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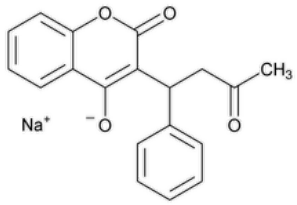
Warfarin Sodium



$C_{19}H_{15}NaO_4$ 330.31

2*H*-1-Benzopyran-2-one, 4-hydroxy-3-(3-oxo-1-phenylbutyl)-, sodium salt;

3-(α-Acetonylbenzyl)-4-hydroxycoumarin sodium salt;

Sodium 2-oxo-3-(3-oxo-1-phenylbutyl)-2*H*-chromen-4-olate CAS RN®: 129-06-6; UNII: 6153CWM0CL.

DEFINITION

Warfarin Sodium is an amorphous solid or a crystalline clathrate. The crystalline form consists principally of warfarin sodium and isopropyl alcohol in a 2:1 molecular ratio. It contains NLT 8.0% and NMT 8.5% of isopropyl alcohol. Warfarin Sodium contains NLT 97.0% and NMT 102.0% of warfarin sodium ($C_{19}H_{15}NaO_4$), calculated on the anhydrous basis for the amorphous form or on the anhydrous and isopropyl alcohol-free basis for the crystalline form.

IDENTIFICATION

Change to read:

• **A.** ▲**Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*: 197K**▲ (CN 1-May-2020)

Standard: Use USP Warfarin RS.

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.

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

• **C.**

Methoxyphenylacetic reagent: Dissolve 2.7 g of methoxyphenylacetic acid in 6 mL of 10% tetramethylammonium hydroxide aqueous solution, and add 20 mL of absolute alcohol.

Diluted ammonia: Dilute 41 g of ammonium hydroxide with water to 100 mL.

Ammonium carbonate solution: 158 mg/mL of ammonium carbonate in water

Sample solution: Dissolve 30 mg of Warfarin Sodium in 0.5 mL of water.

Analysis: Add 1.5 mL of *Methoxyphenylacetic reagent* to the *Sample solution*, and cool in ice water for 30 min.

Acceptance criteria: A voluminous, white, crystalline precipitate is formed. Place the precipitate in a water bath at 20° and stir for 5 min. The precipitate does not disappear. Add 1 mL of *Diluted ammonia*. The precipitate dissolves completely. Add 1 mL of *Ammonium carbonate solution*. No precipitate is formed.

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: 0.376 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* and 15 mL of *Buffer* into a conical flask, and mix.

Sample stock solution: 0.4 mg/mL of Warfarin Sodium prepared as follows. Transfer 100 mg of Warfarin Sodium, accurately weighed, to a 250-mL volumetric flask, and dissolve in 97.8 mL of 0.1 N sodium hydroxide. Add 62.5 mL of 0.2 M monobasic potassium phosphate, and dilute with water to volume.

Sample solution: Transfer 5 mL of *Sample stock solution* and 15 mL of *Buffer* into a conical flask, and mix.

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 warfarin sodium ($C_{19}H_{15}NaO_4$) in the portion of Warfarin Sodium 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 = 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: 97.0%–102.0% on the anhydrous basis for the amorphous form or on the anhydrous and isopropyl alcohol-free basis for the crystalline form

IMPURITIES

• **Organic Impurities**

Diluent: Methanol and water (25:75)

Mobile phase: Acetonitrile, glacial acetic acid, and water (32:1:68)

Standard stock solution: 0.12 mg/mL each of USP Warfarin RS and USP Warfarin Related Compound A RS prepared as follows. Transfer USP Warfarin RS and USP Warfarin Related Compound A 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. Dilute with water to volume.

Standard solution: 2.4 µg/mL each of USP Warfarin RS and USP Warfarin Related Compound A RS in *Diluent*, from *Standard stock solution*

Sample solution: 0.8 mg/mL of Warfarin Sodium in *Diluent*

System suitability solution: 2.4 µg/mL of USP Warfarin Related Compound A RS and 0.8 mg/mL of Warfarin Sodium in *Diluent* prepared as follows. Transfer 0.5 mL of *Standard stock solution* to a 25-mL volumetric flask and dilute with *Sample solution* to volume.

Chromatographic system

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

Mode: LC

Detector: UV 260 nm

Column: 4.6-mm × 25-cm; packing L10

Column temperature: 35°

Flow rate: 1.5 mL/min

Injection volume: 50 µL

Run time: NLT 2 times the retention time of the warfarin peak

System suitability

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

Suitability requirements

Resolution: NLT 3 between warfarin and warfarin related compound A peaks, *System suitability solution*

Relative standard deviation: NMT 5.0% for the warfarin and warfarin related compound A peaks, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of Alice's ketone (sodium salt of warfarin related compound A) in the portion of Warfarin Sodium 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 related compound A from the *Sample solution*

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

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

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

M_{r1} = molecular weight of Alice's ketone, 286.30

M_{r2} = molecular weight of warfarin related compound A, 264.32

Calculate the percentage of any other individual impurity in the portion of Warfarin Sodium 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 any other individual impurity 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 each individual impurity (see *Table 1*)

Acceptance criteria: See *Table 1*. Disregard any impurity peak less than 0.06%.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
4-Hydroxycoumarin[a]	0.4	2.0	0.3
Benzalacetone[b]	0.6	2.0	0.3
Warfarin	1.0	—	—
Alice's ketone[c]	1.2	1.0	0.3
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.0

[a] 4-Hydroxy-2*H*-chromen-2-one.

[b] (*E*)-4-Phenylbut-3-en-2-one.

[c] Sodium salt of warfarin related compound A; 3-(*o*-Hydroxyphenyl)-5-phenyl-2-cyclohexen-1-one sodium salt.

SPECIFIC TESTS

• **Isopropyl Alcohol Content (crystalline clathrate form)**

Internal standard solution: 4.25 mg/mL of *n*-propyl alcohol in water

Standard stock solution: 4.25 mg/mL of isopropyl alcohol in water

Standard solution: Transfer 2.0 mL of the *Standard stock solution* and 2.0 mL of the *Internal standard solution* to a headspace vial, seal, and mix.

Sample solution: Transfer 100 mg of Warfarin Sodium, 2.0 mL of water, and 2.0 mL of the *Internal standard solution* to a headspace vial, seal, and mix.

Blank solution: Transfer 2.0 mL of water and 2.0 mL of the *Internal standard solution* to a headspace vial, seal, and mix.

Chromatographic system

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

Mode: GC

Detector: Flame ionization

Column: 0.32-mm × 30-m; 1.8-µm coating of phase G43

Temperatures

Injector: 140°

Detector: 240°

Column: See *Table 2*.

Table 2

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	0	40	20
40	10	240	10

Carrier gas: Nitrogen

Flow rate: 1 mL/min

Injector type: Headspace; split ratio, 80:1

Temperatures

Equilibration: 65°

Needle: 75°

Transfer line: 85°

Headspace carrier pressure: 15 psi

Times

Equilibration: 20 min

Pressurization: 3.0 min

Loop fill: 0.2 min

Injection: 0.1 min

System suitability

Sample: *Standard solution*

[Note—The relative retention times for isopropyl alcohol and *n*-propyl alcohol are about 0.66 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 5.0 between isopropyl alcohol and *n*-propyl alcohol

Tailing factor: NMT 1.3 for the isopropyl alcohol peak

Relative standard deviation: NMT 2.0%, peak response ratio of isopropyl alcohol to *n*-propyl alcohol

Analysis

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

Calculate the percentage of isopropyl alcohol in the portion of Warfarin Sodium taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of isopropyl alcohol to *n*-propyl alcohol from the *Sample solution*

R_S = peak response ratio of isopropyl alcohol to *n*-propyl alcohol from the *Standard solution*

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

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

Acceptance criteria: 8.0%–8.5%

- **pH** ⟨791⟩

Sample solution: 10 mg/mL

Acceptance criteria: 7.2–8.3

- **Water Determination** ⟨921⟩, *Method I*: NMT 4.5% for the amorphous form; NMT 0.3% for the crystalline clathrate form
- **Absorbance in Alkaline Solution**

Sample solution: 125 mg/mL in sodium hydroxide solution (1 in 20). Pass through a membrane filter.

Blank: Sodium hydroxide solution (1 in 20)

Analysis: Determine the absorbance of the solution in a 1-cm cell at 385 nm, with a suitable spectrometer, within 15 min.

Acceptance criteria: NMT 0.1

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed, light-resistant containers.
- **Labeling:** Label it to indicate whether it is the amorphous or the crystalline form.
- **USP Reference Standards** ⟨11⟩

USP Warfarin RS

USP Warfarin Related Compound A RS

3-(*o*-Hydroxyphenyl)-5-phenyl-2-cyclohexen-1-one.

$C_{18}H_{16}O_2$ 264.32

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

Topic/Question	Contact	Expert Committee
WARFARIN SODIUM	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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