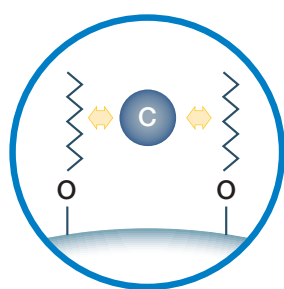


Exploring LC Selectivity Using Steroids in a Clinical Research Setting

Separation Mechanisms



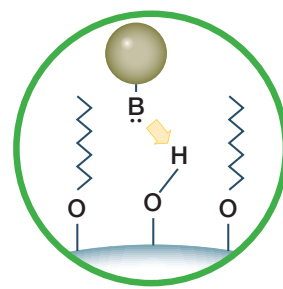
Hydrophobicity

The ability of a phase to hydrophobically interact with carbon groups



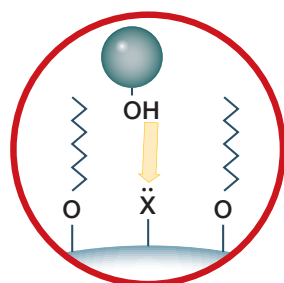
Steric Interaction

The ability of a phase to separate compounds based on structural differences



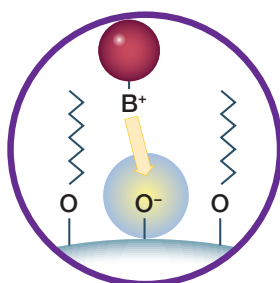
Hydrogen Bond Donating Capacity

The ability of a phase to hydrogen-bond with proton accepting groups



Hydrogen Bond Accepting Capacity

The ability of a phase to hydrogen-bond with proton donating groups

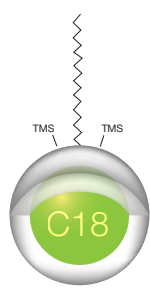


Cation Selectivity at pH 2.8

The ability of a phase to interact with cation groups at acidic pH

Cation Selectivity at pH 7.0

The ability of a phase to interact with cation groups at neutral pH

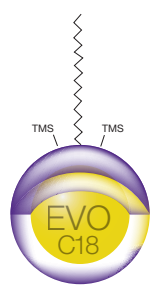


Kinetex C18

pH Range:
1.5 - 8.5*

USP Classification:
L1

Effective Carbon Load:
12%

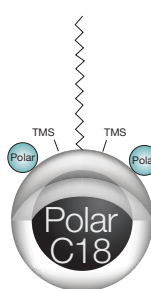


Kinetex EVO C18

pH Range:
1 - 12

USP Classification:
L1

Effective Carbon Load:
11%

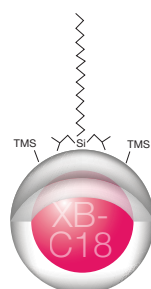


Kinetex Polar C18

pH Range:
1.5 - 8.5*

USP Classification:
L1

Effective Carbon Load:
9%

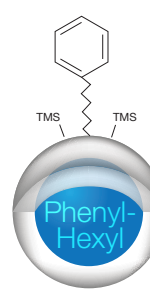


Kinetex XB-C18

pH Range:
1.5 - 8.5*

USP Classification:
L1

Effective Carbon Load:
10%

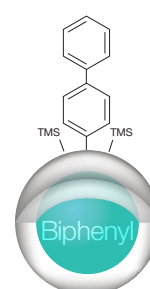


Kinetex Phenyl-Hexyl

pH Range:
1.5 - 8.5*

USP Classification:
L11

Effective Carbon Load:
11%



Kinetex Biphenyl

pH Range:
1.5 - 8.5*

USP Classification:
L11

Effective Carbon Load:
11%

Separation Mechanisms

Column Screening

- Six phases of Kinetex columns
 - C18
 - EVO C18
 - Polar C18
 - XB-C18
 - Phenyl-Hexyl
 - Biphenyl
- LC parameters kept constant
- Particle size and column dimensions kept constant
- 19 steroid analytes

Mobile Phase: A: 0.5 mM Ammonium Fluoride (aq)

B: MeOH

Gradient:	Time (min)	%B
	0.0	40
	6.5	95
	7.0	95
	7.5	95
	8	40

Flow Rate: 600 μ m/min

Injection Vol.: 5 μ m

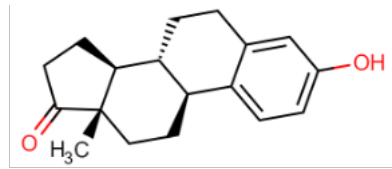
Temperature: 40 $^{\circ}$ C

Instrument: Agilent 1260

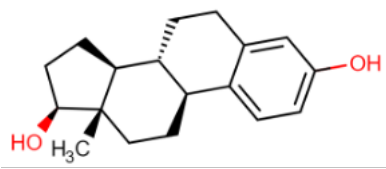
Detection: MS/MS, Sciex Triple Quad™ 7500

Analytes

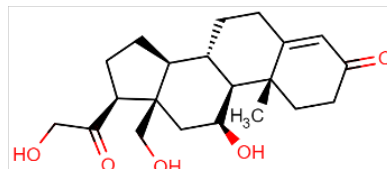
Estrone



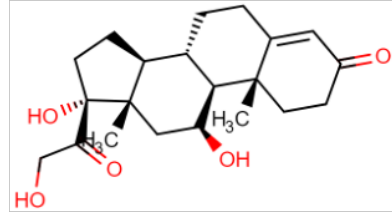
Estradiol



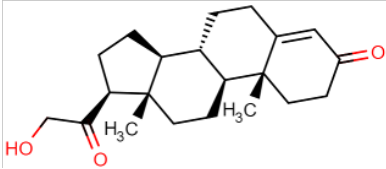
18-OH-Corticosterone



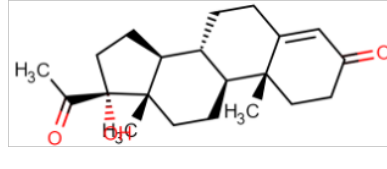
Cortisol



11-Deoxycorticosterone



17-OH-Progesterone



Discussion

- Selectivity is greatly influenced by stationary phase
- All four alkyl phases and Biphenyl achieved separation between all isomers in the steroid panel.
- Phenyl-Hexyl did not separate two sets of isomers:
 - 11-Deoxycortisol and Corticosterone
 - 11-Deoxycorticosterone and 17-OH-Progesterone
- Biphenyl has best selectivity for Estrone and Estradiol
- No separation was achieved between Estrone and Estradiol using EVO C18, Polar C18, and XB-C18
- Adjust mobile phase gradient

