Why Inertness Matters in Gas Phase Analysis

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What we will cover today

• Inertness, what does it mean?
• Where is it most important
• Flow path diagram, activity focal areas
• Five tips to optimize inertness
• Inertness testing and it’s significance
• Real life examples showing the importance of inertness
• Essential tools for your inert flow path
• Brief summary-take home message
Some Basics

Inertness, what does it mean?
- Lack of peak tailing
- Lack of active compound adsorption
- Consistent results for trace level active analytes
- Better data integrity

How did this effort get started?
- Customer focused innovation
- Manufacturing process development
- Testing procedures
Where is inertness most important?

Trace level analysis of active analytes
Advanced GC techniques
  • GC/MS
  • GC/MS/MS
  • GC/MS TOF
  • GC/MS QTOF
  • GC x GC
Samples in difficult matrices
Critical samples
Using glass wool can trap non-volatiles and extend column lifetime. But improperly deactivated liner and glass wool can result in loss of analytes due to active sites. For samples with labile or active compounds use only highly deactivated liners.

Active sites within columns that are not optimally deactivated can negatively affect peak shape and peak response. Using columns that are tested with the most demanding test probes provides the highest confidence in sensitivity and accuracy.

Analyte breakdown products can be indicative of active sites in the detector which can compromise data.
Maintain the inlet

Preventative maintenance helps ensure peak instrument performance and productivity. Inspect and replace worn or dirty flow path supplies—such as syringe needle, septa, ferrules, and inlet seals—to eliminate leaks and minimize downtime. Using certified vials, caps, septa, ferrules, and gold inlet seals also extends the flow path maintenance interval.
Prevent sample loss at injection

Inlet liners are a critical link in the sample flow path, and can be a source of activity and analyte loss. Liner design and chemistry impact the transfer of compounds into the column, so you should always use a reliably deactivated liner suited to your injection technique. Change the liner when there is visible discoloration indicating non-volatile residue buildup from samples. This can be challenging to detect; so when in doubt, change the liner. This will maximize sample transfer and minimize sample loss.
Tip Number 3

Select a column with optimized inertness

Optimized column inertness minimizes compound loss and degradation for more accurate quantitation of active analytes, especially at trace levels. To ensure consistent column inertness, choose a column that has been tested with a rigorous test probe mixture for in-depth evaluation and certification of inertness. When installing the column, start with high quality ferrules and examine column ends for chips or burrs under magnification. Make sure the column is positioned the recommended depth into the inlet and detector.
Tip Number 4

Remember your detector

To ensure accurate quantification and high sensitivity, the flow path must be highly inert, including detector surfaces. This is especially true of mass spectrometers, where an inert ion source is necessary to prevent active compounds from attaching to metal surfaces. The best inert sources are constructed of a solid inert material, as opposed to an inert coating which can wear away over time.
Tip Number 5

Use gas purifiers

A clean, high quality gas supply that is free of oxygen and contaminants reduces the risk of column damage, sensitivity loss, and downtime, improving performance and increasing productivity.
Grob-type Test Mix Results Competitor’s Premium 5ms

1. 1-Octanol
2. n-Undecane
3. 2,6-Dimethylphenol
4. 2,6-Dimethylaniline
5. n-Dodecane
6. Naphthalene
7. 1-Decanol
8. n-Tridecane
9. Methyl decanoate

Sampler: Agilent 7683B, 5 µL syringe (Agilent part # 5181-1273), 1.0 µL split injection, 4 ng each component
Carrier: Hydrogen constant pressure 37 cm/s
Inlet: Split/splitless; 250 °C, 1.4 ml/min. column flow, split flow 140 ml/min.
Liner: Deactivated single taper w glass wool (Agilent part # 5183-4647)
Oven: 120 °C isothermal
Detection: FID at 325 °C, 450 ml/min. air, 40 ml/min. hydrogen, 45 ml/min. nitrogen makeup

not probative
Weak Probes  versus  Strong Probes

2,6-Dimethylphenol

1-Propionic acid

2,6-Dimethylaniline

4-Picoline
Carefully selected probes designed to test inertness effectively

<table>
<thead>
<tr>
<th>Probe</th>
<th>(ng on column)</th>
<th>Column functional test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 1-Propionic acid</td>
<td>1.0</td>
<td>Basicity</td>
</tr>
<tr>
<td>2. 1-Octene</td>
<td>0.5</td>
<td>Polarity</td>
</tr>
<tr>
<td>3. n-Octane</td>
<td>0.5</td>
<td>Hydrocarbon marker</td>
</tr>
<tr>
<td>4. 4-Picoline</td>
<td>1.0</td>
<td>Acidity</td>
</tr>
<tr>
<td>5. n-Nonane</td>
<td>1.0</td>
<td>Hydrocarbon marker</td>
</tr>
<tr>
<td>6. Trimethyl phosphate</td>
<td>1.0</td>
<td>Acidity</td>
</tr>
<tr>
<td>7. 1,2-Pentanediol</td>
<td>1.0</td>
<td>Silanol</td>
</tr>
<tr>
<td>8. n-Propylbenzene</td>
<td>1.0</td>
<td>Hydrocarbon marker</td>
</tr>
<tr>
<td>9. 1-Heptanol</td>
<td>1.0</td>
<td>Silanol</td>
</tr>
<tr>
<td>10. 3-Octanone</td>
<td>1.0</td>
<td>Polarity</td>
</tr>
<tr>
<td>11. n-Decane</td>
<td>1.0</td>
<td>Hydrocarbon marker</td>
</tr>
</tbody>
</table>
UI Mix Results on a Competitor’s “Premium” 5ms Column

1. 1-Propionic acid
2. 1-Octene
3. n-Octane
4. 4-Picoline
5. n-Nonane
6. Trimethyl phosphate
7. 1,2-Pentanediol
8. n-Propylbenzene
9. 1-Heptanol
10. 3-Octanone
11. n-Decane

Sampler: Agilent 7683B, 0.5 µL syringe (Agilent part # 5188-5246), 0.02 µL split injection
Carrier: Hydrogen constant pressure, 38 cm/s
Inlet: Split/splitless; 250 °C, 1.4 ml/min. column flow, split flow 900 ml/min., gas saver flow 75 ml/min. on at 2.0 min.
Liner: Deactivated single taper w glass wool (Agilent part # 5183-4647)
Oven: 65 °C isothermal
Detection: FID at 325 °C, 450 ml/min. air, 40 ml/min. hydrogen, 45 ml/min., nitrogen makeup
UI Mix Results on an Agilent J&W DB-5ms Ultra Inert

1. 1-Propionic acid
2. 1-Octene
3. n-Octane
4. 4-Picoline
5. n-Nonane
6. Trimethyl phosphate
7. 1,2-Pentanediol
8. n-Propylbenzene
9. 1-Heptanol
10. 3-Octanone
11. n-Decane

Sampler: Agilent 7683B, 0.5 µL syringe (Agilent part # 5188-5246), 0.02 µL split injection
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Liner: Deactivated single taper w glass wool (Agilent part # 5183-4647)
Oven: 65 °C isothermal
Detection: FID at 325 °C, 450 ml/min. air, 40 ml/min. hydrogen, 45 ml/min., nitrogen makeup
Agilent J&W DB-UI 8270D testing conditions and probe significance

- Ultra low volume solvent test mix eliminates solvent masking of activity
- Low concentration test probe mixture (~5-10 ng on column)
- 45°C low test temp more stringent test for inertness by allowing us to test small molecule (stronger) acid probe, smaller molecule (stronger) basic probe and smaller molecule (more demanding) primary alcohol plus a demanding –diol to measure silanol activity.
- m & p- xylene are surrogate representatives of aromatic isomers and optimized phase selectivity (RI measurement) for getting highest resolution (resolution measurement) for semi-volatile aromatic isomers.
- Halogenated aromatic to measure for reproducible selectivity of the column for similar semivolatiles found in typical analyses.

Propanoic acid

Pyridine

Selectivity and resolution of m&p xylene

1-Chloro-2-fluorobenzene
Resolution of benzo-b & k fluoranthene isomers

Positional isomers

Column: Agilent DB-UI 8270D, 30 m x 0.25 mm, 0.25 µm (p/n 122-9732)
Liner: Dual taper direct connect liner (p/n G1544-80700)
Inlet: MMI in non-pulsed splitless mode 1 µL at 275 °C
Carrier: He, 1.2 mL/min, constant flow
Septum purge flow: 3 mL/min, purge time on 0.7 min 50 mL/min
Oven program: 30 °C (1.0 min), 15 °C/min to 100 °C, 20 oC/min to 240 °C (0.5 min), 15 °C/min to 325 °C (6.7 min) Gas saver Off
GC/MSD: Agilent 7890/5975C, 325 °C transfer line, 280 °C source, 150 °C quad, 35-500 AMU range
Sampler: Agilent 7693, 10.0 µL syringe (p/n G4513-80216)
Ultra Inert Forensics and Food Examples using the Agilent J&W DB-35ms Ultra Inert GC columns

Drugs of Abuse
   - Key opiate derivative separation

Challenging Pesticide analyses
   - Organo-phosphorus pesticides
   - Analysis in a red snapper fish tissue matrix
Agilent J&W DB-35ms Ultra Inert Fast Toxicology Analyzer Checkout

Instrument Conditions

Carrier: Helium fixed pressure 35.0 PSI
Inlet: splitless 1 µl 280 °C, total flow 56.4 ml/min, 3 ml/min switched septum purge, gas saver off, 50 ml/min after 0.4 minutes
Sample: Agilent GC/MS Toxicology Checkout Mixture (Agilent p/n 5190-04710)
Inlet Liner: dual taper deactivated (Agilent p/n 5181-3315)
Column: Agilent J&W DB-35msUl 15m x 0.25mm x 0.25µm (Agilent p/n 122-3812Ul)
Back-flush: post run: 1 min. 1 psi inlet, 75 psi aux EPC
Oven: 100 °C (0.25 min) to 345 °C (40 °C/min, 2.25 min hold)
MSD: transfer line 300 °C, source 300 °C, quadrapole 180 °C scan mode
NPD: Blos bead 300 °C H2 3 ml/min, 60 ml/min air, 11 ml/min makeup + col flow
CFT Device: 2-Way splitter with solvent venting between MSD and NPD

Application note 5990-6577EN
Oxymorphone (OMOR) and Oxycodone (OCOD) derivatives unresolved on 5 % Phenyl Column

Data courtesy of Christine Giffin of the Delaware Office of the Chief Medical Examiner
Oxymorphone (OMOR) and Oxycodone (OCOD) derivatives resolved on DB-35msUI

- 536 d3 OMOR
- 533 OMOR
- 462 d3 OMOR
- 459 OCOD common ion
- 459 OMOR common ion
Separation of Pesticide Analyzer Checkout Solution with an Agilent J&W DB-35msUI Column

Sample: 1 µg/mL Pesticide Analyzer Checkout solution (Agilent part #5190-0468)
GC/MSD: 7890/5975B with purged ultimate union
Column: DB-35ms UI 20 m 0.18 mm 0.18 µm (Agilent part #121-3822UI)
MMInlet: 1µL, splitless, 50°C (0.02 min), 400°C/min to 250°C
  purge flow 50mL/min at 1.5 min
  gas saver 30mL/min at 2.25 min
Carrier: Helium, 1.3 mL/min cnst flow
Oven: 50°C (1.3 min) to 135°C (50°C/min),
  15°C/min to 200°C, 20°C/min to 310°C (2.35 min)
Restrictor: 0.7m x 0.15mm ID Deactivated capillary column tubing
PCM 1: 3.8 psi constant pressure
Backflush: Post column, Postrun backflush 5 min @310°C
  70 psi Backflush Pressure
  2 psi Inlet Pressure during Backflush
MSD: Transfer line 320°C Source 320°C Quad 150°C

GC/MS Chromatogram of 1 ng on column loading of pesticides

Application note 5990-6595EN
Separation of 11 Pesticides with an Agilent J&W DB-35ms UI Column

Sample: 1µg/mL Custom Standard (Ultra Scientific)
GC/MSD: 7890/5975B with purged ultimate union
Column: DB-35ms UI 20 m 0.18 mm 0.18 μm (Agilent part #121-3822UI)
MMInlet: 1µL, splitless, 50°C (0.02 min), 400°C/min to 250°C
          purge flow 50mL/min at 1.5 min
          gas saver 30mL/min at 2.25 min
Carrier: Helium, 1.3 mL/min cnst flow
Oven:  50°C (1.3 min) to 135°C (50°C/min),
       15°C/min to 200°C, 20°C/min to 310°C (2.35 min)
Restrictor: 0.7m x 0.15mm ID Deactivated capillary column tubing
PCM 1:  3.8 psi constant pressure
Backflush: Post column, Postrun backflush 5 min @310°C
          70 psi Backflush Pressure
          2 psi Inlet Pressure during Backflush
MSD:  Transfer line 320°C Source 320°C Quad 150°C

Application note 5990-6595EN
**GC/MS Chromatogram of Red Snapper fish extracts**

**Blank Relative Vs. Spiked Sample (26 components)**

| 1. Dichlorvos | 14. Methyl parathion |
| 2. Vernolate | 15. Malathion |
| 4. Ethalfluranil | 17. Bromacil |
| 5. Trifluralin | 18. p,p'-DDE |
| 7. Prometon | 20. Propargite isomers |
| 9. Lindane | 22. Leptophos |
| 10. β-BHC | 23. Mirex |
| 11. Heptachlor | 24. Cypermethrin isomers |
| 12. Chlorpyrifos-methyl | 25. Fluvalinate isomers |
| 13. Chlorothalonil | 26. Fenvalerate isomers |

**GC/MS Chromatogram of 0.5 ng on column loading of pesticides in Red Snapper**

**Application Note 5990-6595EN**
Agilent J&W DB-624 UI vs. Competitor 624ms
Organic acid performance at 200 ppm

Column: Agilent J&W DB-624UI 30 m x 0.32 mm x 1.8 um vs Rxi-624Sil MS
Oven: 35°C 7.45 min hold, 6.72°/min to 100°C (2.23 min hold), 10.08 °/min to 220°C (4.47 min hold), 16.79 °/min (4.17 min hold)
Carrier: Helium 39.6 cm/s (approx. 2.6 mL/min) set at 35°C, EPC-Constant Flow
Inlet: Split, 20:1 at 250°C (total flow approx 51 mL/min, and 11.2 psi)
Inlet liner: Ultra Inert with wool
Detector: FID at 280°C, H2 @ 40 mL/min, Air @ 400 mL/min, N2 makeup @ 30 mL/min

Acetic acid
propionic acid
butanoic acid
Octanoic acid

Severe tailing on competitor’s 624ms
No detection on Competitor’s 624ms
Agilent J&W DB-624 UI-Organic Acid
Proof of Performance 25 to 200 ppm

Column: Agilent J&W DB-624UI 30 m x 0.32 mm x 1.8 um (p/n 123-1334UI)
Oven: 35°C 7.45 min hold, 6.72 °C/min to 100°C(2.23 min hold), 10.08 °C/min to 220°C(4.47 min hold), 16.79 °C/min (4.17 min hold)
Carrier: Helium 39.6 cm/s (approx. 2.6 mL/min) set at 35°C, EPC-Constant Flow
Inlet: Split, 20:1 at 250°C (total flow approx 51 mL/min, and 11.2 psi)
Inlet liner: Ultra Inert with wool
Detector: FID at 280°C, H2 @ 40 mL/min, Air @ 400 mL/min, N2 makeup @ 30 mL/min

all four acids observed at each level
Signal to Noise Comparison Residual Solvents
Class 1 Standard at Target Limit

DB-Select 624UI <467>

- Benzene/1,2 dichloroethane
  - R = 1.82

S/N = 6.85

Carbon Tetrachloride

Vendor R G43

- S/N = 3.78
- R = 1.59

Vendor P G43

- S/N = 2.65
- Below 3.0
- R = 1.38

Carbon Tetrachloride
Acetonitrile (2)/ Dichloromethane (3) Resolution
Residual Solvents Class 2A Standard at Limit

DB-Select 624UI <467>

Vendor R G43

Vendor P G43

$R_s = 2.13$

$R_s = 2.02$

$R_s = 1.20$
Pyridine Peak Shape Comparison Residual Solvents Class 2 B Standard at Target Limit

DB-624UI Select<467>
Vendor R G43
Vendor P G43

USP Tailing = 1.3
USP Tailing = 2.5
USP Tailing = ND

Pyridine
Agilent Ultra Inert deactivation passes Endrin/DDT decomposition test after 100 injections due to better stability and inertness than competitor’s deactivated liner.

Robustness Endrin decomposition Test:

<table>
<thead>
<tr>
<th>Peak Identification</th>
<th>1st injection</th>
<th>101st injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>DDE*</td>
<td>1.2</td>
<td>12.2</td>
</tr>
<tr>
<td>Endrin</td>
<td>2.5</td>
<td>3.0</td>
</tr>
<tr>
<td>DDD*</td>
<td>1.1</td>
<td>33.8</td>
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<tr>
<td>Endrin aldehyde*</td>
<td>1.7</td>
<td>2.2</td>
</tr>
<tr>
<td>DDT</td>
<td>2.5</td>
<td>3.0</td>
</tr>
<tr>
<td>Endrin ketone*</td>
<td>2.2</td>
<td></td>
</tr>
</tbody>
</table>

* Breakdown products

Peak breakdown

Agilent Ultra Inert single taper liner (p/n 5190-2292)
Even with **glass wool**, Agilent Ultra Inert deactivated liners provide high responses for sensitive semivolatile acidic compounds. Competitor’s deactivated liners show activity and adsorption.

Agilent Ultra Inert single taper liner **with wool** (p/n 5190-2293)

- Peaks:
  1. 2,4-Dinitrophenol
  2. 4-Nitrophenol
  3. 4,6-Dinitro-2-methylphenol
  4. 4-Aminobiphenyl
  5. Pentachlorophenol

**IS1**. Acenaphthene-d10
**IS2**. Phenanthrene-d10

Competitor’s deactivated gooseneck liner with deactivated wool

Even with glass wool, Agilent Ultra Inert deactivated liners provide high responses for sensitive semivolatile acidic compounds. Competitor’s deactivated liners show activity and adsorption.
Drug of abuse are shown on GC/MS SIM chromatograms. 5 ng of checkout standards on column.

**Agilent UI single taper liner with wool (p/n 5190-2293)**

- Higher response
- Better peak shape

Peaks:
1. Oxycodone
2. Temazepam
3. Flunitrazepam
4. Heroin
5. Nitrazepam
6. Clonazepam
7. Alprazolam

**Competitor’s deactivated gooseneck liner with deactivated wool**
Agilent Ultra Inert Inlet Liners

Get a robust, reproducible, and reliable inert flow path with Agilent Ultra Inert Inlet liners

- Higher sensitivity, accuracy and reproducibility
- Exceptional batch-to-batch uniformity
- Low to no bleed or background contamination
- Exclusive Agilent touchless packaging removes the risk of contamination
- Available in economical bulk packs; 5, 25 and 100 packs
Industry Leading Agilent J&W Ultra Inert GC Columns

- Leading the industry standards for consistent column inertness and exceptionally low column bleed
- Lower detection limits and more accurate data for difficult analytes
- Tested with demanding Ultra Inert test probe mixtures
- UI Columns available:
  
  - DB-1ms UI, HP-1ms UI
  - DB-5ms UI, HP-5ms UI
  - DB-35ms UI
  - DB-624UI, DB-Select 624UI for <467>
  - DB-UI 8270D
Additional productivity enhancing tools

- Free method translation software
- Gas Clean filters
- CFT fittings and devices
- Turn-key analyzer solutions
Take Away Message

• Be aware of potential active sites in the flow path and how to minimize their impact
• Rigorous inertness testing is necessary to assure that flow path components are inert
• Use Ultra Inert liners and columns for critical and trace-level applications
• Ultra Inert liners and columns deliver excellent performance over a range of applications and challenging analyte sets
• Stay tuned for future flow path inertness innovations

To learn more please visit www.agilent.com/chem/ultrainert
Questions?