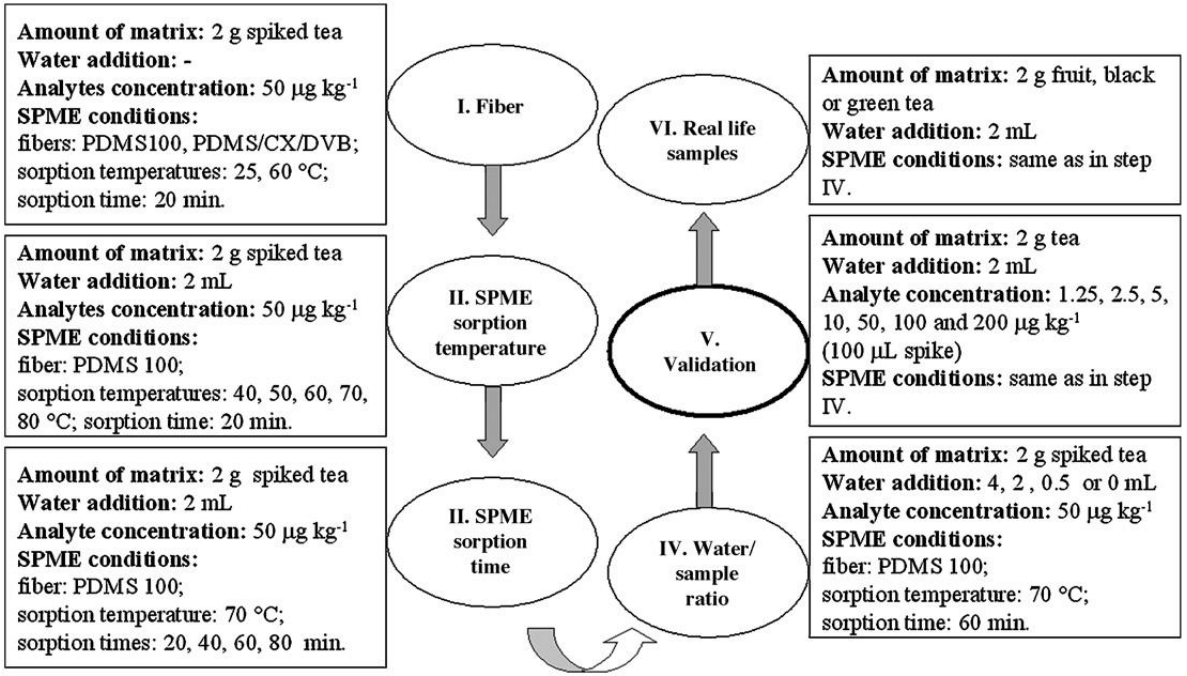
Gas Chromatography

Let's begin with an example problem: SPME head space analysis of pesticides in tea and follow-up analysis by high speed GC.



Samples in 10mL sealed glass vials were placed in the MPS-2 autosampler for HS-SPME extraction. Extracted compounds were thermally desorbed from the fiber after its insertion into the hot (270 ◦C) splitless GC injection port. Desorption time was 2min.

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Gas Chromatography

Conventional **HS-SPME**

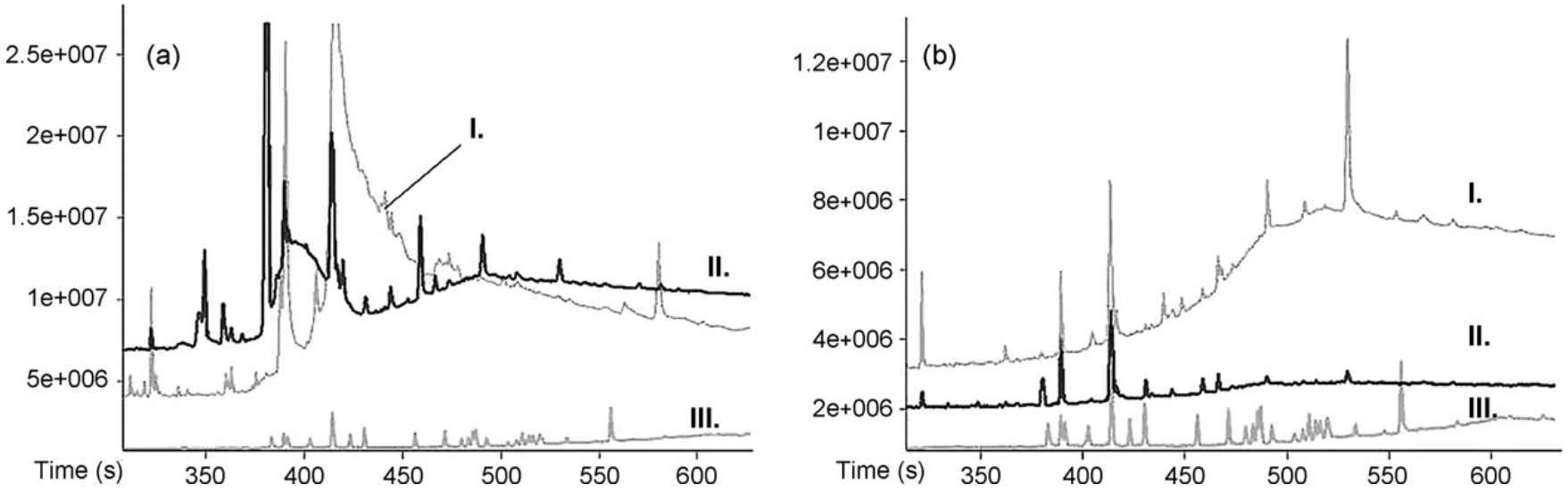
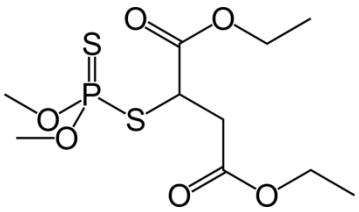
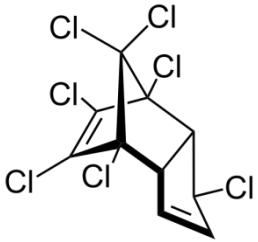


Fig. 3 – Comparison of conventional (a) and SPME-based (b) approach in analysis of pesticide residues in tea samples. GC/TOF MS chromatograms of blank (non-spiked) black (I) and fruit (II) teas; pesticide standard solution (200g kg^{-1}) (III).



Malathion



Heptachlor

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Gas Chromatography

Detection Figures of Merit (36 pesticides tested)

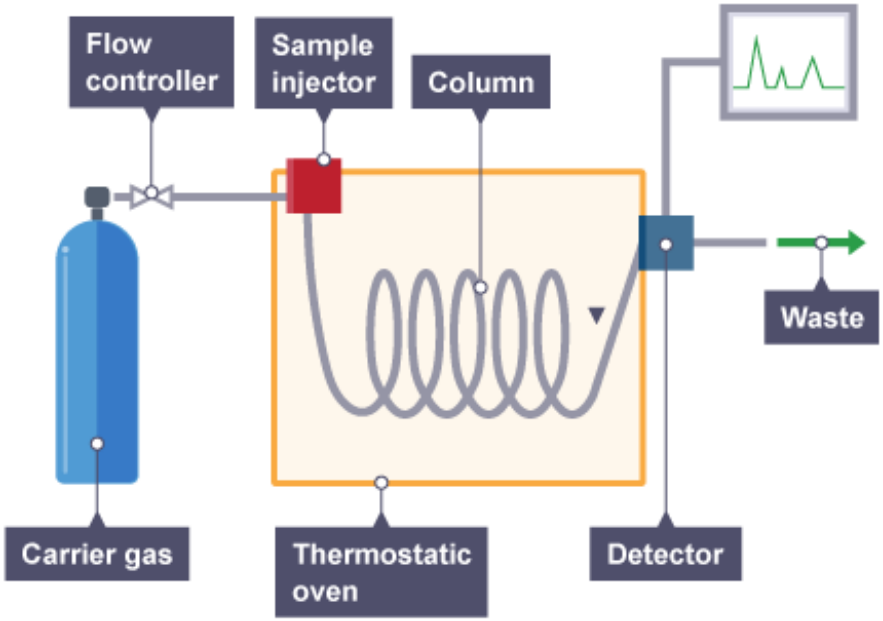
Pesticide	MRL (µg/kg)	Linearity (r^2)	Dynamic Range (µg/kg)	LOQ (µg/kg) S/N = 12	RSD (%) 50 µg/kg N=5
HS-SPME					
Malathion	500	0.9838	50-200	26	13
Heptaclor	20	0.9870	10-200	8	9
Ethyl acetate extraction	*Very time consuming !*				
Malathion	500	0.9977	10-200	8	11
Heptaclor	20	0.9983	1.25-200	1	5

MRL = maxiumum residue limit

Gas Chromatography - Instrument

A gas chromatograph (GC) is an analytical instrument that measures the content of various volatile components in a sample. The analysis performed by a gas chromatograph is called gas chromatography.

Principle of gas chromatography: The sample solution injected into the instrument enters a gas stream which transports the sample into a separation tube known as the "column." (Helium or nitrogen is used as the so-called carrier gas.) The various components are separated inside the column. The detector measures the quantity of the components that exit the column.



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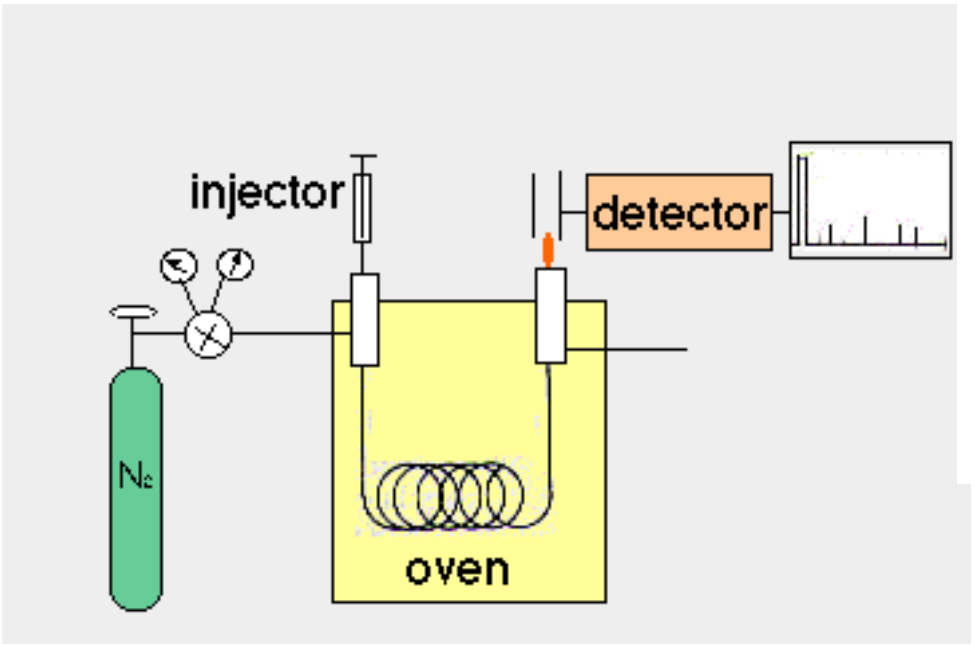
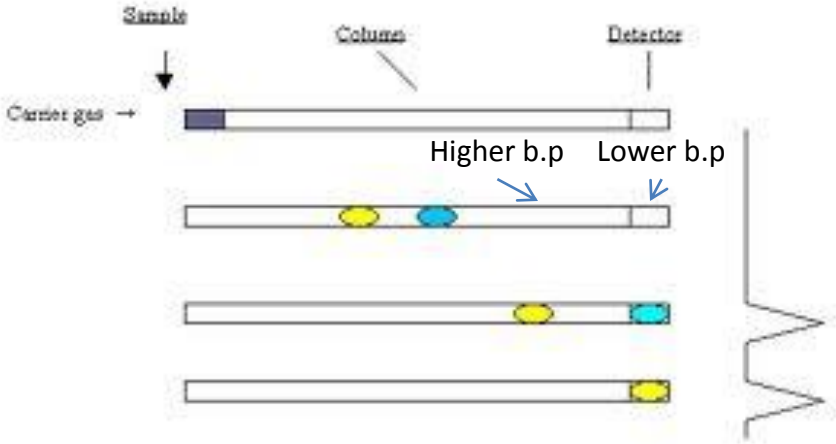
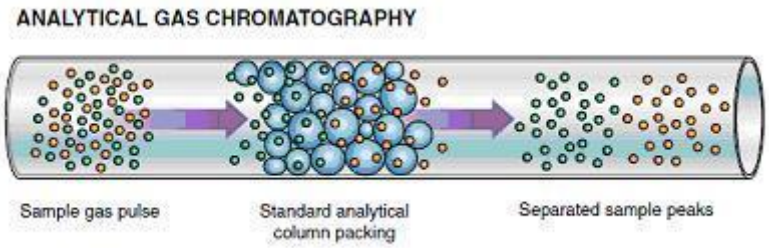
Gas Chromatography

Column separation (gas-liquid, gas-solid) used for separating and analyzing compounds that can be vaporized without decomposition.







Separations generally based on differences in boiling points!

Conditions

- Carrier gas (mobile phase) does NOTHING in GC but transport the compounds. Not involved in separation mechanism (H_2 and He common).
- Injection volume (0.1 – 10 µL generally). Temperature of injector is 50 ^oC greater than least volatile (highest boiling point compound). All compounds must be vaporized before transport onto column.
- Fixed temperature separation – average boiling point of all analytes is a good starting point.
- Carrier gas is often dried by passage over molecular sieves as they strongly retain water. Activated by heating to 300 ^oC in vacuum.
- Gaseous mobile phase carries gaseous compounds (analytes) through a long column with a stationary phase.

Thermodynamics of Separations

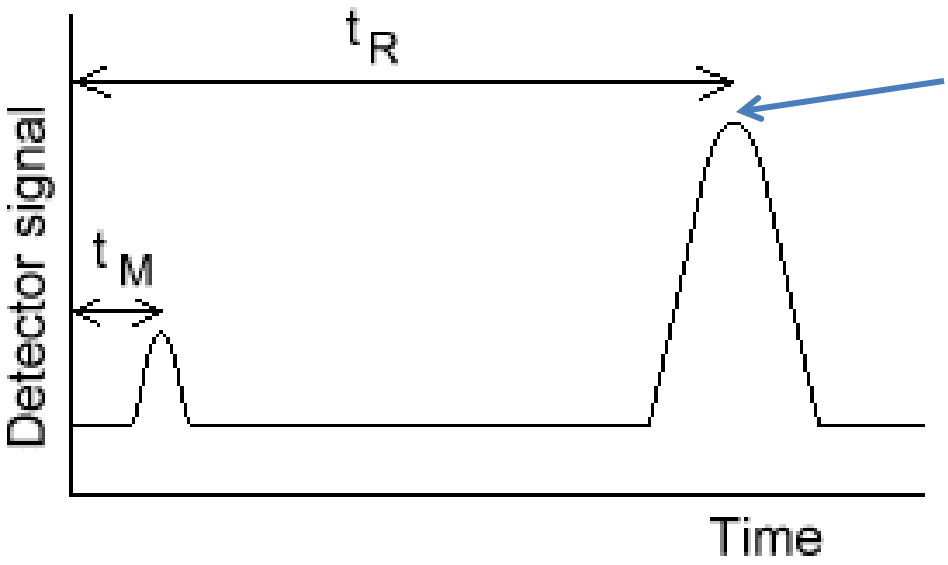
The application of **thermodynamics** to a chromatographic separations explains how the distribution coefficient (which itself determines the magnitude of retention) is controlled by the **standard energy of distribution** and the **absolute temperature**.

$$K_D = \frac{C_{Stat}}{C_{Mobile}}$$

$$A_{mobile} \rightleftharpoons A_{Stationary}$$

$$-\frac{\Delta G_D}{RT} = \ln K_D$$

The Chromatogram



Average velocity of moving analyte zone

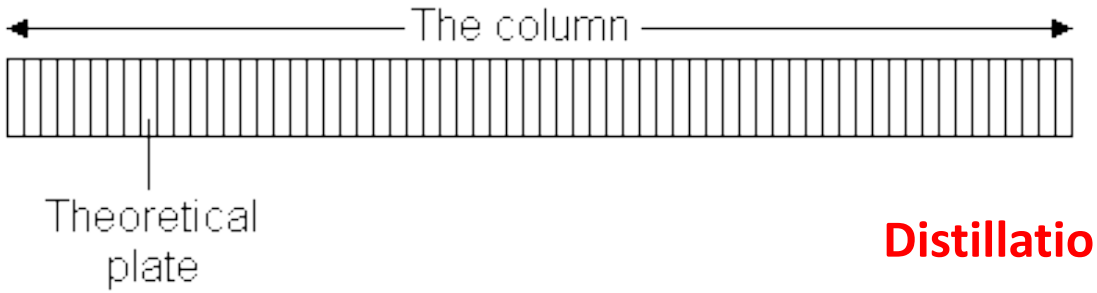
$$k' = \frac{t_r - t_m}{t_m}$$

Capacity Factor (2-10)

$$\alpha = \frac{k_b(last)}{k_a(first)}$$

Selectivity Factor (>1)

Plate Height – Equilibration Zones



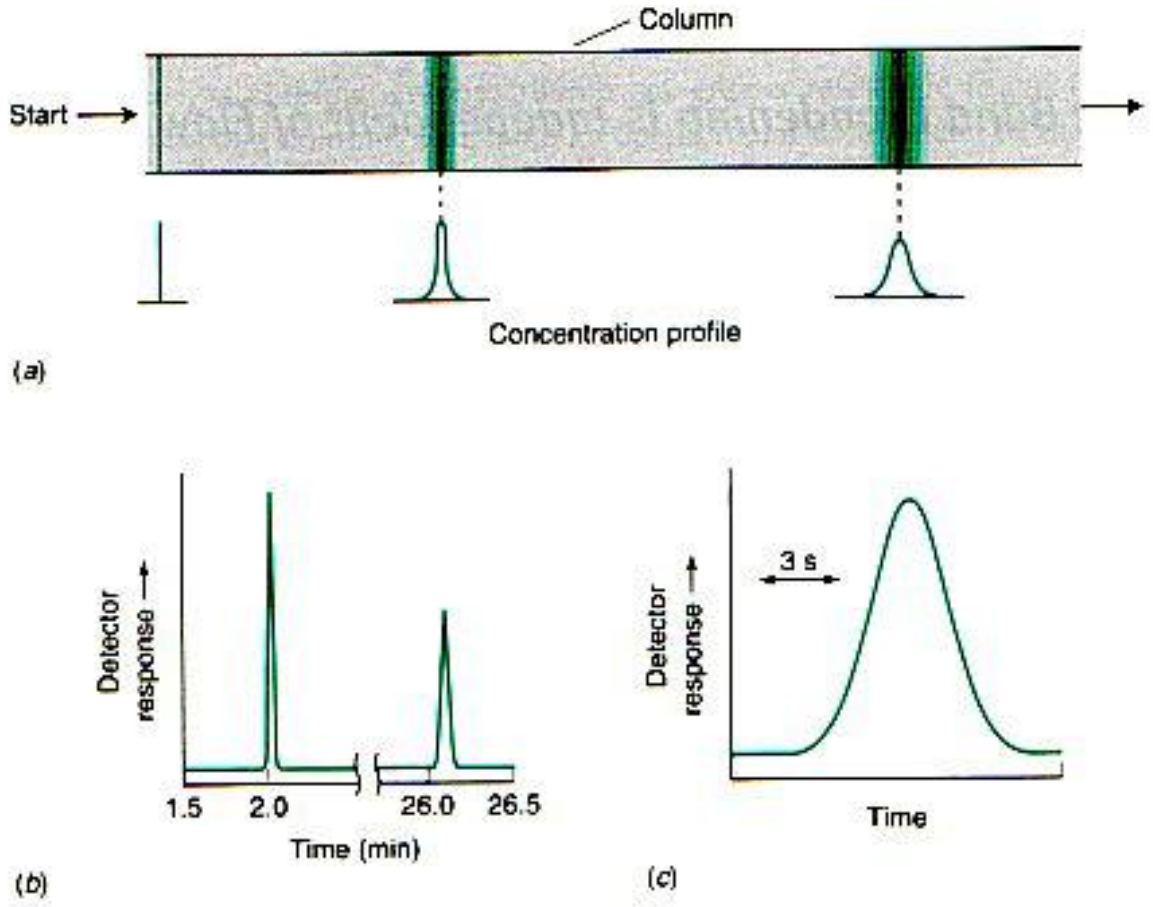
Distillation theory

Height equivalent to a theoretical plate (H)

$$N(\,platenumber) = \frac{L(cm)}{H(cm\,/\,plate)}$$

$$N = 16\left(\frac{t_r}{W}\right)^2 \qquad N = 5.55\left(\frac{t_r^2}{W_{1/2}^2}\right)$$

Why Do Bands Separate and Broaden with Time on Column?



Longer on the column, longer the retention time (t_r) and the broader the peaks.

Why Does Peak Width Matter?

Although the selectivity factor, α, describes the separation of band centers, it does not take into account peak widths. Another measure of how well species have been separated is provided by measurement of the *resolution* (R). The resolution of two species, A and B, is defined as:

$$R_s = \frac{2\left[t_{r_b} - t_{r_a}\right]}{w_b + w_a}$$

w = baseline peak width of two neighboring peaks.

Baseline resolution, R > 1.5

Rate Theory of Chromatography

A more realistic description of the processes at work inside a column takes account of the time taken for the solute to equilibrate between the stationary and mobile phase (unlike the plate model, which assumes that equilibration is infinitely fast). The resulting band shape of a chromatographic peak is therefore affected by the rate of elution. It is also affected by the different paths available to solute molecules as they travel between particles of stationary phase. If we consider the various mechanisms which contribute to band broadening, we arrive at the **Van Deemter** equation for plate height;

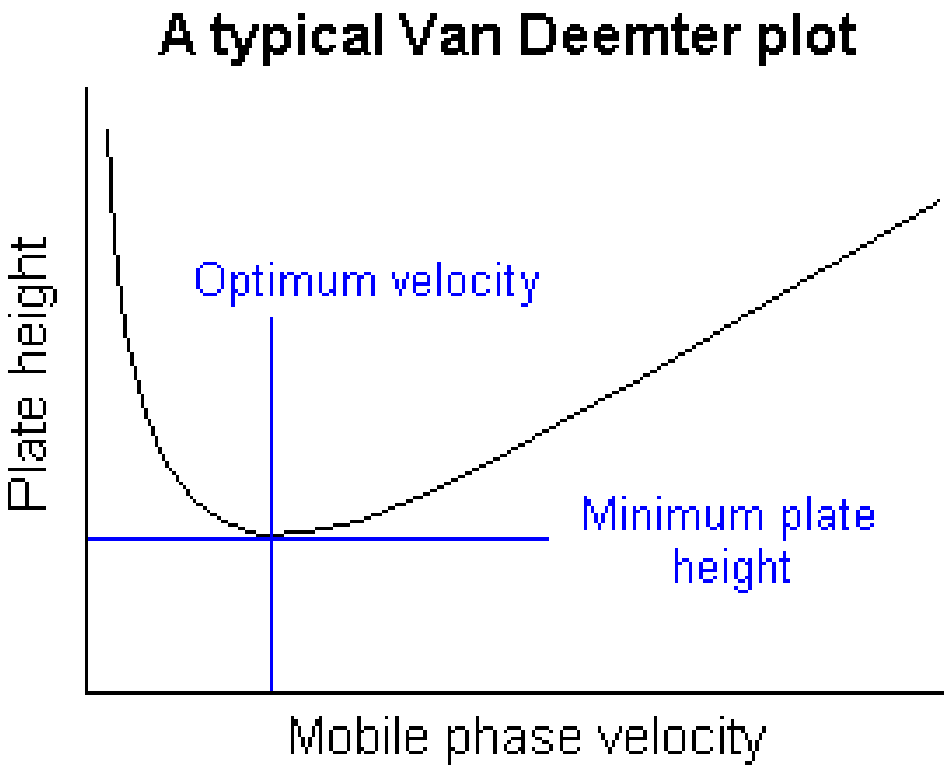
$$H = A + \frac{B}{u} + Cu$$

where *u* is the average velocity of the mobile phase. *A, B,* and *C* are factors which contribute to band broadening.

Rate Theory of Chromatography

Van Deemter plot -

A plot of plate height (H) vs. average linear velocity (u) of mobile phase.



Rate Theory of Chromatography

***A* - Eddy diffusion**

The mobile phase moves through the column which is packed with stationary phase. Solute molecules will take different paths through the stationary phase at random. This will cause broadening of the solute band, because different paths are of different lengths.

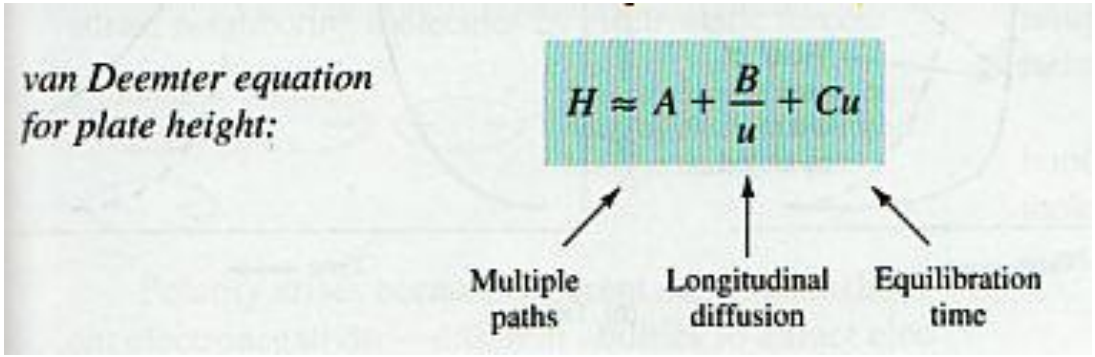
***B* - Longitudinal diffusion**

The concentration of analyte is less at the edges of the band than at the center. Analyte diffuses out from the center to the edges. This causes band broadening. If the velocity of the mobile phase is high then the analyte spends less time on the column, which decreases the effects of longitudinal diffusion.

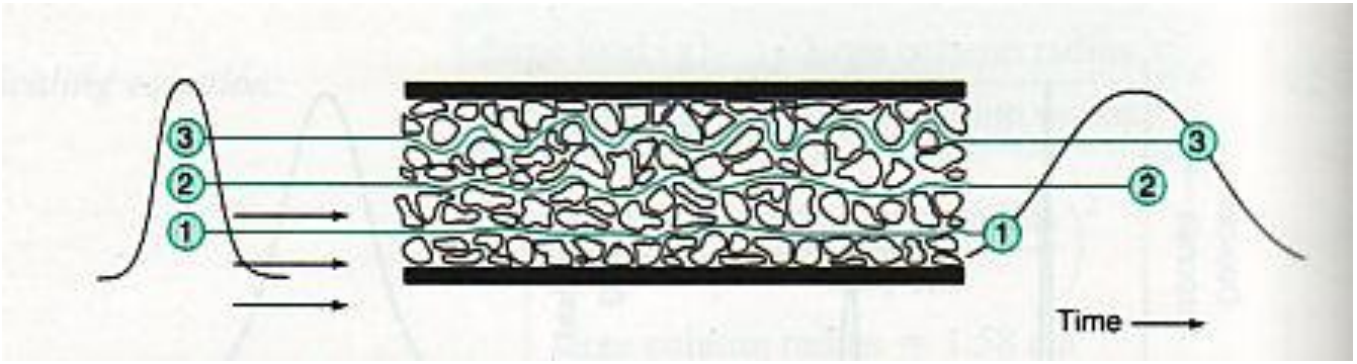
***C* - Resistance to mass transfer**

The analyte takes a certain amount of time to equilibrate between the stationary and mobile phase. If the velocity of the mobile phase is high, and the analyte has a strong affinity for the stationary phase, then the analyte in the mobile phase will move ahead of the analyte in the stationary phase. The band of analyte is broadened. The higher the velocity of mobile phase, the worse the broadening becomes.

Efficiency of Separation (plate number)



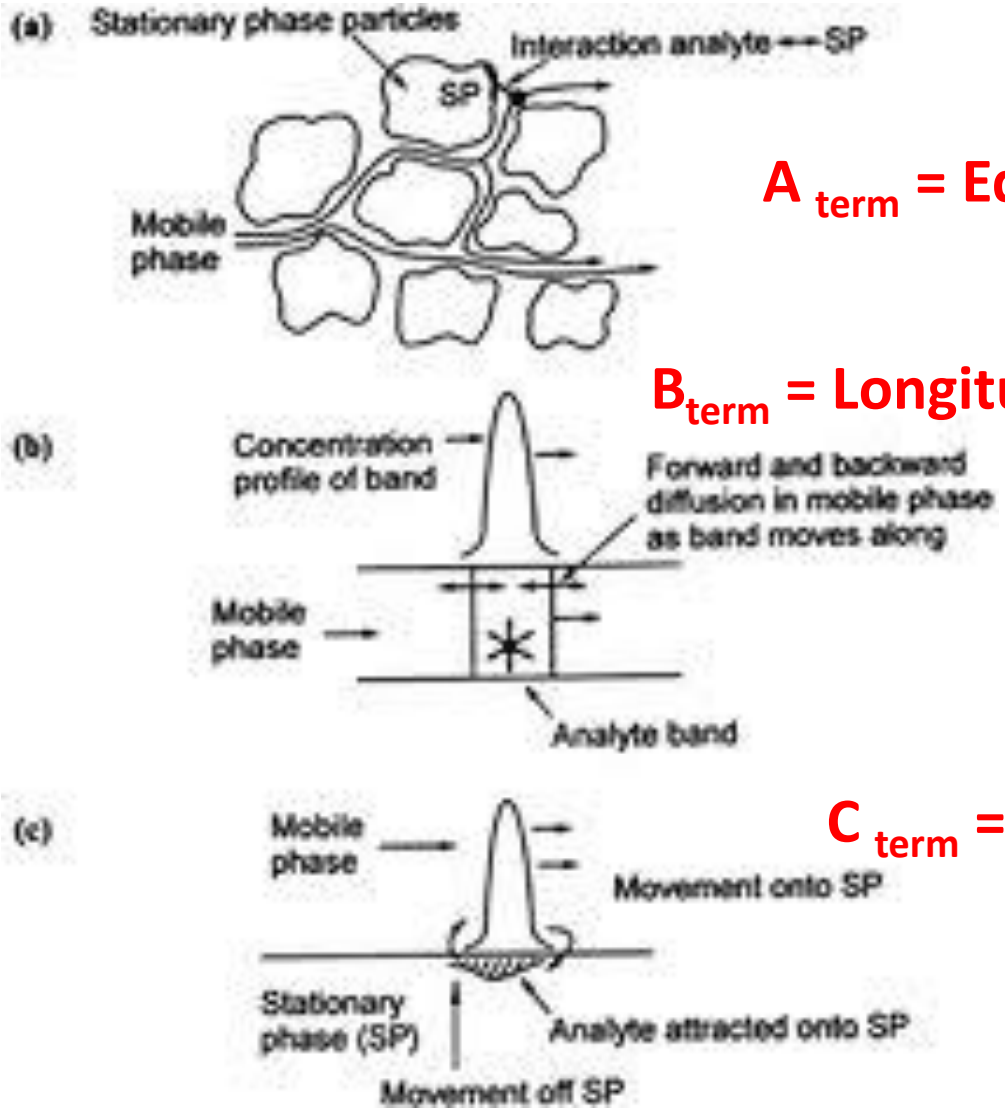
H = L/N Small plate height = narrow peaks = better separation



Multiple Flow Path Term

One wants a small value for H! Plate numbers 100s-1000s for conventional HPLC. Can be 10^6 for some high efficiency separations!!!!

Zone Broadening Terms



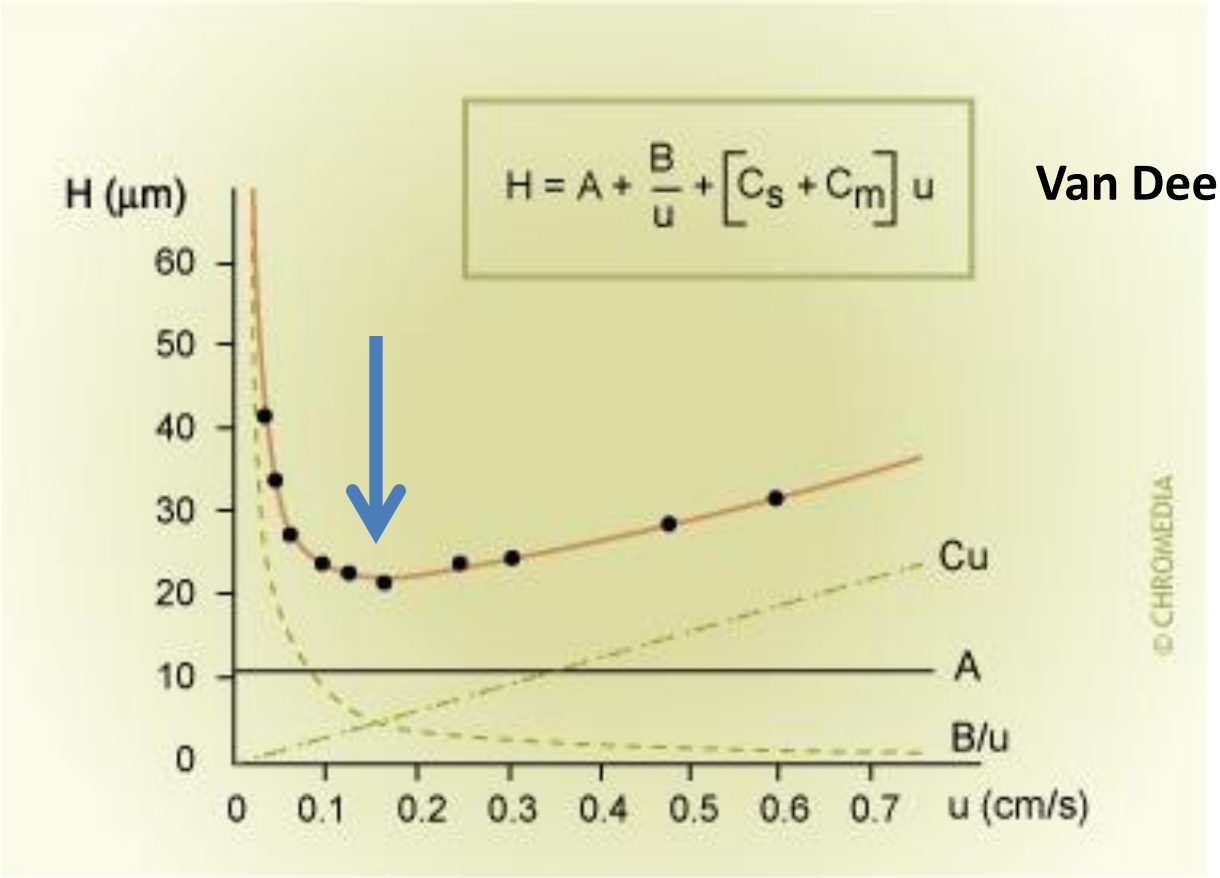
A_{term} = Eddy Diffusion

B_{term} = Longitudinal Diffusion

C_{term} = Stationary Phase Mass Transport

Why Do We Care About Flow Rate?

N (efficiency) = L/H **N α R_s**



Van Deemter Eq.

Flow Velocity (cm/s) = Flow Rate (cm^3/s)/Cross sectional area (cm^2)

Rate Theory of Chromatography

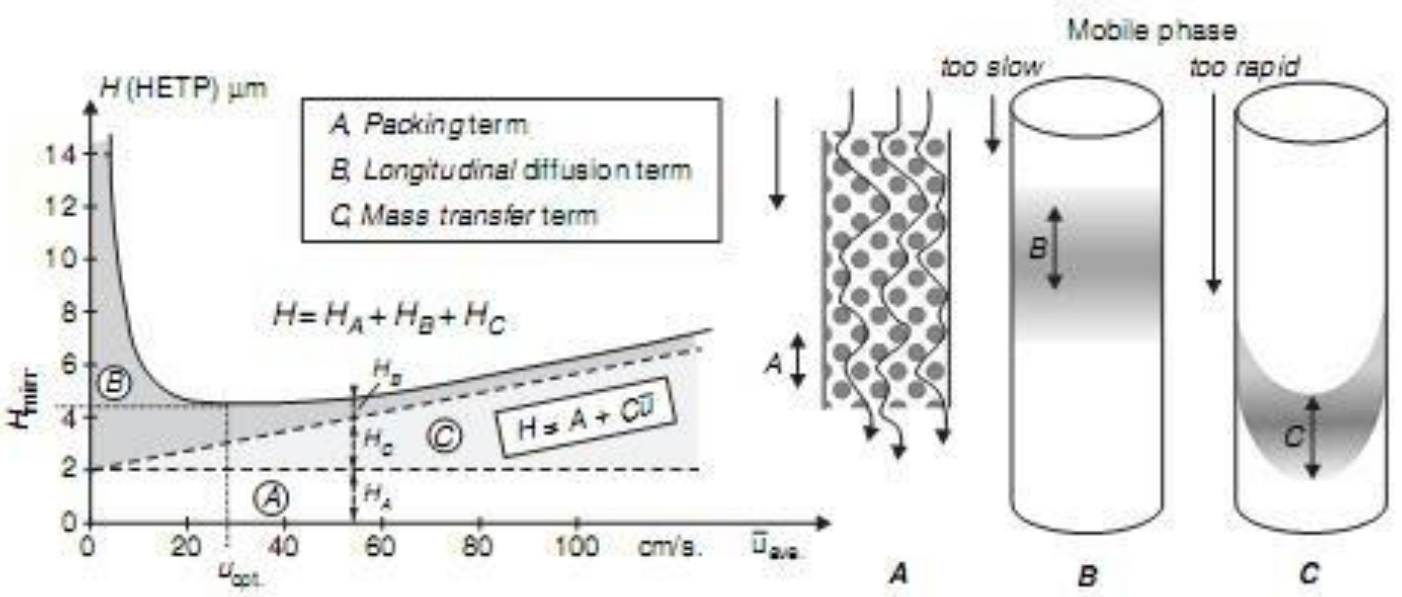


Figure 1.11 *Van Deemter's curve in gas chromatography with the domains of parameters A, B and C indicated.* There exists an equation similar to that of Van Deemter that considers temperature: $H = A + B/T + CT$.

Internet image

Rate Theory of Chromatography

$$H = 2\lambda d_p + \frac{2\gamma D_m}{u} + \frac{\omega(d_p \text{ or } d_c)^2 u}{D_m} + \frac{R d_f^2 u}{D_s}$$

H is plate height
λ is particle shape (with regard to the packing)
d_p is particle diameter
γ, ω, and R are constants (function of packing and k')

D_m is the diffusion coefficient of the mobile phase
d_c is the capillary diameter
d_f is the film thickness
D_s is the diffusion coefficient of the stationary phase.
u is the linear velocity

Goal: To achieve the fastest separation with the highest plate number and resolution!!!