Method 23.1

Ethylene Oxide (EtO) and Ethylene Chlorohydrin Residue in Black Pepper

Purpose: To determine levels of Ethylene Oxide (EtO) and Ethylene Chlorohydrin Residues in Ground Black Pepper (Piper nigrum). (Note 1)

A. Apparatus:

1. Gas chromatograph with flame ionization detector and integrator.
2. Waring blender with semi-micro metal blender cup (Thomas Scientific, Cat. #3392-G05).
3. Centrifuge: 50 mL tube capacity.
4. Syringes, 10 μL and 25 μL syringes (Hamilton #701 and 702).
5. Syringe 10.0 mL with luer-lock tip (Thomas Scientific Cat. #8932 A-18).
6. Syringe filters 0.45 micron (Nalgene: 191-2045).
7. Volumetric Flasks, 10 mL and 100 mL.
8. Pipettes, volumetric, 1.00 mL and 10.00 mL.
9. 125 mL Erlenmeyer flask with ground glass stopper.
10. Centrifuge tubes, 50 mL round bottom.
11. Balance, readable to 0.1 g.
12. Fume hood.
13. GC Column: glass 6' x 1/8' i.d. Porapak Q 80/100 mesh.
14. Graduated cylinder, 50.00 mL.

B. Reagents:

1. Ethylene oxide (Aldrich #38761-4). (Note 2)
2. Propylene oxide (Aldrich #11020-5). (Note 2)

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3. Ethylene chlorohydrin (2-Chloroethanol: Aldrich B6558-6). (Note 2)

C. Preparation of Sample:

1. Use as is.

D. Procedure:

1. Weigh 10.0 grams of spice into a tared semi-micro metal blender cup.

2. Add 50.0 mL of distilled H_2O to the blender cup. Cover and mix at high speed for 30 seconds and quickly pour this mixture into a 125 mL Erlenmeyer flask. Stopper the flask and allow the mixture to stand for 30 minutes. Swirl intermittently.

3. Pour the mixture into a 50 mL centrifuge tube and spin at high speed for 10 minutes.

4. Decant the upper water layer into a 10 mL syringe that is attached to a 0.45 micron syringe filter.

5. Inject approximately 5.0 mL of the sample solution into a 10.0 mL volumetric flask. With a 25 microliter syringe inject 10.0 microliters of 1000 ppm propylene oxide standard into the flask and mix well (see Standard Solutions). Dilute to the mark with filtered sample solution.

6. Chromatograph Conditions:
   a. Temperature
      a. Injector and detector: 180°C
      b. Column: 130°C - ethylene oxide
         165°C - ethylene chlorohydrin
   b. Gas
      a Helium - carrier gas: 40 mL/min
      b. Hydrogen: 40 mL/min
      c. Air: 200 mL/min
   c. Column - glass 6' x 1/8' i.d.: porapak Q 80/100 mesh. (Note 3)
      Detector - flame ionization
      Injector - on-column
   d. Integrator Parameters* - for peak area:
      a. Peak width = width at half peak height.
      b. Peak threshold = 1.0 mV or half peak height.

c. Peak sensitivity: 0.1 mV or equivalents.
d. Chart speed: 0.5 cm/min.

*Actual integration parameters will depend on system used.

7. Standard Solutions (Note 2):
   a. Tare a 100.00 mL volumetric flask with stopper that contains approximately 80 mL of distilled H_2O.
   b. Place the flask on a balance that has been placed inside a ventilating hood and add 1.0 gram of ethylene oxide to the flask. Stopper, mix well, vent, then re-weigh. Record this weight.
   c. Dilute to volume with fresh distilled H_2O (or deionized H_2O). The concentration of the standard is approximately 10,000 ppm ethylene oxide.
   d. To make a 1000 ppm working std.: Pipette 10.00 of the 10,000 ppm standard into a 100.00 mL volumetric flask containing about 80 mL of distilled H_2O. Stopper, mix well, then dilute to mark.
   e. Repeat procedures 7a - 7d for propylene oxide and ethylene chlorohydrin.
   f. Final solutions will be made by diluting the1000 ppm standards to approximately that of the sample concentration.
   g. Store all solutions on ice or in the refrigerator.

8. Determination:
   a. Ethylene Oxide - Column temperature 130°C.
      1. Approximate the concentration of ethylene oxide in sample by injecting 4.0 microliters of sample/propylene oxide solution into the chromatograph.
      2. Prepare a standard solution of ethylene oxide/propylene oxide by adding 10 microliters of propylene oxide to 5 mL of ethylene oxide standard whose concentration is equal to sample. Mix solution well then dilute to mark the ETO standard.
      3. Record samples/propylene oxide and ethylene oxide/propylene oxide ratios.
   b. 2-Chloroethanol - Column temperature 165°C. Repeat procedure as written above using 2-Chloroethanol instead of ethylene oxide.

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E. Calculations:

$$\text{Relative peak area ratio (RPAR)} = \frac{\text{[Sample (X)/propylene oxide] Area}}{\text{[Standard (X)/propylene oxide] Area}}$$

$$\text{(X) Concentration is} = \frac{\text{(RPAR) x (X) standard concentration x 50}}{\text{10.0 grams (sample weight)}}$$

(X) = ethylene oxide or 2-chloroethanol

F. Statistics:

TBD

G. Notes:

1. This method may be used with other spices. The addition of 10.0 mL carbon disulfide to 50 mL water will break emulsions that form when leafy spices are tested. Inject 5.0 mL of the carbon disulfide layer. Repeat with an appropriate standard.

2. All standards are toxic. Prepare, store and use standards only in well ventilated areas. Wear gloves and safety glasses. Keep all solutions and samples refrigerated or on ice until ready to use. Prepare standard solutions only as needed. Properly store and dispose of standard materials .

3. This method may also use column 6' x ¼" glass packed with 20% Carbowax 20 M on A.W. Chromsorb W 80/100. Retention times however, will be shifted.

H. Reference:

N/A