- **LABELING:** If a test for *Organic Impurities* other than *Procedure 1* is used, then the labeling states with which *Organic Impurities* test the article complies.
- **USP REFERENCE STANDARDS ⟨11⟩**

USP Tacrolimus RS
15,19-Epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylethenyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-8-(2-propenyl)-, monohydrate, [3*S*-[3*R**,*E*(1*S**,3*S**,4*S**)],4*S**,5*R**,8*S**,9*E*,12*R**,14*R**,15*S**,16*R**,18*S**,19*S**,26a*R**]]-.
$C_{44}H_{69}NO_{12} \cdot H_2O$ 822.03

USP Tacrolimus Related Compound A RS
(*E*)-8-Ethyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-Hexadecahydro-5,19-dihydroxy-3-[(*E*)-2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-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.
$C_{43}H_{69}NO_{12}$ 792.01

USP Tacrolimus System Suitability Mixture RS
This is a mixture of tacrolimus, ascomycin (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-[(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl]-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.
$C_{43}H_{69}NO_{12}$ 792.01
and tacrolimus 8-propyl analog (3*S*,4*R*,5*S*,8*S*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26a*S*)- 5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-{(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl}-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-8-propyl-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone.
$C_{44}H_{71}NO_{12}$ 806.03

Tacrolimus Capsules

DEFINITION
Tacrolimus Capsules contain NLT 93.0% and NMT 105.0% of the labeled amount of tacrolimus ($C_{44}H_{69}NO_{12}$).

IDENTIFICATION
- 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—Allow the *Standard solution* and the *Sample solution* to stand for 3 h at ambient temperature before use. Protect the solutions from light by using low-actinic glassware.]

Solution A: 6 mM phosphoric acid

Mobile phase: Acetonitrile, *tert*-butyl methyl ether, and *Solution A* (335:55:600)

Solution B: 50 g/L polyoxyethylene (23) lauryl ether. [NOTE—Polyoxyethylene (23) lauryl ether is also called Brij-35.]

Solution C: Acetonitrile and *Solution B* (7:3)

Standard solution: 50 µg/mL of USP Tacrolimus RS in *Solution C*

Sample solution: Equivalent to 50 µg/mL of tacrolimus, from NLT 10 Capsules, in *Solution C*. [NOTE—Sonicate and stir with a magnetic stirrer.]

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 205 nm
Column: 4.0-mm × 5.5-cm; 3-µm packing L1
Column temperature: 60°
Flow rate: 1 mL/min
Injection size: 5 µL

System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for tacrolimus 19-epimer and tacrolimus are 0.67 and 1.0, respectively.]
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 3.0% for the sum of the tacrolimus and tacrolimus 19-epimer peaks

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of tacrolimus ($C_{44}H_{69}NO_{12}$) in the portion of Capsules taken:

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

r_U = sum of the peak responses of tacrolimus and tacrolimus 19-epimer from the *Sample solution*
r_S = sum of the peak responses of tacrolimus and tacrolimus 19-epimer from the *Standard solution*
C_S = concentration of USP Tacrolimus RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–105.0%

PERFORMANCE TESTS
- **DISSOLUTION ⟨711⟩**

Test 1
Medium: Hydroxypropylcellulose in water ($1:2 \times 10^4$); adjusted with 6% phosphoric acid to a pH of 4.5; 900 mL
Apparatus 2: 50 rpm with sinker (see *Dissolution* ⟨711⟩, *Figure 2a*)
Time: 90 min
Mobile phase: Acetonitrile, methanol, water, and 6% phosphoric acid (46:18:36:0.1)
Standard stock solution: (*L*/360) mg/mL in acetonitrile, where *L* is the Capsule label claim in mg
Standard solution: To 20.0 mL of the *Standard stock solution* add 50.0 mL of *Medium* and mix to obtain solutions with known concentrations as indicated in *Table 1*. Allow the solution to stand for NLT 6 h at 25° before use.
Sample solution: Pass 10 mL of the solution under test through a G4 glass filter. To 5.0 mL of the filtrate add 2.0 mL of acetonitrile and mix. Allow the solution to stand for NLT 1 h at 25° before use.

Table 1

Capsule Strength (mg)	Final Concentration (µg/mL)
0.5	0.4
1	0.8
5	4

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: 210 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Temperature: 50°
Flow rate: Adjust the flow rate so that the retention time of tacrolimus is approximately 14 min.

Injection size: See *Table 2*.

Table 2

Capsule Strength (mg)	Injection Volume (µL)
0.5	800
1	400
5	80

[NOTE—For products with strengths other than those listed in *Table 2*, adjust the injection volume to deliver an equivalent amount of tacrolimus into the column.]

System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between tacrolimus 19-epimer and tacrolimus
Tailing factor: NMT 1.5
Relative standard deviation: NMT 1.5%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of tacrolimus ($C_{44}H_{69}NO_{12}$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times D \times V \times (100/L)$$

r_U = peak response of tacrolimus from the *Sample solution*
r_S = peak response of tacrolimus from the *Standard solution*
C_S = concentration of USP Tacrolimus RS in the *Standard solution* (mg/mL)
D = dilution factor of the *Sample solution*
V = volume of *Medium*, 900 mL
L = label claim (mg/Capsule)

Tolerances: NLT 80% (Q) of the labeled amount of tacrolimus ($C_{44}H_{69}NO_{12}$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
[NOTE—Allow the *Standard solution* to stand for 3 h at ambient temperature before use. Protect the solutions from light by using low-actinic glassware.]
Buffer: Dissolve 6 g of sodium dodecyl sulfate and 8.28 g of monobasic sodium phosphate in 6000 mL of water. Adjust with 2 N sodium hydroxide to a pH of 7.0.
Medium: *Buffer*, 900 mL
Apparatus 2: 50 rpm, with sinkers
Time: 60 min
Standard stock solution: 0.2 mg/mL of USP Tacrolimus RS in alcohol and *Medium* (3:7). [NOTE—Dissolve USP Tacrolimus RS in alcohol using 30% of the final volume. Sonicate until dissolved and dilute with *Medium* to volume.]
Standard solution: Dilute the *Standard stock solution* with *Medium* to obtain a final concentration of 5 µg/mL.
Sample solution: Pass a portion of the solution under test through a suitable filter.
Solution A: 6 mM phosphoric acid
Mobile phase: Acetonitrile, *tert*-butyl methyl ether, and *Solution A* (335:50:600)
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 205 nm
Column: 4.0-mm × 5.5-cm; 3-µm packing L1
Column temperature: 60°
Flow rate: 1.2 mL/min
Injection size: 100 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for tacrolimus 19-epimer and tacrolimus are 0.67 and 1.0, respectively.]
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 5.0% for the sum of the areas of tacrolimus and tacrolimus 19-epimer
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of tacrolimus ($C_{44}H_{69}NO_{12}$) dissolved:

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

r_U = sum of the peak responses of tacrolimus and tacrolimus 19-epimer from the *Sample solution*
r_S = sum of the peak responses of tacrolimus and tacrolimus 19-epimer from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of tacrolimus ($C_{44}H_{69}NO_{12}$) is dissolved.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Medium: 50 mg/L of hydroxypropyl cellulose in water. Adjust with phosphoric acid to a pH of 4.5; 900 mL
Apparatus 2 (without sinker), Time, and **Sample solution:** Proceed as directed for *Test 1*.
Buffer: 3.6 g/L of monobasic potassium phosphate in water. Adjust with diluted phosphoric acid to a pH of 2.5.
Mobile phase: *Buffer* and acetonitrile (1:1)
Standard stock solution: 0.1 mg/mL of USP Tacrolimus RS in acetonitrile
Standard solution: Dilute the *Standard stock solution* with *Medium* to obtain a final concentration of (L/900) mg/mL, where L is the Capsule label claim.
Sample solution: Pass a portion of the solution under test through a suitable filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 10-cm; 5-µm packing L1
Column temperature: 60°
Flow rate: 1.3 mL/min
Injection size: 100 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for tacrolimus 19-epimer, tacrolimus open ring, and tacrolimus are 0.67, 0.79, and 1.0, respectively.]
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of tacrolimus ($C_{44}H_{69}NO_{12}$) dissolved:

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

r_U = sum of the peak responses of tacrolimus, tacrolimus 19-epimer, and tacrolimus open ring from the *Sample solution*
r_S = sum of the peak responses of tacrolimus, tacrolimus 19-epimer, and tacrolimus open ring from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*, 900 mL

Tolerances: NLT 75% (Q) of the labeled amount of tacrolimus ($C_{44}H_{69}NO_{12}$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS ⟨905⟩:** Meets the requirements

IMPURITIES

Organic Impurities

- **PROCEDURE 1**

[NOTE— Use *Organic Impurities, Procedure 1* when the impurity profile includes tacrolimus diene and tacrolimus regioisomer. It is suggested that new columns be conditioned with about 500 mL of ethanol before use to meet the resolution criterion.]

Mobile phase: Hexane, *n*-butyl chloride, and acetonitrile (7:2:1). Add *n*-butyl chloride to hexane and mix well before adding acetonitrile. After adding acetonitrile, mix the *Mobile phase* for 2 h to get a clear solution. Any deviations from the ratio of components in the *Mobile phase* and the order of mixing will result in a two-phase solution.

System suitability solution: 0.1 mg/mL each of USP Tacrolimus RS and USP Tacrolimus Related Compound A RS in *Mobile phase*

Sample solution: Transfer the contents of a suitable number of Capsules (equivalent to about 5 mg of tacrolimus for 0.5-mg Capsules or 10 mg of tacrolimus for 1-mg and 5-mg Capsules) into a centrifuge tube. Add 1.5 mL of a mixture of *n*-butyl chloride and acetonitrile (2:1), sonicate in an ultrasonic bath for 2 min, add 3.5 mL of *n*-hexane, and mix. Centrifuge this solution and collect the supernatant or pass the solution through a 0.5-µm membrane filter. Use the solution within 30 min of preparation.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: Two 4.6-mm × 25-cm columns; 5-µm packing L20
Column temperature: 28 ± 2°
Flow rate: 1.5 mL/min. [NOTE— Adjust the flow rate so that the retention time of tacrolimus is approximately 15 min.]
Injection size: 20 µL
Run time: Three times the retention time of tacrolimus

System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 1.1 between tacrolimus and tacrolimus related compound A
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%

Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/F_i) \times [1/\Sigma(r_U/F_i)] \times 100$$

r_U = peak response of each impurity in the *Sample solution*
F_i = relative response factor for each corresponding impurity (see *Table 3*)

Acceptance criteria: See *Table 3*. Disregard peaks due to the solvent.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Tacrolimus diene[a]	0.79	2.2	0.3
Tacrolimus regioisomer[b]	0.88	1.0	0.5
Tacrolimus impurity 1[c]	0.96	1.0	0.3
Tacrolimus related compound A[d]	0.96	—	—
Tacrolimus	1.0	—	—
Tacrolimus 19-epimer[d,e]	1.1	—	—
Tacrolimus open ring[d,f]	1.3	—	—
Any individual unspecified impurity	—	1.0	0.2
Total impurities[g]	—	—	1.0

[a] (14*E*,18*E*)-17-Allyl-1-hydroxy-12-[(*E*)-2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0 ^{4,9}] octacosa-14,18-diene-2,3,10,16-tetrone.

[b] (4*E*,11*E*)-10-Allyl-7,8,10,13,14,15,16,17,18,19,20,21,26,22,28,28a-hexadecahydro-7,21-dihydroxy-3-(4-hydroxy-3-methoxycyclohexyl)-16,18-dimethoxy-4,6,12,14,20-pentamethyl-17,21-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclopentacosine-1,9,22,23(6*H*,25*H*)-tetrone.

[c] Tacrolimus impurity 1 is a specified, unidentified impurity.

[d] For information only. Not to be reported.

[e] (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*S*,26a*S*)-8-Allyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-{(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl}-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.

[f] (3*S*,4*R*,5*S*,8*R*,12*S*,14*S*,15*R*,16*S*,18*R*,26a*S*,*E*)-8-Allyl-5,6,11,12,13,14,15,16,17,18,24,25,26,26a-tetradecahydro-5,15,20,20-tetrahydroxy-3-{(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl}-14,16-dimethoxy-4,10,12,18-tetramethyl-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,19,21(4*H*,8*H*,20*H*,23*H*)-tetrone.

[g] Total impurities limit does not include tacrolimus open ring and tacrolimus 19-epimer.

- **PROCEDURE 2**

[NOTE— Use *Organic Impurities, Procedure 2* when the impurity profile includes tacrolimus 21-carboxylic acid and tacrolimus 8-epimer. It is suggested to equilibrate the column overnight with a mixture of *Solution C* and *Solution D* (17:3) before performing this procedure. Allow the *System suitability solution*, *Standard solution*, and *Sample solution* to stand for 3 h at ambient temperature before use. Protect the solutions from light by using low-actinic glassware.]

Solution A: 6 mM phosphoric acid
Solution B: Acetonitrile and *tert*-butyl methyl ether (81:19). [NOTE—The ratio of acetonitrile to *tert*-butyl methyl ether is critical.]
Solution C: *Solution A* and *Solution B* (4:1)
Solution D: *Solution A* and *Solution B* (1:4)
Mobile phase: See *Table 4*.

Table 4

Time (min)	Solution C (%)	Solution D (%)
0	74	26
45	74	26
60	15	85
75	15	85
76	74	26
85	74	26

Solution E: 50 g/L polyoxyethylene (23) lauryl ether in *Solution A*. [NOTE—Polyoxyethylene (23) lauryl ether is also called Brij-35.]
Diluent: Acetonitrile and *Solution E* (7:3)
System suitability solution: 1.5 mg/mL of USP Tacrolimus System Suitability Mixture RS in *Diluent*
Standard solution: 7.5 µg/mL of USP Tacrolimus RS in *Diluent*
Sensitivity solution: 1.5 µg/mL of USP Tacrolimus RS in *Diluent* from *Standard solution*
Sample solution: Equivalent to 1.5 mg/mL of tacrolimus in *Diluent*. [NOTE—Shake the mixture on a mechanical shaker for 30 min and pass through a suitable filter.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm; 3-µm packing L1
Column temperature: 60°
Flow rate: 1.5 mL/min
Injection size: 40 µL
System suitability
Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*
Suitability requirements
Signal-to-noise ratio: NLT 10.0, *Sensitivity solution*
Resolution: NLT 3.0 between tacrolimus and ascomycin, *System suitability solution*
Relative standard deviation: NMT 10.0% for the sum of the responses of tacrolimus and tacrolimus 19-epimer, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Capsules taken:

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

r_U = peak response of each impurity from the *Sample solution*
r_S = sum of the peak responses for tacrolimus 19-epimer and tacrolimus from the *Standard solution*
C_S = concentration of USP Tacrolimus RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of tacrolimus in the *Sample solution* (mg/mL)
P = potency of tacrolimus in USP Tacrolimus RS (mg/mg)

Acceptance criteria: See *Table 5*. Disregard peaks that are smaller than the tacrolimus peak in the *Sensitivity solution*.

Table 5

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tacrolimus 21-carboxylic acid[a]	0.18	0.5
Tacrolimus open ring[b,c]	0.49	—
Ascomycin 19-epimer[d]	0.52	—
Tacrolimus 19-epimer[b,e]	0.62	—
Ascomycin[f,g]	0.84	—
Desmethyl tacrolimus[f,h]	0.91	—
Tacrolimus	1.0	—
Tacrolimus 8-epimer[i]	1.28	0.2
Tacrolimus 8-propyl analog[f,j]	1.30	—
Any individual unspecified impurity	—	0.2
Total impurities	—	1.5

[a] 2-[(2*R*,3*R*,5*S*,6*R*)-6-{(1*S*,3*S*,5*E*,7*R*,10*S*,11*R*,12*S*,13*E*)-7-Allyl-10-hydroxy-14-{(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl}-1-methoxy-3,5,11,13-tetramethyl-8-oxo-12-[(*S*)-piperidine-2-carbonyloxy]tetradeca-5,13-dienyl}-2-hydroxy-5-methoxy-3-methyltetrahydro-2*H*-pyran-2-yl]-2-oxoacetic acid.

[b] Tacrolimus open ring and tacrolimus 19-epimer are isomers of tacrolimus, which are present in equilibrium with the active ingredient. They are not to be reported as degradation products.

[c] (3*S*,4*R*,5*S*,8*R*,12*S*,14*S*,15*R*,16*S*,18*R*,26a*S*,*E*)-8-Allyl-5,6,11,12,13,14,15,16,17,18,24,25,26,26a-tetradecahydro-5,15,20,20-tetrahydroxy-3-{(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl}-14,16-dimethoxy-4,10,12,18-tetramethyl-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,19,21(4*H*,8*H*,20*H*,23*H*)-tetrone.

[d] (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*S*,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-[(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl]-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.

[e] (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*S*,26a*S*)-8-Allyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-{(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl}-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.

[f] These are process impurities that are controlled in the drug substance. They are not to be reported in the drug product.

[g] (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-[(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl]-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.

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

[i] (3*S*,4*R*,5*S*,8*S*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26a*S*)-8-Allyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-{(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl}-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.

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

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **LABELING:** If a test for *Organic Impurities* other than *Procedure 1* is used, then the labeling states with which *Organic Impurities* test the article complies. When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Tacrolimus RS
  USP Tacrolimus Related Compound A RS
  (*E*)-8-Ethyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-[(*E*)-2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-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.
$C_{43}H_{69}NO_{12}$ 792.01

USP Tacrolimus System Suitability Mixture RS
It contains tacrolimus, ascomycin (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-[(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl]-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.
$C_{43}H_{69}NO_{12}$ 792.01
and tacrolimus 8-propyl analog (3*S*,4*R*,5*S*,8*S*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26a*S*)-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-{(*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl}-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-8-propyl-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)tetrone.
$C_{44}H_{71}NO_{12}$ 806.03

Add the following:

▲Tacrolimus Oral Suspension

DEFINITION

Tacrolimus Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of tacrolimus ($C_{44}H_{69}NO_{12}$).

Prepare Tacrolimus Oral Suspension 0.5 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Tacrolimus capsules[a] equivalent to	50 mg
Vehicle: a 1:1 mixture of Ora-Plus[b] and Syrup, *NF*, a sufficient quantity to make	100 mL

[a] Prograf 5-mg capsules, Astellas Pharma US, Inc., Deerfield, IL.
[b] Paddock Laboratories, Minneapolis, MN.

Calculate the quantity of each ingredient required for the total amount to be prepared. Empty the required number of capsules in a suitable mortar. Add the *Vehicle* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make a tacrolimus liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well. Tacrolimus powder is not interchangeable with *Tacrolimus capsules* and should not be used.

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile and deionized distilled water (65:35). Filter and degas.

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

Standard solution: Pipet 1.0 mL of *Standard stock solution* into a 10-mL volumetric flask, and dilute with *Mobile phase* to volume to obtain a solution having a nominal concentration of 50 µg/mL of tacrolimus. [NOTE—The *Standard solution* is relatively unstable, and the *Assay* should proceed immediately.]

Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Pipet 1.0 mL of Oral Suspension to a 10-mL volumetric flask, and dilute with *Mobile phase* to volume to obtain a solution having a nominal concentration of 50 µg/mL of tacrolimus. [NOTE—The *Sample solution* is relatively unstable, and the *Assay* should proceed immediately.]

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.7 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
[NOTE—The retention time for tacrolimus is about 6.4 min.]
Suitability requirements
Column efficiency: NLT 2500 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% for replicate injections

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of tacrolimus ($C_{44}H_{69}NO_{12}$) in the portion of Oral Suspension taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of tacrolimus in the *Standard solution* (µg/mL)
C_U = nominal concentration of tacrolimus in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

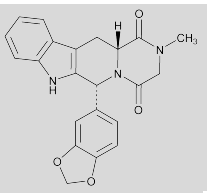
- **pH** ⟨**791**⟩**:** 4.1–5.1

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature.
- **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.
- **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored at controlled room temperature
- **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Tacrolimus RS*▲USP36*

Add the following:

▲Tadalafil



$C_{22}H_{19}N_3O_4$ 389.40

Pyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione, 6-(1,3-benzodioxol-5-yl)-2,3,6,7,12,12a-hexahydro-2-methyl-, (6*R*-12a*R*)-;
(6*R*,12a*R*)-2,3,6,7,12,12a-Hexahydro-2-methyl-6-[3,4-(methylenedioxy)phenyl] pyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione [171596-29-5].