APPENDIX

1. Linear regression

For n individual points (x_1, y_1), (x_2, y_2), $(x_3, y_3)\ldots(x_i, y_i)\ldots(x_n, y_n)$, a product-moment correlation coefficient, r, which represents how excellent the experimental points fit a straight line, is defined by the formula:

$$r = \frac{\sum_i \{(x_i - \bar{x})(y_i - \bar{y})\}}{\left\{\left[\sum_i (x_i - \bar{x})^2\right]\left[\sum_i (y_i - \bar{y})^2\right]\right\}^{\frac{1}{2}}} \qquad \text{(A-1)}$$

where $\bar{x}$ and $\bar{y}$ are the mean of $x-$values and $y-$values, respectively. The $r-$value closed to $|1|$ indicates a great linearity. The straight line calculated from these points is called as the line of regression of y on x whose slope (b) and intercept on the $y-$axis (a) are shown as follow :

$$b = \frac{\sum_i \{(x_i - \bar{x})(y_i - \bar{y})\}}{\sum_i (x_i - \bar{x})^2} \qquad \text{(A-2)}$$

$$a = \bar{y} - b\bar{x} \qquad \text{(A-3)}$$

Errors from the regression line are expressed as a standard deviation of the slope (s_b) and intercept (s_a) which are described by the equation:

$$s_b = \frac{s_{y/x}}{\left\{\sum_i (x_i - \bar{x})^2\right\}^{\frac{1}{2}}} \qquad \text{(A-4)}$$

and

$$s_a = s_{y/x}\left\{\frac{\sum_i x_i^2}{n\sum_i (x_i - \bar{x})^2}\right\}^{\frac{1}{2}} \qquad \text{(A-5)}$$

where $s_{y/x} = \left\{\frac{\sum_i (y_i - \hat{y}_i)^2}{n-2}\right\}^{\frac{1}{2}}$ when the $\hat{y}_i$ for a given value of x_i is calculated from the regression line: $y = bx + a$.

The value of s_b and s_a are important for calculation of a confidence interval. The confidence limit for the slope is given by $b \pm ts_b$,where the t – value has a number of $(n-2)$ degree of freedom at a desired confidence level. The confidence limit for the intercept is similarly taken by $a \pm ts_a$.

Calculation of a concentration

Once a straight line has been established, an $x-$value or a concentration (x_o) corresponding to any measured $y-$value or an analytical signal (y_o) can be calculated from both the slope and intercept:

$$y_o = bx_o + a \qquad \text{(A-6)}$$

with the error in x_o

$$s_{x_o} = \frac{s_{y/x}}{b}\left\{\frac{1}{m}+\frac{1}{n}+\frac{(y_o-\bar{y})^2}{b^2\sum_i (x_i-\bar{x})^2}\right\}^{\frac{1}{2}} \qquad \text{(A-7)}$$

where m is a number of readings to obtain y_o. The confidence limit is also calculated as $x_o \pm ts_{x_o}$, $(n-2)$ degree of freedom.

Limit of detection

Limit of detection (LOD) is generally defined as an analyte concentration which gives an instrumental signal significantly different from a blank or background signal. The widely used definition is that limit of detection is the analyte concentration giving a detector signal (y) equal to the blank signal (y_B) plus two (or three) standard deviations of the blank (s_B). When used, the definition of LOD must be provided.

The description of limit of quantification (LOQ), the lower limit recommended for quantification analysis, is similar to that of LOD but ten standard deviations was adopted instead of two (or three) ones.

To acquire the LOD, calculation at three signal to noise ratio is employed.

$$y \ at \ LOD = y_B + 3s_B \qquad \text{(A-8)}$$

The value of y_B and s_B are estimated from a and $s_{y/x}$, respectively, $y \ at \ LOD$ is then obtained. Inserting it to the regression equation, $y = bx + a$, the detection limit ($x-$value) is finally received.

An example of worksheet used for calculation for parameters in linear regression analysis is displayed in Table 4.8. Note that during a calculation, it is always useful to use the maximum available number of significant figures, rounding only at the end. More information about statistical calculations, please see J.C. Miller and J.N. Miller, 1993, Statistics for Analytical Chemistry, 3rd edn, Great Britain, Ellis Horwood Limited.

2. Precision

Precision is present in term of a relative standard deviation (RSD) which is given by

$$RSD\ (\%) = \frac{SD}{\bar{x}} \times 100 \qquad \text{(A-9)}$$

where SD and $\bar{x}$ is a standard deviation and an arithmetic mean, respectively.

3. Recovery

By addition of known amounts of an analyte to a sample, recovery is calculated from the analyte concentration before and after addition.

$$\text{Recovery (\%)} = \frac{\text{Total found analyte} - \text{sample content of analyte}}{\text{Added amount of analyte}} \times 100 \qquad \text{(A-10)}$$

4. Sampling frequency

A speed of analysis in FIA system is given by the relationship:

$$\text{Sample throughput (sample/h)} = \frac{3600\ (s)}{t_{base}\ (s)} \times \frac{1\ \text{sample}}{1\ \text{h}} \qquad \text{(A-11)}$$

where t_{base} is referred to the time from an appearance of the interested peak to the end.

Table 4.8 Linear regression worksheet for fluoride determination by the ternary aluminium method. (showing LOD of 0.02 ppm and LOQ 0.05 ppm)

n = 4 t = 4.30 (n-2) degree of freedom at 95% confidence limit

x_i	y_i	$x_i - \bar{x}$	$(x_i - \bar{x})^2$	$y_i - \bar{y}$	$(y_i - \bar{y})^2$	$(x_i - \bar{x})(y_i - \bar{y})$	x_i^2	$\hat{y}_i$	$\lvert y_i - \hat{y}_i \rvert$	$\lvert y_i - \hat{y}_i \rvert^2$
0.05	9	-0.2625	0.0689063	-27.75	770.0625	7.284375	0.0025	8.568	0.432	0.186624
0.20	24	-0.1125	0.0126563	-12.75	162.5625	1.434375	0.04	24.672	0.672	0.451584
0.40	46.3	0.0875	0.0076563	9.55	91.2025	0.835625	0.16	46.144	0.156	0.024336
0.60	67.7	0.2875	0.0826563	30.95	957.9025	8.898125	0.36	67.616	0.084	0.007056
sum 1.25	147		0.171875		1981.73	18.4525	0.5625			0.6696

$\bar{x}$ = 0.3125 $\bar{y}$ = 36.75

r	0.999831	b	107.36	s_b	1.3956817	Confidence limit	6.0014311
$s_{y/x}$	0.578619	a	3.2	s_a	0.5233806	Confidence limit	2.2505367

Calculation of a concentration

m = 3 (how many readings of y_o)

y_o	67.7	x_o	0.6007824	s_{x_o}	0.0055668	Confidence limit	0.0239372

Limit of detection	
y at LOD	4.9358571
LOD	0.0161686

Limit of quantification	
y at LOQ	8.9861905
LOQ	0.0538952

VITA

Name : Mr. Romran Chusie

Date of birth : December 17, 1977

Academic status :

- B.Sc. (Chemistry), Prince of Songkla University, 1999.

Scholarship :

- The Development and Promotion of Science and Technology Talents Project (DPST)
- The Postgraduate Education and Research Program in Chemistry (PERCH)

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