Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed (Text with EEA relevance)

ANNEX III

METHODS OF ANALYSIS TO CONTROL THE COMPOSITION OF FEED MATERIALS AND COMPOUND FEED

E.DETERMINATION OF VOLATILE NITROGENOUS BASES

I. BY MICRODIFFUSION

1. Purpose and scope

This method makes it possible to determine the content of volatile nitrogenous bases, expressed as ammonia, in feed.

2. Principle

The sample is extracted with water and the solution clarified and filtered. The volatile nitrogenous bases are displaced by microdiffusion using a solution of potassium carbonate, collected in a solution of boric acid and titrated with sulphuric acid.

3. Reagents

3.1. Trichloroacetic acid, solution 20 % (w/v).

3.2. Indicator: dissolve 33 mg of bromocresol green and 65 mg of methyl red in 100 ml of 95 %-96 % (v/v) of ethanol.

3.3. Boric acid solution: in a 1 litre graduated flask dissolve 10 g of boric acid in 200 ml of 95 %-96 % (v/v) ethanol and 700 ml of water. Add 10 ml of indicator (3.2). Mix and, if necessary, adjust the colour of the solution to light red by adding a solution of sodium hydroxide. 1 ml of this solution will fix a maximum of 300 μg of NH_3.

3.4. Saturated potassium carbonate solution: dissolve 100 g of potassium carbonate in 100 ml of boiling water. Leave to cool, filter.

3.5. Sulphuric acid 0,01 mol/litre.

4. Apparatus

4.1. Mixer (tumbler): approximately 35 to 40 r.p.m.

4.2. Glass or plastic Conway cells (see diagram).

4.3. Microburettes graduated in 1/100 ml.

5. Procedure

Weigh 10 g of sample to the nearest 1 mg and place with 100 ml of water in a 200 ml graduated flask. Mix or stir in the tumbler for 30 minutes. Add 50 ml of trichloroacetic acid solution (3.1), make up to volume with water, shake vigorously and filter through a pleated filter.

Using a pipette, introduce 1 ml of boric acid solution (3.3) into the central part of the Conway cell and 1 ml of the sample filtrate into the crown of the cell. Cover partially with the greased lid. Drop 1 ml of saturated potassium carbonate solution (3.4) quickly into the crown and close the lid so that the cell is airtight. Turn the cell carefully rotating it in a horizontal plane so that the two reagents are mixed. Leave to incubate either for at least four hours at room temperature or for one hour at 40 ^{o}C.

Using a microburette (4.3), titrate the volatile bases in the boric acid solution with sulphuric acid (3.5).

Carry out a blank test using the same procedure but without a sample to be analysed.

6. Calculation of results

1 ml of H_2SO_4 0,01 mol/litre corresponds to 0,34 mg of ammonia.

Express the result as a percentage of the sample.
Repeatability

The difference between the results of two parallel determinations carried out on the same sample shall not exceed:

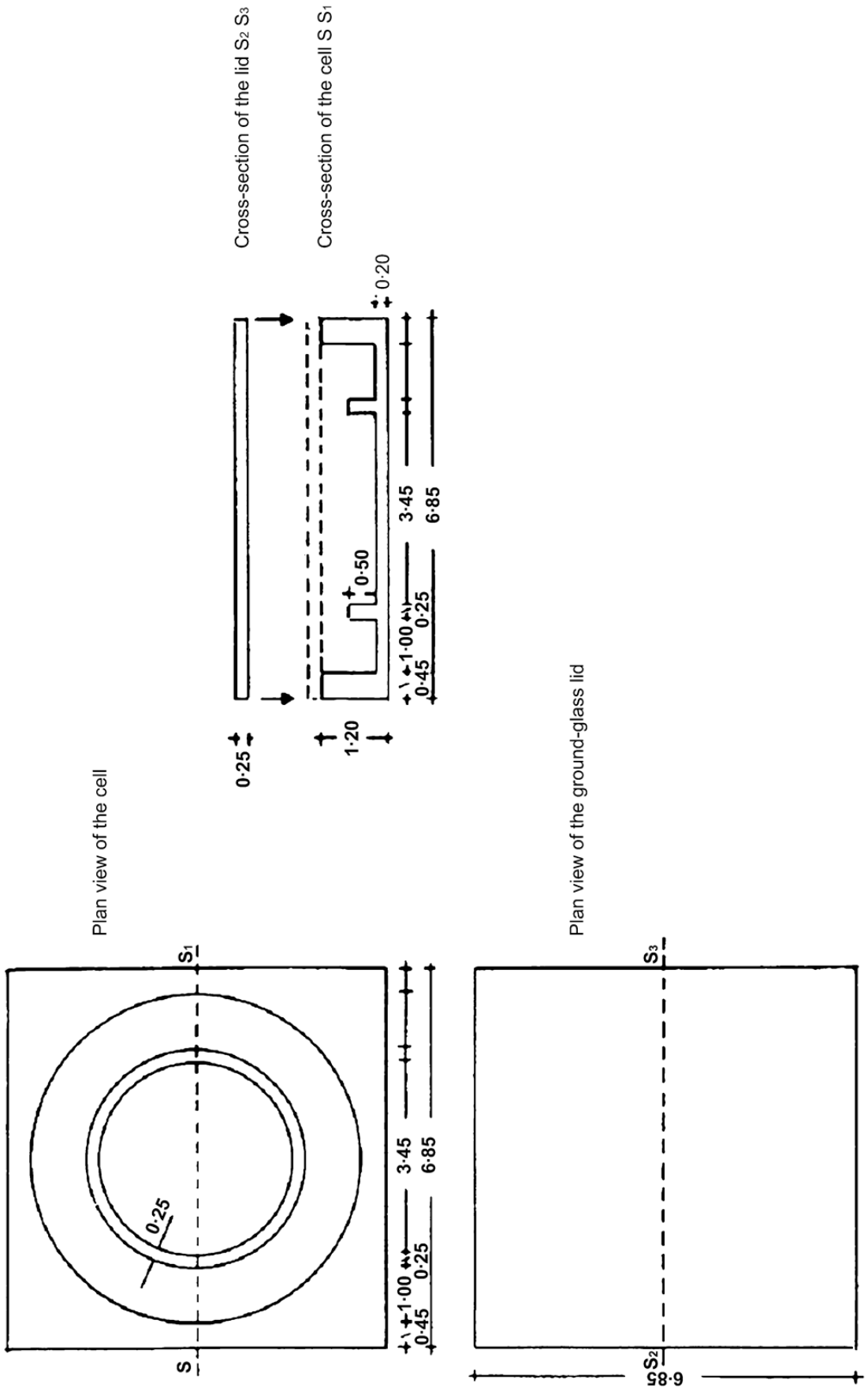
— 10 %, in relative value, for ammonia contents of less than 1,0 %,

— 0,1 %, in absolute value, for ammonia contents of 1,0 % or more.

7. Observation

If the ammonia content of the sample exceeds 0,6 %, dilute the initial filtrate.

CONWAY Scale 1/1
CELL



II. BY DISTILLATION

1. Purpose and Scope

This method makes it possible to determine the content of volatile nitrogenous bases, expressed as ammonia, in fish-meal containing practically no urea. It is applicable only to ammonia contents of less than 0,25 %.

2. Principle

The sample is extracted with water and the solution clarified and filtered. The volatile nitrogenous bases are displaced at boiling point by adding magnesium oxide and collected in a specific quantity of sulphuric acid, the excess of which is back-titrated with a solution of sodium hydroxide.

3. Reagents

3.1. Trichloroacetic acid, solution 20 % (w/v).

3.2. Magnesium oxide.

3.3. Anti-foaming emulsion (e.g. silicone).

3.4. Sulphuric acid 0,05 mol/litre.

3.5. Sodium hydroxide solution 0,1 mol/litre.

3.6. Methyl red solution 0,3 % in 95 %-96 % (v/v) ethanol.

4. Apparatus

4.1. Mixer (tumbler): approximately 35 to 40 r.p.m.

4.2. Distilling apparatus of the Kjeldahl type.

5. Procedure

Weigh 10 g of the sample to the nearest 1 mg and place with 100 ml of water in a 200 ml graduated flask. Mix or stir in the tumbler for 30 minutes. Add 50 ml of trichloroacetic acid solution (3.1), make up to volume with water, shake vigorously and filter through a pleated filter.

Take a quantity of clear filtrate appropriate for the presumed content of volatile nitrogenous bases (100 ml is usually suitable). Dilute to 200 ml and add 2 g of magnesium oxide (3.2) and a few drops of anti-foaming emulsion (3.3). The solution must be alkaline to litmus paper; otherwise add some magnesium oxide (3.2). Proceed according to 5.2 and 5.3 of the method of analysis for the determination of the crude protein content (Part C of this Annex).

Carry out a *blank test* using the same procedure but without a sample to be analysed.

6. Calculation of results

1 ml of $H_2SO_4$0,05 mol/litre corresponds to 1,7 mg of ammonia.

Express the result as a percentage of the sample.
Repeatability

The difference between the results of two parallel determinations carried out on the same sample shall not exceed, in relative value, 10 % of ammonia.

Changes to legislation:
There are currently no known outstanding effects for the Commission Regulation (EC) No 152/2009, Division E..