“Excuse me sir, how pure is that white powder ?”

By **Alex J. Roche**
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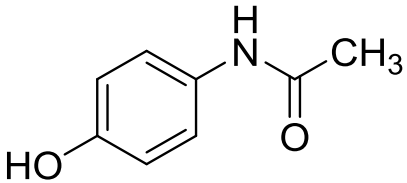
SPRING 2016

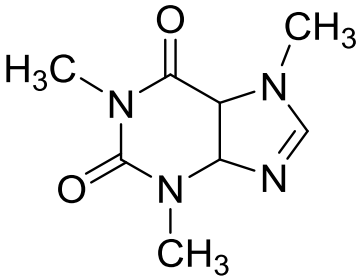
Separations

- water from sea water...?
- sand from sea water...?
- glass from sugar...?
- acetone from water...?
- gasoline and water...?

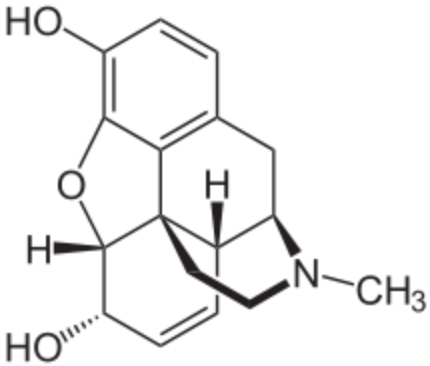
Separation

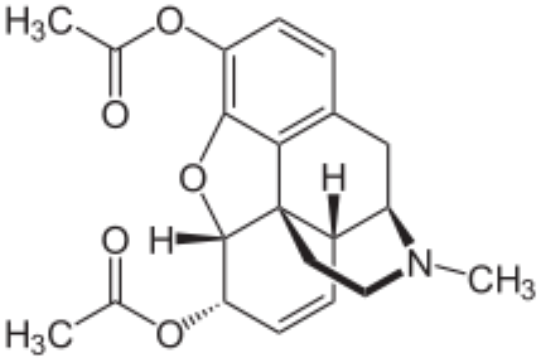
- paracetamol (acetaminophen) and caffeine...?





- morphine and heroin...?





Separation

- Boiling point *(factors: molecular interactions)*
- solubility / miscibility *(factors: **inter**molecular interactions)*
- chromatography *(factors: polar/non-polar; lipophilicity, "organic-ness")*
  Bio/Biochem: Size; Charge, etc.)

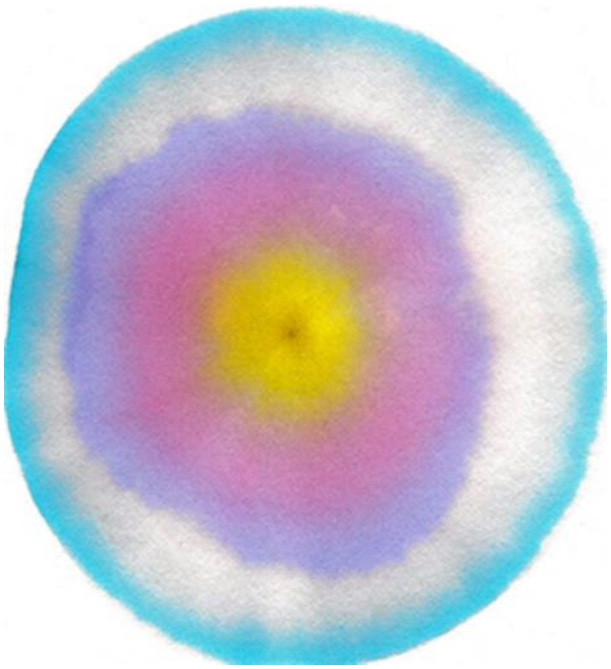
- Mixture (Analytes)
- *Stationary* phase
- *Mobile* phase
- **Partitioning** based on *affinity*

$\Rightarrow$ Different things carried along at different speeds.

(Radial) Chromatography

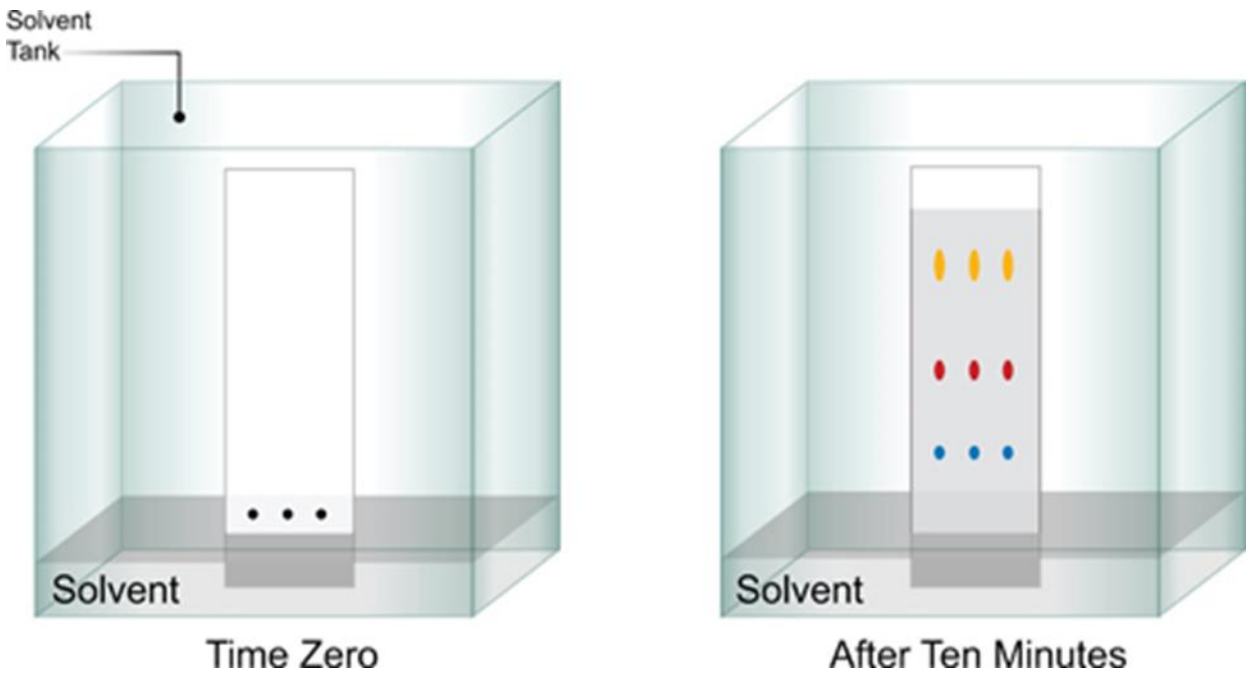
- Classic “black ink dot on filter paper” experiment.

$\Rightarrow$



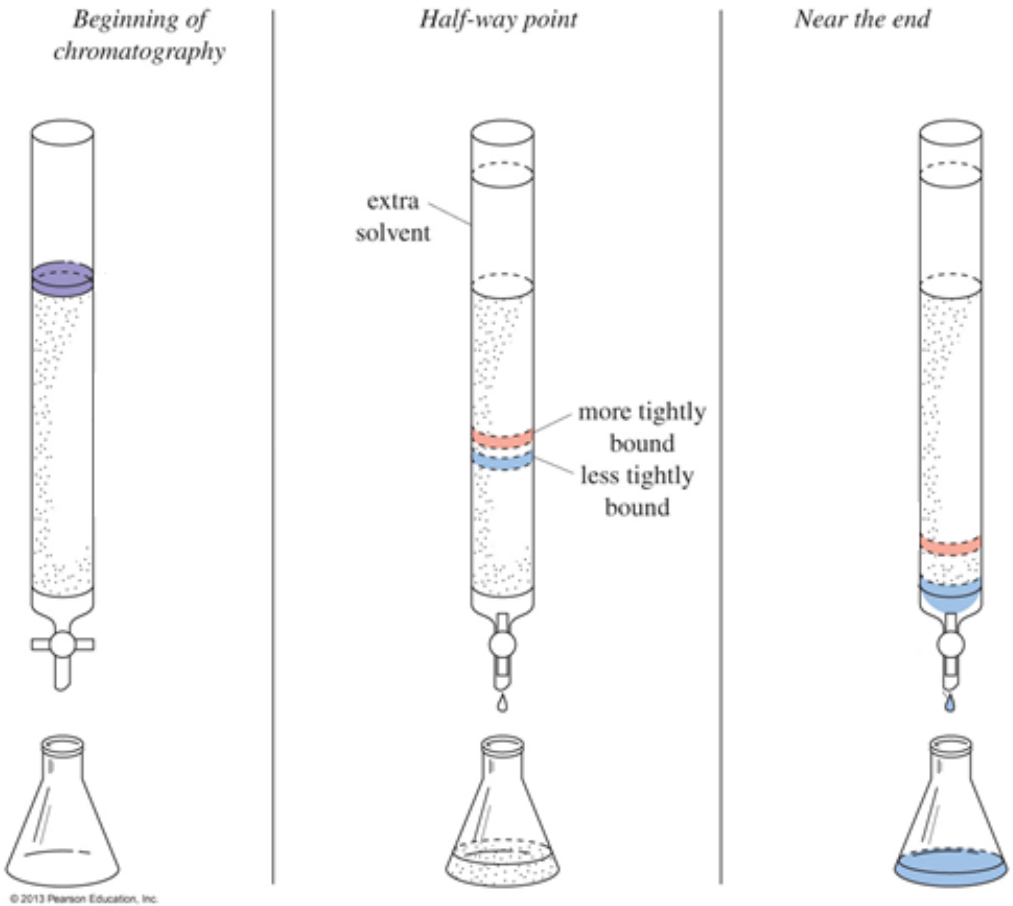
(Thin Layer) Chromatography

- Solvent front progresses up the plate.



(Column) Chromatography

- Solvent front progresses down the column (packed with stationary phase material).



Columns

- These come in different sizes, for different **purposes**, and different amounts of compounds.

- "**Analytical**"

- HPLC-MS Column

20mm in length, 1mm ID

- GC-MS Column

30m in length, 0.25mm ID

Such columns can separate

Picograms pg = 10^{-12}g

Femtograms fg = 10^{-15}g

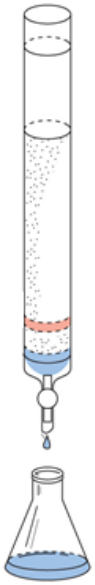
Columns

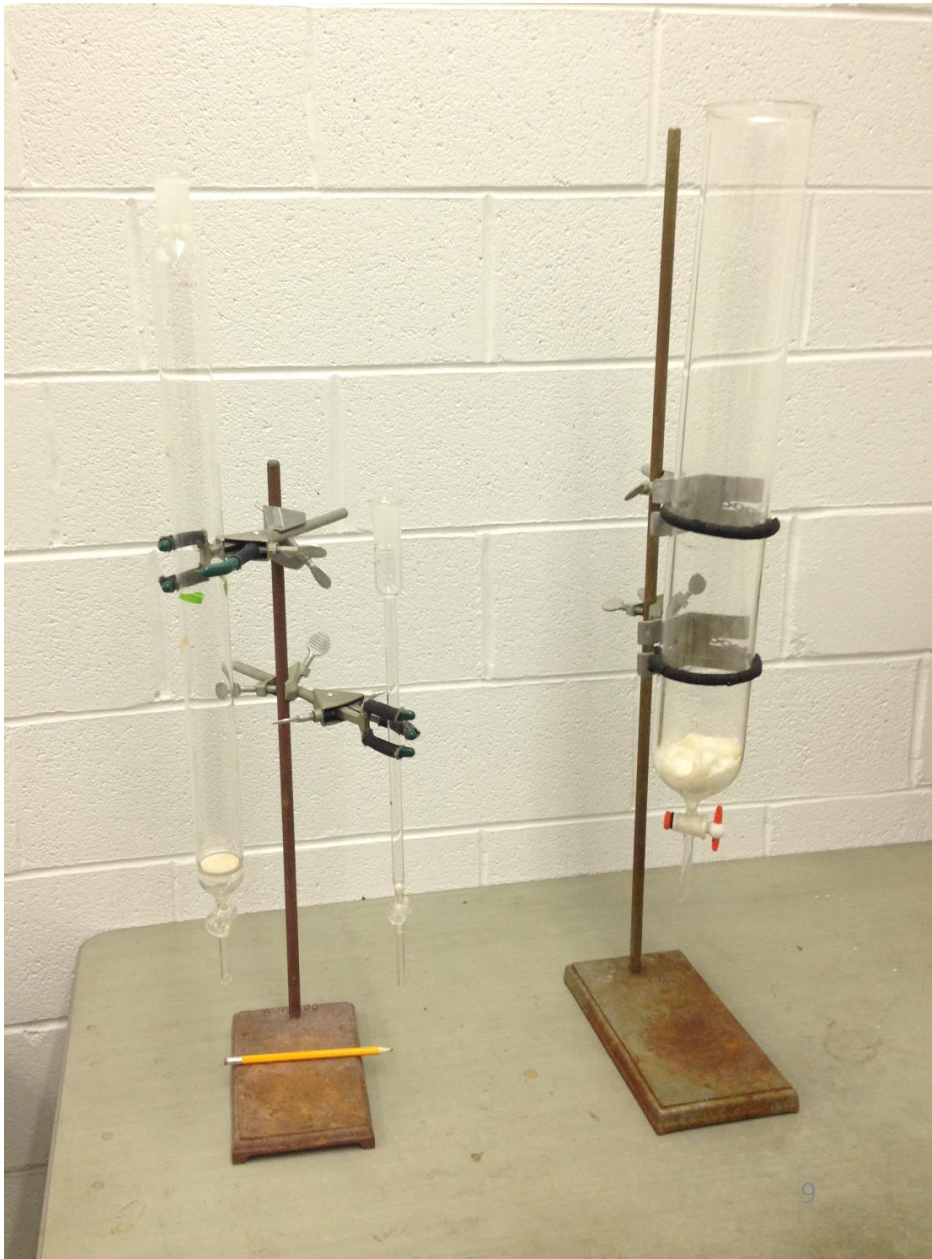
- These come in different sizes, for different **purposes**, and different amounts of compounds.

- "**Preparative**"

Glass Columns to be half filled with Silica gel.

- Maybe purify 1g; < 0.5g and 5g of product.





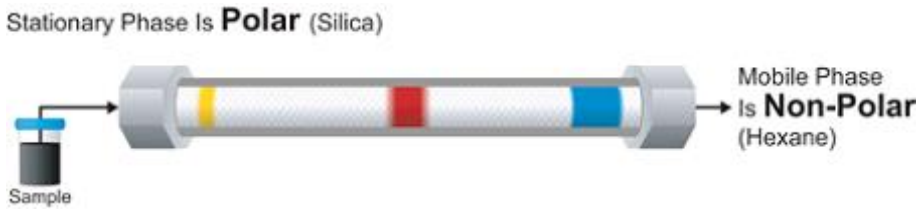
Order of Elution ?

- The order of elution is controlled by the **affinity** of your analytes for the mobile phase *versus* the stationary phase.

- *Polar likes polar.*
- *Non-polar likes non-polar.*

- Always set up a combination of **polar** versus **non-polar**.

- Here stationary phase is polar; mobile phase is non-polar. (***Normal phase*** Chromatography)



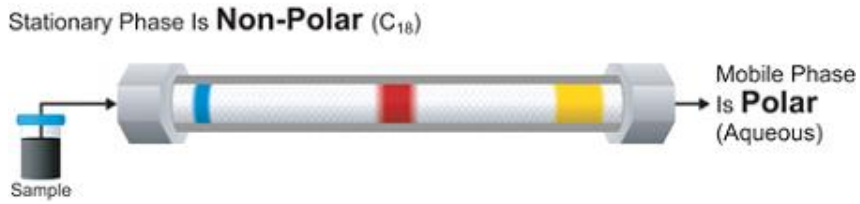
- Yellow is **most** polar compound, sticks to polar column the most (moves the **slowest**).

- Blue is the **least** polar compound, sticks to the column the least (moves the **fastest**; 1st compound collected from column).

Order of Elution ?

- Always set up a combination of **polar** versus **non-polar**.

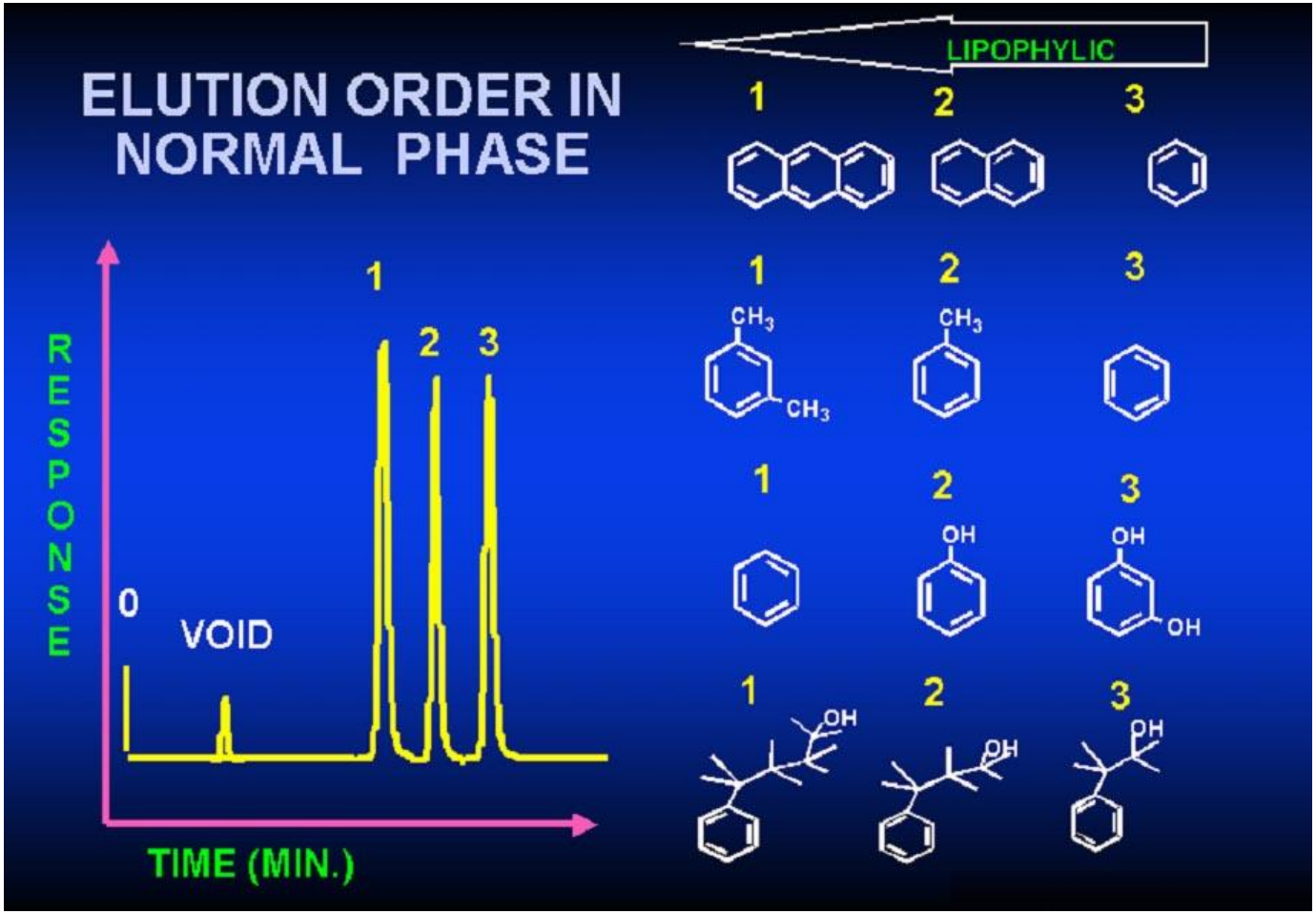
- Here stationary phase is non-polar; mobile phase is polar. (***Reversed phase*** Chromatography)



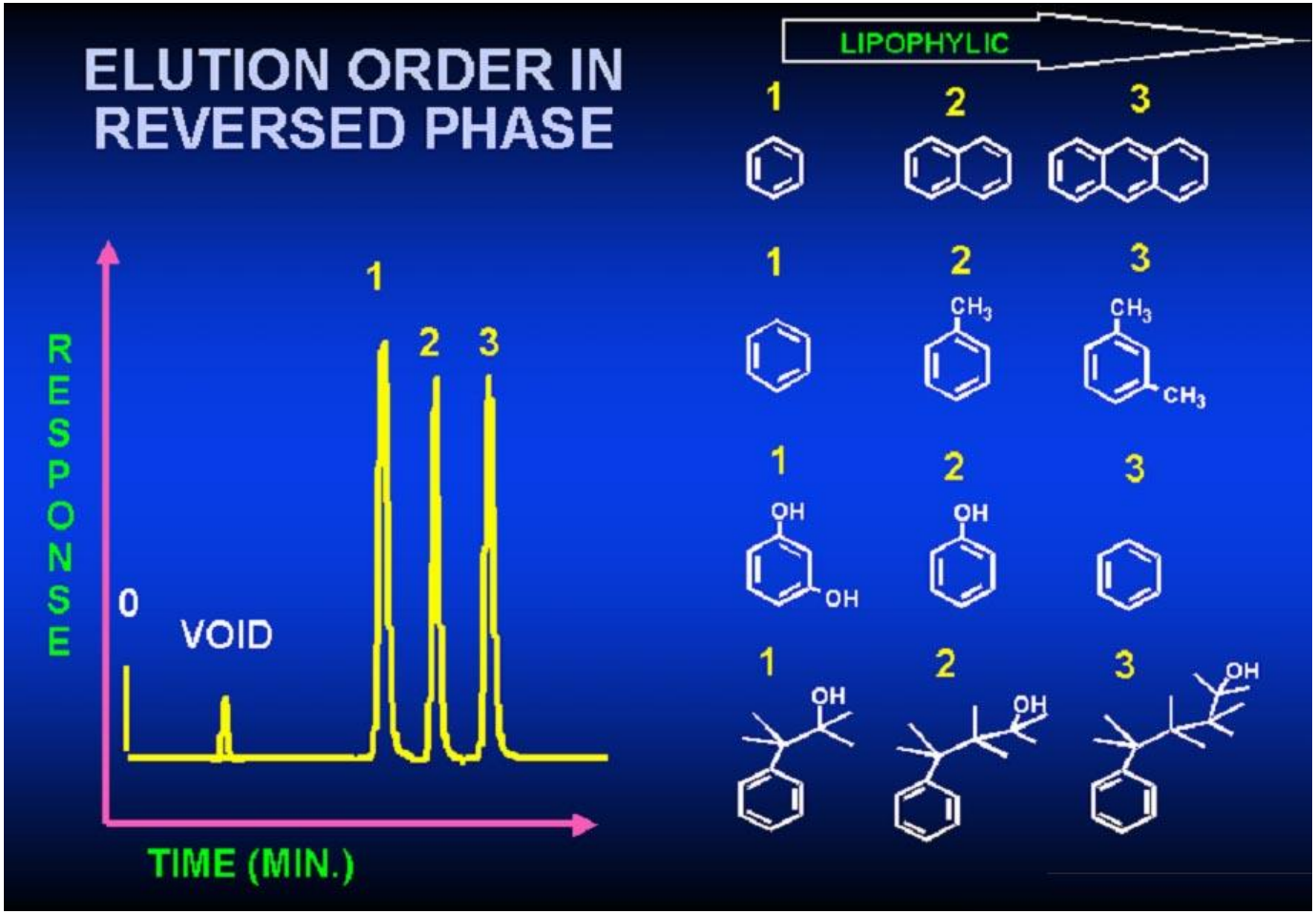
- Yellow is **most** polar compound, sticks to non-polar column the least
  (moves the **fastest**).

- Blue is the **least** polar compound, sticks to the non-polar column the most
  (moves the **slowest**; last compound collected from column).

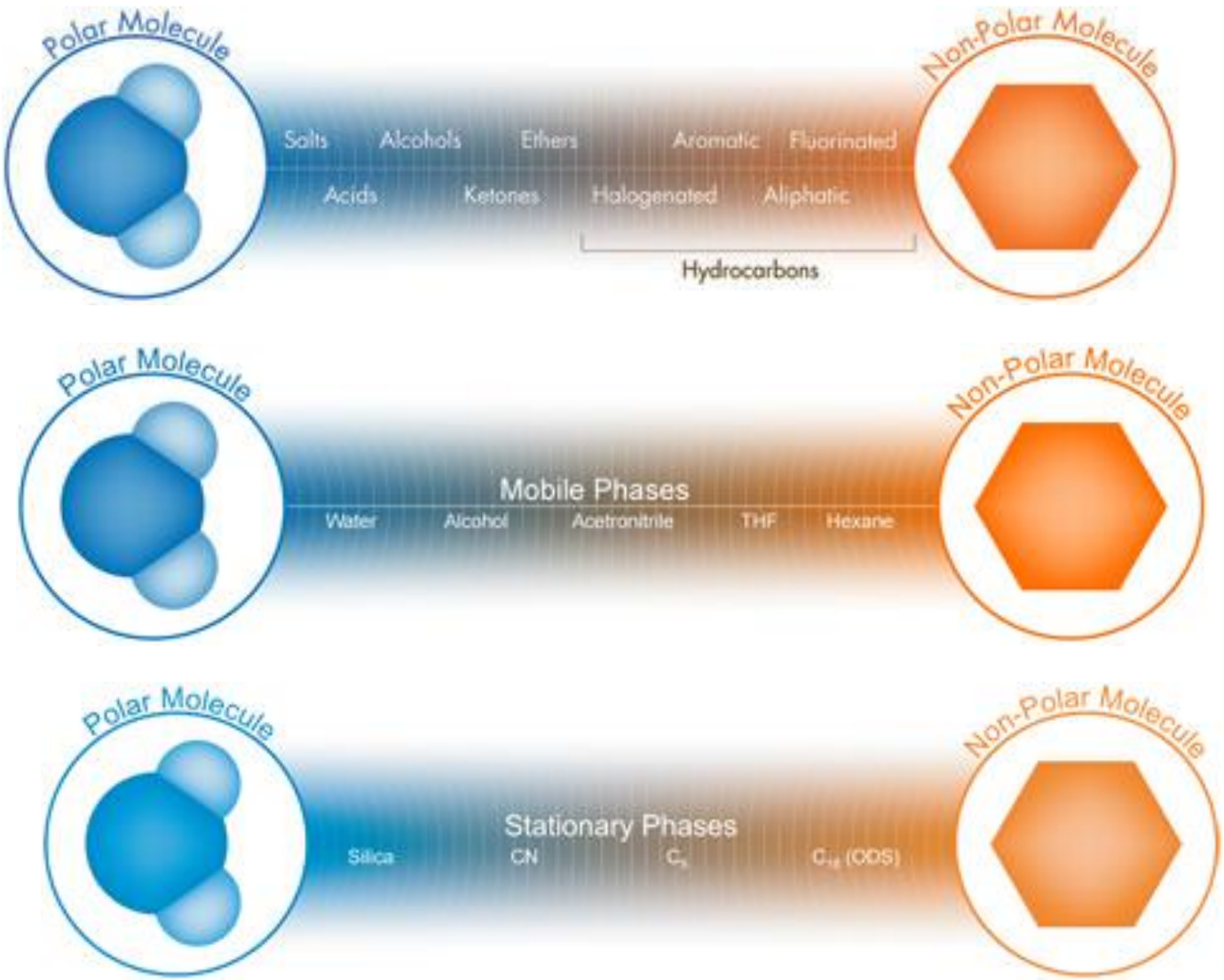
Normal phase order



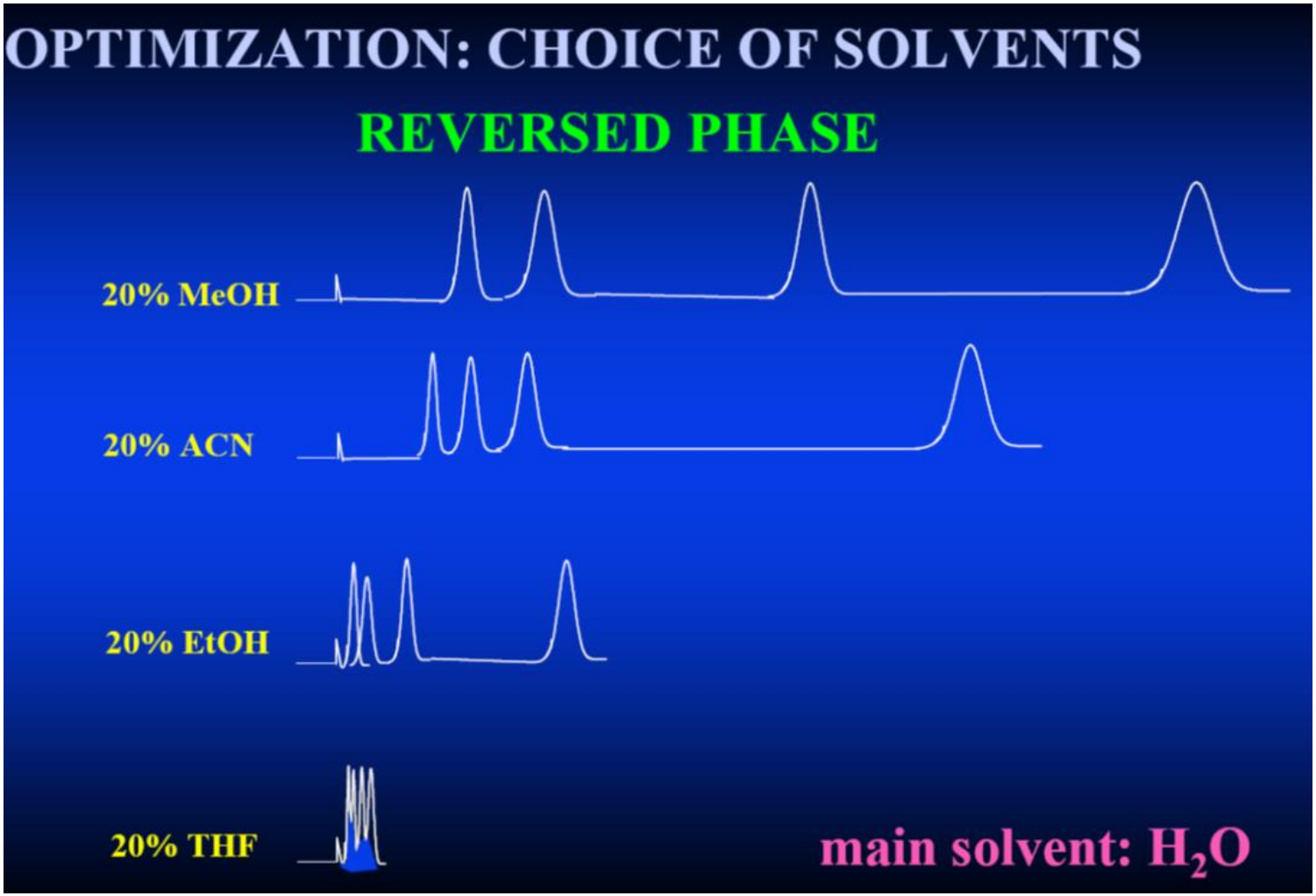
Reversed phase order



Analyte/Mobile/Stationary

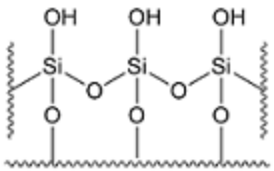


Find Conditions that “work”

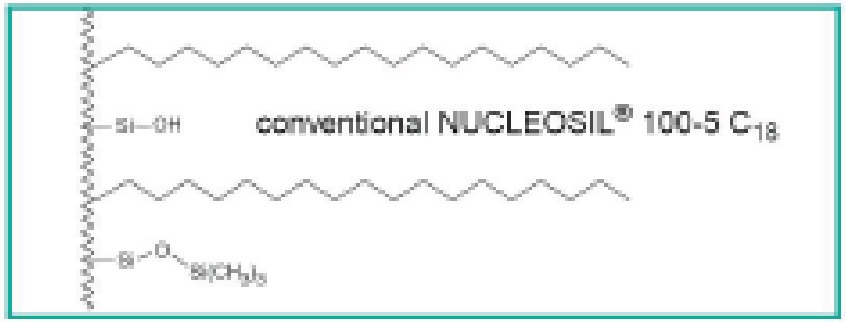


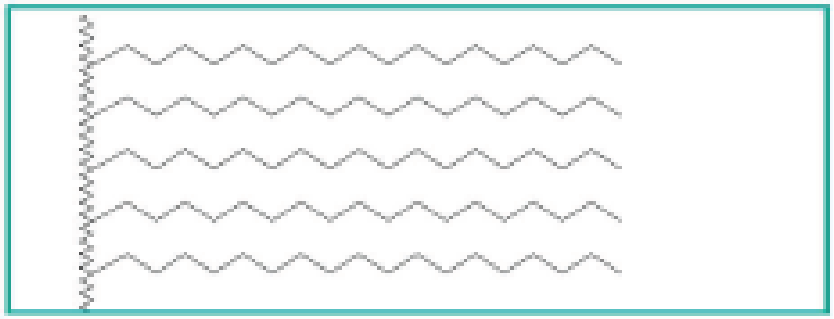
Column Materials

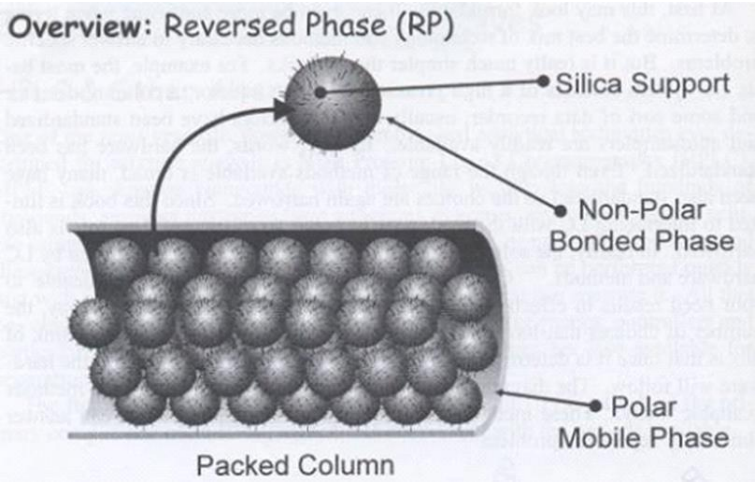
- **Polar**: Silica gel, "SiO_2"



- **Non-polar**: Surface coated with various dangling Alkyl groups

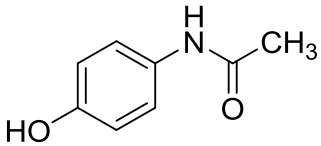


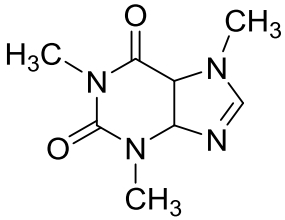


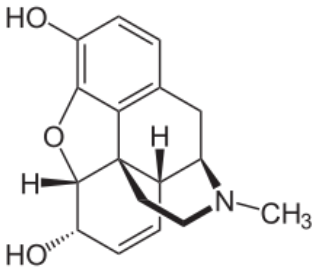


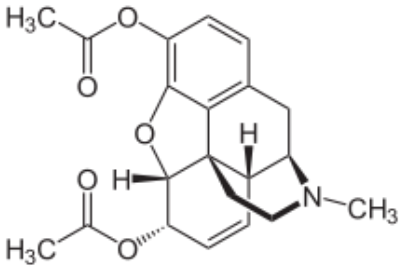
Recall this Separation…?

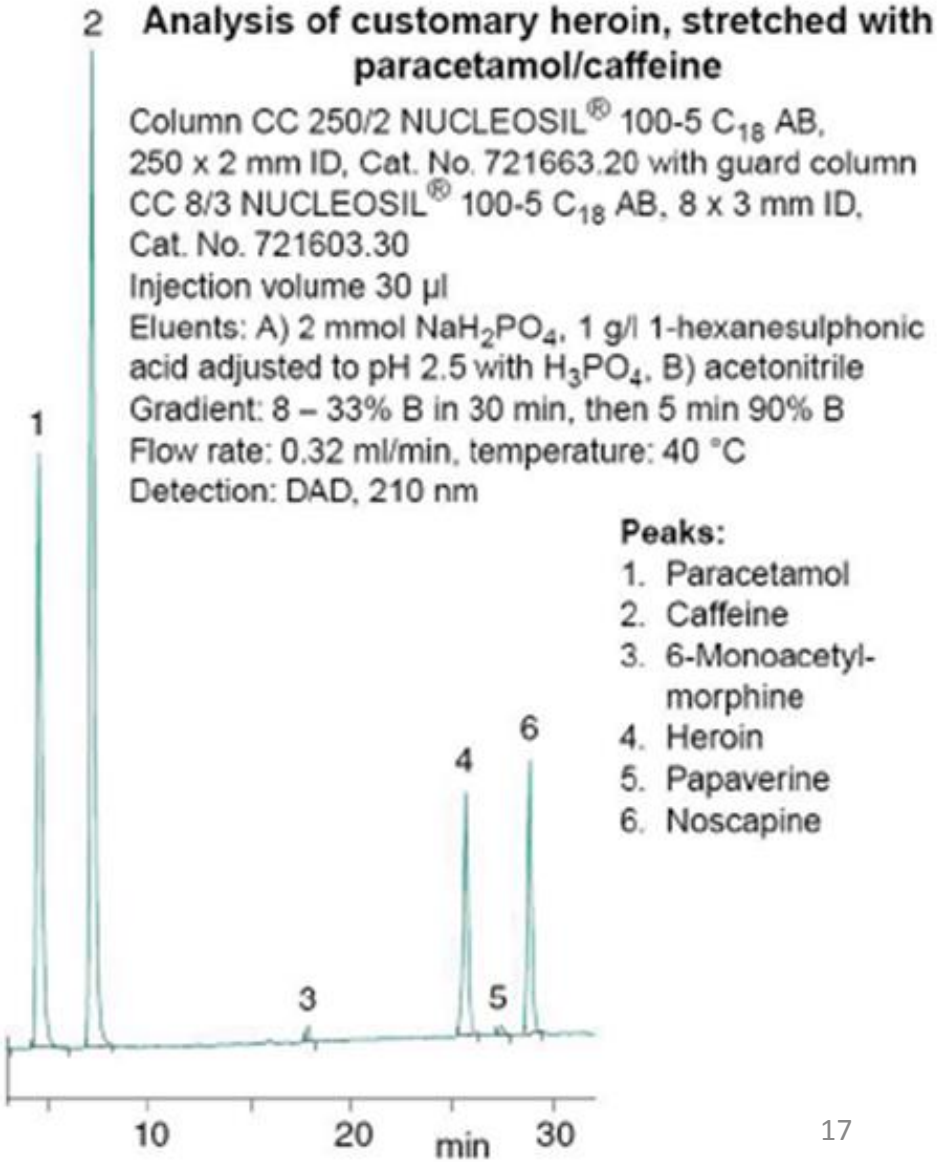
- Paracetamol/caffeine/morphine/heroin











Summary

- Chromatography is the main technique for ***separating*** complex (similar) mixtures.

- GC and HPLC are the most common.
  (Probably the most common lab skill used in “industry”).

- This is *modern* standard Chemistry.

“Excuse me sir, how pure is that white powder ?”

(part II)

By **Alex J. Roche**
Department of CHEMISTRY
QSTEP

SPRING 2016

Characterization

- After separation comes *identification.*
- We are back to "*What is that white powder ?*"
- It depends if the compound is currently Known or Unknown.
- Known: Academic = NMR and **MS**
- Known: Law (Drug testing) = **MS** - really HPLC/GC (**MS**)^2
- Unknown: NMR, **MS** and **HRMS**

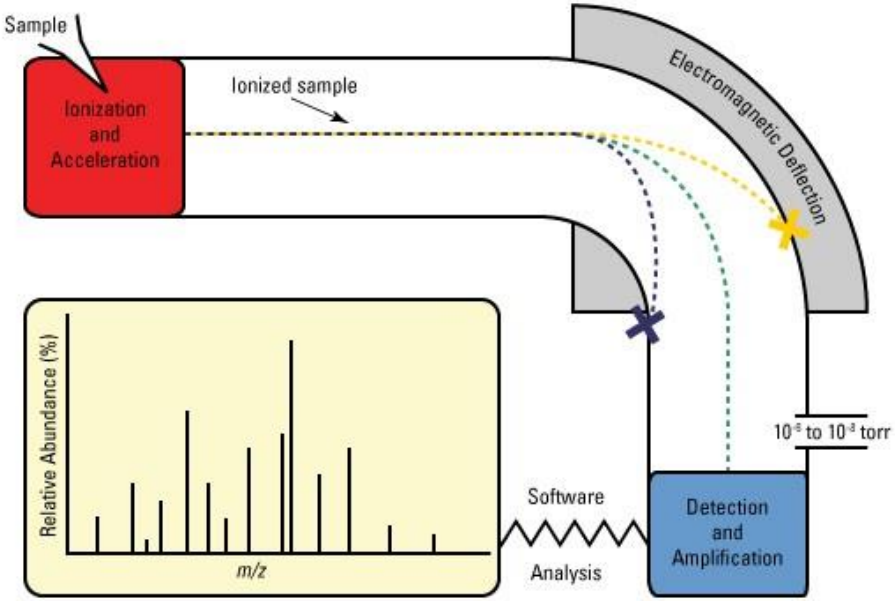
(NMR = Nuclear Magnetic Resonance; **MS** = **Mass Spectrometry**)

Mass Spectrometry

- General principle: moving *ions* generate a **magnetic field**.
- These moving **ions** can be deflected by another magnetic field
- The amount of *deflection* varies according to **m/z ratio**

  (where **m** = mass, **z** = charge)

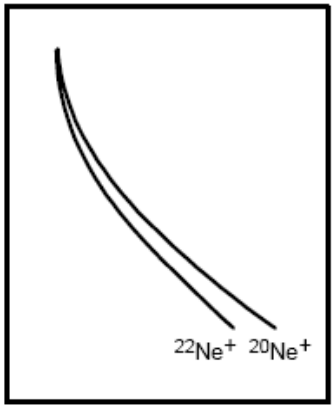
⇒ Information about **Molecular Weight**



Mass Spectrometry (trivia)

- 1803 Dalton "*Mass consists of atoms...*"
- 1911 Thompson 1st mass spectrometer
- 1940 Berry Electron Impact Ionization for organics
- 1980 ESI, FAB, MALDI
- Up to current day, MS fundamental characterization tool.
- 2002 Nobel Prize MALDI / ESI (Tanaka / Fenn)
- (4th MS area Nobel Prize)

Lighter ions are deflected more.



- Mass is measured in a.m.u. = Daltons, Da.
  (^{12}C =12amu = 12Da)

- m/z is measured in Thompsons

MS Detectors

- Quadrupole (LOWEST COST)
  up to 4000 amu, accuracy 0.1 or 0.2 amu, scan speed up to 5000 amu per sec.

- Ion Trap (LOW COST)
  similar to quad, although can increase resolution if focus on a reduced mass range.

- Triple Quad (MEDIUM COST)
  same as quad, but much more sensitive (less noise) and approaching quantitative.

- Time of Flight (HIGH COST)
  upto to 500,000 amu, accuracy can be 5 parts per million, HRMS or accurate mass.

- Fourier Transform – Ion Cyclotron Resonance MS (FTICR)
  (MOST and VERY EXPENSIVE)
  Unsurpassed mass resolving power & accuracy (1 - 20 ppm)

HPLC-MS and GC-MS

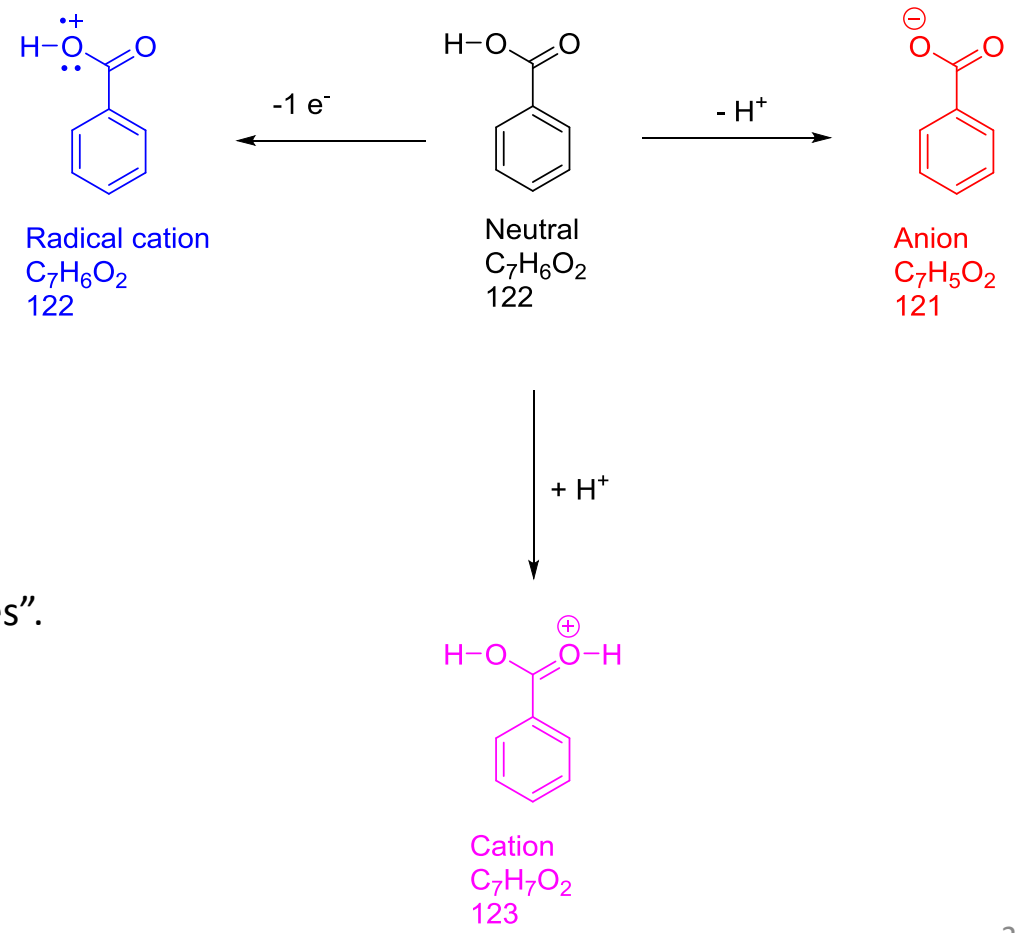
- The Chemistry department has new MS instruments in SCI-319.

Mass Spectrometry

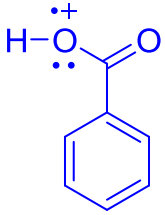
- **Ions** can be generated many different ways.

- Electron ionization
- Protonation
- Deprotonation

- **Many** other “ionization techniques”.



Mol. weight AND Structure

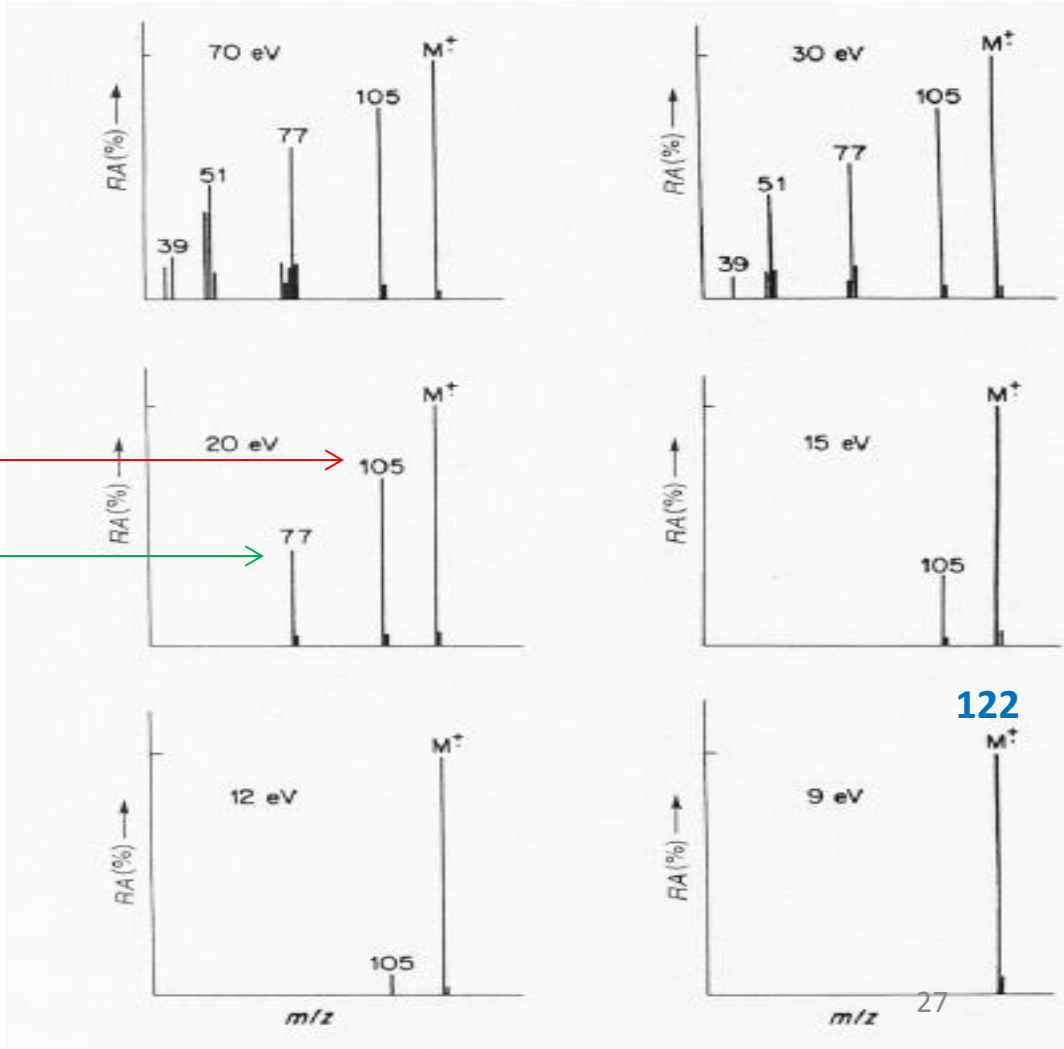


Radical cation
$C_7H_6O_2$
122

- Ionization can also lead to fragmentation inside the MS.
- This can provide **structural** information.
- $C_7H_6O_2$ = 122
- "OH" = 17
- "CO_2H" = 45

C = 12; O=16; H=1

(Higher Energy leads to more fragmentation)



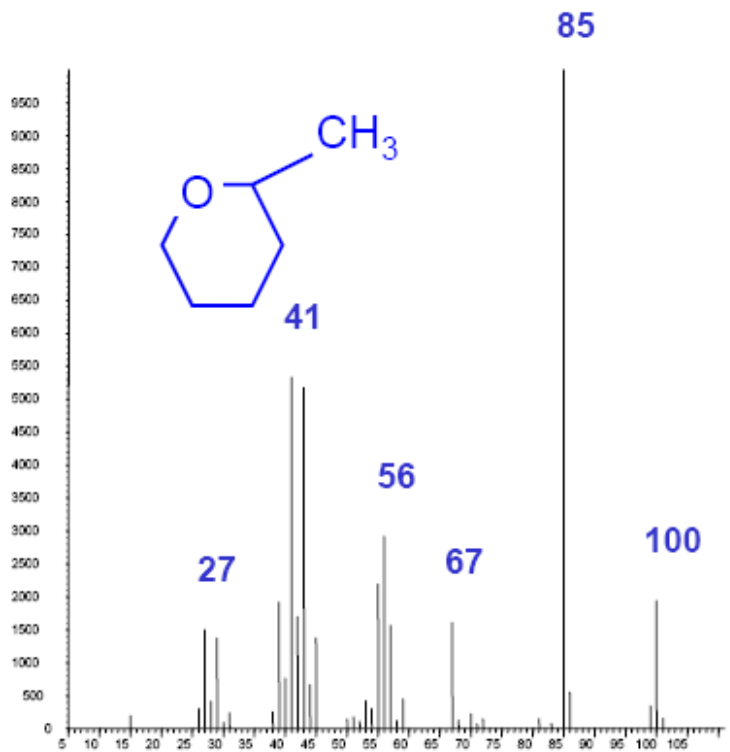
Isomer Differentiation

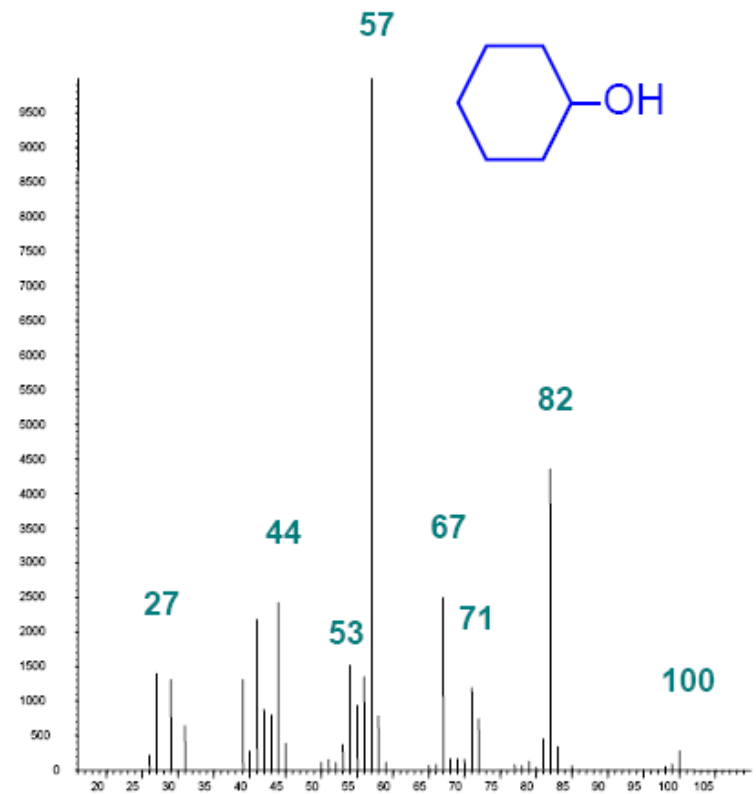
- Isomers have the same molecular formula, which means same molecular weight.
- Both these compounds are $C_6H_{12}O$ = 100.

- Isomers can be differentiated by their *fragmentation* (if you understand **Chemistry**).

- *"CH_3" = 15*

"H_2O" = 18





Different Ionizations

- **EI** gives M^+ but **CI** gives $[M+H]^+$ meaning M+1 peak.

- **$C_5H_9NO_2$ = 115**

- EI removes an electron
- CI adds a H^+

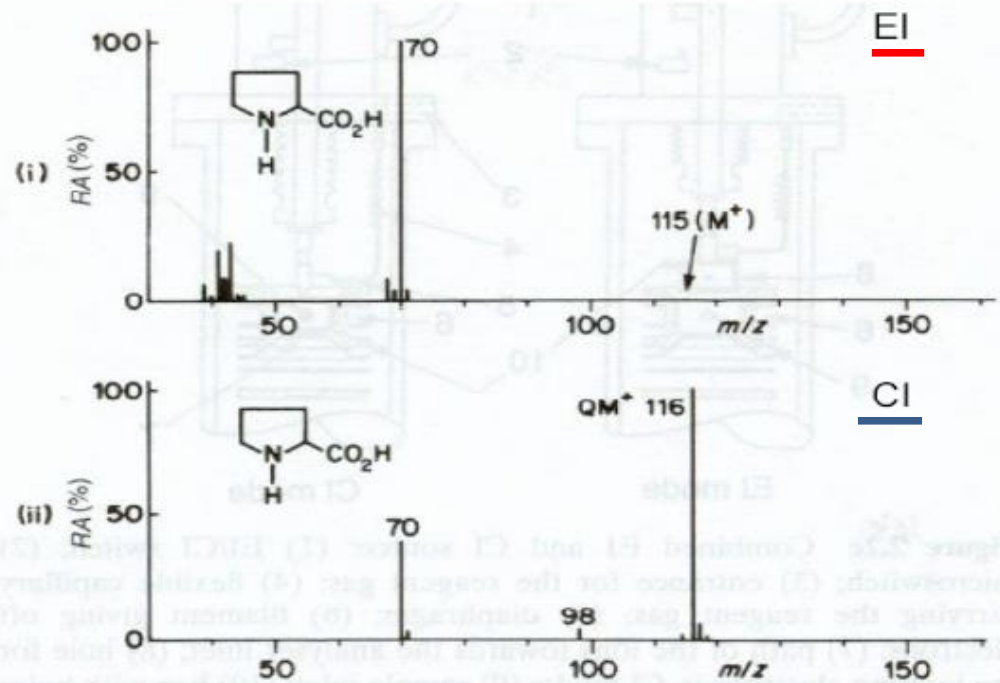


Figure 2.2b Mass spectra of proline obtained by using different ion sources: (i) electron impact; (ii) chemical ionisation

- Fragmentation can be *good* or *bad*.

- Complementary techniques covering all outcomes.

Masses

- When we say atomic mass and its sum to give molecular weight,
  it raises the questions of...

  ...decimal places ?
  ...isotopes ?

- ^{1}H = 1 or 1.0079 amu ?
- Does it matter ?

- What does the MS instrument actually detect ?

most abundant natural isotope

Element	Monoisotopic mass	Average mass
^{1}H	1.007825	1.0079
^{12}C	12.000000	12.0110
^{14}N	14.003074	14.0067
^{16}O	15.994915	15.9994
^{31}P	30.973763	30.9738
^{32}S	31.972073	32.0660
^{35}Cl	34.968853	35.4527

MS or HRMS ?

- Regular MS says **MW = 98**, which gives many possible formulae…

$C_3H_6N_4$	98.0594	
$C_4H_4NO_2$	98.0242	
$C_4H_6N_2O$	98.0480	
$C_4H_8N_3$	98.0719	
$C_5H_6O_2$	98.0368	← gives us the exact formula
C_5H_8NO	98.0606	
$C_5H_{10}N_2$	98.0845	
C_7H_{14}	98.1096	

- **HRMS** says MW = 98.0372 gives only one formula
- HRMS provides molecular formula
- (< 5ppm for publication!)
- HRMS machines are very expensive

Isotope Weights

- Which figures to use ?

- **Average** Cl is 35.45, but, average Cl's do not exist in real life!

Real Cl's are one of two isotopes, either
^{35}Cl =34.96885 (75.7%)
or
^{37}Cl =36.96590 (24.3%)

most abundant natural isotope

Element	Monoisotopic mass	Average mass
^{1}H	1.007825	1.0079
^{12}C	12.000000	12.0110
^{14}N	14.003074	14.0067
^{16}O	15.994915	15.9994
^{31}P	30.973763	30.9738
^{32}S	31.972073	32.0660
^{35}Cl	34.968853	35.4527

- Mass spectrometer measures mass of real species (Not of averages)

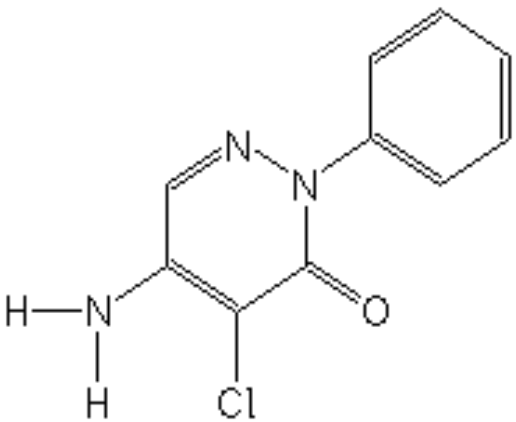
Molecular Weights

- MW of **$C_{10}H_8N_3OCl$** = **221.6463**
  (e.g. if you wanted one mole of cpd)

- Mass spectrometer measures mass of real species

- HRMS of **$C_{10}H_8N_3OCl$** = **221.0350**

  really the mass of:
  10 x ^{12}C
  8 x ^{1}H
  3 x ^{14}N
  1 x ^{16}O
  1 x ^{35}Cl

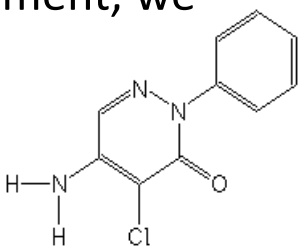


- Notice that the two "masses" are different.

Mass Spectrum Peaks

- Depending if we use positive or negative ionization mode on the instrument, we may not detect M^+, but $[M+H]^+$, or $[M-H]^-$

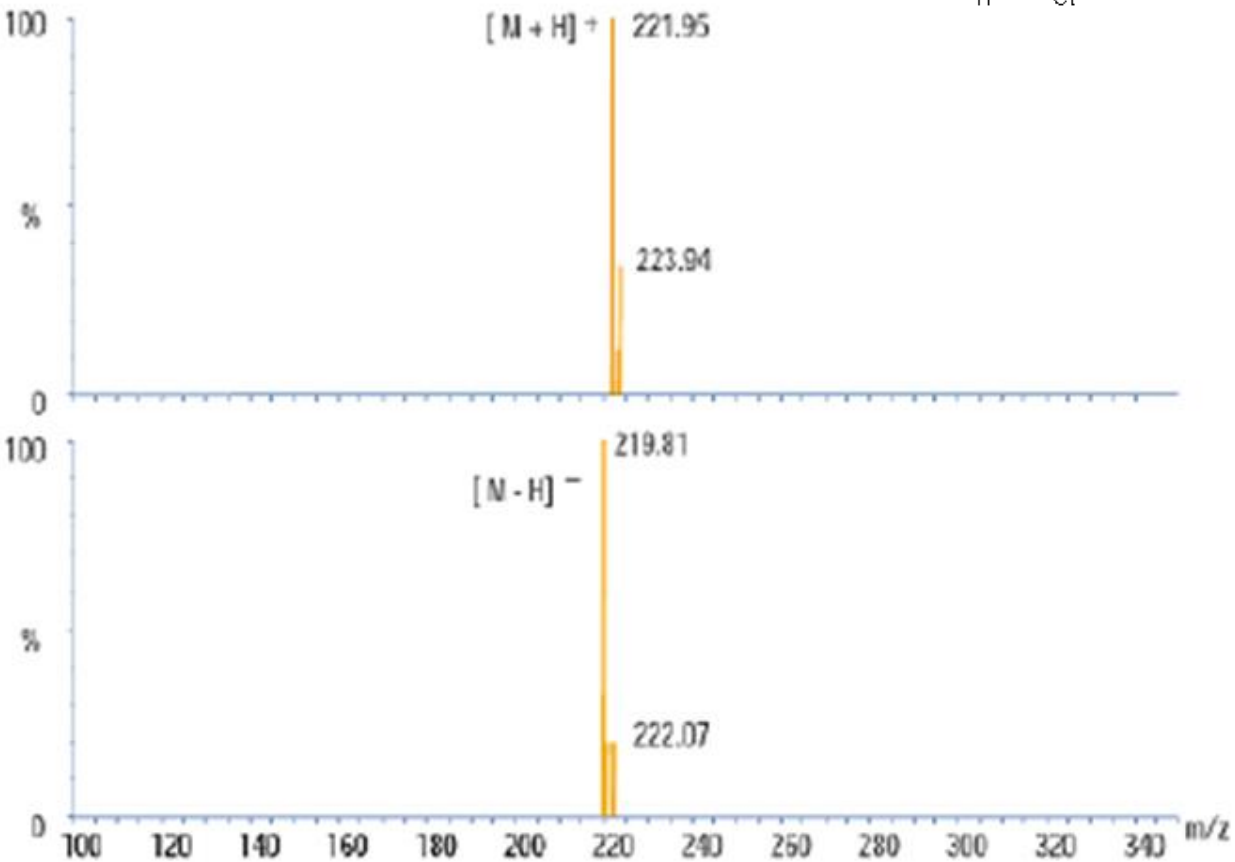
$M^+ = C_{10}H_8N_3OCl$ = **221.03**



$[M+H]^+ = C_{10}H_9N_3OCl$ = 222.0

(This shows typical quadrupole accuracy)

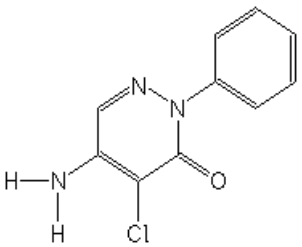
$[M-H]^- = C_{10}H_7N_3OCl$ = 220.0



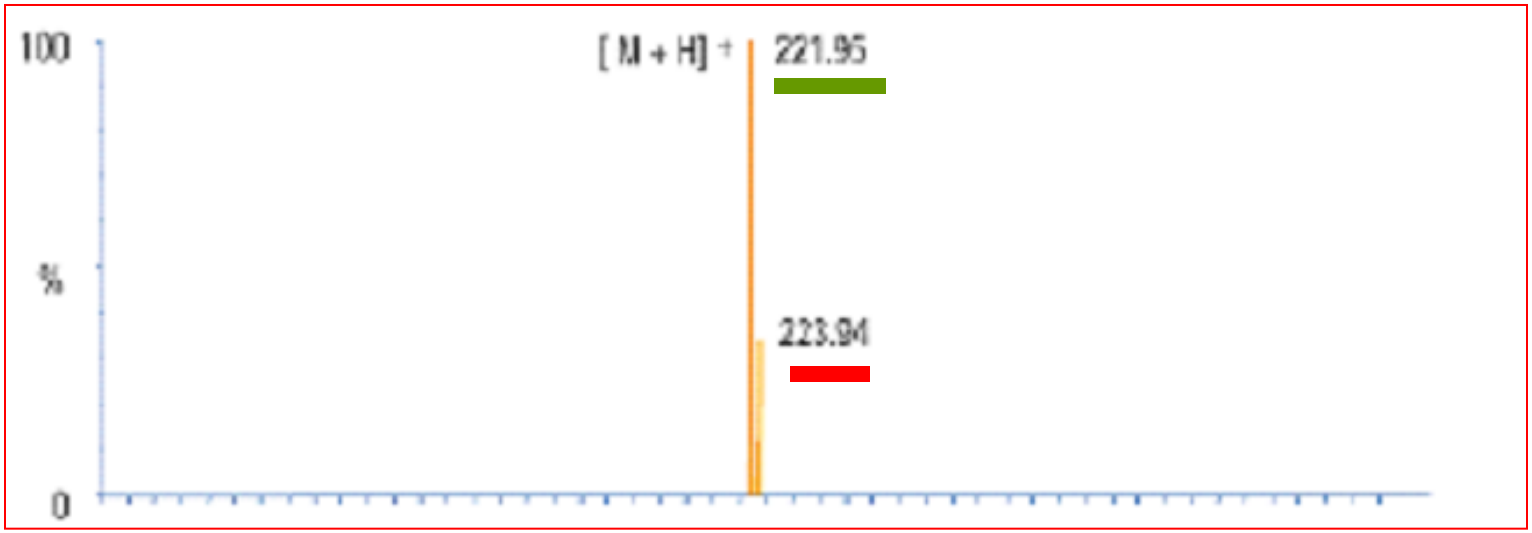
Isotope Peaks

- Average MW for $C_{10}H_8N_3OCl$ = **221.03**, so in *CI mode* $[M+H]^+$ = 222.

- Real Cl's are either ^{35}Cl = 76%
  ^{37}Cl = 24%



$[M+H]^+$ = $C_{10}H_9N_3O^{35}Cl$ = 222.0
$C_{10}H_9N_3O^{37}Cl$ = 224.0



A 30% [X+2] peak is characteristic of a Chlorine being present.

Summary

- Chromatography is the main technique for ***separating*** complex (similar) mixtures.

- GC and HPLC are the most common.
  (Probably the most common lab skill used in “industry”).

- Mass Spectrometry provides insight into the molecular structure.
  (Confirm/ determine/probe)

- Without it, Chemists are “blind”.

- Current instrumentation makes these experiments easy and accessible.

- This is *today’s* Chemistry.