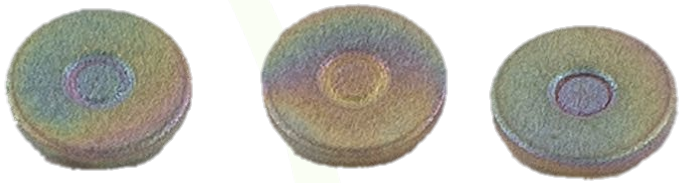
Dursan® Coated HPLC Columns



Key Facts

- **Year founded:** 2009
- **Mission:** To create game-changing coatings.
- **Purpose:** SilcoTek exists to change the game for our customers; to solve their toughest material problems, help them beat the competition, and take their innovations to the next level.

Brief History

New coatings are created for a variety of applications as demand grows for “Restek Performance Coatings” beyond just chromatography (Restek’s core business).

SilcoTek builds a 36,000 ft^2 state-of-the-art coating facility, tripling previous coating capacity.

1990-2008

2013

1987

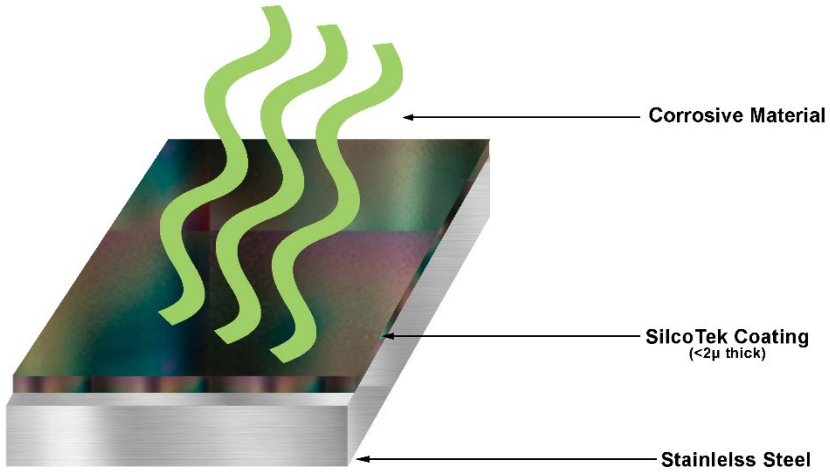
Restek® invents SilcoSteel® to make stainless steel GC capillary columns inert like glass columns.

2009

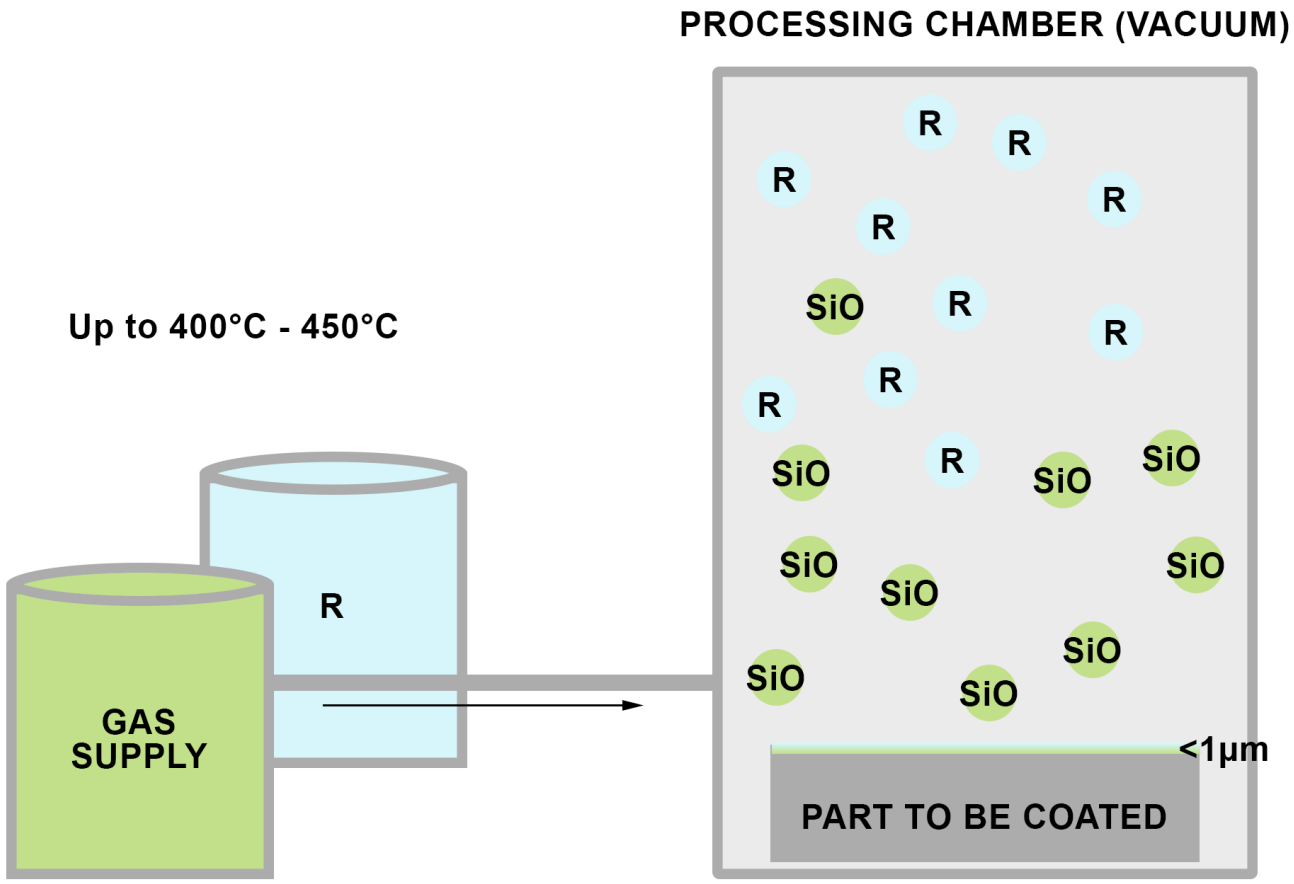
14 Restek Performance Coatings Employees spin-off to form SilcoTek®, an independent company dedicated solely to coatings.

What SilcoTek® does

- Thermal CVD materials onto substrates like stainless steel, titanium, super alloys, glass, ceramics, etc.
- Additional steps for more advanced surface properties
- Also able to directly functionalize substrate without coating



The CVD Process



The Dursan® Coating Process

- The Dursan® coating process results in a chemically protective barrier of amorphous silicon, oxygen, and carbon that is further functionalized to resist adsorption of corrosive, reactive, and otherwise unwanted molecules.

Coating Structure:	Functionalized silica-like coating (a-SiO_x:CH_y)
Maximum Temperature:	500°C (Inert atmosphere) 450°C (Oxidative)
Substrate:	Compatability: Stainless steel, exotic allows, ceramics Size: Up to 78” (198 cm) Geometry: Any shape, including complex geometries
Typical Thickness:	400 – 1600 nm
Hydrophobicity (contact angle):	≥81°
Allowable pH Exposure:	0 - 14

Dursan® for HPLC

- Coat columns and frits for an inert surface like PEEK, but as robust as stainless steel.
- Without an inert flow path analytes can be lost, peaks can be broad and tailing, and testing results can be inconsistent.
- Dursan® has no impact on routine analysis. However, we have found exceptionally improved performance with compounds like chelating agents, proteins, and biologics.

HPLC Data

The proof is in the peaks.

Packability of columns

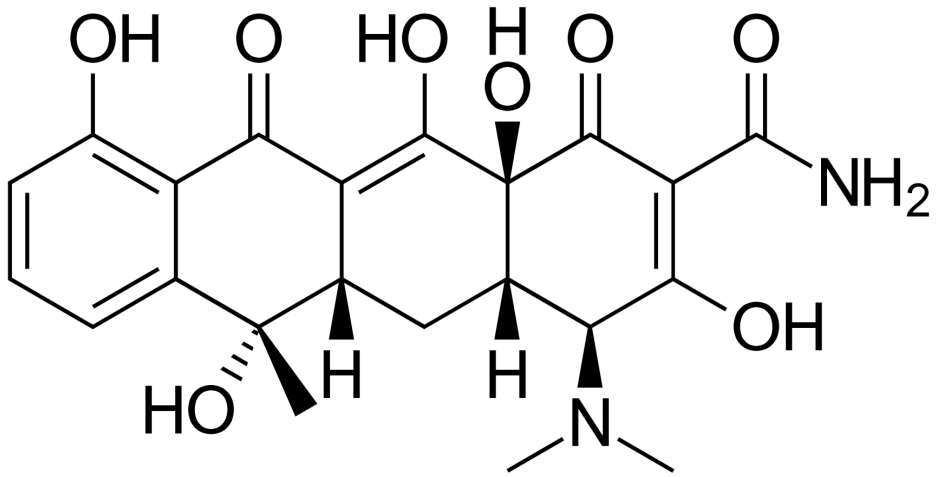
- Three 2.1 mm ID x 100 mm columns were packed of each of the following:
  - All Stainless Steel (A)
  - All Dursan coated (B)
  - Dursan coated frit and stainless steel column (C)
  - Titanium frit and stainless steel column (D)
- Biphenyl peak in standard HPLC mix was used to measure efficiency of column

Column type	Efficiency (avg of 3)	Asym (avg) of 3
A	15195 plates	1.028
B	15233 plates	1.026
C	15069 plates	1.020
D	14263 plates	1.044

- Columns made by Shepard's Machine Shop
- Frits purchased from Mott Corporation
- Packed with ACME PLUS C18 and analysis done by Phase Analytical Technoogy, LLC
- Shows no detrimental effects in packing efficiency
  - Since the coating is quite thin, this was expected.

Tetracycline

- Tetracycline is an antibiotic, commonly used for acne and skin infections
- The molecule has numerous chelating groups that bind readily to metal sites
- Dursan can make the steel column more inert toward metal loving molecules like tetracycline

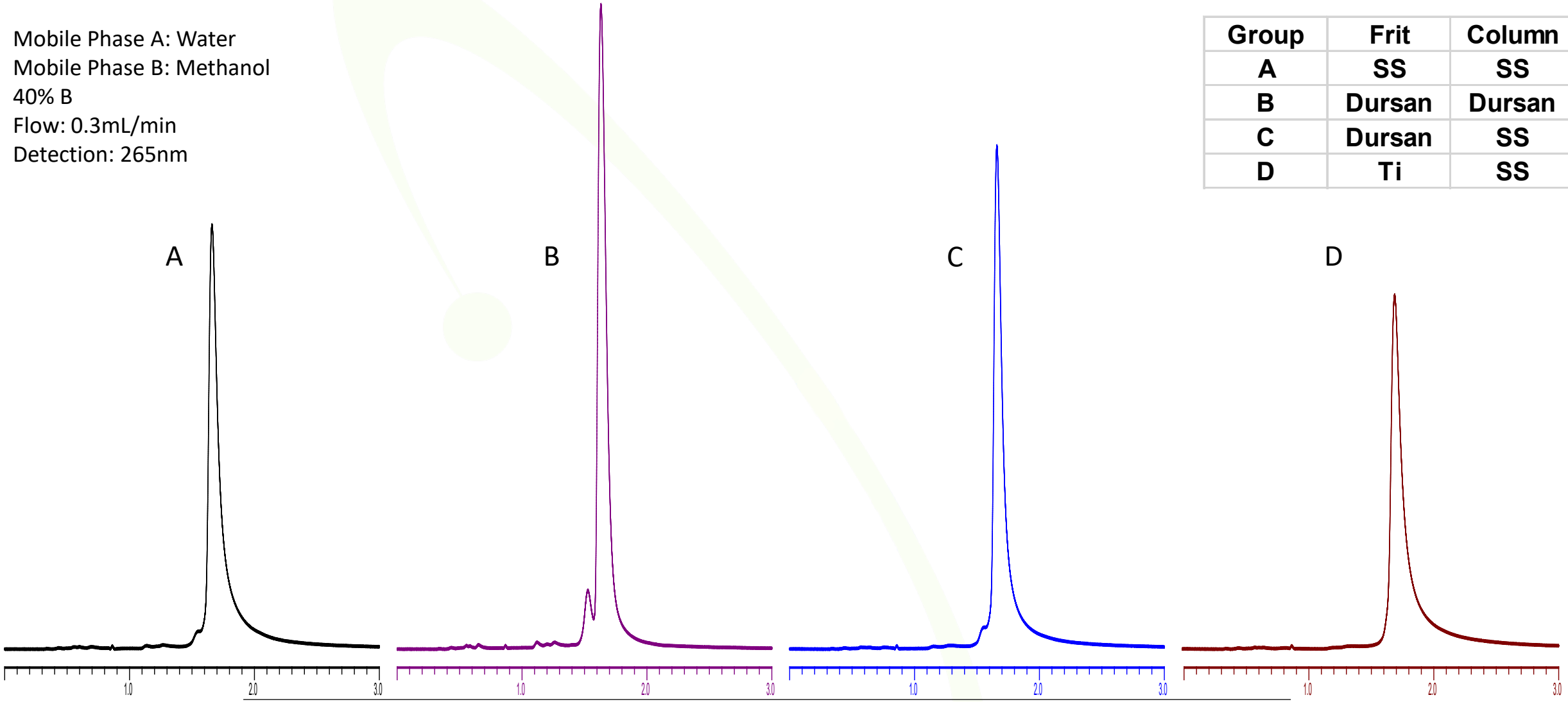


Molecular structure of tetracycline

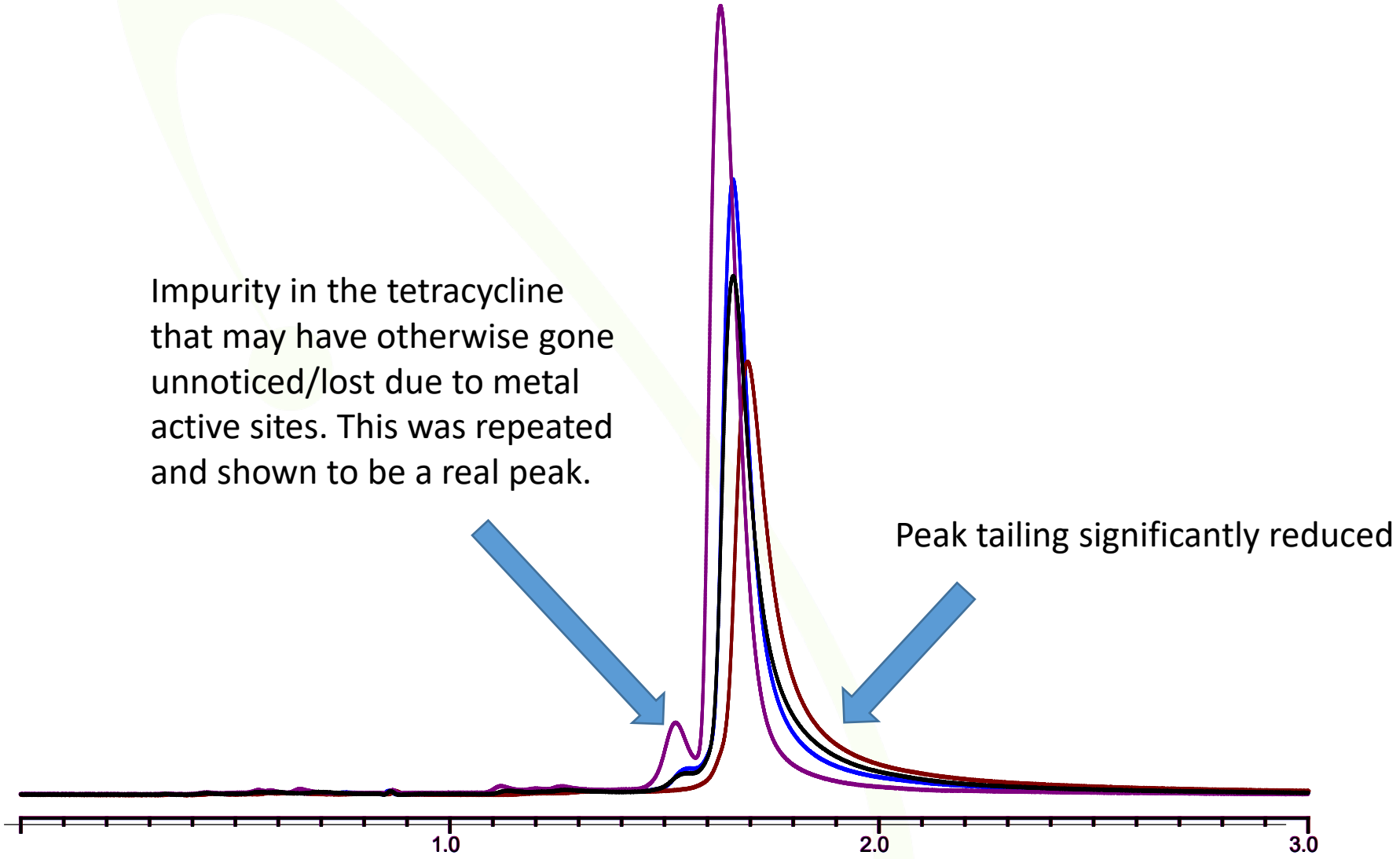
Results:

Mobile Phase A: Water
Mobile Phase B: Methanol
40% B
Flow: 0.3mL/min
Detection: 265nm

Group	Frit	Column
A	SS	SS
B	Dursan	Dursan
C	Dursan	SS
D	Ti	SS



Overlay of all 4 columns:



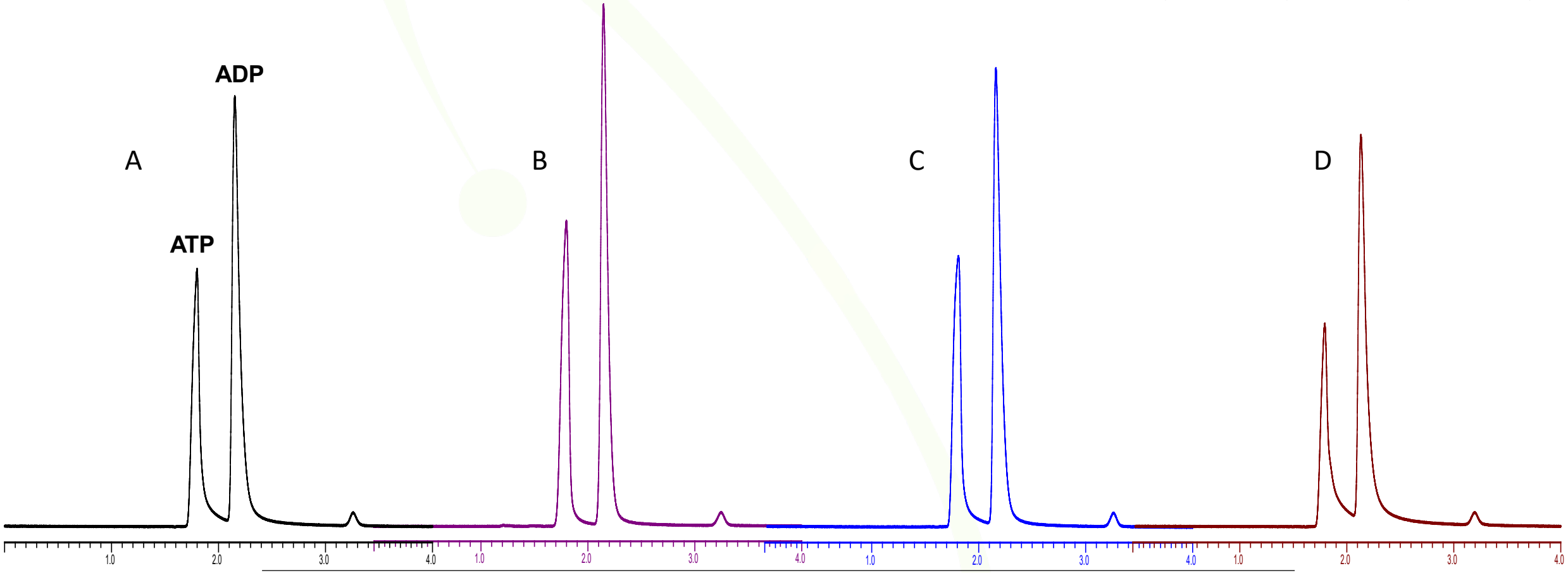
ADP and ATP

- ATP provides energy to drive numerous processes in living cells
- It is typically converted to ADP or AMP
- Phosphates are well known to have severe peak tailing during HPLC analysis due to the phosphate-iron interaction

Results:

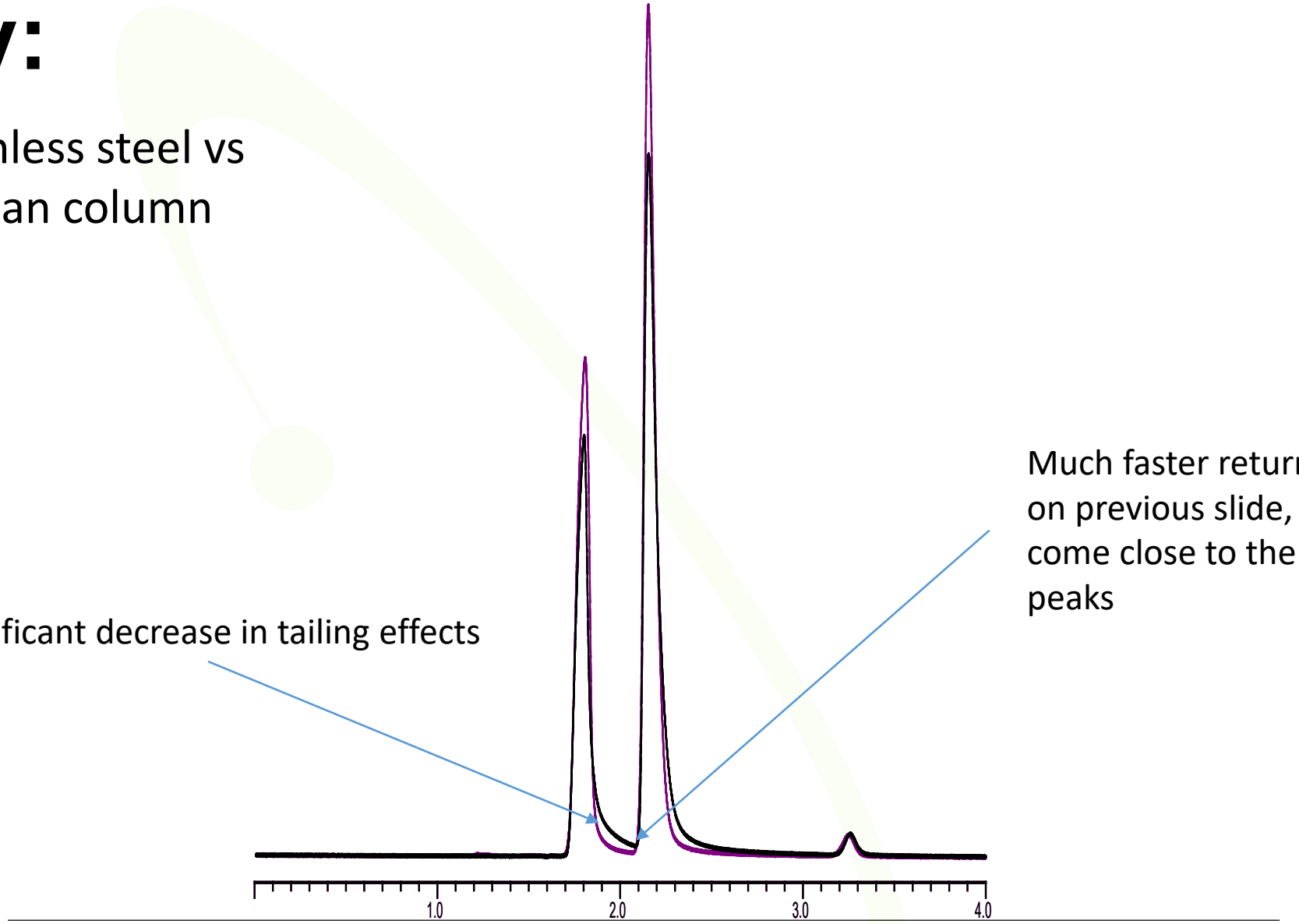
Mobile phase: Water / 10mM Ammonium Acetate
Flow: 0.2mL/min
Detection: 254nm

Group	Frit	Column
A	SS	SS
B	Dursan	Dursan
C	Dursan	SS
D	Ti	SS



Overlay:

All stainless steel vs
all Dursan column



Significant decrease in tailing effects

Much faster return to baseline. Note on previous slide, titanium does not come close to the baseline between peaks

	Peak Name	Ret.Time	Height	Width (50%)	Asym.	Plates
A	ATP	1.799	50.71	0.072	1.45	3440
Stainless Steel	ADP	2.152	84.45	0.072	2.03	4918
B	ATP	1.804	60.09	0.072	0.95	3583
Dursan Dursan	ADP	2.152	102.4	0.066	1.5	5831
C	ATP	1.8	53.3	0.08	1.16	2821
Dursan Frits only	ADP	2.151	90.13	0.073	1.93	4771
D	ATP	1.783	39.96	0.068	2.28	3770
Ti Frits	ADP	2.122	76.74	0.074	2.45	4582

Theoretical Plates

$$TP = \left(\frac{t_R}{w_{1/2}} \right)^2$$

Asymmetry

$$A = \frac{RW_{5\%} + LW_{5\%}}{2 \times LW_{5\%}}$$

2-pyridinol-1-oxide

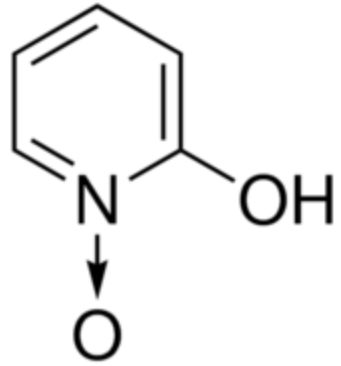
- Ciclopirox is an antifungal agent typically used in topical fingernail and toenail infections
- 2-pyridinol-1-oxide is the chelating part of this antifungal agent
  - It is a very powerful metal chelating agent
- The chromatograms show significant loss of signal due to metal interactions in the separation
  - This highlights the interaction that the column wall has with the analyte and there is a need for column coating as this interaction is not negligible.

A: Water no buffer
Flow: 0.3mL/min
Detection: 230nm

100 X 2.1

Group	Frit	Column
A	SS	SS
B	Dursan	Dursan
C	Dursan	SS
D	Ti	SS

2-Pyridinol-1-Oxide



SilcoTek

Protein analysis

- We know through our collaborative study with Abbott Laboratories, Dursan is an excellent non-specific protein anti-fouling coating.
- We have performed separations on HPLC peptide standard mixture, ribonuclease, cytochrome C, bovine serum albumin, and protein A.
  - We do not see a huge benefit in reverse phase HPLC separations of these compounds/proteins (minor peak narrowing, but nothing to write home about).

Future studies

- Durability tests are underway
  - What do the tetracycline and 2-pyridinol-1-oxide peaks look like after 100, 200, 500, 1000+ injections?
- Compare to PEEK hardware with the same silica packing material
- Continue exploring other molecules of interest
  - Other phosphates
  - Chelating proteins
  - Cannabis
- Explore other parts of the HPLC system
  - Sampling needles
  - Tubing
  - Pump parts

How to get Dursan® for HPLC

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