

**Volatile Oil in Mustard Seed and Flour**

*Purpose: To determine the volatile oil (steam-volatile isothiocyanates) present in mustard seed or flour. (Titration Method)*

**A. Apparatus:**

1. Erlenmeyer flask, 250 mL with T.S. 24/40 ground joint.
2. West type condenser, 400 mm. in length, with T.S. 24/40 ground joints.
3. Glass tube with two right angle bends, with T.S. 24/40 ground joint on the long arm and T.S. 24/40 ground joint on the short arm.
4. Delivery tube, with T.S. 24/40 ground joint.
5. Volumetric flask, 100 mL with ground glass stopper.
6. Water bath, maintained at 37°C.

**B. Reagents:**

1. Anti-foam agent.
2. Authentic yellow mustard flour (*Brassica hirta*).
3. Ammonium hydroxide (NH<sub>4</sub>OH) solution (dilute 1 vol. of concentrated ammonium hydroxide, ACS grade, with two volumes of distilled water).
4. Ethyl alcohol, denatured, 95%.
5. Silver nitrate solution 0.1 N, standardized against ACS grade potassium chloride (KCl) with Mohr titration method.
6. Nitric acid, concentrated, ACS grade.
7. Ferric ammonium sulfate solution, 10-12% by weight, of FeNH<sub>4</sub>(SO<sub>4</sub>)<sub>2</sub> • 12H<sub>2</sub>O ACS grade.
8. Ammonium thiocyanate solution, 0.1 N standardized by titrating with standard Silver Nitrate (AgNO<sub>3</sub>) solution using reagent ferric ammonium sulfate.

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**C. Preparation of Sample:**

1. Mustard flour should be used as is.
2. Prepare mustard seed as directed under Method 1.0.

**D. Procedure:**

1. Weigh 5.0 g of sample into the 250 mL Erlenmeyer flask. If mustard flour is to be analyzed, add 100 mL of water at room temperature. If mustard seed is to be analyzed, add 100 mL of boiling water and four or five drops of anti-foam emulsion, swirl the mixture, boil it for 2 min., and cool it to room temperature.
2. Add about 1.0 g of authentic yellow mustard flour to the mixture, stopper the flask tightly, mix the contents thoroughly and place the flask in a 37°C. water bath for 2 hours (Note 1&2). Mix the contents by swirling the flask every 15 min. (The yellow mustard flour should be omitted in the analysis of mustard flour).
3. Add 20 mL of 95% ethyl alcohol and assemble the apparatus for distillation.
4. Distill ca. 60 mL into the 100 mL. volumetric flask containing 10 mL. of the NH<sub>4</sub>OH solution. (Note 3)
5. After distillation, rinse off the delivery tube with distilled water and add 20 mL of the 0.1 N. AgNO<sub>3</sub> solution. Mix well and allow to stand 4 hours.
6. Dilute to 100 mL with distilled water and filter using a dry filter paper.
7. Acidify a 50 mL. aliquot of the filtrate by adding ca. 5 mL. of conc. HNO<sub>3</sub>, add 5 mL ferric ammonium sulfate solution as an indicator and titrate with the standard ammonium thiocyanate solution.

**E. Calculation:**

$$\text{Volatile oil \%} = \frac{((\text{mL} \times \text{N.}) \text{AgNO}_3 - 2(\text{mL} \times \text{N})\text{NH}_4\text{SCN}) \times 0.04958}{\text{Wt. of Sample (g)}} \times 100$$

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**F. Statistics:**

TBD

**G. Notes:**

1. Authentic yellow mustard flour is added to provide a source of the enzyme, myrosinase, which liberates the bound allyl isothiocyanate.
2. An incubator at 37°C can be used in place of hot water bath.
3. Frothing during distillation can be reduced by adding a non-volatile anti-foaming agent.

**H. References:**

AOAC Official Methods of Analysis (1995) 43.1.15 (970.55).  
J. Assoc. Official Anal. Chemists, 51, 633-636, 1968.

# ASTA ANALYTICAL METHODS

