Reagecon

"Delivering The Correct Result..."

A Guide to Method Validation

A REAGECON GUIDE

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1.0 A Guide to Method Validation

1.1 Introduction

Virtually every aspect of society is supported in some way by analytical measurement. There are innumerable reasons for making these measurements, for example: in-process and final inspection or 'quality control' of manufactured products; supporting healthcare; checking the quality of drinking water; metallurgical analysis of an alloy to confirm its suitability for use in aircraft construction; forensic analysis of body fluids in criminal investigations.

The cost of carrying out these measurements is huge cost implications arise from decisions made on the basis of the results. Analytical results may be used in evidence and challenged in a court of law: tests showing food to be unfit for consumption my result in compensation claims; test confirming the presence of banned drugs could result in fines, imprisonment or even, in some countries, execution. Clearly it is important to determine the correct result and be able to show that it is correct.

1.2 What is method validation?

Method validation is the process of defining an analytical requirement, and confirms that the method under consideration has performance capabilities consistent with what the application requires. Use of equipment that is within specification, working correctly and adequately calibrated is fundamental to the method validation process. Likewise the operator carrying out the studies must be competent in the analysis under study and have sufficient knowledge of the method/analysis to draw conclusions from the observations as the validation work proceeds.

Quite often method validation evolves from method development and so the two activities are often closely tied, with the validation study employing the techniques and steps in the analysis as defined by the method development.

1.3 When should methods be validated?

A method should be validated when it is necessary to verify that its performance parameters are adequate for use for a particular analytical problem. For example:

- Method just developed
- Revised method or established method adapted to a new problem;
- When a review of quality control indicates an established method is changing with time;
- When an established method is used in a different laboratory, with different analysts or with different equipment
- Demonstration of the equivalence between two methods, e.g. a new method and a standard.

Certain areas of analytical practices, such as in clinical chemistry will specify validation requirements relevant to the method. This ensures that particular validation terminology together with the statistics used is interpreted in a manner consistent within the relevant sector. Official recognition of a method may require characterisation using a collaborative study.

1.4 Method Development

As stated above method validation often evolves from method development. Method development can take a number of forms. At one extreme, it may involve adapting an existing method, making minor changes so that it is suitable for a new application.
At the other extreme the analytical chemist may start out with little information and apply expertise and experience to devise a suitable method. This can involve significant innovation based on novel exploitation of known properties of the analyte or measurand. Often a considerable amount of effort is required and initially at least a degree of doubt as to whether the final method will be successful. Frequently method development involves working on a number of different ideas simultaneously and eventually picking one of these.

1.5 The essential components of Method Validation

Confirmation of identity and selectivity/specificity

In general analytical methods consist of a measurement stage which may be preceded by an isolation stage. It is necessary to establish that the signal or reaction produced at the measurement stage is only due to the analyte and not due to something chemically or physically similar or arising as a coincidence. This is **confirmation of identity**. Whether or not other compounds interfere with the measurement of the analyte will depend on the effectiveness of the isolation stage if it was part of the method, as well as the selectivity/specificity of the measurement stage.

1.6 Selectivity and Specificity

Selectivity and specificity are measures of the reliability of measurements in the presence of interferences. Where the measurement stage is non-specific, method development should indicate which analytes do not interfere. There will be cases where chemical interferences can be identified for a particular method but the chances of encountering them in real life may be improbable. The analyst has to decide at what point it is reasonable to stop looking for interferences. These parameters apply to both qualitative and quantitative analysis.

The selectivity of a method is usually investigated by studying its ability to measure the analyte of interest in test portions to which specific interferences have been deliberately introduced (those thought likely to be present in samples). Where it is unclear whether or not interferences are already present, the selectivity of the method can be investigated by studying its ability to measure compared to other independent method/techniques.

Another aspect of selectivity which must be considered is where an analyte may exist in the sample in more than one form such as: free or complexed; inorganic or organometallic; or the possibility of a component such as Chromium ion being present in different oxidation states such as Cr^{3+} or Cr^{6+}.

1.7 Limit of Detection

In quantitative analysis, and where the analyte is present is small concentrations, it is important to know what is the lowest concentration of the analyte or property value that can be confidently detected by the method. Frequently the detection of the analyte does not simply cease at a threshold level, but tends to 'fade' from a predictable signal/concentration ratio gradually to an indiscernible response.

For qualitative measurements, there is likely to be concentration threshold below which specificity becomes unreliable. The threshold may vary if the experiment is repeated at another time with different reagents, fortification, spiking materials, etc. In the example shown in Table 1, positive identification of the analyte has ceased to be 100% reliable below 100 $\mu g.g^{-1}$.

Table 1: Qualitative Analysis – Illustration of how cut-off (i.e. threshold) concentration is determined.

Concentration/$\mu g.g^{-1}$	No. of replicates	Positive/negative results
200	10	10/0
100	10	10/0
75	10	5/5
50	10	1/9
25	10	0/10

1.8 Limit of Quantitation

The 'limit of quantitation' (LoQ) is the least concentration of analyte that can be determined with an acceptable level of repeatability precision and trueness. It is also sometimes known as 'limit of determination'. LoQ is an indicative value and should not normally be used in decision making.

Note that neither 'limit of detection' LoD nor LoQ represent levels at which quantitation is impossible. It is simply that the uncertainty of measurement and the result approach the same magnitude in the region of the LoD.

1.9 Working & Linear Ranges

For any quantitative method, the range of analyte concentrations (i.e. the range of concentrations or property values in the solutions actually measured rather than in the original samples) over which the method may be applied must be known. At the lower end of the concentration range the limiting factors are the values of the limits of detection and/or quantitation. At the upper end of the concentration range limitations may be imposed by a 'shouldering' of the linear range depending on the instrument response system.

Within the working range there may exist a linear response range. Within the linear range signal response will have a linear relationship to analyte concentration or property value. The extent of this range may be established during the evaluation of the working range. Note that regression calculations on their own are insufficient to establish linearity. To do this a visual inspection of the line and residuals may also be necessary. Objective tests, such as 'goodness-of-fit' tests, are better still. In general linearity checks require 10 or more points at different concentrations/property values.

Evaluation of the working and linear ranges will also be useful for planning the calibration required when using the method is in routine use. It is advisable to investigate the variance across the

working range. Within the linear range, two calibration points may be sufficient, to establish the slope of the calibration line. Elsewhere in the working range, multi-point calibration will be necessary. The relationship of instrument response to concentration does not have to be perfectly linear for a method to be effective but the curve should be repeatable from day to day. Note that the working and linear range may be different for different matrices according to the effect of interferences arising from the matrix.

1.10 Accuracy

'Accuracy' expresses the closeness of a result to a true value. Method validation seeks to quantify the likely accuracy of results by assessing systematic and random effects on results. Accuracy is, therefore, normally studied as two components: 'trueness' and 'precision'. The 'trueness' (of a method) is an expression of how close the mean of a set of results (produced by the method) is to the true value. **Trueness** is normally expressed in terms of bias. **Precision** is a measure of how close results are to one another, and is usually expressed by measures such as standard deviation, which describe the spread of results. In addition, an increasingly common expression of accuracy is 'measurement uncertainty', which provides a single figure expression of accuracy. These three different parameters will be discussed in turn.

Practical assessment of **trueness** relies on comparison of mean results from a method with known values, that is trueness is assessed against a reference value (i.e. true value or conventional true value). Two basic techniques are available: checking against reference values for a characterised material or from another characterised method. Reference values are ideally traceable to international standards. Certified reference materials are generally accepted as providing traceable values; the reference value is then the certified value of the CRM. To check trueness using a reference material, determine the mean and standard deviation of a series of replicate test, and compare with the characterised value for the reference material. The ideal reference material is a certified, natural matrix reference material, closely similar to the samples of interest. Clearly, the availability of such materials is limited. Reference materials for validation may accordingly be:

- Prepared by spiking typical materials with pure certified reference materials or other materials of suitable purity and stability;
- Commercially available secondary standards, with certified traceability, whose preparation is ILAB, accredited.
- Typical, well-characterised materials checked in-house for stability and retained for in-house QC.

Validation needs to fit the purpose, so the choice of reference material may be affected by the end use. The reference material must be *appropriate to the use*. For regulatory work, a relevant certified material should be used, ideally matrix matched. For methods used for long-term in-house work, a secondary standard material or certified reference material should be used. For short-term or non-critical work, a prepared standard or spike is often sufficient.

To check against an alternative method, compare results from the two methods for the same sample or samples. The sample(s) may be CRMs, commercially available traceable standard, or simply typical samples. There are advantages to using CRMs, since these have known stability and homogeneity, and additionally give an indication of bias with respect to international standards. On the other hand, CRMs are costly and may not be representative of typical samples.

1.11 Precision

Precision is method and concentration specific, which in practice can be very varied. The two most common precision measures are 'repeatability' and reproducibility'. They represent the two extreme measures of precision, which can be obtained. Repeatability (the smallest expected precision) will give an idea of the sort of variability to be expected when a method is performed by a single analyst on one piece of equipment over a short timescale, i.e. the sort of variability to be expected between results when a sample is analysed in duplicate. If a sample is to be analysed by a number of laboratories for comparative purposes then a more meaningful precision measure is reproducibility (this is the largest measure of precision normally encountered). It may be that some in-between measure is the most useful in particular cases; for example precision measured between different analysts, over extended timescales, within a single laboratory. This is sometimes known as 'intermediate precision', but the exact conditions should be stated. Precision is usually stated in terms of standard deviation or relative standard deviation. Both repeatability and reproducibility are generally dependent on analyte concentration, and so should be determined at a number of concentrations and if relevant, the relationship between precision and analyte concentration should be established

1.12 Repeatability

From the repeatability standard deviation σ_r or s_r it is useful to calculate the 'repeatability limit 'r'', which enables the analyst to decide whether the difference between duplicate analyses of a sample, determined under repeatability conditions, is significant.

1.13 Reproducibility

From the reproducibility standard deviation σ_R or s_R it is useful to calculate the 'reproducibility limit 'R', 'which enables the analyst to decide whether the difference between duplicate analyses of a sample, determined under reproducibility conditions, is significant. These calculations can be performed directly with the built-in statistics function of the instrument, if available, or by using a pocket calculator or a PC (Personal Computer) with a suitable software package (e.g. spreadsheet program).

1.14 Measurement uncertainty

Measurement uncertainty is a single parameter (usually a standard deviation with a coverage factor or confidence interval) expressing the range of values possible on the basis of the measurement result. A measurement uncertainty estimate takes account of all recognised effects operating on the result; the uncertainties associated with each effect are combined according to well-established procedures.

An uncertainty estimate for analytical chemistry is often termed an 'uncertainty budget' and should take into account:

- The overall, long-term precision of the method;
- Bias and its uncertainty, including the statistical uncertainty involved in the bias measurements, and the reference material or method uncertainty. It may be necessary to increase the estimate where a significant bias is detected but left uncorrected.
- Calibration uncertainties. As most equipment calibration uncertainties will be negligibly small by comparison with overall precision and uncertainty in the bias; this needs only to be verified;

- Any significant effects operating in addition to the above. For example, temperature or time ranges permitted by the method may not be fully exercised in validation studies, and their effect may need to be added. Such effects can be usefully quantified by robustness studies (see 'Ruggedness' below) or related studies which establish the size of a given effect on the result.

Where the contribution of individual effects is important, for example in calibration laboratories, it will be necessary to consider the individual contributions from all individual effects separately.

Note that, subject to additional consideration of effects outside the scope of a collaborative trial, the reproducibility standard deviation forms a working estimate of a measurement uncertainty provided that the laboratory's bias, measured on relevant materials, is small with respect to the reproducibility standard deviation, the in-house repeatability precision is comparable to the standard method repeatability and the laboratory's intermediate precision is not large than the published reproducibility standard deviation.

1.15 Sensitivity

This is effectively the gradient of the response curve, i.e. the change in instrument response, which corresponds, to a change in analyte concentration. Where the response has been established as linear with respect to concentration, i.e. within the linear range of the method, and the intercept of the response curve has been determined, sensitivity is a useful parameter to calculate and use in formulae for quantitation. Sensitivity is sometimes used to refer to limit of detection but this use is not generally approved.

1.16 Ruggedness (or Robustness)

Ruggedness is normally evaluated during method development, typically by the originating laboratory, before collaborating with other laboratories and is a measure how well a method stands up to less than perfect implementation. In any method there will be certain stages, which, if not carried out sufficiently carefully, will have a severe effect on method performance, and may even result in the method not working at all. These stages should be identified, usually as part of method development, and if possible, their influence on method performance evaluated using 'ruggedness tests', sometimes also called 'robustness tests'. This involves making deliberate variations to the method, and investigating the subsequent effect on performance. It is then possible to identify the variables in the method, which have the most significant effect and ensure that, when using the method, they are closely controlled. Where there is a need to improve the method further, improvements can probably be made by concentrating on those parts of the method known to be critical. Ruggedness tests are normally applied to investigate the effect on either precision or accuracy.

1.17 Recovery

Analytical methods do not always measure all of the analyte of interest present in the sample. Analytes may be present in a variety of forms in samples not all of interest to the analyst. The method might be deliberately designed to determine only a particular form of the analyte. However, a failure to determine all of the analyte present may reflect an inherent problem in the method. Either way, it is necessary to assess the efficiency of the method in detecting all of the analyte present.

Because it is not usually known how much of a particular analyte is present in a test portion it is difficult to be certain how successful the method has been at extracting it from the matrix. One way to determine the efficiency of extraction is to spike test portions with the analyte at various concentrations, then extract the fortified test portions and measure the analyte concentration. The inherent problem with this is that analyte introduced in such a way will probably not be held as strongly as that which is naturally present in the test portion matrix and so the technique will give an unrealistically high impression of the extraction efficiency. It is however the most common way of determining recovery efficiency, and it is recognised as an acceptable way of doing so. However the drawback of the technique should be borne in mind. Alternatively it may be possible to carry out recovery studies on reference materials, if suitable materials are available. Provided these have been produced by characterisation of natural materials rather than by characterisation of synthetic materials into which the analyte has been spiked, then the recovery study should represent the extraction of real test portions.

1.18 The Validation Tools

(1) Reagent blanks**:** Reagents used during the analytical process (including solvents used for extraction or dissolution) are analysed in isolation in order to see whether they contribute to the measurement signal. The measurement signal arising from the analyte can then be corrected accordingly.

(2) Sample blanks: These are essentially matrices with no analyte. They are difficult to obtain but such materials are necessary to give a realistic estimate of interference that would be encountered in the analysis of test samples.

(3) Samples / test materials: Test materials taken from real samples are useful because of the information they yield on interferences *etc.* which could be realistically encountered in day-to-day work. If the true analyte content of a test material is accurately know it can be used as a way of assessing the accuracy of the method. However the true analyte content is usually difficult to determine unless there is the possibility of using other methods which are known to show negligible bias.

(4) Spiked material: These are material or solutions, which have been fortified with the analyte(s) of interest. These materials or solutions may already contain the analyte of interest so care is needed lest fortification inadvertently leads to levels outside of the range of applicability of the method. Fortification with a known amount of analyte enables the increase in response to the analyte to be measured and calculated in terms of the amount added (assuming 100% recovery), even though the absolute amounts of analyte present before and after the fortification are not know. Note that most methods of fortification add the analyte in such a way that it will not be as closely bound to the sample matrix as it would be if it was present naturally. Therefore, recovery determinations obtained by fortification can be expected to be over-optimistic. The nature of the spike obviously needs to be identified.

(5) (Measurement) Standards: These are traditionally thought of as solutions of single substances but in practice can be anything in which a particular parameter or property has been characterised to a sufficient extent it can be used for reference or calibration purposes.

(6) Reference materials**:** frequently confused with ***certified reference materials***. Reference materials can be virtually any material used as a basis for reference, and could include laboratory reagents of known purity, industrial chemicals, or other artefacts. The property or analyte of interest

needs to be stable and homogenous but the materials does not need to have the high degree of characterisation, traceability and certification more properly associated with certified reference materials.

(7) Certified reference materials: The characterisation of the parameter of interest in a ***certified reference material*** is generally more strictly controlled than for a ***reference material***, and in addition the characterised value is certified with a stated uncertainty by a recognised institution. Characterisation is normally done using several different methods, so that as far as possible, any bias in the characterisation is reduced or even eliminated.

APPENDIX 4

VALIDATION CASE STUDY

VALIDATION AND CONTROL PROTOCOL FOR THE TITRIMETRIC ANALYSIS OF COMPONENTS OF VARIOUS NON AQUEOUS SOLUTIONS

INTRODUCTION

This case study is based on a recent validation exercise conducted by Reagecon, as part of a new product introduction. It details below the steps taken in the validation and samples of the methods, which were validated in the form of Test procedures. Finally an example spreadsheet shows the data analysis, results and conclusions.

SUMMARY

The following general protocol is designed to provide traceability to primary standards and measures of accuracy, linearity and precision for the determination of chemical raw materials: TCA, DCA and Acetic anhydride, and active ingredients in Reagecon manufactured products by pH and REDOX titration. In addition it is intended to provide ongoing control of the methods.

The chart 'Validation Summary data' is the key to the validation plan. It tabulates those Products and Ingredients, which form the scope of the study, the procedure names, reference to the procedures on which the methods are based, the location of raw and calculated data and a summary of the results.

As a condensed example of the practice and evolution of this exercise, the procedures and results of the necessary steps to validate the testing of TCA/DCM are contained in this report. These are Validation Procedure for Sodium Hydroxide Burette (Burette 2), Validation Procedure for the assay of TCA (the active ingredient) and Validation Procedure for TCA/DCM (the finished product).

STEPS FOR VALIDATION AND CONTROL

1 CALIBRATION OF ELECTRODES

For aqueous pH titrations perform a calibration of the electrode (Dolmen 10) to check the electrode parameters. Fresh buffer solutions (specified value ± pH 0.01) must be used for this purpose. Use CALIBRATION PROCEDURE FOR pH ELECTRODE - Reagecon document code: CALPH.

Calibration requirements:

Slope > 0.97
pH(as) 6.9...7.1
(with Dolmen 10 comb. glass electrode)

In the case of non aqueous titrations, perform a calibration of the electrode pair: pH Glass electrode/ Double Junction sleeve junction reference with an outer salt bridge

composed of 2 mol/l Lithium chloride in propan-2-ol, to check the electrode parameters. Fresh buffer solutions (specified value ± pH 0.01) must be used for this purpose. Use CALIBRATION PROCEDURE FOR pH ELECTRODE - Reagecon document code:- CALPH.

Calibration requirements:

Slope > 0.97
pH(as) 4.9...9.1

In the case of redox titrations, calibration of the combined Platinum/reference electrode (Dolmen 23) is not required.

2 VALIDATION OF AUTOTITRATOR BURETTE

Five Burettes/'Exchange Units' will be employed in the validation studies

1. HCl 1.0 mol/l
2. NaOH 1.0 mol/l
3. TBAH (IPA/Methanol) 0.1 mol/l
4. Acetous perchloric acid 0.1 mol/l
5. Sodium thiosulphate 0.1 mol/l

Each burette will be validated according to the procedure given for each Burette Validation. This will quantify the volumetric accuracy, reproducibility and linearity of the burette and standardise the burette against a NIST traceable reference material.

3 VALIDATION OF INGREDIENT ASSAY

When steps one and two have been completed accuracy of the components of the autotitrator is ensured. The concentration of the titrant is accurately know and is traceable to a primary standard.

For each of the raw materials and products, method development is concerned with the specific conditions of titration. These are optimised by experiment with typical product samples, and the optimised conditions are stored in the memory of the Metrohm 702SM Titrino autotitrator with method names given in the validation procedures.

The methods are now validated using the specific validation procedure with laboratory prepared amounts of the products and ingredients. This procedure measures the mean of ten replicate titrations, linearity in the range of interest (typically, target value, low and high values ± 25% of target value), standard deviation and a measure of any systematic error that may be present. It also provides similarly comprehensive data for a control material for subsequent use in routine analysis to ensure adequate Quality Control of results obtained on production batches.

The validation procedures so developed and employed will subsequently be issued as controlled documents and will be the QC test procedures in the future.

CONTROL PROCEDURE

The determinations by automated potentiometric titration have now been validated. To maintain the validation each time a production batch is tested, an individually sealed 100ml Control Sample, directly traceable to the validation study will also be tested. The results of this Control test will then be assessed and must be within 3 standard deviations of the result achieved in the validation to be acceptable. After the initial control batch has been consumed, another batch of control samples will be produced and compared to the previous Control Batch, for traceability, and used for a further year.

2. VALIDATION OF AUTOTITRATOR BURETTE

Guidelines

Summary

As a guideline for the preparation of standard operating procedures to check a titration system comprising a titrator, dispensing unit, measuring chain and possibly a sample changer, use the procedure described below. The limiting values specified must be considered as recommendations. Specific limiting values must be defined in the particular standard operating procedure regarding in-house requirements to the demanded accuracy of the measurement system.

Test intervals

Annual . A special validation is advisable when one or more components of the titration system are replaced.

Maintenance/Service

An indispensable requirement to assure operation conforming to GLP for all instruments used in the laboratory is careful maintenance and cleaning. Particular attention should also be paid to the accurate handling of such instruments. The instructions for use supplied with the instrument should be accessible to all workers in the laboratory.

Method

Apparatus required

- Titrator with dosing unit and stirrer (rod or magnetic stirrer)
- Combined pH glass electrode (Dolmen 10).
- Analytical balance, resolution min. 0.1 mg
- 10 clean 100 mL titration vessels or beakers
- Calibrated thermometer or temperature sensor

Chemicals required

- Primary standard, potassium hydrogen phthalate, certified, declared content min. 99.95 %, dried for 2 h at 120°C and then allowed to cool off in a desiccator, where it is stored

- Fresh titrant c(NaOH) = 1 mol/L

Sodium hydroxide readily absorbs carbon dioxide from the ambient atmosphere. Protect your titrant solution against the ingress of CO_2 by attaching a drying tube filled with CO_2 absorber.

Requirements

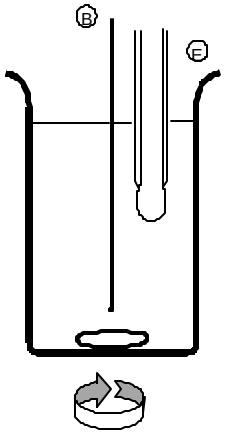
Protect experimental setup against direct sunlight and avoid draughts. The system must be in thermal equilibrium.

The balance should be in calibration.

The time interval between the titrations of a series should be kept to a minimum.

When performing the titrations, ensure optimum mixing of the sample solution. The setup illustrated below has proved its worth in practice.

Arrangement in titration beaker



B ***Burette tip***
E ***Electrode***

The primary standard must be dried in a flat and allowed to cool off in a desiccator for at least 1 h. Standard substances must always be stored in a desiccator.

With pH titrations, it is advisable first to perform a calibration of the electrode to check the electrode parameters. Fresh buffer solutions (specified value ± pH 0.01) must be used for this purpose.

Calibration requirements:

Slope > 0.97
pH(as) 6.9...7.1
(with Dolmen 10 glass electrode and 3 M KCl/AgCl as electrolyte)

In end-point titrations (SET) to a preset pH value, a calibration is essential. Further, it is advisable to enter the working temperature for compensation in the titrator or attach a Pt100 or Pt1000 sensor to the titrator. The titrant solution should be in thermal equilibrium with the surroundings.

Procedure

1. Calculation formula for the titrant molarity

$$\text{Titer} = RS1 = \frac{C00 * C01}{C02 * EP1} \quad \text{or} \quad C00 * C01 / (C02 * EP1) \quad \textit{with 4 decimal places}$$

C00 Sample size of primary standard in g
C01 Theoretical consumption of titrant for 1 mole primary standard in mL (1000 with 1 molar titrant)
C02 Molar mass of primary standard (potassium hydrogen phthalate 204.23 g/mol)
EP1 Consumption of titrant in mL

Setting titration parameters

The settings of the titration parameters depend on the titration mode. The mode which is used most frequently should be selected.
A table is available listing the recommended relevant parameters for the instruments and modes for the titration of potassium hydrogen phthalate with c(NaOH)=1.0 mol/L.

Method

10 titrations are performed with the same instrument settings and different weights of the primary standard (e.g. potassium hydrogen phthalate). The sample size should be varied in random order and result in a consumption of titrant of ca. 0.1 to 1.0 cylinder volume. Refilling of the cylinder should be avoided, except between samples.

The weighed samples are dissolved in ca. 60 mL distilled or deionised water and then immediately titrated.

Interpretation of the results

The relevant parameters for the validation of measuring instruments are the reproducibility (precision) and the accuracy of the measurement results. To assess these quantities, proceed as follows:

The values obtained from the 10 determinations (***molarity*** of the titrant) are used for the calculation of the mean value $\bar{x}$ and the absolute standard deviation s_{abs}. These calculations can be performed directly with the built-in statistics function of the instrument, if available, or by using a pocket calculator or a PC (Personal Computer) with a suitable software package (e.g. spreadsheet program).

Mean value

$$\bar{x} = \frac{x_1 + x_2 + \ldots + x_n}{n} = \frac{1}{n}\sum_{i=1}^{n} x_i = \frac{\text{Sum of the individual values}}{\text{Number of individual values}}$$

Standard deviation $s_{abs} = \sqrt{\frac{1}{n-1}\sum_{i=1}^{n}\left(x_i - \bar{x}\right)^2} = \sqrt{\frac{\sum_{i=1}^{n} x_i^2 - \frac{\left(\sum_{i=1}^{n} x_i\right)^2}{n}}{n-1}}$

Reproducibility, scatter (precision)

The reproducibility of the measurement is expressed by the relative standard deviation.

rel. standard deviation $s_{rel} = \frac{s * 100}{\bar{x}} = \frac{\text{abs. standard deviation} * 100}{\text{mean value}}$

Requirement: The relative standard deviation should be $\leq$ 0.3 %.

(While the limiting value of 0.3 % for the rel. standard deviation is a limit conforming to practice and can easily be met in the normal case, under optimum conditions rel. standard deviations of 0.1 % and lower are obtainable.)

Accuracy

The accuracy of the results obtained depends on the content of the primary standard guaranteed by its producer (assumption: 100.00%).

*a. Calculation of the theoretical **molarity** value as a function of temperature*
The theoretical ***molarity*** value of the titrant solution at 20°C is 1.000 with a reduction in ***molarity*** of 0.02 % per degree temperature rise (with aqueous solutions, see warranty of the chemical producer).

$molarity_{theo}$ (at X°C) = 1.000 + 0.0002 * (20 – x)

b. Calculation of the systematic deviation d_{rel}

The systematic deviation is calculated from

$$d_{rel} = \frac{\text{molarity}_{mean} - \text{molarity}_{theo}}{\text{molarity}_{theo}} * 100$$

Requirement: The systematic deviation should be max. ± 0.5 %.

Note: In sample titrations, reproducibility and linearity (volume vs sample size) are important. There are normally no problems with the accuracy as long as all titrant solutions are subjected to a regular ***molarity*** determination and the ***molarity*** and the sample are determined with the same titration settings.

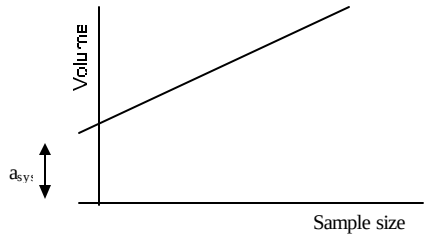
2. Systematic errors

a. Linear regression volume/sample size

To discover systematic errors, e.g. disturbing influences due to the method or solvent blank values, a linear regression of volume (in mL) against sample size (in g) can be calculated. This requires use of a powerful pocket calculator or a statistics package or spreadsheet program on a personal computer. The sample size is plotted as the x-co-ordinate (independent variable) and the volume as the y-co-ordinate (dependent variable).

The linear regression draws a line through the experimental points, which minimises the sum of the squares of the individual deviations. The regression line is described by the formula: $y = bx + a$, where a represents the intercept on the y-axis and b is the slope of the line (see diagram below).

Systematic errors of the titration method are manifested in a significant deviation of the zero point co-ordinates of the y-axis (intercept), i.e. the regression line calculated from the value pairs volume/sample size does not intercept the y-axis exactly at the origin of the system of co-ordinates.



a_{sys} as a measure of the systematic error is calculated from the mean values of the x values, the mean values of the y values and the regression coefficient b (slope).

The calculation formulae:

$$\mathbf{b} = \frac{\sum_{i=1}^{n}\left(x_i - \bar{x}\right)\left(y_i - \bar{y}\right)}{\sum_{i=1}^{n}\left(x_i - \bar{x}\right)^2} = \frac{\sum_{i=1}^{n} x_i y_i - \frac{\sum_{i=1}^{n} x_i * \sum_{i=1}^{n} y_i}{n}}{\sum_{i=1}^{n} x_i^{\,2} - \frac{\left(\sum_{i=1}^{n} x_i\right)^2}{n}}$$

$$\mathbf{a_{sys}} = \text{y-intercept} = \bar{x} - b * \bar{y}$$

Assessment

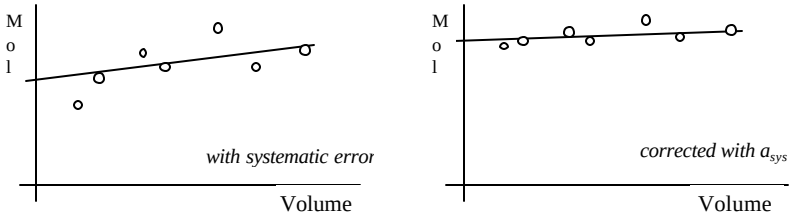
If $a_{sys} > \pm 0.010$ mL (or ± 10 µL), it must be assumed that a systematic error is present. A check on the titration method and other possible disturbing influences due to the system is then imperative. If no optimisation of the validation method is possible, the individual values of the consumption in mL must be corrected by the value of a_{sys}
(volume–a_{sys} in mL) to ensure that the systematic error associated with the method is not incorporated in the assessment of the titrator. The relevant characteristic data for the reproducibility and the accuracy of the titration results must then be recalculated with the corrected consumption values.

3. *b. Linear regression **molarity**/volume*

4. A further possible method to discover systematic errors involves plotting the regression line (scatter diagram) of the value pairs ***molarity***/volume. It is advisable to plot such a diagram as it also provides a good visual impression of the scatter of the results.

5. A significant positive or negative slope of the regression line indicates a fictitious dependence of the ***molarity*** on the magnitude of the volume or the sample size. This can also be an indication of systematic disturbing influences due to the method.

The slope $b_{T/Vol}$ (regression coefficient b, calculation formula, see p. 9) from the equation of the linear function $y = bx + a$ should here be 0.000 in the ideal case, i.e. the line should be horizontal through y=1.000.



Assessment
If $b_{T/Vol}$ is greater than ± 0.0010, a systematic error due to the method must also be assumed here. A correction of the consumption values by a_{sys} (volume in mL – a_{sys} in mL) and a subsequent recalculation of the ***molarity*** shows a dramatic improvement when the regression line (***molarity*** against volume) is replotted.

Conclusion
If systematic errors are found, an attempt must be made to optimise the titration method and adapt the standard operating procedure (SOP) accordingly. If no optimisation is possible or a specified method must be used unchanged, the relevant characteristic data must be calculated with corrected consumption values (volume in mL – a_{sys} in mL).