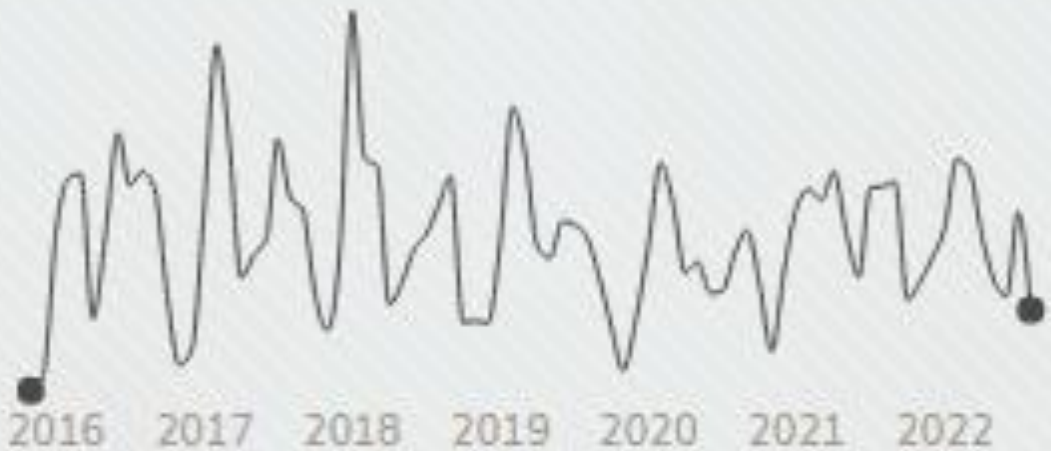
BIOLOGIST

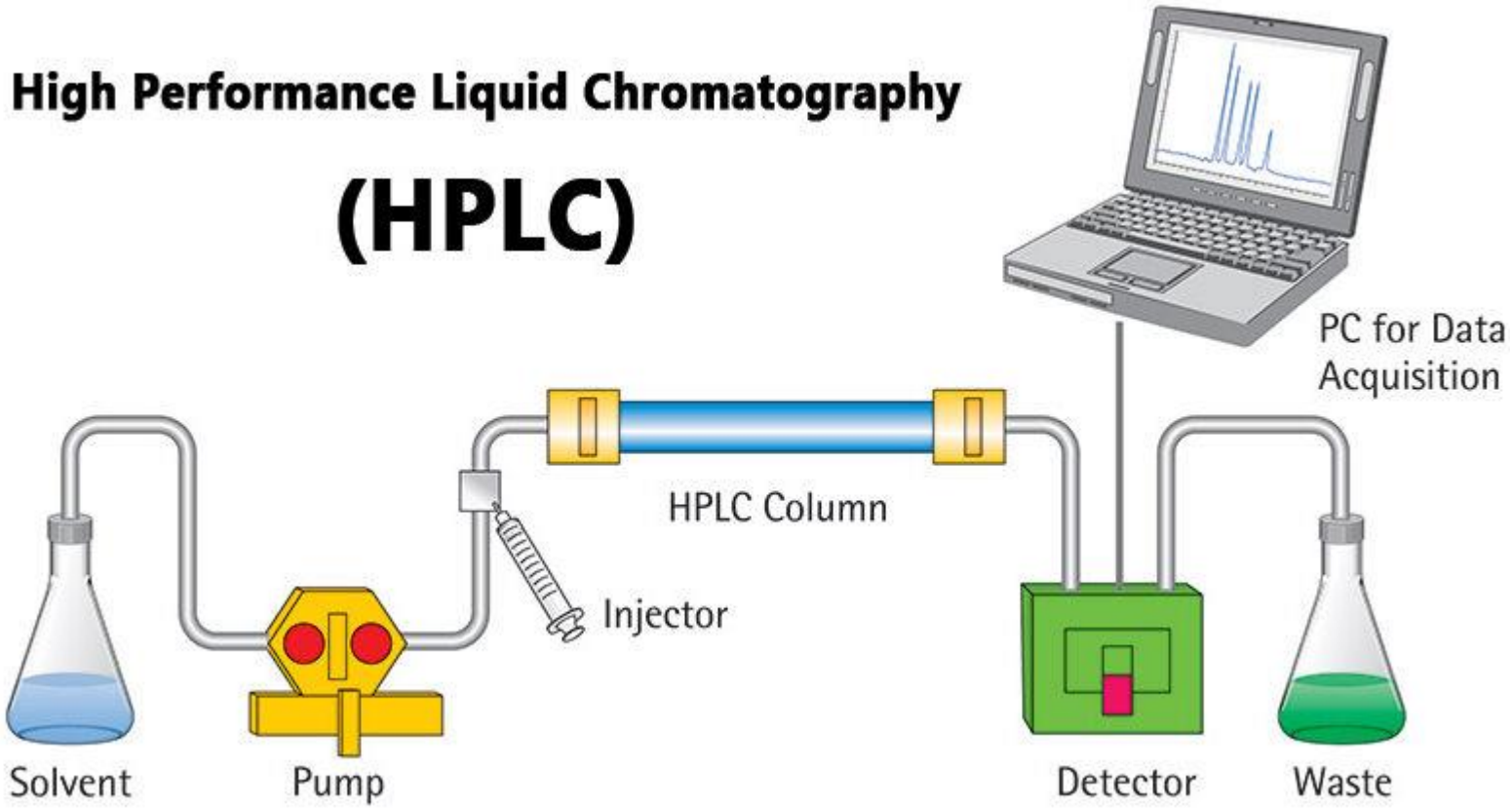
MIKHAIL TSVET

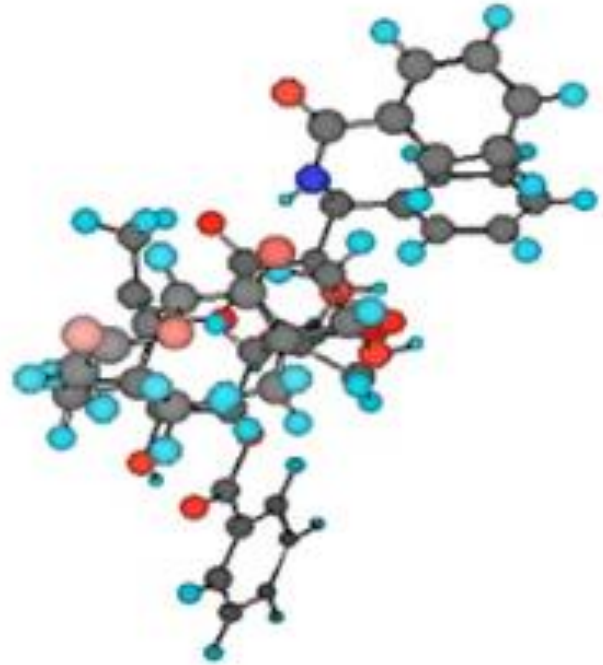
1872 - 1919



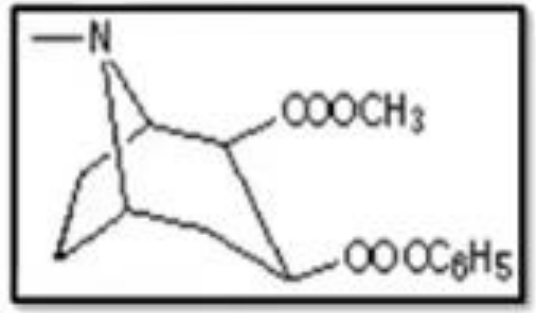
EN.WIKIPEDIA PAGE VIEWS (PV)

المركز العراقي لبحوث السرطان والوراثة الطبية / الجامعة المستنصرية
د. زينب سعد عبد الغني
قسم الاحياء الجزيئي



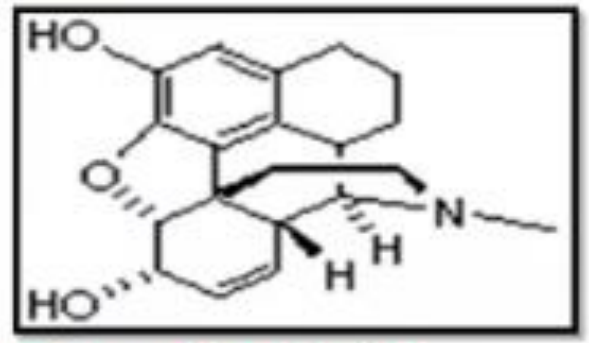


The primary source of Taxol is the **bark of yew trees;** however, the accumulated concentration of Taxol is very low. The evergrowing demand for Taxol greatly exceeds the supply through isolation from its natural source.



Cocaine (narcotic)

Cocaine is a tropane alkaloid and stimulant drug obtained primarily from the leaves of two coca species native to South America, Erythroxylum coca and Erythroxylum novogranatense.



Morphine (narcotic analgesic)

From Opium

Morphine is a pain medication of the opiate family that is found naturally in a dark brown, resinous form, from the poppy plant (**Papaver somniferum**).

Chromatography

The most widely used means of performing analytical separations is chromatography, a method that finds application to all branches of science.

Chromatographic techniques are commonly applied for the separation, identification, and determination of the chemical components in complex mixtures including gases, and volatile substances; nonvolatile and polymeric material as well as biological substances.

Definition of chromatography:

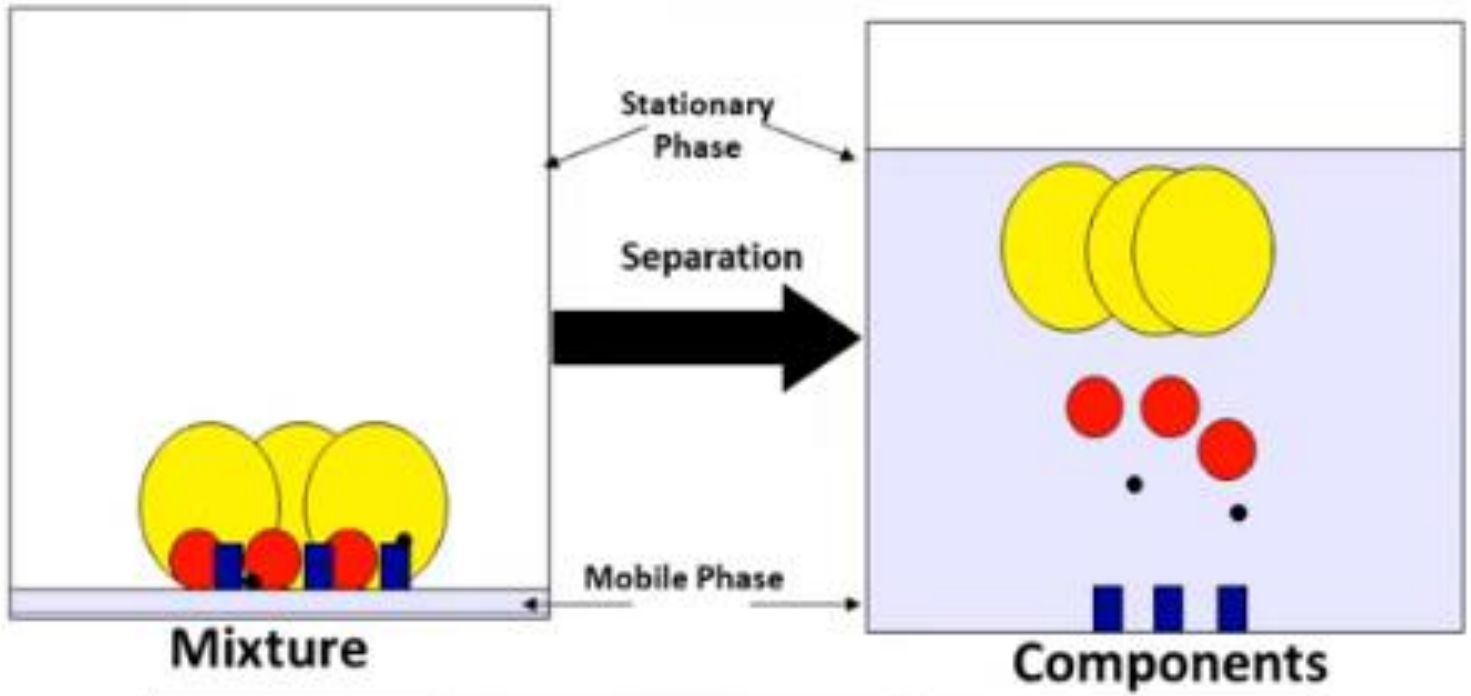
Tswett (1906) stated that " chromatography is a method in which the components of a mixture are separated on adsorbent column in a flowing system".

IUPAC definition:

(International Union of pure and applied Chemistry) (1993): Chromatography is a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary while the other moves in a definite direction.

The stationary phase may be a solid, or a liquid supported on a solid or gel, the mobile phase may be either a gas or a liquid.

Illustration of Chromatography



Components	Affinity to Stationary Phase	Affinity to Mobile Phase
Blue	----------	Insoluble in Mobile Phase
Black	✓✓✓✓✓✓	✓✓
Red	✓✓	✓✓✓✓✓
Yellow	✓	✓✓✓✓✓✓✓✓✓✓✓

كروماتوغرافيا HPLC

- تقنية الكروماتوغرافيا السائلة عالية الأداء HPLC من أفضل التقنيات المنتشرة في جميع الصناعات المختلفة فهي مشهورة جداً ويزيد البحث عنها كثيراً، كما أن أنواعها مختلفة وجميعها لها أهمية كبيرة، فمن أن أهم أنواعها على سبيل المثال كروماتوغرافيا HPLC الغاز وكذلك كروماتوغرافيا HPLCالسائل وغيرها من العديد من الأنواع المختلفة

history

- Mikhail Semyonovich Tsvet (14 May 1872 – 26 June 1919) was a Russian-Italian botanist who invented chromatography. His last name is Russian for "color" and is also the root word of "flower.“
- Mikhail Tsvet invented chromatography in 1900 during his research on plant pigments.
- He used liquid-adsorption column chromatography with calcium carbonate as adsorbent and petrol ether/ethanol mixtures as eluent to separate chlorophylls and carotenoids

Historical Background:

Russian scientist Tswett in 1906 used a glass columns packed with finely divided $CaCO_3$ to separate plant pigments extracted by hexane. The pigments after separation appeared as colour bands that can come out of the column one by one.

12 Nobel prizes were awarded between 1937 and 1972 alone for work in which chromatography played a vital role

Important contributors include: A.J.P. Martin and R.L.M Synge (Nobel Prize 1952 for partition chromatography); A. T. James and Martin (gas-liquid chromatography, 1952); Porath (Size exclusion chromatography, 1958 and affinity chromatography, 1967).

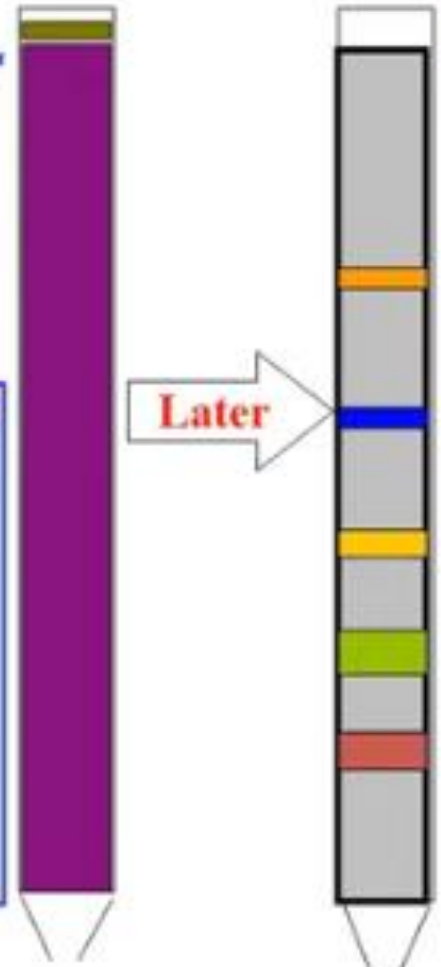
M.S. Tswett

Original Chromatography Experiment

Start: A glass column is filled with powdered limestone ($CaCO_3$).

An EtOH extract of leaf pigments is applied to the top of the column.

EtOH is used to flush the pigments down the column.



Later

End: A series of colored bands is seen to form, corresponding to the different pigments in the original plant extract. These bands were later determined to be chlorophylls, xanthophylls and carotenoids.

كروماتوغرافيا HPLC

- أنواع هذه التقنية يمكن تحديدها على حسب نوع الطور: فمثلًا يمكن تحديدها على حسب الطور الحامل وكذلك الطور الثابت،
- كما أن كروماتوغرافيا السائل تعتمد بشكل كبير على الطور الحامل الثابت : وذلك يتم من خلال عملها حيث أن عملها يتم فيه استخدام هذه التقنية وذلك من أجل تحديد هوية المركبات بالإضافة إلى هوية السوائل، خاصة مجموعة السوائل التي تكون درجة غليانها مرتفعة نسبياً
- والفرق بين كروماتوغرافيا سائلة و الكروماتوغرافيا الغازية أنه السائلة تحتاج إلى ضغوط مرتفعة وذلك مقارنة بالغازية حيث أنها تحتاج إلى ضغط منخفض بشكل نسبي،
- وفي نهاية السبعينات قد لقى هذا الطور عملية إقبال شديدة من قبل كثير من الناس وكذلك من الباحثين حيث أنهم قد قاموا باستخدامه كوسيلة رئيسية في عملية الفصل وتحليل المواد الكيميائية الكيميائية.

Principles of Chromatography:

- Chromatography is a physical process.
- Any Chromatography system is composed of three Components :

-Stationary phase
-Mobile phase
-Mixture to be separated

We can only control stationary and mobile phase as mixtures are the problem we have to deal with.

- Chromatography is a dynamic process in which the mobile phase moves in definite direction.

How Does Chromatography Work?

In all chromatographic separations, the sample is transported in a **mobile phase**. The mobile phase can be a gas, a liquid, or a supercritical fluid.

The mobile phase is then forced through a **stationary phase** held in a column or on a solid surface. The stationary phase needs to be something that **does not react** with the mobile phase or the sample.

The **sample** then has the **opportunity to interact** with the stationary phase as it moves past it. Samples that interact greatly, then appear to move more slowly.

Samples that interact weakly, then appear to move more quickly. Because of this difference in rates, the samples can then be **separated into their components**.

Theoretical basis of Chromatography:

The following theoretical concepts are applicable to all types of chromatographic techniques.

i.e.

1- It is a technique used to separate and identify the components of a mixture.

2- It works by allowing the molecules present in the mixture to distribute themselves between a stationary and a mobile phases.

3- Molecules that spend most of their time in the mobile phase are carried along faster.

Distribution equilibria and rate of travel:

- During chromatography a given solute finds itself either in the stationary phase, which acts as a **"retarder"** or in the mobile phase, which acts as a "**carrier**".
- This distribution is based on distribution equilibria and is expressed by the rate of travel.
- Substances that are distributed preferentially in the moving phase pass through the chromatographic system faster than those that are distributed preferentially in the stationary phase.

I. Distribution equilibria:

The distribution of solutes between the two phases is governed by an equilibrium constant known as the **distribution coefficient, K** (or partition coefficient in certain types of chromatography). This allows quantifying the distribution of a compound between the stationary and mobile phases.

$$K = C_{stationary} / C_{mobile}$$

Where,

$C_{stationary}$ = Concentration of solute in the stationary phase

C_{mobile} = Concentration of solute in the mobile phase

The K value determines the relative population in the two phases **i.e. large K value means that more time is spent in the stationary phase, and vice versa if the K value is small the solute will be eluted very fast with the mobile phase.**

K is a thermodynamic constant that is characteristic for a given compound under specified chromatographic conditions.

II. Rate of travel:

Factors limiting the rate of travel:

The rate of travel of a solute in a chromatographic system is limited by:

1. The velocity of the mobile phase (or carrier).
2. The ratio of volume of the stationary phase to the volume of the mobile phase.
3. The value of the distribution coefficient that is characteristic for each component.

Determination of the rate of travel:

In a chromatographic experiment, all sample constituents started to be distributed between the two phases from the same point at the same time, but each component moves at a different rate.

Determination of the rate of travel could be performed by:

1. Measuring the distance traveled by each solute after a fixed time as in planar chromatography.
2. Measuring the time interval at which each component appears after a fixed distance as detected in columnar chromatography.

- The rate of travel or retention behavior of a solute (t_R) varies with flow rate (temp., etc.

Classification of Chromatographic Techniques:

A) According to separation theory.

B) According to the method of holding the stationary phase.

C) According to purpose of uses.

D) According to mobile phase:

A) According to separation theory:

The relative affinity of the individual components of the mixture for the stationary phase is determined by the differences in their physical and chemical properties and this leads to differences in their rate of migration through the system with the mobile phase. The most common techniques are:

1) Adsorption chromatography:

Adsorption is defined as intermolecular forces between moving molecules in the mobile phase and the atoms of a solid phase. The intermolecular forces thought to be involved are:

3. Ion Exchange Chromatography (IEC):

It is used for separation of charged molecules. The stationary phase is an ion exchange resin to which a cationic or anionic groups are covalently bonded. Ions of opposite charges (counter ions) in the mobile phase will be attracted to the resin and compete with the components of the mixture for the charged group on the resin. Both the mixture components and the mobile phase must be changed. Mixture of Alkaloids (compounds with positive charges) can be separated on anionic exchanger, while mixture of organic acids (negative charges) can be separated using cationic exchanger. Both types are used for desalination of water.

4. Molecular Exclusion Chromatography (Gel permeation or Gel filtration):

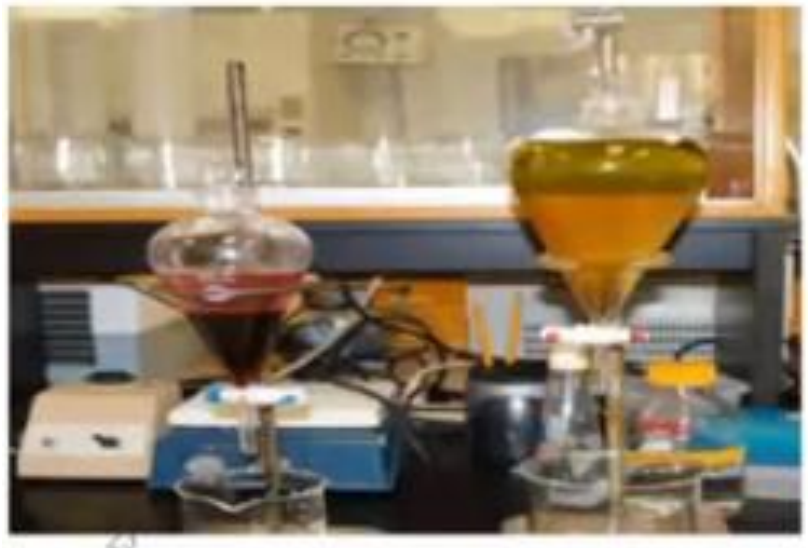
In this case a highly porous gel is used to separate substances according to their molecular size and shape. The mobile phase (liquid or gas) passes through a porous gel, which separates the molecules according to their size. The pores are small and exclude the larger solute molecules, but allow smaller molecules to enter the gel, causing them to flow through a larger volume. This causes the larger molecules to pass through the column at a faster rate than the smaller ones.

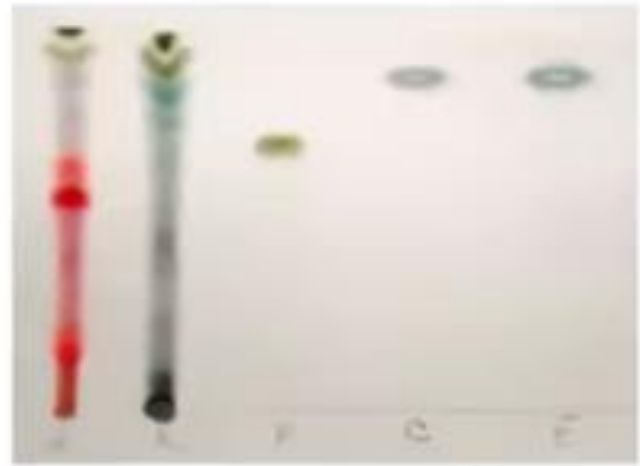
5. Affinity Chromatography:

This is the most selective type of chromatography. It is based on the specific interaction between one kind of solute molecule and a second molecule that is immobilized on a stationary phase.

6- Chiral Chromatography:

In this type we can separate enantiomers – we used chiral stationary phase that react with one enantiomer more than the other so separation takes place





B) According to method of holding the stationary phase:

In this respect chromatographic techniques are classified into two groups: of paper or a layer of adsorbent spread on glass, plastic or aluminum sheets.

1- Planar or Plane Chromatography: In this type of chromatography the stationary phase is used in the form of layer. Plane chromatography is further classified into:

a- Thin Layer Chromatography (TLC):

The stationary phase in the form of fine powder is spread on glass or plastic or aluminum sheets.

b- Paper Chromatography (PC):

A specific type of papers is used as stationary phase in the form of sheets.

2- Columnar or Column Chromatography (CC):

The stationary phase is held in to a tube made of glass or metal.

C)-ACCORDING TO PURPOSE OF USE:

Chromatography can be used for analytical work and also to obtain pure materials from mixtures.

1- Analytical Chromatography:

a- Qualitative Chromatography

In this case Chromatography can be used to:

1- Confirm the absence or probable presence of certain constituent in the sample under investigation

2- Give an idea about the complexity of the mixture and the least number of compounds present.

3- Check purity and identity of any compound.

4- Establish a (finger print) pattern for extracts , volatile oils or pharmaceutical preparations. These finger prints can be then used to check the identity and purity in the future.

5- Monitor both column chromatography and organic chemical reactions.

b- Quantitative Chromatography:

The development of modern instruments enable the use of chromatography to determine the amount of any component in a mixture as absolute amount or relative to another component HPLC/ GC/ HPTLC can be used for there applications.

2- Preparative application:

This was the first and is the main application of chromatography. The technique was developed primarily for this purpose.

Chromatography is used to obtain reasonable quantities of pure compounds from mixtures.

D) Classification of chromatography according to mobile phase:

1- Gas Chromatography (GC)

Where the mobile phase is inert gas nitrogen or helium. Again if the stationary phase is solid it is called: Gas–Solid Chromatography (GSC). When stationary phase is liquid it is called: Gas-Liquid Chromatography (GLC).

2 Liquid Chromatography (LC):

adsorption The mobile phase is liquid. In case of separation by the stationary phase is solid so it is called: Liquid-Solid Chromatography (LSC). If separation occurs through partition the stationary phase is liquid so it is called: Liquid -Liquid Chromatography (LLC).

3- Supercritical fluid chromatography (SFC):

Supercritical fluid ex. CO_2

High-performance Liquid Chromatography (HPLC)

Intoduction

High-performance liquid chromatography (or **High pressure liquid chromatography, HPLC**)is a form of column chromatography used to separate, identify, and quantify compounds. HPLC utilizes a column that holds chromatographic packing material (stationary phase), **a pump** that moves the mobile phase(s) through the column, and a detector that shows the **retention times of the molecules. Retention time varies depending on the interactions between the stationary phase, the molecules being analyzed, and the solvent(s) used.**

مكونات الجهاز

- يتكون هذا الجهاز من العديد من المكونات الأساسية

١. نظام توصيل المذبذب

وهذا النظام عبارة عن مضخة تعمل على ضمان السريان الحر وذلك الأمر يتم بشكل مستمر على الطور الحامل.

٢. نظام إدخال العينة

وهذا النظام يمكننا استخدامه إما بشكل يدوي أو آلي حيث أنه من خلاله يتم استخدام العديد من الصمامات المختلفة، حيث أنه عندما نقوم بفتح هذه العينة يتم من خلالها ملئ تجويف العينة بشكل كبير. ولكن في حال أن يتم غلق جميع الصمامات فهنا يتم ذهاب العينة إلى الطور الحامل، خصوصاً ذي الضغط العالي الذي عندما يتم إرساله إلى العمود وبعد ذلك يتم عملية تحليلها.

٣. وعاء الطور الحامل

- يعتبر هذا الوعاء عبارة عن دورق ولابد أن يكون هذا الوعاء نظيف ونقي جداً.

- كما أنه يجب أن يكون مفرغ بشكل نهائي من جميع الغازات التي فيه وذلك من أجل تفادي الخطأ بشكل نهائي وذلك عند القيام بعملية التحليل، حيث أنها تعتبر من العمليات المهمة التي يجب علينا القيام بها.

٤. العمود

- وهذا يعتبر من الأجزاء المهمة في هذا الجهاز، بالإضافة إلى أن هذا العمود يعتبر مضاد للتآكل كما أنه ينقسم إلى نوعين رئيسيين هما أعمدة تحليلية وأعمدة أولية.

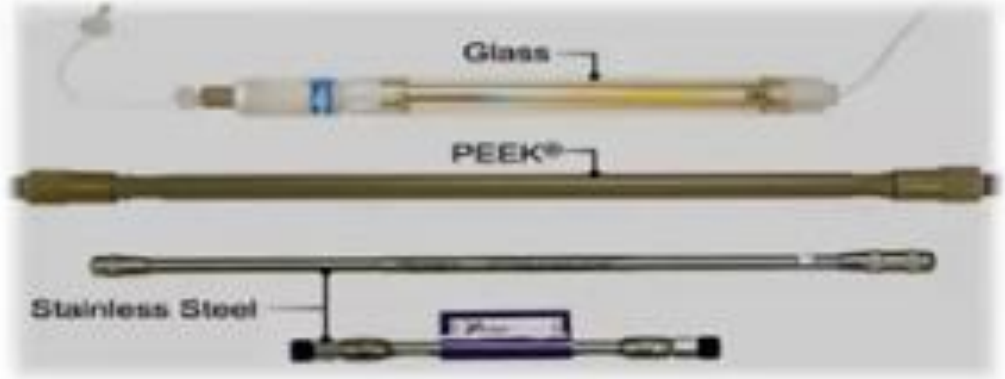
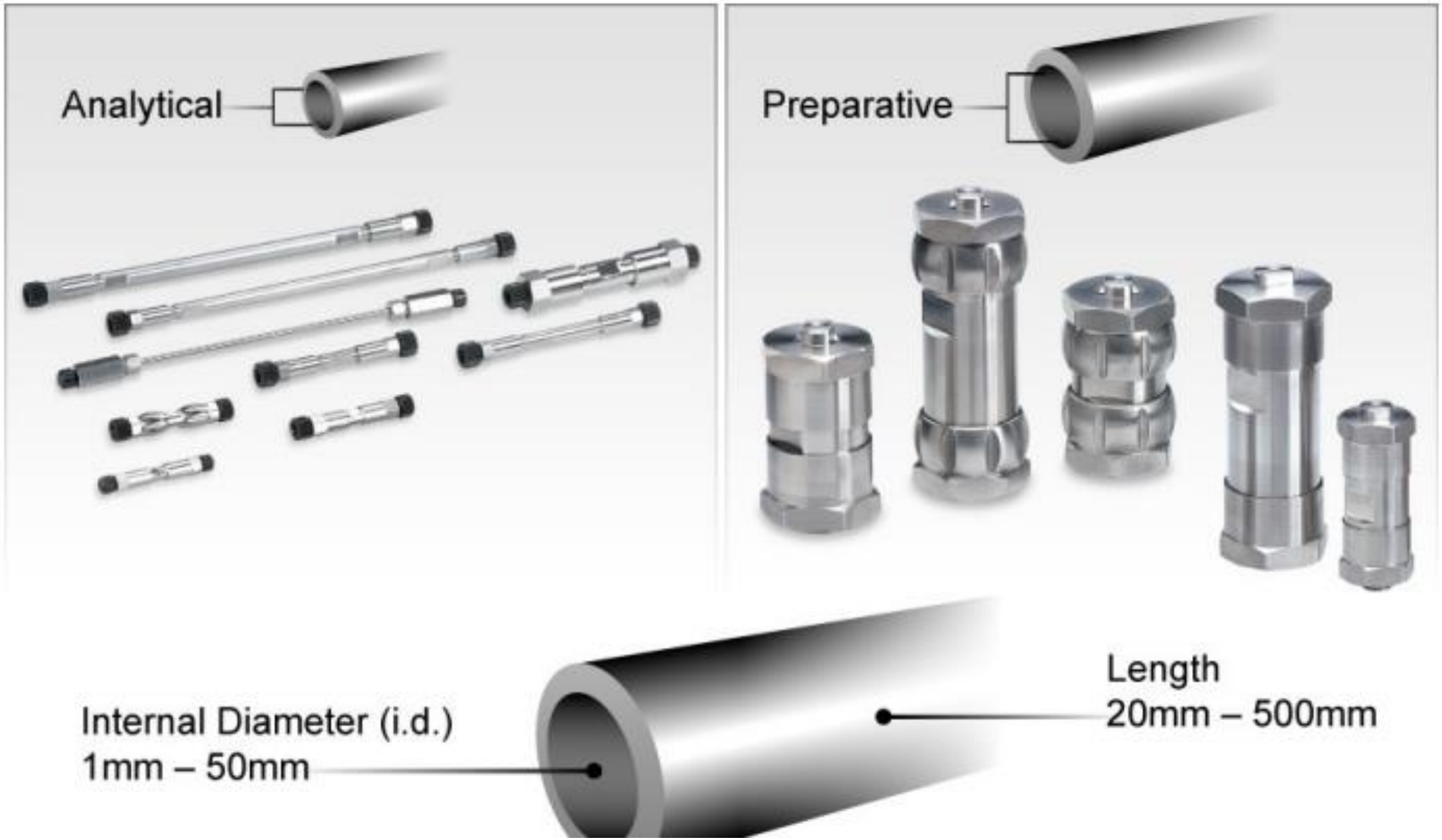


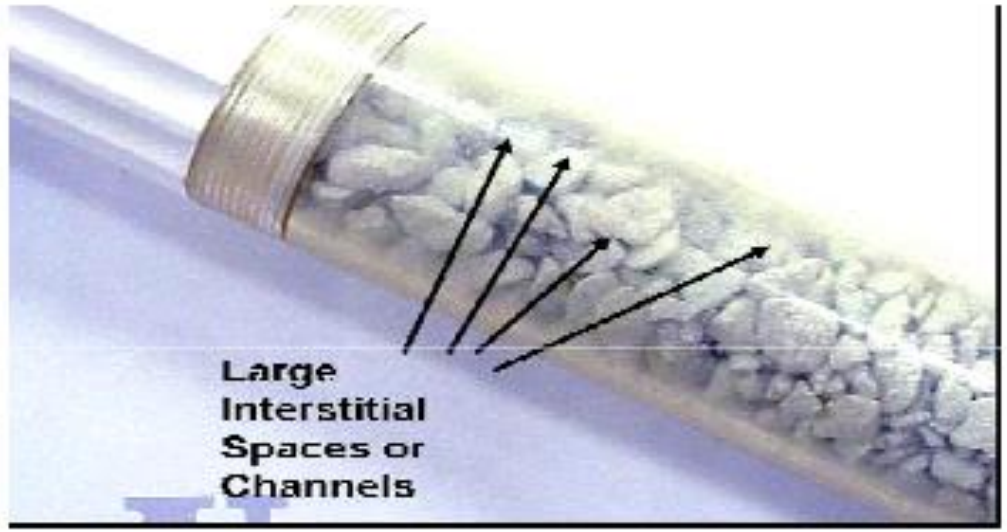
Figure M-1: Column Hardware Examples

A glass column wall offers a visual advantage. In the photo in Figure M-2, flow has been stopped while the sample bands are still in the column. You can see that the three dyes in the injected sample mixture have already separated in the bed; the yellow analyte, traveling fastest, is just about to exit the column.

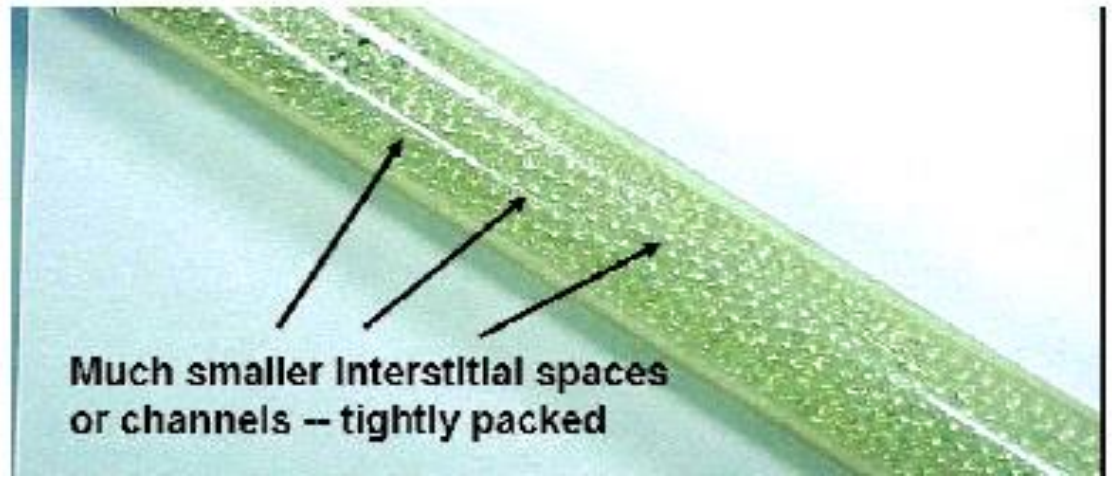
Figure M-2: A Look Inside a Column


Analytical
Preparative
Internal Diameter (i.d.)
1mm – 50mm
Length
20mm – 500mm

1974 helped enable technology for modern HPLC
Irregular shape, large diameter, wide particle size distribution



80's and 90's spherical shape, smaller diameter
5µm and 3µm, narrow particle size distribution



Types of Compounds	Mode	Stationary Phase	Mobile Phase
Neutrals Weak Acids Weak Bases	Reversed Phase	C18, C8, C4 cyano, amino	Water/Organic Modifiers
Ionics, Bases, Acids	Ion Pair	C-18, C-8	Water/Organic Ion-Pair Reagent
Compounds not soluble in water	Normal Phase	Silica, Amino, Cyano, Diol	Organics
Ionics Inorganic Ions	Ion Exchange	Anion or Cation Exchange Resin	Aqueous/Buffer Counter Ion
High Molecular Weight Compounds Polymers	Size Exclusion	Polystyrene Silica	Gel Filtration-Aqueous Gel Permeation-Organic

Separation Performance – Resolution:

The degree to which two compounds are separated is called chromatographic resolution [RS]. Two principal factors that determine the overall separation power or resolution that can be achieved by an HPLC column are: **mechanical separation** power, **created by the column length, particle size, and packed-bed uniformity, and chemical separation power, created by the physicochemical competition for compounds between the packing material and the mobile phase. Efficiency is a measure of mechanical separation power, while selectivity is a measure of chemical separation power.**

Mechanical Separation Power – Efficiency:

If a column bed is stable and uniformly packed, **its mechanical separation power is** determined by the **column length and the particle size**. Mechanical separation power, also called **efficiency,** is often measured and compared by a plate number [symbol = N]. **Smaller-particle chromatographic beds have higher efficiency and higher backpressure**. For a given particle size, more mechanical separation power is gained by **increasing column length.** However, the trade-offs are longer chromatographic run times, greater solvent consumption, and higher backpressure. **Shorter column lengths minimize** all these variables but also reduce mechanical separation power, as shown in Figure N.

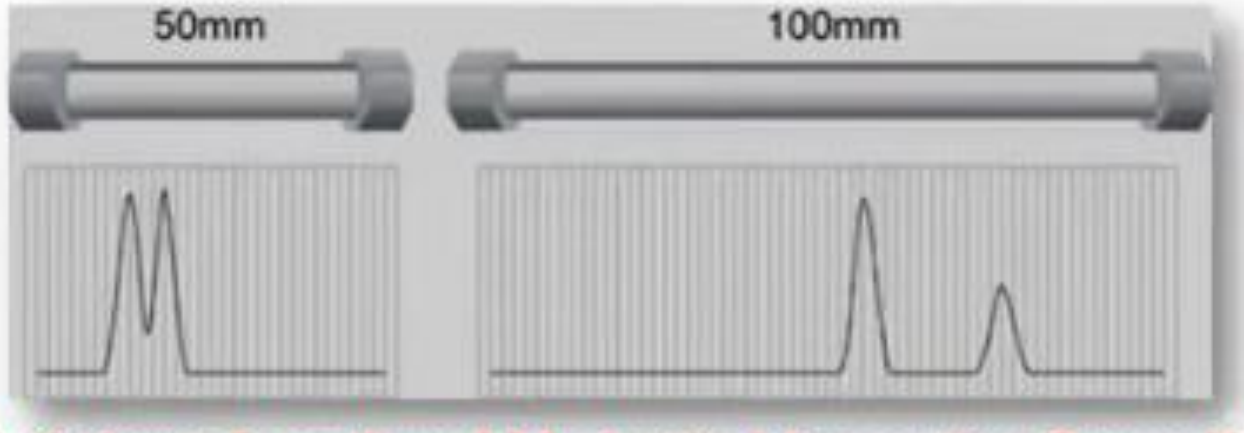


Figure N: Column Length and Mechanical Separating Power [Same Particle Size]

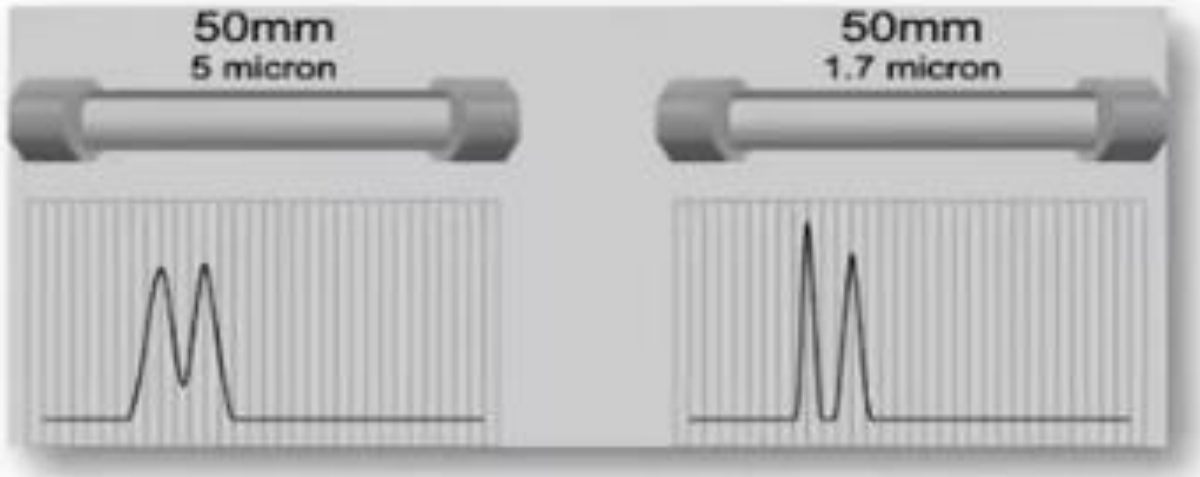
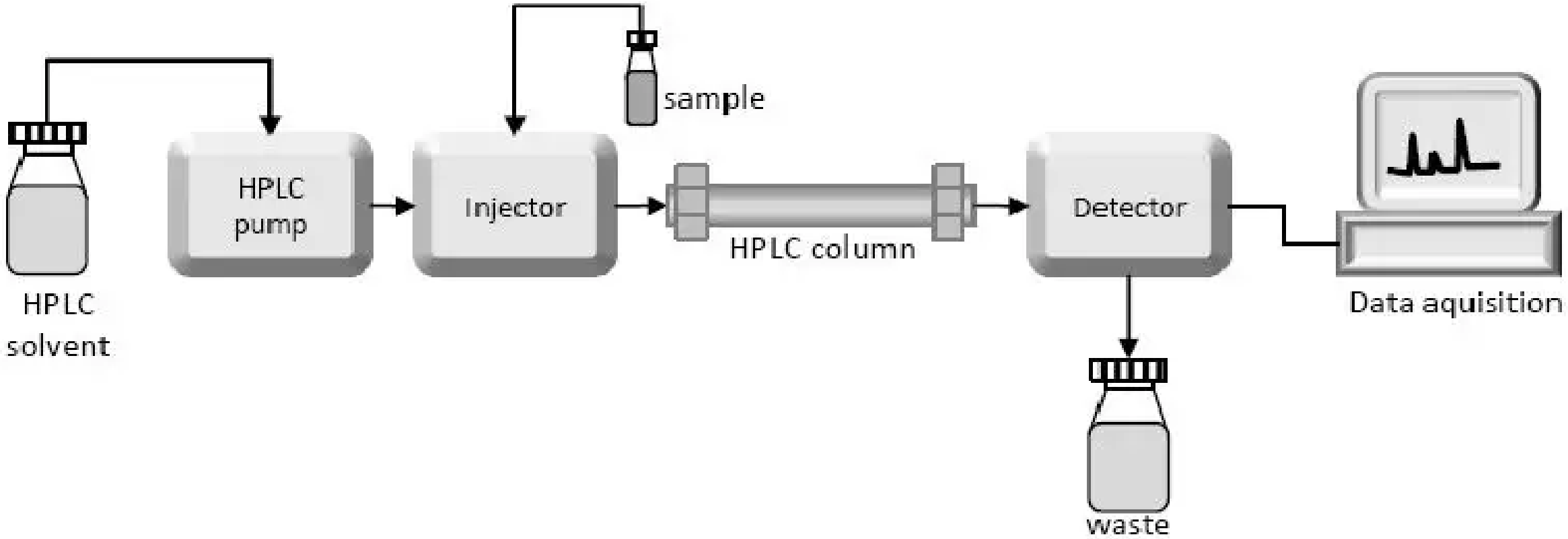


Figure O: Particle Size and Mechanical Separating Power [Same Column Length]


sample
HPLC
pump
Injector
HPLC column
Detector
Data aquisition
HPLC
solvent
waste

٥. **الكاشف**

- هو يعتبر من الأجزاء المهمة وله العديد من الوظائف المهمة في الجهاز منها على سبيل المثال:

- أنه يعمل على مراقبة المواد المذابة.
- بالإضافة إلى أنه يقوم بعملية استخلاصها وذلك يكون بمجرد خروجها من العمود.

- كما أنه يقوم بعملية بعث مجموعة من الإشارات المختلفة التي تتناسب مع مستوى خاصية من الخصائص المعينة.

- وهناك العديد من الكواشف منها على سبيل المثال:

كاشف الأشعة فوق البنفسجية، والذي يعتبر من أشهر أنواع الكواشف على الإطلاق وله أهمية كبيرة.

HPLC Detectors

- UV/Vis
- Refractive index
- Fluorescence
- Evaporative light scattering (ELSD)
- MS
- Diode Array Detector (DAD)

Electrochemical Detectors

- **Gold** for carbohydrates.
- **Platinum** for chlorite, sulfate, hydrazine, etc.
- **Carbon** for phenols, amines.
- **Silver** for chloride, bromide, cyanide.

كيفية عمل HPLC

- تم اذابة المركبات الكيميائية المراد فصلها في مذيب حيث يتم ادخال هذا الخليط في ا لطور الحامل وبالاعتماد على طبيعة الجزيئات، فإنها تتفاعل اكثر او اقل مع الطور التابث الموجود في الانبوب المسمى بالعمود الكزوماتوغرافي chromatography column

- كروماتوغرافيا السائل عالية الدقة طريقة تمزج بين ماهو فزيائي وما هو كيميائي وتعتمد بالاساس على الاختلاف والتنوع في التفاعلات بين المذاب، ا لطور الحامل والطورالتابث. نتيجة لهذه التفاعلات يحصل الفصل المطلوب.

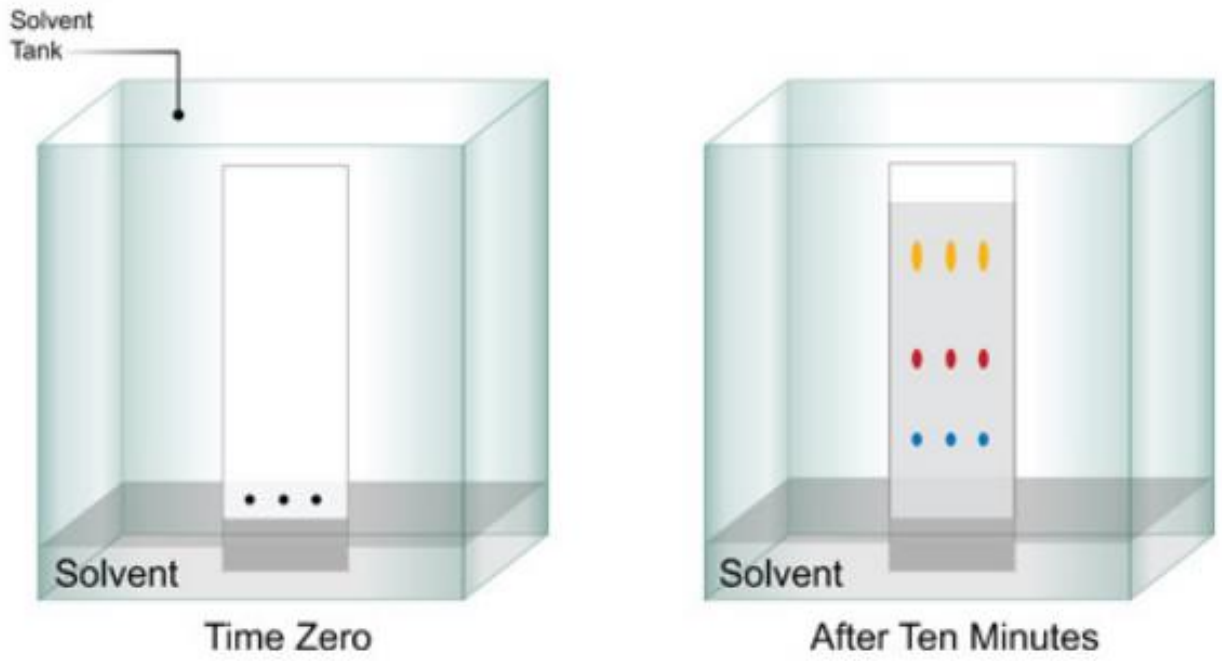
- حيث يتم بداية ضخ الوسط المتحرك داخل العمود باستخدام مضخة تستطيع ذات ضغط عالي ومن ثم يتم حقن العينة المراد فصلها من خلال حاقن، لتنتقل العينة الى العمود، حيث تتم عملية الفصل ويقوم الكاشف باعطاء اشارة لكل مكون من مكونات العينة ، لتظهر النتيجة على هيئة كروماتوغرام ، اذا نجح الفصل فكل ذروة يمثل مكونا من الخليط المراد فصله .كل الذروات المسجلة تسمى او يطلق عليها كروماتوغرام . للحصول على نتيجة ممتازة يجب تطبيق ضغط عال يفوق . 100 bars

- على مستوى العمود يتم توزيع المركبات في المحلول وفقا للانسجام بين الطور المتحرك والطور الثابت.

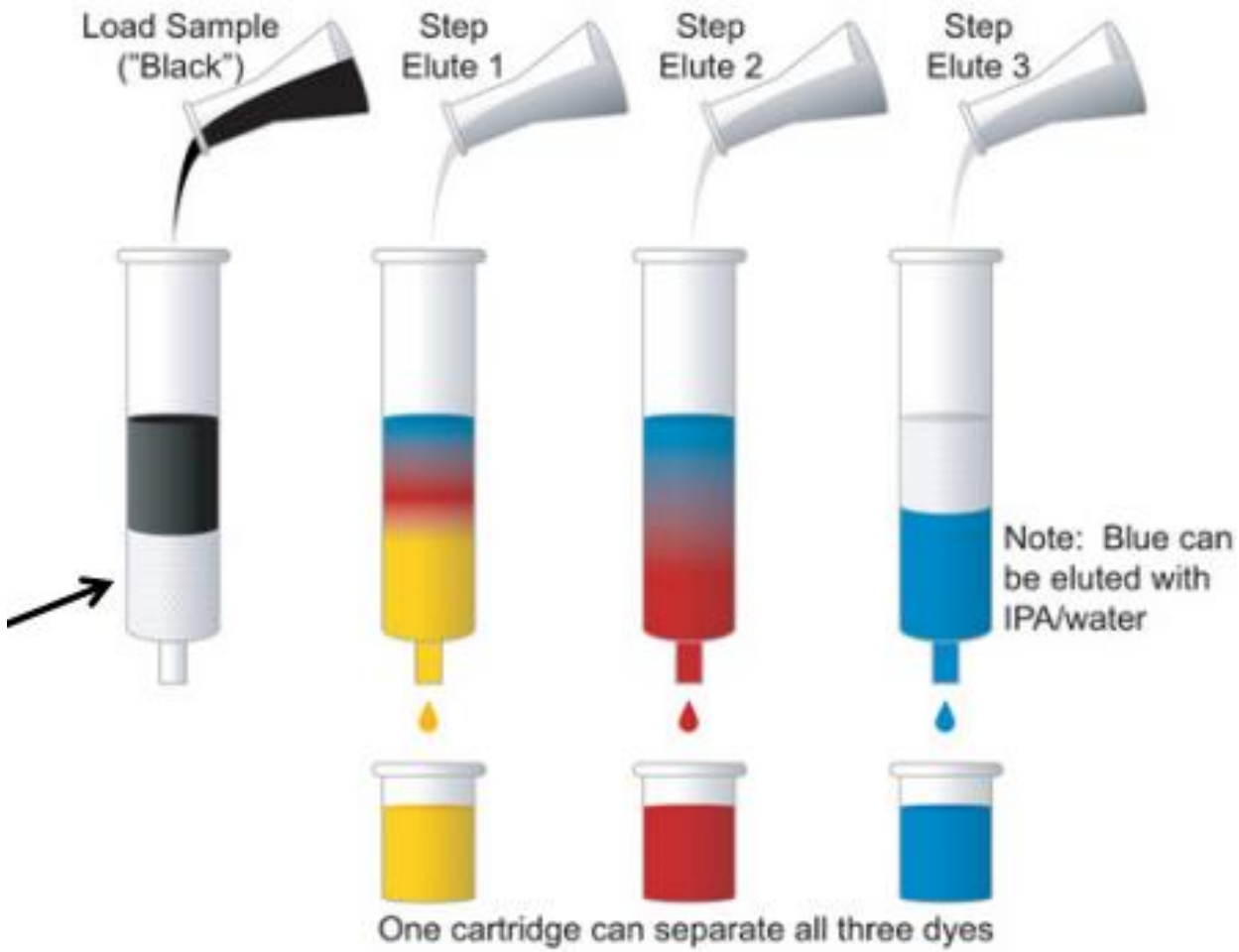
- تعرفنا سابقا ان جهاز ال HPLCيقوم بفصل مكونات العينة ثم التعرف عليها ويتم الفصل عن طريق توزيع العينة ما بين الوسط المتحرك ويكون سائل والاخر ثابت ويكون سائل او صلب

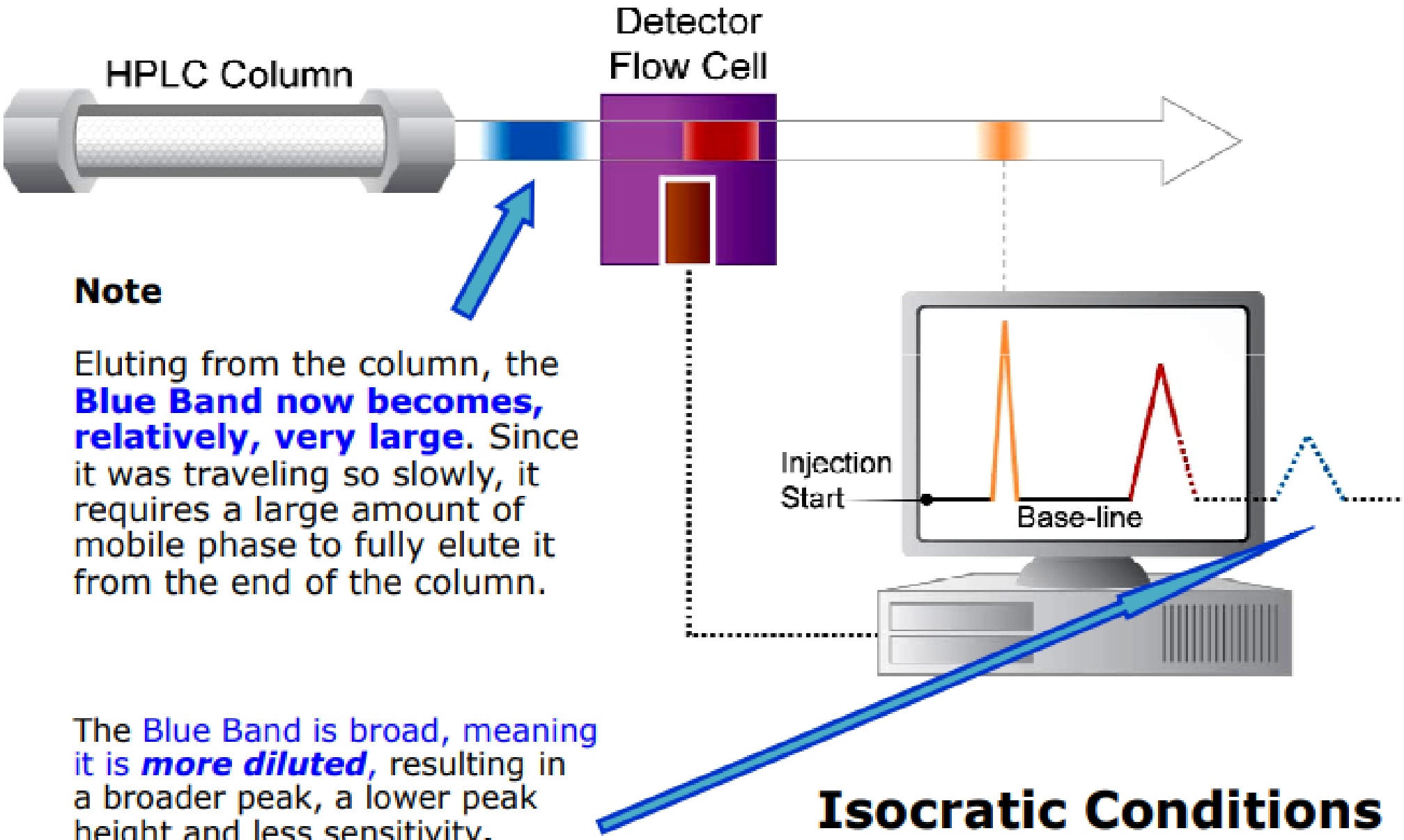
انواع اجهزة الفصل السائل

- Thin Layer Chromatography (TLC)



- Column Chromatography





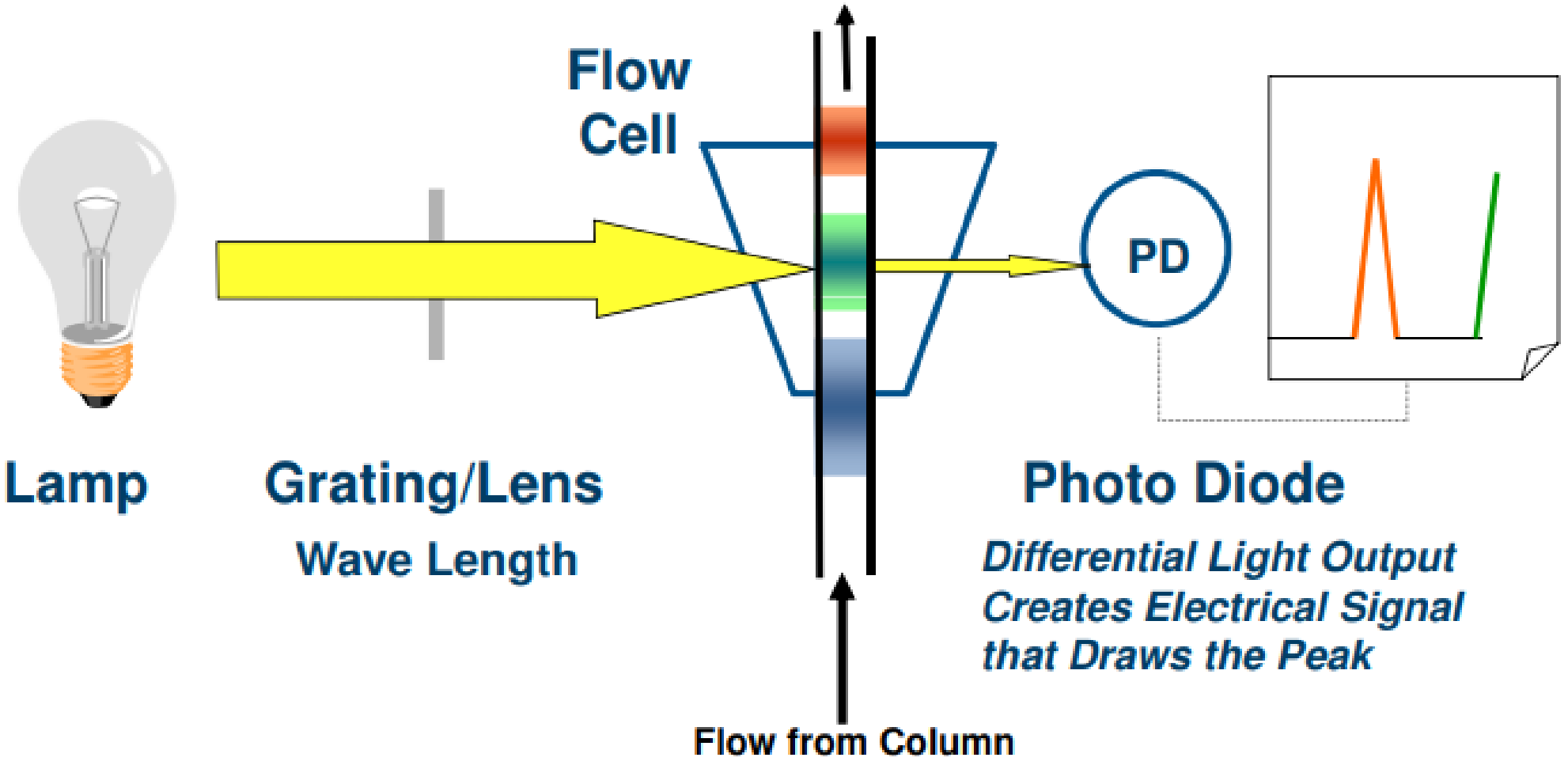
Note

Eluting from the column, the **Blue Band now becomes, relatively, very large**. Since it was traveling so slowly, it requires a large amount of mobile phase to fully elute it from the end of the column.

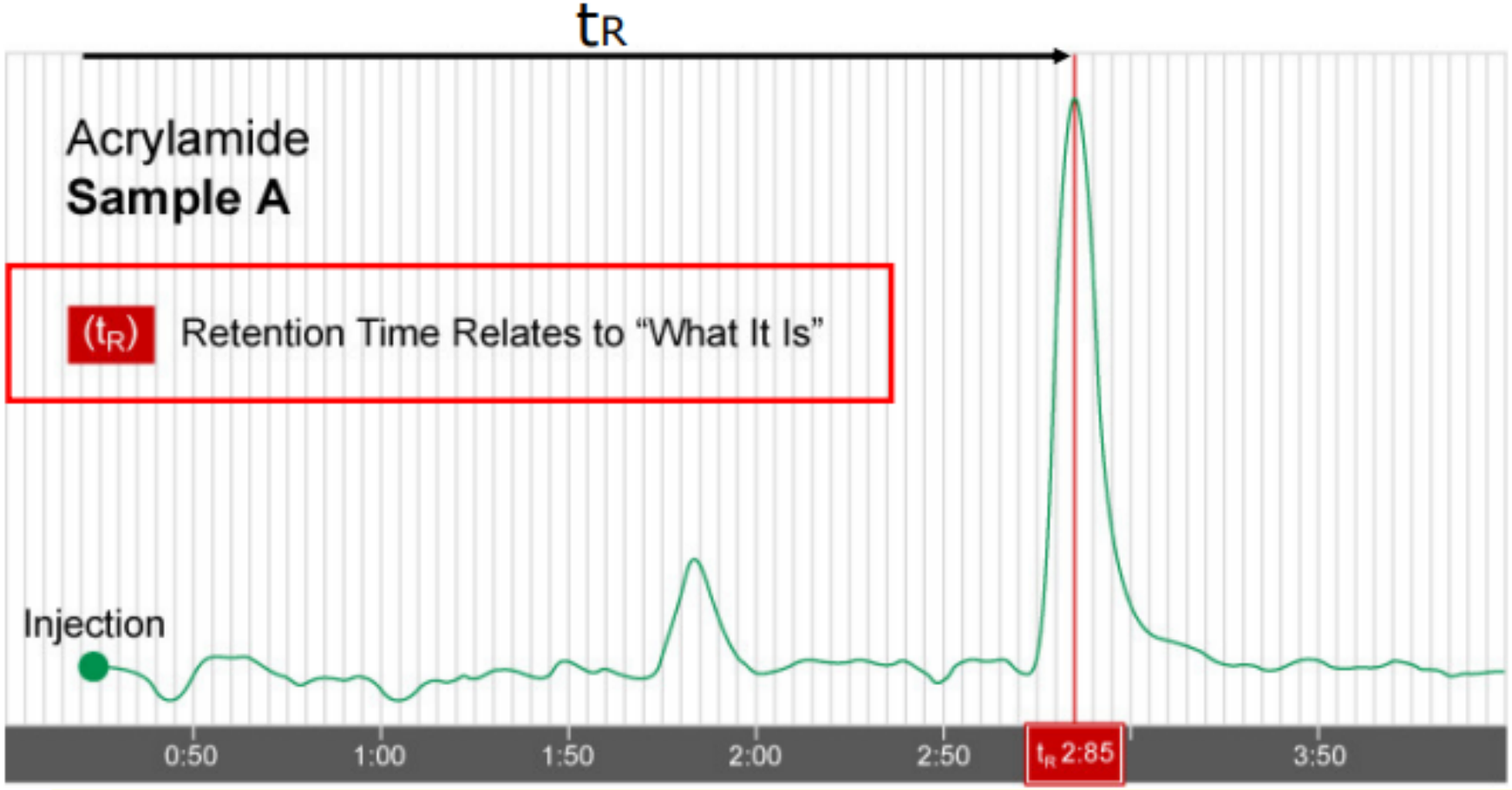
The Blue Band is broad, meaning it is ***more diluted***, resulting in a broader peak, a lower peak height and less sensitivity.

Isocratic Conditions

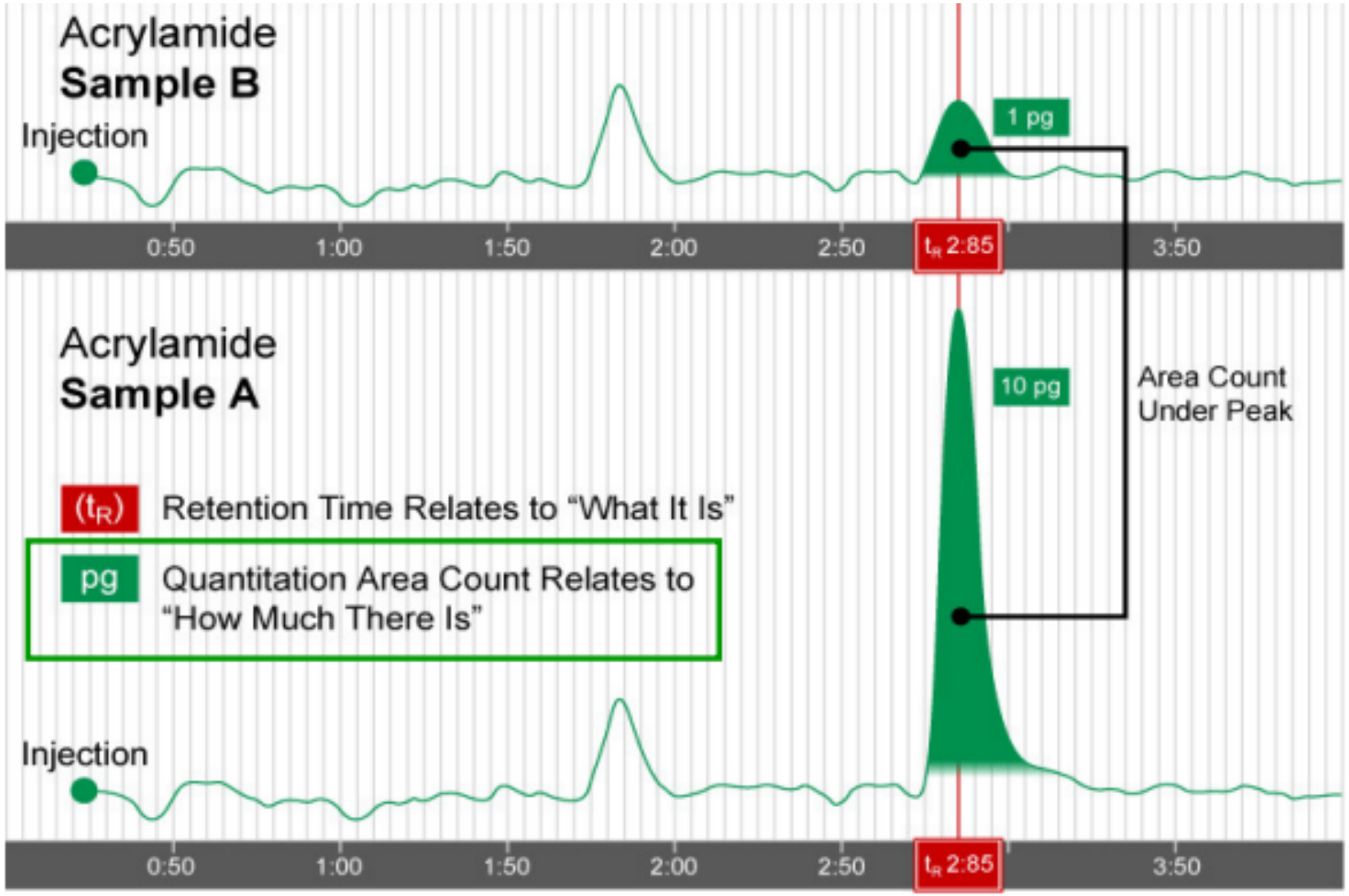
Will only work for compounds that **absorb** UV Light



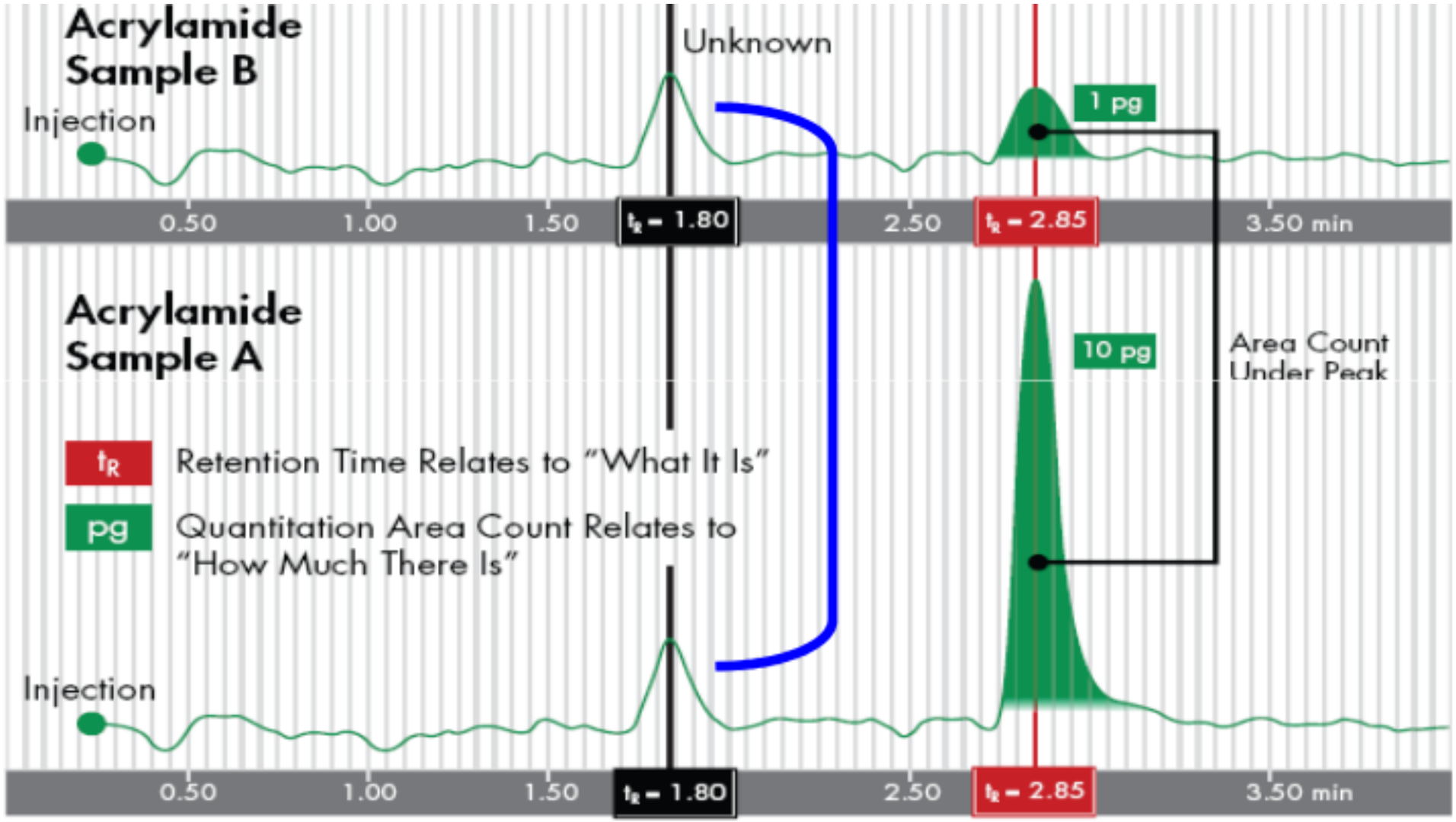
Compound Identification Based on Retention Time



For a given mobile phase, at a given flow rate with a given column, a ***known pure standard of acrylamide*** elutes at 2.85 minutes.
Whenever a real sample is injected that contains acrylamide, you will see a peak at 2.85 minutes.



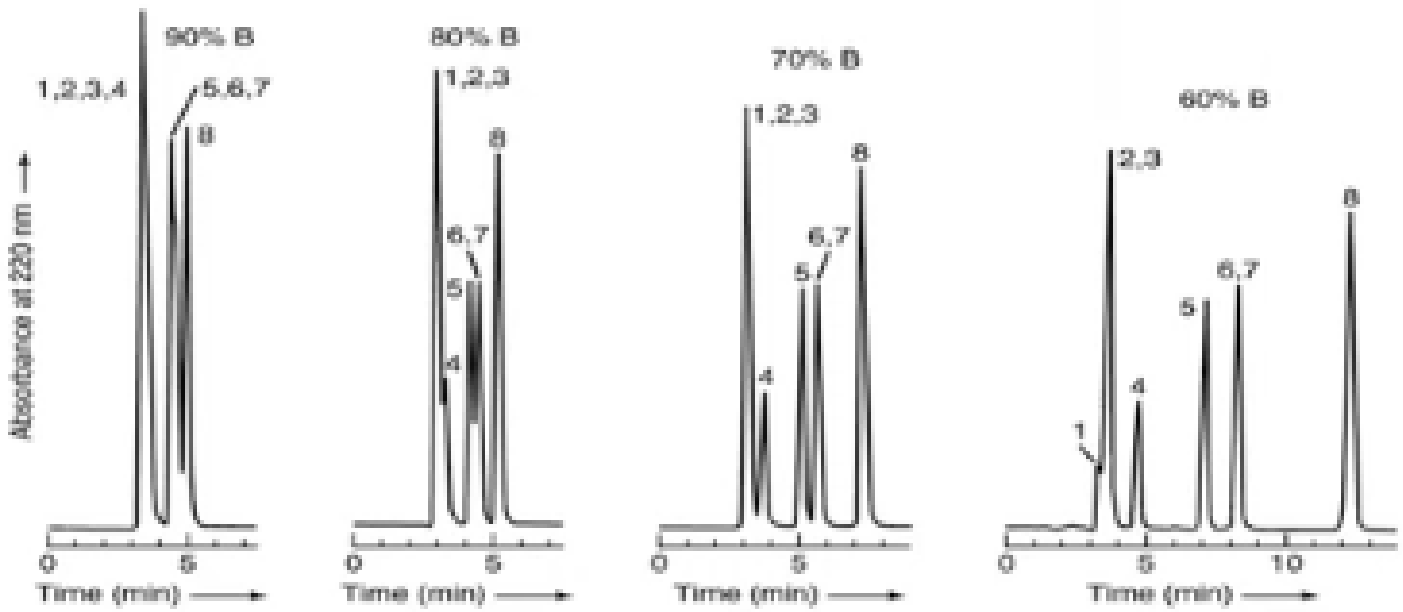
How much is present is measured by the AREA under the peak, which is related to how much was there. Both samples contain acrylamide, however, Sample B has only 1/10 the concentration



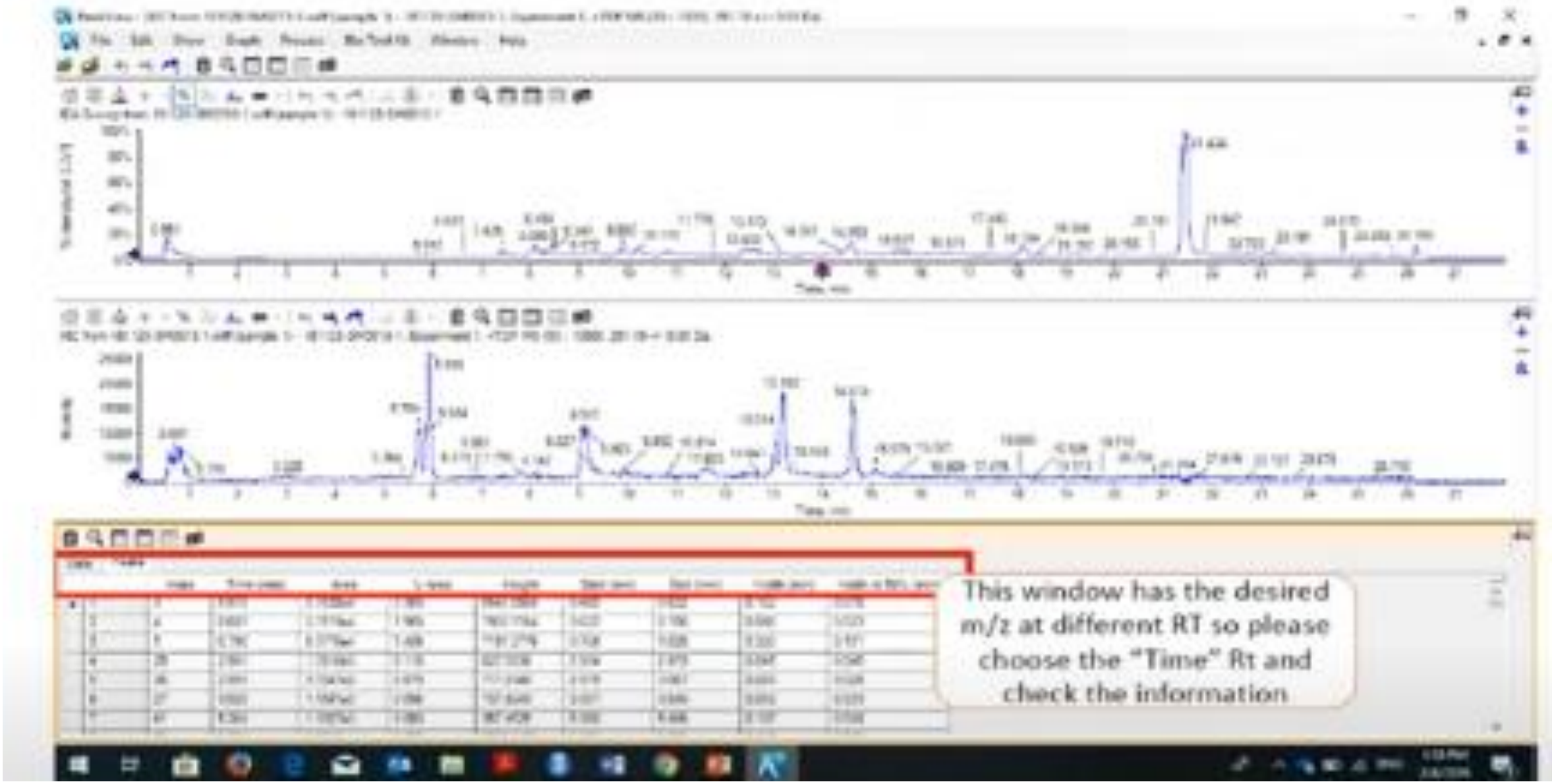
Both samples have ~ SAME amount of this unknown compound

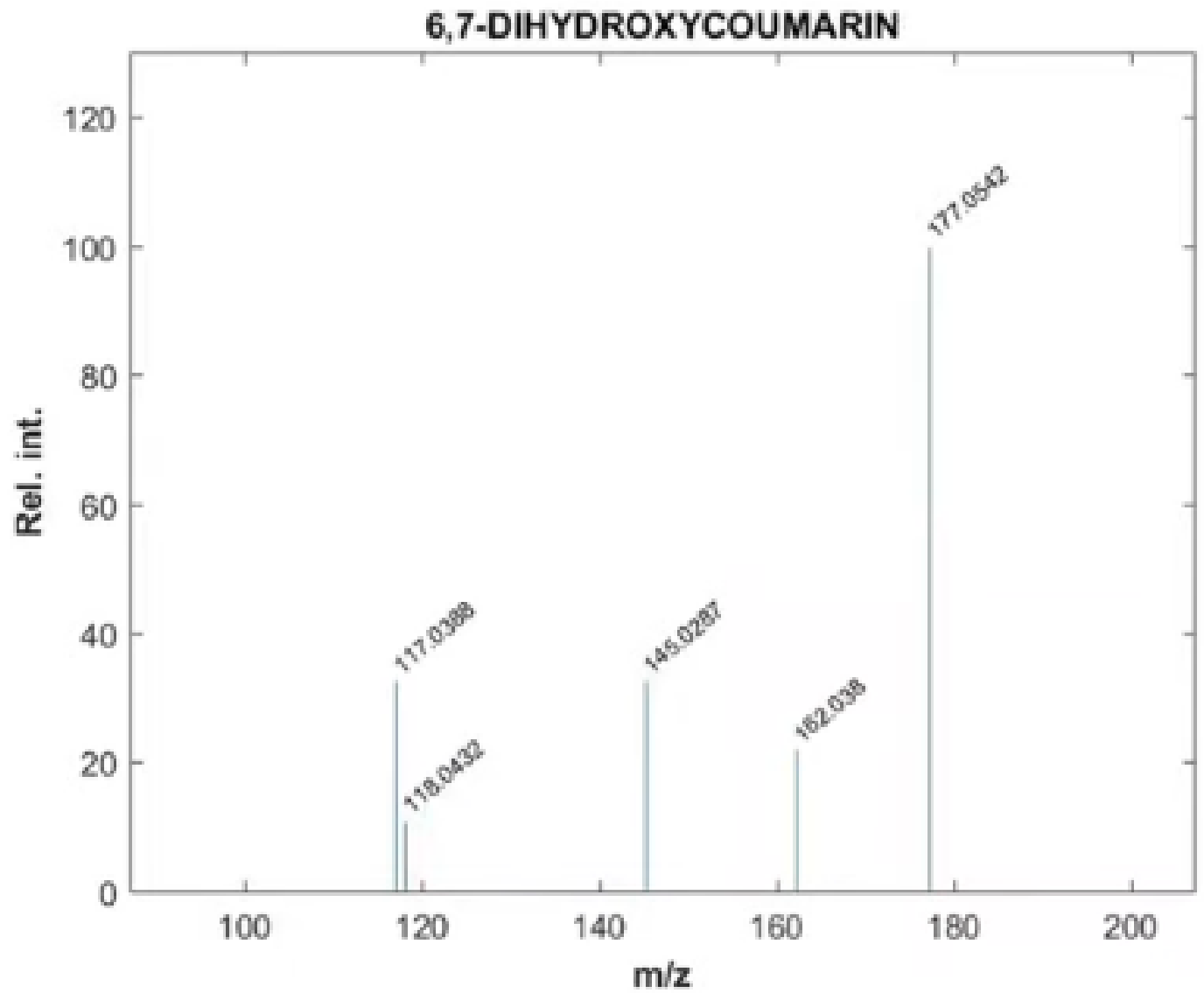
Retention Time:

The **retention time** of a solute is taken as the elapsed time between the time of injection of a solute and the time of elution of the peak maximum of that solute.



HPLC CHROMATOGRAM


This window has the desired m/z at different RT so please choose the "Time" Rt and check the information


6,7-DIHYDROXYCOUMARIN
Rel. int.
m/z
0
20
40
60
80
100
120
100
120
140
160
180
200
177.0542
117.0388
145.0287
162.038
118.0432

The factors which influence the HPLC performance:

1. **Internal diameter of column**

- the smaller in diameter, the higher in sensitivity

2. Pump pressure

- the higher in pressure, the higher in separation

3. Sample size

4. The polarity sample, solvent and column

5. Temperature

HPLC Applications

Chemical

Bioscience

Pharmaceuticals

Consumer Products

Environmental

Clinical

Application of HPLC:

1. Pharmaceuticals industry To control the drug stability

Quantity of drug determination from pharmaceutical dosage forms, ex. Paracetamol determination in panadol tablet

Quantity of drug determination from biological fluids, ex: blood glucose level

2. Analysis of natural contamination

- Phenol & Mercury from sea water

3. Forensic test:Determination of steroid in blood, urine & sweat.

4. Clinical test: Monitoring of hepatic chirosis patient .

5. Food and essence manufacture

- sweetener analysis in the fruit juice
- preservative analysis in sausage.- Detection of psychotropic drug in plasma

Advantages of HPLC:

1- Very rapid technique (few minutes) (Speedy separation).
2- Continuous monitoring of the column effluent.
3- No need for derivatization (C.F. Gas Chromatography).
4- Polar & Ionic compounds are easily analyzed by HPLC then by GLC.
5- It carried out at room temperature or temp. a limit of 80 °C.
6- High resolution (High resolving power).
7- Sensitivity.
8- Reproducibility of +/- 1% (Repetitive and reproducible analysis using the same column.
9- Accuracy (Accurate quantitative measurement).
10- Automation of the analytical procedure and data handling.

Disadvantages of HPLC:

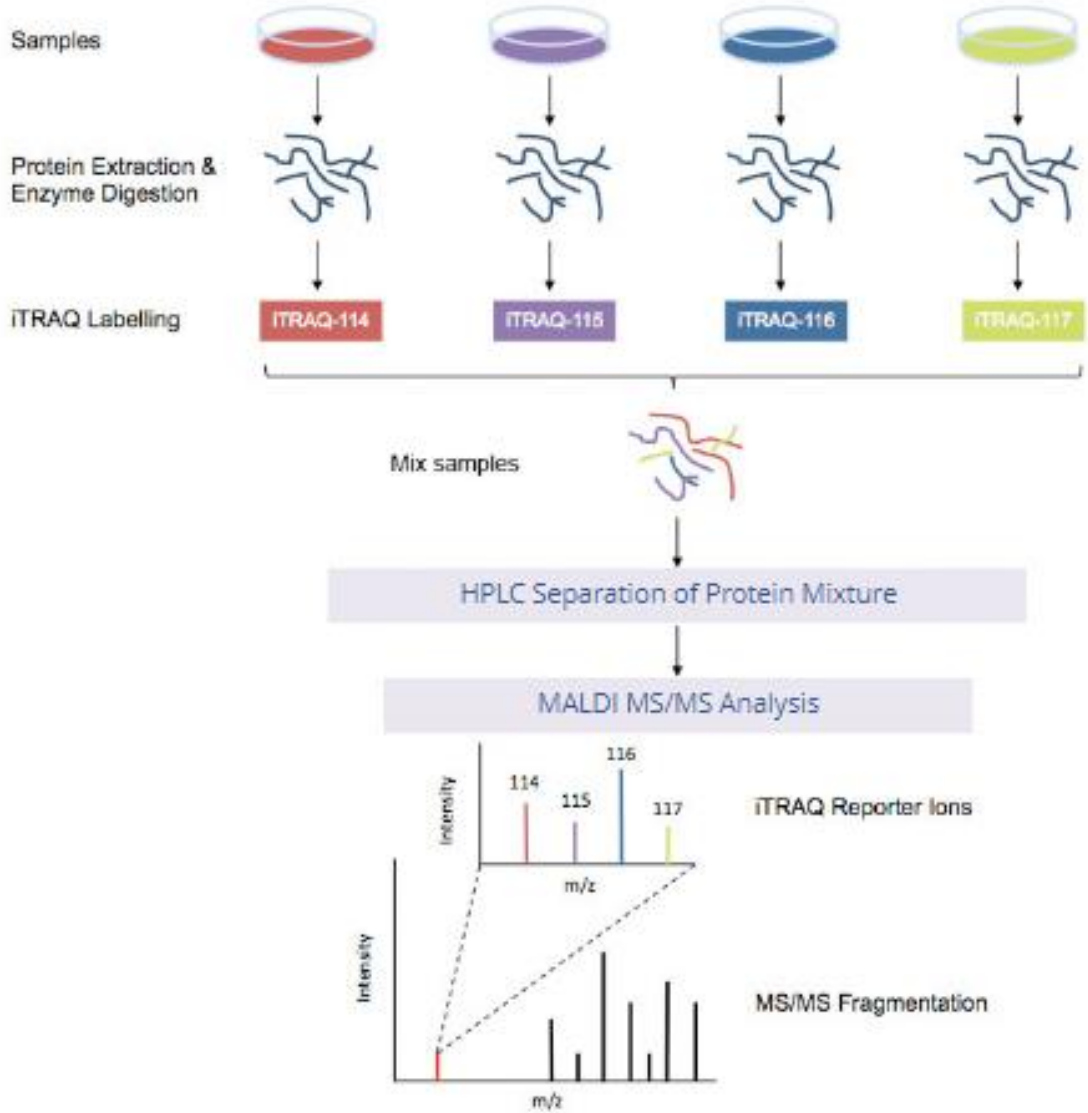
1- Cost
2- Complexity
3-Low sensitivity for some compounds

مميزاتها

- تعتمدها بشكل رسمي إدارة الأغذية والعقاقير FDAللتحكم بالمعايير الصناعية، والتحقق من فاعلية المُنتجات، وللتحليل النوعي والكمي، لذا هي ضرورية في المُختبرات والشركات.
- اختبار كل من المواد الخام والسلع النهائية.
- الاستخدام في الصناعات الطبية والصناعات التجارية والصناعية،
- وفي صناعة الدواء والطلاء وصناعة البوليمرات.
- اختبار المواد الخام للتأكد من المنتجات الصحيحة ودرجات النقاء.
- تُستخدم في المُختبرات لحل مشكلات فشل المُنتجات، والبحث عن الشوائب والملوثات، وكذلك لإجراء تحليلات للمنتجات المنافسة.
- التأكد من نسب ومحتويات المُنتج النهائي الموجود على مُلصق المادة بعد الانتهاء من عمليات التصنيع.
- تحديد مدة صلاحية واستقرار المنتجات عبر تحليلها.
- إمكانية الفحص باستخدام عينات صغيرة.
- تطوير مُنتجات كيميائية أفضل.
- المُساعدة بحل أو منع أو تقليل سحب مُنتجك من السوق.

عيوبها:

- **السرعة**: سرعة ظهور نتائج الفصل الكيميائي بسبب سرعة الدوران ميزة، ولكنَّها عيب بنفس الوقت، بعض المُركبات لها أقطاب وبُنية تكوينية متشابهة، وحين خروجها من الجهاز يصعب تحديد أي منها نحتاج بالضبط، لأنَّها ببساطة تخرج بنفس الوقت.
- **الكثافة**: يستخدم الجهاز مواد حُبيبية للفصل تُصنع من مواد مُختلفة، والمواد الكيميائية الموجودة في العمود ترتبط بهذه الحُبيبات بخواص الكثافة ، وبعض المواد بسبب هذا الارتباط لا يتم إطلاقها أبدًا من العمود، أو لا يتم قياسها من المحلول الخارج من العمود.
- **التكلفة**: عمليات الفصل يتم تنفيذ العديد من الاختبارات لها بشكل فردي لكُل محلول، ممَّا يزيد من التكاليف لهذه الاختبارات، كذلك تُكلِّف عمليات تطوير جهاز HPLC ليُصبح ذو كفاءة أكبر.
- **التعقيد**: بعض عمليات الفصل تستخدم خليط خلوي خاص لعزل بروتينات بعض المواد، وهذه العملية مُعقدة وصعبة التنفيذ في العديد من المُختبرات.



iTRAQ workflow (4-plex) is shown above. Samples to be quantified are prepared under various treatmer conditions followed by cell lysis to extract proteins. After using a standard protein assay to estimate the protein concentration of each sample, proteins are digested using an enzyme, such as trypsin, to genera proteolytic peptides. Each peptide digest is labeled with a different iTRAQ reagent and then the labeled

Thank you for attending!