

# High Performance Liquid Chromatography - HPLC

## Introduction

### High Performance Liquid Chromatography (HPLC) for Clinical and Biomedical Applications

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Homepage: <http://www.forumsci.co.il/HPLC>

## HPLC COURSE LAYOUT

- Introduction & Applicability
- Modes of Chromatography
- Quantitative work and System Qualification.

What does HPLC mean?

High pressure liquid chromatography

High priced liquid chromatography

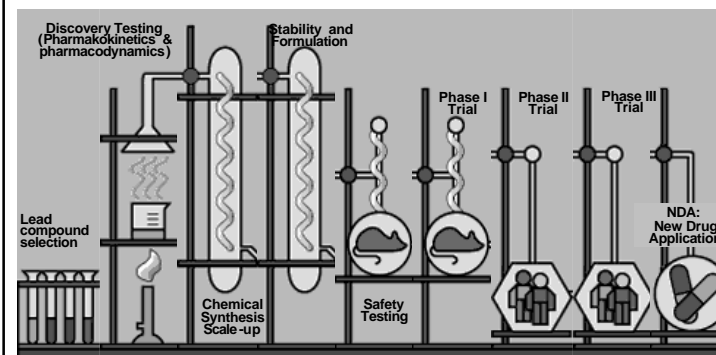
Hewlett-Packard liquid chromatography

High performance liquid chromatography

Hocus pocus liquid chromatography

High patience liquid chromatography

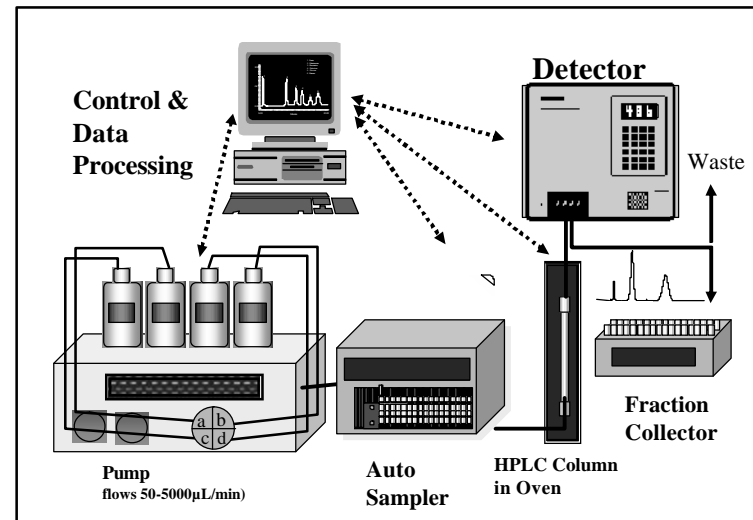
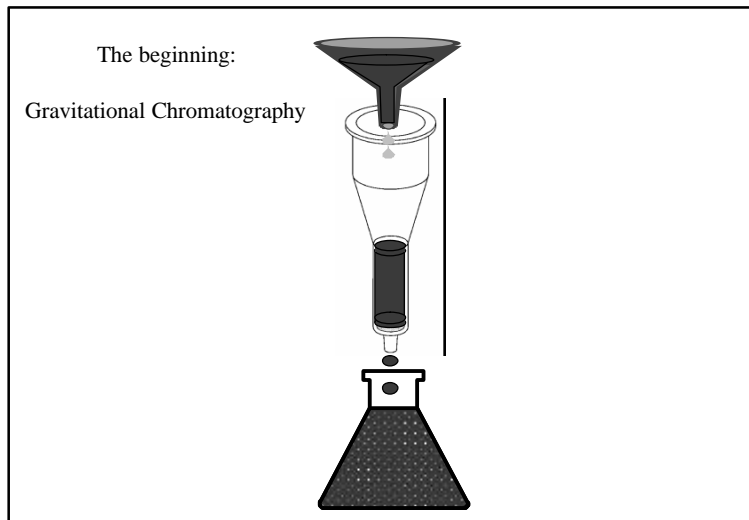
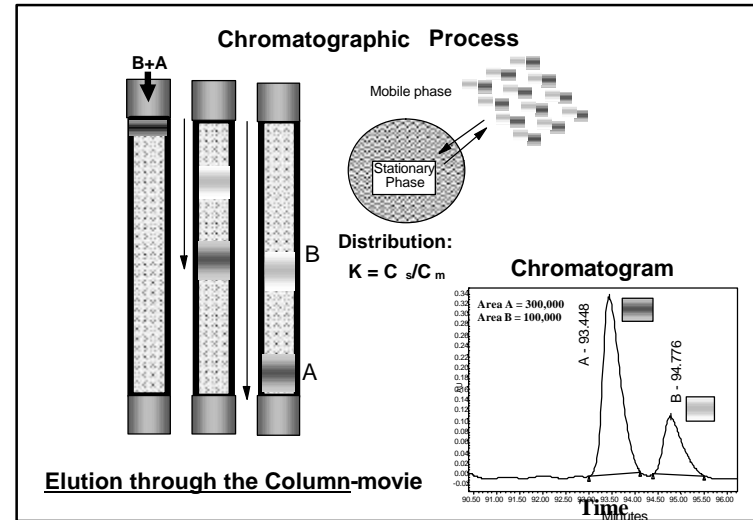
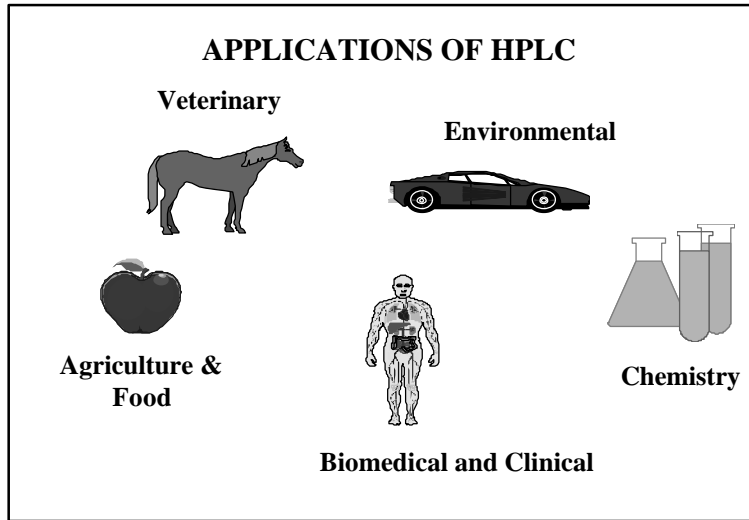
## HPLC in Pharmaceutics Technique No 1



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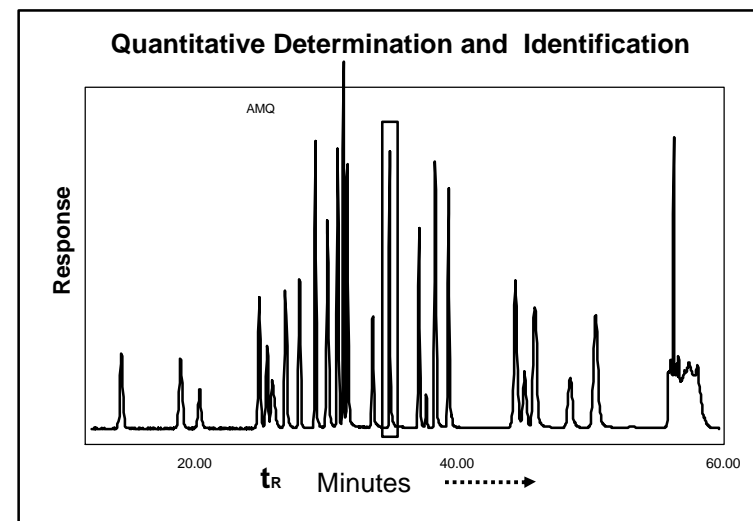
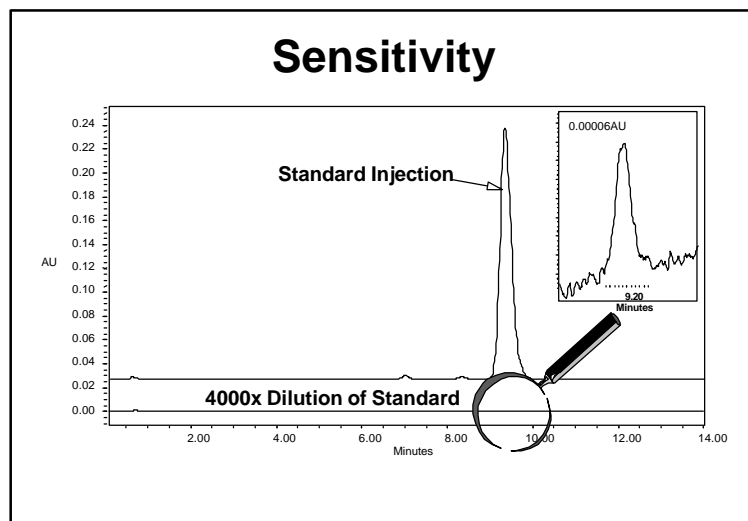
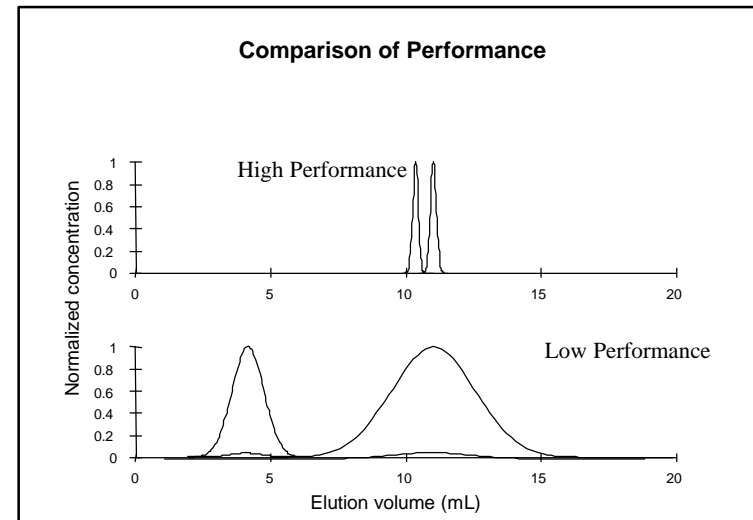
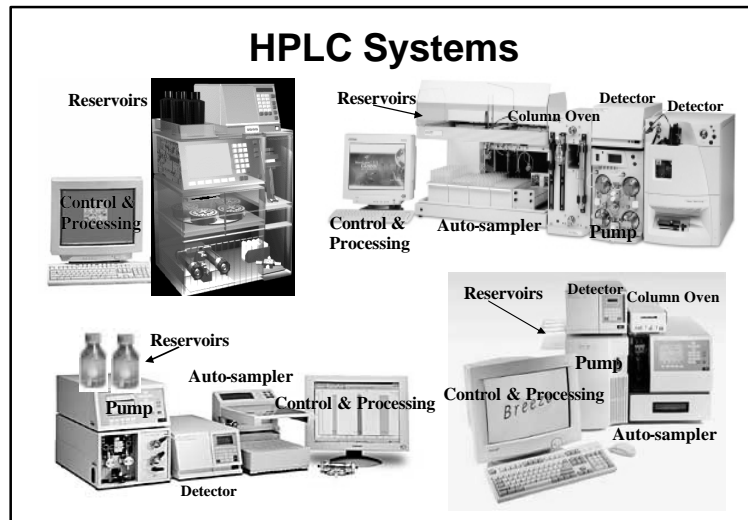
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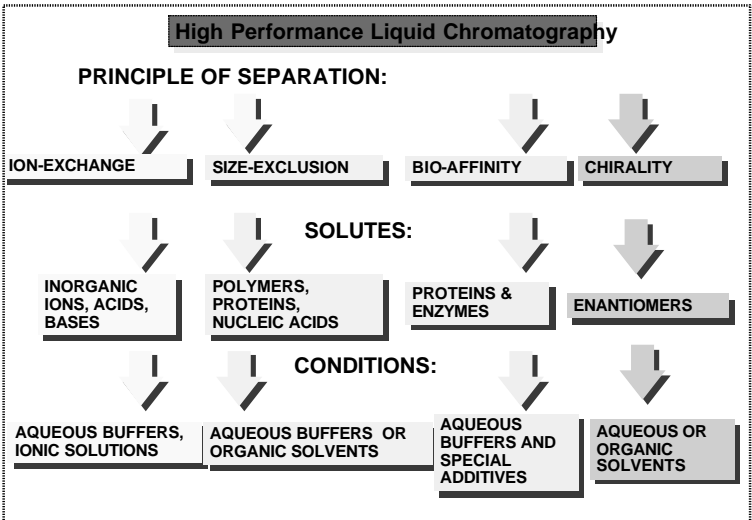
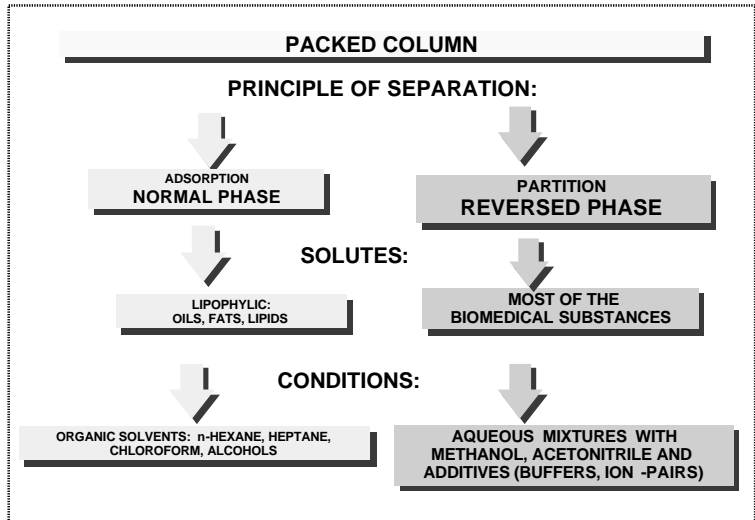
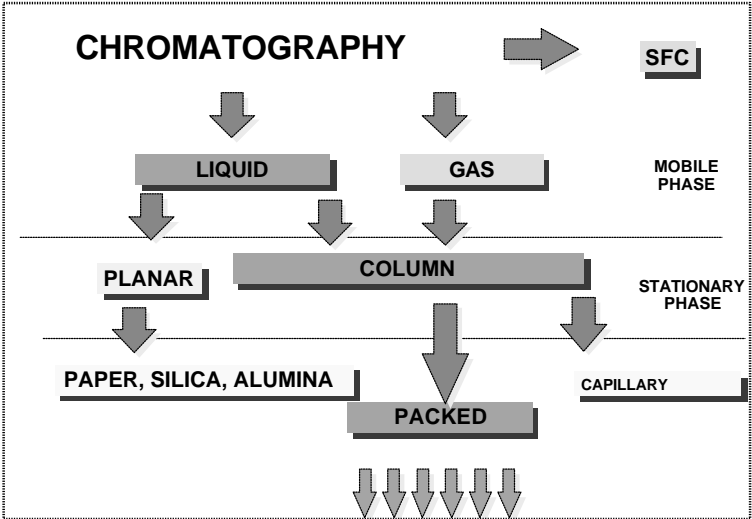


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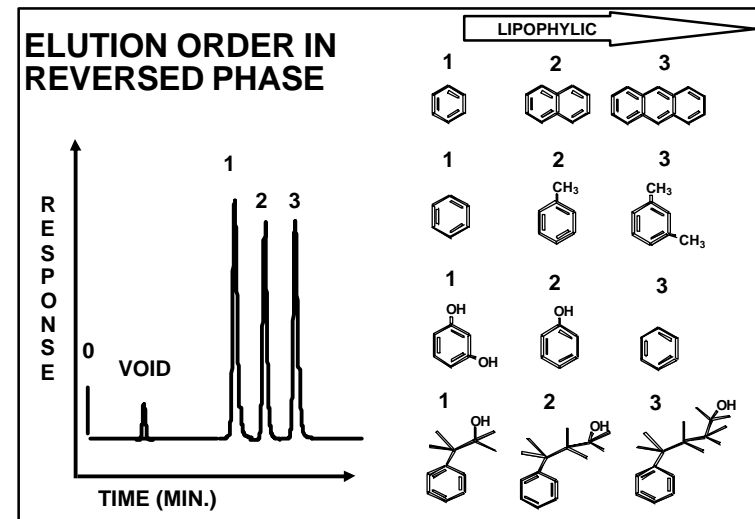
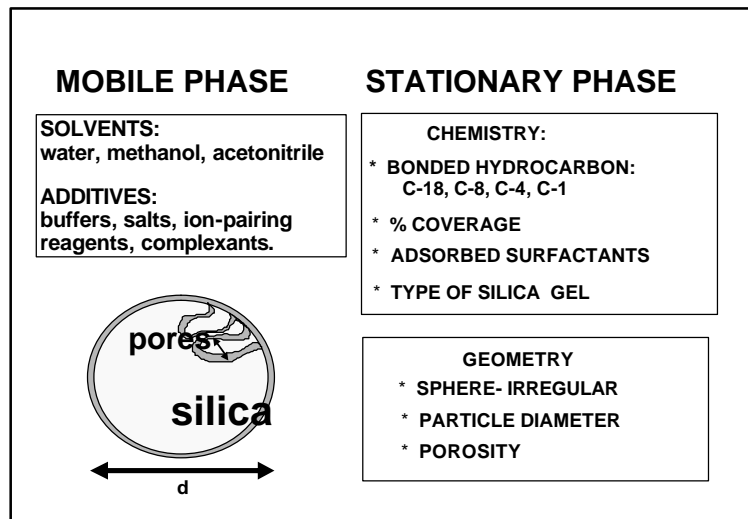
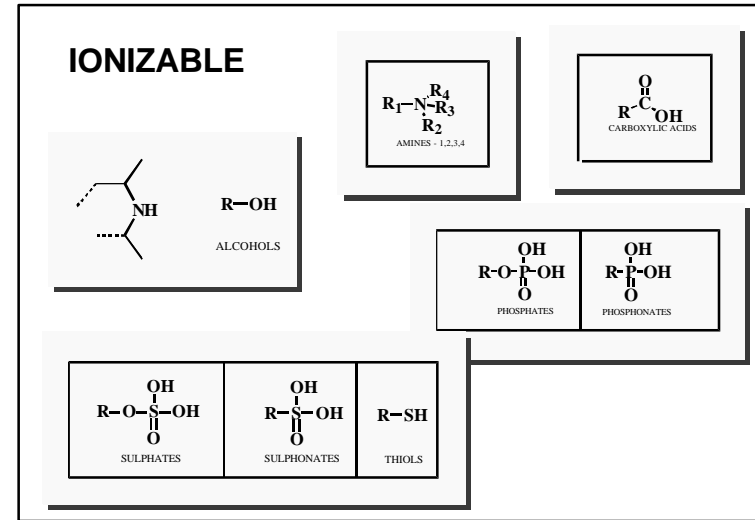
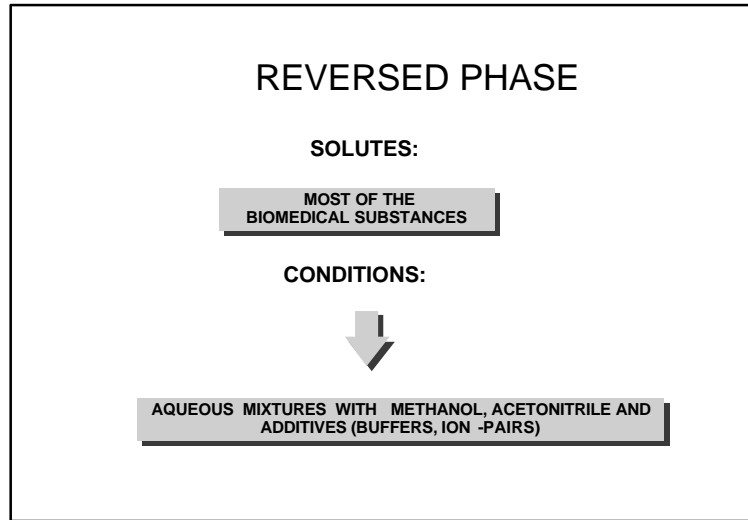
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- Introduction & Applicability
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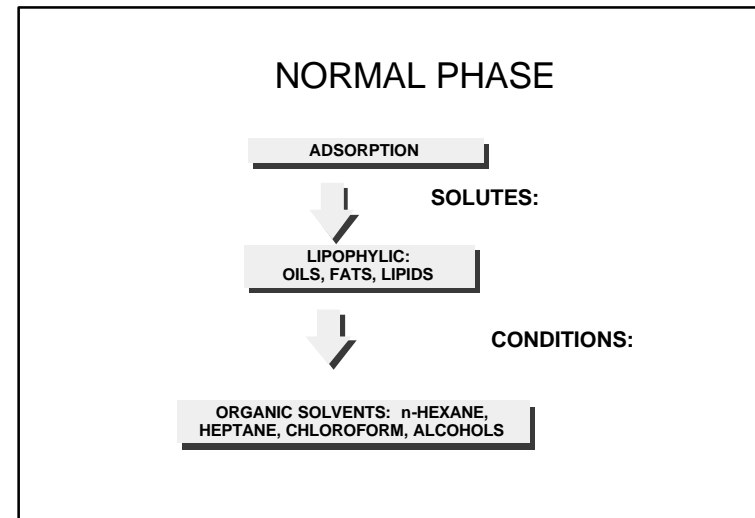
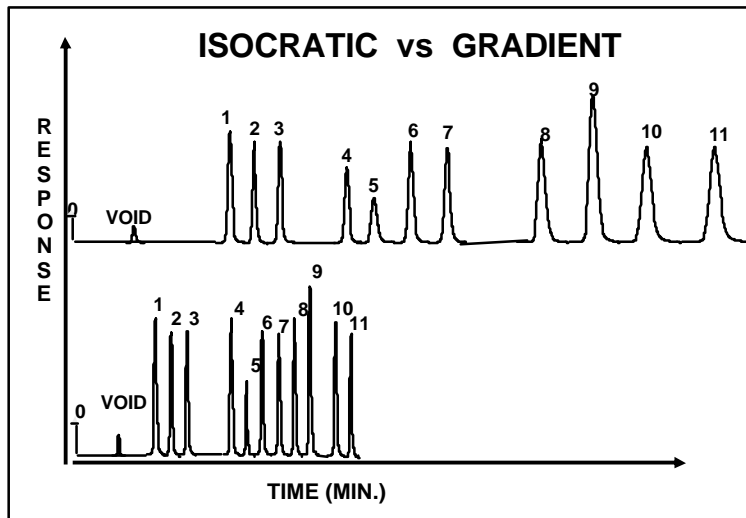
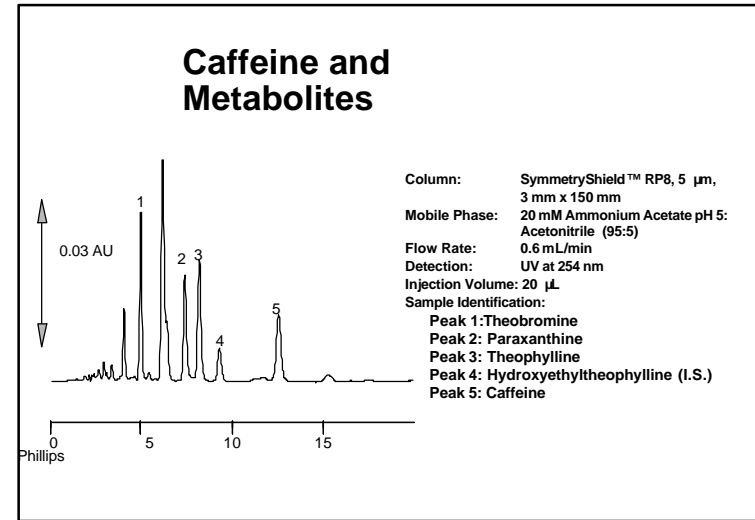
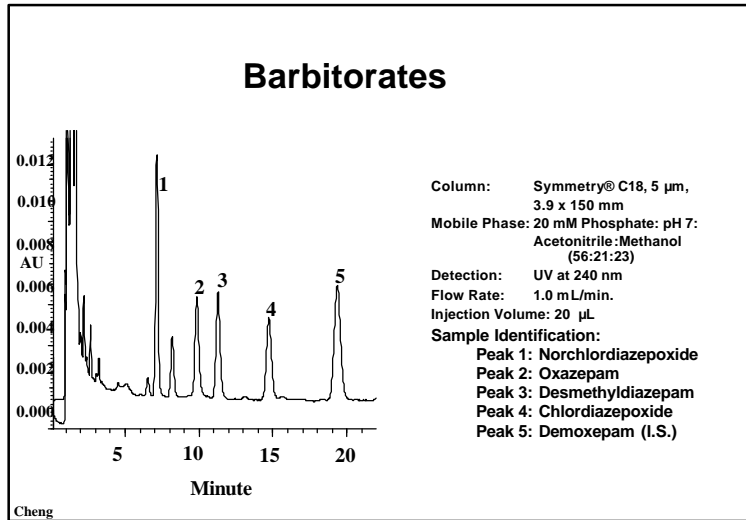
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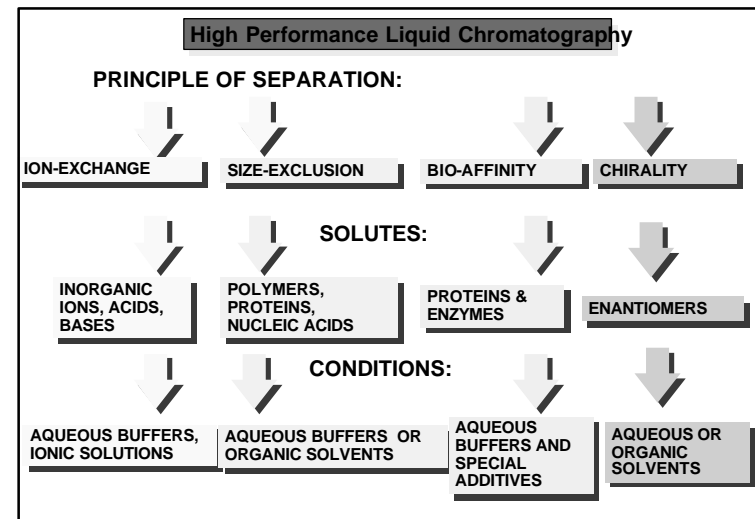
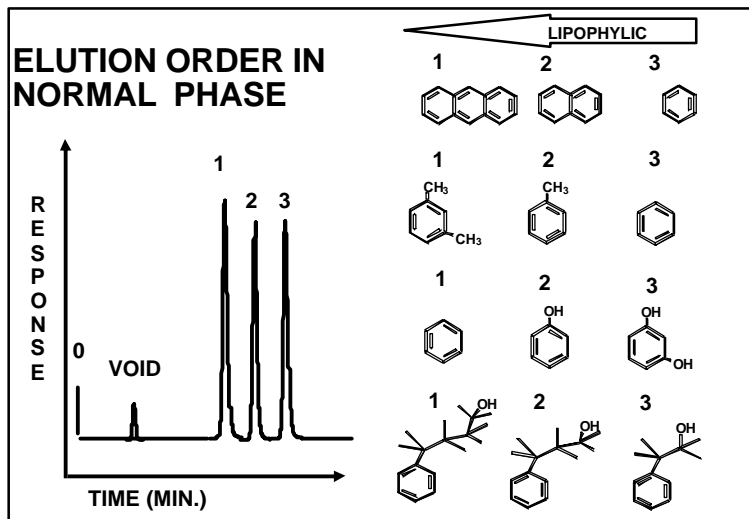
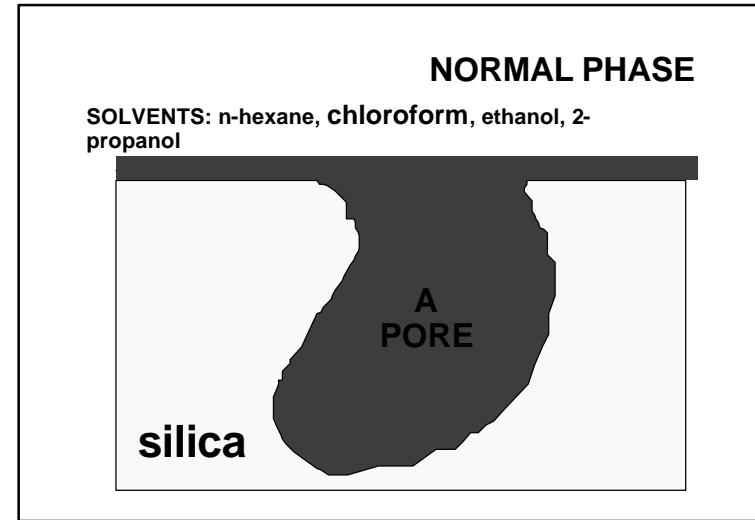
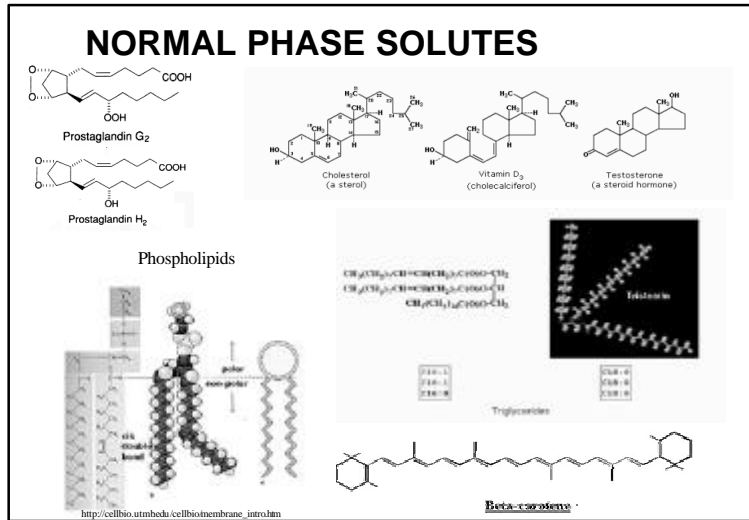
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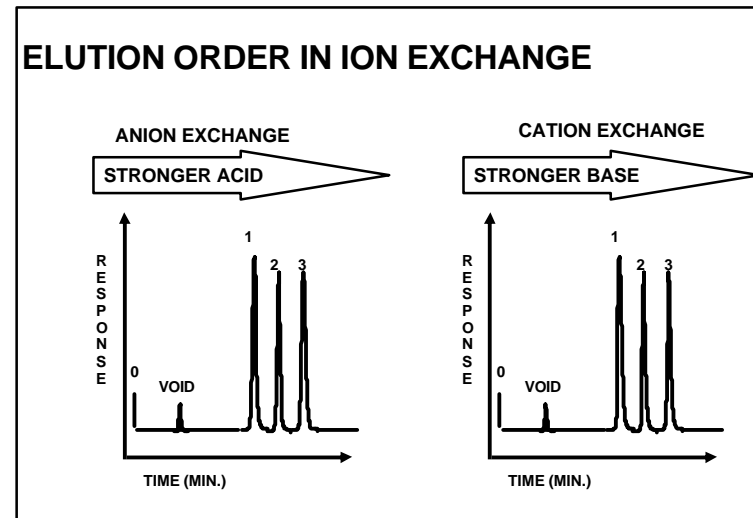
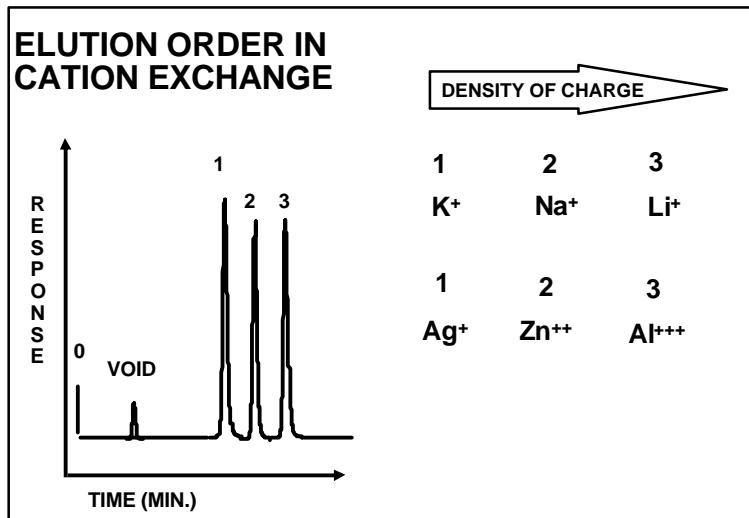
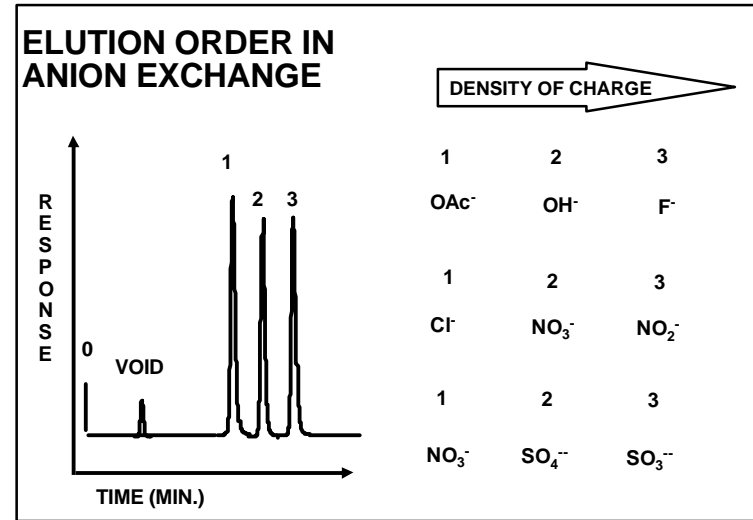
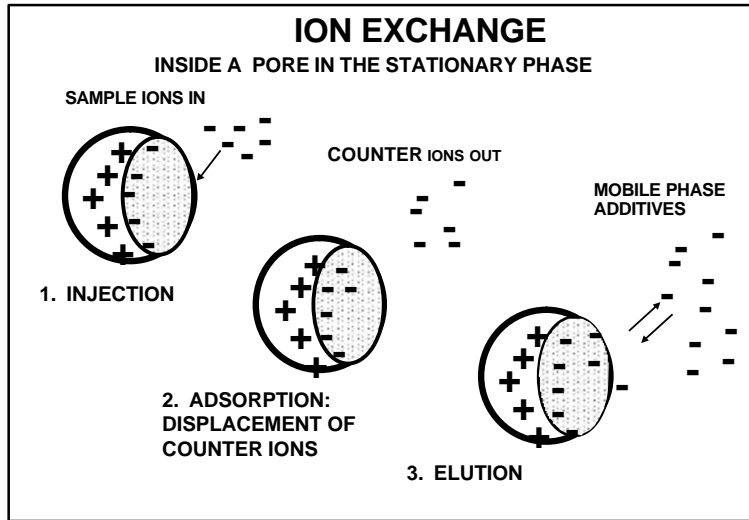
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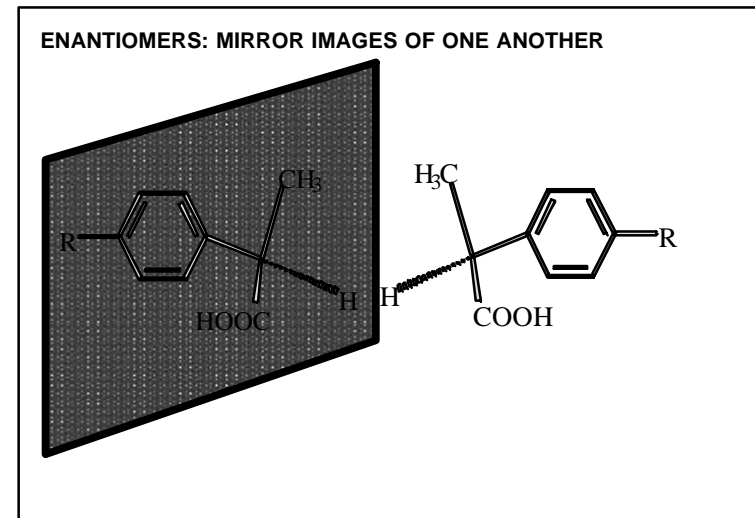
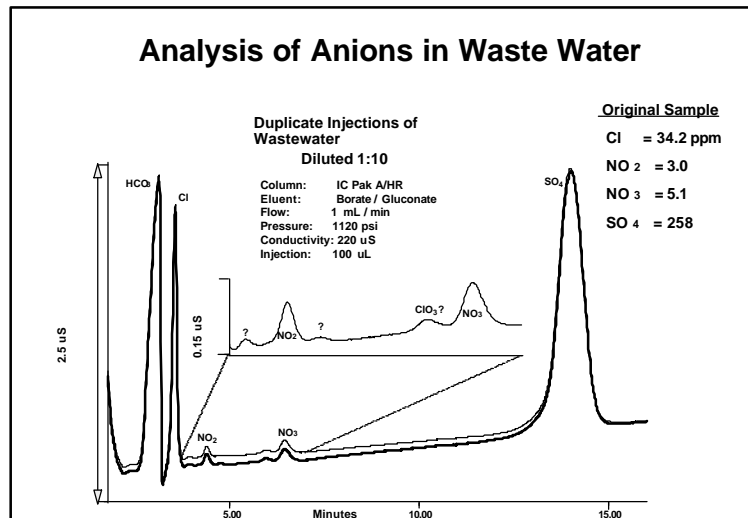
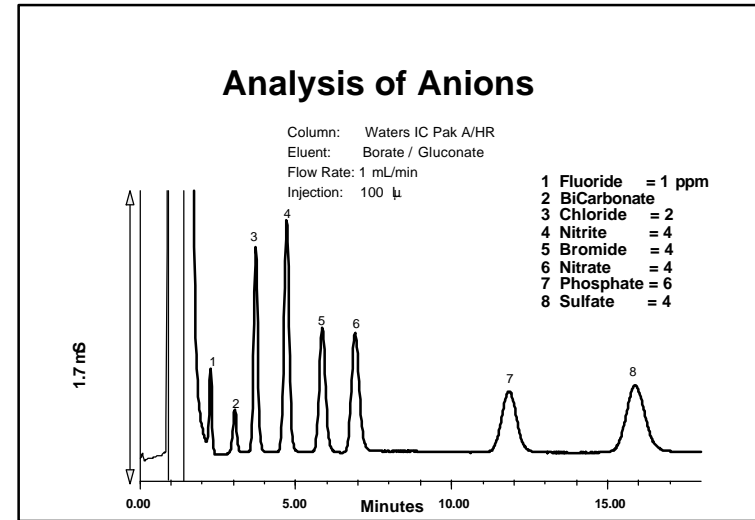
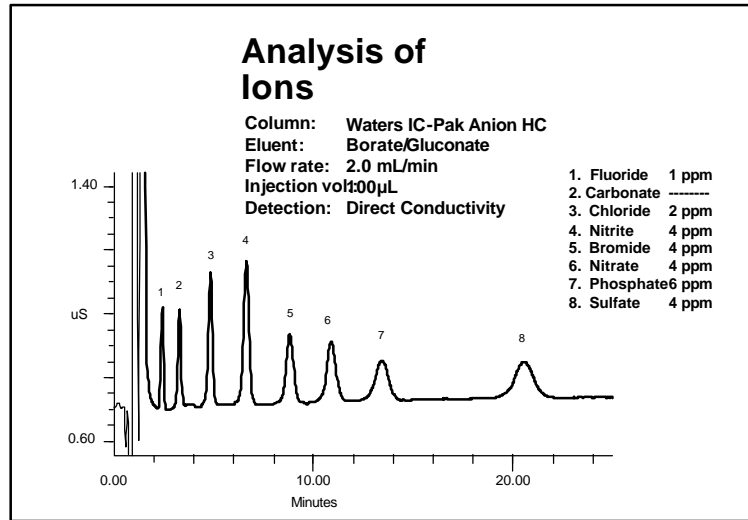
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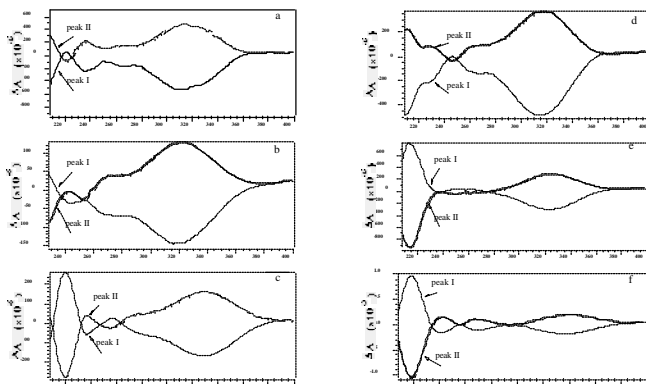
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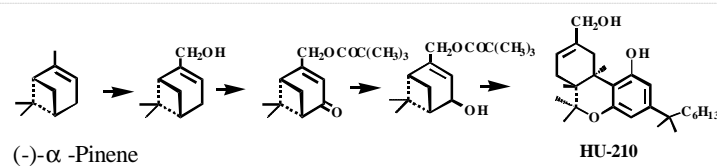
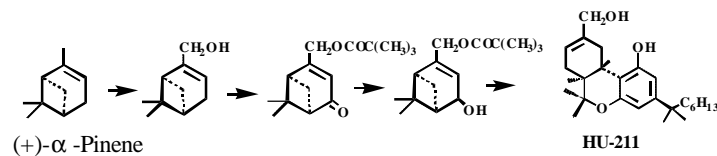
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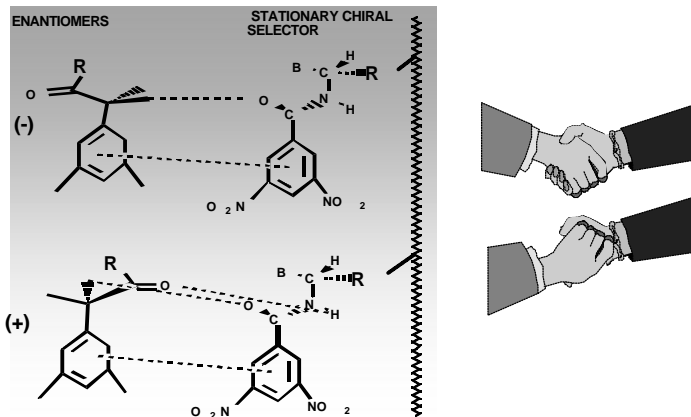
### Circular Dichroism SPECTRA



### Asymmetric Synthesis



### BASIS FOR SEPARATION: CHIRAL RECOGNITION

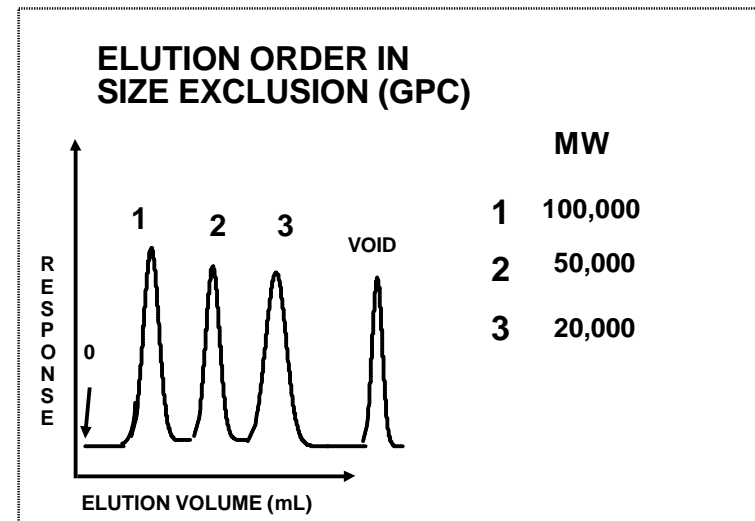
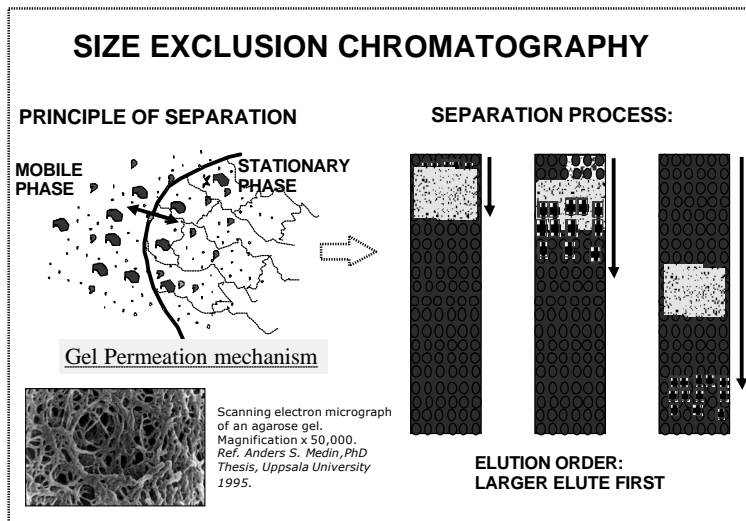
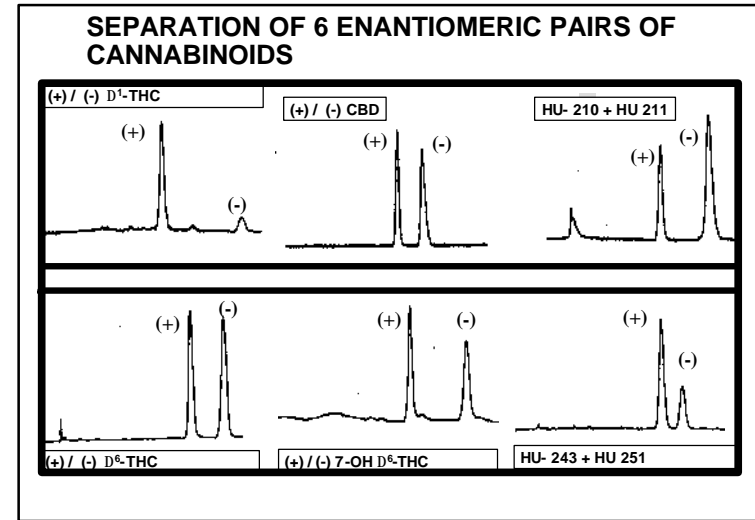
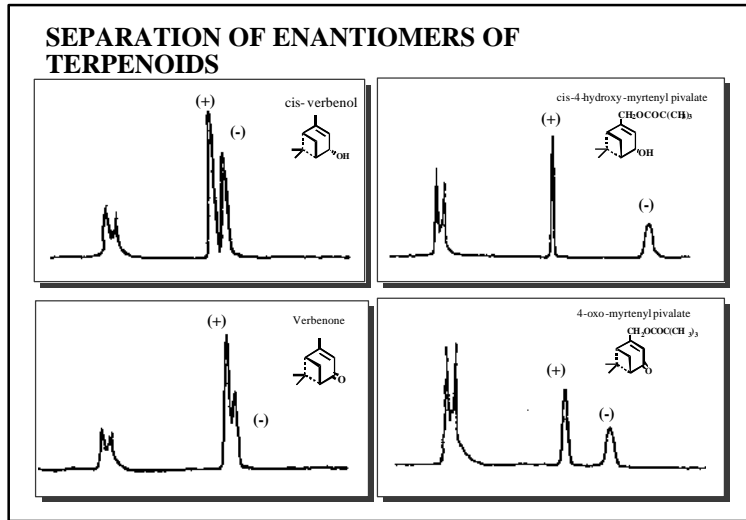


### Chiral stationary phases:

- \* Ligand exchange
- \* p-Donor p-acceptor (Pirkle)
- \* Chiral Host-guest (cyclodextrin)
- \* Immobilized proteins
- \* Immobilized polysaccharides

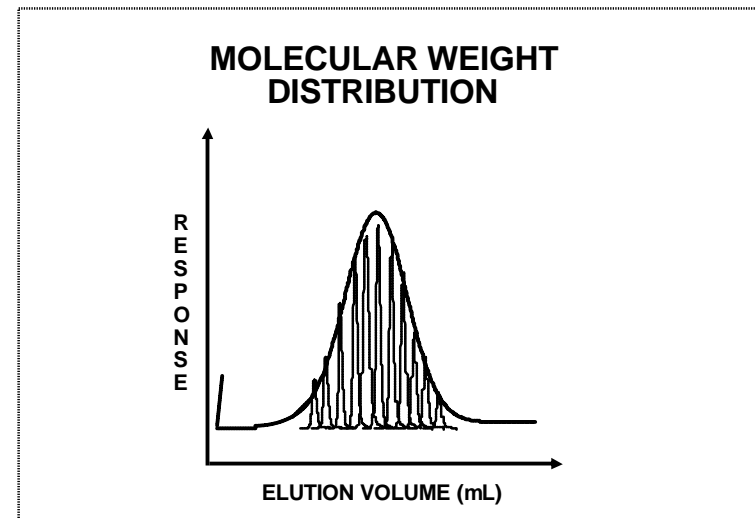
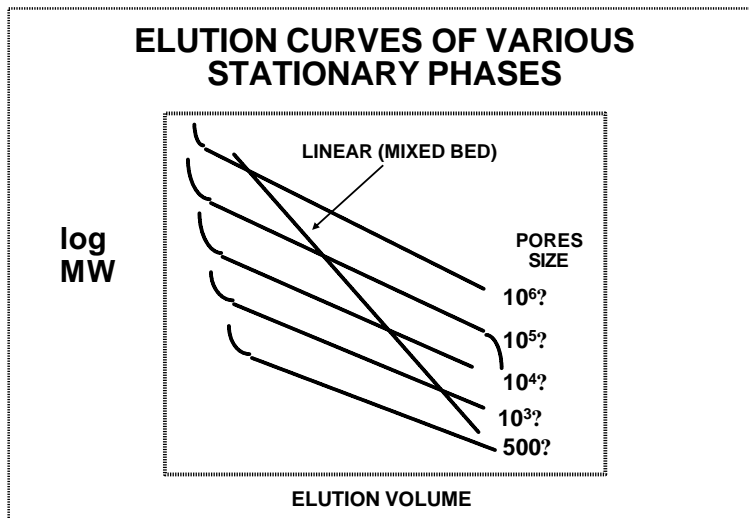
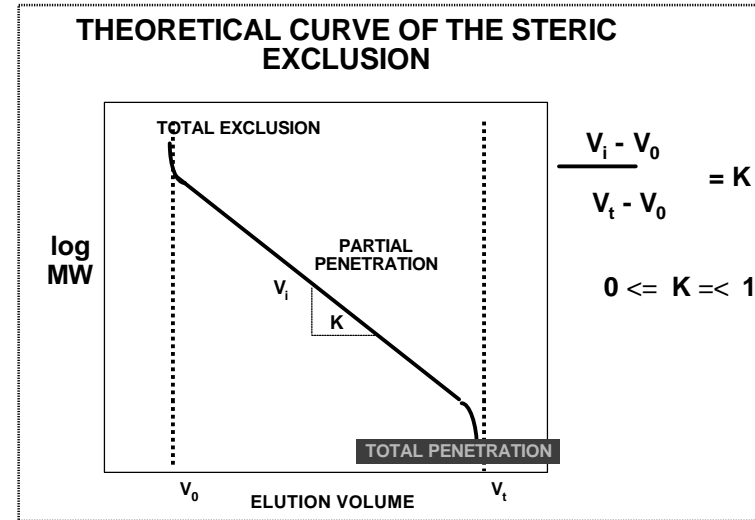
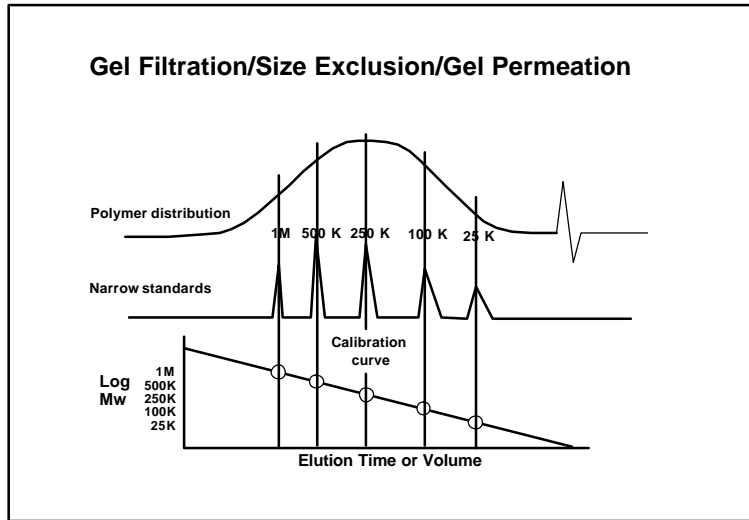
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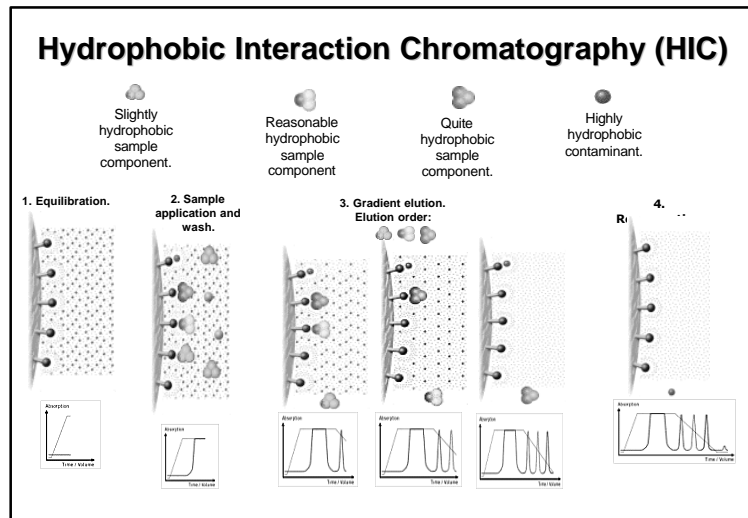
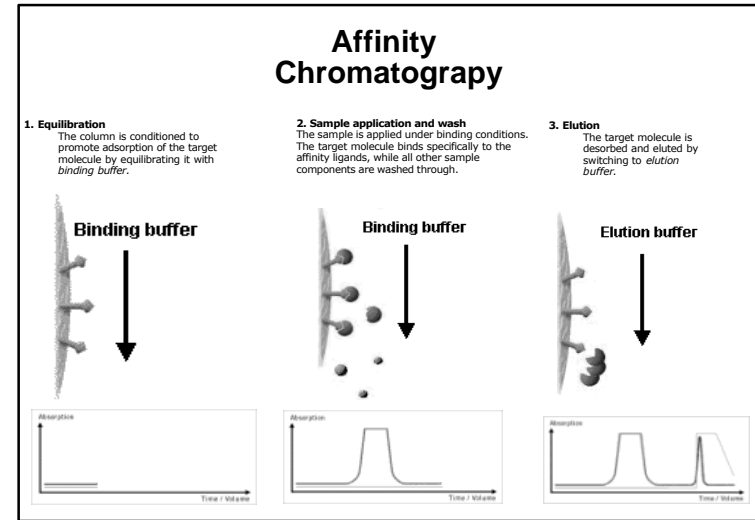
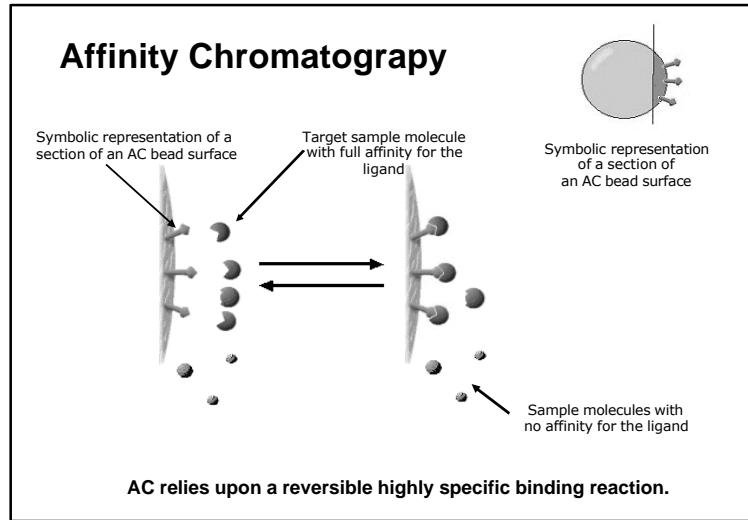
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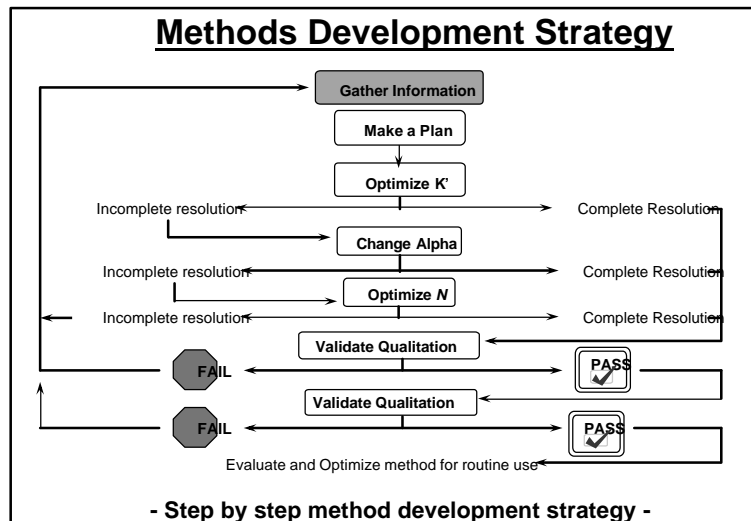
## Introduction



- ### Seven Basic Considerations in Choosing HPLC Operating Parameters
- 1) Solubility** - Hexane, Chloroform, Methanol, Water (buffer pH), other?
  - 2) Molecular Weight** - Would GPC be useful in either the analysis or sample prep?
  - 3) Functional Groups** - Any ionizable groups? Acidic, Basic, or Neutral?
  - 4) Sample Matrix** - What amounts are expected in matrix for either analytical or preparative isolation?
  - 5) Levels in Matrix** - What amounts are expected in matrix for either analytical or preparative isolation?
  - 6) Detectability** - Any chromophores or fluorophores? Consider Redox or derivatization. Together with point #5, an appropriate detector is chosen.
  - 7) How Do Species Differ** - An important clue to manipulate selectivity in the separation, especially if compounds are similar in their structure.

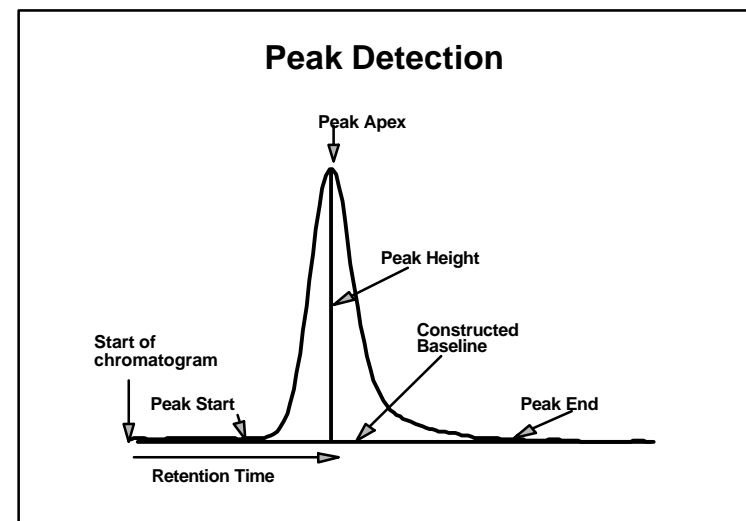
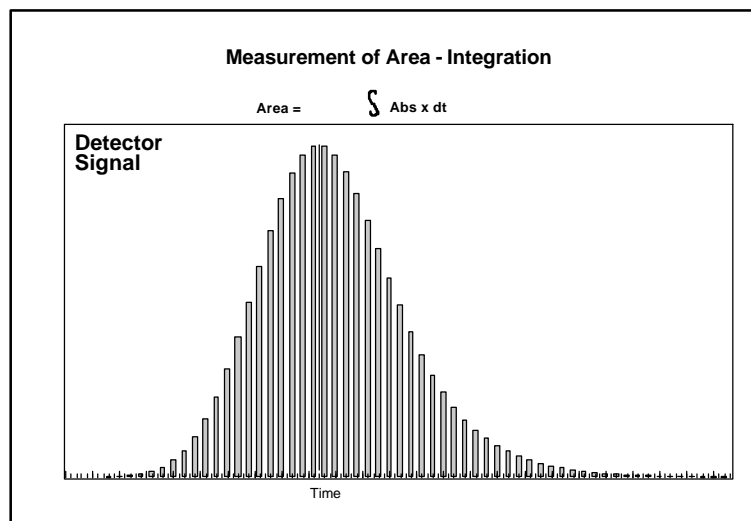
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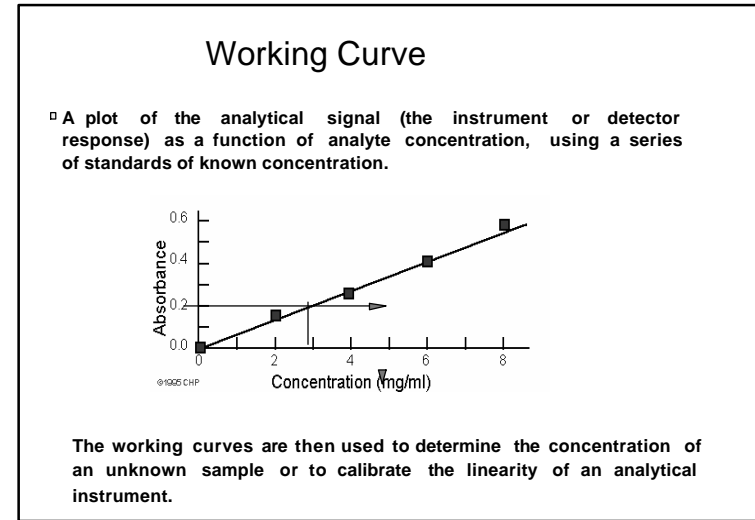
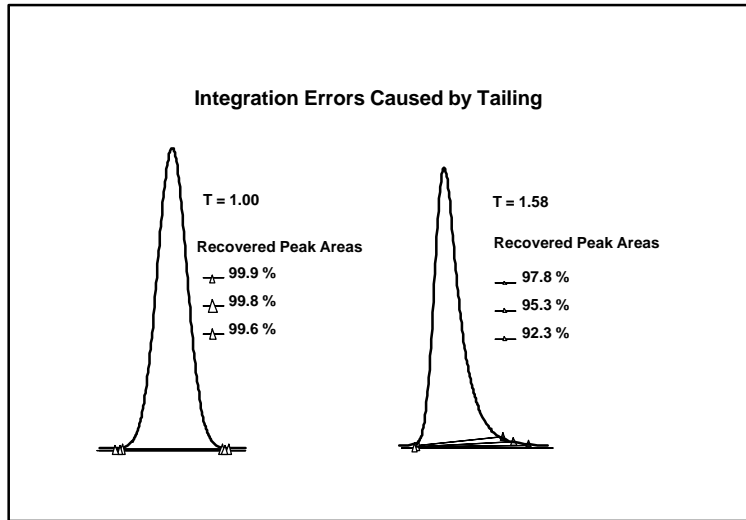
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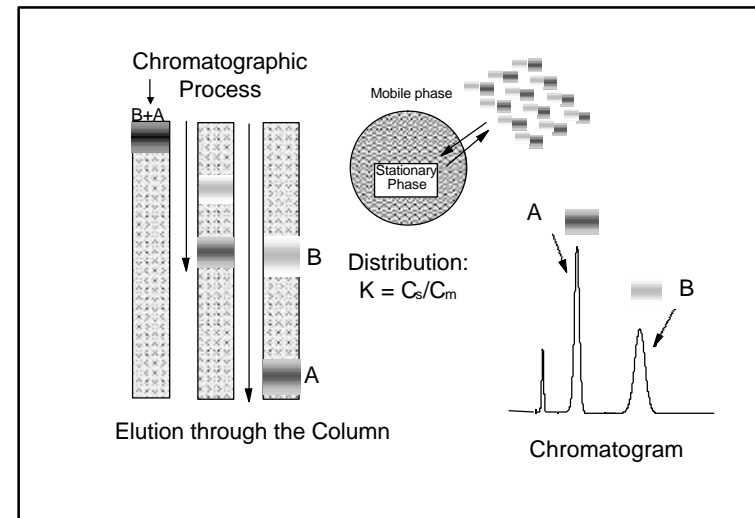
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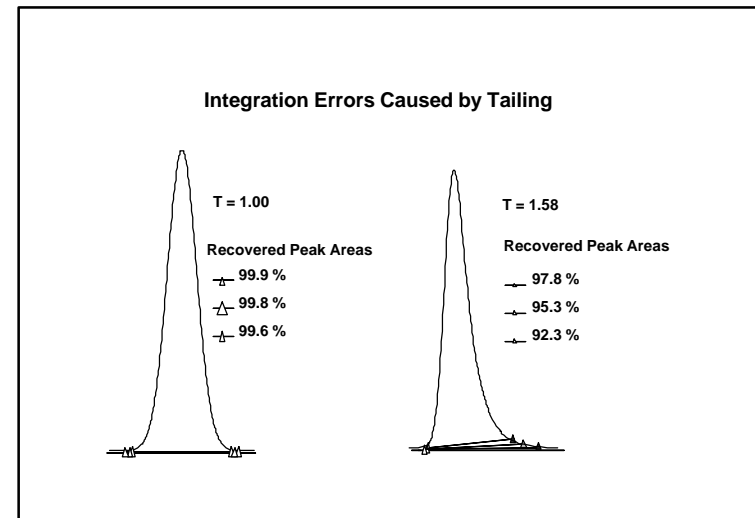
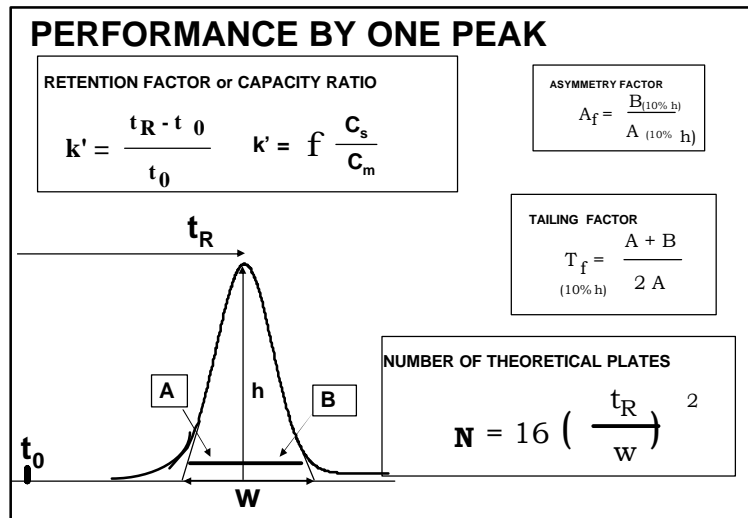
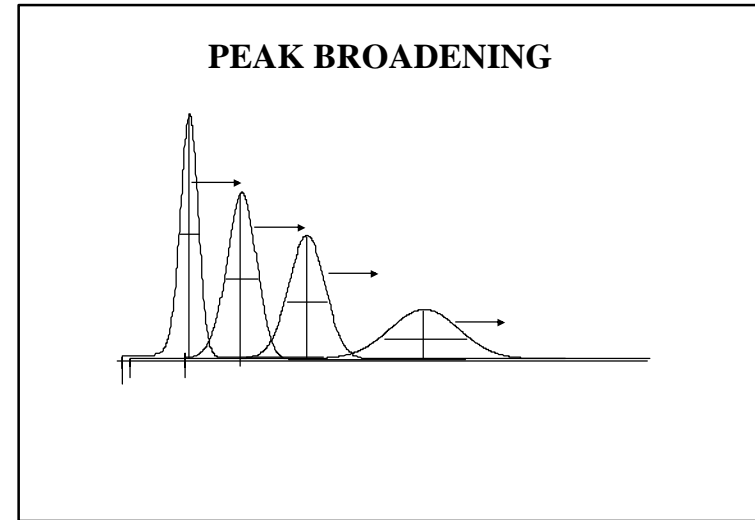
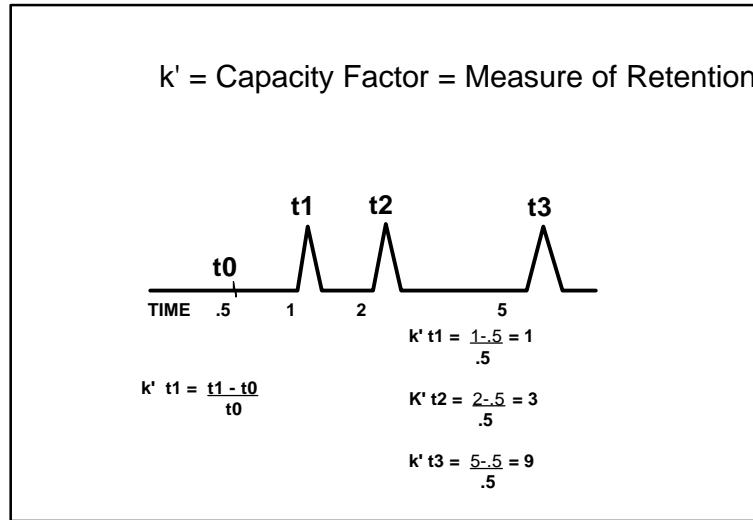
### Quality Control

VIAL	SAMPLE NAME	INJ VOL.	No of Inj	Function	Method	Run Time	Sample Weight	Dilution
1	Blank	20.0	1	Inject Samples	LC Demo Method Set	10.00	1.00000	1.00000
2	System Suitability	20.0	1	Inject Samples	SST Method Set	10.00	1.00000	1.00000
				Clear Calibration	LC Demo Method Set			
3	Std1	20.0	5	Inject Standards	LC Demo Method Set	10.00	1.00000	1.00000
4	Std2	20.0	2	Inject Standards	LC Demo Method Set	10.00	1.00000	1.00000
				Report	LC Calibration Report			
				Report	Standard Comparison			
				Clear Calibration	LC Demo Method Set			
1	Std1	20.0	1	Inject Standards	LC Demo Method Set	10.00	1.00000	1.00000
2	Unk 1	20.0	2	Inject Samples	LC Demo Method Set	10.00	1.00000	1.00000
3	Unk 2	20.0	2	Inject Samples	LC Demo Method Set	10.00	1.00000	1.00000
4	Unk 3	20.0	2	Inject Samples	LC Demo Method Set	10.00	1.00000	1.00000
5	Unk 4	20.0	2	Inject Samples	LC Demo Method Set	10.00	1.00000	1.00000
6	Unk 5	20.0	2	Inject Samples	LC Demo Method Set	10.00	1.00000	1.00000
7	Unk 6	20.0	2	Inject Samples	LC Demo Method Set	10.00	1.00000	1.00000
1	Std1	20.0	1	Inject Standards	LC Demo Method Set	10.00	1.00000	1.00000
				Clear Calibration	LC Demo Method Set			
				Calibrate	LC Demo Method Set			



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