Understanding GC: What is Really Going on Inside the Box

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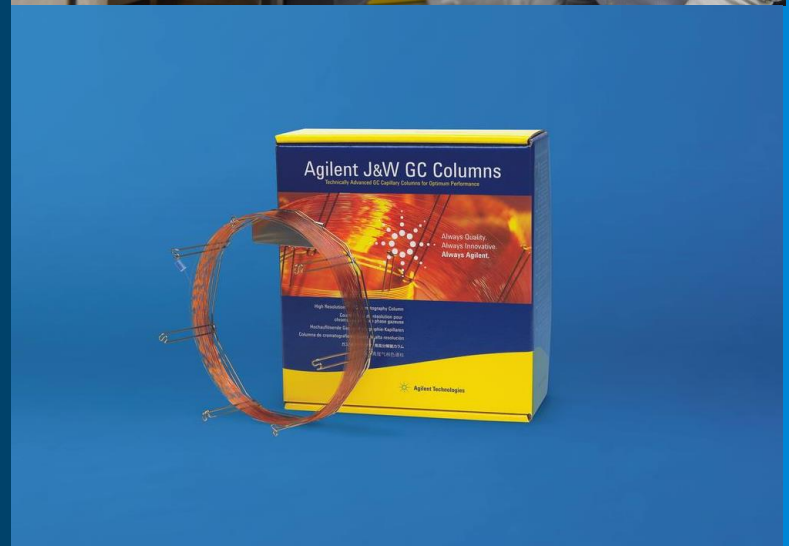
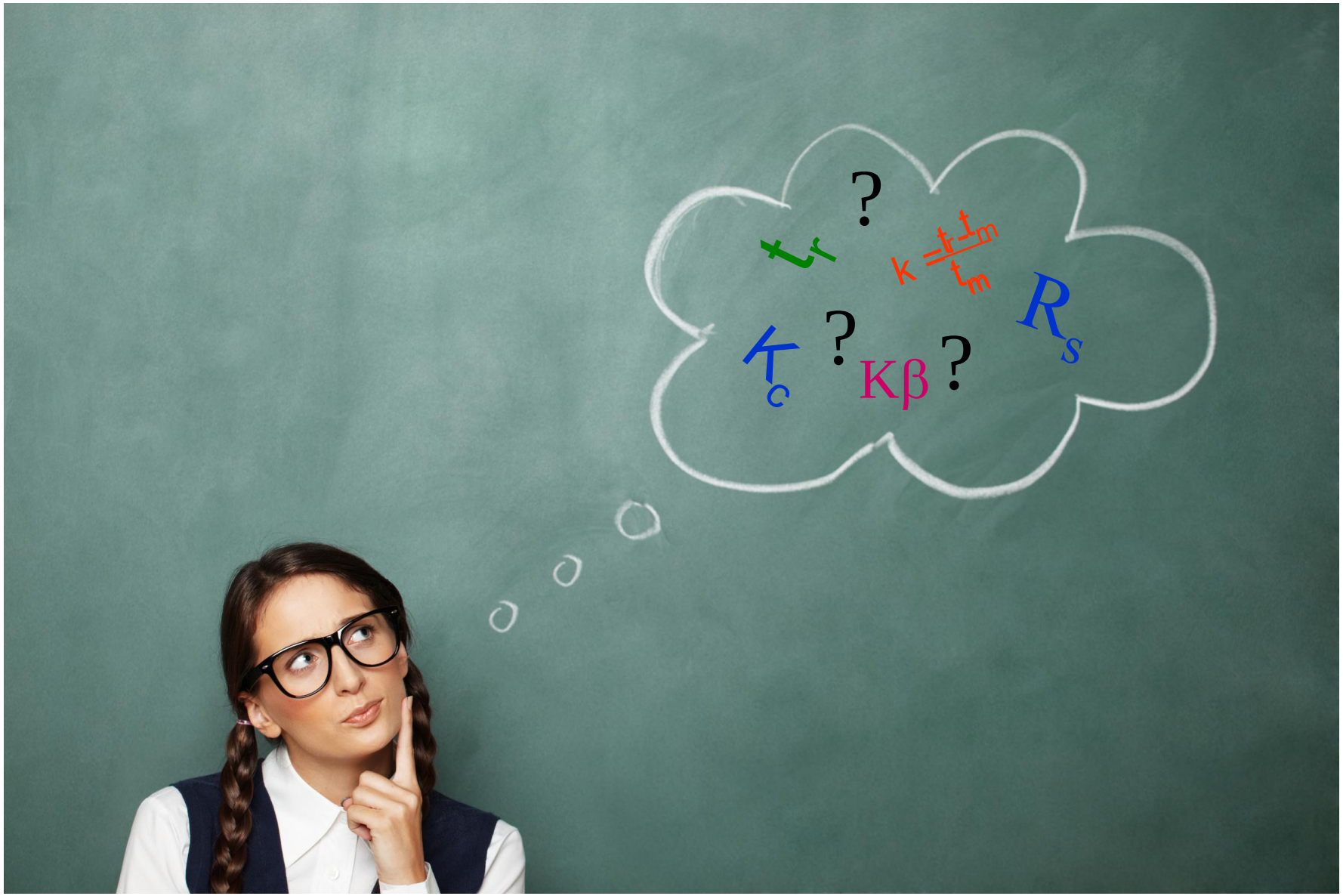


Agilent
Trusted Answers

Introduction to Capillary GC



Compound Requirements for GC

Only 10 to 20% of all compounds are suitable for GC analysis

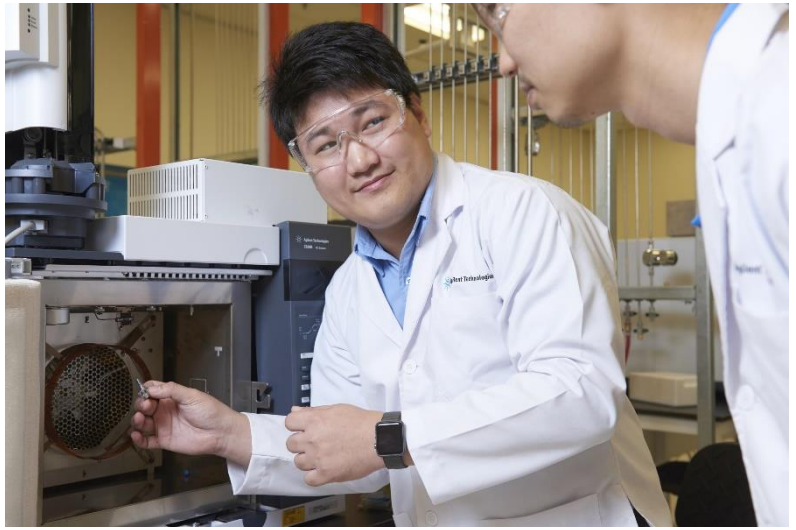
The compounds must have:

- Sufficient volatility
- Thermal stability

No inorganic acids and bases

Be mindful of salts or other non-volatiles (polymers)

Typical GC System



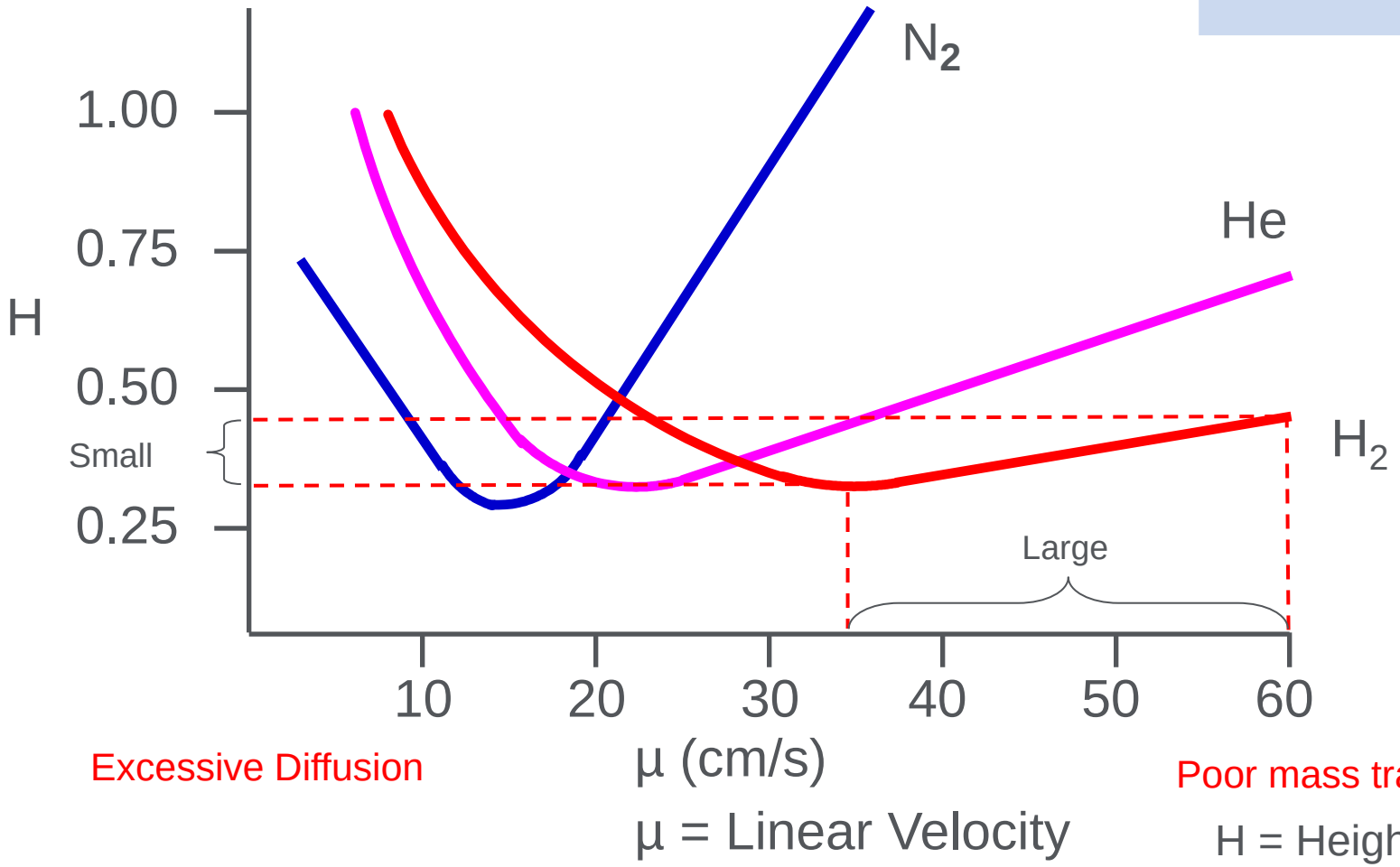
Carrier Gas

- Carries the solutes down the column
- Selection and velocity influences efficiency and retention time

Van Deemter Curves

Type	Velocity Range (u_{opt} – OPGV*)
Nitrogen	8-16
Helium	20-40
Hydrogen	30-55

*OPGV = Optimum practical gas velocity



μ = Linear Velocity

H = Height equivalent of theoretical plate

Sample Introduction

Purpose: To introduce a **representative** portion of sample onto the column in a **reproducible** manner, while **minimizing sample bandwidth.**

Manual Syringe injection

Autosampler injection

Valve injection

- **Gas sampling valve**
- **Liquid sampling valves**

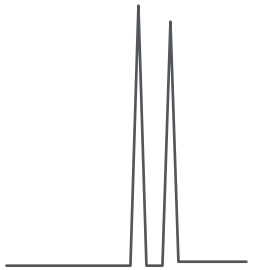
Objective: The sample must not be chemically altered, unless desired (for example, derivatization). Success is not contamination, degradation, or discrimination.

Inlet Choices

Inlet	Column	Mode	Sample Concentration	Comments	Sample to Column
Split / Splitless	Capillary	Split Purged Split Splitless Purged Splitless	High High Low Low	Most commonly used inlet. Very Flexible	Very Little Very Little All All
Multi-Mode	Capillary	Split Pulsed Split Splitless Pulsed Splitless Solvent Vent	High High Low Low Low	Flexibility of standard S/SL inlet and PTV	Very Little Very Little All All Most
Cool-On-Column	Capillary	N/A	Low or labile	Minimal discrimination and decompositoin	All
Packed	Packed Large Capillary	N/A N/A	Any Any	OK if resolution is not critical	All All
Programmed Temperature Vaporizaton	Capillary	Split Pulsed Split Splitless Pulsed Splitless Solvent Vent	High High Low Low Low	Not great for HOT injections. Can concentrate analytes and vent solvent	Very Little Very Little All All Most
Volatiles Interface	Capillary	Direct Split Splitless	Low High Low	Purge & Trap / Headspace	All Very Little All

Influence of Injection Efficiency

Short concentrated



Solute bands

Long diffuse

Same column, same chromatographic conditions

Split Injection
Major variables

Split ratio – determines the fraction of sample on-column and efficiency of injection (sensitivity versus peak width)

Liner – influences efficiency of vaporization/discrimination

Temperature – hot enough to vaporize sample without degradation or causing backflash

Injection volume – typically 0.2–2 µL, increasing it does not have as much of an effect as one might think (smaller is usually always better provided you can meet RSD requirements)

Split Injection

Split ratios

- Too low
  - Poor peak shape
  - Column overload
  - Inlet shut down*
- Too high
  - Poor sensitivity
  - Wastes carrier gas (use gas saver!)
- Responses are usually nonlinear when comparing different split ratios
  - Cannot use split ratio as a “dilution factor”

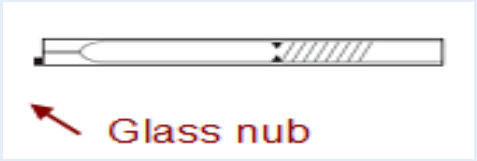
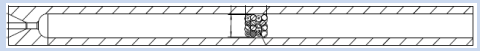
Higher flow rates

Id (mm)	Lowest ratio*
0.10	1:50 - 1:75
0.18 - 0.25	1:10 - 1:20
0.32	1:8 - 1:15
0.53	1:2 - 1:5

*keep total inlet flow ≥ ~20 ml/min to prevent inlet shut-down

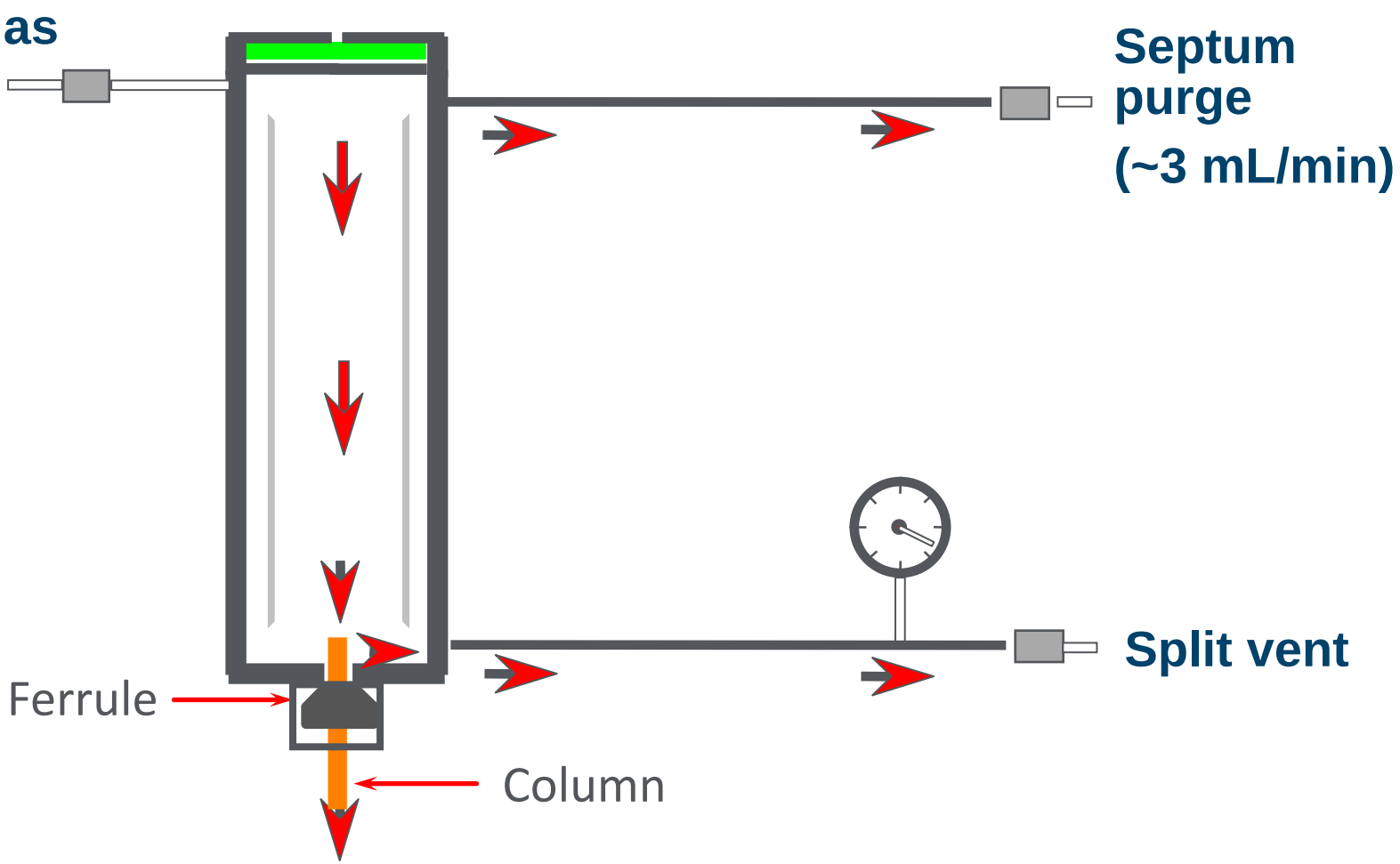
Inlet

Liners – split injection

Liner	Part Number Each 5/pk 25/pk	Comments
	5190-2294 (EA) 5190-3164 (5 pk) 5190-3168 (25 pk)	Simplest split liner, glass wool, UI deactivation, large volume (990 µL). Use for general purpose, can be used in splitless mode
	5190-2295 (EA) 5190-3165 (5 pk) 5190-3169 (25 pk)	Glass wool, UI deactivation, 870 µL volume. Glass nub ensures that a gap remains below liner for split injection. Efficient for most applications
	5190-5105 5190-5105-005 5190-5105-025	Sintered glass frit, UI deactivation. Ideal for basic drugs analysis. Sintered glass frit more reproducible than glass wool.
	18740-80190	Liner with Jennings cup, no wool. 800 µL volume. Reduces inlet discrimination.

Split Injection

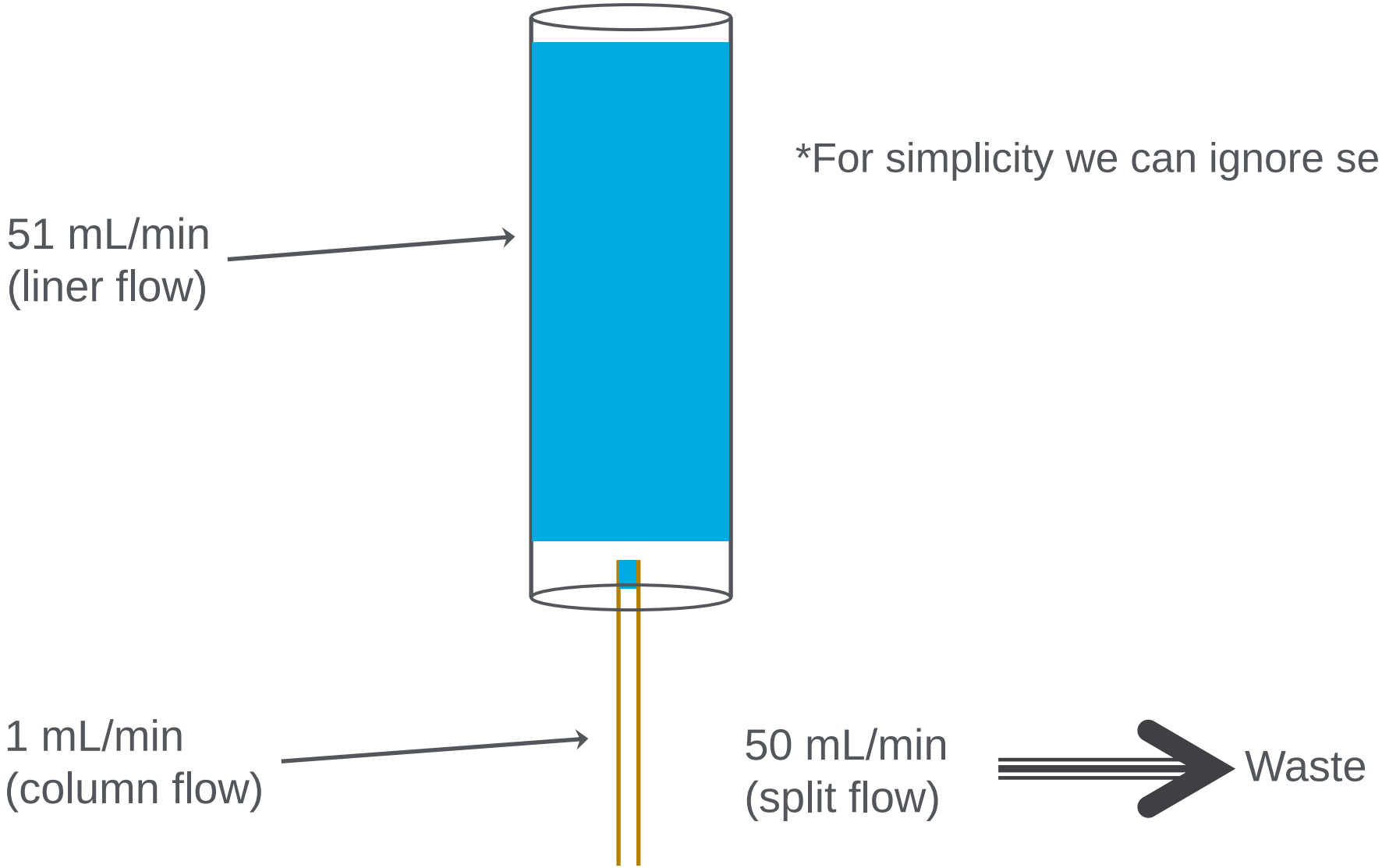
3 Flow paths



Flow through injector = Column flow + split vent flow

Split Injection
Animation

50:1 split ratio



*For simplicity we can ignore septum purge

Splitless Injection

More challenging the SPLIT

Most of the sample is introduced into the column

Used for low concentration samples

Poor injection efficiency = wider peaks = less resolution

Sample refocusing may be necessary

Splitless Injection

For trace level analysis

- Use split/splitless injection port in the splitless mode (split vent closed during injection; opened later)
- Sample is injected, the sample is volatilized, and most of the analytes and solvent are introduced to the column
- Later, the split vent is opened, and residual solvent is vented out (purge time/flow)
- Timing, carrier/split vent flows, and the oven temperature program are important
- Sample has longer residence time in the heated inlet giving more opportunity to vaporize high boiling sample components compared to split injection, so wool is less critocal
- Typical splitless parameters:
  - Purge flow of 50 mL/min
  - Purge time of 0.5–2.0 minutes

Splitless Injection
Major variables

Purge activation time – determines amount of sample onto column and efficiency of injection (sensitivity versus peak shape)

Liner – preventing backflash more critical than vaporization properties (wool is less important…)

Injection volume – typically 1 µL or less (backflash: 0.5 µL max for water!)

Temperature – long residence times allow for lower temperatures

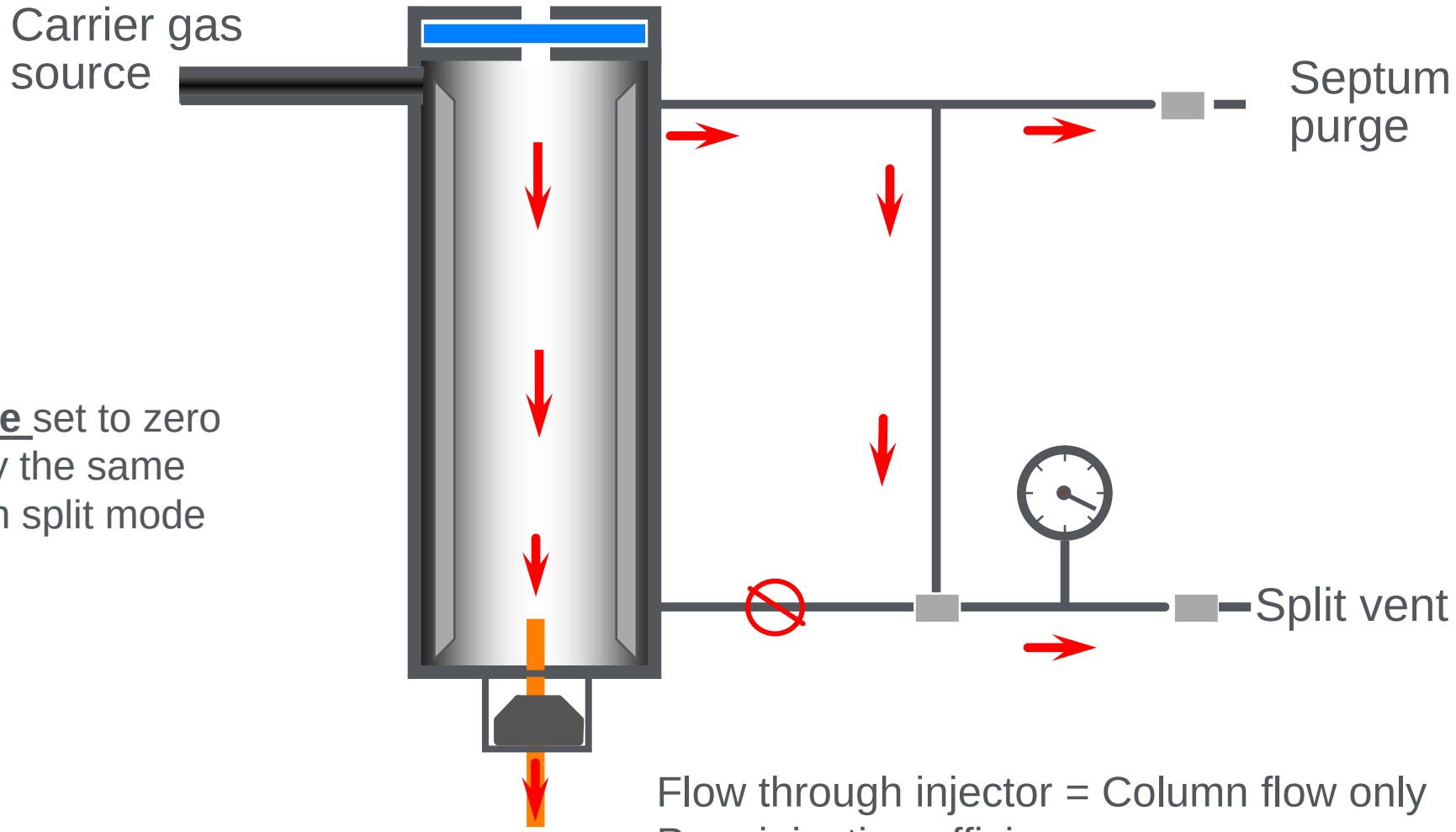
Inlet

Liners – splitless injection

Liner	Part Number Each 5/pk 25/pk	Comments
	5190-2292 (EA) 5190-3162 (5 pk) 5190-3166 (25 pk)	Single taper, UI deactivated, 900 µL volume. Taper isolates sample form gold seal, reducing breakdown of active compounds. Trace samples, general applications.
	5190-2293 (EA) 5190-3163 (5 pk) 5190-3167 (25 pk)	Single taper, UI deactivated, glass wool, 900 µL volume. Glass wool aides with volatilization of heavier compounds and protects the column. Trace, dirty samples.
	5190-5112 5190-5112-005 190-5112-025	Singer taper, UI deactivated, sintered glass frit. Glass frit acts like glass wool but is more reproducible.
	5190-3983 (EA) 5190-4007 (5 pk)	Double taper, UI deactivated, 800 µL volume. Taper on inlet reduces backflash. High efficiency for trace, active samples.
	5190-7011 (5/pk) 5190-7012 (5/pk) 5190-7013 (5/pk) 5190-7014 (5/pk) 5190-7020 (5/pk)	Direct Connect liners, single and dual taper, original deactivation. Column press fits into liner. Focuses almost all sample onto column and reduces exposure to inlet. Ultimate for trace, active samples. Various hole placements for use with EPC

Splitless Injection

Purge off at injection

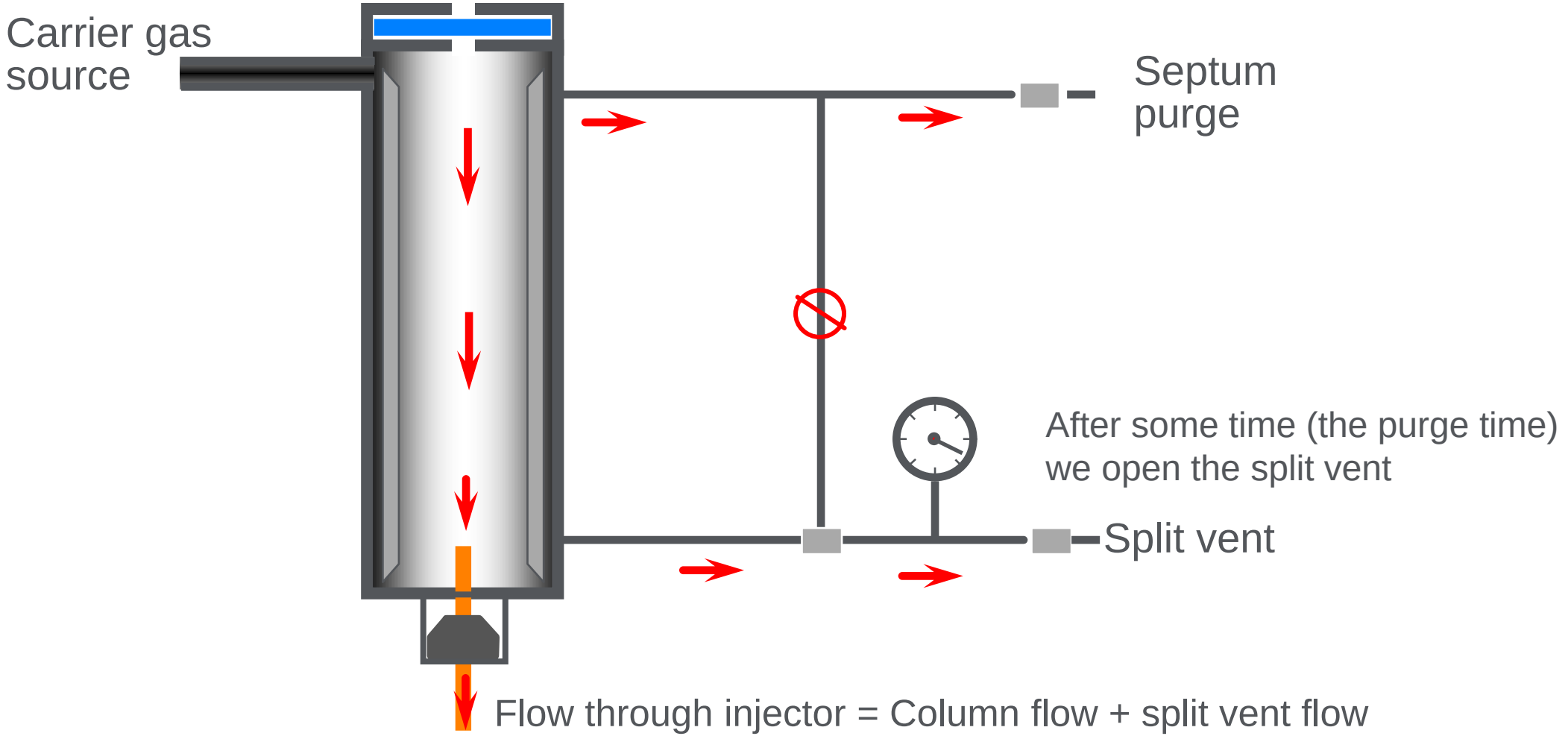


A **purge time** set to zero is essentially the same as running in split mode

Flow through injector = Column flow only
Poor injection efficiency

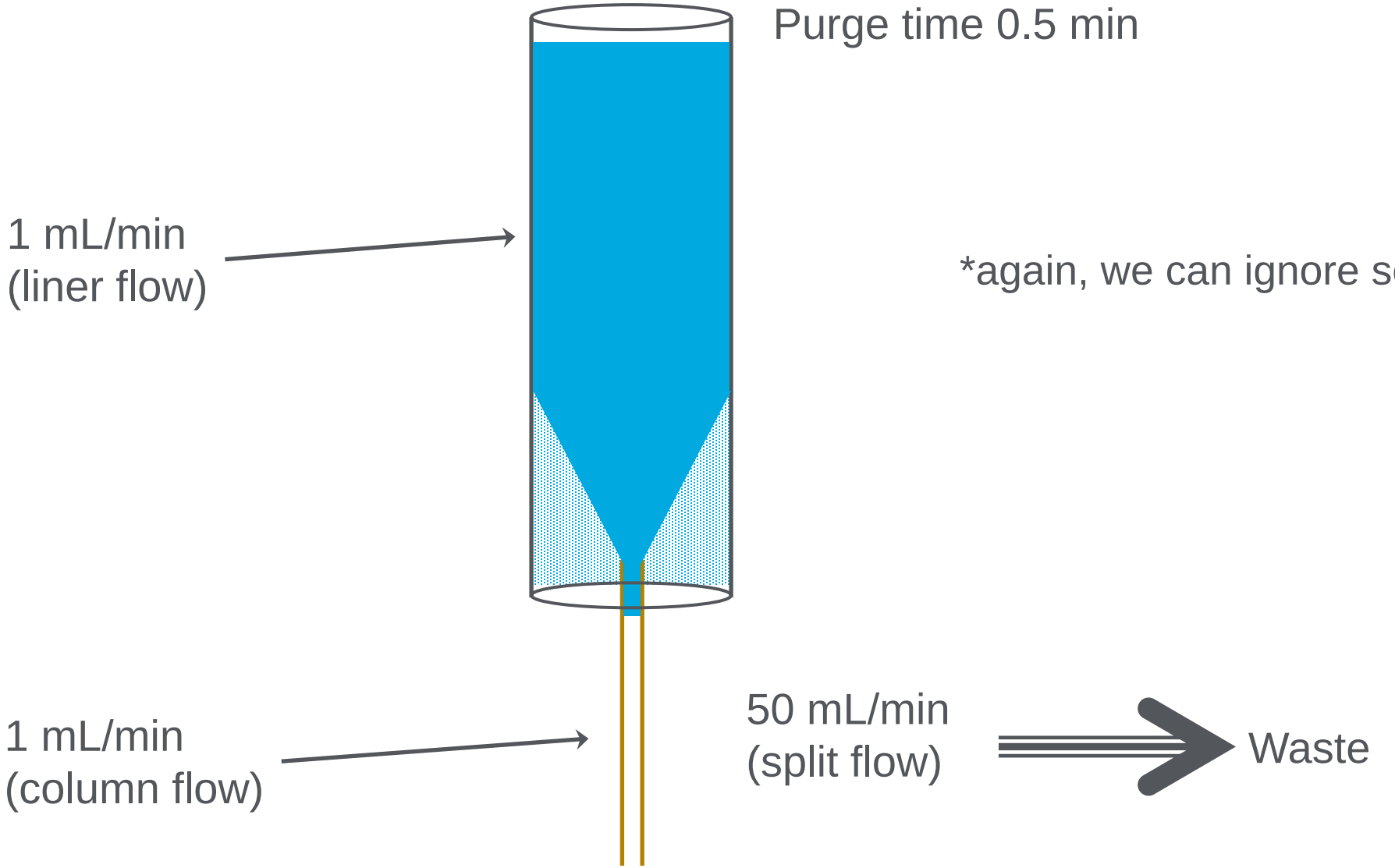
Splitless Injection

Purge on after injection



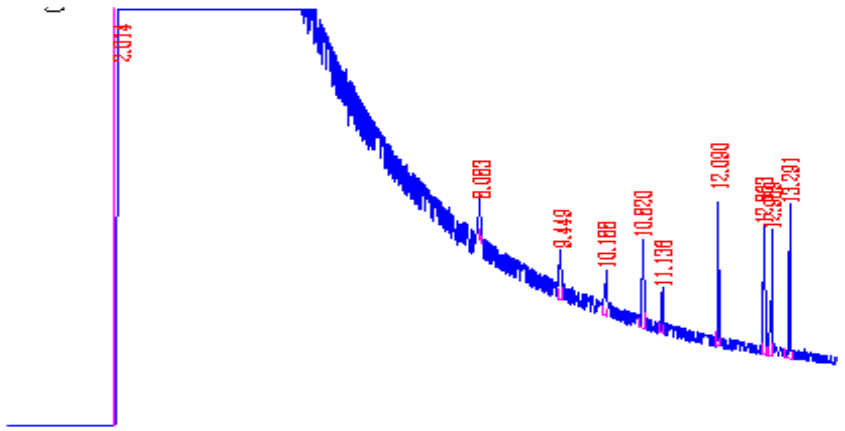
Splitless Injection
Animation

Purge flow 50 mL/min
Purge time 0.5 min

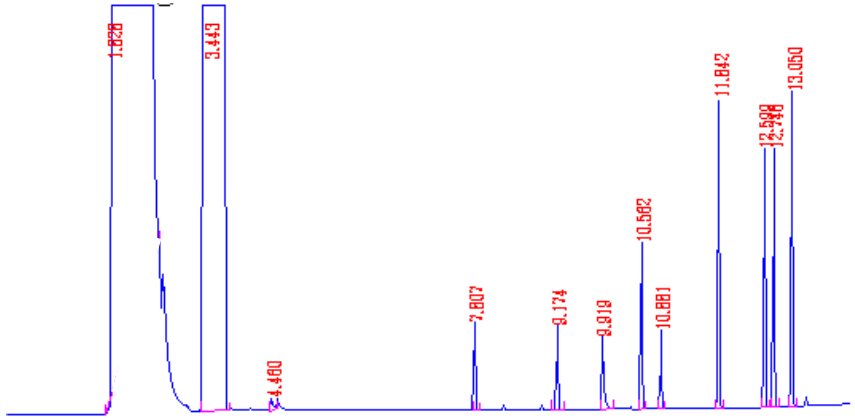


*again, we can ignore septum purge

Splitless Injections – Splitless Time (Purge Time)



Purge time too long results in large solvent tail



0.75 min purge time clips solvent tail

Splitless: Sample Re-focusing and the “Solvent Effect”

- Splitless injections are inherently inefficient
- Sample refocusing
  - Also known as the “solvent effect”
  - Condenses sample as a thin film on the head of the column
  - Initial oven temperature must be at least 10 °C below the solvent B.P.
  - Increases separation efficiency and resolution and better peak shape
    - Especially for low boiling analytes

- “Cold trapping” is a version of sample re-focusing for high boiling analytes
  - Occurs when the starting oven temperature is ~150 °C below the boiling point of analytes of interest
  - Condenses the analytes on the head of the column
  - Results in better peak shapes

- Solvent effect and cold trapping can occur in same sample
  - When looking at analytes with a wide distribution of B.P.s

Splitless Injector: Solvent Effect

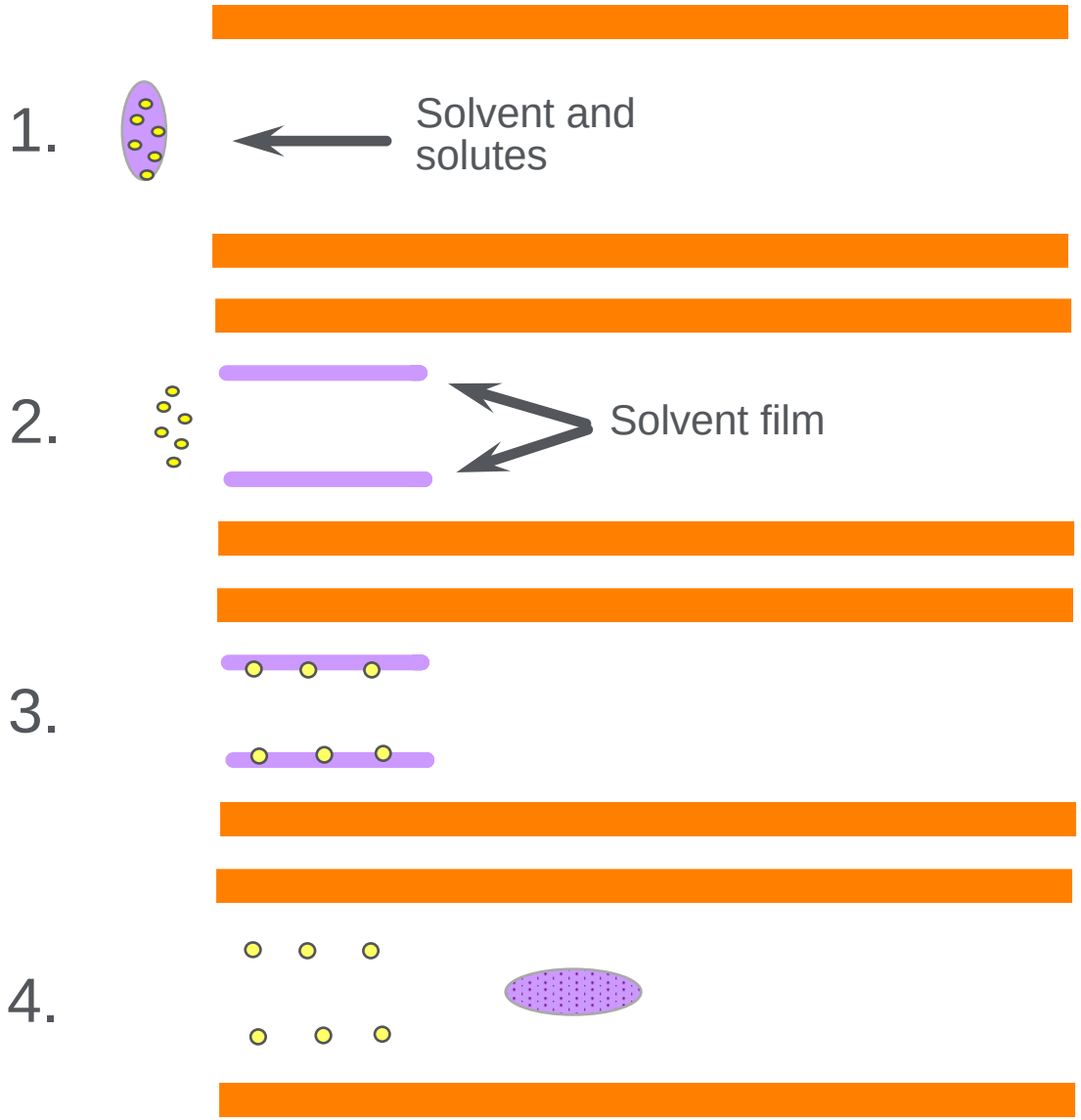
Initial column temperature at least ***10°C below*** sample solvent boiling point

Solvent should be the 1st eluting peak and best if it has similar polarity to that of the stationary phase

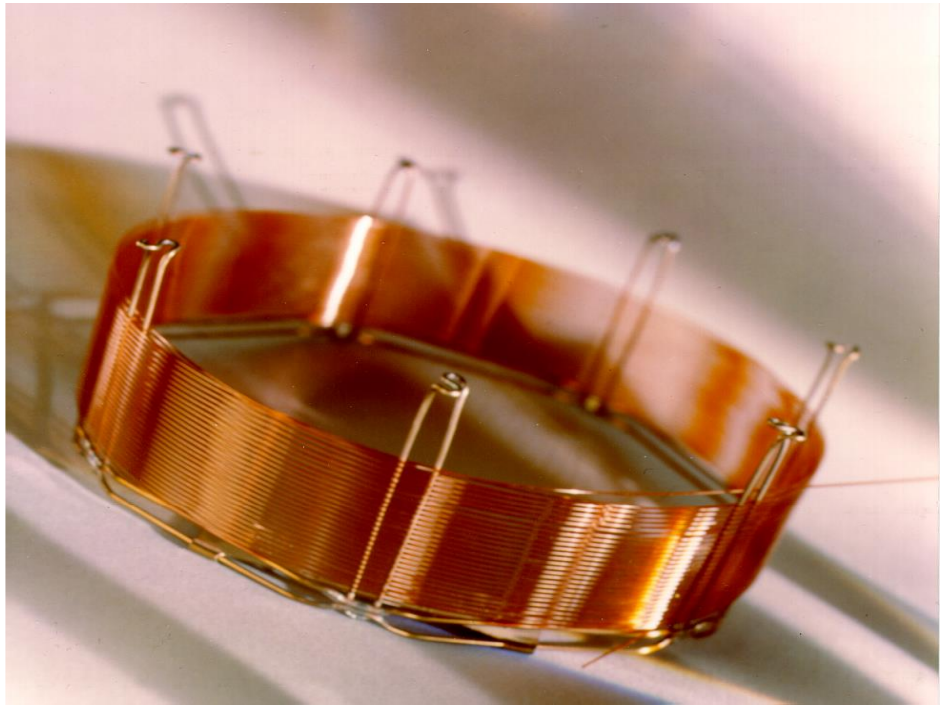
Required to obtain good peak shapes unless cold trapping occurs (large Δ in BP)

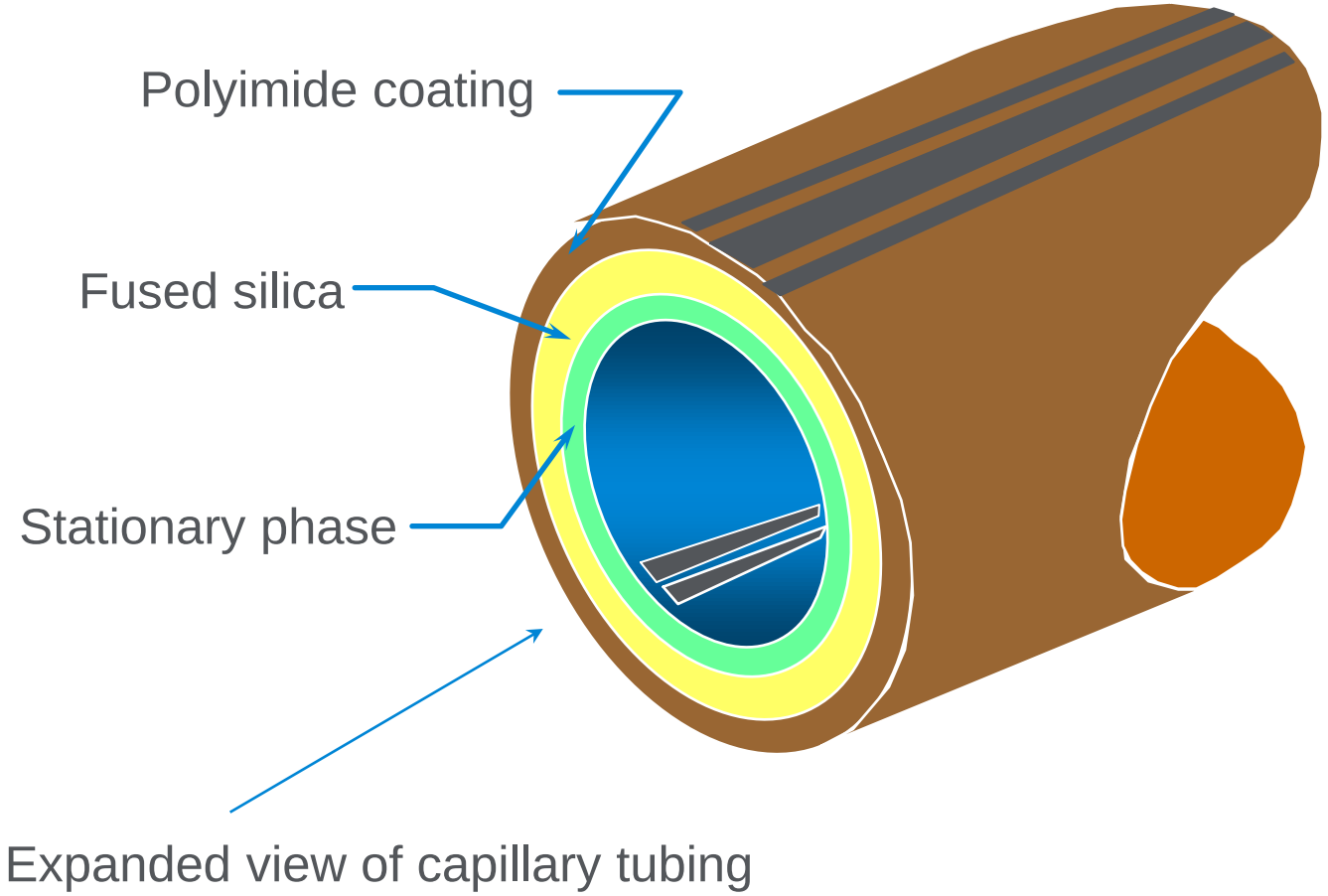
Rule of thumb, if solute BP >150°C above initial column temperature, the solute will cold trap

Cold trapping has greater efficiency than solvent effect

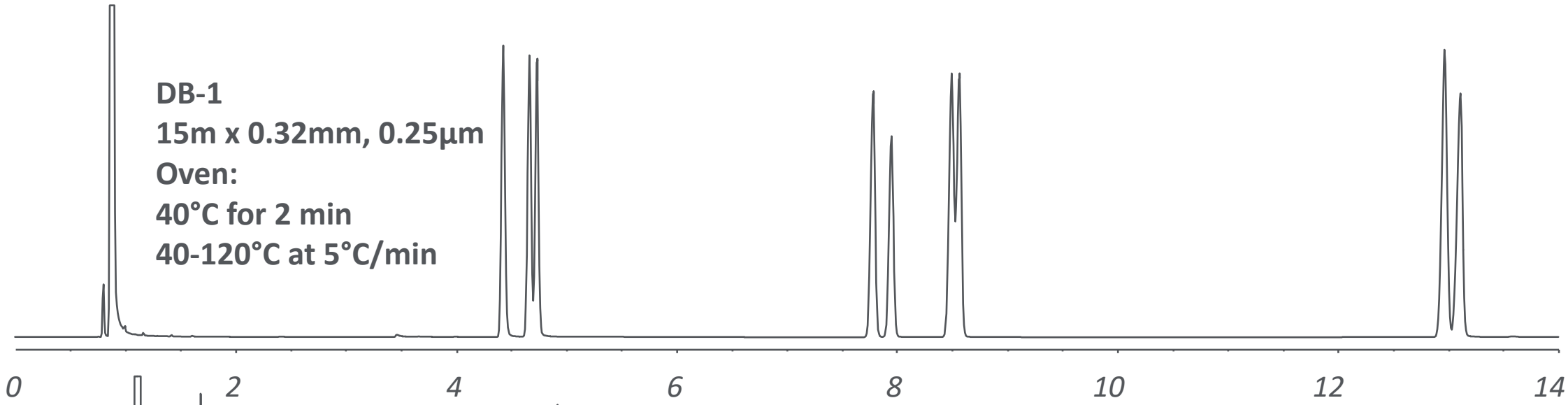


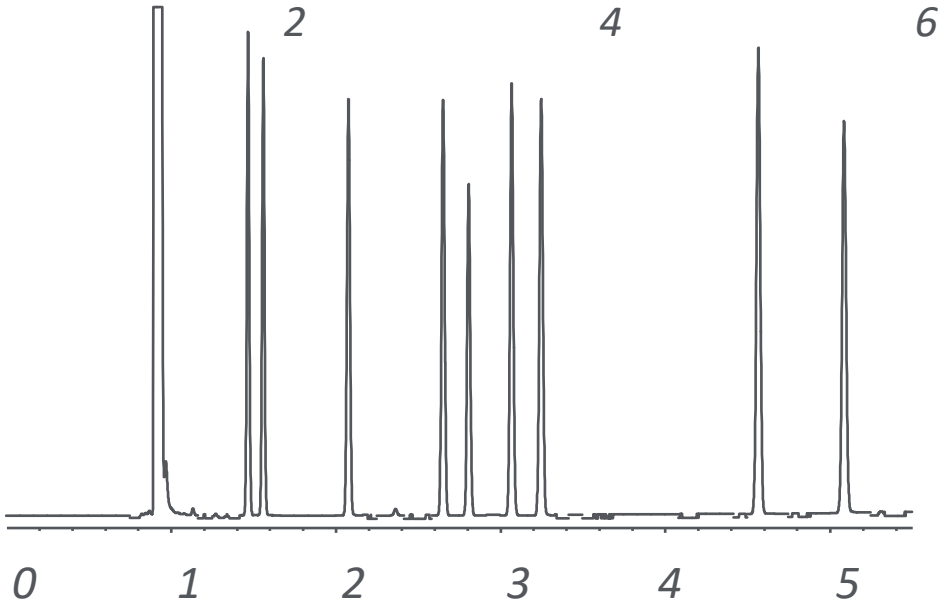
Typical Capillary Column





Start with the Right Phase – “like dissolves like”



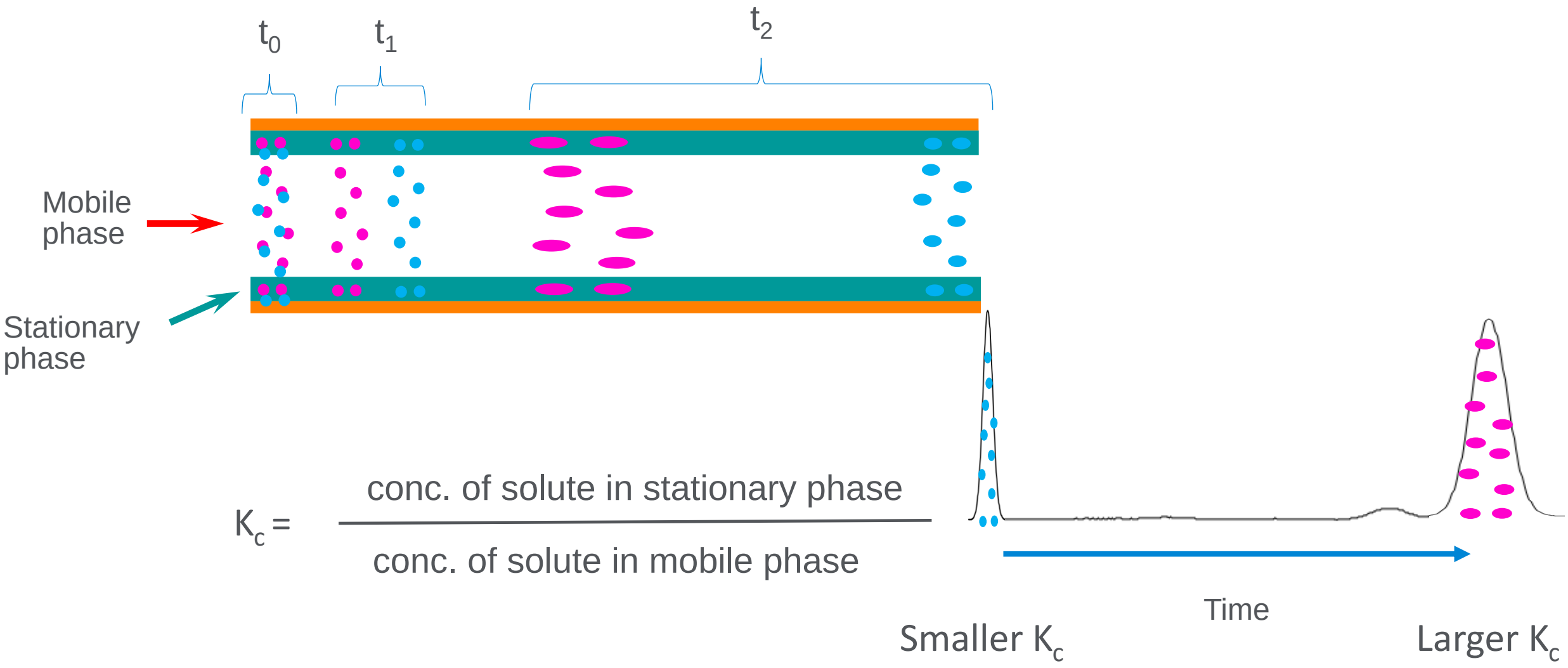


DB-Wax
15m, 0.32mm, 0.25µm
Oven:
80-190°C at 20°C/min

Two Phases

Solute molecules distribute into the two phases
based on their degree of solubility/distribution in the stationary phase (K_c)

Distribution Constant (K_C)



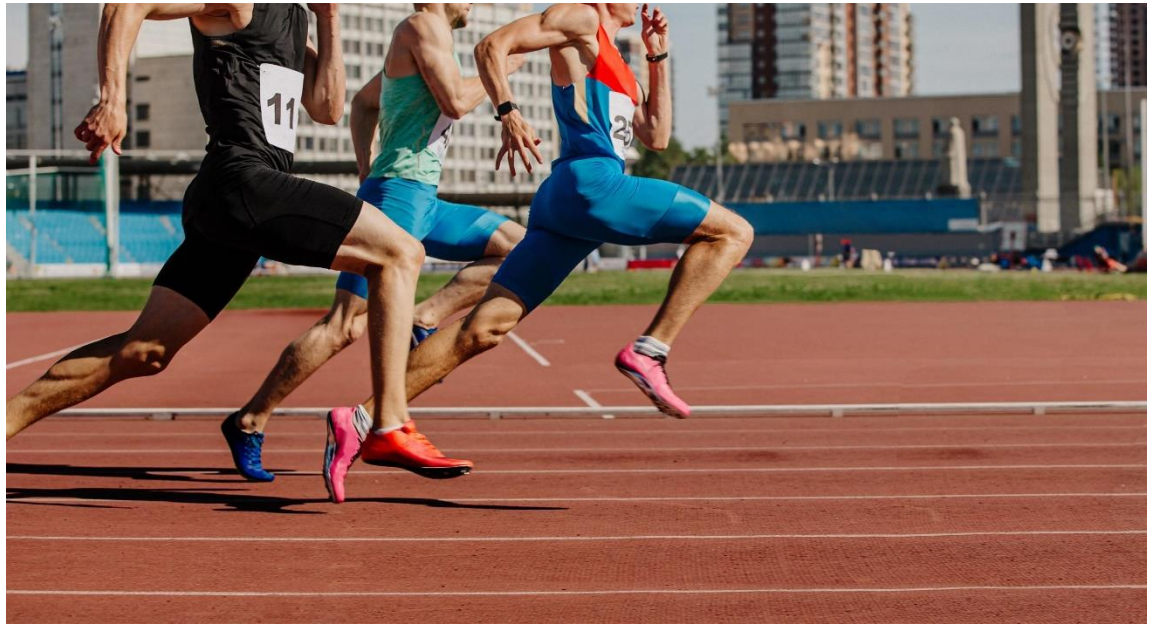
$$K_c = \frac{\text{conc. of solute in stationary phase}}{\text{conc. of solute in mobile phase}}$$

Solute Location

In stationary phase = Not moving down the column

In mobile phase = Moving down the column

All solutes spend the same amount of time in the mobile phase

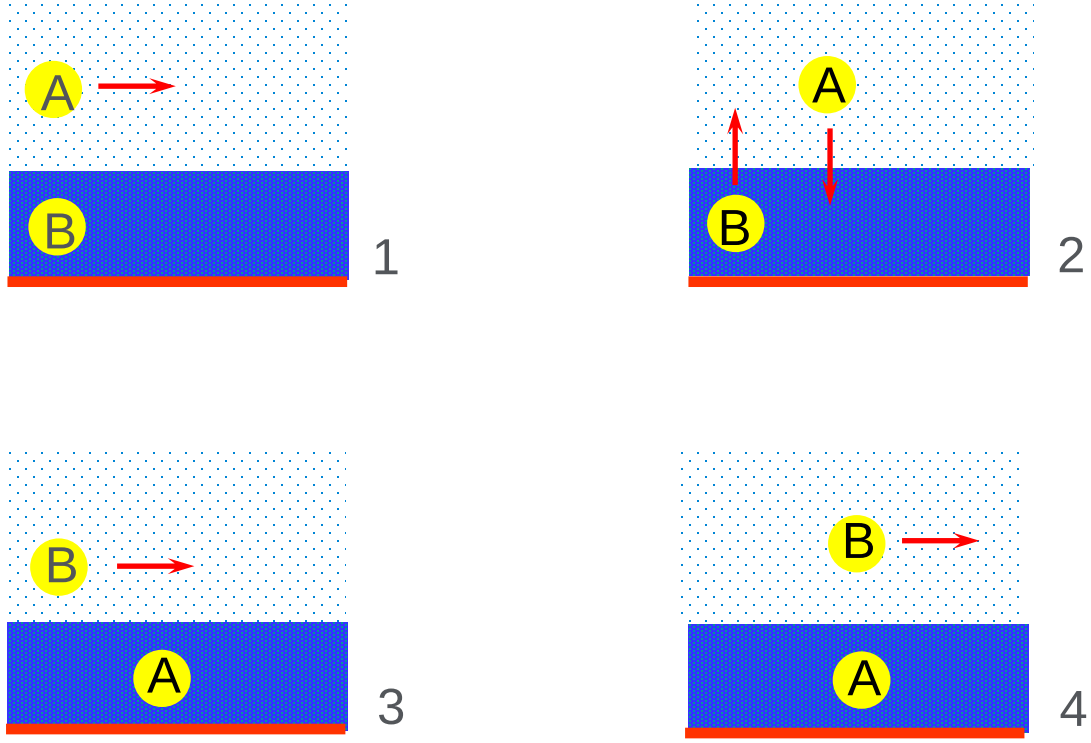


Separation Process: Solute Movement Down the Column

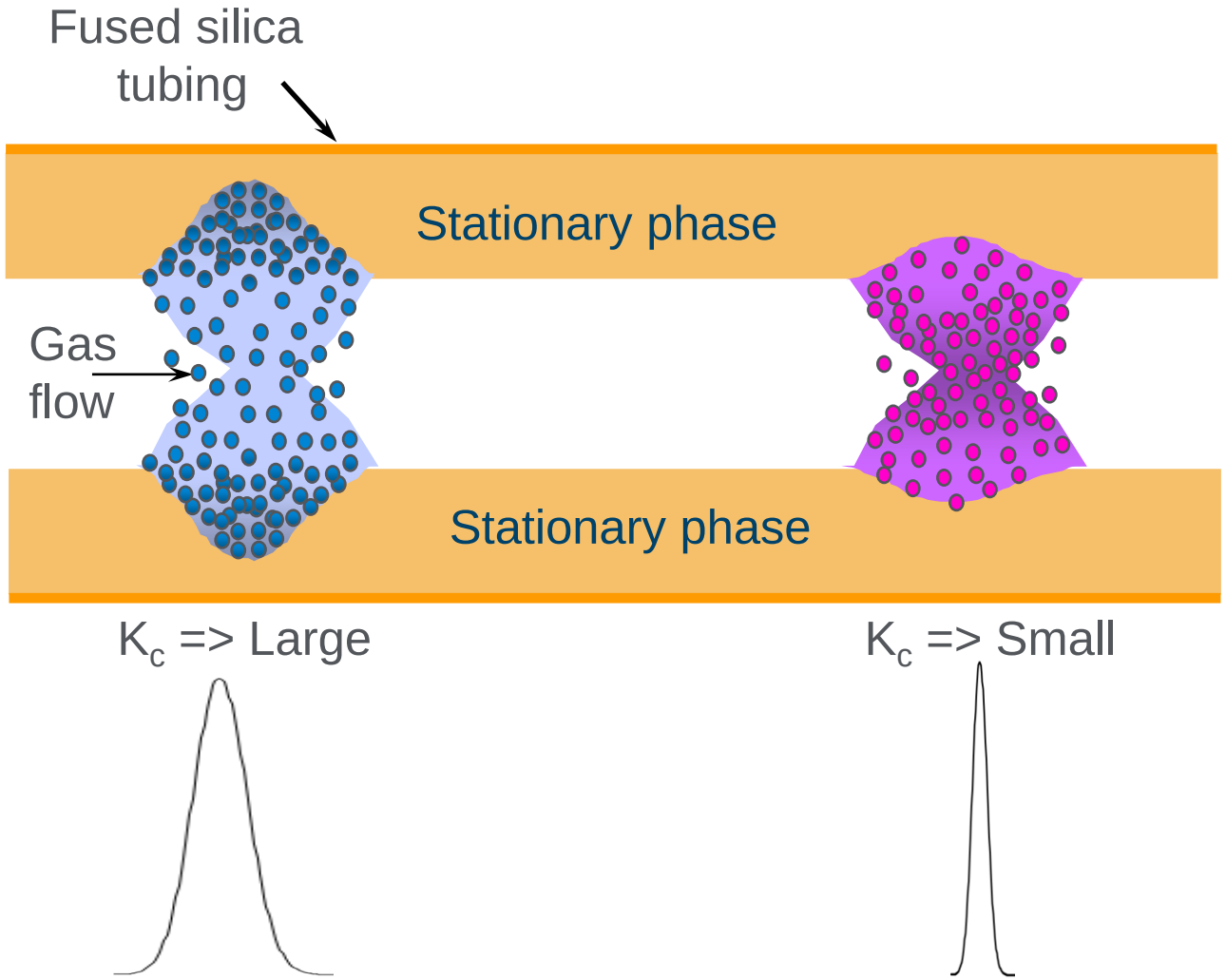
A and B are the same solute

Mobile phase

Stationary phase

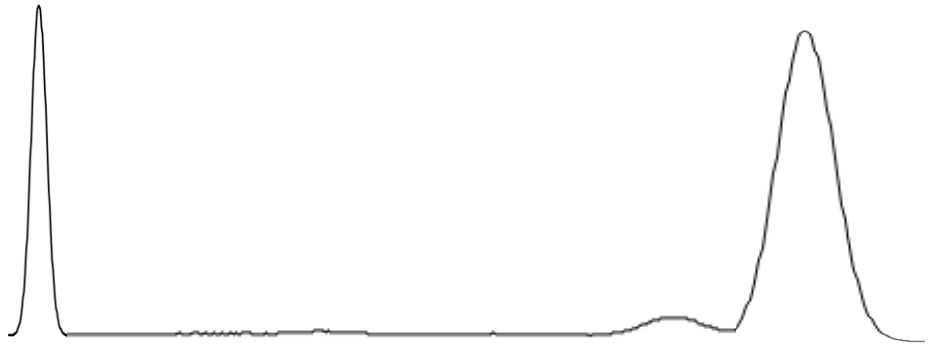


K_c and Peak Width



retention

retention



Band broadening

$$K_c = \frac{\text{conc. of solute in stationary phase}}{\text{conc. of solute in mobile phase}}$$

Retention Time: t_r

Time for a solute to travel through the column

Three Parameters That Affect K_C

Solute:
different solubilities in a stationary phase; unique interactions with stationary phase

Stationary phase:
different solubilities of a solute in that phase based on unique interactions of functional groups

Temperature:
K_C decreases as temperature increases; for better separation/resolution of earlier eluting compounds, start with a lower column temperature

Retention Factor (k)

Ratio of the time the solute spends in the stationary to t
the mobile phases

$$k = \frac{t_r - tm}{t_m}$$

t_r = retention time
t_m = retention time of non-retained compound
Also called “capacity factor” or “partition ratio”

Measure of the magnitude of solute retention

Inversely proportional with column temperature

Phase Ratio: (β)

$$\beta = \frac{r}{2df}$$

r = radius (µm)
d_f = film thickness (µm)

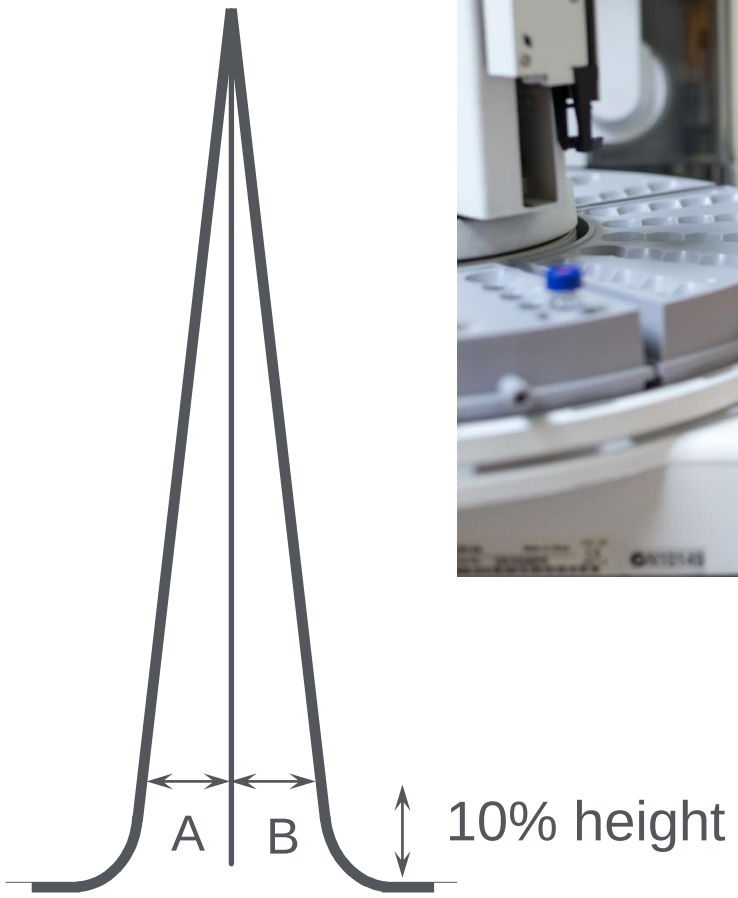
To maintain column selectivity when adjusting diameter, you need to maintain the same **phase radio**

DB-624

ID (mm)	β	Length (m)	Film (µm)
0.18	44	20	1.00
0.20	44	25	1.12
0.25	44	30	1.40
		60	1.40
0.32	44	30	1.80
		60	1.80
0.45	44	30	2.55
		75	2.55
0.53	44	15	3.00
		30	3.00
		60	3.00
		75	3.00

Peak Symmetry

$$\text{Symmetry} = \frac{A}{B}$$



Tailing : Symmetry <1 (Activity)
Fronting : Symmetry >1 (Over-load)

Efficiency: Theoretical Plates (N)

Large number implies a better column

Often a measure of column quality

Relationship between retention time and width

Theoretical Plates: (N)

$$N = 5.545\left(\frac{t_r}{w_h}\right)^2$$

t_r = retention time
W_h = peak width at half height (time)

Measurements of Efficiency: Cautions

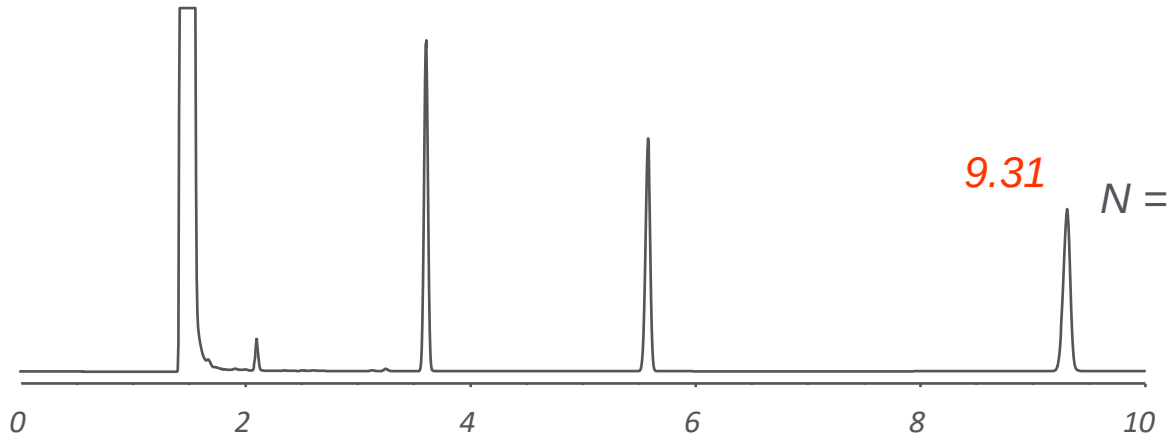
In reality, it is a measurement of the entire GC system

Condition-dependent

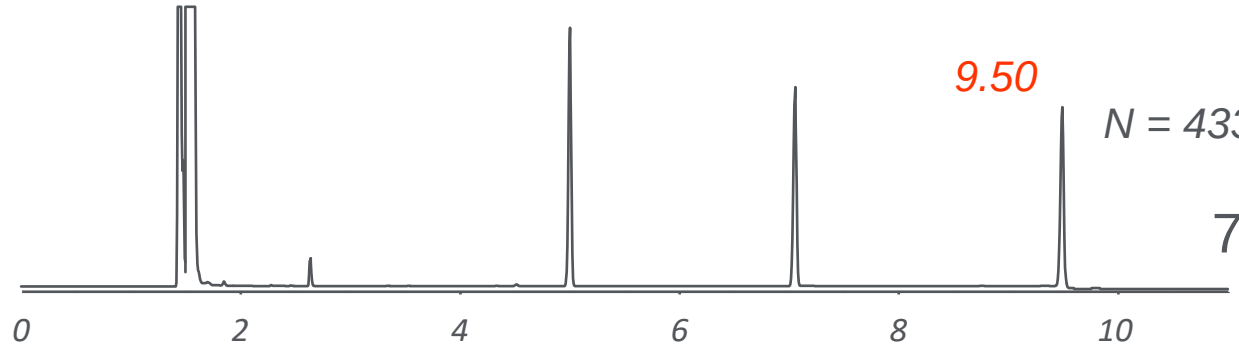
Use a peak with k>5

Isothermal

Isothermal vs Temperature Programming: Efficiency



100 °C isothermal



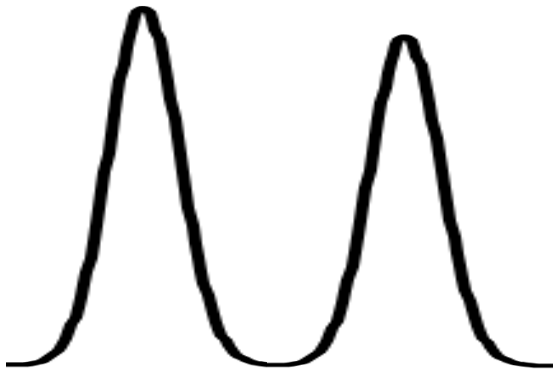
75-135 °C at 5°C/min

DB-1, 30 m x 0.25 mm id, 0.25 µm
He at 37 cm/s
C10, C11, C12

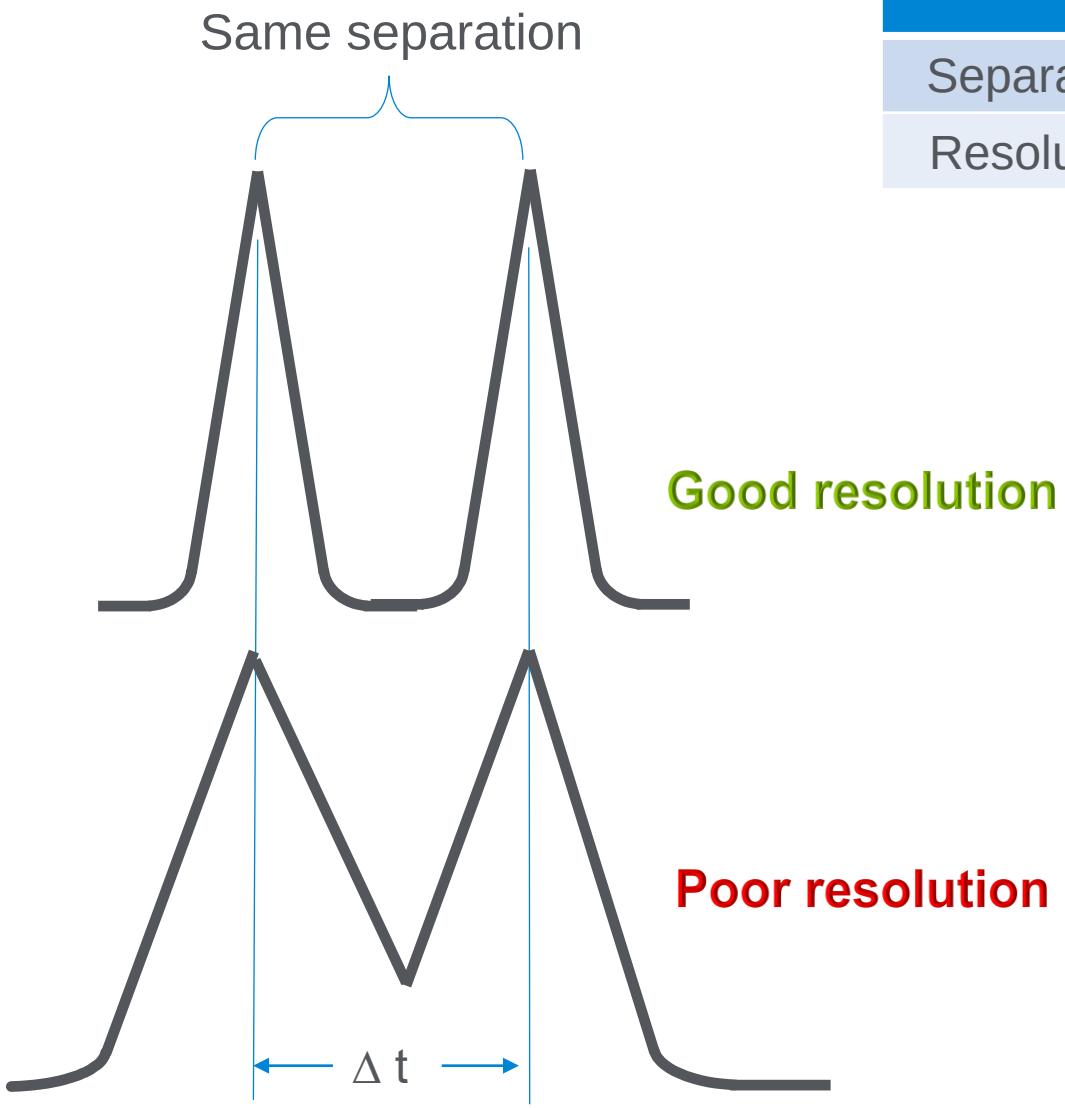
Resolution: (R_s)

$$R_S = 1.18\left(\frac{t_{r2} \quad -t_{r1}}{w_{h2} + w_{h1}}\right)$$

t_r = retention time
W_h = peak width at half height (time)
R_s of $\geq$1.5 is considered baseline resolution



Separation vs Resolution



Separation vs Resolution	
Separation	Absolute time between peak apex
Resolution	Time between peak with respect to peak widths

Resolution

$$R_S = \frac{\sqrt{N}}{4}\left(\frac{k}{k+1}\right)\left(\frac{\alpha-1}{\alpha}\right)$$

Factors effecting R_s	
Efficiency	N = f (gas, L, r_c)
Retention	k = f (T, d_f, r_c)
Selectivity	α = f (T, phase)

Variables	
L	**L**ength
r_c	**r**adius
d_f	film thickness
T	**T**emperature

Column Length and Resolution

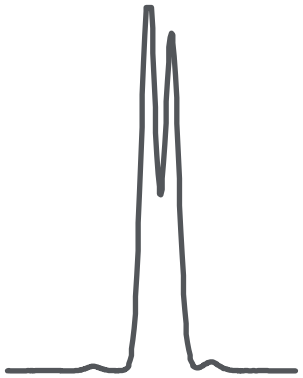
$$R \alpha \sqrt{n} \alpha \sqrt{L}$$

Length X 4 = Resolution X 2

$$t \alpha L$$

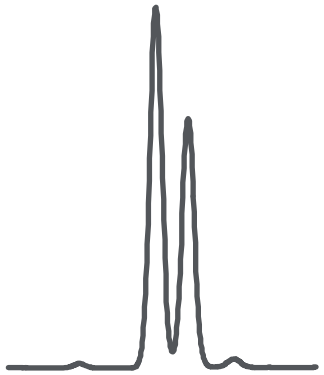
Column Length VS Resolution and Retention: Isothermal

R=0.84
2.29 min



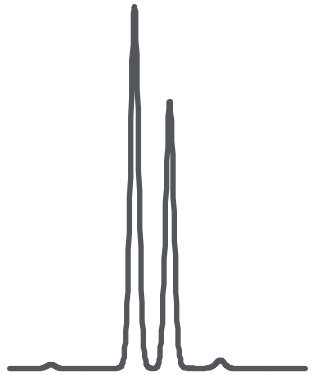
15 m

R=1.16
4.82 min



30 m

R=1.68
8.73 min

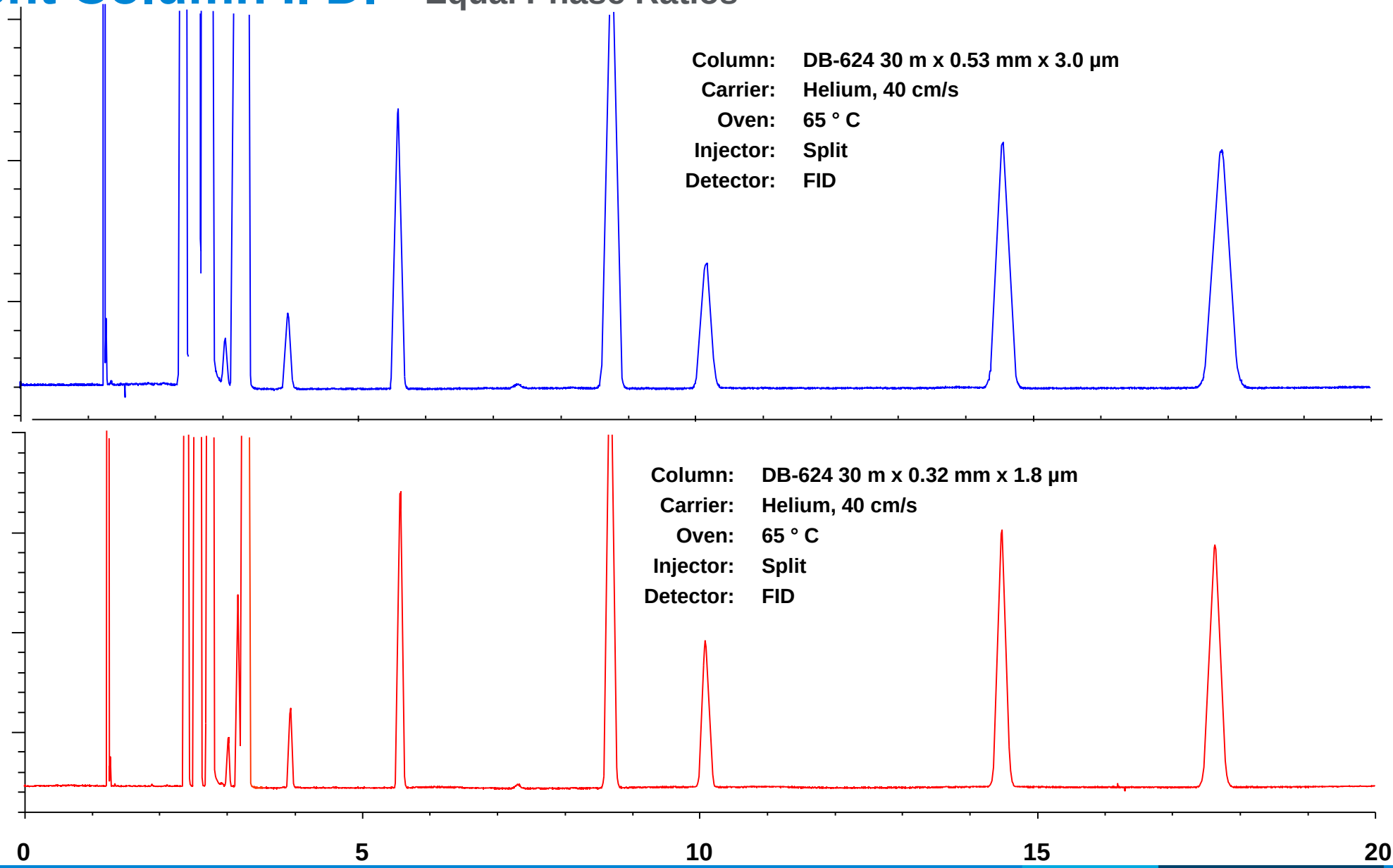


60 m

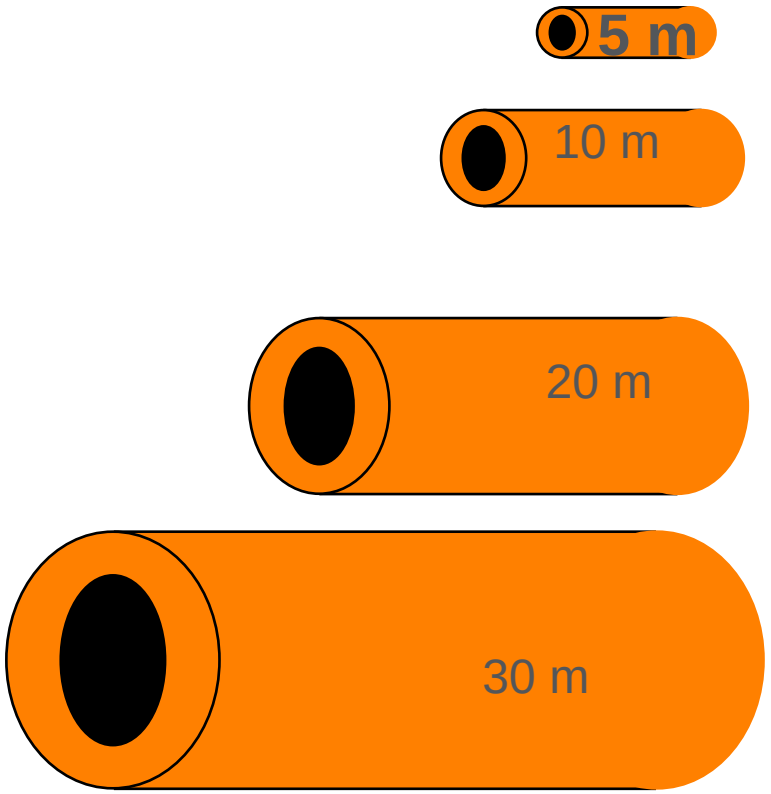
Double the length/plates = double the analysis time, but not double the resolution

However….
we can use this relationship to our advantage

Different Column I. D. - Equal Phase Ratios



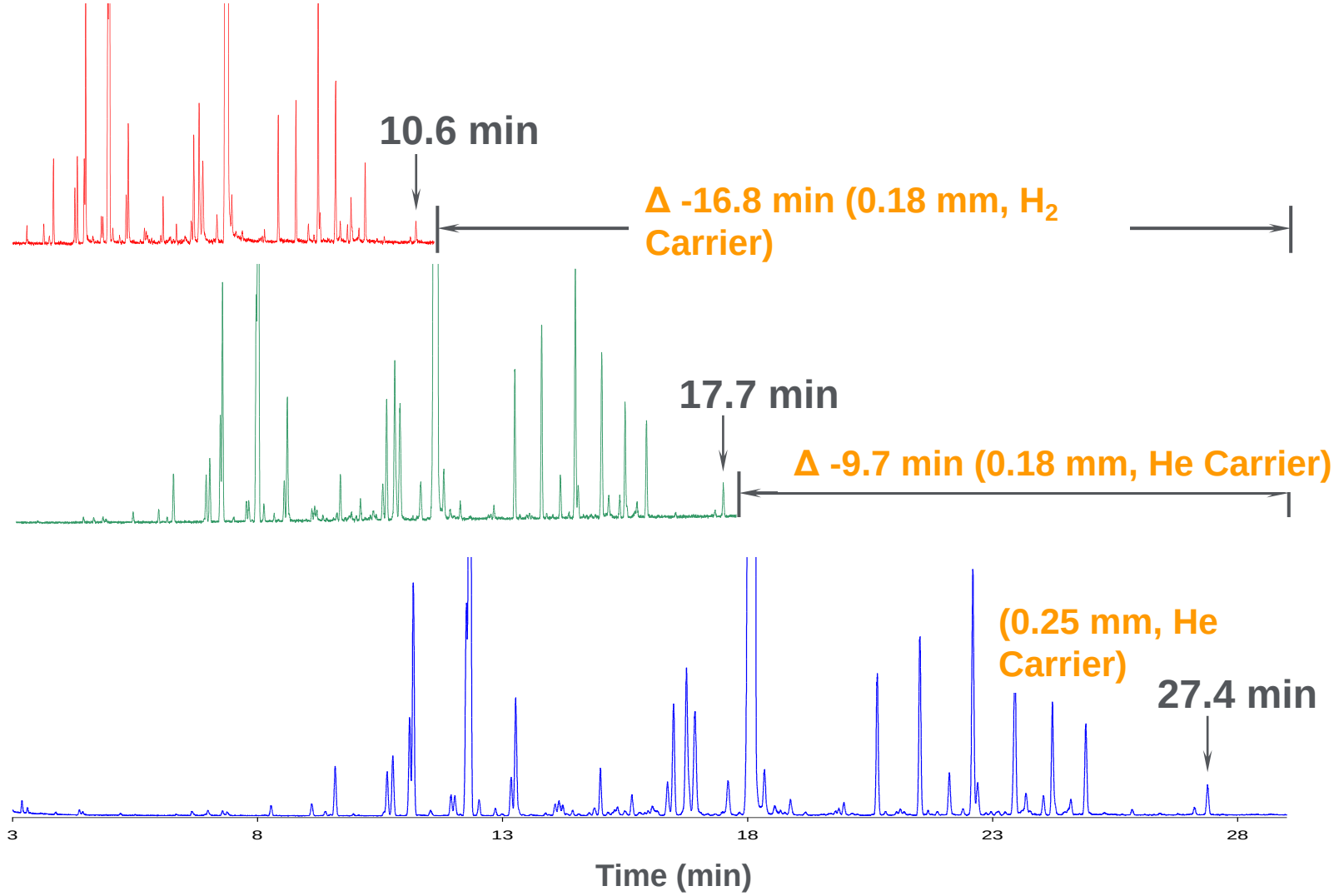
Column Diameter - Theoretical Efficiency



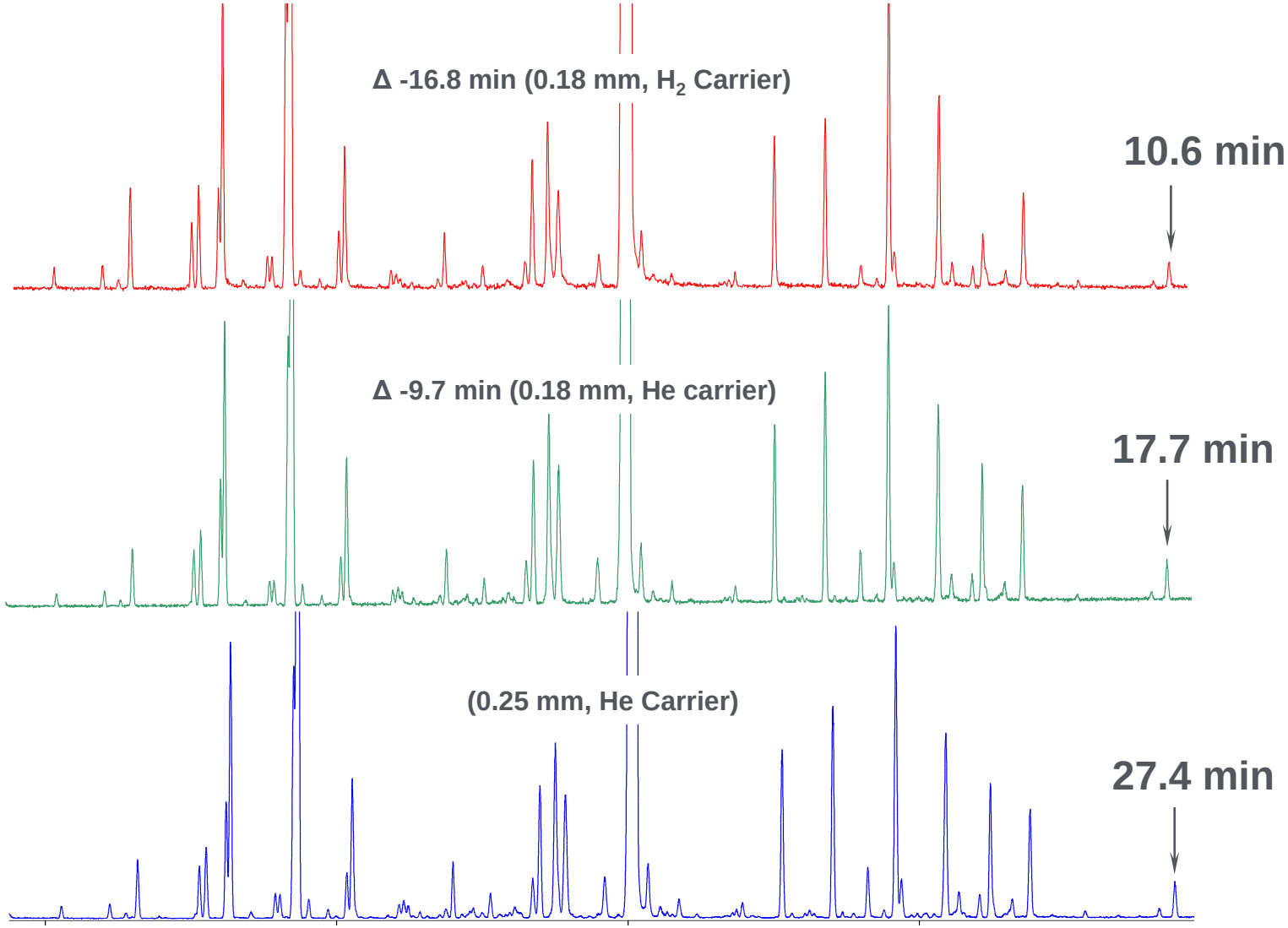
Total Plates	I.D. (mm)	n/m
N ~ 112,000	0.05	23,160
N ~ 112,000	0.10	11,580
	0.18	6,660
N ~ 112,000	0.20	5830
	0.25	4630
N ~ 112,000	0.32	3660
	0.45	2840
	0.53	2060

k = 5

Spearmint Oil



Spearmint Oil – Resolution Check



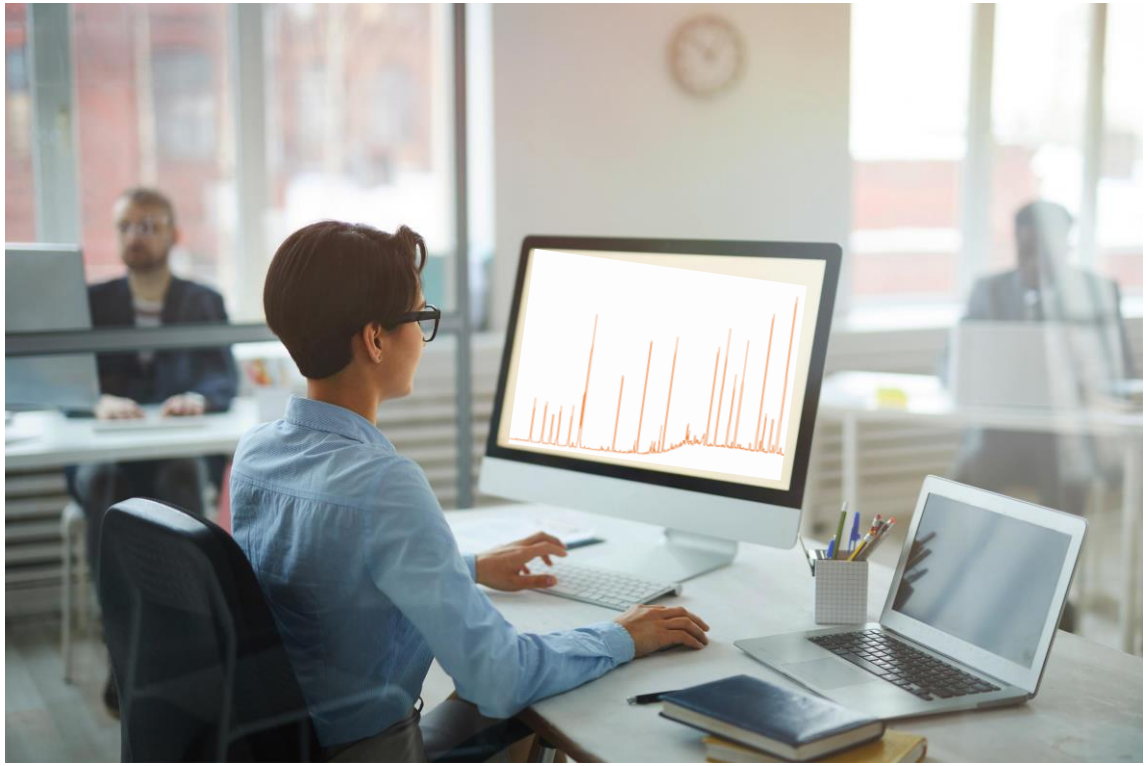
Detectors

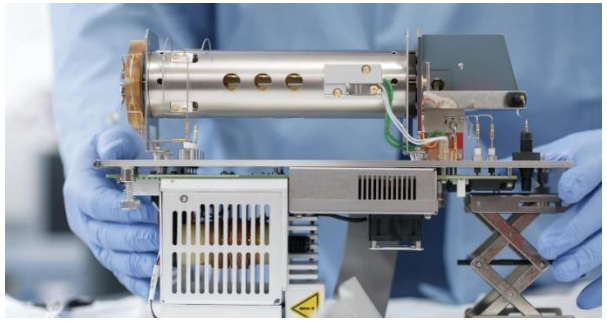
Responds to some property of the solutes

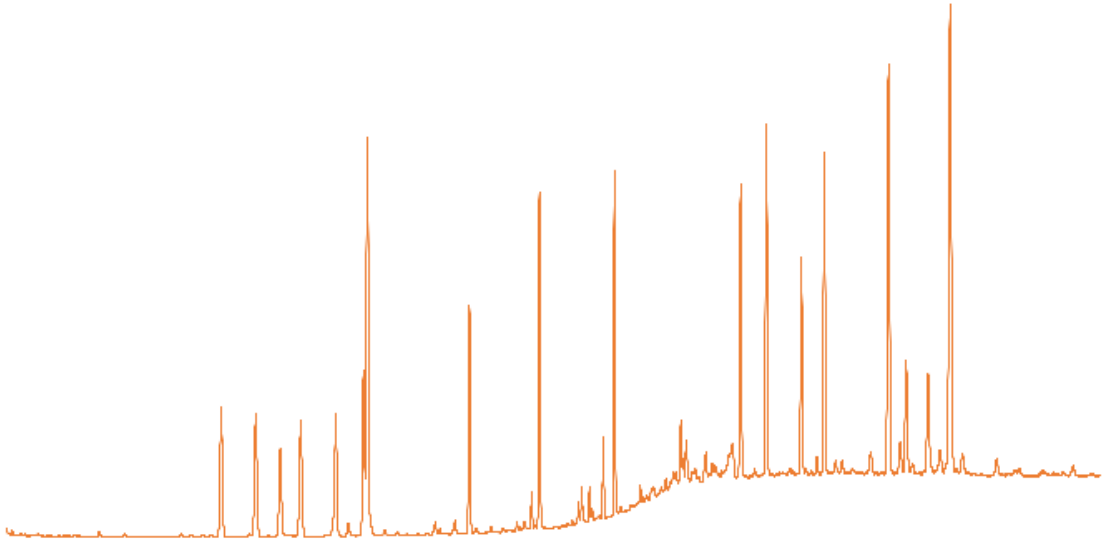
Converts the interaction into a signal

Immediate

Predictable







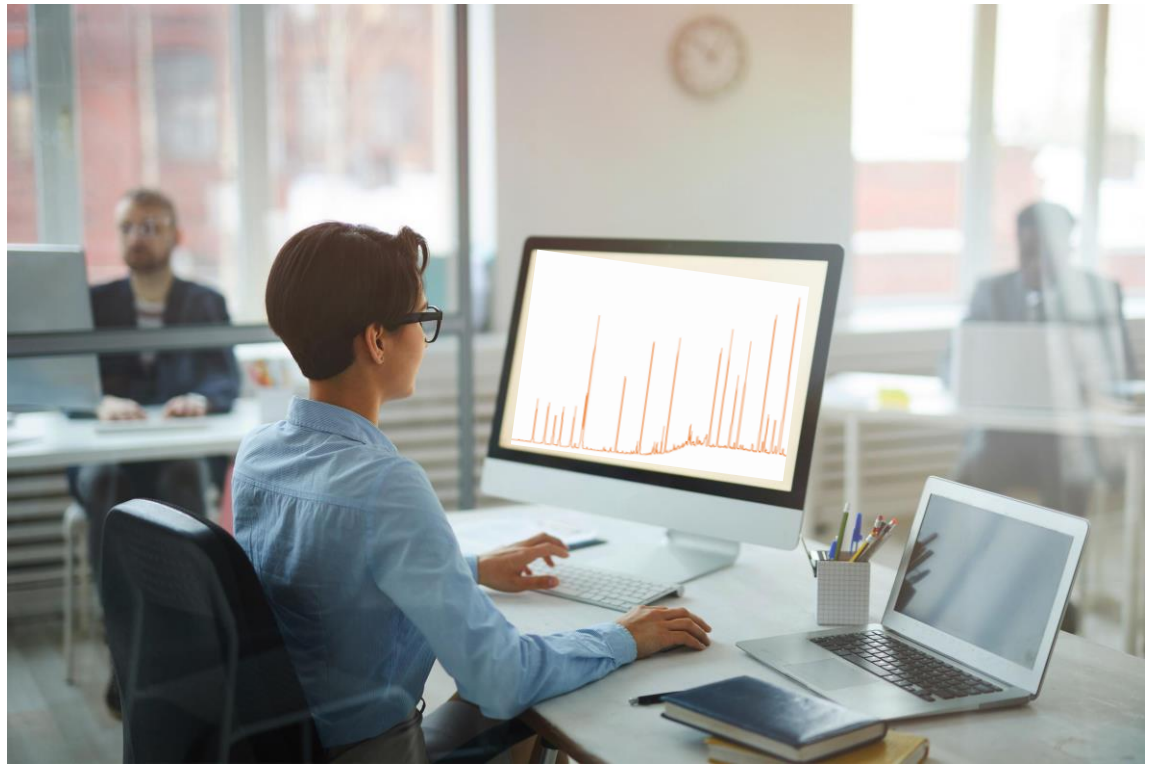
Detectors – Current Specifications for Agilent 8890 GC System

Detector	Dynamic Range	Responds To	MDL
TCD	10^5	Universal	400 pg Tridecane/mL (He)
FID	10^7	C-H bonds/organic	1.2 pg C/s Tridecane
ECD	5 e10^4	Halogens	3.8 fg/mL Lindane
NPD	10^5	Nitrogen/phosphorus	0.08 pg N/s 0.01 pg P/s
FPD	10^5S, 10^5P	Sulfur/phosphorus	60 fg P/s 3.6 pg S/s
SCD	10^4	Sulfur	0.5 pg S/s
NCD	10^4	Nitrogen	3 pg N/s
MSD	Varies	Universal	Varies

Data Handling

Converts the detector signal into a chromatogram

- Integrator
- Software program



Conclusions

The GC is comprised of an inlet, column, and detector that all work together to produce good chromatography.

Good injection efficiency is critical (narrow sample band).

Start with the right phase!

Use column dimensions to your advantage

Separation (via K_c) is based on three things:

- Solute: different solubilities/interaction in a given stationary phase
- Stationary phase: different solubilities/interaction of a solute (correct column selection is critical)
- Temperature: K_C decreases as temperature increases

When in doubt, contact Agilent Technical Support

Contact Agilent Chemistries and Supplies Technical Support

1-800-227-9770 option 3, option 3:

Option 1 for GC and GC/MS columns and supplies

Option 2 for LC and LC/MS columns and supplies

Option 3 for sample preparation, filtration, and QuEChERS

Option 4 for spectroscopy supplies

Option 5 for chemical standards

Available in the U.S. and Canada 8–5, all time zones

gc-column-support@agilent.com

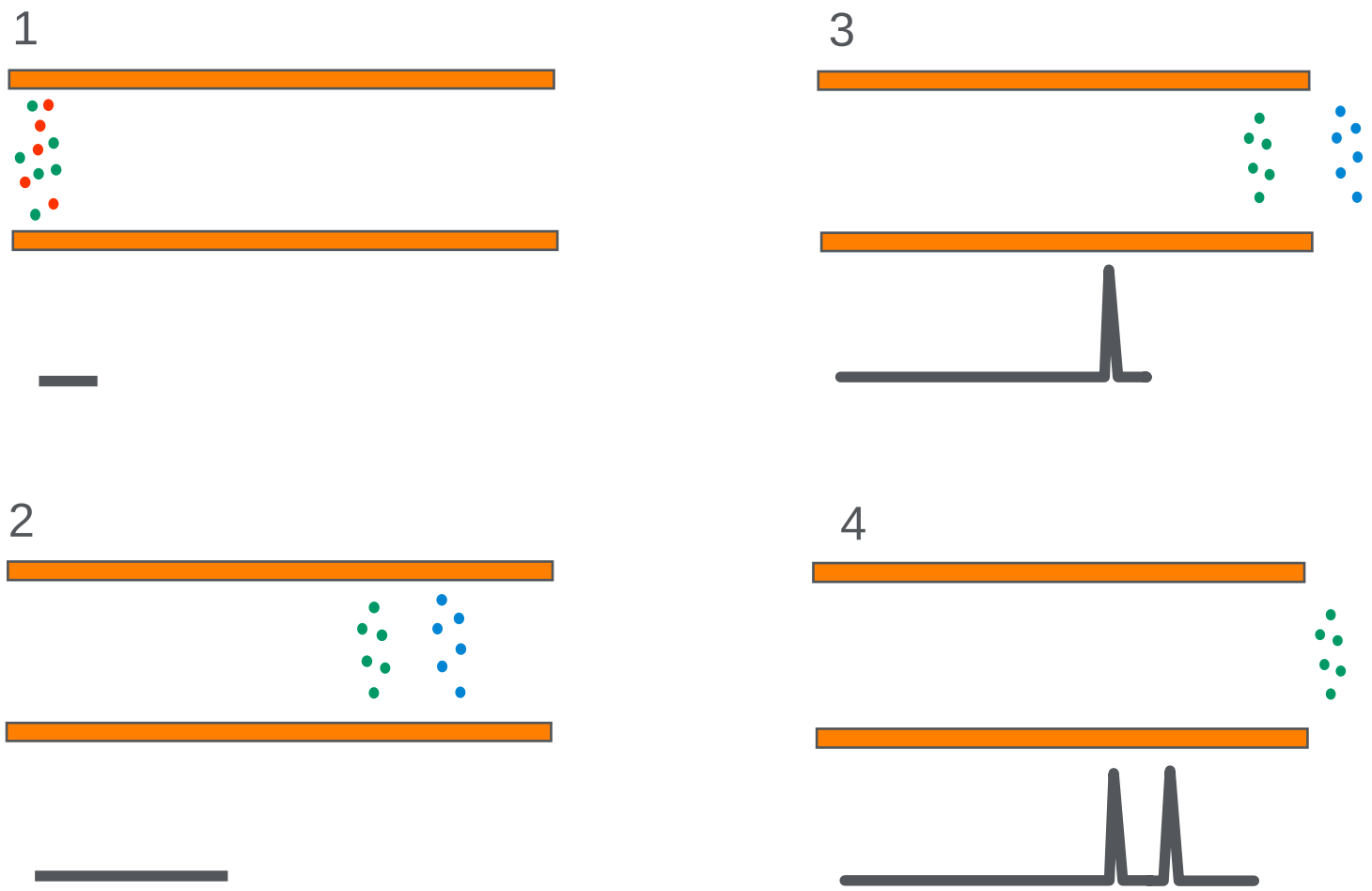
lc-column-support@agilent.com

spp-support@agilent.com

spectro-supplies-support@agilent.com

chem-standards-support@agilent.com

Separation Process



Sample Introduction Goals

Introduce sample into the column

- Narrow band is critical

Reproducible

Minimal efficiency losses

Representative of sample

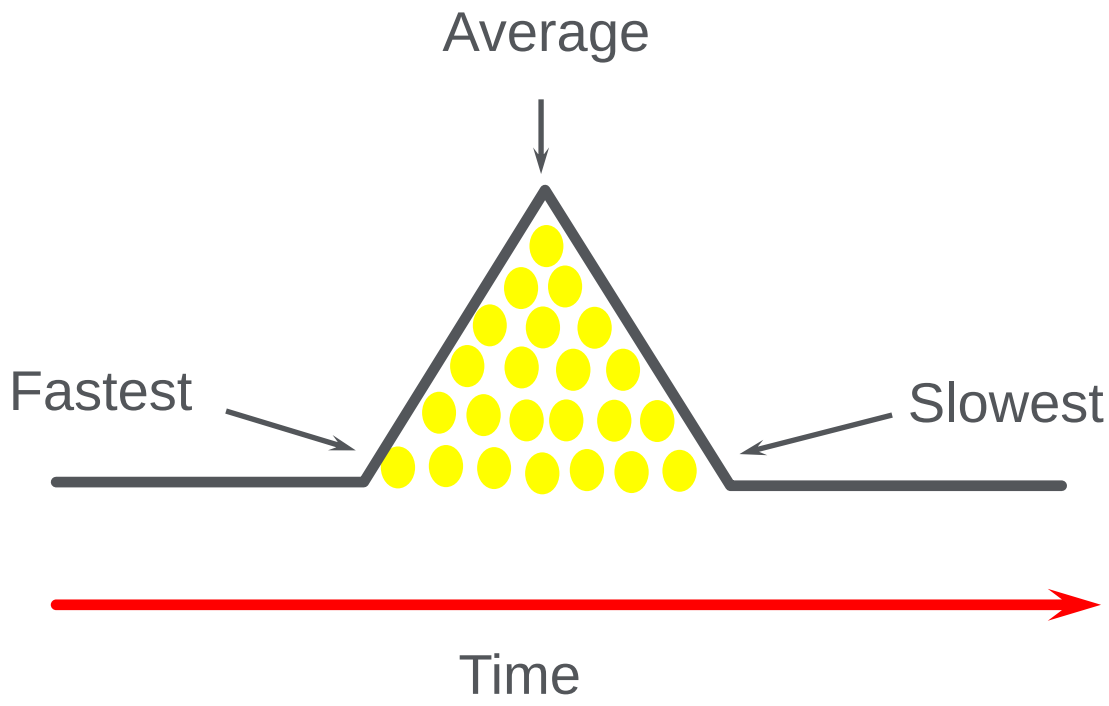
Separation Factor: (α)

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

co-elution: $\alpha = 1$

k_2 = retention factor of 2nd peak
k_1 = retention factor of 1st peak

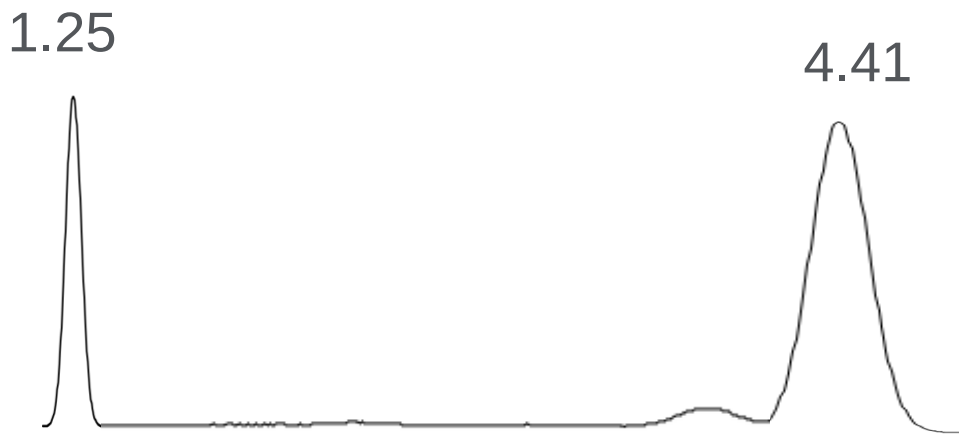
Range of Retention

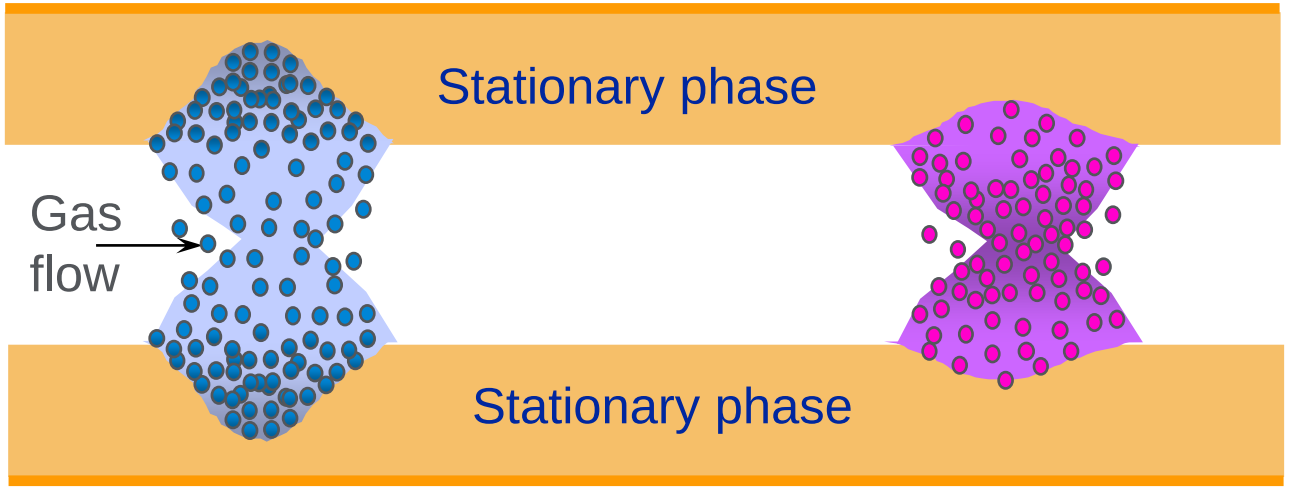


Distribution Constant: K_c

$$K_c = k\beta$$

$$k = \frac{t'_r}{t_m} \qquad \beta = \frac{r}{2df}$$

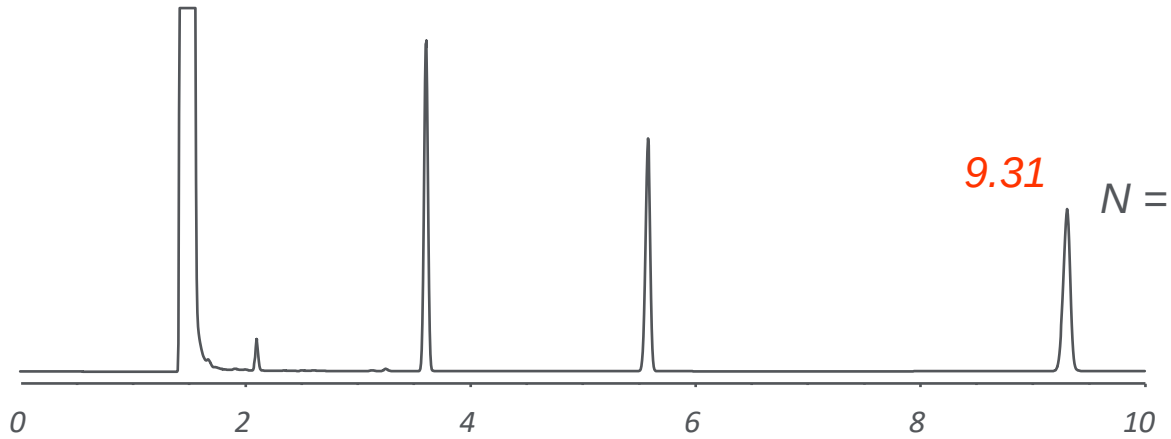




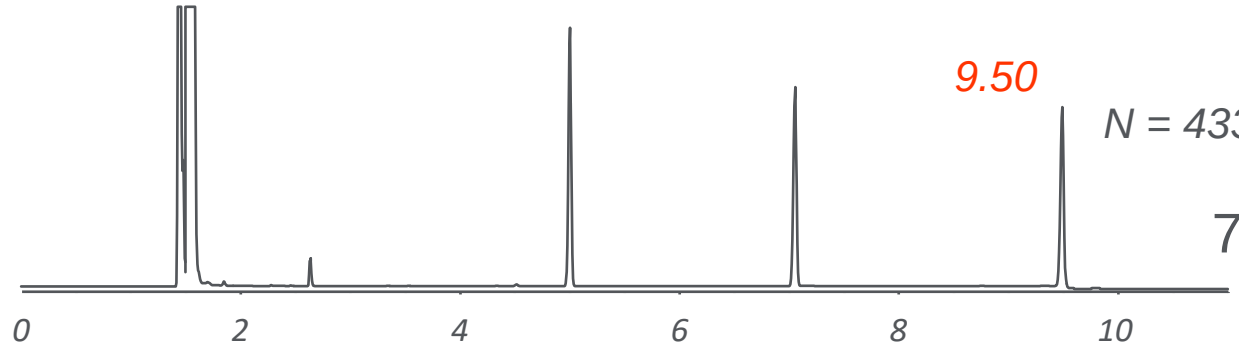
K_c => Large

K_c => Small

Isothermal vs Temperature Programming: Efficiency



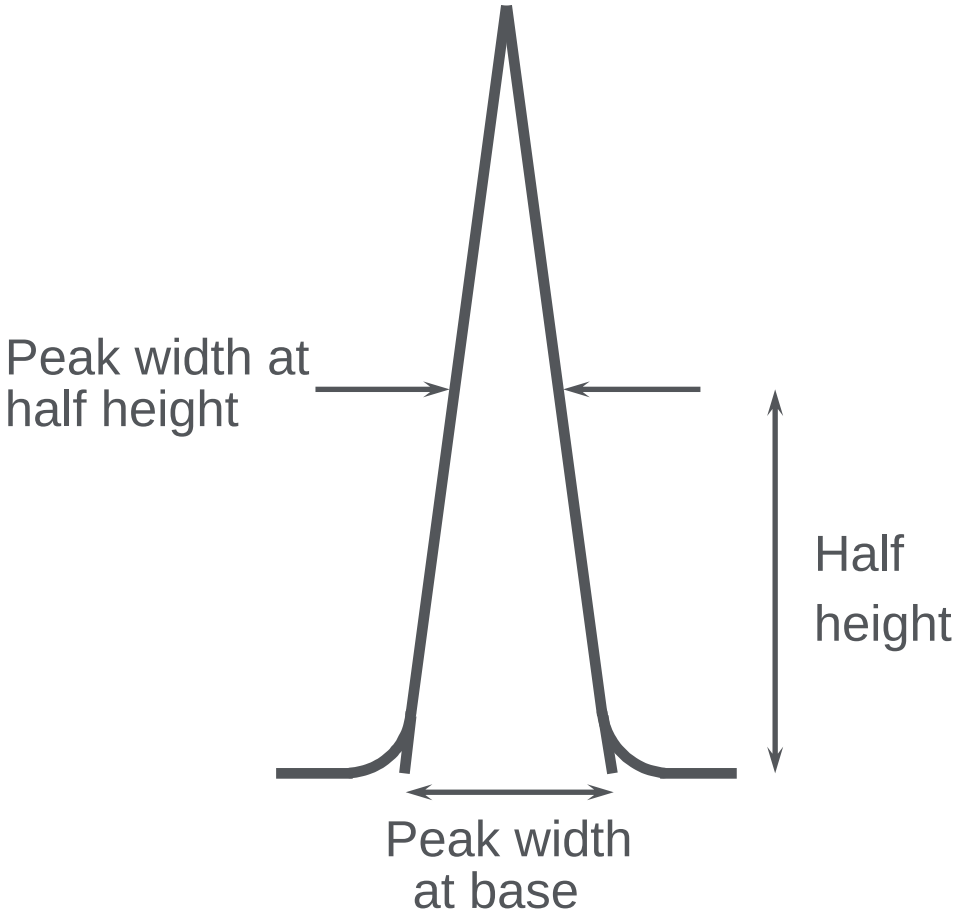
100 °C isothermal



75-135 °C at 5°C/min

DB-1, 30 m x 0.25 mm id, 0.25 µm
He at 37 cm/s
C10, C11, C12

Peak Width

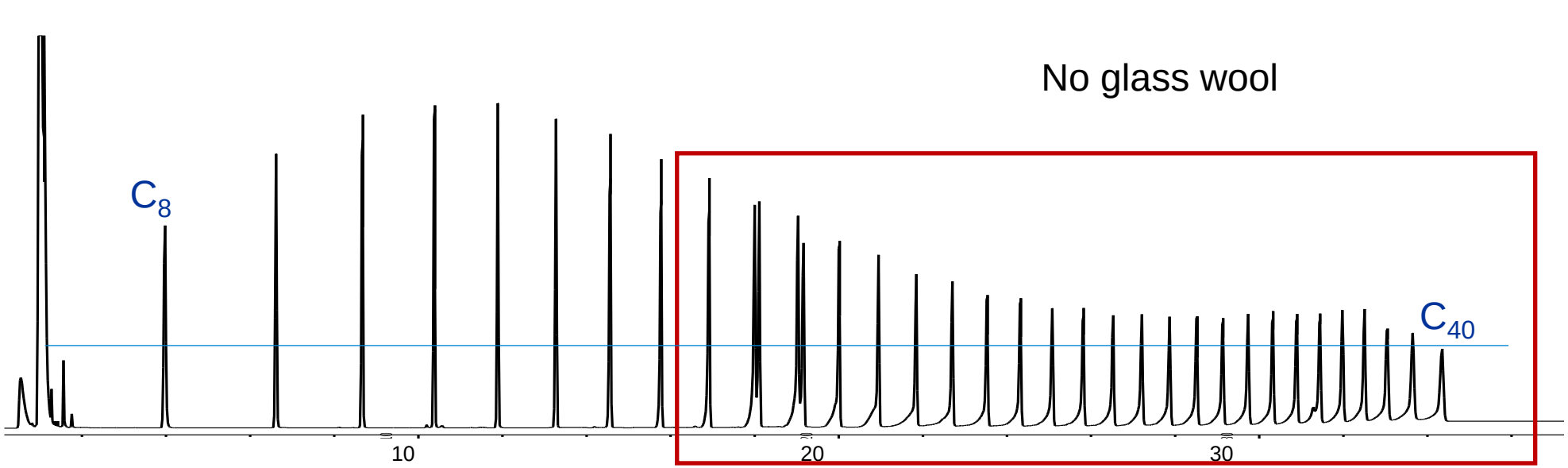


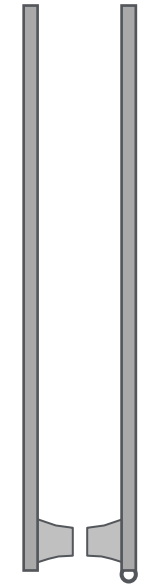
Column Length and Efficiency (Theoretical Plates)

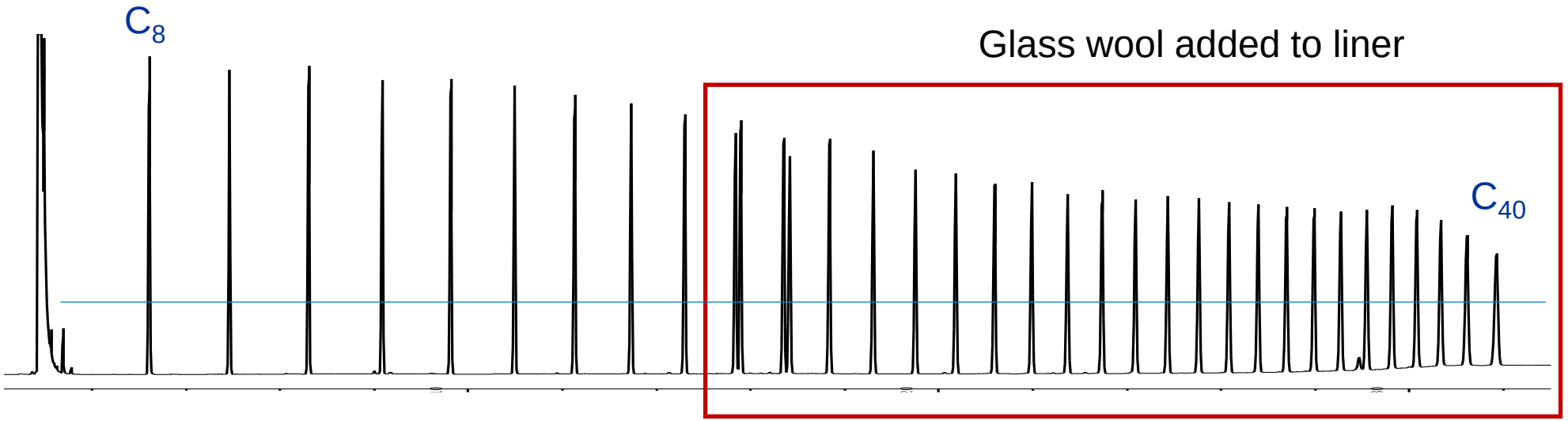
Length (m)	α	n
15		69,450
30		138,900
60		277,800

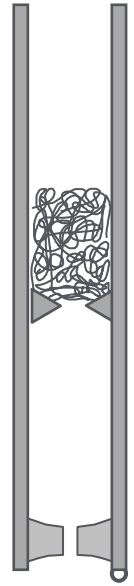
0.25 mm ID
n/m = 4630 (for k = 5)

What Does Inlet Discrimination Look like?

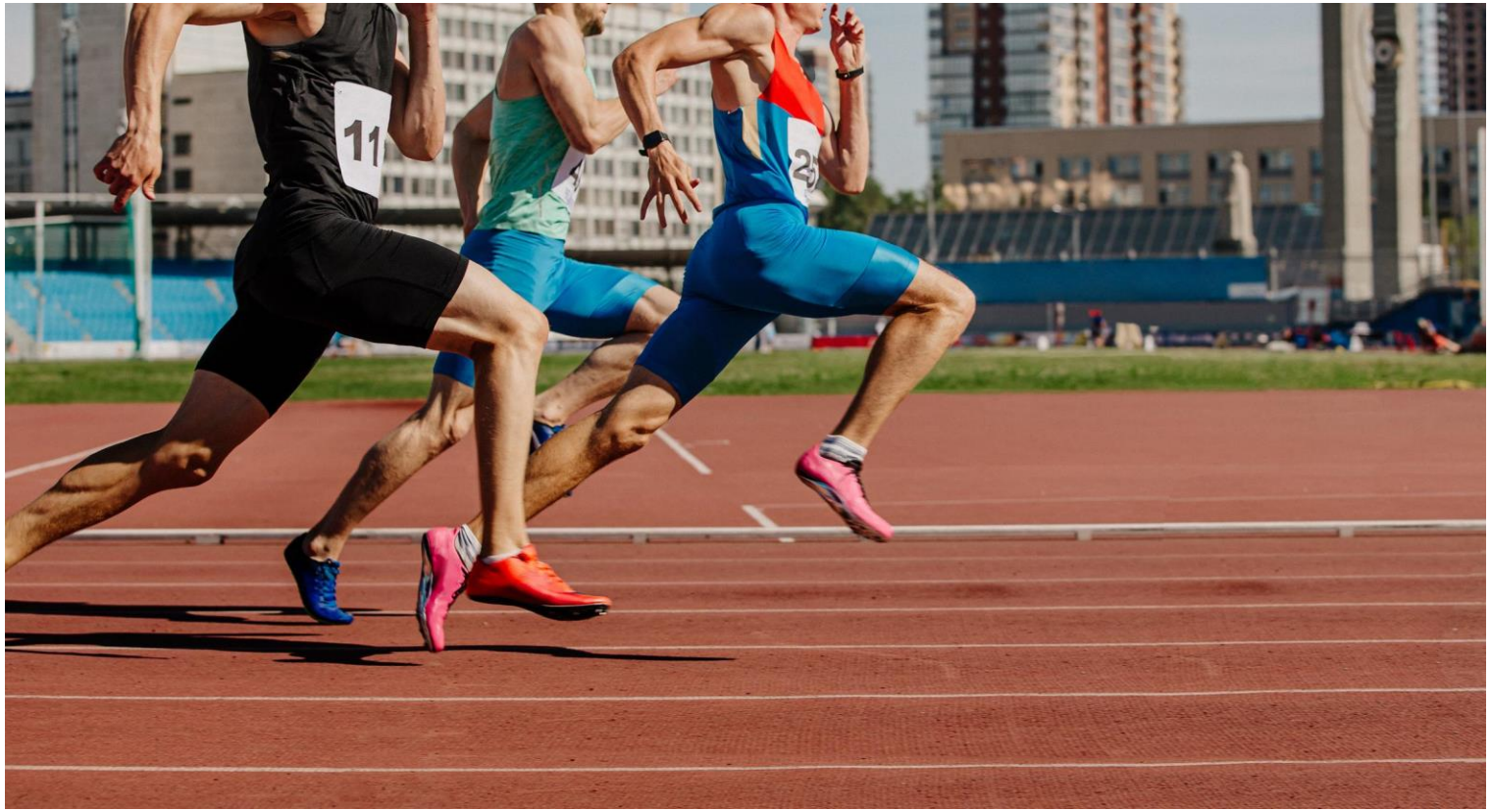


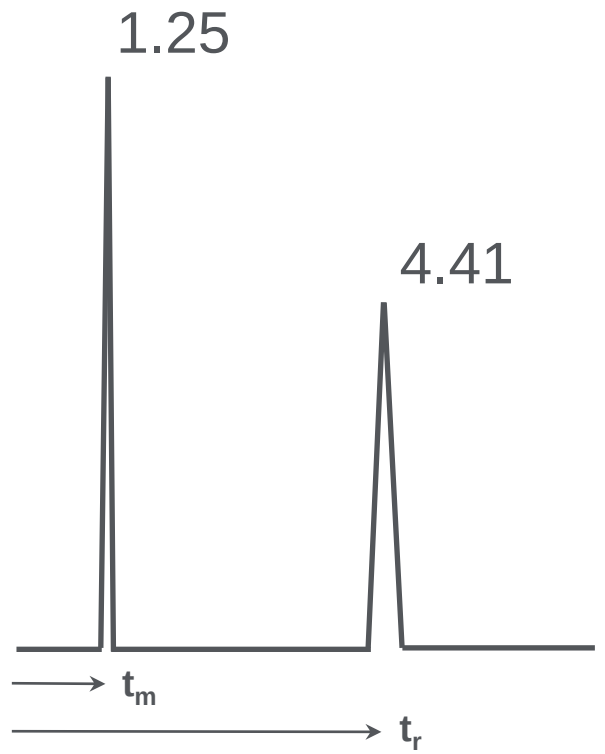






Adjusted Retention Time: t_r'





$t'_r = tr - tm$

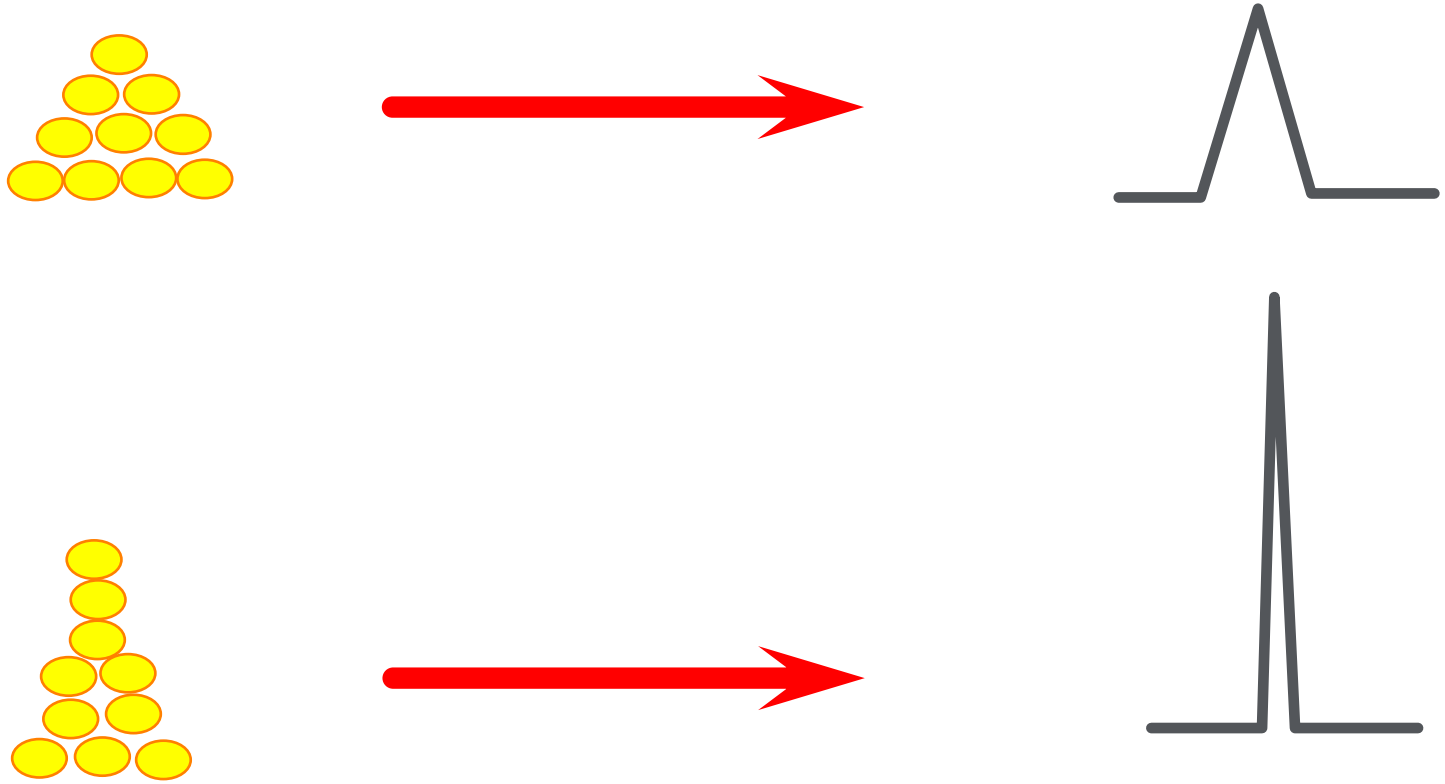
$t'_r = 4.41 - 1.25$

$t'_r = 3.16$ min = time spent in stationary phase

Time Spent in Mobile Phase

All solutes spend the same amount of time in the mobile phase

Peak Width



Adjusted Retention Time: t_r'

Actual time the solute spends in the stationary phase

$$t_r' = t_r - t_m$$

t_r = retention time
t_m = retention time of a non-retained solute