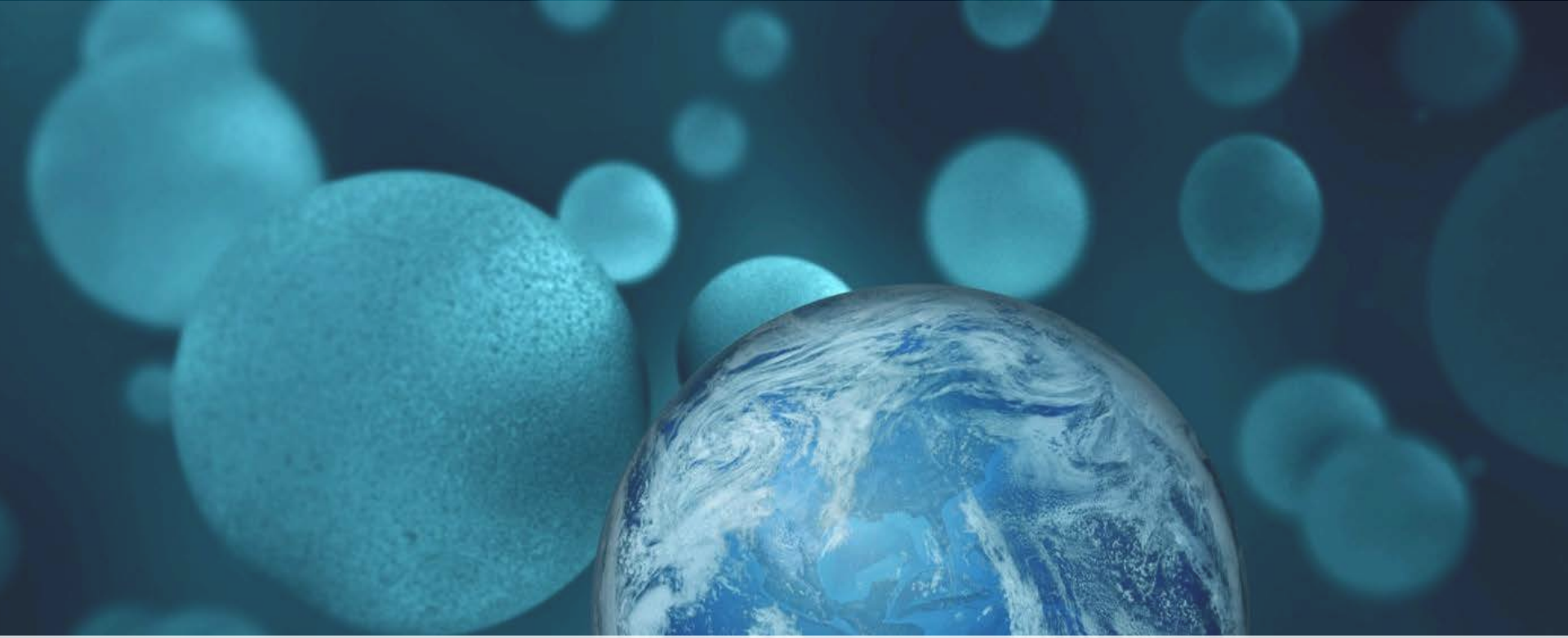

NASA
Enterprise
United States



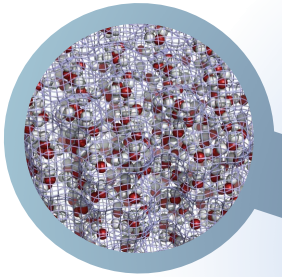
SciSpec

Connected Solution to Small Molecules

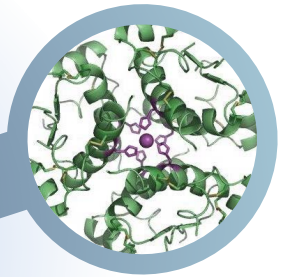
Your Scientific Specialist

The worlds biggest analytical challenges...

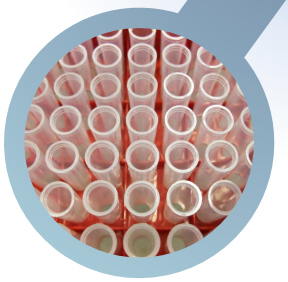
Small
Sample Amounts



Complex Matrices



Complicated
Structures



Large Sample
Numbers

Low
Concentration

deserve even bigger solutions—

OUTLINE


Pharmaceutical


Environmental


Clinical/Toxicology/Forensics


Food Safety

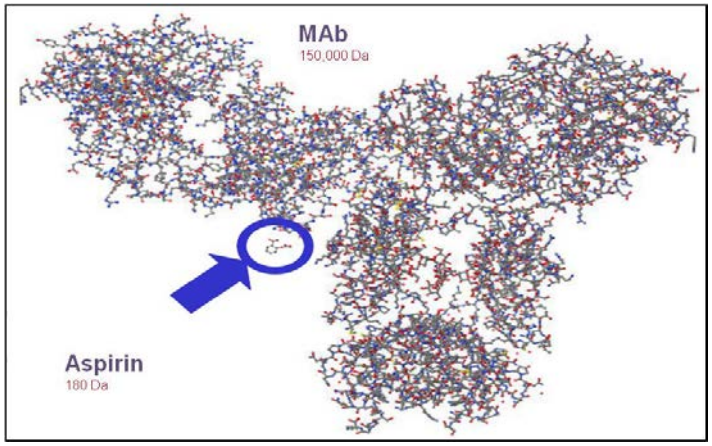
Pharmaceuticals and Biopharmaceuticals – What is the Difference?

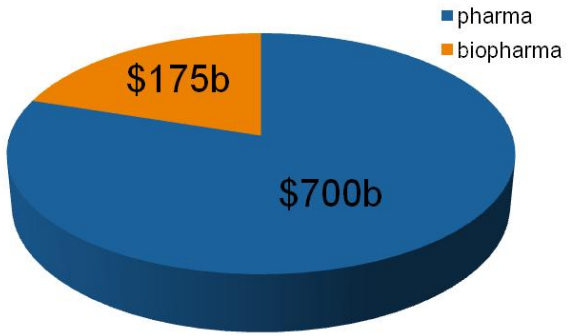
Biopharmaceuticals

- Genetically engineered/produced in living cells
- Large to very large (10 - 2000 reactive groups)
- Complex; A mixture of closely related variants

Pharmaceuticals

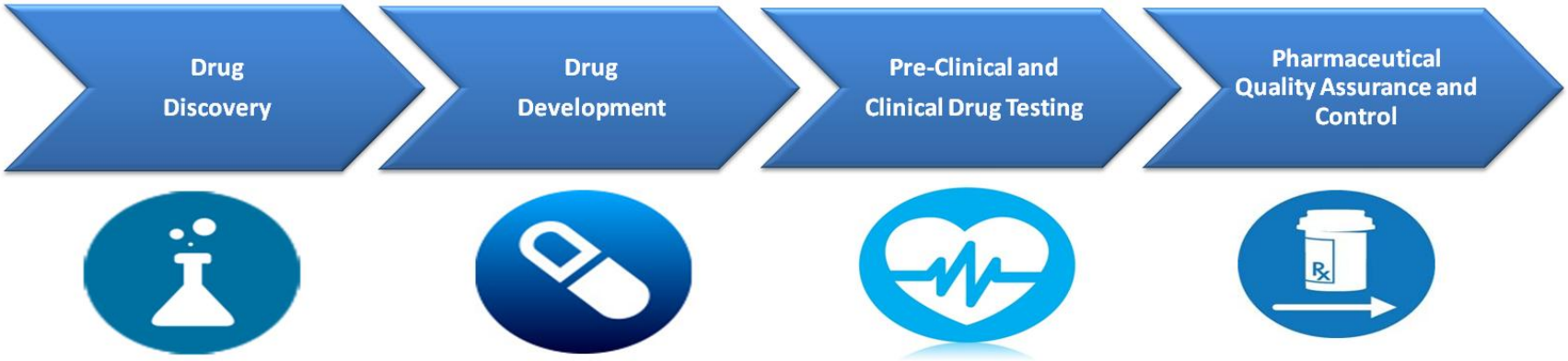
- Produced by chemical synthesis
- Small (1 - 5 reactive groups)
- Precisely defined chemical entities
- Small molecule pharmaceutical market is flat or declining





Small molecule Pharma represents a significant revenue opportunity

Market Opportunity for Classic Pharma



Drug Discovery

Aim:

Identify drugs candidates

Workflow requirements:

Speed and efficiency
"fail fast, fail cheap"

Drug Development

Aim:

Modify drugs candidates for safety and efficacy

Workflow requirements:

Fast, robust, targeted methods

Commercialization

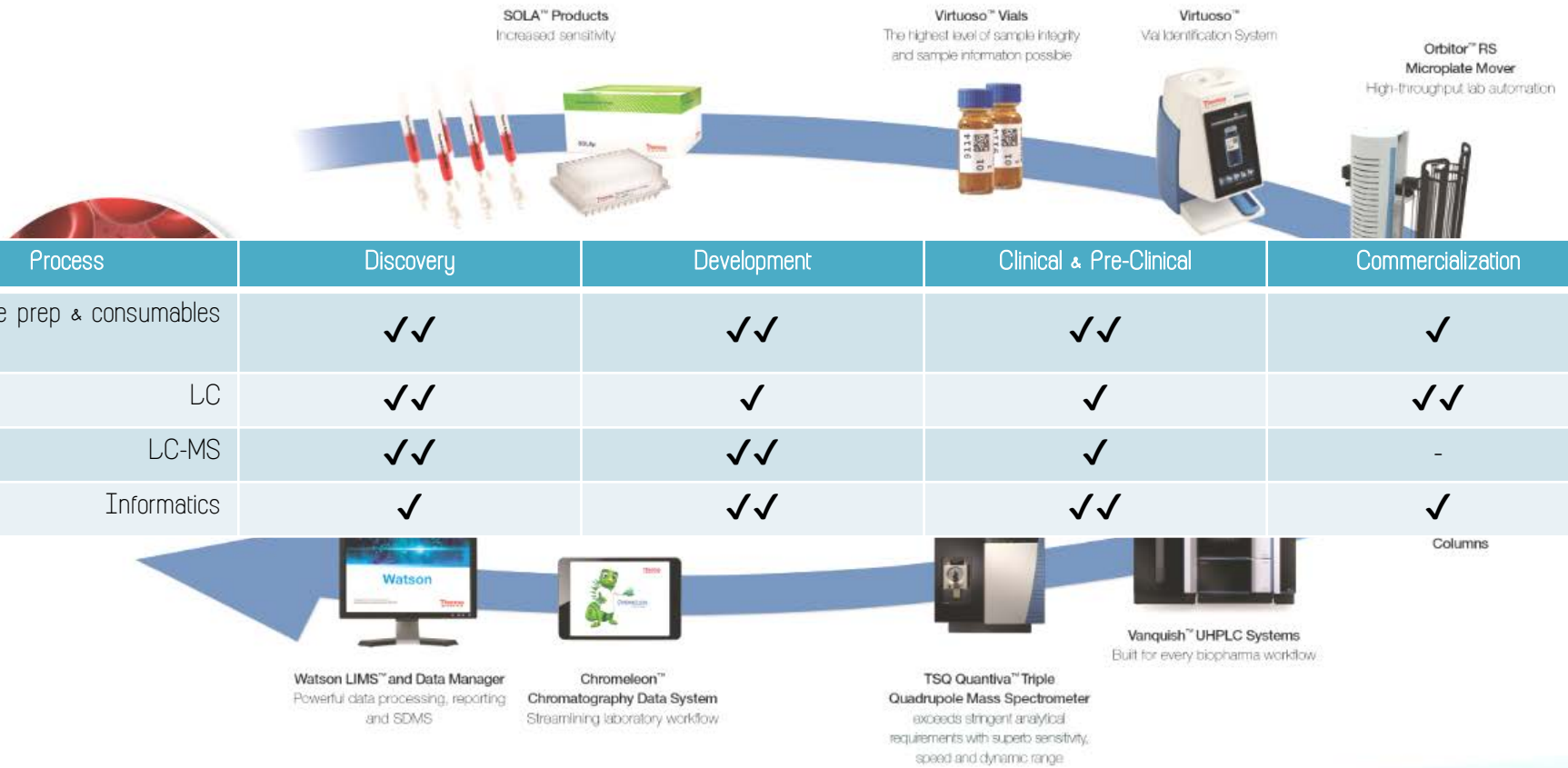
Aim:

Ensure final product is of expected quality

Workflow requirements:

Robust, reproducible methods

Pharma Workflow Solutions



Process	Discovery	Development	Clinical & Pre-Clinical	Commercialization
Sample prep & consumables	✓✓	✓✓	✓✓	✓
LC	✓✓	✓	✓	✓✓
LC-MS	✓✓	✓✓	✓	-
Informatics	✓	✓✓	✓✓	✓

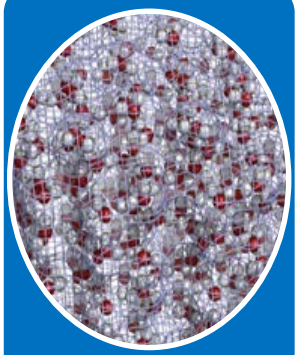
A Reminder: What *is* Biopharma?

"The design and production of biologically-based therapeutics (biotherapeutics)"

Major Biopharmaceutical Groups Using LC/MS

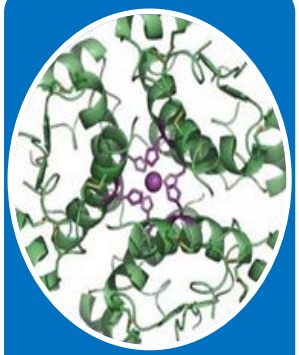
Research

- New Candidates
- Disease pathways
- Biomarker discovery



Process Analytical

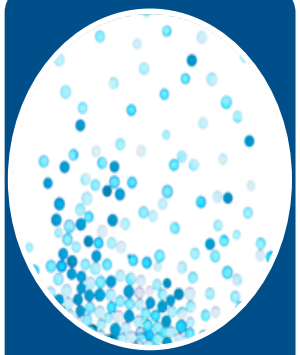
- Cell and Clone selection
- Optimization
- Environment
- Output



Analytical Science

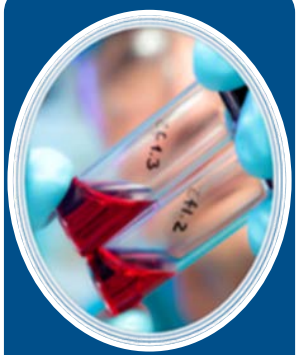
- Complex analysis
- Diverse characterizations and measurements
- Speed of analysis
- Confidence in results

CI Quantitation



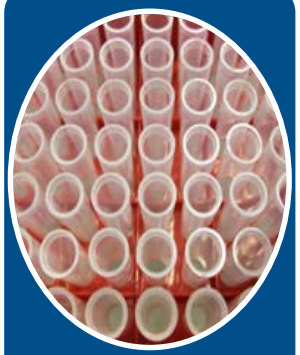
Discovery

- Biomarker discovery & validations
- Throughput & Screening effectiveness



Metabolism

- New area of interest
- Blending of biopharma characterization and pharma structural ID
- Complex samples
- Complex matrices
- Unknown analytes



Bioanalysis

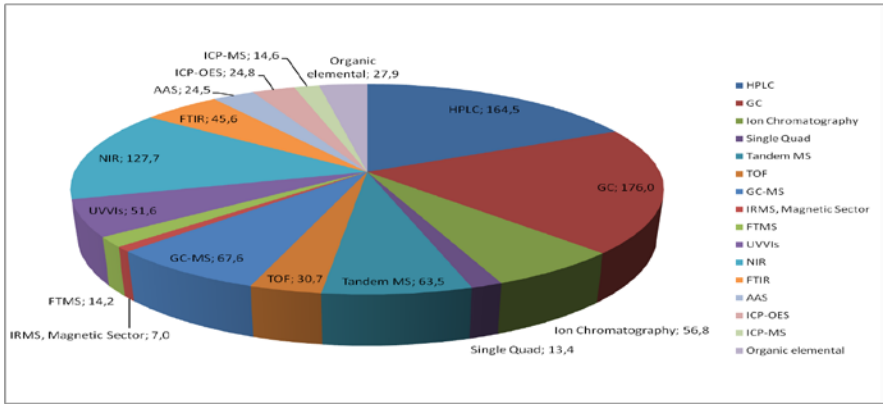
- Newer area of interest for LC/MS
- Complex matrices
- Complex analytes
- Regulations
- Small ample volume
- Many samples
- Speed of analysis
- Low limits of quantitation
- Precision
- Throughput

Global competition, Regulatory compliance, Scientific complexity, and Expense

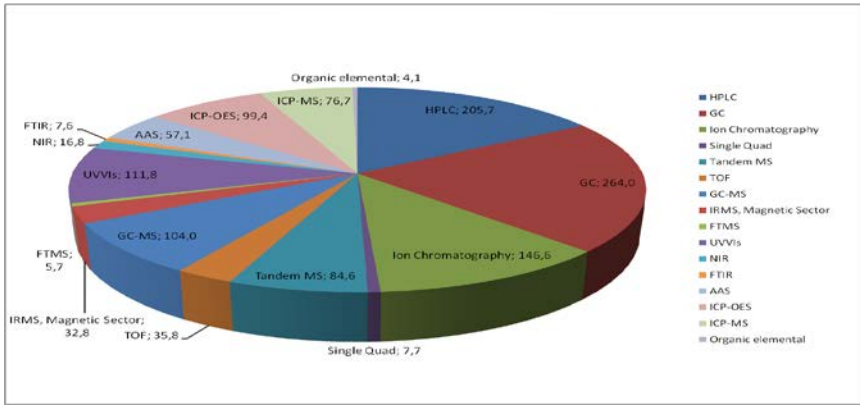
BioPharma Workflow Solution

Environmental and Food Analysis Market opportunity

Food Safety Market



Environmental Market



Environmental and Food Safety markets represent a significant revenue opportunity

Food Safety: The Global Food Chain

- A long food chain is very difficult to control
- Food safety cultures and standards differ across the world
- Modern food production involves large number of ingredients procured globally (traceability issues)
- *When things go wrong tracking and tracing is increasingly difficult*

Greater dependence on testing to provide confidence in food safety

What is Environmental Analysis?

Determination of contaminants in:

- Waters – drinking, surface, ground, waste
- Soils – soils, sediments, foliage, biota
- Sludges – solid and digested waste
- Air – chimney exhaust filters, air filters of contaminated sites, dusts

Environmental Workflow Solution

Industry challenges:

- Regulatory requirements
- Wide range of contaminants
- Emerging contaminants
- Complex matrices
- Cost of analysis

Laboratory requirements:

- Robustness
- Sensitivity
- Throughput
- Applications support

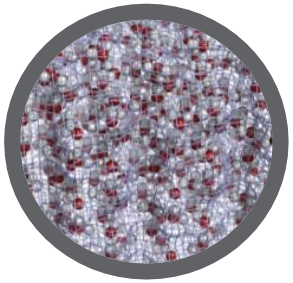
Food Workflow Solution

Why LC-MS for Both Forensic Screening and Confirmation?

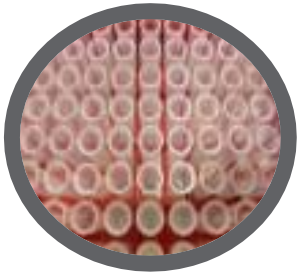
Mass Spectrometry has been proven to be more specific and sensitive than many immunoassay tests

But now, Mass Spectrometry is even .....

Easier



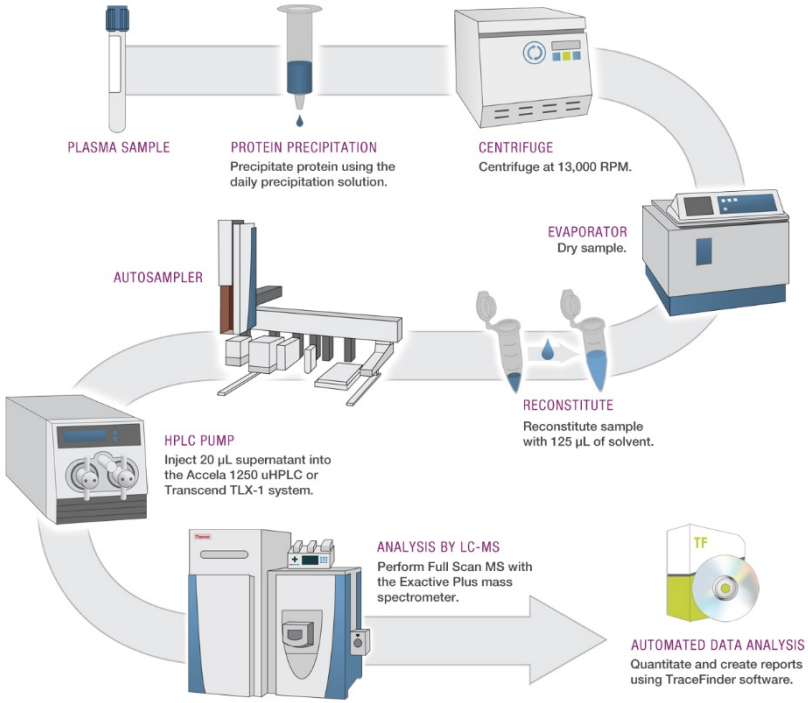
More Robust

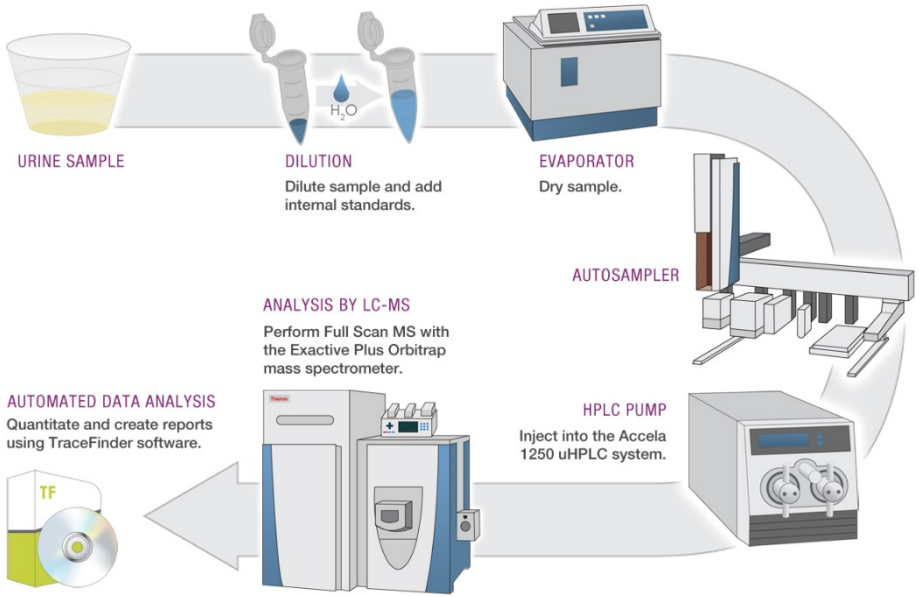


Faster

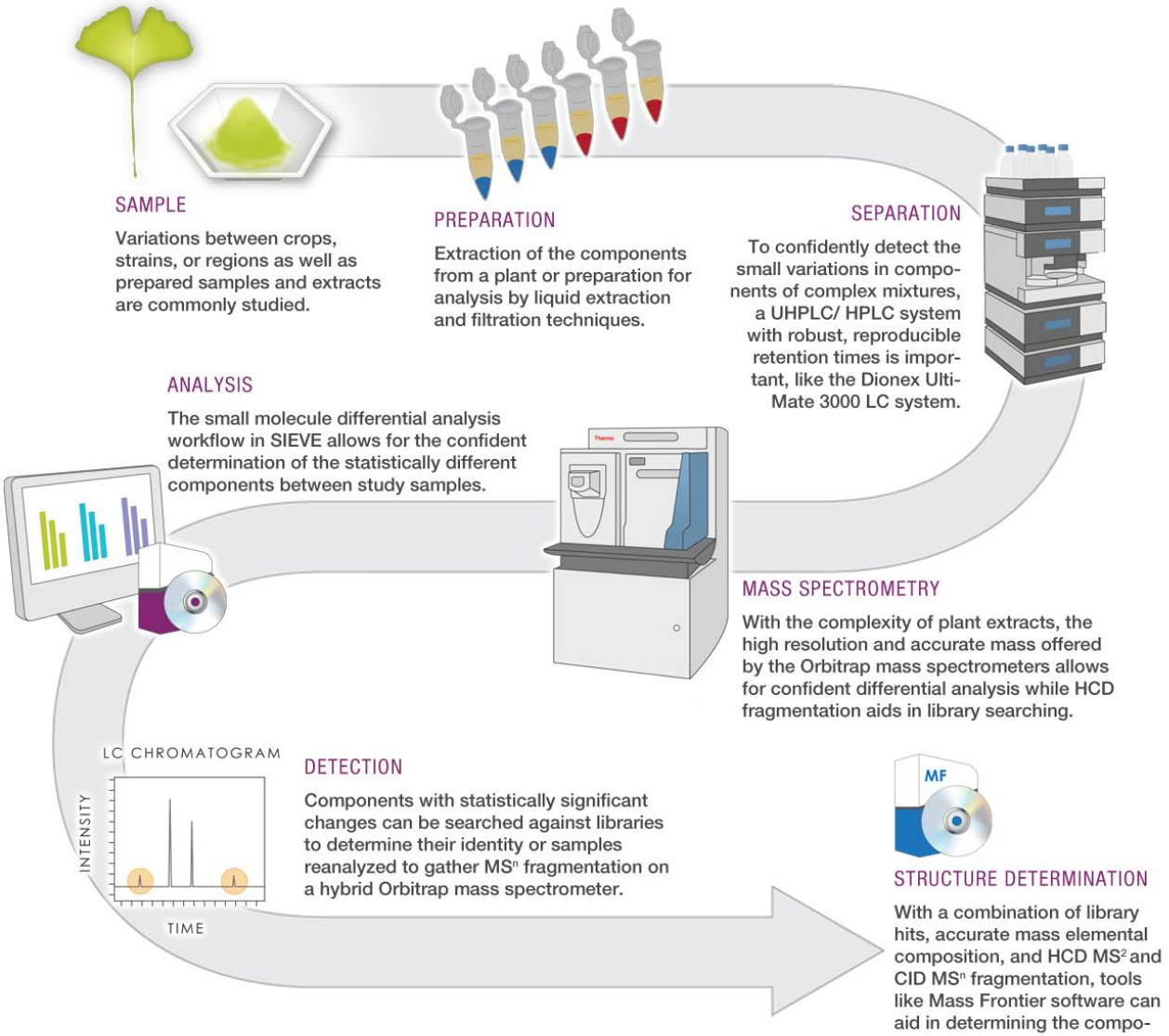
More Efficient

Toxicology/Clinical Workflow Solutions : Screening





Toxicology/Clinical Workflow Solutions : Compounds elucidations



A Total Solution from Thermo Fisher Scientific

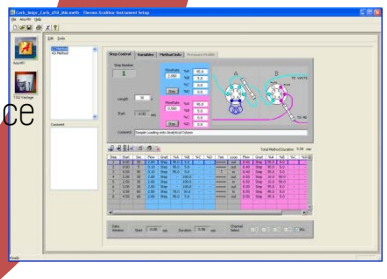
Mass Spec grade mobile and consumables

Certified System ISO 13485 QMI-SAI Global

Prelude SPLC System and Triple Quad MS

TraceFinder Software production workflows

ThermoFisher SCIENTIFIC



Quick Start Methods reduce research methods development time

Unity Lab Services
Service
Parts
IQ/OQ/PV

Now offered as

Unity Lab Services
Part of Thermo Fisher Scientific

Technical Phone Support
Remote Instrument Diagnostics
On site-training

What's important to customers

Market research show that three most criteria when choosing column are

- Technical Performance (peak shape etc.)
- Ruggedness/durability
- Lot-to-lot reproducibility

Thermo Scientific's Syncronis Column

- Consistent, reproducible separation from time to time
- High purity, high surface area silica
- Dense bonding and double endcapping
- Strict manufacturer and QC

Syncronis Phase

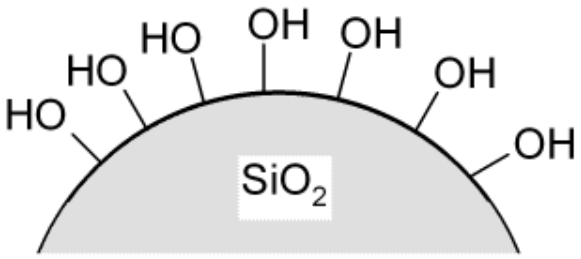
Reverse Phase

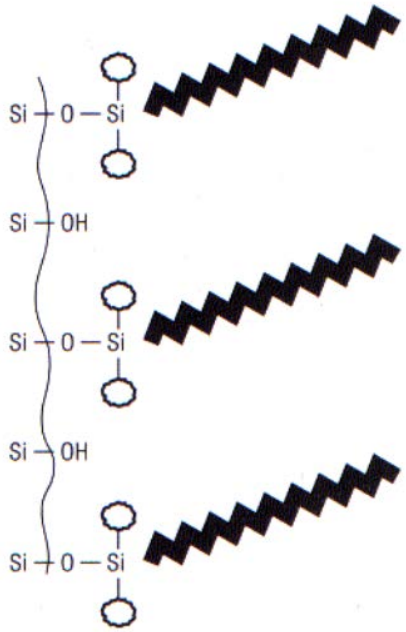
- C18
- C8
- aQ (polar endcapping)
- Phenyl
- Amino
- HILIC (Hydrophilic interaction)

Normal Phase

- Silica

Wait a minute…..what's column chemistry???!!!



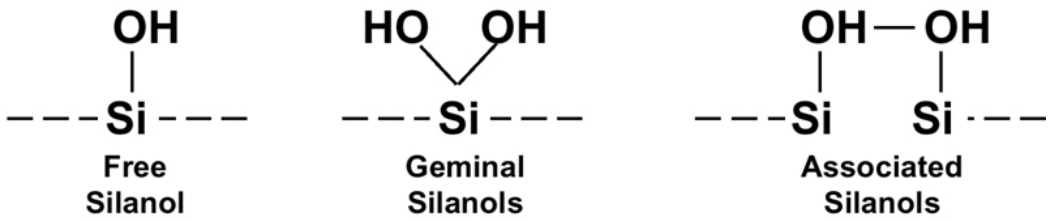


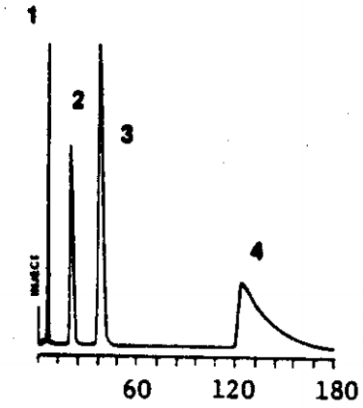
- Diol
- C1, C4, C8, C18
- Aminopropyl
- Nitrile
- Phenyl
- Pentafluorophenyl
- Cation Exchanger
- Anion Exchanger
- etc.

Chemistry is variant. Is all C18 the same ?

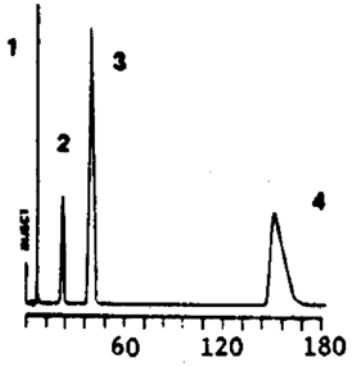
NO !

Un-reactioned: Interaction with Basic Molecules

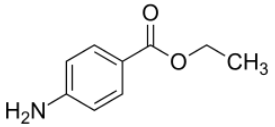
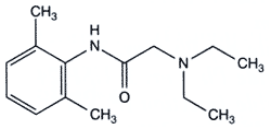
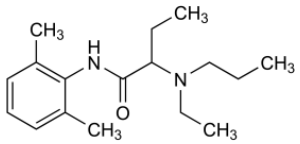
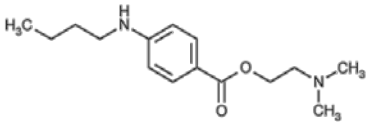




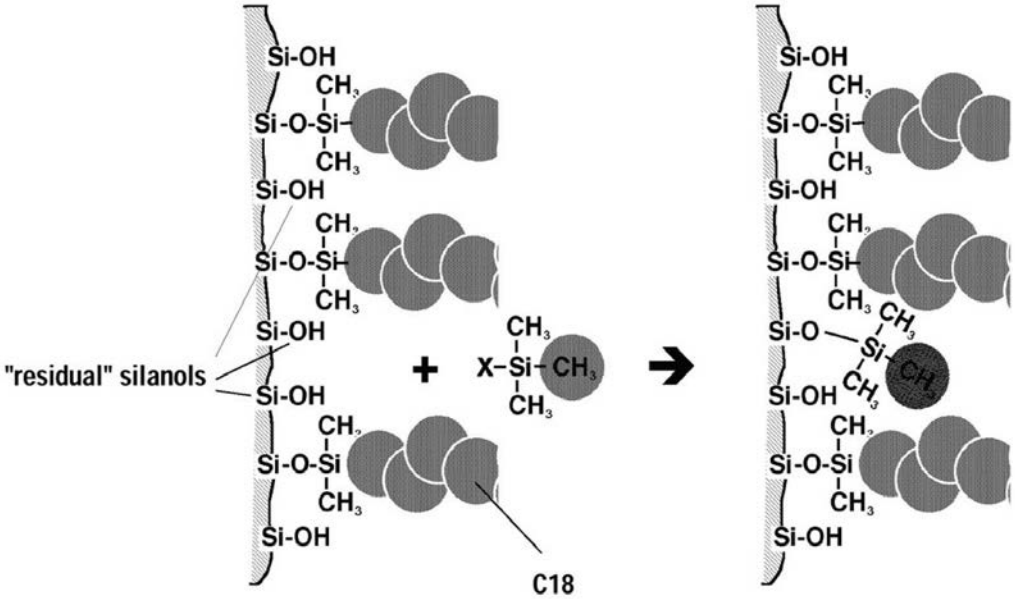
No endcapping

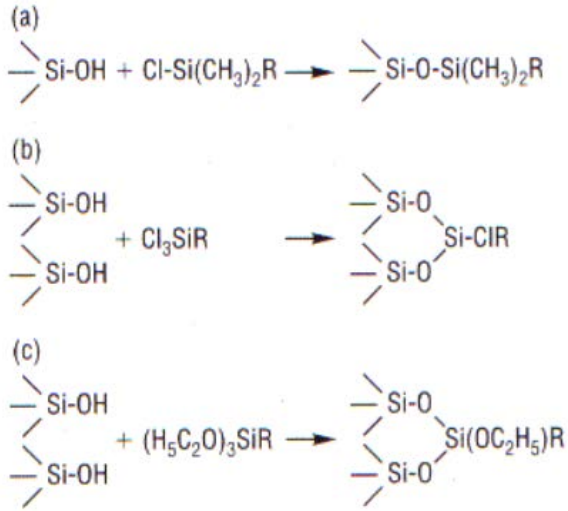


Endcapping

	pKA
	2.8
	7.9
	7.9
	8.5

Endcapping : Prevent peak Tailing & interaction with alkaline



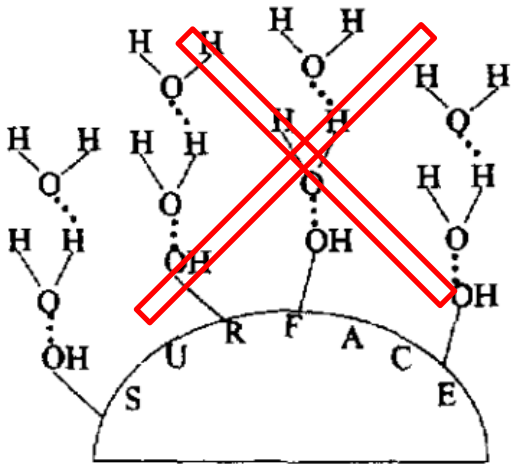


- Polar; amide, urea ,ether
- Hydrophilic
- Trimethylsilyl
- etc.

- Dimethyl silane
- Chloro silane
- Trifunction alkoxysilan
- etc.

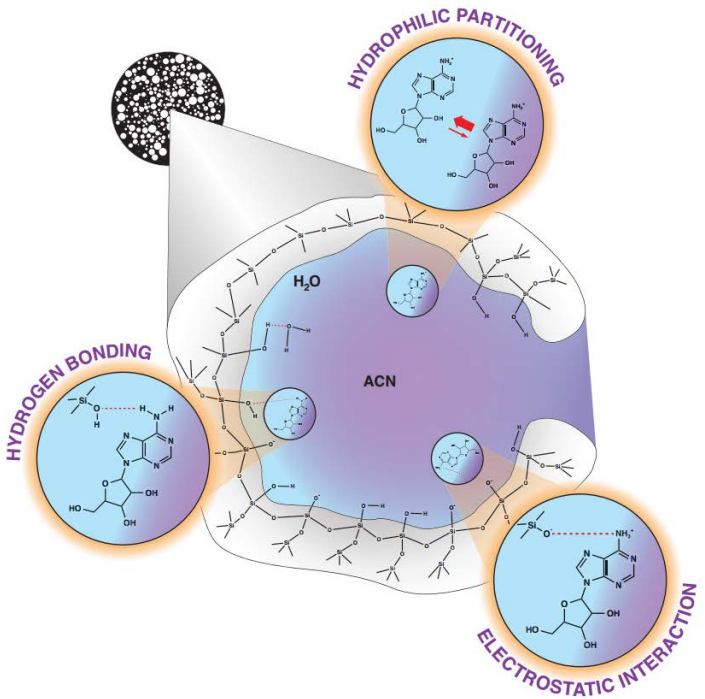
aQ Column and HILIC

aQ



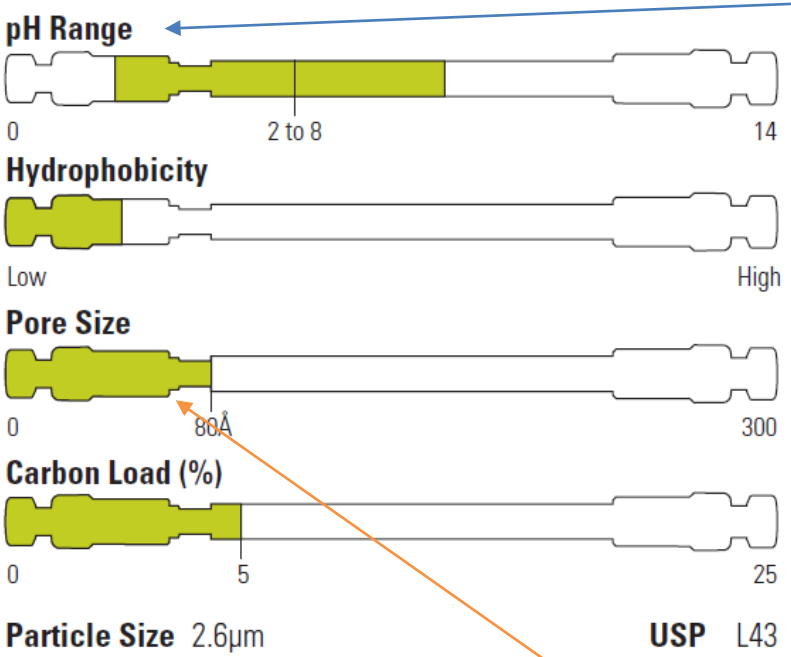
Stable in 100 % Aqueous with polar endcapping, Enhance retention of polar compounds

HILIC



Retain highly polar and hydrophilic compound, no endcapping → can't use with more than 50% aqueous

Column Properties



Working pH of Mobile Phase

Correlated to % carbon, reflected to polarity of column

US Pharmacopeia defined

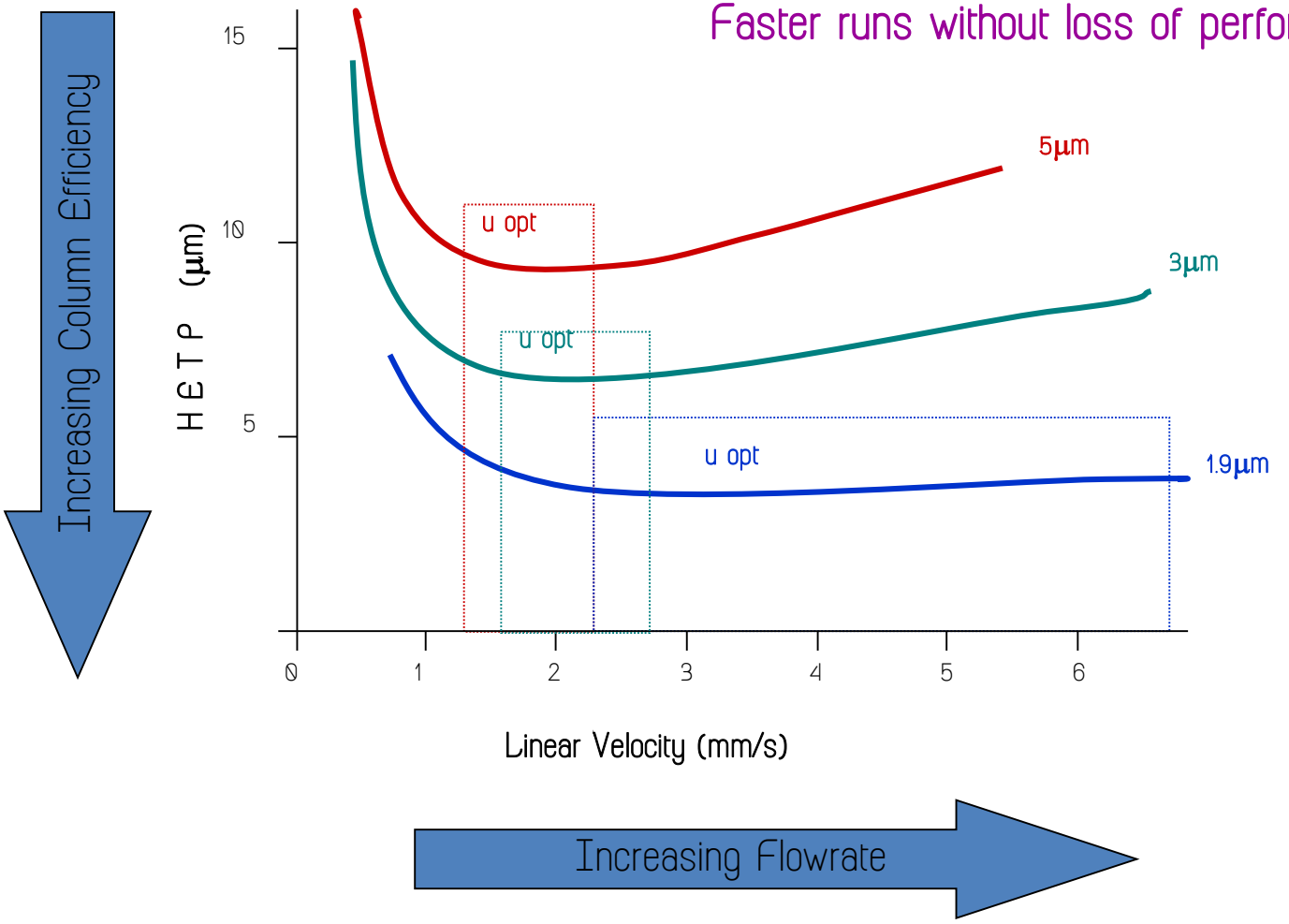
% carbon content, higher is not always better resolution. Higher is more hydrophobic surface that resistance to high pH

60-90 for small molecule
90-120 for both small molecule and peptide
120-300 for peptide or protein

What is the Small Particle Advantage ?

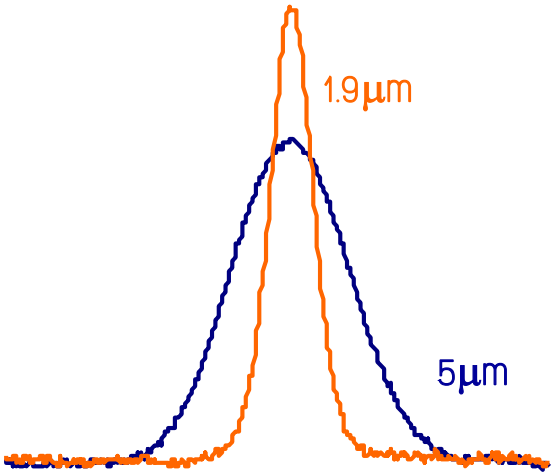
Higher efficiency, independent of flow rate means...

Faster runs without loss of performance



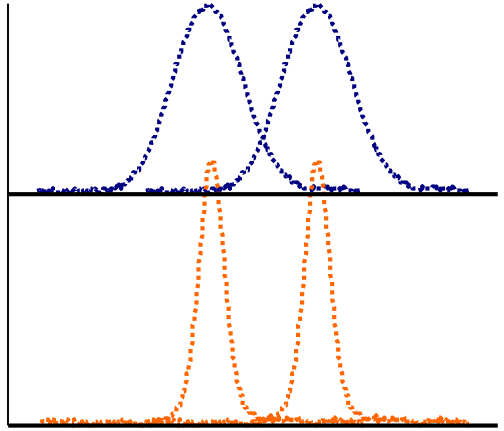
Efficiency is the key!!!

Small Particle Advantage



N = 142,000 plates/m
(189% higher)

N = 75,000 plates /m

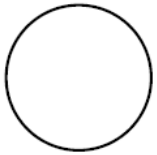
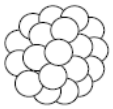


$$R_s = \frac{1}{4}\,\frac{(\alpha-1)}{\alpha}\,\sqrt{N}\,\frac{k}{1+k}$$

Selectivity | Efficiency | Retention

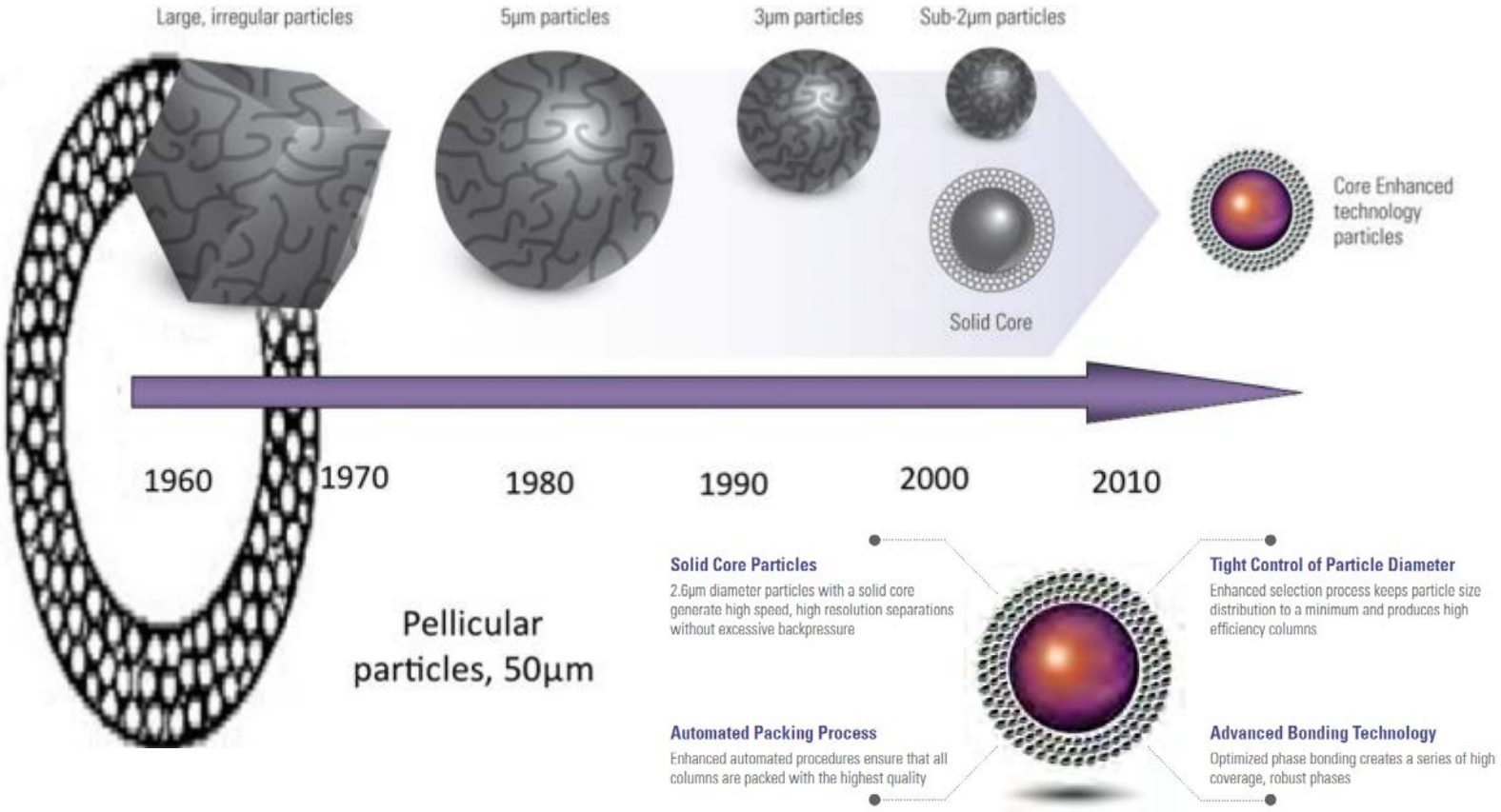
- Higher resolution – narrower peaks
- Higher sensitivity – taller peaks
- Higher peak capacity (more peaks / unit time) – narrower peaks

History of Silica-based Column

Year(s) of Acceptance	Particle Size	Most Popular Nominal Size	Plates / 15cm
1950's		100µm	200
1967		57µm (pellicular)	1,000
1972		10µm	6,000
1985		5µm	12,000
1992		3.5µm	22,000
2003		≤2µm*	>30,000

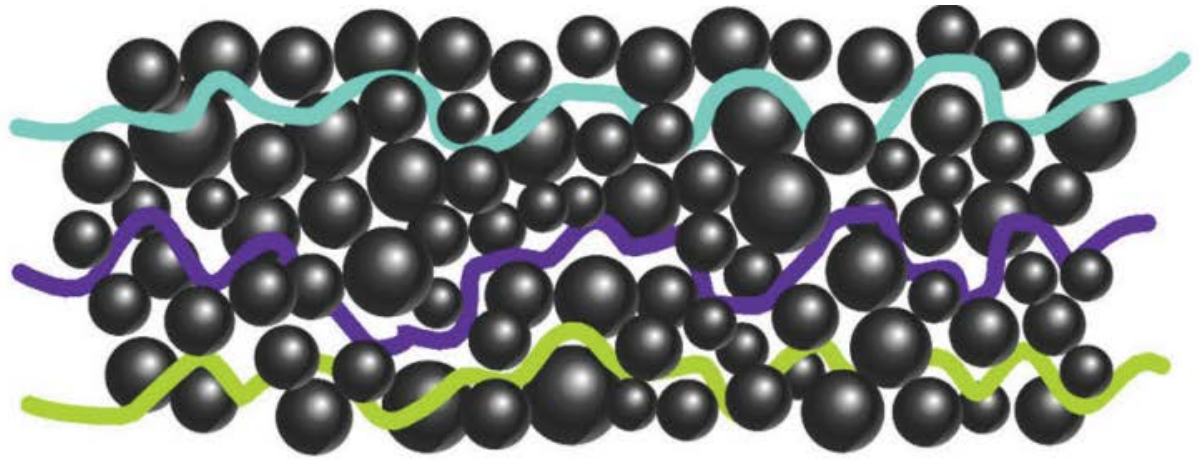
These all called " Fully Porous Silica"

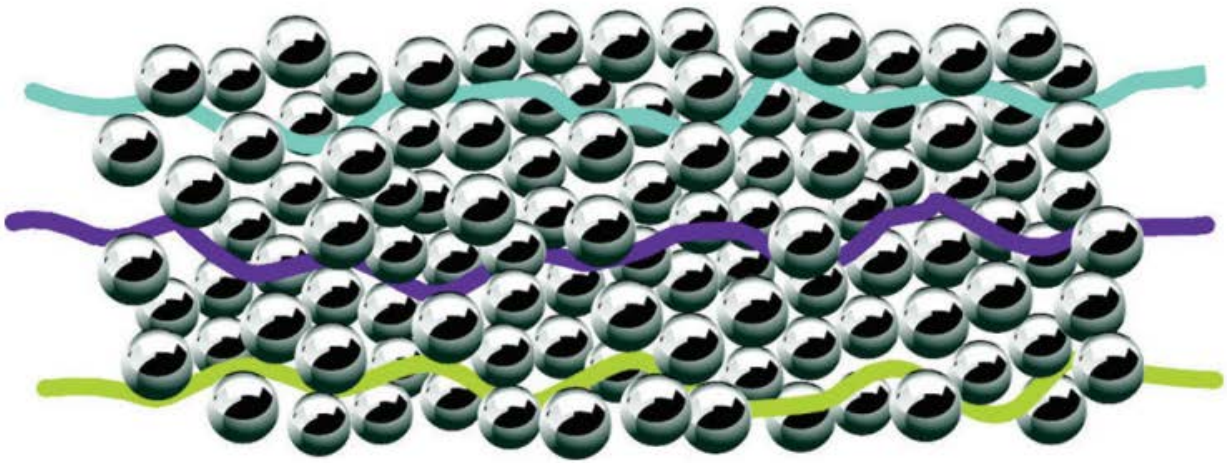
Solid Core Silica



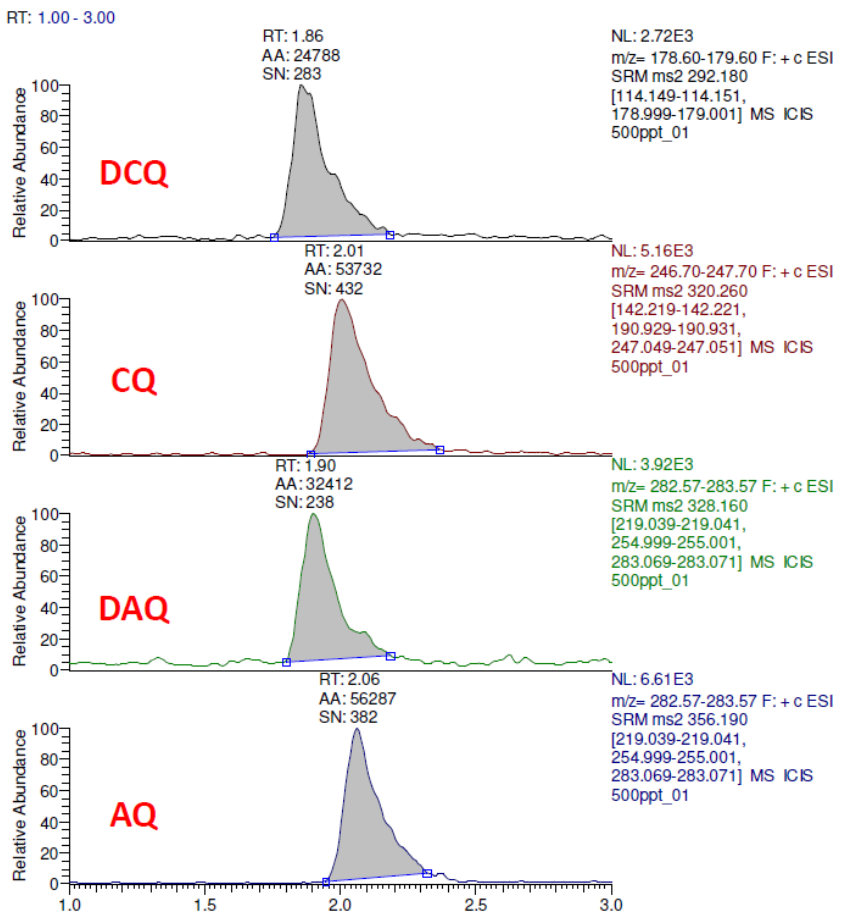
THIS CALLED "SOLID CORE SILICA". Next Generation of Column. High Resolution without excessive pressure

Separation Mechanism

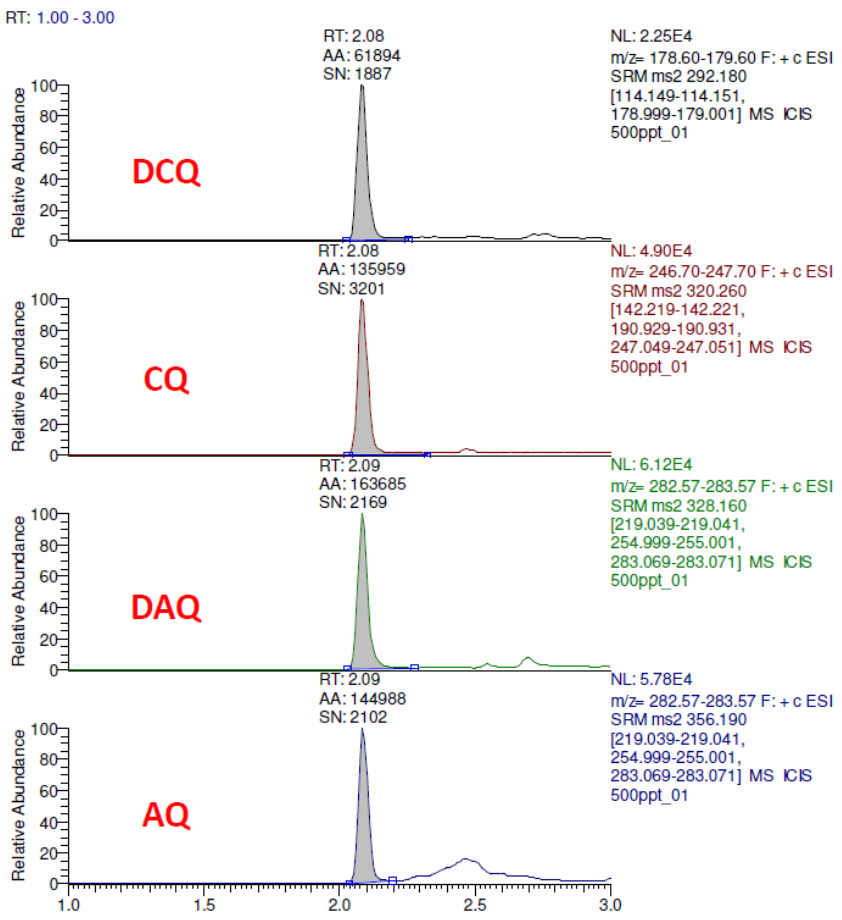




Accucore Performance



Porous Silica C18



Accucore C18

Thermo Scientific's Accucore Column

Ultimate Core Performance

- Solid core particle
- Tight control of particle diameter
- Faster and narrower than 5 or 3 micron column

Accucore Phase

- Vanquish 1.5 micron
- RP-MS
- C18
- C8
- aQ
- Polar Premium
- Phenyl-X
- PFP
- C30
- HILIC
- Urea HILIC
- XL C18

Thermo Scientific's Hypersil GOLD

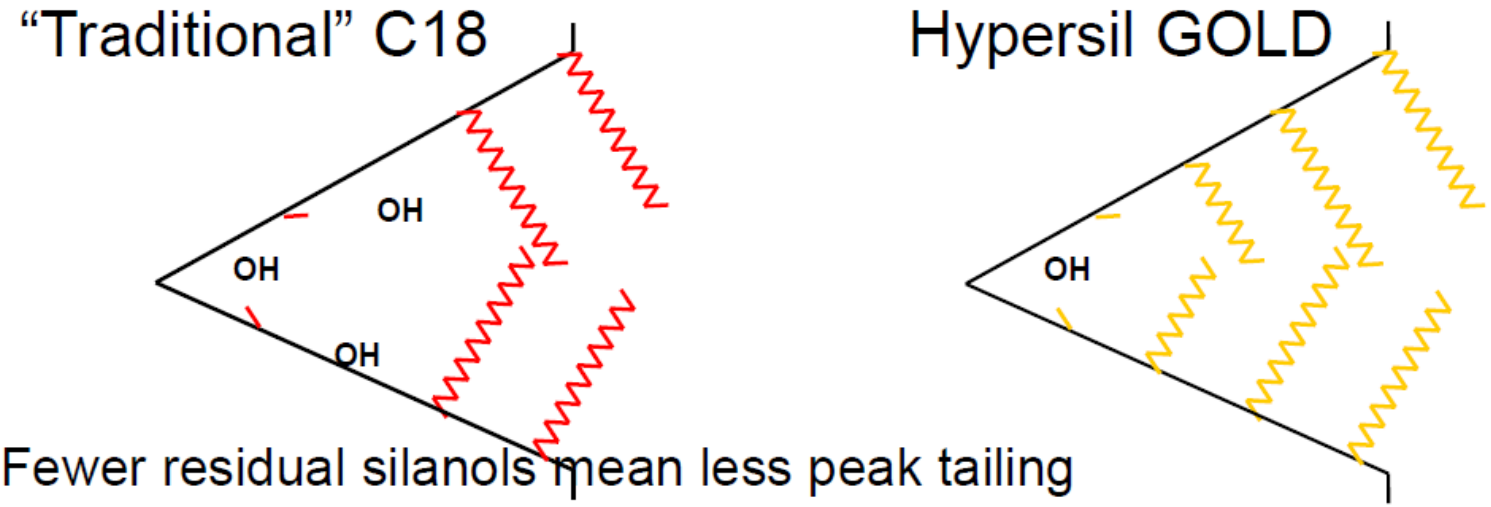
Outstanding Peak Shape for Separation

- Solution for all separation needs
- Improve sensitivity
- Enhance resolution
- pH stability

Hypersil GOLD phase

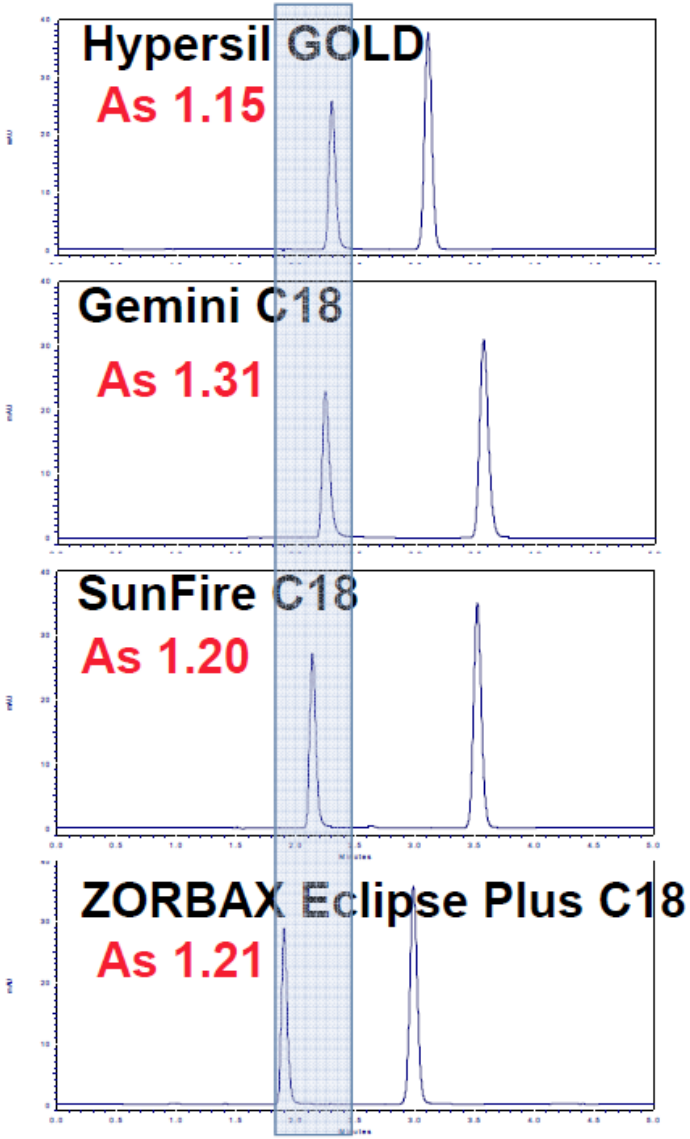
- C18
- C8
- C4
- aQ
- PFP
- Phenyl
- CN
- Amino
- AX
- SAX
- Silica
- HILIC

Hypersil GOLD concept



- Fewer residual silanols mean less peak tailing

Peak symmetry



Almostly the same retention

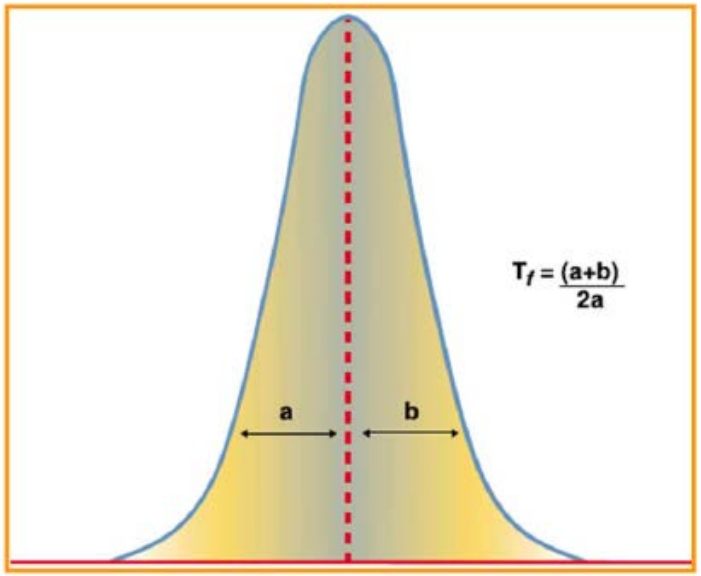
BUT improve peak symmetry

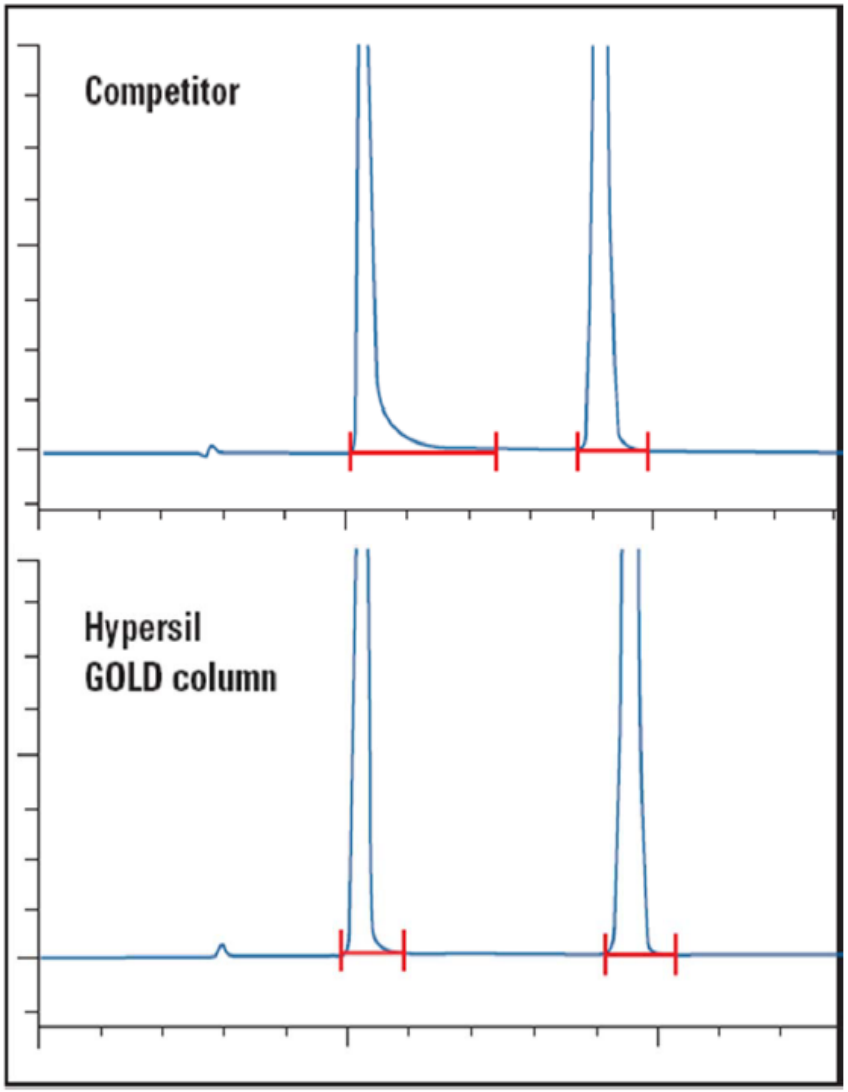
All columns are 150x4.6, 5 uM

Design for Peak Shape

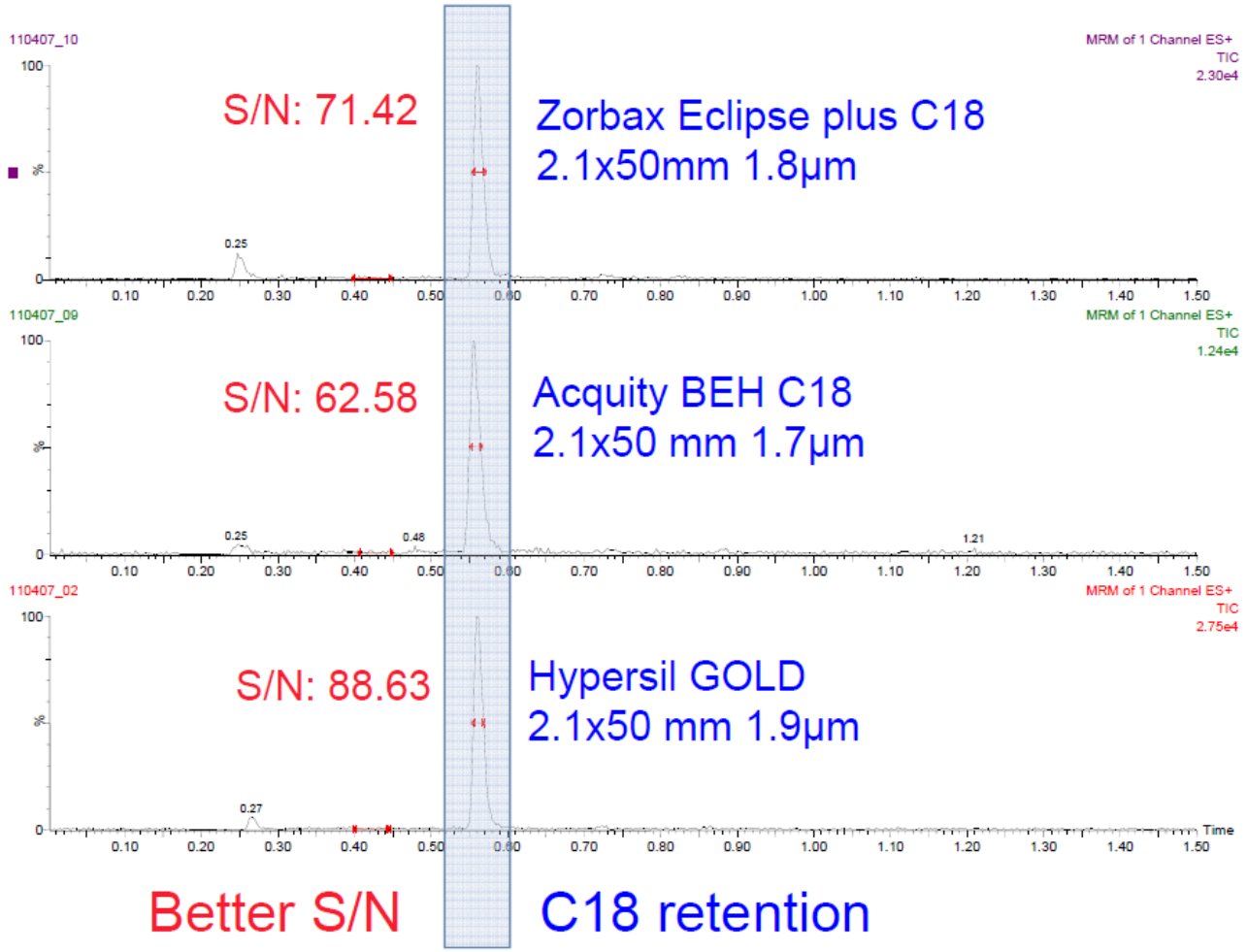
Highly pure (99.999%) silica

- Novel bonding and endcapping
- Even surface
- Reduced silanols
- Less peak tailing
- Outstanding peak symmetry





Sensitivity



Mobile phase A: 0.1%FA
Mobile phase B: ACN

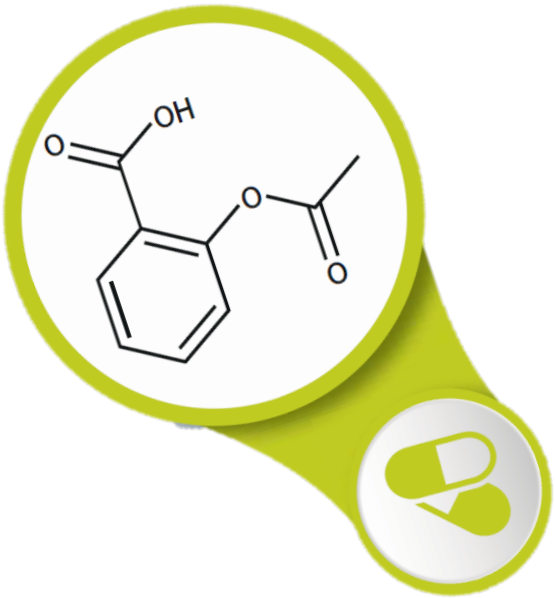
Time (min)	%A	%B
0.0	90	10
0.4	60	40
0.75	60	40
0.8	90	10

Flow rate: 0.7mL/min
Detection: +ESI
Injection volume: 5µL

Thermo Scientific's Acclaim Column

Optimal Selectivity Through Innovative Chemistry

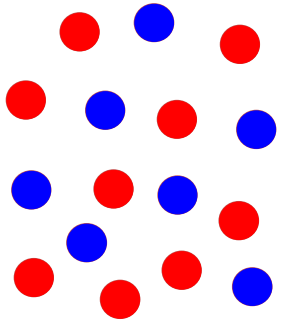
- Diversified selectivity
- Reproducible and reliable
- High Efficiencies
- Ultra-pure porous silica

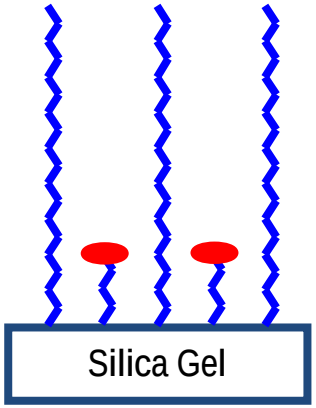


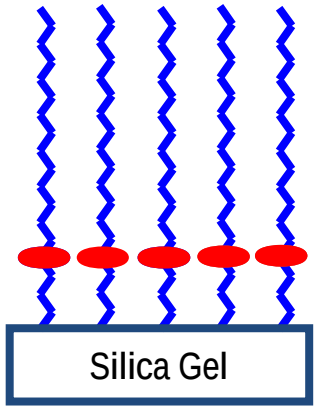
Acclaim Phase

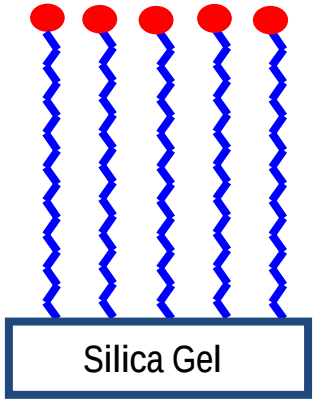
- C18 120, 300
- C8 120
- C30
- Phenyl-1
- PolarAdvantage I & II
- HILIC-10
- Mixed modes
- OmniPAC
- SEC
- Surfactant
- Carbonyl C18
- Organic Acid/Sugar/Vitamin
- Explosive
- Carbamate
- PepMap
- Trinity P1

Mixed Mode Chemistry









I. Blend Packing

Hypersil Duet C18/SAX

II. Mixed Bonding

Alltech Mixed-Mode (Grace)

III. "Embedded"

Primesep Columns (SIELC)

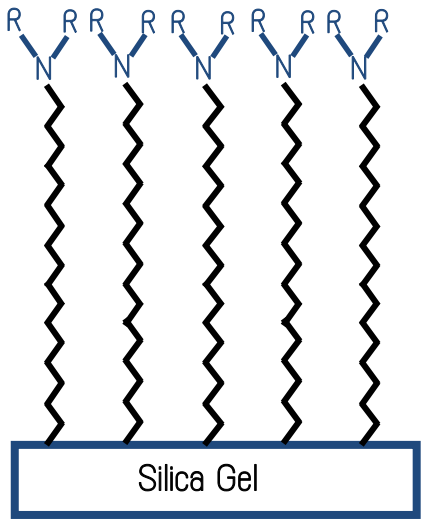
IV. "Tipped"

Acclaim Mixed-Mode Columns

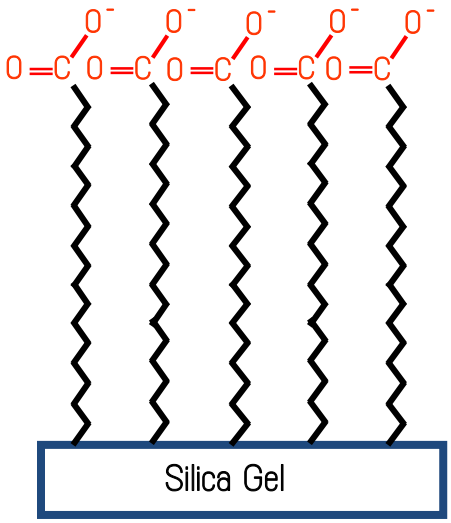
RP – BLUE; IEX – RED

Acclaim Mixed Mode

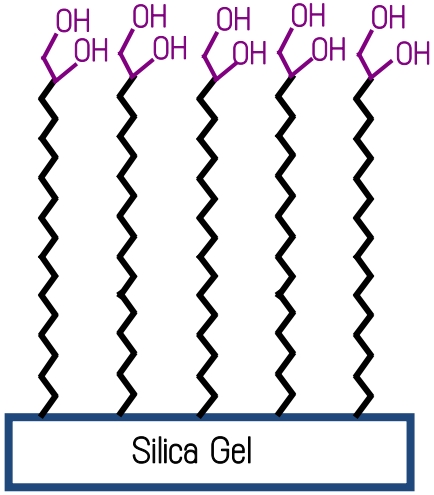
Acclaim Mixed-Mode WAX-1



Acclaim Mixed-Mode WCX-1



Acclaim Mixed-Mode HILIC-1



Silica gel:	high-purity, porous, spherical
Particle size:	3 or 5 µm
Surface area:	300 m^2/g
Pore size:	120 Å

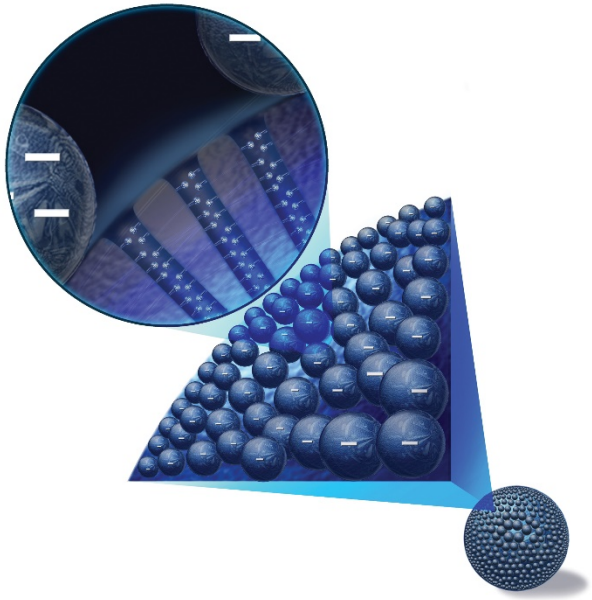
Separation Challenges

- Pharmaceutical counterions (both anions and cations)
- Drug substance and respective counterion (regardless of hydrophobicity)
- Mixtures of basic and acidic drugs with respective counterions
- Flexibility in method development

- Need for an "ideal" column that concurrently provides:
  - Reversed-phase
  - Anion-exchange
  - Cation-exchange

Acclaim Trinity P1 – Column Chemistry

- High-purity, spherical, porous silica substrate
- Nano-polymer Silica Hybrid (NSH) technology



- Silica particles coated with nano-polymer beads
- Inner-pore: RP/WAX
- Outer surface: SCX

Acclaim Trinity P1 – Features, Values & Applications

- Features
  - Multi-mode retention mechanism: RP, AEX, and CEX
  - Adjustable selectivity
- Values
  - Optimal selectivity and greater flexibility in method development
  - Retain ionic and ionizable compounds without ion-pairing reagents
  - A broad range of applications
- Applications
  - Pharmaceutical counterions (both anions and cations)
  - Drug substance and respective counterion (regardless of hydrophobicity)
  - Mixtures of basic and acidic drugs with respective counterions

Thermo Scientific's Classical Column

Exceptional General Propose Column

- Excellent Reproducibility
- Very Robust and Rugged
- Long Column Life Time

Classical Phase

- BDS
- ODS/ODS-2 (C18)
- MOS/MOS-2 (C8)
- SAS (C1)
- Phenyl/Phenyl-2
- CPS/CPS-2
- Silica
- SAX

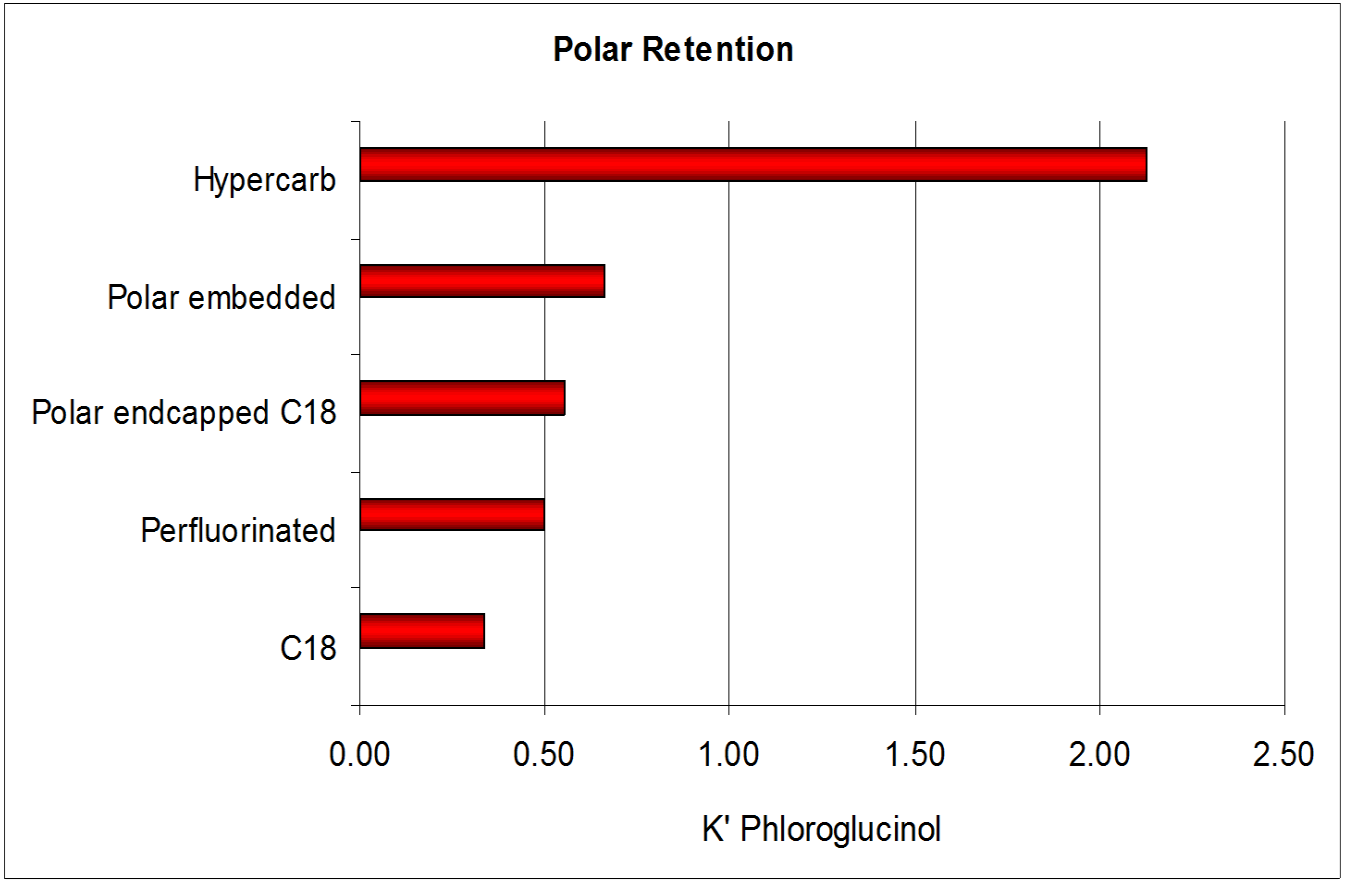
Columns for Fast and Analytical LC

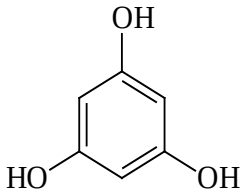
Family (Hydrophobicity)		Attribute	Achieved through
Accucore (Low)		**Ultimate Performance**	**Core Enhanced Technology with 2.6 & 4 µm solid core particles**
Hypersil GOLD (Low)		**Selectivity & Peak Shape**	**Ultra-high purity 1.9, 3 & 5 µm silica Wide range of conventional phases**
Acclaim (Med/High)		**Speciality & Application Specific**	**Ultra-high purity 2.2, 3 & 5 µm silica Innovative and unique stationary phases**
Syncronis (High)		**Ruggedness & Reproducibility**	**High surface area 1.7, 3 & 5 µm silica, Dense bonding, double endcapping, rigorous testing**
Hypercarb (Very High)		**Extended separation capabilities**	**100% porous graphitic carbon**

Hypercarb HPLC Columns

Thermo Scientific Hypercarb HPLC Columns provides HPLC users with extended separation capabilities for difficult applications through unique mechanisms such as polar retention effect on graphite (PREG) and high temperature LC

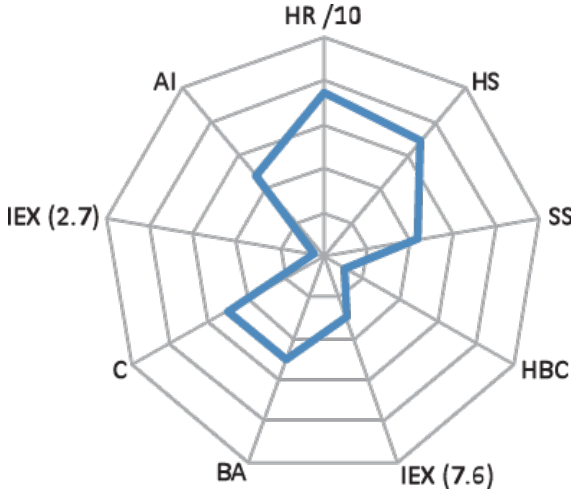
Polar Retention Comparison





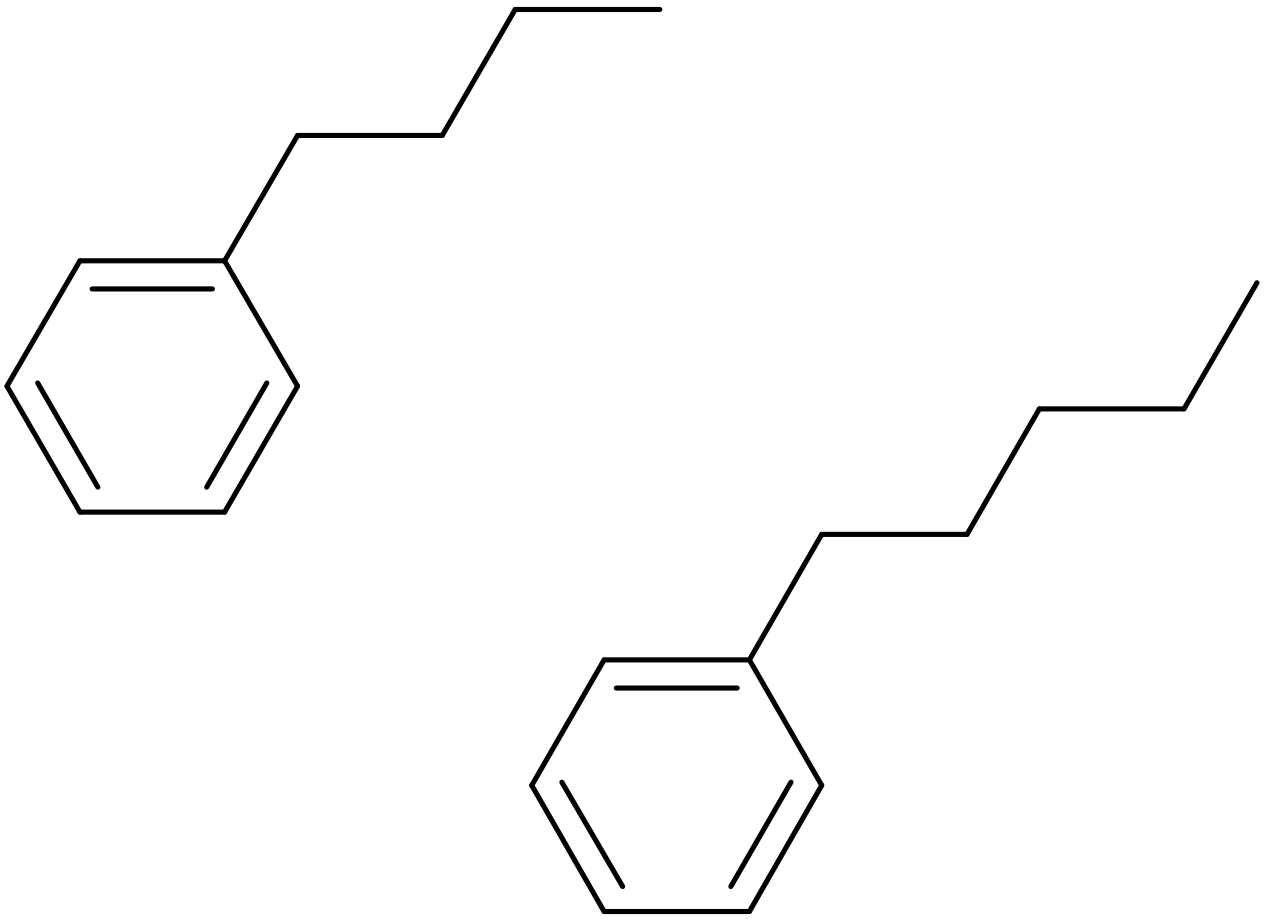
Column Characterisation – Radar Plot

HR	k'
HS	α
SS	α
HBC	α
BA	tf
C	tf
IEX(7.6)	α
AI	tf
IEX(2.7)	α



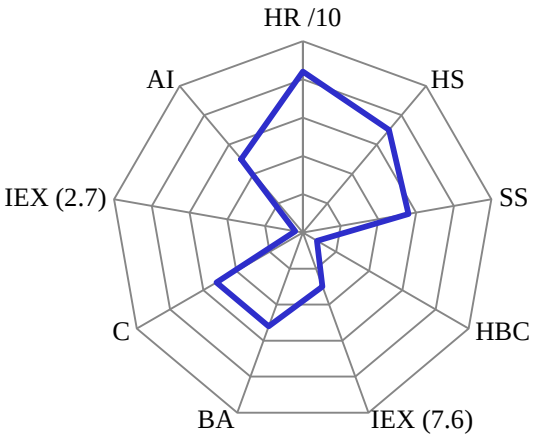
Hydrophobic Interactions	Secondary Interactions	Acidic Interactions
Hydrophobic retention HR	Base activity BA	Acid interaction AI
Hydrophobic selectivity HS	Chelation C	Ion exchange capacity pH 2.7 IEX (2.7)
Steric selectivity SS	Ion exchange capacity (pH 7.6) IEX (7.6)	
Hydrogen bonding capacity HBC		

Compounds to Separate

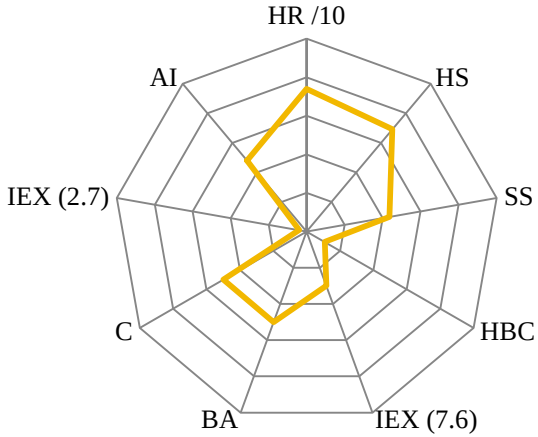


Which is the Right One?

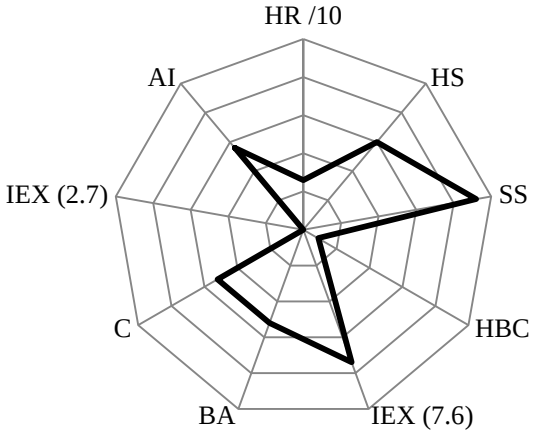
Accucore C18



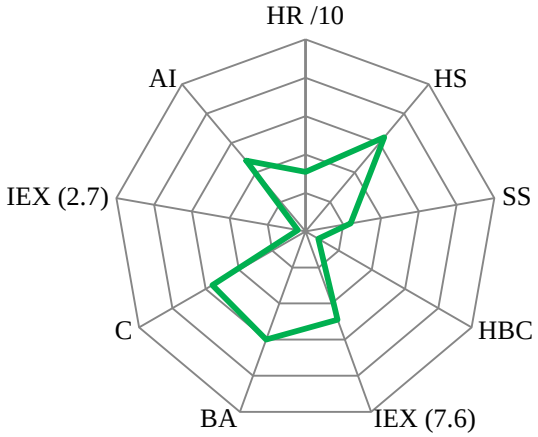
Accucore RP-MS



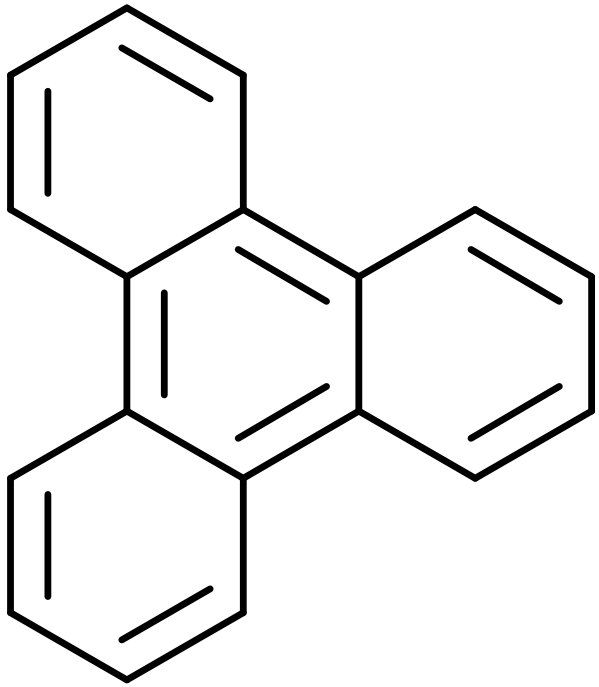
Accucore PFP

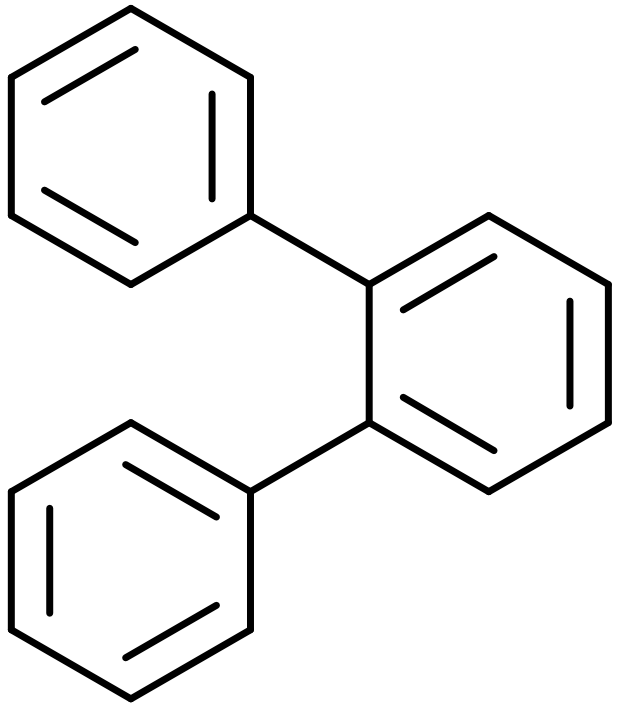


Accucore Phenyl-Hexyl



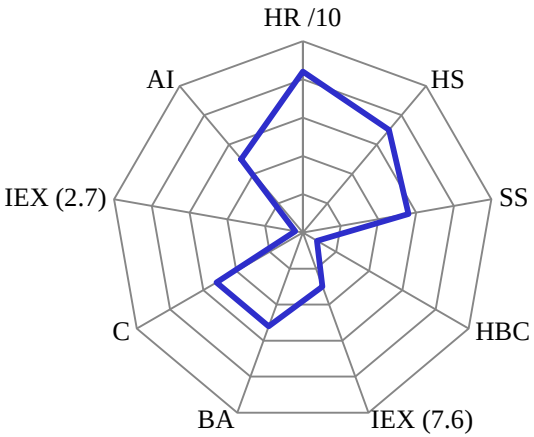
Compounds to Separate



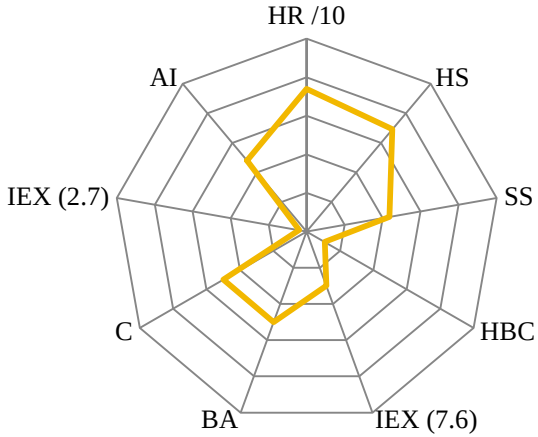


Which is the Right One?

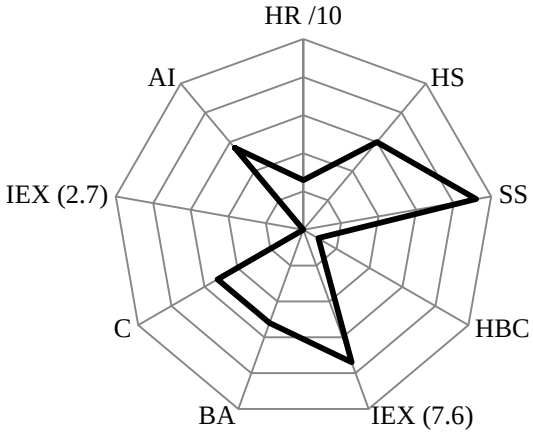
Accucore C18



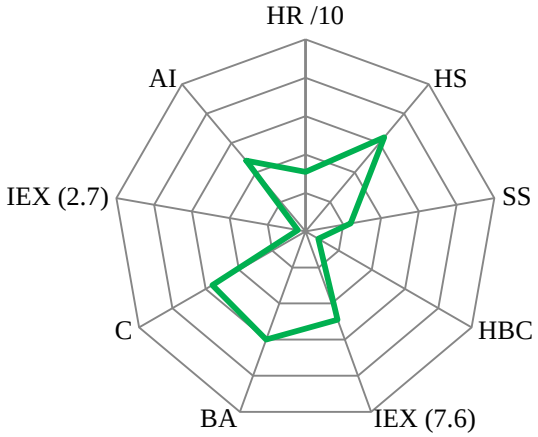
Accucore RP-MS



Accucore PFP

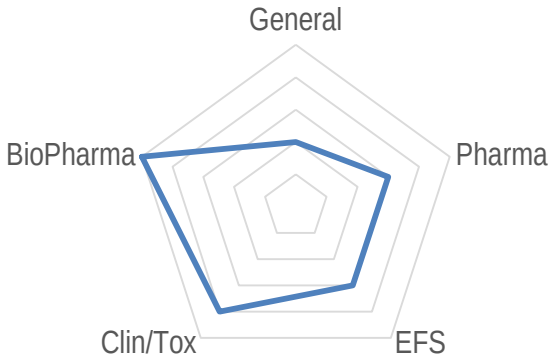


Accucore Phenyl-Hexyl

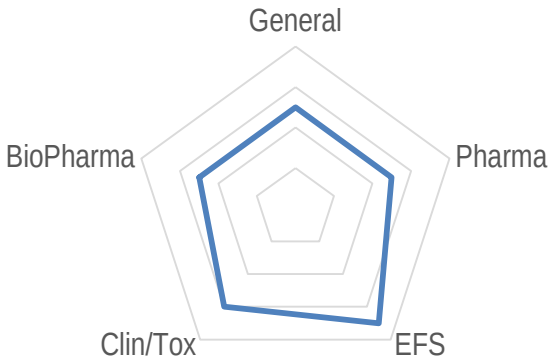


Summary

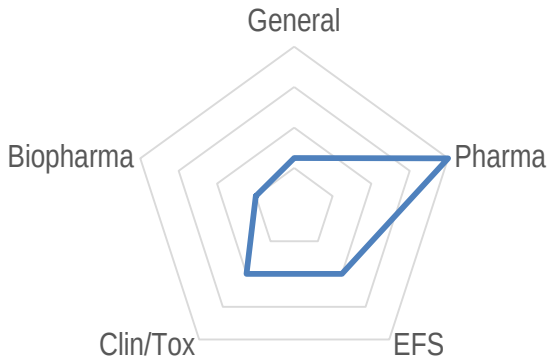
Accucore



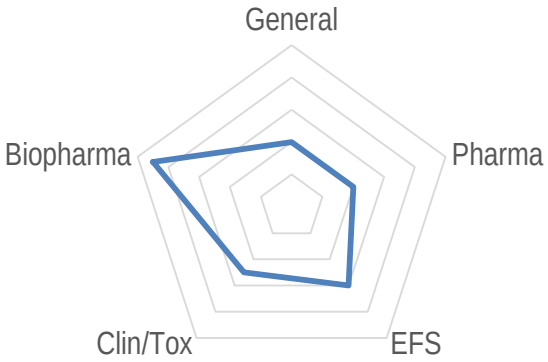
Hypersil Gold



Syncronis



Acclaim



Hypersil Classic