

## Enantiomeric and Diastereometric Purity of Tadalafil per USP Monograph

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### Overview

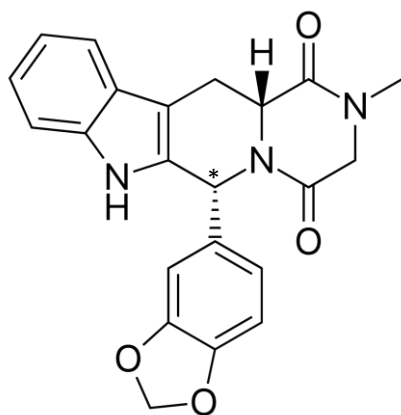
Tadalafil is a phosphodiesterase-5 enzyme (PDE-5) inhibitor that relaxes certain muscles and blood vessels to aid in erectile dysfunction. The development of a quick and efficient analysis of Tadalafil is of interest for generic drug manufacturers. In this application note, we report the separation of Tadalafil and its 6R, 12aS Diastereomer using a Lux 5 µm Amylose-1 column according to the USP monograph for Tadalafil.

The helical structure of the amylose on the surface of the stationary phase particles creates a complex steric environment that provides stronger interactions and better chiral recognition abilities. Additionally, the selector's constituents produce hydrogen bonding, dipole, and pi-pi interactions that all affect the stationary phase's ability to separate diastereomers effectively.

System suitability per USP Monograph for Tadalafil is a resolution no less than (NLT) 2.0 between the 6R, 12aS Diastereomer and Tadalafil, a symmetry factor NLT 0.8 and no more than (NMT) 1.5, a percent relative standard deviation (%RSD) of NMT 10.0 %, and a signal-to-noise ratio (S/N) of NLT 20. System suitability requirements for Tadalafil were met by the Lux 5 µm Amylose-1 column.

All solutions were prepared as indicated in the USP Monograph for Diazoxide. USP Tadalafil RS (Catalog No. 1642879) was purchased from USP.

Figure 1. Tadalafil Structure



### LC-UV Conditions

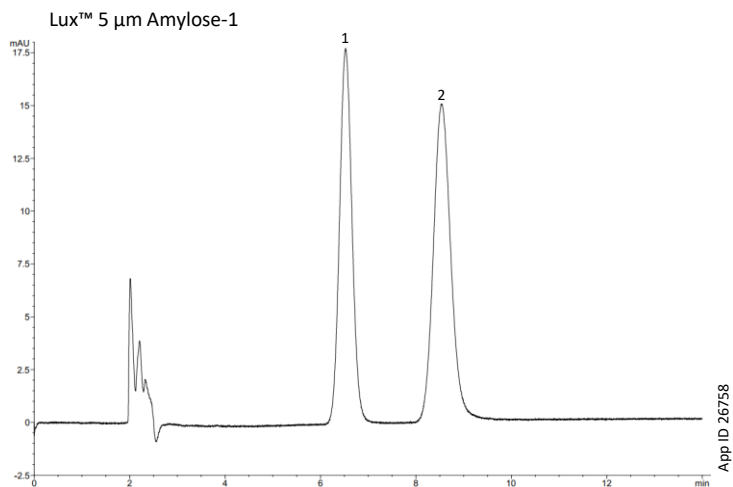
**Column:** Lux™ 5 µm Amylose-1  
**Dimensions:** 150 x 4.6 mm  
**Part No.:** [00F-4732-E0](#)  
**Mobile Phase:** LC Grade Hexanes / Isopropanol (50:50, v/v)  
**Flow Rate:** 0.75 mL/min (Isocratic)  
**Injection Volume:** 10 µL  
**Temperature:** 30 °C  
**Detector:** UV @ 222 nm  
**System:** Agilent® 1260 Binary UHPLC

Table 1. Preparation of Solutions

Solution	Composition
Diluent	Hexanes / Isopropanol / Acetonitrile (40:40:20, v/v/v)
Stock Standard Solution	50 µg/mL of USP Tadalafil RS in Diluent
Standard Solution	0.5 µg/mL of USP Diazoxide RS in Diluent from Stock Standard Solution
System Suitability Stock Solution	To generate the 6R,12aS Diastereomer of Tadalafil, dissolve 25 mg of Tadalafil in 40 mL of Diluent. Add 1.0 mL of 1.0 M Tetrabutylammonium Hydroxide in Methanol and allow to stand at room temperature for 20 min. Add 1.0 mL of Trifluoroacetic Acid and dilute with Diluent to 50 mL.
System Suitability Solution	Transfer 1.0 mL of the System Suitability Stock Solution and 10 mL of the Standard Stock Solution to a 50 mL volumetric flask and dilute with Diluent to volume.
Sensitivity Solution	0.25 µg/mL USP Tadalafil RS; perform a 1:2 dilution of Standard Solution with Diluent

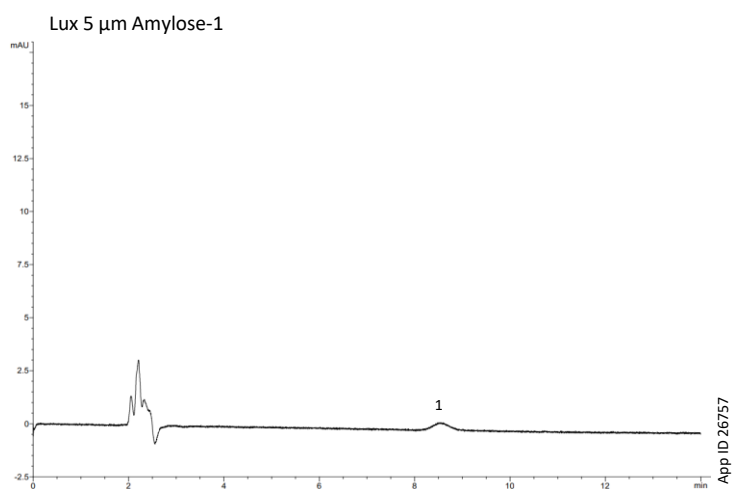


**Figure 2. System Suitability Solution**



Peak No.	Analyte	Retention Time (min)	Area	Height	Resolution
1	6R, 12aS Diastereomer	6.525	318.3	17.7	3.57
2	Tadalafil	8.539	370.8	14.9	
N = 6 Injections					

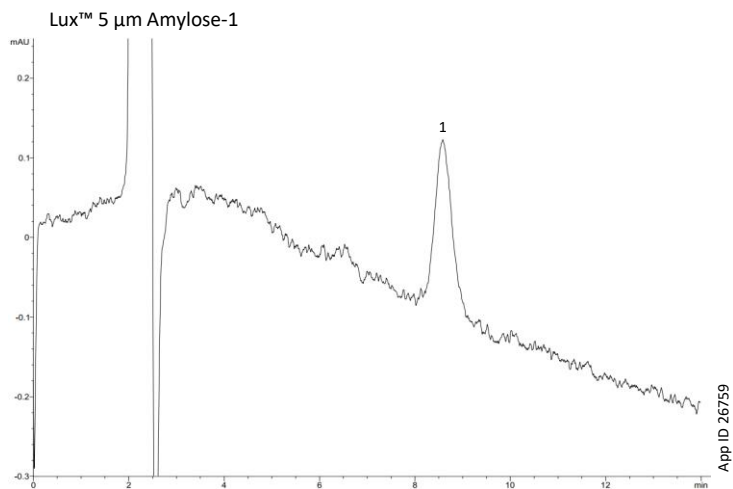
**Figure 3. Standard Solution**



Peak No.	Analyte	Retention Time (min)	Area	Height	Area %RSD	Symmetry Factor
1	Tadalafil	8.538	8.9	3.8E-1	3.889	1.073
N = 6 Injections						



**Figure 4. Sensitivity Solution**



Peak No.	Analyte	Retention Time (min)	Area	Height	S/N
1	Tadalafil	8.586	5.9	2.2E-1	22.5

N = 6 Injections



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