Mi a kromatográfia?

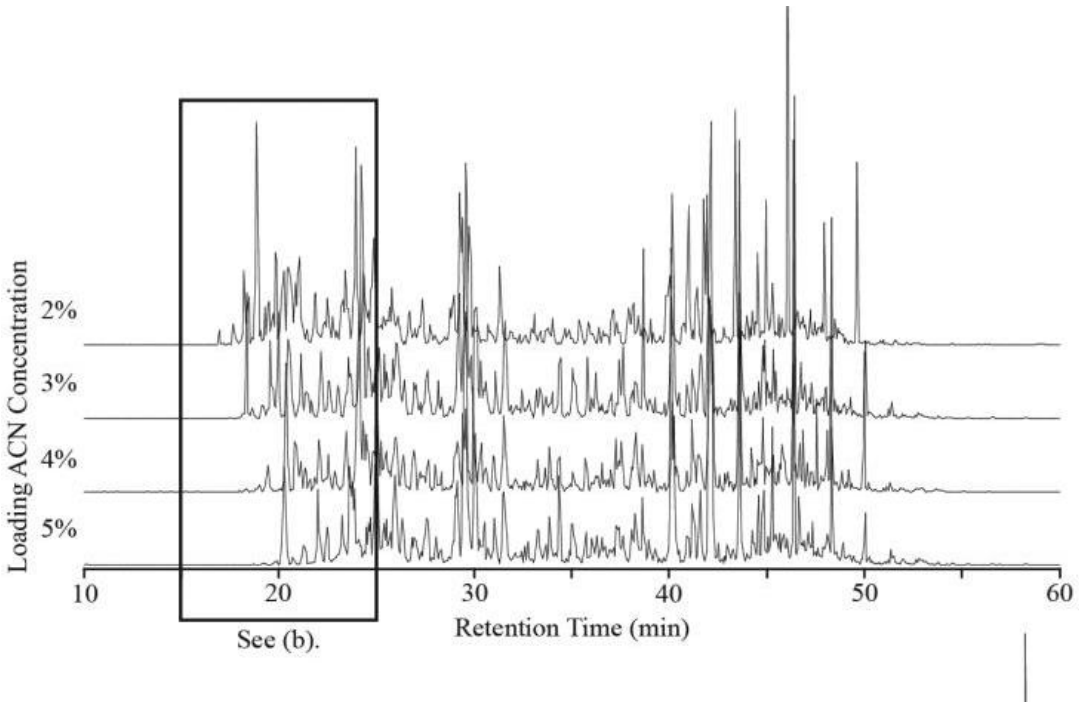
- Nagyon hatékony elválasztási módszer

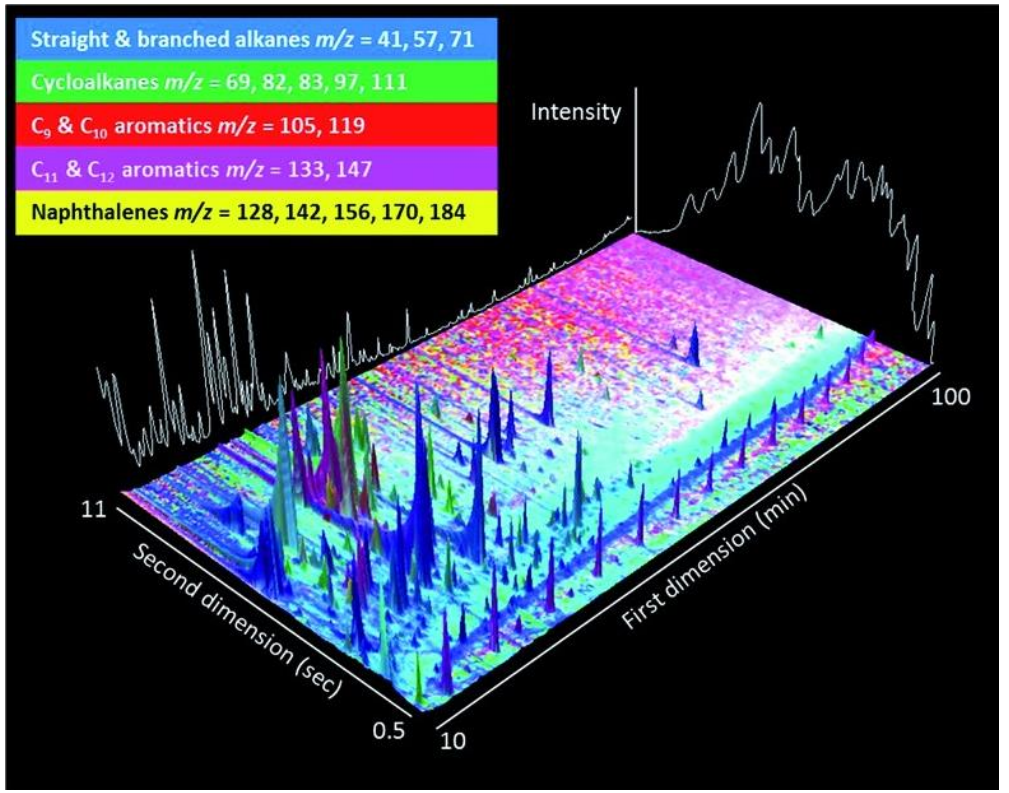
Egyéb elválasztási módszerek

- Oldás
- Szűrés
- Kristályosítás
- Desztilláció
- Adszorpció
- Folyadék-folyadék extrakció

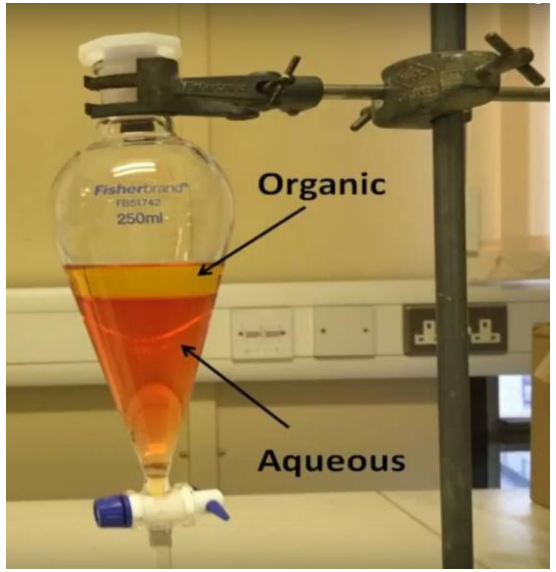
Mi a kromatográfia?

Nagyon hatékony elválasztás + mennyiségi meghatározás és szerkezetazonosítás





Folyadék-folyadék extrakció



$$K = \frac{c_{szerves}}{c_{vizes}} \qquad F = \frac{V_{szerves}}{V_{vizes}}$$

$$R = 100 \cdot \frac{n_{szerves}}{n_{vizes} + n_{szerves}} = 100 \cdot \frac{K \cdot F}{1 + K \cdot F}$$

K – megoszlási hányados
c – a komponens koncentrációja
V – az adott fázis térfogata
F – fázisarány
R – kinyerési hatásfok (%)

99%-os extrakciós hatásfok eléréséhez K=100 kell.

Mi a helyzet, ha két anyagot szeretnénk elválasztani?

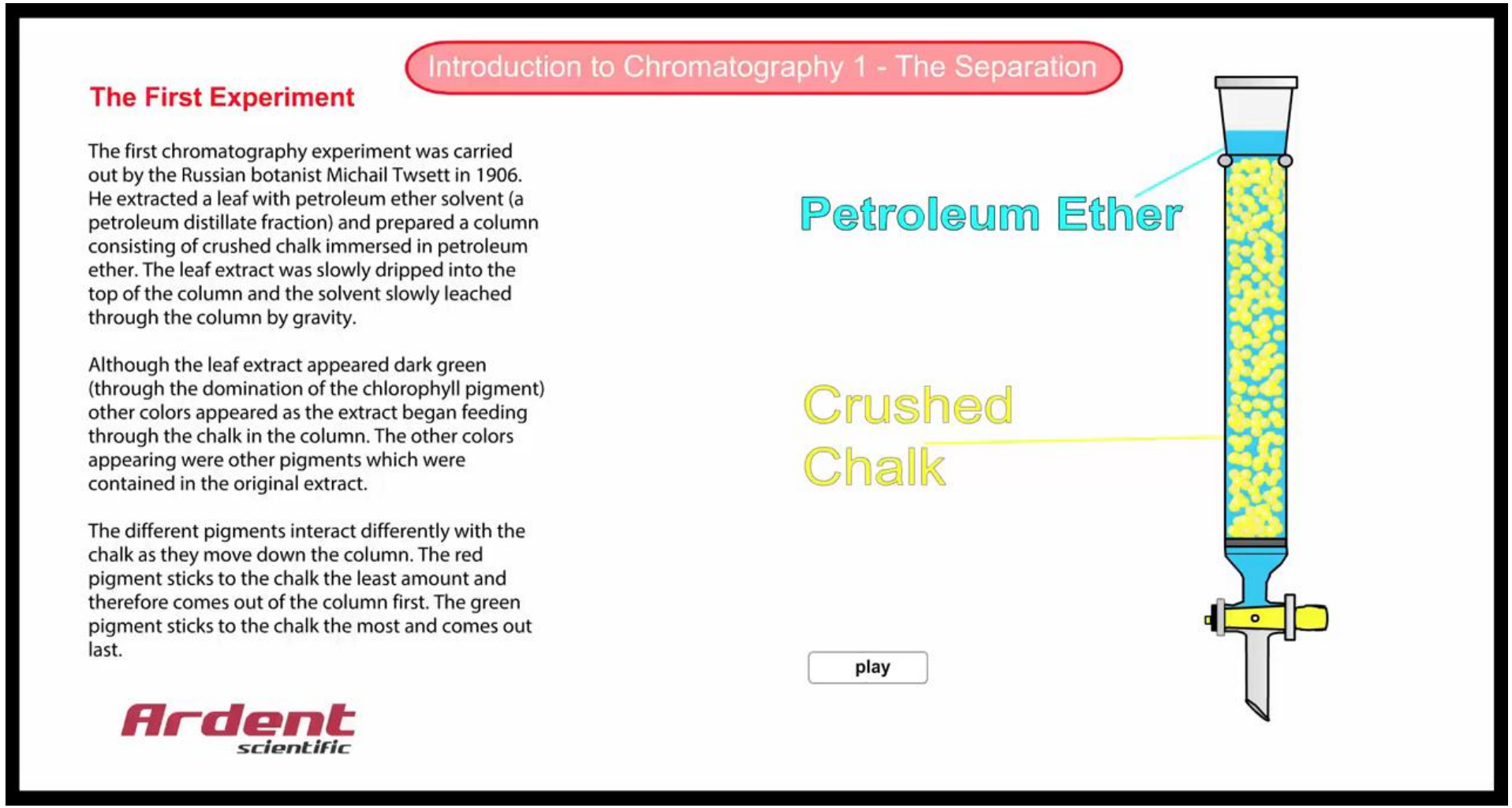
Folyadék-folyadék extrakció

Ahhoz, hogy 99% tisztaságot elérjünk 2 anyagra, az kell, hogy K1=100 és K2=0,01 legyen, vagyis K1 10.000-szer nagyobb, mint K2

KROMATOGRÁFIA

Ahhoz, hogy 99% tisztaságot elérjünk 2 anyagra, elegendő ha $K1=1{,}1 \cdot K2$
vagyis K2 10%-kal nagyobb, mint K1

Az elválasztás



https://www.youtube.com/watch?v=DptfKQI6lUs&list=PLmRpOX9aPBrLF1VPi45PLYs4d68EMf1tQ&index=2&t=0s

A mozgófázis

Introduction to Chromatography 2 - Mobile Phase

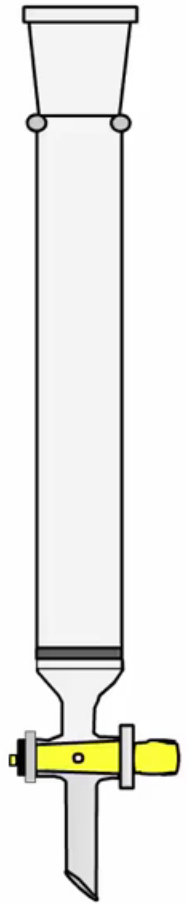
Mobile Phase

Now let's define some terms which are very often used in chromatography. We have already seen that in the first experiment there was a liquid and a solid involved. These two media are known as phases.

The liquid in the column is called the mobile phase. This should be easy to remember as this is the phase that is constantly moving when we have opened the stopcock. In liquid chromatography the mobile phase is a liquid. In gas chromatography the mobile phase is a gas.

In this animation, the mobile phase is fed into the column and will sit there until the stopcock is opened. In the first experiment in 1906 the mobile phase was petroleum ether.

play



Ardent scientific

https://www.youtube.com/watch?v=1KJSq5w1LRc&list=PLmRpOX9aPBrLF1VPi45PLYs4d68EMf1tQ&index=2

Az állófázis

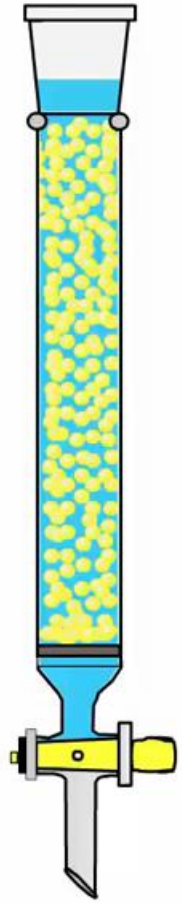
Introduction to Chromatography 3 - Stationary Phase

Stationary Phase

The second phase is the crushed chalk (calcium carbonate) in this example and is known as the Stationary Phase.

The stationary phase retains the components for various periods of time depending on the chemistry of the components in the mixture. The more the component in a mixture is held up in the stationary phase, the longer it will take to drip from the end of the column.

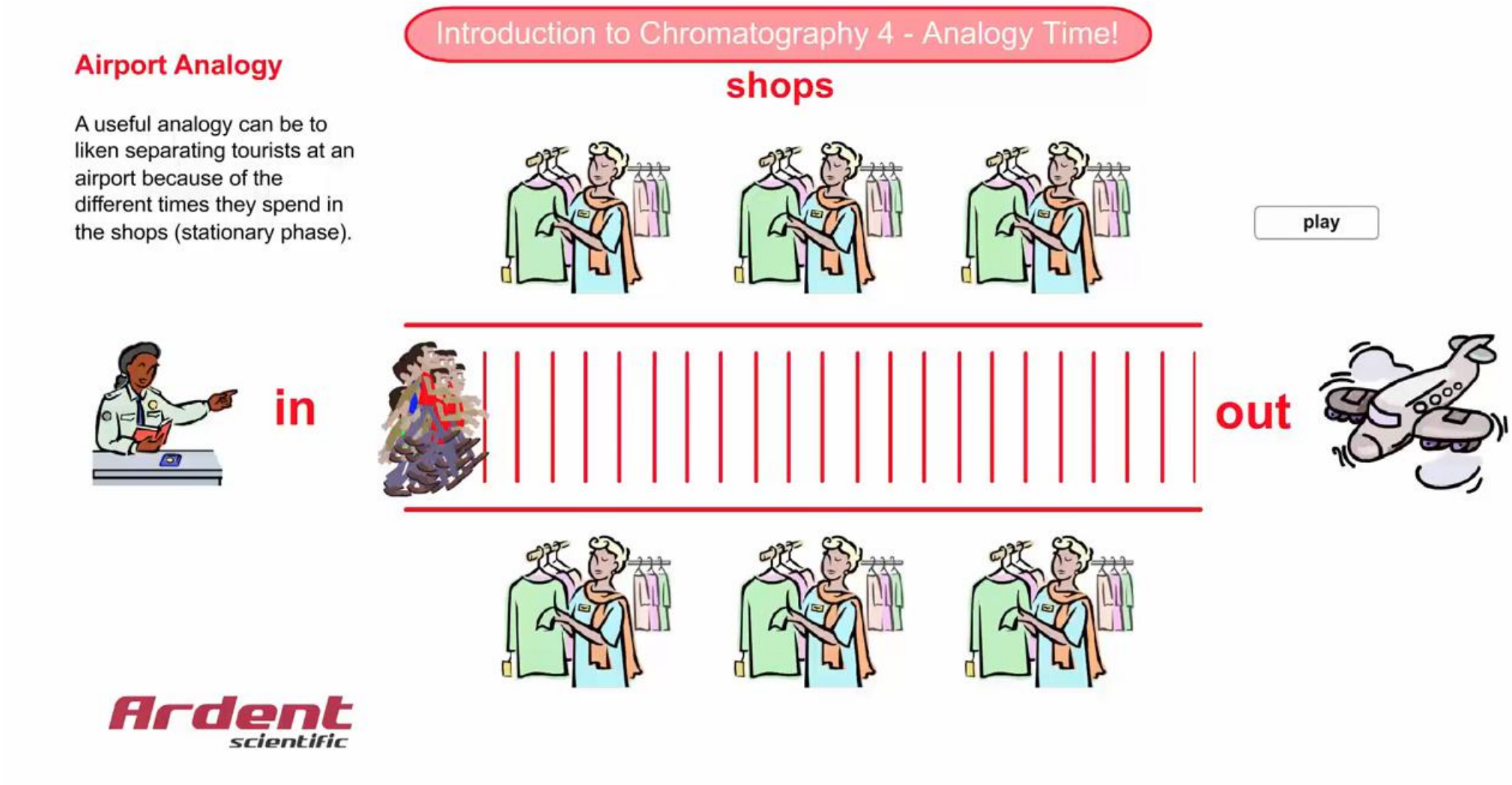
play



Ardent scientific

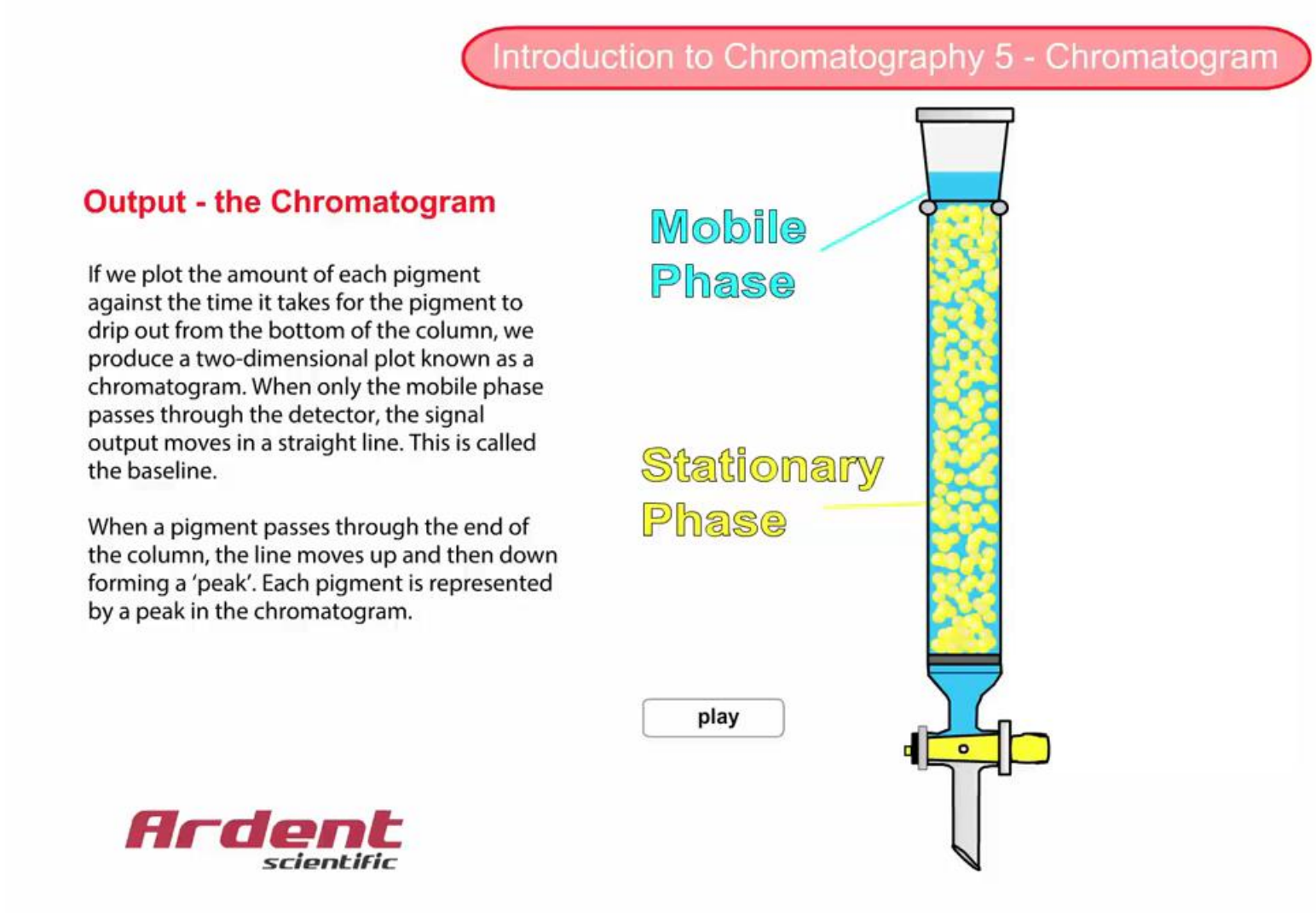
https://www.youtube.com/watch?v=DZUq908S1v8&list=PLmRpOX9aPBrLF1VPi45PLYs4d68EMf1tQ&index=3

A kromatográfia reptéri hasonlata



https://www.youtube.com/watch?v=e8i374hrWQg&list=PLmRpOX9aPBrLF1VPi45PLYs4d68EMf1tQ&index=4

A kromatogram



https://www.youtube.com/watch?v=zrJM3YzauNE&list=PLmRpOX9aPBrLF1VPi45PLYs4d68EMf1tQ&index=5

A retenciós idő

Introduction to Chromatography 6 - Retention Time

Defining Retention Time (t_R)

Eventually a compound comes out (elutes) of the end of the column. The time from the start of the separation to the exit from the system is known as the retention time (t_R). The time is taken at the top (apex) of the peak.

The retention time is very important in chromatography as we use this time to identify the component. Firstly we inject a known standard and identify its retention time in the chromatogram . We then compare this time to a peak in the sample chromatogram. If the times match then there is a high probability of identifying the sample peak.

play

Ardent scientific

https://www.youtube.com/watch?v=WwiRkn9NbQ8&list=PLmRpOX9aPBrLF1VPi45PLYs4d68EMf1tQ&index=6

A kromatográfiás oszlop

Introduction to Chromatography 7 - HPLC Column

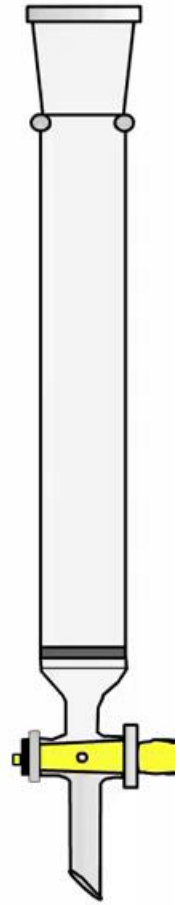
Glass Column and a HPLC Column

The glass column is quite useful because we see the colored pigments separate in the column and it is a useful learning aid. However you might have seen a typical HPLC (High Performance Liquid Chromatography) column which is usually about 250mm long and 4.6mm internal diameter.

The glass column is still used for separations in some labs and these separations are carried out at low pressure. The stainless steel jacket is used for the HPLC column to withstand the high pressures associated with HPLC. These pressures can be higher than 1000 Bar.

play

HPLC - stainless steel column



glass column

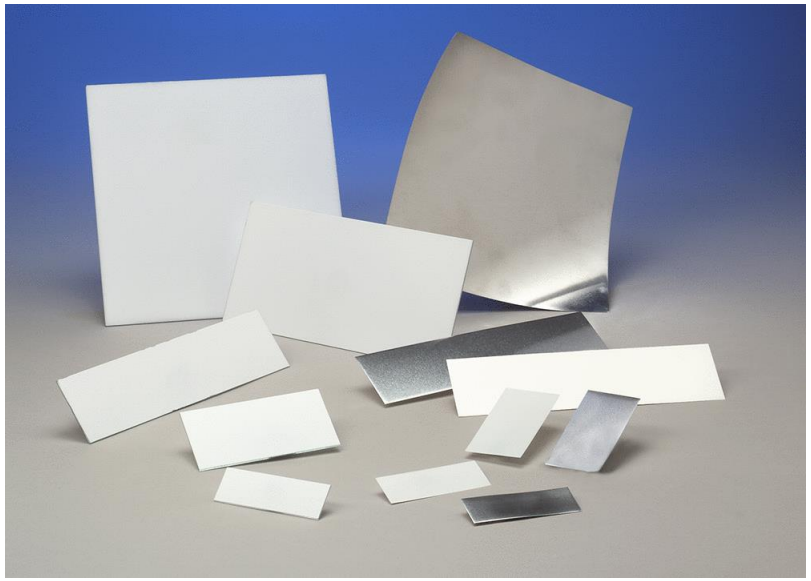
Ardent scientific

https://www.youtube.com/watch?v=wyM5rx3yevo&list=PLmRpOX9aPBrLF1VPi45PLYs4d68EMf1tQ&index=7

folyadékkromatográfiás oszlopok

gázkromatográfiás oszlopok

vékonyrétegkromatográfiás lapok



A folyadékkromatográfiás készülék

Introduction to Chromatography 8 - Instrument

HPLC - The Hardware

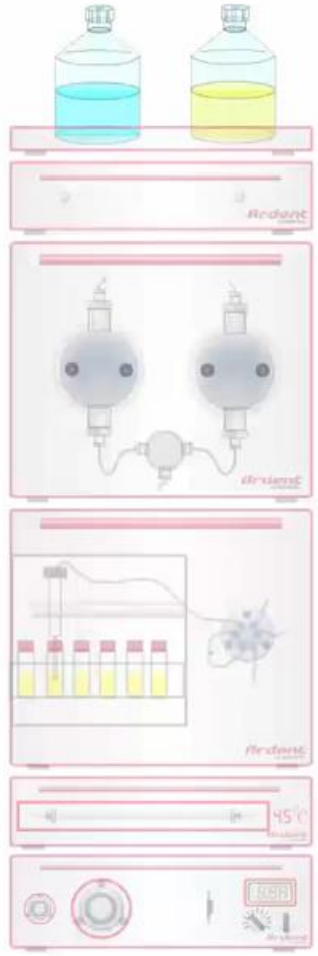
From the first chromatography to today's modern High Performance Liquid Chromatograph. The principle is exactly the same but each of the parts has been replaced with sophisticated equipment.

The data is collected and manipulated through software. The chromatography software also controls the instrumentation.

Roll the mouse over each of the system's components for further explanation.

'HPLC Hardware' is covered fully in a module in this suite of eLearning products with full explanations of their workings together with animations.

Ardent scientific



https://www.youtube.com/watch?v=3YulfePP_A8&list=PLmRpOX9aPBrLF1VPi45PLYs4d68EMf1tQ&index=8

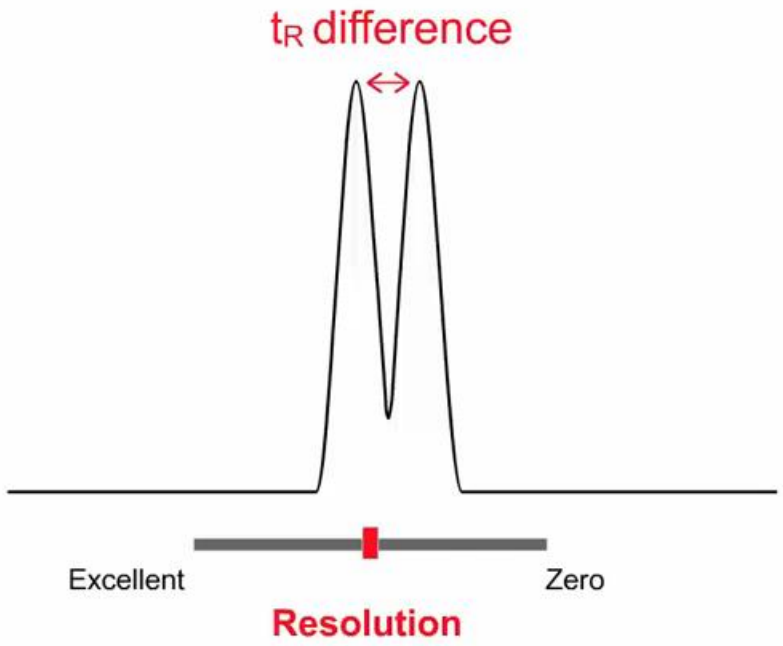
A felbontás és visszatartás (retenció)

Fundamentals of HPLC 1 - Resolution

Resolution and Retention Time (t_R) Difference

Peaks often don't appear equally spaced apart in the chromatogram. Some compounds might come out of the column close with one another. The term defining the degree of separation is known as Resolution.

As the retention time difference increases, the peaks get further apart and the resolution increases.



Ardent scientific

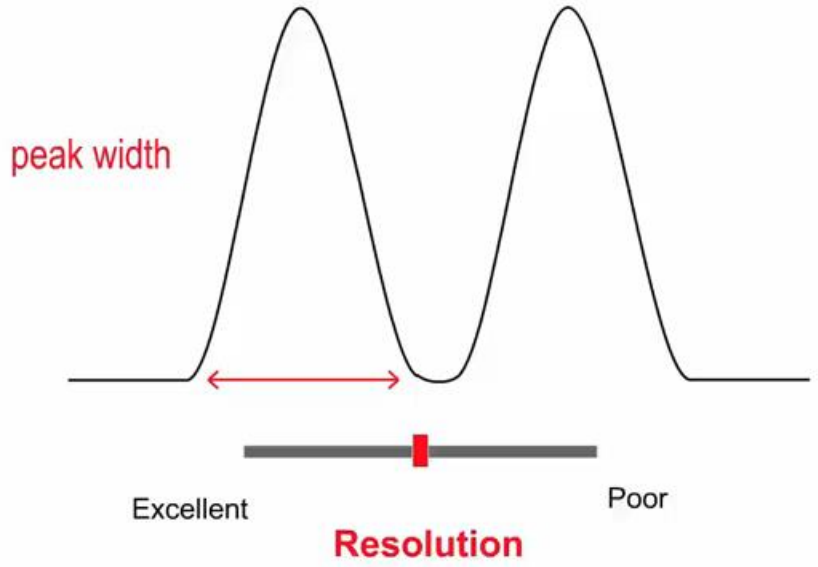
https://www.youtube.com/watch?v=HVQrTSYxLNI&list=PLmRpOX9aPBrLcKidp3cy4D29w5RTnkde4

A felbontás és csúcs szélesség

Fundamentals of HPLC 2 - Resolution

Resolution and Peak Width

As you use the slider bar to change the resolution, you will notice that as the peaks get fatter (increase of peak width), resolution gets worse.



Ardent scientific

https://www.youtube.com/watch?v=Y6kfaJrCVOs&list=PLmRpOX9aPBrLcKidp3cy4D29w5RTnkde4&index=2

A felbontás (R, resolution)

Fundamentals of HPLC 3 - Resolution Value

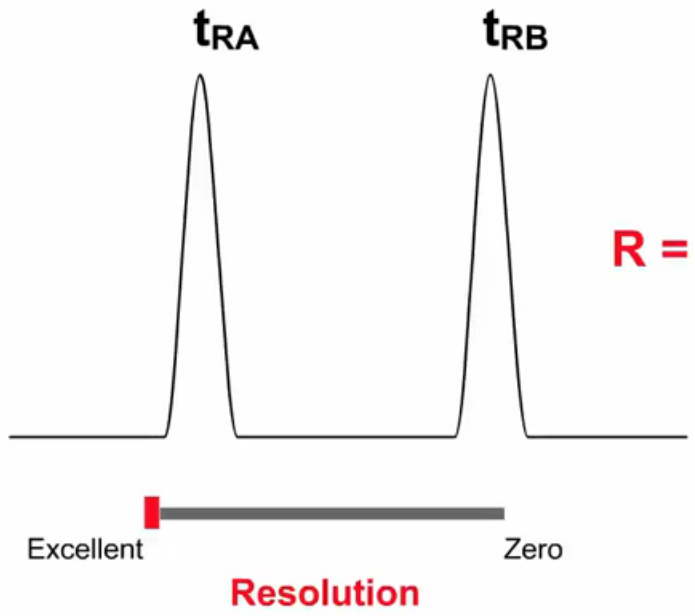
Resolution Value

As we have just seen, Resolution (**R**) is a function of the difference in retention times of the two peaks (t_{RA} and t_{RB}) and the widths of the peaks at the base (W_A and W_B). There is a quantitative measure of Resolution as shown in the equation below.

Use the slider bar to change the Retention Times of these two peaks and see the effect on the '**R**' value. Basline resolution is achieved when R = 1.5.

$$R = \frac{t_{RA} - t_{RB}}{0.5\,(W_A + W_B)}$$

Ardent scientific



https://www.youtube.com/watch?v=o-GYFTD8F1M&list=PLmRpOX9aPBrLcKidp3cy4D29w5RTnkde4&index=3

A felbontást befolyásoló tényezők

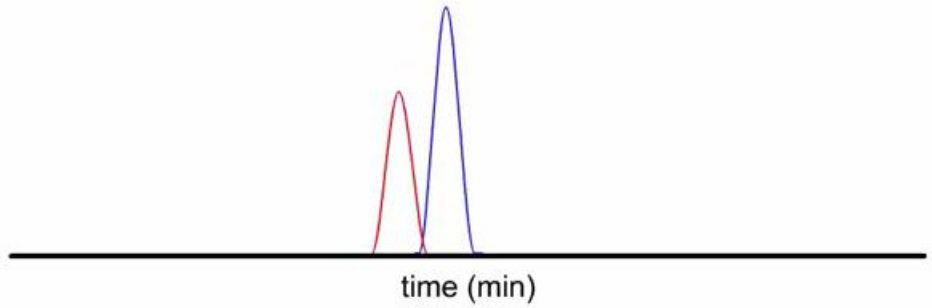
Fundamentals of HPLC 4 - Resolution

Factors Affecting Resolution

There are only three factors in chromatography which affect Resolution. These are:

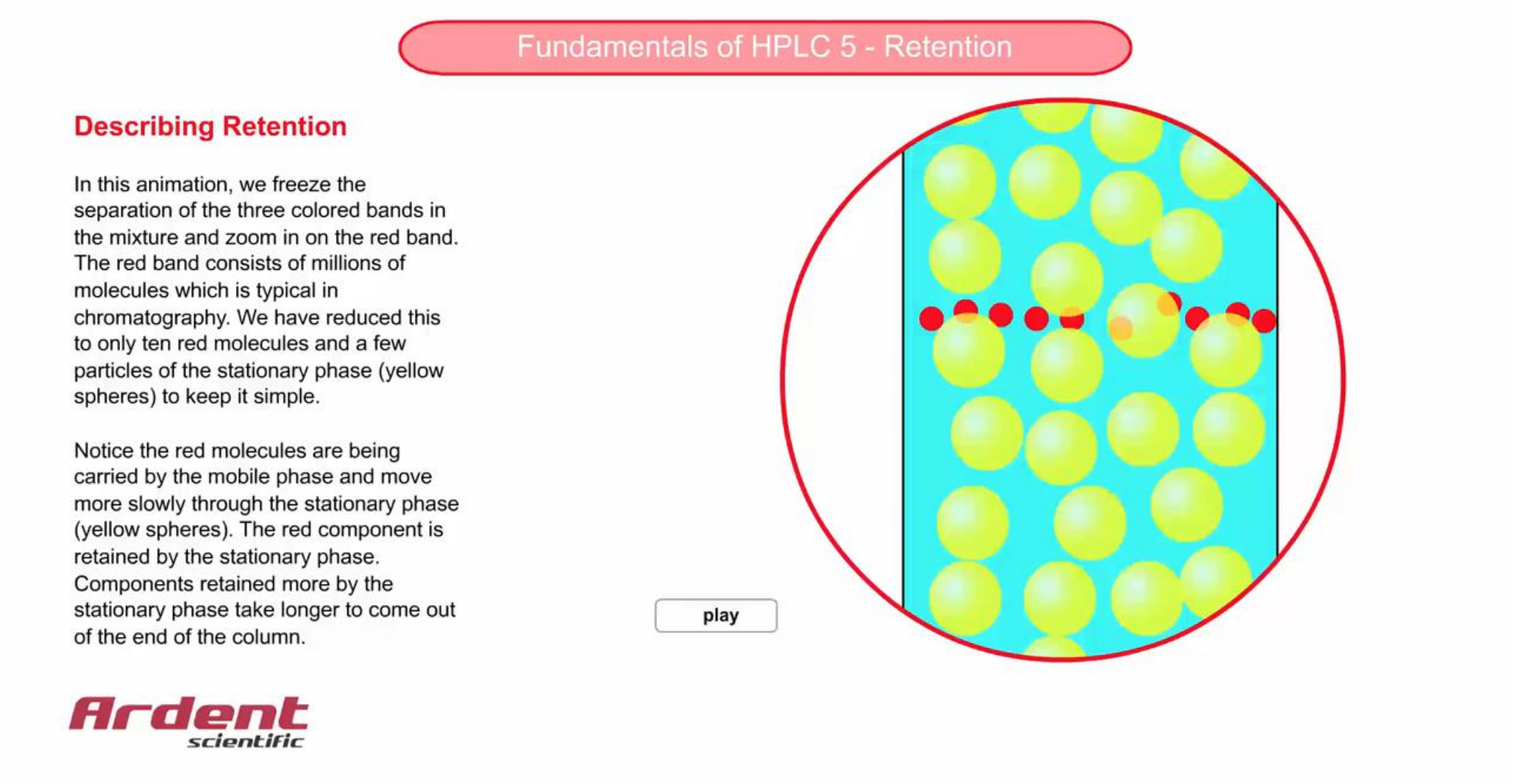
- **Retention** - a measure of the degree of interaction with the stationary phase.
- **Selectivity** - a measure of the differences in the chemistries of the components in the mixture.
- **Efficiency** - a measure of the sharpness of the peaks.

Ardent scientific



https://www.youtube.com/watch?v=vFENzNj6Jq4&list=PLmRpOX9aPBrLcKidp3cy4D29w5RTnkde4&index=4

A visszatartás (retenció)



https://https://www.youtube.com/watch?v=VGvLcvLYvdE&list=PLmRpOX9aPBrI7AhzM0RttLIWJbIg9C_lz

A holtidő (t_0)

Fundamentals of HPLC 6 - Retention

The Unretained Time (t_0)

Any component has to get from the start to the end of the system whether or not there is any interaction with the stationary phase. If there is no interaction of a component with the stationary phase then the time for this unretained component to exit the system is called the unretained time (t_0).

An unretained component moves through the column at the same speed as the mobile phase. The t_0 time can be measured from the injection of a component which has no interaction with the stationary phase.

In this animation, the pink band traveling through the column represents an unretained component *i.e.* no interaction with the stationary phase. This component moves at the same speed as the mobile phase.

play

Ardent scientific

https://www.youtube.com/watch?v=UfV65qMD_gE&list=PLmRpOX9aPBrI7AhzM0RttLIWJbIg9C_Iz&index=2

A nemkötődő komponens

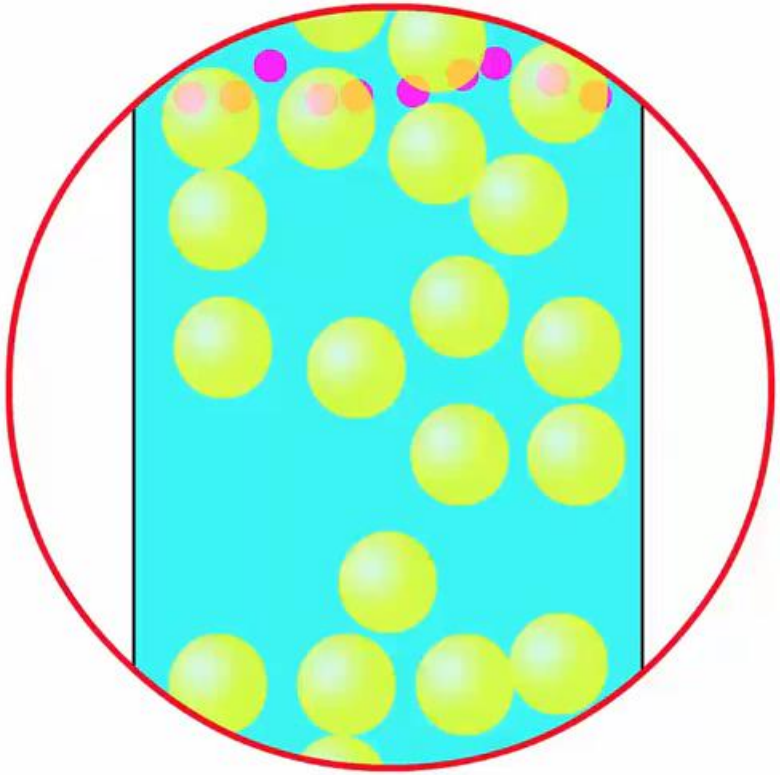
Fundamentals of HPLC 7 - Retention

An Unretained Component

The unretained component has no interaction with the stationary phase. As you can see in this animation the molecules of the unretained component (pink spheres) are running through the column all at the same speed even if they have to pass through the stationary phase.

The unretained component has no interaction with the stationary phase.

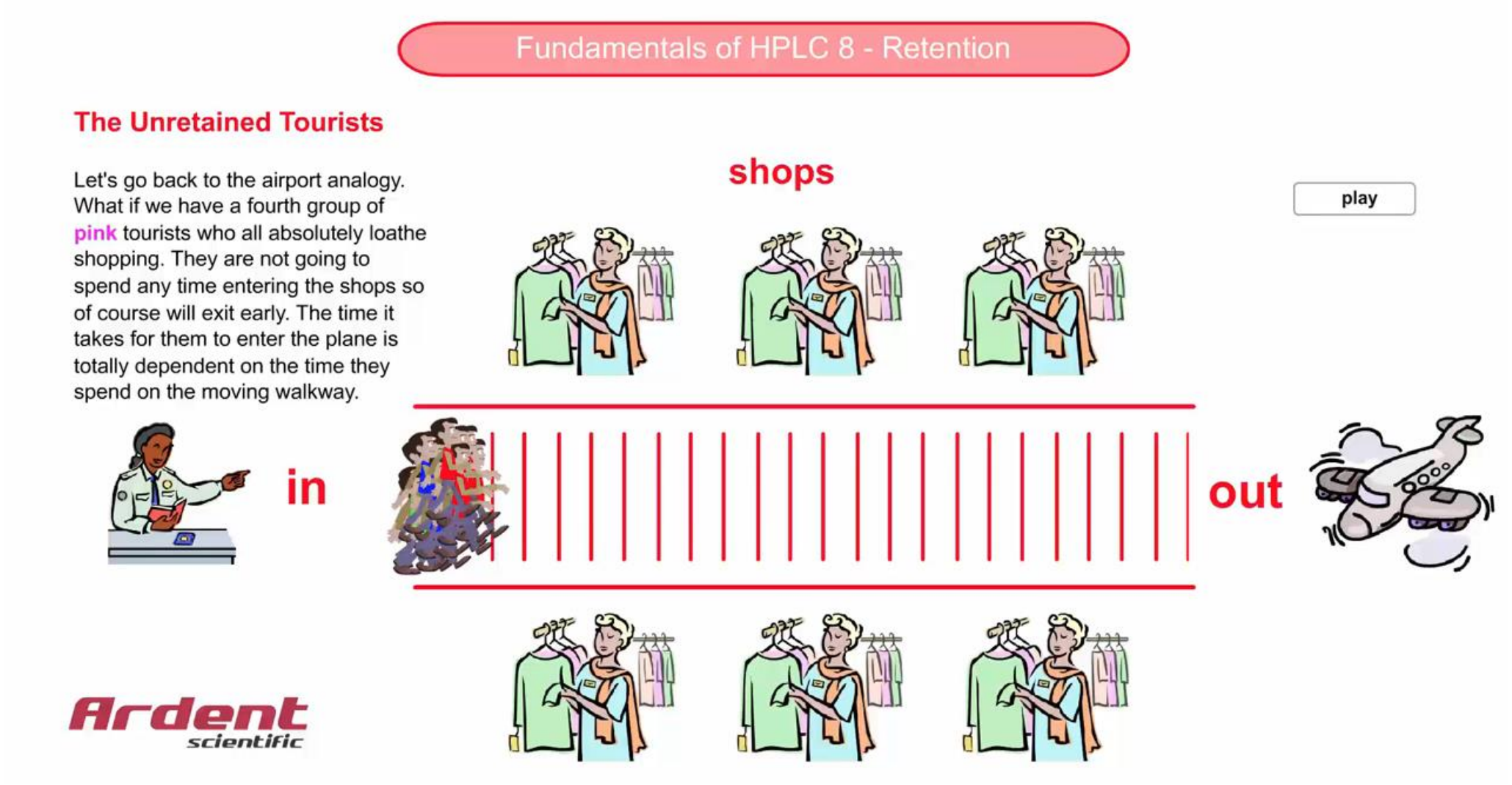
play



Ardent scientific

https://www.youtube.com/watch?v=TZ9VtwohnG4&list=PLmRpOX9aPBrI7AhzM0RttLIWJbIg9C_lz&index=3

A „nemkötődő” turisták



https://www.youtube.com/watch?v=smjOT5jKI_4&list=PLmRpOX9aPBrI7AhzM0RttLIWJbIg9C_Iz&index=4

A relatív retenciós idő (t'_R)

Fundamentals of HPLC 9 - Retention

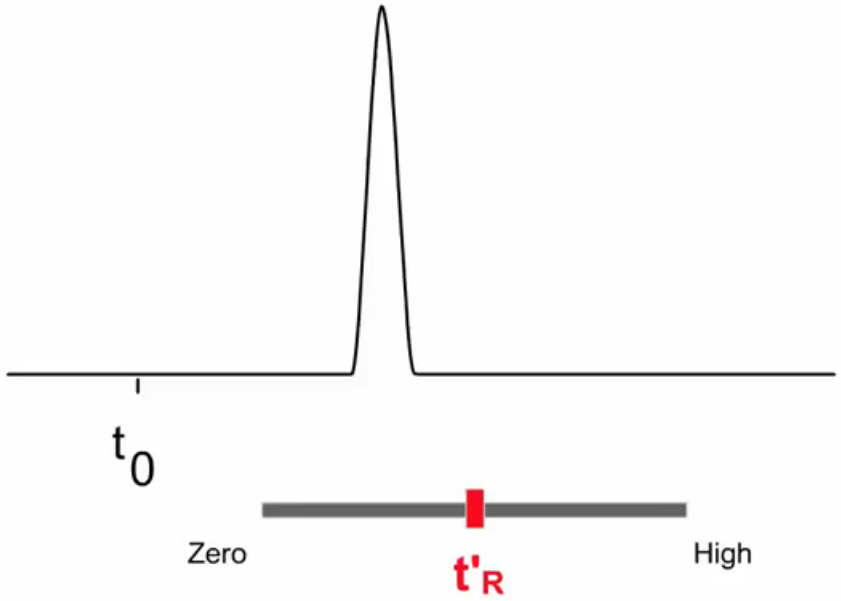
Adjusted Retention Time (t'_R)

The retention time (t_R) is the sum of the time the component interacts with the stationary phase as well as the time it takes for a component to get from the start to the end of the system without any interaction (t_0).

But what if we want to know only the time the component has spent interacting with the stationary phase? Well, we can work it out. For example, if the retention time is 10 minutes and the unretained time is 1 minute, then it makes sense that the component has interacted with the stationary phase for 10 - 1 = 9 minutes . This 9 minutes is called the adjusted retention time (t'_R).

If there is no retention *i.e.* no interaction of the component with the stationary phase, the component elutes at the t_0 time.

Ardent scientific



$t'_R = t_R - t_0 =$ min retention time $t_R =$ min

https://www.youtube.com/watch?v=St1xMEsHwsw&list=PLmRpOX9aPBrI7AhzM0RttLIWJbIg9C_lz&index=5

A retenciós idő tényező (k)

Fundamentals of HPLC 10 - Retention

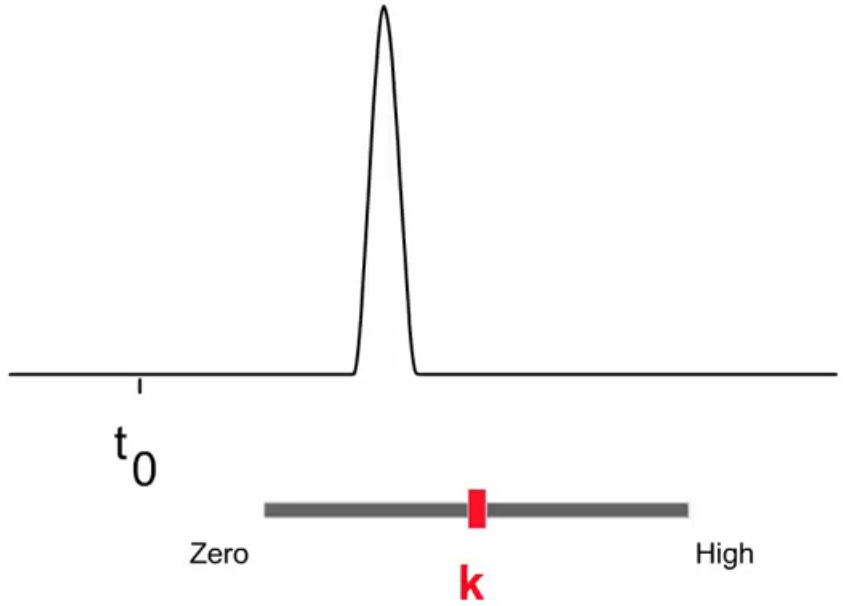
Retention Factor (k) Calculation

The retention factor (**k**) is the time the component interacts with the stationary phase (t'_R) relative to the time the component spends in the mobile phase (t_0).

To arrive at this retention factor we divide t'_R by t_0.

If the mobile phase flow rate is doubled then both the retention time and the unretained time are halved. However, the retention factor value remains unchanged.

If there is no retention, **k** = 0, i.e. the component elutes at the t_0 time.



$$k = \frac{t_R - t_0}{t_0} = \frac{t'_R}{t_0} =$$

retention time t_R = min

https://www.youtube.com/watch?v=SO9dmFbbCEY&list=PLmRpOX9aPBrI7AhzM0RttLIWJbIg9C_Iz&index=6

A retenciós idő tényező (k)

$$k = \frac{n_s}{n_m} = \frac{c_s V_s}{c_m V_0} = K \frac{V_s}{V_0}$$

ahol n_s és n_m a komponens móljainak száma az álló, illetve mozgófázisban
c_s és c_m a komponens koncentrációja az álló, illetve mozgófázisban
K a megoszlási hányados
V_s az állófázis térfogata
V_0 a mozgófázis térfogata
V_s/V_0 a fázisarány

Csak a *komponens*, az *állófázis* és *mozgófázis* anyagi minőségétől függ, illetve a *fázisaránytól*, de ez porózus szemcsékkel töltött oszlop esetén állandó.
(Meg persze a *hőmérséklettől*.)

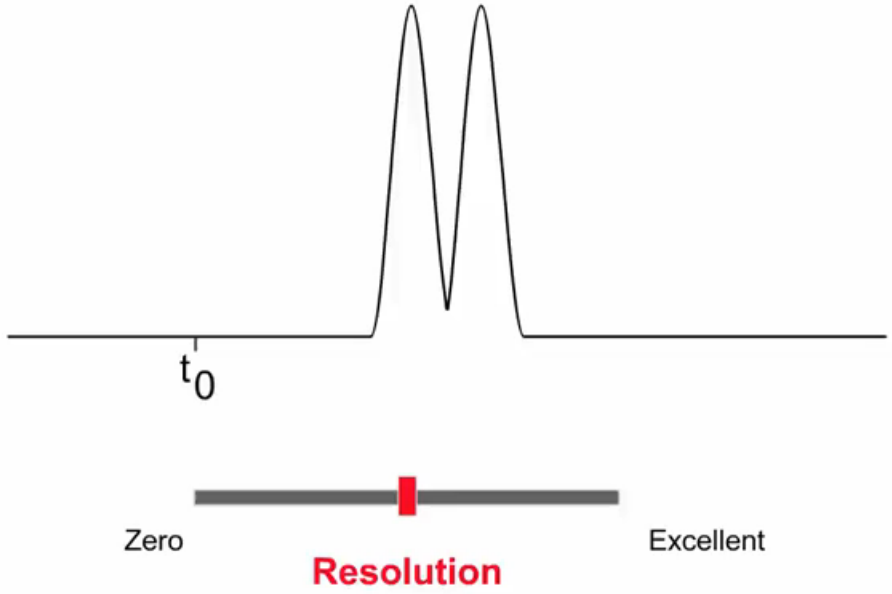
Retenció és felbontás

Fundamentals of HPLC 11 - Retention

Retention and Resolution

The retention factor (**k**) greatly affects resolution. More retention results in better resolution. The different chemistries of the components will be magnified the longer the components are retained in the stationary phase. The relationship between Resolution and Retention, however, is not linear - as shown in this equation.

Improving Resolution by increasing retention is much more effective for low **k** values than for high **k** values. Ideal values are between 1 and 5. Increasing **k** over 5 has minimal effect on Resolution but increases run times.



$$R \propto \frac{k}{1+k} \quad = \quad k =$$

https://www.youtube.com/watch?v=JUwkB418I90&list=PLmRpOX9aPBrI7AhzM0RttLIWJbIg9C_Iz&index=7

A szelektivitás

Fundamentals of HPLC 12 - Selectivity

Describing Selectivity

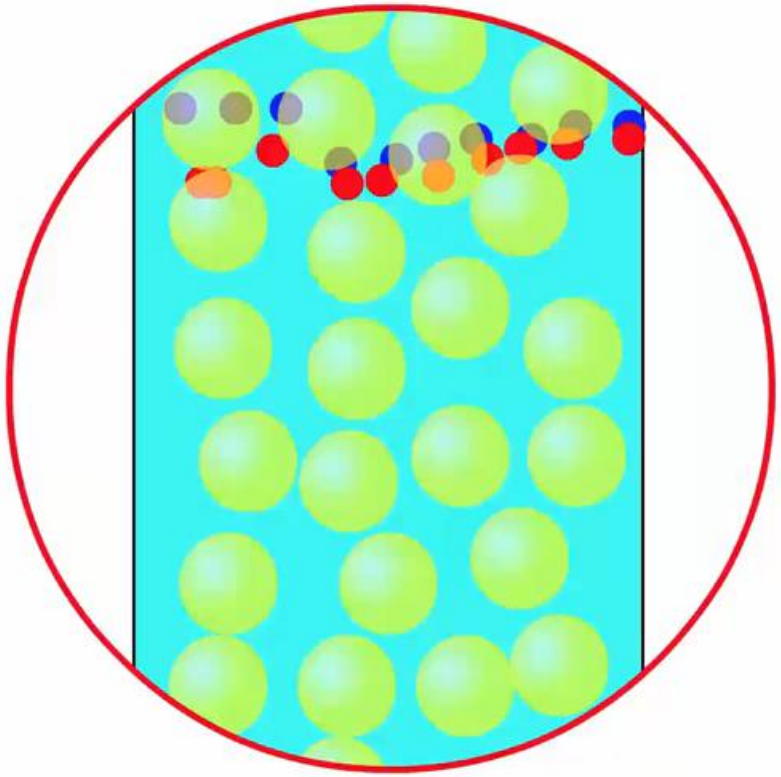
In this animation, as can be seen, the blue and red components are moving through the stationary phase (yellow spheres) at different speeds - there are different retentions of the two components with the stationary phase. This causes elution at the end of the column at different times and hence separation.

The red molecules elute first and then the blue molecules. All the molecules are moving through the mobile phase at the same speed.

The different degrees of retention with the stationary phase is the essence of selectivity.

play

Ardent scientific



https://www.youtube.com/watch?v=7wL3u6CfPnc&list=PLmRpOX9aPBrIPGcrsJnWuw25fikkugxQL

A szelektivitási (elválasztási) tényező

Fundamentals of HPLC 13 - Selectivity

Selectivity Calculation

The selectivity factor is a measure of different retentions of two components in the mixture. Two components will move through the mobile phase at the same speed but have different degrees of interaction with the stationary phase. Selectivity (α) is the measure of this.

As we have seen, the retention time alone does not show us the actual retention of a component so it is better to use retention factors (**k**) to ratio as shown in this equation. k_B and k_A are the retention factors of components 'B' and 'A', respectively.

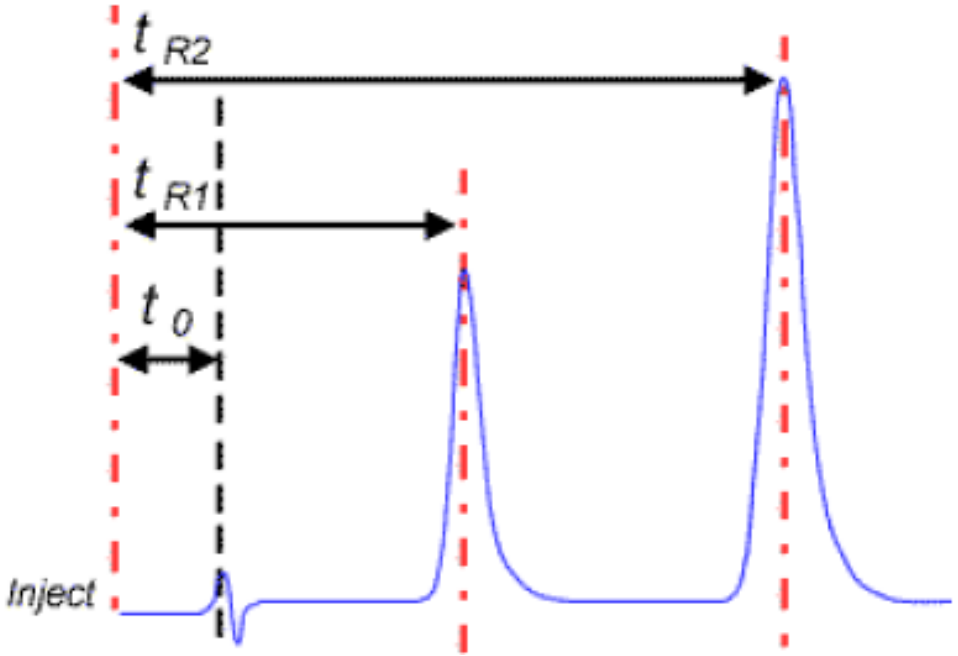
Roll the mouse over each of the k's to view the full equations.

Ardent scientific

$$\alpha = \frac{k_B}{k_A}$$

https://www.youtube.com/watch?v=Mj1U6_oiwoQ&list=PLmRpOX9aPBrIPGcrsJnWuw25fikkugxQL&index=2

A szelektivitási tényező



$$\alpha = \frac{k_2}{k_1} = \frac{K_2 \frac{V_s}{V_m}}{K_1 \frac{V_s}{V_m}} = \frac{K_2}{K_1}$$

Egymás melletti csúcsokra vonatkozik.

Szelektivitás és felbontás

Fundamentals of HPLC 14 - Selectivity

Selectivity (α) and Resolution I

In this animation, the retention factor (k) of both components is increasing. However, half way through, the red peak moves further away from the blue peak as the selectivity increases. This is most likely caused by a change in the mobile phase composition and is discussed further in the module 'Reversed Phase HPLC'.

Use the slider part to examine the relationship between retention, selectivity and resolution.

$$\alpha = \frac{k_B}{k_A}$$

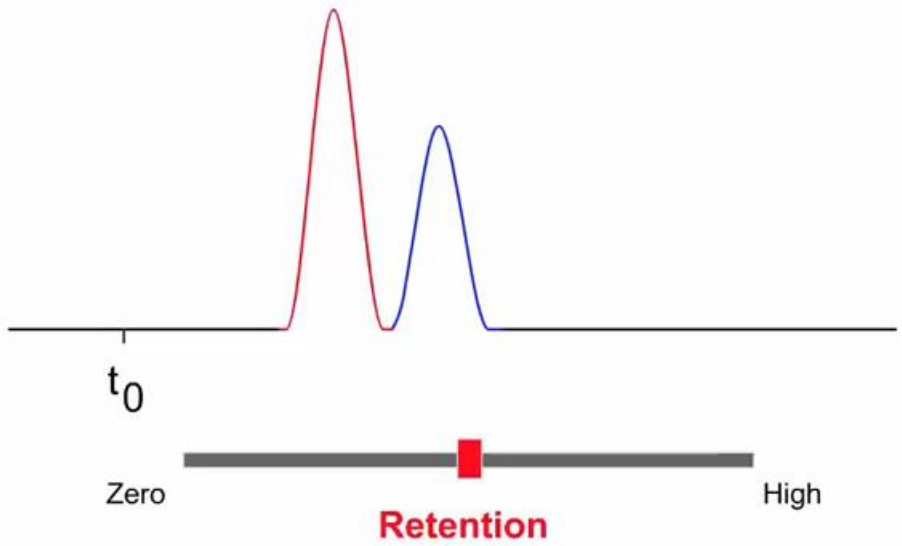
Ardent scientific

k (blue peak) =

k (red peak) =

Selectivity Factor (α) =

Resolution =



https://www.youtube.com/watch?v=kj8RPxM8-Ts&list=PLmRpOX9aPBrIPGcrsJnWuw25fikkugxQL&index=3

Szelektivitás változtatása

Fundamentals of HPLC 16 - Selectivity

Selectivity (α) and Resolution II

In this animation, the retention factor (k) of the two components is also increasing, however, note:

- About half way down the slider bar, the relative k values begin to change leading to a change in selectivity.
- When the k values are equal, selectivity = 1.0 and Resolution = 0 (equation below).
- The peaks swap elution order which can easily happen in HPLC when the mobile phase conditions change and selectivity changes.

$$R \propto \frac{\alpha - 1}{\alpha}$$

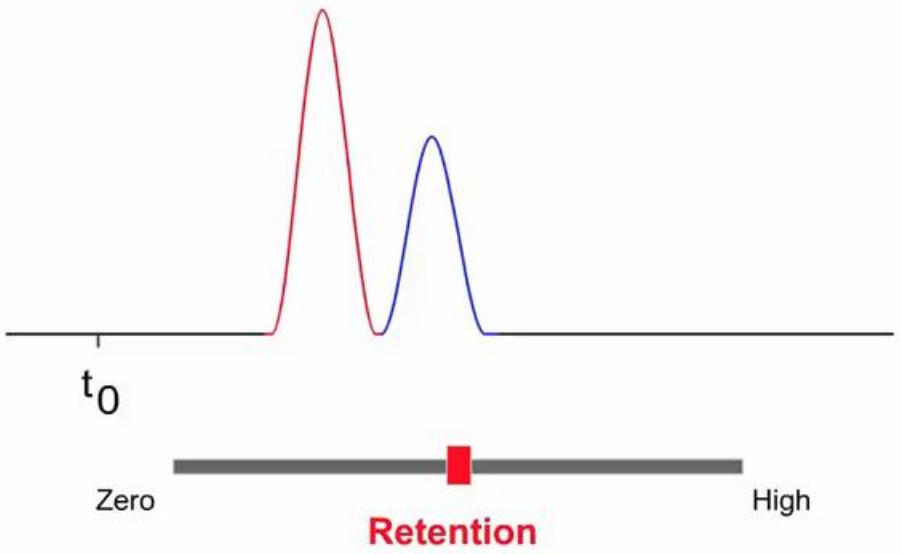
Ardent scientific

k (blue peak) =

k (red peak) =

Selectivity Factor (α) =

Resolution =



https://www.youtube.com/watch?v=Gfu0mxPdAYk&list=PLmRpOX9aPBrIPGcrsJnWuw25fikkugxQL&index=5

Hatékonyság

Fundamentals of HPLC 18 - Efficiency

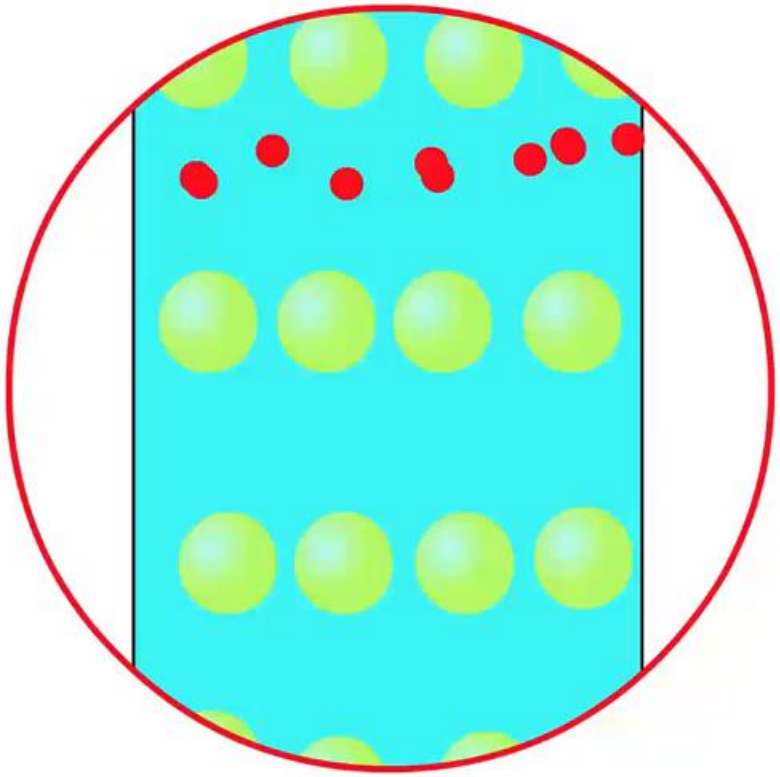
Describing Efficiency

The third and final factor affecting resolution is efficiency.

The efficiency of the separation is related to the number of times the molecules interact between the stationary phase and the mobile phase. One of these interactions is described as one plate.

In this animation, the molecules (red circles) are moving from particle to particle (yellow spheres) of the stationary phase. There are two plates shown in this animation. The group of red molecules is moving into and then out of the first row of particles - one plate. Then the molecules continue on and move in and out of the second row of particles - the second plate.

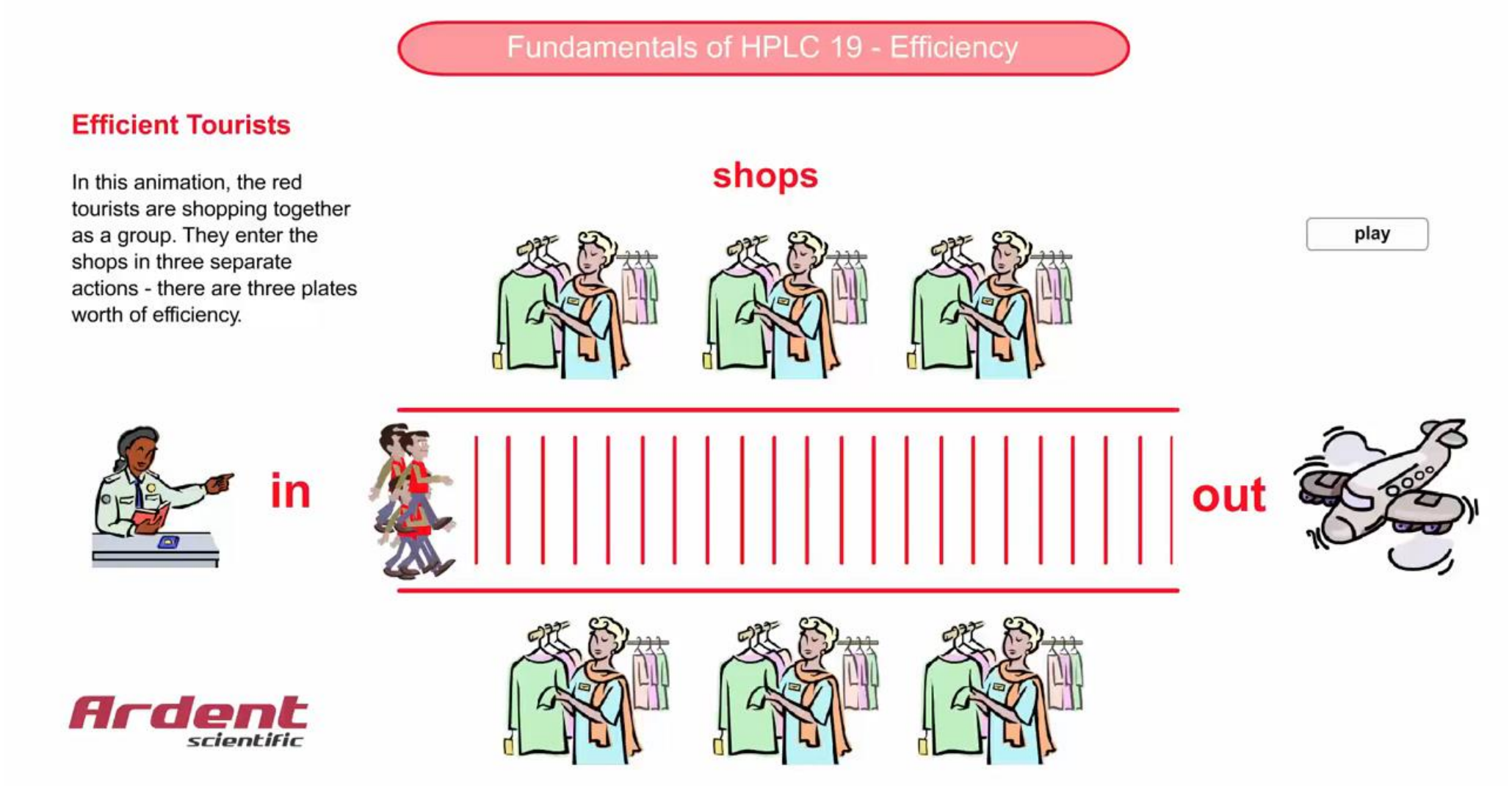
play



Ardent scientific

https://www.youtube.com/watch?v=cDZ6BXxa7GA&list=PLmRpOX9aPBrIQBFEjvr1mfk-XXOooXklT

Hatékony turisták



https://www.youtube.com/watch?v=rvI-gNJm4us&list=PLmRpOX9aPBrIQBFEjvr1mfk-XXOooXklT&index=2

Miért tányérszám?

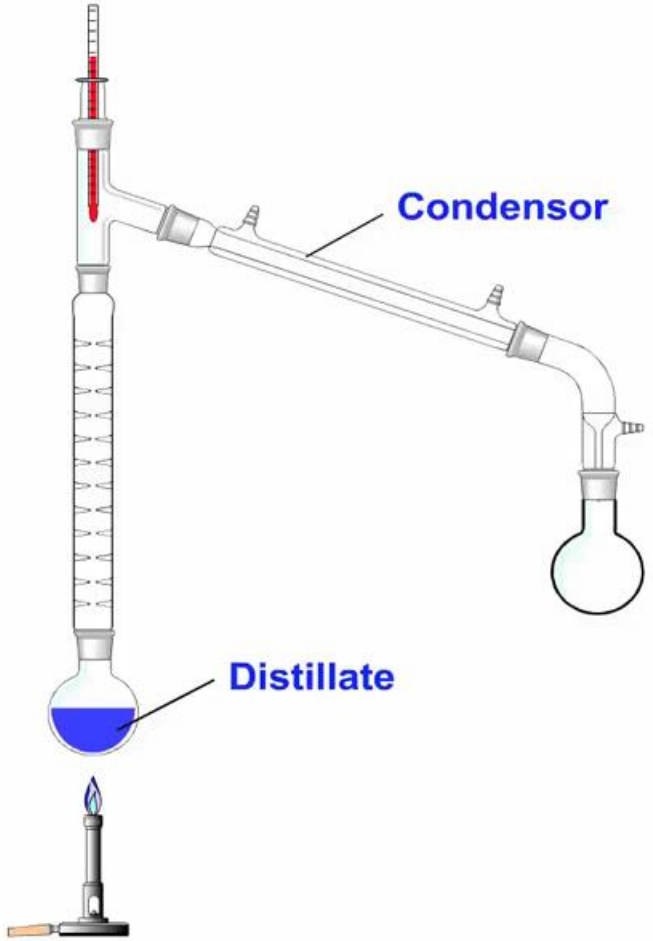
Fundamentals of HPLC 20 - Efficiency

Why Plates?

As we have just seen, the measure of Efficiency in Chromatography is called a Plate. To understand this term we need to see why the word 'plate' is used and a little history.

Petroleum chemists were interested in the proportion of a volume of petroleum distillate that boiled between two boiling points. For example, if a 100mL of distillate was measured, petroleum chemists wanted to know of that 100mL, what volume boiled between 60 and 70ºC. The technique used was a simple distillation apparatus and the distillate (mixture) was heated, boiled and the fraction boiling between these two boiling points was condensed, collected and weighed. It was a time-consuming practice which GC has replaced.

Ardent scientific



play

https://www.youtube.com/watch?v=BU-XGEOYR0M&list=PLmRpOX9aPBrIQBFEjvr1mfk-XXOooXklT&index=3

Desztilláció és tányérszám

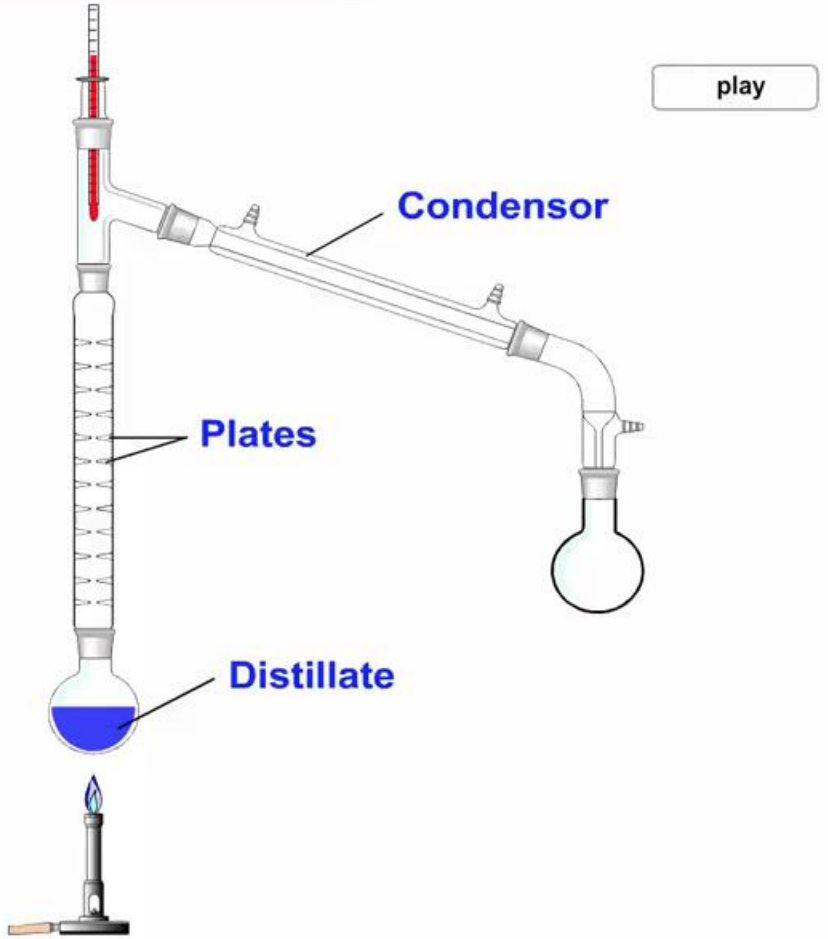
Fundamentals of HPLC 21 - Efficiency

Distillation and Plates

In this animation we 'zoom' in on the distillation glass column which is separating the flask from the condenser. This column contains the plates. In this column the plates are formed glass inside the vertical column.

The vapor of the various fractions rises up the glass column as it is heated. The vapor then condenses on the first plate (which is colder than the flask) before evaporating further. This process continues at each plate. One plate represents one equilibration between the vapor and the liquid phase.

Ardent scientific



https://www.youtube.com/watch?v=mshALfUPrLM&list=PLmRpOX9aPBrIQBFEjvr1mfk-XXOooXklT&index=4

Kromatográfiás oszlop és tányérszám

Fundamentals of HPLC 22 - Efficiency

HPLC Column and Plates

In a HPLC column, there aren't any physical plates and we refer to these as theoretical plates. Therefore, we describe the number of theoretical plates in a column.

One plate in chromatography is one interaction (called a partition) between the stationary phase and the mobile phase. The more partitions in a column, the less spread out the molecules will become and the sharper the peak.

Typically, the number of theoretical plates (N) in a HPLC column is:

250mm x 4.6mm diameter (5 micron particle) - **20,000**
150mm x 4.6mm diameter (5 micron particle - **12,000**
150mm x 4.6mm diameter (3.5 micron particle) - **20,000**

play

Click the Play Button to view the theoretical plates in an HPLC column.

https://www.youtube.com/watch?v=BU-XGEOYR0M&list=PLmRpOX9aPBrIQBFEjvr1mfk-XXOooXklT&index=3

Hatékonyság és kromatogram

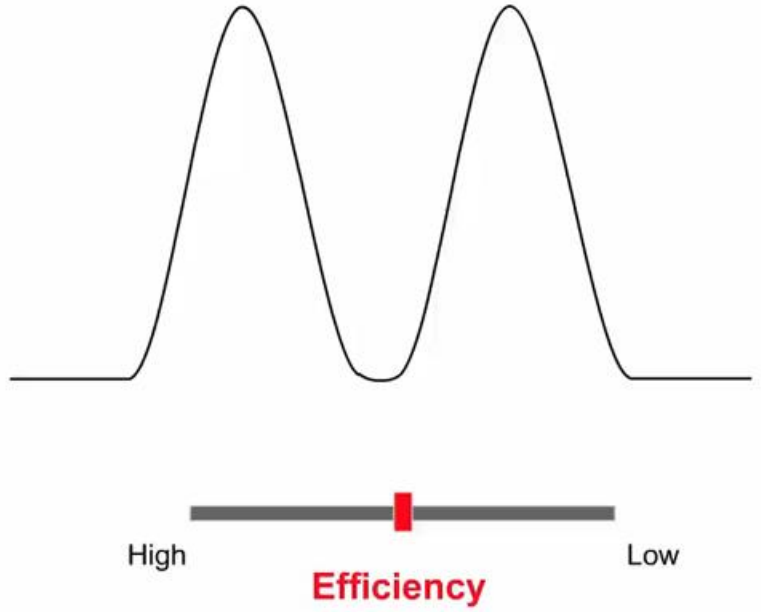
Fundamentals of HPLC 23 - Efficiency

Efficiency and the Chromatogram

As the number of plates in a column increases, the molecules will spread out less and less. This results in a sharper peak. Therefore the more efficient the column and the higher the number of plates - the sharper the peaks will be.

Efficiency is effectively about the sharpness of the peaks.

- High Efficiency = Sharp Peaks
- Low Efficiency = Wide Peaks



Ardent scientific

https://www.youtube.com/watch?v=7XOSoNlfNvk&list=PLmRpOX9aPBrIQBFEjvr1mfk-XXOooXklT&index=6

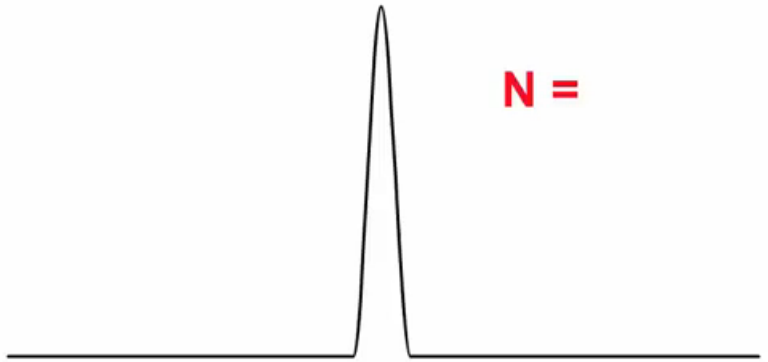
Az elméleti tányérszám kiszámítása

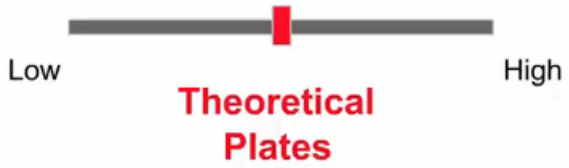
Fundamentals of HPLC 24 - Efficiency

Calculating Plates

Unlike the distillation column, we can't directly count the number of plates in a chromatographic column so we measure the plate number indirectly through examination of the chromatogram. As the system becomes more inefficient (loss of plates), the peak will broaden for a given retention time. Thus we can use the combination of retention time and peak width ($W_{1/2}$ = peak width at half the peak height) to calculate the plate number (**N**) in a system (equation below).

$$\text{Plates (N)} = 5.54 \left(\frac{t_R}{W_{1/2}} \right)^2$$





Ardent scientific

https://www.youtube.com/watch?v=-O2C6B1ghIA&list=PLmRpOX9aPBrIQBFEjvr1mfk-XXOooXklT&index=7

Az elméleti tányérszmagasság

Fundamentals of HPLC 25 - Efficiency

Another Way of Calculating Plates - HETP

There is yet another way to calculate plates. Instead of considering the total number of plates in the column, we can view the length of just one plate. So for example if there were 50 total plates in the column and the length of the column was 200 mm, then each plate length would be simply 4 mm (200/50). This plate length is termed HETP which stands for Height Equivalent of a Theoretical Plate. The Height term again comes from distillation theory where the columns are in a vertical orientation.

We shall refer to HETP in later videos on Band Broadening.

play

Ardent scientific

https://www.youtube.com/watch?v=XowstaYtiOo&list=PLmRpOX9aPBrIQBFEjvr1mfk-XXOooXklT&index=8

Elméleti tányérszám és felbontás

Fundamentals of HPLC 26 - Efficiency

Plates and Resolution

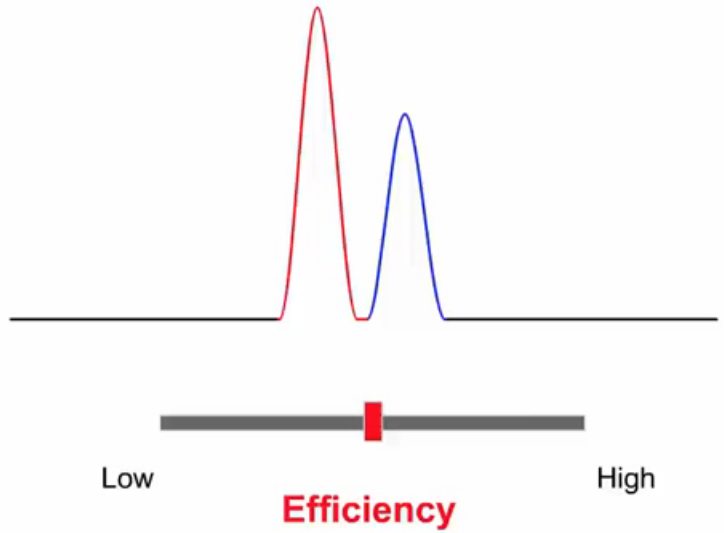
Resolution is proportional to the square root of the Plate number as shown in the equation below.

Efficiency (plates) in HPLC can be increased by:

- increasing column length. Doubling column length doubles the plate number but only increases resolution by 40%.
- decreasing particle size of the packing
- optimizing flow rate

$$R \propto \sqrt{N}$$

Ardent scientific



Resolution =

Plates =

https://www.youtube.com/watch?v=_ZPgkLQPuKI&list=PLmRpOX9aPBrIQBFEjvr1mfk-XXOooXklT&index=9

A felbontás (összefoglalás)

Fundamentals of HPLC 27 - Efficiency

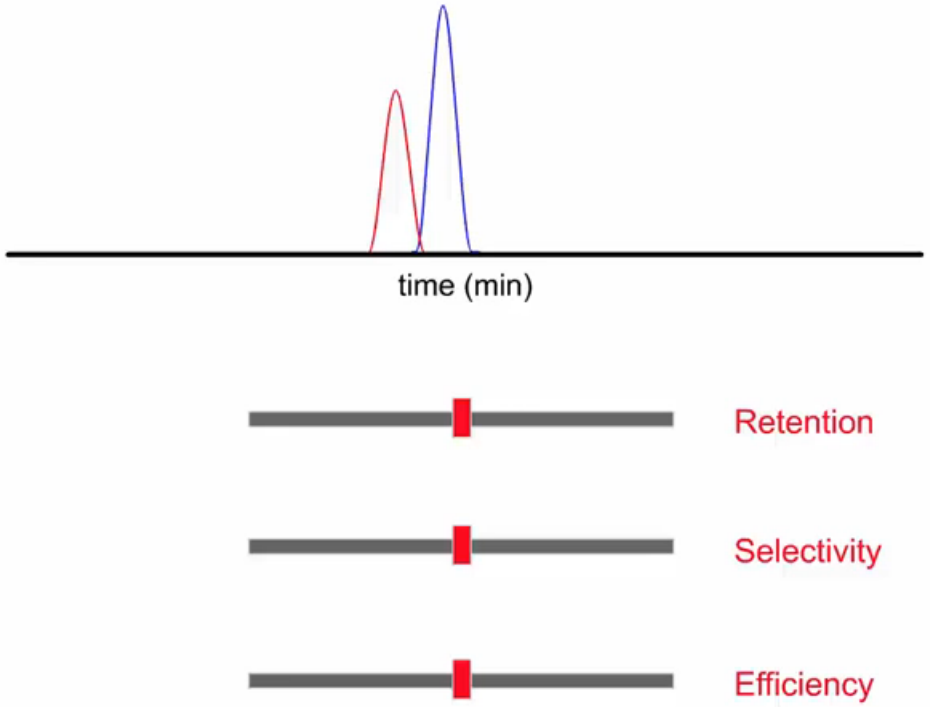
Retention, Selectivity and Efficiency

Now let's combine these three factors we have been sudying and summarise them together in the same chromatogram.

In chromatography, the goal is to separate the compounds in the mixture in the shortest time. The measure of this separation is Resolution.

- **Retention** - a measure of the degree of interaction with the stationary phase.
- **Selectivity** - a measure of the differences in the chemistries of the components in the mixture.
- **Efficiency** - a measure of the sharpness of the peaks.

Ardent scientific



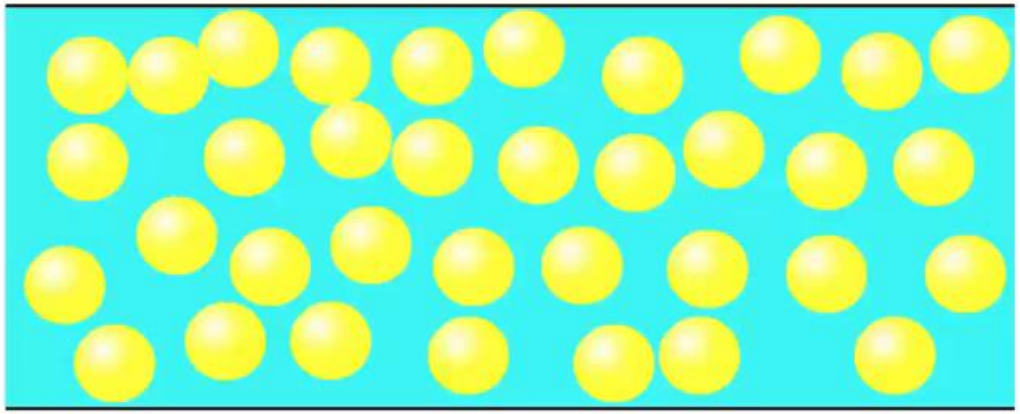
https://www.youtube.com/watch?v=UGvxH7TzZRg&list=PLmRpOX9aPBrIQBFEjvr1mfk-XXOooXklT&index=10

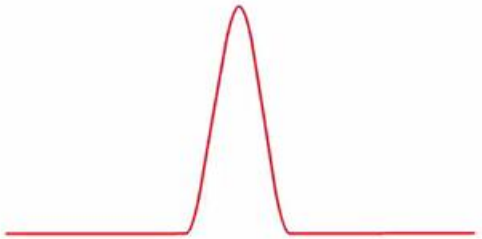
Csúcsszélesedés – eddy (örvény) diffúzió

Fundamentals of HPLC 28 - Band Broadening

Eddy Diffusion

The first of the band broadening mechanisms we will look at is called Eddy Diffusion. I often describe this as either taking the route to work (shortest path) or the scenic route (longest path). So although the molecules can start out close together, by the end of the column they have spread apart i.e. the band has broadened. Play the animation to see this in action.





play

Ardent scientific

https://www.youtube.com/watch?v=p2KvzK81s2g&list=PLmRpOX9aPBrL3ha0tZ15HyDQTlo27W59V

Örvény diffúzió lineáris áramlási sebesség függése

Fundamentals of HPLC 29 - Band Broadening

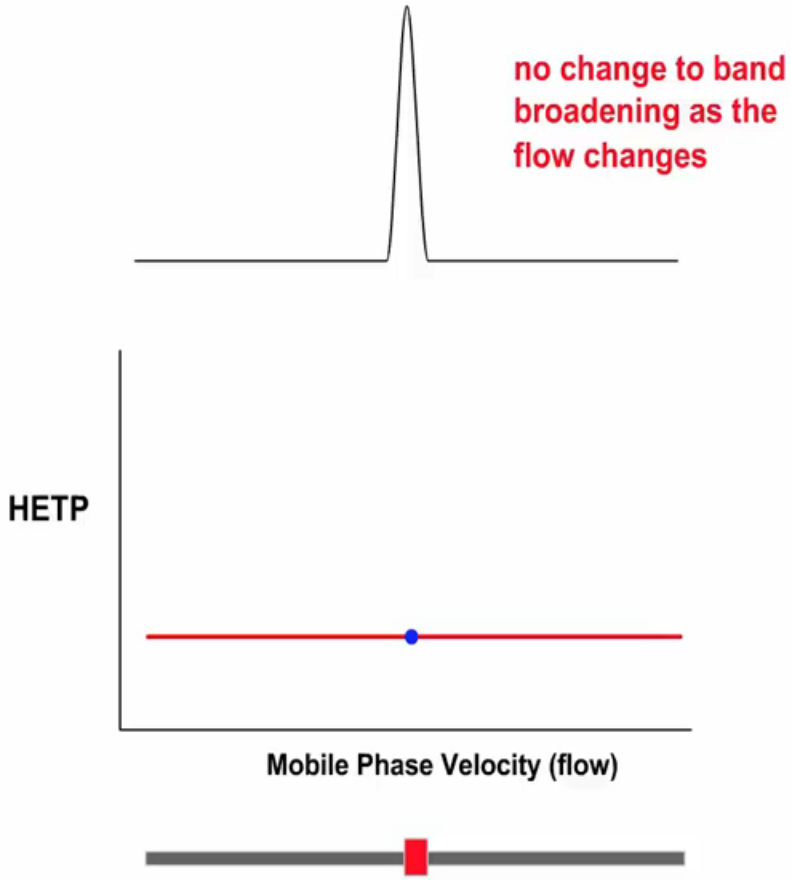
Eddy Diffusion - Mobile Phase Velocity

In this section we are going to compare each of the ways the band can spread apart against the speed (velocity) of the mobile phase.

In the Efficiency section under 'Fundamentals of HPLC', we introduced the term plates and then HETP. If you can't remember what these mean, it might be worth reviewing these terms again. However, the lower the HETP value, the better. A low HETP value equates to a high plate number and means minimal band broadening.

If we plot HETP versus Mobile Phase Velocity, we get a straight line. This is to be expected because changing the flow rate doesn't change the paths the molecules take along the column.

Ardent scientific



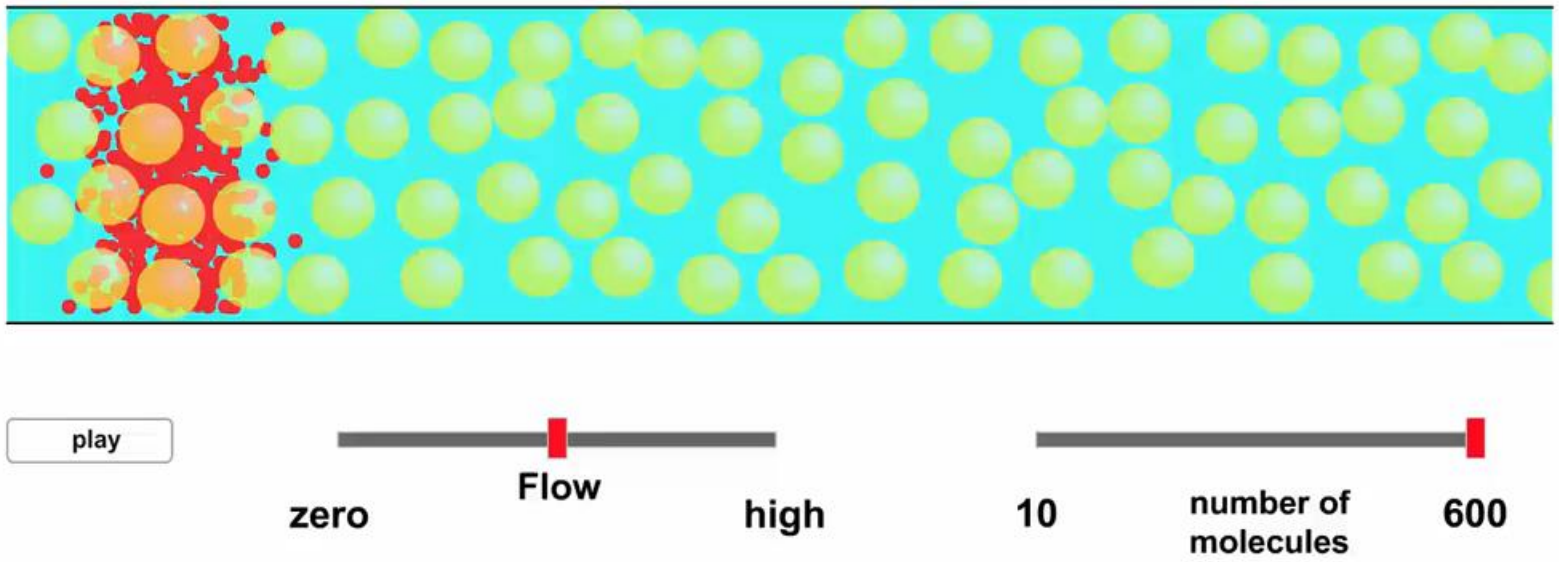
https://www.youtube.com/watch?v=Izg8Xd4Wsek&list=PLmRpOX9aPBrL3ha0tZ15HyDQTlo27W59V&index=2

Hosszirányú diffúzió

Fundamentals of HPLC 30 - Band Broadening

Longitutinal Diffusion

Play the animation and watch especially as the molecules spread apart as the flow is lowered. You may even want to stop the flow altogether. The faster the flow, the less time the molecules will have to spread out in the column. High flow equals sharp peaks.



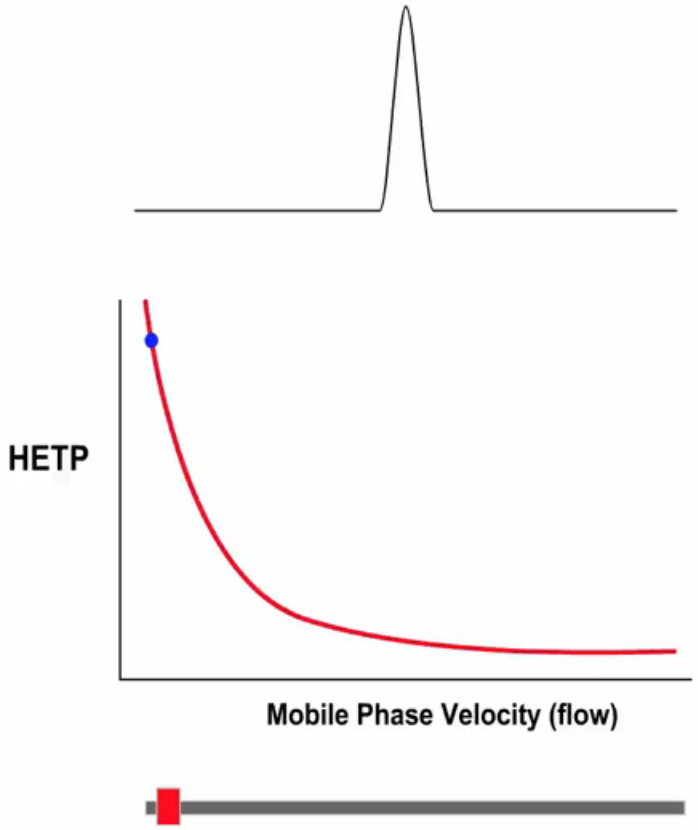
https://www.youtube.com/watch?v=wG5nDzKuGDU&list=PLmRpOX9aPBrL3ha0tZ15HyDQTlo27W59V&index=3

Hosszirányú diffúzió lineáris áramlási sebesség függése

Fundamentals of HPLC 31 - Band Broadening

Longitudinal Diffusion - Mobile Phase Velocity

If we plot the HETP value vs Flow for longitudinal diffusion, you see that it is an inverse relationship. The faster the flow, lower the HETP value and the sharper the peak. And you will remember that sharp peaks means better resolution.



Ardent scientific

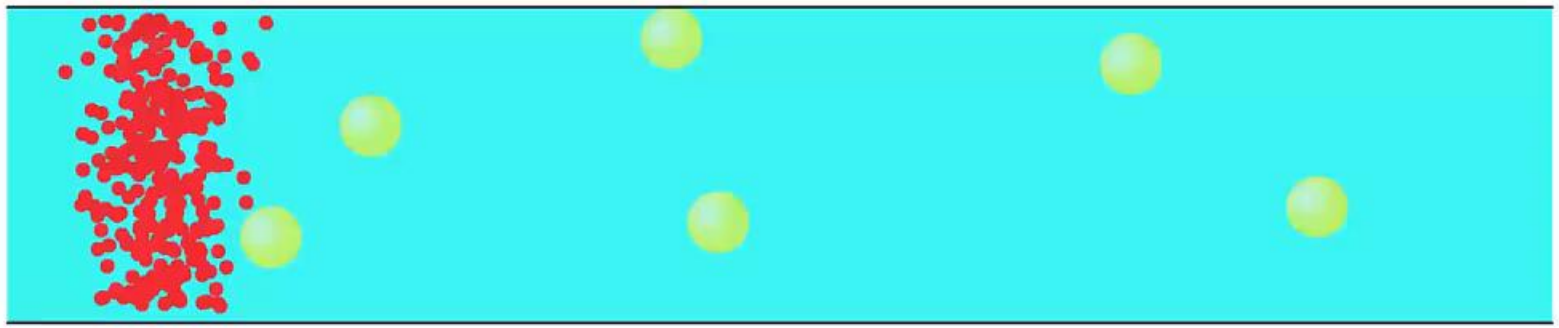
https://www.youtube.com/watch?v=gdaAassaAaA&list=PLmRpOX9aPBrL3ha0tZ15HyDQTlo27W59V&index=4

Anyagátadási ellenállás

Fundamentals of HPLC 32 - Band Broadening

Mass Transfer

There are deliberately less packing particles in this animation to demonstrate the effect more clearly. Play the animation and watch especially as the molecules spread apart as the flow is increased. The slower the flow, the more time the molecules will have to diffuse out of the particle and stay with the other molecules which are in the mobile phase and have not entered the particle. Low flow equals sharp peaks.



play

zero — Flow — high

10 — number of molecules — 600

Ardent scientific

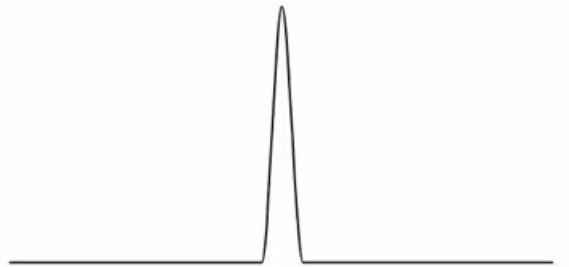
https://www.youtube.com/watch?v=u7EPAPQDLlY&list=PLmRpOX9aPBrL3ha0tZ15HyDQTlo27W59V&index=5

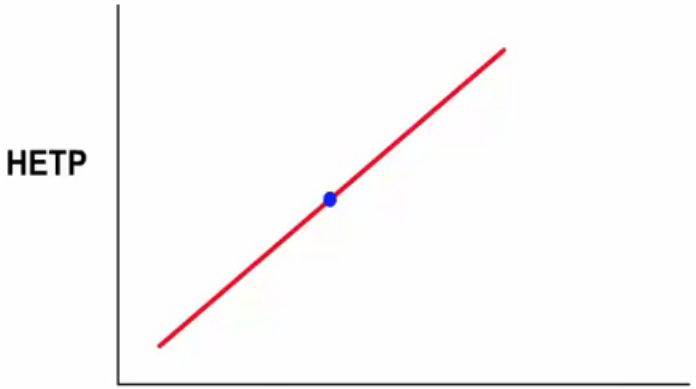
Anyagátadási ellenállás lineáris áramlási sebesség függése

Fundamentals of HPLC 33 - Band Broadening

Mass Transfer - Mobile Phase Velocity

You can see from the grap that the relationship between HETP and flow is a linear one. Increasing the flow in this case is a bad thing and leands to band broadening.





Ardent scientific

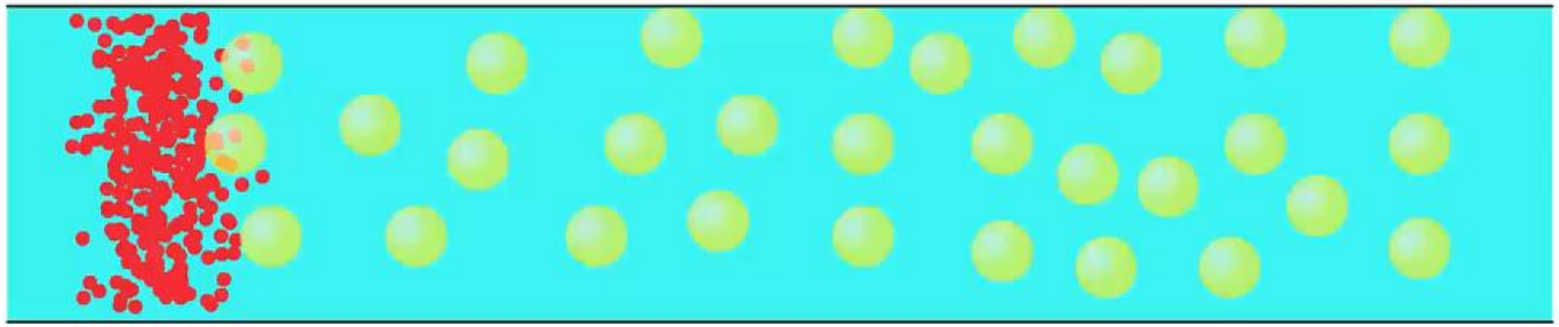
https://www.youtube.com/watch?v=KXg4FrRiEq0&list=PLmRpOX9aPBrL3ha0tZ15HyDQTlo27W59V&index=6

Hosszirányú diffúzió és anyagátadási ellenállás

Fundamentals of HPLC 34 - Band Broadening

A Happy Medium!

This animation combines longitudinal diffusion and mass transfer. As we play around with the flow slider bar, you'll notice that if the flow is too low, longitudinal diffusion dominates. If the flow is too fast, mass transfer dominates.



play

zero — Flow — high

10 — number of molecules — 600

Ardent scientific

https://www.youtube.com/watch?v=vCt0C5OvsxM&list=PLmRpOX9aPBrL3ha0tZ15HyDQTlo27W59V&index=7

A lineáris áramlási sebesség optimalizálása

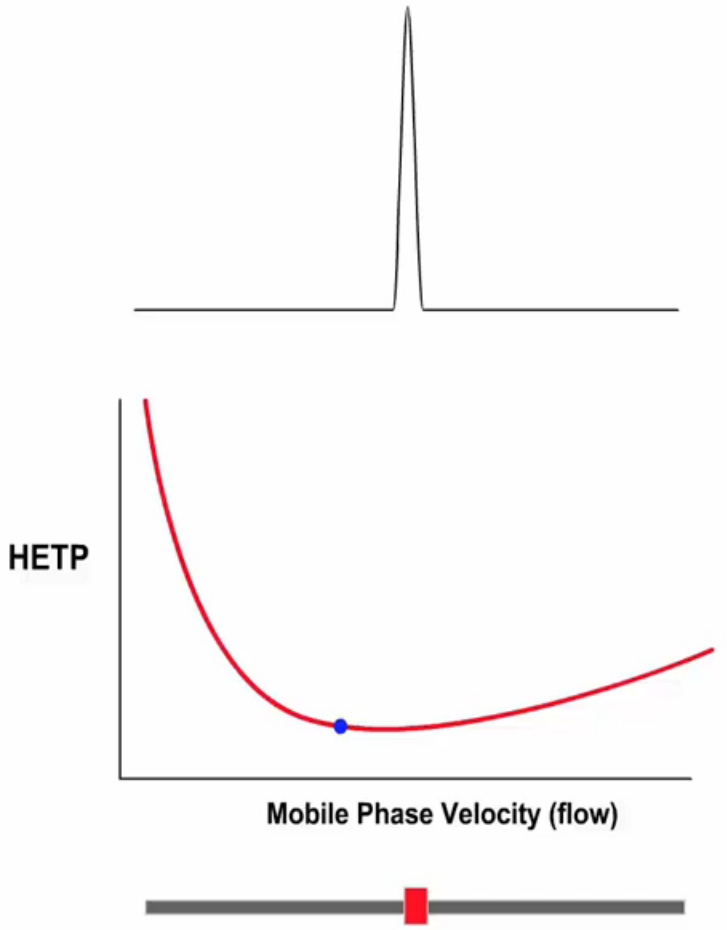
Fundamentals of HPLC 35 - Band Broadening

The Reality!

So let's put all this together and understand what it means for the practising chromatographer. The reality is there is always an optimal flow rate for a given column with a given particle size. Too low a flow results in a broader peak (longitudinal diffusion) and two high a flow results in a broader peak (mass transfer).

There is an optimal flow that takes into account the band broadening mechanisms that we have seen. I like to refer to this as the 'Goldilocks' value - not too hot and yet not too cold; just right!

Ardent scientific



https://www.youtube.com/watch?v=vG3mcMgFNv0&list=PLmRpOX9aPBrL3ha0tZ15HyDQTlo27W59V&index=8