Holistic HPLC Analytical Strategies for Pharmaceutical Labs

UHPLC+ Focused LC Product Portfolio

RSLCnano

RSLC

CMS Software

Viper Fitting System

UHPLC+ focused

Standard LC

Basic Automated

Corona *ultra*

Acclaim Columns

ThermoFisher SCIENTIFIC

Overview

1. Column Efficiency and Selectivity

2. Your Workflow and Our Automation Tools

3. Boosting Nebulizer Based Detectors

Overview

1. Column Efficiency and Selectivity

2. Your Workflow and Our Automation Tools

3. Boosting Nebulizer Based Detectors

Goals in Modern HPLC

- Faster Analysis (typically 10X faster)
  - Increased productivity (more samples/day)
  - Higher instrument ROI (more samples/system)
  - Faster results (e.g. decreased time to market)
  - Increased data content (more data in the same time)
- Less/Faster Method Development
  - Automated Method Scouting
  - Higher Resolution for More Generic Methods
  - Quick Project Turnover
- Reduced Solvent Consumption (typically 10X reduction)
  - Lower solvent acquisition costs
  - Lower solvent disposal costs
  - Reduced environmental impact

Harnessing Efficiency Improvement?

Efficiency
-Using Improved Diffusion Properties And Plates-

Efficiency
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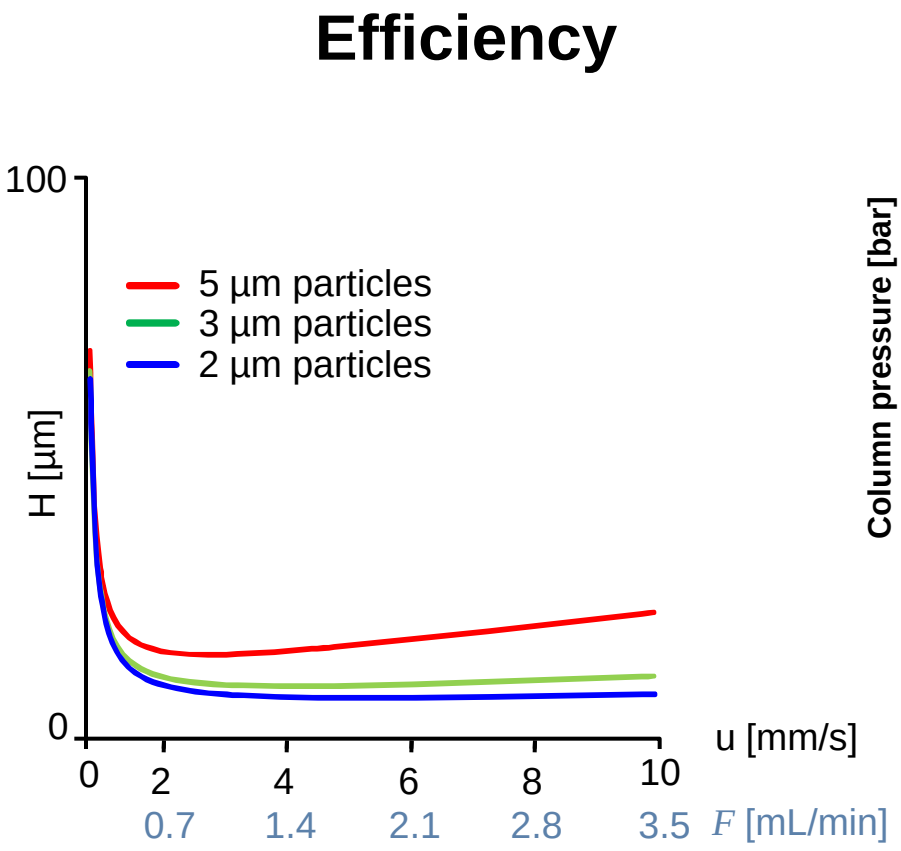
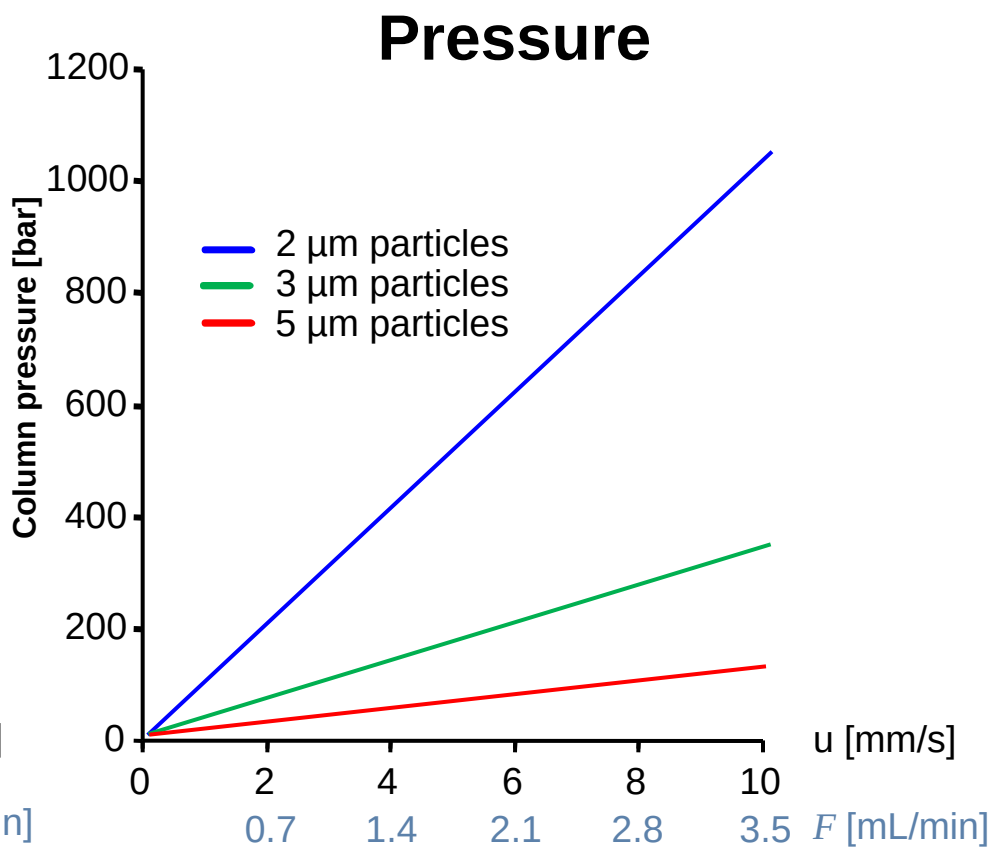
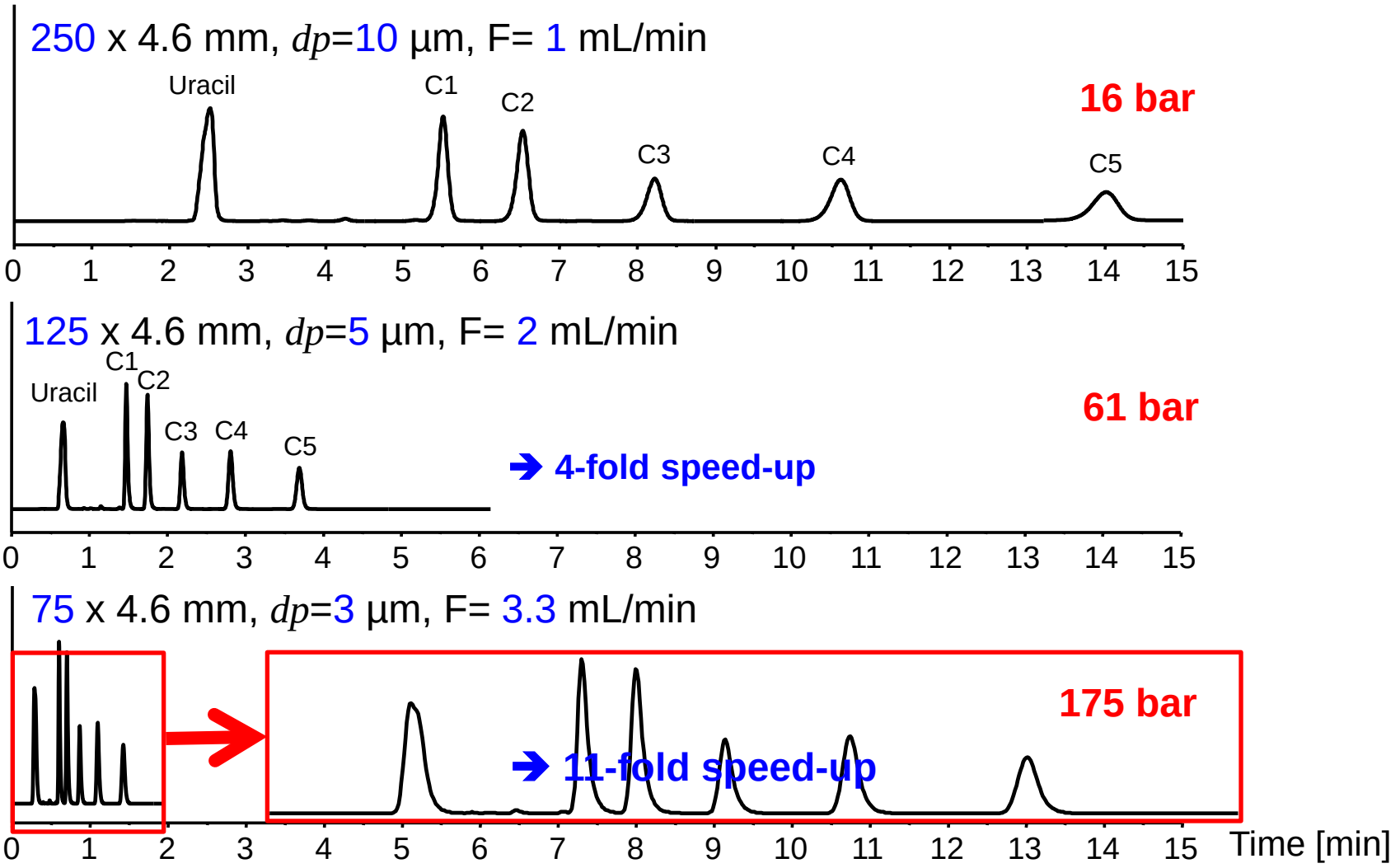




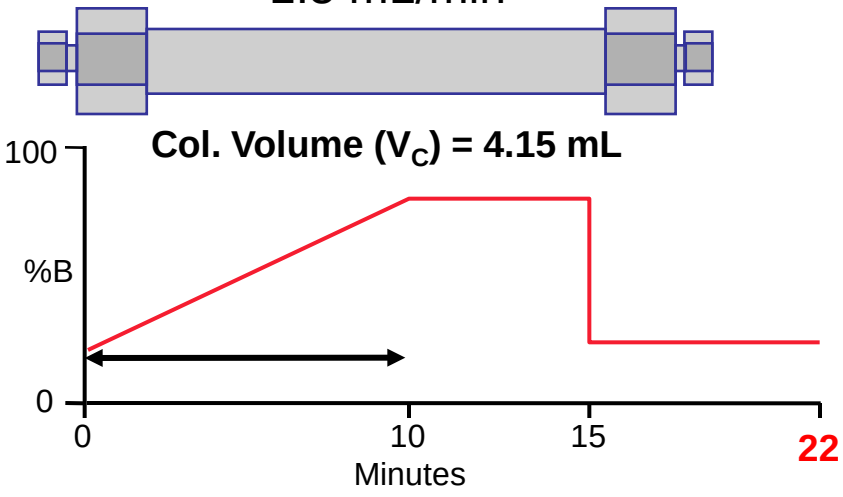
*Linear velocity translated to flow rate for a 3 mm i.d. column

Reducing L and dp for Faster Separations

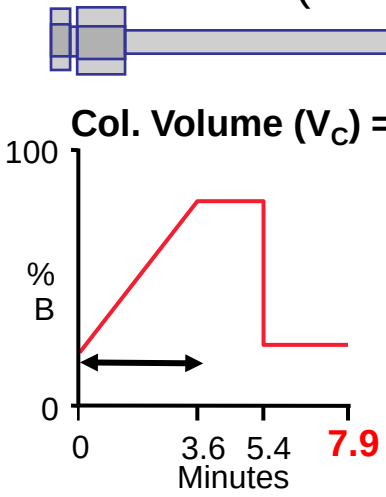


How to Adapt the Gradient Program?

4.6 x 250 mm Column, 5 µm
1.5 mL/min



2.1 x 150 mm Column, 3 µm
0.52 mL/min (Increased *u*)



Original First Gradient Step 0–10 min

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

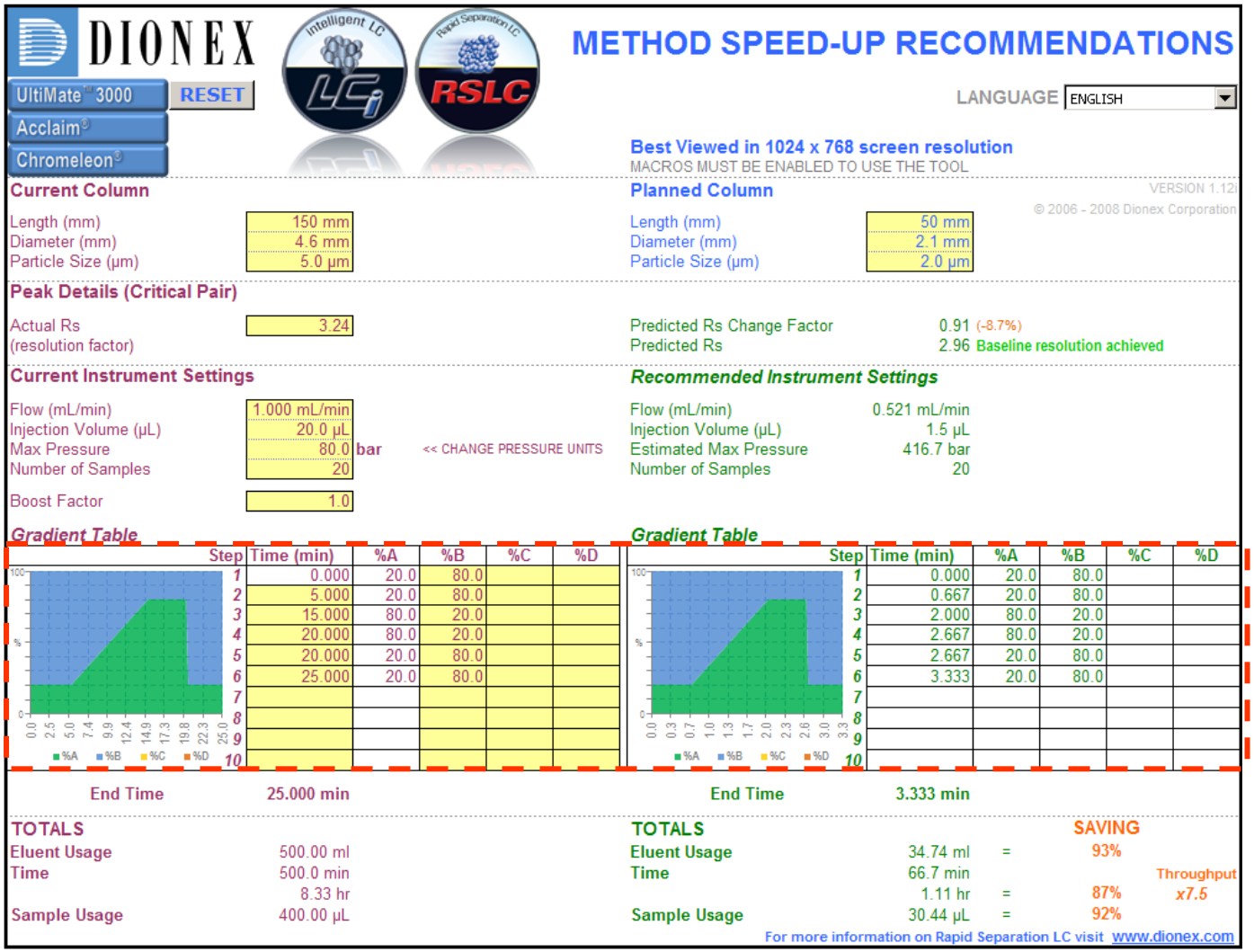
t_G = 10 min
F = 1.5 mL/min
V_C = 4.15 mL

New First Gradient Step 0–? min

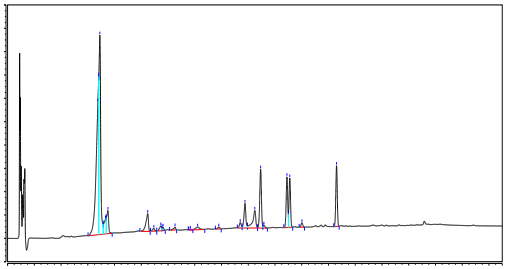
$$t_{G2} = 10 \cdot \frac{1.5}{0.52} \cdot \frac{0.52}{4.15}$$

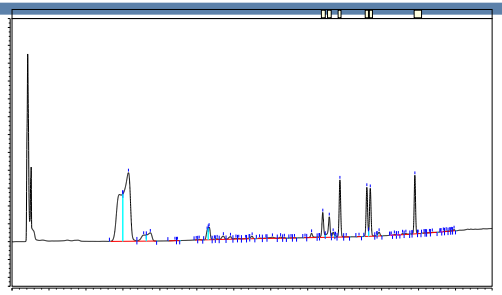
= **3.6 min**

Dionex RSLC Method Speed-Up Calculator

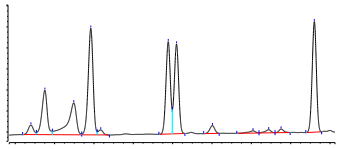


Dionex.com > Products > Liquid Chromatography > LC Solutions > Rapid Separation LC





Potency RS Method Before and After



Harnessing Selectivity Improvement

Selectivity
-Using Increased Chemical Interaction-

Selectivity
-Using Increased Chemical Interaction-

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

R_s – Resolution
N – Theoretical plates
α – Selectivity
k – Retention factor

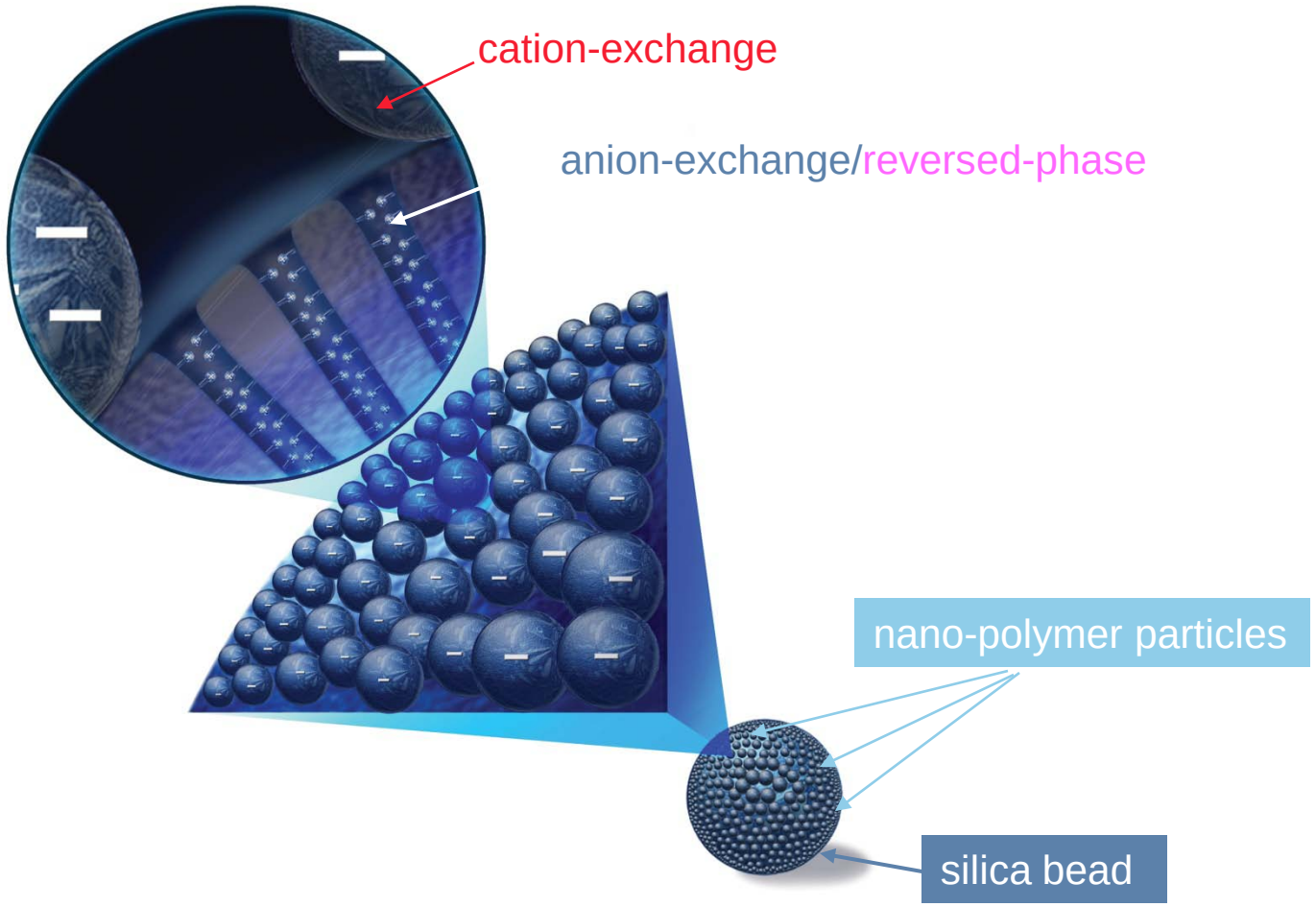
Example:

To separate two analytes on a 5-μm,150 mm C18 column
Assuming N = 10,000 plates/column, k >> 1, and α = 1.04 $\Rightarrow$ R_s = 1

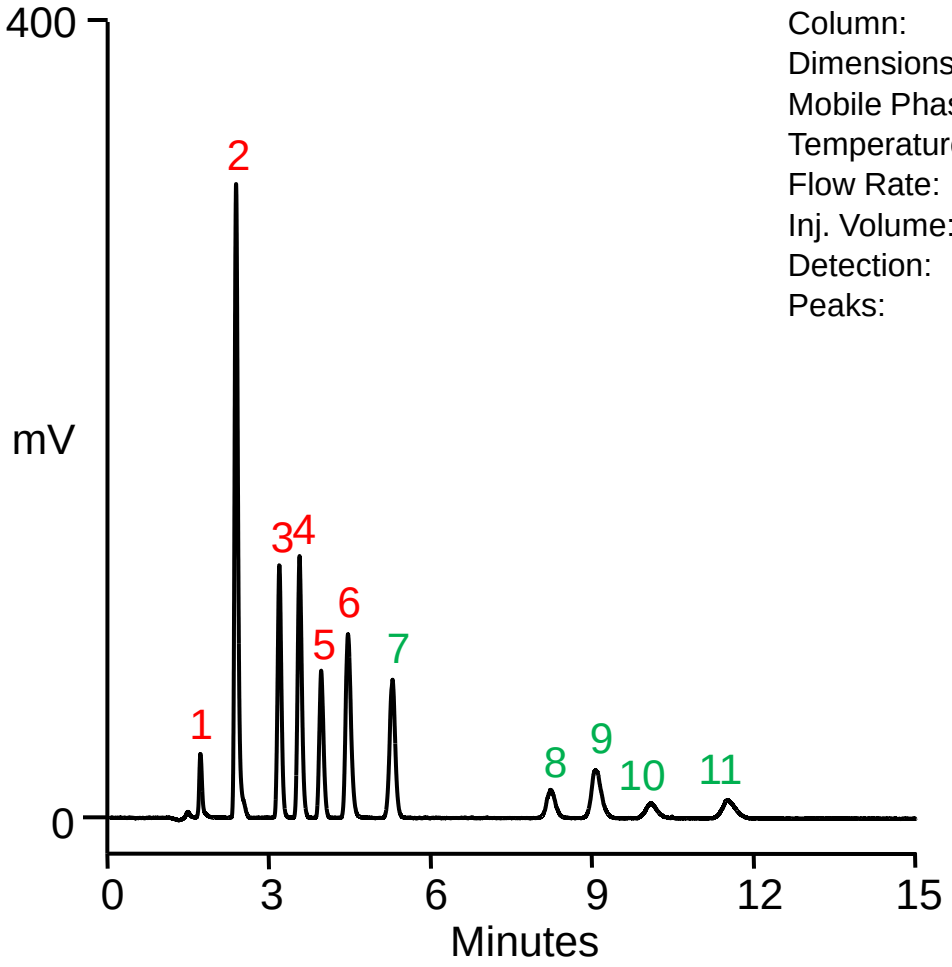
Objective: increase R_s to 2

Approach 1 – increase N needs ***400%*** increase or *N = 40,000* plates
Approach 2 – increase α requires only ***4%*** increase or *α = 1.08*

Acclaim Trinity Nanopolymer Silica Hybrid (NSH) technology



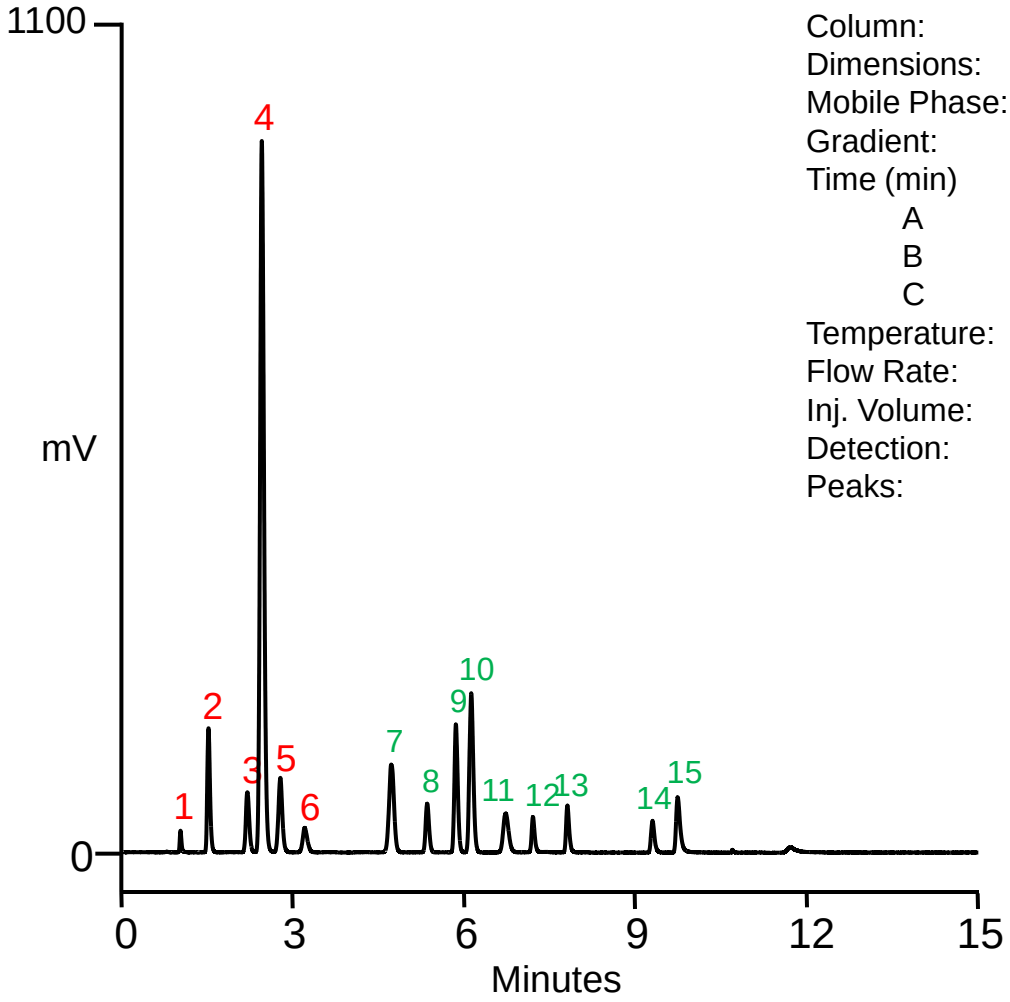
Isocratic Separation of Pharmaceutical Counterions



Column:	Acclaim Trinity P1, 3 µm
Dimensions:	3.0 x 100 mm
Mobile Phase:	60/40 v/v CH_3CN/20 mM (total) NH_4OAc, pH5
Temperature:	30 °C
Flow Rate:	0.5 mL/min
Inj. Volume:	5 µL
Detection:	ELS detector or CAD
Peaks:	(100 to 150 ppm)

1. Procaine
2. Choline
3. Tromethamine
4. Sodium
5. Potassium
6. Meglumine
7. Mesylate
8. Nitrate
9. Chloride
10. Bromide

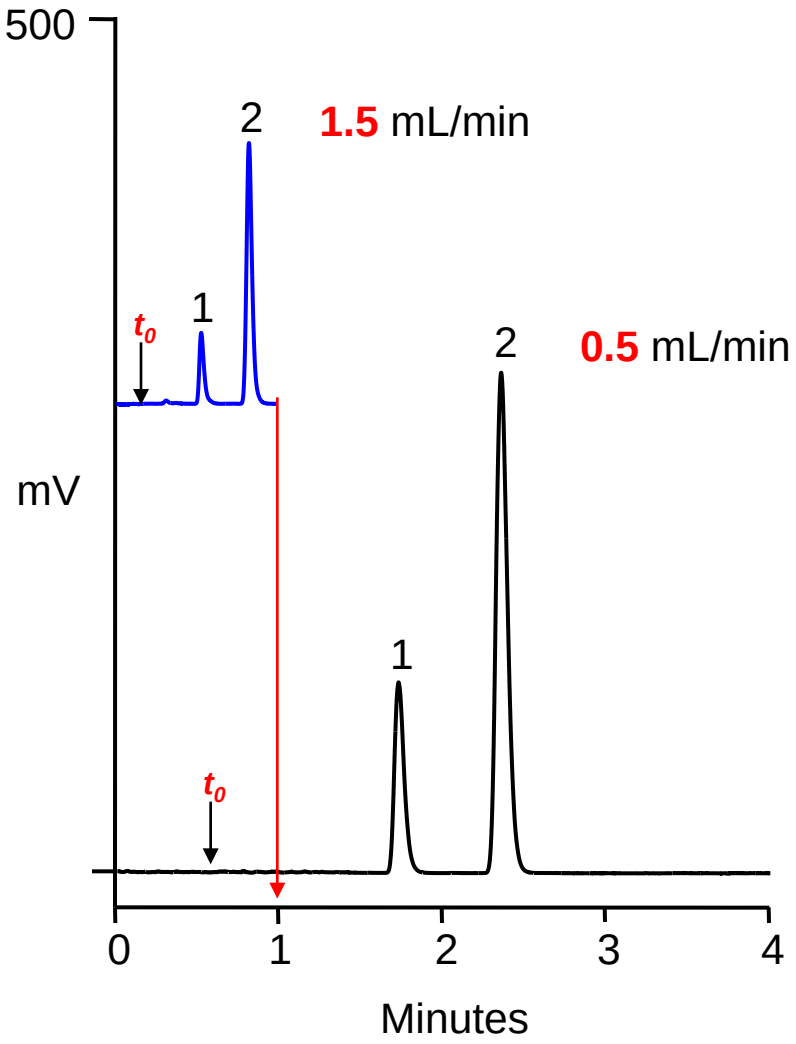
Gradient Separation of Pharmaceutical Counterions



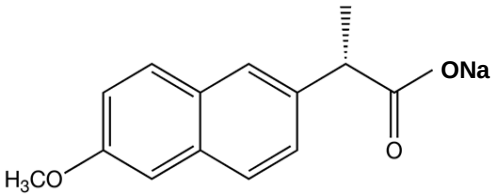
Column:	Acclaim Trinity P1, 3 µm				
Dimensions:	**3.0 x 50 mm**				
Mobile Phase:	A - CH_3CN, B - D.I. H_2O, C – 0.2 M NH_4OAc, pH4				
Gradient:					
Time (min)	-10	0	2	7	15
A	60	60	60	10	10
B	35	35	35	0	0
C	5	5	5	90	90
Temperature:	30 °C				
Flow Rate:	0.5 mL/min				
Inj. Volume:	5 µL				
Detection:	ELS detector				
Peaks:	(80 to 150 ppm)				

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

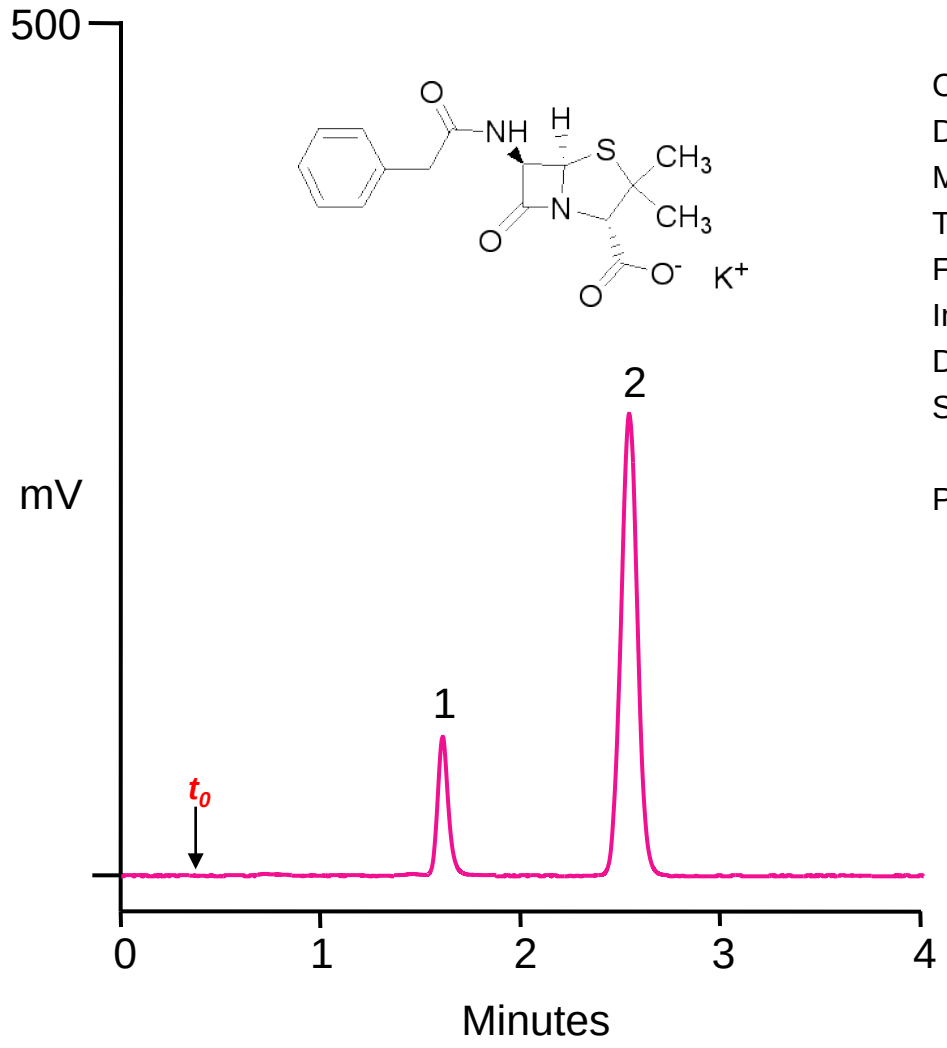
Hydrophobic Acidic API and Counterion – Na, Naproxen



Column:	Acclaim Trinity P1, 3 µm
Dimensions:	3.0 x 50 mm
Mobile Phase:	75/25 v/v CH_3CN/30 mM (total) NH_4OAc, pH5
Temperature:	30 °C
Flow Rate:	0.5 and 1.5 mL/min
Inj. Volume:	2.5 µL
Detection:	ELS detector or CAD
Sample:	Na, Naproxen (0.5 mg/mL in mobile phase)
Peaks:	
	1. Na^+
	2. Naproxen

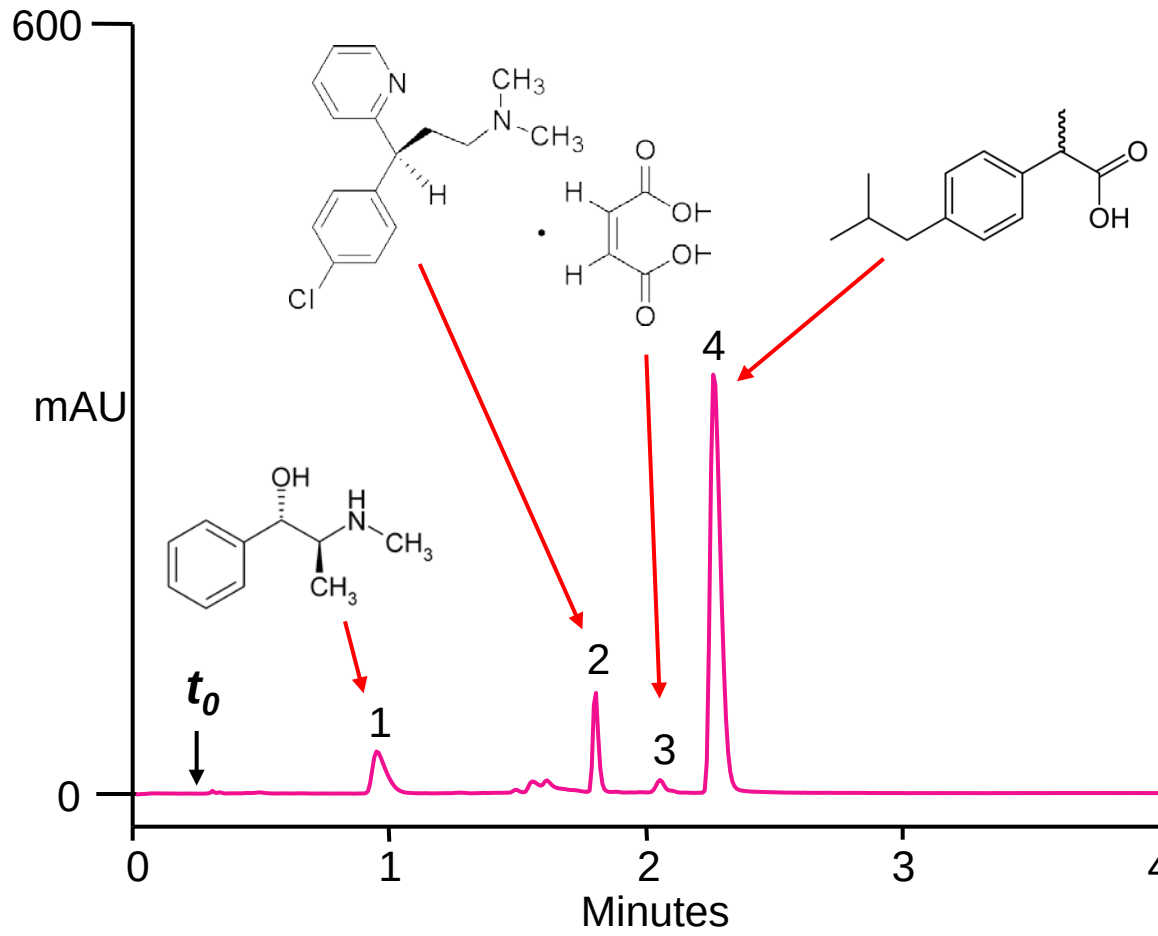


Hydrophilic Acidic Drug and Counterion – Penicillin G, K



Column:	Acclaim Trinity P1, 3 µm
Dimensions:	3.0 x 50 mm
Mobile Phase:	60/40 CH_3CN/20 mM (total) NH_4OAc, pH5.2
Temperature:	30 °C
Flow Rate:	0.6 mL/min
Inj. Volume:	2.0 µL
Detection:	ELS detector or CAD
Sample:	Penicillin G, Potassium Salt (0.5 mg/mL in mobile phase)
Peaks:	1. K^+ 2. Penicillin G

Over-The-Counter Medicine – *Advil ALLERGY and SINUS*



Column: Acclaim Trinity P1, 3 µm
Dimensions: 3.0 x 50 mm
Mobile Phase: A: CH_3CN
B: D.I. H_2O
C: 0.2 M NH_4OAc, pH4.1

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

Temperature: 30 °C
Flow Rate: 0.5 mL/min
Inj. Volume: 2 µL
Detection: UV, 254 nm
Peaks:

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

* Background subtraction applied

Overview

1. Column Efficiency and Selectivity

2. Your Workflow and Our Automation Tools

3. Boosting Nebulizer Based Detectors

All UltiMate 3000 Systems Now Fully UHPLC Compatible

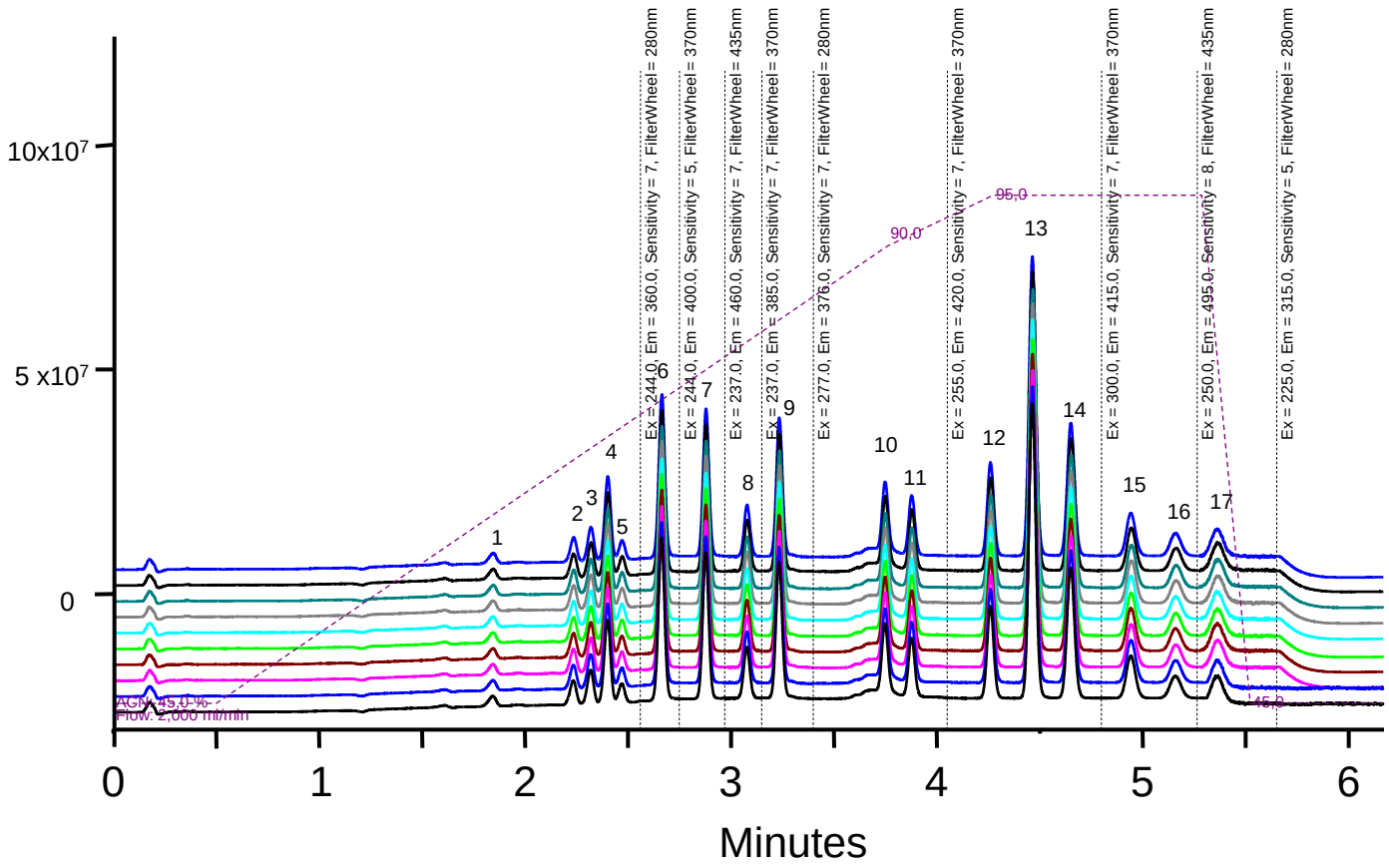
ThermoFisher SCIENTIFIC

•UHPLC Compatible Standard System

- Pressures up to **620 bar** across entire flow range of **10 mL/min**
- Binary, Quaternary, and Dual-Gradient Pumps
- Data Collection Rate of **100 Hz**
- Column Temperatures from **5 – 80 °C**
- Optional switching valves for advanced workflows
- Versatile system for almost any lab

Environmental Application on Binary Standard System

Separation of EPA-PAHs on a 3 µm C18 PAH Column



1. Naphthalene
2. 1-Methylnaphthalene
3. 2-Methylnaphthalene
4. Acenaphthene
5. Fluorene
6. Phenanthrene
7. Anthracene
8. Fluoranthene
9. Pyrene
10. Benzo(a)anthracene
11. Chrysene
12. Benzo(b)fluoranthene
13. Benzo(k)fluoranthene
14. Benzo(a)pyrene
15. Dibenzo(a,h)anthracene
16. Benzo(g,h,i)perylene
17. Indeno(1,2,3-cd)pyrene

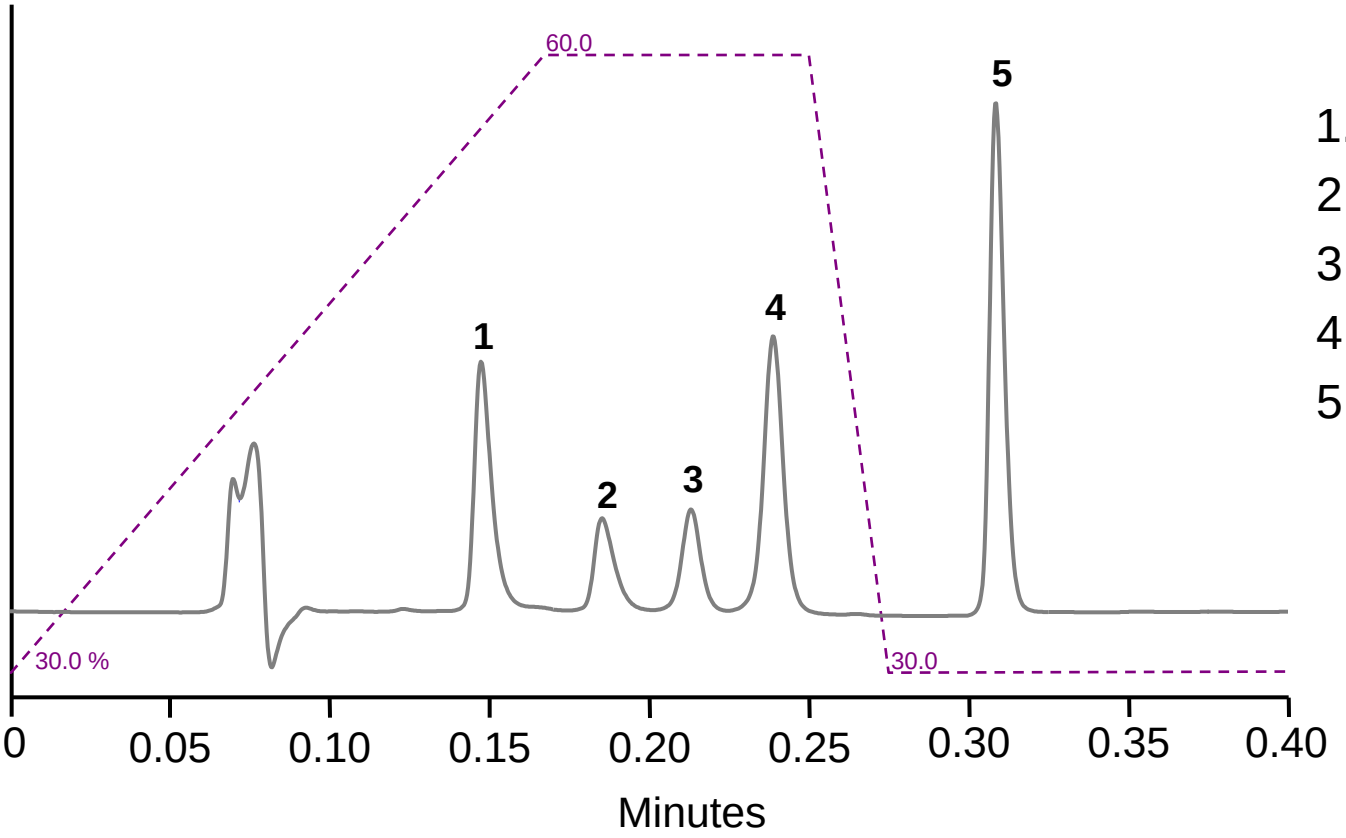
SD Method Parameters:

Analysis time: **6.0 min**, Back pressure: ***510 bar,*** Column: MN Nucleodur C18 PAH, 3 µm, 100 x 3.0 mm
Flow rate: 2.00 mL/min, Column temperature: 30 °C Mobile Phase: A: Water, B: ACN

Maximum UHPLC Performance With The RSLC System

- Pressures up to **1034 bar** at flow rates up to **5 mL/min**
- Pressures of **800 bar** at flows from **5 – 8 mL/min**
- Binary, Quaternary, and Dual-Gradient Pumps
- Data Collection Rate of **200 Hz**
- Column Temperatures from **5 – 110°C**
- Optional switching valves for advanced workflows
- Maximum Performance for Demanding Laboratories

Ultrafast Analysis with the RSLC System



1. Triprolidine
2. Tetracaine
3. Cortisone
4. Dibucaine
5. Furosemide

UHPLC and Standard Pump Capabilities

Binary RSLC and SD modules

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

Quaternary RSLC and SD modules

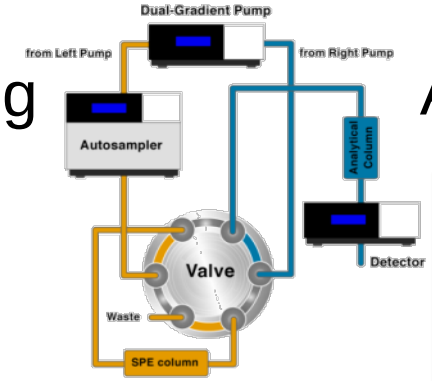
- Worlds first quaternary UHPLC
- Gradient flexibility
- Cost-effectiveness

x2 Dual RSLC and SD modules

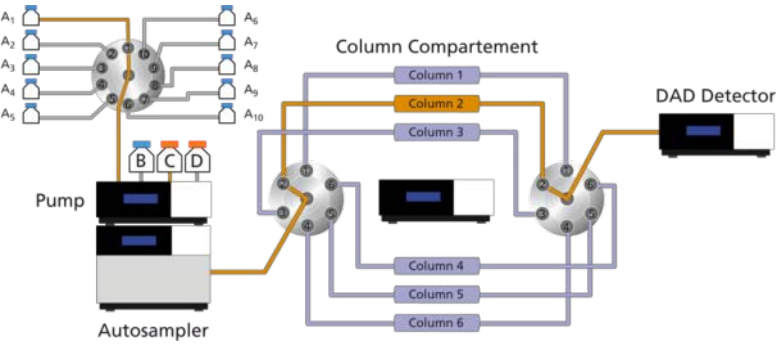
- Unique UHPLC platform
- Advanced chromatographic techniques
- Boost system use time and throughput

UHPLC+ x2 Dual Applications Summary

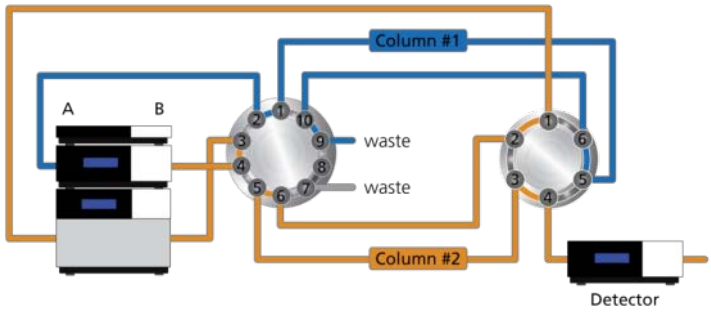
Online SPE

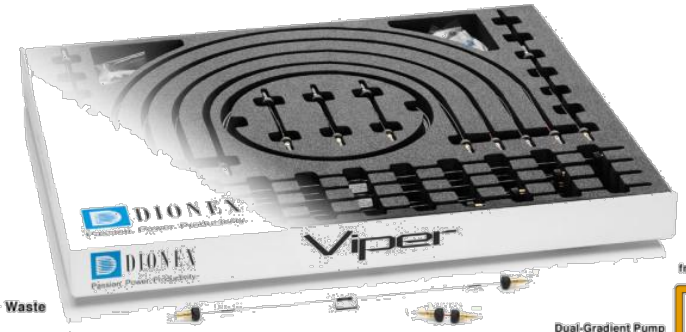


Automated Method Scouting

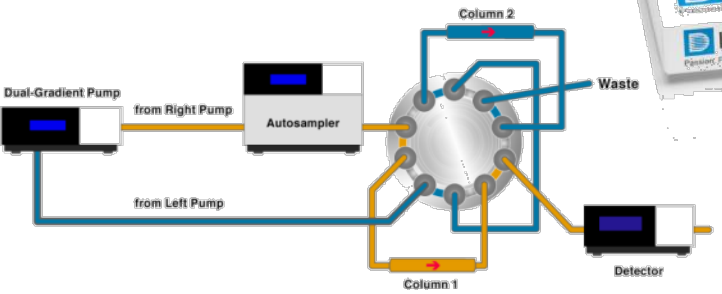


Application Switching

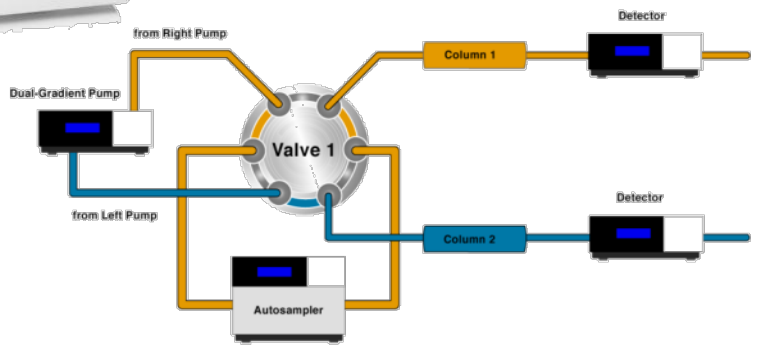




Tandem LC



Parallel LC



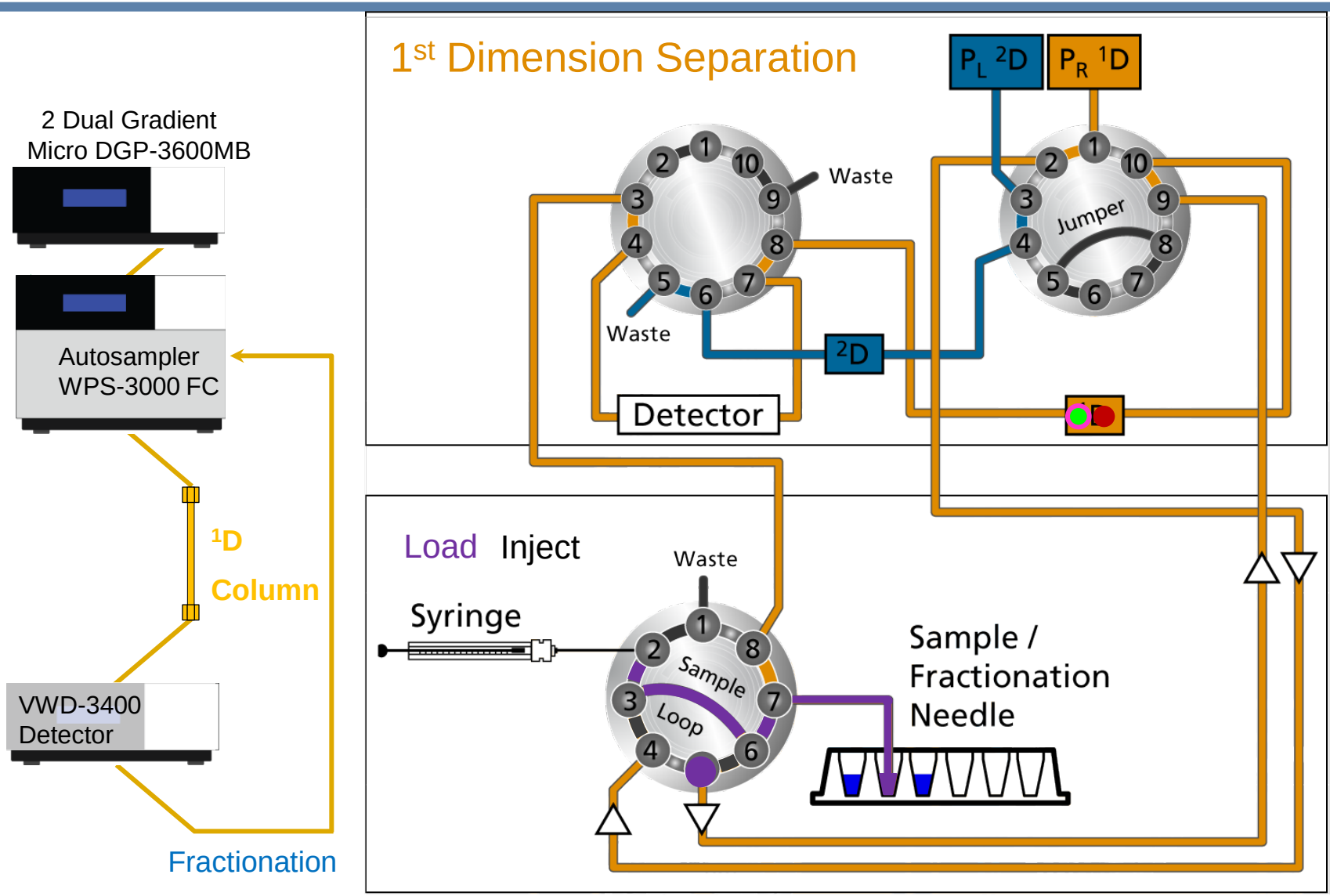
ThermoFisher SCIENTIFIC

WPS-3000 FC
Well-plate Sampler with Fractionation Capabilities

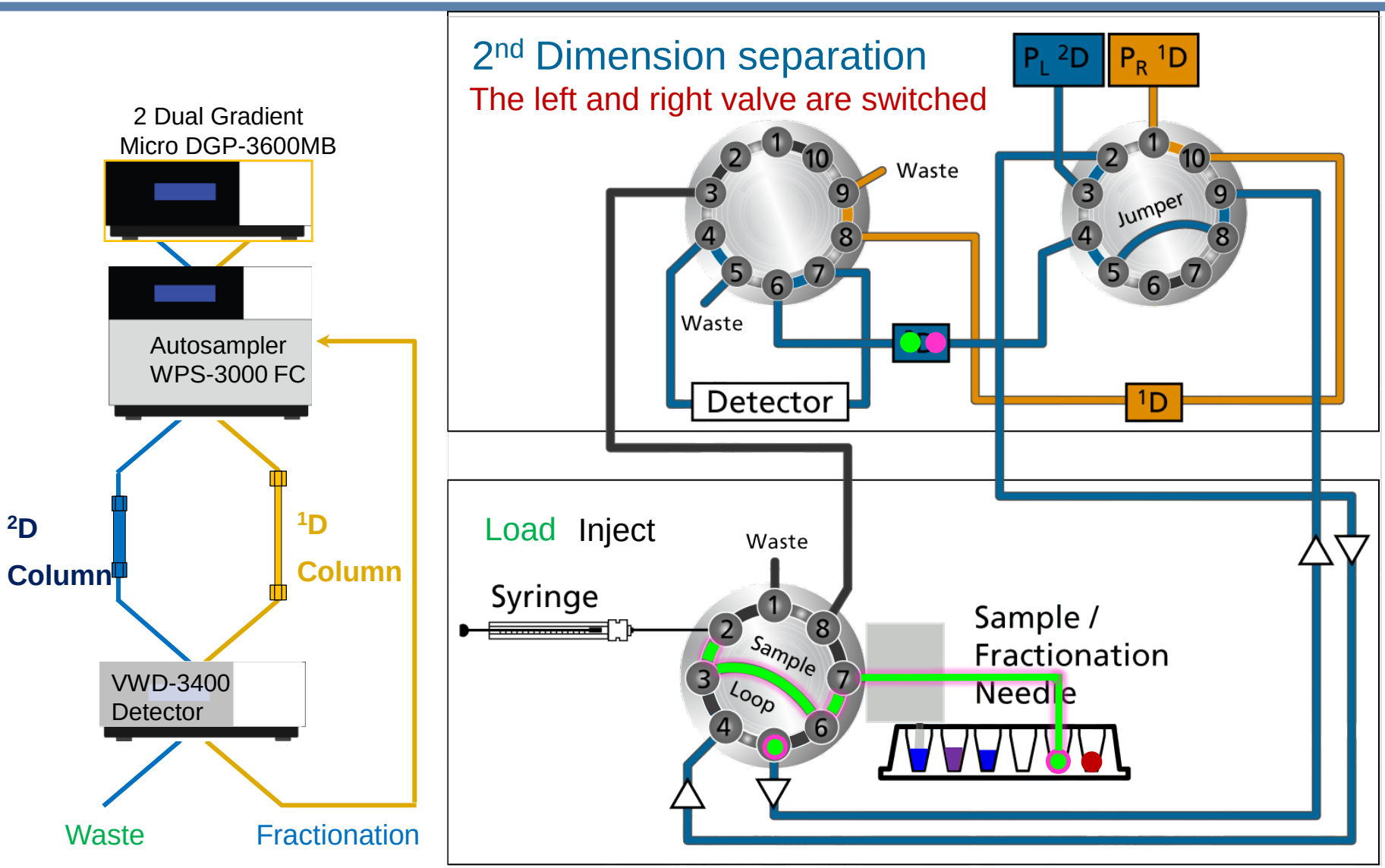
- Dual valve autosampler (bioinert fluidics)
- Optimized for automated workflows *e.g.*:
  - (Off-line) 2D-LC
  - Protein purification combining different modes of LC and Protein purification recovery studies.
  - Sample fractionation and desalting prior to MS detection
  - Sample derivatization, *e.g.*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

Key Technology for Advanced 2D Offline Workflows

Automated Offline 2-D LC Setup

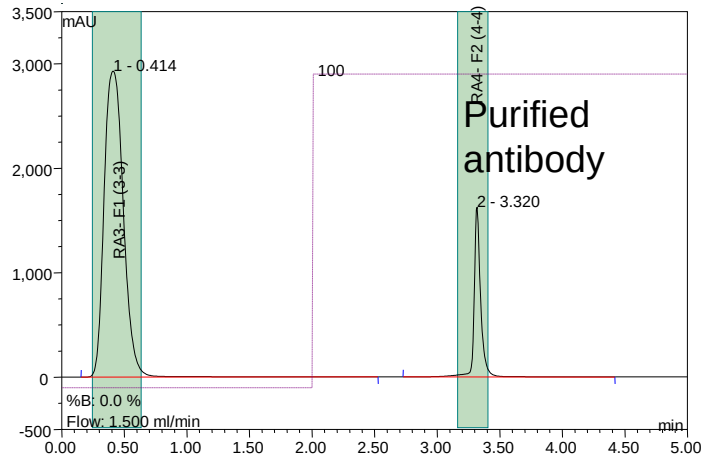


Automated Offline 2-D LC Setup (Cont.)



25053

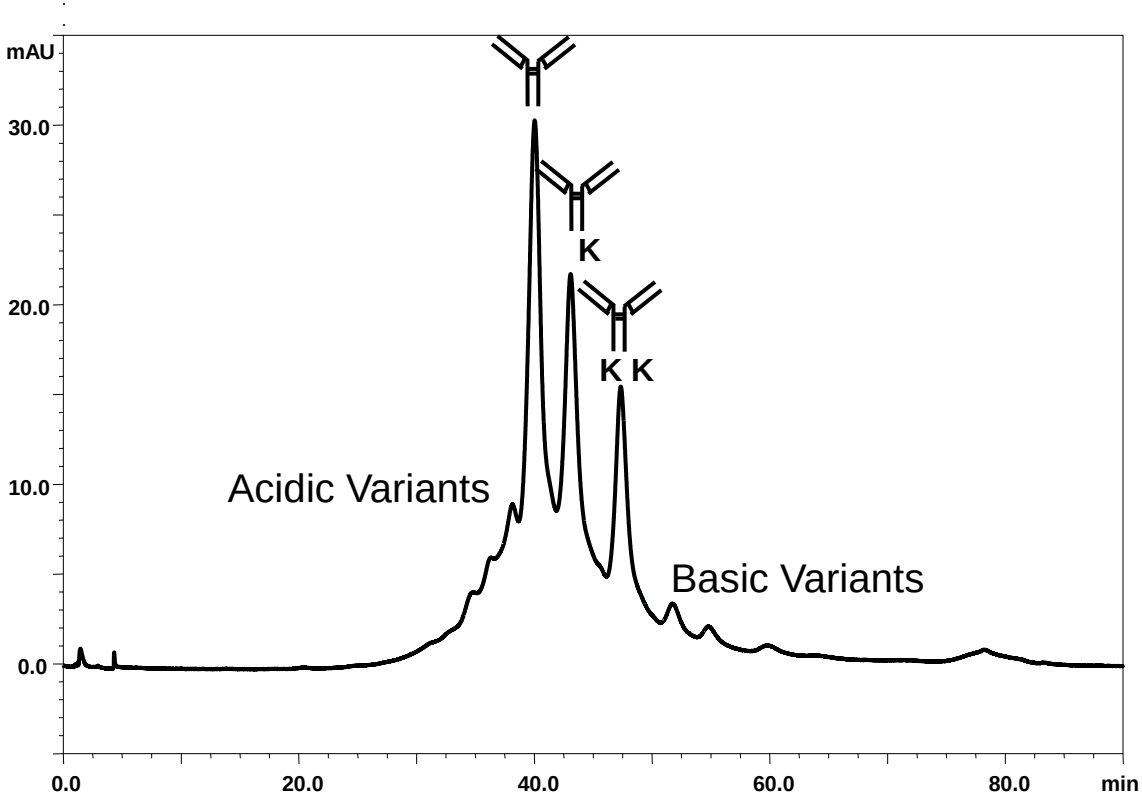
Automated Antibody Affinity//IEX: Antibody Purification and Isoform Analysis



Purified antibody

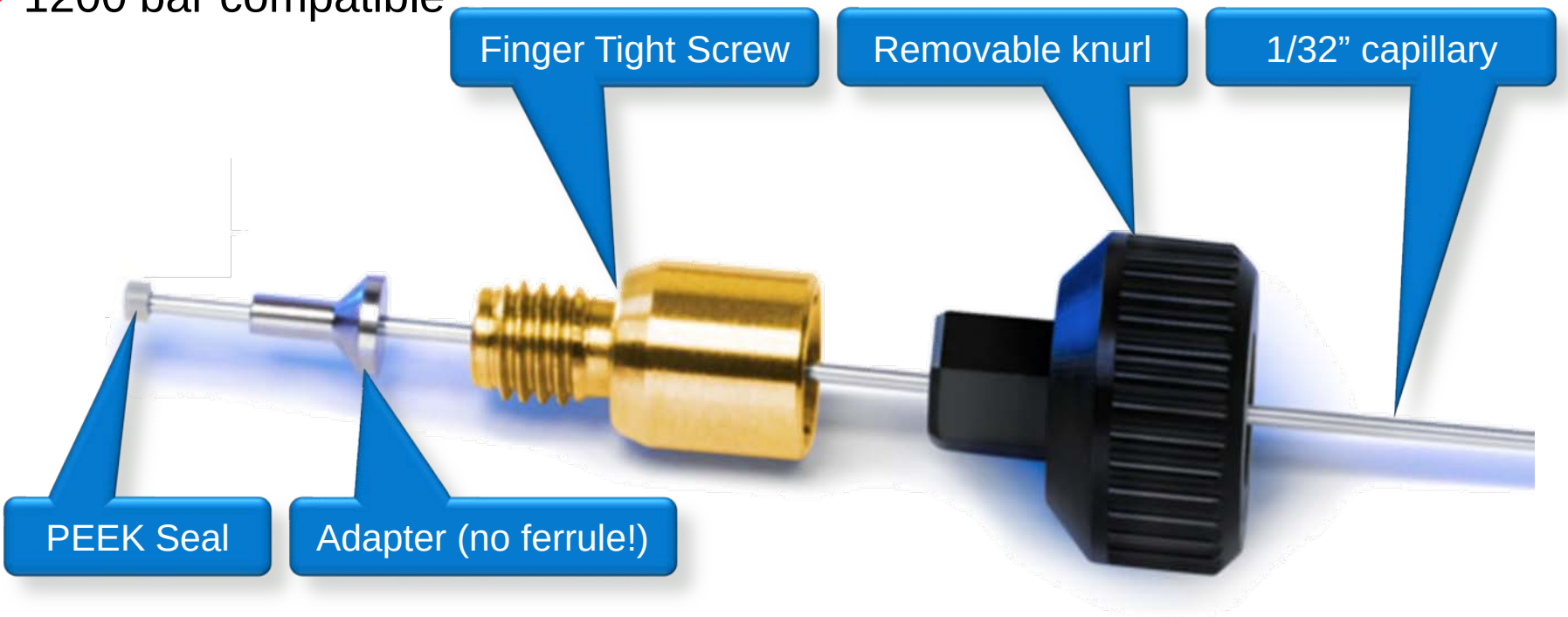
first dimension – protein A
antibody affinity purification and collection

second dimension
WCX ion-exchange analysis



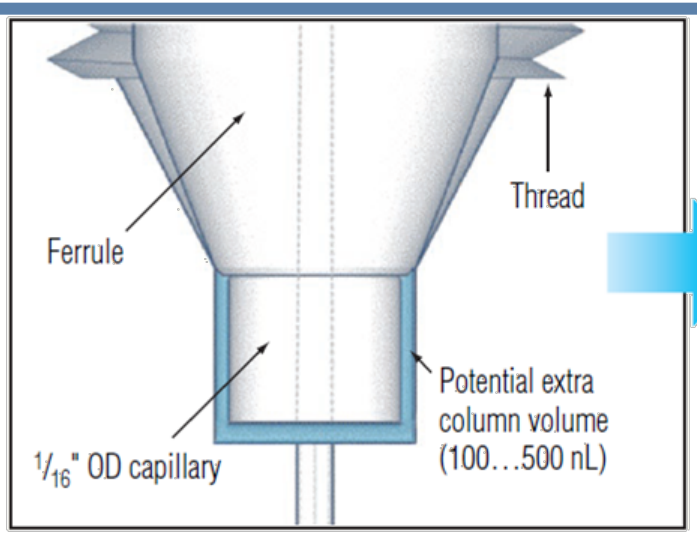
Viper™ Finger Tight Fitting System

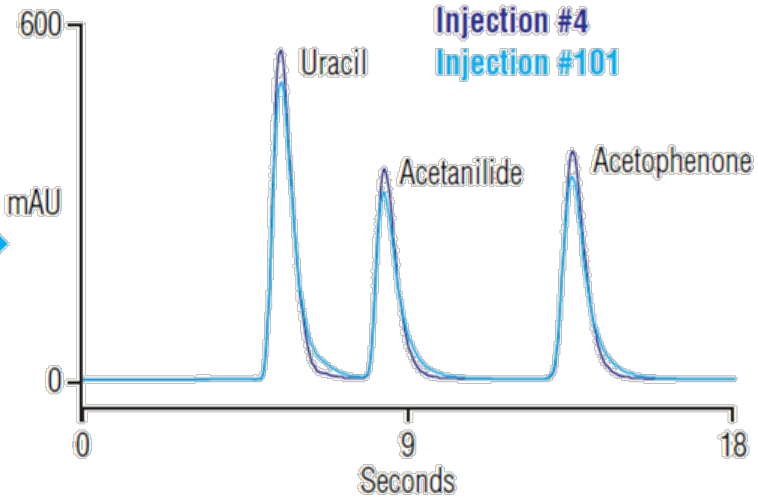
- Finger tight, correct fit every time!
- Universal (fits to Rheodyne, Valco, Swagelok...)
- No dead volume as soon as tightly connected
- Ease of use, no sleeve for small capillaries
- 1200 bar compatible



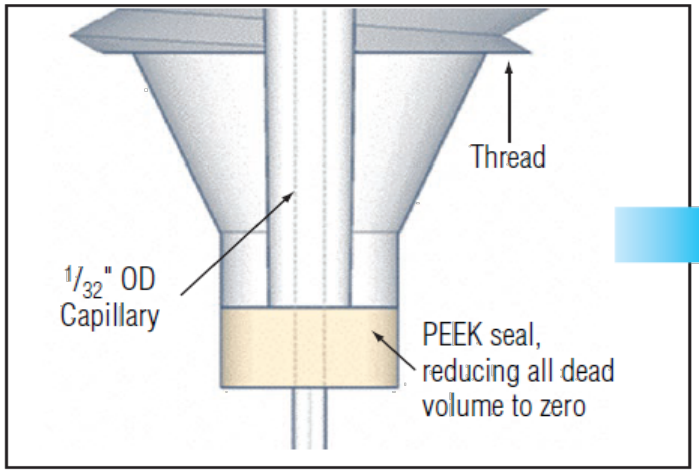
Ease of Use and Ergonomic

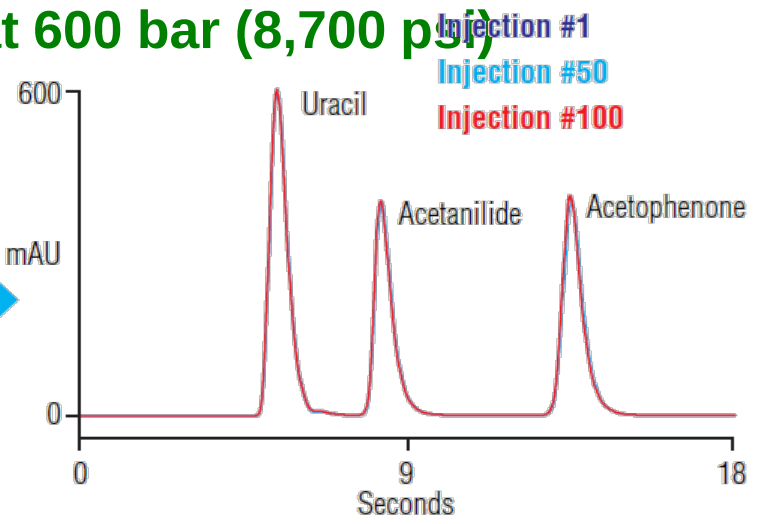
Viper™ Performance





Standard finger tight fitting at 600 bar (8,700 psi)

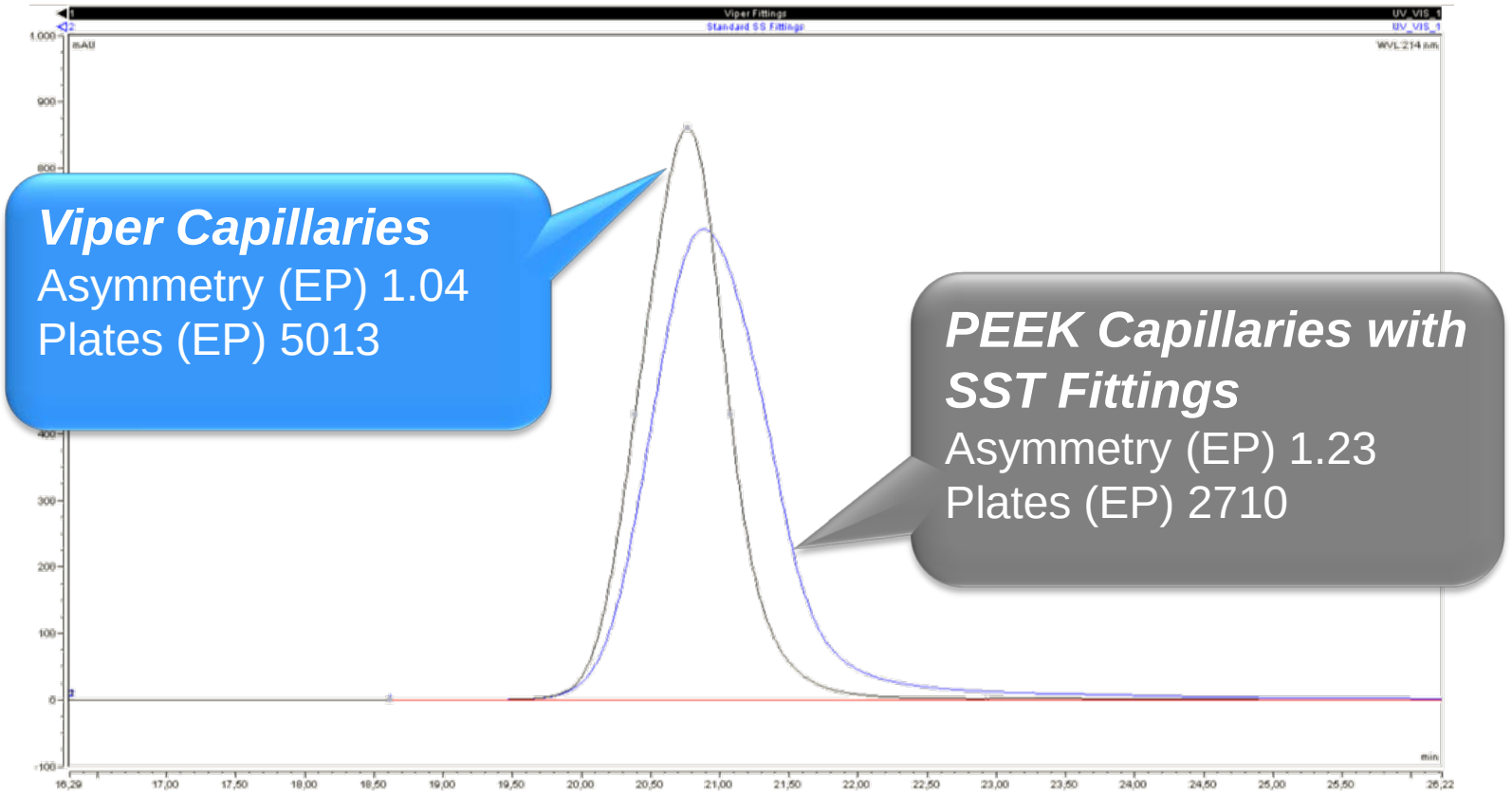




VIPER at 600 bar (8,700 psi)

ZERO Dead Volume

Customer Experience with Viper (I)



Both capillary sets at 180 µm i.d.

Theoretical plates were almost doubled

Overview

1. Column Efficiency and Selectivity

2. Your Workflow and Our Automation Tools

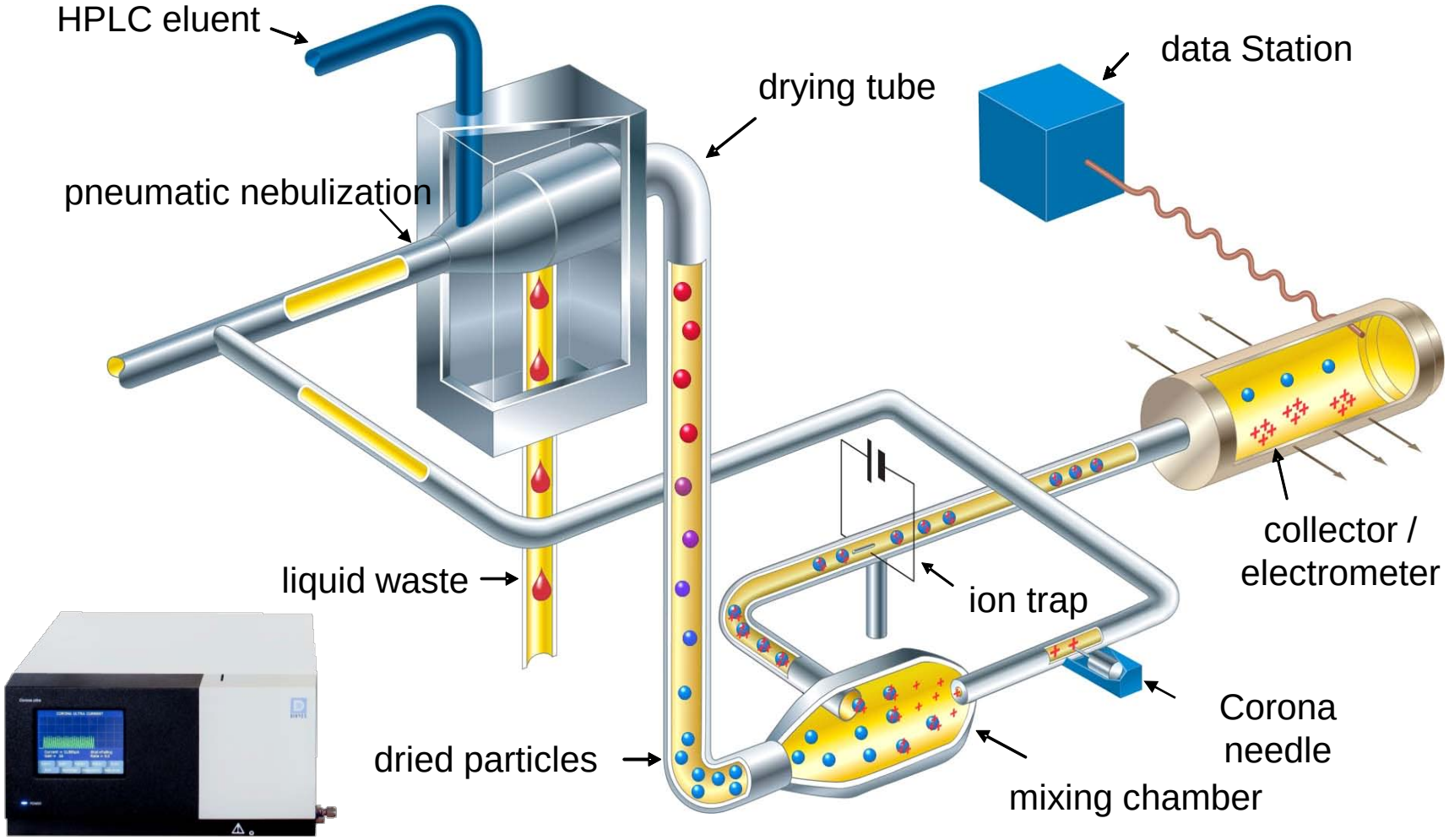
3. Boosting Nebulizer Based Detectors

Thermo MS and UltiMate for Highest Throughput

Advantages of UHPLC for MS Detection

- Increased speed of analysis
- Improved chromatographic resolution
- Maximum sensitivity
- Higher confidence in quantification
- Simple and easy to use
- Accelerated LC/MS method development

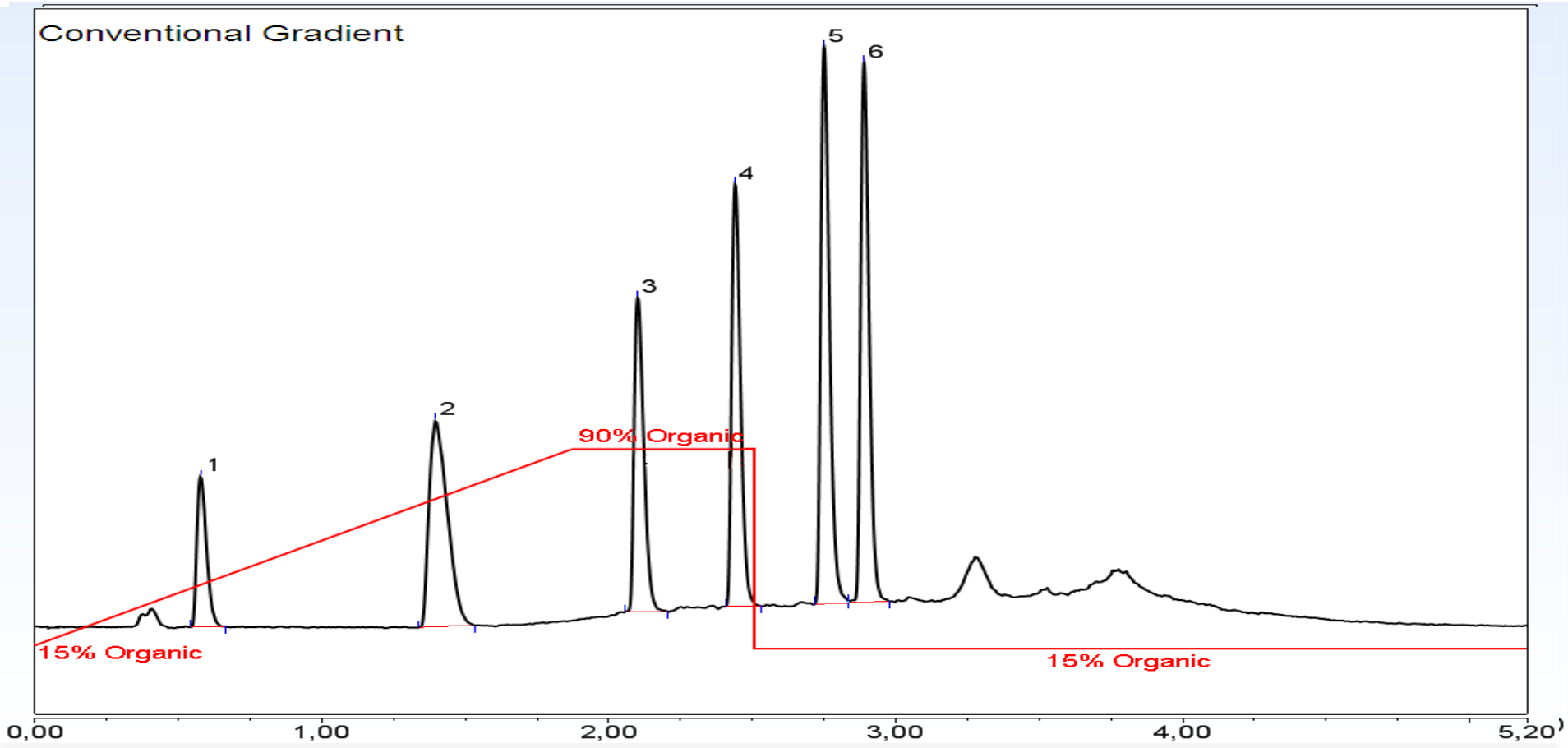
Corona CAD (Charged Aerosol Detector)



Boosting Nebulizer Detectors (MSx, CAD, ELSD)

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

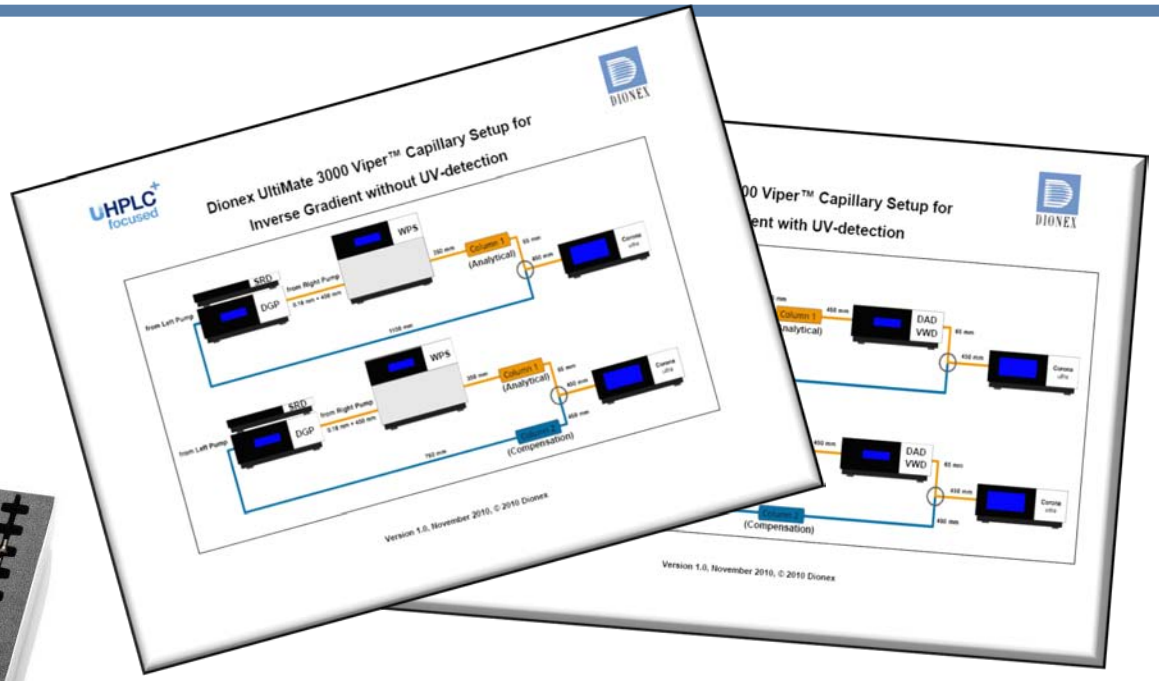
Comparison of Conventional and Inverse Gradient



Gradient: 0 min: 15% acetonitrile / 85% water each with 0.1% formic acid
1.8 min: 90% acetonitrile / 10% water each with 0.1% formic acid
2.5 min: 90% acetonitrile / 10% water each with 0.1% formic acid
2.5 min: 15% acetonitrile / 85% water each with 0.1% formic acid
5.2 min: 15% acetonitrile / 85% water each with 0.1% formic acid

Inverse Gradient: 0 min: 85% acetonitrile / 15% water each with 0.1% formic acid
1.8 min: 10% acetonitrile / 90% water each with 0.1% formic acid
2.5 min: 10% acetonitrile / 90% water each with 0.1% formic acid
2.5 min: 85% acetonitrile / 15% water each with 0.1% formic acid
5.2 min: 85% acetonitrile / 15% water each with 0.1% formic acid

Viper Inverse Gradient Kit for Uniform Response



Ready to Use Viper UHPLC+ Solution Kit:

- All Viper Capillary parts
- Laminated card with flow schemes
- Quick installation guide
- Operation Instructions
- Template files for CM 6 and CM 7

UHPLC+ Focused LC Product Portfolio

RSLCnano

RSLC

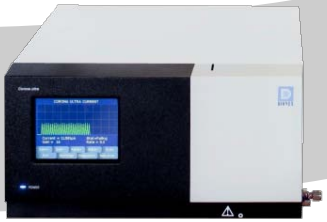
CMS Software

Viper Fitting System

UHPLC+ focused

Standard LC

Basic Automated



Corona *ultra*

Acclaim Columns

Thank you!

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