

# Role of Headspace in Gas Chromatography

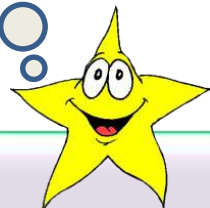
(its importance and Limitation)



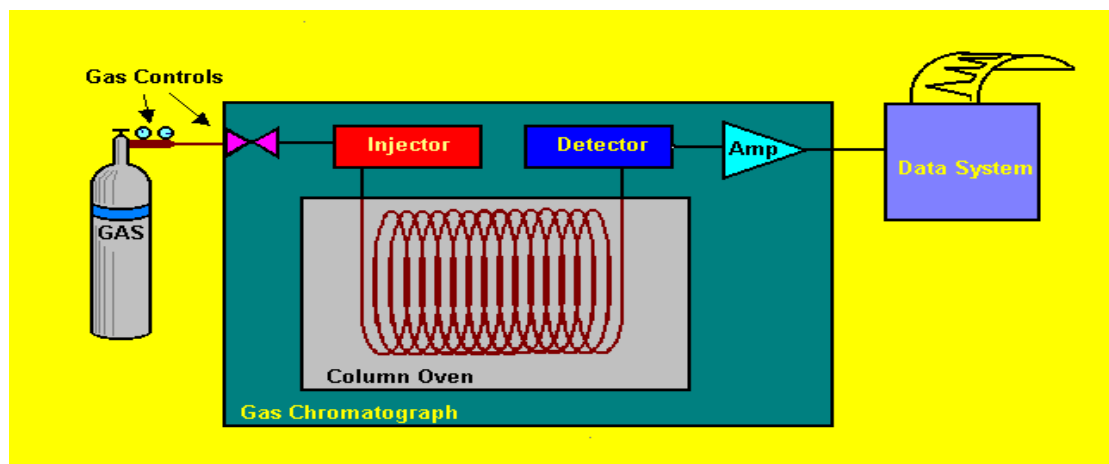
Headspace

# Gas chromatography (GC)

Sample must be capable of being volatilized without undergoing decomposition



An analytical technique for the investigation of **volatile compounds**.



# Injection technique



**Manual injection**

Liquid sample



**Autosampler**

Liquid sample



**Headspace sampler**

Gas sample

**Carrier gas**

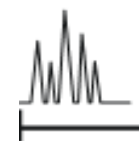
**GC Inlet @ Higher Temperature**

**Volatile Sample with carrier gas**

**Column**

**FID**

**Signal**



# Disadvantage of Manual injection/Autosampler

Column damage  
& Increase  
Interfere



If all sample  
components  
are not volatile

Sample



A solid residue will  
remain in the inlet  
system

Heated inlet system



The solid residue may **eventually decompose, creating volatile breakdown products** which get into the column and thus into the chromatogram.

Sample residue may interfere with subsequent injections due to **adsorption** and / or **catalytic decomposition**.

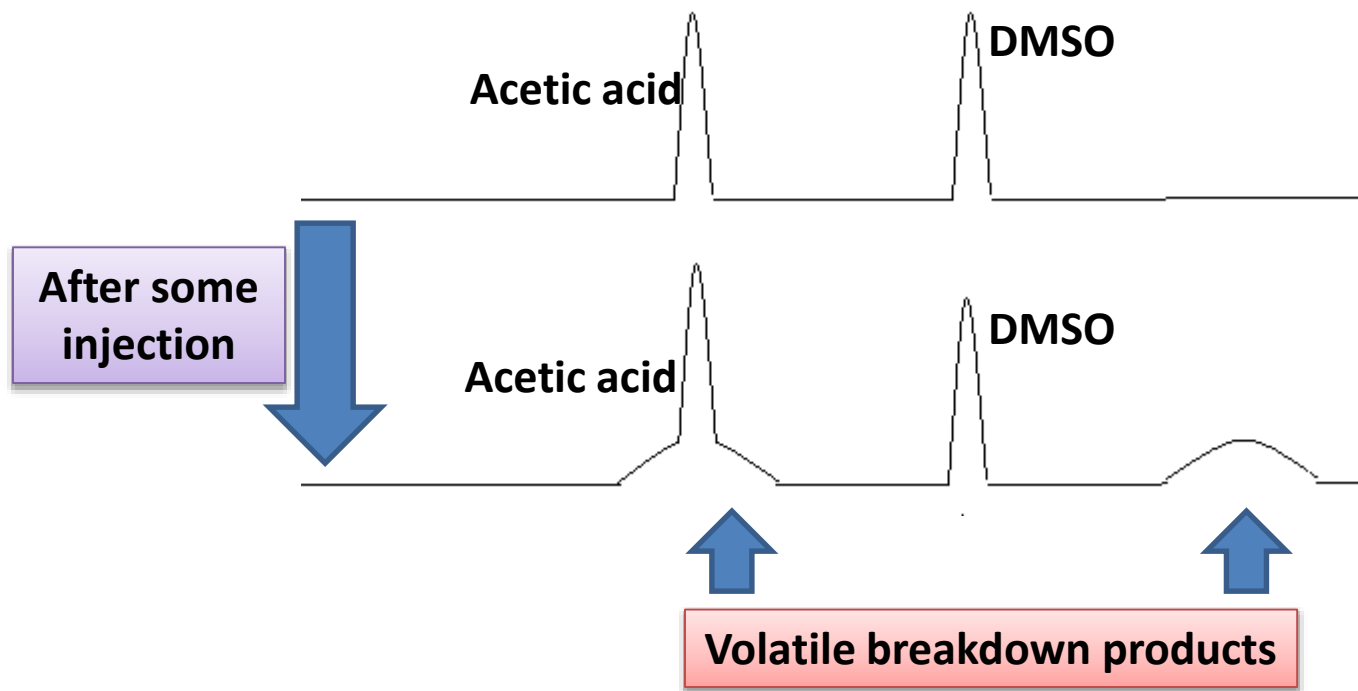


Creating the impression that these compounds  
were present as such in the original sample.

Interfere in analysis



Disadvantage



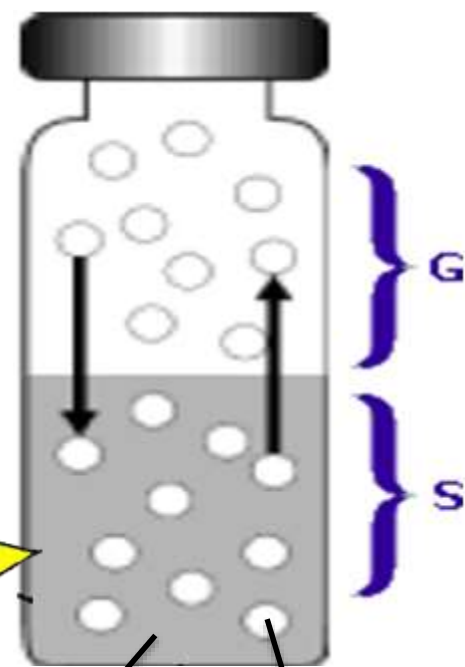
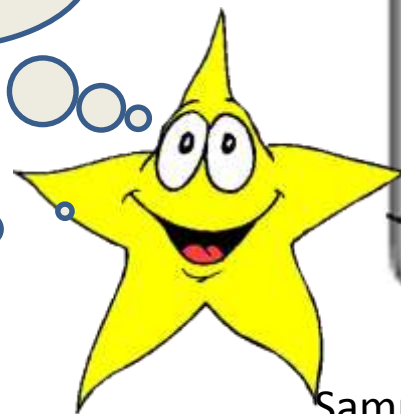
Samples containing constituents which are **unsuitable for injection with a syringe** can not be analyzed e.g. **solid** (Polymers, Resins, Pharmaceutical powders, Soils etc), **highly viscous liquids** (Oils, Emulsions, Gels, Ointments, etc).

# Headspace Gas Chromatography

The 'headspace' is the gas space in a chromatography vial above the sample.

Headspace analysis is therefore the analysis of the components present in space above the sample.

Static or Equilibrium Headspace analysis



Sample and diluted solvent

Volatile analytes

## Sample

**Liquids:** Aqueous samples, Oils, Emulsions, Gels, Ointments, etc.

**Solids :** Polymers, Resins, Pharmaceutical powders, Soils.



## HS and it's advantage

Headspace analysis is the analysis of the vapor lying in equilibrium over a solid/liquid sample in a sealed container.



A defined amount of the vapor is taken and carried to the column in the gas chromatograph for analysis.

Analysis without interference  
of the nonvolatile matrix

Advantage



Only volatile substances reach the column, the non-volatile substances remain in the sample vial.

Advantage



Samples containing constituents which are unsuitable for injection with a syringe can be analyzed (e.g. soil, polymers, highly viscous liquids).

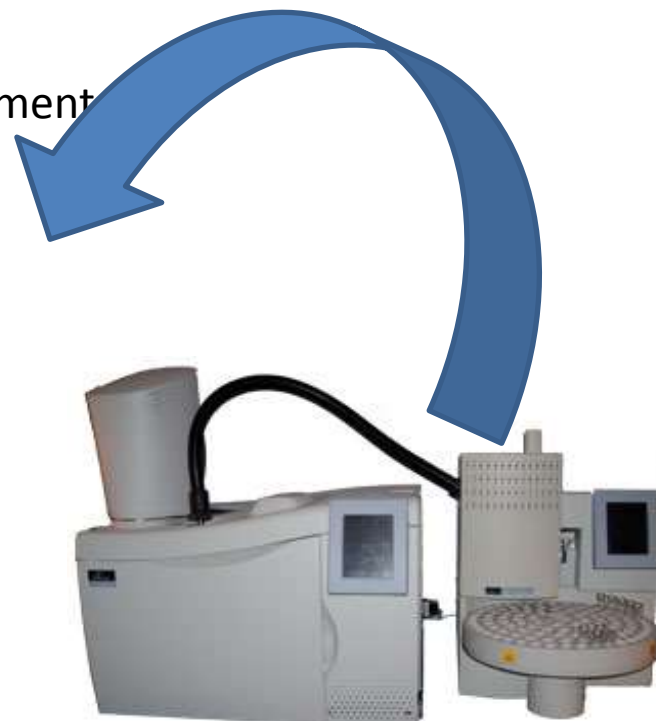
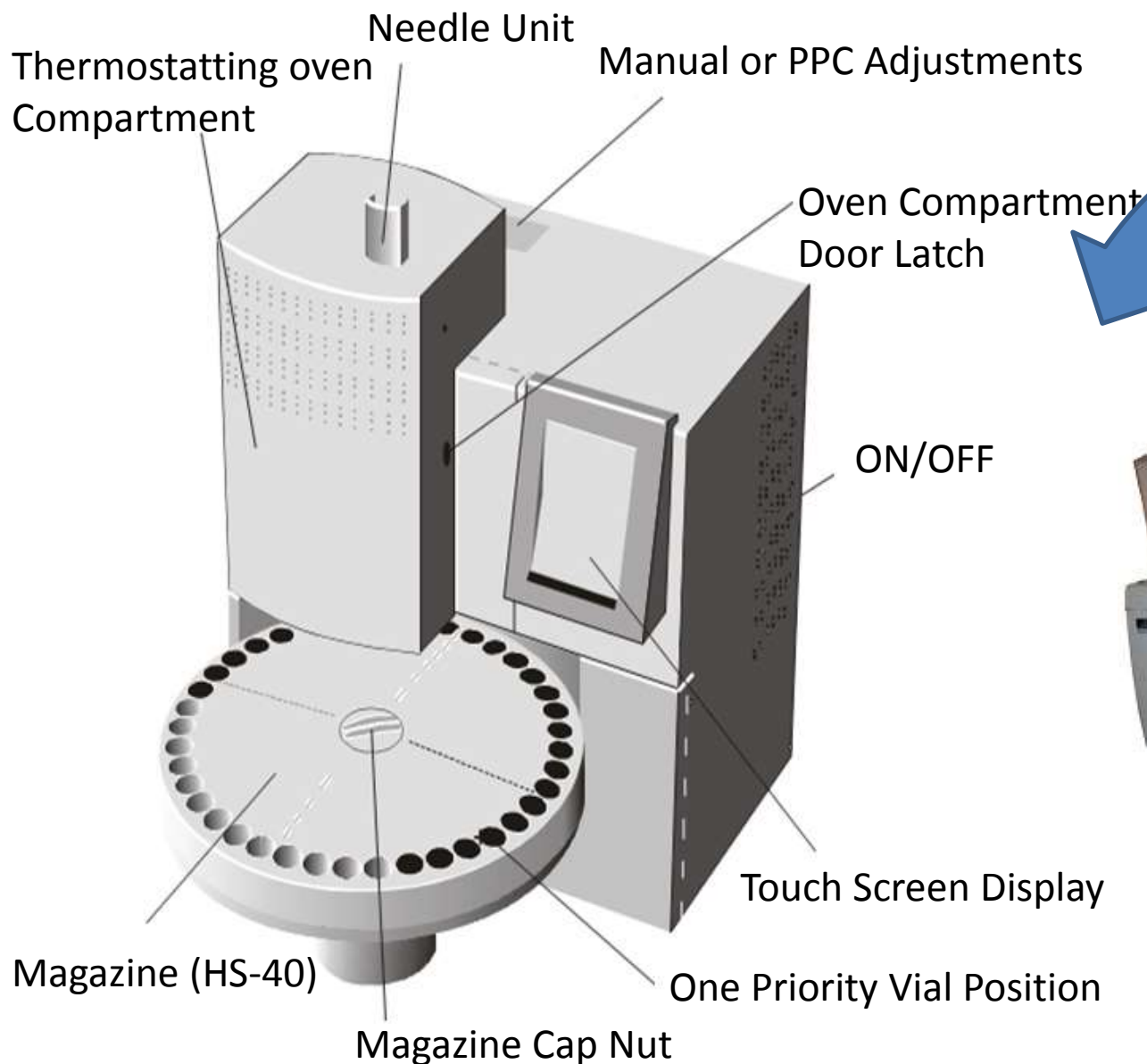


Column Saving  
& Residue  
Interfere

Variety of  
sample can be  
injected.

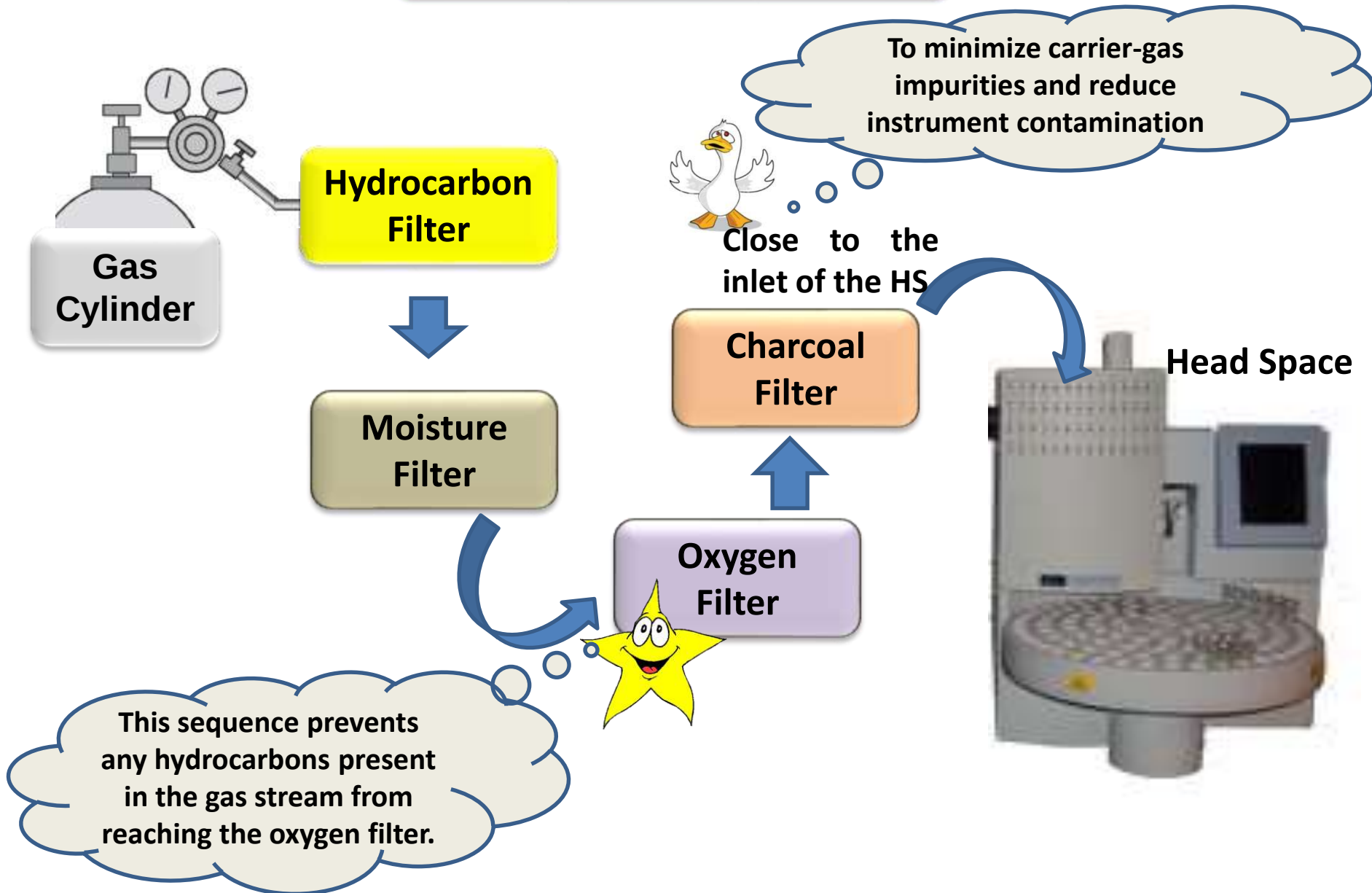


## Layout of TurboMatrix Headspace Sampler



**GC-HS**

## Filter sequence for Head Space



# Headspace Sampling

Sample  
(Gas, Liquid or solid)

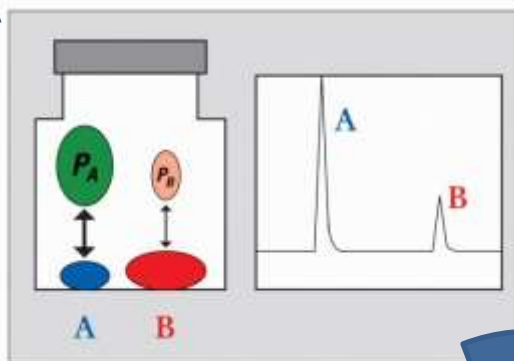


Closed immediately

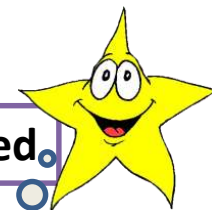
Headspace vial



Pressurized



Equilibrated



Temperature, Time  
& Shaker play a  
important role.

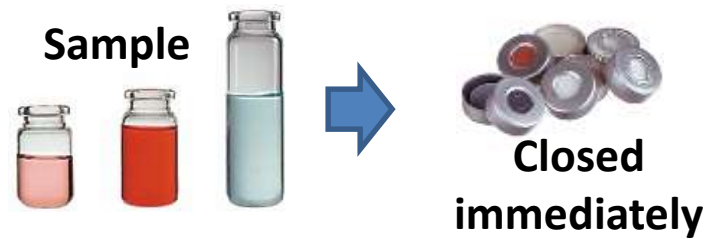
An Aliquot is then  
withdrawn



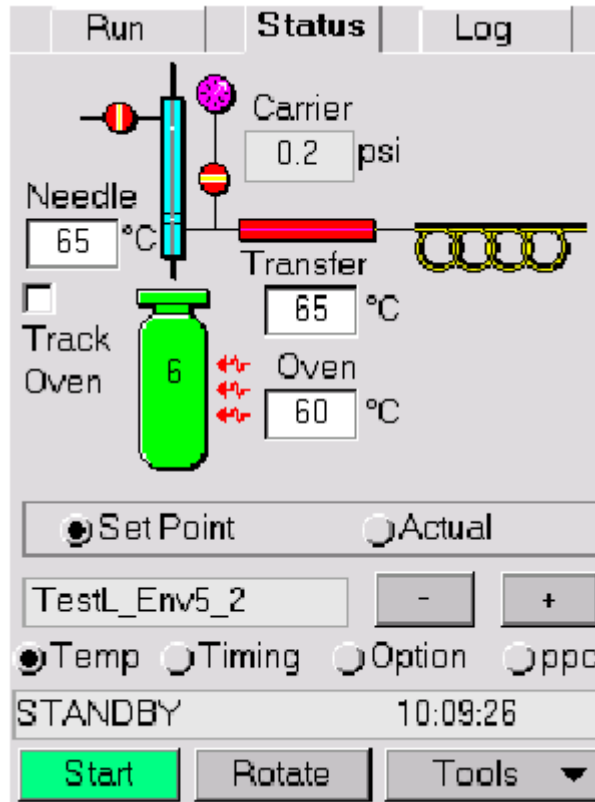
Gas Chromatography

Per concentrated

Cryofocusing (Cold trapping)



**Temperature**



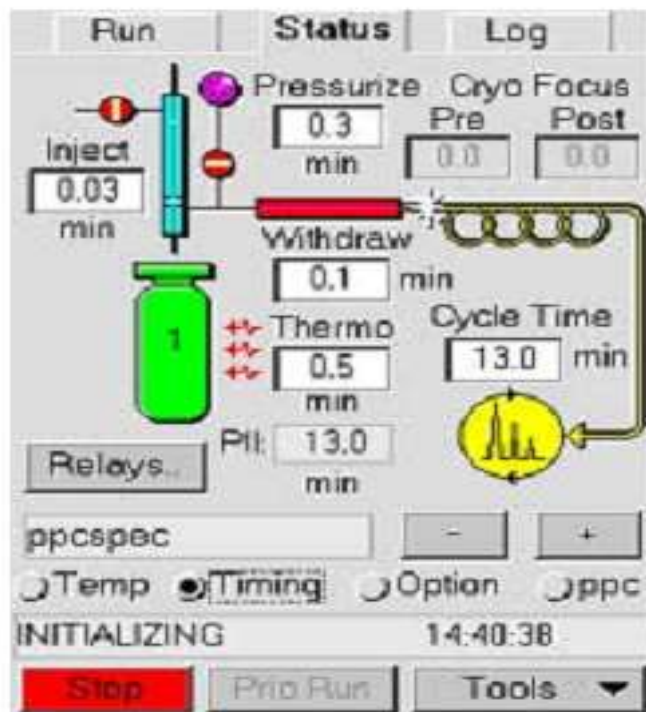
**Thermostating Temperature**

**Needle Temperatures**

**Transfer Line Temperatures**



## Timing



Thermostatting Time

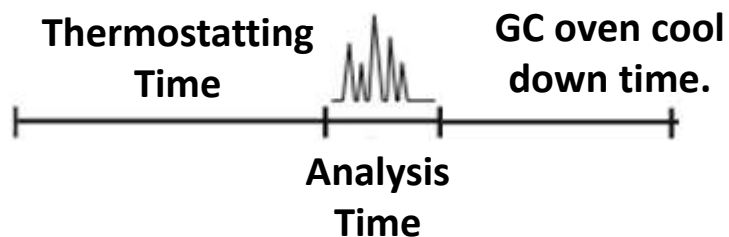
Pressurization Time

Withdrawal Time

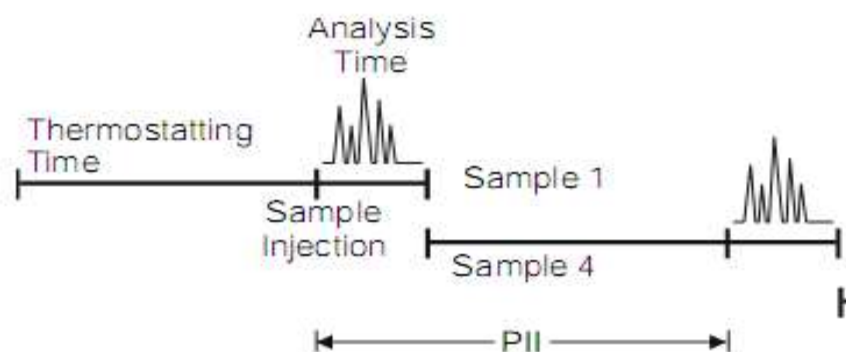
Injection Time

## GC Cycle time

The time at which the GC is ready for the next injection



## PII value : Period from Injection to Injection



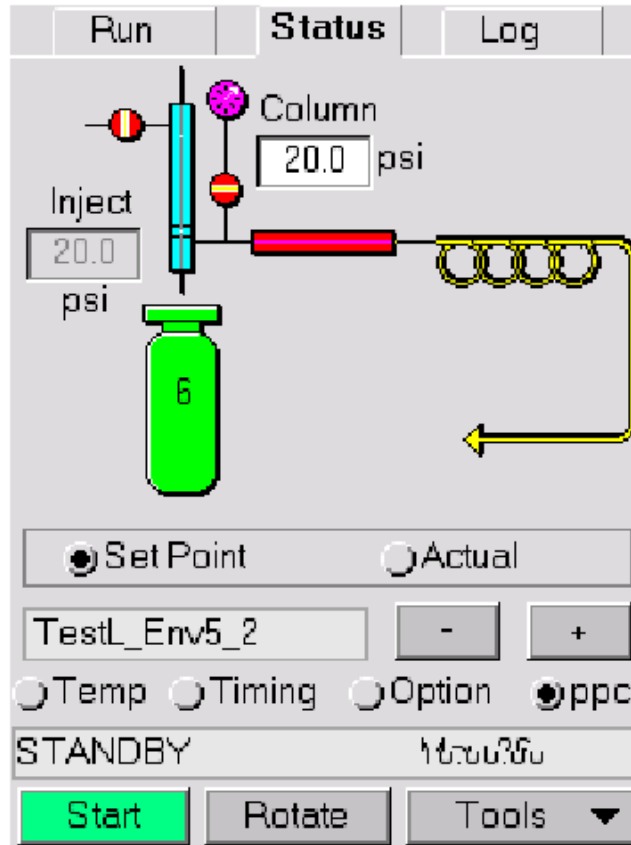
# Programmed Pneumatic Control (PPC)

The electronic control of the carrier gas pressure

## Inject Pressure



Applied to the sampling needle and the column head pressure from start of the pressurization time until the end of the withdrawal time.

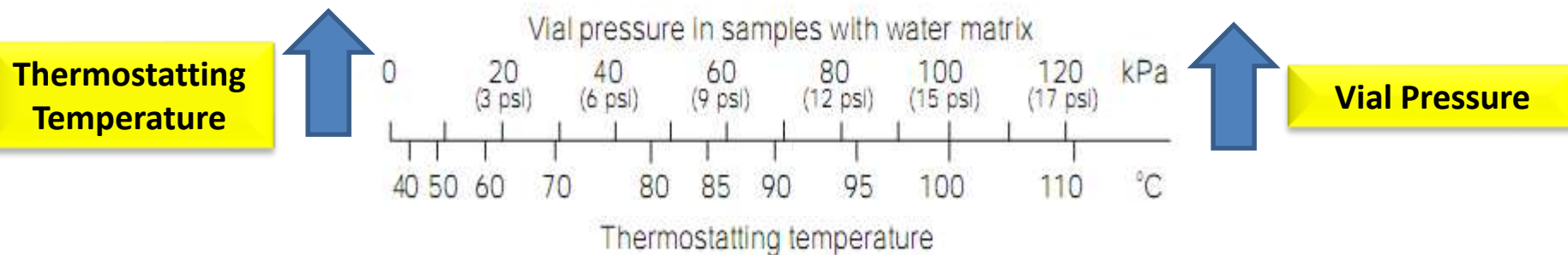


## Column Pressure



The carrier gas is used to pressurize the vials and then carry the sample through the transfer line to the GC injector or column.

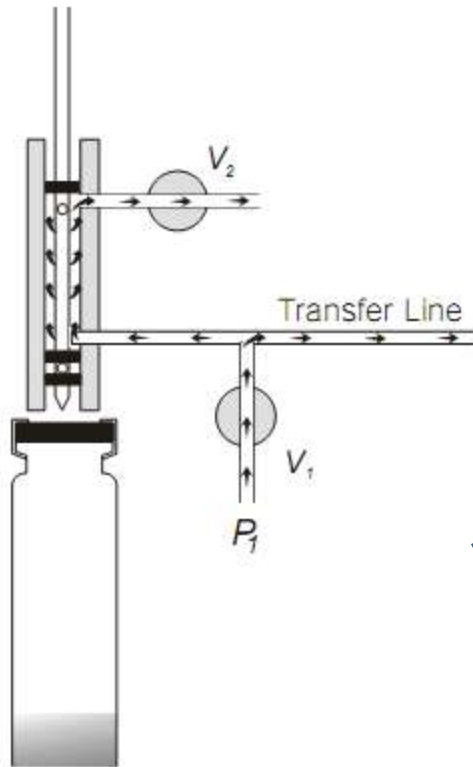
## Relationship Between Vial Pressure and Thermostating Temperature in Samples with a Water Matrix



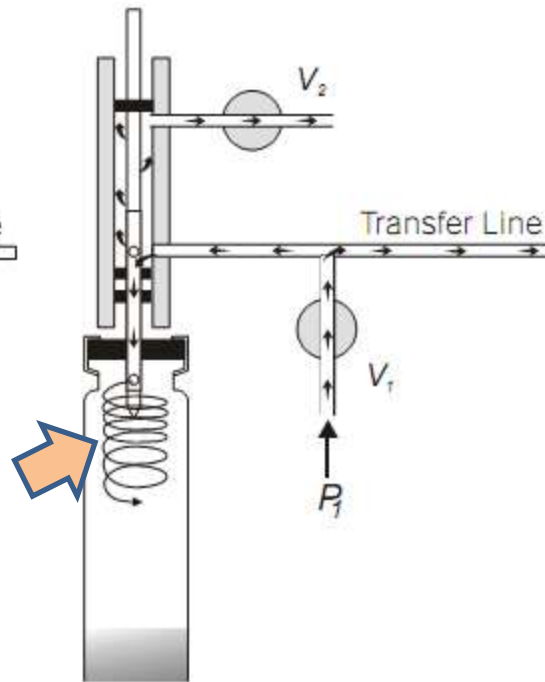
In order to prevent double injections, the column head pressure should be greater than the vial pressure (Carrier Pressure in PPC tab) at the preselected thermostating temperature, by at least a factor of 1.2 + 20 kPa



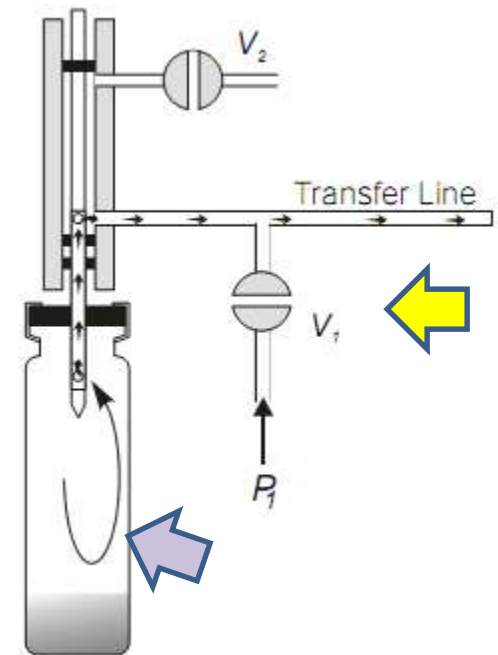
# The HS Sampling Technique



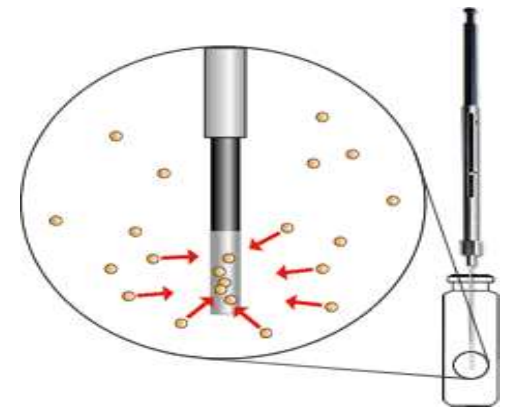
**Thermostatting  
Phase**



**Pressurization  
Phase**



**Injection  
Phase**



# Operating Modes

For Thermostating time, Temperature & Injection

Static HS analysis

**Constant Mode**

All vial have same  
thermostating  
temperature and time.

**Progressive Mode**

All vial have same  
thermostating  
temperature but  
different time.

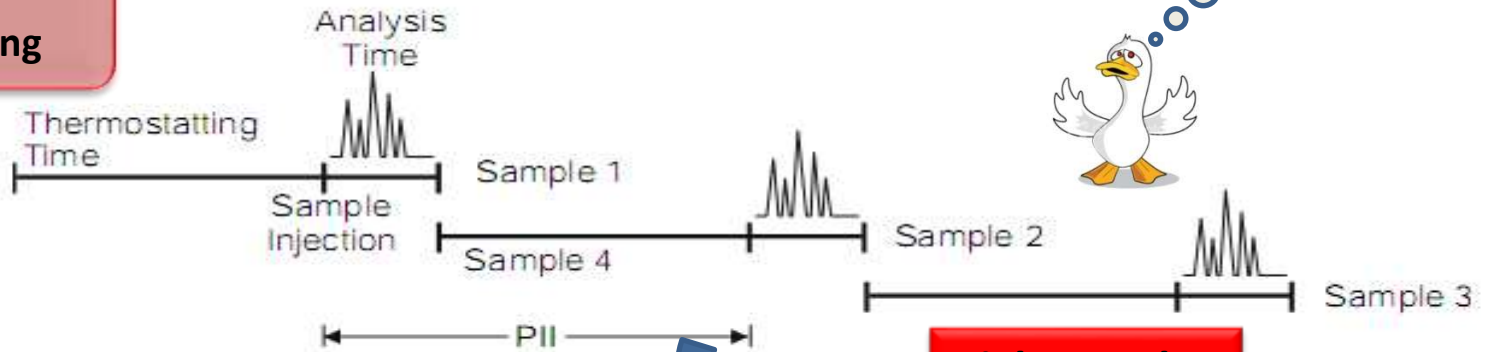
Dynamic HS analysis

**Multiple Headspace  
Extraction Mode**

All vial have same  
thermostating  
temperature and time  
but single vial injected  
more than one time.

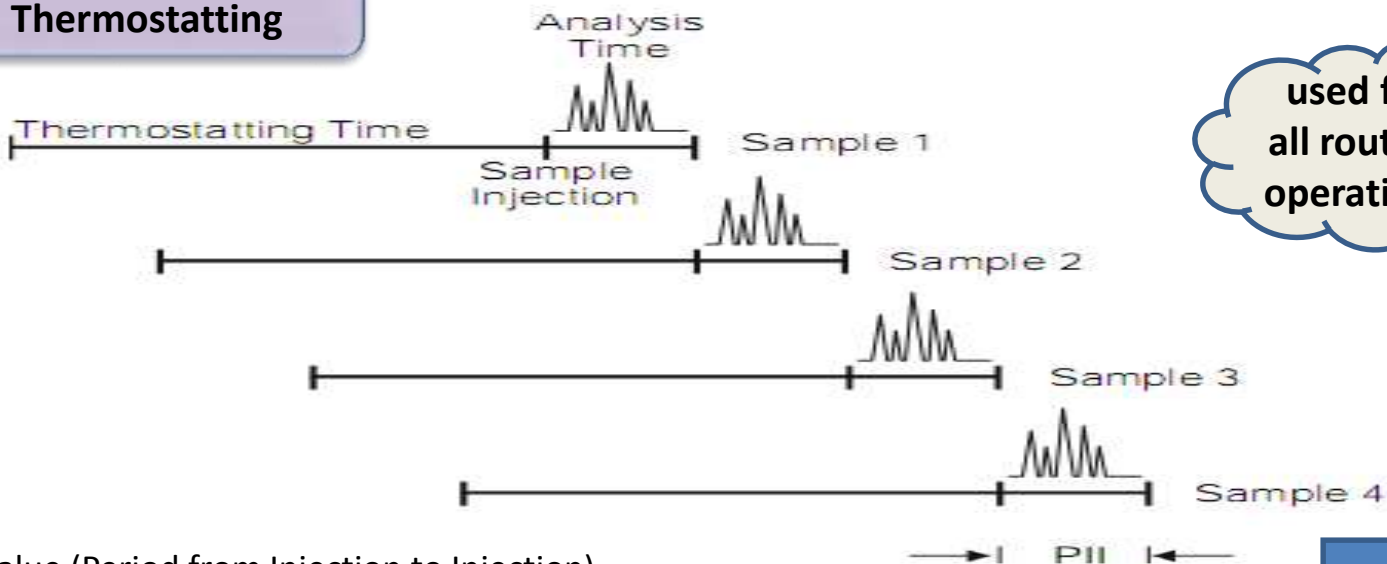
## Constant Mode

### Sequential Thermostating



High PII value

### Overlapping Thermostating



used for  
all routine  
operations

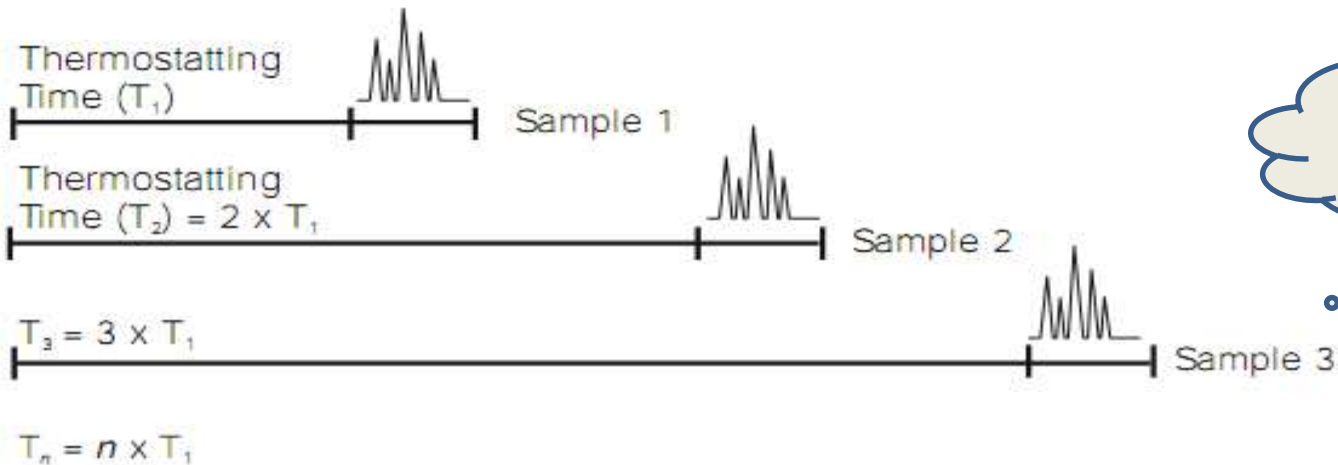
Time  
saving

PII value (Period from Injection to Injection)

Low PII value

## Progressive Mode

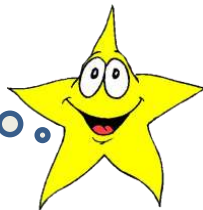
Used for initial method development to determine the thermostating time required for a specific application.



Don't use in routine analysis, time taking.



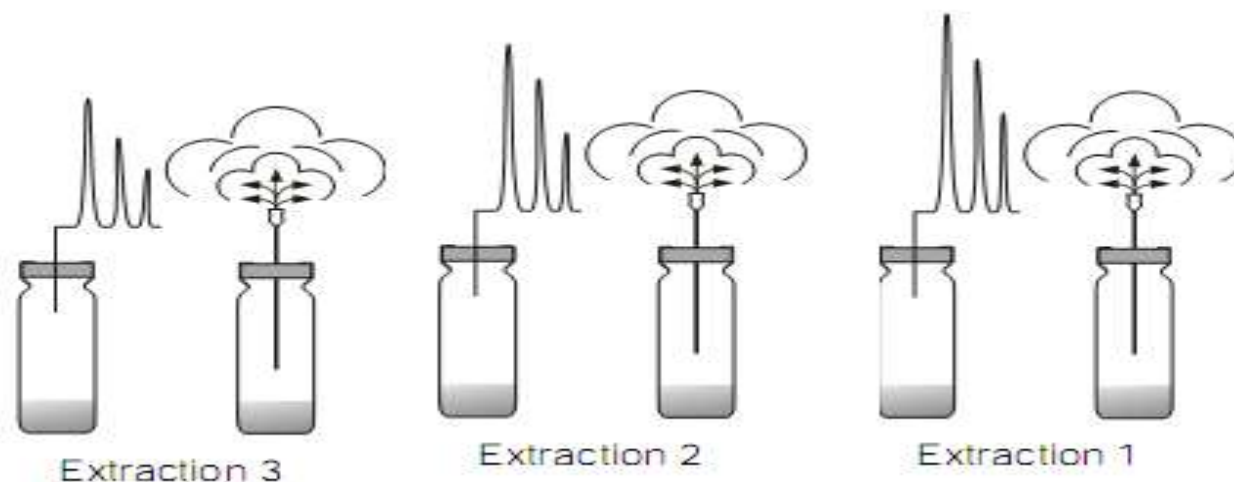
Time saving during method development.



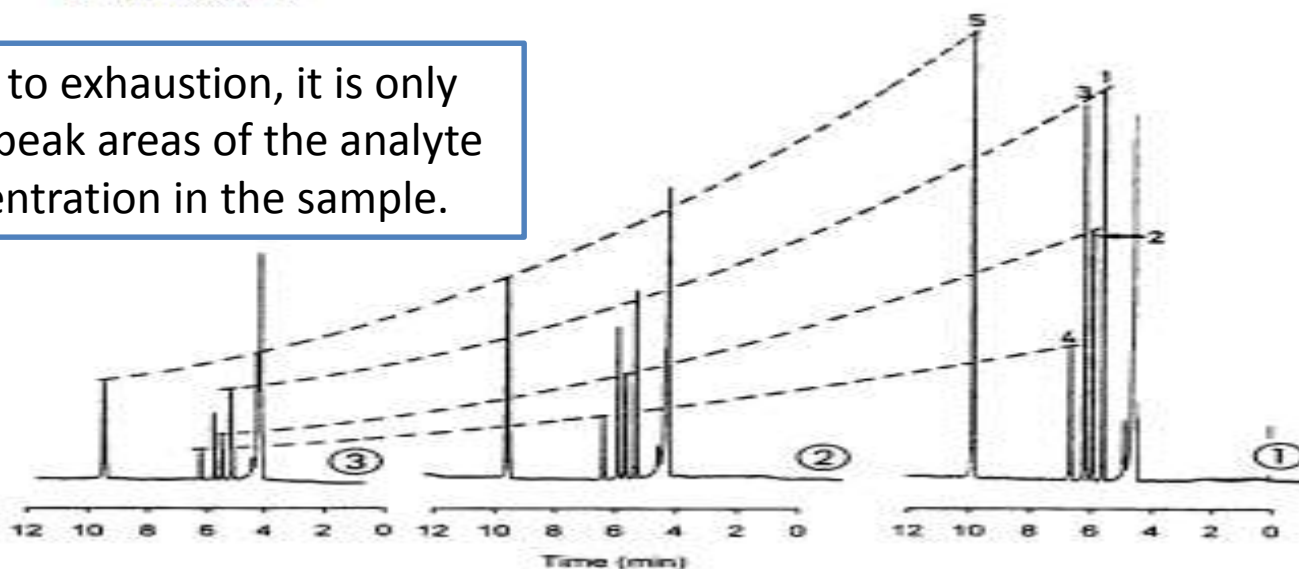
Can also be used for kinetic studies.

## Multiple Headspace Extraction Mode

As each successive extraction and analysis is run, the peak areas of the constituents decrease.

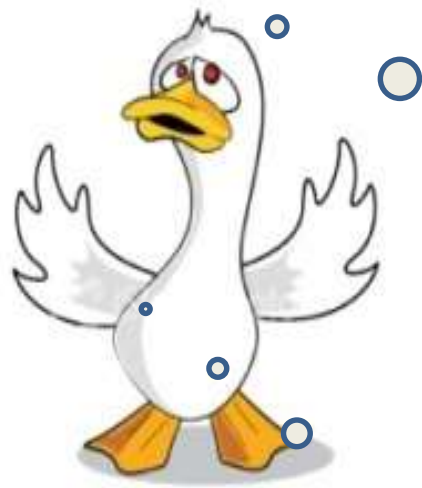


If the extraction is run to exhaustion, it is only necessary to sum the peak areas of the analyte to determine its concentration in the sample.



In practice it is not necessary to run the extraction to exhaustion. After a minimum of two extraction steps, the sum of the peak areas can be calculated from the geometric progression as the decrease in concentration of the compound in subsequent extraction steps follows the **mathematical relationship of a first-order reaction**.

It is especially useful in the analysis of volatiles in insoluble samples. Such samples cannot be quantitatively analyzed directly by headspace gas chromatography, since it is not possible to prepare reference solutions.



This function is used for method development and validation as well as for quantitative analysis of difficult solid samples.

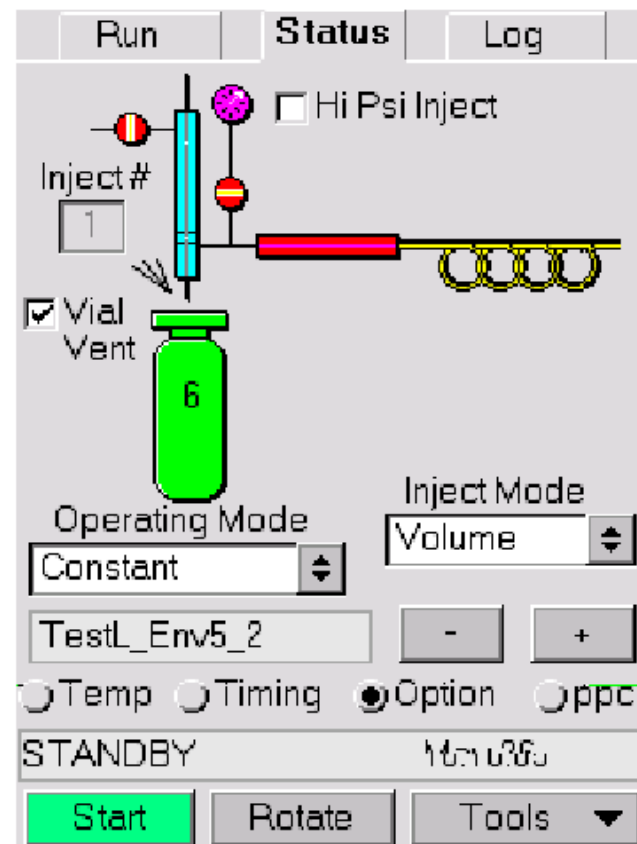
Ex. Ethylene oxide in  
Surgical material (PVC)

## Shaker & Vial Venting

**Shaker** : When applying the shaker, it is important that the sample be in resonance with the shaker frequency to obtain the desired mechanical mixing effect.

In the case of liquid samples, the resonance frequency depends on the sample viscosity and its volume.

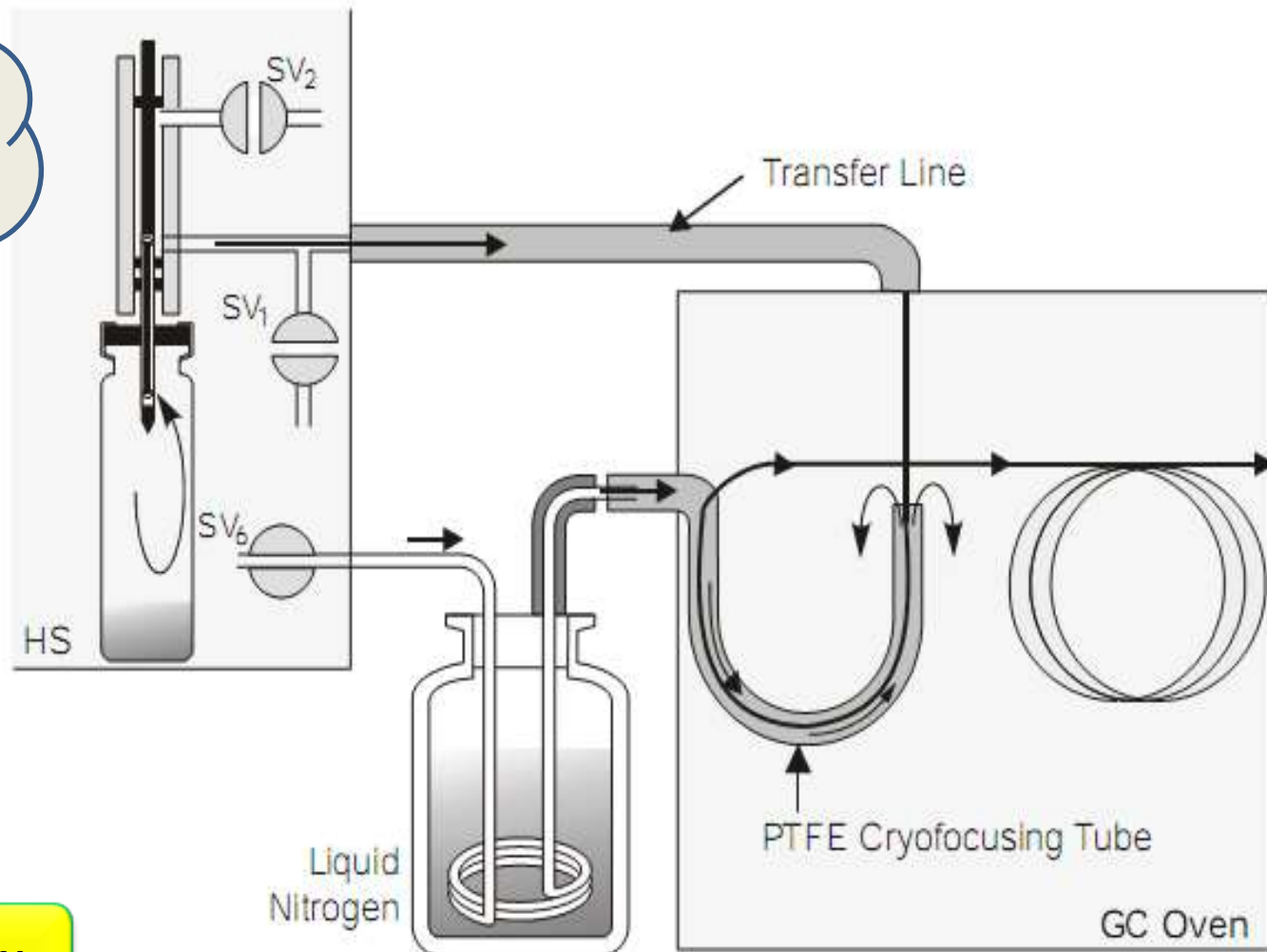
**Vial Venting** : The vial is vented after the withdrawal time. Venting is used to allow for the safe handling of spent vials after analysis.



# Enrichment techniques in HS-GC

## Per concentrated Sampling By Cryofocusing (Cold trapping)

Sample concentration through the application of low temperature.



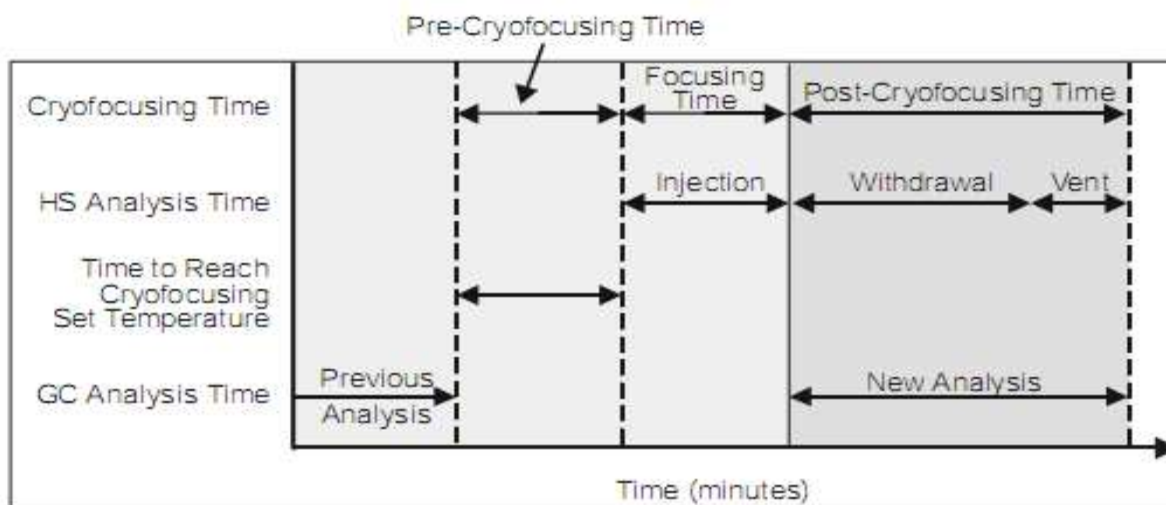
**Cryofocusing Accessory**

The pre-cryofocusing duration allows the head of the GC column to cool to the set cryofocusing temperature.

The post-cryofocusing time maintains the head of the column at the low temp until the sample has been collected and unretained compounds have cleared the column.

The total cryofocusing time comprises the pre- (before sample injection), focusing time (during injection) and post- (after injection) cryofocusing times

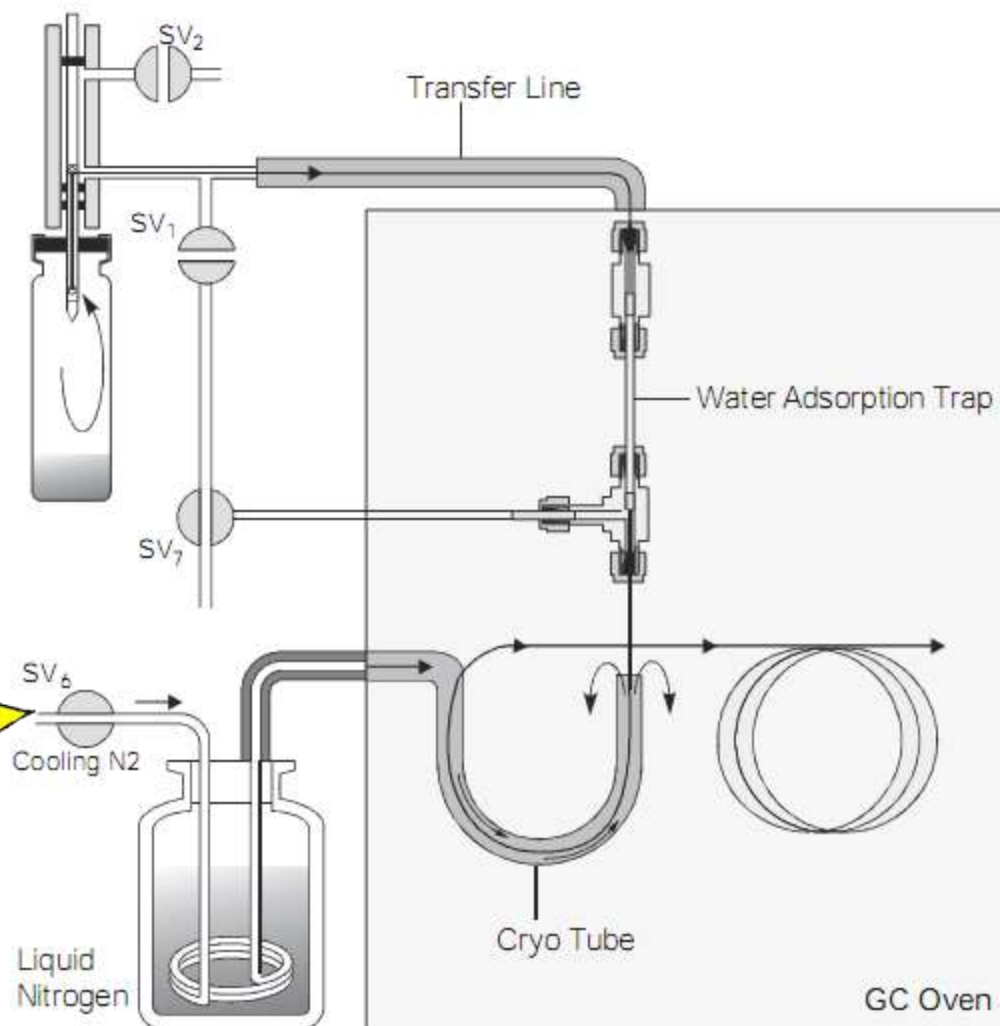
### Setting the Pre and Post Cryofocusing Time



# Cryofocusing with the Water Adsorption Trap

water is removed  
from the sample  
prior to enrichment.

Done by  
adsorption onto  
a hygroscopic  
salt.



# Basic Theory of Headspace analysis

## Phase Ratio ( $\beta$ ) :

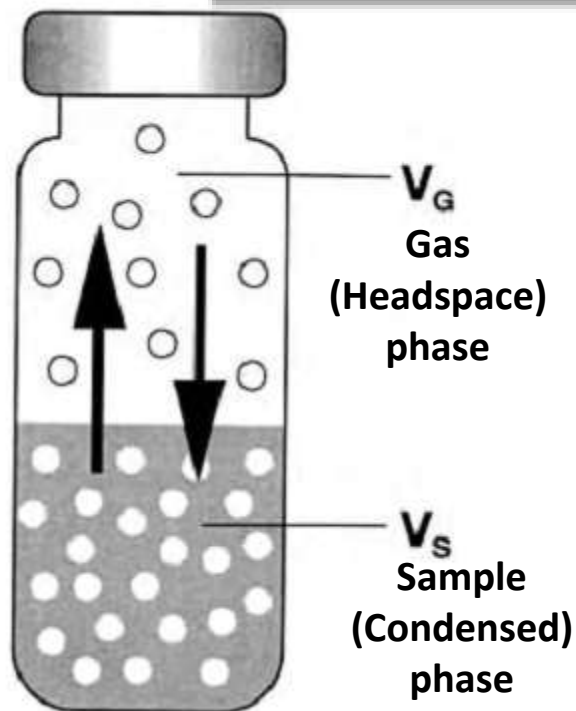
The relative volumes of the two phases in the vial are characterized by the phase ratio  $\beta$ , representing the ratio of the volumes of the two phases present.

$$\beta = V_g/V_s$$

## Partition (distribution) coefficient(K)

The partition coefficient is a fundamental parameter that expresses the mass distribution in the two phase system. It depends on the solubility of the analyte in the condensed phase.

$$K = C_s/C_g$$



$C_g$  = Concentration in gas phase

$C_s$  = Concentration in condensed phase

$(C_s \gg C_g)$ ; The value of K, very high.

Compounds with little solubility,  $C_s$  will be close to  $C_g$  and may even be less than its value : hence, K will be small.

$$C_G = \frac{C_o}{K + \beta}$$

**C<sub>G</sub> = Concentration in gas phase**  
**C<sub>o</sub> = Total Concentration of analyte**

In a given system and under given conditions, both K and β are constants; thus, (K + β) and its reciprocals will also be constants.

$$C_G = (const.) \cdot C_o$$

**The concentration in the headspace α original sample concentration.**

The basic rules of GC that the peak area obtained for a given analyte is proportional to the concentration of the analyte in the analyzed sample.

$$A = (const.) \cdot C_G$$

In our case, an aliquot of the head space is analyzed in which the analyte's concentration is C<sub>G</sub>. Thus, for the obtained peak area A .

$$A = (const.) \cdot C_o$$

# Basic Physicochemical Relationships

Three basic laws : Dalton's law, Raoult's law, and Henry's law.

**Dalton's law**, the total pressure of a gas mixture is equal to the sum of the partial pressures  $p_i$  of the gases present in the mixture.

$$p_{total} = \sum p_i$$

The fraction of the pressure exerted by a gas is equal to the fraction of its total number of moles present in the gas mixture.

$$\frac{p_i}{p_{total}} = \frac{n_i}{n_{total}} = x_{G(i)}$$
$$p_i = p_{total} \cdot x_{G(i)}$$

Where,  $n$  represents the number of moles present and  $x_{G(i)}$  is the mole fraction of the Particular component in the gas mixture.

$$x_{G(i)} = (const.) \cdot C_{G(i)}$$

$$p_i \propto C_{G(i)}$$

The concentration of the analyte in the headspace is proportional to its partial pressure.

**Raoult's law** states that the vapor pressure of a dissolved solute (i.e., its partial pressure,  $p_i$ ) over its solution is directly proportional to its mole fraction in the solution  $x_{S(i)}$  and the proportionality constant is the vapor pressure  $p_i^o$  of the pure analyte.

$$p_i = p_i^o \cdot x_{S(i)}$$

Raoult's law is valid only for ideal mixtures, and in most cases there is a deviation from Raoult's law. To compensate for this deviation, another factor is introduced.

$$p_i = p_i^o \cdot \gamma_i \cdot x_{S(i)}$$

This parameter is called the activity coefficient ( $\gamma_i$ ) of compound  $i$ . The activity coefficient can be considered a correction factor to the concentration (molality) which modifies it to the true "active concentration".

The activity coefficient depends on the nature of component  $i$  and reflects the intermolecular interaction between the analyte and other sample components, particularly the matrix (solvent). Thus, the so-called matrix effect.

For higher concentrations of the analyte, the activity coefficient also becomes a function of the concentration,

**while in dilute solutions it is constant and independent of the analyte's concentration. In such a so-called ideal dilute solution there is a general linearity between the partial vapor pressure and the mole concentration of the analyte, which is expressed by**

**Henry's law**

$$p_i = H \cdot x_i$$

where H is Henry's law constant. In an ideal solution ( $\gamma_1=1$ ),  $H = p_i^o$ . Henry's law is the basis of GC and of head space analysis.

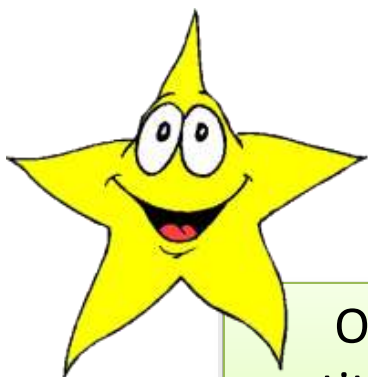
$$p_i = p_{total} \cdot x_{G(i)} = p_i^o \cdot \gamma_i \cdot x_{S(i)}$$

$$\frac{x_{S(i)}}{x_{G(i)}} = \frac{p_{total}}{p_i^o \gamma_i}$$

$$\frac{p_{total}}{p_i^o \cdot \gamma_i} = \frac{x_{S(i)}}{x_{G(i)}} = \frac{C_{S(i)}}{C_{G(i)}} = K$$



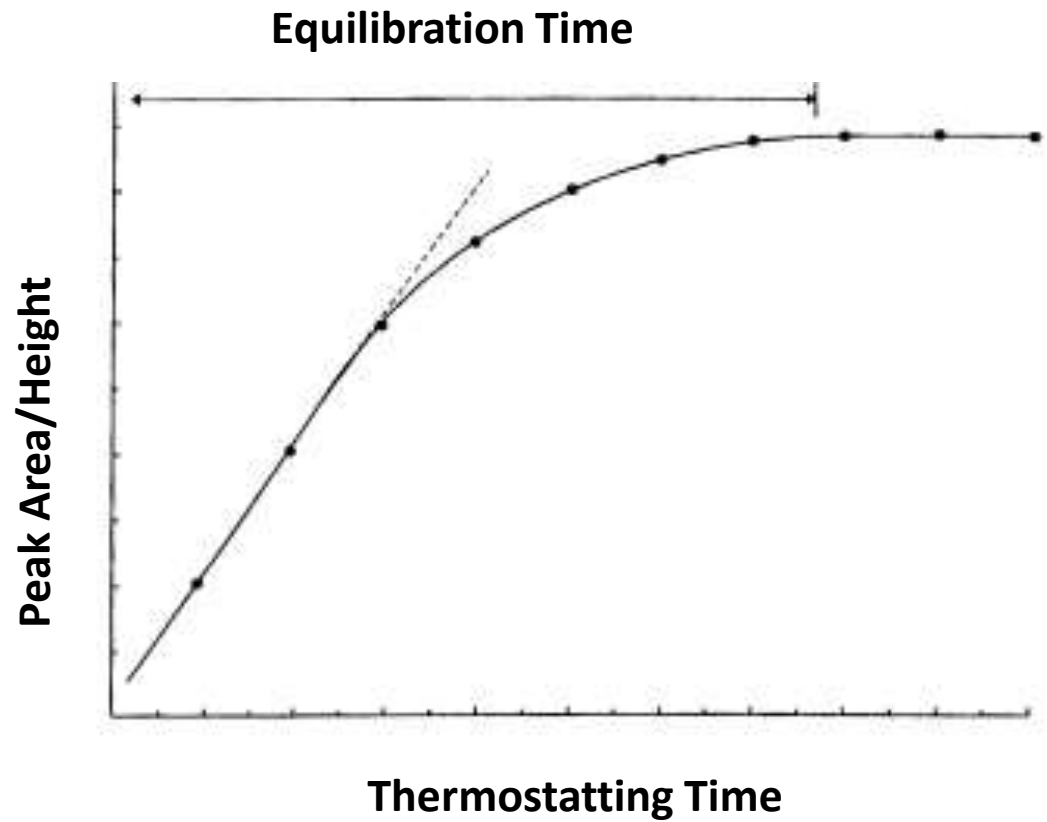
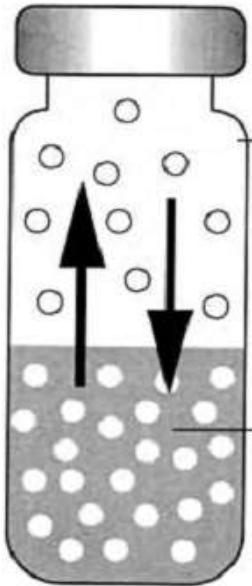
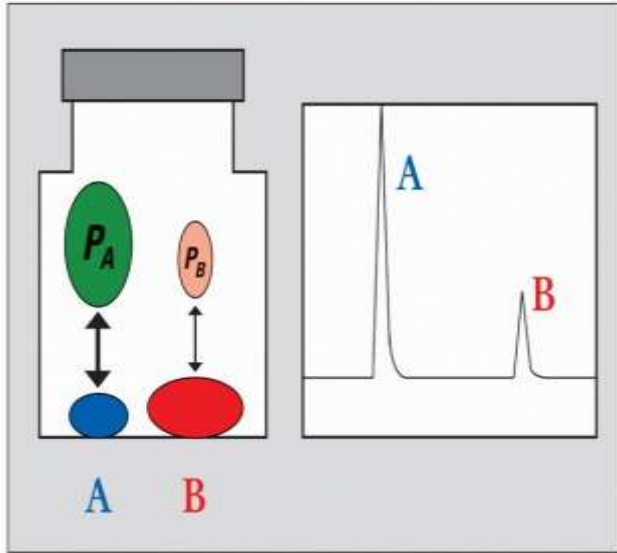
The partition coefficient is proportional to the reciprocal of the vapor pressure and the activity coefficient of the analyte : increasing these values will decrease the value of the partition coefficient.



$$K \propto \frac{1}{p_i^o \cdot \gamma_i}$$

On the other hand, according to a decrease in the partition coefficient will increase the concentration of the analyte in the headspace at equilibrium, and this results in an increase of headspace sensitivity.

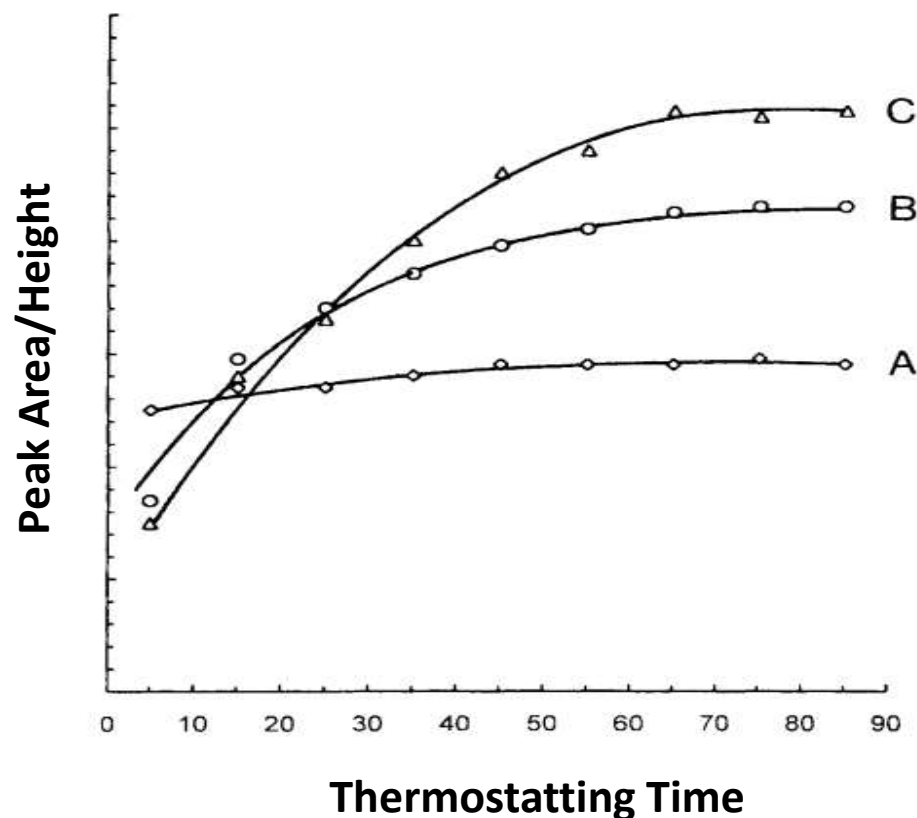
# Equilibration Time



Thermostating time Vs Peak area (Height)

# Effect of Vial volume on Thermostatting time and Peak area /Height

For aqueous solution of toluene, Increase in vial volume, increase response and thermostatting time.

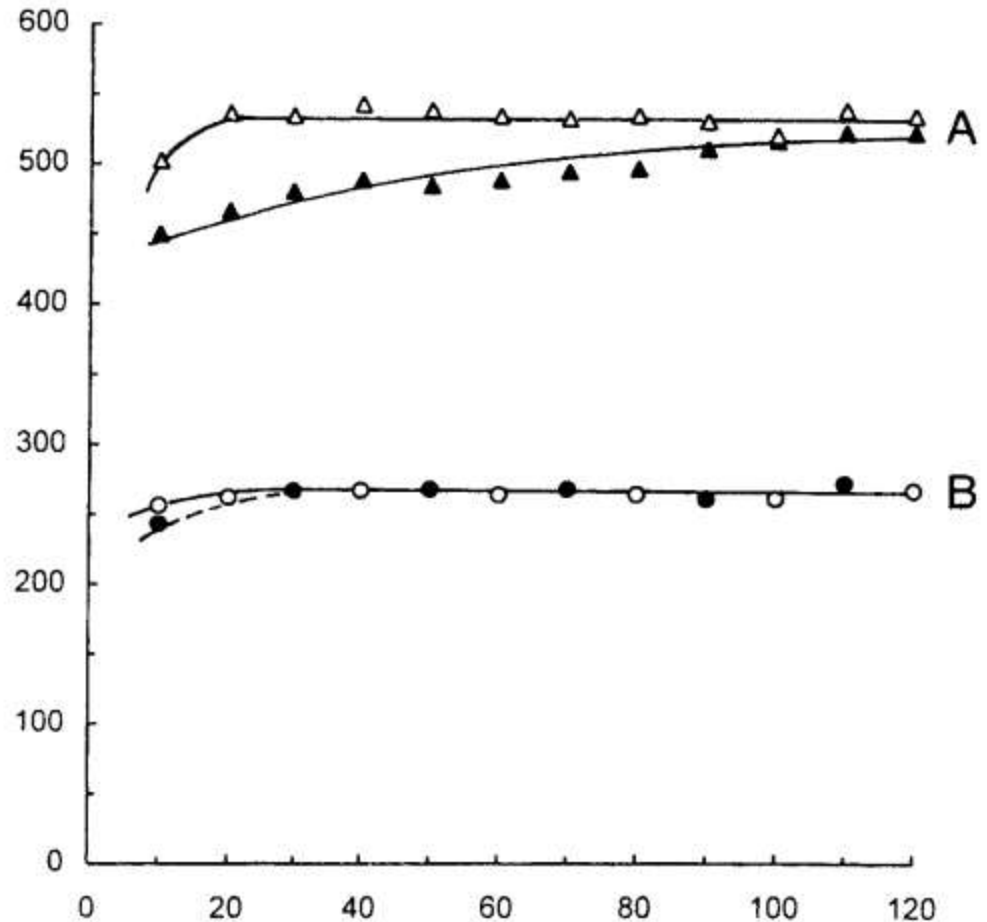


Equilibration time of aqueous solutions of **toluene** (2ppm) with varying volumes.  
Thermostatting temperature :60°C. Headspace vial volume :22.3 mL.  
Sample volume : A = 2.0mL, B = 5.0mL, C = 10.0mL

# Reduction of thermostating Time

1. By increasing Temperature :
2. By Shaking

An increase in sample temperature from 60°C to 90°C reduces the equilibration time by only about 8%.



Equilibration times of **aqueous solutions of toluene (A) and acetone (B)** without (solid symbols) and with (open symbols) **shaking**. Thermostating temperature 60°C. Sample volume : 5.0 mL of A (8 ppm solution of toluene) and B (150 ppm acetone solution).

# Headspace Sensitivity

In headspace analysis we want to obtain a reasonable peak for reliable quantization when analyzing a given sample.

**Peak area  $\propto$  Aliquot of the headspace sample  $\propto$  The concentration of the analyte.**

$$A \propto C_G = \frac{C_o}{K + \beta}$$

Head space sensitivity (the obtained peak area) depends on the combined effect of K and  $\beta$ .

**Partition coefficient (K)**  
**Phase ratio  $\beta$  (Sample volume)**

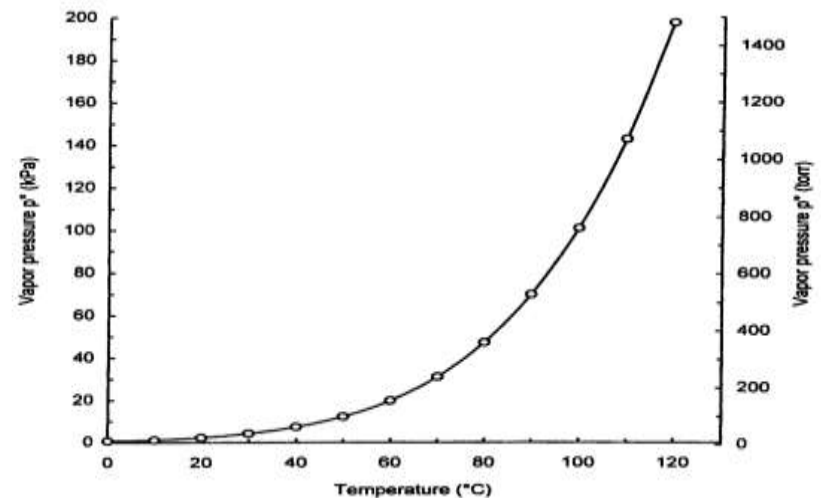
**There are two parameters we can easily change : the sample volume in a headspace vial and its temperature.**

Additional possibilities for changing the whole system include modification of the chemical properties of the sample matrix (e.g., by adding a salt to an aqueous sample) and of the analyte (by preparing more volatile derivatives).

# Influence of Temperature on Vapor Pressure and Partition Coefficient

$$\log p_i^o = -\frac{B}{T} + C$$

An increase of 20 C in the thermostating temperature can more than double the vapor pressure.

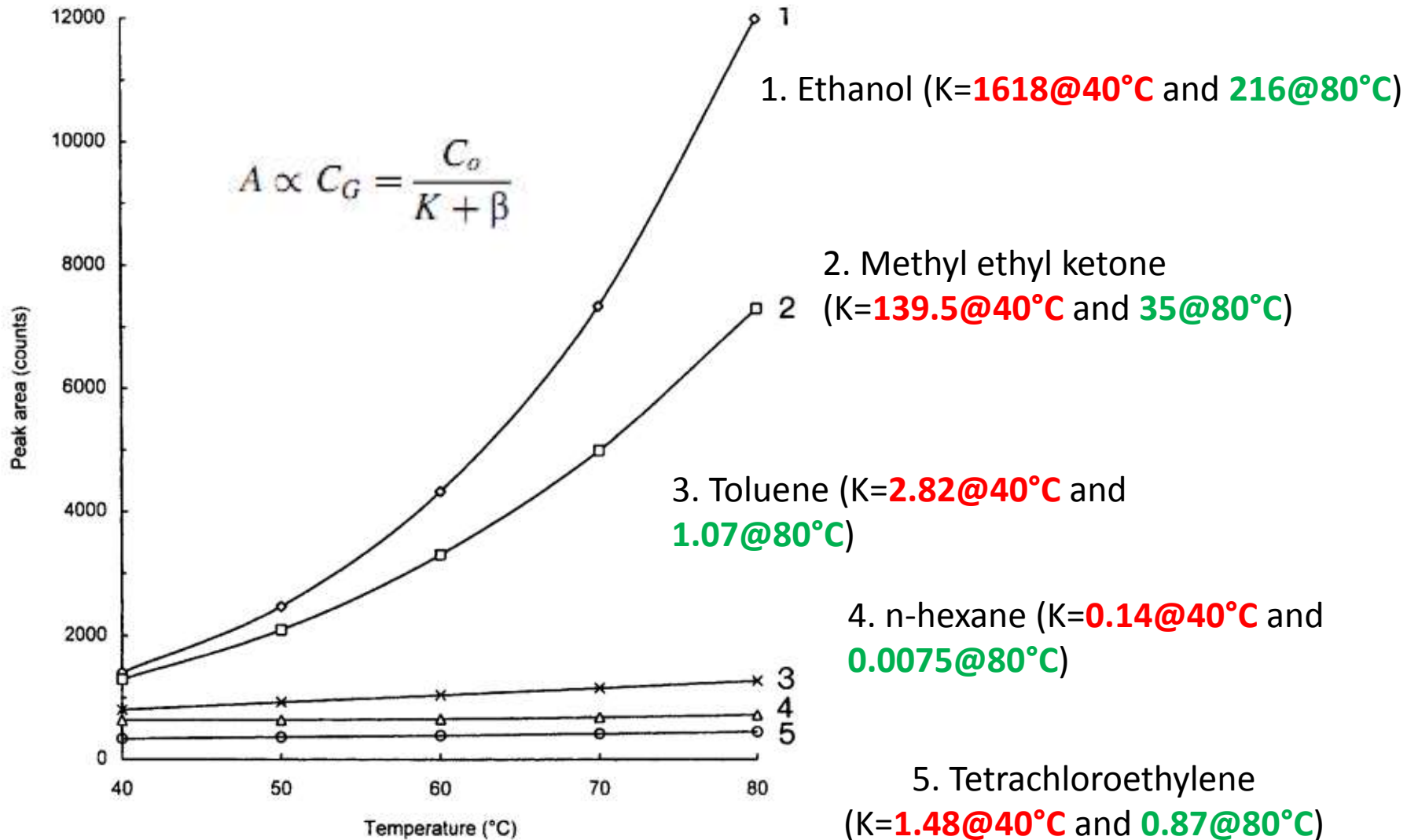


Dependence of the saturation vapor pressure  $p^\circ$  of water on temperature

| °C  | $p^\circ$ (kPa) | $p^\circ$ (torr) [3a] | $d$ (µg/mL) [3b] |
|-----|-----------------|-----------------------|------------------|
| 10  | 1.2             | 9.2                   | 9.4              |
| 20  | 2.3             | 17.5                  | 17.3             |
| 30  | 4.2             | 31.8                  | 30.3             |
| 40  | 7.4             | 55.3                  | 51.1             |
| 50  | 12.3            | 92.5                  | 83.2             |
| 60  | 19.9            | 149.4                 | 130.5            |
| 70  | 31.1            | 233.7                 | 198.4            |
| 80  | 47.2            | 355.1                 | 293.8            |
| 90  | 69.9            | 525.8                 | 424.1            |
| 100 | 101.1           | 760.0                 | 598.0            |
| 110 | 142.9           | 1074.5                | 826.5            |
| 120 | 198.1           | 1489.1                | 1122.0           |

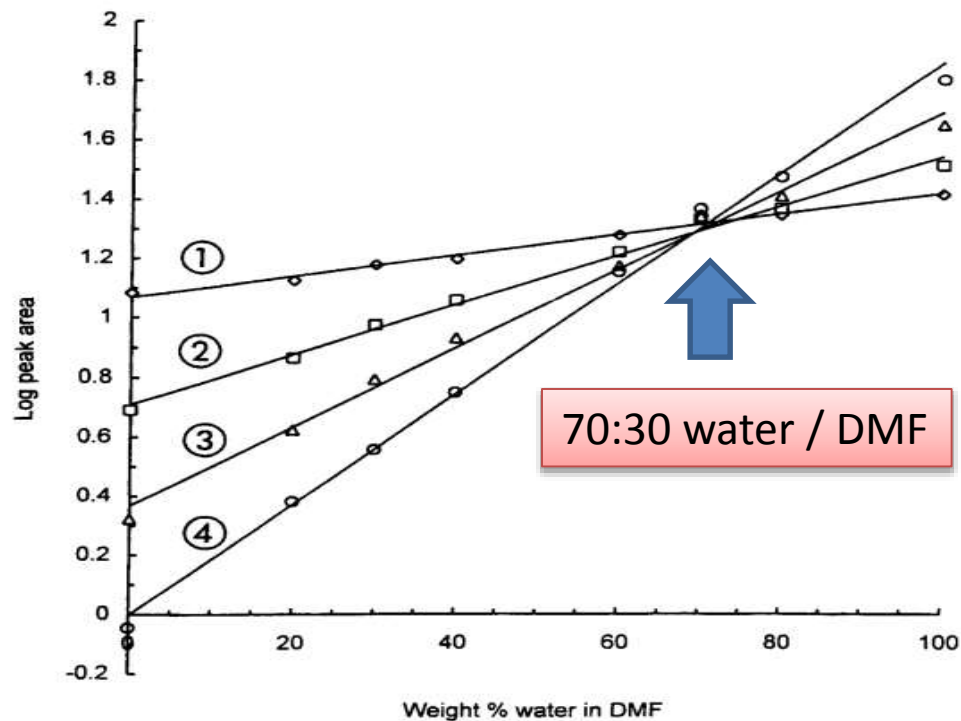
Vapor pressure ( $p^\circ$ ) and density ( $d$ ) data for saturated water vapor as a Function of temperature.

# Influence of temperature on headspace sensitivity (peak area values, counts) as a Function of the partition coefficient K from an aqueous solution with $\beta = 3:46$ .



## Diluents effect

| Solvent Composition(%) |       | Peak Area (in Arbitrary Units) |                    |                   |                    |
|------------------------|-------|--------------------------------|--------------------|-------------------|--------------------|
| DMF                    | Water | Ethanol                        | <i>n</i> -Propanol | <i>n</i> -Butanol | <i>n</i> -Pentanol |
| 100                    | —     | 12.1                           | 4.9                | 2.1               | 0.9                |
| 80                     | 20    | 13.3                           | 7.3                | 4.2               | 2.4                |
| 60                     | 40    | 15.7                           | 11.4               | 8.5               | 5.6                |
| 40                     | 60    | 18.9                           | 16.6               | 14.9              | 14.2               |
| 20                     | 80    | 22.1                           | 23.3               | 25.7              | 29.7               |
| —                      | 100   | 26.0                           | 32.5               | 44.5              | 63.2               |



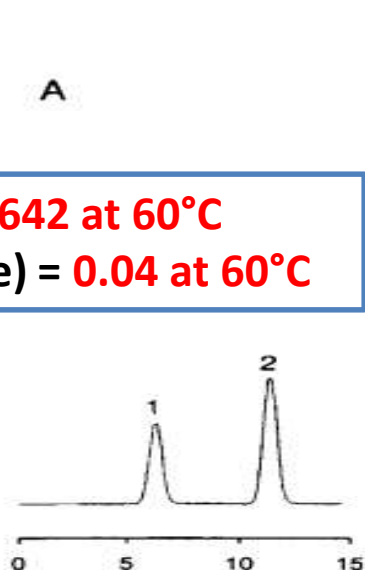
**Headspace Sensitivity for C2–C5 Alcohols as a Function of the Water Content of the Solvent (120 ppm of each analyte in the solution.)**

## Vial Volume and Salt addition

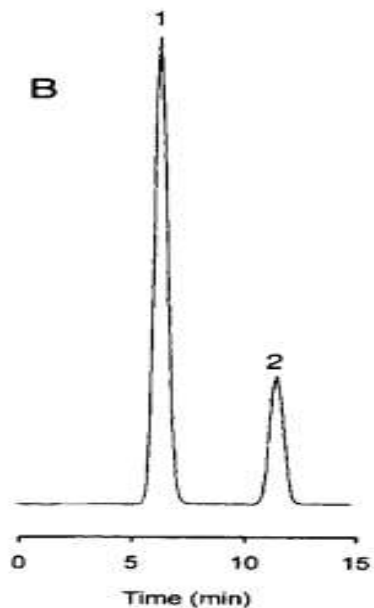
**Peaks:**

**1 = Cyclohexane,  
2 = 1,4-dioxane.**

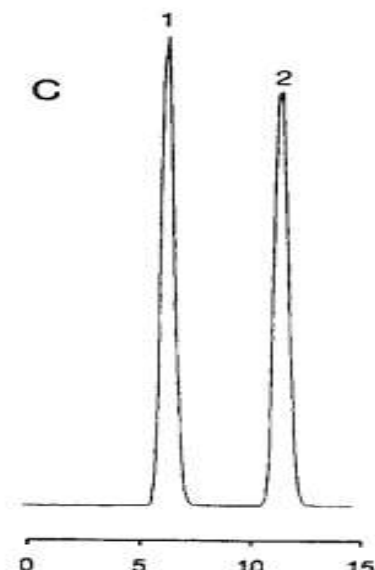
|   |                            | Peak Area (Counts) |         |
|---|----------------------------|--------------------|---------|
|   |                            | Cyclohexane        | Dioxane |
| A | 1.0 mL solution            | 42,882             | 71,848  |
| B | 5.0 mL solution            | 237,137            | 72,800  |
| C | 5.0 mL solution + 2 g NaCl | 240,287            | 234,312 |



(A) 1.0 mL solution  
( $\beta = 21.3$ )



(B) 5.0 mL solution  
( $\beta = 3.46$ )



(C) 5.0 mL solution + 2 g of NaCl  
( $\beta = 3.46$ )

**K (Dioxane) = 642 at 60°C**  
**K (Cyclohexane) = 0.04 at 60°C**

**Analysis of three samples of an aqueous solution of cyclohexane (0.002vol-%) and 1,4 dioxane (0.1vol-%) in a 22.3 mL vial.**

## Headspace Linearity

### **The theoretical Headspace Linearity**

Partition coefficient and the activity coefficient are constant and independent of analyte concentration.

The actual linear range depends on the analyte's solubility (i.e., its partition coefficient) and its activity coefficient.

Generally, it covers concentrations below 0.1–1%, which is usually the range analyzed by HS-GC.

The linear range of HS-GC for a given analyte can not be predicted.

It must be determined by actual measurements.

Carried out automatically by the MHE technique.

In some cases, the linear range extends to higher concentrations. A typical case is the analysis of aqueous ethanol solutions, where the line range extends upto 25–30%. This observation permits the practical conclusion that the direct determination of the ethanol content of blood or wines is possible by HS-GC. On the other hand, in the case of hard liquor (brandies, gin, vodka, whiskies), the sample must first be diluted.

## When to Use It?

1. When performing quantitative analysis of volatiles.
2. When entire sample should not be injected into GC.
3. When minimum sample handling is desirable.
4. When high sample throughput is desirable.
5. For trace levels & low to medium concentrations.

## Main Applications

1. Organic Volatile Impurities (OVI's) in Pharmaceutical Preparations.
2. Residual Monomers and Solvents In Polymers
3. Volatiles (e.g. Residual Printing Ink Solvents) In Packaging Materials
4. Volatiles In Biological Fluids (e.g. Blood Alcohol)
5. Food & Beverage Quality control
6. Volatiles in Water, Soil, and Waste

# Limitations

1. Very limited use of low boiling solvents (like Methanol, DCM, Acetone etc.)
2. Response of high boiling point analyte are low.
3. Run time long.
4. One sample can not be injected more than one time.
5. Limited higher temperature can be applied for thermostating.
6. Prolong heating leads to sample decomposition.
7. Method development is critical and time taking.
8. Commonly used organic solvent (like DMSO,DMF, NMP and DMA)are carcinogenic and extremely hazardous.
9. Sample preparation takes proper care.
- 10.Contamination are higher during sample preparation and analysis.
- 11.Needs high purity gasses.
- 12.Costly technique.

**THANKS**