KALİBRASYON TEKNİKLERİ

ENSTRÜMANTAL ANALİZ LAB.

2022-2023 BAHAR

Tek Standart ile Karşılaştırma

- Aörnek/Astandard = cörnek/cstandard

Question 1: A 2.0 g of tea sample was analyzed to determine its caffeine content. First the tea sample was dissolved in 100 mL of distilled water and 1.0 mL of this solution was diluted to 10.0 mL. The diluted sample gave a peak area as 1433381. Another measurement was conducted for caffeine standard solution which recorded as 1402518. It was prepared by 0.10 g of caffeine diluted to 50.0 mL of distilled water and 2.0 mL of this solution was filled to 25 mL with distilled water. Please calculate percent caffeine amount in tea sample.

Q1)

$$\frac{A_{standard}}{A_{sample}} = \frac{C_{standard}}{C_{sample}} \qquad \frac{1402518}{1433381} = \frac{1.6\times10^{-4}\ g/mL}{C_{sample}}$$

$C_{standard}$

$$\frac{0.1\ g}{50\ mL} = 2\times10^{-3}\ g/mL$$

$M_1V_1 = M_2V_2$

$$2\times10^{-3}\ \frac{g}{mL} \times 2mL = M_2 \times 25mL$$

$$M_2 = 1.6\times10^{-4}\ g/mL$$

$$C_{sample} = 1.635\times10^{-4}\ g/mL$$

$1\ mL \quad 1.635\times10^{-4}\ g$

$10\ mL \rightarrow \boxed{1.635\times10^{-3}\ g}$

$1.635\times10^{-3}\ g\ /\ 1mL$

$1\ mL \quad 1.635\times10^{-3}\ g$

$100\ mL \rightarrow \boxed{1.635\times10^{-1}\ g}$

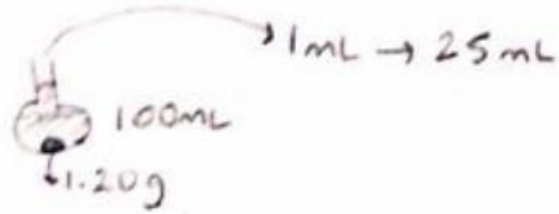
$$\begin{array}{ll} 2.0\ g \rightarrow 1.635\times10^{-1}\ g & \\ 100 \qquad\quad x & \end{array} \qquad \boxed{x = 8.175\ \%}$$

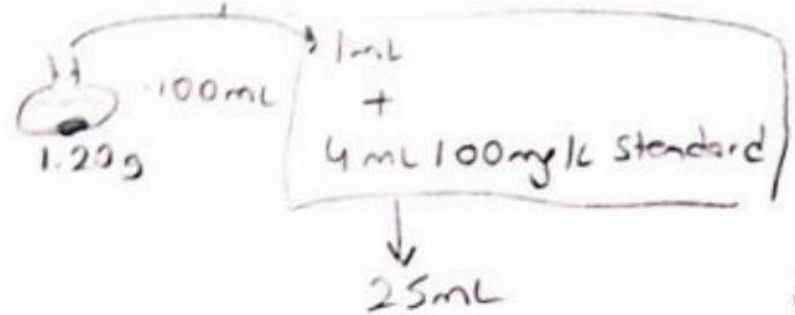
Tekli Standart Katma Yöntemi

- Aörnek/(Aörnek+Astandart) = cörnek/(cörnek+standart)

1) 1.20 g sample including caffeine was dissolved in water and then volume was set to 100 mL with deionized water. 1.0 mL of this solution is diluted to 25 mL. Absorbance value of caffeine was measured as 0.235 at 272 nm. Another 1.20 g was dissolved in water and volume was set to 100 mL. 1.0 mL of this solution was taken and mixed with 4.0 mL of 100 mg/L of caffeine and last volume was set to 25 mL. Absorbance of this solution was measured as 0.710 at 272 nm. Calculate the caffeine amount in sample .

1)





$$\frac{A_{sample}}{A_{sample} + A_{std}} = \frac{C_{sample}}{C_{sample} + C_{std}}$$

$$\frac{0.235}{0.710} = \frac{C_{sample}}{C_{sample} + 16}$$

$$0.235\,C_{sample} + 3.76 = 0.710\,C_{sample}$$

$$C_{sample} = \frac{3.76}{0.475} = 7.916\ mg/L$$

$C_{standard}$

$4\ mL \times 100\ mg/L = 25\ mL \times C_{std}$

$C_{std} = 16\ mg/L$

1000 mL — 7.916 mg
25 mL — ?

$? = 0.1979\ mg / 25\ mL$

$= 0.1979\ mg / 1\ mL$

1 mL — 0.1979 mg
100 mL — ?

$? = 19.79\ mg / 100\ mL$

$\% = \frac{19.79\ mg}{1200\ mg} \cdot 100$

$= \%\ 1.65$

İç Standart Katma Yöntemi

- (Aörnek/Aiçstandart)/(Astandart/Aiçstandart) = cörnek/cstandard

- A 1.0 g tea sample is added to 25 mL volumetric flask and 1.0 mL internal standard is also added to the flask. The flask is completed with distilled water and chromatogram is obtained for the solution. Peak area at 2.0 min retention time is found as 1514 and peak area for internal standard is recorded as 1428.

- A 2.0 mL of 250 mg/L theophylline solution is added into another 25 mL volumetric flask and 1.0 mL internal standard is added. The flask is completed with distilled water and chromatogram is obtained for the solution. Peak area for theophylline at 2.0 min retention time is found as 1689 and peak area for internal standard is recorded as 1543. Calculate theophylline percentage found in tea sample.

$$\frac{\frac{1514}{1428}}{\frac{1689}{1543}} = \frac{\frac{C_s}{C_{std}}}{\frac{C_{std}}{C_s}}$$

$$\frac{1.06}{1.095} = \frac{C_s}{20\ mg/L} \qquad C_s = 19.36\ mg/L$$

$$2mL \times 250\ mg/L = 25mL \times C_{std}$$

$$C_{std} = 20\ mg/L$$

1000 mL — 19.36 mg

25 mL — ?

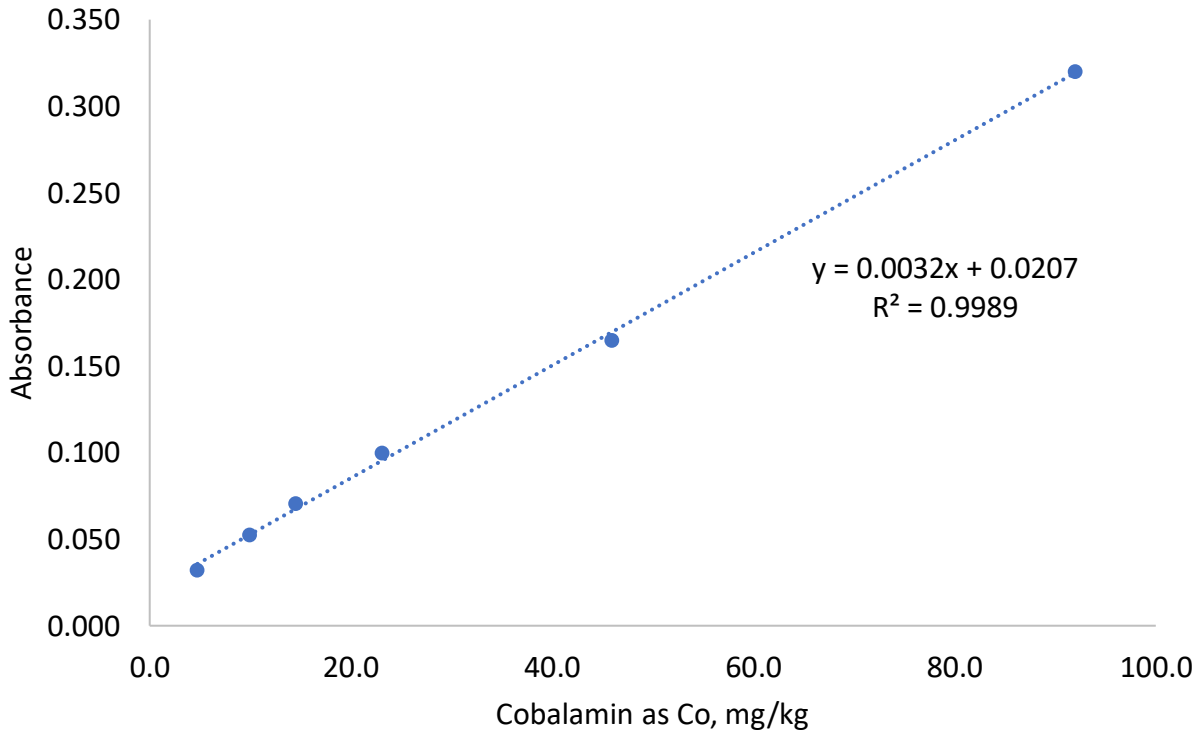
? = 0.484 mg / 25mL

1000 mg sample — 0.484 mg

100 — ?

? = % 0.0484

Dış Kalibrasyon Yöntemi



Derişim, mg/kg	Absorbans
4.7	0.032
9.9	0.053
14.5	0.071
23.1	0.100
45.9	0.165
92.0	0.320

LOD: 3*s/m
LOQ: 10*s/m

Determination of a pesticide was performed by spectrometric method and the following equation was obtained for linear calibration plot obtained the standards given in the table;

$y = 0{,}1126x + 0{,}0096$

Pesticide (mg/L)	Number of measurement	Mean Result	Standard Deviation
1.0	20	0.111	0.01
2.0	5	0.224	0.05
5.0	8	0.61	0.07
10	5	1.12	0.30

Using the data given above, calculate;

a) Limit of detection and quantification
a) Analytical sensitivity for 5.0 mg/L pesticide
b) Calibration sensitivity

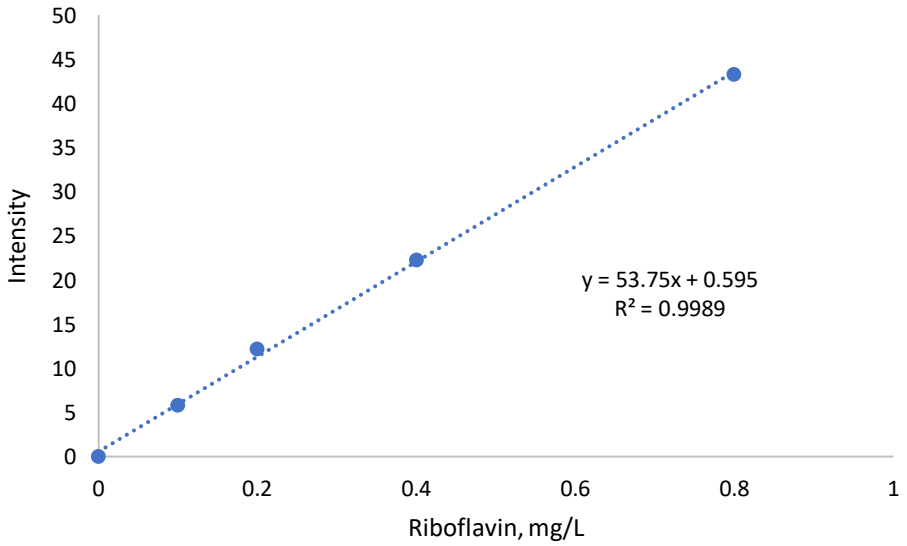
$$LOD = \frac{3s}{m} = \frac{3 \times 0.01}{0.1126} = 0.266 \text{ mg/L}$$

$$LOQ = \frac{10s}{m} = \frac{10 \times 0.01}{0.1126} = 0.888 \text{ mg/L}$$

$$\text{Analytical sensitivity} = \frac{\text{Slope}}{\text{Std. dev}} = \frac{0.1126}{0.07} = 1.609$$

Calibration sensitivity = Slope = 0.1126

Question 2: Riboflavin (vitamin B2) is determined in a cereal sample by measuring its fluorescence intensity in 5% acetic acid solution. A calibration curve was prepared by measuring the fluorescence intensities of a series of standards of increasing concentrations. The following data were obtained. Use the method of least squares to obtain best straight line for the calibration curve and to calculate the concentration of riboflavin in the sample solution. The sample fluorescence intensity was 15.4.



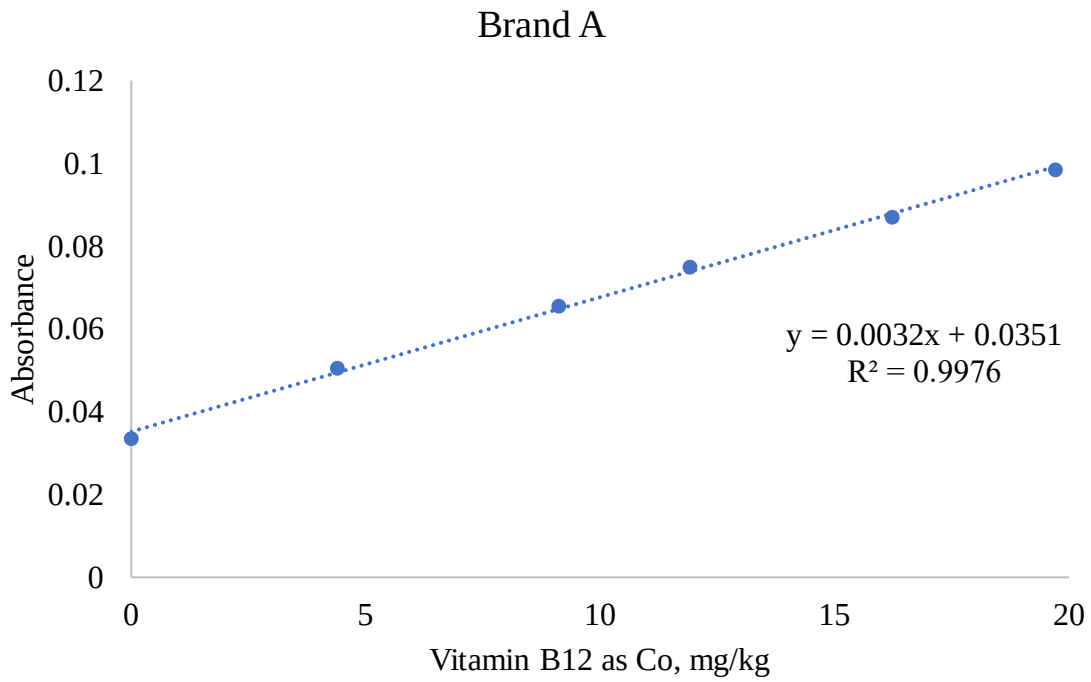
Riboflavin, mg/L	Floerescence Intensity
0.00	0.0
0.100	5.8
0.200	12.2
0.400	22.3
0.800	43.3

Q2) $y = 53.75x + 0.595$

$15.4 = 53.75x + 0.595$

$$x = \frac{15.4 - 0.595}{53.75} = 0.275 \text{ mg/L}$$

Standart Katma Kalibrasyon Yöntemi



Derişim, mg/kg	Absorbans
0	0.0335
4.4	0.0505
9.1	0.0655
11.9	0.075
16.2	0.087
19.7	0.0985

0=0.0032x+0.0351
X=-11.0

Exactly 5.00-mL aliquots of a solution containing phenobarbital were measured into 50.00-mL volumetric flasks and made basic with KOH. The following volumes of a standard solution of phenobarbital containing 2.000 μg/mL of phenobarbital were then introduced into each flask and the mixture was diluted to volume: 0.000, 0.500, 1.00, 1.50, and 2.00 mL. The fluorescence of each of these solutions was measured with a fluorometer, which gave values of 3.26, 4.80, 6.41, 8.02, and 9.56, respectively.

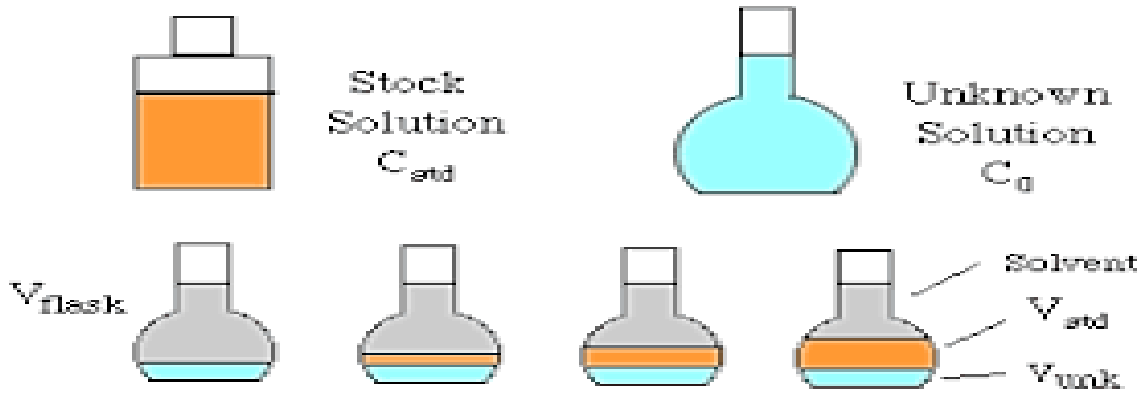
(a) Plot the data.

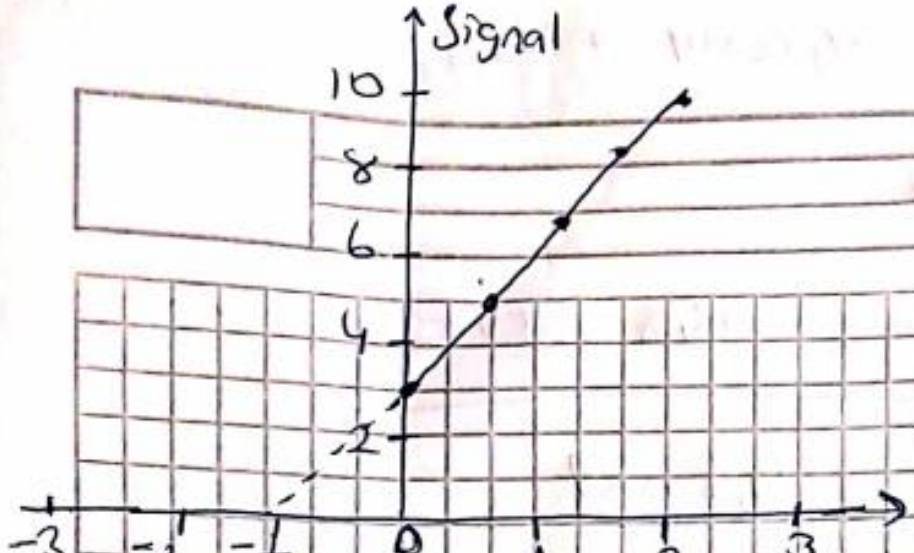
*(b) Using the plot from (a), calculate the concentration of phenobarbital in the unknown.

*(c) Derive a least-squares equation for the data.

*(d) Find the concentration of phenobarbital from the equation in (c).

(e) Calculate a standard deviation for the concentration obtained in (d).





Volume of standard	Signal
0	3.26
0.5	4.80
1.0	6,41
1.5	8.02
2.0	9.56

After linear regression;

Slope = m = 3.164

Intercept = b = 3.246

$y = 3.164x + 3.246$

in order to find the x intercept → $y = 0$

$0 = 3.164x + 3.246$

$x = -1.026$ mL

$C_1V_1 = C_2V_2$

$2\ \mu g/mL \times 1.026\ mL = C_2 \cdot 50\ mL$

$C_2 = 0.041\ \mu g/mL$

$1\ mL \longrightarrow 0.041\ \mu g$

$50\ mL \longrightarrow x$

$x = 2.05\ \mu g$

$C_{sample} = \frac{2.05\ \mu g}{5\ mL} = 0.41\ \mu g/mL$

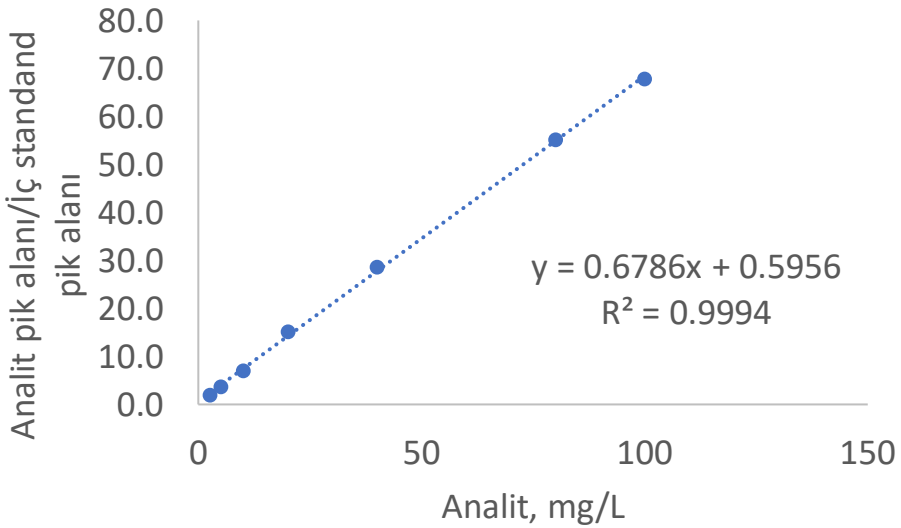
OR

$\text{Dilution factor} = \frac{50\ mL}{5\ mL} = 10\ \text{times}$

$C_2 = 0.041\ \mu g/mL$

$C_{sample} = 0.041\ \mu g/mL \times 10 = 0.41\ \mu g/mL$

İç Standart Katma Kalibrasyon Yöntemi



Analit, mg/L	Analit pik alanı	İç std. Pik alanı	Oran
2.5	100	55	1.8
5	200	56	3.6
10	400	58	6.9
20	800	53	15.1
40	1600	56	28.6
80	3200	58	55.2
100	4000	59	67.8
Örnek	1500	56	26.8

26.8=0,6786x+0,5956
X=38,6 mg/L