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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• [DISSOLUTION <711>](#)**Test 1**

Medium: [Hydroxypropylcellulose](#) in [water](#) ($1:2 \times 10^4$), adjusted with 6% [phosphoric acid](#) to a pH of 4.5; 900 mL

Apparatus 2: 50 rpm with sinker (see [Dissolution <711>](#), [Figure 2a](#))

Time: 90 min

Mobile phase: [Acetonitrile](#), [methanol](#), [water](#), and 6% [phosphoric acid](#) (46: 18: 36: 0.1)

Standard stock solution: ($L/360$) mg/mL in [acetonitrile](#), where L is the Capsule label claim in mg

Standard solution: To 20.0 mL of the *Standard stock solution* add 50.0 mL of *Medium*, and mix to obtain solutions with known concentrations as indicated in [Table 1](#). Allow the solution to stand for NLT 6 h at 25° before use.

Sample solution: Pass 10 mL of the solution under test through a G4 glass filter. To 5.0 mL of the filtrate add 2.0 mL of [acetonitrile](#), and mix. Allow the solution to stand for NLT 1 h at 25° before use.

Table 1

Capsule Strength (mg)	Final Concentration (µg/mL)
0.5	0.4
1	0.8
5	4

Chromatographic system

(See [Chromatography <621>](#), [System Suitability](#).)

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
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*.



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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

