Lower Detection Limits and Quantitate with Confidence with Breakthrough Ultra Inert Technology

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#### **Goals of the Presentation**

1. Understand what it means to be *INERT*, and why it is important to have an inert flow path

- 2. Talk about the pieces of the flow path
  - Liner
  - Column
- 3. Inert MS Source
- 4. ?????



#### **Snapshot of Flowpath**





### What is meant by inert?

According to Wikipedia....

To be in a state of doing little or nothing!

As it Pertains to Chromatography....

Not Chemically Reactive



### What Does GC System Inertness Look Like?

Easier question: What does poor inertness look like?

Symptoms of poor GC system inertness:

- \* Tailing peaks
- \* Reduced peak response
- \* No peak response
- \* Extra peaks!
- \* Poor linearity of a peak usually at low concentrations
- \* Unstable detector baseline



#### GC System Inertness What do we mean?

Problems with poor inertness usually limited to —ative" solutes.

Tailing or breakdown of "benign" solutes is symptomatic of a more generalized system problem, usually related to gross contamination.



#### GC System Inertness What do we mean?

Problems with poor inertness usually limited to —ative" solutes.

For example: Alcohols & Diols (-OH), Phenols ( $\langle \_ \rangle^{-OH}$ ), Amines (-NH3), Acids (COOH), Thiols & Sulfur in general like to tail.

Thermally labile and structurally -strained" solutes will breakdown or rearrange, e.g., DDT, Endrin, Carbamates, Nitroglycerines.



#### **Possible Inertness Problem Areas**

#### Inlet

- liner, liner packing, gold seal, stainless steel
   Consumables
- septa, syringe, vial, caps, inserts, solvents
   Column

#### GC Detector

- source geometry, material, column interface, acquisition rates
   Temperatures
- inlet, transfer line, source, quads, oven

Other method factors i.e. samples and standards preparation



#### What is the Surface Area Contribution to Overall <u>Flowpath</u> Inertness?

#### GC Flowpath Surface Areas

	L (cm)	d (cm)	π	Surface Area (cm <sup>2</sup> )
Liner	7.85	0.4	3.142	9.86
Gold Seal		0.8	3.142	0.5
Column	3000	0.025	3.142	235.6



### Let's Start at the Inlet





#### **Liner Problems**

Many chromatographic problems are blamed on the column.

Often, an active liner is the culprit.

Symptoms include:

Poor peak shape Irregular baselines Poor resolution Poor response

Extra Peaks



## **Degradation in the Liner**



Pub: 5990-7596EN



#### **Pesticides**





## **Liner Deactivation**

- 1. Surface PreparationTreatment
- 2. Drying of the liner
- 3. Coating
- 4. Drying



## LINER DEACTIVATION

Prior to deactivation, surface must be cleaned with an acid leach step:

- Place liner in clean test tube
- Cover liner with 1N HCl or HNO<sub>3</sub> solution
- Soak for at least 8 hours (overnight is preferred)
- If acid solution is highly discolored, replace with clean solution and continue to soak until no color change is noted
- Do not soak liners for longer than 24 hours
- Rinse with deionized water followed by methanol
- Dry the liner at 100-150°C. Do not exceed 150°C.



#### LINER DEACTIVATION

#### **Solution Silylation Procedure**

Place liner in screw cap test tube

Cover liner with 10% TMCS or DMCS in toluene

Tightly seal with PTFE-lined cap

Allow to stand for at least 8 hours

Remove from solution and thoroughly rinse with toluene, then methanol

Dry the liner at 75-100°C

NOTE: Several liners can be done in one test tube, but rotate the tube several times to ensure that all surfaces are exposed to the solution.



# <u>Ultra Inert</u> GC Inlet Liners – ultimate deactivation performance

- Response levels / Inertness : 2,4-Dinitrophenol recovery
  - peak shape and signal to noise at trace levels (0.5 ppm)
- Robustness : Endrin / DDT Breakdown
- < 20% breakdown of Endrin after a sequence of <u>100</u> <u>injections</u> -- not just the first injection

Reliability / Linearity: Response factors of active compounds

• over low level calibration range – 2 ng to 80 ng on column



#### **Reliability / Quality Assurance : Ultra Inert Liner Certificate of Performance**

#### Lot to Lot Liner Reproducibility assured:

Each Ultra Inert deactivation lot is *Certified* to ensure consistent and efficient coverage using both acidic and basic probes at trace (2 ng) levels on column

Certificate of Performance with every liner is printed on a label ready to peel and stick into your laboratory notebook for easier compliance.

Traceability: Deactivation lot number and glass lot numbers are on the Certificate Part Number is permanently identified on the liner for fast and easy re-ordering

#### Certificate of Performance

5190-2293 Ultra I	nert Liner
Splitless, Sngl taper, (	Gass Wool
Liner Body Lot:	0023A
Deactivation Lot:	B11002





## More Benefits of Ultra Inert Deactivated Liners

#### **Unequalled Reproducibility**

- •Lot testing ensures reproducible coverage of deactivation
  - QC test with probes selected to reveal activity
  - QC method tailored to test liner -- not column or system -- inertness
- Lab notebook friendly Certificate of Performance on a sticker shipped with each liner
- Ease of Use with exclusive -Touchless" packaging...
- •Plasma treated Non-Stick O-ring is preinstalled on the liner
- •Packaging is Pharmaceutical grade PTEG tubing approved by GCMS extraction testing for cleanliness
- •Install new liner with O-ring without touching or risk contaminating the new, clean Ultra Inert liner





#### **Robustness: Endrin Decomposition Test**



#### Pass/Fail criteria : < 20% degradation

Agilent Ultra Inert deactivation passes Endrin/DDT decomposition test after 100 injections



#### Semi Volatile critical component : 2, 4 DNP Comparison of splitless single taper liners without wool



Response Factors (FID) over calibration range (2-80 ng on column)



## Glass Wool, or No Glass Wool?

Provides a lot of additional surface area to help with sample mixing and volatilization

Helps trap non-volatile residues which minimizes the amount that gets into the column

## Provides a lot of additional surface area



## **Semivolatile Activity Comparison**



Figure 6. Performance comparison of Agilent Ultra Inert deactivated liner with wool (p/n 5190-2293) and Ultra Inert deactivated liner without wool (p/n 5190-2292).

PUB: 5990-7381EN



#### **Robustness: Endrin Breakdown on Liners with Wool**





Exceptional inertness maintained through a sequence of 100 injections



# Just Because you can't see it, doesn't mean it's not there.....





#### **Ultra Inert Liners Available for non-Agilent GC's**



#### We currently support:

- Bruker, Varian\*
- CTC
- PerkinElmer
- Shimadzu
- Thermo Scientific
- And more coming soon





### Where does column activity come from?



Sterically difficult to —ap? all of them—estimates 40-65% capped with traditional deactivation.

Non-traditional sources such as trace impurities in starting materials and manufacturing lines.



#### **Traditional Deactivations**

Dichlorodimethylsilane, various silizanes, etc... -endqas"

Traditional deactivation has gaps in surface coverage due to bulky TMS type moieties, and tight fused silica lattice, and is somewhat inert and chemically resistant.





#### DB-5ms and HP-5ms Engineered Deactivations

**Polymeric Deactivation Technology** 

-Bids" at multiple points with many silanols



-Blanetts" sterically hindered active silanols, fewer silanols



### What does Column Activity look like?





#### What are the specific benefits of High Inertness?

Greater sensitivity for traditional trace active analytes meet RRF requirements with greater ease more runs before maintenance

Greater reliability for ultra-trace non-traditionally active analytes (<100 ppb PAHs, Chlorinated dioxins, etc...)



#### Who benefits from 'Ultra' Inert Columns?

Anyone doing trace analysis of active analytes

- Environmental semivolatile analysts
- Pesticide residue analysts
- Forensic/Drugs of abuse analysts
- Anyone in Industry, Government, or Academia interested in ultratrace amounts of even modestly active analytes



## **Test Probes and Column Activity QC Testing**

- Test probes are vital to ensure the quality and reproducibility of GC columns
  - Properly deactivated
  - Contain the correct amount of stationary phase
  - consistent batch-to-batch relative retention time
- Test probes can either highlight or mask the deficiencies of a column, normally include:
  - An organic acid (peak tailing or lost response of acid indicates the column is basic)
  - A base (peak tailing or lost response of base indicates the column is acidic)
  - An alcohol (gives indication of any oxygen damage or exposed silanols)
  - Non-active probes (e.g. alkanes)
- Good test probes allows the probative portion of the test module to penetrate and fully interact with the columns stationary phase and surface.
  - Low molecular weight
  - Low boiling points
  - No steric shielding of active group



## Weak Probes vs. Strong Probes



#### **Grob-Type Mix - QC Testing of the 80s**



ion: FID at 325 °C, 450 ml/min. air, 40 ml/min. hydrogen, 45 ml/min. nitrogen makeup

#### **DB-5ms Test Mix – QC Testing of the 90s**



Competitor X Inert 5ms 30m x 0.25mm x 0.25um



1. 2-Ethylhexanic acid

2. 1,6-Hexanediol

3. 4-Chlorophenol

4. Tridecane

5. 1-Methylnaphthalene

6. 1-Undecanol

7. Tetradecane

8. Dichlorohexylamine

 Carrier:
 Hydrogen constant pressure 38 cm/s

 Inlet:
 25°0C Split flow 75 mL/min

 Liner:
 Deactivated single taper w/wool (5183-4647)

 Oven:
 125°C Isothermal

 Detector:
 FID, 320°C. 450 mL/min Air, 40 mL/min H<sub>2</sub>, 45 mL/min N<sub>2</sub> Makeup



# Ultra Inert Test Mix – QC Testing for Today's Demanding Applications

		Column	
Probe	(ng on column)	functional test	Carefu
1. 1-Propionic acid	1.0	Basicity	deman
2 1-Octene	0.5	Polarity	depth
3. n-Octane	0.5	Hydrocarbon marker	inertne
4. 4-Picoline	1.0	Acidity	<ul> <li>Test te</li> </ul>
5. n-Nonane	1.0	Hydrocarbon marker	(isothe
6. Trimethyl phosphate	1.0	Acidity	norma
7. 1,2-Pentanediol	1.0	Silanol	tests
8. n-Propylbenzene	1.0	Hydrocarbon marker	
9. 1-Heptanol	1.0	Silanol	
10. 3-Octanone	1.0	Polarity	
11. n-Decane	1.0	Hydrocarbon marker	

Carefully selected very demanding test probes for indepth evaluation of column inertness

Test temperature 65° C (isothermal), well below that normally used in conventional tests

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



### **Ultra Inert Test Mix on Competitor X Inert 5ms**



#### All highlighted peaks have poor peak shape – poor column deactivation

- The Competitor X column showed very poor performance when tested against the Über One test mix.
- Less demanding test probes masked the column activity for this column.
- The same column performed well with Grob-type test mix and DB-5ms test mix



#### **Ultra Inert Test Mix on Agilent J&W DB-5ms Ultra Inert**



Increased peak heights for accurate integration and detection of trace samples
 Routine analysis of demanding analytes now feasible



#### Same Selectivity – No Method Re-Development

- DB-5ms Ultra Inert columns have the same selectivity as their DB-5ms counterparts
- HP-5ms Ultra Inert columns have the same selectivity as their HP-5ms counterparts





#### **DB-35ms Ultra Inert Exhibits the Same Selectivity as DB-35ms**





## **Application Examples**

- Semi Volatile Analysis
- Brominated Fire Retardants
- Drugs of abuse
- Pesticides in Orange Oil
- PAHs
- PBDEs



#### **Semi Volatile Analysis**

1. 2. 3. 4. 5. 6.	N-nitrosodimethylamine Aniline 1,4 dichlorobenzene-D4 Benzoic acid Naphthalene- D8 Acenapthene-D10	GC : Sampler : Carrier: Inlet: Inlet Liner: Column: Oven: Detection:	Agilent 6890 Agilent 7683 column Helium cons Split/splitles off Deactivated DB-5ms Ultr 40% C (1 min MSD source	N/5975B MSD B, 5.0 μL syrin stant flow 30 c s; 260% C, 53 single taper v a Inert 30m x n) to 100%C (1 at 300% C, qu	nge (Agilent part # 5188-5246), m/s .7 ml/min. total flow, purge flow y glass wool (Agilent part # 518 0.25mm x 0.25µm (Agilent part 5% C/min), 10% C to 210% C ( adrupole at 180% C, transfer li	1.0 μL splitless injection w 50 ml/min. on at 0.5 μ 33-4647) # 122-5532UI) 1 min), 5% C/min. to 3 <sup>7</sup> ine at 290% C, scan ra	on, 5 ng on nin., gas saver 10% C (8 min) nge 50-550 AMU
7.	2,4-dinitrophenol		6	11	12	16	17
8.	4-nitrophenol	:	5	11	12		
9.	2-methyl-4,6-dinitrophenol						
10.	pentachlorophenol	3				14	18
11.	4-aminobiphenyl						
12.	Penanthrene-D10						
13.	Benzidine						
14.	Chrysene-D12						
15.	3,3'-dichlorobenzidine			10	13	15	
16.	Benzo [b] fluoroanthene			10			
17.	Benzo [k] fluoroanthene						
18.	Perviene-D12						
				9			
	1			8			
			7				
		4	1				
	,					I	



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#### "Large Mix" 5 ng on Column AccuStandard 8270 Mixes 1,2,3,4a,4b,5 &6 (93 Compounds) Select compound highlighted

- 1. n-Nitrosodimethylamine
- 2. 2-methyl pyridine
- 3. Benzidene
- 4. Flouranthene
- 5. Benzo (g,h,i) perylene

GC/MSD ConditionsColumn: DB-5ms Ultra Inert 30 m x 0.25 μm part # 122-5532UICarrier: He 30 cm/sec constant flowOven: 40% C (1min) to 100 % C (15 % C/min), 10 % C /min to 210% C (1min), 5 % C/min to 310% C (8 min)Inlet: splitless 260 % C purge flow 50 % ml/min at 0.5 min, gas saver 80 ml/min on at 1 minuteMSD: transfer line 290 % C, source 300 % C, quad 180 % C





#### **Pesticides and Fire Retardants (US EPA 527)**

	GC/MSD Conditions			
1,2-Dimethyl-2-nitrobenzene Acenaphthalene-D10	Sample:	Pesticide/PBDE standards 1 ng with 5ng IS/SS on column		
Dimethoate	Column:	DB-5MS Ultra Inert 30m x 0.25mm x 0.25um (Agilent part # 122-5532UI)		
Propazine	Carrier:	Helium 52cm/sec, constant flow		
Anthracene-D10	Oven:	60°C (1min) to 210°C (25º/min), 20ºC/min to 310ºC (3 min)		
Prometryne	Injection:	Splitless, 250°C, purge flow 50ml/min at 1min, gas saver 80ml/min on at 3 min		

9. Bromacil

1.

2.

3.

4.

5.

6.

7.

8.

- 10. Malathion
- 11. Thiazopyr
- 12. Dursban
- 13. Benthiocarb
- Parathion 14.
- 15. **Terbus sulfone**
- 16. **Bioallethrin**
- 17. Oxychlordane
- 18. Fenamiphos
- 19. Nitrophen
- 20. Norflurazone
- 21. Kepone
- 22. Hexazinone
- **Triphenyl phosphate** 23.
- 24. Bifenthrin
- 25. Chrysene-D12
- 26. BDE-47
- 27. Mirex
- 28. **BDE-100**
- 29. **BDE-99**
- 30. Perylene-D12
- 31. Fenvalerate
- 32. Esfenvalerate
- 33. Hexabromobiphenyl
- 34. **BDE-153**



9.00

Transfer Line 290°C, Source 300°C, Quad 180°C

9-14

17

15,16

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18

19

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2

MSD:

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28 29

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32

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31

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34

23

24

20

121

22

11.00

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#### **Drugs of Abuse**

Column:	DB-5ms Ultra Inert 30 m x 0.25 mm x 0.25 µm (Agilent part # 122-5532UI)
Carrier:	Helium 43.8 cm/sec constant flow
Oven:	120% C (2min) 20 % C/min to 180 % C (6 min hold), 18 % C /min to 270% C (2min),
	25 % C/min to 325% C (2 min)
Inlet:	split 30:1, ~ 1 ng on column 250 %C, single taper liner (Agilent # 5181-3316)
MSD:	transfer line 300 % C, source 280 % C, quad 200 % C, full scan m/z 50-450





#### **Bezodiazepines**





# **Pesticides in Orange Oil**

Analysis was carried out on the Agilent 7890A/5975 GC/MS or 7890A/7000 GC/MS/MS equipped with either a 7683 or 7683B Series ALS, split/splitless injection port and triple-axis detector. An Agilent J&W DB-5ms Ultra Inert 15 m x 0.25 mm x 0.25 um column (Agilent part # 122-5512UI) was used. The initial GC oven temperature was 70° C, which was held for 0.67 minutes. The oven was then ramped by 75° C/minute to 150° C, held for 0 minutes and ramped by 9° C/minute to 200° C and held for 0 minutes before ramping by 24° C/minute to 280° C and holding for 3 minutes. A six-minute post-run at 320° C was used. Pressure was held constant at 10 psi throughout the run and a split ratio of 10:1 for a 1uL injection. An open ended 4 mm helical liner was used (Agilent #5188-5396). The inlet temperature was 250° C and transfer line was set to 280° C. In the case of both detectors the source temperature was set to 300° C and the analyzer to 180° C.





#### **PAH Analysis**

#### **GC/MSD** Conditions

Sample:

1.

2. 3.

4.

5.

6.

7.

8.

9.

10.

11.

12.

13.

14.

15. 16. 10ug/ml PAH Standard

DB-5ms Ultra Inert 30m x 0.25mm x 0.25um (Agilent part # 122-5532UI) Column:

- Carrier: Helium 45cm/sec, constant flow
- 55°C (1min) to 320°C (25°/min), hold 3 min Oven:

Injection: Pulsed splitless, 300°C, 40psi until 0.2 min, purge flow 30ml/min at 0.75 min

Gas saver 80ml/min on at 3 min

MSD: Transfer Line 280°C, Source 300°C, Quad 180°C





### **PBDE Analysis**

#### **GC/MS** conditions

Column:	DB-5ms Ultra Inert 15 m × 0.25 mm × 0.25 µm (Agilent part # 122-5512UI)
Carrier:	Carrier Helium 72 cm/s, constant flow
Oven:	150 to 325 °C (17 °C/min), hold 5 min
Injection:	Pulsed splitless; 325 °C, 20 psi until 1.5 min, purge flow 50 mL/min at 2.0 min
MSD:	Source at 300 °C, Quadrupole at 150 °C, transfer line at 300 °C, scan range 200–1000 amu





## **PBDE Analysis**

#### **GC/MS** conditions

Column:	DB-5ms Ultra Inert 15 m × 0.25 mm × 0.25 µm (Agilent part # 122-5512UI)
Carrier:	Carrier Helium 72 cm/s, constant flow
Oven:	150 to 325 °C (17 °C/min), hold 5 min
Injection:	Pulsed splitless; 325 $^\circ$ C, 20 psi until 1.5 min, purge flow 50 mL/min at 2.0 min
MSD:	Source at 300 °C, Quadrupole at 150 °C, transfer line at 300 °C, scan range 200–1000 amu



Linearity is excellent across the range studied (0.5 ng/mL to 1,000 ng/mL, except for BDE-209 at 2.5 to 1,000 ng/mL range), giving R<sup>2</sup> values of 0.997 or greater in all cases and demonstrating highly inert surface of the column.



#### **Inert MSD Source**



Mass chromatograms for the pesticide Fenitrothion acquired via the inert source (upper) and a standard source design (lower). The black line indicates the ion abundance of the molecular ion of Fenitrothion (m/z 277) and the green line is attributed to a degradation product (m/z 247).



#### **Inert MSD Source**



Improved spectral integrity. New inert source eliminates surface activity reactions, resulting in more reliable library matches.



#### Don't Forget About....

- Sample Discrimination
- Sample Stability
- Carrier Gas
- Sample Prep
- Sample Vials



#### Conclusions.....

Inert flow path gives better peak shapes for \_active'compounds allowing for lower detection limits

Agilent Ultra Inert liners are packed with <u>Touchless</u>' packaging \*\*Available for non-Agilent Systems

Both the Agilent UI Liners and Columns go through rigorous testing to ensure performance as well as column to column, or liner to liner reproducibility.

#### For more information, please visit this website:

http://www.chem.agilent.com/en-US/Products/columns-supplies/gc-gcmscolumns/Pages/ultrainerthome.aspx



## **Agilent/J&W Technical Support**

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

#### \* Select option 3..3..1

866-422-5571 (fax)



email: gc-column-support@agilent.com

www.agilent.com/chem



