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Sulfamethoxazole and Trimethoprim Injection

DEFINITION

Sulfamethoxazole and Trimethoprim Injection is a sterile solution of Sulfamethoxazole and Trimethoprim in Water for Injection, which, when diluted with Dextrose Injection, is suitable for intravenous infusion. It contains NLT 90.0% and NMT 110.0% of the labeled quantities of sulfamethoxazole ($C_{10}H_{11}N_3O_3S$) and trimethoprim ($C_{14}H_{18}N_4O_3$).

IDENTIFICATION

• **A.** The R_F values of the principal spots of the *Sample solution* correspond to those of the *Standard solutions* of USP Trimethoprim RS (R_F about 0.5) and USP Sulfamethoxazole RS (R_F about 0.7), as obtained in the *Impurities* tests.

ASSAY

• **Procedure**

Mobile phase: Mix 1400 mL of water, 400 mL of acetonitrile, and 2.0 mL of triethylamine in a 2000-mL volumetric flask. Allow to equilibrate to room temperature, and adjust with 0.2 N sodium hydroxide or dilute glacial acetic acid (1 in 100) to a pH of 5.9 ± 0.1. Dilute with water to volume, and pass through a filter of 0.45-µm pore size.

Standard stock solution: 0.32 mg/mL of USP Trimethoprim RS and 0.32*J* mg/mL of USP Sulfamethoxazole RS in methanol, where *J* is the ratio of the labeled amount, in mg, of sulfamethoxazole to the labeled amount, in mg, of trimethoprim in the dosage form

Standard solution: 0.032 mg/mL of USP Trimethoprim RS per mL and 0.032*J* mg/mL of USP Sulfamethoxazole RS per mL in *Mobile phase* from *Standard stock solution*

Sample stock solution: Transfer a volume of Injection, equivalent to 80 mg of sulfamethoxazole, to a 50-mL volumetric flask, and dilute with methanol to volume.

Sample solution: Nominally 0.16 mg/mL of sulfamethoxazole in *Mobile phase* from *Sample stock solution*

Chromatographic system

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

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[Note—The relative retention times for trimethoprim and sulfamethoxazole are 1.0 and 1.8, respectively.]

Suitability requirements

Resolution: NLT 5.0 between sulfamethoxazole and trimethoprim

Tailing factor: NMT 2.0 for trimethoprim and sulfamethoxazole

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of trimethoprim ($C_{14}H_{18}N_4O_3$) and sulfamethoxazole ($C_{10}H_{11}N_3O_3S$) in the portion of Injection taken:

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

r_U = peak response of trimethoprim or sulfamethoxazole from the *Sample solution*

r_S = peak response of trimethoprim or sulfamethoxazole from the *Standard solution*

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

C_U = nominal concentration of trimethoprim or sulfamethoxazole in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% each of sulfamethoxazole ($C_{10}H_{11}N_3O_3S$) and trimethoprim ($C_{14}H_{18}N_4O_3$)

IMPURITIES

• **Limit of Trimethoprim Degradation Product**

Solution A: 0.06 N hydrochloric acid solution

Standard solution A: 48 mg/mL of USP Trimethoprim RS in chloroform and methanol (1:1)

Standard solution B: 240 µg/mL of USP Trimethoprim RS in a mixture of chloroform and methanol (1:1) from *Standard solution A*

Sample solution: Transfer a volume of Injection, equivalent to 48 mg of trimethoprim and 240 mg of sulfamethoxazole, to a glass-stoppered, 50-mL centrifuge tube. Add 15 mL of *Solution A*, and mix. Add 15 mL of chloroform, shake for 30 s, and centrifuge at high speed for 3 min. Transfer the supernatant layer to a 125-mL separator. Extract the chloroform layer in the centrifuge tube with 15 mL of *Solution A*, centrifuge at high speed, and add the extract to the separator. Add 2 mL of sodium hydroxide solution (100 mg/mL) to the solution in the separator, and extract with three 20-mL portions of chloroform, collecting the organic layer in a 125-mL conical flask. Evaporate the chloroform under a stream of nitrogen to dryness. Dissolve the residue in 1 mL of a mixture of chloroform and methanol (1:1).

Chromatographic system

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

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 10 µL

Developing solvent system: Chloroform, methanol, and ammonium hydroxide (97:7.5:1)

Spray reagent: Freshly prepared mixture of ferric chloride solution (100 mg/mL) and potassium ferricyanide solution (50 mg/mL) (1:1)

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Apply the *Samples* to separate points on the plate. Develop the chromatogram using *Developing solvent system* until the solvent front has moved at least 12 cm. Remove the plate from the developing chamber, air-dry, and spray with *Spray reagent*. Locate the bands by viewing under short-wavelength UV light. Trimethoprim produces a spot at about R_F 0.5, and the trimethoprim degradation product produces a spot at about R_F 0.6–0.7. [Note—There may be spots due to concentrate excipients at about R_F 0.1.]

Acceptance criteria: Any spot from the *Sample solution* at about R_F 0.6–0.7 is not greater in size and intensity than the spot produced by *Standard solution B* at about R_F 0.5, corresponding to NMT 0.5%.

• **Limit of Sulfanilamide and Sulfanilic Acid**

Solution A: Methanol and dehydrated alcohol (5:95)

Solution B: Dilute 1 mL of ammonium hydroxide with *Solution A* to 100 mL

Standard solution A: 10 mg/mL of USP Sulfamethoxazole RS in *Solution B*

Standard solution B: 0.05 mg/mL of USP Sulfanilamide RS in *Solution B*

Standard solution C: 0.03 mg/mL of USP Sulfanilic Acid RS in *Solution B*

Sample solution: Transfer a volume of Injection, equivalent to 32 mg of trimethoprim and 160 mg of sulfamethoxazole, into a 25-mL graduated cylinder. Dilute with *Solution B* to 16 mL.

Chromatographic system

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

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 10 µL

Developing solvent system: *Solution A*, heptane, chloroform, and glacial acetic acid (30:30:30:10)

Spray reagent (Modified Ehrlich's reagent): 100 mg of *p*-dimethylaminobenzaldehyde in 1 mL of hydrochloric acid. Dilute with alcohol to 100 mL.

Analysis

Samples: *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Sample solution*

Apply the *Samples* to separate points on the plate. Develop the chromatogram in the *Developing solvent system* until the solvent front has moved NLT 12 cm. Remove the plate from the developing chamber, air-dry, spray with *Spray reagent*, and allow the plate to stand for 15 min. Sulfamethoxazole produces a spot at about R_F 0.7.

Acceptance criteria: Any spots from the *Sample solution* at about R_F 0.5 or 0.1 are not greater in size or intensity than spots produced by *Standard solution B* and *Standard solution C*, respectively, corresponding to NMT 0.5% of sulfanilamide and 0.3% of sulfanilic acid.

SPECIFIC TESTS

- **pH ⟨791⟩:** 9.5–10.5
- **Pyrogen Test ⟨151⟩:** Meets the requirements, the test dose being 0.5 mL/kg
- **Particulate Matter in Injections ⟨788⟩:** Meets the requirements for small-volume injections
- **Other Requirements:** It meets the requirements in *Injections and Implanted Drug Products ⟨1⟩*.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in single-dose, light-resistant containers, preferably of Type I glass. It may be packaged in 50-mL multiple-dose containers.
- **Labeling:** Label it to indicate that it is to be diluted with 5% Dextrose Injection before administration.
- **USP Reference Standards ⟨11⟩**

USP Sulfamethoxazole RS

USP Sulfanilamide RS

p-Aminobenzenesulfonamide.

$C_6H_8N_2O_2S$ 172.20

USP Sulfanilic Acid RS

Benzenesulfonic acid, 4-amino-.

$C_6H_7NO_3S$ 173.19

USP Trimethoprim RS

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
SULFAMETHOXAZOLE AND TRIMETHOPRIM INJECTION	Documentary Standards Support	SM12020 Small Molecules 1
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	SM12020 Small Molecules 1

Chromatographic Database Information: Chromatographic Database

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