Add the following:

•• **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.• (RB 1-Jun-2011)

• **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Sertraline Hydrochloride RS
USP Sertraline Hydrochloride Racemic Mixture RS
(1*RS*,4*RS*)-4-(3,4-Dichlorophenyl)-*N*-methyl-1,2,3,4-tetrahydro-1-naphthylamine hydrochloride.
$C_{17}H_{17}Cl_2N \cdot HCl$ 342.69

Delete the following:

▪Sincalide for Injection

DEFINITION
Sincalide for Injection is a sterile, synthetically prepared C-terminal octapeptide of cholecystokinin and sodium chloride. It contains NLT 85.0% and NMT 125.0% of the labeled amount of sincalide ($C_{49}H_{62}N_{10}O_{16}S_3$).

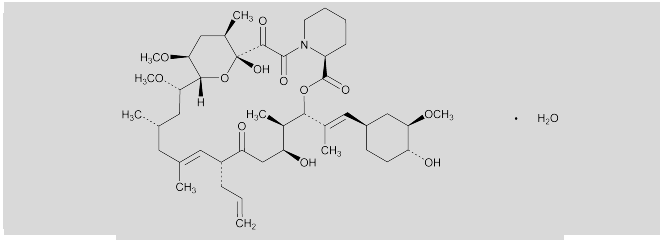
ASSAY
• **PROCEDURE**
Test animals: Select male guinea pigs, each weighing at least 500 g, but restrict selection so that no guinea pig is more than 30% heavier than the lightest. Withdraw food, but not water, from each animal.
Sodium chloride solution: Sodium Chloride Injection containing 0.9% of NaCl
Standard Stock solutionA: 10 µg/mL of USP Sincalide RS in *Sodium chloride solution*
Standard solutions: 0.0624 µg of sincalide/kg of the animal's body weight in each 0.1 mL from *Standard Stock solution*. Prepare a series of 1-in-2 dilutions of this solution with *Sodium chloride solution* to contain 0.0312, 0.0156, and 0.0078 µg of sincalide/kg of body weight. [NOTE—Other dose levels may be used if so indicated by the responses obtained in the *Procedure*.]
Sample Stock solution: Constitute 1 vial of Sincalide for Injection in a sufficient volume of Water for Injection to obtain a solution having a concentration of about 1 µg of sincalide/mL.
Sample solutions: 0.0624 µg of sincalide/kg of the animal's body weight in each 0.1 mL from *Sample Stock solution*. Prepare a series of 1-in-2 dilutions of this solution with *Sodium chloride solution* to contain 0.0312, 0.0156, and 0.0078 µg of sincalide/kg of body weight. [NOTE—Other dose levels may be used if so indicated by the responses obtained in the *Procedure*.]
Analysis: Anesthetize each guinea pig by injecting it, subcutaneously, with 2.25 g of urethane/kg of body weight, administered as a 25% solution. Perform a tracheotomy, then expose a jugular vein, and cannulate with a polyethylene catheter. Tie a thin silk line to the free pole or fundus of the gallbladder, or attach a thin hook with connecting silk line to the wall of the fundus. Gallbladder contractile responses, transmitted through the silk line, cause a change in the line tension. Connect the free end of the silk line to a force transducer, and impose on the system an initial tension of about 2 *g*. Connect the force transducer to a polygraph, which records the contractile responses. Determine the sensitivity or the responsiveness of the guinea pig's gallbladder by making a few trial injections through the jugular vein catheter, then select two nonconsecutive dose levels (e.g., 0.0624 and 0.0156) for the *Assay*. Use the same dose levels for the *Sample solutions* as for the *Standard solutions*. Administer the selected dose levels of the *Standard solutions* and the *Assay* preparations as 0.1-mL dose volumes in random order, taking 2–3 s to inject each dose volume and flushing each through the catheter with about 0.5 mL of *Sodium chloride solution*. Make injections at about 10-min intervals or when the gallbladder has returned to approximately the initial 2 *g* of tension.
[NOTE—Three injections of each dose level may be made. As many as three different samples can be tested on the same animal before retiring the animal.]
Calculation: Calculate the potency of each vial (see *Design and Analysis of Biological Assays* ⟨111⟩), using a log transformation, straight-line method with a least-squares fitting procedure, and a test for linearity.
Acceptance criteria: 85.0%–125.0%

SPECIFIC TESTS
• **pH** ⟨**791**⟩
Sample solution: Contents of 1 vial in 5 mL water
Acceptance criteria: 5.0–7.5
• **PARTICULATE MATTER IN INJECTIONS** ⟨**788**⟩**:** Meets the requirements for small-volume injections
• **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements for *Injections* ⟨1⟩, *Constituted Solutions*.
• **BACTERIAL ENDOTOXINS TEST** ⟨**85**⟩**:** NMT 83.3 USP Endotoxin Units/µg of sincalide
• **OTHER REQUIREMENTS:** It meets the requirements under *Injections* ⟨1⟩.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type I glass.
• **LABELING:** Label it to state that it is to be used within 24 h after constitution.
• **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Endotoxin RS
USP Sincalide RS▪1S (*USP35*)

Add the following:

▪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-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**]]-;
(–)-(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 $C_{44}H_{69}NO_{12}$, 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**

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. Protect from light by using low-actinic glassware.
Standard solution: 3 mg/mL of USP Tacrolimus RS in *Diluent*. Allow the solution to stand for 3 h at ambient temperature before use. Protect from light by using low--actinic glassware.
Sample solution: 3 mg/mL of Tacrolimus in *Diluent*. Allow the solution to stand for 3 h at ambient temperature before use. Protect from light by using low-actinic glassware.
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°
Autosampler temperature: 4°
Flow rate: 1.5 mL/min
Injection size: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
[Note—The relative retention times for tacrolimus open ring, tacrolimus 19-epimer, ascomycin, and tacrolimus are 0.52, 0.63, 0.87, and 1.0, respectively.]
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 $C_{44}H_{69}NO_{12}$ 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%, calculated on the anhydrous and solvent-free basis

IMPURITIES

Inorganic Impurities

- **Residue on Ignition** ⟨281⟩**:** NMT 0.1%
- **Heavy Metals,** *Method II* ⟨231⟩**:** NMT 10 ppm

Organic Impurities

- **Procedure 1**

[Note—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 size: 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:

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

r_U = peak response for each peak in the *Sample solution*
F = relative response factor for the corresponding peak (see *Table 2*)

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 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-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26,26a,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 ascomycin, desmethyl tacrolimus, tacrolimus 8-epimer, and tacrolimus 8-propyl analog.]

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. Protect from light by using low-actinic glassware.

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

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, tacrolimus open ring, and tacrolimus 19-epimer, *Standard solution*

Analysis

Samples: *Sample solution*, *Standard solution*, and *Reporting threshold solution*

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 for each impurity peak 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)

Acceptance criteria: See *Table 3*. 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[c]	0.54	0.1
Tacrolimus 19-epimer[b,d]	0.63	—
Ascomycin[e]	0.87	0.50
Desmethyl tacrolimus[f]	0.94	0.1
Tacrolimus	1.00	—
Tacrolimus 8-epimer[g]	1.28	0.1
Tacrolimus 8-propyl analog[h]	1.33	0.1
Any individual unspecified impurity	—	0.1
Total impurities	—	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] (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.

[e] (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.

[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-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.

[g] (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.

[h] (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*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.

SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨**781S**⟩**:** –110° to –115° on an "as is" basis

Sample solution: 10 mg/mL in *N,N*-dimethylformamide

- **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 *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■1S (*USP35*)

Add the following:

■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*.)