

# Installation Instruction for ProfIC Vario 9 Anion

ProfIC Vario 9 – Anion is a Professional IC Vario system with variable sample preconcentration and Matrix Elimination for trace analysis of anions or cations with sequential suppression. With the «ProfIC Vario 9», concentrations down to the sub-ppb range ( $\mu\text{g/L}$ ) in various matrixes can reliably be determined. The intelligent 800 Dosino carries out the high-precision sample preconcentration on the preconcentration column, which replaces the sample loop and removes all sample matrix using the Matrix Elimination technique.



### Table of contents

1.	Delivery Package .....	2
	IC .....	2
	Detector .....	2
	Sample processor .....	2
	Accessoires .....	2
	Optional .....	2
2.	Installation .....	2
2.1.	Installation of the software .....	2
2.2.	Accessory Kit: Vario/Flex Basic (6.5000.000) .....	2
2.3.	Accessory Kit: Vario/Flex ONE (6.5000.010) .....	2
2.4.	858 Professional Sample Processor .....	2
2.4.1.	Liquid Handling Station (LQH Station) .....	3
2.4.1.1.	Rinsing unit .....	3
2.4.2.	800 Dosino .....	3
	2.4.2.1. LH Dosino .....	3
	2.4.2.2. UPW Dosino .....	4
2.5.	Interconnection of devices .....	4
2.6.	940 Professional IC Vario .....	4
3.	MagIC Net .....	5
3.1.	Configuration .....	5
3.2.	Method adjustment .....	7
3.3.	Purge of the system .....	7
3.3.	User defined determination .....	8
4.	Exemplary measurement .....	9
5.	Optional equipment .....	10
5.1.	Eluent Production Module .....	10
5.2.	Alternative MSM rinsing and regeneration methods .....	10

## 1. Delivery Package

Delivered with ProfIC Vario 9 Anion package:

Nr	Article no.	Article designation
<b>IC</b>		
1	2.940.1500	Professional IC Vario ONE/SeS/PP
<b>Detector</b>		
1	2.850.9010	IC Conductivity Detector
<b>Sample processor</b>		
1	2.858.0010	Professional Sample Processor
<b>Accessoires</b>		
2	2.800.0010	800 Dosino
1	6.5330.160	IC Equipment: MiPCT-ME
<b>Optional</b>		
1	6.6059.312	MagIC Net 3.1 Professional CD - 1 license
1	6.6059.313	MagIC Net 3.1 Multi - 3 licenses
1	6.9920.191	Sample rack 3 x 60.6 mm + 35 x 41 mm
20	6.1608.100	PE bottle 50 mL
1	6.2820.000	Al foil
1	6.1030.420	Metrosep A Supp 15 - 150/4.0
1	6.1030.500	Metrosep A Supp 15 Guard/4.0
1	6.1052.420	Metrosep C Supp 1 – 150/4.0
1	6.1052.500	Metrosep C Supp 1 Guard/4.0
1	6.1006.310	Metrosep A PCC 1 HC/4.0
1	6.1010.310	Metrosep C PCC 1 HC/4.0
1	6.2842.200	MSM-HC Rotor C
1	6.2832.000	MSM Rotor A
1	6.2842.000	MSM-HC Rotor A
1	6.2844.000	MSM-LC Rotor A
1	6.2842.020	Adapter Vario to MSM and MSM-LC
1	2.941.0010	Eluent Production Module

## 2. Installation

Following is a detailed description of how to install ProfIC Vario 9 Anion.

We strongly recommend that the individual steps are carried out in the order given below.

### 2.1. Installation of the software

All programs must be shut down first. Make sure no Metrohm instrument is connected to the PC. Install MagIC Net with the help of the MagIC Net - CD. The Microsoft Installation Wizard

is accepted and executed. All the standard directories proposed by the program should be accepted. Restart windows.

Now as soon as you connect a new Metrohm instrument via USB connection to the PC, the driver is installed and a window will pop up in MagIC Net, asking you if you'd like to store this device in your configuration. If you do, please answer with yes. The names will be checked later in this installation instruction.

### 2.2. Accessory Kit: Vario/Flex Basic (6.5000.000)

Using the Accessory Kit Vario/Flex Basic, install the Professional IC Vario. Remove handle, place the detector block in the instrument and connect the detector cable, remove the transport locking screws, connect the leak sensor cable and connect the drainage tubing. Then please mount the holder for Dosinos (6.2057.210) on the IC by removing the bottle holder on top of the instrument, placing the Dosino holder on the side of the IC and remounting the bottle holder on top.

In continuation, set up the waste collector by assembling the cap and screwing it onto the vessel. Then hang the waste collector holder on the side of the IC, so you can observe the droplets coming out of the capillaries later connected to the collector. Attach the waste tube to the vessel and lead it to the waste canister. When the tube is too long, please shorten it, because it is important to have a high level difference for the liquid to drain.

The power cable and USB cable (6.2151.020) are plugged into the rear of the Professional IC Vario. Please don't switch on the instrument yet. This step will follow after the completed installation.

### 2.3. Accessory Kit: Vario/Flex ONE (6.5000.010)

In the box with the Accessory Kits ONE, you will find all the accessories for setting up the Eluent bottle. Please lead the aspiration tube for the Eluent through the M8 stopper, the earring and the eluent cap. Then fix the white weight (6.2744.210), the adaptor (6.2744.210) and the aspiration filter (6.2821.090) on the eluent aspiration tube, all the while being careful not to touch the filter and its connections with bare hands in order to avoid cross contamination. Also fix the filled adsorber tube on the eluent cap. Please refer to the 940 Professional IC Vario manual for a detailed description.

### 2.4. 858 Professional Sample Processor

For a detailed description, please refer to the 858 Professional Sample Processor manual. In general, you will have to do the following: Plug in the Swing Head connection cable and the power supply cable. The controller cable (6.2151.000) is plugged into the plug "Contr." on the 858 and connected to the IC device via USB. The sample processor

initializes and lifts its Swing Head, once the IC device it is connected to is recognized by the software. For this, you have to plug the USB cable of the IC into the PC and turn it on. Afterwards, it is possible to mount the retaining plate, the needle and the safety shield (for detailed instructions please refer to the 858 Professional Sample Processor manual).

Please remove one element of the energy chain close to the swing head by using a screwdriver to unfix it. In its place, connect the transfer tubing PFA, 20 mL (6.1825.280).

For the following installations, please shut down the IC again and disconnect the 858 from the power supply.

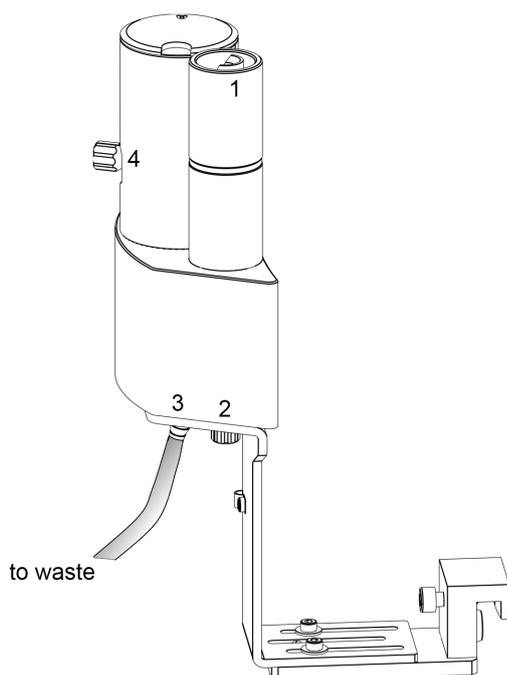
### 2.4.1. Liquid Handling Station (LQH Station)

The Liquid Handling Station consists of two function units: The rinsing unit is equipped with a two-pipe system and is used for rinsing the sample tube from the inside and outside or for fresh ultra pure water pick-up. The second unit is used for dilution of samples and standards. In the present case, the dilution unit won't be used.

The LQH Station is mounted onto the left side of the Sample Processor. For installation instructions, please refer to the Liquid Handling Station manual. Please make sure that the Liquid Handling Station is aligned properly before continuing the setup. All angle settings will be configured in a later step.

#### 2.4.1.1. Rinsing unit

For this application, ultrapure water for rinsing will be supplied by a Dosino. The installation of this Dosino will be explained in one the next paragraphs. The waste tubing (6.1801.120) is fixed at the bottom of the rinsing station to drain the waste water.



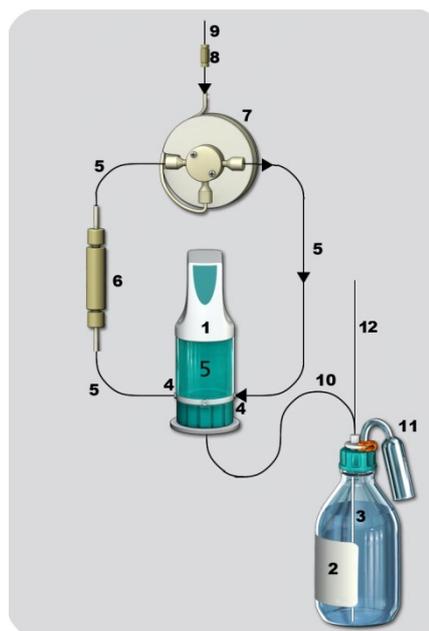
### 2.4.2. 800 Dosino

#### 2.4.2.1. LH Dosino

The liquid handling for this installation will be done with an 800 Dosino. The transfer tubing (6.1825.280) is used to avoid sample contact with the Dosing Unit. In the IC Equipment: MiPCT-ME, the transfer tubing PFA is included. If fluoride is required to be analysed in the sub-ppb ( $\mu\text{g/L}$ ) range, it is recommended to use the transfer tubing 10 mL PEEK, 1/8 (6.1825.270) instead, as Fluoride can leach out of the PFA material.

Accurate dosing is performed via a trap column (port 1) to guarantee excellent water quality. Aspiration and filling steps are carried out via port 3 (no trap column) to reduce back pressure. Tight screwing of all connections at the Dosino and the transfer tubing is of utmost importance for accurate results.

For installation details, please refer to the following schematics:



1. Dosino with 5 mL dosing unit: Place it on the Dosino holder that has been mounted to the side of the IC in the beginning of this installation and secure it with the help of the thread adapter (6.1618.020).
2. Ultrapure water reservoir (2 L bottle). To avoid air bubbles in the dosing unit, degas the water before use.
3. FEP aspiration tubing to canister (6.1819.110) to be inserted into the M6 hole of the eluent cap and to be cut to the appropriate length.
4. M6 threads / UNF 10/32 coupling (6.2744.080) to connect capillaries to the Dosino ports.

5. PEEK capillaries (i.D. 0.5 mm, 6.1831.180 included in 6.5330.160).
6. Metrosep I Trap 1 - 100/4.0 column (6.1014.200), to be connected to the Dosino port 1. The flow direction is from the Dosino to the transfer tubing (n°7).
7. Transfer tubing (20 mL, 6.1825.280) with T-connector.
8. Coupling (6.2744.310) to connect the transfer tubing to a capillary.
9. PEEK capillary (i.D. 0.5 mm, 6.1831.180 included in 6.5330.160) leading to the injection valve position 2 in the IC device.
10. FEP Tubing M6 100 cm (6.1805.120) to connect the LH Dosino port 2 with the T-connector (6.1808.060) splitting the water coming from the 2 L bottle of ultrapure water.
11. Adsorber tube consists of two parts, the adapter for the adsorber tube (6.1624.000) and the adsorber tube (6.1619.000) which can be found in the accessories of the Dosing Unit. It is recommended to fill the adsorber tube with cotton and CO<sub>2</sub> adsorber material.
12. FEP Tubing M6 100 cm (6.1805.120) to connect the UPW Dosino port 2 with the T-connector (6.1808.060) splitting the water coming from the 2 L bottle of ultrapure water (see next paragraph).

The Dosino is plugged in at the back of the Sample Processor. The method ProfIC Vario 9 Anion uses the MSB1 port of the IC for the Dosino

#### 2.4.2.2. UPW Dosino

Continuous fresh ultrapure water supply is very essential for this technique as every impurity is visible on the chromatogram or could accumulate in the system. Therefore, an 800 Dosino is used to feed fresh ultrapure water to the Liquid Handling Station for rinsing the needle and for fresh ultrapure water supply. This second Dosino is called "UPW Dosino" and is connected to the MSB2 Port of the Sample Processor.

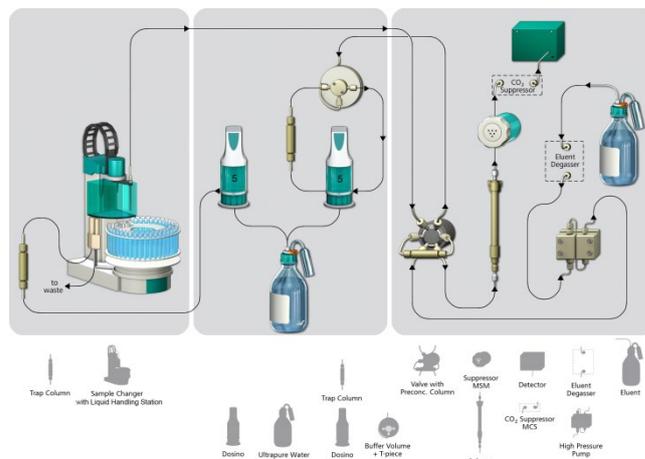


Second Dosino with 5 mL dosing unit: Place it on the Dosino holder that has been mounted to the side of the IC in the beginning of this installation and secure it with the help of the thread adapter (6.1618.020).

1. Connection of the UPW Dosino to the Liquid Handling Station on the No. 2 PEEK pressure screw (see chapter 2.4.1.1). PEEK capillaries (i.D. 0.5 mm, 6.1831.180 included in 6.5330.160) are used to connect the Dosino Port 1 with the LQH Station, installing a Metrosep I Trap 1 - 100/4.0 column (6.1014.200) in between the two elements in order to purify the water further before use.
2. Connection to the ultrapure water reservoir (2 L bottle). FEP Tubing M6 100 cm (6.1805.120) to connect the UPW Dosino port 2 with the T-connector (6.1808.060) splitting the water coming from the 2 L bottle of ultrapure water.

#### 2.5. Interconnection of devices

The whole setup of the ProfIC Vario 9 Anion packet is depicted here:



Prepare and degas an eluent suitable for the column you want to use (see column manual).

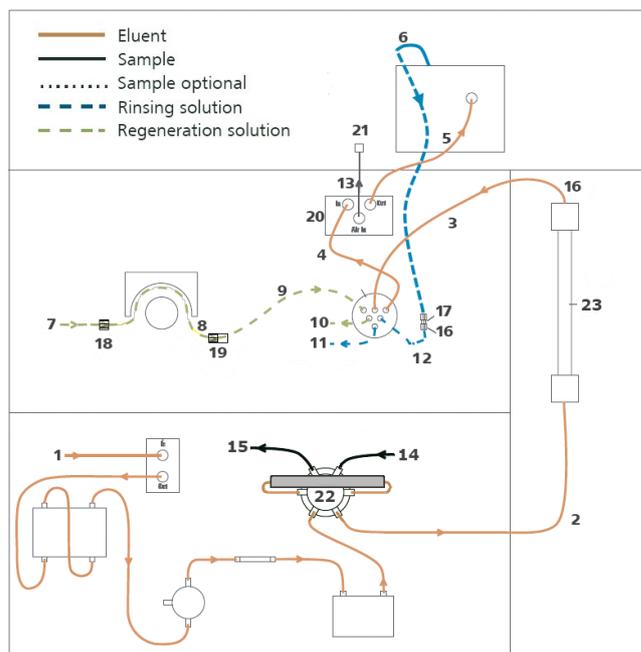
Provide the regeneration solution for the MSM Suppressor (100 mmol/L sulfuric acid for anions or 70 mmol/L Na<sub>2</sub>CO<sub>3</sub>, 70 mmol/L NaHCO<sub>3</sub> for cations).

#### 2.6. 940 Professional IC Vario

Capillaries are connected according to the following list and the diagram below:

1. Connection to the eluent bottle
2. Capillary to Column inlet – 0.25 mm ID
3. MSM inlet capillary – labeled with *in*
4. MSM outlet capillary – labeled with *out* can be connected to the MCS or directly to the detector using a coupling (6.2744.040)

5. Detector inlet capillary
6. Detector outlet capillary connected to the MSM inlet capillary – labeled with *rinsing solution* with the help of a coupling (6.2744.040)
7. Regeneration solution aspiration capillary – PTFE, 0.5 mm ID, connected to the bottle with 100 mmol/L sulfuric acid and the orange/yellow pump tubing (8). This pump tubing is, on the other side, relied to the regeneration solution capillary of the MSM - labeled with *regenerant* - with a security lock and filter (19)
8. Peristaltic pump tubing with orange/yellow stoppers (6.1826.420)
9. MSM regeneration inlet capillary – labeled with *regenerant*
10. MSM regeneration solution outlet capillary – labeled with *Waste reg.* – to be connected to the waste collector
11. MSM rinsing solution outlet capillary – labeled with *waste rins.* - to be connected to the waste collector
12. MSM rinsing solution inlet capillary - labeled with *rinsing solution*– connected to the detector outlet capillary with a coupling (6.2744.040)
13. MCS air aspiration capillary – connected to the CO<sub>2</sub>-adsorber cartridge
14. PEEK capillary connection (i.D. 0.5 mm) to the transfer tubing (6.1831.180 included in 6.5330.140)
15. PEEK capillary connection (i.D. 0.5 mm) to the needle of the Sample Processor
16. PEEK pressure screw short (6.2744.070)
17. Coupling 2xUNF 10/32 PEEK (6.2744.040)
18. Coupling nozzle - UNF 10/32 (6.2744.034)
19. Pump tubing connector with security lock and filter (6.2744.180)
20. PEEK pressure screw long (6.2744.090)
21. Luer coupling – connect with short PEEK pressure screw to CO<sub>2</sub>-adsorber cartridge
22. Metrosep A PCC 1 HC/4.0 (6.1006.310) – IC preconcentration column, to be connected to the valve with PEEK capillaries (i.D. 0.25 mm), instead of a loop, flow direction should go with the eluent (left to right)
23. The UNF 10/32 coupling (6.2744.040) is installed instead of the column to rinse the system with eluent. After rinsing the column is installed.

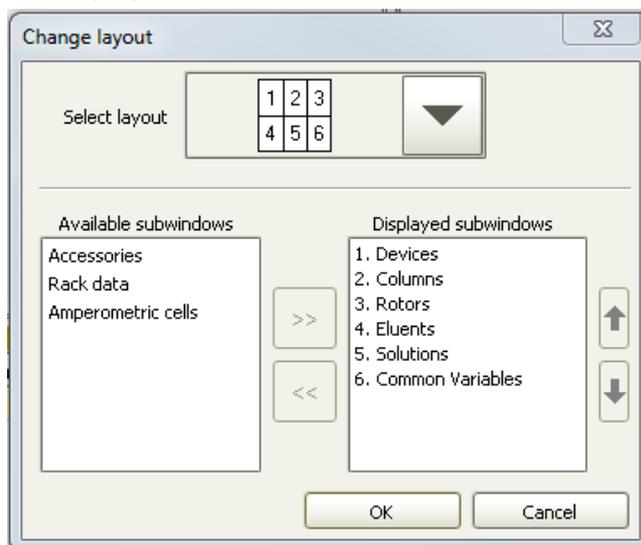


Make sure that all outlet capillaries are put into the waste collector.

### 3. MagIC Net

#### 3.1 Configuration

Please adjust the “view layout” of the configuration. Please configure it in the following way (the order of the sub windows is up to you):

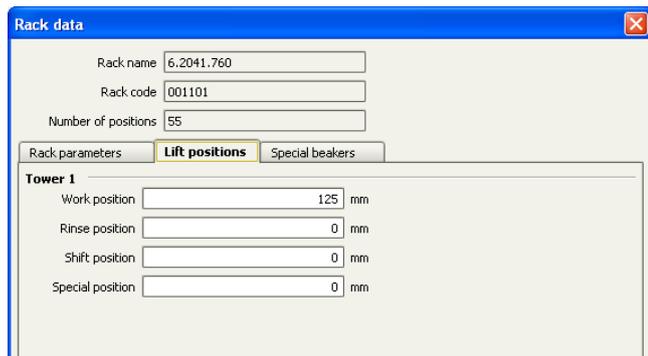


Now please connect the USB cables from the instruments and turn their power on. Connected USB devices are automatically recognized when MagIC Net is started. After confirmation of the automatically generated requests, the devices and columns are stored in the configuration. The devices are predefined as “940 Professional IC Vario 1” and “858 Professional Sample Processor 1”. Name them accordingly, if other names appear in your configuration (e.g.

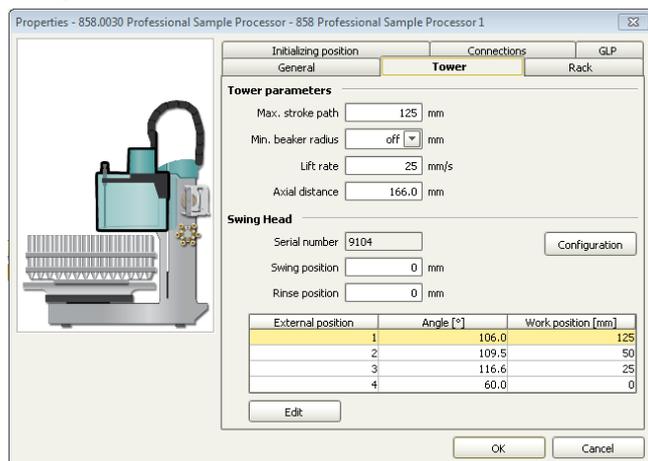
due to changed settings on your computer). The Dosino labeled as “LH Dosino”, connected to the MSB1 of the 858 and the Dosino called “UPW Dosino”, connected to the MSB2 of the 858 can be found in the sub window “solution”.

In the window configuration, the 940 Professional IC Vario, the 858 Professional Sample Processor and the column are visible. Add and define the eluent, the suppressor solution and the MSM Rotor.

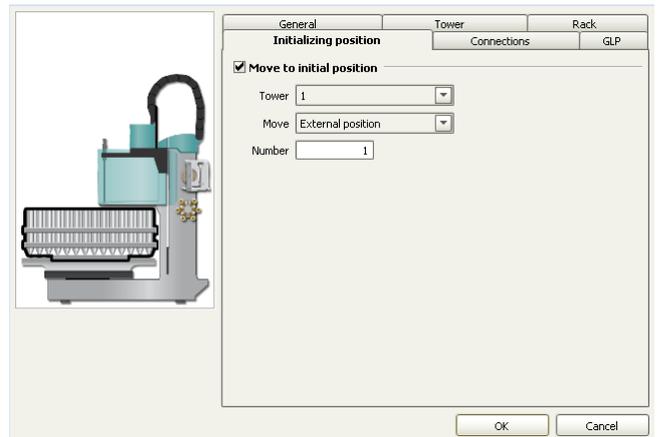
The settings for the rack require a “work position” fitting to the respective rack (e.g. 125 mm for rack 6.2041.760).



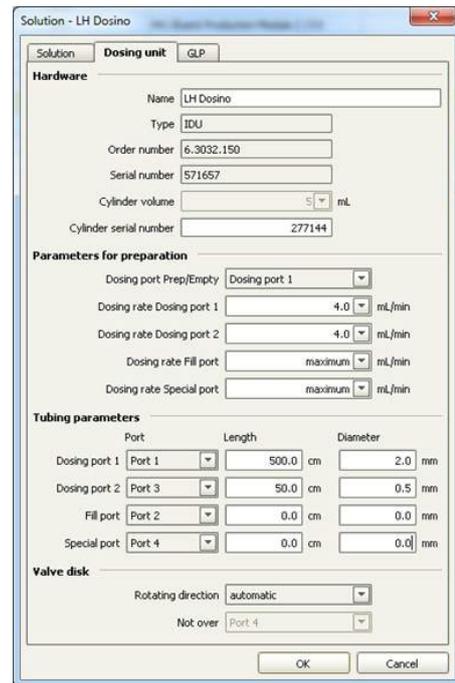
For using the Liquid Handling Station, it is necessary to define external positions for the needle. This is done in the configuration of the Sample Processor in the subtab “Tower”. Please define the external positions 1 and 2 according to the picture below. Attention: The angles are only approximate values, for fine tuning, please use the manual control to find the correct values for this system and adjust them then in the configuration.



In the sub-ppb analysis all contaminations have to be avoided. To have a possible source of contamination under control, it is important to move the needle of the Sample Processor to a defined position after initialization. For this, please add an initial position which is equal to the external position 1 in your configuration:



In the configuration of the solution LH Dosino, define the settings of the Dosing Unit. In the here present setup, an increased backpressure due to many capillary connections, Trap column and preconcentration column can be observed. For this reason it is important to define a maximum dosing rate for the Dosino. Also adjust length and diameter of your capillaries connected to the Dosing Unit. This is important to ensure complete rinsing of all connections when executing the prepare command.



In the UPW Dosino, define the settings of the Dosing Unit. Adjust length and diameter of your capillaries connected to the Dosing Unit. This is important to ensure complete rinsing of all connections when executing the prepare command.

Port	Length	Diameter
Dosing port 1	100.0 cm	0.5 mm
Dosing port 2	0.0 cm	0.0 mm
Fill port	100.0 cm	2.0 mm
Special port	0.0 cm	0.0 mm

### 3.2 Method adjustment

The method for this configuration can be found in the example method folder on your MagIC Net - CD. In the window method, import the method for ProfIC Vario 9 Anion from the installation CD: Go to File → method manager and choose your method group. Afterwards, click on edit → import and choose the pathway on the installation CD: MagICNet/examples/methods/ProfIC Vario \ProfIC Vario\_9\_Anion.imet

In the window "Method", open the ProfIC Vario 9 Anion method and assign the following equipment: Assign the suppressor solution to the IC peristaltic pump and set the speed rate to 1, add the MSM Rotor you've defined in the configuration, link the rack of the connected sample processor, assign the eluent to the IC pump and assign the column to the analysis. Adjust the temperature of the column oven to 45°C. Check the analytes in the components table and adapt if necessary.

Perform a method test and save the method.

### 3.3. Purge of the system

Before inserting the column, disconnect the Out capillary of the suppressor connection piece from the MCS or from the detector and put it into a waste beaker. The suppressor needs to be rinsed with the system first and its waste should not pass through the sensitive MCS and/or detector in the beginning, as loose particles could be flushed out.

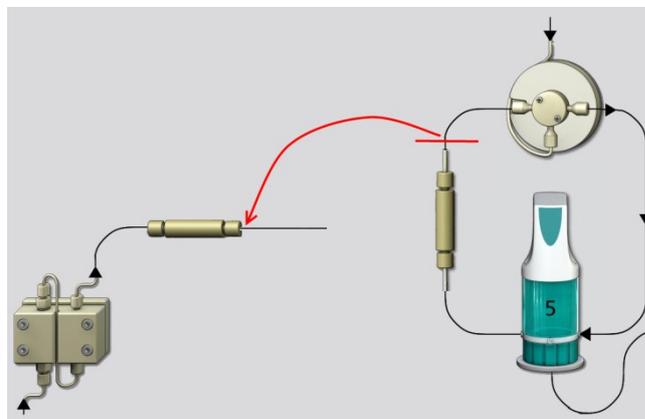
Now, please flush the system for about 10 minutes and get rid of air bubbles (by using the purge valve and syringe).

During these 10 minutes, step the MSM three times in order to flush all three chambers.

As soon as the whole system is purged, reconnect the suppressor Out capillary back to the MCS or the detector.

Now insert and rinse the precolumn for 10 minutes by leading the outlet directly into the waste. Afterwards connect the analytical column and flush it likewise for 10 minutes. Only afterward the column is completely connected to the flow path of the eluent (see also 940 manual). Start the peristaltic pump and adjust the pressure of the lever on the tubing, in order to see the droplets of suppressor regeneration solution drop into the waste collector.

In order to confirm the tightness of the sample flow path and to rinse the capillaries, a test will be performed. Please replace the inline filter after the high pressure pump with the preconcentration column (PCC), making sure the flow direction corresponds to the markings on the PCC. Now disconnect the capillary leading from the Trap column on the Dosino (No.6 in the chapter 2.4.2.) to the T-connector on the Sample Processor after the Trap column. Connect this capillary to the outlet of the PCC after the high pressure pump. Be careful not to use the Trap column as part of this installation! If it comes in contact with the eluent, it will be contaminated and you won't be able to use it any more in this installation.



In the manual control window, make sure the needle at the Sample Processor is immersed in the Liquid Handling Station (external position number 2). Set the pump speed of the high pressure pump to 2 mL/min to check the resulting backpressure. It should be below 0.5 MPa. If the pressure is too high, check your installation for any obstruction in the flow path. If no such hindrance can be found, reduce the flow of the high pressure pump gradually until the backpressure is below 0.5 MPa. The respective flow value has to be transferred to the method. In the subprograms "Sample", "Standard" and "Blank" adjust the dosing rate of the time dosing program lines with the determined value.

Leave the pump running for at least 30 minutes.

If during this rinsing procedure no leaks have appeared, reconnect the capillary where it was before (after the Trap column) and reinstall the PCC on the injection valve. Rinse the Dosinos and fill the transfer tubing by applying the “prepare” button under manual control for both Dosinos. Now dose 100 mL with the Dosino with a flow of 4 mL/min, through port 1 to rinse the Trap column and the flow path with ultrapure water.

Subsequently to this rinsing step, raise the needle out of the LQH Station so you can see its tip. Now dose 0.1 mL of ultrapure water through port 1 (flow of 2 mL/min). When the Dosino has finished dosing the water, observe the needle tip. If the water droplets not stop in the following 5 seconds, but continue dripping there is a connection in the flow path which is not completely tight. Retighten all connections including the needle at the Sample Processor and repeat this dosing test. When the dripping test has been passed, the installation is good to go.

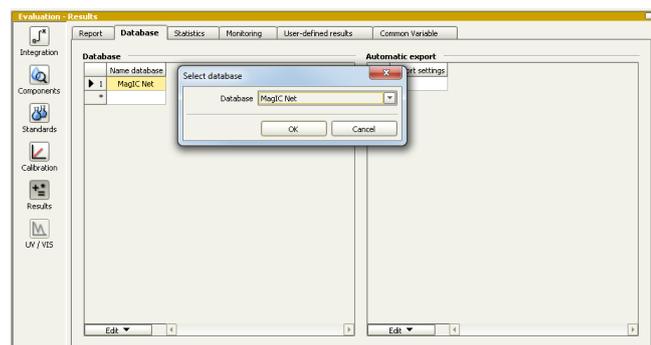
In order to start the equilibration, go to the window work place, load the anion method, and press “Start HW”.

Equilibrate the system until the baseline is stable.

### 3.3 User defined determination

In the window method, enter the ions and the concentrations of the required standards. The standard with the highest concentration (standard 1) has to be prepared when using the method ProfIC\_Vario\_9\_Anions.imet. All other standards have to be a divisor of standard 1, e.g. all components of standard 2 have half the concentration of those in standard 1.

Now please add the correct Database in the Evaluation window (Evaluation - Results - Database) for the method.

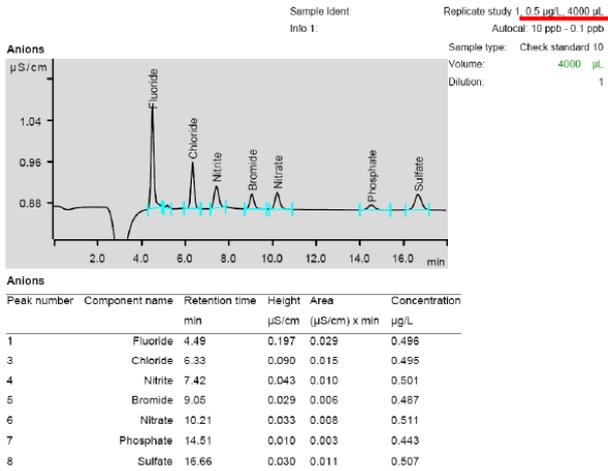


In the window work place, set up a “determination series”, describing your samples by ident, vial number, sample type (standard, blank, check standard or sample etc.). Please note that for standards, always 2000 µL has to be written in the column “volume” of the sample table, because the method itself calculates the volume that actually has to be injected. After putting the analyte solutions onto the rack, press “start”.

For evaluation and after recording the first chromatogram, check the retention times of your compounds. Since they depend on the performance of your column, you may have to adjust them in your method.

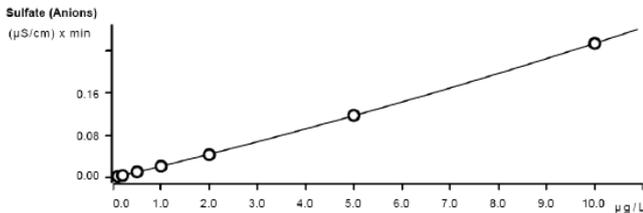
#### 4. Exemplary measurement

Fluoride, chloride, nitrite, bromide, nitrate, sulfate, and phosphate (0.5 µg/L of each compound) were measured with preconcentration of 4000 µL.



The calibration was performed within the concentration range of 0.1 – 10 µg/L, and a quadratic curve type was used for evaluation. Correlation coefficients and standard deviations are listed in the following table. The calibration curve for sulfate is shown as an example.

	Correlation coefficient	Percentage standard deviation [%]
Fluoride	0.999868	2.66
Chloride	0.999974	1.29
Nitrite	0.999976	1.23
Bromide	0.999997	0.39
Nitrate	0.999995	0.56
Phosphate	0.999889	2.52
Sulfate	0.999983	1.01



Function: .....  $A = -3.69238E-4 + 5.39462E-6 \times Q + 2.43628E-11 \times Q^2$   
 Relative standard deviation ..... 1.013491 %  
 Correlation coefficient ..... 0.999983

Sample type	Index	Conc.	Volume	Area	Ident	Date	Used
Standard 100	1	0.100	4000.0	0.002138	Cal STD 100	2010-02-24 11:29:49 UTC+1	used
Standard 50	1	0.200	4000.0	0.003838	Cal STD 50	2010-02-24 11:52:46 UTC+1	used
Standard 20	1	0.500	4000.0	0.010877	Cal STD 20	2010-02-24 12:15:34 UTC+1	used
Standard 10	1	1.000	4000.0	0.021567	Cal STD 10	2010-02-24 12:38:30 UTC+1	used
Standard 5	1	2.000	4000.0	0.043307	Cal STD 5	2010-02-24 13:01:53 UTC+1	used
Standard 2	1	5.000	4000.0	0.117875	Cal STD 2	2010-02-24 13:25:03 UTC+1	used
Standard 1	1	10.000	4000.0	0.254287	Cal STD 1	2010-02-24 13:49:03 UTC+1	used

The precision of 6 injections of 0.5, 5 and 1000 ppb standard anions was determined. For these measurements, a volume of 4000 µL was preconcentrated. The results are shown in the following table.

0.5 µg/L, 4000 µL

Ion	Mean value [µg/L]	Standard deviation relative [%]	Recovery [%]
Fluoride	0.496	0.6	99.1
Chloride	0.496	0.6	99.2
Nitrite	0.494	2.6	98.8
Bromide	0.487	0.7	97.4
Nitrate	0.512	0.5	102.4
Phosphate	0.473	4.8	94.6
Sulfate	0.505	1.9	101.0

5 µg/L, 4000 µL

Ion	Mean value [µg/L]	Standard deviation relative [%]	Recovery [%]
Fluoride	5.00	0.2	100.0
Chloride	4.95	0.1	99.0
Nitrite	4.84	1.3	96.7
Bromide	4.94	0.2	98.7
Nitrate	4.96	0.2	99.2
Phosphate	5.00	4.6	99.9
Sulfate	4.93	1.6	98.7

1000 µg/L, 4000 µL

Ion	Mean value [µg/L]	Standard deviation relative [%]	Recovery [%]
Fluoride	992	0.4	99.2
Chloride	1011	0.3	101.1
Nitrite	1011	1.7	101.1
Bromide	1006	0.3	100.6
Nitrate	1001	0.3	100.1
Phosphate	989	3.0	98.9
Sulfate	958	2.0	95.8

## **5. Optional equipment**

### **5.1. Eluent Production Module**

The 941 Eluent Production Module creates fresh new eluent out of eluent concentrate and ultrapure water. For installation instructions and further information, please refer to the 941 Eluent Production Module Manual.

### **5.2. Alternative MSM rinsing and regeneration methods**

For alternative suppressor rinsing and regeneration methods and setups, please refer to the AB-395.