine stream and then 10 ml of the concentrated nitric acid. The mixture shall continue to be boiled for 10 minutes and shall then be cooled, transferred to the 500 ml volumetric flask and diluted to volume at 20°C with distilled water. The contents of the flask shall be mixed well and filtered through a dry filter paper in the dry filter funnel into the dry flat bottom flask, the first 10 to 20 ml of filtrate being discarded.

(3) The phosphate determination shall then proceed in the manner prescribed in subsections (b)(iv), (v), (vi), (vii), (viii) and (c)(v) of this rule.

10. (1) The following apparatus and reagents in addition to those specified under rule 8(1) of these Rules shall be used for the determination of total phosphate in a fertilizer containing organic material or in an animal foodstuff—

(a) a silica capsule or dish about 55 mm diameter;

(b) a hot plate with an energy regulator heat control;

(c) a laboratory oven set to operate at 100°C;

(d) a laboratory furnace;

(e) beakers 400 ml;

(f) a volumetric flask 500 ml, minimum specification B.S. 1792, Class B;

(g) a graduated safety pipette 23 ml type 2;

(h) a safety pipette 5 ml;

(i) an 8 cm diameter glass funnel;

(j) 12.5 cm diameter medium fine filter papers;

(k) a watch glass 9 cm diameter;

(l) calcium oxide;

(m) concentrated nitric acid; and

(n) concentrated hydrochloric acid.

(2) Between 4.9 and 5.1 gm weighed to the nearest milligram of the sample which has been prepared in the manner prescribed under these Rules shall be placed in the silica capsule or dish. 1 gm of calcium oxide shall then be added and mixed with the sample and the mixture shall be thoroughly wetted with water. The wet mixture shall then be dried in the oven. It shall then be heated gently and finally incinerated in the furnace to destroy as much organic matter as possible, but the temperature in the furnace during that time shall not be allowed to exceed 500°C.

(3) The incinerated material shall then be allowed to cool and shall be transferred to the 400 ml beaker with 10 ml of distilled water. 12 ml of the concentrated hydrochloric acid shall then be added, the addition being made sufficiently slowly to avoid loss by effervescence. 5 ml of the concentrated nitric acid shall then be added. The mixture shall then be heated to incipient boiling and kept at that temperature for 10 minutes. About 10 ml of water shall then be added and the solution filtered through a 12.5 cm filter paper in an 8 cm filter funnel into a 400 ml beaker, any insoluble material remaining being transferred to the filter with minimum amount of water and washed twice with small quantities of water. The filtrate on the beaker shall then be protected with the watch glass.

(4) The filter paper and the insoluble matter it contains shall then be transferred to the original capsule or dish and dried in the oven. It shall then be heated gently and finally incinerated in furnace, the incineration being continued until all the carbon is destroyed, but the temperature in the furnace during that time shall not be allowed to exceed 500°C.

(5) The resultant ash shall then be combined with the filtrate which had been protected with the watch glass in a beaker and the whole shall be heated to boiling. The solution shall then be cooled to 20°C, transferred to a 500 ml volumetric flask diluted to volume, mixed well and filtered through a dry 32 cm filter paper in the dry 20 cm filter funnel into the dry flat bottom flask, the first 10 to 20 ml of filtrate being discarded.

(6) The phosphate determination shall then proceed in the manner prescribed under this rule.

11. (1) The following apparatus shall be used for the determination of the percentage material passing through a Standard Test sieve—

(a) a laboratory spoon for drawing the sample;

(b) a weighing bottle or boat;

(c) a laboratory balance sensitive to 1 cg or less, and its weights;

(d) a laboratory oven set to operate at 100°C;

(e) a standard sieve, minimum specification B.S. 140, of the mesh prescribed for the determination, with a fitted lid and lower receiver;

(f) a time piece;

(g) a small weighed beaker;

(h) a smooth clean dry hardwood surface;

(i) a sharp edged ruler;

(j) a camel hair dabbing brush; and

(k) a camel hair flat brush.

(2) A large portion of the Official Sample shall be mixed thoroughly on the hardwood surface and a representative portion thereof obtained by applying the procedure prescribed under rule 15 of the Fertilizer and Animal Foodstuffs (Sampling) Rules, 1972 (L.N. 214/1972), for obtaining a sub-sample and weighting at least 25 gm, shall be dried at 100°C and cooled. About 20 gm, weighed to the nearest centigram, of the dried sample shall be transferred to the sieve with the lower receiver attached. The lid shall then be fitted and the sieve shall then be shaken for five minutes with frequent tapping of the sides. The powder which collects in the lower receiver during the shaking shall then be brushed with the flat brush into the small weighed beaker and weighed to the nearest centigram. The sieving unit shall then be reassembled and shaking and tapping shall be continued for two minutes. The powder which has collected in the lower receiver during this second shaking period shall then be added to the first portion and the weighing repeated. The shaking, tapping and weighing processes shall be continued until no more than four centigrams of powder passes through the sieve in a two-minute shaking period.

(3) The fibres of the dabbing brush shall be applied to any lumps remaining on the sieve after each shaking period so as to cause them to disintegrate but care shall be taken that the hard parts of the brush do not make contact with the lumps during the disintegration or that the brush is not used to brush particles through the sieve.

(4) The percentage material reported as passing through the sieve shall be taken as the total weight of powder in centigrams collected in the lower receiver, divided by the weight in grams and parts of a gram of dried sample taken for the determination.

12. (1) The following apparatus and reagents shall be used for the determination of free acid in Sulphate of ammonia—

(a) a laboratory spoon for drawing the sample;

(b) a weighing bottle or boat;

(c) a laboratory balance sensitive to 1 centigram or less, and its weights;

(d) burettes of capacity appropriate to the percentage of acid believed to be present in the sample, minimum specification B.S. 846, Class B;

(e) a flat bottom flask 400 ml;

(f) a 10 cm diameter glass funnel;

(g) 15 cm diameter general purpose filter papers;

(h) glass rods;

(i) 0.1N standard sodium hydroxide, standardized against 0.1N sulphuric or hydrochloric acid which had been standardized just previously against 0.1N sodium carbonate solution;

(j) methyl red solution prepared by adding 0.5 ml of 0.1N sodium hydroxide to 5 ml of 90 per cent industrial methylated spirits, dissolving in this solution 25 mgm of methyl red and diluting the resultant solution to 250 ml with 50 per cent industrial methylated spirits; and

(k) about 20 gm weighed to the nearest centigram, of the sample which has been prepared for analysis in the manner prescribed in rule 2(a) of these Rules, shall be dissolved in about 50 ml of water and filtered, the filtrate being collected in the flat bottom flask.

(2) Any insoluble matter retained in the filter shall be washed repeatedly and the combined filtrate and washing shall be made up to about 250 ml. This solution shall then be titrated from a burette of suitable capacity with the standard sodium hydroxide, using two or three drops of methyl red solution as indicator, the titration being made to the nearest 0.05 ml. The per cent free acid found in the fertilizer shall be taken as the number of millilitres and parts of a millilitre, of the standard sodium hydroxide solution used to neutralize the fertilizer solution, multiplied by 4.0 times the normality of the aforesaid standard sodium hydroxide solution and divided by the weight in grams and parts of a gram of sample taken for the analysis.

(3) Where an allowance for change in moisture content under these Rules must be made, the percentage free acid content reported shall be the percentage found divided by 100 minus the percentage moisture content of the sample determined as prescribed under these Rules and multiplied by 100 minus the percentage moisture content of the Official Sample. Otherwise the percentage found shall be the percentage reported.

13. (1) The following apparatus and reagents shall be used for the determination of biuret in a urea containing fertilizer—

(a) a laboratory spoon for drawing the sample;

(b) a weighing bottle or boat;

(c) a laboratory balance which is sensitive to 1 mgm weight or less;

(d) a laboratory balance with a capacity of up to 500 gm which is sensitive to 1 decigram weight or less;

(e) balance weights;

(f) a hot plate with energy regulator heat control;

(g) a time piece;

(h) a water bath regulated to maintain a water temperature between 32.9°C and 33.1°C;

(i) a spectrophotometer with a monochrometer capable of being set to give a source of light with wavelength of 5500A° or a colorimeter or absorptiometer capable of being fitted with a 5400 or 5500A° or a yellow/green light filter and with paired cells of the same optical length;

(j) a measuring cylinder 25 ml;

(k) volumetric flasks 100 and 1,000 ml, minimum specification B.S. 1972, Class B;

(l) burettes 50 ml minimum specification B.S. 846, Class B. Pipette 50 ml minimum specification B.S. 1583, Class B;

(m) a beaker 1 litre;

(n) 25 cm diameter general purpose filter papers;

(o) 40 cm diameter general purpose filter papers;

(p) a centigrade thermometer reading to 100°C in one degree units;

(q) glass rods;

(r) graph papers 1 mm rulings;

(s) approximately 0.1N sodium hydroxide or approximately 0.1N sulphuric acid as prescribed under this rule;

(t) sodium potassium tartarate solution prepared by dissolving 50.8 gm of sodium potassium tartarate tetrahydrate and 25.7 gm sodium hydroxide in water and diluting to 1 litre;

(u) a copper sulphate solution prepared by dissolving 15 gm crystalline copper sulphate in water and diluting to 1,000 ml; and

(v) pure biuret which has been dried and stored in a dessicator over anhydrous calcium chloride.

(2) A portion of the sample which has been prepared in the manner prescribed under these Rules and which is believed to contain between 0.4 and 1.2 gm of biuret shall be weighed to the nearest decigram.

(3) The portion taken shall be placed in the litre beaker, together with 700 ml of distilled water. The mixture shall be heated to 70° to 80°C with stirring and then allowed to cool to 30°C. A piece of litmus paper shall then be added and the solution shall be neutralized with the approximately 0.1N sodium hydroxide (if the paper turns red) or the approximately 0.1N sulphuric acid (if the paper turns blue) until the paper just starts to change colour. The solution shall then be filtered into the 1,000 ml volumetric flask. The residue shall be washed three times with water and the combined washings and filtrate diluted to volume.

(4) 50 ml of the samples solution so obtained shall be measured by pipette into a 100 ml volumetric flask.

(5) About 2 gm, weighed to the nearest milligram, of the pure biuret shall be treated in the manner prescribed in this rule.

(6) Into a series of six 100 ml volumetric flasks shall be measured by burette, 10.0, 14.0, 18.0, 22.0, 26.0 and 30.0 ml of the pure biuret solution so obtained. The total volume of each of the pure biuret solutions shall then be adjusted to 50 ml by suitable addition of water from a second burette.

(7) To each of these 50 ml samples of pure biuret solution and to the 50 ml aliquot of sample solution, 20 ml of the sodium potassium tartarate solution and 20 ml of the copper sulphate solution shall be added with constant swirling. The solutions shall then be diluted to volume and stood for 15 to 30 minutes in the water bath at between 32.9°C and 33.1°C while their colour develops. While the solutions are standing in the water bath the spectrophotometer shall be set to operate at 5500A° (or the colorimeter or absorptiometer shall be brought into operation as the case may be). Then, the standing period having been completed and following the procedures which are appropriate to the operation of the instrument used, the optical densities of its cells be compared, using the coloured standard solution containing 10.0 ml of the pure biuret solution. If the comparison reveals that there is a small difference between the two cells being used, the cell with the lower optical density shall be used as the standard reference cell. The apparent optical densities of the coloured standard solutions and of the coloured sample solution relative to the coloured standard containing 10.0 ml of pure biuret solution per 100 ml shall then be determined.

(8) The apparent optical densities of the coloured standard solution shall be plotted on a calibration graph against the number of millilitres of pure biuret solution per 100 ml which they contain. The number of millilitres of pure biuret solution per 100 ml in a coloured standard solution having the same optical density as the coloured sample solution shall then be determined by interpolation on the calibration graph. The determination shall be made to the nearest millilitre.

(9) A new pure biuret solution, a new set of coloured standards prepared from it and a new calibration graph shall be prepared for each biuret determination.

(10) If the number of millilitres of pure biuret solution per 100 ml of a coloured standard solution having the same optical density as the coloured sample solution is

expressed at x, and the number of grams and parts of a gram of pure biuret which was weighed out for preparing the pure biuret solution is expressed as y, the percentage biuret found in the fertilizer shall be taken as x times y multiplied by 2 and divided by the weight in grams and parts of a gram of sample taken for the analysis.

(11) Where an allowance for change in moisture content under these Rules must be made, the percentage biuret reported shall be the percentage found, divided by 100 minus the percentage moisture content of the sample determined as prescribed under these Rules and multiplied by 100 minus the percentage moisture content of the Official Sample. Otherwise the percentage found shall be the percentage reported.

14. (1) The following apparatus and reagents shall be used for the determination of salt in an animal foodstuff—

(a) a laboratory spoon for drawing the sample;

(b) a weighing bottle or boat;

(c) a laboratory balance which is sensitive to 1 mg or less, and weights;

(d) a laboratory oven set to operate at 100°C;

(e) a laboratory furnace;

(f) a silica dish about 50 mm diameter;

(g) a porcelain mortar 8.9 to 11.5 cm outside diameter and pestle;

(h) a volumetric flask 250 ml, minimum specification B.S. 1792, Class B;

(i) pipettes 5 and 100 ml minimum specification B.S. 1583, Class B;

(j) burettes 10 ml minimum specification B.S. 846, Class B;

(k) 10 cm diameter filter funnels;

(l) 15 cm diameter general purpose 15 cm diameter rapid double acid washed filter papers;

(m) glass rods;

(n) a conical flask 250 ml;

(o) a flat bottom flask 500 ml;

(p) a wash bottle with its neck and mouthpiece insulated for handling hot liquids;

(q) calcium oxide, finely ground, free from chloride. 0.1N silver nitrate standardized against 0.1N sodium chloride (which contains 5.846 grams pure sodium chloride per litre);

(r) 0.1N ammonium or potassium thiocyanate standardized against the 0.1N silver nitrate;

(s) dilute nitric acid, prepared by adding 1 part of concentrated nitric acid to 4 parts of water;

(t) clarified nitric acid solution prepared by diluting concentrated nitric acid with about $^{1}/_{4}$ of its volume of water and boiling until practically colourless; and

(u) ferric indicator solution, prepared by adding 5 ml of concentrated nitric acid to 100 ml of saturated aqueous ferric ammonium sulphate.

(2) About 5 gm weighed to the nearest milligram of the sample which has been prepared in the manner prescribed under these Rules shall be placed in the silica dish. 1 gm of the calcium oxide shall then be added and shall be mixed with the sample and the mixture shall be wetted with water to a thick paste. The mixture shall be dried in the oven, allowed to cool and then ground to a fine powder and shall then be heated gently and finally incinerated in the furnace until all the organic matter has been thoroughly charred, but the temperature in the furnace during that time shall not be allowed to exceed 500°C.

(3) The residue shall then be extracted with repeated portions of hot water and filtered through a general filter paper. The filtrate shall be cooled and diluted to volume in a 250 ml volumetric flask. 100 ml of this solution shall be measured by pipette into the conical flask and acidified with the dilute nitric acid. A known volume of the 0.1N silver nitrate solution shall then be measured into the solution in the conical flask from a burette, the silver nitrate solution being added until no further precipitate is formed and a slight excess of silver nitrate is present. The volume of 0.1N silver nitrate added shall be measured to the nearest 0.1 ml. The precipitate shall then be stirred well, filtered through a rapid filter paper into the flat bottom flask and washed thoroughly. 5 ml measured by pipette, of the ferric indicator solution and a few millilitres of the clarified nitric acid solution shall then be added to the combined filtrate and washings in the flat bottom flask. The excess of the silver nitrate remaining in the filtrate shall then be determined by titration with 0.1N ammonium or potassium thiocyanate from a burette until a permanent light brown colour appears. The titration shall be made to the nearest 0.1 ml.

(4) If the number of millilitres and parts of a millilitre of 0.1N silver nitrate added to the solution in the conical flask is expressed as x and if the number of millilitres and parts of a millilitre of 0.1N thiocyanate solution used in the titration to determine the excess of silver nitrate is expressed as y, the percentage salt found be taken as x-y multiplied by 1.461 and divided by the weight in grams and parts of a gram of sample taken for the analysis.

(5) Where an allowance for change in moisture content under these Rules must be made, the percentage salt reported shall be the percentage found, divided by 100 minus the percentage moisture content of the sample determined as prescribed under these Rules and multiplied by 100 minus the percentage moisture content of the Official Sample. Otherwise the percentage found shall be the percentage reported.

15. (1) The following apparatus and reagents shall be used for the determination of ash, sand, silicious material and other insoluble mineral matter in an animal foodstuff—

(a) a laboratory spoon for drawing the sample;

(b) a weighing bottle or boat;

(c) a laboratory balance which is sensitive to 1 mg or less, and weights;

(d) a hot plate with an energy regulator heat control;

(e) a laboratory furnace;

(f) silica capsules or dishes about 55 mm diameter;

(g) an 11 cm diameter funnel;

(h) 7 cm diameter general purpose ashless filter papers; wash bottles with their necks and mouth-pieces insulated for handling hot liquids;

(i) glass rods;

(j) concentrated hydrochloric acid; and

(k) dilute hydrochloric acid, prepared by diluting 240 ml of concentrated hydrochloric acid with water to 1 litre.

(2) Between 2 and 5 g weighed to the nearest milligram of the sample which has been prepared in the manner prescribed under these Rules shall be placed in a silica capsule or dish and shall then be heated gently and finally incinerated in the furnace until all the carbon has been destroyed but the temperature in the furnace during that time shall not be allowed to exceed 500°C. The ash shall then be cooled and moistened with the concentrated hydrochloric acid. The moist ash shall then be evaporated to dryness and baked on the hot plate. The dried ash shall then be extracted repeatedly with the hot dilute hydrochloric acid. The extract each time shall be decanted through a filter paper in the filter funnel. After the extraction has been completed, the residue shall be transferred to the aforesaid filter paper in the filter funnel and shall be washed thoroughly with hot water. The filter paper and the residue it contains shall then be placed in a silica capsule or dish

whose weight is known to the nearest milligram. These shall be dried in the oven, and shall then be heated gently and finally incinerated in the furnace until all the carbon is destroyed, but the temperature in the furnace during that time shall not be allowed to exceed 500°C. The silica capsule and the ash it contains shall be allowed to cool and shall be reweighed, the weight being determined to the nearest milligram. The percentage of sand, silicious matter and other insoluble mineral material found shall be taken as the weight in milligrams of ash so found, multiplied by 100 and divided by the weight in milligrams of samples taken for analysis.

(3) Where an allowance for change in moisture content under these Rules must be made, the percentage of sand, silicious material and other insoluble matter reported shall be the percentage found, divided by 100 minus the percentage moisture content of the sample determined as prescribed under these Rules and multiplied by 100 minus the percentage moisture content of the Official Sample. Otherwise the percentage found shall be the percentage reported.

15A. (1) The following reagents shall be used for the determination of sugar in an animal foodstuff—

(a) Potassium oxalate solution—dissolve 50 g of potassium oxalate in water and dilute to 1 litre.

(b) Zinc acetate solution—dissolve 219 g of crystallized zinc acetate and 30 ml of glacial acetic acid in water and dilute to 1 litre.

(c) Potassium ferrocyanide solution—dissolve 106 g of crystallized potassium ferrocyanide in water and dilute to 1 litre.

(d) N. hydrochloric acid.

(e) Phenolphthalein indicator solution—dissolve 250 mg of phenolphthalein in 150 ml of industrial methylated spirit and dilute with water to 250 ml.

(f) 10 per cent sodium hydroxide solution—dissolve 100 g of sodium hydroxide in water and dilute to 1 litre.

(g) Fehling's solution—mix equal volumes of a solution of copper sulphate and a solution of sodium potassium tartrate prepared as follows—

Copper sulphate solution—dissolve 69.28 g of copper sulphate ($CuSO_4 5H_2O$) in water and dilute to 1 litre.

The strength of the Fehling's solution should be such that 10 ml is equivalent to 0.0525 g of invert sugar. It should be checked by titrating with a solution of pure sucrose inverted by, and using, the procedure described in subparagraph (f) of subrule (2) of this rule.

(h) Sodium potassium tartrate solution—dissolve 346 g of sodium potassium tartrate and 100 g of sodium hydroxide in water and dilute to 1 litre.

(i) Methylene blue solution—dissolve 2.5 g of methylene blue in water and dilute to 250 ml.

(2) The following procedure and apparatus shall be used for the determination of sugar in an animal foodstuff—

(a) When the substance is in solid form weigh to the nearest centigram about 20 g of the sample or a sufficient quantity to contain about 2 g of sugar. Grind in a mortar with hot water (temperature not to exceed 60°C.) and transfer with the aid of water to a 250 ml beaker using in all about 120 ml of water. Stir well and decant through muslin into a 250 ml volumetric flask, allowing to drain until the liquid is substantially removed, and then squeeze the residue on the muslin. Return the residue to the beaker, add about 50 ml of water, mix and decant through the muslin into the volumetric flask, again squeezing the residue after draining. Repeat this treatment with a

further 50 ml of water, and finally squeeze the residue on the muslin. Add 5 ml of potassium oxalate solution to the contents of the volumetric flask followed by 5 ml of zinc acetate solution; mix well and then add 5 ml of potassium ferrocyanide solution, dilute to 250 ml, mix well and filter. Determine the sugar in 50 ml of the filtrate by the procedure described in subparagraph (c) of this paragraph.

(b) When the substance is in liquid form weigh to the nearest mg about 5 g of the sample and wash with water into a 250 ml volumetric flask using about 200 ml of water. To clear the solution add 5 ml of zinc acetate solution. Mix, dilute to 250 ml of potassium ferrocyanide solution, again mix, dilute to 250 ml, mix and filter. Determine the sugar in 25 ml of the filtrate by the procedure described in subparagraph (c) of this paragraph.

(c) In order to determine the sugar content transfer the measured volume of filtrate obtained as described in subparagraph (a) or (b) of this paragraph to a 300 ml beaker, add 15 ml of N. hydrochloric acid, dilute to 150 ml with water, cover with a glass and heat to boiling point. Continue to boil for 2 minutes, cool, add 2 or 3 drops of phenolphthalein indicator solution, just neutralize with 10 per cent sodium hydroxide solution, transfer to a 200 ml volumetric flask and dilute to 200 ml. Filter if necessary.

(d) A preliminary estimation is usually necessary where the percentage of sugar is unknown, in which case transfer exactly 10 ml of Fehling's solution to a 250 ml conical flask and add 20 ml of water. Add from a burette approximately 10 ml of the filtrate obtained as described in subparagraph (c) of this paragraph, heat to boiling point, and boil briskly for 1 minute. Add 3 drops of methylene blue solution and titrate from the burette at the rate of 1 ml per 15 seconds until the blue colour is discharged, the contents of the flask being kept boiling throughout the titration. Note the total number of ml required and call this X ml. This titration should not be outside the range of 15-40 ml otherwise the determination should be repeated using a more appropriate volume of the filtrate.

(e) To achieve an exact determination proceed as follows: To 10 ml of Fehling's solution in a 250 ml conical flask add, from a burette, (X-1) ml of the filtrate obtained as described in subparagraph (c) of this paragraph together with sufficient water to make a total volume of 60 ml. Heat to boiling point, boil briskly for 1½ minutes and add 3 drops of methylene blue solution. Titrate from the burette at the rate of approximately 0.25 ml per 15 seconds until the blue colour is discharged, the contents of the flask being kept boiling briskly throughout the titration which must not take more than 1½ minutes. Then the total number of mil. used in the determination equals the sugar equivalent of 10 ml of Fehling's solution. 10 ml Fehling's solution = 0.0525 g invert sugar. Not more than 1 ml of the filtrate should be required for the completion of the titration. If more than 1 ml is required, then the determination should be repeated using a more closely calculated volume of filtrate for the original addition. The time taken from the initial boiling point until the end of the titration should be about 3 minutes. If this time is exceeded by more than 20 seconds, the titration should be repeated.

(f) The Fehling's solution shall be standardized as follows: Dissolve 2.375 g sucrose (dried at 100°C) in about 100 ml of water in a 300 ml beaker, add 15 ml of N. hydrochloric acid and sufficient water to give a volume of 150 ml. Heat to boiling point, boil for 2 minutes, cool, add 2 or 3 drops of phenolphthalein solution, just neutralize with 10 per cent sodium hydroxide solution, transfer to a 500 ml volumetric flask and dilute to 500 ml. Then follow the procedure described in subparagraph (e) of this paragraph. 1 ml of this solution = 0.00475 g sucrose = 0.005 g invert sugar, i.e. 10 ml of

Fehling's solution = 10.5 ml of this standard invert sugar solution. The total copper reducing power should finally be determined in terms of sugar ($C_{12}H_{22}O_{11}$).

[L.N. 292/194.]

16. The Certificate of Analysis issued under these Rules shall be in the form set out in the Schedule to these Rules.

17. The Analyst shall report in this Certificate the percentage, to the first decimal place, of each of the constituents of an Official Sample which an inspector, in pursuance of rule 21 of the Fertilizers and Animal Foodstuffs (Sampling) Rules, 1972, shall have stated (in the Certificate he affixed to the container or package containing that sample) in addition to the particulars printed or marked in accordance with the requirements of rule 3 of the Fertilizers and Animal Foodstuffs (Packing of Approved Fertilizers) Rules, 1972 (L.N. 210/1972), or rule 4 of the Fertilizers and Animal Foodstuffs (Packing of Approved Animal Foodstuffs) Rules, 1972 (L.N. 212/1972), on the containers or packages from which the Official Sample was drawn or on the labels attached to those containers. Where the Official Sample is stated to be drawn from packages or containers marked or labelled as being Sulphate of ammonia, he shall also report the percentage of free acid. Where the animal foodstuff from which the Official Sample was drawn is sold for poultry mash he shall also report the percentage of salt. Where he finds the Official Sample to contain any deleterious substance whatsoever, he shall report on its presence. Where the Official Sample is an animal foodstuff and where he suspects deleterious proportions of sand, silicious matter or other insoluble matter to be present, he shall also report the total percentage of these materials.

18. The Analyst shall send copies of the Certificate of Analysis of each Official Sample which he analyses to the Inspector who drew the sample, to the person in possession of the fertilizer or animal foodstuff at the time the sample was taken, and also to the person, if any, under whose instructions the sample was collected and to the person who last sold the fertilizer or animal foodstuff.

19. The aforesaid analysis may be undertaken under the supervision of an Analyst by any person or persons whom the said Analyst shall instruct to undertake the analysis but the Certificate of Analysis shall be signed and certified only by an Analyst appointed by the Minister under section 8 of the Act.

SCHEDULE

[r. 15.]

CERTIFICATE OF ANALYSIS

[1] ..... duly appointed by *Gazette* Notice No.[2] ..... to be an analyst under the Fertilizers and Animal Foodstuffs Act, hereby certify that a sample in a sealed container to which was attached a certificate on which was included the following information concerning the sample[3]—

The name and full postal and business addresses of the manufacturer where known and of the seller or the person who was in possession of the fertilizer and animal foodstuff at the time the sample was taken:

.....

.....

1. Here insert the name of the analyst signing the Certificate and the capacity in which he acts in undertaking the analysis.
2. Here insert the particulars of the *Gazette* Notice under which the analyst signing the Certificate was appointed an Analyst under the Fertilizers and Animal Foodstuffs Act.
3. Here insert full particulars taken from the Certificate affixed to the container containing the Official Sample whose analysis is here reported for the Fertilizer or Animal Foodstuff whence the Official Sample was drawn to be recognized.

SCHEDULE—*continued*

The name of the fertilizer or animal foodstuff:

.....

The analysis guaranteed by the manufacturer or seller:

.....

The name and full postal address of the Inspector who took the sample:

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The name and full postal and business address of the person, if any, under whose instructions the sample was taken:

.....

.....

.....

The date and place at which the sample was taken:

.....

Other identifying marks or particulars:

.....

.....

.....

has been analysed by me or under my direction and I declare the result of the analysis to be as follows—

Moisture by [4, 5]		..... %
Nitrogen	Total	..... %
Phosphoric acid as—		
P_2O_5—water soluble		..... %
soluble in 2 per cent citric acid		..... %
soluble in mineral acid or total		..... %
Material passing through Standard Test sieve B.S. 410, having apertures (Minimum Specification)[6] ..... mm square—		
Oil		..... %
Protein		..... %
Fibre		..... %
Biuret		..... %
Sodium Chloride		..... %
Sand, silicious and other insoluble mineral matter		..... %
Other analysis and remarks (if any)— [7]		..... %

4. Here report the moisture content and those particulars in respect of which a specification is laid down or guarantee required and given under the Fertilizers and Animal Foodstuffs Act, in respect of the fertilizer or animal foodstuff whose analysis is here reported.

   The result of the analysis shall be reported to the first decimal point.

5. Here state the drying procedure followed in the determination.

6. Here state the aperture size of the Standard Test Sieve that was used and that was required to be used in determining the percentage of material passing through the Standard Test Sieve.

7. Here report the presence of deleterious substance.

SCHEDULE—*continued*

The analyses were made in accordance with the methods prescribed by the Fertilizers and Animal Foodstuffs (Analysis) Rules, 1972.

Witnessed under my hand this ..... day of ....., 20 .....

.....

Signature

Address of Analyst .....