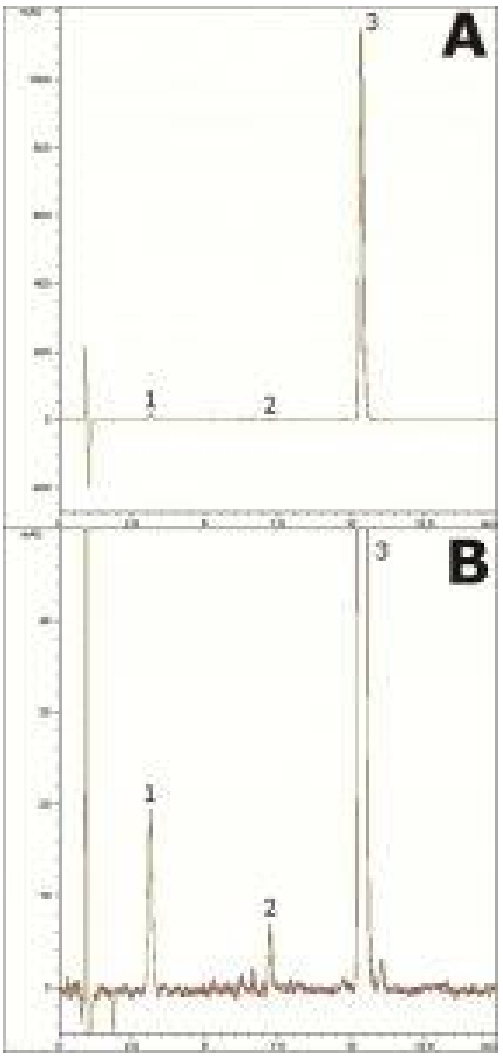
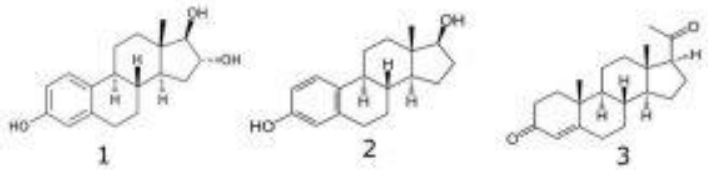
Hormone Replacement Capsule Analysis by HPLC - AppNote

Separation of Estriol, Estradiol, & Progesterone

This gradient method features a separation of the three components of a hormone replacement formulation. *Figure A* shows a five run overlay of the formulation extract injections. *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.





Peak:

1. Estriol
2. Estradiol
3. Progesterone

Method Conditions

Column: Cogent UDC Cholesterol™, 4µm, 100Å
Catalog No.: 69069-7.5P
Dimensions: 4.6 x 75mm
Mobile Phase:
A: DI Water / 0.1% Formic Acid (v/v)
B: Acetonitrile / 0.1% Formic Acid (v/v)
Gradient:

Time *(minutes)*	%B
0	33
2	33
11	65
12	33

Post Time: 3 minutes
Flow rate: 1.0 mL / minute
Detection: UV @ 210nm
Injection vol.: 1µL
Sample Preparation: 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 minutes. Then A portion was filtered with a 0.45µm Nylon Syringe Filter (MicroSolv Tech. Corp.). Peak identities were confirmed by individual standards of 0.1 mg / mL in a Solvent B diluent.

COGENT HPLC Columns™

Attachment

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MicroSolv Technology Corporation
9158 Industrial Blvd. NE, Leland, NC 28451
Tel: (732) 380-8900
Fax: (910) 769-9435
Email: customers@mtc-usa.com
Website: www.mtc-usa.com