Deacon's Challenge

No 119 - Answer

A chromatographic method for a drug (A) described in the literature, appears satisfactory for routine use. However, when you set up the method in your laboratory you discover that one of the drug's metabolites (B) co-elutes with the drug. On further investigation you observe that A and B have over-lapping absorption spectra with maxima at 580 nm and 630 nm respectively. Fortunately your HPLC system is equipped with a diode array detector.

Use the following data to calculate the urinary drug concentration:

Sample	*Absorbance (mA)*	
	580 nm	*600 nm*
Drug A standard solution (100 µmol/L)	*100*	*50*
Metabolite B standard solution (100 µmol/L)	*25*	*50*
Urine	*50*	*40*

Abbreviations:

A_{580} = milliabsorbance at 580 nm; A_{600} = milliabsorbance at 600 nm;

C_A = concentration of drug A (µmol/L); C_B = concentration of metabolite B (µmol/L);

$\varepsilon_{A.580}$ = µmolar absorptivity of A at 580 nm; $\varepsilon_{A.600}$ = µmolar absorptivity of A at 600 nm;

$\varepsilon_{B.580}$ = µmolar absorptivity of B at 580 nm; $\varepsilon_{B.600}$ = µmolar absorptivity of B at 600 nm

Two approaches can be used:

Method 1

Since the absorbances of A and B are identical at 600 nm when their concentrations are also the same it follows that 600 nm is an ISOBESTIC point (i.e. their molar absorptivities are equal at this wavelength). Therefore the total concentration of A and B can be calculated from the absorbance of the mixture measured at this wavelength using either A or B as the standard:

$$\frac{\text{Urine } C_A + C_B}{\text{Urine } A_{600}} = \frac{\text{Standard } C_A \text{ (or } C_B)}{\text{Standard } A_{600} \text{ (A or B)}}$$

$$\frac{\text{Urine } C_A + C_B}{40} = \frac{100}{50}$$

$$\text{Urine } C_A + C_B = \frac{100 \times 40}{50} = 80 \text{ µmol/L}$$

Since the absorbance of each compound (A or B) is proportional to concentration and are also additive it follows that the ratio of the total absorbance at 580 nm to that at 600 nm (i.e. A_{580}/A_{600}) is a linear function of the percentage of A (or B) in the mixture. The lowest A_{580}/A_{600} (when A = 0% and B = 100%) is 25/50 = 0.5 and the highest value (when A = 100% and B = 0%) is 100/50 = 2. The slope of a plot of A_{580}/A_{600} is therefore (2.0 - 0.5)/100 = 0.015 so that the overall relationship is:

$$A_{580}/A_{600} = 0.5 + 0.015\ \%A$$

Substituting absorbances for the urine sample (A_{580} = 50, A_{600} = 40) and solving for %A:

$$\frac{50}{40} = 0.5 + 0.015\ \%A$$

$$1.25 = 0.5 + 0.015\ \%A$$

$$0.015\ \%A = 1.25 - 0.5 = 0.75$$

$$\%A = \frac{0.75}{0.015} = 50\%$$

Therefore C_A = Total concentration ($C_A + C_B$) x 50/100

= 80 x 0.5 = **40 µmol/L**

Method 2

Since absorbances are additive at each wavelength and $A = C \times \varepsilon$ (path-length is constant and can be ignored) the following simultaneous equations can be set up for the total absorbance at each wavelength:

at 580 nm: $A_{580} = (C_A \times \varepsilon_{A.580}) + (C_B \times \varepsilon_{B.580})$

at 600 nm $A_{600} = (C_A \times \varepsilon_{A.600}) + (C_B \times \varepsilon_{B.600})$

Calculation of µmolar absorptivities (dividing absorbance by concentration of standard):

$\varepsilon_{A.580}$ = 100/100 = 1.0 $\varepsilon_{A.600}$ = 50/100 = 0.5

$\varepsilon_{B.580}$ = 25/100 = 0.25 $\varepsilon_{B.600}$ = 50/100 = 0.5

Substituting these values together with the absorbances for the urine peaks at both wavelengths gives:

$$50 = 1.0\ C_A + 0.25\ C_B$$

$$40 = 0.5\ C_A + 0.5\ C_B$$

Multiplying the first equation by 2 then subtracting the second equation eliminates terms for C_B enabling C_A to be determined:

$$100 = 2.0\ C_A + 0.5\ C_B$$

$$40 = 0.5\ C_A + 0.5\ C_B$$

$$60 = 1.5\ C_A$$

$$C_A = \frac{60}{1.5} = \mathbf{40\ µmol/L}$$

Question 120

Use the following data (obtainsed on paired specimens) to calculate the percentage of filtered urea reabsorbed by the renal tubules:

Plasma urea	=	7.5 mmol/L
Urine urea	=	360 mmol/L
Plasma creatinine	=	150 µmol/L
Urine creatinine	=	12 mmol/L