Application Bulletin

Of interest to: General analytical chemistry

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

Guidelines

Summary

GLP (**G**ood **L**aboratory **P**ractice) 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 componentries 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. Instruments from the Metrohm company are also supplied with built-in diagnostic programs which enable the user to check the functioning of certain componentries in the event of malfunctions or faulty behaviour and to localise the fault. Diagnostic programs may also be integrated in a validation procedure.

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:

Titroprocessors
Titrinos
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 instrument 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.
In the case of the 670 Titroprocessor, the presence and operational readiness of a Dosimat is verified. 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_2O_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 CO_2 by attaching a drying tube (6.1609.000) filled with CO_2 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 CO_2 and air. If at all possible, aqueous solutions should be selected as titrants and these must also be highly stable to the influences of CO_2, air and light.

Apparatus required

- Titrator with dosing unit and stirrer (rod or magnetic stirrer)
- Exchange unit with anti-diffusion burette tip (6.1543.050)

- Suitable measuring chain, e.g. combined pH glass electrode (6.0233.100)
- 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. 99.95 %, 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

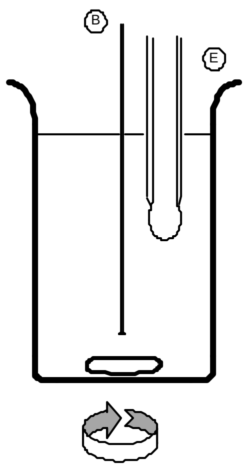
Protect experimental setup against direct sunlight and avoid draughts. The system must be in thermal equilibrium.

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



B *Burette tip*
E *Electrode*

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, it is advisable 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 for 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 mole 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 670 Titroprocessor

$$\text{Titer} = \text{R1} = \text{S0} * \text{C1} / \text{C2} / \text{E1}$$ *with 4 decimal places*

S0 Sample size of primary standard in g
C1 Theoretical consumption of titrant for 1 mole primary standard in litres (1000 or 10 000 with 1 molar/ 0.1 molar titrant)
C2 Molar mass primary standard (TRIS 121.14 g/mol)
E1 Consumption of titrant in litres

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.
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.

Instrument	**Parameter**		
702, 716, 719, 720, 718 Titrino	**DET pH** meas.pt.density 4 min. incr. 10.0 µl start V: rel. factor 50 stop pH 2.8 signal drift 50 mV/min	**MET pH** V step 0.10 ml titr.rate max start V: rel. factor 50 stop pH 2.8 signal drift 50 mV/min	**SET pH** EP1 at pH 5.1 dynamics 3 max. rate 5 ml/min min. rate 0.5 µl/min start V rel. factor 50 stop drift 20 µl/min
686, 682 and 678 Titroprocessor	**GET pH** titr.rate 0.10 ml/min anticip. 0 stop pH 3.0 start pH 7.0 EP crit. 3	**MET pH** drift 50 mV/min stop pH 2.8 start pH 8	**SET pH** EP1 pH 5.1 dyn. ΔpH1 3.0 drift1 10,0 mV/s t(delay)1 10s dV/dt 2
670 Titroprocessor	**DYNT** MEAS1 QUANTITY pH DRIFT /min 20 mV M.DELAY 38 s TBEG VOLUME 0 DOS.RATE MAX. M.VALUE pH 8.0 DYNT 1 MPT.DENSITY 4 DOS.RATE /min 5.0 mL TSTOP M.VALUE pH 3.0 END	**MONT** MEAS1 QUANTITY pH DRIFT /min 20 mV M.DELAY 38 s TBEG VOLUME 0 DOS.RATE/min 150 mL M.VALUE pH 8.0 MONT 1 V.MONOT 100 uL DOS.RATE /min 5.0mL TSTOP M.VALUE pH 3.0 END	**SET** MEAS1 QUANTITY pH DRIFT /min 20 mV M.DELAY 38 s TBEG VOLUME 0 DOS.RATE MAX. M.VALUE pH 8.0 SET1 EP.Value 5.1 DOS.RATE MAX TSTOP s.DELAY 25 V.STOP 25 mL END

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 volume	Weight range for TRIS	c(HCl)=
5 mL	120...550 mg	1 mol/L
10 mL	250...1100 / 25...110 mg	1 mol/L / 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

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

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

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 ≤ 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 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).

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

b. Calculation of the systematic deviation d_{rel}

The systematic deviation is calculated from

$$\mathbf{d_{rel}} = \frac{titer_{mean} - titer_{theo}}{titer_{theo}} * 100$$

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 titer determination and the titer and the sample are determined with the same titration settings.

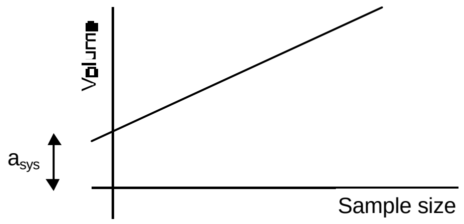
3. Systematic errors

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:

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

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

Assessment

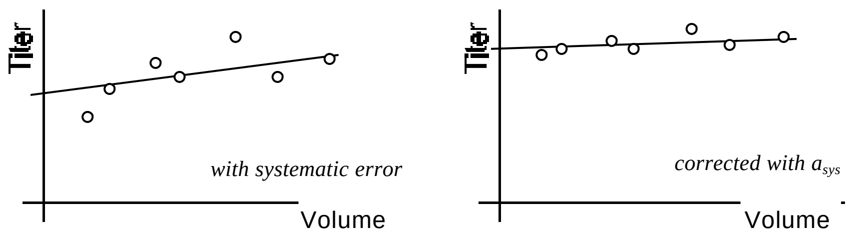
If $a_{sys} > \pm$ 0.010 mL (or $\pm$ 10 µL), it must be assumed that a systematic error is present. A check on the titration method and other possible disturbing influences due to the system is then imperative. If no optimisation of the validation method is possible, the individual values of the consumption 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.

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 ± 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 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
• Titrator	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).

The Titrinos include a test data set in the pertinent menu program to check the internal evaluation algorithms of incremental titration methods (DET, MET). Numerous users have testified to the worth of these algorithms in daily use. If irregular results are obtained, prime consideration should be given to defects in the titration method or the working technique.

Most Metrohm titrators have a special diagnostic program which enables malfunctions of the instruments to be traced and localised. If a fault in the instrument is suspected (repeated appearance of errors, large scatter of results), the diagnostic program must be run through carefully, step by step. The exact procedure is described in the instructions for use of the titrator in question.

If a malfunction can be localised in the diagnostic tests, this must be reported to Metrohm service and the instrument removed from service until the fault has been rectified.

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.

The last page can be used as a master for copies of the validation record.

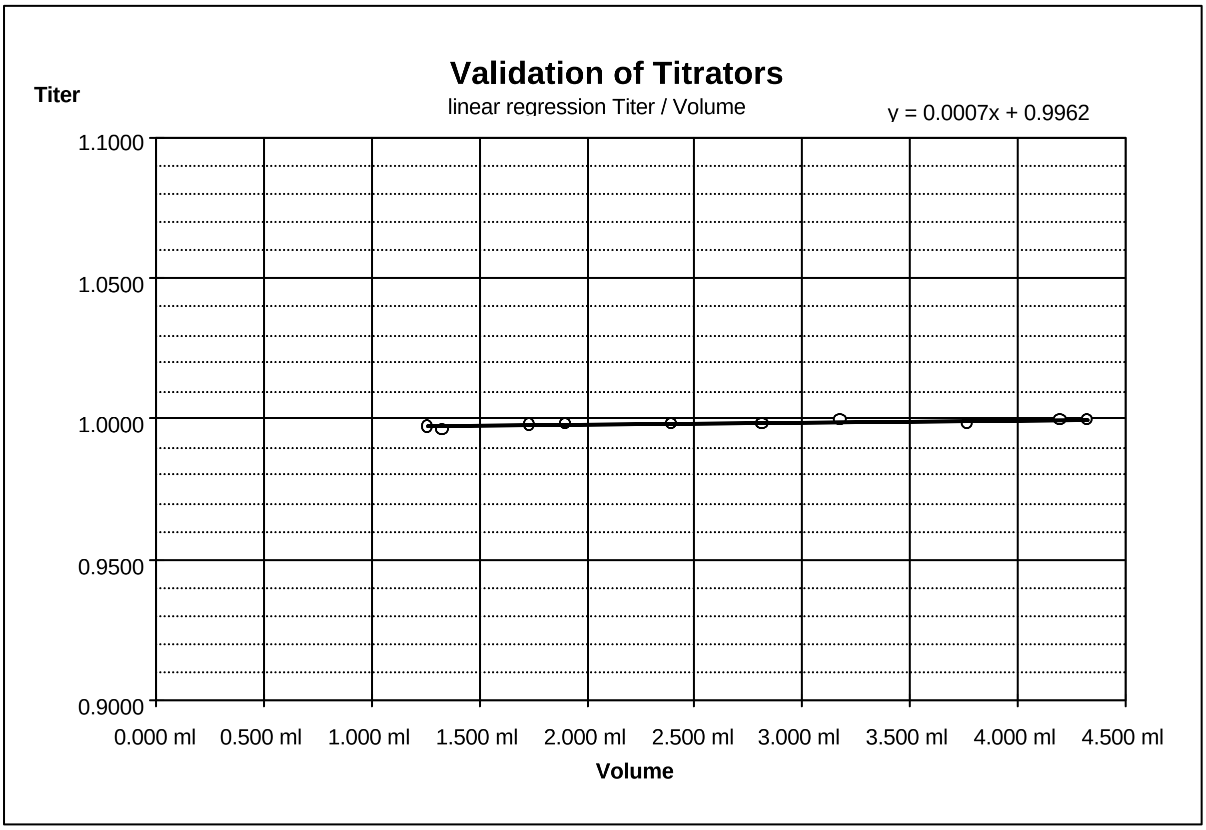
PC users can purchase a 3.5“ diskette from Metrohm with a worksheet for the statistical evaluations in file formats of Excel 5.0 and Excel 4.0 (Application Bulletin 252/1 Disk 8.000.8003).

Validation Record Titrators		Company :	*Metrohm AG*
		Division :	*Chem.Lab 1*
Temperature in °C :	*24.5*	Instrument :	*DMS Titrino 716 /OE9 135*
Titrant :	*HCl*	Electrode :	*6.0219.100/II*
Conc. in mol/L :	*1.0*	Slope :	*0.998*
Lot / Date of manufact. :	*4.09.94*	pH (as) :	*7.03*
Primary standard:	*TRIS*	Exchange Unit :	*6.3011.253/ I*
Molar mass in g/mol	*121.14*	Burette size :	*5 mL*
Titration parameters :		Mode :	*DET pH*
meas.pt.density :	*4*	*stop pH :*	*2*
min.incr.	*10 µL*	*signal drift :*	*50 mV/min*
start V	*rel.*		
factor	*5*		

Smpl size :	Volume :	Titer :	No.	Remark :			
0.1608 g	*1.332 ml*	*0.9962*	1				
0.3398 g	*2.810 ml*	*0.9983*	2				
0.1520 g	*1.258 ml*	*0.9973*	3	*Calculations :*			
0.4558 g	*3.769 ml*	*0.9984*	4	s_{rel} = 0.0010 * 100 / 0.9983 = 0.10 %			
0.2301 g	*1.902 ml*	*0.9985*	5	$Titer_{ref}$ = 1.000 + 0.0002 * (20 -24.5°C) = 0.9991			
0.2895 g	*2.394 ml*	*0.9982*	6	d_{rel} = (0.9983 - 0.9991) / 0.9991 * 100 = -0.08 %			
0.5237 g	*4.325 ml*	*0.9995*	7	Mean =	*0.9983*	s_{abs} =	*0.0010*
0.3848 g	*3.179 ml*	*0.9993*	8	$Titer_{theo}$ =	*0.9991*	s_{rel} =	*0.10 %*
0.2089 g	*1.729 ml*	*0.9976*	9	d_{rel} =	*-0.08 %*	a_{sys} =	*0.0043 ml*
0.5082 g	*4.198 ml*	*0.9993*	10			$b_{T/Vol}$ =	*0.0007*

Result : *ok*

Date :	*5.09.94*	Signature :	D. Möckli	Vis. :	VS



Validation of Titrators
linear regression Titer / Volume
y = 0.0007x + 0.9962
Titer
1.1000
1.0500
1.0000
0.9500
0.9000
0.000 ml
0.500 ml
1.000 ml
1.500 ml
2.000 ml
2.500 ml
3.000 ml
3.500 ml
4.000 ml
4.500 ml
Volume

Validation Record Titrators	Company :
	Division :
Temperature in °C :	Instrument :
Titrant :	Electrode :
Conc. in mol/L :	Slope :
Lot / Date of manufact. :	pH (as) :
Primary standard :	Exchange unit :
Molar mass in g/mol	Burette size :
Titration parameters :	Mode :

Smpl size :	Volume :	Titer :	No.	Remark :	
			1		
			2		
			3		
			4		
			5		
			6		
			7	Mean = *#DIV/0!*	s_{abs} = *#DIV/0!*
			8	$Titer_{ref}$ = *1.0040*	s_{rel} = *#DIV/0!*
			9	d_{rel} = *#DIV/0!*	a_{sys} = *#DIV/0!*
			10		$b_{T/Vol}$ = *#DIV/0!*

Result :

Date :	Signature :	Visa :