

S.O.S. IN ENVIRONMENTAL CHEMISTRY

JIWAJI UNIVERSITY, GWALIOR(M.P.)



Presentation on HPLC



Contents

- Introduction (HPLC)
- Instrumentation
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- Reaction monitoring
- Reaction Monitoring of Sugammadex
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Introduction:

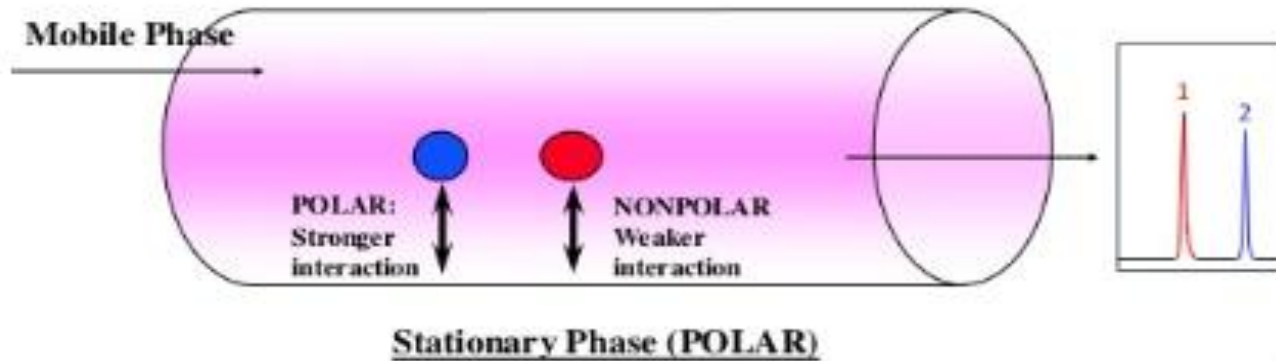
HPLC is a separation technique that involves:

- The injection of a small volume of liquid sample into a tube packed with tiny particles where individual components of the sample are moved down the packed tube (column) with a liquid (mobile phase) forced through the column by high pressure delivered by a pump.

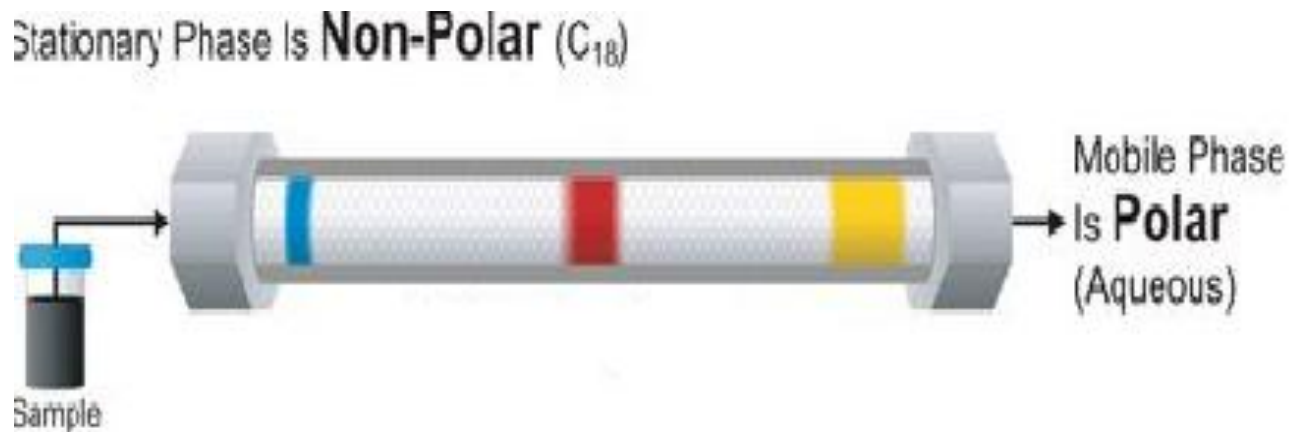


Types of HPLC modes:

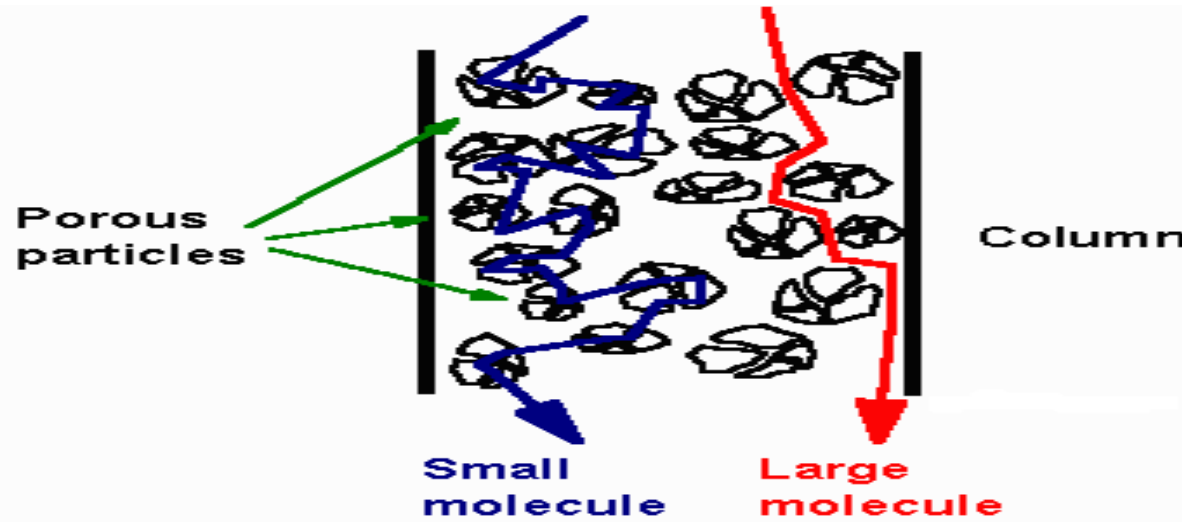
1) Normal phase:



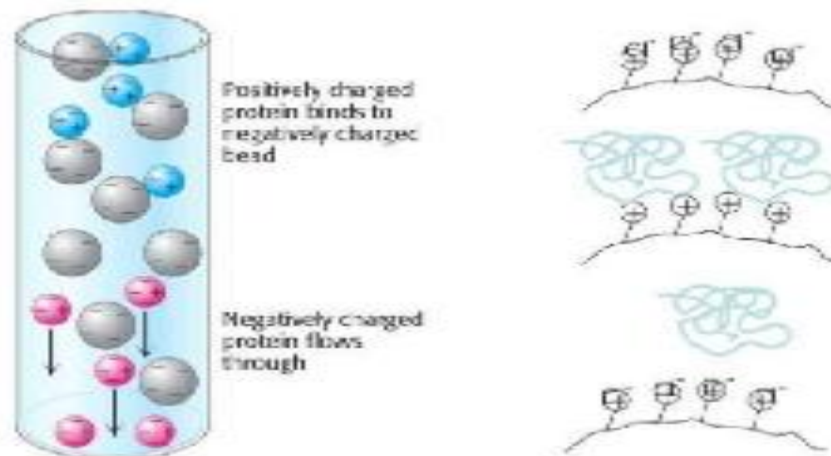
2) Reverse phase:



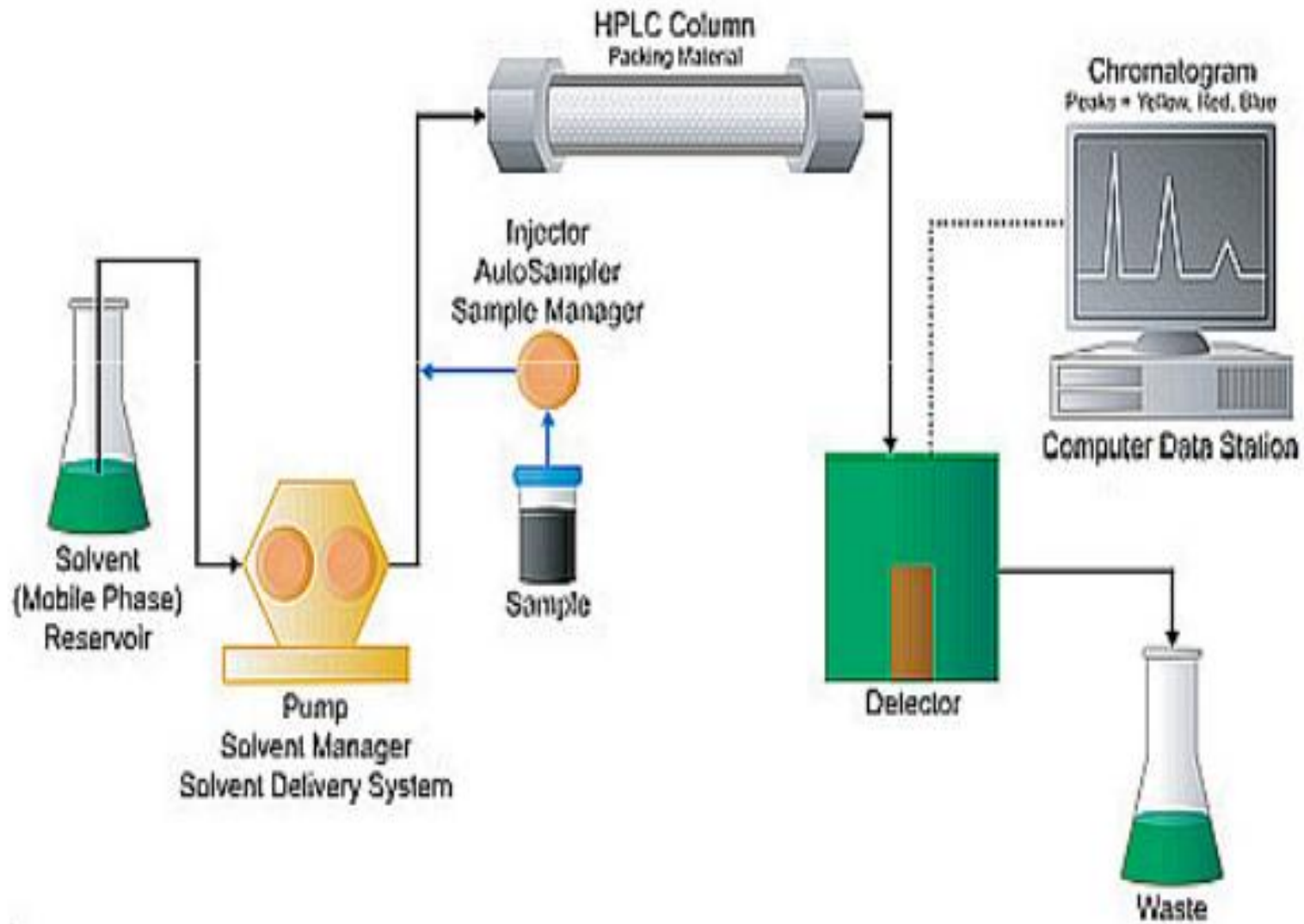
Size Exclusion:



Ion Exchange:

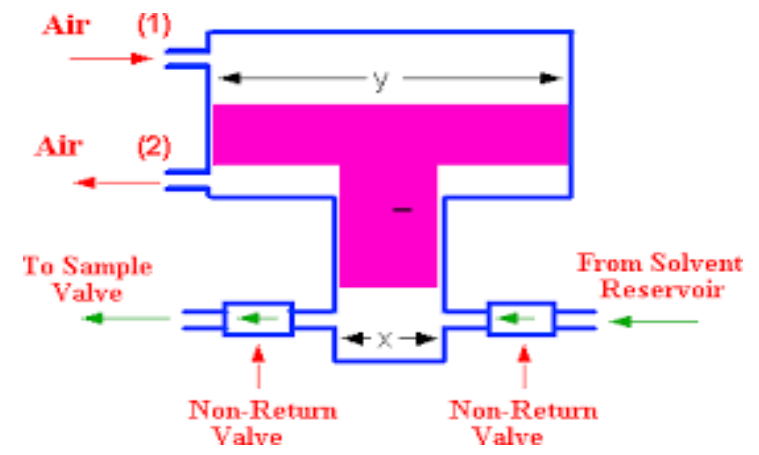
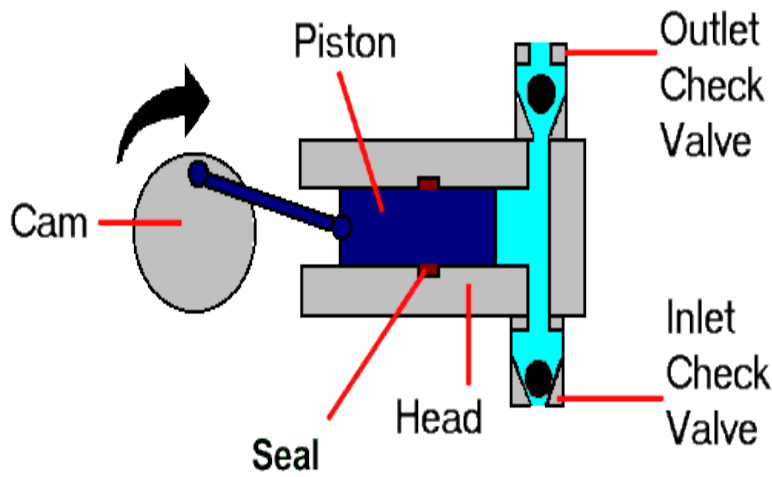
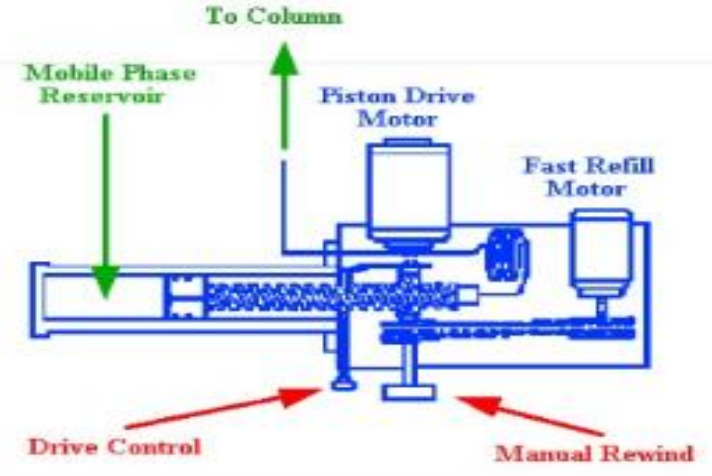


Instrumentation:

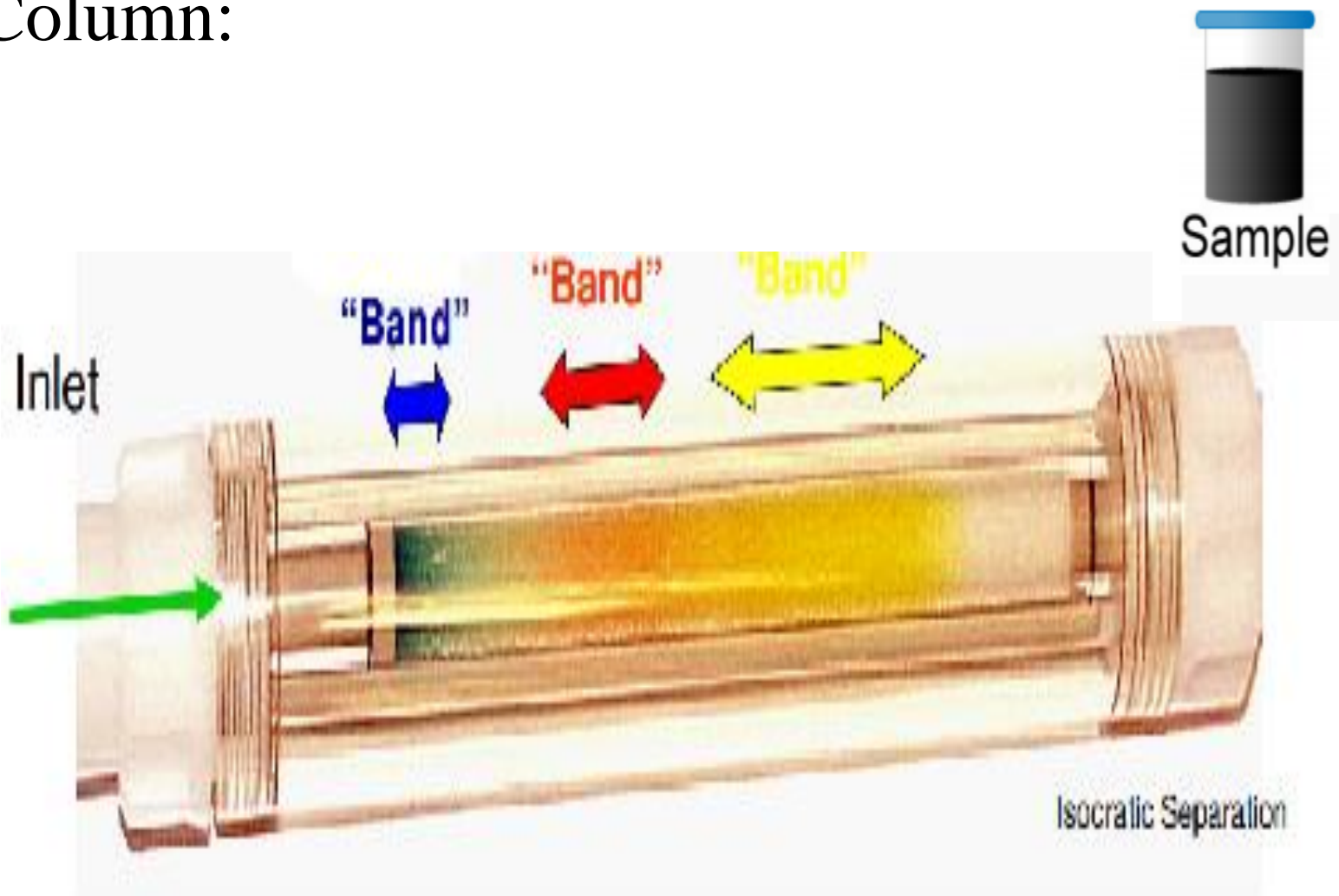


Types of pumps:

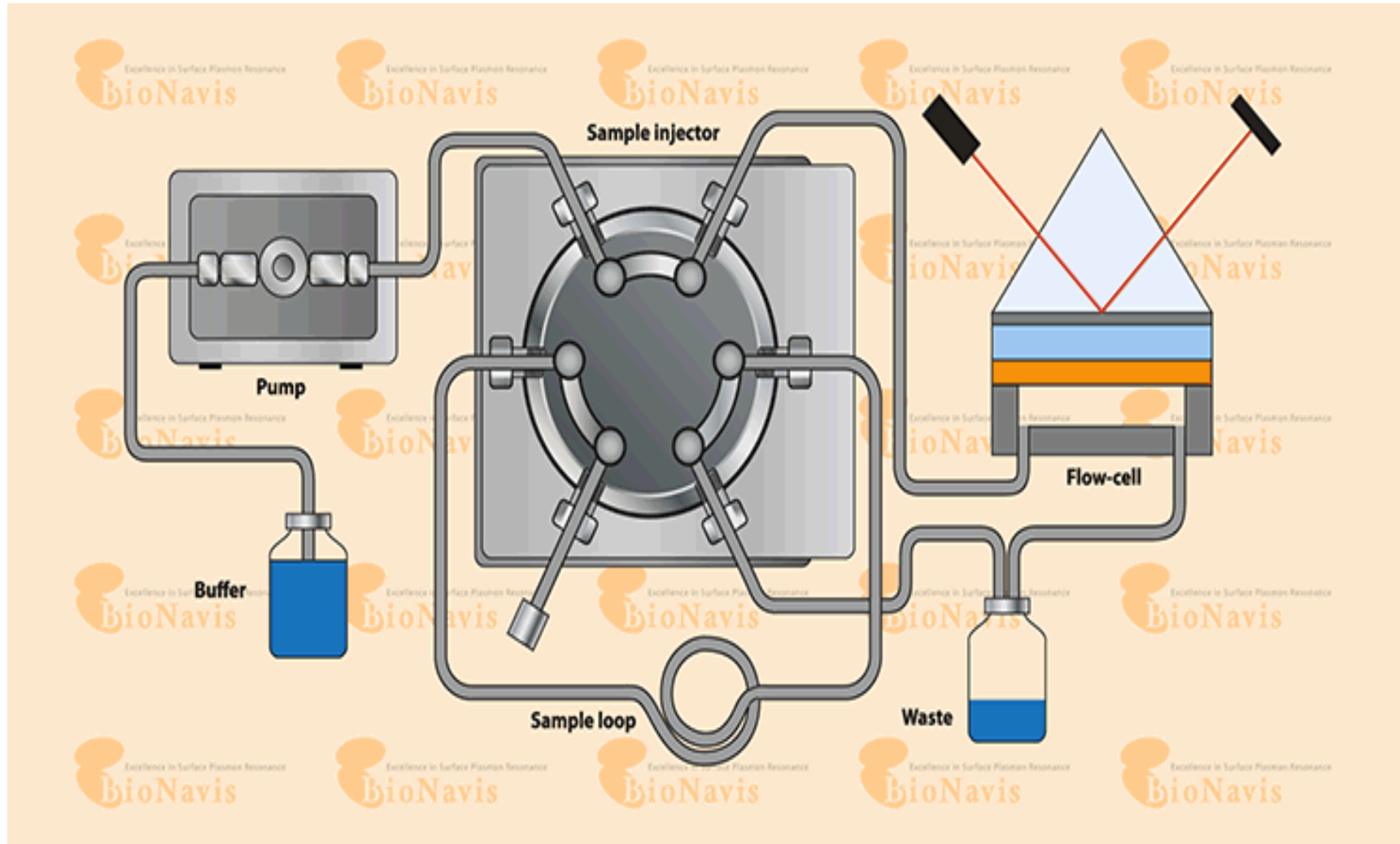
- 1) Displacement pump
- 2) Reciprocating pump
- 3) Pneumatic pump



Column:



Sample Injection System:



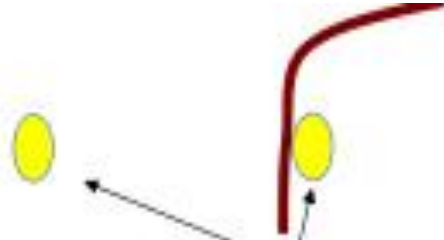
Detectors:

Different compounds require different detectors to be “seen” .

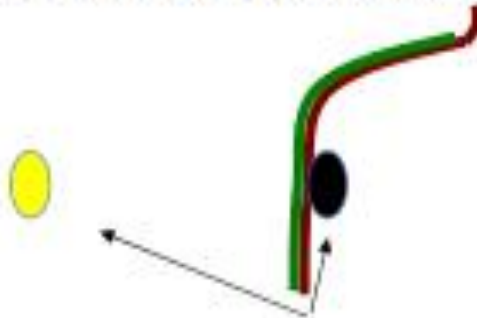
- UV
- Fluorescence
- Evaporative Light Scattering
- Refractive Index
- Electrochemical



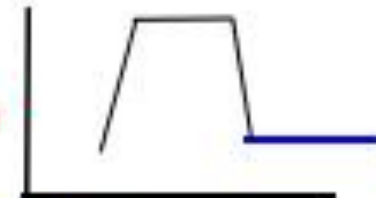
Concept of UV absorbance detector:



Light Sensors measure the SAME amount of Light - **NO SIGNAL**

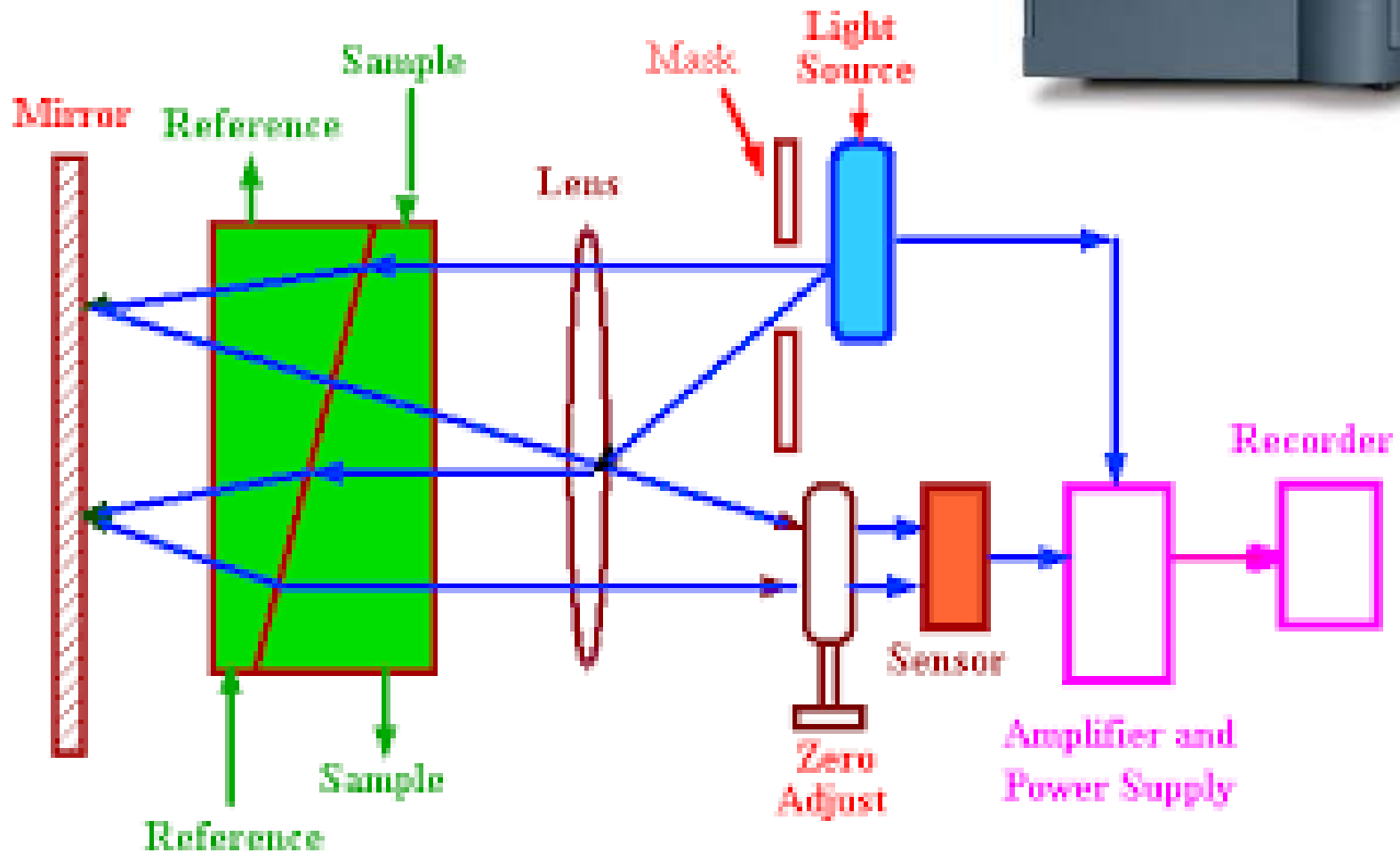


**Wash Off
SunScreen**



Light Sensors measure the SAME amount of Light - **NO SIGNAL**

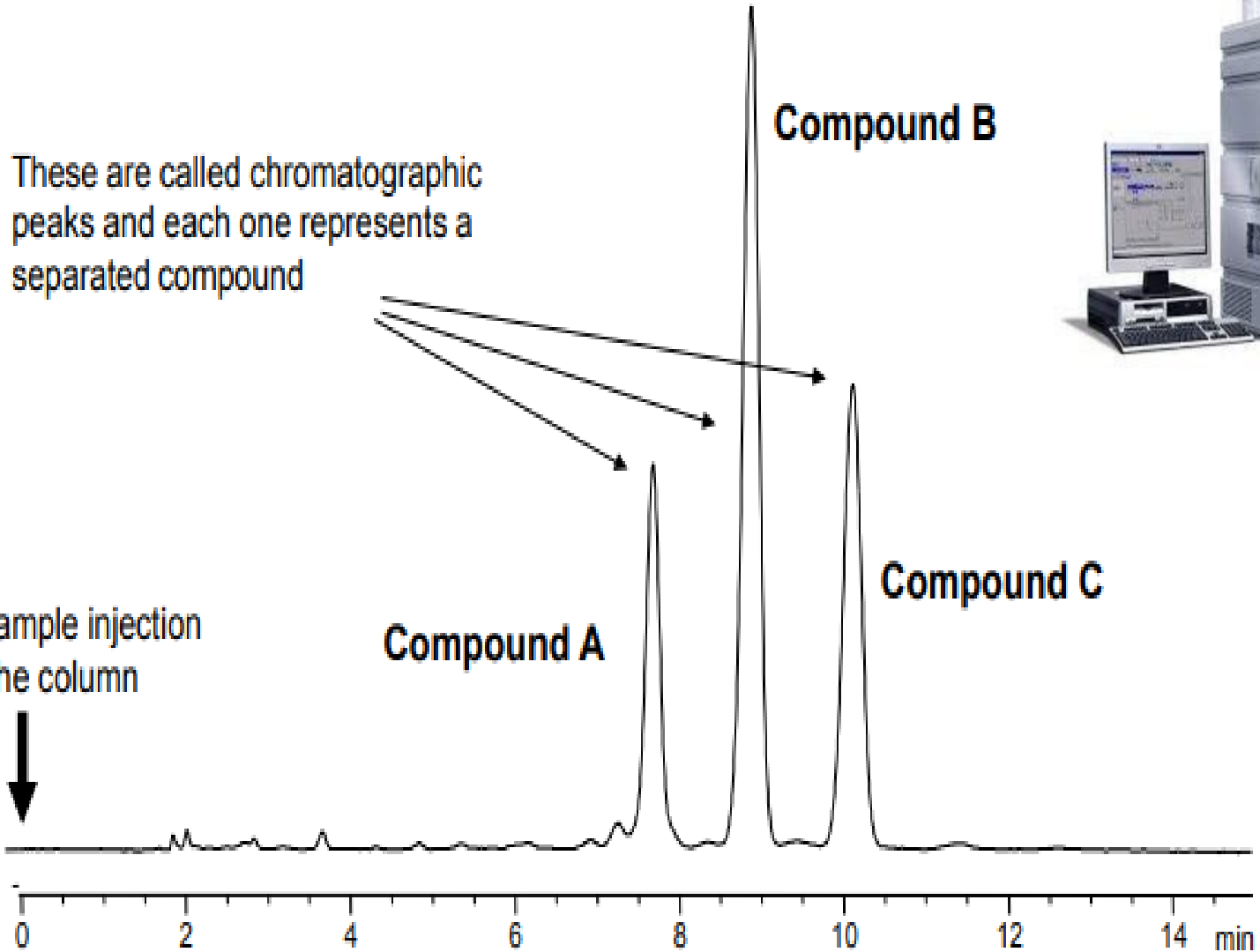
RI Detector:



HPLC chromatogram:

These are called chromatographic peaks and each one represents a separated compound

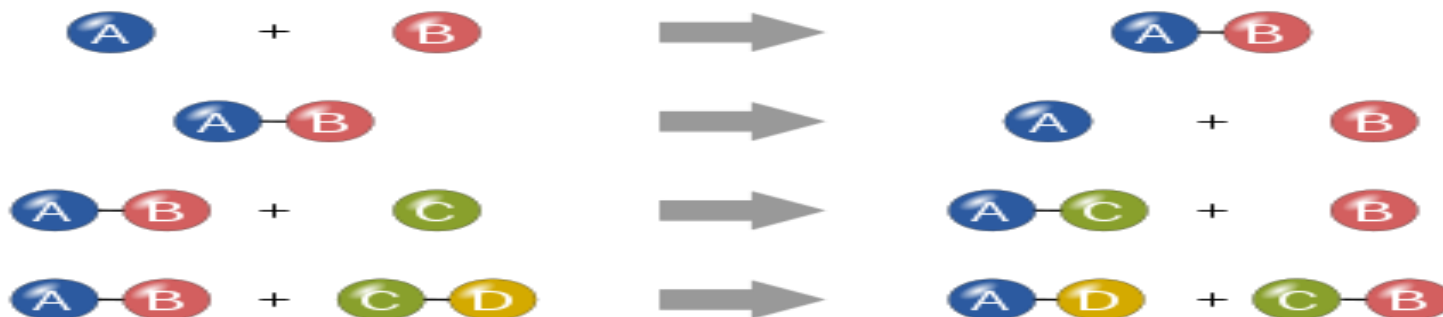
Point of sample injection
into the column



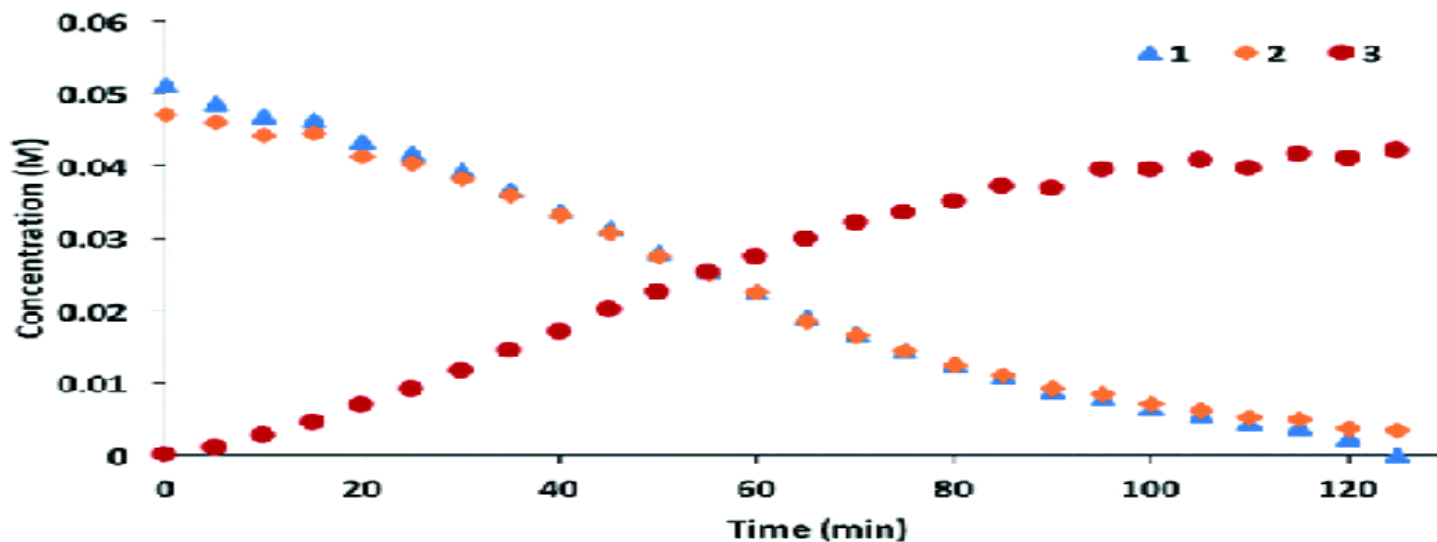
Reaction monitoring of API by HPLC

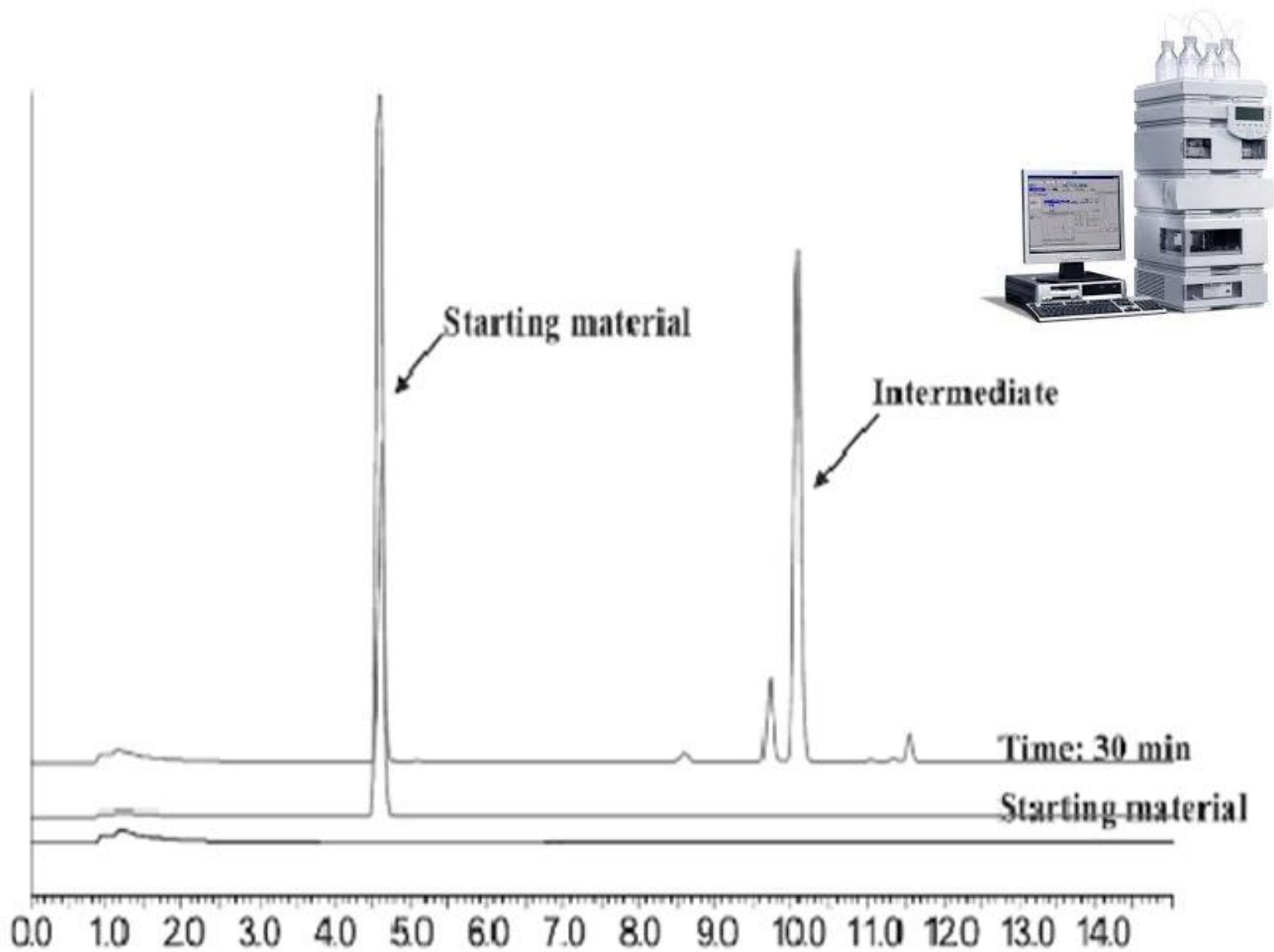
Reaction monitoring:

During process development of a new compound, many samples are generated which include reaction mixtures from process monitoring, batch and waste layers from extractions



- During R&D, reaction monitoring can be used to optimize the process conditions.
- In the manufacturing process, reaction monitoring can help to make sure that the reaction is progressing as expected.



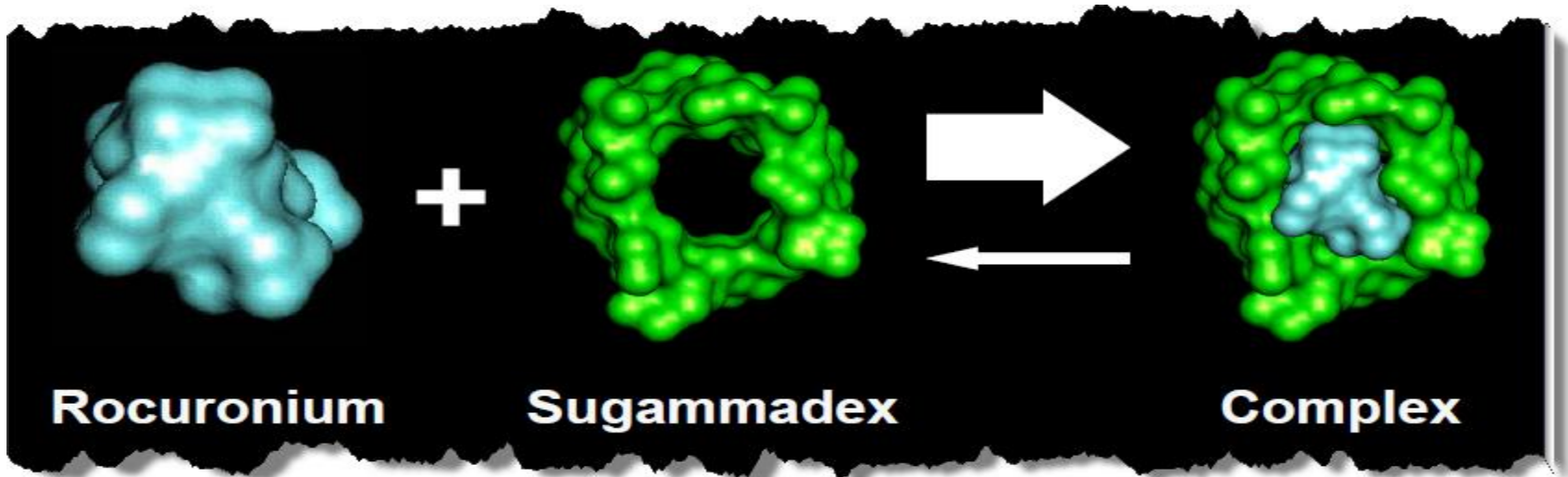


Reaction conversion of starting material to intermediate.

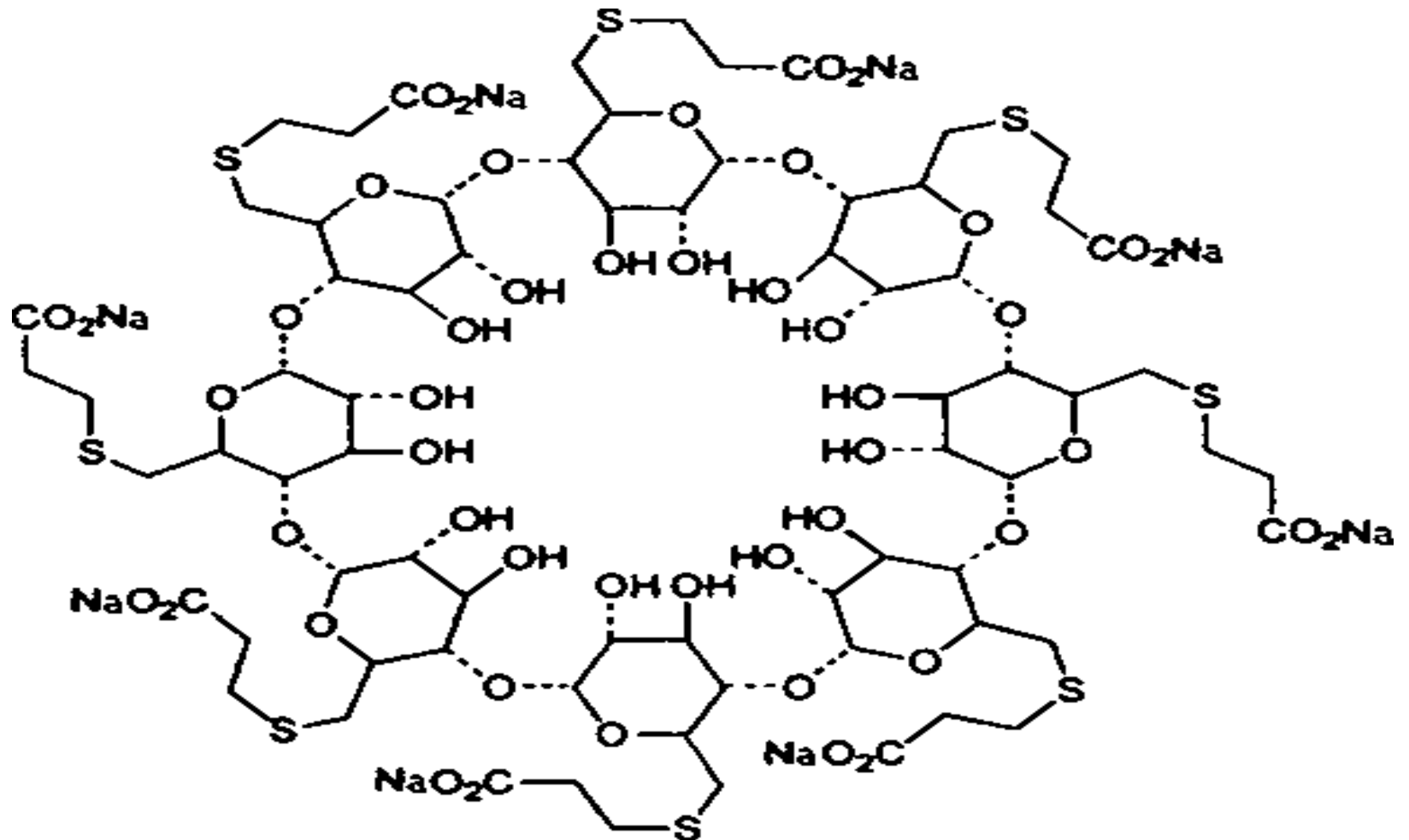
Reaction Monitoring of Sugammadex

Sugammadex

Sugammadex (tradename Bridion) is an agent for reversal of neuromuscular blockade by the agent rocuronium in general anaesthesia



Structure of Sugammadex:



Chromatographic analysis of Sugammadex

Preparation of mobile phase:

Mobile phase-A: 0.1% Ortho-Phosphoric Acid

Mobile phase-B: 100% Acetonitrile

Diluent for sample preparation: Water

Concentration of sample for analysis: 5000 ppm

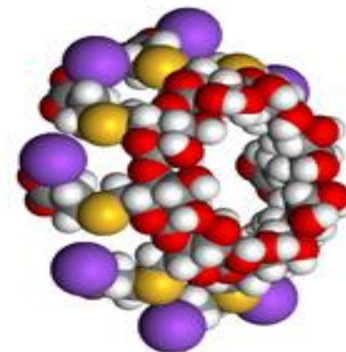
Wavelength for detector: 210 nm

Sample & Column temperature: 25°C

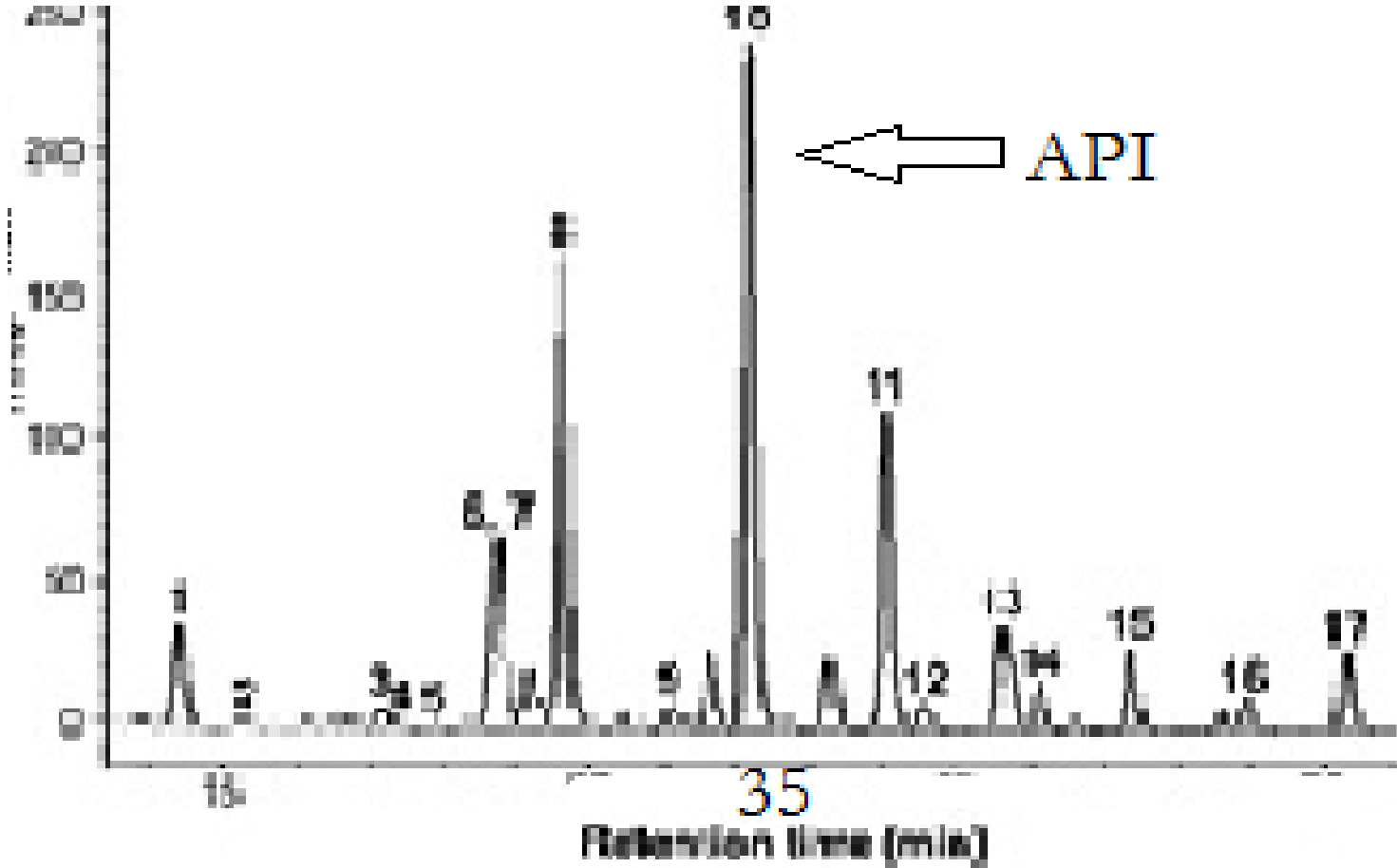
Elution programming: Gradient

Run time: 60 minutes

Injection volume: 10 μ L



Obtained Chromatogram:



Conclusion

Monitoring of reaction vessels by HPLC allows for the simultaneous quantification of APIs and process impurities for maximum product yields and purity. It involves taking timely measurements throughout the production process to verify the quality of in-process batches and to understand performance in each of the critical steps of that process. It is a more selective and sensitive technique, with a broader linear dynamic ranges, and has the ability to quantify multiple components within complex samples, including low level impurities.

References

1. Beckett A.H., Stenlake J.B. Practical Pharmaceutical chemistry. 4th edition part two.
2. Connors K.A. A textbook of pharmaceutical analysis. Third edition. New York: A Wiley-interscience publication.
3. Hanai T., HPLC A Practical Guide. Published by The Royal Society of Chemistry Cambridge.
4. hplc.chem.shu.edu/NEW/HPLC_Book/Detectors/det_uv.html. Accessed

Thank You

The text "Thank You" is rendered in a highly decorative, cursive script. The letters are a deep red color with a prominent gold outline, giving them a three-dimensional appearance. The text is embellished with clusters of vibrant red roses and green foliage. Two white doves are depicted in flight, one positioned above the letter 'h' and the other above the letter 'y'. The entire graphic is set against a plain white background.