Identification—

A: *Infrared Absorption* ⟨197K⟩—

Test specimen—Transfer an amount of Capsules, equivalent to about 100 mg of tacrine, to a 250-mL separatory funnel containing 100 mL of water, and mix. Add 2 mL of 1 N sodium hydroxide, and shake. Add 30 mL of ether, and shake. Allow the layers to separate, transfer the top ether layer to a beaker, and evaporate in a hood under a stream of nitrogen. Allow the solid to air-dry, and then dry at 105° for 90 minutes. Prepare a mixture of about 0.5% to 1.0% of the isolated solid in potassium bromide.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay*.

Dissolution ⟨711⟩**—**

Medium: 0.1 N hydrochloric acid; 900 mL.

Apparatus 2: 50 rpm.

Time: 30 minutes.

Procedure—Determine the amount of $C_{13}H_{14}N_2$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 240 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, in comparison with a Standard solution having a known concentration of USP Tacrine Hydrochloride RS in the same *Medium*.

Tolerances—Not less than 85% *(Q)* of the labeled amount of $C_{13}H_{14}N_2$ is dissolved in 30 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

PROCEDURE FOR CONTENT UNIFORMITY—

Standard solution—Dissolve an accurately weighed quantity of USP Tacrine Hydrochloride RS in 0.1 N hydrochloric acid, and dilute quantitatively, and stepwise if necessary, with 0.1 N hydrochloric acid to obtain a solution having a known concentration of about 4.1 µg per mL.

Test solution—Place 1 intact Capsule in a 100-mL volumetric flask, add about 70 mL of 0.1 N hydrochloric acid, and sonicate until the gelatin capsule shell has dissolved completely (about 15 minutes). [NOTE—Periodically swirl the flask during the sonication to loosen the Capsule from the bottom of the flask and to dissolve a floating Capsule.] Shake mechanically for about 30 additional minutes, dilute with 0.1 N hydrochloric acid to volume, and mix. Pass a portion of the solution through a suitable filter, and dilute quantitatively with 0.1 N hydrochloric acid to obtain a solution having a concentration of about 4.1 µg of tacrine hydrochloride per mL. [NOTE—Do not use nylon filters.] Immediately prior to removing an aliquot for analysis, mix the solution vigorously.

Blank—Place an empty Capsule of each Capsule strength into a separate 100-mL volumetric flask and prepare as directed for *Test solution.*

Procedure—Concomitantly determine the absorbances at 240 nm of the *Blank*, the *Standard solution*, and the *Test solution* with a suitable spectrophotometer. Calculate the quantity, in mg, of tacrine ($C_{13}H_{14}N_2$) in the Capsule taken by the formula:

$$1000L(C_S / C_U)(198.27/234.73)(A_U / A_S)$$

in which L is the labeled quantity, in mg, of tacrine hydrochloride in the Capsule; C_S is the concentration, in µg per mL, of USP Tacrine Hydrochloride RS in the *Standard solution;* C_U is the concentration, in µg per mL, of tacrine hydrochloride in the *Test solution*, based on the labeled quantity per Capsule and the extent of dilution; 198.27 and 234.73 are the molecular weights of tacrine and tacrine hydrochloride, respectively; and A_U and A_S are the absorbances obtained from the *Test solution* and the *Standard solution*, respectively.

Assay—

0.1 M Triethylamine phosphate solution—Transfer 28 mL of triethylamine to a 2000-mL volumetric flask containing about 1800 mL of water, and mix. Adjust with phosphoric acid to a pH of 3.25, dilute with water to volume, and mix.

Mobile phase—Prepare a filtered and degassed mixture of *0.1 M Triethylamine phosphate solution* and methanol (85:15). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Tacrine Hydrochloride RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 100 µg of tacrine per mL.

Assay preparation—Transfer 10 Capsules to a 1000-mL volumetric flask containing 500 mL of *Mobile phase*. Sonicate for about 45 minutes until the gelatin capsule shells have dissolved. Periodically swirl the flask during sonication to loosen any Capsules sticking to the bottom of the flask and to dissolve floating Capsules. Add an additional 300 mL of *Mobile phase*, shake for 30 minutes on a mechanical shaker, dilute with *Mobile phase* to volume, and mix. Pass an aliquot of this solution through an appropriate filter presaturated with the solution, and dilute, if necessary, with *Mobile phase* to obtain a solution containing about 100 µg of tacrine per mL.

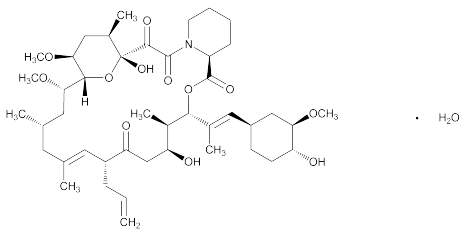
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a variable wavelength detector and a 4.6-mm × 15-cm column that contains 5-µm packing L1. The flow rate is about 2.5 mL per minute. Initially, the detector is maintained at a wavelength of 240 nm. At 7.0 minutes, the wavelength is changed to 260 nm. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the column efficiency is not less than 3500 theoretical plates; the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 1.0%.

Procedure—Separately inject equal volumes (about 30 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of tacrine ($C_{13}H_{14}N_2$) in the portion of Capsules taken by the formula:

$$1000C(198.27/234.73)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Tacrine Hydrochloride RS in the *Standard preparation;* 198.27 and 234.73 are the molecular weights of tacrine and tacrine hydrochloride, respectively; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Tacrolimus



$C_{44}H_{69}NO_{12} \cdot H_2O$ 822.03

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-hy-

droxy-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**]]-;
(–)-(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-4,10,12,18-tetramethyl-15,19-epoxy-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone monohydrate [109581-93-3].

DEFINITION

Tacrolimus contains NLT 98.0% and NMT 102.0% of tacrolimus ($C_{44}H_{69}NO_{12}$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

- **A. Infrared Absorption** ⟨197M⟩
- **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 solutions containing tacrolimus from light.

Solution A: 6 mM phosphoric acid
Solution B: Acetonitrile and *tert*-butyl methyl ether (81:19)
Solution C: *Solution A* and *Solution B* (4:1)
Solution D: *Solution A* and *Solution B* (1:4)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution C (%)	Solution D (%)
0	72	28
30	72	28
53	15	85
54	72	28
60	72	28

Diluent: Acetonitrile and water (7:3)
System suitability solution: 3 mg/mL of USP Tacrolimus System Suitability Mixture RS in *Diluent*. Allow the solution to stand for 3 h at ambient temperature before use.
Standard solution: 3 mg/mL of USP Tacrolimus RS in *Diluent*. Allow the solution to stand for 3 h at ambient temperature before use.
Sample solution: 3 mg/mL of Tacrolimus in *Diluent*. Allow the solution to stand for 3 h at ambient temperature before use.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm; 3-µm packing L1
Temperatures
Column: 60°
Autosampler: 4°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 3* for relative retention times.]
Suitability requirements
Resolution: NLT 3.0 between ascomycin and tacrolimus, *System suitability solution*
Relative standard deviation: NMT 1.0% for the sum of the responses of tacrolimus, tacrolimus open ring, and tacrolimus 19-epimer, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of tacrolimus ($C_{44}H_{69}NO_{12}$) in the portion of Tacrolimus taken:

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

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

Delete the following:

- **Heavy Metals,** *Method II* ⟨231⟩**:** NMT 10 ppm (Official 1-Jan-2018)
- **Organic Impurities, Procedure 1**

Use *Organic Impurities, Procedure 1* when the impurity profile includes tacrolimus methylacrylaldehyde and tacrolimus diene. It is suggested that new columns be conditioned with about 500 mL of alcohol 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: 2.0 mg/mL of Tacrolimus in *Mobile phase*
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. Adjust the *Flow rate* so that the retention time of tacrolimus is approximately 15 min.
Injection volume: 20 µL
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 Tacrolimus taken:

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

r_U = peak response of each impurity from the *Sample solution*
F_i = relative response factor for each corresponding impurity (see *Table 2*)

USP Monographs

r_T = peak response of tacrolimus from the *Sample solution*

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Tacrolimus methylacryl aldehyde[a]	0.55	16.7	0.2
Tacrolimus diene[b]	0.79	2.2	0.2
Tacrolimus impurity 1[c]	0.96	1.0	0.2
Tacrolimus related compound A[d]	0.96	—	—
Tacrolimus	1.0	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]	—	—	0.3

[a] (*E*)-3-[[(1*R*,3*R*,4*R*)-4-Hydroxy-3-methoxycyclohexyl]-2-methylacrylaldehyde.

[b] (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.

[c] Specified unidentified impurity.

[d] For informational purposes 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.

- **ORGANIC IMPURITIES, PROCEDURE 2**

Use *Organic Impurities, Procedure 2* when the impurity profile includes ascomycin, desmethyl tacrolimus, tacrolimus 8-epimer, and tacrolimus 8-propyl analog. Protect solutions containing tacrolimus from light.

Solution A, Solution B, Solution C, Solution D, Mobile phase, Diluent, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard solution: 30 µg/mL of USP Tacrolimus RS in *Diluent*. Allow the solution to stand for 3 h at ambient temperature before use.

Reporting threshold solution: 1.5 µg/mL of USP Tacrolimus RS in *Diluent*

Peak identification solution 1: 10 µg/mL of USP Tacrolimus 8-epimer RS in acetonitrile

Peak identification solution 2: 10 µg/mL of USP Tacrolimus 8-propyl Analog RS in acetonitrile

System suitability

[NOTE—Identify the related compounds by the relative retention times provided in *Table 3*.]

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

Suitability requirements

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: *Sample solution*, *Standard solution*, *Reporting threshold solution*, *Peak identification solution 1*, and *Peak identification solution 2*

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

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

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

r_S = sum of the peak responses of tacrolimus 19-epimer and tacrolimus from the *Standard solution*

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

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

Acceptance criteria: See *Table 3*. Identify tacrolimus 8-epimer and tacrolimus 8-propyl analog using *Peak identification solution 1* and *Peak identification solution 2*. Report impurity peaks with responses NLT that of the peak in the *Reporting threshold solution* (0.05%). Disregard peaks with retention times less than 3 min.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tacrolimus open ring[a,b]	0.52	—
Ascomycin 19-epimer (if present)[c,d]	0.54	0.1
Tacrolimus 19-epimer[b,e]	0.63	—
Ascomycin[f]	0.87	0.50
Desmethyl tacrolimus (if present)[d,g]	0.94	0.1
Tacrolimus	1.00	—
Tacrolimus 8-epimer[h]	1.28	0.15

[a] (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.

[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*,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.

[d] If possible from the manufacturing process.

[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*,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.

[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-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]oxaaza-cyclotricosine-1,7,20,21-(4*H*,23*H*)-tetrone.

[h] (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.

[i] (3*S*,4*R*,5*S*,8*R*,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]oxaaza-cyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone.

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

Table 3 *(Continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tacrolimus 8-propyl analog[i]	1.33	0.15
Any individual unspecified impurity	—	0.1
Total impurities[j]	—	1.0

[a] (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.

[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*,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.

[d] If possible from the manufacturing process.

[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*,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.

[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-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.

[h] (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.

[i] (3*S*,4*R*,5*S*,8*R*,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.

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

SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨**781S**⟩
  Sample solution: 10 mg/mL in *N,N*-dimethylformamide
  Acceptance criteria: –110° to –115° on the "as-is" basis
- **WATER DETERMINATION,** *Method I* ⟨**921**⟩**:** NMT 4.0%

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 test for *Organic Impurities* 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 8-epimer RS
  (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.
  $C_{44}H_{69}NO_{12}$ 804.02
  USP Tacrolimus 8-propyl Analog RS
  (3*S*,4*R*,5*S*,8*R*,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
  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*R*,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

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV absorption spectrum of the major peak of the *Sample solution* and that of the *Standard solution* exhibit maxima and minima at the same wavelengths, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  Allow the *Standard solution* and *Sample solution* to stand for 3 h at ambient temperature before use. Protect solutions containing tacrolimus from light.
  Solution A: 6 mM phosphoric acid
  Solution B: 50 g/L of polyoxyethylene (23) lauryl ether. [NOTE—Polyoxyethylene (23) lauryl ether is also called Brij-35.]
  Solution C: Acetonitrile and *Solution B* (7:3)
  Mobile phase: Acetonitrile, *tert*-butyl methyl ether, and *Solution A* (335:55:600)
  Standard solution: 50 µg/mL of USP Tacrolimus RS in *Solution C*