method, we typically demonstrate that requirements are met for specificity, linearity, accuracy, precision, range, limit of detection, limit of quantitation, and robustness. Specificity is the ability to distinguish analyte from anything else. Linearity is usually measured by the square of the correlation coefficient for the calibration curve. Types of precision include instrument precision, intra-assay precision, intermediate precision, and, most generally, interlaboratory precision. The "Horwitz trumpet" is an empirical statement that precision becomes poorer as analyte concentration decreases. Range is the concentration interval over which linearity, accuracy, and precision are acceptable. The detection limit is usually taken as 3 times the standard deviation of the blank. The lower limit of quantitation is 10 times the standard deviation of the blank. The reporting limit is the concentration below which regulations say that analyte is reported as "not detected," even when it is observed. Robustness is the ability of an analytical method to be unaffected by small changes in operating parameters.

A standard addition is a known quantity of analyte added to an unknown to increase the concentration of analyte. Standard additions are especially useful when matrix effects are important. A matrix effect is a change in the analytical signal caused by anything in the sample other than analyte. Use Equation 5-7 to compute the quantity of analyte after a single standard addition. For multiple standard additions to a single solution, use Equation 5-9 to construct the graph in Figure 5-6, in which the x-intercept gives us the concentration of analyte. For multiple solutions made up to the same final volume, the slightly different graph in Figure 5-8 is used. Equation 5-10 gives the x-intercept uncertainty in either graph.

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. Internal standards are useful when the quantity of sample analyzed is not reproducible, when instrument response varies from run to run, or when sample losses occur in sample preparation. The response factor in Equation 5-11 is the relative response to analyte and standard.

Efficient experimental design decreases the number of experiments needed to obtain required information and an estimate of uncertainty in that information. A tradeoff is that the fewer experiments we do, the greater the uncertainty in the results.

Exercises

5-A. *Detection limit.* In spectrophotometry, we measure the concentration of analyte by its absorbance of light. A low-concentration sample was prepared, and nine replicate measurements gave absorbances of 0.004 7, 0.005 4, 0.006 2, 0.006 0, 0.004 6, 0.005 6, 0.005 2, 0.004 4, and 0.005 8. Nine reagent blanks gave values of 0.000 6, 0.001 2, 0.002 2, 0.000 5, 0.001 6, 0.000 8, 0.001 7, 0.001 0, and 0.001 1.

(a) Find the absorbance detection limit with Equation 5-3.

(b) The calibration curve is a graph of absorbance versus concentration. Absorbance is a dimensionless quantity. The slope of the calibration curve is $m = 2.24 \times 10^4\ M^{-1}$. Find the concentration detection limit with Equation 5-5.

(c) Find the lower limit of quantitation with Equation 5-6.

5-B. *Standard addition.* An unknown sample of Ni^{2+} gave a current of 2.36 μA in an electrochemical analysis. When 0.500 mL of solution containing 0.028 7 M Ni^{2+} was added to 25.0 mL of unknown, the current increased to 3.79 μA.

(a) Denoting the initial, unknown concentration as $[Ni^{2+}]_i$, write an expression for the final concentration, $[Ni^{2+}]_f$, after 25.0 mL of unknown were mixed with 0.500 mL of standard. Use the dilution factor for this calculation.

(b) In a similar manner, write the final concentration of added standard Ni^{2+}, designated as $[S]_f$.

(c) Find $[Ni^{2+}]_i$ in the unknown.

5-C. *Internal standard.* A solution was prepared by mixing 5.00 mL of unknown element X with 2.00 mL of solution containing 4.13 μg of standard element S per milliliter, and diluting to 10.0 mL. The signal ratio in atomic absorption spectrometry was (signal from X)/(signal from S) = 0.808. In a separate experiment, with equal concentrations of X and S, (signal from X)/(signal from S) = 1.31. Find the concentration of X in the unknown.

5-D. In Figure 5-6, the x-intercept is –2.89 mM and its standard deviation is 0.09_8 mM. Find the 90% and 99% confidence intervals for the intercept.

5-E. *Control chart.* Volatile compounds in human blood serum were measured by purge and trap gas chromatography/mass spectrometry. For quality control, serum was periodically spiked with a constant amount of 1,2-dichlorobenzene and the concentration (ng/g = ppb) was measured. Find the mean and standard deviation for the following spike data and prepare a control chart. State whether or not the observations (Obs.) meet each criterion for stability in a control chart.

Day	Obs. (ppb)	Day	Obs. (ppb)	Day	Obs. (ppb)	Day	Obs. (ppb)	Day	Obs. (ppb)
0	1.05	91	1.13	147	0.83	212	1.03	290	1.04
1	0.70	101	1.64	149	0.88	218	0.90	294	0.85
3	0.42	104	0.79	154	0.89	220	0.86	296	0.59
6	0.95	106	0.66	156	0.72	237	1.05	300	0.83
7	0.55	112	0.88	161	1.18	251	0.79	302	0.67
30	0.68	113	0.79	167	0.75	259	0.94	304	0.66
70	0.83	115	1.07	175	0.76	262	0.77	308	1.04
72	0.97	119	0.60	182	0.93	277	0.85	311	0.86
76	0.60	125	0.80	185	0.72	282	0.72	317	0.88
80	0.87	128	0.81	189	0.87	286	0.68	321	0.67
84	1.03	134	0.84	199	0.85	288	0.86	323	0.68

SOURCE: D. L. Ashley, M. A. Bonin, F. L. Cardinali, J. M. McCraw, J. S. Holler, L. L. Needham, and D. G. Patterson, Jr., "Determining Volatile Organic Compounds in Blood by Using Purge and Trap Gas Chromatography/Mass Spectrometry," Anal. Chem. ***1992****, 64, 1021.*

Problems

Quality Assurance and Method Validation

5-1. Explain the meaning of the quotation at the beginning of this chapter: "Get the right data. Get the data right. Keep the data right."

5-2. What are the three parts of quality assurance? What questions are asked in each part and what actions are taken in each part?

5-3. How can you validate precision and accuracy?

5-4. Distinguish *raw data*, *treated data*, and *results*.

5-5. What is the difference between a *calibration check* and a *performance test sample*?

5-6. What is the purpose of a blank? Distinguish *method blank*, *reagent blank,* and *field blank*.

5-7. Distinguish *linear range*, *dynamic range*, and *range*.

5-8. What is the difference between a *false positive* and a *false negative*?

5-9. Consider a sample that contains analyte at the detection limit defined in Figure 5-2. Explain the following statements: There is approximately a 1% chance of falsely concluding that a sample containing no analyte contains analyte above the detection limit. There is a 50% chance of concluding that a sample that really contains analyte at the detection limit does not contain analyte above the detection limit.

5-10. How is a control chart used? State six indications that a process is going out of control.

5-11. Here is a use objective for a chemical analysis to be performed at a drinking water purification plant: "Data and results collected quarterly shall be used to determine whether the concentrations of haloacetates in the treated water demonstrate compliance with the levels set by the Stage 1 Disinfection By-products Rule using Method 552.2" (a specification that sets precision, accuracy, and other requirements). Which one of the following questions best summarizes the meaning of the use objective?

(a) Are haloacetate concentrations known within specified precision and accuracy?

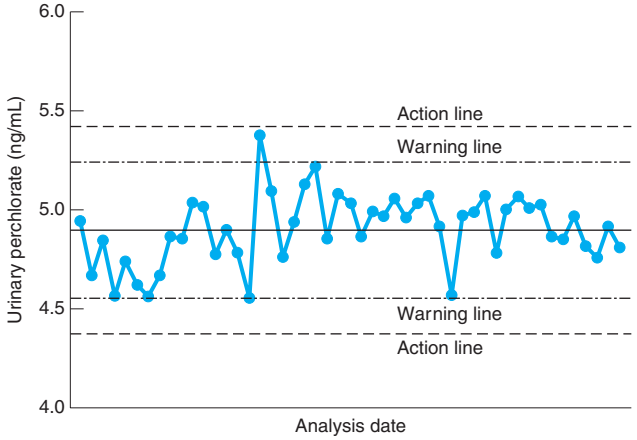
(b) Are any haloacetates detectable in the water?

(c) Do any haloacetate concentrations exceed the regulatory limit?

5-12. What is the difference between an instrument detection limit and a method detection limit? What is the difference between robustness and intermediate precision?

5-13. Define the following terms: instrument precision, injection precision, intra-assay precision, intermediate precision, and interlaboratory precision.

5-14. *Control chart.* A laboratory monitoring perchlorate (ClO_4^-) in urine measured quality control samples made from synthetic urine spiked with (ClO_4^-). The graph shows consecutive quality control measurements. Are any troubleshooting conditions from Box 5-1 observed in these data?



Control chart for ClO_4^- in urine. [Data from L. Valentin-Blasini, J. P. Mauldin, D. Maple, and B. C. Blount, "Analysis of Perchlorate in Human Urine Using Ion Chromatography and Electrospray Tandem Mass Spectrometry," *Anal. Chem.* **2005**, *77*, 2475.]

5-15. *Correlation coefficient and Excel graphing.* Synthetic data are given below for a calibration curve in which random Gaussian noise with a magnitude of 80 was superimposed on y values that follow the equation $y = 26.4x + 1.37$. This exercise shows that a high value of R^2 does not guarantee that data quality is excellent.

(a) Enter concentration in column A and signal in column B of a spreadsheet. Prepare an XY (Scatter) chart of signal versus concentration without a line as described in Section 2-11. Use LINEST (Section 4-7) to find the least-squares parameters including R^2.

(b) Now insert the Trendline by following instructions in Section 4-9. In the Options window used to select the Trendline, select Display Equation and Display R-Squared. Verify that Trendline and LINEST give identical results.

(c) Add 95% confidence interval y error bars following the instructions at the end of Section 4-9. The 95% confidence interval is $\pm ts_y$, where s_y comes from LINEST and Student's t comes from Table 4-2 for 95% confidence and $11 - 2 = 9$ degrees of freedom. Also, compute t with the statement "=TINV(0.05,9)".

Concentration (x)	Signal (y)	Concentration (x)	Signal (y)
0	14	60	1 573
10	350	70	1 732
20	566	80	2 180
30	957	90	2 330
40	1 067	100	2 508
50	1 354		

5-16. In a murder trial in the 1990s, the defendant's blood was found at the crime scene. The prosecutor argued that blood was left by the defendant during the crime. The defense argued that police "planted" the defendant's blood from a sample collected later. Blood is normally collected in a vial containing the metal-binding compound EDTA as an anticoagulant with a concentration of ~4.5 mM after the vial has been filled with blood. At the time of the trial, procedures to measure EDTA in blood were not well established. Even though the amount of EDTA found in the crime-scene blood was orders of magnitude below 4.5 mM, the jury acquitted the defendant. This trial motivated the development of a new method to measure EDTA in blood.

(a) *Precision and accuracy.* To measure accuracy and precision of the method, blood was fortified with EDTA to known levels.

$$\text{Accuracy} = 100 \times \frac{\text{mean value found} - \text{known value}}{\text{known value}}$$

$$\text{Precision} = 100 \times \frac{\text{standard deviation}}{\text{mean}} \equiv \textit{coefficient of variation}$$

For each of the three spike levels in the table, find the precision and accuracy of the quality control samples.

EDTA measurements (ng/mL) at three fortification levels

Spike:	22.2 ng/mL	88.2 ng/mL	314 ng/mL
Found:	33.3	83.6	322
	19.5	69.0	305
	23.9	83.4	282
	20.8	100.0	329
	20.8	76.4	276

SOURCE: *R. L. Sheppard and J. Henion, Anal. Chem.* ***1997,*** *69, 477A, 2901.*

(b) *Detection and quantitation limits.* Low concentrations of EDTA near the detection limit gave the following dimensionless instrument

readings: 175, 104, 164, 193, 131, 189, 155, 133, 151, and 176. Ten blanks had a mean reading of 45.$_0$. The slope of the calibration curve is $1.75 \times 10^9\ M^{-1}$. Estimate the signal and concentration detection limits and the lower limit of quantitation for EDTA.

5-17. **(a)** From Box 5-2, estimate the minimum expected coefficient of variation, CV(%), for interlaboratory results when the analyte concentration is (i) 1 wt% or (ii) 1 part per trillion.

(b) The coefficient of variation within a laboratory is typically ~0.5–0.7 of the between-laboratory variation. If your class analyzes an unknown containing 10 wt% NH_3, what is the minimum expected coefficient of variation for the class?

5-18. *Spike recovery and detection limit.* Species of arsenic found in drinking water include AsO_3^{3-} (arsenite), AsO_4^{3-} (arsenate), $(CH_3)_2AsO_2^-$ (dimethylarsinate), and $(CH_3)AsO_3^{2-}$ (methylarsonate). Pure water containing no arsenic was spiked with 0.40 μg arsenate/L. Seven replicate determinations gave 0.39, 0.40, 0.38, 0.41, 0.36, 0.35, and 0.39 μg/L.[15] Find the mean percent recovery of the spike and the concentration detection limit (μg/L).

5-19. *Detection limit.* Low concentrations of Ni^{2+}-EDTA near the detection limit gave the following counts in a mass spectral measurement: 175, 104, 164, 193, 131, 189, 155, 133, 151, 176. Ten measurements of a blank had a mean of 45 counts. A sample containing 1.00 μM Ni^{2+}-EDTA gave 1 797 counts. Estimate the detection limit for Ni-EDTA.

5-20. *Detection limit.* A sensitive chromatographic method was developed to measure sub-part-per-billion levels of the disinfectant by-products iodate (IO_3^-), chlorite (ClO_2^-), and bromate (BrO_3^-) in drinking water. As the oxyhalides emerge from the column, they react with Br^- to make Br_3^-, which is measured by its strong absorption at 267 nm. For example, each mole of bromate makes 3 mol of Br_3^- by the reaction

$$BrO_3^- + 8Br^- + 6H^+ \rightarrow 3Br_3^- + 3H_2O$$

Bromate near its detection limit gave the following chromatographic peak heights and standard deviations (s). For each concentration, estimate the detection limit. Find the mean detection limit. The blank is 0 because chromatographic peak height is measured from the baseline adjacent to the peak. Because blank = 0, relative standard deviation applies to both peak height and concentration, which are proportional to each other. Detection limit is 3s for peak height or concentration.

Bromate concentration (μg/L)	Peak height (arbitrary units)	Relative standard deviation (%)	Number of measurements
0.2	17	14.4	8
0.5	31	6.8	7
1.0	56	3.2	7
2.0	111	1.9	7

SOURCE: *H. S. Weinberg and H. Yamada, "Post-Ion-Chromatography Derivatization for the Determination of Oxyhalides at Sub-PPB Levels in Drinking Water," Anal. Chem.* ***1998,*** *70, 1.*

5-21. Olympic athletes are tested to see if they are using illegal performance-enhancing drugs. Suppose that urine samples are taken and analyzed and the rate of false positive results is 1%. Suppose also that it is too expensive to refine the method to reduce the rate of false positive results. We do not want to accuse innocent people of using illegal drugs. What can you do to reduce the rate of false accusations even though the test always has a false positive rate of 1%?

5-22. *Blind samples.* The U.S. Department of Agriculture provided homogenized baby food samples to three labs for analysis.[3] Results agreed well for protein, fat, zinc, riboflavin, and palmitic acid. Results for iron were questionable: Lab A, 1.59 ± 0.14 (13); Lab B, 1.65 ± 0.56 (8); Lab C, 2.68 ± 0.78 (3) mg/100 g. Uncertainty is the standard deviation, with the number of replicate analyses in parentheses. Use two separate t tests to compare results from Lab C with those from Lab A and Lab B at the 95% confidence level. Comment on the sensibility of the t test results. Offer your own conclusions.

Standard Addition

5-23. Why is it desirable in the method of standard addition to add a small volume of concentrated standard rather than a large volume of dilute standard?

5-24. An unknown sample of Cu^{2+} gave an absorbance of 0.262 in an atomic absorption analysis. Then 1.00 mL of solution containing 100.0 ppm (= μg/mL) Cu^{2+} was mixed with 95.0 mL of unknown, and the mixture was diluted to 100.0 mL in a volumetric flask. The absorbance of the new solution was 0.500.

(a) Denoting the initial, unknown concentration as $[Cu^{2+}]_i$, write an expression for the final concentration, $[Cu^{2+}]_f$, after dilution. Units of concentration are ppm.

(b) In a similar manner, write the final concentration of added standard Cu^{2+}, designated as $[S]_f$.

(c) Find $[Cu^{2+}]_i$ in the unknown.

5-25. *Standard addition graph.* Tooth enamel consists mainly of the mineral calcium hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$. Trace elements in teeth of archeological specimens provide anthropologists with clues about diet and diseases of ancient people. Students at Hamline University measured strontium in enamel from extracted wisdom teeth by atomic absorption spectroscopy. Solutions were prepared with a constant total volume of 10.0 mL containing 0.750 mg of dissolved tooth enamel plus variable concentrations of added Sr.

Added Sr (ng/mL = ppb)	Signal (arbitrary units)
0	28.0
2.50	34.3
5.00	42.8
7.50	51.5
10.00	58.6

SOURCE: *V. J. Porter, P. M. Sanft, J. C. Dempich, D. D. Dettmer, A. E. Erickson, N. A. Dubauskie, S. T. Myster, E. H. Matts, and E. T. Smith, "Elemental Analysis of Wisdom Teeth by Atomic Spectroscopy Using Standard Addition," J. Chem. Ed.* ***2002,*** *79, 1114.*

(a) Find the concentration of Sr and its uncertainty in the 10-mL sample solution in parts per billion = ng/mL.

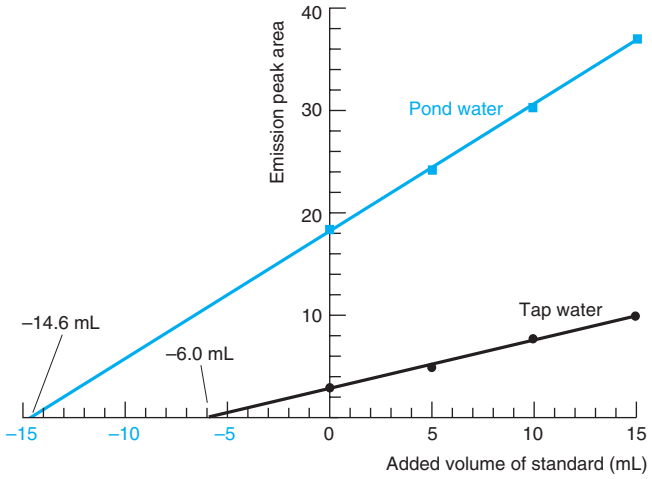
(b) Find the concentration of Sr in tooth enamel in parts per million = μg/g.

(c) If the standard addition intercept is the major source of uncertainty, find the uncertainty in the concentration of Sr in tooth enamel in parts per million.

(d) Find the 95% confidence interval for Sr in tooth enamel.

5-26. Europium is a lanthanide element found in parts per billion levels in natural waters. It can be measured from the intensity of orange light emitted when a solution is illuminated with ultraviolet radiation. Certain organic compounds that bind Eu(III) are required

to enhance the emission. The figure shows standard addition experiments in which 10.00 mL of sample and 20.00 mL containing a large excess of organic additive were placed in 50-mL volumetric flasks. Then Eu(III) standards (0, 5.00, 10.00, or 15.00 mL) were added and the flasks were diluted to 50.0 mL with H_2O. Standards added to tap water contained 0.152 ng/mL (ppb) of Eu(III), but those added to pond water were 100 times more concentrated (15.2 ng/mL).



Standard addition of Eu(III) to pond water or tap water. [Data from A. L. Jenkins and G. M. Murray, "Enhanced Luminescence of Lanthanides," *J. Chem. Ed.* **1998**, *75*, 227.]

(a) Calculate the concentration of Eu(III) (ng/mL) in pond water and tap water.

(b) For tap water, emission peak area increases by 4.61 units when 10.00 mL of 0.152 ng/mL standard are added. This response is 4.61 units/1.52 ng = 3.03 units per ng of Eu(III). For pond water, the response is 12.5 units when 10.00 mL of 15.2 ng/mL standard are added, or 0.082 2 units per ng. How would you explain these observations? Why was standard addition necessary for this analysis?

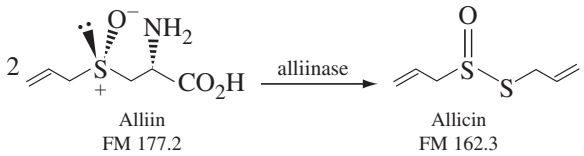
5-27. *Standard addition graph*. Students performed an experiment like that in Figure 5-7 in which each flask contained 25.00 mL of serum, varying additions of 2.640 M NaCl standard, and a total volume of 50.00 mL.

Flask	Volume of standard (mL)	Na^+ atomic emission signal (mV)
1	0	3.13
2	1.000	5.40
3	2.000	7.89
4	3.000	10.30
5	4.000	12.48

(a) Prepare a standard addition graph and find $[Na^+]$ in the serum.

(b) Find the standard deviation and 95% confidence interval for $[Na^+]$.

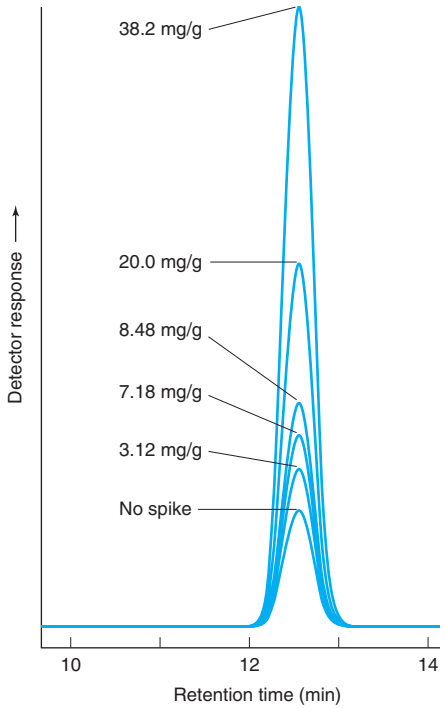
5-28. *Standard addition graph*. Allicin is a major component in garlic (0.4 wt% in fresh garlic) with antimicrobial and possibly anticancer and antioxidant activity. It is unstable and therefore difficult to measure. An assay was developed in which the stable precursor alliin is added to freshly crushed garlic and rapidly converted to allicin by the enzyme alliinase found in garlic. Components of the crushed garlic are extracted and measured by liquid chromatography. The graph shows standard additions reported as mg alliin added per gram of garlic. The chromatographic peak is allicin from the conversion of alliin.



Alliin
FM 177.2

Allicin
FM 162.3

(a) The standard addition procedure has a constant total volume. Prepare a graph to find how much alliin equivalent was in the unspiked garlic. The units of your answer will be mg alliin/g garlic. Include the standard deviation in your answer.



Chromatographic measurement of alliin after standard addition to garlic. [From M. E. Rybak, E. M. Calvey, and J. M. Harnly, "Quantitative Determination of Allicin in Garlic: Supercritical Fluid Extraction and Standard Addition of Alliin," *J. Agric. Food Chem.* **2004**, *52*, 682.]

(b) Given that 2 mol of alliin are converted to 1 mol of allicin in the assay, find the allicin content of garlic expressed as mg allicin/g garlic, including the standard deviation.

Internal Standards

5-29. State when standard additions and internal standards, instead of a calibration curve, are desirable, and why.

5-30. A solution containing 3.47 mM X (analyte) and 1.72 mM S (standard) gave peak areas of 3 473 and 10 222, respectively, in a chromatographic analysis. Then 1.00 mL of 8.47 mM S was added to 5.00 mL of unknown X, and the mixture was diluted to 10.0 mL. This solution gave peak areas of 5 428 and 4 431 for X and S, respectively.

(a) Calculate the response factor for the analyte.

(b) Find the concentration of S (mM) in the 10.0 mL of mixed solution.

(c) Find the concentration of X (mM) in the 10.0 mL of mixed solution.

(d) Find the concentration of X in the original unknown.

5-31. Chloroform is an internal standard in the determination of the pesticide DDT in a polarographic analysis in which each compound is reduced at an electrode surface. A mixture containing 0.500 mM chloroform and 0.800 mM DDT gave signals of 15.3 μA for chloroform and 10.1 μA for DDT. An unknown solution (10.0 mL) containing DDT was placed in a 100-mL volumetric flask and 10.2 μL of chloroform (FM 119.39, density = 1.484 g/mL) were added. After dilution to the mark with solvent, polarographic signals of 29.4 and 8.7 μA were observed for the chloroform and DDT, respectively. Find the concentration of DDT in the unknown.

5-32. *Verifying constant response for an internal standard.* When we develop a method using an internal standard, it is important to verify that the response factor is constant over the calibration range. Data are shown below for a chromatographic analysis of naphthalene ($C_{10}H_8$), using deuterated naphthalene ($C_{10}D_8$ in which D is the isotope ^2H) as an internal standard. The two compounds emerge from the column at almost identical times and are measured by a mass spectrometer, which distinguishes them by molecular mass. From the definition of response factor in Equation 5-11, we can write

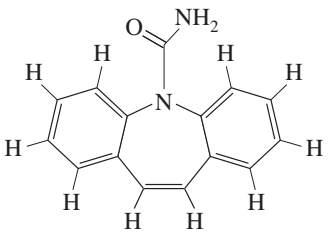
$$\frac{\text{Area of analyte signal}}{\text{Area of standard signal}} = F\left(\frac{\text{concentration of analyte}}{\text{concentration of standard}}\right)$$

Prepare a graph of peak area ratio ($C_{10}H_8/C_{10}D_8$) versus concentration ratio ($[C_{10}H_8]/[C_{10}D_8]$) and find the slope, which is the response factor. Evaluate F for each of the three samples and find the standard deviation of F to see how "constant" it is.

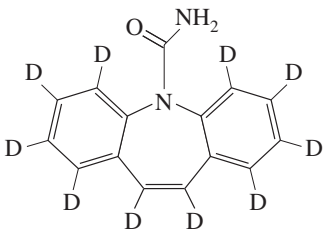
Sample	$C_{10}H_8$ (ppm)	$C_{10}D_8$ (ppm)	$C_{10}H_8$ peak area	$C_{10}D_8$ peak area
1	1.0	10.0	303	2 992
2	5.0	10.0	3 519	6 141
3	10.0	10.0	3 023	2 819

The volume of solution injected into the column was different in all three runs.

5-33. *Correcting for matrix effects with an internal standard.* The appearance of pharmaceuticals in municipal wastewater (sewage) is an increasing problem that is likely to have adverse effects on our drinking water supply. Sewage is a complex matrix. When the drug carbamazepine was spiked into sewage at a concentration of 5 ppb, chromatographic analysis gave an apparent spike recovery of 154%.[16] When deuterated carbamazepine was used as an internal standard for the analysis, the apparent recovery was 98%. Explain how the internal standard is used in this analysis and rationalize why it works so well to correct for matrix effects.



Carbamazepine



Carbamazepine-d_{10}

Experimental Design

5-34. Acid-base titrations similar to those in Section 5-5 had volumes and results shown in the table.[14] Use Excel LINEST to find the concentrations of acids A, B, and C and estimate their uncertainty.

Acid volume (mL)			mmol of OH^- required
A	B	C	
2	2	2	3.015
0	2	2	1.385
2	0	2	2.180
2	2	0	2.548
2	2	2	3.140