Tacrolimus Capsules

<table>
<thead>
<tr>
<th>Type of Posting</th>
<th>Notice of Intent to Revise</th>
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<tr>
<td>Posting Date</td>
<td>20-Nov-2020</td>
</tr>
<tr>
<td>Targeted Official Date</td>
<td>To Be Determined, Revision Bulletin</td>
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<tr>
<td>Expert Committee</td>
<td>Small Molecules 1</td>
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</table>

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 \( \text{C}_{44}\text{H}_{69}\text{NO}_{12} \).

**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:** 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 x 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 \( \text{C}_{44}\text{H}_{69}\text{NO}_{12} \) in the portion of Capsules taken:

  \[
  \text{Result} = \left( \frac{r_U}{r_S} \right) \times \left( \frac{C_S}{C_U} \right) \times 100
  \]

  \( r_U \) = sum of the peak responses of tacrolimus and tacrolimus 19-epimer from the Sample solution
\[ r_S = \text{sum of the peak responses of tacrolimus and tacrolimus 19-epimer from the Standard solution} \]

\[ C_S = \text{concentration of USP Tacrolimus RS in the Standard solution (mg/mL)} \]

\[ C_U = \text{nominal concentration of the Sample solution (mg/mL)} \]

Acceptance criteria: 93.0%–105.0%

PERFORMANCE TESTS

Change to read:

- **Dissolution (711)**
  
  **Test 1**
  
  **Medium:** Hydroxypropylcellulose in water \((1:2 \times 10^4)\), 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>
<thead>
<tr>
<th>Capsule Strength (mg)</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>

<table>
<thead>
<tr>
<th>Chromatographic system</th>
</tr>
</thead>
<tbody>
<tr>
<td>(See Chromatography (621), System Suitability.)</td>
</tr>
<tr>
<td><strong>Mode:</strong> LC</td>
</tr>
<tr>
<td><strong>Detector:</strong> UV 210 nm</td>
</tr>
<tr>
<td><strong>Column:</strong> 4.6-mm (\times) 15-cm; 5-µm packing L7</td>
</tr>
<tr>
<td><strong>Column temperature:</strong> 50°</td>
</tr>
<tr>
<td><strong>Flow rate:</strong> Adjust the flow rate so that the retention time of tacrolimus is approximately 14 min.</td>
</tr>
<tr>
<td><strong>Injection volume:</strong> See Table 2.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Capsule Strength (mg)</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>Capsule Strength (mg)</td>
<td>Injection Volume (µL)</td>
</tr>
<tr>
<td>-----------------------</td>
<td>-----------------------</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 (C_{44}H_{69}NO_{12}) dissolved:

\[
\text{Result} = \left( \frac{r_U}{r_S} \right) \times C_S \times D \times V \times (100/L)
\]

- \(r_U\) = 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:

\[ \text{Result} = \left( \frac{r_U}{r_S} \right) \times \left( \frac{C_S}{L} \right) \times V \times 100 \]

- \( r_U \) = 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\textsubscript{44}H\textsubscript{69}NO\textsubscript{12}) dissolved:

\[
\text{Result} = \left( \frac{r_U}{r_S} \right) \times \left( \frac{C_S}{L} \right) \times V \times 100
\]

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

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

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

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

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

**Tolerances:** NLT 75% (Q) of the labeled amount of tacrolimus (C\textsubscript{44}H\textsubscript{69}NO\textsubscript{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>Capulet 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>

**Table 3**

**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>Capulet 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>
</tbody>
</table>

**Table 4**
<table>
<thead>
<tr>
<th>Capsule Strength (mg)</th>
<th>Concentration (mg/mL)</th>
</tr>
</thead>
<tbody>
<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 5**

<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°

**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:

\[
\text{Result} = \left( \frac{r_U}{r_S} \right) \times \left( \frac{C_S}{L} \right) \times V \times 100
\]

\( r_U \) = 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 (C44H69NO12) dissolved:

\[
\text{Result} = \left( \frac{r_U}{r_S} \right) \times \left( \frac{C_S}{L} \right) \times V \times 100
\]

\[r_U\] = 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 (C44H69NO12) 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 x 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:

  \[\text{Result} = \left(\frac{r_U}{r_S}\right) \times \left(\frac{C_S}{L}\right) \times V \times 100\]

  - \(r_U\) = 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
<table>
<thead>
<tr>
<th>Capsule Strength (mg)</th>
<th>Final Concentration (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>19</td>
</tr>
<tr>
<td>1.0</td>
<td>22</td>
</tr>
<tr>
<td>5.0</td>
<td>110</td>
</tr>
</tbody>
</table>

**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-µm 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°C
- **Flow rate:** 1.0 mL/min
- **Injection volume:** 100 µL
- **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 \((C_{44H_{69}NO_{12}})\) in the sample withdrawn from the vessel at each time point \((i)\):

\[
\text{Result}_i = \left(\frac{r_U}{r_S}\right) \times C_S
\]

\(r_U\) = 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 \((C_{44H_{69}NO_{12}})\) dissolved at each time point \((i)\):

\[
\text{Result}_1 = C_i \times V \times (1/L) \times 100
\]

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

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

\(C_i\) = 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>
<thead>
<tr>
<th>Time Point ((i))</th>
<th>Time ((\text{min}))</th>
<th>Tolerances ((%))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>NLT 20</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>NMT 75</td>
</tr>
<tr>
<td>3</td>
<td>90</td>
<td>NLT 80 (Q)</td>
</tr>
</tbody>
</table>

▲ (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:

\[
\text{Result} = \left( \frac{r_j/F_j}{r_T} \right) \times \left( \frac{1}{1 + \sum (r_j/F_j)} \right) \times 100
\]

- \( r_j \) = peak response of each impurity from the Sample solution
- \( F_j \) = relative response factor for each corresponding impurity (see Table [TBD])
- \( r_T \) = peak response of tacrolimus from the Sample solution

**Acceptance criteria:** See Table [TBD]. Disregard peaks due to the solvent.

<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(^a)</td>
<td>0.79</td>
<td>2.2</td>
<td>0.3</td>
</tr>
<tr>
<td>Tacrolimus regioisomer(^b)</td>
<td>0.88</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Tacrolimus impurity 1(^c)</td>
<td>0.96</td>
<td>1.0</td>
<td>0.3</td>
</tr>
<tr>
<td>Tacrolimus related compound A(^d)</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(^e,f)</td>
<td>1.1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Tacrolimus open ring(^g)</td>
<td>1.3</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Any individual unspecified impurity</td>
<td>—</td>
<td>1.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Total impurities</td>
<td>—</td>
<td>—</td>
<td>1.0</td>
</tr>
</tbody>
</table>

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

\(^b\) (4E,11E)-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-3H-pyrido[2,1-c][1,4]oxazacyclotetrasine-1,9,22,23(6H,25H)-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.

\(^f\) (3S,4R,5S,8R,9E)-12S,14S,15S,18S,18R,26aS)-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-[(1R,3R,5R)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3H-pyrido[2,1-c][1,4]oxazacyclotetrasine-1,7,19,21(4H,23H)-tetrone.

\(^g\) (3S,4R,5S,8R,12S,14S,15S,16S,18S,26aS,3E)-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-[(1R,3R,4R)-4-hydroxy-3-methoxycyclohexyl]-1-methylvinyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-3H-pyrido[2,1-c][1,4]oxazacyclotetrasine-1,7,19,21(4H,8H,20H,23H)-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 A9 (TBD).

### Table A9 (TBD)

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Solution C (%)</th>
<th>Solution D (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>74</td>
<td>26</td>
</tr>
<tr>
<td>45</td>
<td>74</td>
<td>26</td>
</tr>
<tr>
<td>60</td>
<td>15</td>
<td>85</td>
</tr>
<tr>
<td>75</td>
<td>15</td>
<td>85</td>
</tr>
<tr>
<td>76</td>
<td>74</td>
<td>26</td>
</tr>
<tr>
<td>85</td>
<td>74</td>
<td>26</td>
</tr>
</tbody>
</table>

**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 x 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:

\[
\text{Result} = \left( \frac{r_U}{r_S} \right) \times \left( \frac{C_S}{C_U} \right) \times P \times (1/F) \times 100
\]

- \( r_U \) = 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 A10\((\text{TBD})\))

**Acceptance criteria:** See Table A10\((\text{TBD})\). 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>
<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 hydroxy acid(^a)</td>
<td>0.18</td>
<td>1.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Tacrolimus open ring(^b,c)</td>
<td>0.49</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ascomycin 19-epimer(^d,e)</td>
<td>0.52</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Tacrolimus 19-epimer(^b,f)</td>
<td>0.62</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ascomycin(^s,g)</td>
<td>0.84</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Desmethyl tacrolimus(^s,h)</td>
<td>0.91</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>1.0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Tacrolimus 8-epimer(^l)</td>
<td>1.28</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Name</td>
<td>Relative Retention Time</td>
<td>Relative Response Factor</td>
<td>Acceptance Criteria, NMT (%)</td>
</tr>
<tr>
<td>------</td>
<td>-------------------------</td>
<td>--------------------------</td>
<td>-------------------------------</td>
</tr>
<tr>
<td>Tacrolimus 8-propyl analog&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.30</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Any individual unspecified impurity</td>
<td>—</td>
<td>1.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Total Impurities</td>
<td>—</td>
<td>—</td>
<td>1.5</td>
</tr>
</tbody>
</table>


<sup>b</sup> 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.

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

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

<sup>e</sup> These are process impurities that are controlled in the drug substance. They are not to be reported in the drug product.


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


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

<sup>j</sup> (3S,4R,5S,8R,9E,12S,14S,15R,16S,18R,26aS)-5,6,8,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-{[(E)-2-{(1R,3R,4R)-4-hydroxy-3-methoxycyclohexyl]1-methylvinyl}-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-8-propyl-3H-pyrido[2,1-c][1,4]oxazacrotocinosine-1,7,20,21(4H,23H)-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 I is used, then the labeling states 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 I is not used.

- **USP Reference Standards (11)**
  - USP Tacrolimus RS
  - USP Tacrolimus Related Compound A RS
  - USP Tacrolimus 8-epimer RS

- C<sub>43</sub>H<sub>69</sub>N<sub>12</sub>O<sub>12</sub> 792.01
(3S,4R,5S,8S,9E,12S,14S,15R,16S,18R,19R,26aS)-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-[(1R,3R,4R)-4-hydroxy-3-methoxy cyclohexyl]-1-methylvinyl\}-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3H-pyrido[2,1-c][1,4]oxaaazacyclotricosine-1,7,20,21(4H,23H)-tetrone.

\[\text{C}_{44}\text{H}_{69}\text{NO}_{12}\] 804.02

**USP Tacrolimus 8-propyl Analog RS**


\[\text{C}_{44}\text{H}_{71}\text{NO}_{12}\] 806.03

**USP Tacrolimus System Suitability Mixture RS**

It contains tacrolimus, ascomycin


\[\text{C}_{43}\text{H}_{69}\text{NO}_{12}\] 792.01

and tacrolimus 8-propyl analog


\[\text{C}_{44}\text{H}_{71}\text{NO}_{12}\] 806.03

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**Page Information:**

Not Applicable

**DocID:**

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