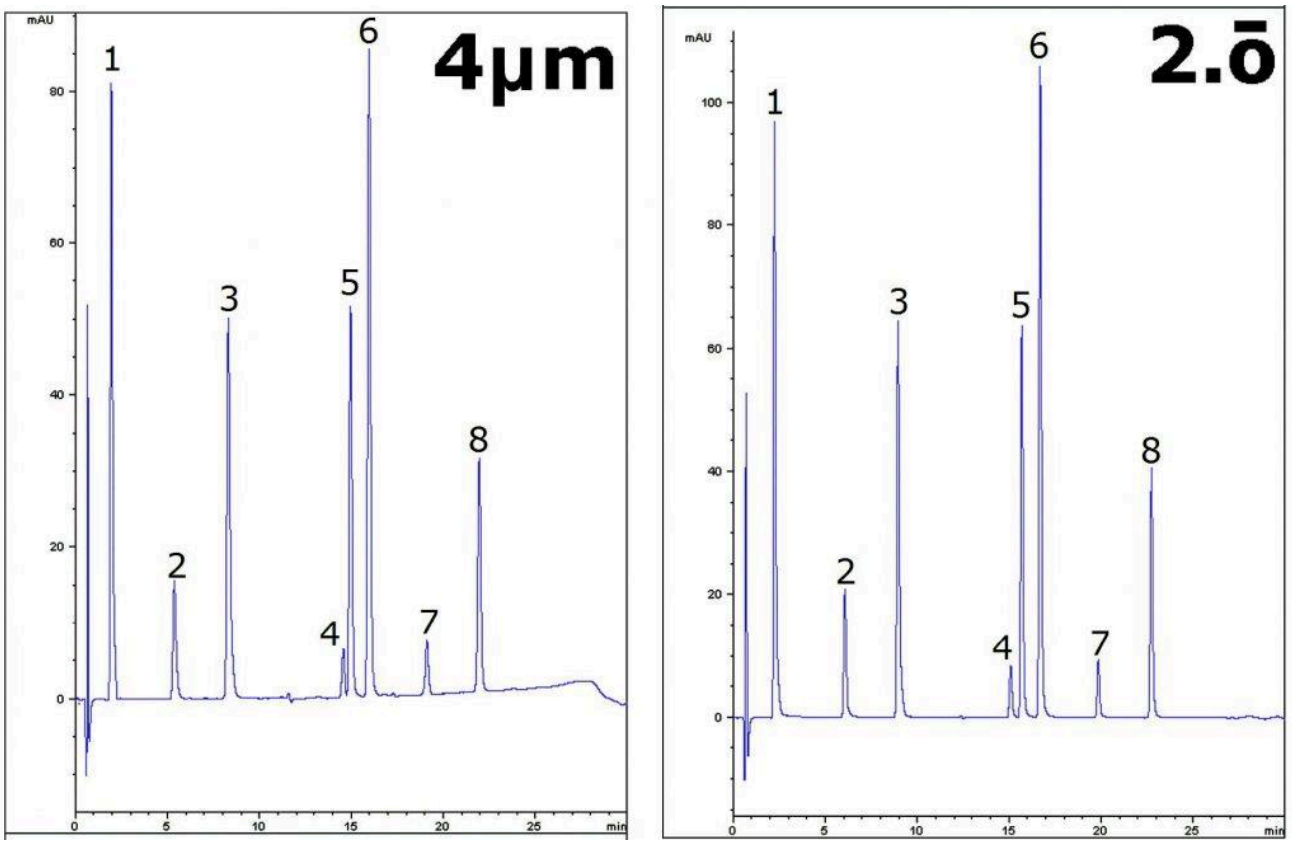
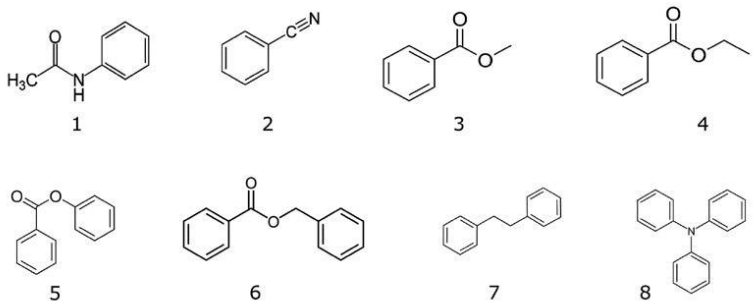
# Transfer HPLC Method to UHPLC with Hydrophobic Compounds - AppNote

## Separation of Hydrophobic Compounds by HPLC & UHPLC

This AppNote shows separation of analytes within a range of hydrophobicity. A simple gradient is used to elute all the compounds and baseline separation is obtained for the critical pair (*peaks 4 and 5*) and the least hydrophobic compound is adequately retained.

A comparison is shown in the figure below with a 4µm Cogent Bidentate C18 Column and a similar 2.o (2.2µm) Column. The retention profiles are quite comparable, meaning Method Transfer from one Column to the other will be easy to achieve.



## Peaks:

1. Acetanilide, 2. Benzonitrile, 3. Methyl Benzoate, 4. Ethyl Benzoate,
5. Phenyl Benzoate, 6. Benzyl Benzoate, 7. Bibenzyl 8. Triphenylamine

## Method Conditions

**Columns**: Cogent Bidentate C18 2.o™, 2.2µm, 120Å; Cogent Bidentate C18™, 4um, 100Å
**Catalog Nos.**: 40218-05P-2; 40018-05P-2
**Dimensions**: 2.1 x 50mm for both Columns
**Mobile Phase**:
A: DI Water / 0.1% Formic Acid (v/v)
B: Acetonitrile / 0.1% Formic Acid (v/v)
**Gradient**:

| Time *(minutes)* | %B |
|---|---|
| 0 | 20 |
| 1 | 20 |
| 25 | 80 |
| 26 | 80 |
| 27 | 20 |

**Injection vol.**: 1 µL
**Flow rate**: 0.3mL / minute
**Detection**: UV @ 254nm
**Sample Preparation:** Mixture of solutes in 80:20:0.1 Acetonitrile / DI Water / Formic Acid Diluent. Peak identities were confirmed with individual standards.
**$t_0$**: 0.7 minutes

COGENT HPLC Columns™

**Attachment**

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**MicroSolv Technology Corporation**
9158 Industrial Blvd. NE, Leland, NC 28451
Tel: (732) 380-8900
Fax: (910) 769-9435
Email: customers@mtc-usa.com
Website: www.mtc-usa.com