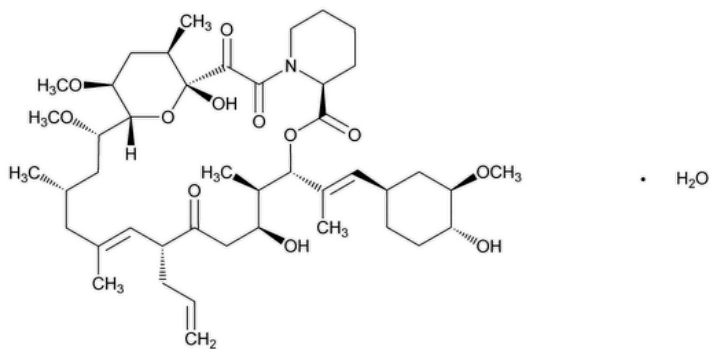


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Tacrolimus



$C_{44}H_{69}NO_{12} \cdot H_2O$ 822.03
15,19-Epoxy-3H-pyrido[2,1-c][1,4]oxaazacyclotricosine-1,7,20,21(4H,23H)-tetrone-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylethenyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-8-(2-propenyl)-, monohydrate, [3S-[3R*,E(1S*,3S*,4S*)],4S*,5R*,8S*,9E,12R*,14R*,15S*,16R*,18S*,19S*,26aR*]]-; (-)-(3S,4R,5S,8R,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-methoxycyclohexyl]-1-methylvinyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3H-pyrido[2,1-c][1,4]oxaazacyclotricosine-1,7,20,21(4H,23H)-tetrone monohydrate CAS RN®: 109581-93-3; UNII: WM0HAQ4WNM.

DEFINITION
Tacrolimus contains NLT 98.0% and NMT 102.0% of tacrolimus ($C_{44}H_{69}NO_{12}$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

- **A. [SPECTROSCOPIC IDENTIFICATION TESTS \(197\)](#), [Infrared Spectroscopy: 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**
Protect solutions containing tacrolimus from light.
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 1

Time (min)	Solution C (%)	Solution D (%)
0	72	28
30	72	28
53	15	85
54	72	28

Time (min)	Solution C (%)	Solution D (%)
60	72	28

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.

Standard solution: 3 mg/mL of [USP Tacrolimus RS](#) in *Diluent*. Allow the solution to stand for 3 h at ambient temperature before use.

Sample solution: 3 mg/mL of Tacrolimus in *Diluent*. Allow the solution to stand for 3 h at ambient temperature before use.

Chromatographic system

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

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 15-cm; 3-μm packing L1

Temperatures

Column: 60°

Autosampler: 4°

Flow rate: 1.5 mL/min

Injection volume: 20 μL

System suitability

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

[NOTE—See [Table 3](#) for relative retention times.]

Suitability requirements

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 tacrolimus ($C_{44}H_{69}NO_{12}$) in the portion of Tacrolimus taken:

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

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

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

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

C_U = concentration of Tacrolimus in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

• [RESIDUE ON IGNITION \(281\)](#): NMT 0.1%

• **ORGANIC IMPURITIES, PROCEDURE 1**

Use *Organic Impurities, Procedure 1* when the impurity profile includes tacrolimus methylacrylaldehyde 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 225 nm