obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Assay for citrate—

Mobile phase, Standard Preparation 1, and *Chromatographic System*—Proceed as directed under *Assay for Citric Acid/Citrate and Phosphate* ⟨345⟩.

Assay preparation—Pipet 15 mL of Oral Solution into a suitable volumetric flask, and proceed as directed for *Assay Preparation for Citric Acid/Citrate Assay* under *Assay for Citric Acid/Citrate and Phosphate* ⟨345⟩.

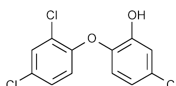
Procedure—Proceed as directed for *Procedure* under ⟨345⟩, and calculate the concentration, in mg per mL, of citrate ($C_6H_5O_7$) in the Oral Solution taken by the formula:

$$0.001 C_S (D/V)(r_U / r_S) - A(189.10 / 210.14)$$

in which C_S is the concentration, in µg per mL, of citrate in *Standard Preparation 1; D* is the dilution factor; *V* is the volume of Oral Solution used in the preparation of the *Assay preparation;* r_U and r_S are the citrate peak areas obtained from the *Assay preparation* and *Standard Preparation 1,* respectively; 189.10 is the molecular weight of citrate ($C_6H_5O_7$); 210.14 is the molecular weight of citric acid monohydrate ($C_6H_8O_7 \cdot H_2O$); and *A* is the concentration of citric acid monohydrate, in mg per mL, determined in the *Assay for citric acid*.

Assay for citric acid—Transfer 15 mL of Oral Solution, accurately measured, to a 250-mL volumetric flask, dilute with water to volume, and mix. Pipet 5 mL of this solution into a suitable flask, add 25 mL of water and 5 drops of phenolphthalein TS, and titrate with 0.02 N sodium hydroxide VS to a pink endpoint. Record the buret reading, and calculate the volume (*A*) of 0.02 N sodium hydroxide consumed. Each mL of 0.02 N sodium hydroxide is equivalent to 1.401 mg of $C_6H_8O_7 \cdot H_2O$.

Triclosan



$C_{12}H_7Cl_3O_2$ 289.54
Phenol, 5-chloro-2-(2,4-dichlorophenoxy)-.
2,4,4′-Trichloro-2′-hydroxydiphenyl ether   [3380-34-5].

» Triclosan contains not less than 97.0 percent and not more than 103.0 percent of $C_{12}H_7Cl_3O_2$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP 2,4-Dichlorophenol RS
USP Parachlorophenol RS
USP Triclosan RS
USP Triclosan Related Compounds Mixture A RS

Identification—

A: *Infrared Absorption* ⟨197K⟩.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Water, *Method I* ⟨921⟩**:** not more than 0.1%.

Completeness of solution ⟨641⟩—A solution of 1.40 g of Triclosan in 10 mL of acetone is clear.

Heavy metals, *Method II* ⟨231⟩**:** 0.002%.

Related compounds—

Chromatographic system—Proceed as directed in the *Assay*.

Test solution—Use the *Assay preparation.*

Procedure—Inject a volume (about 0.5 µL) of the *Test solution* into the chromatograph, increase the column temperature by 20° per minute to 140°, then increase column temperature by 4° per minute to 240°, maintain this temperature for not less than 5 minutes, record the chromatogram, and measure the peak responses. Calculate the percentage of each impurity in the portion of Triclosan taken by the formula:

$$100(r_i / r_s)$$

in which r_i is the peak response for each impurity; and r_s is the sum of the responses of all of the peaks: not more than 0.1% of any individual impurity is found; and not more than 0.5% of total impurities is found.

Limit of monochlorophenols and 2,4-dichlorophenol—

Phosphate buffer—Transfer about 1.38 g of anhydrous monobasic sodium phosphate and about 1.42 g of dibasic sodium phosphate to a 1-L volumetric flask, dissolve in and dilute with water to volume, and mix.

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile and *Phosphate buffer* (1:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard solution—Quantitatively dissolve accurately weighed quantities of USP Parachlorophenol RS and USP 2,4-Dichlorophenol RS in acetonitrile, dilute with an equal volume of water, and mix. Transfer a portion of this solution to a suitable container, and dilute quantitatively, and stepwise if necessary, with a mixture of acetonitrile and water (1:1) to obtain a solution having known concentrations of about 0.5 µg of parachlorophenol and 0.1 µg of 2,4-dichlorophenol per mL.

Test solution—Transfer about 250 mg of Triclosan, accurately weighed, to a 25-mL low-actinic volumetric flask, dissolve in 20 mL of acetonitrile, dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a coulometric electrochemical detector with electrode 1 set at 0.45 V and electrode 2 set at 0.75 V, both having a positive (oxidative) polarity and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 9.0% for 2,4-dichlorophenol.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. The peak responses for parachlorophenol and 2,4-dichlorophenol in the chromatogram of the *Test solution* are not greater than the corresponding peaks in the chromatogram of the *Standard solution*.

Limit of 1,3,7-trichlorodibenzo-*p*-dioxin, 2,8-dichlorodibenzo-*p*-dioxin, 2,8-dichlorodibenzofuran, and 2,4,8-trichlorodibenzofuran—

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile, water, and glacial acetic acid (70:30:0.1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Test solution—Transfer about 2.0 g of Triclosan, accurately weighed, to a screw-capped centrifuge tube, add 5 mL of 2 N potassium hydroxide, and shake for 10 minutes to dissolve. Add 3 mL of *n*-hexane, shake for 10 minutes, and allow the phases to separate. Transfer the organic layer to a

suitable container, add another 3 mL of *n*-hexane to the aqueous layer, shake for 10 minutes, and allow the phases to separate. Transfer the organic layer to the previous extract, discard the aqueous layer, add 3 mL of 2 N potassium hydroxide to the combined organic layers, shake for 10 minutes, and allow the phases to separate. Discard the aqueous layer, add another 3 mL of 2 N potassium hydroxide to the combined organic layers, shake for 10 minutes, and allow the phases to separate. Transfer the organic layer to a suitable container, and evaporate with the aid of a stream of nitrogen to dryness. Dissolve the residue in 1.0 mL of methanol, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the USP Triclosan Related Compounds Mixture A RS, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.59 for 2,8-dichlorodibenzofuran, 0.71 for 2,8-dichlorodibenzo-*p*-dioxin, 0.88 for 2,4,8-trichlorodibenzofuran, and 1.0 for 1,3,7-trichlorodibenzo-*p*-dioxin; and the relative standard deviation for replicate injections is not more than 15.0%, determined from the 2,8-dichlorodibenzo-*p*-dioxin peak.

Procedure—Inject a volume (about 20 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the concentration of each analyte in the portion of Triclosan taken by the formula:

$$(C / W)(r_i / r_S)$$

in which C is the concentration, in µg per mL, of the respective analyte in the USP Triclosan Related Compounds Mixture A RS; W is the weight, in g, of Triclosan taken; and r_i and r_S are the peak responses for the respective analyte obtained from the *Test solution* and the USP Triclosan Related Compounds Mixture A RS, respectively: not more than 0.25 ppm of 2,8-dichlorodibenzofuran is found; not more than 0.5 ppm of 2,4,8-trichlorodibenzofuran is found; not more than 0.25 ppm of 1,3,7-trichlorodibenzo-*p*-dioxin is found; and not more than 0.5 ppm of 2,8-dichlorodibenzo-*p*-dioxin is found.

Limit of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and 2,3,7,8-tetrachlorodibenzofuran—[*Caution—2,3,7,8-tetrachlorodibenzo-p-dioxin and 2,3,7,8-tetrachlorodibenzofuran are extremely toxic substances. Exercise all necessary precautions in the conduct of this procedure.*]

Stationary phase A—Transfer about 10 g of silica gel to a suitable container, add about 3 mL of 1 N sodium hydroxide, and mix.

Stationary phase B—Transfer about 60 g of silica gel to a suitable container, add about 74 mL of concentrated sulfuric acid, and mix.

Chromatographic column A—Transfer 5.1 g of *Stationary phase A*, 0.5 g of silica gel, 6.2 g of *Stationary phase B*, and 3.2 g of sodium sulfate to a glass chromatographic column having an internal diameter of 10 mm. Wash the column with 50 mL of *n*-hexane, and discard the eluate.

Chromatographic column B—Transfer 2.5 g of alumina and 2.5 g of sodium sulfate to a glass chromatographic column having an internal diameter of 6 mm. Wash the column with 30 mL of *n*-hexane, and discard the eluate.

Internal standard solution—Transfer accurately measured quantities of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, ^{13}C-labeled, and 2,3,7,8-tetrachlorodibenzofuran, ^{13}C-labeled, in nonane, and dilute quantitatively, and stepwise if necessary, with 2,2,4-trimethylpentane to obtain a solution having known concentrations of about 1.0 pg of each per µL.

Test solution—Transfer about 30 g of Triclosan, accurately weighed, to a separatory funnel, add 30 µL of *Internal standard solution*, dissolve in 200 mL of 1 N sodium hydroxide, extract with four 30-mL portions of *n*-hexane, and combine the extracts. Wash the combined extracts with 20 mL of water, extract the washing with 15 mL of *n*-hexane, and add the extract to the other combined extracts. Add about 3 g of anhydrous sodium sulfate to the combined extracts, allow to stand for 30 minutes, quantitatively transfer to an appropriate round-bottom flask, and distill, using a distillation apparatus with a vigreux column, until about 1 mL remains. Transfer this solution to the top of *Chromatographic column A*, and elute with 50 mL of *n*-hexane. Collect the eluate on top of *Chromatographic column B*, and elute with 30 mL of a mixture of *n*-hexane and methylene chloride (98:2), discarding the eluate. Elute with 40 mL of a mixture of *n*-hexane and methylene chloride (1:1), collecting the eluates in a round-bottom flask. Distill the combined eluates, using a distillation apparatus with a vigreux column, until about 1 mL remains. Further concentrate this solution with the aid of a stream of nitrogen to about 50 µL, evaporate at room temperature to dryness, and dissolve in 10 µL of 2,2,4-trimethylpentane.

Chromatographic system (see *Chromatography* ⟨621⟩ and *Mass Spectrometry* ⟨736⟩)—The gas chromatograph is equipped with a high-resolution mass spectrograph with an electron-impact ionization source and a 0.25-mm × 60-m capillary column coated with phase G48. The carrier gas is helium. The chromatograph is programmed as follows. Initially the temperature of the column is equilibrated at 80°, then, 1 minute after the injection, the temperature is increased at a rate of 20° per minute to 220°, then increased at a rate of 2° per minute to 270°, and maintained at 270° for not less than 20 minutes. The injection port temperature is maintained at 280°. Chromatograph the *Internal standard solution*, and record the peak responses as directed for *Procedure:* the signal-to-noise ratio at a mass-to-charge ratio of 321.89 is not less than 50.

Procedure—Inject a volume (about 1 µL) of the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses at mass-to-charge ratios of 319.90, 321.89, 331.88, 333.93, 303.90, 305.90, 315.94, and 317.94. The peak response for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin at a mass-to-charge ratio of 319.90 is not more than the peak response of the associated internal standard at a mass-to-charge ratio of 331.88; the peak response for 2,3,7,8-tetrachlorodibenzofuran at a mass-to-charge ratio of 303.90 is not more than the peak response of the associated internal standard at a mass-to-charge ratio of 315.94.

Assay—

Standard preparation—Dissolve an accurately weighed quantity of USP Triclosan RS in ethyl acetate, and dilute quantitatively, and stepwise if necessary, with ethyl acetate to obtain a solution having a known concentration of about 0.4 mg per mL.

Assay preparation—Transfer about 40 mg of Triclosan, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with ethyl acetate to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector and a 0.53-mm × 15-m capillary column with phase G3. The carrier gas is helium maintained at about 6 psi. The injection port temperature is maintained at 34° and is increased rapidly to 200° immediately after the injection, the column temperature is maintained at 34°, and the detector temperature is maintained at 260°. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.

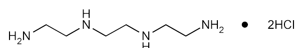
Procedure—Separately inject equal volumes (about 2.0 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, increase the column temperature by 20° per minute to 140°, then increase the column temperature by 4° per minute to 240°, maintain this temperature for not less than 5 minutes, record the chromatograms, and

measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{12}H_7Cl_3O_2$ in the portion of Triclosan taken by the formula:

$$100C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Triclosan RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Trientine Hydrochloride



$C_6H_{18}N_4 \cdot 2HCl$ 219.16
1,2-Ethanediamine, *N,N′*-bis(2-aminoethyl)-, dihydrochloride.
Triethylenetetramine dihydrochloride [38260-01-4].

» Trientine Hydrochloride contains not less than 97.0 percent and not more than 103.0 percent of $C_6H_{18}N_4 \cdot 2HCl$, calculated on the dried basis.

Packaging and storage—Preserve under an inert gas in tight, light-resistant containers, and store in a refrigerator.

USP Reference standards ⟨11⟩—
USP Trientine Hydrochloride RS

Identification, *Infrared Absorption* ⟨197M⟩.

pH ⟨791⟩: between 7.0 and 8.5, in a solution (1 in 100).

Loss on drying ⟨731⟩—Dry it in vacuum at a pressure not exceeding 5 mm of mercury at 40° for 4 hours: it loses not more than 2.0% of its weight.

Residue on ignition ⟨281⟩: not more than 0.15%.

Heavy metals, *Method II* ⟨231⟩: 0.001%.

Chromatographic purity—The sum of the intensities of all secondary spots obtained from the *Test preparation* in *Part I* and *Part II* corresponds to not more than 2.0%.

Part I—

*Spray reagent—*Dissolve 300 mg of ninhydrin in a mixture of 100 mL of butyl alcohol and 3 mL of glacial acetic acid.

Standard preparation A—[NOTE—Use low-actinic glassware.] Dissolve an accurately weighed quantity of USP Trientine Hydrochloride RS in methanol to obtain a solution containing 10 mg per mL.

Standard preparation B—[NOTE—Use low-actinic glassware.] Dissolve an accurately weighed quantity of diethylenetriamine in methanol to obtain a solution containing 1.0 mg per mL. Transfer 3.0 mL of this solution to a 100-mL volumetric flask, dilute with methanol to volume, and mix.

Standard preparation C—[NOTE—Use low-actinic glassware.] Dissolve an accurately weighed quantity of 1-(2-aminoethyl)piperazine in methanol to obtain a solution containing 1.0 mg per mL. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with methanol to volume, and mix.

Standard preparation D—[NOTE—Use low-actinic glassware.] Transfer 5.0 mL of *Standard preparation C* to a 10-mL volumetric flask, dilute with methanol to volume, and mix.

Test preparation—[NOTE—Use low-actinic glassware.] Dissolve an accurately weighed quantity of Trientine Hydrochloride in methanol to obtain a solution containing 10 mg per mL.

*Procedure—*Apply separately 3 µL each of the *Test preparation*, of *Standard preparation B,* and of *Standard preparation C* to a suitable unwashed, high performance thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) having a 1.5-cm preadsorbent zone and coated with a 0.15-mm layer of chromatographic silica gel mixture. To a fourth spot, apply 3 µL each of *Standard preparations A*, *B*, and *C*. To a fifth spot, apply 3 µL each of *Standard preparations A*, *B*, and *D*. Allow the spots to dry, place the plate in a chromatographic chamber, and develop the chromatograms in a solvent system consisting of a mixture of isopropyl alcohol and ammonium hydroxide (3:2) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and dry the plate with the aid of a current of air. Spray the plate with *Spray reagent*, dry at 105° for 5 minutes, and observe the plate under long-wavelength UV light. Determine the locus of the diethylenetriamine and the 1-(2-aminoethyl)piperazine spots from the chromatograms of *Standard preparations B* and *C*, respectively. Determine the concentration of diethylenetriamine in the *Test preparation* by comparing the size and intensity of any secondary spot from the chromatogram of the *Test preparation* having an R_F value corresponding to the R_F value of diethylenetriamine with the diethylenetriamine spots obtained from the chromatograms of the *Standard preparation* mixtures. Determine the concentration of any other observed impurities in the *Test preparation* by comparing the size and intensity of any other secondary spots from the chromatogram of the *Test preparation* with the 1-(2-aminoethyl)piperazine spots obtained from the chromatograms of the *Standard preparation* mixtures.

Part II—

*Spray reagent—*Dissolve 200 mg of ninhydrin in 100 mL of alcohol.

Tris(2-aminoethyl)amine stock solution—[NOTE—Use low-actinic glassware.] Dissolve an accurately weighed quantity of tris(2-aminoethyl)amine in methanol to obtain a solution containing 1.0 mg per mL.

Standard preparation A—[NOTE—Use low-actinic glassware.] Dissolve an accurately weighed quantity of USP Trientine Hydrochloride RS in methanol to obtain a solution containing 10 mg per mL.

Standard preparation B—[NOTE—Use low-actinic glassware.] Transfer 1.0 mL of *Tris(2-aminoethyl)amine stock solution* to a 10-mL volumetric flask, dilute with methanol to volume, and mix.

Standard preparation C—[NOTE—Use low-actinic glassware.] Transfer 0.5 mL of *Tris(2-aminoethyl)amine stock solution* to a 10-mL volumetric flask, dilute with methanol to volume, and mix.

Test preparation—[NOTE—Use low-actinic glassware.] Dissolve an accurately weighed quantity of Trientine Hydrochloride in methanol to obtain a solution containing 10 mg per mL.

*Procedure—*Apply separately 3 µL each of the *Test preparation* and of *Standard preparation A* to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture and previously washed with methanol. To a third spot apply 3 µL each of *Standard preparations A* and *B.* To a fourth spot, apply 3 µL each of *Standard preparations A* and *C.* Allow the spots to dry, place the plate in a chromatographic chamber, and develop the chromatograms in a solvent system consisting of a mixture of ammonium hydroxide and alcohol (2:1) at a temperature of 2° to 6° until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and dry the plate with the aid of a current of air. Spray the plate with *Spray reagent*, dry at 105° for 5 minutes, and observe the plate under long-wavelength UV light. Determine the concentration of tris(2-aminoethyl)amine in the *Test preparation* by comparing the size and intensity of any secondary spot from the chromatogram of the *Test preparation* having an R_F value corresponding to the R_F value of tris(2-aminoethyl)amine with the tris(2-ami-