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

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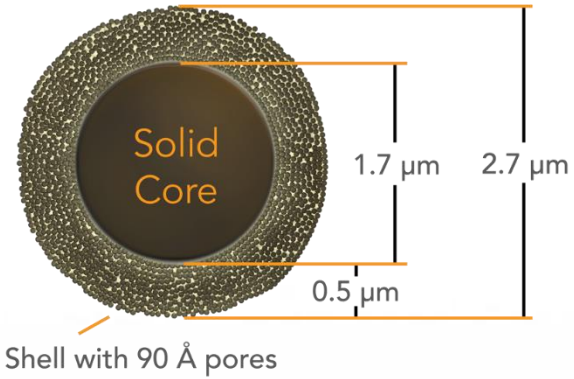
# Outline

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

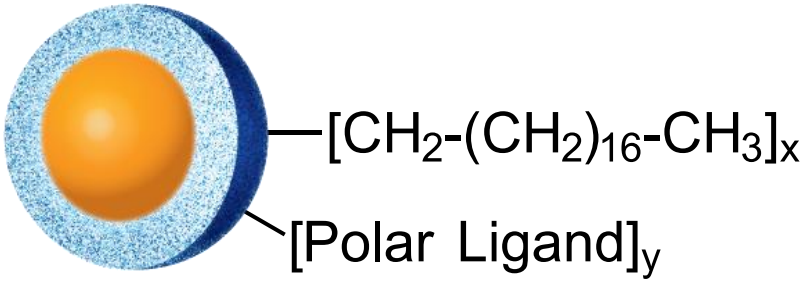
## Fused-Core® Specifications:



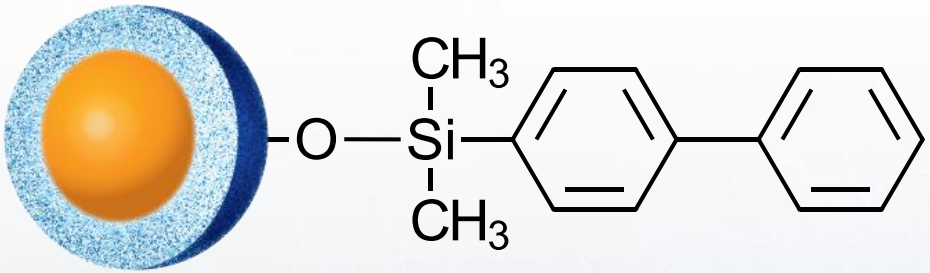

- Superficially Porous (SPP Type)
- Type B silica (highly pure and low acidity)
- Available in 2, 2.7, and 5 µm total size*
- Porous shell with controlled thickness for short diffusion paths
- 90 Å pores for unrestricted diffusion and high surface area for good retention and high loadability of small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

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

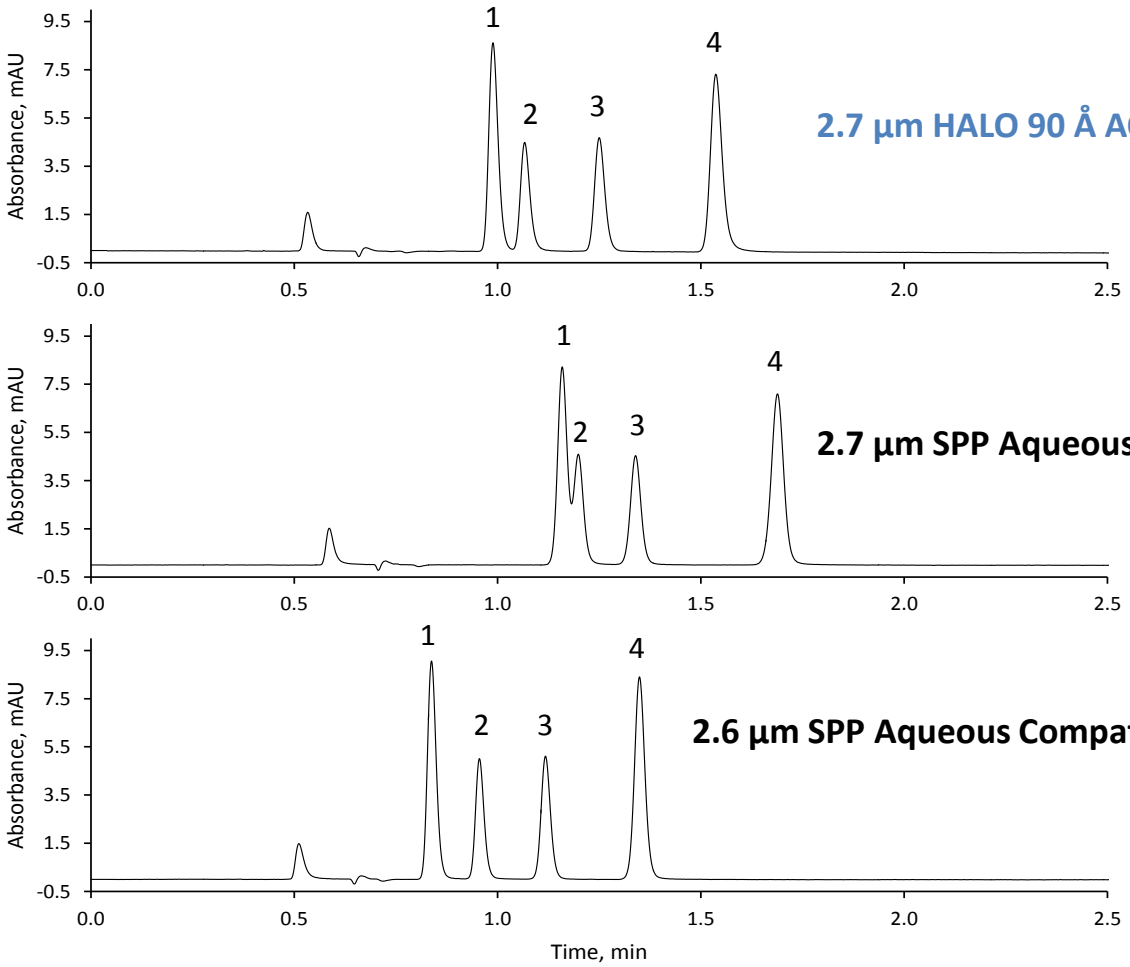
Data provided online by Stoll group at Gustavus Adolphus College. www.hplccolumns.org

# 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

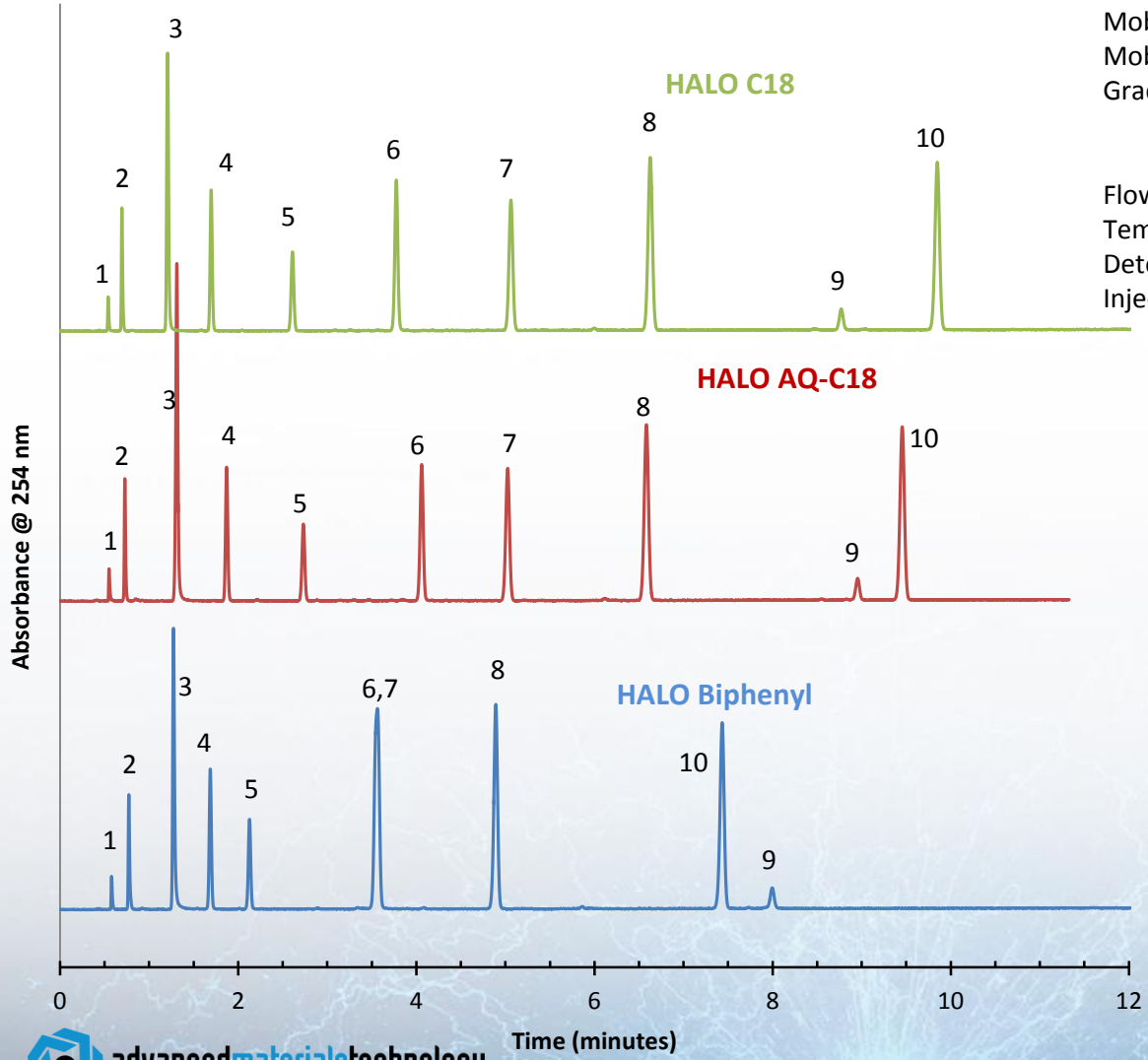
2.1 x 100 mm, 0.1% DFA in water, 0.35 mL/min, 35 °C, 1 µl @ 10 mM each in 0.1% DFA, UV detection @ 265 nm




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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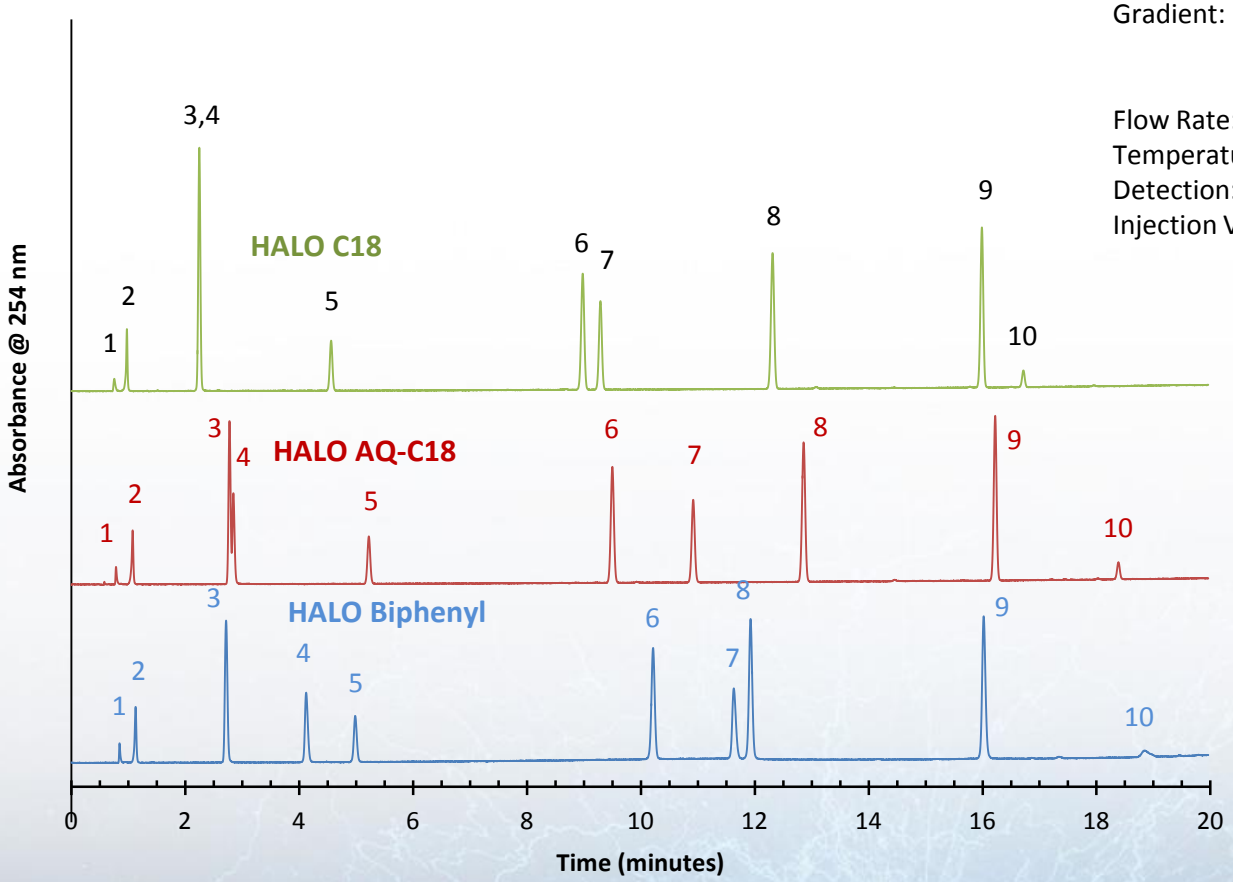
# Comparison of Aromatic Test Mix in Methanol




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

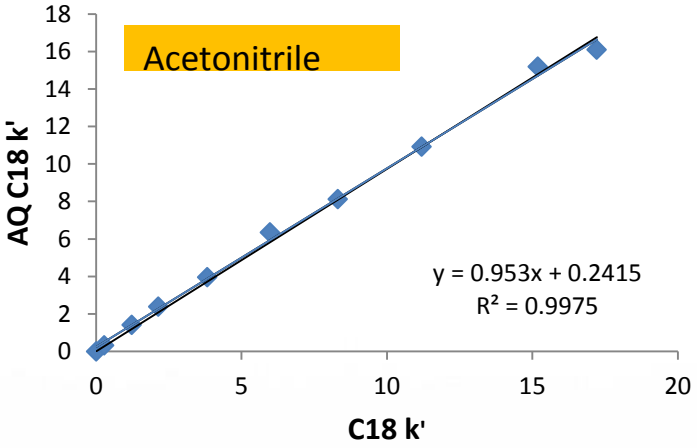
| Gradient: | Time | %B |
|---|---|---|
| | 0 | 45 |
| | 20 | 55 |

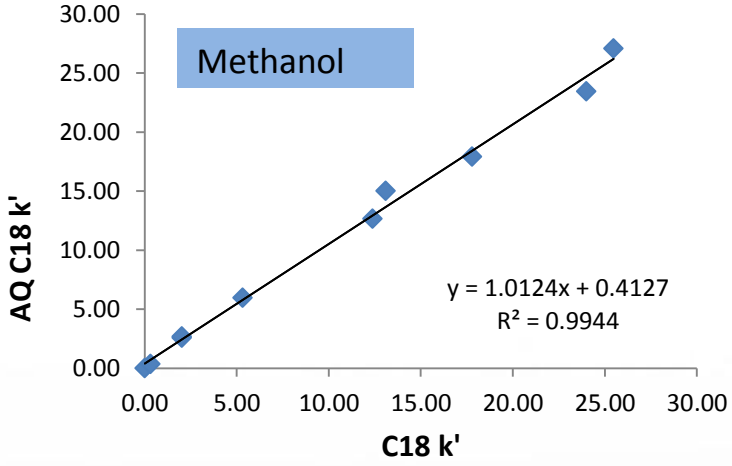
Flow Rate: 1.2 mL/min
Temperature: 30 ° C
Detection: UV 254 nm, VWD
Injection Volume: 2 µL

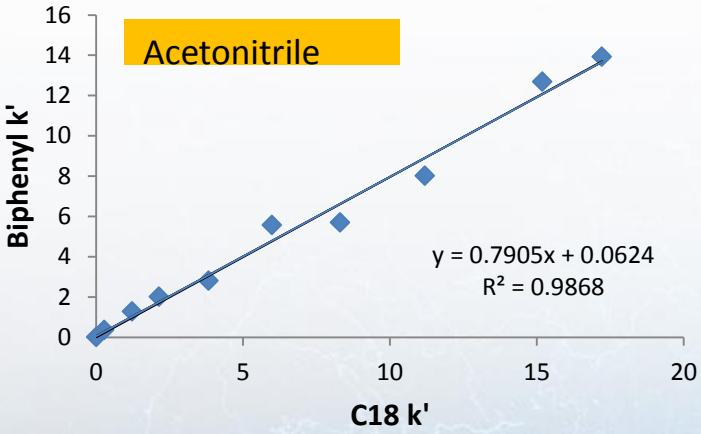
Peak Identities:

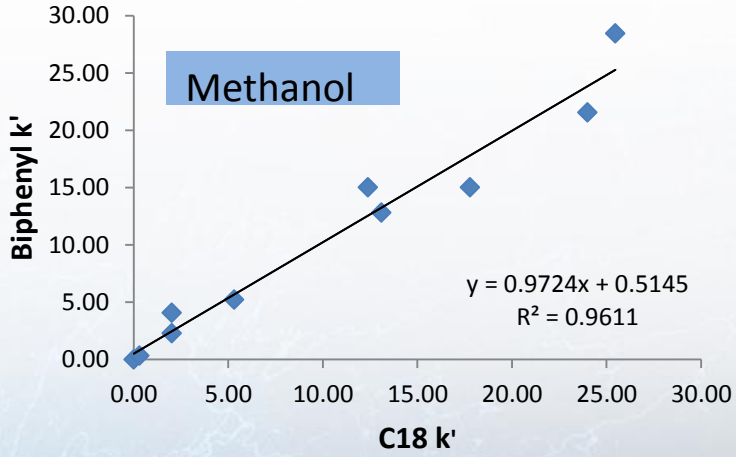
1. uracil
2. resorcinol
3. 4-nitrophenol
4. benzonitrile
5. anisole
6. valerophenone
7. trans-stilbene
8. biphenyl
9. o-terphenyl
10. impurity

# Selectivity Comparison for Aromatic Test Mix



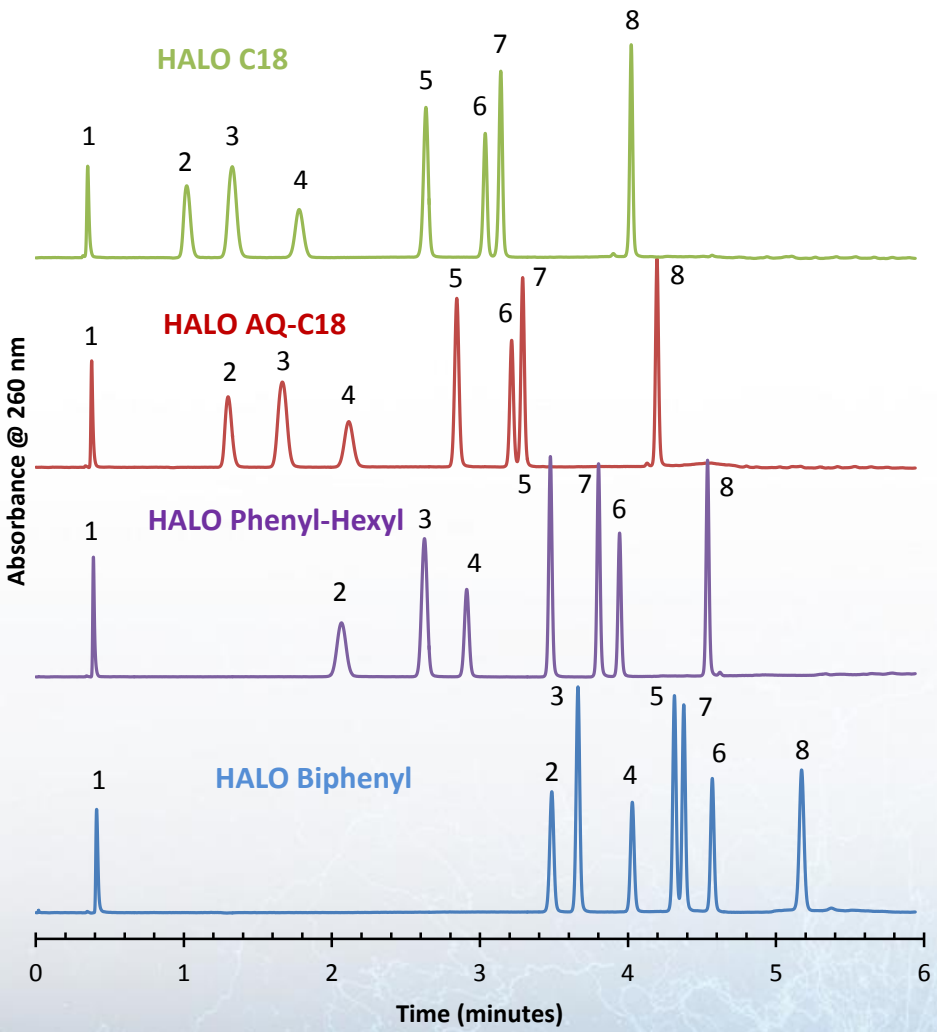




See previous two slides for conditions

# 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:

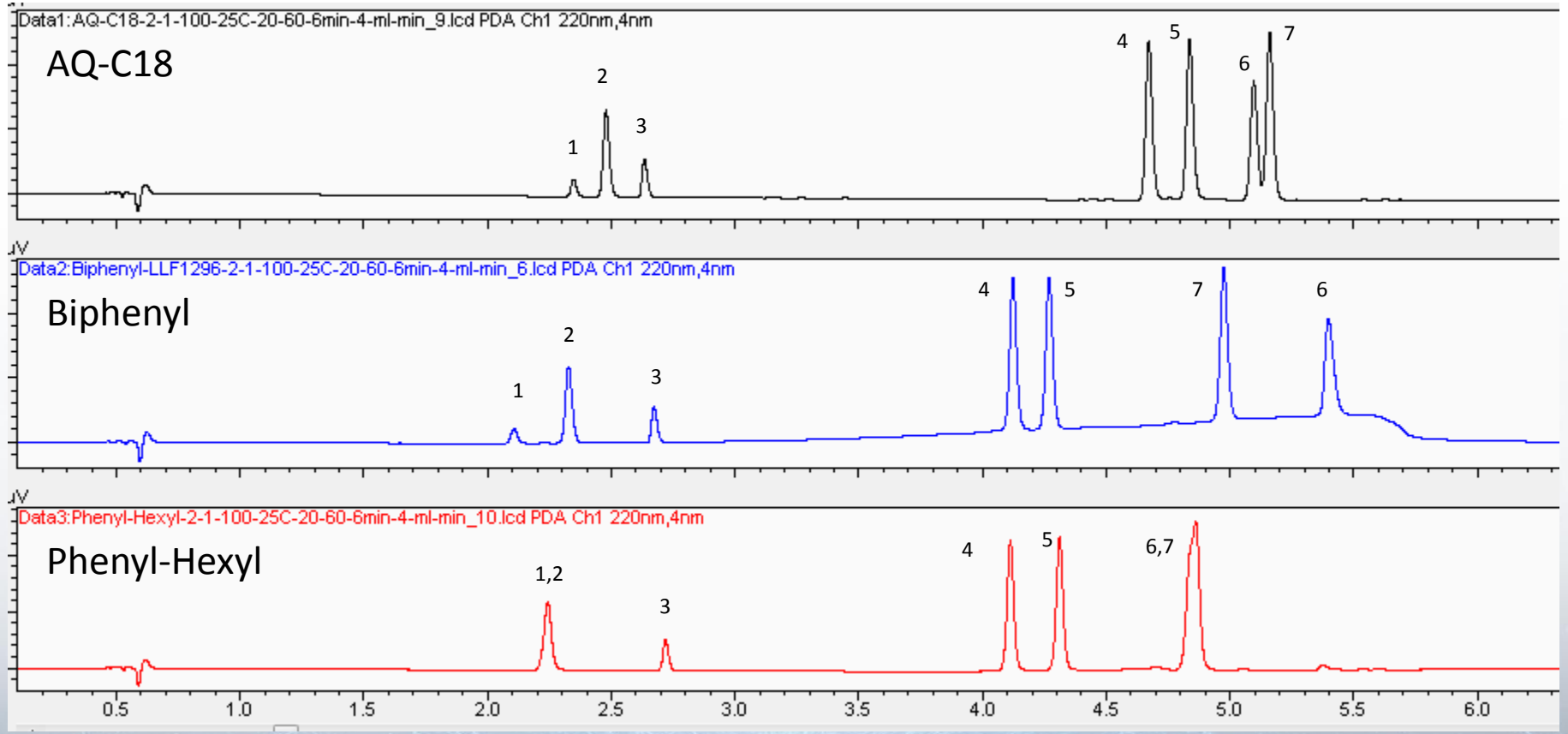
1. uracil
2. sulfadiazine
3. sulfathiazole
4. sulfamerazine
5. sulfamethizole
6. sulfachloropyridazine
7. sulfamethoxazole
8. sulfadimethoxin

# Estrogen Steroid Separation on Three Phases

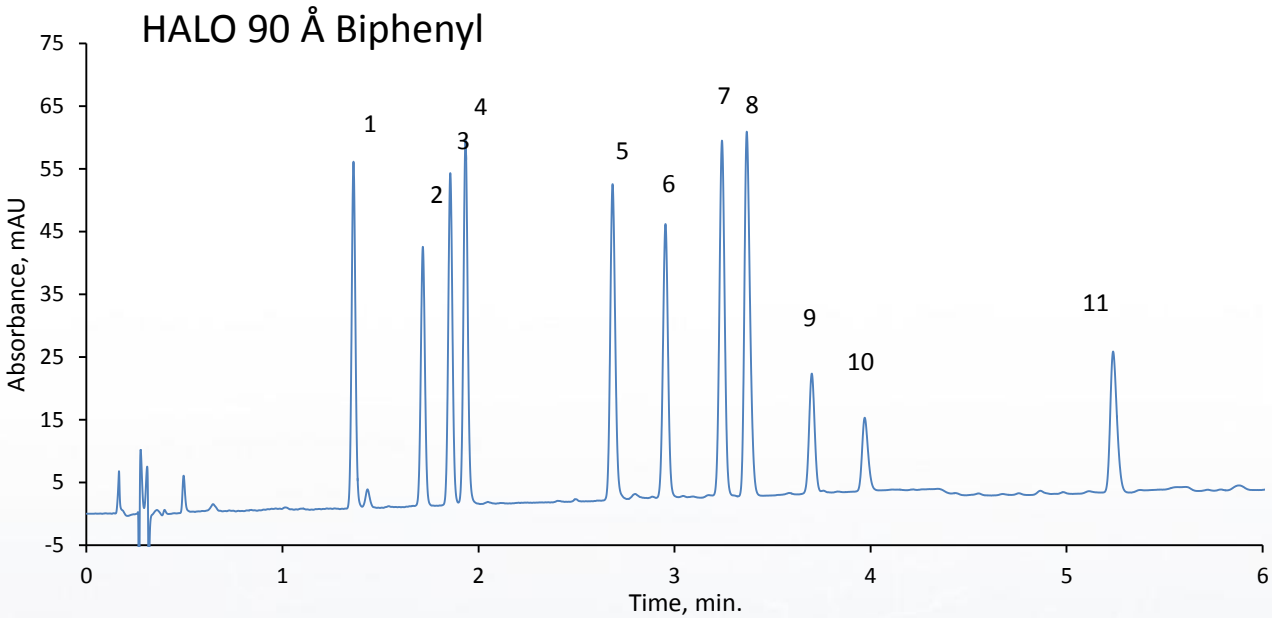
Mobile Phase A: 10mM Potassium Phosphate pH: 7
B: Acetonitrile
Gradient: 20-60%B in 6 min.
Instrument: Nexera
Wavelength: 220nm

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



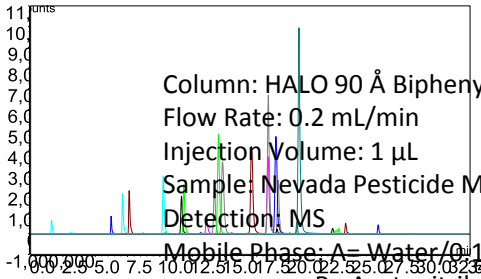

PEAK IDENTITIES:

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

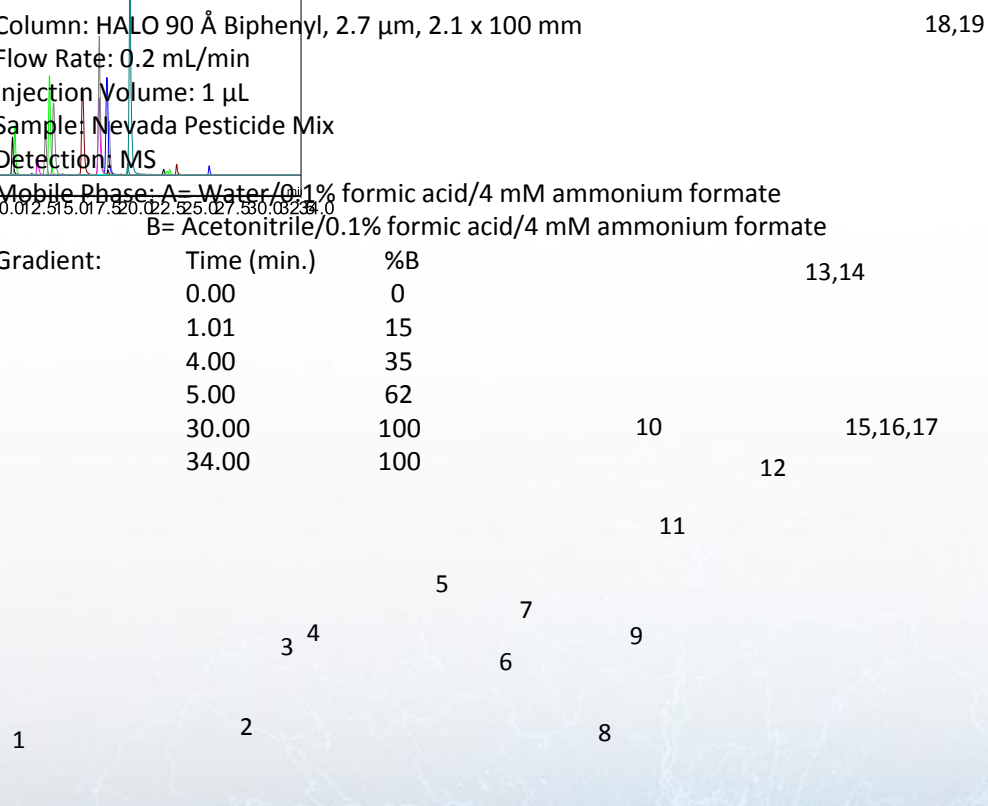
## HALO® Biphenyl



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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