

## Application Bulletin 255/3 e

# Validation of Metrohm KF Titrators according to GLP/ISO9001

Of interest to  
General analytical chemistry  
B 1

### 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 the electronic and mechanical components of measuring instruments can and should be undertaken as part of regular servicing. Manufacturer-trained personnel should perform these checks. All Metrohm instruments have automatic start-up test routines that check whether the relevant assemblies are working correctly when the instrument is turned on. If no error message is displayed, it can be assumed that the instrument is functioning correctly. Metrohm instruments are also supplied with built-in diagnostic programs that allow the user to test the functions of certain components in the event of malfunction or faulty behavior and to localize 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:

Titrandos with KF Mode (e.g. 835, 836, 841, 857)  
Titrimos with KF Mode (e.g. 701, 720, 751, 758, 784, 795, 799)

Of course, older KF titrators of the 600 series can also be checked in a similar way.

### Test intervals

Annual repetition of the test is recommended. If an instrument is used in continuous operation, it may be advisable to carry out the test more frequently, e.g. every six or even every three months. A special validation is recommended when one or more components of the titration system have been replaced.

### Maintenance/Serviceing

Careful maintenance and cleaning are indispensable requirements to assure compliance to GLP for all instruments used in the laboratory.

Particular attention should also be paid to the proper handling of the instruments. The Instructions for Use supplied with the instrument should be available to all laboratory personnel. We recommend annual servicing of all measuring instruments. Many Metrohm agencies offer servicing agreements for their instruments.

### Accessories and reagents required

- Analytical balance (minimum resolution 0.1 mg)
- Syringe with long, thin needle
- Karl Fischer reagents for volumetric water determination

It is recommended that all titrations should be performed using standard solutions with certified water content. They can be obtained from all leading chemical manufacturers. Solid substances such as disodium tartrate dehydrate may also be used, however, special attention is required to assure complete dissolution in the working medium. Standard solution is available with a water content of  $10,0 \pm 0,1$  mg/g .

### Requirements

KF instruments should not be set up in areas that are subject to large temperature fluctuations. They must not be placed in the proximity of heating or cooling devices. If water baths or sinks are in the immediate vicinity then adequate protection against splashes must be provided.

The drying tube of the titration cell must be filled with fresh molecular sieve.

The test setup must be protected against direct sunlight and drafts. The system must be in thermal equilibrium.

The balance should be validated before use and also close to the instrument.

The measurement series must be processed without any interruptions.

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

Only well mixed sample solutions should be used.

## Analysis

### 1. Calculation formula for titer determination

For standard solutions in mg/g:

$$\text{Titer} = \text{RS1} = \frac{\text{C00} * \text{C01}}{\text{EP1}} \text{ with 4 decimal places}$$

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

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

#### Ipol:

titration direction: -  
I(pol): 50  $\mu$ A  
EP at U: 250 mV  
dynamics: 100 mV  
drift correction: no

#### Upol:

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

### 3. Procedure

6 to 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 titrant consumption of approx. 0.2 to 0.9 cylinder volumes. Avoid refilling of the cylinder during the titration.

Recommended quantities to be weighed in for the water standard solution 10 mg/g when using the 5 mg/mL KF reagent, depending on the cylinder volume of the dosing element:

Cylinder volume	Recommended quantities to be weighed in
5 mL	0.5...2.25 g
10 mL	1.0...4.5 g
20 mL	2.0...8 g
50 mL	not recommended

Recommendations for the practice:

Water is everywhere. Pay careful attention to insure that the test procedures are performed without water contamination. Plastic tubing may allow infiltration of water vapor. Thoroughly rinse the tubing of the Dosing Unit with titrant before starting the determination series. Insure the tightness of the titration cell and regularly check the seal integrity.

Condition the cell and carefully shake it back and forth from time to time to remove water adhering to the walls of titration cell. It is important to obtain a **constant** drift that is as low as possible.

Immediately after breaking open the standard vial, fill the syringe with about half the solution. Rinse the syringe by turning it round and holding the needle point up, then expel the entire contents (air and standard solution). Thus, the whole inner surface of the syringe is moistened. Refill the syringe with no aspiration of air. Wipe off the needle, tare the syringe on the balance and start the KF titrator. Inject part of the standard solution with one stroke. This can be done in two different ways:

- Inject the contents of the syringe directly under the surface of the KF solution.
- Inject the contents of the syringe without dipping in the needle. Aspirate the last drop back into the syringe.

Pull out the syringe and weigh it back. The sample weight can be transmitted directly from the balance to the KF titrator or can be entered manually.

Organic solvents (methanol, xylene, etc.) have a high thermal expansion coefficient (approx. 0.1% per °C). Care must be taken to ensure that the apparatus (balance, Dosing Unit, etc.) and the required reagents are in thermal equilibrium. Efficient operation without interruption can avoid disturbing influences of ambient temperature changes. When using standard solutions, 5 to 10 determinations can be carried out without changing the solvent.

Carefully consider recommendations of the primary standard manufacturer.

Exact analytical technique is indispensable for a validation of Karl Fischer titrators.

### Evaluation and interpretation of the results

The precision or repeatability and the accuracy of the measured values are relevant for the validation of measuring instruments. The calculation and assessment of these statistical parameters is performed as follows:

The values obtained from the 6 to 10 determinations are used for the calculation of the mean value and the absolute standard deviation  $s_{abs}$ . These calculations can be carried out directly with the built-in statistics function of the instrument or by using a PC with suitable software package (e.g. spreadsheet program). Since slightly differing results may be obtained in complex calculations using different computing aids with different calculation accuracies, preference should always be given to the values calculated by the measuring instrument itself.

#### Mean value:

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

#### Absolute standard deviation:

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

#### 1. Precision or repeatability

The repeatability of the measurement is expressed by the relative standard deviation  $s_{rel}$ :

#### Relative standard deviation:

$$s_{rel} = \frac{s_{abs} \cdot 100}{\bar{x}} = \frac{\text{Abs. standard deviation} \cdot 100}{\text{Mean value}}$$

#### Recommendation:

The relative standard deviation to be expected also depends on the standard solution used. Using standards with low water contents (e.g. 0.1 mg/g), you must reckon with higher relative standard deviations because here there are more influences exercised on the result. Our recommendations are as follows:

Standards  $\geq 10.00$ mg/g:  $s_{rel} \leq 1\%$

Standards  $< 1.00$ mg/g:  $s_{rel} \leq 2\%$

Requirements can, of course, be set lower.

#### 2. Accuracy

Since producers of KF reagents do not guarantee any exact titer, the accuracy of the results obtained depends on the water content of the standard, which must be guaranteed by its producer.

Accuracy can be assumed if the titer values of all titrants are regularly checked and the titer values as well as the samples are determined with the same titration parameters.

#### 3. Systematic error

##### 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 that minimizes 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.

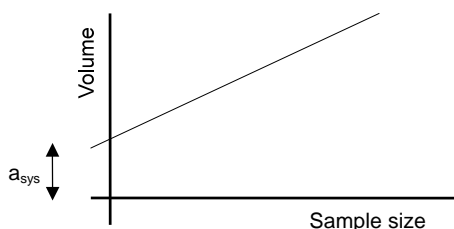
$a_{\text{sys}}$  as a measure of the systematic error is calculated from the mean of the x values, the mean of the y values and the regression coefficient b (slope).

**Calculation formulae:**

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

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

Any blank has to be subtracted from  $a_{\text{sys}}$ .



**Assessment:**

If  $a_{\text{sys}} > \pm 0.050 \text{ mL}$  or  $50 \mu\text{L}$ , 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 optimization of the validation method is possible, the individual values of the consumption in mL must be corrected by the value of  $a_{\text{sys}}$  (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 repeatability and the accuracy of the titration results must then be recalculated with the corrected titrant consumption values.

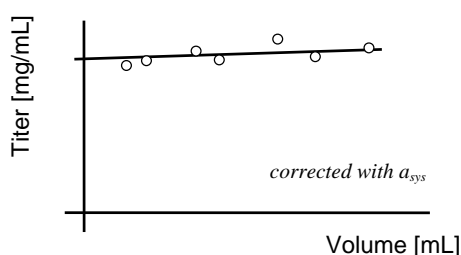
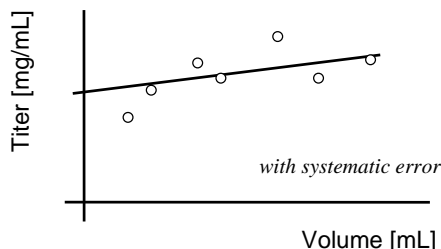
It may also be necessary to apply the correction of the systematic error to sample titrations.

*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, that is the line should be horizontal through  $y = \text{theoretical titer}$ .



**Assessment:**

If  $b_{T/Vol}$  is greater than  $\pm 0.0100 \text{ mg/mL}^2$ , 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 optimize the method and adapt the Standard Operating Procedure accordingly. If an optimization is not possible or a specific method has to be used unchanged, the calculation of the relevant characteristic data ought to be carried out using the values corrected by  $a_{sys}$ .

**Possible error sources**

Standard	impure, not guaranteed primary standard quality, no certification
Balance/weighing	balance inaccurate, drafts, temperature influences, contaminated balance, temperature gradient titration vessel/balance, careless weighing, sample weight too low, check the table in point 3
Titration cell	contaminated, leaky septum or connections, exhausted molecular sieve
Solvent	impure
Titrant	impure
Dosing Unit	tubing connections not tight, contaminated cylinder (visible corro-

sion), 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, unfavorable arrangement of buret tip and electrode, excessive response time of electrode
Titration	wrong measurement parameters, titration rate too fast or too slow

**Recommendations for troubleshooting**

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

- Optimize arrangement of titration and control parameters (see literature references in Metrohm Application Bulletins and literature references given below).
- Check tubing connections.
- Remove Exchange/Dosing 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.
- Use fresh titrant (possibly other production batch).

*With rel. systematic deviation too high (accuracy unsatisfactory)*

- Use fresh titrant (use a different production batch, if possible).
- Visually inspect the Exchange/ Dosing Unit and replace it if needed..
- 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 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.

Most Metrohm titrators have a special diagnostic program that enables the user to trace and localize malfunctions of the instruments. If an instrument fault is suspected (repeated appearance of errors, large scatter of results, etc.), 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 localized with the diagnostic tests, this must be referred 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 Monograph "Water determination by Karl Fischer Titration. 8.026.5003 – 2003-09

HYDRANAL Multi Media Guide. Version 2.0, Sigma-Aldrich / Riedel-de Haën 2004

HYDRANAL Manual. Eugen Scholz, Reagents for Karl Fischer Titration, Sigma-Aldrich / Riedel-de Haën 2001

Apura Application data base for water determinations acc. to Karl Fischer, Version 1/2004, Merck

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

<b>Validation Record</b>		Company :		<i>Metrohm AG</i>	
<b>KF Titrators</b>		Division :		<i>Appl- Lab.</i>	
Titrant :	<i>Composite 5</i>	Instrument :	<i>795 KF Titrino</i>		
Water capacity :	<i>5 mg/mL</i>	Mode :	<i>KF ipol</i>		
Lot :	<i>31300.0</i>	Electrode :	<i>6.0338.100</i>		
Primary standard :	<i>H2O-Std</i>	Exchange unit :	<i>6.3026.210</i>		
Water content :	<i>10.04 mg/g</i>	Burette size :	<i>10 mL</i>		
Density :		Temperature in °C :	<i>24°C</i>		
Lot :	<i>33430</i>	Blank value :			
<u>Titration parameters :</u>					
<i>I(Pol) 50 µA</i>					
<i>EP at U 250 mV</i>					
<i>Dynamics 100 mV</i>					
Smpl size :	Volume :	Titer :	No.	Remark :	
<i>3.4113 g</i>	<i>6.933 ml</i>	<i>4.9401</i>	1		
<i>0.9857 g</i>	<i>2.009 ml</i>	<i>4.9260</i>	2	<i>Titer will be calculated automatically</i>	
<i>2.5098 g</i>	<i>5.114 ml</i>	<i>4.9273</i>	3	<i>Enter the blank value in cell G9, ev.</i>	
<i>1.4971 g</i>	<i>3.049 ml</i>	<i>4.9298</i>	4	<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>	5	<i>a<sub>syscorr</sub> is calculated from the blank</i>	
<i>4.3717 g</i>	<i>8.932 ml</i>	<i>4.9140</i>	6		
<i>3.0076 g</i>	<i>6.110 ml</i>	<i>4.9421</i>	7		
<i>2.0358 g</i>	<i>4.138 ml</i>	<i>4.9394</i>	8	Mean = <i>4.9350 mg/mL</i>	S <sub>abs</sub> = <i>0.011 mg/mL</i>
<i>3.8737 g</i>	<i>7.862 ml</i>	<i>4.9468</i>	9	a <sub>sys</sub> = <i>-0.004 mL</i>	S <sub>rel</sub> = <i>0.22 %</i>
<i>1.1621 g</i>	<i>2.364 ml</i>	<i>4.9355</i>	10	a <sub>syscorr</sub> = <i>-0.004 mL</i>	b <sub>T/Vol</sub> = <i>-0.0009</i>
Result :					
Date :	<i>06.03.2005</i>	Signature :	<i>D. Möckli</i>	Vis. :	<i>VS</i>

