CHE680
Advanced Analytical Chemistry
Chapter 11

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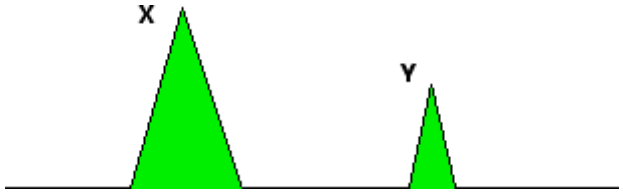
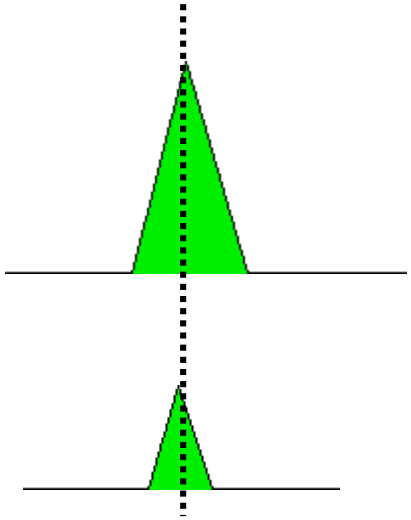
Interpretation of Data: Basic Idea

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

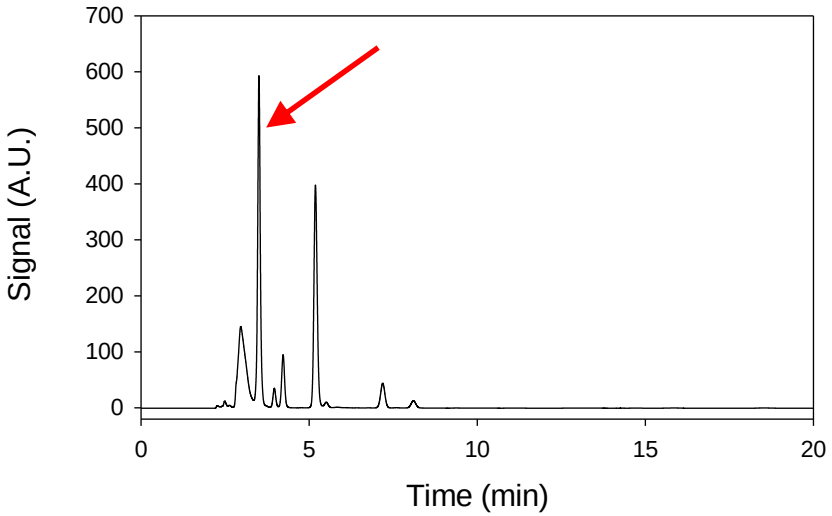


The area under the peak is proportional to the amount of X which has passed the detector, and this area can be calculated automatically by the computer linked to the display.

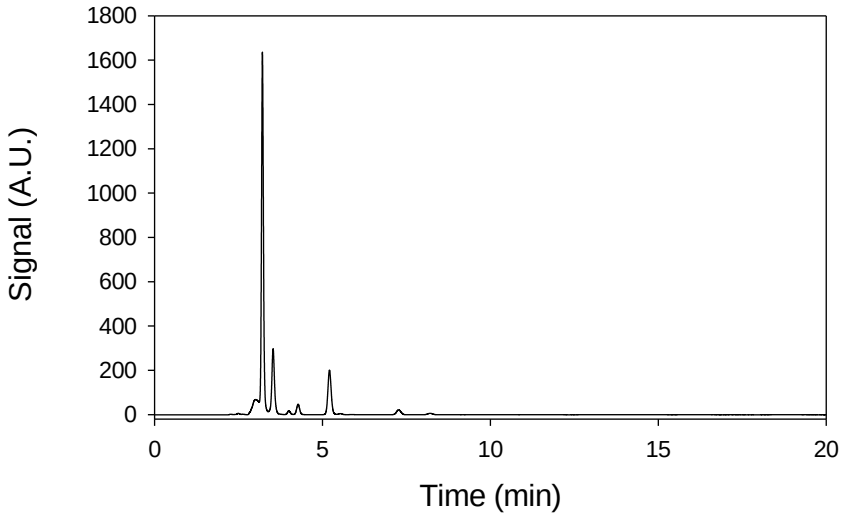
If the solution of X was less concentrated, the area under the peak would be less - although the retention time will still be the same.

Qualitative Analysis: Identification of Target Compounds

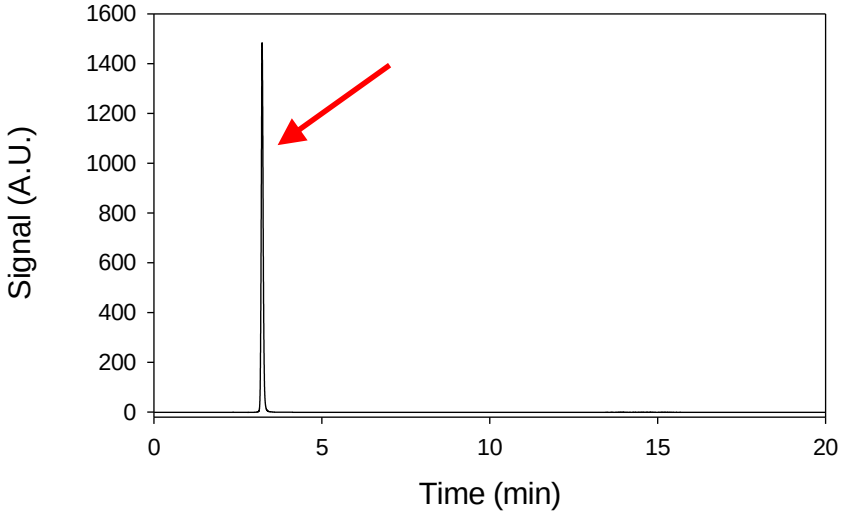
sample containing ascorbic acid

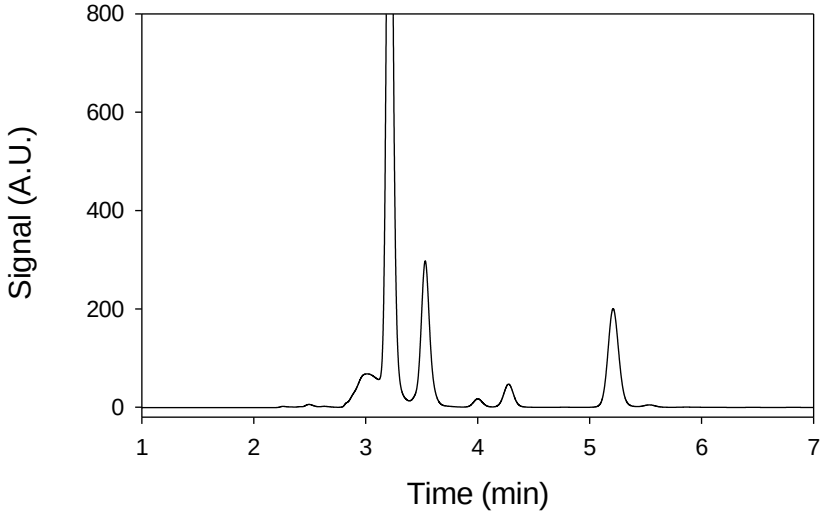


sample spiked with ascorbic acid

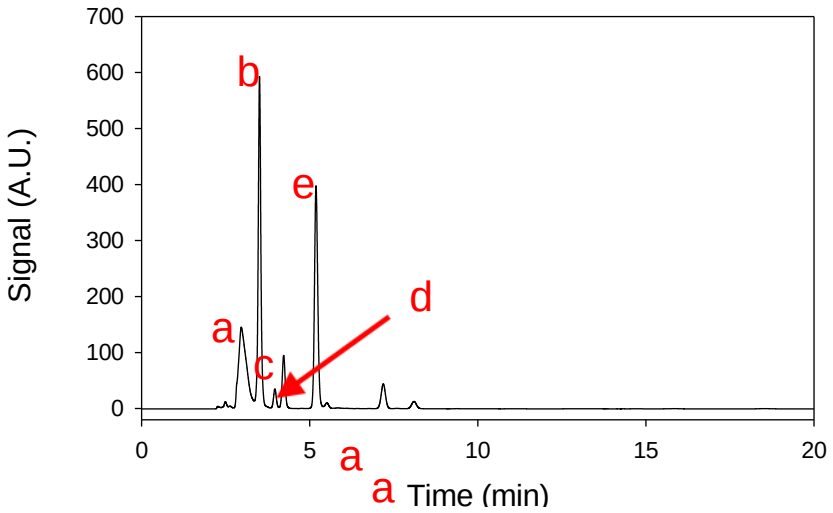


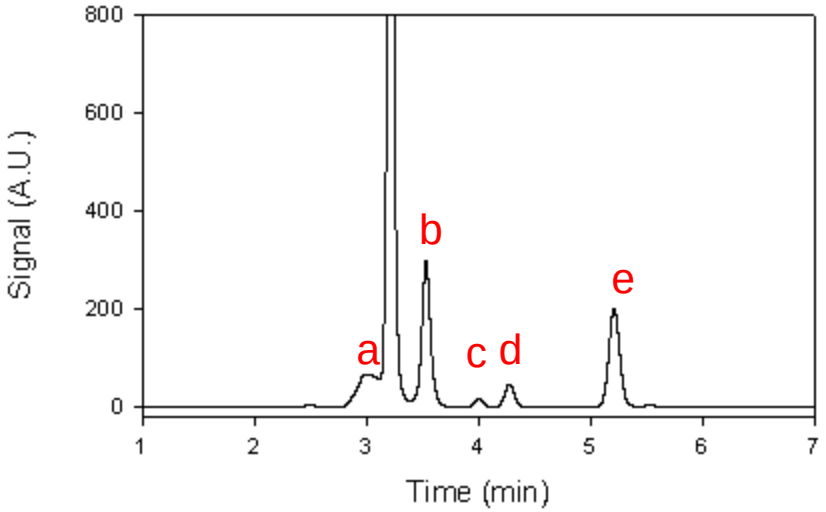
ascorbic acid





Can’t Believe?





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*

Problem

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

Quantification Calculation

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

Percent (%): Uncalibrated Procedure

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

- Assumes that all compounds respond equally to the detector
- Area % is close to the relative amounts of each component

Reference Sample

Problem

- Each sample does not respond equally to the detector
- Absolute response factor (RF)

$$m_i = RF_i A_i$$

- 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
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	

$$RF_i = m_i/A_i$$

$$Unknown = RF*A$$

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 does mean a larger amount*

- *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

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

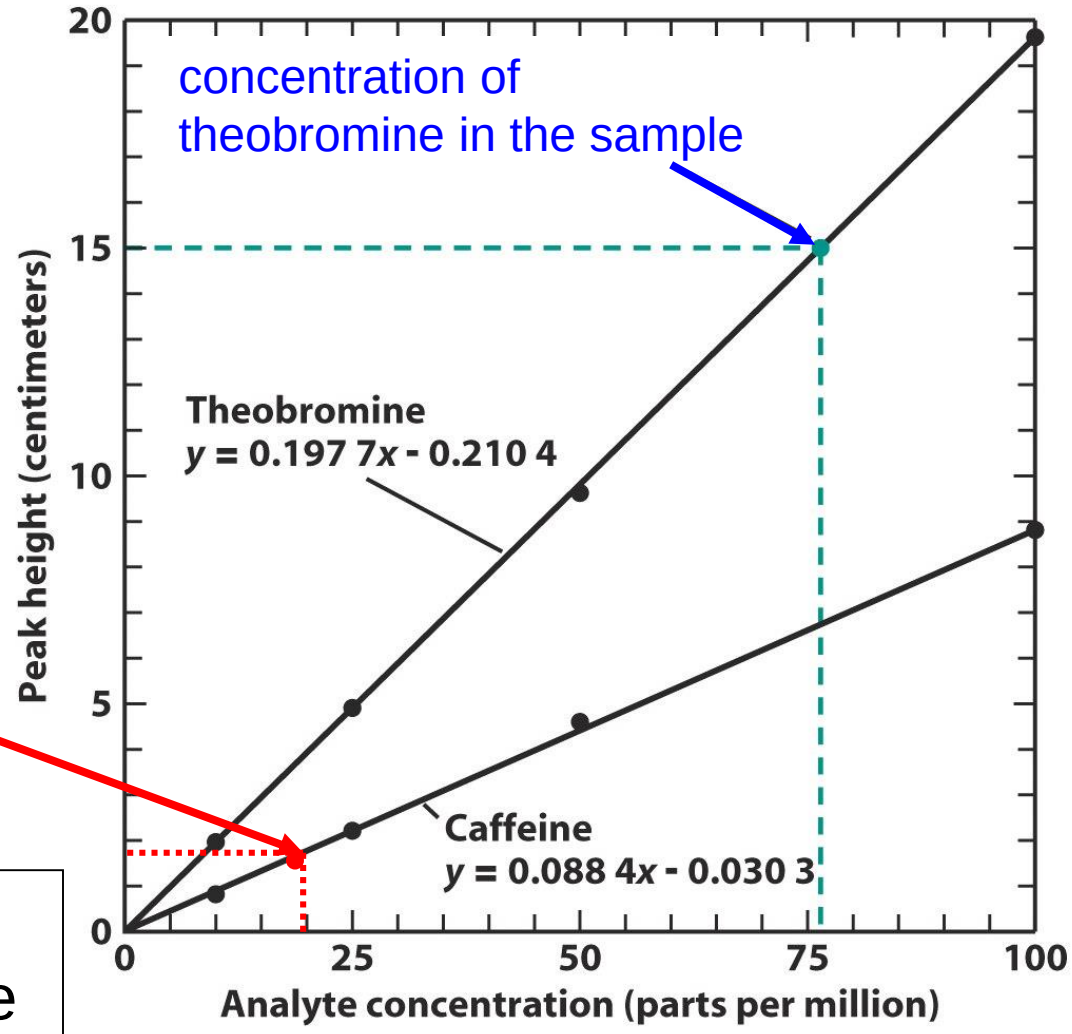
Problem

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

Calibration Curve Using Multiple External Standards

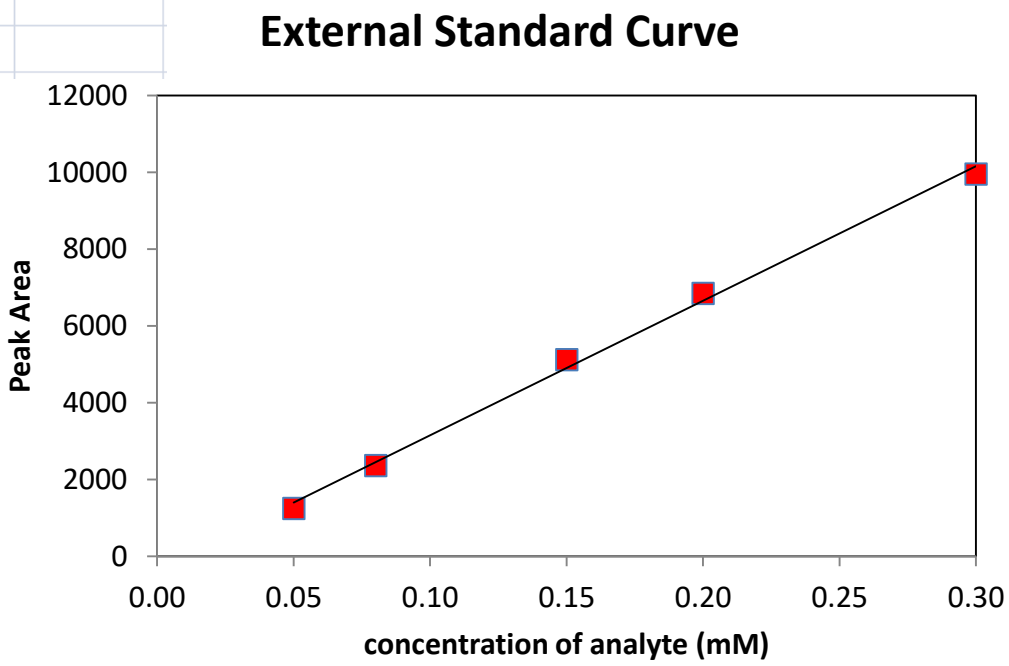
external standards

- 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 (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

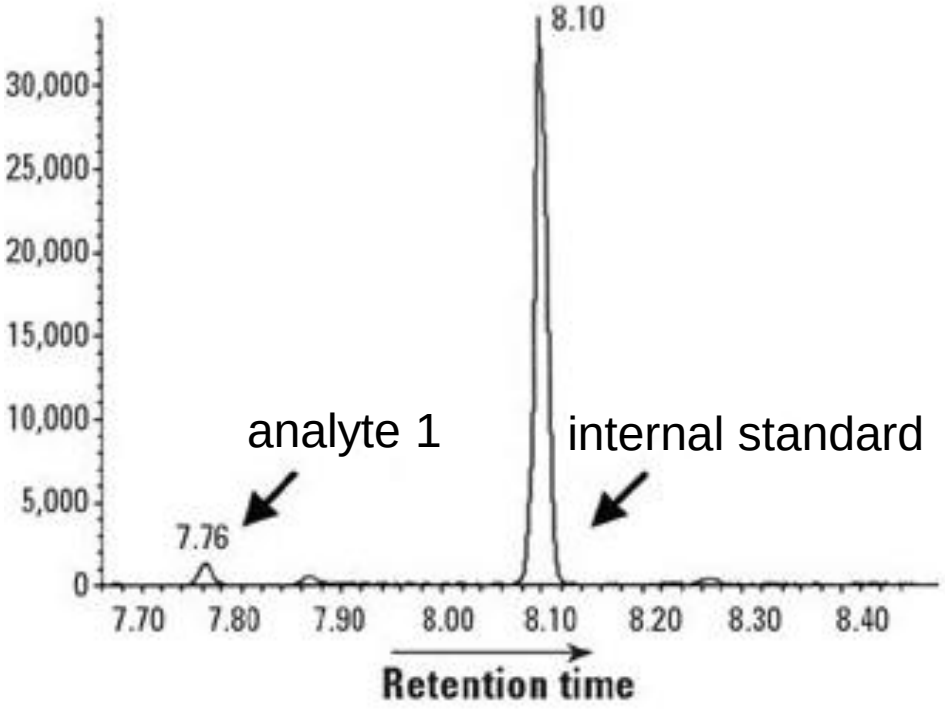
$$m_{IS} = RF_{IS}A_{IS}$$

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

$$m_1 = RF_1A_1$$

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

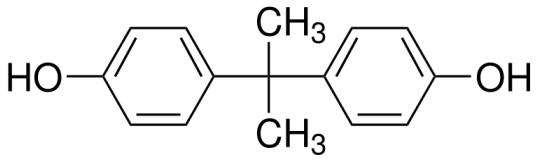
Internal Standard Method



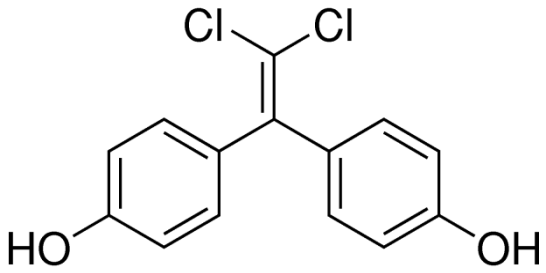
$$\frac{m_{IS}}{m_1} = \left(\frac{RF_{IS}}{RF_1}\right)\left(\frac{A_{IS}}{A_1}\right)$$

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

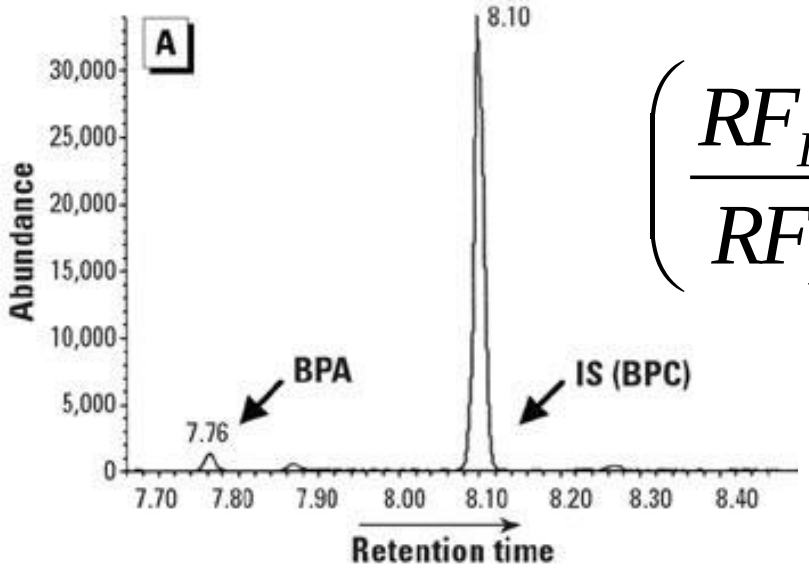
Internal Standard Method



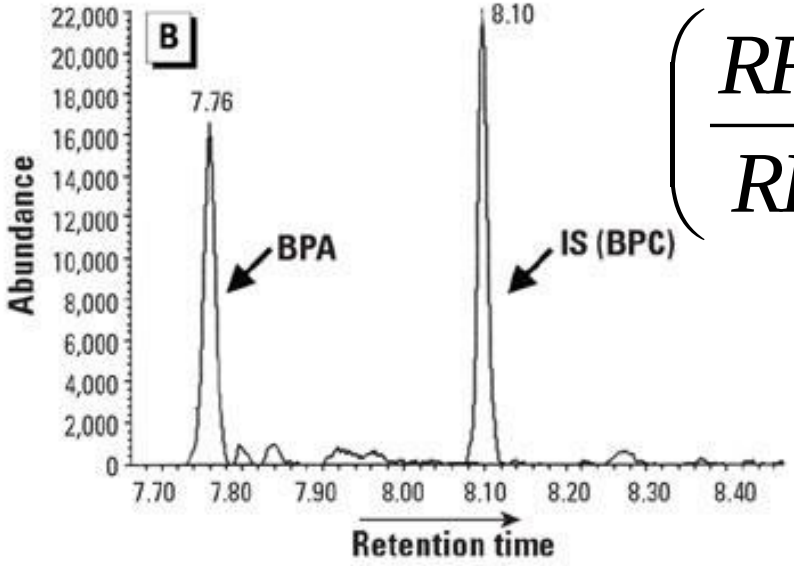
Bisphenol A (BPA)



Bisphenol C (BPC)

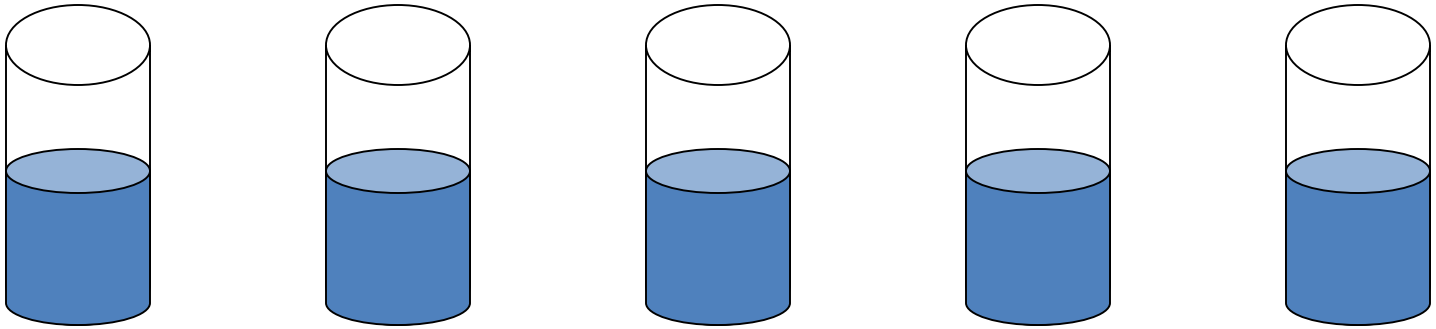


$$\left(\frac{RF_{IS}}{RF_1}\right) \cong 100$$

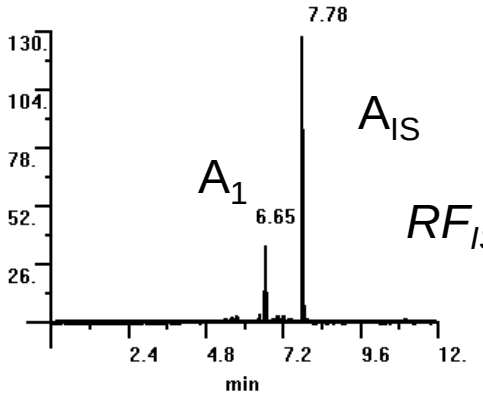


$$\left(\frac{RF_{IS}}{RF_1}\right) \cong 1.5$$

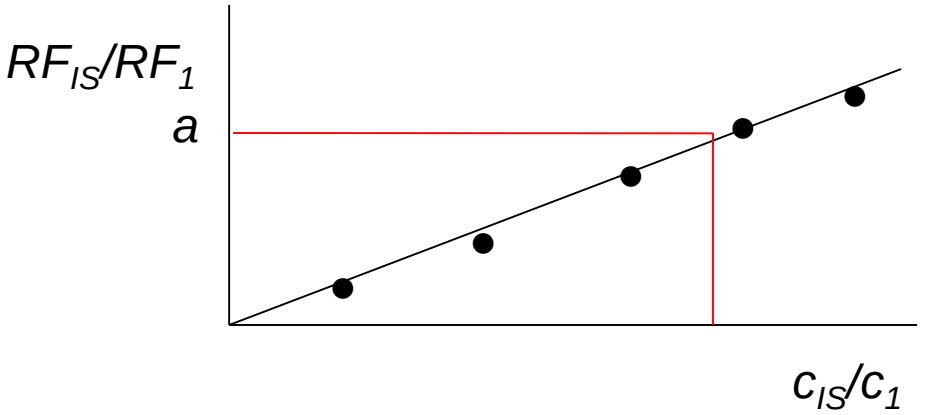
Internal Standard Method



	A	B	C	D	E
Analyte (ppm)	5	10	40	50	100
ISD (ppm)	100	100	100	100	100
K_{IS}/K_1	198	101	24	19	9



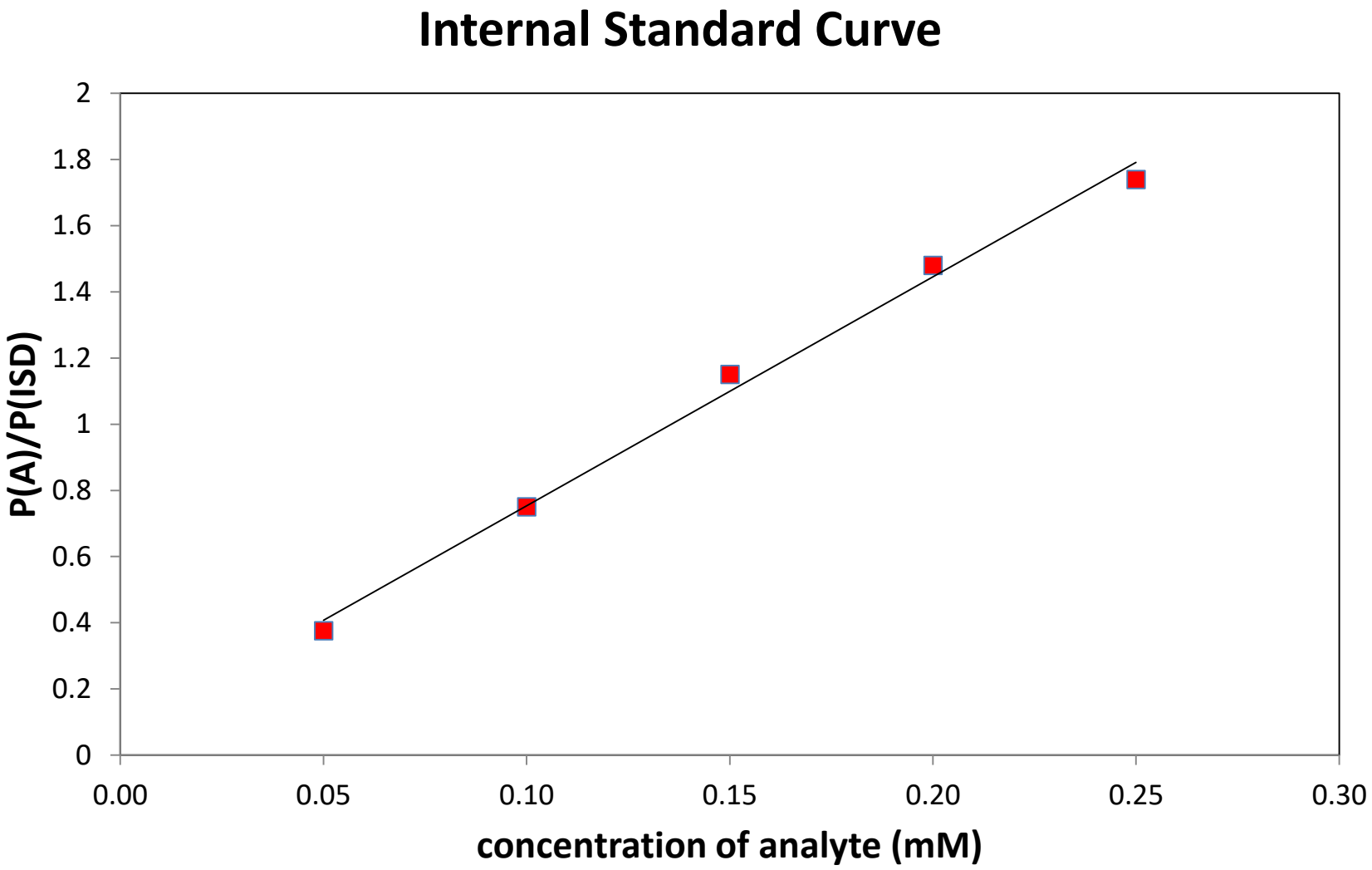
$RF_{IS}/RF_1 = a$



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



Matrix Addition

- Good for samples contain other than analyte which affects signals from analyte (**matrix effect**)
- Cocaine samples of same concentration in methanol and blood produce different intensities in mass spectrum
- Deuterated cocaine can be used as an internal standard (cocaine-d3)

Matrix Effect