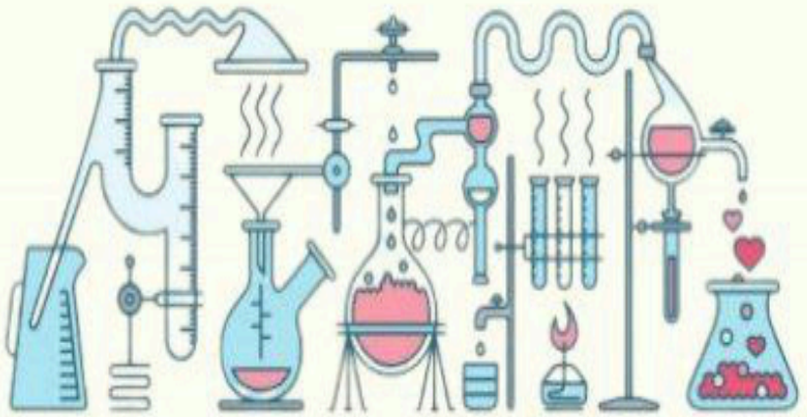
تفريغ تحليل آلي



اسم الموضوع: *Introduction*
Part 3
Calibration Techniques

إعداد الصيدلاني/ـة: *Sara Jaber*

ادعولي ♡
ولكم بالمثل

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لجان الدفعات

Calibration Techniques

LOD = 3SB / slope

LOQ = 10SB / slope

LOL → ماهكينا كيف نفلعه → Slide (33-

Calibration Techniques

- 1. External Calibration Curve Method

اسهل اشي

- 2. Standard Additions Method

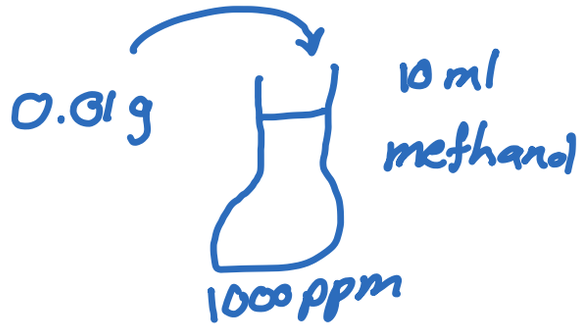
- 3. Internal Standard Method

External Calibration Curve Method

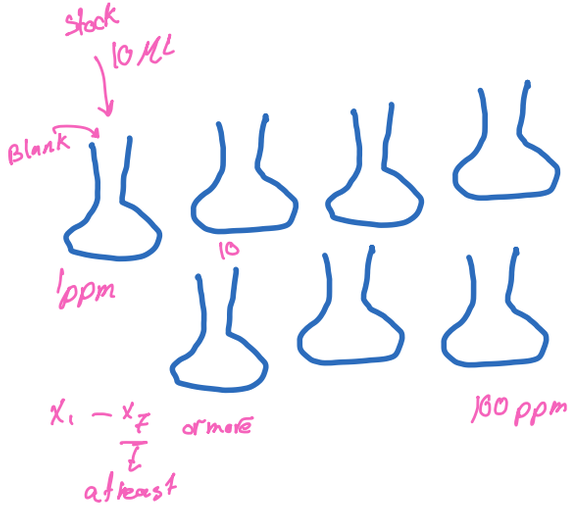
1. Most convenient when a large number of similar samples are to be analyzed.
2. Most common technique.
3. Facilitates calculation of Figures of Merit.

الخطوات :

1- Stock Solution



2- Standerd Solution "dilution"



3- بدخلهم للجهاز ⟶ $x \longrightarrow y$

$x \longrightarrow y$

$x_1 \longrightarrow y_1$

$y_7 \rightarrow y_7$

S_{xy} / S_{xy}

$m = \frac{S_{xy}}{S_{xy}}$

$b = \bar{y} - m\bar{x}$

$y = mx \pm b$

LOD = 3 SDB / m

LOQ = 10 SDB / m

R^2

4- باخذ عينة من المريض و بحطها بالجهاز و باخذ القراءة ومن المعادلة بطلع x

External Calibration Curve Procedure

1. Prepare a series of standard solutions (analyte solutions with known concentrations).

2. Plot [analyte] vs. Analytical Signal.

3. Use signal for unknown to find [analyte].

Example: Pb in Blood by GFAAS

Atomic absorption
لاع ناخذ عينات بجربن

X	y
[Pb]	Signal
(ppb)	(mAbs)
0.50	3.76
1.50	9.16
2.50	15.03
3.50	20.42
4.50	25.33
5.50	31.87

تركيز الرصاص بالوحدة

قراءة الجهاز

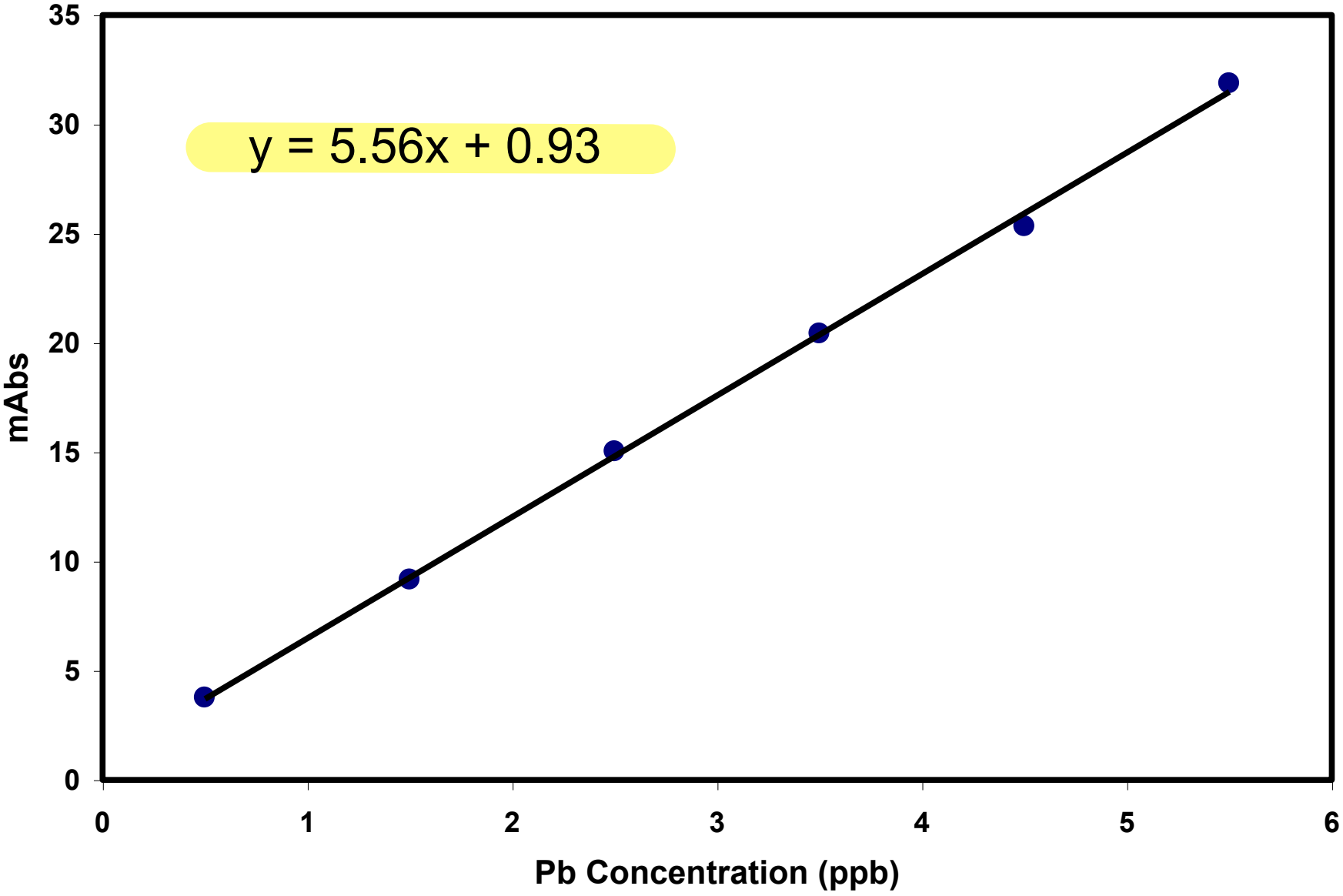
Results of linear regression:

$$S = mC \pm b$$

$m = 5.56$ mAbs/ppb

$b = 0.93$ mAbs

$y = 5.56x + 0.93$


y = 5.56x + 0.93
mAbs
Pb Concentration (ppb)
0
5
10
15
20
25
30
35
0
1
2
3
4
5
6

Calculate the LOD for Pb

20 blank measurements gives an average signal

Blank جيبت
Solution
واد خلته للجهاز 20
موة واعذت القراءات

$\bar{X}_{Blank}$ = **0.92 mAbs**

with a standard deviation (Sb) of

$$\sigma_{bl} = 0.36 \text{ mAbs}$$

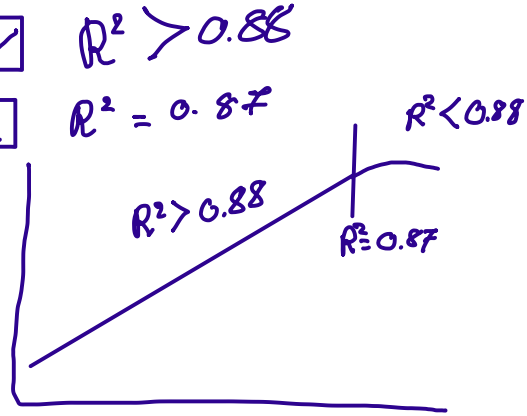
LOD = 3 Sb/m = 3 x 0.36 mAbs / 5.56 mAbs/ppb

5.56

LOD = 0.2 ppb

$LOD = \frac{10 + 0.36}{5.56} =$ ppb

Find the LOL for Pb



Lower end = LOD = 0.2 ppb

(include this point on the calibration curve)

$S_{LOD} = 5.56 \times 0.2 + 0.93 = 2.0$ mAbs

(0.2 ppb (X) , 2.0 mAbs (Y))

Find the LOL for Pb

طريقة الأرقام

Upper end = collect points beyond the linear region and estimate the 95% point.

Suppose a standard containing 18.5 ppb gives rise to s signal of (Y practical= 98.52 mAbs)

بالعملي

This is approximately 5% below the expected value of (Y theoretical=103.71 mAbs)

النظري

لما يرتفع التركيز بتصير الجزيئات تحجب الضوء عن بعضها ← قراءة خاطئة

(18.50 ppb , 98.52 mAbs)

لحوض x في المعادلة وبوجد y د (theory)

وبعمل التجربة وباخذ ← إذا كان الفرق بينهم ±٥٪ معناها التركيز مش داخل مجي

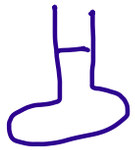
القراءة

Standard Addition Method
Reduce matrix effect

Plasma of Rat.

1. Most convenient when a small number of samples are to be analyzed.

2. Useful when the analyte is present in a complicated matrix and no ideal blank is available.



Stock
1000ppm

Sample
Pb is water
من النهر

Pb in water distellet
10 ml H_2O
0.01g

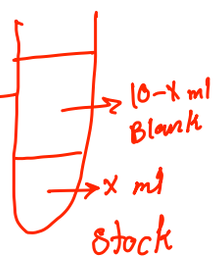
Stock	x_1 0ml	x_2 1ml	x_3 2ml	x_4 3	4 ml
Sample	1ml	1ml	1ml	1ml	1ml
	x_1	x_2	x_3	x_4	x_5
	y_1	y_2	y_3	y_4	y_5

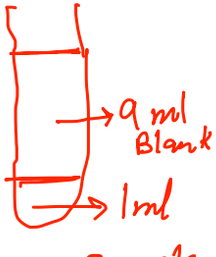
عينة غير معلومة

at least 5

بكل مرة

بكمل بـ 10ml لـ Blank
بحيث يكونوا لهم نفس Volume





25. للـ Stock

when $x_1 = 0$ ml
$y_{respons\ to\ 0} = y_0$
$x_2 = 1$ ml
$y_{respons\ 1} = y_1$
:

Response to Stock

$$y = mx \pm b$$

$$C_x = b \times \frac{C_s}{m.V}$$

- b: intercept
- m: slop
- C_x: Sample Con
- C_s: Conc stock
- V: Volume of Added sample

Standard Addition Procedure

1. Add one or more increments of a standard solution to sample aliquots of the same size. Each mixture is then diluted to the same volume.

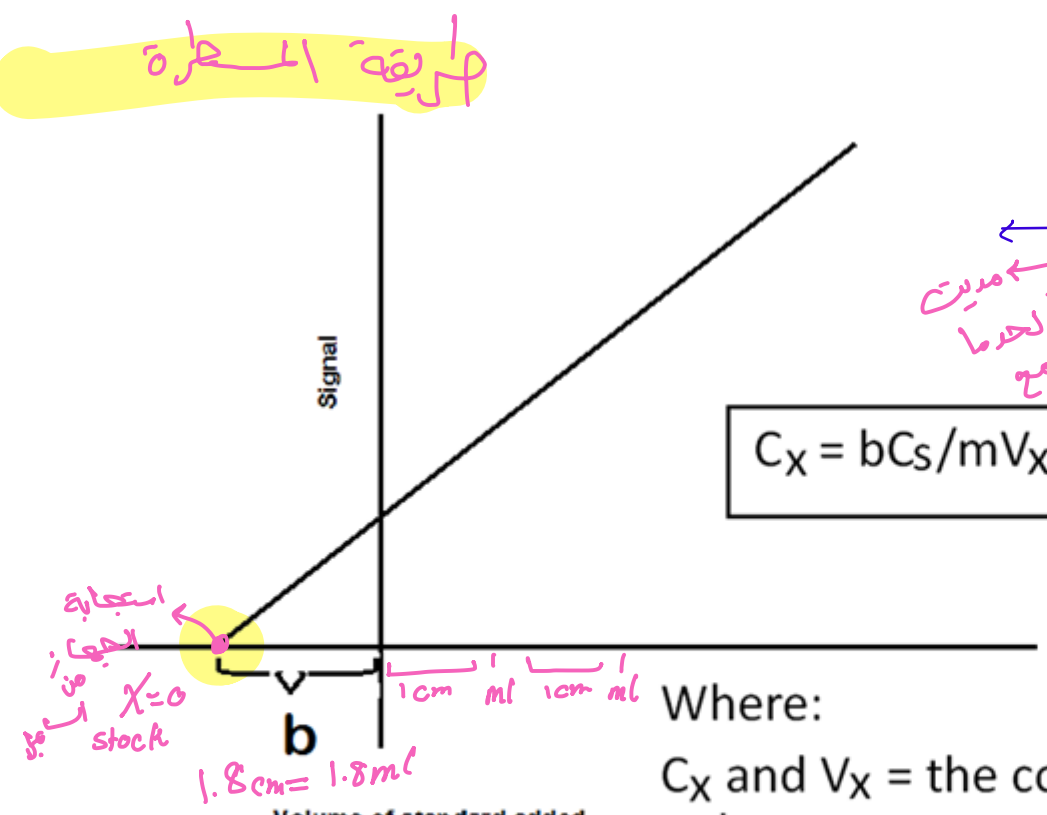
2. Prepare a plot of Analytical Signal versus:
   a) volume of standard solution added, or
   b) concentration of analyte added.

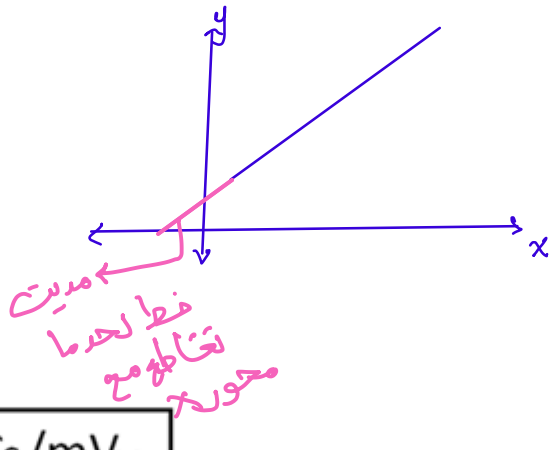
Standard Addition Procedure

3. The x-intercept of the standard addition plot corresponds to the amount of analyte that must have been present in the sample (after accounting for dilution).

4. The standard addition method assumes:
   a) the curve is linear over the concentration range
   b) the y-intercept of a calibration curve would be 0

Calculation of standard addition



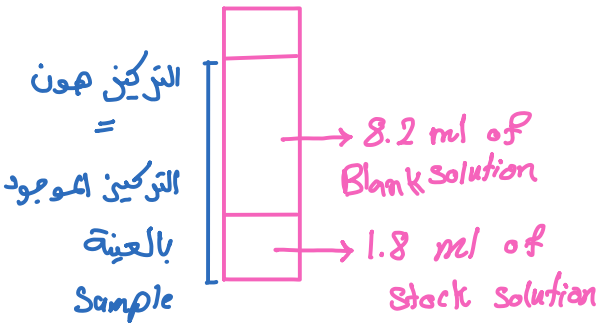


$$C_X = bC_S/mV_X$$

Where:

C_X and V_X = the concentration and the volume of unknown

C_S and V_S = the concentration and the volume of added standard



Example: Fe in Drinking Water

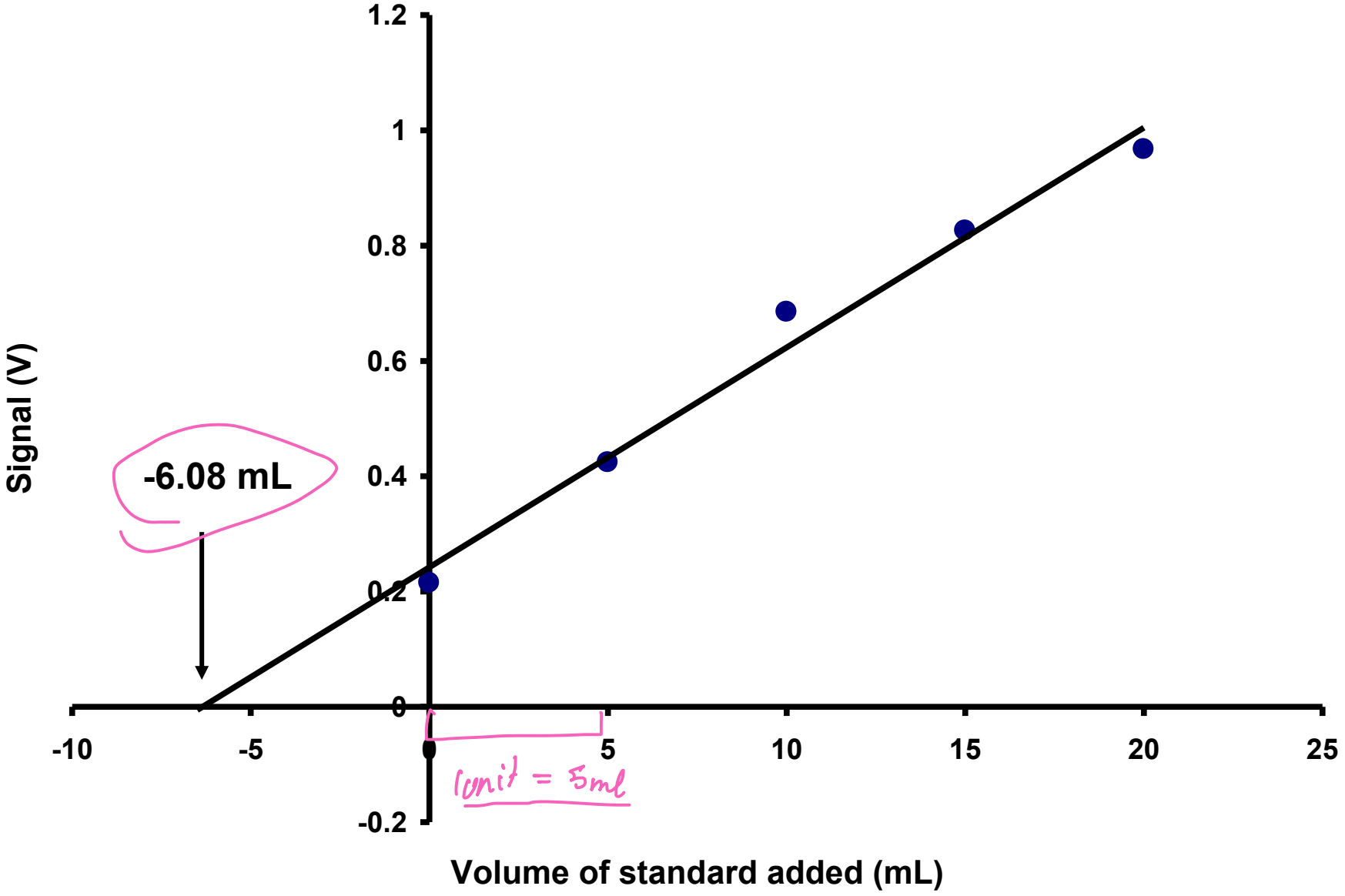
Sample Volume (mL)	Standard Volume (mL)	Signal (V)
10	0	0.215
10	5	0.424
10	10	0.685
10	15	0.826
10	20	0.967

The concentration of the Fe standard solution is 11.1 ppm

All solutions are diluted to a final volume of 50 mL

$$C_x = b C_s / m V_x$$

$$C_x = 6.08 * 11.1 / * 10$$


1.2
1
0.8
0.6
0.4
0.2
0
-0.2
-10
-5
0
5
10
15
20
25
Signal (V)
Volume of standard added (mL)
-6.08 mL
1 unit = 5 ml

[Fe] = ?

x-intercept = -6.08 mL

Therefore, 10 mL of sample diluted to 50 mL would give a signal equivalent to 6.08 mL of standard diluted to 50 mL.

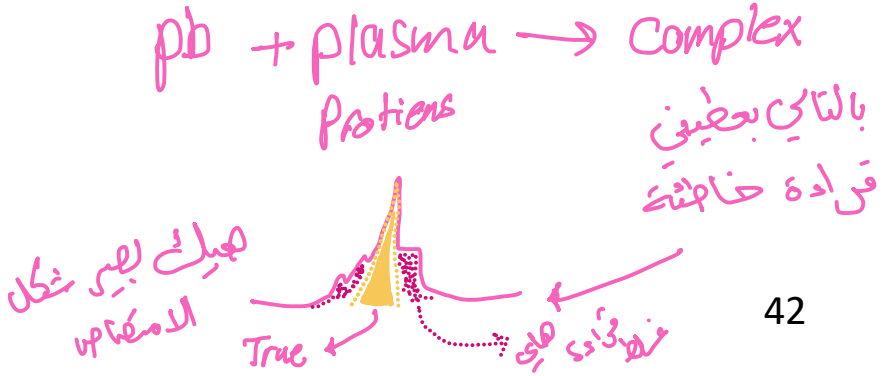
$$V_{sam} \times [Fe]_{sam} = V_{std} \times [Fe]_{std}$$

10.0 mL x [Fe] = 6.08 mL x 11.1 ppm

[Fe] = 6.75 ppm

Internal Standard Method
Reduce matrix and instrument effects

1. Most convenient when variations in analytical sample size, position, or matrix limit the precision of a technique.
2. May correct for certain types of noise.



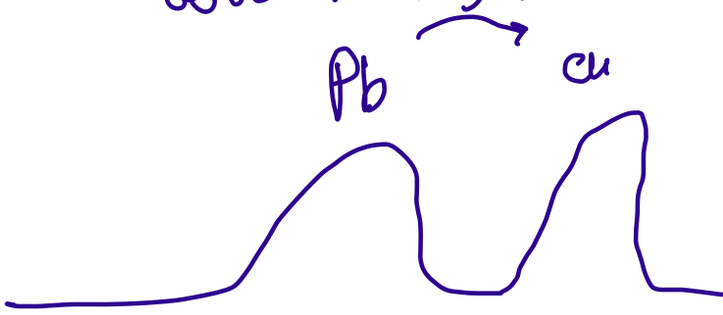
100 ppm
Cu

100 ppm
Pb

هون تركيز مش Y	X_1	X_2	X_3	X_4	.....
Conc Pb -ppm-	20	40	60	100	
Conc Cu -ppm-	100	100	100	100	100
y_{final}	$y_1 = \frac{y\ Pb}{y\ Cu}$	$y_2 = \frac{y\ Pb}{y\ Cu}$	...	---	--

$y = mx + b$

بشبحوا بحلن بالخصائص



فبضيف النحاس على العينة وبطلع
قراءة الرصاص بدلالة قراءة النحاس

+100 ppm Cu

Sample
(Pb فيها)

$\longrightarrow y_{sample} = \frac{y_{Pb}}{y_{Cu}}$

Internal Standard Procedure

بالمثال كان Pb

1. Prepare a set of standard solutions for analyte (A) as with the calibration curve method, but add a constant amount of a second species (B) to each solution.

بالمثال Cu

2. Prepare a plot of S_A/S_B versus [A].

3. External calibration equation

$$S_A/S_B = mC + b$$

$y_{final} = mc + b$

Notes

1. The resulting measurement will be independent of sample size and position.

Position , Size لا تعتمد على

2. Species A & B must not produce signals that interfere with each other. Usually they are separated by wavelength or time.

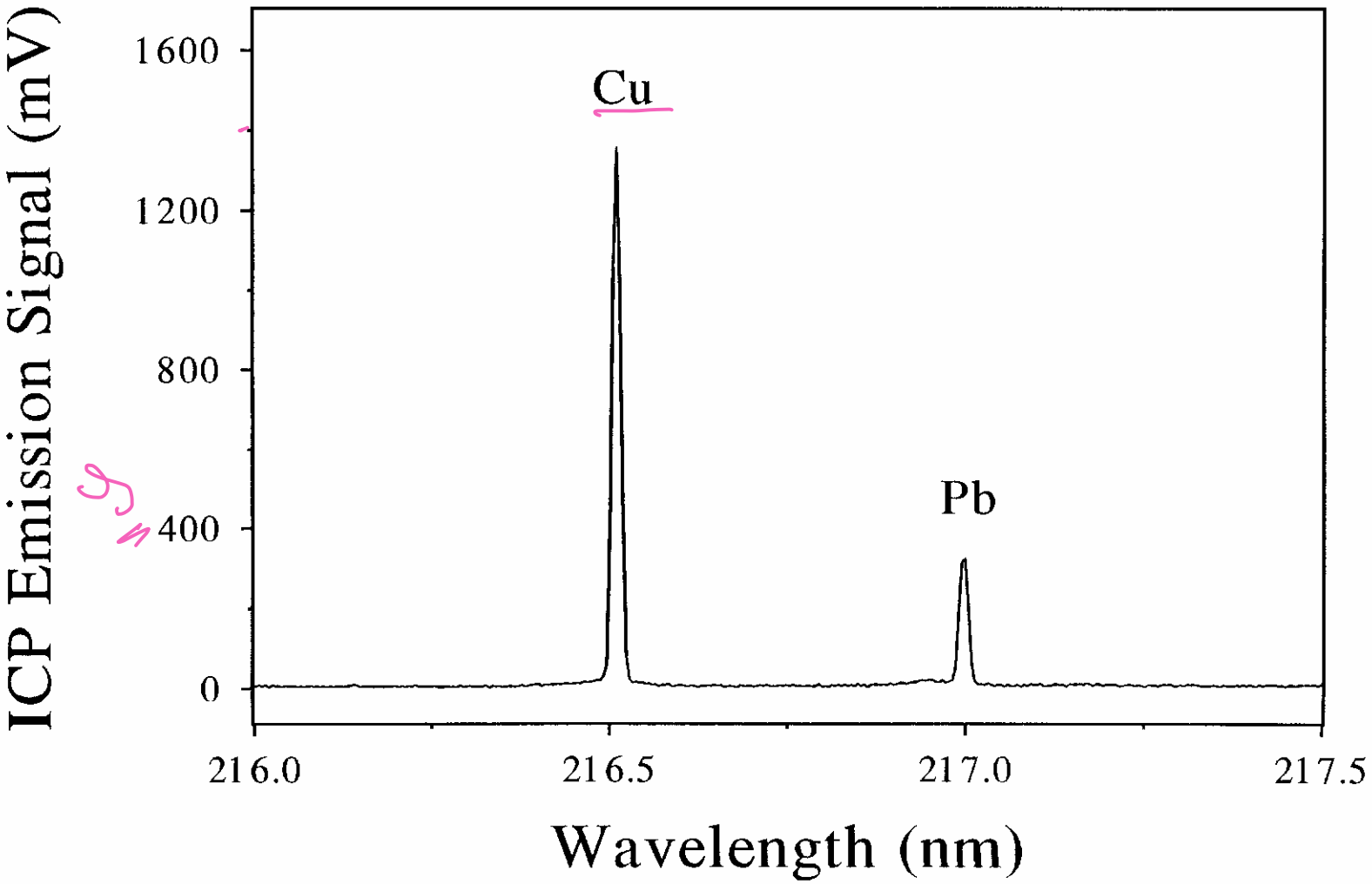
Example: Pb by ICP Emission

نركز انه هون كرد التجربة وآخذ الثمن مادة فنحتمد الطريقة هاي بالحل

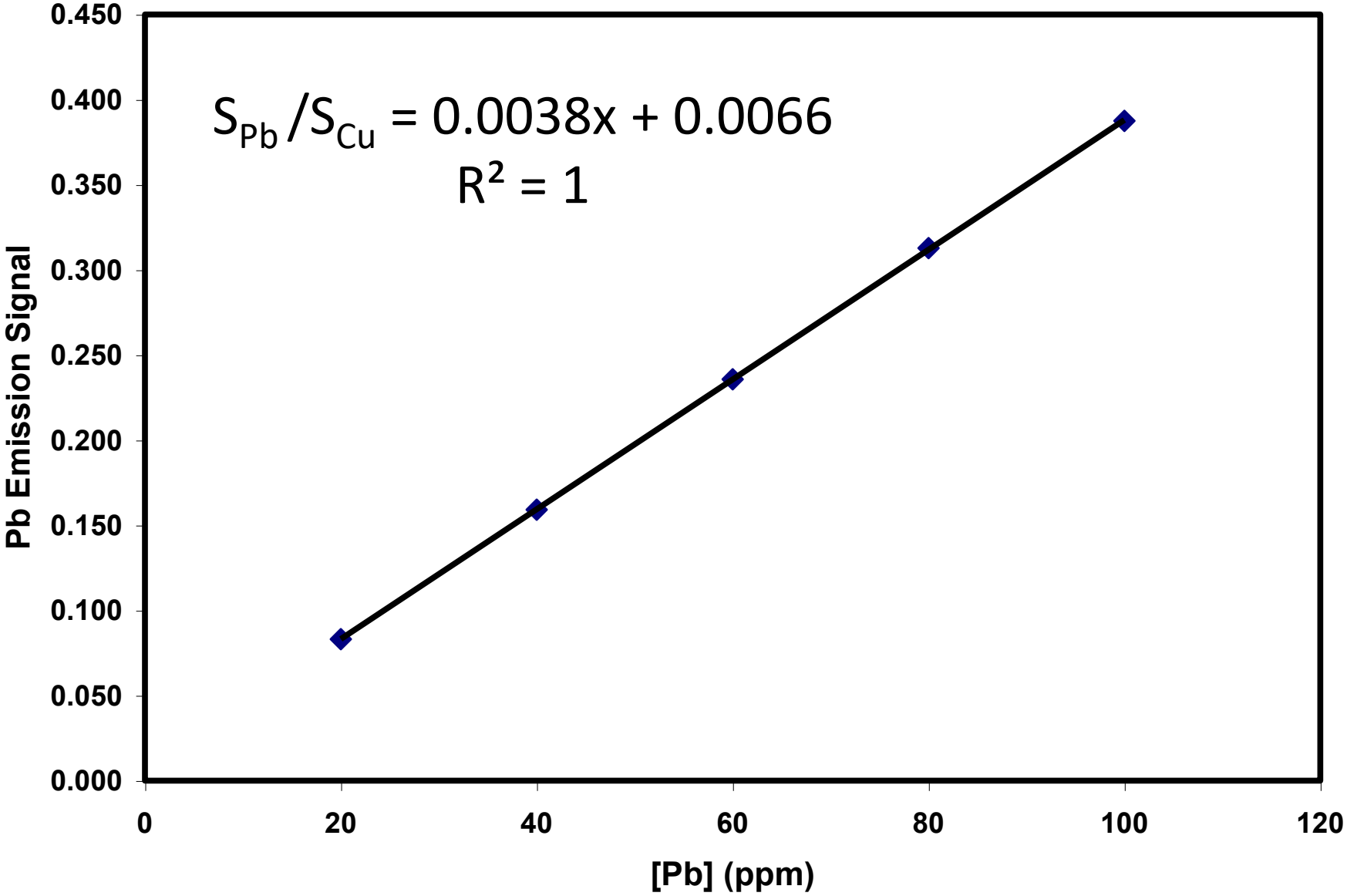
لكن في طريقة أحرى لو اعطاني مادة وحده موجودة في "Part 4

Each Pb solution contains 100 ppm Cu.

	Signal		
[Pb] (ppm)	Pb	100 ppm Cu	Pb/Cu
x			y
20	112	1347	0.083
40	243	1527	0.159
60	326	1383	0.236
80	355	1135	0.313
100	558	1440	0.388


ICP Emission Signal (mV)
1600
1200
800
400
0
Cu
Pb
216.0
216.5
217.0
217.5
Wavelength (nm)

Internal Standard Correction



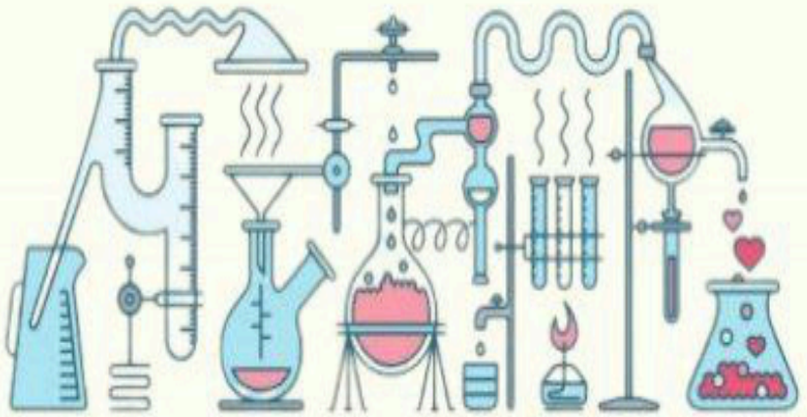
Results for an unknown sample after adding 100 ppm Cu

	Signal		
Run	Pb	Cu	Pb/Cu
	x		y
1	346	1426	0.243
2	297	1229	0.242
3	328	1366	0.240
4	331	1371	0.241
5	324	1356	0.239
mean	325	1350	0.241 → y

$$0.241 = 0.0038\ X + 0.0066$$

$$X = 61.684 \text{ ppm of Pb}$$

تفريغ تحليل آلي



اسم الموضوع: *Introduction Part 4*

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ادعولي ♡
ولكم بالمثل

بداية المحاضرة الدكتور
كمل المثال السابق

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Another way for Internal Standard method calculation

لو اعطاني قراءة واحدة مش جدول
نتبع الطريقة هاي ↓

- In a single-point internal standardization, we prepare a single standard containing the analyte and the internal standard and use it to determine the value of *K*.

تركيز المادة internal المادة المي اضفتها

$$K = \left(\frac{C_{IS}}{C_A}\right)_{std} \times \left(\frac{S_A}{S_{IS}}\right)_{std}$$

factor

Response to the Analyte

Response to the internal

تركيز المادة Analyte المادة المي بدي اوجدها

- Where, CA is the standard concentration of the analyte, SA instrument response of analyte, CIS, and SIS the are the concentration and the response of internal added compound , respectively.

① analyte = Pb^{2+}

A sixth spectrophotometric method for the quantitative analysis of Pb^{2+} in blood uses Cu^{2+} as an internal standard. A standard containing 1.75 ppb Pb^{2+} and 2.25 ppb Cu^{2+} yields a ratio of $(S_A/S_{IS})_{std}$ of 2.37. A sample of blood is spiked with the same concentration of Cu^{2+}, giving a signal ratio, $(S_A/S_{IS})_{samp}$, of 1.80. Determine the concentration of Pb^{2+} in the sample of blood.

SOLUTION allows us to calculate the value of K using the data for the standard

$$K = \left(\frac{C_{IS}}{C_A}\right)_{std} \times \left(\frac{S_A}{S_{IS}}\right)_{std} = \frac{2.25 \text{ ppb Cu}}{1.75 \text{ ppb } Pb^{2+}} \times 2.37 = 3.05 \frac{\text{ppb } Cu^{2+}}{\text{ppb } Pb^{2+}}$$

The concentration of Pb^{2+}, therefore, is

$$C_A = \frac{C_{IS}}{K} \times \left(\frac{S_A}{S_{IS}}\right)_{samp} = \frac{2.25 \text{ ppb } Cu^{2+}}{3.05 \frac{\text{ppb } Cu^{2+}}{\text{ppb } Pb^{2+}}} \times 1.80 = 1.33 \text{ ppb } Cu^{2+}$$

Standerd

analyte → Pb

Conc Pb = 1.75 ppm

internal → Cu

Conc Cu = 2.25

$$\frac{S_A}{S_{IS}} = 2.37 \rightarrow \text{Standerd}$$

تفصيل الحل

المعطيات

Sample

$$\frac{S_A}{S_{IS}} = 1.80$$

فيها نفس تركيز Cu

المطلوب: بده تركيز Pb في العينة

Step ① ⟵ نعوض معطيات الـ Standerd بالقانون

$$K = \frac{C_{Cu}}{C_{Pb}} * \frac{S_{Pb}}{S_{Cu}}$$

$$= \frac{2.25 \text{ ppm } Cu^{+2}}{1.75 \text{ ppm } Pb^{+2}} * 2.37$$

$$K = 3.05 \quad \frac{\text{ppm } Cu^{+2}}{\text{ppm } Pb^{+2}}$$

Step ② ⟵ نعوض معطيات الـ Sample + K اللي اوجدناها فوق بالقانون

$$K_{std} = K_{sample}$$

$$3.05 \frac{\text{ppm } Cu^{+2}}{\text{ppm } Pb^{+2}} = \frac{2.25 \text{ ppm } Cu^{+2}}{X \text{ ppm } Pb^{+2}} * 1.80$$

Step (3) نخلي تركيز الـ Pb^{+2} موضوع المجهول

$$3.05 \frac{ppm\ Cu^{+2}}{ppm\ Pb^{+2}} = \frac{4.05\ ppm\ Cu^{+2}}{x\ ppm\ Pb^{+2}}$$

$$x\ ppm\ Pb^{+2} = \frac{4.05\ ppm\ Cu^{+2}}{3.05 \frac{ppm\ Cu^{+2}}{ppm\ Pb^{+2}}}$$

يعني بالبسط

$$x = 1.3278\ ppm\ Pb^{+2}$$

$$x \simeq 1.33\ ppm\ Pb^{+2}$$