

**Volatile Oil in Mustard Seed and Flour
(Alternate Method)**

Purpose: To determine the volatile oil present in mustard seed and flour. (Gas Chromatographic Method)

A. Apparatus:

1. Gas chromatograph.
 - a. Column, 10' X 1/8" stainless steel packed with 10% Carbowax 20 M and 5% DEGS on A.W. Chromosorb W 60/80 mesh. (Note 1)
 - b. Temperature-Column 150°C; Detector 250°C; Injector 250°C.
 - c. Carrier Gas-Helium at 50 ml/min.
2. Recorder or integrator.
3. Graduated centrifuge tube 50 mL, with glass stopper.
4. High speed coffee grinder.
5. Shaker table.
6. Vial 10 mL with cap.
7. Glass wool, Pasteur pipet.

B. Reagents:

1. Carbon disulfide (CS₂), ACS grade. (Note 2)
2. Allyl isothiocyanate (AIC), ACS grade.

C. Preparation of Sample:

1. Mustard Flour:
 - a. Weight 1.0 g flour into a centrifuge tube.
 - b. Add 25 mL distilled water.
 - c. Add 10 mL CS₂.
 - d. Stopper with greased stopper and mix one hour on shaker table.
 - e. Go to D.

Method 15.1

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2. Mustard Seed:
 - a. Grind seed to pass 30 mesh sieve.
 - b. Weigh 1.0 g ground seed into a centrifuge tube.
 - c. Add 25 mL boiling distilled water, a little anti-foam and boil 5 more minutes, then cool to room temperature. Add 0.2 g yellow flour.
 - d. Add 10 mL CS₂.
 - e. Stopper with greased stopper and mix two hours on shaker table.
 - f. Go to D.

D. Procedure:

Prepared Samples from C1 or C2:

1. Centrifuge sample at 3,000 rpm for five minutes.
2. Filter the CS₂ layer through a Pasteur pipette plugged with a small amount of glass wool at the end.
3. Use a clean Pasteur pipette to transfer the CS₂ layer of each sample to a 10 mL vial. Allow the CS₂ layer to pass through the wool plug. (Note 3)
4. Inject 5 µl of CS₂ filtrate or adequate volume.
5. When sample has eluted, inject 5 µl of standard (Std.). (Note 4)
6. Keep vial tightly capped when not in use.

E. Calculation:

$$\text{Relative area} = \frac{\text{Sample area}}{\text{Std. Area}}$$

$$\% \text{ Volatile Oil} = \frac{\text{Rel. area} \times 10 \text{ mL extract} \times \mu\text{L AIC/mL std.}}{\text{Sample wt.} \times 1000} \times 100$$

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$$= \frac{\text{Rel. area} \times \mu\text{L AIC/mL std.}}{\text{Sample wt.}}$$

F. Statistics:

TBD

G. Notes:

1. Another column that can be used for this analysis:
Column 6' X 1/4" glass packed with 20% Carbowax 20 M on A.W. Chromosorb W 60/80 mesh.
2. Do all work with carbon disulfide in a well ventilated area of fume hood.
3. Filtering the sample prevents mustard from clogging the syringe.
4. Standard should be made fresh daily (AIC) approximately the same concentration as the extract--usually 0.2-0.1 $\mu\text{L/mL}$.

H. References:

AOAC Official Methods of Analysis (1995) 43.1.15 (970.55).
JAOAC 4,525 (1921); 53,1 (1970).