
Application Bulletin

Of interest to: General analytical chemistry

Validation of Metrohm KF Titrators and KF Oven according to GLP/ISO9001

Guidelines

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 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 with KF mode (672, 682, 678)
Titrinos with KF mode (701, 720)
KF Processor (658)
KF Oven (707)

Test intervals

Annual repetition of the testing of Karl Fisher titrators appears appropriate. If a titrator 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 and of the oven are replaced.

Internal instrument test routines

The Metrohm titrators have an internal instrument start-up check and test routines. In the start-up test, the display elements are checked and the content of the program memory is 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/Serviceing

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

It is recommended to work with certified standard solutions with definable guaranteed water contents. It can be bought from all leading chemical producers. Solid substances, as disodium tartrate dihydrate, can be used too. Pay attention to complete dissolution in the working medium.

Standard solutions are available with water contents of $10,0 \pm 0,1$ mg/g (for volumetric determinations of water content), $0,1 \pm 0,03$ mg/g or $0,1 \pm 0,005$ mg/g (for coulometric determinations).

If only a few specific samples are operated, a specific standard solution or substance with defined, exactly known water content can be used for proceeding a sample series and for the determination of the refunding. However the requirement is, that the conditions, which a primary standard (specific, absolute water content, high purity, resistance) sets, must be 100 % fulfilled. If this is the case, a validation for the operation, which is used in the daily work, can be established.

If a KF Oven is included in the validation, the same procedure can be performed. In particular the KF Oven demands the same conditions relating to the checking of consistency and maintenance. Standard solutions and primary standards are both applicable. The heating temperature and duration for solid standard substances must be observed carefully.

Apparatus required

- Titrator with dosing unit and magnetic stirrer
- Exchange unit with anti-diffusion burette tip (6.1543.050)
- Double Pt electrode (6.0338.100) or adequate
- KF Cell, complete
- Weighing adds (syringe with a long, possibly a thin needle 6.2816.030)
- Printer
- KF Oven 707 with glass or aluminium sample boat, ev.

Chemicals required

- Water standard solution (10,0 mg/g water content), recommended
- or primary standard, e.g. as disodium tartrate dihydrate (pay attention to the solubility, not unlimited recommended)
- Karl Fisher reagent as titrant (titer 5 or 2 mg/ml) with solvent or combined reagent, with methanol as recipient
- Molecular sieve (6.2811.000)

Requirements

KF titrators should not be installed in rooms with high temperature fluctuations. They should not be near cooling thermostats or heaters. If water baths or wash basins are in direct proximity, a splash guard must be guaranteed.

The drying tube of the titration cell must be hard-faced with a drying substance or molecular sieve, resp.

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

The balance should first have been validated.

The KF Oven should be tested with a calibrated thermometer during 2 hours, checking if the adjusted temperature will be held (standard value $\pm 5^{\circ}\text{C}$).

The weighing adds should be stored in dessicator.

The series of determinations should be carried out without a break.

Weighing and sample boats should be washed with unaqueous solvents, dried and stored in a desiccator.

During execution of the determination an effective mixing of the working medium must be guaranteed.

Fresh reagents should be used (titrant, standard solutions).

Procedure

1. Calculation formula for titer determination

For Titrinos and Titroprocessors

For standard solutions in mg/g:

$$\text{Titer} = \text{RS1} = \frac{\text{C00} * \text{C01}}{\text{EP1}} \quad \text{with 3 decimal places}$$

C00 Sample size of primary standard in g
 C01 Water content of the standard in mg/g
 EP1 Consumption of titrant in mL

*** The 701 KF Titrino has a fixed integrated formula.

For more information see the specific Instructions for use.

2. Setting the titration parameters

The settings of the titration parameters depend on the instrument and titration mode. In general the default parameters of the KF mode can be used. Usually the Ipol method with given polarisation current is to be applied. It is also possible to select Upol mode, which uses a selectable polarisation voltage. The relevant parameters are:

Titrinos

Ipol:

titration direction: -
 I(pol): 50 μA
 EP at U: 250 mV
 dynamics: 100 mV

drift correction: no

Upol:

titration direction: +
 U(pol): 400 mV
 EP at I: 50 μ A
 dynamics: 5 - 10 μ A
 drift correction: no

Titroprocessors

titration rate
 of the Dosimat: $dV/dt = \sim 2$

EP at U: 250 mV
 (t)delay: 5 s
 extr.time: 0 s

KF Drying Oven

temperature: 160°C
 purge time: min. 300 sec
 flow rate: ca. 80 mL/min

3. Method

10 titrations are performed with the same instrument settings and different sample sizes of the primary standard. We propose to weigh the sample size instead of dosing a certain volume. With an appropriate weighing technique a more precise sampling is possible. The sample size should be varied in random order and result in a consumption of titrant of 0.2 to 0.9 cylinder volume approx. Avoid refilling of the cylinder.

Using a KF Oven it is recommended to dose the methanol standard solution in a preheated sample boat through the septum stopper with the help of a syringe and afterwards to weigh back the syringe. Solid primary substances will be weighed in a weighing add (e.g. weighing spoon) and after removing the cap on the KF Oven passed in the preheated sample boat.

Do not open the KF Drying Oven during operation or only as less as possible. Therefore a determination of a blank value can be dispensed. Though a reproduction of a blank value should not meet the expected results, it is recommended to apply a sufficient heating period and to consider a blank value for the determination, which will be subtracted from the consumption of titrant.

Recommendations for the practice:

Water is everywhere. Pay attention that the operation is absolute water free. Plastic tubings are pervious for water vapour. Rinse thoroughly the tubing of the dosing unit with titrant before starting the determination se-

ries. The tightness of the titration cell must be guaranteed and should be checked regularly. Particularly pay attention to the O-ring. The KF cell should show a small dead volume. It should be equipped either with a drying tube (6.1609.000) filled with molecular sieve, or an absorption tube (6.1612.003) filled with KF reagent. Do not open the KF cell frequently.

The sample addition should, if possible, be effectuated with a syringe, which goes through the septum stopper. Before using the syringe (recommendation: 5 mL syringe) rinse it with standard solution. After breaking the vial aspirate approx. 1 mL standard solution and fill up the rest of the syringe volume with air. By holding upward the pinpoint and expelling the content (air and solution) the syringe will be rinsed and the whole inner surface of the syringe will be moistened. Afterwards fill up the syringe with the desired standard solution, without aspiration of air. After wiping off the syringe put it on the balance, tare it and start the titrator. The sample quantity should be added with one stroke, without dipping the syringe. Aspirate the last drop. Reweigh the syringe. The sample weight may be transmitted directly from the balance to the titrator or can be introduced by hand.

Organic solvents (methanol, xylol, etc.) show a high thermal expansion coefficient (ca. 0,1 % per °C). Pay attention that the apparatus (balance, dosing unit, etc.) and the needed reagents are in thermal equilibrium. Efficient operation without interruption can avoid disturbing influences of ambient temperature changes for a period of determination series of 10 titrations. When using standard solutions 5 to 10 determinations can be carried out without changing the solvent.

Pay attention to the recommendations of the primary standard manufacturer.

Neat and exact operation is self-evident but indispensable for a validation of Karl Fischer titrators.

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

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

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

1. Reproducibility, scatter (precision)

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

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

Requirement. The relative standard deviation should be $\leq 0.5 \%$.

Using a KF Oven a higher limit (recommendation 1.0 %) may be appropriate, as more influences can affect the result.

2. Accuracy

As producers of KF reagents don't guarantee any exact titer, the absolute accuracy of the results obtained cannot be assessed. Therefore the accuracy of the results obtained depends on the content of the primary standard only which must be guaranteed by its producer.

It can be assumed that the accuracy is given, in case the titer of all titrants are regularly checked and the titer as well as the samples are determined with the same titration parameters.

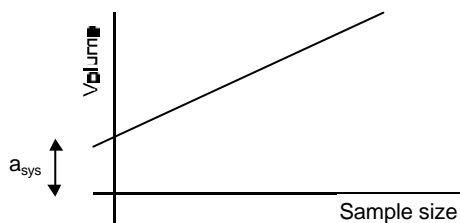
3. Systematic errors

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-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 formula:

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

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

A casual blank value must be subtracted from a_{sys}

Assessment

If $a_{\text{sys}} > \pm 0.020 \text{ mL}$ or $20 \mu\text{L}$ (with KF Oven $\pm 0,050 \text{ mL}$), 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 $- a_{\text{sys}}$ 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.

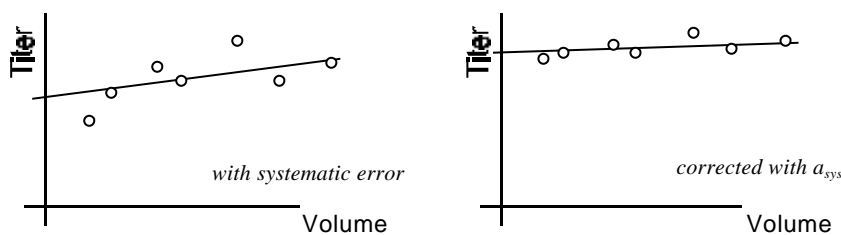
Using a KF Oven over a longer heating period an automatic drift value deduction or a consideration of a blank value is indicated.

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 above) from the equation of the linear function $y = bx + a$ should here be 0.000 in the ideal case, e.g. the line should be horizontal through $y = \text{theor. titer}$.



Assessment

If $b_{T/Vol}$ is greater than ± 0.0100 , 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 impure, no guaranteed primary standard quality, no certification
- Balance/weighing balance too inaccurate, draughts, temperature influences, contaminated balance, temperature gradient titration vessel/balance, careless weighing, sample weight too low
- KF Oven leaky connections, wrong heating temperature
- Titration cell contaminated, leaky septum or connections, consumed molecular sieve
- Solvent impure
- Titrant impure
- Exchange unit tubing connections not tight, contaminated cylinder (visible corrosion), 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 or broken electrode, loose contact at connector, faulty cable, poor mixing of sample solution, unfavourable arrangement of burette tip and electrode, excessive response time of electrode
- Titrator wrong measurement parameters, titration rate too fast or too slow
- Temperature temperature fluctuations

Recommendations for troubleshooting

With rel. standard deviation too high (poor reproducibility))

- Optimise arrangement of titration and control parameters (see Metrohm Application Bulletins literature notice below).
- Check tubing connections.
- Remove Exchange Unit, clean and possibly change tubing as well as piston and/or cylinder. Dry the unit carefully before assembling.
- Weigh out sample only after temperature equilibrium established between balance and titration vessel.
- Use fresh solvent (new vial).
- Use fresh titrant (possibly other production batch).
- Check tightness of the KF oven

With rel. systematic deviation too high (accuracy unsatisfactory)

- Use fresh titrant (use different production batch, if possible).
- Visual inspection and replacement if need to be of Exchange unit.
- Check electrode and titration parameter, regenerate or replace electrode.
- Check balance.
- Check heating temperature of KF Drying Oven.

Procedure with values not conforming to specifications

All non-conforming values must be commented on 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).

Most Metrohm titrators have a special diagnostic program which enables to trace and localise malfunctions of the instruments. 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 KF titrations can be found in the following publications:

- Metrohm Application Bulletin No. 77, Karl Fischer water determinations
- Hydranal®, Practical course, Water reagents according to Eugen Scholz, Riedel de Haën, 1987
- Water Determination by Karl Fischer Titration, G. Wieland, GIT Verlag, 1985
- Instrumental Titration Techniques, *F. Oehme and W. Richter*, Hüthig Verlag, Heidelberg, 1987
- Practical Aspects of Modern Titration, *W. Richter and U. Tinner*, Monographs Metrohm Ltd. 1988
- Metrohm Application Bulletin No. 238, Check of Dosimat according to GLP/ISO

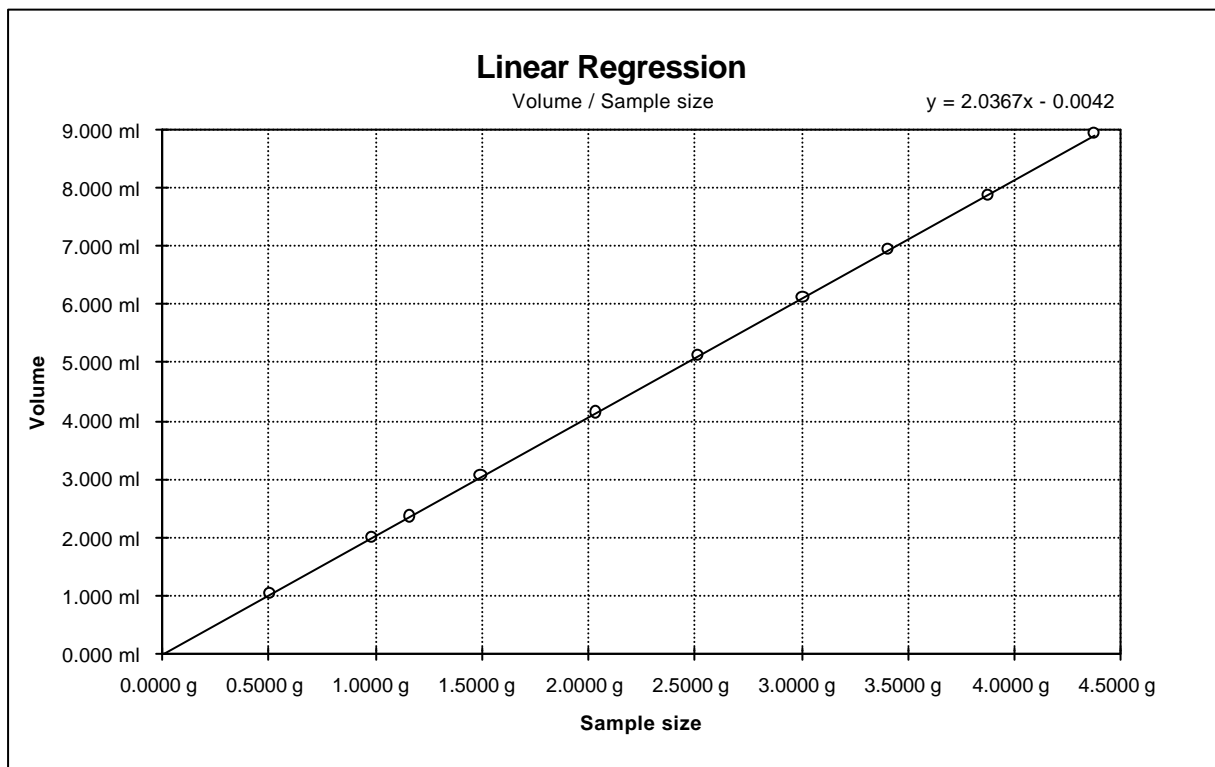
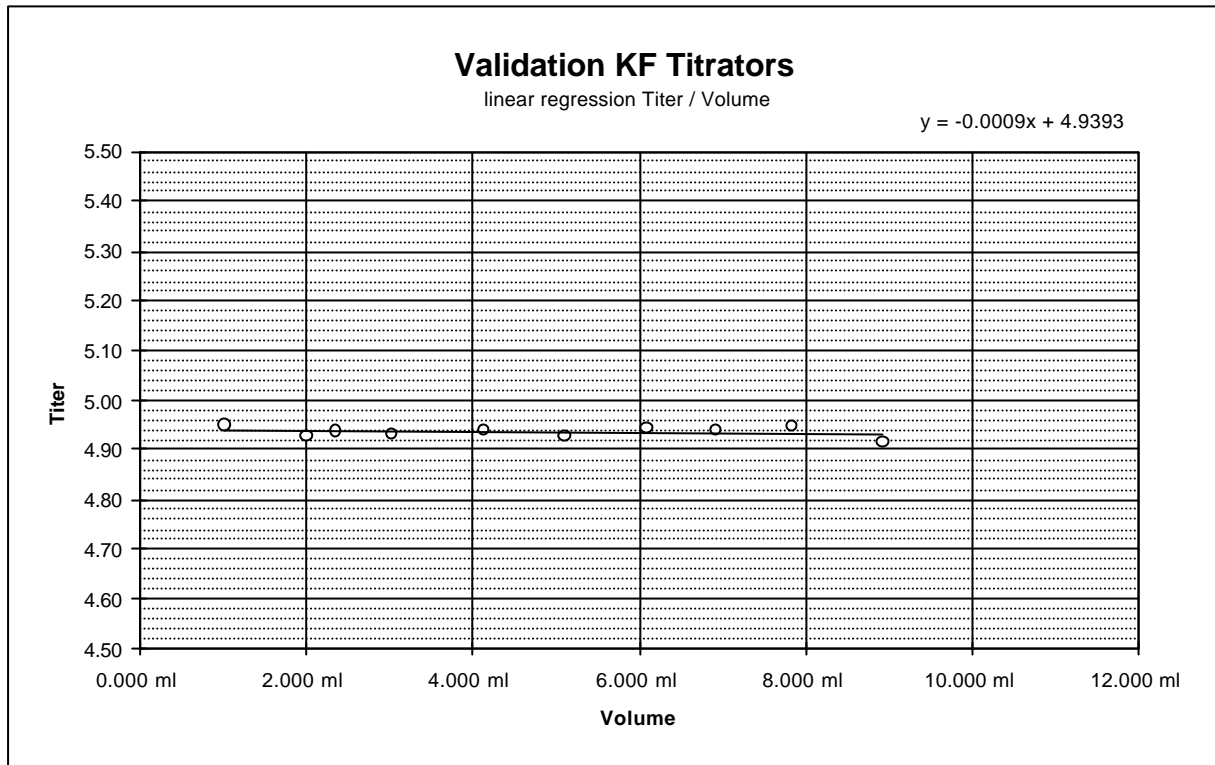
On the following pages you will find an example of a validation record and a diagram of the linear regression mentioned above.

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 (Application Bulletin 255/1, Application Disk 8.000.8013, GLP KF Titrators).

14.1.96, D. Möckli

Validation Record				Company : <i>Metrohm AG</i>		
KF Titrators				Division : <i>Appl. Lab.</i>		
Titrant :	<i>Composite 5</i>			Instrument :	<i>720 KFS-Titrino OP1/108</i>	
Water capacity :	<i>5.0 mg/mL</i>			Mode :	<i>KFTIPol</i>	
Lot :	<i>31300.0</i>			Electrode :	<i>6.0338.100 / II</i>	
Primary standard :	<i>H2O-Std</i>			Exchange unit :	<i>6.3012.213</i>	
Water content :	<i>10.04 mg/g</i>			Burette size :	<i>10 mL</i>	
Density :				Temperature in °C :	<i>24</i>	
Lot :	<i>33430</i>			Blank value :		
<u>Titration parameters :</u>						
<i>I(Pol) :</i>	<i>50 µA</i>					
<i>EP at U :</i>	<i>250 mV</i>					
<i>Dynamics :</i>	<i>100 mV</i>					
Smpl size :	Volume :	Titer :	No.	Remark :		
<i>3.4113 g</i>	<i>6.933 mL</i>	<i>4.9401</i>	<i>1</i>			
<i>0.9857 g</i>	<i>2.009 mL</i>	<i>4.9260</i>	<i>2</i>	<i>- Titer will be calculated automatically</i>		
<i>2.5098 g</i>	<i>5.114 mL</i>	<i>4.9273</i>	<i>3</i>	<i>- Enter blank value in cell G9, ev.</i>		
<i>1.4971 g</i>	<i>3.049 mL</i>	<i>4.9298</i>	<i>4</i>	<i>- The blank value will be considered in the titer calculation</i>		
<i>0.5043 g</i>	<i>1.023 mL</i>	<i>4.9493</i>	<i>5</i>	<i>- a_{syscorr} is calculated from the blank value</i>		
<i>4.3717 g</i>	<i>8.932 mL</i>	<i>4.9140</i>	<i>6</i>			
<i>3.0076 g</i>	<i>6.110 mL</i>	<i>4.9421</i>	<i>7</i>			
<i>2.0358 g</i>	<i>4.138 mL</i>	<i>4.9394</i>	<i>8</i>	Mean =	<i>4.9350 mg/mL</i> s _{abs} = <i>0.011 mg/mL</i>	
<i>3.8737 g</i>	<i>7.862 mL</i>	<i>4.9468</i>	<i>9</i>	a _{sys} =	<i>-0.004 mL</i> s _{rel} = <i>0.22 %</i>	
<i>1.1621 g</i>	<i>2.364 mL</i>	<i>4.9355</i>	<i>10</i>	a _{syscorr} =	<i>-0.004 mL</i> b _{T/Vol} = <i>-0.0009</i>	
Result : <i>ok</i>						
Date :	<i>4.12.95</i>	Signature :			<i>D. Möckli</i>	Vis. : <i>VS</i>



Validation Record				Company :	
KF Titrator's				Division :	
Titrant :			Instrument :		
Water capacity :			Mode :		
Lot :			Electrode :		
Primary standard :			Exchange unit :		
Water content :			Burette size :		
Density :			Temperature in °C :		
Lot :			Blank value :		
<u>Titration parameters :</u>					
Smpl size :	Volume :	Titer :	No.	Remark :	
			1		
			2		
			3		
			4		
			5		
			6		
			7		
			8	Mean =	S _{abs} =
			9	a _{sys} =	S _{rel} =
			10	a _{syscorr} =	b _{T/Vol} =
Result :					
Date :		Signature :			Vis. :

