Acetaminophen and Related Substances

In this study, we analyze acetaminophen and related substances with a new HPLC particle technology based on monodisperse fully porous particles. We explore the impact of modifying the stationary phase and mobile phase on selectivity.

Experimental Conditions

Time (min)	%B	Flow Rate (ml/min)
0	5	0.4
5	95	0.4
15	95	0.4
20	5	0.4

Mobile Phase A: 10 mM Ammonium Formate (pH 3.0)

Mobile Phase B: 10 mM Ammonium Formate pH 3.0 in Acetonitrile: Water 9:1 v/v

Mobile Phase B1: 10mM Ammonium Formate pH 3.0 in Methanol: Water 9:1 v/v

Temp: 40 °C

Detection: 254 nm

Screening Experiment – 8 columns
Ballistic Gradient – 2 Solvent Systems

Evosphere 100 Å, 3 µm, 2.1 x 100 mm Column

C18/AR

C18/PFP

Phenyl-Hexyl

RP18-Amide

C12

Aqua

Diphenyl

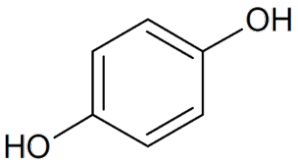
PFP

Peak Identities

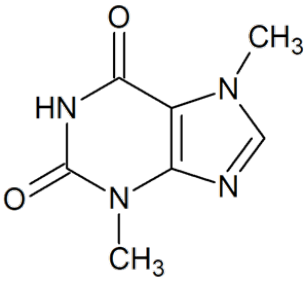
1. Hydroquinone
2. Theobromine
3. Acetaminophen
4. Theophylline
5. Paraxanthine
6. 4-Hydroxybenzoic acid
7. 2-Acetamidophenol
8. Caffeine
9. Phenol
10. Aspirin
11. 2-Hydroxybenzoic acid
12. 4-Nitrophenol

Peak Identities- Phase Structures

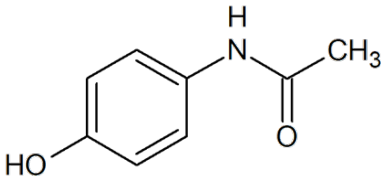
1. Hydroquinone



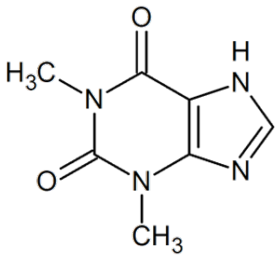
2. Theobromine



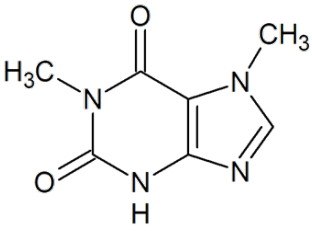
3. Acetaminophen



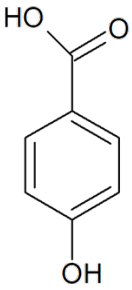
4. Theophylline



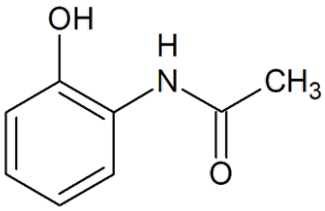
5. Paraxanthine



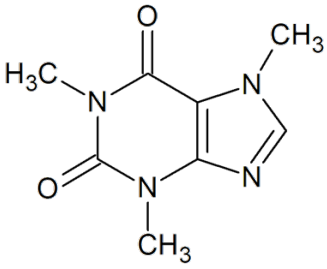
6. 4-Hydroxybenzoic acid



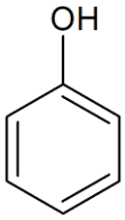
6. 2-Acetamidophenol OH



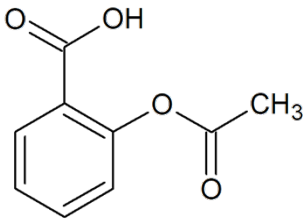
8. Caffeine



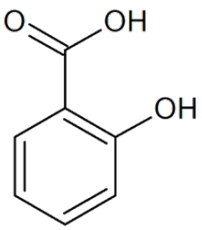
9. Phenol



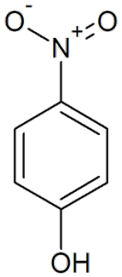
10. Aspirin



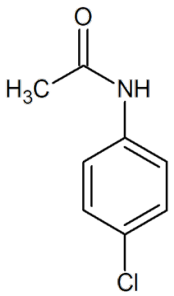
11. 2-Hydroxybenzoic acid



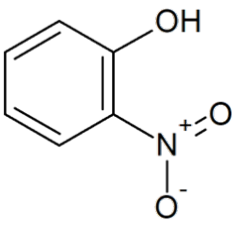
12. 4-Nitrophenol



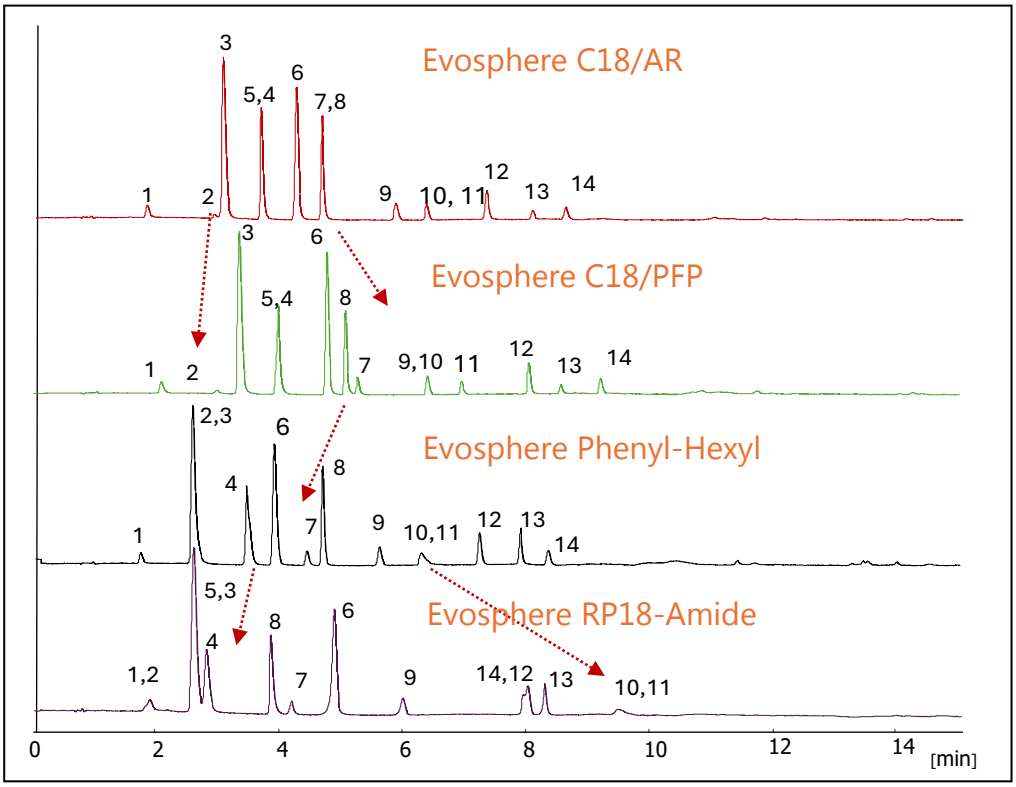
13. 4-Chloroacetanilide

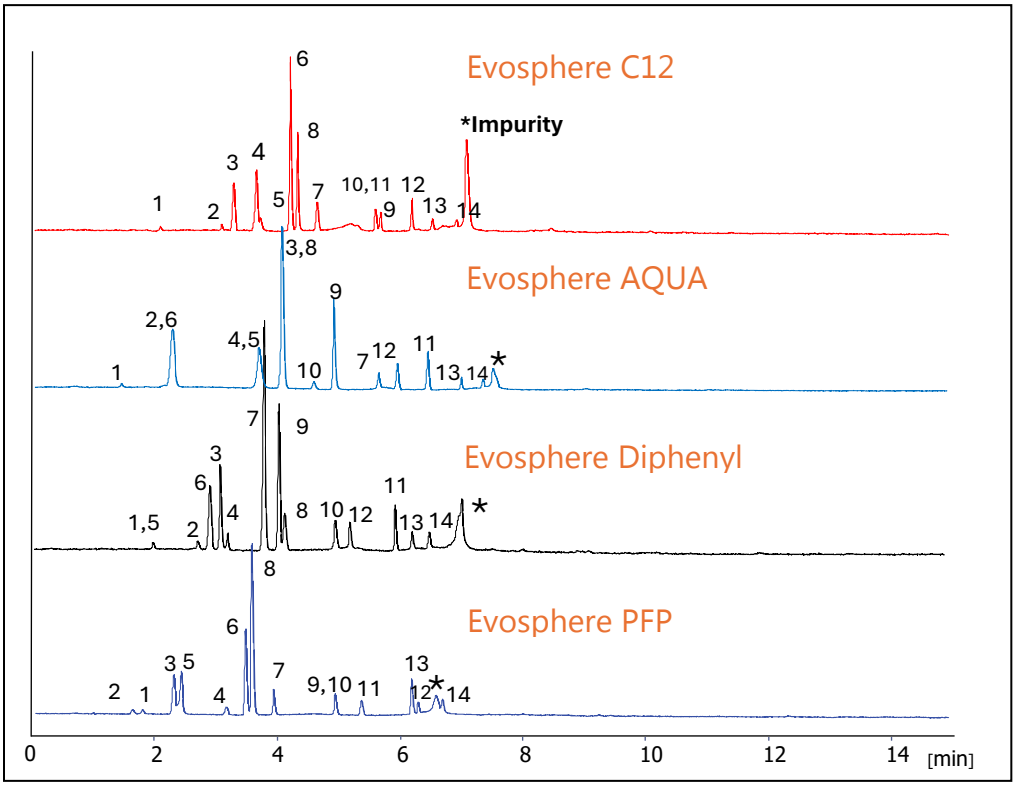


14. 2-Nitrophenol

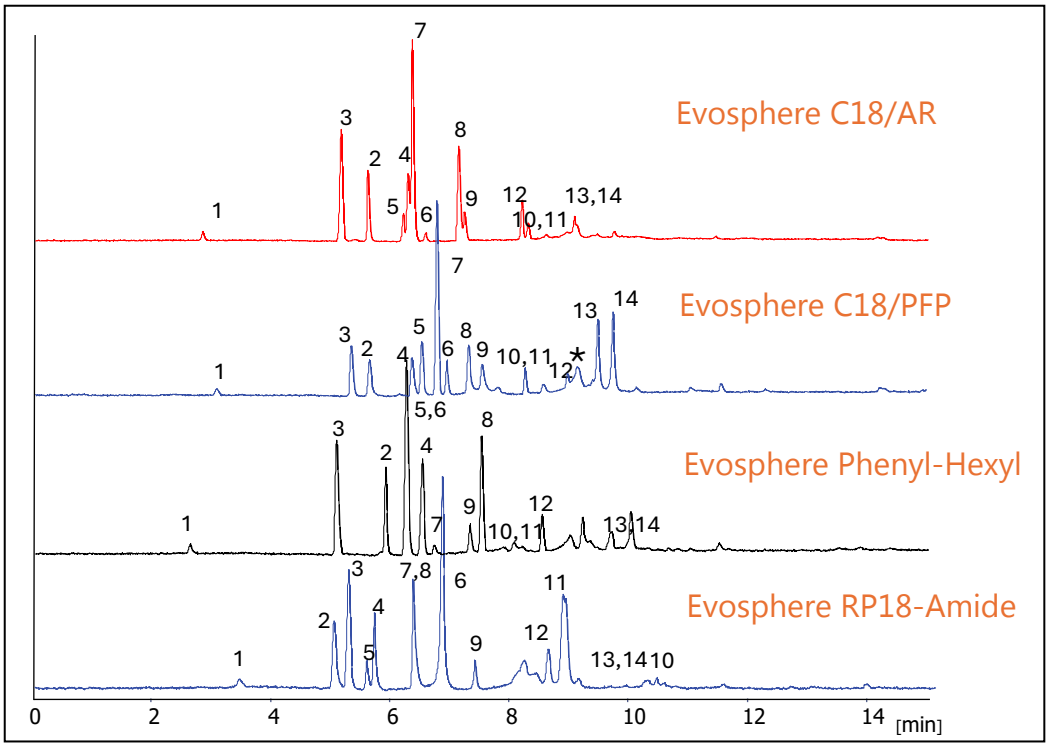


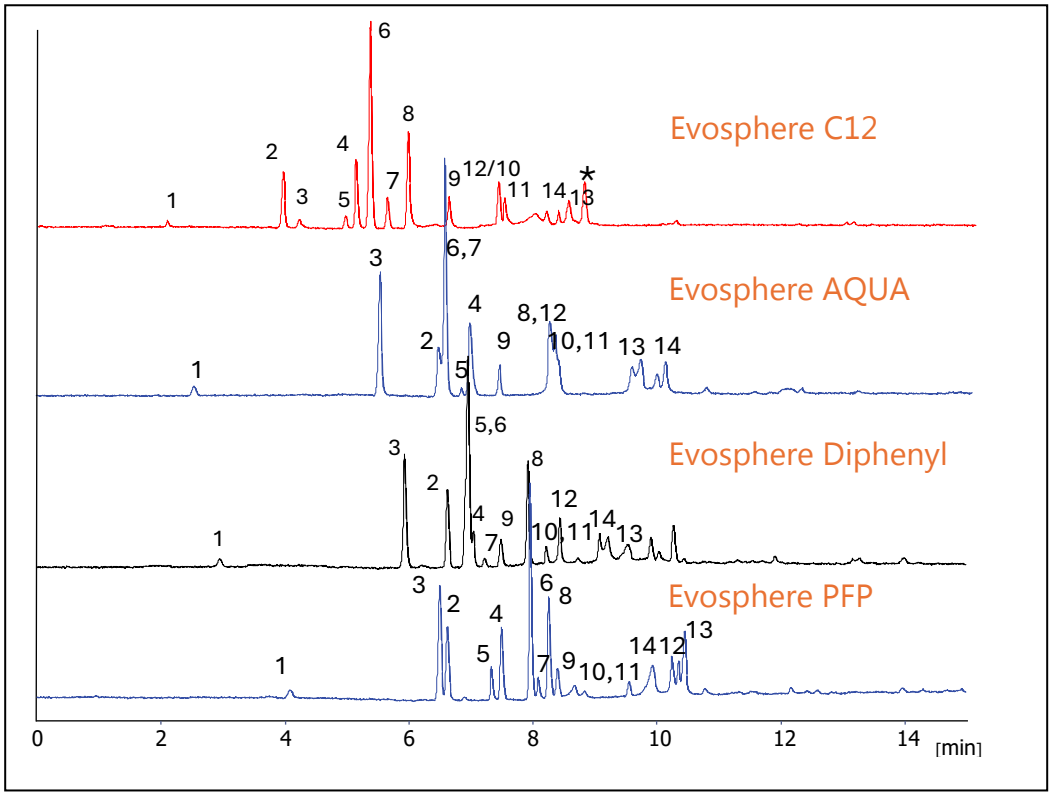
The first set of 8 chromatographic traces is with MeCN as the strong solvent in reversed-phase mode. Based on the results, the C18/PFP, Diphenyl, RP18-Amide and PFP are most promising for further method development optimization depending on the critical peak pairs.





The second set of 8 chromatographic traces is with MeOH as the strong solvent in reversed-phase mode. Based on the results, the C18/PFP, C12 and Phenyl-Hexyl are most promising for further method development optimization depending on the critical peak pairs.





Future work:

Explore other MS friendly buffer systems like ammonium acetate

Explore different Temperatures including 45 °C and 50 °C

Based on retention, suggest exploring shallower gradients to adjust selectivity of key critical peak pairs

EVOSPHERE

Application Note

Acetaminophen and Related Substances

For technical support or applications contact: info@mac-mod.com

For more information VISIT: www.mac-mod.com

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