Tacrolimus Capsules

DEFINITION
Tacrolimus Capsules contain NLT 93.0% and NMT 105.0% of the labeled amount of tacrolimus (C₄₄H₆₉NO₁₂).

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.

ASSAY
• PROCEDURE
[NOTE—Allow the 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: 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
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 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 (C₄₄H₆₉NO₁₂) in the portion of Capsules taken:

Result = (r₀/r₅) × (Cₛ/C₀) × 100

r₀ = sum of the peak responses of tacrolimus and tacrolimus 19-epimer from the Sample solution
r₅ = sum of the peak responses of tacrolimus and tacrolimus 19-epimer from the Standard solution
Cₛ = concentration of USP Tacrolimus RS in the Standard solution (mg/mL)
C₀ = 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 × 10³); 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>
<thead>
<tr>
<th>Sample</th>
<th>Final Concentration (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.4</td>
</tr>
<tr>
<td>1</td>
<td>0.8</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

Chromatographic system
(See Chromatography (621), System Suitability.)
Mode: LC
Detector: 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>
<thead>
<tr>
<th>Sample</th>
<th>Injection Volume (μL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>800</td>
</tr>
<tr>
<td>1</td>
<td>400</td>
</tr>
<tr>
<td>5</td>
<td>80</td>
</tr>
</tbody>
</table>

[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 (C₄₄H₆₉NO₁₂) dissolved:

Result = (rₒ/r₅) × Cₛ × D × V × (100/L)
Tacrolimus

\[ r_o = \text{peak response of tacrolimus from the Sample solution} \]
\[ r_s = \text{peak response of tacrolimus from the Standard solution} \]
\[ C_s = \text{concentration of USP Tacrolimus RS in the Standard solution (mg/mL)} \]
\[ D = \text{dilution factor of the Sample solution} \]
\[ V = \text{volume of Medium, 900 mL} \]
\[ L = \text{label claim (mg/Capsule)} \]

**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 mL 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:** 4.6-mm × 10-cm; 5-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 2.0% for 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:

\[ \text{Result} = (r_o/r_s) \times (C_s/L) \times V \times 100 \]

\[ r_o = \text{sum of the peak responses of tacrolimus and tacrolimus 19-epimer from the Sample solution} \]

\[ r_s = \text{sum of the peak responses of tacrolimus and tacrolimus 19-epimer from the Standard solution} \]

\[ C_s = \text{concentration of the Standard solution (mg/mL)} \]

\[ L = \text{label claim (mg/Capsule)} \]

\[ V = \text{volume of Medium, 900 mL} \]

**Tolerances:** NLT 75% (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 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:

\[ \text{Result} = (r_o/r_s) \times (C_s/L) \times V \times 100 \]

\[ r_o = \text{sum of the peak responses of tacrolimus, tacrolimus 19-epimer, and tacrolimus open ring from the Sample solution} \]

\[ r_s = \text{sum of the peak responses of tacrolimus, tacrolimus 19-epimer, and tacrolimus open ring from the Standard solution} \]

\[ C_s = \text{concentration of the Standard solution (mg/mL)} \]

\[ L = \text{label claim (mg/Capsule)} \]

\[ V = \text{volume of Medium, 900 mL} \]

**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>
<thead>
<tr>
<th>Capsule Strength (mg)</th>
<th>Volume of Medium (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>500</td>
</tr>
<tr>
<td>1</td>
<td>900</td>
</tr>
<tr>
<td>5</td>
<td>900</td>
</tr>
</tbody>
</table>

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**UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements.

**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>
<thead>
<tr>
<th>Capsule Strength (mg)</th>
<th>Concentration (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>L/25</td>
</tr>
<tr>
<td>1</td>
<td>L/45</td>
</tr>
<tr>
<td>5</td>
<td>L/45</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Capsule Strength (mg)</th>
<th>Concentration (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>L/500</td>
</tr>
<tr>
<td>1</td>
<td>L/900</td>
</tr>
<tr>
<td>5</td>
<td>L/900</td>
</tr>
</tbody>
</table>

**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°C  
**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 (C44H69NO12) dissolved:  
Result = \( \frac{r_0}{r_s} \times \frac{(C_s/L)}{V} \times 100 \)  

- \( r_0 \) = 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 (C44H69NO12) is dissolved.  

**IMPURITIES**  
**Change to read:**  

- **ORGANIC IMPURITIES, PROCEDURE 1**  
[NOTE—Use Organic Impurities, Procedure 1 when the impurity profile includes tacrolimus diene and tacrolimus regiosomer. 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°C  
**Flow rate:** 1.5 mL/min. [NOTE—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:  
Result = \( \frac{(r_u/F)}{[1/\Sigma(r/F)]} \times 100 \)  

- \( r_u \) = peak response of each impurity in the Sample solution  
- \( F \) = relative response factor for each corresponding impurity (see Table 6)  

**Acceptance criteria:** See Table 6. Disregard peaks due to the solvent.
**Organic Impurities, Procedure 2**

**Relative Retention Time**

<table>
<thead>
<tr>
<th>Name</th>
<th>Relative Retention Time</th>
<th>Relative Response Factor</th>
<th>Acceptance Criteria, NMT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tacrolimus diene</td>
<td>0.79</td>
<td>2.2</td>
<td>0.3</td>
</tr>
<tr>
<td>Tacrolimus regiosomer</td>
<td>0.88</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Tacrolimus impurity 1</td>
<td>0.96</td>
<td>1.0</td>
<td>0.3</td>
</tr>
<tr>
<td>Tacrolimus related compound</td>
<td>0.96</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>1.0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Tacrolimus 19-epimer</td>
<td>1.1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Tacrolimus open ring</td>
<td>1.3</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

**Total impurities**

| —                           | 1.0                     | —                        |

**Change to read:**

**Organic Impurities, Procedure 2**

**Relative Retention Time**

<table>
<thead>
<tr>
<th>Name</th>
<th>Relative Retention Time</th>
<th>Relative Response Factor</th>
<th>Acceptance Criteria, NMT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tacrolimus diene</td>
<td>0.79</td>
<td>2.2</td>
<td>0.3</td>
</tr>
<tr>
<td>Tacrolimus regiosomer</td>
<td>0.88</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Tacrolimus impurity 1</td>
<td>0.96</td>
<td>1.0</td>
<td>0.3</td>
</tr>
<tr>
<td>Tacrolimus related compound</td>
<td>0.96</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>1.0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Tacrolimus 19-epimer</td>
<td>1.1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Tacrolimus open ring</td>
<td>1.3</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

**Total impurities**

| —                           | 1.0                     | —                        |

**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 x 15-cm; 3-µm packing L1

**Column temperature:** 40 °C

**Flow rate:** 1.5 mL/min

**Injection volume:** 40 µL

**System suitability**

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

**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**

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

\[
\text{Result} = \left( \frac{r_0}{r_3} \right) \times \left( \frac{C_l}{C_0} \right) \times P \times 100
\]

where:

- \( r_0 \) = peak response of each impurity from the Sample solution
- \( r_3 \) = sum of the peak responses for tacrolimus 19-epimer and tacrolimus from the Standard solution
- \( C_l \) = concentration of USP Tacrolimus RS in the Standard solution (mg/mL)
- \( C_0 \) = nominal concentration of tacrolimus in the Sample solution (mg/mL)
- \( P \) = potency of tacrolimus in USP Tacrolimus RS (mg/mg)

**Acceptance criteria:** See Table 8. (Continued) Disregard peaks that are smaller than the tacrolimus peak in the Sensitivity solution.
Tacrolimus - Table 80

<table>
<thead>
<tr>
<th>Name</th>
<th>Relative Retention Time</th>
<th>Acceptance Criteria, NMT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tacrolimus 21-carboxylic acida</td>
<td>0.18</td>
<td>0.5</td>
</tr>
<tr>
<td>Tacrolimus open ringb</td>
<td>0.49</td>
<td>—</td>
</tr>
<tr>
<td>Ascomycin 19-epimerc</td>
<td>0.52</td>
<td>—</td>
</tr>
<tr>
<td>Tacrolimus 19-epimerd</td>
<td>0.62</td>
<td>—</td>
</tr>
<tr>
<td>Ascomycinf</td>
<td>0.84</td>
<td>—</td>
</tr>
<tr>
<td>De-methyl tacrolimusf</td>
<td>0.91</td>
<td>—</td>
</tr>
<tr>
<td>Tacrolimus 8-epimerf</td>
<td>1.0</td>
<td>—</td>
</tr>
<tr>
<td>Tacrolimus 8-propyl analogf</td>
<td>1.28</td>
<td>0.5</td>
</tr>
<tr>
<td>Any individual unspecified impurity</td>
<td>—</td>
<td>0.2</td>
</tr>
<tr>
<td>Total impurities</td>
<td>—</td>
<td>1.5</td>
</tr>
</tbody>
</table>

a 2-[(2,3R,5S,5′R)-2,6,7,10,13,14,15,16,17,18,19,24,25,26,26a-tetrahydroxy-5,19-dihydroxy-3-[(E)-(E)-2-[(1R,3R,4R)-4-hydroxy-3-methoxyxocyclohexyl]-1-methoxy]-14,16-dimethyl-8-oxo-12-(3-piperidine-2-carboxyloxy)tetradeca-5,13-dienyl]-2-hydroxy-5-methoxy-3-methyltetrahydro-2H-pyran-2-yl]-2-oxoacetic acid.

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

c USP Tacrolimus Related Compound A RS

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