Selectivity Choices on Superficially Porous Columns

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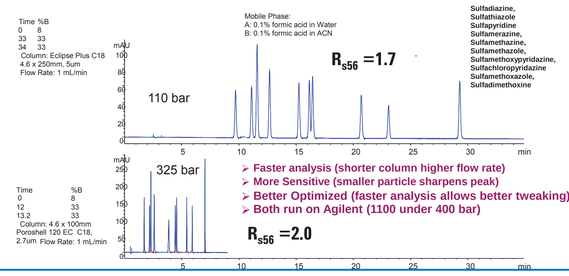
Introduction

Poroshell 120 columns were introduced in 2008. 10 phases have been introduced, allowing easy transition from totally porous to superficially porous particles.

As can be seen below, the highly efficient Poroshell 120 EC-C18 replaces a longer 5 micron column, In this case the separation of 10 compounds is better than the original separation and is complete before the first peak elutes on the longer column. Columns such as Poroshell 120 EC-C18 should be a first choice in separations because they work. However if they don't there are many other phase options.

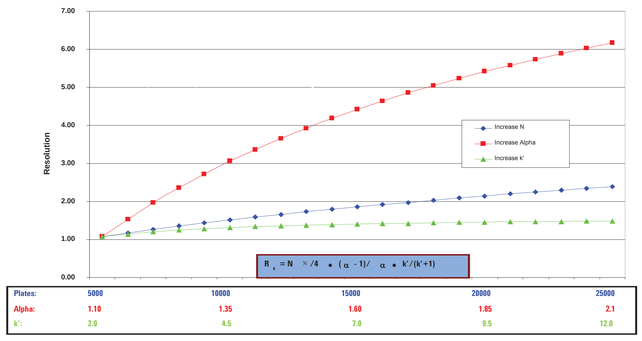
Comparison of 4.6 x 250 mm 5 um to 4.6 x 100 mm, 2.7 µm Poroshell 120 EC-C18



- Faster analysis (shorter column higher flow rate)
- More Sensitive (smaller particle sharpens peak)
- Better Optimized (faster analysis allows better tweaking)
- Both run on Agilent (1100 under 400 bar)

Of all method development parameters, selectivity is the most powerful. As shown in Figure 2 selectivity effects resolution far more than efficiency (driven by particle size)or k' (driven by solvent strength. Changing the column (phase) or changing the mobile phase are the best options.

Typical Method Development Parameters-Effects of Selectivity, Efficiency, and Retention on Resolution



One way of evaluating potential column choices is to use the Hydrophobic Subtraction Model (HSM) The Hydrophobic Subtraction Model data shown below. In this work, 15 compounds are injected in one of two mobile phases (50 % ACN/Buffer) either 60 mM phosphate buffer pH 2.8 or 7.0. Values are determined by fitting to a published model. This model classifies columns by attributes such as Hydrophobicity and Steric Influence. In addition an "F" value can be calculated which estimates the differences between the phases in 5 dimensions.

Hydrophobic Subtraction Model Data from Stoll Lab

Column	H	S*	A	B	C	Ethyl benzene k'	F
Poroshell 120 EC-C18	1.023	0.008	-0.13	-0.004	0.16	6.92	0
Poroshell 120 EC-C8	0.877	0.011	-0.23	0.023	0.13	4.84	12
Poroshell 120 EC-CN	0.421	-0.06	-0.48	0.002	0.045	0.953	15
Poroshell 120 Phenyl Hexyl	0.752	-0.08	-0.39	0.018	0.14	3.59	17
Poroshell 120 Bonus RP	0.686	-0.03	-0.57	0.18	-0.67	3.98	63
Poroshell 120 SB-C18	0.956	-0.04	0.17	0.025	0.21	5.44	21
Poroshell 120 SB-C8	0.726	-0.09	0.068	0.044	0.087	3.56	15
Poroshell 120 SB-AQ	0.581	-0.12	-0.13	0.051	-0.014	2.15	16
Poroshell 120 PFP	0.63	-0.52	-0.52	0.43	-11	2.3	59

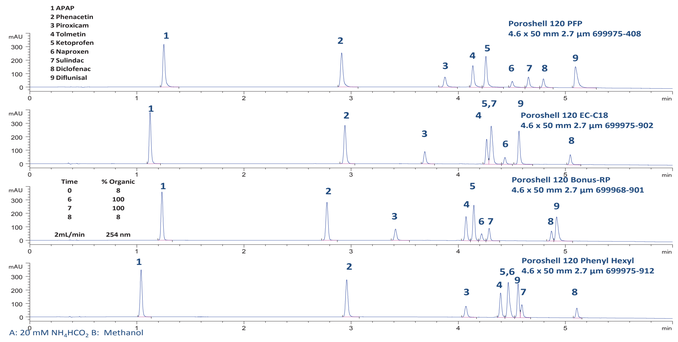
$$Fs=\{[12.5\ (H_2-H_1)]^2+[100(S^*_2-S^*_1)]^2+[30(A_2-A_1)]^2+[143(B_2-B_1)]^2+[83(C_2-C_1)]^2\}^{1/2}$$

Dissimilarity from Poroshell 120 EC-C18*

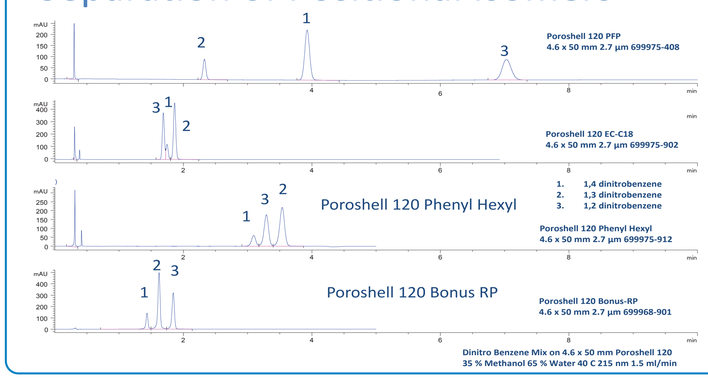
Results and Discussion

Poroshell 120 PFP Using the HSM Data as a starting point we see that Poroshell 120 Bonus RP and Poroshell 120 PFP are the most different from Poroshell 120 EC-C18. While C18 column interactions are predominantly hydrophobic, PFP columns utilize π-π, dipole, and hydrogen bonding interactions. The PFP ring structure imparts π-π interactions, however since the electronegative Fluorine atoms create an electron deficient ring that seeks analytes that are capable of donating electrons, the polar PFP phase also interacts through dipole and hydrogen bonding through the electronegative Fluorine atoms. Positional isomers or Halogenated compounds are ideal candidates for Poroshell 120 PFP.

Analysis of Analgesics

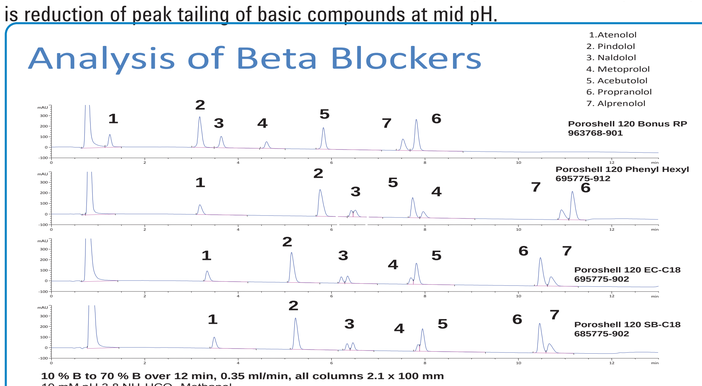


Separation of Positional Isomers



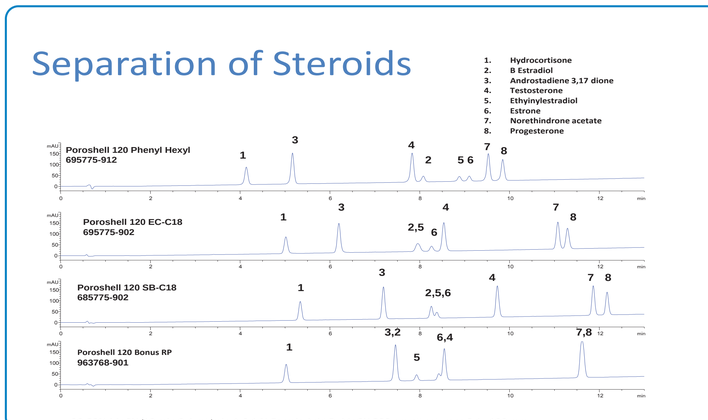
Poroshell 120 Bonus RP, is a Polar embedded column. In this phase the amide group acts a strong H-bond donor, and as such interacts with substituted phenols. The highly polar column can be used with 100 % aqueous mobile phase. And an important property is reduction of peak tailing of basic compounds at mid pH.

Analysis of Beta Blockers



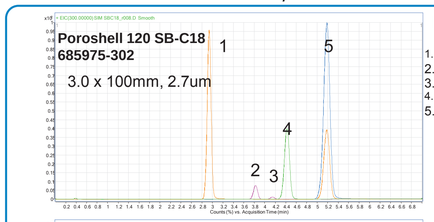
Poroshell 120 Phenyl-Hexyl column primarily interact through π-π interactions. The Phenyl ring structure much like the PFP phase, however unlike the electronegative PFP ring, the Phenyl ring is electron rich. The Phenyl-Hexyl phase also has more hydrophobic interactions than Phenyl propyl phases due to the longer linking chain. The π-π interactions of the Poroshell 120 Phenyl Hexyl phase can be overwhelmed by Acetonitrile.

Separation of Steroids



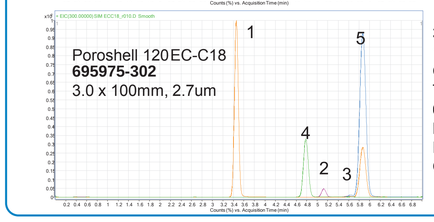
Results and Discussion

Poroshell 120 SB-C18 is an alternative C18 phase. This phase uses a di isopropyl protected C18 without end-capping. This phase is highly resistant to hydrolysis and is recommend when very low pH mobile phases are used. The exposed silanols can also offer some alternative selectivity.



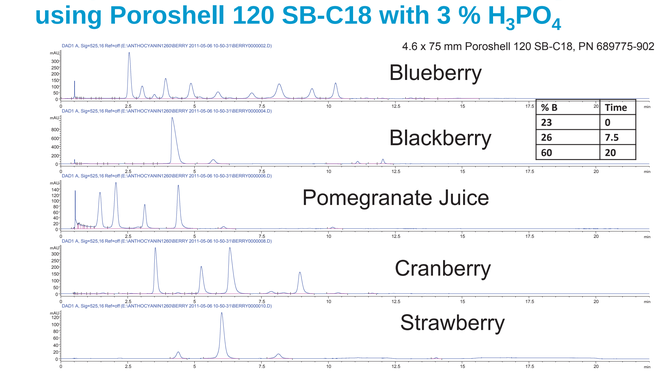
Endocannabinoids

1. Anandamide (AEA)
2. 2-arachidonoylglycerol (2-AG)
3. Impurity
4. Palmitoylethanolamide (PEA)
5. Oleoylethanolamide (OEA)



35/65 Water/ ACN
1 mL/min
Columns: 3 x 100 mm,
TCC: 30 C
6410 QQQ
MS Source Gas Temp 350 C
Flow 12L/min Nebulizer 40 psi
Capillary Voltage = 4000V

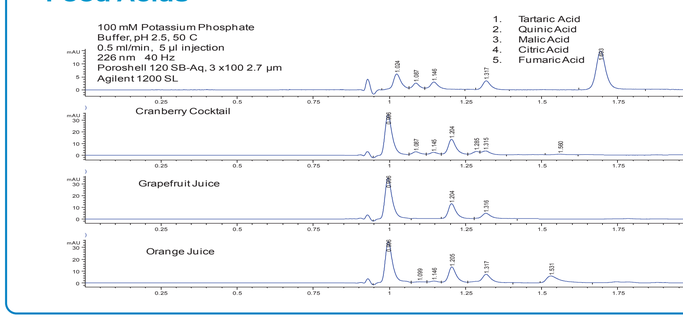
Analysis of various Anthocyanin Containing Fruits using Poroshell 120 SB-C18 with 3 % H_3PO_4



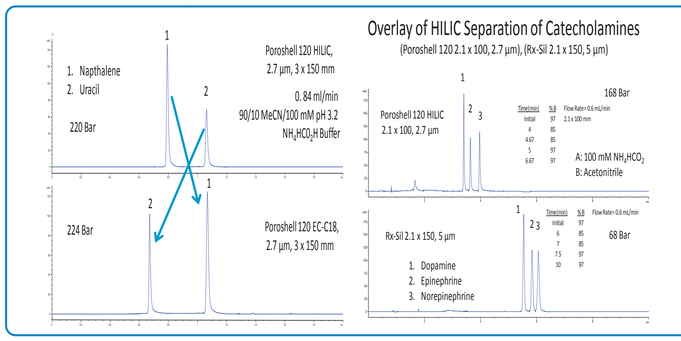
Various Anthocyanin Containing Samples using Poroshell 120 SB-C18 of varied samples using a H_3PO_4 gradient

Poroshell 120 SB-Aq can be used with 100 % aqueous mobile phases. Like the other StableBond phases, it is useful at low pH, for example,. TFA or Perchloric acid mobile phases. Using low pH mobile phase will allow acidic compounds to be fully protonated and allow them to be better retained.

Food Acids



Poroshell 120 HILIC column offers an alternative selectivity to reverse phase columns. They are especially useful in separating difficult to retain polar compounds. The mobile phases used in HILIC separations are high in organic phase and can allow more sensitive LC MS analyses.



Conclusions

A wide range of Poroshell Phases are available
- First choice for method development should be Poroshell 120 EC-C18 It works!
- HSM model suggests next method development choice should be Poroshell 120 Bonus RP or Poroshell 120 PFP

Thank you to Professor Dwight Stoll of Gustavus Augustus College for the HSM Data