

## ACETYL

### PRINCIPLE

The determination of acetyl is based on the reaction between acetyl groups and hydroxylamine to produce acetohydroxamic acid and the formation of a soluble red complex with ferric ions. The amount of the ferric acetohydroxamic complex is determined spectrophotometrically at 510 nm (Note 1).

### SCOPE

The method is applicable to acetylated carbohydrate polymers.

### SPECIAL APPARATUS

1. Spectrophotometer: An instrument capable of accurate absorbance measurements at 510 nm and equipped with a 1 cm cuvette, or a matched pair if needed.
2. Magnetic stirrer

### REAGENTS

1. Water, Purified: Use deionized or distilled water throughout.
2. Sodium Hydroxide Solution: Dissolve 94 g of reagent grade sodium hydroxide in water, dilute to 1 L and mix thoroughly.
3. Hydroxylamine Hydrochloride Solution: Dissolve 37.5 g of hydroxylamine hydrochloride in water solution and dilute to 1 L.
4. Ferric Perchlorate Solution: Dissolve 2.53 g of ferric perchlorate, non-yellow in water and dilute to 100 mL (Note 2). When stored in a refrigerator, this solution is stable for at least one month. Transfer 60 mL of this ferric perchlorate stock solution to a 500 mL volumetric flask, chill in an ice bath, and add 8.3 mL of 70% perchloric acid. Cool in an ice bath. Make to volume with chilled reagent grade absolute methanol. Allow this

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solution to stabilize under refrigeration for three days before using. The solution is then stable for at least one week if kept refrigerated.

5. **Glucose Pentaacetate Standard:** Dissolve 108.9 mg of  $\alpha$ -D-glucose pentaacetate in 5 mL of ethyl alcohol with gentle heating, and dilute to 100 mL with water. Take 2, 4, 5 and 7 mL aliquots of this solution and dilute to 50 mL with water. Five mL aliquots of these diluted aqueous solutions represent 120, 240, 300 and 420  $\mu$ g of acetyl.
6. **Acid-Methanol Solution:** Chill 35.2 mL of 70% perchloric acid and dilute to 500 mL with chilled reagent grade absolute methanol.

**PROCEDURE**

Calibration Curve: Pipet accurately 2 mL of a freshly prepared 1:1 mixture of sodium hydroxide and hydroxylamine hydrochloride into each of four 25 mL volumetric flasks. To these add, while stirring, 5.0 mL of the diluted standard glucose pentaacetate solutions containing 120, 240, 300 and 420  $\mu$ g of acetyl, respectively. Allow the solutions to stand 30 mins., add 5.0 mL of acid-methanol solution and mix thoroughly. Dilute to volume with the ferric perchlorate solution, adding it in small increments with thorough mixing after each addition (Note 2). Allow the color to develop for five minutes, and determine the absorbance at 510 nm vs. a reference solution prepared by using 5.0 mL water for the sample solution. Plot  $\mu$ g of acetyl against absorbance readings.

Sample Analysis: Weigh accurately a sample estimated to contain 2-10 mg acetyl into a 150 mL beaker. Maximum sample size is 1 g. Add a stirring bar and while stirring on a magnetic stirrer, add by pipet 25 mL of the hydroxylamine solution. Over a period of 10-15 mins. add from a buret, 25 mL of sodium hydroxide solution. Cover with a watch glass and continue stirring until the sample is dissolved. Usually, this is accomplished in 10 mins. Pipet accurately 2 mL of the solution into a 25 mL volumetric flask, add 5.0 mL water, 5.0 mL acid-methanol and mix. Dilute to volume with the ferric perchlorate solution, adding it in small increments and with mixing after each addition. After 5 mins., filter the solution through Whatman No. 2 filter paper. After 15 mins., determine the absorbance vs. a reference solution prepared as previously described under Calibration Curve.

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$$\text{Acetyl, \% (as is)} = \frac{\text{Acetyl, } \mu\text{g (From Graph)} \times 25 \times 100}{\text{Sample Wt. } (\mu\text{g})}$$

If results are to be reported on dry basis, correct results for moisture.

**NOTES AND PRECAUTIONS**

1. The procedure is adapted from that described by E. A. McComb and R. M. McReady, "Determination of Acetyl in Pectin and in Acetylated Carbohydrate Polymers," *Anal. Chem.*, **1957**, 5, 819. Extreme care and caution must be exercised in the handling of perchlorate solutions.
2. The order of addition of reagents is important, see reference in Note 1.