Get Your Peaks into Shape

General GC Column
Troubleshooting

Alexander Ucci
October 5, 2021
“Everything Was Just Fine... and Then This Happened!”

“How do I troubleshoot?”

Track your actions/keep a logbook of events:

- Changed column, liner, septum, or syringe
- Injected samples, or used another method
- Carried out maintenance, cut column, or inlet flush

Logic

\[ \text{Something changed (slowly or suddenly)} = \text{Something is different} \]
Logical Troubleshooting

Troubleshooting starts with isolating the problem.
• There are five basic areas from where problems can arise:
  - Injector
  - Flow
  - Column
  - Detector
  - Electronics
Or…
  - A combination of these

Knowing what can and cannot cause the symptom is key, and most importantly DON’T PANIC!
Common Peak Shape Issues

• Peak tailing – flow path or activity
• Bonus peaks – in sample or back flash (carry-over)
• Split peaks – injector problems, mixed solvent
• No peaks – wasn’t introduced, wasn’t detected
• Response changes – activity, injector discrimination, detector problem
• Peak fronting – overload or solubility mismatch, injector problems
• Shifting retention – leaks, column aging, contamination, or damage
• Loss of resolution – separation decreasing, peak broadening
• Baseline disturbances – column bleed, contamination, electronics
• Noisy or spiking baseline – electronics or contaminated detector
• Quantitation problems – activity, injector, or detector problems
• Other
Peak Tailing

**Injector or column is active**
- Reversible adsorption of active compounds (-OH, -NH, -SH)

**Flow problem**
- Dead volume, obstruction, poor installation, or severe column contamination

**Miscellaneous** - overloading of PLOT columns, coelution, polarity mismatch between phase, solute or solvent, and some compounds always tail

*Tip:* Inject a light hydrocarbon. Should not tail unless flow path problem.
**Agilent Inert Flow Solution**

Modified Agilent J&W DB-WAX UI mix on DB-WAX UI, 122-7032UI

<table>
<thead>
<tr>
<th>Compound ID</th>
<th>Compound Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>5-Nonanone</td>
</tr>
<tr>
<td>2.</td>
<td>Decanal</td>
</tr>
<tr>
<td>3.</td>
<td>Propionic acid</td>
</tr>
<tr>
<td>4.</td>
<td>Ethylene glycol</td>
</tr>
<tr>
<td>5.</td>
<td>Heptadecane</td>
</tr>
<tr>
<td>6.</td>
<td>Aniline</td>
</tr>
<tr>
<td>7.</td>
<td>Methyl dodecanoate</td>
</tr>
<tr>
<td>8.</td>
<td>2-Chlorophenol</td>
</tr>
<tr>
<td>9.</td>
<td>1-Undecanol</td>
</tr>
<tr>
<td>10.</td>
<td>Nonadecane</td>
</tr>
<tr>
<td>11.</td>
<td>2-Ethylhexanoic acid</td>
</tr>
<tr>
<td>12.</td>
<td>Ethyl maltol</td>
</tr>
</tbody>
</table>

*Every column is tested individually*

[Chemical peaks diagram]
Peak Tailing from Contaminated Consumables

Before gold seal replacement

Sym: 0.108

After gold seal replacement

Sym: 0.816

Peak
0  Methane
1  2-Nonanone
2  Decanal
3  2,3-Butanediol
4  Ethyleneglycol
5  Dicyclohexylamine
6  Heptadecane
7  Aniline
8  Methyl Dodecanoate
9  2-Chlorophenol
10 1-Undecanol
11  Nonadecane
Effect of Oxygen on Peak Shape of 2-ethylhexanoic Acid

250°C/H₂ with <0.3 ppm O₂

As = 1.08

250°C/H₂ with 10 ppm O₂

As = 1.19

260°C/H₂ with 10 ppm O₂

As = 1.44

280°C/H₂ with 10 ppm O₂

As = 2.38
Self Tightening Nuts: No Leaks, No Downtime, No Frustration

- Spring-driven piston continuously presses against ferrule
- Automatically retightens when ferrule shrinks
- Wing design for finger tightening
- No tools needed
- Works only with graphite/vespel ferrules

<table>
<thead>
<tr>
<th>Part Number</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>G3440-81013</td>
<td>Column Nut, Collared Self-Tightening MSD</td>
</tr>
<tr>
<td>G3440-81011</td>
<td>Column nut, Collared Self Tightening Inlet/Detect</td>
</tr>
<tr>
<td>G3440-81012</td>
<td>Collar for Self Tightening Nut</td>
</tr>
</tbody>
</table>

Peak Tailing from Thermal Degradation

40 Hours at 400 °C

Peak | Name
---|---
0 | Methane
1 | Decane
2 | 1-Octanol
3 | 2,6-Dimethylphenol
4 | 2,6-Dimethylaniline
5 | Naphthalene
6 | 1-Decanol
7 | Tridecane
8 | Methyl Decanoate
Column Efficiency Over 120 Hours at 400 °C

Time at 400°C (hours)

Brand X-5ht
30 m x 0.25 mm x 0.10 µm

Agilent J&W DB-5ht
30 m x 0.25 mm x 0.10 µm

Peak Name
0 Methane
1 Decane
2 1-Octanol
3 2,6-Dimethylphenol
4 2,6-Dimethylaniline
5 Naphthalene
6 1-Decanol
7 Tridecane
8 Methyl Decanoate

Agilent Publication 5994-1013EN
Bonus or Ghost Peaks

Contamination in injector, column, or flow (carrier gas)

- Carryover from a backflash or previous sample
- Bad tank of gas, or traps have expired
- Septum bleed

**Tip:** Run a blank run… it should be blank!
What Are These Repeating Peaks?

Septa contamination in wash vials or inlet liners can be diagnosed by looking for siloxane polymers in your total ion chromatogram. Each peak in the chromatogram corresponds to a cyclized (ring structure) siloxane molecule. These molecules fragment with similar patterns.

Example spectrum:

**Common ions for siloxane molecules:**
- 73
- 147
- 207
- 281
- 355

Is it column bleed? No!
Multiple Injections from the Same Vial: Siloxanes!

Acquisition Time (min)

Run 1
Run 6
Does Your Baseline Look Like This? Do You See Extra Peaks?
The Matrix

If your target ions are buried beneath matrix peaks, it might be time to trim the column or do sample clean-up.

500 ppb ethenoprox in black tea

m/z 163 EIC

Matrix TIC

10 ppb linalool in shampoo

m/z 93 EIC

...(or improve your sample cleanup)
The Importance of Sample Cleanup

For sample cleanup help, please contact us!  spp-support@agilent.com
Offline Options for Sample Matrix Removal

Bond Elut Solid Phase Extraction cartridges and plates

Filter vials

QuEChERS

Captiva EMR-Lipid filtration cartridges and plates

Chem Elut S

Captiva syringe filters

SPME

October 5, 2021
Split Peaks

Injector (poor sample introduction)
- Injecting the sample twice (somehow?)
- Mixed sample solvent (polarity difference)
- Sample in syringe needle (manual inject)

Injector (activity)
- Breakdown (not really a split peak, two peaks)
- Sample degradation in injector

Volatility
- High boilers dropping out on cold spots
- Transfer line temperatures
- Unions or fittings not tracking column temperature
No Peaks

Detector (not on, or not operational)
Injector (not working)
Plugged syringe/plunger not moving
  • Wrong injector (or detector)
  • Huge leak (older systems)
  • No carrier gas flow

Not the column unless…
  • Broken column or no column

MISSING

Last Seen Yesterday
Peak Response

All change in size

Injector
- Leaky syringe
- Split ratio set incorrectly
- Wrong purge activation time
- Septum purge flow too high
- Injector temperature too low*

Detector (response problem)
- Settings or flows changed
- Electronics failing

*Tip: Ask is it all of them or some of them, if all then injector or detector
50 ng/mL before 50 Injections of Salmon Oil

After 50 Injections of Dilute Salmon Oil
Injector or column is active/contaminated

- Irreversible adsorption of active compounds (-OH, -NH, -SH)

Decomposition of sample

- Temperature change – discrimination
- Evaporation from sample

Run 1
Run 26 (after 25 matrix injections)
Example of Reduction in Response for One Peak

phenylacetylene

phenylacetylene
Change in Response: Pyraclostrobin in Spinach on Run 1
Change in Response: Pyraclostrobin in Spinach on Run 65
Change in Response: Pyraclostrobin in Spinach on Run 1 vs Run 65
Change in Response: Pyraclostrobin in Spinach with New Liner

Counts vs. Acquisition Time (min)
Peak Fronting
Shark fin-shaped or just slight

Column (contaminated)
- Overload (more pronounced with large solute and phase polarity differences)

Injector
- Compound soluble in injection solvent (need retention gap)
- Mixed sample solvent

Other
- Coelution
- Breakdown
Retention Time Shift

**Injector**
- Leak in the septum
- Change in injection solvent
- Large change in sample concentration

**Flow**
- Change in gas velocity

**Column**
- Contamination
- Damaged stationary phase
- Loss of stationary phase
- Change in temperature
Thermal Stability and Retention Time Shifting on Standard WAX Column

<table>
<thead>
<tr>
<th>Peak</th>
<th>Compound</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Methanol</td>
</tr>
<tr>
<td>2</td>
<td>Benzene</td>
</tr>
<tr>
<td>3</td>
<td>Toluene</td>
</tr>
<tr>
<td>4</td>
<td>Ethylbenzene</td>
</tr>
<tr>
<td>5</td>
<td>p-Xylene</td>
</tr>
<tr>
<td>6</td>
<td>m-Xylene</td>
</tr>
<tr>
<td>7</td>
<td>o-Xylene</td>
</tr>
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</table>

Initial BTEX Injection

- 50 hours at 280 °C
- 25 hours at 280 °C

Application note 5991-9035EN
### DB-HeavyWAX

<table>
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<th>Compound</th>
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<tr>
<td>1</td>
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<td>6</td>
<td>m-Xylene</td>
</tr>
<tr>
<td>7</td>
<td>o-Xylene</td>
</tr>
</tbody>
</table>

**Application note 5991-9035EN**

- **Initial Injection**
  - 100 hours at 280 °C
  - 75 hours at 280 °C
  - 50 hours at 280 °C
  - 25 hours at 280 °C
Resolution is a function of separation and peak width.
Loss of Resolution - Separation Decrease (Retention Times Changed)

Column
- Different column temperature
- Contamination (more phase?)
- Matrix components coeluting

Flow
- Change in velocity?

![Diagram showing separation and peak width]
Loss of Resolution - Peak Broadening (Retention Times Unchanged)

Flow
• Make-up gas

Column
• Contamination
• Phase degradation

Injector (efficiency)
• Settings, liner, installation, etc.
Peak Broadening: Omethoate in Avocado in Run 1
Peak Broadening: Omethoate in Avocado in Run 65
Peak Broadening: Omethoate in Avocado in Run 1 versus Run 65
Peak Broadening: Recover Peak Shape with New Liner
Example of Column Contamination and Broad Peaks

Agilent J&W DB-624 QC Test Mix
After 75 injections of oily sample

*Temperature program// 35 °C hold 1.50 min //  30°/min to 65 °C, hold 10 min
Example of Column Contamination

Removed $1\frac{1}{2}$ m from injector end

*Before column rinse and bake

Temperature program // 35 °C hold 1.50 min // 30° C/min to 65 °C, hold 10 min
Example of Column Contamination

We have more semivolatile contamination!

1$\frac{1}{2}$ m removed*

QC test mix to upper temperature limit

*Before column bake

Temperature program // 35 °C, hold 1.50 min // 30 °C/min to 65 °C, hold 15 min // 20 °C/min to 260 °C, hold 50 min
Agilent J&W DB-624 Column

QC Test Mix

Column: Agilent J&W DB-624
30 m x 0.53 mm id, 3.0 µm

Carrier: Helium at 40 cm/sec
measured at 35 °C

Injector: Direct, 260 °C

Detector: FID, 300 °C

Oven: 35 °C for 1.50 min
30 °C/min to 65 °C for 10 min

1. 1,2-Dichloropropane
2. Octane
3. Tetrachloroethylene
4. Chlorobenzene
5. Nonane
Changing to a Higher Split Ratio Improves Peak Sharpness

5:1 Split ratio

10:1 Split ratio
Baseline Disturbances
Sudden changes, wandering, or drifting

Drifting/wandering/weird disturbances

Column or detector
- Not fully conditioned or stabilized (electronics)
- Contamination

Flow
- Changes in carrier and/or detector gas flows
- Valves switching, leaks
Jittery Baseline Example

October 5, 2021  Get Your Peaks Into Shape  DE44470.3469212963
Jittery Baseline Example

Baseline is not normal
- Potential issues with filaments
- Run a Tune Evaluation

Jittery baseline + failed Tune = check your filaments
Noisy Baseline

Flow
- Contaminated gas
- Incorrect detector settings

Column
- Bleed if at high temperature
- In detector flame (poor installation)

Detector
- Air leak - ECD, TCD
- Electronics malfunction

Mild

Severe
Spiking Baseline

Detector

- Particles entering the detector
- Random: poor connection
- Regular: nearby "cycling" equipment (electronics)

Application note 5991-2975EN
Weird Peak Shape Due to Lack of Analyte Refocusing

Free fatty acids in water on DB-FATWAX UI

150 °C

40 °C
Quantitation Problems

Detector
- Poor stability (electronics) or baseline disturbances (contamination)
- Outside detector's linear range or wrong settings
- Integration parameters

Activity (adsorption) in injector or column

Injector
- Technique, settings, conditions
- Syringe worn

Other
- Coelution
- Matrix effects
- Sample evaporation – leaky vials
- Sample decomposition
What is Not Caused by a Column?

• Peaks
  - Any reproducible sharp chromatographed peak
• Siloxanes (even though it looks like bleed spectrally)
• Degradation product peaks: Endrin Aldehyde, endrin ketone, DDE, DDD…
• Carryover of sample compounds
• Splitting of peaks
Troubleshooting Techniques
Bleed profile (non-injection): *baseline problems*
Inject a nonretained peak: *peak shape problems*
Test mix: *all problems*
Isolate the components: *all problems*

Condensation test: *baseline problems*
Jumper tube test: *baseline problems*
Generating a Bleed Profile

Produce when the column is new (for future reference) when there is a baseline problem

(Simply remove syringe from ALS)

Agilent J&W DB-1, 30 m x 0.32 mm id, 0.25 µm
Temperature program // 40 °C, hold 1 min // 20 °C/min to 320 °C, hold 10 min.
Inject a Nonretained Compound to Check Flow Path

Used to check flow path

Potential explanations:
• Injector or septum leak
• Too low of a split ratio
• Liner problem
  - (broken, leaking, misplaced)
• Column position in injector and detector
Test Mix – Make Your Own!

A test mix is used to determine how “good” the column is, or whether the problem is related to the chemical properties of the analytes.

It is simplest to use your own standard.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrocarbons</td>
<td>Efficiency, Retention</td>
</tr>
<tr>
<td>Alcohols</td>
<td>Activity</td>
</tr>
<tr>
<td>FAMEs, PAHs</td>
<td>Retention</td>
</tr>
<tr>
<td>Acids</td>
<td>Acidic Character Activity</td>
</tr>
<tr>
<td>Bases</td>
<td>Basic Character Activity</td>
</tr>
</tbody>
</table>

Test Conditions

<table>
<thead>
<tr>
<th>Inlet:</th>
<th>Split (250 °C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detector:</td>
<td>FID(320 °C)</td>
</tr>
<tr>
<td>Flow:</td>
<td>37.3 cm/sec (1.8 mL/min)</td>
</tr>
<tr>
<td>Carrier gas:</td>
<td>Hydrogen</td>
</tr>
<tr>
<td>Holdup compound:</td>
<td>Methane (0.671 min)</td>
</tr>
<tr>
<td>Temperature program:</td>
<td>Isothermal (110 °C)</td>
</tr>
</tbody>
</table>
ULTRA Scientific is Now Part of Agilent Technologies

Agilent ULTRA Chemical Standards have:

- Best in class online search, compare, and ordering capabilities
- Rapid shipping: 99.9% of orders dispatched within 24 to 48 hours (continental US only, as of now)
- Custom standard solutions including our new online custom quoting tool, enabling customers to upload recipe formulations to and to modify the recipe before submitting it
  - Tool will allow customers to see the quote pricing instantly and allow them to check quote pricing based on quantity range
- Rigorously tested and manufactured under ISO 9001, ISO 17025, and ISO 17034 accreditation
- Sample preparation materials, columns, supplies, instrumentation, and reference materials from a single source
Perform a Noninjection “Blank”

Remove syringe from autosampler

Run your program

If you see peaks, it is likely that there is inlet contamination
Condensation Test

A condensation test is used to isolate the cause of:

- Erratic baselines
- Ghost peaks or carryover

For use when problems are worse after periods of GC nonuse.
Condensation Test

Procedure

• Leave GC at 40–50 °C for > 8 hours
• Blank run
• Repeat a blank run immediately after the first blank run is complete
• Compare the two blank runs
Condensation Test

Results

• First blank run is worse: Contaminants (from injector, lines, traps, or carrier gas) carried into the column.

• Blank runs the same: **Contaminants are not strongly focused on the front of the column.**
Jumper Tube Test

Purpose

- Helps to locate the source of contamination or noise
- Isolates GC components
Jumper Tube Test

Isolate the detector

- Remove column from the detector
- Cap detector and turn on
- Blank run
Jumper Tube Test

Isolation of detector – results:

Detector OK

Detector is the problem
Jumper Tube Test

Isolate the injector

• Connect the injector and detector
  - 1–2 meters deactivated fused silica tubing
• Turn on carrier gas
• Blank run
Jumper Tube Test

Isolate the injector – results:

Injector OK

Injector, lines, or carrier gas contaminated
Jumper Tube Test

Isolate the column

• Reinstall the column

• Set up as before

• Blank run
Jumper Tube Test

Isolate the column – results:

• Problem returns? It’s the column

• Problem gone? Previous leak, solid debris, or installation problem
Have a Good Troubleshooting Story? Let Us Know!

Please call or email us today to share a troubleshooting success story or if you need help troubleshooting!
Agilent University
Why training? What can we help with?

Agilent University:
• Trained over 38K students FY19
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Staff turnover
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Daily consistency with output and results
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- On-site or virtual on-site

Flexible and convenient training options when and where you need them:

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Troubleshooting Tips

1. Isolate the problem
   (blank run, inject unretained compound, jumper tube test)
2. Change only one variable at a time
3. Compare before/after chromatograms
   (Peak shape, response, retention, baseline rise, background, look for trends, etc.)
4. Utilize technical support
Remember

Complete system = carrier gas + injector + column + detector + data system

- Multiple causes and effects
- Do not change too many variables at once
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 USA and Canada 8–5, all time zones

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spectro-supplies-support@agilent.com
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