Nitrate and Nitrite in Water and Seawater (MT519A)

1 SCOPE

This method covers the determination of Nitrate-N and Nitrite-N in drinking water and seawater. Additional sample pre-treatment might be required.

2 CALIBRATION RANGE

Low Range:
Sample low: 0.18 – 3.6 µmol/L (2.5 – 50.4 µg/L as N)
Sample high: 2.5 – 50 µmol/L (35 – 700 µg/L as N)

High Range
Sample low: 1.8 – 36 µmol/L (25 – 504 µg/L as N)
Sample high: 25 – 500 µmol/L (0.35 – 7 mg/L as N)

3 TYPICAL PERFORMANCE DATA

	Sample low		***Sample high***	
	Low Range	High Range	Low Range	High Range
Sampling	60 samples/h: 3:1		60 samples/h: 3:1	
Highest Calibrant	3.6 µmol/L	36 µmol/L	50 µmol/L	500 µmol/L
Blank variation (SD of 10 sequential blanks)	**0.002 µmol/L**	**0.007 µmol/L**	**0.009 µmol/L**	**0.027 µmol/L**
Detection limit (EPA procedure 40 CFR Part 136, App. B)	**0.006 µmol/L**	**0.019 µmol/L**	**0.086 µmol/L**	**0.077 µmol/L**

These performance statistics were generated using genuine SEAL Analytical parts and consumables. Performance may vary depending on system components and the number of channels selected.
Performance values are pooled from independent runs. Refer to section 17 for details.

4 METHOD PRINCIPLE

This automated procedure for the determination of nitrate and nitrite uses the procedure whereby nitrate is reduced to nitrite at pH 7.5 in a copperized cadmium reduction coil. The nitrite reduced from nitrate plus any nitrite react under acidic conditions with sulfanilamide to form a diazo compound that then couples with N-1-naphthylethylenediamine dihydrochloride (NEDD) to form a reddish-purple azo dye.

5 REFERENCES

1. Standard methods for the examination of water and waste water, 23rd edition, 2017

6 HARDWARE REQUIREMENTS

Chemistry Hardware: 2.0 mm glass, 5 mL heating bath, 4-way-valve, Cd-coil
Detector: LED photometer at 540 nm, 10 mm flowcell
Pump tubes: 6 + 2 air + sampler wash

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7 LIST OF REQUIRED CHEMICALS

All chemicals must be of analytical grade quality (ACS grade, pro analysi, …). DI water refers to high grade quality distilled or deionized water, free from organic contamination (e.g. ISO 3696 Grade 1 or ASTM standard D 1193 Type I/II)
Persons using this method should be familiar with normal laboratory practice. This method does not purport to address all safety risks, if any, associated with its use. It is the responsibility of the user to establish safety and health practices and to ensure compliance with local regulatory conditions.

Compound	CAS No.	Safety classification
Copper sulfate, pentahydrate	7758-99-8	*GHS07, GHS09*
Hydrochloric acid, 36.5-38%	7647-01-1	*GHS05, GHS07*
Imidazole, highest purity	228-32-4	*GHS05, GHS07, GHS08*
iso-propanol	67-63-0	*GHS02, GHS07*
N-(1-Naphthyl)ethylenediamine dihydrochloride	1465-25-4	*GHS07*
Nitric acid	7727-37-9	*GHS03, GHS05*
Phosphoric acid, conc.	7664-38-2	*GHS05*
Potassium nitrate	7757-79-1	*GHS03*
Sdoium chloride	7647-14-5	--
Sodium hydroxide	1310-73-2	*GHS05*
Sodium nitrite	7632-00-0	*GHS03, GHS06, GHS09*
Sulfanilamide	63-74-1	--
Triton X-100	9002-93-1	*GHS05, GHS07, GHS09*
Nitrogen gas	7727-37-9	*GHS04*

GHS01: Danger - Explosive; GHS02: Danger - Flammable; GHS03: Danger - Oxidizing; GHS04: Warning - Compressed gas; GHS05: Warning/Danger - Corrosive; GHS06: Danger - Toxic; GHS07: Irritant; GHS08: Danger - Health hazard; GHS09: Warning/Danger - Environmentally Damaging

8 REAGENT PREPARATION

To reach the stated performance values, standard, reagents and sampler wash must be free of solids and dissolved air.
For best performance vacuum filter all reagents through a 45 µm filter or glass filter paper. If necessary, vacuum filter all DI water used in the preparation of standards and for the sampler wash or degas the water in another way.
The total volume of each reagent can be varied, if the concentrations of its ingredients remain the same. It is recommended to only prepare the required amount of reagent, which can be calculated from the flowrate of its corresponding reagent pump tube

8.1 SYNTHETIC SEAWATER

Raw Material	Amount	Reagent Label
Sodium chloride	35 g	N/A
DI water	to 1000 mL	
Storage: plastic container at room temperature Stability: one week		

Dissolve 35 g of sodium chloride in about 800 mL of DI water. Dilute to 1000 mL with DI water and mix thoroughly.

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8.2 TRITON X-100, 50% SOLUTION

Raw material	Amount	Reagent Label
Triton X-100	50 mL	 Danger
Iso-propanol	50 mL	
Storage: glass or plastic bottle at room temperature Stability: six months, replace if turbid or cloudy		

Thoroughly mix 50 mL of Triton X-100 and 50 mL of iso-propanol.

8.3 SYSTEM WASH SOLUTION

Raw material	Amount	Reagent Label
DI water	1000 mL	N/A
Triton X-100, 50% solution	1 mL	
Storage: glass or plastic bottle at room temperature Stability: infinite, replace if turbid or cloudy		

Add 1 mL of Triton X-100 solution to 1000 mL of DI water and mix thoroughly.

8.4 SODIUM HYDORXIDE 1 mol/L

Raw material	Amount	Reagent Label
Sodium hydroxide	40 g	 Warning
DI water	to 1000 mL	
Storage: Plastic bottle at room temperature Stability: Infinite, replace if turbid or cloudy		

Dissolve 40 g of sodium hydroxide in about 700 mL of DI water. Cool to room temperature. Dilute to 1000 mL with DI water and mix thoroughly.

8.5 HYDROCHLORIC ACID 1 mol/L

Raw material	Amount	Reagent Label
Hydrochloric acid, 36.5 – 38%	85 mL	 Warning
DI water	to 1000 mL	
Storage: Plastic bottle at room temperature Stability: Infinite, replace if turbid or cloudy		

Add 85 mL of hydrochloric acid to about 700 mL of DI water. Cool to room temperature. Dilute to 1000 mL with DI water and mix thoroughly.

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8.6 HYDROCHLORID ACID, 2 mol/L

Raw material	Amount	Reagent Label
Hydrochloric acid, 36.5-38%	16.5 mL	 Danger
DI water	to 100 mL	
Triton X-100, 50% solution	0.5 mL	
Storage: glass or plastic bottle at room temperature Stability: infinite, replace if turbid or cloudy		

Cautiously, while stirring, add 16.5 mL of hydrochloric acid to about 60 mL of DI water. Dilute to 100 mL with DI water. Add 0.5 mL of Triton X-100 solution and mix thoroughly.

8.7 NITRIC ACID, 2 mol/L

Raw material	Amount	Reagent Label
Nitric acid, 69-71%	12.5 mL	 Danger
DI water	to 100 mL	
Storage: glass or plastic bottle at room temperature Stability: infinite, replace if turbid or cloudy		

Cautiously, while stirring, add 12.5 mL of nitric acid to about 60 mL of DI water. Dilute to 100 mL with DI water and mix thoroughly.

8.8 STOCK SOLUTION COPPER SULFATE

Raw material	Amount	Reagent Label
Copper sulfate	0.5 g	N/A
DI water	to 200 mL	
Storage: glass or plastic bottle at room temperature Stability: infinite, replace if turbid or cloudy		

Dissolve 0.5 g of copper sulfate in about 150 mL of DI water. Dilute to 200 mL with DI water and mix thoroughly.

8.9 COPPER ACTIVATION SOLUTION

Raw material	Amount	Reagent Label
Stock solution copper sulfate	50 mL	N/A
DI water	to 100 mL	
Triton X-100, 50% solution	0.5 mL	
Storage: glass or plastic bottle at room temperature Stability: infinite, replace if turbid or cloudy		

Add 50 mL of DI water to 50 mL of copper sulfate stock solution. Add 0.5 mL of Triton X-100 solution and mix thoroughly.

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8.10 STOCK SOLUTION IMIDAZOLE

Raw material	Amount	Reagent Label
Imidazole	16.3 g	 Danger
Hydrochloric acid, 36.5-38%	as required	
DI water	to 1000 mL	
Storage: amber bottle at room temperature Stability: infinite, replace if turbid or cloudy		

Dissolve 16.3 g of imidazole in about 900 ml of DI water. Adjust pH to 7.5 with hydrochloric acid. Dilute to 1000 mL with DI water and mix thoroughly.

8.11 WORKING IMIDAZOLE

Raw material	Amount	Reagent Label
Stock solution imidazole	375 mL	 Danger
Stock solution copper sulfate	0.9 mL	
DI water	to 500 mL	
Triton X-100, 50% solution	0.5 mL	
Storage: amber bottle at room temperature Stability: one week		

Add 0.9 mL of stock solution copper sulfate to 375 mL of stock imidazole buffer. Dilute to 500 mL with DI water, add 0.5 mL 50% Triton X-100 and mix thoroughly.

8.12 COLOR REAGENT

Raw material	Amount	Reagent Label
Sulfanilamide	10 g	 Danger
Phosphoric acid	100 mL	
N-(1-Naphthyl)ethylenediamine dihydrochloride (NEDD)	0.5 g	
DI water	to 1000 mL	
Storage: amber bottle at 4℃ Stability: one month		

Add 100 mL of phosphoric acid to about 800 mL of DI water. If necessary, cool to room temperature. Add 10 g of sulfanilamide and 0.5 g of NEDD and dissolve completely. Dilute to 1000 mL with DI water and mix thoroughly. The reagent should be colorless: if it is pink the phosphoric acid is probably impure.

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9 STANDARDS

These formulas assume that samples are preserved with the described preserving solution (see section 10). If other preservation methods are used, the sample and standard matrix and the sampler wash solution should be similar.

9.1 STOCK STANDARD A – 1000 MG/L NO3-N

Raw material	Amount	Reagent Label
Potassium nitrate	7.219 g	N/A
DI water	to 1000 mL	
Storage: glass or plastic bottle at 4℃ Stability: one year		

Dissolve 7.219 g of potassium nitrate in about 600 mL of DI water. Dilute to 1000 mL with DI water and mix thoroughly.

9.2 STOCK STANDARD B – 3.6 mmol/L

Raw material	Amount	Reagent Label
Stock standard A – 1000 mg/L NO3-N	5 mL	N/A
DI water	to 100 mL	
Storage: glass or plastic bottle at 4℃ Stability: one year		

Add 5 mL of stock standard A to about 70 mL of DI water. Dilute to 100 mL with DI water and mix thoroughly.

9.3 STOCK STANDARD C – 360 µmol/L

Raw material	Amount	Reagent Label
Stock standard A – 1000 mg/L NO3-N	0.5 mL	N/A
DI water	to 100 mL	
Storage: glass or plastic bottle at 4℃ Stability: one year		

Add 0.5 mL of stock standard A to about 80 mL of DI water. Dilute to 100 mL with DI water and mix thoroughly.

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9.4 WORKING STANDARDS

Prepare working standards fresh every day before use. For example:

9.4.1 SAMPLE HIGH

Low range	
mL of Stock C	**µmol/L**
0.14	5.04
0.35	12.6
0.7	25.2
1.05	37.8
1.4	50.4

High range	
mL of Stock A	**µmol/L**
0.07	50.4
0.175	126
0.35	252
0.525	378
0.7	504

Pipette each aliquot of Stock standard A or C into a 100 mL volumetric flask. Dilute to volume with synthetic seawater or water and mix thoroughly

9.4.2 SAMPLE LOW

Low range	
mL of Stock C	**µmol/L**
0.1	0.36
0.25	0.9
0.5	1.8
0.75	2.7
1	3.6

High range	
mL of Stock B	**µmol/L**
0.1	3.6
0.25	9
0.5	18
0.75	27
1	36

Pipette each aliquot of Stock standard B or Stock standard C into a 100 mL volumetric flask. Dilute to volume with synthetic seawater or water and mix thoroughly.

10 SAMPLE PRESERVATION AND STORAGE

Samples containing particles > 0.1 mm must be filtered. Strongly acidic or alkaline samples must be approximately neutralized before analysis.
The sample may be filtered through activated carbon, provided changes of the nitrite or nitrate concentration in the sample can be ruled out.

11 INTERFERENCES

Oil, oxidizing agents and organic matter can deactivate the cadmium coil as well as higher concentrations of copper and iron.

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12 START-UP PROCEDURE

1. Switch on all modules
2. Open the nitrogen supply
3. Start pumping:
   - DI water through the sampler wash
   - System wash solution through the reagent lines
4. Wait for a stable bubble pattern
5. Switch the reagent lines from wash solution to their corresponding reagent
6. When the imidazole buffer has reached the 4-way-valve, open the valve to insert the cadmium coil into the liquid stream
7. Wait for the baseline and bubble pattern to stabilize again.

13 SHUTDOWN PROCEDURE

1. Switch the 4-way-valve to remove the cadmium coil from the liquid stream
2. Switch the reagent lines:
   - DI water through the sampler wash
   - System wash solution through the reagent lines
3. Pump for at least 20 minutes
4. Release the pump platen
5. Switch of all modules.

14 SYSTEM CLEANING PROCEDURE

DAILY:
Follow the shutdown procedure.

WEEKLY:
With the coil being bypassed pump sodium hydroxide 1mol/L for 10 minutes through all reagent lines and DI water through the sample line. Flush out the NaOH by pumping system wash solution through the reagent lines for 10 minutes.

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15 CADMIUM COIL HANDLING

When starting with a new coil, clean it and then perform the activation procedure. When the recovery of the nitrate falls below 90%, repeat the activation procedure. If activating the column fails to increase the recovery rate of nitrate, clean the column and activate it again. Oil, oxygen and high concentrations of organic compounds reduce the reduction efficiency quickly.
For both procedures it is important that the liquid passes the coil quickly. Otherwise you can easily dissolve too much cadmium during the cleaning procedure or block the coil with colloidal copper during the activation procedure.

15.1 CLEANING PROCEDURE

Use this procedure sparingly, because it dissolves a lot of cadmium from the coil and shortens its life.

1. Remove the Tygon sleeve from one end of the cadmium coil. Keep the sleeving and some PE tubing at the other end.
2. Use a water vacuum pump or a 25 mL plastic syringe to suck the cleaning liquids through the coil. Connect them to the end of the Cadmium coil which has no tubing. Always suck the liquid through the coil rather than pushing it through and make sure to suck air at the end of each stroke.
3. Place 20 mL of nitric acid 2 mol/L in a small glass container and suck the acid through the coil by dipping the PE tubing of the open coil into the acid. The coil will become warm to the touch.
4. Place 20 mL of concentrated hydrochloric acid in a beaker and 20 mL of DI water in another beaker. Suck about 5 mL of acid then about 5 mL of DI water through the coil by dipping the PE tubing of the open coil into the liquid. Repeat until both liquids are used up.
5. Suck additional 20 mL of DI water through the coil in the same manner.
6. Replace the Tygon sleeve and ensure that you have a tight joint between the ends of the coil and the Tygon tubing.

15.2 ACTIVATION PROCEDURE

1. Start pumping:
   - DI water through the sample line
   - Buffer solution through the buffer line
   - System wash solution through all reagent lines after the cadmium coil
2. When the buffer reagent reaches the switch valve, pump:
   - Hydrochloric acid 2 mol/L for one minute
   - Copper activation solution for two minutes
   - Hydrochloric acid 2 mol/L for five minutes
3. Pump buffer solution through its reagent line for 5 to 10 minutes
4. Aspirate the top standard through the sample line for 5 to 10 minutes to condition the coil.
5. Place the sample line in DI water for 10 minutes again.
6. Close the switch valve.
7. Start up the system normally.

16 OPERATING NOTES

1.
2. Nitrogen gas is used for the segmentation of the liquid stream to prevent oxidation of the cadmium coil's active surface. The nitrogen has to be injected at room pressure to provide a correct bubble pattern. The use of a nitrogen cushion (P/N 178-N000-01) is recommended.

3. A cadmium coil with ten turns (p/n 165-0301-02) is used to obtain sufficient reduction efficiency. Alternatively, two cadmium coils with six turns (p/n 165-0301-01) can be used.

4. The setup can be used to measure nitrite only. In this case keep the 4-way-valve closed, so that the cadmium coil is bypassed.

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17 PERFORMANCE VALIDATION

17.1 TEST CONDITIONS

Sampler type	XY-3
Sample probe	High-Tech 1.0 mm
Sample tubing and length	PE15, 90 cm
Total sample flow rate (incl. by-pass and other channels)	2.4 mL/min
Wash pot type	Fixed
Wavelength	540 nm
Reference energy	
Sample:ref ratio	
Light power	98%
Room temperature	22 – 25℃

17.2 PERFORMANCE DATA IN SEAWATER – OVERVIEW

	Sample low		***Sample high***	
	Low range	High range	Low range	High range
Highest calibrant	**3.6 mmol/L**	**36 µmol/L**	**50 µmol/L**	**500 µmol/L**
Sensitivity *(in specified flowcell)*	0.07 – 0.09 AU	0.76 – 0.84 AU	0.09 – 0.10 AU	0.85 – 0.87 AU
Correlation coefficient *(linear, six points)*	0.999	0.999	0.999	0.999
Reagent absorbance	0.01 – 0.02 AU		0.01 – 0.02 AU	
Coefficient of variation	0.005 µmol/L; 0.3%	0.014 µmol/L; 0.1%	0.046 µmol/L; 0.2%	0.253 µmol/L; 0.1%
Pooled SD	0.003 µmol/L	0.020 µmol/L	0.075 µmol/L	0.517 µmol/L
Blank variation (SD of 10 sequential blanks)	**0.002 µmol/L**	**0.007 µmol/L**	**0.009 µmol/L**	**0.027 µmol/L**
Detection limit (EPA, spikes)	0.004 µmol/L	0.006 µmol/L	**0.086 µmol/L**	**0.077 µmol/L**
Detection limit (EPA, blanks)	**0.006 µmol/L**	**0.019 µmol/L**	0.030 µmol/L	0.067 µmol/L

These performance statistics were generated using genuine SEAL Analytical parts and consumables.
Performance may vary depending on system components and the number of channels selected.
Performance values are pooled from independent runs. The **bold** values are reported on the first page of this method document.

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17.3 PERFORMANCE DATA IN WATER – OVERVIEW

	Sample low		***Sample high***	
	Low range	High range	Low range	High range
Highest calibrant	**3.6 µmol/L**	**36 µmol/L**	**50 µmol/L**	**500 µmol/L**
Sensitivity *(in specified flowcell)*	0.07 – 0.09 AU	0.76 – 0.84 AU	0.08 – 0.09 AU	0.73 – 0.85 AU
Correlation coefficient *(linear, six points)*	0.999	0.999	0.999	0.999
Reagent absorbance	0.01 – 0.02 AU		0.01 – 0.02 AU	
Coefficient of variation	0.008 µmol/L; 0.4%	0.122 µmol/L; 0.6%	0.041 µmol/L; 0.1%	0.249 µmol/L; 0.1%
Pooled SD	0.006 µmol/L	0.132 µmol/L	0.115 µmol/L	0.747 µmol/L
Blank variation (SD of 10 sequential blanks)	0.001 µmol/L	0.002 µmol/L	0.019 µmol/L	0.021 µmol/L
Detection limit (EPA, spikes)	0.003 µmol/L	0.005 µmol/L	0.094 µmol/L	0.042 µmol/L
Detection limit (EPA, blanks)	0.003 µmol/L	0.007 µmol/L	0.227 µmolL/	0.060 µmol/L

These performance statistics were generated using genuine SEAL Analytical parts and consumables.
Performance may vary depending on system components and the number of channels selected.
Performance values are pooled from independent runs. The **bold** values are reported on the first page of this method document.

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17.4 CALIBRATION DATA

17.4.1 SAMPLE LOW

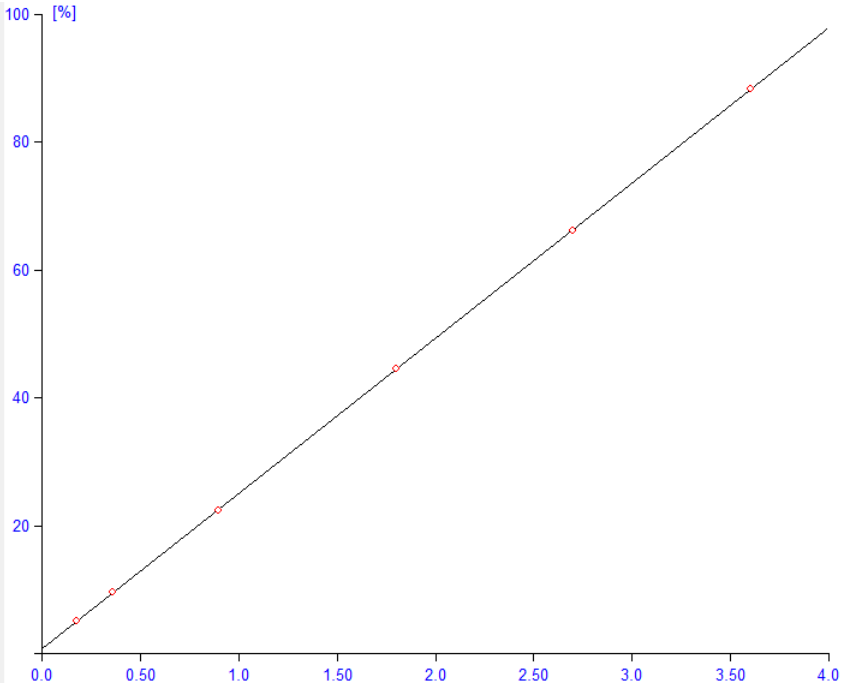
NOx

Kalibr. Typ: linear
Baseline not in cal.
Korr. Koeff.: 1.0000
Gain/AUFS: 100 / 0.1000
Gleichung: y = bx + a
y= Peakhöhe in dig.We
x = Konz. in<umol/L>
a = 3.5746E+003
b = 1.5926E+004

Korrekturen:
Basislinie: ja
Drift: ja
Carryover: ja 0.22 %

Kalibr. Werte:

Typ	Berechnet	Zielwert	Diff. (Konz.)	Diff. (%)
1C	0.1802	0.1800	0.0002	0.09
2C	0.3655	0.3600	0.0055	1.52
3C	0.8911	0.9000	-0.0089	-0.99
4C	1.8065	1.8000	0.0065	0.36
5C	2.6935	2.7000	-0.0065	-0.24
6C	3.6033	3.6000	0.0033	0.09



Low calibration range: 0.18 µmol/L to 3.6 µmol/L. Absorbance of top standard: 0.088 AU

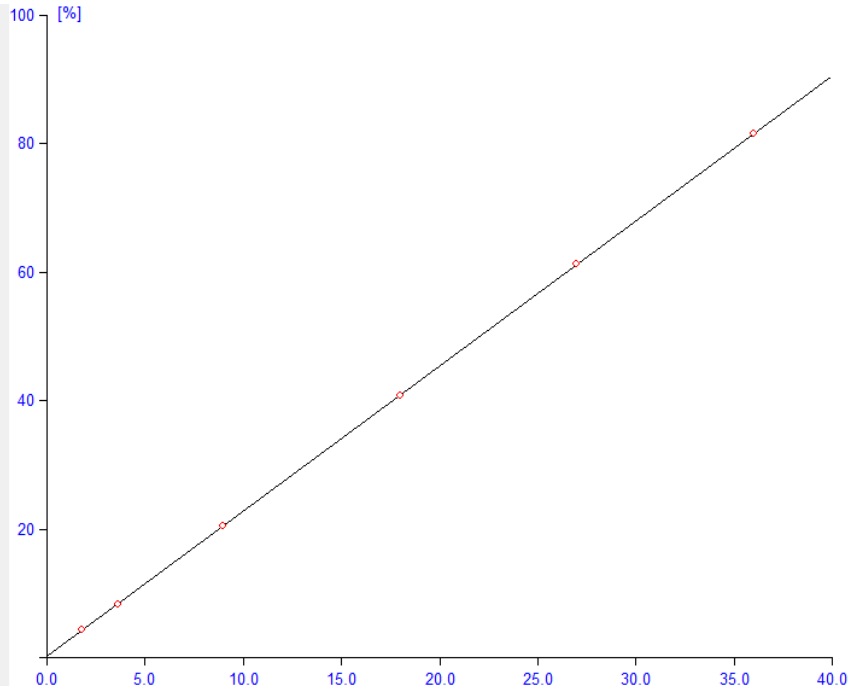
NOx

Kalibr. Typ: linear
Baseline not in cal.
Korr. Koeff.: 1.0000
Gain/AUFS: 10 / 1.0000
Gleichung: y = bx + a
y= Peakhöhe in dig.We
x = Konz. in<umol/L>
a = 3.2430E+003
b = 1.4804E+003

Korrekturen:
Basislinie: ja
Drift: ja
Carryover: nein

Kalibr. Werte:

Typ	Berechnet	Zielwert	Diff. (Konz.)	Diff. (%)
1C	1.804	1.800	0.004	0.20
2C	3.608	3.600	0.008	0.23
3C	8.984	9.000	-0.016	-0.18
4C	18.004	18.000	0.004	0.02
5C	26.996	27.000	-0.004	-0.02
6C	36.004	36.000	0.004	0.01



High calibration range: 1.8 µmol/L to 36 µmol/L. Absorbance of top standard: 0.82 AU

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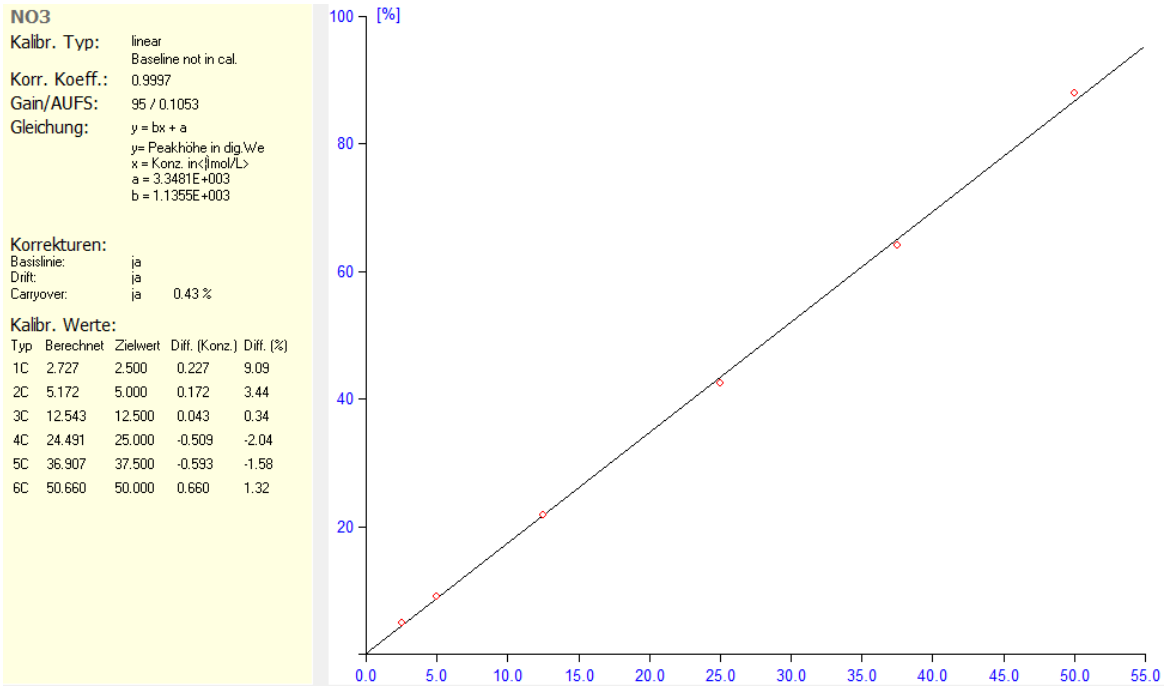
17.4.2 SAMPLE HIGH



NO3

Kalibr. Typ: linear
Baseline not in cal.

Korr. Koeff.: 0.9997

Gain/AUFS: 95 / 0.1053

Gleichung: y = bx + a
y= Peakhöhe in dig.We
x = Konz. in<[]mol/L>
a = 3.3481E+003
b = 1.1355E+003

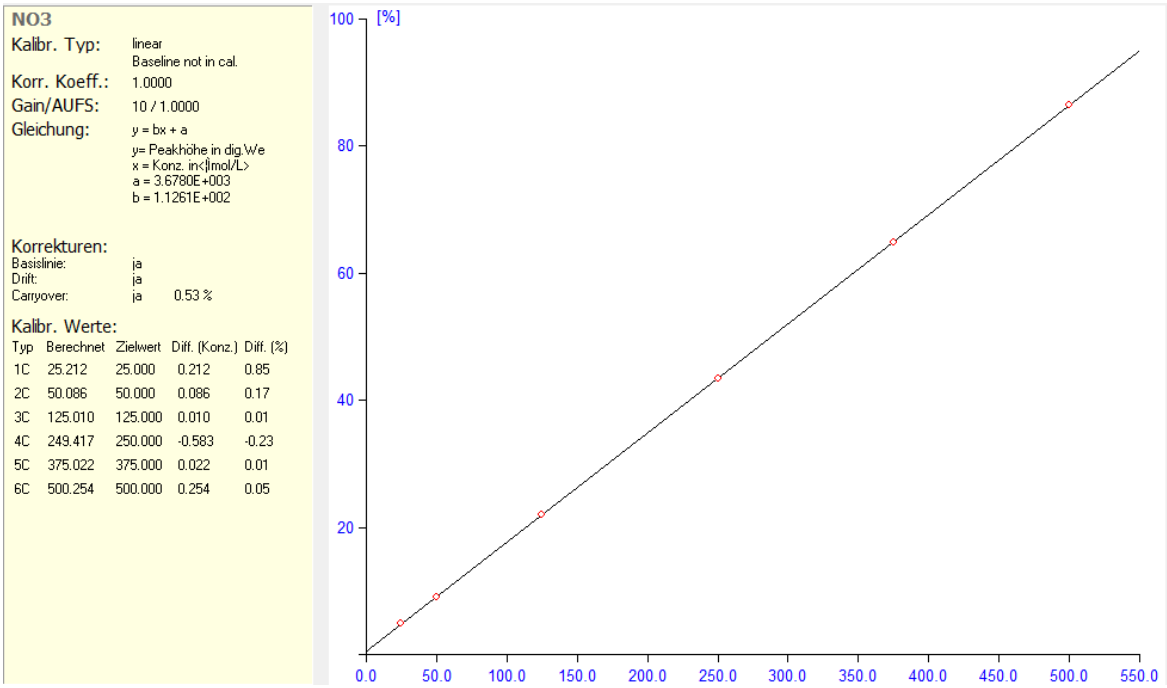
Korrekturen:

Basislinie:	ja	
Drift:	ja	
Carryover:	ja	0.43 %

Kalibr. Werte:

Typ	Berechnet	Zielwert	Diff. (Konz.)	Diff. (%)
1C	2.727	2.500	0.227	9.09
2C	5.172	5.000	0.172	3.44
3C	12.543	12.500	0.043	0.34
4C	24.491	25.000	-0.509	-2.04
5C	36.907	37.500	-0.593	-1.58
6C	50.660	50.000	0.660	1.32

Low calibration range: 2.5 µmol/L to 50 µmol/L. Absorbance of top standard: 0.090 AU



NO3

Kalibr. Typ: linear
Baseline not in cal.

Korr. Koeff.: 1.0000

Gain/AUFS: 10 / 1.0000

Gleichung: y = bx + a
y= Peakhöhe in dig.We
x = Konz. in<[]mol/L>
a = 3.6780E+003
b = 1.1261E+002

Korrekturen:

Basislinie:	ja	
Drift:	ja	
Carryover:	ja	0.53 %

Kalibr. Werte:

Typ	Berechnet	Zielwert	Diff. (Konz.)	Diff. (%)
1C	25.212	25.000	0.212	0.85
2C	50.086	50.000	0.086	0.17
3C	125.010	125.000	0.010	0.01
4C	249.417	250.000	-0.583	-0.23
5C	375.022	375.000	0.022	0.01
6C	500.254	500.000	0.254	0.05

High calibration range: 25 µmol/L to 500 µmol/L. Absorbance of top standard: 0.86 AU

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17.5 REPRODUCIBILITY – SAME CONCENTRATION

The reproducibility is checked by measuring 20 replicates of a 50% calibration range standard. Three runs are performed on three different days. Baseline and sensitivity drift correction are applied. The coefficient of variation is calculated by dividing the standard deviation of the replicates by the mean and then multiplying with 100.

17.5.1 SAMPLE LOW

Peak no.	Low Range			High Range		
	1	2	3	1	2	3
	µmol/L			µmol/L		
Nominal	*1.800*			*18.000*		
1	1.813	1.828	1.813	18.067	18.047	18.005
2	1.813	1.828	1.812	18.062	18.043	18.009
3	1.808	1.830	1.809	18.063	18.056	18.004
4	1.810	1.828	1.811	18.079	18.056	17.997
5	1.810	1.828	1.812	18.043	18.058	17.992
6	1.807	1.828	1.809	18.070	18.046	17.987
7	1.807	1.828	1.806	18.022	18.046	17.973
8	1.803	1.826	1.809	18.045	18.053	17.988
9	1.806	1.826	1.807	18.050	18.041	18.008
10	1.800	1.825	1.807	18.050	18.037	18.002
11	1.799	1.826	1.805	18.035	18.048	17.989
12	1.798	1.825	1.807	18.027	18.033	17.998
13	1.796	1.827	1.805	18.052	18.033	17.998
14	1.794	1.824	1.803	18.045	18.051	18.020
15	1.792	1.824	1.803	18.031	18.045	17.994
16	1.790	1.821	1.802	18.019	18.051	17.998
17	1.794	1.823	1.806	18.041	18.070	18.013
18	1.787	1.822	1.808	18.043	18.070	18.013
19	1.801	1.822	1.809	18.051	18.077	17.987
20	1.794	1.822	1.808	18.041	18.073	17.971
Mean	1.801	1.826	1.807	18.047	18.052	17.997
Std. deviation	0.008	0.003	0.003	0.016	0.013	0.013
Coefficient of variation	0.4%	0.1%	0.2%	0.1%	0.1%	0.1%

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17.5.2 SAMPLE HIGH

Peak no.	Low Range			High Range		
	1	2	3	1	2	3
	µmol/L			µmol/L		
Nominal	*25.000*			*250.000*		
1	24.710	24.761	24.630	250.506	249.796	250.241
2	24.682	24.786	24.630	250.681	249.734	250.536
3	24.717	24.826	24.649	250.804	250.098	250.563
4	24.749	24.813	24.691	251.102	250.204	250.580
5	24.710	24.774	24.674	251.269	250.408	250.937
6	24.708	24.762	24.599	250.786	250.319	250.482
7	24.676	24.760	24.479	250.453	249.965	250.536
8	24.709	24.770	24.634	250.997	249.858	250.125
9	24.721	24.846	24.665	250.935	249.991	250.661
10	24.748	24.825	24.653	250.918	249.903	250.429
11	24.766	24.815	24.669	251.128	250.363	250.634
12	24.725	24.768	24.645	250.480	250.266	250.527
13	24.739	24.751	24.595	250.418	249.965	250.768
14	24.717	24.764	24.665	250.839	249.646	249.938
15	24.778	24.777	24.613	250.988	250.036	250.090
16	24.861	24.781	24.691	251.155	250.018	250.268
17	24.603	24.754	24.699	251.041	250.124	250.081
18	24.717	24.733	24.692	250.427	249.956	250.411
19	24.761	24.691	24.683	250.497	249.690	250.759
20	24.739	24.708	24.671	250.734	249.770	250.420
Mean	24.727	24.773	24.646	250.808	250.006	250.449
Std. deviation	0.049	0.039	0.050	0.273	0.225	0.259
Coefficient of variation	0.2%	0.2%	0.2%	0.1%	0.1%	0.1%

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17.6 REPRODUCIBILITY – VARYING CONCENTRATION

Reproducibility is checked by running ten replicates of five different standards in pseudo-random order. Three runs are performed on three different days. Baseline, sensitivity drift and carryover correction are applied.

17.6.1 SAMPLE LOW

	Low Range				**High Range**			
		1	**2**	**3**		**1**	**2**	**3**
Group	Nominal concentration µmol/L	Standard deviation in µmol/L			Nominal concentration µmol/L	Standard deviation in µmol/L		
1	3.6	0.004	0.003	0.005	36	0.024	0.038	0.032
2	2.7	0.010	0.003	0.003	27	0.018	0.023	0.020
3	1.8	0.004	0.002	0.004	18	0.020	0.014	0.023
4	0.9	0.002	0.002	0.002	9	0.010	0.011	0.012
5	0	0.003	0.002	0.003	0	0.013	0.013	0.010
Pooled SD		0.005	0.002	0.003	Pooled SD	0.018	0.022	0.021

17.6.2 SAMPLE HIGH

	Low Range				**High Range**			
		1	**2**	**3**		**1**	**2**	**3**
Group	Nominal concentration µmol/L	Standard deviation in µmol/L			Nominal concentration µmol/L	Standard deviation in µmol/L		
1	50.0	0.175	0.108	0.077	500	0.362	0.995	0.823
2	37.5	0.072	0.084	0.065	375	0.655	0.536	0.618
3	25.0	0.067	0.056	0.064	250	0.425	0.451	0.409
4	12.5	0.053	0.048	0.040	125	0.397	0.395	0.455
5	0	0.025	0.022	0.034	0	0.136	0.125	0.097
Pooled SD		0.094	0.070	0.058	Pooled SD	0.428	0.575	0.537

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17.7 DETECTION LIMIT DATA (EPA SPIKE METHOD)

The detection limit MDL_s is determined from ten replicates of spikes. Three runs are performed on three different days. Baseline and sensitivity drift are applied. The detection limit is calculated by multiplying the standard deviation of the replicates by the student factor for 10 replicates (T = 2.821).

17.7.1 SAMPLE LOW

Peak no.	Low Range			High Range		
	1	2	3	1	2	3
	µmol/L			µmol/L		
Nominal	*0.072*			*0.720*		
1	0.064	0.065	0.060	0.553	0.533	0.604
2	0.063	0.065	0.059	0.553	0.533	0.608
3	0.065	0.065	0.060	0.556	0.535	0.611
4	0.066	0.065	0.060	0.551	0.533	0.607
5	0.064	0.065	0.059	0.549	0.533	0.605
6	0.067	0.067	0.058	0.549	0.53	0.605
7	0.065	0.066	0.058	0.55	0.532	0.606
8	0.066	0.065	0.058	0.551	0.529	0.605
9	0.068	0.065	0.059	0.546	0.529	0.605
10	0.069	0.066	0.059	0.544	0.531	0.604
Mean	0.066	0.065	0.059	0.550	0.532	0.606
Std. deviation	0.002	0.001	0.001	0.003	0.002	0.002
Detection Limit	0.005	0.003	0.003	0.008	0.005	0.005

17.7.2 SAMPLE HIGH

Peak no.	Low Range			High Range		
	1	2	3	1	2	3
	µmol/L			µmol/L		
Nominal	*1.000*			*10.000*		
1	1.139	1.004	0.945	6.258	7.591	7.388
2	1.135	1.011	0.949	6.249	7.600	7.352
3	1.126	1.007	0.955	6.276	7.574	7.361
4	1.135	1.012	0.971	6.276	7.583	7.379
5	1.132	1.013	0.970	6.293	7.583	7.397
6	1.132	1.013	1.000	6.311	7.618	7.379
7	1.127	1.007	0.971	6.311	7.600	7.397
8	1.130	1.016	0.970	6.302	7.600	7.415
9	1.116	1.007	0.976	6.302	7.618	7.361
10	1.142	1.014	0.986	6.337	7.636	7.433
Mean	1.131	1.010	0.969	6.292	7.600	7.386
Std. deviation	0.007	0.004	0.017	0.027	0.019	0.025
Detection Limit	0.021	0.011	0.047	0.076	0.054	0.072

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17.8 DETECTION AND REPORTING LIMIT DATA (EPA BLANK METHOD)

The detection limit MDL_b is determined from ten replicates of blanks. Three runs are performed on three different days. Baseline and sensitivity drift are applied. The detection limit is calculated by multiplying the standard deviation of the blanks by the student factor for 10 replicates (T = 2.821) and adding the mean value. if it is positive.

17.8.1 SAMPLE LOW

Peak no.	Low Range			High Range		
	1	2	3	1	2	3
	µmol/L			µmol/L		
1	-0.017	-0.028	-0.028	-0.127	-0.154	-0.100
2	-0.018	-0.028	-0.027	-0.129	-0.157	-0.105
3	-0.017	-0.028	-0.028	-0.133	-0.159	-0.106
4	-0.019	-0.029	-0.027	-0.135	-0.164	-0.110
5	-0.020	-0.030	-0.025	-0.137	-0.167	-0.111
6	-0.020	-0.028	-0.027	-0.139	-0.170	-0.109
7	-0.022	-0.028	-0.024	-0.141	-0.174	-0.111
8	-0.024	-0.027	-0.026	-0.143	-0.177	-0.113
9	-0.024	-0.027	-0.025	-0.145	-0.178	-0.116
10	-0.023	-0.025	-0.024	-0.144	-0.180	-0.115
Mean	-0.020	-0.028	-0.026	-0.137	-0.168	-0.110
Std. deviation	0.003	0.001	0.002	0.006	0.009	0.005
Detection Limit	0.008	0.003	0.006	0.017	0.025	0.014

17.8.2 SAMPLE HIGH

Peak no.	Low Range			High Range		
	1	2	3	1	2	3
	µmol/L			µmol/L		
1	0.135	-0.113	-0.141	-3.946	-2.679	-2.978
2	0.136	-0.111	-0.144	-3.981	-2.697	-2.942
3	0.128	-0.131	-0.152	-3.972	-2.714	-2.995
4	0.129	-0.131	-0.168	-3.998	-2.714	-2.978
5	0.135	-0.120	-0.149	-3.990	-2.705	-3.058
6	0.124	-0.128	-0.148	-3.998	-2.714	-3.004
7	0.124	-0.137	-0.156	-3.990	-2.705	-3.058
8	0.127	-0.135	-0.155	-3.998	-2.732	-3.058
9	0.129	-0.133	-0.166	-4.007	-2.697	-3.049
10	0.136	-0.129	-0.179	-4.007	-2.705	-3.022
Mean	0.130	-0.127	-0.156	-3.989	-2.706	-3.014
Std. deviation	0.005	0.009	0.012	0.019	0.014	0.041
Detection Limit	0.144	0.026	0.034	0.052	0.039	0.116

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17.9 OTHER METHOD DATA AND SETTINGS

Parameter	Value	Notes
Lag time	8 minutes	Time between sample cup and detector. Depends on number of channels in use.
Carryover	0.3% - 0.5%	See AACE manual for calculation.
Reagent absorbance	0.01 – 0.02 AU	See AACE manual for how reagent absorbance is calculated.
Smoothing	none	See AACE manual for further information.

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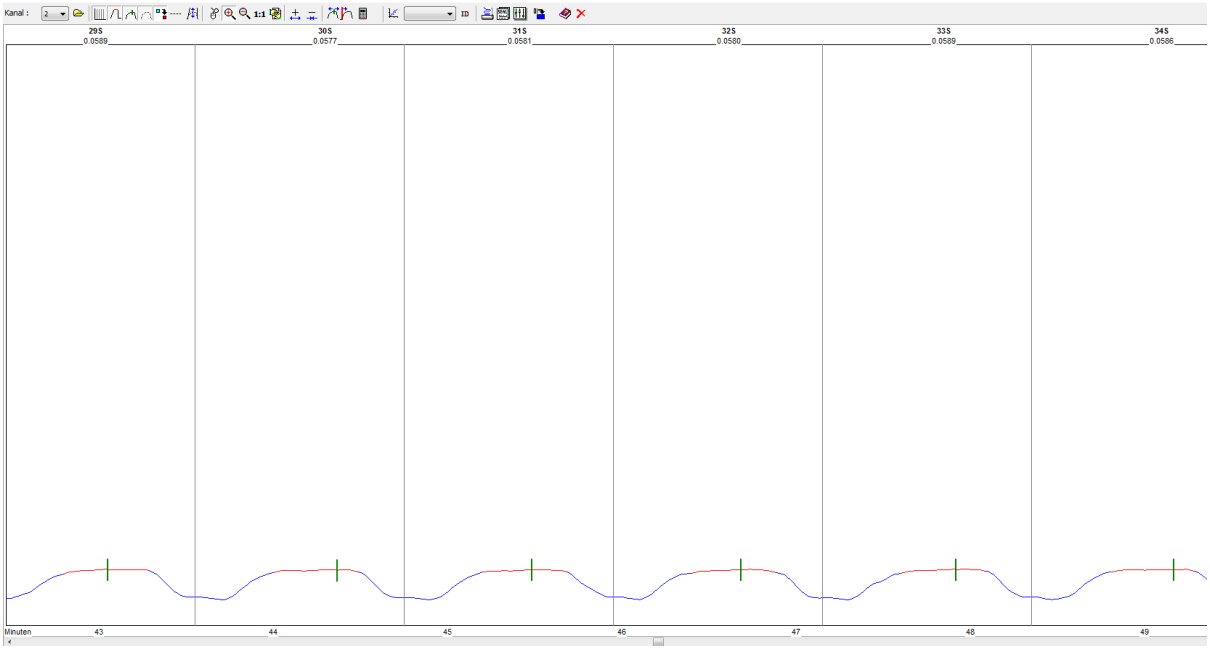
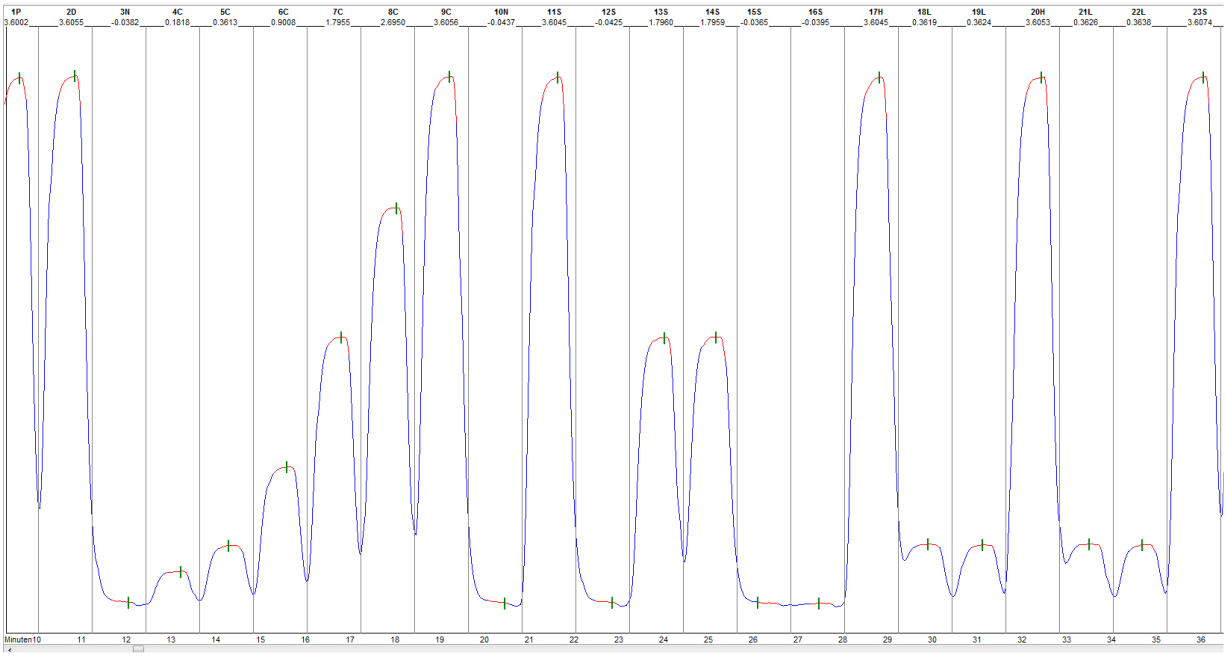
17.10 TYPICAL PEAK SHAPES

17.10.1 SAMPLE LOW– LOW RANGE



Low range: Spike replicates at 0.072 µmol/L (expanded view)



Low range: Excerpt from a test run at 5 concentrations

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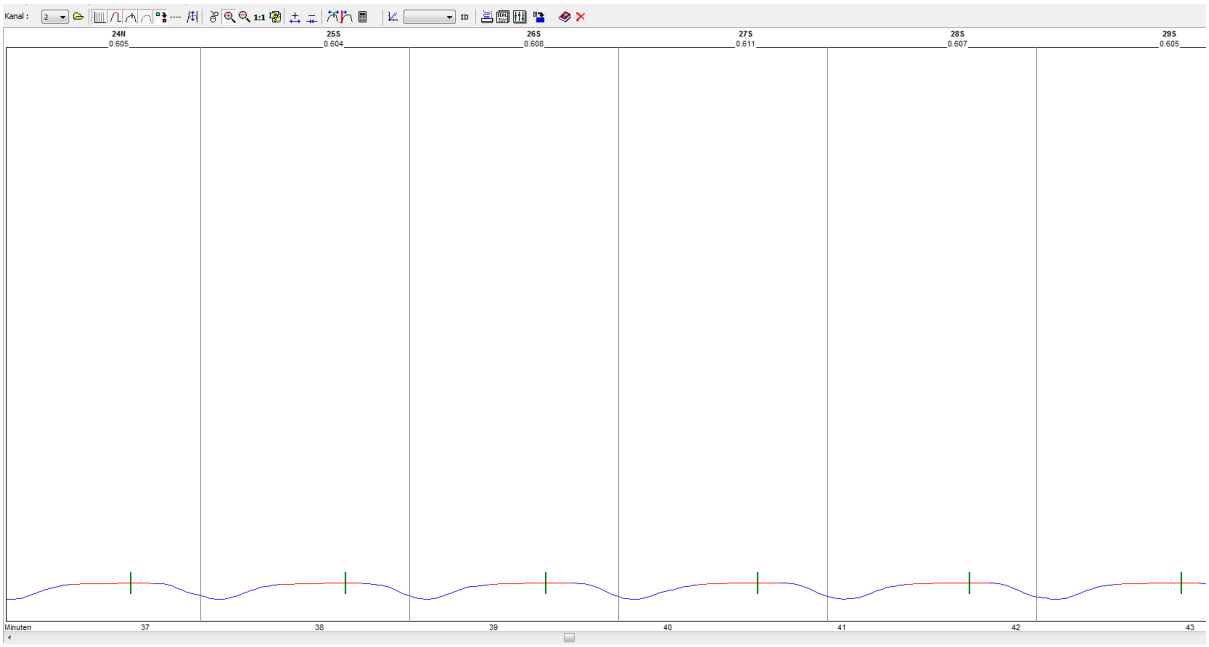
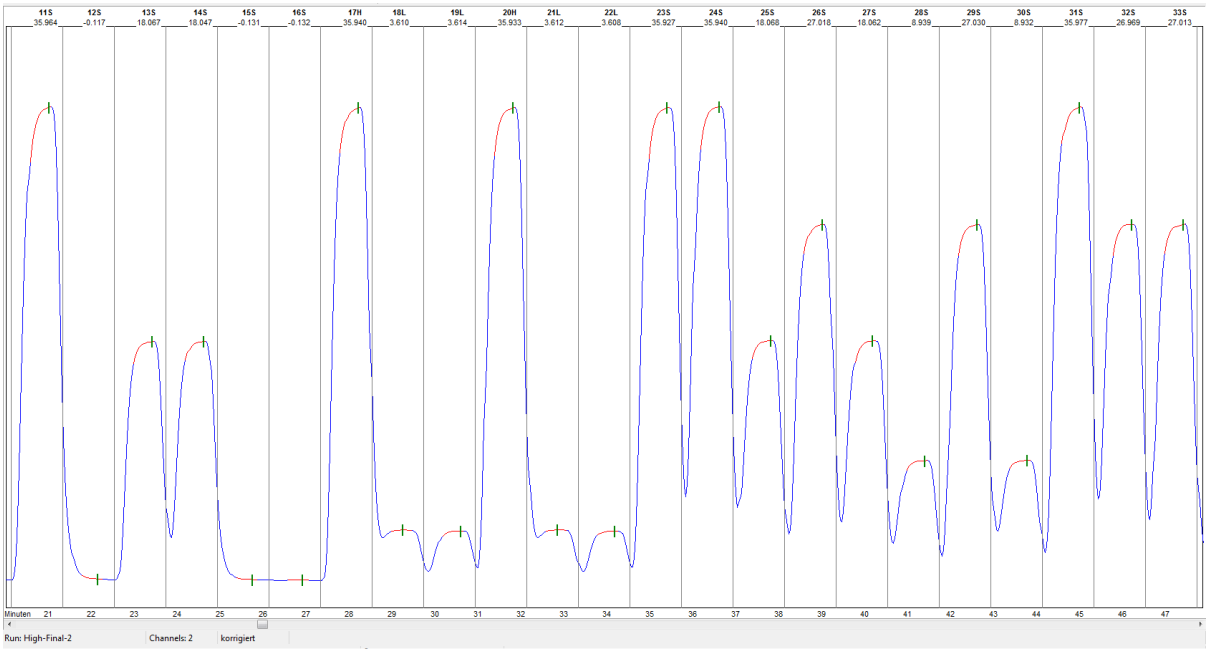
17.10.2 SAMPLE LOW – HIGH RANGE



High range: Spike replicates at 0.72 µmol/L (expanded view)



High range: Excerpt from a test run at 5 concentrations

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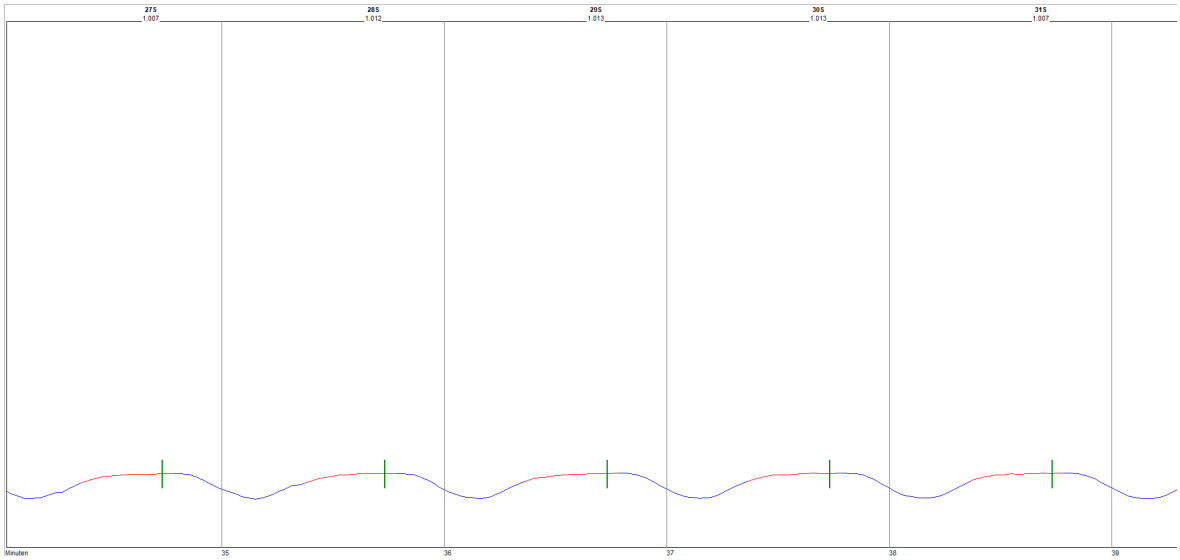
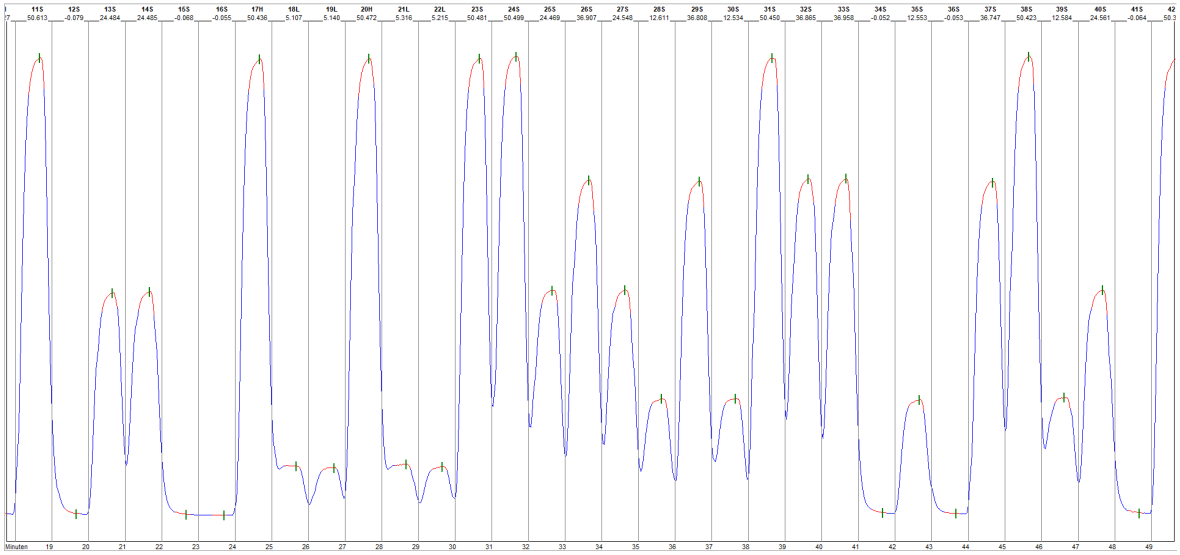
17.10.3 SAMPLE HIGH – LOW RANGE



Low range: Spike replicates at 1 µmol/L (expanded view)



Low range: Excerpt from a test run at 5 concentrations

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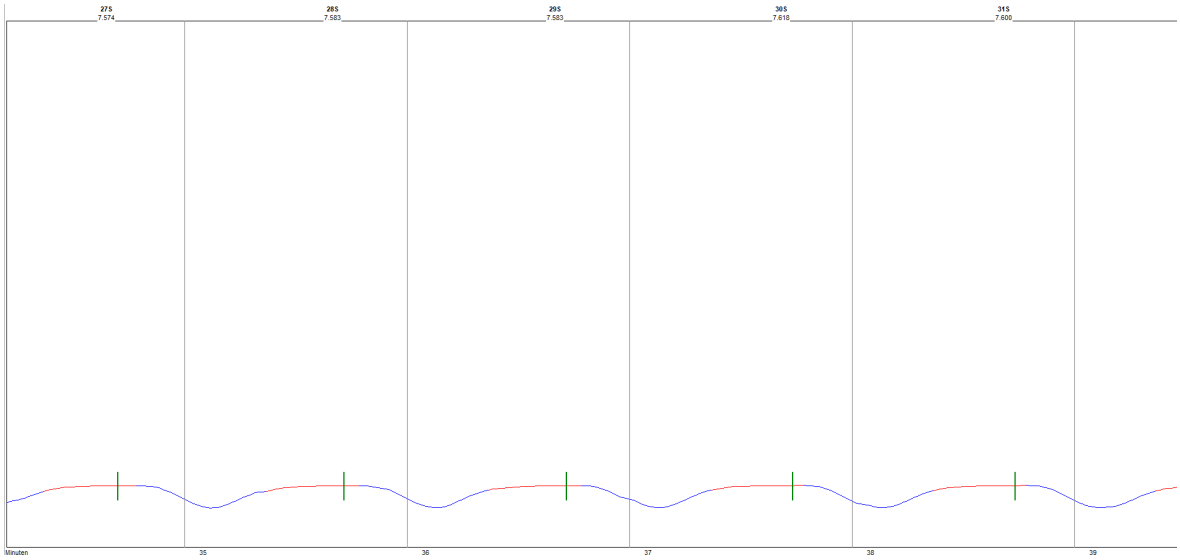
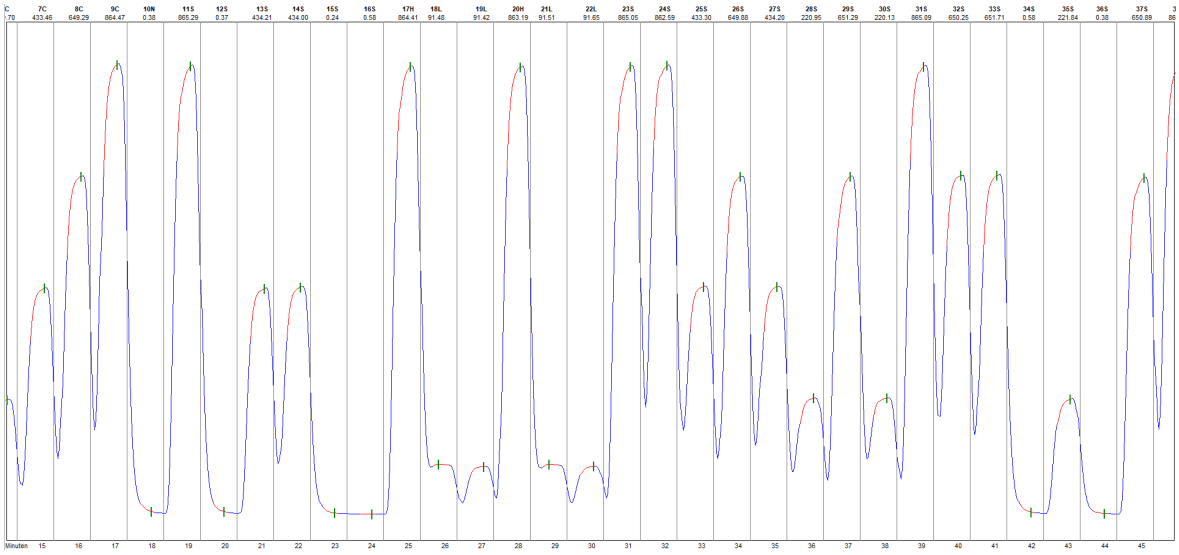
17.10.4 SAMPLE HIGH – HIGH RANGE



High range: Spike replicates at 10 µmol/L (expanded view)



High range: Excerpt from a test run at 5 concentrations

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18 REVISIONS

Revision	Date	Changes
0	April 2019	New method
1	June 2019	Flowchart updated
2	July 2019	Performance data for water removed, performance data and flowchart for sample high in seawater added. Flowcharts and parts list updated.
3	August 2019	Flowchart updated
4	March 2020	Logo changed, introduction for section 8 updated, minor text changes, weekly cleaning procedure corrected, parts list corrected. Added synthetic seawater in section 8 and sodium chloride in section 7. Section 15 corrected.
5	August 2021	Air valves tube changed. Consumables list and flowchart updated.

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19 PARTS LIST

Only use genuine SEAL parts and consumables with the SEAL logo or the “SEAL Tec” stamp on the package or the part itself. Performance cannot be guaranteed if parts from other sources are used and warranty might be lost If repairs are carried out with non-genuine spare parts or by unauthorized personnel.

19.1 CONSUMABLES KIT – 12 MONTHS

Description	Legend	Part Number	Kit Content
ORN/GRN. 0.10 mL/min		116-0549-04	1 pkg./12
ORN/WHT. 0.23 mL/min		116-0549-06	2 pkg./12
BLK/BLK. 0.32 mL/m		116-0549-07	2 pkg./12
ORN/ORN. 0.42 mL/min		116-0548-08	1 pkg./12
WHT/WHT. 0.60 mL/min		116-0549-09	1 pkg./12
YEL/YEL. 1.20 mL/min		116-0549-12	1 pkg./12
Tubing Norprene	PM07	117+0540-07	2.4 m

19.2 ADDITIONAL TUBINGS

Description	Legend	Part Number	Sales Unit
Tubing polyethylene	PE02	562-2002-01	1 m
Tubing polyethylene	PE15	562-2015-01	1 m
Tubing Tygon	T11	116-0536-11	1 m
Tubing Tygon	T16	116-0536-16	1 m
Tubing reagent small, clear	R01S	116+1101-01	1 m
Tubing reagent small, red	R02S	116+1101-02	1 m
Tubing reagent small, blue	R03S	116+1101-03	1 m
Tubing Norprene	PM07	117+0540-07	1 m

19.3 SPARES KIT

Description	Legend	Part Number	Kit Content
Injection fitting 3 pt.	a	116-0489-01	1 pc
Coil 5 turns + Pt Nipple	5TM+NE	163+G003-05	1 pc
Coil 10 turns	10TM	163+G001-10	1 pc
Coil 10 turns + Pt Nipple	10TM+NE	163+G003-10	1 pc
Glass tubing. l = 44 mm	d	116-G004-02	1 pc
Glass fitting. L = 51 mm	e	116-G004-03	1 pc
Glass tubing. HB in/out	cl	163+G020-01	1 pc
Glass tubing U	ae	116-0223-48	1 pc
Debubbler/Rebubbler	cx	163+G035-03	1 pc
Nipple N5	N5	116-0002-01	6 pcs
Nipple N8	N8	116-0003-01	6 pcs
Nipple R13	R13	116+B152-01	6 pcs
Connector Y-Form ID 1.1mm	tw	822624	1 pc

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19.4 PHOTOMETER PARTS

Description	Legend	Part Number	Sales Unit
Flowcell 10 mm		169+B045-10	1 pc
LED Assy. 540 nm		161+B661-54	1 pc
Optic Assy 10 mm		161+B650-10	1 pc
Glass coil 7 turn		161+G500-01	1 pc

19.5 OTHER SPECIAL PARTS

Description	Legend	Part Number	Sales Unit
Coil Assy. 13 turns (5 ml). fix		163+B410-11	1 pc
Cadmium Coil, 6T		165-0301-03	1 pc
Cadmium Coil 10T		165-0301-02	1 pc
4-way-valve		161+B340-01	1 pc

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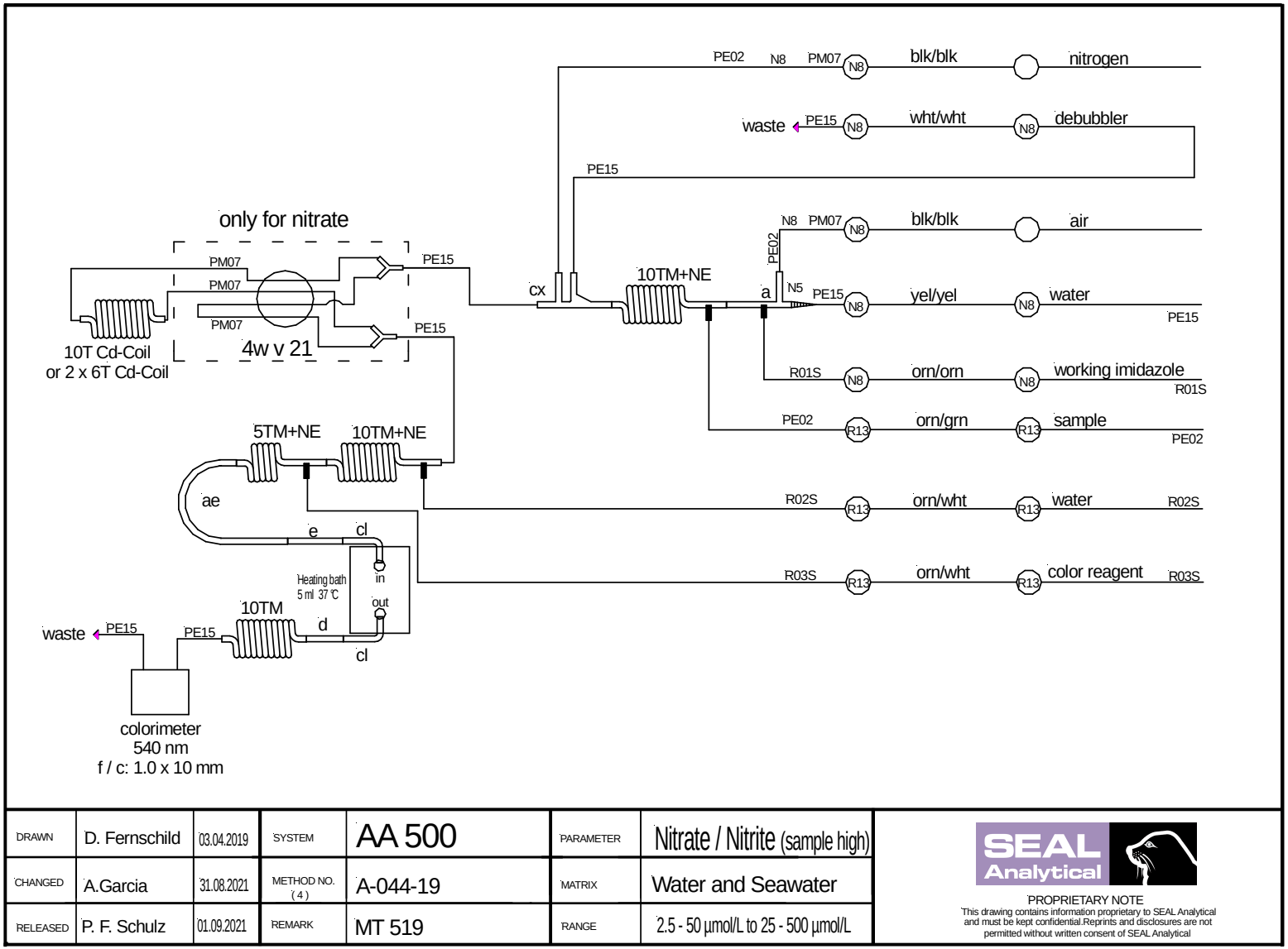
20 FLOWCHART



DRAWN	D. Fernschild	03.04.2019	SYSTEM	AA 500	PARAMETER	Nitrate / Nitrite (sample high)
CHANGED	A.Garcia	31.08.2021	METHOD NO. (4)	A-044-19	MATRIX	Water and Seawater
RELEASED	P. F. Schulz	01.09.2021	REMARK	MT 519	RANGE	2.5 - 50 µmol/L to 25 - 500 µmol/L

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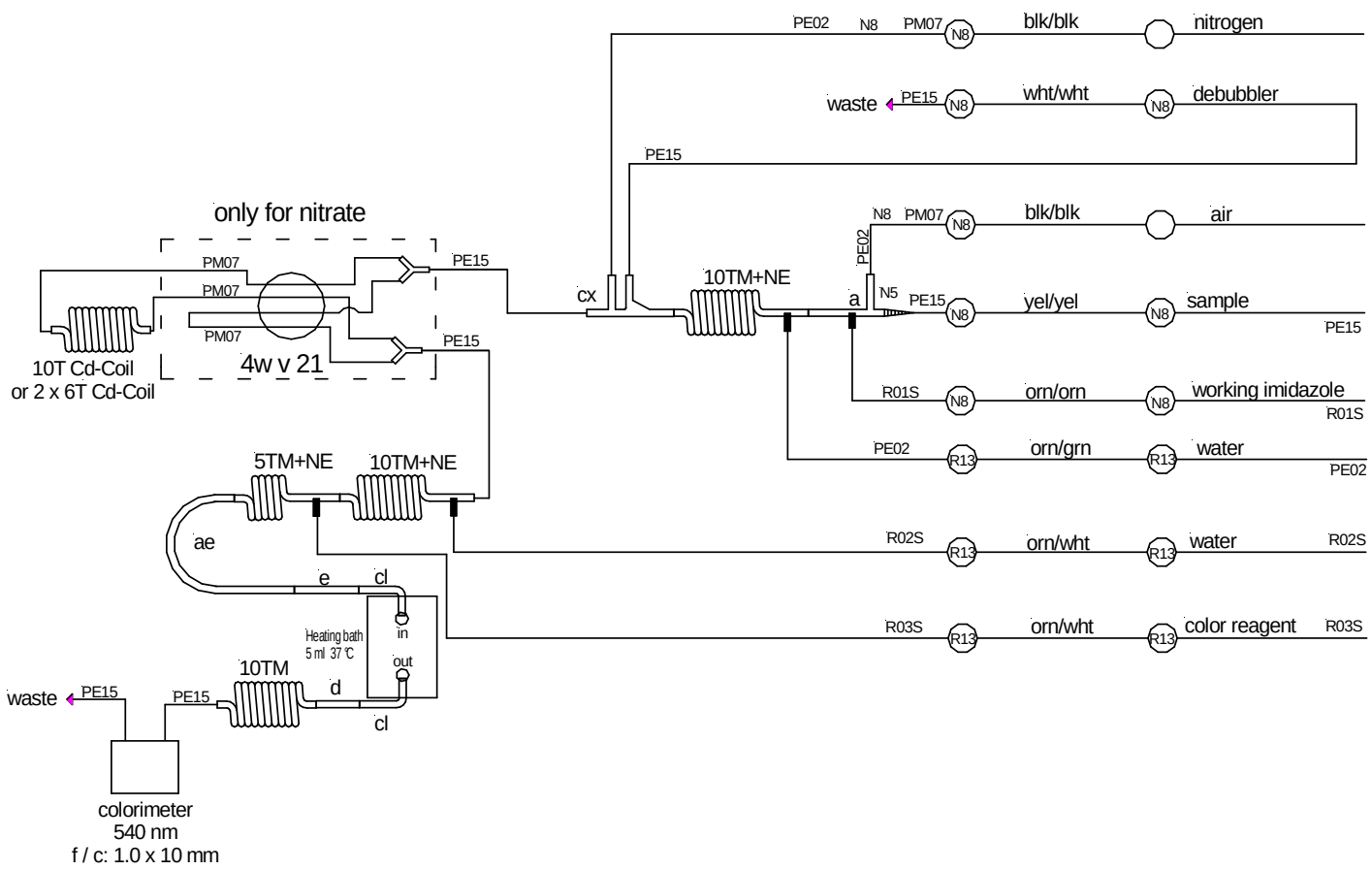

DRAWN	D. Fernschild	03.04.2019	SYSTEM	AA 500	PARAMETER	Nitrate / Nitrite (sample low)
CHANGED	A.Garcia	31.08.2021	METHOD NO. (4)	A-044-19	MATRIX	Water and Seawater
RELEASED	P. F. Schulz	31.08.2021	REMARK	MT 519	RANGE	0.18 - 3.6 µmol/L to 1.8 - 36 µmol/L

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