A Systematic Approach to Developing Stability Indicating Methods (SIMs)

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Outline

- Purpose
- Regulatory Requirements
- Development Steps
- Method Acceptability
- Case Study

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Purpose of SIM

- 1970s, rising concerns over drug product stability
- 1975, USP added drug product expiration dating
- 1987, FDA issued guidelines on submission of stability data
- 2009, WHO issued its own guidelines

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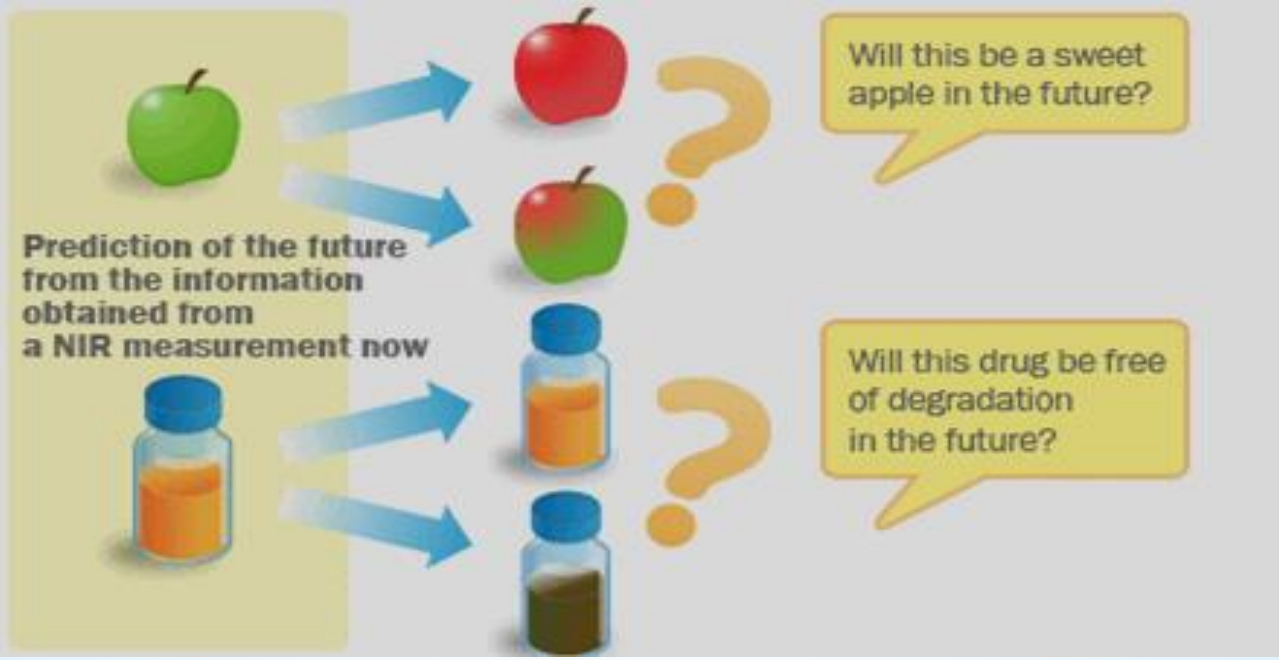
Purpose of SIM

Necessary to establish drug substance or drug product stability over shelf-life



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Regulatory Requirements

-multiple guidance documents (see references)

-new definition as of July 2015

-some detail provided

-what constitutes SIM open for interpretation

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Regulatory Definition (August 2000)

“accurately measures the active ingredients, without interference from degradation products, process impurities, excipients, or other potential impurities.”

Guidance for Industry, Analytical Procedures and Method Validation, U.S. Department of Health and Human Services FDA, August 2000

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New Definition (July 2015)

"If a procedure …can detect changes in a quality attribute(s) of the drug substance and drug product during storage, it is considered a stability-indicating test."

Guidance for Industry, Analytical Procedures and Methods Validation for Drugs and Biologics, U.S. Department of Health and Human Services FDA, July 2015

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New Definition (July 2015)

Specificity – more detail given

- Samples spiked with target analytes and all known interferences
- Samples that have undergone various laboratory stress conditions
- Actual product samples (produced by the final manufacturing process) that are aged or stored under accelerated temperature/ humidity conditions

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Development Steps

- Analytical Methodology
- Method Development Strategy
- Current Best Practices

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Development Steps

- **Analytical Methodology**

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Examples

HPLC/UPLC

LCMS

GCMS

TLC/HPTLC

CE

UV spectroscopy

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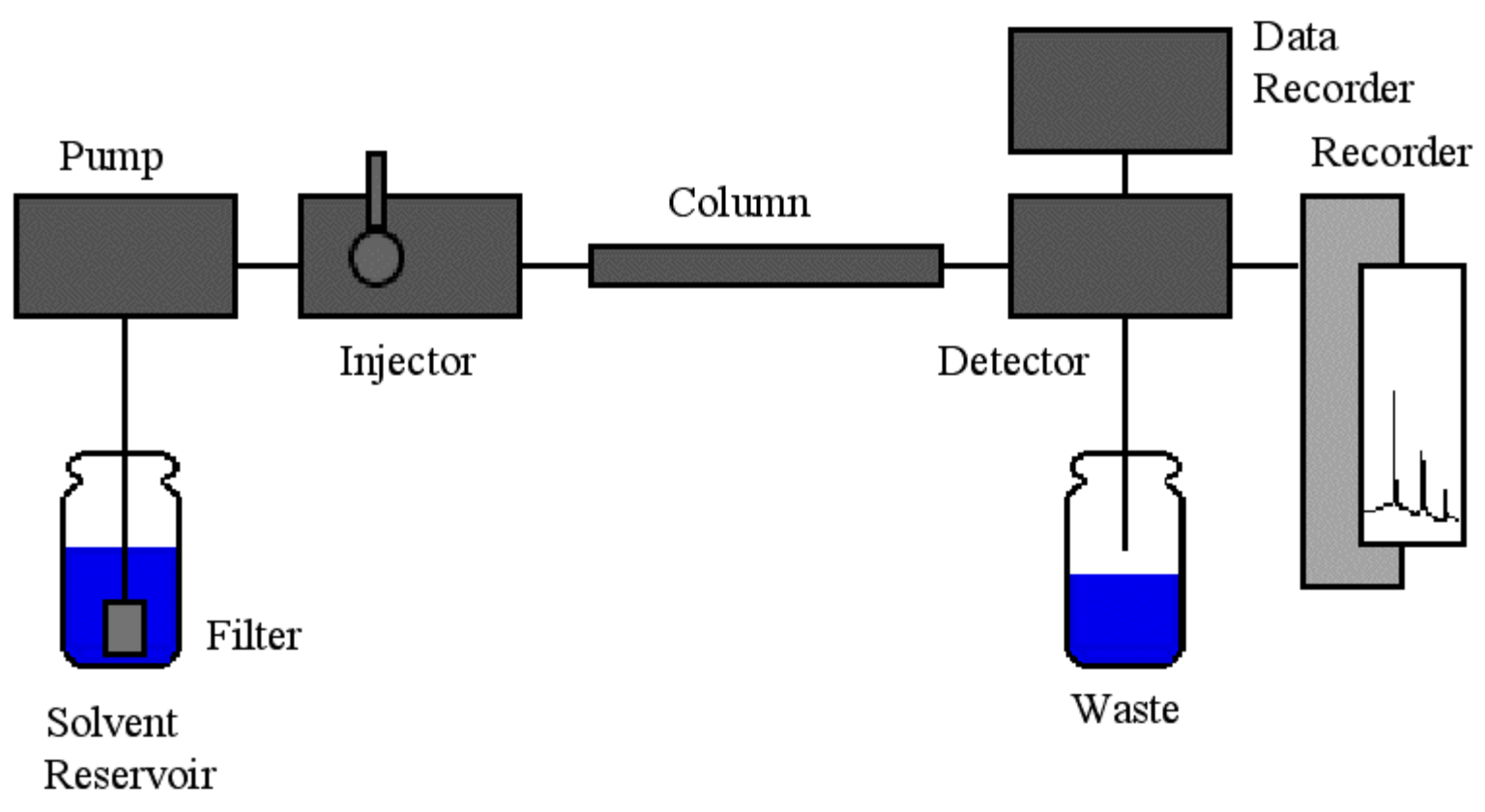
Majority of SIMS are HPLC



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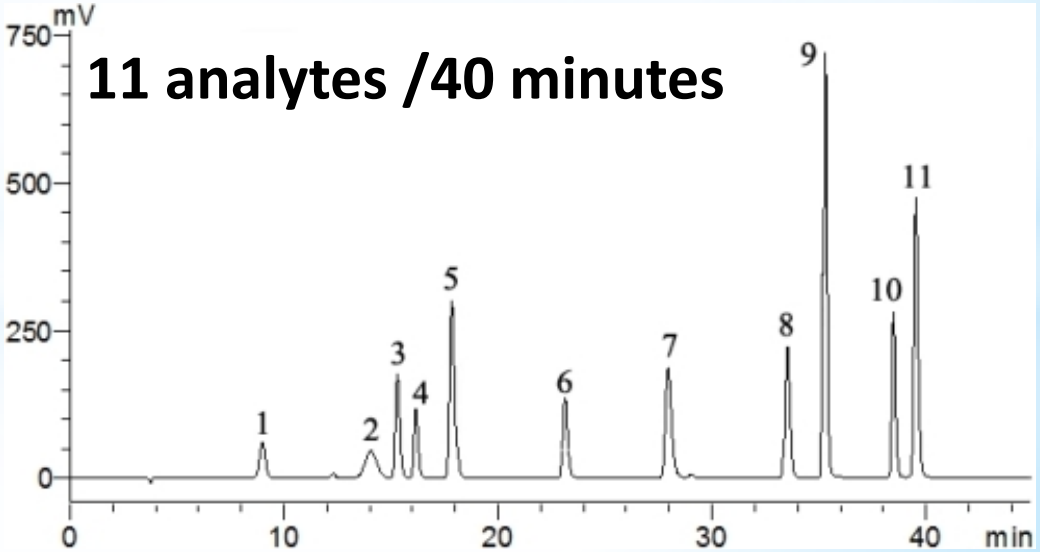
HPLC Advantages

- For impurity profiling – power of separation
- Good sensitivity / multiple detectors
- Diverse range of analytes
- Continuous innovations
- Automated



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HPLC Advantages

Multiple modes of separation base on chemical characteristics of compound:

Polarity

Electrical Charge

Molecular Size

Stereochemistry

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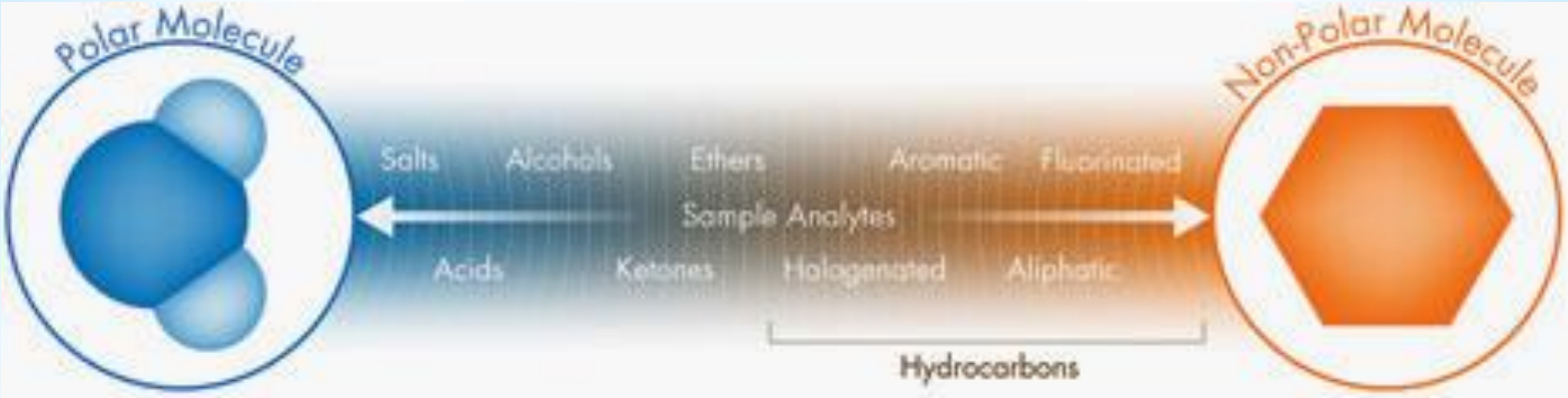
Polarity



Reverse Phase

Normal Phase

Hydrophobic Interaction (HIC)

Hydrophilic Interaction (HILIC)

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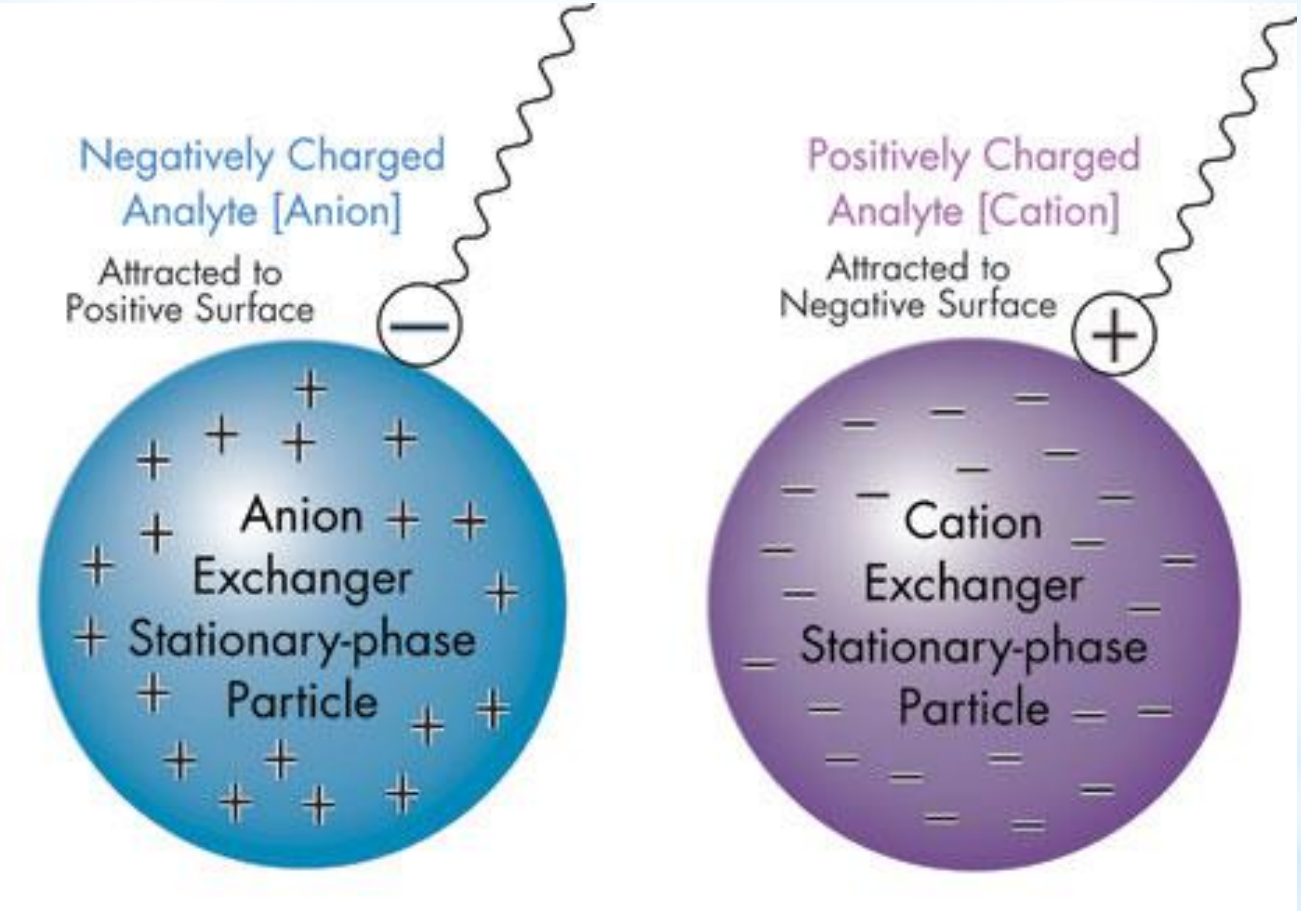
Ion-Exchange



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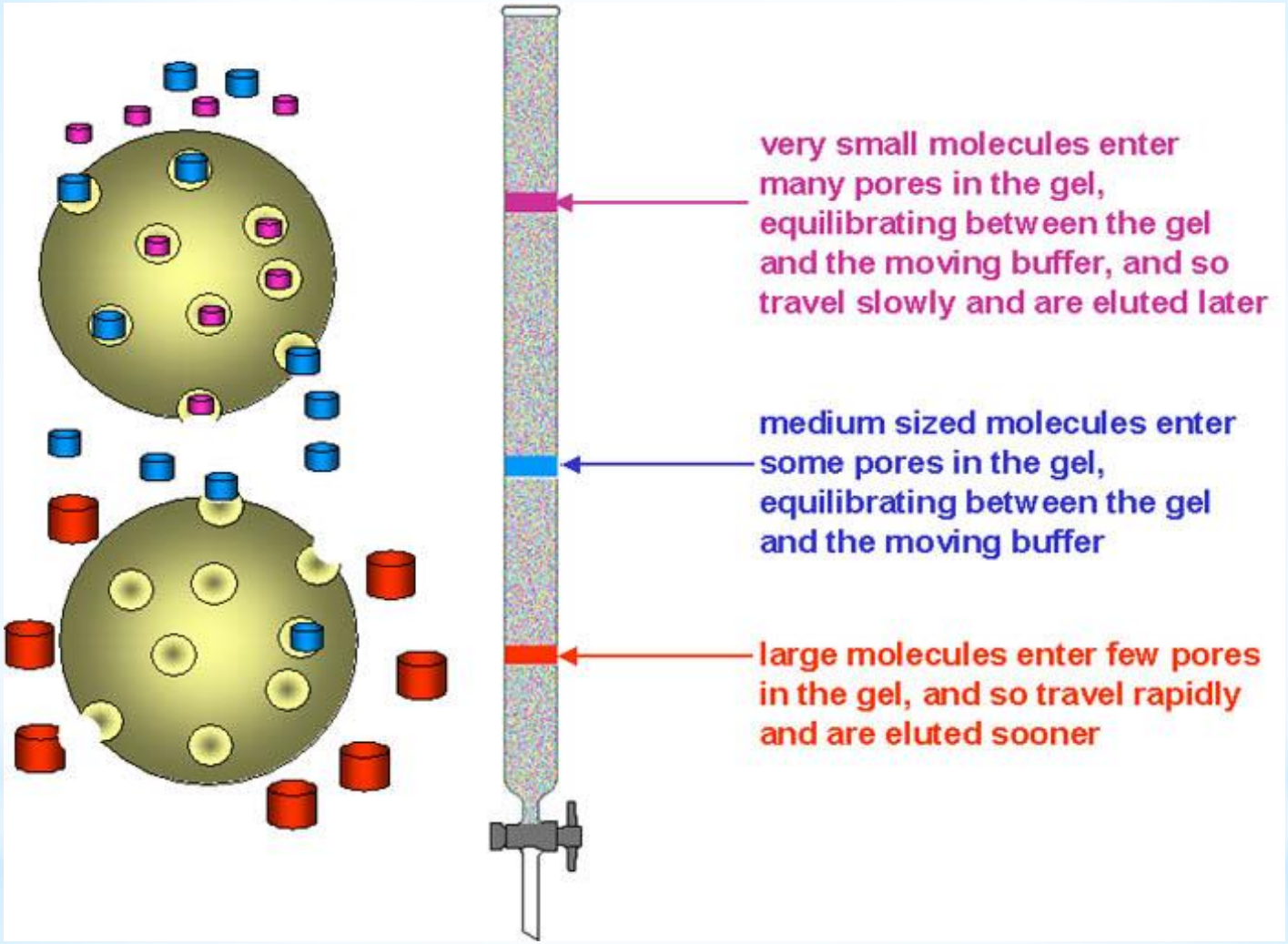
Size Exclusion



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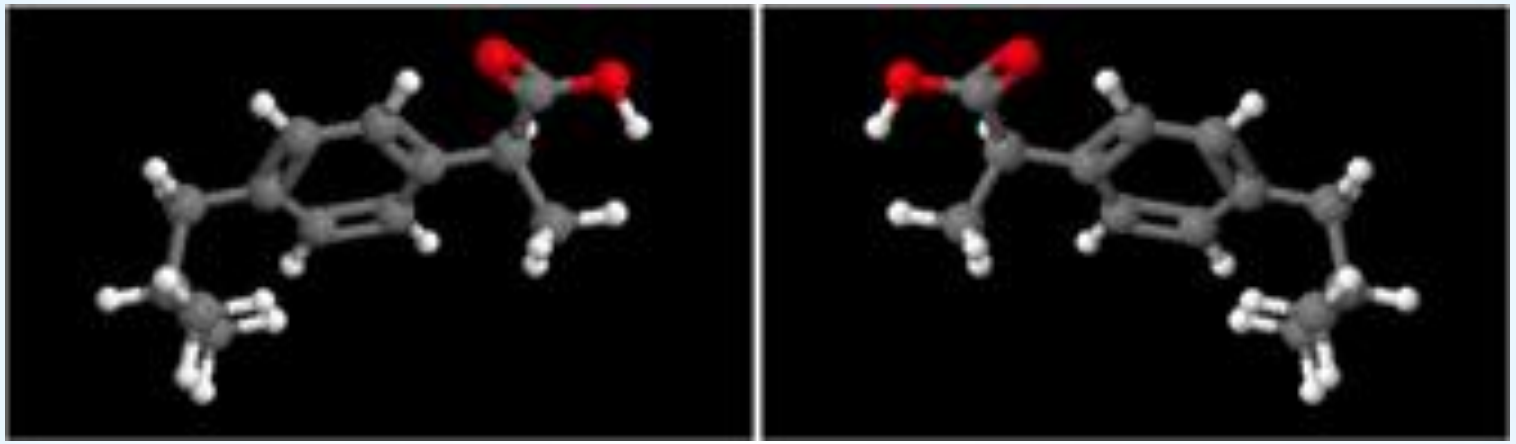
Chiral chromatography

-well known example is ibuprofen



S+ibuprofen (left) and R–ibuprofen (right) showing their mirror image relationship

Chiral chromatography

Chiral stationary phase. Requires 3 interactions through:

- **H-bonding**
- **π-π interactions**
- **Dipole stacking**
- **Inclusion complexing**
- **Steric bulk**

Conducted in both normal / reverse phase modes

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75% of all HPLC methods are Reverse Phase

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Reverse Phase HPLC

Definition: mobile phase is more polar than stationary phase. Stationary phase hydrophobic.

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Selectivity in Reverse Phase HPLC

Three equilibria involved:

1. Solute interaction with mobile phase
2. Solute interaction with solid phase
3. Solute interaction with support

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Selectivity in Reverse Phase HPLC

Molecular interactions

-Dispersion /hydrophobic

-Charge Transfer (π-π) / aromatic

-Hydrogen bonding / acidic

-Dipole-dipole / basic

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Reverse Phase HPLC

Detection Method

Stationary Phase

Isocratic or Gradient Mode

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RP HPLC Detection

- Most popular UV/Vis, diode array
- Mass Spectrometry – increasing use
- Additional – refractive index (RI), fluorescence, charged aerosol detector (CAD), evaporative light scattering (ELSD), amperometry, and conductivity

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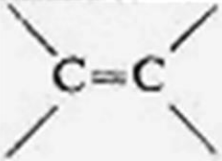
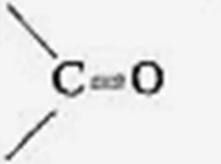
UV/Vis Detection

Example Chromophores

Chromophore	Example	Transition	λ_{max}/nm	ε/mol^{-1} m^2
	ethylene	$\pi \rightarrow \pi^*$	165	1500
	acetone	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$	188 279	90 1.5
—N=N—	azomethane	$n \rightarrow \pi^*$	347	0.45
—N=O	nitrosobutane	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$	300 665	10 2
	benzene	$\pi \rightarrow \pi^*$	200 255	800 21.5

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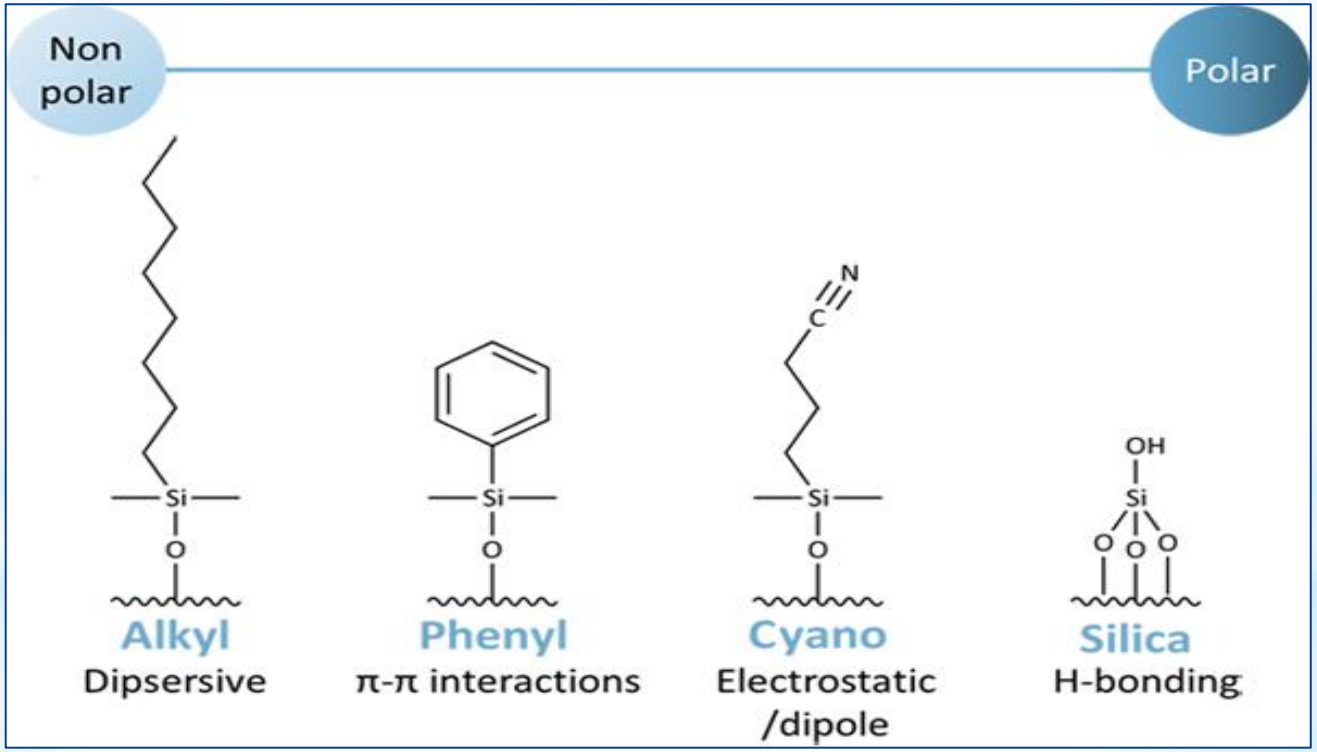
Stationary Phase

- Variety phases: alkyl, fluoroalkyl, amide, amine, cyano, diol, phenyl, etc.
- Most widely used is C18 alkyl phase



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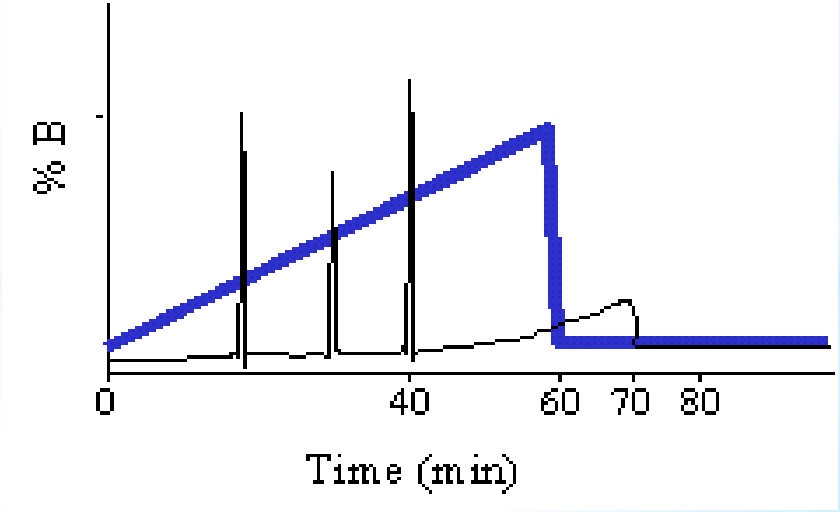
Isocratic or Gradient Elution

Isocratic: composition of mobile phase remains constant throughout the run. Most used for assay, dissolution. Separating 2 or 3 compounds in a single run.

Gradient: Mobile phase composition varies over run. Most used for impurity profiling and degradation studies.



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Development Steps

- **Method Development Strategy**

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Method Stages

- Pure API
- Changing API process
- Changing salt form
- Formulation concerns
- It is transferable?

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Method Development

Starting considerations:

- Assay only, or assay & related substances? Multiple actives?
- Special sample type such as inorganic ions, enantiomers, biomolecules, polymers, carbohydrates, isomers?
- Is it feasible to determine all related substances in one method?

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Method Development

1. **Gather information on chemical & physical properties of analytes**
    - Literature search
    - Synthetic process
    - pKa (pH 50% of compound protonated) and log P (lipophilicity) values
    - Check availability of method development software (DryLab)

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2. Evaluate analyte mixture

- Range of pKa and log P values
- Determine critical pairs
- Presence of amine-containing compounds (tailing)
- Presence of amphoteric compounds (presence of both acidic and basic functional groups)

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3. Develop starting conditions

- Based on step 2, select 3-4 stationary phases (commercial kits are available)
- Start at low pH
- Run gradient 5 -100% over 20-40min
- Use diode array detection for λmax values
- Use test mixtures of critical pairs

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Selectivity

Changed when we change the chemistry of chromatographic system

Variables that affect selectivity:

-solvent strength and type

-temperature of the column

-gradient steepness

-buffer and other additives

-type of column packing

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Organic Solvents

Solvent	UV cut off (nm)	Viscosity (mPa s)	B.P. (°C)	Polarity (P')[1]
Water	200	1.00	100	9.0
Methanol	205	0.6	65	6.6
Acetonitrile	190	0.37	82	6.2
THF	215	0.55	65	4.2
IPA	210	2.35	83	4.3
Hexane	195	0.313	69	0

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Common Buffers

Buffer	pK_a	pH range	UV cut off (nm)
Phosphate, pK_a1	2.1	1.1 - 3.1	<200
Phosphate, pK_a2	7.2	6.2 - 8.2	<200
Phosphate, pK_a3	12.3	11.3 - 13.3	<200
Citrate, pK_a1	3.1	2.1 - 4.1	230
Citrate, pK_a2	4.7	3.7 - 5.7	230
Citrate, pK_a3	6.4	5.4 - 7.4	230
Carbonate, , pK_a1	6.1	5.1 - 7.1	<200
Carbonate, pK_a2	10.3	9.3 - 11.3	<200
Formate	3.8	2.8 - 4.8	210 (10 mM)
Acetate	4..8	3.8 - 5.8	210 (10 mM)
Ammonia	9.3	8.3 - 10.3	200 (10 mM)
Borate	9.2	8.2 - 10.2	N/A
TFA	0.5		210 (0.1%)

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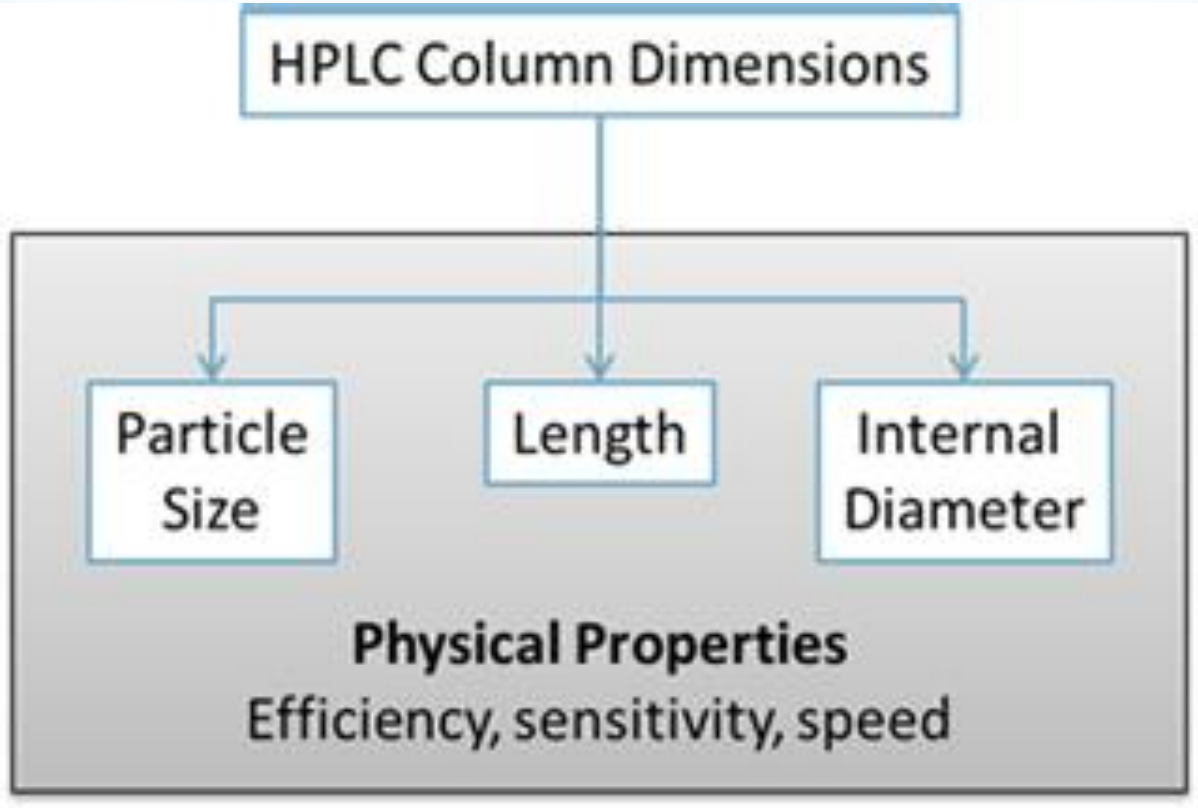
Column Dimensions



$$HETP = L / N$$

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Column Dimensions

Column Length, L (mm)	Particle Size, d_p (µm)	Resolution Capacity L/d_p
300	10	30,000
150	5	30,000
100	3	33,300
50	1.7	29,500
100	1.7	58,820
150	1.7	88,230

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4. Optimization

- Most time-consuming step
- Speed vs. resolution
- Early stage development *an iterative process that is repeated several times to accommodate unexpected impurities that arise*

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5. Method merits

- Resolution between peaks of 1.0 or better (API peaks usually broad due to their higher concentration in sample)
- Peak shape (tailing factor 0.9-1.5) & plate number (>4000)
- Repeatability – RTs ±0.1 min and R_s ±0.2 units

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5. Method merits

- No interference from blank
- No interference from sample matrix
- Linear response for all degradants
- Able to achieve desired limit of quantitation (LOQ) for degradants
- Able to recover API & degradants from sample matrix

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Sample Extraction Method

Development Steps

- **Current Best Practices**

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Current Best Practices

- Thorough understanding of drug substance chemistry - vulnerability assessment
- Degradation target range of 5-10%; careful not to under or over-degrade sample
- Perform “complete” identification of unknowns that exceed ICH thresholds

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Current Best Practices

- Pre-qualify method to ensure it is “validatable”/ method development is not GMP- want successful GMP testing
- Phase-specific strategy for validation
- Adopt USP “life cycle management” approach to SIM

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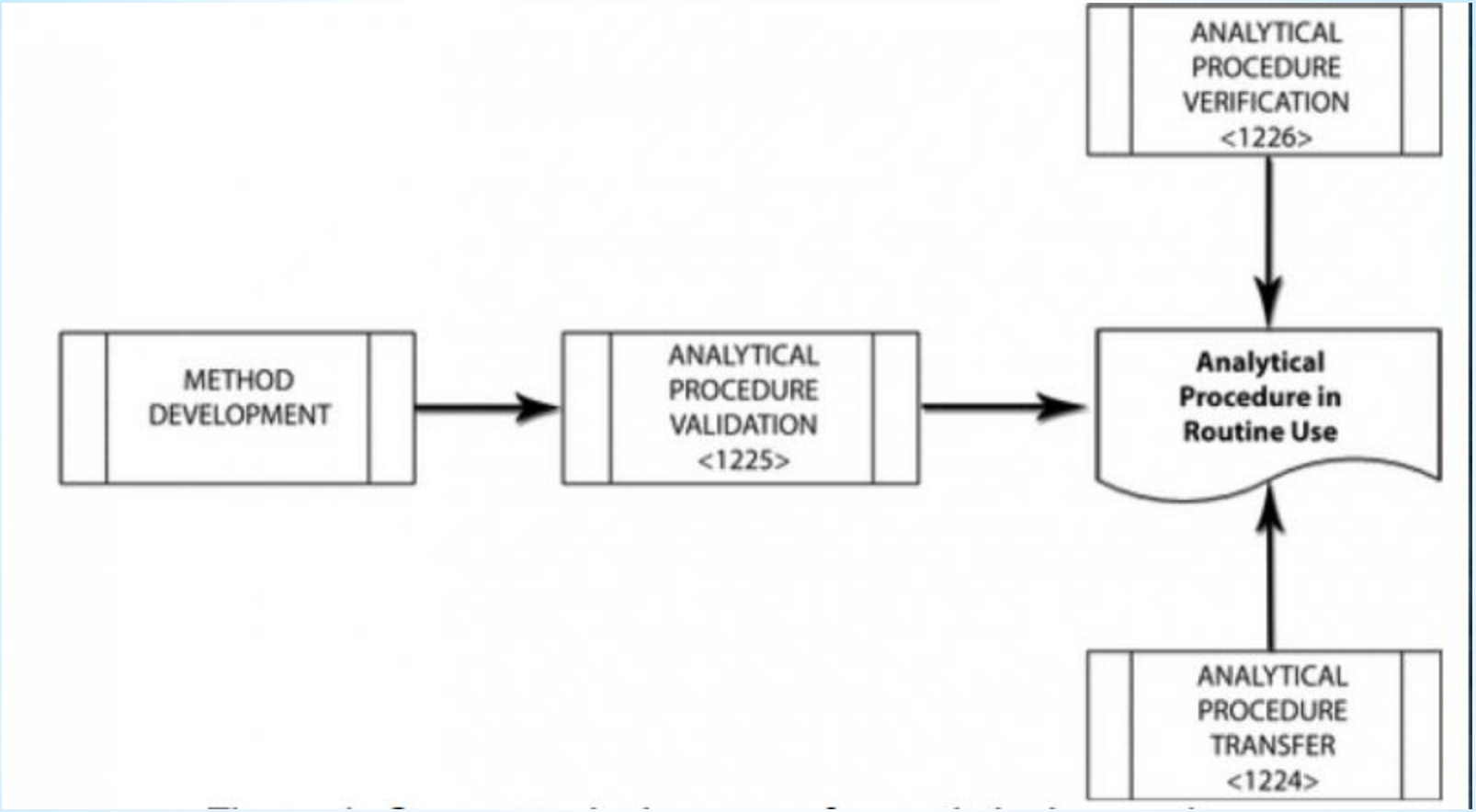

Typical Process

Lifecycle Approach

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Case Study

Goal

Develop single method for assay and related substances for monolayer tablet

- Assay 2 actives in tablet
- 9 related substances

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Case Study

History

- Prior method developed not working after transfer
- Reported problems reported were column failures, excessive back pressure, shifting retention times, loss of resolution, difficulty obtaining LOQ

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Case Study

Simple method corrections

- Appropriate wavelength for detection
- Type of detector and path length
- Buffer mobile phase (stay within buffering range)
- Select column suitability for high aqueous starting conditions

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-Dual therapy product: separating 2 actives and all associated related substances (14 compounds total)

-Not all compounds neutral, ionizable groups (pKa values should be evaluated

-Several amine-containing compounds (peak tailing is a concern)

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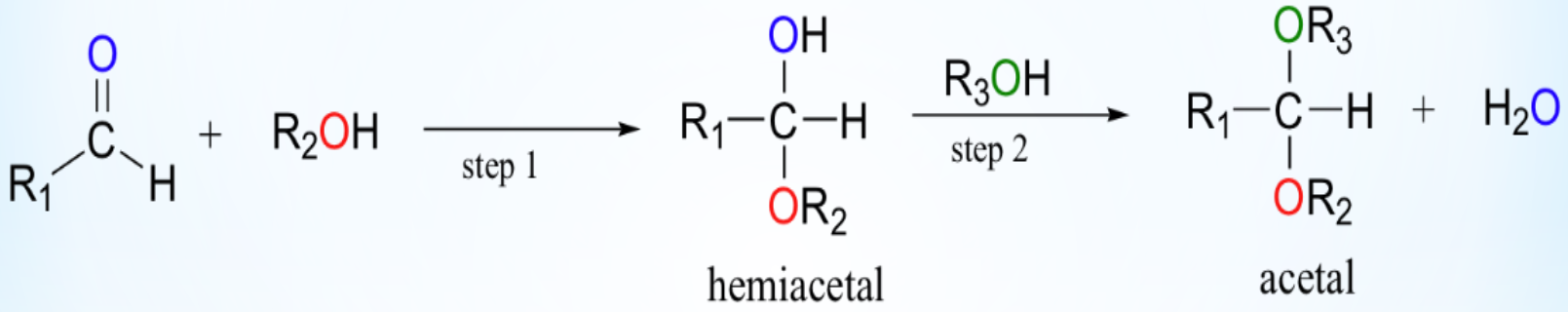
Case Study

-Ketal chemistry a concern for 3 of the compounds (better to use aprotic solvent)



Case Study

Evaluation

- pKa values: 3.98, 8.2, 9.6, 10.6

  -available pH 2.5, 5.5-7, high pH
- Log P: wide range from -0.2 to 4
- All compounds contain phenyl ring

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Case Study

Evaluation

- Two critical pairs with similar structures
- Target specification levels of 0.3%
- Large difference in levels of actives; 8 mg active 1 / 90 mg active 2
- One degradant has isomer

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Case Study

Rules of thumb retention behavior:

- Increases as #carbon atoms increases
- Branched-chain compounds elute more rapidly
- Decreases with increasing unsaturation
- Order of elution generally follows:

aliphatics > weak Lewis bases (**esters**, **aldehydes**, **ketones**) > strong **Lewis bases** (amines) > weak **Lewis acids** (**alcohols, phenols**) > strong Lewis acids (**carboxylic acids**)

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Case Study

Establish starting separation conditions:

HPLC column needs to:

- operate under highly aqueous (avoid hydrophobic collapse)
- work for mixture neutral, acidic, basic compounds
- prevent tailing (do not want to add ion-pairing reagent, if possible)

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Case Study

Column dimensions – 4.6 mm x 25 cm, 5µm
Both columns designed for high aqueous

1. *Supelcosil ABZ plus - alkyl amide phase for mixtures of neutral, acidic, basic compounds, use with low ionic strength buffers without need for ion-suppressing modifier*

2. *ACE C18AR – C18-phenyl phase extra resolving power with π-π selectivity of the phenyl functionality, highly inert for amines*

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Case Study

Results of screening exercise

-50% of compound eluted in first 20 minutes

-50% of compounds eluted after 40 minutes

-20 minutes of “dead time”

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Case Study

Results of screening exercise

-at low pH, all retention times decreased, one compound elutes in void volume of column

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Case Study

Results of screening exercise

-pH > 6.0, lack of resolution between critical pair below that differs only in the addition of a hydroxyl group

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Final separation conditions:

1. Buffer: 20 mM ammonium acetate @ pH 5.8 vs. prior 100 mM sodium phosphate @ pH 6.1 (actually buffering)
2. Based on UV/Vis spectra collected using PDA – wavelength set at 229 nm vs. 280 nm which was *not* detecting one of related substances

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Case Study

Final separation conditions:

3. Organic modifier acetonitrile for best peak shape
4. Incorporated 2 gradients into method using of a step function:

    -100% MP A – 75% MP A

    -step to 60% MP A

    -60% MP A – 0% MP A

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Case Study

Final separation conditions:

3. Column selected was ABZ plus – see chromatograms on following slides

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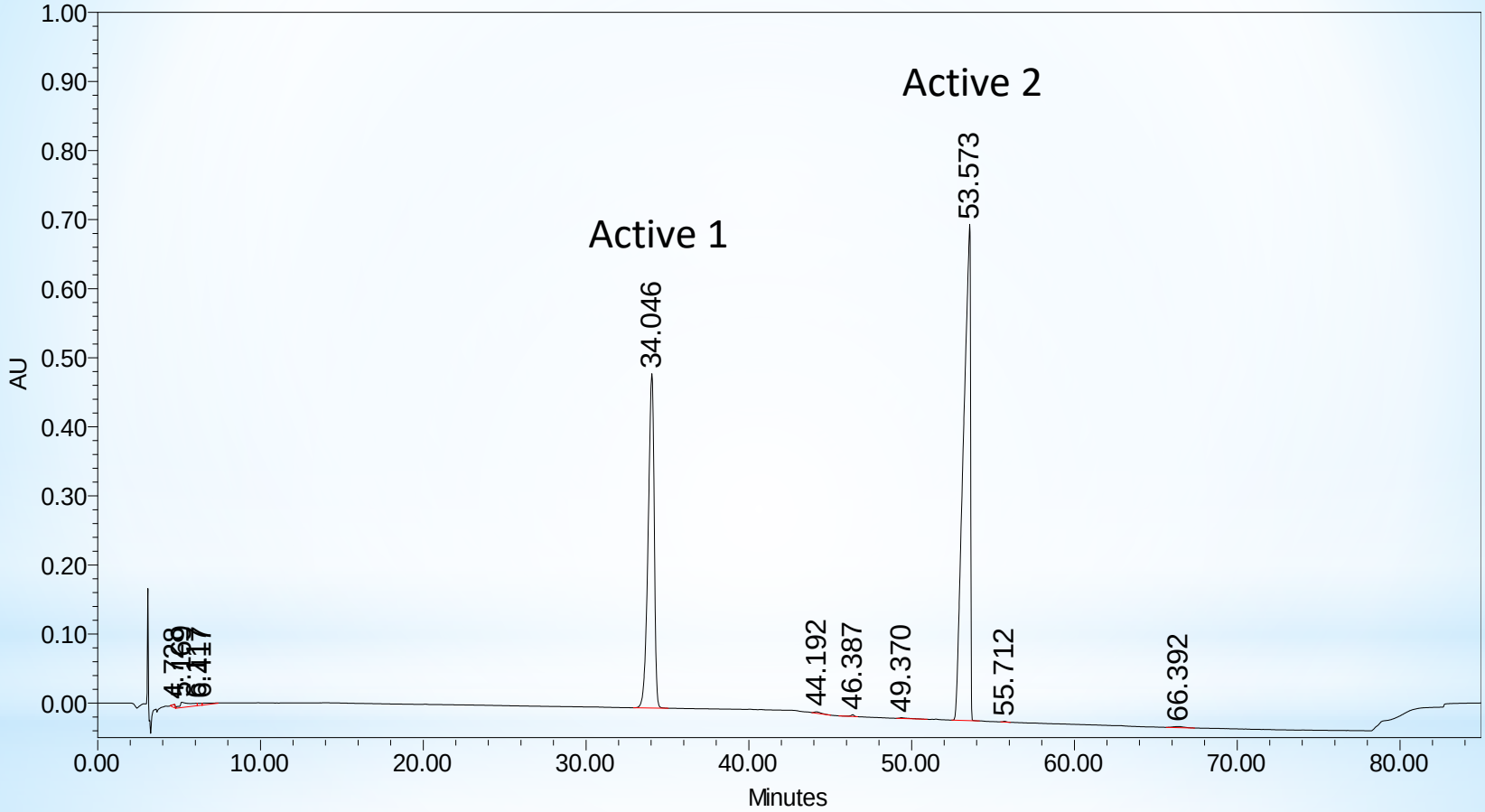
Case Study

ACE C18AR Column



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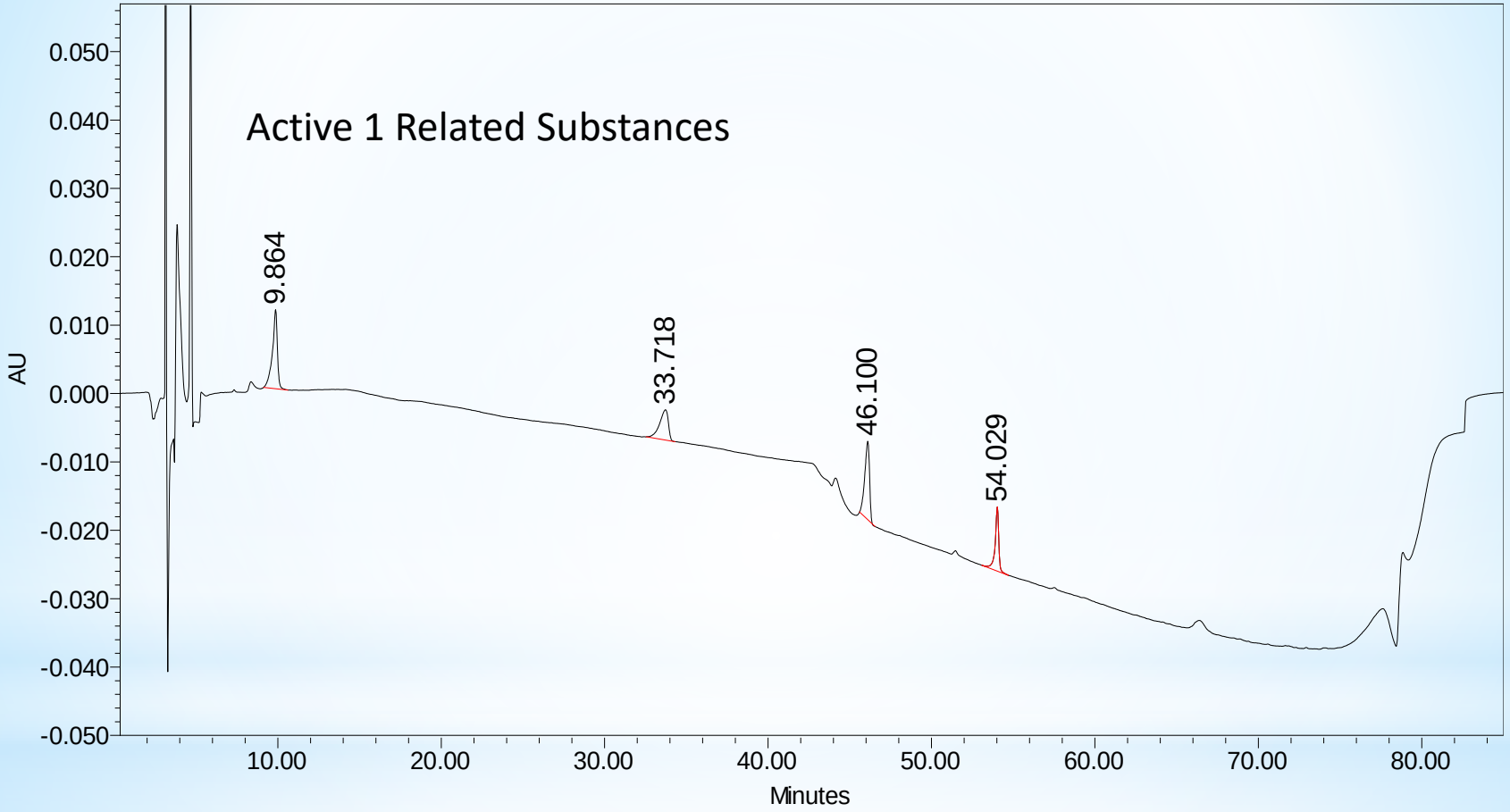
ACE C18AR Column



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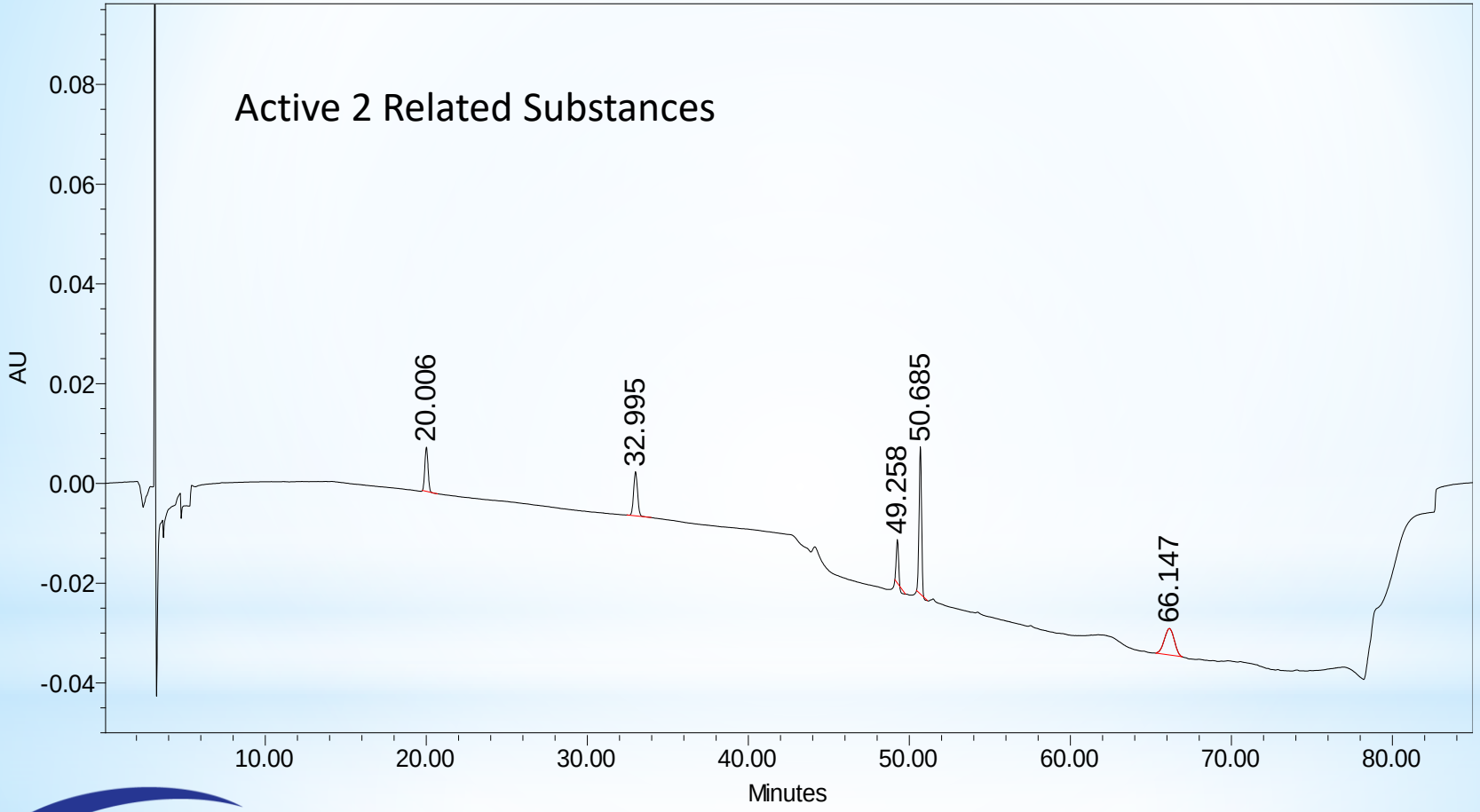
Case Study

ACE C18AR Column



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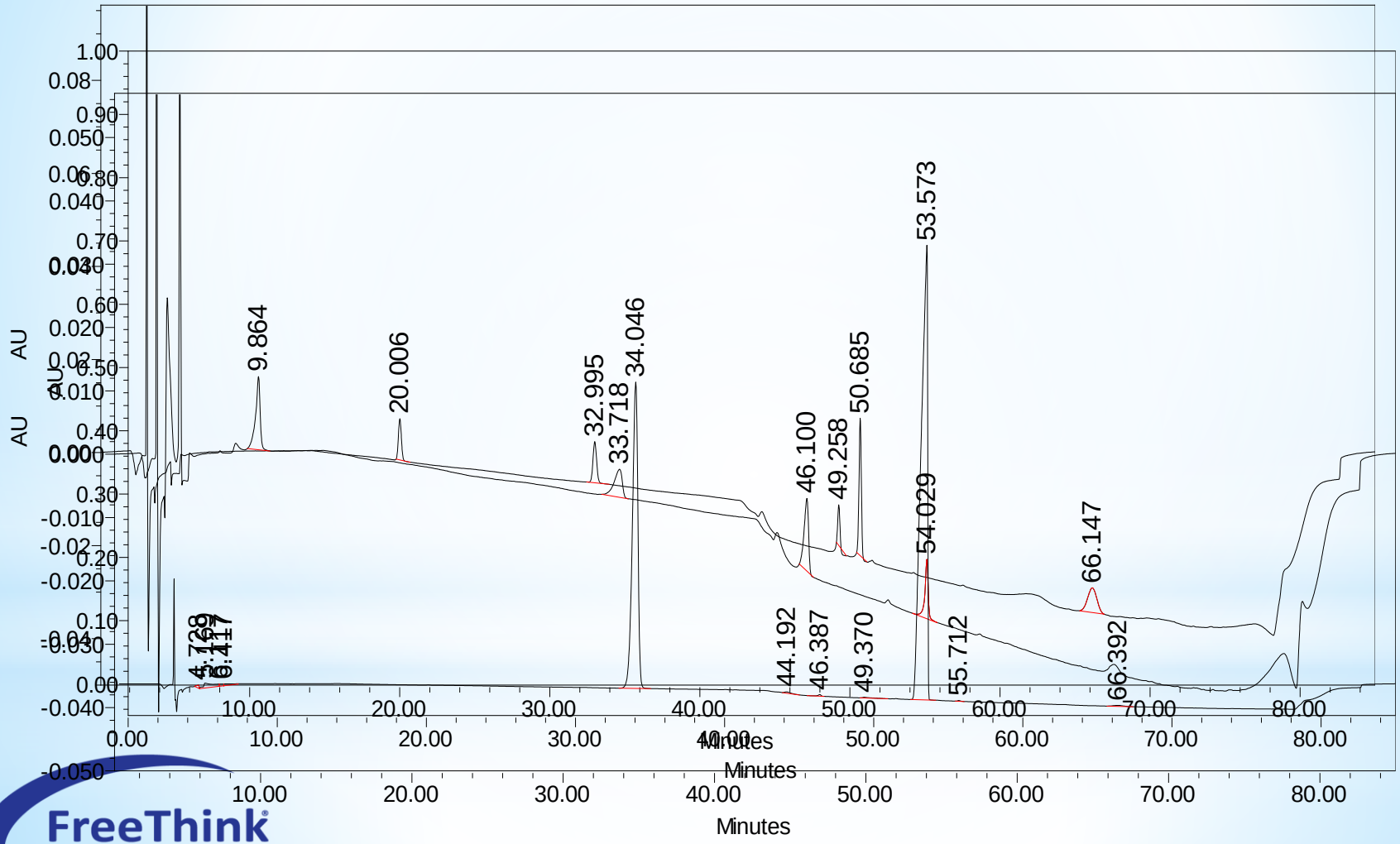
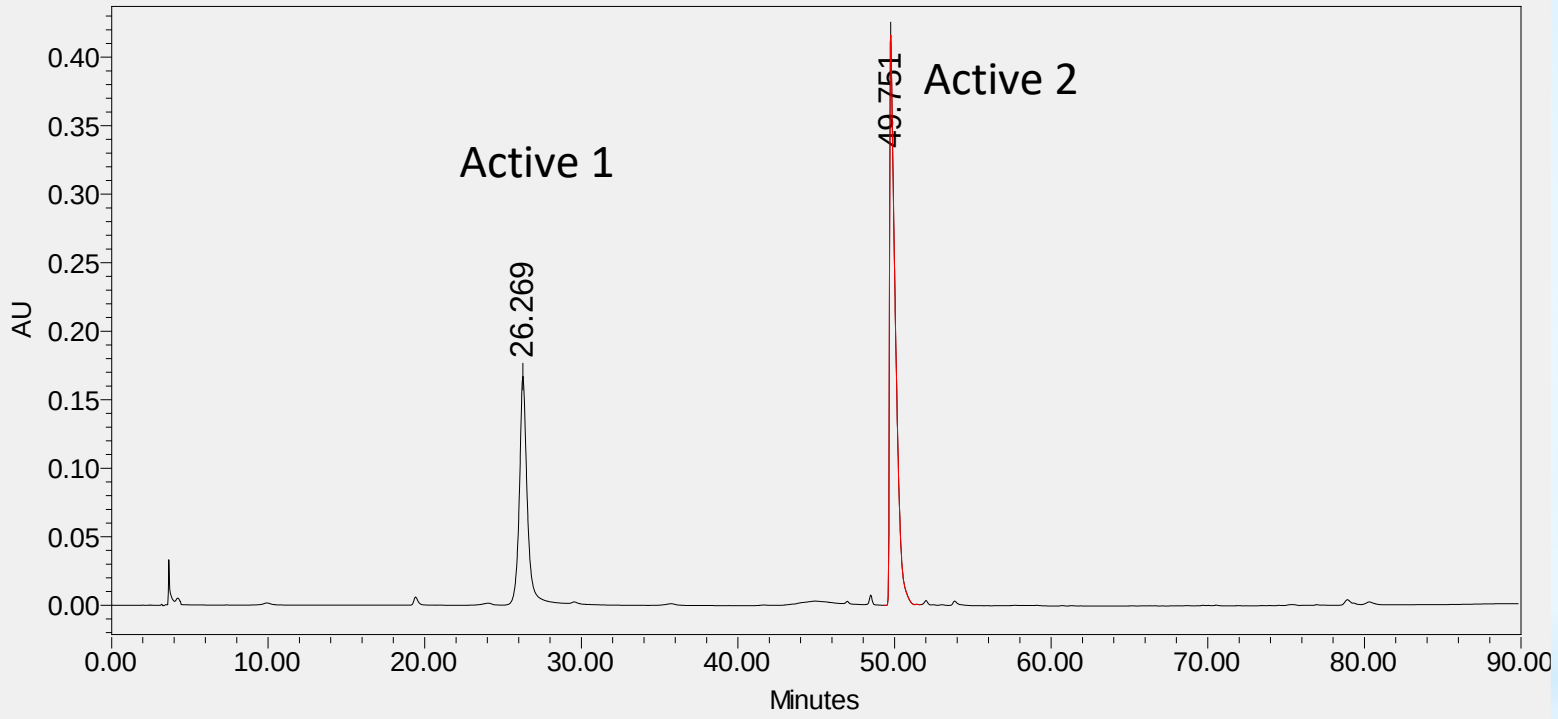
ACE C18AR Column



Case Study

ABZ Plus Column



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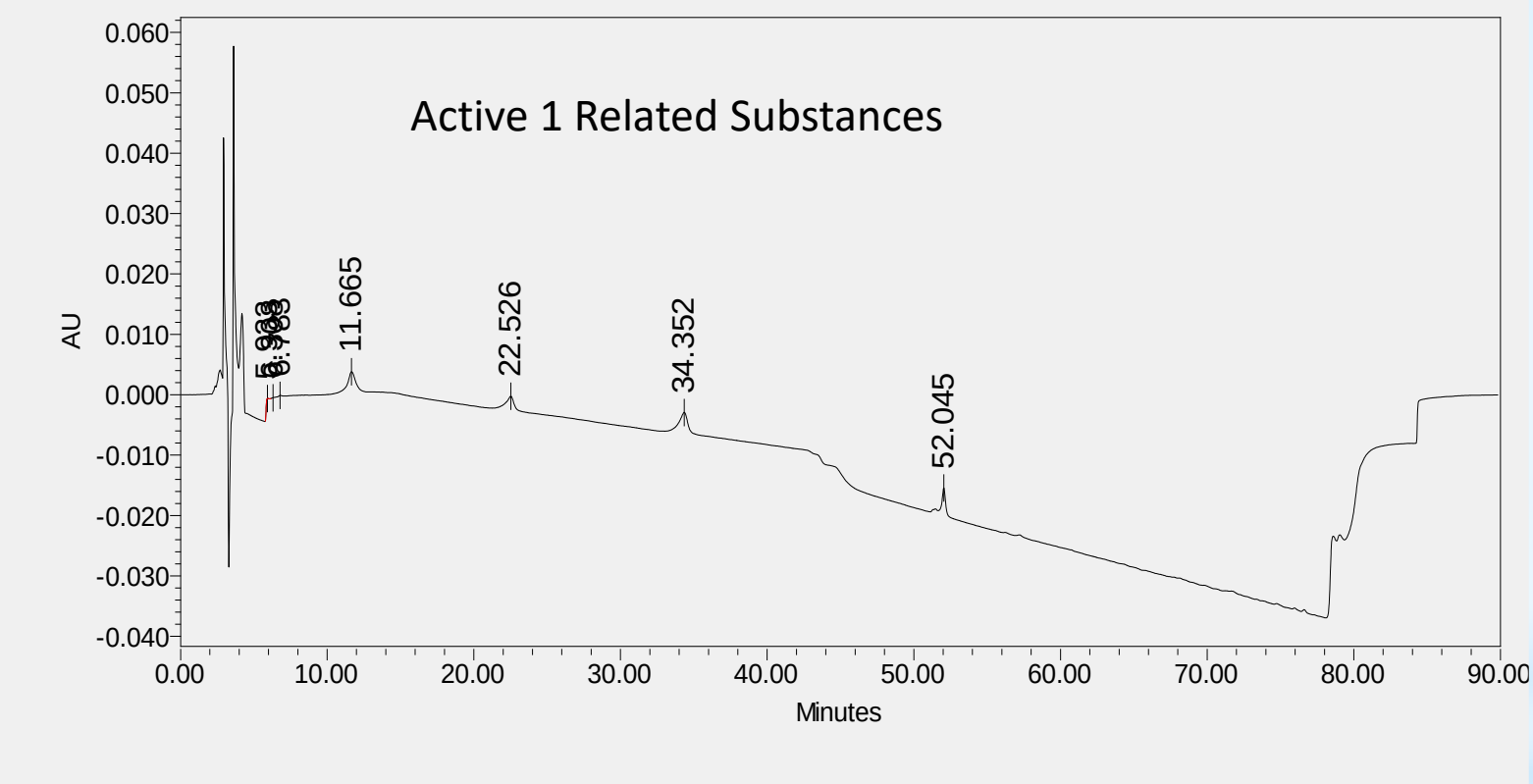
ABZ Plus Column



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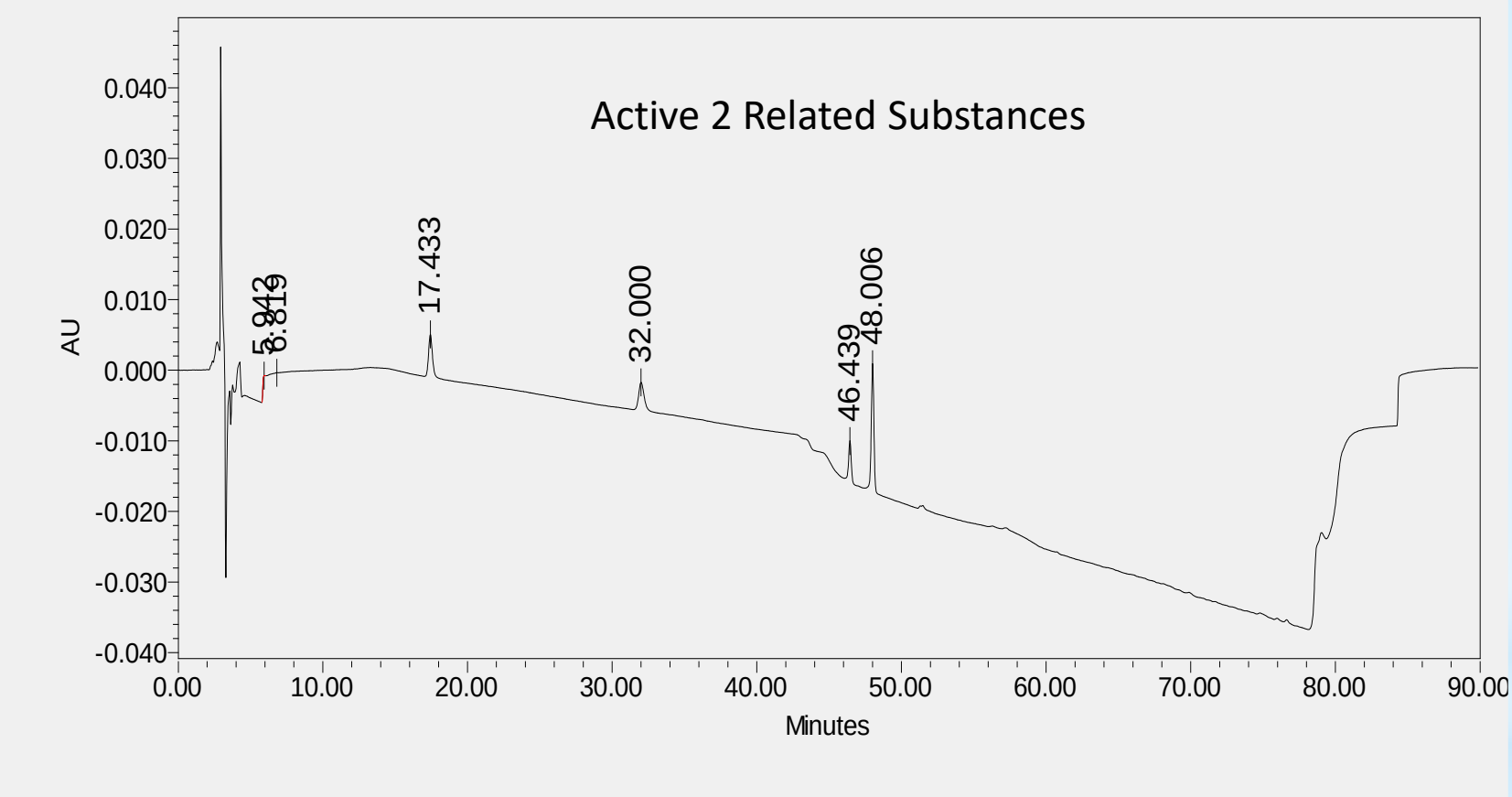
ABZ Plus Column



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Case Study

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