

2.4.20

AOAC Official Method 959.03

Urea in Fertilizers

Urease Method

First Action 1959

Final Action 1960

A. Reagent

Neutral urease solution.—Use fresh commercial 1% urease solution, or dissolve 1 g urease powder in 100 mL H₂O, or shake 1 g jack bean meal with 100 mL H₂O 5 min. Transfer 10 mL solution to 250 mL Erlenmeyer, dilute with 50 mL H₂O, and add 4 drops methyl purple (available from Fisher Scientific Co.; No. So-I-9). Titrate with 0.1M HCl to reddish purple; then back-titrate to green with 0.1M NaOH. From difference in mL, calculate volume 0.1M HCl required to neutralize remainder of solution (usually ca 2.5 mL/100 mL), add this amount of acid, and shake well.

Verify enzyme activity of urease source periodically. Discard any source which does not produce solution capable of hydrolyzing 0.1 g urea/20 mL solution.

B. Determination

Weigh 10 ± 0.01 g test sample (≤ 1.0 g of urea) and transfer to 15 cm Whatman No. 12 fluted filter paper. Leach with ca 300 mL H₂O into 500 mL volumetric flask. Add 75–100 mL saturated Ba(OH)₂ solution to precipitate phosphates. Let settle and test for complete precipitation with few drops saturated Ba(OH)₂ solution. Add 20 mL 10% Na₂CO₃ w/v solution to precipitate excess Ba and any soluble Ca salts. Let settle and test for complete precipitation. Dilute to volume, mix, and filter through 15 cm Whatman No. 12 fluted paper. Transfer 50 mL aliquot (equivalent to 1 g sample) to 200 or 250 mL Erlenmeyer and add 1–2 drops of methyl purple. Acidify with 2M HCl and add 2–3 drops excess. Neutralize solution with 0.1M NaOH to first change in color of indicator. Add 20 mL neutral urease solution, close flask with rubber stopper, and let stand 1 h at 20°–25°C. Cool flask in ice-H₂O slurry and titrate at once with 0.1M HCl to full purple; then add ca 5 mL excess. Record total volume added. Back-titrate excess HCl with 0.1M NaOH to neutral end point.

$$\text{Percent urea} = \frac{(\text{mL } 0.1\text{M HCl} - \text{mL } 0.1\text{M NaOH}) \times 0.3003}{\text{g test portion}}$$

References: *Ind. Eng. Chem. Anal. Ed.* **7**, 259(1935).

JAOAC **41**, 637(1958); **42**, 494(1959);

43, 123(1960).

CAS-57-13-6 (urea)