Deacon's Challenge

No 184 - Answer

A new assay is being devised for the measurement of phenytoin. In an assessment of recovery, aliquots of a solution of phenytoin sodium (1 mg in 1 mL) are added to separate 1 mL aliquots of a serum sample and the aliquots mixed then re-assayed with the following results:

Aliquot	*Added aliquot of phenytoin standard (µL)*	*Apparent phenytoin concentration measured by new assay (µmol/L)*
A	*0*	*40*
B	*10*	*80*
C	*20*	*125*
D	*30*	*176*

Calculate the recovery for aliquots A, B, C and D and give an explanation for the pattern observed (Mol. W. of phenytoin sodium 274).

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When a specimen (already containing some of the analyte in question) is spiked by adding a known amount of analyte the recovery can be defined as:

Total measured concentration – Endogenous concentration

The endogenous concentration is obtained using an aliquot of specimen in which no exogenous analyte has been added. Analytical imprecision always limits the performance of recovery experiments – particularly if single measurements are used (see Question 160). The recovery may also be expressed as a proportion or percentage of the amount of added analyte.

As the concentration of phenytoin in the stock solution is given as 1 mg/mL this must first be converted to µmol/L to be compatible with the units of measurement. Multiply by 1,000 to convert from mg to µg, by 1,000 to convert from mL to L and divide by the molecular weight of phenytoin sodium (274):

$$\text{Phenytoin (µmol/)} = \frac{1\text{ mg/mL} \times 1{,}000 \times 1{,}000}{274} = 3650\text{ µmol/L}$$

Addition of exogenous stock phenytoin to serum dilutes the endogenous phenytoin and this will be different for each aliquot since a different volume is added:

$$\text{Endogenous phenytoin} = \frac{\text{Measured phenytoin in A} \times \text{Volume of serum (1 mL)}}{\text{Volume of serum (1mL)} + \text{Volume added phenytoin (mL)}}$$

The added phenytoin will also be diluted by the serum and since a different volume was added each time then the dilution will be different for each aliquot:

$$\text{Added phenytoin} = \frac{\text{Stock phenytoin (3650 µmol/L)} \times \text{Volume of stock phenytoin (mL)}}{\text{Volume of serum (1 mL)} + \text{Volume added phenytoin (mL)}}$$

The difference between the measured and endogenous phenytoin is the recovery (which can also be expressed as a ratio to the added phenytoin – or as a percentage) but it is often more useful to compare the recovery directly to the amount added.

These calculations are performed for aliquots B, C and D. Note that it is impossible to calculate a recovery for aliquot A since no phenytoin was added!!

Aliquot	**Added phenytoin µmol/L**	**Endogenous phenytoin µmol/L**	**Measured phenytoin µmol/L**	**Recovered phenytoin µmol/L**
A	0	40	40	0
B	$\frac{0.01 \times 3650}{1.010}$ = **36.1**	$\frac{40 \times 1}{1.010}$ = **39.6**	80	80 - 39.6 = **40.4**
C	$\frac{0.02 \times 3650}{1.020}$ = **71.6**	$\frac{40 \times 1}{1.020}$ = **39.2**	125	125 - 39.2 = **85.8**
D	$\frac{0.03 \times 3650}{1.030}$ = **106.3**	$\frac{40 \times 1}{1.030}$ = **38.8**	176	176 - 38.8 = **137.2**

All aliquots show a proportional over-recovery. There are two likely explanations:

1. Calibration error. If this is the case then the true value for aliquot A will be lower than the observed value. If the phenytoin recovered is plotted against phenytoin added then there is a good linear relationship but the projected line crosses the axis for zero added phenytoin at a concentration of approximately 11 µmol/L so that the actual concentration in aliquot A would be 29 µmol/L.
2. There was an error in preparing the 1 mg/1 mL sodium phenytoin solution used to spike the aliquots – possibly by using phenytoin rather than its sodium salt.

Question 185

A new drug (A) is inactivated by a deaminase in plasma to form metabolite (B). A rare deficiency in the enzyme can easily result in toxic plasma concentrations of the drug. To assay the enzyme 10 µL of plasma was added to 2 mL of buffer then the reaction initiated by adding 100 µL of substrate (A). The reaction was monitored by following the change in absorbance in a cell with a 1 cm path-length. Unfortunately both the product and substrate absorb strongly at the wavelength used with the following molar absorptivities:

Drug (A) = 3.0×10^5 L.mol^{-1}.cm^{-1}
Metabolite (B) = 1.2×10^5 L.mol^{-1}.cm^{-1}

Calculate the enzyme activity (in µmol /min/L plasma) for an initial rate of change in absorbance of -0.021/min.