Deacon's Challenge

No 95 - Answer

Reproduced below are peak area data from an HPLC analytical run set up to measure plasma phenylalanine. The assay is used to monitor adequacy of dietary control in patients with phenylketonuria, good control being regarded as maintaining plasma phenylalanine between 120 and 360 µmol/L.

N-methyl phenylalanine has been used as the internal standard. 200 µL of internal standard has been added to 200 µL aliquots of samples and standards prior to analysis.

Standard concentration = 500 µmol/L
N-methyl phenylalanine (NMP) concentration = 100 µmol/L
QC target: 180 – 210 µmol/L

Sample	*Peak area*	
	NMP	*Phenylalanine*
Standard	*20,000*	*81,000*
QC	*22,000*	*35,000*
Patient	*21,000*	*140,000*

a) Is the assay in control?
b) What is the patient's plasma phenylalanine concentration?
c) What comment would you make about the patient's control from this result?

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The peak area ratio (PAR) is proportional to concentration.

$$\text{PAR} = \frac{\text{Peak area of analyte (phenylalanine)}}{\text{Peak area of internal standard (NMP)}}$$

There is no need to correct for the dilution of sample and internal standard (0.5) since all samples are treated the same and the dilution factor cancels.

Sample	Peak areas		PAR
	NMP	Phenylalanine	
Standard	20,000	81,000	81,000/20,000 = 4.05
QC	22,000	35,000	35,000/22,000 = 1.59
Patient	21,000	140,000	140,000/21,000 = 6.67

It is necessary to assume that the peak area ratio is proportional to phenylalanine concentration, so that their ratio is constant:

$$\frac{\text{Phenylalanine concentration}}{\text{PAR}} = \text{Constant}$$

and that this relationship holds across the concentration range of the data (presumably verified when the method was set up). Therefore at two concentrations (corresponding to standard and unknown samples):

$$\frac{\text{Sample phenylalanine concentration}}{\text{Sample PAR}} = \frac{\text{Standard phenylalanine concentration}}{\text{Standard PAR}}$$

which can be rearranged to calculate the phenylanine concentration in the sample:

Sample phenylalanine concentration (µmol/L)

$$= \frac{\text{Standard phenylalanine concentration (500 µmol/L)} \times \text{Sample PAR}}{\text{Standard PAR}}$$

a) For the QC sample:

$$\text{Phenylalanine (µmol/L)} = \frac{500 \times 1.59}{4.05} = \mathbf{196\ \mu mol/L}$$

Since this value is well within the quoted range (180 – 210 µmol/L) the assay is in control.

b) For the patient:

$$\text{Phenylalanine (µmol/L)} = \frac{500 \times 6.67}{4.05} = 823\ \mu mol/L$$

c) The patient's result is well above the recommended range so is consistent with sub-optimal control.

Question 96

A patient who is known to have diabetes insipidus is admitted in a semicomatose state. His serum sodium concentration is 155 mmol/L. His admission weight is 79 Kg. Estimate his water deficit, indicating clearly any assumptions you make.

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