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Metrohm

Validation of Metrohm Ion Chromatography Systems by using Standard Operating Procedures (SOP)

Of interest to:
general analytical laboratories; water analysis
I 1, 2, 4, 7, 8, 9, 10, 11, 12, 14,16

Summary

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

The operator is recommended to validate the Metrohm ion chromatography system (IC system) as a complete and integral measuring system, i.e. by carrying out an ion chromatography determination on standard solutions of known content and critically assessing the results by statistical means.

Checking the electronic and mechanical functional groups can and should be carried out within the framework of a regular service visit by the manufacturer's own service technicians. All Metrohm instruments are provided with start-up check routines which check that the instrument is functioning properly when it is switched on. If no error messages appear then it can be assumed that the instrument is working properly. Metrohm also supplies its instruments with built-in diagnosis programs which allow the operator to check the functioning of particular assemblies if faults should occur in order to localize the fault. These diagnosis programs can also be integrated in a validation method.

Metrohm recommends that you use the method described below as a guideline for drawing up a Standard Operating Procedure for checking your IC system. The limits mentioned are to be regarded as examples. Depending upon the demands placed upon the accuracy of your measuring system, it may be necessary to define other limits in your Standard Operating Procedure.

Range of applications

These instructions are intended for use with IC systems consisting of combinations of the following ion chromatography instruments:

- 732 IC Detector
- 733 IC Separation Center
- 709 IC Pump
- 752 IC Pump Unit
- 753 IC Suppressor Module
- 754 Dialysis Unit
- 750 Autosampler and 766 Sample Processor

as well as Compact IC instruments.

- 761 Compact IC
- 790 Personal IC
- 792 Basic IC

Validation of older ion chromatography systems

Older Metrohm ion chromatography systems consisting of the 690 Ion Chromatograph in combination with a 697 IC Pump and possibly a 698 IC Sample Changer can also be checked in a similar manner.

Checking intervals

It is a good idea to check the IC system at annual intervals. However, if an instrument is used continuously then a more frequent check is indicated, e.g. every six months or even at monthly intervals.

A special validation should be carried out whenever one or more components of the IC system are exchanged.

Internal check routines

Metrohm ion chromatography instruments are provided with their own internal switch-on tests and check routines. In the switch-on test the display elements are checked and the contents of the program memory are checked via a check-sum test. The functioning of the data storage area is tested with a read-write test.

If IC systems are checked within the framework of a regular service visit then it is possible to dispense with a special validation of the instrument electronics.

The Compact IC instruments additionally contain an integral diagnosis program which allows the service technician to check the functions of particular assemblies if faults or errors occur in order to localize the fault.

Maintenance / Service

An essential requirement for ensuring the GLP-conform operation of all instruments used in the laboratory is that they are properly cared for and cleaned; in particular they must also be handled properly. The 'Instructions for Use' supplied with the instruments must be available to all laboratory staff. We also recommend that all relevant instruments are serviced once per year. Your local Metrohm agency can provide you with favorably-priced service contracts.

Methods

In many cases only a few particular ion chromatography methods are used in day-to-day work. We recommend that you select an ion chromatography method for validating your IC system which corresponds as closely as possible to one of the methods which you frequently use. In addition, sources of error inherent to the method should be excluded as far as possible.

Metrohm recommends that you use the standard systems described below as a guideline for drawing up a Standard Operating Procedure for checking your IC system.

Remarks

We repeat with emphasis that the operator is recommended to validate the IC system as a complete and integral measuring system. This means that checking the calibration curve and the reproducibility are essential in the validation of an IC system (Part 1 and Part 2). Part 3 should be carried out to check the pump, particularly after maintenance work, while Part 4 can be carried out to check the detector block.

Reagents

- Ultrapure water, degassed
- Chloride ion chromatography standard solution*
- Nitrate ion chromatography standard solution*
- Sulfate ion chromatography standard solution*

  (*commercially available standard solutions with a concentration ρ(Anion) = 1000 mg/L are used.)
- Sodium carbonate, anhydrous, extra pure analytical grade
- Sodium hydrogen carbonate (sodium bicarbonate), extra pure analytical grade
- Sulfuric acid, extra pure analytical grade
- Phthalic acid, extra pure analytical grade
- Acetonitrile, HPLC-quality
- Tris(hydroxymethyl)-aminomethane (TRIS), extra pure analytical grade

Requirements

Any balances and test agents used must have been previously validated.

Multi-element-standard solution A

1.0 mL of each of the chloride, nitrate and sulfate standards (commercially available standard solutions ρ(Anion) = 1000 mg/L) is pipetted into a 100mL volumetric flask and made up to the mark with ultrapure water. The working concentration of the multi-element standard solution is 10 mg/L chloride, nitrate and sulfate.

A. Standard system with chemical suppression

Metrosep Anion Dual 1 IC glass cartridge (6.1006.020) with glass cartridge holder (6.2828.100)

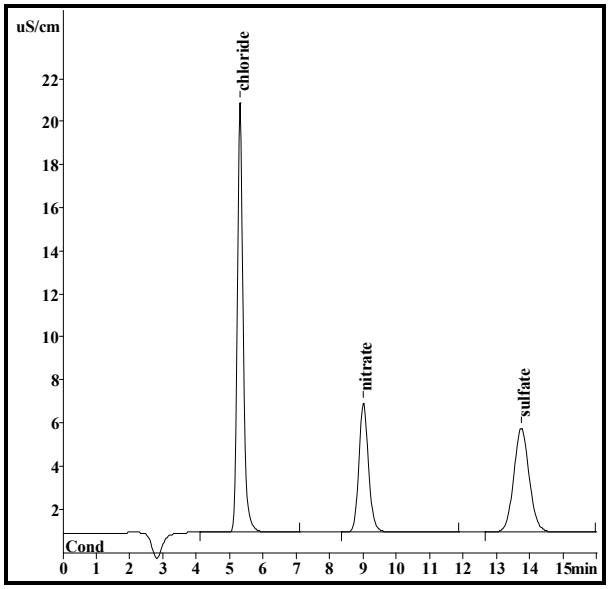
Carbonate/Hydrogen carbonate eluent

Composition: 2.5 mmol/L sodium carbonate, 2.4 mmol/L sodium hydrogen carbonate
(conductivity after chemical suppression approx. 16 µS/cm)

Preparation: 530 mg sodium carbonate (anhydrous) and 403 mg sodium hydrogen carbonate are dissolved in ultrapure water and then made up to 2 L with degassed ultrapure water. The eluent is filtered before use (0.45 µm).

Multi-element standard solution A

Flow:	0.5 mL/min
Injection volume:	20 µL
Detection:	conductivity after chemical suppression
Full scale:	20 µS/cm
Polarity:	+



Retention time [min]	Ion	Conc. [mg/L]
5.3	Chloride	10
9	Nitrate	10
13.7	Sulfate	10

B. Standard system without chemical suppression:

Metrosep Anion Dual 1 IC glass cartridge (6.1006.020) with glass cartridge holder (6.2828.100)

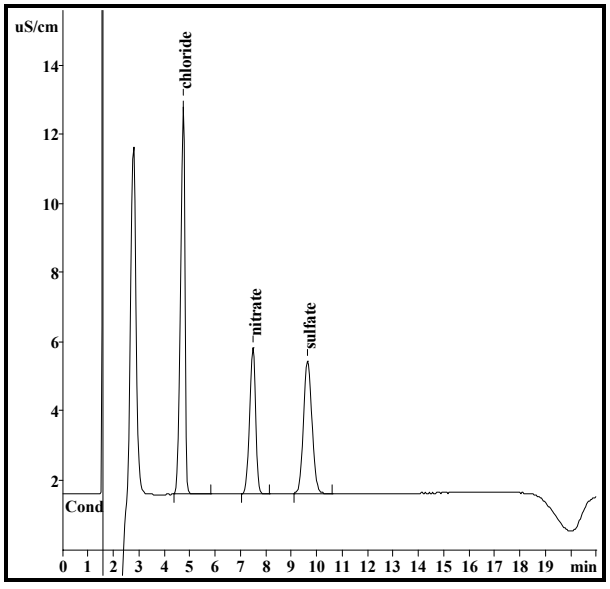
Phthalic acid eluent

Composition: 8 mmol/L phthalic acid, 2% acetonitrile; pH = 4.1 (TRIS)
(conductivity approx. 400 µS/cm)

Preparation: 2.658 g phthalic acid is dissolved in 40 mL acetonitrile under stirring and then made up to 2 L with ultrapure water; the pH is adjusted to 4.0 with tris(hydroxymethyl)aminomethane (TRIS). The eluent is filtered before use (0.45 µm) and degassed by applying a vacuum.

Multi-element standard solution A

Flow:	0.5 mL/min
Injection volume:	100 µL
Detection:	conductivity
Full scale:	20 µS/cm
Polarity:	+

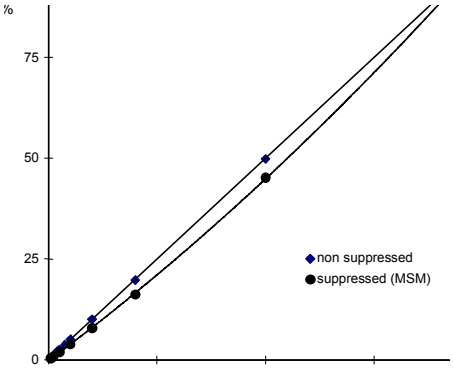


Retention time [min]	Ion	Conc. [mg/L]
4.7	Chloride	10
7.5	Nitrate	10
9.6	Sulfate	10

Part 1: Linearity of the calibration curves

An important parameter for analytical determination methods is the linearity of the calibration. If chemical suppression is used then slightly curved calibration curves are usually obtained. In this case the calibration values should be matched as closely as possible to the content of the sample in order to obtain results which are as accurate as possible. In contrast, calibration curves without chemical suppression are linear throughout several decimal powers. The following illustration shows an example of the different linearities.

Calibration curves for nitrate with and without chemical suppression



Apart from this curvature, which indicates only a slight variation from linearity, the linear working ranges with and without the use of a chemical suppressors do differ considerably. In the following given working range the curvature of the calibration curve for systems with chemical suppression can be ignored.

Instruments and solutions required

- Multi-element standard solution A
- 10.0 mL and 25.0 mL volumetric pipets
- 50 mL volumetric flasks

Procedure

- Start up the IC system and condition the column according to the 'Instructions for Use'
- Prepare multi-element standard solution A
- Use a volumetric pipet to pipet 25.0 mL multi-element standard solution A into a 50 mL volumetric flask and make the solution up to the mark with ultrapure water. (Standard solution B: working concentration 5 mg/L chloride, nitrate and sulfate)
- Use a volumetric pipet to pipet 10.0 mL multi-element standard solution A into a 50 mL volumetric flask and make the solution up to the mark with ultrapure water. (Standard solution C: working concentration 2 mg/L chloride, nitrate and sulfate)
- Prepare the integration system
- Inject standard solution C, standard solution B and multi-element standard solution A. A double injection is made for each concentration.
- Evaluate the recorded chromatograms and assess the linearity.

Assessment of the results

The linear regression is calculated in order to assess the results. This is done by using a powerful pocket calculator or a statistics package or spreadsheet program on a PC. The concentration of the standard solution is plotted as the x-coordinate (independent variable) and the peak areas obtained as the y-coordinate (dependent variable). The linear regression places a straight line through the measuring points so that the sum of the squares of the individual variations is at a minimum.

Requirement: the coefficient of regression should be ≥ 0.998.

Part 2: Reproducibility

The reproducibility of the injection system is checked by 2 series of 5 injections each.

Instruments and solutions required

- Multi-element standard solution A

Procedure

- Start up the IC system and condition the column according to the 'Instructions for Use'
- Prepare the multi-element standard solution A
- Prepare the integration system
- Inject 2 series a of 5 injections each of the multi-element standard solution
- Evaluate the recorded chromatograms and assess the results.

Assessment of the results

For the validation of measuring instruments the reproducibility (precision) of the measurements is relevant. These quantities are assessed as follows:

The values obtained from the 2 x 5 determinations (peak area and retention time) are used to calculate the mean value $\bar{x}$ and the absolute standard deviation s_{abs}. These calculations can be carried out on a PC (Personal Computer) with suitable software (e.g. spreadsheet program) or by using a pocket calculator.

For complex calculations using different calculating aids results may be obtained which differ slightly from one another owing to the different accuracies of the calculating aids.

Mean value:

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

Standard deviation:

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

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 for the peak areas and the retention time should be ≤ 2%.

Assessment	S_{rel} Rt	S_{rel} Fläche
very good reproducible	< 0.5%	< 1.0%
reproducibility okay	< 2.0%	< 3.0%
not reproducible	>2.0%	>3.0%

Part 3: Checking the IC Pump flow

The 709 IC Pump is a double-piston pump which has been specially developed for use in ion chromatography; it works with minimal residual pulsation and features an outstandingly constant flow. The mobile phase is displaced directly by the piston, the flow direction is controlled by ball valves. The piston seal and ball valves are highly stressed and must be serviced at regular intervals. As variations in flow cause an unstable baseline and can produce peak areas which are not reproducible it is necessary to check the flow rate (Flow) at regular intervals.

Instruments required

- Stopwatch
- Graduated 10 mL measuring cylinder
- Analytical balance

Procedure

After maintenance work on the pump head and/or the pump valves you have to condition the system for one hour, after replacement of piston seals for two hours at the working flow rate.

- Start up the IC system and condition the column according to the 'Instructions for Use'
- Set the required flow rate (Flow)
- We recommend that you check the normal working flow rate as well as flow rates which are 50% lower and 50% higher than the working flow rate, e.g.:

Flow:	0.25 / 0.5 / 0.75 mL/min
Pmin:	0.0 MPa
Pmax:	10.0 MPa
Flow-corr.:	1.0

- Set the pump to the relevant flow rate and allow it to pump for at least 5 min before starting the measurement.
- Collect the eluent is in a graduated and tared 10 mL measuring cylinder at the detector outlet for 10 min.
- Calculate the pump flow rate in [mL/min] from the volume or weight obtained.
- Repeat the measurement (2x)

Assessment of the results

The variation between the displayed and actual flow rate must not exceed ± 3%.

Assessment	Deviation [%]
flow okay.	<3%
flow not okay	>3%

You have the possibility of adjusting smaller variations in the flow rate of the 709 IC Pump by entering a correction factor of 0.9 - 1.1.

$$\textit{Correction factor} = \frac{\textit{displayed flow rate (in mL/min)}}{\textit{measured flow rate (in mL/min)}}$$

Part 4: Checking the conductivity detector

In ion chromatography it is normally only the relative alteration in the conductivity which is of interest and not an absolute value. The accuracy of the absolute value of the conductivity detector can only be checked electronically, i.e. by a Metrohm service technician. However, in ion chromatography this is only of secondary importance as the peaks required for the evaluation are produced by changes in conductivity. The following conductivity detector check can be used to check the functions of the measuring cell in the detector block.

Instruments and solutions required

- 6.2301.060 Conductivity Standard
- 5.0 mL volumetric pipet
- 100 mL volumetric flask

oder

- Cell Simulator (3.496.8580)

Procedure

with Conductivity Standard

- Start up the IC system without column and suppressor according to the 'Instructions for Use'
- Set the pump flow rate to 1.0 mL/min and the max. pump pressure to 5 MPa
- The following parameters must be set on the IC Detector: range 1.00 mS/cm, full scale 1.00 mS/cm, temperature 35ºC, the cell constant given on the detector block (if none is given then the default value is 16.7 µS/cm).
- Rinse the system with a solution of 20% methanol in ultrapure water for 10 min.
- 5.0 mL Conductivity Standard is pipetted from a volumetric pipet into a 100 mL volumetric flask and then made up to the mark with ultrapure water. (test solution 0.005 mol KCL).
- Rinse with test solution (0.005 mol KCL) until the display on the 732 IC Detector remains stable for at least 1 min (change of conductivity 2µS/cm per minute). Read off the displayed value.
- Then rinse the system with rinsing solution (20% methanol in water) until the displayed value is less than 5 µS/cm.

with Cell Simulator

- Connect the Cell Simulator to the socket „Detektor Block“
- Set the temperature of the Cell simulator to 20ºC
- Check the values according to the following table.

Range [µS/cm]	Cell Simulator [µS/cm]	Tolerance
50 -100	50	∀ 1µS
100-500	250	∀ 5µS
500 -1000	500	∀ 10µS
> 1000	1000	∀ 20µS

Assessment of the results

Check with Conductivity Standard

The value must be 580 .. 780 µS/cm.

Check with Cell Simulator

The value must be within the tolerance

Part 5: General information

The following information should be obtained from the chromatograms and evaluated as the test result:

1. The retention time as a criterion for the pump quality.
2. The peak area as a measure for the injection accuracy of the injection valve or the Autosampler.
3. The calibration curve for checking the linearity of the detection system.

The subsequent statistical evaluation of the data combinations allow rapid conclusions to be drawn about the performance of the components.

Recommendations for fault remediation

Information about fault remediation can also be found in the 'Instructions for Use' for the particular instruments. The following list is not exhaustive and is only intended to show examples of possible sources of error.

Fault	Cause	Remedy
Inadequate linearity	contaminated standard solutions	check by injecting 1-component standard solutions
	contaminated volumetric flasks	clean volumetric flasks
	contaminated or inaccurate pipets	check pipets (clean)
Differing peak areas	IC Pump output inadequate	adjust pump head
	eluent poorly degassed	degas eluent with vacuum or ultrasonics
	loop badly mounted (dead volume) or loop contaminated	check assembly and/or exchange loop
	faulty injector	adjust injector (Metrohm service)

	suction capillary blocked or contaminated	exchange suction capillary
	sample changer needle blocked or contaminated	exchange needle
Varying retention times	contaminated pump valves	clean valves & carry out Part 3
	faulty piston seals	exchange piston seals & carry out Part 3
	eluent poorly degassed	degas eluent with vacuum or ultrasonic
Baseline drift	thermal equilibrium not yet reached	condition system with heating switched on
	leak in system	check and tighten connections
	evaporation of organic solvent from eluent	seal eluent storage container more efficiently
Unstable baseline 	interfering pressure-variations owing to leaking connections, blocked filters or capillaries, pump faults.	check IC system and replace affected parts.

Procedure if values do not meet the requirements

All anomalous values must be recorded in the validation protocol and any further procedures noted.

If anomalous values are obtained then the various points under 'Recommendations for fault remediation' must be carefully checked and the disturbing influences removed. The validation process must be repeated. If unsatisfactory results are still obtained when the validation process is repeated then the validation should be carried out again by a different operator.

Literature

You will find an example of a validation protocol on the following pages.

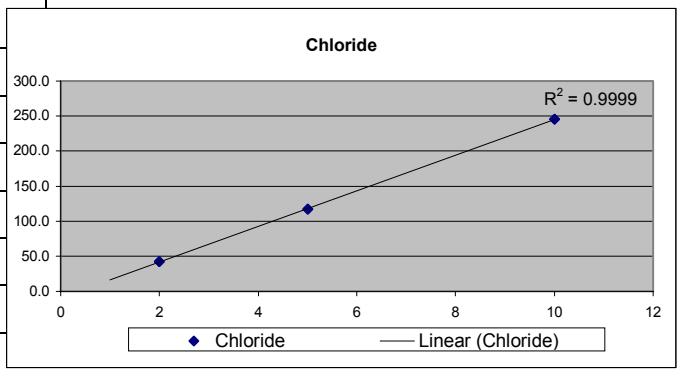
Appendix

Validation protocol IC System		*Company:*	Metrohm AG
		Division:	*IC Marketing*
Date:	10.04.00	*Operator:*	Urs Waldburger
Time:	11:00		
Instrument:	761 Compact IC	*Serial number:*	02122
	766 Sample Processor		04138
Eluent:	2.5mmol/L $NaHCO_3$, 2.4 mmol/L Na_2CO_3		
Column:	Metrosep Anion Dual 1 IC glass cartridge		

Calibration curve linearity

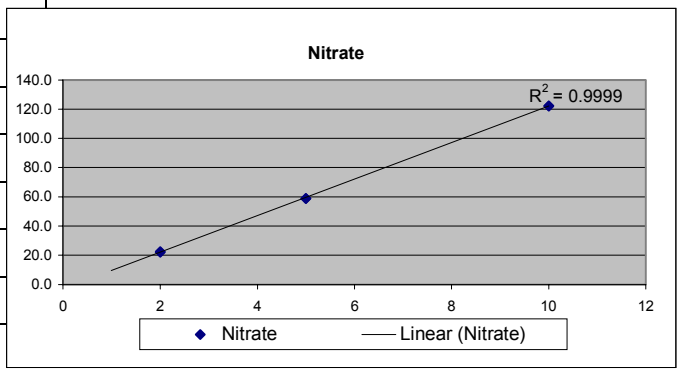
Chloride

Concentration [ppm]	Area
2	41.9
2	42.4
5	117.0
5	117.0
10	245.4
10	245.7



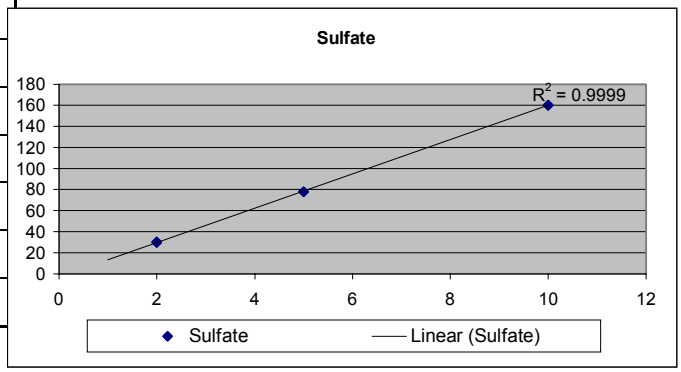
Nitrate

Concentration [ppm]	Area
2	22.1
2	22.4
5	59.0
5	58.9
10	122.2
10	122.3



Sulfate

Concentration [ppm]	Area
2	30
2	30
5	78
5	78
10	160
10	160



Reproducibility

Series I	Chloride		Nitrate		Sulfate	
	Rt	**Area**	**Rt**	**Area**	**Rt**	**Area**
	5.3	*243.2*	*9.0*	*121.7*	*13.7*	*158.6*
	5.3	*243.6*	*9.0*	*122.0*	*13.7*	*160.0*
	5.3	*243.3*	*9.0*	*126.4*	*13.7*	*163.5*
	5.3	*246.9*	*9.0*	*123.5*	*13.7*	*160.2*
	5.3	*246.3*	*9.0*	*123.2*	*13.7*	*160.9*
Mean value	**5.3**	**244.6**	**9.0**	**123.4**	**13.7**	**160.6**
S_{rel}	**0.1**	**0.7**	**0.1**	**1.5**	**0.0**	**1.1**

Series II	Chloride		Nitrate		Sulfate	
	Rt	**Area**	**Rt**	**Area**	**Rt**	**Area**
	5.3	*245.9*	*9.0*	*122.9*	*13.7*	*160.3*
	5.3	*243.9*	*9.0*	*121.6*	*13.7*	*158.9*
	5.3	*244.4*	*9.0*	*121.9*	*13.7*	*159.9*
	5.3	*244.7*	*9.0*	*122.0*	*13.7*	*159.9*
	5.3	*242.6*	*9.0*	*121.2*	*13.7*	*157.5*
Mean value	**5.3**	**244.3**	**9.0**	**121.9**	**13.7**	**159.3**
S_{rel}	**0.0**	**0.5**	**0.1**	**0.5**	**0.1**	**0.7**

Validation passed: **Yes** ☐ **No** ☐

Signature: **Name:**