FOR414
Chapter 4
Data Analysis in Chromatography

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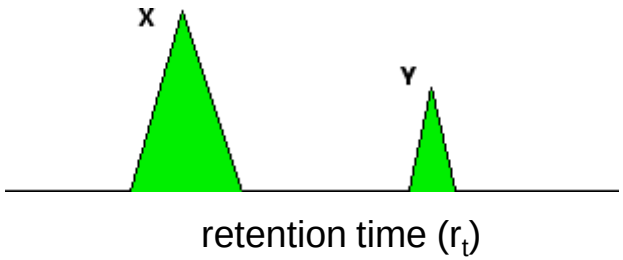
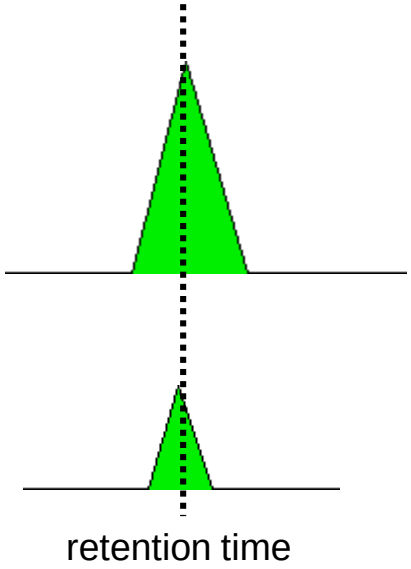
Interpretation of Data: Basic Idea

1. Qualitative analysis



If you had two different substances in the mixture (X and Y), you could say nothing about their relative amounts.

2. Quantitative analysis

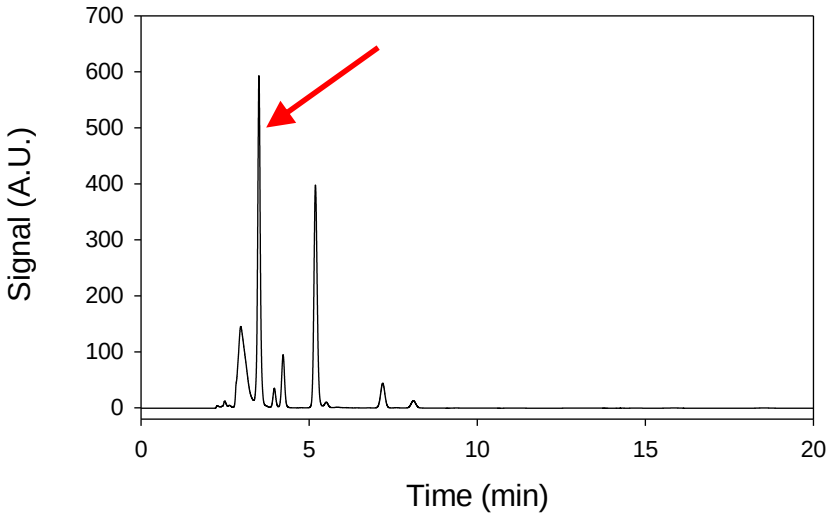


The area under the peak is proportional to the amount (or concentration) of X which has passed the detector, and this area can be calculated automatically by the computer linked to the display.

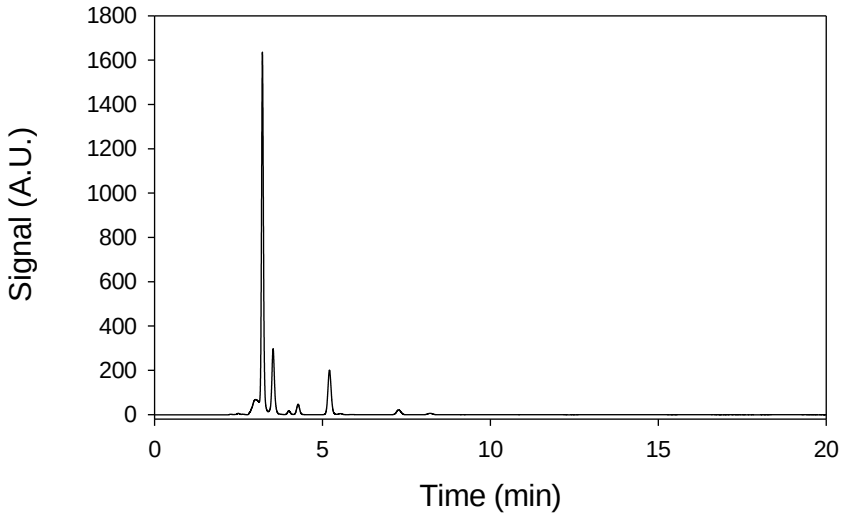
If the solution of X was less concentrated, the area under the peak would be less - although the retention time will still be the same.

Qualitative Analysis: Identification of Target Compounds

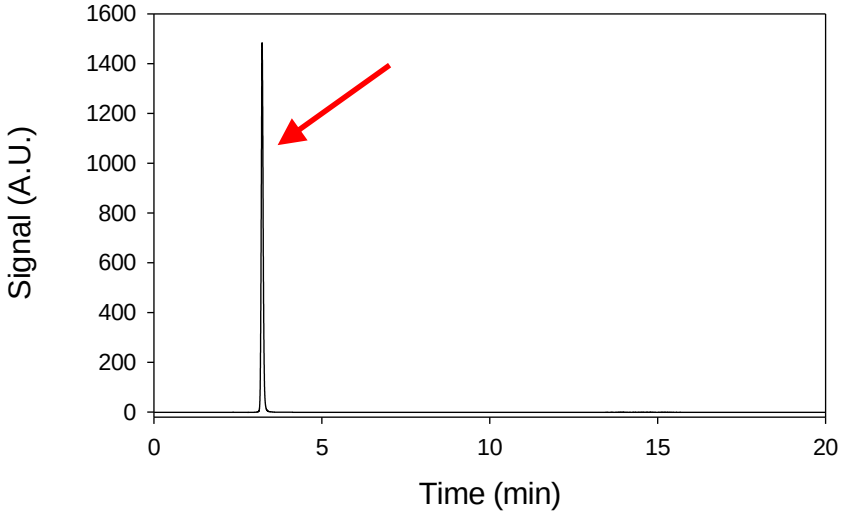
sample containing ascorbic acid

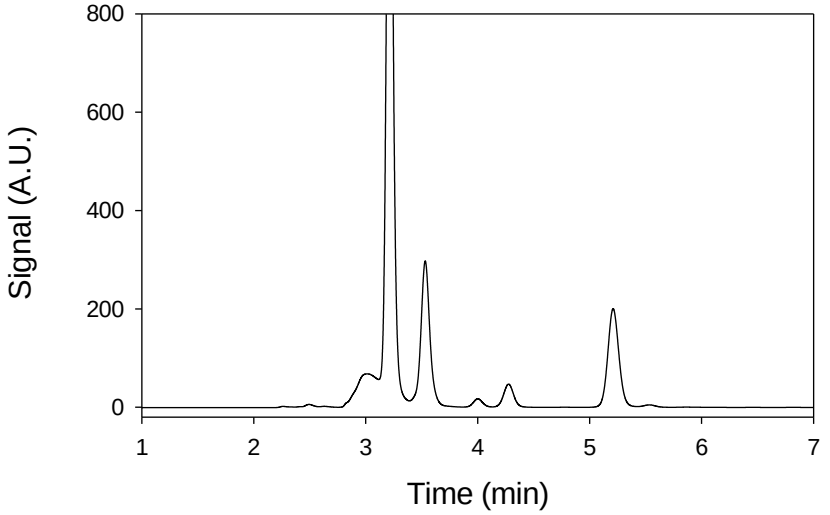


sample spiked with ascorbic acid

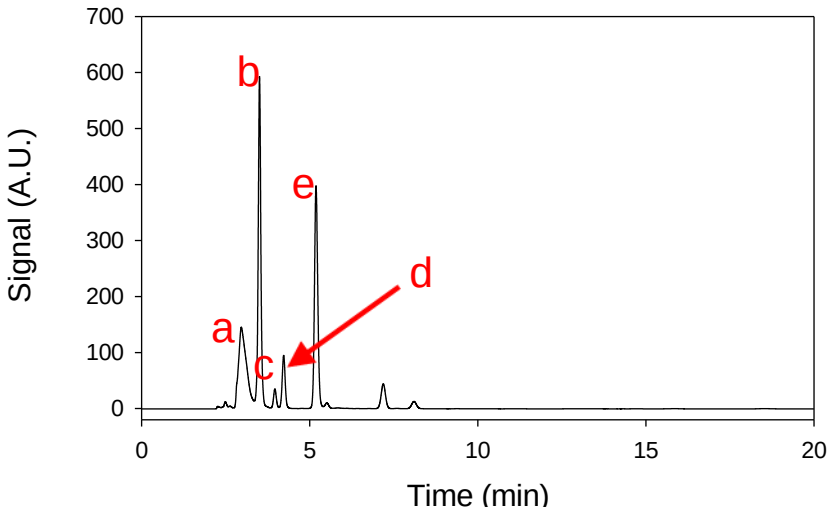


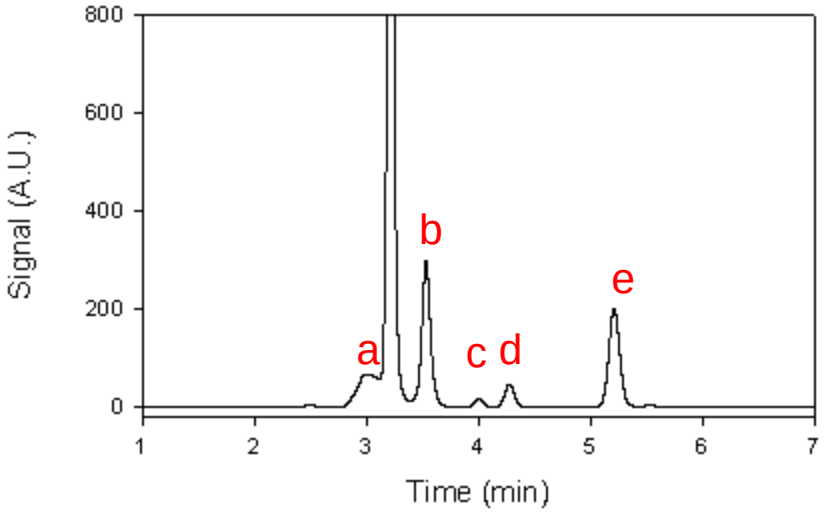
ascorbic acid





Be Careful and Meticulous



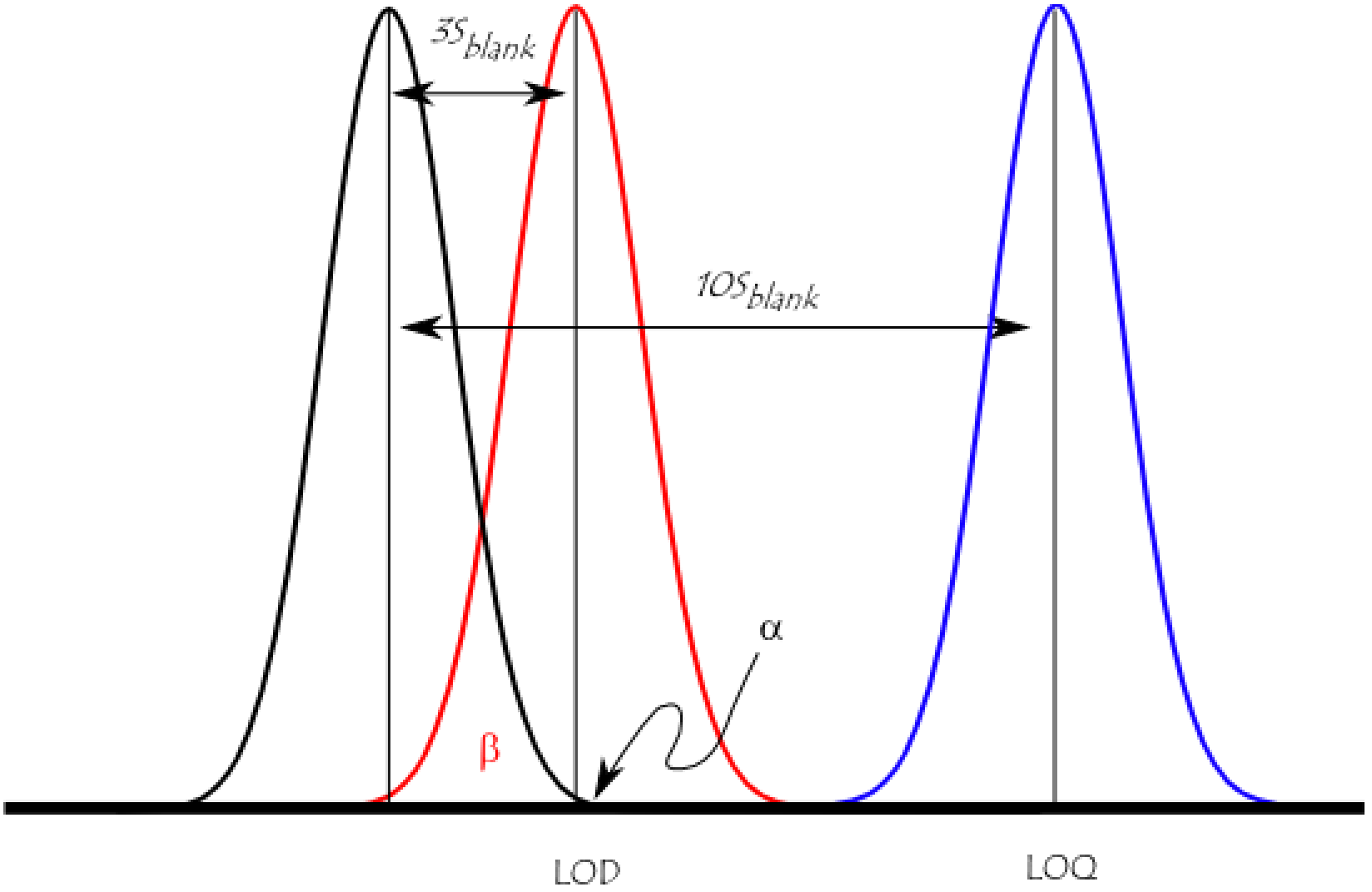


LOD and LOQ

- LOD (Limit of Detection): the lowest quantity of a substance that can be distinguished from the absence of that substance (a *blank value*) within a stated confidence limit (~3 times of blank std).

- LOQ (Limit of Quantification): the limit at which we can reasonably tell the difference between two different values (~10 times of blank std).

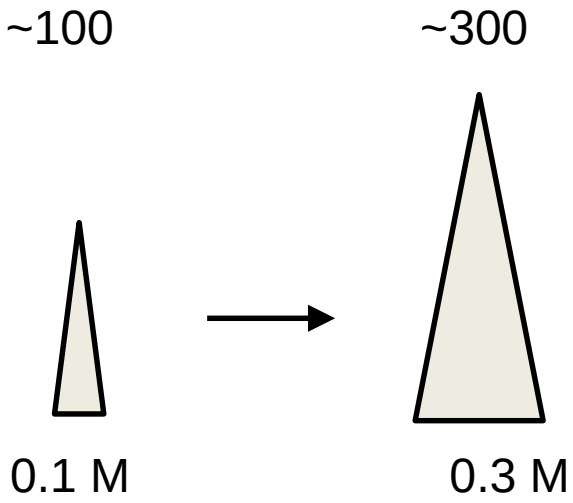
LOD and LOQ



Quantitative Analysis by Chromatography

$$m_i = RF_i \cdot A_i$$

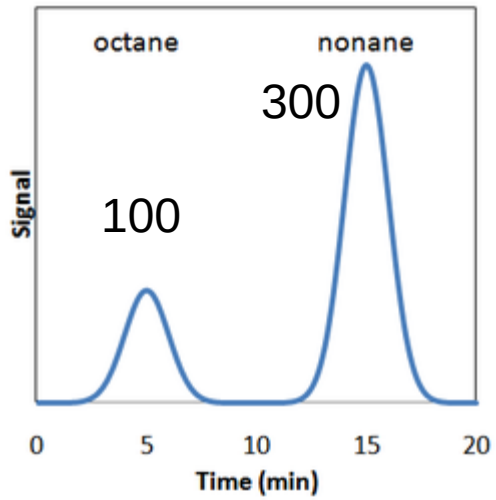
- m_i: quantity of compound *i* injected into the column
- RF_i: Absolute response factor for compound *i*
- A_i: area of the eluting peak for compound *I*
- A larger amount of injection $\rightarrow$ a larger peak area



Potential Problems

$$m_i = RF_i \cdot A_i$$

- RF_i: depends on the compounds, instruments, and concentrations
- Difficult to know exact injected quantity of compound *i* (instrumental error, personal error, etc)
- Recovery of a target compound during preliminary sample preparation processes is not ~100%



Does this mean concentration ratio 1:3?

Quantification Calculation

- Percent
- External standard (ESD)
- Internal standard (ISD)
- Standard addition (SA)

Percent (%): Uncalibrated Procedure

Compound	Integrated Area	Area (%)	Amount (%)
A	280	26.2	26.2
B	250	23.4	23.4
C	220	20.6	20.6
D	320	29.9	29.9
Total	1070	100	100

- Assumes that all compounds respond equally to the detector (RF_i is same)
- Area % is proportional to the relative amounts of each component in a sample

Reference Sample

Problems

- Each sample does not respond equally to the detector ($RF_a \neq RF_b \neq RF_c$, etc)
- Each sample has its own absolute response factor

$$m_i = RF_i A_i$$

- m_i: quantity of compound *i* injected into the column
- RF_i: Absolute response factor for compound *i*
- A_i: area of the eluting peak for compound *i*

Percent (%): Reference (25 mg per Each Compound)

Compound	Integrated Area	Area %	RF (mg/A)
A	250	20.2	25/250=0.100
B	290	23.4	25/290=0.086
C	330	26.6	25/330=0.076
D	370	29.8	25/370=0.068
Total	1240	100	

$RF_i = m_i/A_i$ for each compound can be calculated

Unknown quantity = $RF_i * A_i$ can be calculated

Percent (%): Unknown Sample

Compound	Area	RF	Amount (mg)
A	140	0.100	140*0.100 = 14.0
B	360	0.086	360*0.086 = 31.0
C	230	0.076	230*0.076 = 17.5
D	420	0.068	420*0.068 = 28.6

- *Unknown = RF*A*
- *A larger peak for a given compound does NOT mean a larger amount in a sample mixture*

- *RF is constant for all concentrations?*

External Standard Method

$$m_{ref} = RF \cdot A_{ref}$$

- m_{ref}: known quantity of an analyte injected into the column
- RF: Absolute response factor of an analyte
- A_{ref}: area of the eluting peak

$$m_{sample} = RF \cdot A_{sample}$$

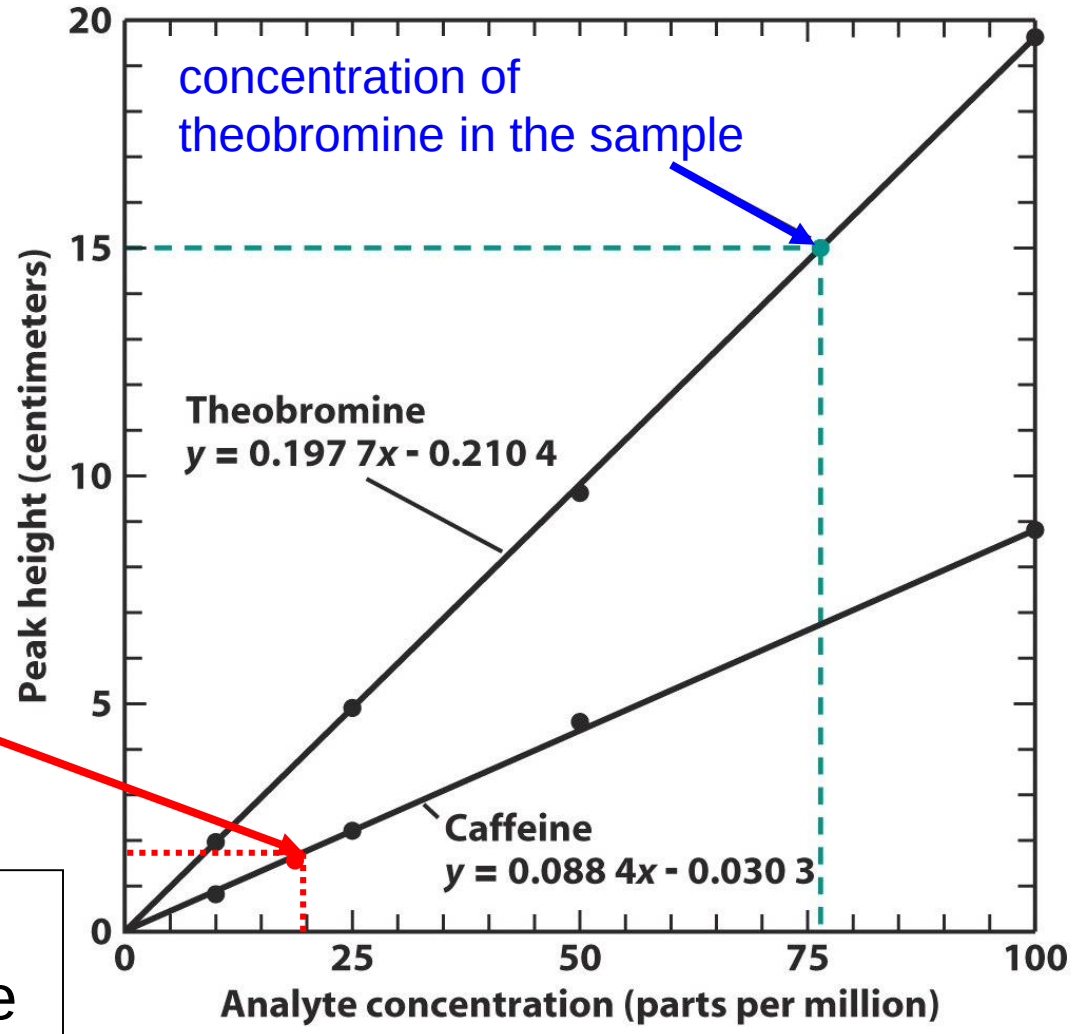
Potential Problems

- Difficult to know exactly injected quantity of an analyte (instrumental error, personal error, etc)
- RF: should be constant over the concentrations

Calibration Curve Using Multiple External Standards

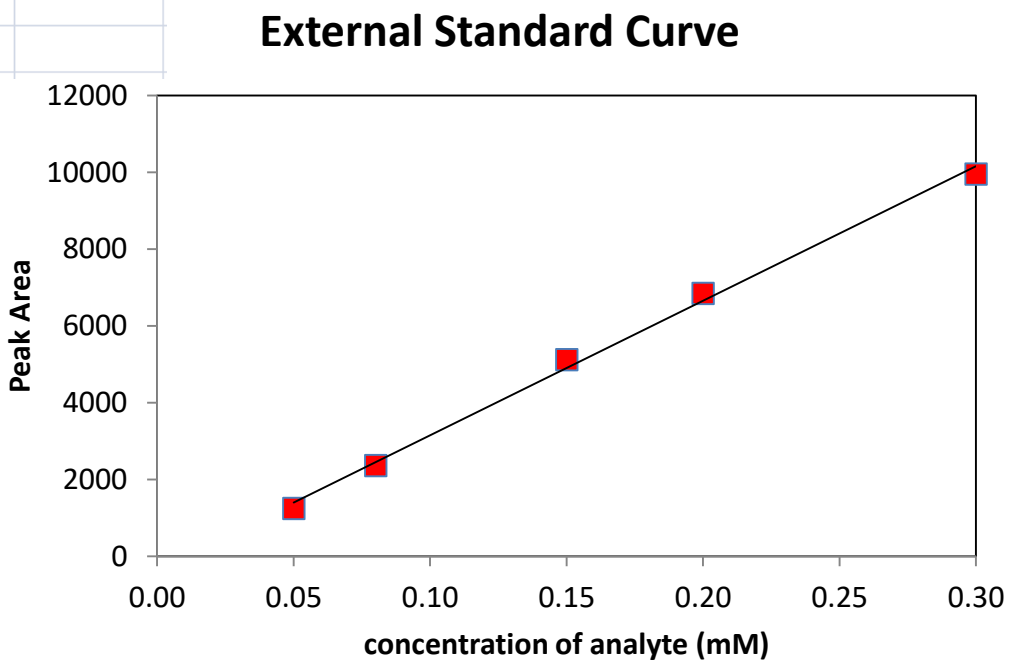
external standards

- Caffeine
  10, 25, 50, and 100 ppm
- Theobromine
  10, 25, 50, and 100 ppm



can find concentration ranges with linear response

Sample	ethanol (%)		peak area of ethanol
1	0.30		9954
2	0.20		6846
3	0.15		5127
4	0.08		2366
5	0.05		1248
unknown1	0.041463527		1095
unknown2	0.034385623		847
	y = ax + b		
	a =	35038.62034	
	b =	-357.8247734	



Internal Standard

Conditions

- Why?: Quantity of samples vary (auto-injector), instrument is not stable (detector), etc.
- Internal standard (ISD): a known amount of compound, different from analyte, that is added to the unknown sample.
- Must be pure and not present initially in the sample
- Its elution peak must be well resolved from the other compounds in the sample.
- Retention time should be close to that of target compound (chemically and physically similar, but should be different)
- Should be chemically inert to all compounds in a sample.
- Chemically/physically stable over the time

Internal Standard Method

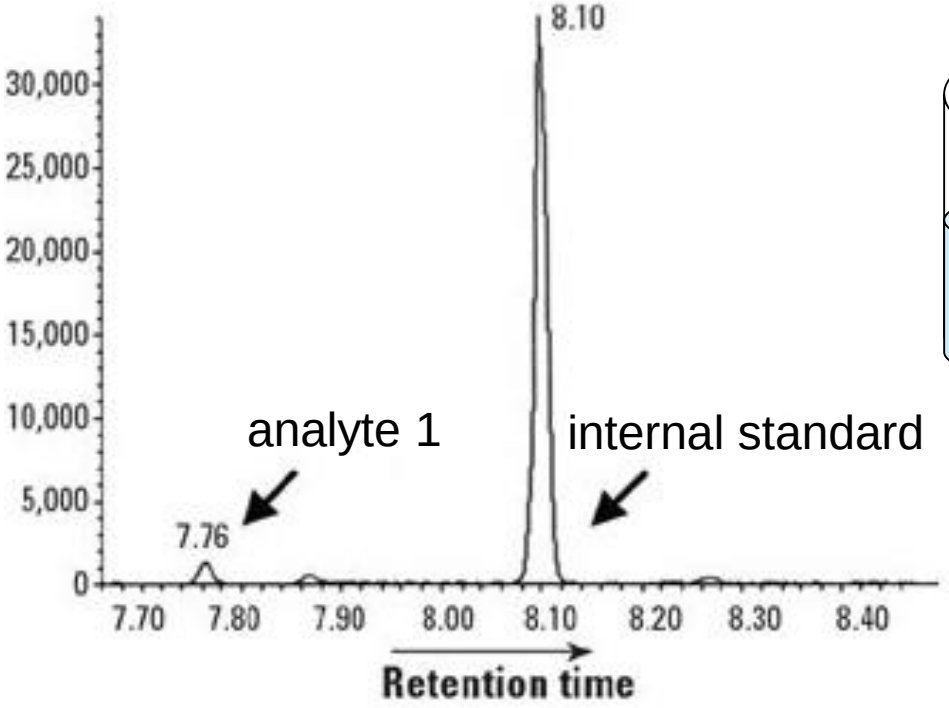
$$m_{ISD} = RF_{ISD}A_{ISD}$$

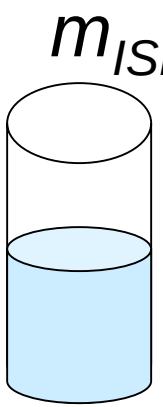
- m_{ISD}: known quantity of internal standard (ISD) injected into the column
- RF_{ISD}: Absolute response factor of ISD
- A_{ISD}: area of the eluting peak for ISD

$$m_1 = RF_1A_1$$

- m_1: known quantity of an analyte 1 injected into the column
- RF_1: Absolute response factor of an analyte 1
- A_1: area of the eluting peak for an analyte 1

Internal Standard Method





From Data

$$\frac{m_1}{m_{ISD}} = \left(\frac{RF_1}{RF_{ISD}}\right)\left(\frac{A_1}{A_{ISD}}\right)$$

- RF_{ISD}/RF_1 can be calculated from a known sample
- From unknown sample (m_x) with m_{ISD}, (A_x/A_{ISD}) can be calculated data, m_1/m_{ISD} can be calculated because we know RF_1/RF_{ISD} from a known sample.

Example 1

In an HPLC experiment, a known mixture containing 0.1 M of analyte A and 0.01 M of internal standard S was injected onto a column and the area of the two chromatographic peaks was found to be 8.17 and 0.14 units respectively. Next, 1.0 mL of the 0.01 M internal standard solution was added to 4.50 mL of a solution containing only the analyte A of unknown concentration. The peak areas for A and S for the unknown mixture were found to be 0.88 and 0.57 units respectively. What's the concentration of analyte A in unknown sample?

$$\frac{m_1}{m_{ISD}} = \left(\frac{RF_1}{RF_{ISD}}\right)\left(\frac{A_1}{A_{ISD}}\right)$$

$$\frac{0.1M}{0.01M} = \left(\frac{RF_1}{RF_{ISD}}\right)\left(\frac{8.17}{0.14}\right)$$

$$\left(\frac{RF_1}{RF_{ISD}}\right) = 0.171$$

$$\frac{m_1}{m_{ISD}} = \left(\frac{RF_1}{RF_{ISD}}\right)\left(\frac{A_1}{A_{ISD}}\right)$$

$$\frac{0.818xM}{0.00182M} = 0.171\left(\frac{0.88}{0.57}\right)$$

$$x = 0.000589M$$

Example 2

In an experiment, a known mixture containing 0.0837 M of analyte X and 0.0666 M of internal standard S was injected onto a column and the area of the two chromatographic peaks was found to be 423 and 347 units respectively. Next, 10.0 mL of the 0.146 M internal standard solution was added to 10 mL of a unknown solution containing analyte X. This mixture was diluted to 25 mL. The peak areas for X and S for the mixture were found to be 553 and 582 units respectively. What's the concentration of analyte A in unknown sample?

$$\frac{m_1}{m_{ISD}} = \left(\frac{RF_1}{RF_{ISD}}\right)\left(\frac{A_1}{A_{ISD}}\right)$$

$$\frac{0.0837M}{0.0666M} = \left(\frac{RF_1}{RF_{ISD}}\right)\left(\frac{423}{347}\right)$$

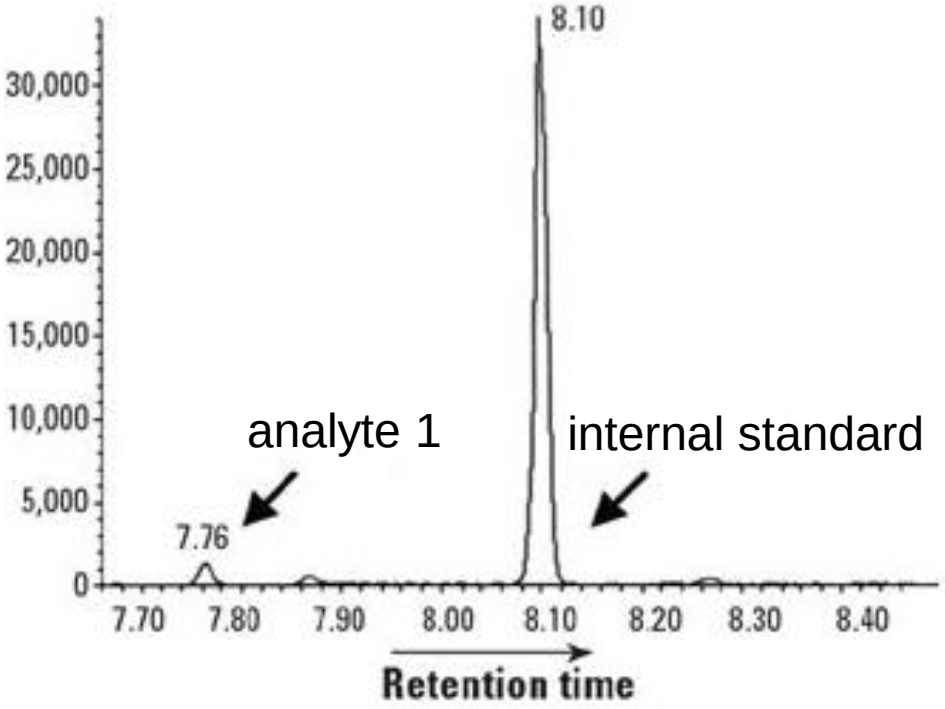
$$\left(\frac{RF_1}{RF_{ISD}}\right) = 1.031$$

$$\frac{m_1}{m_{ISD}} = \left(\frac{RF_1}{RF_{ISD}}\right)\left(\frac{A_1}{A_{ISD}}\right)$$

$$\frac{0.4xM}{0.0584M} = 1.031\left(\frac{553}{582}\right)$$

$$x = 0.143M$$

Internal Standard Method



$$\frac{m_1}{m_{ISD}} = \left(\frac{RF_1}{RF_{ISD}}\right)\left(\frac{A_1}{A_{ISD}}\right)$$

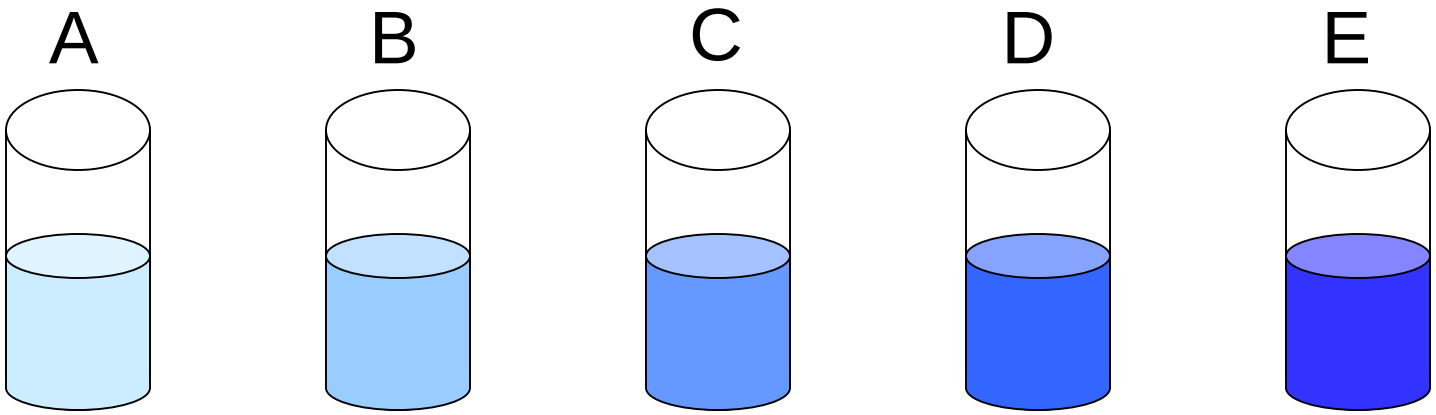
- Good news: Regardless of the amount of sample injected, the ratio of m_1/m_{ISD} is same.
- Question: is RF_1/RF_{ISD} always constant over the concentration?

Internal Standard Method

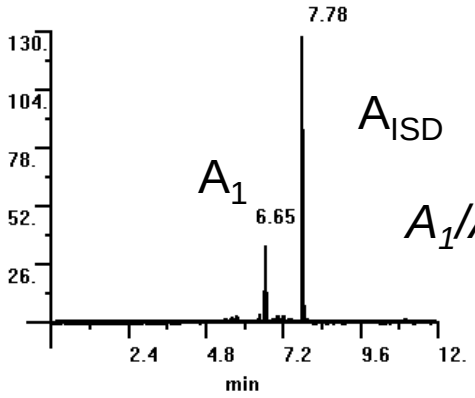
$$\frac{m_1}{m_{ISD}} = \left(\frac{RF_1}{RF_{ISD}}\right)\left(\frac{A_1}{A_{ISD}}\right)$$

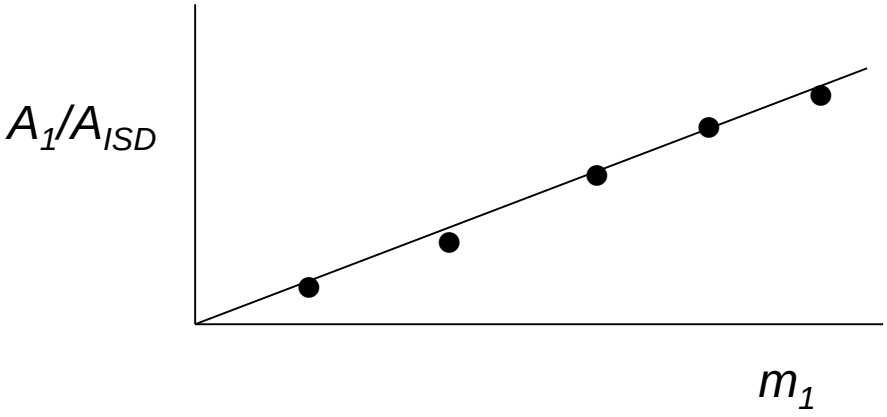
- How can we check that RF_1/RF_{ISD} always constant over the concentration?
- m_1 vs. (A_1/A_{ISD}) straight line if RF_1/RF_{ISD} is constant

Internal Standard Method



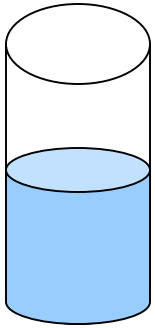
	A	B	C	D	E
Analyte (ppm)	5	10	40	50	100
ISD (ppm)	100	100	100	100	100
A_1/A_{ISD}	9	19	24	101	198

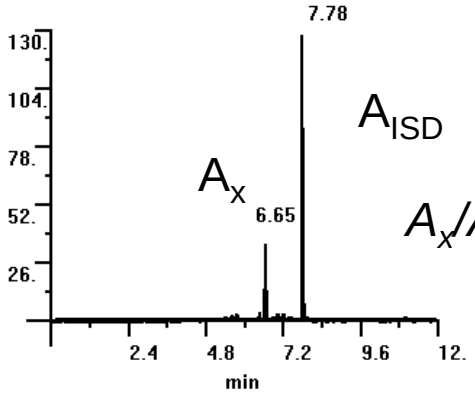




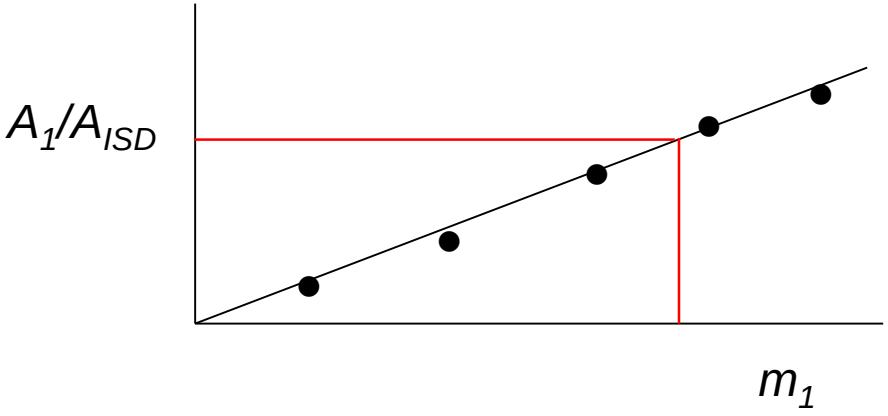
Internal Standard Method

unknown sample, $m_x + m_{ISD}$





$A_x/A_{ISD} = a$



Internal Standard Method

Sample	Analyte	ISD		Peak area of Analyte	Peak Area of ISD	P(A)/P(ISD)
1	0.05	0.30		18.80	50.00	0.376
2	0.10	0.30		48.10	64.10	0.75039
3	0.15	0.30		63.40	55.10	1.150635
4	0.20	0.30		63.20	42.70	1.480094
5	0.25	0.30		93.60	53.80	1.739777
6	unknown	0.30		58.90	49.40	1.192308
	y = ax + b					
	a =	6.914515129				
	b =	0.062201901				

Internal Standard Method