GC Method Development

Column and Phase Selection – Series 3

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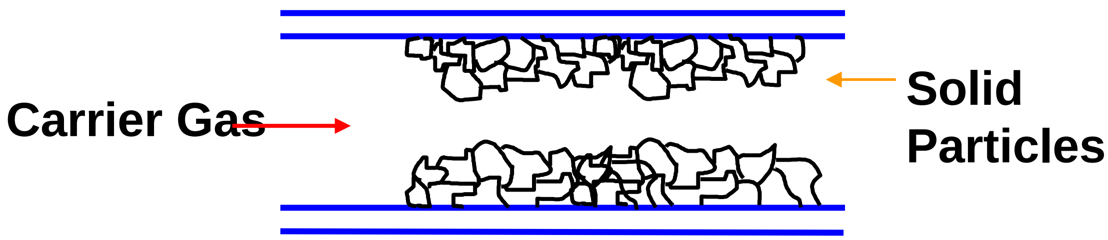
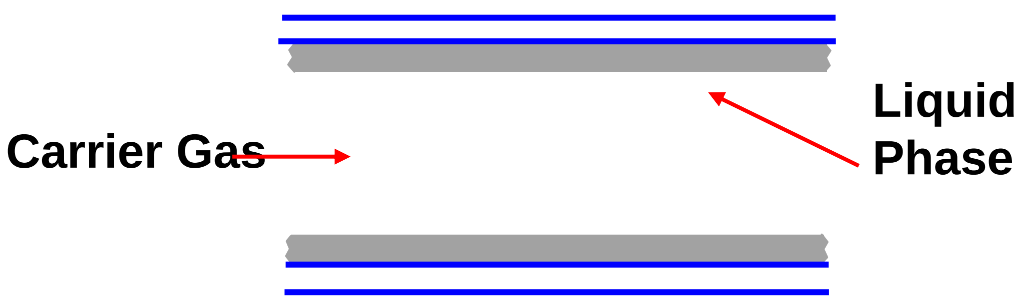
Application Engineer
March 24, 2011

CAPILLARY COLUMN TYPES

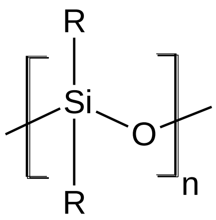
Porous Layer Open Tube (PLOT)



Wall Coated Open Tube (WCOT)

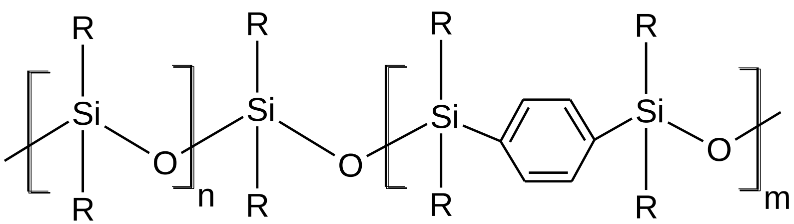


STATIONARY PHASE POLYMERS

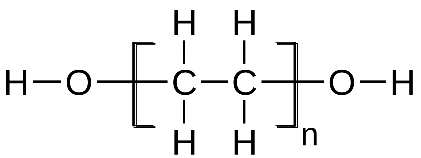


Siloxane

R=methyl, phenyl, cyanopropyl, trifluoropropyl



Siarylene backbone

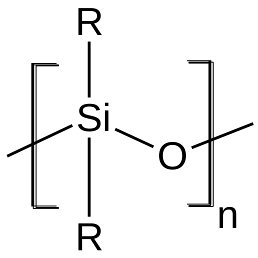


Polyethylene Glycol

Stationary Phase
% Substitution -- polysiloxanes

% = # of sites on silicon atoms occupied

Balance is methyl



Siloxane

R=methyl, phenyl, cyanopropyl, trifluoropropyl

Stationary Phase
Poly(ethylene) Glycol

$$HO - (CH_2 - CH_2 - O) - H$$

100% PEG (DB-WAX)
Less stable than polysiloxanes
Unique separation characteristics

Poly(Ethylene) Glycol
Modified

- Base deactivated (CAM)
- Acid Modified (DB-FFAP)
- Extended Temperature Range

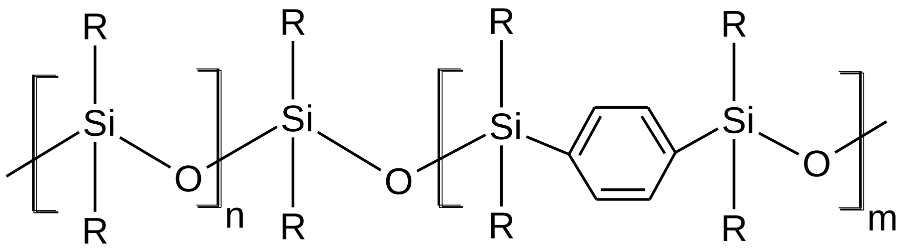
Specialty Phases

Columns developed for particular applications

Examples: DB-VRX, DB-MTBE, DB-TPH, DB-ALC1, DB-ALC2, DB-HTSimDis, DB-Dioxin, Select Low Sulfur, CP-Volamine, Select PAH, DB-EUPAH

Three Types Of Low Bleed Phases

- Phases tailored to "mimic" currently existing polymers
  Examples: DB-5ms, DB-35ms, DB-17ms



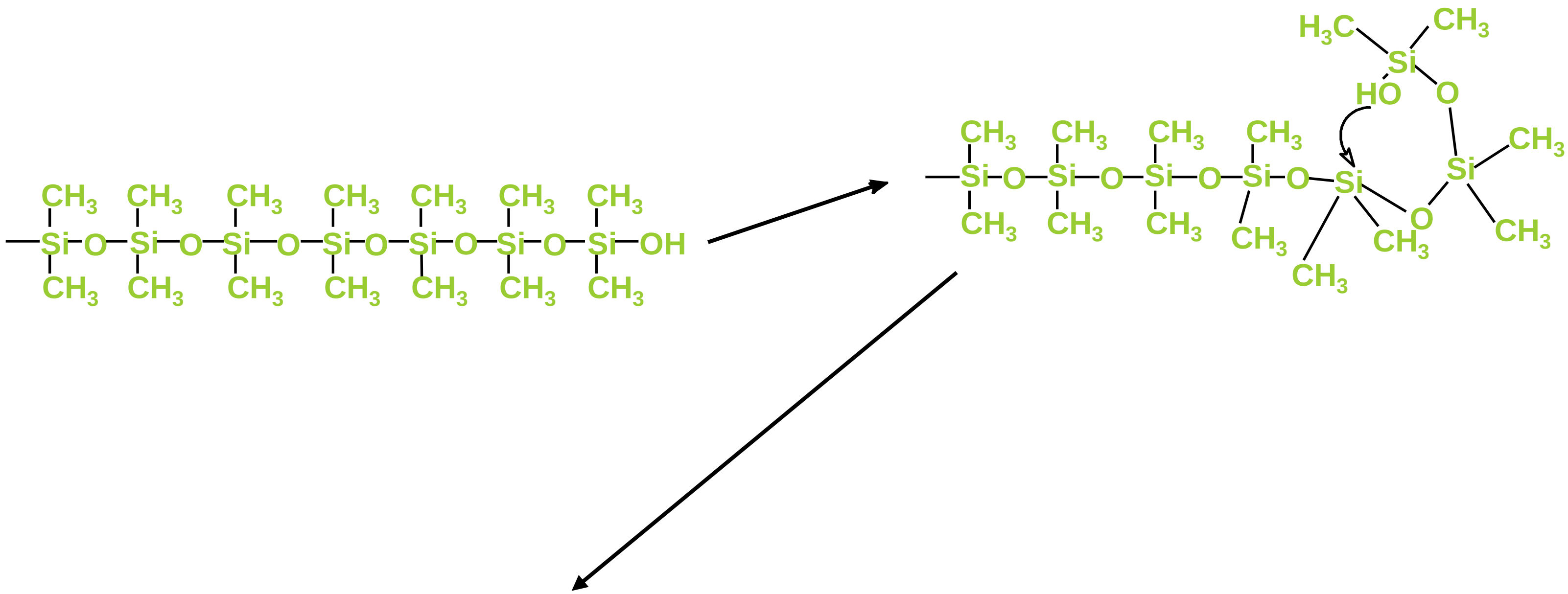
Siarylene backbone

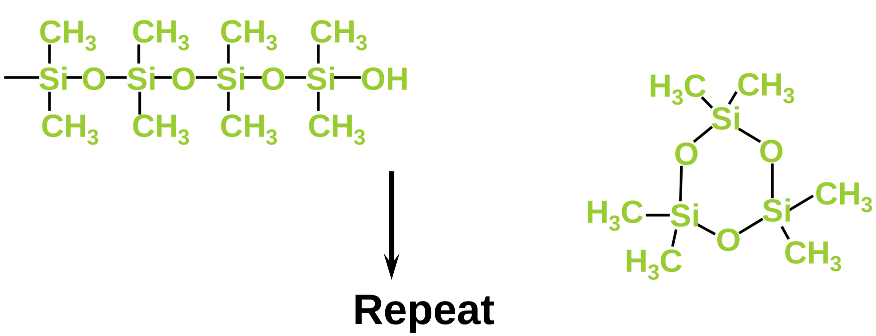
- New phases unrelated to any previously existing polymers
  Examples: DB-XLB

- Optimized manufacturing processes
  DB-1ms, HP-1ms, HP-5ms

What is Column Bleed???

“Back Biting” Mechanism of Product Formation

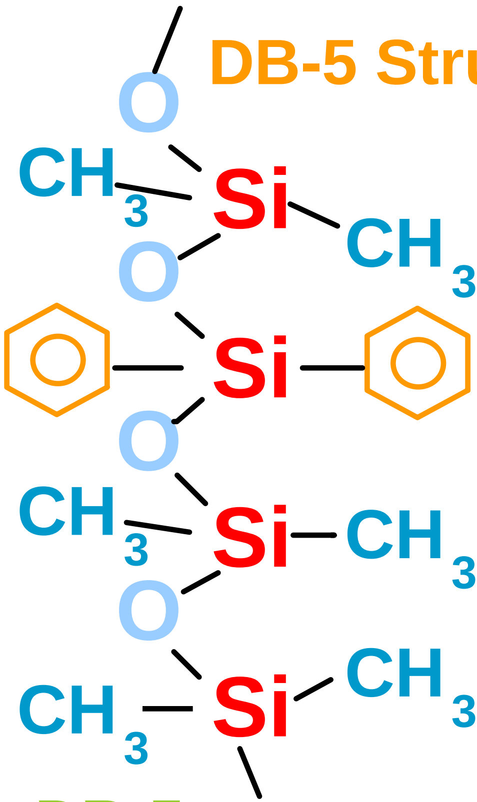




Cyclic products are thermodynamically more stable!

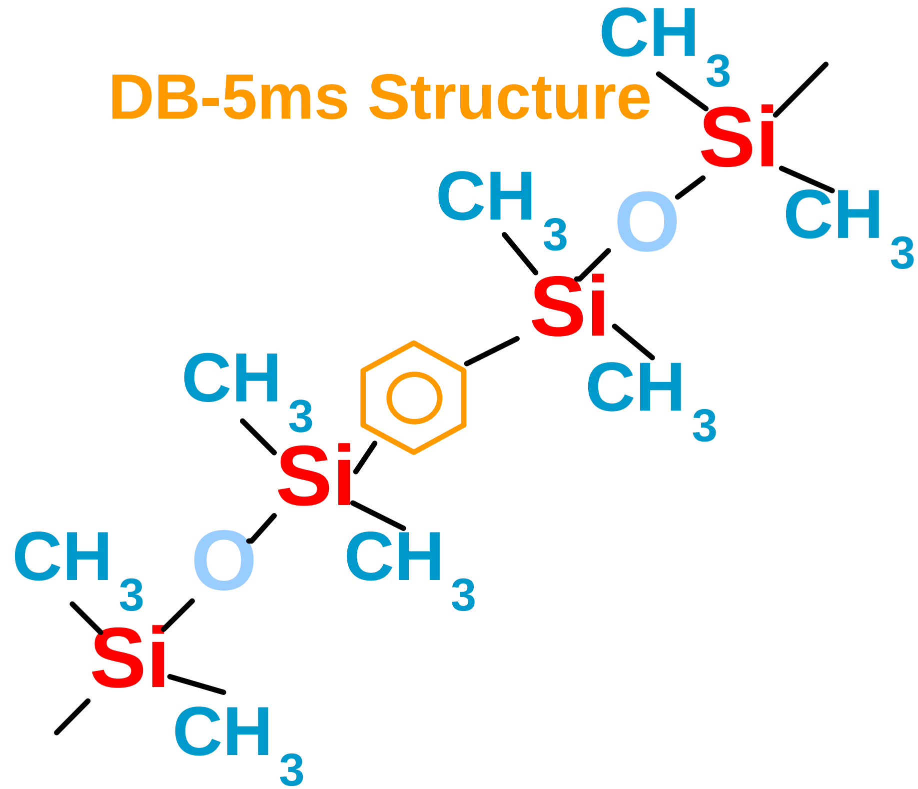
DB-5ms Structure

DB-5 Structure



DB-5
5% Phenyl

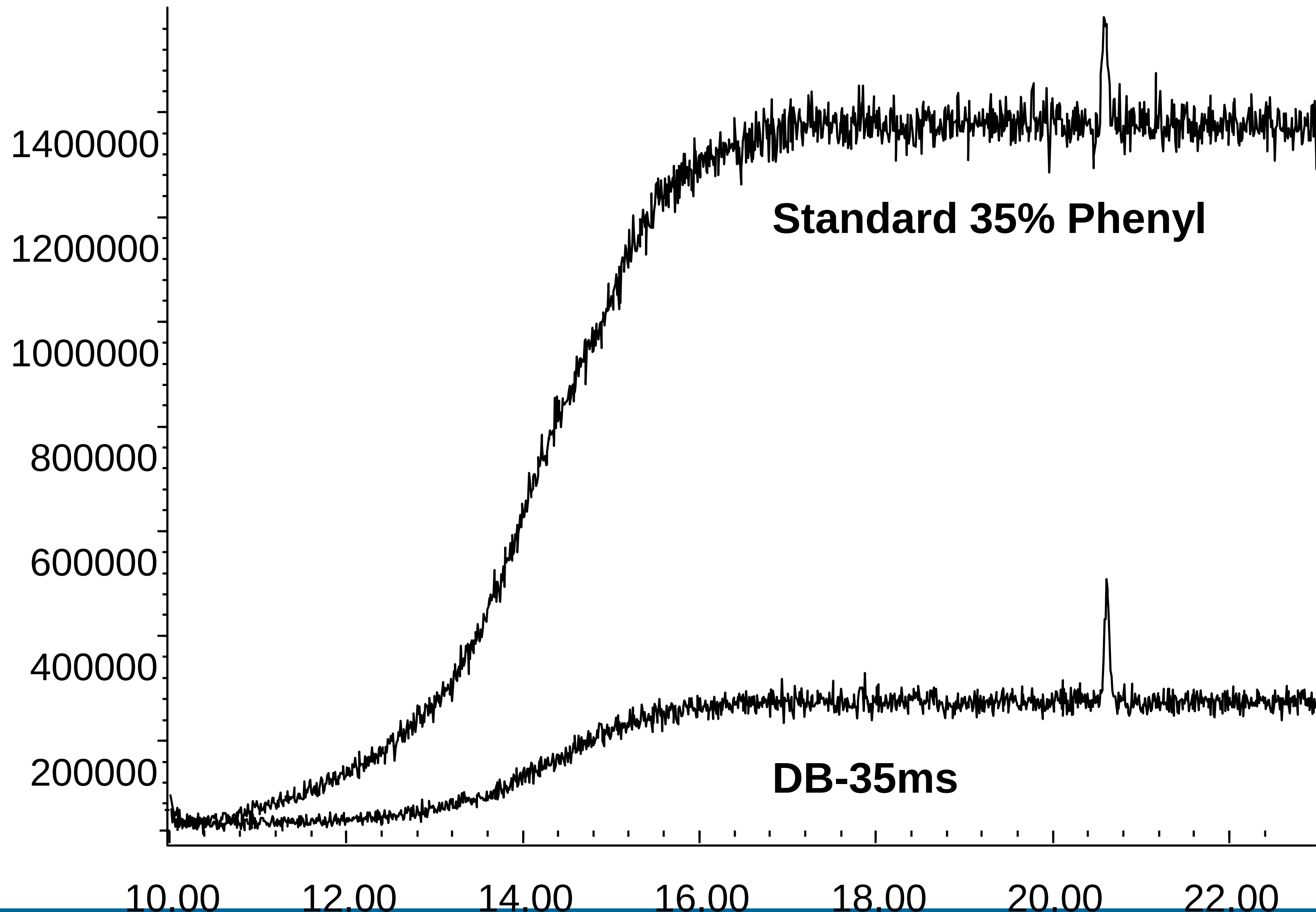
DB-5ms Structure



DB-5ms
1. Increased stability
2. Different selectivity
3. Optimized to match DB-5

DB-35MS VS STANDARD 35% PHENYL

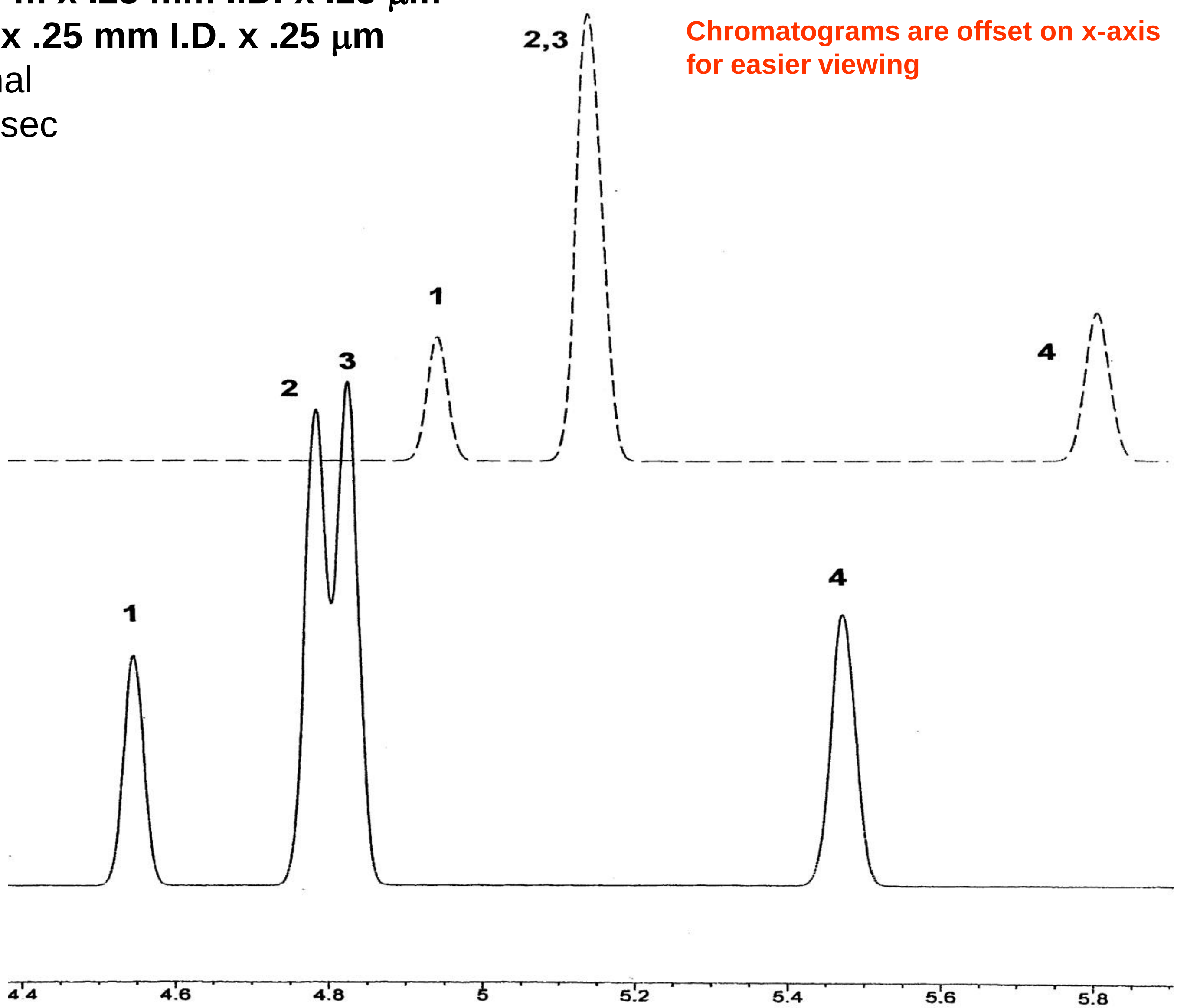
Benzo[g,h,i]perylene, 1ng



Solid line: **DB-5ms 30 m x .25 mm I.D. x .25 μm**
Dashed line: **DB-5 30 m x .25 mm I.D. x .25 μm**
Oven: 60º C isothermal
Carrier gas: H_2 at 40 cm/sec

1: Ethylbenzene
2: m-Xylene
3: p-Xylene
4: o-Xylene

Chromatograms are offset on x-axis for easier viewing



Agilent Technologies

Why is stationary phase type important?

Influence on α

$$\alpha = \frac{k_2}{k_1}$$

k_2 = partition ratio of 2nd peak
k_1 = partition ratio of 1st peak

Selectivity

- Relative spacing of the chromatographic peaks
- The result of all non-polar, polarizable and polar interactions that cause a stationary phase to be more or less retentive to one analyte than another

Optimizing Selectivity (α)

Match analyte polarity to stationary phase polarity

- 'like dissolves like'

Take advantage of unique interactions between analyte and stationary phase functional groups

Analyte Polarity

Nonpolar Molecules - generally composed of only carbon and hydrogen and exhibit no dipole moment (Straight-chained hydrocarbons (n-alkanes))

Polar Molecules - primarily composed of carbon and hydrogen but also contain atoms of nitrogen, oxygen, phosphorus, sulfur, or a halogen (Alcohols, amines, thiols, ketones, nitriles, organo-halides, etc. Includes dipole-dipole interactions and H-bonding)

Polarizable Molecules - primarily composed of carbon and hydrogen, but also contain unsaturated bonds (Alkenes, alkynes and aromatic compounds)

Selectivity Interactions

- Dispersion
- Dipole
- Hydrogen bonding

Dispersion Interaction

ΔH_{vap}

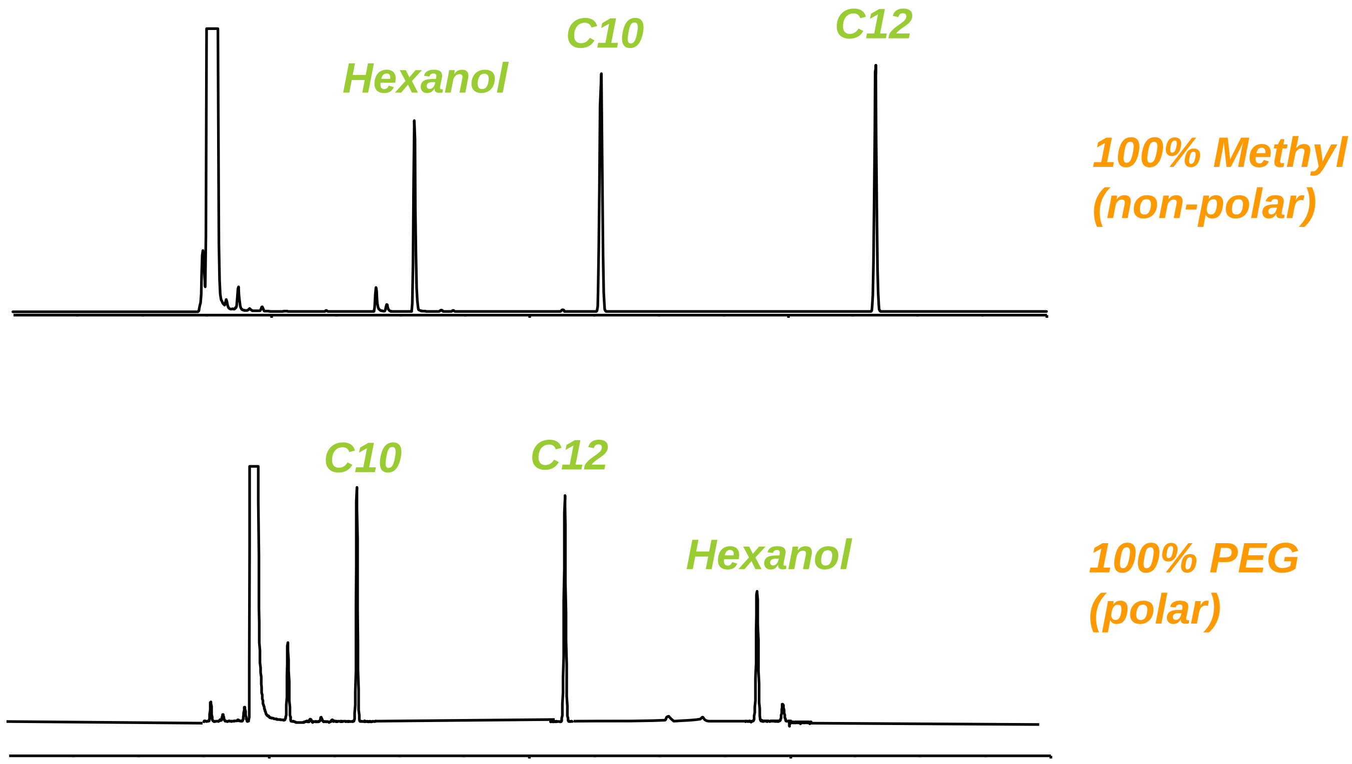
- Separation by differences in analyte heat of vaporizations (DH_{vap})

- Heat necessary to convert a liquid into a gas (at the same temperature)

Dispersion Interaction

Solubility And Retention

Hexanol	158°C
Decane	174°C
Dodecane	216°C



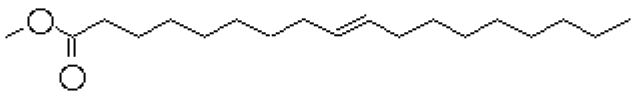
30 m x 0.32 mm ID, 0.25 µm
He at 35 cm/sec
50-170°C at 15°/min

Dispersion Interaction

ΔH_{vap}

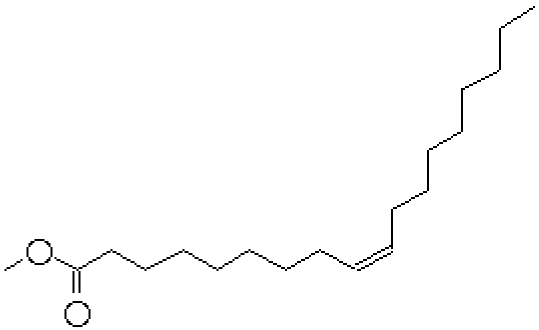
- ✓ **Vapor pressure: good approximation**
- ✓ **Boiling point: poor approximation**

Dipole Interaction



C18:1 (Methyl *trans*-9-octadecenoate)

B.Pt. 186°C



C18:1 (Methyl *cis*-9-octadecenoate)

B.Pt. 186°C

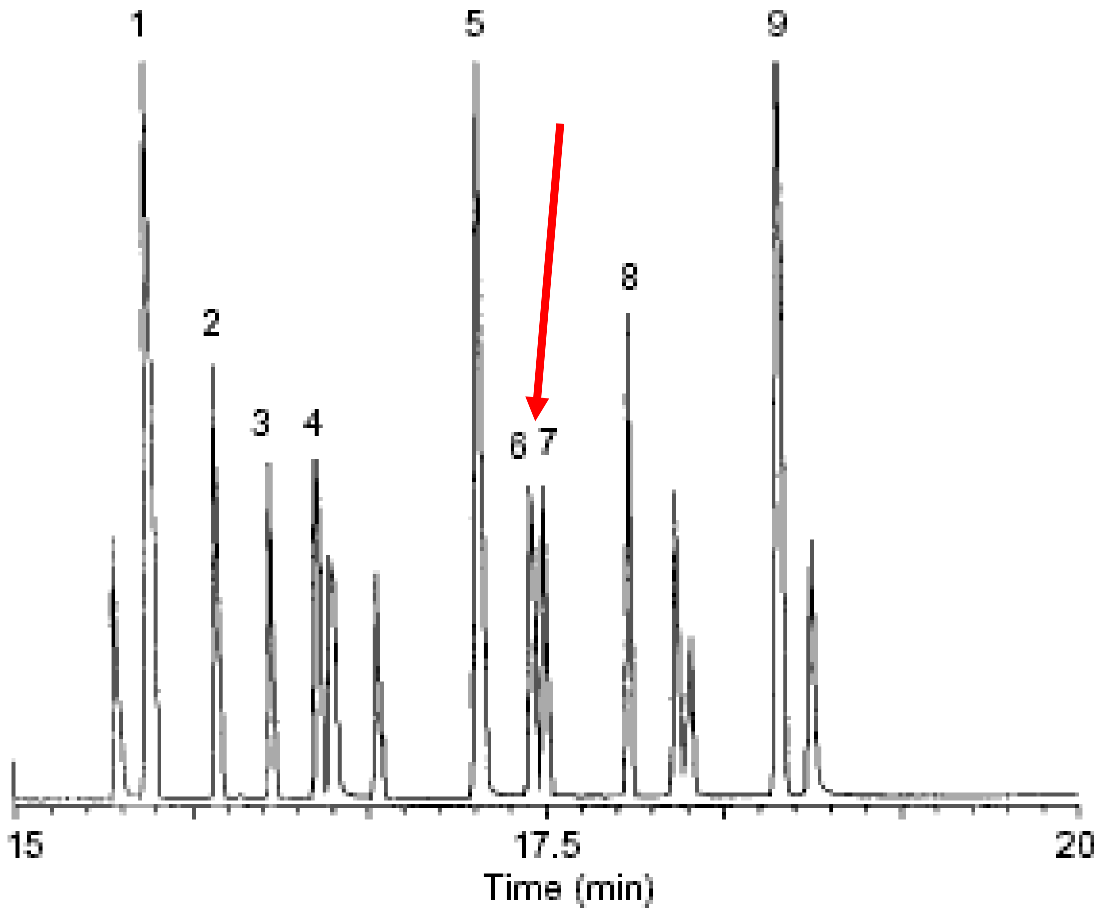
Smaller differences require a stronger dipole phase

Agilent Technologies

Bacterial Fatty Acid Methyl Esters

Column:	**DB-23**
	30 m x 0.25 mm I.D., 0.25 µm
J&W P/N:	122-2332
Carrier:	Hydrogen at 40 cm/sec
Oven:	90°C for 6 min
	90-210°C at 10°/min
Injector:	Splitless, 45 sec purge activation time, 1 µL
Detector:	Finnigan INCOS 50 MSD

1. 14-Me C15:0 14-Methyl pentadecanoate
2. C16:1 *cis*-9-Hexadecanoate
3. 14-Me C16:0 14-Methyl hexadecanoate
4. C17:0 Heptadecanoate
5. 15-Me C17:0 15-Methyl heptadecanoate
6. C18:1 *cis*-Octadecenoate
7. C18:1 *trans*-Octadecenoate
8. C19:0 Nonadecanoate
9. C20:0 Arachidate

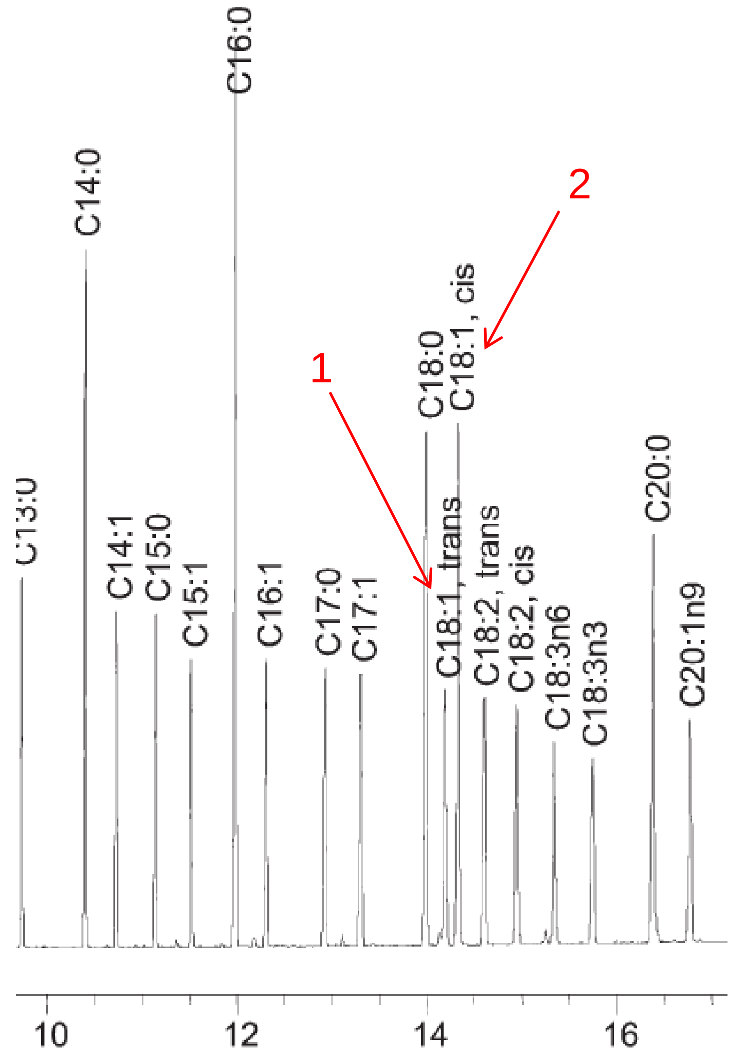


Fames – 37 Component Standard

Column: DB-23
60 m X 0.25 mm X 0.15 µm

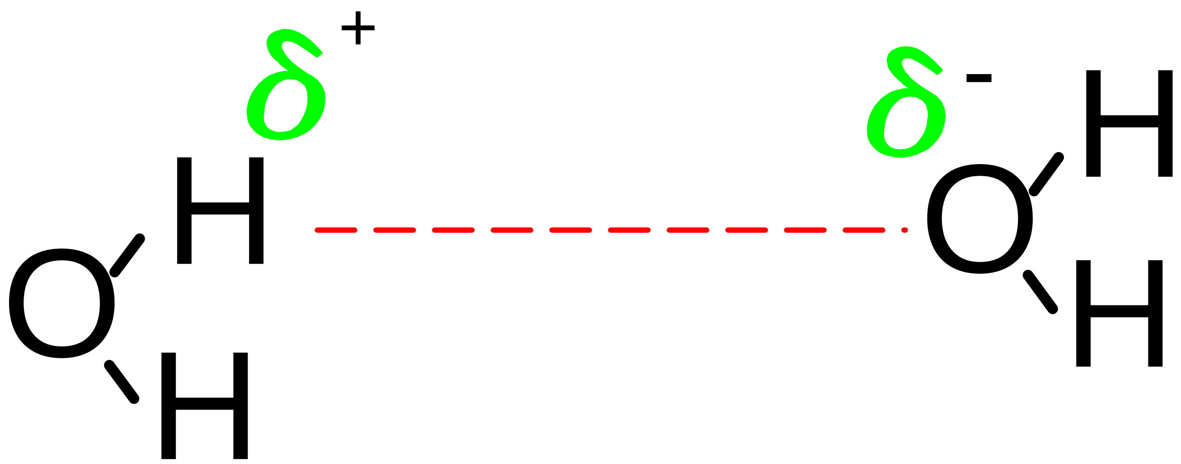
Agilent P/N 122-2361
Carrier: He , 33 cm/sec @ 50°C
Oven: 50°C for 1 min
25°C/min to 175 (no hold)
4°C/min to 230°C hold 5 min
Injector: 250°C, Split 50:1, 1uL
Detector: FID, 250°C

1 C18:1 (Methyl *trans*-9-octadecenoate)
2 C18:1 (Methyl *cis*-9-octadecenoate)



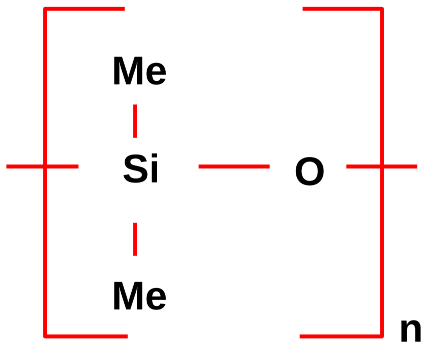
Hydrogen Bonding Interaction

Dipole-Dipole interaction with H bound to O or N interacting with an O or N



NONPOLAR PHASES

Typified by 100% polydimethylsiloxanes such as HP-1, DB-1, DB-1ms, HP-1ms,VF-1ms, CP-Sil 5 CB

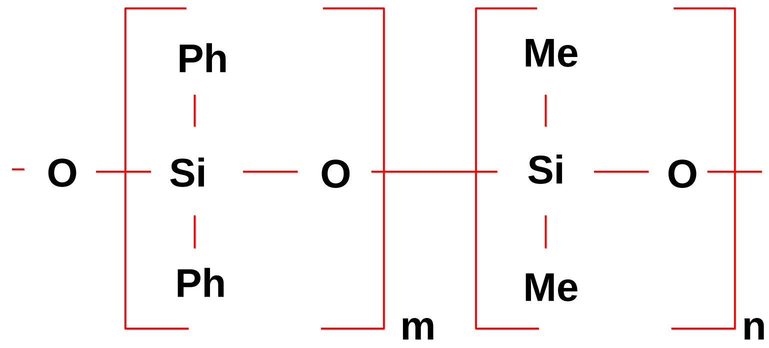


Separation Mechanisms:

- Dispersion only

POLARIZABLE PHASES

Typified by phenyl substituted siloxanes, substituted at 5-50% (HP-5, HP-5ms, DB-35, DB-35ms, DB-17, DB-17ms)



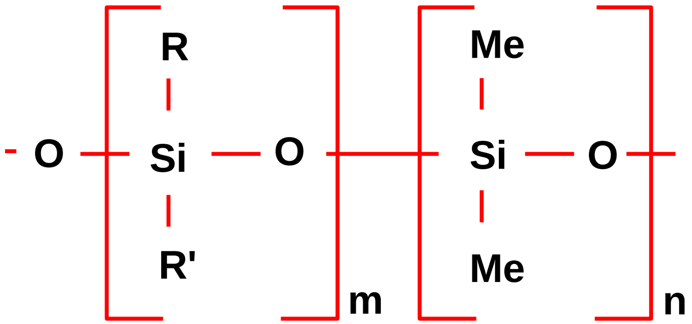
5%--weakly polar, rest--mid polar

Separation Mechanisms:

- Dispersion
- Inducible dipole at phenyl groups

STRONG DIPOLE PHASES

Typified by cyanopropyl or trifluoropropyl substituted siloxanes, substituted 6-50% (DB-1701, DB-1301, DB-200, DB-23, DB-225)



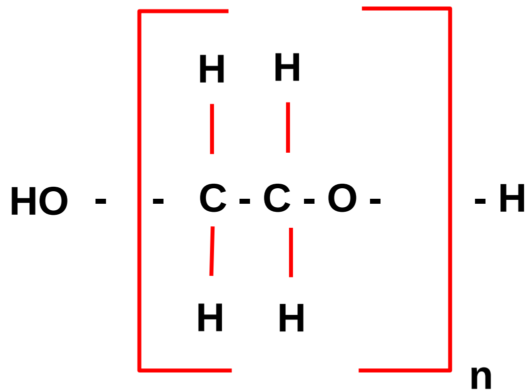
R = cyanopropyl or trifluoropropyl
R′ = phenyl or methyl

Separation Mechanisms:

- Dispersion
- Inducible dipole at phenyl groups
- Strong permanent dipole
- Hydrogen bonding

HYDROGEN BONDING PHASES

Typified by polyethylene glycol polymers (Carbowax, HP-INNOWax, DB-WAX, DB-FFAP, CAM)



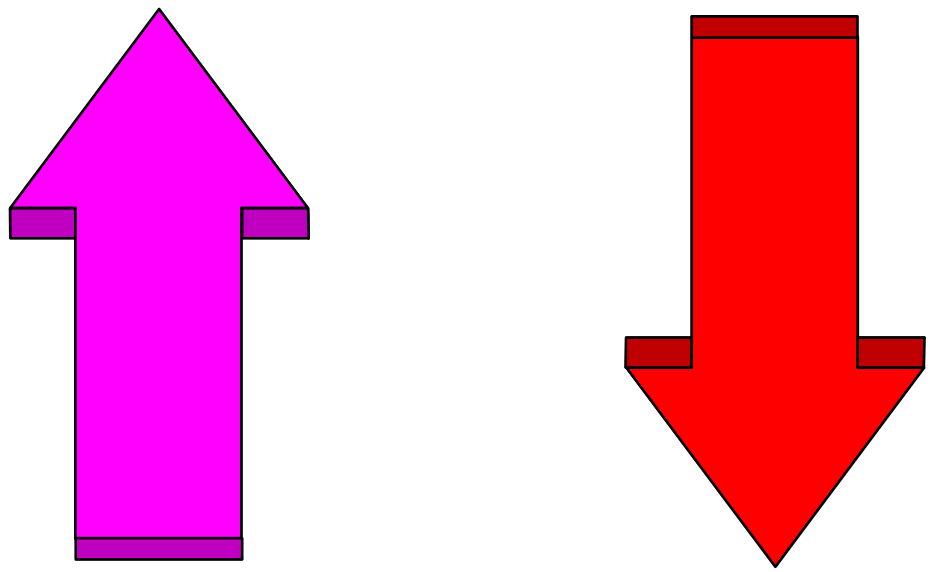
Separation Mechanisms:

- Dispersion
- Strong permanent dipole
- Hydrogen bonding

Selectivity
Interaction Strengths

Phase	Dispersion	Dipole	H Bonding
Methyl	Strong	None	None
Phenyl	Strong	None	Weak
Cyanopropyl	Strong	Strong	Moderate
Trifluoropropyl	Strong	Moderate	Weak
PEG	Strong	Strong	Moderate

Polarity



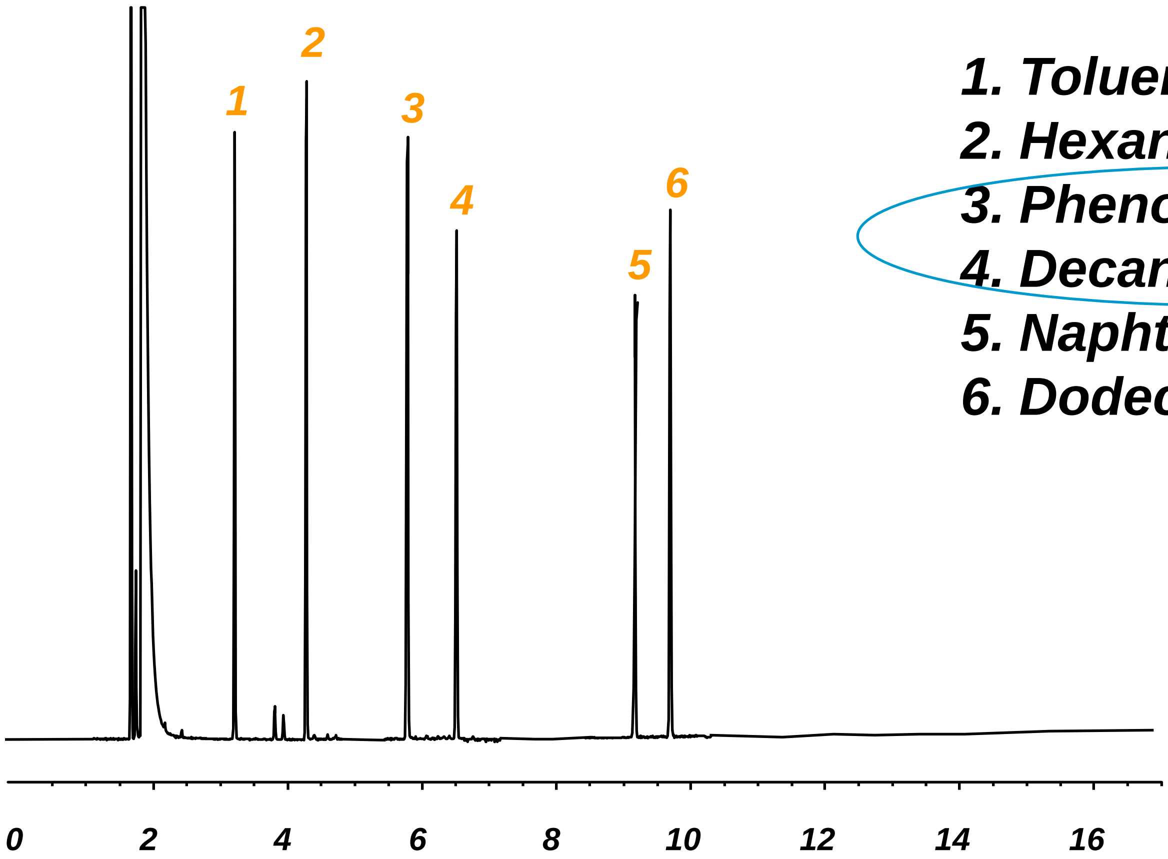
Polarity

Stability
Temperature Range

Compounds Properties

Compounds	Polar	Aromatic	Hydrogen Bonding	Dipole
Toluene	no	yes	no	induced
Hexanol	yes	no	yes	yes
Phenol	yes	yes	yes	yes
Decane	no	no	no	no
Naphthalene	no	yes	no	induced
Dodecane	no	no	no	no

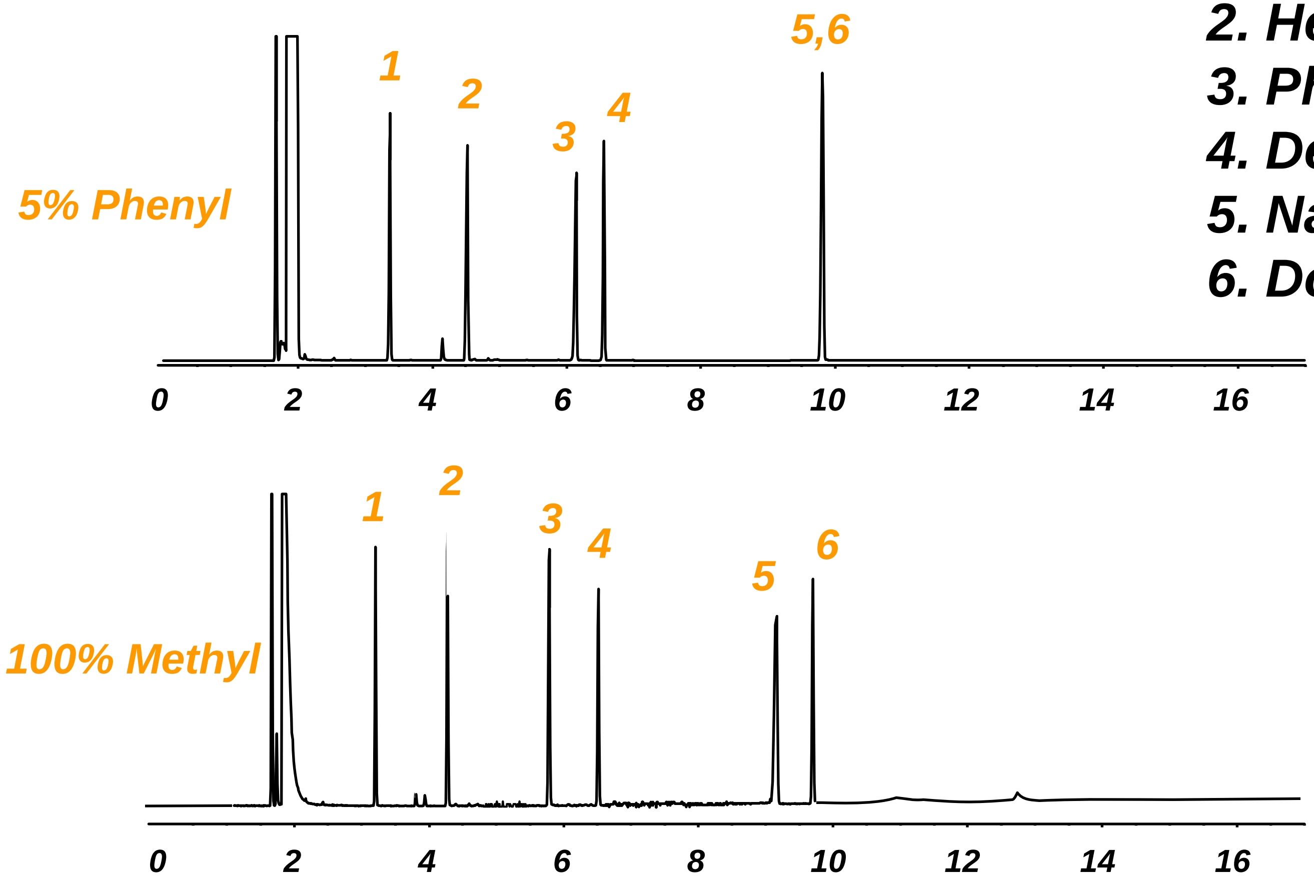
100% Methyl Polysiloxane



1. Toluene 110°C
2. Hexanol 158°C
3. Phenol 181°C
4. Decane (C10) 174°C
5. Naphthalene 218°C
6. Dodecane (C12) 216°C

Strong Dispersion
No Dipole
No H Bonding

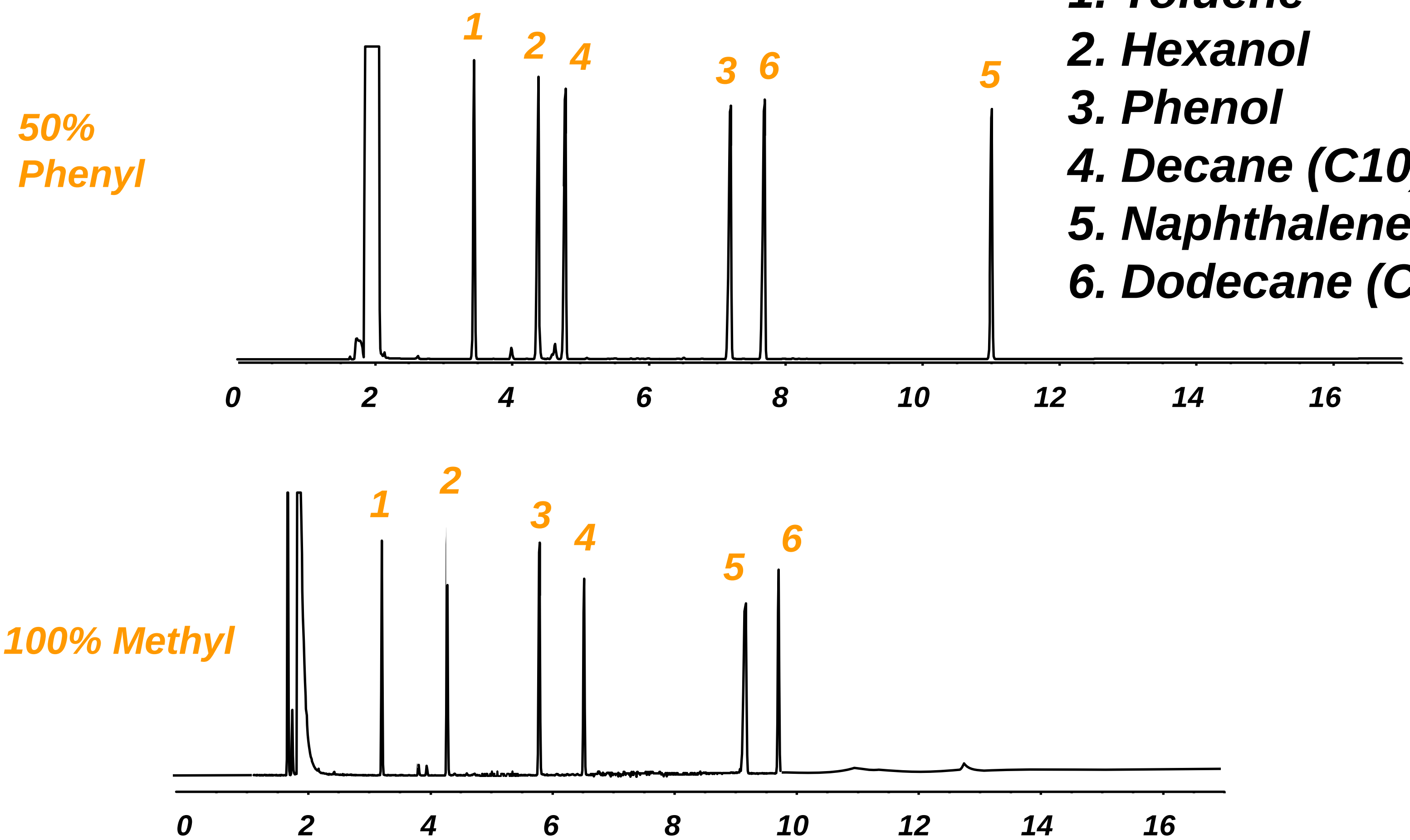
5% Phenyl



1. Toluene 110°C
2. Hexanol 158°C
3. Phenol 181°C
4. Decane (C10) 174°C
5. Naphthalene 218°C
6. Dodecane (C12) 216°C

Strong Dispersion
No Dipole
Weak H Bonding

50% Phenyl

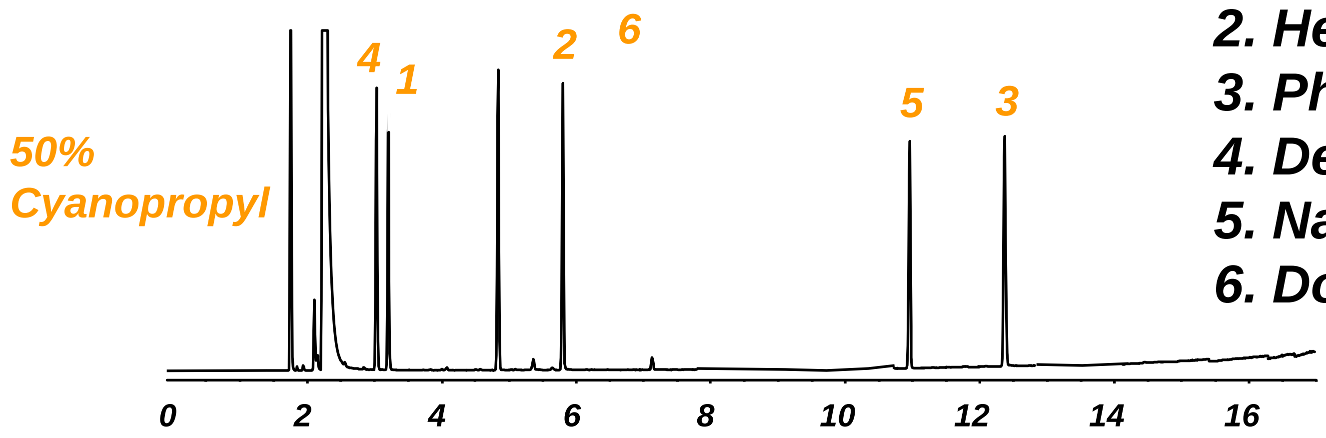


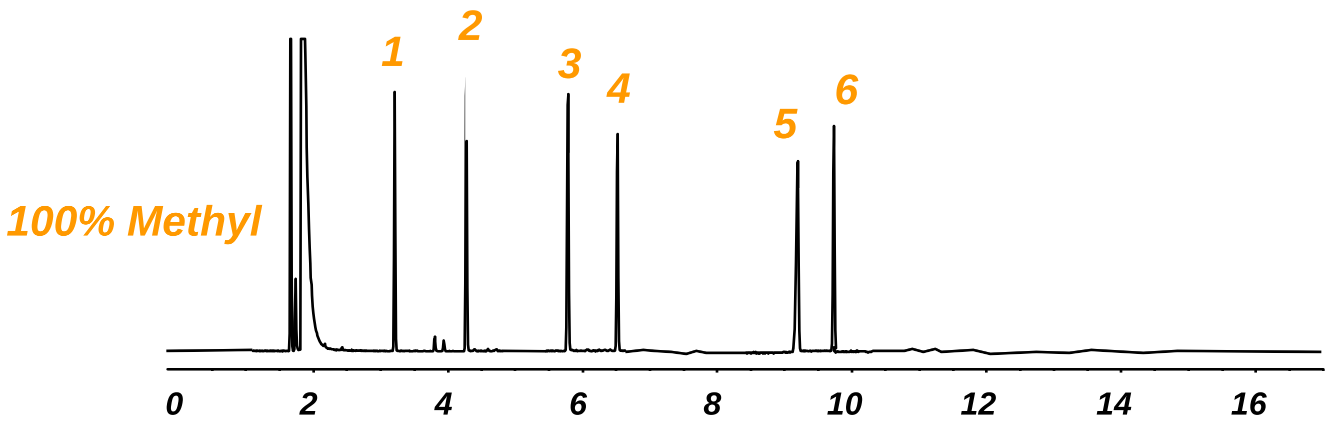
1. Toluene 110°C
2. Hexanol 158°C
3. Phenol 181°C
4. Decane (C10) 174°C
5. Naphthalene 218°C
6. Dodecane (C12) 216°C

Strong Dispersion
No Dipole
Weak H Bonding

50% Cyanopropyl

1. Toluene 110°C
2. Hexanol 158°C
3. Phenol 181°C
4. Decane (C10) 174°C
5. Naphthalene 218°C
6. Dodecane (C12) 216°C

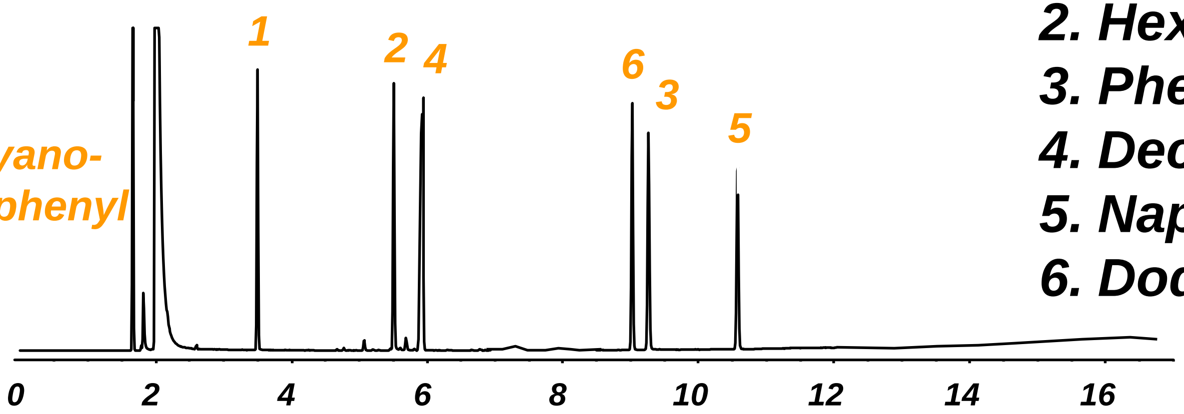




Strong Dispersion
Strong Dipole
Moderate H Bonding

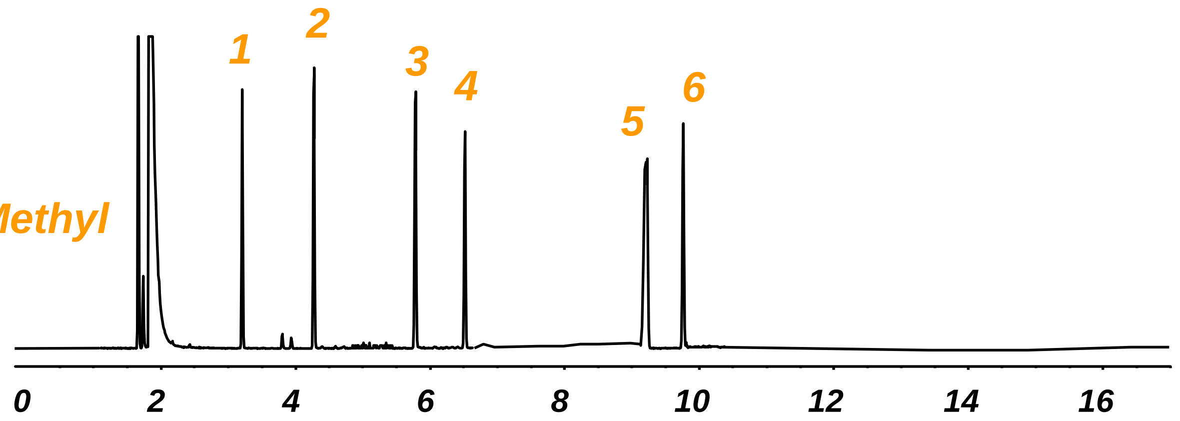
14% Cyanopropylphenyl

14% Cyano-propylphenyl



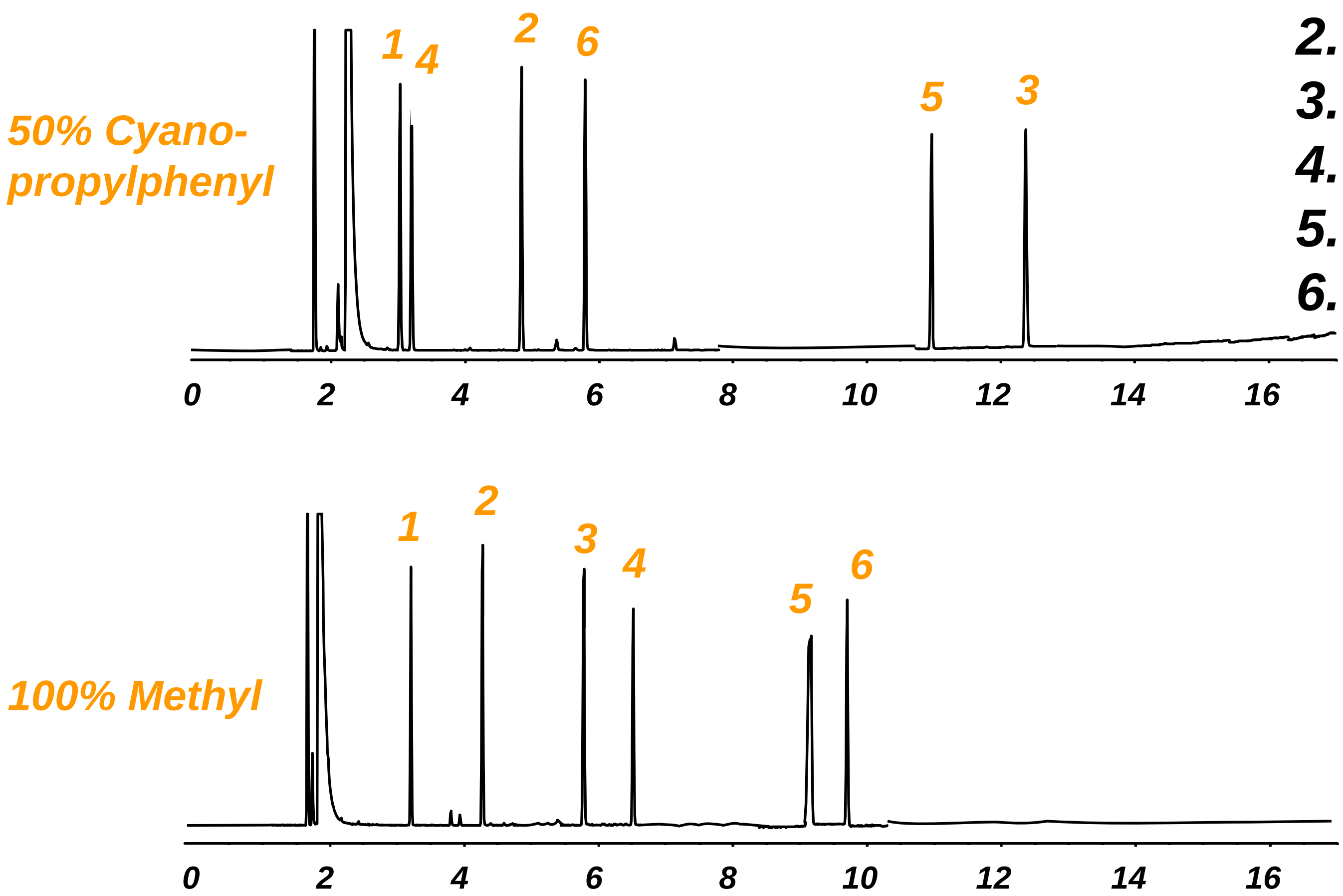
1. Toluene ***110°C***
2. Hexanol ***158°C***
3. Phenol ***181°C***
4. Decane (C10) ***174°C***
5. Naphthalene ***218°C***
6. Dodecane (C12) ***216°C***

100% Methyl



Strong Dispersion
None/Strong Dipole (Ph/CNPr)
Weak/Moderate H Bonding (Ph/CNPr)

50% Cyanopropylphenyl



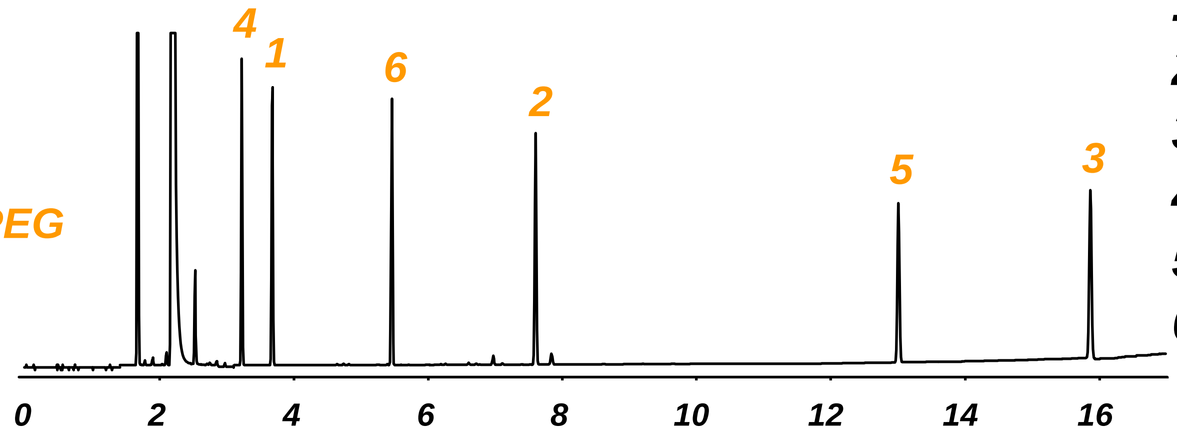
1. Toluene
2. Hexanol
3. Phenol
4. Decane (C10)
5. Naphthalene
6. Dodecane (C12)

Strong Dispersion
None/Strong Dipole (Ph/CNPr)
Weak/Moderate H Bonding (Ph/CNPr)

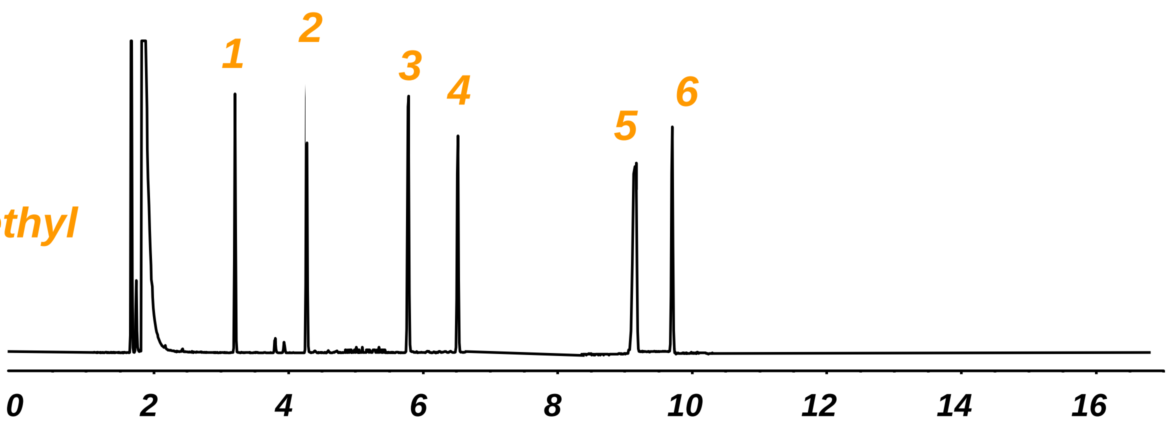
100% Polyethylene Glycol

1. Toluene
2. Hexanol
3. Phenol
4. Decane (C10)
5. Naphthalene
6. Dodecane (C12)

100% PEG



100% Methyl



Strong Dispersion
Strong Dipole
Moderate H Bonding

Stationary Phase Selection
Part 1

- Existing Information
- Selectivity
- Polarity
- Critical Separations
- Temperature Limits

Stationary Phase Selection
Part 2

- Capacity
- Analysis Time
- Bleed
- Versatility
- Selective Detectors

Column Dimensions

- Inner Diameter
- Length
- Film Thickness

Column Diameter
Capillary Columns

I.D. (mm)	Common Name
0.53	Megabore
0.45	High speed Megabore
0.32	Wide
0.20-0.25	Narrow
0.18	Minibore

Column Diameter
Theoretical Efficiency

I.D. (mm)	N/m
0.10	11905
0.18	6666
0.20	5941
0.25	4762
0.32	3717
0.53	2242

k = 5

Efficiency and Resolution

Relationship

$$\sqrt{N} \propto R_s$$

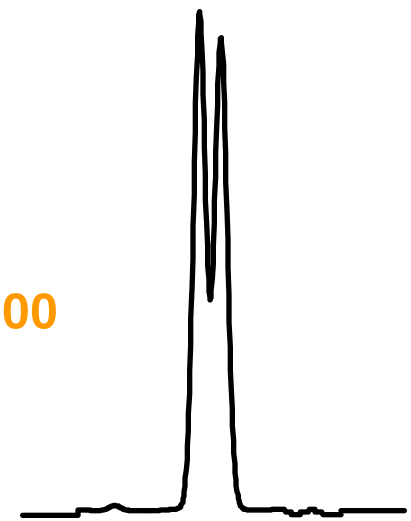
Efficiency X 4 = Resolution X 2

Column Diameter

Resolution
180°C isothermal

R=0.87

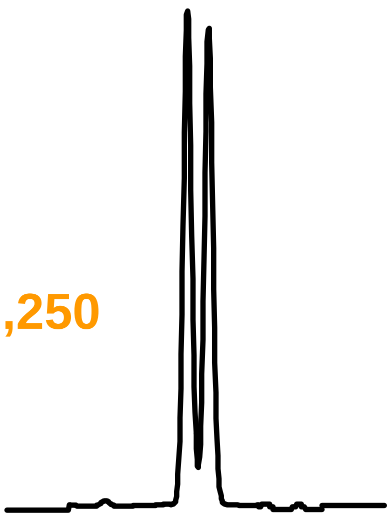
n =58,700



0.53 mm

R=1.01

n =107,250



0.32 mm

Square root of resolution is inversely proportional to column diameter

Column Diameter

Inlet Head Pressures
Helium

I.D (mm)	Pressure (psig)
0.10	225-250
0.20	25-35
0.25	15-25
0.32	10-20
0.53	2-4

30 meters
Hydrogen pressures x 1/2

Column Diameter

Capacity

Like Polarity Phase/Solute

I.D. (mm)	Capacity (ng)
0.20	50-100
0.25	75-150
0.32	125-250
0.53	200-400

0.25 µm film thickness

Column Diameter
Carrier Gas Flow Rate

- Smaller diameters for low flow situations (e.g., GC/MS)
- Larger diameters for high flow situations (e.g., purge & trap, headspace, gas sample valve)

Column Length

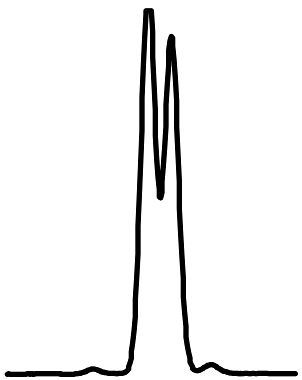
Most common: 15-60 meters

Available: 5-150 meters

Column Length

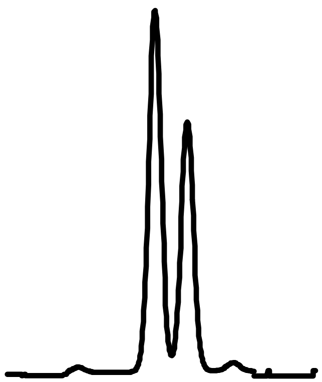
Resolution and Retention
210°C isothermal

R=0.84
2.29 min



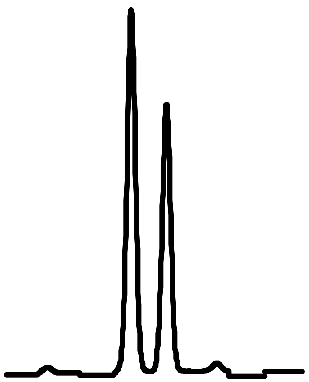
15 m

R=1.16
4.82 min



30 m

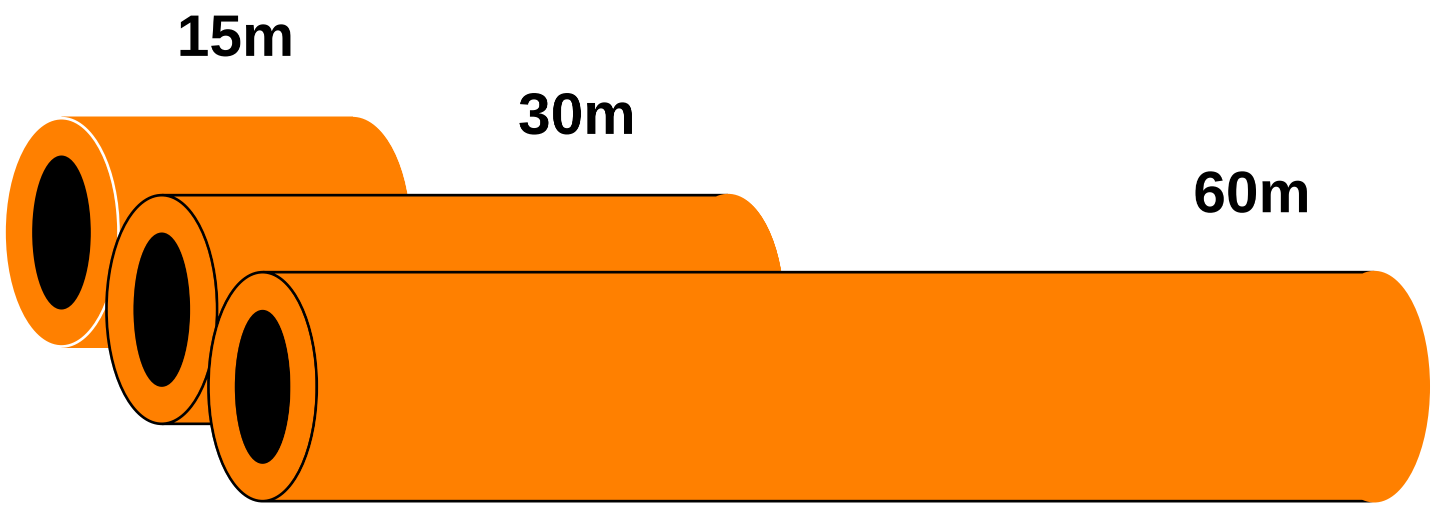
R=1.68
8.73 min

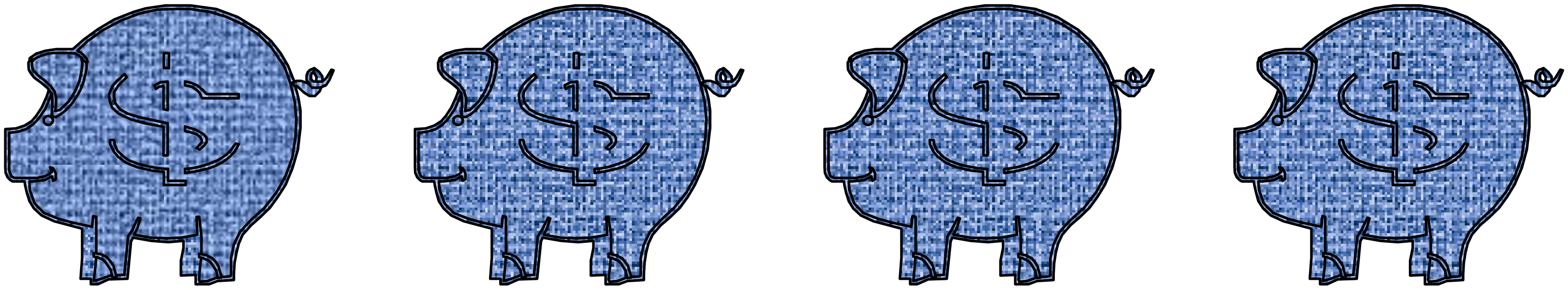


60 m

Resolution is proportional to the square root of column length
Isothermal: Retention is proportional to length
Temperature program: 1/3-1/2 of isothermal values

Column Length
Cost



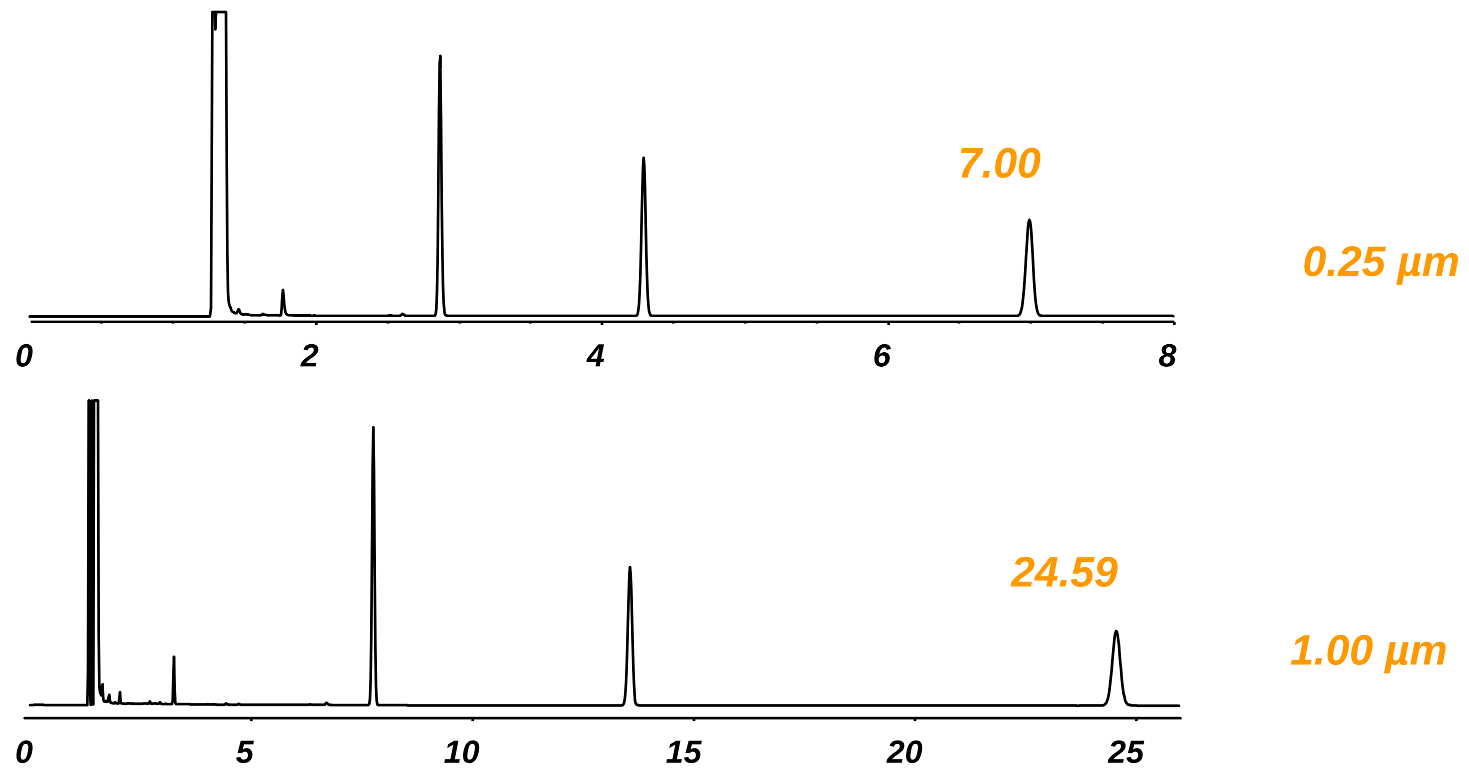


Film Thickness

- Most common: 0.1-3.0 µm
- Available: 0.1-10.0 µm

Film Thickness

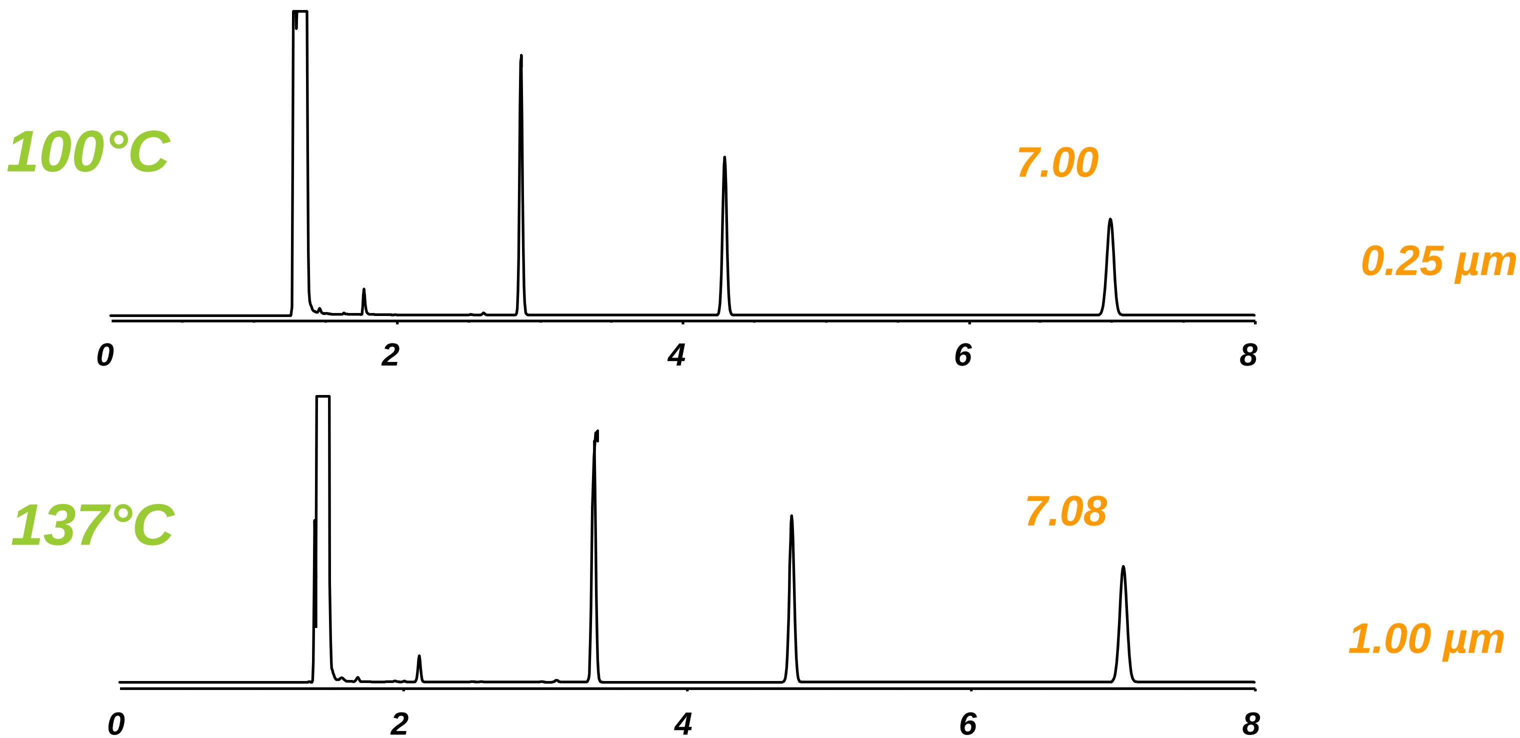
Retention
100°C Isothermal



Isothermal: Retention is proportional to film thickness
Temperature program: 1/3-1/2 of isothermal values

Agilent Technologies

Film Thickness
Equal Retention: Isothermal



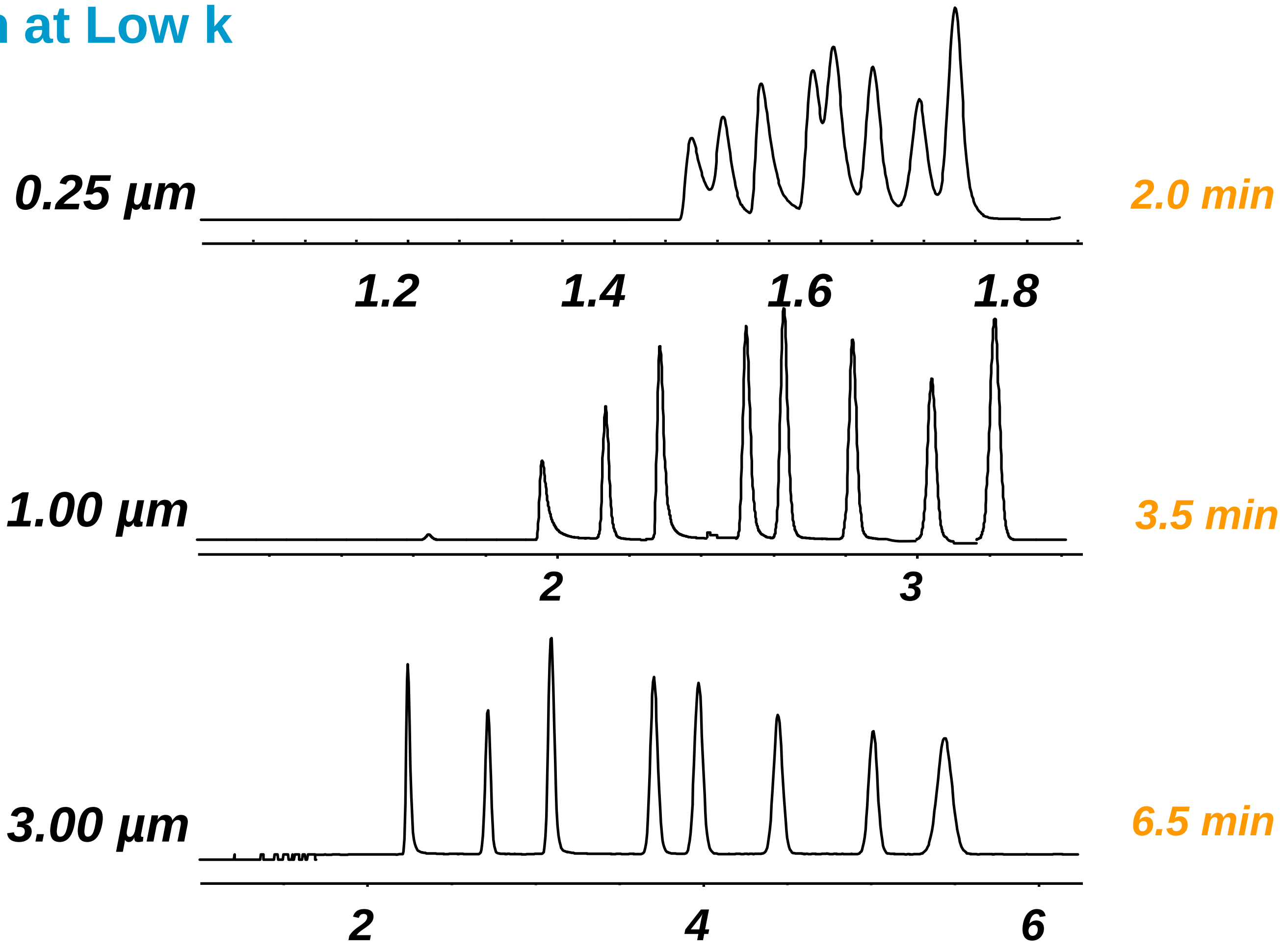
DB-1, 30 m x 0.32 mm ID
He at 37 cm/sec
C10, C11, C12

Film Thickness
Resolution

When solute k < 5 d_f ↑ R ↑

When solute k > 5 d_f ↑ R ↓

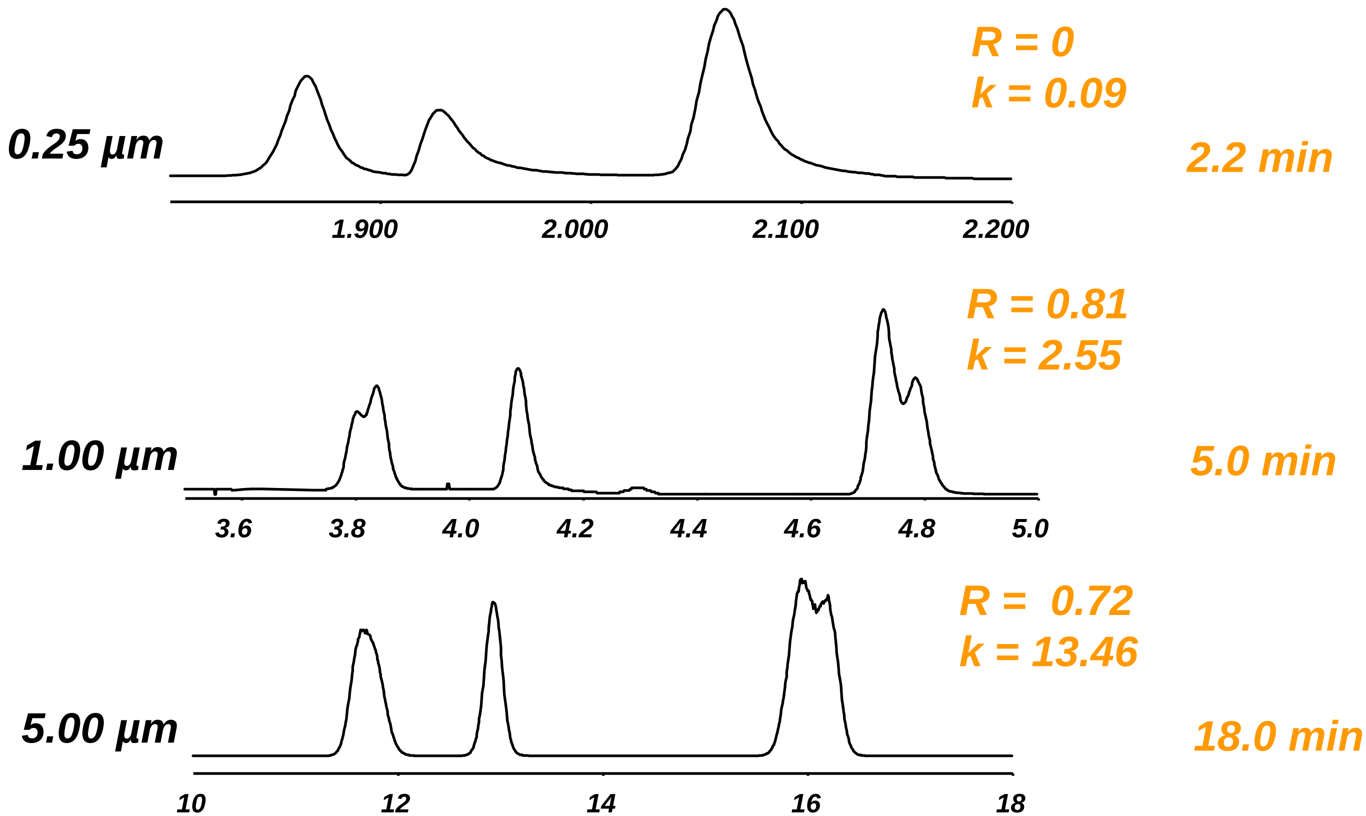
Film Thickness
Resolution at Low k



DB-1, 30 m x 0.32 mm ID
40°C isothermal, He at 35 cm/sec
Solvent mixture

Film Thickness

Resolution at High k



DB-1, 30 m x 0.32 mm ID
40°C isothermal, He at 35 cm/sec
Solvent mixture

Film Thickness

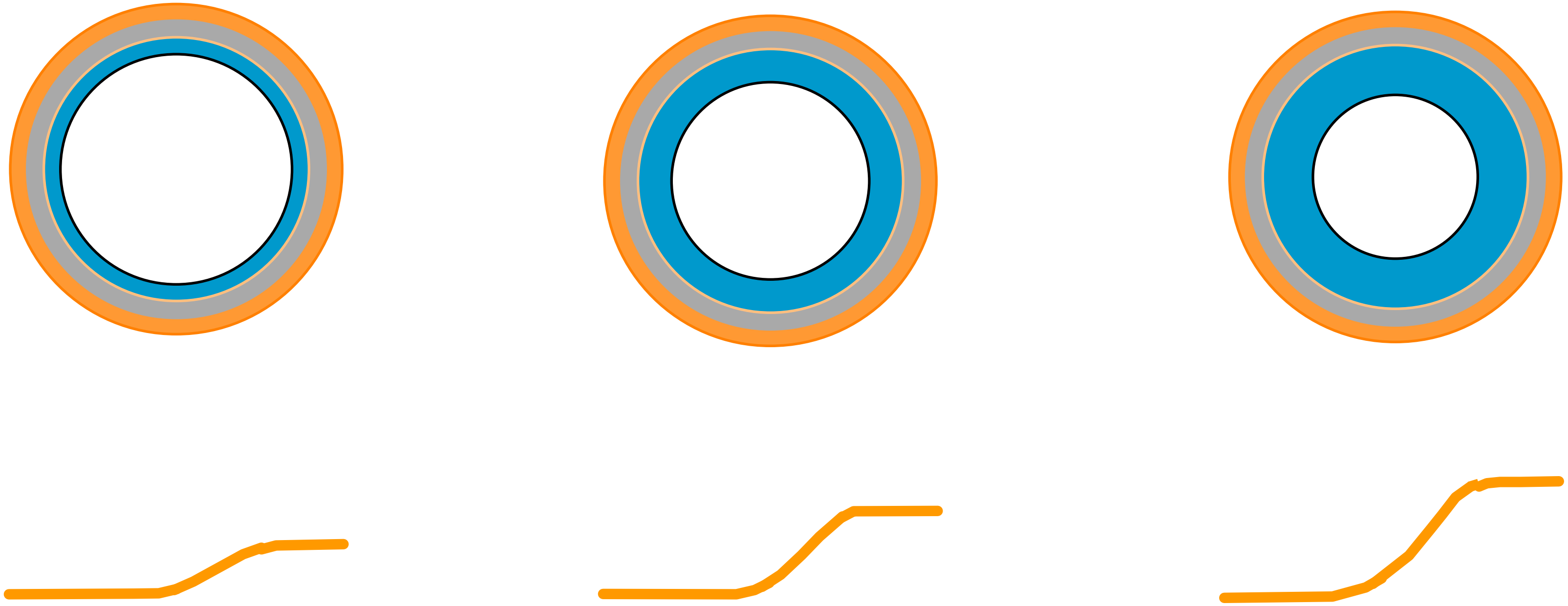
Capacity
Like Polarity Phase/Solute

Thickness (um)	Capacity (ng)
0.10	50-100
0.25	125-250
1.0	500-1000
3.0	1500-3000
5.0	2500-5000

0.32 mm I.D.

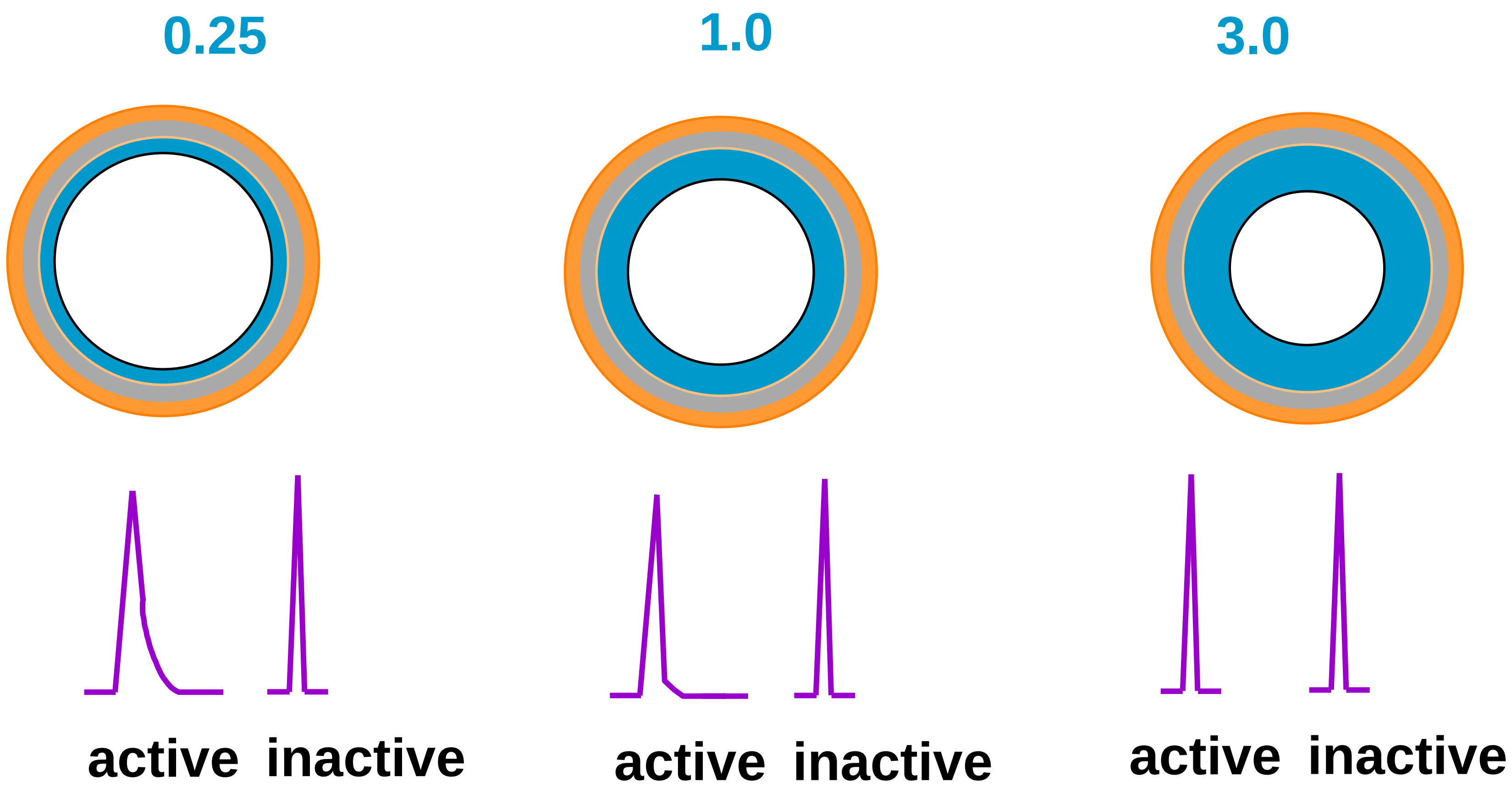
Film Thickness
Bleed

More stationary phase = More degradation products



Film Thickness

Inertness Summary



Column Dimensions
Diameter Summary

To Increase	Make Diameter
Resolution	Smaller
Retention	Smaller
Pressure	Smaller
Flow rate	Larger
Capacity	Larger

Column Dimensions
Length Summary

To Increase	Make Length
Resolution	Longer
Retention	Longer
Pressure	Longer
Cost	Longer

Column Dimensions
Film Thickness Summary

To Increase	Make Film
Retention	Thicker
Resolution (k<5)	Thicker
Resolution (k>5)	Thinner
Capacity	Thicker
Inertness	Thicker
Bleed	Thicker

Agilent/J&W Technical Support

800-227-9770 (phone: US & Canada)*

- *Select option 3, then option 3, then option 1.*
- **866-422-5571 (fax)**

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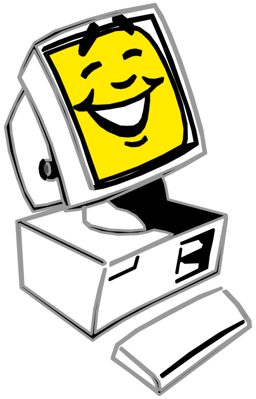

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