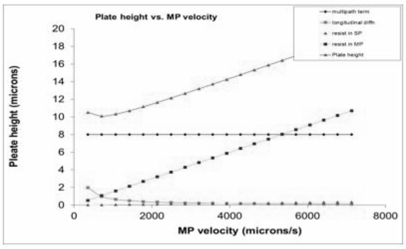
# Liquid Chromatography Cheat Sheet

by shaylannxd via cheatography.com/149855/cs/32584/

## Basic Theory

| | |
|---|---|
| **Liquid Chromatography** | MP ➔ Liquid<br>‣ Actively participate in equilibrium process |
| | SP ➔ Quasi/porous solid<br>‣ Most common do reverse-phase chromatography SP<br>‣ Film thickness ➔ Very small (monolayer) |
| | $D_m$ ~ $10D_s$<br>‣ B/U ~ 0 |
| | $d_f$ ~ 0<br>‣ $C_sU$ ~ 0 |
| | Always carried-out in packed columns |
| **Advantage** | More versatile than GC |
| | Adaptable to needs |
| **Disadvantage** | Much less efficient than GC |
| | Diffusion coefficient of analyte is orders of magnitude smaller than in GC<br>‣ Bounced into other molecules ➔ Diffusion rate is small |
| | In liquid phase (not gas) |
| **Improve efficiency** | Use small particle ➔ Narrow range velocity<br>‣ Dependent on particle diameter ($d_p$)<br>‣ Smaller particle size = smaller plate height at any given velocity |
| | Compensate for travel distance of analyte to reach surface of SP<br>‣ Minimize the space between the particles that the analyte have to diffuse across |
| | Analytical LC ➔ < 5µm |
| | HPLC ➔ >3µm |
| | UHPLC ➔ < 3µm |

## Theory Equations

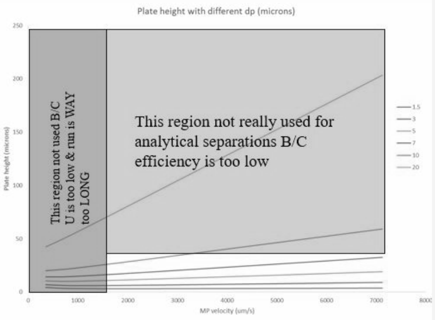


$$A = 2\lambda d_p \qquad \frac{B}{U} = \frac{2\gamma D_m}{U} \approx 0$$

$$C_sU = \frac{f_s(k')d_f^2}{D_s}U \approx 0$$

$$C_mU = \frac{f_m(k')d_p^2}{D_m}U$$

## LC Velocity Range



## Column

| | |
|---|---|
| **Packed Column** | Packed full of particles |
| | Usually composed of stainless steel, etc. |
| | Length<br>‣ 2-20cm(analytical)<br>‣ Large column ➔ Use for preparative scale<br>‣ Small column ➔ Packaged inside a capillary (75-100um diameter) ➔ Couple efficiently to MS |

## Column (cont)

Efficiency (N)

- 3000-20000
- Dramatically lower than GC
- Rule of thumb (GUESTIMATE ONLY): N~ 3500 *L(cm) / $d_p$(um)

Sample Capacity

- Depending on size of column and packing
- ~5mg/g {[fa-arrow-right]} C18/silica
- ~10mg → "vanilla" column

Thickness of SP

- ~ 1-2nm

Resistance to mass transfer in MP and multipath terms dominate

## HPLC Column

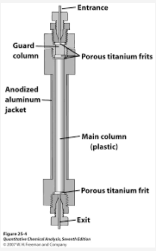


Figure 25-4
*Quantitative Chemical Analysis, Seventh Edition*
© 2007 W. H. Freeman and Company

## HPLC System

| | |
|---|---|
| **MP reservoirs** | Stores MP in inert glass bottles<br>▸ Platic coated Pyrex bottle (common) → $300/pc |
| | Degas solvent<br>▸ Add element for filtering/degas<br>▸ Minimize amount of oxygen dissolved into MP → Oxygen reactive in high pressure (increase oxidation of analyte)<br>▸ Small bubbles can form → Result intensive undesirable peaks |
| | Connected to a computer(pump)<br>▸ Control mixing value to produces desired MP mixture |

## HPLC System (cont)

| | |
|---|---|
| **Analytical Column** | Wide variety<br>▸ Diameter → ~0.5cm (general)<br>▸ Length → 10-20cm (general) |
| **Injector** | Manual<br>▸ Syringe with sample<br>▸ Inject needle into port and release<br>▸ Liquid flow into loop (at atmospheric pressure)<br>▸ When rotate lever to 60 degree → Rearrange injector (set of valves)<br>▸ Switches loop into flow path = swept down into analytical column |
| | Operates at very high pressure<br>▸ If inject sample into septum →Shatter syringe |
| | Autosampler<br>▸ Prepare in vials → Tightly seal for sample to not evaporate<br>▸ Program computer<br>▸ Runs separation overnight<br>▸ ~100 samples |
| **Detector** | Record data and integrate peak area<br>▸ Quantitation |
| **High-Pressure Pump** | Direct MP through system |
| | Use high pressure<br>▸ Analytical column is filled with fine particles |
| **Dynamic Mixer** | Needed to blend the different fluids (MP) |
| | Provides the correct percentages of fluids dynamically as the separation goes |

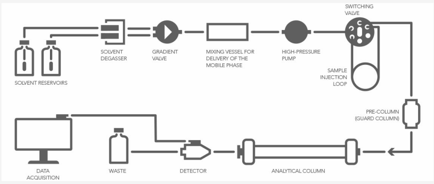
# Liquid Chromatography Cheat Sheet

by shaylannxd via cheatography.com/149855/cs/32584/

## HPLC System (cont)

| | |
|---|---|
| **Guard Column** | Avoid column killers<br>▸ Species that strongly store in SP → Never eluted<br>▸ Contaminated column<br>▸ Can change separation → Destroy separation in terms of its analytical quality |
| | Very small column<br>▸ Contains same type of SP as analytical column |
| | Contamination is trapped inside<br>▸ Periodically replace cartridges → Preserves analytical column |
| | *Optional* |
| **Narrow Bore Tubing** | Tubes that connects components |
| | Has to be rated for HPLC<br>▸ Can handle high pressure<br>▸ Has to be narrow bore → Don't want MP to mixing + dilute sample peaks |
| | Use short length as much as possible |
| | If fitting not installed correctly<br>▸ Can result in dead volume → Analyte that gets trapped in dead volume = gets broaden |
| **Thermostat Oven** | Constant temperature<br>▸ 30-45 degrees<br>▸ For a given MP → Equilibrium is constant |
| **Fraction Collector** | Robots that periodically move tubes of the eluted species from detector |
| | Collect in vials<br>▸ Sophisticated → Deposit 1 peak per vial<br>▸ Less sophisticated → Periodically move from one vial to the next |

## HPLC System Diagram



## Stationary Phase

| | |
|---|---|
| **Control Retention** | Control retention → Control distribution constant(K) |
| | Control by:<br>▸ Adjust type of MP and SP<br>▸ Adjust "strength" of MP and/or SP<br>▸ Add additives to MP → Interact specifically with analyte, SP, MP<br>▸ MP velocity → Does not alter retention (K or K') |
| **Stationary Phase** | Most use silica support particles<br>▸ Not great in high pH → Use alumina(high pH resistance)<br>▸ Low pH → SP can come off of support (hydrolyzed) → Use polymera support |
| | Almost always a "pure" SP<br>▸ Not mixed |
| | Use chemistry reaction to anchor SP to wall/surface |
| | Almost always a "monolayer" SP<br>▸ `df`~0 |
| | Wide range of polarities<br>▸ Use chemical reactions to adjust the SP |
| | Can use chiral SP<br>▸ Separate enantiomers<br>▸ Reasonable environment conditions |

By **shaylannxd**

cheatography.com/shaylannxd/

Not published yet.
Last updated 20th June, 2022.

| Stationary Phase (cont) | |
|---|---|
| **Silane Reaction** | Use to anchor/bond silicones to silica surfaces<br>▸ In packing materials (particles)<br>▸ FS capillaries |
| | Use to deactivate silanols |
| | Silanol<br>▸ Very reactive<br>▸ Highly polar<br>▸ Expose on surface of silica |
| | Deactivate silanol<br>▸ Use chloro silane<br>▸ Ex: C18<br>▸ Result in silanization of surface |
| | Residual silanol<br>▸ SP is usually of a different polarity (non-polar)<br>▸ Results in tailing of analytical peak |
| | After reacting surface with SP<br>▸ Use a short chain alkyl<br>▸ Take care of residual silanol |
| | If silanol peaks present<br>▸ Column is old<br>▸ Molecules of SP are desorbs or removed from surface |
| **Silanol Interactions** | “Standard” silica → SP support particles<br>▸ Has silanols on surface → Si-OH<br>▸ ~50% of Si-OH are reacted to Si-O-Si-C18 |

| Stationary Phase (cont) | |
|---|---|
| | Residual Si-OH<br>▸ When close to a metal in the silica<br>▸ Are “acidic” and deprotonate easily leaving a Si-O$^-$ on the surface |
| | Act as ion-exchange sites for basic analyte<br>▸ Reverse-Phase MP is not suited to ion-exchange separation<br>▸ Very poor peaks are obtained for basic analyte → Tailing peak |
| | Alternative options<br>▸ Use a high purity silica column → Less acidic silanol<br>▸ Purchase a deactivated silica column |
| | Affects only basic compounds<br>▸ Neutral and acidic compounds does not show tailing |
| **Particle and Surface Area** | Terms that dominate:<br>▸ Overall plate height<br>▸ Overall plate number<br>▸ $d_p$ → VD eq.: A and $C_mU$ |
| | Spherical Particle<br>▸ Surface area/Volume scales with 1/$d_p$<br>▸ ↑ A/V = ↓ Retention<br>▸ More SP packed = ↑ Retention (K') = ↑ Resolving Power (R') |
| | Size<br>▸ Nearly all SP are um scale silica particles<br>▸ Impacts VD equations |

### Stationary Phase (cont)

| | |
|---|---|
| | Porous Particles<br>▸ ↑ Surface area per particle<br>▸ ↑Amount of SP inside column<br>▸ ↑ Retention and sample capacity = Better R'<br>▸ Smaller the pore→ the larger the surface area/g of support |
| | Diffusional Trap of small pores<br>▸ Loss of analyte → Tailing peaks<br>▸ Large MW analyte go into small pores → Never gets eluted |
| | Separation molecules<br>▸ Small molecules ~ 80Å<br>▸ Large proteins ~120-300Å |
| **Normal Phase (NP)** | Developed initially<br>▸ Used raw silica as SP → Polar silanol |
| | Separation based<br>▸ Polar-polar interactions with silanol<br>▸ Non-polar elute earlier<br>▸ Polar analyte elute later |
| | In general<br>▸ MP is opposite polarity to SP<br>▸ Works well for polar species only |
| **Reverse Phase (RP)** | Use non-polar SP → Silane reaction<br>▸ Use polar MP (Water based) |
| | Separation based<br>▸ Nonpolar-Nonpolar interactions<br>▸ Reverse separation of normal phase<br>▸ Polar species elute first<br>▸ Non-polar species elute later |

### Stationary Phase (cont)

More popular
▸ Organic solvents used for MP → Expensive/dangerous
▸ Most analytes are made our of biological origin → Soluble in water-based MP

### Controlling Retention

| | |
|---|---|
| **SP Polarities** | Retention depends on<br>▸ Mass of SP<br>▸ Type of SP |
| | Mass<br>▸ Control by chain length<br>▸ Density of SP on silica→ % of silanol reacted<br>▸ Surface area →Porisity |
| | Selectivity depends on<br>▸ Type of SP<br>▸ Chain length<br>▸ Linker type/length |
| **Chain Length** | ↑Chain length and/or % organic (carbon) load of SP = ↑ Retention (K') |
| | Example<br>▸ C4 → C8 chain<br>▸ Double chain length → Double volume of SP → Double retention |
| | If within the same type of SP<br>▸ Ex: Alkyl chains<br>▸ No significant changes in selectivity<br>▸ Only shrinking/expanding the chromatogram about $t_m$<br>▸ Shrink c-gram = reduce carbon<br>▸ Stretch c-gram = Increase carbon load |

## Controlling Retention (cont)

| | |
|---|---|
| | If analyte is not retained<br>▸ Increase % carbon of SP/chain length |
| | If analyte is excessively retained<br>▸ Reduce % carbon |
| | Significant changes in selectivity ➔ Resolving power<br>▸ By changing the type of SP<br>▸ Overall retention should remain roughly constant<br>▸ Keeping the % carbon constant |
| **Effect of MP Strength** | MP plays an active role in retention<br>▸ Distribution constant |
| | Common solvents can be sorted according to their polarity |
| | Polarity of MP<br>▸ Main factors of controlling K ➔ K' |
| | When changing MP strenght<br>▸ Can calculate the retention under new MP |
| | Equation (Only for RP):<br>▸ $K'_{new}/K'_{old} = 10((P'_{new}-P'_{old})/2)$ |
| | Equation (Polarity) $P'_{MP}$= Weighted Polarity = $\%_A * PI_A + \%_B * PI_B$<br>▸ PI= Polarity |
| | Equation (Only for NP):<br>▸ $K'_{old}/K'_{new} = 10((P'_{old}-P'_{new})/2)$ |
| **Example** | 1. Look at K' of first and last peak<br>▸ $K'_{old}$ = (last peak - first peak)/first peak ➔ K' = (2.8-1.8)/1.8 = 0.5<br>▸ $K'_{new}$ ➔ Want it at 10 |
| | 2. Replace terms in equation<br>▸ $K'_{new}/K'_{old}$ = 10/0.5 = 20 |

## Controlling Retention (cont)

| | |
|---|---|
| | 3. Old MP polarity<br>▸ If old MP is 20% water/80% Acetonitrile<br>▸ $P'_{old}$= (0.2)(10.2)+(0.8)(5.8) = 6.68 |
| | 4. New MP polarity<br>▸ $P'_{new} = 2\log(K'_{new}/K'_{old}) + P'_{old}$<br>▸ $P'_{new}$= 2log(20) +6.68 = 9.28 |
| | 5. Solve new MP components<br>▸ $P'_{new}$ = (x)(10.2) + (1-x)(5.8) = 9.98<br>▸ 79.1% water and 20.9% ACN |
| | Rule of 3<br>▸ Tool to check/estimate results (not used in calculations)<br>▸ Change of 20% water ~ 3x change in K' |
| **MP Gradient** | Dynamically adjust MP |
| | Some sample contain wide range of analytes<br>▸ Low or high retention<br>▸ no single MP that will elute them all in a satisfactory range of k’ |
| | MP gradient<br>▸ MP strength is initially "weak"➔Analyte well retained<br>▸ Those with low retention➔Elute at reasonable K'<br>▸ Strengthen MP over the course of separation<br>▸ Strongly retained species can be eluted➔At a reasonable K" and R' |
| | MP≠constant ➔ Changing strength<br>▸ K and K' ≠ constant<br>▸ Can no longer be predicted |

## Controlling Retention (cont)

| | |
|---|---|
| **MP Selectivity** | Alter selectivity (α) by changing type of solvent |
| | Resolving power<br>▸ $R = (\alpha-1)(K'/(1+K'))(\sqrt{N}/4)$<br>▸ Sensitive to selectivity → Critical pairs in peaks |
| | Selectivity<br>▸ Depends on nature of MP<br>▸ Change selectivity = change type of MP |
| | Try and keep<br>▸ $P'_{old} \sim P'_{new}$<br>▸ Overall retention is roughly the same<br>▸ Selectivity of peaks change |
| | Selectivity changes cannot be predicted |

## Chiral Separation

| | |
|---|---|
| **Basic Theory** | Important to bioanalyses and pharmaceutical separation |
| | Separation of chiral species<br>▸ Enantio selective |
| | Chiral SP or chiral additives to MP → Separation of enantiomers |
| | Possible to separate structural isomers → Strength of interaction changes as a function of isomer |

## Ion-Exchange

| | |
|---|---|
| **Ionic Species** | Small "hard" ions<br>▸ Inorganic ions<br>▸ Cannot use ion pairing<br>▸ Ions can interact with appropriate SP → Ionic SP |

## Ion-Exchange (cont)

| | |
|---|---|
| **Basic Theory** | Ion-exchange<br>▸ Equilibrium-based separation<br>▸ Discreet soption and displacement process<br>▸ Carry throughout column |
| | Column<br>▸ Does not use silica particles<br>▸ Use polymer resin<br>▸ Attach with a strong anion or cation |
| **Example** | Using a strong anion SP<br>▸ Cation exchange column |
| | 1. SP sulfonic acid (IEX resin) → Anion surface particle ($SO_3^-$)<br>▸ Wash column with acid solution {{fa-arrow-right}} Cations ($H^+$)<br>▸ Sulfonic acid is protonated ($SO_3H$) |
| | 2. Inject sample with cation analytes → Metal ion ($M^{2+}$)<br>▸ Metal ions interact with SP<br>▸ Metal ions displace some of $H^+$ from resin |
| | 3. Unbind analyte from SP<br>▸ Introduce a higher concentration of protons behind analytes → MP gradient<br>▸<br>▸ $H^+$ displace weakly bound analytes the move onto strongly bound analyte (cation) → Exchange process<br>▸ Cation analytes is displace off of surface and solubilize in MP |

# Liquid Chromatography Cheat Sheet

by shaylannxd via cheatography.com/149855/cs/32584/

## Ion-Exchange (cont)

| | |
|---|---|
| | 4. Analytes move down the column in strength of MP<br>▸ Each type of analyte elute as a peak<br>▸ MP ahead of each analyte is too weak → Bound<br>▸ MP behind each analyte is too strong → Fully displace |
| **Equilibrium Constant** | If it behaves like an equilibrium → There is an equilibrium constant<br>▸ Expect to behave like an LC → Produce peaks |
| | Ion-exchange equilibrium constant would behave like a distribution constant<br>▸ Obtain similar result of chromatogram peaks<br>▸ MP controls retention |
| | Equation<br>▸ $K_{iex}=[exchange\&analyte]_{s}/[analyte]_{m} = C_{s}/C_{m}$ |

## Optimization

| | |
|---|---|
| **Process of Separation** | 1. Carry out initial separation<br>▸ Choose a strong MP<br>▸ Ensure everything is eluted and fast separation |
| | 2. Adjust MP strength<br>▸ Retention of last peak is within the right region<br>▸ Depending on the complexity of sample<br>▸ Simple sample → K' ~ 10<br>▸ Complex sample → K'~ 20<br>▸ Do calculations for an estimate adjusting needed |

## Optimization (cont)

| | |
|---|---|
| | 3. Examine if peaks are within the acceptable region<br>▸ Examine if all analytes are well resolved |
| | 4. Consider if a gradient is required<br>▸ Presence of large area of empty baseline |
| | 5. If needed<br>▸ Switch MP type to alter selectivity<br>▸ Gradient to reach acceptable retention and resolution |
| | 6. Consider using additives in MP<br>▸ Help alter selectivity |
| | If MP type/mix strength does not achieve required separation<br>▸ Change SP type<br>▸ May consider type of separation |
| **Summary of MP Effects** | Very powerful tool → Versatile<br>▸ Control retention and selectivity |
| | Directly affects distribution constant |
| | ↑ MP strength = ↓ K' |
| | MP "strength" is polarity<br>▸ Effect are opposite in RP vs NP<br>▸ RP → Non-polar solvent (organic) = Stronger solvent<br>▸ NP → Polar solvent = Stronger solvent |
| | Ramped MP → Gradient<br>▸ Helps dynamically adjust K' |
| | Useful to make separation less intuitive → R↓ = K' ↓<br>▸ Gradient runs → R ↑ = K' ↓ |

## LC Detectors

| | |
|---|---|
| **Ideal Detectors** | High sensitivity<br>▸ Steep slope |
| | High stability<br>▸ Minimal drift<br>▸ Minimal noise on baseline |
| | Very low DL |
| | Long LDR |
| | Can accept MP over wide range<br>▸ Need reference to null out MP gradients |
| | Fast response<br>▸ Independent of MP |
| | Easy to use, maintain and repair |
| | Inexpensive |
| | Selective/universal<br>▸ Can be either depending on properties |
| | Non destructive<br>▸ Can collect fractions |
| **1λ: UV- Vis Detector** | Volume<br>▸ ~1-10 uL (very small)<br>➔If V is too large, the signal becomes constant and we see a square shaped peak |
| | Pathlengths<br>▸ ~5-10mm<br>▸ Longer = better<br>Bigger absorbance for same concentration (beer-lambert law) |
| | Window material<br>▸ Quartz |

## LC Detectors (cont)

| | |
|---|---|
| | $D_2$ lamps<br>▸ Good broad UV source<br>▸ 185-400nm<br>▸ Spectrometer to isolate narrow band of wavelength<br>▸ More simple than FAA<br>➔UV does not have to compensate for a flame |
| | 2 sensors<br>▸ Sample diode (I)<br>➔Intensity coming through the sample<br>▸ Reference diode ($I_o$)<br>➔ Intensity from the light source<br>▸ Equation<br>➔$A = -\log(I/I_o) = -\log(T)$ |
| | Chromatogram<br>▸ Abs vs time<br>▸ Use peak area for quantitation |
| **Many λ: Photodiode array (PDA) Detector** | Chromatogram<br>▸ Collect many chromatograms across many wavelength (a spectrum) |
| | Sensitivity<br>▸ Can choose/use chromatogram that provides the greatest sensitivity for each analyte<br>▸ Find wavelength where analyte has least interference from neighbouring peaks |

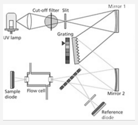
## LC Detectors (cont)

| | |
|---|---|
| | Application<br>▸ Useful to verify which peaks is which when MP is changed |
| **Refractive Index (RI) Detector** | Uses refractive index of analyte compared to MP<br>▸ Snell’s law<br>➔The rays will bend if there is a mismatch in refractive indices of the outside and the inside<br>▸ When refractive indices match<br>➔Rays not refracted |
| | Chromatogram<br>▸ If RI match (only MP)<br>➔Full intensity reaches sensor<br>▸ If RI does not match (analyte eluting)<br>➔Reduced intensity reaches sensor<br>▸ Plot signal vs time |
| | Properties<br>▸ Universal<br>▸ Sensitivity<br>➔~3 orders of magnitude less sensitive than UV<br>▸ Absorbance<br>➔Optically silent<br>▸ Reference flow<br>➔Limited gradient capability |

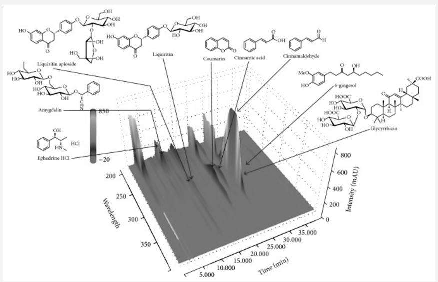
## LC Detectors (cont)

| | |
|---|---|
| **Evaporative Light Scatter (ELS) Detector** | How it works<br>▸ Uses nebulizer to produce aerosol<br>▸ MP evaporates<br>➔Leaves behind analyte fine crystals<br>▸ Scattering of light (usually laser)<br>➔Only when crystals are present |
| | Analyte<br>▸ Needs to produce crystals<br>➔ Very low volatility<br>▸ Can work for non-absorbing analytes<br>▸ Response is nearly uniform for all analytes |
| | Buffers (MP)<br>▸ Must be volatile<br>➔Restricts choices<br>➔Can’t use inorganic buffers: Leads to buffer salts |
| | Better than RI detector<br>▸ Higher sensitivity<br>▸ Longer LDR |

## UV-Vis Detector Diagram



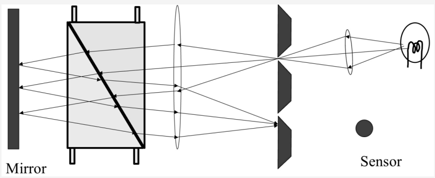
## Photodiode Array Chromatogram



# Liquid Chromatography Cheat Sheet

by shaylannxd via cheatography.com/149855/cs/32584/

## Refractive Index Detector Diagram



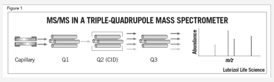
## LC-MS

| | |
|---|---|
| **Properties** | Electrospray<br>▸ Sample goes through nebulizer<br>▸ High voltage is applied ➔ Produces charged droplets |
| | Fine metal capillary tube<br>▸ ~0.5-1mm<br>▸ Connected to the outlet<br>▸ Charged with high voltage |
| | Signal<br>▸ MP is pumped<br>▸ Charged droplets are attracted to MS interface<br>▸ Droplets dry down in flight<br>➔ charge density ⬆ until charge repulsion causes coulombic explosion |
| **Single Quadrupole MS** | Mass spectrum<br>▸ Simple<br>▸ MP evaporates away<br>➔ Leaves $[M+H]^+$ ions ➔ no fragments |
| | Problem<br>▸ Difficult for definitive ID<br>▸ Potential m/z overlap |
| **Triple Quadrupole MS** | Allows the production of fragments<br>▸ Contains Q1, Q2(CID) and Q3 |
| | Q1 ➔ Parent ions are selected<br>Q2(CID) ➔ Collision induced dissociation ➔ Selected ions collide with Ar/He/N2 (Creates fragments)<br>Q3 ➔ Fragment ions are filtered/scanned ➔ Detected to produce mass spectrum |

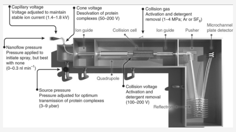
## LC-MS (cont)

| | |
|---|---|
| | Mass spectrum<br>▸ Scanning mode ➔ Produce full spectrum ➔ For method development<br>▸ Multiple reaction monitoring (MRM) ➔ Only selected fragments are measured ➔ For quantitation |
| | Better than single quadrupole<br>▸ Lower DL<br>➔ Less interferences<br>▸ Longer LDR<br>▸ Allows positive ID of analyte<br>▸ Better selectivity with MRM |
| | Problem<br>▸ Q3 scans across m/z range pretty slowly (1-30 spectra/s)<br>▸ Lowering resolution allows faster scanning ➔ Can't get a full detailed spectrum |
| **QTOF MS** | Quadrupole time of flight MS |
| | Advantage<br>▸ Can scan 10000 spectra/s<br>➔ Many are averaged together to improve quality (better than QQQ)<br>▸ Allows more analytes to be measured simultaneously<br>▸ Higher mass accuracies and resolution<br>➔ Permits greater ID power<br>▸ LDR>5 orders of magnitude |
| | Problem<br>▸ Not as precise as QQQ<br>▸ Expensive |

## Triple Quadrupole Detector Diagram



## QTOF MS Detector Diagram



## Summary and Applications

| | |
|---|---|
| **Advantage** | MP plays a critical role in controlling separation<br>▸ Retention ➔"Strength"<br>▸ Selectivity ➔"Type" |
| | Wide range of MP available<br>▸ Diverse set of separation conditions<br>▸ Within the same SP and column<br>▸ Allow to quickly try different separation conditions<br>▸ Allow to quickly arrive to a newer optimization separation |
| | No requirements of volatile analyte<br>▸ Needs to be soluble in MP |
| | Wider range of SP available<br>▸ Can choose type<br>▸ Can change particle size<br>▸ Can choose the amount of SP/unit of column |
| | Easy to collect purified analyte |
| **Disadvantage** | Much lower N compared to GC-FSOT<br>▸ Degrades R and ↑ Overlapping peaks<br>▸ Many LC have low N ➔ 1000-5000 |
| **Detector Comparison** | Selective or universal |
| | DL |
| | LDR |

## Summary and Applications (cont)

| | |
|---|---|
| | Cost<br>▸ Purchase<br>▸ Maintenance |
| | Sample capacity |
| | Immune from MP gradients? |
| | Amendable to using IS? |
| **Key Factors if LC is useful** | 1. Analytes soluble in liquid MP |
| | 2. Concentration of analytes are high enough<br>▸ Can load larger volumes/concentraton on columns<br>▸ Combine with sensitive detectors |
| | 3. Does sample require a high R' separation<br>▸ GC favored over LC |
| | 4. Need to recover analyte<br>▸ LC > GC |
| | 5. Slower than GC |
| **Applications** | Anti-dopping and forensics |
| | Pharmaceutical<br>▸ Process control<br>▸ Quality control<br>▸ R&D<br>▸ Metabolic<br>▸ Proteomic |
| | Food and Beverages<br>▸ Vitamins<br>▸ Pesticides<br>▸ Contaminants |
| | Environmental<br>▸ Pesticides<br>▸ Industrial materials |

## Summary and Applications (cont)

R&D

- Organic synthesis
- Catalysis

Industrial

- Feedstock