Rapid HPLC Method Development *with* C18 and Phenyl Columns

Toshi Ono[1], Ken Tseng[1], Tsunehisa Hirose[2], Kazuhiro Kimata[2]
[1]Nacalai USA, Inc., San Diego, United States, [2]Nacalai Tesque, Inc., Kyoto, Japan

Introduction

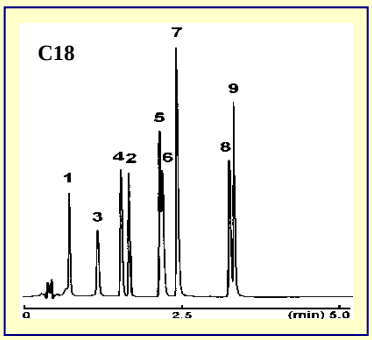
When developing separation methods, the use of orthogonal selectivity in the HPLC column screening is an effective way for choosing the right column. We propose a configuration of C18 column in acetonitrile/water and a phenyl column in methanol/water for the initial screening. Depending on how well the samples are separated, the experimental parameters can be further refined by adjusting the mobile phase conditions or using other columns. When the C18 column with water/acetonitrile works better, columns such as Cholester, C8, or C4 can be used for further refinement. When the phenyl column with water/methanol works better, πNAP or PYE column can be tested. Examples are show in each situation when a column with the right selectivity improves the resolution and minimizes the method development time.

Experimental and Results

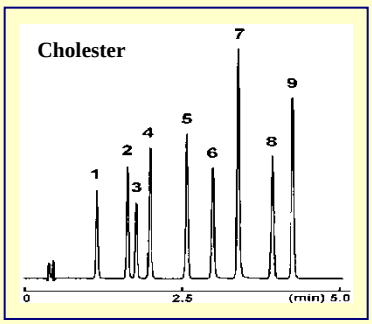
Catechin Mixture

1. Gallocatechin (GC)	(0.40mg/ml)
2. Caffeine	(0.04mg/ml)
3. Epigallocatechin (EGC)	(0.40mg/ml)
4. Catechin (C)	(0.20mg/ml)
5. Epicatechin (EC)	(0.20mg/ml)
6. Epigallocatechin gallate (EGCG)	(0.10mg/ml)
7. Gallocatechin gallate (GCG)	(0.20mg/ml)
8. Epicatechin gallate (ECG)	(0.10mg/ml)
9. Catechin gallate (CG)	(0.10mg/ml)

C18 column achieved better separations than the phenyl column (not shown)

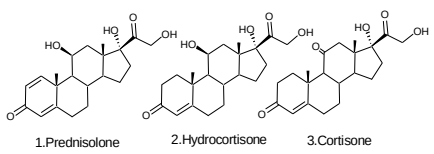


Further refinement with Cholester column achieved baseline separation

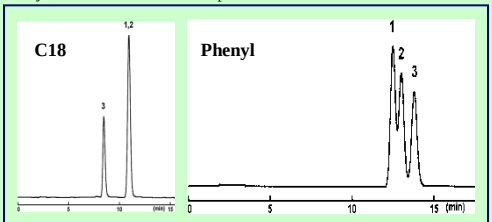


Column size: 3.0 x 75 mm, 2.5µm
Gradient: A: ACN/ 20 mmol/l phosphate (pH 2.5) = 10/90
B: ACN/ 20 mmol/l phosphate (pH 2.5) = 30/70
B: 0→100% 5 min linear gradient
Mixer 0.5 ml
Flow rate: 1.0 ml/min
Temperature: 40°C
Detection: UV 280nm
Injection Vol. 1.0 µl

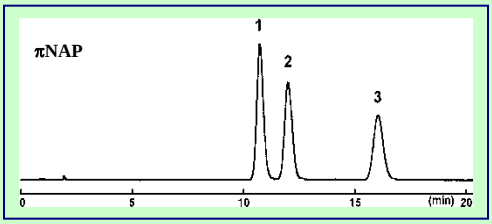
Adrenal Cortical Hormones



Phenyl column achieved better separations than the C18 column

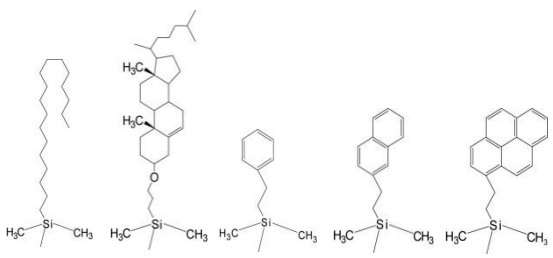


Further refinement with the πNAP column achieved baseline separation

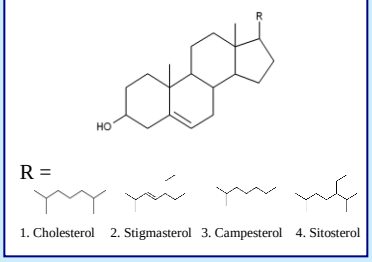


Column size: 4.6 x 150mm
Mobile phase: $5C_{18}$-MS-II Methanol/ H_2O = 50/50
5PE-MS Methanol/ H_2O = 50/50
πNAP Methanol/ H_2O = 60/40
Flow rate: 1.0 ml/min
Temperature: 30°C
Detection: UV254nm

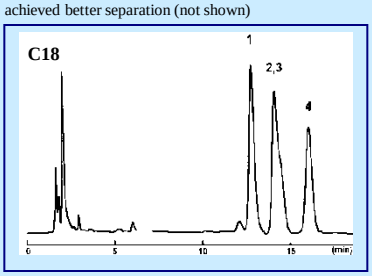
Stationary Phase Functional Groups



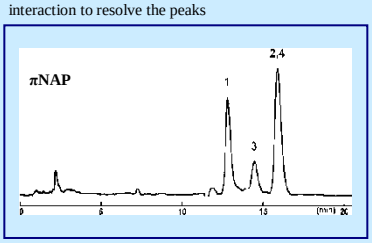
Sterol Mixture



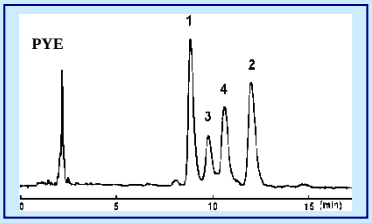
Poor Separation with a C18 column, a phenyl column achieved better separation (not shown)



πNAP column still does not have enough π–π interaction to resolve the peaks



PYE column with its four-ring structure has enough π–π interaction to resolve the peaks



Column size: 4.6 x 150mm
Mobile phase: $5C_{18}$-MS-II Methanol/ H_2O = 98/2
πNAP Methanol/ H_2O = 90/10
5PYE Methanol/ H_2O = 95/5
Flow rate: 1.0 ml/min
Temperature: 30°C
Detection: UV210nm

Conclusions

- Orthogonal selectivity in C18 with acetonitrile/water and phenyl with methanol/water allows good initial screening
- Cholester, πNAP, and PYE columns from Nacalai Cosmosil provide unique selectivity for further method refinement
- Better resolution and minimal method development time were achieved in the three examples shown