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Everolimus

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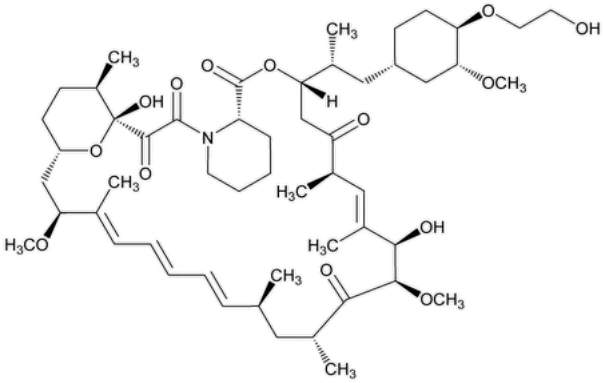

$C_{53}H_{83}NO_{14}$ ▲958.24▲ (CN 1-Aug-2024)

(1*R*,9*S*,12*S*,15*R*,16*E*,18*R*,19*R*,21*R*,23*S*,24*E*,26*E*,28*E*,30*S*,32*S*,35*R*)-1,18-Dihydroxy-12-[(1*R*)-2-[(1*S*,3*R*,4*R*)-4-(2-hydroxyethoxy)-3-methoxycyclohexyl]-1-methylethyl]-19,30-dimethoxy-15,17,21,23,29,35-hexamethyl-11,36-dioxa-4-azatricyclo[30.3.1.$0^{4,9}$]hexatriaconta-16,24,26,28-tetraene-2,3,10,14,20-pentaone;
(3*S*,6*R*,7*E*,9*R*,10*R*,12*R*,14*S*,15*E*,17*E*,19*E*,21*S*,23*S*,26*R*,27*R*,34a*S*)-9,10,12,13,14,21,22,23,24,25,26,27,32,33,34,34a-Hexadecahydro-9,27-dihydroxy-3-[(1*R*)-2-[(1*S*,3*R*,4*R*)-4-(2-hydroxyethoxy)-3-methoxycyclohexyl]-1-methylethyl]-10,21-dimethoxy-6,8,12,14,20,26-hexamethyl-23,27-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclohentriacontine-1,5,11,28,29(4*H*,6*H*,31*H*)-pentone CAS RN®: 159351-69-6.

DEFINITION

Everolimus contains NLT 95.0% and NMT 102.0% of everolimus ($C_{53}H_{83}NO_{14}$), calculated on the anhydrous and solvent-free basis. It may contain a suitable antioxidant.

IDENTIFICATION

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

• **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**

Protect everolimus from exposure to oxygen. Protect solutions containing everolimus from light.

Solution A: 0.27 g/L of monobasic potassium phosphate

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	60	40
5	43	57
23	36	64
31	0	100
36	0	100
37	60	40

Time (min)	Solution A (%)	Solution B (%)
45	60	40

System suitability solution: 0.5 mg/mL of USP Everolimus System Suitability Mixture RS in acetonitrile

Standard solution: 0.5 mg/mL of USP Everolimus RS in acetonitrile

Sample solution: 0.5 mg/mL of Everolimus in acetonitrile

Chromatographic system

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

Mode: LC

Detector: UV 275 nm

Column: 3-mm × 25-cm; 5-µm packing L1

Column temperature: 50°

Flow rate: 1.1 mL/min

Injection volume: 10 µL

System suitability

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

[Note—The relative retention times for 9-*O*-hydroxyethyl sirolimus, everolimus, and everolimus oxepane isomer are about 0.92, 1.0, and 1.1, respectively.]

Suitability requirements

Resolution: NLT 1.2 between 9-*O*-hydroxyethyl sirolimus and everolimus and NLT 1.5 between everolimus and everolimus oxepane isomer, *System suitability solution*

Relative standard deviation: NMT 2.0% for the sum of the everolimus and everolimus oxepane isomer peaks, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of everolimus ($C_{53}H_{83}NO_{14}$) in the portion of Everolimus taken:

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

r_U = sum of the peak responses of everolimus and everolimus oxepane isomer from the *Sample solution*

r_S = sum of the peak responses of everolimus and everolimus oxepane isomer from the *Standard solution*

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

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

F = percentage of sirolimus from the test for *Limit of Sirolimus*

Acceptance criteria: 95.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

• **Residue on Ignition ⟨281⟩:** NMT 0.1%

• **Limit of Alcohol**

Analysis: See *Residual Solvents ⟨467⟩*.

Acceptance criteria: NMT 1.5%

• **Organic Impurities**

Protect everolimus from exposure to oxygen. Protect solutions containing everolimus from light and inject them within 2 h of preparation to minimize the effects of isomerization between everolimus and everolimus oxepane isomer.

Solution A, Solution B, and **Mobile phase:** Prepare as directed in the *Assay*.

System suitability solution: 5 mg/mL of USP Everolimus System Suitability Mixture RS in acetonitrile. Use the solution within 24 h.

Sensitivity solution: 0.0025 mg/mL of USP Everolimus RS in acetonitrile

Standard solution: 0.05 mg/mL of USP Everolimus RS in acetonitrile

Sample solution: 5 mg/mL of Everolimus in acetonitrile

Chromatographic system

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

Mode: LC

Detector: UV 210 nm

Column: 3-mm × 25-cm; 5-µm packing L1

Column temperature: 50°

Flow rate: 1.1 mL/min

Injection volume: 10 µL

System suitability

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

[NOTE—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 1.2 between 9-*O*-hydroxyethyl sirolimus and everolimus; NLT 1.5 between everolimus and everolimus oxepane isomer, *System suitability solution*

Relative standard deviation: NMT 5% for everolimus, *Standard solution*

Signal-to-noise ratio: NLT 10 for everolimus, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Everolimus taken:

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

r_U = peak response of each impurity from the *Sample solution*

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

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

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

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Everolimus-19-ene open ring[a]	0.4	1.25	0.5
Specified unidentified impurity 1	0.75	1.0	0.4
Specified unidentified impurity 2	0.78	1.0	0.4
Specified unidentified impurity 3	0.81	1.0	0.8
10-Desmethyl everolimus[b]	0.87	1.0	0.8
9-*O*-Hydroxyethyl sirolimus[c]	0.92	1.0	0.8
Everolimus	1.0	—	—
Everolimus oxepane isomer[d,e]	1.1	—	—
Butylated hydroxytoluene[f]	1.2	—	—
Formyl sirolimus[g] and specified unidentified impurity 4[h]	1.4	1.0	0.3
Everolimus cyclic hemiacetal[i]	1.6	1.25	0.2
Any individual unspecified impurity	—	1.0	0.20
Total impurities	—	—	2.5

[a] (*S*)-1-{2-[(2*R*,3*R*,6*S*)-2-Hydroxy-6-{(2*S*,3*E*,5*E*,7*E*,9*S*,11*R*,13*R*,14*R*,15*E*,17*R*,19*E*,21*R*)-14-hydroxy-22-[(1*S*,3*R*,4*R*)-4-(2-hydroxyethoxy)-3-methoxycyclohexyl]-2,13-dimethoxy-3,9,11,15,17,21-hexamethyl-12,18-dioxodocosa-3,5,7,15,19-pentaen-1-yl}-3-methyltetrahydro-2*H*-pyran-

2-yl]-2-oxoacetyl}piperidine-2-carboxylic acid.

[b] (3*S*,6*R*,7*E*,9*R*,10*R*,12*R*,14*S*,15*E*,17*E*,19*E*,21*S*,23*S*,26*R*,27*R*,34a*S*)-9,10,12,13,14,21,22,23,24,25,26,27,32,33,34,34a-Hexadecahydro-9,10,27-trihydroxy-3-[(1*R*)-2-[(1*S*,3*R*,4*R*)-4-(2-hydroxyethoxy)-3-methoxycyclohexyl]-1-methylethyl]-21-methoxy-6,8,12,14,20,26-hexamethyl-23,27-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclohentriacontine-1,5,11,28,29(4*H*,6*H*,31*H*)-pentone.

[c] (3*S*,6*R*,7*E*,9*R*,10*R*,12*R*,14*S*,15*E*,17*E*,19*E*,21*S*,23*S*,26*R*,27*R*,34a*S*)-9,10,12,13,14,21,22,23,24,25,26,27,32,33,34,34a-Hexadecahydro-27-hydroxy-9-(2-hydroxyethoxy)-3-[(1*R*)-2-[(1*S*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylethyl]-10,21-dimethoxy-6,8,12,14,20,26-hexamethyl-23,27-epoxy-3*H*-pyrido[2,1-*c*][1,4]-oxaazacyclohentriacontine-1,5,11,28,29(4*H*,6*H*,31*H*)-pentone.

[d] 3*S*,6*R*,7*E*,9*R*,10*R*,12*R*,14*S*,15*E*,17*E*,19*E*,21*S*,23*S*,26*R*,28*R*,34a*S*)-9,10,12,13,14,21,22,23,24,25,26,28,32,33,34,34a-Hexadecahydro-9,28-dihydroxy-3-[(1*R*)-2-[(1*S*,3*R*,4*R*)-4-(2-hydroxyethoxy)-3-methoxycyclohexyl]-1-methylethyl]-10,21-dimethoxy-6,8,12,14,20,26-hexamethyl-23,28-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclohentriacontine-1,5,11,27,29(4*H*,6*H*,31*H*)-pentone.

[e] Everolimus oxepane isomer is formed in polar protic solvents. It is listed here for information only; it is not to be reported.

[f] Butylated hydroxytoluene is an antioxidant that may be used to stabilize everolimus. It is listed here for information only; it is not to be reported.

[g] (3*S*,6*R*,7*E*,9*R*,10*R*,12*R*,14*S*,15*E*,17*E*,19*E*,21*S*,23*S*,26*R*,27*R*,34a*S*)-9,10,12,13,14,21,22,23,24,25,26,27,32,33,34,34a-Hexadecahydro-9,27-dihydroxy-3-[(1*R*)-2-[(1*S*,3*R*,4*R*)-4-formyloxy-3-methoxycyclohexyl]-1-methylethyl]-10,21-dimethoxy-6,8,12,14,20,26-hexamethyl-23,27-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclohentriacontine-1,5,11,28,29(4*H*,6*H*,31*H*)-pentone.

[h] The system may resolve formyl sirolimus and specified impurity 4. The limit is for the sum of the two peaks.

[i] (3*S*,6*R*,7*E*,9*R*,10*R*,11*R*,12*R*,14*S*,15*S*,16*E*,19*E*,21*S*,23*S*,26*R*,27*R*,34a*S*)-9,10,12,13,14,15,18,21,22,23,24,25,26,27,32,33,34,34a-Octadecahydro-9,11,27-trihydroxy-3-[(1*R*)-2-[(1*S*,3*R*,4*R*)-4-(2-hydroxyethoxy)-3-methoxycyclohexyl]-1-methylethyl]-10,21-dimethoxy-18-ethoxy-6,8,12,14,20,26-hexamethyl-23,27:11,15-diepoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclohentriacontine-1,5,28,29(4*H*,6*H*,31*H*)-tetrone.

• **Limit of Sirolimus**

Protect everolimus from exposure to oxygen. Protect solutions containing everolimus from light.

Solution A: 0.32 g of ammonium formate dissolved in 450 mL of water, 100 mL of methanol, 450 mL of acetonitrile, and 0.05 mL of formic acid

Solution B: 0.32 g of ammonium formate dissolved in 330 mL of water, 100 mL of methanol, 570 mL of acetonitrile, and 0.05 mL of formic acid

Mobile phase: See *Table 3*. [Note—The procedure is sensitive to the percentage of methanol in the *Mobile phase*.]

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	100	0
14	100	0
23	0	100
26	0	100
26.5	100	0
32	100	0

System suitability solution: 2 mg/mL of USP Everolimus System Suitability Mixture RS and 0.016 mg/mL of USP Sirolimus RS in acetonitrile

Sensitivity solution: 0.001 mg/mL of USP Everolimus RS in acetonitrile

Standard solution: 0.016 mg/mL of USP Everolimus RS in acetonitrile

Sample solution: 2 mg/mL of Everolimus in acetonitrile

Chromatographic system

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

Mode: LC

Detector: UV 278 nm

Column: 2.1-mm × 15-cm; 2.7-µm packing L1

Temperatures

[Note—A detector temperature of 40° was shown to improve the chromatography.]

Autosampler: 6°

Column: 60°

Flow rate: 0.9 mL/min

Injection volume: 3.5 µL

System suitability

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

[NOTE—See *Table 4* for relative retention times.]

Suitability requirements

Resolution: NLT 1.2 between sirolimus and everolimus, *System suitability solution*

Relative standard deviation: NMT 5% for the sum of the peak responses for everolimus and everolimus oxepane isomer, *Standard solution*

Signal-to-noise ratio: NLT 10 for the everolimus peak, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of sirolimus in the portion of Everolimus taken:

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

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

r_S = sum of the peak responses of everolimus and everolimus oxepane isomer from the *Standard solution*

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

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

Acceptance criteria: See *Table 4*. The reporting threshold is 0.05%.

Table 4

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Everolimus-19-ene open ring[a]	0.4	—
10-Desmethyl everolimus[a]	0.7	—
Sirolimus[b]	0.9	0.8
Everolimus	1.0	—
Everolimus oxepane isomer[c]	1.1	—

[a] These impurities are controlled in the test for *Organic Impurities*. They are listed here for information only.

[b] (3*S*,6*R*,7*E*,9*R*,10*R*,12*R*,14*S*,15*E*,17*E*,19*E*,21*S*,23*S*,26*R*,27*R*,34a*S*)-9,10,12,13,14,21,22,23,24,25,26,27,32,33,34,34a-Hexadecahydro-9,27-dihydroxy-3-[(1*R*)-2-[(1*S*,3*R*,4R)-4-hydroxy-3-methoxycyclohexyl]-1-methylethyl]-10,21-dimethoxy-6,8,12,14,20,26-hexamethyl-23,27-epoxy-3H-pyrido[2,1-c][1,4]-oxaazacyclohentriacontine-1,5,11,28,29(4*H*,6*H*,31*H*)-pentone.

[c] Everolimus oxepane isomer is formed in polar protic solvents. It is listed here for information only; it is not to be reported.

SPECIFIC TESTS

- **WATER DETERMINATION ⟨921⟩, *Method I*:** NMT 1.5%
- **OPTICAL ROTATION ⟨781S⟩, *Procedures, Specific Rotation***

Sample solution: 10 mg/mL in methanol

Acceptance criteria: −141.0° to −153.0° on the anhydrous basis

- **MICROBIAL ENUMERATION TESTS ⟨61⟩** and **TESTS FOR SPECIFIED ORGANISMS ⟨62⟩:** The total aerobic microbial count does not exceed 10^3 cfu/g. The total yeasts and molds count does not exceed 10^2 cfu/g.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers in an atmosphere of protective gas. Store at refrigerated temperatures.
- **LABELING:** Label it to indicate the nature and the content of the antioxidant, if present.
- **USP REFERENCE STANDARDS ⟨11⟩**

USP Everolimus RS

USP Everolimus System Suitability Mixture RS

This is a mixture of everolimus, everolimus oxepane isomer, and 9-*O*-hydroxyethyl sirolimus. Other minor components may be present.

USP Sirolimus RS

Topic/Question	Contact	Expert Committee
EVEROLIMUS	Documentary Standards Support	SM12020 Small Molecules 1

Chromatographic Database Information: Chromatographic Database

Most Recently Appeared In:
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