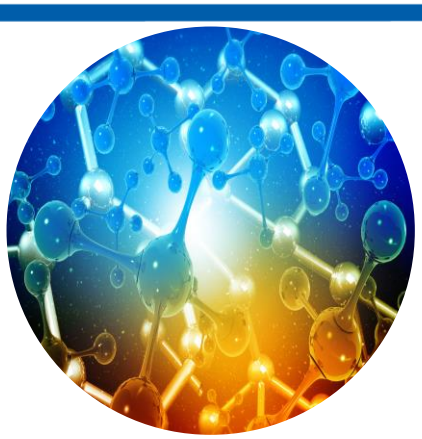

# Ph. Eur. Monograph 2244: Tacrolimus Monohydrate Related Substances on Luna® 3 µm C18(2) and Luna Omega 3 µm C18

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## Overview

Tacrolimus is an immunosuppressive drug. The molecule is an inhibitor of calcineurin, a phosphatase regulating the interleukin-2 production.

In this application note we show the separation of Tacrolimus from its related substances following Ph. Eur. monograph 2244. The Luna 3 µm C18(2) and Luna Omega 3 µm C18 used for this study met the system suitability criteria of a R ≥ 3 between Tacrolimus ($t_R$ ≅ 23 min) and impurity A ($t_R$ ≅ 20 min).

All reference solutions were prepared as indicated in Ph. Eur. monograph 2244 for Tacrolimus monohydrate. All solutions were prepared in glassware protected from light and allowed the test and reference solutions to stand for 3 hours at room temperature before use. The following certified reference standards (CRS) were purchased from the European Directorate for the Quality of Medicines & HealthCare (EDQM) – Council of Europe; Postal address: Allee Kastner CS 30026 F - 67081 Strasbourg (France):

- Y0001926, batch 1 Tacrolimus monohydrate CRS
- Y0001933, batch 1 Tacrolimus for system suitability CRS

## LC-UV Conditions

**Columns:** Luna 3 µm C18(2) (00F-4251-E0)
Luna Omega 3 µm C18 (00F-4784-E0)
**Dimension:** 150 x 4.6 mm
**Mobile Phase:** A = **Mobile Phase 1**/**Buffer Solution** (20:80, v/v)
B = **Mobile Phase 1**/**Buffer Solution** (80:20, v/v)

**Gradient:**

| Time (min) | %B |
|---|---|
| 0 | 28 |
| 30 | 28 |
| 53 | 85 |
| 55 | 28 System equilibration |
| 60 | 28 System equilibration |

**Injection:** 20 µL
**Temperature:** 60 °C
**Detector:** UV @ 220 nm
**System:** Agilent® 1260 Infinity I

**Table 1.** Preparation of Buffer Solution, Mobile Phase Solution, and Solvent

| Solution | Composition |
|---|---|
| **Buffer Solution** | Add 0.4 mL phosphoric acid to 500 mL water, then dilute to 1000 mL |
| **Mobile Phase 1** | 1,1-Dimethylether/acetonitrile (19:81, v/v) |
| **Solvent** | Water/acetonitrile (30:70, v/v) |

**Table 2.** Preparation of Test and Reference Solutions

| Solution | Composition |
|---|---|
| **Reference Solution (a) = Sample/Test Solution** | Dissolve 30.0 mg reference substance Tacrolimus monohydrate CRS in **Solvent** and dilute to 10.0 mL with the same **Solvent**. Mix well (SaS). Allow the sample solution to stand for 3 h at room temperature before use (RSa). |
| **Reference Solution (b)** | Dissolve approx. 3.0 mg reference substance Tacrolimus for system suitability CRS (containing impurity A) in **Solvent** and dilute to 1.0 mL with the same **Solvent**. Mix well. |
| **Reference Solution (c)** | Transfer 1.0 mL of **Reference Solution (a)** into a 100 mL volumetric flask, fill up to volume with **Solvent** and mix well (RSa-1). Transfer 1.0 mL of RSa-1 into a 10 mL volumetric flask, fill up to volume with **Solvent** and mix well. |

**Figure 1.** System Suitability Test using Reference Solution (b) on Luna® 3 µm C18(2)

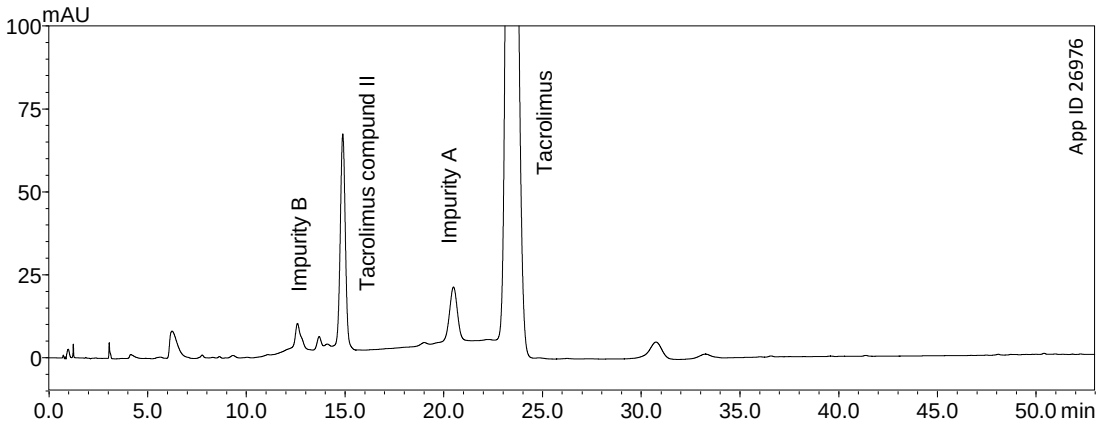


**Table 3.** System Suitability Test using Reference Solution (b) on Luna 3 µm C18(2)

| | Impurity B | | Tacrolimus Compound II | | Impurity A | | Tacrolimus | | | R Tacrolimus/ Impurity A |
|---|---|---|---|---|---|---|---|---|---|---|
| Inj. No. | $t_R$ | Area | $t_R$ | Area | $t_R$ | Area | $t_R$ | Area | Tailing Factor | |
| 1 | 12.592 | 142433 | 14.887 | 1136649 | 20.496 | 440305 | 23.394 | 12843556 | 1.24 | 3.73 |
| 2 | 12.596 | 142624 | 14.894 | 1136618 | 20.503 | 439897 | 23.402 | 12842176 | 1.24 | 3.73 |
| 3 | 12.607 | 141228 | 14.907 | 1136898 | 20.523 | 440548 | 23.426 | 12836480 | 1.24 | 3.73 |
| 4 | 12.612 | 139698 | 14.916 | 1136346 | 20.535 | 439356 | 23.441 | 12830299 | 1.24 | 3.73 |
| 5 | 12.618 | 139487 | 14.926 | 1137175 | 20.549 | 439833 | 23.454 | 12833823 | 1.24 | 3.73 |
| 6 | 12.631 | 138135 | 14.942 | 1136265 | 20.572 | 439412 | 23.483 | 12822469 | 1.24 | 3.73 |
| Average | 12.609 | 140601 | 14.912 | 1136659 | 20.530 | 439892 | 23.433 | 12834801 | 1.24 | 3.73 |
| % RSD | 0.11 | 1.27 | 0.14 | 0.03 | 0.14 | 0.11 | 0.14 | 0.06 | 0.08 | 0.06 |

**Figure 2.** Reference Solution (a) on Luna® 3 µm C18(2)

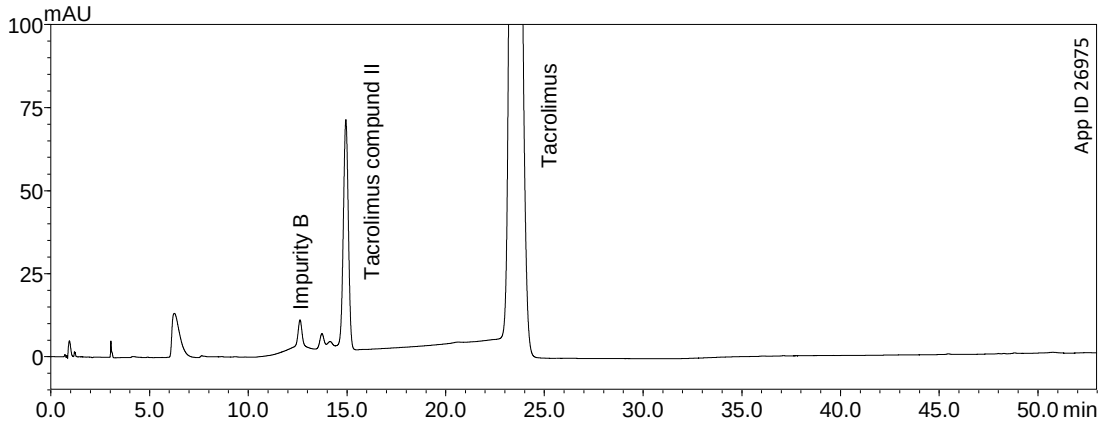


**Table 4.** Reference Solution (a) on Luna 3 µm C18(2)

| | Impurity B | | Tacrolimus Compound II | | Tacrolimus | | |
|---|---|---|---|---|---|---|---|
| Inj. No. | $t_R$ | Area | $t_R$ | Area | $t_R$ | Area | Tailing Factor |
| 1 | 12.631 | 121958 | 14.952 | 1238528 | 23.485 | 13837607 | 1.26 |
| 2 | 12.640 | 120184 | 14.964 | 1197193 | 23.506 | 13829646 | 1.25 |
| 3 | 12.638 | 119503 | 14.959 | 1199238 | 23.490 | 13822786 | 1.26 |
| 4 | 12.606 | 119101 | 14.922 | 1197651 | 23.430 | 13802206 | 1.26 |
| 5 | 12.606 | 118229 | 14.932 | 1196083 | 23.452 | 13799546 | 1.26 |
| 6 | 12.652 | 116425 | 14.990 | 1193951 | 23.547 | 13796236 | 1.26 |
| Average | 12.629 | 119233 | 14.953 | 1203774 | 23.485 | 13814671 | 1.26 |
| % RSD | 0.15 | 1.56 | 0.16 | 1.42 | 0.18 | 0.13 | 0.12 |

**Figure 3.** System Suitability Test using Reference Solution (b) on Luna® Omega 3 µm C18

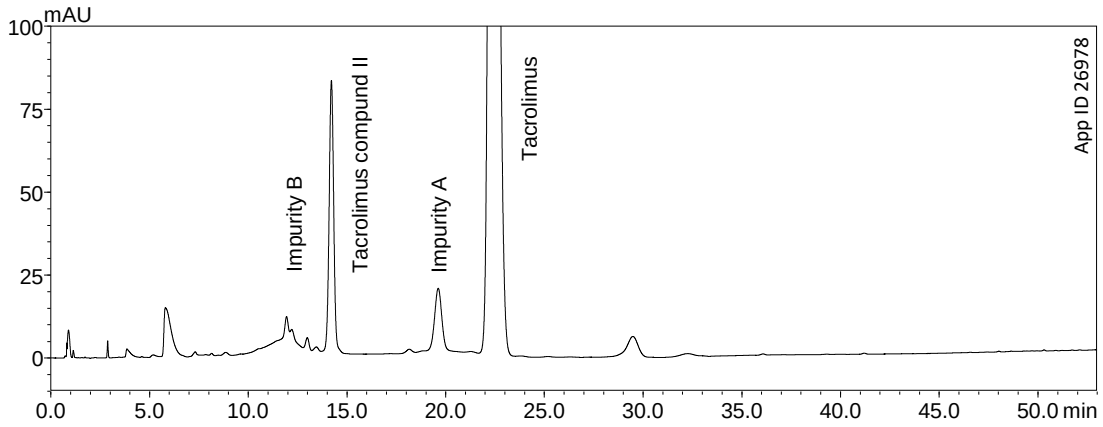


**Table 5.** System Suitability Test using Reference Solution (b) on Luna Omgea 3 µm C18

| | Impurity B | | Tacrolimus Compound II | | Impurity A | | Tacrolimus | | | R Tacrolimus/ Impurity A |
|---|---|---|---|---|---|---|---|---|---|---|
| Inj. No. | $t_R$ | Area | $t_R$ | Area | $t_R$ | Area | $t_R$ | Area | Tailing Factor | |
| 1 | 11.953 | 55691 | 14.223 | 1306845 | 19.634 | 466575 | 22.376 | 13764864 | 1.32 | 3.85 |
| 2 | 11.952 | 55444 | 14.223 | 1306289 | 19.632 | 467499 | 22.374 | 13759471 | 1.32 | 3.85 |
| 3 | 11.957 | 55624 | 14.228 | 1307104 | 19.642 | 466395 | 22.385 | 13746057 | 1.32 | 3.85 |
| 4 | 11.967 | 55414 | 14.243 | 1303581 | 19.660 | 464397 | 22.407 | 13720535 | 1.32 | 3.85 |
| 5 | 11.973 | 55491 | 14.253 | 1303009 | 19.677 | 464309 | 22.428 | 13717911 | 1.32 | 3.86 |
| 6 | 11.983 | 55342 | 14.267 | 1305552 | 19.695 | 464679 | 22.448 | 13728662 | 1.32 | 3.85 |
| Average | 11.964 | 55501 | 14.240 | 1305397 | 19.657 | 465642 | 22.403 | 13739583 | 1.32 | 3.85 |
| % RSD | 0.10 | 0.24 | 0.13 | 0.13 | 0.13 | 0.29 | 0.13 | 0.15 | 0.13 | 0.09 |

**Figure 4.** Reference Solution (a) on Luna® Omega 3 µm C18

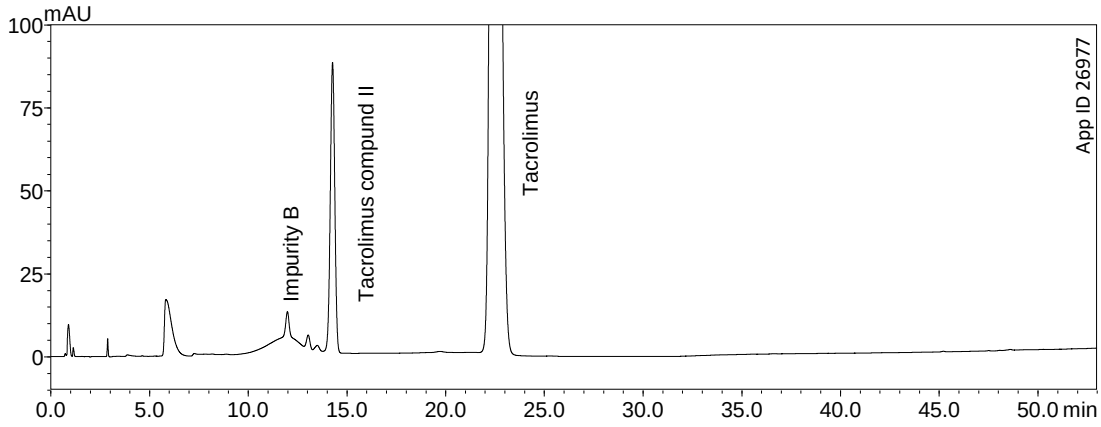


**Table 6.** Reference Solution (a) on Luna Omega 3 µm C18

| | Impurity B | | Tacrolimus Compound II | | Tacrolimus | | |
|---|---|---|---|---|---|---|---|
| Inj. No. | $t_R$ | Area | $t_R$ | Area | $t_R$ | Area | Tailing Factor |
| 1 | 11.994 | 102163 | 14.281 | 1380416 | 22.455 | 14784437 | 1.35 |
| 2 | 12.005 | 101120 | 14.295 | 1380206 | 22.475 | 14777072 | 1.35 |
| 3 | 12.030 | 99050 | 14.328 | 1379529 | 22.536 | 14776594 | 1.35 |
| 4 | 12.061 | 96605 | 14.368 | 1378994 | 22.602 | 14778160 | 1.35 |
| 5 | 12.088 | 94767 | 14.400 | 1378935 | 22.659 | 14768606 | 1.35 |
| 6 | 12.131 | 92101 | 14.456 | 1378421 | 22.747 | 14770702 | 1.35 |
| Average | 12.052 | 97634 | 14.355 | 1379417 | 22.579 | 14775928 | 1.35 |
| % RSD | 0.44 | 3.96 | 0.46 | 0.06 | 0.50 | 0.04 | 0.07 |

## Conclusion

Both columns used for this study showed good separation of all Tacrolimus related compounds according to Ph. Eur. monograph 2244. The Luna 3 µm C18(2) column met the system suitability criteria with a resolution of 3.73 between Impurity A and Tacrolimus (**Table 3**). The Luna Omega 3 µm C18 column achieved a resolution of 3.85 between Impurity A and Tacrolimus (**Table 5**). Therefore, both columns are suitable for the analysis of Tacrolimus and related substances following the Ph. Eur. monograph 2244.

## Need a different column size or sample preparation format?

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