Chlordiazepoxide Hydrochloride and Clidinium Bromide Capsules

DEFINITION

Chlordiazepoxide Hydrochloride and Clidinium Bromide Capsules contain NLT 90.0% and NMT 110.0% of the labeled amounts of chlordiazepoxide hydrochloride ($C_{16}H_{14}ClN_3O \cdot HCl$) and clidinium bromide ($C_{22}H_{26}BrNO_3$).

IDENTIFICATION

• **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

[NOTE—Use low-actinic glassware.]

Buffer: Dissolve 1.92 g of sodium 1-pentanesulfonate in 900 mL of water in a 1-L volumetric flask. Adjust with 1 N sulfuric acid to a pH of 3.8 ± 0.1. Dilute with water to volume.

Mobile phase: Methanol, tetrahydrofuran, and *Buffer* (6:24:70)

Diluent: Methanol and water (1:1)

Standard solution: 0.1 mg/mL of USP Chlordiazepoxide Hydrochloride RS and 0.05 mg/mL of USP Clidinium Bromide RS in *Diluent*

Sample solution: Weigh the contents of NLT 20 Capsules, and calculate the average weight per Capsule. Mix the combined contents of the Capsules, and transfer an amount equivalent to about 5 mg of chlordiazepoxide hydrochloride ($C_{16}H_{14}ClN_3O \cdot HCl$) to a 50-mL volumetric flask. Add about 25 mL of *Diluent*, sonicate for 5 min, and shake by mechanical means for 10 min. Dilute with *Diluent* to volume, and filter, discarding the first 20 mL of the filtrate.

Chromatographic system

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

Mode: LC

Detector: UV 212 nm

Column: 8-mm × 10-cm; packing L1

Flow rate: 3 mL/min

Injection size: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for clidinium bromide and chlordiazepoxide hydrochloride are about 0.5 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 5.0 between the clidinium bromide and chlordiazepoxide hydrochloride peaks

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of chlordiazepoxide hydrochloride ($C_{16}H_{14}ClN_3O \cdot HCl$) in the portion of Capsules taken:

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

r_U = peak response of chlordiazepoxide hydrochloride from the *Sample solution*

r_S = peak response of chlordiazepoxide hydrochloride from the *Standard solution*

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

C_U = nominal concentration of chlordiazepoxide hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of the labeled amount of clidinium bromide ($C_{22}H_{26}BrNO_3$) in the portion of Capsules taken:

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

r_U = peak response of clidinium bromide from the *Sample solution*

r_S = peak response of clidinium bromide from the *Standard solution*

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

C_U = nominal concentration of clidinium bromide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION,** *Procedure for a Pooled Sample* ⟨**711**⟩

Medium: Water; 900 mL

Apparatus 1: 100 rpm

Time: 30 min

Buffer: Dissolve 1.92 g of sodium 1-pentanesulfonate in 900 mL of water in a 1-L volumetric flask. Adjust with dilute sulfuric acid to a pH of 3.8 ± 0.1. Dilute with water to volume.

Mobile phase: Methanol, tetrahydrofuran, and *Buffer* (6:18:75)

Standard solution: Prepare a solution having known concentrations of USP Chlordiazepoxide Hydrochloride RS and USP Clidinium Bromide RS in *Medium*.

Sample solution: Pass a portion of the solution under test through a suitable filter. Combine equal volumes of the filtered solutions and use the pooled sample for the analysis. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*, if necessary.

Chromatographic system

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

Mode: LC

Detector: UV 212 nm

Column: 4-mm × 25-cm; packing L1

Flow rate: 2 mL/min

Injection size: 100 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for clidinium bromide and chlordiazepoxide hydrochloride are about 0.6 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 5.0 between the clidinium bromide and chlordiazepoxide hydrochloride peaks

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the average percentage of chlordiazepoxide hydrochloride ($C_{16}H_{14}ClN_3O \cdot HCl$) or clidinium bromide ($C_{22}H_{26}BrNO_3$) dissolved:

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

r_U = peak response of chlordiazepoxide hydrochloride or clidinium bromide from the *Sample solution*

r_S = peak response of chlordiazepoxide hydrochloride or clidinium bromide from the *Standard solution*

C_S = concentration of USP Chlordiazepoxide Hydrochloride RS or USP Clidinium Bromide RS in the *Standard solution* (mg/mL)

L = chlordiazepoxide hydrochloride or clidinium bromide label claim (mg)

V = volume of *Medium* (mL), 900

Tolerances: NLT 75% (*Q*) each of the labeled amounts of chlordiazepoxide hydrochloride ($C_{16}H_{14}ClN_3O \cdot HCl$) and clidinium bromide ($C_{22}H_{26}BrNO_3$) are dissolved.

• **Uniformity of Dosage Units ⟨905⟩:** Meet the requirements

IMPURITIES

• **Limit Of Chlordiazepoxide Related Compound A and 2-Amino-5-Chlorobenzophenone**

Standard solution A: 1 mg/mL of USP Chlordiazepoxide Related Compound A RS in acetone

Standard solution B: 50 µg/mL of USP 2-Amino-5-chlorobenzophenone RS in acetone

Sample solution: Transfer an amount equivalent to 25 mg of chlordiazepoxide hydrochloride from Capsule contents to a 10-mL conical flask, add 2.5 mL of acetone, and shake. Allow any undissolved particles to settle, and use the supernatant.

Chromatographic system

(See *Chromatography ⟨621⟩, Thin-Layer Chromatography*.)

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 50 µL for the *Sample solution*, 15 µL for *Standard solution A*, and 10 µL for *Standard solution B*

Developing solvent system: Ethyl acetate

Spray reagent: 2 N sulfuric acid

Analysis

Samples: *Standard solutions* and *Sample solution*

Proceed as directed in the chapter. Develop the chromatogram in a chromatographic chamber (not previously saturated with the developing solvent) in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with *Spray reagent*. Dry at 105° for 15 min, and then spray in succession with sodium nitrite solution (1 in 1000), ammonium sulfamate solution (1 in 200), and *N*-(1-naphthyl)ethylenediamine dihydrochloride solution (1 in 1000).

Acceptance criteria: Any spots from the *Sample solution* are not greater in size or intensity than the spots at the respective R_F values produced by the *Standard solutions*, corresponding to NMT 3.0% of chlordiazepoxide related compound A and to NMT 0.1% of 2-amino-5-chlorobenzophenone.

Delete the following:

•• **Limit of 3-Quinuclidinyl Benzilate**

Adsorbent: 0.25-mm layer of chromatographic silica gel

Sample solution: Place a glass wool plug in the bottom of a 2.5-cm × 35 ± 5-cm glass chromatographic tube, add 2 g of chromatographic siliceous earth triturated with 1 mL of 1 N hydrochloric acid, and lightly pack with a glass tamping rod. Empty a number of Capsules, equivalent to 15 mg of clidinium bromide, into a 100-mL beaker, add 3 mL of 1 N hydrochloric acid, and swirl to dissolve. Add 4 g of chromatographic siliceous earth, mix with a spatula, and add the Capsule–siliceous earth mixture to the chromatographic tube. Dry-wash the beaker with an additional 0.5–1.0 g of chromatographic siliceous earth, adding the washing to the top of the column. Lightly pack with the tamping rod, and overlay the column with glass wool. Insert the lower exit tube of the column into a 125-mL separator, and elute the column with 100 mL of chloroform previously distilled over 1 N sulfuric acid and saturated with water. Extract the chloroform eluate with 20 mL of freshly prepared ascorbic acid solution (1 in 20), preserving the extract. Extract the eluate with a second 15-mL portion of the ascorbic acid solution, combine the extracts in the separator, and discard the chloroform layer. Neutralize the acid extracts by adding sodium bicarbonate until the solution is slightly alkaline to the pH paper. Extract the slightly alkaline solution with two 25-mL portions of chloroform, combine the chloroform extracts, and pass through dry, fluted filter paper into a 100-mL beaker. Evaporate the chloroform to dryness with the aid of a stream of nitrogen, and transfer the residue to a glass-stoppered 1.0 mL volumetric flask, using methanol to facilitate the transfer. Dilute with methanol to volume.

Identification solution: 30 µg/mL of USP 3-Quinuclidinyl Benzilate RS in methanol

Spray reagent: Potassium iodoplatinate TS

Application volume: 100 µL for the *Sample solution*, 15 µL for the *Identification solution*

Developing solvent system: Methanol

Analysis

Samples: *Sample solution* and *Identification solution*

Place the plate in a paper-lined, methanol-saturated chromatographic chamber, and proceed as directed for *Chromatography ⟨621⟩, Thin-layer Chromatography*. Remove the plate, air-dry, spray with *Spray reagent*, and allow the spots to develop for 10 min. Any spot in the chromatogram of the *Sample solution* occurring at an R_F value of 0.3 is not greater in size or intensity than the spot in the chromatogram of the *Identification solution*.

Acceptance criteria: NMT 0.03% of 3-quinuclidinyl benzilate•• (RB 1-Oct-2010)

• **Limit of Clidinium Bromide Related Compound A**

Extracting solvent mixture: Dehydrated alcohol and cyclohexane (1:1)

Identification solution: Dissolve 50 mg of *USP Clidinium Bromide RS* in 1 mL of 0.1 N methanolic hydrochloric acid. To this solution add 20 µL of a solution of 25 mg/mL of USP Clidinium Bromide Related Compound A RS in methanol. Prepare this solution at the time of use.

Standard solution: 50 mg/mL of USP Clidinium Bromide RS in 0.1 N methanolic hydrochloric acid. [NOTE—Prepare this solution at the time of use.]

Sample solution: Empty a number of Capsules, equivalent to 25 mg of clidinium bromide, into a glass-stoppered centrifuge tube, and add 5 mL of the *Extracting solvent mixture*. Heat the tube gently, with shaking, to 50°, centrifuge, and decant the clear supernatant into a second tube. Repeat the addition of *Extracting solvent mixture* twice, heating, centrifuging, and decanting as before, and combine the three extracts in a single tube. Gently heating, evaporate the combined extracts under a stream of nitrogen to dryness. Dissolve the residue in 0.5 mL of methanol.

Chromatographic system:

(See *Chromatography ⟨621⟩, Thin-Layer Chromatography*.)

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 20 µL

Developing solvent system: Acetone, methanol, water, and hydrochloric acid (70:20:5:5)

Spray reagent: Dissolve 850 mg of bismuth subnitrate in a mixture of 10 mL of glacial acetic acid and 40 mL of water. In a separate container, dissolve 20 g of potassium iodide in 50 mL of water. Mix the two solutions, and dilute with dilute sulfuric acid (1 in 10) to 500 mL. Add 7.5 ± 2.5 g of iodine, and mix until solution is complete

Chromatographic plates: Predevelop suitable thin-layer chromatographic plates by placing in a chromatographic chamber saturated with *Developing solvent system*, and allow the *Developing solvent system* to move 15 cm. Remove the plates from the chamber, dry at 105° for 15 min, and cool.

Analysis

Samples: *Standard solution*, *Identification solution*, and *Sample solution*

Proceed as directed in the chapter. Place the plates in an unsaturated chromatographic chamber containing freshly prepared *Developing solvent system*, and develop the chromatogram until the solvent front has moved 15 cm. Remove the plates, and dry at 105° for 10 min. Cool to room temperature, and spray with *Spray reagent*. Any spot in the chromatogram of the *Sample solution* occurring at an R_F value of 0.4 is not greater in size or intensity than the corresponding spot in the chromatogram of the *Identification solution*; and the *Standard solution* shows no spot at the R_F value corresponding to that of clidinium bromide related compound A.

Acceptance criteria: NMT 1.0% of clidinium bromide related compound A

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩

USP 2-Amino-5-chlorobenzophenone RS
2-Amino-5-chlorobenzophenone.
$C_{13}H_{10}ClNO$ 231.68

USP Chlordiazepoxide Hydrochloride RS

USP Chlordiazepoxide Related Compound A RS
7-Chloro-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one 4-oxide.
$C_{15}H_{11}ClN_2O_2$ 286.72

USP Clidinium Bromide RS

USP Clidinium Bromide Related Compound A RS
3-Hydroxy-1-methylquinuclidinium bromide.
$C_8H_{16}BrNO$ 222.13)

- (RB 1-Oct-2010)