APPLICATION NOTES

Cogent™ UDC Cholesterol™ with TYPE-C Silica™

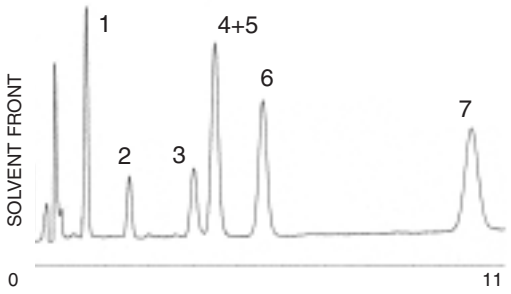
HPLC

No. 2 of Series C

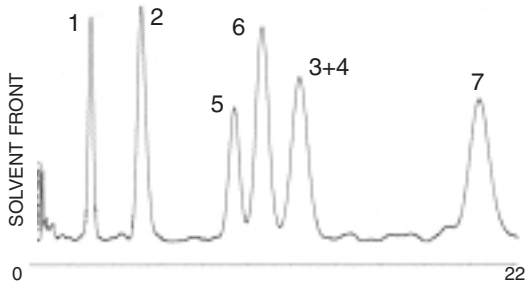
Different Solvent...Different Selectivity Mechanism

Polarity or Shape Recognition – YOU CHOOSE!

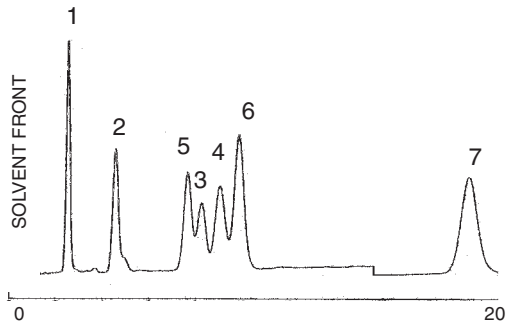
Chromatogram A:



Chromatogram B:



Chromatogram C:



Steroid Compound Type

Peak 1: Prednisolone
Peak 2: Corticosterone
Peak 3: Estradiol
Peak 4: Ethinyl Estradiol
Peak 5: Estrone
Peak 6: Norgesterel
Peak 7: Progesterone

Method Conditions

Mobile Phase

Chromatogram A: 60% Aqueous (0.1%TFA)
40% Acetonitrile (ACN)
Chromatogram B: 45% Aqueous (0.1%TFA) 55% MeOH
Chromatogram C: 56% Aqueous (0.1%TFA)
24% MeoH and 20% ACN

Column	Cogent UDC-Cholesterol™
Catalog No.	69069-75R
Dimensions	75 x 4.6mm id
Flow Rate	1.0ml/minute
Detection	UV, 240 nm
Temperature	15° C

Discussion of Chromatograms

An FDA requirement for Birth Control Product Analysis is the resolution of hormonal steroids ETES and ESTN.

- With Cogent Bidentate C18™ and Cogent UDC-Cholesterol™ HPLC columns, peaks ETES and ESTN are resolved with polarity-based, Reverse Phase, ACN as the organic modifier.
- With other C18 columns, these same peaks are not resolved.
- When eluting with the shape recognition in Methanol on the Cogent UDC Cholesterol™ column, ETES and ESTN are extremely well resolved. However, in this test mix ETES now co-elutes with ESTD.
- By mixing the selectivity mechanisms, polarity (ACN) and shape recognition (MeOH), partial resolution was achieved on all compounds using the UDC Cholesterol™ column. Scaling up to a 250x4.6mm column resulted in baseline resolution of all peaks.

Implications, Advantages & Uses

Using a single UDC Cholesterol™ column with orthogonal selectivity, polarity in ACN and shape recognition in Methanol can lead to unique problem solving.

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