New Developments in GC, HPLC and Sample Prep at Supelco

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sigma-aldrich.com/analytical

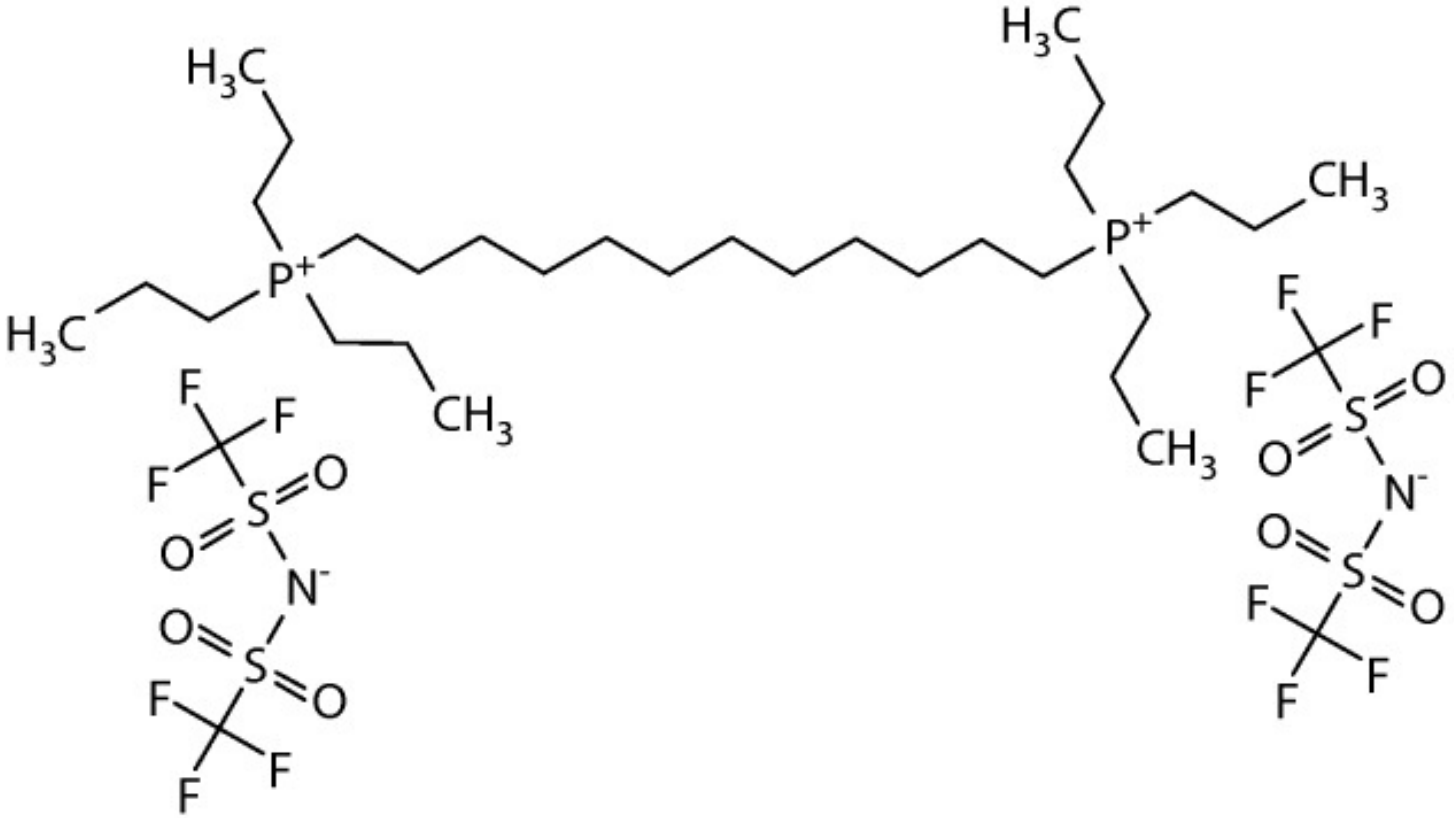
Overview of Presentation

- **Ionic Liquid Capillary Columns**
- **SPME**
- **Titan HPLC**
- **SPE Developments**
- **Asset Air Sampler**
- **Conclusions**

SLB-IL60

Phase Structure

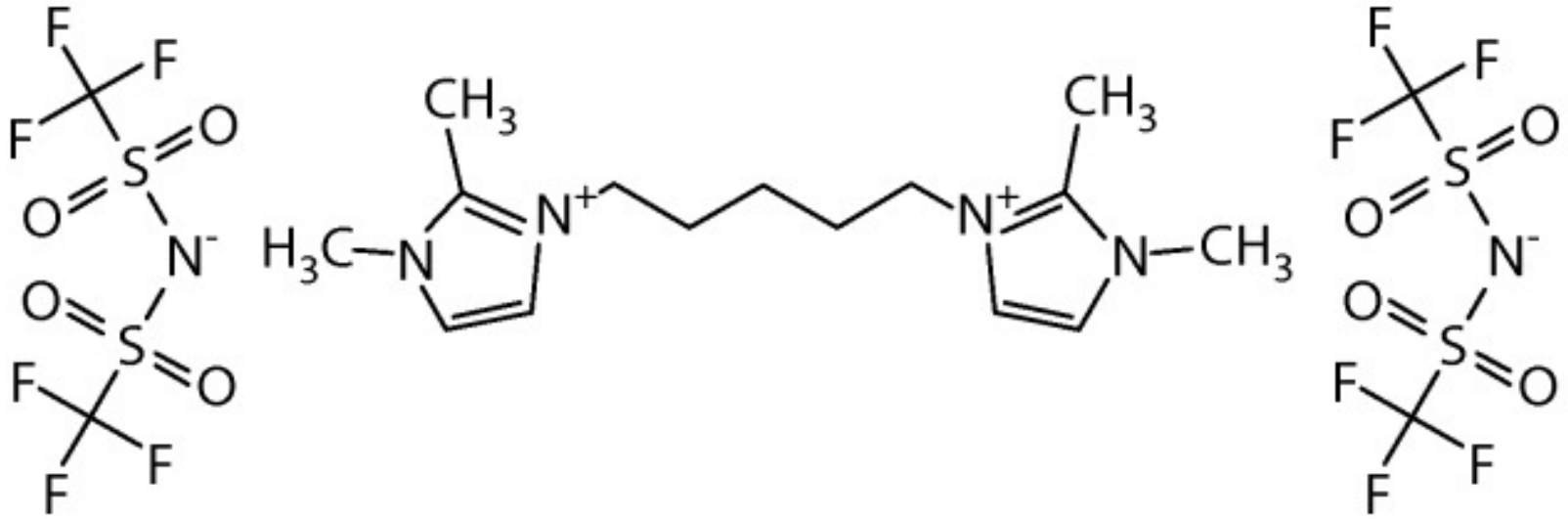
1,12-Di(tripropylphosphonium)dodecane bis(trifluoromethylsulfonyl)imide



SLB-IL111

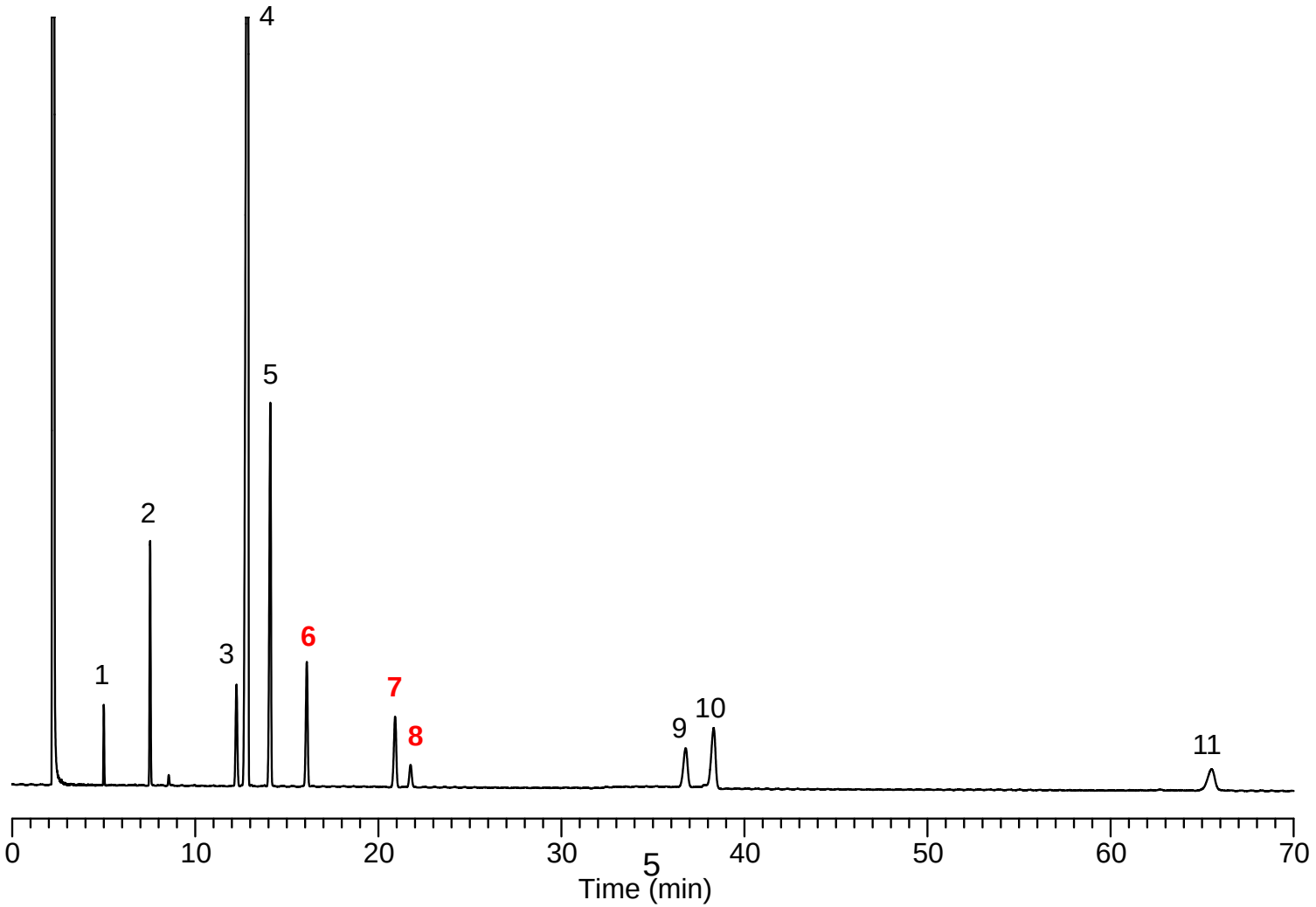
Phase Structure

1,5-Di(2,3-dimethylimidazolium)pentane bis(trifluoromethylsulfonyl)imide



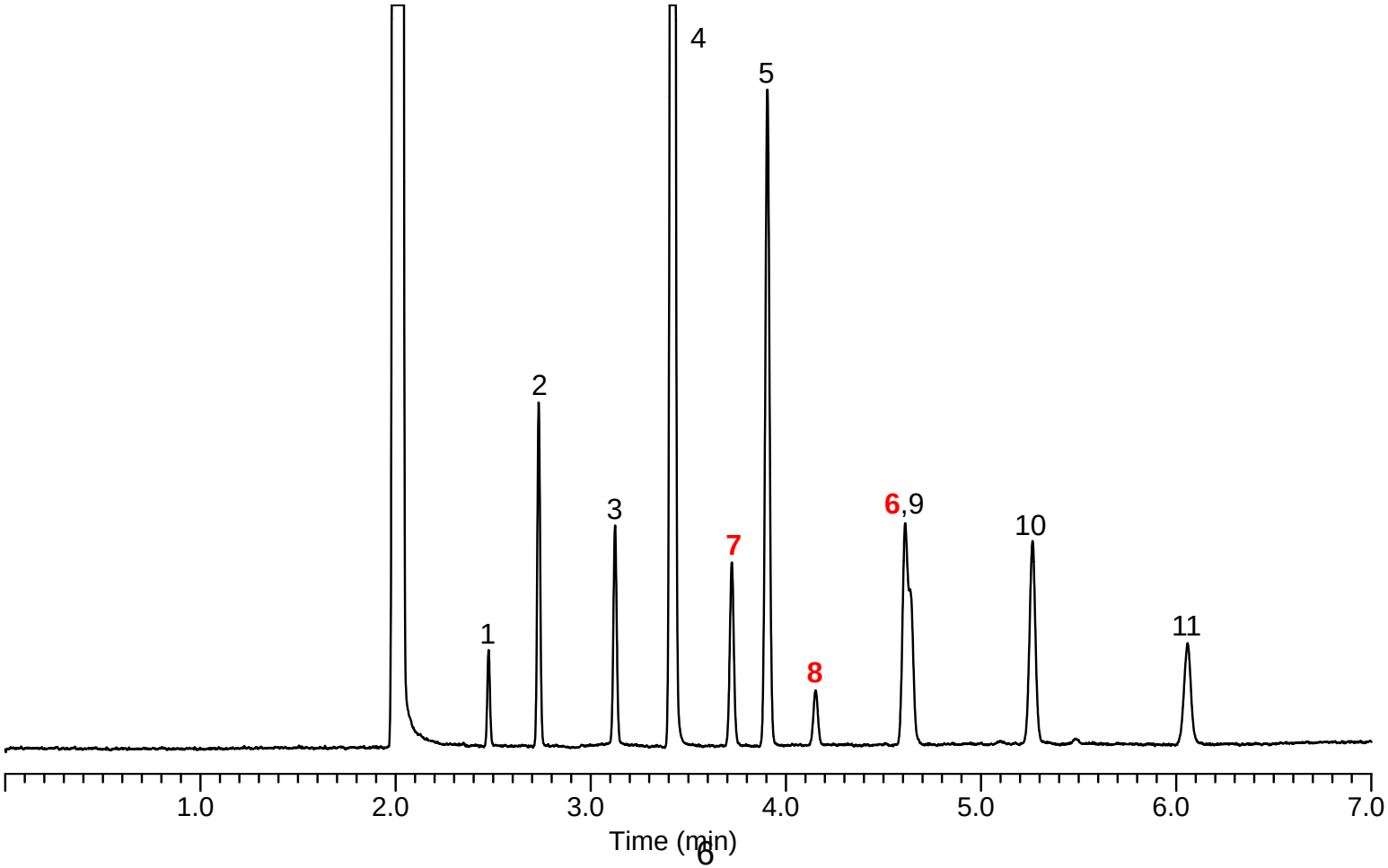
Column Selectivity

SLB-IL60, 30 m x 0.25 mm I.D., 0.20 µm (29505-U)



Column Selectivity

SLB-IL111, 30 m x 0.25 mm I.D., 0.20 µm (28927-U)



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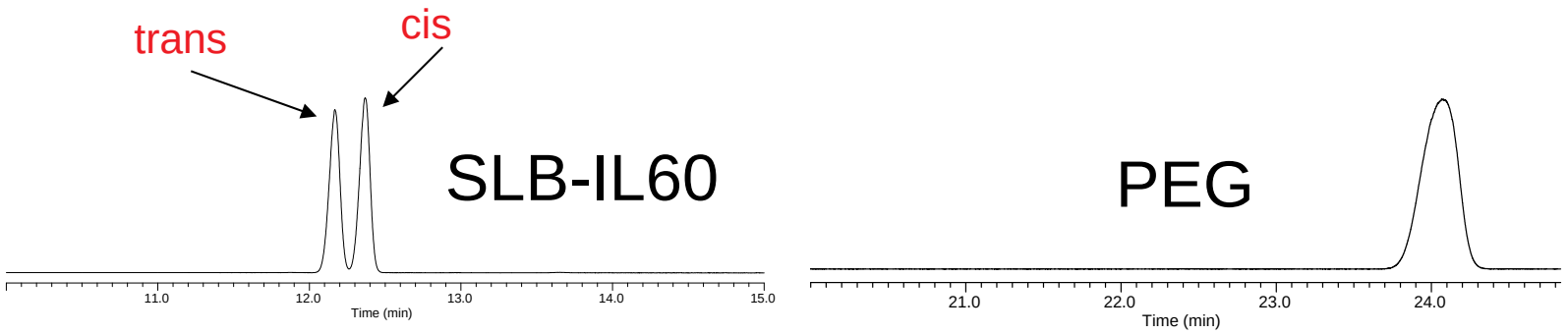
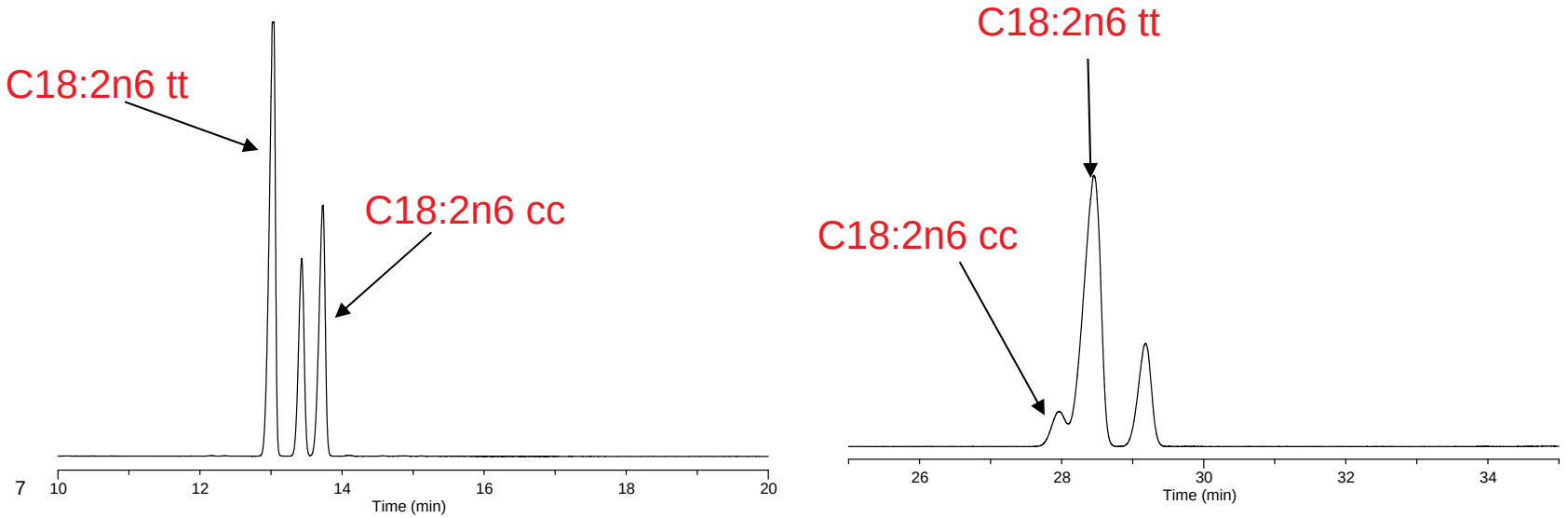
Cis/ trans FAMES on SLB-IL60 vs. PEG Type Phase

C18:1n9 cis / trans FAMEs @ 180°C



C18:2n6 cis & trans FAME Isomers- 180°C



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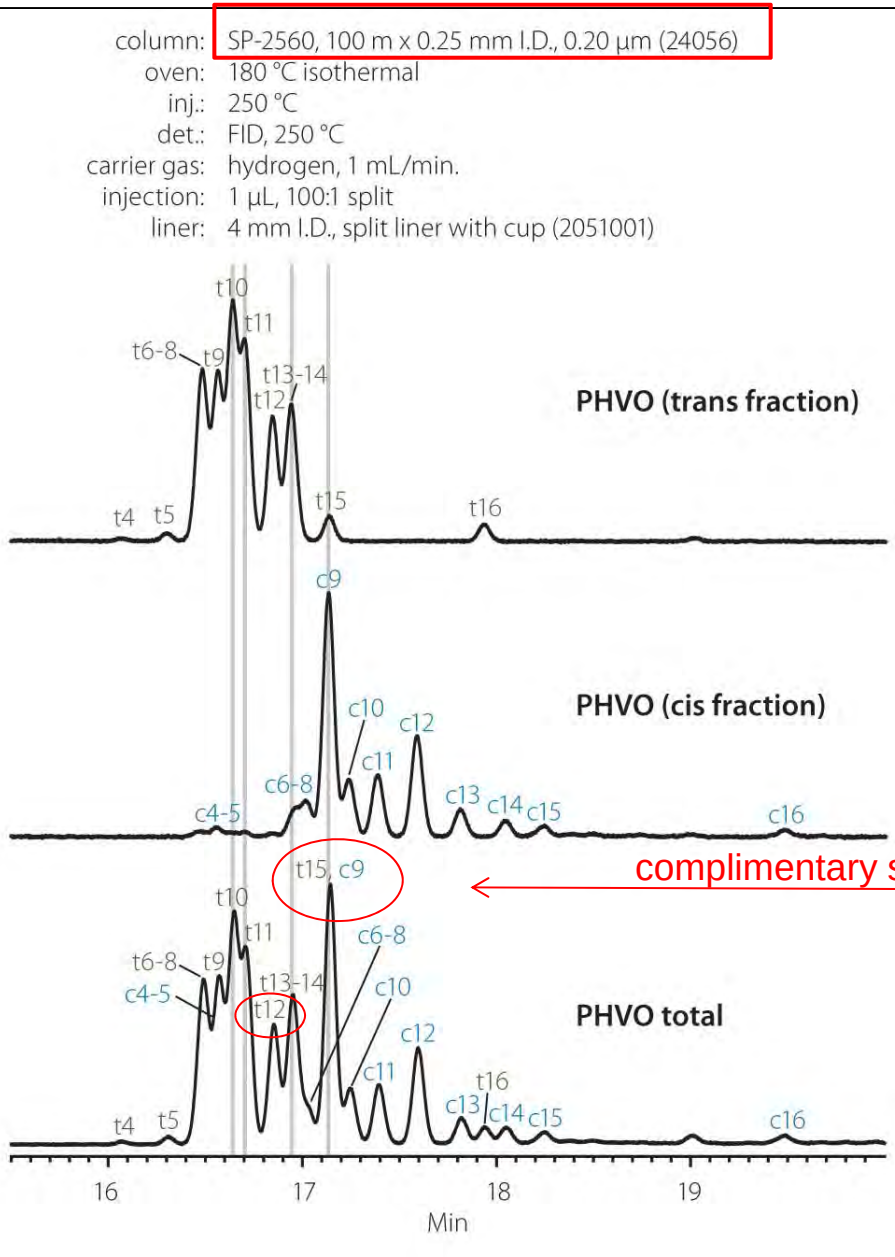
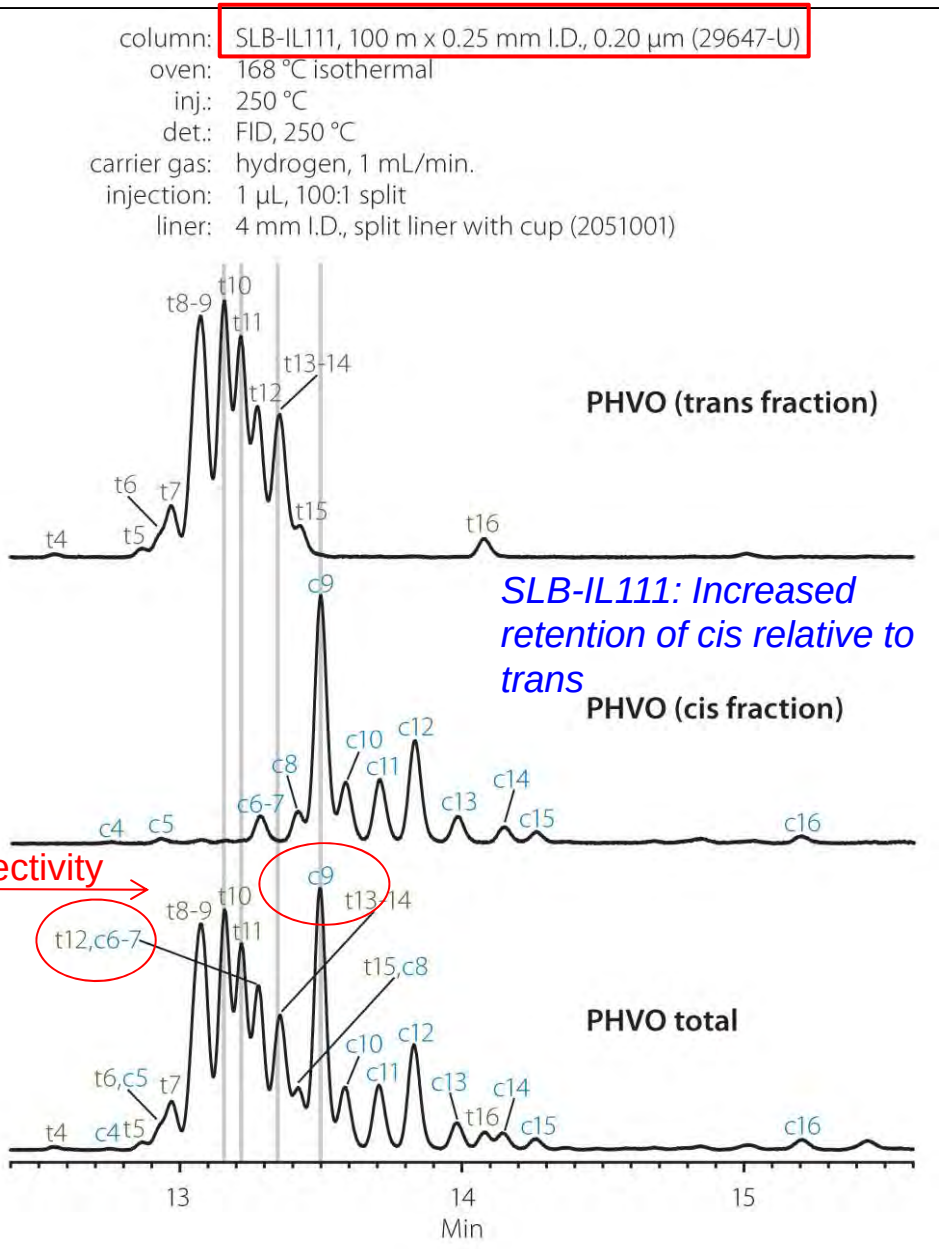
C18:1 cis/trans FAME Isomers in Partially Hydrogenated Vegetable Oil (PHVO) SLB-IL111 vs. SP-2560: 100 m columns





complimentary selectivity

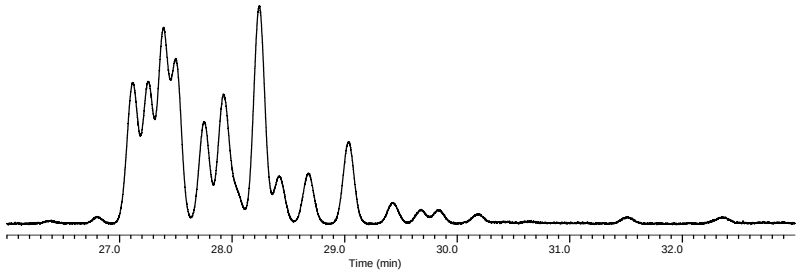
SLB-IL111: Increased retention of cis relative to trans

Positional cis/trans FAME Isomers

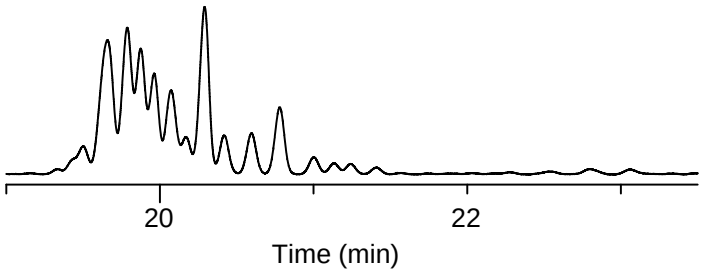
column:	SP-2560, 200 m x 0.25 mm I.D., 0.20 µm
oven:	180 ° C isothermal
inj.:	250 ° C
det.:	FID, 250 ° C
carrier gas:	hydrogen, 1 mL/min.
injection:	1 µL, 100:1 split
liner:	4 mm I.D., split liner with cup (2051001)

column:	SLB-IL111, 200 m x 0.25 mm I.D., 0.20 µm
oven:	168 ° C isothermal
inj.:	250 ° C
det.:	FID, 250 ° C
carrier gas:	hydrogen, 1 mL/min.
injection:	1 µL, 100:1 split
liner:	4 mm I.D., split liner with cup (2051001)

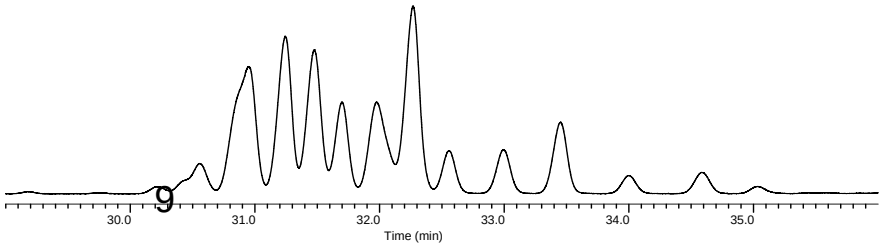
PHVO total FAMEs



PHVO total FAMEs



PHVO total FAMEs on SLB-IL111 @ 150 ° C isothermal



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PAHs on SLB-IL 59 20m x 0.18mm x 0.04umd$_f$

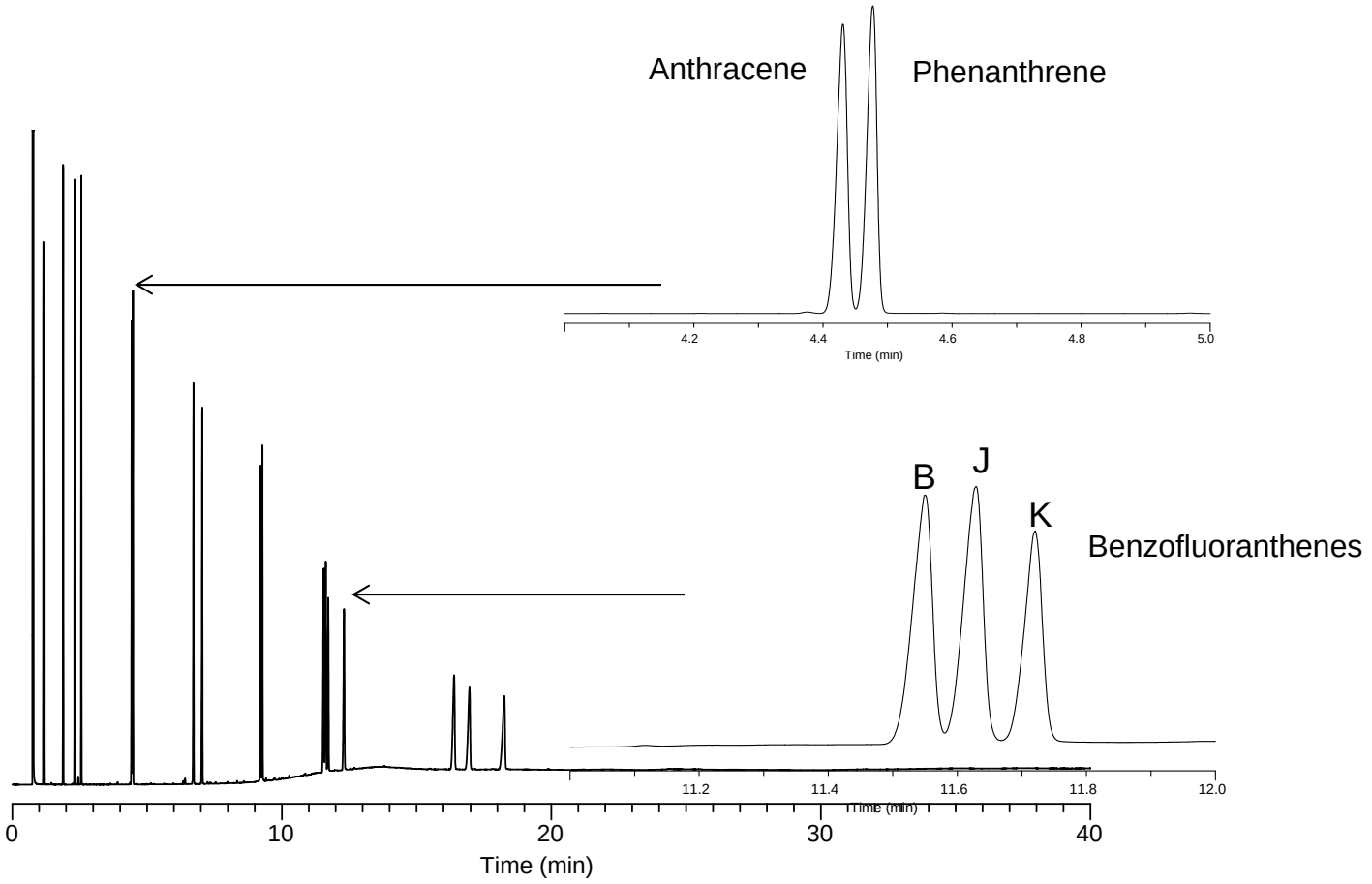
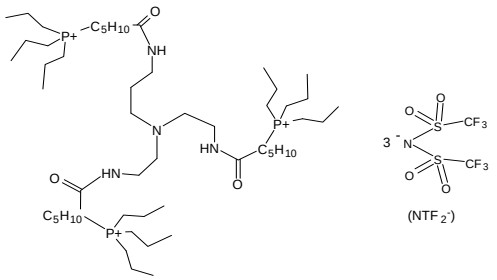


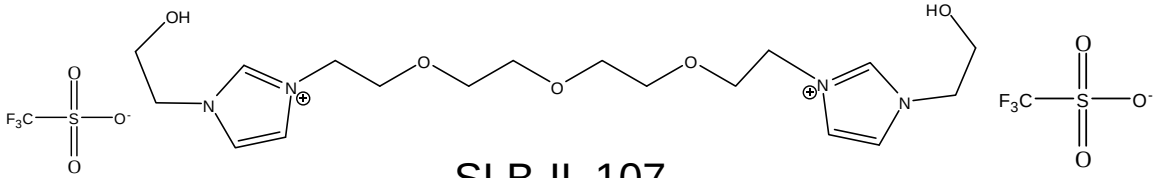
Figure 6. TCL PAHs on SLB-IL 59, 20m x 0.18mm x 0.04umdf, H_2 carrier gas; Expanded views show anthracene/phenanthrene and benzofluoranthene isomers

Ionic Liquid Water Separations

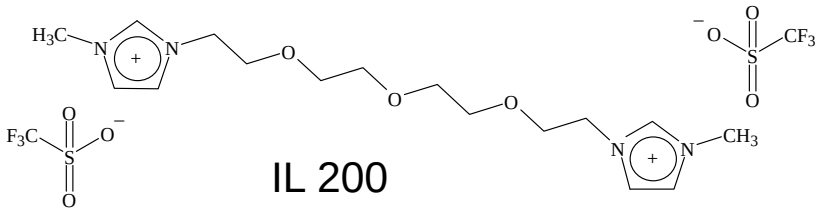
Column: SLB-IL 94, SLB-IL 107, SLB-IL 200 30m x 0.25mm x 0.20umd$_f$
Oven: 35˚C, 4˚C/min to 125˚C, 125˚(2min)
Det: TCD, 300˚C
Flow Rate: 25cm/sec constant pressure He
Inj: 250˚C,1uL, split, 100:1
Liner: 4mm ID cup design split liner
Samples: IL Solvent Test Mix: MeOH, EtOH, Acetone, IPA, n-propanol, 1-butanol, 1,4-Dioxin in water



SLB-IL 94



SLB-IL 107



IL 200

IL Solvent Mix on SLB-IL 94 30m x 0.25mm x 0.20umd_f

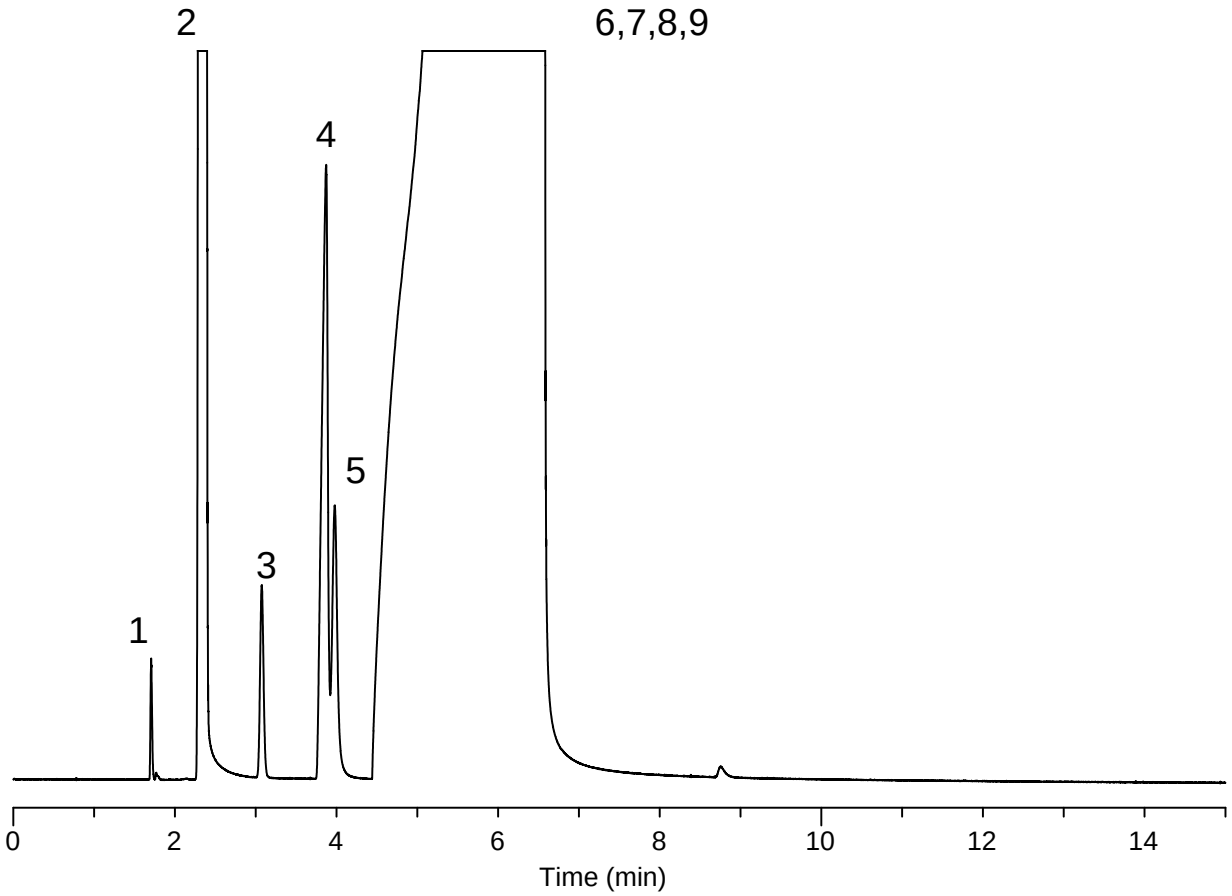


Figure 9. Solvent test standard programmed separation on SLB-IL 94; 1) MeOH, 2) $MeCl_2$ 3) acetone, 4) ethanol, 5) IPA, 6) n-Propanol, 7) 1,4dioxane, 8) butanol 9) water

IL Solvent Mix on SLB-IL 107 30m x 0.25mm x 0.20umd_f

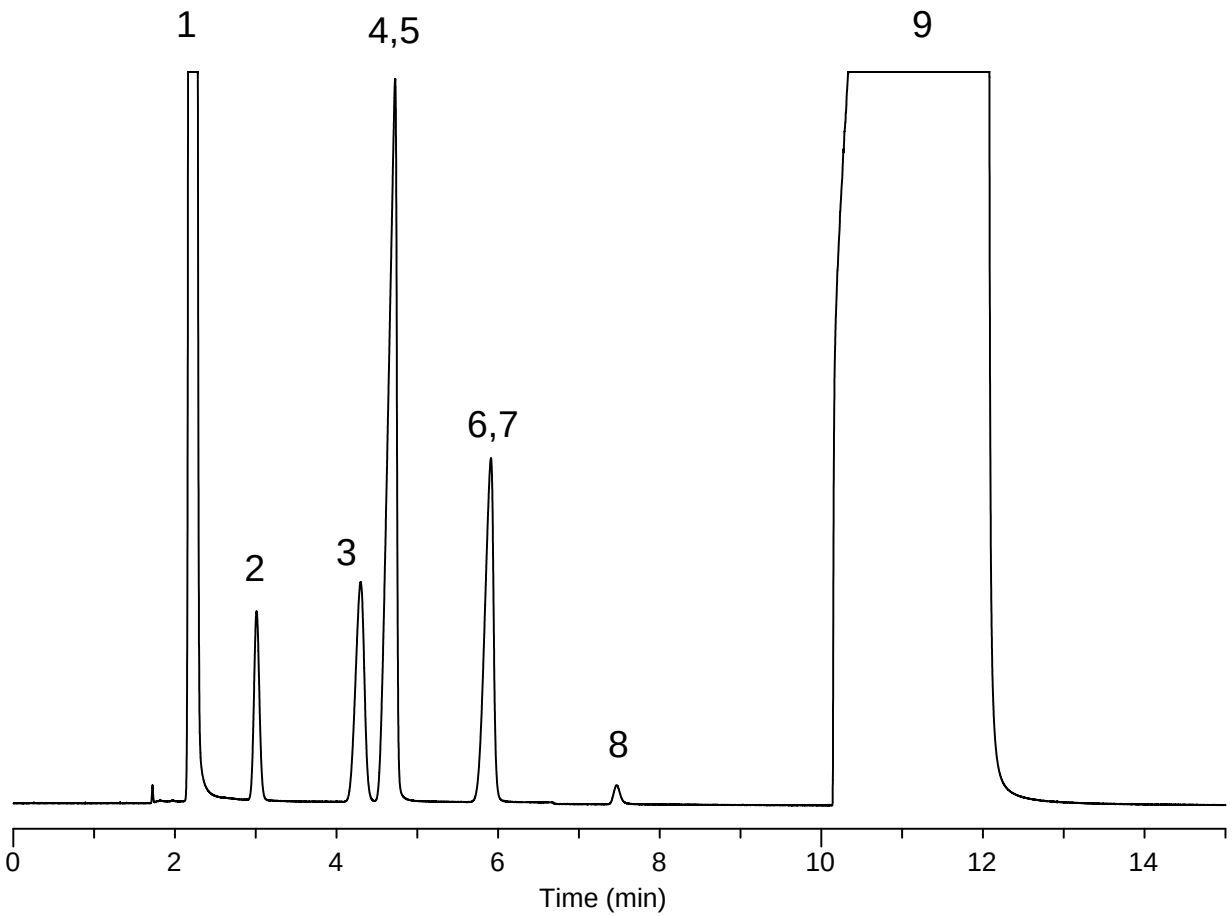
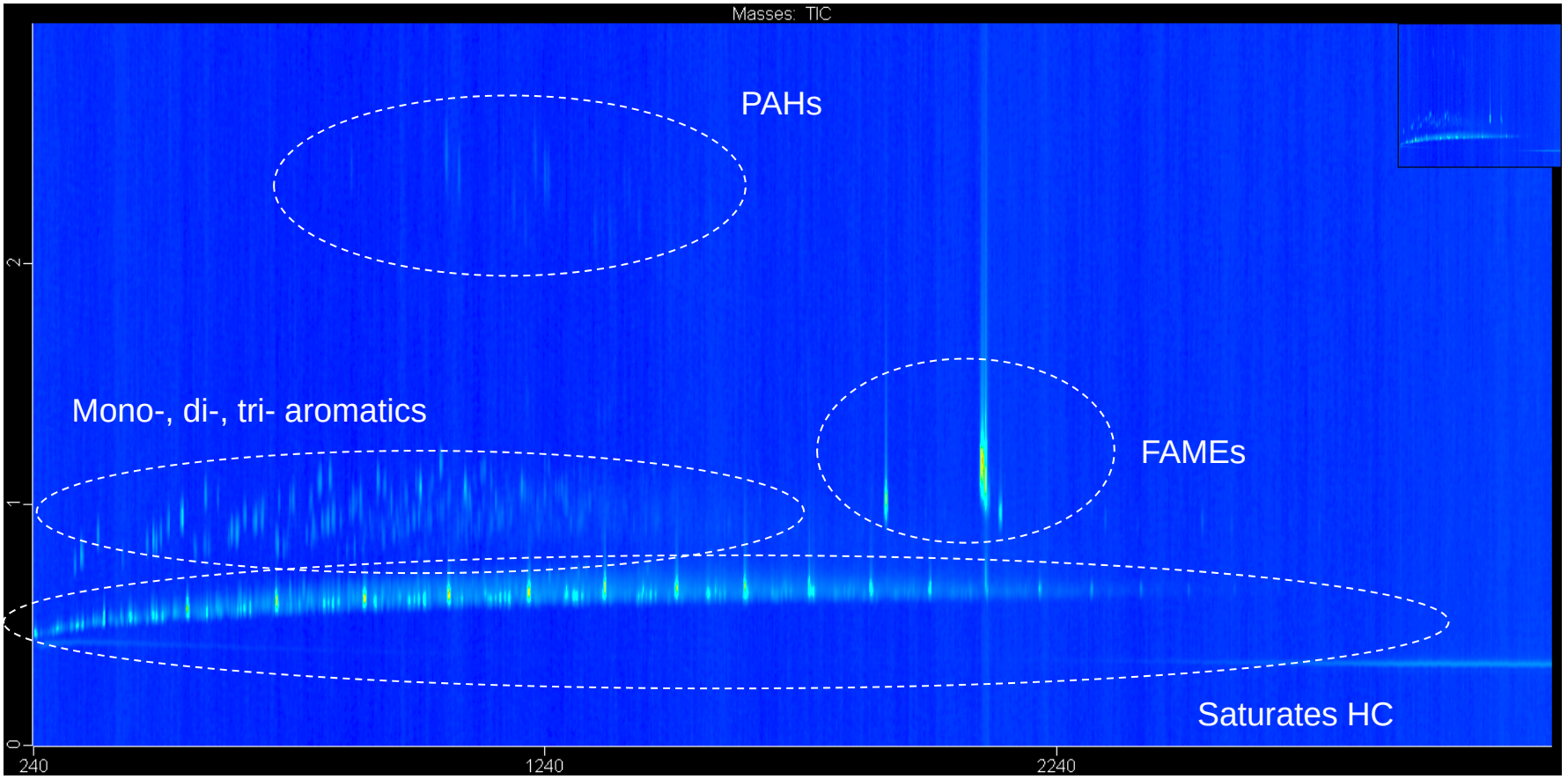


Figure 8. Solvent test standard programmed separation on SLB-IL 107; 1) MeOH/$MeCl_2$, 2) acetone, 3) IPA, 4) ethanol, 5)methanol, 6) n-Propanol, 7) 1,4dioxane 8) butanol, 9) water

NON-POLAR – POLAR STRATEGY

Biodiesel 20



^{2}D (sec) – **SLB IL60** (1 m x 0.10 mm x 0.08 µm df)

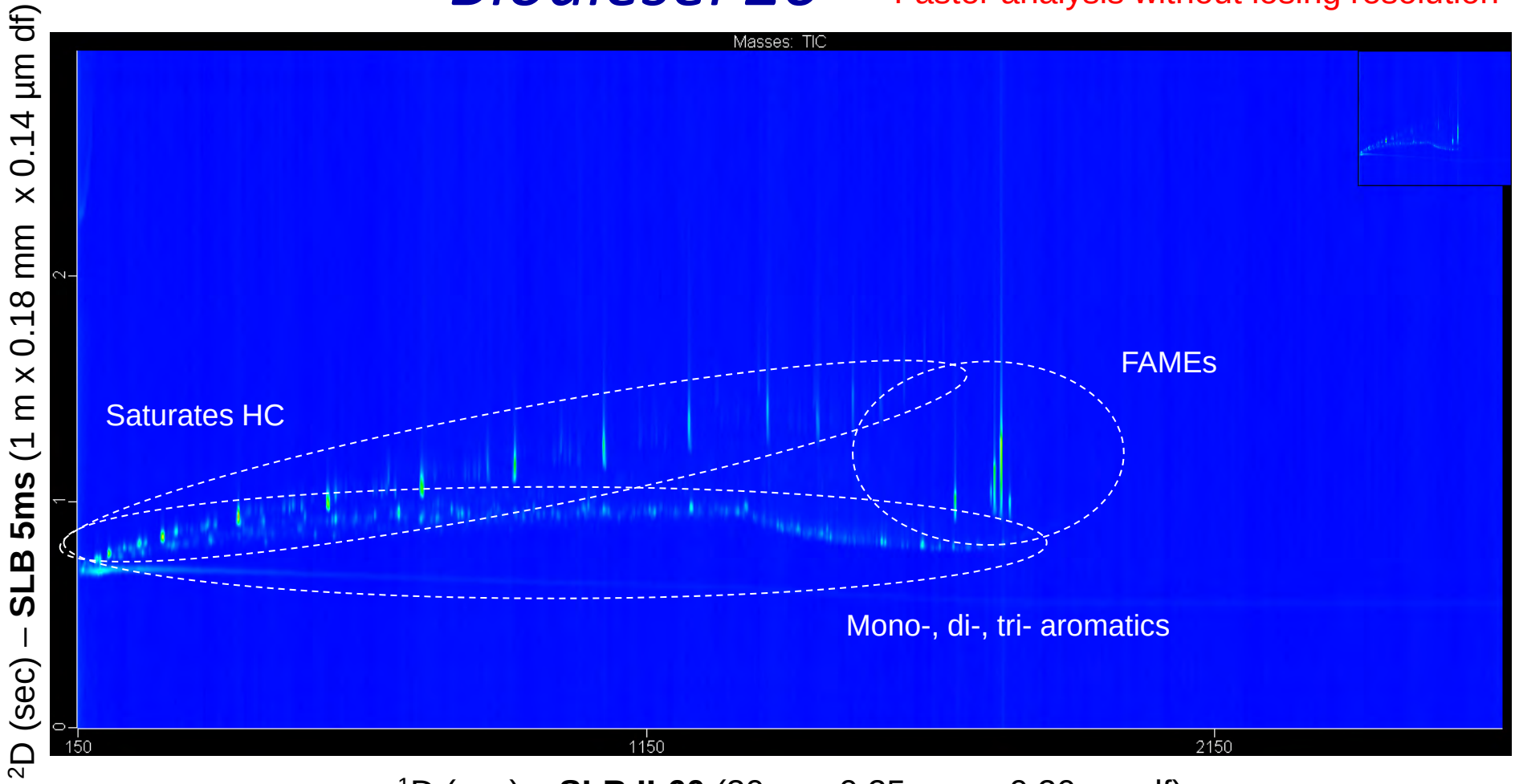
^{1}D (sec) – **SLB 5ms** (30 m x 0.25 mm ID x 0.25 µm df)

Equipment: LECO PEGASUS GC × GC/TOFMS. Carrier gas: Helium set @ 1.0 mL/min. Sample: 1 µL, split ratio 50:1, inlet temp. 250 ºC. GCxGC method temp. program: Primary column: 40 ºC (2 min), ramped @ 4 ºC/min to 270 ºC (20min). Secondary column: 55 ºC (2 min), ramped @ 4 ºC/min to 280 ºC (20min). Modulator temp. offset: 30 ºC. Modulation Period: 3 s. TOFMS method parameters: mass range 35–450 m/z., acquisition rate 200 spectra/s, ion source temp. 250 ºC.

POLAR – NON-POLAR STRATEGY

Biodiesel 20

Faster analysis without losing resolution



Equipment: LECO PEGASUS GC × GC/TOFMS. Carrier gas: Helium set @ 1.2 mL/min. Sample: 1 µL, split ratio 50:1, inlet temp. 250 ºC. GCxGC method temp. program: Primary column: 60 ºC, ramped @ 10 ºC/min to 225 ºC (5 min). Secondary column: 75 ºC, ramped @ 10 ºC/min to 240 ºC (5 min). Modulator temp. offset: 30 ºC. Modulation Period: 3 s. TOFMS method parameters: mass range 35–450 m/z., acquisition rate 200 spectra/s, ion source temp. 250 ºC.

SIGMA-ALDRICH®

Goals of Development of SPME Fibers for Solvent Desorption

Fiber coating must be durable and reproducible

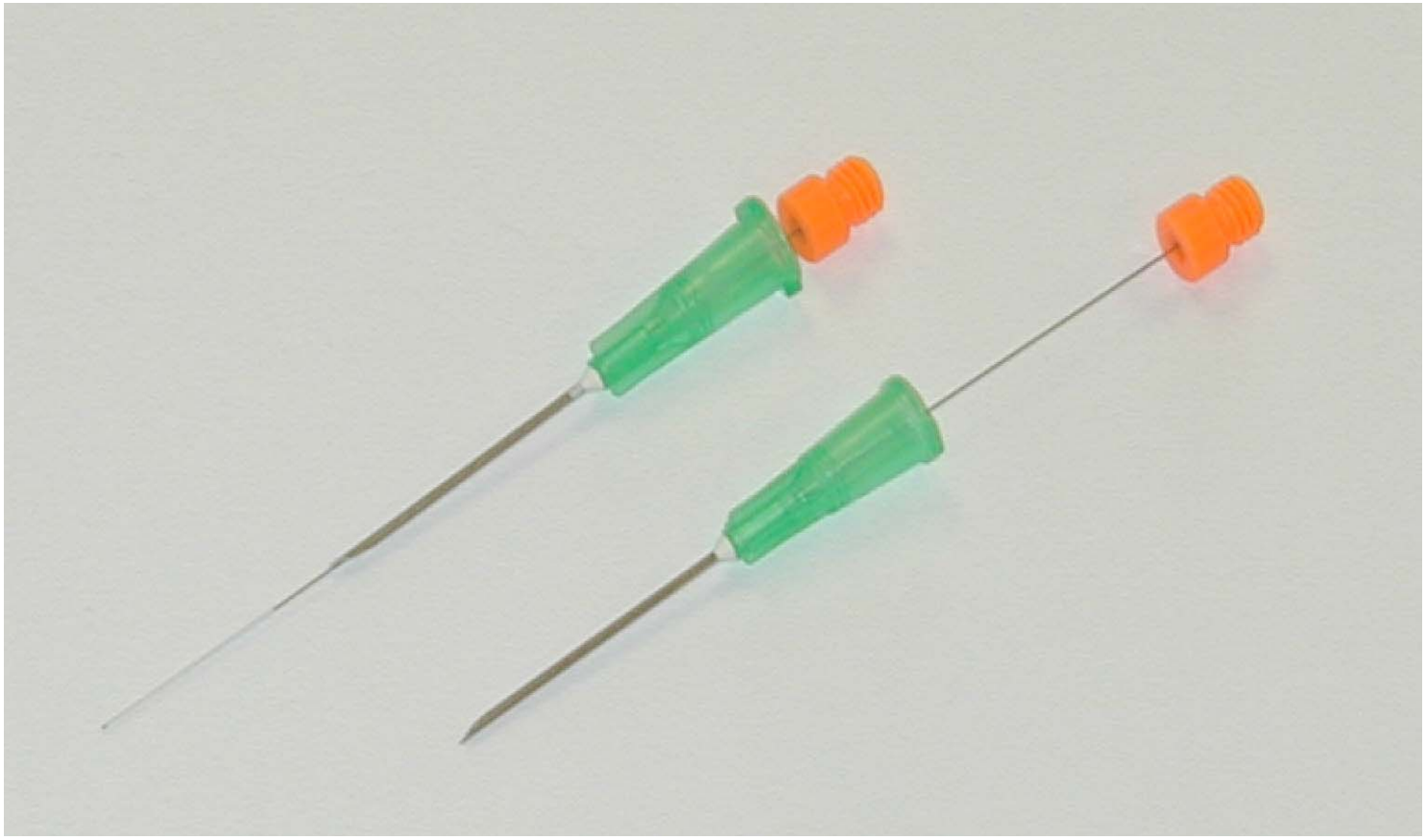
Fiber coating must not swell in water or organic solvents

To coat HPLC particles on fiber the Binder

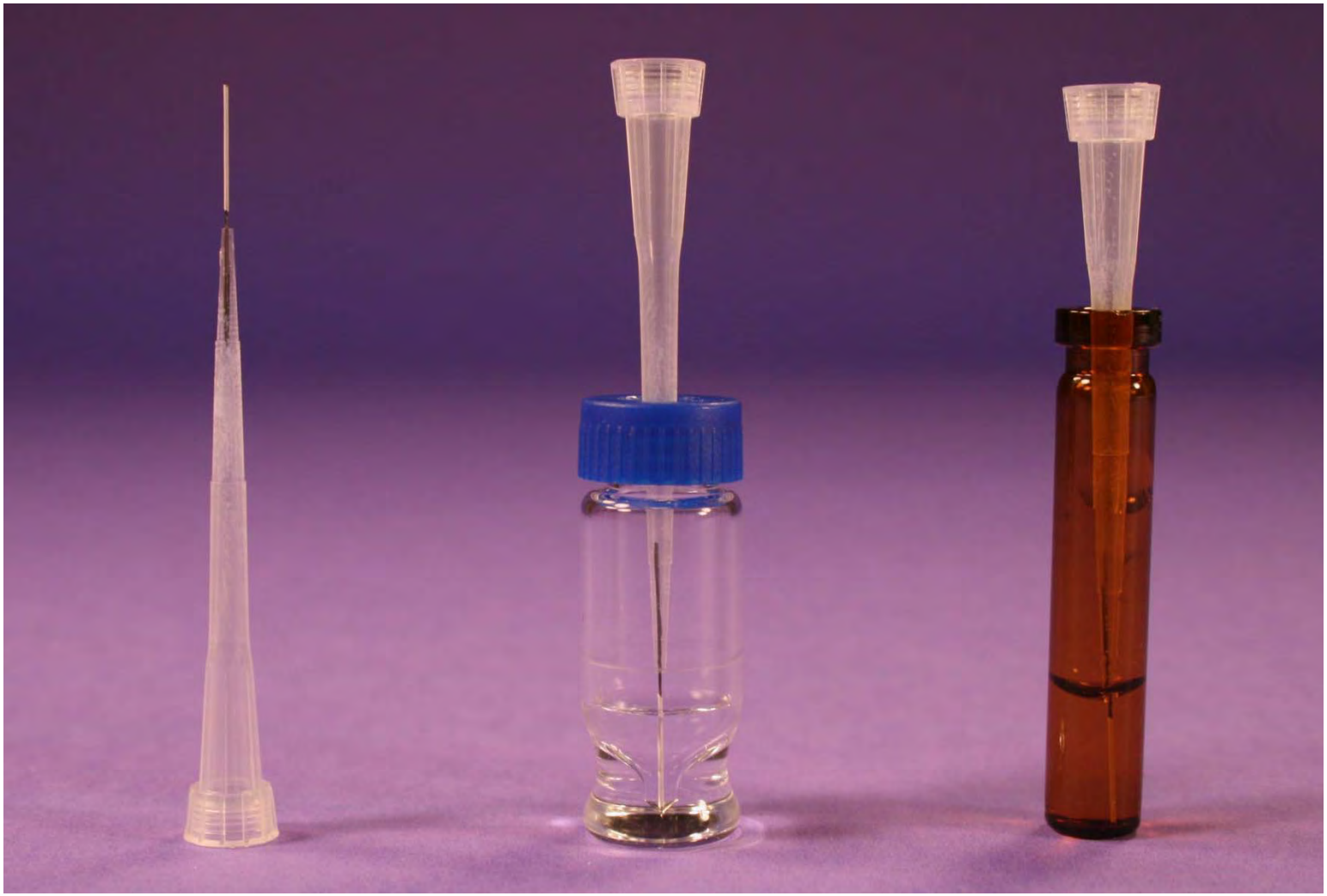
- should not affect uptake of analytes
- should be biocompatible
  - Resists large (macro)molecules
  - For in-vivo type experiments without harm to organism

Device needs to be affordable e.g. for single use analysis

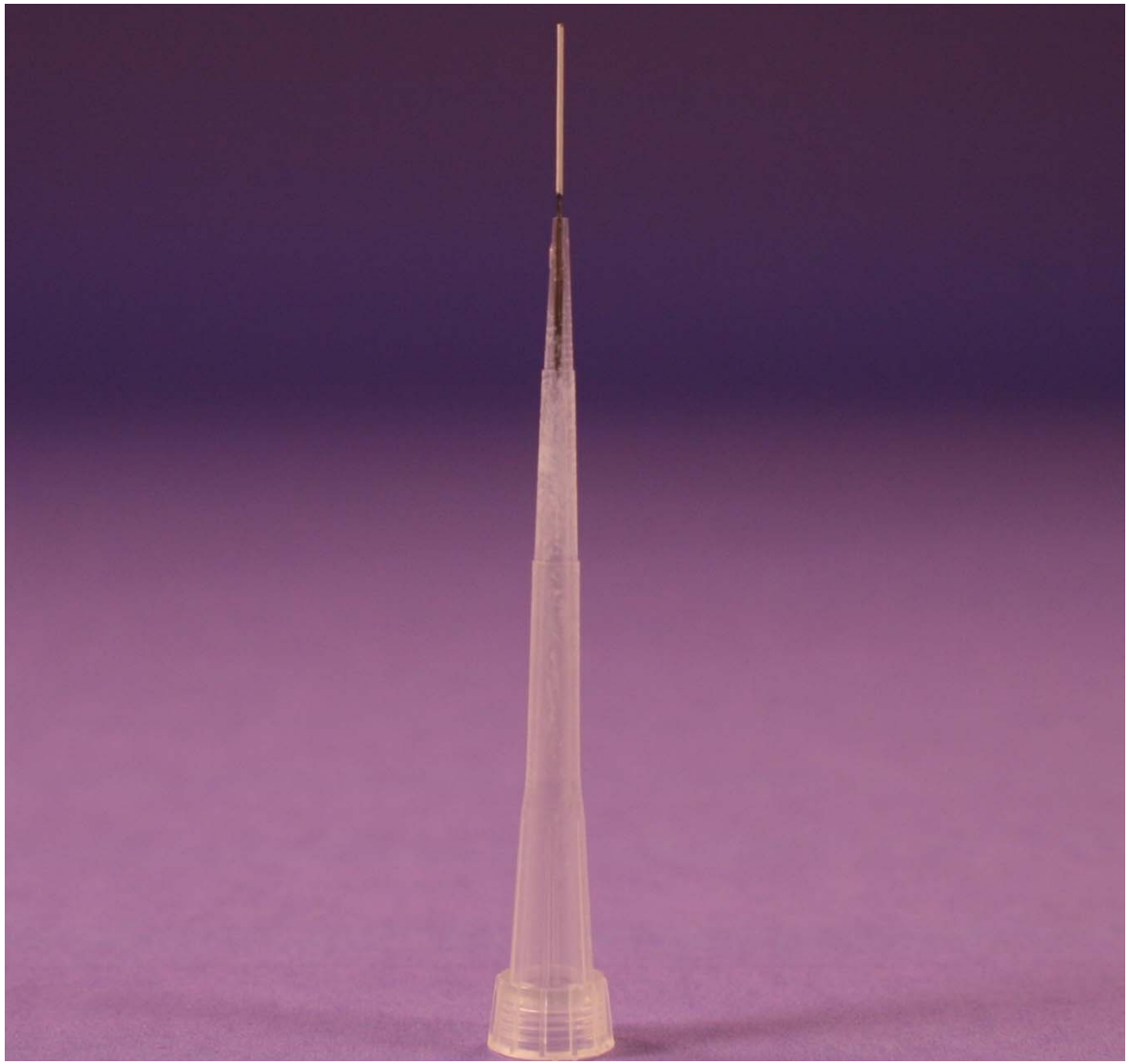
Single Use Biocompatible Fiber Probes for *in-vivo* Analysis



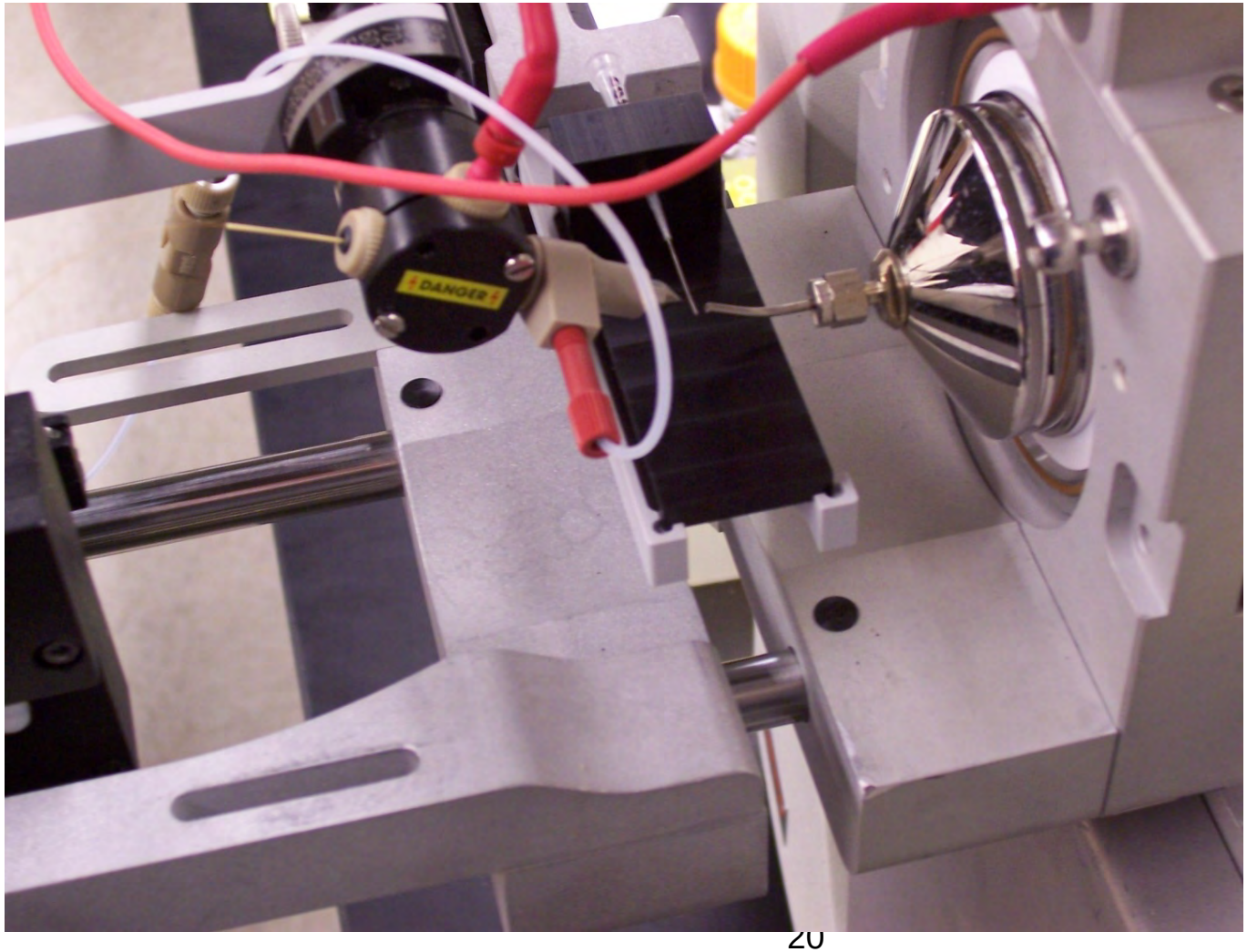
Fiber Pipette Tips



Fiber Tip for HPLC Analysis

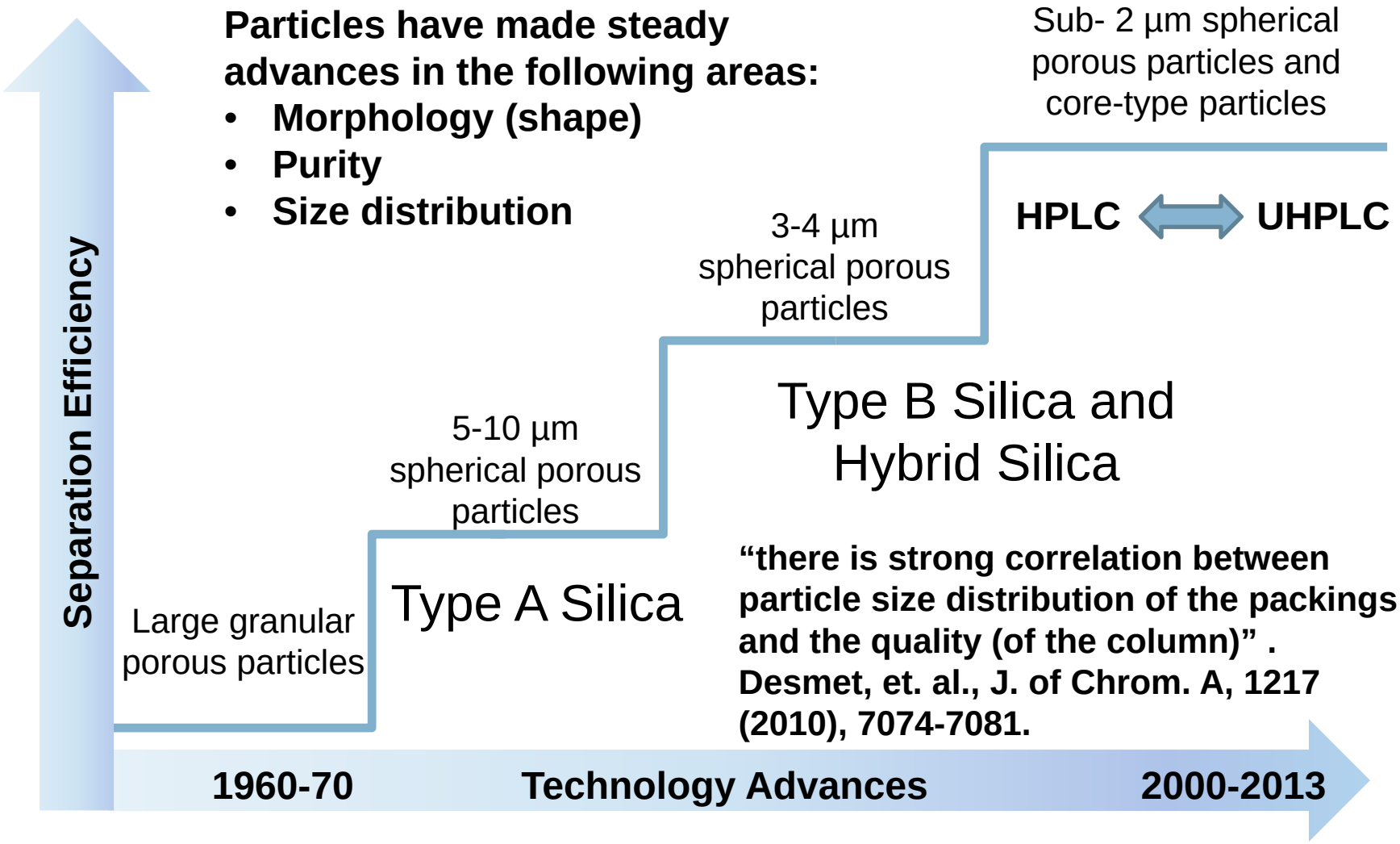


SPME fiber Holder with Automated DESI-1D Source



Courtesy of Joseph Kennedy of Prosolia

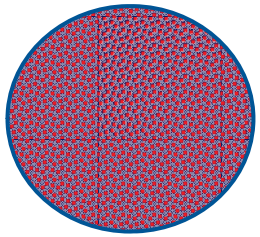
Evolution of HPLC Column and Particle Technology



Introduction to Titan™ UHPLC Columns

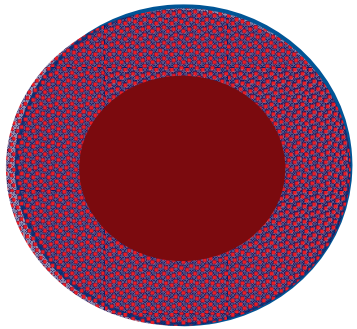
High Purity Monodisperse Silica Particles from Supelco

Titan Porous

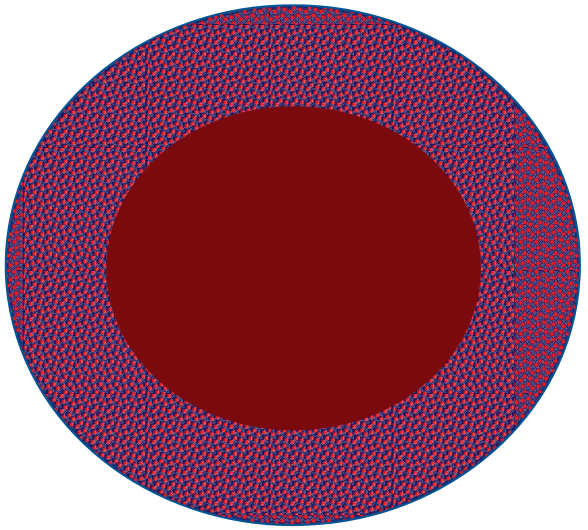


1.9 µm

Fused-Core® (Ascentis® Express and BIOshell™)



2.7 µm

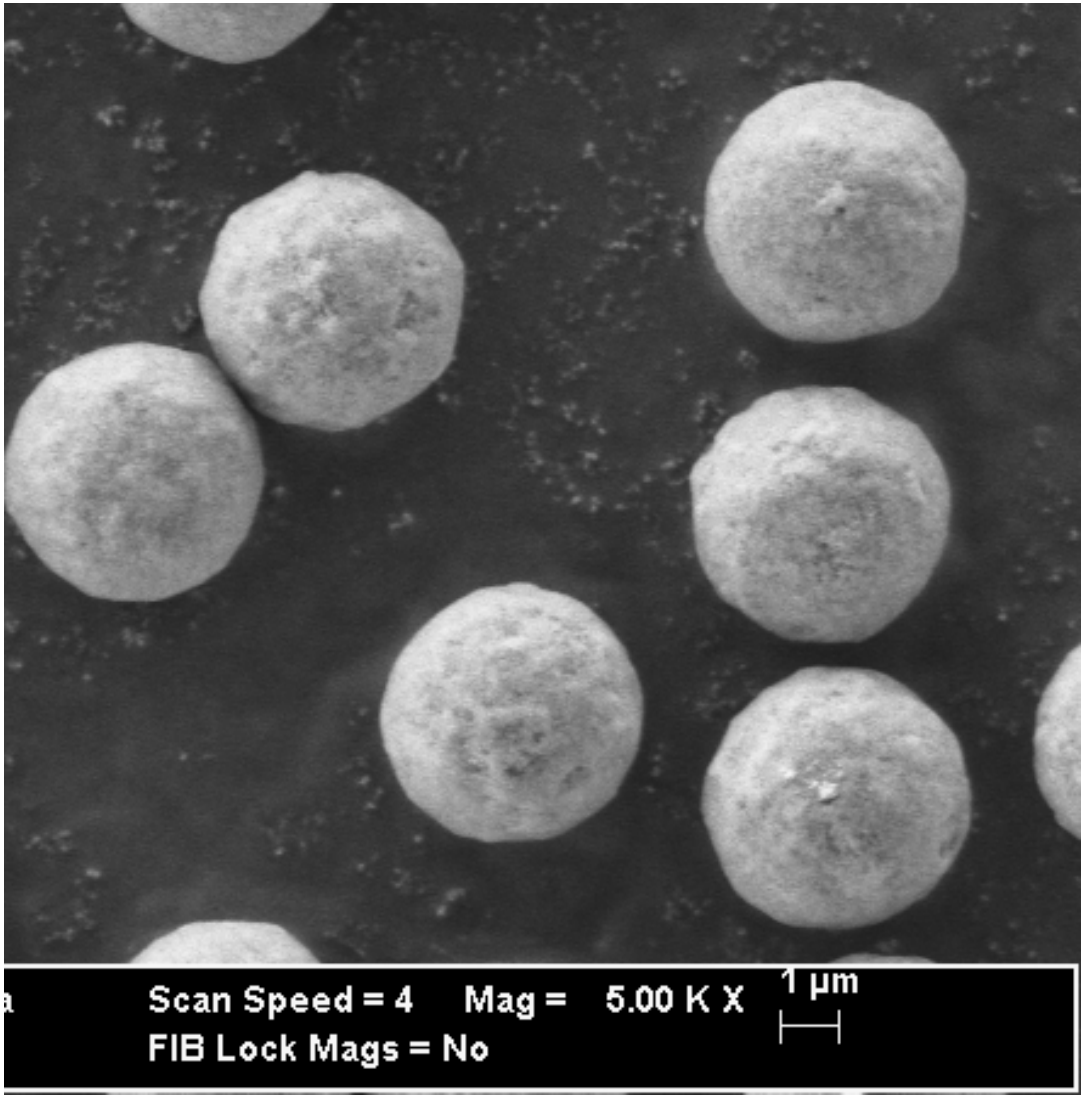


5 µm

Supelco HPLC and UHPLC particles feature very narrow PSD

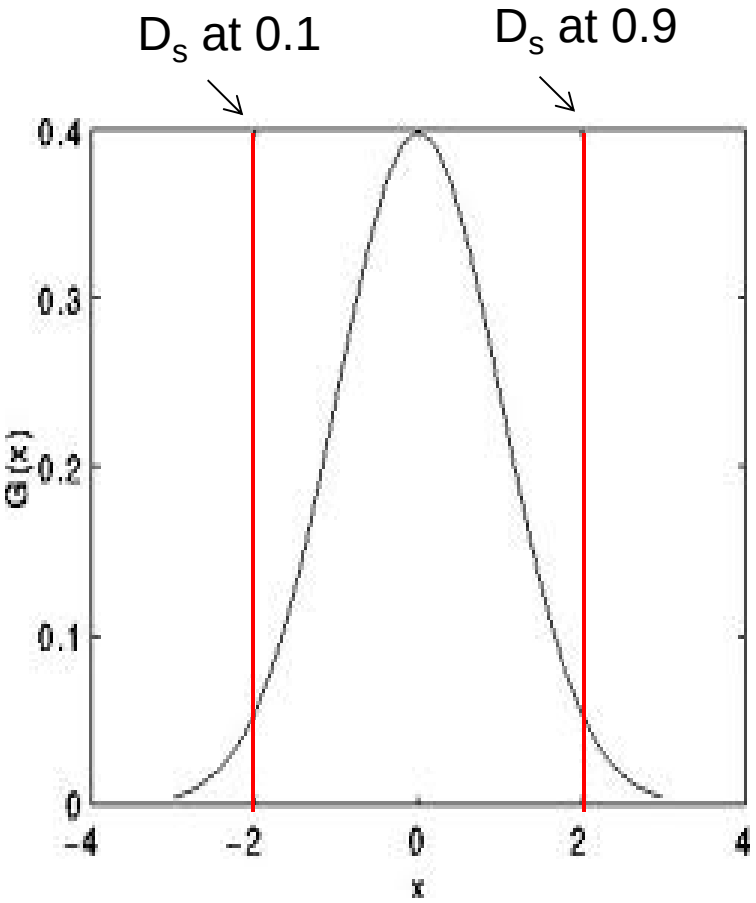
Fused-Core Monodispersity was the Key Advantage

*

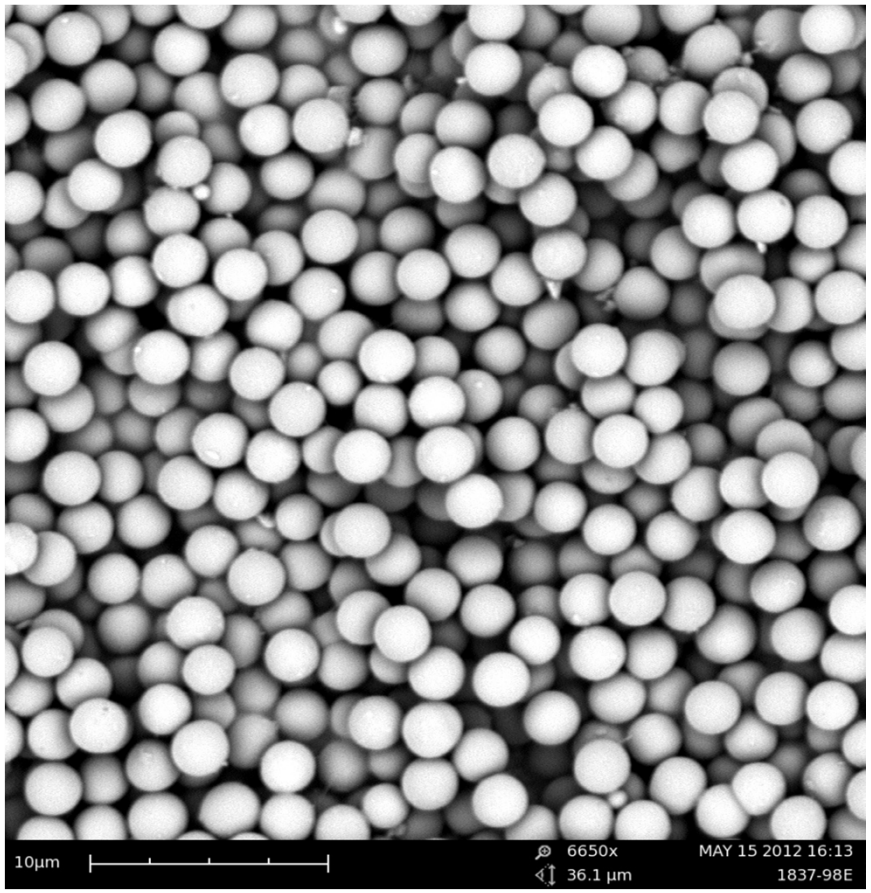


Slide courtesy of AMT

Titan Porous Silica- Narrow PSD Like Fused-Core

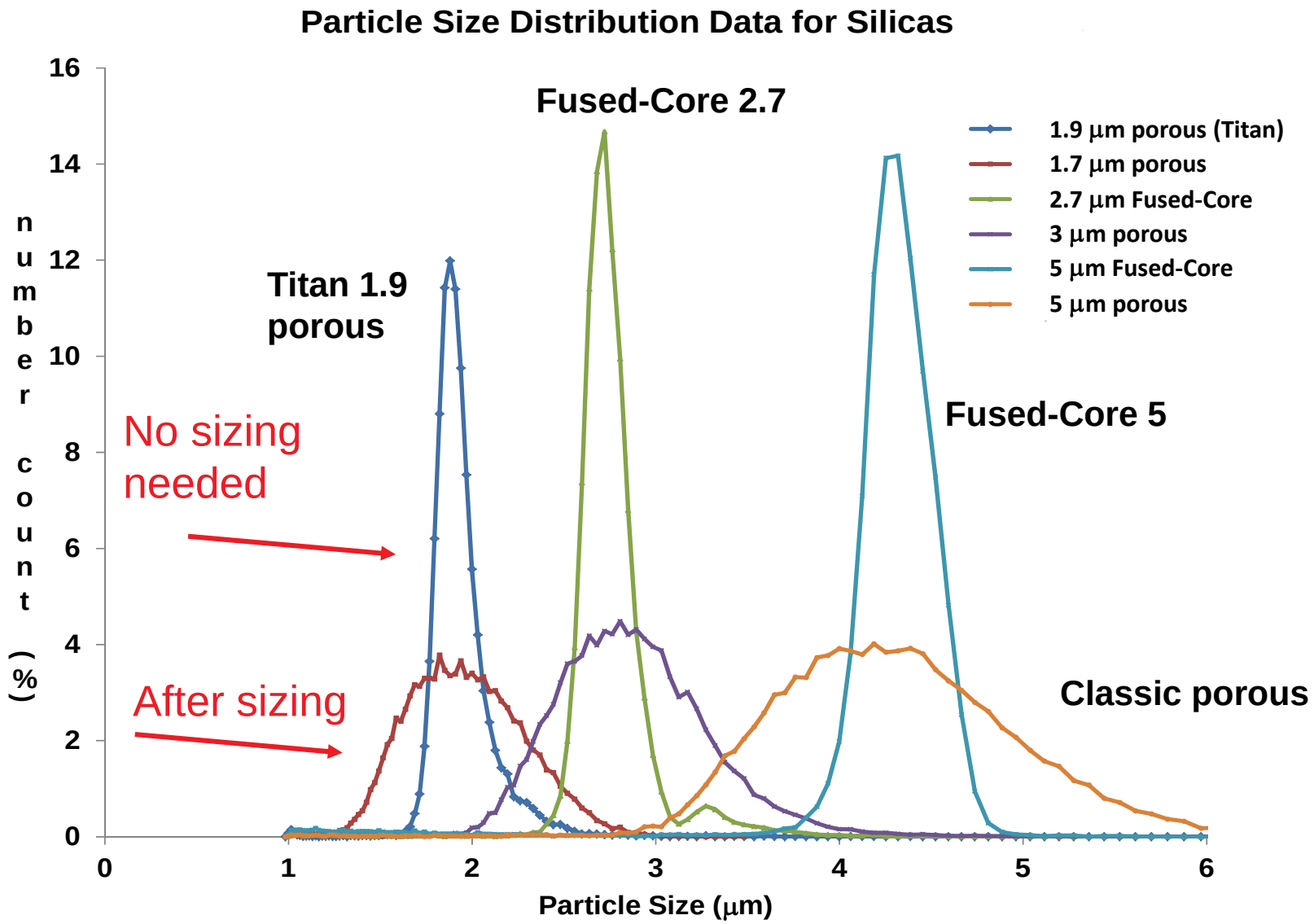


D_s (90/10) = particle size at 0.9 divided by particle size at 0.1; scale units arbitrary.

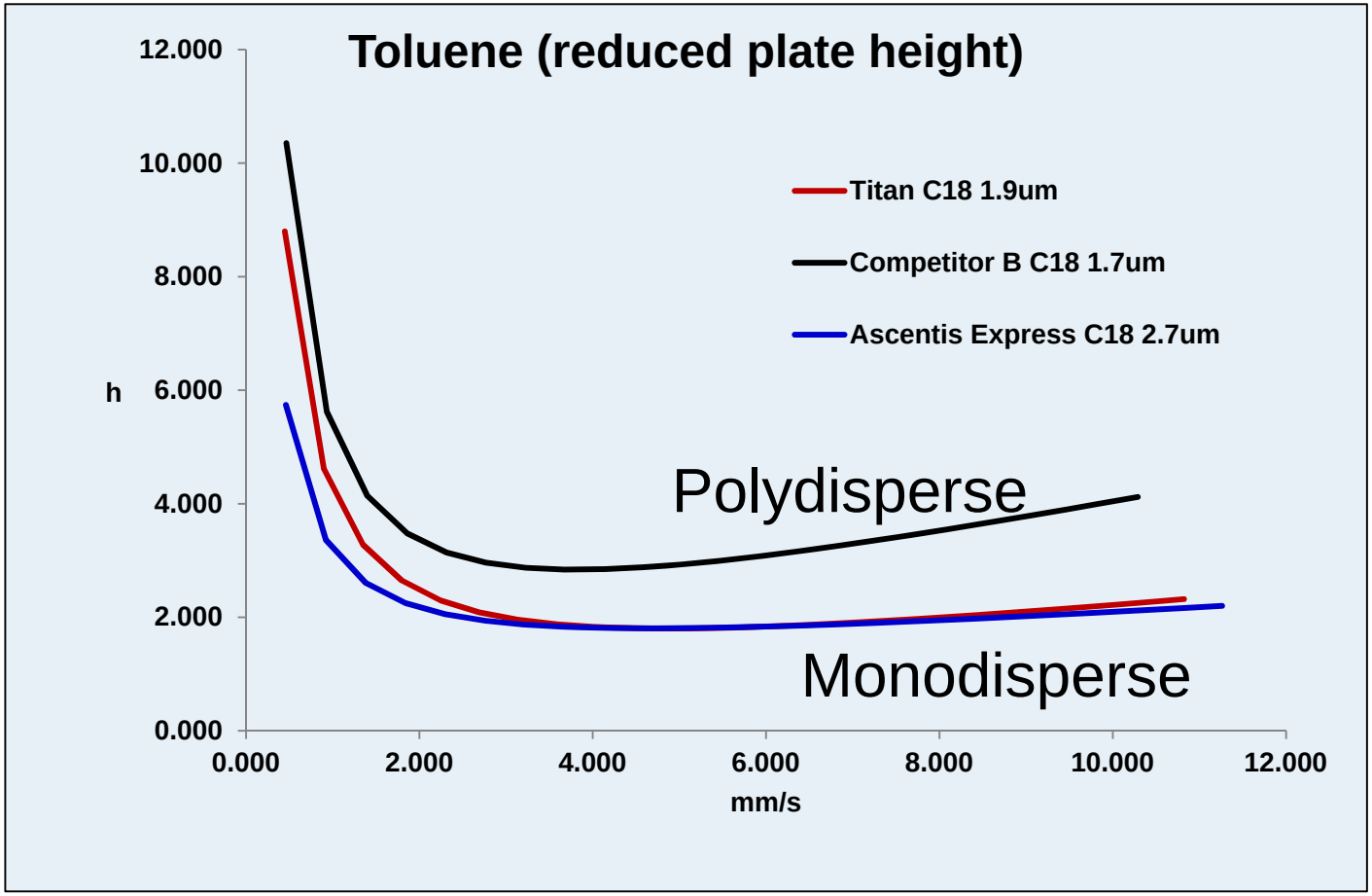


Ecoporous process results in very narrow distribution ($D_{90/10}$ < 1.15) without additional sizing.

Size Distribution Comparison for Range of Silicas



Evidence for the Monodispersity Advantage



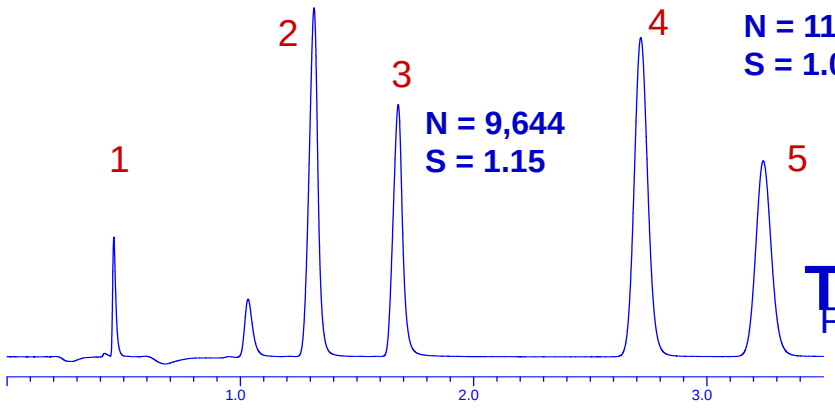
Titan C18 Performance Comparison in MeOH

Dionex 3000 (Low D tubing)
Column: 50 x 2.1 mm
Mobile Phase:
60% Methanol
40% 0.1% Ammonium Acetate (pH= 7.1)
Temp: 35 ° C
Flow Rate: 0.25 mL/min
Detection: 220 nm

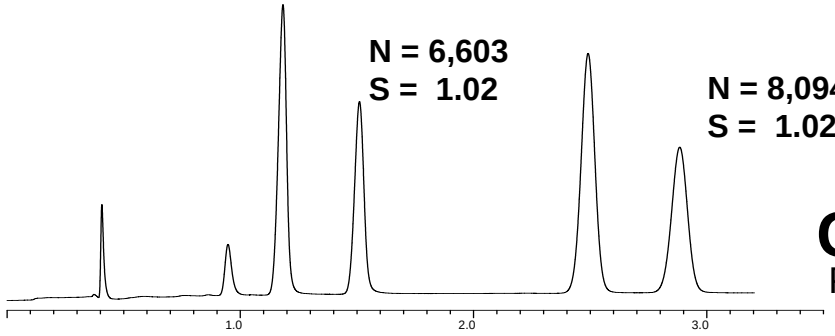
1.Uracil
2.Quinidine
3.Diphenhydramine
4.Nordiazepam
5.Diazepam

Efficiency and asymmetry reported on peaks 3 and 5

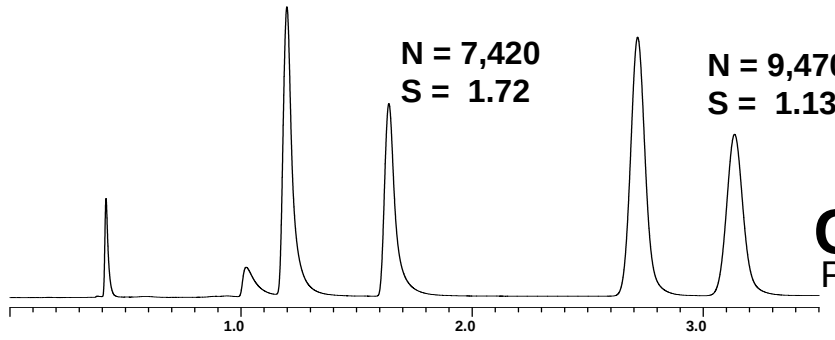
*system pressures include instrument blank pressure



Titan C18 1.9 µm
Pressure= 4,210 psi*



Competitor A 18 1
Pressure= 5,220 psi*



Competitor B C18
Pressure= 5,370 psi*

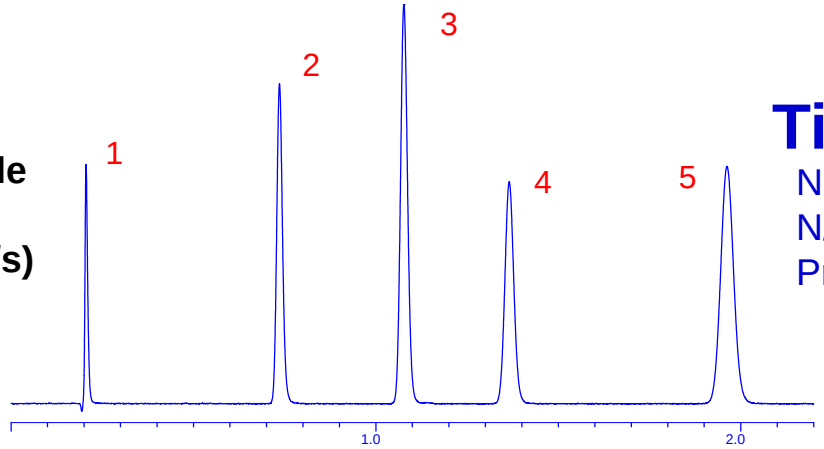
Titan C18 Performance Comparison in ACN

Dionex 3000 (Low D tubing)
Column: 50 x 3.0 mm
Mobile Phase: 60% Acetonitrile
Temp: 35 ° C
Flow Rate: 0.9 mL/min (4 mm/s)
Detection: 254 nm

1.Uracil
2.Diazepam
3.Toluene
4.Naphthalene
5.Biphenyl

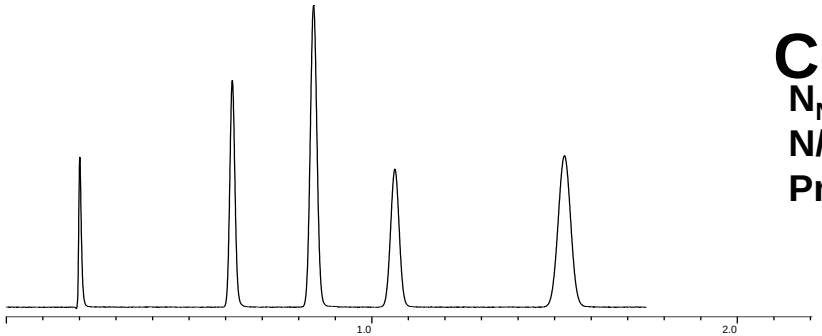
Data used in subsequent van Deemter plots

*system pressures include instrument blank pressure; optimized instrument is necessary.



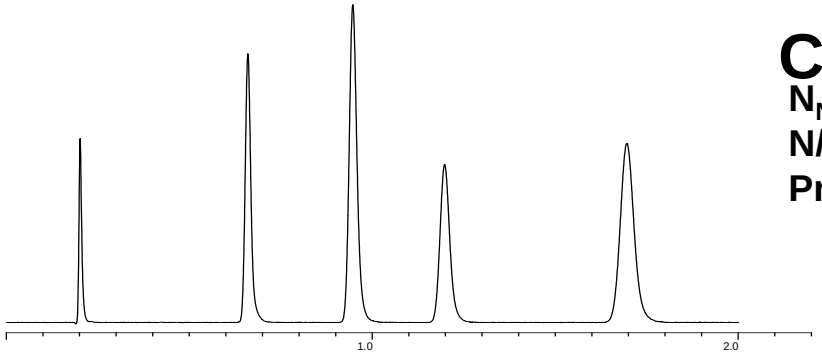
Titan C18 1.9 µm

N_N = 14,710
N/m = 294,200
Pressure = 4,100 psi*



Competitor A 18 1.8

N_N = 9,260
N/m = 185,200
Pressure = 4,650 psi*



Competitor B C18 1

N_N = 9,783
N/m= 195,700
Pressure = 4,900 psi*

Conclusions

- A new process called Ecoporous™ has been developed for making porous silica that matches the narrow size distribution of Fused-Core particles; no extra sizing step is required; no silica is wasted; a new standard has been established.
- Particles with 80 Å pores and 410 m^2/g have been prepared in 1.9 µm with a 6% standard deviation in PSD; larger pores and a range of particles sizes can be created by the process.
- Efficiency matches or exceeds porous particles of 1.7 and 1.8 µm size while pressure drop for the larger Titan particle is lower.
- Titan™ C18 columns with uniform particles are stable over a range of UHPLC flow, pressure and mobile phase conditions. .
- Higher sample loads can be injected without loss of efficiency.
- Titan columns are designed for enhanced performance with UHPLC instruments having very low dispersion and fast detection.

HybridSPE-Phospholipid (HybridSPE-PL)

96-well SPE plates and cartridges
Zirconia-coated silica particles

Features:

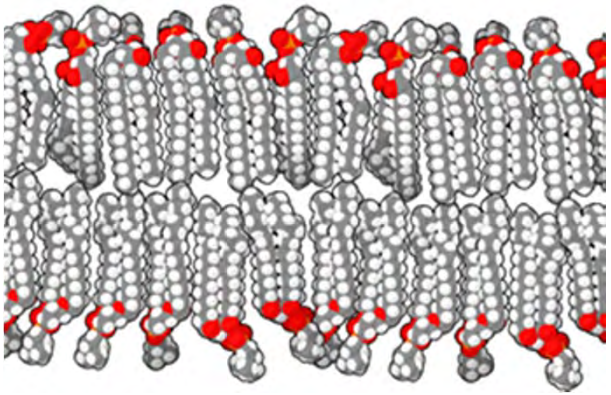
- Selective removal of phospholipid interferences and precipitated proteins
- Simple 2-3 step procedure

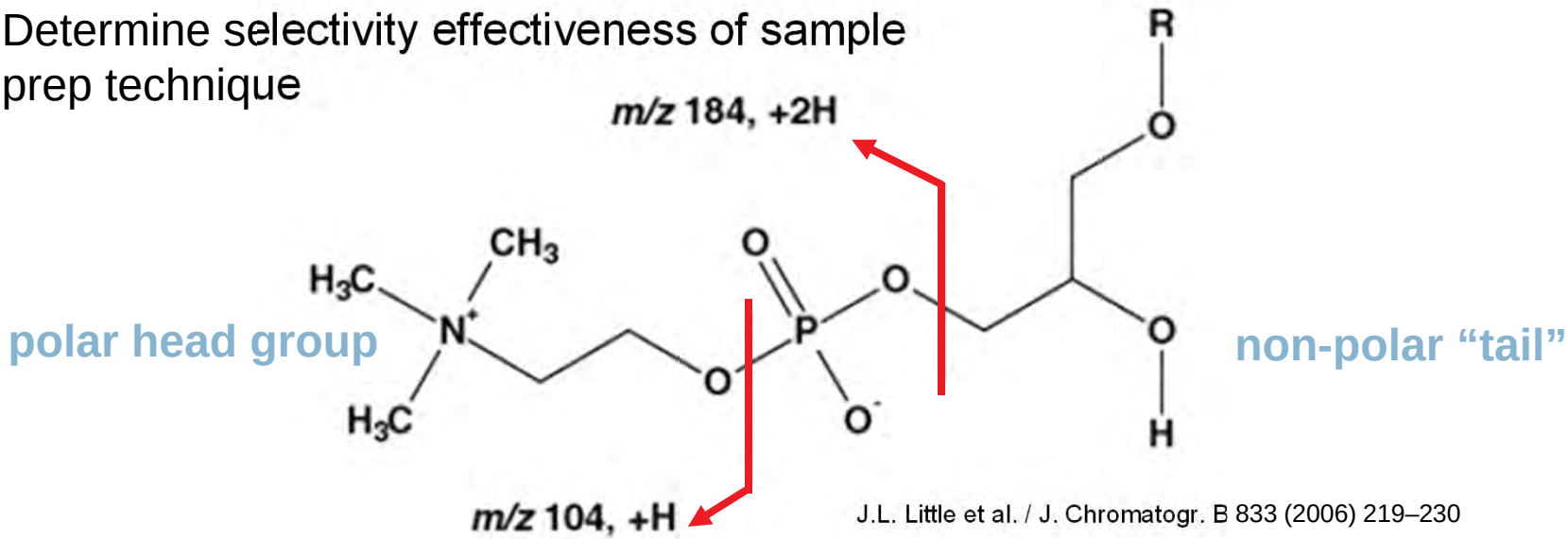
Benefits

- Improved LC-MS sensitivity (reduced matrix effect)
- Enhanced column lifetime
- Gradients not needed to clean column

Monitoring Phospholipid Contamination

- PLs major component of cell membranes
- Polar head group, non-polar tail
- Largest subclass (phosphatidylcholine) monitored using m/z 184 or m/z 104 fragment ions
- Used as a marker for ion-suppression risk assessment during LC-MS/MS
- Determine selectivity effectiveness of sample prep technique

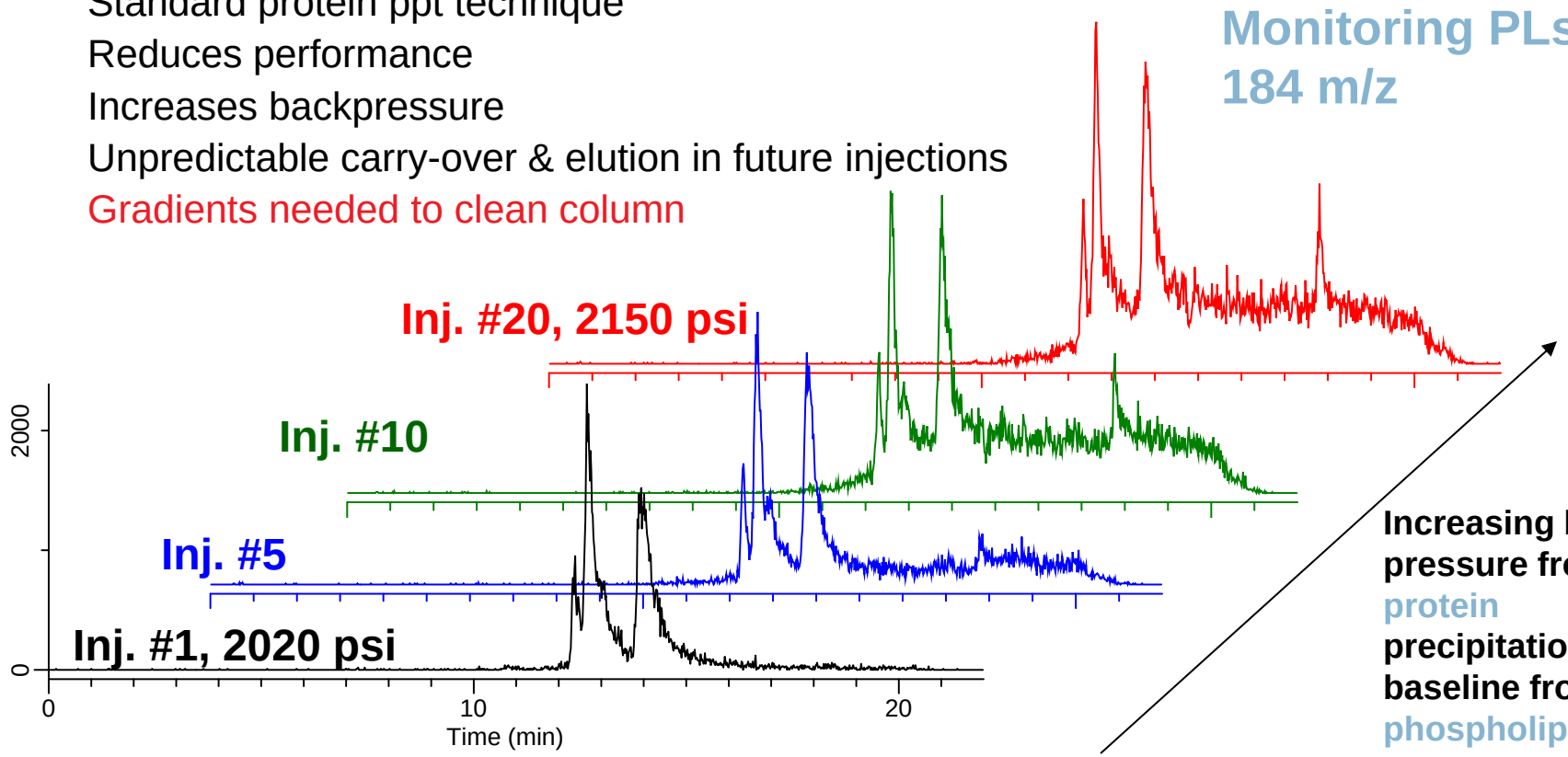




J.L. Little et al. / J. Chromatogr. B 833 (2006) 219–230

Problem: Protein and Phospholipid Accumulation on HPLC Column

Standard protein ppt technique
Reduces performance
Increases backpressure
Unpredictable carry-over & elution in future injections
Gradients needed to clean column



HPLC column: Sub-2um C18, 5 cm x 2.1 mm I.D.

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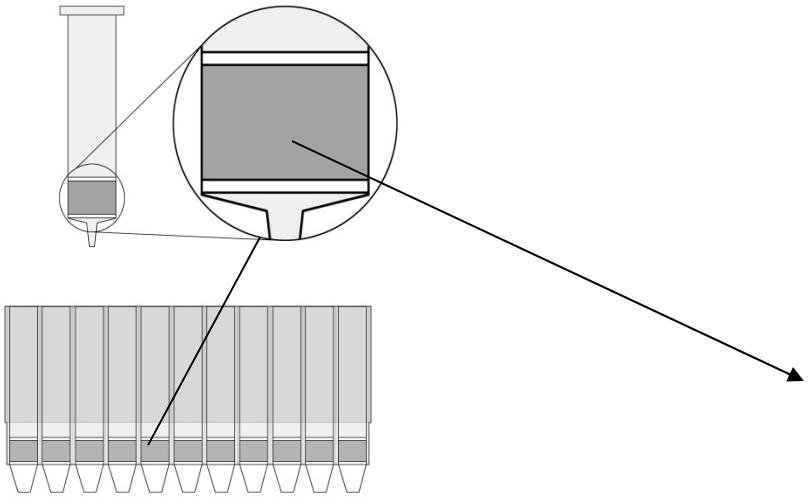
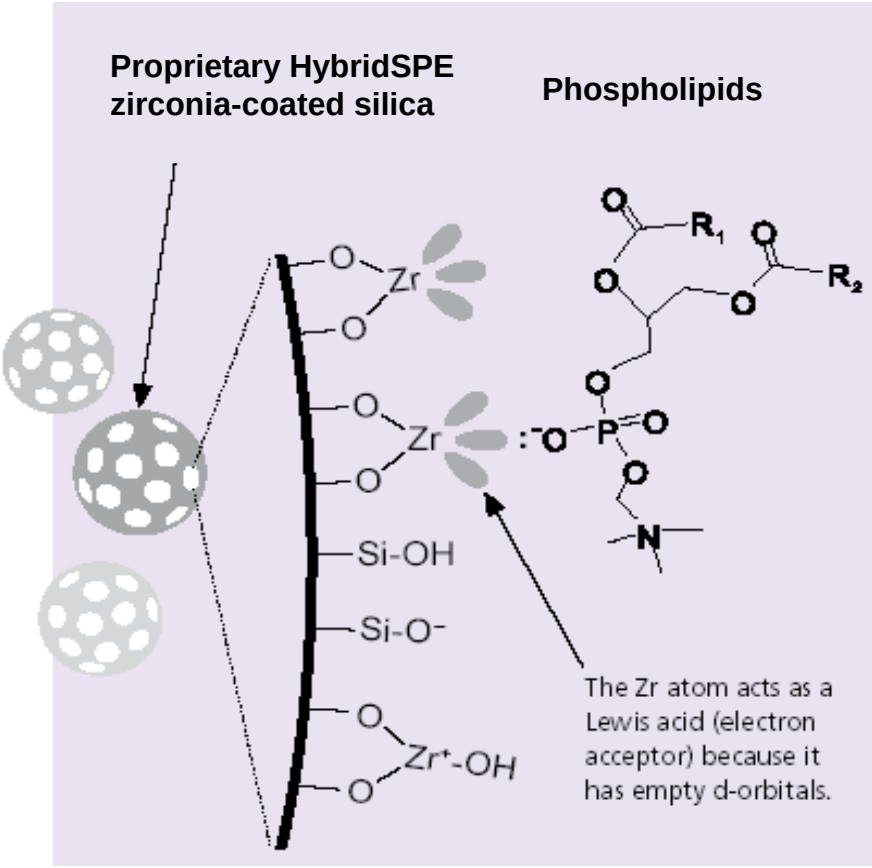
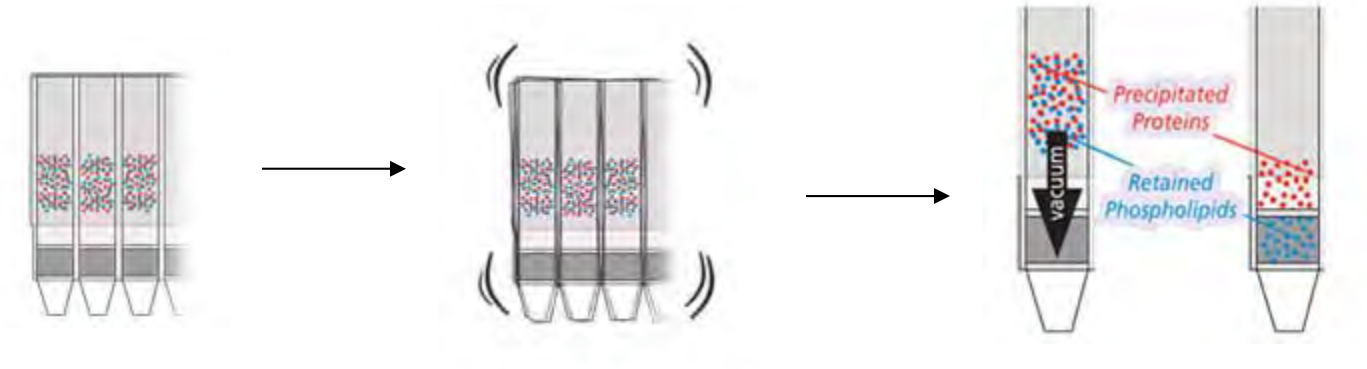
Solution: Phospholipids Selectively Removed using HybridSPE-PL Technology





- The Zr atom on the particle acts as a Lewis acid
- The phosphate groups on the phospholipids are strong Lewis bases and complex with the zirconium atoms
- Analytes are eluted free of phospholipids

HybridSPE-PL Method (96-Well Format)



Precipitate proteins in well

Mix

Apply vacuum

- 100 µL plasma/serum
- 300 µL 1% formic acid in acetonitrile
- Add I.S. as necessary

Resulting filtrate/eluate is free of proteins and phospholipids, ready for LC-MS

Improved Situation: No Protein or Phospholipid Accumulation Using HybridSPE-PL

Consistent column performance

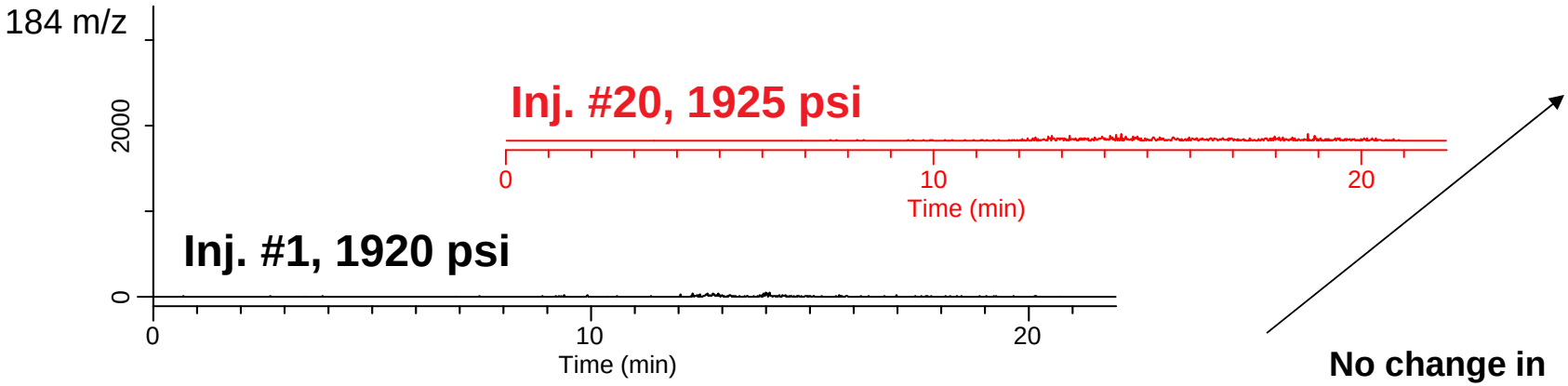
No increase in backpressure

Eliminates carry-over & elution in future injections

Extends column lifetime

Gradients are not needed to clean column

Monitoring PLs at 184 m/z

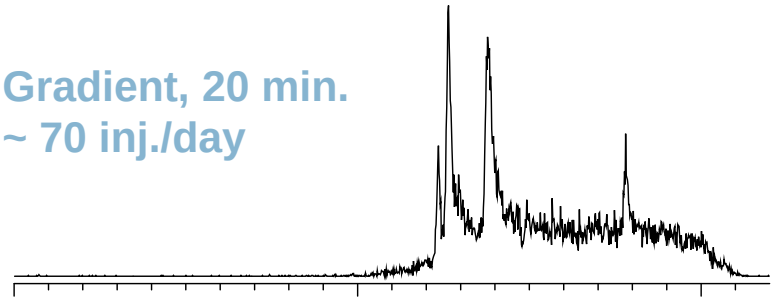


No change in back-pressure and baseline

Improved Through-put with HybridSPE-PL

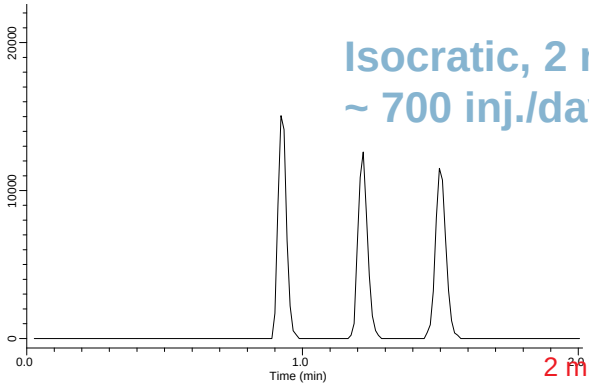
Elimination of need for post-gradient HPLC column clean-up improves sample throughput

Gradient, 20 min.
~ 70 inj./day

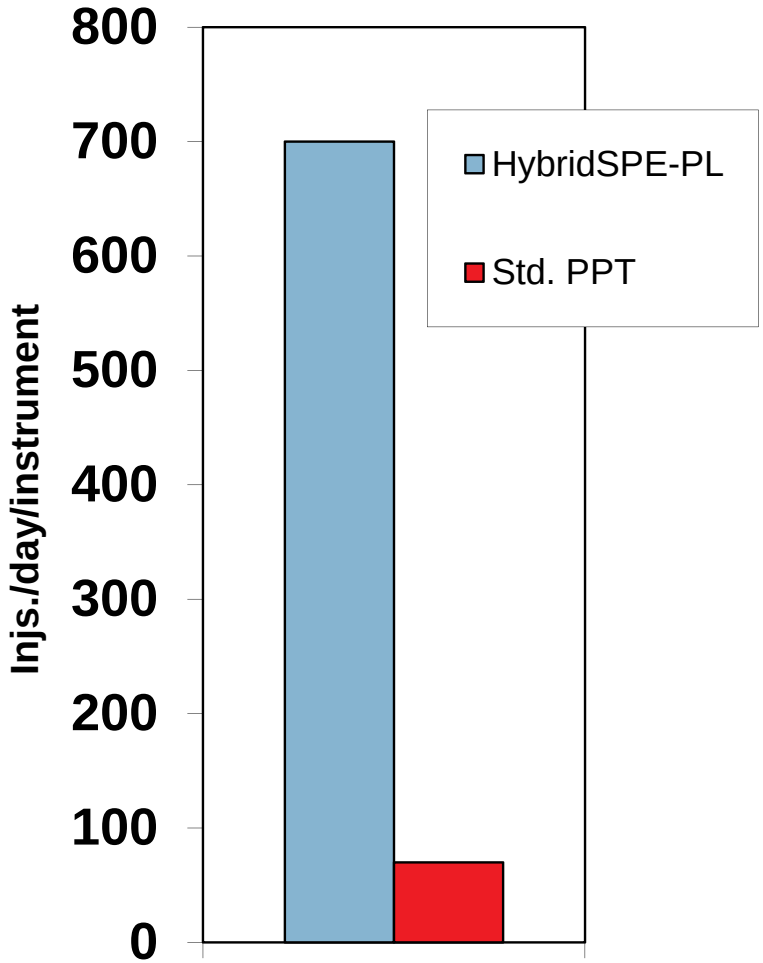


20 min.

Isocratic, 2 min.
~ 700 inj./day



2 min.



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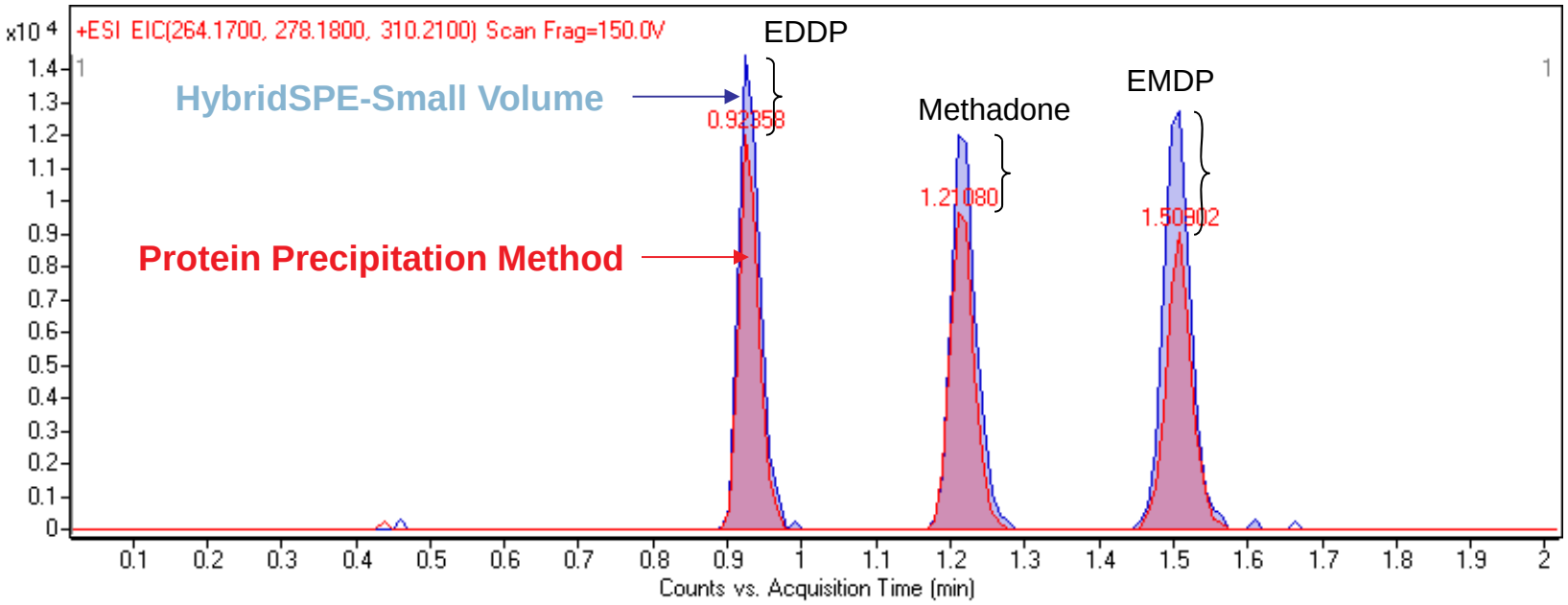
Overlay of HybridSPE-Small Volume and Protein Precipitation Samples

Methadone and metabolites from plasma

Sample was extracted using HybridSPE-PL small volume (20 uL of plasma was used) or standard PPT (100 uL of plasma was used)

High concentration (1200 ng/mL), still shows suppression with standard ppt method



Column: Ascentis Express RP-Amide 10 cm X 2.1, mm I.D., 2.7um; ESI+ detection

HybridSPE-PL Technology

- Fast and convenient SPE method uses Interference Removal strategy
- Complete removal of precipitated proteins and phospholipids for analysis of pharmaceutical compounds
- Reduces matrix effects, improves HPLC column lifetime and method throughput
- Can be used to extract and concentrate phospholipids in lipidomics application

For more information, please visit sigma-aldrich.com/hybridspe-pl.

QuEChERS Method: Pesticides in Food

Qu*ick*

E*asy*

Ch*eap*

E*ffective*

R*ugged*

S*afe*

Pesticides in

- fruit
- vegetable
- further food & feed

QuEChERS Method: the choice of sorbents

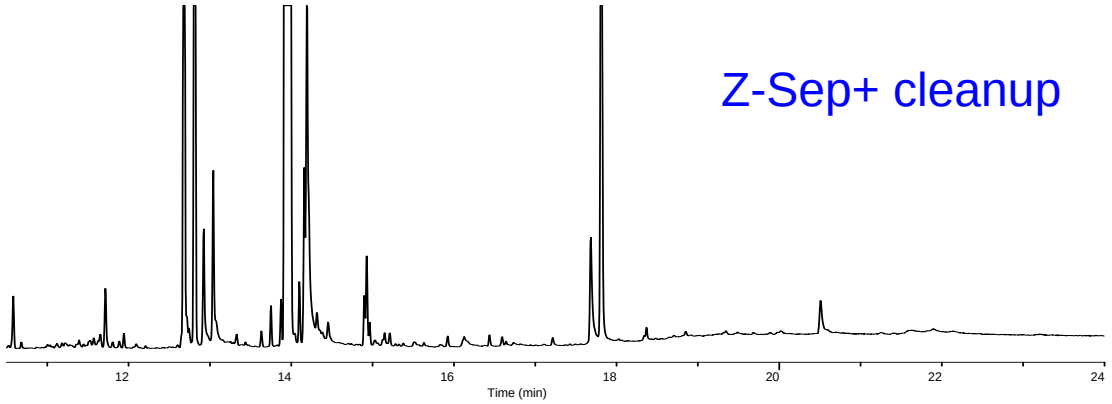
interference	PSA	C18	C18/PSA	ENVI-Carb	ENVI-Carb/PSA	PSA/C18/ ENVI-Carb	Z-Sep	Z-Sep+	Z-Sep/C18
Fats		X	X			X	**X**	**X**	**X**
Pigments	X			X	X	X	**X**	**X**	**X**
Sugars	X		X		X	X			
Acids	X		X		X	X			

New choice of cleanup sorbents for Fat-containing and pigmented samples:

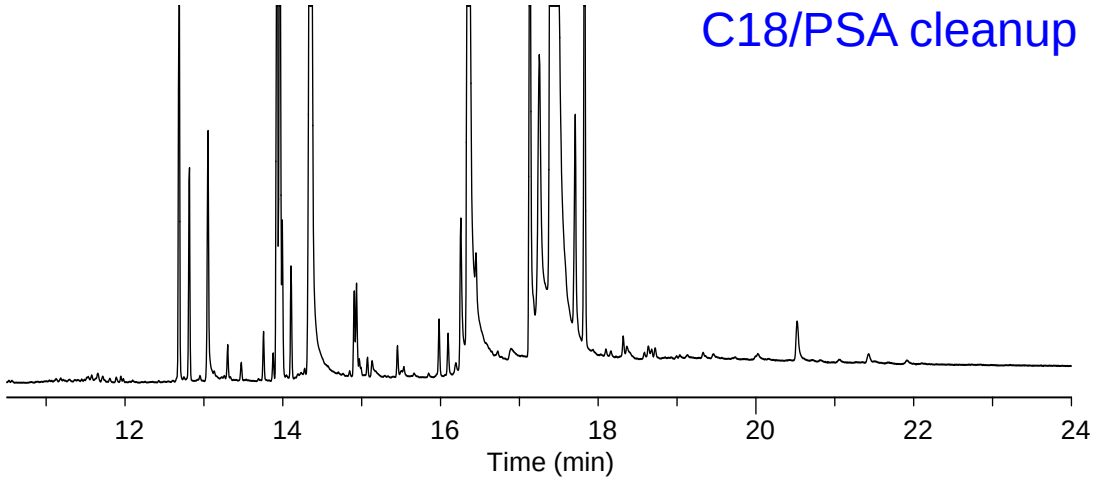
- Supel Que Z-Sep for hydrophobic analytes
- Supel QuE Z-Sep/C18 (Discovery® DSC-18 + Z-Sep) for samples containing <15% fat
- Supel QuE Z-Sep+ (C18 and zirconia dual bonded to silica) for samples containing >15% fat

Analysis of avocado extracts

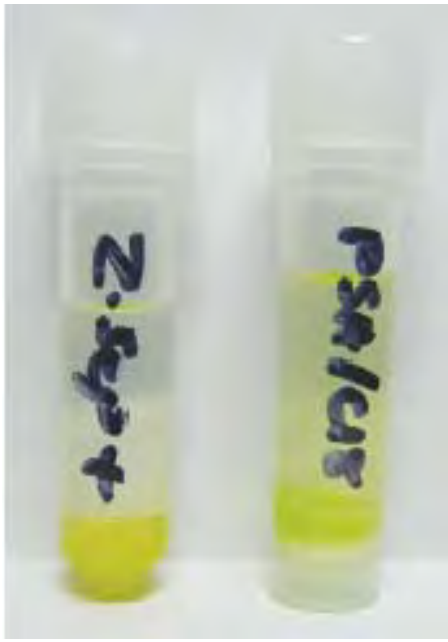
Scan mode



Z-Sep+ cleanup



C18/PSA cleanup



SUPELCO
Solutions within.

A New Dry Sampler for Isocyanates

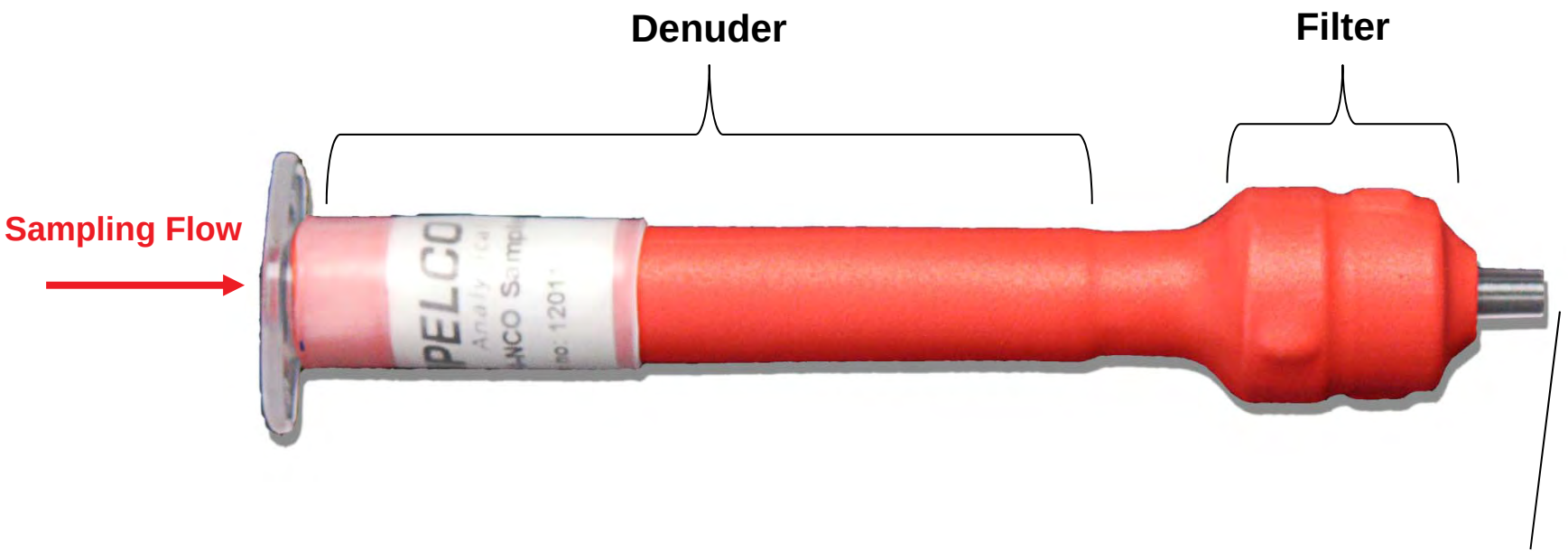
sigma-aldrich.com/analytical

SIGMA-ALDRICH®

Existing methods for isocyanate sampling and analysis

Method	**DBA** (Dibutylamine)	**1-2PP** (1-(2-pyridyl piperazine))	**2-MP or MOPP** (2-methyoxyphenyl piperazine)	**MAP** (9-(1-methyl anthracenyl piperzine))	**MOPIP and MAMA (**9-(N-methylaminomethyl) anthracene)
Media type	Filter - ASSET NCO	Filter	Impinger	Impinger + GFF	ISO-CHEK Filter System
Isocyanates	*2,4 TDI; 2,6-TDI, MDI, IPDI, HPDI, PhI, ICA, MIC, PIC, EIC; HMDI; HDI Adducts*	*2,4; 2-6 TDI. HDI; or MDI only*	*2,4-TDI; 2,6-TDI, MDI, HDI, NDI, HMDI, IPDI*	*2,4-TDI; 2,6-TDI, MDI, HDI, NDI, HMDI, IPDI*	*2,4-TDI; 2,6-TDI, HDI, IPDI, MDI, HMDI*
Ease of Use	No field reagent addition; no field extraction; stable	Easy	Personal sampling not recommended	Personal sampling not recommended	15 min sampling time; requires field derivitization; may require field reagent addition depending on system purchased
Storage	No storage issues	Refrigerate before use	Refrigerate	Refrigerate	MAMA Reagent Light-Sensitive
Sample Prep	Evaporate; recover	Evaporate; recover	Evaporate; recover	Evaporate; recover	Short sample prep time
Results	Quantifies aromatic and aliphatic isocyanates with LC-MS, MS/MS at low detection limits	LC-UV; Underestimates concentrations; incomplete derivatization	Difficulty identifying oligomeric isocyanates	Quantifies polyisocyanates w/LC-UV; Derivatives unstable; artifact peaks	Cannot collect isocyanates and then derivatize in solution; pre-polymers may react on first filter; complicated review of results - correction factors required

Dry Sampler - ASSET™EZ4-NCO



About the size and weight of a fat pen

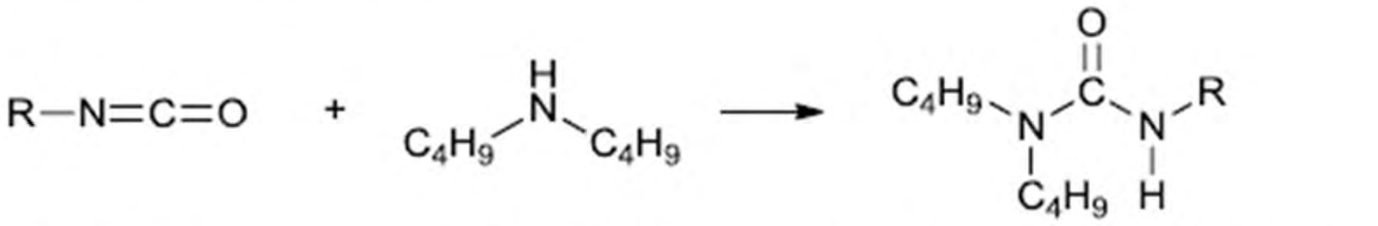
Connects to Air Sampling Pump

Dibutylamine (DBA) Impregnated Media

Vapor phase isocyanates are collected in the denuder

The isocyanate particles are collected on the filter

Derivatization Reaction



Isocyanate + Dibutylamine → Isocyanate dibutylamine derivative (urea derivative)

Dibutylamine as the Derivative Agent

Advantages

- DBA reacts quickly with the isocyanates
- The derivatives are stable
- No special storage of the sampler is required
- No field extraction of the sampler required
- DBA evaporates during Sample Prep.

Disadvantage

- DBA doesn't contain a UV chromophore so it doesn't enhance the LC-UV response of the isocyanates. $\Rightarrow$ requires LCMS analysis

ASSET EZ4-NCO Dry Sampler for Isocyanates

Flow Rate Range 100-250 mL/min (200 mL/min suggested)

Low back-pressure (suitable for most air sampling pumps)

- *~9 inches of water @ 200 mL/min*

Sampling Time Range 5 min. to 8 h (15 minutes is typical)

After sampling,

- Put the caps back on the sampler & send to lab
- ASSET can be stored at room temp. at any time

Analysis by LC-MS

Calibration Standards are available

- Calibration solution
- Deuterated Internal Standard Solution
- Kit containing both sets of standards

HDI adducts standards are now available, polymeric MDI Standards will be released in the next few weeks

sigma-aldrich.com/asset

ASSET EZ4-NCO Dry Sampler

Provides more reliable results due to permanent derivatisation of isocyanates both in the vapour and particulate phase.

More stable derivative - Easier used and handling

Suitable for sampling of various Isocyanates

Aliphatic monomers:

- Ethyl isocyanate (EIC)
- Isophorone diisocyanate (IPDI)
- Hexamethylene diisocyanate (HDI)
- Methyl isocyanate (MIC)
- Propyl isocyanate (PIC)
- Isocyanic acid (ICA)

Aromatic monomers:

- 4,4'-Methylenediphenyl diisocyanate (MDI)
- Phenyl isocyanate (PhI)
- 2,4-Toluene diisocyanate (2,4-TDI)
- 2,6-Toluene diisocyanate (2,6-TDI)

Acknowledgements

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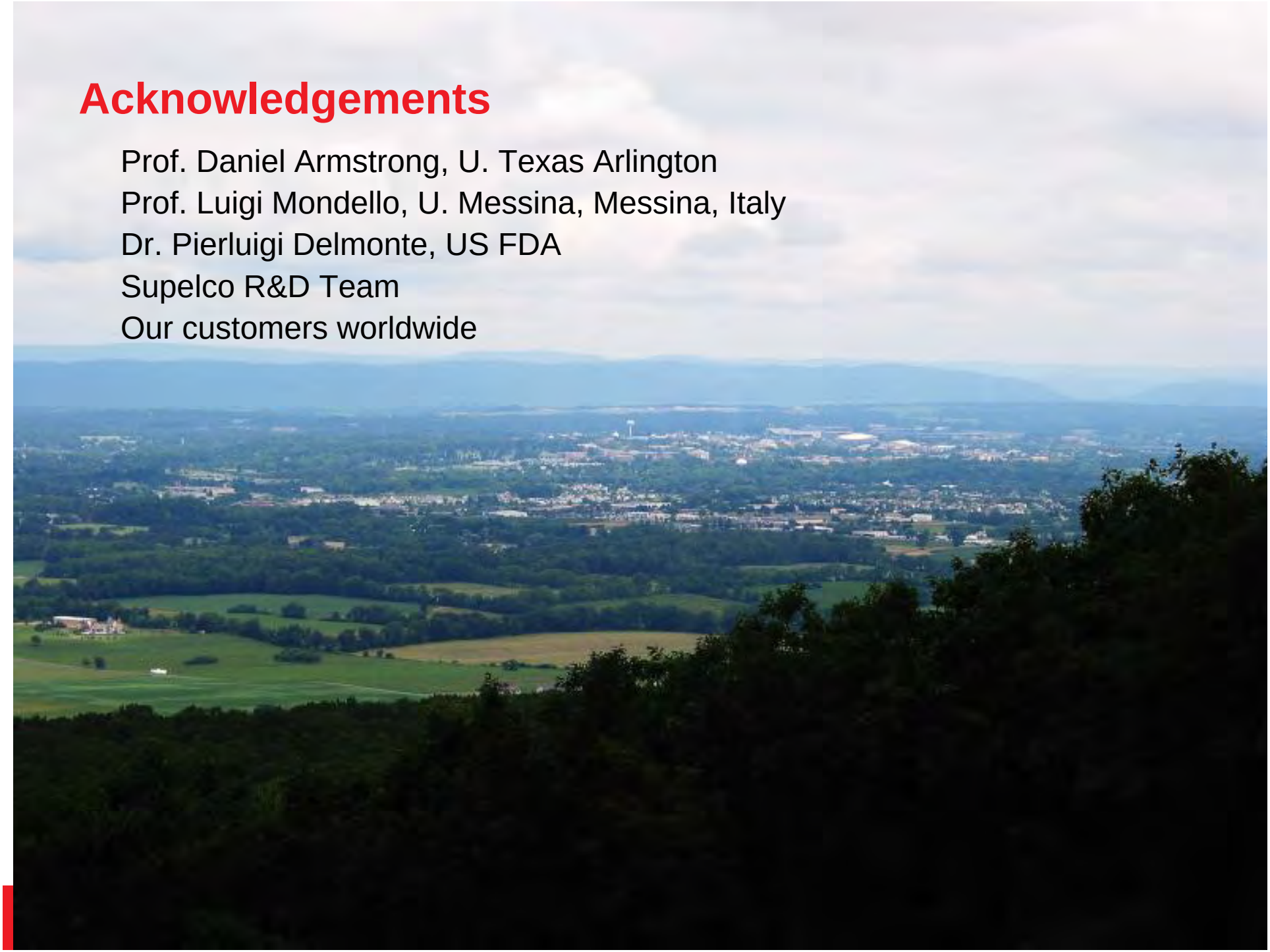
Dr. Pierluigi Delmonte, US FDA
Supelco R&D Team
Our customers worldwide



Thank You