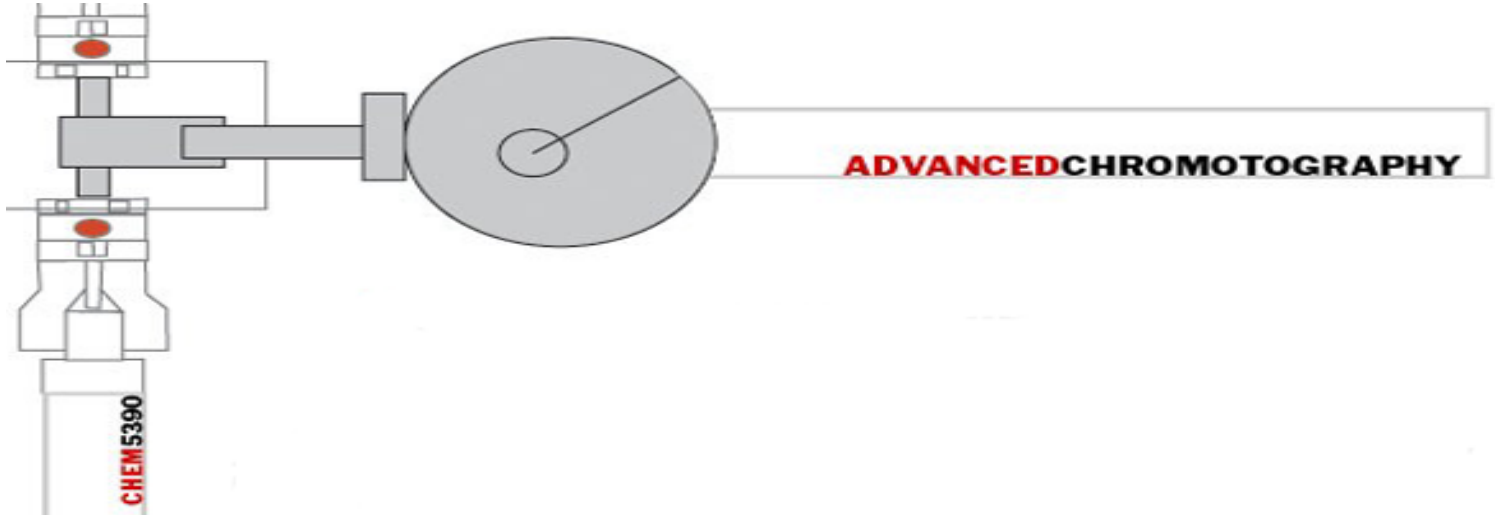
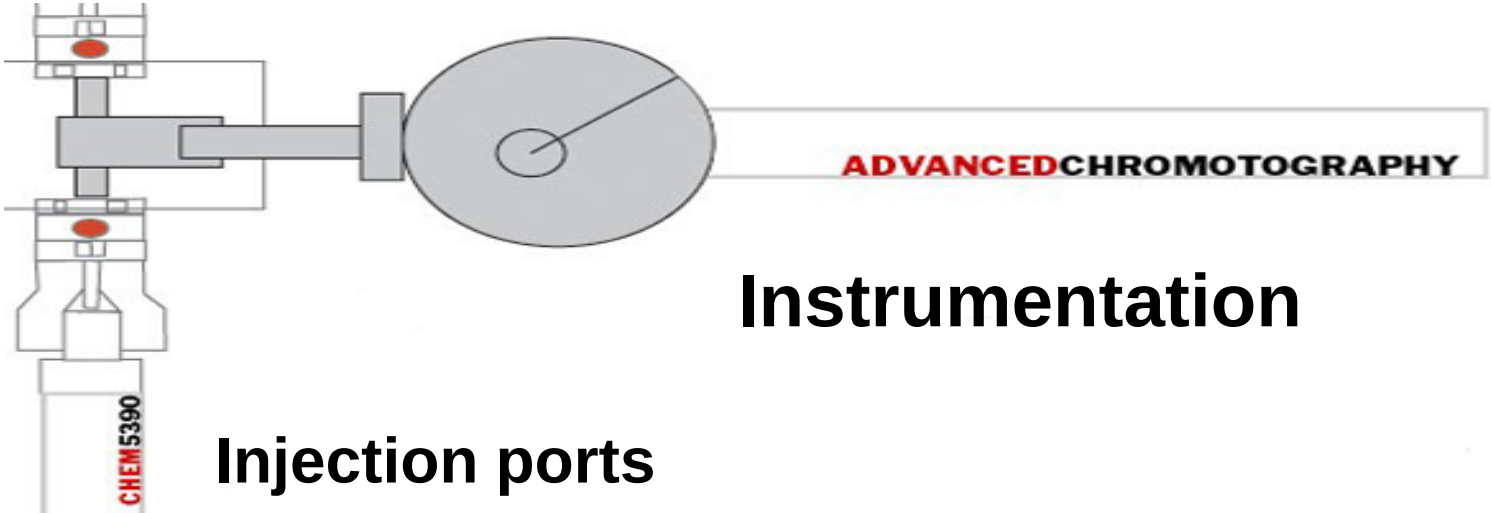


Lecture 5: Gas Chromatography Injection

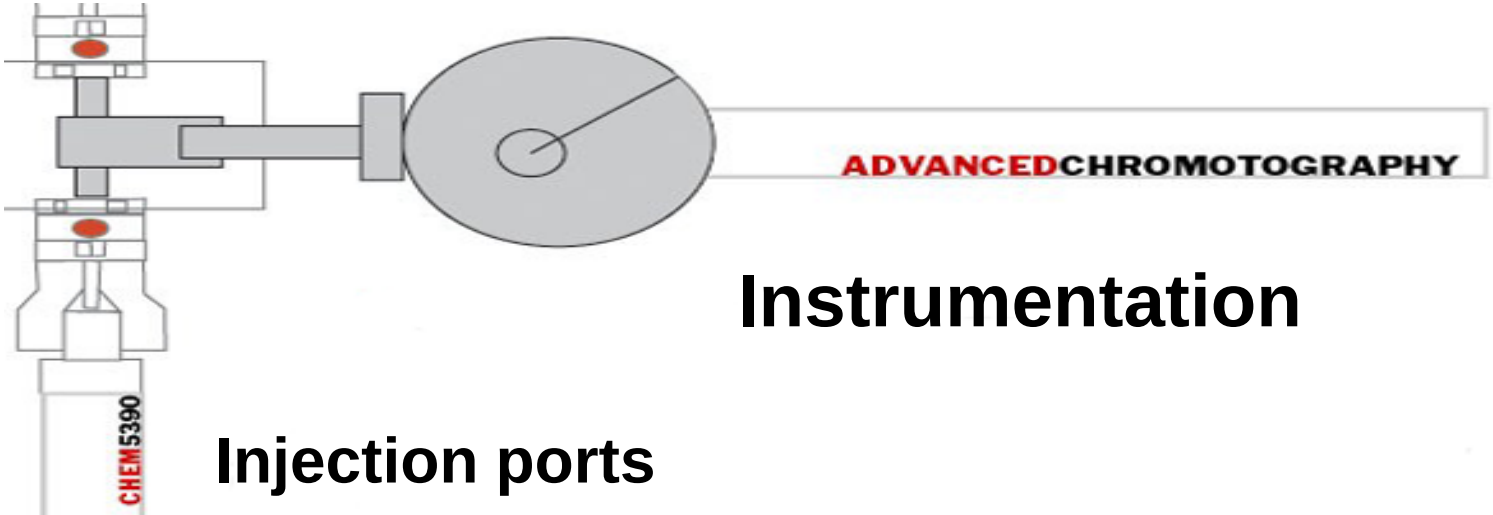


Instrumentation

Injection ports

For optimum column efficiency, the sample should not be too large, and should be introduced onto the column as a "plug" of vapour - slow injection of large samples causes band broadening and loss of resolution.

The most common injection method is where a microsyringe is used to inject sample through a rubber septum into a flash port at the top of the column.



Instrumentation

Injection ports

The temperature of the sample port is usually about 50°C higher than the boiling point of the least volatile component of the sample.

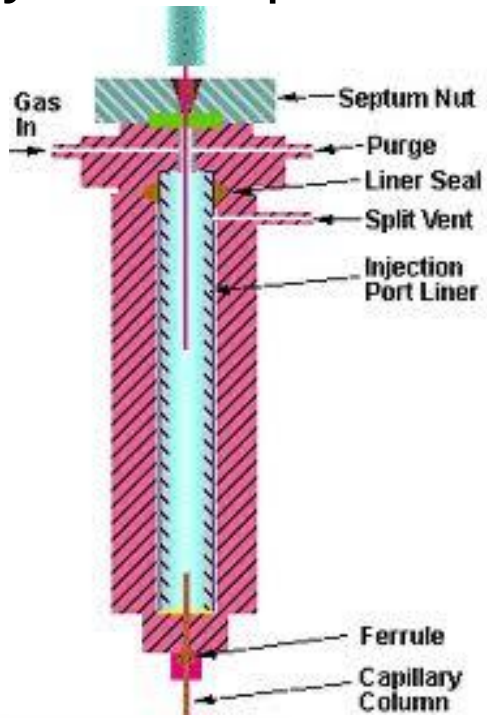
For packed columns, sample size ranges from tenths of a microliter up to 20 microliters.

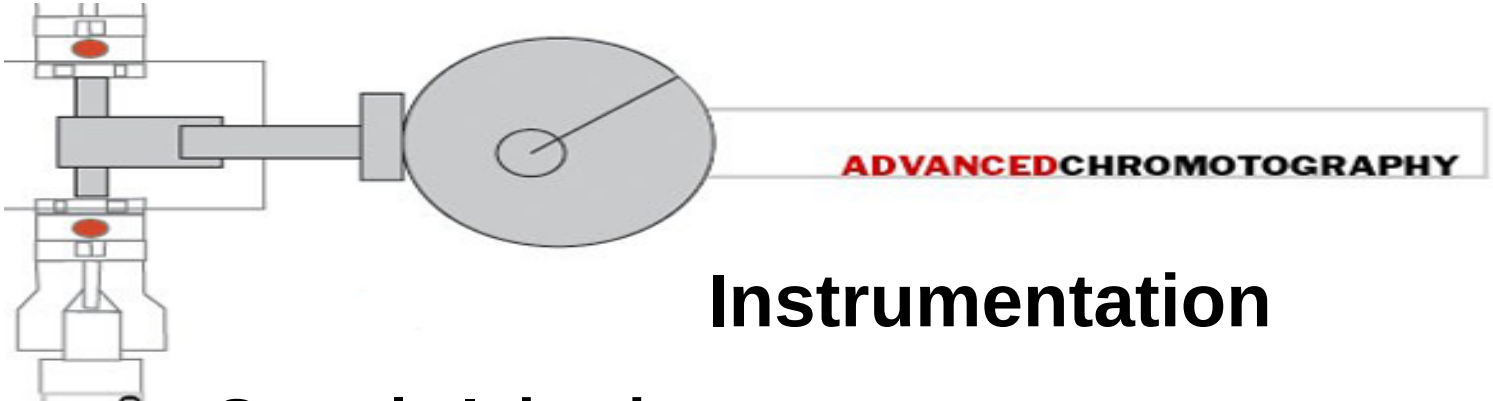
Capillary columns, on the other hand, need much less sample, typically around 10-3 μL, split/splitless injection.

Instrumentation

Injection ports

Purpose of port is to flash evaporate your sample and introduce it into the column.



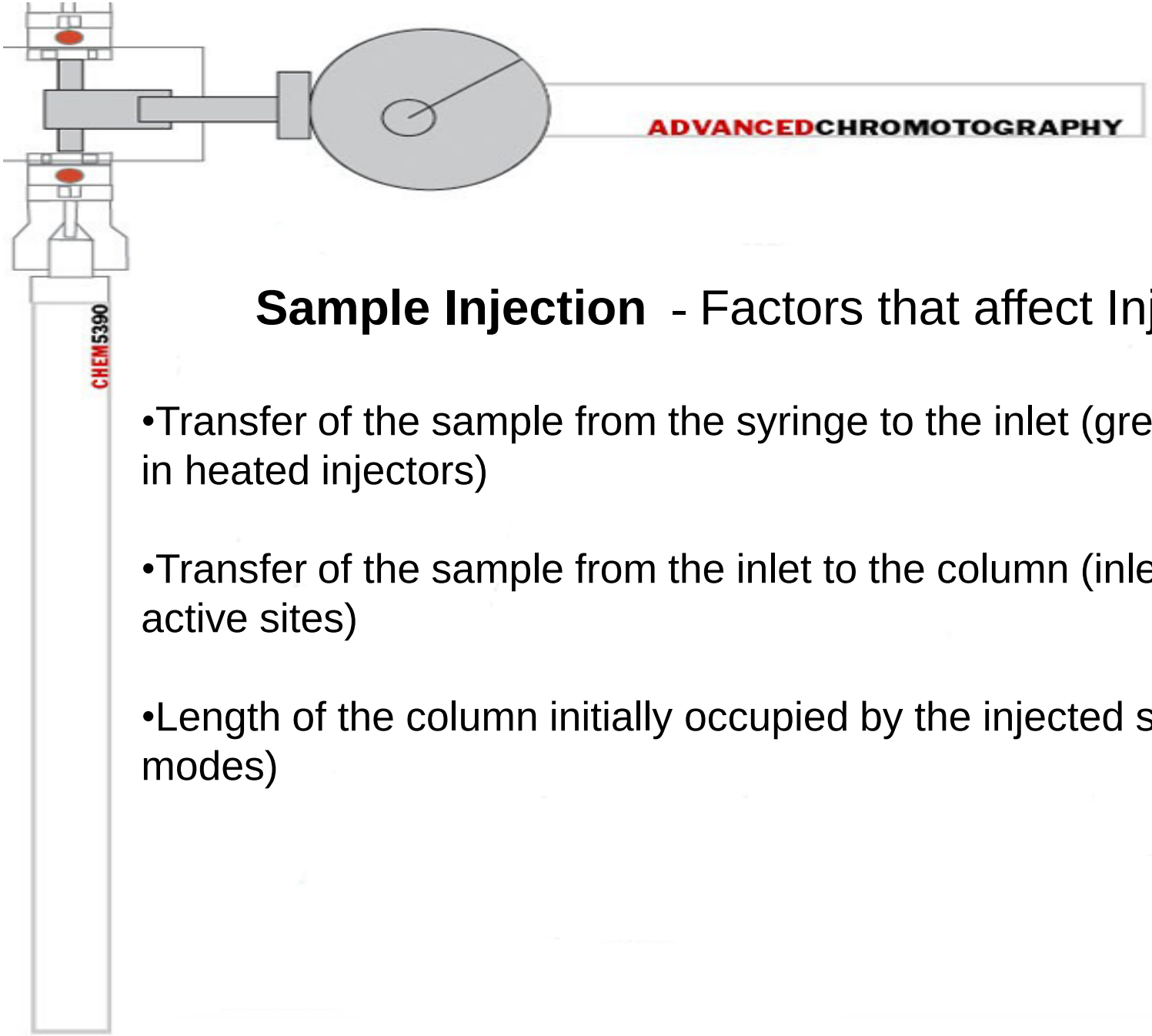


Instrumentation

Sample Injection

•Peak widths are influenced by the efficiency of the injection process. The shorter the length of the column occupied by the injected sample, the shorter the band as it begins and completes the chromatographic process.

•The critical function of the injection process is to introduce the sample so that it occupies the shortest possible length of column.

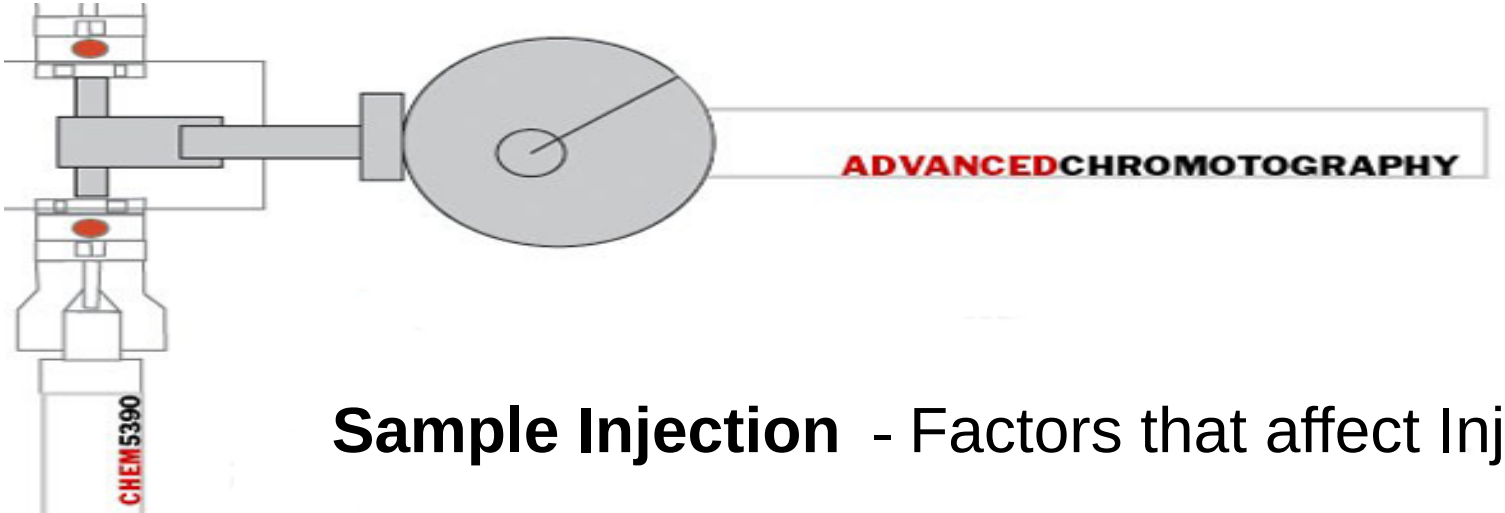


Sample Injection - Factors that affect Injection

•Transfer of the sample from the syringe to the inlet (greater concern in heated injectors)

•Transfer of the sample from the inlet to the column (inlets have active sites)

•Length of the column initially occupied by the injected sample (all modes)



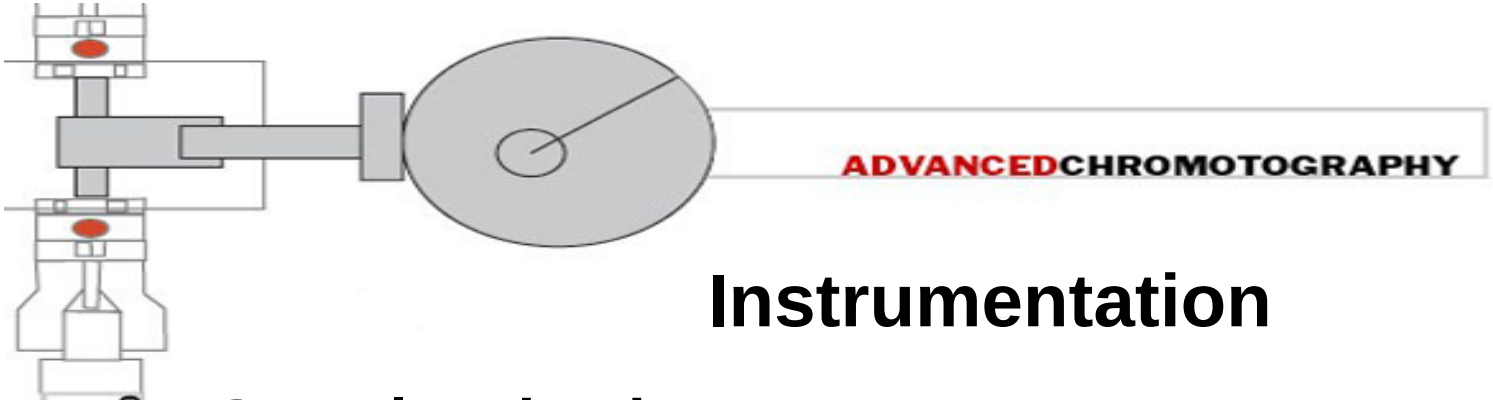
Sample Injection - Factors that affect Injection

•Rate and efficiency of sample vaporization in the inlet (split and splitless)

•Speed of sample transport from the inlet to the column (split, splitless, and PTV)

•Completeness of sample transport from the inlet (all modes)

•Homogeneity of temperature and phase ratio for sample band



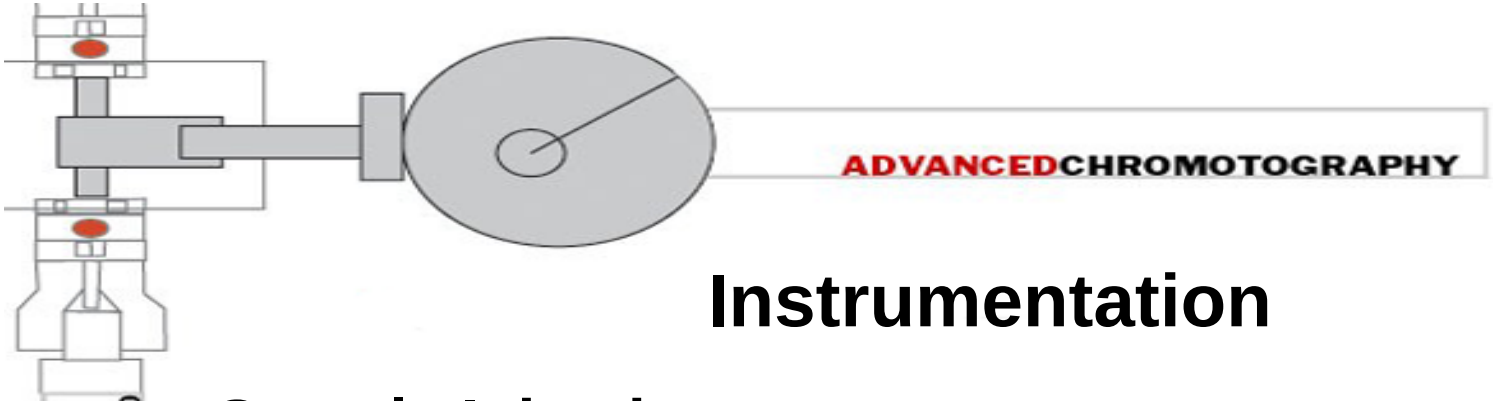
Instrumentation

Sample Injection

Syringe Technique

- **Universal method of introduction is with a microsyringe through a septum.**

- **Reproducibility with gas samples is poor since the volume of gas is temperature dependent – use of a sampling valve increases precision. Most samples are injected as liquids.**

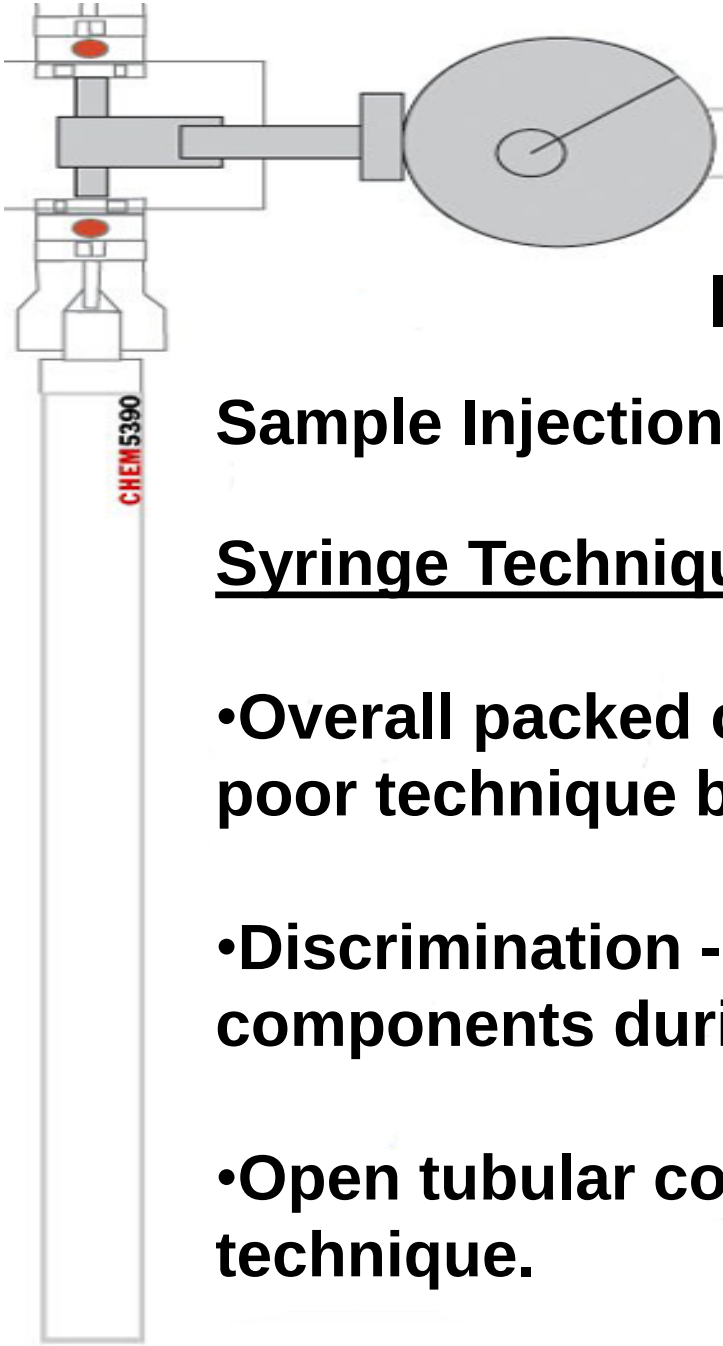


Instrumentation

Sample Injection

Syringe Technique

- For packed columns, injection of the sample solution is into a heated port at a higher temperature than the column to assist vaporization or the sample can be deposited directly onto the end of the column housed inside the injection port. The first few cm of the column packing may need to be replaced due to absorbed sample and decomposition – washing away w/ mobile phase and temperature.



Instrumentation

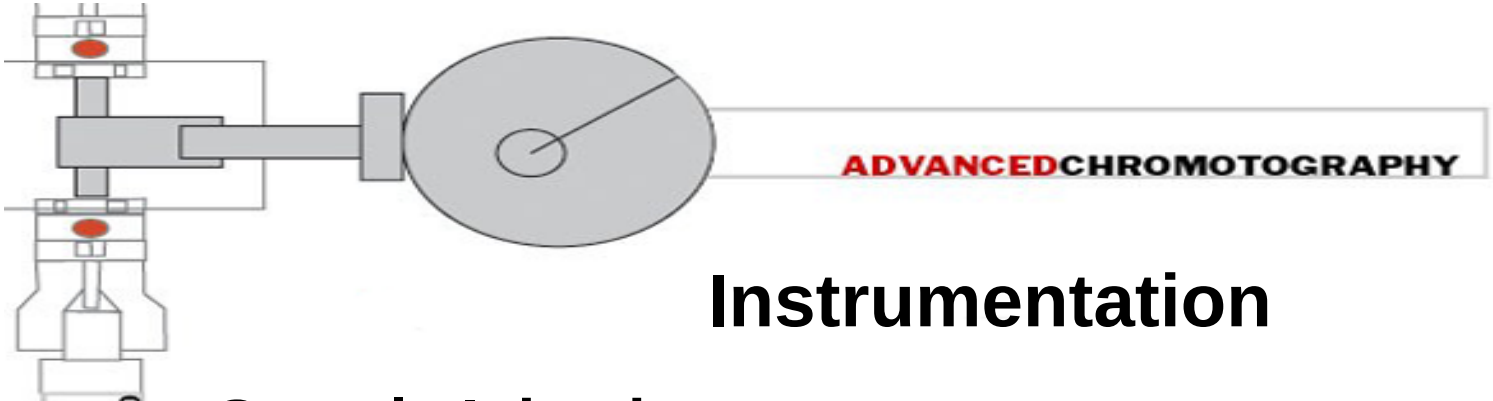
Sample Injection

Syringe Technique

- Overall packed columns are relatively forgiving of poor technique because of large sample size.

- Discrimination - selective loss of some sample components during injection.

- Open tubular columns are more demanding on technique.



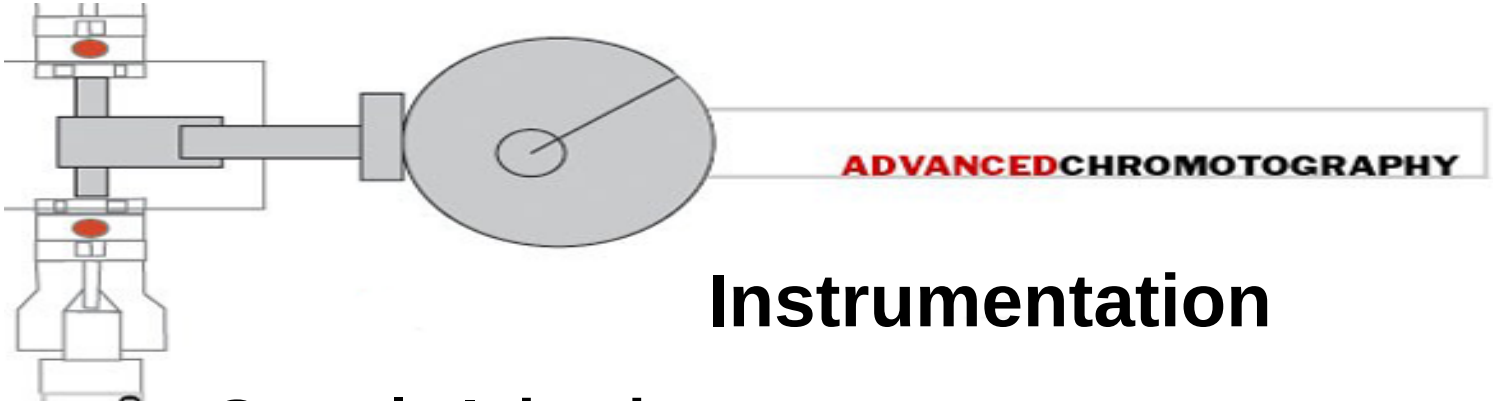
Instrumentation

Sample Injection

Syringe Technique

Several techniques are used to inject the sample:

•A vaporizing injection can retain the sample in the needle when introduced into the injection port. Most common and simple but poorest method. Variations include hot needles, solvent flush (or air), cold needles and filled needles.



Instrumentation

Sample Injection

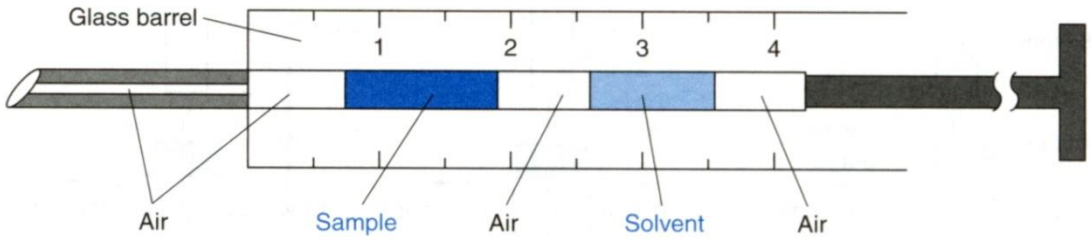
Syringe Technique

Hot needle - sample in barrel only (temperature allowed to equilibriate).
Cold needle - sample in barrel only (immediate injection).
Filled needle - sample in barrel and needle.
Solvent flush - solvent + sample + solvent or solvent + air + sample + air.

Instrumentation

Sample Injection

Syringe Technique – Solvent flush or Sandwich injection



Air bubble prevents depletion of most volatile compounds before sample injection is complete (barrier between oven and sample during injection)

Solvent is used to pushes out sample, but bubble prevents mixing

Final air bubble pushes out solvent

Gas-tight syringe is required for gas samples

Injection volume is typically 0.1-2 μL

Sample Injection

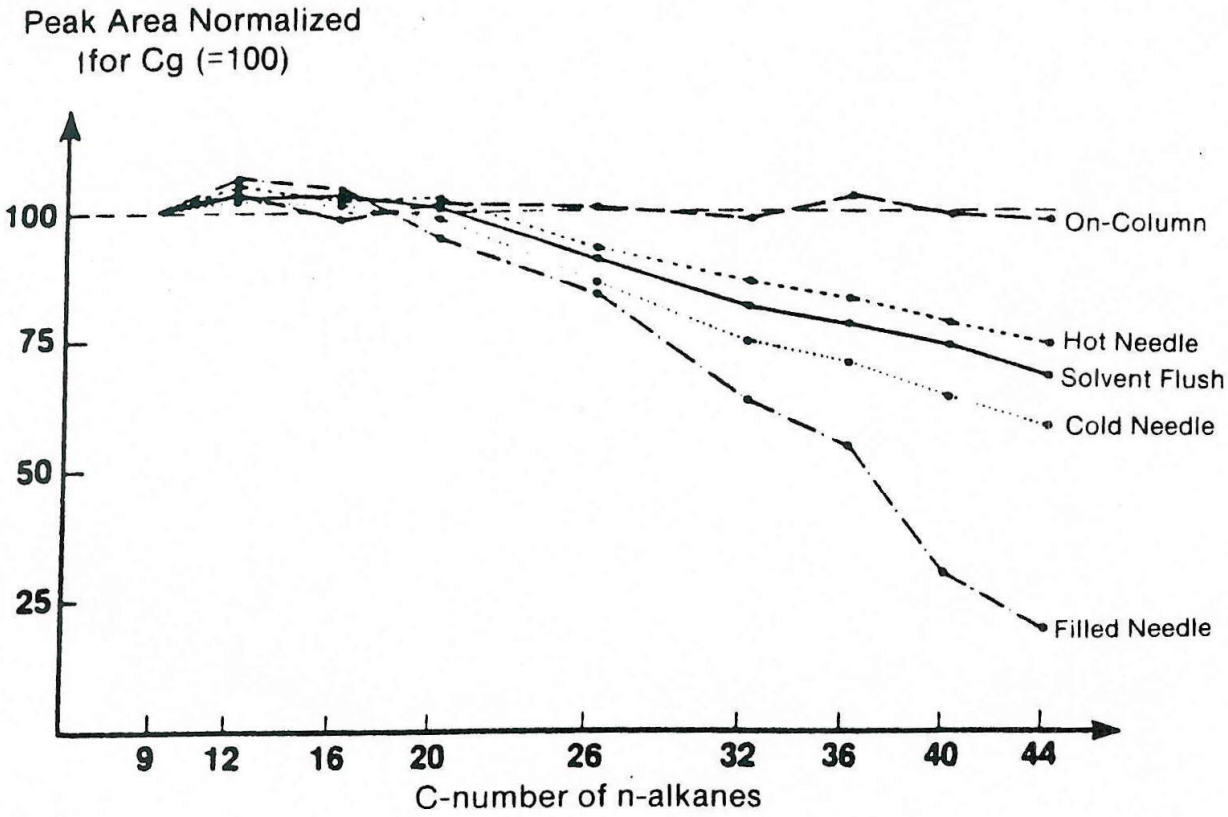
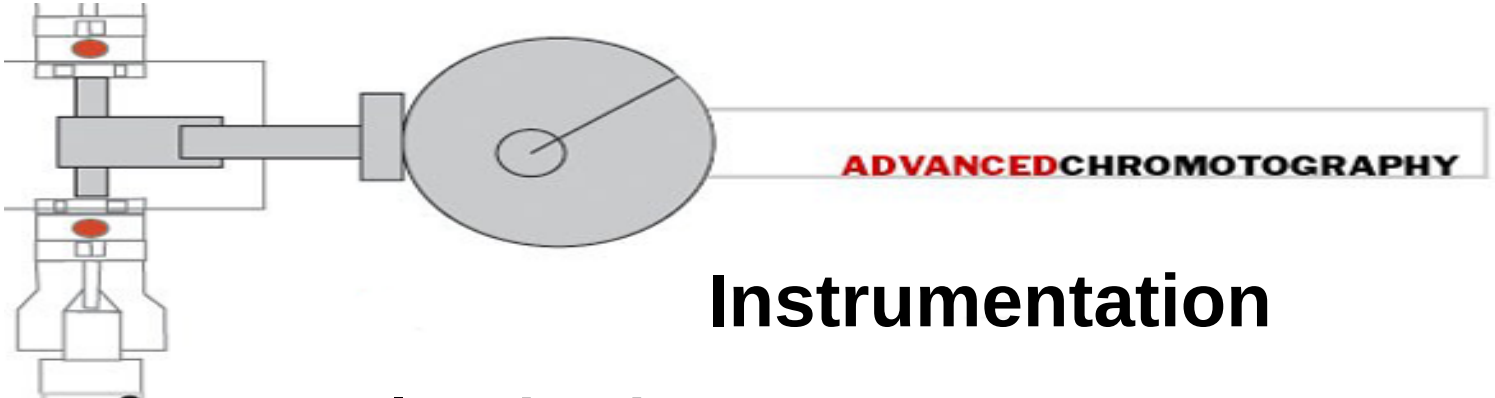


Fig. 3.5. Discrimination of *n*-alkanes obtained by different injection techniques using a split injection (1 μl; split ratio 1:15) and injection port temperature of 350°C. Methods of syringe handling are described in the text. Reproduced with permission from Grob and Neukom (1979). *J. High Resol. Chromatogr., Chromatogr. Comm.*, **2**: 15.



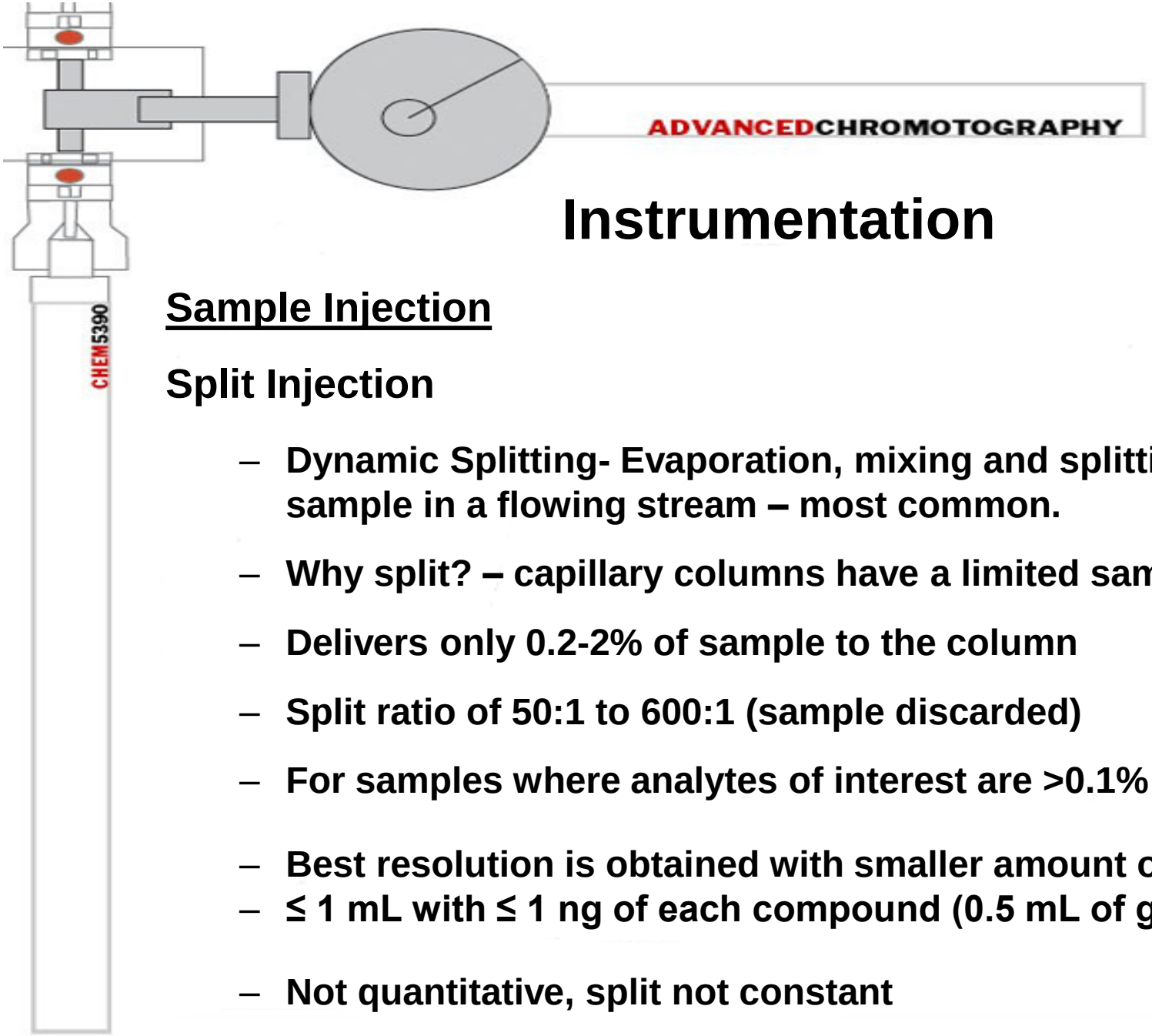
Instrumentation

Sample Injection

Split Injection

- First open tubular column injection technique used.

- Sample is injected using solvent flush or hot needle technique and after evaporation and mixing with carrier gas, the sample is split into two unequal portions – the smaller one passes to the column while the rest is vented to waste.



Instrumentation

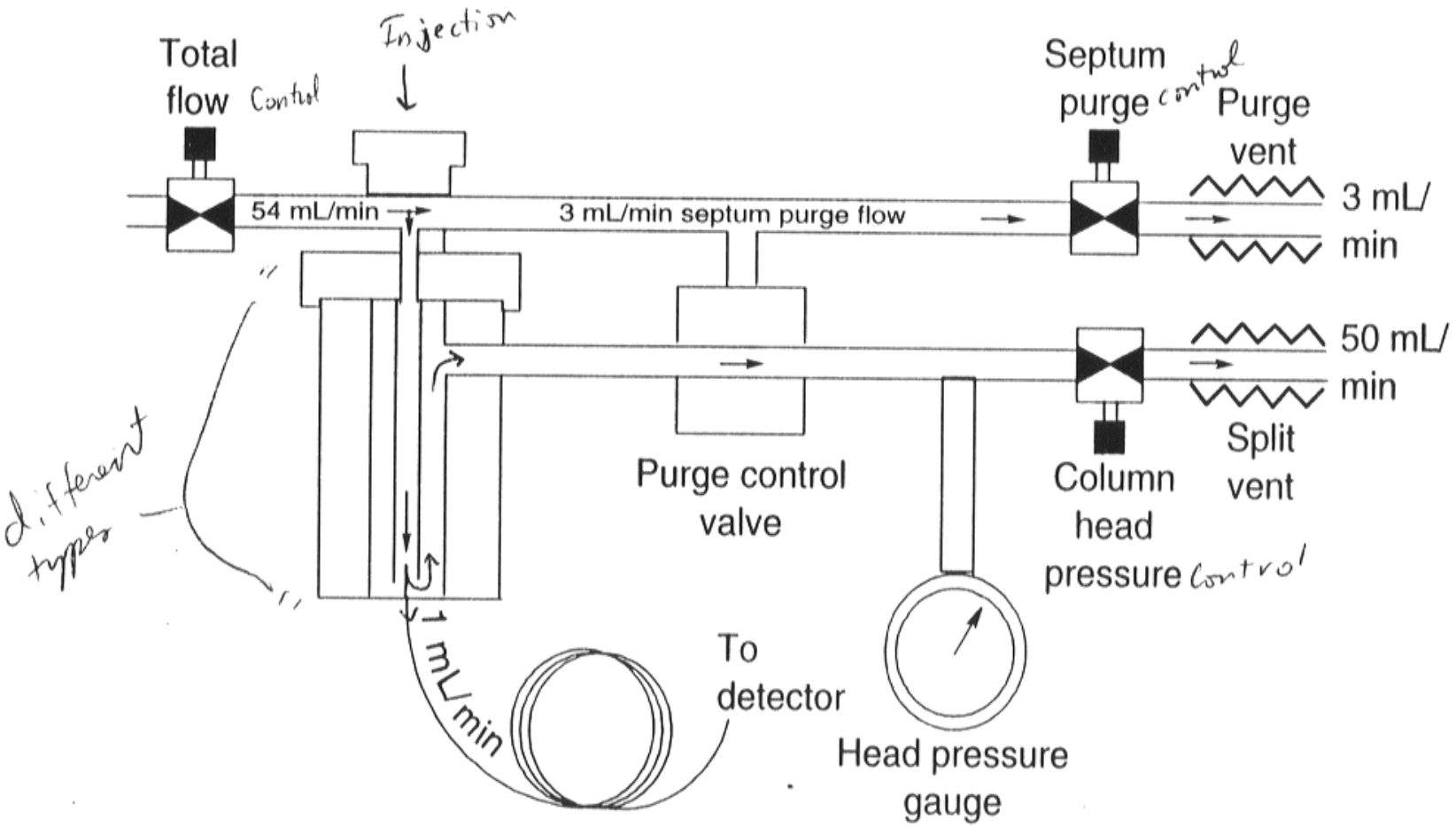
Sample Injection

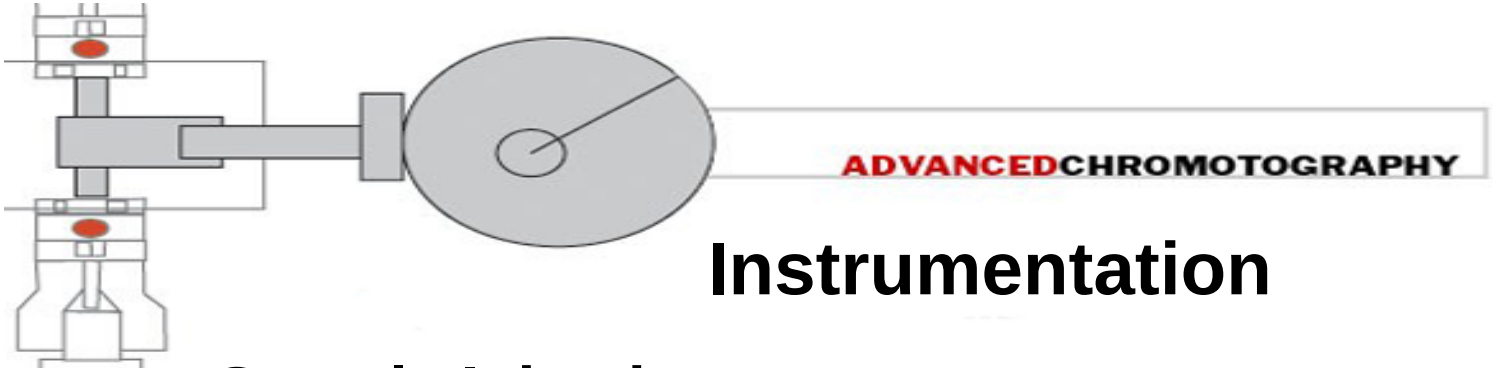
Split Injection

- Dynamic Splitting- Evaporation, mixing and splitting of the sample in a flowing stream – most common.
- Why split? – capillary columns have a limited sample capacity.
- Delivers only 0.2-2% of sample to the column
- Split ratio of 50:1 to 600:1 (sample discarded)
- For samples where analytes of interest are >0.1% of sample
- Best resolution is obtained with smaller amount of sample
- ≤ 1 mL with ≤ 1 ng of each compound (0.5 mL of gas volume)
- Not quantitative, split not constant

Sample Injection - Split Injection

Figure 8.2 Split inlet of the HP5890 GC. *(Courtesy of Hewlett-Packard Company.)*



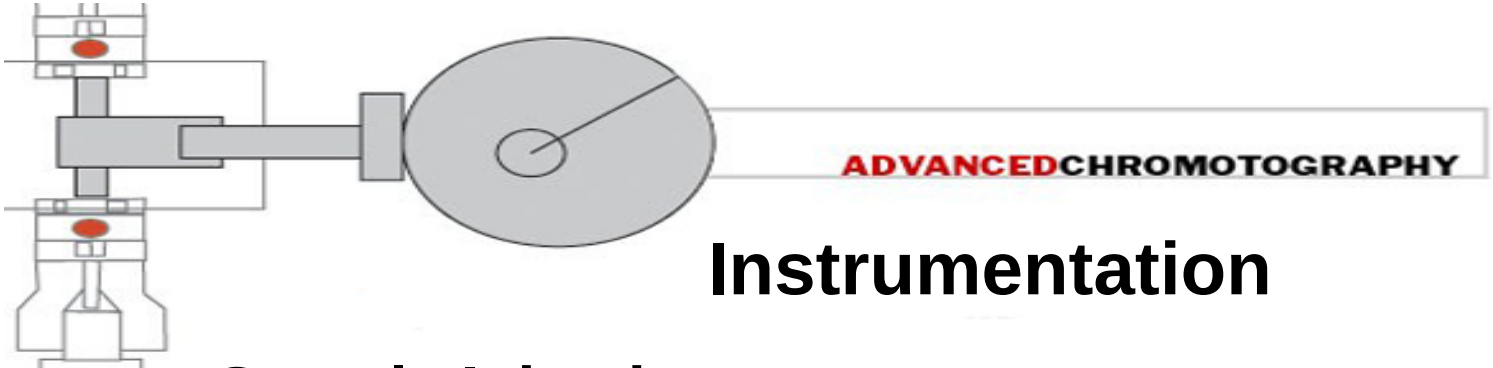


Instrumentation

Sample Injection

Split Injection

- Total carrier gas flow into the inlet is split into three portions as it passes the inlet port.
  - 1st portion – 1 to 3 ml/min – passes past the septum to sweep away contaminates from the septum to purge vent.
  - 2nd and 3rd portion then flow into the inlet of the injection port.
  - At the bottom of the injection port a split occurs
    - a very small portion flows down into the column ~ 1 ml/min
    - the rest flows out the split vent



Instrumentation

Sample Injection

Split Injection

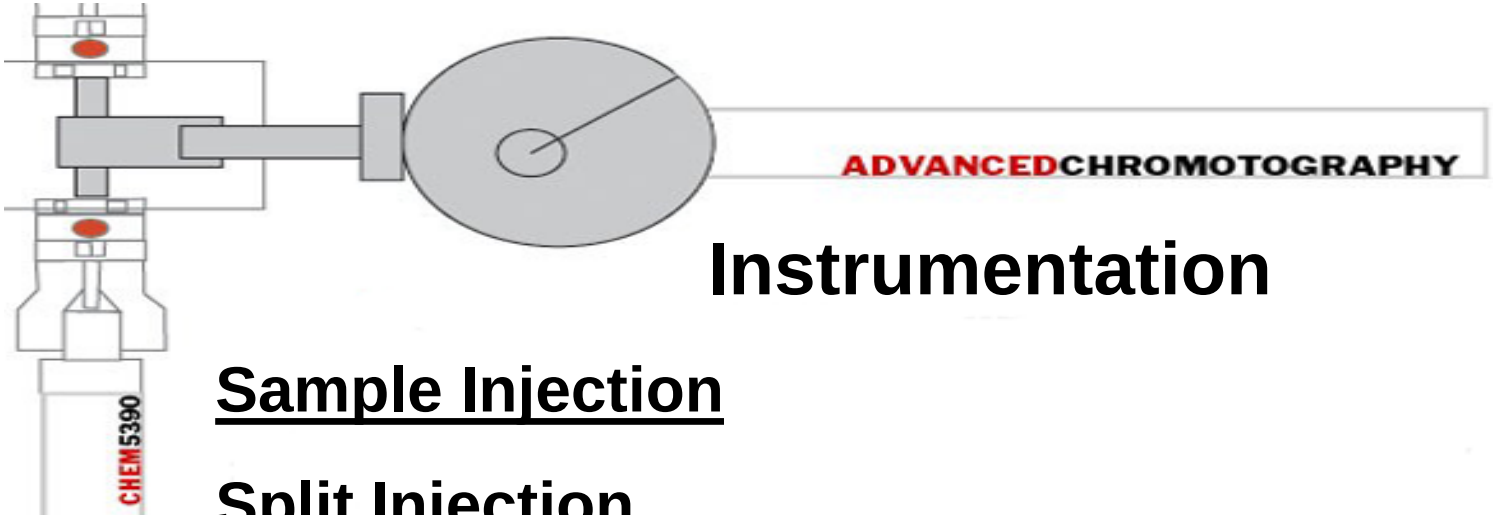
So ~90% of the sample is thrown away, however for quantitative analysis the proportion of the sample analyzed must be known.

$$\text{Split Ratio} = \frac{\text{split vent flow + column flow}}{\text{column flow}}$$

Split ratios range from 10:1 to 500:1.

For our figure:

$$\text{Split Ratio} = \frac{50+1}{1} = 51{:}1$$



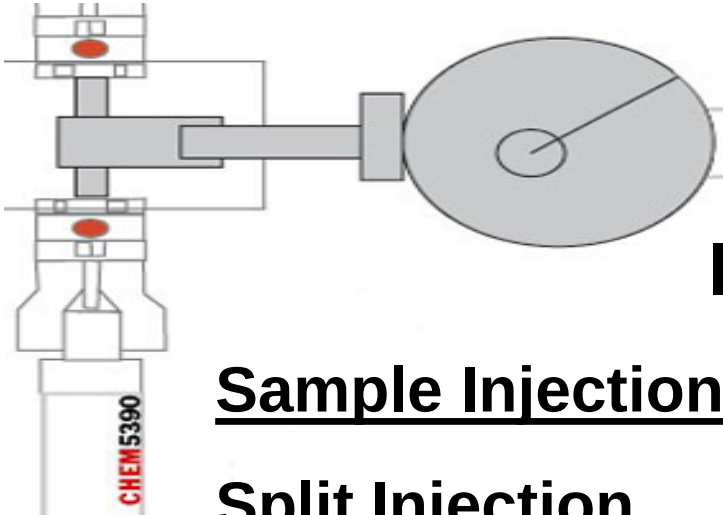
Instrumentation

Sample Injection

Split Injection

The smaller the column the higher the split ratio used to keep from overloading the column.

Disadvantage: split inlets have a high probability of suffering from sample discrimination. So it is important to select the appropriate liner. (Variables – molecular size, polarity of analytes, injected volume, diameter of split liner, viscosity)



Instrumentation

Sample Injection

Split Injection

- For a split system quantitation is actually not very reliable unless the operator is an expert, because of all the different variables involved.

- Split injection can usually provide a small initial band of solute for many samples.

Liners

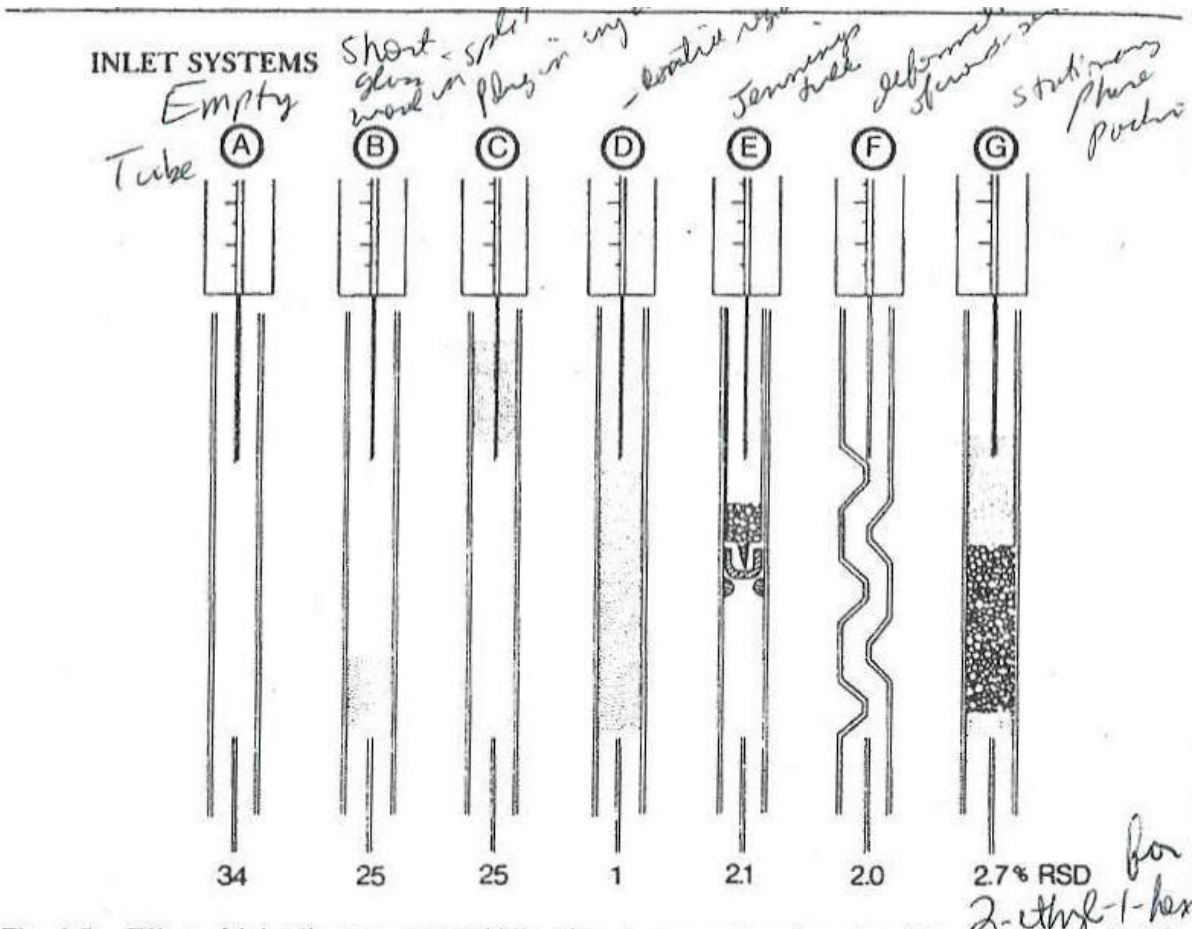
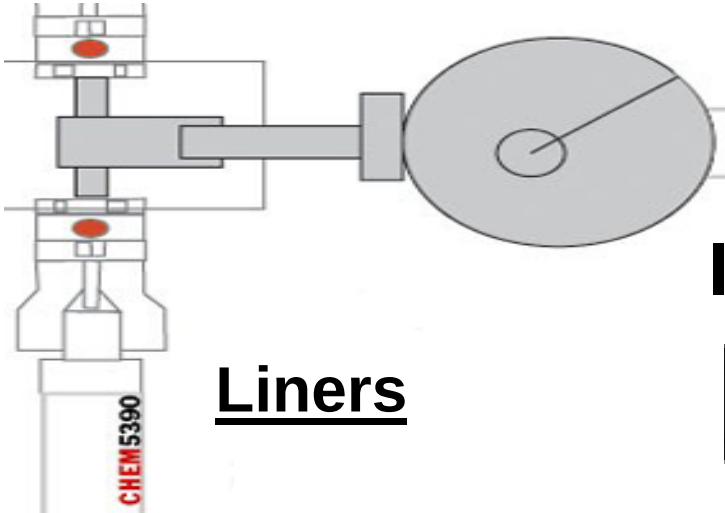


Fig. 3.7. Effect of inlet liner on repeatability of peak area ratios of methanol:2-ethyl-1-hexanol. (A) empty tube, (B) short glass wool plug in the splitting region, (C) short glass wool plug in the injection region, (D) long and tight glass wool plug, (E) Jennings tube, (F) deformation of cross-section, (G) chromatographic support packing. Reprinted with permission from Schomburg *et al.* (1977). *J. Chromatgr.*, **142**: 87.



Instrumentation

Liners

Straight

Function: Low surface area for less activity

Recommended for: Volatiles

Advantages

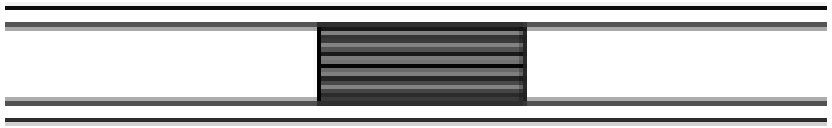
- Simple to use
- Least expensive
- Low activity

Disadvantages

- Possible inlet discrimination
- More frequent seal maintenance from exposure to sample contamination
- Possible inconsistency if sample injection bypasses split ratio

Instrumentation

Liners



Glass Wool (middle)

Function: Traps non-volatiles; mixes sample; vaporizes sample above the column

Recommended for: Dirty samples, volatiles, high initial oven temperatures

Advantages

- Reduces seal contamination and maintenance
- More reproducible results
- Can help focus analytes
- Extends column life

Disadvantages

- Higher surface area that can become active
- Glass wool can become dislodged

Instrumentation

Liners

Glass Wool (bottom)

Function: Traps non-volatiles; mixes sample; vaporizes sample above the column

Recommended for: Dirty samples

Advantages

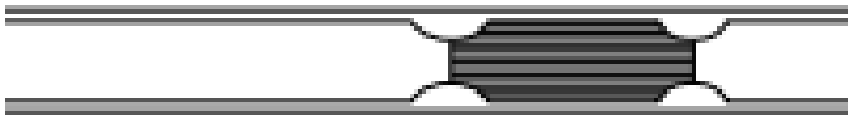
- Reduces seal contamination and maintenance
- More reproducible results
- Can provide higher responses than wool in middle

Disadvantages

- Higher surface area that can become active
- Glass wool can become dislodged

Instrumentation

Liners



Glass Wool (top)

Function: Keeps glass wool in place; wipes syringe needle clean

Recommended for: Pressure pulsed injections, dirty samples, volatiles, high initial oven temperatures

Advantages

- Reduces seal contamination and maintenance
- More reproducible results
- Can help focus analytes
- Extends column life

Disadvantages

- Higher surface area that can become active

Instrumentation

Liners

Taper/Gooseneck (top)

Function: Limits the expansion of the solvent to the inlet

Recommended for: Water injections

Advantages

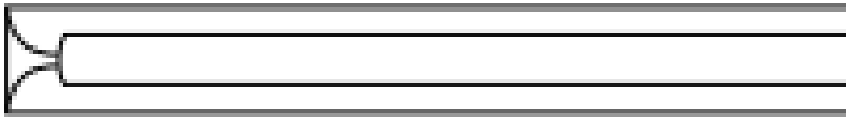
- Allows for larger injection volumes
- Decrease backflash

Disadvantages

- Higher risk of needle breakage
- Increased cost
- Cannot self-pack with glass wool

Instrumentation

Liners



Taper/Gooseneck (bottom)

Function: Directs flow onto column; low surface area

Recommended for: Pesticides (without wool), semi-volatiles (with wool)

Advantages

- Reduces seal contamination and maintenance
- Improved sensitivity
- Lower activity

Disadvantages

- Increased cost

Instrumentation

Liners

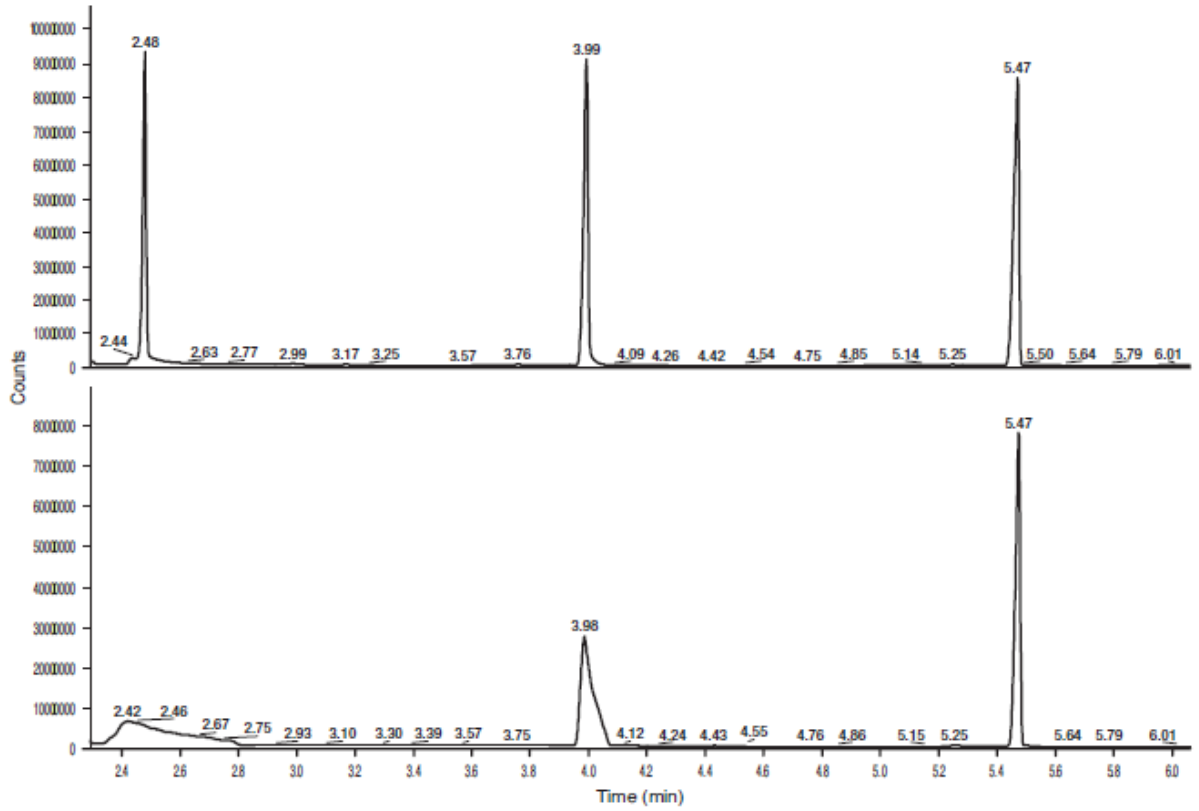
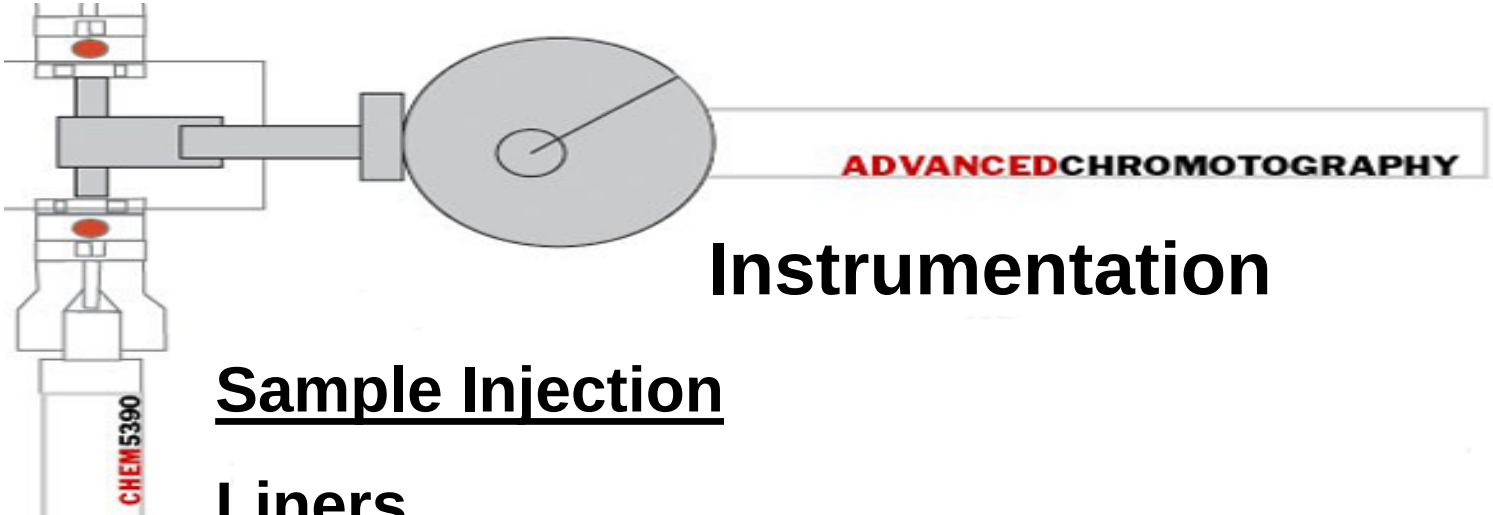


Figure 3: The effect of using the wrong liner in splitless mode (n-alkanes). Top chromatogram, splitless (correct) liner, bottom chromatogram split (incorrect) liner



Instrumentation

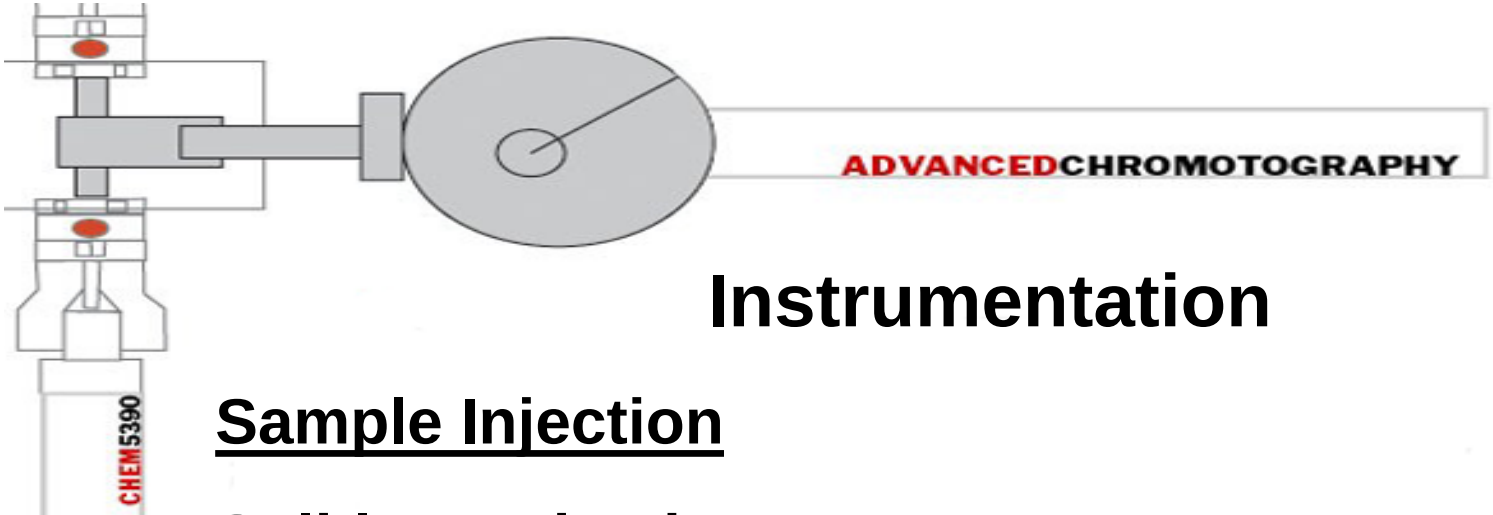
Sample Injection

Liners

- For high boiling temperature solutes – a packed liner works well – increases the liner's heat capacity and assists in sample vaporization.

- For labile solutes – packed liners can contribute to solute decomposition.

- For very volatile solutes – packed liner will increase band broadening because of the multipath diffusion through the packing – unpacked liners are better.



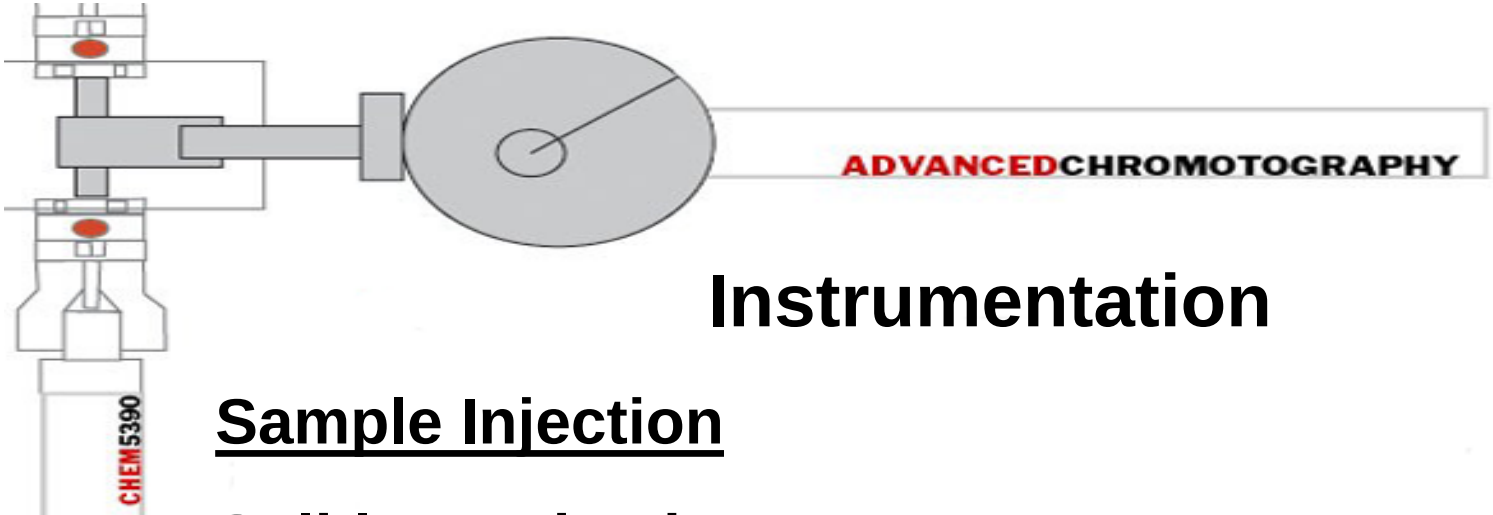
Instrumentation

Sample Injection

Splitless Injection

- For ultratrace analysis, very complex samples with wide range of boiling points – splitless injection is better.

- Delivers ~80% of sample to the column



Instrumentation

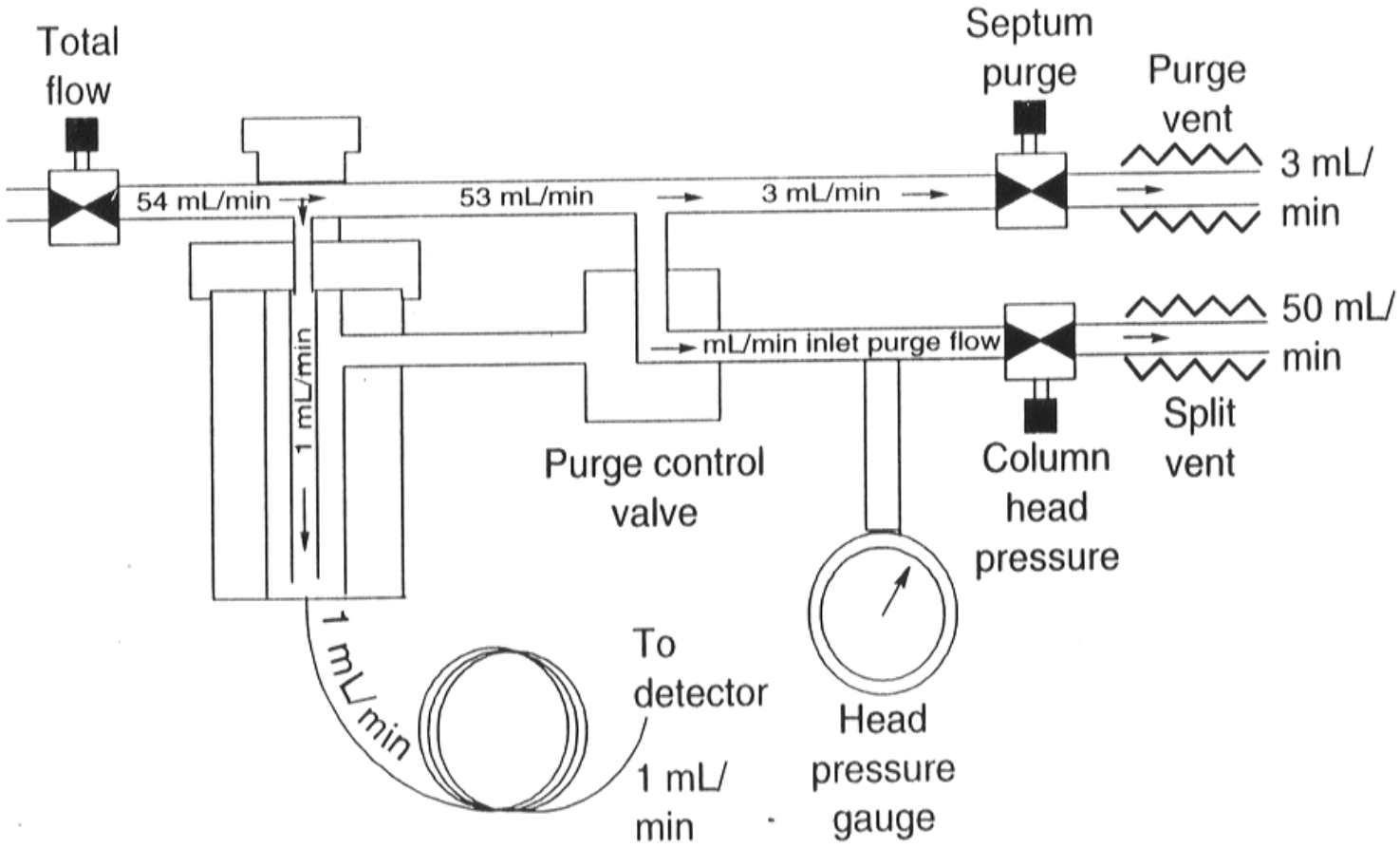
Sample Injection

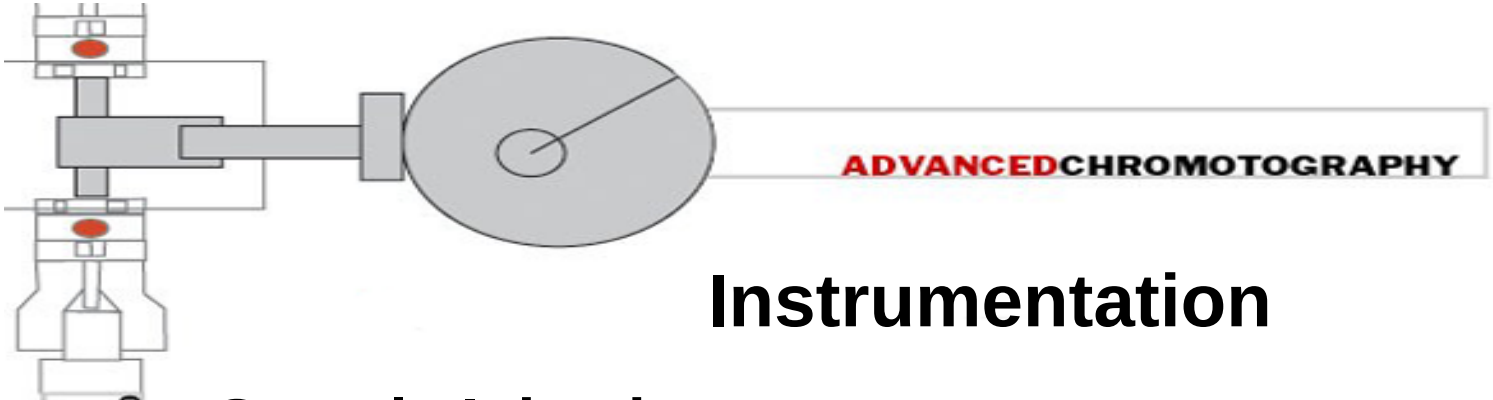
Splitless Injection

- There are two types
  - evaporation and trapping (PTV – programmed temperature vaporizer)
  - cold-on-column injection or direct on column
    - Delivers ~100% of sample to the column
    - For samples that decompose above their boiling points
    - Solution injected directly on column
    - Warming column initiates chromatography

Sample Injection - Splitless Injection

Figure 8.3 Splitless configuration. *(Courtesy of Hewlett-Packard Company.)*





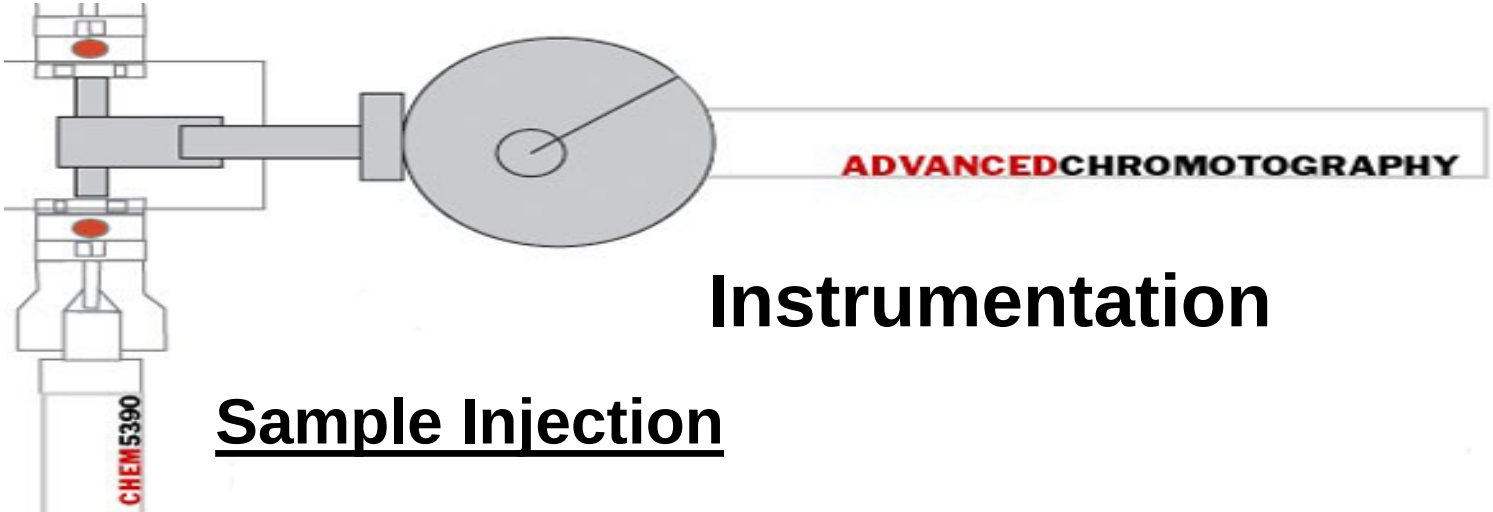
Instrumentation

Sample Injection

Splitless Injection

- For splitless – a large volume (1-5 ml) of dilute sample is introduced. The carrier gas velocity is lower for splitless than for split.

- Residence time of sample in injection port liner is longer for splitless mode (15 s) than for split mode (less than 1 sec).



Instrumentation

Sample Injection

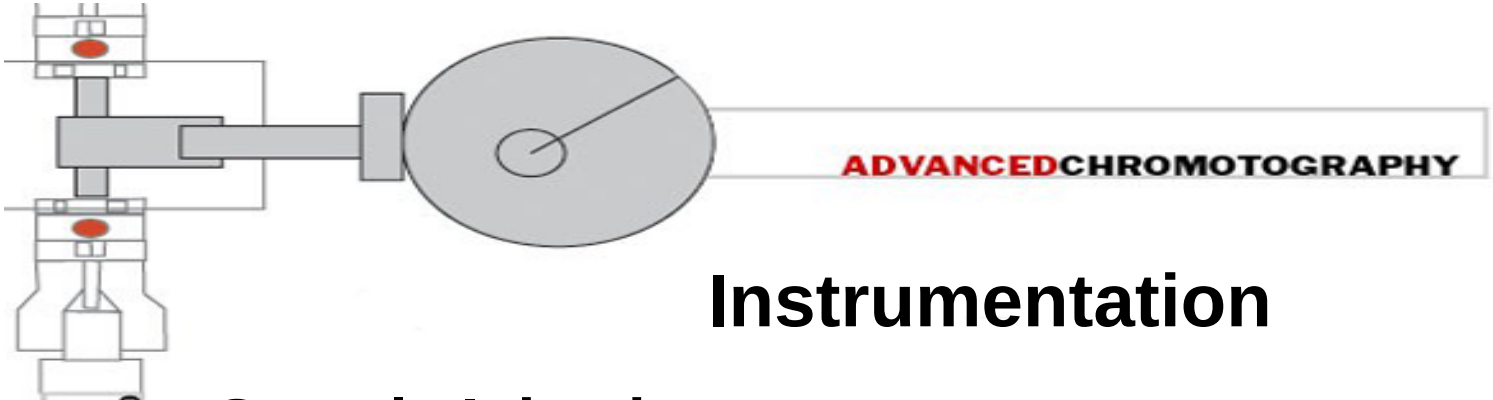
Splitless Injection

•Splitless work best for bonded phase columns since a high solvent load is placed on the column.

•Cold-on-column injection reduces solute decomposition and discriminatory effects if the injection is rapid. One disadvantage is that non-volatile material in the sample will enter the column and remain there.

•So the programmed temperature vaporizer (PTV) was developed using a cold split/splitless injection port, which can be rapidly heated. Non-volatile materials remain in the inlet and discrimination from the needle is minimized since the port is initially cold.

PTV is a universal injection system – program – split/splitless.



Instrumentation

Sample Injection

Programmed Temperature Vaporizing (PTV)

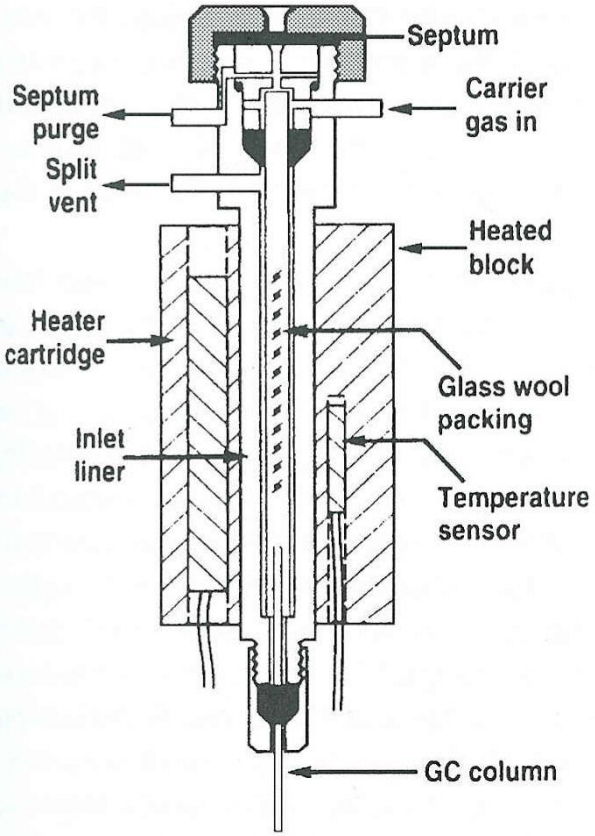
PTV is a universal injection system that can be used in split, splitless or direct mode.

The initial inlet temperature is below the boiling point of all components including the solvent.

After the sample is injected, the temperature of the inlet is programmed to increase at a high rate.

Linearity up to C28 hydrocarbon.

Sample Injection



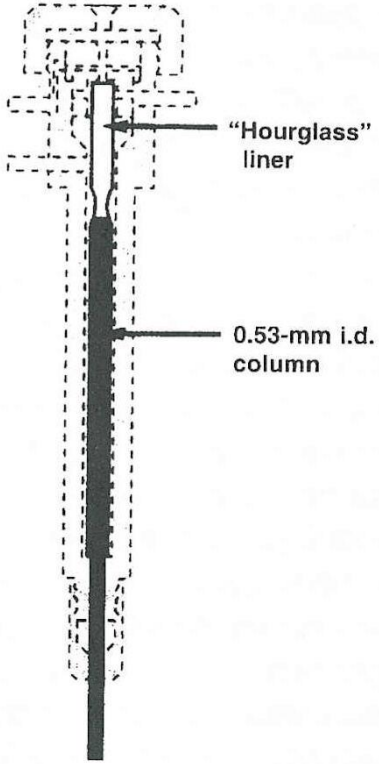
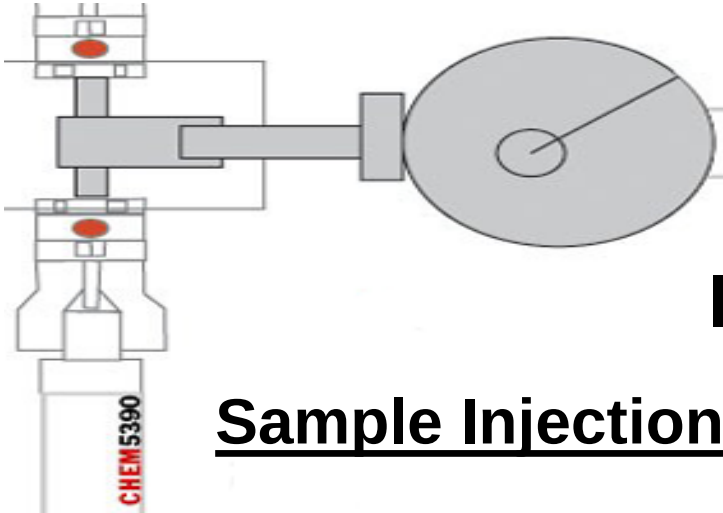


Fig. 3.7. Left, a Schematic representation of a PTV injector configured for split/splitless injection. The cooling coils were omitted for clarity. Right, the same injector modified for on-column PTV injection. The glass insert helps guide the needle directly into the column. *Taken from, J. Hinshaw, LC–GC, **10**:748 (1992)* with permission.



Instrumentation

Sample Injection

Other techniques with specific applications

Purge-and-Trap Method

Headspace method – Static and Dynamic

SPME – Solid Phase Microextraction

Instrumentation

Sample Injection

Purge-and-Trap Method (Headspace – Dynamic)

Effective way for sampling and analyzing low levels of volatile organic compounds from matrices such as drinking water, waste water, soil, and sludge.

Preferred method for evaluation of water purity in the US using EPA methods. One method can quantitate 82 analytes at a detection limit of 0.1 ppb in a single analysis.

Instrumentation

Sample Injection

Purge-and-Trap Method

A sample (liquid) is purged for a specific time and temperature with a purge gas, usually helium.

The volatile analytes are swept by the purge gas to a trap and absorbed.

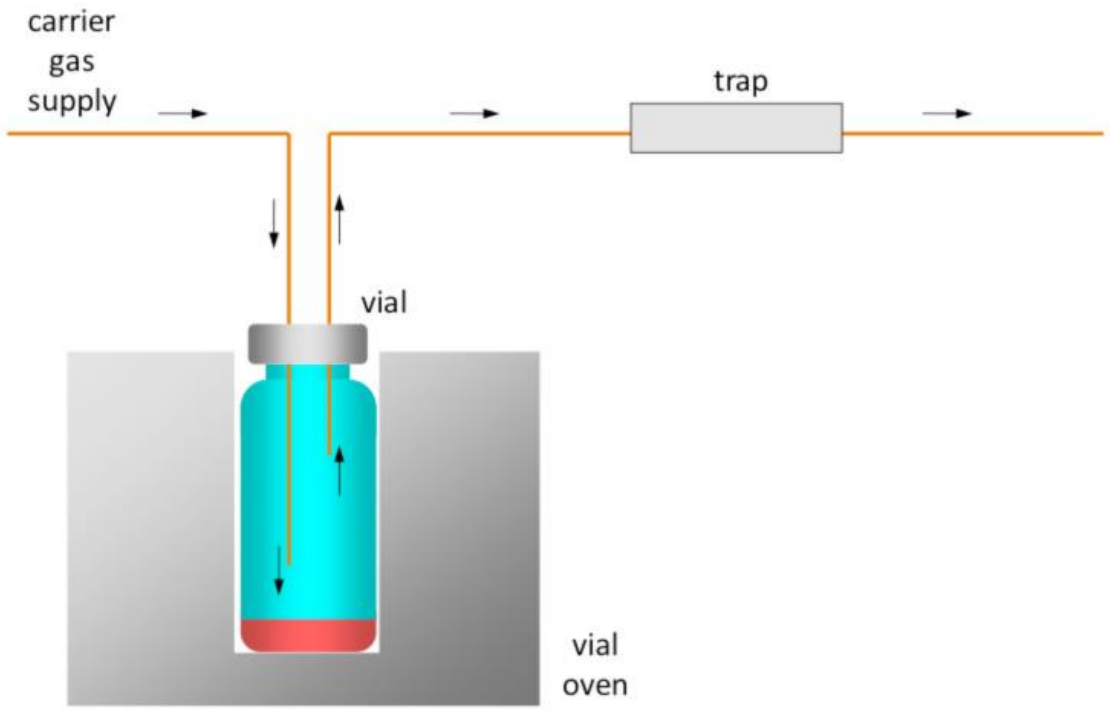
The trap material (usually Tenax - 2,6-diphenylene-oxide polymer resin) is an absorbant material.

After a specified trapping time, the trap is rapidly heated and the analytes are desorbed and swept into the GC column by the carrier gas.

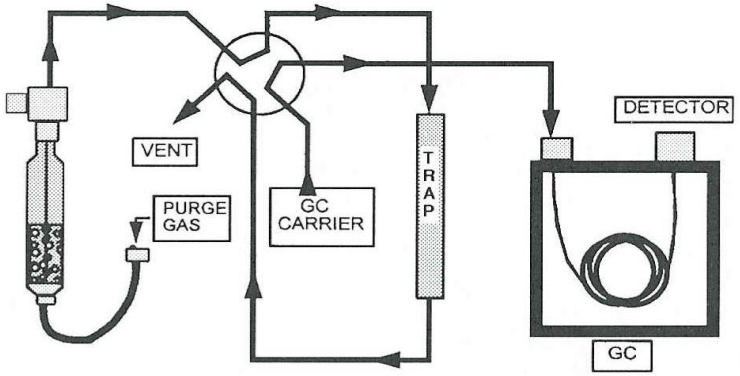
Instrumentation

Sample Injection

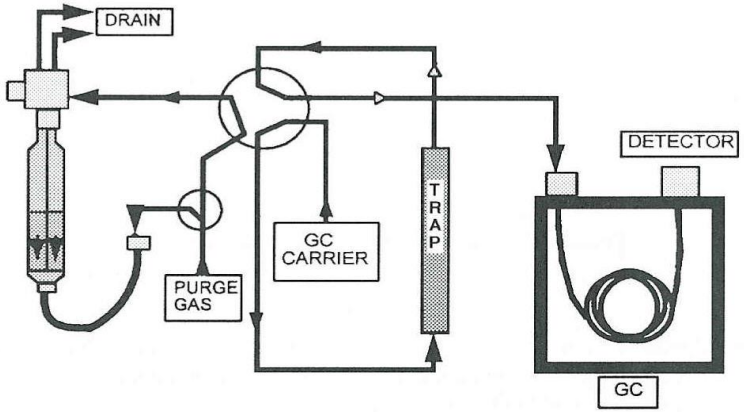
Purge-and-Trap Method



Purge-and-Trap Method

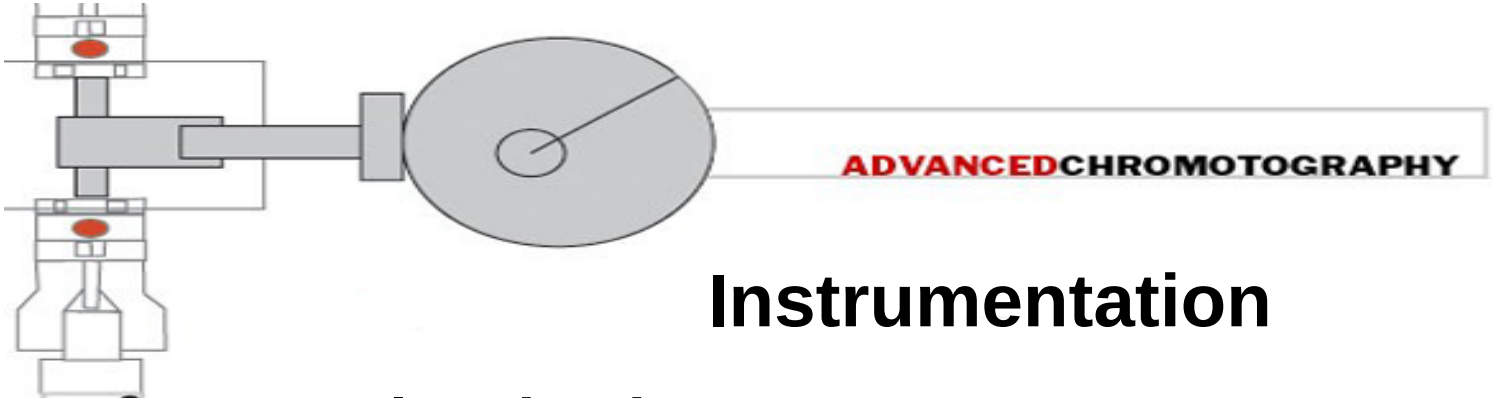


PURGE STATE



DESORPTION & DRAIN

Fig. 3.16. Schematic representation of a purge and trap concentrator in the (top) purge, and (bottom) desorb modes. The six port valve is automated.



Instrumentation

Sample Injection

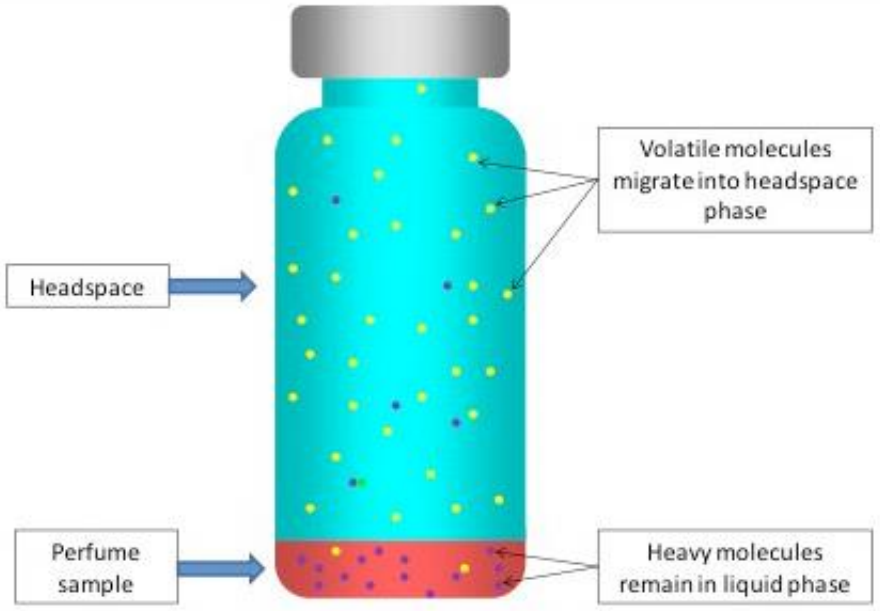
Headspace - Static

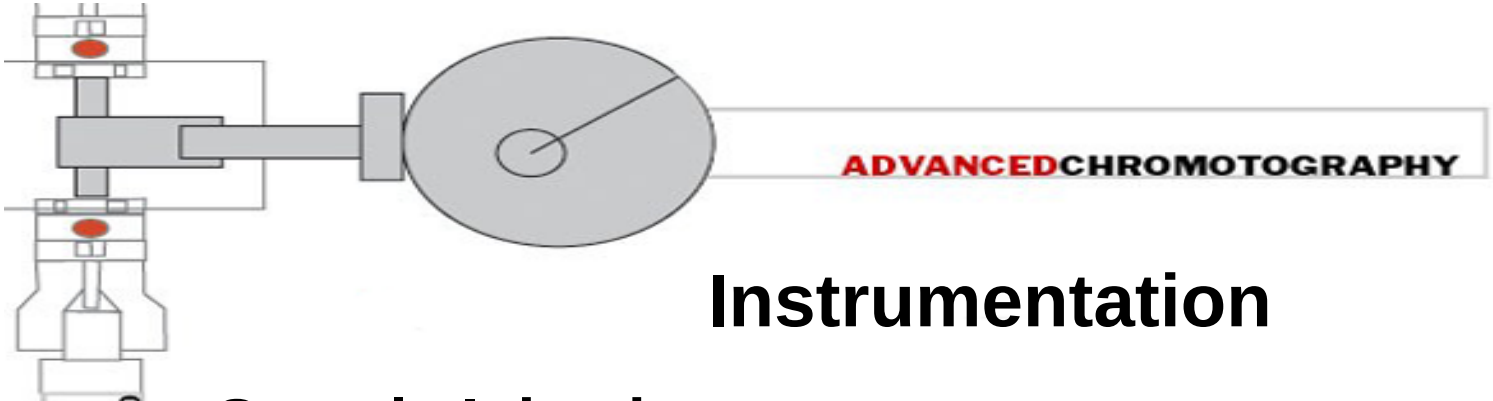
Put liquid or solid sample into sealed container.
Heat and/or shake container.
The more volatile compounds will tend to move into the gas phase (or headspace) above the sample.
The more volatile the compound, the more concentrated it will be in the headspace.
The less volatile components that represent the bulk of the sample will tend to remain in the liquid phase.
Extract some of the headspace vapor with a gas tight syringe and inject it.

Instrumentation

Sample Injection

Headspace





Instrumentation

Sample Injection

Headspace

The partition coefficient is proportional to the ratio of the concentration of molecules between the two phases when at equilibrium.

$K = C_S/C_G$

Where:

K is the partition coefficient of a given compound between sample (liquid) phase and the gas (headspace) phase

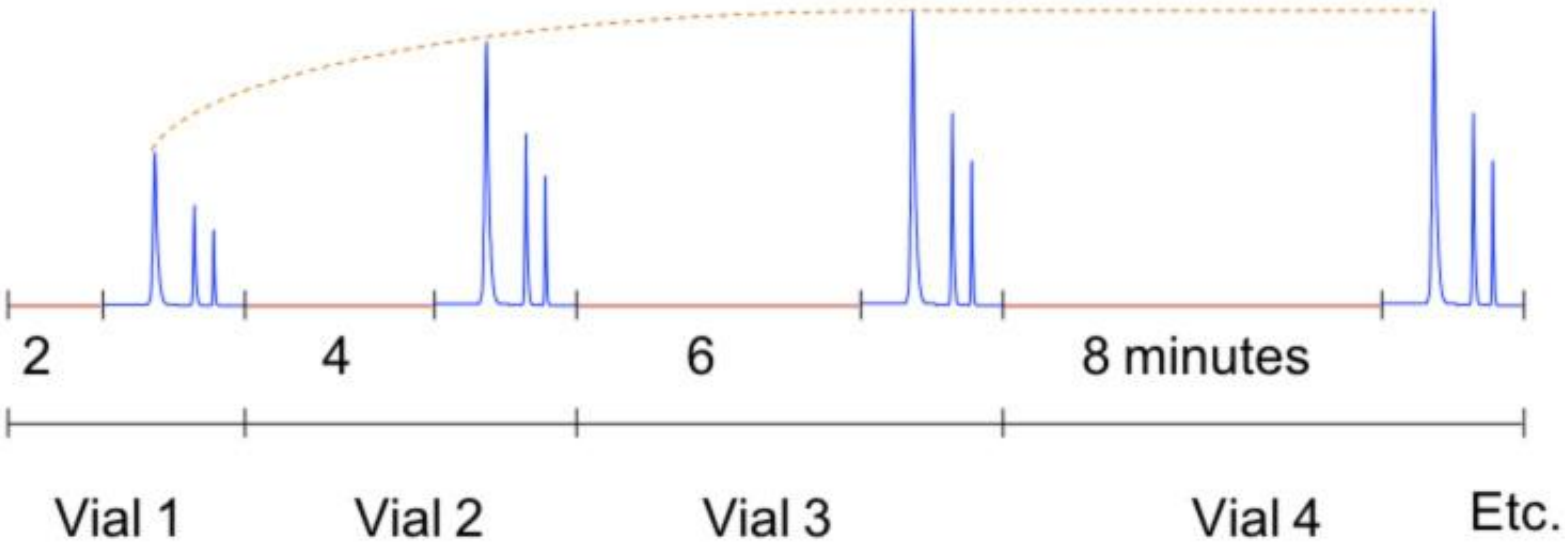
C_S is the concentration of that compound in the sample (liquid) phase

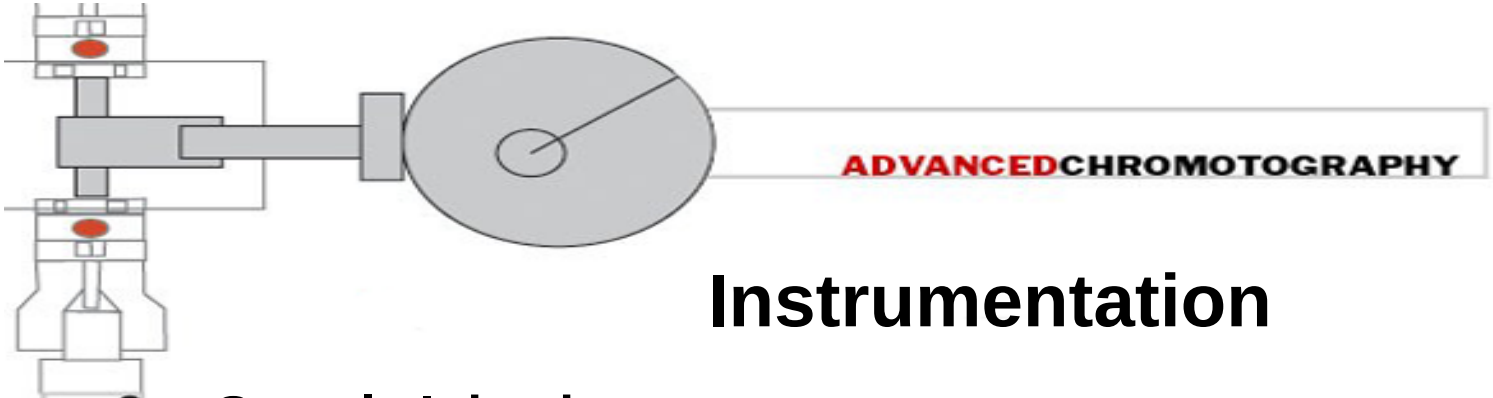
C_G is the concentration of that compound in the gas (headspace) phase

Instrumentation

Sample Injection

Headspace – Effect of Equilibrium Time



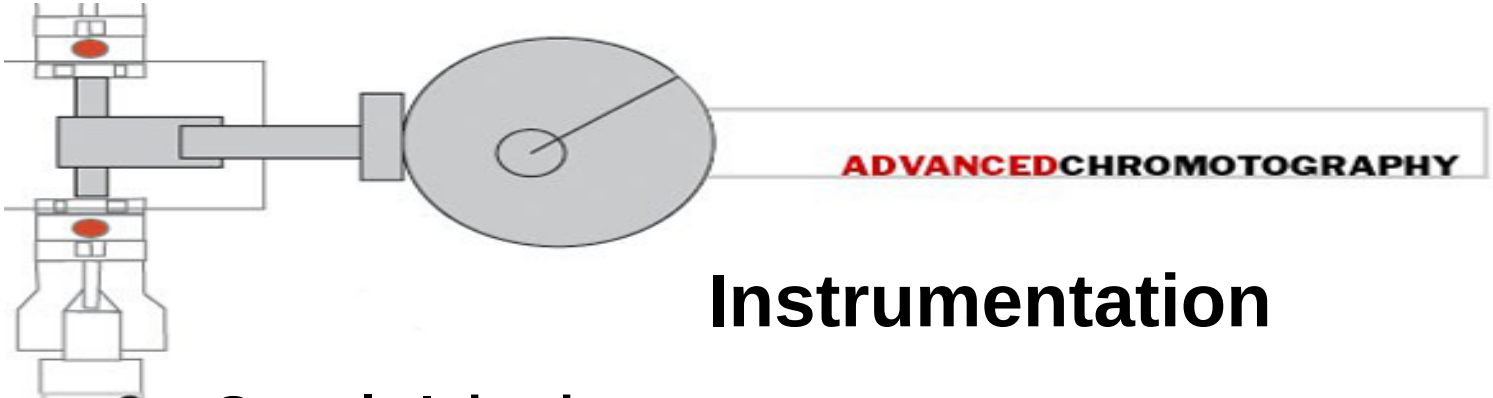


Instrumentation

Sample Injection

Solid Phase Microextraction (SPME)

- **Extract analytes from complex mixture without solvent**
- **Uses a fused-silica fiber coated with stationary phase**
- **Stationary phase similar to those used in GC**
- **Expose Fiber to sample to extract compounds and then inject fiber into GC to evaporate analytes**
- **Forensic Analysis**



Instrumentation

Sample Injection

Solid Phase Microextraction

SPME is another technique that can be used to extract and concentrate compounds from headspace vapor.

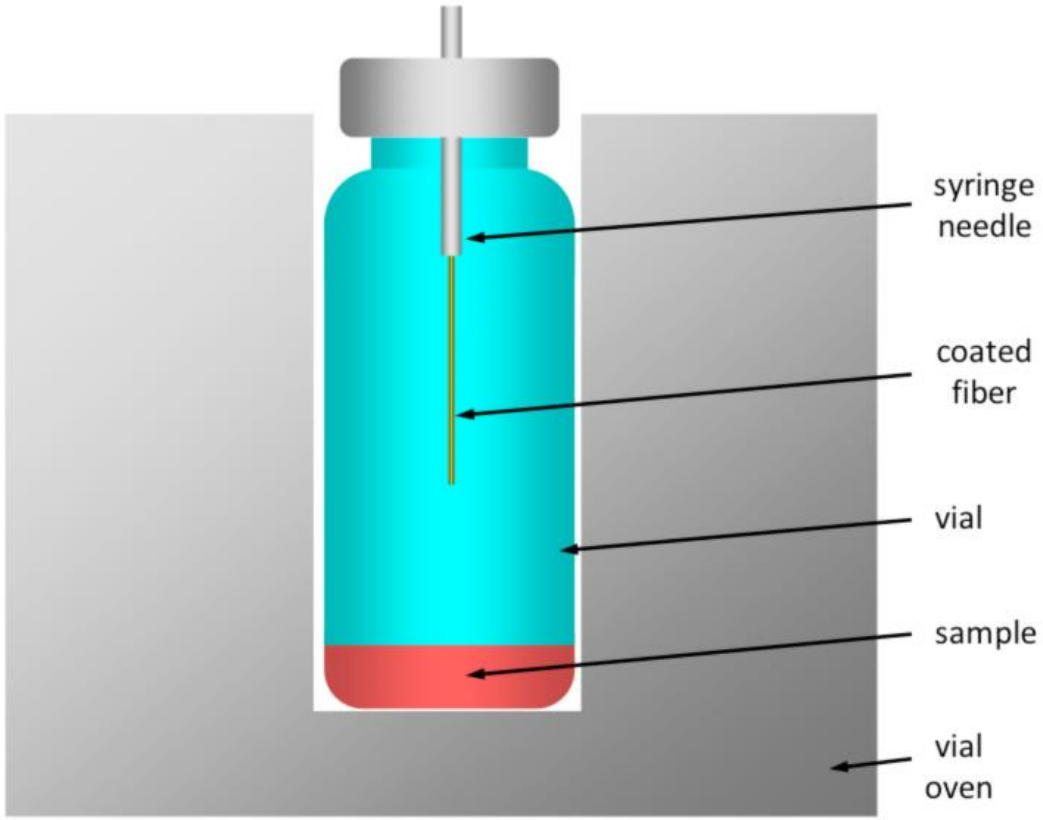
Instead of using carrier gas to sweep the headspace vapor out of the sample vial, SPME inserts a ‘trap’ into the headspace vapor inside the vial.

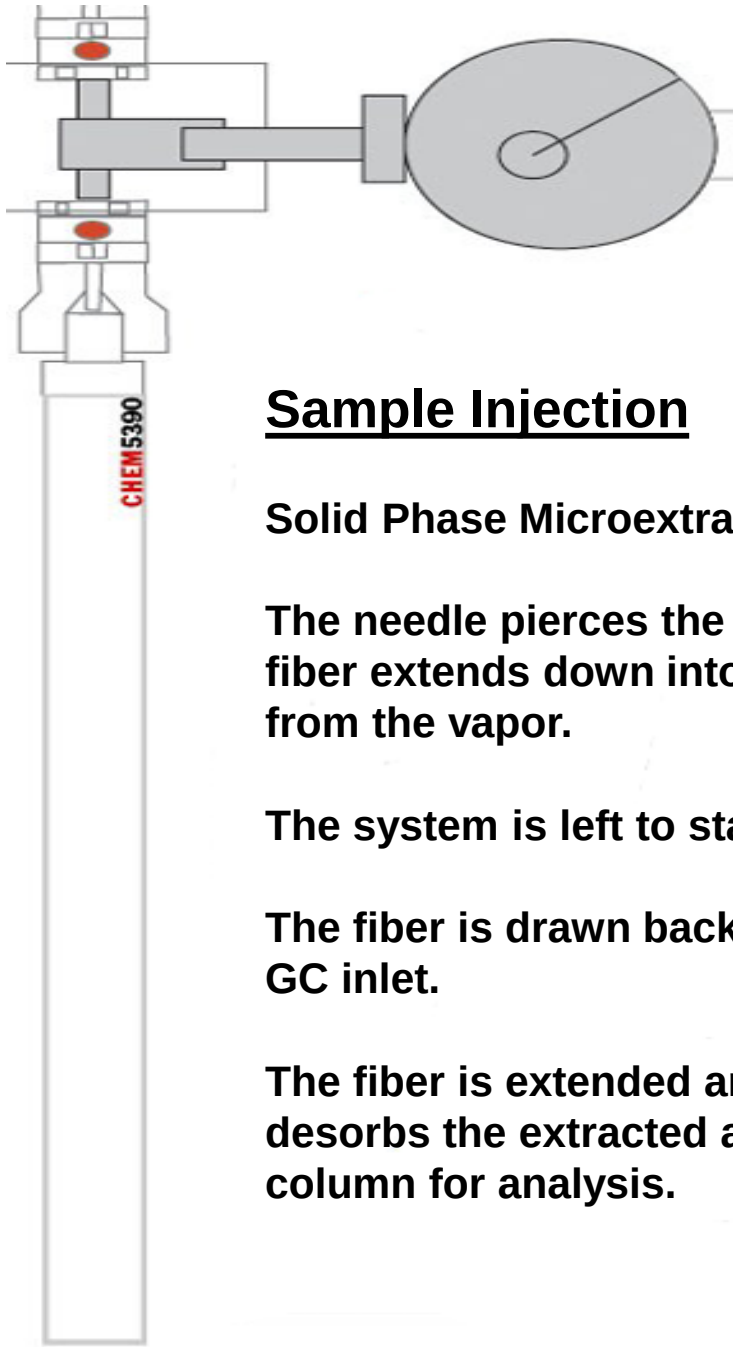
This ‘trap’ is in the form of a retentive coating applied to a narrow fused silica fiber which is located within the needle of a special syringe.

Instrumentation

Sample Injection

Solid Phase Microextraction (SPME)





Instrumentation

Sample Injection

Solid Phase Microextraction

The needle pierces the seal of a vial containing the sample and the coated fiber extends down into the headspace and starts to adsorb compounds from the vapor.

The system is left to stabilize or equilibrate for a period of time.

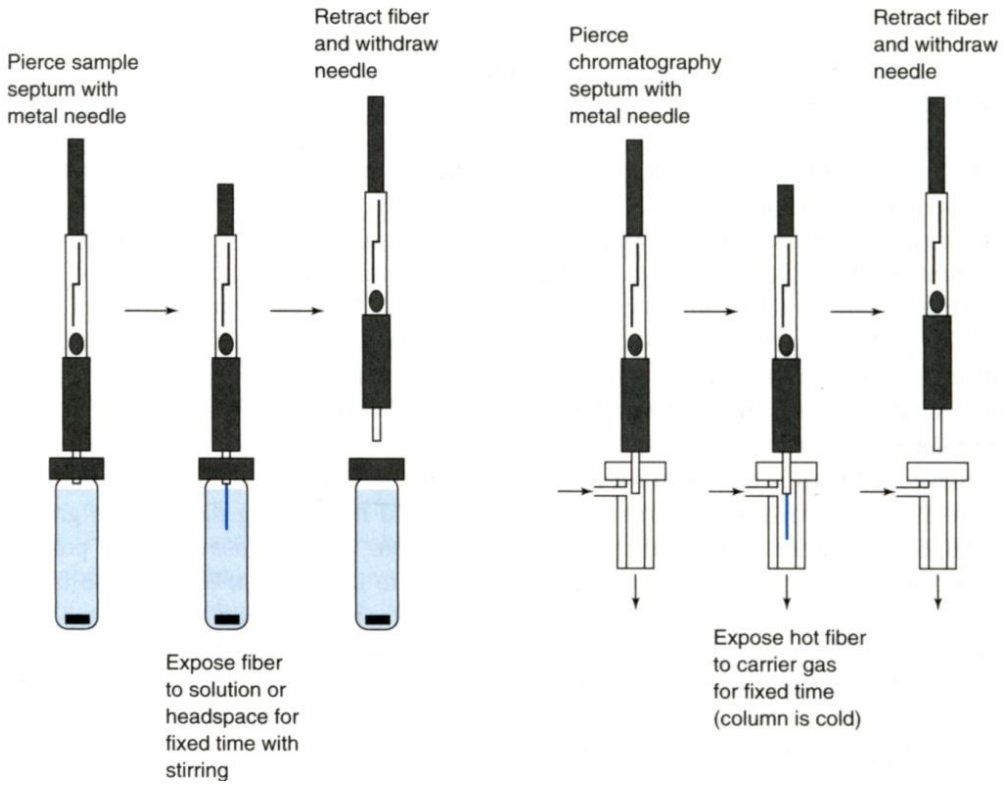
The fiber is drawn back into the syringe needle and inserted into a heated GC inlet.

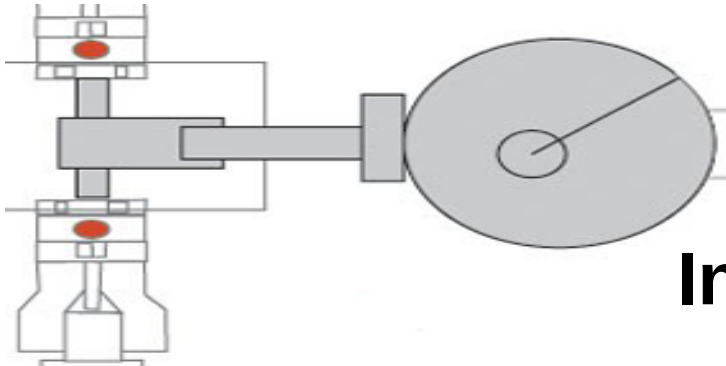
The fiber is extended and absorbs heat from the injector liner which desorbs the extracted analytes and carrier gas transfers them to the GC column for analysis.

Instrumentation

Sample Injection

Solid Phase Microextraction





Instrumentation

Sample Injection

Solid Phase Microextraction

SPME simplifies the extraction technique – no gases or plumbing are required.
It provides a good degree of analyte pre-concentration for many analytes and is very effective at eliminating the effects of water.

However, the theory is much more complex. SPME is a 3-phase system. The headspace vapor phase will interact with the sample phase and with the SPME fiber coating.
Two thermodynamic systems are at work: analytes will seek to achieve an equilibration between the sample and the headspace vapor and also between the headspace vapor and the fiber coating.

Thus two partition coefficients are involved to achieve a final equilibration in the system.

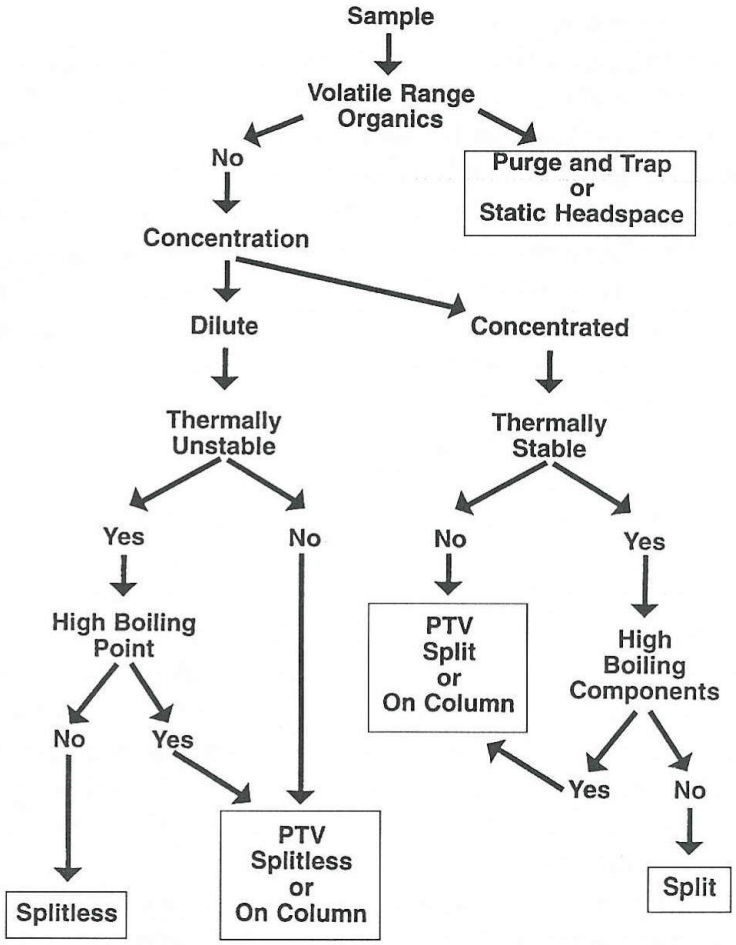
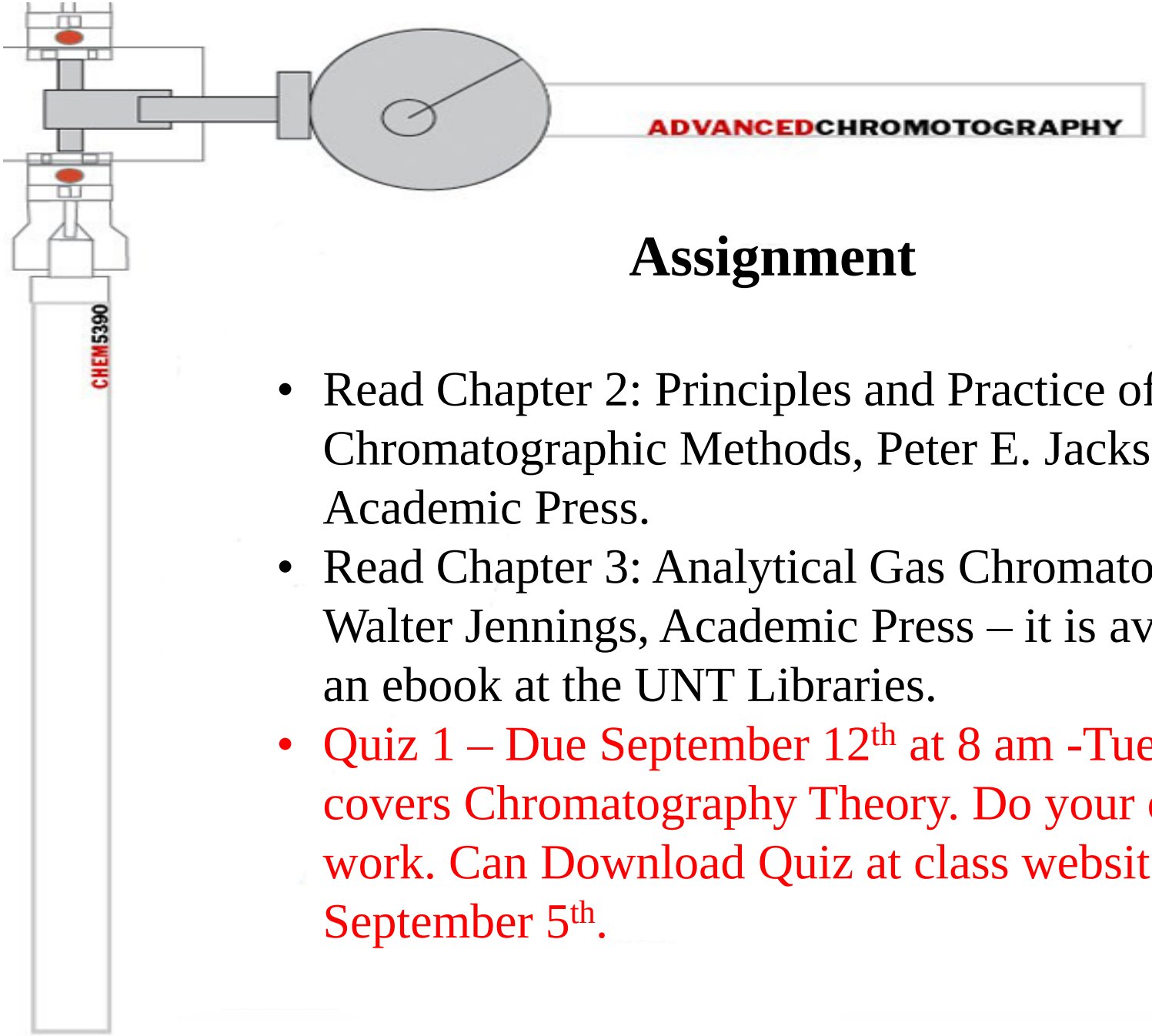


Fig. 3.19. A sample-based flow chart for selecting the injection mode. As an example, if the sample did not contain volatile range organics, was reasonably concentrated, thermally stable, and lacked high boiling components, the injection mode of choice is split.



Assignment

- Read Chapter 2: Principles and Practice of Modern Chromatographic Methods, Peter E. Jackson, Academic Press.
- Read Chapter 3: Analytical Gas Chromatography, Walter Jennings, Academic Press – it is available as an ebook at the UNT Libraries.
- Quiz 1 – Due September 12th at 8 am -Tuesday – covers Chromatography Theory. Do your own work. Can Download Quiz at class website on September 5th.