Tacrolimus Capsules

Type of Posting	Notice of Intent to Revise
Posting Date	20-Nov-2020
Targeted Official Date	To Be Determined, Revision Bulletin
Expert Committee	Small Molecules 1

In accordance with the Rules and Procedures of the Council of Experts and the Pending Monograph Guideline, this is to provide notice that the Small Molecules 1 Expert Committee intends to revise the Tacrolimus Capsules monograph.

- Based on the supporting data received from a manufacturer awaiting FDA approval, the Expert Committee proposes to add *Dissolution Test* 8 to the monograph. The Notice of Intent to Revise regarding *Dissolution Test 7* is previously posted.

The revision also necessitates a change in the table numbering in the tests for *Organic Impurities, Procedure 1* and *Organic Impurities, Procedure 2*.

The proposed revision is contingent on FDA approval of a product that meets the proposed monograph specifications. The proposed revision will be published as a Revision Bulletin and an official date will be assigned to coincide as closely as possible with the FDA approval of the associated product.

See below for additional information about the proposed text.[1]

Should you have any questions, please contact Praveen Pabba, Scientific Liaison (301-816-8540 or pkp@usp.org).

[1] This text is not the official version of a *USP–NF* monograph and may not reflect the full and accurate contents of the currently official monograph. Please refer to the current edition of the *USP–NF* for official text.

USP provides this text to indicate changes that we anticipate will be made official once the product subject to this proposed revision under the Pending Monograph Program receives FDA approval. Once FDA approval is granted for the associated revision request, a Revision Bulletin will be posted that will include the changes indicated herein, as well as any changes indicated in the product's final approval, combined with the text of the monograph as effective on the date of approval. Any revisions made to a monograph under the Pending Monograph Program that are posted without prior publication for comment in the *Pharmacopeial Forum* must also meet the requirements outlined in the USP Guideline on Use of Accelerated Processes for Revisions to the *USP–NF*.

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*

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. When this procedure is used for *Identification* test *B*, use a diode array detector set at 200–400 nm.

Column: 4.0-mm × 5.5-cm; 3-µm packing L1

Column temperature: 60°

Flow rate: 1 mL/min

Injection volume: 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 the labeled amount 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

Change to read:

- **Dissolution** ⟨711⟩

Test 1

Medium: Hydroxypropylcellulose in water (1:2 × 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: UV 210 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Column temperature: 50°

Flow rate: Adjust the flow rate so that the retention time of tacrolimus is approximately 14 min.

Injection volume: See *Table 2*.

Table 2

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

Capsule Strength (mg)	Injection Volume (µL)
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 solutions containing tacrolimus from light.]

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 volume: 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 hydroxypropylcellulose in water. Adjust with phosphoric acid to a pH of 4.5; 900 mL.
Apparatus 2 (without sinker) and **Time:** Proceed as directed in *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 in mg.
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 volume: 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.

Test 4: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.

Medium: Hydroxypropylcellulose in water (1 in 20,000), adjusted with phosphoric acid to a pH of 4.5. See *Table 3* for the volume.

Table 3

Capsule Strength (mg)	Volume of Medium (mL)
0.5	500
1	900
5	900

Apparatus 2: 50 rpm, with sinkers

Time: 120 min

Diluent: 1 mg/mL of hydroxypropylcellulose in water. Sonicate as needed to dissolve.

Buffer: To a solution of 1 g/L of sodium 1-hexanesulfonate in water add 0.1 mL/L of trifluoroacetic acid.

Mobile phase: Acetonitrile, methanol, and *Buffer* (550:50:400)

Standard stock solution: Dissolve USP Tacrolimus RS in acetonitrile. See *Table 4* for the concentrations (L is the Capsule label claim in mg).

Table 4

Capsule Strength (mg)	Concentration (mg/mL)
0.5	$L/25$
1	$L/45$

Capsule Strength (mg)	Concentration (mg/mL)
5	*L*/45

Standard solution: Dilute the *Standard stock solution* with *Diluent*. See *Table 5* for the concentrations (*L* is the Capsule label claim in mg).

Table 5

Capsule Strength (mg)	Concentration (mg/mL)
0.5	*L*/500
1	*L*/900
5	*L*/900

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 × 15-cm; 5-µm packing L1

Column temperature: 60°

Flow rate: 1 mL/min

Injection volume: 100 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 3.0%

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 = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

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

L = label claim (mg/Capsule)

V = volume of *Medium* (mL) (see *Table 3*)

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

Test 5: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.

Medium: 0.05 g/L hydroxypropylcellulose in water. Adjust with phosphoric acid to a pH of 4.5; 900 mL.

Apparatus 2: 50 rpm, with sinkers

Time: 90 min

Solution A: 0.1 mL/L of trifluoroacetic acid in water

Mobile phase: Acetonitrile and *Solution A* (50:50)

Standard stock solution: 0.22 mg/mL of USP Tacrolimus RS in acetonitrile

Standard solution: (*L*/900) mg/mL of USP Tacrolimus RS from the *Standard stock solution* in *Medium*, where *L* is the label claim in mg/Capsule

Sample solution: Centrifuge a portion of the solution under test. Use the supernatant.

Chromatographic system

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

Mode: LC

Detector: UV 205 nm

Column: 2.1-mm × 15-cm; 3.5-µm packing L7

Column temperature: 60°

Flow rate: 0.8 mL/min

Injection volume: 750 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for tacrolimus 19-epimer (tautomer 1), tacrolimus open-ring (tautomer 2), and tacrolimus are 0.55, 0.79, and 1.0, respectively.]

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 4.0% for the peaks due to tautomer 1, tautomer 2, and tacrolimus.

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 open-ring, and tacrolimus 19-epimer from the *Sample solution*

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

C_S = concentration of USP Tacrolimus RS in 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.

Test 6: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 6*.

Dilute phosphoric acid: Transfer 7.1 mL of phosphoric acid to a 100 mL volumetric flask, and dilute with water to volume.

Medium: 50 mg/L of hydroxypropyl cellulose in water. Adjust with *Dilute* phosphoric acid to a pH of 4.5; 900 mL.

Apparatus 2: 50 rpm

Time: 60 min

Buffer: 3.6 g/L of monobasic potassium phosphate in water. Adjust with *Dilute* phosphoric acid to a pH of 2.5.

Mobile phase: Acetonitrile and *Buffer* (1:1)

Standard stock solution: 0.11 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 label claim in mg/Capsule.

Sample solution: Centrifuge a portion of the solution under test. Use the supernatant.

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 volume: 100 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for tacrolimus 19-epimer, tacrolimus open ring, and tacrolimus are 0.77, 0.89, and 1.0, respectively.]

Suitability requirements

Tailing factor: NMT 2.0 for tacrolimus

Relative standard deviation: NMT 2.0% for the sum of tacrolimus 19-epimer, tacrolimus open ring, and tacrolimus

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 USP Tacrolimus RS in 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 8:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 8*.

Medium: 50 mg/L of hydroxypropylcellulose in water. Adjust with 6% phosphoric acid to a pH of 4.5; 500 mL.

Apparatus 2: 75 rpm with sinker

Times: 15, 30, and 90 min

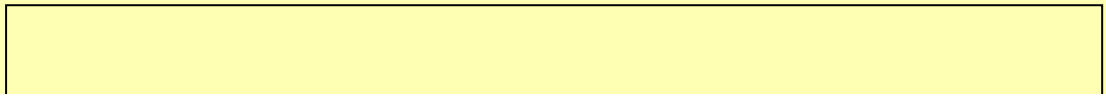
Diluent: 1 mg/mL of hydroxypropylcellulose in water

Buffer: Dissolve 1 g of sodium 1-hexanesulfonate in 1 L of water and add 0.1 mL of trifluoroacetic acid.

Mobile phase: Acetonitrile, methanol, and *Buffer* (55:5:40)

Standard stock solution: Dissolve USP Tacrolimus RS in acetonitrile. See *Table 6* for the concentrations.

Table 6



Capsule Strength (mg)	Final Concentration (µg/mL)
0.5	19
1.0	22
5.0	110

Standard solution: Dilute the *Standard stock solution* with *Diluent* to obtain a final concentration of (*L*/500) mg/mL, where *L* is the label claim in mg/Capsule. Using the *Standard stock solution* to prepare the final *Standard solution*, acetonitrile will not exceed 10%.

Sample solution: Pass 10 mL of the solution under test through a 1-um glass filter. Replace the portion of solution withdrawn with an equal volume of *Medium*.

Chromatographic system

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

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Column temperature: 60°

Flow rate: 1.0 mL/min

Injection volume: 100 uL

Run time: NLT 1.6 times the retention time of tacrolimus

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i) of tacrolimus ($C_{44}H_{69}NO_{12}$) in the sample withdrawn from the vessel at each time point (*i*):

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

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)

Calculate the percentage of the labeled amount of tacrolimus ($C_{44}H_{69}NO_{12}$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$

$$\text{Result}_3 = \{(C_3 \times V) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of tacrolimus in the portion of sample withdrawn at each time point (mg/mL)

V = volume of *Medium*, 500 mL

L = label claim (mg/Capsule)

V_S = volume of the *Sample solution* withdrawn at each time point (mL)

Tolerances: See *Table 7*.

Table 7

Time Point (*i*)	Time (min)	Tolerances (%)
1	15	NLT 20
2	30	NMT 75
3	90	NLT 80 (*Q*)

▲ (TBD)

- **Uniformity of Dosage Units ⟨905⟩:** Meet the requirements

IMPURITIES

Change to read:

- **Organic Impurities, Procedure 1**

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

Columns: 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

Run time: 3 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:

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

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

F_i = relative response factor for each corresponding impurity (see Table ▲8▲ (TBD))

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

Acceptance criteria: See Table ▲8▲ (TBD). Disregard peaks due to the solvent.

Table ▲8▲ (TBD)

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[e,f]	1.1	—	—
Tacrolimus open ring[e,g]	1.3	—	—
Any individual unspecified impurity	—	1.0	0.2
Total impurities	—	—	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] Tacrolimus related compound A is listed here to indicate the relative retention time of this compound. It is used in the procedure to evaluate system suitability and is not to be reported. It is not to be included in total impurities.

[e] 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 and are not included in total impurities.

[f] (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.

[g] (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.

Change to read:

• **Organic Impurities, Procedure 2**

Use *Organic Impurities, Procedure 2* when the impurity profile includes tacrolimus hydroxy 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 solutions containing tacrolimus from light.

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 ▲9▲ (TBD).

Table ▲9▲ (TBD)

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 of 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*

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

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

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 volume: 40 µL

System suitability

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

Signal-to-noise ratio: NLT 10.0, *Sensitivity solution*

Analysis

Samples: *Standard solution*, *Peak identification solution 1*, *Peak identification solution 2*, and *Sample solution*

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

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

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 = nominal concentration of tacrolimus in the *Sample solution* (mg/mL)

P = potency of tacrolimus in USP Tacrolimus RS (mg/mg)

F = relative response factor (see Table ▲*10*▲ (TBD))

Acceptance criteria: See Table ▲*10*▲ (TBD). Identify tacrolimus 8-epimer and tacrolimus 8-propyl analog using *Peak identification solution 1* and *Peak identification solution 2*. Disregard peaks that are smaller than the tacrolimus peak in the *Sensitivity solution*.

Table ▲10▲ (TBD)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Tacrolimus hydroxy acid[a]	0.18	1.5	0.5
Tacrolimus open ring[b,c]	0.49	—	—
Ascomycin 19-epimer[d,e]	0.52	—	—
Tacrolimus 19-epimer[b,f]	0.62	—	—
Ascomycin[e,g]	0.84	—	—
Desmethyl tacrolimus[e,h]	0.91	—	—
Tacrolimus	1.0	—	—
Tacrolimus 8-epimer[i]	1.28	1.0	0.5

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Tacrolimus 8-propyl analog[e,j]	1.30	—	—
Any individual unspecified impurity	—	1.0	0.2
Total impurities	—	—	1.5

[a] (3*S*,4*R*,5*S*,8*R*,12*S*,14*S*,15*R*,16*S*,18*R*,25a*S*,*E*)-8-Allyl-5,15,19-trihydroxy-3-{(*E*)-1-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]prop-1-en-2-yl}-14,16-dimethoxy-4,10,12,18-tetramethyl-1,7,20-trioxo-1,3,4,5,6,7,8,11,12,13,14,15,16,17,18,19,20,22,23,24,25,25a-docosahydropyrido[2,1-*c*][1]oxa[4]azacyclodocosine-19-carboxylic 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 and are not included in total impurities.

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

[f] (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.

[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*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.

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. 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 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

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*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

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