

Validation of Metrohm titrators (potentiometric) according to GLP/ISO9001

Guidelines

Of interest to: General analytical chemistry

Summary

GLP (Good Laboratory Practice) requirements include the periodic check of analytical instruments for reproducibility and accuracy using **standard operating procedures (SOP)**.

The user is advised to validate the Metrohm titrators as a complete, integrated titration system, i.e. to perform a series of titrations using standard titrimetric substances (primary standards) and critically assess the results using statistical methods.

Checking of the electronic and mechanical components of measuring instruments can and should be undertaken by qualified personnel of the manufacturing company as part of regular servicing. All Metrohm instruments are provided with start-up test routines which check that the relevant assemblies are working perfectly when the instrument is switched on. If no error message is displayed, it can be assumed that the instrument is functioning faultlessly.

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, Metrohm suggests 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.

Application range

These test specifications are applicable to the following Metrohm titrators:

- Titrandos
- Titrimos
- Titroprocessors
- Potentiographs

Test intervals

Annual repetition of the testing of titrators appears appropriate. If a dispensing unit is used in continuous operation or if the work involves frequent use of caustic, corrosive or precipitate-forming titration solutions which have a considerable adverse effect on the dispensing and/or measuring device, it may be advisable to decrease the time between testing to, e.g. every 6 or even 3 months.

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

Internal instrumental test routines

The Metrohm titrators have an internal instrument start-up test and test routines. In the start-up test, the display elements are checked and the contents of the program memories are tested by means of a checksum test. Proper functioning of the data memory area is tested with a write/read test.

With the Titrino instrument series, the RS232 interface is also subjected to an exhaustive test.

If the titrators are regularly maintained, it is generally possible to dispense with the specific validation of the instrument electronics.

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. We also recommend regular servicing of all relevant measuring instruments once a year. Many Metrohm agencies offer favourably priced servicing agreements for their instruments.

Method

If the daily work involves only a few, specific titration methods, for the validation of the titrator it is advisable to select a combination of titrant and sample as similar as possible to those used in one of the frequently employed methods and for which a primary substance of specified, high purity is available. In addition, it should be possible to eliminate any error sources due to the method.

From the multiplicity of all possible combinations of titrants and measuring chains, the pH titration with hydrochloric acid has been selected as an example. The primary standard tris(hydroxymethyl)-aminomethane (TRIS) available from specialist dealers is titrated as a sample. The result is calculated as a titer determination.

Other possible combinations:

Titrant	Primary standard	Comments
HCl	TRIS	Trouble-free
NaOH	Potassium hydrogen phthalate	Carbonate-free proceeding required *
AgNO ₃	Sodium chloride, NaCl	NaCl hygroscopic
Sodium thiosulphate	Potassium hydrogen diiodate	Mind pH-value
Ce(IV) sulphate, Ce ⁴⁺	As ₂ O ₃	Mind pH-value
Potassium permanganate	Disodium oxalate	Mind pH-value
TBAOH	Benzoic acid	Carbonate-free proceeding required *
Perchloric acid	Potassium hydrogen phthalate	Mind temperature
NaNO ₂	Sulphanilic acid	MET mode
EDTA	CaCO ₃	Buffering required

* Sodium hydroxide readily absorbs carbon dioxide from the ambient atmosphere. Protect your titrant solution against the ingress of CO₂ by attaching a drying tube (6.1609.000) filled with CO₂ absorber or an absorption tube (6.1612.003) filled with NaOH.

Other possibilities can be found in the relevant literature, e.g. Metrohm Application Bulletin No. 206.

It is essential to ensure that only highly pure, dried primary substances (content min. 99.5%) are used. Primary standards should not be hygroscopic and must be virtually completely insensitive to CO₂ and air.

If at all possible, aqueous solutions should be selected as titrants and these must also be highly stable to the influences of CO₂, air and light.

Apparatus required

- Titrator with dosing unit and stirrer (rod or magnetic stirrer)
- Exchange unit with anti-diffusion burette tip (6.1543.200)
- Suitable measuring chain, e.g. combined pH glass electrode (6.0232.100)
- Analytical balance, resolution min. 0.1 mg
- 10 clean 100 mL titration vessels or beakers
- Calibrated thermometer or temperature sensor

Chemicals required

- Primary standard, e.g. TRIS, certified, declared content min. 99.5 %, dried for 2 h at 105°C and then allowed to cool off in a desiccator, where it is stored
- Fresh titrant c = 0.1 mol/L, possibly c = 1 mol/L (e.g. c(HCl) = 0.1 mol/L). Titer deviation <0.2 % (commercially available in bottles as certified, ready to use reagent solution)

Requirements

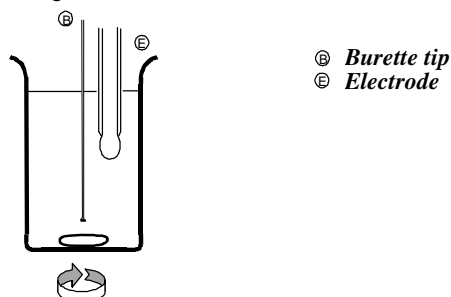
Protect experimental setup against direct sunlight and avoid draughts. The system must be in thermal equilibrium.

The balance should first have been validated.

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



The primary standard must be dried in a flat dish (e.g. 2 h at 105°C, depending on the type of primary standard) 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 (especially in the SET-mode), it is strongly recommended first to perform a calibration of the electrode to check the electrode parameters. Fresh buffer solutions (specified value ± pH 0.02) must be used for this purpose.

Calibration requirements:

Slope > 0.97
 pH(as) 6.9...7.1
 (with comb. glass electrode and 3 M KCl 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 of the titer

For Titrinos

$$\text{Titer} = \text{RS1} = \frac{\text{C00} * \text{C01}}{\text{C02} * \text{EP1}} \text{ or } \text{C00} * \text{C01} / (\text{C02} * \text{EP1})$$

with 4 decimal places

C00 Sample size of primary standard in g
 C01 Theoretical consumption of titrant for 1 mol primary standard in mL (1000 or 10 000 with 1 molar/ 0.1 molar titrant)

C02 Molar mass of primary standard (TRIS 121.14 g/mol)
 EP1 Consumption of titrant in mL

For Titrandos

$$\begin{aligned} \text{Titer} &= \text{R2} = \text{C00} * 1000 / \text{CONC} / \text{CI2} / \text{EP1} \\ \text{mean value} &= \text{R3} = \text{SMN1} \\ \text{abs. Std. Deviation} &= \text{R4} = \text{SSA1} \\ \text{rel. Std. Deviation} &= \text{R5} = \text{SSR1} \end{aligned}$$

C00 Sample size of primary standard in g
 CONC Concentration of the titrant in mol/L
 CI2 = ID2 = Molar mass of primary standard (TRIS 121.14 g/mol)
 EP1 Consumption of titrant in mL

All result variables and statistic functions are available as result templates.

For Titroprocessor 796

$$\text{Titer} = \text{RT1} = \text{SS} * 1000 / \text{C} / \text{ID2} / \text{EP1}$$

SS Sample size of primary standard in g
 C Concentration of the titrant in mol/L
 ID2 Molar mass of primary standard
 EP1 Consumption of titrant in mL

2. Setting titration parameters

The settings of the titration parameters depend on the instrument and titration mode. The mode which is used most frequently should be selected.

If a start volume is used, the required factor has to be calculated for each sample:

For 796 Titroprocessor:

$$\text{factor} = \frac{1000 * \text{C02}}{\text{C} * \text{ID2}} = \frac{1000 * 0.6}{0.1 * 121.14} = 49.5 \approx 50$$

1000 conversion in mL
 ID2 molar mass of primary standard in g/mol
 C concentration of titrant in mol/L
 C02 percentage of volume at EP (e.g 0.6)

For Titrino

$$\text{factor} = \frac{1000 * C02}{C01 * ID2} = \frac{1000 * 0.6}{0.1 * 121.14} = 49.5 \approx 50$$

1000 conversion in mL
 ID2 molar mass of primary standard in g/mol

C01 concentration of titrant in mol/L
 C02 percentage of volume at EP (e.g 0.6)

For Titrande:

The start volume is calculated at the beginning of the method and saved as result R1

$$R1 = \frac{C00 * 1000 * CV02}{CV01 * CI2}$$

1000 conversion in mL
 CI2 molar mass of primary standard in g/mol
 C00 sample size in g
 CV01 concentration of titrant in mol/l
 CV02 percent of volume at EP

The following table lists the recommended, relevant parameters for the instruments and modes for the titration of TRIS with c(HCl)=0.1 mol/L.

Parameter	Instrument		
	719, 794, 798, 799 Titrino	796 Titro- processor	808,809 Titrande
DET pH			
meas.pt.dens.	4	4	4
min. incr.	10.0 µl	10.0 µl	10.0 µl
start V	rel.	rel.	R1
factor	50	50	
stop pH	2.8	2.8	2.8
signal drift	50 mV/min	50 mV/min	50 mV/min
MET pH			
V step	0.10 ml	0.10 ml	0.10 ml
titr.rate	max.	max.	max.
start V	rel.	rel.	R1
factor	50	50	
stop pH	2.8	2.8	2.8
signal drift	50 mV/min	50 mV/min	50 mV/min
SET pH			
EP1 at pH	5.1	5.1	5.1
dynamics	3	3	3
max. rate	5 ml/min	5 ml/min	5 ml/min
min. rate	0.5 µl/min	0.5 µl/min	0.5 µl/min
start V	rel.	rel.	R1
factor	50	50	
stop drift	20 µl/min	20 µl/min	20 µl/min

3. Method

10 titrations are performed with the same instrument settings and different weights of the primary standard (e.g. TRIS). The sample size

should be varied in random order and result in a consumption of titrant of ca. 0.2 to 0.9 cylinder volume. Refilling of the cylinder should be avoided, except between samples.

The recommended sample weight ranges for TRIS are given in the following table:

Cylinder vol.	Weight of TRIS	c(HCl)=
5 mL	120...550 mg	1 mol/L
10 mL	250...1100 mg	1 mol/L
10 ml	25...110 mg	0.1 mol/L
20 mL	50...220 mg	0.1 mol/L
50 mL	120...550 mg	0.1 mol/L

As low sample weights increase the weighing error and hence the scatter of the results, it is advisable to avoid these by using 1.0 molar titrant solutions with 5 mL and possibly 10 mL cylinders.

The weighed samples are dissolved in ca. 40 mL distilled or deionised water and then immediately titrated. The preparation of stock solutions and titration of an aliquot introduces a further source of error (pipetting error) and is thus not recommended.

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 (titer 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). As slightly different results can be obtained in complex calculations with different computing aids owing to the different calculation accuracies, preference should always be given to values calculated in the instrument itself.

Mean value

$$\bar{x} = \frac{x_1 + x_2 + \dots + x_n}{n} = \frac{1}{n} \sum_{i=1}^n x_i$$

= $\frac{\text{Sum of the individual values}}{\text{Number of individual values}}$

Standard deviation

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

$$= \sqrt{\frac{\sum_{i=1}^n x_i^2 - \frac{\left(\sum_{i=1}^n x_i\right)^2}{n}}{n-1}}$$

1. Reproducibility, scatter (precision)

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

rel. standard deviation

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

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 practise and can easily be met in the normal case, under optimum conditions rel. standard deviations of 0.1 % and lower are obtainable with Metrohm titrators.)

2. 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 titer value as a function of temperature

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

$$\text{Titer}_{\text{theo}} (\text{at } X^\circ\text{C}) = 1.000 + 0.0002 * (20 - x)$$

b. Calculation of the systematic deviation d_{rel}

The systematic deviation is calculated from

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

Requirement: The systematic deviation should be max. $\pm 0.5\%$.

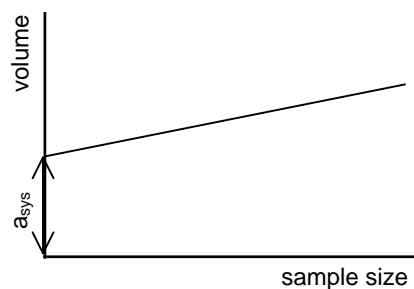
Note: In sample titrations, reproducibility and linearity (volume vs sample size) are important. There are normally no problems with the accuracy as long as all titrant solutions are subjected to a regular titer determination and the titer and the sample are determined with the same titration settings.

3. 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-coordinate (independent variable) and the volume as the y-coordinate (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 coordinates 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 coordinates.



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:

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

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

Assessment

If $a_{\text{sys}} > \pm 10 \mu\text{L}$ for 1 mL-burettes or $a_{\text{sys}} > \pm 50 \mu\text{L}$ for 5, 10, 20 and 50 ml burettes, 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.

If they are necessary, these time-consuming calculations should be performed only with a computer or powerful calculator. However, it must be noted that slightly different results can also be obtained here on different computing systems owing to the different calculation accuracy.

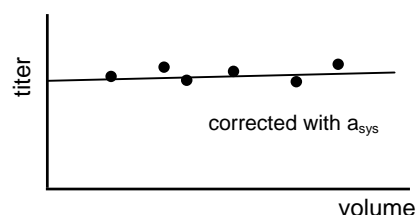
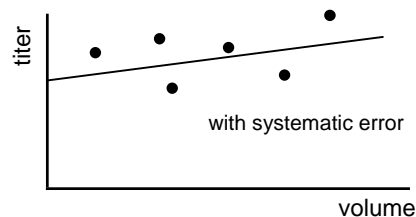
b. Linear regression titer/volume

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

A significant positive or negative slope of the regression line indicates a fictitious dependence

of the titer 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 $\pm 0.0010 \text{ ml}^{-1}$, 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 titer shows a dramatic improvement when the regression line (titer 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).

Possible error sources

• Primary standard	unsuitable, impure, moist, inhomogeneous, no guaranteed primary standard quality
• Balance / weighings	balance too inaccurate, draughts, temperature influences, contaminated balance, temperature gradient titration vessel/balance, careless weighing, sample weight too low
• Titration vessel	contaminated
• Solvent	impure (blank value), poor solubilising power
• Titrant	contains CO ₂ , impure
• Dispensing unit	tubing connections not tight, contaminated cylinder (visible corrosion marks), leaky piston (liquid film or crystals below the piston), filling rate too high, leaking burette tip, air in tubing system, three-way stopcock leaking
• Measurement	contaminated electrode, blocked diaphragm, loose contact at connector, faulty cable, poor mixing of sample solution, unfavourable arrangement of burette tip and electrode, excessive response time of electrode
• Titration	unsuitable titration mode, wrong measurement parameters, titration rate too fast or too slow
• Temperature	temperature fluctuations, especially perceptible with titrants in organic solvents

Recommendations for troubleshooting

With rel. standard deviation too high (poor reproducibility)

- Finely grind fresh the primary standard substance in a mortar, dry and allow to cool off in a desiccator (possibly grind again).
- Ensure complete dissolution of the weighed sample in the solvent.
- Optimise arrangement of burette tip, electrode and stirrer.
- Regenerate or change electrode.
- Optimise titration parameters (see Metrohm Application Bulletins).
- Remove Exchange Unit, clean and possibly change tubing as well as piston and/or cylinder.
- Weigh out sample only after temperature equilibrium established between balance and titration vessel.
- Possibly increase sample weight.

With rel. systematic deviation too high (accuracy unsatisfactory)

- Use pure solvent (without blank value), boil out water if necessary.
- Dry standard primary substance.
- Ensure complete dissolution of the weighed sample in the solvent.
- Use fresh titrant (possibly use different production batch).
- Visual inspection of Exchange unit and replacement if need be.
- Check electrode and titration parameters, regenerate or replace electrode.
- Check balance.

Procedure with values not conforming to specifications

All non-conforming values must be commented on in the validation record and the subsequent procedure noted.

If excessive deviations are found, the different points under the sections “Possible error sources” and “Recommendations for troubleshooting” must be carefully checked and the disturbing influences eliminated. It is essential to repeat the validation. If unsatisfactory results are still obtained when the test series is repeated, the validation must be performed again by a different person on a different day.

If doubt exists regarding the precision of the dosing unit, this can be checked separately (see Metrohm Application Bulletin No. 238).

Literature

Further information on titrations and titer determinations can be found in the following publications:

- Metrohm Application Bulletin No. 206, Titer determinations in potentiometry
- Metrohm Application Bulletin No. 238, Check of Dosimat according to GLP/ISO
- Instrumental Titration Techniques, *F. Oehme and W. Richter*, Hüthig Verlag, Heidelberg, 1987
- Practical Aspects of Modern Titration, *W. Richter and U. Tinner*, Monographs Metrohm AG, 1988
- Electrodes in potentiometry, *U. Tinner*, Monographs Metrohm AG, 1989

On the following pages you will find an example of a validation record and a diagram of the linear regression mentioned above.

Validation Record				Company :	<i>Metrohm Ltd.</i>	
Titrators				Division :	<i>Ti.-Marketing</i>	
Temperature in °C :	25	Instrument :	<i>809 Titrande</i>			
Titrant :	<i>HCl</i>	Electrode :	<i>6.1231.000</i>			
Conc. in mol/L :	<i>1.0</i>	Slope :	<i>99.0</i>			
Lot / Date of manufact. :	<i>22.01.2002</i>	pH (as) :	<i>6.961</i>			
Primary standard :	<i>TRIS</i>	Exchange Unit :	<i>800</i>			
Molar mass in g/mol	<i>121.14</i>	Burette size :	<i>10 ml</i>			
Titration parameters :			Mode :	<i>DET pH</i>		
Stop pH	<i>off</i>	signal drift	<i>50 mv/min</i>			
Stop EP	<i>1</i>	measr. Pt. Density	<i>4</i>			
Vol. after EP	<i>2 ml</i>	min. increment	<i>10 µl</i>			
			EP criterion	<i>5</i>		
Smpl size :	Volume :	Titer :	No.	Remark :		
<i>0.5883 g</i>	<i>4.858 ml</i>	<i>0.9996</i>	1			
<i>0.3439 g</i>	<i>2.843 ml</i>	<i>0.9987</i>	2			
<i>0.7883 g</i>	<i>6.511 ml</i>	<i>0.9994</i>	3			
<i>0.9979 g</i>	<i>8.243 ml</i>	<i>0.9994</i>	4			
<i>1.0701 g</i>	<i>8.837 ml</i>	<i>0.9997</i>	5			
<i>0.8896 g</i>	<i>7.347 ml</i>	<i>0.9995</i>	6			
<i>0.4897 g</i>	<i>4.048 ml</i>	<i>0.9987</i>	7	Mean =	<i>0.9993</i>	S _{abs} = <i>0.0004</i>
<i>0.3691 g</i>	<i>3.031 ml</i>	<i>0.9987</i>	8	Titer _{ref} =	<i>0.9990</i>	S _{rel} = <i>0.04 %</i>
<i>0.2604 g</i>	<i>2.150 ml</i>	<i>0.9997</i>	9	d _{rel} =	<i>0.03 %</i>	a _{sys} = <i>-0.0044 ml</i>
<i>0.6814 g</i>	<i>5.628 ml</i>	<i>0.9994</i>	10			b _{T/Vol} = <i>0.0001</i>
Result :						

Date :	23.01.2002	Signature :	Haider	Vis.:	
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