〈228〉 Ethylene Oxide and Dioxane

The following procedure is used to determine the contents of residual ethylene oxide and dioxane in the products prepared from ethylene oxide. Unless otherwise directed in the individual monograph, use *Method I*.

Method I

[**CAUTION**—Ethylene oxide is toxic and flammable. Prepare these solutions in a well-ventilated fume hood, using great care. Protect both hands and face by wearing polyethylene protective gloves and an appropriate face mask. Store all solutions in hermetic containers, and refrigerate between 4° and 8°.]

[NOTE—Before using the polyethylene glycol 200 in this test, remove any volatile components from it by placing 500 mL of polyethylene glycol 200 in a 1000-mL round-bottom flask and attaching the flask to a rotary evaporator maintained at 60° and under a vacuum of 10–20 mm Hg for 6 h.]

Acetaldehyde solution: 10 µg/mL of acetaldehyde. [NOTE—Prepare immediately before use.]

Ethylene oxide stock solution: 2.5 mg/g of ethylene oxide. Prepare as follows: Tare a glass-stoppered conical flask, add 50 mL of polyethylene glycol 200, and reweigh the flask. Transfer 5 mL of the liquid ethylene oxide to a 100-mL beaker chilled in a mixture of sodium chloride and ice (1:3). Transfer 300 µL (corresponding to 250 mg) of liquid ethylene oxide to the polyethylene glycol 200, and swirl gently to mix. Replace the stopper, reweigh the flask, and determine the amount of ethylene oxide absorbed by weight difference. Adjust the weight of the mixture with polyethylene glycol 200 to 100.0 g, replace the stopper, and swirl gently to mix. [NOTE—Fill a chilled pressure bottle with liquid ethylene oxide, and store in a freezer when not in use. Use a small piece of polyethylene film to protect the liquid from contact with the rubber gasket. Use an adequately chilled apparatus where appropriate. Prepare this stock solution immediately prior to use, and store in a refrigerator after preparation.]

Ethylene oxide solution: Tare a glass-stoppered conical flask, and chill it in a refrigerator. Add 35 mL of polyethylene glycol 200, and reweigh the flask. Transfer 1 g of chilled *Ethylene oxide stock solution* to the tared conical flask. Adjust the weight of the solution with polyethylene glycol 200 to 50.0 g, replace the stopper, and swirl gently to mix. Transfer 10 g of this solution to a 50-mL volumetric flask. Add 30 mL of water, and mix. Dilute with water to volume, and mix to obtain a solution containing 10 µg/mL of ethylene oxide. [NOTE—Use an adequately chilled apparatus where appropriate. Prepare immediately before use.]

Dioxane solution: 500 µg/mL of dioxane

Standard solution A: Transfer 0.1 mL of *Ethylene oxide solution* to a 10-mL pressure headspace vial. [NOTE—Other sizes such as a 22-mL pressure headspace vial may be used, depending on operating conditions; however, the same size must be used for *Standard solution A*, *Standard solution B*, and the *Sample solution*.] Add 0.1 mL of *Acetaldehyde solution* and 0.1 mL of *Dioxane solution*, seal the vial, and mix.

Standard solution B: Transfer 1.0 g of the test substance to a 10-mL pressure headspace vial, and add 0.1 mL of *Ethylene oxide solution*, 0.1 mL of *Dioxane solution*, and 1.0 mL of *N*,*N*-dimethylacetamide. Seal the vial, and mix.

Sample solution: Transfer 1.0 g of the test substance to a 10-mL pressure headspace vial, and add 1.0 mL of *N*,*N*-dimethylacetamide and 0.2 mL of water. Seal the vial, and mix.

Chromatographic system
(See *Chromatography 〈621〉, System Suitability*.)

Mode: Headspace GC

Detector: Flame ionization

Column: 0.32-mm × 30-m glass or quartz capillary; 1.0-µm layer of phase G1

Temperature
Injector port: 150°

Detector: 250°

Column: See the column temperature table below.

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
50	—	50	5
50	5	180	—
180	30	230	5

Carrier gas: Helium

Linear velocity: 20 cm/s

Injection volume: 1 mL (gaseous headspace)

Injection type: Split ratio 20:1

Headspace sampler
Temperature equilibration time: 45 min

Equilibration temperature
70° for *Standard solution A*
90° for *Standard solution B*
90° for *Sample solution*

Transfer line temperature: 150°

Pressurization time: 1 min

Injection time: 12 s

System suitability
Sample: *Standard solution A*
[NOTE—The relative retention times for acetaldehyde and ethylene oxide are 0.94 and 1.0, respectively.]

Suitability requirements
Resolution: NLT 2.0 between acetaldehyde and ethylene oxide
Signal-to-noise ratio: NLT 5, determined from the dioxane peak
Relative standard deviation: NMT 15%

Analysis
Samples: *Standard solution B* and *Sample solution*
[NOTE—The relative retention times for ethylene oxide and dioxane are 1.0 and 2.5, respectively.]
Calculate the content of ethylene oxide, in ppm, in the portion of the test substance taken:

$$\text{Result} = A_E \times r_U/[(r_S \times W_U) - (r_U \times W_S)]$$

A_E = quantity of ethylene oxide added to *Standard solution B* (µg)
r_U = ethylene oxide peak responses from the *Sample solution*
r_S = ethylene oxide peak responses from *Standard solution B*
W_U = weight of the test substance taken to prepare the *Sample solution* (g)
W_S = weight of the test substance taken to prepare *Standard solution B* (g)

Calculate the content of dioxane, in ppm, in the portion of the test substance taken:

$$\text{Result} = A_D \times r_U/[(r_S \times W_U) - (r_U \times W_S)]$$

A_D = quantity of dioxane added to *Standard solution B* (µg)
r_U = dioxane peak responses from the *Sample solution*
r_S = dioxane peak responses from *Standard solution B*
W_U = weight of the test substance taken to prepare the *Sample solution* (g)
W_S = weight of the test substance taken to prepare *Standard solution B* (g)

Method II

Ethylene oxide standard solution: Dilute 0.5 mL of ethylene oxide in methylene chloride (50 mg/mL)[1] with water to 50.0 mL. [NOTE—The solution is stable for 3 months if stored in vials with polytetrafluoroethylene (polytef)-coated silicon membrane crimped caps at −20°.] Allow to reach room temperature. Dilute 1.0 mL with water to 250.0 mL to obtain a solution having a concentration of 2 µg/mL of ethylene oxide. [NOTE—Use this solution immediately after preparation.]

Dioxane standard solution: 0.05 µL/mL of dioxane

Acetaldehyde standard solution: 10 µg/mL of acetaldehyde. [NOTE—Prepare immediately before use.]

Resolution solution: Add 2.0 mL of *Acetaldehyde standard solution* and 2.0 mL of *Ethylene oxide standard solution* to a 10-mL headspace vial. Seal the vial immediately with a polytef-coated silicon membrane and an aluminum cap, and mix carefully.

Standard solution A: 0.48 µg/mL of ethylene oxide, from *Ethylene oxide standard solution*, and 0.005 µL/mL of dioxane, from *Dioxane standard solution*, in water

Standard solution B: Transfer 1.0 g of the test substance into a 10-mL headspace vial. Add 2.0 mL of *Standard solution A*, seal the vial immediately with a polytef-coated silicon membrane and an aluminum cap, and mix carefully.

Sample solution: Transfer 1.0 g of the test substance into a 10-mL headspace vial. Add 2.0 mL of water, seal the vial immediately with a polytef-coated silicon membrane and an aluminum cap, and mix carefully.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: Headspace GC

Detector: Flame ionization

Column: 0.53-mm × 50-m fused-silica capillary column; 5.0-µm layer of phase G27

Temperature

Injector port: 85°

Detector: 250°

Column: See the column temperature table below.

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
70	10	250	5

Carrier gas: Helium

Flow rate: 4 mL/min

Injection volume: 1 mL (gaseous headspace)

Injection type: Split ratio 3.5 : 1

Headspace sampler

Temperature equilibration time: 30 min

Equilibration temperature: 80°

System suitability

Sample: *Resolution solution*
[NOTE—The relative retention times for acetaldehyde and ethylene oxide are 0.9 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between acetaldehyde and ethylene oxide

Analysis

Samples: *Standard solution B* and *Sample solution*
[NOTE—The relative retention times for ethylene oxide and dioxane are 1.0 and 1.9, respectively.]
Calculate the content of ethylene oxide, in ppm, in the portion of the test substance taken:

$$\text{Result} = C_E \times V \times r_U/[(r_S \times W_U) - (r_U \times W_S)]$$

C_E = concentration of ethylene oxide in the *Standard solution A* (µg/mL)
V = volume of *Standard solution A* added to *Standard solution B* (2.0 mL)
r_U = ethylene oxide peak responses from the *Sample solution*
r_S = ethylene oxide peak responses from *Standard solution B*
W_U = weight of the test substance taken to prepare the *Sample solution* (g)
W_S = weight of the test substance taken to prepare *Standard solution B* (g)

Calculate the content of dioxane, in ppm, in the portion of the test substance taken:

$$\text{Result} = C_D \times V \times \rho \times F \times r_U/[(r_S \times W_U) - (r_U \times W_S)]$$

C_D = concentration of dioxane in *Standard solution A* (µL/mL)
V = volume of *Standard solution A* added to *Standard solution B* (2.0 mL)
ρ = density of dioxane (1.03 g/mL = 1.03 mg/µL)
F = conversion factor (1000 µg/mg)
r_U = dioxane peak responses from the *Sample solution*
r_S = ethylene oxide peak responses from *Standard solution B*
W_U = weight of the test substance taken to prepare the *Sample solution* (g)
W_S = weight of the test substance taken to prepare *Standard solution B* (g)

---

[1] This is a commercially available solution.

⟨231⟩ HEAVY METALS

This test is provided to demonstrate that the content of metallic impurities that are colored by sulfide ion, under the specified test conditions, does not exceed the *Heavy metals* limit specified in the individual monograph in percentage (by weight) of lead in the test substance, as determined by concomitant visual comparison (see *Visual Comparison* in the section *Procedure* under *Spectrophotometry and Light-Scattering* ⟨851⟩) with a control prepared from a *Standard Lead Solution.* [NOTE—Substances that typically will respond to this test are lead, mercury, bismuth, arsenic, antimony, tin, cadmium, silver, copper, and molybdenum.]

Determine the amount of heavy metals by *Method I,* unless otherwise specified in the individual monograph. *Method I* is used for substances that yield clear, colorless preparations under the specified test conditions. *Method II* is used for substances that do not yield clear, colorless preparations under the test conditions specified for *Method I,* or for substances that, by virtue of their complex nature, interfere with the precipitation of metals by sulfide ion, or for fixed and volatile oils. *Method III,* a wet-digestion method, is used only in those cases where neither *Method I* nor *Method II* can be used.

Special Reagents

Lead Nitrate Stock Solution—Dissolve 159.8 mg of lead nitrate in 100 mL of water to which has been added 1 mL of nitric acid, then dilute with water to 1000 mL. Prepare and store this solution in glass containers free from soluble lead salts.

Standard Lead Solution—On the day of use, dilute 10.0 mL of *Lead Nitrate Stock Solution* with water to 100.0 mL. Each mL of *Standard Lead Solution* contains the equivalent of 10 µg of lead. A comparison solution prepared on the basis of 100 µL of *Standard Lead Solution* per g of substance being tested contains the equivalent of 1 part of lead per million parts of substance being tested.

Method I

pH 3.5 Acetate Buffer—Dissolve 25.0 g of ammonium acetate in 25 mL of water, and add 38.0 mL of 6 N hydrochloric acid. Adjust, if necessary, with 6 N ammonium hydroxide or 6 N hydrochloric acid to a pH of 3.5, dilute with water to 100 mL, and mix.

Standard Preparation—Into a 50-mL color-comparison tube pipet 2 mL of *Standard Lead Solution* (20 µg of Pb), and dilute with water to 25 mL. Using a pH meter or short-range pH indicator paper as external indicator, adjust with 1 N acetic acid or 6 N ammonium hydroxide to a pH between 3.0 and 4.0, dilute with water to 40 mL, and mix.

Test Preparation—Into a 50-mL color-comparison tube place 25 mL of the solution prepared for the test as directed in the individual monograph; or, using the designated volume of acid where specified in the individual monograph, dissolve in and dilute with water to 25 mL the quantity, in g, of the substance to be tested, as calculated by the formula:

$$2.0/(1000L)$$

in which L is the *Heavy metals* limit, as a percentage. Using a pH meter or short-range pH indicator paper as external indicator, adjust with 1 N acetic acid or 6 N ammonium hydroxide to a pH between 3.0 and 4.0, dilute with water to 40 mL, and mix.

Monitor Preparation—Into a third 50-mL color-comparison tube place 25 mL of a solution prepared as directed for *Test Preparation,* and add 2.0 mL of *Standard Lead Solution.* Using a pH meter or short-range pH indicator paper as external indicator, adjust with 1 N acetic acid or 6 N ammonium hydroxide to a pH between 3.0 and 4.0, dilute with water to 40 mL, and mix.

Procedure—To each of the three tubes containing the *Standard Preparation,* the *Test Preparation,* and the *Monitor Preparation,* add 2 mL of *pH 3.5 Acetate Buffer,* then add 1.2 mL of thioacetamide–glycerin base TS, dilute with water to 50 mL, mix, allow to stand for 2 minutes, and view downward over a white surface*: the color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation,* and the color of the solution from the *Monitor Preparation* is equal to or darker than that of the solution from the *Standard Preparation.* [NOTE—If the color of the *Monitor Preparation* is lighter than that of the *Standard Preparation,* use *Method II* instead of *Method I* for the substance being tested.]

Method II

NOTE—This method does not recover mercury.

pH 3.5 Acetate Buffer—Prepare as directed under *Method I.*

Standard Preparation—Prepare as directed under *Method I.*

Test Preparation—Use a quantity, in g, of the substance to be tested as calculated by the formula:

$$2.0/(1000L)$$

in which L is the *Heavy metals* limit, in percentage. Transfer the weighed quantity of the substance to a suitable crucible, add sufficient sulfuric acid to wet the substance, and carefully ignite at a low temperature until thoroughly charred. (The crucible may be loosely covered with a suitable lid during the charring.) Add to the carbonized mass 2 mL of nitric acid and 5 drops of sulfuric acid, and heat cautiously until white fumes no longer are evolved. Ignite, preferably in a muffle furnace, at 500° to 600°, until the carbon is completely burned off. Cool, add 4 mL of 6 N hydrochloric acid, cover, digest on a steam bath for 15 minutes, uncover, and slowly evaporate on a steam bath to dryness. Moisten the residue with 1 drop of hydrochloric acid, add 10 mL of hot water, and digest for 2 minutes. Add 6 N ammonium hydroxide dropwise until the solution is just alkaline to litmus paper, dilute with water to 25 mL, and adjust with 1 N acetic acid to a pH between 3.0 and 4.0, using short-range pH indicator paper as an external indicator. Filter if necessary, rinse the crucible and the filter with 10 mL of water, combine the filtrate and rinsing in a 50-mL color-comparison tube, dilute with water to 40 mL, and mix.

Procedure—To each of the tubes containing the *Standard Preparation* and the *Test Preparation,* add 2 mL of *pH 3.5 Acetate Buffer,* then add 1.2 mL of thioacetamide–glycerin base TS, dilute with water to 50 mL, mix, allow to stand for 2 minutes, and view downward over a white surface*: the color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation*.

Method III

pH 3.5 Acetate Buffer—Prepare as directed under *Method I.*

Standard Preparation—Transfer a mixture of 8 mL of sulfuric acid and 10 mL of nitric acid to a clean, dry, 100-

---

* In those countries or jurisdictions where thioacetamide cannot be used, add 10 mL of freshly prepared hydrogen sulfide TS to each of the tubes, mix, allow to stand for 5 minutes, and view downward over a white surface.