



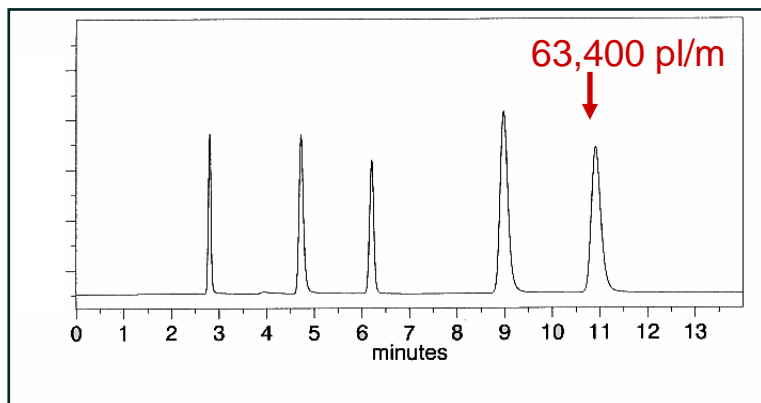
Introducing reproducible
selectivity changes to HPLC



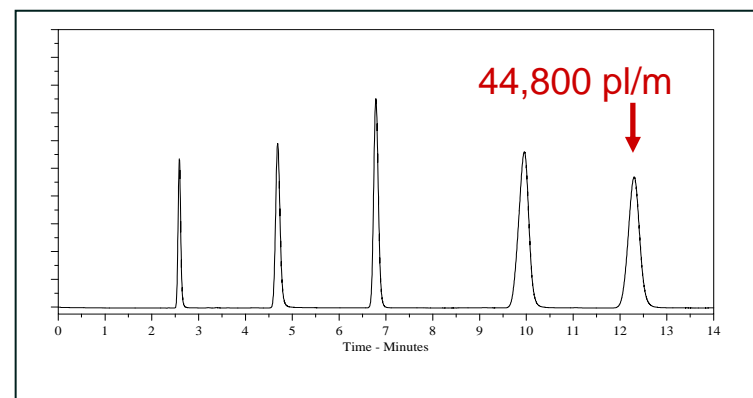


Antidepressants Separation – Leading C18 Columns

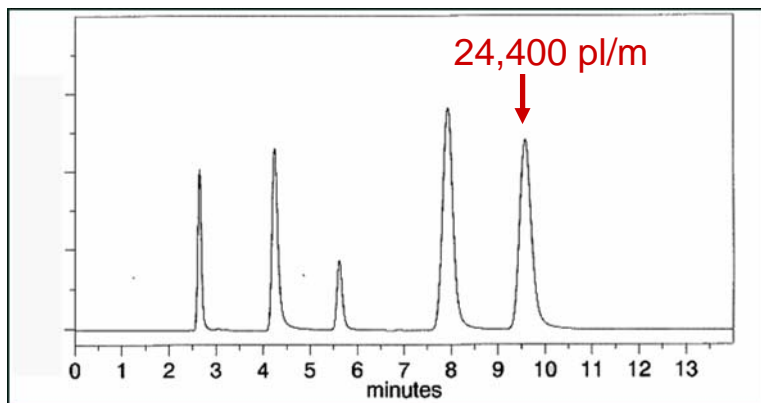
ACE 5 C18



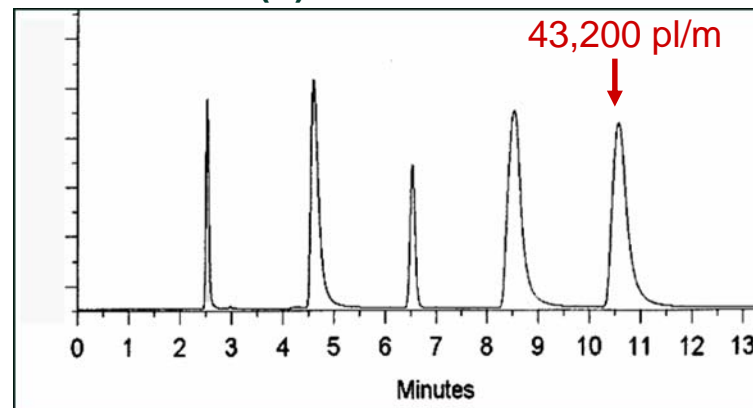
SunFire 5 C18



XTerra MS 5 C18



Luna 5 C18 (2)

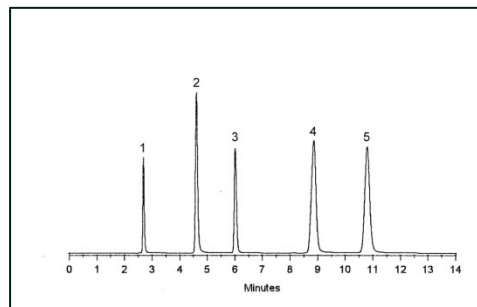


Leading high purity C18 brands show similar selectivity

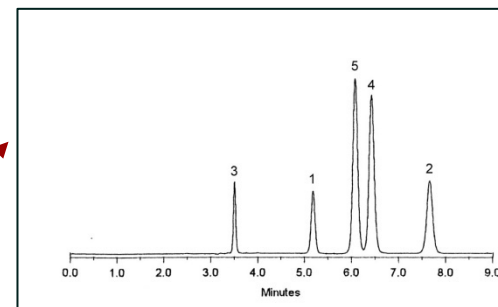


Changing Selectivity – Bonded Phase Effects

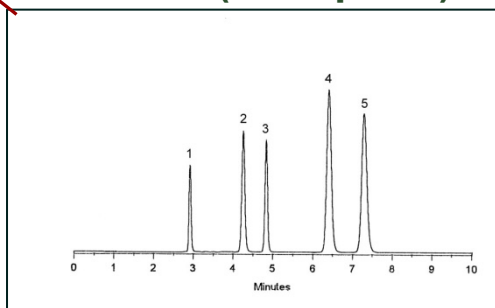
ACE C18 – Increase Retention



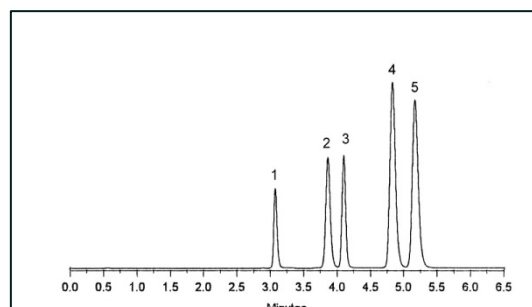
ACE CN – Alternate Selectivity



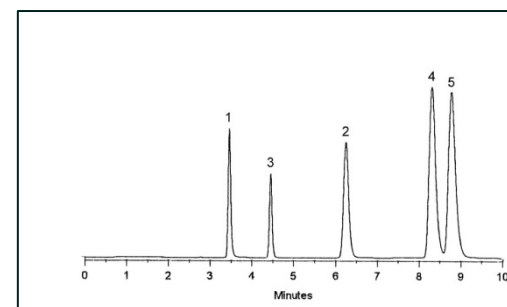
ACE C8 (start point)



ACE C4 – Decrease Retention



ACE Phenyl – Alternate Selectivity

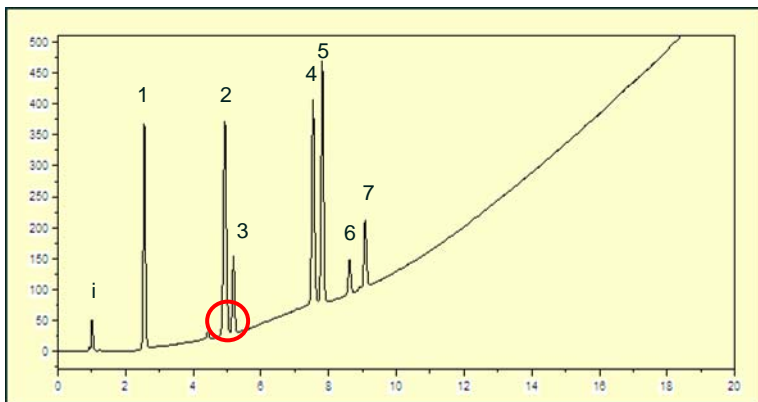


Change selectivity using different bonded phases
Use ultra high purity silica for good chromatography and reproducibility

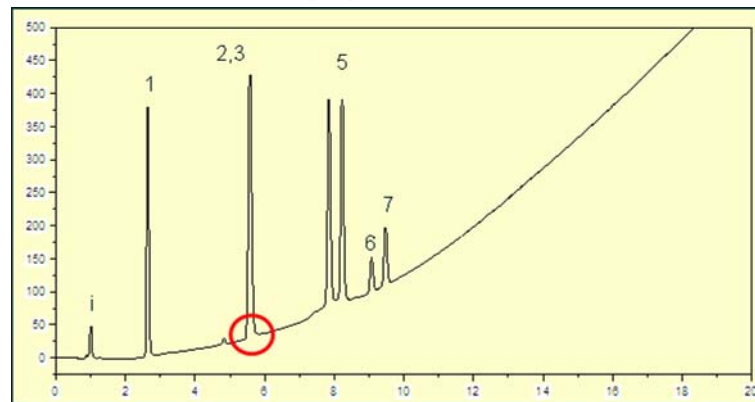


Vitamin Separation – Leading C18 Columns

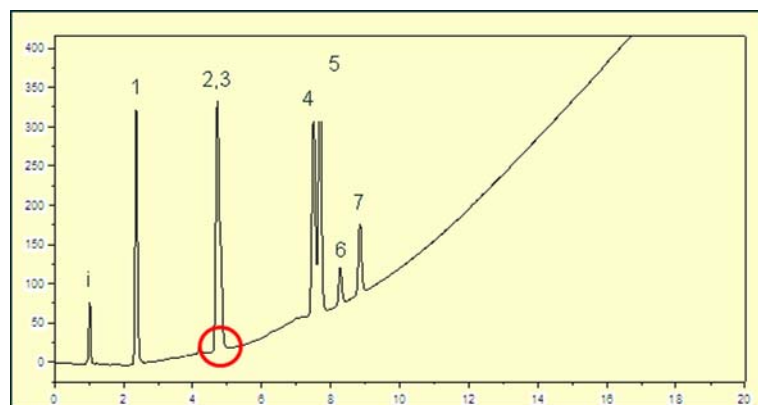
ACE 5 C18



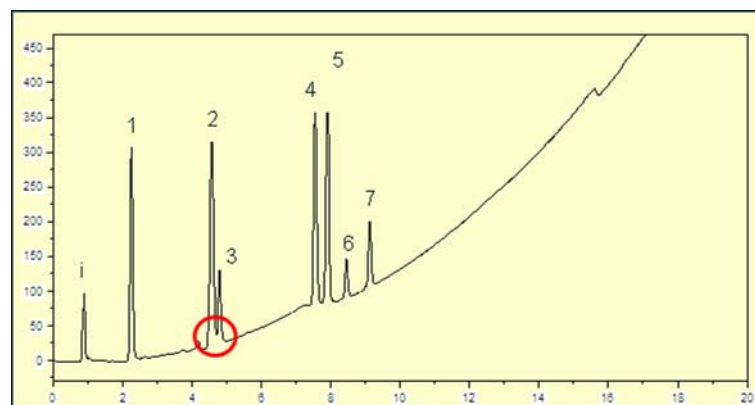
Luna 5 C18(2)



XTerra MS 5 C18



Zorbax XDB 5 C18



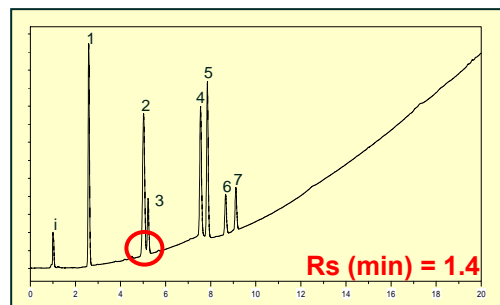
Leading high purity C18 brands show similar selectivity

Column: 150 x 4.6mm Mobile phase: MeOH/20mM KH₂PO₄ (pH2.9) , linear gradient, 5 to 95% MeOH in 20 mins Flow: 1.50ml/min
 Components; 1: Pyridoxine, 2: Pantothenic acid, 3: p-Aminobenzoic acid, 4: Cyanocobalamine, 5: Folic acid, 6: d-Biotin, 7: Riboflavin, i: impurity

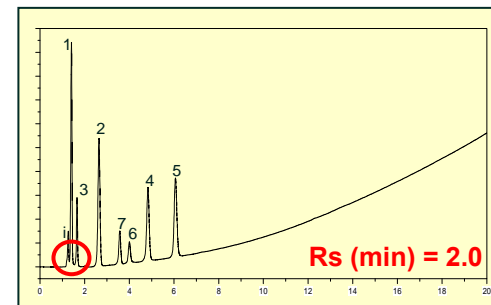


Changing Selectivity – Bonded Phase Effects

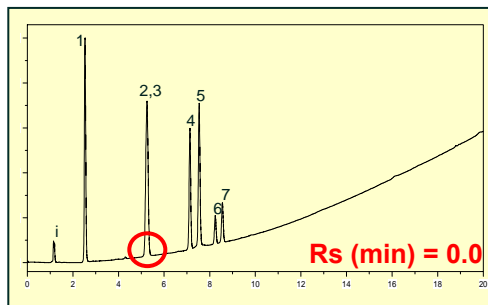
ACE 5 C18



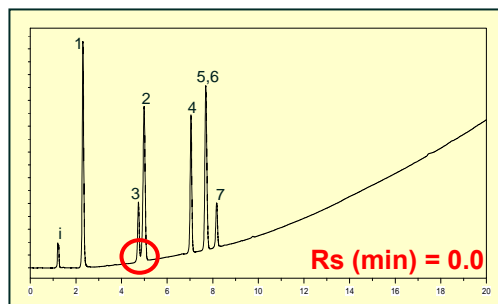
ACE 5 CN



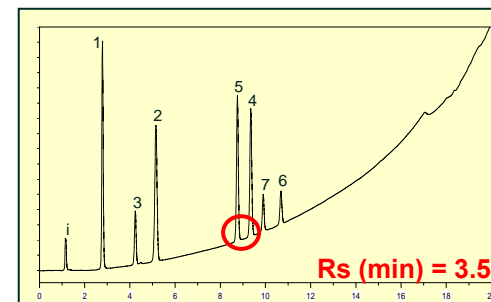
ACE 5 C8



ACE 5 C4



ACE 5 Phenyl



**Change selectivity using different bonded phases.
Use ultra high purity silica for good chromatography and reproducibility.**



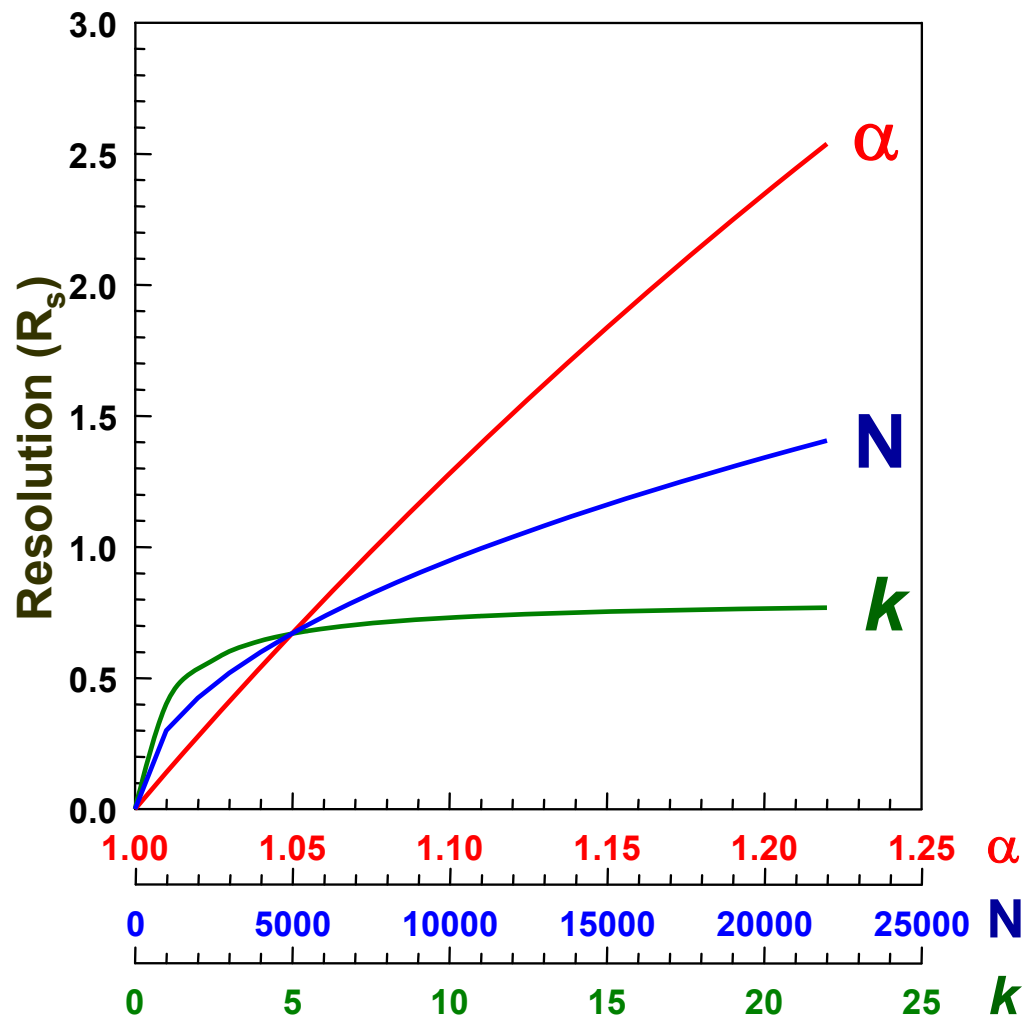
Selectivity: The Key to HPLC Resolution

Selectivity (α) has the greatest impact on improving resolution; however, improvements can still be made in all three areas.

Efficiency Selectivity Retention

$$R_s = \frac{\sqrt{N}}{4} \cdot \frac{\alpha - 1}{\alpha} \cdot \frac{k}{k + 1}$$

$$\alpha = \frac{k_j}{k_i}$$

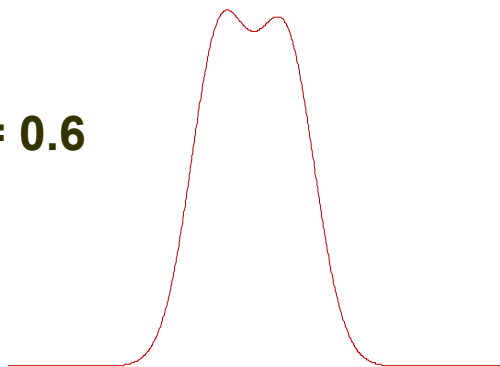




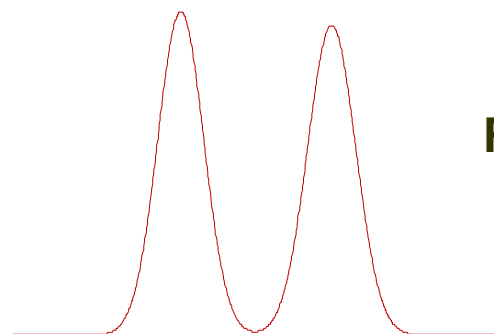
Resolution

50% impurity \longrightarrow

$R_s = 0.6$

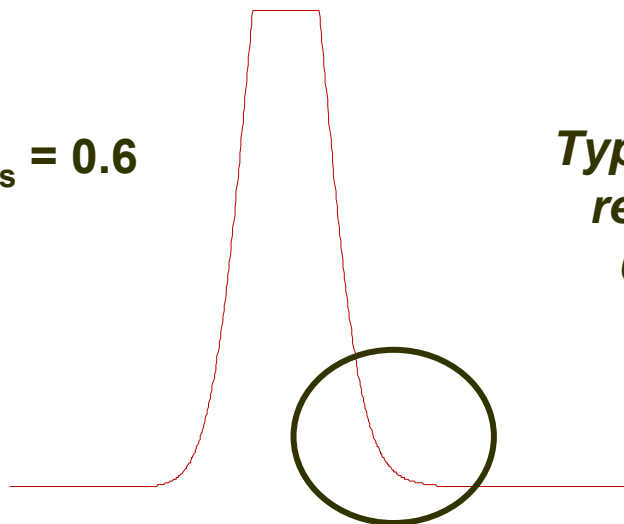


$R_s = 1.6$



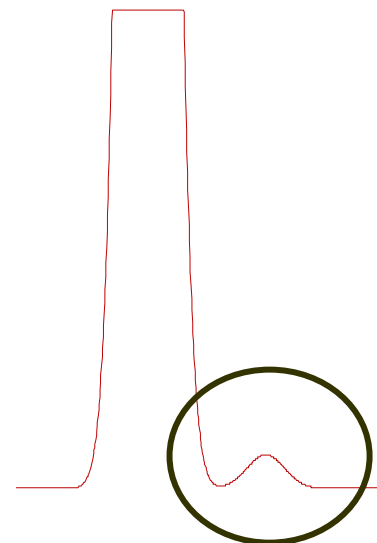
1% impurity \longrightarrow

$R_s = 0.6$



Typically need to resolve @ the 0.05% level

$R_s = 1.6$





Test mix 1 – LC/MS friendly conditions

1. **Metronidazole**
2. **4-Hydroxybenzoic acid**
3. **3-Hydroxybenzoic acid**
4. **Caffeine**
5. **1,3-Dinitrobenzene**
6. **Acetophenone**
7. **Dimethylphthalate**
8. **Ethyl parabens**
9. **Piroxicam**
10. **Sulindac**
11. **Chrysin**
12. **Piperine**
13. **Flurbiprofen**
14. **Ibuprofen**

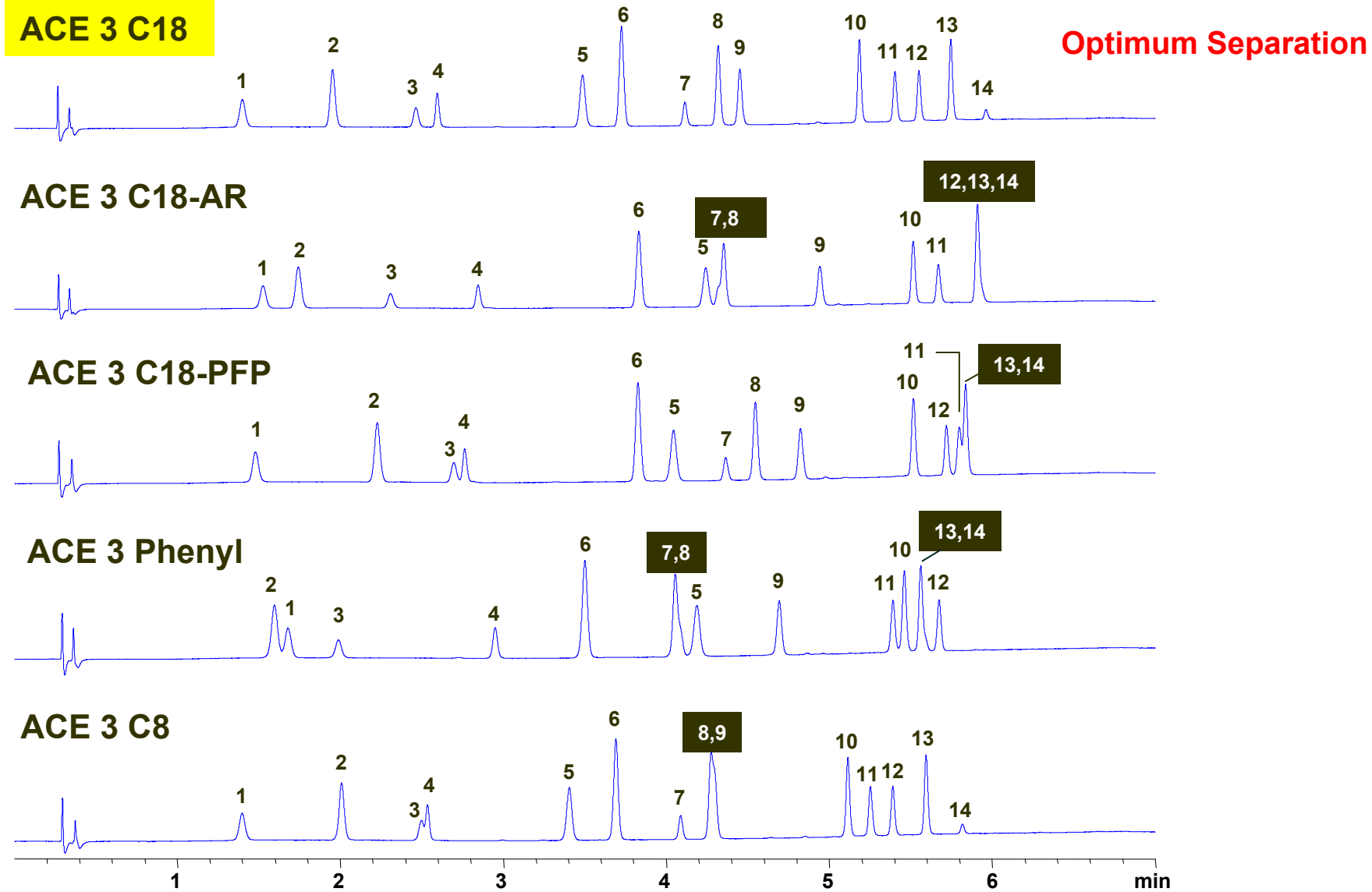
Gradient

Time (min)	% B
0	3
6.5	100
7.5	100
8.0	3
10.5	3

MPA: 0.1% v/v formic acid in water
MPB: 0.1 % v/v formic acid in MeOH
Temperature: 40°C
Flow 0.6 ml min⁻¹
Detection: 254nm



Test mix 1 – LC/MS friendly conditions





Test mix 2 – LC/MS friendly conditions

1. **3-Hydroxybenzoic acid**
2. **Methylphenylsulphoxide**
3. **Quinoxaline**
4. **Salicylic acid**
5. **Benzylcyanide**
6. **1,2-Dimethoxybenzene**
7. **Ethyl parabens**
8. **1,4-Dimethoxybenzene**
9. **Bendroflumethazide**
10. **Piroxicam**
11. **Benzylchloride**
12. **Thioanisole**
13. **Sulindac**
14. **Chrysin**
15. **Ibuprofen**
16. **1,2,3-Trichlorobenzene**
17. **Meclofenamic acid**

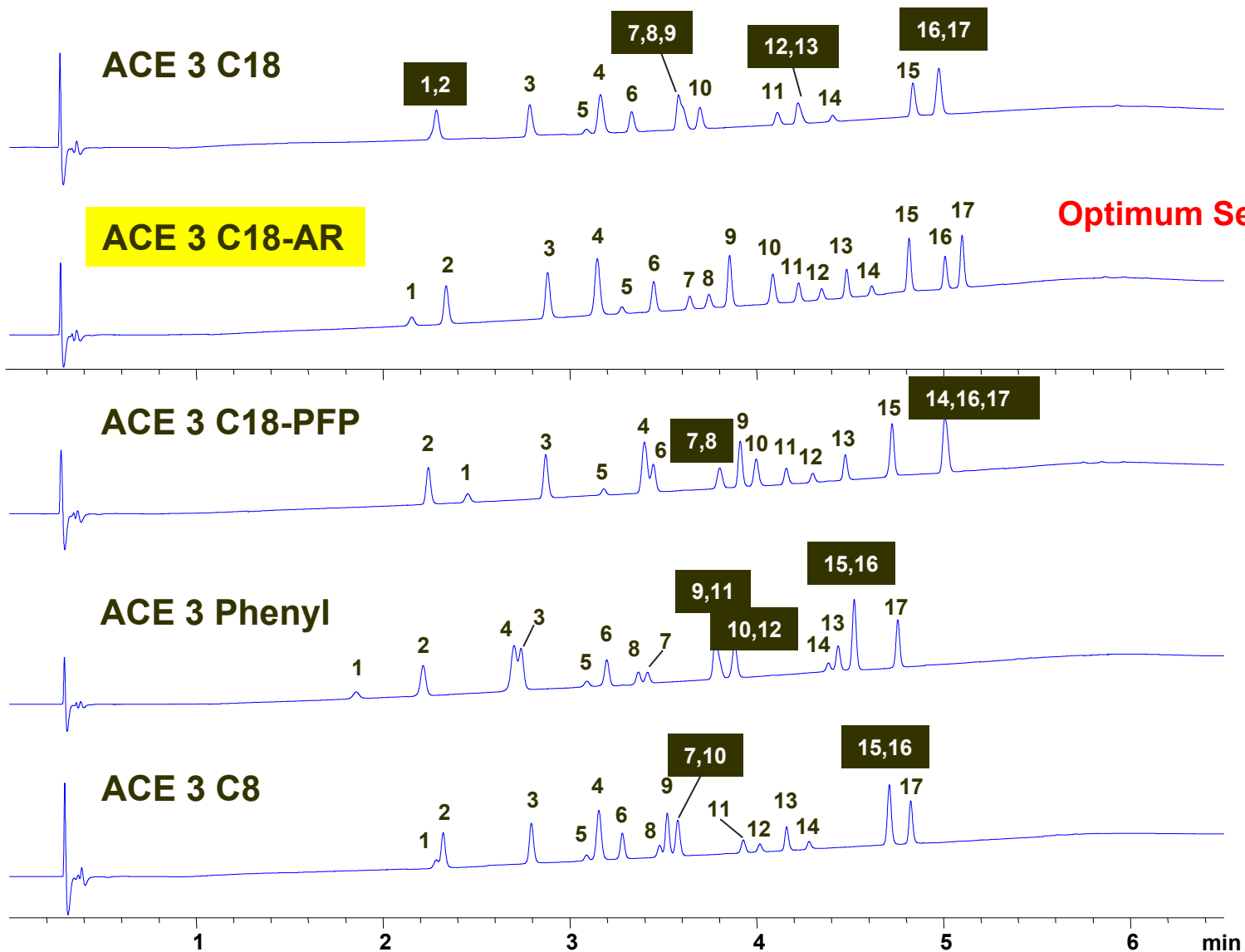
Gradient

Time (min)	% B
0	3
5	100
6	100
6.5	3
9	3

MPA: 0.1% v/v formic acid in water
MPB: 0.1 % v/v formic acid in MeOH
Temperature: 40°C
Flow 0.6 ml min⁻¹
Detection: 214nm



Test mix 2 – LC/MS friendly conditions





Summary

Optimisation of Efficiency (N) and Selectivity (α)

		R_s	Analysis Time Reduction
Efficiency (N) (change particle size)	5 μ m	2.00	—
	3 μ m	2.53	38%
	2 μ m	2.83	50%
Selectivity (α) (change bonded phase) (change temperature)	$\alpha = 1.10$	2.00	—
	$\alpha = 1.20$	4.00	75%
	$\alpha = 1.32$	6.40	90%

Selectivity (α) usually has a much greater impact on resolution and thus reducing analysis times than efficiency (N)



Conclusion

- ◆ **high purity, ultra inert materials are strongly recommended due to improved chromatography, reproducibility and lifetime**
- ◆ **if alternate selectivity is required, try a different surface chemistry (phase) – based on an ultra high purity silica**
- ◆ **always try to improve selectivity (α) before efficiency (N)**