

## Batch-to-Batch Comparison: Steroid Mixture on Luna® Omega 3 µm C18

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### Overview

Analysis of a mixture containing six steroid compounds was used to assess the overall separation and retention time reproducibility for three different batches of Luna Omega 3 µm C18 in comparison to multiple batches of XBridge 3.5 µm C18 and ZORBAX 3.5 µm SB-C18 columns.

The Luna Omega C18 columns provided narrower peak widths for all six steroids when compared to the XBridge C18 (ca. 4 – 5% narrower) and ZORBAX SB-C18 (ca. 12 – 15% narrower) columns. This is in part due to the smaller particle size and thermally modified silica for the Luna Omega 3 µm C18 media, versus 3.5 µm for the XBridge C18 and ZORBAX SB-C18 media.

The Luna Omega C18 exhibits a modest increase in overall retention when compared with the XBridge C18, and both columns provide a similar elution profile for the six steroids, indicating a very similar overall selectivity. However, the ZORBAX SB-C18 exhibits a significant increase in retention for the last three steroids in the mixture (21-hydroxyprogesterone, 17- $\alpha$ -hydroxyprogesterone, and progesterone), which is indicative of a slightly different selectivity associated with the ZORBAX SB-C18 column.

All three batches of the Luna Omega C18 column tested gave similar retention times for all peaks (RSD  $\leq$  1.1%), indicating excellent batch-to-batch reproducibility.

### LC-UV Conditions

**Column:** Luna Omega 3 µm C18 ([00F-4784-E0](#))  
Waters® XBridge® 3.5 µm C18  
Agilent® ZORBAX® 3.5 µm SB-C18

**Dimension:** 150 x 4.6 mm

**Mobile Phase:** A = 0.1% Formic Acid in Water  
B = 0.1% Formic Acid in Acetonitrile

Gradient: Time (min)	% B
0	20
1.5	20
16.5	45
19.5	45
22.5	20
25	20

**Flow Rate:** 2.0 mL/min

**Temperature:** 30 °C

**Detector:** UV @ 254 nm

**Injection:** 1 µL

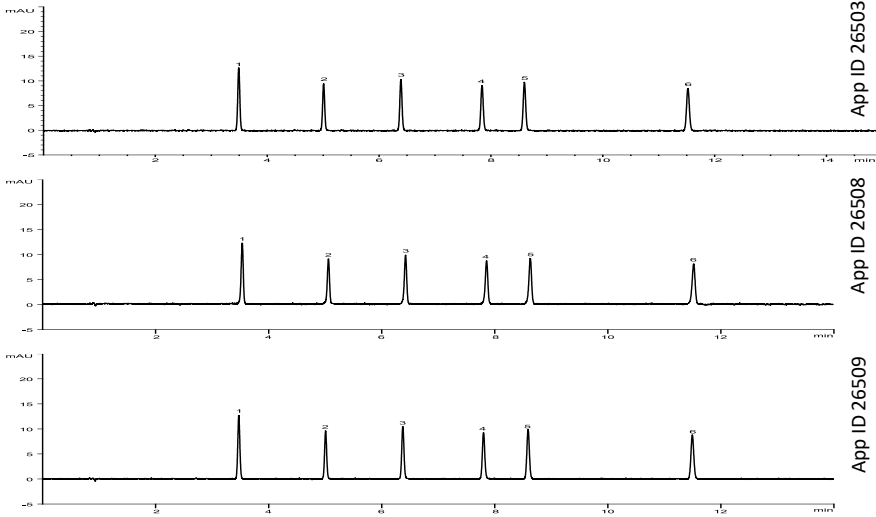
**System:** Agilent® 1260 (binary)

**Sample:** Steroid Mixture (0.1 mg/mL each)

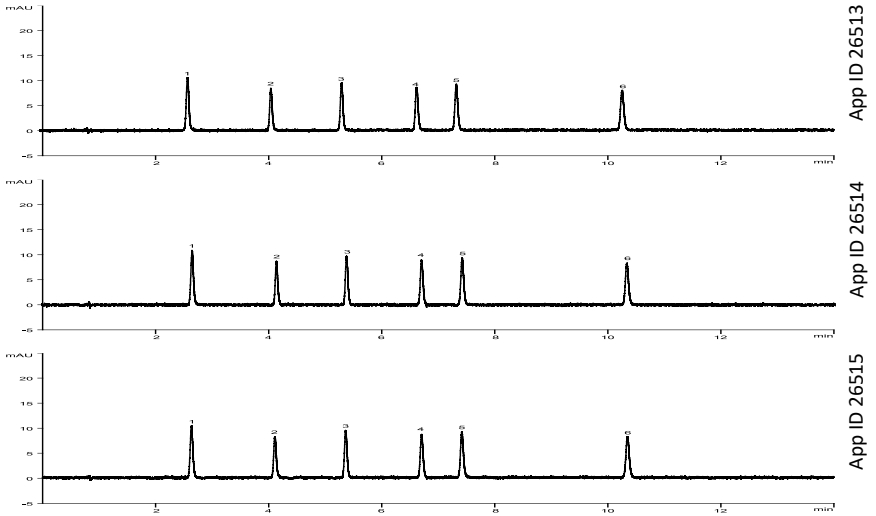
1. Hydrocortisone
2. Dexamethasone
3. 11- $\alpha$ -hydroxyprogesterone
4. 21-hydroxyprogesterone
5. 17- $\alpha$ -hydroxyprogesterone
6. Progesterone



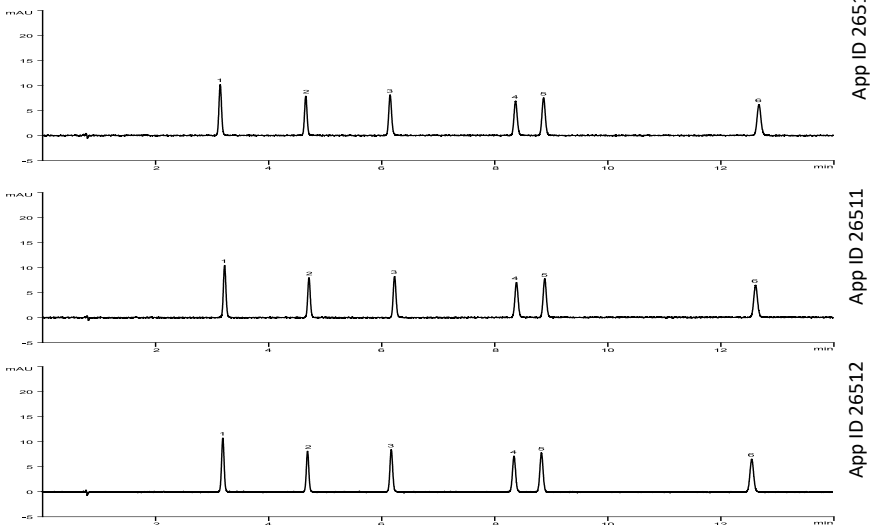
**Luna Omega 3 µm C18**



**XBridge 3.5 µm C18**



**ZORBAX 3.5 µm SB-C18**



Analyte	Avg. Peak Width, min
Hydrocortisone	0.0426
Dexamethasone	0.0416
11-α-hydroxyprogesterone	0.0429
21-hydroxyprogesterone	0.0453
17-α-hydroxyprogesterone	0.0467
Progesterone	0.0508

Analyte	Avg. Peak Width, min
Hydrocortisone	0.0443
Dexamethasone	0.0437
11-α-hydroxyprogesterone	0.0462
21-hydroxyprogesterone	0.0472
17-α-hydroxyprogesterone	0.0491
Progesterone	0.0530

Analyte	Avg. Peak Width, min
Hydrocortisone	0.0479
Dexamethasone	0.0480
11-α-hydroxyprogesterone	0.0502
21-hydroxyprogesterone	0.0517
17-α-hydroxyprogesterone	0.0534
Progesterone	0.0586



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