DEPARTMENT OF CHEMISTRY

UNIVERSITY OF OXFORD

GRADUATE COURSE IN MASS SPECTROMETRY: LECTURE 5

Separation methods (chromatography) – Mass spectrometry

Dr. James Wickens, 11th November 2015

Chromatography
History and milestones

Paper Chromatography

Column Chromatography

Tsvet

Partition Chromatography

New developments all the time

Erica Cremer

The Nobel Prize in Chemistry 1952

Archer John Porter Martin

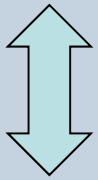
Prize share: 1/2

Richard Laurence Millington Synge

Prize share: 1/2

Chromatography

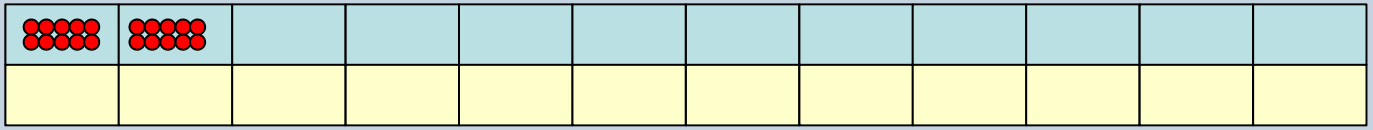
Mobile Phase



Stationary phase

Chromatography

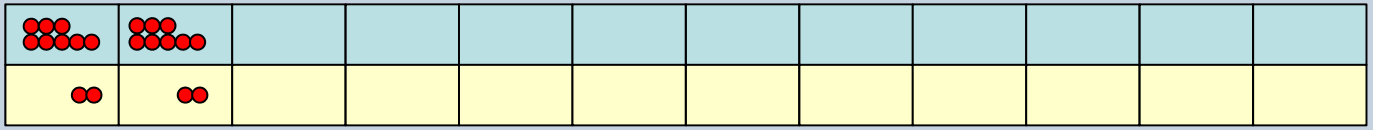
- Injection



80 : 20
Mobile : Stationary

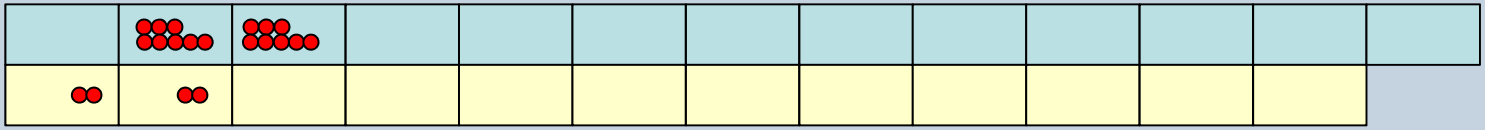
Chromatography

- Equilibration



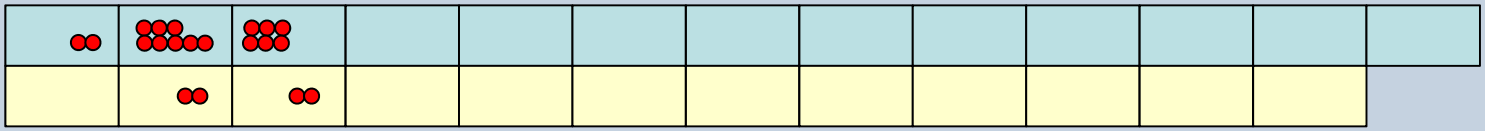
Chromatography

- Mobile phase moves



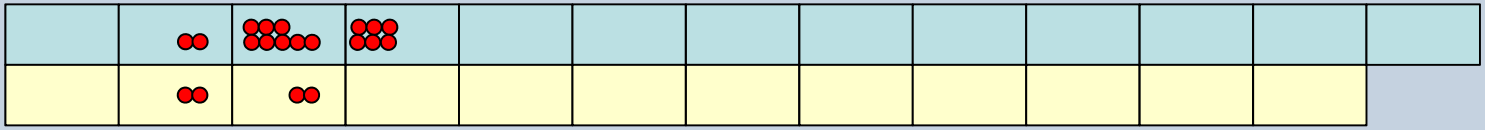
Chromatography

- Equilibration



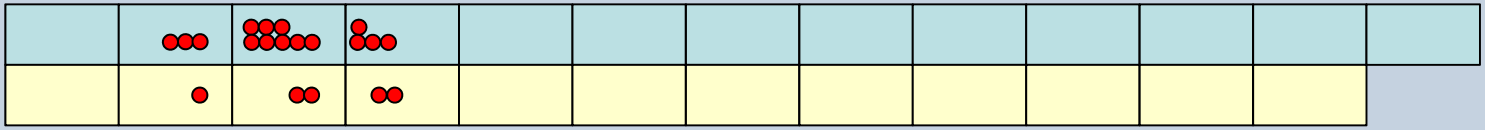
Chromatography

- Mobile phase moves



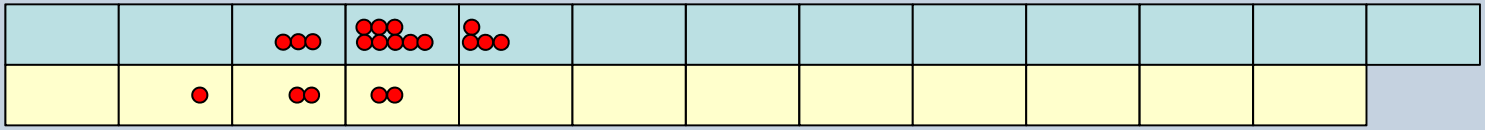
Chromatography

- Equilibration



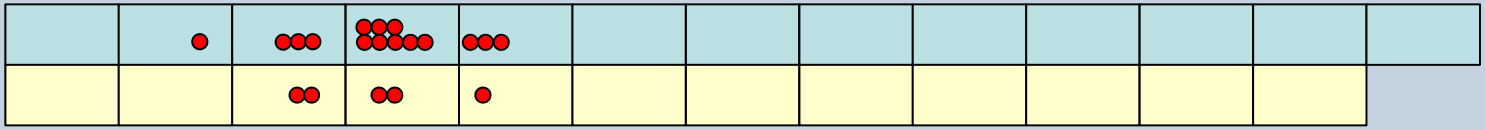
Chromatography

- Mobile phase moves



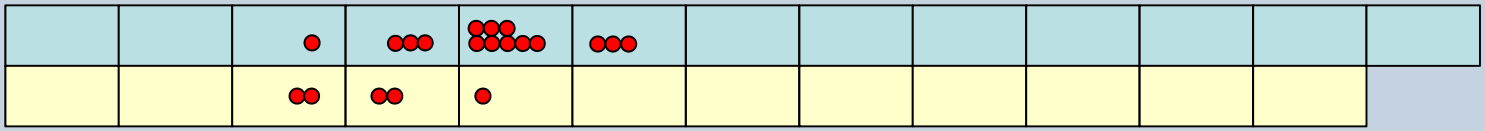
Chromatography

- Equilibration



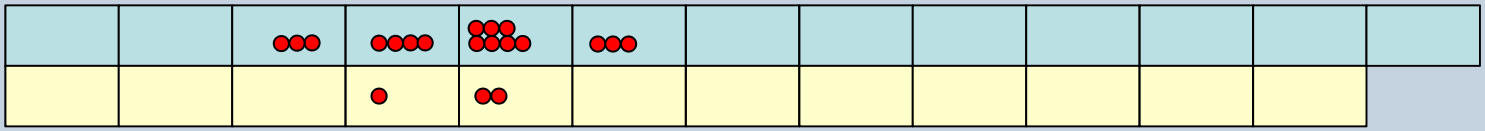
Chromatography

- Mobile phase moves



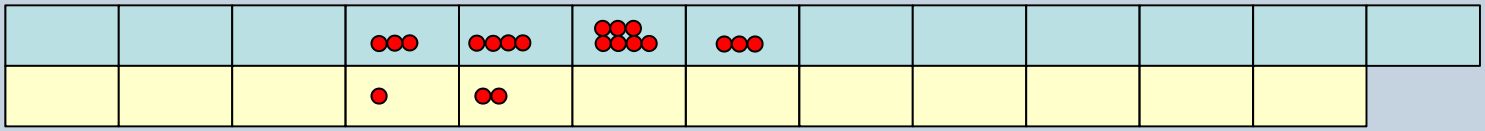
Chromatography

- Equilibration



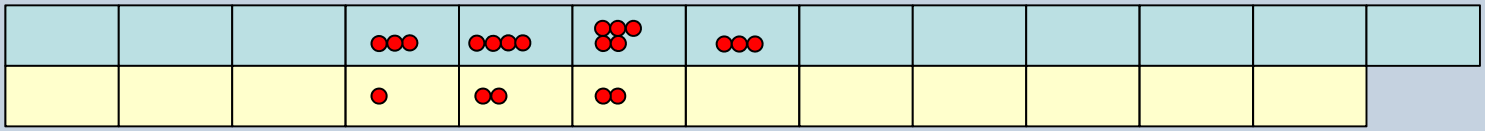
Chromatography

- Mobile phase moves



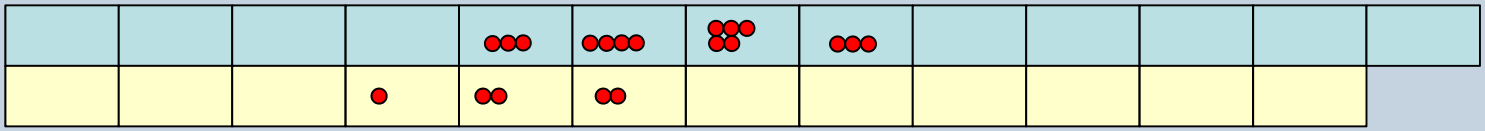
Chromatography

- Equilibration



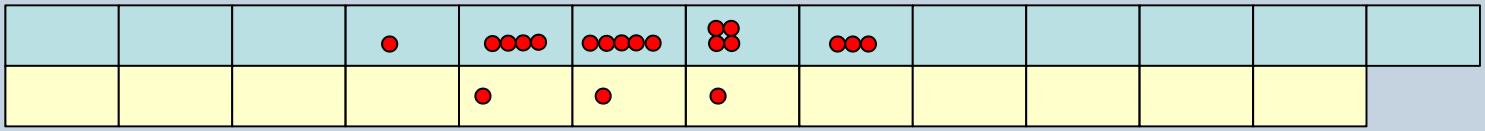
Chromatography

- Mobile phase moves

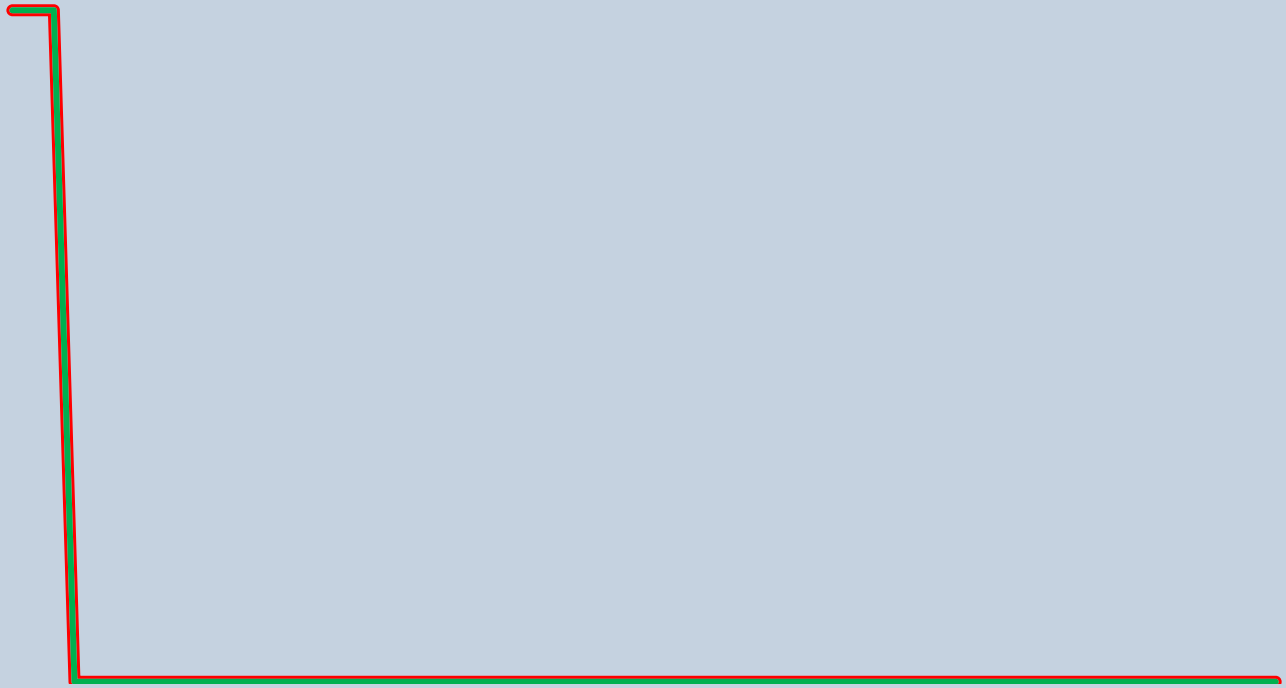


Chromatography

- Equilibration



Chromatography: Separation



Chromatography: Separation

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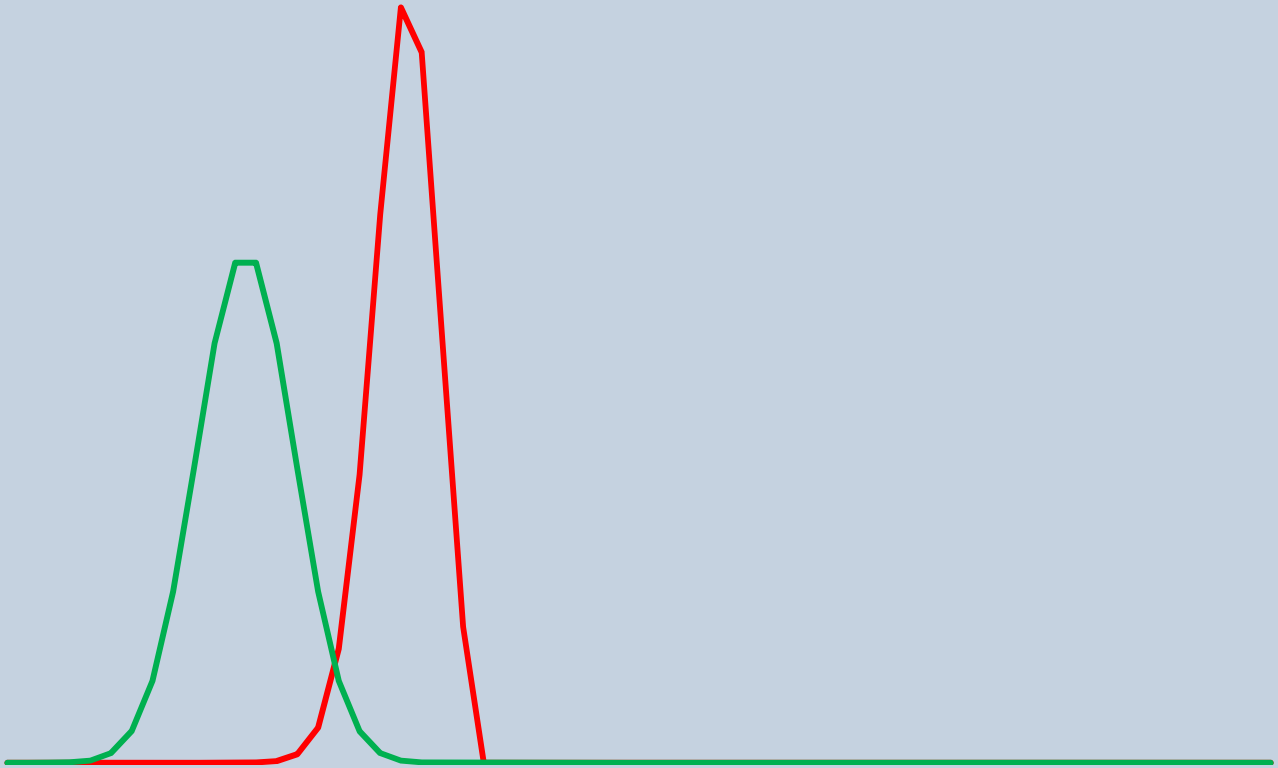
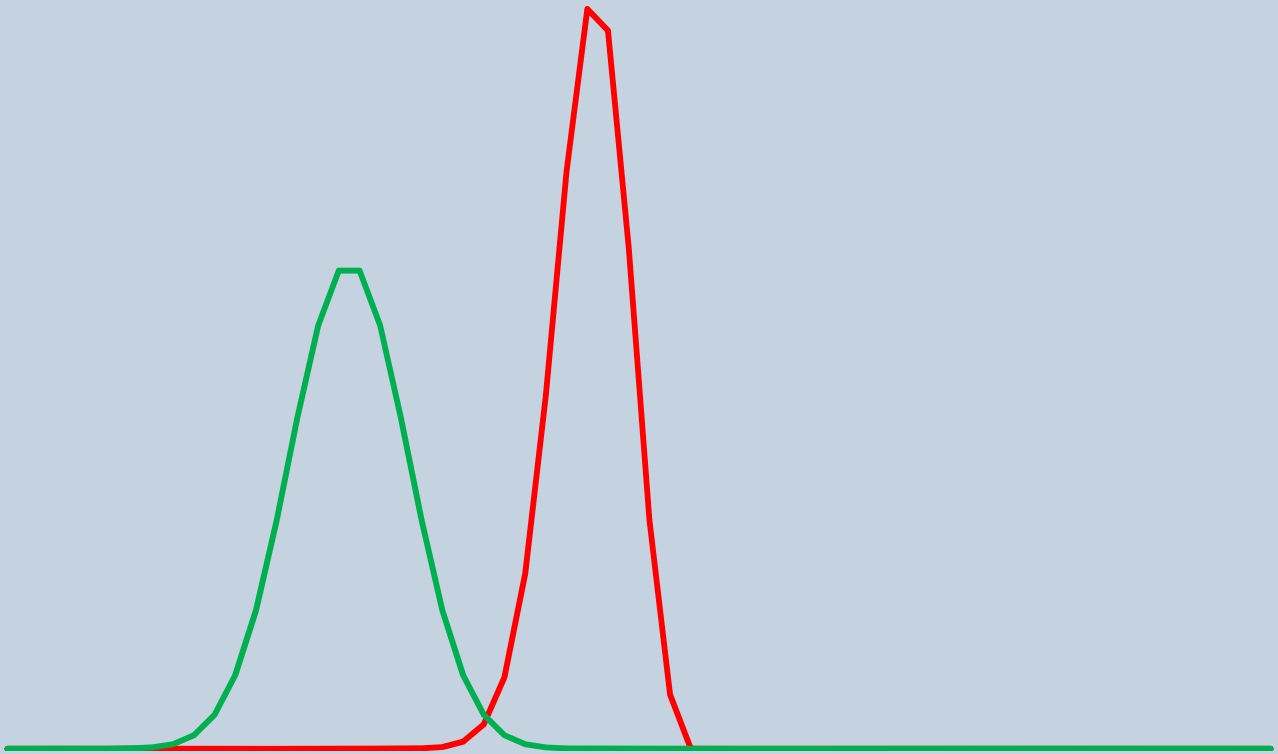
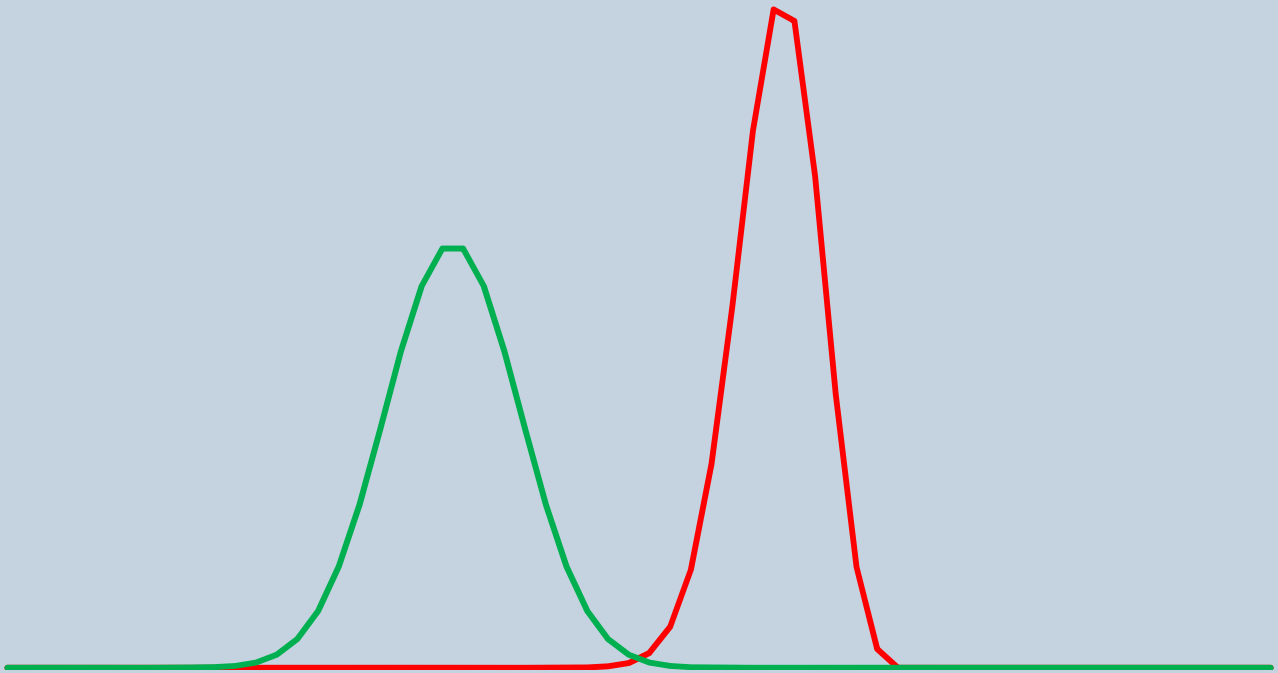


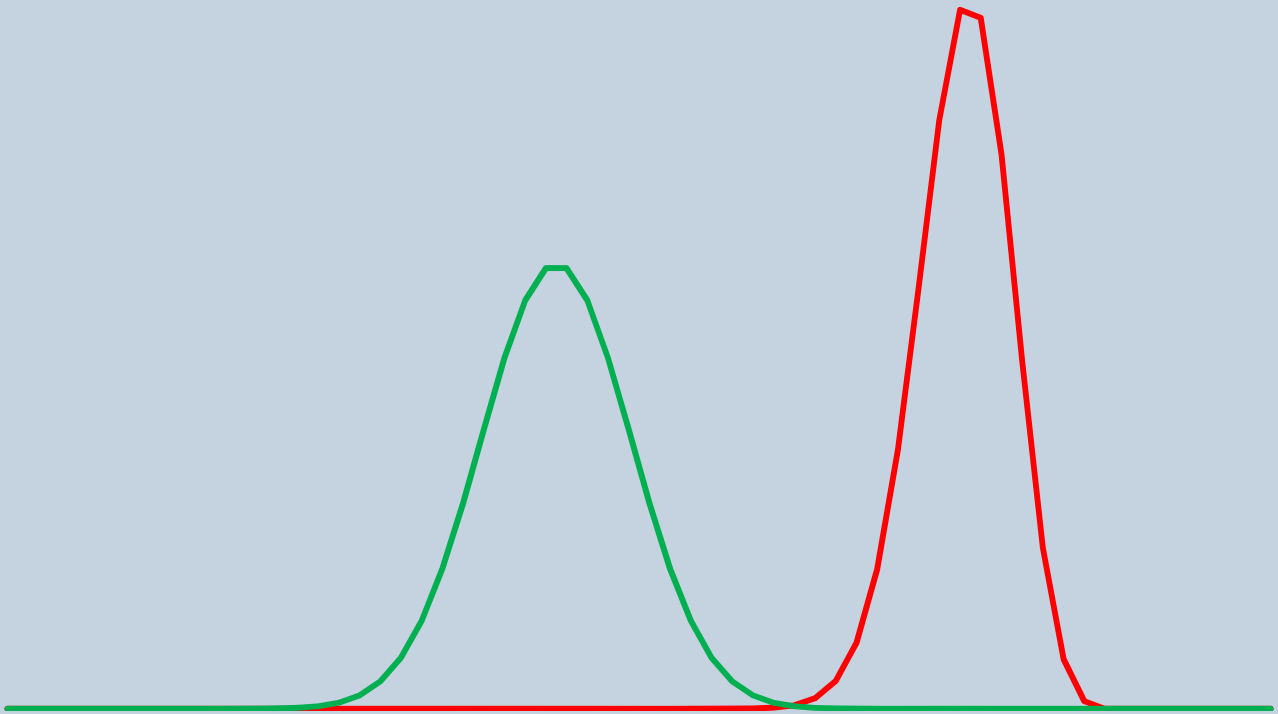
Chromatography: Separation



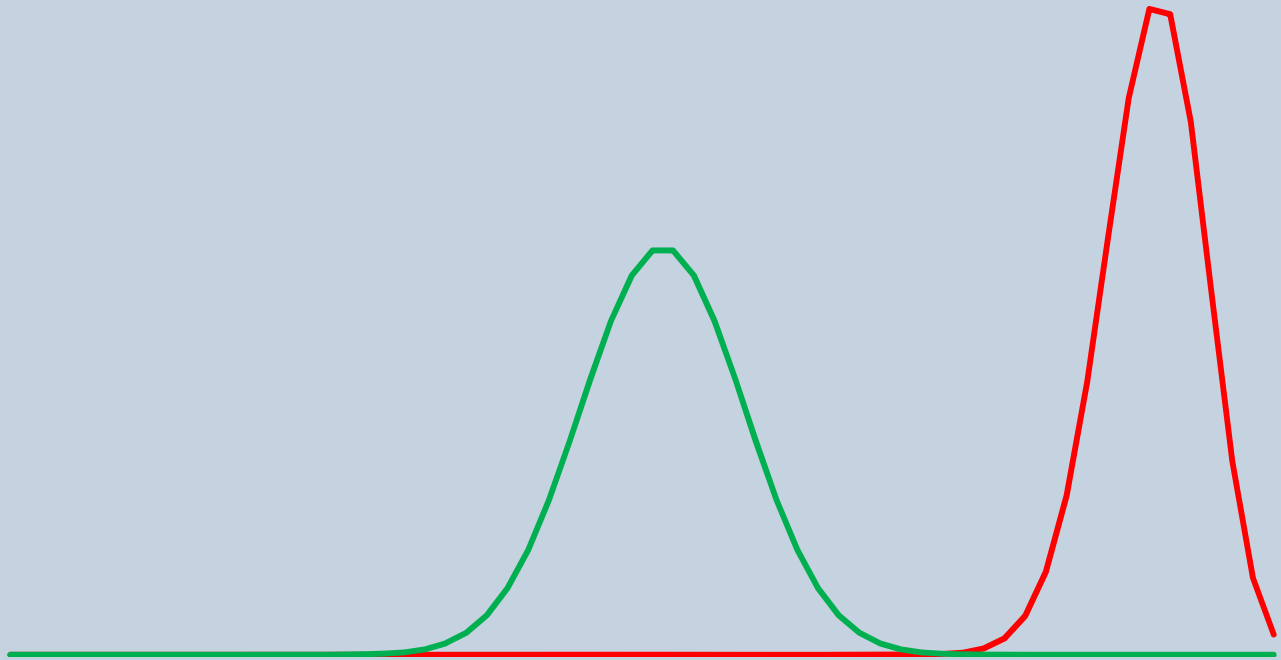
Chromatography: Separation



Chromatography: Separation



Chromatography: Separation

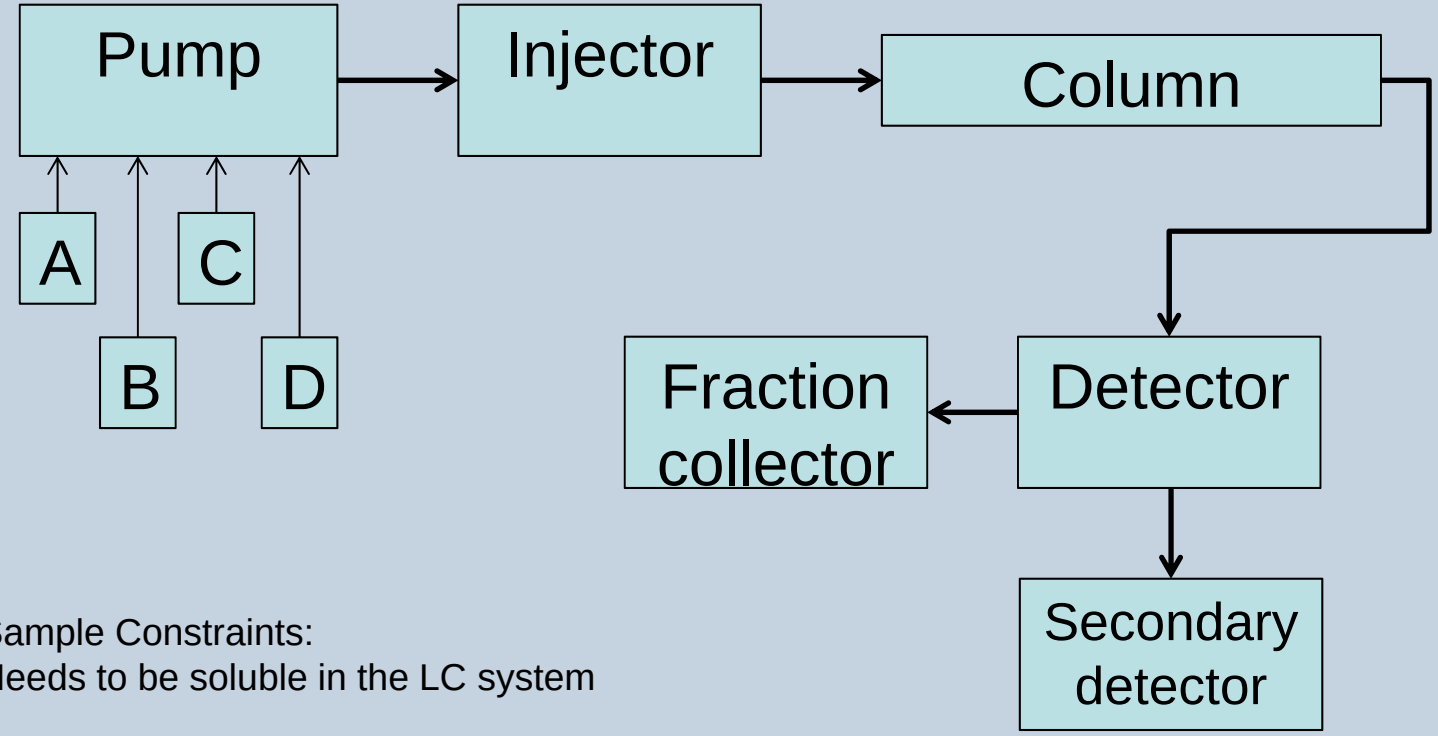


Liquid Chromatography

- Basics
  - Experimental description, sample constraints etc.
- Scale
  - Preparative - Nano
- Hardware
  - LC
  - Physical description of the column
- Separation mechanisms
- MS interfaces

Liquid Chromatography Basics

- High Performance Liquid Chromatography



Sample Constraints:
Needs to be soluble in the LC system

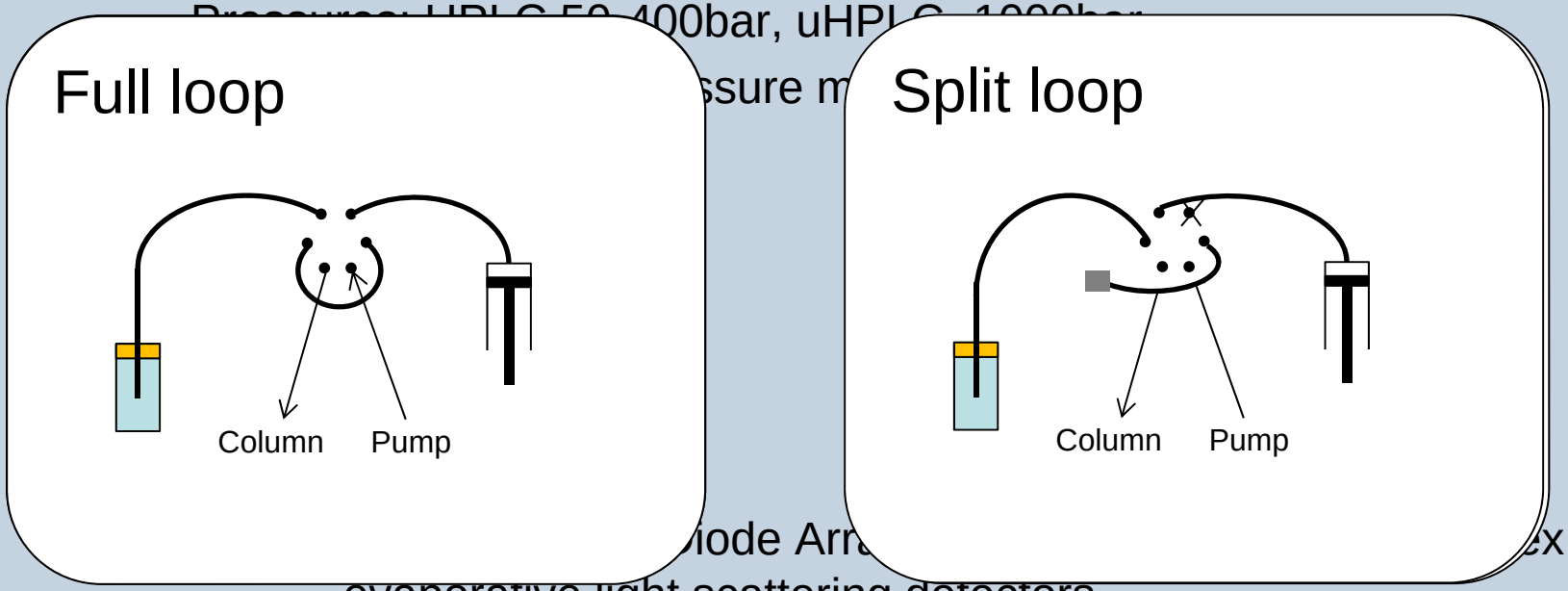
Liquid Chromatography Scale

Column Diameter	Description	Flow rate
>100mm	Industrial	~ 1 L/min
10 - 50mm	Preparative	~ 20 mL/min
2 - 4.6mm	Analytical	~ 0.5 mL/min
0.5 – 1 mm	Microflow	~ 100 µL/min
0.05 – 0.1 mm	Nanoflow	~ 100 nL/mim

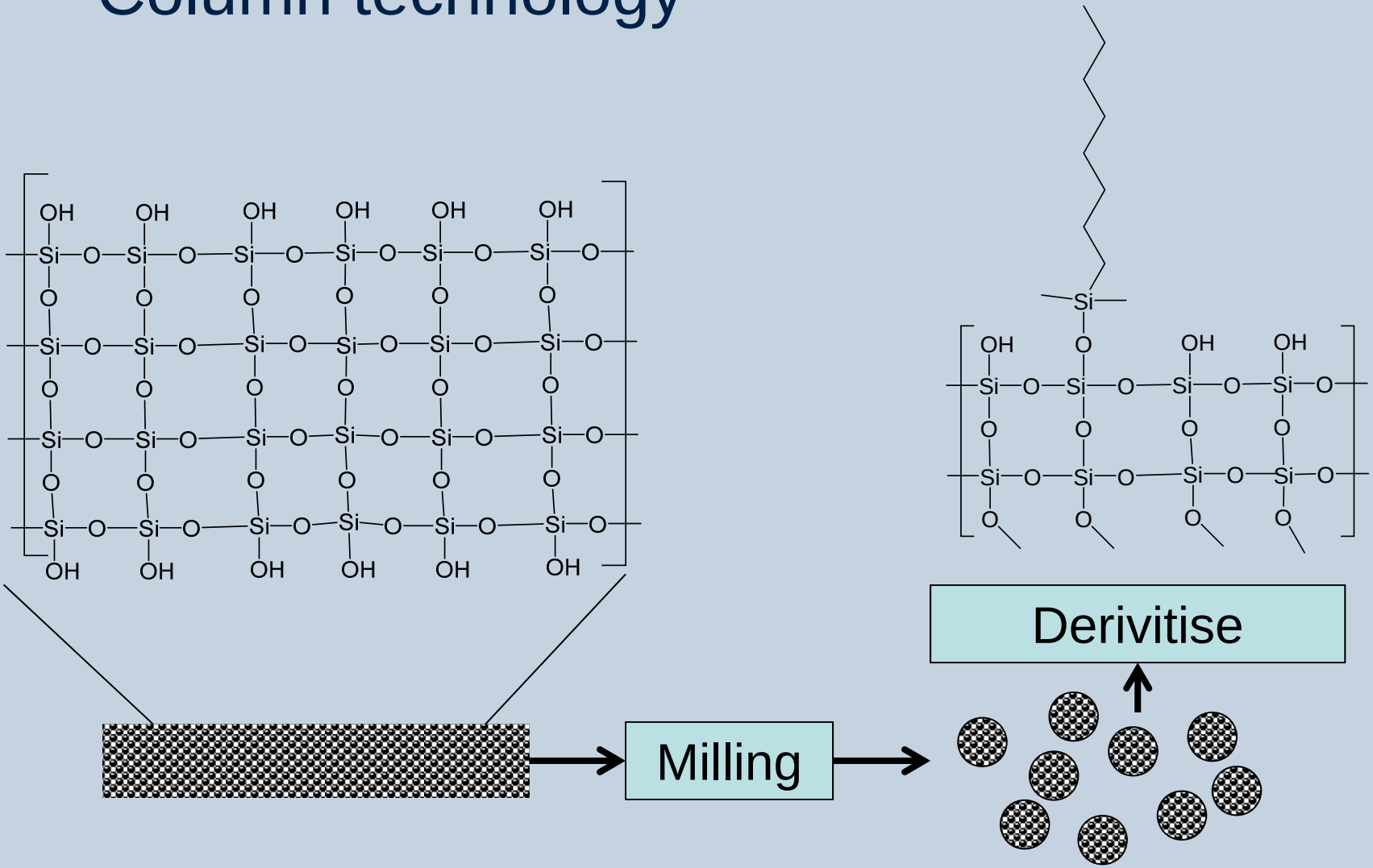
Liquid Chromatography Hardware

- Pump
  - Pressures: HPLC 50-400bar, uHPLC 1000bar
  - ssure m
  - iode Arr
  - ex,

evaporative light scattering detectors.



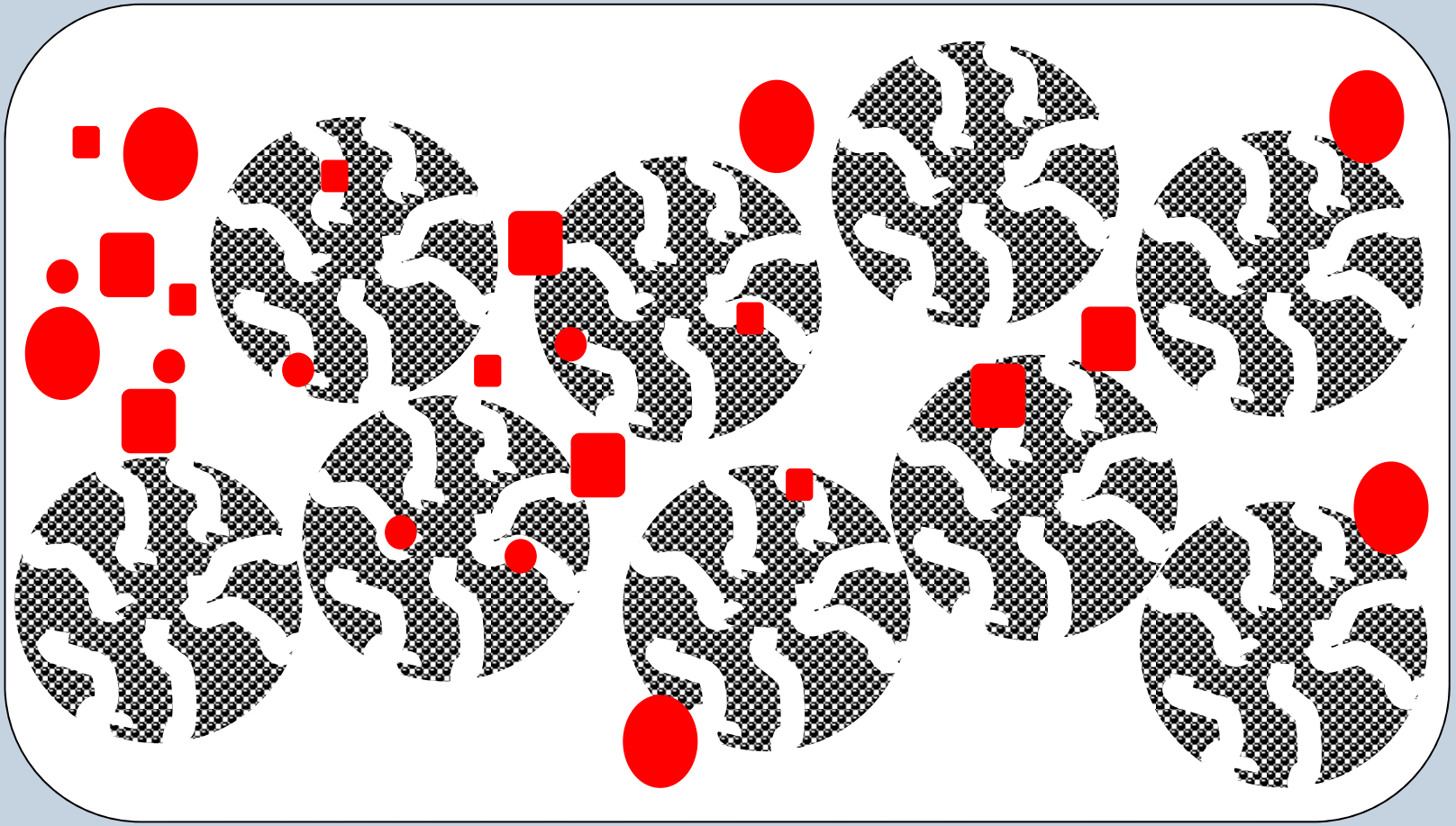
Column technology



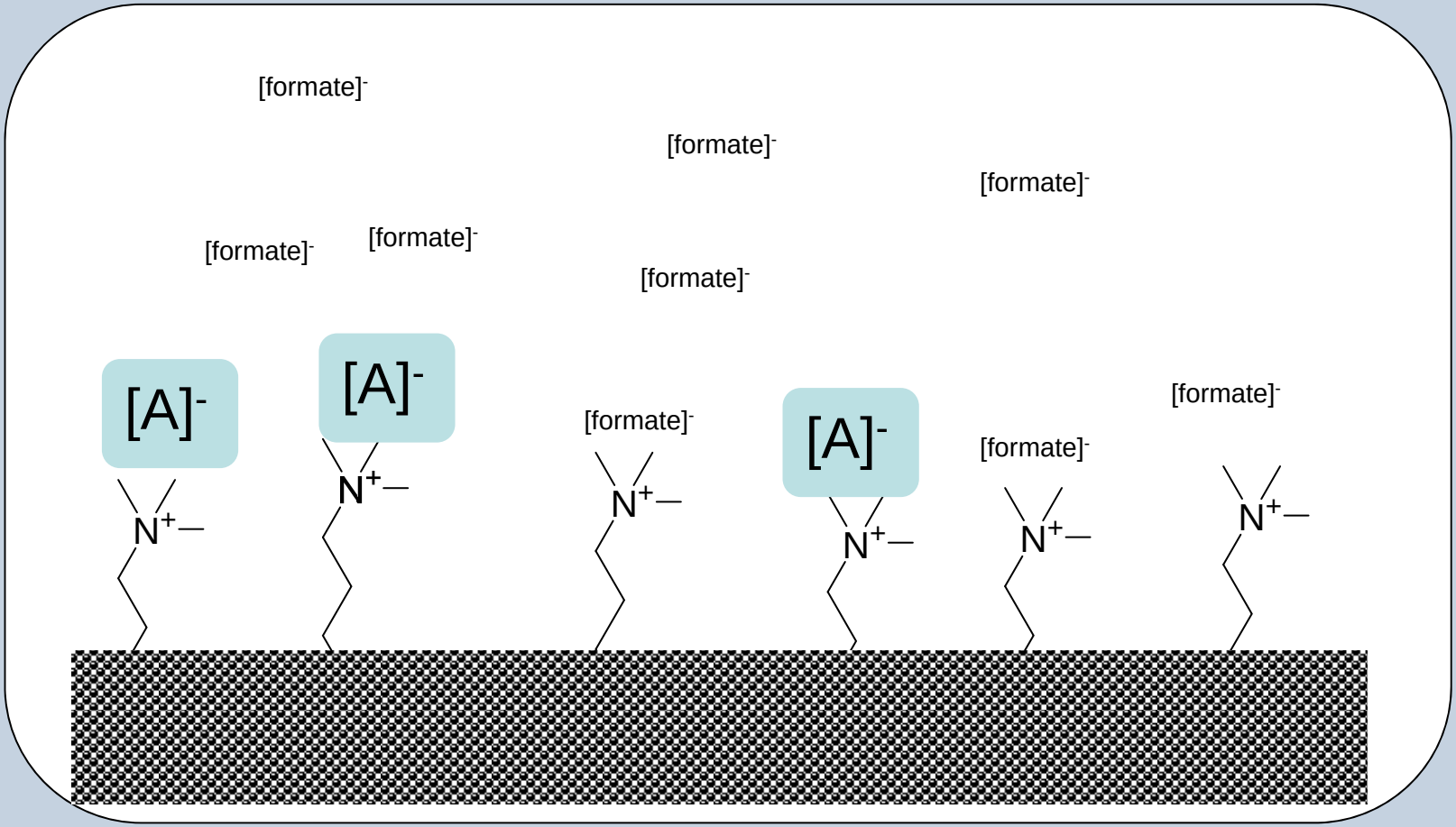
Liquid Chromatography: Separation mechanisms:

- Size exclusion
- Ion exchange
- Normal Phase
  - Classic
  - Aqueous normal phase
  - SCF
- Reversed phase
  - Detailed discussion later

Size exclusion chromatography



Ion exchange chromatography



Normal phase liquid chromatography

- Non-polar Mobile phase
  - Hexane, DCM, ethyl-acetate
- Polar stationary phase
  - Bare silica, amino, amide, and chiral phases
- Separation mechanisms
  - H-bond, Dipole interactions (stearic interactions)
- Ideal for:
  - Very hydrophobic analytes, very polar analytes, structural isomers, chiral separations
- Problems
  - Intolerant to impurities (water, protic solvents, salts)
- Variants
  - Aqueous normal phase/HILIC, super-critical fluid chromatography

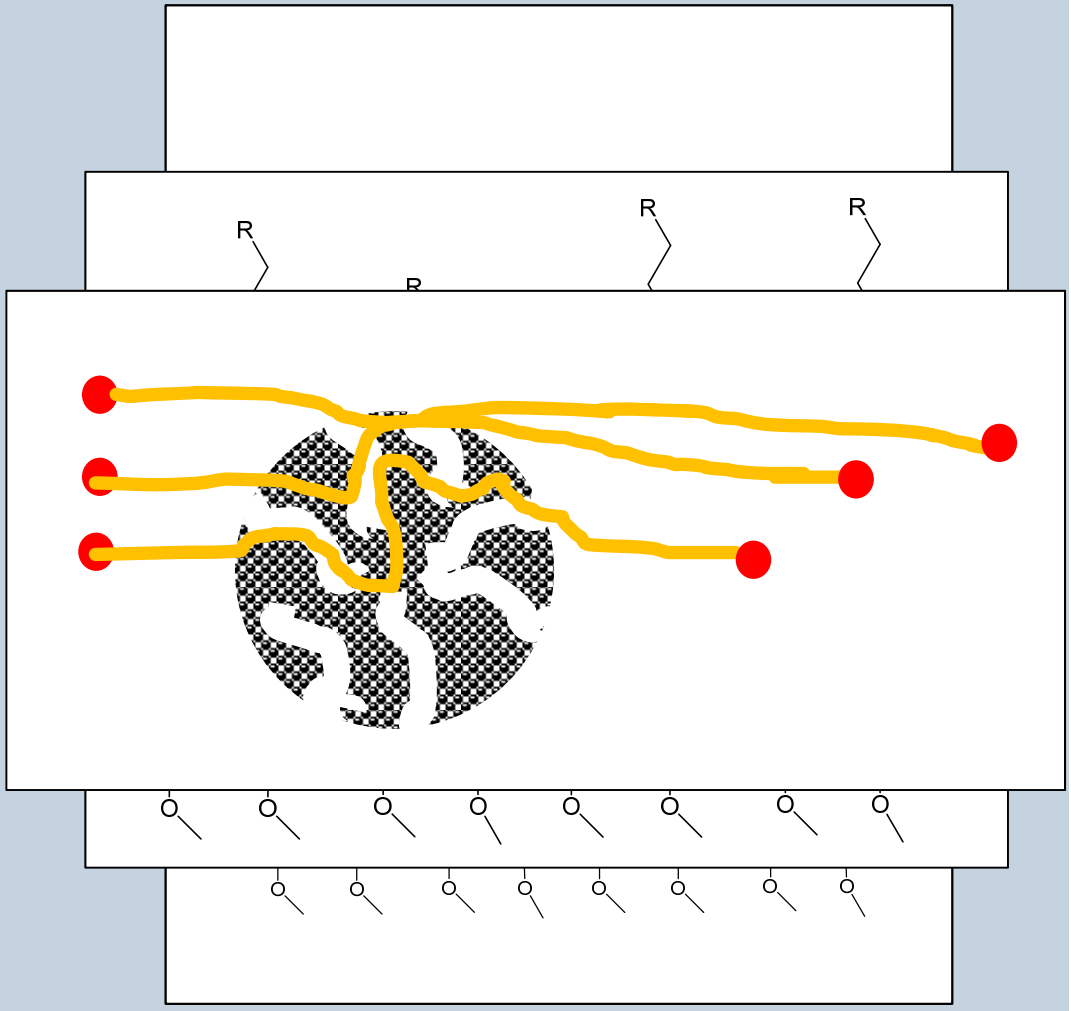
Reversed phase liquid chromatography

The most popular type of liquid chromatography

- Polar Mobile phase
  - Water, Methanol, Acetonitrile
- Non-polar stationary phase
  - C18, C8, C4, phenyl etc.
- Separation mechanisms
  - Hydrophobicity/lipophilicity
- Ideal for:
  - Very wide range of analytes
  - High loading capacity
- Problems
  - Next slide

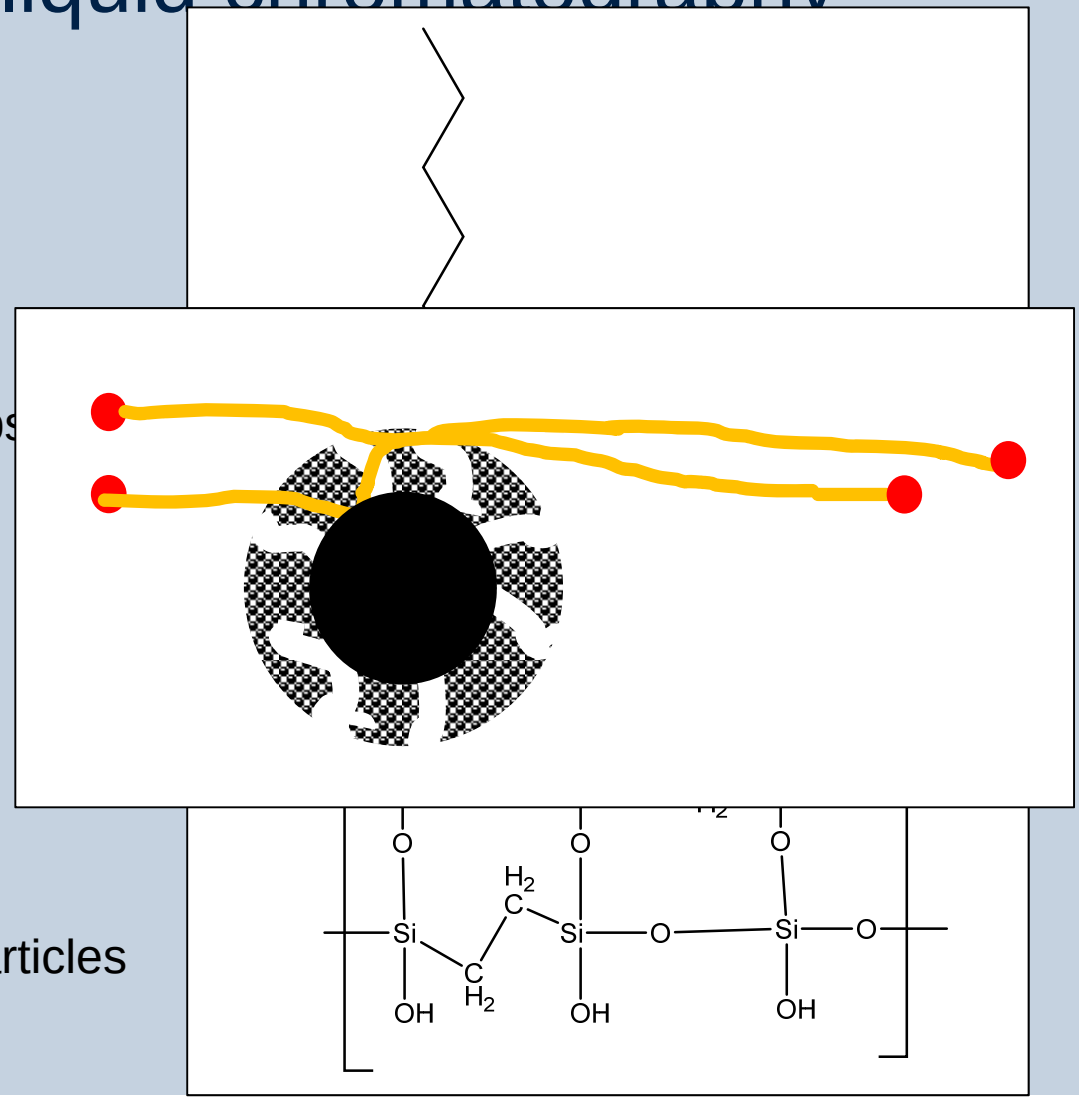
Reversed phase liquid chromatography Problems

- Very polar analytes
  - Ligand collapse
- Mixed mode interactions
  - Si-OH
- pH instability
  - ‘Normal’ silica pH 2-8
- Mechanical dispersion
  - Multiple paths
  - Size exclusion effects



Reversed phase liquid chromatography solutions

- ~~Ion Pairing~~
- ligand redesign
  - Polar embedded groups
  - End-capping
  - Multi dentate
- Hybrid silica
- Silica support redesign
  - Smaller particles
  - Superficially porous particles
  - monoliths



Connecting LC to MS

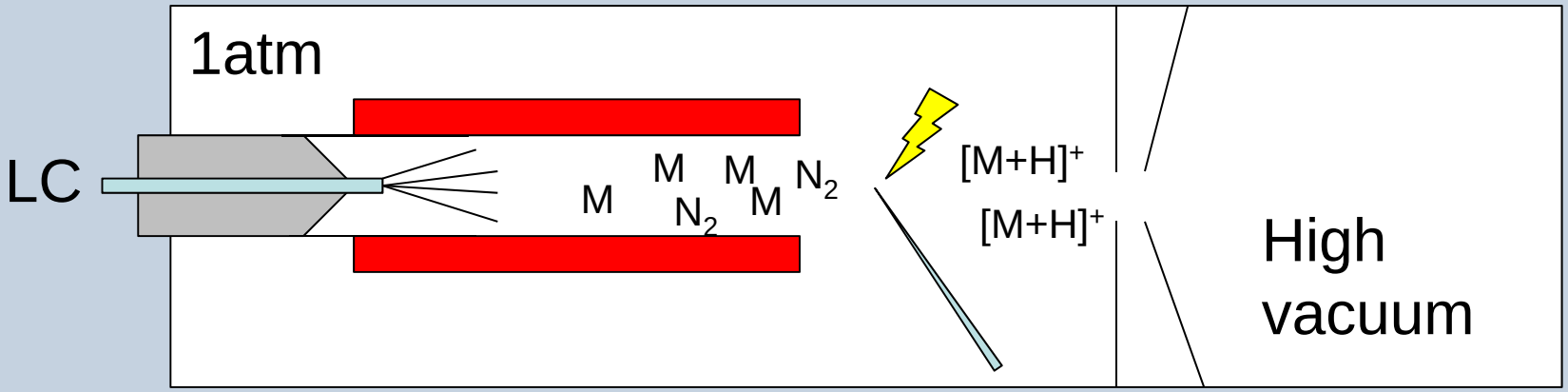
Many older and less frequently used techniques:

- APPI, Thermospray, Moving belt interface, continuous flow FAB

Two major techniques:

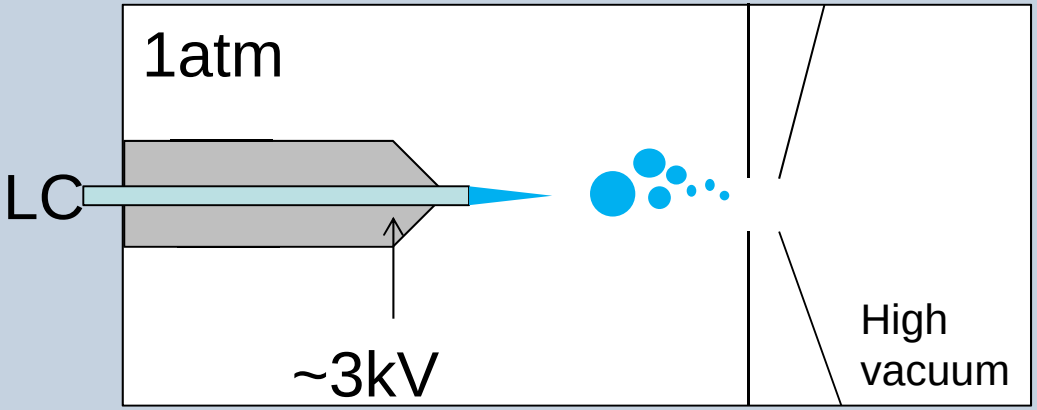
- APCI
- Electrospray

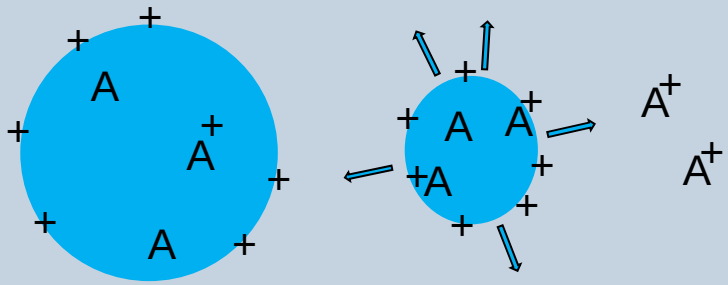
LC-APCI



- Flow rate: ~1 mL/min design dependant
  - Sufficient to ensure nebulisation
- Solvent: compatible with non-polar solvents
  - Make sure your source gas is nitrogen… BANG!
- Tolerant to matrix introduction
- Problems
  - Soot formation, limited chemical range

LC-Electrospray





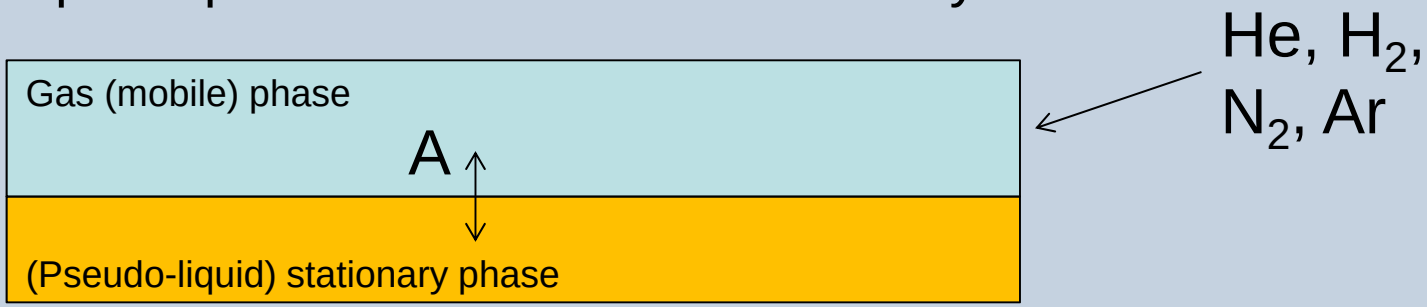
- Flow rate: The lower the better
  - ‘Standard’ sources <0.2 mL/min. → flow spliting
  - Nano flow has incredible sensitivies
- Solvent: water miscible, protic
- Problems
  - Matrix effects

Gas Chromatography

- Basics
- Hardware
  - GC
  - Packed column vs. capillary
  - Stationary phase chemistries
    - Non-polar
    - Polar
    - Specialist
- GC-MS interfacing

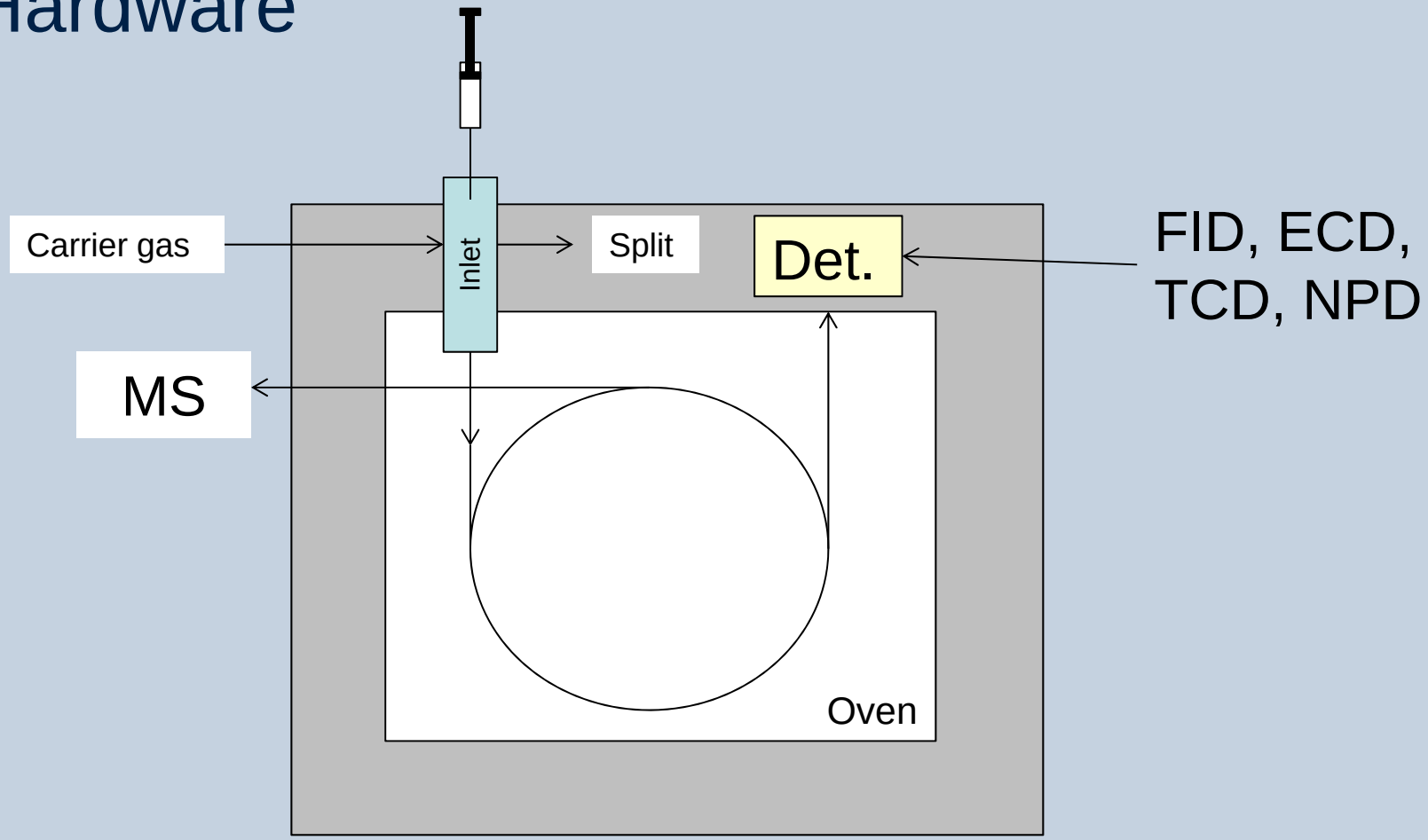
Gas Chromatography Basics

- Principle separation mechanism is volatility



- Samples must be volatile or capable of being made volatile
- Can be packed column or Capillary column
- Can be very efficient (narrow peaks)
  - Fast diffusion
  - Open tubular column; no multiple path diffusion

Gas Chromatography Hardware

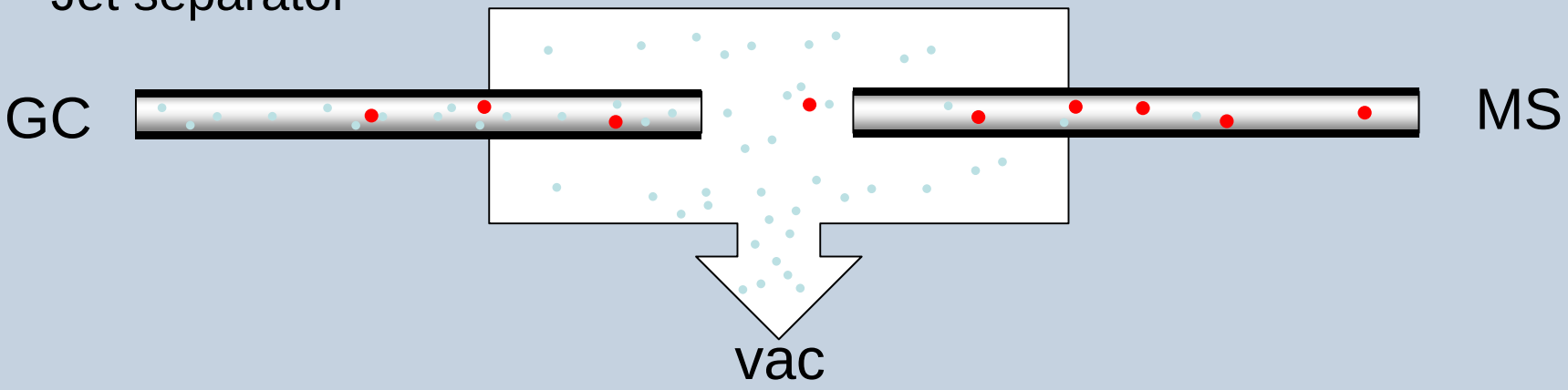


Gas Chromatography
Stationary phase chemistries

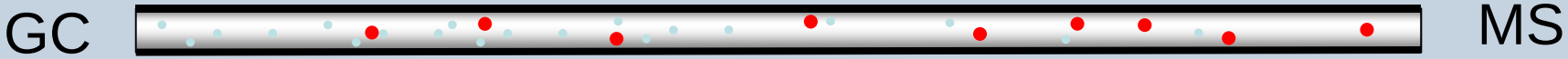
- NonPolar
  - "1"s (HP1, ZB1) — Poly-dimethylsiloxane
  - "5"s (HP5, ZB5) — Poly-diphenylsiloxane
  - "50"s — Poly-diphenylsiloxane
  - "1701"s (DB1701) — Poly-cyanopropyl-methylsiloxane
- Polar
  - "Wax" — Polyethylene glycol

GC-MS interface

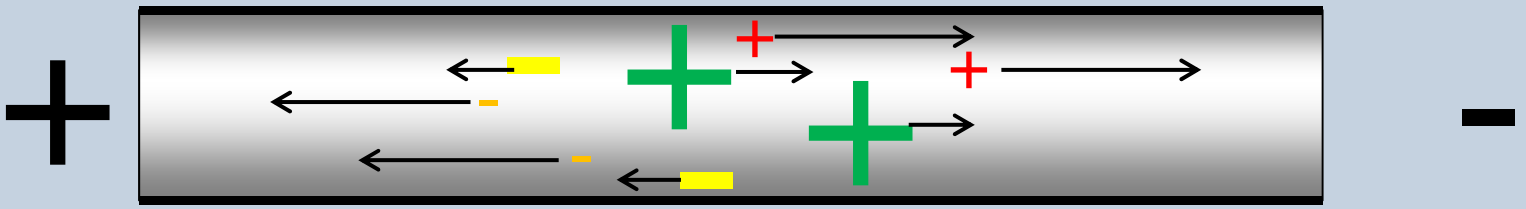
Packed column & capillaries > 0.53 mm
Jet separator

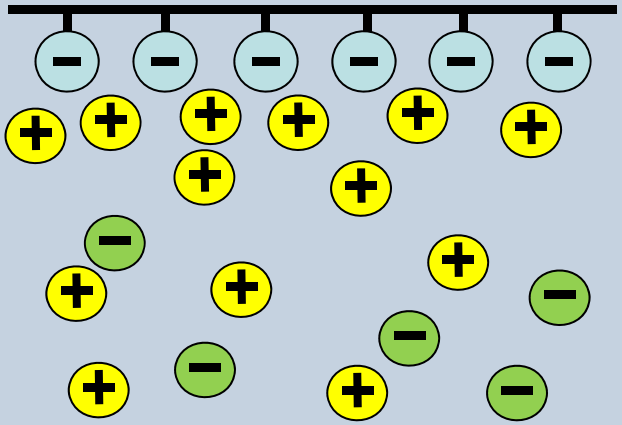


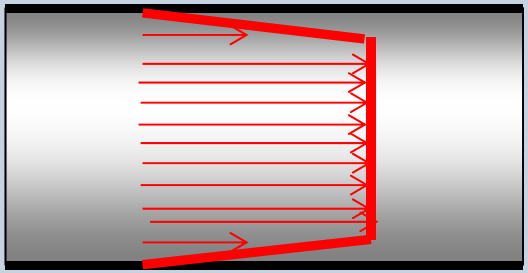
0.32, 0.25, 1mm capillaries

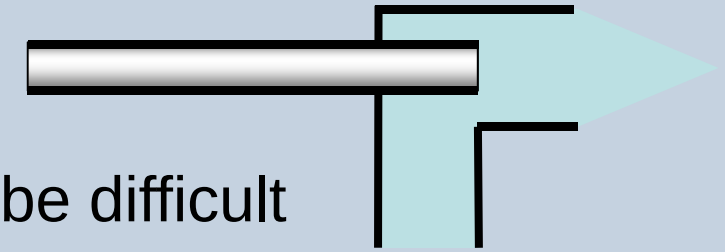


Capillary electrophoresis









MS interfacing can be difficult

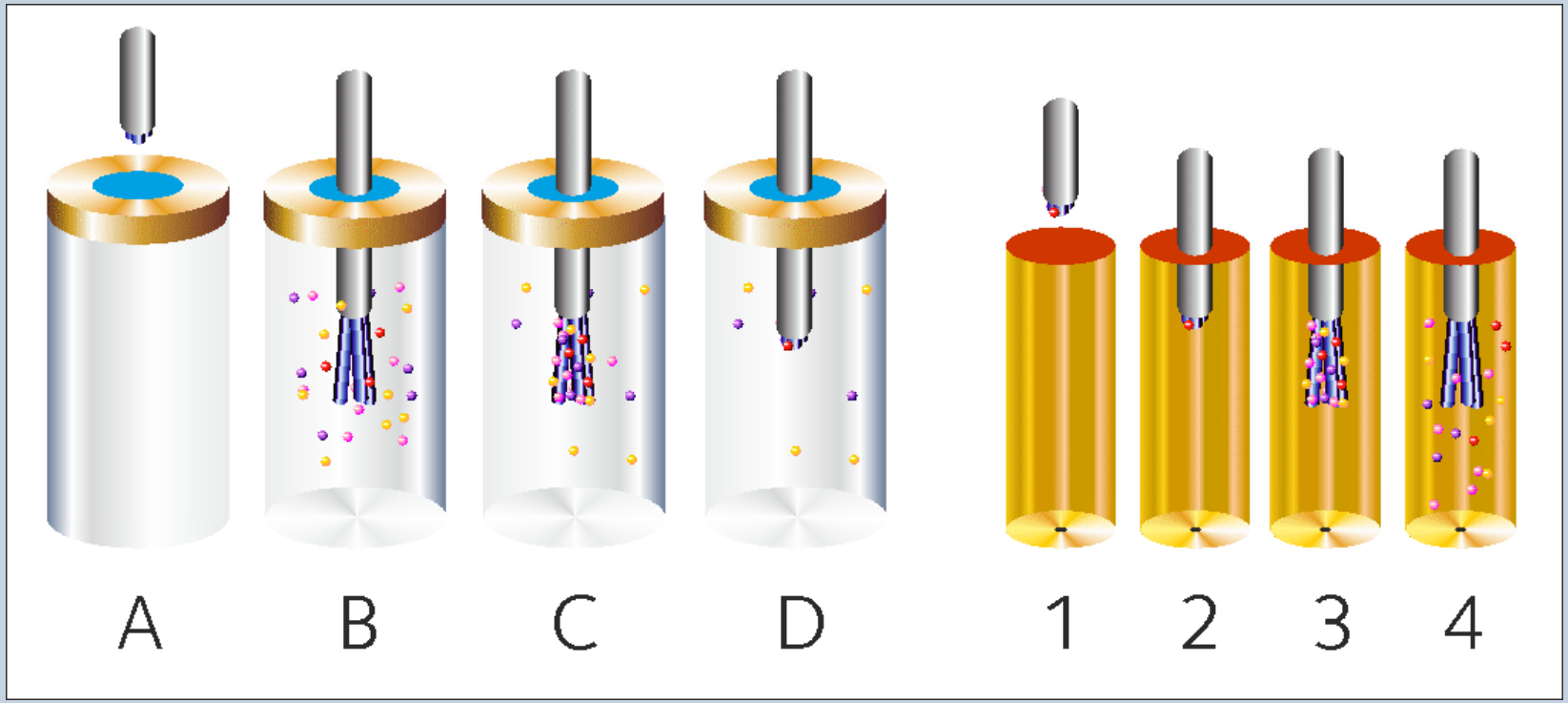
Example 1

Introduction

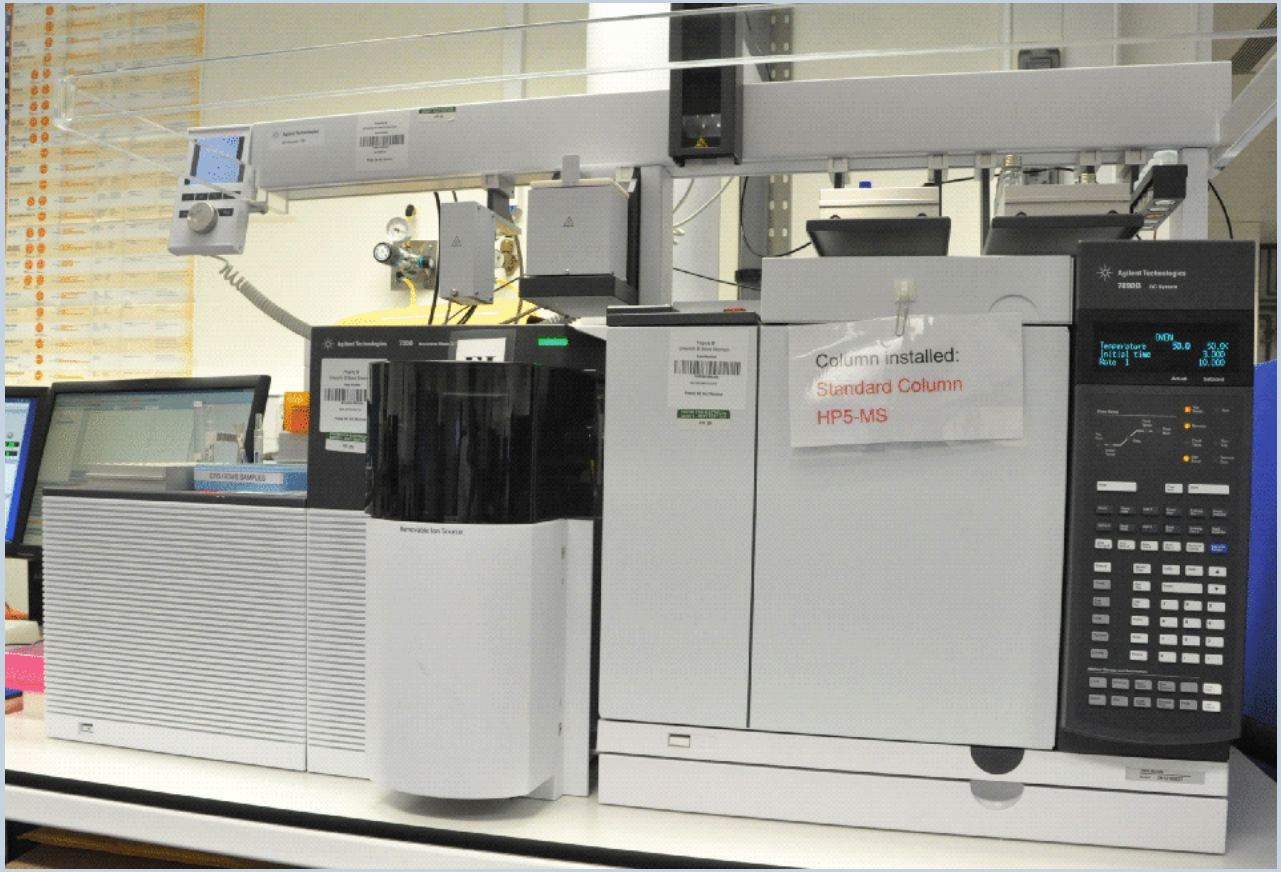
The new GC-MS system in the mass spectrometry reseach facility, department of Chemistry enables enrichment and analysis of volatile compounds. Here we present analysis of scent from individual sweetpea flowers

Example 1

SPME



Example 1



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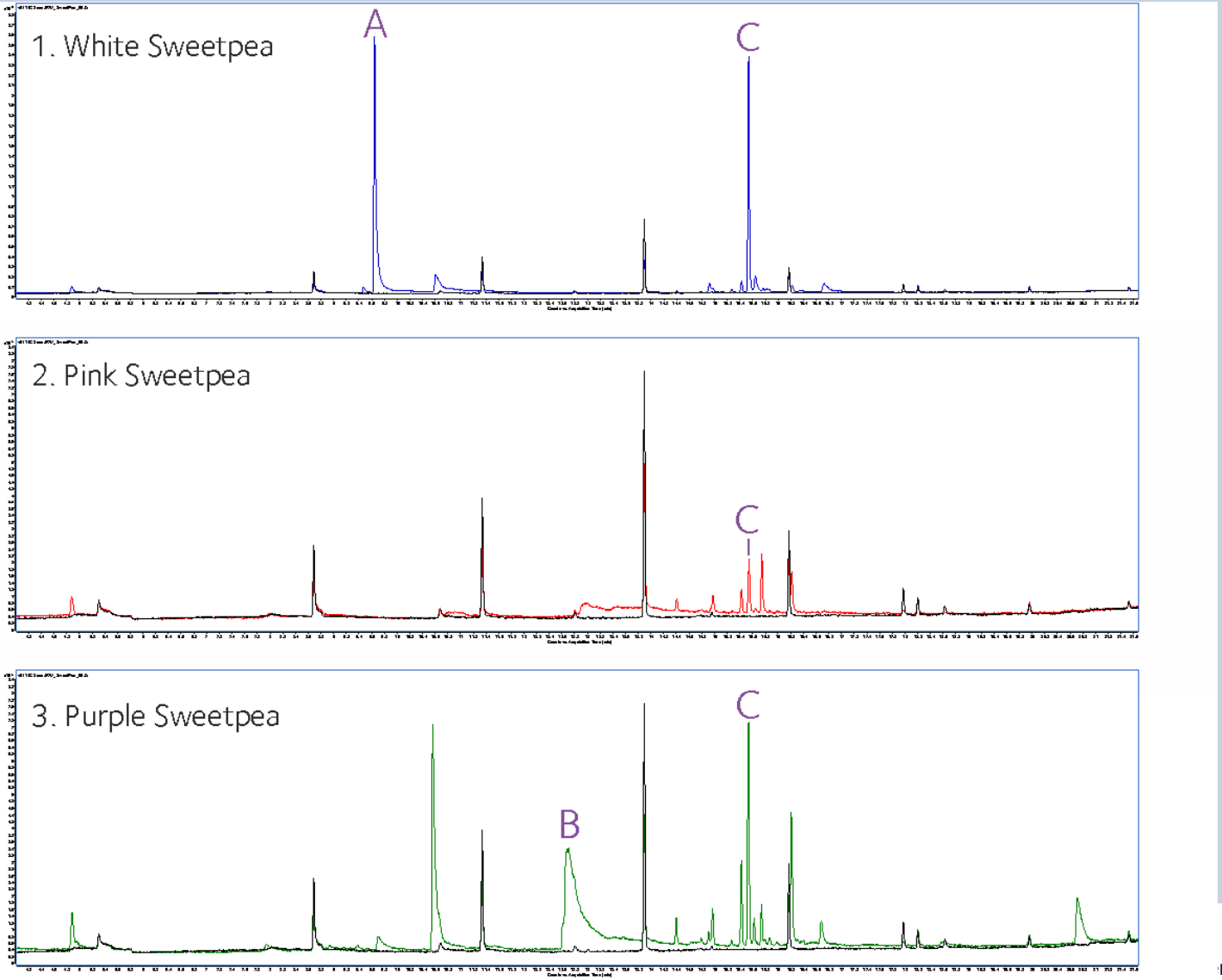
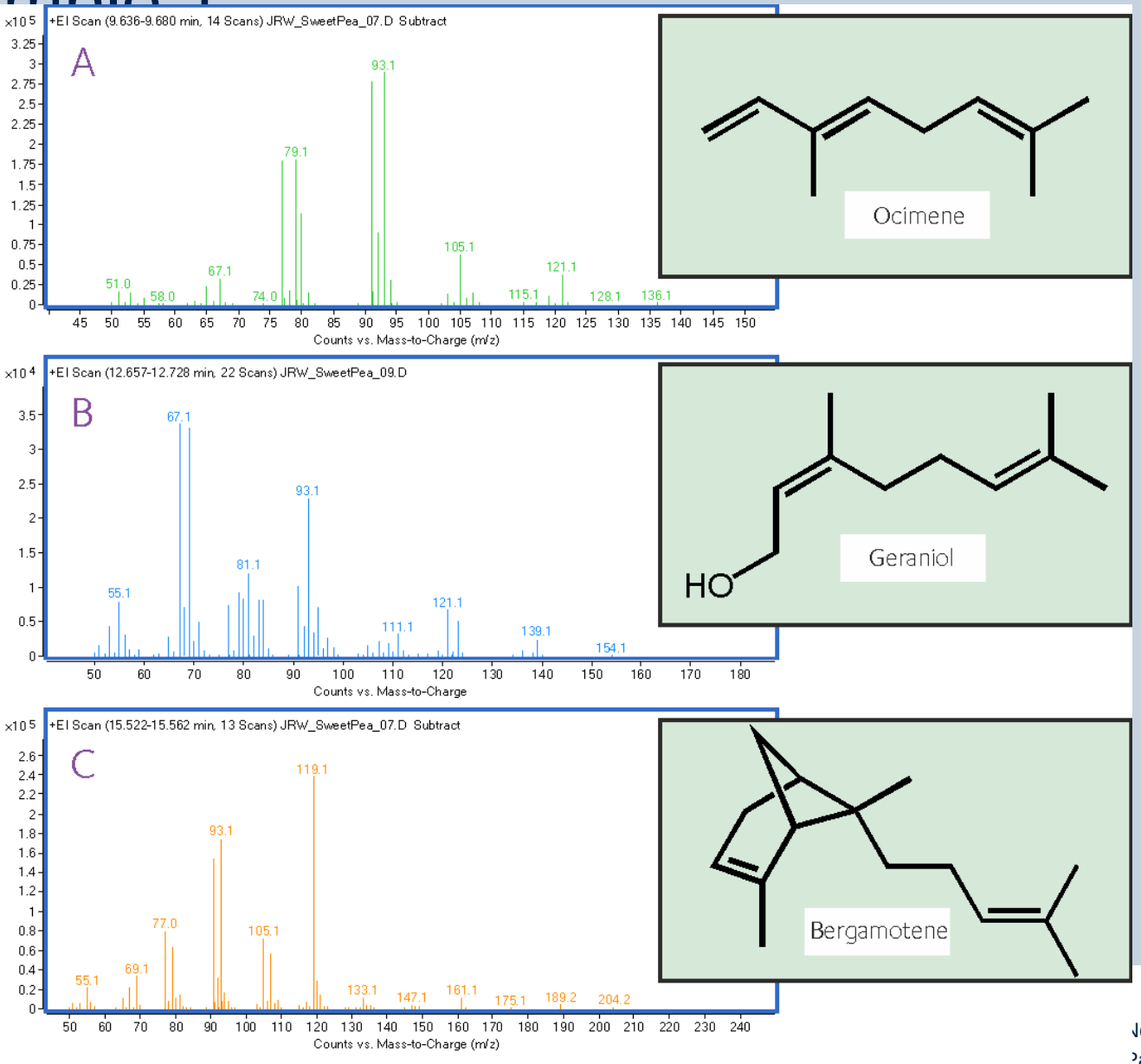
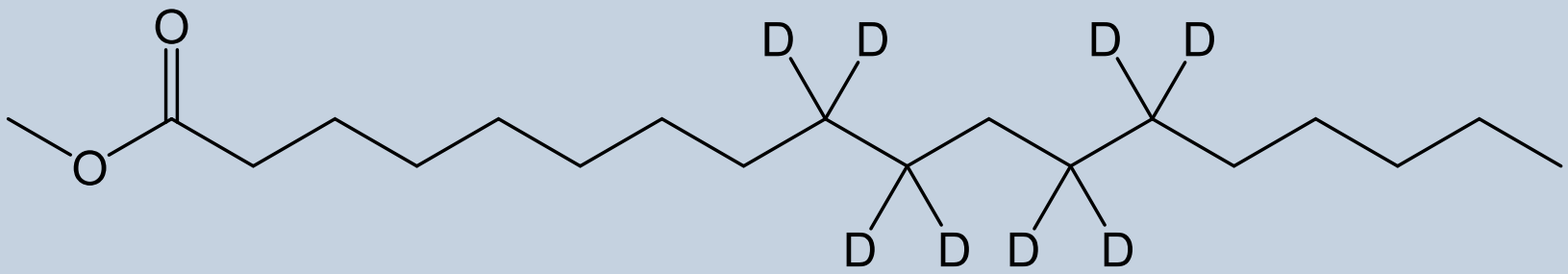


Example 1



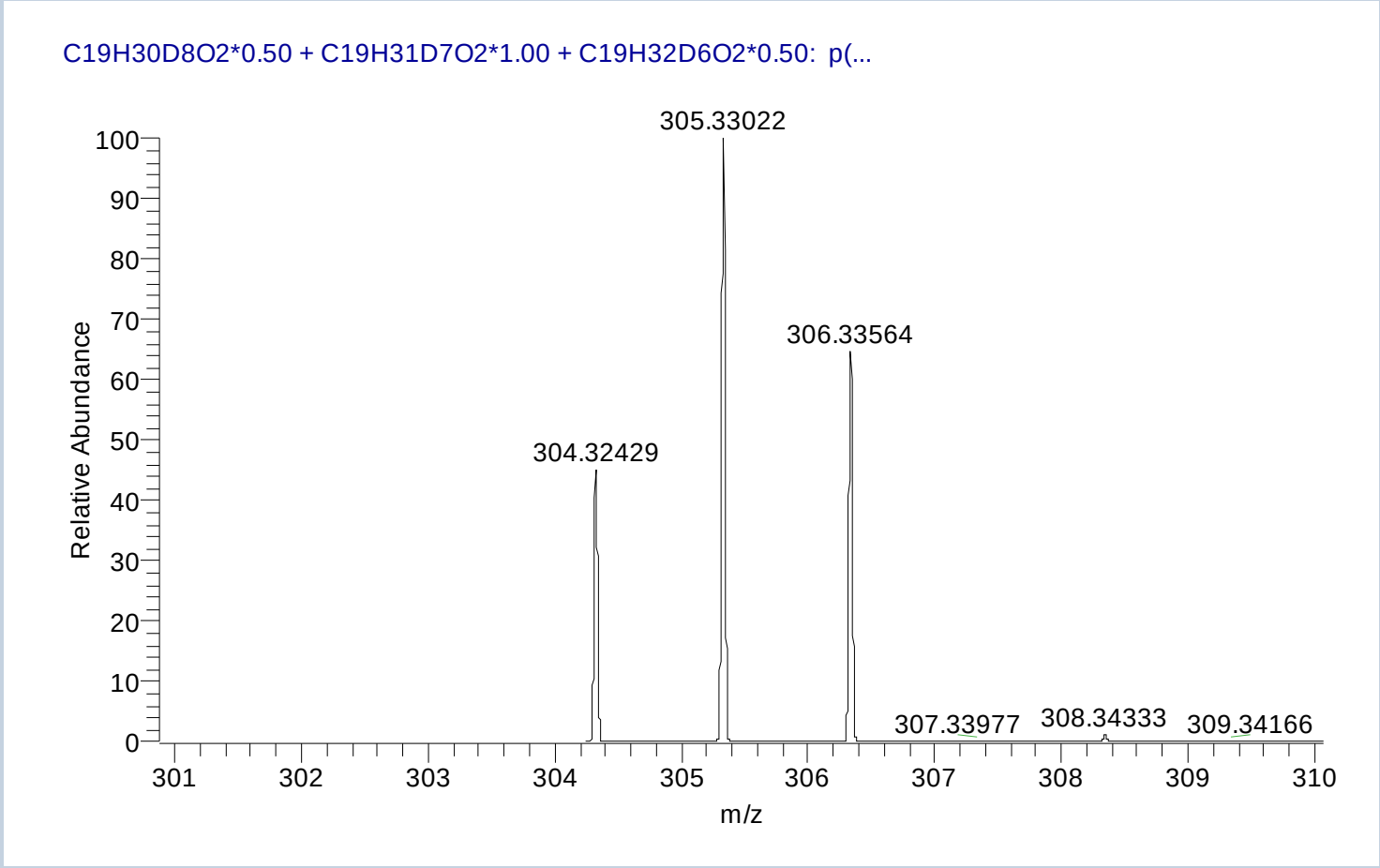
Mass spectra of volatile compounds from the peaks labelled in the chromatograms above. Chemical structures are tentative.

Deuteration investigation

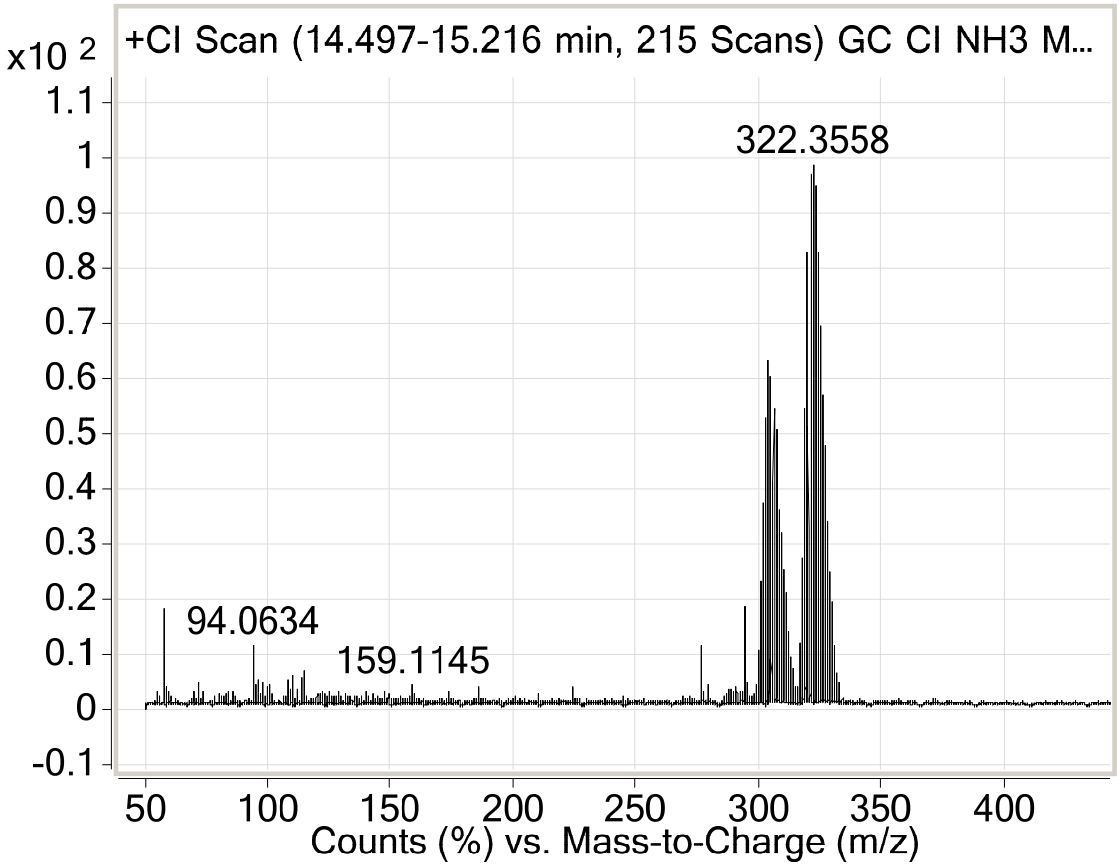


D_8? - $C_{19}H_{30}D_8O_2$ rmm=306
D_7? - $C_{19}H_{31}D_7O_2$ rmm=305
D_6? - $C_{19}H_{32}D_6O_2$ rmm=304

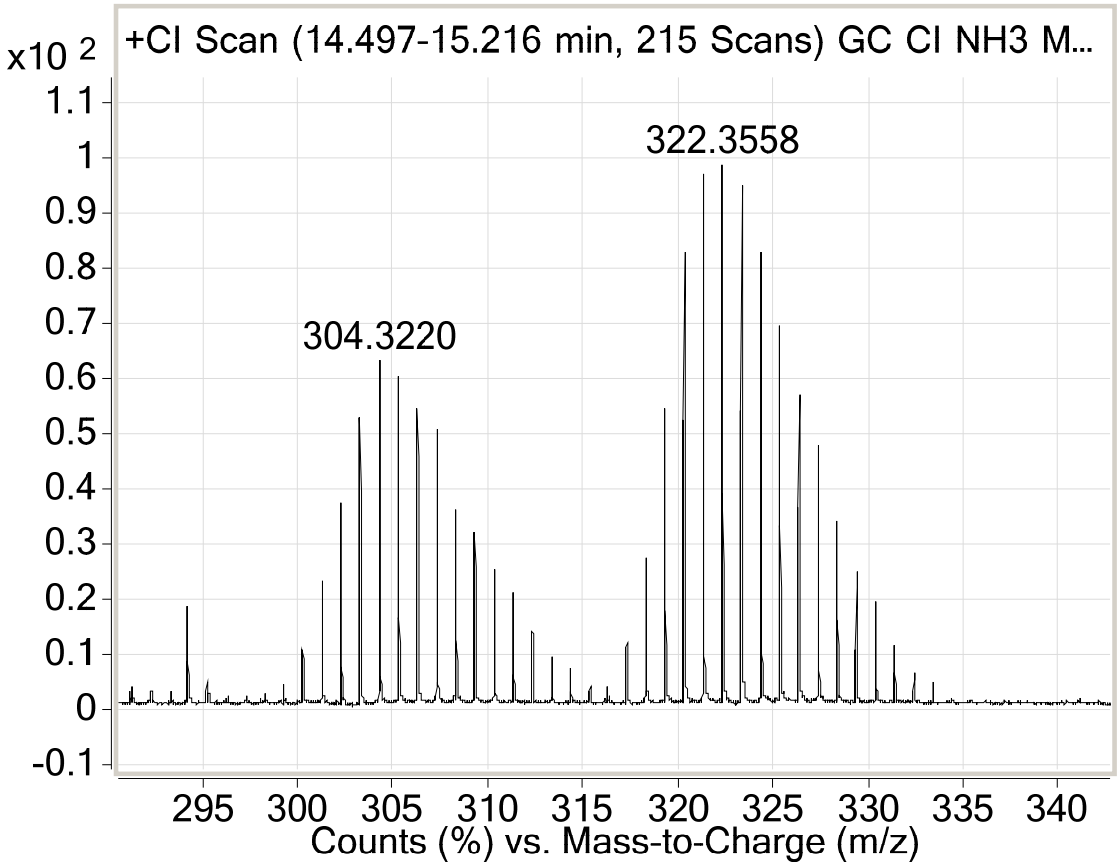
Simulation
$1\text{-}d_6 : 2\text{-}d_7 : 1d_8$



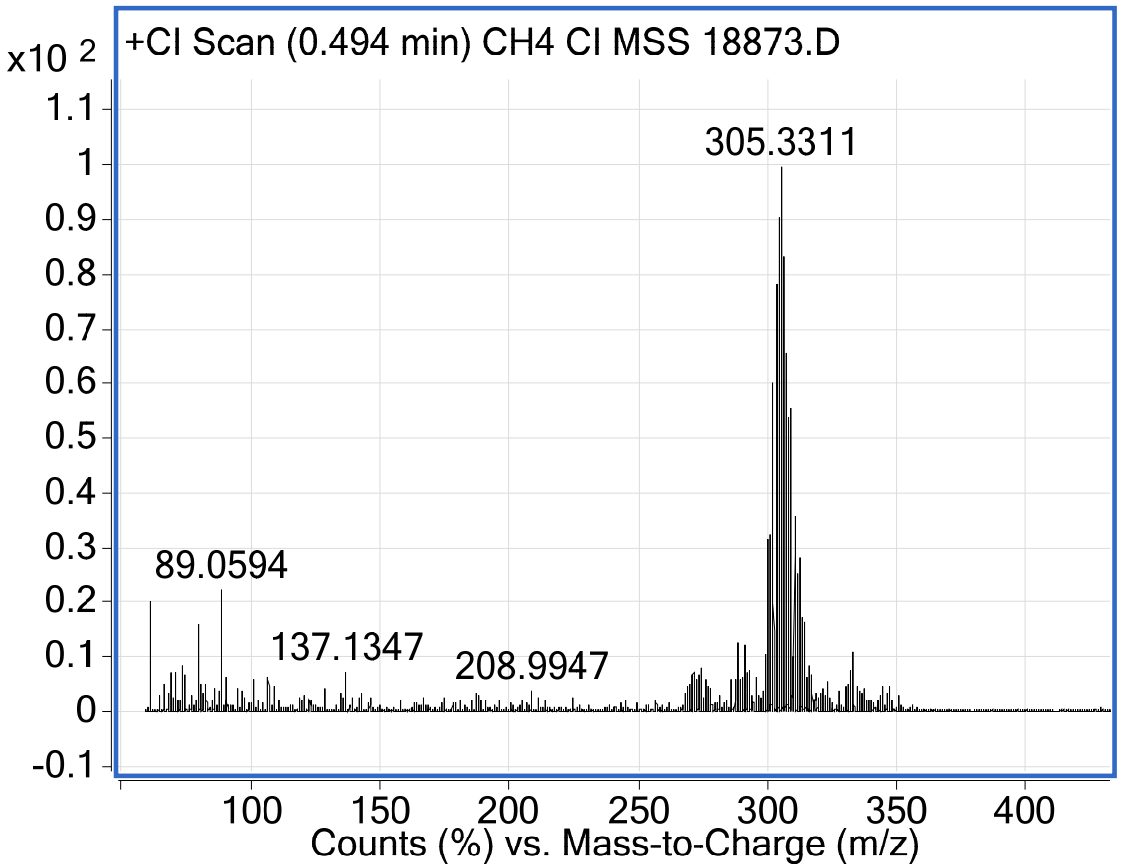
Ammonia CI spectrum



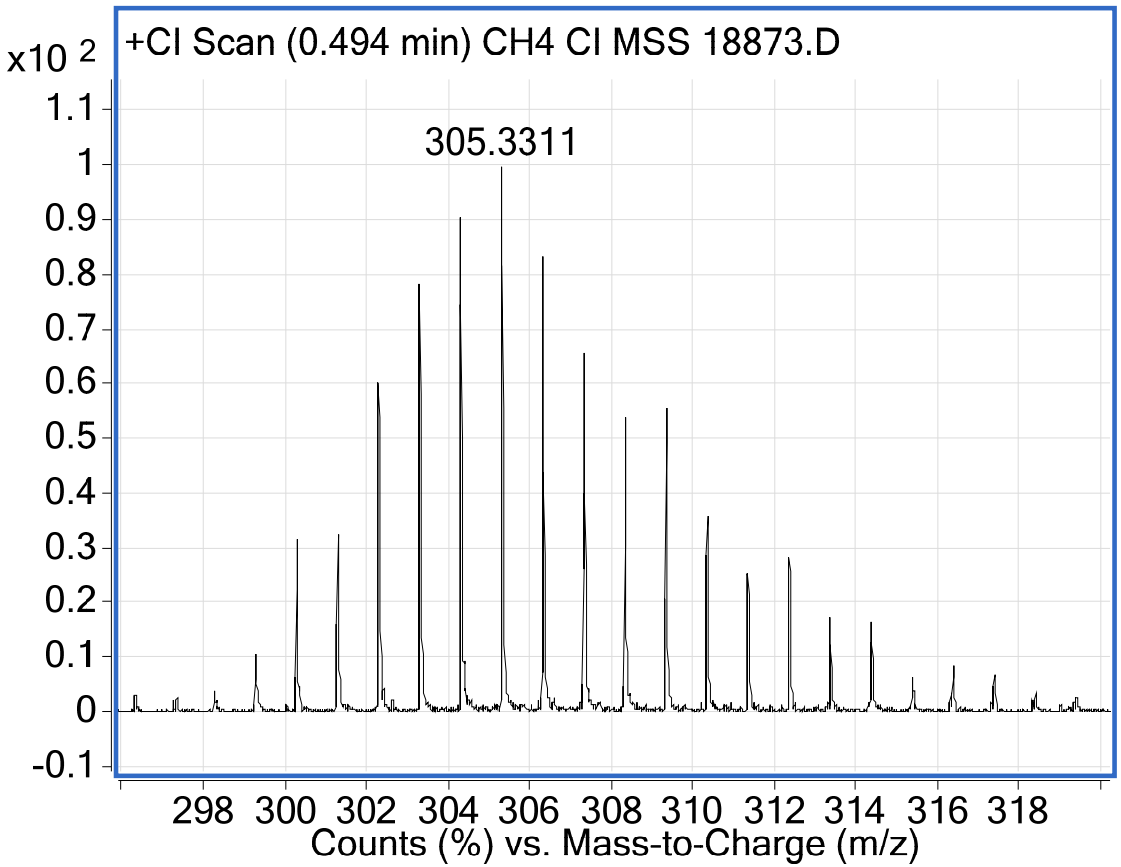
Ammonia CI spectrum



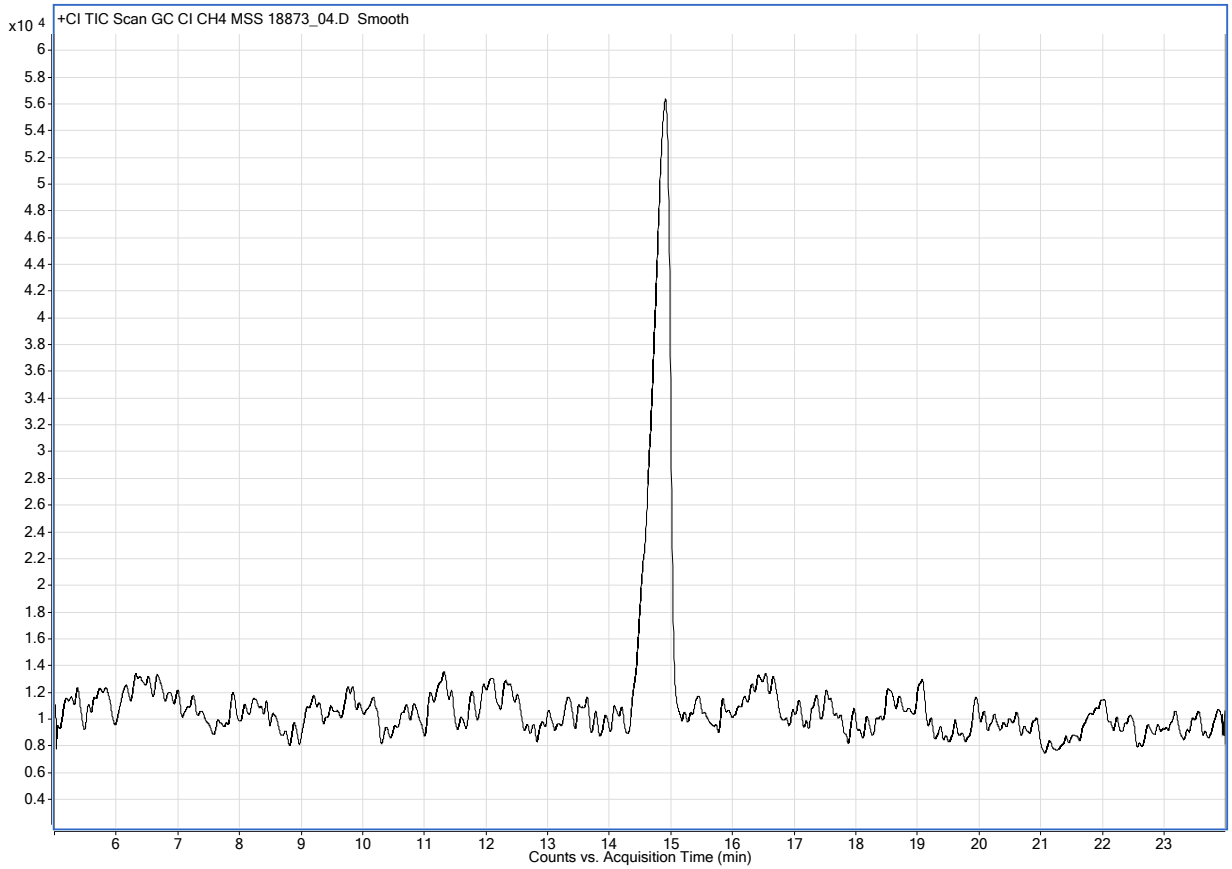
Methane CI spectrum



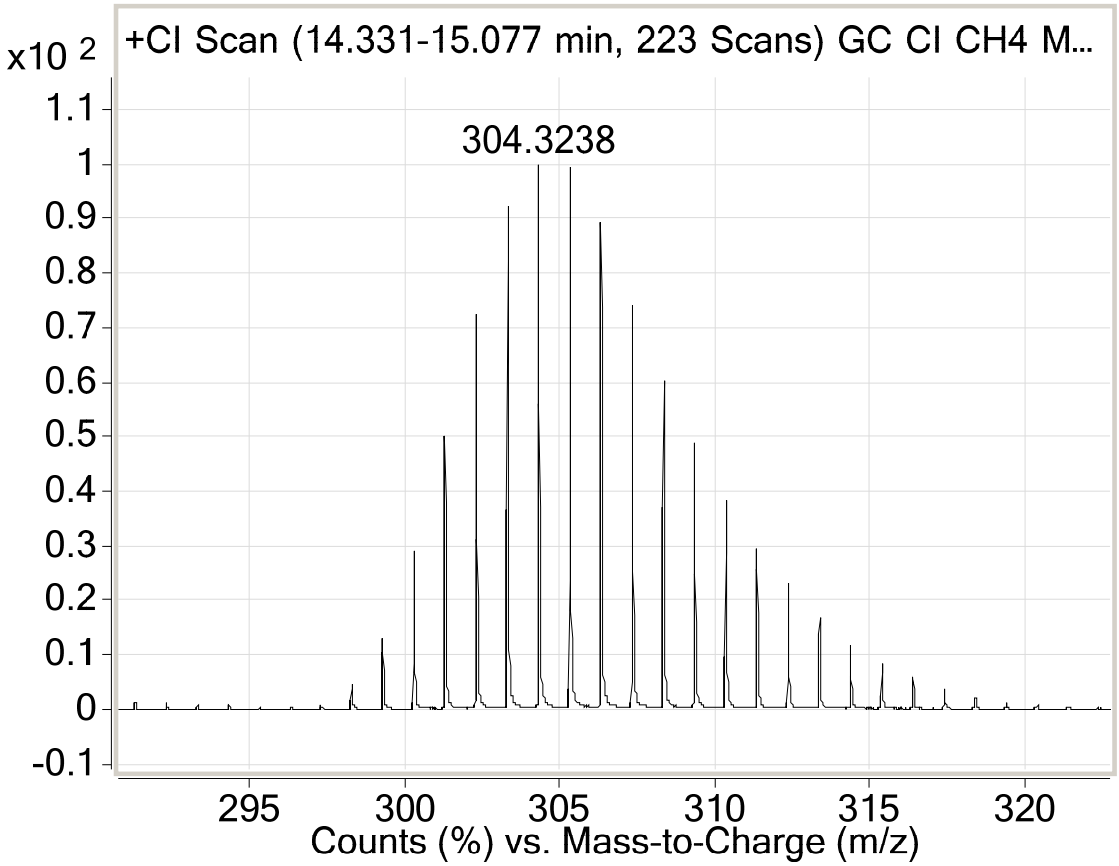
Methane CI spectrum



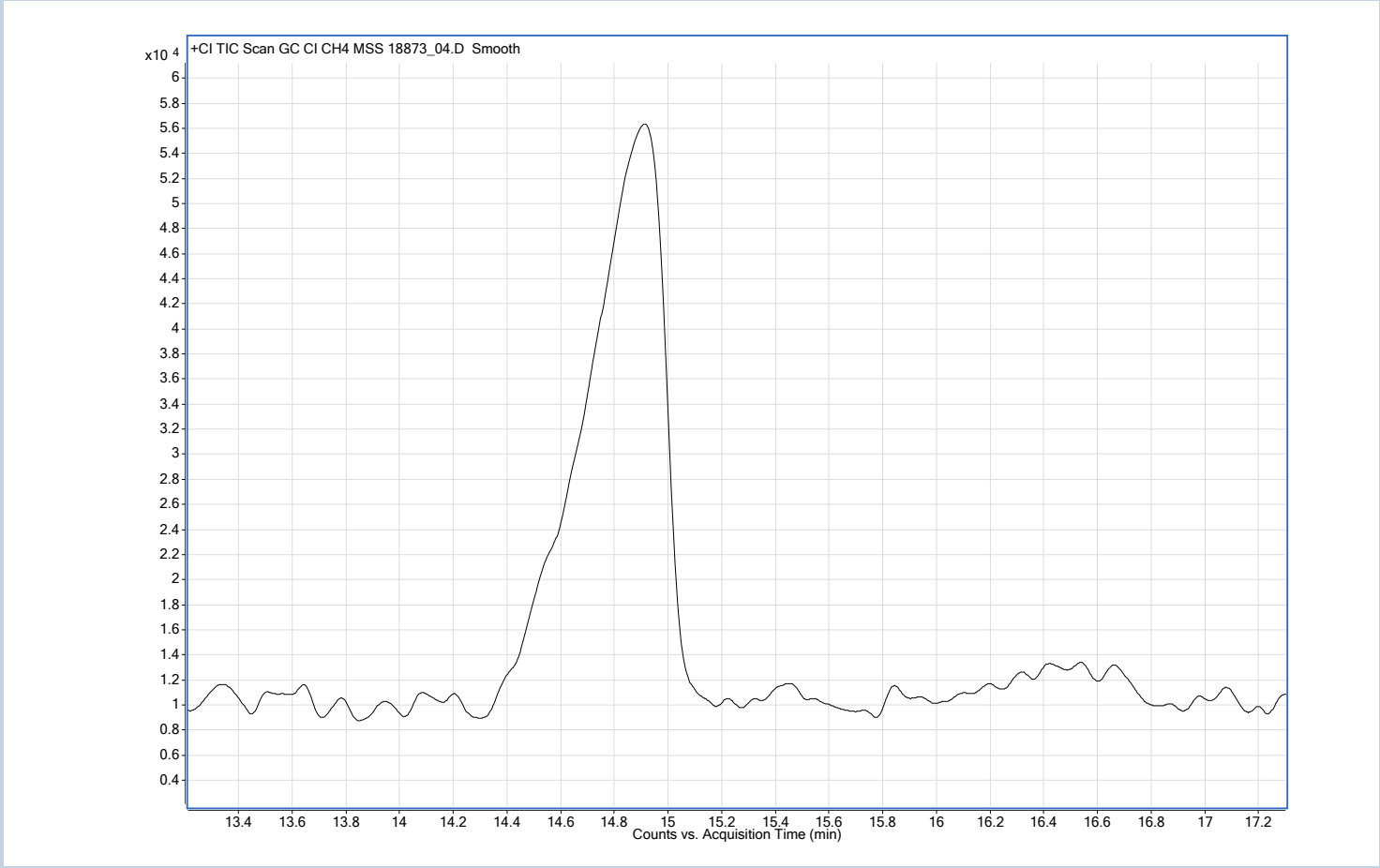
GC Chromatogram first glance



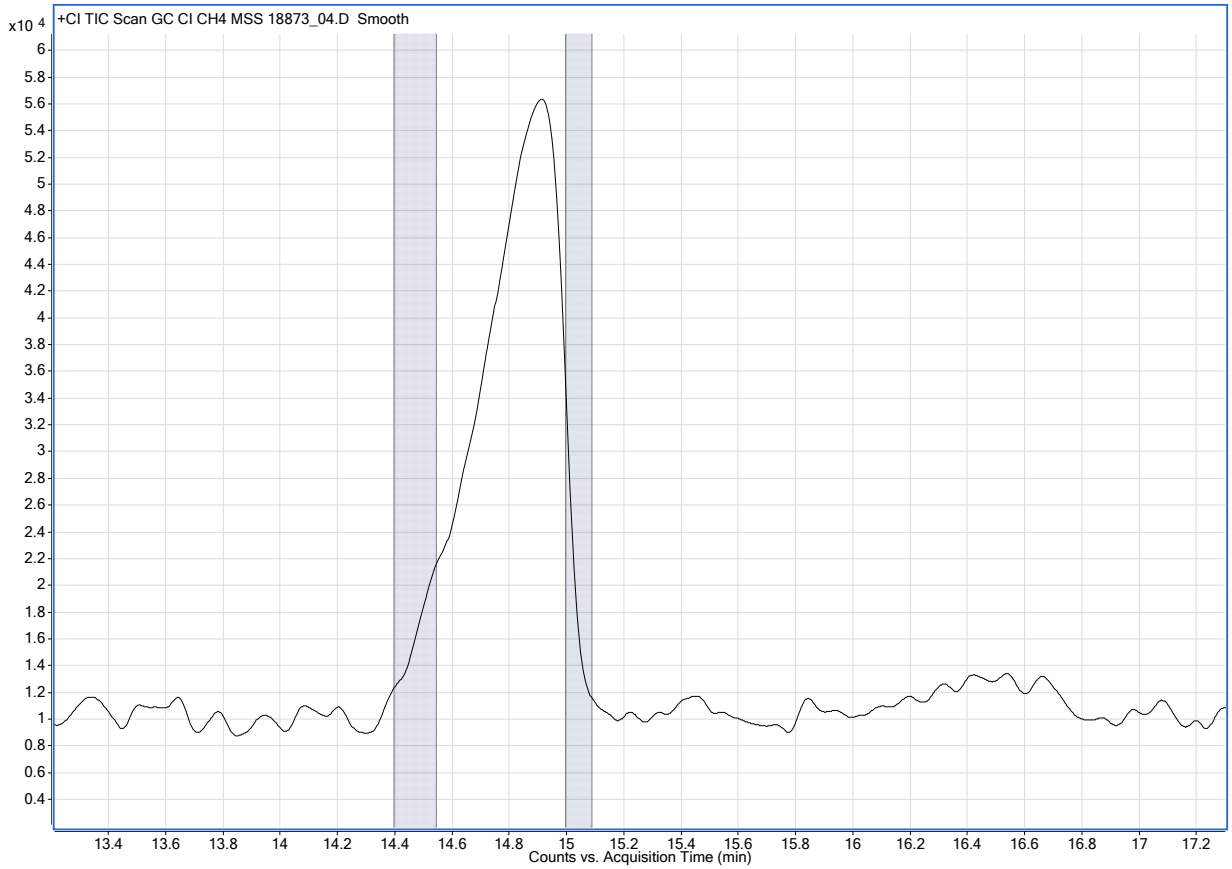
GC Methane CI spectrum first glance



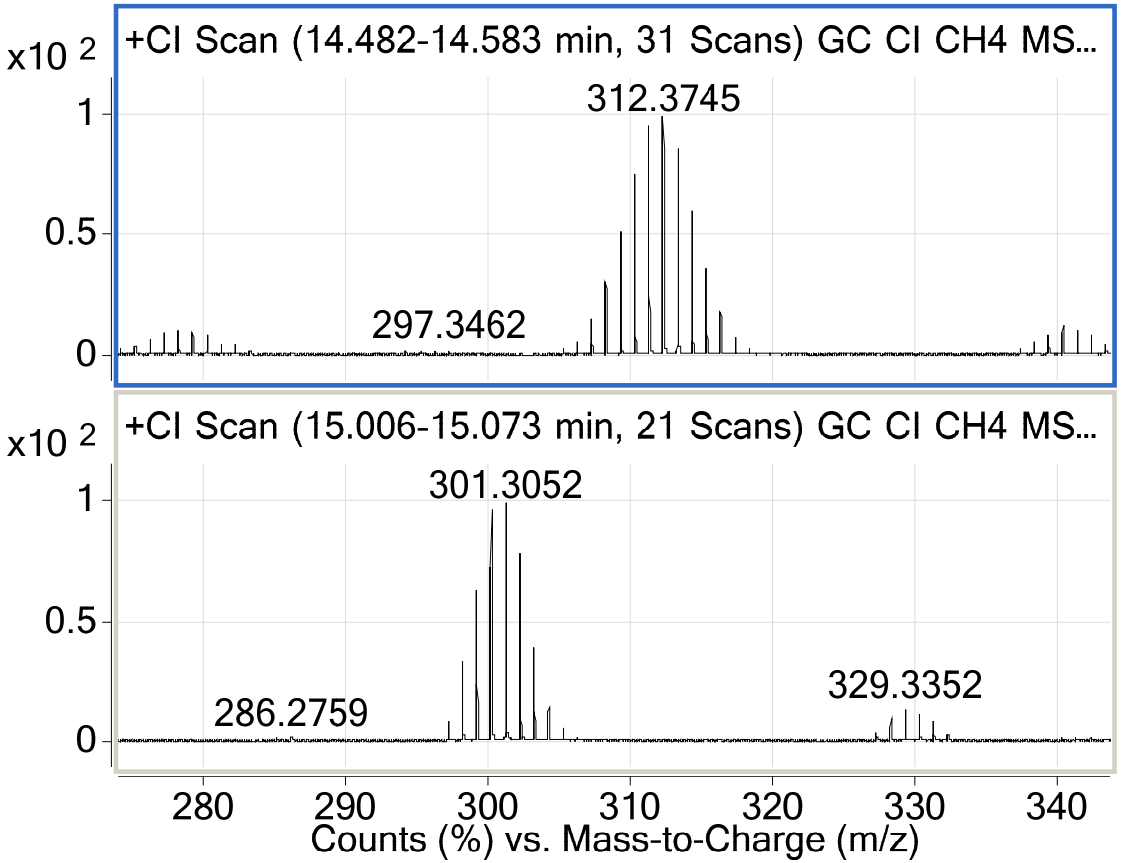
GC Chromatogram



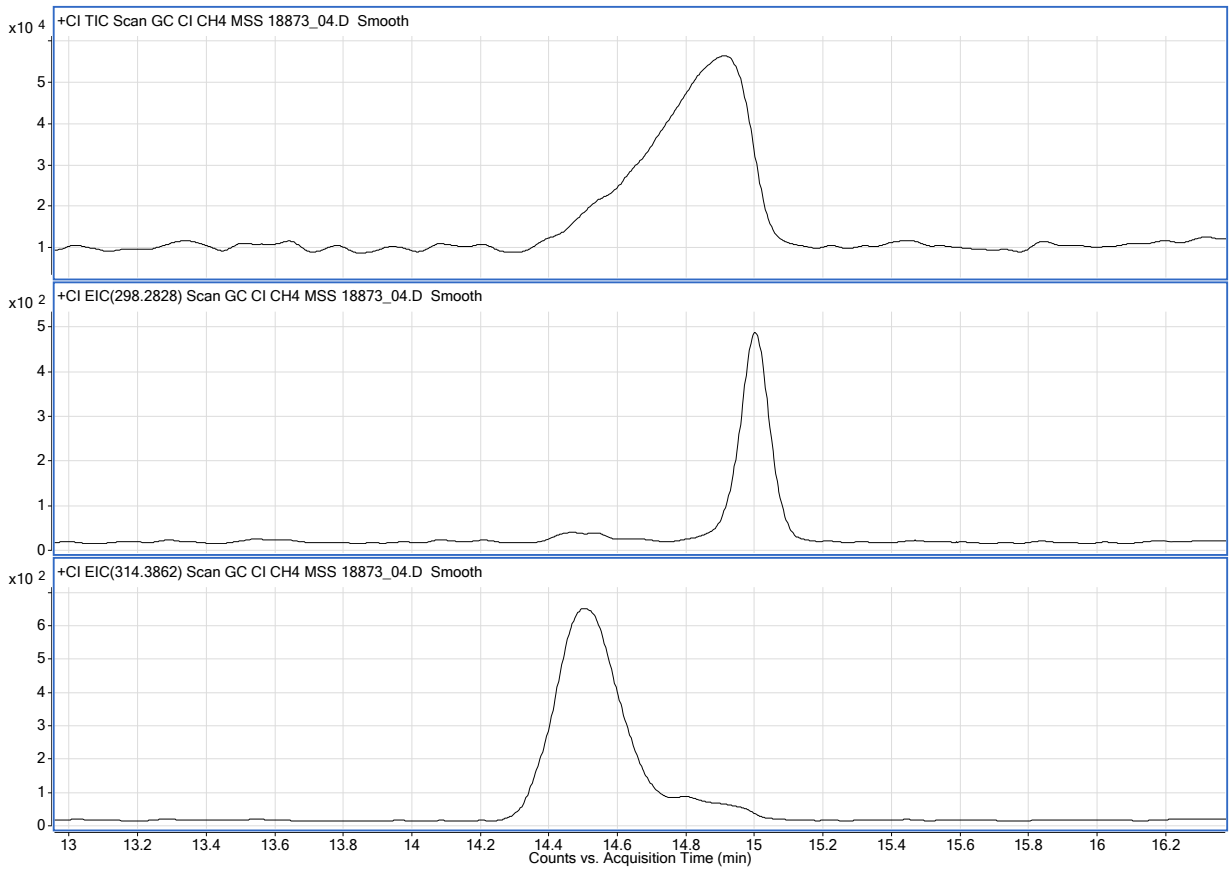
GC Chromatogram



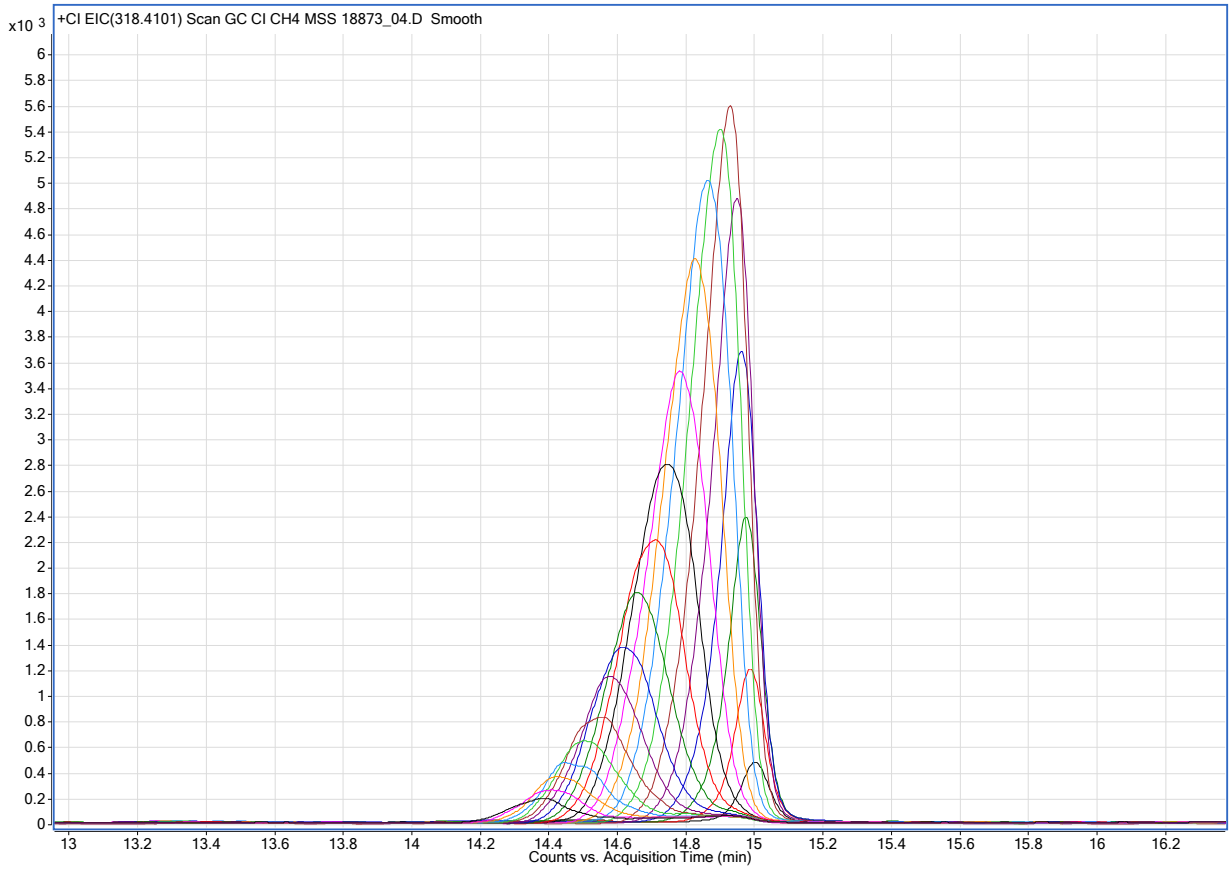
GC Methane CI spectrum



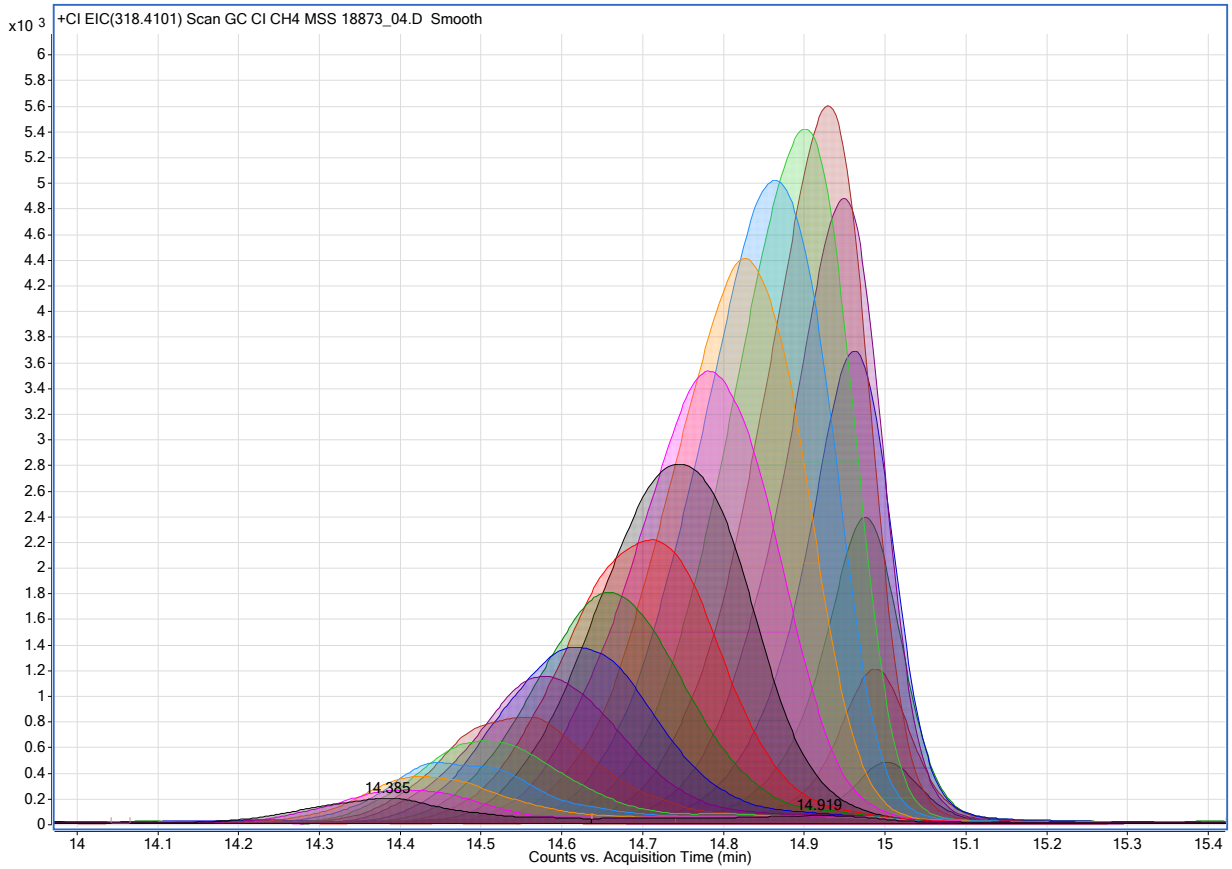
GC Chromatogram extracted ion *m/z*=298 and *m/z*=314



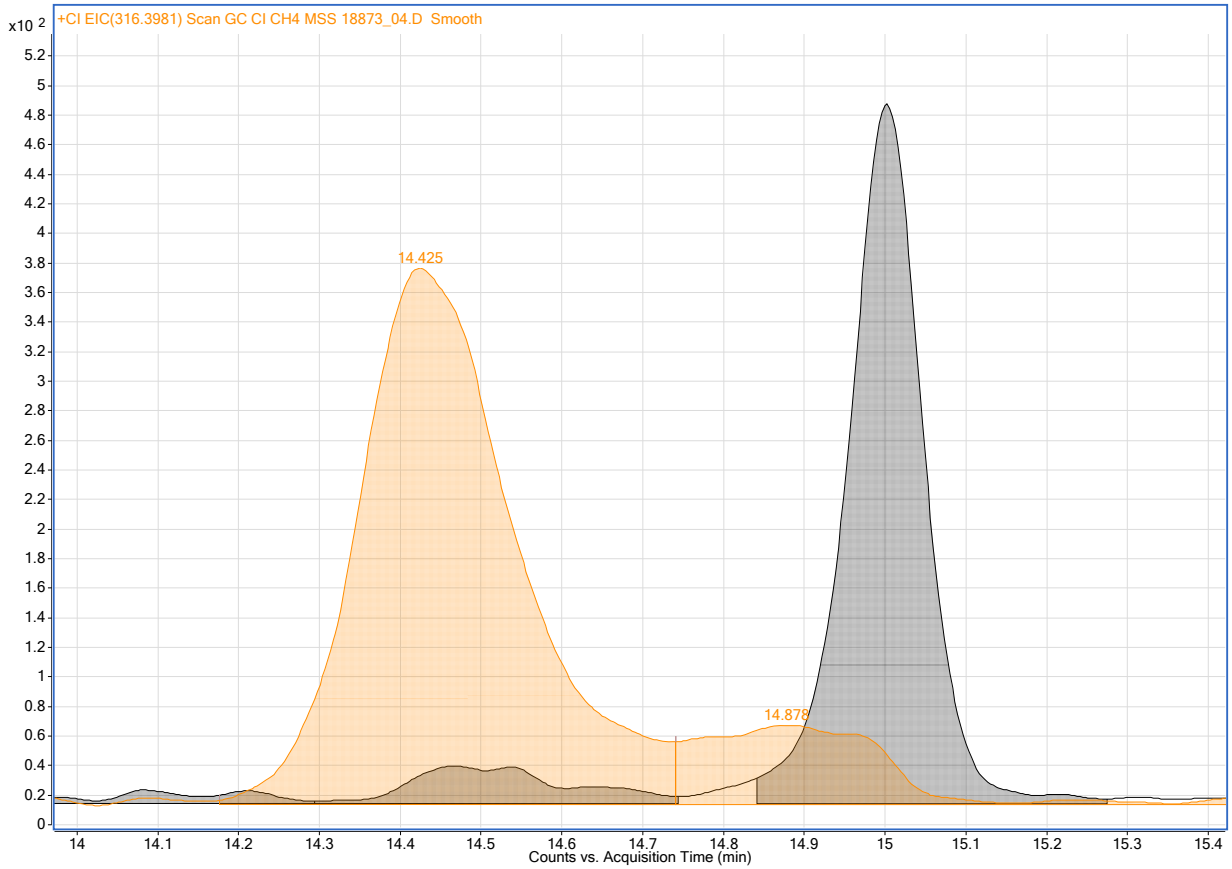
GC Chromatogram extracted ion
every isotopologue



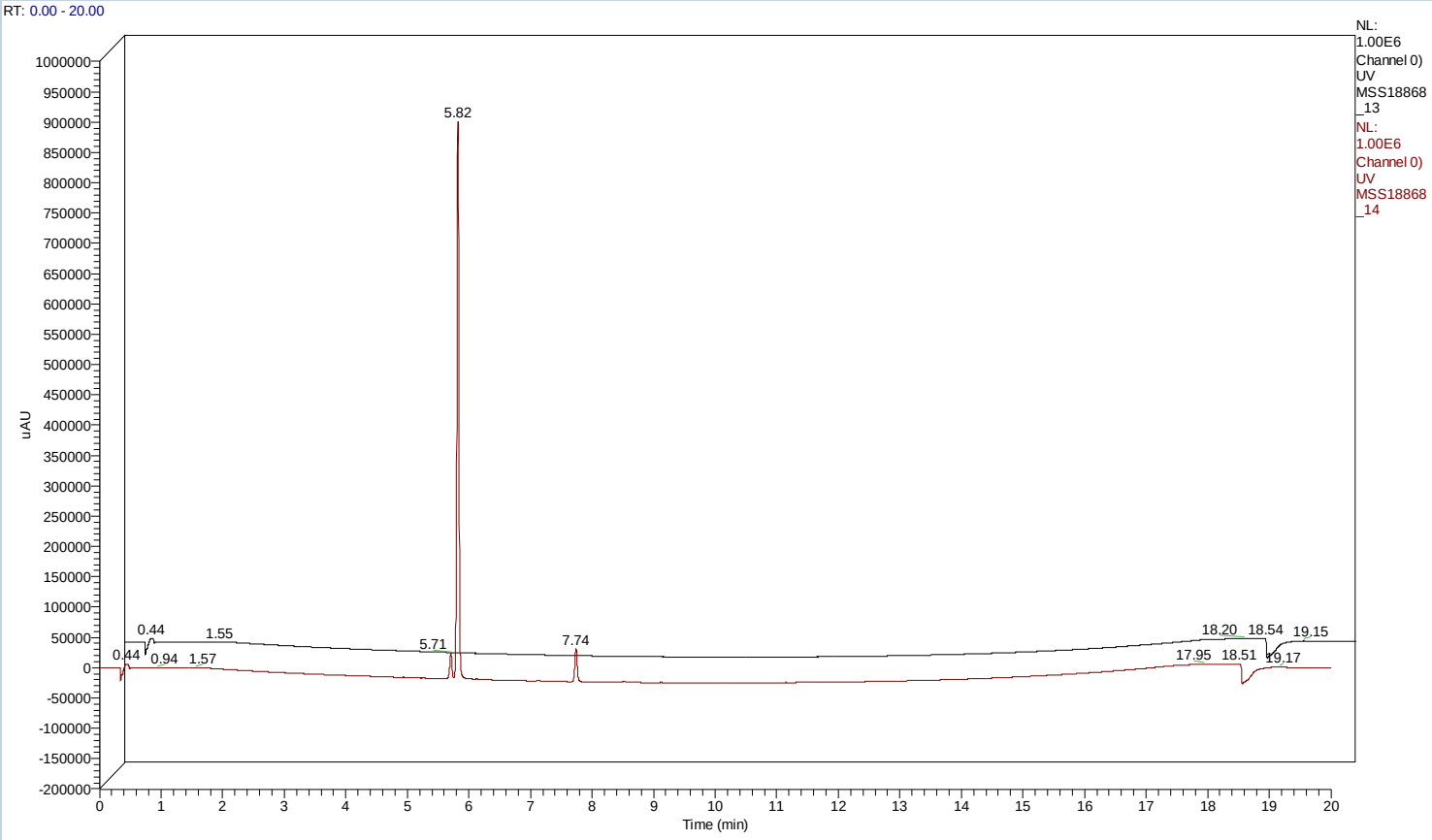
GC Chromatogram extracted ion
every isotopologue



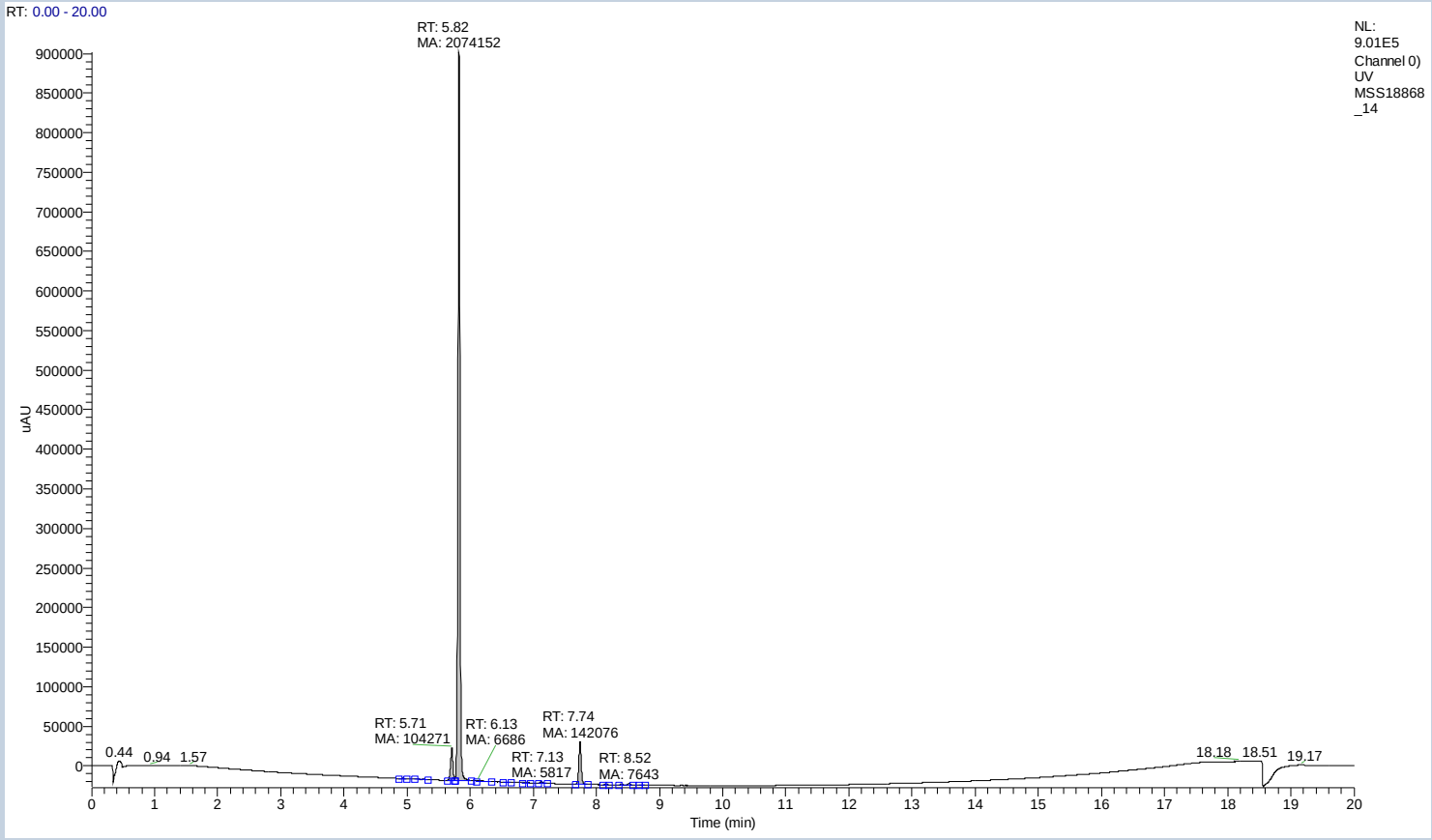
GC Chromatogram extracted ion
m/z=298 and *m/z*=314 peak width



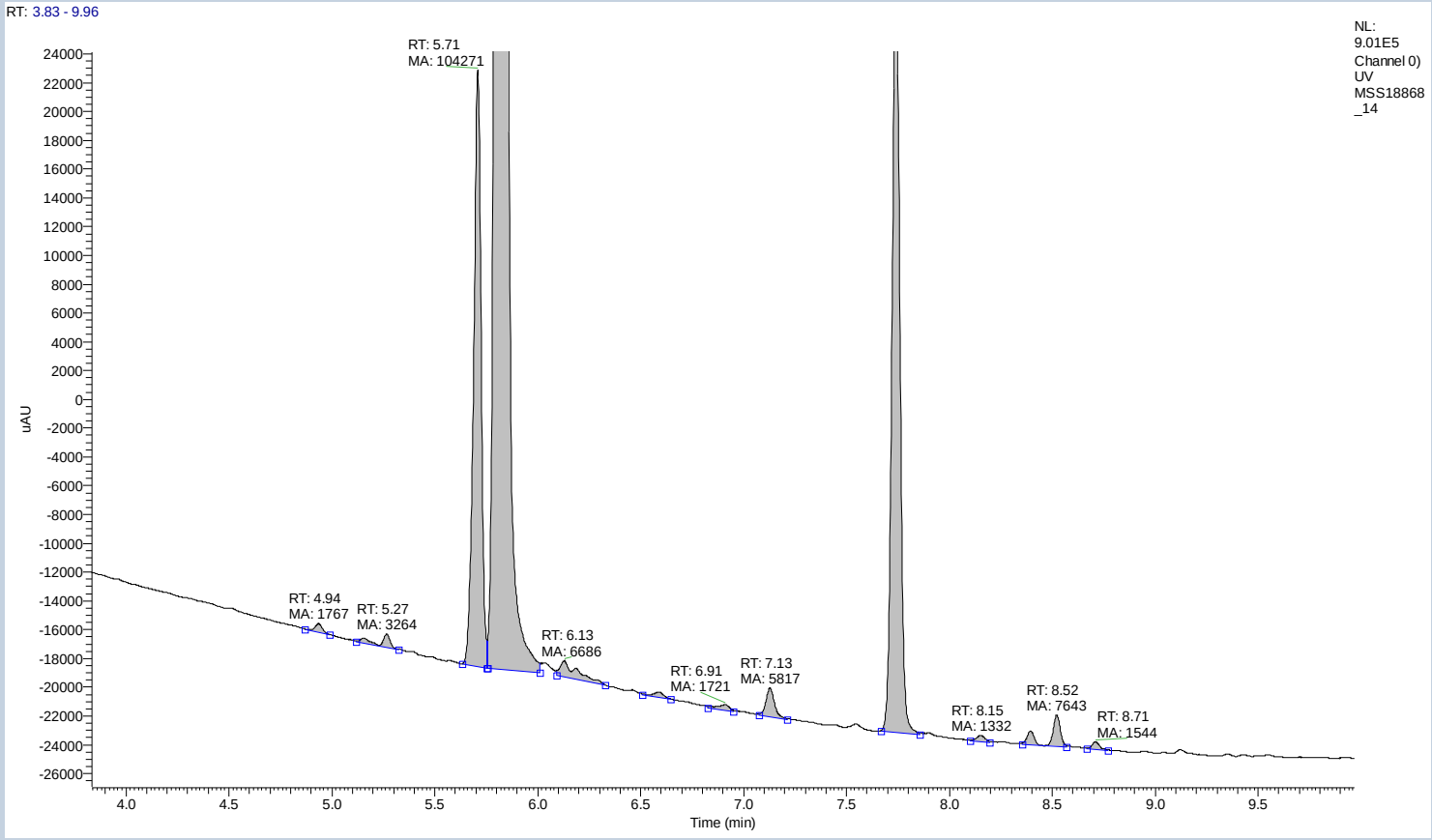
“Purity” by LC-MS
a can of worms



UV Chromatogram at 322nm



UV Chromatogram at 322nm



Peak area table (322nm)

PEAK LIST

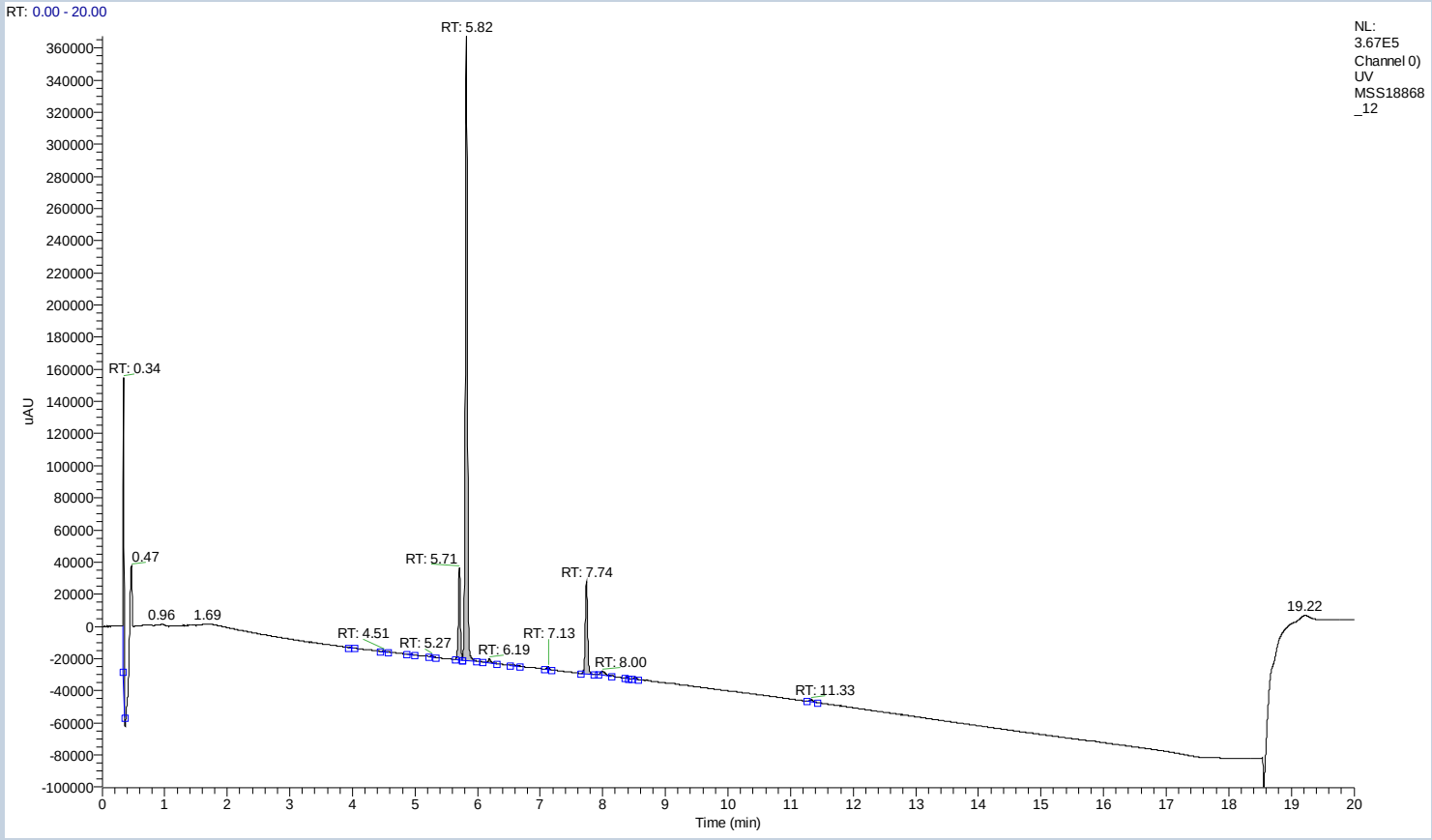
MSS18868_14.raw

RT: 3.83 - 9.96

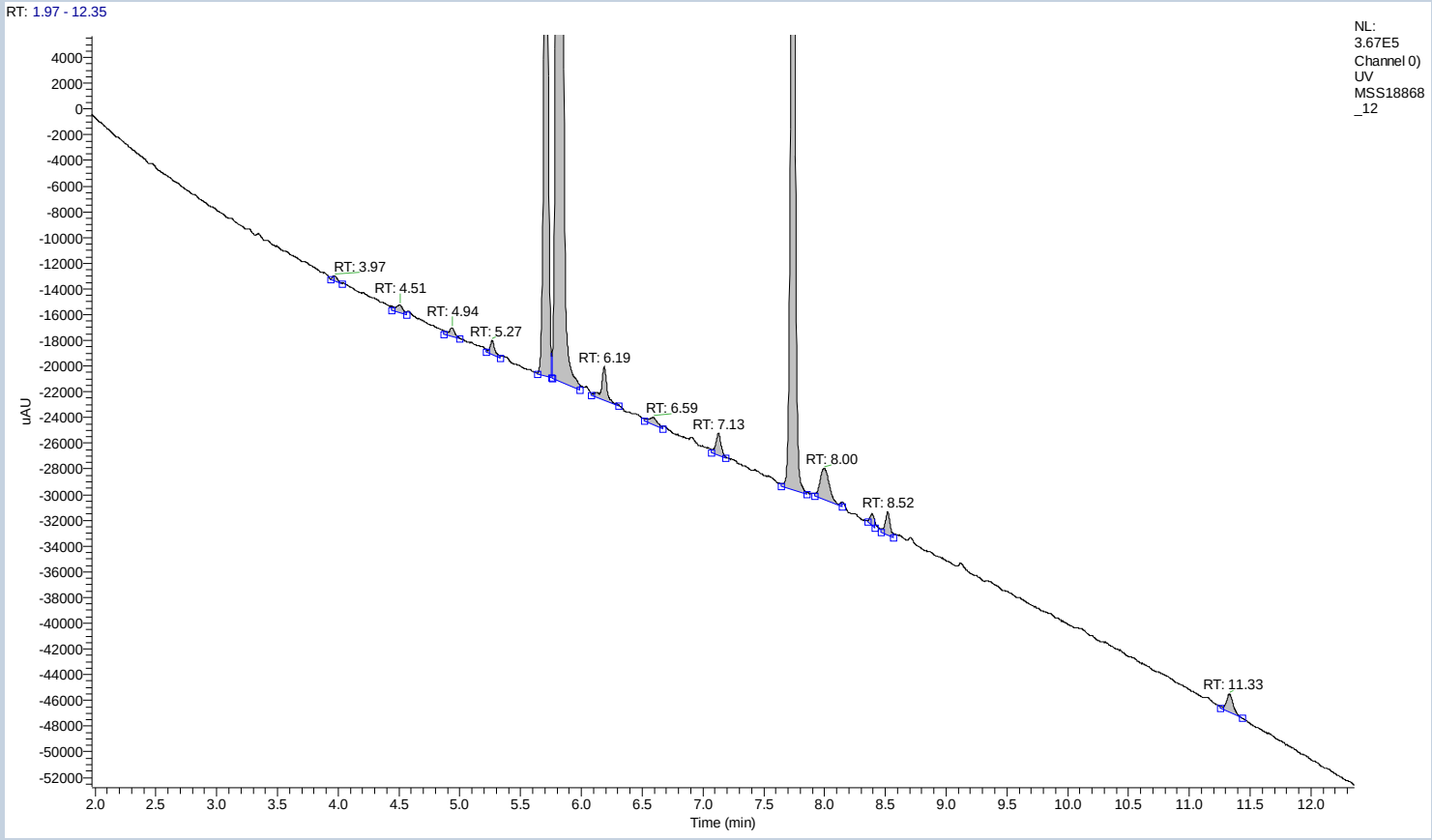
Number of detected peaks: 12

Apex RT	Area	%Area
4.94	1766.772	0.08
5.27	3264.045	0.14
5.71	104270.7	4.43
5.82	2074152	88.19
6.13	6685.573	0.28
6.59	1527.32	0.06
6.91	1720.855	0.07
7.13	5817.416	0.25
7.74	142076.2	6.04
8.15	1331.607	0.06
8.52	7642.639	0.32
8.71	1544.385	0.07

UV Chromatogram at 240nm



UV Chromatogram at 240nm

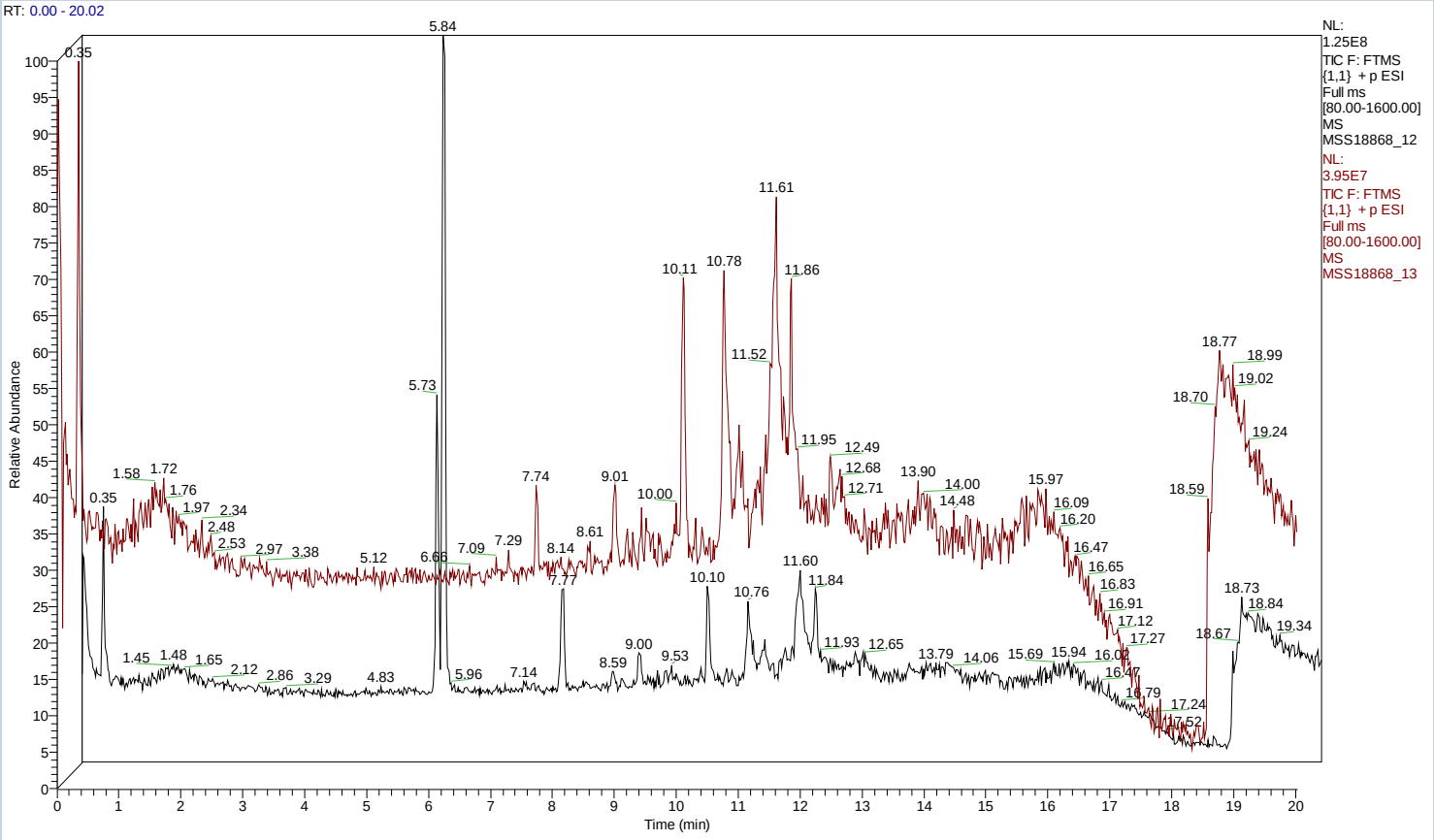


Peak area table (240nm)

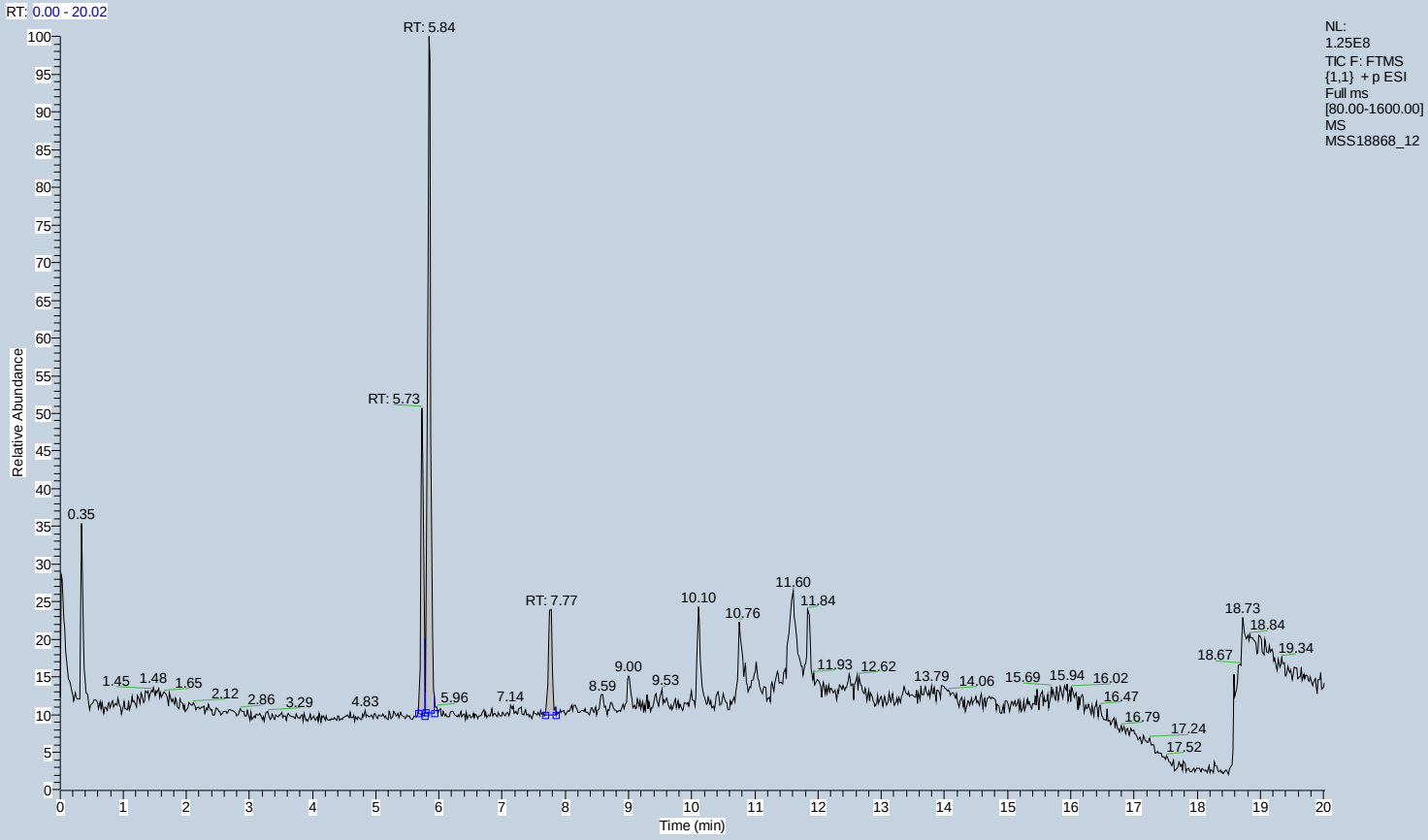
PEAK LIST
MSS18868_12.raw
RT: 1.97 - 12.35
Number of detected peaks: 15

Apex RT	Area	%Area
0.34	190741.58	13.47
3.97	1165.396	0.08
4.51	2693.562	0.19
4.94	2527.462	0.18
5.27	3057.909	0.22
5.71	129118.483	9.12
5.82	892036.507	63.01
6.19	8201.617	0.58
6.59	2892.024	0.2
7.13	5260.836	0.37
7.74	151938.723	10.73
8	13224.811	0.93
8.39	1976.459	0.14
8.52	5189.208	0.37
11.33	5682.965	0.4

Mass chromatogram TIC pos



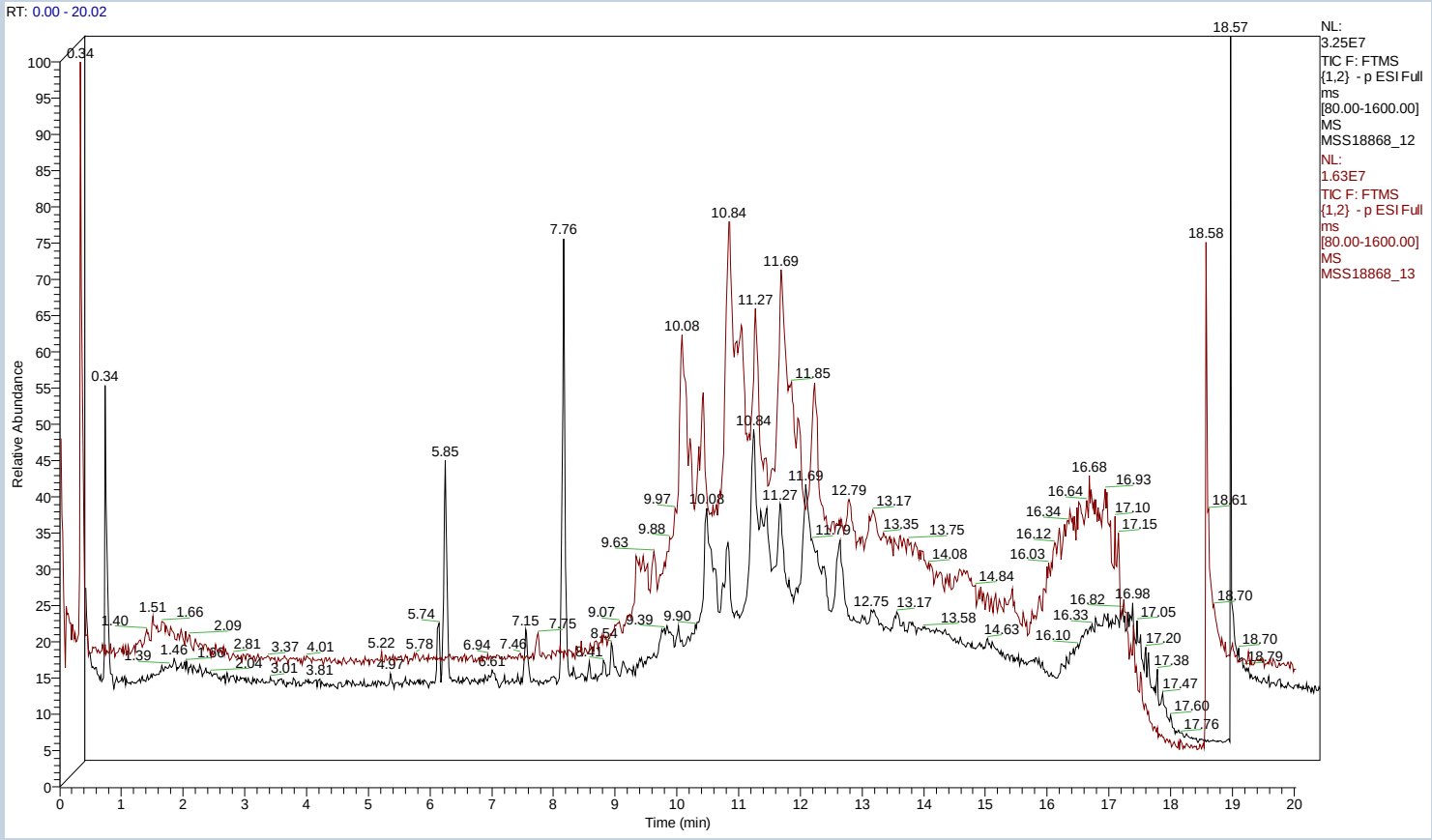
Mass chromatogram TIC pos



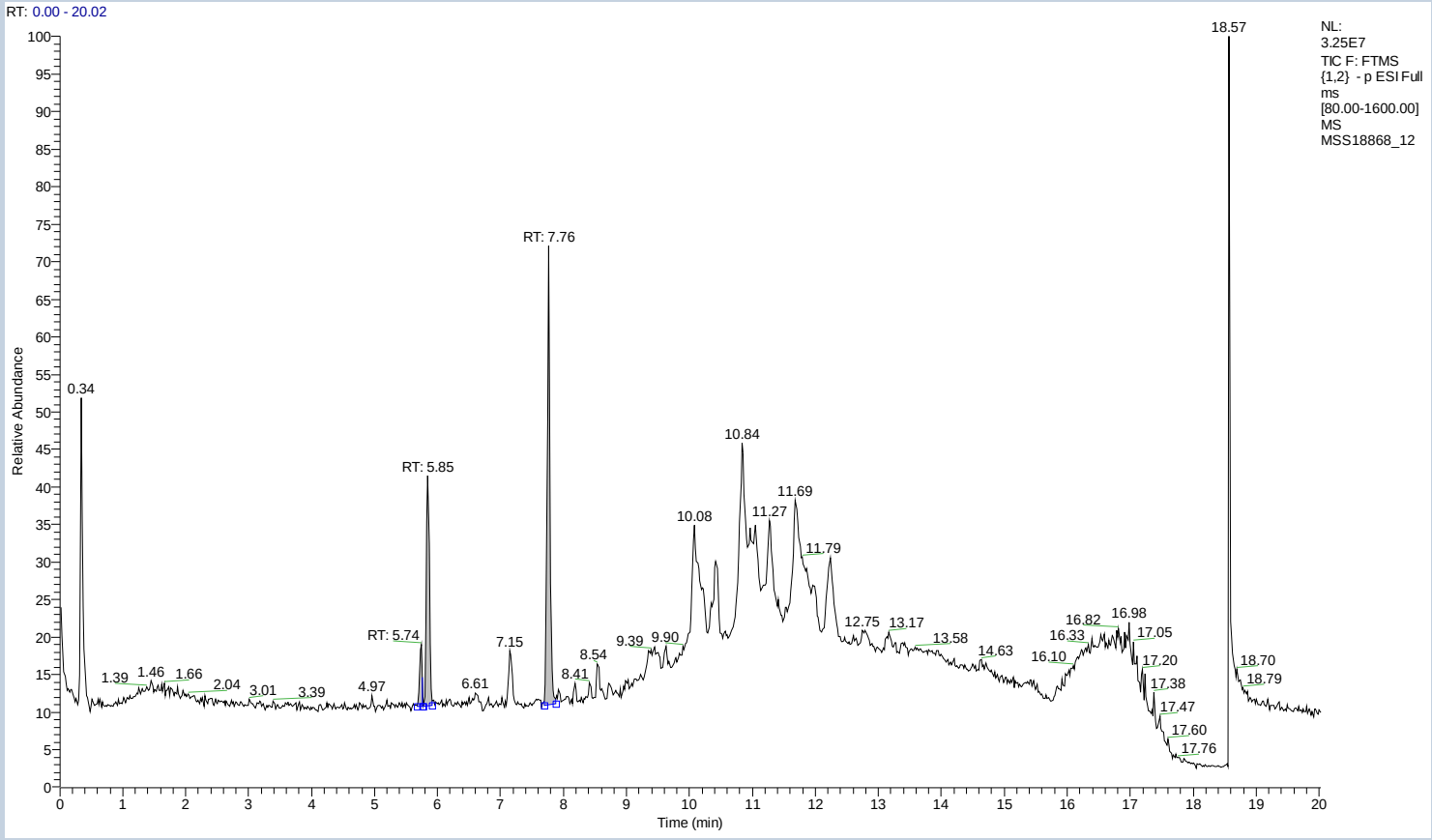
Peak area table (positive TIC)

	PEAK LIST			
	MSS18868_12.raw			
	RT: 0.00 - 20.02			
	Number of detected peaks: 3			
	Apex RT	Area	%Area	
	5.73	153838800.8	24.35	
	5.84	404375386.6	64.02	
	7.77	73471112.5	11.63	

Mass chromatogram TIC neg



Mass chromatogram TIC neg



Peak area table (negative TIC)

	PEAK LIST			
	MSS18868_12.raw			
	RT: 0.00 - 20.02			
	Number of detected peaks: 3			
	Apex RT	Area	%Area	
	5.74	6983375.211	6.69	
	5.85	34258905.82	32.8	
	7.76	63216928.44	60.52	

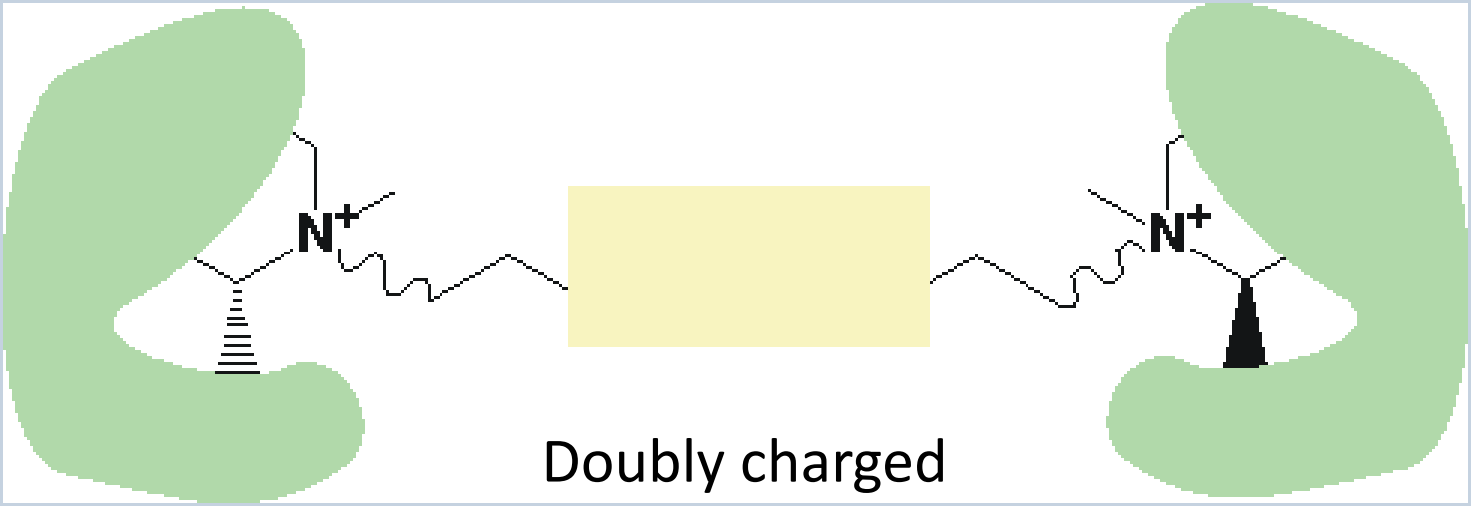
Round up

- Mass spectrometry and separation science is a great combo
- Many problems have be faced and solved
  - Check the literature and commercial application notes!
- Many more problems to be investigated

Extra examples

Example 2

- Legacy compound
- Wanted to reformulate/new therapeutic area



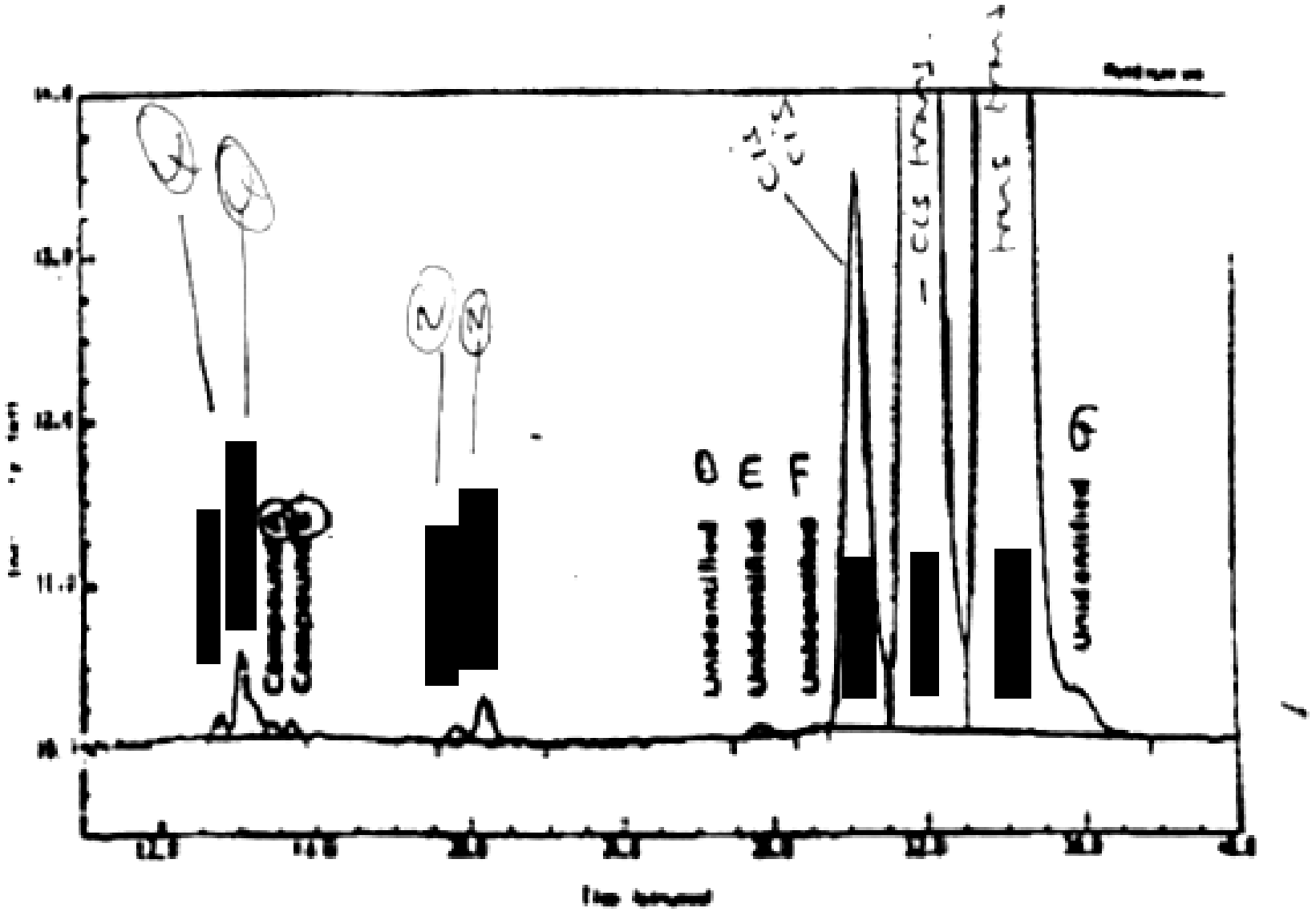
Doubly charged

4 chiral centres

RMM 1014

m/z 514

Example Sample Chromatogram

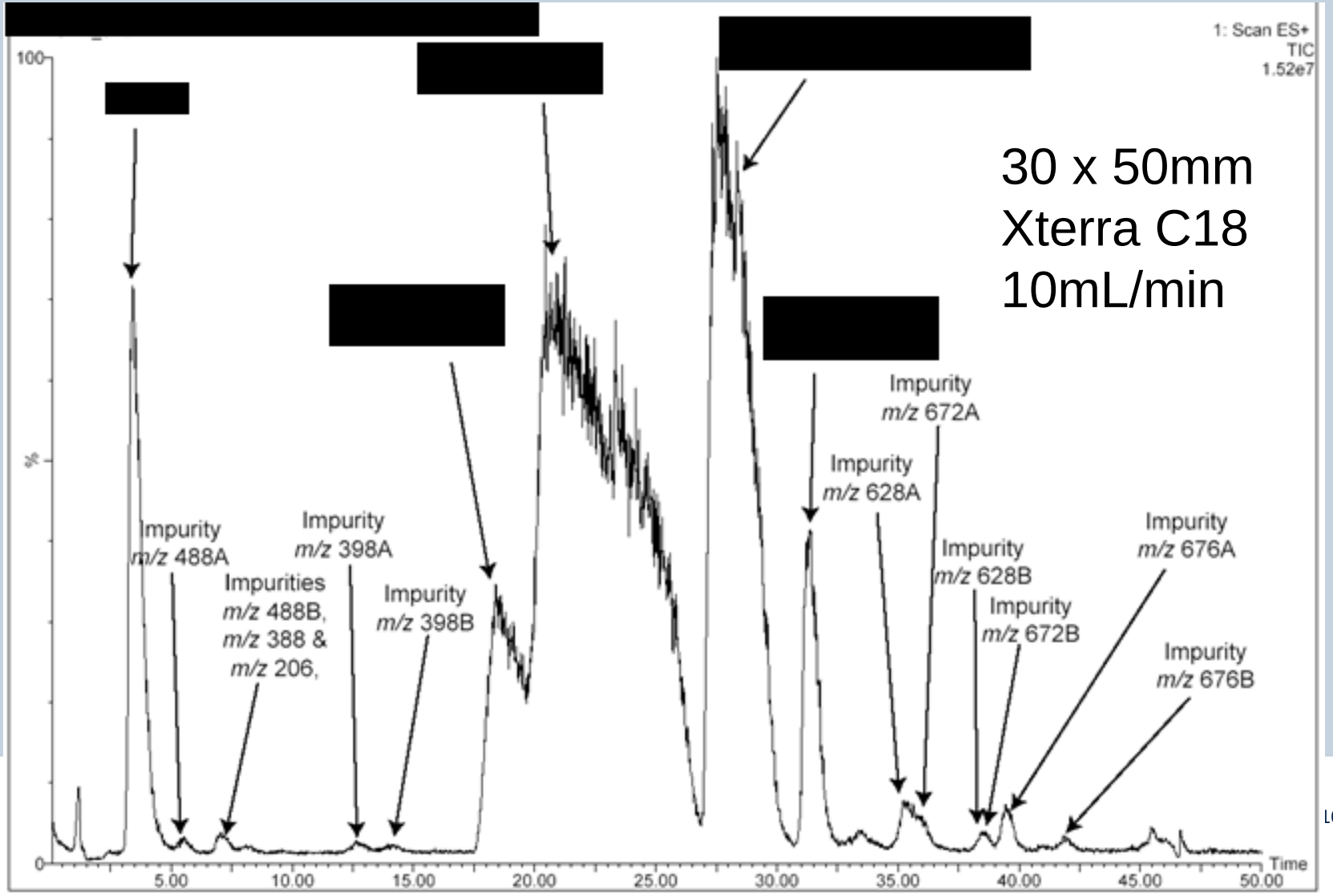


Example 2

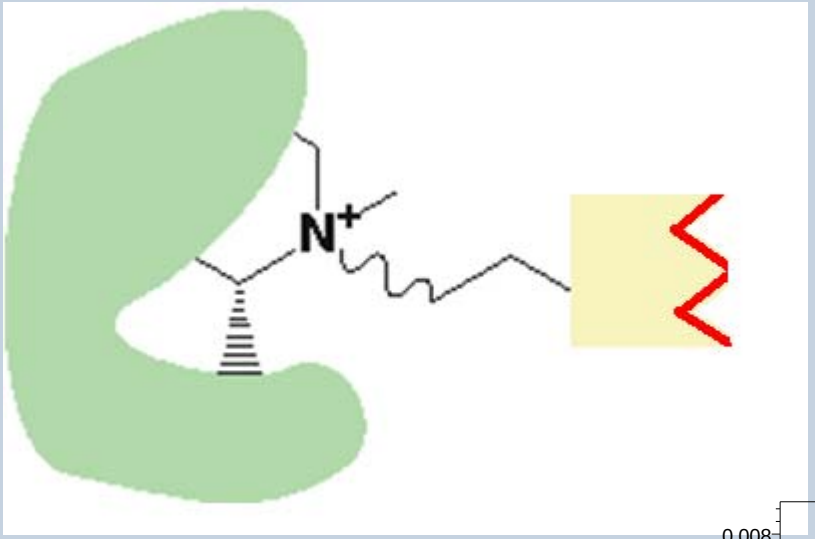
- The original LC method

- 0.4 mL.min^{-1}
- 84 acetonitrile to 16 water
  - + 1% conc Phosphoric acid
- Whatman Partisil-5, 25cm x 4.6mm
- Bare silica

Example 2
Prep Reversed phase LC

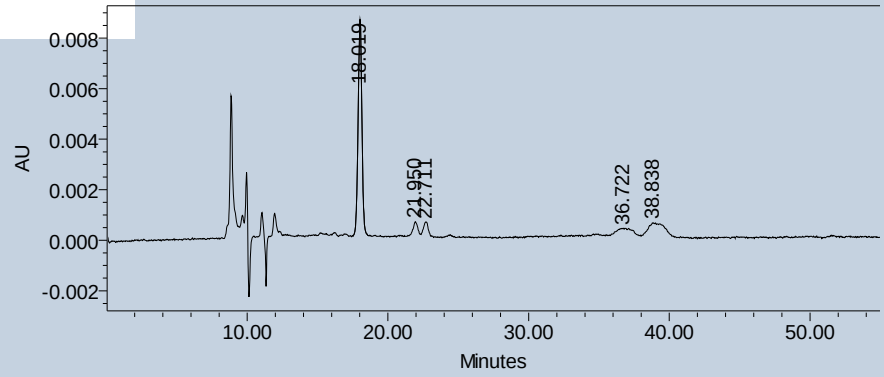


Example 2



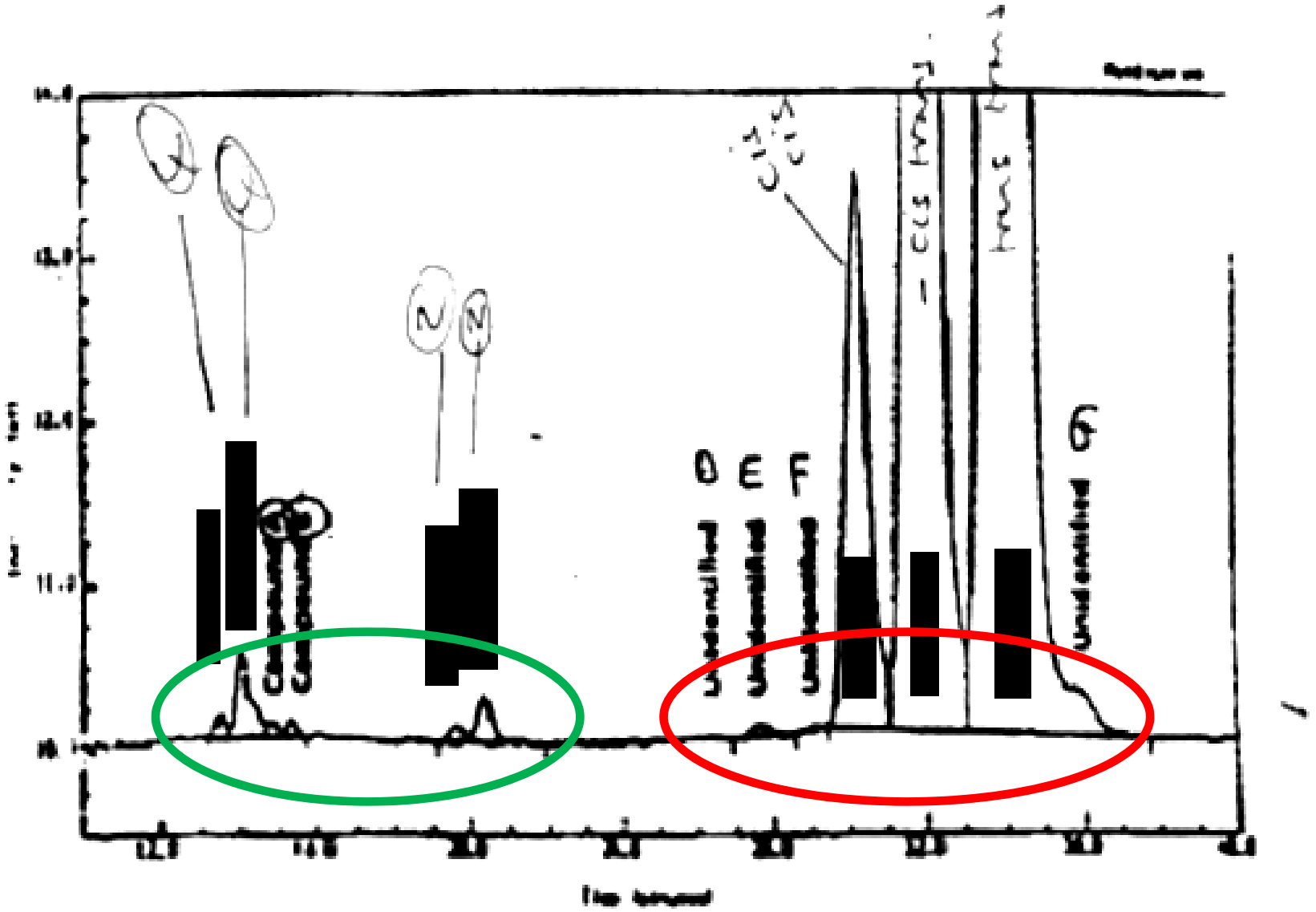
NMR

Original LC

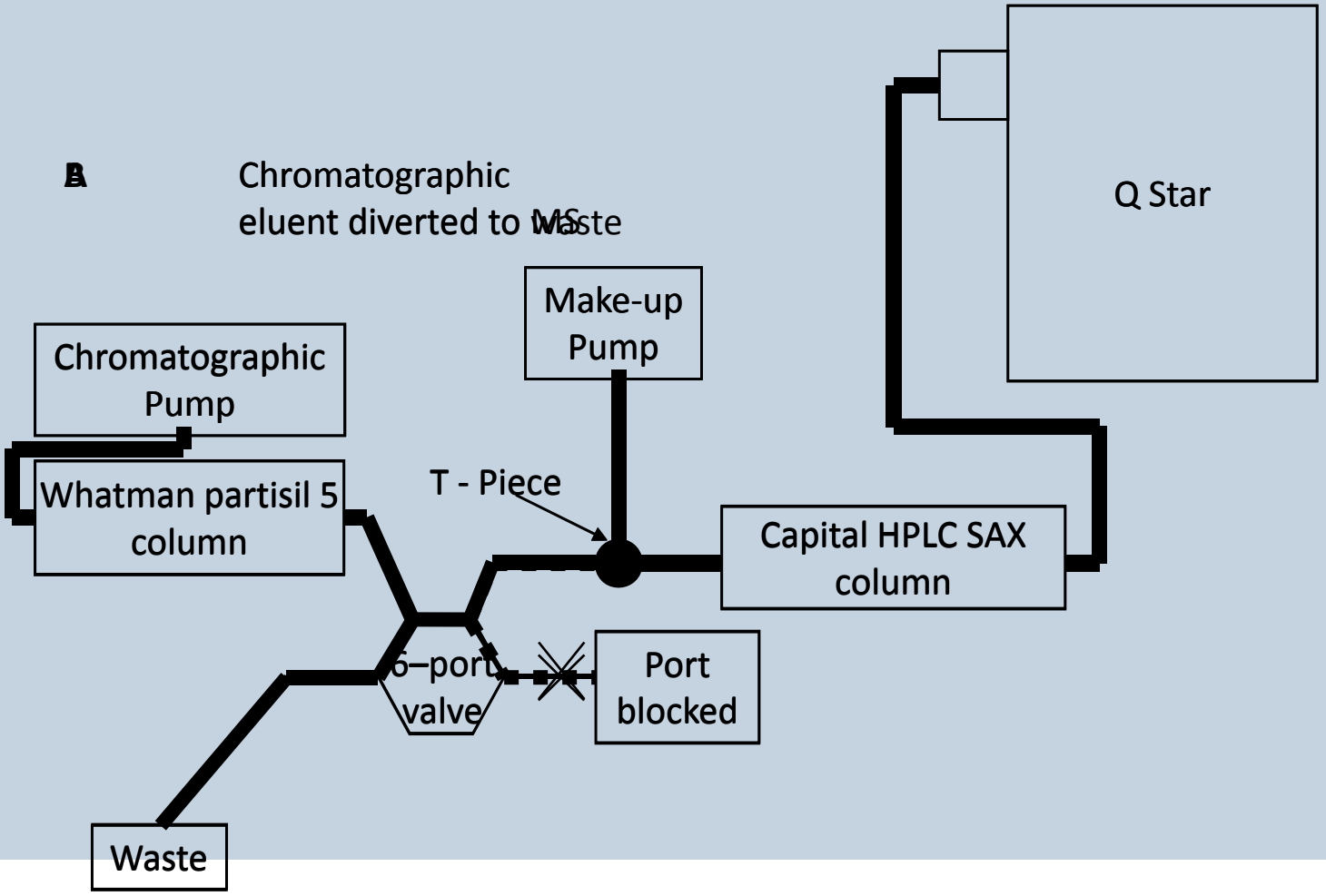


One of early eluting imps

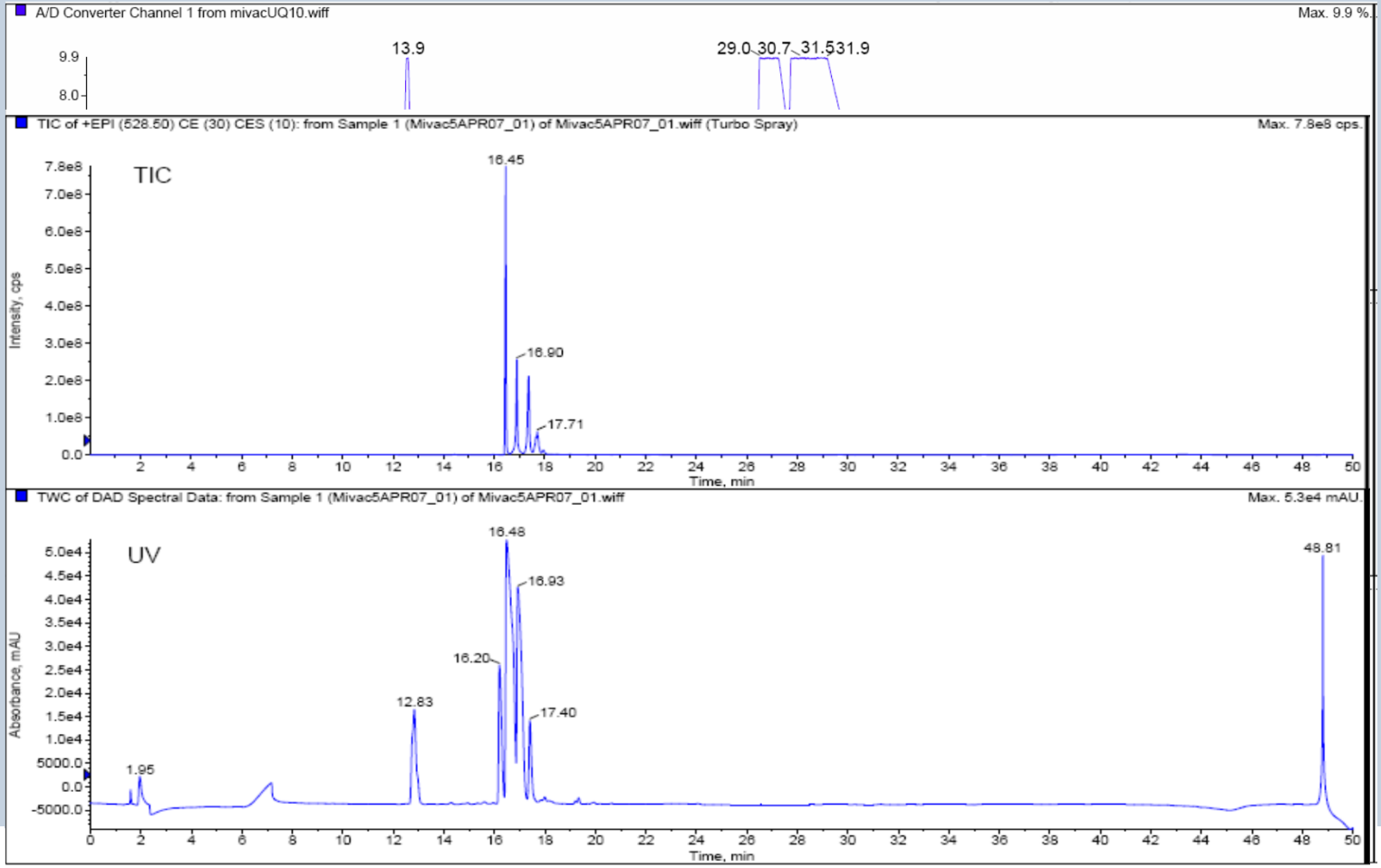
Example Sample Chromatogram



Example 2


Chromatographic eluent diverted to MS
Waste
Q Star
Make-up Pump
Chromatographic Pump
Whatman partisil 5 column
T - Piece
Capital HPLC SAX column
6–port valve
Port blocked
Waste

Example 2


A/D Converter Channel 1 from mivacUQ10.wiff
Max. 9.9 %
13.9
29.0
30.7
31.5
31.9
TIC of +EPI (528.50) CE (30) CES (10): from Sample 1 (Mivac5APR07_01) of Mivac5APR07_01.wiff (Turbo Spray)
Max. 7.8e8 cps.
TIC
16.45
16.90
17.71
Intensity, cps
Time, min
TWC of DAD Spectral Data: from Sample 1 (Mivac5APR07_01) of Mivac5APR07_01.wiff
Max. 5.3e4 mAU
UV
16.48
16.93
16.20
12.83
17.40
1.95
48.81
Absorbance, mAU
Time, min