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Theoretical Aspects of One-Point Calibration: Causes and Effects of Some Potential Errors, and Their Dependence on Concentration

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The potential errors of the one-point calibration technique are described mathematically. I examine the theoretical causes and effects of analytical errors in absorbance and of errors in determining calibration concentration, and discuss techniques for minimizing their impact. The dependence of these errors on the calibration concentration and on the size of the result is considered, and some conclusions are drawn about the choice of calibration concentration.

Additional Keyphrases: *statistics · analytical error*

In various analyzers the principle of one-point calibration is used. After the instrument is zeroed on reagent or diluent, it then aspirates in turn a calibration standard of assigned concentration C (see Table 1) and then a series of unknown samples, which may be patients' specimens or quality-control sera. If the absorbances given by the standard and a sample are A_c and A_s, respectively, then the sample concentration, S, is estimated by

$$S = (A_s/A_c) \cdot C \quad (1)$$

This method, particularly useful for the measurement of analytes in complex matrices, has been used in a variety of analytical systems, including peak-drawing continuous-flow instruments, true equilibrium chemistries, and (with modification) some kinetic methods for the measurement of concentrations or enzyme activities.

The validity of this procedure depends on the familiar assumptions of zero blank, linear calibration curve, and similarity of response to analyte in standards and in unknown samples. Failure to meet these conditions is a source of error; one example that has been discussed elsewhere (*1*) is non-zero reagent blank. All these assumptions are tested during method development, but it may be difficult to know how large a deviation from ideal can be tolerated unless we know how the overall error is related to its components.

In this paper I attempt to provide a theoretical analysis of these errors, their effects on overall accuracy and precision, and their dependence on the concentrations of sample and standard.

Methods

Systematic errors can be expressed as either absolute or relative quantities, and these can be represented by dx and dx/x, respectively. Similarly, random errors can be expressed either absolutely as a standard deviation, $SD(x)$, or relatively as a coefficient of variation, $CV(x)$. In what follows dx/x and $CV(x)$ will be understood as fractions, not percentages.

The theory of error transmission (*2*) permits the bias and imprecision in S (that is, the systematic and random error, respectively) to be expressed in terms of those in its components. From equation 1, and assuming all errors are small, we have

$$dS/S = dA_s/A_s - dA_c/A_c + dC/C \quad (2a)$$

$$CV^2(S) = CV^2(A_s) + CV^2(A_c) + CV^2(C) \quad (2b)$$

Table 1. Symbols Used in This Paper

dx, dx/x	Absolute and relative bias (inaccuracy) in x.
$SD(x)$, $CV(x)$	Standard deviation and coefficient of variation of x; squares of these expressed as $SD^2(x)$, etc.
S, C	Sample and standard concentrations.
S_a, P_a	Concentrations of a sample and of the preceding sample, affected by carryover.
S_m, S_b, S_t	For unimodal distribution, the mean and the bottom and top reference limits.
S_1, S_2	For bimodal distribution, the lower and upper modal concentrations.
A_s, A_c, A_b	Absorbances of sample, standard, and blank.
A', $A(t)$	Absorbances corrected for blank, and measured at time t, respectively.
A_{sm}, A_{s1}	Absorbances of sample at concentrations S_m, S_1, etc.
T, G	Unimodal "relative width"; bimodal "relative separation."
B, E	Constant and proportional errors.
B_m, E_m	Maximum values of baseline and slope drift.
P, Q, f	Nonlinear error constants.
U, V, W	General error constants.
K_p, K_r	Equilibrium constants.
k, k'	"Analytical" constants.
K_t, n	Turbidity constants.
h, r	Carryover constant and reagent concentration.
Z_1, Z_2	Bichromatic wavelengths.

In examining the dependence of these errors on the values of C and S, it is useful to consider two idealized distributions of values of S for patients.

Unimodal distribution. Unimodal distribution is the "normal" distribution, with a mean value S_m, and it has a conventional reference interval, whose top and bottom limits (S_t and S_b) are, respectively, two population standard deviations above and below S_m. We can characterize it by a quantity T, which measures its "relative width." By definition,

$$T = 2 \cdot (\text{population standard deviation})/S_m \quad (3a)$$

where the factor 2 is chosen to simplify subsequent expressions. From this, we can write the following:

$$T = (S_t - S_b)/2 \cdot S_m \quad (3b)$$

$$S_t = S_m \cdot (1 + T) \text{ and } S_b = S_m \cdot (1 - T) \quad (3c)$$

In most cases, equation 3b is the easiest way to calculate T, and can never yield a value greater than unity. Many analytes in clinical chemistry are roughly of this type, and Table 2 shows some examples, along with their T-values, as calculated from this laboratory's reference intervals.

Bimodal distribution. Bimodal distribution will be thought of as two frequency peaks at concentrations S_1 and S_2, where S_2 is larger. We can characterize it by a quantity G, which measures the "relative separation" of the peaks. By definition,

$$G = (S_2 - S_1)/S_1 \quad (4a)$$

which we can write as follows:

$$S_2 = S_1 \cdot (1 + G) \quad (4b)$$

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Table 2. Typical Values of S_m and T for Some Unimodal Analytes

Analyte	S_m	T
Na	139 mmol/L	0.04
Cl	101 mmol/L	0.05
Ca	2.35 mmol/L	0.11
Protein	72 g/L	0.13
HCO_3^-	28.5 mmol/L	0.16
Albumin	43 g/L	0.16
K	4.25 mmol/L	0.18

In laboratories serving a renal unit, this distribution gives a reasonable fit to measured concentrations of urea, creatinine, and phosphate. Certain other analytes, e.g., bilirubin, glucose, and alkaline phosphatase, show a proportion of values much higher than the reference interval. It may be useful to think of these as "pseudo-bimodal" analytes, with a very broad upper peak. Any bimodal analyte will of course have a T-value for the "normal" peak; because this will usually be the lower peak, then $S_m = S_1$. Some values of T and G are shown in Table 3. For the bimodal analytes proper, S_2 is a genuine second mode, whereas for the "pseudo-bimodal" analytes, one or two representative sample values have been used to stand in for the second peak.

It may be useful to express errors in terms of concentrations as well as absorbances. If the assumptions of the one-point technique are valid, then $A_s = k \cdot S$ and $A_c = k \cdot C$, where k is an analytical constant. This includes information about sample dilution, dialysis fraction, the stoichiometry of the analytical reaction, and the absorbance of the final colored product.

Errors in Calibration Concentration

We need to know how errors in the calibration concentration are likely to depend on its value. The systematic error, dC/C, has four components:

(*a*) Deterioration or loss of analyte during use or storage.
(*b*) Evaporation of matrix during use or storage.
(*c*) Gravimetric and dispensing errors during vial filling.
(*d*) Volumetric errors during vial reconstitution.

In the increasingly rare case of calibration with pure aqueous standards, errors (*c*) and (*d*) are replaced by the gravimetric and volumetric errors involved in the preparation of stock solutions and working dilutions.

Errors (*b*), (*c*), and (*d*) will all give rise to relative bias, which is independent of the value of C. Error (*a*) will also do so if the decay or loss process obeys first-order kinetics, as it well may. The total bias, dC/C, is therefore likely to be independent of C.

Table 3. Typical Values of S_m (S_1), S_2, T, and G for Some Bimodally Distributed Analytes and Pseudo-Bimodal Analytes[a]

Analyte	S_m (S_1)	T	Approx. S_2	Approx. G
Bimodal				
Phosphate	1.1 mmol/L	0.55	2 mmol/L	0.8
Urea	5.8 mmol/L	0.47	25 mmol/L	3
Creatinine	78 μmol/L	0.55	900 μmol/L	11
Pseudo-bimodal				
Glucose	4.5 mmol/L (fasting)	0.51	15 mmol/L	2
			30 mmol/L	6
Alk. phosphatase	64 U/L	0.66	800 U/L	12
Bilirubin	11 μmol/L	1.0	50 μmol/L	4
			250 μmol/L	22

[a] For pseudo-bimodal analytes the values of S_2 and G are only examples.

These factors also contribute to imprecision: deterioration and evaporation act "within vial," and filling/reconstitution errors act "between vials." The overall within- and between-vial imprecision is therefore likely to be independent of C.

Errors in Measured Absorbance

These are of two kinds: photometric and analytical. Photometric absorbance errors have been comprehensively examined in ref. *3*. Their dependence on A is complex, and they will not be discussed here. If necessary, a photometric term can be added to any of the error expressions derived below.

Analytical absorbance errors are those due to partial failure to fulfill the necessary conditions of the one-point technique, and the possible types of error will be discussed separately.

Constant Errors

Here a constant increment, with mean B and population standard deviation SD(B), is added to the true absorbance. It follows that the resulting errors in A are given by

$$dA/A = B/A \text{ and } CV(A) = SD(B)/A \quad (5)$$

Examples of this type include the errors due to baseline drift (*4*), sample or reagent blank (*1*), and nonspecificity.

Another interesting case is that of uncorrected carryover (*5*). In a unimodal patient distribution each sample is preceded, on average, by samples with a mean concentration and standard deviation equal to those of the parent population. If h is the carryover constant, then the absorbance, A_s', given by a sample whose true absorbance is A_s, will on average be

$$A_s' = A_s \cdot (1 - h) + h \cdot A_{sm} \quad (6a)$$

The resulting average bias is given by

$$dA/A = h \cdot (A_{sm}/A_s - 1) = h \cdot (S_m/S - 1) \quad (6b)$$

Variation in A_s' will arise as a result of population variation in the absorbance of the preceding sample. From equation 6a we can calculate the resulting standard deviation, $SD(A_s)$, for any given S:

$$SD(A_s) = h \cdot (\text{standard deviation of preceding absorbance}) = h \cdot k \cdot (\text{population standard deviation}) \quad (6c)$$

From the definition of T, we can express this as

$$SD(A_s) = \tfrac{1}{2}h \cdot k \cdot S_m \cdot T = \tfrac{1}{2}h \cdot A_{sm} \cdot T \quad (6d)$$

and so

$$CV(A_s) = \tfrac{1}{2}h \cdot T \cdot A_{sm}/A_s = \tfrac{1}{2}h \cdot T \cdot S_m/S \quad (6e)$$

Proportional Errors

Here the true absorbance is multiplied by a factor (1 + E), where E is a mean value taken from a population whose standard deviation is SD(E). The resulting errors in A are given by

$$dA/A = E \text{ and } CV(A) = SD(E) \quad (7)$$

Examples of this type include volumetric errors [including those due to sample viscosity (*6*), for nonprotein analytes], slope drift (*7*), simple errors in the rate and extent of dialysis, errors due to failure to reach equilibrium, and certain types of peak-drawing errors in continuous-flow systems.

Nonlinearity

In the previous two cases, the calibration curve remains linear, but with an incorrect intercept and slope, respectively. Of the many possible errors due to nonlinearity of the calibration curve, two will be discussed here.

Noncompletion errors. In equilibrium methods, these errors result from the failure of the analytical reaction to go to completion. They depend on the concentrations of analyte and reagent, and on the equilibrium constant. They must be distinguished from errors due to the failure to reach equilibrium, which depend on time and on the rate constant, and which will usually give rise to proportional error.

In the simplest case, reagent and analyte react in a 1:1 ratio to form a colored product, proceeding in one step with an equilibrium constant K_r. If the reagent and analyte concentrations are r and S, respectively, it can be shown that the resulting error in the measured analytical absorbance is given by

$$dA/A = -1/[(r - S) \cdot K_r] \qquad (r > S) \qquad (8a)$$

if we make the reasonable assumption that $(1/K_r)^2$ is negligible.

Product-dissociation errors. These errors are produced by partial dissociation of the colored product into one or more less strongly absorbing species. In the simplest case of a one-step breakdown (with equilibrium constant K_p) into two species, it can be shown that the resulting error in A is (for small S) given by

$$dA/A = -M \cdot K_p/(K_p + k' \cdot S) \qquad (8b)$$

In this expression, M is a term containing the absorbances of the colored product and its subproducts (so that if the subproducts do not absorb, M = 1), and k' relates the concentrations of the original analyte and the colored product.

General nonlinearity. Equations 8a and 8b can be put into the following general form:

$$dA/A = -P/(Q + f \cdot A) \qquad (9a)$$

In this expression, P and Q are constants, and f is a parameter that takes the value −1 for noncompletion errors (so that the error increases with A), and +1 for product-dissociation errors (where bias decreases with increasing A). This expression is still awkward, and so if A/Q is small we make a further approximation:

$$dA/A = -(P/Q) + A \cdot (f \cdot P/Q^2) \qquad (9b)$$

How good are these general forms? Equation 9a is always valid for noncompletion errors; it is quite good for dissociation errors if $4 \cdot k' \cdot S/K_p$ is less than 1, and not very bad if this term is less than 2. Equation 9b is reasonably good if S/r is less than 0.2 (noncompletion), or if $4 \cdot k' \cdot S/K_p$ is less than 0.3 (dissociation); it is not very bad if these quantities are less than 0.5 and 1, respectively. All these approximations tend to underestimate dA/A. Because K_r is likely to be large and K_p is not, these equations are likely to work best for noncompletion errors.

Composite Errors

At fairly low absorbances, where equation 9b is valid, we can combine constant, proportional, and nonlinear errors to find a general expression for dA/A. Gathering terms and defining three new constants, we have

$$dA/A = U + V/A + W \cdot A \qquad (10a)$$

If all the constants are positive, then a minimum bias of $U + 2 \cdot (V \cdot W)^{1/2}$ is reached at $A = +(V/W)^{1/2}$. If one or more of the constants is negative, then dA/A may reach zero at some value of A.

At low A, the V-term predominates. As A increases, the W-term increases, but the approximate equation 9b becomes increasingly inaccurate, and must eventually be replaced by 9a to give

$$dA/A = U' + V/A - P/(Q + f \cdot A) \qquad (10b)$$

where U' is a new constant.

If equation 10a holds, and if U, V, and W are mean values from populations with respective standard deviations of SD(U), SD(V), and SD(W), then the imprecision in A is given by

$$CV^2(A) = SD^2(U) + SD^2(V)/A^2 + SD^2(W) \cdot A^2 \qquad (11)$$

Again, the V-term predominates at low A; then as A increases, the W-term becomes larger until the approximation breaks down.

Finally, it is not uncommon for standards, and sometimes samples, to be run in replicate. For any serum run j times, $CV^2(A)$ is multiplied by $1/j$ while dA/A is left unchanged.

Errors in Calculated Concentration

With these expressions for errors in absorbance, we can calculate the consequences for the accuracy and precision of S. The constants U, V, and W may or may not be identical for standards and samples. Standards are often animal sera; they are usually elaborately processed, and they may even be pure solutions, so it is perhaps to be expected that their error constants will in fact differ from those of most human serum samples. I will consider the various cases in turn.

Constant error. In the general case, if standards and samples are subject to constant errors of B and $B \cdot (1 + q)$, respectively, the resulting bias in S is given by

$$dS/S = B \cdot [(1 + q)/A_s - (1/A_c)] + dC/C \qquad (12a)$$

of which the result given in ref. *1* is the special case where q and dC/C are both zero. If the CV of the blank absorbance is independent of its size, then the imprecision of S is given by

$$CV^2(S) = SD^2(B) \cdot [(1 + q)^2/A_s^2 + (1/A_c)^2] + CV^2(C) \qquad (12b)$$

An interesting case is that of baseline drift after an error-free calibration. If this drift is such that its size at any sample is simply proportional to the cup-number, we can calculate the mean bias for a sample inserted randomly into a run. If the runs are fairly long, this is given by

$$dS/S = dA_s/A_s + dC/C = \tfrac{1}{2}B_m/A_s + dC/C \qquad (13a)$$

where B_m is the maximum drift, reached at the last sample of the run. This calculated bias is the expected mean of a long series of randomly inserted samples. The expected standard deviation of that series can be calculated from first principles, and measures the imprecision due to drift. When the runs are fairly long, this is given by

$$CV^2(S) = SD^2(B)/A_s^2 + CV^2(C) = (1/12) \cdot (B_m/A_s)^2 + CV^2(C) \qquad (13b)$$

Another special case is that of uncorrected carryover. Usually this will only affect samples, and so, using the earlier results,

$$dS/S = h \cdot (S_m/S - 1) + dC/C \qquad (14a)$$

$$CV^2(S) = (1/4) \cdot (h \cdot T)^2 \cdot (S_m/S)^2 + CV^2(C) \qquad (14b)$$

Proportional errors. It is reasonable to define the standards as showing no proportional error and so, using the earlier results,

$$dS/S = E + dC/C \qquad (15a)$$

$$CV^2(S) = SD^2(E) + CV^2(C) \qquad (15b)$$

One special case is that of slope drift after an error-free calibration. If this occurs so that E is proportional to cup-number, and if the run is fairly long, then working as before we can calculate the resulting errors for a randomly inserted sample:

$$dS/S = \tfrac{1}{2}E_m + dC/C \qquad (16a)$$

$$CV^2(S) = (1/12) \cdot E_m^2 \qquad (16b)$$

where E_m is the size of E at the last cup of a run.

Nonlinearity. If standards and samples are subject to the same error, then

$$dS/S = (f \cdot P/Q^2) \cdot (A_s - A_c) + dC/C \quad (17)$$

The expressions for imprecision, and for bias when the error constants are not identical, can also be written if necessary.

Composite errors. With the earlier expressions for errors in absorbance, we can write general equations for errors in the calculated sample concentration, S (the values of the error constants may of course be different for samples and standards). The results of any combination of absorbance errors can be derived from these equations, and the results are usually obvious from the components. For example, if both baseline and slope drifts occur as described, then

$$dS/S = \tfrac{1}{2}(E_m + B_m/A_s) + dC/C \quad (18a)$$
$$CV^2(S) = (1/12) \cdot [E_m^2 + (B_m/A_s)^2] + CV^2(C) \quad (18b)$$

Minimizing Errors by Technique

Several techniques can be used to minimize the effects of some constant absorbance errors. A detailed treatment would be inappropriate, but a few points are relevant here.

Sample Blank Measurement

The effects of turbidity (but not analytical nonspecificity) can be removed by running a sample blank, measuring its absorbance as A_b, and calculating the sample concentration as

$$S = (A_s - A_b) \cdot C/(A_c - A_b) = (A_s'/A_c') \cdot C \quad (19)$$

If A_b is equal to the blank error, B, the error is eliminated. Proportional and nonlinear errors may still exist. The latter now depend on the corrected absorbance, A'. Photometric errors still depend on the total absorbance, A.

It can be shown that the imprecision that results from the use of a sample blank is

$$CV^2(S) = CV^2(A_s') + CV^2(A_c') + CV^2(C) + 2 \cdot SD^2(A_b) \cdot [(1/A_s')^2 + (1/A_c')^2] \quad (20)$$

The improvement in accuracy is therefore paid for by a decrease in precision, which may be significant at low concentrations.

Bichromatic Analysis

Measurement of absorbance at two wavelengths, Z_1 and Z_2, can be used to correct for sample turbidity (*8*). The correction depends on an assumed value of K_t, which is the ratio of the absorbances due to turbidity at Z_2 and Z_1. It is known that $K_t = (Z_2/Z_1)^n$, where n is a constant with quite a large population standard deviation. As a result of this uncertainty in n, K_t is subject to error:

$$dK_t/K_t = \log_e(Z_2/Z_1) \cdot dn \quad (21a)$$
$$CV(K_t) = \log_e(Z_2/Z_1) \cdot SD(n) \quad (21b)$$

This has a complex effect on the total error in S for any particular sample, but it may be difficult to ignore unless Z_2 and Z_1 are very close together.

Carryover Correction

When carryover is expected, a correction can be applied. It is necessary, of course, to use the correct formula (*5*). Even then, uncertainty in the carryover constant, h, is transmitted to the corrected absorbance: if h is small, it can be shown that the resulting uncertainty in S is approximately

$$CV^2(S) = CV^2(S_a) + CV^2(h) \quad (22a)$$
$$dS/S = dS_a/S_a - dh/h \quad (22b)$$

where S_a is the uncorrected sample concentration.

Even when h is known without error, its use can still lead to increased bias or imprecision in S. If h is not negligible, then

$$dS/S = (dS_a - h \cdot dP_a)/(S_a - h \cdot P_a) \quad (23a)$$
$$CV^2(S) = [SD^2(S_a) + h^2 \cdot SD^2(P_a)]/(S_a - h \cdot P_a)^2 \quad (23b)$$

where P_a is the uncorrected absorbance of the preceding sample. If $dS_a/S_a = dP_a/P_a$, as it may well be, then $dS/S = dS_a/S_a$, and the carryover correction adds no extra bias. However, if $CV(S_a) = CV(P_a)$, which is also quite possible, then

$$CV^2(S) = CV^2(S_a) \cdot (S_a^2 + h^2 \cdot P_a^2)/(S_a - h \cdot P_a)^2 \quad (24)$$

which is always greater than $CV^2(S_a)$. In effect, use of the carryover correction, even when h is known precisely, is a source of imprecision in S. Once again, increased accuracy costs precision.

Kinetic Methods

This is a large subject, involving a wide variety of methodologies (*9*). I will discuss only one here.

The one-point calibration can be adapted to kinetic use by making absorbance measurements twice, at times t_1 and t_2, before equilibrium is reached. If $A(t)$ stands for the absorbance at time t, then the sample concentration, S, is calculated as

$$S = C \cdot [A_s(t_2) - A_s(t_1)]/[A_c(t_2) - A_c(t_1)] \quad (25)$$

This procedure eliminates constant error, and removes noncompletion as a source of nonlinearity.

The imprecision in S is given by

$$CV^2(S) = \{SD^2[A_s(t_1)] + SD^2[A_s(t_2)]\}/[A_s(t_2) - A_s(t_1)]^2 + \{SD^2[A_c(t_1)] + SD^2[A_c(t_2)]\}/[A_c(t_2) - A_c(t_1)]^2 + CV^2(C) \quad (26)$$

The SD(A) terms should no longer contain any constant-error component. However, the total imprecision may well be larger than in the nonkinetic technique. This will depend on what other errors are present, and on the size of the absorbance changes; this in turn depends on C and S, on the analytical constant k, and on the interval $t_2 - t_1$.

Minimizing Errors by Choice of Calibration Concentration

A laboratory has some control over the value of C, subject to the availability of commercial sera, and so it will be useful to consider the dependence of errors in S on C. In what follows, dC/C will be ignored; if necessary, it can be taken into account, but the equations become rather more complicated. The unimodal and bimodal cases will be treated separately.

Unimodal Distribution

Zero bias is achieved when $dA_s/A_s = dA_c/A_c$. When the error constants are identical for standards and samples, such is the case at $S = C$, and so for a unimodal distribution the average bias is minimized by setting C equal to S_m, the population mean. It is interesting to estimate the resulting bias at other parts of the distribution; one way to do this is to calculate dS/S at S_t and S_b, the top and bottom limits of the reference interval. Some expressions for these errors are shown in Table 4; this also includes the case of drift errors, which of course do not change with calibration concentration and therefore cannot be minimized by choosing C.

Notice that, when C is between S_t and S_b, the sum of the absolute magnitudes of the biases at S_t and S_b is indepen-

Table 4. Bias in S at S_t, S_b, and S_m, when $C = S_m$

Type of error	dS_b/S_b	dS_m/S_m	dS_t/S_t
Constant blank	$(B/A_{sm}) \cdot T/(1 - T)$	0	$-(B/A_{sm}) \cdot T/(1 + T)$
Carryover	$h \cdot T/(1 - T)$	0	$-h \cdot T/(1 + T)$
Nonlinearity	$-A_{sm} \cdot T \cdot f \cdot P/Q^2$	0	$A_{sm} \cdot T \cdot f \cdot P/Q^2$
Slope drift	$\frac{1}{2}E_m$	$\frac{1}{2}E_m$	$\frac{1}{2}E_m$
Baseline drift	$\frac{1}{2}B_m/A_{sm} \cdot (1 - T)$	$\frac{1}{2}B_m/A_{sm}$	$\frac{1}{2}B_m/A_{sm} \cdot (1 + T)$

dent of C, and is given by

$$|dS_t/S_t| + |dS_b/S_b| = |dA_{st}/A_{st} - dA_{sb}/A_{sb}| \quad (27)$$

A similar equation holds for any two boundary values of S.

Error constants can, of course, be different for standards and samples, and drift is an extreme example in which standards are not subject to error at all. In other cases, where error constants differ, it may still be possible to make the bias zero, but this will no longer be so at $S = C$. Consider, for example, the case of nonidentical blank errors, described by equations 12a and 12b. Bias is zero at $C = S/(1 + q)$; we can therefore minimize average bias by setting C equal to $S_m/(1 + q)$. If we do this, the resulting errors at S_t and S_b are found by multiplying those of the identical-error case (given in Table 4) by $(1 + q)$. If we calibrate at $C = S_m$, regardless, we can calculate the resulting errors at S_m, S_t, and S_b:

$$\begin{aligned} dS_m/S_m &= B \cdot q/A_{sm} \\ dS_t/S_t &= B \cdot (q - T)/A_{sm} \cdot (1 + T) \\ dS_b/S_b &= B \cdot (q + T)/A_{sm} \cdot (1 - T) \end{aligned} \quad (28)$$

It may be useful to estimate the consequences for precision of particular values of C. If, for example, we have identical blank errors, and calibrate at $C = S_m$, the imprecisions at S_m, S_t, and S_b are given by

$$\begin{aligned} CV^2(S_m) &= 2 \cdot SD^2(B)/A_{sm}^2 + CV^2(C) \\ CV^2(S_t) &= [SD(B)/A_{sm}]^2 \cdot \{1/[(1 + T)^2] + 1\} + CV^2(C) \\ CV^2(S_b) &= [SD(B)/A_{sm}]^2 \cdot \{1/[(1 - T)^2] + 1\} + CV^2(C) \end{aligned} \quad (29)$$

Bimodal Distribution

Here the problem is to apportion the inevitable error sensibly between the two peaks at S_1 and S_2. Notice that, by analogy with equation 27, the sum of the magnitudes of the biases at S_1 and S_2 is independent of C, and is given by

$$|dS_1/S_1| + |dS_2/S_2| = |dA_{s2}/dA_{s2} - dA_{s1}/A_{s1}| \quad (30)$$

provided that C is between S_1 and S_2.

The general problem is to find C so that the size of the bias at S_2 is N times that at S_1, where N is some number to be decided on after considering the clinical function of the analysis. If dC/C is negligible, this value of C is found by solving an equation relating the absorbance errors at S_1, S_2, and C:

$$dA_c/A_c = [(N \cdot dA_{s1}/A_{s1}) + (dA_{s2}/A_{s2})]/(N + 1) \quad (31)$$

For example, for nonidentical blank errors, as discussed previously, this optimum value of C is given by

$$1/C = (1 + q) \cdot (N/S_1 + 1/S_2)/(N + 1) \quad (32a)$$

and for simple nonlinear errors, as in equation 17,

$$C = (N \cdot S_1 + S_2)/(N + 1) \quad (32b)$$

The errors resulting from this choice of C are set out in Table 5.

It is interesting to calculate the result of calibrating at C $\frac{1}{2}(S_1 + S_2)$ if the error is in fact of the constant type. In this case

$$\begin{aligned} dS_1/S_1 &= (B/A_{s1}) \cdot [G/(G + 2) + q] \\ dS_2/S_2 &= (B/A_{s1}) \cdot [q - G/(G + 2)]/(1 + G) \end{aligned} \quad (33)$$

Table 5. Bias in S at S_1 and S_2 When C Is Optimal (for Blank and Nonlinear Errors) and for Any C

Unequal blank errors

$$\begin{aligned} dS_2/S_2 &= -[B \cdot (1 + q)/A_{s1}] \cdot [N/(N + 1)] \cdot [G/(G + 1)] \\ dS_1/S_1 &= [B \cdot (1 + q)/A_{s1}] \cdot [1/(N + 1)] \cdot [G/(G + 1)] \end{aligned}$$

Nonlinearity

$$\begin{aligned} dS_2/S_2 &= [f \cdot P \cdot A_{s1}/Q^2] \cdot G \cdot [N/(N + 1)] \\ dS_1/S_1 &= -[f \cdot P \cdot A_{s1}/Q^2] \cdot G \cdot [1/(N + 1)] \end{aligned}$$

Combined slope and baseline drift

$$\begin{aligned} dS_2/S_2 &= \tfrac{1}{2}E_m + \tfrac{1}{2}B_m/A_{s1} \cdot (1 + G) \\ dS_1/S_1 &= \tfrac{1}{2}E_m + \tfrac{1}{2}B_m/A_{s1} \end{aligned}$$

In the case of a composite error, it may be difficult to find an optimum value of C. It is worth noting that for some errors dS/S may reach zero at more than one C; for example, if the bias in absorbance is as described by equation 10a, and if the error constants are identical for samples and standards, then dS/S is zero at $C = S$, and also at $C = V/W \cdot k^2 \cdot S$.

Discussion

This paper provides a theoretical framework for understanding the "analytical" causes of bias and imprecision in the one-point calibration procedure. The results can be used in three ways.

- To describe the consequences of known or suspected errors, and their dependence on C and S, and to quantify this if numerical data are available. This may help to decide the appropriateness of a method for the one-point technique, and to assess the need for special procedures to reduce bias.
- To choose a calibration concentration that will minimize average bias (for a unimodal distribution of patients' results) or apportion it sensibly between the two sample peaks (for a bimodal distribution). The most obvious answer may well be correct, but if not we can calculate the consequences of oversimplification. In the absence of some analysis of this sort there is no rational basis for preferring any value of C to any other.
- To assist in method development by suggesting the kind of information that needs to be collected, the way it should be processed, and the actions that should be taken on the results.

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