Control of the quality of analytical methods

Box 1.1 Questions pharmaceutical analysis methods are used to answer

- Is the identity of the drug in the formulated product correct?
- What is the percentage of the stated content of a drug present in a formulation?
- Does this formulation contain solely the active ingredient or are additional impurities present?
- What is the stability of a drug in the formulation and hence the shelf-life of the product?
- At what rate is the drug released from its formulation so that it can be absorbed by the body?
- Do the identity and purity of a pure drug substance to be used in the preparation of a formulation meet specification?
- Do the identity and purity of excipients to be used in the preparation of a formulation meet specification?
- What are the concentrations of specified impurities in the pure drug substance?
- What is the concentration of the drug in a sample of tissue or biological fluid?
- What are the p*Ka* value(s), partition coefficients, solubilities and stability of a drug substance under development?

Introduction

Pharmaceutical analysis procedures may be used to answer any of the questions outlined in Box 1.1 above. The quality of a product may deviate from the standard required but in carrying out an analysis one also has to be certain that the quality of the analysis itself is of the standard required. Quality control is integral to all

modern industrial processes and the pharmaceutical industry is no exception. Testing a pharmaceutical product involves chemical, physical and sometimes microbiological analyses. It has been estimated that £10 billion is spent each year on analyses in the UK alone and such analytical processes can be found in industries as diverse as those producing food, beverages, cosmetics, detergents, metals, paints, water, agrochemicals, biotechnological products and pharmaceuticals. With such large amounts of money being spent on analytical quality control, great importance must be placed on providing accurate and precise analyses. Thus it is appropriate to begin a book on the topic of pharmaceutical analysis by considering, at a basic level, the criteria which are used to judge the quality of an analysis. The terms used in defining analytical quality form a rather elegant vocabulary that can be used to describe quality in many fields and in writing this book the author would hope to describe each topic under consideration with accuracy, precision and most importantly with reproducibility so that the information included in it can be readily assimilated and reproduced where required by the reader. The following sections provide an introduction to the control of analytical quality. More detailed treatment of the topic is given in the reference cited at the end of the chapter.[1]

Control of errors in analysis

A quantitative analysis is not a great deal of use unless there is some estimation of how prone to error the analytical procedure is. Simply accepting the analytical result could lead to rejection or acceptance of a product on the basis of a faulty analysis. For this reason it is usual to make several repeat measurements of the same sample in order to determine the degree of agreement between them. There are three types of errors which may occur in the course of an analysis: gross, systematic and random. Gross errors are easily recognised since they involve a major breakdown in the analytical process such as samples being spilt, wrong dilutions being prepared or instruments breaking down or being used in the wrong way. If a gross error occurs the results are rejected and the analysis is repeated from the beginning. Random and systematic errors can be distinguished in the following example:

A batch of paracetamol tablets are stated to contain 500 mg of paracetamol per tablet; for the purpose of this example it is presumed that 100% of the stated content is the correct answer. Four students carry out a spectrophotometric analysis of an extract from the tablets and obtain the following percentages of stated content for the repeat analysis of paracetamol in the tablets:

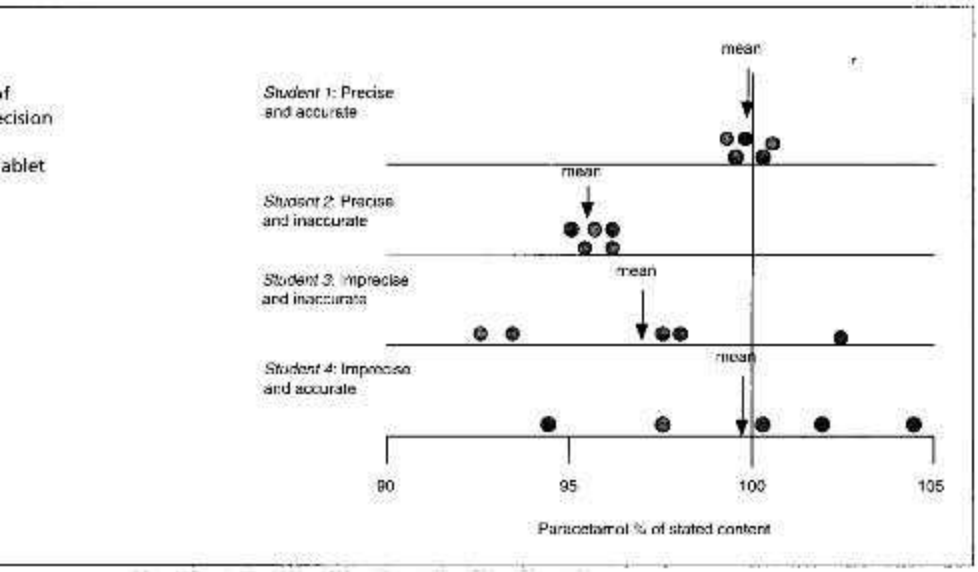
Student 1: 99.5%, 99.9%, 100.2%, 99.4%, 100.5%
Student 2: 95.6%, 96.1%, 95.2%, 95.1%, 96.1%
Student 3: 93.5%, 98.3%, 92.5%, 102.5%, 97.6%
Student 4: 94.4%, 100.2%, 104.5%, 97.4%, 102.1%

The means of these results can be simply calculated according to the formula:

$$\bar{x} = \sum_i \frac{x_i}{n} \qquad \textbf{[Equation 1]}$$

Where $\bar{x}$ is the arithmetic mean, x_i is the individual value and n is the number of measurements.

These results can be seen diagrammatically in Figure 1.1.



Student 1 has obtained a set of results which are all clustered close to 100% of the stated content and with a mean for the five measurements very close to the correct answer. In this case the measurements made were both precise and accurate and obviously the steps in the assay have been controlled very carefully.

Student 2 has obtained a set of results which are closely clustered but give a mean which is less than the correct answer. Thus although this assay is precise it is not completely accurate. Such a set of results indicates that the analyst has not produced random errors which would produce a large scatter in the results but has produced an analysis containing a systematic error. Such errors might include repeated inaccuracy in the measurement of a volume or failure to zero the spectrophotometer correctly prior to taking the set of readings. The analysis has been mainly well controlled except for probably one step which has caused the inaccuracy and thus the assay is precisely inaccurate.

Student 3 has obtained a set of results which are widely scattered and hence imprecise, and which give a mean which is less than the correct answer. Thus the analysis contains random errors or possibly, looking at the spread of the results, three defined errors which have been produced randomly. The analysis was thus poorly controlled and it would require more work than that required in the case of student 2 to eliminate the errors. In such a simple analysis the random results might simply be produced by, for instance, a poor pipetting technique where volumes both higher and lower than that required were measured.

Student 4 has obtained a set of results which are widely scattered yet a mean which is close to the correct answer. It is probably only chance that separates the results of student 4 from those of student 3 and although the answer obtained is accurate, it would not be wise to trust it to always be so.

The best assay was carried out by student 1 and student 2 produced an assay which might be improved with a little work.

In practice it might be rather difficult to tell whether student 1 or student 2 had carried out the best analysis since it is rare, unless the sample is a pure analytical standard, that the exact content of a sample is known. In order to determine whether

student 1 or 2 had carried out the best assay it might be necessary to get other analysts to obtain similar sets of precise results in order to be absolutely sure of the correct answer. The factors leading to imprecision and inaccuracy in assay results are outlined in Box 1.2.

Box 1.2 Some factors giving rise to imprecision and inaccuracy in an assay

- Incorrect weighing and transfer of analytes and standards
- Inefficient extraction of the analyte from a matrix, e.g. tablets
- Incorrect use of pipettes, burettes or volumetric flasks for volume measurement
- Measurement carried out using improperly calibrated instrumentation
- Failure to use an analytical blank
- Selection of assay conditions that cause degradation of the analyte
- Failure to allow for or to remove interference by excipients in the measurement of an analyte

Self-test 1.1

Suggest how the following might give rise to errors in an analytical procedure:

(i) Analysis of a sucrose-based elixir using a pipette to measure aliquots of the elixir for analysis.
(ii) Weighing out 2 mg of an analytical standard on a four-place analytical balance which weighs a minimum of 0.1 mg.
(iii) Use of an analytical standard that absorbs moisture from the atmosphere.
(iv) Incomplete powdering of coated tablets prior to extraction.
(v) Extraction of an ointment with a solvent in which it is poorly soluble.
(vi) Use of a burette that has not been rinsed free of traces of a detergent.

Answers: (i) Viscosity leads to incomplete drainage of the pipette; (ii) In any weighing there is an uncertainty of ± 0.05 mg which in relation to 2 mg is ± 2.5%; (iii) The degree of moisture absorption is uncertain; (iv) Poor recovery of the analyte; (v) Poor recovery of the analyte; (vi) Loss of meniscus making reading of the burette inaccurate

Accuracy and precision

The most fundamental requirements of an analysis are that it should be accurate and precise. It is presumed, although it cannot be proven, that a series of measurements (y) of the same sample will be normally distributed about a mean (μ) i.e. they fall into a Gaussian pattern as shown in Figure 1.2.

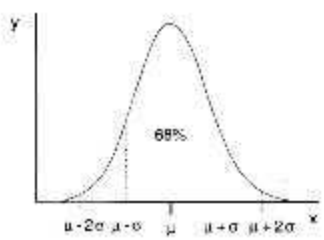


Fig. 1.2 The Gaussian distribution.

The distance σ shown in Figure 1.2 appears to be nearly 0.5 of the width of distribution, however, because the function of the curve is exponential it tends to

zero and does not actually meet the y axis until infinity where there is an infinitesimal probability that there may be a value for x. For practical purposes approximately 68% of a series of measurements should fall within the distance σ either side of the mean and 95% of the measurements should lie with 2σ of the mean. The aim in an analysis is to make σ as small a percentage of the value of μ as possible. The value of σ can be estimated using the Equation 2:

$$s = \sqrt{\frac{\sum(x_i - x)^2}{(n-1)}} \qquad \textbf{[Equation 2]}$$

s = standard deviation
n = number of samples
x_i = values obtained for each measurement
x = mean of the measurements

Sometimes n rather than $n - 1$ is used in the equation but, particularly for small samples, it tends to produce an underestimate of σ. For a small number of values it is simple to work out s using a calculator and the above equation. Most calculators have a function which enables calculation of s directly and on calculators σ estimated using the above equation is usually labelled as σ_{n-1}. For instance if the example of results obtained by student 1, where the mean is calculated to be 99.9%, are substituted into equation 2 the following calculation results:

$$s = \sqrt{\frac{\sum(99.5 - 99.9)^2 + (99.9 - 99.9)^2 + (100.2 - 99.9)^2 + (99.4 - 99.9)^2 + (100.5 - 99.9)^2}{(5-1)}}$$

$$= \sqrt{\frac{\sum(-0.4)^2 + (0)^2 + (0.3)^2 + (-0.5)^2 + (0.6)^2}{4}}$$

$$= \sqrt{\frac{0.16 + 0 + 0.09 + 0.25 + 0.36}{4}} = \sqrt{\frac{0.86}{4}} = \sqrt{0.215} = 0.46$$

s = 0.46% of stated content

The calculated valve for s provides a formal expression of the scatter in the results from the analysis rather than the visual judgement used in Figure 1.1. From the figure obtained for the standard deviation (SD) we can say that 68% of the results of the analysis will lie within the range 99.9 ± 0.46% (± σ) or within the range 99.44–100.36%. If we re-examine the figures obtained by student 1 it can be seen that 60% of the results fall within this range, with two outside the range including one only very slightly below the range. The range based on ± σ defines the 68% confidence limits; for 95% confidence ± 2σ must be used, i.e. 95% of the results of student 1 lie within 99.9 ± 0.92% or 98.98–100.82%. It can be seen that this range includes all the results obtained by student 1.

The precision of an analysis is often expressed as the ± relative standard deviation (± RSD) (Equation 3).

$$\text{RSD} = \frac{s}{x} \times 100\% \qquad \textbf{[Equation 3]}$$

The confidence limits in this case are often not quoted but since it is the SD that is an estimate of σ which is being used they are usually 68%. The advantage of

expressing precision in this way is that it eliminates any units and expresses the precision as a percentage of the mean. The results obtained from the assay of paracetamol tablets are shown in Table 1.1.

Table 1.1 Results obtained for the analysis of paracetamol tablets by four analysts

Student	Mean (% of stated content)	S (% of stated content)	± RSD (68% confidence)
1	99.9	0.5	± 0.5%
2	95.6	0.5	± 0.5%
3	96.9	4.0	± 4.4%
4	99.7	4.0	± 4.0%

Self-test 1.2

Four analysts obtain the following data for a spectrophotometric analysis of an injection containing the local anaesthetic bupivacaine. The stated content of the injection is 0.25% weight in volume (w/v).
Analyst 1: 0.245% w/v, 0.234% w/v, 0.263% w/v, 0.261% w/v, 0.233% w/v.
Analyst 2: 0.236% w/v, 0.268% w/v, 0.247% w/v, 0.275% w/v, 0.285% w/v.
Analyst 3: 0.248% w/v, 0.247% w/v, 0.248% w/v, 0.249% w/v, 0.253% w/v.
Analyst 4: 0.230% w/v, 0.233% w/v, 0.227% w/v, 0.230% w/v, 0.229% w/v.
Calculate the mean percentage of stated content and RSD for each set of results at the 68% confidence level. Assuming the content really is as stated on the label, comment on the accuracy and precision of each set of results. Calculate the precision of each assay with regard to 95% confidence limits.

Answers: *Analyst 1*: 98.9% ± 5.8%; accurate and but imprecise; At 95% confidence RSD = ± 11.6%; *Analyst 2*: 104.9 ± 7.7%; inaccurate and imprecise; At 95% confidence RSD = ±15.4%; *Analyst 3*: 99.6% ± 0.9%; accurate and precise; At 95% confidence RSD = ± 1.8%; *Analyst 4*: 91.9% ± 0.9%; inaccurate and precise; At 95% confidence RSD = ±1.8

Repeatability and reproducibility

In order for an assay to be valid it must be possible to get it to work on every occasion. The terms of repeatability and reproducibility are often rather poorly defined to the extent that they don't add very much to the general concept of precision. In order to prevent overlap of the terms it is necessary to extend their scope. First it is necessary to define in more specific terms what is meant by the precision of an assay.

Within-assay precision

The precision of an assay is a measure of its ability to produce close agreement between the results for several determinations of the same sample. Precision is a fairly general term since it applies to the assay as it is described and the extent of the details given for an assay may vary. There is no general agreement as to whether all the operations within an assay are being assessed in determining precision. For instance in a spectrophotometric assay a single weighing and extraction of a sample may be made and then several aliquots of the extract may be taken and diluted to the concentration required for analysis in order to assess the precision of the method. The precision of the weighing and extraction steps is not assessed. This is understandable since if the precision of every aspect of an assay were assessed then a very large number of samples would be generated. For instance if five samples of tablets were powdered and from each batch of powdered tablets five samples were

weighed out and extracted separately and then five dilutions were prepared from each extract, a total of 125 samples would be generated, which is rather an overkill. Even in relatively simple assays such as titrations the question arises as to whether or not one should make up several solutions of the titrant and standardise all of them before carrying out the assay. Thus precision of an assay cannot be a fixed term and it is an assessment of the assay as it was carried out and as such should be called the *within-assay precision*. If the number of operations which were assessed in order to determine the within-assay precision was inadequate then it would exhibit poor repeatability. For instance, if it really was necessary to assess the precision of the extraction step in the assay of paracetamol tablets then this would confound attempts to repeat the analysis on another occasion and achieve the same accuracy and precision. Thus it is useful to distinguish between *repeatability* and *within-assay precision* since it enables the routine assay to be limited to a sensible number of repetitive steps.

Repeatability

This differs from simple within-assay precision in that it might be considered to compare the sum total of the operations carried out in an assay of a particular sample from the beginning, carried out by a single operator, with the same sum total of all critical operations, carried out by the same operator, probably in sequence with the initial assay and certainly within the same day.

Where repeatability is being assessed the sum total of operations in an assay might be considered to include the following:

(i) The weighing out of all standards and samples
(ii) Fresh preparation and standardisation of all solutions involved in the quantitative aspects of the assay
(iii) All dilution and extraction steps involved in the assay being carried out from the beginning.

Thus repeatability is a catch-all term and allows for the assessment of the precision of some of the steps which may have been missed from the routine assay. If repeatability is poor some extra operations may within the routine assay need to be assessed for precision.

Between-day repeatability

This concept is simply as suggested in the term that the repeatability is assessed on separate days or even separated by several days by the same operator.

Within-laboratory reproducibility

Reproducibility is often used in the way that repeatability has been defined above but this does not leave room for a term defining what happens when an analytical procedure is handed over to another analyst. Since in the art world reproduction relates to copying of an original by another artist it would seem appropriate to use the term in the same way in analytical chemistry. If an assay is carried out in a laboratory by several analysts it is unlikely that these analysts will weigh out identical amounts of sample and use identical items of equipment. A clearly defined assay procedure should be capable of being reproduced by a number of analysts in a laboratory. Furthermore, having confidence in its reproducibility should facilitate

staff training and give confidence in the overall control of other parts of the analytical process such as calibration and handling of a range of equipment.

Between-laboratory reproducibility

If a laboratory was fully confident in a particular assay it might submit it for testing by several laboratories, which would give a measure of how reproducible the assay was in a wider sense with different operators and equipment. For an assay to succeed in this type of exercise it would have to very *robust*. For example pharmacopoeial monographs are designed, in theory, to be sufficiently robust to be reproduced relatively easily by many laboratories. However, the tolerances for the precision of such assays might be quite wide.

Standard operating procedure (SOP) for the assay of paracetamol tablets

The terms defined above are perhaps illustrated by using the example of the simple assay which we have mentioned before. The assay in Box 1.3 is laid out in the style of a SOP. This particular section of the operating procedure describes the assay itself but there would also be other sections in the procedure dealing with safety issues, the preparation and storage of the solutions used for extraction and dilution, the glassware required and a specification of the instrumentation to be used.

Box 1.3 Extract from a standard operating procedure for the analysis of paracetamol tablets

8. *Assay procedure*
8.1 Use a calibrated balance
8.2 Weigh 20 tablets
8.3 Powder the 20 paracetamol tablets and weigh *by difference* a quantity of tablet powder equivalent to 125 ± 10 mg of paracetamol
8.4 Shake the tablet powder sample with *ca* 150 ml of acetic acid (0.05 M) for 10 min in a 500 ml volumetric flask and then adjust the volume to 500 ml with more acetic acid (0.05 M).
8.5 Filter *ca* 100 ml of the solution into a conical flask and then transfer five separate 5 ml aliquots of the filtrate to 100 ml volumetric flasks and adjust the volumes to 100 ml with acetic acid (0.05 M)
8.6 Take two readings of each dilution using a UV spectrophotometer and using the procedure specified in **Section 9**

The assay described in Box 1.3 assesses the precision of some of the operations within the assay. If a single analyst was to assess the *repeatability* of the assay, instructions might be issued to the effect the assay as described was to be repeated five times in sequence, i.e. completing one assay before commencing another. If *between-day repeatability* were to be assessed the process used for determining the repeatability would be repeated on two separate days. If the *within-laboratory reproducibility* were to be assessed two or more analysts would be assigned to carry out the *repeatability* procedure. In arriving at a SOP such as the one described in Box 1.3 there should be some justification in leaving out certain steps in the complete assay. For instance, weighing is often the most precise step in the process and thus repeat weighings of samples of tablet powder would not be necessary to guarantee precision; the precision of the extraction might be more open to question.

Each of the sections within an assay would have other SOPs associated with them governing, for instance, the correct use and care of balances as listed in Box 1.4.

Box 1.4 Procedure for the use of a calibrated balance SOP/001A/01

This balance is a high-grade analytical balance. It carries out internal calibration but as a double check it is checked with certified check weights. Any deviation of the check weight values from those expected indicates need for servicing of the balance. Check weight calibration should be carried out once a week according to the instructions in SOP/001C/01.

Caution The logbook (form SOP/001 AR/01) must be filled in. Any spillages on the balance must be cleaned up immediately and recorded in the log. This balance is to be used only for analytical grade weighings.

Operation

1. When carrying out weighing of amounts < 50 mg use tweezers to handle the weighing vessel.
2. Make sure the door of the balance is shut. Switch on the balance and allow it to undergo its internal calibration procedure. When it is ready the digital read-out will be 0.0000. Wait 30 s to ensure that the reading has stabilised.
3. Introduce the weighing vessel onto the balance pan. Close the door. Wait 30 s to ensure that the reading has stabilised and then send the reading to the printer.
4. If the tare is used in the weighing procedure, press the tare button and wait until the balance reads 0.0000. Wait 30 s to ensure that the reading has stabilised. If it drifts, which under normal circumstances it should not, press the tare button again and wait for a stable reading.
5. Remove the weighing vessel from the balance, introduce the sample into the vessel and put it back onto the balance pan. Close the door and note the reading.
6. Remove the sample and adjust the sample size to bring it closer to the required amount. Re-introduce the sample onto the balance pan. Close the door and note the reading.
7. Repeat step 5 until the target weight is reached. When the required weight is reached wait 30 s to ensure that the reading has stabilised. Send the reading to the printer.

N.B. An unstable reading may indicate that moisture is being lost or gained and that the sample must be weighed in a capped vessel.

Date of issue: 6/10/95 **Signature:**

Compound random errors

Systematic errors in analysis can usually be eliminated but true random errors are due to operations in an assay which are not completely controlled. A common type of random error arises from the acceptance of manufacturers' tolerances for glassware. Table 1.2 gives the RSD values specified for certain items of grades A and B glassware.

Table 1.2 Manufacturers' tolerances on some items of glassware

Item of glassware	Grade A	Grade B
1 ml bulb pipette	± 0.7%	± 1.5%
5 ml bulb pipette	± 0.3%	± 0.6%
100 ml volumetric flask	± 0.08%	± 0.15%
500 ml volumetric flask	± 0.05%	± 0.1%
full 25 ml burette	± 0.2%	± 0.4%

An estimate of compound random errors is obtained from the square root of the sum of the squares of the RSDs attributed to each component or operation in the analysis. If the analysis of paracetamol described in Box 1.3 is considered then, assuming the items of glassware are used correctly. Assuming the items of glassware are used correctly the errors involved in the dilution steps can be simply estimated from the tolerances given for the pipette and volumetric flasks. The British Standards Institution (BS) tolerances for the grade A glassware used in the assay are as follows:

500 ml volumetric flask 500 ml ± 0.05%
100 ml volumetric flask 100 ml ± 0.08%
5 ml one mark pipette 5 ml ± 0.3%

Standard deviation of error from glassware =

$$\sqrt{0.05^2 + 0.08^2 + 0.3^2} = \sqrt{0.0989} = 0.31\%$$

Thus it can be seen that the compound error from the glassware differs little from the largest error in the process. Of course the glassware errors can be eliminated by calibration of the glassware prior to use but in general analysts will accept manufacturers' tolerances. The tolerated random error from glassware could be readily eliminated; other random errors such as variation in the extraction efficiency are more difficult to control.

Self-test 1.3

Estimate the compound random error in the following assay with respect to the dilution steps described and calculate the error as SD of the w/v percentage of the injection assuming it is exactly 2% w/v.

A 2% w/v injection was diluted twice using grade A 5 ml bulb pipettes and grade A 100 ml volumetric flasks as follows:

Dilution 1: 5 to 100 ml
Dilution 2: 5 to 100 ml
The uncertainty in the spectrophotometric reading was ± 0.2%.

Answer ± 0.48% and ± 0.01% w/v

Reporting of results

In calculating an answer from the data obtained in an analysis it is important to not indicate a higher level of precision than was actually possible in the assay. As mentioned the previous section, when considering the accuracy of glassware used with the assumption that it complied with the BS grade A standard, it was obvious that there was some uncertainty in any figure < 1%. It might be possible to improve on this degree of precision by calibrating glassware; however, any improvement in precision in the real world would take time and hence have cost implications. Thus for the purposes of most analyses, and for the purposes of the calculations in this book, it would seem sensible to report four significant figures, i.e. to 0.1%. In the process of carrying out calculations, five figures can be retained and rounded up to four figures at the end of the calculation. Since in pharmaceutical analyses the percentage of the stated content of a drug in a formulation may be reported as being between 90 and 99.9%, if the first significant figure is 9 then at the end of the calculation a more realistic estimate of precision is given by rounding the answer up to three significant figures. The SD or RSD reported with the answer should reflect

the number of significant figures given; since there is usually uncertainty in figures < 1% of the answer the RSD should not be reported to > 0.1%. Taking this into consideration the correct and incorrect ways of reporting some answers are given in Table 1.3.

Table 1.3 Significant figures in the reporting of analytical results

Answer ± S Incorrect	RSD	Answer ± S Correct	RSD
% of stated content = 99.2 ± 0.22	0.22	% of stated content = 99.2 ± 0.2	0.2
% of stated content = 101.15 ± 0.35	0.35	% of stated content = 101.2 ± 0.4	0.4
0.2534 ± 0.00443% w/v	1.75	0.2534 ± 0.0044% w/v	1.7
1.0051 ± 0.0063% w/w	0.63	1.005 ± 0.006% w/w	0.6
1.784 ± 0.1242 μg/ml	6.962	1.784 ± 0.124 μg/ml	7.0

Other terms used in analytical procedures control

Analytical blank

This consists of all the reagents or solvents used in an analysis without any of the analyte being present. A true analytical blank should reflect all the operations to which the analyte in a real sample is subjected. It is used for example in checking that reagents or indicators do not contribute to the volume of titrant required for a titration, including zeroing spectrophotometers or in checking for chromatographic interference.

Calibration

The calibration of a method involves comparison of the value or values of a particular parameter measured by the system under strictly defined conditions with pre-set standard values. Examples include: calibration of the wavelength and absorbance scales of a UV/visible spectrophotometer (Ch. 4), calibration of the wavelength scale of an IR spectrometer (Ch. 5) and construction of chromatographic calibration curves (Ch. 12).

Limit of detection

This is the smallest amount of an analyte which can be detected by a particular method. It is formally defined as follows:

$$x - x_B = 3s_B$$

Where x is the signal from the sample, x_B is the signal from the analytical blank and s_B is the SD of the reading for the analytical blank. In other words the criterion for a reading reflecting the presence of an analyte in a sample is that the difference between the reading taken and the reading for the blank should be three times the SD of the blank reading. The SD of the signal from the sample can be disregarded since the sample and the blank should have been prepared in the same manner so that it and the sample produce a similar SD in their readings. A true limit of detection should reflect all the processes to which the analyte in a real assay is subjected and not be a simple dilution of a pure standard for the analyte until it can no longer be detected.

Self-test 1.5

In which of the following cases has the limit of detection been reached?

Signal from sample	Sample SD	Signal from analytical blank	Analytical blank SD
1. Abs 0.0063	0.0003	0.0045	0.0003
2. Abs 0.0075	0.0017	0.0046	0.0018
3. 0.335 ng/ml	0.045 ng/ml	0.045 ng/ml	0.037 ng/ml

Answer 2

Linearity

Most analytical methods are based on processes where the method produces a response that is linear and which increases or decreases linearly with analyte concentration. The equation of a straight line takes the form:

$$y = a + bx$$

where a is the intercept of the straight line with the y axis and b is the slope of the line. Taking a simple example, a three-point calibration curve is constructed through readings of absorbance against procaine concentration (Table 1.4).

Table 1.4 Data used for the construction of a calibration curve for the spectrophotometric determination of procaine

Procaine concentration mg/100 ml	Absorbance reading
0.8	0.604
1.0	0.763
1.2	0.931

The best fit of a straight line through these values can be determined by determining a and b from the following equations:

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

$$a = \bar{y} - b\bar{x}$$

where x_i are the individual values for x, $\bar{x}$ is the mean value of x_i, y_i are the individual values for y and $\bar{y}$ is the mean of y_i.

From the data in Table 1.3:

$$\bar{x} = \frac{0.8 + 1.0 + 1.2}{3} = 1.0$$

$$\bar{y} = \frac{0.604 + 0.763 + 0.931}{3} = 0.766$$

$$b = \frac{(0.8 - 1.0)(0.604 - 0.766) + (1.0 - 1.0)(0.763 - 0.766) + (1.2 - 1.0)(0.931 - 0.766)}{(0.8 - 1.0)^2 + (1.0 - 1.0)^2 + (1.2 - 1.0)^2}$$

$$= \frac{0.0324 + 0 + 0.033}{0.04 + 0.04} = 0.818$$

$$a = 0.766 - 0.818 \times 1.0 = -0.052$$

Thus the equation for the best fit is:

$$y = 0.818x - 0.052$$

The statistical measure of the goodness of fit of the line through the data is the correlation coefficient r. A correlation coefficient of > 0.99 is regarded as indicating linearity. The correlation coefficient is determined from the following equation:

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

Substituting the values from Table 1.3:

$$r = \frac{(0.8 - 1.0)(0.604 - 0.766) + (1.0 - 1.0)(0.763 - 0.766) + (1.2 - 1.0)(0.931 - 0.766)}{\sqrt{[(0.8 - 1.0)^2 + (1.0 - 1.0)^2 + (1.2 - 1.0)^2][(0.604 - 0.766)^2 + (0.763 - 0.766)^2 + (0.931 - 0.766)^2]}}$$

$$r = \frac{0.0324 + 0 + 0.033}{\sqrt{0.08 \times 0.0534}} = 1.00$$

Thus to three significant figures the straight line fit through the values in Table 1.3 is perfect. For a fuller treatment of the mathematical determination and significance of a correlation coefficient see reference 1. The equation for the correlation coefficient is very useful in that it can be applied to correlations between curves of any shape and thus it can be used for spectral comparisons such as those carried out between diode array spectra obtained during high-pressure liquid chromatography (Ch. 12 p. 251).

Range

The range of a method is related to its *sensitivity*, although there are methods such as immunoassays which are capable of measuring very small amounts of material, but are not very sensitive in that they measure over a restricted range of low concentration. Thus, some types of detection have very wide dynamic ranges and others may only function over a restricted range before *linearity* is lost. A UV detector has a dynamic range of about 1×10^3 and for a particular compound it might measure concentrations between 0.1 and 100 μg/ml. In contrast a flame photometer has a range not much greater than 1×10. Sample concentrations must be adjusted so that they fall into the range of the equipment used to make the measurement.

Robustness

This term refers to how resistant the precision and accuracy of an assay is to small variations in the method, e.g. changes of instrumentation, slight variations in extraction procedures, sensitivity to minor impurities in reagents, etc. Robust assays may not be capable of the highest precision or specificity but they are regarded as fit for the purpose for which they are designed.

Selectivity

The selectivity of a method is a measure of how capable it is of measuring the analyte alone in the presence of other compounds contained in the sample. The most selective analytical methods involve a chromatographic separation. Detection methods can be ranked according to their selectivity. A simple comparison is

between fluorescence spectrophotometry and UV spectrophotometry; there are many more compounds which exhibit UV absorption than there are those which exhibit strong fluorescence, thus fluorescence spectrophotometry is a more selective method. Because selective methods are based on more complex principles than non-selective methods they may be less robust, e.g. fluorescence spectrophotometry is more affected by changes in the analytical method than UV spectrophotometry.

Sensitivity

The sensitivity of method indicates how responsive it is to a small change in the concentration of an analyte. It can be viewed as the slope on a response curve and may be a function of the method itself or of the way in which the instrumentation has been calibrated. In Figure 1.3 the method having a *linear* response $y = 2.5 \times$ is 5 times more sensitive than the method exhibiting a linear response $y = 0.5x$. Sensitivity and the *limit of detection* of a method are often confused. The limit of detection is due to a combination of *range* and *sensitivity*.

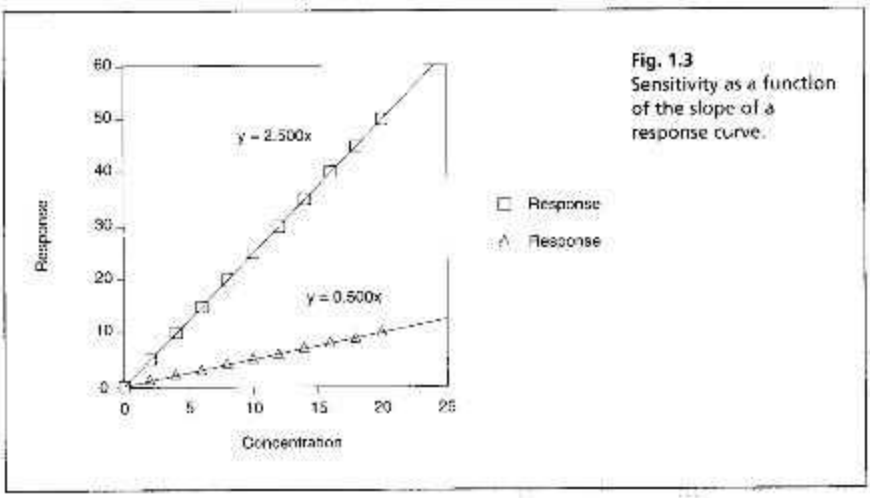


Fig. 1.3
Sensitivity as a function of the slope of a response curve.

Weighing by difference

Weighing by difference is used to minimise weighing errors in an analytical procedure. The sample is weighed in a suitable vessel, e.g. a glass weighing boat with a spout, and then transferred immediately to the vessel in which it is going to be analysed or dissolved. The weighing vessel is then reweighed and the difference between the weights before and after transfer gives the weight of the sample. This method of weighing minimises errors due to, for example, the absorption of moisture onto the surface of the vessel. It also means that there is not a requirement for complete transfer of the sample that is to be analysed.

The points listed in Boxes 1.5 and 1.6 indicate how pharmaceutical preparations may come to be out of specification.

Box 1.5 Sources of impurities in pharmaceutical manufacture

- During the course of the manufacture of a pure drug substance, impurities may arise as follows:
  (i) Present in the synthetic starting materials
  (ii) Result from residual amounts of chemical intermediates used in the synthetic process and from unintended side reactions
  (iii) Result from reagents, solvents and catalysts used in manufacture
- The process used to produce the formulated drug substance may introduce impurities as follows:
  (i) Particulate matter from the atmosphere, machines and devices used in the manufacturing process and from containers
  (ii) Impurities that are present in the excipients used in the formulation
  (iii) Cross contamination may occur from other processes carried out using the same equipment, e.g. from mixers
  (iv) Microbial contamination may occur
  (v) The drug may react with the excipients used in the formulation
  (vi) Impurities may be introduced from packaging, e.g. polymeric monomers.

Box 1.6 Processes leading to the deviation of the actual content from the stated content of a drug in a formulation

- Incomplete mixing of drug with formulation excipients prior to compression into tablets or filling into capsules
- Physical instability of the dosage form: tablets that disintegrate too readily; creams or suspensions that separate and over- or undercompression of tablets leading to deviation from the required weight
- Chemical breakdown of the drug resulting from its reaction with air, water, light, excipients in a formulation or with packaging materials
- Partitioning of the drug into packaging materials.

References

1. J.C. Miller and J.N. Miller. Statistics for Analytical Chemistry. 3rd Edn. Ellis Horwood (1993).