Installation, Care and Maintenance of Capillary Gas Chromatography Columns

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Application Engineer
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Happy New Year!

Installation, Care and Maintenance of Capillary Gas Chromatography Columns

or....

"It's not what your column can do for you, but what you can do for your column"

Column Installation

"Getting off to a good start"

Column Installation Procedure

- Install the column
- Leak and installation check
- Column conditioning
- Setting linear velocity or flow rate
- Bleed profile
- Test mix

Column Installation

What type of ferrule should I use?

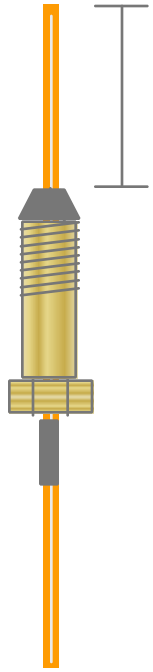
- **Graphite**
- **Graphite/Vespel**
- **Metal Ferrules UltiMetal Plus Flexible Metal Ferrules from Agilent for Capillary Flow Tech. and other GC connections**

Column Installation

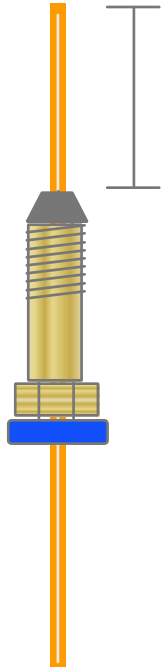
Measuring the right distance

Refer to manufacturers recommendations

White out



Septa



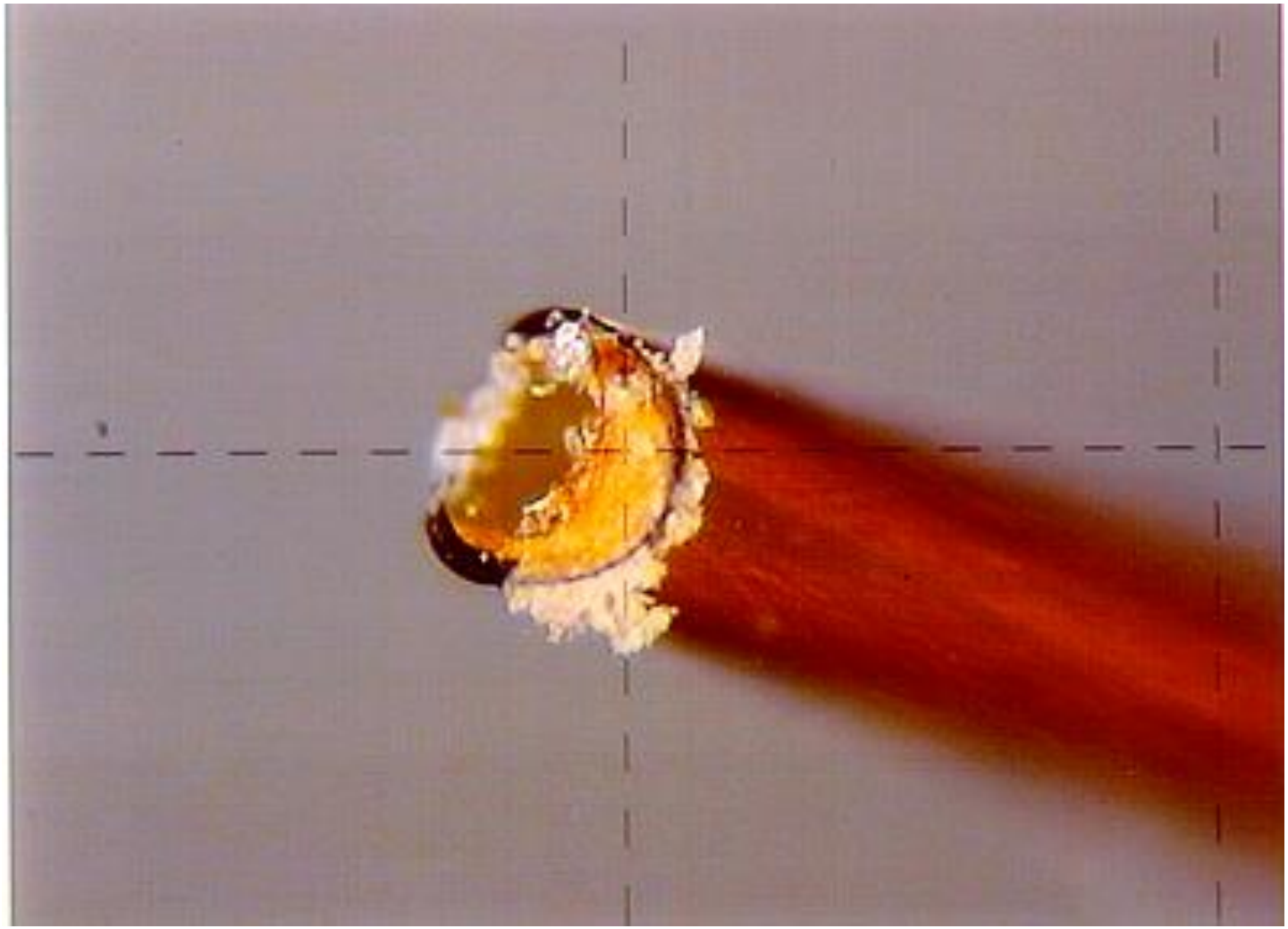
Cutting The Column

Gently scribe through the polyimide coating.
Do not attempt to cut the glass.

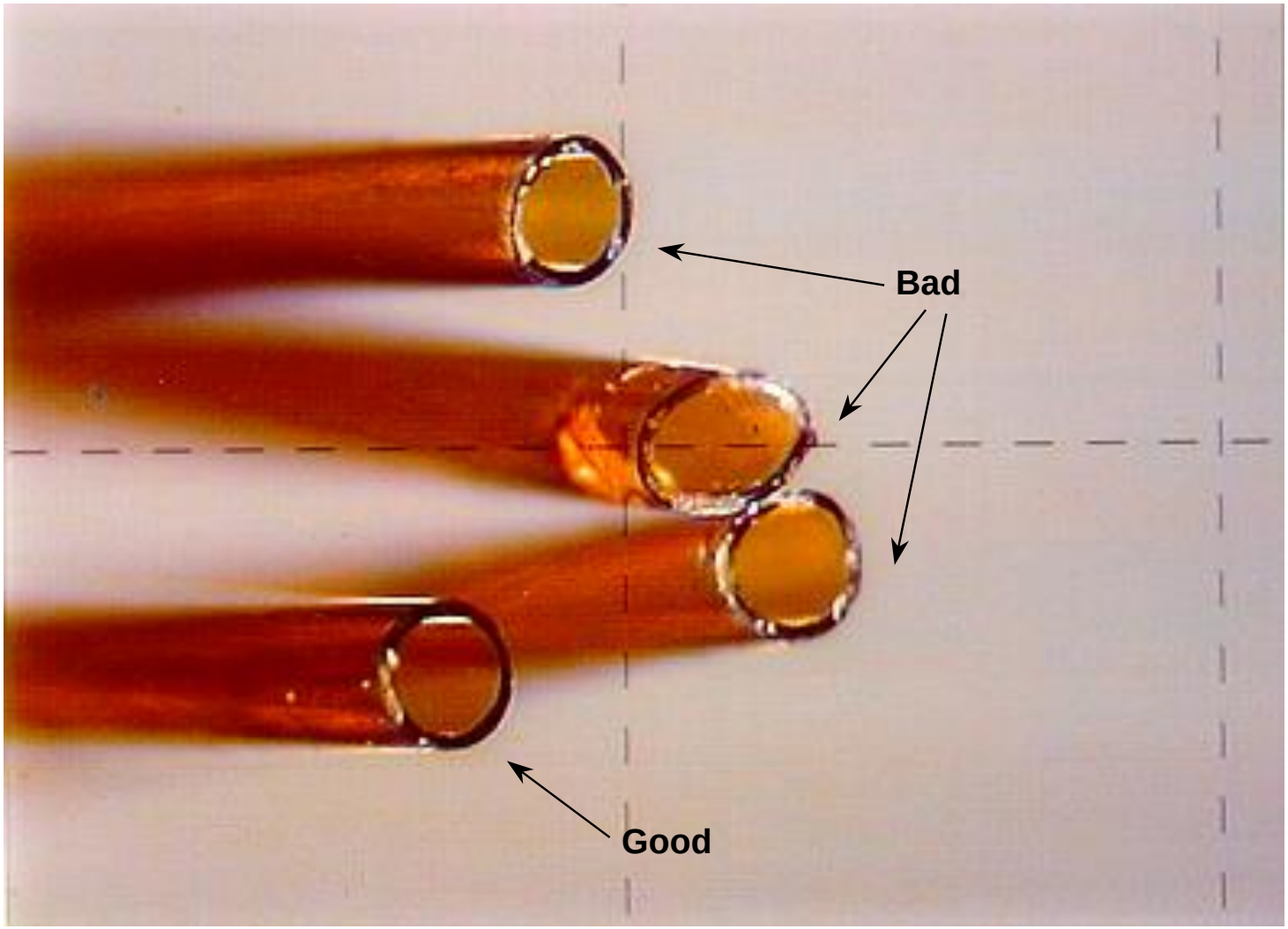
<u>Recommended tools:</u>
Diamond or carbide tipped pencil; or sapphire cleaving tool, ceramic wafer
Ocular or 6x magnification glass

<u>Do not use:</u>
Scissors, file, etc.

Example of a Bad Cut

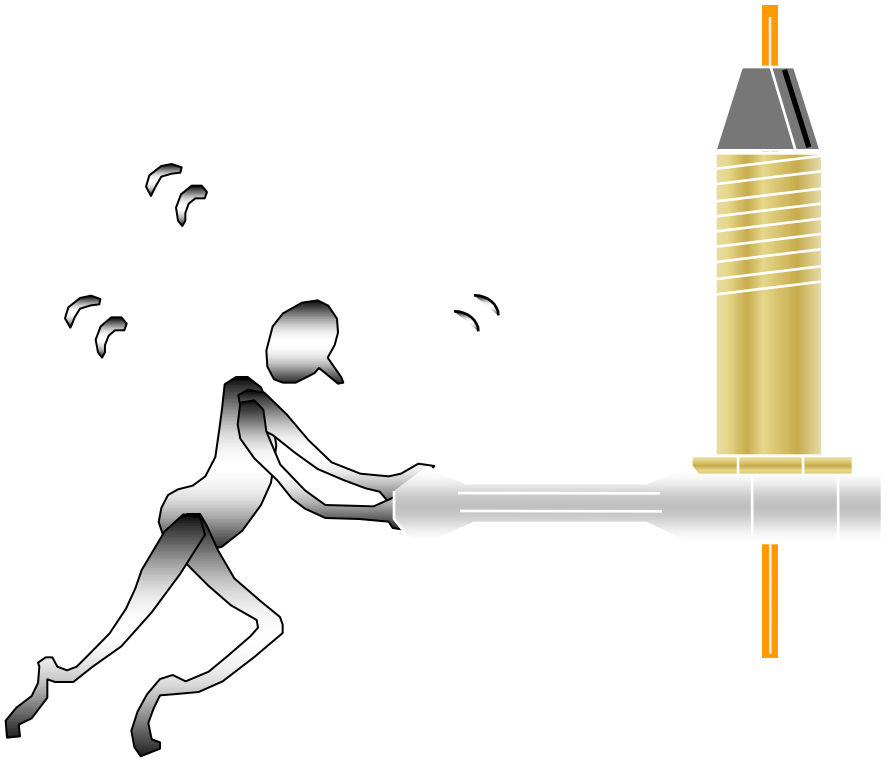


Examples of Column Cuts

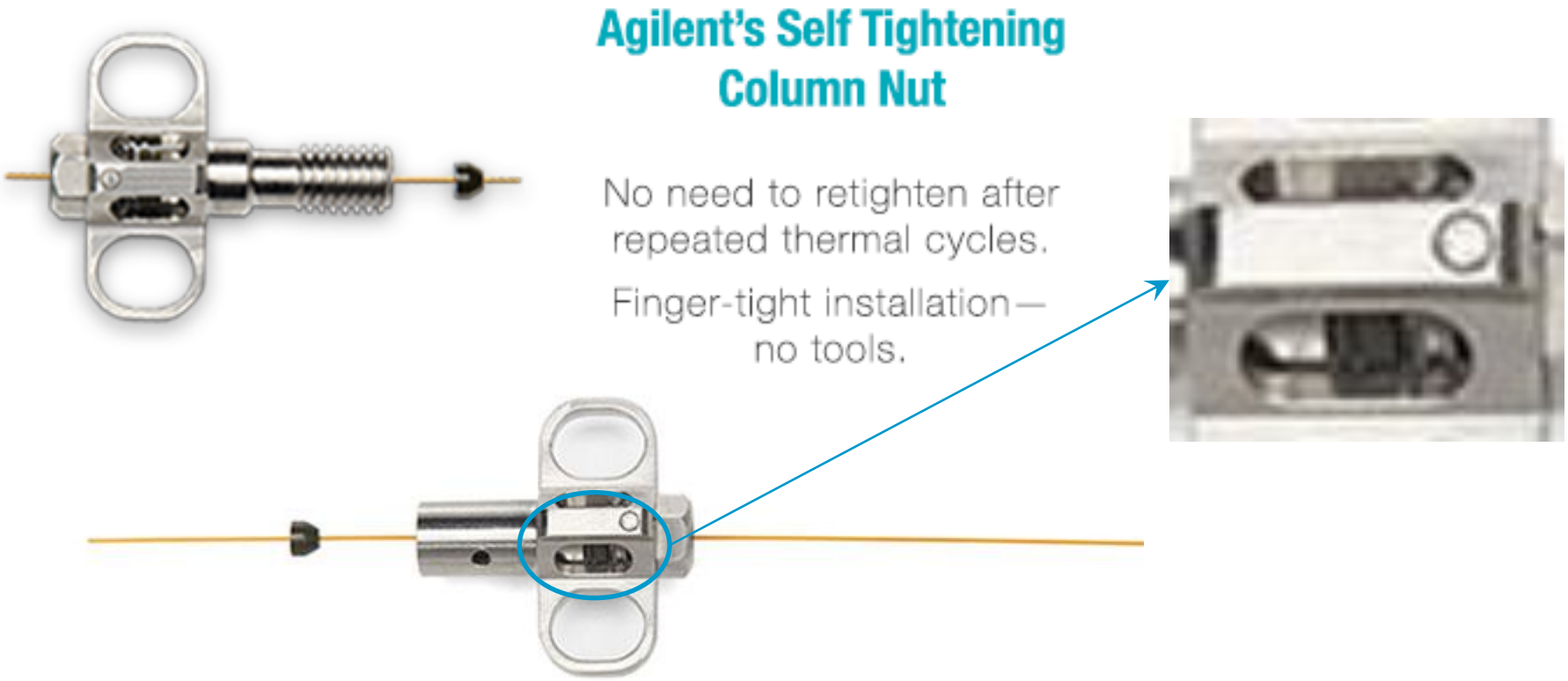


Column Installation

How tight is tight?



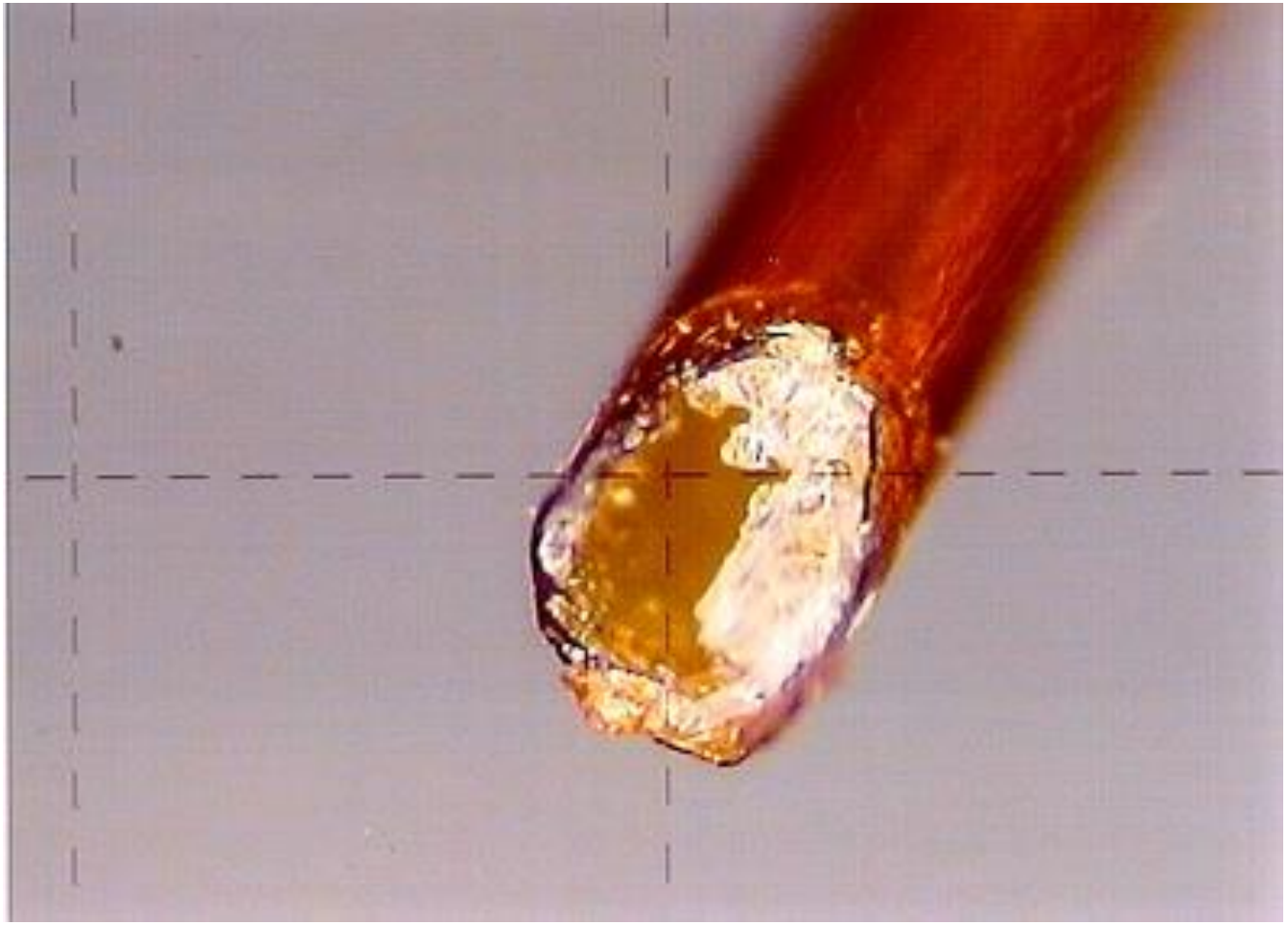
New self tightening column nuts



http://www.chem.agilent.com/en-US/products-services/Parts-Supplies/Chromatography-Spectrometry/GC-and-GC-MS/Self-Tightening-Column-Nuts/Pages/default.aspx

(easiest to google search: Agilent "self tightening column nut")

Over tightened Ferrule



Column Installation

Leak Check

DO NOT USE SNOOP on GC Columns

Electronic leak detector
IPA/Water
Inject a non-retained peak

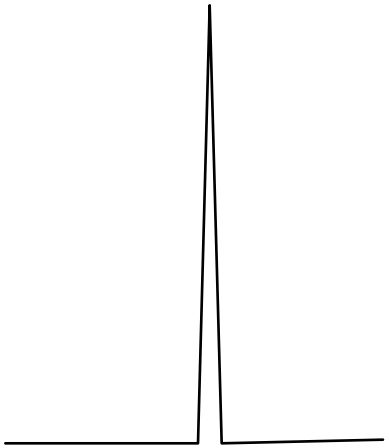
Leak and Installation Check

Inject a non-retained compound on a DB-1

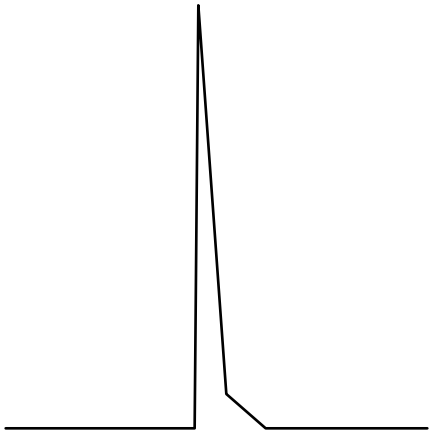
Detector	Compound
FID	Methane or Butane
ECD	$MeCl_2$ (headspace or diluted)
NPD	CH_3CN-acetonitrile (headspace or diluted)
TCD	Air
MS	Air or Butane

The peak should be sharp and symmetrical

Non-Retained Peak Shapes



Good Installation



Improper Installation or Injector Leak

Check for: -Too low of a split ratio

-Injector or septum leak

-Liner problem:

(broken, leaking, misplaced)

-Column position in injector and detector

Calculating Linear Velocity

Inject a non-retained compound and obtain the retention time:

$$\bar{\mu} = \frac{L}{t_o}$$

$\bar{\mu}$ = Average linear velocity (cm/sec)
L = Column length (cm)
t_o = Retention time (sec)

-$\bar{\mu}$ is *dependent* on column temperature
-$\bar{\mu}$ is *independent* of column diameter

He 35-40 cm/sec
H_2 45-60 cm/sec

Calculating Flow Rate

Inject a non-retained compound and obtain the retention time:

$$\bar{F} = \frac{\pi r^2 L}{t_o}$$

$\bar{F}$ = Flow rate (mL/min)
r = Column radius (cm)
L = Column length (cm)
t_o = Retention time (min)

$\bar{F}$ is dependent on column temperature
Measuring flow with a flow meter is often inaccurate

Column Conditioning

System must be leak free before conditioning column

Follow Manufactures Recommendations

Heat the column to the lower of:

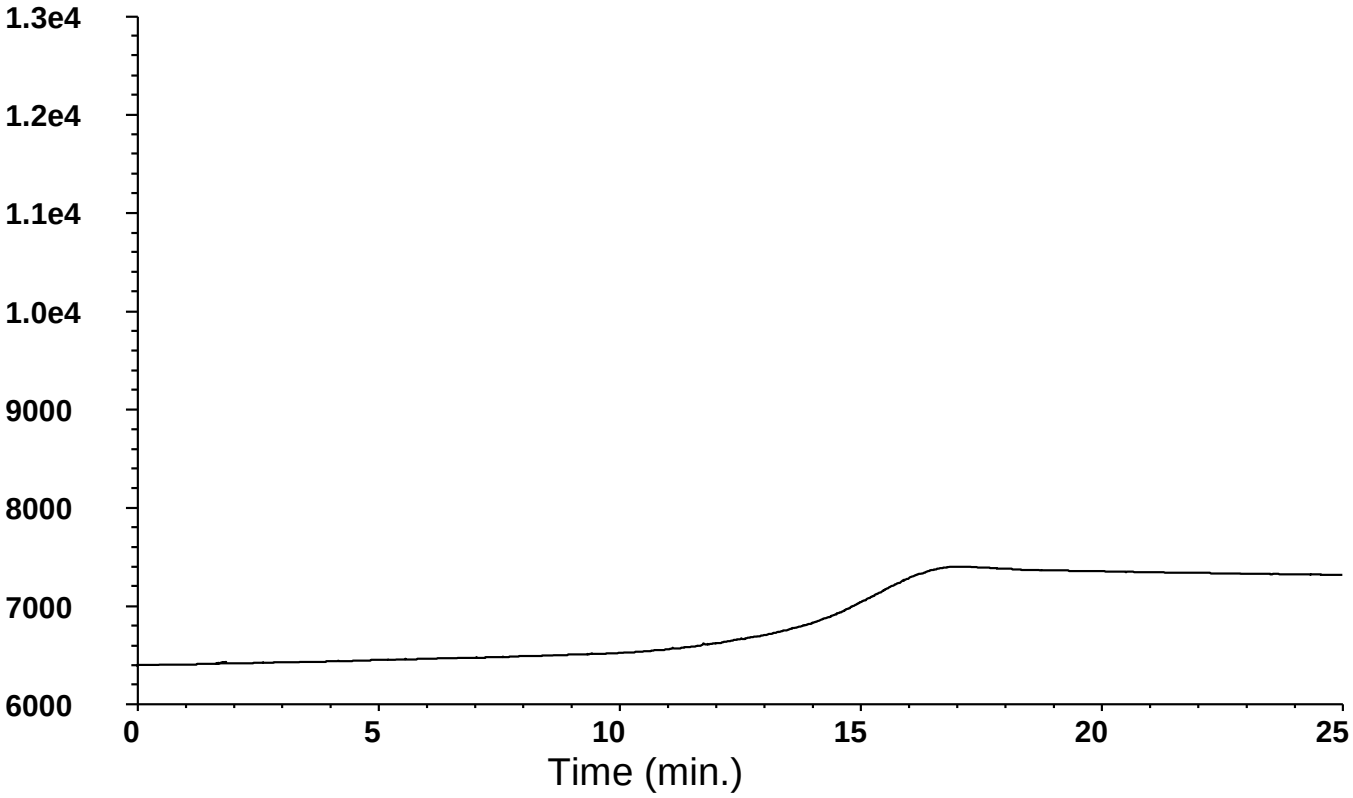
- **Isothermal maximum temperature OR**
- **20° to 30°C above highest operation temperature**
- **Temperature programming is not necessary**

Stop conditioning when the stable baseline is obtained:

- **1 to 2 hours in most cases**

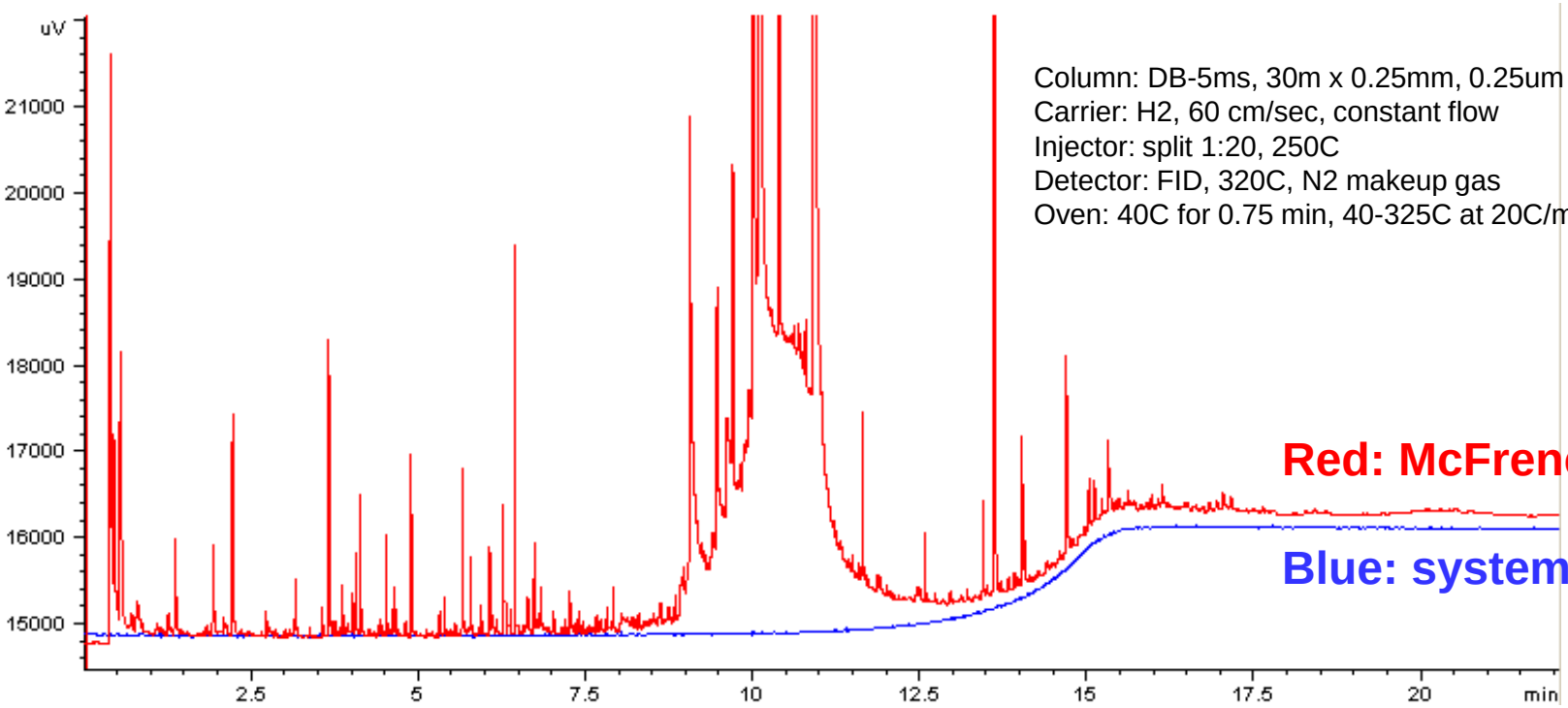
Generating a Bleed Profile

Temperature program the column without an injection*



***DB-1 30m x .32mm I.D., .25µm**
Temperature program // 40°C, hold 1 min // 20°/min to 320°C, hold 10 min.

Contamination from French Fry Grease



Column: DB-5ms, 30m x 0.25mm, 0.25um
Carrier: H2, 60 cm/sec, constant flow
Injector: split 1:20, 250C
Detector: FID, 320C, N2 makeup gas
Oven: 40C for 0.75 min, 40-325C at 20C/min, 325C for 30 min

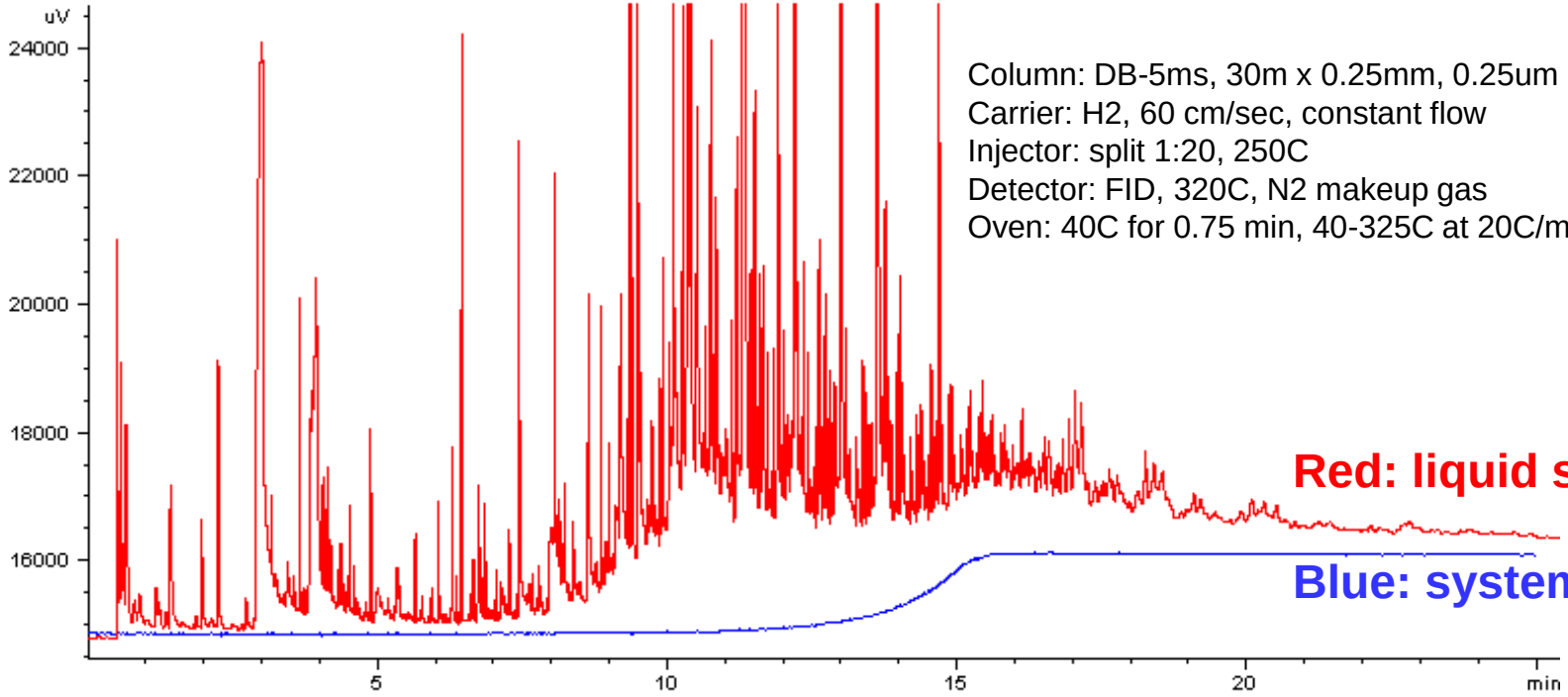
Red: McFrench Fry

Blue: system blank

Procedure:

(1) Held french fry for 5 seconds.
(2) Fingertip was wiped with paper towel to remove as much of the offending material as possible.
(3) Lightly touched the part of the column sticking up above the ferrule.
(4) Installed column into injector.
(5) Set oven temperature to 40C.
(6) Started oven temperature program as soon as oven reached 40C.

Contamination from Liquid Soap



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Carrier: H2, 60 cm/sec, constant flow
Injector: split 1:20, 250C
Detector: FID, 320C, N2 makeup gas
Oven: 40C for 0.75 min, 40-325C at 20C/min, 325C for 30 min

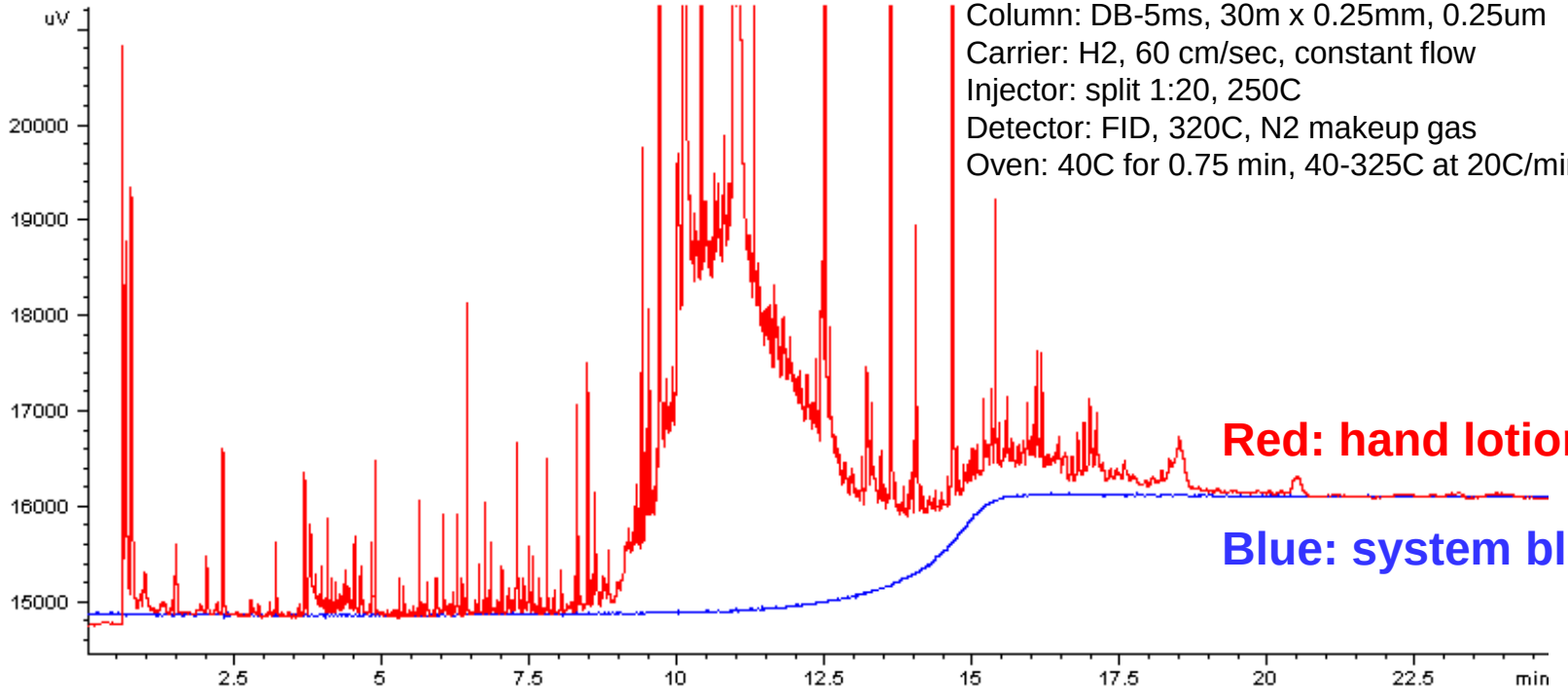
Red: liquid soap

Blue: system blank

Procedure:

(1) One very small drop of liquid placed on one fingertip.
(2) Fingertip was wiped with paper towel to remove as much of the offending material as possible.
(3) Lightly touched the part of the column sticking up above the ferrule.
(4) Installed column into injector.
(5) Set oven temperature to 40C.
(6) Started oven temperature program as soon as oven reached 40C.

Contamination from Hand Lotion



Column: DB-5ms, 30m x 0.25mm, 0.25um
Carrier: H2, 60 cm/sec, constant flow
Injector: split 1:20, 250C
Detector: FID, 320C, N2 makeup gas
Oven: 40C for 0.75 min, 40-325C at 20C/min, 325C for 30 min

Red: hand lotion

Blue: system blank

Procedure:

(1) One very small drop of liquid placed on one fingertip.
(2) Fingertip was wiped with paper towel to remove as much of the offending material as possible.
(3) Lightly touched the part of the column sticking up above the ferrule.
(4) Installed column into injector.
(5) Set oven temperature to 40C.
(6) Started oven temperature program as soon as oven reached 40C.

Test Mixes

Used to determine how "good" the column is

Column Performance Summary

PART NO: 1225032
COLUMN I.D. NO.: 3303121
LIQUID PHASE: DB-5
FILM THICKNESS: 0.25 µm
COLUMN DIMENSIONS:
30 m X 0.252 mm
TEMPERATURE LIMITS:
-60° C TO 325° C 350° C PROGRAM)

THEORETICAL PLATES/METER:	MIN SPEC	ACTUAL
PENTADECANE	3900	4389
COATING EFFICIENCY:		
PENTADECANE	90.0	95.5

RETENTION INDEX:	MIN SPEC	MAX SPEC	ACTUAL
1-UNDECANOL	1371.04	1372.04	1371.43
ACENAPHTHYLENE	1459.34	1460.34	1459.53

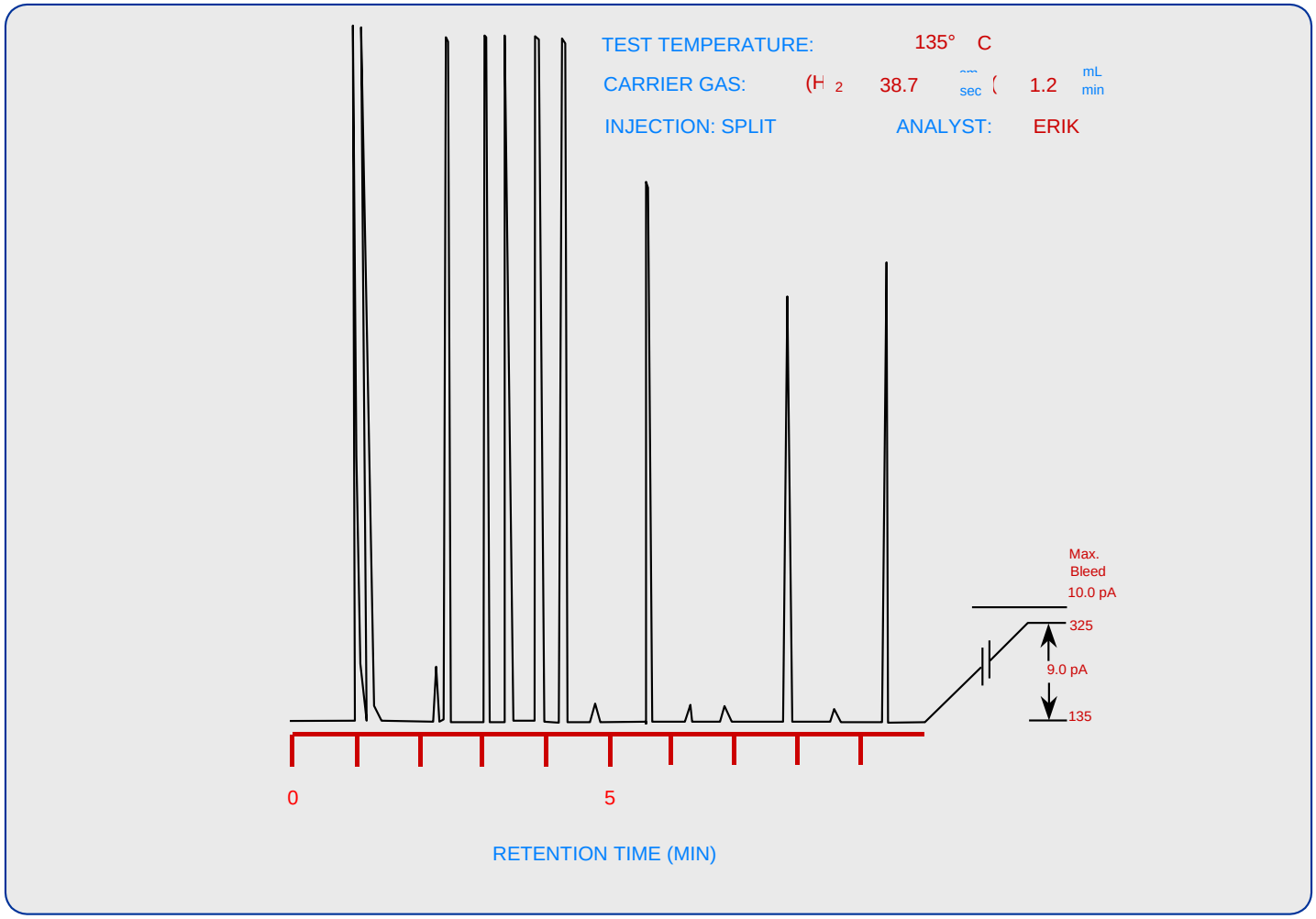
PEAK HEIGHT RATIO:	
4-CHLOROPHENOL/ METHYL NONANOATE	0.83
4-PROPYLANILINE/ METHYL NONANOATE	1.14

COMPOUND IDENTIFICATION	RETENTION TIME (t_R)	PARTITION RATIO (k)	PEAK WIDTH (W 1/2)
1,6-HEXANEDIOL	2.51	0.9	0.019
4-CHLOROPHENOL	2.95	1.3	0.022
METHYL NONANOATE	3.21	1.5	0.022
4-PROPYLANILINE	3.81	1.9	0.026
TRIDECANE	4.20	2.2	0.027
1-UNDECANOL	5.52	3.3	0.036
ACENAPHTHYLENE	8.00	5.2	0.053
PENTADECANE	9.58	6.4	0.062

Approximately 5-10 ng on column

o 1.29

Chromatographic Performance



Test Mixture Components

Compounds	Purpose
Hydrocarbons	Efficiency
	Retention
FAME's, PAH's	Retention
Alcohols	Activity
Acids	Acidic Character
Bases	Basic Character

Own Test Mixture

- More specific to your application
- Selective detectors
- Concentrations specific to your application
- Use same instrument conditions
- Easiest to simply inject a calibration standard
- Store for future measure of column performance

An Ounce of Prevention.....

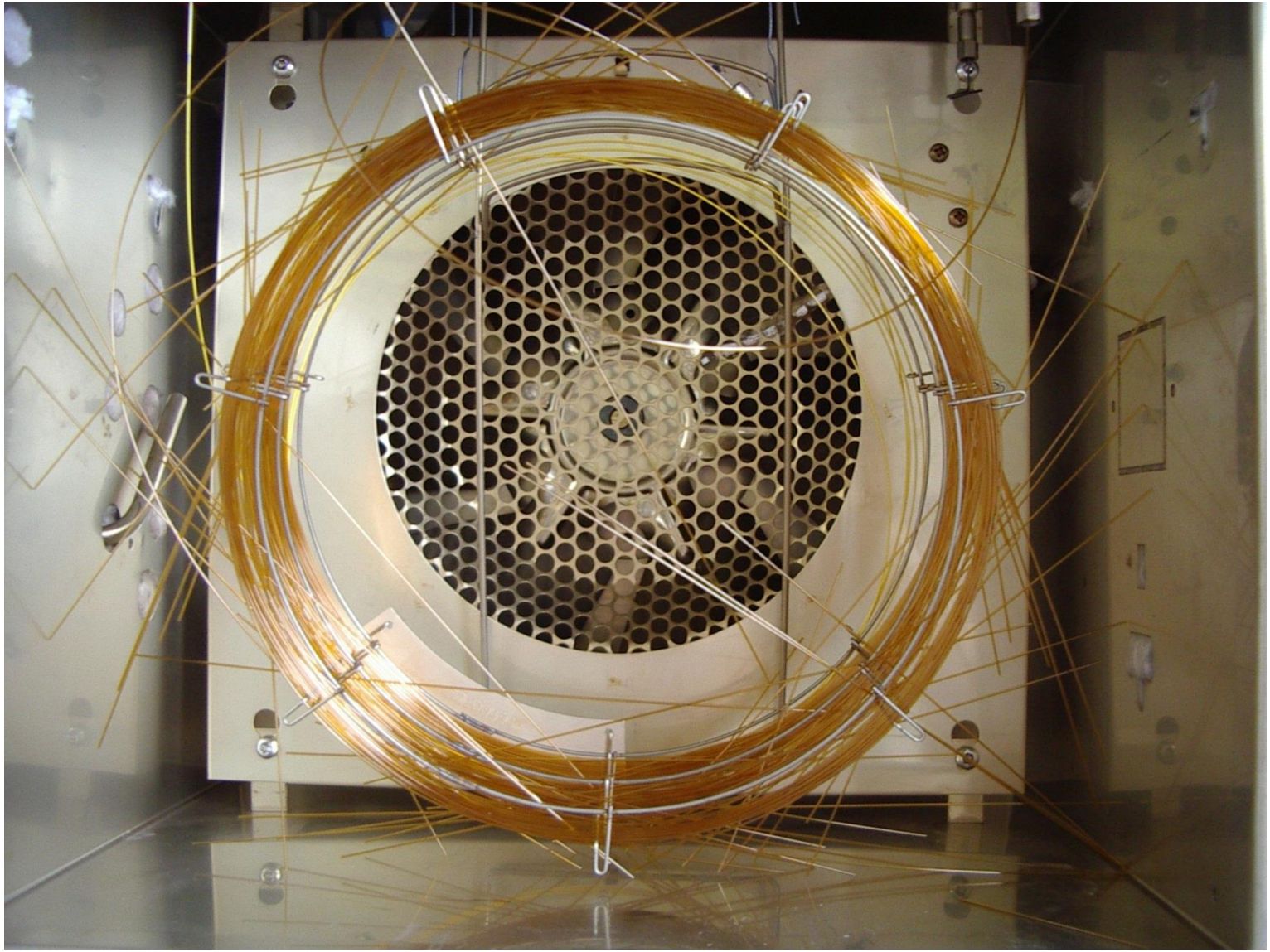
Common Causes of Column Performance Degradation

- **Physical damage to the polyimide coating**
- **Thermal damage**
- **Oxidation (O_2 damage)**
- **Chemical damage by samples DCM and H2**
- **Contamination**

Physical Damage to The Polyimide Coating

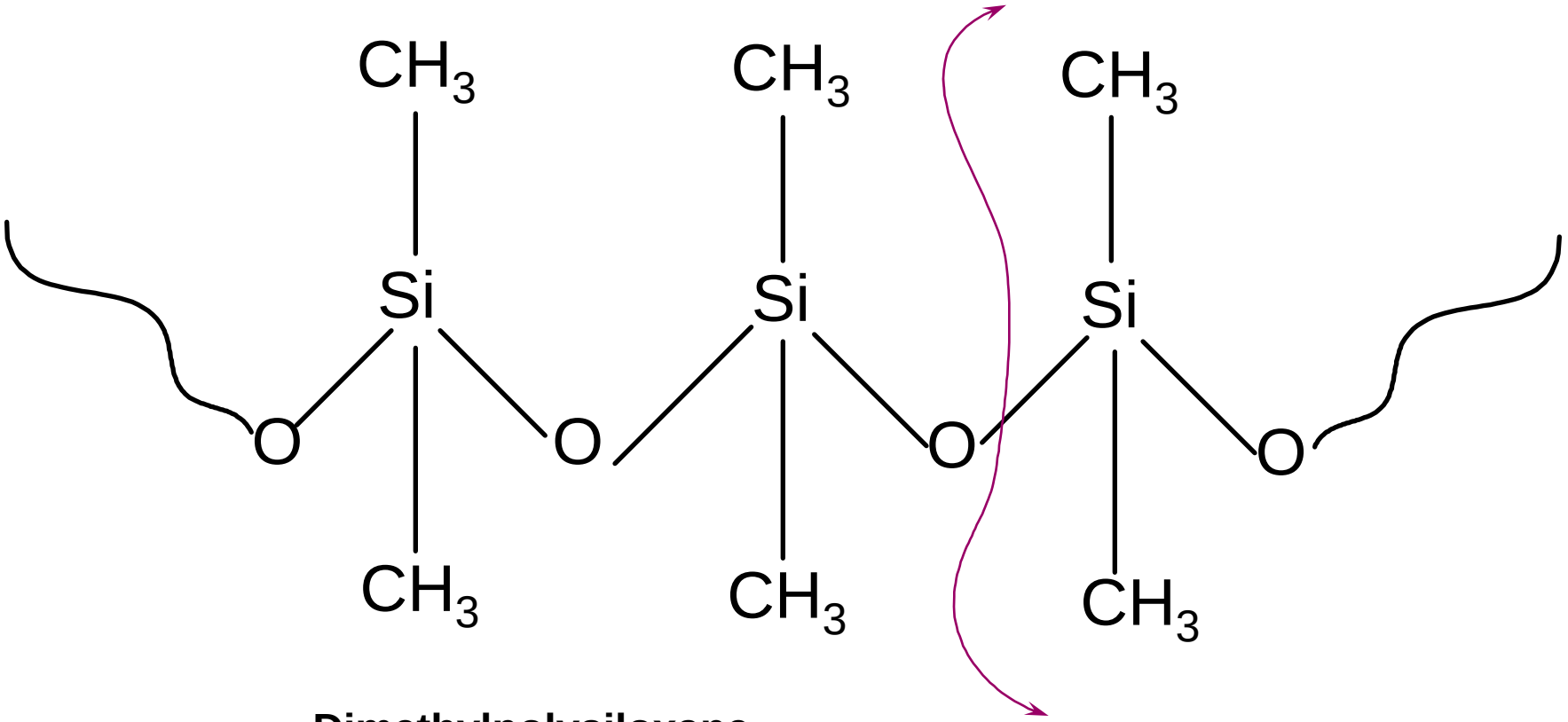
- **Smaller diameter tubing is more flexible than larger diameter tubing.**
- **Avoid scratches and abrasions**
- **Immediate breakage does not always occur upon physical damage**

NOT what you want your column to look like!



Thermal Damage

Degradation of the stationary phase is increased at higher temperatures. Breakage along the polymer backbone.



Dimethylpolysiloxane

Thermal Damage
What To Do If It Happens

- Disconnect column from detector
- “Bake out” overnight at isothermal limit
- Remove 10-15 cm from column end
- On most columns one wrap = 1/2 meter

Thermal Damage

- **Rapid degradation of the stationary phase caused by excessively high temperatures**

  Isothermal limit = Indefinite time

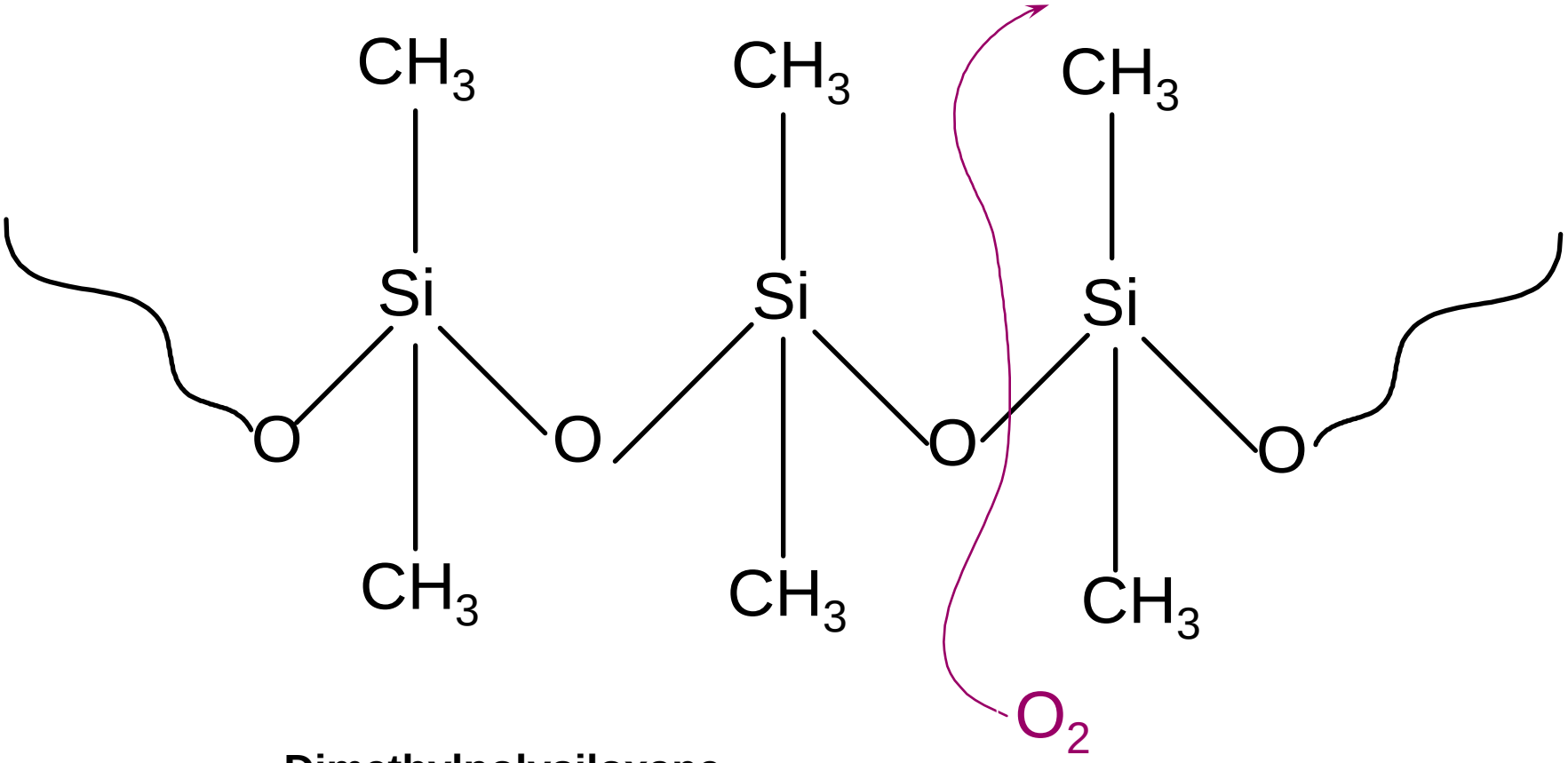
  Programmed limit = 5-10 minutes 325/350

- **Temporary "column failure" below lower temperature**

  Polar or Specialty columns have lower oven temps.

Oxidation (O2 Damage)

Oxygen in the carrier gas rapidly degrades the stationary phase. The damage is accelerated at higher temperatures. Damage along the polymer backbone is irreversible.



Dimethylpolysiloxane

Oxygen Damage

- **Causes rapid damage to the column**
- **Usually results in irreversible column damage**

How to Prevent Column Damage by Oxygen

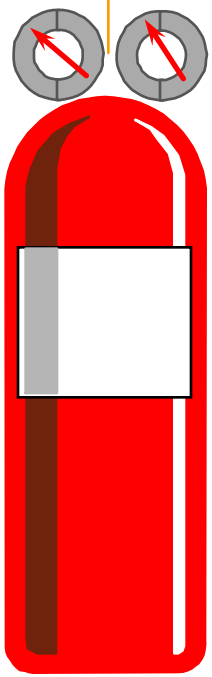
- **High quality carrier gas (4 nine's or greater)**
- **Leak free injector and carrier lines**
  - **Change septa**
  - **Maintain gas regulator fittings**
- **Appropriate impurity traps**
- **Use Gas Clean Filters from Agilent**

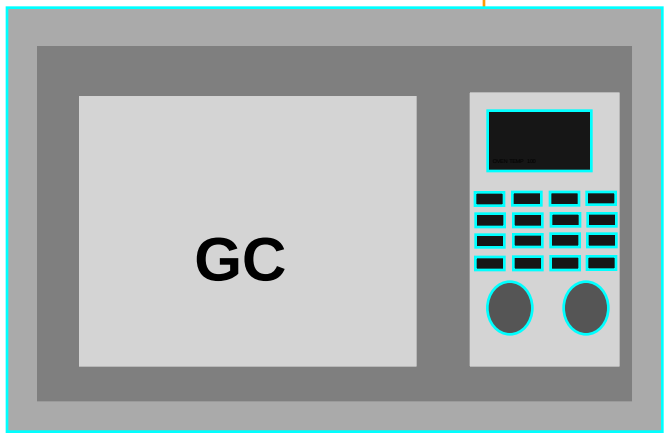
Configurations for Carrier Gas Purifiers

Indicating Moisture Trap

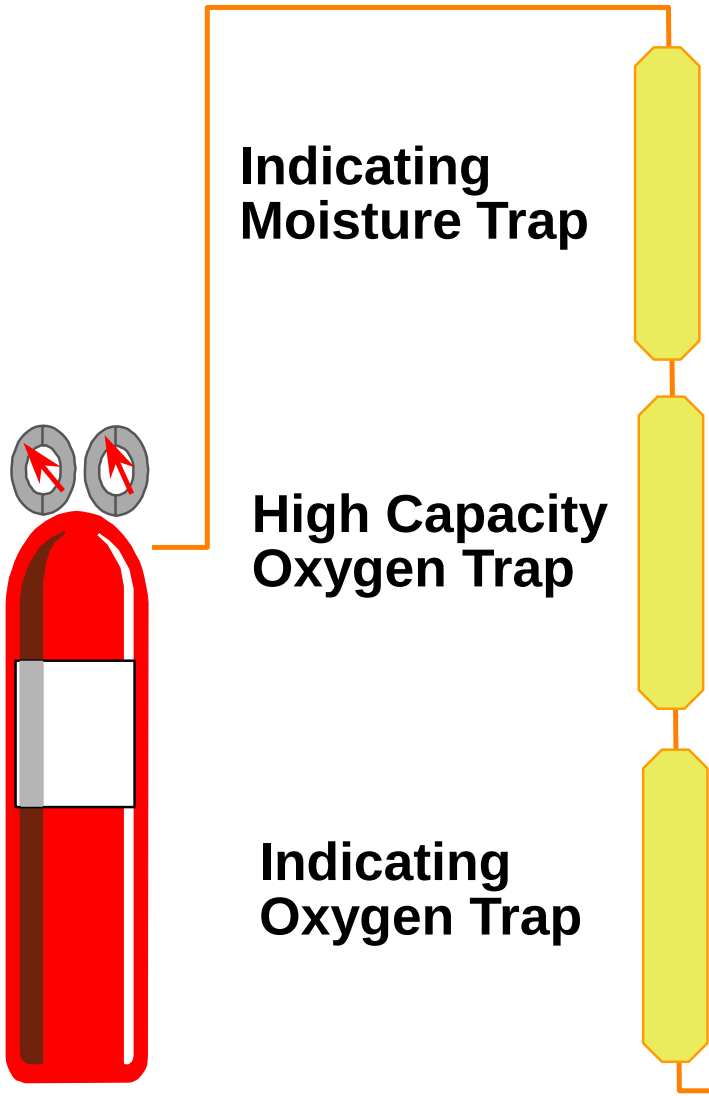
High Capacity Oxygen Trap

Indicating Oxygen Trap





Configurations for Carrier Gas Purifiers



Chemical Damage

Bonded and cross-linked columns have excellent chemical resistance except for inorganic acids and bases

HCl NH_3 KOH NaOH

H_2SO_4 H_3PO_4 HF etc.

Chemical damage will be evident by excessive bleed, lack of inertness or loss of resolution/retention lack of inertness or loss of resolution/retention

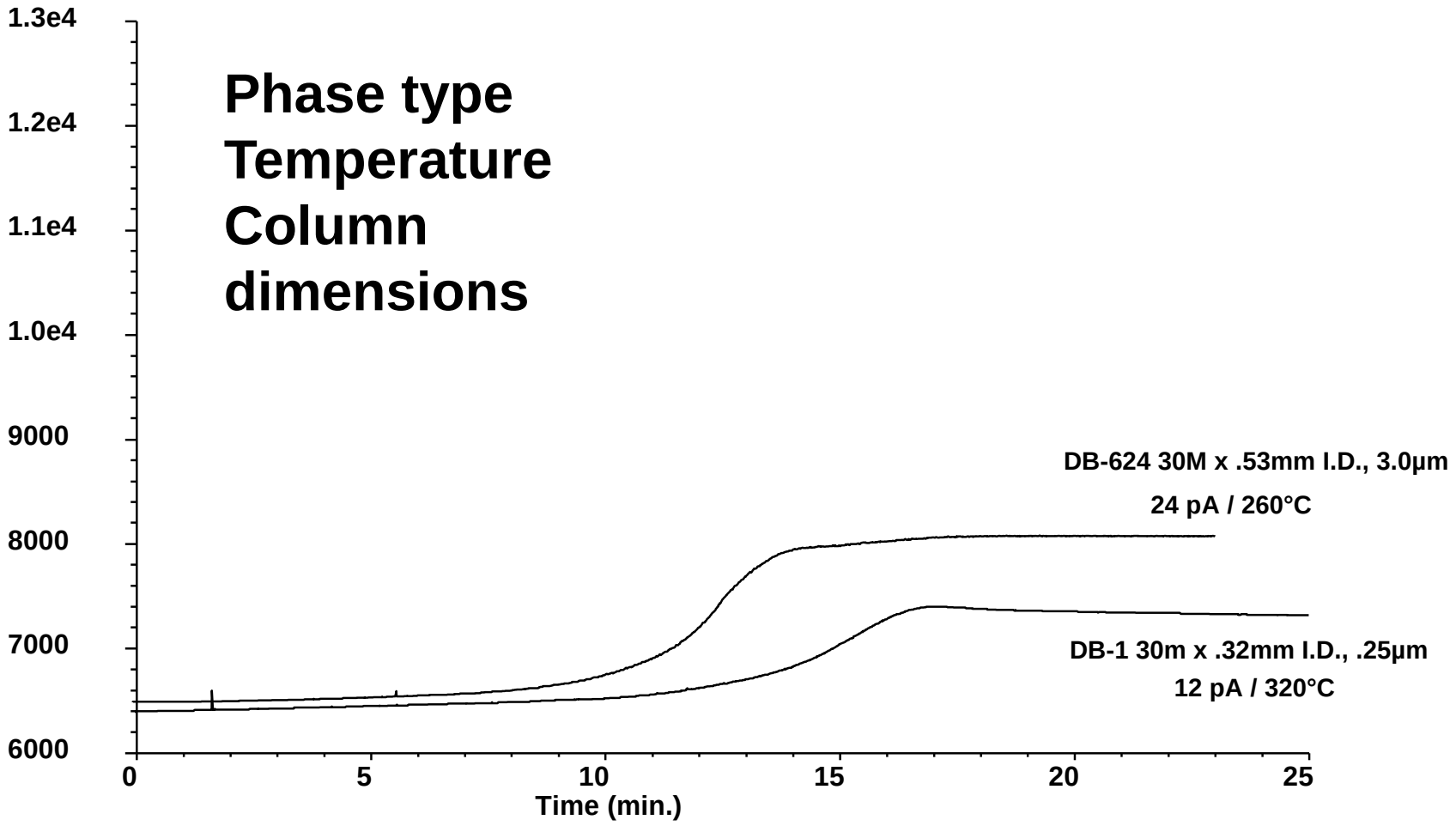
Chemical Damage
What To Do If It Happens

- Remove 1/2 - 1 meter from the front of the columns
- Removing one wrap is = to 1/2 meter on a 7” column
- Severe cases may require removal of up to 5 meters

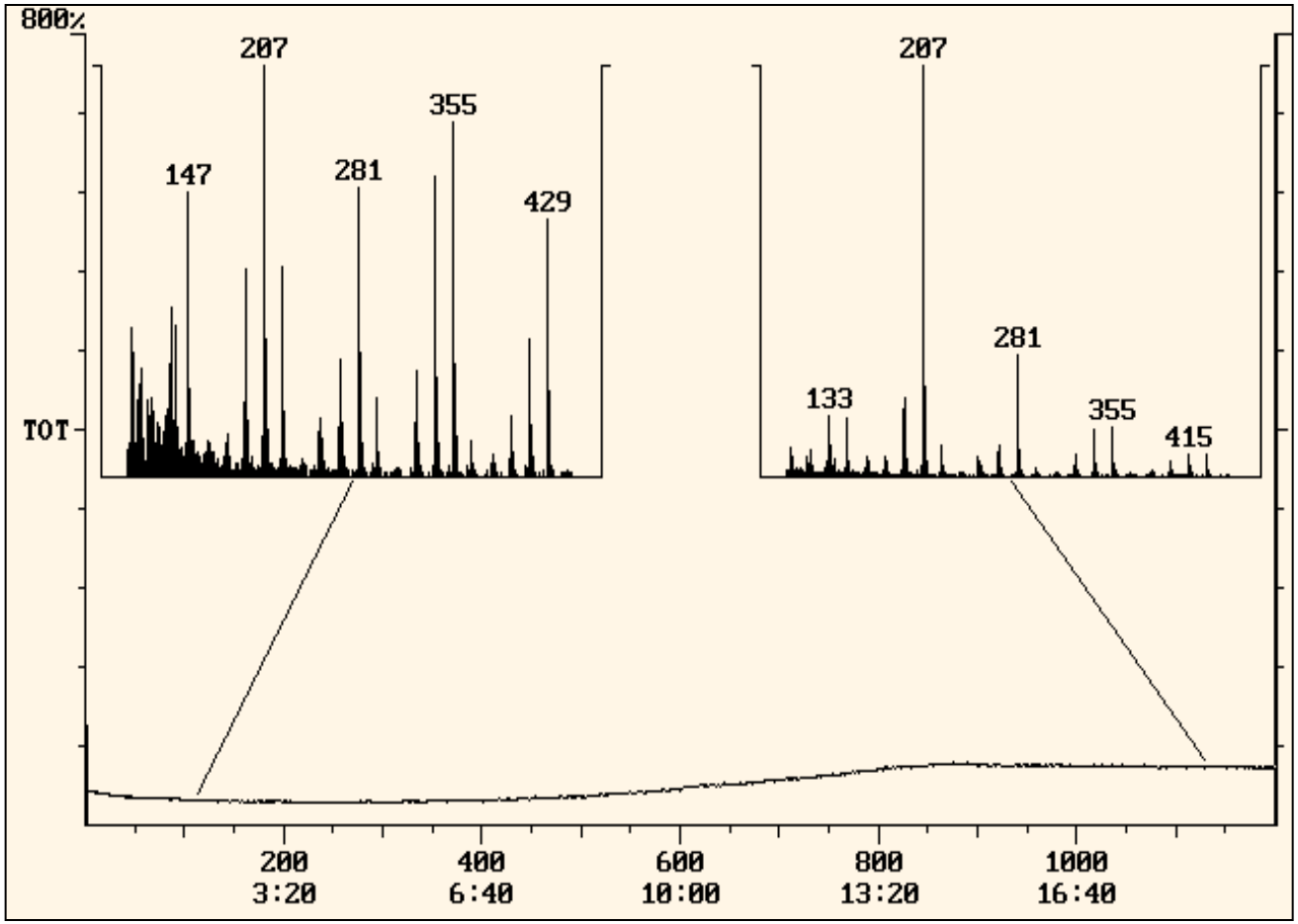
What is Normal Column Bleed

Normal background signal generated by the elution of normal degradation products of the column stationary phase

Column Bleed is Influenced by:



Mass Spectrum of Phenylmethylpolysiloxane Column Bleed (Normal Background)



Mass spectral library search is not always accurate

What is a Bleed Problem?

IT IS:

An abnormal elevated baseline at High Temperature

IT IS NOT:

A high baseline at Low Temperature

Wandering or drifting baseline at any temperature

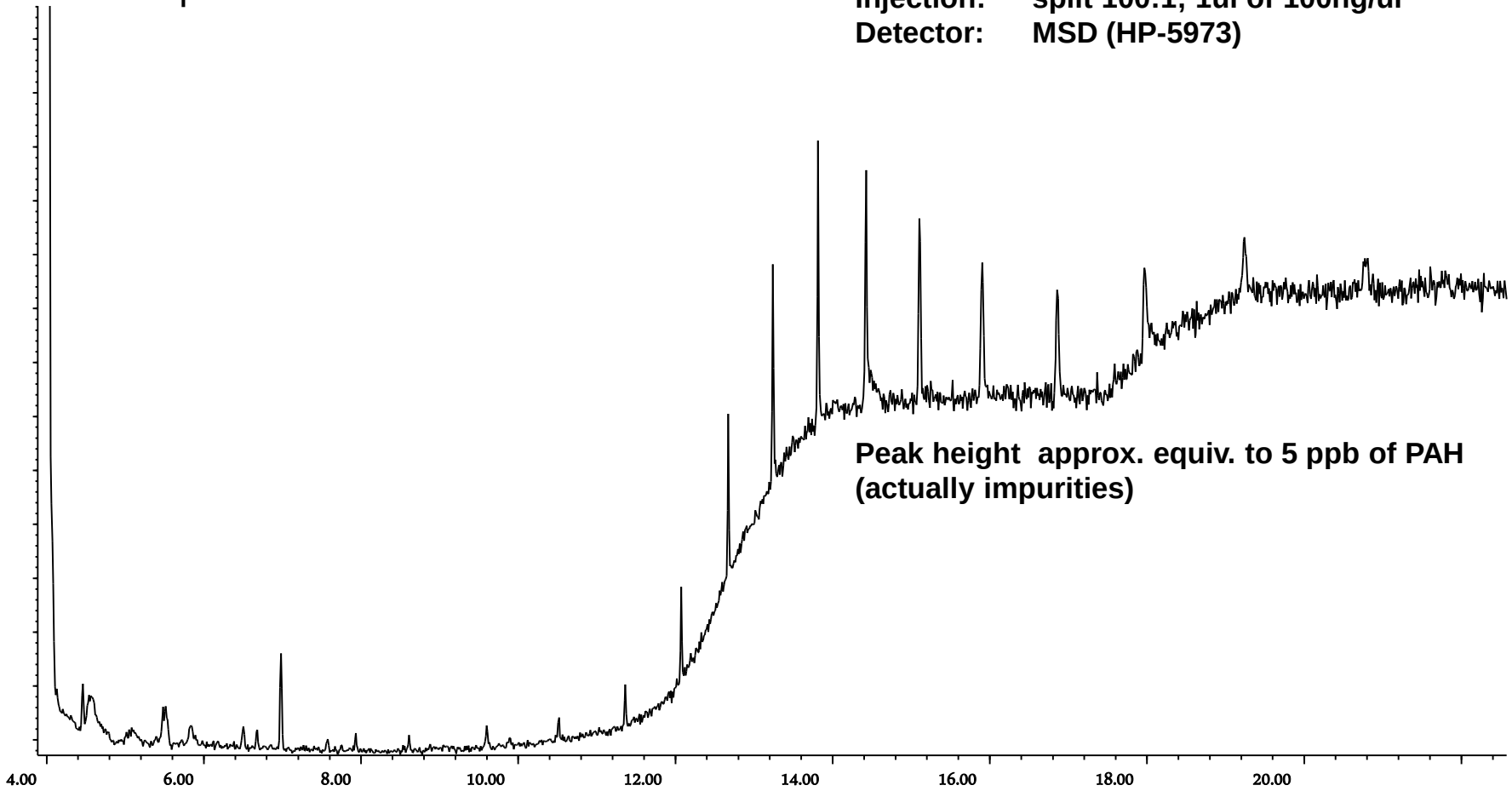
Discrete peaks

Bleed Profiles (MSD)

Discrete peaks are NOT bleed

Source of peaks from outside of the column

Columns:	**HP 5MS 30mx0.25mmx0.25um**
Oven:	**80 to 160C at 25 C/min, 160 to 320 C at 3 C/min(4), 320 to 325 C at 20C/min(4)**
Injection:	**split 100:1; 1ul of 100ng/ul**
Detector:	**MSD (HP-5973)**



Peak height approx. equiv. to 5 ppb of PAH (actually impurities)

Column Contamination

- **Fouling of GC and column by contaminants**
- **Mimics nearly every chromatographic problems**

Symptoms of Contamination

- **Poor peak shape**
- **Loss of separation (resolution)**
- **Changes in retention**
- **Reduced peak size**
- **Baseline disturbances (semi-volatiles only)**

Typical Samples That Contain a Large Amount of Residues

Biological (Blood, Urine, Tissue, Plants)

Soils **Foods**

Waste Water **Sludges**

<u>All</u> samples contain residues!! (even standards!)

Other Sources of Contamination

- **Septum and ferrule particles**
- **Gas and trap impurities**
- **Unknown sources (vials, syringes, etc.)**

Non-Volatile Residues

Any portion of the sample that does not elute from the column or remains in the injector.

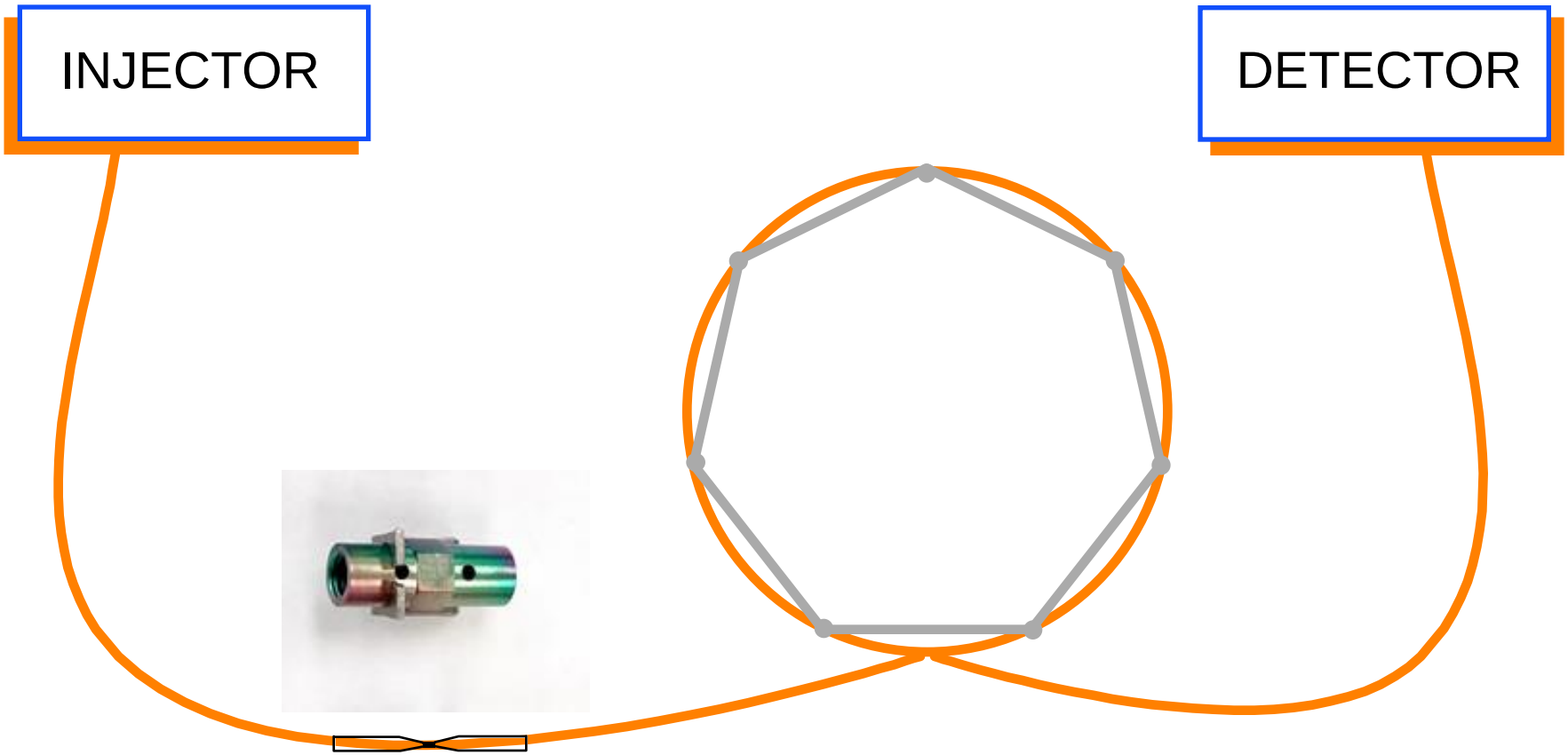
Semi-Volatile Residues

Any portion of the sample that elutes from the column after the current chromatographic run.

Methods to Minimize Non-Volatile Residue Problems

- **Sample cleanup**
- **Packed injection port liners**
- **Guard columns**

Guard Column or Retention Gap



The guard column is 3 - 5 meters of deactivated fused silica tubing with the same diameter as the analytical column. It is connected with a zero dead volume union.

Non-Volatile Contamination

What To Do If It Happens

- Do not “bake out” the column
- Front End Maintenance

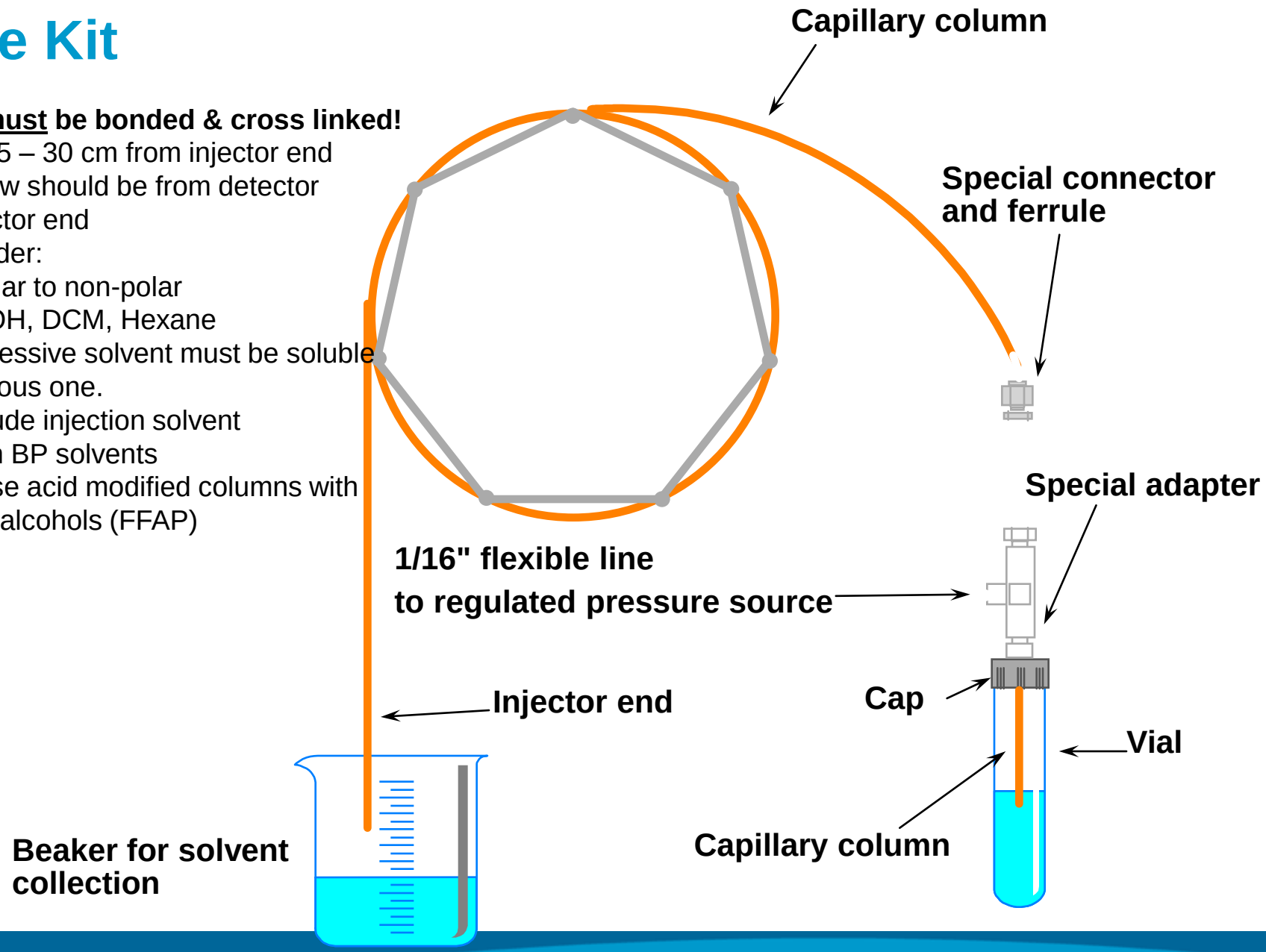
  clean or change the injector liner

  clean the injector

  cut off 1/2 -1 meter of the front of the column
- Turn the column around
- Solvent rinse the column
- Cut the column in half

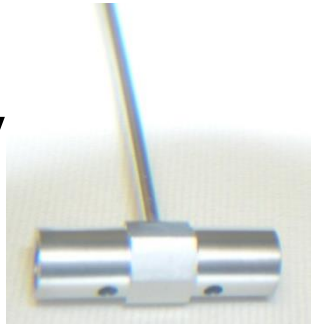
Rinse Kit

>**Column <u>must</u> be bonded & cross linked!**
>Remove 15 – 30 cm from injector end
>Solvent flow should be from detector end to injector end
>Solvent order:
-from polar to non-polar
-i.e. MeOH, DCM, Hexane
>Each successive solvent must be soluble in the previous one.
>Try to include injection solvent
>Avoid High BP solvents
>Do not rinse acid modified columns with water or alcohols (FFAP)



Semi-Volatile Contamination
What To Do If It Happens

- "Bake out" the column
  - Limit to 1-2 hours
  - Longer times may polymerize some contamination and reduces column life
- Solvent rinse the column
- Consider utilizing Back Flush Technology



Column Storage

- Place septa over the ends
- Return to column box

Always Remember to:

- **Start with a good installation**
- **Maintain an oxygen free system**
- **Avoid physical, thermal, and chemical damage**
- **Take steps to prevent contamination**

Thank you and remember to call Technical Support!!

1-800-227-9770 Select Opt. #3, #3 then #1

1-866-422-5571 (FAX)

e-mail gc-column–support@agilent.com