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Report

Method Validation Revisited: A Chemometric Approach

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A validation procedure is presented that satisfies the FDA requirements of accuracy (including precision repeatability), sensitivity, linearity, dynamic range, and homoscedasticity, all with a single set of data. The procedure utilizes the corrigible error correction (CEC) technique comprised of three response curves—standard, Youden one-sample, and method of standard additions (MOSA) plots, from a total of 15 to 18 X,Y data pairs. For the bias component of accuracy, the systematic bias error of the method is quantitatively separated into its constant and proportional error components. The overall constant systematic error is further separated into the system (blank) and analyte-matrix (sample) components. The CEC data also provide an internal, i.e., *in situ* corrected assay for the sample for comparison with alternative method data. Statistical diagnostic tests are used for the final evaluation of the method acceptability, specifically in deciding whether or not the systematic error indicated requires a root source search for its removal or is simply a calibration constant of the method.

KEY WORDS: accuracy; method validation; systematic error; statistical diagnostic tests; corrigible error correction technique.

INTRODUCTION

In a method validation program, there is a variety of intra- and interlaboratory analytical procedures that are capable of detecting systematic (bias) error. A discussion of these procedures and the statistical diagnostic tests that are used in the evaluation of the data produced is available (1). Only one of these procedures, the corrigible error correction (CEC) (2,3) technique, not only is capable of detecting bias error but also is able to characterize it quantitatively into its constant and proportional error components and at the same time yield a corrected assay result on the actual sample under analysis. The CEC technique, then, has been selected for examination of some of the chemometrics involved.

Rather than a review of all the chemometric tests that are available and useful in a validation program, which would be beyond the scope of this paper, we emphasize only those statistical tests and considerations which, although not new to statisticians, may be new to most practicing pharmaceutical analysts. With these, it is our intent to demonstrate the power of chemometrics as an aid in some of the decision-making processes that are involved throughout a validation program. Finally, we will make the point that a method validation program based on the CEC technique provides all of the required new drug application (NDA) documentation data and does so more efficiently and more effectively than do the present conventional validation approaches.

STATISTICAL TESTS

Simple Linear Regression Statistics

One of the requisites in the use of simple linear regression analysis is that a simple linear model, namely, $Y = mX + b$, must be shown to be applicable. This requires that the data pass an appropriate linearity test and also that the data be homoscedastic (constant variance over the range). Tests for both of these requirements are given below. Two of the various statistics that come out of a simple linear regression analysis are either underused or perhaps unknown to analytical chemists. These are based on the intercept and the standard error of estimate, $s_{y \cdot x}$ (4,5).

Relative Intercept. The use of the intercept as a parameter of the line for analytical calculations is, of course, basic to analytical practice. The calculation of the confidence interval for the intercept at some selected confidence level to compare, for example, against an expected value of zero is also a well-known significance test. If zero is included in the confidence interval, the intercept is statistically not significantly different from zero. The analyst however, does not equate it to zero but still utilizes the actual value as a calibration constant. There is no inference of a bias error in the calibration or sample response system. However, if the intercept value is statistically significantly different from zero, then such an inference can be made and there is justification for a *first-principles* search for the root source of the bias error.

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Not so familiar to analytical chemists, however, is the relative intercept statistic, $b/\overline{Y}$ (5). This statistic has basic significance to analytical chemists but is of no concern or interest to statisticians. Their interest in the intercept is in its confidence interval, as already mentioned. Analytically, however, the intercept as a percentage of the response signal is of value to the analyst in judging the degree of error resulting from the bias especially when considered simultaneously with the relative variation statistic (Rel Var), discussed below. [Concepts and applications of the relative intercept have been discussed (5).]

Relative Variation (Rel Var). This statistic, $s_{y\cdot x}/\overline{Y}$ is the standard error of estimate as a percentage of the mean (4,5) and may be used in at least two ways, if the data pass an appropriate linearity test as demonstrated in Appendix 1. (If the data cannot pass a linearity test, simple linear regression analysis cannot be used. This situation is beyond the scope of this paper.)

The first usage involves its characteristic as a precision measure. If its magnitude is acceptable when assessed against the precision required in the proposed method, then the linear dynamic range (that has first been tested and passed) is acceptable. For example, if the Rel Var for the Youden one-sample plot (2,3) were 0.80% and a final method precision of less than 1% were the goal, then the response curve procedure would be acceptable since the Youden one-sample plot incorporates all of the procedural steps except for the Kaiser inverse operation (6) via the standard response curve. [There is some precision deterioration in this operation as Mitchell and Garden (7) have pointed out.]

However, if the magnitude of the Rel Var is unacceptable, the linear dynamic range should be shortened, dropping the high and low values, if necessary and rerunning the new range span with an adequate number of X, Y data pairs. (This procedure of shortening the usable range is based partly on our observations that the Rel Var decreases as the $\overline{X}, \overline{Y}$ centrum point is approached. We have been unable to produce a rigorous statistical explanation of this behavior. Also, any undetected curvilinearity in the line decreases as the range is shortened, with a resultant increase in precision). If the Rel Var of the new redetermined linear regression is still unacceptable, the process is repeated until an acceptable value of the Rel Var is obtained provided that the remaining linear range is still analytically useful. If an analytically useful range with an acceptable Rel Var cannot be found, then a first principles restudy of the precision variables in the methodology is required. If the search does not uncover a root source of the variability or if, after the method has been revised, the rerun of the regression still results in an unacceptable magnitude of the Rel Var, the response curve procedure must be abandoned.

The second usage of the Rel Var statistic is to answer the question, Is the linear dynamic range homoscedastic? Examination of Fig. 1 shows that the Rel Var is analogous to the RSD precision measure. After testing and accepting linearity, the postulation is then made that if the linear dynamic range is homoscedastic, then $\sigma^2 = \sigma^2_{y\cdot x}$ (4). An illustration is shown in the data in Table III. The computation is shown in Appendix 2 and since the assumption of homoscedasticity is not rejected for the dynamic range, then simple linear regression analysis may be used. (If the range were shown to be heteroscedastic, then weighted regression analysis would have to be used, a situation beyond the scope of this paper.)

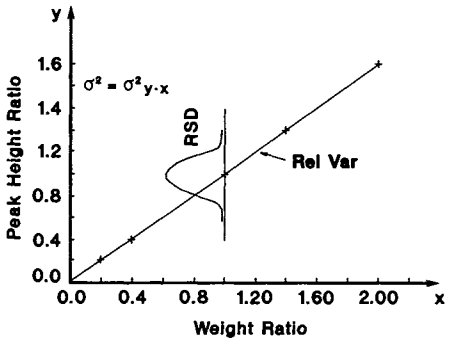

Fig. 1. Response curve precisions: Rel Var (line) and RSD (single level).

Corrigible Error Correction (CEC) Technique Concepts and Statistical Tests

There are three response curves utilized in the CEC technique, each of which is subjected to simple linear regression analysis. These are shown in Fig. 2 as the standard plot, the Youden one-sample plot, and the method of standard additions (MOSA) plot. In each, the slopes are, respectively, m_s, m_Y, and m_M, with the intercepts, respectively, SB, TYB and A. The significance of each of these mathematical parameters has been discussed (2,3). One of the most important concepts that has been proven (2,3) is that the overall functionality of the MOSA is as shown in Fig. 2B, where the relationship is $Y = m_M W_r + \text{TYB}$, a relationship wherein the MOSA is simply an extension of the Youden one-sample

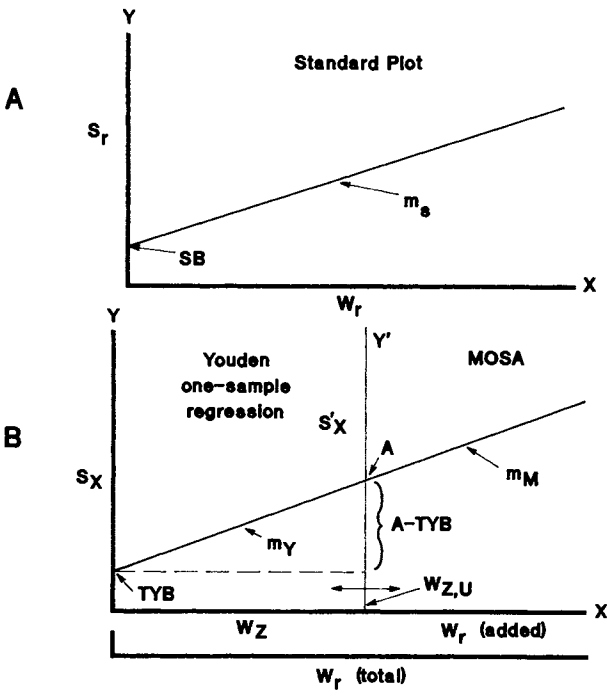

Fig. 2. Response curves in the corrigible error correction technique.

curve. This relationship shows that in the MOSA, the unspiked sample analyte signal response S'_x is equal to the MOSA intercept, A, and that for a correct MOSA calculation, the true sample blank, i.e., the total Youden blank (TYB), must be subtracted.

Another important concept is the term expressed in the relationship, TYB − SB = YB. Since the TYB is the intercept of a response curve in a matrix, while the SB is the intercept of a matrixless standard response curve, the difference between the two intercepts, namely, the Youden blank (YB), is statistically equivalent to zero, if there is no analyte–matrix interaction effect. It is important to note that each intercept, SB and TYB, can be individually tested for equivalence to zero by a calculation of its respective confidence interval at the desired confidence level. If both intercept confidence intervals include the value of zero, then the values of the SB and TYB are not statistically different from zero and the conclusion can be drawn that the intercepts are simply calibration constants to be used in assay calculations. More importantly, however, is the case where either the SB or the TYB, or both, may be statistically different from zero. In this case, it is the difference between the intercepts that must be statistically tested against zero. A calculation of the confidence interval of the difference is made. If the confidence interval includes the value of zero, then the SB and TYB are simply calibration constants. If the confidence interval does not include the value of zero, a constant systematic error in the sample system is indicated that can justify a first-principles search for the root source of the error. The significance test for the difference between two intercepts is shown in Appendix 3.

Finally, another concept is the ratio of the MOSA slope to that of the standard slope, m_M/m_s, defined as the proportional error factor, P. The value of P is equal to unity unless the matrix causes an interaction effect on the MOSA slope. If the value of P is not unity, a statistical significance test is again needed to decide when the value is significantly different from unity. If the slope ratio confidence interval includes the value of unity, then the value of P is simply a calibration constant. If it does not, a proportional systematic error is indicated that can justify a first-principles search for the root source of the error. This significance test is shown in Appendix 4.

EXPERIMENTAL

A steroid cream dosage formulation was assayed for the active steroid by an HPLC method using a single extraction procedure for the removal of matrix components and other interferents and measuring the peak height responses (PH) of the steroid analyte.

For the standard calibration curve-based procedure, the respective weighed samples were taken up in solvent, the solutions extracted with a specified volume of immiscible solvent, and the separated aqueous layer diluted to volume. Standard solutions were prepared in the same manner except that the extraction step was omitted, the solutions being diluted directly to volume.

In the MOSA procedure, for the incremental spike solutions, each of the unspiked sample solutions, $W_{z,u}$ of 7.432 ± 0.015 mg/ml concentration (± 0.2% variation in the g sample weight) were treated with an appropriate volume of standard solution before extraction and completion of the procedure above. The data are shown in Table I.

RESULTS

In Table I, the X,Y data pairs for the respective response curves are given, each with pertinent parameters from simple linear regression analysis, the intercept and slope, and their respective standard (deviations) errors. These statistics are those needed for the calculation of the confidence interval for the difference in the Youden one-sample and standard curve intercepts, and the MOSA/standard curve slope ratio as mentioned above, as shown in Appendices 3 and 4.

In Table II, standard curve assay results are calculated for the various Youden one-sample range points, uncorrected for the revealed total Youden blank, TYB, and corrected for the TYB using the formulae in the footnotes to Table II, as given in reference 8, Fig. 2, Code A3.2.1, for the uncorrected value and in reference 2, Fig. 1, II, for the corrected value. Similarly, the uncorrected and corrected MOSA values, respectively, utilize Code A5.2 in Ref. 8, Fig. 2, and formula IV in Ref. 2, Fig. 1. The CEC statistics (2,3) are also shown in Table II except that the relative total average bias (Rel TAB) is herewith defined as [(uncorrected - corrected) / corrected] (100).

The data in Tables I and II furnish the method documentation information required by the regulatory agencies (e.g., the FDA for an NDA) ascertaining to the accuracy method performance characteristic that a method validation procedure must provide.

DISCUSSION

The regulatory requirements for the validation of a new method as part of a new drug application are set out in the

Table I. Determination of Steroid in a Steroid Cream by an HPLC Method

Standard curve[a]		Youden curve[a]		MOSA curvea[a,b]	
X (W_r), μg/ml	Y (S_r), PH	X (W_z), mg sple/ml	Y (S_x), PH	X (W_r), μg/ml	Y (S'_x), PH
30.01	0.2296	2.680	0.2274	0.00	0.6231
60.01	0.4578	5.252	0.4419	10.00	0.7094
80.02	0.6067	7.432	0.6231	30.01	0.8672
100.03	0.7487	9.908	0.8405	50.01	1.0377
125.03	0.9527	11.940	1.0185	70.02	1.2002
150.03	1.1502	13.970	1.1802		
$n_s = 6$		$n_Y = 6$		$n_M = 5$	
$b_s = -0.00373$		$b_Y = -0.00287$		$b_M = 0.6241$	
$s_{bs} = 0.007582$		$s_{bY} = 0.005047$			
$m_s = 0.007646$				$m_M = 0.008231$	
$s_{ms} = 0.00007643$				$s_{mM} = 0.00005443$	

[a] Dilution volume was 250 ml; dilution factor, 1; symbols for axes are as used in Fig. 2.

[b] Sample solution concentration for spiking, $W_{z,u}$, was 7.432 mg/ml.

Table II. HPLC Method Assay Results by Standard Curve and MOSA Procedures

	mg/g			
	Standard curve		MOSA curve	
mg sple/ml	Uncorr.[a]	Corr.[b]	Uncorr.[c]	Corr.[d]
2.680	11.279	10.439		
5.252	11.097	10.289		
7.432	11.031	10.233	10.20	10.25
9.908	11.144	10.341		
11.940	11.197	10.393		
13.970	11.084	10.289		
Mean	11.14	10.33		
RSD	0.80%	0.73%		
Rel Var	—	0.71%		
	CEC statistics			
Rel TYB	—	−0.40%		
Rel YB	—	0.12%		
P	—	1.08		
Rel TAB	—	7.84%		

[a] mg/g = (PH − SB)/(m_s) (W_z).
[b] mg/g = (PH − TYB)/(m_s) (W_z) (P).
[c] mg/g = PH/(m_M) ($W_{z,u}$).
[d] mg/g = (PH − TYB)/(m_M) ($W_{z,u}$).

Good Manufacturing Practices (GMPs) (9). The definitions and data requirements for the method performance characteristics, accuracy, sensitivity, specificity, and reproducibility [and ruggedness (10)] that are called for in the regulations, which attest to the reliability of the method and which have been generally accepted by analytical chemists in the pharmaceutical and chemical industries, are well-known (11,12).

The CEC technique addresses only the accuracy method performance characteristic. Although the PMA guidelines (12) and GMPs (9) use the term accuracy as synonymous to the term bias, inaccuracy is now generally accepted as meaning total error, i.e., a composite of both bias and imprecision (Refs. 1–9 in Ref. 1). The other method performance characteristics all involve individually designed experiments of which the PMA guidelines are representative. The exception is the sensitivity criterion that is omitted in the PMA guidelines. In its place are the limit of detection and limit of quantitation criteria that are only requirements for methods in Category II (12), that is, low-level determinations of impurities or degradants. There is no problem posed, however, in meeting the GMP sensitivity criterion since its generally accepted definition is that it is provided by the slope of the analytical response curve, a datum that all methodology provides (11). In Table I, it is m_s = 0.007646.

The essence of the CEC technique is that the entire accuracy criterion is met by a single set of data (Table II), 17 data points in all. The precision component of accuracy is provided both by the Rel Var statistic discussed in the preceding section (0.71%, Table II) and by the RSD of the Youden one-sample range points (0.73%, Table II). These are, of course, the precision repeatability criterion since the data are within a set. This is, however, currently accepted analytical practice and it should be noted that the PMA guidelines do not explicitly call for the reproducibility precision criterion. To provide a reproducibility precision estimate, the data set need only be repeated on another day and the individual RSD estimates statistically pooled.

The bias components of accuracy are provided by the statistics in Table II: the total constant error in the overall sample system is the TYB and the constant error from the analyte–matrix interaction is the YB. The proportional error is P and since an assay corrected for these systematic errors is provided, an overall net systematic error, the Rel TAB, can be calculated. The Rel TAB can be mostly one-sided, as in this example where almost all the bias error is proportional in nature. In other cases, it can be mostly constant error, and in others, it can be almost zero when the errors arbitrarily cancel each other. In the latter case, in conventional validation practice, systematic error can remain undetected in an apparently bias free method, a situation that can cause otherwise unexplainable aberrant results even when obtained under normally variable laboratory conditions.

In the CEC validation procedure, an appropriate linearity test has been included which some or even many analytical chemists may reject as redundant or unnecessary since the correlation coefficient, r, is, in fact, acceptable to the FDA for this purpose. The correlation coefficient and its related coefficient of determination, R^2, are *not* indicators of the linearity of a functional response curve and this has been recognized for some time (7,13,14). A high degree of correlation in a functional relationship does not necessarily mean that a straight line relationship exists. Thus, a perfectly correlated straight line has an r value of ±1 but so does a perfectly correlated curvilinear function. In analyses where a straight-line calibration curve is based on a physicochemical relationship such as the Beer–Lambert law, the redundancy is in the citation of r as proof of the linearity of an already established function.

A more appropriate linearity test for data as represented in the response curves in Table I is an F test for lack of fit utilizing replicated X,Y data pairs along the response curve (7). This option is somewhat unsatisfactory because of the replication requirement, whereas the only somewhat inferior but adequate option (7) selected in Appendix 1 requires none. A signs test for linearity based on residuals as suggested by Thompson (15) requires a minimum of 19 or 20 data pairs for a conclusive estimate, a somewhat impractical criterion for a response curve. However, a residuals plot interpreted visually can be of value in assessing linearity (16).

In summary, tests are provided that qualify the use of simple linear regression statistics and help select the span of the linear dynamic range, which will provide acceptable precision for the intended usage. The intercepts of the standard and Youden one-sample curves and the MOSA to a standard curve slope ratio can be statistically tested to determine whether or not these regression line parameters are simply calibration constants or whether they indicate an underlying root source of bias which should be sought for and eliminated. The bias component of the accuracy criterion is separated quantitatively into its constant and proportional error components, a unique capability of the CEC validation procedure. This capability furnishes additional assistance in decisions concerning systematic error root sources searches.

The use of the systematic error constants to correct, *in situ*, the assay is especially helpful when comparing alternate method results. Thus, the data set provides a more effective diagnosis of the overall method performance than is possible with conventional alternative validation approaches with less data.

APPENDIX 1: LINEARITY TEST

Premise

$$Y = b_o + b_1X + \underbrace{b_2X^2}_{\text{quadratic term}}$$

Test the value of b_2 from fitted data; if it differs significantly from zero, nonlinearity is demonstrated (at the confidence level chosen).

Procedure

1. Input X, Y data into
   Part A: a polynomial regression program such as SAS [Statistics Analysis Systems (17)] or
   Part B: the manual algorithms provided by Burnett (18) and the authors (19).
2. From the quadratic parameter b_2 and its standard error, s_{b2}, as obtained from either Part A or Part B, calculate the experimental t:

$$t = b_2/s_{b2}.$$

3. Obtain the tabular t for $n - 3$ df at the confidence level selected [usually 95% (i.e., $\alpha = 0.05$)].

Interpretation

If calculated (experimental) $t >$ tabular t, then b_2 is indicated to be significantly different from zero and the quadratic model equation is required, i.e., nonlinearity is demonstrated.

Note. Martin (20) points out that the same conclusion is derived from an appropriate F test.

Example

Use the X, Y Youden curve data pairs in Table I.

Part A: the SAS program direct readout provides the experimental t value:
experimental $t = 0.17$
tabular $t_{(1-\alpha/2)(n-3\ \mathrm{df})}$, $n = 6$, $\alpha = 0.05$
$t_{0.975,\ 3\mathrm{df}} = 3.18$

Part B: manual algorithms
$i = 1, 2, 3, \ldots, n$
Make the calculations using the following algorithms, in order.

1. Calculate the b_0, b_1, and b_2 parameters.

$$F = [\Sigma(X_i - \bar{X})^2]\,[\Sigma(X_i^2 - \Sigma(X_i^2)/n)^2$$

$$G = \Sigma[X_i^2 - \Sigma(X_i^2)/n]\,(Y_i - \bar{Y})$$

$$H = \Sigma(X_i - \bar{X})\,[X_i^2 - \Sigma(X_i^2)/n]$$

$$J = \Sigma(X_i - \bar{X})\,(Y_i - \bar{Y})$$

$$b_1 = \frac{[\Sigma(X_i^2 - \Sigma(X_i^2)/n)^2]J - HG}{F - H^2}$$

$$b_2 = \frac{[\Sigma(X_i - \bar{X})^2]G - HJ}{F - H^2}$$

$$b_o = \bar{Y} - b_1\bar{X} - b_2[\Sigma(X_i^2)/n]$$

2. $\hat{Y}_i = b_o + b_1X_i + b_2X_i^2$
3. $S^2 = \dfrac{\Sigma(Y_i - \hat{Y}_i)^2}{n - 3}$
4. $m_{33} = \left[C - B^2/A - \dfrac{A^2E^2 - 2ABDE + B^2D^2}{A^2B - AD^2}\right]^{-1}$

   where
   $A = n = 6$, $B = \Sigma X_i^2$, $C = \Sigma X_i^4$
   $D = \Sigma X_i$, $E = \Sigma X_i^3$
5. Calculation of s_{b2} can now be made from the following relationship:

$$s_{b2} = \sqrt{S^2 m_{33}}$$

Conclusion

The values of b_2 and s_{b2} from either Part A or Part B are 0.0000325 and 0.000189, giving an experimental t of 0.17. Since the experimental t is less than the tabular t (Part A above), then the b_2 quadratic parameter is not indicated to be significantly different from zero, therefore we conclude that the quadratic equation is not required.

Note. When $b_2 = 0$ by the above test, the parameters for b_o and b_1 from the quadratic model are not used. Use the parameters from the simple linear regression model.

Comment

The SAS program in Part A is very sophisticated and is used by statisticians for many purposes. The polynominal regression algorithms in Part B are specifically for the purpose of the test described. Although the manual calculations using the algorithms are very tedious, with a personal computer and a program, the t test described could be no more complicated, in effect, than a standard deviation calculation now routinely made with an electronic calculator, simply the input of the X, Y data pairs.

APPENDIX 2: HOMOSCEDASTICITY TEST

Question: Is $s = s_{y\cdot x}$? (Is the standard deviation equivalent to the std-err-est?

H_o: $\sigma = \sigma_{y\cdot x}$ (null hypothesis)

F test

Compute experimental value (data from Table III)

Table III. Homoscedasticity Test

Rel Var[a]		RSD		
X, W_z (mg sple/ml)	Y, S_x (PH)	Samples: 2.225 g ± 1 mg (8.90 mg sple/ml) Y PH responses		
2.680	0.2274	0.7496	0.7572	0.7484
5.252	0.4419	0.7562	0.7536	0.7547
7.432	0.6231	0.7581	0.7510	
9.908	0.8405			
11.940	1.0185			
13.970	1.1802			
$\bar{X}$ 8.530				
$\bar{Y}$	0.7219	$\bar{Y}$ 0.7536		
$n_1 = 6$		$n_2 = 8$		
$s_{y \cdot x} = 0.005094$		$s = 0.003605$		
Rel Var = 0.71%		RSD = 0.48%		

[a] Data from Table I (Youden curve).

$$F_{expt} = s^2_{y \cdot x}/s^2$$
$$= 0.005094^2/0.003605^2$$
$$= 2.00$$

Obtain the two-tailed tabular t statistic at the confidence level, e.g., 95% ($\alpha = 0.05$).

$$F_{tab(1-\alpha/2),(df_1,df_2)} \qquad df_1 = n - 2$$
$$df_2 = n - 1$$

$$F_{0.975,(4,7df)=5.52}$$

Since $F_{expt} < F_{tab}$, we cannot reject the null hypothesis. We conclude that the variances are not significantly different, therefore, there is no evidence that the range is not homoscedastic.

APPENDIX 3: SIGNIFICANCE TEST FOR DIFFERENCE BETWEEN INTERCEPTS (21)

$$YB = TYB - SB$$
$$= b_Y - b_s$$
$$C.I._{YB} = (b_Y - b_s) \pm t_g \sqrt{s^2_{bY} + s^2_{bs}}$$

where the b_Y, b_s, s_{bY}, and s_{bs} terms are those provided by the simple linear regression data in Table I.

t_g is the two-tailed critical value from the Student's t distribution with g degrees of freedom calculated as follows:

$$C = s^2_{bY}/(s^2_{bY} + s^2_{bs})$$
$$g = 1/[C^2/(n_Y - 2) + (1 - C)^2/(n_s - 2)]$$
$$g = 6.963\ (\sim 7)$$
t_g at the 95% confidence level, $\alpha = 0.05$
$$t_{g(1-\alpha/2)7df} = 2.365$$

then

$$C.I._{YB} = 0.00086 \pm 0.02154$$

or

$$-0.0207 \text{ to } 0.0224$$

Note. There are several approximate solutions to t_g in the statistical literature.

APPENDIX 4: SIGNIFICANCE TEST FOR RATIO OF SLOPES, P

$$P = m_M/m_s$$

The confidence interval is

$$C.I._P = P \pm (t/m_s)[s^2_{mM} + P^2 s^2_{ms} - (t^2 s^2_{ms} s^2_{mM})/m^2_s]^{1/2}/(1 - (t^2 s^2_{ms})/m^2_s)$$

In the simplified Fieller's theorem equation (22) above, all covariance terms are omitted for this case since the regression functions are totally independent.

The slopes, m_s and m_M, and the standard error of the slopes, s_{ms} and s_{mM}, are those provided by the simple linear regression analysis data in Table I.

t is the two-tailed Student's value for the df as follows:

$$df = (n_s - 2) + (n_M - 2)$$
$$= 4 + 3$$

Tabular t for $\alpha = 0.05$

$$t_{(1-\alpha/2)7df} = 2.365$$

Then

$$C.I._P = (1.0765 \pm 0.0305)/0.9994$$
$$= 1.046 \text{ to } 1.107$$

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