GC Method Development
What to Consider

The Sample
Method of injection
Inlet
Detector
Carrier Gas
Column
COMPOUND REQUIREMENTS FOR GC

Only 10-20% 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!
Sample Considerations

1. Sample matrix residues? dirty samples?

2. Analyte Composition
   1. Isomers?
   2. Polar vs. non-Polar?
   3. Organic Acids?
   4. Light Gases?
   5. Noble Gases?
   6. Halogens?
Sample Residues

Semi-volatile residues
  Bake out
  Back flush

Non-volatile residues
  Guard column
  Bake out
  Back flush

Dirty Samples
  Sample clean up?
  Back flush
Use What You Know About the Analytes

Complex Mixture?
Few analytes?
Homologous Series?
Mixture of polar and non-polar?
Labile analytes?
Volatility?
Gas or Liquid Sample?
We have thought about the sample
...What’s next?
Let’s Get the Sample Onto the Column...

- Manual Injection
- Liquid Injection
- Headspace
- Purge & Trap
- Gas Sampling Valve
- SPME
- Thermal Desorption
- Custom
The Inlet

Volatile Interface
Cool-On-Column
Purged Packed
PTV
Split / Splitless
Multi-Mode
Volatile Interface

Used for ‘volatile’ samples
Sample is already a vapor
Headspace
Purge & Trap
# Volatiles Interface

<table>
<thead>
<tr>
<th>Mode</th>
<th>Sample Concentration</th>
<th>Sample to Column</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Split</td>
<td>High</td>
<td>Very little, most is vented</td>
<td>Can switch to split mode electronically</td>
</tr>
<tr>
<td>Splitless</td>
<td>Low</td>
<td>All</td>
<td>Must physically disconnect split vent, plug the interface, and reconfigure the GC. Maximizes sample recovery and eliminates possibility of contamination to pneumatic system.</td>
</tr>
<tr>
<td>Direct</td>
<td>Low</td>
<td>All</td>
<td></td>
</tr>
</tbody>
</table>
Cool-On-Column

* Good for Labile Samples
  Sample is deposited “ON” the column
  Temperature of inlet follows Oven Temperature

• Good for ‘Active’ analytes
  • Minimizes inlet discrimination
  • No inlet Liner*

• Good for Trace Analysis
• Guard Column Highly Recommended
Purged Packed

Good for HIGH flow applications

Used with Packed columns

Can be used with 0.53 mm and 0.32 mm ID columns

**Has a minimal capacity for sample expansion**

**Back Flash**
# PTV

*(Programmable Temperature Vaporization)*

<table>
<thead>
<tr>
<th>Mode</th>
<th>Sample Concentration</th>
<th>Sample to Column</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Split</td>
<td>High</td>
<td>Very Little</td>
<td></td>
</tr>
<tr>
<td>Pulsed Split</td>
<td>High</td>
<td>Very Little</td>
<td></td>
</tr>
<tr>
<td>Splitless</td>
<td>Low</td>
<td>All</td>
<td></td>
</tr>
<tr>
<td>Pulsed Splitless</td>
<td>Low</td>
<td>All</td>
<td></td>
</tr>
<tr>
<td>Solvent Vent</td>
<td>Low</td>
<td>All</td>
<td>Multiple injections concentrate analytes and vent solvent.</td>
</tr>
</tbody>
</table>
## Split / Splitless

<table>
<thead>
<tr>
<th>Mode</th>
<th>Sample Concentration</th>
<th>Sample to Column</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Split</td>
<td>High</td>
<td>Very Little</td>
<td></td>
</tr>
<tr>
<td>Pulsed Split</td>
<td>High</td>
<td>Very Little</td>
<td>Useful with large injections</td>
</tr>
<tr>
<td>Splitless</td>
<td>Low</td>
<td>All</td>
<td>Useful with large injections</td>
</tr>
<tr>
<td>Pulsed Splitless</td>
<td>Low</td>
<td>All</td>
<td><em>better transfer of sample to column</em></td>
</tr>
</tbody>
</table>
SPLIT INJECTOR

Split Ratio

• Too low: Poor peak shape
  - Column overload

• Too high: Poor sensitivity
  - Wastes carrier gas (gas saver)

• Usually non-linear
  - **Do not** use ratio as a dilution factor
# Minimum Recommended Split Ratio

<table>
<thead>
<tr>
<th>mm I.D.</th>
<th>Lowest ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.10</td>
<td>1:50 - 1:75</td>
</tr>
<tr>
<td>0.18 - 0.25</td>
<td>1:10 - 1:20</td>
</tr>
<tr>
<td>0.32</td>
<td>1:8 - 1:15</td>
</tr>
<tr>
<td>0.53</td>
<td>1:2 - 1:5</td>
</tr>
</tbody>
</table>

*Want to have 20 mL/min flow through the inlet*
## Multimode

<table>
<thead>
<tr>
<th>Mode</th>
<th>Sample Concentration</th>
<th>Sample to Column</th>
<th>Discussion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Split</td>
<td>High</td>
<td>Low</td>
<td></td>
</tr>
<tr>
<td>Pulsed Split</td>
<td>High</td>
<td>Low</td>
<td></td>
</tr>
<tr>
<td>Splitless</td>
<td>Low</td>
<td>All</td>
<td></td>
</tr>
<tr>
<td>Pulsed Splitless</td>
<td>Low</td>
<td>All</td>
<td></td>
</tr>
<tr>
<td>Solvent Vent</td>
<td>Low</td>
<td>All</td>
<td>Multiple Injections concentrate sample and vent solvent</td>
</tr>
<tr>
<td>Direct</td>
<td>Low</td>
<td>All</td>
<td></td>
</tr>
</tbody>
</table>
Sample Expansion...Liners?

Split / Splitless Inlet
Multimode Inlet
Packed inlet
PTV
Inlet Liners - Purpose

Glass Inlet Liners provide an “inert” space for liquid samples to be uniformly vaporized to a gas and moved to the column.

Liquid-gas phase change involves a significant change in volume.

Gaseous sample volume depends on

- the solvent type
- column head pressure
- temperature of inlet

These aspects should be optimized for your sample volume and application.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Volume (µL at 250°C and 20psig)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Hexane</td>
<td>140</td>
</tr>
<tr>
<td>Acetone</td>
<td>245</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>350</td>
</tr>
<tr>
<td>Methanol</td>
<td>450</td>
</tr>
<tr>
<td>Water</td>
<td>1010</td>
</tr>
</tbody>
</table>

Liners - 3 Key Aspects Govern Applications

Liner Volume

Liner Treatments or Deactivation

Special Characteristics (glass wool, cup, taper, etc.)

When choosing a liner for your application, consider all three aspects to give you the best chromatography.

You must also determine what type of inlet is in your GC.

Then consider the application itself, and the types of liners and injection techniques used for it:

- Split
- Splitless
Choose a liner with enough volume to accommodate the vaporized sample.

Important, especially for polar solvents with large vapor volumes.

If vapor volume of sample exceeds liner volume, samples may back up (backflash) into carrier gas supply lines, causing ghost peaks and reproducibility problems in chromatography.
Liner Volume (contd.)

Agilent liners are primarily 2mm or 4mm in inner diameter (without tapers and additional features) and 78mm long.

- Thus, 2mm liners hold approx. 0.245 mL or 245 µL of vapor
  4mm liners hold approx. 0.972 mL or 972 µL of vapor

Recommended injection volumes are 1-2µL or less for organic solvents, 0.5µL for water.
Liner Volume

How Do we Calculate the Vapor Volume?

Pressure / Flow Calculator

Free download from our Website

www.chem.agilent.com

Pressure / Flow Calculator

Hewlett-Packard FlowCalc 2.0

File

Pressure Flow...
Vapor Volume...

Help
Exit
Determine what the inlet pressure will be:
Test Inlet Conditions For Solvent Expansion
Water as Solvent
Water as Solvent
Cut Injection Volume in Half

[Image of a software interface for calculating solvent vapor volume with water as the solvent, showing an injection volume of 750 ul and an 88% vaporization rate.]

Agilent Technologies
Water as Solvent
Pulsed Injection
Liner Treatments or Deactivation

Minimizes possibility of active sample components from adsorbing on active sites on the liner or glass wool surface.

Unwanted sample adsorption leads to tailing peaks and loss of response for polar compounds.

Although not necessary for all applications, deactivated liners provide added insurance against possible sample adsorption.

Deactivation of borosilicate glass liners is often done with a silylating reagent like Dimethyldichlororosilane (DMDCS)
Special Characteristics

Some liners have special features that are necessary for different injection techniques. For example:

**Taper** (gooseneck), minimizes sample contact with gold seal.

**Dual taper**, also minimizes sample contact with inlet weldment and reduces potential for backflash.

Glass wool and shelf to hold it in place, prevents non-volatiles from reaching column and removes residual sample from needle. Glass wool should be deactivated.

**Jennings cup**, normally used for efficient sample mixing in split inlets, reduces sample discrimination and prevents non-volatiles from reaching the column. Not for very dirty samples.

**Press fit (direct) connection** end to hold capillary column firmly (virtually all sample goes onto the column). Side hole needed for Electronic Pressure Control with direct connect liners.
Special Characteristics (contd.)

Other special characteristics include:

• Baffles
• Spiral paths
• Glass or ceramic frits or beads
• Laminar cups (elongated version of Jennings cups)
• Column packings with stationary phases

All designed to provide:

• a turbulent sample flow path for sample mixing
• protrusions, barriers, or adsorbents to collect high molecular weight sample components or particles
• surfaces for efficient vaporization of sample components.
## Split Injection Liners

<table>
<thead>
<tr>
<th>Liner</th>
<th>Part No.</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Image" /></td>
<td>19251-60540</td>
<td>Simplest split liner, glass wool, no-deactivation, large volume, 990µL volume. Use for general purpose applications for compounds with low glass adsorption activity. Also used for Splitless mode.</td>
</tr>
<tr>
<td><img src="image2.png" alt="Image" /></td>
<td>5183-4647</td>
<td>Glass wool (held near needle entrance to remove residual sample on needle), deactivated, 870µL volume. Glass nub ensures that gap remains below liner for split injection. Efficient, for most applications, including active compounds. Fail-safe insertion into injection port. Needle length is important.</td>
</tr>
<tr>
<td><img src="image3.png" alt="Image" /></td>
<td>18740-80190</td>
<td>Liner with Jennings cup, no glass wool, 800µL volume. For manual injection only. Use for general purpose applications, high and low MW compounds. Reduces inlet discrimination.</td>
</tr>
<tr>
<td><img src="image4.png" alt="Image" /></td>
<td>18740-60840</td>
<td>Liner with Jennings cup, glass wool, and column packing, 800µL volume. For manual injection only. For dirty samples, traps non-volatiles and particulates well. For high and low MW compounds. Not recommended for use with EPC.</td>
</tr>
</tbody>
</table>
# Splitless Injection Liners

<table>
<thead>
<tr>
<th>Liner</th>
<th>Part No.</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Liner Image" /></td>
<td>5181-3316</td>
<td>Single taper, deactivated, 900 µL volume. Taper isolates sample from metal seal, reducing breakdown of compounds that are active with metals. For trace samples, general application.</td>
</tr>
<tr>
<td><img src="image2.png" alt="Liner Image" /></td>
<td>5062-3587</td>
<td>Single taper, deactivated, with glass wool, 900 µL volume. Glass wool aides volatilization and protects column. For trace (dirty) samples.</td>
</tr>
<tr>
<td><img src="image3.png" alt="Liner Image" /></td>
<td>5181-3315</td>
<td>Double taper, deactivated, 800 µL volume. Taper on inlet reduces chance for backflash into carrier gas lines. High efficiency liner for trace, active samples.</td>
</tr>
<tr>
<td><img src="image4.png" alt="Liner Image" /></td>
<td>G1544-80730 G1544-80700</td>
<td>Direct connect liners, single and dual taper, deactivated. Capillary column press fits into liner end, eliminating sample exposure to inlet. Ultimate protection for trace, active samples. Side hole permits use with EPC.</td>
</tr>
</tbody>
</table>
GLASS WOOL
Liner Packing Recommendations

Amount, size and placement must be consistent for consistent results

Can be broken upon installation into the liner, exposing active sites

Liner deactivation with glass wool plug in place is ideal
GLASS WOOL Placement in Liner

Near top of liner:
- Wipes syringe needle of sample
- Can improve injector precision
- Helps to prevent backflash

Near bottom of liner:
- Helps in volatilization of high MW components
- Increases mixing

Both positions help retain *some* non-volatile residues from reaching the column
Carrier Gas Considerations

- Carries the solutes down the column
- Selection and velocity influences efficiency and retention time
RESOLUTION VS. LINEAR VELOCITY
Helium
Resolution of 1.5 = baseline resolution

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

$R = 1.46$
30 cm/sec
4.4 psig

$R = 1.31$
35 cm/sec
5.1 psig

$R = 0.97$
40 cm/sec
5.8 psig
VAN DEEMTER CURVE

\[ H = \frac{u_{opt}}{OPGV} \]
$\bar{u}_{\text{opt}}$ and OPGV

$\bar{u}_{\text{opt}}$: Maximum efficiency

OPGV: Optimal practical gas velocity
Maximum efficiency per unit time

$1.5 - 2x \bar{u}_{\text{opt}}$
VAN DEEMTER CURVES

The graph shows the Van Deemter curves for different gases. The x-axis represents the carrier gas flow rate in cm/sec, and the y-axis represents the plate height in cm. The curves are labeled with different gases:

- **He**: Helium curve
- **N₂**: Nitrogen curve
- **H₂**: Hydrogen curve

The graph highlights:

- A small Δ (delta) indicating a small difference in plate height for helium and nitrogen at lower flow rates.
- A large Δ (delta) indicating a significant difference in plate height for hydrogen at lower flow rates compared to helium and nitrogen.

The curves demonstrate the relationship between the carrier gas flow rate and the plate height, which is crucial in gas chromatography for optimizing the separation efficiency.
What Happens to the Flow as Oven Temp Increases?
Carrier Gas: Constant Pressure
Carrier Gas: Constant Flow

[Image of Column Pressure/Flow Calculator]

- **Column Parameters**
  - Length (m): 30.0
  - i.d. (mm): 0.320
  - Temp (C): 325

- **Split Ratio**
  - Split vent flow: 0.0
  - Split Ratio (vent flow/col flow): 1

- **Hold up time**: 1.20 minutes

- **Inlet**
  - Inlet Temperature (C): 175
  - Inlet Flow (mL/min): 1.24

- **Carrier Gas Parameters**
  - Inlet Pressure (gauge): 194
  - Outlet Flow (mL/min): 1.75
  - Average Velocity (cm/s): 41.6
  - Outlet Pressure (Absolute): 147

- **Carrier gas**
  - Opt. Vel. range: 20-40

- **Pressure Units**
  - Options: KPa, psi, bar
## Detectors

<table>
<thead>
<tr>
<th>Detector</th>
<th>Dynamic Range</th>
<th>MDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCD</td>
<td>$10^5$ Universal</td>
<td>400 pg Tridecane</td>
</tr>
<tr>
<td>FID</td>
<td>$10^7$ Responds to C-H bonds</td>
<td>1.8 pg Tridecane</td>
</tr>
<tr>
<td>ECD</td>
<td>$5 \times 10^5$ Responds to free electrons</td>
<td>6 fg/mL Lindane</td>
</tr>
<tr>
<td>NPD</td>
<td>$10^5$ Specific to N or P</td>
<td>0.4 pg N/s 0.06 pg P/s</td>
</tr>
<tr>
<td>FPD</td>
<td>$10^3 S, 10^4 P$ Specific to S or P</td>
<td>60 fg P/s 3.6 pg S/s</td>
</tr>
<tr>
<td>SCD</td>
<td>$10^4$ Specific &amp; Selective to S</td>
<td>0.5 pg S/s</td>
</tr>
<tr>
<td>NCD</td>
<td>$10^4$ Specific &amp; Selective to N</td>
<td>3 pg N/s</td>
</tr>
<tr>
<td>MSD</td>
<td>Universal</td>
<td>S/N 400:1 1 pg/uL OFN</td>
</tr>
</tbody>
</table>
Selecting the RIGHT Column

Understanding the Stationary Phase
CAPILLARY COLUMN TYPES

Porous Layer Open Tube (PLOT)

Carrier Gas → Solid Particles

Wall Coated Open Tube (WCOT)

Carrier Gas → Liquid Phase
STATIONARY PHASE POLYMERS

R = methyl, cyanopropyl, cyanopropylphenyl, trifluoropropyl

Siloxane

Arylene

Polyethylene glycol backbone
Selectivity Interactions

- Dispersion
- Dipole
- Hydrogen bonding
## Selectivity Interaction Strengths

<table>
<thead>
<tr>
<th>Phase</th>
<th>Dispersion</th>
<th>Dipole</th>
<th>H Bonding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl</td>
<td>Strong</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Phenyl</td>
<td>Strong</td>
<td>None</td>
<td>Weak</td>
</tr>
<tr>
<td>Cyanopropyl</td>
<td>Strong</td>
<td>Strong</td>
<td>Moderate</td>
</tr>
<tr>
<td>Trifluoropropyl</td>
<td>Strong</td>
<td>Moderate</td>
<td>Weak</td>
</tr>
<tr>
<td>PEG</td>
<td>Strong</td>
<td>Strong</td>
<td>Moderate</td>
</tr>
</tbody>
</table>
Now Let’s Apply What We learned
<table>
<thead>
<tr>
<th></th>
<th>Drug Name</th>
<th>Chemical Structure</th>
<th>Drug Name</th>
<th>Chemical Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cadaverine</td>
<td><img src="image" alt="Cadaverine" /></td>
<td>11</td>
<td>Phenelzine</td>
</tr>
<tr>
<td>2</td>
<td>Cyclopentamine</td>
<td><img src="image" alt="Cyclopentamine" /></td>
<td>12</td>
<td>Phenylpropanolamine</td>
</tr>
<tr>
<td>3</td>
<td>Amphetamine</td>
<td><img src="image" alt="Amphetamine" /></td>
<td>13</td>
<td>Clortermine</td>
</tr>
<tr>
<td>4</td>
<td>Phenethylamine</td>
<td><img src="image" alt="Phenethylamine" /></td>
<td>14</td>
<td>Chlorphentermine</td>
</tr>
<tr>
<td>5</td>
<td>Phentermine</td>
<td><img src="image" alt="Phentermine" /></td>
<td>15</td>
<td>Ephedrine</td>
</tr>
<tr>
<td>6</td>
<td>Propylhexedrine</td>
<td><img src="image" alt="Propylhexedrine" /></td>
<td>16</td>
<td>Pseudoephedrine</td>
</tr>
<tr>
<td>7</td>
<td>Methamphetamine</td>
<td><img src="image" alt="Methamphetamine" /></td>
<td>17</td>
<td>Phendimetrazine</td>
</tr>
<tr>
<td>8</td>
<td>Methenamine</td>
<td><img src="image" alt="Methenamine" /></td>
<td>18</td>
<td>MDA</td>
</tr>
<tr>
<td>9</td>
<td>Amantidine</td>
<td><img src="image" alt="Amantidine" /></td>
<td>19</td>
<td>Ecgonine methyl ester</td>
</tr>
<tr>
<td>10</td>
<td>Mephentermine</td>
<td><img src="image" alt="Mephentermine" /></td>
<td>20</td>
<td>diethylpropion</td>
</tr>
</tbody>
</table>
Starting Method Parameters

Column: DB-5 30m X 0.32mm X 0.25um
S/Si Inlet: Split 50:1 Temp 250°
FID: Temp 350°
Carrier: He

Constant flow 30cm/sec

Oven: 50°C Hold for 5 min
10°C/min to 325°C Hold for 5 min
Am I Going to Have Backflash?
Injection Volume / Solvent Expansion
Developing Temperature Program
Initial Run

Initial Temp 50°C Hold for 5 min
Ramp 10°C/min to 325°C Hold for 5 min
Developing Temperature Program
Initial Run - Define Areas for Improvement
Next Step…

When does the first peak come out?
~9 minutes

What temperature does it come out at?

Temp program:
50°C for 5 minutes
10°C to 325°C

1st Peak comes out at 90°C
Developing Temperature Program

2nd Try

Initial Temp 90°C Hold for 5 min
Ramp 10°C/min to 325°C Hold for 5 min

From 9 min to ~4

Actually looks better
Developing Temperature Program
3rd Try

Initial Temp 100°C
Hold for 5 min
Ramp 10°C/min to 325°C
Hold for 5 min

Time to resolve these peaks
Resolve Co-elutions

Add a hold 20-30° below the elution temperature

Co-elutions occur at 10 minutes

100°C hold for 5 minutes
10°C/min to 325°C

Co-elutions occur at 150°C

Set hold at 130°C
Developing a Temperature Program

Oven: 100°C Hold for 10 minutes
10°C/min to 130°C hold for 5 min
10°C/min to 325°C
Developing a Temperature Program
Conclusions:

Think about the sample first

**Is it chromatographable by GC?**

- sample composition
- sample clean up
- level of detection

Use information sources first when choosing a column

Mild oven program to begin with

*Utilize Technical Support*
Agilent J&W Scientific Technical Support

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

* Select option 3, then 3, then 1.

866-422-5571 (fax)

GC-Column-support@agilent.com

www.chem.agilent.com