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Warfarin Sodium for Injection

DEFINITION

Warfarin Sodium for Injection is a sterile, freeze-dried mixture of Warfarin Sodium and suitable added substances. It contains NLT 95.0% and NMT 105.0% of the labeled amount of warfarin sodium ($C_{19}H_{15}NaO_4$). It may contain a suitable buffer.

IDENTIFICATION

- **A. Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*: 197K**

  Sample: Dissolve 100 mg in 25 mL of water, and adjust with hydrochloric acid to a pH of less than 3, using short-range pH indicator paper. Stir the mixture, and allow the precipitate to coagulate. Filter the mixture. Wash the precipitate with four 5-mL portions of water, and dry under vacuum over phosphorus pentoxide for 4 h. Use the warfarin obtained.

  Standard: Use USP Warfarin RS.

- **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: Transfer 1.36 g of monobasic potassium phosphate to a 200-mL volumetric flask, and dissolve in 50 mL of water. Add 39.1 mL of 0.2 N sodium hydroxide, and dilute with water to volume. Adjust with sodium hydroxide or phosphoric acid to a pH of 7.4 ± 0.1.

  Mobile phase: Methanol, glacial acetic acid, and water (64:1:36)

  Standard stock solution: 0.94 mg/mL of USP Warfarin RS prepared as follows. Transfer USP Warfarin RS to a suitable volumetric flask, and dissolve in 0.1 N sodium hydroxide equivalent to 39% of the final volume. Add 0.2 M monobasic potassium phosphate, equivalent to 25% of the final volume, and dilute with water to volume.

  Standard solution: Transfer 5 mL of *Standard stock solution* into a 50-mL volumetric flask, and dilute with *Buffer* to volume.

  Sample solution: Nominally 0.1 mg/mL of warfarin sodium in *Buffer* prepared as follows. Dissolve the contents of NLT 10 containers of Warfarin Sodium for Injection in *Buffer*.

  Chromatographic system

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

  Mode: LC

  Detector: UV 280 nm

  Column: 4.6-mm × 25-cm; packing L7

  Flow rate: 1.4 mL/min

  Injection volume: 20 µL

  System suitability

  Sample: *Standard solution*

  Suitability requirements

  Relative standard deviation: NMT 1.0%

  Analysis

  Samples: *Standard solution* and *Sample solution*

  Calculate the percentage of the labeled amount of warfarin sodium ($C_{19}H_{15}NaO_4$) in the portion of Warfarin Sodium for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

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

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

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

C_U = nominal concentration of warfarin sodium in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of warfarin sodium, 330.31

M_{r2} = molecular weight of warfarin, 308.33

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

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

IMPURITIES

• **Organic Impurities**

Diluent: Methanol and water (25:75)

Mobile phase: Methanol, tetrahydrofuran, glacial acetic acid, and water (53:1:1:47)

Standard stock solution 1: 0.126 mg/mL of USP Warfarin RS prepared as follows. Transfer USP Warfarin RS to a suitable volumetric flask, and add 0.1 N sodium hydroxide and methanol equivalent to 2% and 25% of the final volume, respectively. Sonicate to dissolve. Dilute with water to volume.

Standard stock solution 2: 2.4 µg/mL of USP Warfarin RS in *Diluent* from *Standard stock solution 1*

Standard solution: 1.2 µg/mL of USP Warfarin RS in *Diluent* from *Standard stock solution 1*

System suitability stock solution 1: 0.24 mg/mL of USP Warfarin Related Compound A RS in methanol

System suitability stock solution 2: 2.4 µg/mL of USP Warfarin Related Compound A RS in *Diluent* from *System suitability stock solution 1*

System suitability solution: 1.2 µg/mL of each of USP Warfarin RS and USP Warfarin Related Compound A RS in *Diluent* from *Standard stock solution 2* and *System suitability stock solution 2*

Sample solution: Nominally equivalent to 0.5 mg/mL of warfarin sodium in *Diluent* prepared as follows. Transfer the contents of NLT 10 vials of Warfarin Sodium for Injection into a suitable volumetric flask, dissolve, and dilute with *Diluent* to volume.

Chromatographic system

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

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; packing L11

Column temperature: 35°

Flow rate: 1.5 mL/min

Injection volume: 100 µL

Run time: NLT 4 times the retention time of warfarin

System suitability

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

[Note—The relative retention times for warfarin and warfarin related compound A are about 1.0 and 1.4, respectively.]

Suitability requirements

Resolution: NLT 2 between warfarin and warfarin related compound A peaks, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of Alice's ketone in the portion of Warfarin Sodium for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

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

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

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

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

M_{r1} = molecular weight of warfarin sodium, 330.31

M_{r2} = molecular weight of warfarin, 308.33

F = relative response factor for Alice's ketone, 0.9

[NOTE—Alice's ketone is a sodium salt of warfarin related compound A, 3-(*o*-hydroxyphenyl)-5-phenyl-2-cyclohexen-1-one sodium salt.]

Acceptance criteria: NMT 0.5%

SPECIFIC TESTS

• **WATER DETERMINATION, *Method I* ⟨921⟩:** NMT 4.5%

• **pH ⟨791⟩**

Sample solution: 10 mg/mL

Acceptance criteria: 7.2–8.3

• **CONSTITUTED SOLUTION:** Meets the requirements in *Injections and Implanted Drug Products ⟨1⟩, Specific Tests, Completeness and clarity of solutions*

• **BACTERIAL ENDOTOXINS TEST ⟨85⟩:** NMT 24.0 of USP Endotoxin Units/mg of warfarin sodium

• **STERILITY TESTS ⟨71⟩:** Meets the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in light-resistant containers as described in *Packaging and Storage Requirements ⟨659⟩, Injection Packaging, Packaging for constitution*. Store at controlled room temperature.

• **LABELING:** Meets the requirements in *Injections and Implanted Drug Products ⟨1⟩, Specific Tests, Completeness and clarity of solutions*

Change to read:

• **USP REFERENCE STANDARDS ⟨11⟩**

USP Warfarin RS

USP Warfarin Related Compound A RS

3-(▲2▲ (ERR 1-Feb-2022) -Hydroxyphenyl)-5-phenyl-2-cyclohexen-1-one.

$C_{18}H_{16}O_2$ ▲264.32▲ (ERR 1-Feb-2022)

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

Topic/Question	Contact	Expert Committee
WARFARIN SODIUM FOR INJECTION	Documentary Standards Support	SM22020 Small Molecules 2
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	SM22020 Small Molecules 2

Chromatographic Database Information: Chromatographic Database

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