APPLICATION NOTES

Cogent™ UDC Cholesterol™ with TYPE-C Silica™

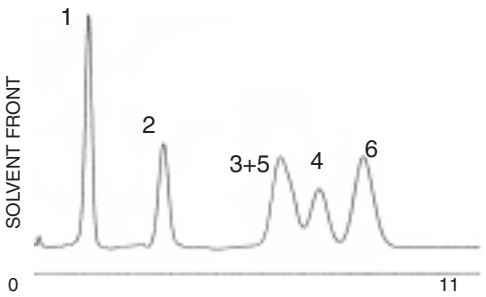
HPLC

No. 3 of Series C

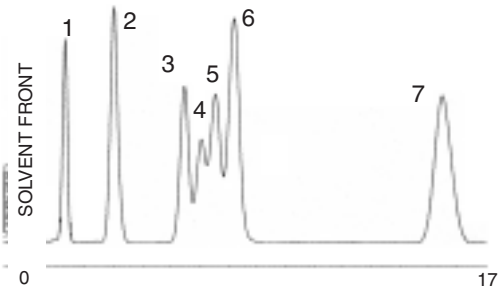
Unique Solutions by Combining Polarity and Shape Recognition

Anti-Inflammatory and Birth Control Steroids by Cogent UDC-Cholesterol™ Column

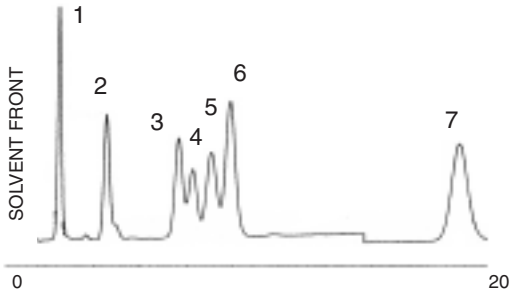
Chromatogram A:



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: 56% Aqueous(0.1%TFA)
20% MeOH /24% Acetonitrile

Chromatogram B: 53% Aqueous (0.1%TFA)
27% MeOH /20% Acetonitrile

Chromatogram C: 56% Aqueous (0.1%TFA)
24% MeOH / 20% Acetonitrile

Column	Cogent UDC-Cholesterol™
Catalog No.	69069-75R
Dimensions	75 x 4.6mm id/250 x 4.6mm id
Flow Rate	1.0ml/minute
Detection	UV, 240 nm
Temperature	15° C

Discussion of Chromatograms

Polarity and shape recognition retention-time profiles were compared to assess the likely impact of varying the ACN and MeOH ratios.

Organic solvent mix percentage and temperature dependence were studied. Temperature changes above 15°C had little effect on the polarity-based, reverse-phase selectivity (ACN) and negative effects on shape selectivity (MeOH). As a result 15°C was used. Initially, 75 x 4.6mm columns were used at 2.0-3.0 ml/min to reduce column equilibration time and to speed method development. Flow rates of 1.0 ml/min were used only on promising mixes for final optimizations.

The chromatograms show that relative retention times can vary when the ratios of the selectivity mechanisms (ACN and MeOH) are manipulated.

Implications, Advantages & Uses

Using the 75 x 4.6 mm UDC-Cholesterol™ column rapidly achieved a partial resolution of all standards. Scaling up to 250 x 4.6 mm allowed baseline resolution of all components. This method rapidly achieved results in a fraction of the time it would have taken if only using the larger size columns.

The different orthogonal selectivities of the UDC-Cholesterol™ column can be utilized to achieve unique solutions to difficult analytical problems.

MICROSOLV™

MicroSolv Technology Corporation
103 Brighton Avenue, Long Branch, NJ 07740 USA
Ph. 1-732-229-3400 Fx. 1-732-229-2403
www.MicroSolvTech.com Email: info@microsolvtech.com