

**Determination of Ethylene Oxide (EtO)**

*Purpose: This method describes a procedure for determining ethylene oxide (EtO) residues in spices.*

**A. Apparatus:**

1. Micropipet, 100 $\mu$ L size and tips.
2. Volumetric Flasks, various sizes.
3. Headspace Vials, 20 mm, Perkin Elmer #B010-4236, or equivalent.
4. Septa, PTFE Butyl, Perkin Elmer #B010-4239.
5. Balance, readable to 0.0001g. Top loading balance, readable to 0.01 g.
6. Volumetric pipets, various sizes.
7. Perkin Elmer Autosystem Gas Chromatograph, equipped with a split/splitless injection port, and a FID Detector, or equivalent.
8. Perkin Elmer Headspace Autosampler, model HS-40 or equivalent.
9. GC Column: GS-Q 0.53 mm I.D. 30 Meter Length (J&W Scientific Co. #115-3432).
10. GC Guard Column: Phenyl Methyl Deactivated, 0.32 mm I.D. (Restek Corp. #10044).
11. Integrator: Perkin Elmer TurboChrom Data Station, or equivalent.

**B. Reagents:**

1. Ethylene Oxide (EtO), Aldrich #38761-4. (Note 1)
2. 1-Octanol, Aldrich #29, 324-5, or equivalent.

**Determination of Ethylene Oxide****C. Preparation of Samples:**

1. Small particle samples.
  - a) Tare a clean 22 mL headspace vial on a top-loading balance.
  - b) Weigh  $1.00 \pm 0.02$  grams of sample into the vial.
  - c) Add 100  $\mu$ L of octanol to the vial. Cap and mix the contents of the vial.
  
2. Large Particle Samples:
  - a) Break up the sample by use of a blender or a hammer.
  - b) Tare a clean 22 mL headspace vial on a top-loading balance.
  - c) Weigh  $1.00 \pm 0.02$  grams of sample into the vial.
  - d) Add 100  $\mu$ L of octanol to the vial. Cap and mix the contents of the vial.

**D. Procedure:**

1. Chromatography Conditions:
  - a. Gas Chromatography Parameters:
    - 1) Injector temperature: 180°C.
    - 2) Detector Temperature: 250°C.
    - 3) Initial Column Temperature/Time: 72°C for 9 minutes.
    - 4) First Temperature Ramp: 72-120°C @ 10°C/minute.
    - 5) Second Temperature Ramp: 120-230°C @ 45°C/minute.
    - 6) Carrier Gas - Helium: Column Flow 7.5 mL/minute.
    - 7) Detector Gases -Hydrogen: 45 mL/minute  
-Air: 450 mL/minute
    - 8) Split Flow: 10 mL/minute.
  - b. Headspace Sampling Parameters:
    - 1) Sample Thermostatting Temperature: 75°C.
    - 2) Sample Thermostatting Time: 20 minutes.
    - 3) Needle Temperature: 95°C.
    - 4) Transfer Line Temperature: 115°C.
    - 5) G.C. Cycle Time: 20 minutes.
    - 6) Pressurization Time: 1 minute.
    - 7) Injection Time: 0.03 minutes
    - 8) Withdraw Time: 0.2 minutes.
  
2. Preparation of Standard Solutions and Matrix Standards:
  - a. Preparation of Standard Solutions. (Notes 1 & 2)
    - 1) Cool all glassware to be used for preparation of standard solutions in a refrigerator.

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- 2) Add approximately 80 mL of octanol to a 100 mL volumetric flask. Tare the octanol and flask on the analytical balance.
- 3) Accurately weigh  $1.00 \pm 0.100$ g of ETO into the flask and record the weight.
- 4) Add octanol to the volume mark, mix and store in the refrigerator at 4°C.  
This stock standard will contain approximately 10,000  $\mu$ g EtO/mL.
- 5) Prepare a 5,000  $\mu$ g EtO/mL intermediate standard by pipetting 5. mL of the stock standard into a 10.0 mL volumetric flask. Dilute to the mark with octanol.
- 6) Prepare the following standards by pipeting the appropriate volumes from the stock and intermediate standard solutions into separate chilled 100 mL volumetric flasks, containing approximately 80 mL of chilled octanol. To mL of the stock standard (10,000  $\mu$ g/mL) into separate volumetric flasks and dilute each to 100 mL with octanol. To prepare the 100, 50, 20, 10, and 5  $\mu$ g EtO/mL standards, pipet 10.0 mL, 5.0 mL, 2.0 mL, 1.0 mL and 0.5 mL, respectively, of the 1,000  $\mu$ g/mL intermediate standard into separate volumetric flasks and dilute each to 100 mL with 1-octanol.

**b. Preparation of Matrix Standards. (Note 2)**

- 1) Tare a clean 22 mL headspace vial on a top-loading balance.
  - 2) Weigh  $1.00 \pm 0.02$  grams of the spice into separate head space vials.
  - 3) Chill the vials containing the spice samples for 10 minutes.
  - 4) Add 100  $\mu$ L of the each standard solution to the appropriate vial. Cap and mix the contents of the vial.
3. Determination by Headspace Gas Chromatography:
- a. *After allowing the instrument time to equilibrate, perform a multi-level calibration curve, using reagent standards.*
  - b. After the solvent standard calibration has been obtained, run a multi-level calibration curve using the spice matrix standards.
  - c. Run an octanol reagent blank during the run to determine if any carry over is occurring.
  - d. Run a standard after every 4-6 samples.
  - e. The retention time for EtO is approximately 8-11 minutes.

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**E. Calculations:**

1. Perform separate linear regression analyses on the reagent standard calibration and the matrix standard calibration to determine the equations of the lines that best fits these points. Force the y-intercepts of the calibration equations to equal zero, and determine the slope, m. Define the x-variable as the peak area of EtO and the y-variable as the concentration in µg EtO.
  - a. R<sup>2</sup> from regression analysis must be greater than 0.95.
  - b. Recovery of EtO from the matrix standard curve should be 70-120%.
  - c. Limit of Detection (LOD)  $\simeq$  1 ppm.
  
2. Calculate the concentration of EtO in the space by using the following formula:

$$\text{Conc } (\mu\text{g/g}) = \frac{(P) (M)}{W}$$

Where

- |   |                                       |
|---|---------------------------------------|
| P | = peak area of EtO found in the spice |
| M | = slope from the calibration curve    |
| W | = sample weight (g) = 1.0g            |

**F. Statistics:**

TBD

**G. Notes:**

1. EtO is toxic. Prepare, store and use EtO only in well ventilated areas. Wear gloves and safety glasses. Keep all solutions and samples refrigerated or on ice until ready for use. Prepare standard solutions only as needed. Properly store and dispose of standard materials .
  
2. Use the solvent standard curve to determine the true EtO recoveries from the spice. Use the matrix standard curve to determine the concentration of EtO in the spice.

**H. Reference:**

P. G. Hoffman, et. al. "Analytical Method Validation Study for Determination of Ethylene Oxide, Ethylene Chlorohydrin, Ethylene Bromohydrin, and Ethylene Glycol Residues in Spices," Report to the Environmental Protection Agency, (1994).