

LC-MS/MS Separation of Sildenafil and its Metabolite N-Desmethylsildenafil Using the Kinetex® 2.6 µm Biphenyl Column and Luna® Omega 1.6 µm C18 Column

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Overview

Sildenafil is a specific inhibitor of phosphodiesterase-5 (PDE5), sold in the market under the brand name Viagra®. The primary and prevalent metabolite is N-Desmethylsildenafil, which has significantly higher pharmacologic activity than Sildenafil (**Figure 1**). This drives the interest in developing a reversed phase HPLC method able to separate and analyze Sildenafil and its metabolite successfully and in a short amount of time. In this application note, we present an LC-MS/MS method to separate Sildenafil and its active metabolite by utilizing Kinetex 2.6 µm Biphenyl and Luna Omega 1.6 µm C18 columns.

The Kinetex Biphenyl column provided separation for Sildenafil and its metabolite through a mixture of pi-pi and polar interactions; and the higher efficiency provided by the core-shell particle morphology also results in narrow peaks and increased MS sensitivity (**Figure 2a**).

The Luna Omega C18 column is a conventional C18 column with TMS endcapping, making this column a good choice for retention of analytes through hydrophobic interactions. Depending on the mobile phase used, there was a shift in elution order observed. Using Acetonitrile instead of Methanol in the mobile phase on the Luna Omega 1.6 µm C18 column gave narrower peaks and the same elution order observed with the Kinetex Biphenyl column (**Figure 2b**). Acetonitrile, which has a stronger elution strength than methanol, was reduced to 25% in the mobile phase in order to achieve comparable retention times when using methanol on the Luna Omega C18 column, (**Figure 2c**). When using methanol in the mobile phase, the run time was much shorter on the Luna Omega 1.6 µm C18 column compared to the Kinetex 2.6 µm Biphenyl column, which is likely due to the multiple interactions between the analytes and the Biphenyl stationary phase.

LC-MS/MS Conditions

Column: Kinetex 2.6 µm Biphenyl ([00B-4622-AN](#))
Luna Omega 1.6 µm C18 ([00B-4742-AN](#))
Dimension: 50 x 2.1 mm
Mobile Phase: A: 0.1 % Formic Acid in Water
B: See Chromatogram
Gradient: See Chromatogram
Flow Rate: 500 µL/min
Injection Volume: 5 µL
Temperature: 40 °C
LC System: Agilent® 1200 Series
Detection: MS/MS
Detector: SCIEX® 4500

MRM Transitions

Analyte	Q1 (m/z)	Q3 (m/z)
Sildenafil-1	475	100
Sildenafil-2	475	283
Sildenafil-3	475	311
N-Desmethylsildenafil-1	461.2	310.8
N-Desmethylsildenafil-2	461.2	283

Figure 1. Sildenafil and N-Desmethylsildenafil

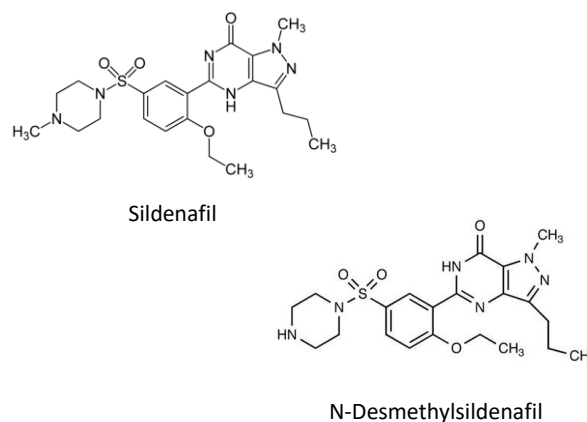


Figure 2a. Kinetex® 2.6 µm Biphenyl

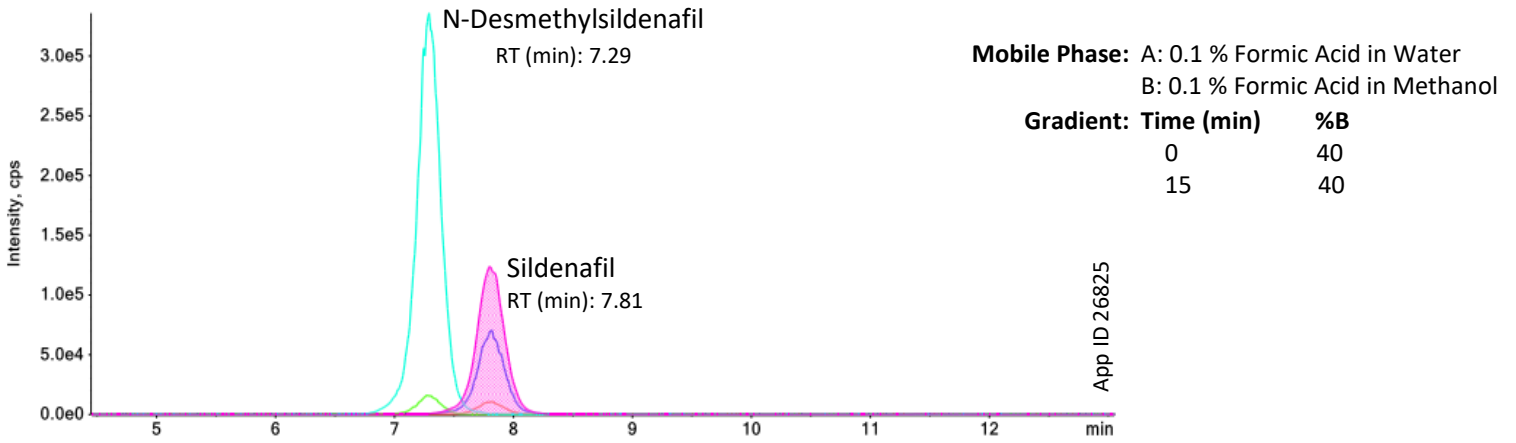


Figure 2b. Luna® Omega 1.6 µm C18

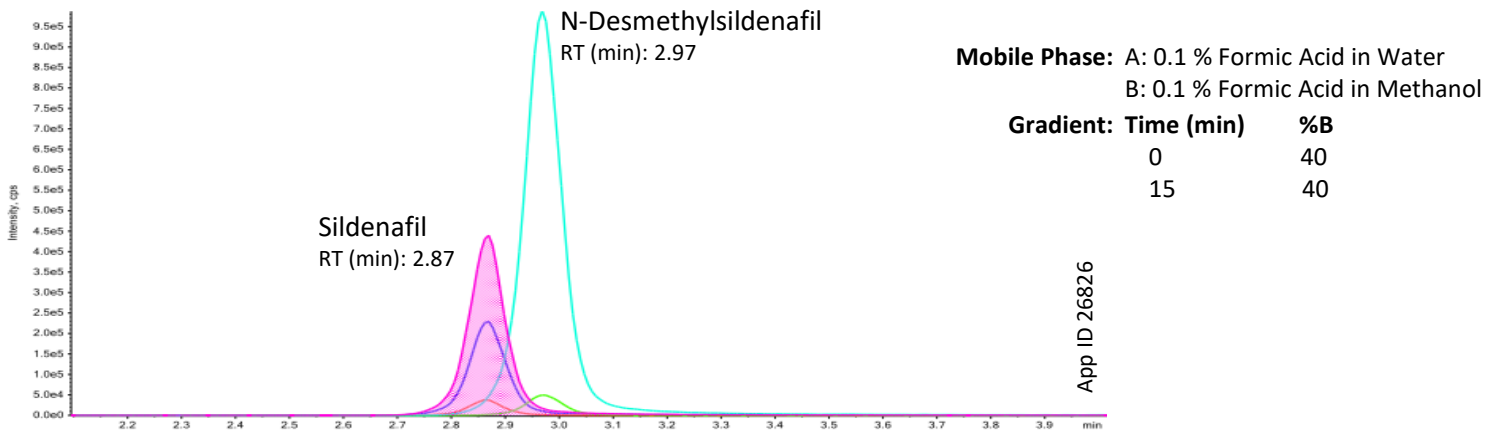
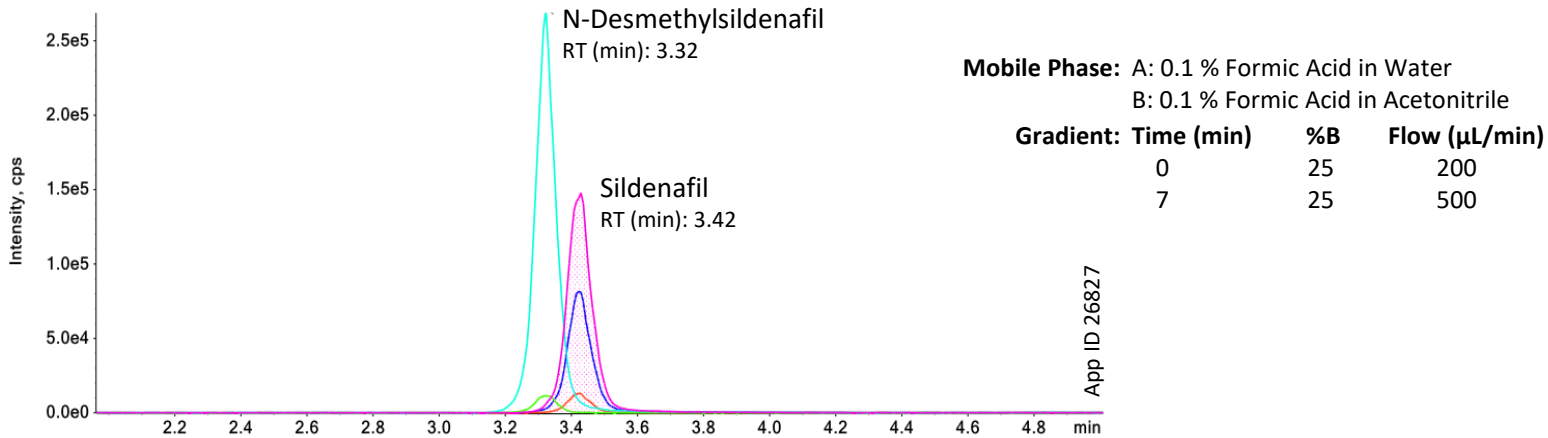


Figure 2c. Luna Omega 1.6 µm C18



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