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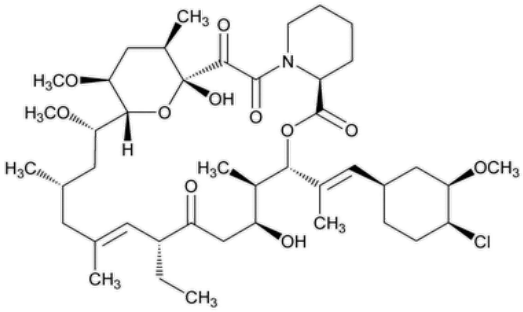
Add the following:

▲Pimecrolimus



$C_{43}H_{68}ClNO_{11}$ 810.46

15,19-Epoxy-3*H*-pyrido[2,1-c][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone, 3-[(1*E*)-2-[(1*R*,3*R*,4*S*)-4-chloro-3-methoxycyclohexyl]-1-methylethenyl]-8-ethyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-14,16-dimethoxy-4,10,12,18-tetramethyl-, (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26a*S*)-;
(3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26a*S*)-3-[(*E*)-2-[(1*R*,3*R*,4*S*)-4-Chloro-3-methoxycyclohexyl]-1-methylvinyl]-8-ethyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone CAS RN®: 137071-32-0; UNII: 7KYV510875.

DEFINITION

Pimecrolimus contains NLT 98.0% and NMT 102.0% of pimecrolimus ($C_{43}H_{68}ClNO_{11}$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

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

[Note—Protect solutions containing pimecrolimus from light and store at 5°. Dissolve quickly with minimum sonication.]

Solution A: 0.1% of phosphoric acid in water

Solution B: 0.1% of phosphoric acid in methanol

Mobile phase: *Solution A* and *Solution B* (29:71)

Diluent: 0.1% of formic acid in acetonitrile

Standard solution: 0.5 mg/mL of USP Pimecrolimus RS in *Diluent*

Sample solution: 0.5 mg/mL of Pimecrolimus in *Diluent*

Chromatographic system

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

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 15-cm; 2.7-µm packing L1

Temperatures

Autosampler: 5°

Column: 60°

Flow rate: 1 mL/min

Injection volume: 10 µL

Run time: NLT 1.8 times the retention time of pimecrolimus

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for pimecrolimus tautomer I and pimecrolimus tautomer II are 0.89 and 0.55, respectively.]

Suitability requirements

Tailing factor: 0.8–1.5 for pimecrolimus

Relative standard deviation: NMT 0.73% for pimecrolimus

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of pimecrolimus ($C_{43}H_{68}ClNO_{11}$) in the portion of Pimecrolimus taken:

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

r_U = sum of the peak response of pimecrolimus, pimecrolimus tautomer I (if present), and pimecrolimus tautomer II (if present) from the *Sample solution*

r_S = sum of the peak response of pimecrolimus, pimecrolimus tautomer I (if present), and pimecrolimus tautomer II (if present) from the *Standard solution*

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

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

Acceptance criteria: 98.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

• **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%

• **ORGANIC IMPURITIES**

[NOTE—Protect solutions containing pimecrolimus from light and store between 2° and 8°. Avoid sonication while preparing.]

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

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	33	67
2	33	67
38	27	73
50	27	73
56	33	67
68	33	67

System suitability solution: 6 mg/mL of USP Pimecrolimus System Suitability Mixture RS in *Diluent*

Standard solution: 0.006 mg/mL of USP Pimecrolimus RS in *Diluent*

Sample solution: 6 mg/mL of Pimecrolimus in *Diluent*

Chromatographic system

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

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 15-cm; 2.7-µm packing L1

Temperatures

Autosampler: 5°

Column: 60°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

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

[NOTE—The relative retention times in Table 2 are provided as information that could aid in peak assignment.]

Table 2

Name	Relative Retention Time
Ascomycin[a]	0.56
Pimecrolimus tautomer II[b]	0.60
Desmethylpimecrolimus[c]	0.87
Pimecrolimus tautomer I[d]	0.93
Pimecrolimus	1.0
Pimecrolimus triene analog	1.2

[a] (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26a*S*)-8-Ethyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-[(1*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylethenyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone.

[b] (3*S*,4*R*,5*S*,8*R*,12*S*,14*S*,15*R*,16*S*,18*R*,19*S*,26a*S*,*E*)-3-{(*E*)-1-[(1*R*,3*R*,4*S*)-4-Chloro-3-methoxycyclohexyl]prop-1-en-2-yl}-8-ethyl-5,19-dihydroxy-14,16-dimethoxy-4,10,12,18-tetramethyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-3*H*-15,19-epoxypyrido[2,1-*c*][1]oxa[4]azacyclotricosine-1,7,20,21(4*H*,23*H*)-tetraone.

[c] (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26a*S*)- 3-[(1*E*)-2-[(1*R*,3*R*,4*S*)-4-chloro-3-methoxycyclohexyl]-1-methylethenyl]-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-14,16-dimethoxy-4,8,10,12,18-pentamethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone.

[d] (3*S*,4*R*,5*S*,8*R*,12*S*,14*S*,15*R*,16*S*,18*R*,26a*S*,*E*)-3-{(*E*)-1-[(1*R*,3*R*,4*S*)-4-chloro-3-methoxycyclohexyl]prop-1-en-2-yl}-8-ethyl-5,15,20,20-tetrahydroxy-14,16-dimethoxy-4,10,12,18-tetramethyl-3,4,5,6,11,12,13,14,15,16,17,18,24,25,26,26a-hexadecahydro-7*H*-pyrido[2,1-*c*][1]oxa[4]azacyclotricosine-1,7,19,21(8*H*,20*H*,23*H*)-tetraone.

Suitability requirements

Resolution: NLT 2.5 between pimecrolimus and pimecrolimus triene analog, *System suitability solution*

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

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any impurity in the portion of Pimecrolimus taken:

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

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

r_S = sum of the peak response of pimecrolimus, pimecrolimus tautomer I (if present), and pimecrolimus tautomer II (if present) from the *Standard solution*

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

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

Acceptance criteria: See Table 3.

Table 3

Name	Acceptance Criteria, NMT (%)
Ascomycin	0.15
Desmethylpimecrolimus	0.5
Pimecrolimus triene analog	0.2
Any unspecified impurity	0.10
Total impurities[a]	1.0

[a] Excludes pimecrolimus tautomer I and pimecrolimus tautomer II.

SPECIFIC TESTS

• **Water Determination** ⟨921⟩, *Method I, Method Ia*: NMT 0.5%

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

Sample solution: 5 mg/mL of Pimecrolimus in chloroform

Acceptance criteria: −45° to −55° at 20°, calculated on the anhydrous and solvent-free basis

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight, light-resistant containers. Store between 2° and 8°.

• **USP Reference Standards** ⟨11⟩

USP Pimecrolimus RS

USP Pimecrolimus System Suitability Mixture RS

Contains a mixture of the following 2 compounds.

Pimecrolimus.

Pimecrolimus triene analog: [3*S*-[3*R*[*E*(1*S*,5*S*)],4*S*,5*R*,8*S*,9*E*,12*R*,14*R*,15*S*,16*R*,18*S*,19*S*,26a*R*]]-8-Ethyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-14,16-dimethoxy-3-[2-(5-methoxy-3-cyclohexen-1-yl)-1-methylethenyl]-4,10,12,18-tetramethyl-15,19-Epoxy-3*H*-pyrido[2,1-c][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone.

$C_{43}H_{67}NO_{11}$ 774.01

Other known impurities may also be present, such as

Desmethylpimecrolimus: (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26a*S*)-3-[(1*E*)-2-[(1*R*,3*R*,4*S*)-4-Chloro-3-methoxycyclohexyl]-1-methylethenyl]-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-14,16-dimethoxy-4,8,10,12,18-pentamethyl-15,19-epoxy-3*H*-pyrido[2,1-c][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone.

$C_{42}H_{66}ClNO_{11}$ 796.44▲ (USP 1-Aug-2024)

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

Topic/Question	Contact	Expert Committee
PIMECROLIMUS	Documentary Standards Support	SM12020 Small Molecules 1
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	SM12020 Small Molecules 1

Chromatographic Database Information: Chromatographic Database

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