GC Method Development

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Application Engineer
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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. Nobel 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>
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</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>
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<th>Mode</th>
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<tbody>
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<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>Useful with large injections. <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>Multiple Injections concentrate sample and vent solvent</td>
</tr>
<tr>
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<td>High</td>
<td>Low</td>
<td></td>
</tr>
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<td>Splitless</td>
<td>Low</td>
<td>All</td>
<td></td>
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<td>Low</td>
<td>All</td>
<td></td>
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<td></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

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

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
Determine what the inlet pressure will be:
Test Inlet Conditions For Solvent Expansion
Water as Solvent
Water as Solvent
Cut Injection Volume in Half
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 Dimethyldichlorosilane (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="image" alt="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>
<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="image" alt="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>
<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="image" alt="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>
<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="image" alt="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>
<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="image" alt="Single taper, deactivated, 900µL volume. Taper isolates sample from metal seal, reducing breakdown of compounds that are active with metals. For trace samples, general application." /></td>
<td>5181-3316</td>
<td>Single taper, deactivated, 900µL 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="image" alt="Single taper, deactivated, with glass wool, 900µL volume. Glass wool aids volatilization and protects column. For trace (dirty) samples." /></td>
<td>5062-3587</td>
<td>Single taper, deactivated, with glass wool, 900µL volume. Glass wool aids volatilization and protects column. For trace (dirty) samples.</td>
</tr>
<tr>
<td><img src="image" alt="Double taper, deactivated, 800µL volume. Taper on inlet reduces chance for backflash into carrier gas lines. High efficiency liner for trace, active samples." /></td>
<td>5181-3315</td>
<td>Double taper, deactivated, 800µL 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="image" alt="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>
<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

$R = 1.46$
30 cm/sec
4.4 psig

$R = 1.31$
35 cm/sec
5.1 psig

$R = 0.97$
40 cm/sec
5.8 psig

DB-1, 15 m x 0.32 mm ID, 0.25 um
60°C isothermal
1,3- and 1,4-Dichlorobenzene
$\bar{u}_{opt}$ and OPGV

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

OPGV: Optimal practical gas velocity
  Maximum efficiency per unit time

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

\[ \text{cm/sec} \]

- **He**
- **N}_2\)**
- **H}_2\)**

H: 0.25, 0.50, 0.75, 1.00

He and N\_2 curves show different behaviors with changes in cm/sec.
What Happens to the Flow as Oven Temp Increases?
Carrier Gas: Constant Pressure
Carrier Gas: Constant Flow
## Detectors

<table>
<thead>
<tr>
<th>Detector</th>
<th>Dynamic Range</th>
<th>MDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCD</td>
<td>$10^5$</td>
<td>400 pg Tridecane</td>
</tr>
<tr>
<td>FID</td>
<td>$10^7$</td>
<td>1.8 pg Tridecane</td>
</tr>
<tr>
<td>ECD</td>
<td>$5 \times 10^5$</td>
<td>6 fg/mL Lindane</td>
</tr>
<tr>
<td>NPD</td>
<td>$10^5$</td>
<td>0.4 pgN/s, 0.06 pg P/s</td>
</tr>
<tr>
<td>FPD</td>
<td>$10^3S, 10^4P$</td>
<td>60 fg P/s, 3.6 pg S/s</td>
</tr>
<tr>
<td>SCD</td>
<td>$10^4$</td>
<td>0.5 pg S/s</td>
</tr>
<tr>
<td>NCD</td>
<td>$10^4$</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)

Wall Coated Open Tube (WCOT)
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
Sample List (drugs)

<table>
<thead>
<tr>
<th></th>
<th>Name</th>
<th>Chemical Structure</th>
<th></th>
<th>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>
<td><img src="image" alt="Phenelzine" /></td>
</tr>
<tr>
<td>2</td>
<td>Cyclopentamine</td>
<td><img src="image" alt="Cyclopentamine" /></td>
<td>12</td>
<td>Phenylpropanolamine</td>
<td><img src="image" alt="Phenylpropanolamine" /></td>
</tr>
<tr>
<td>3</td>
<td>Amphetamine</td>
<td><img src="image" alt="Amphetamine" /></td>
<td>13</td>
<td>Clortermine</td>
<td><img src="image" alt="Clortermine" /></td>
</tr>
<tr>
<td>4</td>
<td>Phenethylamine</td>
<td><img src="image" alt="Phenethylamine" /></td>
<td>14</td>
<td>Chlorphentermine</td>
<td><img src="image" alt="Chlorphentermine" /></td>
</tr>
<tr>
<td>5</td>
<td>Phentermine</td>
<td><img src="image" alt="Phentermine" /></td>
<td>15</td>
<td>Ephedrine</td>
<td><img src="image" alt="Ephedrine" /></td>
</tr>
<tr>
<td>6</td>
<td>Propylhexedrine</td>
<td><img src="image" alt="Propylhexedrine" /></td>
<td>16</td>
<td>Pseudoephedrine</td>
<td><img src="image" alt="Pseudoephedrine" /></td>
</tr>
<tr>
<td>7</td>
<td>Methamphetamine</td>
<td><img src="image" alt="Methamphetamine" /></td>
<td>17</td>
<td>Phendimetrazine</td>
<td><img src="image" alt="Phendimetrazine" /></td>
</tr>
<tr>
<td>8</td>
<td>Methenamine</td>
<td><img src="image" alt="Methenamine" /></td>
<td>18</td>
<td>MDA</td>
<td><img src="image" alt="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>
<td><img src="image" alt="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>
<td><img src="image" alt="diethylpropion" /></td>
</tr>
</tbody>
</table>
Starting Method Parameters

Column:  DB-5 30m X 0.32mm X 0.25um
S/Sl 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

**Solvent Vapor Volume Calculator**

- **Approximate vapor volume (ul):** 669 ul
- **Pressure Units:** KPa
- **Injection Volume (ul):** 1.0
- **Inlet Temp (°C):** 250
- **Inlet Pressure:** 8.6
- **Solvent Properties:**
  - Methanol
  - Boiling Pt (°C): 64.7
  - Density (g/cm³): 0.791
  - Molar Wt. (amu): 32
- **Injection Liner:** 5183-4647 single-t
  - Volume (ul): 850
  - Capacity limits (%): 75, 100

**Agilent Technologies**
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