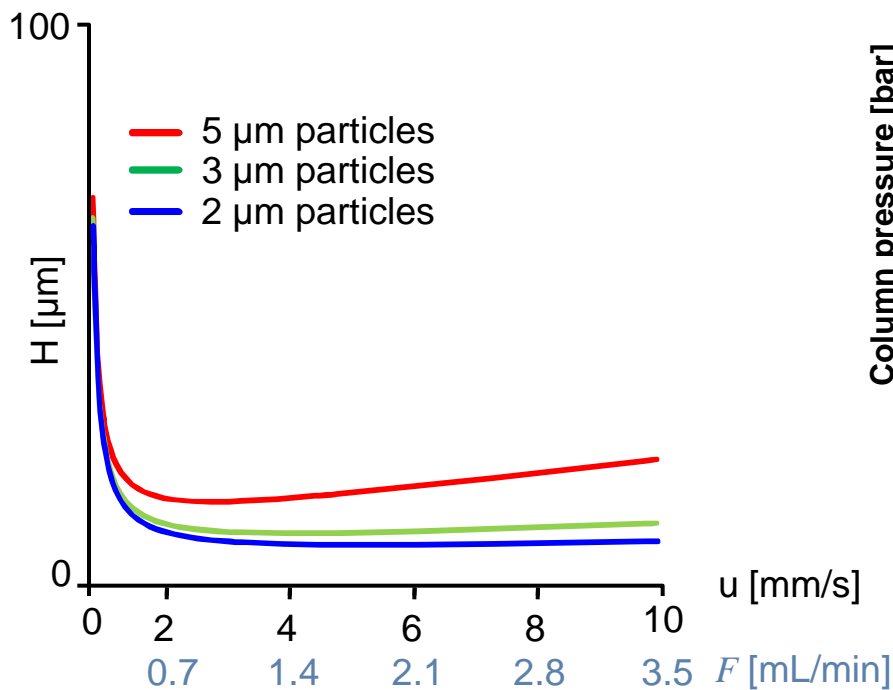


# Holistic HPLC Analytical Strategies for Pharmaceutical Labs

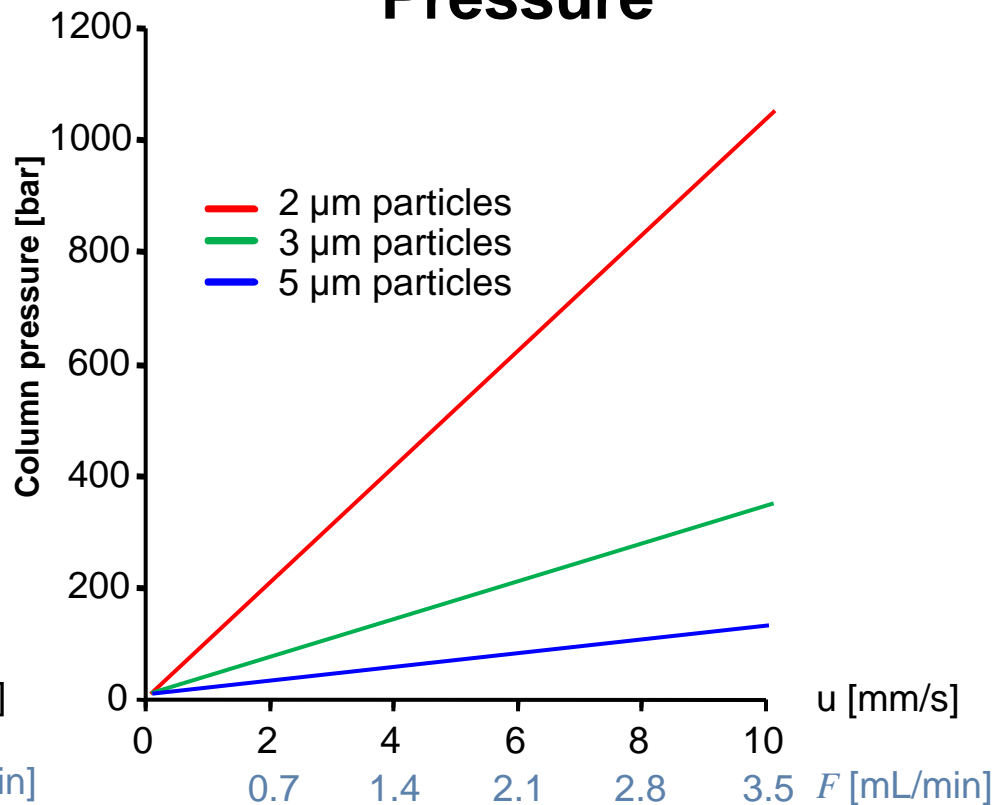
by Rainer Bauder, Solutions Manager Pharmaceutical Markets

1. Column Efficiency and Selectivity
2. Your Workflow and Our Automation Tools
3. Boosting Nebulizer Based Detectors

### Efficiency



### Pressure



\*Linear velocity translated to flow rate for a 3 mm i.d. column

$$R_s = \frac{1}{4} \times \frac{\alpha - 1}{\alpha} \times \frac{k}{k + 1} \times \sqrt{N}$$

$R_s$  – Resolution

$N$  – Theoretical plates

$\alpha$  – Selectivity

$k$  – Retention factor

### Example:

To separate two analytes on a 5- $\mu$ m, 150 mm C18 column

Assuming  $N = 10,000$  plates/column,  $k \gg 1$ , and  $\alpha = 1.04 \Rightarrow R_s = 1$

Objective: increase  $R_s$  to 2

**Approach 1 – increase  $N$**

needs **400%** increase or  $N = 40,000$  plates

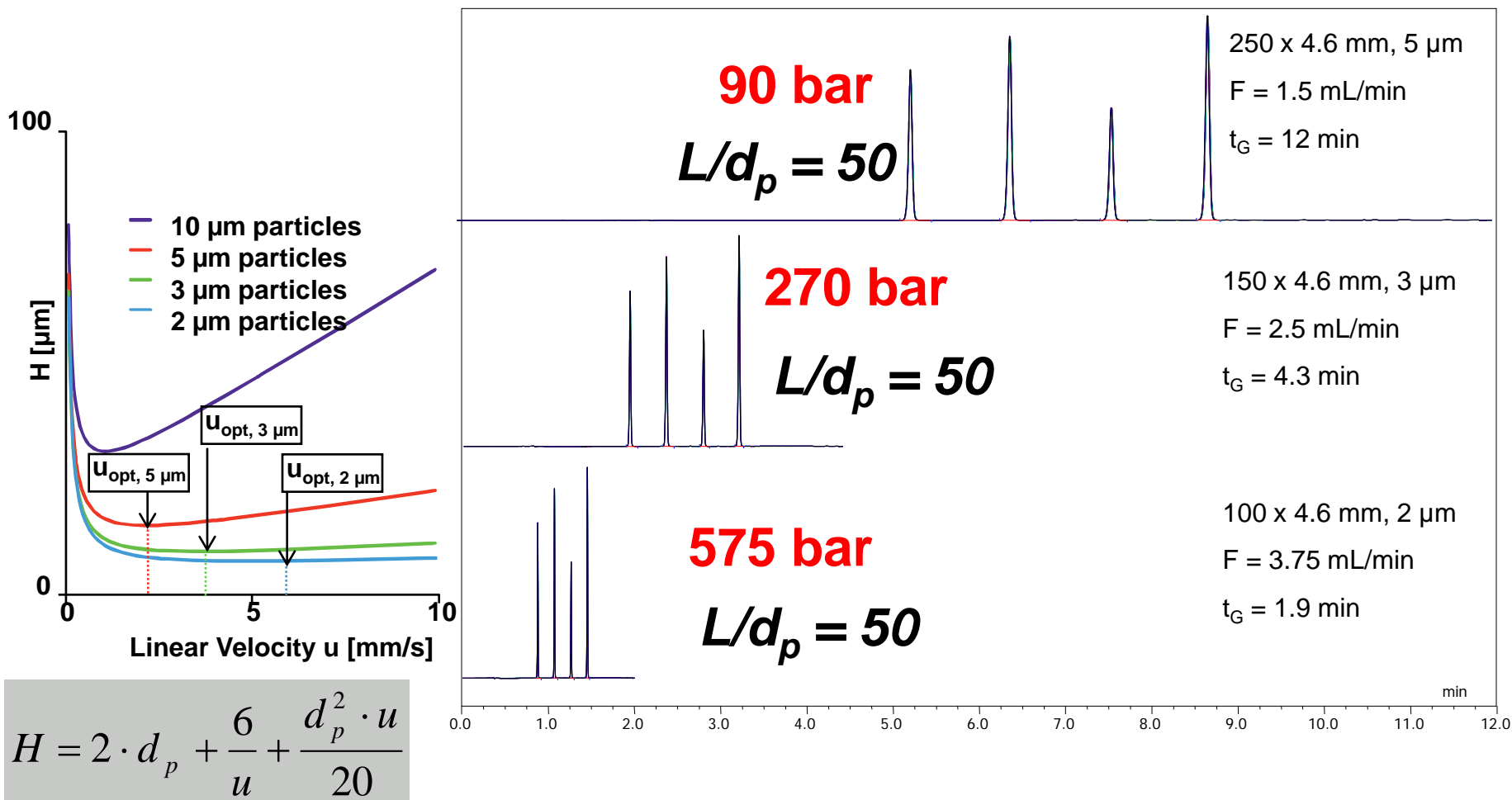
**Approach 2 – increase  $\alpha$**

requires only **4%** increase or  $\alpha = 1.08$

# Speed-Up Strategy

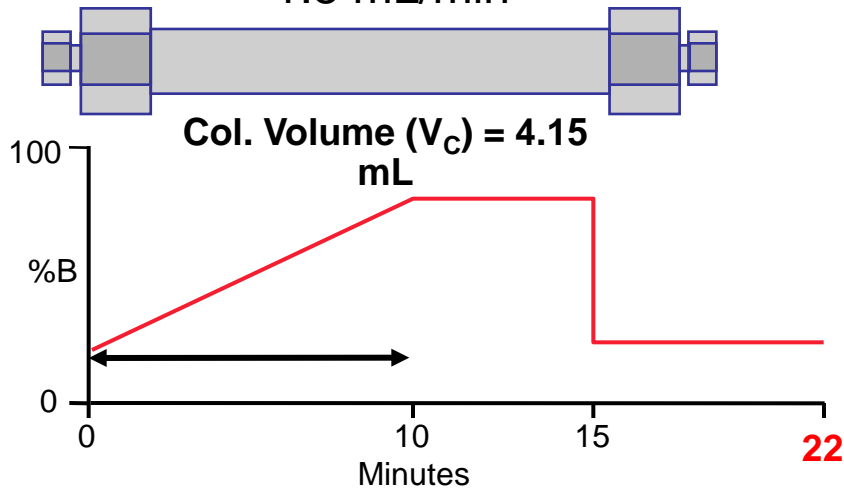
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# Speeding-up Gradient Separation of Parabenes

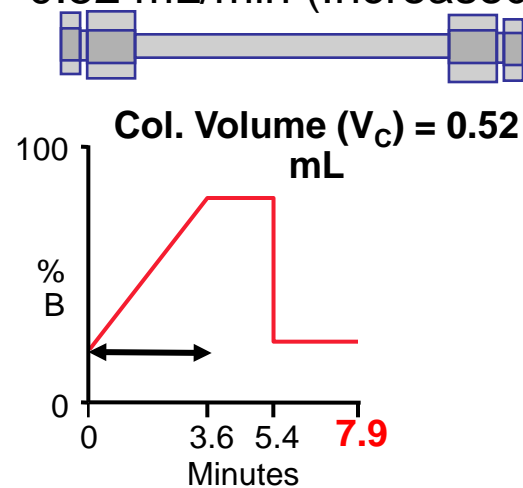


# How to Adapt the Gradient Program?

4.6 x 250 mm Column, 5  $\mu$ m  
1.5 mL/min



2.1 x 150 mm Column, 3  $\mu$ m  
0.52 mL/min (Increased  $u$ )



Original First Gradient Step 0–10 min  
 $t_G = 10$  min  
 $F = 1.5$  mL/min  
 $V_C = 4.15$  mL


$$t_{G2} = t_{G1} \cdot \frac{F_1}{F_2} \cdot \frac{V_{C2}}{V_{C1}}$$

New First Gradient Step 0–? min

$$t_{G2} = 10 \cdot \frac{1.5}{0.52} \cdot \frac{0.52}{4.15}$$

= **3.6 min**



# Dionex RSLC Method Speed-Up Calculator



UltiMate<sup>™</sup> 3000 RESET

Acclaim<sup>®</sup>

Chromleon<sup>®</sup>

## METHOD SPEED-UP RECOMMENDATIONS

LANGUAGE ENGLISH

Best Viewed in 1024 x 768 screen resolution  
MACROS MUST BE ENABLED TO USE THE TOOL

VERSION 1.121  
© 2006 - 2008 Dionex Corporation

---

**Current Column**

Length (mm) 150 mm

Diameter (mm) 4.6 mm

Particle Size (µm) 5.0 µm

**Planned Column**

Length (mm) 50 mm

Diameter (mm) 2.1 mm

Particle Size (µm) 2.0 µm

---

**Peak Details (Critical Pair)**

Actual Rs (resolution factor) 3.24

Predicted Rs Change Factor 0.91 (-8.7%)

Predicted Rs 2.96 Baseline resolution achieved

---

**Current Instrument Settings**

Flow (mL/min) 1.000 mL/min

Injection Volume (µL) 20.0 µL

Max Pressure 80.0 bar << CHANGE PRESSURE UNITS

Number of Samples 20

Boost Factor 1.0

**Recommended Instrument Settings**

Flow (mL/min) 0.521 mL/min

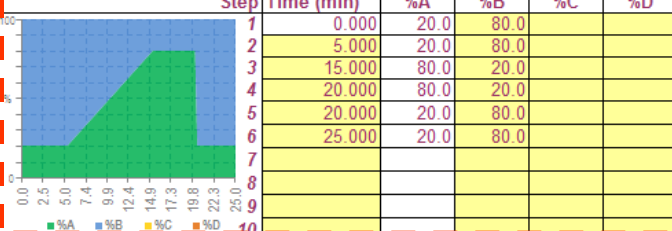
Injection Volume (µL) 1.5 µL

Estimated Max Pressure 416.7 bar

Number of Samples 20

---

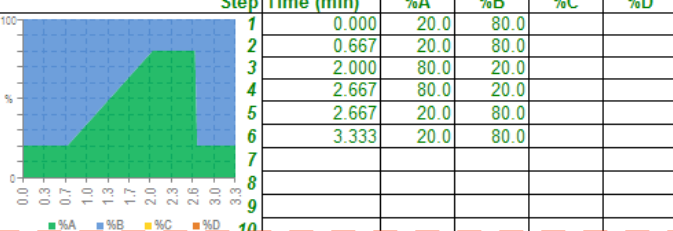
**Gradient Table**



Step	Time (min)	%A	%B	%C	%D
1	0.000	20.0	80.0		
2	5.000	20.0	80.0		
3	15.000	80.0	20.0		
4	20.000	80.0	20.0		
5	20.000	20.0	80.0		
6	25.000	20.0	80.0		
7					
8					
9					
10					

**End Time** 25.000 min

**Gradient Table**



Step	Time (min)	%A	%B	%C	%D
1	0.000	20.0	80.0		
2	0.667	20.0	80.0		
3	2.000	80.0	20.0		
4	2.667	80.0	20.0		
5	2.667	20.0	80.0		
6	3.333	20.0	80.0		
7					
8					
9					
10					

**End Time** 3.333 min

---

**TOTALS**

Eluent Usage 500.00 ml

Time 500.0 min

8.33 hr

Sample Usage 400.00 µL

**TOTALS**

Eluent Usage 34.74 ml = 93% **SAVING**

Time 66.7 min = 87% **Throughput**

1.11 hr = x7.5

Sample Usage 30.44 µL = 92%

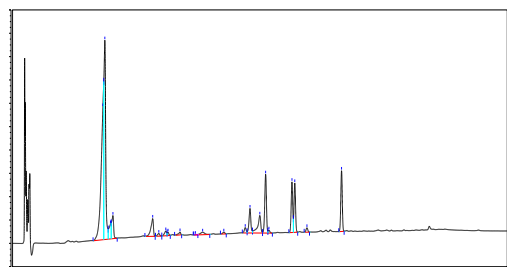
For more information on Rapid Separation LC visit [www.dionex.com](http://www.dionex.com)

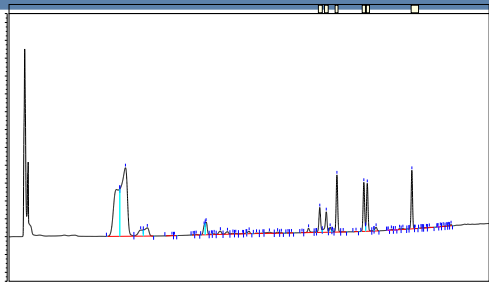


# Harnessing Efficiency Improvement?

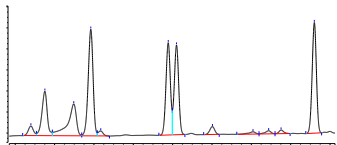
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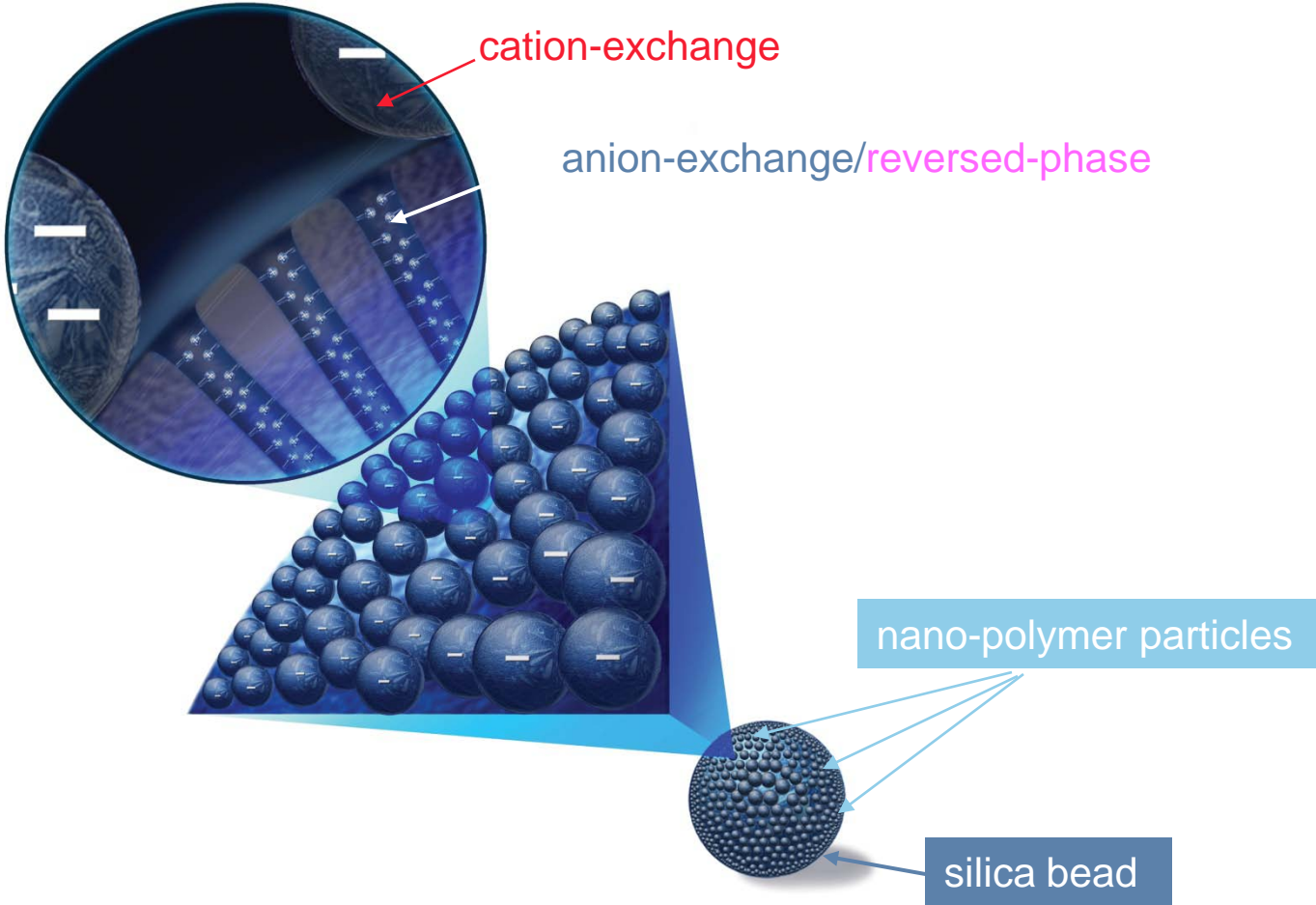
# Potency RS Method Before and After



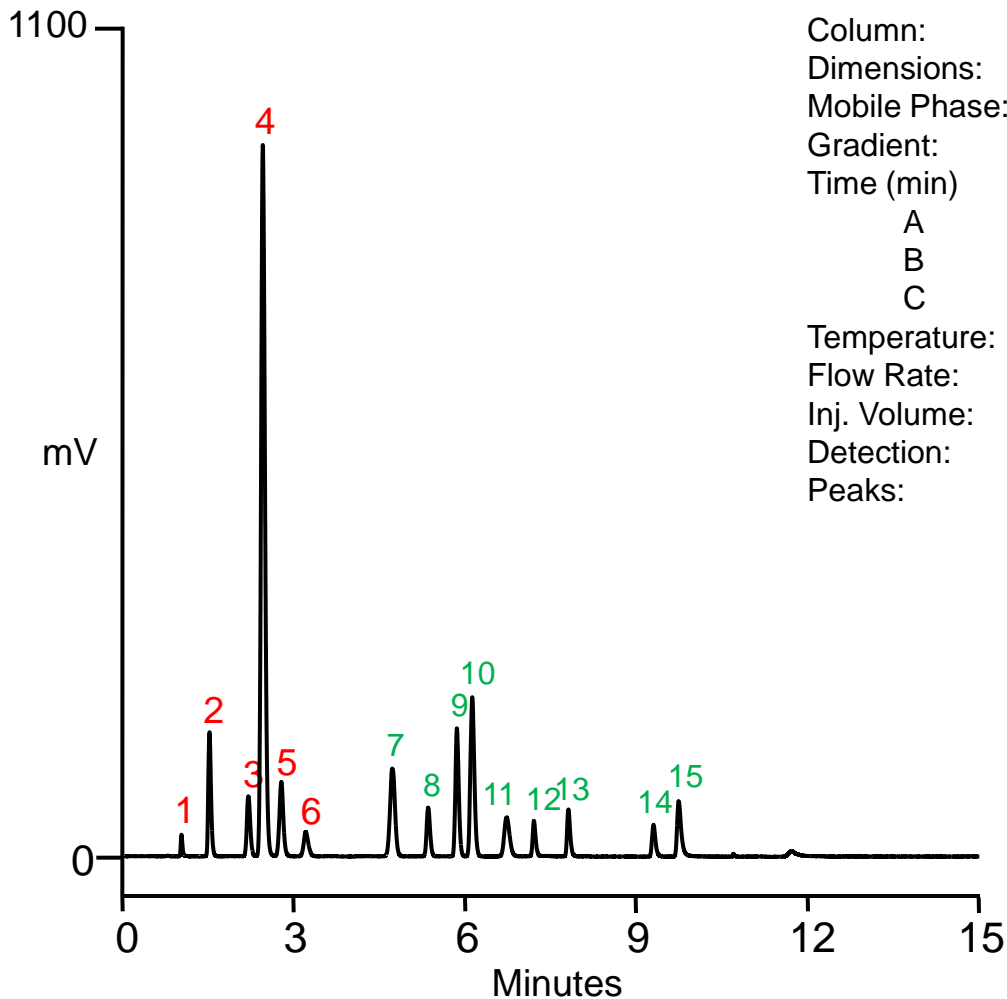
# Harnessing Selectivity Improvement

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# Acclaim Trinity Nanopolymer Silica Hybrid (NSH) technology



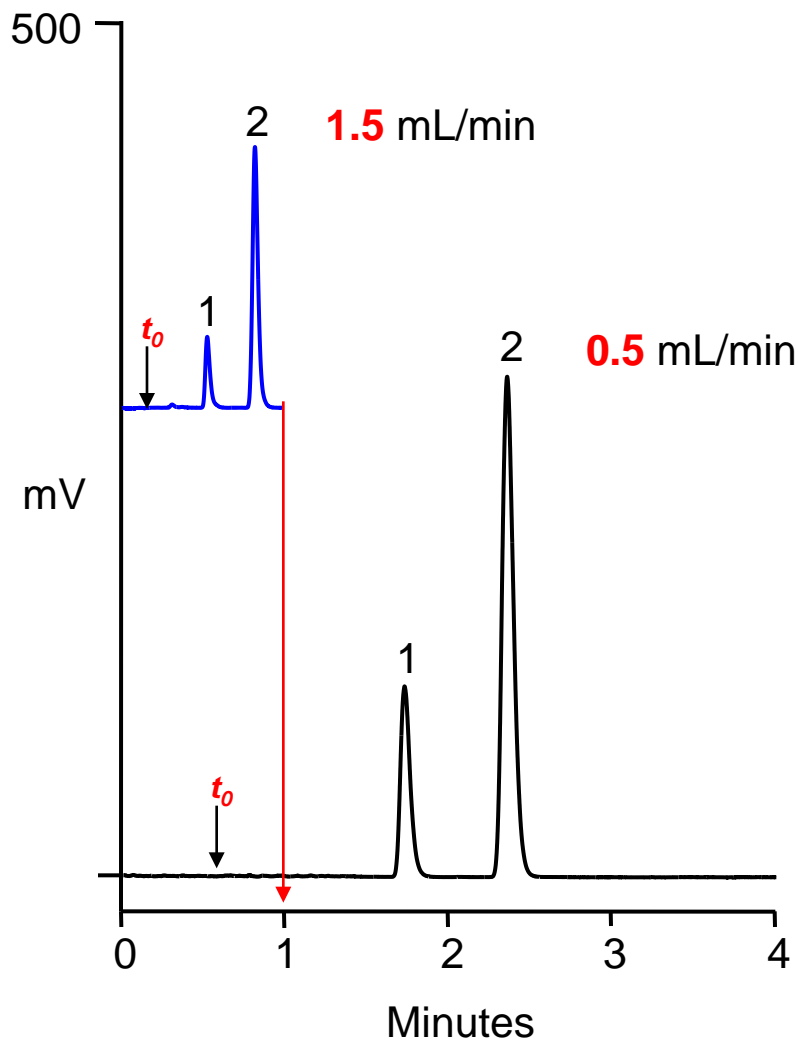
# Gradient Separation of Pharmaceutical Counterions



Column: Acclaim Trinity P1, 3  $\mu$ m  
 Dimensions: **3.0 x 50 mm**  
 Mobile Phase: A - CH<sub>3</sub>CN, B - D.I. H<sub>2</sub>O, C - 0.2 M NH<sub>4</sub>OAc, pH4  
 Gradient:  
 Time (min)    -10    0    2    7    15  
                   A    60    60    60    10    10  
                   B    35    35    35    0    0  
                   C    5    5    5    90    90  
 Temperature: 30 °C  
 Flow Rate: 0.5 mL/min  
 Inj. Volume: 5  $\mu$ L  
 Detection: ELS detector (Charged Aerosol as Preferred Alternative)  
 Peaks: (80 to 150 ppm)

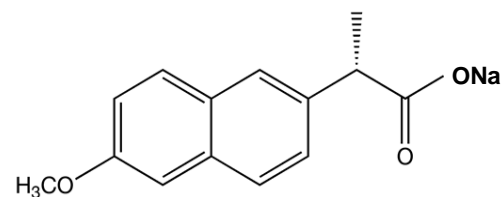
- |                 |               |
|-----------------|---------------|
| 1. Procaine     | 9. Chloride   |
| 2. Choline      | 10. Bromide   |
| 3. Tromethamine | 11. Iodide    |
| 4. Sodium       | 12. Phosphate |
| 5. Potassium    | 13. Malate    |
| 6. Meglumine    | 14. Tartrate  |
| 7. Mesylate     | 15. Citrate   |
| 8. Maleate      | 16. Oxalate   |

## Hydrophobic Acidic API and Counterion – Na, Naproxen



Column: Acclaim Trinity P1, 3  $\mu$ m  
Dimensions: 3.0 x 50 mm  
Mobile Phase: 75/25 v/v CH<sub>3</sub>CN/30 mM (total) NH<sub>4</sub>OAc, pH5  
Temperature: 30 °C  
Flow Rate: 0.5 and 1.5 mL/min  
Inj. Volume: 2.5  $\mu$ L  
Detection: CAD  
Sample: Na, Naproxen (0.5 mg/mL in mobile phase)  
Peaks:

1. Na<sup>+</sup>
2. Naproxen





# Acclaim<sup>®</sup> Trinity Application: Advil ALLERGY and SINUS

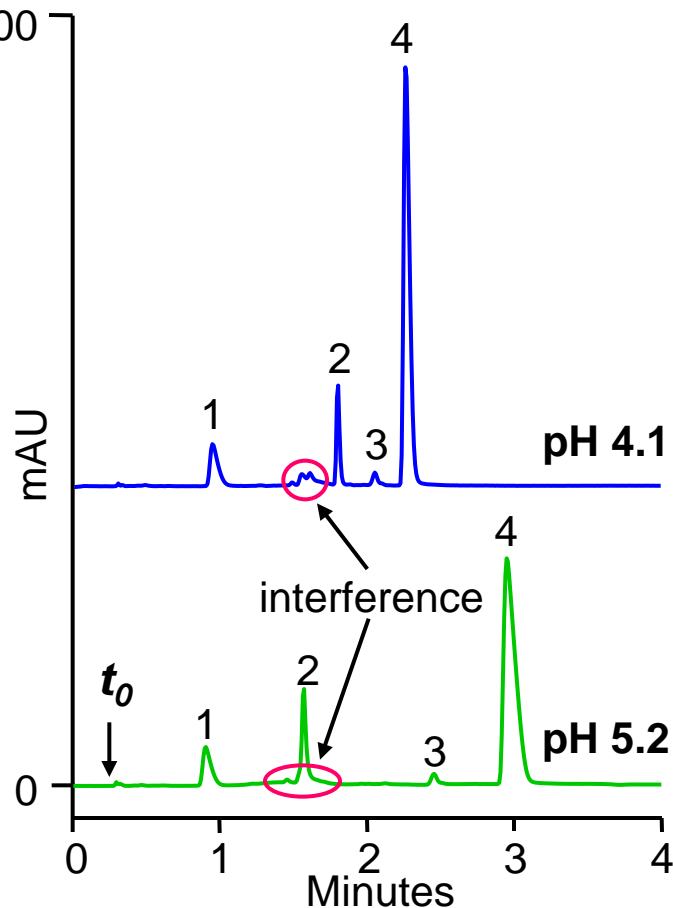
(pH Effect)

Column: Acclaim Trinity P1, 3  $\mu$ m  
 Dimensions: 3.0 x 50 mm  
 Mobile Phase: A: CH<sub>3</sub>CN  
 B: D.I. H<sub>2</sub>O  
 C: 0.2 M NH<sub>4</sub>OAc, pH 5.2  
 Gradient:  
 Time (min)      -4   0   0.1   1   4  
 A: 25 25 25 80 80  
 B: 65 65 65 0 0  
 C: 10 10 10 20 20

Sample Prep: 1. Ground one tablet (~0.49 g) into fine powder  
 2. Weigh 20 mg of above powder in a 20-mL sample vial  
 3. Add 10 mL of MeCN/H<sub>2</sub>O (v/v, 1/1) solution  
 4. Sonicate the vial containing sample suspension at 40 °C for 30 min  
 5. Filter the extract with 0.2- $\mu$ m membrane filter

Temperature: 30 °C  
 Flow Rate: 1.0 mL/min  
 Inj. Volume: 2  $\mu$ L  
 Detection: UV, 254 nm

Peaks: 1. Pseudo-ephedrine  
 2. Chlorpheniramine  
 3. Maleate  
 4. Ibuprofen



\* Background subtraction applied

1. Column Efficiency and Selectivity

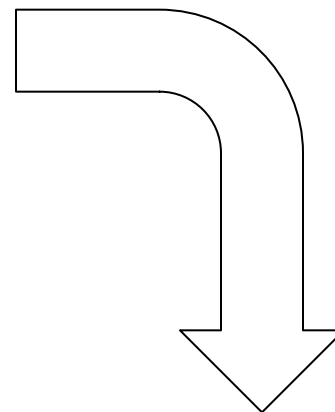
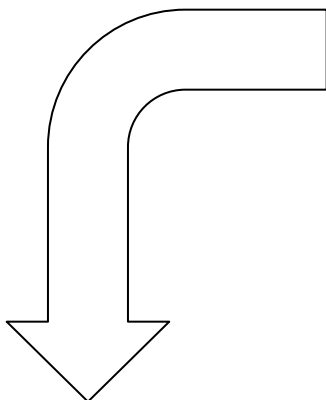
2. Your Workflow and Our Automation Tools

3. Boosting Nebulizer Based Detectors

## (Binary) RSLC (2008)



- Best flow-pressure footprint
- Brought Dionex up to UHPLC
- Full conventional LC capability



## Quaternary RSLC



- Gradient Flexibility
- Cost-Effectiveness

## x2 Dual RSLC



- Boost system use time
- Boost throughput
- Integrate sample prep
- Comprehensive LC

# We Introduced Two Awesome Pump Modules



**LPG-3400RS – A True Quaternary UHPLC Pump**

- Low-pressure gradient proportioning of up to four solvents
- Integrated 4 channel degasser
- Provides full application flexibility
- Supports ultrafast automated method scouting

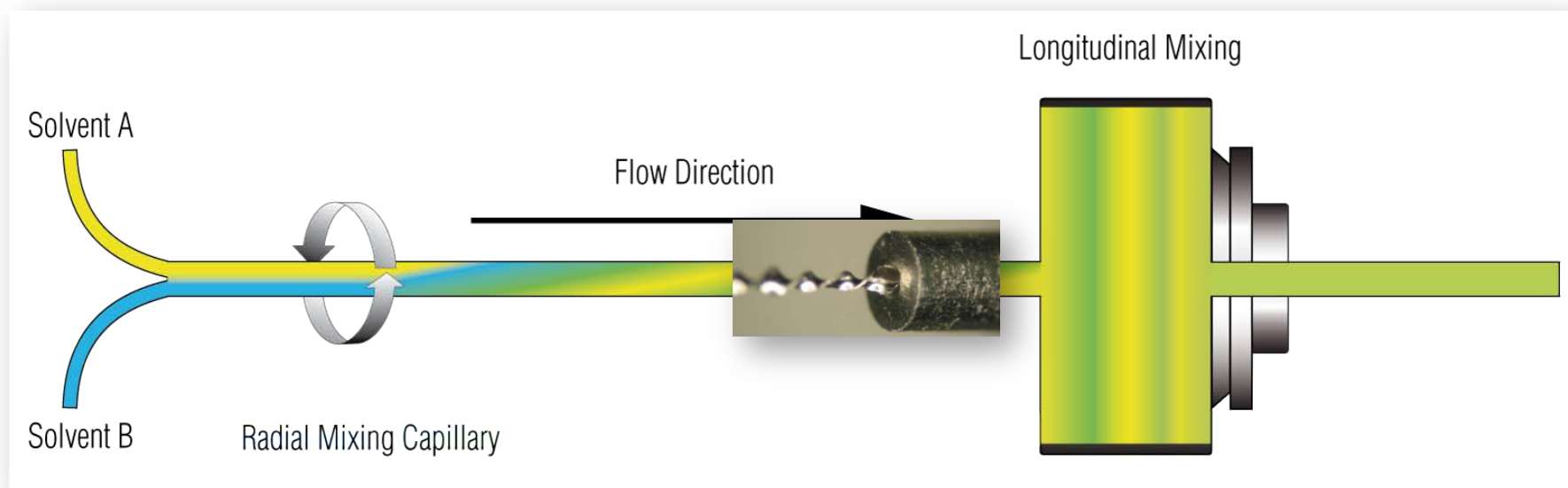
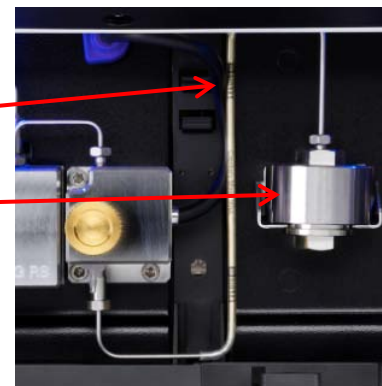


**DGP-3600RS – Unique Dual-Gradient UHPLC Pump**

- Two ternary UHPLC pumps in one
- Supports x2 Dual LC techniques
  - Automated application switching
  - Parallel RSLC (2 systems in one)
  - Tandem RSLC
  - Multidimensional LC (max. resolution in 1<sup>st</sup> dimension and max. speed in 2<sup>nd</sup> dimension)

# The Powerful Two-stage SpinFlow™ Design

- Concept as introduced with HPG-3x00RS
  - Initial small volume radial mixing
  - Variable sized longitudinal mixing
- Outstanding mixing-per-volume performance
- Patent-pending



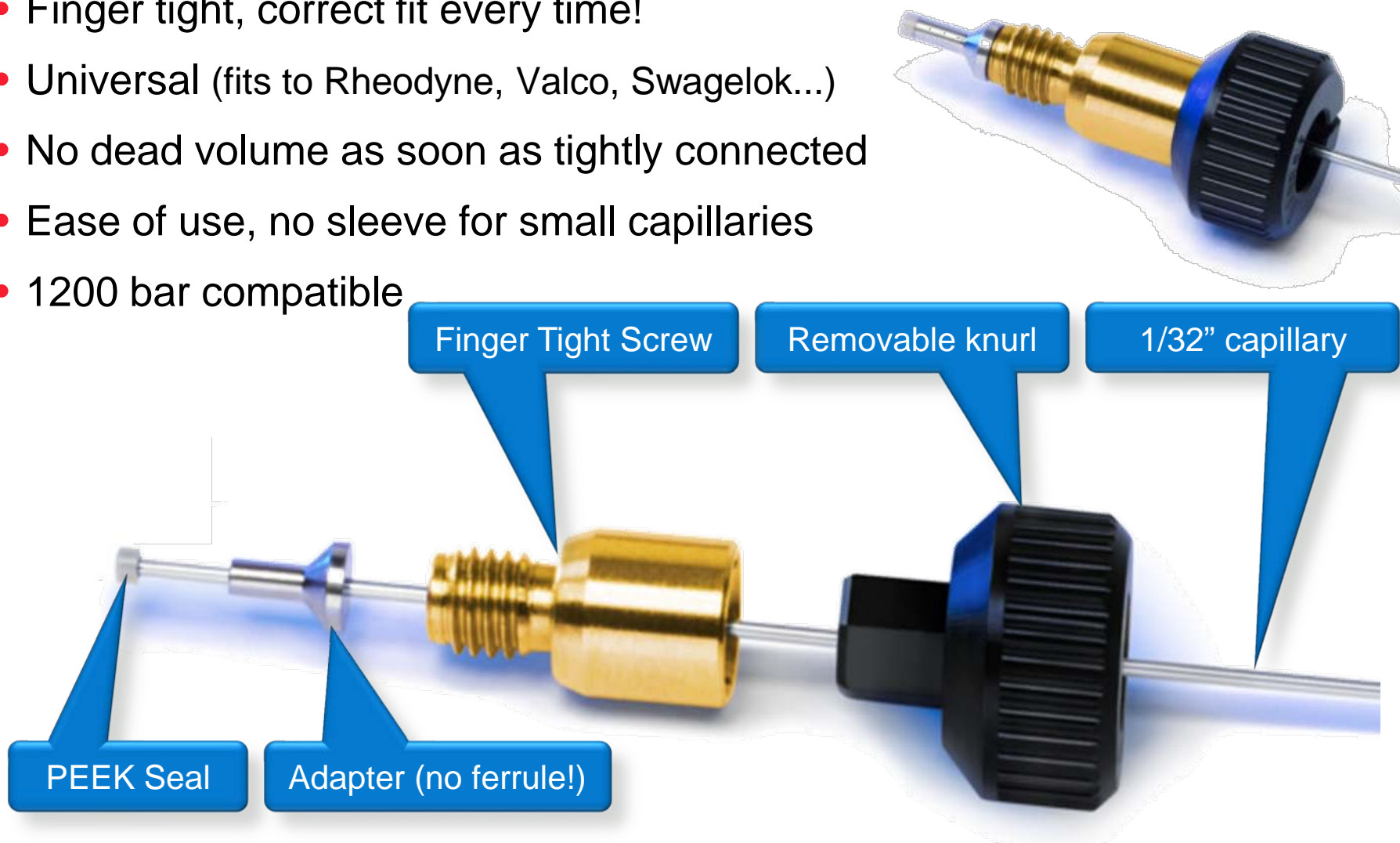
# The Powerful Two-Stage SpinFlow™ Design



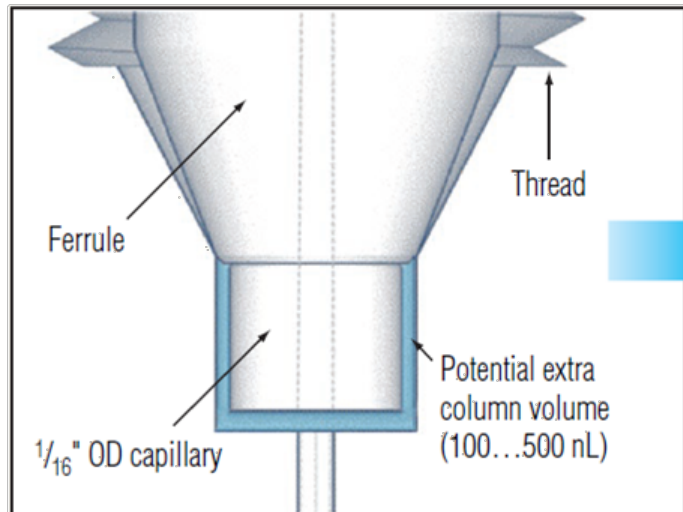
<b>Total Mixing Volume</b>	<b>Capillary Mixer Volume</b>	<b>Static Mixer Volume</b>
35 µL	25 µL	10 µL In-line Filter
100 µL	25 µL	75 µL
200 µL <sup>o</sup>	50 µL	150 µL
400 µL*	50 µL	350 µL
800 µL	50 µL	750 µL
1550 µL	50 µL	1500 µL

# Viper™ Finger Tight Fitting System

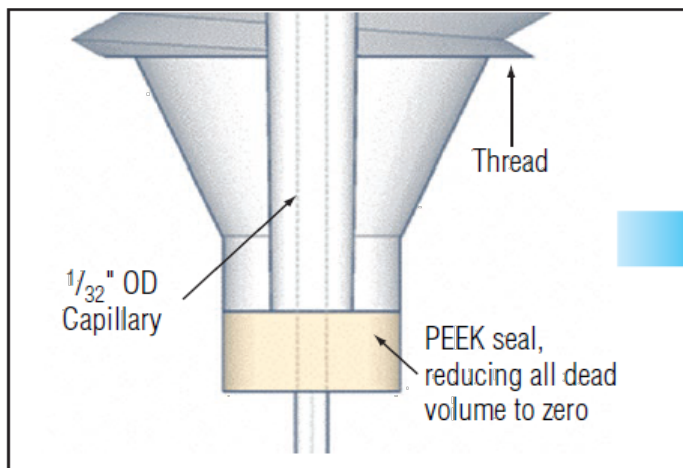
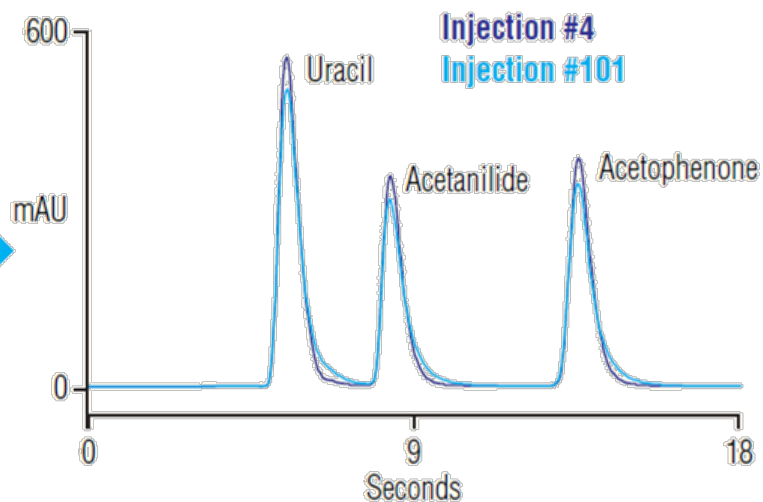
- Finger tight, correct fit every time!
- Universal (fits to Rheodyne, Valco, Swagelok...)
- No dead volume as soon as tightly connected
- Ease of use, no sleeve for small capillaries
- 1200 bar compatible



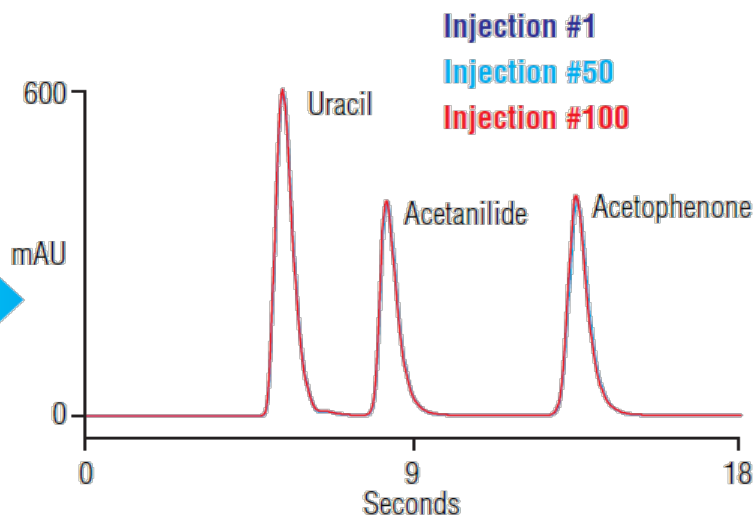
# Viper™ Performance at 600bar/8700psi



Standard finger tight fitting



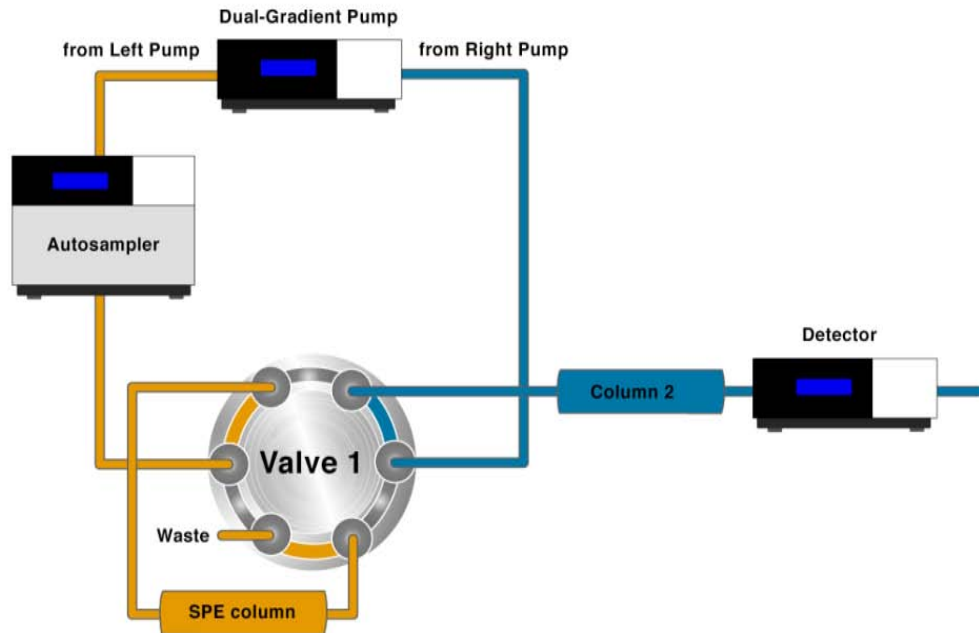
VIPER





- Eliminate Matrix
- 2 x UHPLC Systems in One
- Gradient Methods and Tandem Operation
- (Automated Method Scouting)
- Automated 2D-Offline Fractionation for Biotherapeutics
- System Startup and Shutdown

# Intelligent (UHP)LC Solution for On-line SPE-LC



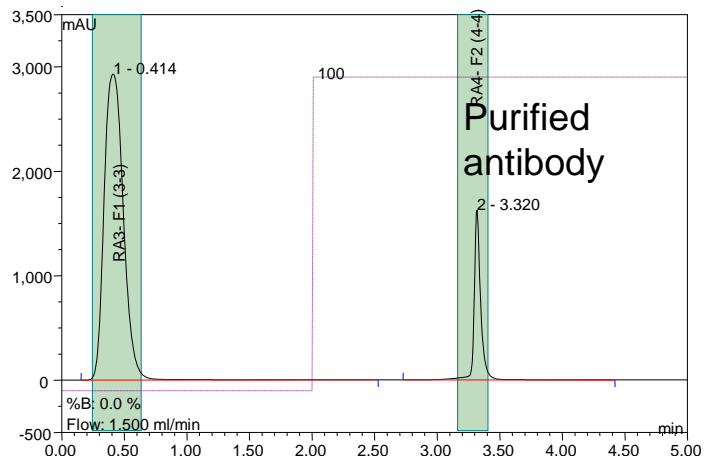
- First dimension SPE (e.g., RAM) column separates analytes from matrix
  - After transfer to an analytical column, analytes are separated and detected
  - Unique Chromeleon on-line SPE-LC wizard helps develop methods
- ⇒ Allows direct injection of complex samples, e.g., biological fluids, surface water
- ⇒ Faster, more precise, cheaper and better automated than alternative techniques

- Dual valve autosampler (bioinert fluidics)
- Optimized for automated workflows e.g.:
  - (Off-line) 2D-LC
  - Protein purification combining different modes of LC and Protein purification recovery studies.
  - Sample fractionation and desalting prior to MS detection
  - Sample derivatization, e.g. digestion, neutralization, in-between LC separations



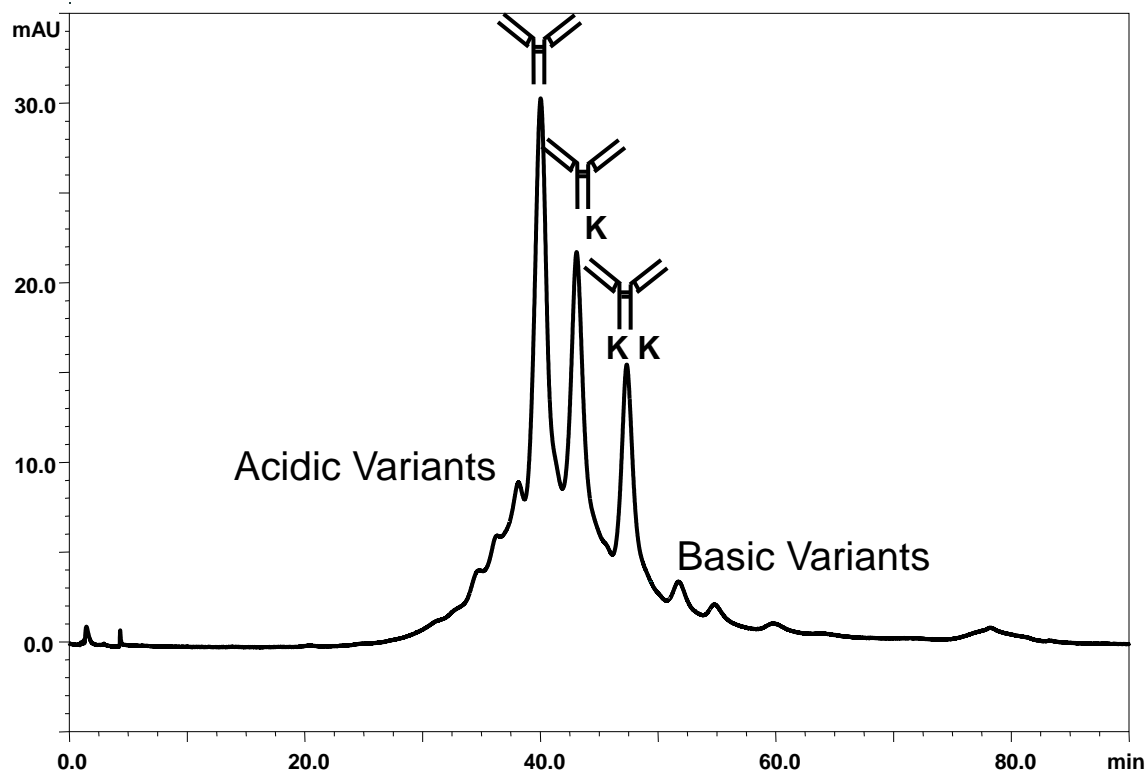
## Key Technology for Advanced 2D Offline Workflows

# Automated Antibody Affinity//IEX: Antibody Purification and Isoform Analysis



second dimension  
WCX ion-exchange analysis

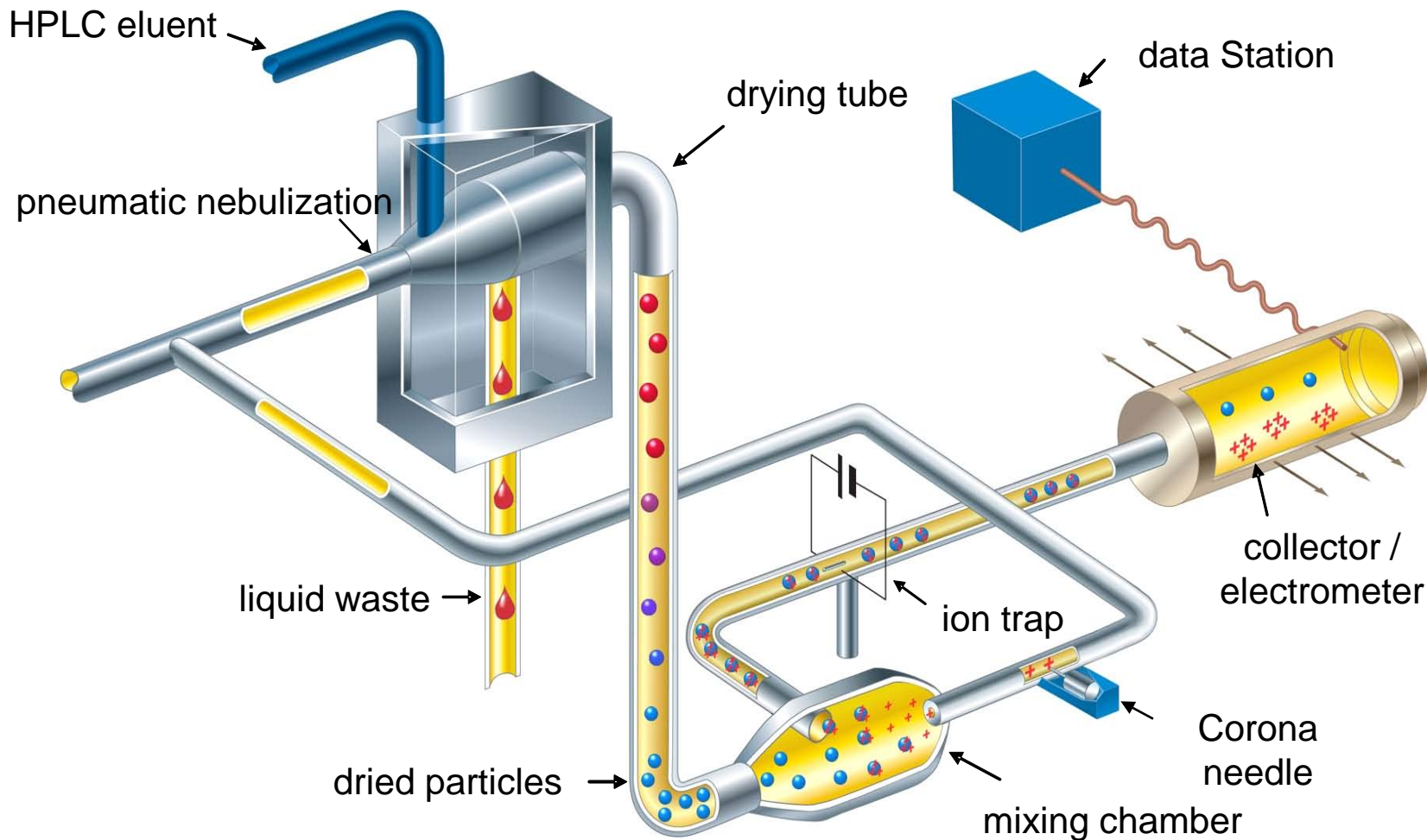
first dimension – protein A  
antibody affinity purification and collection



1. Column Efficiency and Selectivity
2. Your Workflow and Our Automation Tools
3. Boosting Nebulizer Based Detectors

- Stabilize Mobile Phase Viscosity
- Stabilize Nebulizer and Droplet Efficiency
- Stabilize Ionization and Detector Response
- Eliminate Baseline Drifts and Shifts

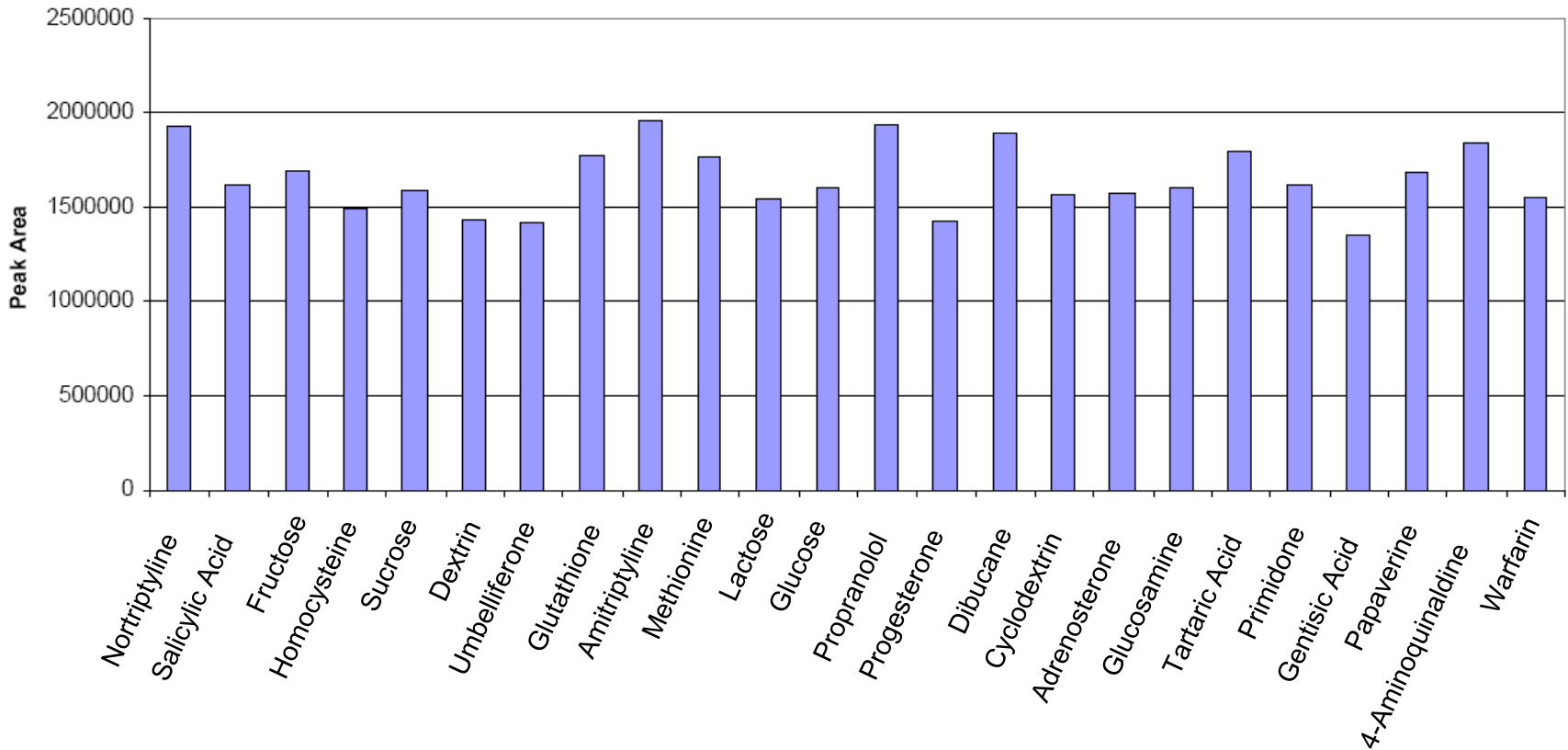
# Corona CAD – Inside The Engine



# Universal Detector for Non-volatiles!

➤ 10.7% RSD variation in CAD response among non-volatile analytes

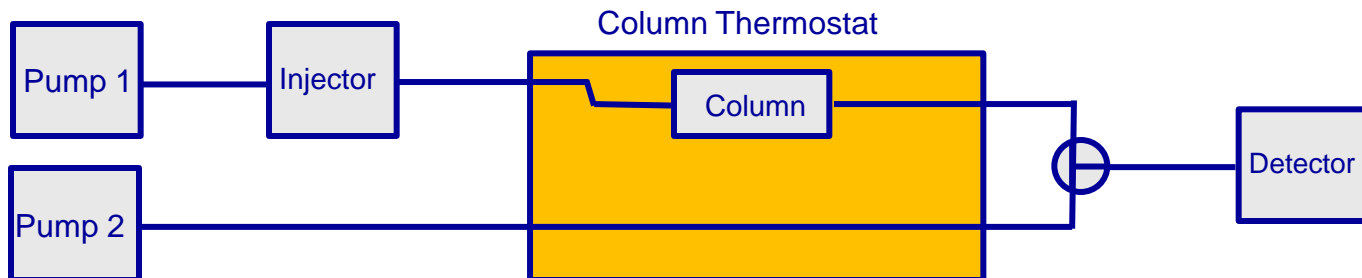
CAD Response, 1.0µg by flow Injection



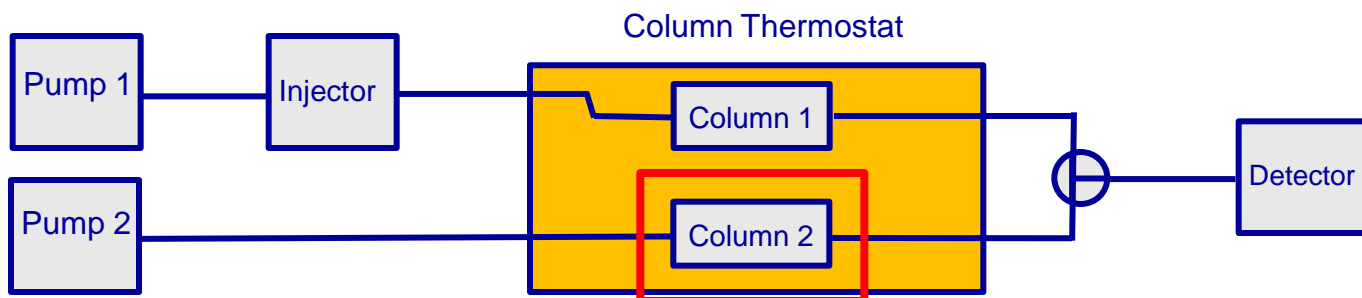


# Implementation of Inverse Compensation Gradient

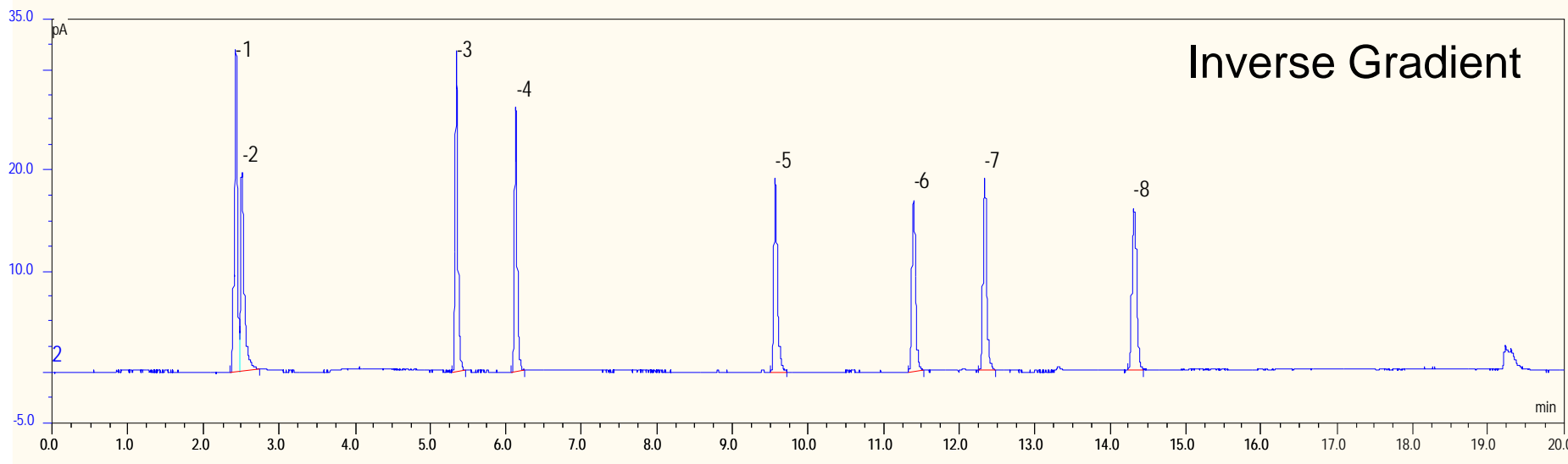
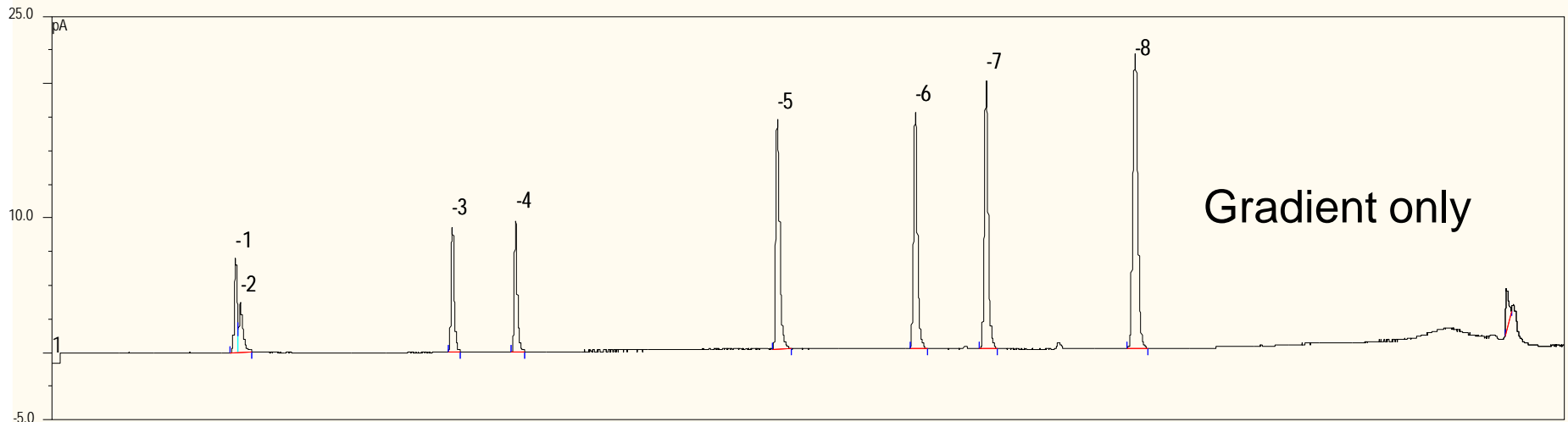
1. Second liquid branch with given tubing volume and delayed gradient profile (to compensate for column)



2. Second liquid branch includes identical column



# Improved Quantification with Inverse Gradient



Smart Method Development Using Column Efficiency And Column Selectivity

Use a Straight Forward and Easy to Establish Strategy to Migrate LC Methods to Faster UHPLC Separations

Utilize a Wide Detection Offering to Separate Chromophores and Non-Chromophores

Clever Hardware and Software Allows Us to Speed Up Our Processes Outside the (UHP)LC (Workflow Integration)

Good Luck and Have Fun!

---

**UHP<sup>+</sup>LC**  
**focused**