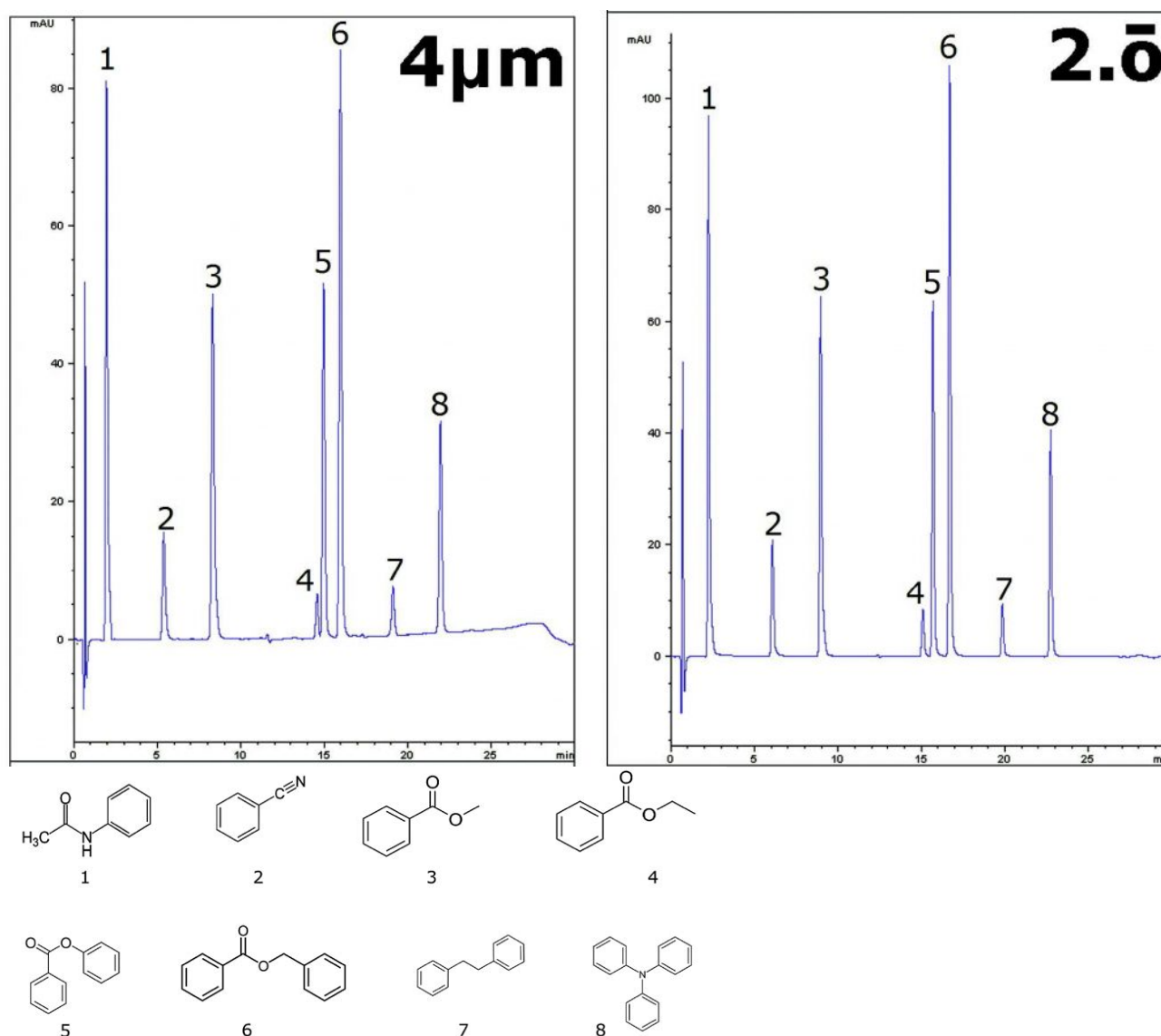


Separation of Hydrophobic Compounds by HPLC & UHPLC

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 4 μ m Cogent Bidentate C18 Column and a similar 2.0 (2.2 μ m) 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

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Columns: Cogent Bidentate C18 2.0™, 2.2µm, 120Å; Cogent Bidentate C18™, 4µm, 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.

t₀: 0.7 minutes



Attachment

No 274 Transfer HPLC Method to UHPLC with Hydrophobic Compounds pdf 1 Mb [Download File](#)

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