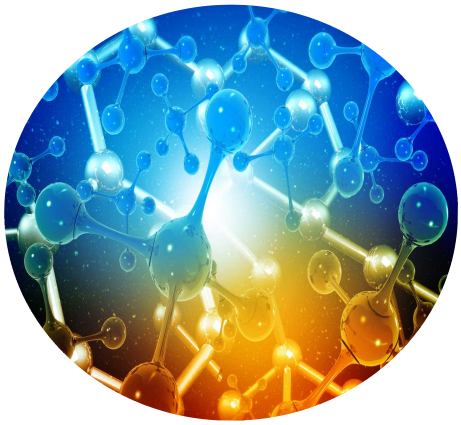

Ph. Eur. Monograph 2918: Test for Everolimus Impurity A on Kinetex® 2.7 µm C18

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Overview

Everolimus is an immunosuppressant used to prevent rejection of organ transplants and in the treatment of renal cell cancer and other tumors.

In this application note we demonstrate the separation of Everolimus from Sirolimus (Impurity A) according to Ph. Eur. Monograph 2918 for the determination of Impurity A.

The chromatogram obtained for reference solution (a) was used to confirm the retention time for Impurity A. Three batches of the Kinetex 2.7 µm C18 column were used to demonstrate excellent batch-to-batch reproducibility, with each column meeting the system suitability requirement for the separation between Impurity A and Everolimus, as determined by the peak-to-valley ratio (minimum 2.0); where H_p = height above the baseline of the peak due to Impurity A and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to Everolimus.

All reference solutions were prepared as indicated in Ph.Eur . monograph 2918 for Everolimus. (NOTE: Plastic labware was not used for solution preparation, as recommended, to minimize the formation of the everolimus tautomer; therefore, the observed peak for the tautomer was significantly larger than expected). The Everolimus (catalog no. Y0002048) certified reference standard (CRS) was purchased from the European Directorate for the Quality of Medicines & HealthCare (EDQM) Council of Europe; and the Sirolimus (Impurity A) reference standard (catalog no. 1612765) was obtained from US Pharmacopeia (USP).

LC-UV Conditions

Column: Kinetex 2.7 µm C18
Dimension: 150 x 2.1 mm (00F-4783-AN)
Mobile Phase: A = Formic acid/Methanol/0.7 g/L Ammonium Formate/Acetonitrile (0.05:100:450:450 v/v/v/v)
B = Formic acid/Methanol/0.96 g/L Ammonium Formate/Acetonitrile (0.05:100:330:570 v/v/v/v)

Gradient:

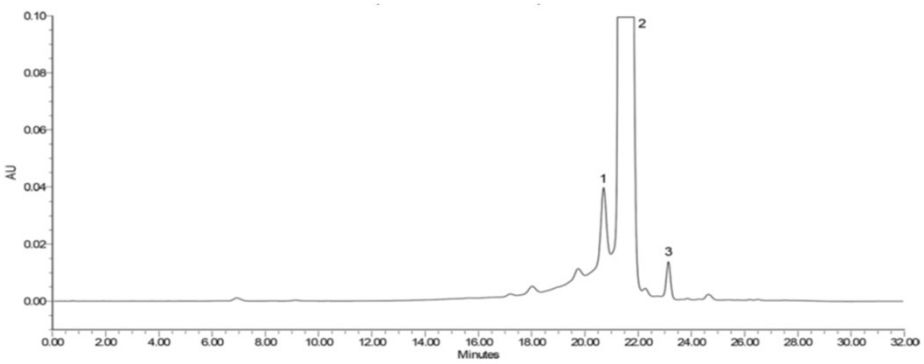
Time (min)	% B
0	0
14	0
23	100
28	100
30	0
34	0

Flow Rate: 0.9 mL/min
Temperature: 60 °C
Detector: UV @ 278 nm
Injection: 3.5 µL
System: Waters® ACQUITY® I-Class
Sample:
1. Sirolimus (Impurity A)
2. Everolimus
3. Everolimus Tautomer

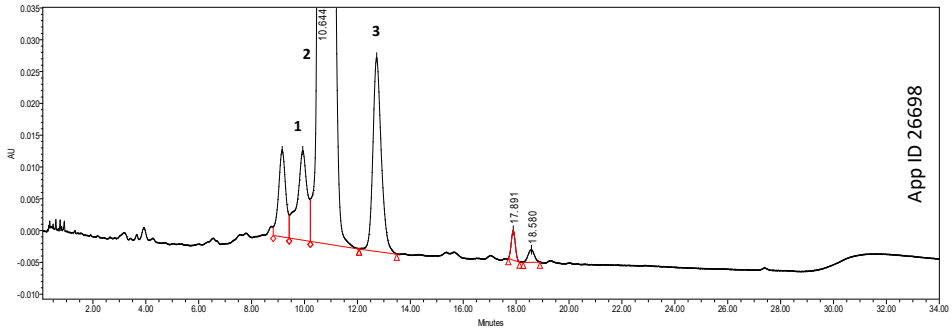
Table 1: Preparation of Test and Reference Solutions

Solution	Composition
Test Solution	Dissolve 50.0 mg of Everolimus CRS (or the substance to be examined) in acetonitrile and dilute to 25.0 mL with acetonitrile.
Reference solution (a)	Dissolve 1 mg of USP Sirolimus RS (impurity A) in 5 mL of acetonitrile. Dilute 0.8 mL of the solution to 10 mL with the test solution.

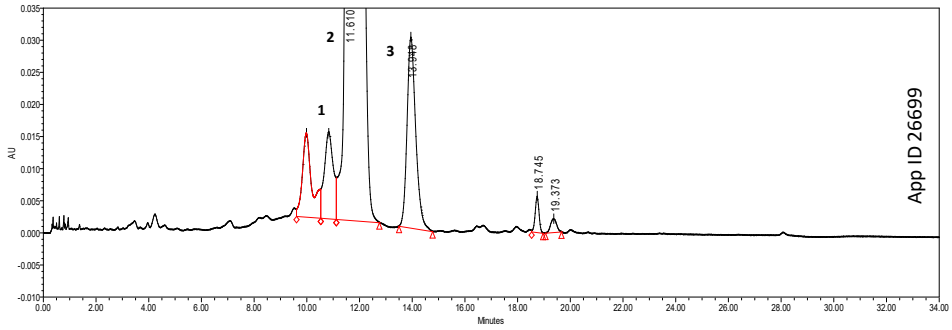
Original chromatogram published in EDQM for reference solution (a)



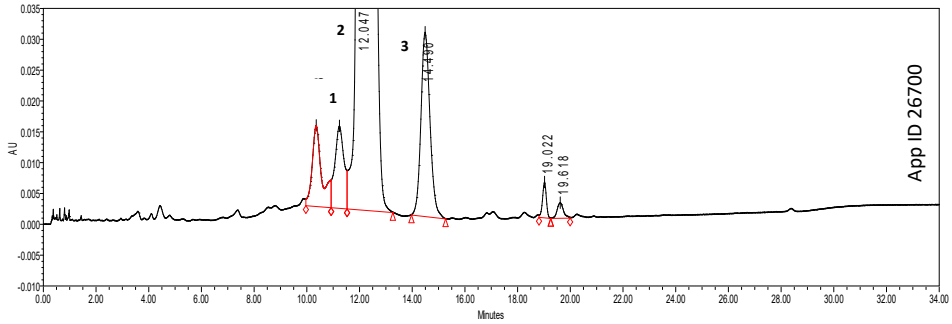
Kinetex® 2.7 µm C18 150 x 2.1 mm (00F-4783-AN); reference solution (a) on 3 different batches



H_p, (Impurity A)	H_v, (Everolimus)	p/v = Hv/Hp
17458	5668	3.08



H_p, (Impurity A)	H_v, (Everolimus)	p/v = Hv/Hp
17299	5751	3.01



H_p, (Impurity A)	H_v, (Everolimus)	p/v = Hv/Hp
17141	5470	3.13

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