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 (C₄₉H₆₂N₁₀O₁₆S₃).

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 Solution: 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.

Sample Stock solution: 10 µg/mL of USP Sincalide RS

Sample solutions: 0.0624 µg of sincalide/kg of the animal's body weight in each 0.1 mL from Sample Stock solution.

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 a thin 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. Make 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.

Acceptance criteria: NMT 85.0%–125.0% of the labeled amount of sincalide.

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 Tacrolimus RS

Tacrolimus

C₆₆H₄₅NO₂₀ · H₂O 822.03

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>
<thead>
<tr>
<th>Time (min)</th>
<th>Solution C (%)</th>
<th>Solution D (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>72</td>
<td>28</td>
</tr>
<tr>
<td>30</td>
<td>72</td>
<td>28</td>
</tr>
<tr>
<td>53</td>
<td>15</td>
<td>85</td>
</tr>
<tr>
<td>54</td>
<td>72</td>
<td>28</td>
</tr>
<tr>
<td>60</td>
<td>72</td>
<td>28</td>
</tr>
</tbody>
</table>

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 °C
AutoSampler temperature: 4 °C
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:
\[ \text{Result} = \left( \frac{r_U}{r_D} \right) \times \left( \frac{C_U}{C_D} \right) \times 100 \]

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 methylycra lactaldehyde 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 220 nm
Column: Two 4.6-mm × 25-cm columns; 5-µm packing L20
Column temperature: 28 ± 2 °C
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:
\[ \text{Result} = \left( \frac{r_U}{r_D} \right) \times \left( \frac{C_U}{C_D} \right) \times 100 \]
\[ r_U = \text{sum of the peak responses of tacrolimus open ring, tacrolimus 19-epimer, and tacrolimus from the Sample solution} \]
\[ r_D = \text{sum of the peak responses of tacrolimus open ring, tacrolimus 19-epimer, and tacrolimus from the Standard solution} \]
\[ C_D = \text{concentration of USP Tacrolimus RS in the Sample solution (mg/mL)} \]
\[ C_U = \text{concentration of Tacrolimus in the Sample solution (mg/mL)} \]

Acceptance criteria: 98.0%–102.0%, calculated on the anhydrous and solvent-free basis
**Acceptance criteria: See Table 2.**

<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 methylacryl aldehyde&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.55</td>
<td>16.7</td>
<td>0.2</td>
</tr>
<tr>
<td>Tacrolimus diene&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.79</td>
<td>2.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Tacrolimus impurity &lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.96</td>
<td>1.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Tacrolimus related compound &lt;sup&gt;1,4&lt;/sup&gt;</td>
<td>0.96</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>1.0</td>
<td>1.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Tacrolimus 19-epimer&lt;sup&gt;4&lt;/sup&gt;</td>
<td>1.3</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Tacrolimus open ring&lt;sup&gt;4&lt;/sup&gt;</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>0.3</td>
</tr>
</tbody>
</table>

<sup>1</sup> (E)-3-[(1R,3R,4R)-4-Hydroxy-3-methoxy-2-cyclohexenyl]-2-methylacrylaldehyde.  
<sup>2</sup>(14E,18S)-17- Allyl-1-hydroxy-12-[(E)-2-(4-hydroxy-3-methoxy-2-cyclohexenyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-18,28-diax-4-azacyclic[22.3.1.0<sup>12,18</sup>]octacos-14,18-diene-3,10,16-tetraol.  
<sup>3</sup> Specified unidentified impurity.  
<sup>4</sup> For information only; not to be reported.

**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 Diluent:

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

\[ \text{Result} = \frac{(r_0/f_0) \times (C_0/C_1) \times 100}{f} \]

**Note:** See Table 2. Report impurity peaks with responses NLT that of the peak in the Reporting threshold solution (0.05%). Disregard peaks with retention times less than 3 min.

**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.

**Specific tests**

**Optical rotation,** Specific Rotation (7815): −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.
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 (33:55:600)
  Solution B: 50 g/L polyoxyethylene (23) lauryl ether
  [NOTE—Polyoxyethylene (23) lauryl ether is also called Brrij 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 x 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.]

**Analysis**
- **Samples:** Standard solution and Sample solution
  Calculate the percentage of tacrolimus (C_{44}H_{69}NO_{12}) in the portion of Capsules taken:

\[
\text{Result} = \left( \frac{f_0}{f_1} \right) \times \left( \frac{C_1}{C_0} \right) \times 100
\]

- \( f_0 \) = sum of the peak responses of tacrolimus and tacrolimus 19-epimer from the Sample solution
- \( f_1 \) = sum of the peak responses of tacrolimus and tacrolimus 19-epimer from the Standard solution
- \( C_1 \) = concentration of USP Tacrolimus RS in the Standard solution (mg/mL)
- \( C_0 \) = 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 x 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 (46:18:34:0.1)
  - **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>

**Chromatographic system**
(See Chromatography (621), System Suitability.)