

Achieving PEEK-like Performance on Stainless Steel HPLC Components with Bio-Inert CVD Coatings

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Outline

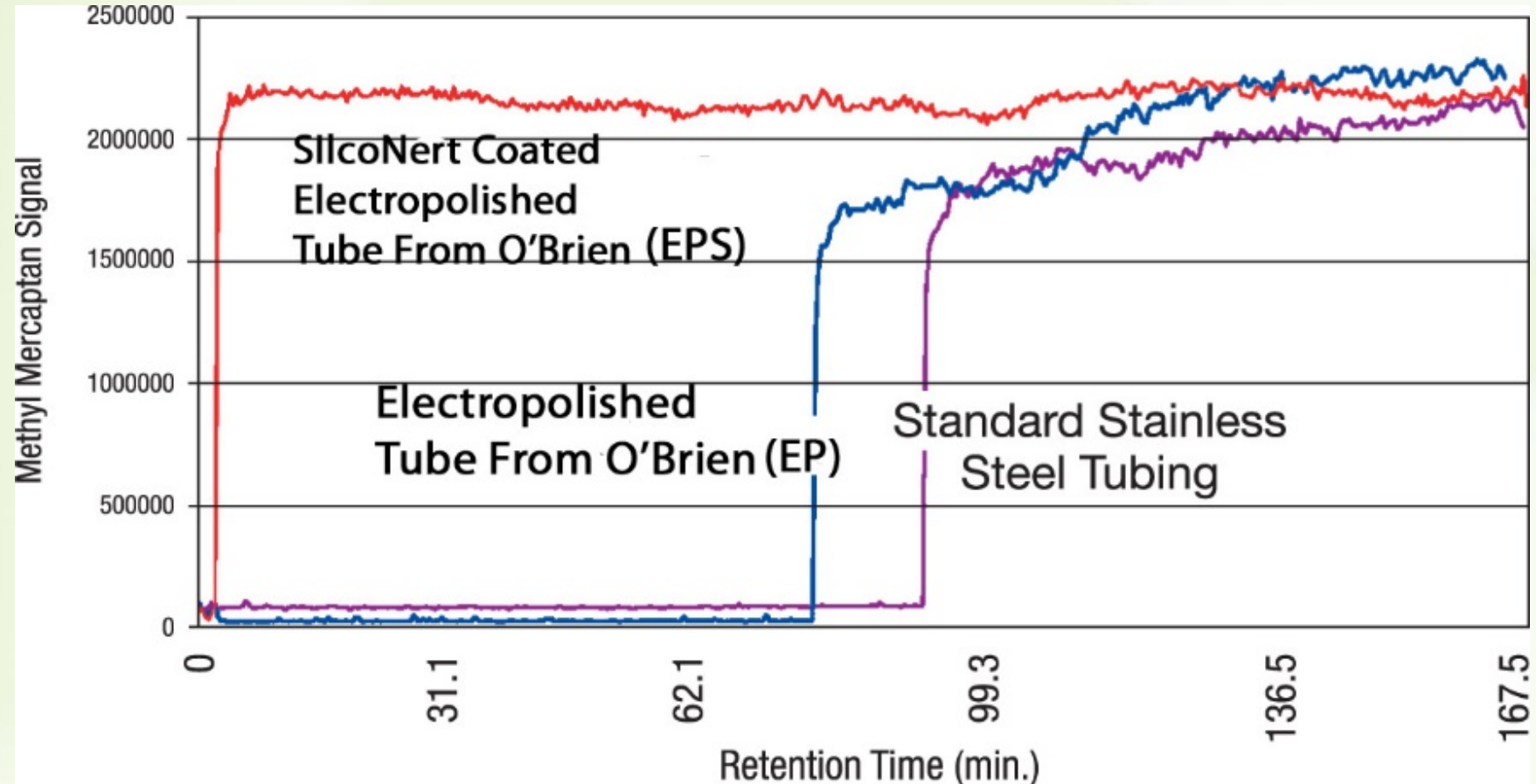
- Why is an inert flow path critical?
- CVD basics and how a CVD coating differs from traditional coatings
- Chemical and physical properties of Dursan[®]:SilcoTek's HPLC coating of choice
- HPLC data highlighting the use of Dursan[®] on HPLC columns

Why is an inert flow path critical?

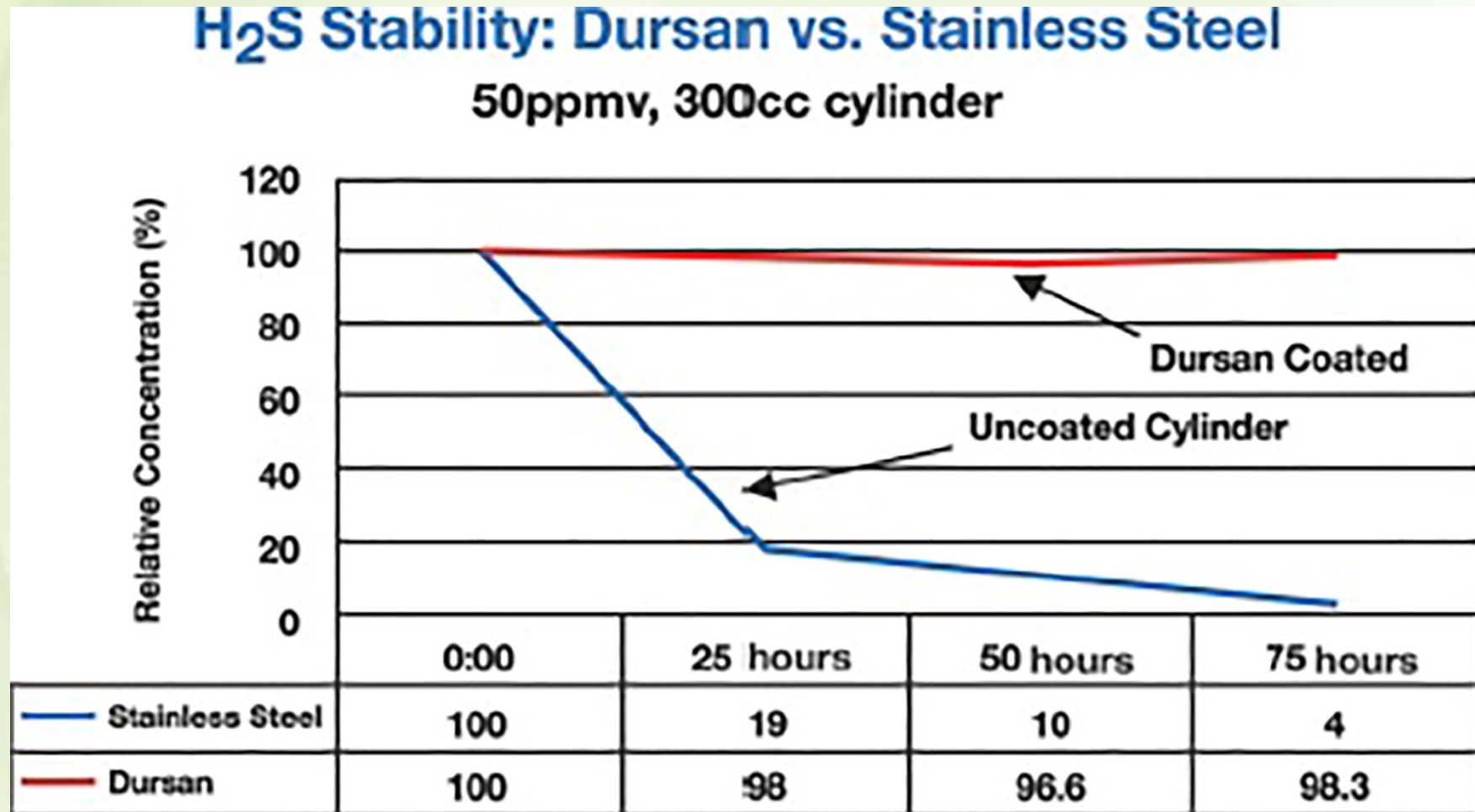
- Allow analytes to get from injection to detector without the risk of adsorption
- Reduce costs and downtime
 - No need to re-test
 - Accurate profile of all components, both reactive and non-reactive
- Trust your results
 - Eliminates false negatives and ghosting
- No additional molecules/ions introduced
 - Stainless steel: chemical reactivity, corrosion, and abrasion can all lead to the introduction of molecules and ions that are not in your sample.

Inertness leads to faster response time

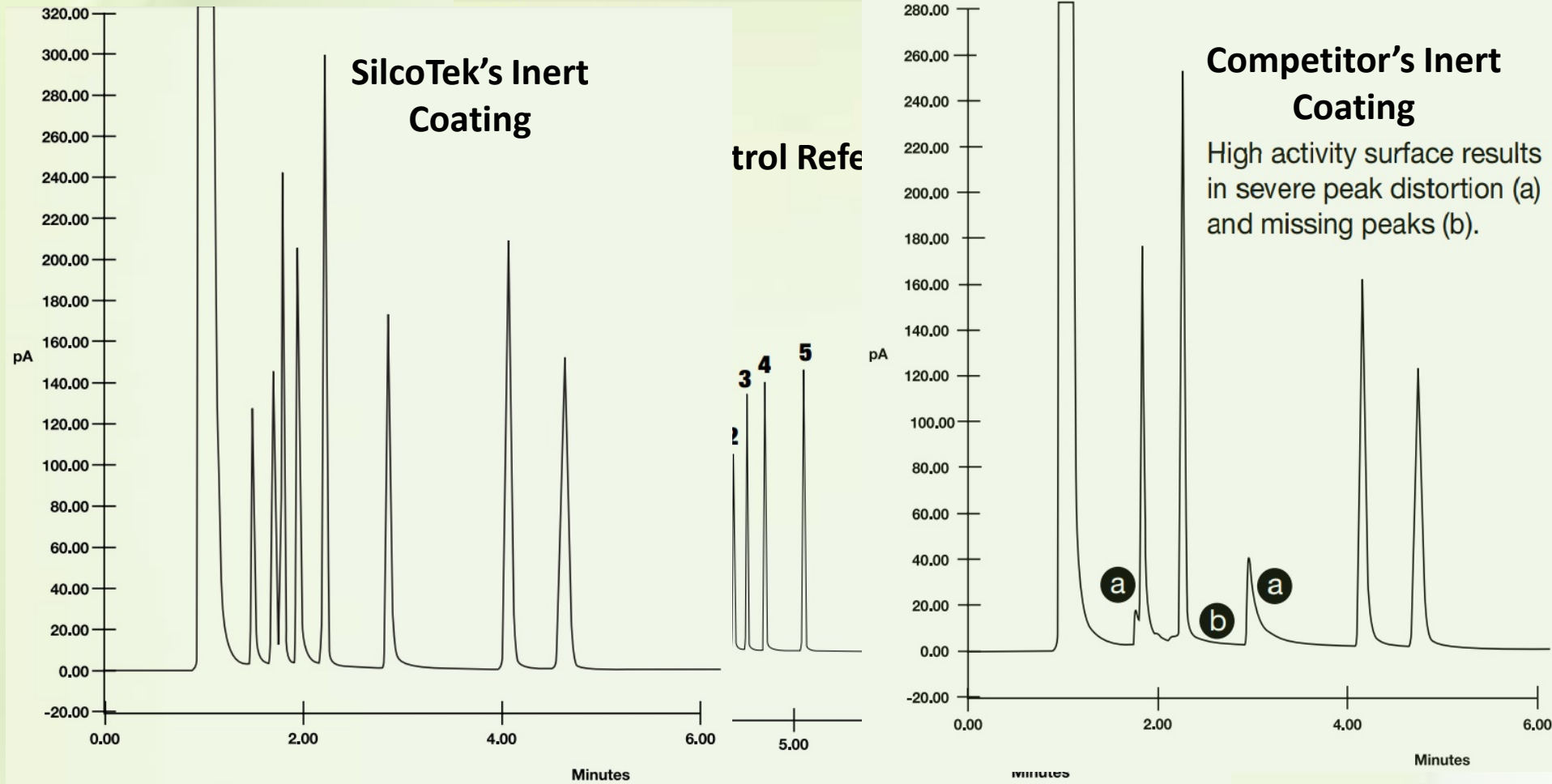
- 500 ppbv methyl mercaptan ran through 100 feet of 1/8 in diameter tubing



Inertness prevents loss of analytes



Inert flow path example for GC

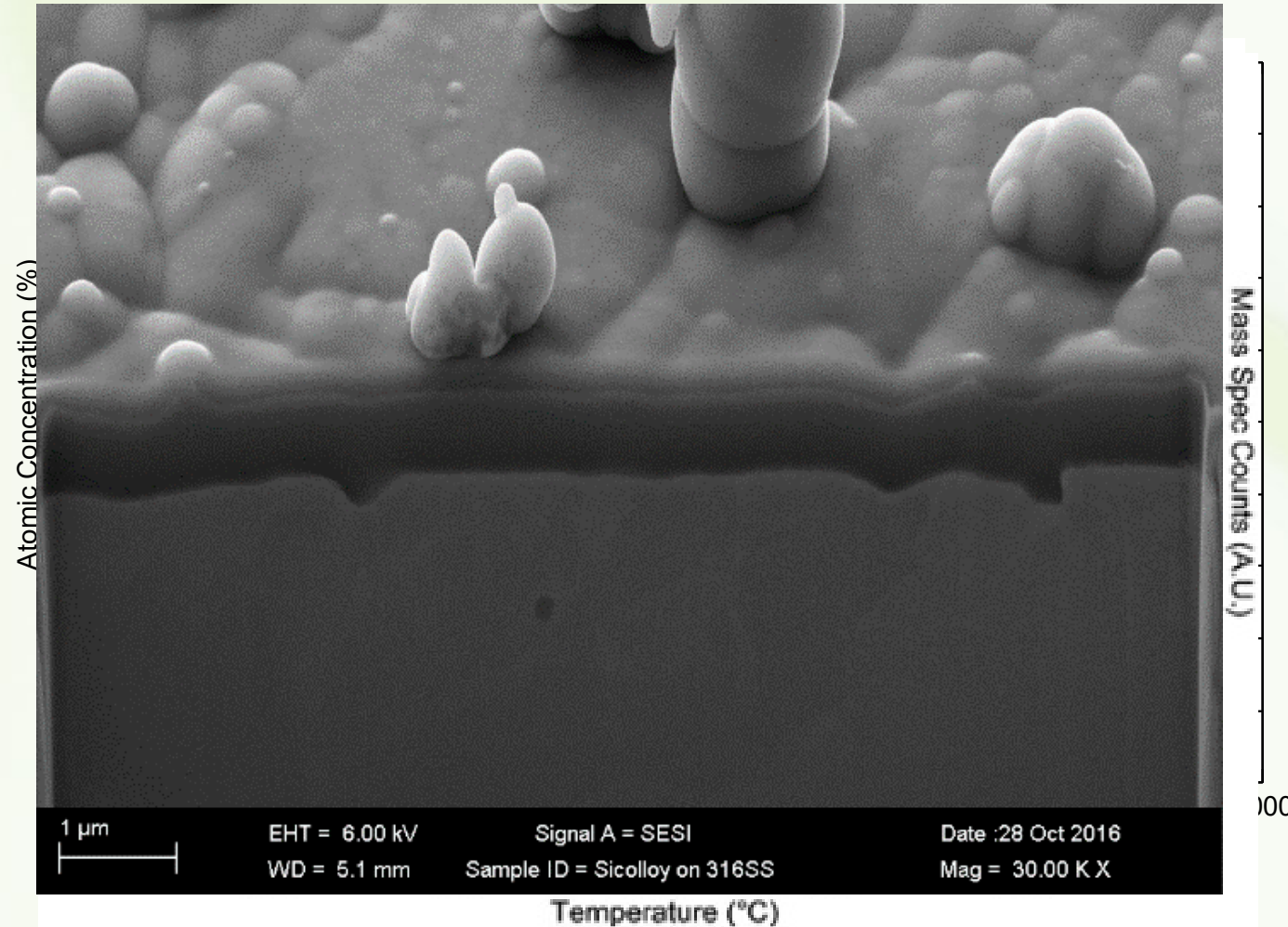


Current inert flow paths for HPLC

Material	Benefits	New issues
Titanium	Bio-inert	Metal surface can still have “hot spots” of reactivity
PEEK	General inertness Flexible Lightweight and easily cut	Solvent limitations Swelling causes back pressure Pressure limitations
Ceramics	Robust Chemically inert	Brittle Can be expensive

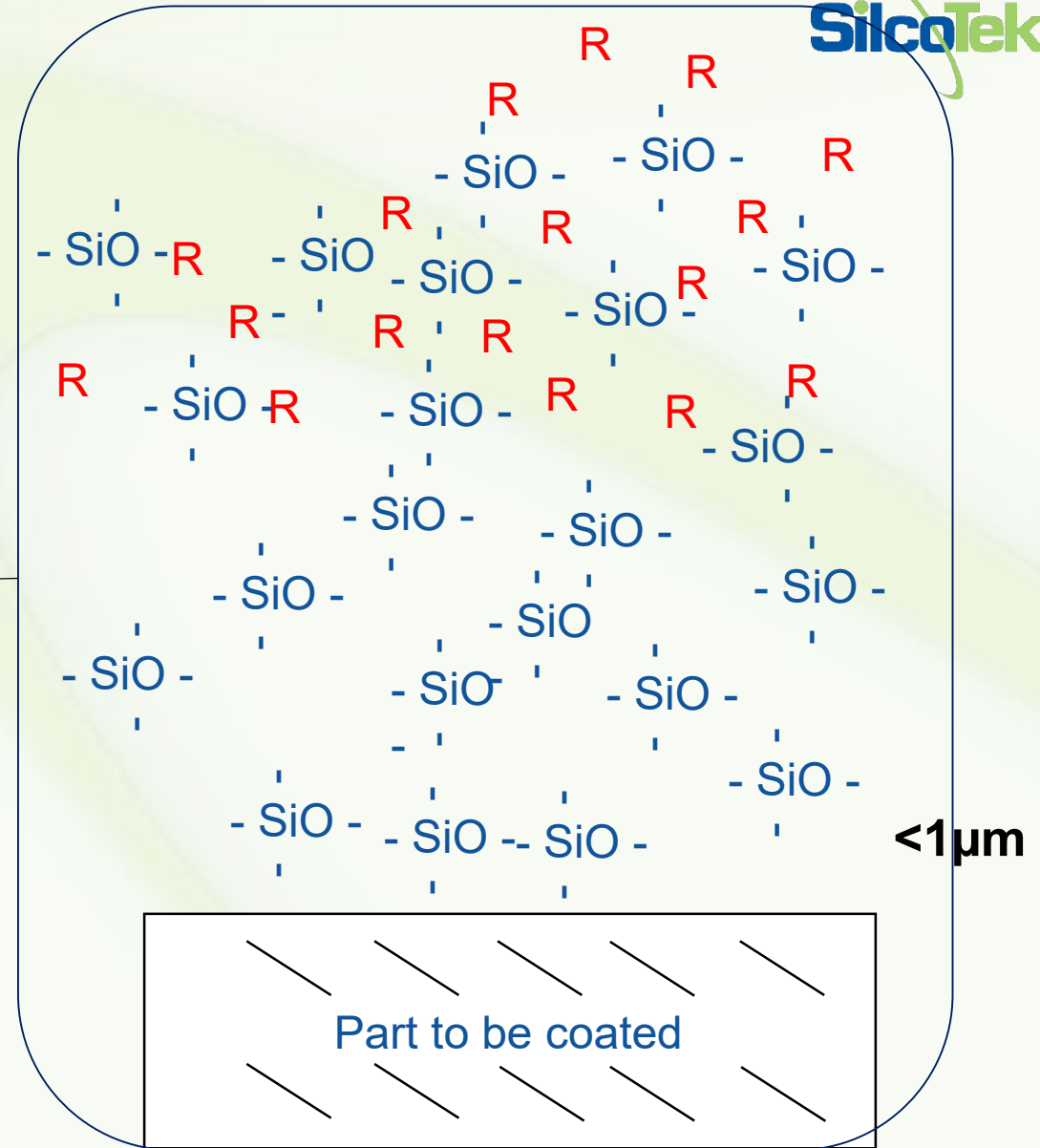
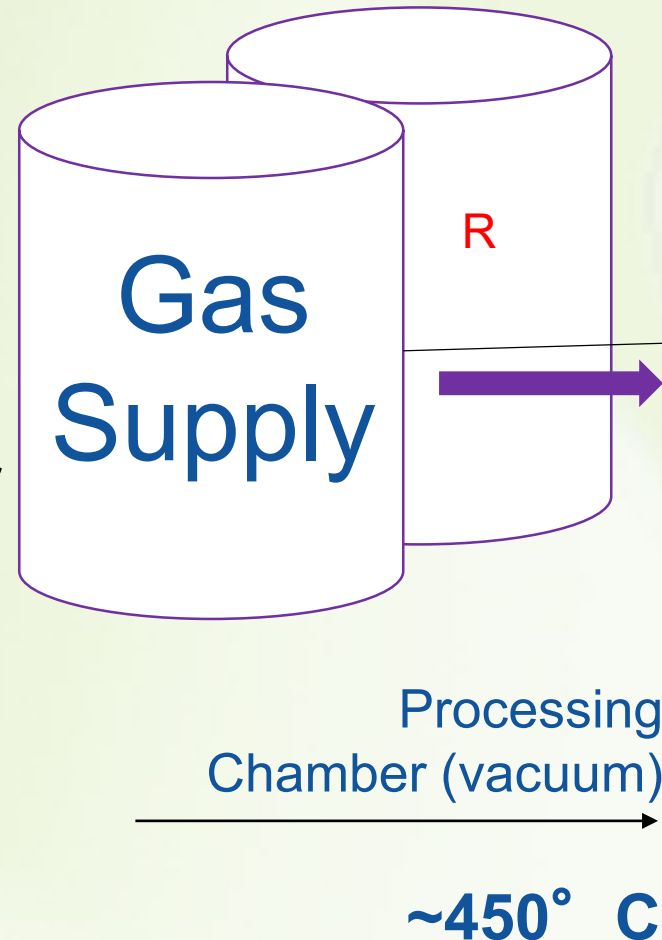
SilcoTek's solution: Dursan®

- Functionalized silica-like coating
- Highly corrosion resistant
- Stable up 450°C
- Provides a barrier between your solvent/analyte, and the substrate
 - Can be applied directly onto existing parts
 - <1 µm thick so will not affect tolerances



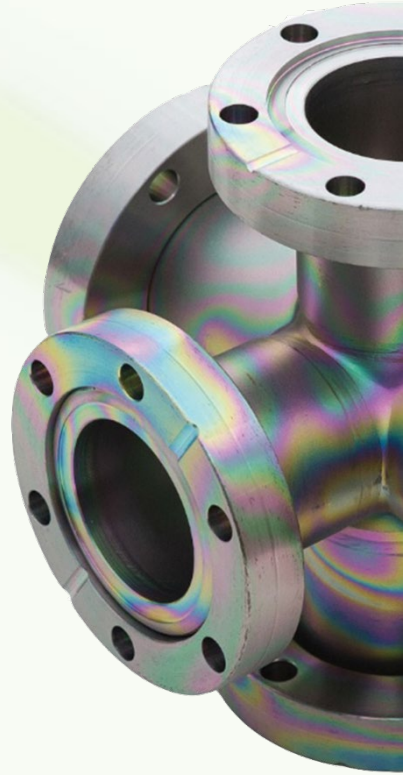
The CVD Coating Process

- Silane based CVD coatings
- Most metal alloys and ceramics can be coated
- Provides an inert barrier between substrate and flow path
- Surface chemistry can be altered by varying **R**.



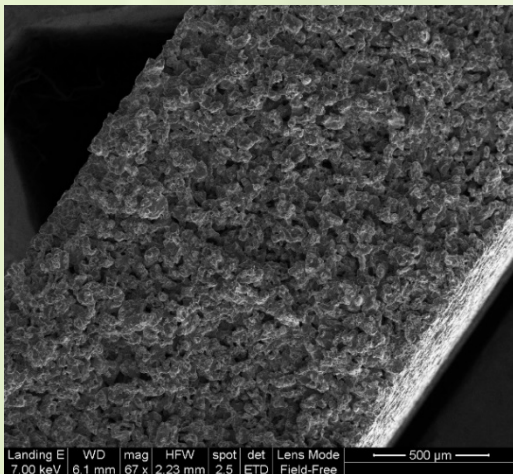
Advantages of CVD Coatings

- Non-line-of-sight deposition; uniformly treats 3D, high aspect ratio part geometries
- Molecular adhesion to base substrate. Won't flake nor delaminate.
- Scalable, versatile, and highly reproducible

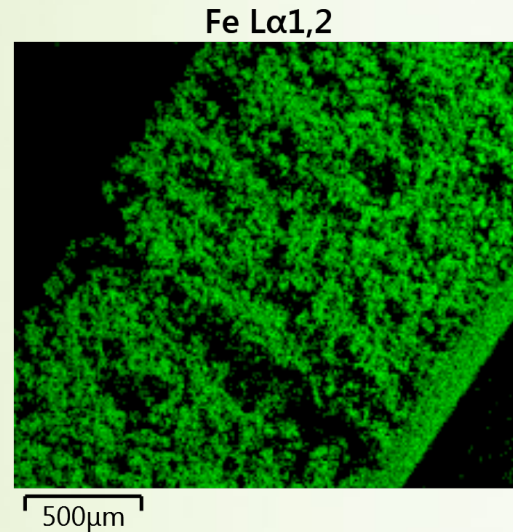


Example of non line of sight coating:

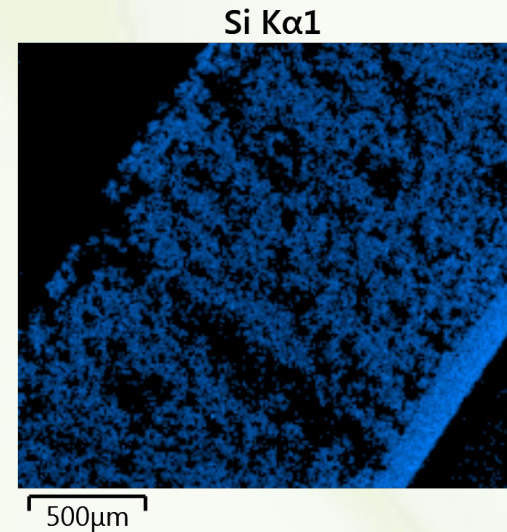
- Cross section of a 2 μ m nominal pore size frit after Dursan coating:



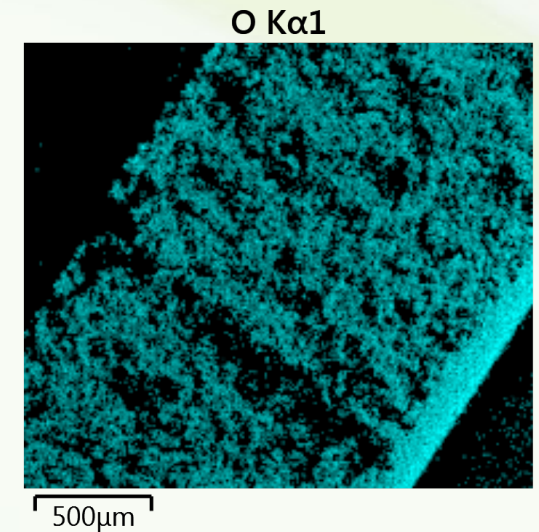
SEM micrograph



Iron EDS map



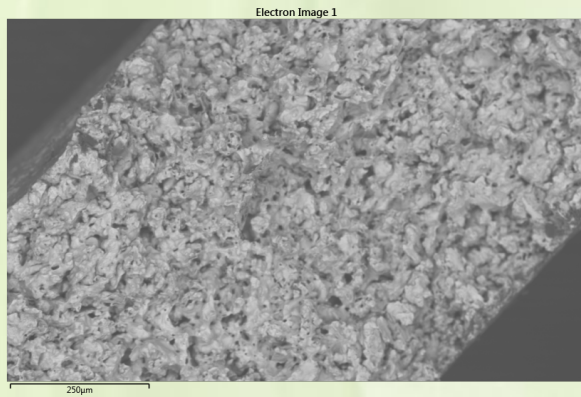
Silicon EDS map



Oxygen EDS map

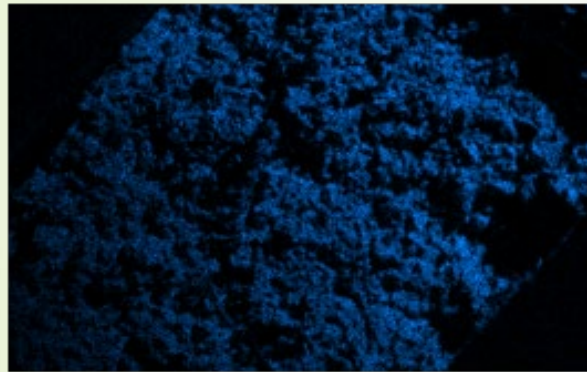
Example of non line of sight coating:

- Cross section of a 0.5 μm nominal pore size frit after Dursan coating:



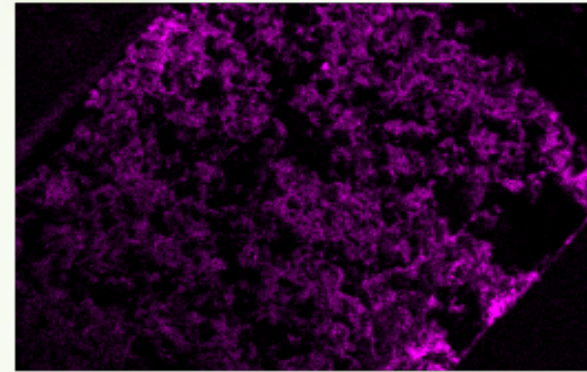
SEM micrograph

Fe $\text{L}\alpha_{1,2}$



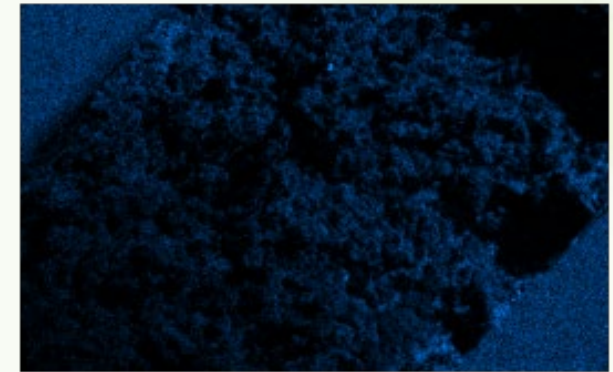
Iron EDS map

Si $\text{K}\alpha_1$



Silicon EDS map

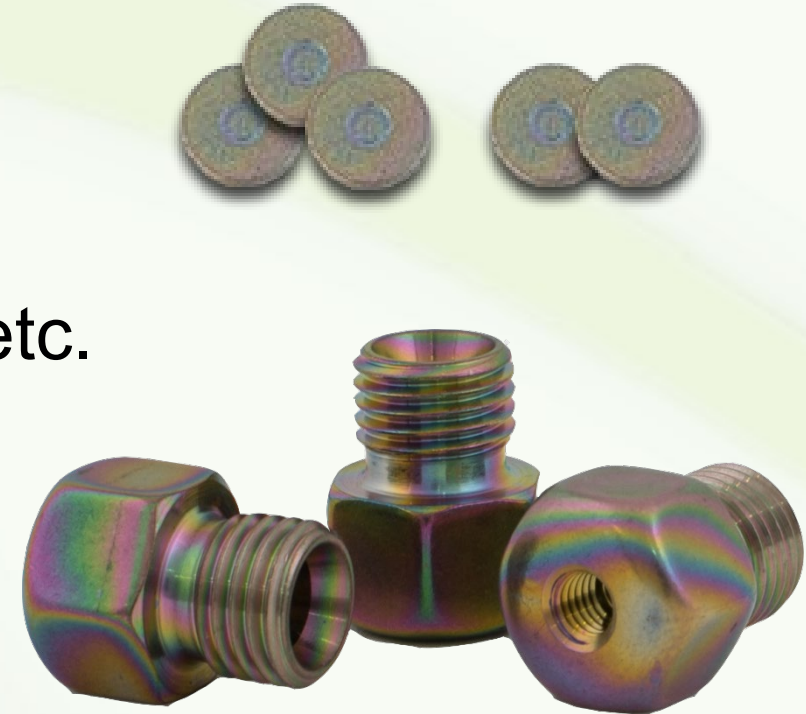
O $\text{K}\alpha_1$



Oxygen EDS map

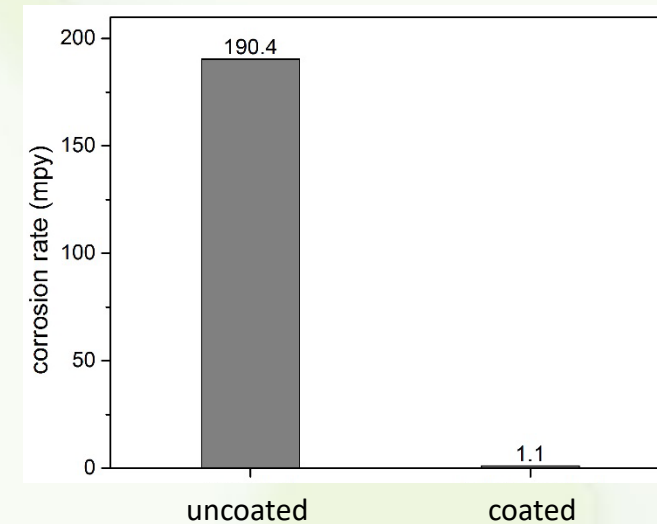
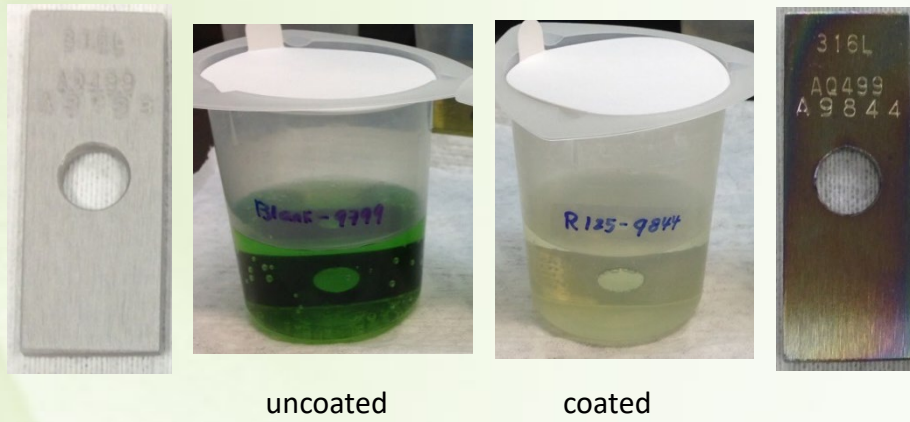
Coating Capabilities

- We apply CVD coatings to:
 - Tubing: as small as 0.10mm ID
 - Internal coating, 600m ++ lengths if coiled
 - Frits, valve components (wetted), manifolds, etc.
 - Entire analytical flow path
 - Parts can be bent or flexed without flaking



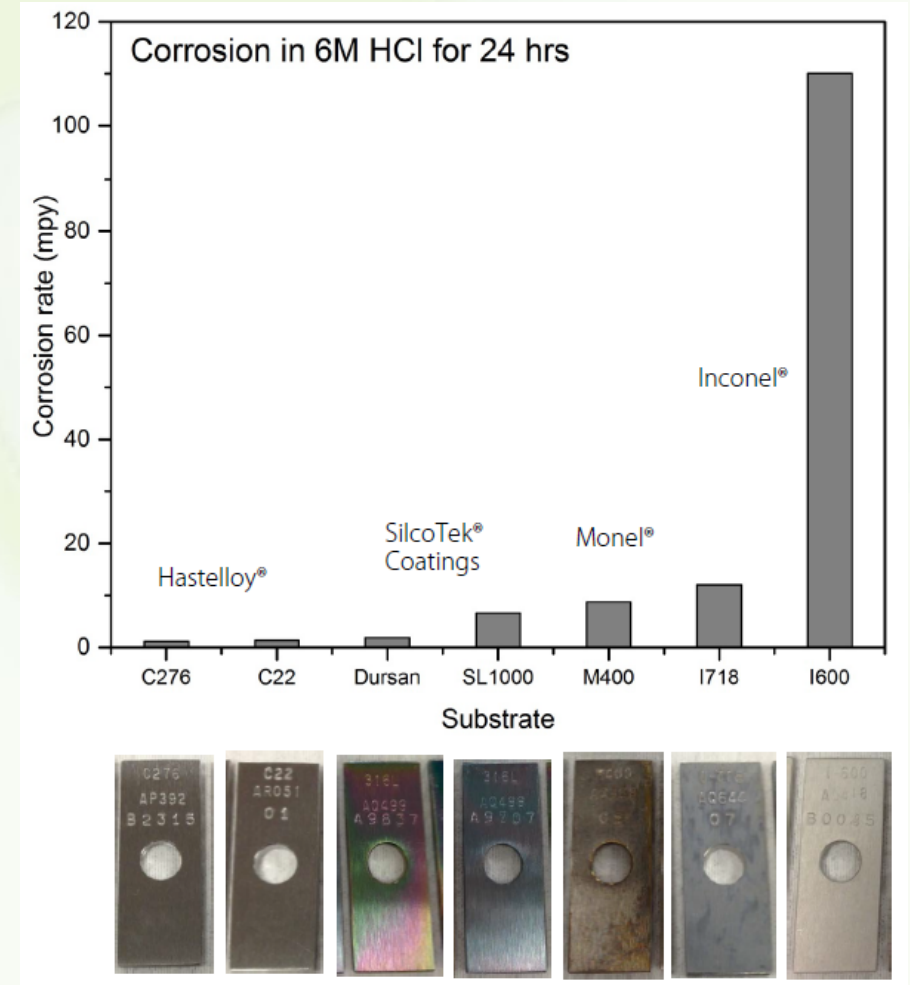
Corrosion resistance in hydrochloric acid

- ASTM G31 guidelines
- 20% (6M) HCl room temperature immersion 24 hours
- Over 170x improvement with coating



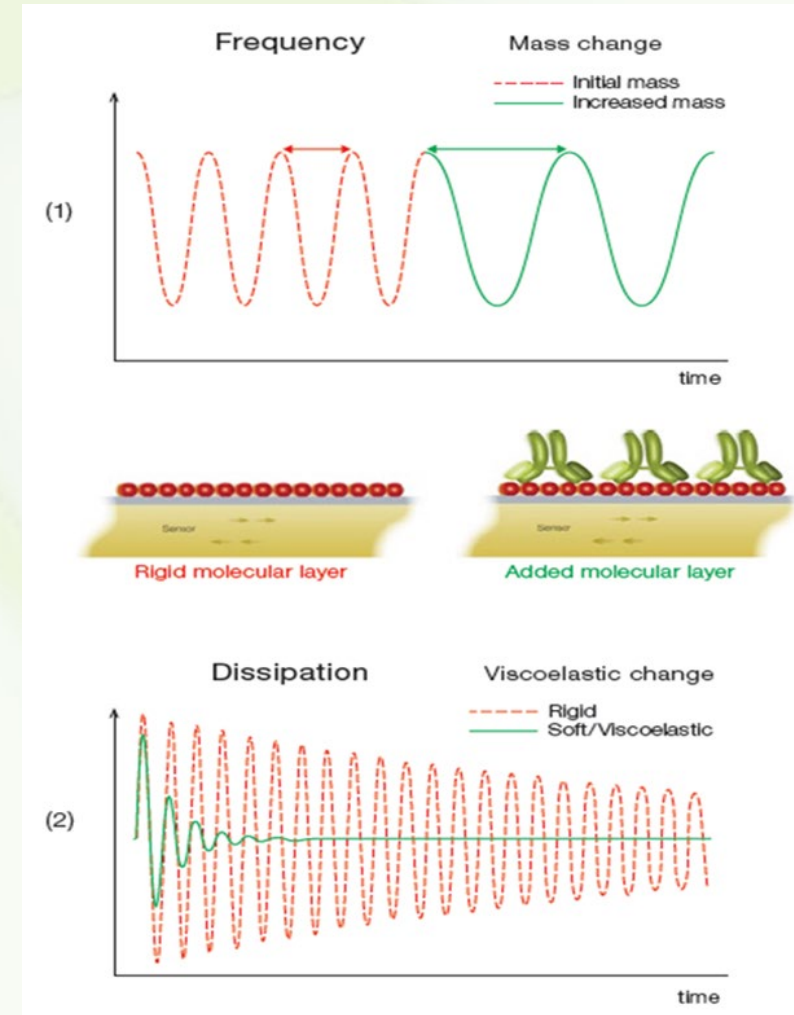
Additional corrosion resistance

Corrosive media	Bare Stainless Steel (MPY)	Dursan coated steel (MPY)	Improvement multiplier
6M HCl @ 50°C	3116.1	23.5	133x
Concentrated H ₂ SO ₄	78.45	0.15	523x
48% HBr	2.05	0.29	7x
Bleach	1.70	0.10	17x
Concentrated H ₃ PO ₄ @ 80°C	2.14	0.53	4x
2% TFA	No corrosion, change in CA	Unaffected	-

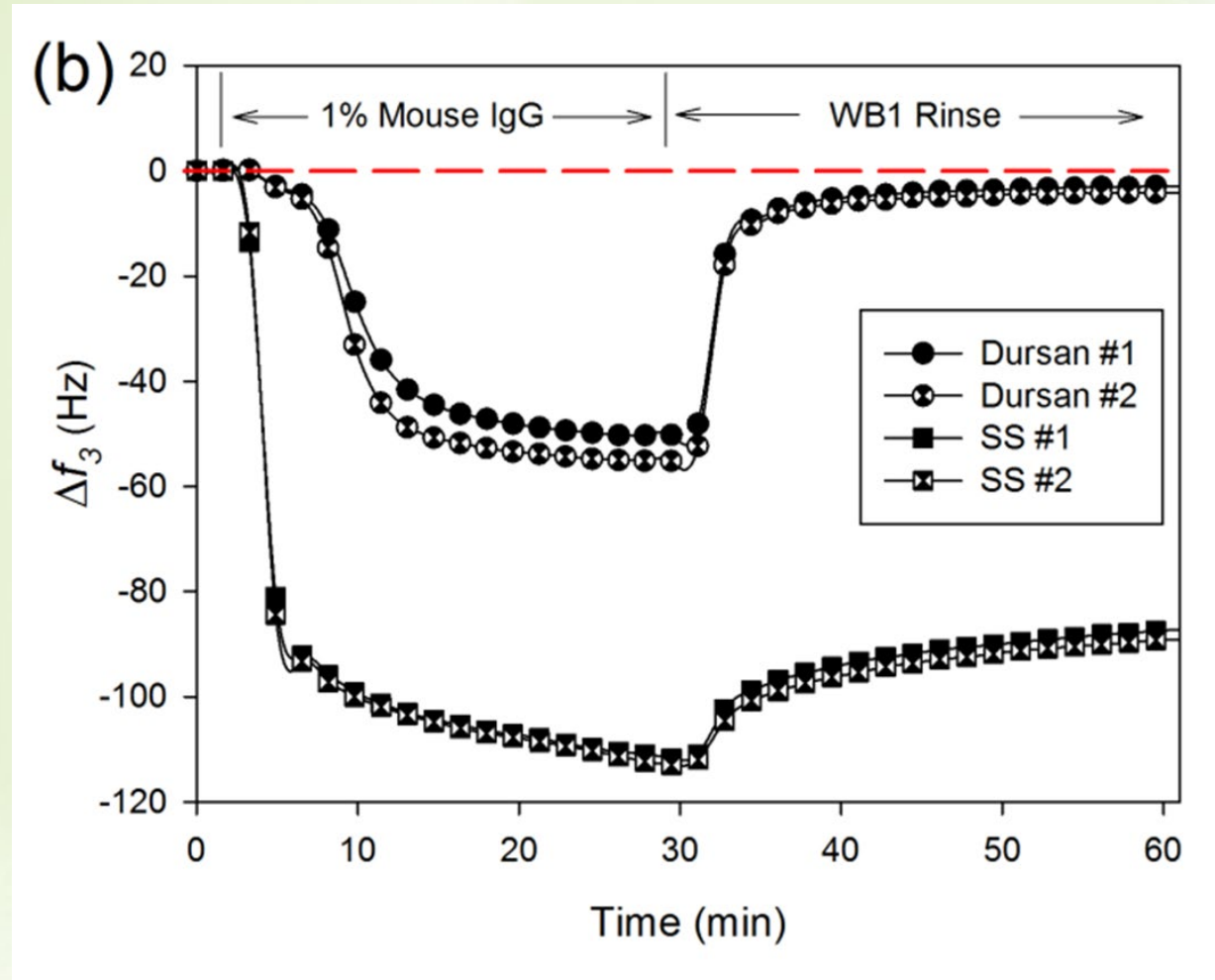


Bio-Inertness: Non-specific protein adsorption studies

- Collaborative study between Abbott Laboratories and SilcoTek on protein adsorption
- QCM-D with a thin layer of 316L SS was coated with Dursan
- Protein solutions were flowed over the sensor and the frequency was monitored over time

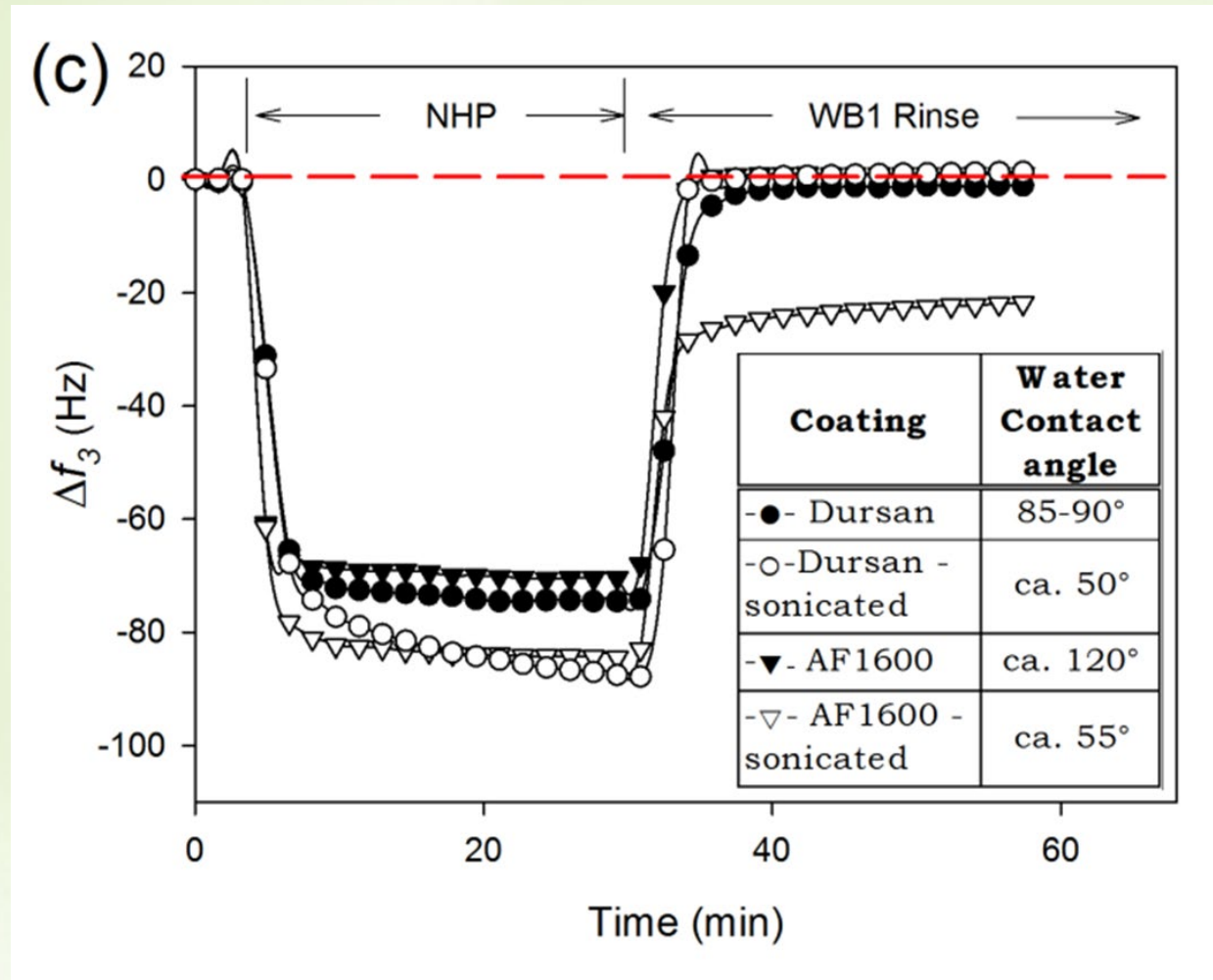


Mouse immunoglobulins do not stick



Vaidya, S.V.; Yuan, M.; Narvaez, A.R.; Daghfal, D.; Mattzela, J.; Smith, D. Appl. Surf. Sci. 2016, 364, 896-908.

Performs as well as fluoroplastic in non-sonicated environment



Dursan[®] coated HPLC columns: Customer testimonials

“The group that was using the coated column to isolate and purify their sensitive novel compounds have completed their study. The LC work was critical as it allowed further characterization of the compounds they synthesized.”

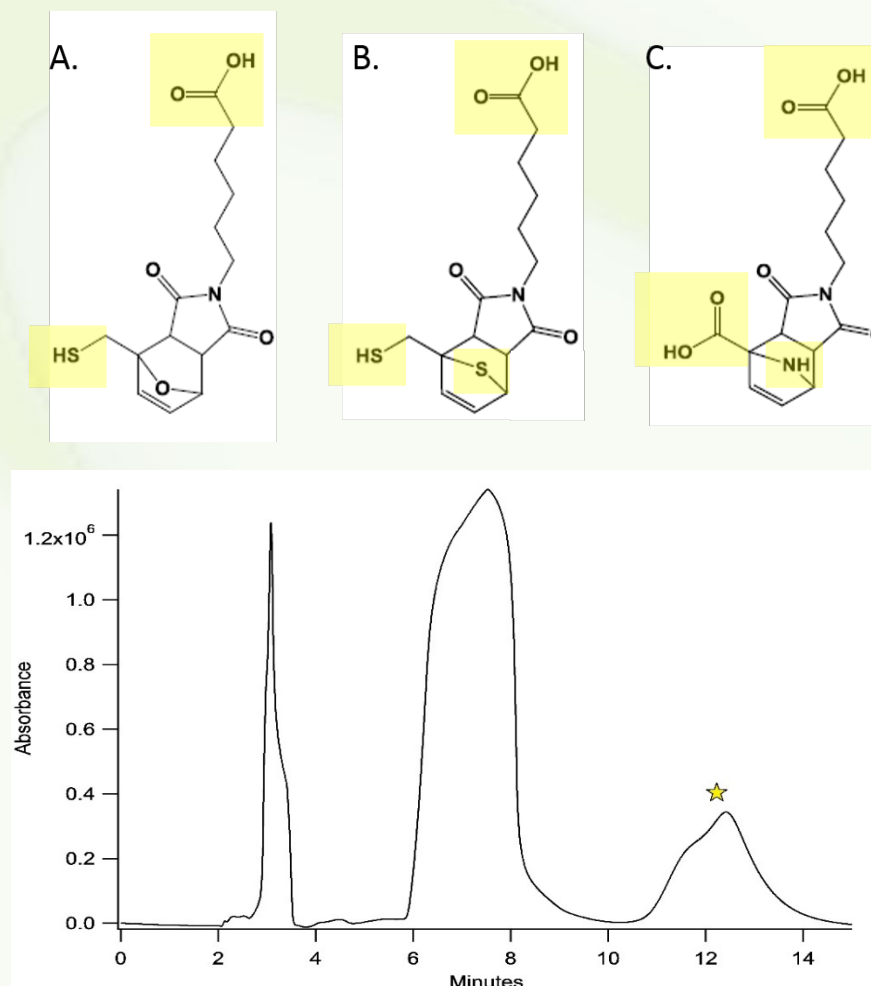
“We have tried the treated tubes on two different column phases and we are seeing an increase in efficiency and improved peak shape.”

“The coated columns have so far passed all tests bravely. The chromatographic separation in standard samples remains unchanged; in the case of biomolecules the results were, as expected, much better than in normal steel columns, but also better than in the case of pure PEEK columns.”

Inertness to sulfur compounds

- In a recent paper¹, Dursan coated columns were used to purify and concentrate molecules that would readily react with stainless steel
- The peak of interest is the furan molecule (A).
 - Note that the researchers were optimizing for clean separation and not necessarily peak efficiency.

1. Abu-Laban, M.; Kumal, R. R.; Casey, J.; Becca, J.; Lamaster, D.; Pacheco, C. N.; Sykes, D. G.; Jensen, L.; Haber, L. H.; Hayes, D. J. *Journal of Colloid and Interface Science* **2018**, 526, 312–321.



Our studies on HPLC columns

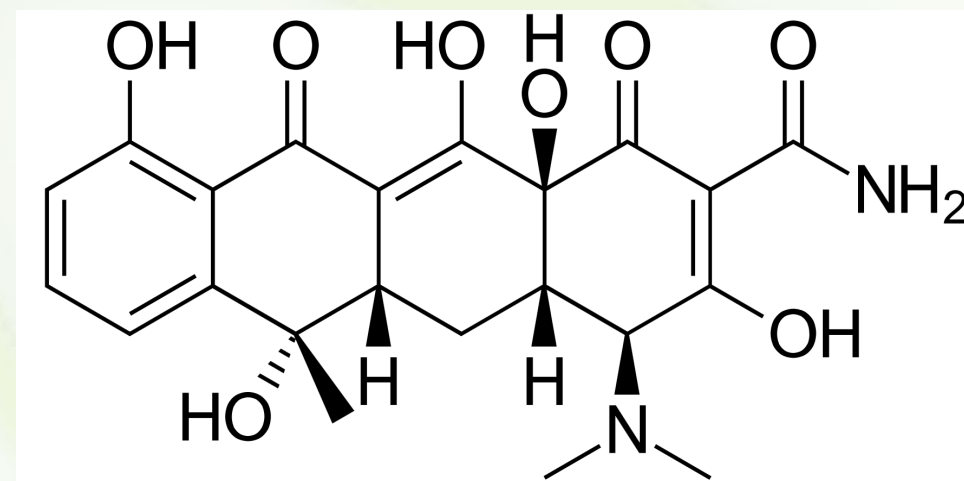
- Three 2.1 mm ID x 100 mm columns were packed of each of the following:
 - All Stainless Steel (A)
 - All Dursan coated (B)
 - Dursan coated frit and stainless steel column (C)
 - Titanium frit and stainless steel column (D)
- Biphenyl peak in standard HPLC mix was used to measure efficiency of column
 - No change in efficiency, asymmetry, or capacity factor after 800 injections

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 Technology, 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

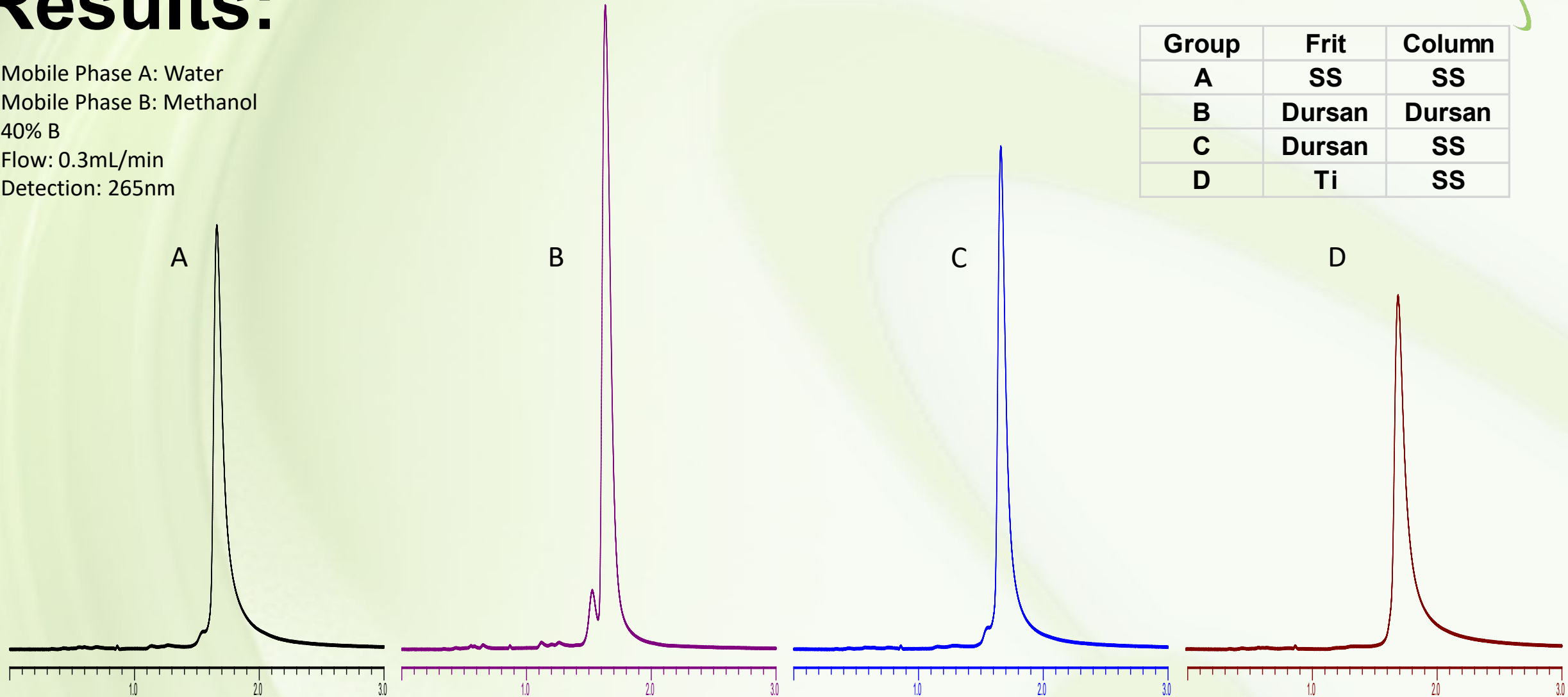


Molecular structure of tetracycline

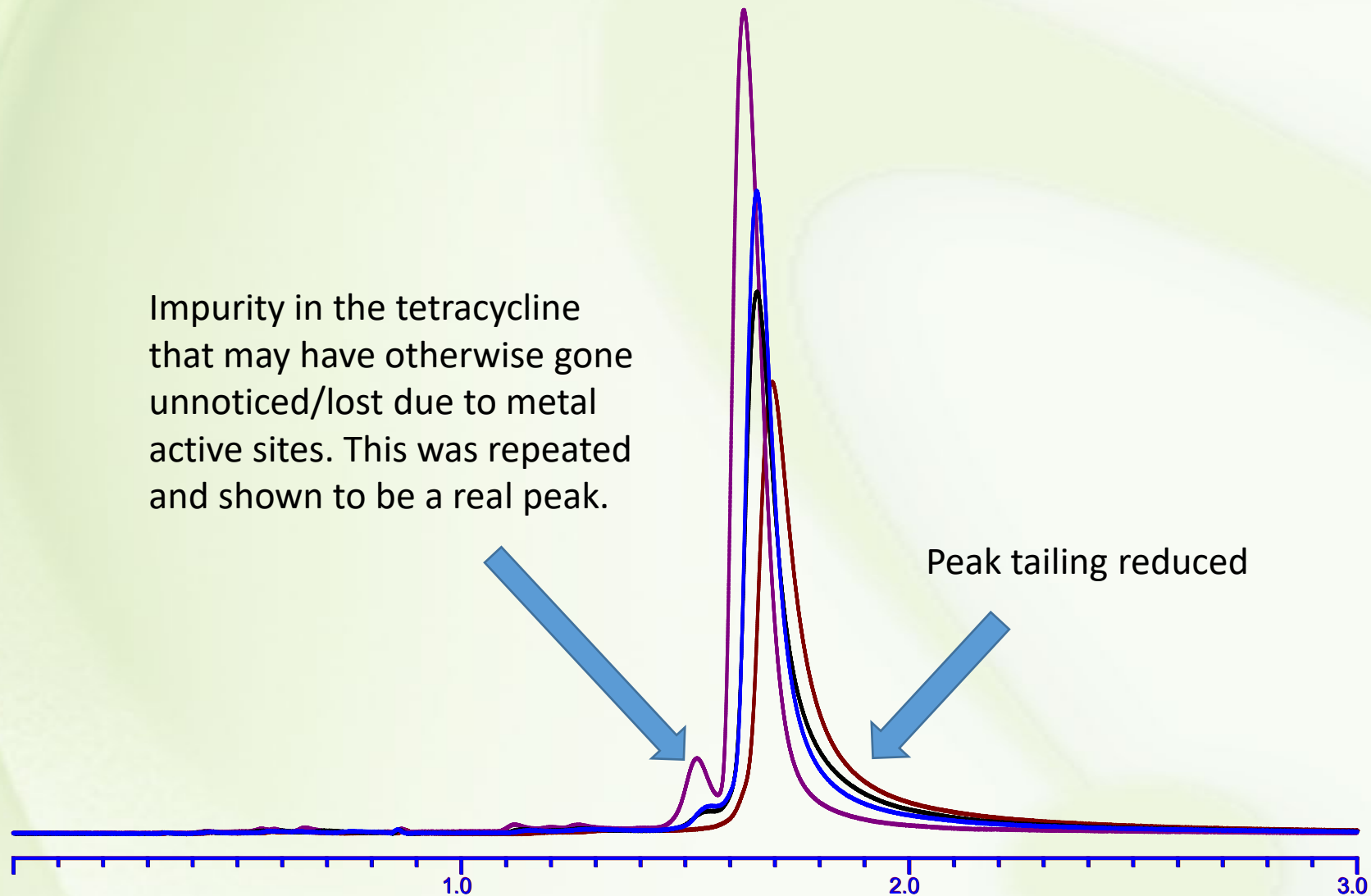
Results:

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

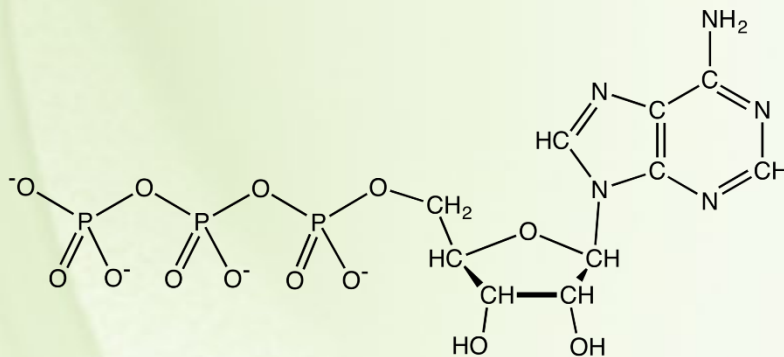


Overlay of all 4 columns:

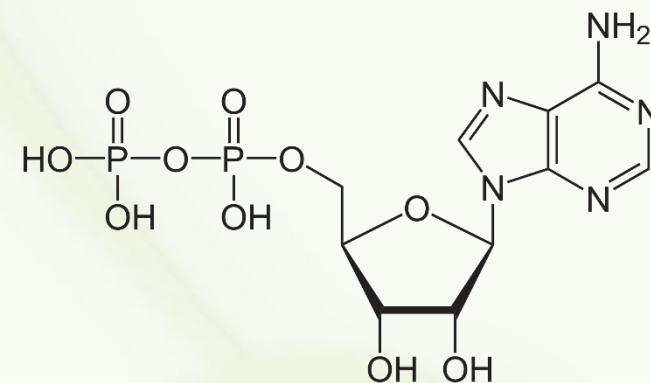


Adenosine triphosphate and diphosphate (ATP and ADP)

- 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



ATP

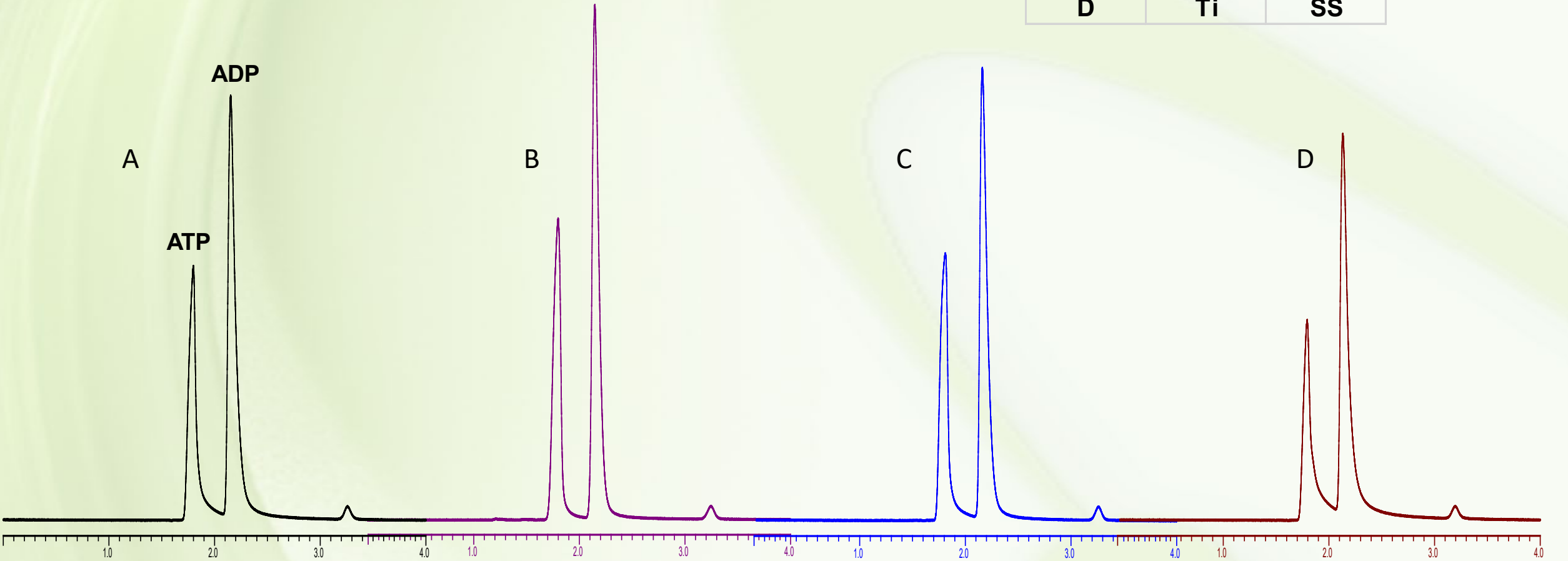


ADP

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

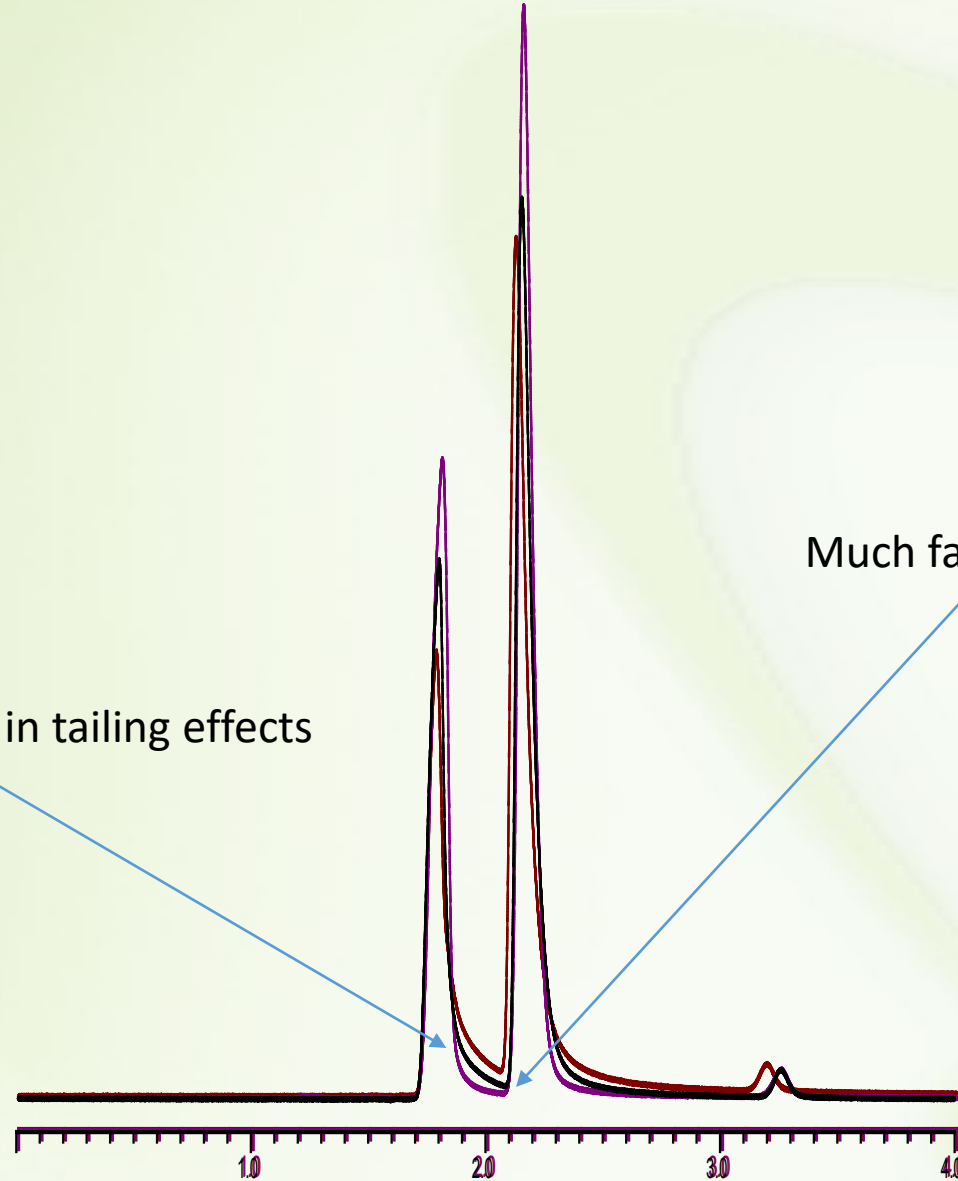


Overlay:

All stainless steel vs
all Dursan column

Significant decrease in tailing effects

Much faster return to baseline.



Peak analysis of ATP and ADP

- Note the increase in height and decrease in asymmetry values for the Dursan system

	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

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.

Results:

Mobile phase: Water no buffer

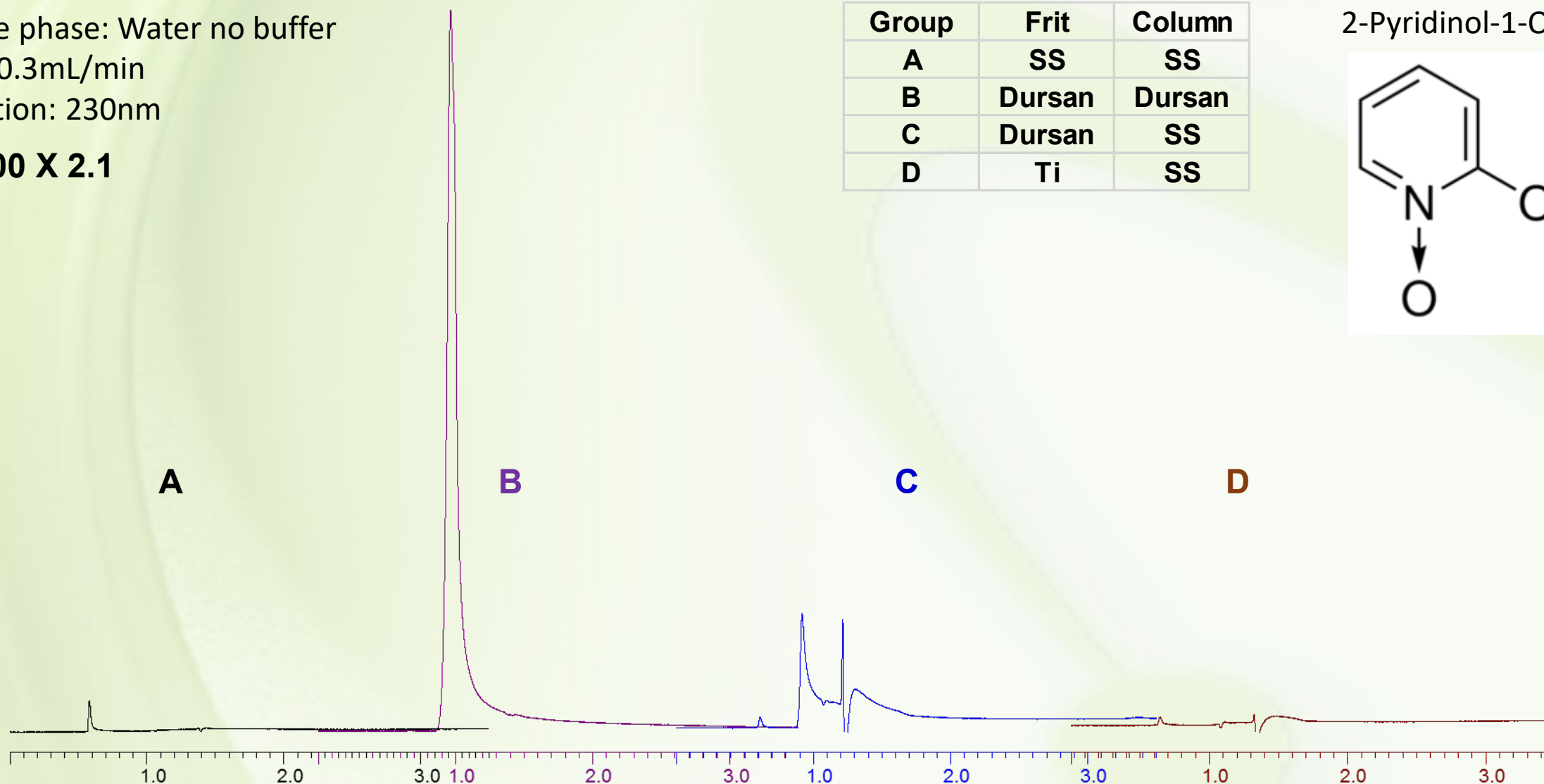
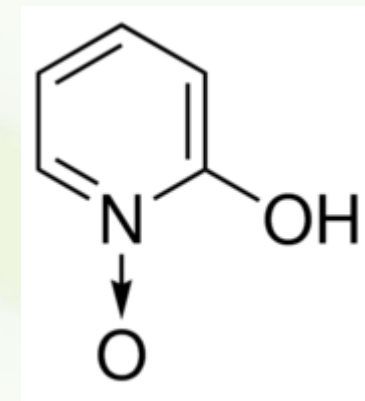
Flow: 0.3mL/min

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



Planned future studies

- PEEK vs Dursan[®]
 - Customers have anecdotally told us results, but we want to test it for ourselves
- Durability studies in harsh environments
 - High and low pH solvents could degrade columns, does Dursan[®] provide sufficient protection?
- More case studies on difficult molecules and/or biologics
 - Antibodies like IgG1
- Moving past reverse phase HPLC
 - Does Dursan[®] help in HILIC, ion exchange, gel filtration, etc.

Acknowledgements



Phase

Analytical Technology LLC

phaseanalytical.net



Conclusions

- Dursan[®] is a robust, inert, and reproducible coating technology that can be applied to as built stainless steel HPLC components
- Corrosion resistance and non-fouling properties of Dursan[®] can be beneficial under harsh testing conditions
- Coating columns and frits have shown benefits for troublesome molecules such as peak narrowing, and faster return to baseline, or in the case of powerful chelating agents, the ability to get a good peak at all.
- You can gain the inertness of PEEK while maintaining the robustness and reliability of stainless steel

Next steps

- You can get parts Dursan-coated exclusively by SilcoTek, or request coated hardware from your preferred vendor
- www.silcotek.com has an array of technical and educational resources for further learning
- To talk directly with a SilcoTek representative about your application, please e-mail silcod@silcotek.com

Questions?