

Ethylene Chlorohydrin Residue in Spices

Purpose: To determine ethylene chlorohydrin residues in spices.

A. Apparatus:

1. Gas Chromatograph with flame ionization detector and integrator.
2. Centrifuge, capable of holding 50 mL tubes at 2000 RPM.
3. Centrifuge tubes, polypropylene, 15 mL graduated with screw caps.
4. Balance, readable to 0.01 g and 0.1 mg.
5. Balance, readable to 0.0001 g.
6. Volumetric flasks, class A, various sizes.
7. Volumetric pipets, class A, various sizes.
8. Mechanical shaker, Glas-Col model S-500 or equivalent.
9. Turbo Vap LV Evaporator or equivalent.
10. Extrelut QE Columns, EM Science #901003-1.
11. Air source - house air or cylinder.
12. Column DB-Wax, 30 m x 0.25 mm ID, 0.25 micron film (J&W Scientific) or equivalent.
13. Disposable serological pipets, various sizes.
14. Dispensing buret, 50 mL size.
15. Vials.

Ethylene Chlorohydrin Residue in Spices

B. Reagents:

1. 2-Chloroethanol (ECH), Aldrich #B6558-6.
2. Ethyl Acetate, ACS reagent grade, free of components that would elute near ECH.

C. Sample preparation:

1. Accurately weigh four (4) g of treated or untreated sample into a 50 mL centrifuge tube. With each set of samples prepare two spikes by weighing four (4) grams of untreated samples into two additional tubes. To one of the tubes pipet in 0.4 mL of the 1000 µg/mL spiking solution, to the other pipet in 0.4 mL of the 100000 µg/mL spiking solution.
2. Dispense in 40.0 mL of deionized water and tightly cap each sample solution.
3. Mix these solutions using a reciprocating shaker for 10 minutes.
4. Centrifuge the samples for 10 minutes at 2000 rpms to clarify the solution, if necessary. (Note 1)

D. Procedure:

1. Chromatograph Conditions:
 - a. Temperatures:
 1. Injector and Detector: 250°C.
 2. Initial column temperature and time: 80°C for 1 minute.
 3. Initial temperature rate: 10°C/minute.
 4. Intermediate temperature and time: 180°C for 0 minutes.
 5. Final temperature rate: 20°C/minute.
 6. Final temperature and time: 240°C for 5 minutes.
 - b. Gas:
 1. Helium, carrier gas: 23 cm/second (column velocity).
 2. Helium, make-up gas: 40 mL/minute.
 3. Hydrogen, detector gas: 30 mL/minute.
 4. Air, detector gas: 300 mL/minute.
 5. Split flow: 10 mL/minute.
 - c. Injection volume: 2 µL

Ethylene Chlorohydrin Residues in Spices

2. Standard Solutions:
 - a. Preparation of standard solutions:
 1. Accurately weigh 1.00 ± 0.10 g of ECH into a 100 mL volumetric flask. Dissolve and bring to volume with deionized water. Mix well before use. This stock standard will contain approximately 10000 µg/mL of ECH.
 2. Pipet a 5.00 mL aliquot of the stock standard solution into two separate 100 mL volumetric flask. Dilute to volume with ethyl acetate and mix well before use. This intermediate standard will contain approximately 500 µg/mL of ECH.
 3. Prepare a series of working standard solution by pipeting 0.4, 2.0, 10.0, and 20.0 mL of the intermediate standard solution into separate 100 mL volumetric flasks. Dilute each flask with ethyl acetate and mix well before use. These working standard solution will contain 2, 10, 50 and 100 µg/mL of ECH, respectively.
 - b. Preparation of spiking solutions:
 1. Accurately weigh 5.00 ± 0.10 g of ECH into a 50 mL volumetric flask. Dissolve and bring to volume with deionized water and mix well before use. This spiking solution will contain approximately 100000 µg/mL ECH.
 2. From the stock standard solution (see 2.a.1), pipet a 10 mL aliquot into a 100 mL volumetric flask. Dissolve and bring to volume with deionized water and mix well before use. This spiking solution will contain approximately 1000 µg/mL of ECH.
3. Determination:
 - a. Label a series of 15 mL centrifuge tubes for each sample and standard solution and attach an Extrelut QE column to each tube.
 - b. Pipet a 3.0 mL aliquot of each solution onto the Extrelut columns and allow the solution to absorb for at least two minutes.
 - c. Add 6 mL of ethyl acetate to each Extrelut column and collect the eluant into the appropriate tube without applying external pressure. Allow the column to drain completely before adding a second 6 mL portion of ethyl acetate. Combine both ethyl acetate fractions.
 - d. Concentrate the ethyl acetate fraction to 3.0 mL using a gentle stream of nitrogen or compressed air.
 - e. Mix thoroughly and transfer a portion to a labeled GC vial.
 - f. After the GC system has equilibrated, run a series of standards followed by the control, spiked samples and treated samples and obtain there corresponding chromatographs.
 - g. Run ethyl acetate reagent blanks frequently to make sure that there is no sample carryover occurring. (Note 2)
 - h. Run a standard after every sixth sample injection.

Method 23.3

Ethylene Chlorohydrin Residues in Spices

- i. At the completion of the GC analysis, perform a multi-level calibration curve using the standard solution run initially.
- j. Retention times of the peak of interest are as follows:
 - a. ECH: RT = 6.0 minutes

E. Calculation:

- 1. Perform a linear regression analysis on the standard calibration to obtain the slope, m, and the intercept of the line that best fits these points. Force this line through the origin and let the peak area (height) of standard be the x-variable and the concentration (µg/mL) be the y-variable.
- 2. Calculate the concentration of the ECH in each sample using the following equation:

$$\text{Conc. (g/g)} = \frac{(\text{P}) (\text{m}) (\text{V})}{\text{W}}$$

Where

- P = peak area (height) for ECH in sample
- m = slope from the calibration curve
- V = volume on extraction, mL
- W = sample weight, g

F. Statistics:

TBD

G. Notes:

- 1. Samples that are highly absorbent may require using a smaller weight or a larger extraction volume.
- 2. When analyzing celery seed, a reagent blank must be run after each sample injection to remove a late eluting peak occurring from the previous sample injection.

Ethylene Chlorohydrin Residues in Spices

H. References:

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).