Method Development for Capillary GC Systems

AREAS TO OPTIMIZE

- Injector
- Carrier gas
- Column temperature

COMMON INJECTOR MODES

- **Vaporization Injection Modes**
  - **Megabore Direct**
  - **Split**
  - **Splitless**
- **Cool Injection Modes**
  - **On-Column**
  - **PTV**

INJECTORS

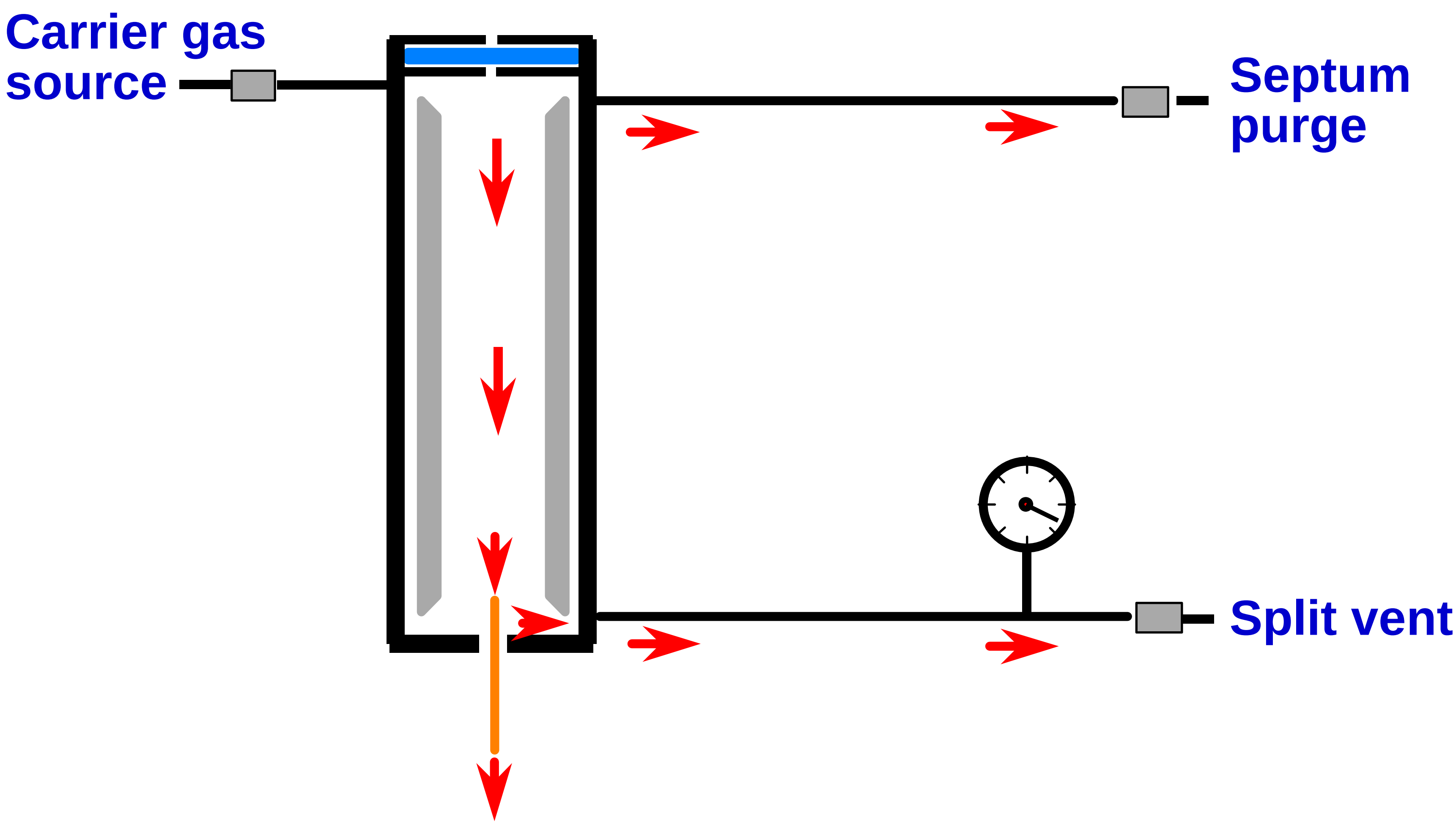
Split

Splitless

SPLIT INJECTOR
Overview

- **Introduces only a small amount of sample into the column**
- **Used for concentrated samples**
- **Produces narrow and sharp peaks**

SPLIT INJECTOR
Flow Path



SPLIT INJECTOR
Major Variables

- Split ratio
- Liner
- Temperature
- Injection volume

SPLIT INJECTOR
Split Ratio

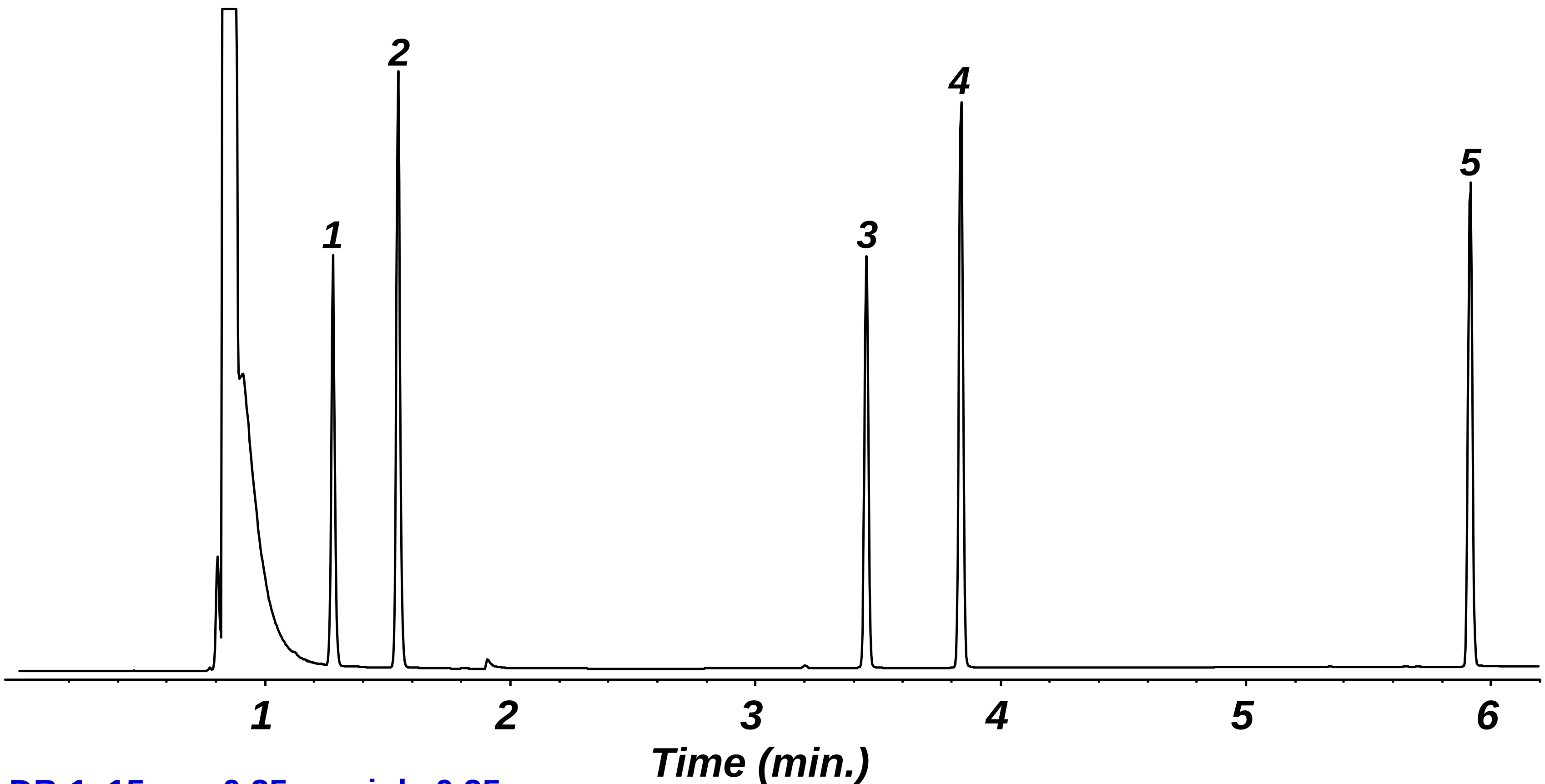
- **Determines the amount of sample entering the column**

- **Typically 20:1 to 100:1**

Higher ratio = Less sample into the column

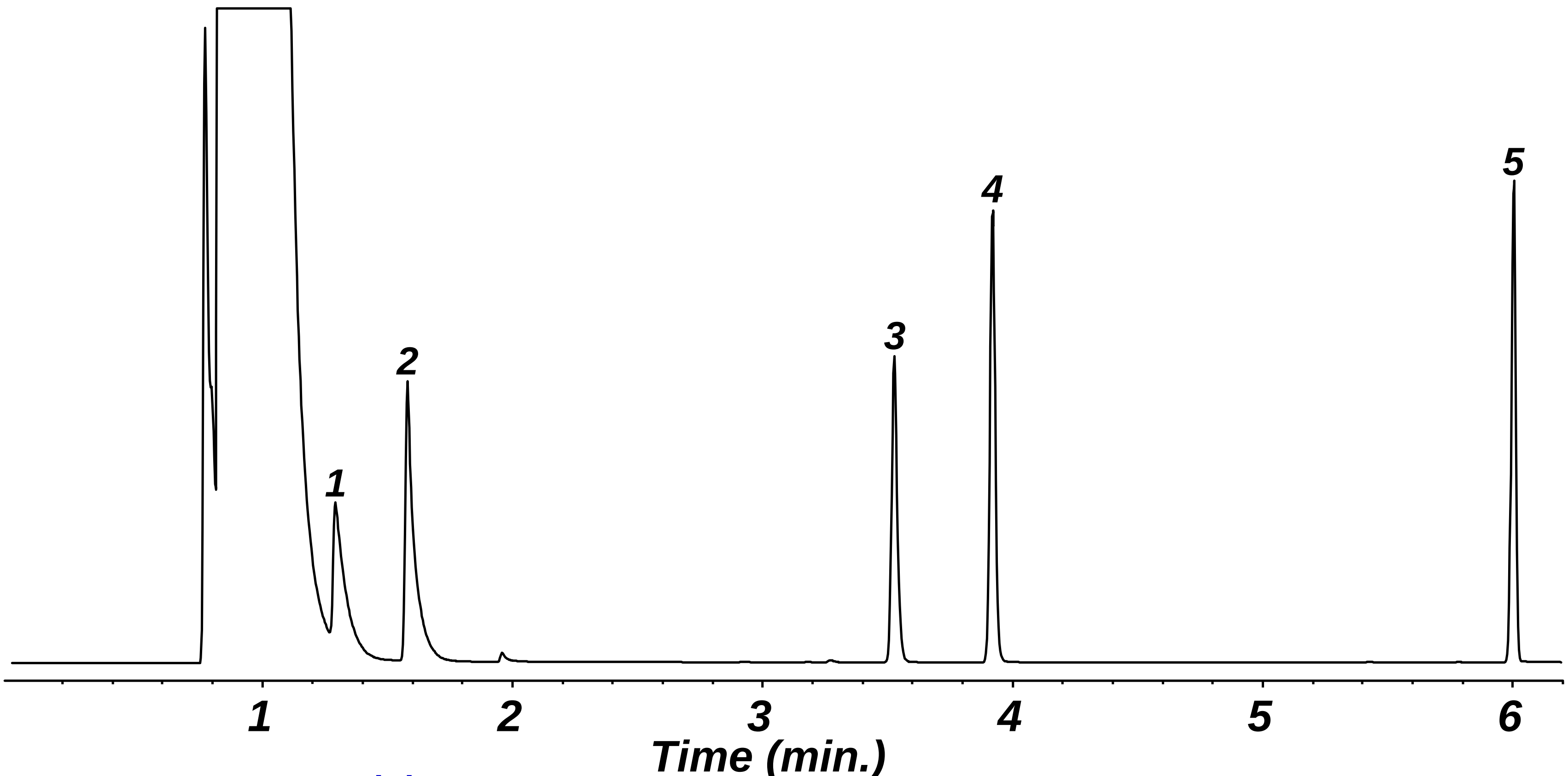
SPLIT INJECTOR

50:1 Split Ratio



DB-1, 15 m x 0.25 mm i.d., 0.25 µm
60°C for 1 min, 60-180°C at 20°/min; Helium at 30 cm/sec
1. n-heptane 2. toluene 3. n-decane 4. n-butylbenzene 5. n-tridecane

SPLIT INJECTOR
5:1 Split Ratio



DB-1, 15 m x 0.25 mm i.d., 0.25 µm
60°C for 1 min, 60-180°C at 20°/min; Helium at 30 cm/sec
1. n-heptane 2. toluene 3. n-decane 4. n-butylbenzene 5. n-tridecane

MINIMUM RECOMMENDED SPLIT RATIO

mm I.D.	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

SPLIT INJECTOR

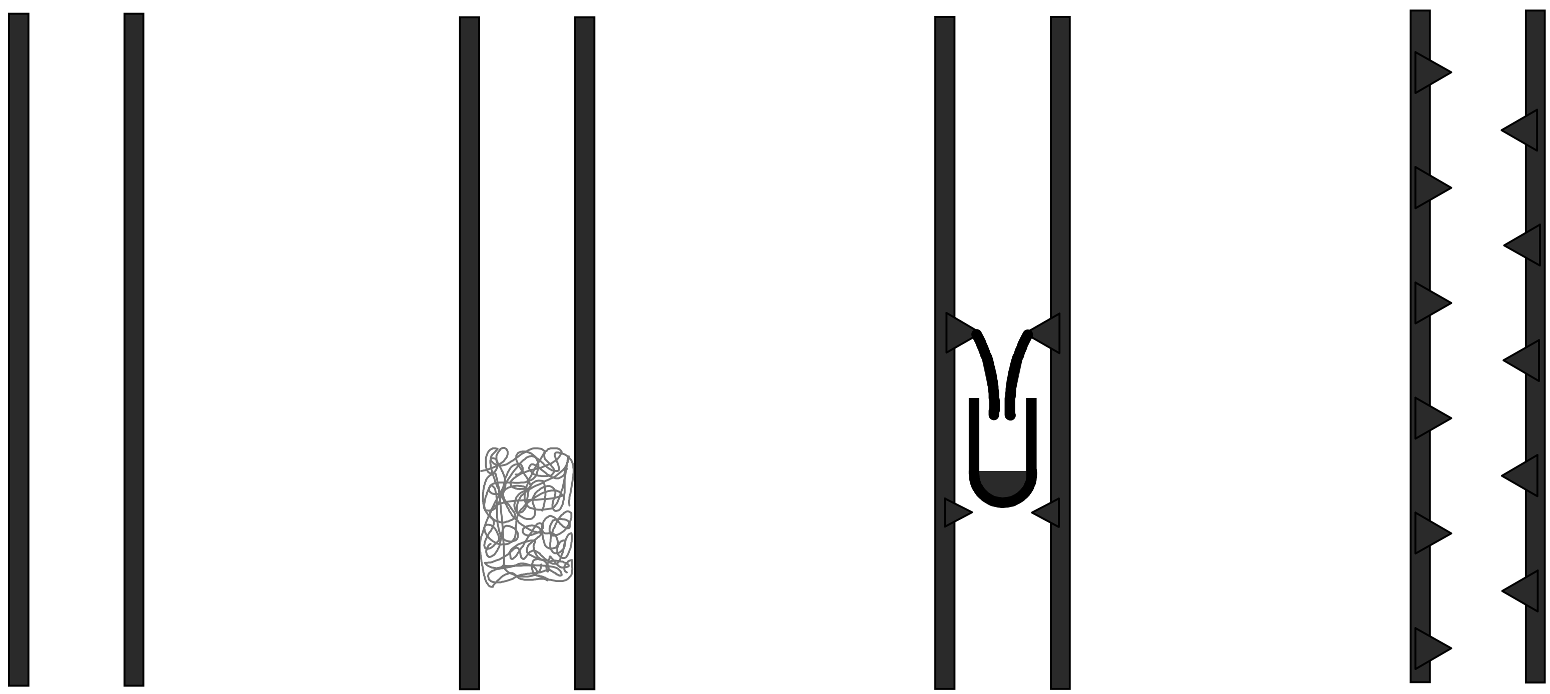
Split Ratio

- **Too low: Poor peak shape**
  Column overload

- **Too high: Poor sensitivity**
  Wastes carrier gas

- **Usually non-linear**

SPLIT INJECTOR
Liner Examples



Straight tube

Straight tube with glass wool

Inverted cup

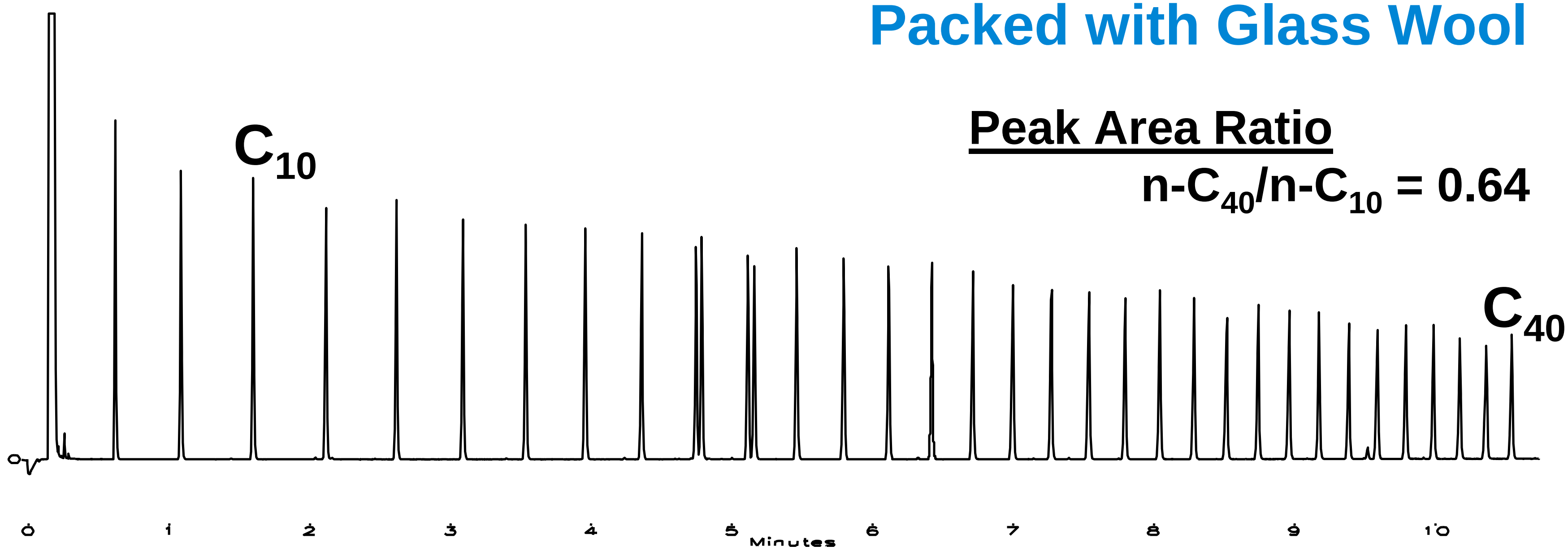
Baffle

SPLIT LINER

Packed with Glass Wool

Peak Area Ratio

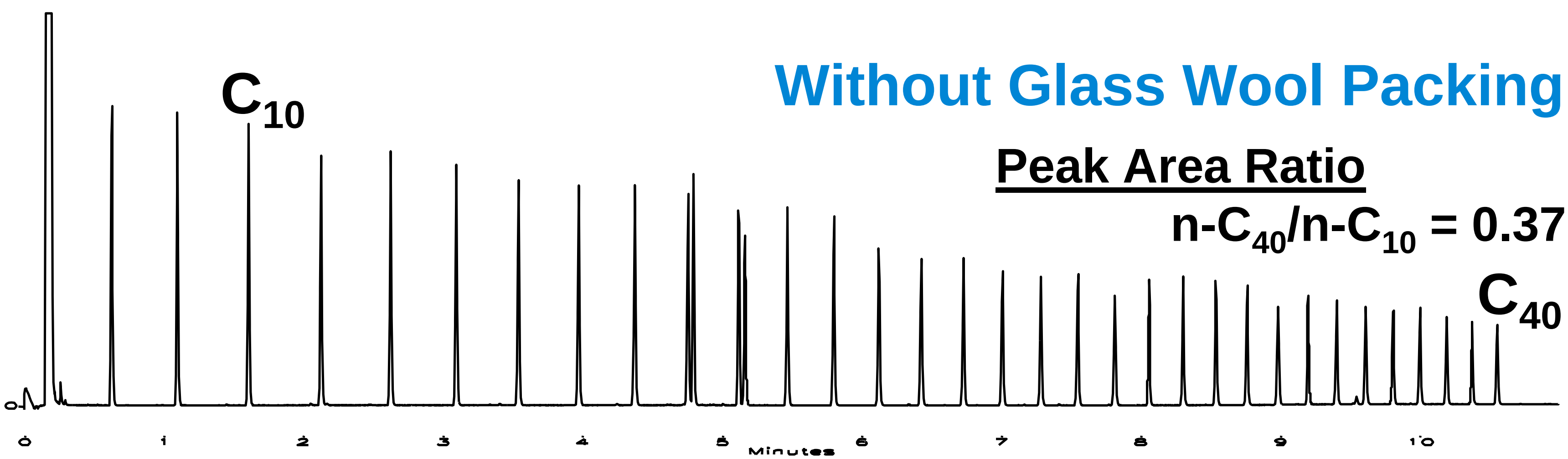
n-C_{40}/n-C_{10} = 0.64



Without Glass Wool Packing

Peak Area Ratio

n-C_{40}/n-C_{10} = 0.37



SPLIT INJECTOR
Temperature

- **Hot enough to rapidly vaporize the sample**
- **May degrade sample or result in injector contamination if too hot**
- **Typically 200-250°C**
- **Injector temperature may not be critical**
- **Use same temperature for reproducible results**

SPLIT INJECTOR
Injection Volume

- Typically 1-3 µl
- Injection volume is not linear
- Inject same volume for all samples and standards for accurate and precise results

Break Number 1

- For Questions and Answers
- Press *1 on Your Phone to
- Ask a Question

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INJECTORS

Split

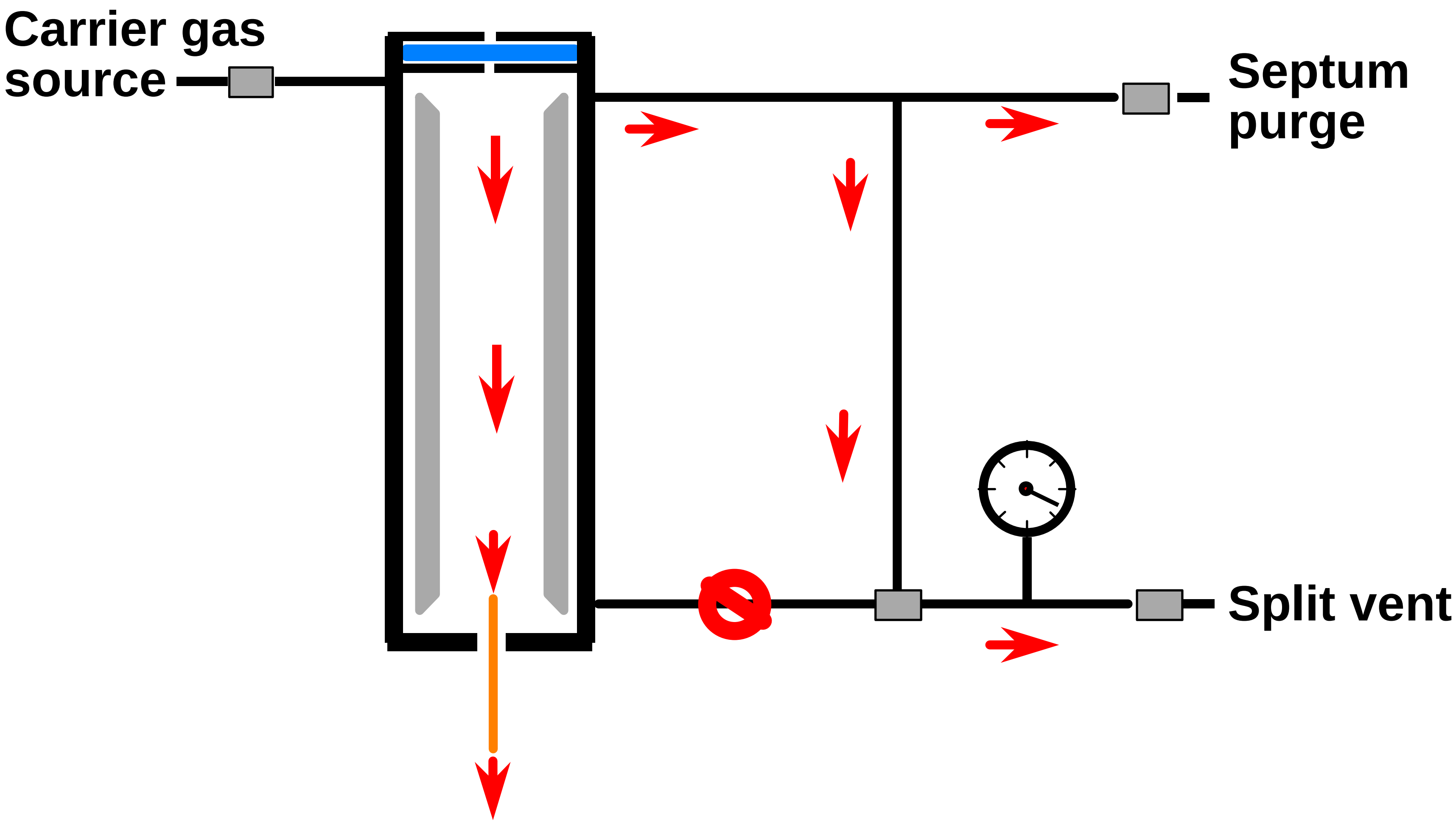
Splitless

SPLITLESS INJECTOR
Overview

- **Most of the sample is introduced into the column**
- **Used for low concentration samples**
- **Wider peaks are obtained than for split injections**

SPLITLESS INJECTOR

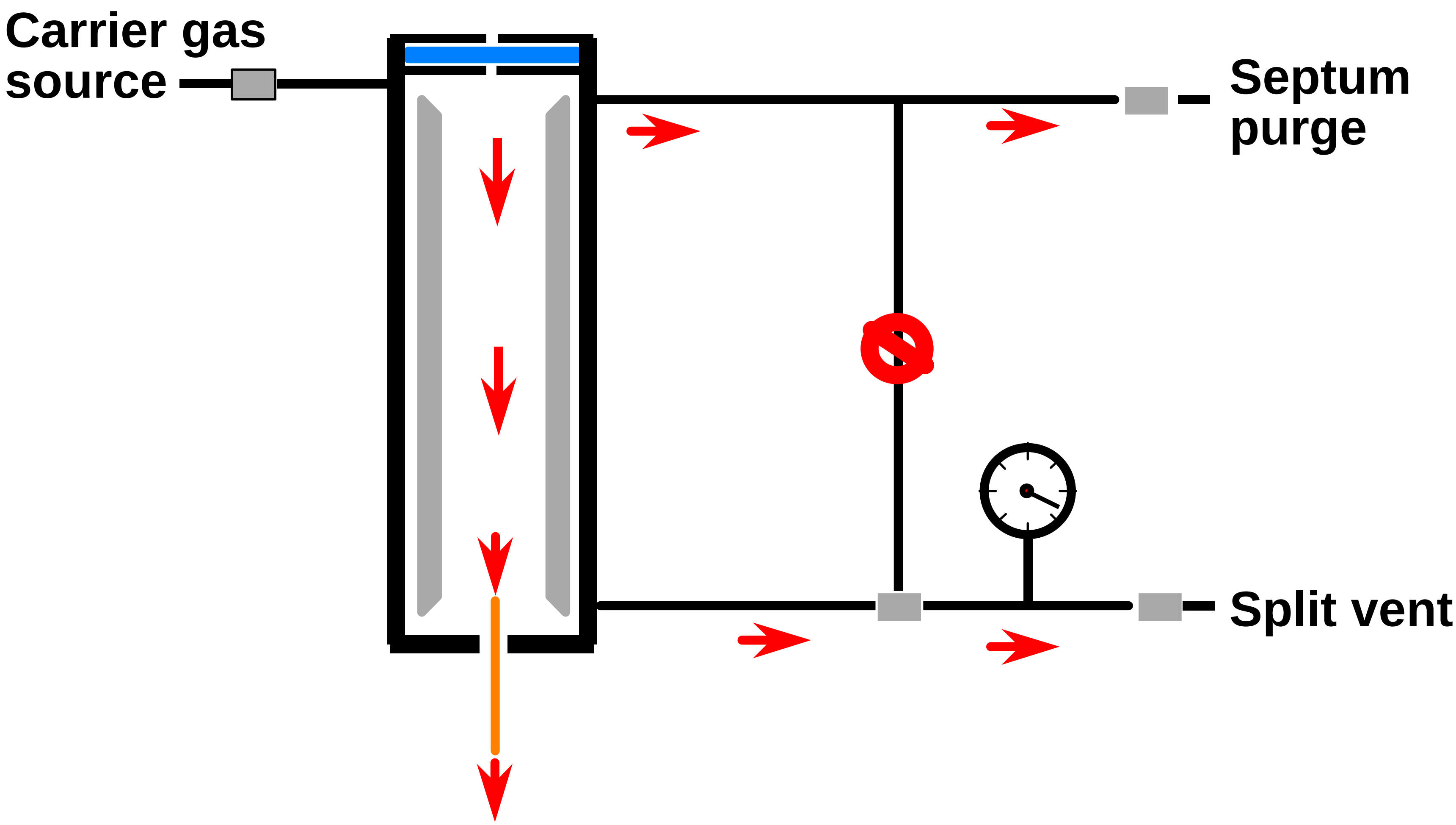
Purge Off At Injection



Flow through injector = Column flow only

SPLITLESS INJECTOR

Purge On After Injection



Flow through injector = Column flow + Split Vent Flow

SPLITLESS INJECTOR
Major Variables

- **Purge activation time**
- **Liner**
- **Injection volume**
- **Temperature**

SPLITLESS INJECTOR
Purge Activation Time

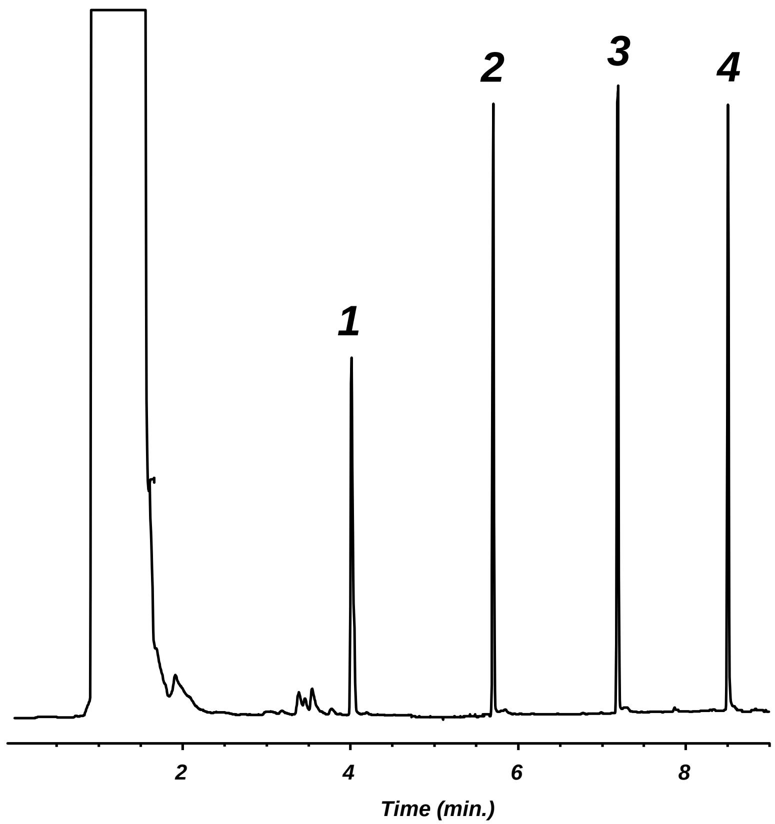
- **Purges injector of residual sample**
- **Reduces solvent front size**
- **Typically 0.25-1.5 minutes**

Longer purge time = More sample in column and larger solvent front

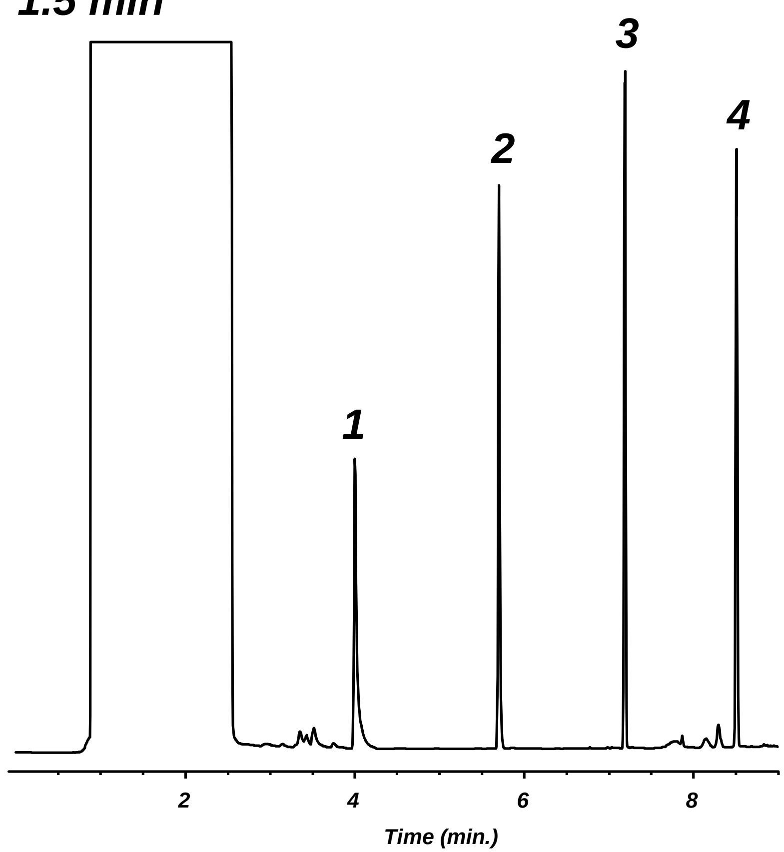
SPLITLESS INJECTOR

Purge Activation Time

0.5 min



1.5 min



DB-1, 15 m x 0.25 mm i.d., 0.25 µm
60°C for 1 min, 60-180°C at 20°/min; Helium at 30 cm/sec
1. n-decane 2. n-dodecane 3. n-tetradecane 4. n-hexadecane

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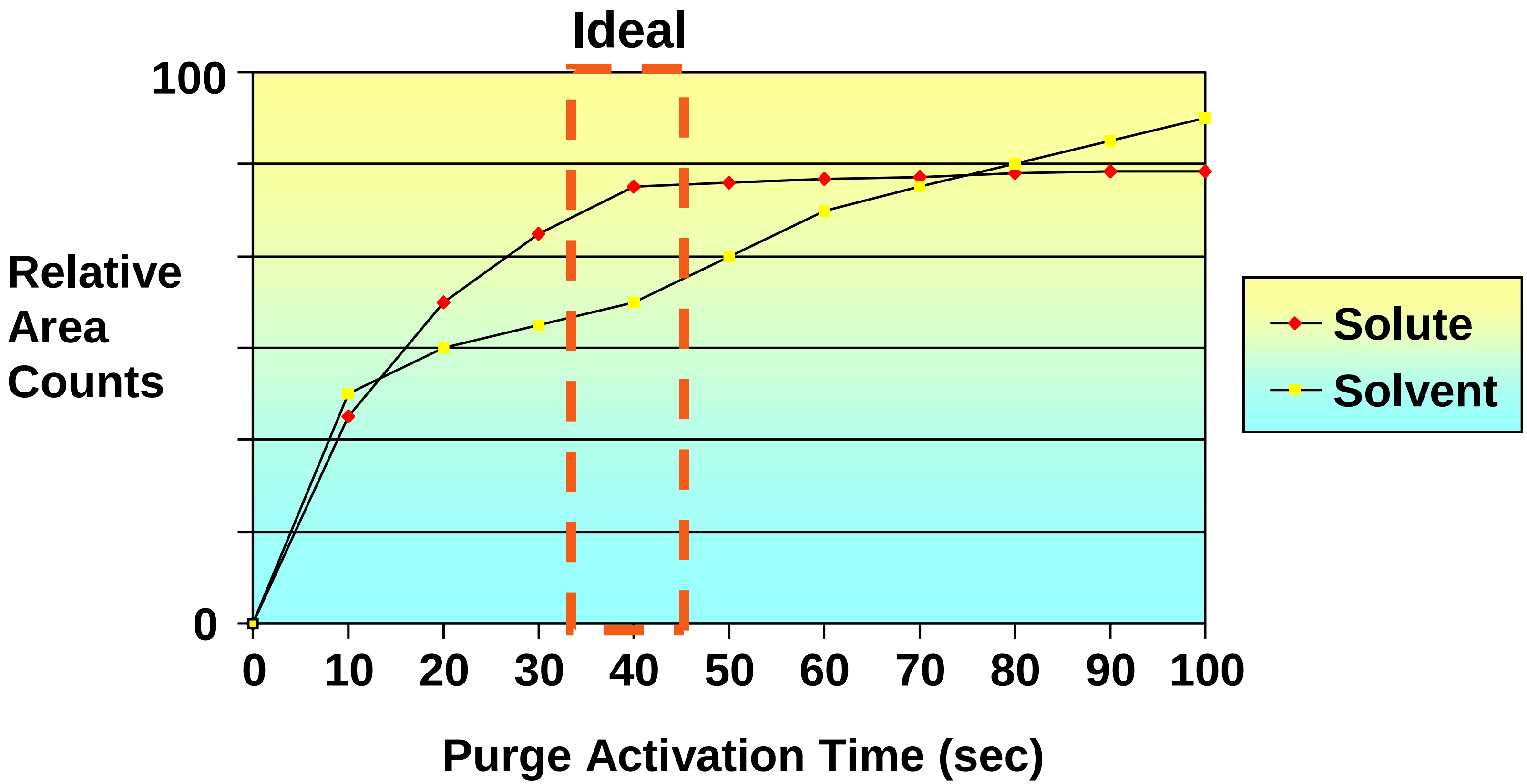
SPLITLESS INJECTOR
Purge Time vs. Peak Size



SPLITLESS INJECTOR
Purge Activation Time

- Longer time introduces more sample into the column
- Not linear
- Very long times result in large solvent fronts

Usually 0.5-0.75 min

SPLITLESS INJECTOR
Liner

- **Usually a straight tube**

- **Top and bottom restriction recommended***

***Sometimes called "double gooseneck"**

SPLITLESS INJECTOR
Injection Volume

- Typically 1-2 µl
- Not linear
- Wider peaks often occur for >2 µL
- Potential backflash problems with larger volumes

SPLITLESS INJECTOR
Injector Temperature

- Hot enough to vaporize the sample
- Long residence time of sample in the injector
- Typically 200-250°C
- Injector temperature may not be critical
- Use same temperature for reproducible results

SPLITLESS INJECTOR

Sample Re-focusing

- **Sample re-focusing improves efficiency**
- **Use low column temperature to refocus solvent**
- **Called the *solvent effect***

SPLITLESS INJECTOR

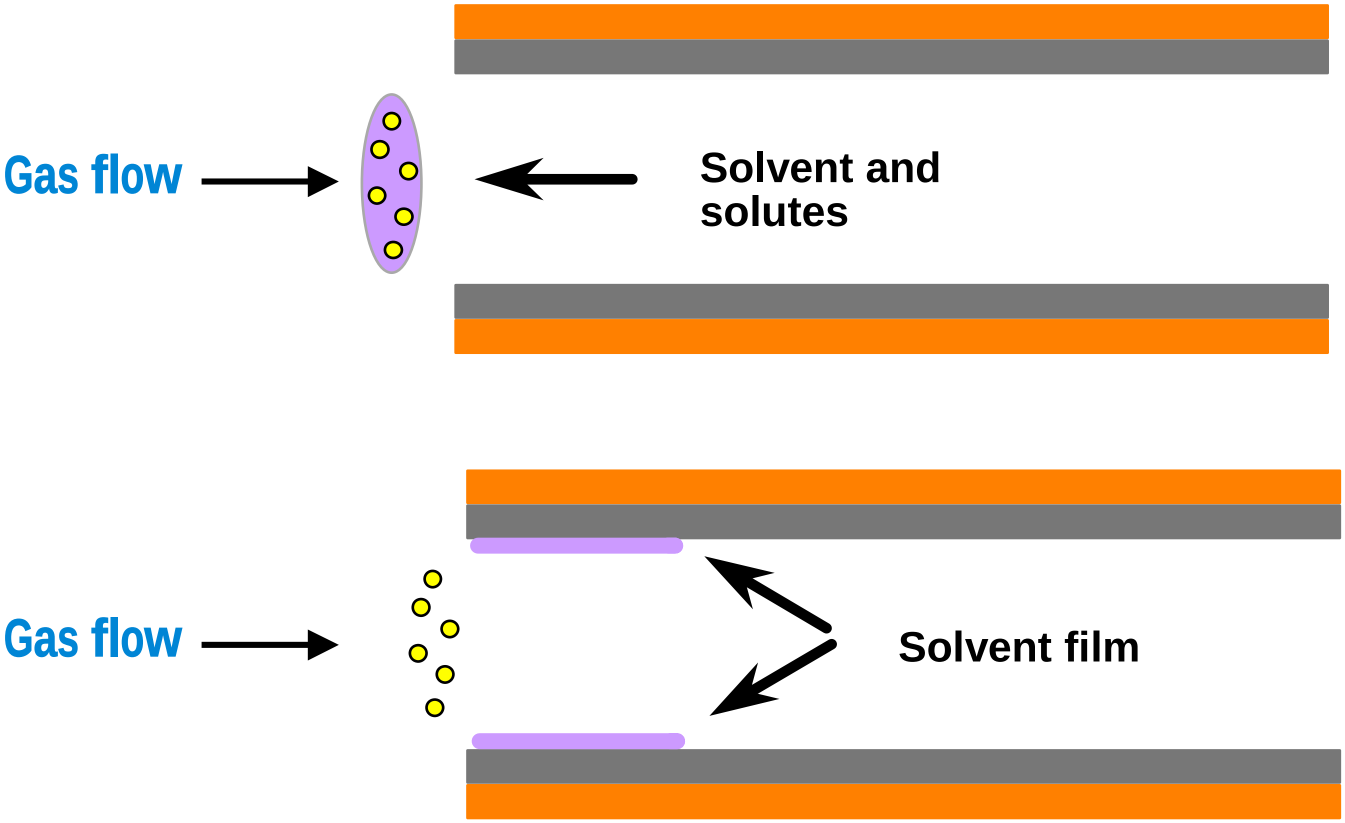
Column Temperature
Solvent Effect

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

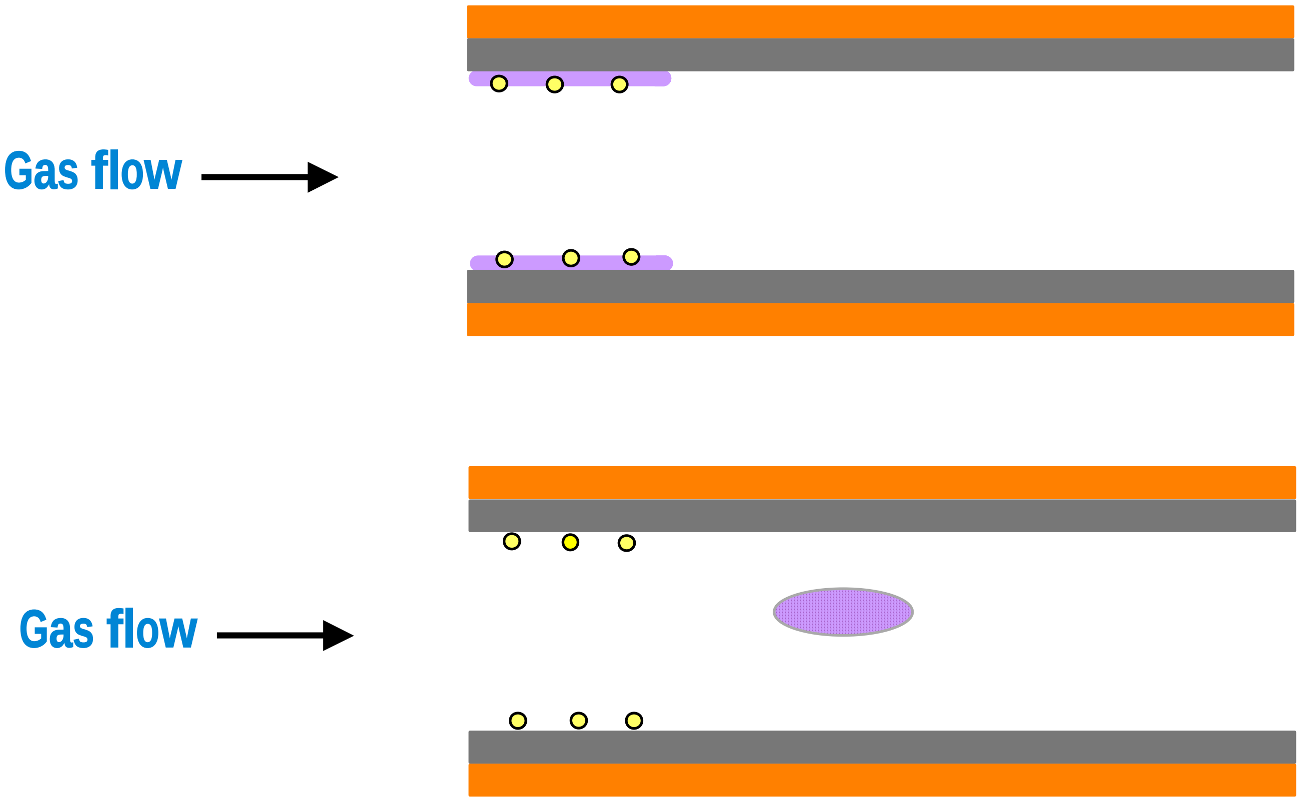
- **Required to obtain good peak shapes***

***Except if cold trapping occurs**

SPLITLESS INJECTOR
Solvent Effect

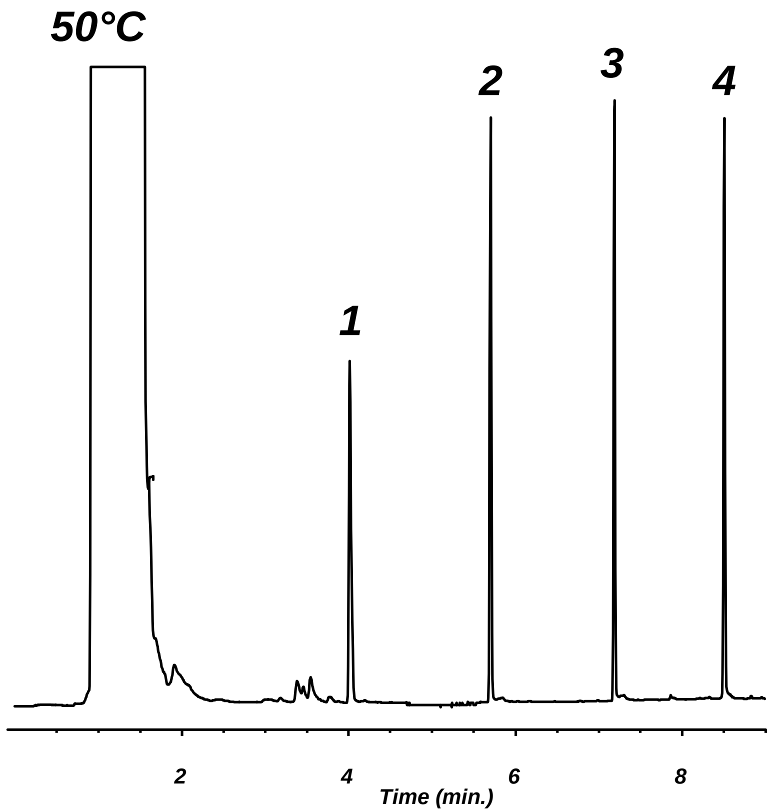


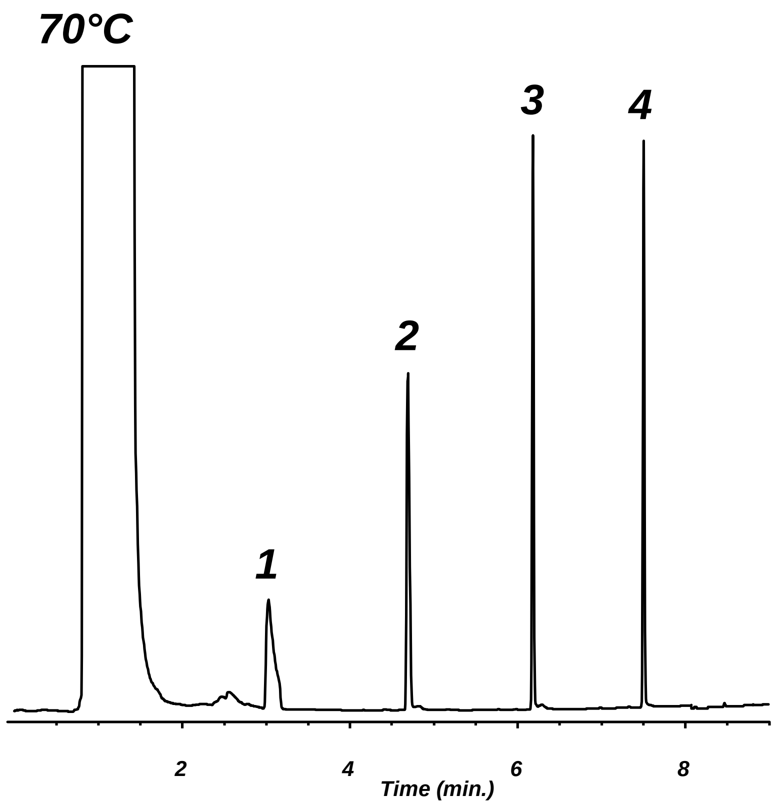
SPLITLESS INJECTOR
Solvent Effect



SPLITLESS INJECTOR

Initial Column Temperature
Hexane Solvent (BP = 68-69°C)





DB-1, 15 m x 0.25 mm i.d., 0.25 µm
50°C or 70°C for 0.5 min, to 210°C at 20°/min; Helium at 30 cm/sec
1. n-decane 2. n-dodecane 3. n-tetradecane 4. n-hexadecane

SPLITLESS INJECTOR
Cold Trapping

- **Solvent effect not always necessary**
- **If solute BP >150°C above initial column temperature, the solute will cold trap**

COLD TRAPPING

- Has the same result as the solvent effect
- Greater efficiency than solvent effect

SPLITLESS INJECTOR
Retention Gap

- Retention gaps often improve peak shapes

- Greatest impact on earlier eluting peaks, especially if there is a polarity mismatch between solvent and phase

SPLITLESS
Sample Solvent

- Avoid very low or high BP solvents

- Solvent should be lowest BP sample component

- Avoid mixed solvents

Break Number 2

- For Questions and Answers
- Press *1 on Your Phone to
- Ask a Question

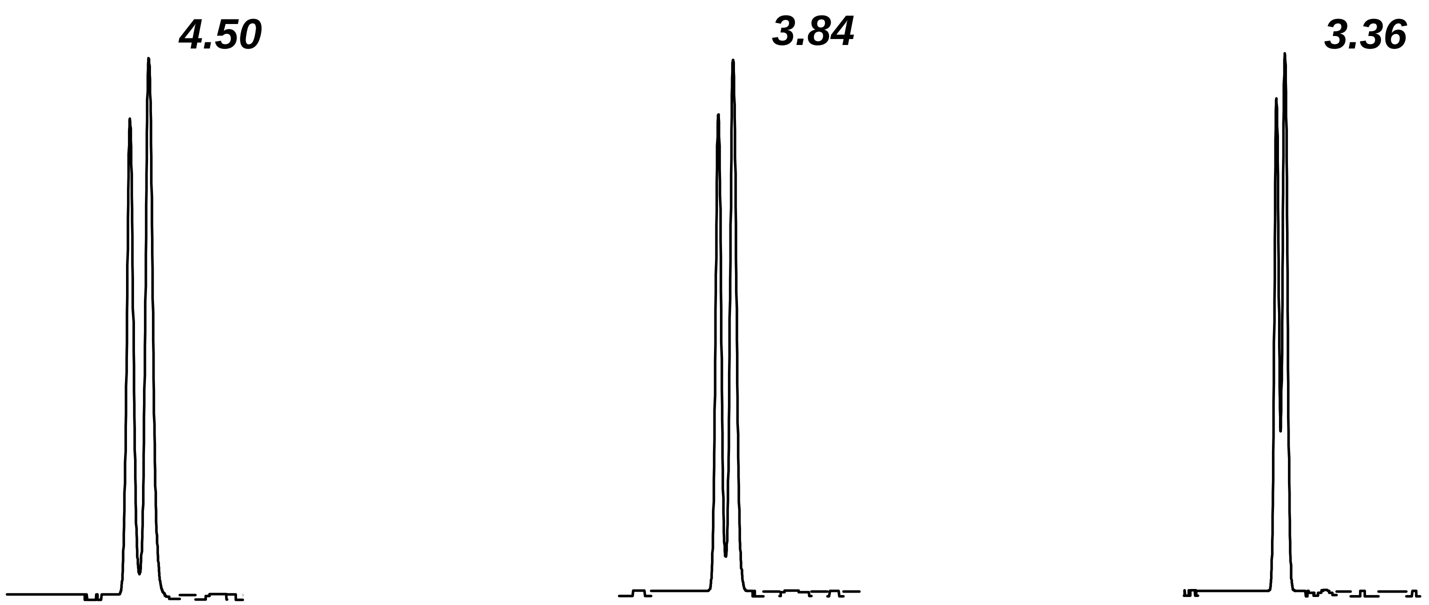
CARRIER GAS

Mobile Phase

CARRIER GAS

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

RESOLUTION VS. LINEAR VELOCITY
Helium



30 cm/sec	35 cm/sec	40 cm/sec
R = 1.46	*R = 1.31*	*R = 0.97*
4.4 psig	*5.1 psig*	*5.8 psig*

DB-1, 15 m x 0.32 mm ID, 0.25 um
60°C isothermal
1,3- and 1,4-Dichlorobenzene

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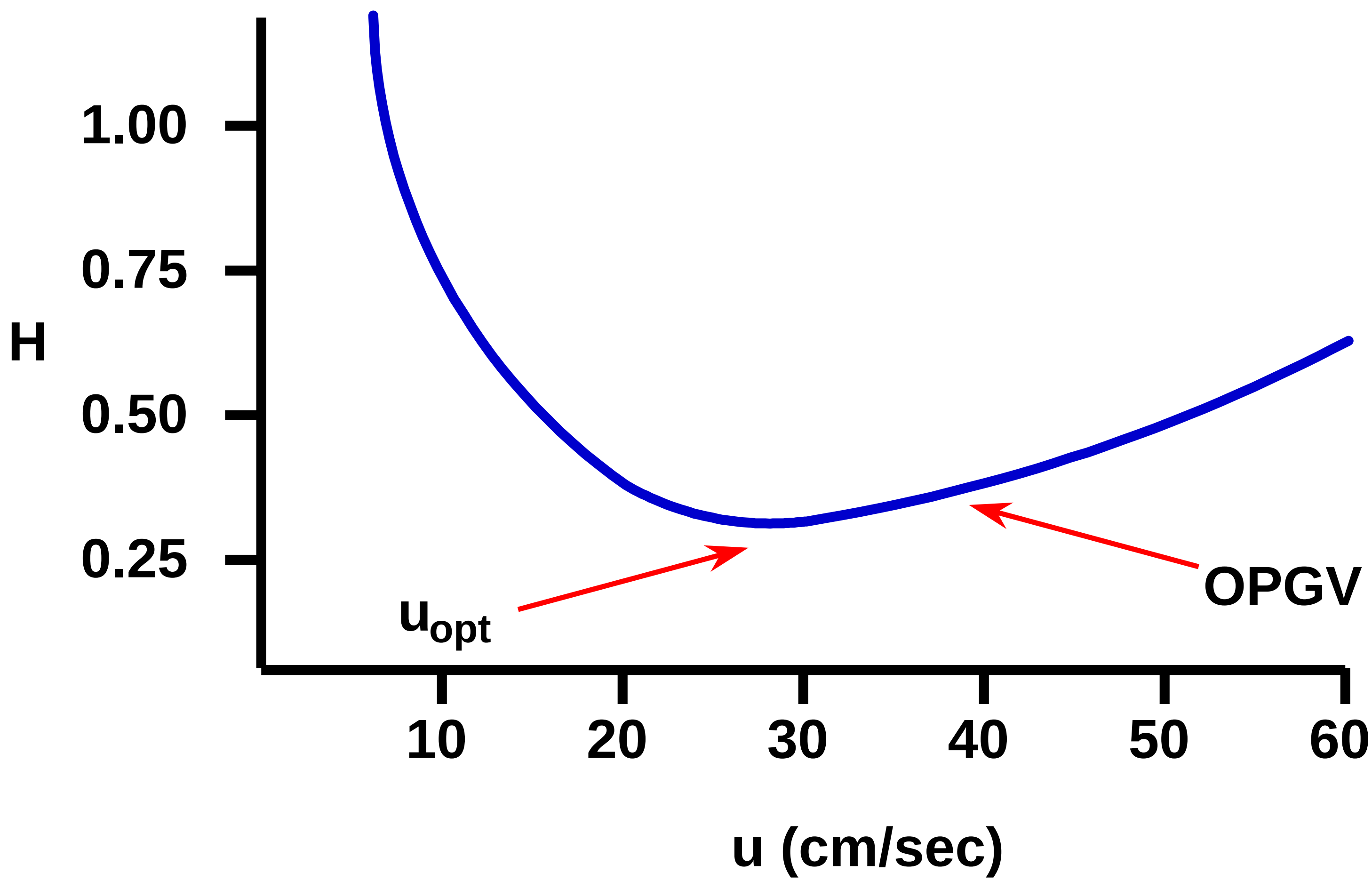
VAN DEEMTER CURVE



$\bar{u}_{opt}$ and OPGV

$\bar{u}_{opt}$:

Maximum efficiency

OPGV:

Optimal practical gas velocity

Maximum efficiency per unit time

1.5 - 2x u_{opt}

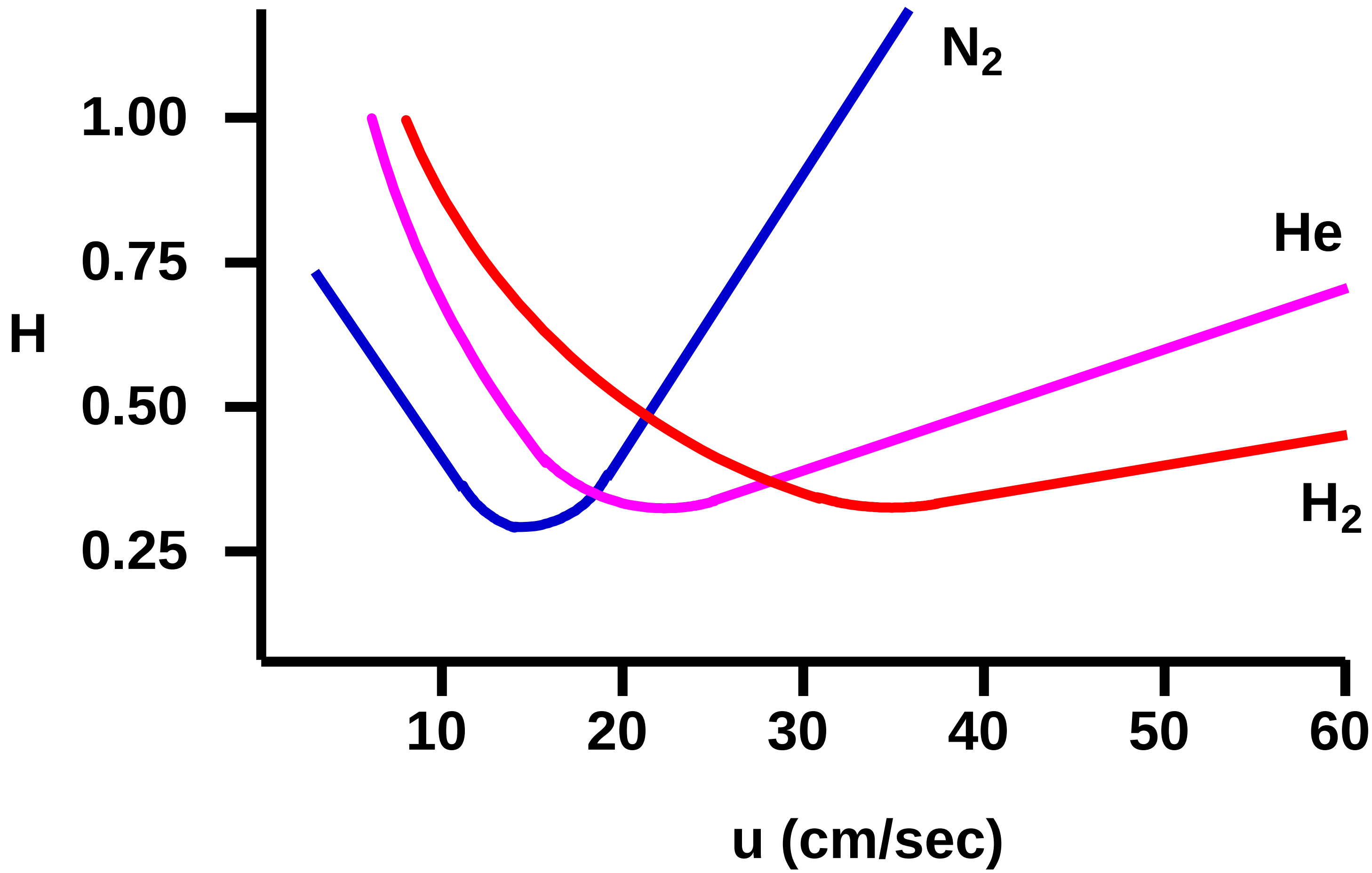
COMMON CARRIER GASES

Nitrogen

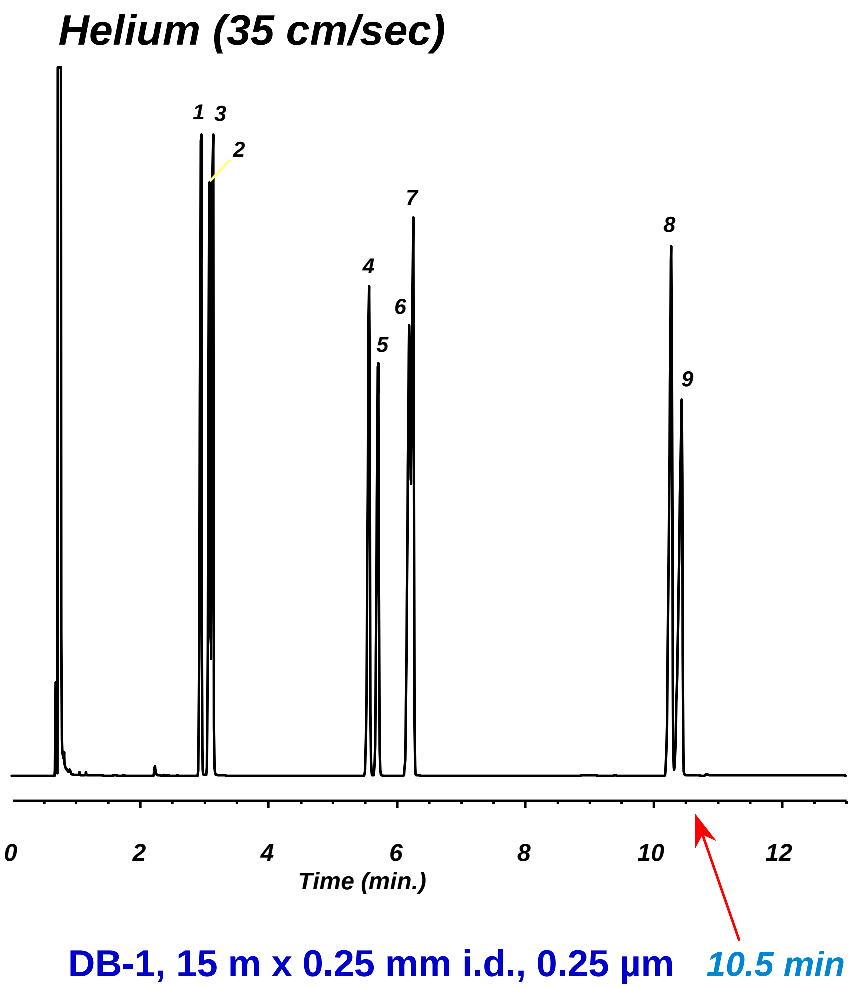
Helium

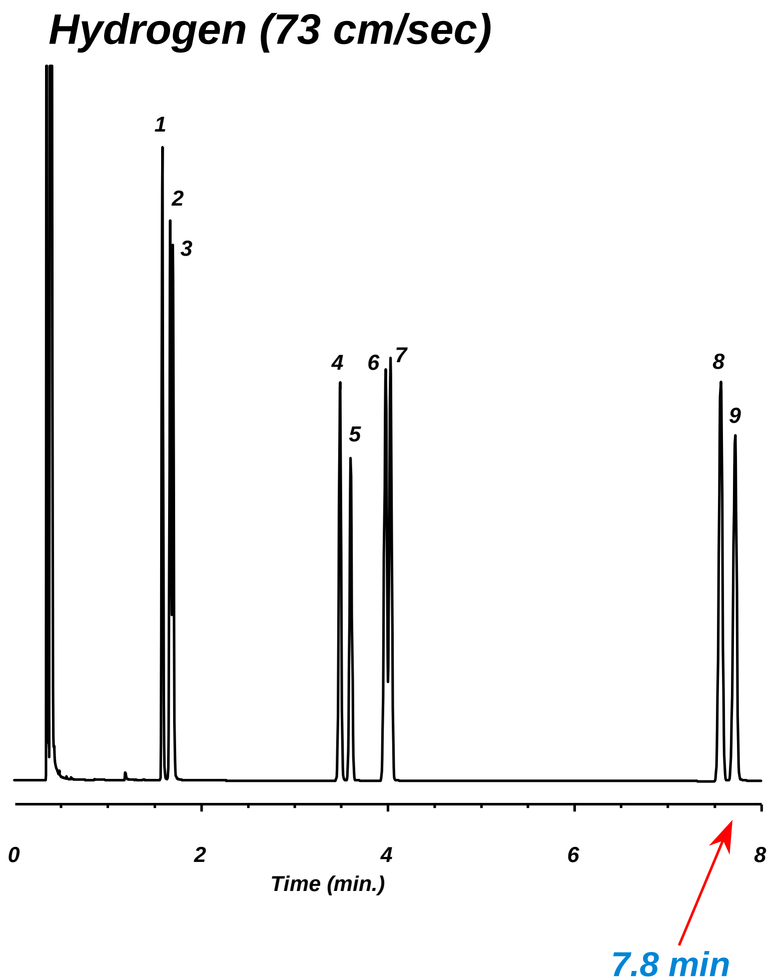
Hydrogen

VAN DEEMTER CURVES



CARRIER GAS
Helium vs. Hydrogen





DB-1, 15 m x 0.25 mm i.d., 0.25 µm
50°C for 2 min, 50-110°C at 20°/min

CARRIER GAS

Gas	Advantages	Disadvantages
Nitrogen	Cheap, Readily available	Long run times
Helium	Good compromise, Safe	Expensive
Hydrogen	Shorter run times, Cheap	Explosive

Hydrogen is difficult to explode under GC conditions

COLUMN TEMPERATURE

- **Most powerful variable**
- **Most difficult to develop**
- **Often involves trial and error**

COLUMN TEMPERATURE

- Isothermal
- Temperature Program

COLUMN TEMPERATURE

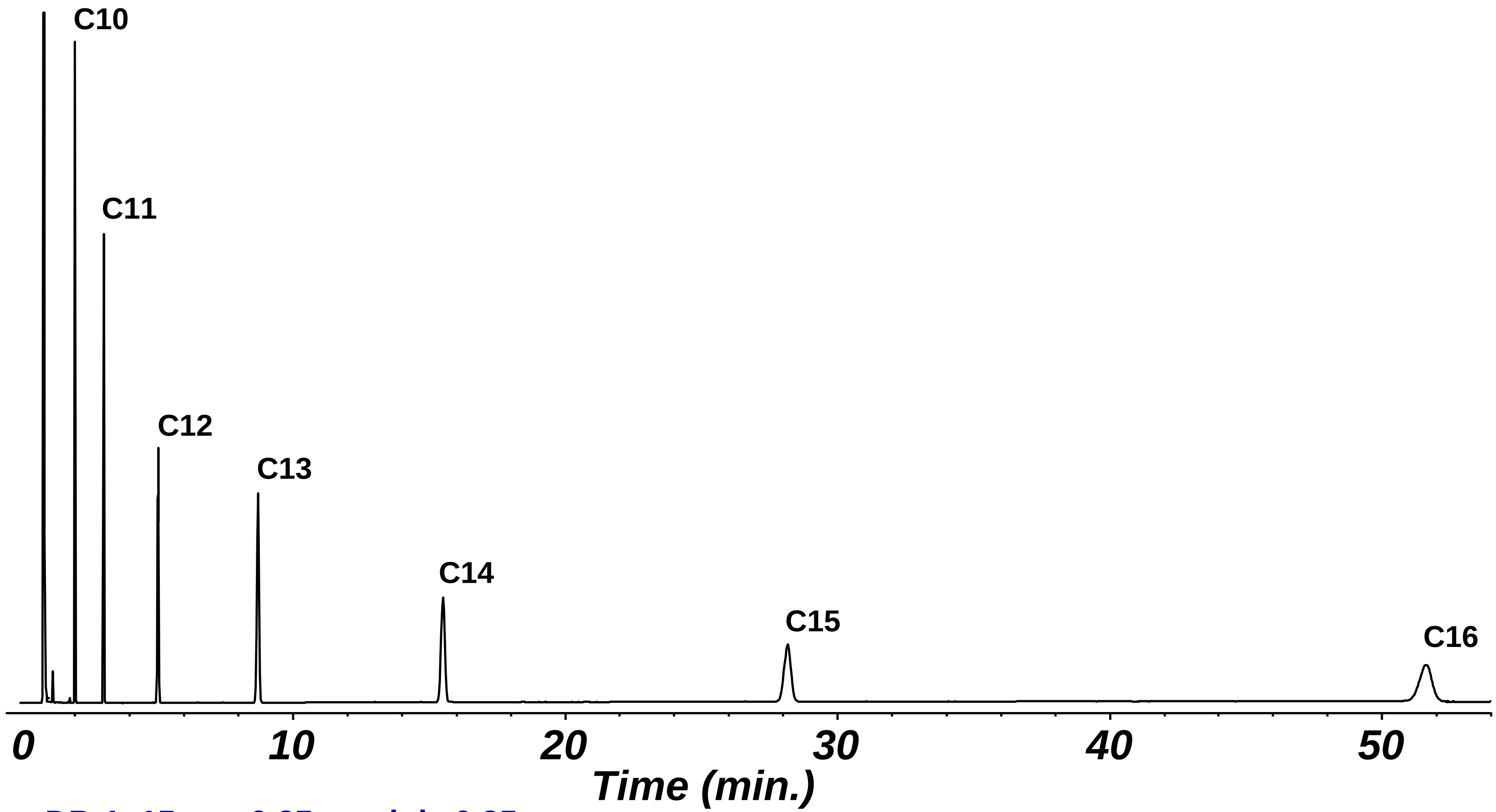
Isothermal

- **For compounds with similar retention**

- **Peak widths increase as retention increases**

COLUMN TEMPERATURE

Isothermal



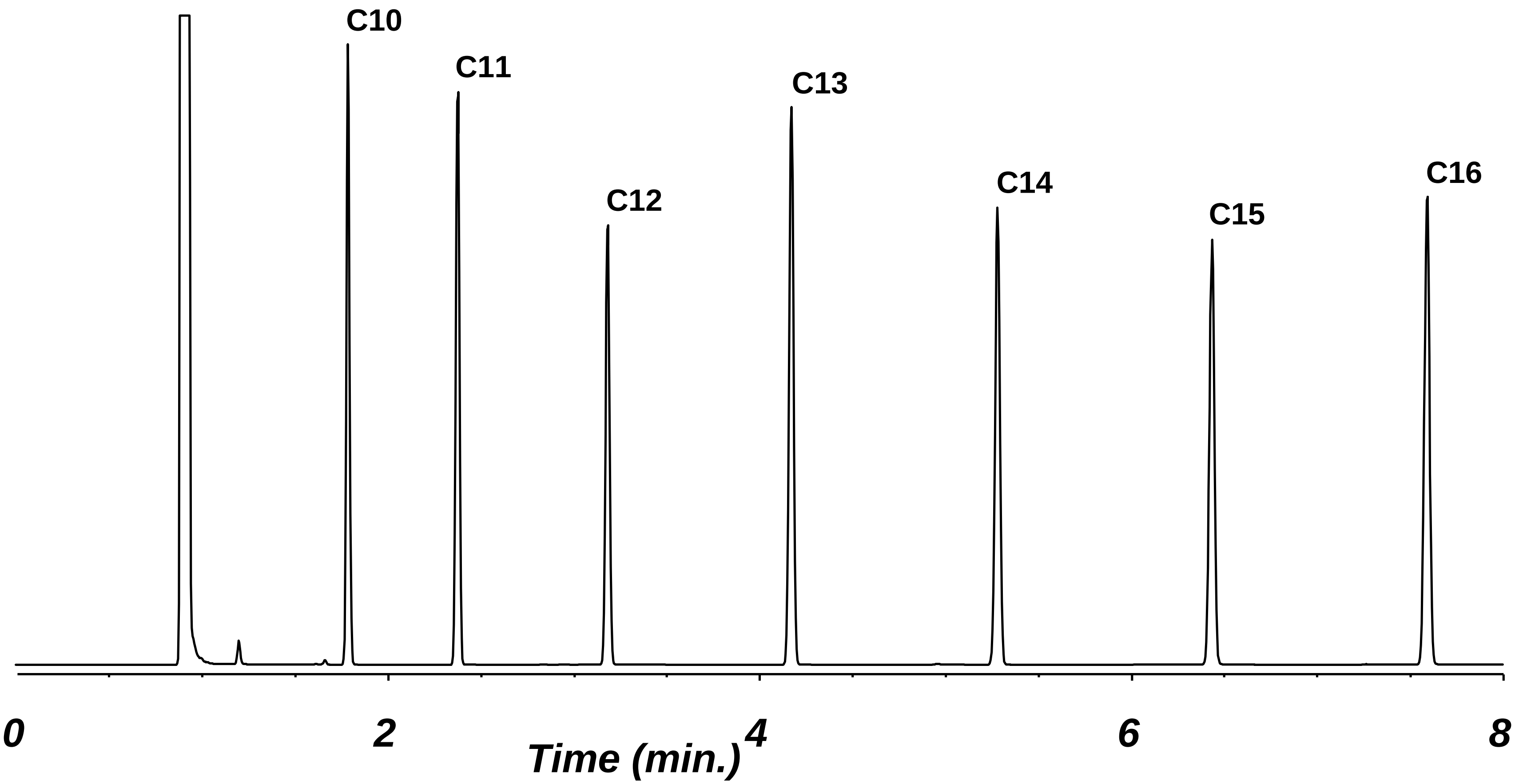
DB-1, 15 m x 0.25 mm i.d., 0.25 µm
100°C; Helium at 30 cm/sec
n-alkanes

COLUMN TEMPERATURE

Temperature Program

- For compounds with dissimilar retention
- Little peak broadening with increasing retention
- Requires cool down between analyses

COLUMN TEMPERATURE
Temperature Program



DB-1, 15 m x 0.25 mm i.d., 0.25 µm
60°C for 1 min, 60-180°C at 20°/min; Helium at 30 cm/sec
n-alkanes

COLUMN TEMPERATURE
Developing Temperature Programs

- More difficult prediction and development
- Natural log (ln) relationship between retention and temperature
- Factor in cool down time

DEVELOPING TEMPERATURE PROGRAMS

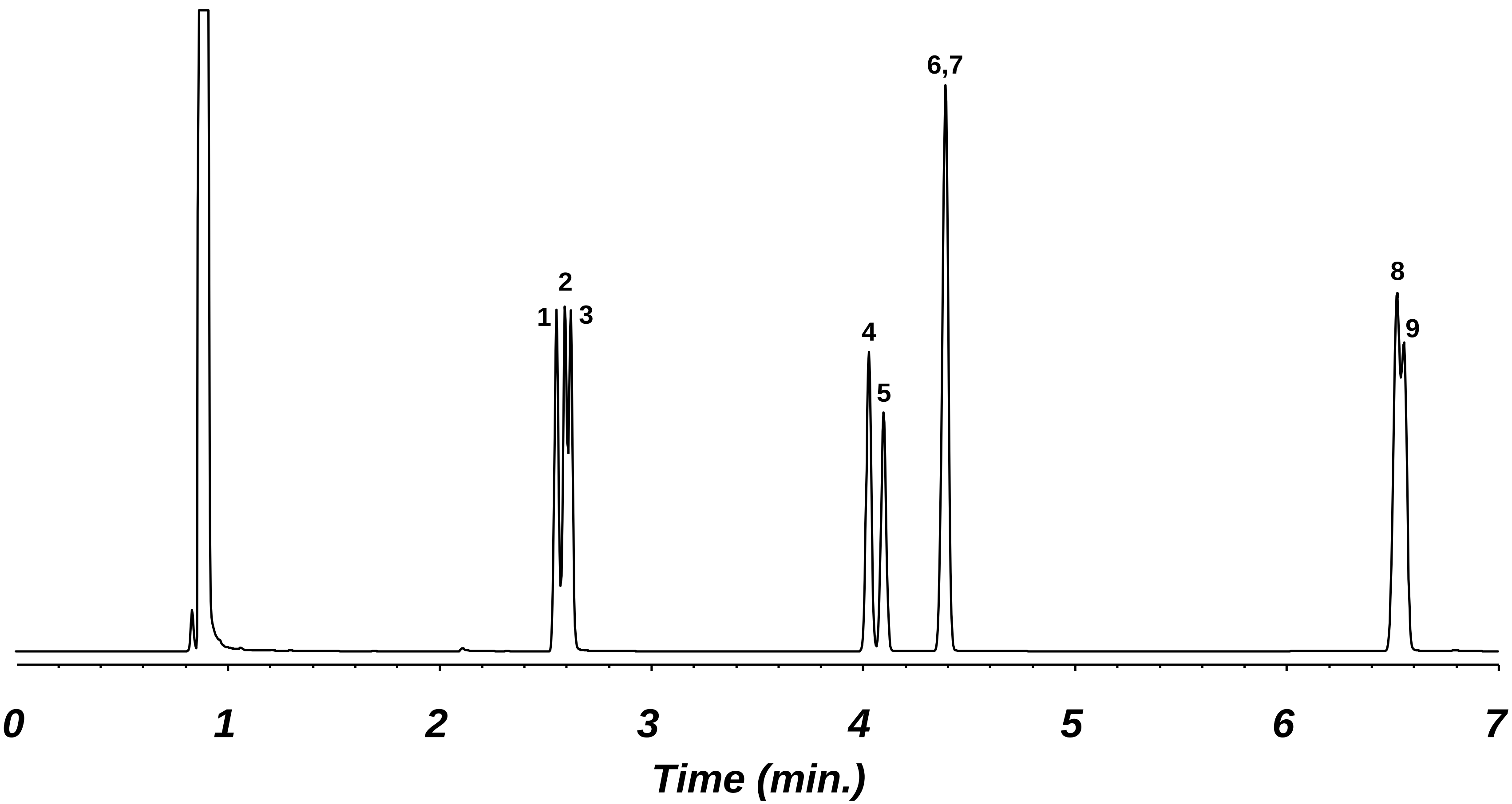
First Step - Linear Program

- **Initial temperature: 40-50°C**
- **Ramp rate: 10°C/min**
- **Final temperature: Column's upper limit***
- **Final hold: Until the last peak elutes**

***Or until the last peak elutes from the column**

DEVELOPING TEMPERATURE PROGRAMS

Linear Program
50-130°C at 10°/min



DB-1, 15 m x 0.25 mm i.d., 0.25 µm

CARRIER GAS
Compound List for Chromatograms

Peak	Compound
1	3-heptanone
2	2-heptanone
3	cyclohexanone
4	1,3-dichlorobenzene
5	1,4-dichlorobenzene
6	1,2-dichlorobenzene
7	iodobenzene
8	naphthalene
9	3-nitrobenzene

DEVELOPING TEMPERATURE PROGRAMS
Second Step

Change initial hold time

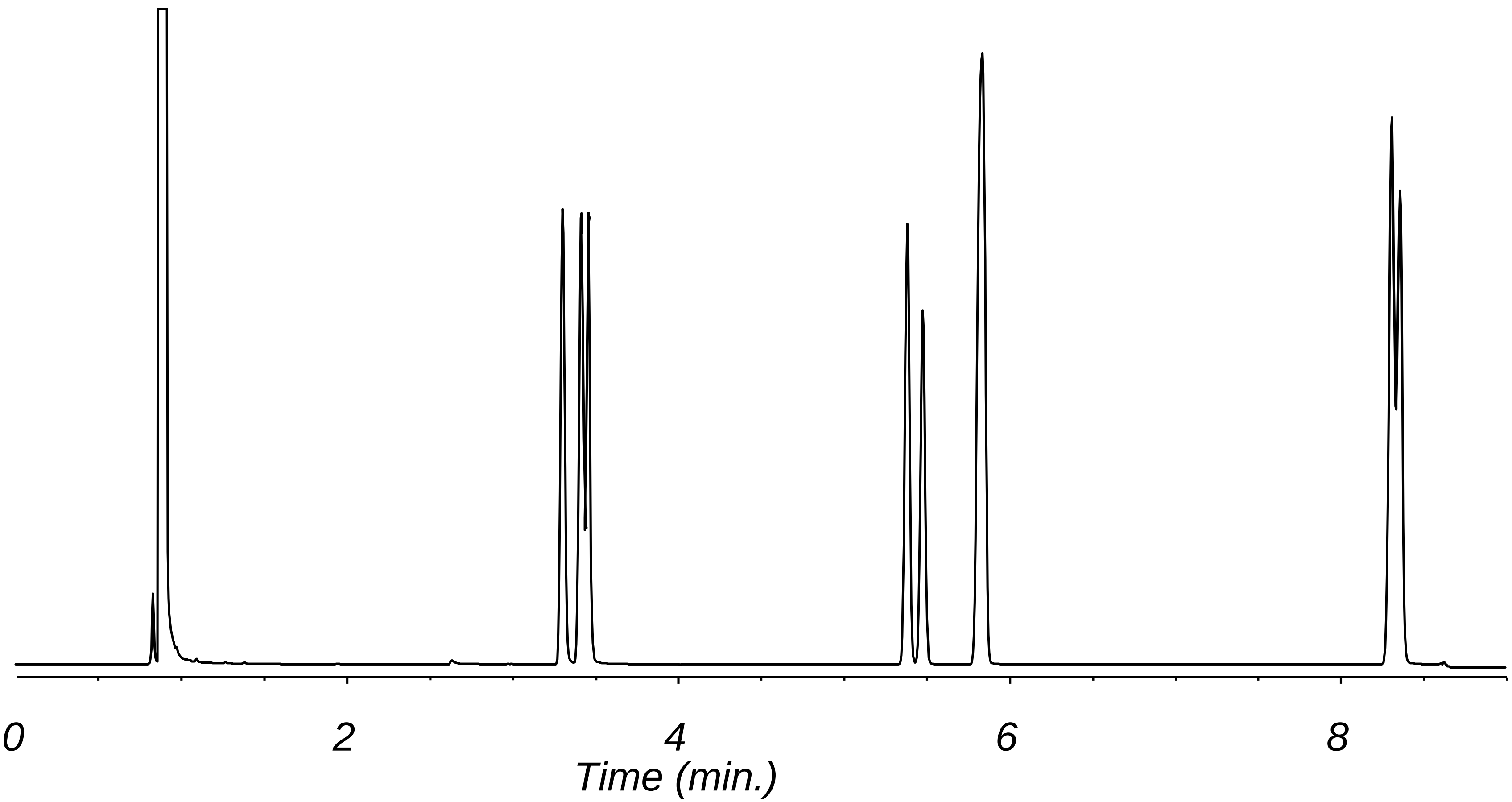
or

Change initial temperature

DEVELOPING TEMPERATURE PROGRAMS

Increase Initial Hold Time

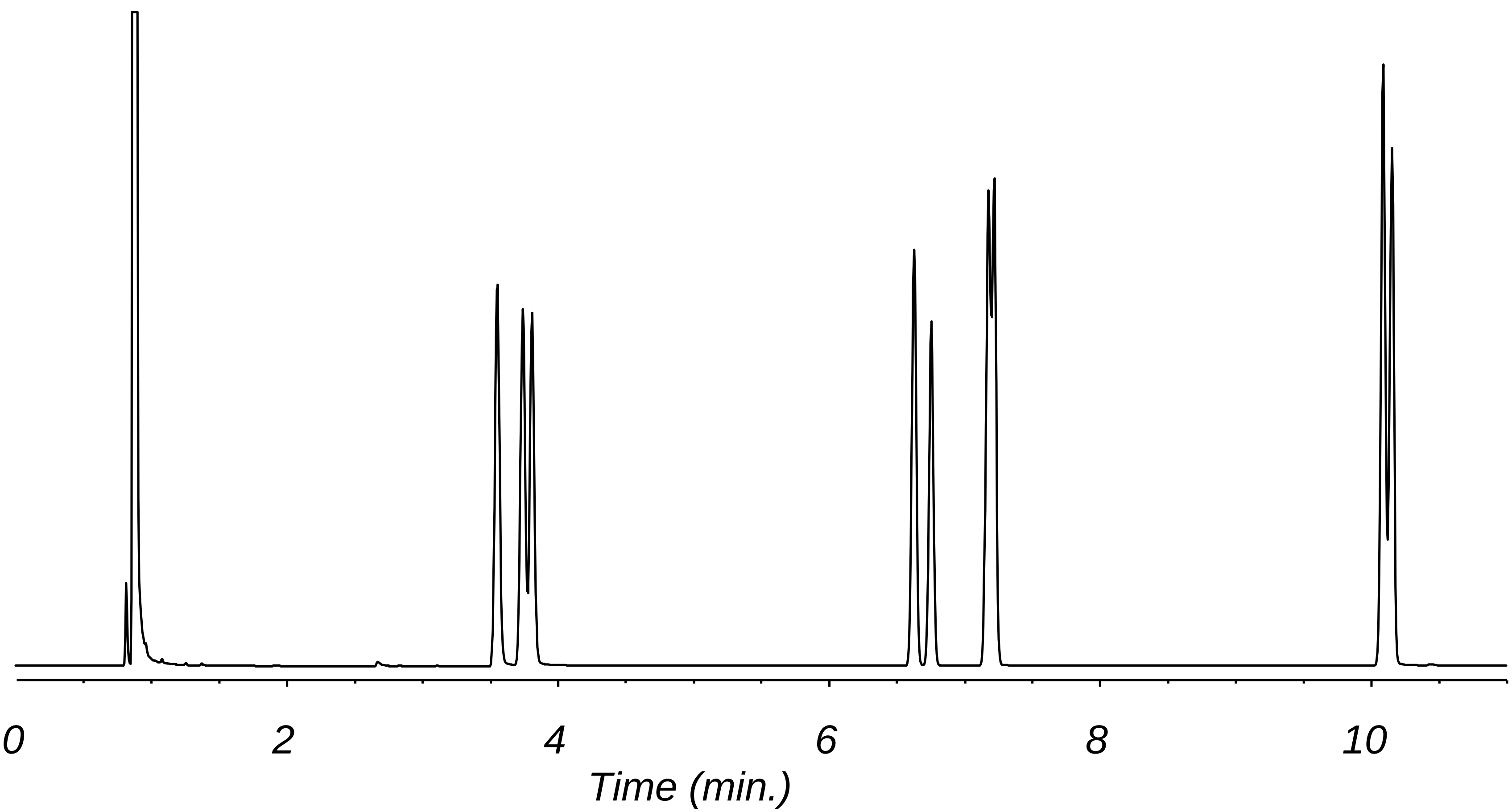
50°C for 2 min, 50-130°C at 10°/min



DEVELOPING TEMPERATURE PROGRAMS

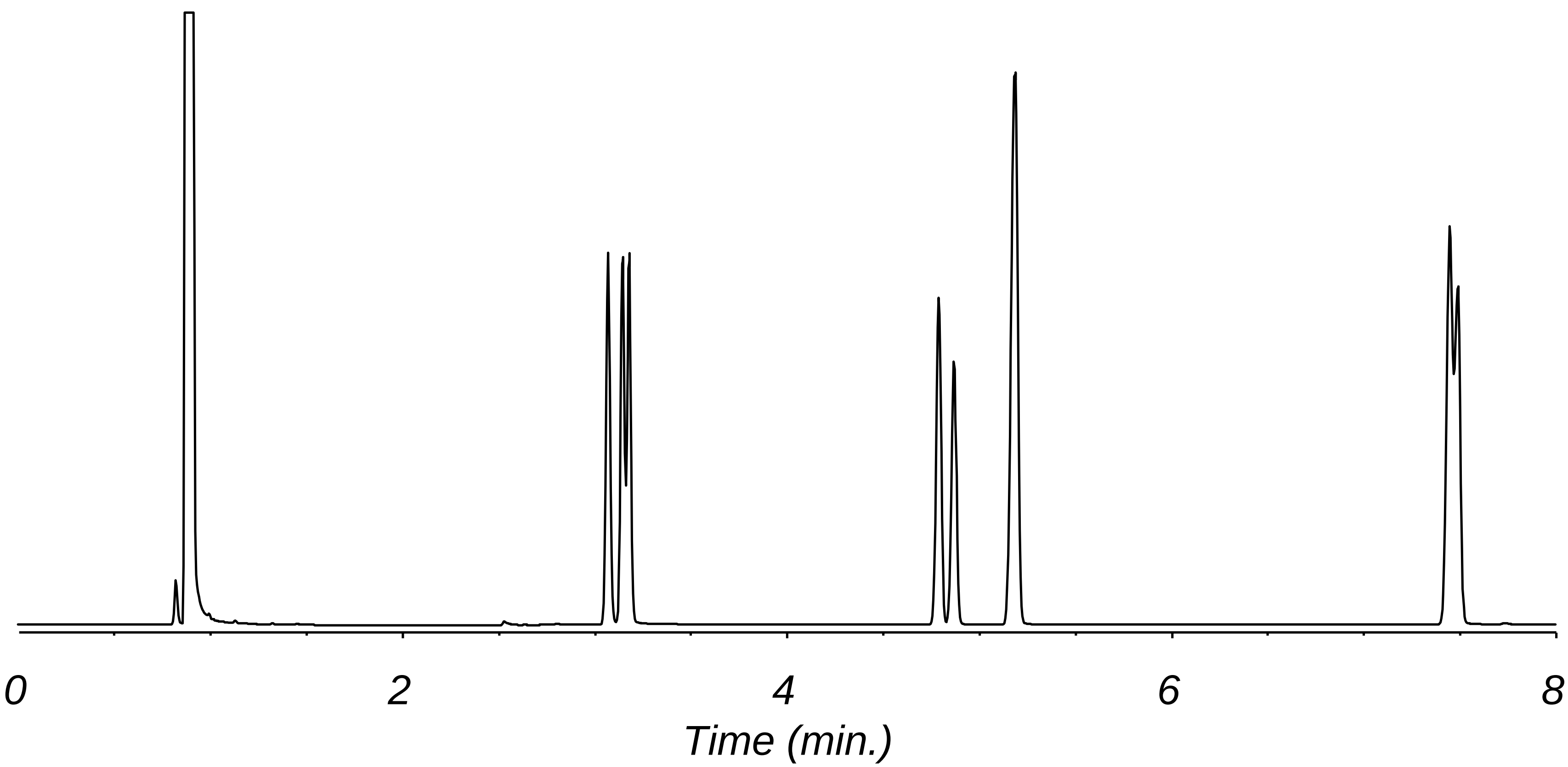
Increase Initial Hold Time

50°C for 4 min, 50-130°C at 10°/min



DEVELOPING TEMPERATURE PROGRAMS

Decrease Initial Temperature
40-130°C at 10°/min



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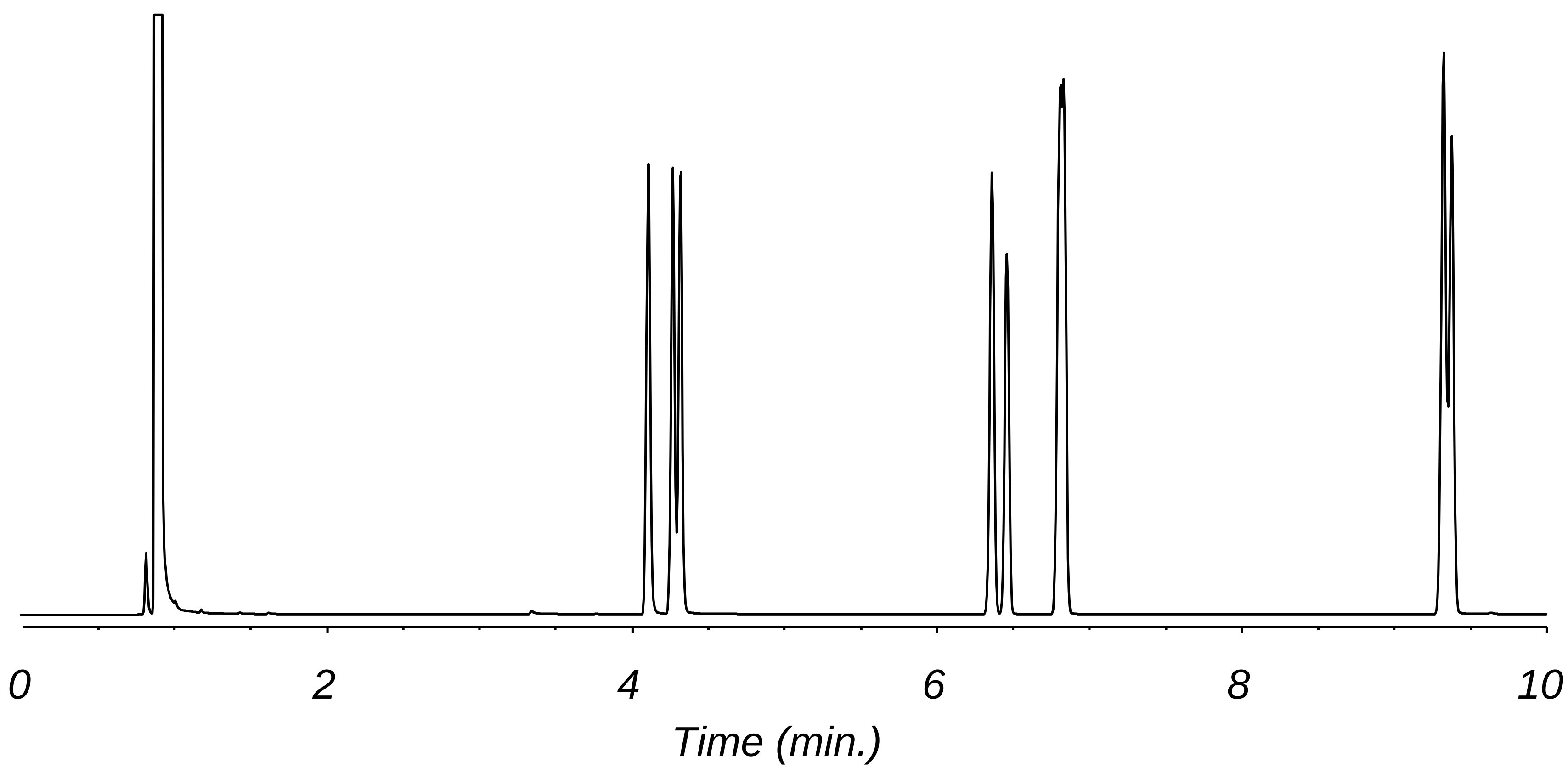
DEVELOPING TEMPERATURE PROGRAMS

Decrease Initial Temperature & Increase hold

40°C for 2 min, 40-130°C at 10°/min

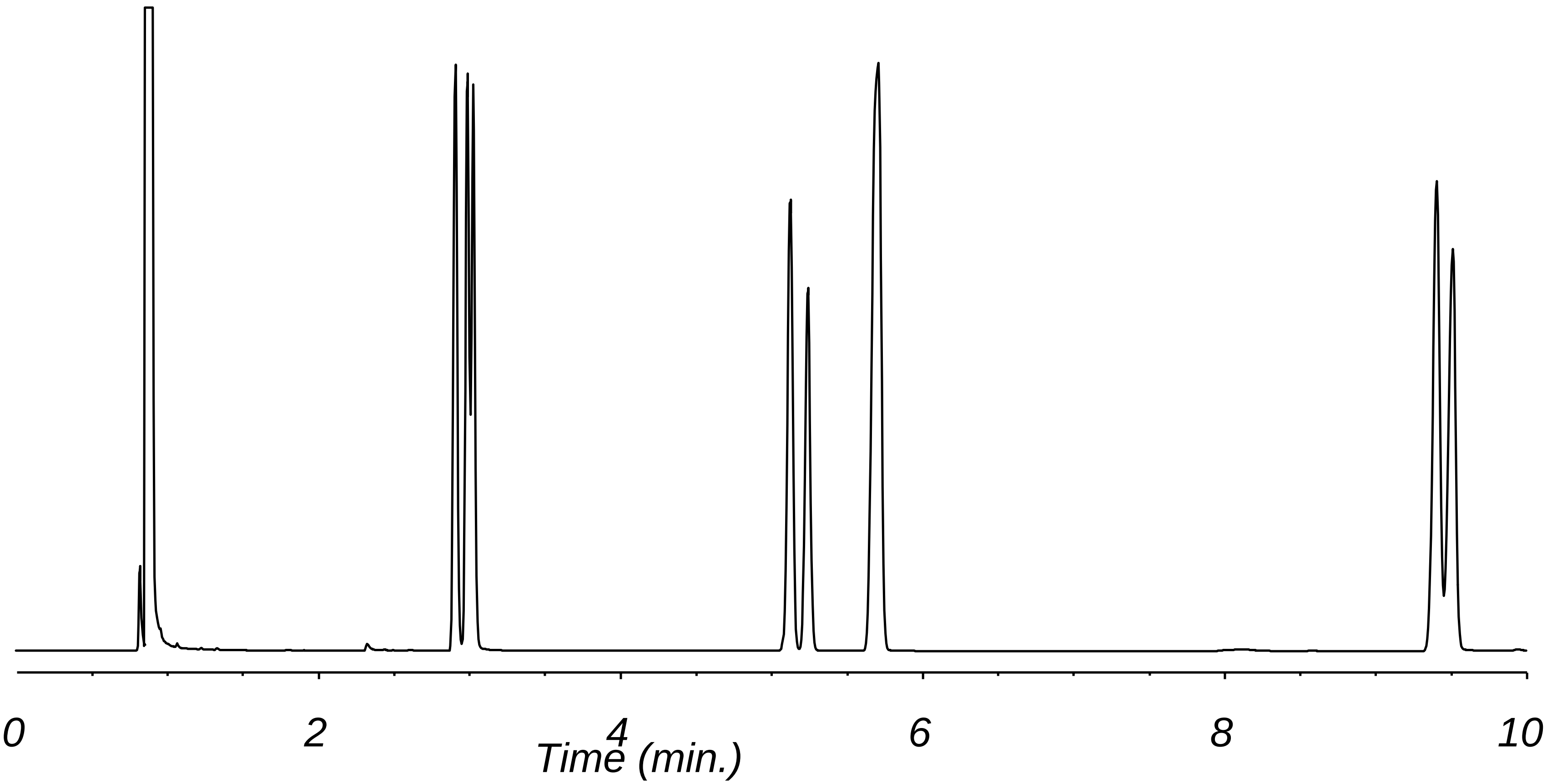


DEVELOPING TEMPERATURE PROGRAMS
Third Step

- Change the ramp rate

- ±5°C/min per change

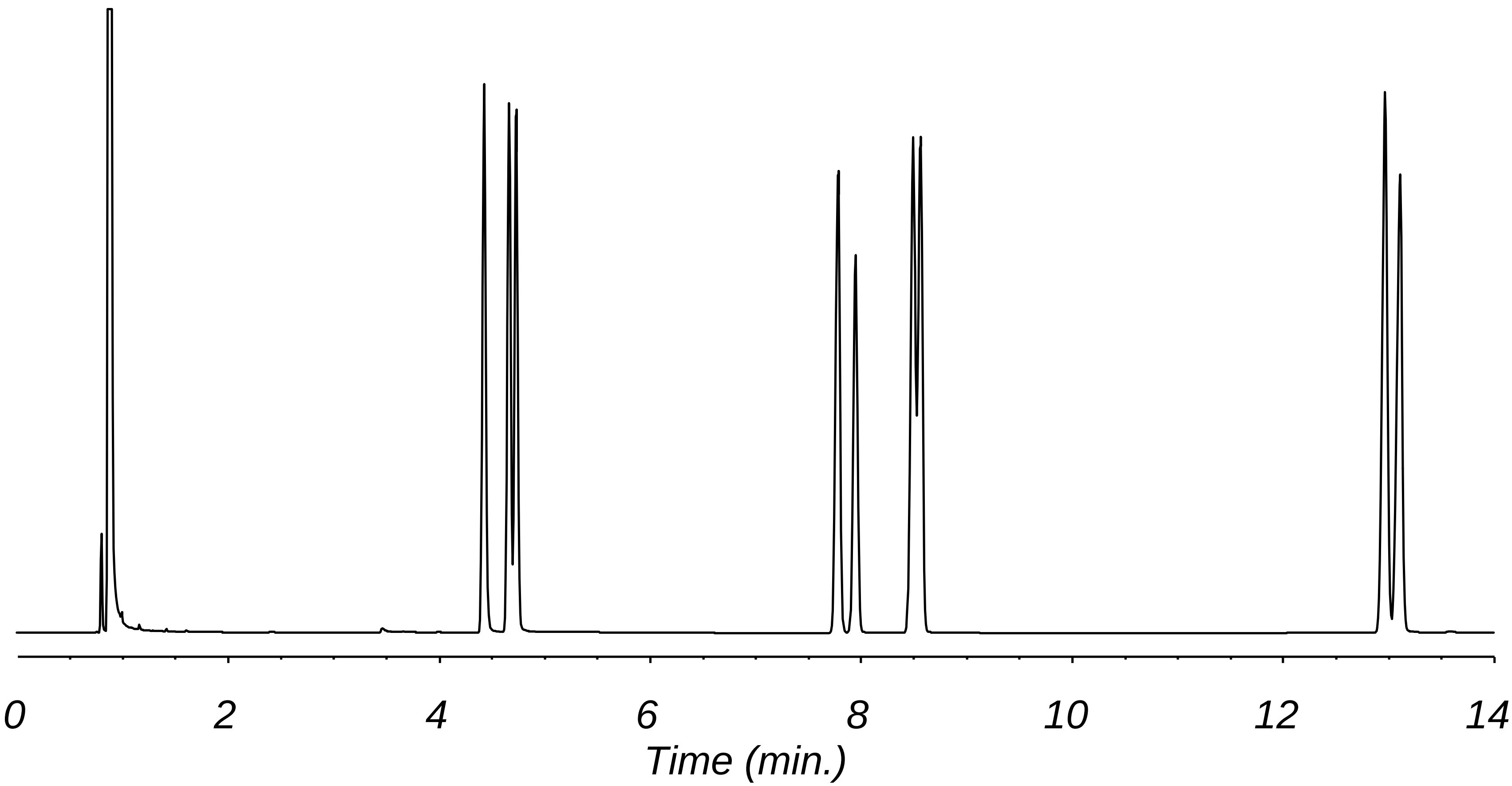
DEVELOPING TEMPERATURE PROGRAMS
50-120°C at 5°/min



DB-1, 15 m x 0.25 mm i.d., 0.25 µm

DEVELOPING TEMPERATURE PROGRAMS

40°C for 2 min, 40-120°C at 5°/min



DB-1, 15 m x 0.25 mm i.d., 0.25 µm

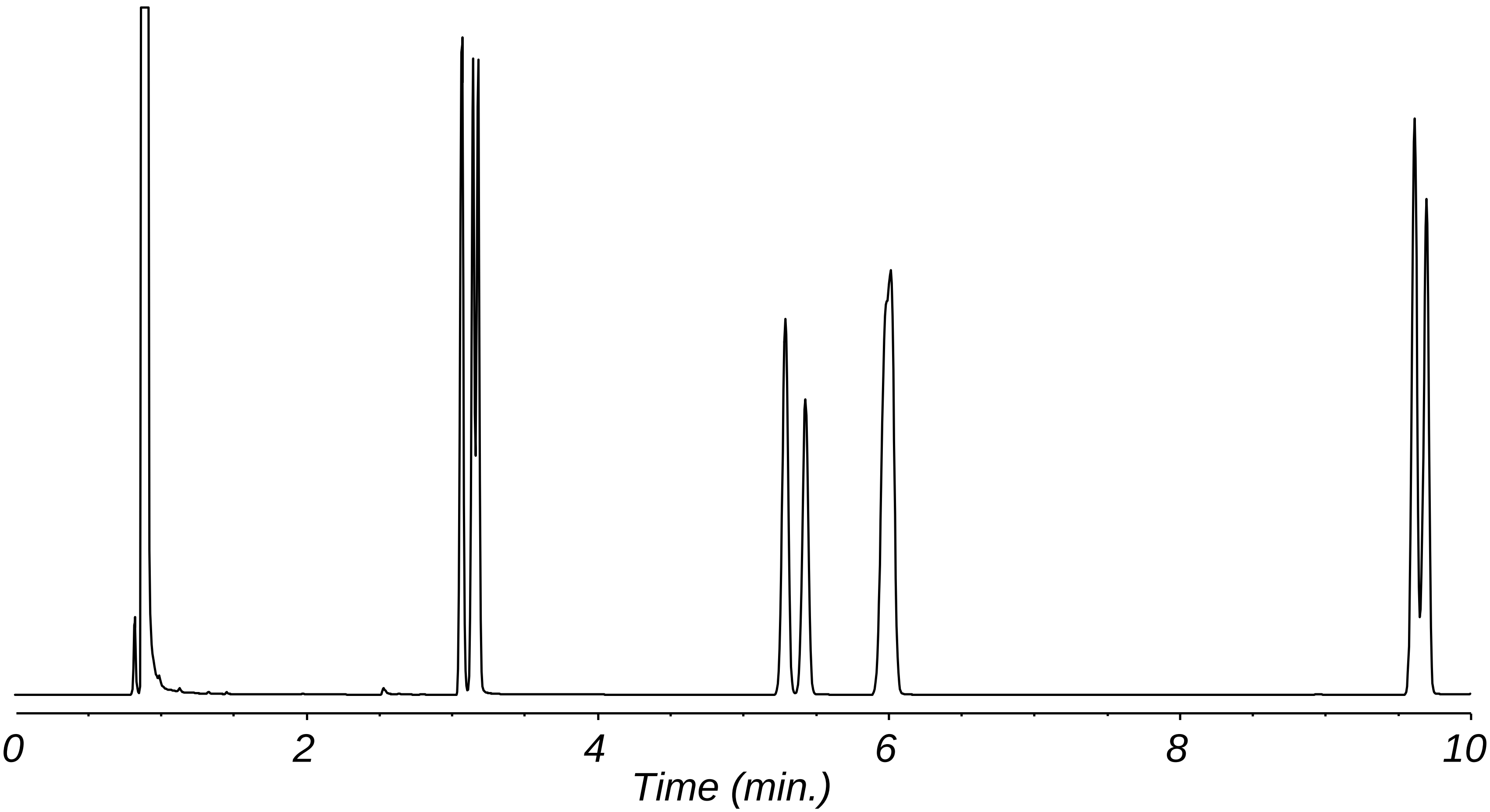
DEVELOPING TEMPERATURE PROGRAMS

Mid Ramp Holds

- Isothermal portion during the temperature program
- 2-5 minute hold
- 20-30°C below elution temperature of peaks

DEVELOPING TEMPERATURE PROGRAMS

40-70°C at 10°/min, 70°C for 3 min, 70-120°C at 10°/min
Hold at 20° below elution of peaks 6 & 7



DB-1, 15 m x 0.25 mm i.d., 0.25 µm

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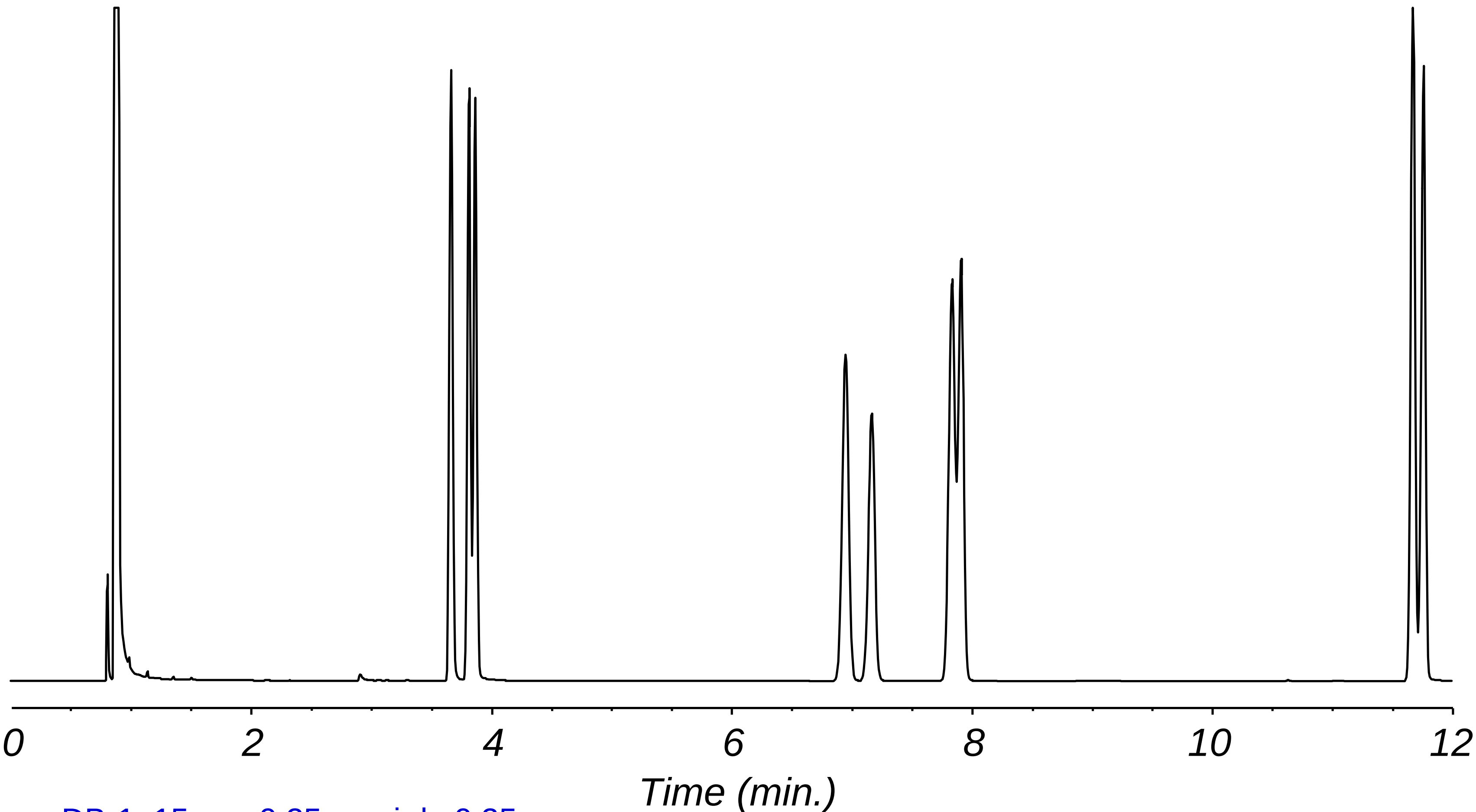
DEVELOPING TEMPERATURE PROGRAMS

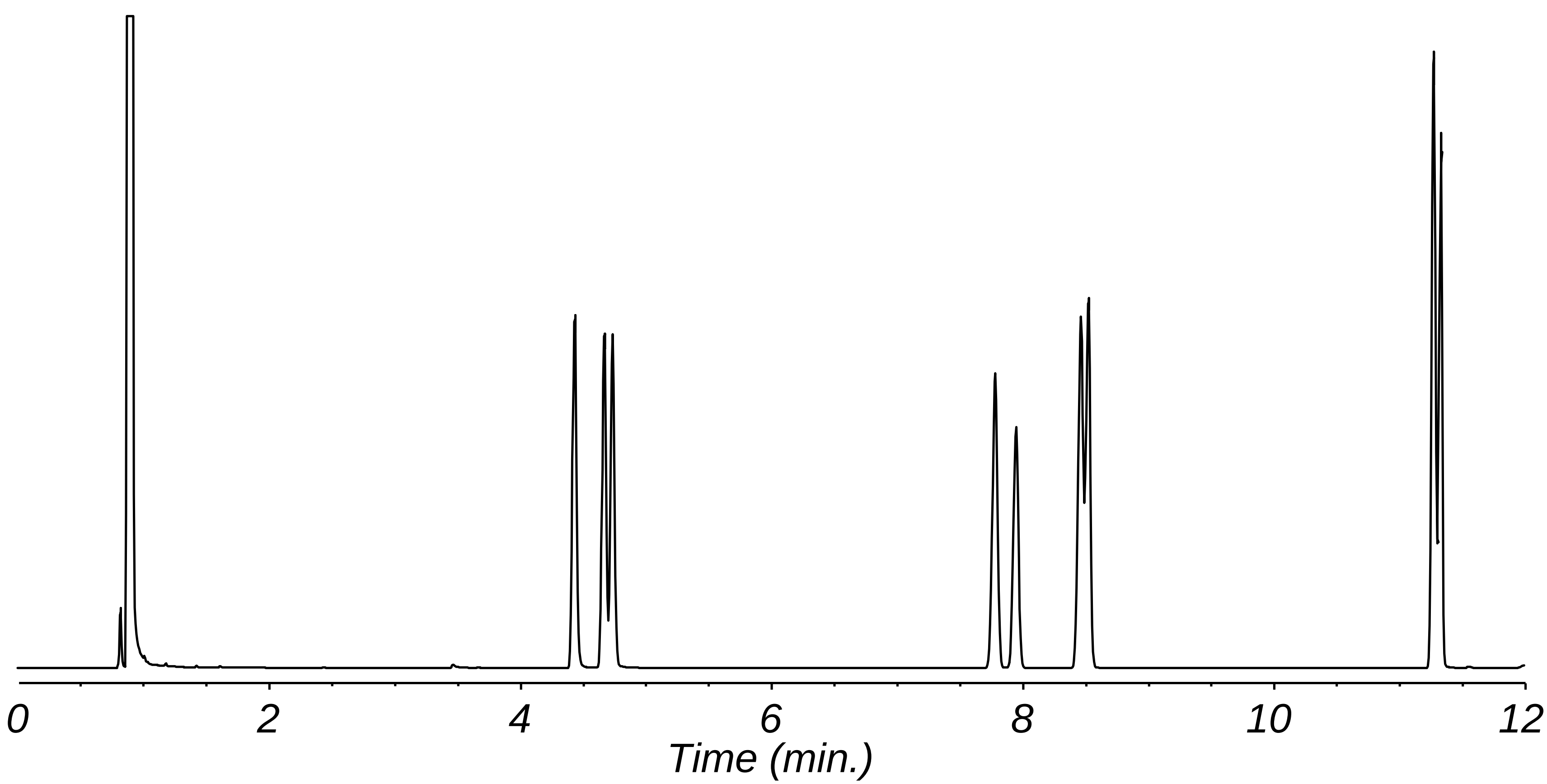
40-60°C at 5°/min, 60°C for 3 min, 60-120°C at 5°/min
Hold at 30° below elution of peaks 6&7



DB-1, 15 m x 0.25 mm i.d., 0.25 µm

DEVELOPING TEMPERATURE PROGRAMS

40°C for 2 min, 40-70°C at 5°/min, 70-130°C at 15°/min



DB-1, 15 m x 0.25 mm i.d., 0.25 µm

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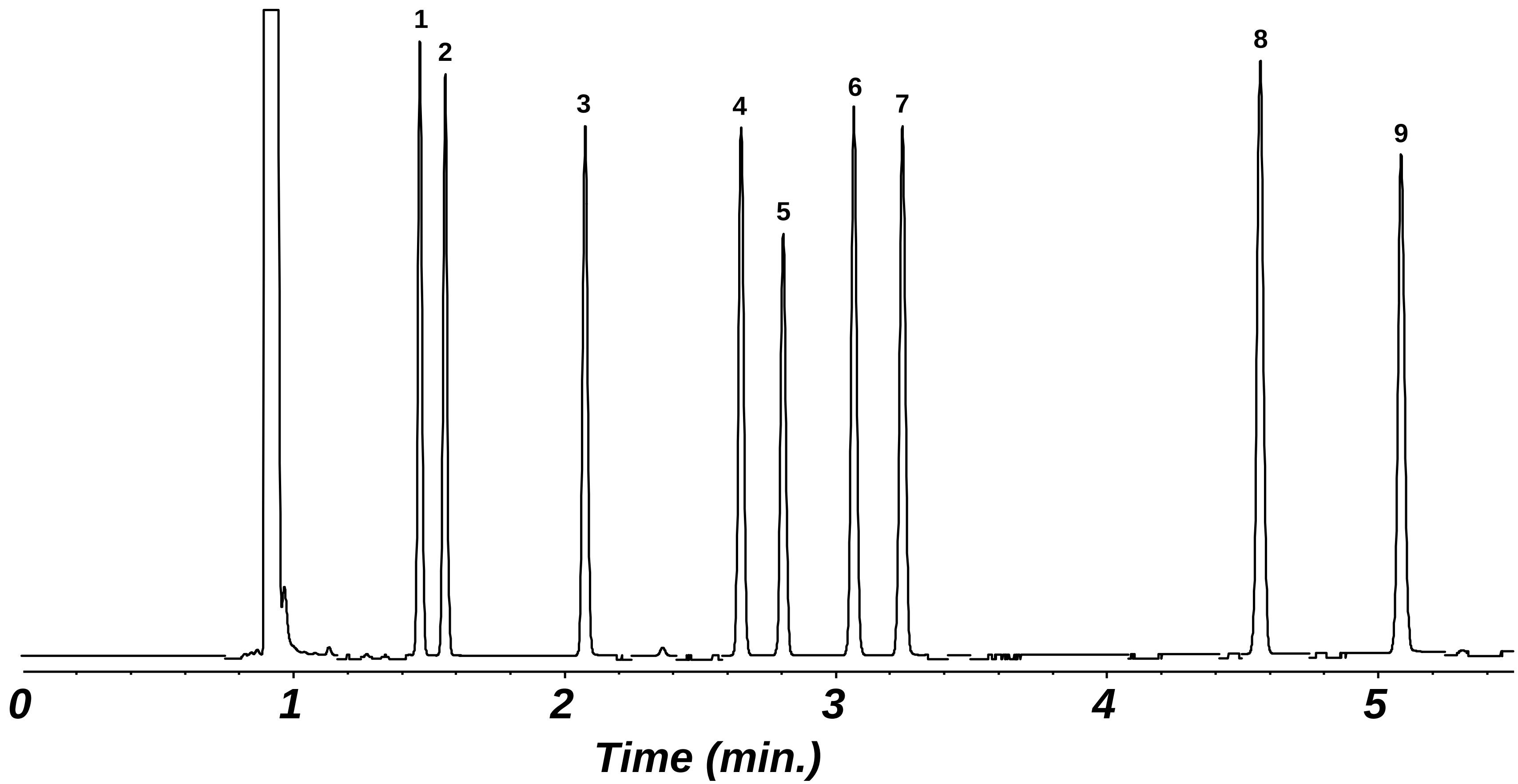
DEVELOPING TEMPERATURE PROGRAMS

80-190°C at 20°/min

DB-WAX, 15 m x 0.32 mm i.d., 0.25 µm



DEVELOPING TEMPERATURE PROGRAMS
Lowering the Initial Temperature

- **Improves resolution of earlier peaks**
- **Smaller resolution improvement of later peaks***

***Resolution increases are smaller for longer columns**

DEVELOPING TEMPERATURE PROGRAMS

Increasing Initial Temperature Hold Time

- **Similar, but smaller effect as lowering the initial temperature**

DEVELOPING TEMPERATURE PROGRAMS

Changing Ramp Rate

- Affects resolution of later peaks
- Minimal effects resolution improvement on earlier peaks
- Substantial changes in analysis time

DEVELOPING TEMPERATURE PROGRAMS
Mid Ramp Hold

- Sometimes improves resolution of co-eluting peaks in the middle of the chromatogram
- May cause peak broadening
- More complicated programs

DEVELOPING TEMPERATURE PROGRAMS
Combining Parameters

- **Offset retention increases by adjusting another parameter**

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*** Select option 4, then option 1.***

916-608-1964 (fax)

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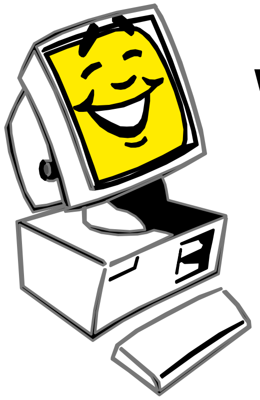

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