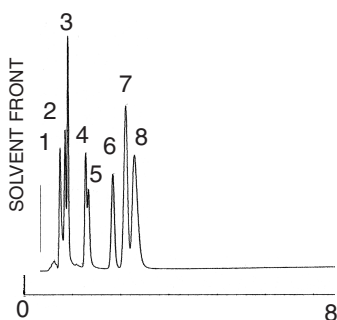


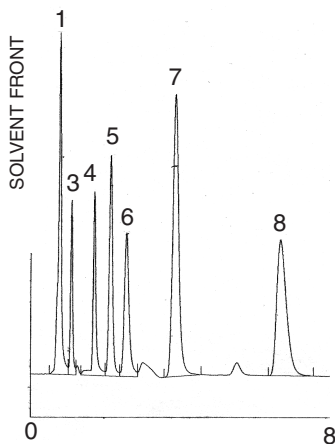
**Rapid Method Development Using
Type-B and TYPE-C™ HPLC Columns**

Finding Successful Long-Term Use of 100% Aqueous Mobile Phase
for Separation of Highly Polar Acids and Bases

Chromatogram A:
UDC-Cholesterol™ Column



Chromatogram B:
Bidentate C-18™ Column



Peaks:

- | | |
|------------------|-------------------|
| 1. Oxalic Acid | 5. Sodium Azide |
| 2. Tartaric Acid | 6. Uracil |
| 3. Formic Acid | 7. Fumaric Acid |
| 4. Acetic Acid | 8. Propionic Acid |

Method Conditions

Mobile Phase 100% Aqueous (0.05% H₃PO₄)

Columns C18, C8, UDC-Cholesterol™ (shown), Amino, Cyano, Bidentate C18™ (shown), Phenyl, Pentafluorophenyl

Dimensions 75 x 4.6mm id; Mini-Scout™

Flow Rate 1.0 ml/min

Detection: UV, 215nm

Discussion of Chromatograms

Eight columns with different selectivities were chosen for this study. Both of the type-B, C18 columns exhibited retention time shifts with prolonged exposure to 100% Aqueous conditions. None of the other type-B phases resolved the mixture.

Chromatogram A: The Cogent UDC Cholesterol™ column does not show retention shifts with prolonged exposure to 100% Aqueous, but its resolution was not optimal for this sample. This column's unique selectivity is best suited to compounds that contain ring structures.

Chromatogram B: The Cogent Bidentate C18™ column produced stability and optimal resolution when used for prolonged periods with 100% Aqueous.

Implications

Using 8 (short) columns with different selectivities allowed for the quick choice of the optimal phase – the Bidentate C18™.

Advantages & Uses

Since columns that are 75x4.6mm can be equilibrated and initially run at 3 ml/min, then at 1 ml/min for final selection, non-suitable phase selectivities can be quickly determined. Once the optimal phase is selected, the appropriate solvent system can be determined...Rapid Method Development.

This process using "Mini Scout™, 75 x 4.6mm id" columns is up to TEN TIMES FASTER than using 250 x 4.6 mm id columns being equilibrated and run at 1 ml/min.