Analytical Science

A course (in 15 Chapters), developed as an Open Educational Resource, designed for use at 2nd year England & Wales undergraduate level and as a CPD training resource

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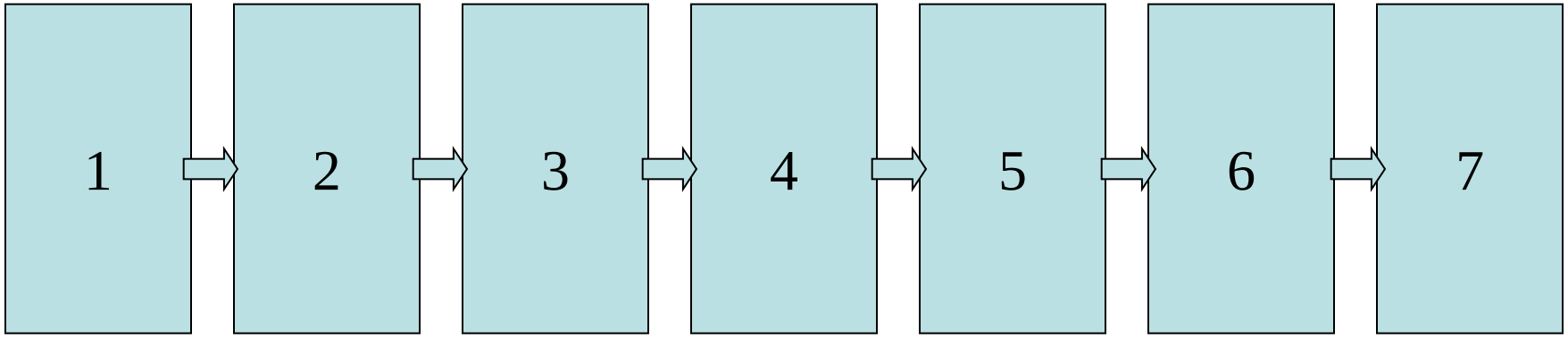
Chapter 5: Analytical Process Unit 7 – Evaluation of Data and Consideration of Objectives

Contents

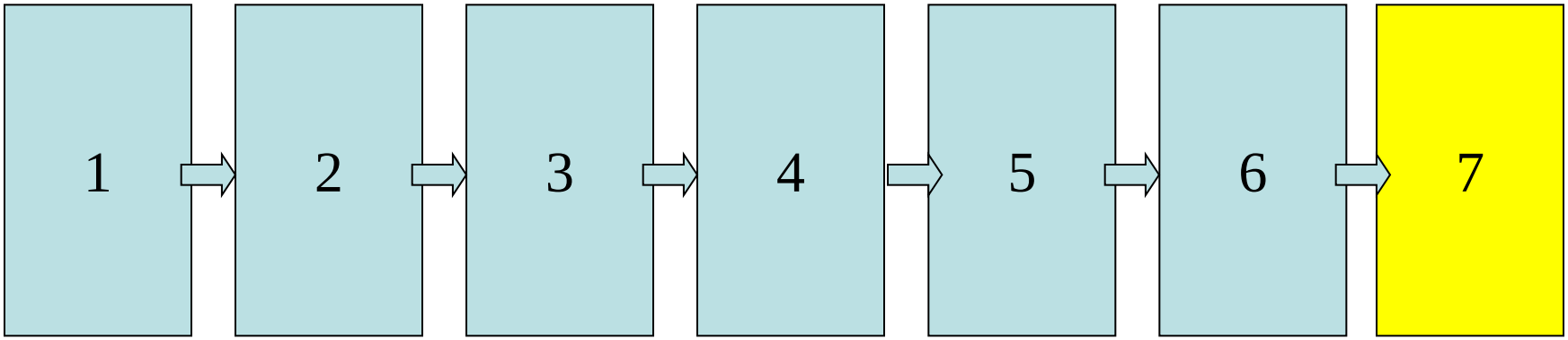
The analytical process model – revision slide

Any analysis may be considered as consisting of a maximum of seven unit processes. These are shown diagrammatically and descriptively below:



Unit 1. Consider the problem and decide on the objectives
Unit 2. Select procedure to achieve objectives
Unit 3. Sampling
Unit 4. Sample preparation
Unit 5. Separation and/or concentration
Unit 6. Measurement of target analytes
Unit 7. Evaluation of the data, have the objectives been met?

Process unit 7



In using the process model to define analysis, units 1 & 2 have been shown to be preliminary steps – deciding on the objectives for carrying out the analysis, and the methods to be employed in order to achieve the objectives. Units 3 – 6 were the practical stages of sampling, preparing the sample for analysis and making analytical measurements. The final stage involves:

- An evaluation of the data obtained, by application of statistical tests;
- An indication of the accuracy of the result through an estimation of measurement uncertainty;
- A decision as to whether the analysis carried out and the results obtained have satisfied the objectives set out in unit 1. If these have not been achieved, then a decision is required on what further work needs to be carried out.

Evaluation of data using statistical methodologies

This initial part of process unit 7 will focus on the handling of data that has resulted from quantitative analytical measurement. The analytical process was designed to produce data that represents the amount or concentration of an analyte in a sample, the sample being representative of the bulk. **Therefore the final result should reflect the amount or concentration of the analyte in the bulk**.

The values resulting from the data analysis step, should be reported appropriately with a certain level of **confidence**. The result is even more useful if there is some estimation of the **uncertainty** associated with the measurement.

How the data is reported, e.g. the number of **significant figures**, and the units, all contribute to the usefulness of the result.

In order to perform statistical calculations, the use of a hand held scientific calculator such as Casio or Sharp, and a spreadsheet such as Microsoft Excel will be useful.

Mean and standard deviation

- When the sample is taken to the lab it is divided up into test portions and thus only a small portion of the sample received by the laboratory is actually analysed.

- Multiple measurements or *replicates* are taken and a value for each of these is recorded. The **mean ($\bar{x}$)** of these values estimates the **true value (μ)**. The 'true' value may never be known, as there is always an element of **uncertainty** associated with the result.

- To obtain the result of the analysis, the sample may have been spiked with a known amount of analyte. A **certified reference material (CRM)** may have been used, or a consensus true value may have been agreed by a number of **accredited** laboratories.

- The **standard deviation** represents the spread of the data - it is an estimate of **precision**. Precision is a measure of random variation associated with a measurement or instrument. Where the **uncertainty** is systematic, we can say there is a **bias** in the results.

Calculating mean and standard deviation

The **mean** and **standard deviation** in analytical science are associated with samples. Where the entire population has been measured we refer to the mean and standard deviation as 'μ' and 'σ' respectively.

However when a sample (rather than the entire population) has been measured, we refer to the mean and standard deviation as '$\bar{x}$' and 's'.

- If each measurement is represented by 'x_i', and there are 'n' measurements, the mean is calculated by [note that the term '$\sum$' represents 'the sum of'']:

  $(\sum x_i) / n$ Equation (5.1)

- and s by:

  $\sqrt{[\sum(x_i - \bar{x})^2 / (n-1)]}$ Equation (5.2)

As hand-held calculators are readily available, it is advisable to learn to use the SD mode and use this to obtain the sample standard deviation. Of the two values offered, the sample standard deviation is often represented as σ_{n-1}. If in doubt this will be the larger of the two values.

Relative standard deviation and variance

Using standard deviation in isolation is not a useful measure of precision. For instance where the mean values for the analysis of a sample performed by two procedures are different, it can be difficult to estimate which procedure offers the better precision. To get a more meaningful result, it is preferable to relate the mean to the standard deviation to produce relative standard deviation (RSD) – equation (5.3)

$s / \bar{x}$ Equation (5.3)

There are cases, such as repeated measurements of peak height in a chromatographic analysis, where 's' can give a measure of an instrument's performance. The RSD may then be expressed as a percentage and is referred to as the coefficient of variation (CV) – equation (5.4):

$100 \times (s / \bar{x})$ Equation (5.4)

Variance (s^2) is a statistical term used to evaluate **measurement uncertainty** and later to compare precisions.

Continued on the next slide

Standard deviation can also be expressed as the standard deviation of the mean (SDM) – equation (5.5):

$s/\sqrt{n}$ Equation (5.5)

As more data is collected, the standard deviation of the means becomes smaller - proportional to n. This is a measure of dispersion of the means. It is also the correct way to express **standard uncertainty** (**see later**).

Table (5.1) below summarises the statistical terms introduced so far.

Term	Formula
Mean	$(\sum x_i)/n$
Standard deviation	$\sqrt{[\sum(\overline{x_i} - x)^2 /(n-1)]}$
Relative standard deviation	$s / \overline{x}$
Coefficient of variation	$100 (s / \overline{x})$
Variance	s^2
Standard deviation of the mean	$s/\sqrt{n}$

Table (5.1) statistical terms

Example (5.i)

Seven replicate samples of a prepared food were analysed for fat content. The results obtained were: 3.080, 3.094, 3.107, 3.056, 3.112, 3.174 & 3.198 % w/w. Calculate the standard deviation (s) and coefficient of variation (CV), of this set of data.

$s = \sqrt{[\sum(x_i - \bar{x})^2/(n-1)]}$ and $RSD = s/\bar{x} \times 100\ \%$

Sample number	Mass % w/w	$(x_i - \bar{x})$	$(x_i - \bar{x})^2$
1	3.080	- 0.037	0.00137
2	3.094	- 0.023	0.00053
3	3.107	- 0.010	0.00001
4	3.056	- 0.061	0.00372
5	3.112	- 0.005	0.000003
6	3.174	0.057	0.00325
7	3.198	0.081	0.00656
Mean	3.117		$\sum$ = 0.01544

$s = \sqrt{0.01544 / 6}$

$= \sqrt{0.00257}$

$= \mathbf{0.051}$

$CV = (0.051/3.117) \times 100$

$= \mathbf{1.63\ \%}$

Example (5.ii) – statistical calculations associated with the weighing of 7 tablets from a single batch

Weighing, or measuring mass is a common procedure in any analytical laboratory. Using a '4-figure' analytical balance, which has been previously calibrated, a sample of seven tablets were weighed. The following data represent the weight of the individual tablets:

X_i : 555.1mg, 556.2mg, 554.8mg, 557.1mg, 556.5mg, 554.7mg and 556.2mg.

Note: There are two sources of variation, the **actual variation** in the mass of the tablets, and the **uncertainty** associated with the measurement process. Typical total (expanded) uncertainty for a 4-figure analytical balance is $\pm$ 0.0004g. Nominal accuracy is 0.0001g (0.01%).

Calculate: $\bar{x}$, s, RSD, CV, SDM and variance for the sample of tablets

$\bar{x}$ = 3890.6/7 = **555.8 mg**
s = $\sqrt{}$ (5.2/6) = **0.93 mg**
RSD = 0.93/555.8 = **0.0017**
CV = RSD X 100 = **0.17**
SDM = 0.93/$\sqrt{}$7 = 0.93/2.64 = **0.35**
Variance = 0.93^2 = **0.87**

The number of significant figures is the number required to express the result consistent with the precision. Quote all certain figures, and the first uncertain one. In this case, given the accuracy, the fourth digit is the first uncertain one.

Note: If the number of tablets taken had been large enough to constitute a 'population' (50+), then the standard deviation would be represented by 'σ' to give a value of 0.86 mg [(n – 1) replaced by 'n']

Confidence limits

- In all analytical measurements there is only one 'true' answer μ, and all values obtained are spread around this value. In the absence of any **bias**, or **systematic error**, the values, x_i, will be randomly clustered around μ. If x_i ,on the 'x' axis, is plotted against the frequency of its occurrence, on the 'y' axis, a normal or Gaussian distribution is obtained as shown in figure (5.1)

- The reason for making replicate measurements is to estimate, with the highest degree of confidence possible, the 'true' answer. As has been shown, the more measurements made the 'better' or more reliable the answer. As the frequency plot, or histogram, is prepared, and more data is plotted the curve 'smoothes out' to its final bell shape – see figure (5.1)

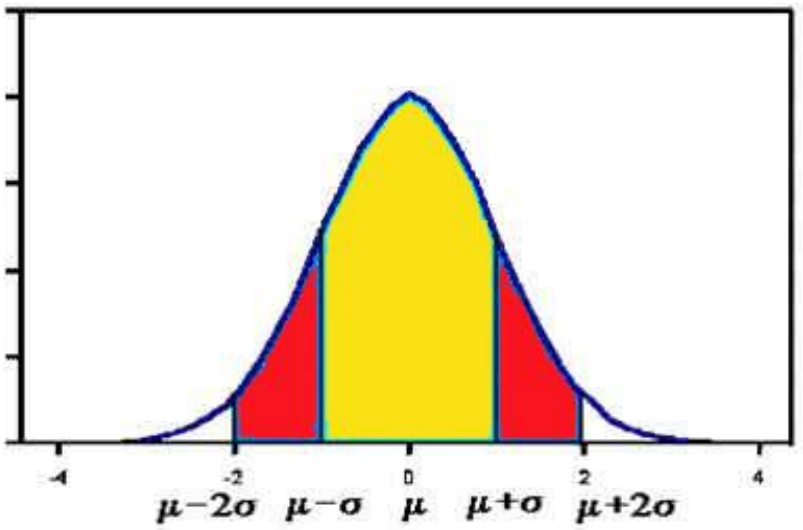


Figure 5.1 – typical statistical histogram

Confidence

A degree of confidence implies a degree of **probability**. Confidence limits define a range within which one may reasonably assume the true value lies. This assumes random variation only. The probability that the true value lies within the range defined can be expressed as a %.

Where there is a normal distribution of data and an infinite number of measurements, 68.3% of the values lie between $\pm$ 1 standard deviation, 95.4% between $\pm$ 2 standard deviations and 99.7% between $\pm$ 3standard deviations.

In a realistic analytical experiment, only a small number of measurements are made, the confidence limits can be described by:

$[\ \bar{x} + t_{n-1}\ s/\sqrt{n}\ ]$ Equation (5.6)

Where (n-1) refers to **degrees of freedom** (υ), the number of independent deviations used to calculate s. **For every mean calculated, the number of degrees of freedom is reduced by 1**. The value of 't' (**see next slide**), also depends on the level of confidence required. For a large sample size at an confidence of 95%, 't' is close to 2 (1.96), for smaller sample sizes, the value of 't' may be obtained from 't test tables'.

In other words 95% of sample means lie between $\mu \pm 1.96\ \sigma/\sqrt{n}$.

‘t’-tables

- The confidence limits are the product of the standard deviation of the mean, $s/\sqrt{n}$ and ‘t’.
- As the level of confidence increases, so does ‘t’, widening the range in which it is probable that the true value lies.
- As ‘n’ increases, both ‘t’ and $s/\sqrt{n}$ decrease. The more measurements made the more confident the analyst is that the range defined will include the true value.
- ‘t’ tables or Students-t distribution is mathematically defined by a probability density function beyond the scope of this unit. The data is tabulated in a form convenient for analysts using relatively small (<30) samples.

A ‘t’ table can be found in most textbooks of Analytical Chemistry and on the following website: http://en.wikipedia.org/wiki/Student%27s_t-distribution

Example (5.iii) - on the use of the 't' tables

The data previously given in example (xx) will be used:

X_i : 555.1 mg, 556.2 mg, 554.8 mg, 557.1 mg, 556.5 mg, 554.7 mg and 556.2 mg.

$\overline{x}$ = 555.8 mg

n = 7
s = 0.93
υ = 6 (n-1)
t_υ, at 95% confidence = 2.447

By using the formula: [t_υ.s/$\sqrt{n}$], we can now calculate the interval surrounding the mean value, where we are 95% confident that the true mean will lie:

[2.447 X 0.93/2.65] = 0.86

Thus, the confidence limits at 95% are expressed: 555.8 $\pm$ 0.86 mg

The analyst is 95% certain, that the true value lies in the range 556.7 to 554.9 mg.

Confidence limits can also test for bias, or systematic error.

Outliers

An outlier refers to any piece of data that appears to be outside the normal data set. At the outset it may be so different as to be obvious that it does not fit with the other data points in the set. However there are occasions when this conclusion is less obvious. Under these circumstances, it is necessary to apply an approved statistical test, such as the Dixon's Q test which is considered on the next slide.

When collecting raw data, it is up to the analyst to ensure the integrity of that data. This is usually achieved by recording the data, in ink, carefully in a lab notebook, or attaching the read-out from an instrument. Once this data is recorded it must never be erased or obscured. Data initially recorded incorrectly is simply scored through and the replacement data entered. In accredited laboratories, or with the use of automated data collection, data cannot be changed without applying an agreed protocol.

Once collected, data should not be discarded without good reason. Often a note is made in the notebook explaining the reason for changing data, e.g. it may have been transposed, or the analyst may have been interrupted. If an error was made during the analysis, e.g. an overfilled flask, then the measurement should have been abandoned and the data not collected in the first place.

Continued on the next slide

Once recorded, any piece of data that appears to be outside the normal data set, should be tested to see if it is an outlier, and if so, then removed and the reason for removal stated in the lab notebook.

It is good practise to append all calculations and keep any rejected values in the data set, but with an explanation as to why they are not included in the data analysis.

Dixons Q test

- A simple test for outliers is the Dixons Q test. Once identified the suspect point is tested:
- Q_{calc} = [suspect value - nearest value]/[largest value - smallest value] [equation (5.7)]
- Note all values are included until they are formally rejected.
- The calculated statistic, is then compared to a published critical value, **see Table (5.2) on the next slide**. If the calculated value is greater than the critical value then the data point is rejected, and no longer included in the data analysis.
- There are situations where more than one test of an outlier (e.g. Grubbs test) should be applied, but Dixons Q test is sufficient for most applications.

Number of values:	3	4	5	6	7	8	9	10
$Q_{90\%}$:	0.941	0.765	0.642	0.560	0.507	0.468	0.437	0.412
$Q_{95\%}$:	0.970	0.829	0.710	0.625	0.568	0.526	0.493	0.466
$Q_{99\%}$:	0.994	0.926	0.821	0.740	0.680	0.634	0.598	0.568

Table 5.2 – critical values of Q at 3 confidence levels

Example (5.iv) – use of the Dixon's test for outliers

In an experiment to determine the concentration of glucose in a sample the following data were obtained:

X_i : 0.48 mM, 0.46 mM, 0.48mM, 0.47mM, 0.47 mM, 0.54 mM.

The most obvious suspect value is 0.54 mM.

Q calc = [0.54 - 0.47]/[0.54 - 0.46] = **0.875**

From the tables for a sample size of 6, Q crit = **0.625** at 95% confidence level.

As Q calc exceeds Q crit, the data point can be rejected.

Significance tests

A significance test establishes if two statistics are the same or if they are significantly different. As with the Q test for outliers a calculated value is compared to a critical value, usually obtained from tables. If the calculated value exceeds the critical value, the difference is deemed to be significant. Examples of where this test could be applied are:

- Comparing the mean of a set of data with the ‘true’ value, could establish if there is a bias in the result.
- The means of two methods could be compared to establish if there is a significant difference between the methods.
- Comparing variance can establish if the precision obtained by one method or instrument is significantly better than that obtained by an alternative technique.

In each case, the statistical test is just one step in a process.

The t-test

A t-test is used for comparing two means. If the means are the same then we can say (rather obviously) there is no difference. In other words the difference $\Delta \overline{x}$, or $(\overline{x}_1 - \overline{x}_2) = 0$.

The t-test is used, to test whether $\Delta \overline{x}$ is significantly different from zero. The value of 't_{calc}' is calculated using the formula – equation (5.8):

$$t_{calc} = (\overline{x}_1 - \overline{x}_2) / s(\sqrt{(1/n_1 + 1/n_2)} \qquad \text{Equation (5.8)}$$

and compared to that of 't_{crit}' at the 95% confidence level. If the calculated value exceeds the critical, then there is a significant difference between the means.

As there is a standard deviation associated with each data set, the standard deviation in this formula is a pooled standard deviation obtained by:

$$S^2 = [(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2] / (n_1 + n_2 - 2) \qquad \text{Equation (5.9)}$$

Note: There are $(n_1 + n_2 - 2)$ degrees of freedom as two means were drawn from the total data set.

Example (5.v) – a 't' test to compare two means

Two methods for the determination of antimony in the atmosphere were compared, and the data shown in table (5.3) was obtained

From this data the following calculations were obtained:

$\overline{x}_1 = 19.98$ mg/m^3
$S_1 = 3.14$ mg/m^3
$\overline{x}_2 = 18.8$ mg/m^3
$S_2 = 2.61$ mg/m^3

Pooled standard deviation = 2.89
't_{calc}' = 0.708
't_{crit}' = 2.23 at 95%

Thus there is deemed to be no significant difference between the two means

Sample no	Standard mg/m^3	Proposed mg/m^3
1	25	22.2
2	19.5	19.2
3	16.6	15.7
4	21.3	20.4
5	20.7	19.6
6	16.8	15.7

Table 5.3

Example (5.vi) - a t-test to compare a mean with a known value

Where the mean of a set of data is being compared with a true value, μ, a t-test is used. The 't_{calc}' value is obtained from equation (5.10):

$(\bar{x}-\mu)(\sqrt{n}/s)$ Equation (5.10)

In this case there is only one value for standard deviation, that associated with the measured value. The true value may have been a quoted value, a consensus true value from a number of labs or the value from a certified reference material, CRM.

From the previous example (5.v), the proposed method is compared to the 'true value', μ = 20 mg/m^3. Is 18.8 mg/m^3, significantly different to 20 mg/m^3 in this case?

$t_{calc} = (18.8 - 20)/(\sqrt{6}/2.61) = 1.27$ [Note: the negative sign is ignored]
t_{crit} (at 95%) = 2.57

Thus, as the calculated value is again lower than the critical value at the 95% Confidence level, the proposed method produces a result that is not significantly different from the true value.

Comparing standard deviations, the F-test.

Comparing the standard deviations of sets of data is carried out by the F-test. The statistic, F_{calc} is compared to F_{crit}, obtained from tables. This test uses a comparison of the two variances from the methods and the calculation is given by equation (5.11):

$$F_{calc} = s_1^2/s_2^2 \qquad \text{Equation (5.11)}$$

Where the variances are chosen such that F_{calc} will always be greater or equal to 1.

If the comparison is being made to establish if the precision of one method is better than another, then a **one-tailed** test is used. If the test is to ascertain whether there is a significantly different between the two standard deviations, a **two tailed** test is used.

An analogy

To remember whether to use a **one** or a **two** tailed test, think of crossing the road. If you have some information and are testing if one method is significantly better, then look one way. If you have not made any assumptions about the data and are simply looking for a significant difference, then look both ways.

The F-tables

For a one-tailed test at 95% confidence, see Table (5.4) on the next slide.
Other tables at different confidence levels, can be found in specialist textbooks on statistical methods.

When comparing two standard deviations to see if they are significantly different, it is necessary to use the one-tailed F-tables.
The set with the larger of the two variances is read horizontally and that with the lower variance is read vertically. From this it is possible to read the value for F_{crit}. The table shown as table (5.4) on the next slide illustrates how an F-test table is to be applied.

Select the column corresponding to the degrees of freedom, υ for data set 1 from the top row of the tables, then drop down that column until you reach the row corresponding to υ for data set 2, and read the value for Fcrit.
When comparing two precisions to determine if one is significantly better than the other, an assumption is being tested, so a one tailed test is applied in this situation.

$\upsilon_1 = n_1 - 1$ → $\upsilon_2 = n_2 - 1$ ↓	2	3	4	5	6	7	8	9	10
2	19.00	19.16	19.25	19.30	19.33	19.35	19.37	19.39	19.40
3	9.55	9.28	9.12	9.01	8.94	8.89	8.85	8.81	8.79
4	6.94	6.59	6.39	6.26	6.16	6.09	6.04	6.00	5.96
5	5.79	5.41	5.19	5.05	4.95	4.88	4.82	4.77	4.74
6	5.14	4.76	4.53	4.39	4.28	4.21	4.15	4.10	4.06
7	4.74	4.35	4.12	3.97	3.87	3.79	3.73	3.68	3.64
8	4.46	4.07	3.84	3.69	3.58	3.50	3.44	3.39	3.35
9	4.26	3.86	3.63	3.48	3.37	3.29	3.23	3.18	3.14
10	4.10	3.71	3.48	3.33	3.22	3.14	3.07	3.02	2.98

Table 5.4 - critical F-values for 95% confidence level

Note: the figures in red relate to example (5.vii) on the next slide

Example (5.vii) of an F-test to compare precisions

Returning to the data used in example (5.v) which is shown again in the table on the right. The F-test can be used to compare the precisions between the two methods.

Standard Method :

$\bar{x}_1$ = 19.98 mg/m^3,

S_1 = 3.14 mg/m^3

S_1^2 = 9.86

Proposed Method:

$\bar{x}_2$ = 18.8 mg/m^3,

S_2 = 2.61 mg/m^3

S_2^2 = 6.81

Sample no	Standard mg/m^3	Proposed mg/m^3
1	25	22.2
2	19.5	19.2
3	16.6	15.7
4	21.3	20.4
5	20.7	19.6
6	16.8	15.7

n1 and n2 = 6

υ1 and υ2 = 5

F_{calc} = 9.86/6.81 = 1.45

F_{crit} = 5.05

As we are testing to see if the precision of the standard method is superior to that of proposed method, then a one tailed test may be employed

Thus as the calculated value is less than the critical value, there is no significant difference between the two precisions of the two procedures.

Example (5.viii) - a t-test to test if means are significantly different

In 1904 Lord Rayleigh won the Noble prize for the discovery of argon. He was measuring the mass of a gas by two different methods, he noticed a discrepancy in the two sets of data, tested it, found it to be significant. If he had not known his expected measurement uncertainty, then the discrepancy may have been attributed to experimental error, and the discovery of argon delayed. The table gives the data for the weight of gas, after removal of oxygen by two different methods, test to see if the two data sets are significantly different.

From air (g)	From chemical decomp.(g)
2.31017	2.30143
2.30986	2.29890
2.31010	2.29816
2.31001	2.30182
2.31024	2.29869
2.31010	2.29940
2.31028	2.29849
	2.29889

For the gas from air:
Mean = 2.31011
Std dev = 1.426×10^{-4}
n = 7, $\upsilon = 6$

For the gas from chemical decomposition:
Mean = 2.29947
Std Dev = 1.379×10^{-3}
n = 8, $\upsilon = 7$

Pooled standard deviation, S = 1.016×10^{-3}

t calc = $[(2.31011 - 2.29947)/(1.016 \times 10^{-3} \times \sqrt{(1/7+1/8)}]$
= **20.25**

t crit = 2.16 (υ =13, 95%)

The means are significantly different. Rayleigh then correctly concluded there was something else in the oxygen free air, not present in the chemically deoxygenated system.

Summary of equations & tests

Test/statistic	Equation
Confidence	$[\bar{x} + t_{n-1}\, s/\sqrt{n}\,]$
Dixons Q test	Q_{calc} = [suspect value - nearest value] / [largest value - smallest value]
't'-test	$t_{calc} = (\bar{x}_1 - \bar{x}_2) / s(\sqrt{(1/n_1+1/n_2)}$
Pooled standard deviation	$S^2 = [(n_1-1)s_1^2 + (n_2-1)s_2^2] /(n_1+n_2-2)$
F-test	$F_{calc} = s_1^2/s_2^2$

Table 5.5– summary of statistical equations & tests

Statistics and calibration curves

The straight line is used regularly in analytical science, one of the more common applications being to determine the concentration of an unknown from a series of standards. This is referred to as the **calibration curve** and will be explained more fully in this section. [**also refer Chapter 4 in this teaching and learning programme**]

Where there are complex matrix effects for example, direct comparison with separate standards may not be applicable, in this case the method of multiple standard additions may be used. In this example the value of the **intercept** is calculated. [**Again refer to Chapter 4**]

Sometimes a value for the **slope** is required, for example when determining molar absorptivity (or molar absorption coefficient) from a Beer-Lambert plot. [**please refer to Chapter 10 of this teaching and learning programme**]

All of the above require a robust statistical method capable of determining the required statistics, with a certain degree of confidence.

Continued on the next slide

Calibration curves

To prepare a calibration curve, data is collected and evaluated in a particular way. Instead of carrying out replicate analysis of a single measurement, measurements are taken over a wide concentration range.

A minimum of 4 calibration standard solutions are prepared. These standards are measured under **exactly** the same conditions as the unknown solution of analyte. A graph is established and the concentrations of unknown samples can be determined. The following questions about the calibration may then be posed

- Is the graph linear?
- What is best straight line (consider error/uncertainty)?
- What is the uncertainty associated with the slope and intercept?
- What is the uncertainty associated the determination of the unknown sample?
- Can we use the data to determine the **Limit of Detection**, LOD for the method?

Preparation of a calibration curve

Please refer to Chapter 4 of this teaching & learning programme

The standards used to prepare calibration curves should “bracket” the unknown, except when using the method of standard additions. Always include a blank. The blank itself is subject to error and therefore should not normally be subtracted from the calibration standards.

The standards are measured, and a direct measurement of response, or the ratio of the response and an appropriate internal standard, is recorded. Instrument response is plotted on the vertical, ‘y axis’, standard concentrations are plotted on the horizontal, ‘x axis’. As the analyst controls the preparation of the standard solutions, assuming that there is no gross error on behalf of the analyst, the uncertainty is assumed to be associated with the instrument response.

When the series of standards have been measured, the plot is constructed as described above. It is always good practise to graphically plot the data and visually inspect it prior to further data analysis.

Continued on the next slide

The regression carried out is referred to as the regression of ‘y on x’, ‘y’ being the independent variable. A linear equation is generally expressed algebraically by the equation:

$y = mx + C$

Using the Linear Regression mode of a hand held calculator will allow **regression** or **correlation coefficient, r** to be calculated. The slope, m, and the intercept,C. It will also allow the calculation of any value of x for a given value of y, and *vice versa*.

Excel, or similar programs can carry out regression calculations as well as plot the data and provide **residual plots**.

It is also possible to prepare a spreadsheet which can be customised to give all of the above information including confidence limits for slope, intercept and determination of unknown, and an estimate of **limit of detection.**

Testing for linearity

The correlation or regression coefficient, r, establishes if there is a linear relationship between the variables x_i and y_i.

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

Equation (5.12)

For a perfect positive correlation, the value for r of +1 or -1 represents a perfect negative correlation. As most analysis suffer from some degree of random variation, values close to 1 are acceptable. Significance tests can be carried out to establish if r is significantly different from 1, but are beyond the scope of this unit.

An r value close to 0 means x and y are not linearly related, but the plot should be inspected for some non-linear correlation.

Example (5.ix)

Riboflavin (vitamin B2) is determined in a cereal sample by fluorescence (see Chapter 11) in 5% acetic acid solution. A calibration curve was prepared by measuring the fluorescence intensities of a series of standards. The data obtained is tabulated and shown graphically below. Show that there is a good linear relationship between the data points. Although this would normally be achieved by using a simple computer or calculator programme, the calculation shown partly as table (5.6) on the next slide shows how the result is achieved.

Riboflavin ($\mu g/cm^3$)	Fluorescence (Units of Intensity)
0.0	0.0
0.1	5.8
0.2	12.2
0.4	22.3
0.8	43.3

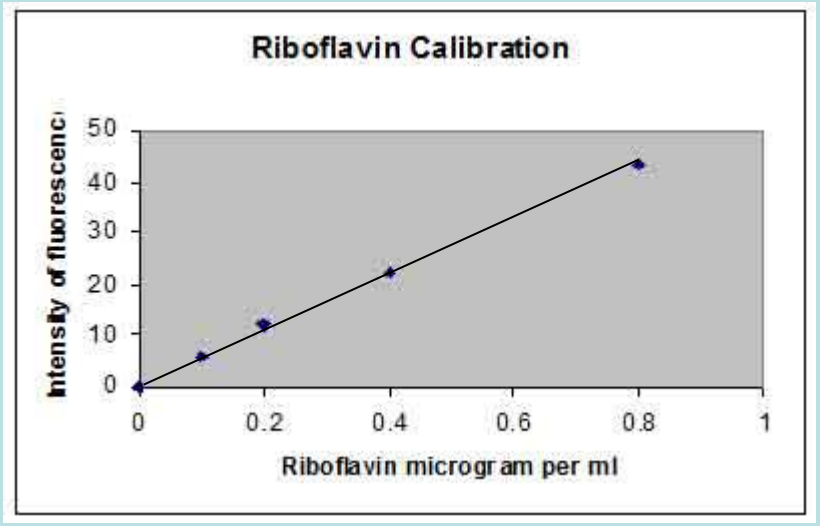


Figure 5.2

X_i	$(x_i - \bar{x})$	$(x_i - \bar{x})^2$	Yi	$(y_i - \bar{y})$	$(y_i - \bar{y})^2$	$(x_i - \bar{x})(y_i - \bar{y})$
0	-0.3	0.09	0	-16.72	279.56	5.016
0.1	-0.2	0.04	5.8	-10.92	119.25	2.184
0.2	-0.1	0.01	12.2	-4.52	20.43	0.452
0.4	0.1	0.01	22.3	5.58	31.36	0.558
0.8	0.5	0.25	43.3	26.58	706.50	13.29
Σ=1.5		Σ=0.40	Σ=83.6		Σ=1157.10	Σ=21.5
$\bar{x}$ = 0.3			$\bar{y}$=16.72			

Table 5.6 – calculation of correlation coefficient

$$r = \frac{\sum_i \{(x_i - \bar{x})(y_i - \bar{y})\}}{\{[\sum_i (x_i - \bar{x})^2][\sum_i (y_i - \bar{y})^2]\}^{\frac{1}{2}}} = \frac{21.5}{\sqrt{(0.40)(1157.1)}} = \mathbf{0.9995}$$

The value of ‘r’ is very close to 1 so there is a good linear relationship between signal and concentration.

Residual plots

In the example (5.ix) both a visual inspection of the plot, and r suggest a very good linear correlation. An alternative technique is to plot the **residuals**.

When the 'best' line has been established it will pass through all the points described by 'x'. However, a new set of 'y' values will have been described. These are referred to as 'fitted' y values, $\hat{y}$. To construct a residual plot use the equation of the line to determine $\hat{y}$ for each value of x. Plot the difference, ($\hat{y}$ - y) against x. For the calibration of riboflavin as shown in figure (5.2), the residual is shown in figure (5.3):

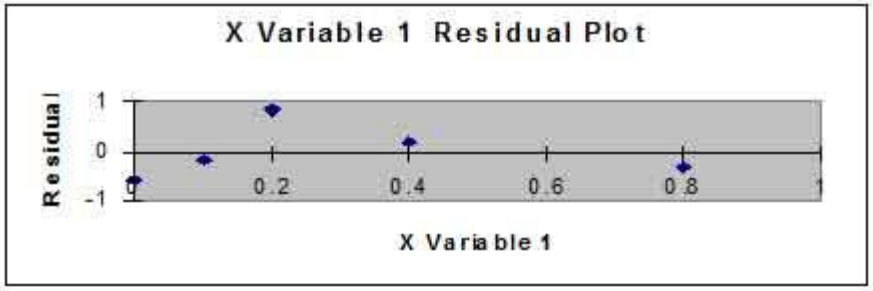


Figure 5.3 - residual plot

Continued on the next slide

Interpreting residual plots

A horizontal line is a perfect linear correlation. As most lines have some random error, a 'normal' residual plot for a straight line will have random scatter about the x axis. As is the case with the example illustrated in figure (5.3).

The main characteristics of a residuals plot are:

- A curved plot implies a non-linear correlation, see examples on the next slide.
- Residuals that increase or decrease with x indicate a 'non-constant' variance.
- Large individual residuals are probably outliers, which can be eliminated by methods beyond the scope of this unit.

Residual plot for a plateau

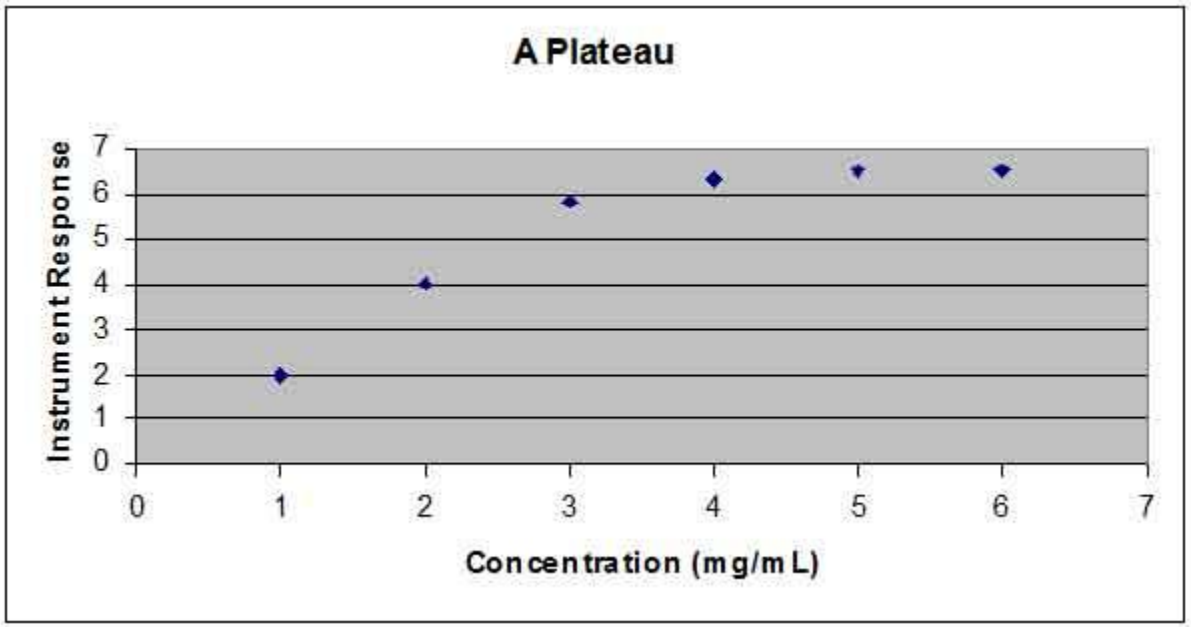


Figure 5.4 - a calibration plot showing a plateau region

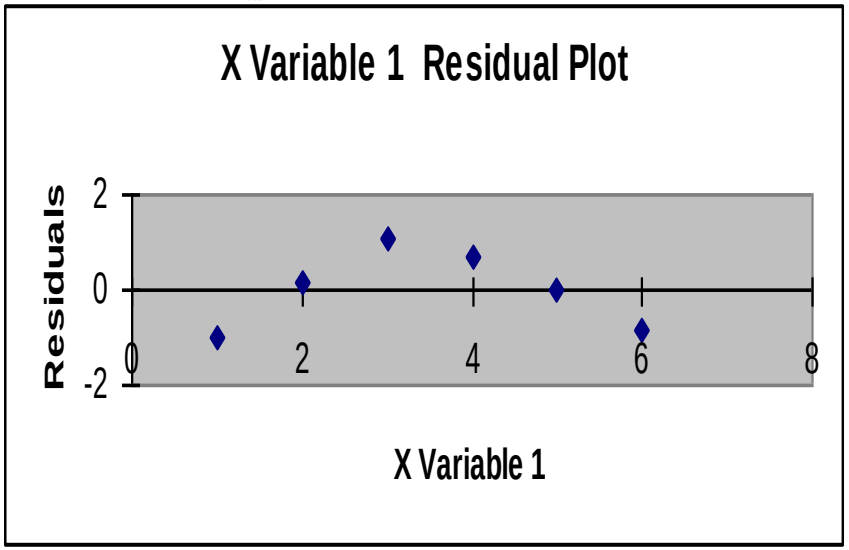


Figure 5.5 - residual plot for a calibration graph containing a plateau region

Residual plots for a curve

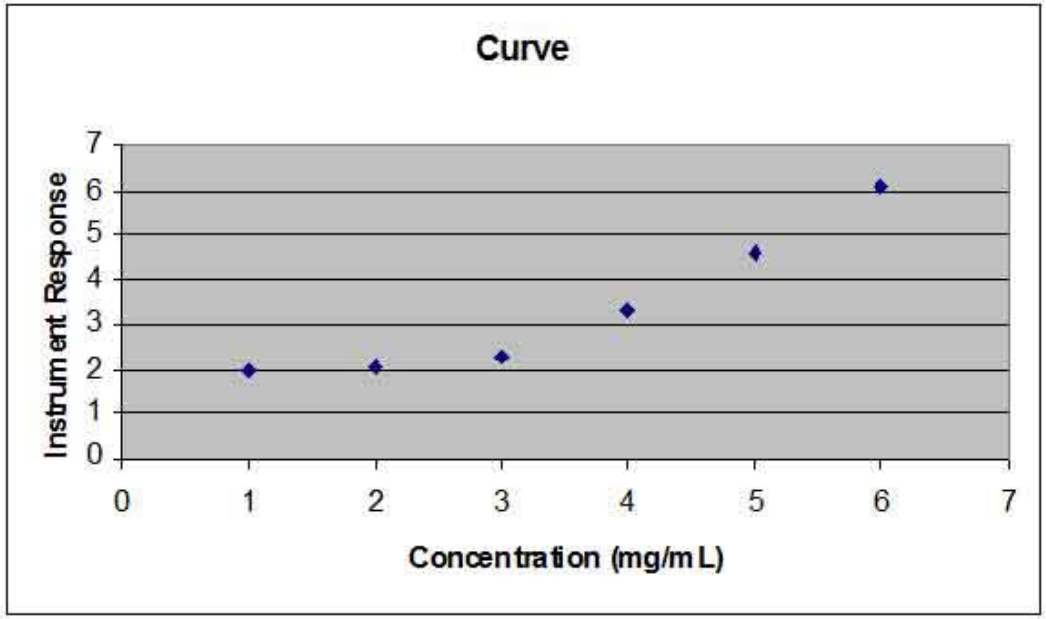
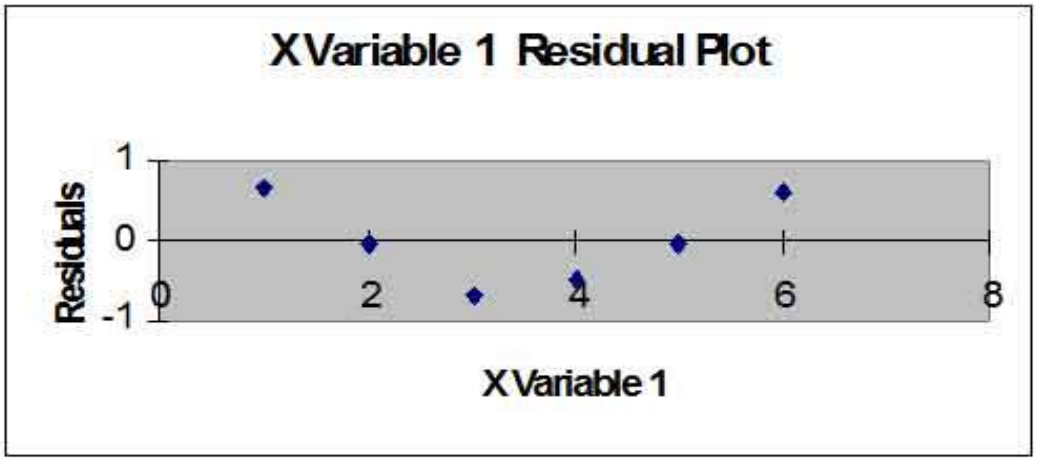


Figure 5.6 - curved calibration plot

Figure 5.7 - residual plot for a curved calibration plot



Determining the slope

As can be seen from the preceding examples, the best straight line is the line that minimises the residuals. It is actually attempting to minimise sum of squares of residuals, and is sometimes referred to as the method of least squares.

To calculate the line of regression of 'y' on 'x' use the formula:

$$b = \frac{\sum_i \{(x_i - \bar{x})(y_i - \bar{y})\}}{\sum_i (x_i - \bar{x})^2}$$ Equation (5.13)

and:

$$a = \bar{y} - b\,\bar{x}$$ Equation (5.14)

Remember 'a' is the intercept and 'b' is the slope in the [y = bx + a] equation

‘Errors’ in the slope and intercept

To determine the uncertainty in the slope and intercept a statistic similar to ‘s’ is calculated:

$$s_{\frac{y}{x}} = \left\{ \frac{\sum_i (y_i - \hat{y}_i)^2}{n-2} \right\}^{1/2}$$

Equation (5.15)

Where the $\hat{y}_i$ values are the points on the calibrated regression line corresponding to the individual ‘x’ values

Then the standard deviation of the slope can be calculated:

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

Equation (5.16)

And the standard deviation for the intercept:

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

Equation (5.17)

Confidence limits for slope and intercept

Having calculated values of standard deviations for slope and intercept, confidence limits can be calculated in the usual way:

Slope: $b \pm t\, s_b / \sqrt{n}$

Intercept: $a \pm t\, s_a / \sqrt{n}$ Equation (5.6)

Where t is taken at desired confidence level and n-2 degrees of freedom. [Note: n-2, because two averages have been calculated from the data – average of both 'x' and 'y' values.]

Both the slope and intercept are used to determine the 'unknown'. If an unknown sample containing x_0, gives an instrument response of y_0 and 'm' replicates were measured, then:

$$s_{xo} = \frac{s_{y/x}}{b}\left\{\frac{1}{m} + \frac{1}{n} + \frac{(y_o - \bar{y})^2}{b^2 \sum_i (x_i - \bar{x})^2}\right\}^{\frac{1}{2}}$$ Equation (5.18)

Summary of equations used in calibration data

Test / statistic	Equation
Testing for linearity	$r = \dfrac{\sum_i \{(x_i - \bar{x})(y_i - \bar{y})\}}{\{[\sum_i (x_i - \bar{x})^2][\sum_i (y_i - \bar{y})^2]\}^{\frac{1}{2}}}$
Determining the slope	$a = \bar{y} - b\,\bar{x}$ $b = \dfrac{\sum_i \{(x_i - \bar{x})(y_i - \bar{y})\}}{\sum_i (x_i - \bar{x})^2}$
Errors in the slope & intercept	$s_{\frac{y}{x}} = \left\{\dfrac{\sum_i (y_i - \hat{y}_i)^2}{n-2}\right\}^{1/2}$ $s_b = \dfrac{s_{y/x}}{\left\{\sum_i (x_i - \bar{x})^2\right\}^{\frac{1}{2}}}$ $s_a = s_{y/x}\left\{\dfrac{\sum_i x_i^2}{n\sum_i (x_i - \bar{x})^2}\right\}^{\frac{1}{2}}$
Confidence limits for slope & intercept	$s_{xo} = \dfrac{s_{y/x}}{b}\left\{\dfrac{1}{m} + \dfrac{1}{n} + \dfrac{(y_o - \bar{y})^2}{b^2 \sum_i (x_i - \bar{x})^2}\right\}^{\frac{1}{2}}$

Confidence limits for an ‘unknown’

From equation (5.18) it can be shown that the confidence limits decrease as we approach the mid point of the calibration plot (median) and thus the closer we are to this central point, the more reliable the result obtained is going to be. Thus to narrow the confidence limits (y_o approaches y) we should:

- Increase the number of calibration points (increase ‘n’)
- Take replicate measurements of unknown (increase ‘m’)

A graphical representation of the relationship between a calibration plot and confidence limits is shown In figure (5.8)

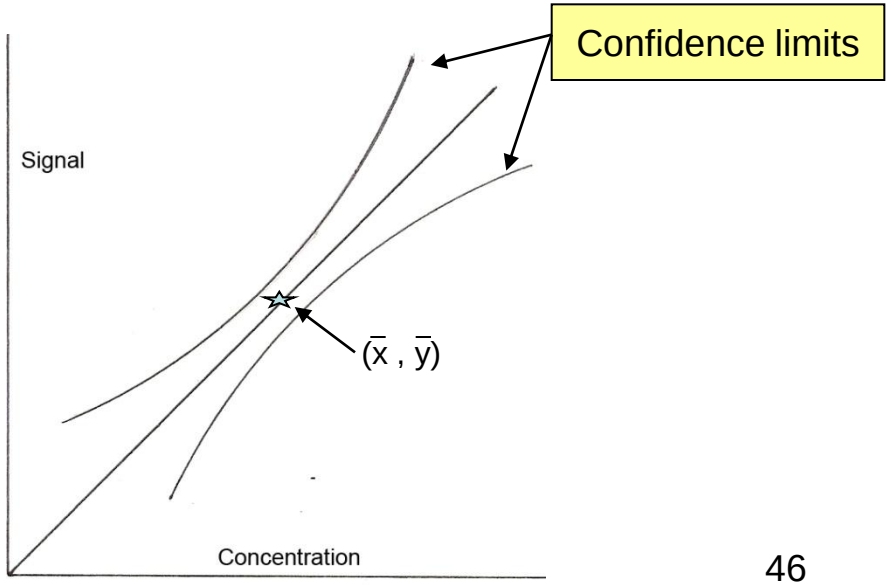


Figure 5.8 – confidence limits for a calibration plot

Limit of detection and quantitation

For an instrumental method, where the response is linear, the **limits of detection and quantitation** can be estimated using the values obtained from linear regression.

The **limit of detection** is the analyte concentration that gives a signal **significantly** different to that of the blank/background. The limit of **quantitation** is defined as the lower limit for precise quantitative measurement

Mathematically, these are represented as:

LOD $y = y_B + 3s_B$ Equation (5.19)
LOQ $y = y_B + 10s_B$ Equation (5.20)

Where y_B is given by *a,* the intercept and s_B, is the standard deviation of the blank. The value of s_B may be obtained by either measuring the blank several times which is time-consuming, or by using the $s_{y/x}$ statistic as given by equation (5.15). This gives a value of y which can then be used to calculate 'x', the concentration.

The method of standard additions

Please refer to Process Unit 6 in Chapter 4 of the teaching and learning programme

In most instrumental methods using calibration, a series of standards are prepared and compared to the unknown. There are situations however where matrix effects can interfere with the analysis and thus the method of standard additions becomes the method of choice. For instance, it may be difficult to extract a sample from the matrix. In this case known amounts of analyte can be added to the sample and matrix and the response measured. The method of standard additions is used routinely in atomic spectrometry and electrochemical analysis to avoid matrix effects.

Equal volumes of sample solution are taken and are spiked with known amounts of analyte. Then **ALL** solutions are diluted to the same volume.

The resultant data is plotted on a graph, with the instrument response on the 'y' axis and the amount added on the 'x' axis.

To calculate sample concentrations from standard additions

To “calculate” the amount (or concentration) of analyte extrapolate to y = 0. This intercept, of the x axis, can be estimated by intercept/slope:

a/b

Both a and b are subject to uncertainty, therefore a standard deviation associated with the extrapolated value of x, ‘x_E’, can be calculated:

$$s_{xe} = \frac{s_{y/x}}{b}\left\{\frac{1}{n} + \frac{\bar{y}^2}{b^2\sum_i (x_i - \bar{x})^2}\right\}^{\frac{1}{2}}$$

Equation (5.21)

Confidence for x_E:

$x_E \pm ts_{xe}/\sqrt{n}$

Reflection on the construction of calibration graphs

Some final rules for the construction of successful calibration curves:

- Calibration curves should bracket the unknown, this is because there is less variation toward the centre of the line, the error is greatest at the extremes, extrapolated values will have larger uncertainties, they may also be outside of the linear range.

- The intercept is a value obtained from an extremity, there will be larger uncertainties associated with it than for a sample ‘read’ from the centre of the graph.

- As the method of standard additions depends on an extrapolated intercept, it will have greater uncertainty than other methods, and should only be used when there is no other method available.

Measurement uncertainty

The term ‘ **uncertainty**’ means doubt and thus in its broadest sense, the uncertainty of a measurement means doubt about the validity of the result.

ISO definition of **measurement uncertainty**
A parameter associated with the results of a measurement that characterises the dispersion of the values that could reasonably be attributed to the measureand (that which is being measured)

The uncertainty of a measurement comprises in general many components associated with the overall analysis and is in effect a statistical value associated with all of the possible errors which could conceivably occur during an analysis.

The essential difference between **uncertainty** and **confidence limits**, is that the latter refers only to the final measurement. They take no account of any of the other parts of the overall method by which the analysis was carried out.

Measurement uncertainty is expressed as a bar on either side of the measured value, giving a band of values within which there is 95% confidence that the real values lies

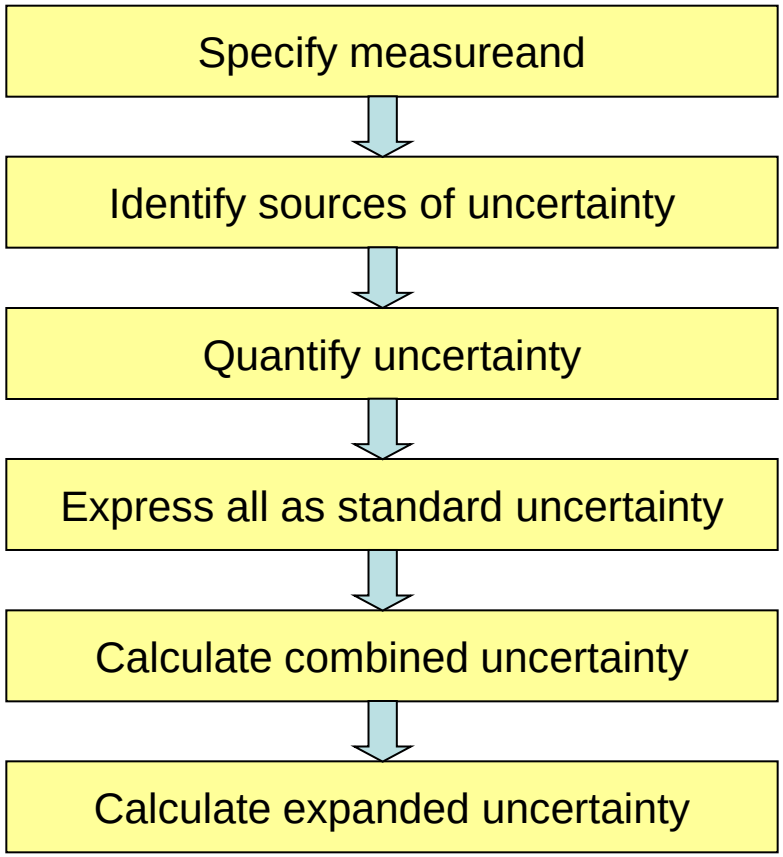
Example (5.x) - the error components associated with a simple acid-base titration

All parts of the procedure will affect the total uncertainty associated with the result, but some will have more effects than others. The potential sources of uncertainty are:

- sampling – was the sample homogeneous?
- matrix effects and interferences;
- loss of sample during transfer;
- standardisation of the titrant;
- uncertaintities in weights and volumes through the equipment used;
- personal bias in the reading of analogue instruments/glass apparatus.

Calculating uncertainty

To calculate measurement uncertainty of a method, all sources of uncertainty must be combined, this is sometimes referred to as **propagation of error**. Figure (5.9) shows the stages in the calculation process.



Each contribution to the uncertainty of a method is referred to as an **uncertainty component**. When they are expressed as a standard deviation, they are referred to as a **standard uncertainty**. For the result of a measurement, the total uncertainty is referred to as **combined standard uncertainty**. Finally, the **expanded uncertainty** is calculated by multiplying the combined uncertainty by a coverage factor.

These terms are explained on the following 5 slides

Figure 5.9 - stages in the estimation of measurement uncertainty

Expressing uncertainty

Standard uncertainty [u(y)] - is the standard deviation of an uncertainty component. Note: In some cases there is a correlation between uncertainty components necessitating the calculation of covariance.

Combined standard uncertainty [$u_c(y)$] – estimated standard deviation equal to the +ve square root of the total variance obtained by combining all variances and covariances.

Expanded uncertainty [U] – provides a range of values within which the true value is believed to lie. U is obtained by multiplying the $u_c y$ value by a coverage factor 'k'. The value of 'k' is dependent upon the level of confidence required. For 95% confidence, the value of 'k' is 2 [actually 1.96 from the 't' distribution table]

Estimating uncertainty

Figure (5.9) on slide 52 highlighted the stages in the estimation process

- **Specification** – write down a clear statement of what is being measured including measurement quantities, constants, calibration standards etc.

- **Identify sources of uncertainty** – list all possible sources

- **Quantify uncertainty** – measure or estimate the size of the uncertainty associated with each potential source of uncertainty. Not all of the components will have a significant effect on the combined uncertainty and those having a minor effect may be disregarded. If the level of uncertainty varies with the quantity then this will need to be taken into account. Individual components may be estimated in a number of ways. For instance:
  - experimental work carried out in the laboratory;
  - analysis of reference materials;
  - utilisation of published data;
  - judgement of the analyst.

Continued on the next slide

Expressing standard uncertainties – all uncertainty components must be expressed as standard uncertainties. This may be achieved in a number of ways.

- Where a value was obtained experimentally, it is possible to express this as a standard deviation;
- Where an uncertainty is obtained from previous results, it may already be in the form of a standard deviation. However if a confidence interval is given then a calculation will need to be performed.

Example (5.xi) – a balance reading is quoted as ± 0.1 mg at 95% confidence.
From statistical tables, 95% confidence refers to 1.96. Thus the SD is 0.1/1.96 = 0.05 mg

- Where no confidence interval is provided, then it is usual to assume a rectangular distribution with a SD of $x/\sqrt{3}$

Example (5.xii) – a grade A 25.0 cm^3 pipette is certified to deliver 25.0 ± 0.2 cm^3.
The standard uncertainty is thus $0.2/\sqrt{3} = 0.11$ cm^3

- Where an estimate is made on judgement, it may be possible to estimate directly as a SD. If this is not possible assume a rectangular distribution

Continued on the next slide

Calculating the combined uncertainty – there are three rules that normally apply for combining together the individual standard deviations.

- **Rule 1** – applies when considering only the sum or differences of quantities. For instance: $y = a + b + c$. The combined uncertainty is then given by:

$$u_c(y) = \sqrt{[u(a)^2 + u(b)^2 + u(c)^2]} \qquad \text{Equation (5.22)}$$

- **Rule 2** – applies when considering a product or a quotient. For instance: $y = abc$ or $y = a/bc$. The combined uncertainty is then given by:

$$u_c(y)/y = \sqrt{\{[u(a)/a]^2 + [u(b)/b]^2 + [u(c)/c]^2\}} \qquad \text{Equation (5.23)}$$

[Note: u(x)/x is a relative SD]

- **Rule 3** – applies when considering an exponent term. For instance: $y = a^n$

$$u_c(y) = \frac{n \times y \times u(a)}{a} \qquad \text{Equation (5.24)}$$

Calculating and reporting expanded uncertainty – multiply the $u_c(y)$ value by the coverage factor 'k'. This is usually k is set to 2, but where there are less than 6 degrees of freedom, for any major uncertainty component, then 'k' should be set at the two tailed t-value for the degrees of freedom of that component, at 95% confidence.

Example (xxxi) – total nitrogen is determined as 3.53 % with a combined uncertainty of 0.08 %.
Allowing for the coverage factor of 2, the result quoted as **3.53 ± 0.16 %** at 95% confidence.

Example (5.xiii) - the use of rule 1

We have 4 objects and we wish to know their combined weight and the uncertainty associated with this weight. The following information is available:

a = 27.72 g, u(a) = ± 0.01 g
b = 32.35 g, u(b) = ± 0.03 g
c = 47.10 g, u(c) = ± 0.12 g
d = 19.86 g, u(d) = ± 0.02g

The model for this estimation of uncertainty is: T (total weight) = a + b + c + d

Using rule 1, the combined uncertainty is:

$u_c(T) = \sqrt{[u(a)^2 + u(b)^2 + u(c)^2 + u(d)^2]}$
$= \sqrt{[0.0001 + 0.0009 + 0.0144 + 0.0004]}$
$= \sqrt{0.0158}$
$= \mathbf{0.126}$

T = 27.72 + 32.35 + 47.10 + 19.86
= **127.03**

Therefore the combined weight and its associated combined uncertainty is:
127.03 ± 0.13 g or **127.03 ± 0.25 g at 95% confidence** as an expanded uncertainty

Example (5.xiv) - the use of rule 2

In a simple titration, an unknown HCl solution is titrated against a standard solution of NaOH. Calculate the concentration of the HCl together with its measurement uncertainty. The Following information is available:

C_{NaOH} = 0.0994 M, $u(C_{NaOH})$ = 0.00017
V_{NaOH} = 25.00 cm^3 $u(V_{NaOH})$ = 0.022
V_{HCl} = 25.40 cm^3 $u(V_{HCl})$ = 0.034

The model for the estimation of the uncertainty is: $C_{HCl} = [C_{NaOH} \times V_{NaOH}]/[V_{HCl}]$
Thus the HCl concentration is calculated to be:
$C_{HCL} = [0.0994 \times 25.00]/25.40$ M = **0.0978 M**

Using rule 2 the combined uncertainty is:

$$u_c(C_{HCl})/C_{HCl} = \sqrt{\{[u(C_{NaOH})/C_{NaOH}]^2 + [u(V_{NaOH})/V_{NaOH}]^2 + [u(V_{HCl})/V_{HCl}]^2\}}$$
$$= \sqrt{[0.0000029 + 0.0000007 + 0.0000017]}$$
$$= 0.00230$$

Thus $u_c(C_{HCl})$ = 0.00230 X 0.0978
= **0.000225**

Concentration of the HCl is 0.0978 ± 0.0002 M or **0.0978 ± 0.0005 at 95% confidence**

Have the objectives been met?

By this stage of the analysis, all measurements have been made and an assessment of their reliability established by statistical means. It is now necessary to look at the data and to see if the initial objectives for carrying out the analysis in the first place have been met and the problem solved.

If the tests and analyses carried out have not totally solved the problem as far as the sample provider is concerned, then it may be necessary to carry out further work. This may involve examining the sample, probably by using further and different techniques, in order to:

- Provide further qualitative information about the sample;
- Provide more quantitative information at lower detection levels for individual analytes;
- Provide quantitative information with lower estimates of uncertainty;
- Develop an analytical method that would be more cost effective for routine usage.

In the end, it is the customer who will determine if the analytical project has been successful.

Question 5.1 The results of determination of Titanium in four samples of an CR alloy by a spectrophotometric method are shown in the table below, 8 measurements were made for each determination. The mean value for each sample is compared to the quoted value of the certified reference material. Is there a significant difference for the value obtained by the spectrophotometric method and the known amount in the certified reference material?

Sample	CRM %Ti	Mean %Ti	sd
1	0.496	0.482	0.0257
2	0.995	1.009	0.0258
3	1.493	1.505	0.0287
4	1.990	2.002	0.0212

Question 5.2 Calculate the combined measurement uncertainty for the following problem –
Y = AB/C.
Where A = 1.76 $\pm$ 0.03 : B = 1.89 $\pm$ 0.02; C = 0.59 $\pm$ 0.03

Outline answer to question 5.1

The equations relating to this calculation are shown on slides 21 - 24

The CRM value is the accepted true value: μ

Use the formula: $\mathbf{(\bar{x}-\mu)(\sqrt{n}/s)}$

$t_{calc} = (0.482-0.496)\sqrt{(8/0.0257)} = 1.54$

$t_{crit} = 2.36$ from tables at 95% confidence.

Conclusion: there is no significant difference between the sample and the CRM

Outline answer to question 5.2

The equations relating to this calculation are shown on slides 53 - 60

1. Combine the values = 5.64

2. Convert each uncertainty to relative uncertainty and square them:
   0.03/1.76 = 0.017, $2.9 \exp^{-4}$
   0.02/1.89 = 0.011 $1.12 \exp^{-4}$
   0.02/0.59 = 0.033 $1.15 \exp^{-4}$

3. Then take the root sum of squares = 0.04, and multiply by 5.64 = 0.225

4. Express as: 5.64 ± 0.23