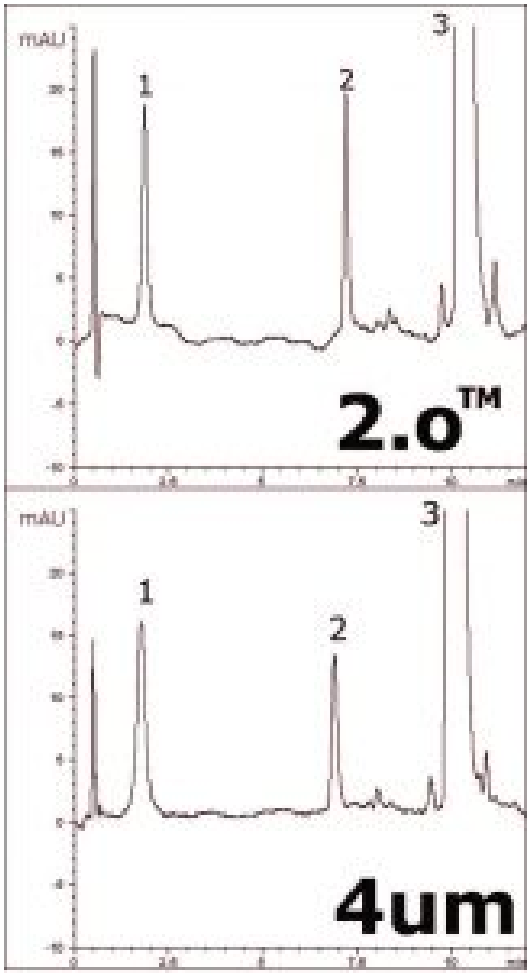
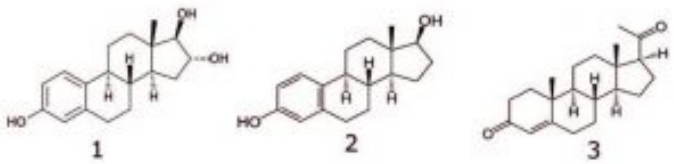
Bi-Est® Method Transfer – AppNote

4µm to Near-UHPLC – Separation of Hormones: Estriol, Estradiol, and Progesterone

Separation of three components of a hormone replacement formulation is demonstrated in this Application Note using the Cogent Bidentate C18 2.o™ Column. The two figures demonstrate how comparable retention can be obtained for both the near-UHPLC column as well as the standard 4µm Column, allowing for easy method transfer.

As an example of the advantages for the 2.o™ Column, the calculated efficiency for peak 2 was 201,360 plates/m for the 4µm Column and 383,800 for the 2.o™.





Peaks:

1. Estriol
2. Estradiol
3. Progesterone

MICROSOLV

Method Conditions

Column: Cogent Bidentate C18 2.o™, 2.2µm, 120Å

Catalog No.: 40218-05P-2

Dimensions: 2.1 x 50 mm

Solvents:

A: 90% DI Water / 10% Acetonitrile / 0.1% Formic Acid (v/v)

B: Acetonitrile / 0.1% Formic Acid (v/v)

Time (minutes)	%B
0	20
2	20
11	80
12	20

Post time: 5 minutes

Injection vol.: 2µL

Flow rate: 0.3mL/minute

Detection: UV @ 210 nm

Sample: 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 5% solvent A / 95% solvent B and sonicated 10 min. Then a portion was filtered with a 0.45µm Nylon Syringe Filter (MicroSolv Tech. Corp.). Peak identities were confirmed by individual standards.

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