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. Nobel Gases?
   6. Halogens?
Sample Residues

Semi-volatile residues
   Bake out
   Back flush

Non-volatile residues
   Guard column
   Back flush

Dirty Samples
   Sample clean up?
   Back flush
Perform Sample Preparation

- To acquire desired sensitivity/selectivity
- To reduce contamination/carryover issues
- Increase inlet maintenance interval
- Use of sensitive and expensive instruments: **Protect your investment!!!**

Pesticides in Avocado *without* SP

Pesticides in Avocado *with* SP
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/MMI
Split / Splitless
Multi-Mode
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**
## 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>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

**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

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

<table>
<thead>
<tr>
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<th>Discussion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Split</td>
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<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

Use the same liners
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 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>

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

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:
Determine what the inlet pressure will be:
Test Inlet Conditions For Solvent Expansion
Water as Solvent

[Image of a software interface for calculating solvent vapor volume with specific settings and properties for water.]
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, UI deactivation, large volume, 990μL volume. Use for general purpose. Also used for Splitless mode." /></td>
<td>5190-2294</td>
<td>Simplest split liner, glass wool, UI deactivation, large volume, 990μL volume. Use for general purpose. 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>5190-2295</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. 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. 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 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 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" alt="Liner Image" /></td>
<td>5190-2292</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="image2" alt="Liner Image" /></td>
<td>5190-2293</td>
<td>Single taper, deactivated, with glass wool, 900μL volume. Glass wool aides volatilization and protects column. For trace (dirty) samples.</td>
</tr>
<tr>
<td><img src="image3" alt="Liner Image" /></td>
<td>5190-3983</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="image4" 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
VAN DEEMTER CURVE

The graph illustrates the VAN DEEMTER CURVE, which is a plot of the plate height (H) against the carrier gas velocity (u). The curve shows the relationship between these two variables, with the optimal carrier gas velocity (u_{opt}) indicated at approximately 30 on the x-axis. The OPGV (Optimum Pressure Gradient Velocity) is also marked on the graph, showing a value around 50 on the x-axis.
$\overline{u}_{\text{opt}}$ and OPGV

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

OPGV: Optimal practical gas velocity
Maximum efficiency per unit time

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

Graph showing Van Deemter curves for different gases: He, N₂, and H₂. The x-axis represents cm/sec and the y-axis represents H. The graph indicates that N₂ has a lower H value than H₂ and He across the cm/sec range. There are regions marked as 'Small △' and 'Large △' on the graph.
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>Universal 400 pg Tridecane</td>
</tr>
<tr>
<td>FID</td>
<td>$10^7$</td>
<td>Responds to C-H bonds 1.8 pg Tridecane</td>
</tr>
<tr>
<td>ECD</td>
<td>$5 \times 10^5$</td>
<td>Responds to free electrons 6 fg/mL Lindane</td>
</tr>
<tr>
<td>NPD</td>
<td>$10^5$</td>
<td>Specific to N or P 0.4 pgN/s 0.06 pg P /s</td>
</tr>
<tr>
<td>FPD</td>
<td>$10^3$S, $10^4$P</td>
<td>Specific to S or P 60 fg P/s 3.6 pg S/s</td>
</tr>
<tr>
<td>SCD</td>
<td>$10^4$</td>
<td>Specific &amp; Selective to S 0.5 pg S/s</td>
</tr>
<tr>
<td>NCD</td>
<td>$10^4$</td>
<td>Specific &amp; Selective to N 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)

Carrier Gas

Solid Particles

Liquid Phase
STATIONARY PHASE POLYMERS

Siloxane

R= methyl, cyanopropyl, cyanopropylphenyl, trifluoropropyl

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>
Selecting the Correct Column

Match analyte polarity to column polarity
‘Like dissolves like’

Look for unique interactions that analytes may have with a phase

Use preexisting information

Use the Agilent GC Application Support Team:

gc-column-support@agilent.com
Now Let’s Apply What We Have Learned
<table>
<thead>
<tr>
<th>Sample List (drugs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Cadaverine</td>
</tr>
<tr>
<td>2. Cyclopentamine</td>
</tr>
<tr>
<td>3. Amphetamine</td>
</tr>
<tr>
<td>4. Phenethylamine</td>
</tr>
<tr>
<td>5. Phentermine</td>
</tr>
<tr>
<td>6. Propylhexedrine</td>
</tr>
<tr>
<td>7. Methamphetamine</td>
</tr>
<tr>
<td>8. Methenamine</td>
</tr>
<tr>
<td>9. Amantidine</td>
</tr>
<tr>
<td>10. Mephentermine</td>
</tr>
<tr>
<td>11. Phenelzine</td>
</tr>
<tr>
<td>12. Phenylpropanolamine</td>
</tr>
<tr>
<td>13. Clortermine</td>
</tr>
<tr>
<td>14. Chlorphentermine</td>
</tr>
<tr>
<td>15. Ephedrine</td>
</tr>
<tr>
<td>16. Pseudoephedrine</td>
</tr>
<tr>
<td>17. Phendimetrazine</td>
</tr>
<tr>
<td>18. MDA</td>
</tr>
<tr>
<td>19. Ecgonine methyl ester</td>
</tr>
<tr>
<td>20. 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 30 cm/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

[Image of a software interface for solvent vapor volume calculation]

- Approximate vapor volume: 669 ul
- Solution: 79%

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
  - Mol Wt. (amu): 32

Injection Liner
- Volume (ul): 850
- Capacity limits (%): 75 - 100

Pressure Units:
- KPa
- psi
- bar

[Buttons: Print, Help, OK]
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°C 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 5 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
Conclusions: Starting Parameters

--Assuming S/Si – FID system

Inlet Temp: 250°C

    Split 50:1

Carrier Gas: Helium ~ 30 cm/sec, Hydrogen ~45 cm/sec

Oven Temp: 40°C hold for 5 minutes

    10°C/min Ramp to Isothermal Limit of column

    hold for 5-10 minutes

Detector Temp: 20°C above the highest oven temp
Questions
Contact Agilent Chemistries and Supplies
Technical Support

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

Option 1 for GC/GCMS Columns and Supplies
Option 2 for LC/LCMS Columns and Supplies
Option 3 for Sample Preparation, Filtration and QuEChERS
Option 4 for Spectroscopy Supplies

Available in the USA 8-5 all time zones

gc-column-support@Agilent.com
lc-column-support@agilent.com
spp-support@agilent.com
spectro-supplies-support@agilent.com
Additional Resources and Application Support

**Sample preparation eSeminar Series**

**Reference Materials and Guides:**
Agilent Enhanced Matrix Removal – Lipid Brochure (Publication Number: 5991-6052EN)


Agilent Sample Preparation Landing Page

Agilent Sample Preparation Catalog (Publication Number: 5991-1057EN)