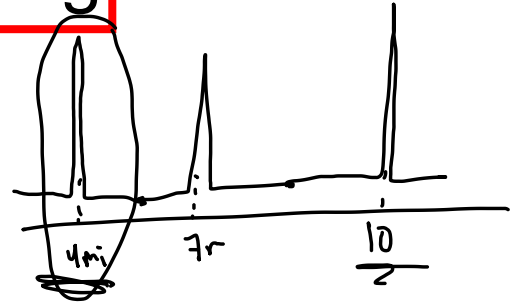
PHMD 221

Asynchronous lecture 10
Chromatography

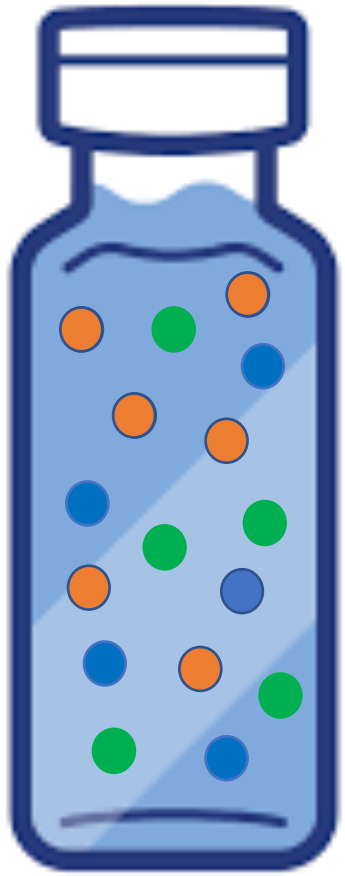
High-performance liquid chromatography (HPLC)

Basic principles of HPLC

A liquid mobile phase is pumped under pressure through a stainless-steel column containing particles of stationary phase with a diameter of 3–10 μm (1.7 μm in ultrahigh-performance liquid chromatography (UPLC)). The analyte is loaded onto the head of the column via a loop valve and separation of a mixture occurs according to the relative lengths of time spent by its components in the stationary phase. It should be noted that all components in a mixture spend more or less the same time in the mobile phase in order to exit the column. Monitoring of the column effluent can be carried out with a variety of detectors.



Separation of a complex mixture



UV/Vis → determine pure sample conc. for

How can you determine the concentration of each component in the mixture?

more one component in mixture.

to determine each component conc. by seperate each on another by using HPLC

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Mobile phase versus stationary phase

Mobile means moving

can be mixture.

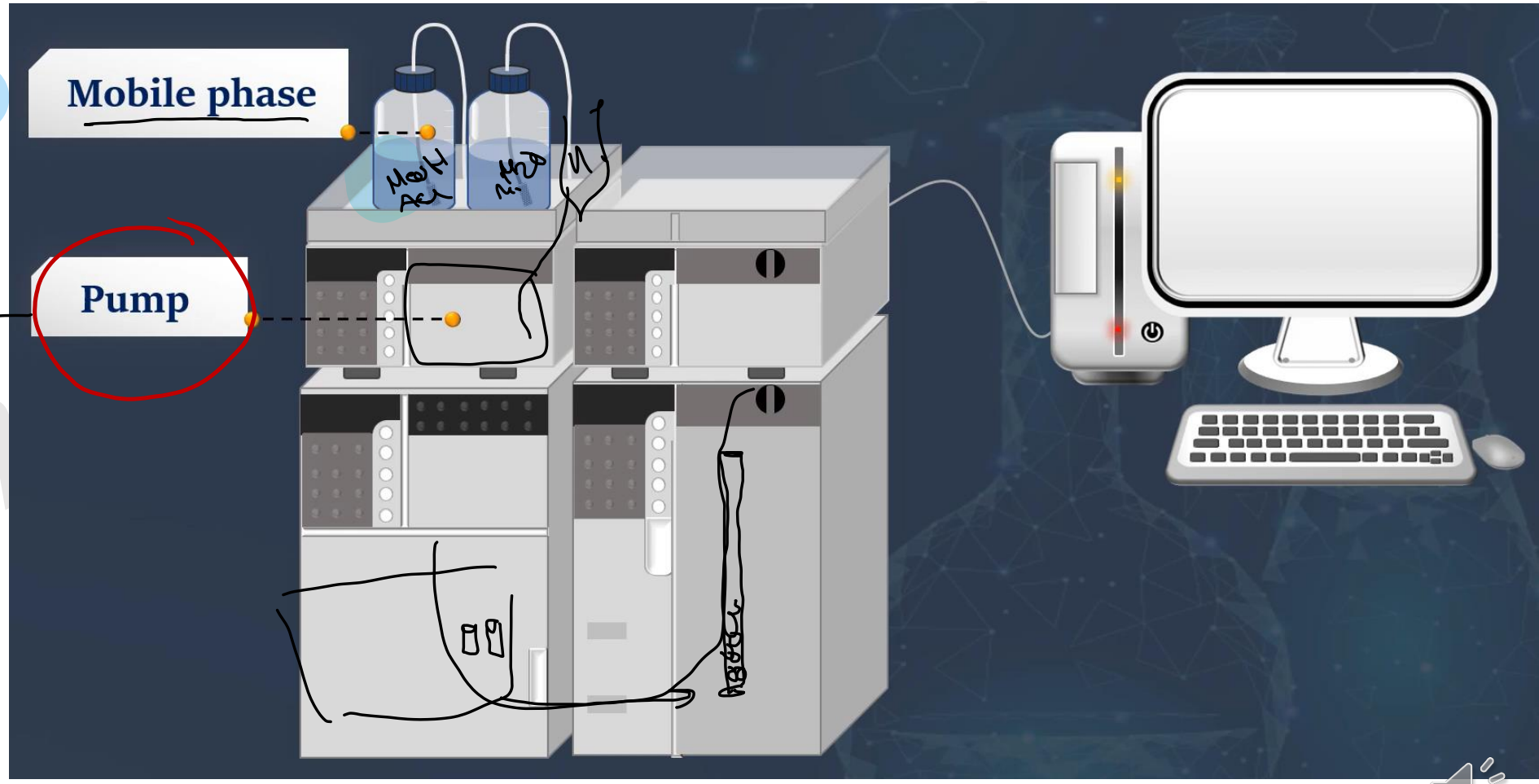
↓

pumped by pump

↓

piping system

through



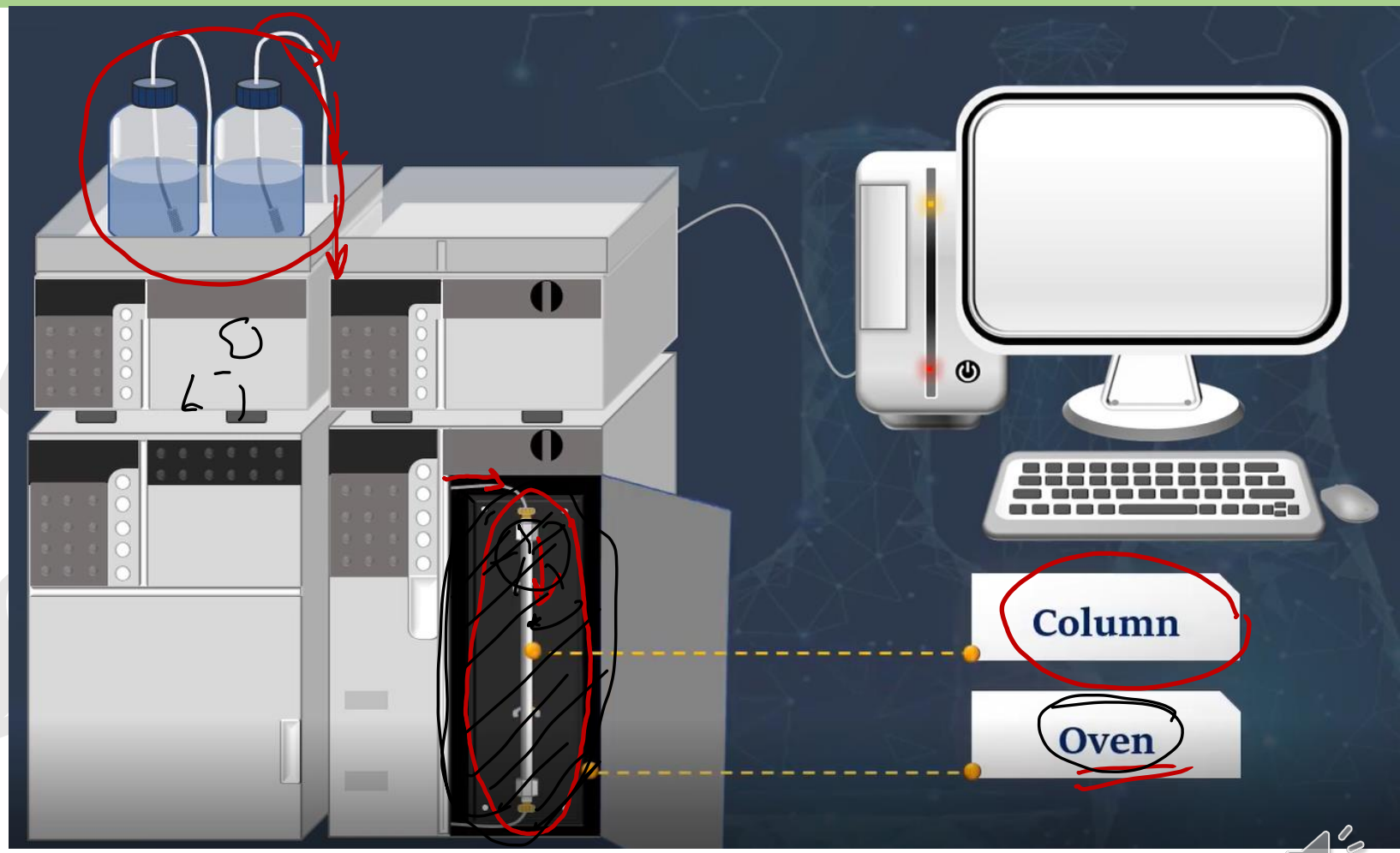
Mobile phase versus stationary phase

Stationary means does not move (static)

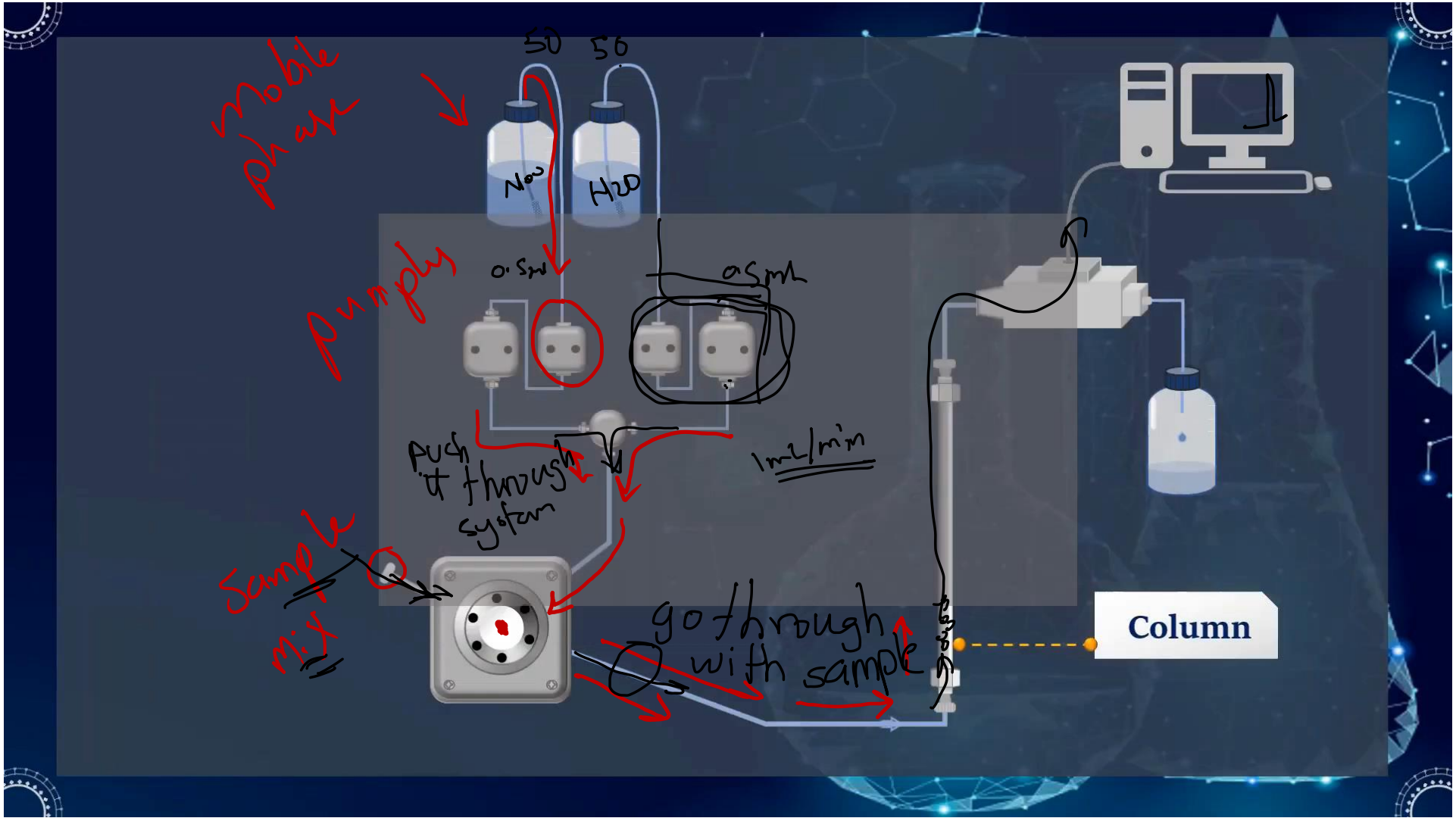
solid → inside the column → can't see.

mobil phase move in column & stationary phase.

oven → control the temperature



Mobile phase versus stationary phase

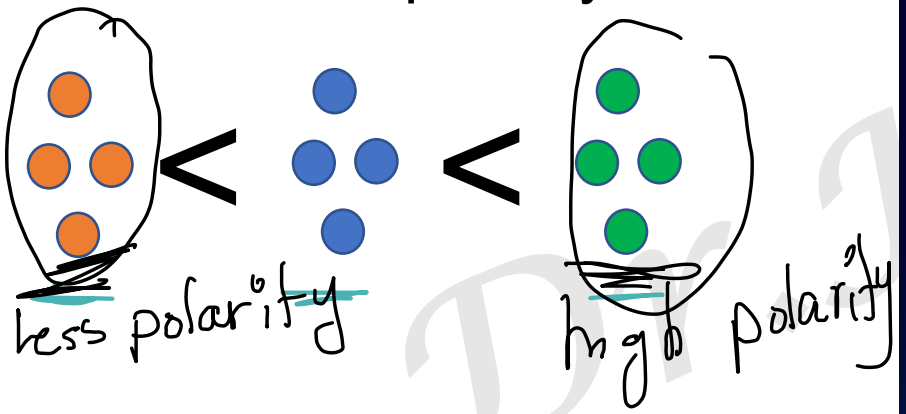


like dissolve like.

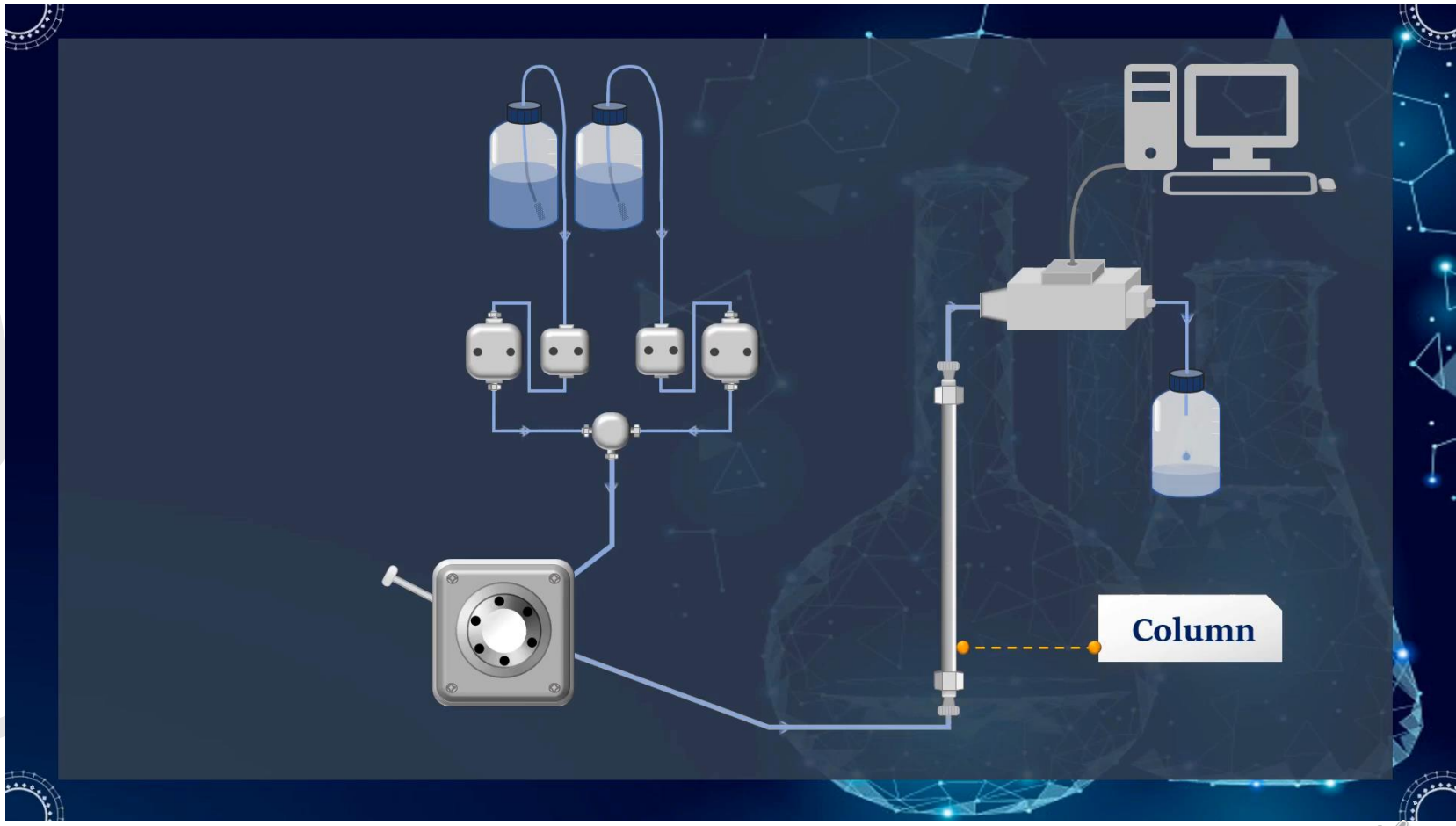
Separation is based on difference in affinity to the mobile and stationary phase

mixture have three different component.

Order of polarity:



In a nonpolar stationary phase



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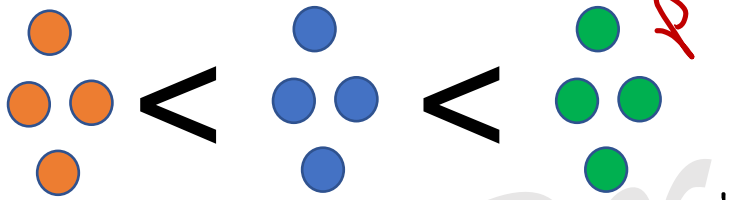
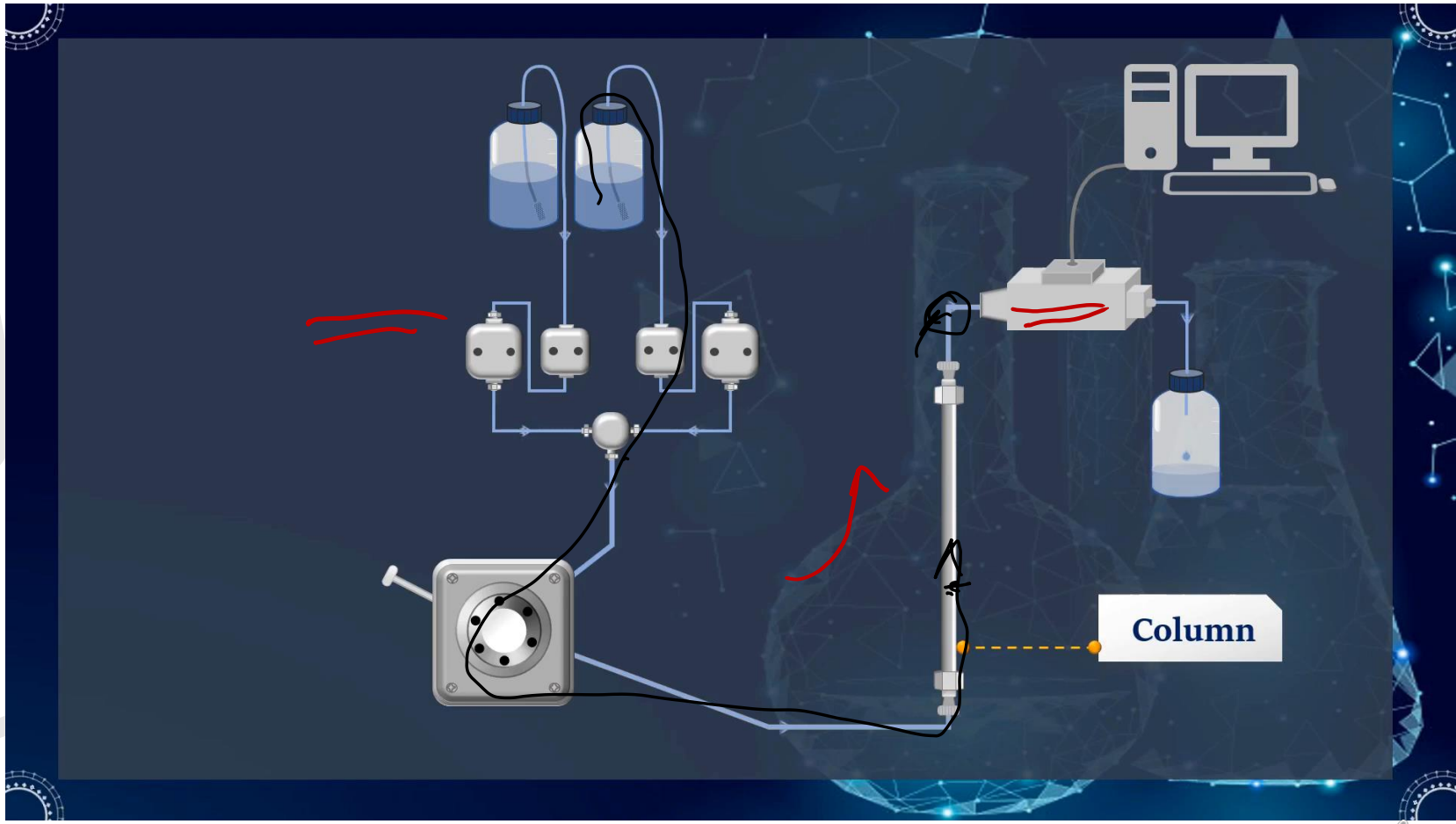
Order of polarity:

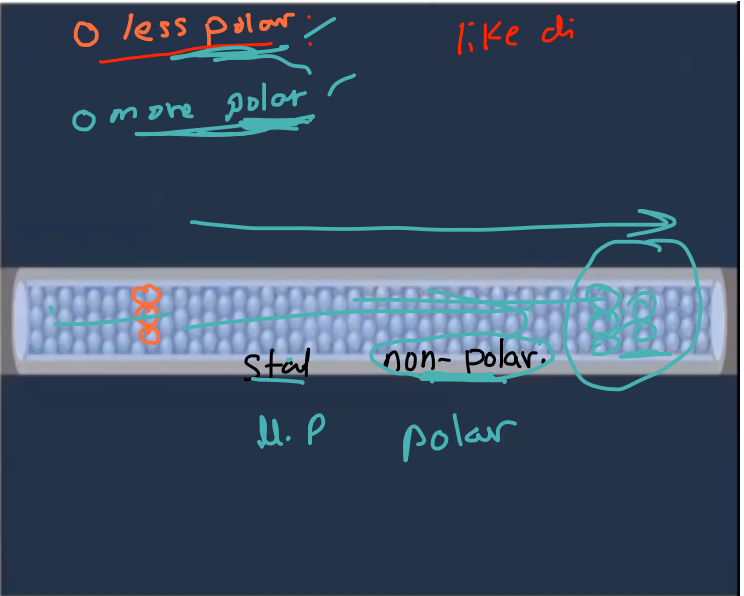


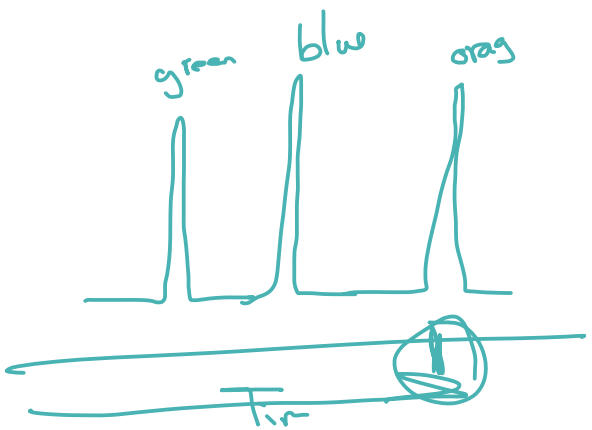
In a nonpolar stationary phase

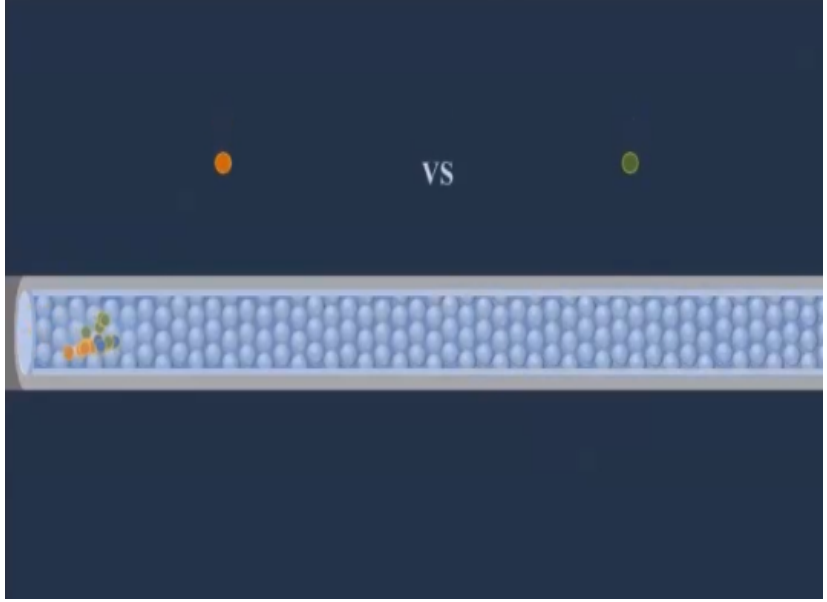
mobile phase

polar



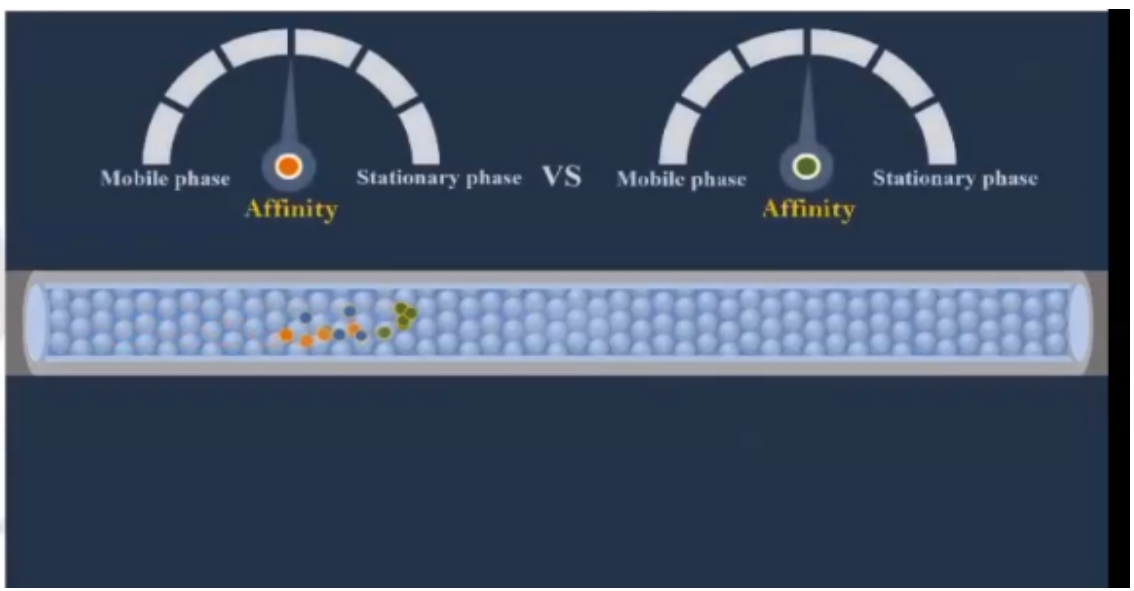


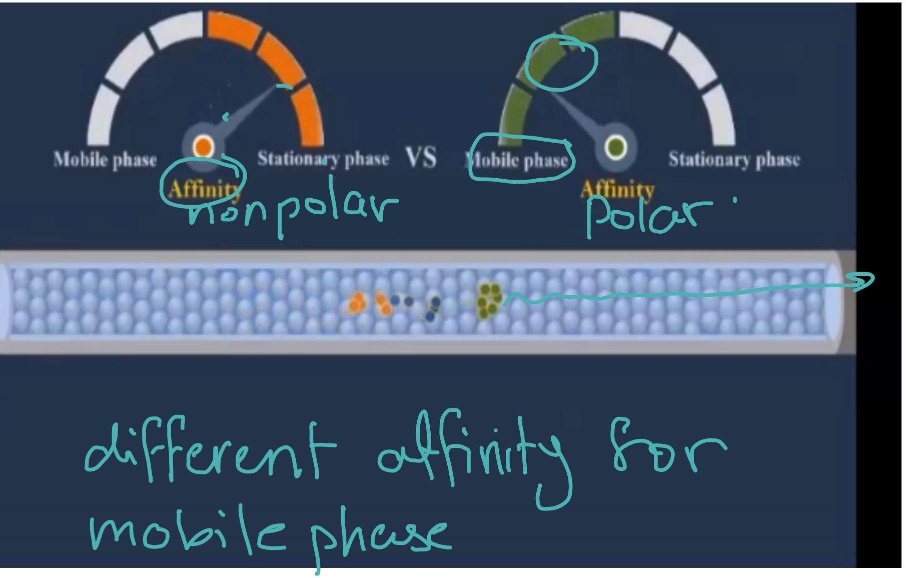




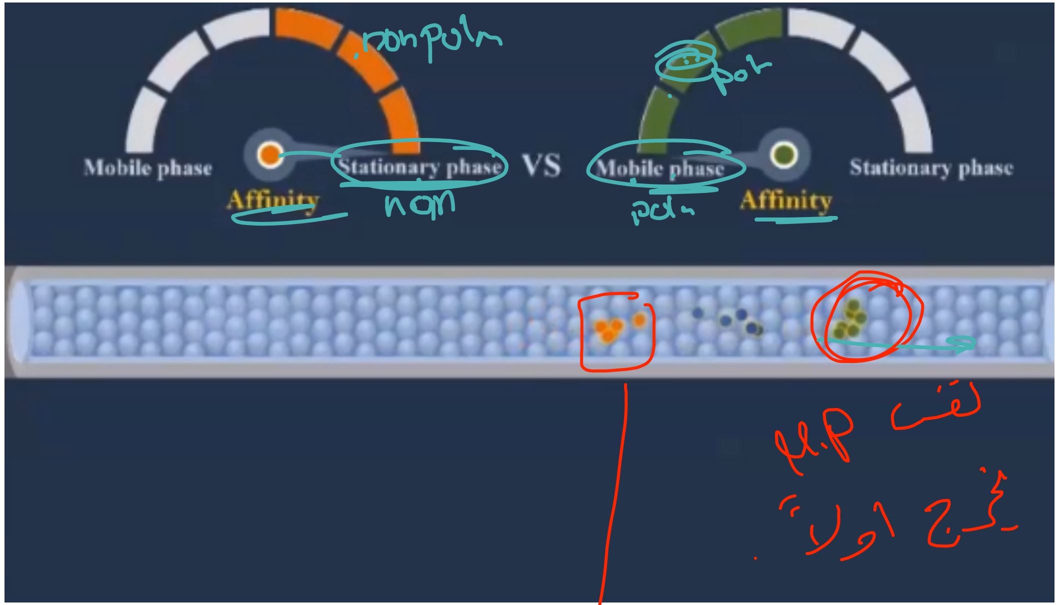
Inside the column is a solid → Fixed (stationary) phase.

* moved solvent → mobile phase.





& stationary phase.



takes more time

ابطئ واحدة

لانها نفس

statio

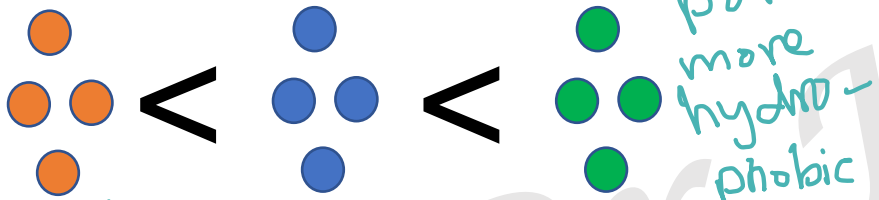
Affinity

لها

s.p اكثر

Separation is based on difference in affinity to the mobile and stationary phase

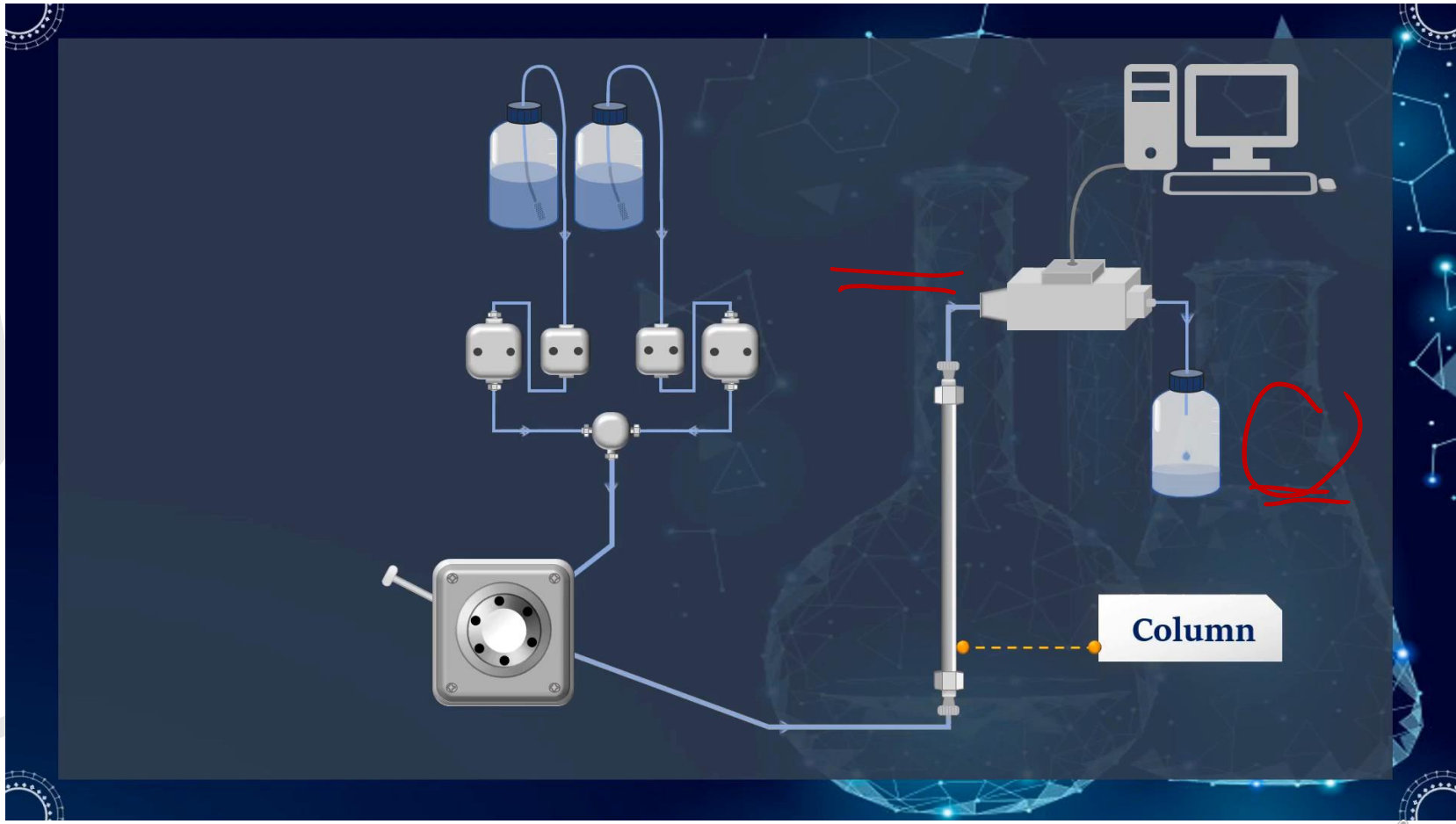
Order of polarity:



In a nonpolar stationary phase

will stay in stationary phase.

* mobile phase polar



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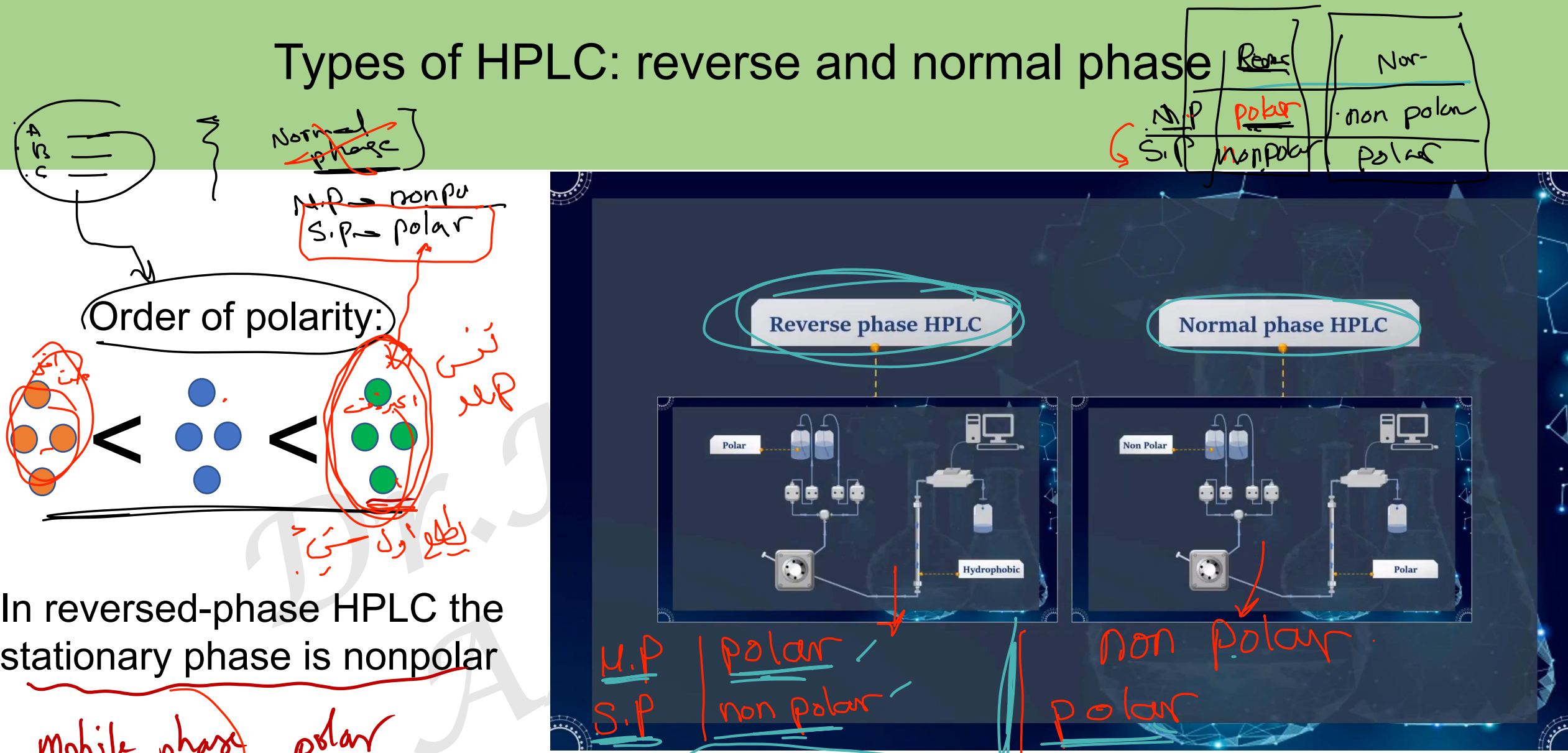
Types of HPLC: reverse and normal phase

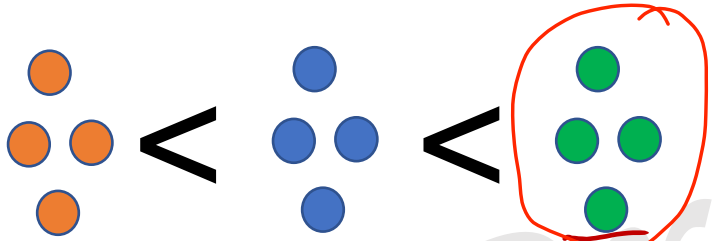


Order of polarity:

In reversed-phase HPLC the stationary phase is nonpolar

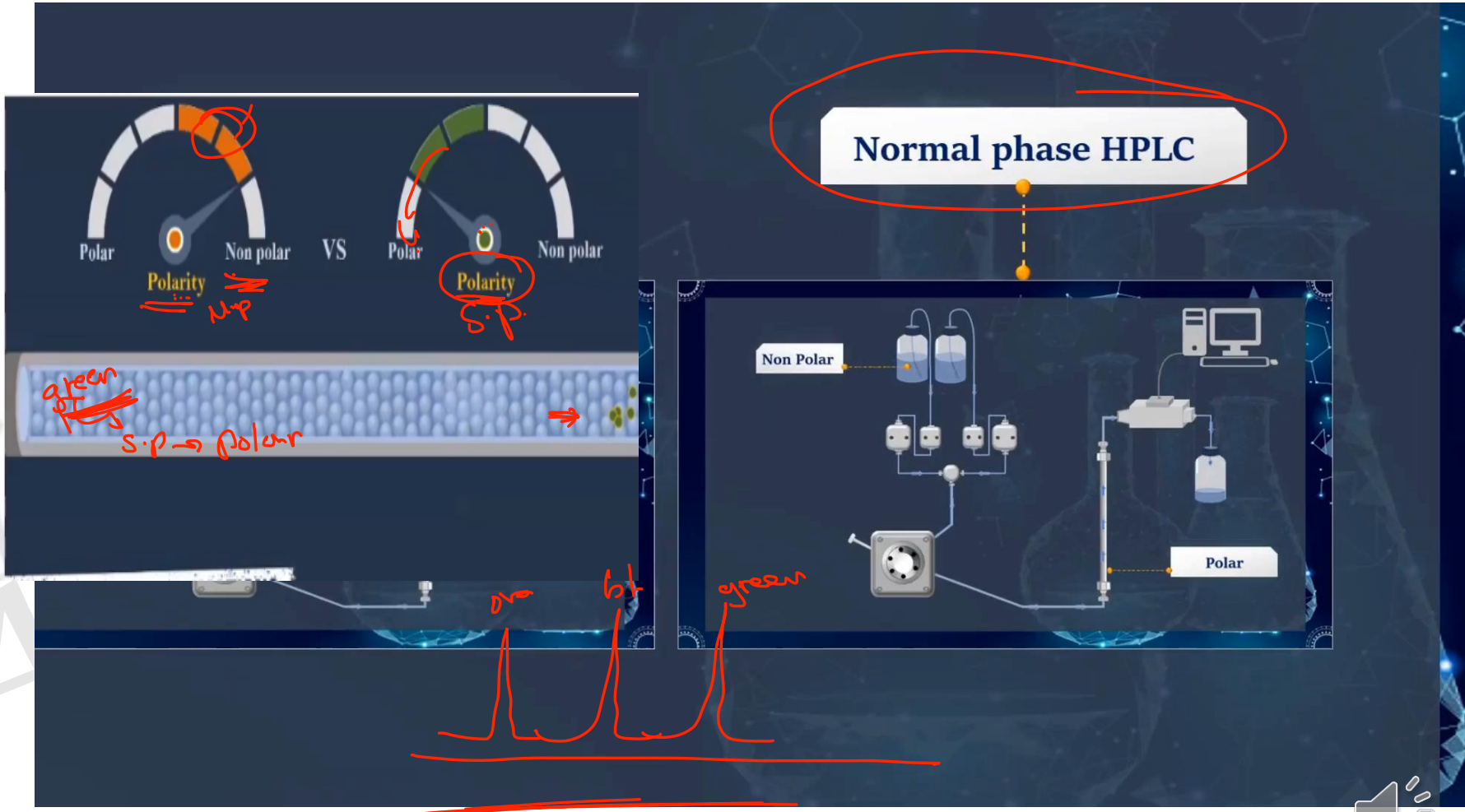
Types of HPLC: reverse and normal phase

Order of polarity:

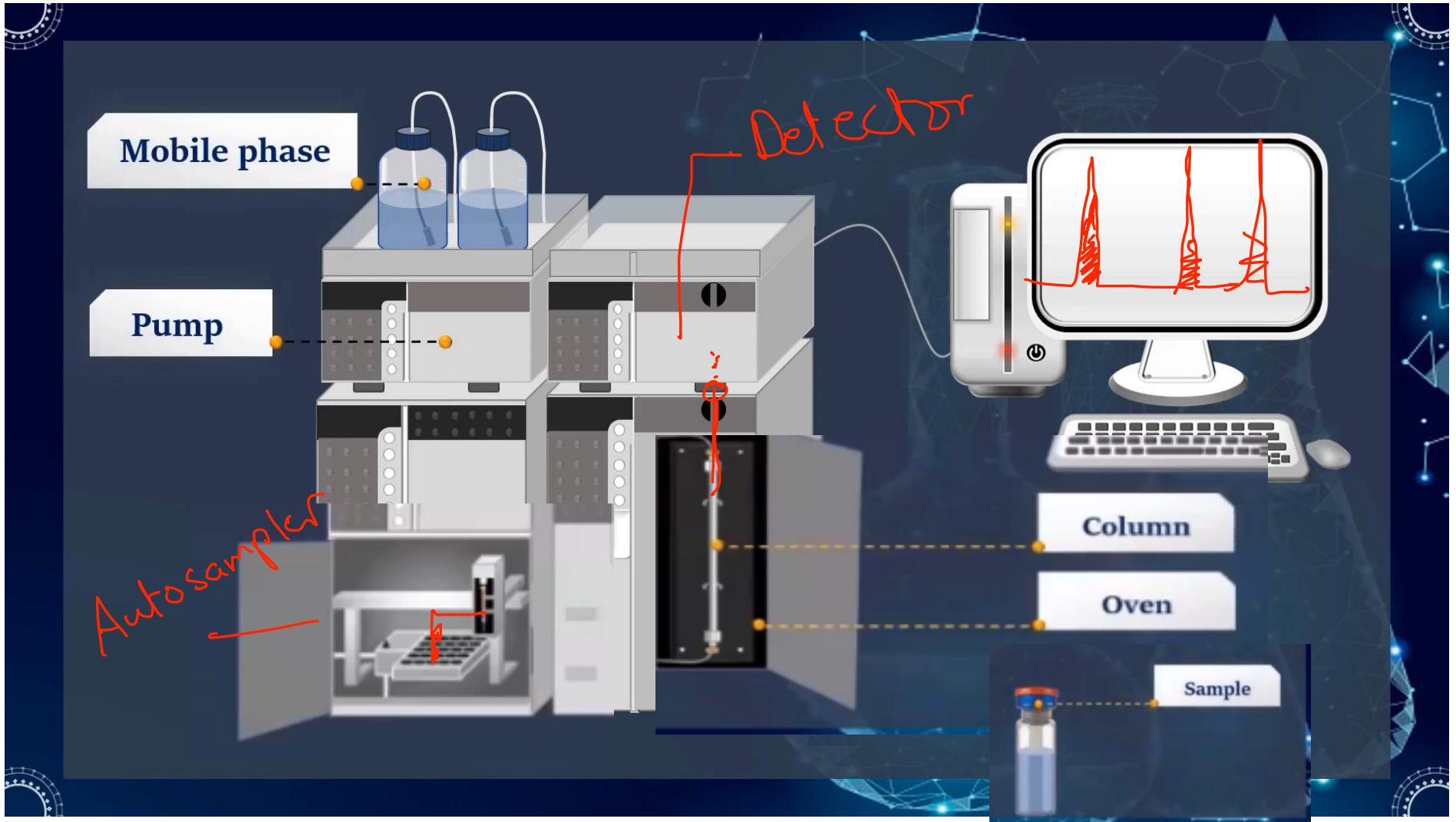


In normal-phase HPLC the stationary phase is polar

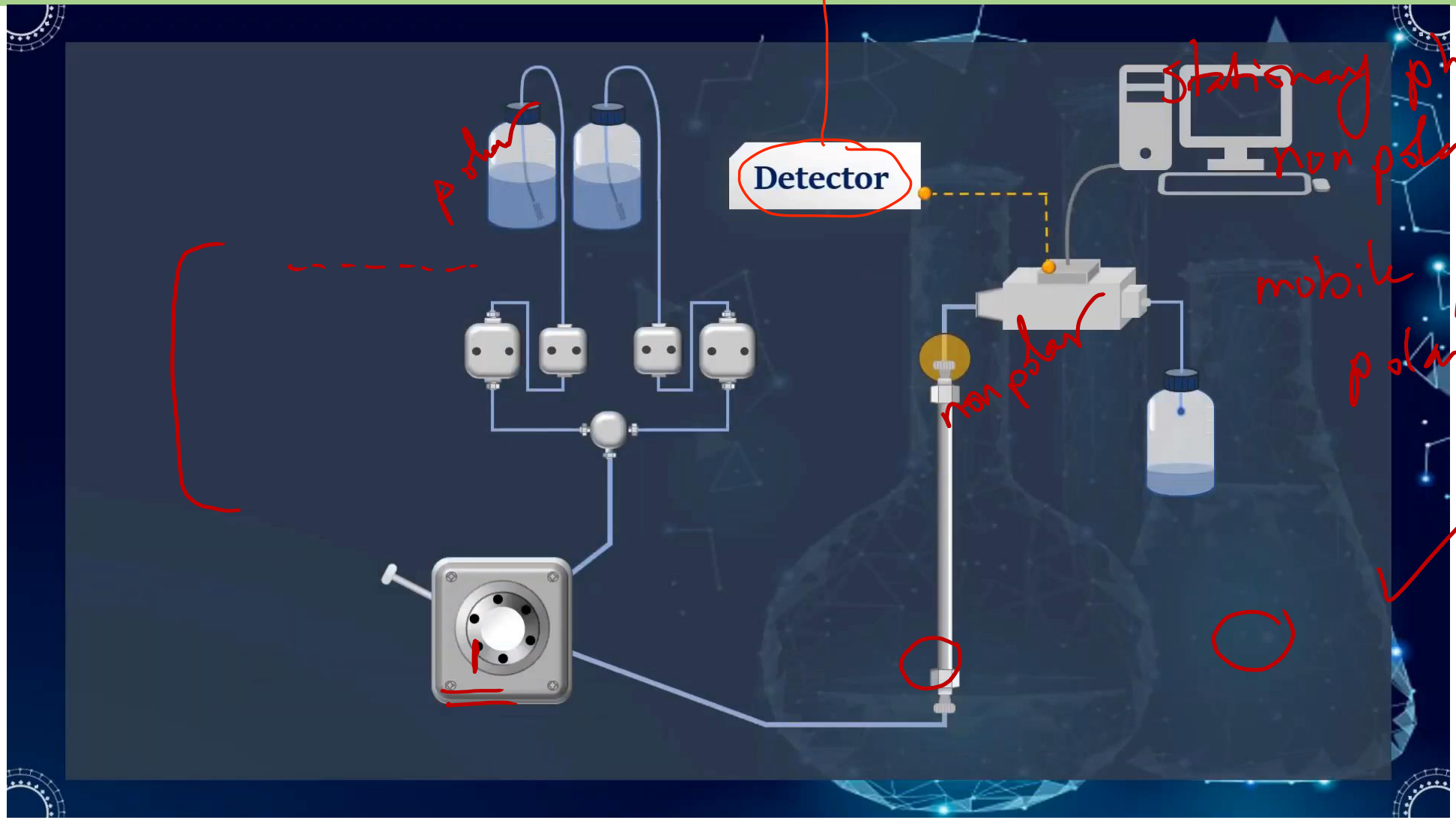
mobile phase non polar

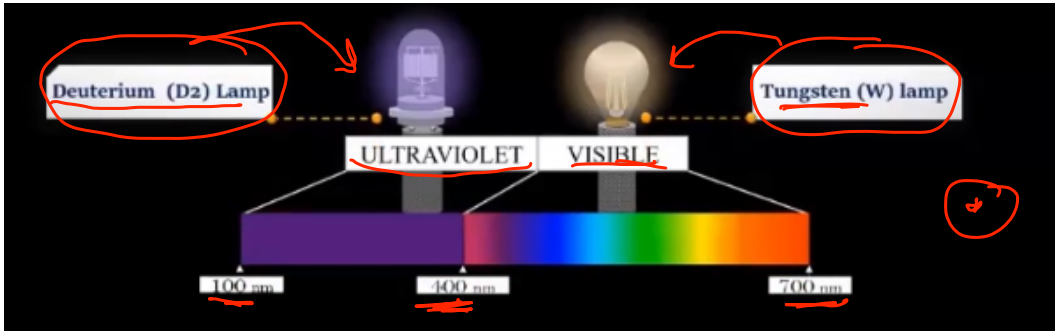


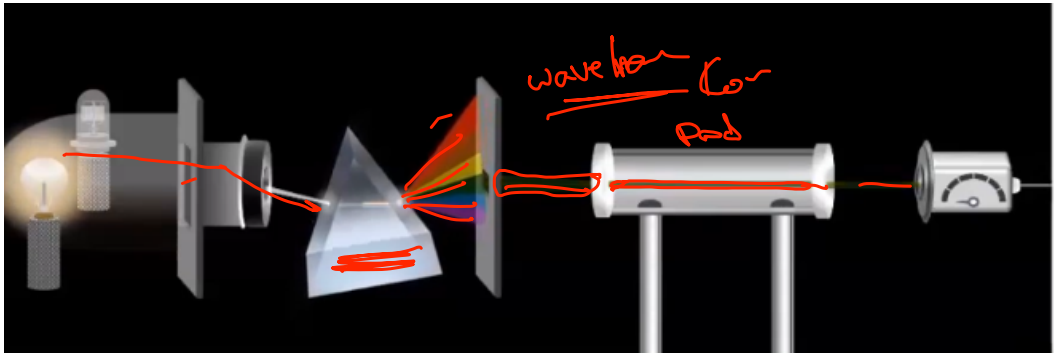
Detection of eluting components

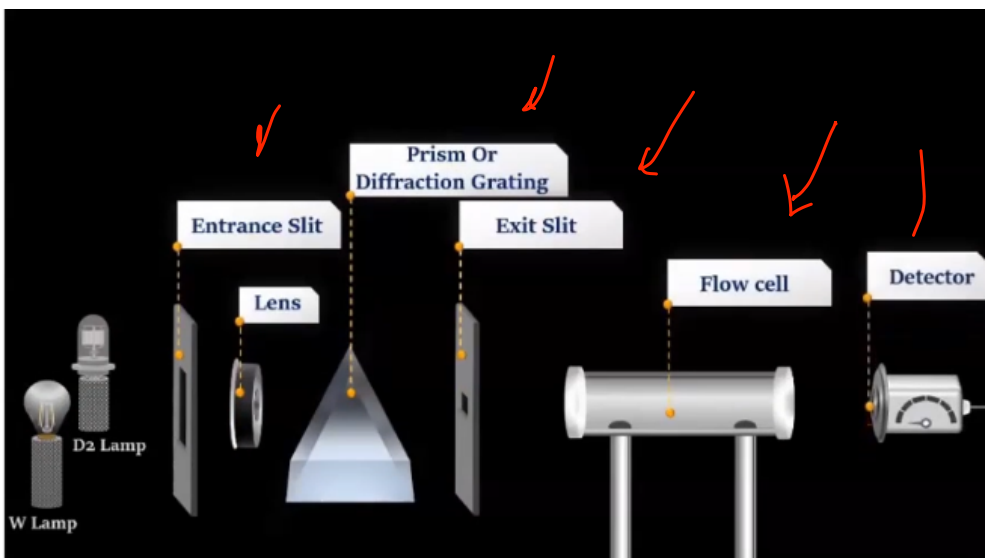


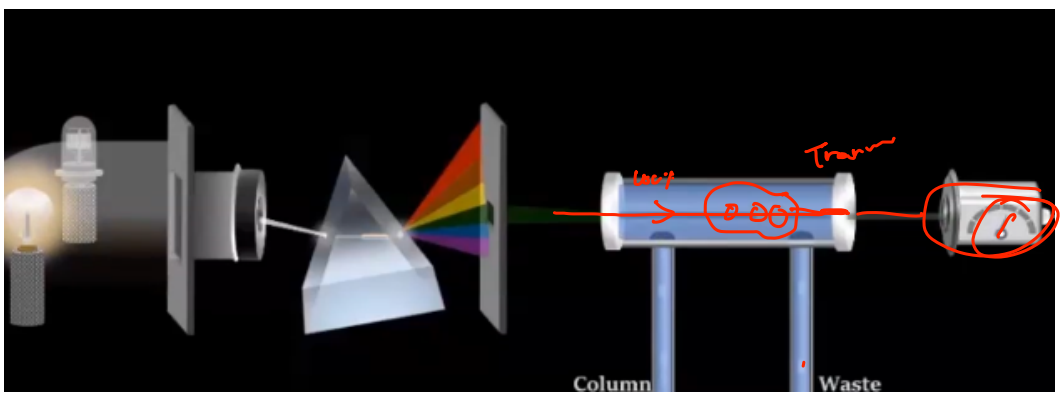
Detection of eluting components

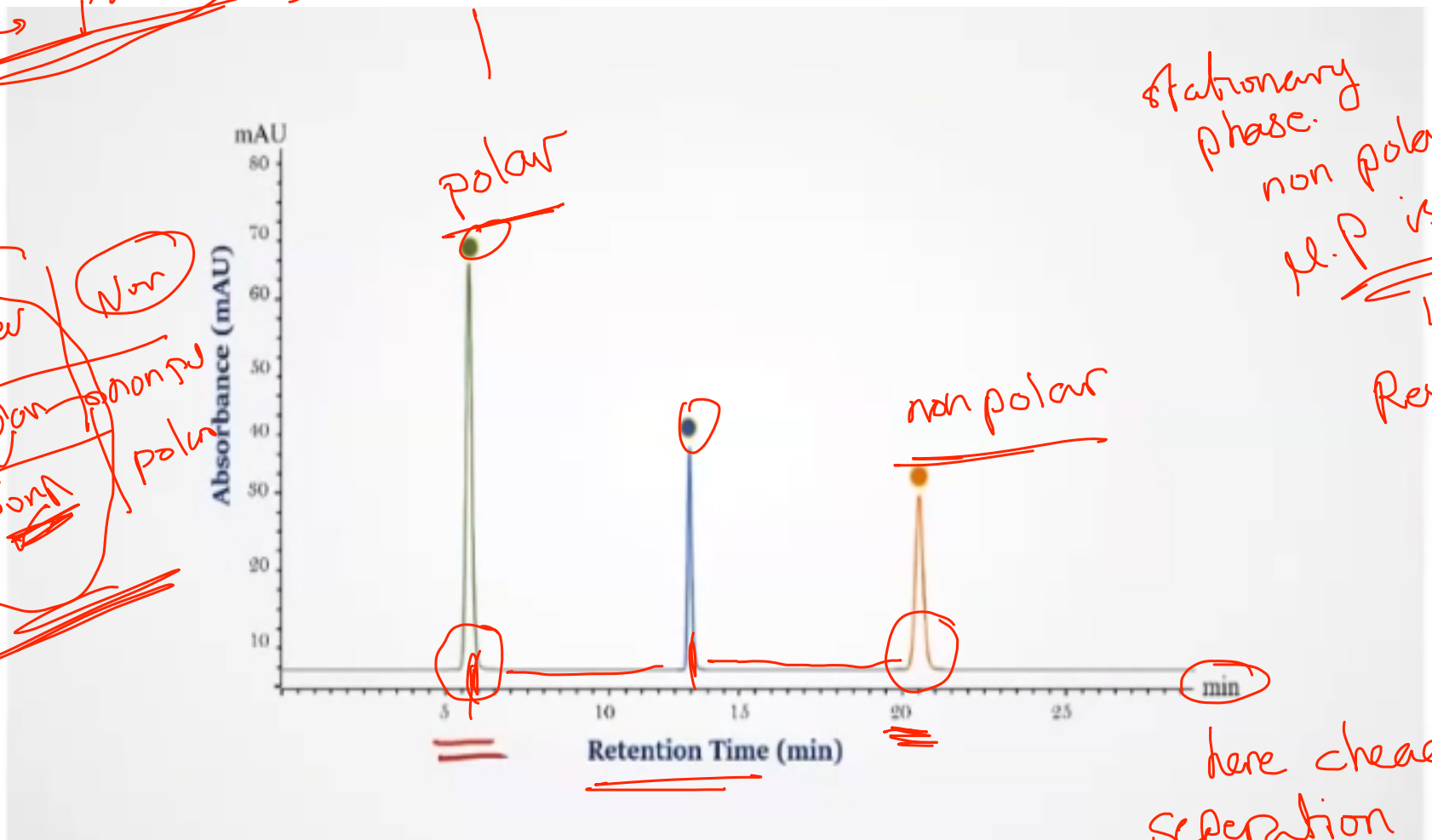



Deuterium (D2) Lamp
Tungsten (W) lamp
ULTRAVIOLET
VISIBLE
100 nm
400 nm
700 nm



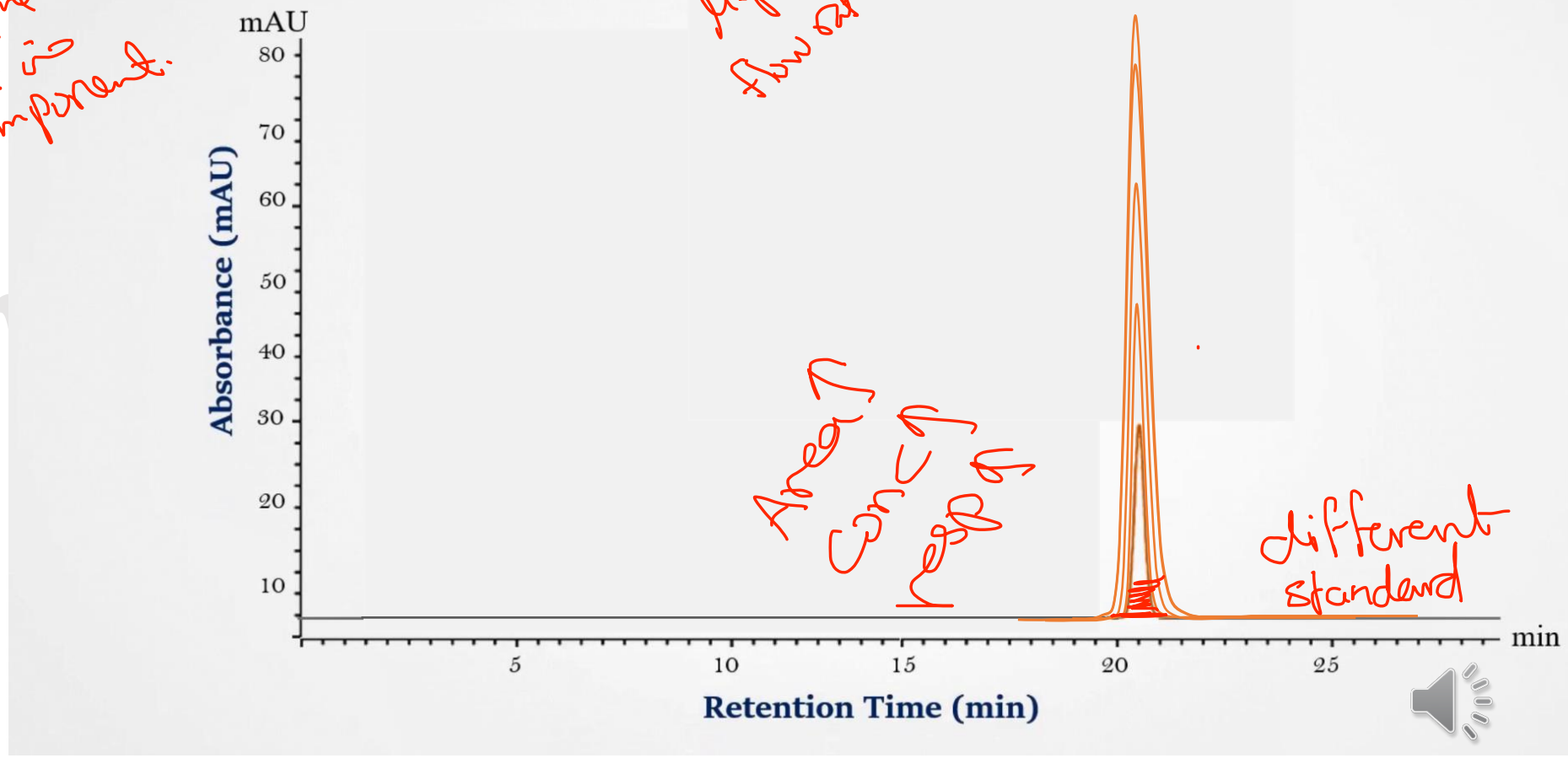

Prism Or
Diffraction Grating
Entrance Slit
Exit Slit
Lens
Flow cell
Detector
D2 Lamp
W Lamp


Column
Waste

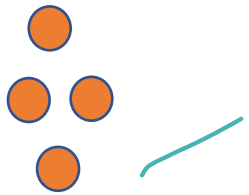

mAU
80
70
60
50
40
30
20
10
Absorbance (mAU)
5
10
15
20
25
min
Retention Time (min)
polar
non polar

To determine the concentration of the components you need to build a calibration curve

Conc. (µM)	Peak area
50	410.3
100	825.1
150	1220.8
200	1637.4
250	2042.6



In HPLC we build a calibration curve by plotting known concentrations versus the area under the curve



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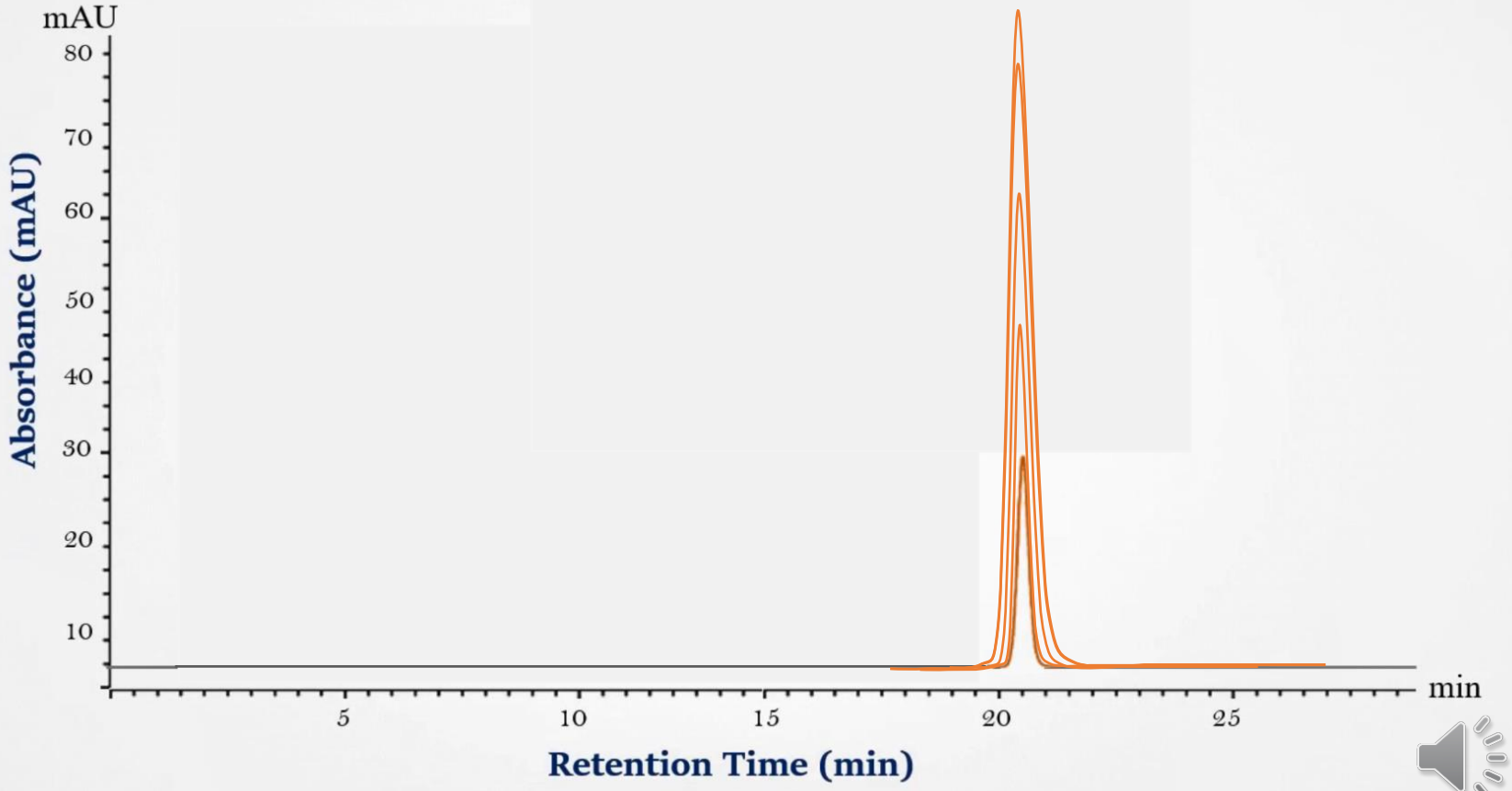

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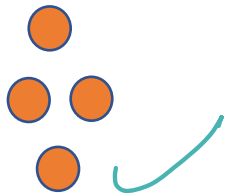
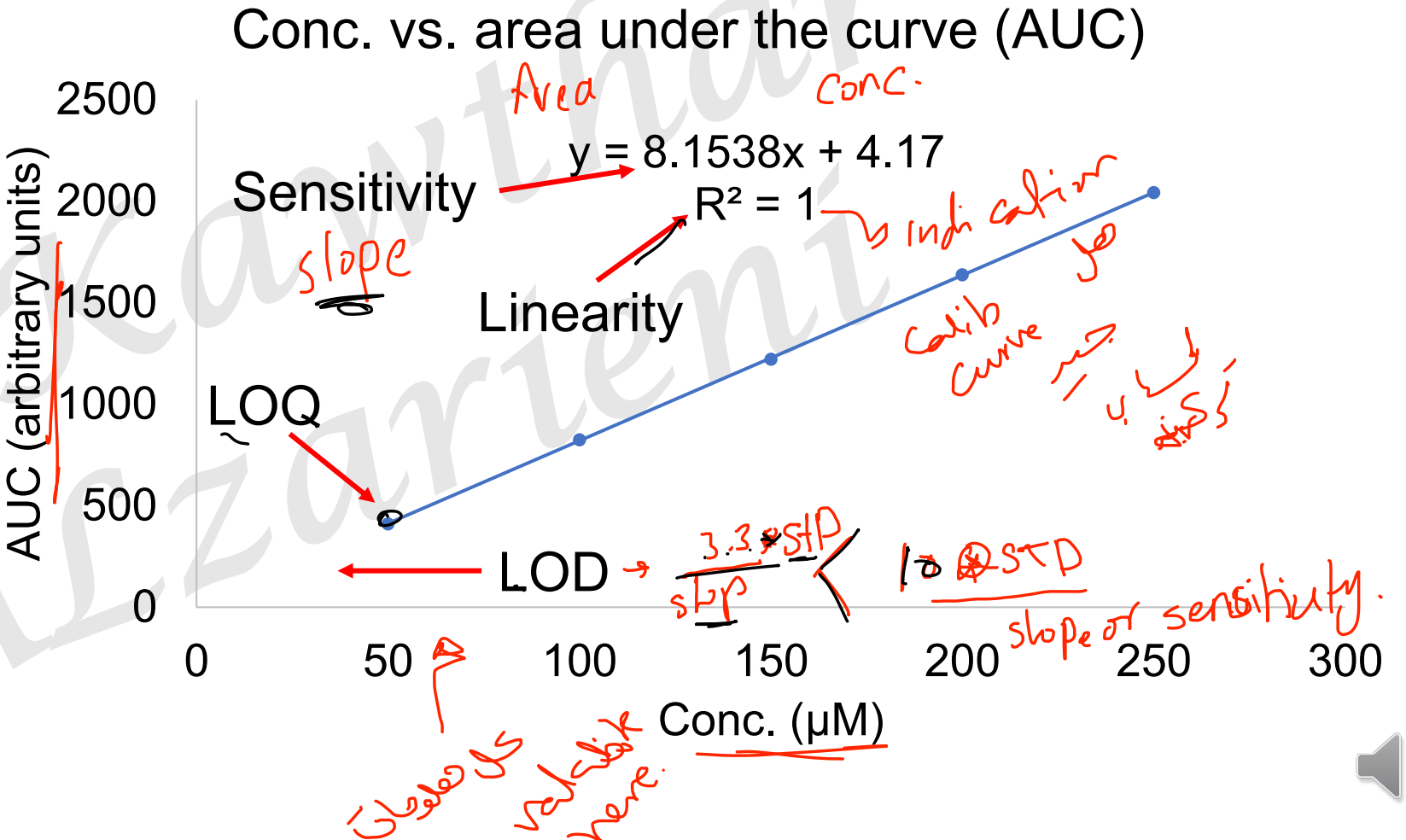


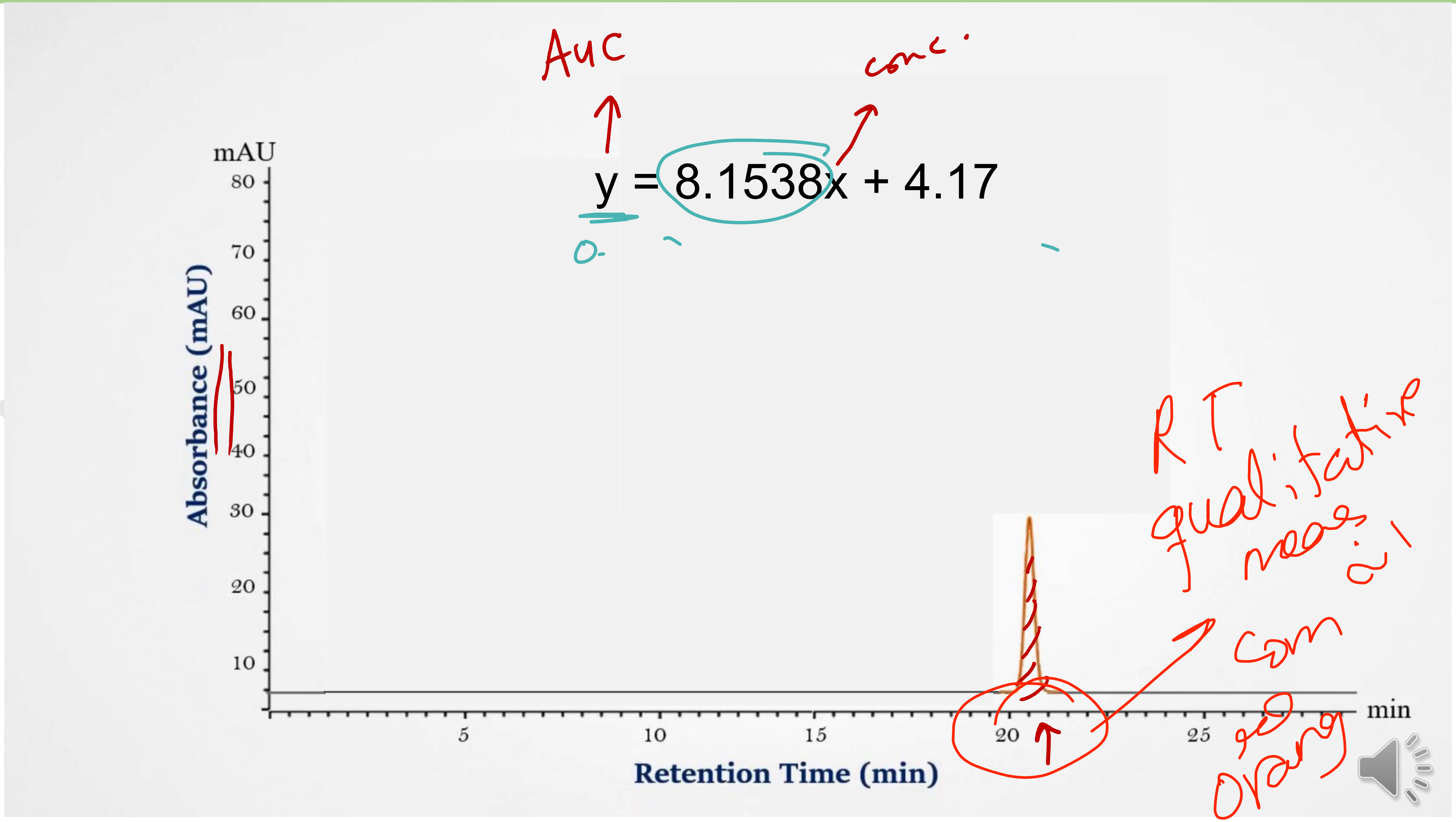
Conc. (µM)	Peak area
50	410.3
100	825.1
150	1220.8
200	1637.4
250	2042.6

you have at least 5 point



Determination of unknown concentration by using the calibration curve equation

Conc. (µM)	Peak area
~~113.6~~	930.5



green → نقطه وصل calibration curve بزن

↓

STD → SBn

↓

Area

↓

creat calib

↓

sampl

↓

Area

↓

Calcu Unkn con ←

Cali b

→ qualit

→ sepu

→ quanti