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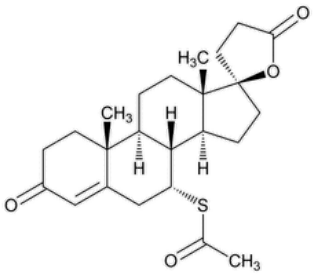
Spironolactone



$C_{24}H_{32}O_4S$ 416.57

Pregn-4-ene-21-carboxylic acid, 7-(acetylthio)-17-hydroxy-3-oxo-, γ-lactone, (7α,17α)-;

17-Hydroxy-7α-mercapto-3-oxo-17α-pregn-4-ene-21-carboxylic acid γ-lactone acetate;

(2'*R*)-7α-(Acetylthio)-3',4'-dihydro-5'*H*-spiro[androst-4-ene-17,2'-furan]-3,5'-dione CAS RN®: 52-01-7; UNII: 27O7W4T232.

DEFINITION

Spironolactone contains NLT 97.0% and NMT 103.0% of spironolactone ($C_{24}H_{32}O_4S$), calculated on the dried basis.

IDENTIFICATION

Change to read:

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

• **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**

Mobile phase: Methanol and water (60:40)

Standard solution: 0.5 mg/mL of USP Spironolactone RS in a mixture of acetonitrile and water (1:1)

Sample solution: 0.5 mg/mL of Spironolactone in a mixture of acetonitrile and water (1:1)

Chromatographic system

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

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: NLT 2 times the retention time of spironolactone

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 1.10%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of spironolactone ($C_{24}H_{32}O_4S$) in the portion of Spironolactone taken:

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

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

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

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

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

Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES

• **Limit of Mercapto Compounds**

Sample solution: Shake 2.0 g with 30 mL of water, and filter.

Analysis: To 15 mL of the *Sample solution* add 3 mL of starch TS, and titrate with 0.010 N iodine. Perform a blank determination, and make any necessary correction.

Acceptance criteria: NMT 0.10 mL of 0.010 N iodine is consumed.

• **Organic Impurities**

Diluent: Acetonitrile and water (50:50)

Mobile phase: Acetonitrile, tetrahydrofuran, methanol, and water (15:20:425:540)

Standard stock solutions: 2 mg/mL of USP Spironolactone RS and 0.2 mg/mL each of USP Spironolactone Related Compound A RS, USP Spironolactone Related Compound B RS, USP Spironolactone Related Compound C RS, USP Spironolactone Related Compound D RS, USP Spironolactone Epimer Mixture RS, and USP Spironolactone Related Compound I RS prepared separately as follows. Dissolve each suitable amount of the corresponding USP Reference Standard in a suitable volumetric flask in about 10% of the total volume of tetrahydrofuran. Dilute with *Diluent* to volume.

Standard solution: 0.002 mg/mL of USP Spironolactone RS and 0.006 mg/mL of USP Spironolactone Related Compound A RS in *Diluent* from the corresponding *Standard stock solutions*

System suitability solution: 0.02 mg/mL of USP Spironolactone RS and 0.006 mg/mL each of USP Spironolactone Related Compound A RS, USP Spironolactone Related Compound B RS, USP Spironolactone Related Compound C RS, USP Spironolactone Related Compound D RS, USP Spironolactone Epimer Mixture RS, and USP Spironolactone Related Compound I RS in *Diluent* from the corresponding *Standard stock solutions*

Sensitivity solution: 0.001 mg/mL of USP Spironolactone RS in *Diluent* from the corresponding *Standard stock solution*

Sample solution: 2 mg/mL of Spironolactone prepared as follows. Dissolve a suitable amount of Spironolactone in about 10% of the total volume of tetrahydrofuran and dilute with *Diluent* to volume.

Chromatographic system

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

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; 3.5-µm packing L1

Column temperature: 40°

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: NLT 2.8 times the retention time of the spironolactone peak

System suitability

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

Suitability requirements

Resolution: NLT 1.2 between spironolactone and spironolactone related compound B, *System suitability solution*

Relative standard deviation: NMT 5.0% for the spironolactone peak, *Standard solution*

Signal-to-noise ratio: NLT 20, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of spironolactone related compound A in the portion of Spironolactone taken:

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

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

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

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

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

Calculate the percentage of each impurity, other than spironolactone related compound A, in the portion of Spironolactone taken:

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

r_U = peak response of each impurity, other than spironolactone related compound A, from the *Sample solution*

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

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

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

Acceptance criteria: See *Table 1*. The reporting threshold is 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Spironolactone related compound B	0.95	0.2
Spironolactone	1.0	—
Spironolactone related compound A	1.2	0.2
Spironolactone related compound C	1.5	0.2
Spironolactone related compound D	1.6	0.3
Spironolactone epimer	1.7	0.3
Spironolactone related compound I	1.9	0.1
Any unspecified impurity	—	0.10
Total impurities	—	1.0

SPECIFIC TESTS

- **Optical Rotation** ⟨781S⟩, *Procedures*, *Specific Rotation*

  Sample solution: 10 mg/mL in alcohol

  Acceptance criteria: −41° to −45°

- **Loss on Drying** ⟨731⟩

  Analysis: Dry at 105° for 2 h.

  Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers.
- **USP Reference Standards** ⟨11⟩

USP Spironolactone RS

USP Spironolactone Related Compound A RS

(2'*R*)-3',4'-Dihydro-5'*H*-spiro[androst-4,6-diene-17,2'-furan]-3,5'-dione.

$C_{22}H_{28}O_3$ 340.46

USP Spironolactone Related Compound B RS

(2'*R*)-7α-(Acetylthio)-5'*H*-spiro[androst-4-ene-17,2'-furan]-3,5'-dione.

$C_{24}H_{30}O_4S$ 414.56

USP Spironolactone Related Compound C RS

(2'*R*)-3',4'-Dihydro-5'*H*-spiro[androst-4-ene-17,2'-furan]-3,5'-dione.

$C_{22}H_{30}O_3$ 342.47

USP Spironolactone Related Compound D RS

(2'*R*)-7α-(Acetyldisulfanyl)-3',4'-dihydro-5'*H*-spiro[androst-4-ene-17,2'-furan]-3,5'-dione.

$C_{24}H_{32}O_4S_2$ 448.64

USP Spironolactone Epimer Mixture RS

It contains a mixture of approximately 39% of spironolactone and 56% of 7-epispironolactone:
(2'*R*)-7β-(Acetylthio)-3',4'-dihydro-5'*H*-spiro[androst-4-ene-17,2'-furan]-3,5'-dione.

$C_{24}H_{32}O_4S$ 416.57

USP Spironolactone Related Compound I RS

S-[17α-(Ethoxymethyl)-17-hydroxy-3-oxoandrost-4-en-7α-yl]ethanethioate.

$C_{24}H_{36}O_4S$ 420.61

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

Topic/Question	Contact	Expert Committee
SPIRONOLACTONE	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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