Sulfamethoxazole and trimethoprim intravenous infusion (Sulfamethoxazoli et trimethoprimi infusio intraveno)

2014-01

Other name. Co-trimoxazole intravenous infusion

Category. Antibacterials.

Requirements

Comply with the monograph for *Parenteral preparations*.

Definition. Sulfamethoxazole and Trimethoprim intravenous infusion is a sterile solution containing Trimethoprim and sodium derivative of Sulfamethoxazole. It is prepared immediately before use by diluting Sulfamethoxazole and Trimethoprim sterile concentrate according to the manufacturers' instructions.

SULFAMETHOXAZOLE AND TRIMETHOPRIM STERILE CONCENTRATE

Description. A colourless or slightly yellow solution.

Storage: Sulfamethoxazole and Trimethoprim sterile concentrate should be kept in tightly-closed, single-dose, light-resistant, glass containers.

Additional information. Strengths in the current WHO Model list of essential medicines: 80 mg per mL Sulfamethoxazole, 16 mg per mL Trimethoprim in 5 mL or 10 mL ampoule.

Strengths in the current WHO Model list of essential medicines for children: 80 mg per mL Sulfamethoxazole, 16 mg per mL Trimethoprim in 5 mL or 10 mL ampoule.

Requirements

Comply with the monograph for *Parenteral preparations*.

Definition. Sulfamethoxazole and Trimethoprim sterile concentrate is a sterile solution containing Trimethoprim and the sodium derivative of Sulfamethoxazole. It contains not less than 90.0% and not more than 110.0% of the amounts of Sulfamethoxazole ($C_{10}H_{11}N_3O_3S$) and Trimethoprim ($C_{14}H_{18}N_4O_3$) stated on the label.

Identity tests

-Either tests A, E and F or tests D, E and F or tests B and C may be applied.

A. Carry out test A.1 or, where UV detection is not available, test A.2.

A.1 Carry out the test as described under *1.14.1. Thin-layer chromatography* using silica gel R6 as the coating substance and a mixture of 100 volumes of dichloromethane R, 10 volumes of methanol R and 5 volumes of dimethylformamide R as the mobile phase. Apply separately to the plate 5 µl of each of the following two solutions in methanol R. For solution (A) evaporate to dryness on a steam bath a volume of the concentrate, containing about 0.16 g of Sulfamethoxazole, shake the residue with 8 mL of methanol R and filter. For solution (B) use 20 mg of sulfamethoxazole RS and 4 mg of trimethoprim RS per mL. After removing the plate from the chromatographic chamber allow it to dry exhaustively in air or in a current of cool air. Examine the chromatogram in ultraviolet light (254 nm).

The principal spots obtained with solution (A) correspond in position, appearance and intensity to those obtained with solution (B).

A.2 Carry out the test as described under *1.14.1 Chromatography*, Thin-layer chromatography using silica gel R5 as the coating substance and the conditions described above under test A.1. Spray the plate with potassium iodobismuthate TS2 solution.

The principal spots obtained with solution (A) correspond in position, appearance and intensity to those obtained with solution (B).

B. Add drop-wise to 75 mL of hydrochloric acid (~3.65 g/l) TS a volume of the concentrate containing about 0.8 g of Sulfamethoxazole, stirring continuously. Allow the suspension to stand for 5 minutes and filter through a sintered-glass filter. Wash the residue with 10 mL of water R, recrystallize from ethanol (~750 g/l) TS and dry at 105 °C. Dissolve the residue in a minimum volume of sodium carbonate (~50 g/l) TS, add hydrochloric acid (~36.5 g/l) TS drop-wise until precipitation is complete, filter, wash the residue sparingly with water R and dry at 105 °C. Carry out the test as described under *1.7 Spectrophotometry in the infrared region*. The infrared absorption spectrum of the residue is concordant with the spectrum obtained from sulfamethoxazole RS or with the *reference spectrum* of sulfamethoxazole.

C. To a volume of the concentrate containing about 80 mg of Trimethoprim add 30 mL of sodium hydroxide (~4 g/l) TS and extract with two quantities, each of 50 mL, of dichloromethane R. Wash the combined extracts with two quantities, each of 10 mL, of sodium hydroxide (~4 g/l) TS and then with 10 mL of water R. Shake with 5 g of anhydrous sodium sulfate R, filter and evaporate the filtrate to dryness. Carry out the test as described under *1.7 Spectrophotometry in the infrared region*. The infrared absorption spectrum of the residue is concordant with the spectrum obtained from trimethoprim RS or with the *reference spectrum* of trimethoprim.

D. See the test described under "Assay", Method A. The retention times of two principal peaks in the chromatogram obtained with solution (1) correspond to those in the chromatogram obtained with solution (2).

E. Dilute a volume of concentrate containing about 80 mg of sulfamethoxazole to 10 mL with water R. Add 1 mL of sodium hydroxide (~4 g/l) TS and 3 mL of 1% copper sulphate (~10g/l) TS drop by drop until the colour change is complete. A green precipitate is produced.

F. Evaporate a volume of concentrate containing 32 mg of trimethoprim to dryness on a water bath. To the residue add a drop of ammonium vanadate TS; a dark brown colour is produced.

pH value (1.13). pH of the solution, 9.5–11.0

Bacterial endotoxins. Carry out the test described under *3.4 Test for bacterial endotoxins*, contains not more than 6 IU of endotoxin per mg Sulfamethoxazole.

Related substances

Trimethoprim related substance

Carry out the test as described under *1.14.1. Thin-layer chromatography* using silica gel R4 as the coating substance and a mixture of 97 volumes of chloroform R, 7.5 volumes of methanol R and 1 volume of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 10 µl of each of the following two solutions. For solution (A) transfer an accurately measured volume of the concentrate, containing about 48 mg of Trimethoprim, to a glass-stoppered, 50 mL centrifuge tube. Add 15 mL of hydrochloric acid (~2.19 g/l) TS and mix. Add 15 mL of dichloromethane R, shake for 30 seconds and centrifuge for 3 minutes. Transfer the supernatant layer to a 125 mL separator. Extract the dichloromethane layer in the centrifuge tube with 15 mL of hydrochloric acid (~2.19 g/l) TS, centrifuge and add the aqueous layer to the separator. Add 2 mL of sodium hydroxide (~100 g/l) TS to the solution in the separator and extract with three 20 mL portions of dichloromethane R, collecting the organic layer in a 125 mL conical flask. Evaporate the dichloromethane under a stream of nitrogen to dryness. Dissolve the residue in 1.0 mL of a mixture of equal volumes of dichloromethane R and methanol R (solvent mixture). For solution (C) use a solution of 240 µg of trimethoprim RS per mL solvent mixture. After removing the plate from the chromatographic chamber allow it dry in air, spray with ferric chloride/potassium ferricyanide TS1 and examine the chromatogram in ultraviolet light (254 nm).

Trimethoprim and related substance have the following R_f values: trimethoprim about 0.5; and the trimethoprim degradation product about 0.6–0.7. In the chromatogram obtained with solution (A) any spot corresponding to the trimethoprim degradation product is not greater in size and intensity than the spot obtained with solution (C) (0.5%).

Sulfamethoxazole related substances

Either test A or test B may be applied.

A. Carry out the test as described under *1.14.1. Thin-layer chromatography* using silica gel R5 as the coating substance. As the mobile phase use a mixture of 25 volumes of ethanol/methanol (95/5) TS, 25 volumes of heptane R, 25 volumes of dichloromethane R and 7 volumes of glacial acetic acid R. Apply separately to the plate 10 µl of each of the following four solutions. For solution (A) transfer an accurately measured volume of the concentrate, containing about 160 mg of Sulfamethoxazole, to an evaporating dish. Evaporate the sample to dryness using a steam bath. Reconstitute the residue with 16 mL of ammonia/ethanol/methanol (1/95/5) TS. For solution (B) use 0.05 mg of sulfanilamide R per mL ammonia/ethanol/methanol (1/95/5) TS. For solution (C) use 0.03 mg of sulfanilic acid R per mL ammonia/ethanol/methanol (1/95/5) TS. For solution (D) dissolve 10 mg of sulfamethoxazole RS in 1.0 mL of a solution containing 0.05 mg of sulfanilamide R and 0.03 mg of sulfanilic acid R per mL of ammonia/ethanol/methanol (1/95/5) TS. After removing the plate from the chromatographic chamber allow it dry in air, spray with 4-dimethylaminobenzaldehyde TS7, allow the plate to stand for 15 minutes and examine the chromatogram.

In the chromatogram obtained with solution (A) any spot corresponding to sulfanilamide is not greater in size or intensity than the spot obtained with solution (B) (0.5%) and any spot corresponding to sulfanilic acid is not greater in size or intensity than the spot obtained with solution (C) (0.3%). The test is not valid unless the chromatogram obtained with solution (D) shows three clearly separated principal spots.

B. Carry out the test described under *1.14.1 Chromatography*, High-performance liquid chromatography using the conditions given below under "Assay", Method A.

Prepare the following solutions. For solution (1) transfer 1.0 mL of the concentrate containing 80 mg of Sulfamethoxazole into a test tube. Add 7 mL of the mobile phase and mix. Transfer 5.0 mL of this solution to a 100 mL volumetric flask, dilute with the mobile phase to volume, mix and filter. For solution (2) prepare 5 mg/mL of sulfanilamide R in ammonia/methanol (10/90) TS. Dilute 5.0 mL of this solution to 50.0 mL with the mobile phase. For solution (3) prepare 3 mg/mL of sulfanilic acid R in ammonia/methanol (10/90) TS. Dilute 5.0 mL of this solution to 50.0 mL with mobile phase. For solution (4) transfer 1.0 mL of each of solution (2) and (3) into a 200 mL volumetric flask and make up to volume with mobile phase. For solution (5) accurately weigh 50 mg of sulfamethoxazole RS in a 100 mL volumetric flask and dilute with solution (4) to volume.

Inject separately 20 µl each of solutions (1), (4) and (5) and record the chromatogram for 1.5 times the retention time of sulfamethoxazole.

In the chromatogram obtained with solution (5) the three principal peaks are eluted at the following relative retention times with reference to sulfamethoxazole (retention time about 11 minutes): sulfanilic acid about 0.2; sulfanilamide about 0.3. The test is not valid unless for solution (5) the resolution factor between the peaks due to sulfanilic acid and to sulfanilamide is at least 5.0 and the resolution factor between the peaks due to sulfanilamide and sulfamethoxazole is at least 10.

Measure the areas of the peak responses obtained in the chromatograms from solution (1) and (4). In the chromatogram obtained with solution (1): the area of any peak corresponding to sulfanilic acid is not more than the area of the peak due to sulfanilic acid in the chromatogram obtained with solution (4) (0.3%) and the area of any peak corresponding to sulfanilamide is not greater than the area of the peak due to sulfanilamide in the chromatogram obtained with solution (4) (0.5%).

Assay

-Either method A or methods B and C may be applied.

A. Carry out the test as described under *1.14.1 Chromatography*, High-performance liquid chromatography using a stainless steel column (25 cm x 4.6 mm) packed with particles of base-deactivated silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl groups (5 µm). As the mobile phase use a solution prepared as follows: mix 1400 mL of water R, 400 mL of acetonitrile R and 2.0 mL of triethylamine R in a 2000 mL volumetric flask. Allow to equilibrate to room temperature and adjust with acetic acid (~10 g/l) TS to pH 5.9. Dilute to volume with water R and filter.

Prepare the following solutions. For solution (1) transfer an accurately measured volume of the concentrate containing about 80 mg of Sulfamethoxazole into a 50 mL volumetric flask. Add methanol R to volume and mix. Transfer 5.0 mL of this solution to a 50 mL volumetric flask, dilute with the mobile phase to volume, mix and filter. For solution (2) prepare a solution of 0.32 mg of trimethoprim RS and 1.60 mg of sulfamethoxazole RS per mL methanol R. Dilute 5.0 mL of this solution to 50.0 mL with the mobile phase.

Operate with a flow rate of 1.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 254 nm.

Inject separately 20 µl each of solutions (1) and (2) and record the chromatogram for 1.5 times the retention time of sulfamethoxazole. In the chromatogram obtained with solution (2) the peak due to trimethoprim is eluted at a relative retention of 0.53 with reference to sulfamethoxazole (retention time about 11 minutes). The test is not valid unless the resolution factor between the peaks due to sulfamethoxazole and to trimethoprim is at least 5.0.

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2) and calculate the content of Sulfamethoxazole ($C_{10}H_{11}N_3O_3S$) and Trimethoprim ($C_{14}H_{18}N_4O_3$) in the concentrate using the declared content of $C_{10}H_{11}N_3O_3S$ and $C_{14}H_{18}N_4O_3$ in sulfamethoxazole RS and trimethoprim RS.

B. To an accurately measured volume of the concentrate, containing about 48 mg of Trimethoprim, add 30 mL of sodium hydroxide (~4 g/l) TS and extract with four quantities, each of 50 mL, of dichloromethane R, washing each extract twice with a quantity of 10 mL of sodium hydroxide (~4g/l) TS. Combine the dichloromethane extracts and extract with four quantities of 50 mL of acetic acid (~60 g/l) TS. Wash the combined aqueous extracts with 5 mL of dichloromethane R and dilute to 250.0 mL with acetic acid (~60 g/l). To 10.0 mL of this solution add 10 mL of acetic acid (~60 g/l) and dilute to 100.0 mL with water R. Measure the absorbance of the resulting solution at the maximum at 271 nm.

Calculate the amount of Trimethoprim ($C_{14}H_{18}N_4O_3$) using the absorptivity value of 20.4 ($A^{1\%}_{1cm}$ = 204).

C. To an accurately measured volume of the concentrate containing about 0.4 g of Sulfamethoxazole add 60 mL

of water R and 10 mL of hydrochloric acid (~420 g/l) TS. Add 3 g of potassium bromide R, cool in ice and titrate slowly with sodium nitrite (0.1 mol/l) VS, stirring constantly and determining the end-point potentiometrically.

Each mL of sodium nitrite (0.1 mol/l) VS is equivalent to 25.33 mg of Sulfamethoxazole ($C_{10}H_{11}N_3O_3S$).