
Application Bulletin

Of interest to: General analytical laboratories

D F L 1, 3, 4, 5, 6, 7, 8, 11, 12, 13, 14

Validation of Metrohm KF coulometers using Standard Operating Procedures

Summary

Among other things, **GLP (Good Laboratory Practice)** requires that the accuracy and precision of analytical instruments are checked at regular intervals using **Standard Operating Procedures (SOPs)**.

The user is advised to validate the KF coulometer as a whole integrated measuring system, i.e. to perform a series of determinations with certified standard solutions and critically assess the results using statistical methods.

Checking of the electronic and mechanical components of the measuring instruments can and should be carried out by qualified personnel of the manufacturing company as part of regular servicing. All Metrohm instruments are equipped with start-up test routines that check the correct functioning of the relevant assemblies upon switch-on of the instrument. If no error message is displayed, it can be assumed that the instrument is functioning perfectly. In addition, Metrohm instruments are supplied with built-in diagnostic programs that enable the user to check the functioning of certain components in the event of malfunctions or erratic behavior and to localize the fault. These diagnostic programs can also be included in a validation procedure.

The procedure described below is meant as a guideline for setting up a Standard Operating Procedure to check the entire measuring system. The limits specified should be regarded as recommendations. In compliance with particular in-house requirements on the accuracy of the measuring system, these limits may have to be redefined in the Standard Operating Procedure.

Application range

These test specifications can be used with the following Metrohm instruments:

737, 756 and 831 KF Coulometer (coulometric cell with or without diaphragm).

Of course, older KF coulometers 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.

Internal instrument test routines

Metrohm instruments are equipped with internal start-up checks and test routines. During 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 checked with a write/read test.

If the KF coulometer is regularly maintained, specific validation of the instrument electronics is usually unnecessary.

Maintenance and service

Careful maintenance and cleaning of all instruments used in the laboratory is an indispensable prerequisite to ensure operation conforming to GLP. Particular attention has to be paid to the correct handling of the measuring instruments. The Instructions for use supplied with the instrument should be accessible to all operators in the laboratory. We also recommend a regular, annual servicing of all relevant measuring instruments. Many Metrohm distributors offer service contracts for their instruments.

Accessories and reagents required

- Analytical balance (minimum resolution 0.1 mg)
- Plastic syringe with long, very thin needle
- Karl Fischer reagents for coulometry
- Certified standard solutions with a water content of approx. 1.00 mg/g and/or 0.10 mg/g. (We recommend you give preference to the 1 mg/g standard. Standard solutions with 0.1 mg/g are difficult to handle for the reasons mentioned below and require much experience in order to obtain satisfactory results.)

If you are working with only a few specific samples, a special standard with defined, exactly known water content can be used in order to carry out a series of determinations and establish the recovery. Prerequisite for this is, however, that it fulfils 100% the conditions set for a primary standard (specified absolute water content, high purity, stability). If this is the case, the method and operating procedure used in daily work can be tested at the same time a validation is performed.

Recommendations for practice

Water is everywhere. (At a relative air humidity of 60% and a temperature of 25 °C 0.5 mL air contains approx. 7 µg H₂O.) Tightness of the titration cell must be guaranteed and should be regularly checked. (Particular attention should be paid to any cell O-rings. Check the drift!)

Immediately after breaking open the corresponding vial, fill the syringe about half with the standard 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. Afterwards fill the syringe with the entire remaining contents of the vial, with no aspiration of air. After wiping off the needle, tare the syringe on the balance and start the KF coulometer. Inject part of the standard solution with one stroke. This can be done in two different ways:

- a) Inject the contents of the syringe directly under the surface of the KF solution.
- b) 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 coulometer or can be entered manually.

Requirements

- The balance should have been validated beforehand.
- The coulometric cell is filled with fresh electrolyte solution(s). The septum stopper must be fitted with a new septum and the drying tube with fresh dehydrating agent or molecular sieve.
- Condition the cell and shake it carefully back and forth from time to time to remove water adhering to the walls. It is important that a **constant** drift is obtained that should be as low as possible.
- If possible, the syringe should be pre-dried and rinsed at least once with the standard solution (see above).

Procedure

6 to 10 determinations are performed with the same instrument settings and different quantities of the standard solution. The quantity of standard weighed in should be varied in random order. Attention has to be paid that at least 50 µg H₂O (at least 0.5 mL of the 0.10 mg/g standard) is injected. Smaller amounts are very difficult to handle and systematic faults become disproportionately larger. (This has nothing to do with the instrument, see above.)

Recommended quantities of standard to be weighed in:

Water standard solution 1.00 mg/g	0.2 ... 2.0 g
Water standard solution 0.10 mg/g	0.5 ... 5.0 g

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 \bar{x} and the absolute standard deviation s_{abs} . These calculations can be carried out directly with the built-in statistics function of the instrument, if available, or by using a pocket calculator or 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:

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

1. Precision or repeatability

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

Relative standard deviation:

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

Requirement:

The relative standard deviation to be expected also depends on the respective standard solution. 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 ≥ 1.00 mg/g $s_{rel} \leq 2\%$

Standards < 1.00 mg/g $s_{rel} \leq 5\%$

Requirements can, of course, be set lower.

2. Accuracy

The determination of the accuracy is based solely upon the water content of the standard guaranteed by the manufacturer. Please be careful to only use the values established and guaranteed by the manufacturer (and not the general, theoretical value printed on the label).

Recommended limits with regard to the recovery (values given by the manufacturers):

Standards ≥ 1.00 mg/g 97 ... 103%

Standards < 1.00 mg/g 90 ... 110%

The manufacturer Riedel-de Haën recommends in its brochure «ISO 9000 und die Karl-Fischer-Titration» (see Literature) the above-mentioned determinations. You will find a corresponding example in the annex of this bulletin. (This brochure contains among other things also an operating procedure with corresponding record.)

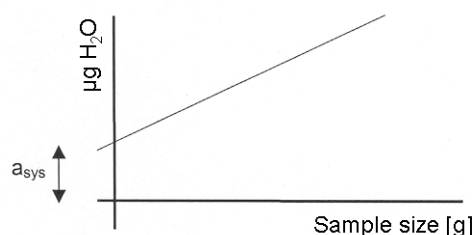
3. Systematic error

3.1 Linear regression: $\mu\text{g H}_2\text{O}$ found as a function of the sample size

To discover systematic errors, e.g. interferences due to the method or problems concerning the operating procedure, a linear regression of the amount of water found versus the sample size can be calculated. This requires use of a powerful pocket calculator or a PC with statistics or spreadsheet program. The sample size is plotted as the x coordinate (independent variable) and the $\mu\text{g H}_2\text{O}$ found as the y coordinate (dependent variable).

The linear regression draws a straight line through the measuring points so that the sum of the squares of the individual deviations is at a minimum. The regression line is described by the equation $y = bx + a$, where «a» stands for the y axis intercept and «b» for the slope of the line (see diagram below).

Systematic errors are indicated by a significant deviation of the zero point coordinate of the y axis (y axis intercept), i.e. the regression line calculated from the values [sample size, $\mu\text{g H}_2\text{O}$ found] does not intersect the y axis exactly at the origin of the coordinate system.



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

Calculation formulae:

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

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

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

Assessment:

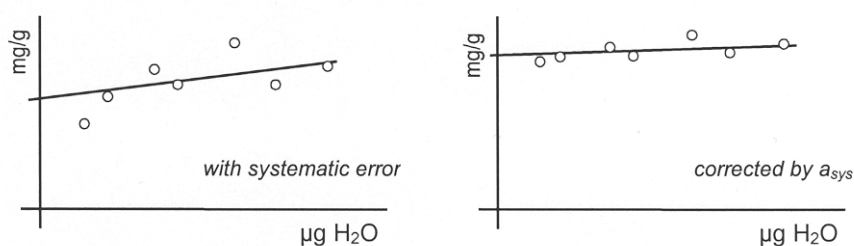
If a_{sys} is greater than $\pm 5 \mu\text{g}$ it must be assumed that a systematic error exists. It is thus imperative to check possible interferences caused by the system. If an optimization of the method and operating procedure is not possible, the individual values found must be corrected by a_{sys} (value found $- a_{\text{sys}}$, in $\mu\text{g H}_2\text{O}$), so that the systematic error of the method is not incorporated in the assessment of the titrator. Afterwards the relevant characteristic data for the repeatability and the accuracy of the titration results have to be recalculated with the corrected values. A correction of the systematic error may also be necessary if titrations of samples are performed.

3.2 Linear regression (scatter diagram): result in mg/g as a function of the $\mu\text{g H}_2\text{O}$ found

A further possibility for revealing systematic errors is based on the calculation and graphical representation of the regression line from the values [$\mu\text{g H}_2\text{O}$ found, result in mg/g]. This diagram also provides a good visual impression of the scatter of the results.

Significant positive or negative slopes of the regression line indicate an apparent dependence of the standardized result (in mg/g) upon the amount of water found, respectively on the sample size. This can also be an indication of systematic interferences due to the method.

In the ideal case the slope b_{scatter} (regression coefficient, calculation formula see above) from the linear function $y = bx + a$ should be 0.000, i.e. the line should run parallel to the x axis and be described by the equation $y = \text{theoretical value}$.



Assessment:

If b_{scatter} is greater than $\pm 0.001 \text{ mg}^{-1}$ then a systematic error in the method must again be assumed. After correction of the values found by a_{sys} (value found $- a_{\text{sys}}$, in $\mu\text{g H}_2\text{O}$) the subsequent recalculation yields a much better regression coefficient. This considerable improvement also becomes visible in the new representation of the regression line (result in mg/g versus $\mu\text{g H}_2\text{O}$ found).

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 Contaminated, poor quality, no certification, an opened or broken bottle/vial was used
- Balance/weighing Balance too inaccurate, draughts, temperature influences, contaminated balance, temperature difference balance/sample, careless weighing, sample size too small
- Syringe Unsuitable syringe, insufficient rinsing, moist syringe (air humidity)
- Titration cell Contaminated, septum and O-rings leaky, molecular sieve worn, poorly conditioned
- Reagents Contaminated, fully spent
- Temperature Temperature variations
- Measurements Contaminated or broken electrodes, loose contact at connector, defect cables, poor mixing of sample solution, too high or unsteady start drift

Recommendations for troubleshooting

If the relative standard deviation is too high (poor repeatability):

- Before weighing in the sample, wait for the temperature equilibrium to establish between balance and syringe.
- Rinse the syringe thoroughly with standard or sample.
- Clean the generator electrode with conc. HNO_3 . Clean the indicator electrode mechanically with moist polishing powder (Alox or toothpaste), rinse well and dry. Align the Pt wires parallel.
- Use only fresh, certified standard solutions.

- Check tightness of the titration cell, pay heed that the cell is free from water (good conditioning).
 - Use fresh reagents; if necessary, replace the electrodes.
 - Increase the quantity of standard solution weighed in.
-

Procedure with values not conforming to specifications

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

If excessive deviations are ascertained, the individual points under sections «Possible error sources» and «Recommendations for troubleshooting» should be conscientiously checked and the interferences eliminated. If unsatisfactory results are still obtained during a repetition of the test series, another person should perform the validation again on a different day.

All Metrohm KF coulometers are equipped with special diagnostic programs that enable a malfunction of the instrument to be traced and localized. If justifiable suspicion exists for a fault in the instrument (repeated appearance of errors, large scatter of results), the diagnostic program must be conscientiously carried out step by step. The precise procedure is described in the Instructions for use of the respective instrument.

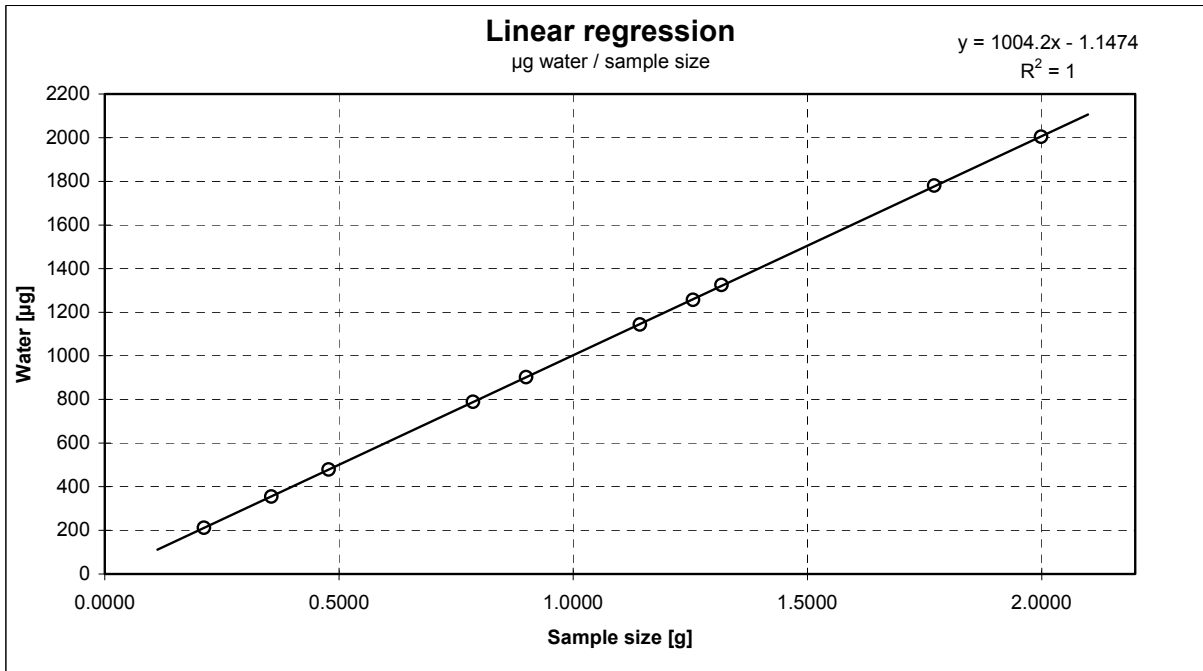
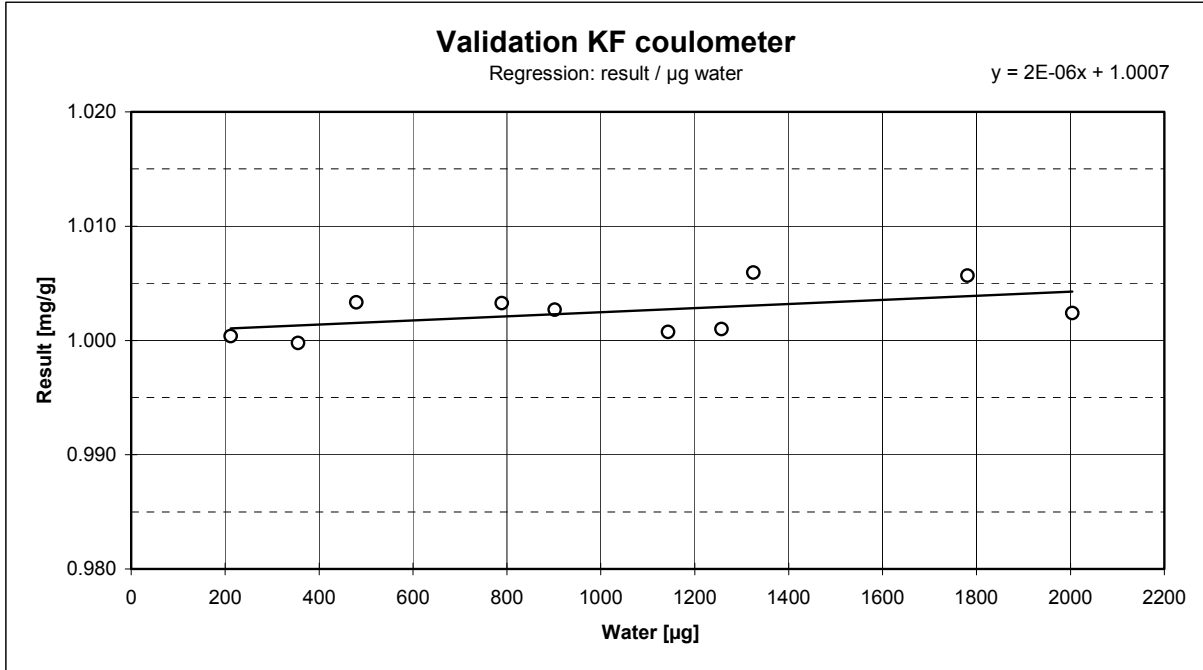
If during the diagnostic tests a malfunction of the instrument is discovered, this must be reported to the local Metrohm service agency and the instrument removed from use until the fault has been rectified.

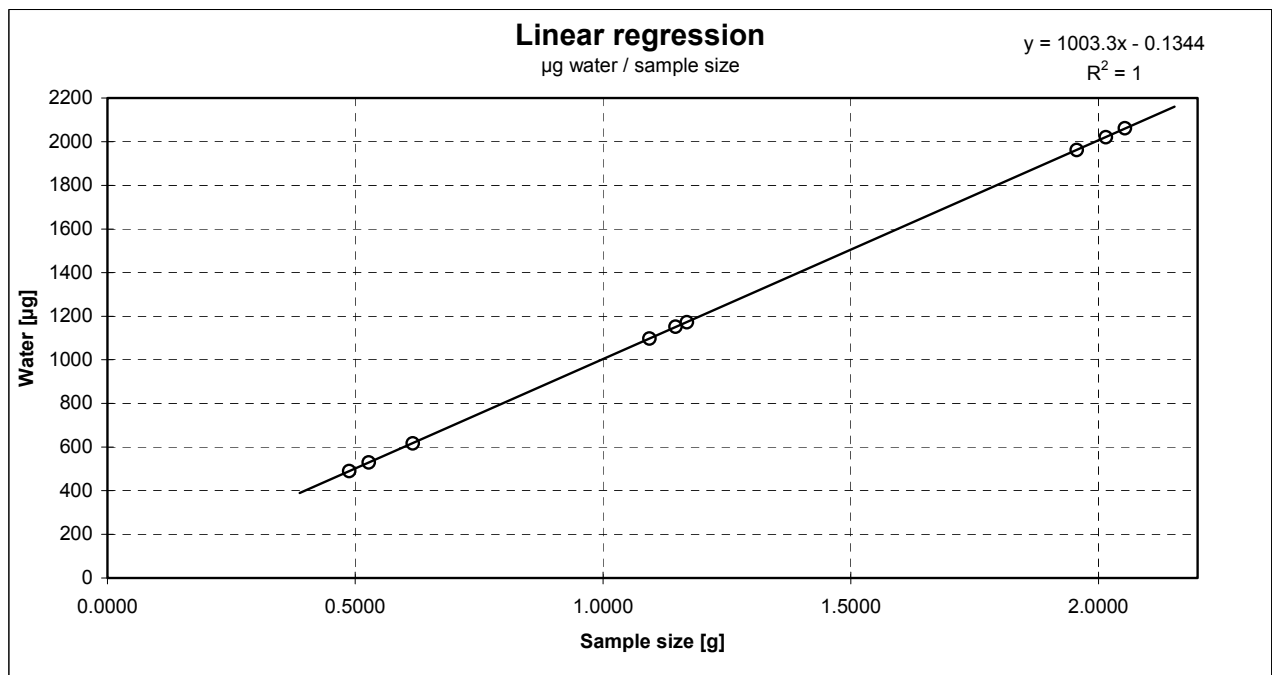
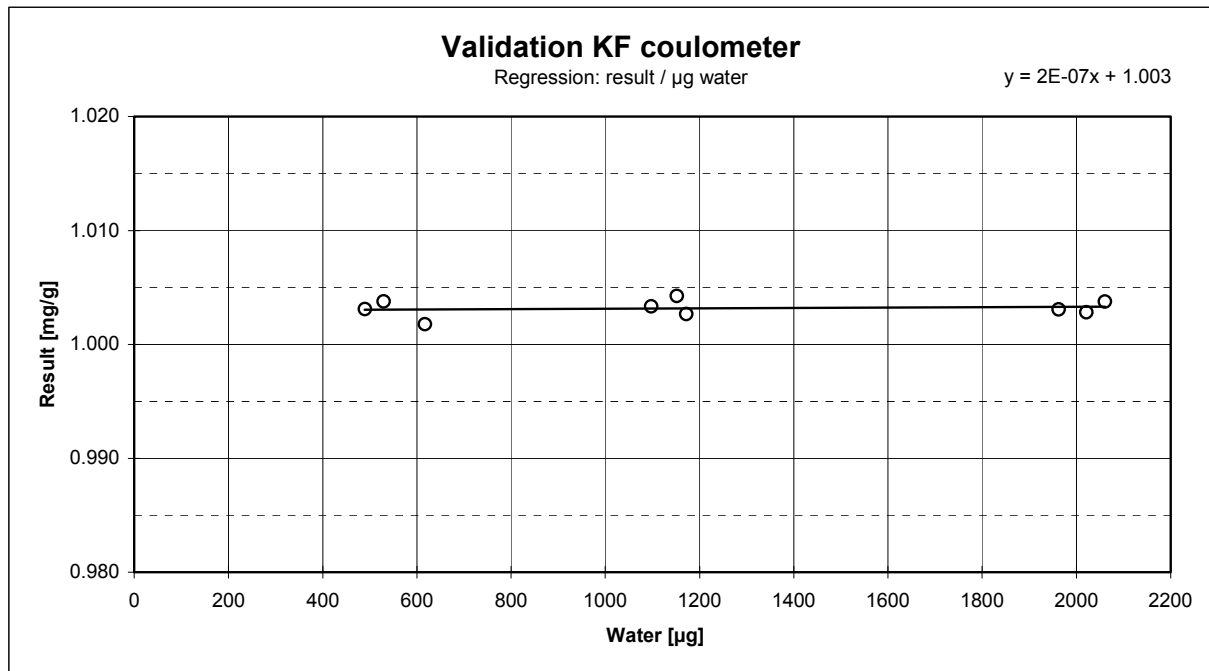
Literature

Further information concerning coulometric KF titration can be found in the following publications:

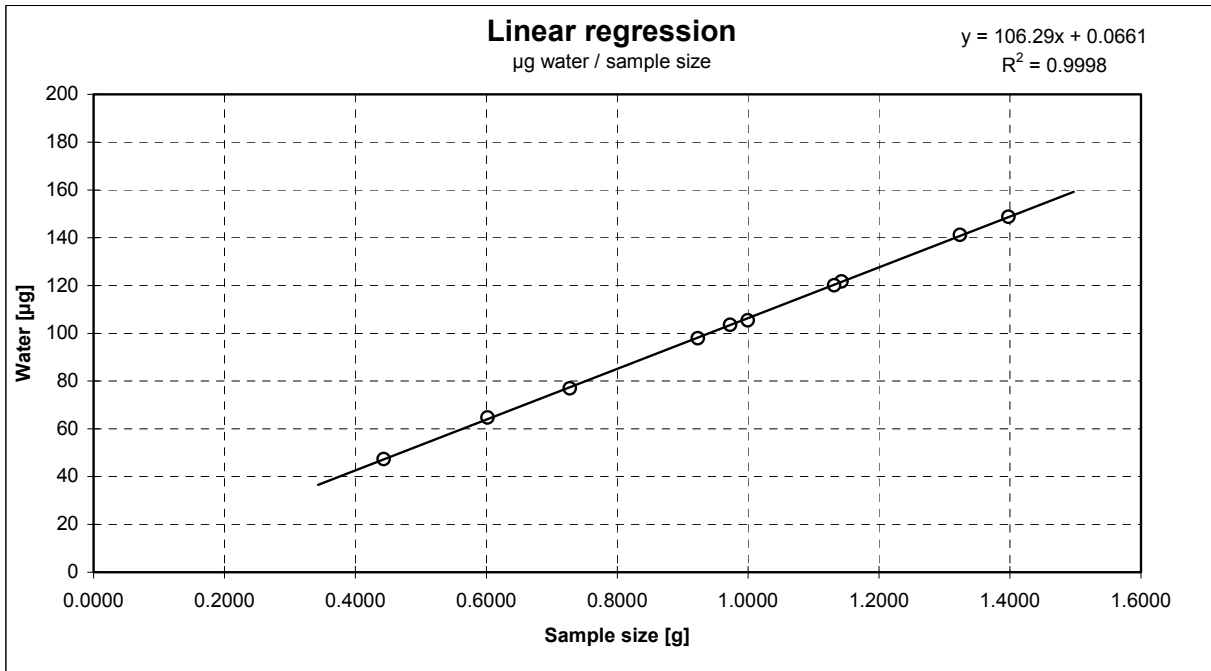
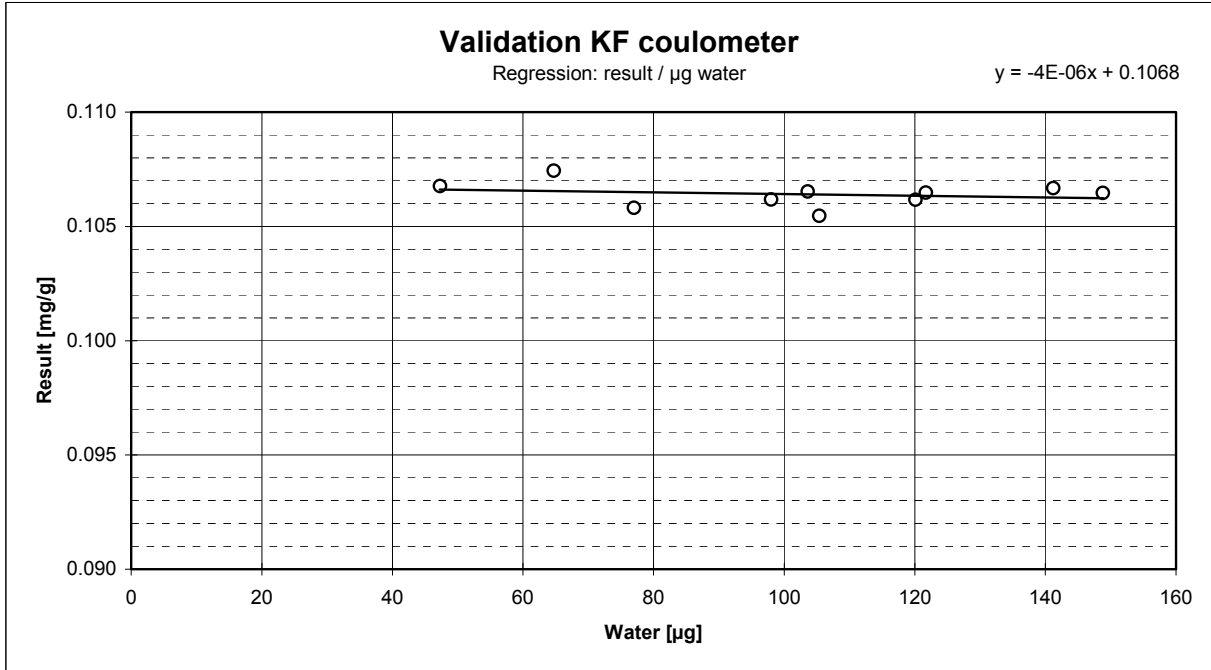
- Metrohm Application Bulletin No. 137, Karl Fischer water determination with the KF coulometer
- Metrohm Application Bulletin No. 141, Analysis of edible fats and oils
- Metrohm Application Bulletin No. 142, Karl Fischer water determination in gases
- Metrohm Application Bulletin No. 209, Coulometric water determination according to Karl Fischer in insulating oils and other hydrocarbon products
- Metrohm Monograph No. 8.026.5003, Water Determination by Karl Fischer Titration, 2003
- HYDRANAL-Praktikum, Wasserreagenzien nach Eugen Scholz für die Karl-Fischer-Titration, Riedel-de Haën, 1996
- HYDRANAL Guide for PC, Riedel-de Haën, 1998
- ISO 9000 und die Karl-Fischer-Titration, Riedel-de Haën, 1996
- G. Wieland, Wasserbestimmung durch Karl-Fischer-Titration, GIT Verlag, 1985

On the following pages you will find examples of validation records as well as diagrams of the described linear regressions. The last page can be used as a master for copies of the validation records.





Validation Record				Company: <i>Metrohm Ltd., Herisau</i>	
KF Coulometer				Division: <i>Appl. Lab.</i>	
Reagent:	<i>HYDRANAL Coulomat AG</i>			Instrument: <i>756 KF Coulometer</i>	
	<i>HYDRANAL Coulomat CG</i>			Mode: <i>GLP</i>	
Batch/lot:	<i>82430/7350B</i>			Electrode: <i>Gen. with diaphragm</i>	
Standard:	<i>HYDRANAL Water Standard 0.10</i>				
Water content:	<i>0.107 mg/g</i>				
std. dev.:	<i>0.002 mg/g (n = 15)</i>			Temperature in °C:	
Batch/lot:	<i>41370</i>			Blank:	
<u>Titration parameters:</u>					
<i>Standard parameters</i>					
Sample size	Water	Result	No.	Remarks:	
<i>1.14289 g</i>	<i>121.7 µg</i>	<i>0.106 mg/g</i>	<i>1</i>		
<i>0.44304 g</i>	<i>47.3 µg</i>	<i>0.107 mg/g</i>	<i>2</i>		
<i>0.60221 g</i>	<i>64.7 µg</i>	<i>0.107 mg/g</i>	<i>3</i>		
<i>1.32374 g</i>	<i>141.2 µg</i>	<i>0.107 mg/g</i>	<i>4</i>		
<i>0.97251 g</i>	<i>103.6 µg</i>	<i>0.107 mg/g</i>	<i>5</i>		
<i>0.99937 g</i>	<i>105.4 µg</i>	<i>0.105 mg/g</i>	<i>6</i>		
<i>1.39758 g</i>	<i>148.8 µg</i>	<i>0.106 mg/g</i>	<i>7</i>	Mean: <i>0.106 mg/g</i>	<i>S_{abs}: 0.0005 mg/g</i>
<i>1.13125 g</i>	<i>120.1 µg</i>	<i>0.106 mg/g</i>	<i>8</i>	<i>a_{sys}: 0.07 µg</i>	<i>S_{rel}: 0.51 %</i>
<i>0.72768 g</i>	<i>77.0 µg</i>	<i>0.106 mg/g</i>	<i>9</i>	<i>a_{sys corr}: 0.07 µg</i>	<i>b_{scatter}: -3.7E-06 /mg</i>
<i>0.92297 g</i>	<i>98.0 µg</i>	<i>0.106 mg/g</i>	<i>10</i>	Recovery: <i>99.44 %</i>	
Result:					
Date:	<i>03.03.1999</i>		Operator:	<i>J. Fässler</i>	
			Supervisor:	<i>Br</i>	



Validation Record				Company:	
KF Coulometer				Division:	
Reagent:				Instrument:	
				Mode:	
Batch/lot:				Electrode:	
Standard:					
Water content: 					
std. dev.:				Temperature in °C:	
Batch/lot:				Blank: 	
<u>Titration parameters:</u>					
Sample size	Water	Result	No.	Remarks:	
			1		
			2		
			3		
			4		
			5		
			6		
			7	Mean:	S _{abs} :
			8	a _{sys} :	S _{rel} :
			9	a _{sys corr} :	b _{scatter} :
			10	Recovery:	
Result:					
Date:		Operator:		Supervisor:	