SCHEDULE 2

Methods of Analysis

ANNEX III

Method for Determining Isobutylidenediurea

Scope and Field of Application

1. This method is for the determination of isobutylidenediurea in feeding stuffs.

Principle

2. The sample is hydrolysed, liberating isobutyraldehyde, the concentration of which is determined by gas chromatography.

Reagents

3.—(3.1) Toluene.

(3.2) Sodium sulphate, anhydrous.

(3.3) Buffer solution pH1: dissolve 27.2 g sodium acetate trihydrate in 300 ml 1M hydrochloric acid and add 700 ml water.

(3.4) Buffer solution pH 0.65: dissolve 27.2 g sodium acetate trihydrate in 400 ml 1M hydrochloric acid and add 600 ml water.

(3.5) Isobutylidenediurea.

(3.6) Internal standard solution: dilute 5 ml isopropyl acetate to 100 ml with toluene (3.1).

Apparatus

4.—(4.1) 250 ml conical flasks with ground glass or PTFE stoppers.

(4.2) Stoppered centrifuge tubes.

(4.3) Gas chromatograph with flame ionisation detector.

(4.4) Column:

either (i) 1.5 m glass column (4 mm internal diameter) packed with 5% OV17 on Gas Chrom Q, 80-100 mesh,

or (ii) 1.5 m glass column (4 mm internal diameter) packed with 5% Carbowax 20M-TPA on Diatomite C-AAW, 80-100 mesh.

(4.5) Water bath: hotplate stirrer on which is placed a 2,000 ml beaker (or suitable vessel) containing water maintained at 40-50°C.

Procedure

Hydrolysis

Hydrolysis

5.—(5.1) Weigh to the nearest 0.001 g, between 3 and 7 g of the prepared sample containing about 0.2 g of isobutylidenediurea into a conical flask (4.1). Add 100 ml buffer solution (3.4) and

20.0 ml toluene (3.1) to the sample and place in the flask a magnetic bar. Stopper firmly to ensure that the flask remains tightly closed during the hydrolysis.

Place the flask in the water bath (4.5) and stir vigorously for 20 minutes. Remove the flask and immerse in an ice-water bath for 5 minutes. Add 15 g sodium sulphate (3.2) and 5.0 ml internal standard solution (3.6) to the contents of the flask. Stopper the flask again, shake, return to the water bath (4.5) and warm for 3 minutes with stirring. Cool in the ice-water bath for 5 minutes. Transfer slowly between 15 and 25 ml of the mixture to the centrifuge tube (4.2), stopper, and centrifuge for 5 minutes to separate the layers. (Repeat the transfer if insufficient toluene is decanted). Transfer a portion of the upper (toluene) layer to a test tube with a pasteur pipette.

Determination

(5.2) Inject between 0.5 and 1.0 ml of the toluene solution (5.1) into the gas chromatograph (4.3).

Suggested conditions:

Column	70°C	Nitrogen	40 ml per minute
Injection	150°C	Hydrogen	30 ml per minute
Detector	150°C	Air	370 ml per minute

Approximate retention times:

Isobutyraldehyde	1 min.
Internal standard	1.5 min.
Toluene	3 min.

Measure the peak heights of the isobutyraldehyde and internal standard. Calculate the peak height ratio, isobutyraldehyde/internal standard, and from this value determine the quantity of isobutylidenediurea present by reference to the calibration curve (5.3).

Calibration curve

(5.3) Weigh to the nearest mg, 100, 200 and 300 mg isobutylidenediurea (3.5) into three conical flasks (4.1). Add 100 ml buffer solution (3.3), 20.0 ml toluene (3.1) and a magnetic bar to each. Stopper the flasks firmly. Continue as in 5.1 from "…. Place the flask in the water bath ….". Inject the toluene solutions into the gas chromatograph (4.3), and measure the peak heights. Calculate the peak height ratios, isobutyraldehyde/internal standard, and plot the calibration curve using peak height ratios as the ordinates and the corresponding weights of isobutylidenediurea as the abscissae.

Expression of the Results

6. The per cent content of isobutylidenediurea in the sample is given by the formula:

$AW \times 10$

where:

A = weight of isobutyldenediurea (mg) read from the calibration curve; and

W = weight of sample in grams.