Notes on experimental procedures and data analysis in LC/GC

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Outline

Molecules/properties: UV-Vis spectra

Chromatographic separation

Resolution

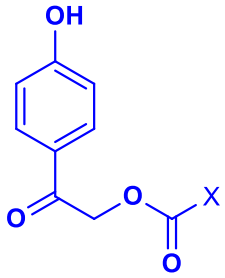
Examples

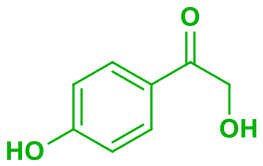
Areas and quantitation

Results

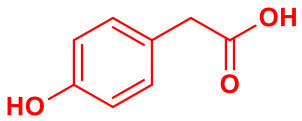
Applications of chromatography

Molecules/properties: UV-Vis spectra, expected m/z values

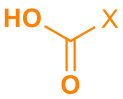


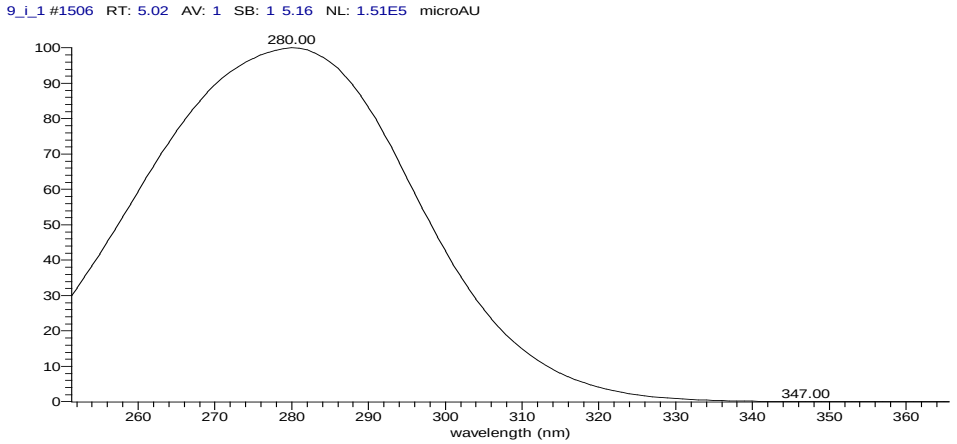


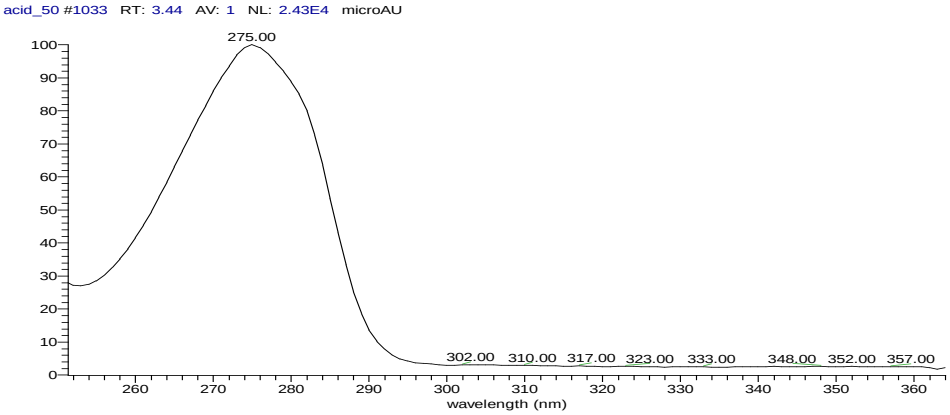
m/z 152



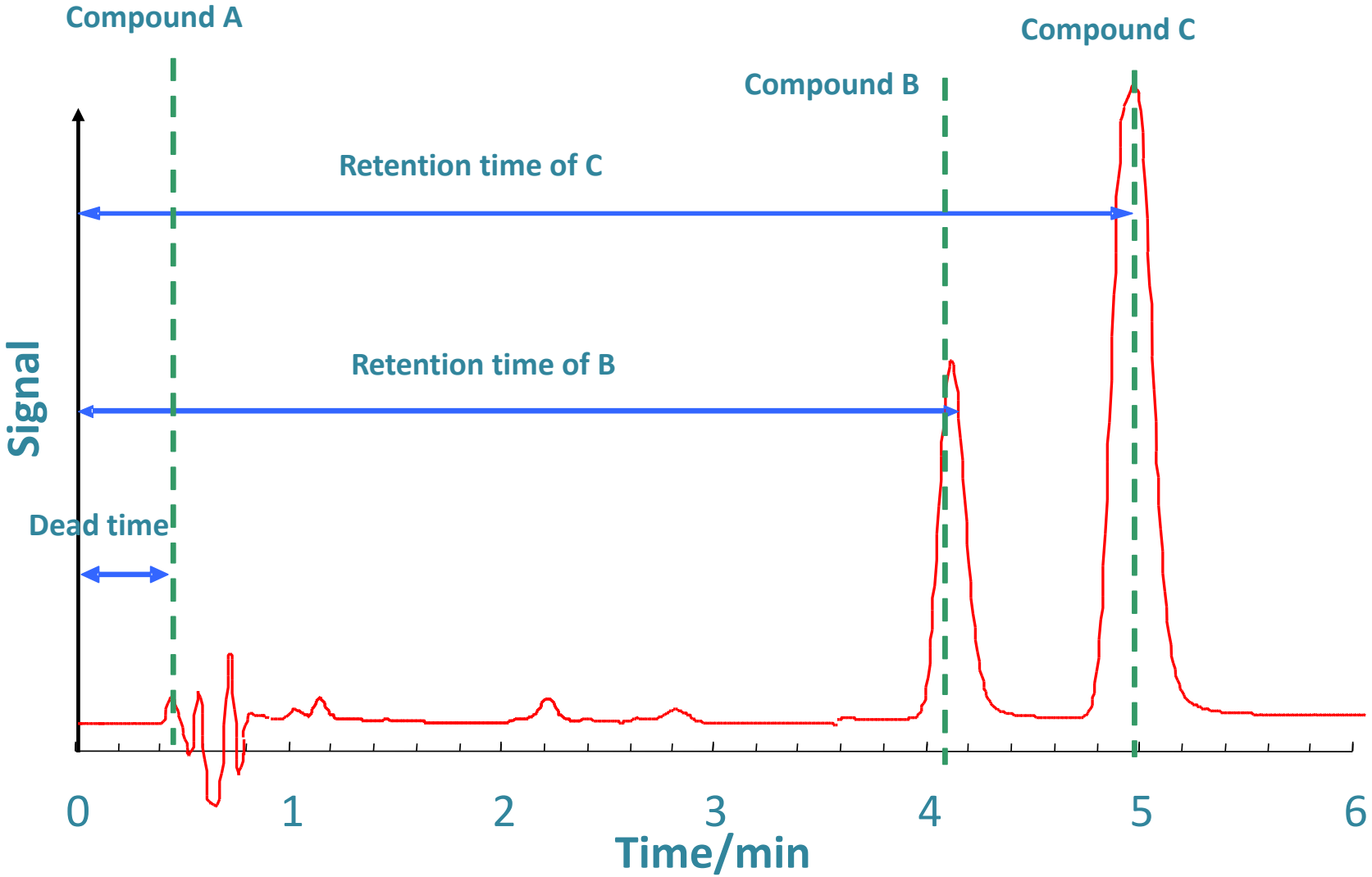
m/z 152



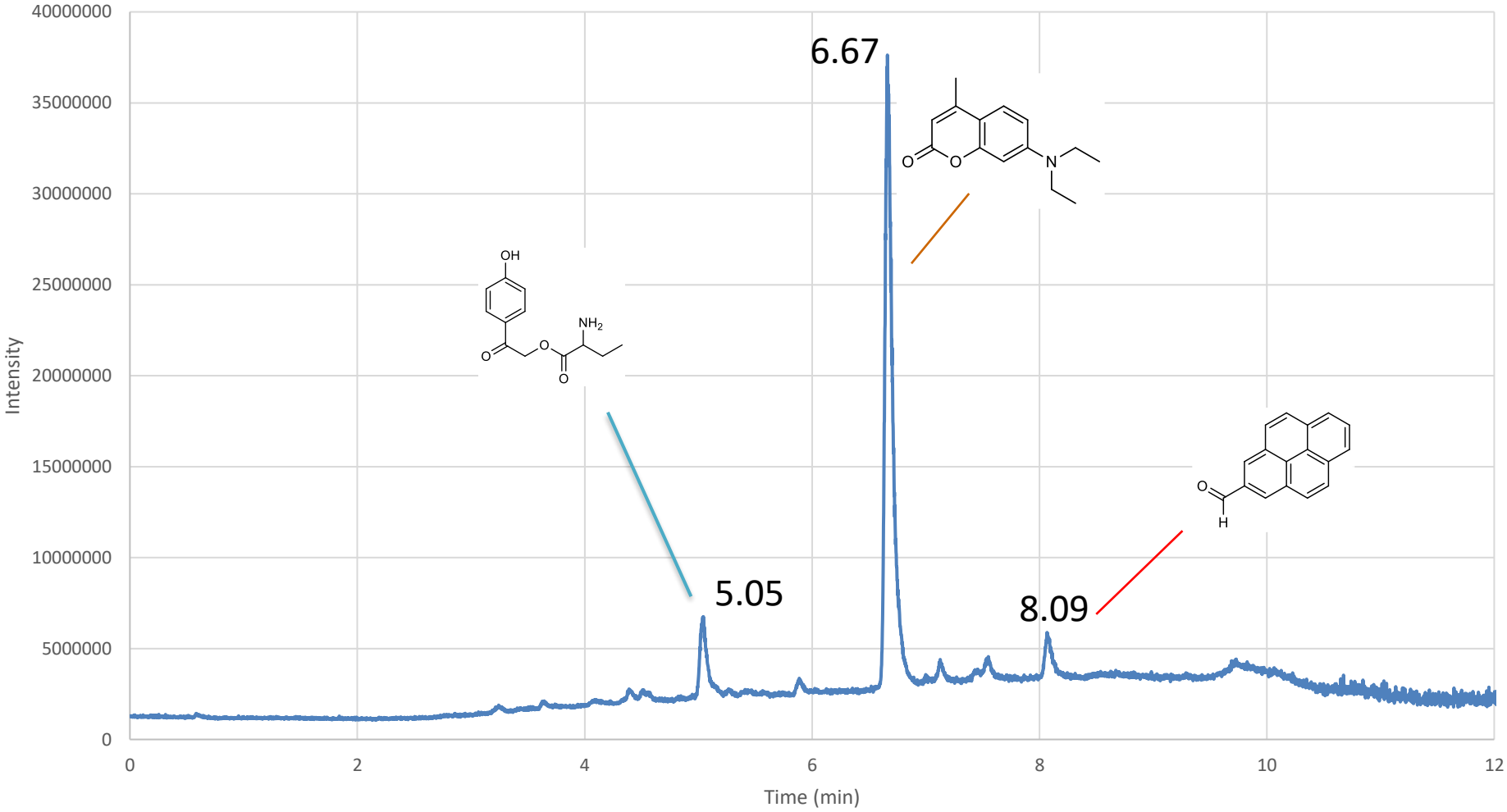




The chromatogram – basic parameters

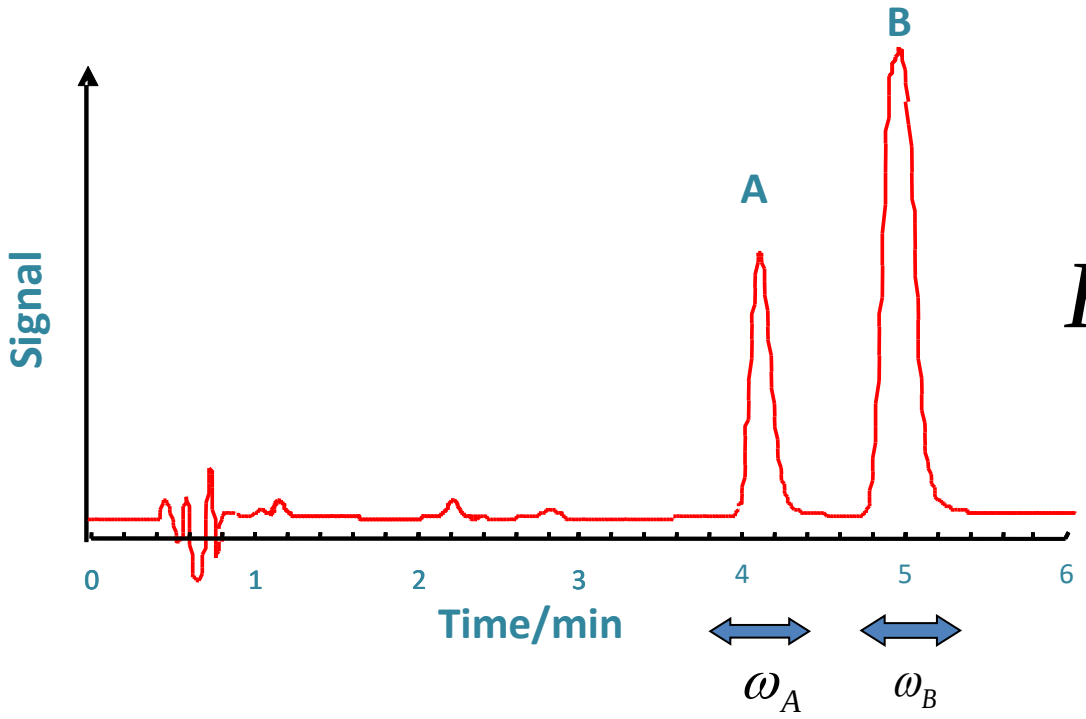


Chromatographic separation

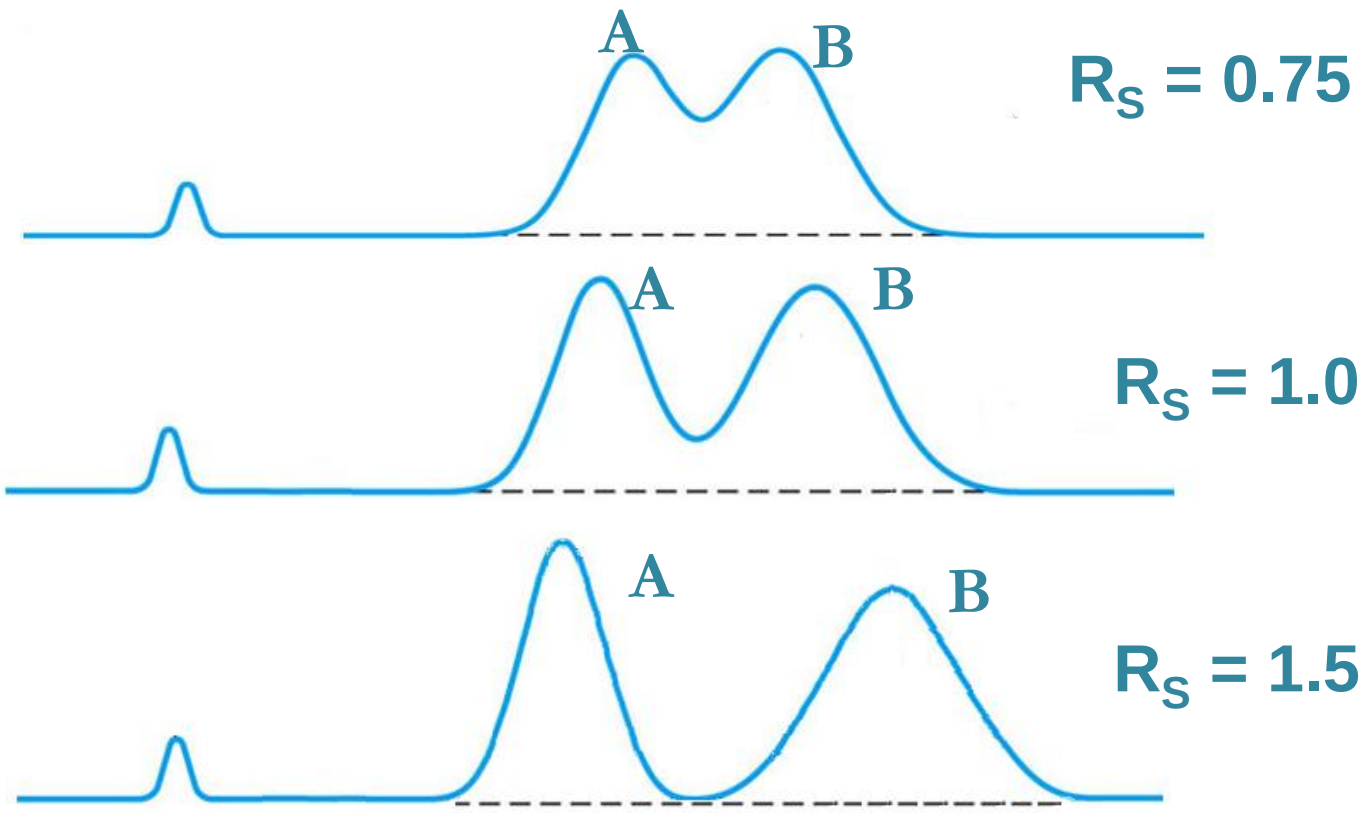


Resolution

The resolution R_s of a column is a quantitative measure of its ability to separate two analytes



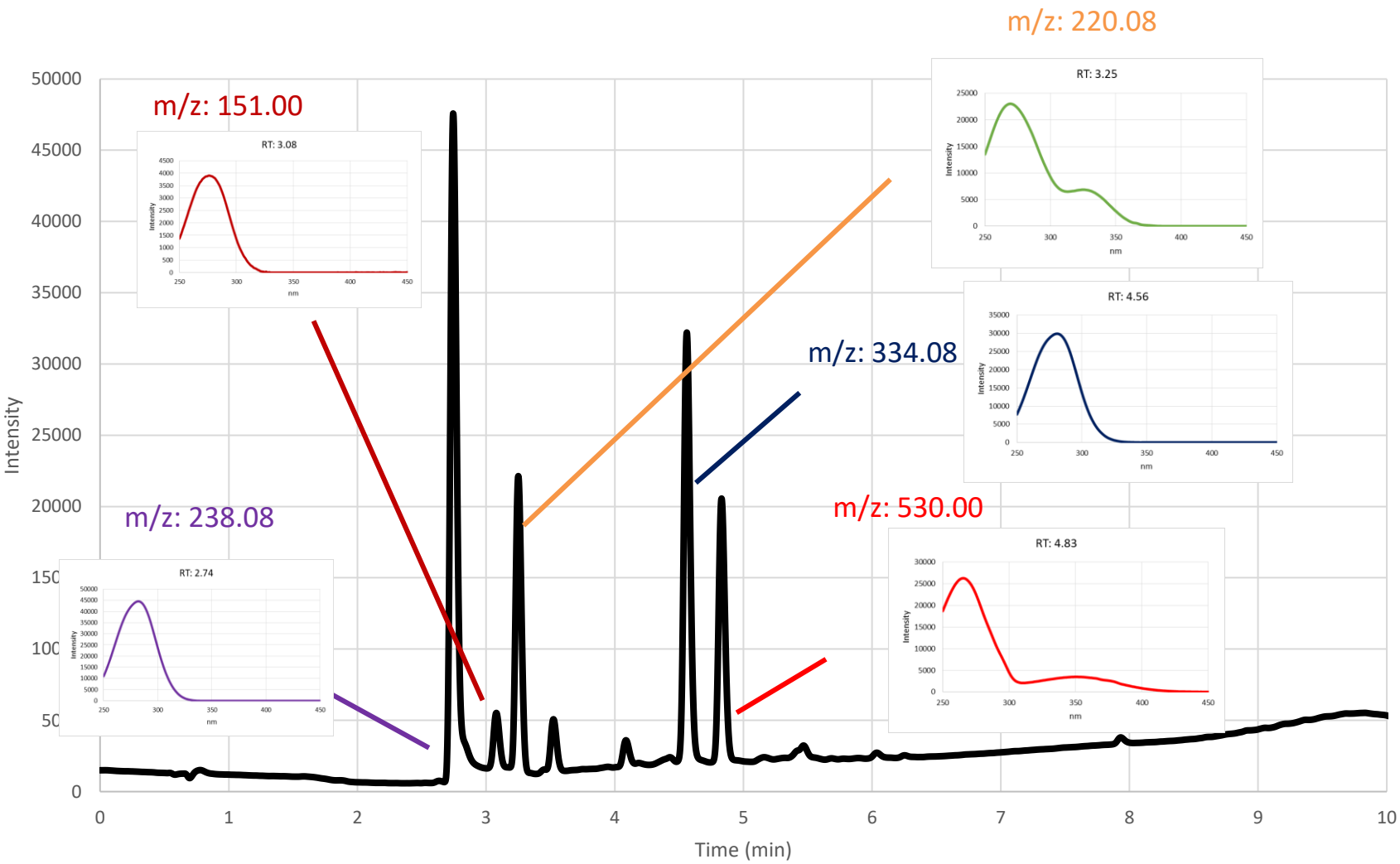
$$R_S = \frac{2\left(t_{RB} - t_{RA}\right)}{\omega_B + \omega_A}$$



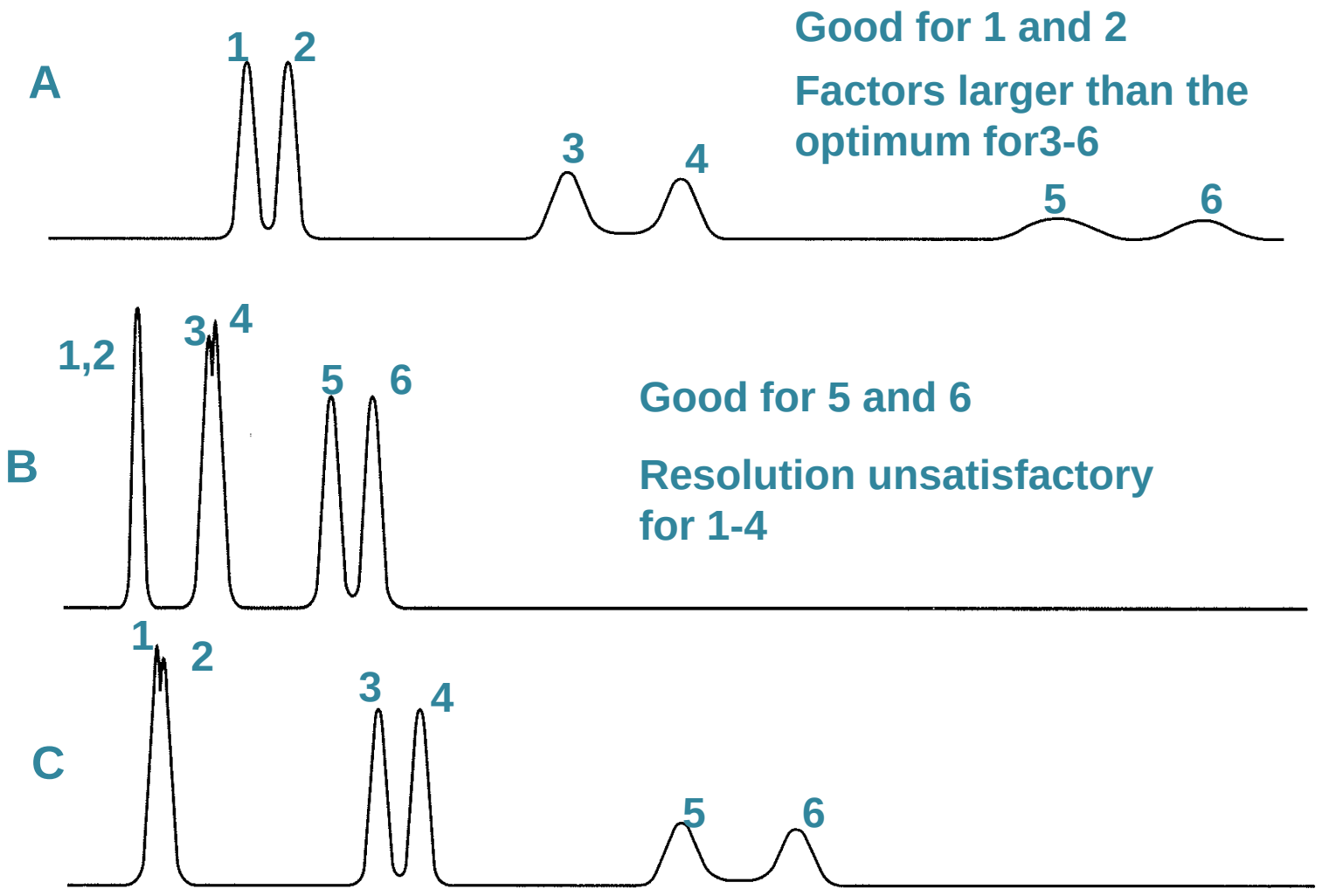
1 - A resolution of 1.5 gives an essentially complete separation of the two components, whereas a resolution of 0.75 does not

2 - At a resolution of 1.0, zone A contains about 4% of B and zone B contains a similar amount of A

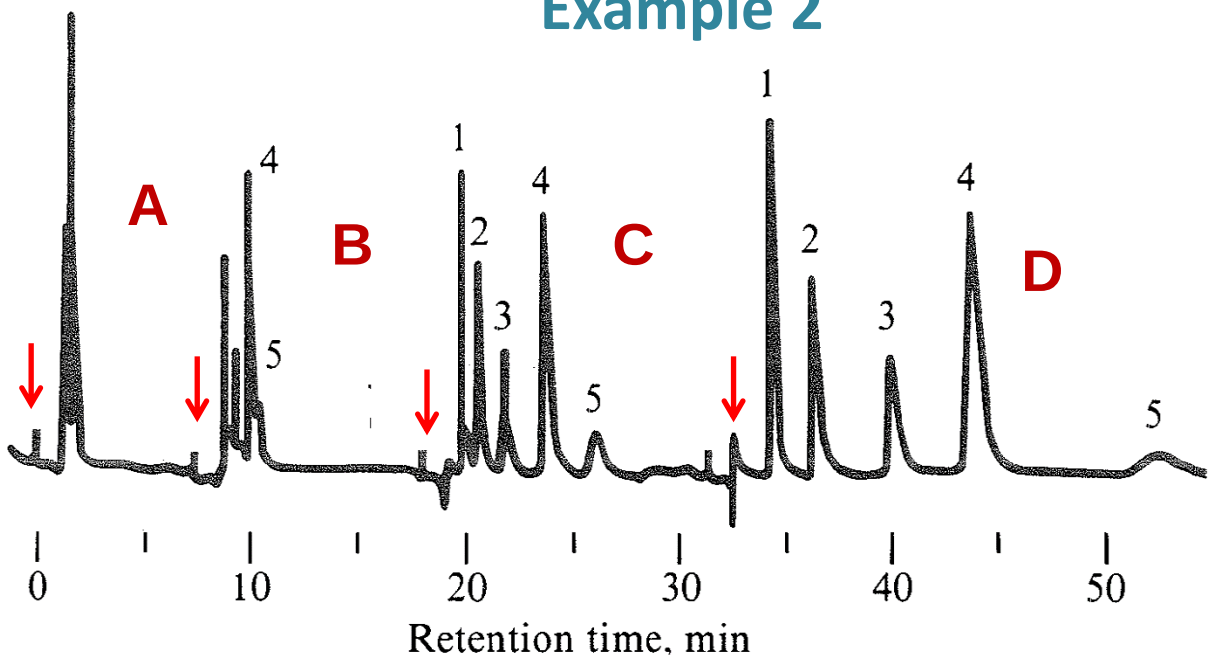
3 - At a resolution for 1.5, the overlap is about 0.3%


m/z: 220.08
m/z: 151.00
m/z: 334.08
m/z: 238.08
m/z: 530.00
RT: 3.25
RT: 3.08
RT: 4.56
RT: 2.74
RT: 4.83
Intensity
Time (min)
nm

Example 1


A
1
2
3
4
5
6
Good for 1 and 2
Factors larger than the optimum for3-6
B
1,2
3
4
5
6
Good for 5 and 6
Resolution unsatisfactory for 1-4
C
1
2
3
4
5
6

Example 2

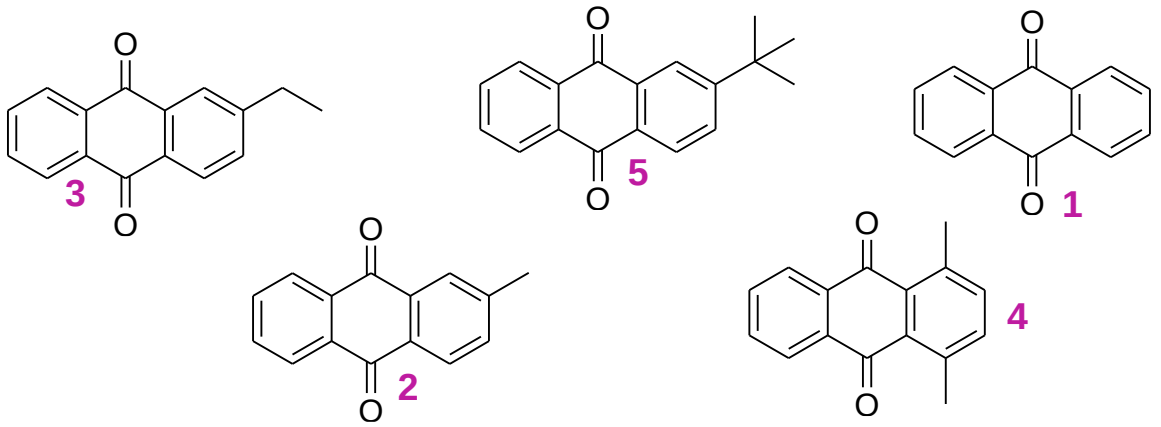


A – Methanol-water 70-30

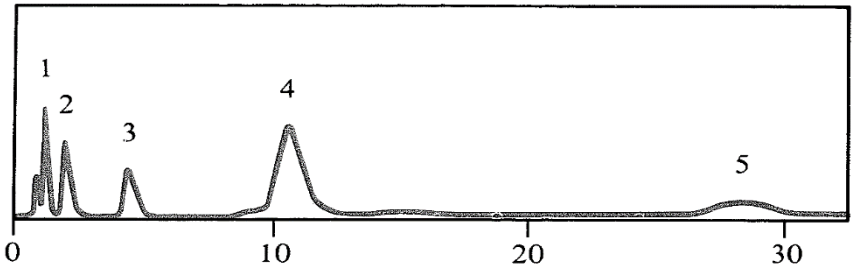
B – Methanol-water 60-40

C – Methanol-water 50-50

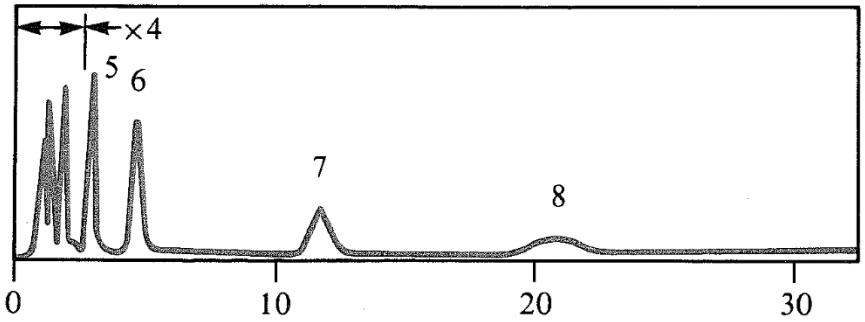
D – Methanol-water 40-60



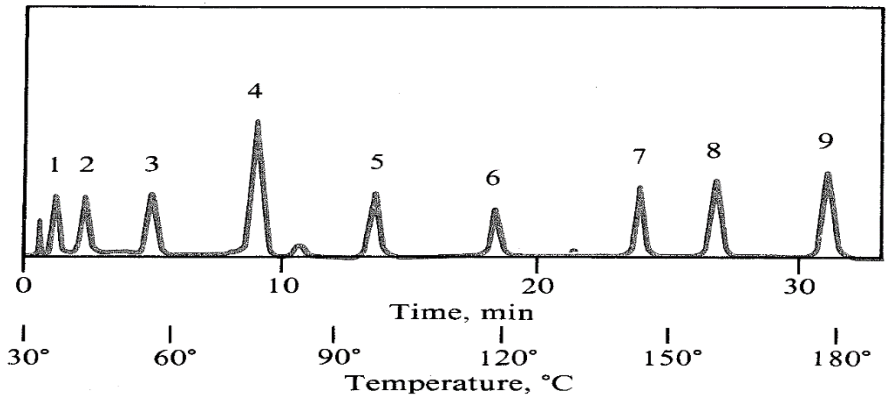
Example 3



Isothermal @ 45 ºC

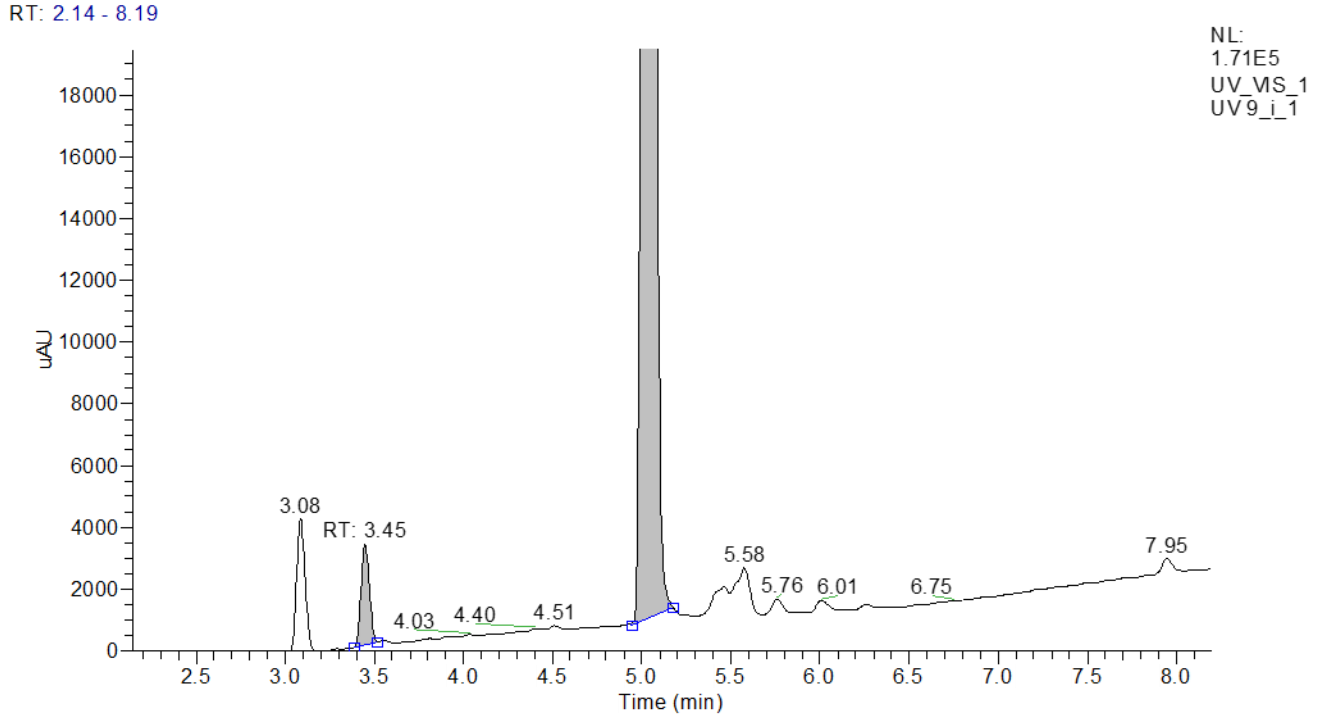


Isothermal @ 145 ºC



Programmed, from 30 ºC to 180 ºC

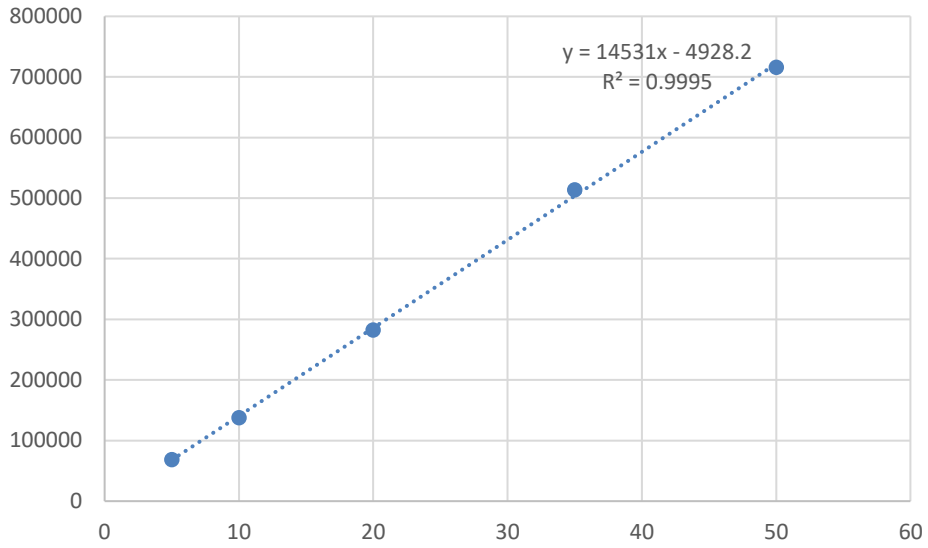
Areas



PEAK LIST						
9_i_1.raw						
RT: 2.14 - 8.19						
Number of detected peaks: 2						
Apex RT	Start RT	End RT	Area	%Area	Height	%Height
3.45	3.39	3.52	**10580.35**	1.72	3253.081	1.87
5.03	4.94	5.18	**604407.1**	98.28	170317.3	98.13

Areas

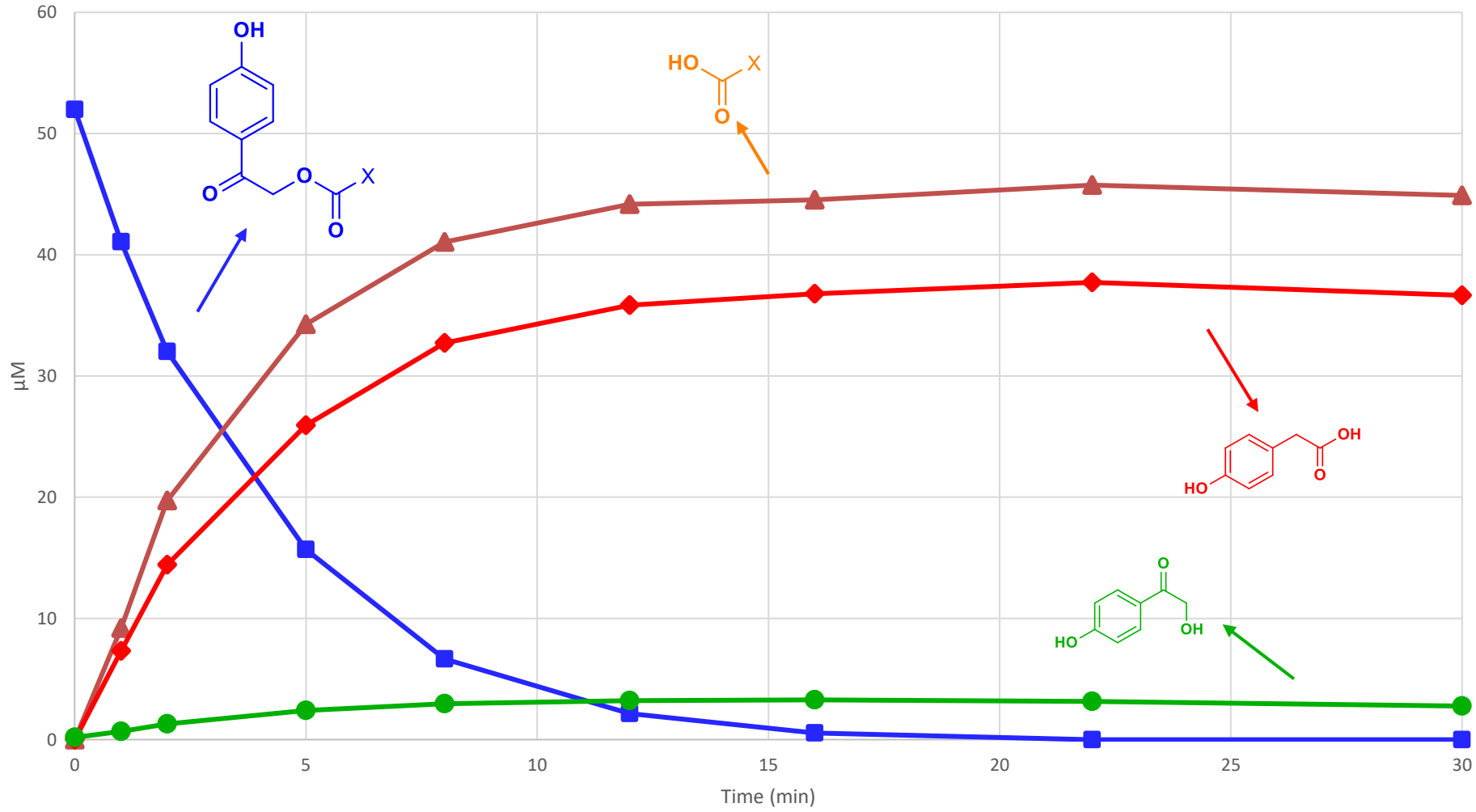
Concentration	Peak Area
5	68758
10	137808
20	282389
35	513981
50	716091



Area of sample= 312147;

Concentration of sample: 21.8 μM

Results


60
50
40
30
20
10
0
µM
0
5
10
15
20
25
30
Time (min)
OH
O
O
X
O
HO
X
O
HO
OH
O
O
HO
OH

Applications of chromatography

A - Separation

B - Qualitative analysis - Identification

A single piece of qualitative information: the retention time, t_R

Much less information that one can get from an NMR or mass spec. It supports however the qualitative spectroscopy analysis by providing the separated compounds

While positive identification of chemical compounds is limited, chromatograms provide a sure evidence of the absence of certain chemical species.

C - Quantitative analysis - Quantification

1 - It usually involves the use of the peak areas (sometimes the peak height)

2 – Needs the comparison of the area (or height) of analyte peak with that of one or more standards.