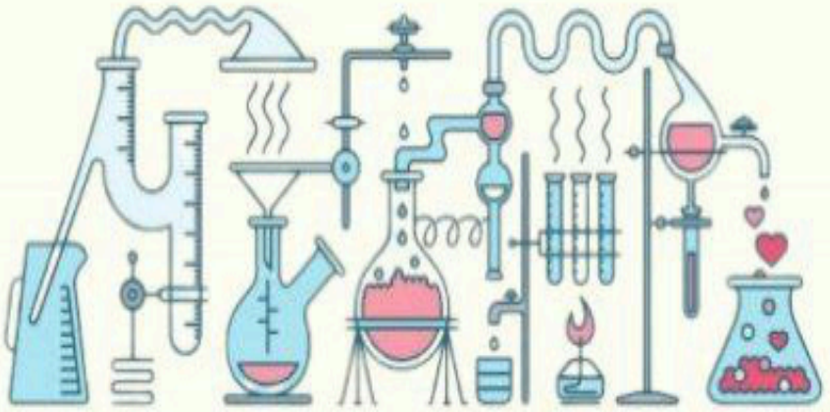
تفريغ تحليل آلي



اسم الموضوع: Chromatography

إعداد الصيدلاني/ـة: Sara Jaber

Soul Academy

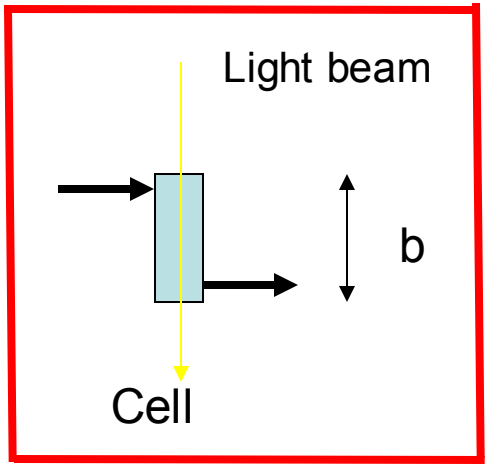
لجان الدفعات

Detectors used in HPLC

Type	Principle	Detection limit	Comments	Destructive	Universal
Spectro-photometer	Measure absorbance of light	<1 ng	Analyte must absorb UV or visible light	No	No
Fluorometers	Measures fluorescence	pg to ng	Analyte must fluoresce	No	No
Electro-chemical detectors	Electrochemically measures oxidized/ reduce analyte	pg to ng	Useful for catecholamines	yes	yes
Mass spectrometer	Detects ions after separation by mass-to-charge ration	fg to ng	Analyte must be converted to ionized form		
Refractometer	Measure change in refractive index	1 µg	Detection of most compounds but relatively low sensitivity	No	yes

• UV Absorption Detectors

- The most common type of detector
- Principle: absorption of ultraviolet (or visible) light
- Follows Beer's Law: $A = -\log(I/I_o) = \varepsilon bC$
  - I = intensity of light (I_o for blank)
  - ε = molar absorptivity (constant)
  - b = path length ≈ 1 cm
  - C = concentration



- Best results for $0.001 < A < 1$
- Fast response – sensitivity trade off in path length (can select cell volumes)

• UV Absorption Detectors

– Sensitivity to Compounds (ε values)

- Best for compounds with conjugated double bonds, aromatic groups or strongly absorbing functional groups (e.g. R-NO_2, R-I, R-Br)
- Poor response for compounds with few or weakly absorbing functional groups (worst for R-CN, R-NH_2, R-F; poor for R-OR', R-OH, R-COOH, R-COOR')

– Solvents:

- Requires use of solvents that absorb poorly in UV

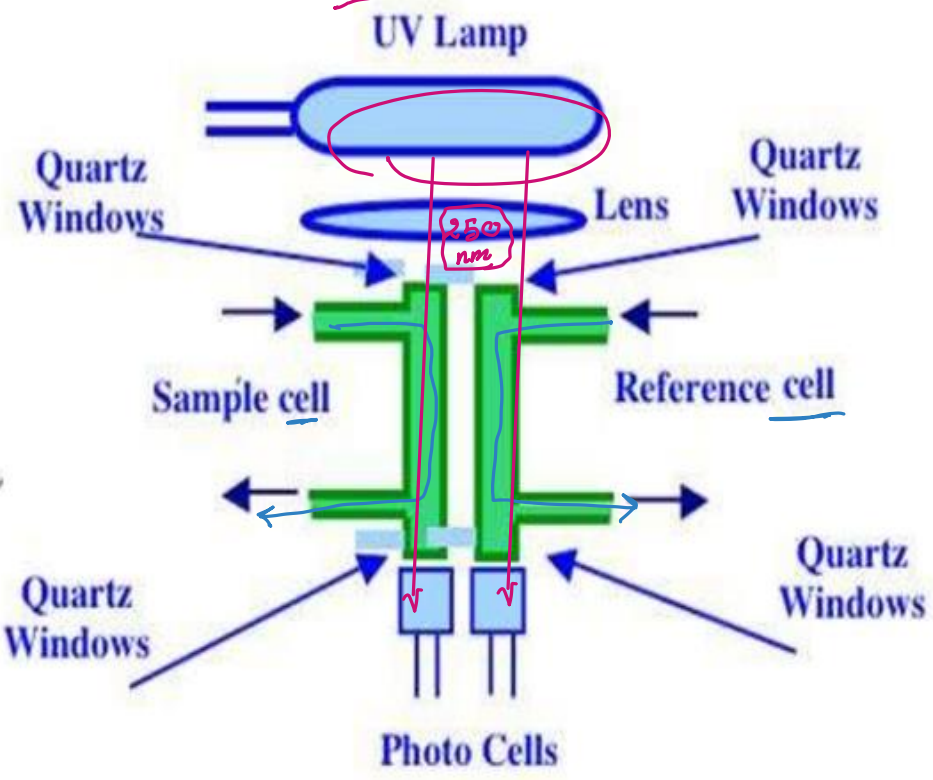
Absorbance detectors

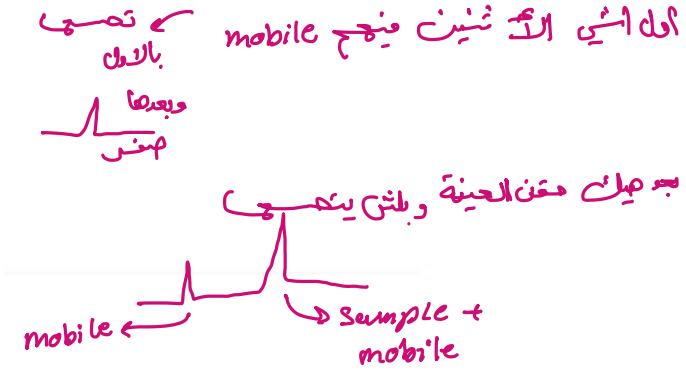
UV/Visible detectors

- Solute property detector
- Three types
  - Fixed wavelength detector
  - Variable wavelength detector
  - Diode array detector

Fixed Wavelength detector

- 254nm
- Higher detection capacity.
- Hg vapour lamp(discharge lamp)
- Focus of light through two absorption cells.
- Volume of cell is kept constant.





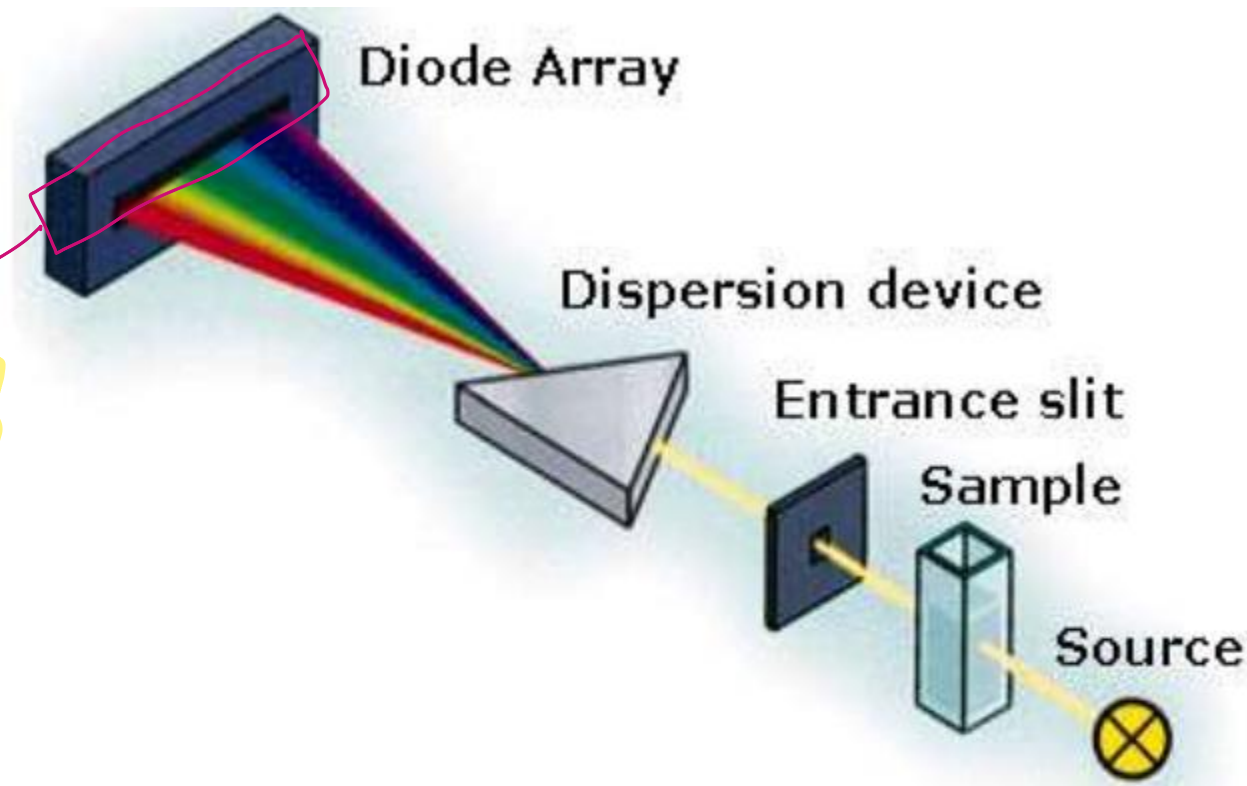
Variable Wavelength Detectors

- Relatively wide band pass UV-Visible spectrophotometer coupled to a chromatographic system.
- Offers a wide selection of UV & Visible wavelengths with increased cost.
- For complete spectrum, eluent flow must be stopped to trap the component of interest in the detector cell.

Diode Array Detector

- Scanning Wavelength Detector
- Required to obtain a real time spectrum of each solute as it elutes
- Work in parallel, monitoring all wavelength
- Xenon lamp
- Complete development of chromatogram

بنقدر احلل كثير Compunds ب one Run



بتفحص كل الاطوال الموجية

Universal not destructive

• Refractive Index Detectors

– Principle:

physical property ← معامل انكسار

- liquids with different refractive index will diffract light differently
- Composition will determine refractive index
- Any compound with a refractive index different than the solvent's is detectable

– Advantage:

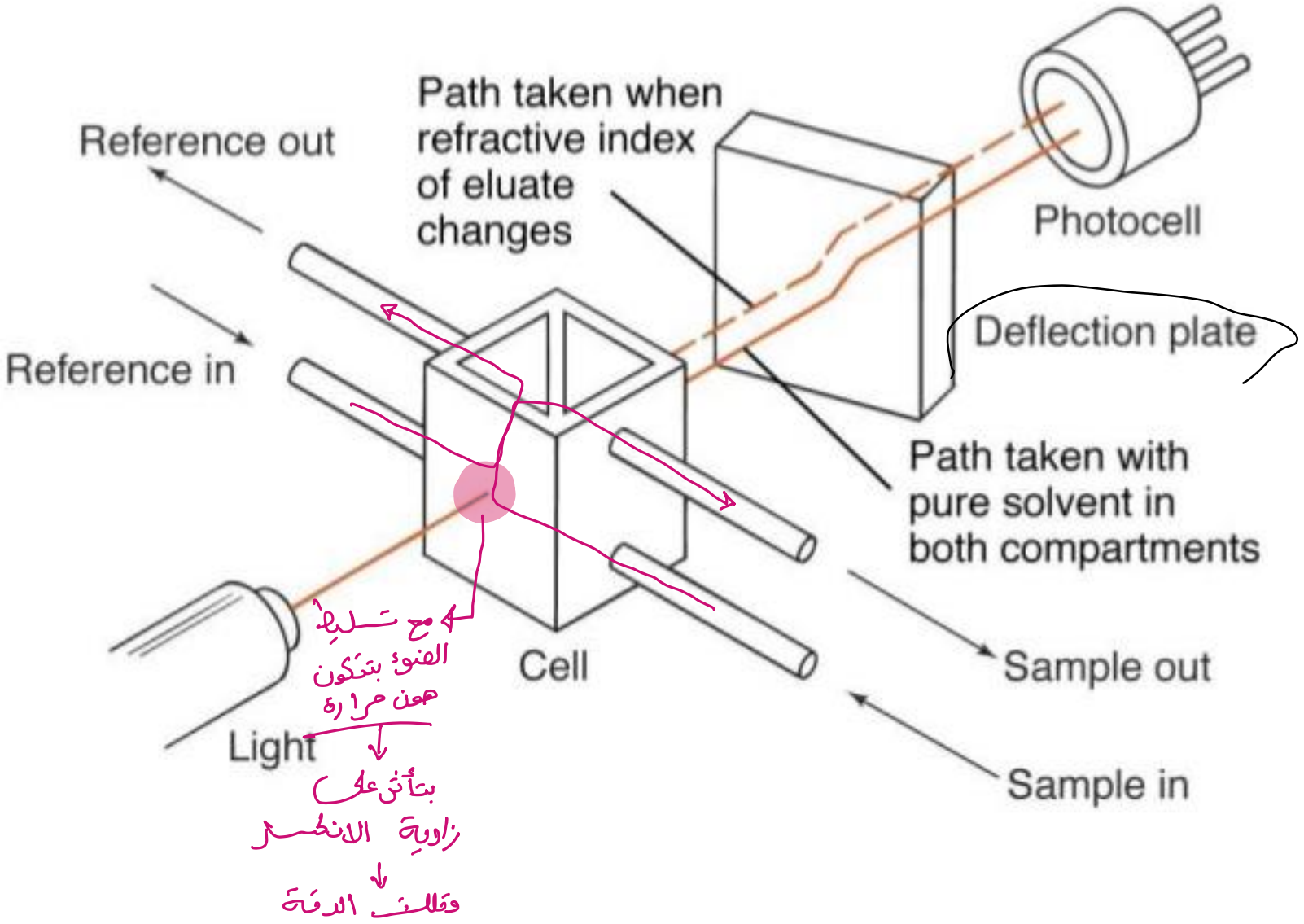
- Most universal detector (can detect weakly absorbing compounds)

– Disadvantages:

- Gradients are not possible
- Requires thermal stability
- Generally not very sensitive

* بعرف زاوية انكسار الـ mobile
وبعيّن الزاوية الناتجة
وبعدها الزاوية الناتجة – زاوية الـ mobile = زاوية المركب المطلوب

pharmacopeia ← بعرفها من الـ


Reference out
Path taken when refractive index of eluate changes
Photocell
Reference in
Deflection plate
Path taken with pure solvent in both compartments
Cell
Sample out
Light
Sample in

3 or more Conjugated double Bond

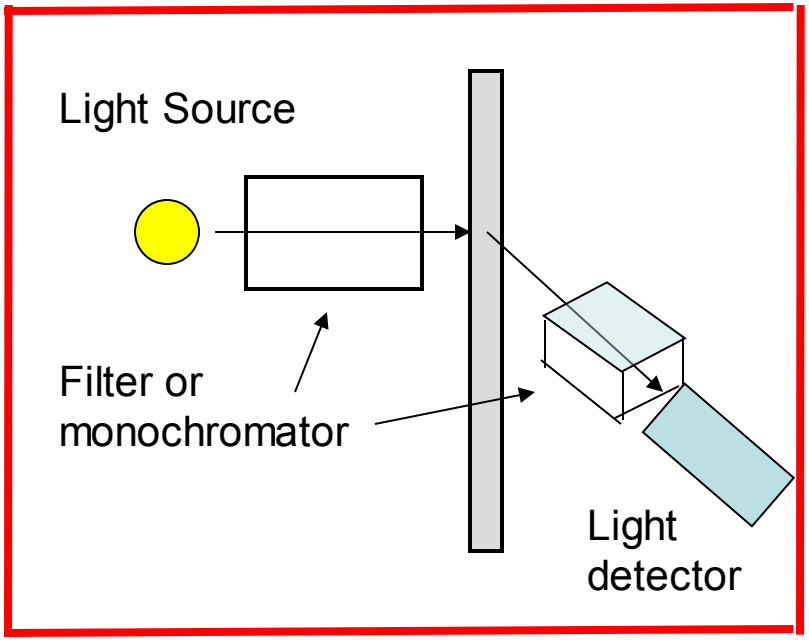
Fluorescence Detectors

- Detection Principle: Fluorophor
  - Light promotes molecules to excited electronic state
  - Excited molecules transition from lowest excited state back to the ground state and emit light in the process

$$M + h\nu \rightarrow M^*$$

$$M^* \rightarrow M^{*\prime} \text{ (lower vibrational level)}$$

$$M^{*\prime} \rightarrow M + h\nu'$$

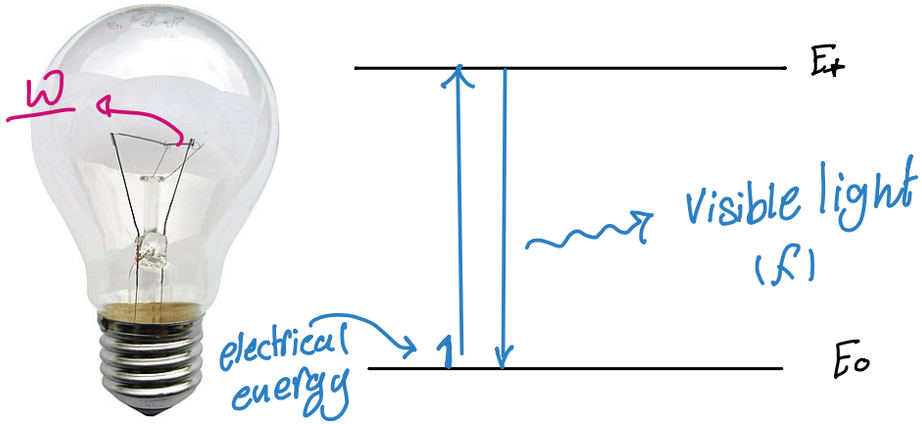


- Equipment:
  - High intensity light source
  - Filters or monochromators to select wavelengths (before and after cell)
  - Sensitive light detector

Sensitivity اعلى

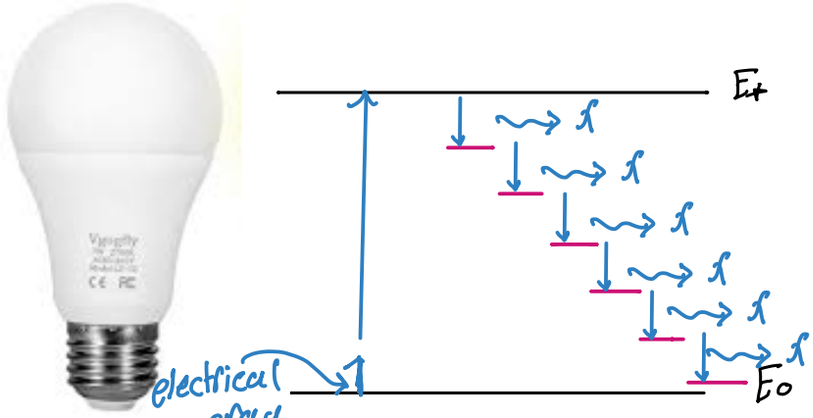
- Advantages:
  - Greater sensitivity possible (for molecules with high fluorescence efficiencies) because easy to detect small signal against zero background (see below)
  - Much greater selectivity because few molecules fluoresce, particularly at selected wavelengths
- Disadvantages:
  - Limited to relatively few molecules (although derivatization is also possible)

اضيف سلسلة فيها conjugated في على العكس اللي بدي اعطله



(الإضاءة القديمة)

بأقل من 1s بحتاج طاقة
كبيرة عشان اقدر اعفز الـ e
(عملية الصعود والنزول لـ e
سريعة فبحتاج اعفزه مرات
كتيرة حتى يعطيني ضوء متصل)



(الإضاءة الحديثة)

بالدقيقة ممكن يتحفز الـ e مرة واحدة
فبحتاج لطاقة اقل ← لأن الـ e اللي
تحفز وصعد لـ E* رجع لـ Eo على
مراحل وكل مرة كان يبعث f بطاقة
ثابتة بالتالي اعطاني ضوء مستمر
بأقل طاقة ممكنة (energy saver)

Fluorescence

مقارنة بين UV و الـ Fluorescence

Fluorescence → More sensitive
- ↳ Can only analyse fluorphor Compounds
- ↳ more intensity peak
- ↳ limited (المركبات اللي تتأثر قليلة)

UV → less sensitive
- ↳ Can analyse fluorophor & Chromophore
- ↳ less intensity peak

+ الاشعين Not universal ,

Not destructive

---

3 or more Conjugated double Bond

لو حطيتها بالـ UV →

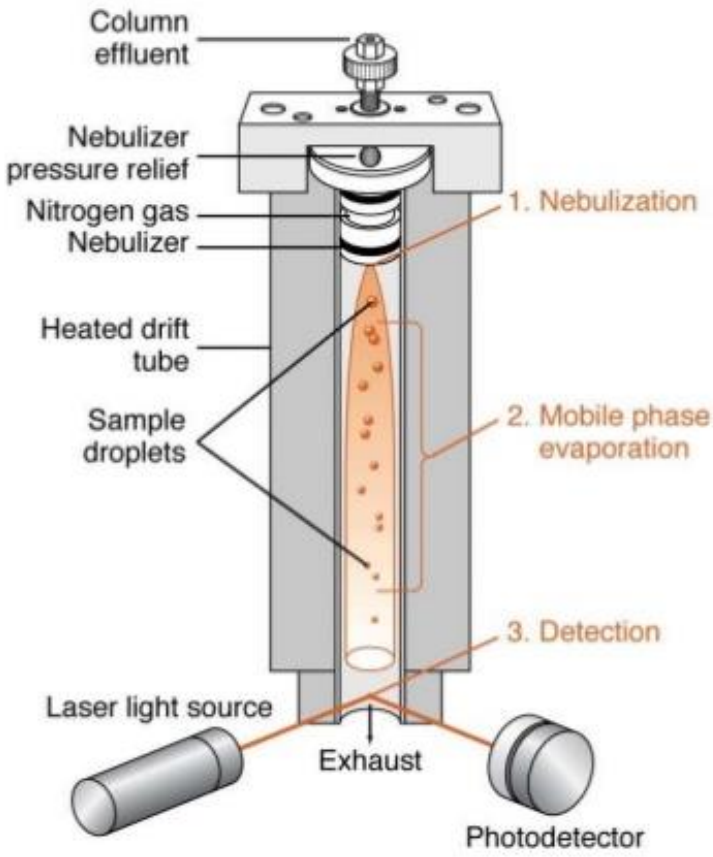
لو حطيتها بالـ Fluorescence →

عالية
حتى لو التركيز قليل
عشان هيك هي more sensitive

لأنه عدد f المنبعثة بتكون الحبي

• ELSD (Evaporative Light Scattering Detector)

- Universal, destructive highly sensitive
- Useful for large molecules and wide linear range.
- Analytes are de-solvated in the detector.
- Molecules pass through a large cuvette for a UV-VIS instrument.
- The reduction in light intensity detected (due to scattering by the analytes) is measured.

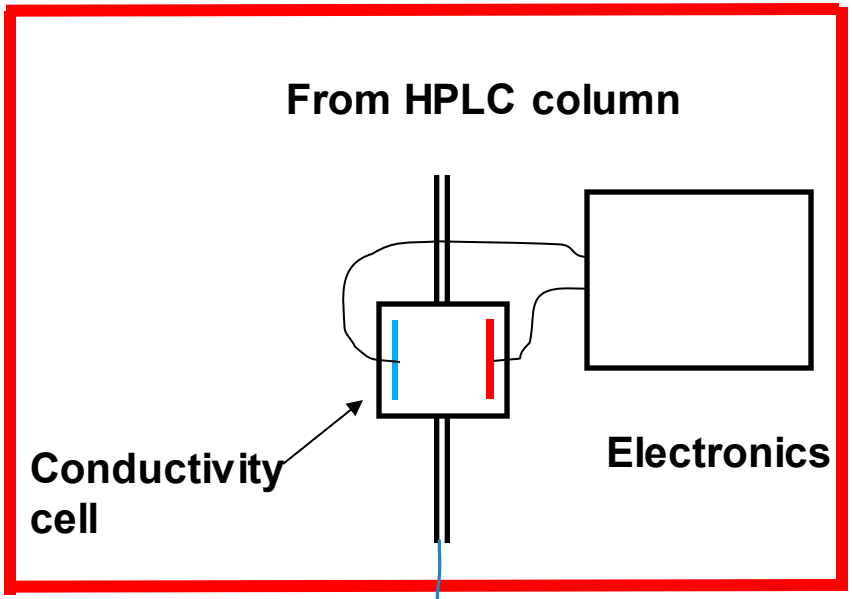


Not destructive
Not universal
ما بتقدر اعلل المركبات اللي بس فيها Pure Covalent

Ion Exchange Chromatography

غالبًا الـ mobile phase غير ناقل الكهرباء والعينة ناقلة او العكس

- Types of Instruments:
  - Single column
  - With analytical plus suppressor columns
- Detection in Single Column Instruments
  - Other detection methods (fairly common)
  - Conductivity detection
- Conductivity Detector
  - Resistance measured (AC circuit)
  - Conductivity = 1/(resistance)
  - Ions in solution create conductance
  - Conductivity depends on ion concentration and size



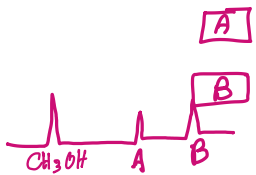
اول اشي فات هو الـ mobile phase فرضًا ← methanol ← Polar ← ناقل e^- ← تحسسها

ثاني اشي فات هو الـ Sample ← ماكانت Polar بنفس درجة الـ methanol ← e^- تعلقت ← مختلفة عنه

اعطت Signal يزعن الـ mobile phase

تم تطوير عمل

على فرضًا
methanol 7 e^-/min
A 3 e^-/min
B 6 e^-/min



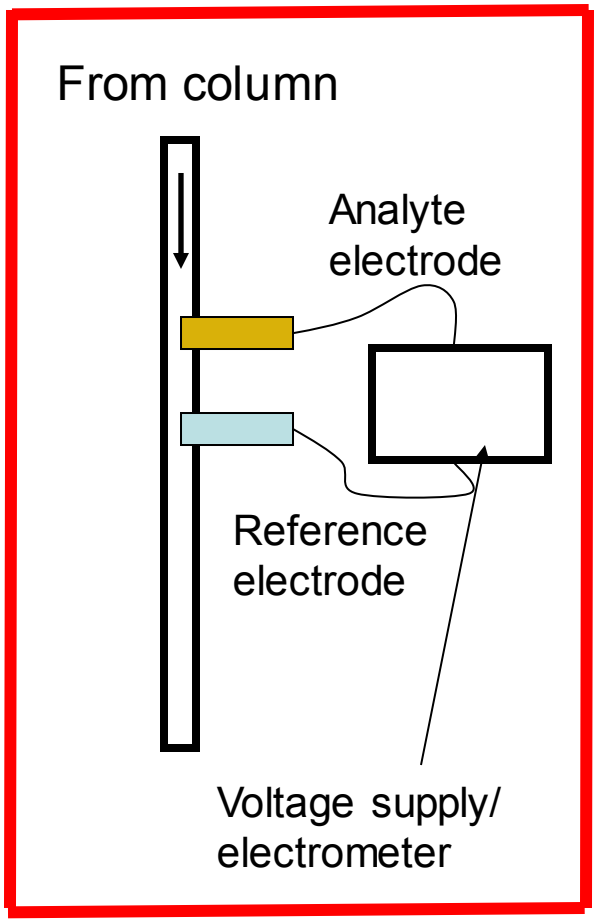
الجهاز

oxidizing the sample

Universal & Destructive

- **Electrochemical Detectors**

  - Principle:
    - Redox reactions occur at electrodes following column
    - Potential cycle used to periodically oxidize/reduce analytes at electrode
    - Current depends on concentration of analyte being reduced or oxidized (similar to A in UV detector)
    - Electrode potential determines classes of compounds that are detectable (similar to λ in UV detector)



• Mass Spectrometry

Bonds in **cation radicals** begin to break (fragment)

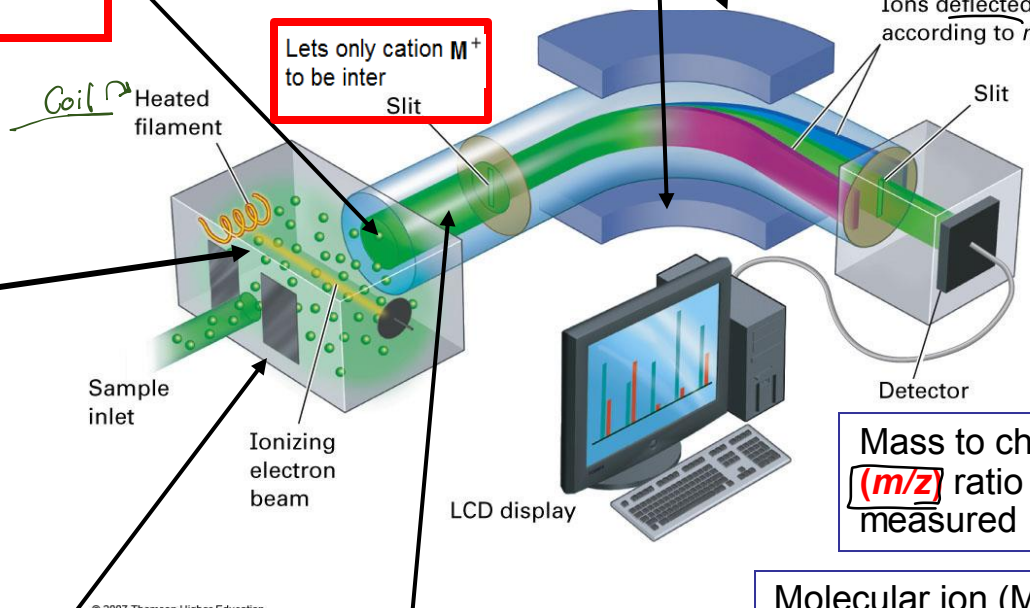
$M^{+\cdot} \longrightarrow M^{+} + M^{\cdot}$

Cation radical — fragment

Sample vaporized and subjected to bombardment by electrons that remove an electron creating a cation radical

$M \xrightarrow{e^-} M^{+\cdot} + e^-$

Organic molecule — Cation radical



Mass to charge (*m/z*) ratio is measured

Molecular ion (M^+) shows **molecular weight**

Acceleration

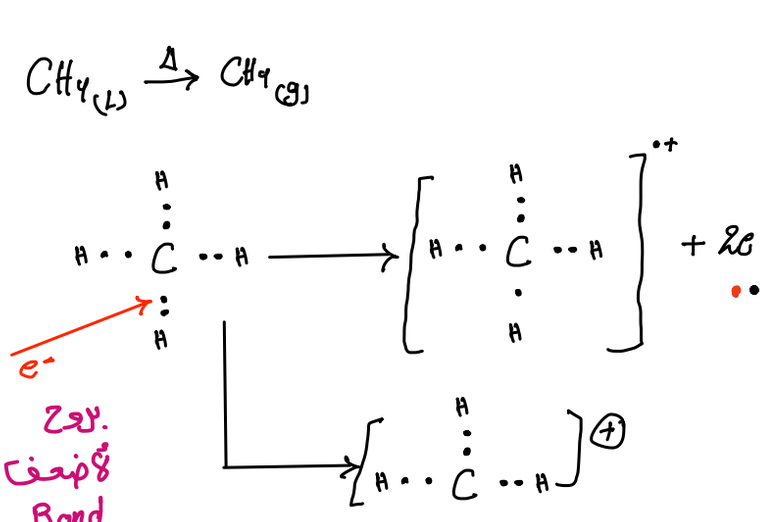
Ionization unit

شرح 2 مبدأ عمله بالتفصيل

1 دخلت العينة على اول Unit اي هي Ionization unit

داخلها في (ملف Coil) بنتج حرارة بسخن ال Sample لحد ما تصير Vapor وبدأ بعدها (يقصف bombardment) ال Vapor باستخدام ال e^-

مثلاً



بعد ما تضعف Bond بيهم وبقتلع e^- وبخرج

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2 Magnatic ← بيميل الجزيئات المشحونة (المتأينة) والجزيئات غير المشحونة بتنحرف عن المسار

أقل الجاذبية ← كان اقدر احدد الوزن

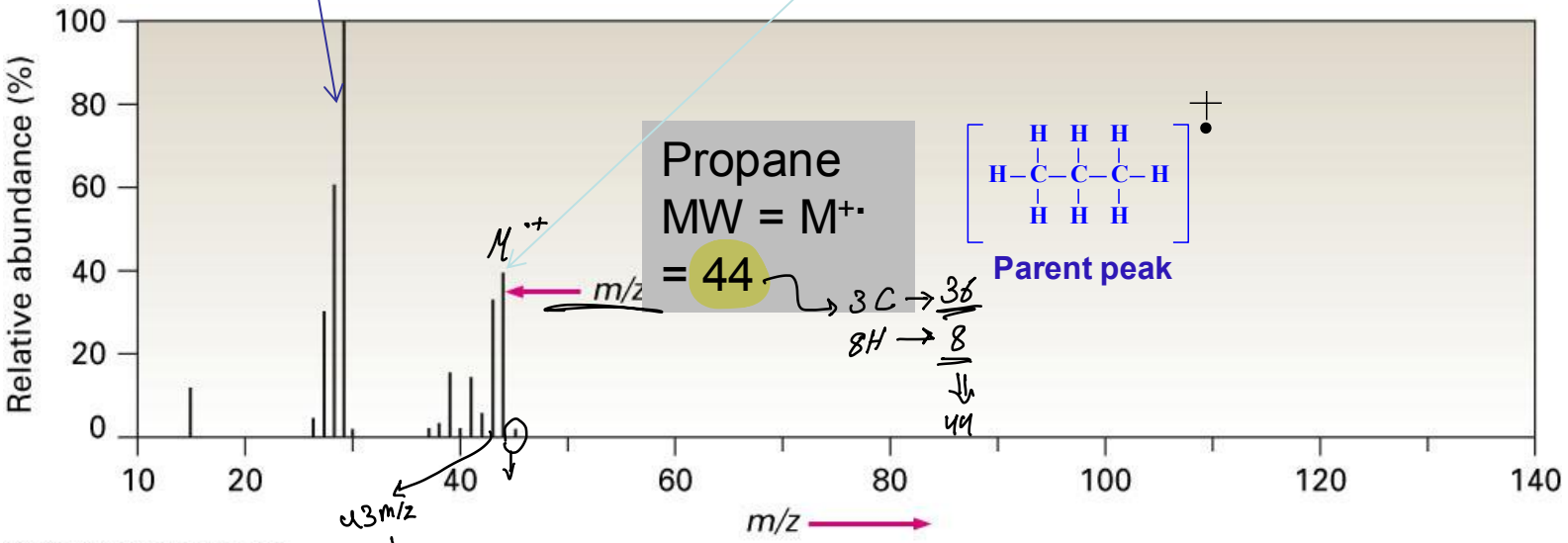
3 المرحلة اللي بعدها هي الميزان ← لازم تكون مشحونة حتى يقدر الميزان يوزنها (M^+ أو M^-)

عبارة عن ⊕ أو ⊖ إذا كانت neutral ما بتنقل شحنة فما بقدر اوزنها

وزن الـ H جدًا قليل فما بأثر على وزن الذرة لأن وزن الذرة متركز في النواة

The Mass Spectrum

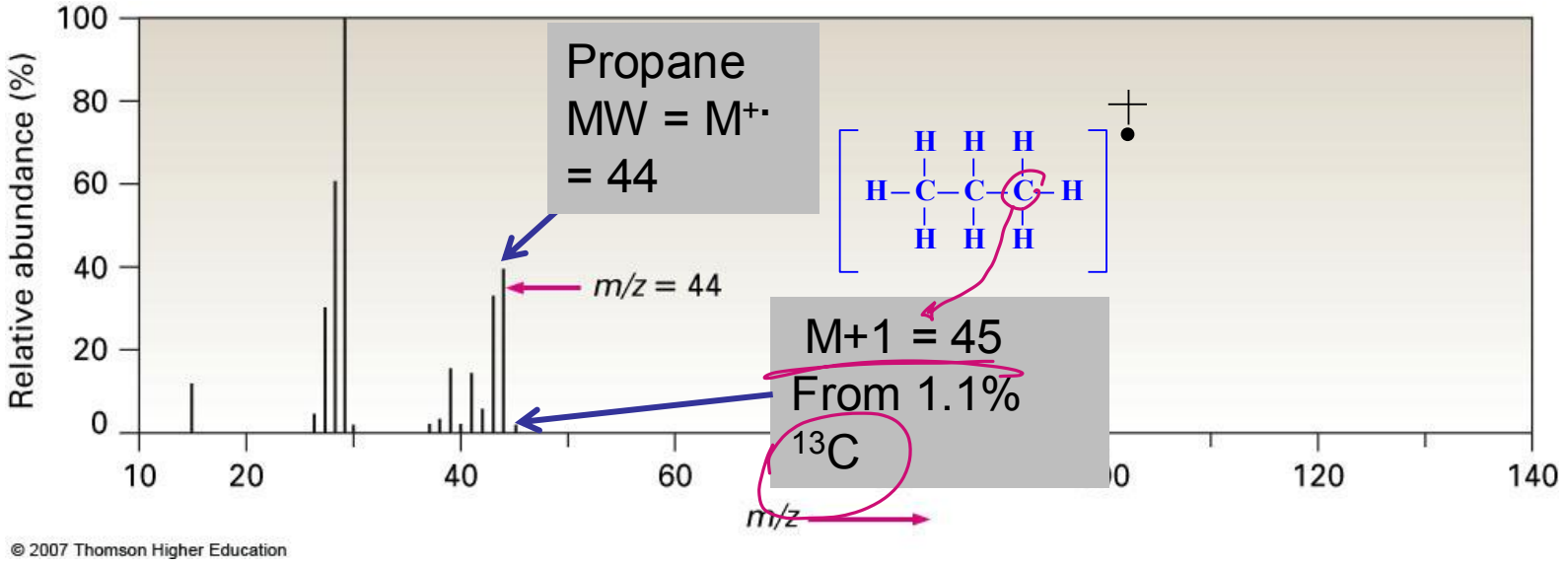
- Plot mass of ions **(m/z) (x-axis)** versus the intensity of the signal (roughly corresponding to the **number of ions) (y-axis)**
- Tallest peak is **base peak** (100%)
  - Other peaks listed as the % of that peak
- Peak that corresponds to the unfragmented **radical cation $[M]^{+\cdot}$ is parent peak**



Determining the molecular formula
M and M+n peak :

Peaks above the molecular weight appear as a result of naturally occurring heavier isotopes in the sample

- M ^{12}C (98.9%) and (M+1) from (1.1%) of ^{13}C in nature
- (M and M+2) in (75.8%) /(24.2%) ratio = ^{35}Cl and ^{37}Cl
- (M and M+2) in (50.7%) /(49.3%) ratio = ^{79}Br and ^{81}Br



Natural abundances of Isotopes of some common elements

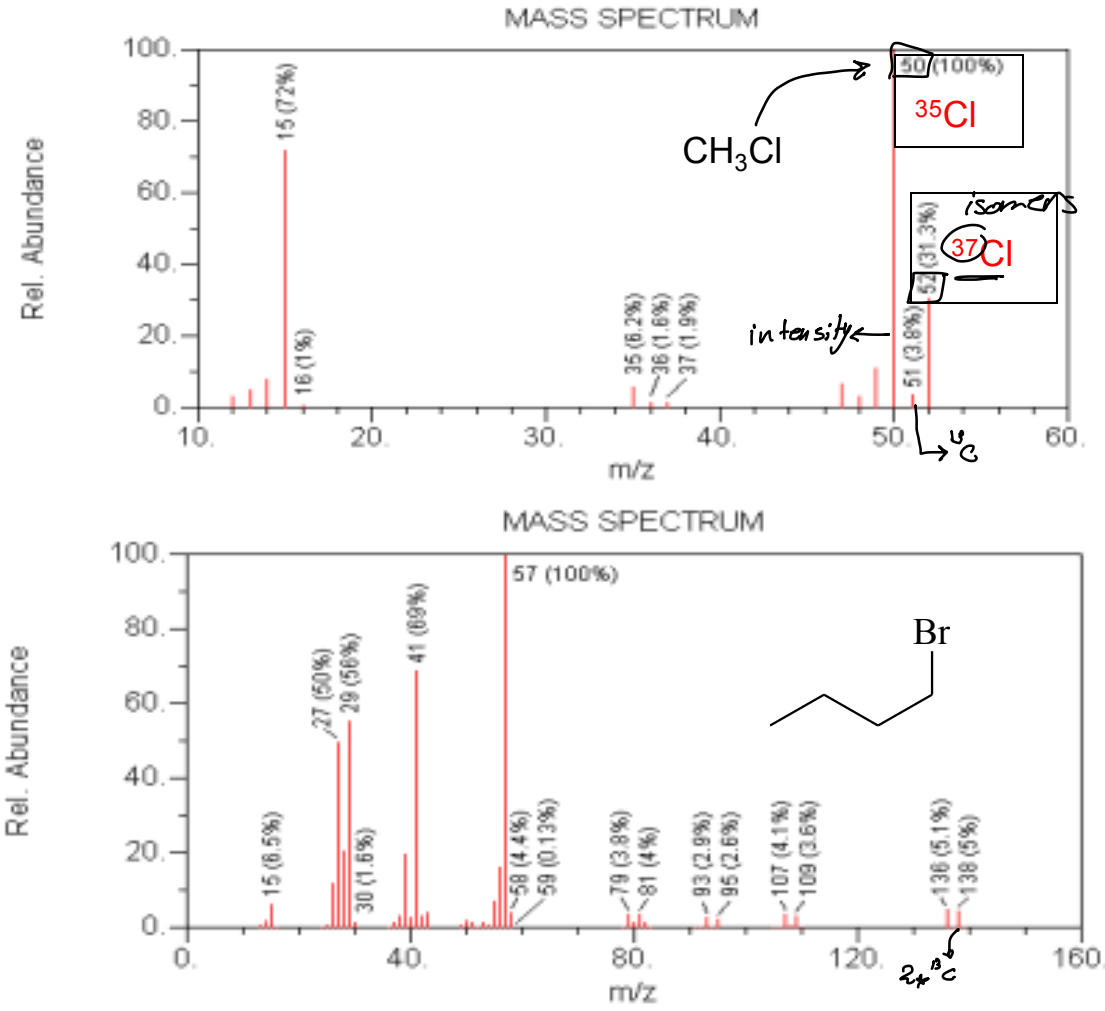
Element	Major Isotope	RA	M + 1 Isotope	RA	M + 2 Isotope	RA
Hydrogen	^{1}H	100				
Carbon	^{12}C	98.9	^{13}C	1.1		
Nitrogen	^{14}N	99.6	^{15}N	0.4		
Oxygen	^{16}O	99.8			^{18}O	0.2
Fluorine	^{19}F	100				
Sulfur	^{32}S	94.8	^{33}S	0.8	^{34}S	4.4
Chlorine	^{35}Cl	75.8			^{37}Cl	24.2
Bromine	^{79}Br	50.7			^{81}Br	49.3
Iodine	^{127}I	100				

The relative abundance (RA) of the most abundant isotope is listed as 100, and the abundances of the other isotopes are listed relative to that number. The M + 1 isotope is the one that is responsible for the peak at *m/z* one unit higher than the peak for M^{+}.

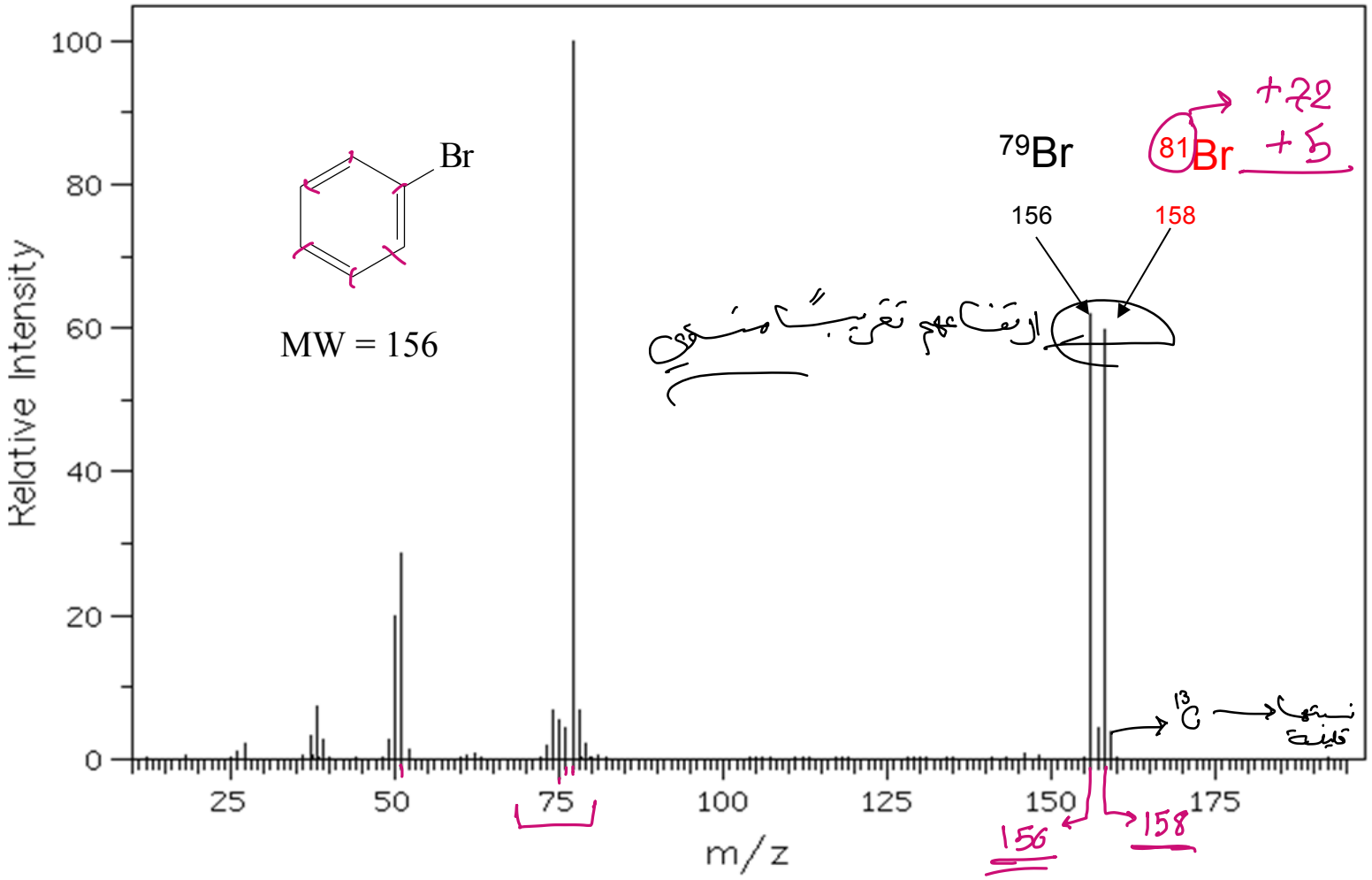
M^+ peak: Halides

M^+ and M+2 in 75.8%:
24.2% (~ 3:1) ratio
= ^{35}Cl and ^{37}Cl

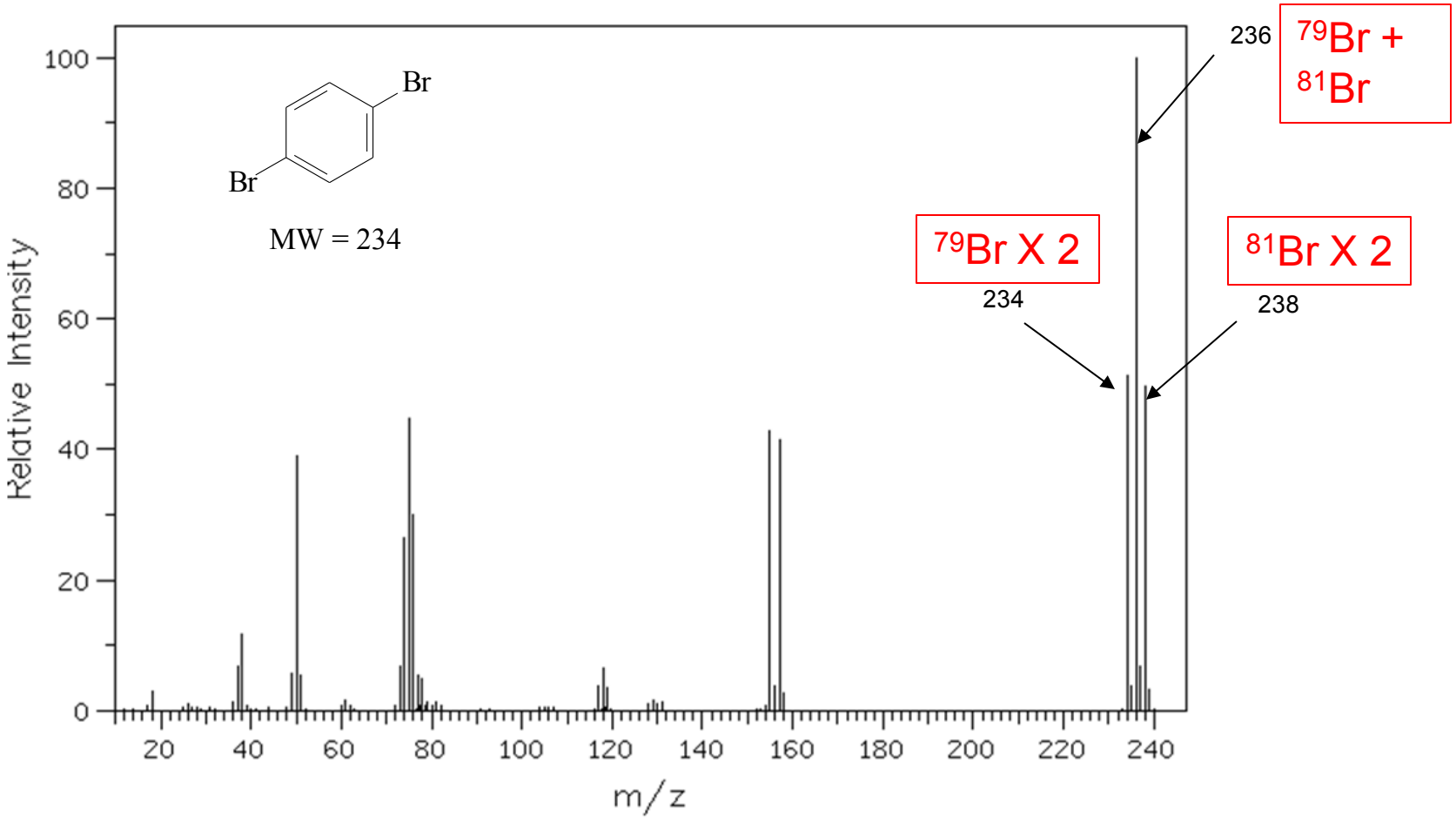
M^+ and M+2 in 50.7%:
49.3% (~ 1:1) ratio
= ^{79}Br and ^{81}Br



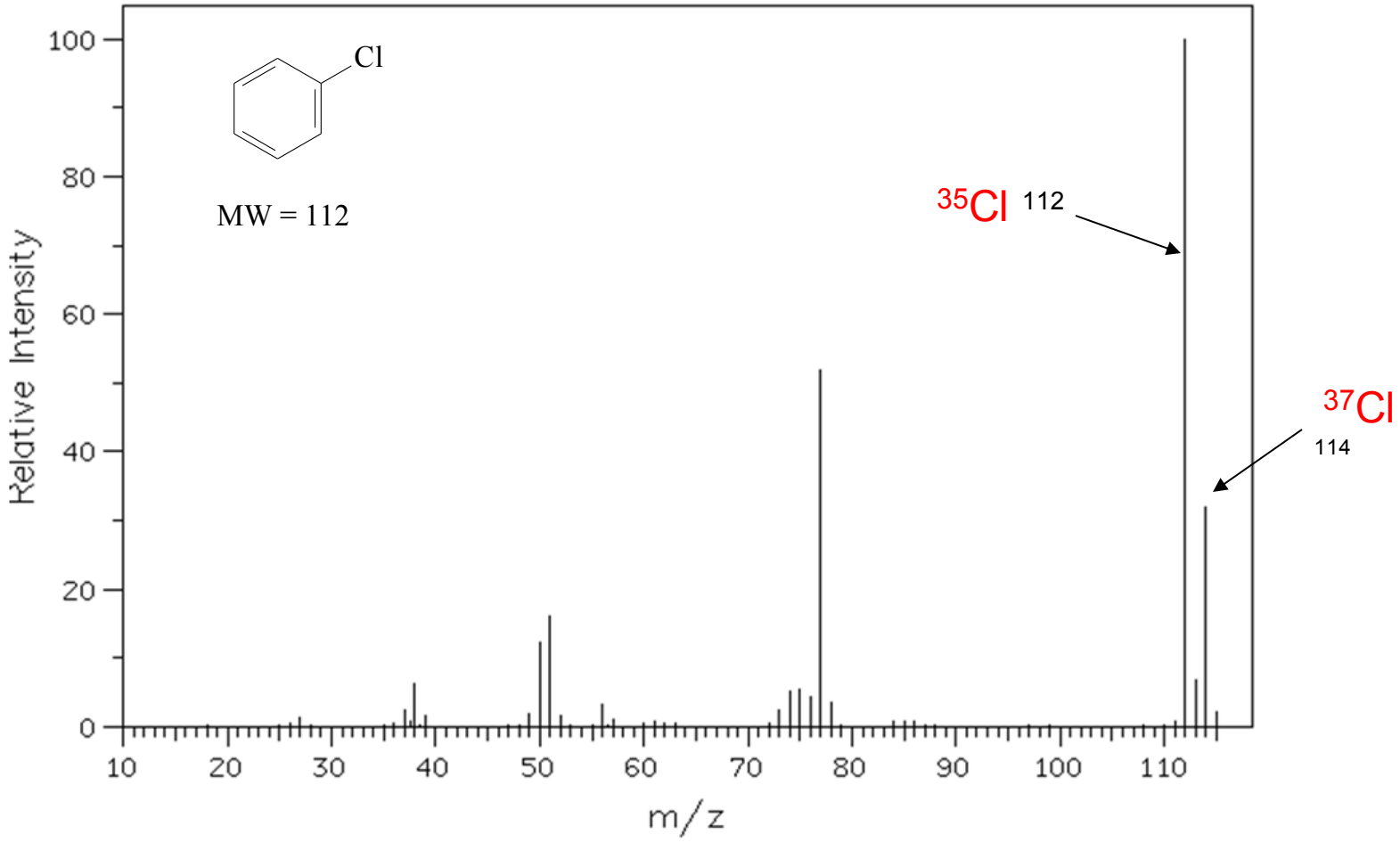
Determining the molecular formula



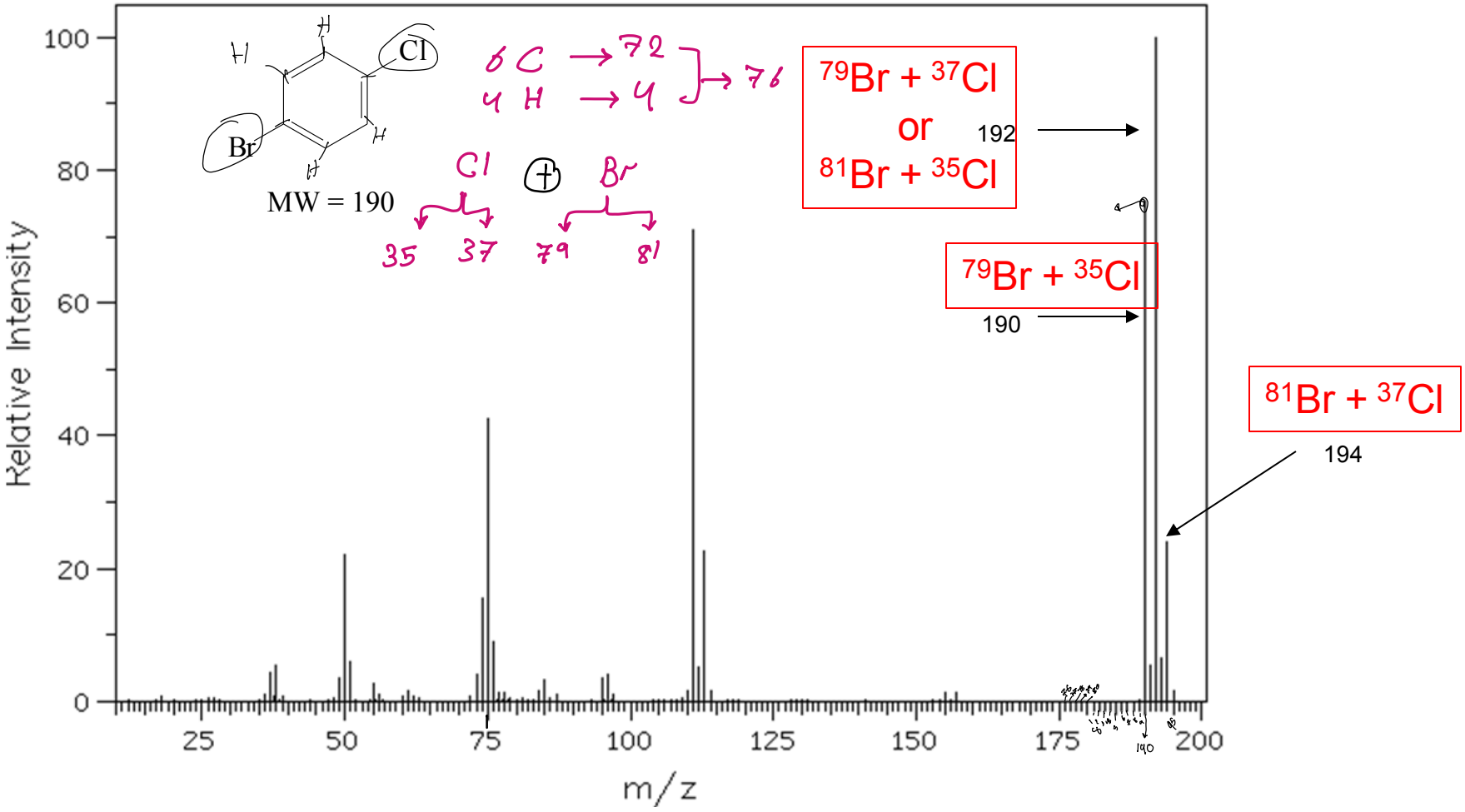
Determining the molecu



Determining the molecular formula



Determining the molecular formula



Determining the molecular formula