5-4 Internal Standards

An **internal standard** is a known amount of a compound, different from analyte, that is added to the unknown. Signal from analyte is compared with signal from the internal standard to find out how much analyte is present.

In *standard addition*, the standard is the same substance as the analyte. An *internal standard* is different from the analyte.

Internal standards are especially useful for analyses in which the quantity of sample analyzed or the instrument response varies slightly from run to run. For example, gas or liquid flow rates that vary by a few percent in a chromatography experiment (Figure 0-10) could change the detector response. A calibration curve is accurate only for the one set of conditions under which it was obtained. However, the *relative* response of the detector to the analyte and standard is usually constant over a range of conditions. If signal from the standard increases by 8.4% because of a change in flow rate, signal from the analyte usually increases by 8.4% also. As long as the concentration of standard is known, the correct concentration of analyte can be derived. Internal standards are used in chromatography because the small quantity of sample injected into the chromatograph is not reproducible.

The assumption that relative response to analyte and standard remains constant over a range of concentrations must be verified.

Internal standards are desirable when sample loss can occur during sample preparation steps prior to analysis. If a known quantity of standard is added to the unknown prior to any manipulations, the ratio of standard to analyte remains constant because the same fraction of each is lost in any operation.

To use an internal standard, we prepare a known mixture of standard and analyte to measure the relative response of the detector to the two species. In the chromatogram in Figure 5-9, the area, A, under each peak is proportional to the concentration of the species injected into the column. However, the detector generally has a different response to each component. For example, if both analyte (X) and internal standard (S) have concentrations of 10.0 mM, the area under the analyte peak might be 2.30 times greater than the area under the standard peak. We say that the **response factor,** F, is 2.30 times greater for X than for S.

Response factor:

$$\frac{\text{Area of analyte signal}}{\text{Concentration of analyte}} = F\left(\frac{\text{area of standard signal}}{\text{concentration of standard}}\right) \qquad (5\text{-}11)$$

$$\frac{A_X}{[X]} = F\left(\frac{A_S}{[S]}\right)$$

If the detector responds equally to standard and analyte, $F = 1$. If the detector responds twice as much to analyte as to standard, $F = 2$. If the detector responds half as much to analyte as to standard, $F = 0.5$.

[X] and [S] are the concentrations of analyte and standard *after they have been mixed together*. Equation 5-11 is predicated on linear response to analyte and standard.

EXAMPLE Using an Internal Standard

In a preliminary experiment, a solution containing 0.083 7 M X and 0.066 6 M S gave peak areas of $A_X = 423$ and $A_S = 347$. (Areas are measured in arbitrary units by the instrument's computer.) To analyze the unknown, 10.0 mL of 0.146 M S were added to 10.0 mL of unknown, and the mixture was diluted to 25.0 mL in a volumetric flask. This mixture gave the chromatogram in Figure 5-9, for which $A_X = 553$ and $A_S = 582$. Find the concentration of X in the unknown.

Solution First use the standard mixture to find the response factor in Equation 5-11:

Standard mixture:

$$\frac{A_X}{[X]} = F\left(\frac{A_S}{[S]}\right)$$

$$\frac{423}{0.083\,7} = F\left(\frac{347}{0.066\,6}\right) \Rightarrow F = 0.970_0$$

In the mixture of unknown plus standard, the concentration of S is

$$[S] = \underbrace{(0.146\text{ M})}_{\text{Initial concentration}}\underbrace{\left(\frac{10.0}{25.0}\right)}_{\text{Dilution factor}} = 0.058\,4\text{ M}$$

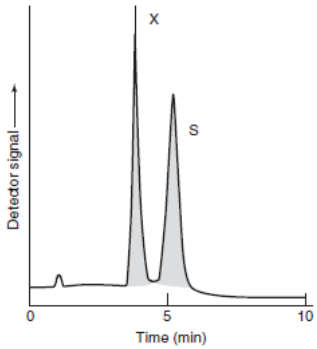

FIGURE 5-9 Chromatographic separation of unknown (X) and internal standard (S). A known amount of S was added to the unknown. The relative areas of the signals from X and S allow us to find out how much X is in the mixture. It is necessary first to measure the relative response of the detector to each compound.

The dilution factor $\frac{\text{initial volume}}{\text{final volume}}$ converts initial concentration into final concentration.

Using the known response factor, substitute back into Equation 5-11 to find the concentration of unknown in the mixture:

Unknown mixture:
$$\frac{A_X}{[X]} = F\left(\frac{A_S}{[S]}\right)$$

$$\frac{553}{[X]} = 0.970_0\left(\frac{582}{0.058\,4}\right) \Rightarrow [X] = 0.057\,2_1\ M$$

Because X was diluted from 10.0 to 25.0 mL when the mixture with S was prepared, the original concentration of X in the unknown was (25.0 mL/10.0 mL)(0.057 2_1 M) = 0.143 M.

Test Yourself Suppose that peak areas for the known mixture were $A_X = 423$ and $A_S = 447$. Find [X] in the unknown. (*Answer:* $F = 0.753_0$, [X] = 0.184 M)

5-5 Efficiency In Experimental Design

Operating parameters usually need to be optimized when we develop an analytical method. The least efficient way to do this is to vary one parameter at a time while keeping everything else constant. More efficient procedures are called *fractional factorial experimental design*[12] and *simplex optimization*.[13] We now discuss one example of experimental design intended to provide maximum information in the fewest number of trials.

Suppose we have three different unknown solutions of acid, designated A, B, and C. If we titrate each one once with base, we find its concentration, but have no estimate of uncertainty. If we titrate each solution three times, for a total of nine measurements, we would find each concentration and its standard deviation.

Acids could be delivered by transfer pipets whose tolerances are given in Table 2-4. So 2 mL means 2.000 mL with uncertainty in the third decimal place.

A more efficient experimental design provides concentrations and standard deviations in fewer than nine experiments.[14] One of many efficient designs is shown in Figure 5-10. Instead of titrating each acid by itself, we titrate mixtures of the acids. For example, in row 5 of the spreadsheet, a mixture containing 2 mL A, 2 mL B, and 2 mL C required 23.29 mL of 0.120 4 M NaOH, which amounts to 2.804 mmol of OH^-. In row 6, the acid mixture contained 2 mL A, 3 mL B, and 1 mL C. Other permutations are titrated in rows 7 and 8. Then row 5 is repeated independently in row 9. Column E gives mmol of base for each run.

	A	B	C	D	E
1	Experimental Design				
2					
3	Volumes of unknown acids (mL)			mL NaOH	mmol
4	A	B	C	(0.1204 M)	NaOH
5	2	2	2	23.29	2.804
6	2	3	1	20.01	2.409
7	3	1	2	21.72	2.615
8	1	2	3	28.51	3.433
9	2	2	2	23.26	2.801
10					
11			[C]	[B]	[A]
12		Molarity	0.8099	0.4001	0.1962
13		Std. dev.	0.0062	0.0062	0.0062
14			0.9994	0.0130	#N/A
15			R^2	S_y	
16	Highlight cells C12:E14				
17	Type "= LINEST(E5:E9,A5:C9,FALSE,TRUE)"				
18	Press CTRL +SHIFT+ENTER (on PC)				
19	Press COMMAND+RETURN (on Mac)				

FIGURE 5-10 Spreadsheet for efficient experimental design uses Excel LINEST routine to fit the function $y = m_A x_A + m_B x_B + m_C x_C$ to experimental data by a least-squares procedure.