Estrone, Estradiol, and Estriol from Water @ pH 2.0 Strata-X via Kinetex XB-C18 150x4.60mm

COLUMN: Kinetex® 2.6 µm XB-C18 100 Å, LC Column 150 x 4.6 mm, Ea
DIMENSIONS: 150 x 4.6 mm ID
ORDER NO: 00F-4496-E0
ELUTION TYPE: Gradient
ELUENT A: 6.9mM Ammonium Acetate pH 4.0 with 25% Acetonitrile (v/v)
ELUENT B: Acetonitrile

GRADIENT:

<table>
<thead>
<tr>
<th>Step No.</th>
<th>Time (min)</th>
<th>Pct A</th>
<th>Pct B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>35</td>
<td>65</td>
</tr>
</tbody>
</table>

FLOW RATE: 1.2 mL/min
COL. TEMP.: ambient
DETECTION: UV-Vis Abs.-Variable Wave.(UV) @ 230 nm (ambient)
ANALYST NOTE: SecurityGuard™ ULTRA Guard Cartridge System extends column lifetime.
- SecurityGuard ULTRA Cartridges, UHPLC C18 for 4.6mm ID Columns, 3/Pk Part No.: AJ0-8768
- Holder Part No.: A30-9000

ANALYTES:
1. Estriol
2. Ethyl paraben (internal standard)
3. 17-beta-Estradiol
4. Estrone
Sample Preparation Details
for UHPLC Application ID No.: 19680

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PRODUCT DESCRIPTION:
Strata™-X 33 µm Polymeric Reversed Phase, 200 mg / 6 mL, Tubes, 30/Pk

Order No.: 8B-S100-FCH

SOLID PHASE EXTRACTION (SPE) PROCEDURE:
Note: The solvent volumes shown below are for a 200 mg bed mass.
The solvent volumes will need to be adjusted for a smaller or larger bed mass.

Condition:

Load:
100mLs of Water @ pH 2.0 spiked with Estrogen probes

Wash:

Dry:
1 Minute

Elute:

Final Prep and Analysis:
Inject: 5 µL on HPLC UV-Vis Abs.-Variable Wave.(UV) @ 230 nm (ambient)

<table>
<thead>
<tr>
<th>ANALYTES</th>
<th>Spiked Conc. (ng/mL)</th>
<th>Log P</th>
<th>pKa</th>
<th>% Rec</th>
<th>%RSC (n=0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Estradiol</td>
<td>30</td>
<td></td>
<td></td>
<td>81</td>
<td></td>
</tr>
<tr>
<td>2 Ethyl paraben</td>
<td>20</td>
<td></td>
<td></td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>3 17-beta-Estradiol</td>
<td>30</td>
<td></td>
<td></td>
<td>98</td>
<td></td>
</tr>
<tr>
<td>4 Estrone</td>
<td>30</td>
<td></td>
<td></td>
<td>88</td>
<td></td>
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</tbody>
</table>

Note: This method is designed as a convenient starting point for further investigation and can be tailored to meet your extraction goals.
Call your local Phenomenex Representative for assistance in method development and optimization techniques.

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For more information contact your Phenomenex Representative at info@phenomenex.com

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