

Increasing Sensitivity in HPLC

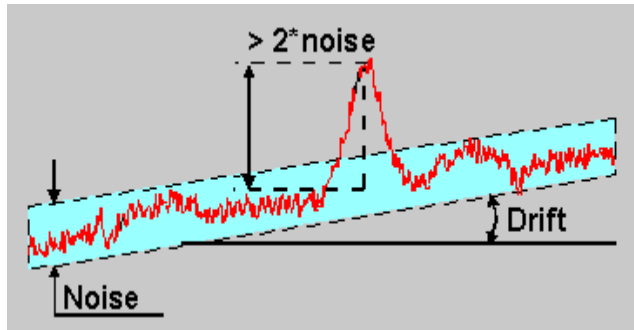
Dr. Shulamit Levin
Medtechnica

levins@medtechnica.co.il
shulal@zahav.net.il

www.forumsci.co.il/HPLC

Noise and drift

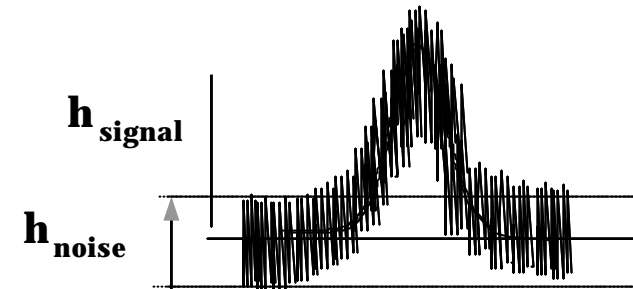
- Noise, drift, and smallest detectable peak.



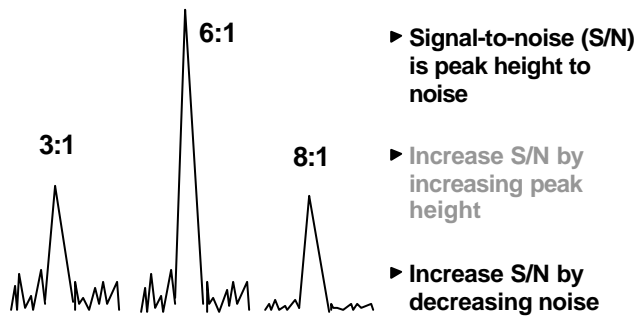
PROPERTIES OF DETECTORS

DETECTION LIMIT

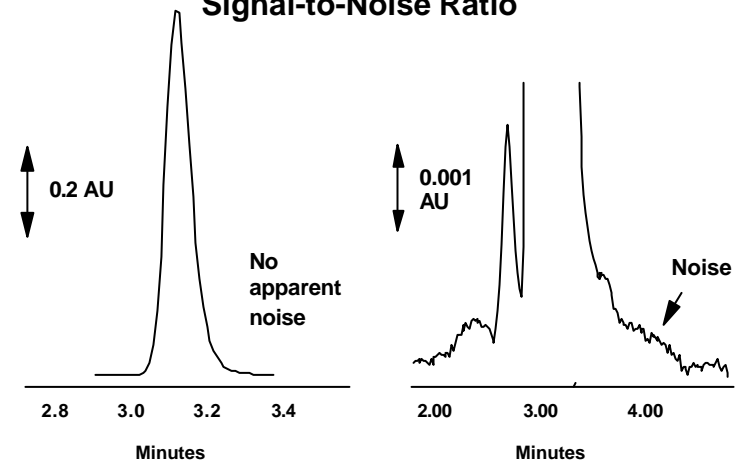
$$h_{\text{signal}} = 2 \times h_{\text{noise}}$$



Increase Signal-to-Noise Ratio



Chromatographic Sensitivity Signal-to-Noise Ratio



Triazine herbicides at detection limit

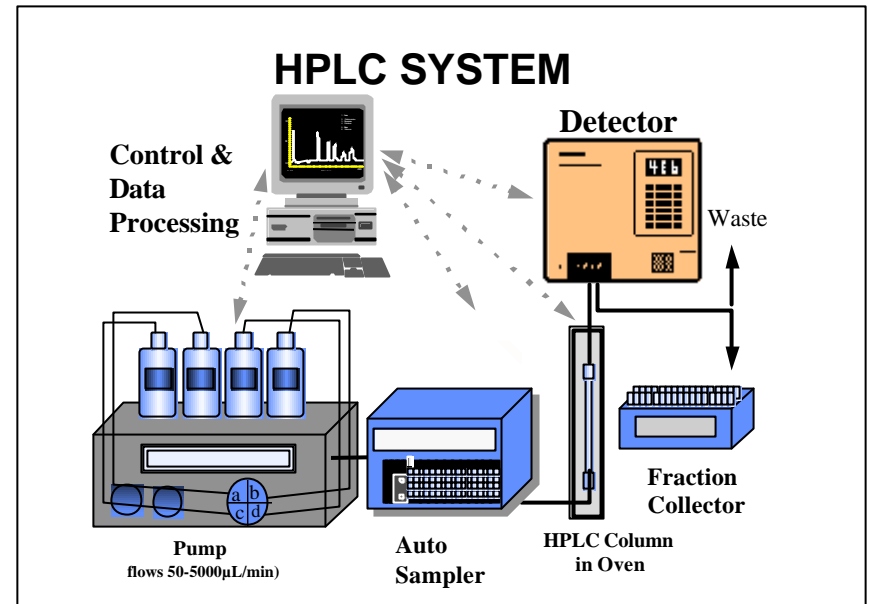
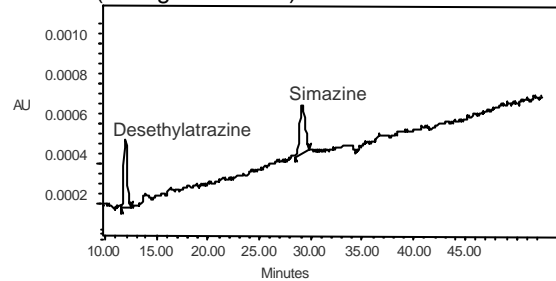
Conditions:

Gradient: Phosphate-Acetonitrile

Column: Novapak 2 x 300 mm

Sample: 2 ppb each pesticide

Injection: 150 µl
(0.3 ng on column)

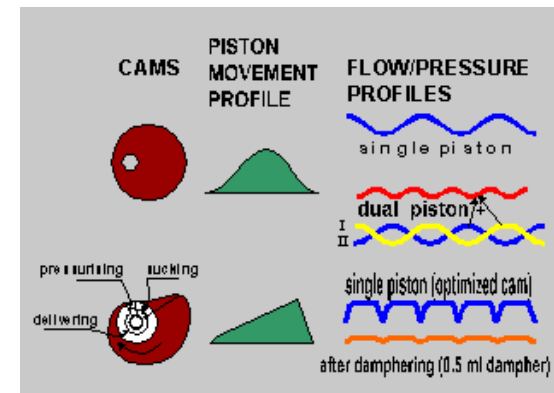


Chromatographic System

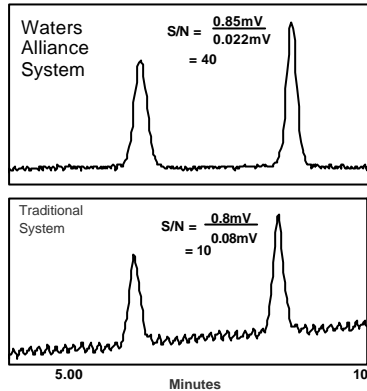
- Pump
- Solvent proportioning
- Detector
- Column Performance & Geometry
- Stationary phases' chemistry
- Mobile phases' chemistry
- Integration events

Pulse Dampers

- Flow and pressure profiles for different types of pumps and cam shape



Smooth Flow Enhances Sensitivity



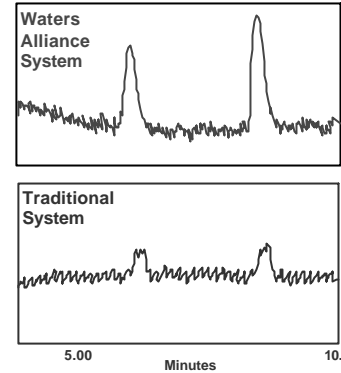
Glucose & Sucrose
2.5ug each

Waters High Performance Carbohydrate Analysis Column

75/25
CH₃CN/H₂O
1.4 mL/min.

Waters 410 Differential Refractometer

Smooth Flow Enhances Detection



Glucose & Sucrose
500ng each

Waters High Performance Carbohydrate Analysis Column

75/25
CH₃CN/H₂O
1.4 mL/min.

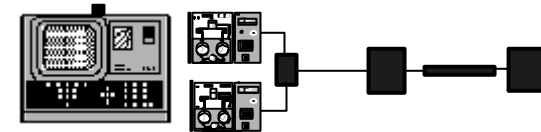
Waters 410 Differential Refractometer

Chromatographic System

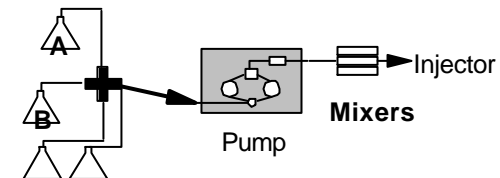
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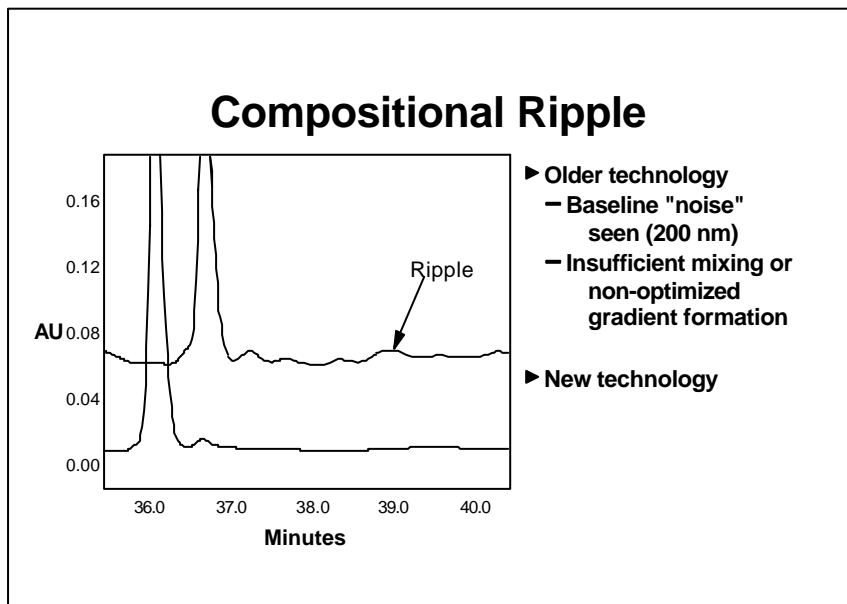
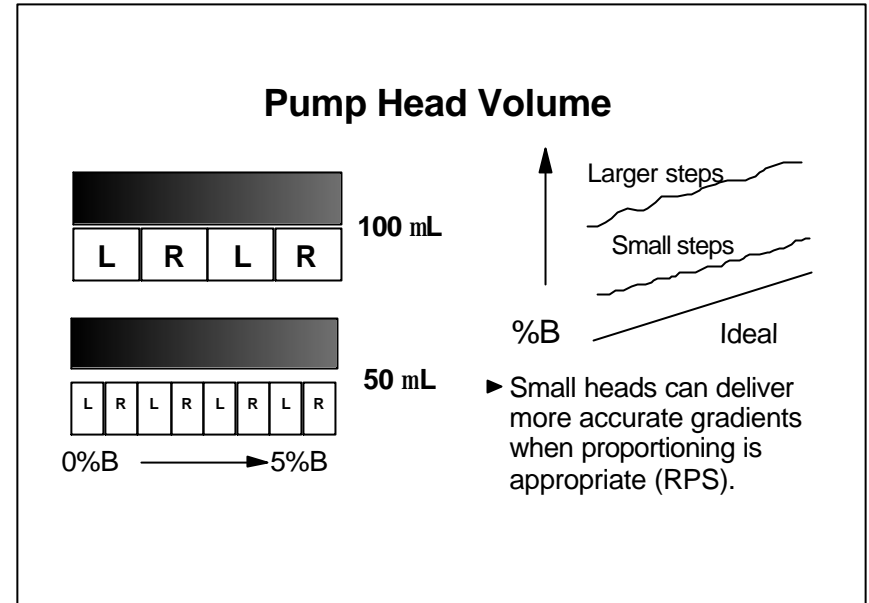
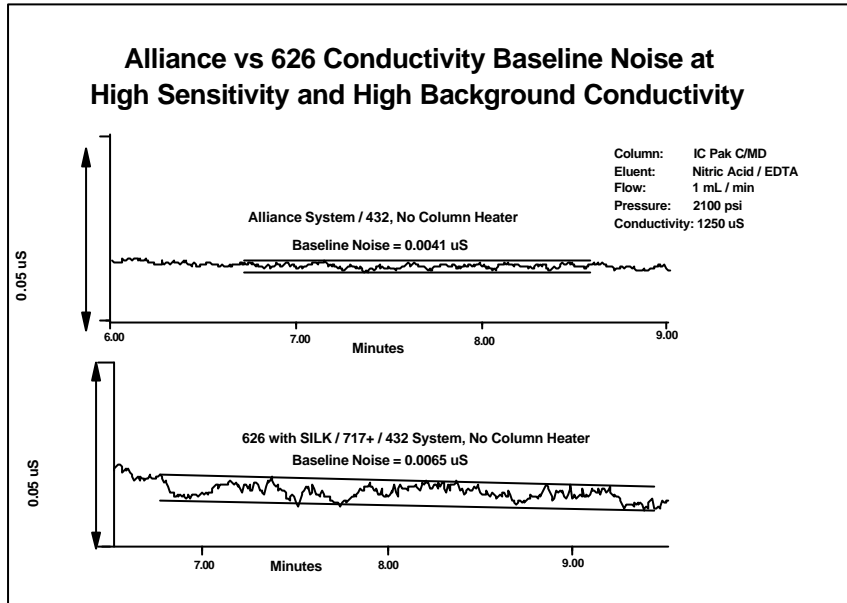
Composition accuracy and consistency

High-Pressure Mixing



Low-Pressure mixing



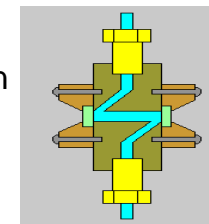


- ### Chromatographic System
- Pump
 - Solvent proportioning
 - Detector
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Detectors

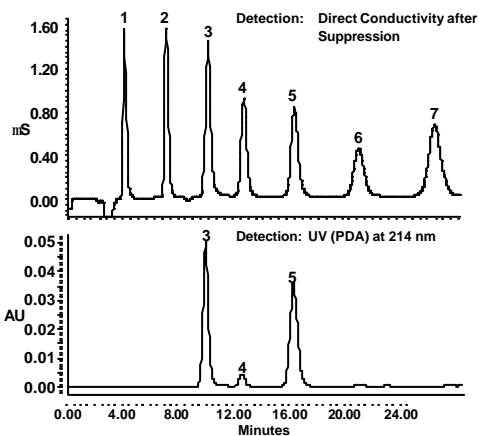
- The most common HPLC detectors
 - UV/Vis
 - Fixed wavelength
 - Variable wavelength
 - Diode array
 - Refractive index
 - Fluorescence
 - Electrochemical
 - - Conductivity
 - Mass-spectrometric (LC/MS)
 - Evaporative light scattering

Factors Increasing Signal



- Increase sample concentration
- Increase injection volume
- Choice of wavelength (s)
- Low volume flow cell
- Flow cell pathlength

432 Conductivity and 996 PDA Detectors in Series

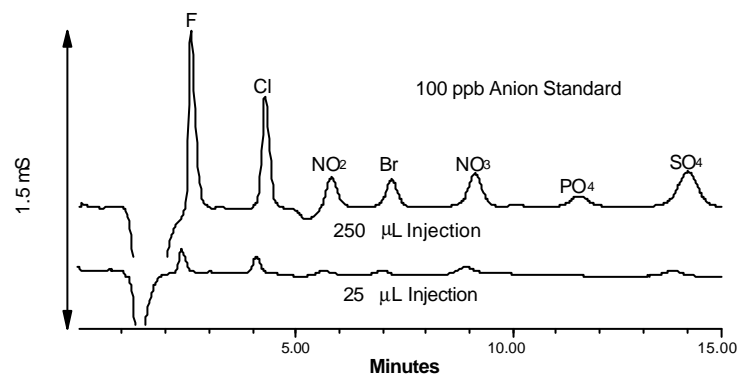


1. Fluoride	1 ppm
2. Chloride	2 ppm
3. Nitrite	4 ppm
4. Bromide	4 ppm
5. Nitrate	4 ppm
6. Phosphate	6 ppm
7. Sulfate	4 ppm

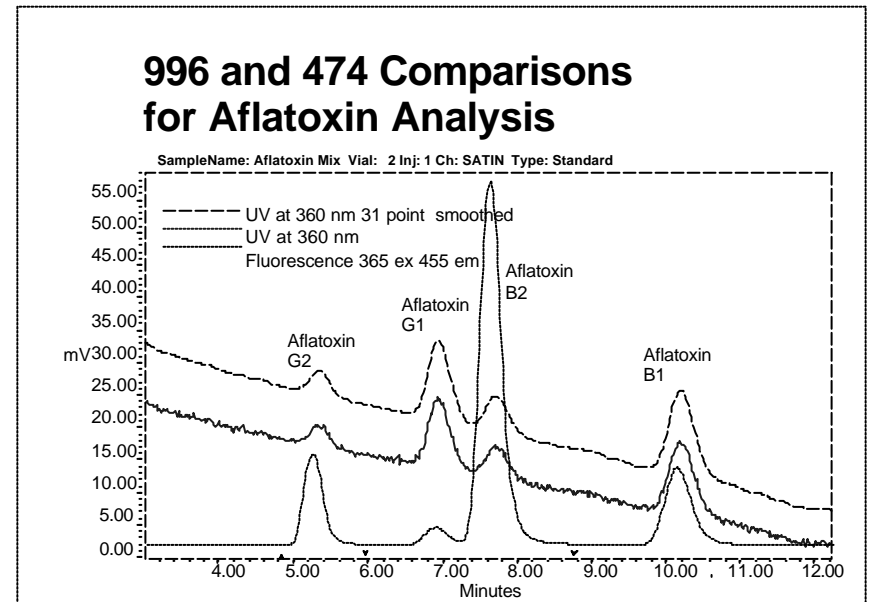
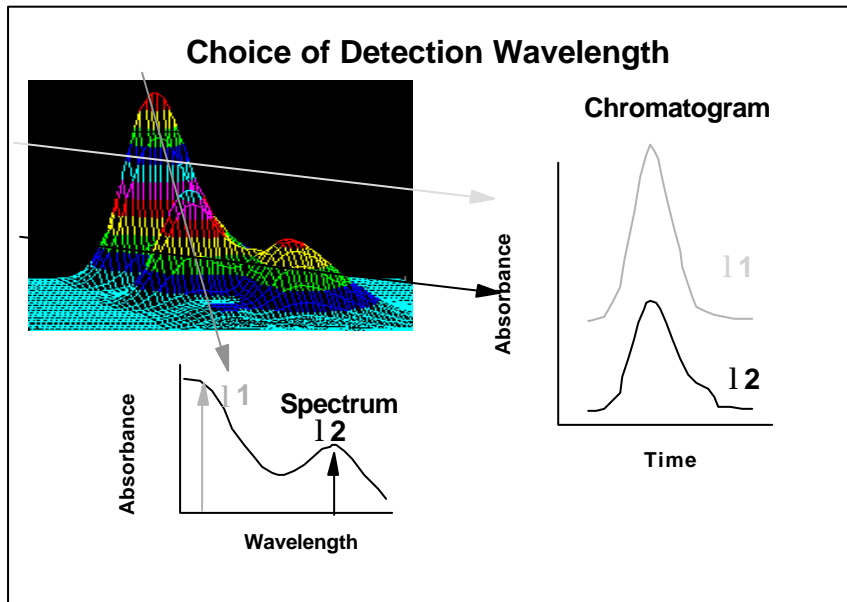
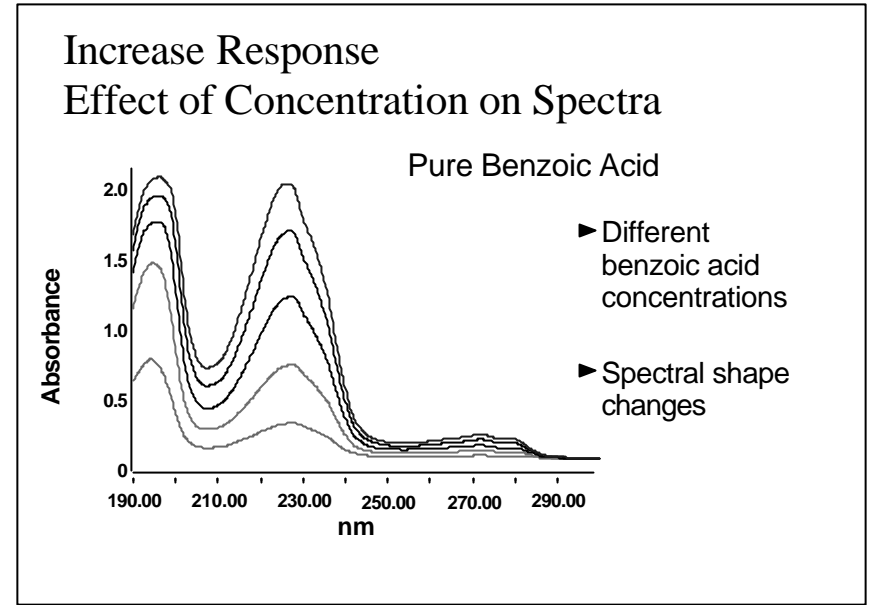
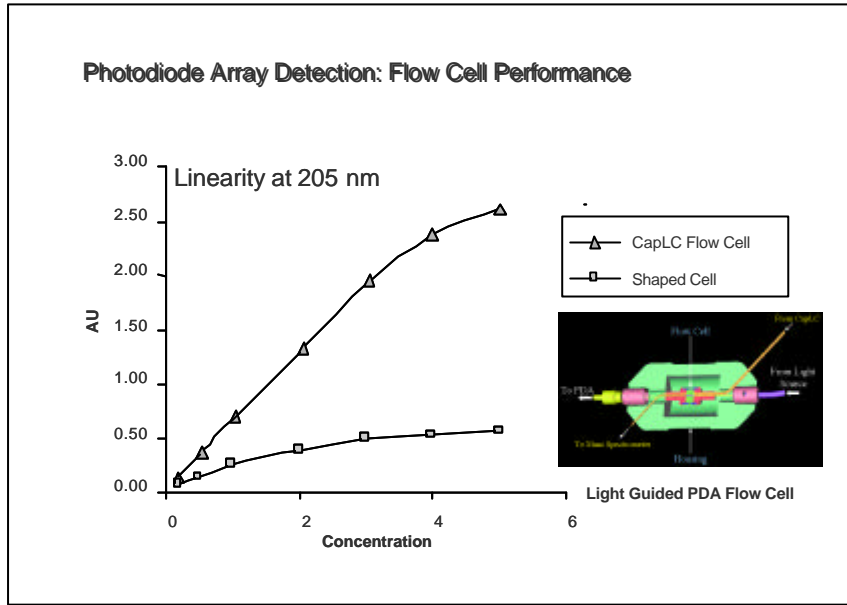
Column: Waters IC-Pak Anion HR
 Eluent: 1.2 mM Sodium Carbonate/
 1.2 mM Sodium Bicarbonate
 Flow rate: 1.0 mL/min
 Injection vol.: 50 µL

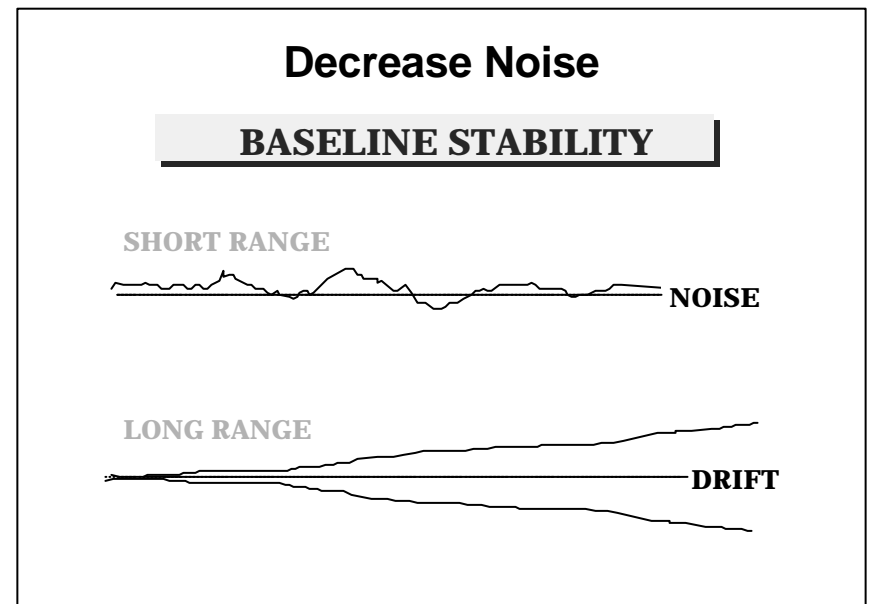
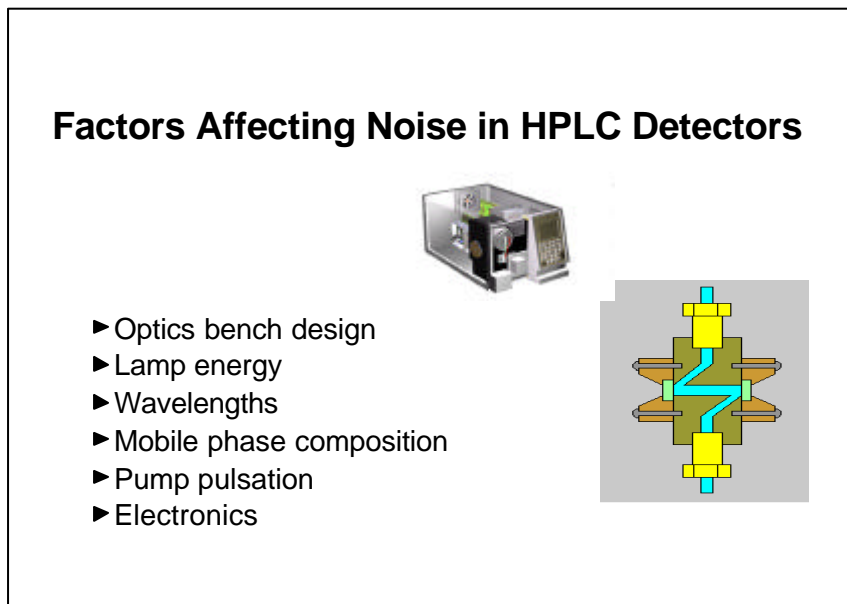
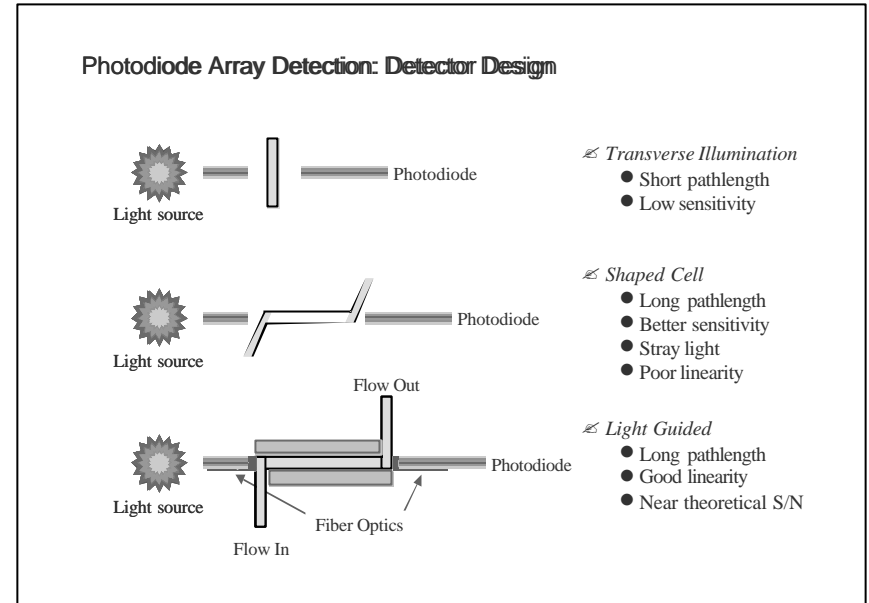
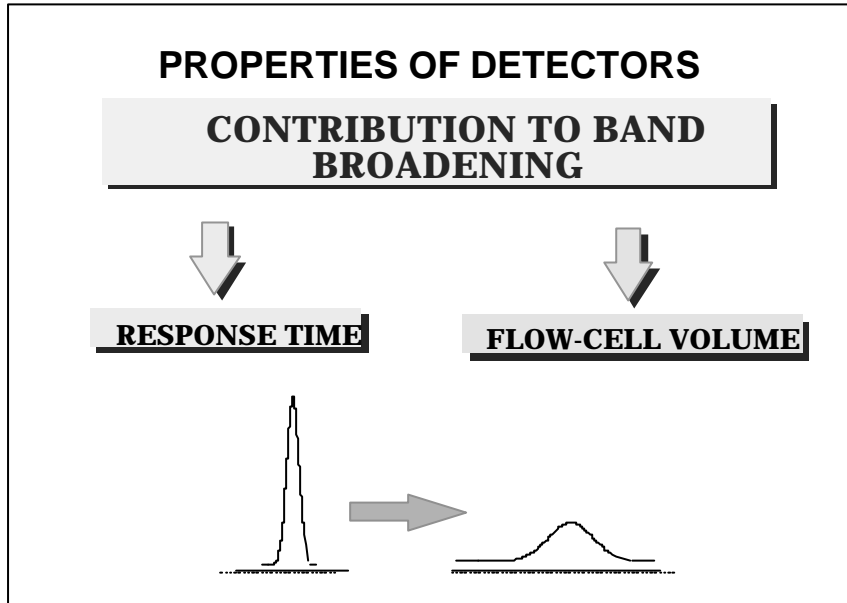
Alliance IC with ERIS 1000

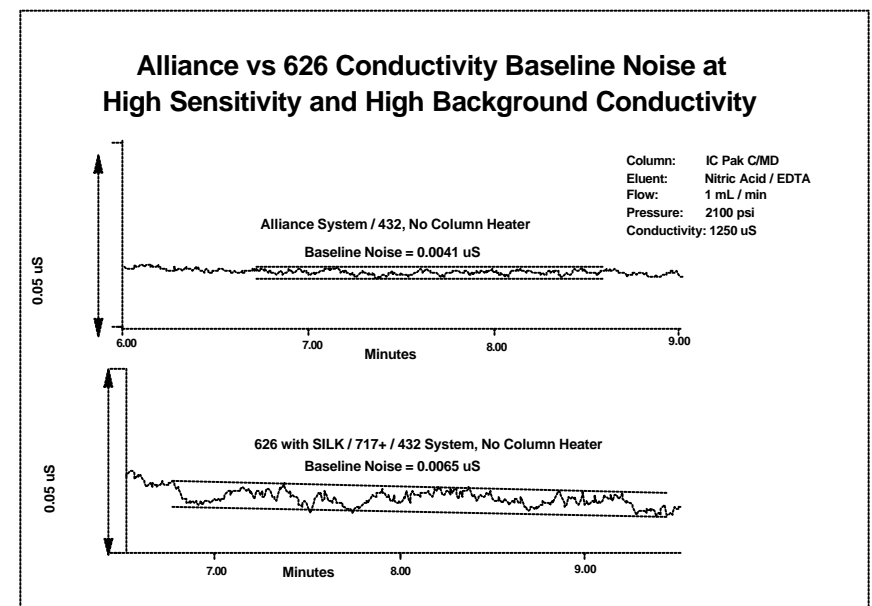
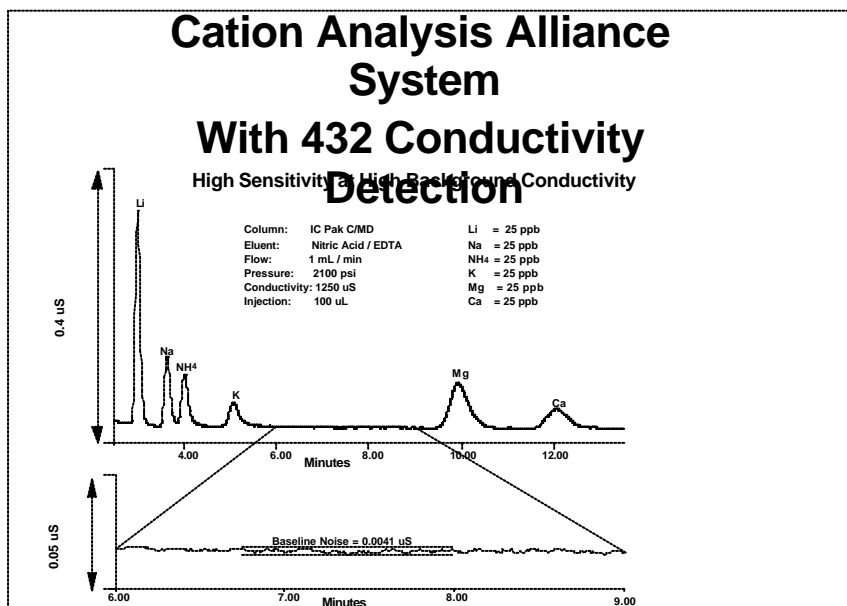
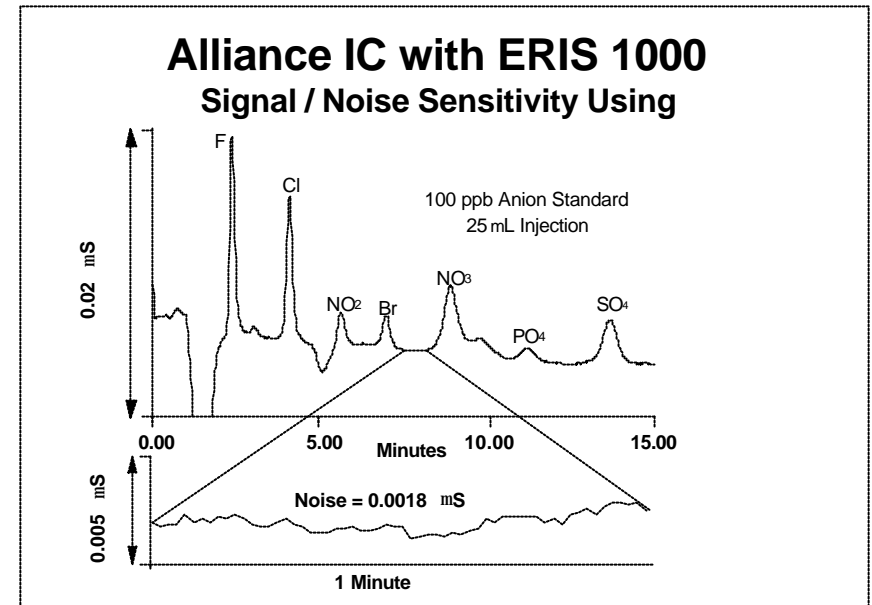
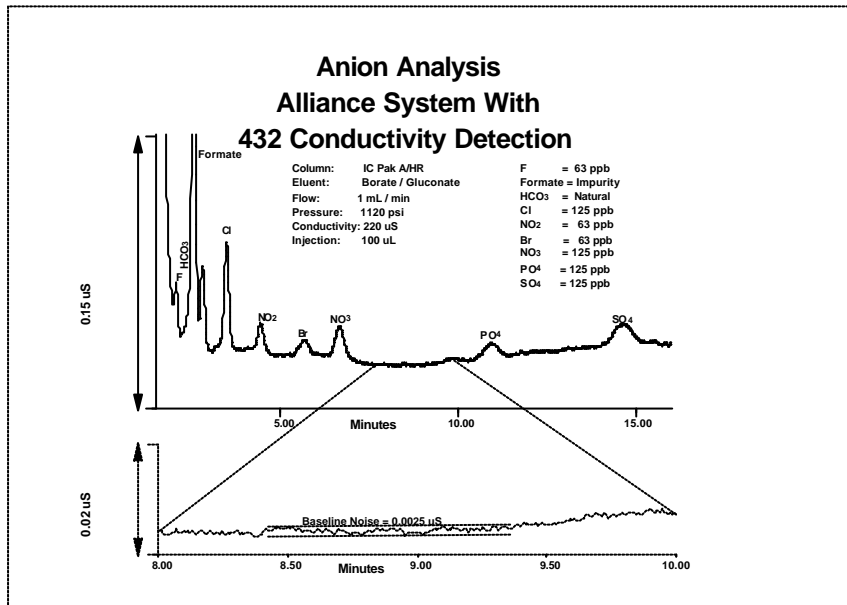
Sensitivity with Large Injection Volume
 IC Pak A/HR



Alliance with Colum Heater at 30 C, 432 Conductivity Detector, ERIS 1000 Suppressor Module, Carbonate / Bicarbonate Eluent



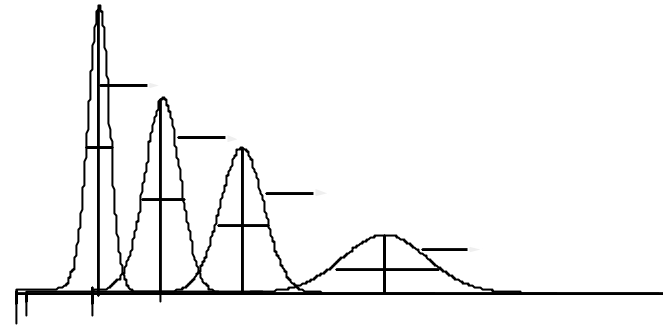




Chromatographic System

- Pump
- Solvent proportioning
- Detector
- Column Performance & Geometry
- Stationary phases' chemistry
- Mobile phases' chemistry
- Integration events

Peak Broadening



LOQ and LOD Relationship to

Column Performances

- LOQ and LOD can be expressed by:

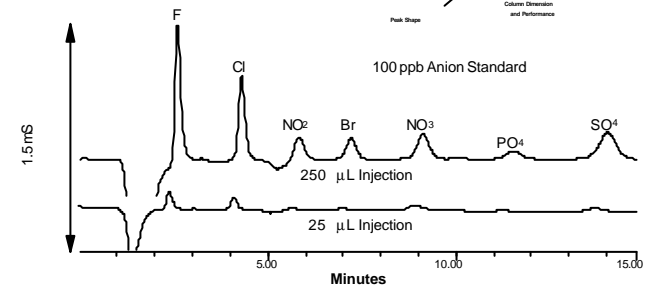
$$C_{\max} = \frac{4}{\epsilon\pi\sqrt{2p}} \frac{1}{[1 + b(T_a - 1)]} \frac{\sqrt{N}}{Ld_c^2} \frac{V_{inj}c_0}{(1 + k')}$$

Peak Shape
Column Dimension and Performance
Retention

Alliance IC with ERIS 1000

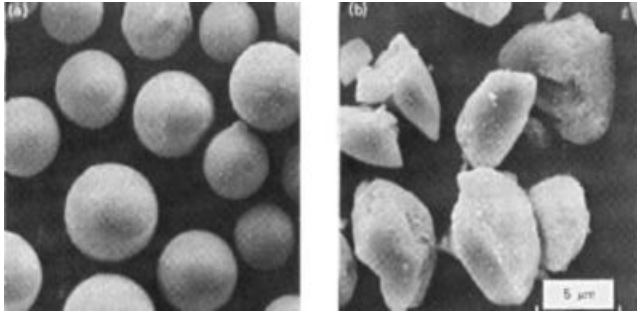
$$C_{\max} = \frac{4}{\epsilon\pi\sqrt{2p}} \frac{1}{[1 + b(T_a - 1)]} \frac{\sqrt{N}}{Ld_c^2} \frac{V_{inj}c_0}{(1 + k')}$$

Peak Shape
Column Dimension and Performance
Retention



Alliance with Colum Heater at 30 C, 432 Conductivity Detector, ERIS 1000 Suppressor Module, Carbonate / Bicarbonate Eluent

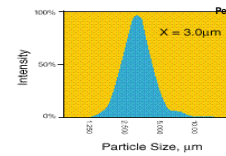
Spherical and Irregular particles



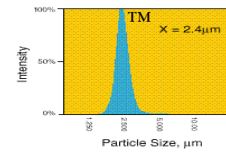
■ Electron microphotograph of spherical and irregular silica particles. [W.R.Melander, C.Horvath, Reversed-Phase Chromatography, in HPLC Advances and Perspectives, V2, Academic Press, 1980]

Challenge of Making “2 µm” Packings

$$C_{max} = \frac{4}{\epsilon\pi \sqrt{2p}} \frac{1}{[1 + b(T_a - 1)]} \frac{\sqrt{N}}{Ld_c^2} \frac{V_{inj} c_o}{(1 + k')}$$



Centered at 3 µm
Wider distribution

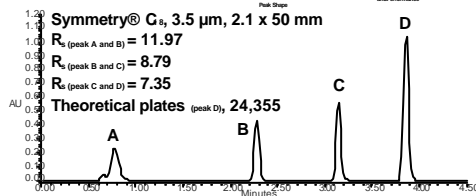


Centered at 2.4 µm
Narrower distribution



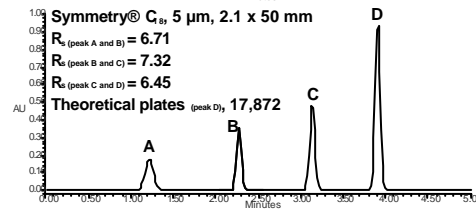
Impact of Particle Size (dp) on Resolution

$$C_{max} = \frac{4}{\epsilon\pi \sqrt{2p}} \frac{1}{[1 + b(T_a - 1)]} \frac{\sqrt{N}}{Ld_c^2} \frac{V_{inj} c_o}{(1 + k')}$$



Conditions:

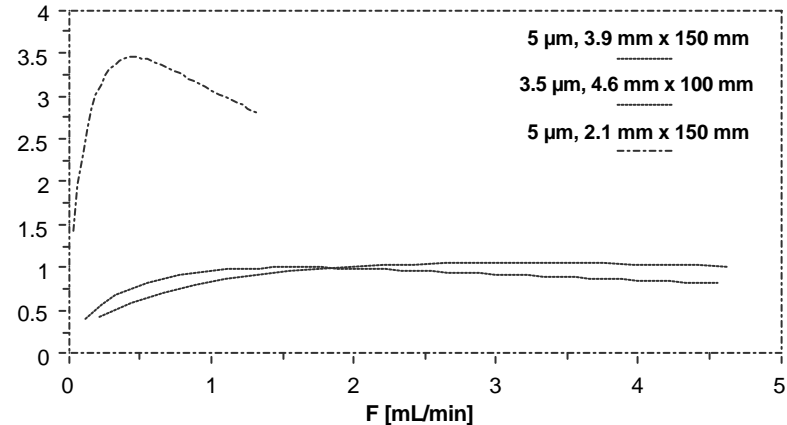
Columns: Symmetry® C₈, 5 µm, 4.6 X 50 mm and Symmetry® C₈, 3.5 µm, 4.6 X 50 mm
 Mobile phases: A=0.1% TFA in water, B=0.1% TFA in acetonitrile
 Gradient: 0-60% B in 4 minutes
 Column temperature: 30.0 °C
 Detector: 254 nm
 Injection volume: 1 µL

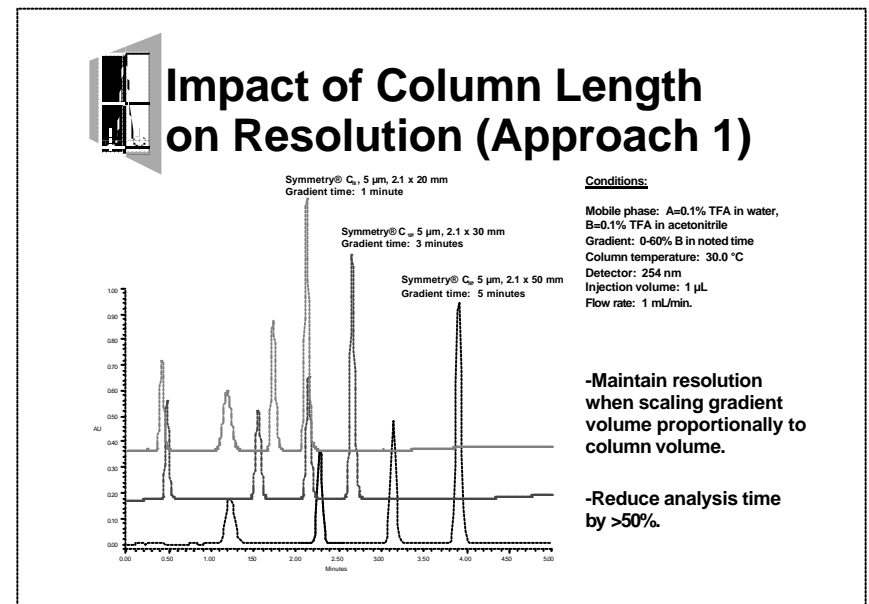
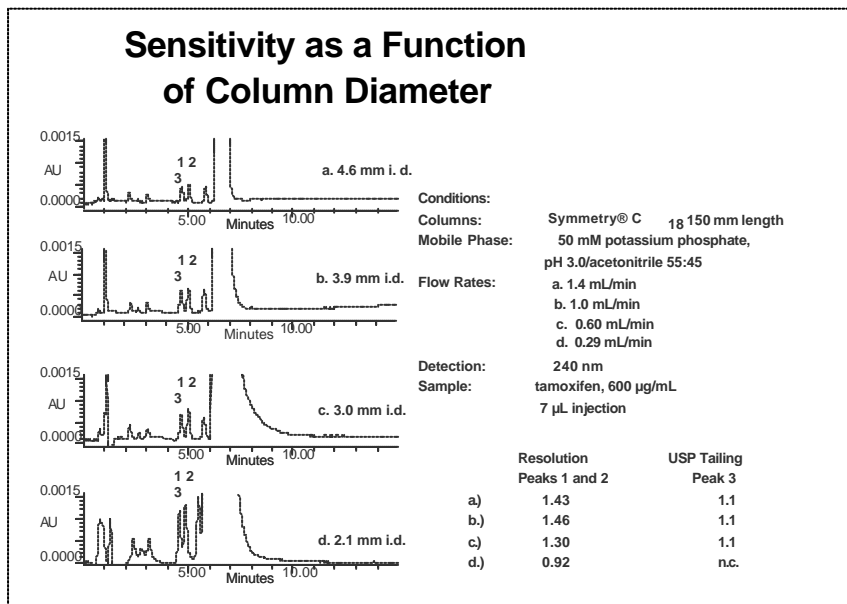
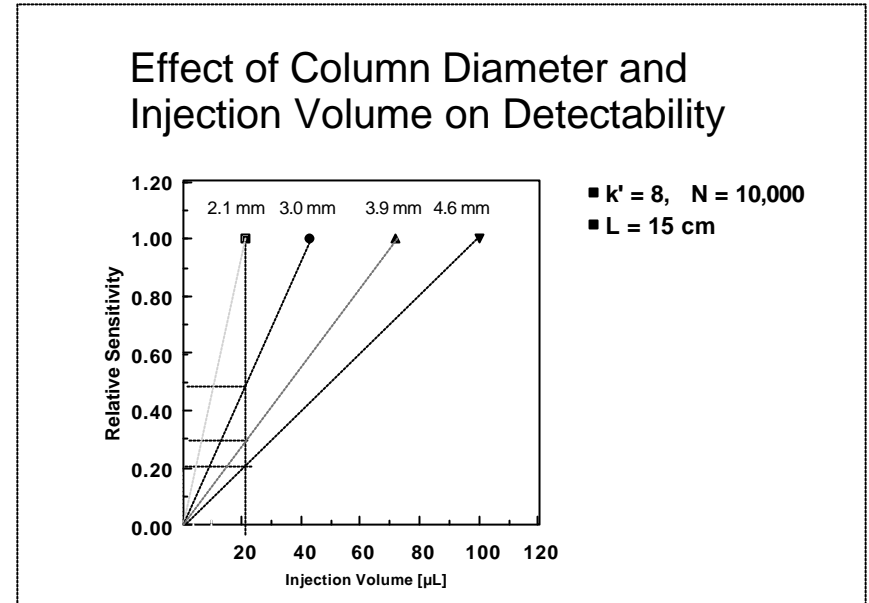
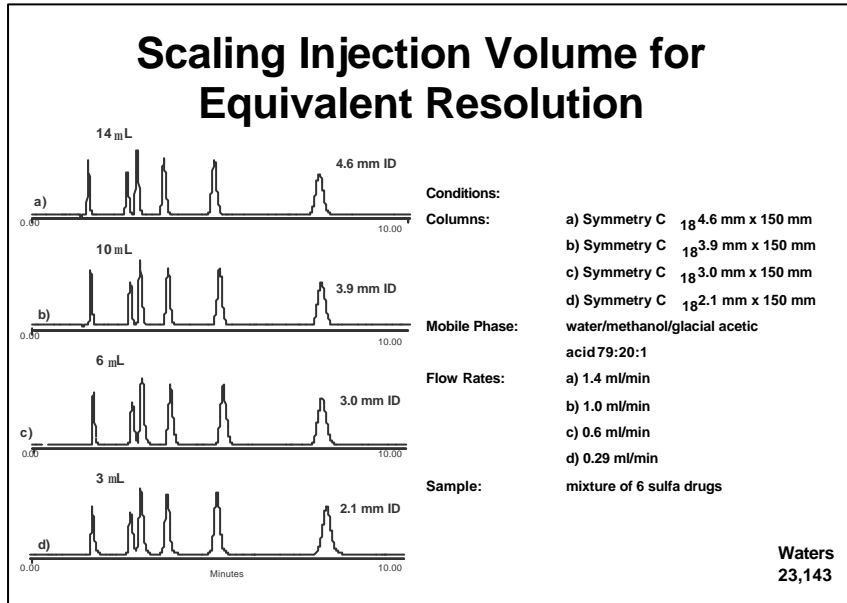


-Achieve increased resolution with the smaller particle size material in the same gradient time

Sensitivity as a Function of Column Dimensions

Relative Sensitivity



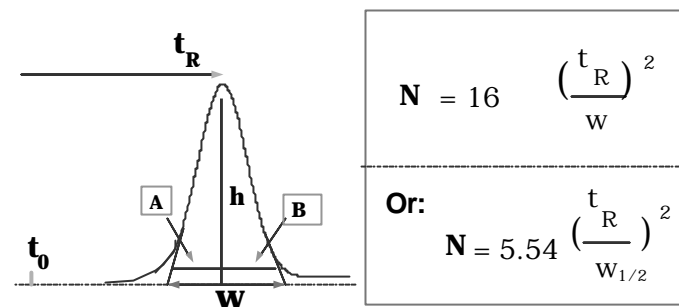


Chromatographic System

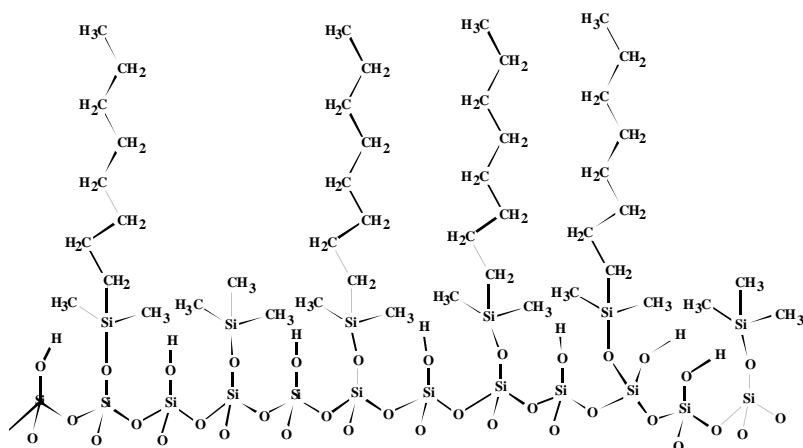
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PERFORMANCE BY ONE PEAK

NUMBER OF THEORETICAL PLATES

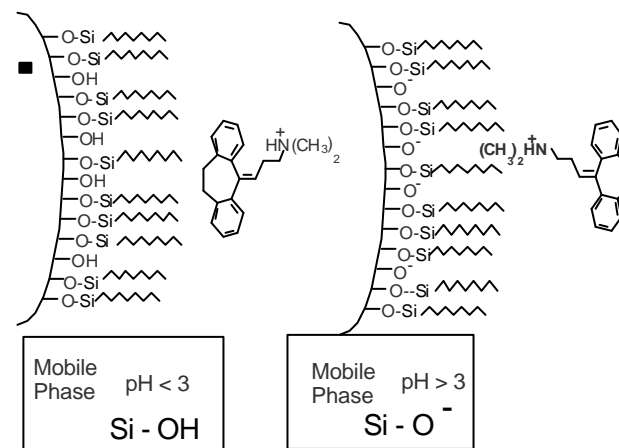


Surface of a Reversed-Phase Packing

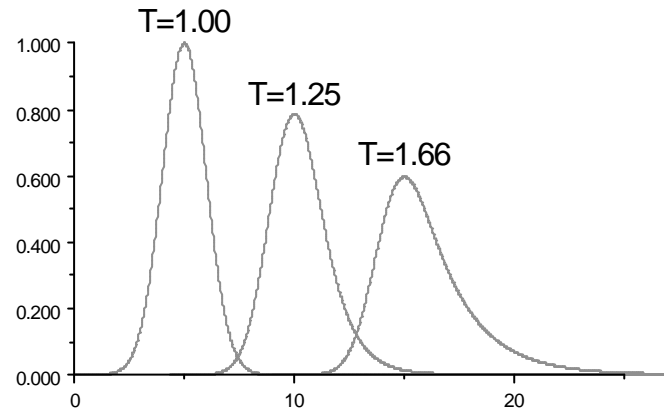


Mixed-Mode Retention:

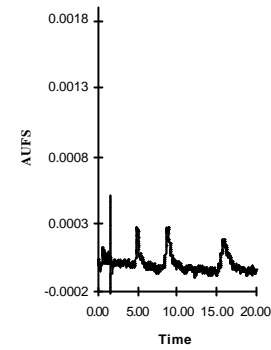
- Hydrophobic Interaction with Bonded Phase
- Ion exchange Interaction with Charged Sites



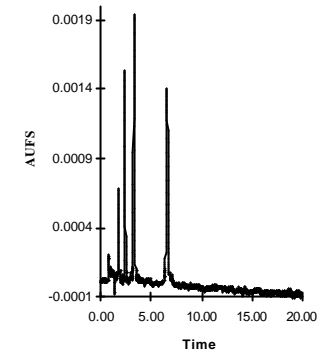
Example of Lowered Peak-Height



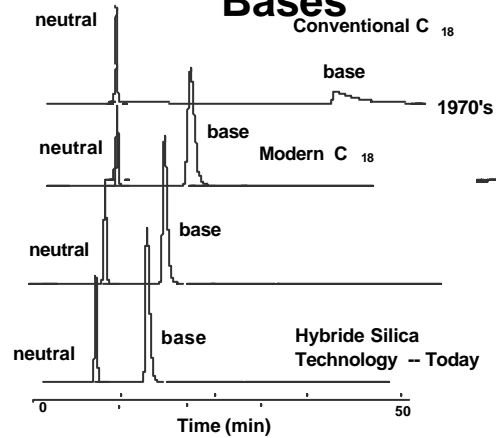
Zorbax 40 nG Procainamide



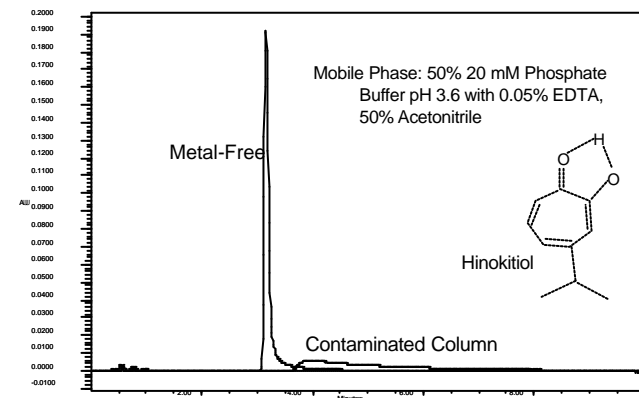
Symmetry 40nG Procainamide



Improvement in Peak Shape for Bases



Peak Forms of Complexing Agent (Hinokitiol)



© Waters Corporation

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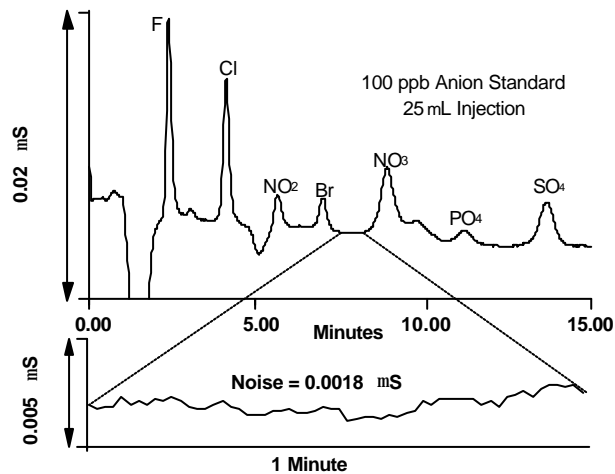
Solvent Effects

	Wavelength nm							
	200	205	210	215	220	230	240	250
Acetonitrile	0.05	0.03	0.02	0.01	0.01	<.01		
Methanol	2.06	1.00	0.53	0.37	0.24	0.11	0.05	0.02
Degassed	1.91	0.76	0.35	0.21	0.15	0.06	0.02	<.01
Isopropyl	1.89	0.68	0.34	0.24	0.19	0.08	0.04	0.03
New THF	2.44	2.57	2.31	1.80	1.54	0.94	0.42	0.21
Old THF	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5
1% HOAc	2.50	2.54	2.47	2.37	2.16	1.01	0.17	0.04
0.1% HPO ₄	0.01	0.01	0.01	<.01				
0.1% TFA	1.82	0.87	0.68	0.36	0.22	0.07	<.01	
1% TEA	2.33	2.42	2.50	2.45	2.37	1.96	0.50	0.12

Absorbance AU

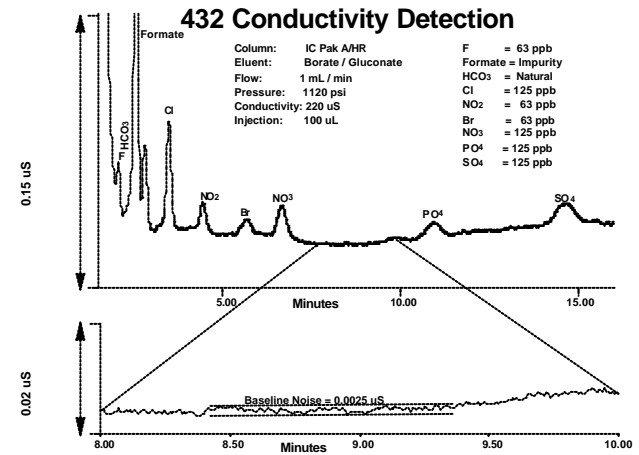
Alliance IC with ERIS 1000

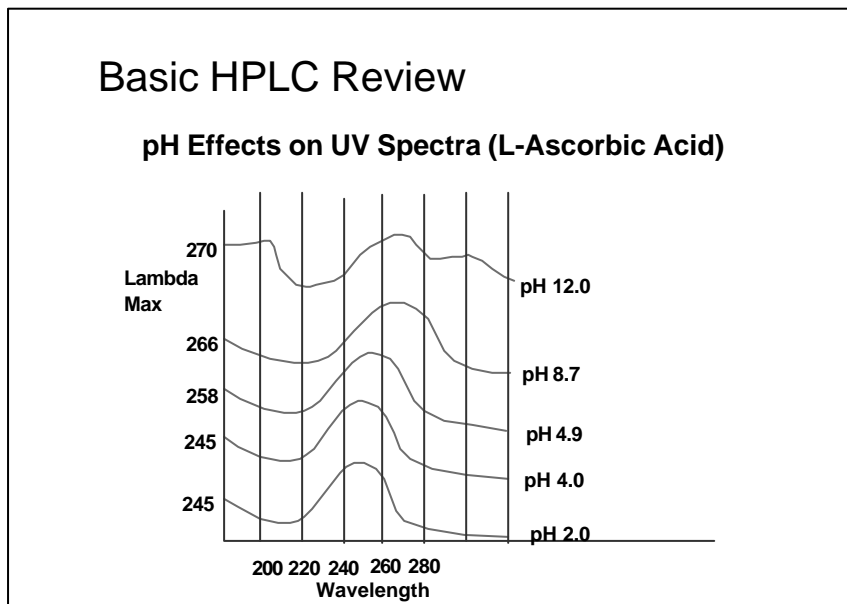
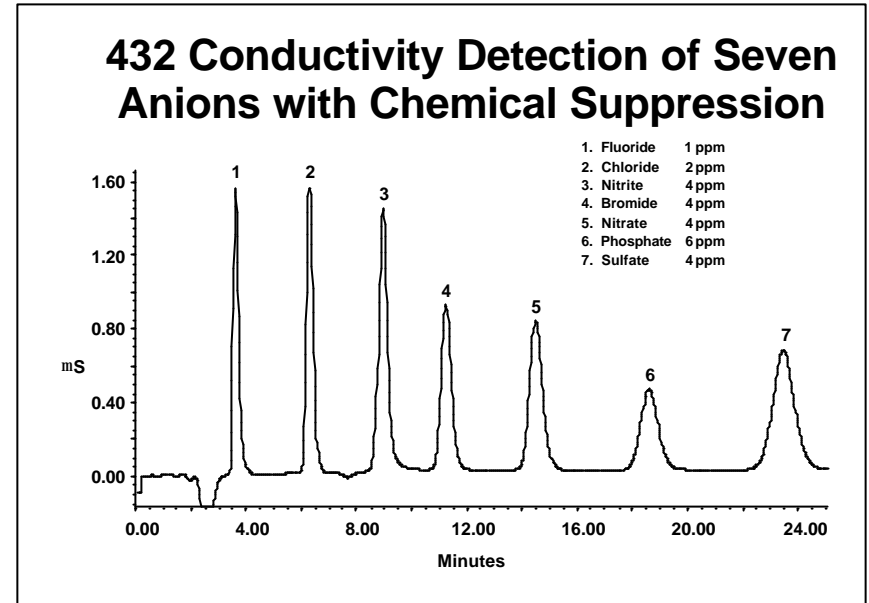
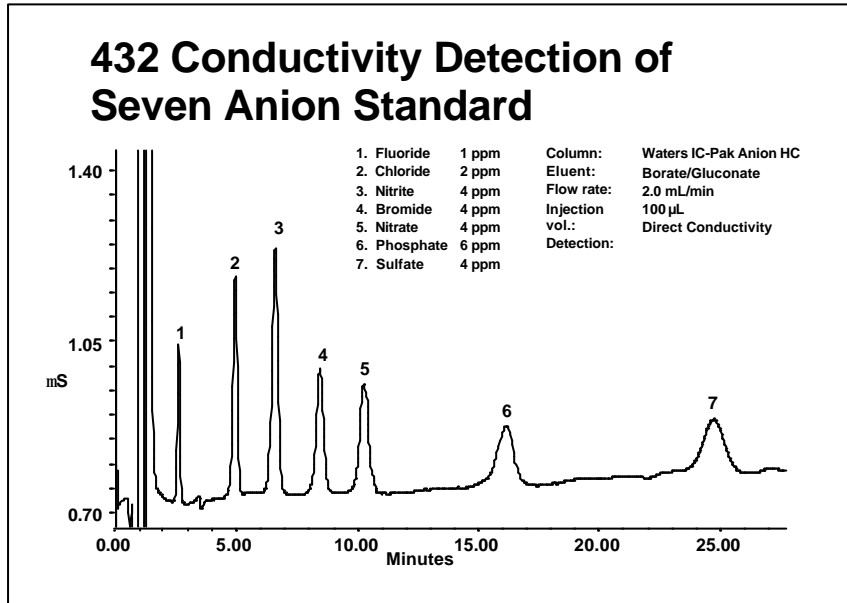
Signal / Noise Sensitivity Using



Anion Analysis

Alliance System With
432 Conductivity Detection





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