this solution to a 100-mL volumetric flask, dilute with 0.01 M sodium hydroxide to volume, mix, and filter.

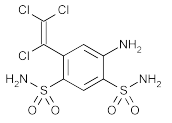
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 230-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses of the major peaks. Calculate the quantity, in mg, of clorazepate dipotassium ($C_{16}H_{11}ClK_2N_2O_4$) in the portion of T ablets taken by the formula:

$$1333C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Clorazepate Dipotassium 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.

Clorsulon



$C_8H_8Cl_3N_3O_4S_2$ 380.66
1,3-Benzenedisulfonamide, 4-amino-6-(trichloroethenyl)-.
4-Amino-6-(trichlorovinyl)-*m*-benzenedisulfonamide [60200-06-8].

» Clorsulon contains not less than 98.0 per cent and not more than 101.0 per cent of $C_8H_8Cl_3N_3O_4S_2$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.
Labeling—Label it to indicate that it is for veterinar y use only.

USP Reference standards ⟨11⟩**—**
USP Clorsulon RS

Identification—

A: *Infrared Absorption* ⟨197M⟩.

B: The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for clorsulon, the retention time of which corresponds to that of the *Standard preparation* obtained as directed in the *Assay*.

Melting range ⟨741⟩: between 197° and 203°.

Loss on drying ⟨731⟩**—**Dry it in vacuum at 100 ° for 4 hours: it loses not more than 0.5% of its weight.

Residue on ignition ⟨281⟩: not more than 0.1%.

Heavy metals, *Method II* ⟨231⟩: 0.003%.

Chromatographic purity—[NOTE—The *Standard solutions* and *Test solutions* should be stored in low-actinic glassware.] Prepare a solution of Clorsulon in methanol containing 10.0 mg per mL *(Test solution)*. Prepare a solution of USP Clorsulon RS in methanol containing 10.0 mg per mL (*Standard solution A*). Transfer 1.0 mL of *Standard solution A* to a 100-mL volumetric flask, dilute with methanol to volume, and mix (*Standard solution B*). Apply 10-µL portions of the *Test solution* and of *Standard solution A*, and 5- and 10- µL portions of *Standard solution B* to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩), coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dr y, and develop the chromatograms in a solvent system consisting of a mixture of chloroform and methanol (4:1) 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, allow the solvent to evaporate, and examine the plate under short-wavelength UV light: the chromatograms show principal spots at about the same R_F value. Estimate the amounts of any additional spots obser ved in the chromatogram of the *Test solution* by comparing them with the spots in the two chromatograms obtained from *Standard solution B*, corresponding to 0.5% and 1.0% of impurity: no spot, other than the principal spot, in the chromatogram of the *Test solution* is larger or more intense than that of the principal spot in the chromatogram obtained from the 5-µL portion of *Standard solution B* (0.5%), and the sum of all such impurities is not more than 2.0%.

Assay—

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

Standard preparation—Dissolve an accurately weighed quantity of USP Clorsulon RS in *Mobile phase* to obtain a solution having a known concentration of about 0.1 mg per mL. Store the solution in low-actinic glassware.

Assay preparation—Transfer about 50 mg of Clorsulon, accurately weighed, to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. T ransfer 5.0 mL of this solution to a second 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Store the solution in low-actinic glassware.

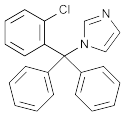
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L7. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the column efficiency is not less than 7400 theoretical plates; the tailing factor is not more than 1.4; and the relative standard deviation for replicate injections is not more than 1.0%.

Procedure—Separately inject equal volumes (about 30 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_8H_8Cl_3N_3O_4S_2$ in the portion of Clorsulon taken by the formula:

$$500C(r_U / r_S)$$

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

Clotrimazole



$C_{22}H_{17}ClN_2$ 344.84
1*H*-Imidazole, 1-[(2-chlorophenyl)diphenylmethyl]-;
1-(*o*-Chloro-α,α-diphenylbenzyl)imidazole [23593-75-1].

DEFINITION
Clotrimazole contains NLT 98.0% and NMT 102.0% of $C_{22}H_{17}ClN_2$, calculated on the dried basis.

IDENTIFICATION
- **A. Infrared Absorption** ⟨197M⟩
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay.*

ASSAY

• **PROCEDURE**

Buffer: 4.35 mg/mL of dibasic potassium phosphate

Mobile phase: Acetonitrile and *Buffer* (3:1). Pass through a membrane filter having a 0.2-µm or finer pore size. The ratio of volumes may be changed to obtain the required resolution.

Standard solution: 0.5 mg/mL of USP Clotrimazole RS in methanol

System suitability solution: 0.1 mg/mL each of USP Clotrimazole RS and USP Clotrimazole Related Compound A RS in methanol

Sample solution: 0.5 mg/mL of Clotrimazole in methanol

Chromatographic system

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

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1.5 mL/min

Injection size: 25 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for clotrimazole and clotrimazole related compound A are 1.0 and 1.2, respectively.]

Suitability requirements

Resolution: NLT 2.0 between clotrimazole and clotrimazole related compound A, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{22}H_{17}ClN_2$ in the portion of Clotrimazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of clotrimazole from the *Sample solution*

r_S = peak response of clotrimazole from the *Standard solution*

C_S = concentration of USP Clotrimazole RS in the *Standard solution* (mg/mL)

C_U = concentration of Clotrimazole in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

Inorganic Impurities

• **RESIDUE ON IGNITION** ⟨**281**⟩**:** NMT 0.1%

• **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 10 ppm

Organic Impurities

• **PROCEDURE 1: LIMIT OF IMIDAZOLE**

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Standard solution: 500 µg/mL of USP Imidazole RS in chloroform

Sample solution: 100 mg/mL of Clotrimazole in chloroform

Application volume: 5 µL

Developing solvent system: Methanol and chloroform (3:2)

Analysis

Samples: *Standard solution* and *Sample solution*

Proceed as directed for *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*. After air-drying the plate for 5 min, place it in a closed container with a dish containing 100 g of iodine in a shallow layer, and allow to remain for 60 min. Remove the plate from the container, and observe the chromatogram.

Acceptance criteria: Any brown spot from the *Sample solution* at an R_F value corresponding to the principal spot from the *Standard solution* is not greater in size or intensity than the principal spot from the *Standard solution:* NMT 0.5% of imidazole.

• **PROCEDURE 2: LIMIT OF CLOTRIMAZOLE RELATED COMPOUND A**

Buffer, Mobile phase, System suitability solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard solution: 50 µg/mL of USP Clotrimazole Related Compound A RS prepared by dissolving in methanol using about 75% of the final flask volume. Dilute with *Buffer* to volume.

Sample solution: Transfer 100 mg of Clotrimazole to a 10-mL volumetric flask, add 5 mL of methanol to dissolve, add 2.5 mL of *Buffer*, dilute with methanol to volume, and mix.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of clotrimazole related compound A in the portion of Clotrimazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of clotrimazole related compound A from the *Sample solution*

r_S = peak response of clotrimazole related compound A from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.5%

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨**731**⟩**:** Dry a sample at 105 ° for 2 h: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** ⟨**11**⟩

USP Clotrimazole RS

USP Clotrimazole Related Compound A RS

(*o*-Chlorophenyl)diphenylmethanol.

$C_{19}H_{15}ClO$ 294.78

USP Imidazole RS

Clotrimazole Cream

DEFINITION

Clotrimazole Cream contains NLT 90.0% and NMT 110.0% of the labeled amount of clotrimazole ($C_{22}H_{17}ClN_2$).

IDENTIFICATION

• The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: 4.35 mg/mL of dibasic potassium phosphate

Mobile phase: Acetonitrile and *Buffer* (3:1)

[NOTE—The ratio of volumes may be changed to obtain the required resolution.]

Standard solution: 0.5 mg/mL of USP Clotrimazole RS in methanol

System suitability solution: 0.1 mg/mL each of USP Clotrimazole RS and USP Clotrimazole Related Compound A RS in methanol

Sample solution: Transfer the equivalent of 25 mg of clotrimazole from the Cream to a 50-mL screw-capped centrifuge tube. Add 25.0 mL of methanol, and heat at 50 ° in a water bath for 5 min, with occasional shaking. Remove the tube from the bath, and shake vigorously for 5 min. Cool in a methanol–ice bath for 15 min, and promptly centrifuge. Transfer the supernatant to a 50-mL volumetric flask. Add 20.0 mL of methanol to the residue in the centrifuge tube, and repeat the extraction starting with "heat at 50 ° in a water bath". Transfer the supernatant to the volumetric flask containing the supernatant from the first extraction, dilute with methanol to volume, and mix.