New Reversed-Phase SPP Columns with Alternate Selectivity for Small Molecules

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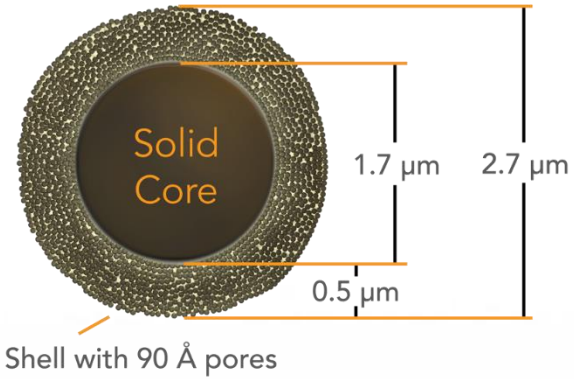
Outline

1. Description of Fused-Core® SPP silica for small molecule HPLC.
2. HALO® AQ-C18: designed with adequate polarity for pores to stay wet in pure water; selectivity toward highly polar analytes is altered very slightly.
3. HALO® Biphenyl: designed with increased aromatic character to improve retention and selectivity for polar analytes over less polar phases like Phenyl-Hexyl.
4. Discussion of column selectivity concepts, Fs equivalency values, and the USP/PQRI database.
5. Selected applications to illustrate and compare selectivity.

The Pioneering Shell Particle for Small Molecules

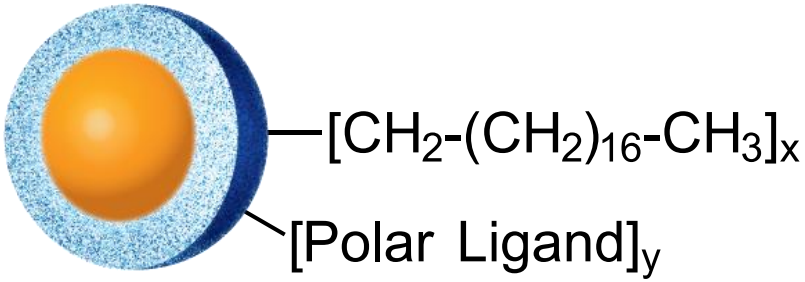
Fused-Core® Specifications:



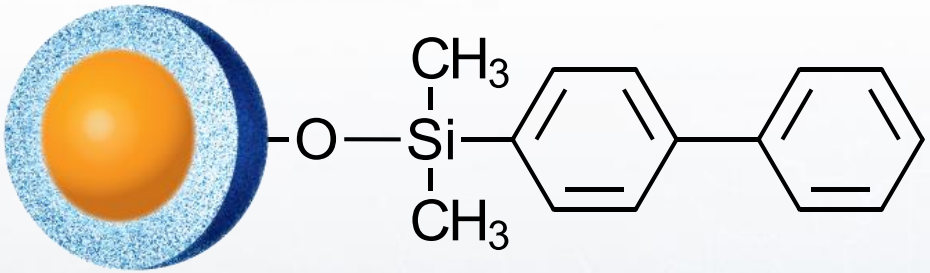
- Superficially Porous (SPP Type)
- Type B silica (highly pure and low acidity)
- Available in 2, 2.7, and 5 µm total size*
- Porous shell with controlled thickness for short diffusion paths
- 90 Å pores for unrestricted diffusion and high surface area for good retention and high loadability of small molecules

* 2 and 5 µm Biphenyl available in 2019

New HALO® AQ-C18 and Biphenyl Phases



AQ-C18



Biphenyl

- Designed to allow phase pores to stay wet in low organic and to add a small, reproducible polar selectivity component
- Designed to change aromatic/aliphatic content and alter selectivity for many polar analytes

F_s Values for 90 Å HALO® Phases vs HALO® C18

Columns shown in descending order of hydrophobicity, H.

F_s	Column	H	S*	A	B	C (pH 2.8)	C (pH 7.0)	ethylbenzene k
0	HALO C18	1.1	0.04	0	-0.05	0.05	0.04	6.1
12.07	HALO AQ-C18	1	-0.036	0.099	-0.048	0.156	0.864	6.7
10.04	HALO C8	0.91	0.02	-0.13	0	-0.01	0.18	4.3
52.83	HALO RP-Amide	0.85	0.08	-0.38	0.19	-0.41	0.31	4.6
17.35	HALO Phenyl-Hexyl	0.78	-0.09	-0.23	0	0.1	0.45	3.5
26.76	HALO Biphenyl	0.708	-0.183	-0.279	0.028	0.047	0.99	3.1
94.45	HALO PFP	0.702	-0.117	-0.073	-0.062	1.17	0.972	2.3
22.78	HALO ES-CN	0.566	-0.11	-0.344	0.021	0.126	1.15	1.88

$$F_s = \sqrt{\left(w_{\mathbf{H}}(\mathbf{H}_1 - \mathbf{H}_2)\right)^2 + \left(w_{\mathbf{S}^*}(\mathbf{S}^*_1 - \mathbf{S}^*_2)\right)^2 + \left(w_{\mathbf{A}}(\mathbf{A}_1 - \mathbf{A}_2)\right)^2 + \left(w_{\mathbf{B}}(\mathbf{B}_1 - \mathbf{B}_2)\right)^2 + \left(w_{\mathbf{C}_{2.8}}(\mathbf{C}_{2.8_1} - \mathbf{C}_{2.8_2})\right)^2}$$

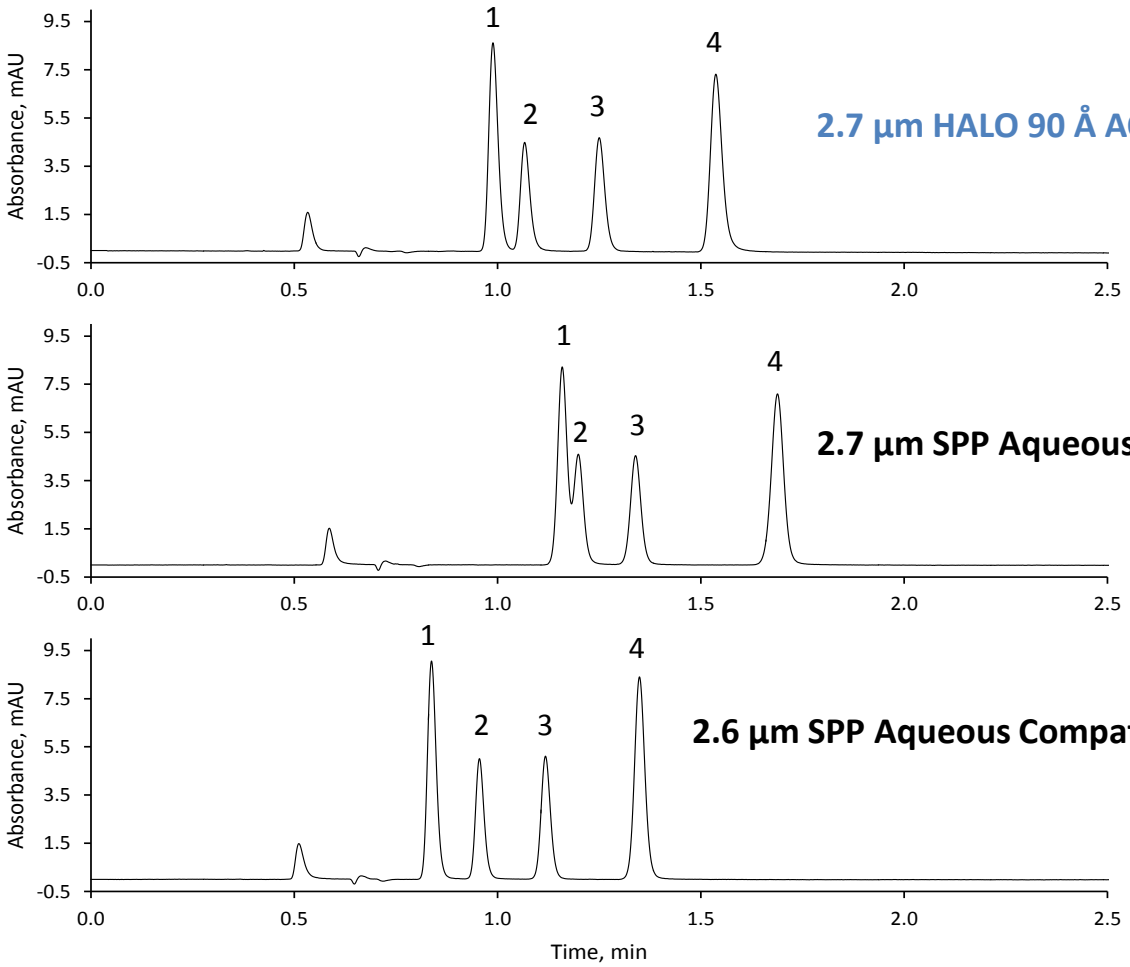
Data provided online by Stoll group at Gustavus Adolphus College. www.hplccolumns.org

Overview of Two New HALO® HPLC Columns

- HALO® AQ-C18
  - Maintains L1 column classification (>90% C18 character).
  - Complements HALO® C18 (in ease of use, stability and reproducibility).
  - Moderate change in retention and selectivity for many polar analytes.
  - Useful in both methanol and acetonitrile mobile phases.
  - Extends mobile phase range to 100% aqueous and low organic for polar analytes, including gradients.
- HALO® Biphenyl
  - Maintains L11 USP column classification (phenyl aromatic phase).
  - Complements HALO® Phenyl-Hexyl with alternate selectivity.
  - Shows significant selectivity differences for polar and aromatic analytes when compared to phenyl phases with alkyl character.
  - Very useful in both methanol and acetonitrile mobile phases.

Purines on Aqueous-Compatible C18 SPP Phases

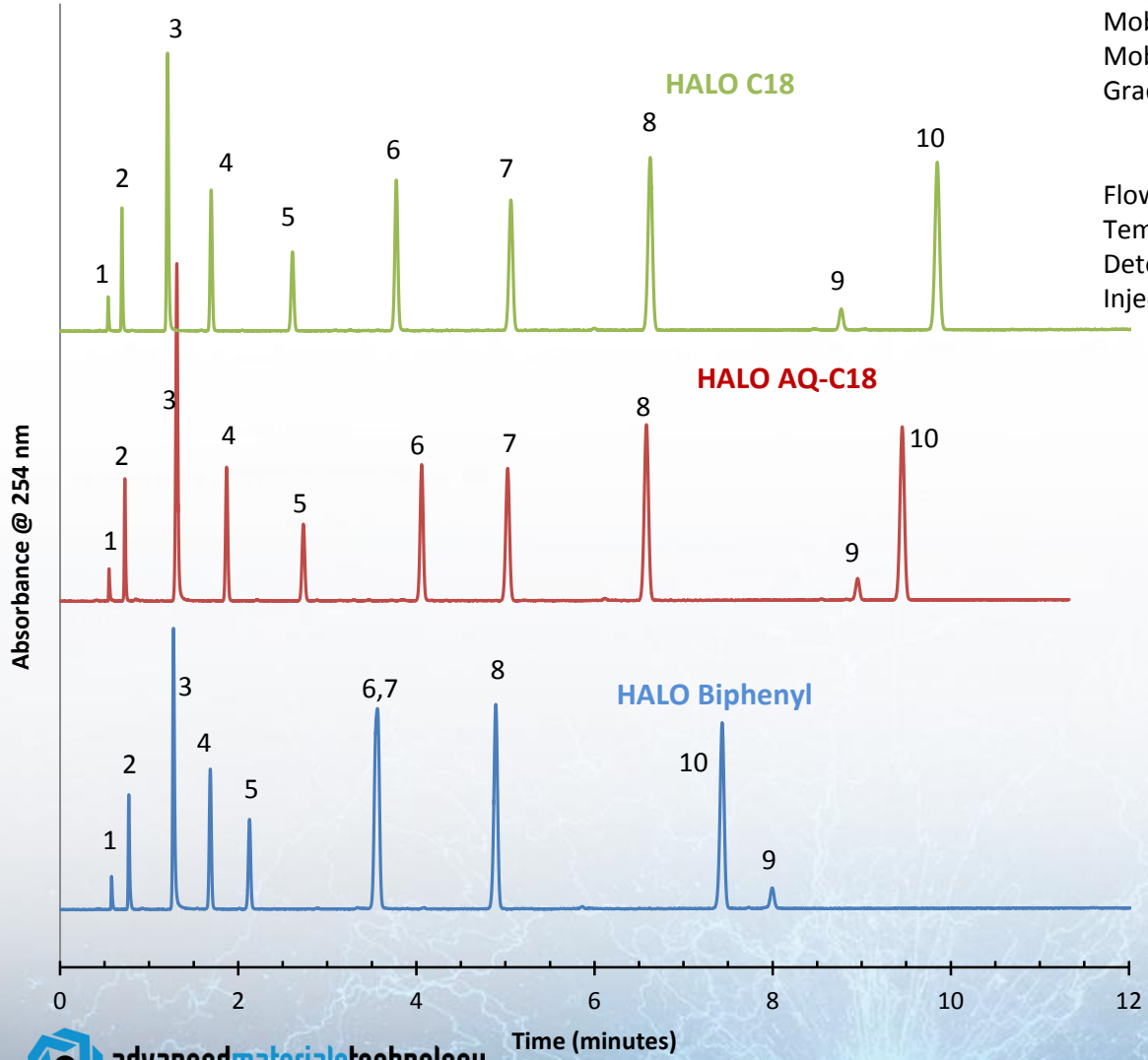
2.1 x 100 mm, 0.1% DFA in water, 0.35 mL/min, 35 °C, 1 µl @ 10 mM each in 0.1% DFA, UV detection @ 265 nm



1. Guanine
2. Hypoxanthine
3. Uracil
4. Xanthine

Comparative results presented here may not be representative for all applications.

Comparison of Aromatic Test Mix in Acetonitrile



Columns: HALO 90 Å C18, 2.7 µm; HALO 90 Å AQ-C18, 2.7 µm; HALO 90 Å Biphenyl, 2.7 µm; all 4.6 x 100 mm
Mobile Phase A: water
Mobile Phase B: ACN
Gradient:

Time	%B
0	40
15	90

Flow Rate: 1.5 mL/min
Temperature: 30 ° C
Detection: UV 254 nm, VWD
Injection Volume: 2 µL

Peak Identities:

1. uracil
2. resorcinol
3. 4-nitrophenol
4. benzonitrile
5. anisole
6. trans-stilbene
7. valerophenone
8. biphenyl
9. impurity
10. o-terphenyl

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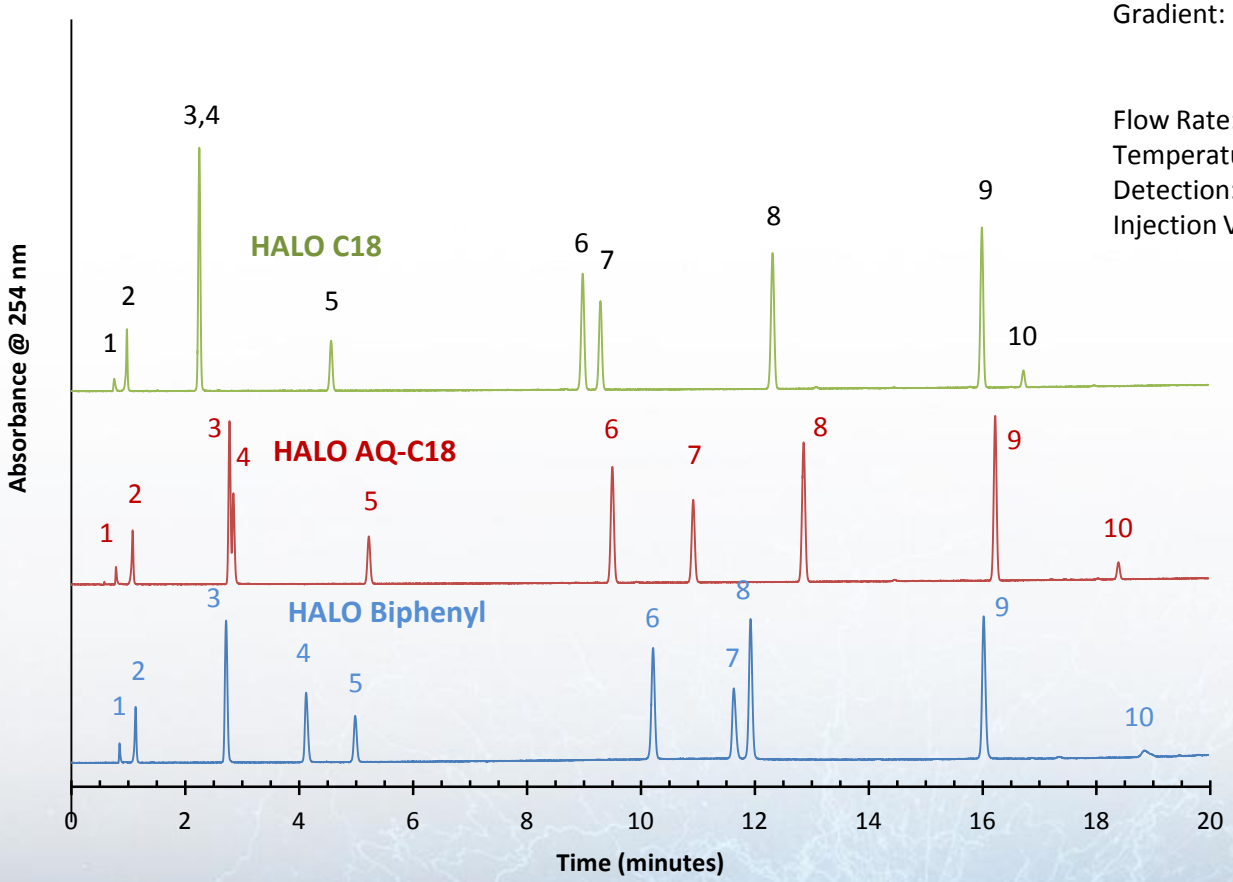
Comparison of Aromatic Test Mix in Methanol



Columns: HALO 90 Å C18, 2.7 µm; HALO 90 Å AQ-C18, 2.7 µm; HALO 90 Å Biphenyl, 2.7 µm; all 4.6 x 100 mm
Mobile Phase A: water
Mobile Phase B: methanol

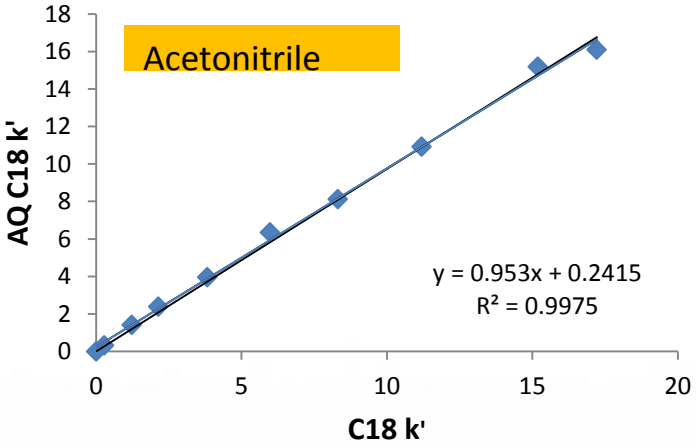
Gradient:	Time	%B
	0	45
	20	55

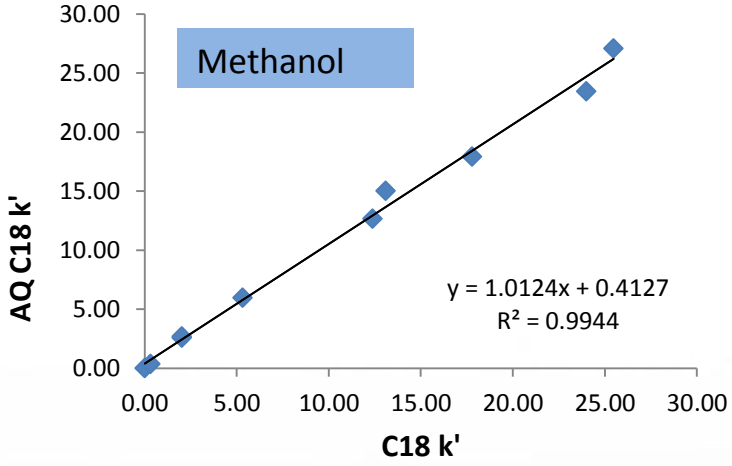
Flow Rate: 1.2 mL/min
Temperature: 30 ° C
Detection: UV 254 nm, VWD
Injection Volume: 2 µL

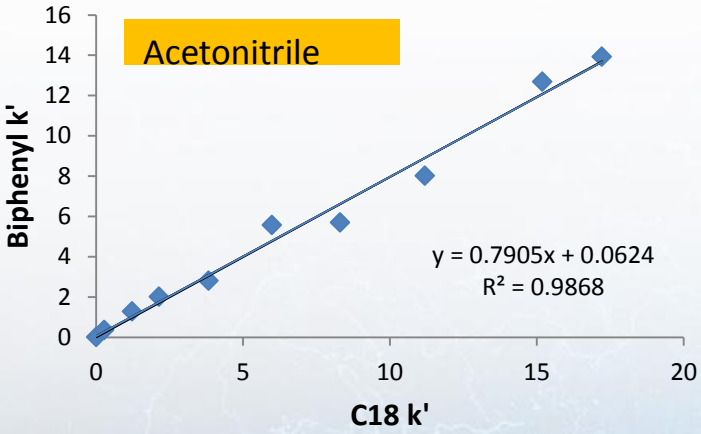
Peak Identities:

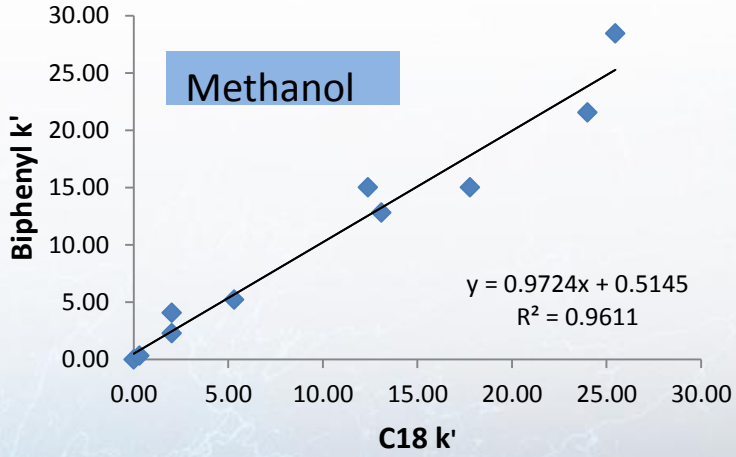
1. uracil
2. resorcinol
3. 4-nitrophenol
4. benzonitrile
5. anisole
6. valerophenone
7. trans-stilbene
8. biphenyl
9. o-terphenyl
10. impurity

Selectivity Comparison for Aromatic Test Mix



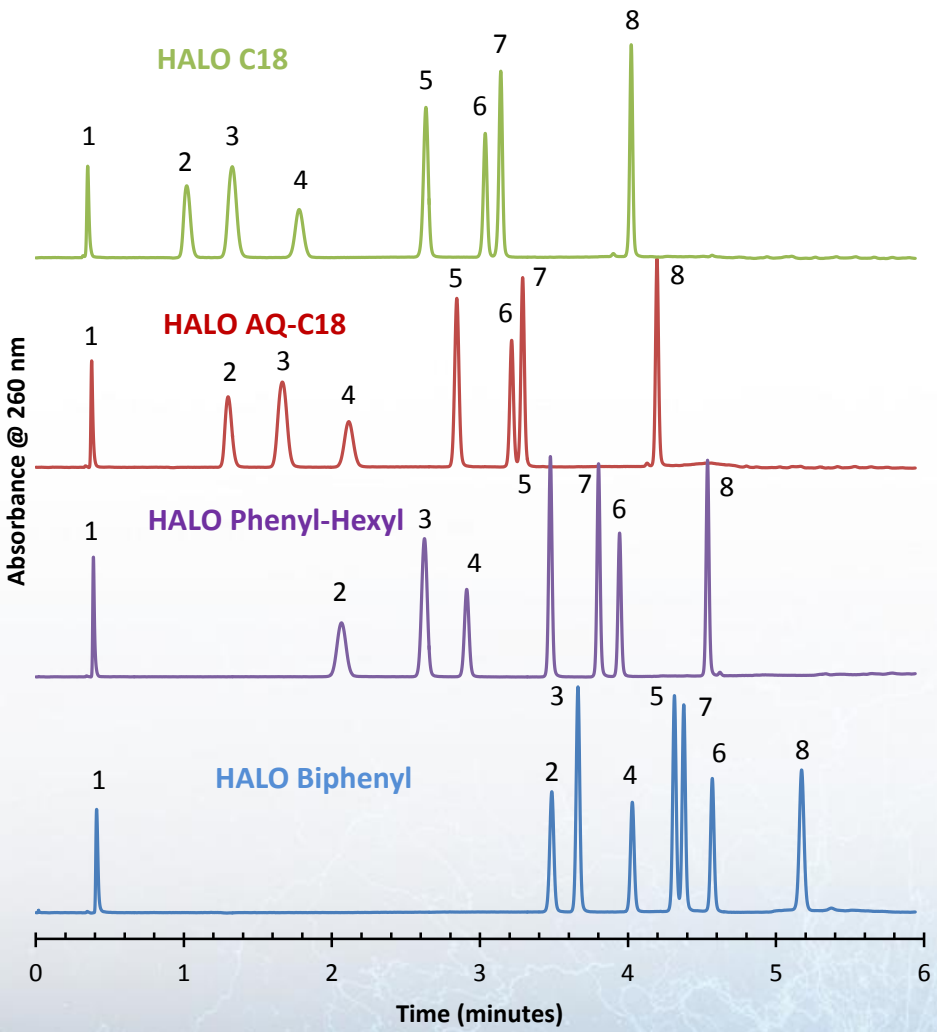






See previous two slides for conditions

Comparison of Sulfa Drugs on Four Phases



Columns: HALO 90 Å C18, 2.7 µm; HALO 90 Å AQ-C18, 2.7 µm; HALO 90 Å Phenyl-Hexyl, 2.7 µm; HALO 90 Å Biphenyl, 2.7 µm; all 3.0 x 100 mm
Mobile Phase A: 20 mM Ammonium Formate, pH 3
Mobile Phase B: methanol

Gradient:

Time	%B
0	12
1.5	13
4.0	50
6.0	60

Flow Rate: 0.65 mL/min
Temperature: 30 ° C
Detection: UV 260 nm, VWD
Injection Volume: 1 µL

Peak Identities:

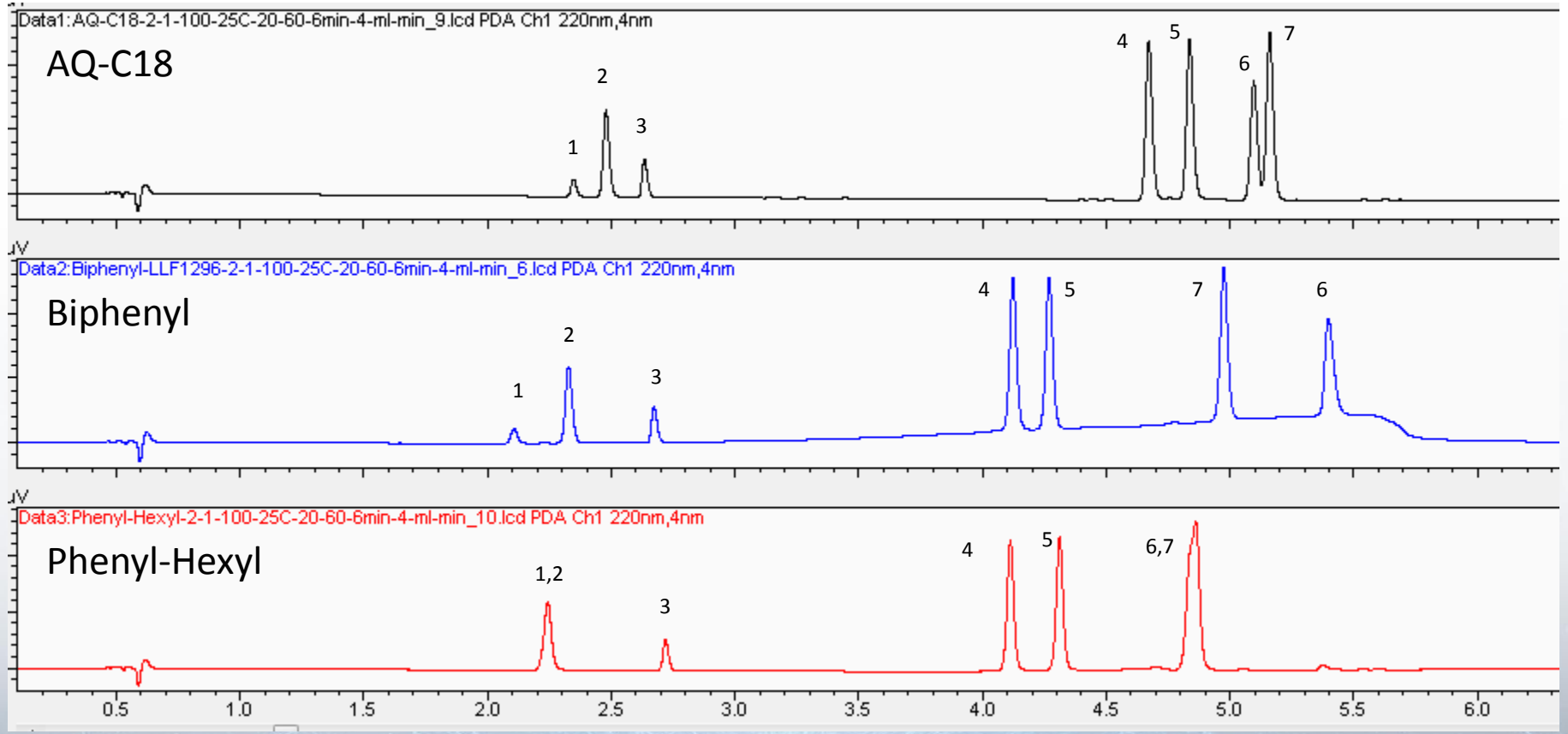
1. uracil
2. sulfadiazine
3. sulfathiazole
4. sulfamerazine
5. sulfamethizole
6. sulfachloropyridazine
7. sulfamethoxazole
8. sulfadimethoxin

Estrogen Steroid Separation on Three Phases

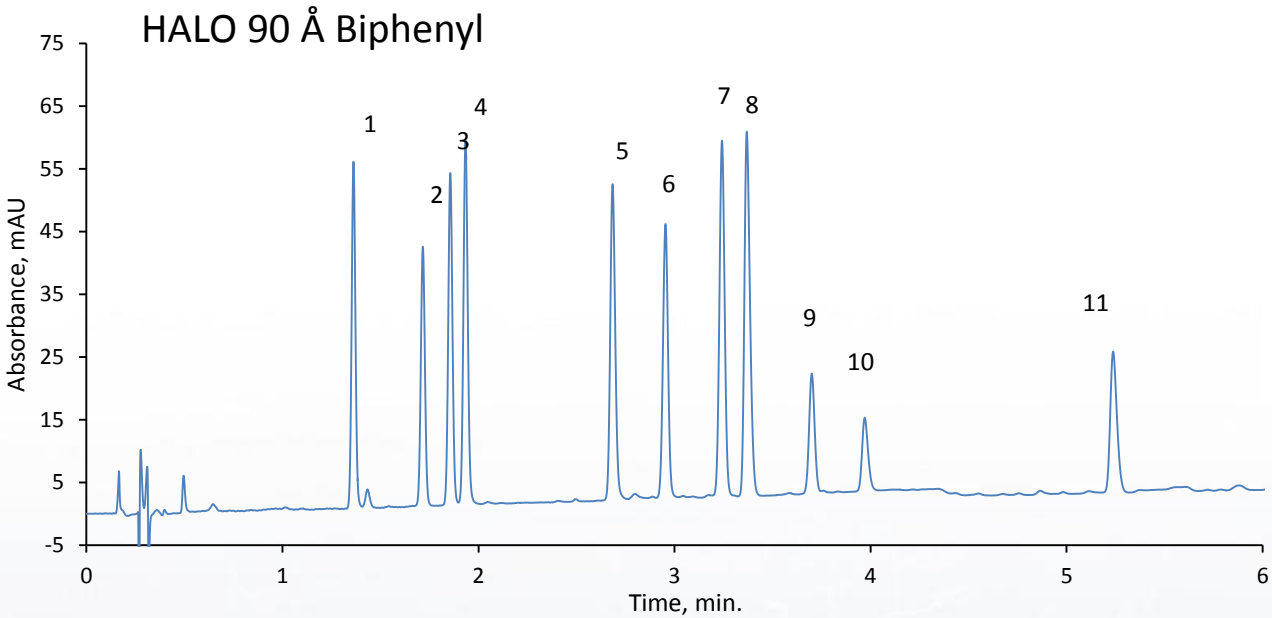
Mobile Phase A: 10mM Potassium Phosphate pH: 7
B: Acetonitrile
Gradient: 20-60%B in 6 min.
Instrument: Nexera
Wavelength: 220nm

Injection: 1 µl
Temperature: 25°C
Flow Rate: 0.4 mL/min.
Column: 2.1x100mm, 2.7µm

Peak Identities
1. B-Estradiol-3-sulfate
2. Estriol
3. Estrone-3-sulfate
4. B-estradiol
5. A-estradiol
6. Androstenedione
7. Estrone



Steroid Separation on HALO® Biphenyl



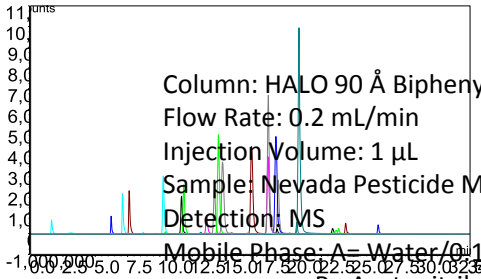
PEAK IDENTITIES:

1. Estriol
2. Hydrocortisone
3. Prednisone
4. Cortisone
5. Corticosterone
6. β-Estradiol
7. Cortisone Acetate
8. Testosterone
9. 17-α-Hydroxyprogesterone
10. 11-Deoxycorticosterone
11. Progesterone

Column: HALO 90Å Biphenyl, 2.7 µm, 4.6 x 50mm
Mobile Phase A: Water
Mobile Phase B: Acetonitrile
Gradient: 20-60% B in 6 minutes
Flow Rate: 1.85 mL/min
Initial Pressure: 344 bar
Temperature: 30 °C
Detection: UV 215 nm, PDA
Injection Volume: 4 µL
Sample Solvent: Acetonitrile:water, 37.5:62.5

LC-MS of Pesticides on Biphenyl

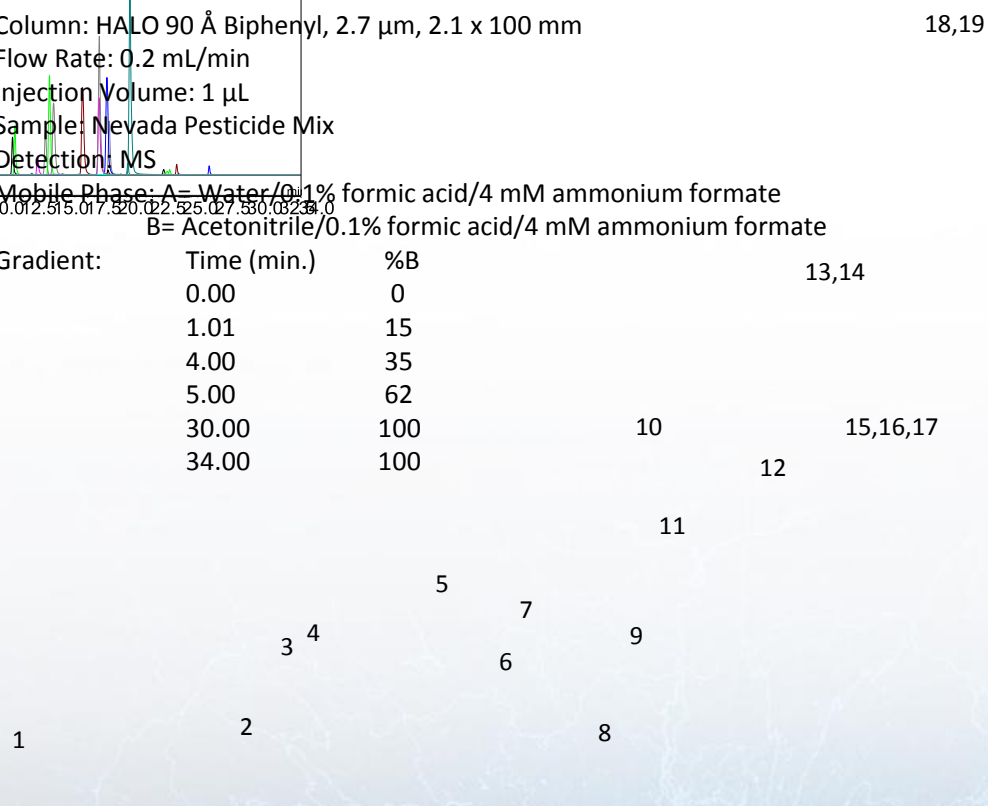
HALO® Biphenyl



Column: HALO 90 Å Biphenyl, 2.7 µm, 2.1 x 100 mm
Flow Rate: 0.2 mL/min
Injection Volume: 1 µL
Sample: Nevada Pesticide Mix
Detection: MS
Mobile Phase: A= Water/0.1% formic acid/4 mM ammonium formate
B= Acetonitrile/0.1% formic acid/4 mM ammonium formate

Gradient:

Time (min.)	%B
0.00	0
1.01	15
4.00	35
5.00	62
30.00	100
34.00	100



PEAK IDENTITIES

1. Daminozide
2. Flonicamid
3. Thiamethoxam
4. Imidacloprid
5. Paclobutrazol
6. Fenhexamid
7. Myclobutanil
8. Bifenazate
9. Dimethomorph Isomer 1
10. Pirotetramat
11. Dimethomorph Isomer 2
12. Spinosad A
13. Spinosad D
14. Trifloxystrobin
15. Spinetoram
16. Pyrethrin II
17. Piperonyl butoxide
18. Pyrethrin I
19. Etoxazole
20. Abamectin A
21. Cypermethrin
22. Bifenthrin
23. Acequinocyl

Summary and Conclusions

- HALO® AQ-C18 allows exploration of a wider solvent range starting with low organic or even 100% aqueous without concern for stationary phase dewetting; major change in C18 selectivity or retention is unlikely because the C18 character greatly dominates.
- Both MeCN and MeOH should be evaluated when HALO® AQ-C18 and Biphenyl columns are selected for method development; closely separated analytes with polar character may increase in resolution and in some cases may even change elution order in MeOH.
- HALO® Biphenyl with dominant aromatic character shows enhanced interactions with polar groups compared to either C18 or AQ-C18 and has even greater aromatic character than HALO® Phenyl-Hexyl, which has about equal aliphatic and aromatic content.

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