ANNEX III

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

D.DETERMINATION OF UREA

1. Purpose and scope

This method makes it possible to determine the level of urea in feed.

2. Principle

The sample is suspended in water with a clarifying agent. The suspension is filtered. The urea content of the filtrate is determined after the addition of 4-dimethylaminobenzaldehyde (4-DMAB) by measuring the optical density at a wavelength of 420 nm.

3. Reagents

3.1. Solution of 4-dimethylaminobenzaldehyde: dissolve 1,6 g of 4-DMAB in 100 ml of 96 % ethanol and add 10 ml of hydrochloric acid (ρ_{20}1,19 g/ml). This reagent keeps for a maximum period of two weeks.

3.2. Carrez solution I: dissolve in water 21,9 g of zinc acetate, $Zn(CH_3COO)_2$ $2H_2O$ and 3 g of glacial acetic acid. Make up to 100 ml with water.

3.3. Carrez solution II: dissolve in water 10,6 g of potassium ferrocyanide, K_4 Fe $(CN)_6$ $3H_2O$. Make up to 100 ml with water.

3.4. Active carbon which does not absorb urea (to be checked).

3.5. Urea, 0,1 % solution (w/v).

4. Apparatus

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

4.2. Test tubes: 160 × 16 mm with ground-glass stoppers.

4.3. Spectrophotometer.

5. Procedure

5.1. Analysis of sample

Weigh out 2 g of the sample to the nearest mg and place with 1 g of active carbon (3.4) in a 500 ml volumetric flask. Add 400 ml of water and 5 ml of Carrez solution I (3.2), mix for approximately 30 seconds and add 5 ml of Carrez solution II (3.3). Mix for 30 minutes in the tumbler. Make up to volume with water, shake and filter.

Remove 5 ml of the transparent colourless filtrates, place in test tubes with ground-glass stoppers, add 5 ml of 4-DMAB solution (3.1) and mix. Place the tubes in a water bath at 20 ^{o}C (+/- 4 ^{o}C). After 15 minutes measure the optical density of the sample solution with the spectrophotometer at 420 nm. Compare with the blank test solution of the reagents.

5.2. Calibration curve

Remove volumes of 1, 2, 4, 5 and 10 ml of the urea solution (3.5), place in 100 ml volumetric flasks and make up the volume with water. Remove 5 ml from each solution, add 5 ml of 4-DMAB solution (3.1) to each of them, homogenise and measure the optical density as shown

above in comparison with a control solution containing 5 ml of 4-DMAB and 5 ml of water free from urea. Plot the calibration curve.

6. Calculation of results

Determine the amount of urea in the sample using the calibration curve.

Express the result as a percentage of the sample.

7. Observations

7.1. In the case of contents of urea exceeding 3 %, reduce the sample to 1 g or dilute the original solution so that there are not more than 50 mg of urea in 500 ml.

7.2. In the case of low contents of urea, increase the sample as long as the filtrate remains transparent and colourless.

7.3. If the sample contains simple nitrogenous compounds such as amino acids, the optical density shall be measured at 435 nm.

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