Separation of Tacrolimus and its Organic Impurities per USP Monograph

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Overview

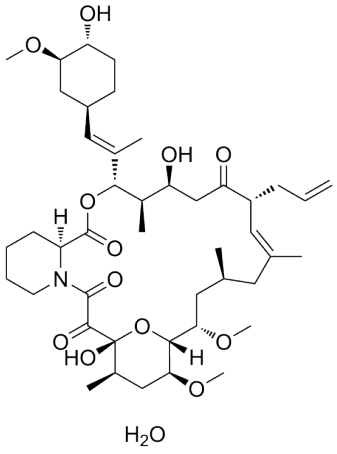
Tacrolimus is an inhibitor of calcineurin, a phosphatase that regulates production of interleukin-2. In this application note, we report the separation of Tacrolimus and its related organic impurities per the USP monograph using a Luna 3 µm C18 column, a Luna 3 µm C18(2) column, and a Luna Omega 3 µm C18 column.

System suitability per USP Monograph for Tacrolimus Assay is a resolution no less than (NLT) 3.0 between Ascomycin and Tacrolimus and a percent relative standard deviation (%RSD) no more than (NMT) 1.0 % for 5 replicates for the sum of the responses of Tacrolimus, Tacrolimus open ring, and Tacrolimus 19-epimer. System suitability per USP Monograph for Tacrolimus Organic Impurities, Procedure 2, is resolution NLT 3.0 between Ascomycin and Tacrolimus and a percent relative standard deviation (%RSD) no more than (NMT) 10.0 % for 6 replicates for the sum of the responses of Tacrolimus and Tacrolimus 19-epimer.

All system suitability requirements for Tacrolimus Assay and Organic Impurities were met by all columns. However, the newer generation Luna Omega C18 column, which delivers an L1 column with increased inertness and reproducibility, provided the greatest resolution and lowest % RSD making it the ideal column selection for this monograph.

All solutions were prepared as indicated in the USP Monograph for Tacrolimus. USP Tacrolimus RS (Catalog No. 1642802), USP Tacrolimus Related Compound A RS (Catalog No. 1642813), USP Tacrolimus System Suitability Test Mix RS (Catalog No. 1642857), USP Tacrolimus 8-epimer RS (Catalog No. 1642824), and USP Tacrolimus 8-propyl analog (Catalog No. 1642835) were purchased from USP.

Figure 1. Tacrolimus Structure.



LC-UV Conditions

Columns: Luna™ 3 µm C18 (00F-4114-E0)
Luna 3 µm C18(2) (00F-4251-E0)
Luna Omega 3 µm C18 (00F-4784-E0)

Dimensions: 150 x 4.6 mm

Mobile Phase: A: **Solution A** / **Solution B** (4:1, v/v)
B: **Solution A** / **Solution B** (1:4, v/v)

Gradient:

Time (min)	%B
0	28
30	28
53	85
54	28
60	28

Flow Rate: 1.5 mL/min

Injection Volume: 20 µL

Temperature: 60 °C

Detector: UV @ 220 nm

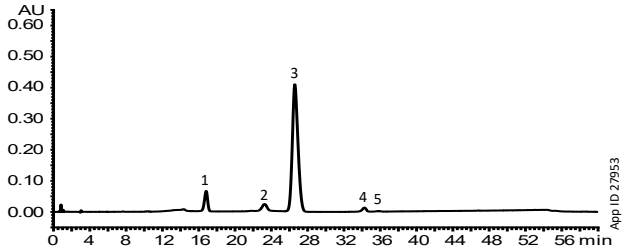
System: Waters® ACQUITY Arc® HPLC

Table 1. Preparation of Test and Reference Solutions

Solution	Composition
Solution A	6 mM Phosphoric Acid
Solution B	Acetonitrile / *tert*-Butyl Methyl Ether (81:19, v/v)
Diluent	Acetonitrile / Water (7:3, v/v).
System Suitability Solution (Assay and Organic Impurities)	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 (Assay)	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.
Standard Solution (Organic Impurities, Procedure 2)	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.

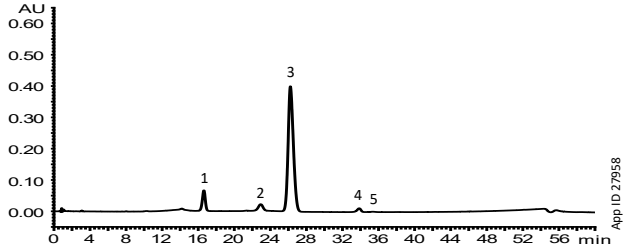
Figure 2. System Suitability Solution – Assay and Organic Impurities

Luna™ 3 µm C18 Column



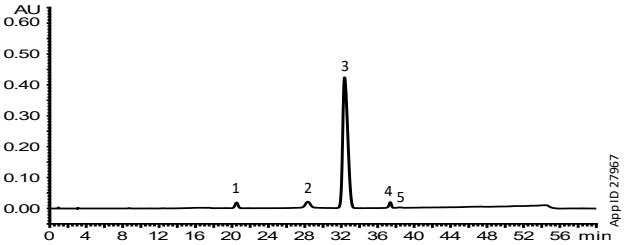
Peak No.	Analyte	Retention Time (min)	RT Ratio	Area	Resolution
1	Tacrolimus 19-epimer	16.82	0.63	1573240	-
2	Ascomycin	23.26	0.87	846856	3.01
3	Tacrolimus	26.59	1.00	17646968	
4	Tacrolimus 8-epimer	34.27	1.29	382551	-
5	Tacrolimus 8-propyl analog	35.78	1.35	45686	-
N=5 Injections					

Luna 3 µm C18(2) Column



Peak No.	Analyte	Retention Time (min)	RT Ratio	Area	Resolution
1	Tacrolimus 19-epimer	16.61	0.63	1560723	-
2	Ascomycin	22.94	0.88	838941	3.23
3	Tacrolimus	26.20	1.00	17172567	
4	Tacrolimus 8-epimer	33.93	1.30	376096	-
5	Tacrolimus 8-propyl analog	35.43	1.35	48686	-
N=5 Injections					

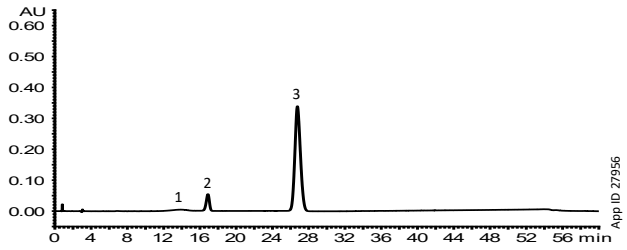
Luna Omega 3 µm C18 Column



Peak No.	Analyte	Retention Time (min)	RT Ratio	Area	Resolution
1	Tacrolimus 19-epimer	20.503	0.63	428618	-
2	Ascomycin	28.327	0.88	760690	3.89
3	Tacrolimus	32.370	1.00	16292286	
4	Tacrolimus 8-epimer	37.382	1.15	349065	-
5	Tacrolimus 8-propyl analog	38.406	1.19	41012	-
N=5 Injections					

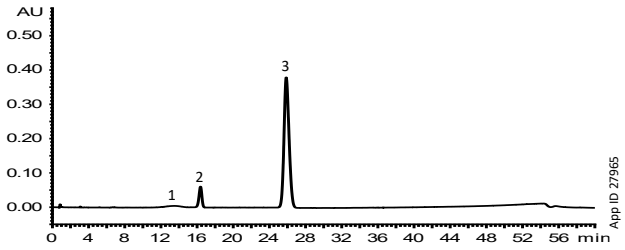
Figure 3. Standard Solution – Assay

Luna™ 3 µm C18 Column



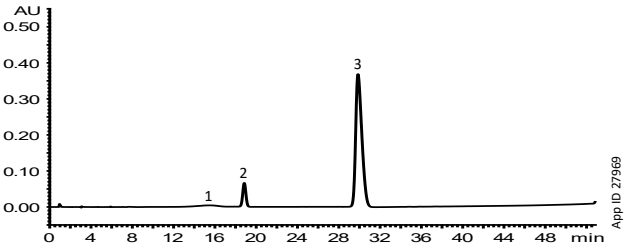
Peak No.	Analyte	Retention Time (min)	Area	Total Area	Total Area %RSD
1	Tacrolimus open ring	13.93	467785		
2	Tacrolimus 19-epimer	16.98	1308637	16347732	0.30
3	Tacrolimus	26.79	14571310		
N=5 Injections					

Luna 3 µm C18(2) Column



Peak No.	Analyte	Retention Time (min)	Area	Total Area	Total Area %RSD
1	Tacrolimus open ring	13.50	497129		
2	Tacrolimus 19-epimer	16.43	1328070	16615330	0.78
3	Tacrolimus	25.74	14790131		
N=5 Injections					

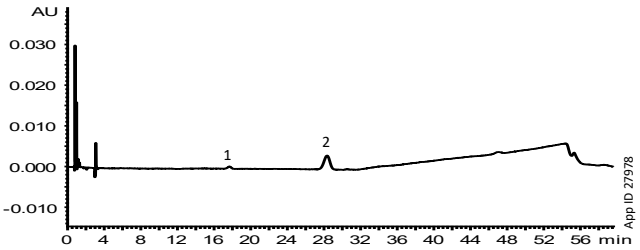
Luna Omega 3 µm C18 Column



Peak No.	Analyte	Retention Time (min)	Area	Total Area	Total Area %RSD
1	Tacrolimus open ring	15.62	521842		
2	Tacrolimus 19-epimer	18.93	1408543	17348029	0.13
3	Tacrolimus	29.98	15417644		
N=5 Injections					

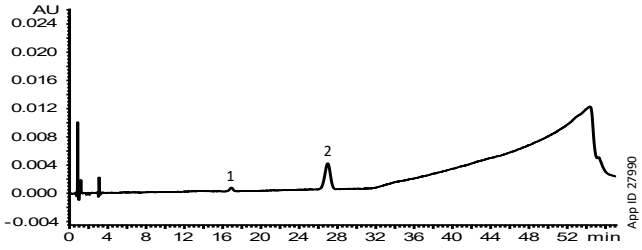
Figure 4. Standard Solution – Assay

Luna™ 3 µm C18 Column



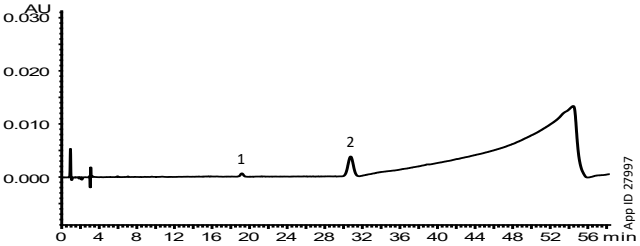
Peak No.	Analyte	Retention Time (min)	Area	Total Area	Total Area %RSD
1	Tacrolimus 19-epimer	17.80	14774	170120	0.91
2	Tacrolimus	28.51	155346		
N=6 Injections					

Luna 3 µm C18(2) Column



Peak No.	Analyte	Retention Time (min)	Area	Total Area	Total Area %RSD
1	Tacrolimus 19-epimer	17.00	14145	166737	1.27
2	Tacrolimus	27.09	152592		
N=6 Injections					

Luna Omega 3 µm C18 Column



Peak No.	Analyte	Retention Time (min)	Area	Total Area	Total Area %RSD
1	Tacrolimus 19-epimer	19.35	14204	161680	1.60
2	Tacrolimus	31.02	147476		
N=6 Injections					

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