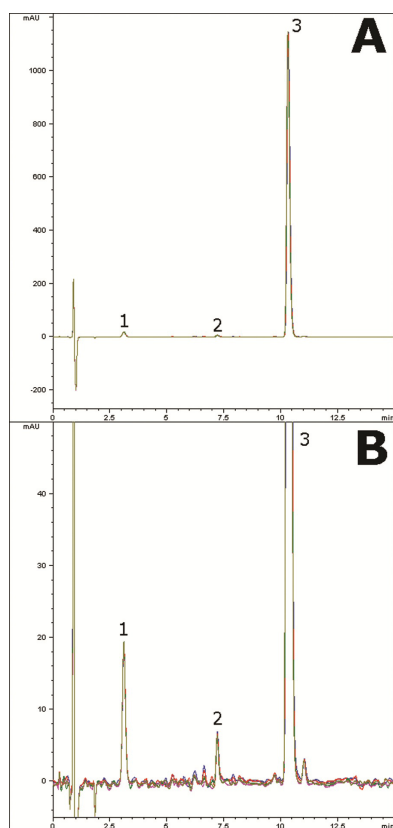
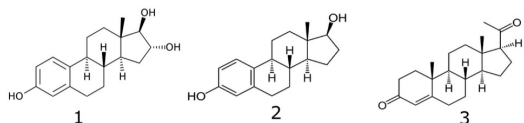


Hormone Replacement Capsule

Separation of Estriol, Estradiol, & Progesterone



Notes: The contents of a capsule containing 0.124 mg estradiol, 1.001 mg estriol, and 50 mg progesterone were added to a 25 mL volumetric flask. The flask was diluted to mark with solvent B and sonicated 10 min. Then a portion was filtered with a 0.45µm nylon syringe filter (Micro-Solv Tech. Corp.). Peak identities were confirmed by individual standards of 0.1 mg/mL in a solvent B diluent.

Method Conditions

Column: Cogent UDC Cholesterol™, 4µm, 100Å

Catalog No.: 69069-7.5P

Dimensions: 4.6 x 75 mm

Solvents: A: DI H₂O/ 0.1% formic acid (v/v)

B: Acetonitrile/ 0.1% formic acid (v/v)

Gradient:	time (min.)	%B
	0	33
	2	33
	11	65
	12	33

Post Time: 3 min

Injection vol.: 10µL

Flow rate: 1 mL/min

Detection: UV 210 nm

Peaks: 1. Estriol
2. Estradiol
3. Progesterone

Discussion

This gradient method features a separation of the three components of a hormone replacement formulation. Excellent separation is obtained between the three compounds using the Cogent UDC-Cholesterol column.

Figure A shows a five run overlay of the formulation extract injections, demonstrating the excellent run-to-run repeatability of the method.

Figure B shows a zoomed-in view so that the estriol and estradiol peaks, which are present in much lower concentration than progesterone, can be seen clearly. Figure B also shows separation of an impurity from the progesterone peak.