Scope

This method is used to determine the concentration of nitrate (NO_3^-) plus nitrite (NO_2^-) or nitrite singly in estuarine and coastal waters (seawater) according to USEPA Method 353.4 and Standard Methods 4500–NO_3^- F. This method can also be used to analyze low-turbidity limnological and freshwater samples. Additionally, this method enables nitrate plus nitrite analysis according to ISO Method 13395.

Summary

Nitrate is reduced quantitatively to nitrite by cadmium metal. Nydahl provides a good discussion of nitrate reduction by cadmium metal. The nitrite formed, in addition to any nitrite originally present in the sample, is diazotized with sulfanilamide (SAN) and subsequently coupled with N-(1-naphthyl)ethylenediamine dihydrochloride (NED). The resulting highly colored azo dye is colorimetrically detected at 540 nm. A calibration curve allows for accurate quantitation of the detected nitrite.

Nitrite singly may be measured without the cadmium reduction. Without the cadmium, nitrate is not reduced to nitrite and is not detected since only nitrite forms the azo dye.

Both nitrate and nitrite may be measured simultaneously by using a two channel flow analyzer. One channel is used to measure nitrate plus nitrite, while the second channel is used to measure nitrite only. Using WinFLOW™ software, the results of the nitrite analysis may be subtracted from the results of the nitrate plus nitrite analysis, thus providing quantitative nitrate results.

The quality of the analysis is assured through reproducible calibration and testing of the Segmented Flow Analysis (SFA) system.

Interferences

Turbid samples may interfere with the photometric detector's ability to measure the true absorbance of the sample. Filter turbid samples prior to analysis.

Iron, copper, and other metals may interfere with the analysis by binding with the nitrate and/or nitrite in the sample, thus blocking the color formation reaction. The imidazole buffer eliminates this interference.

Samples that are outside the functional pH range of the ammonium chloride buffer may affect the results obtained from this method. Adjust the pH of these samples to within a range of 5–9 using either concentrated hydrochloric acid (HCl) or sodium hydroxide (NaOH).

Oil and grease will coat the cadmium surface, thus reducing its reduction efficiency. Extract samples containing large concentrations of oil and grease with an appropriate organic solvent.

Sulfide in the presence of cadmium will form cadmium sulfide (CdS), which will inhibit nitrate reduction. Samples containing sulfide cannot be determined by this method without first removing the sulfide by precipitation with cadmium salts.

Dissolved oxygen and carbonate can react with cadmium to form cadmium hydroxide ($Cd(OH)_2$) and cadmium carbonate ($CdCO_3$) precipitants. Additionally, dissolved oxygen competitively inhibits the reduction of nitrate to nitrite. Care must be taken to ensure that the pH never exceeds 8.5. Also, degass all reagents prior to analysis.

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Chlorine may reduce the reduction efficiency of the cadmium reactor. Samples that may contain residual chlorine should be tested for reduction efficiency through the analysis of Matrix Spike/Matrix Spike Duplicate (MS/MSD) samples. When necessary, dechlorinate samples with sodium thiosulfate ($Na_2S_2O_3$).

Chloride ions retard the reduction of nitrate to nitrite, and are prevalent in seawater matrices. Compensate by using two cadmium coils in tandem during the analysis.

Method interferences may be caused by contaminants in the reagents, reagent water, glassware, etc., which may bias the results. Care should be taken to keep all such items free of contaminants.

Performance Specifications

Range:		1.0–5,000 μg/L
Throughput:		30 samples/hour
Precision:		
	10 μg/L	<3% RSD
	100 μg/L	<1% RSD
	1,000 μg/L	~1.5% RSD
Method Detection Limit (MDL)		0.25 μg/L

Chemicals

Brij®-35 (21% Solution) (Part #A21-0110-33)

Cupric Sulfate Pentahydrate, $CuSO_4{\cdot}5H_2O$

Deionized Water (ASTM Type I or II)

Hydrochloric Acid, concentrated, HCl

Imidazole, $C_3H_4N_2$

Magnesium Sulfate Heptahydrate, $MgSO_4{\cdot}7H_2O$

N-(1-naphythyl)ethylenediamine Dihydrochloride, $C_{12}H_{14}N_2{\cdot}2HCl$

Potassium Nitrate, KNO_3

Potassium Nitrite, KNO_2

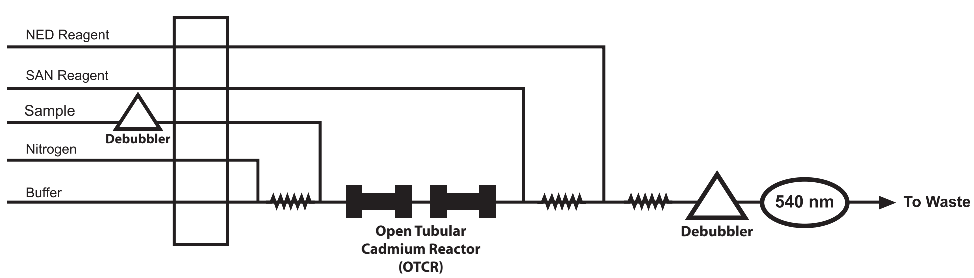
Sodium Bicarbonate, $NaHCO_3$

Sodium Chloride, NaCl

Sodium Hydroxide, NaOH

Sulfanilamide, $C_6H_8N_2O_2S$

Basic Flow Diagram



Note

This method complies with USEPA Method 353.4.

Selected References

Determination of Nitrate and Nitrite in Estuarine and Coastal Waters by Gas Segmented Continuous Flow Colorimetric Analysis. Methods for the Chemical Analysis of Water and Wastes; EPA/600/R-79-020; U.S. Environmental Protection Agency, Office of Research and Development, Environmental Monitoring and Support Laboratory: Cincinnati, OH, 1997; Method 353.4.

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Nydahl, F. Talanta 1976, 23, 349–357.

Standard Methods for the Examination of Water and Wastewater, 21st ed.; American Public Health Association: Washington, D.C., 2005.

Water Quality–Determination of Nitrite Nitrogen and Nitrate Nitrogen and the Sum of Both by Flow Analysis (CFA and FIA) and Spectrometric Detection. *International Standard*; ISO 13395:1996(E); 1st ed.

Figures

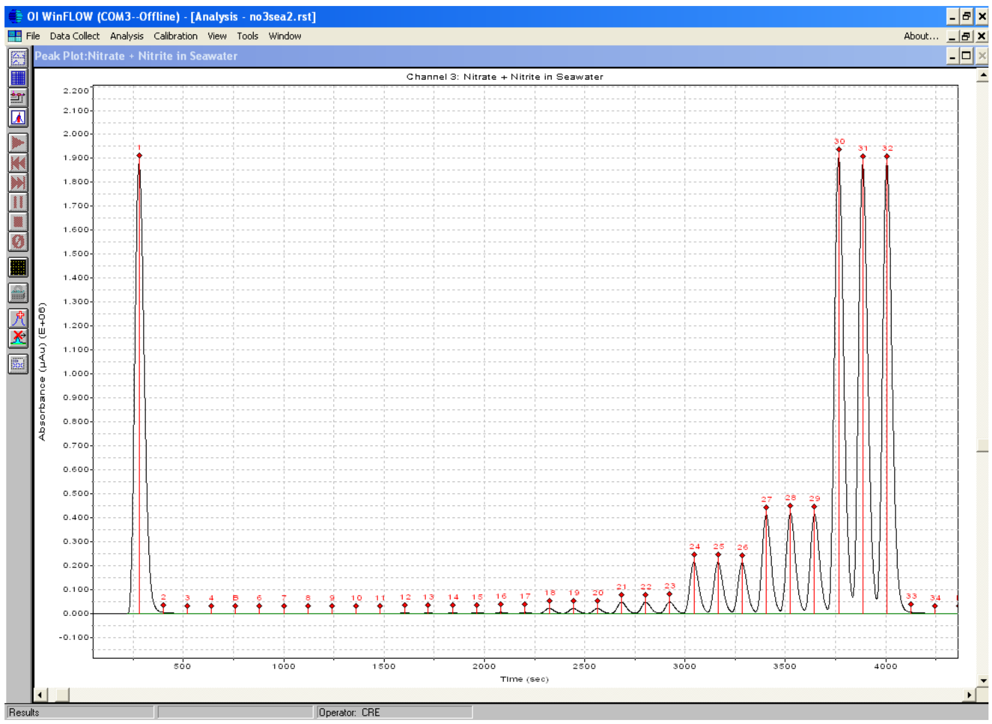

Figure 1. Nitrate Calibration (1.0–5,000 μg/L)

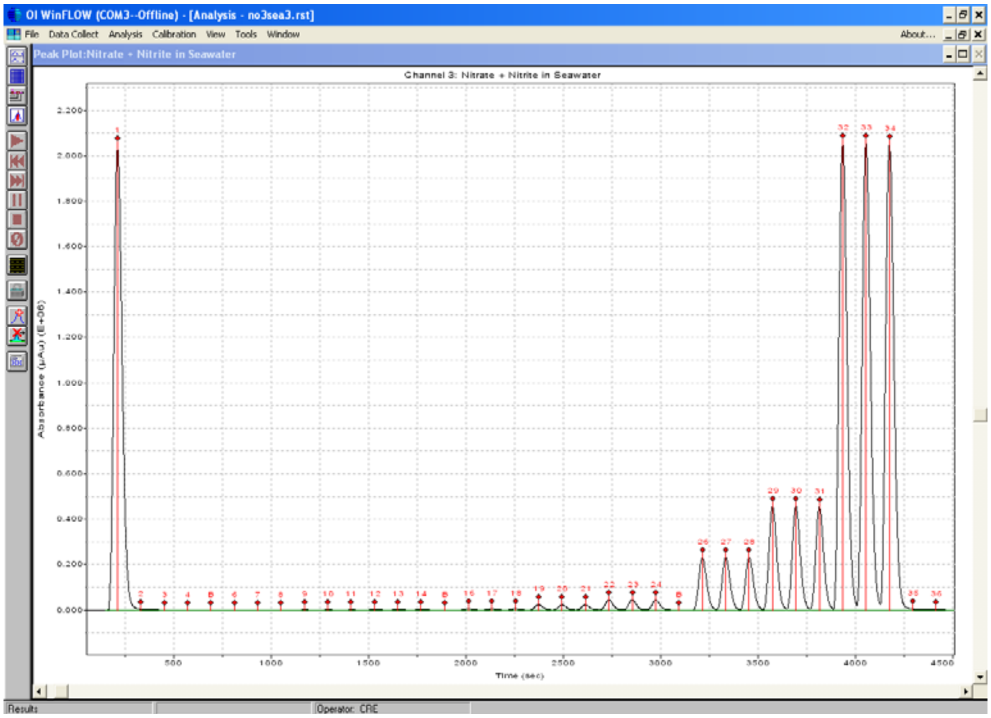

Figure 2. Nitrite Calibration (1.0–5,000 μg/L)

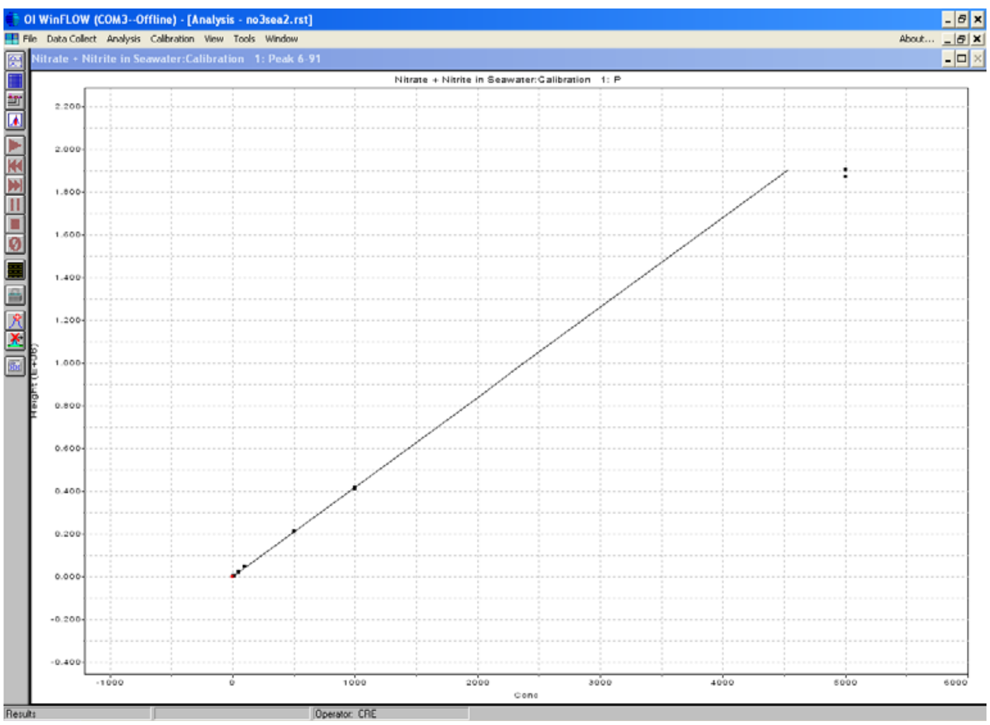

Figure 3. Nitrate Calibration Curve (1.0–5,000 μg/L)

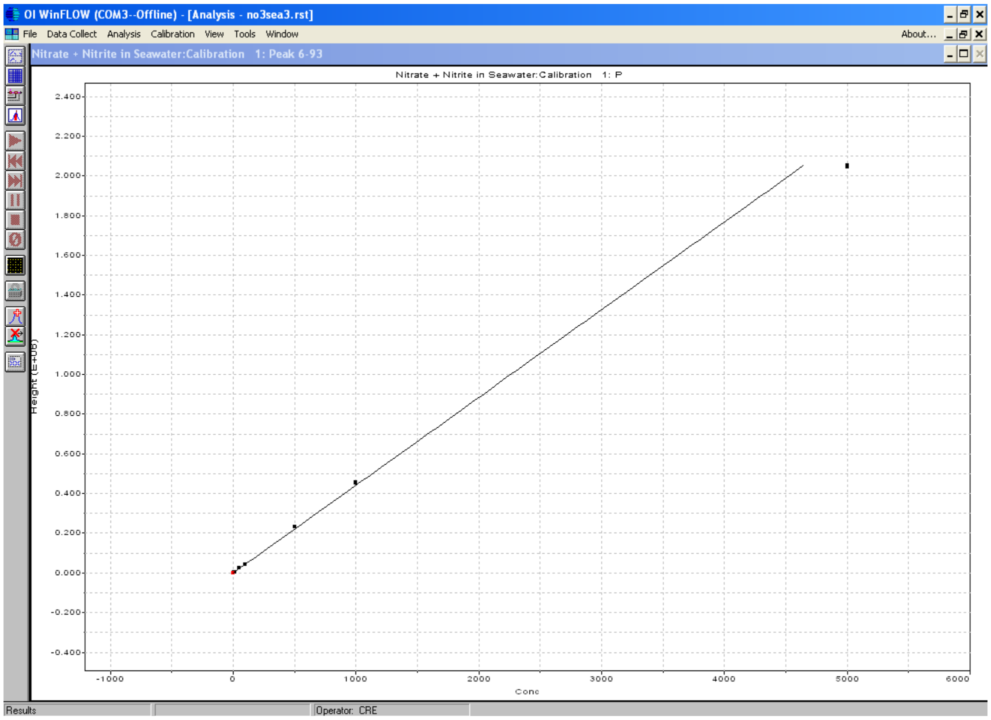

Figure 4. Nitrite Calibration Curve (1.0–5,000 μg/L)

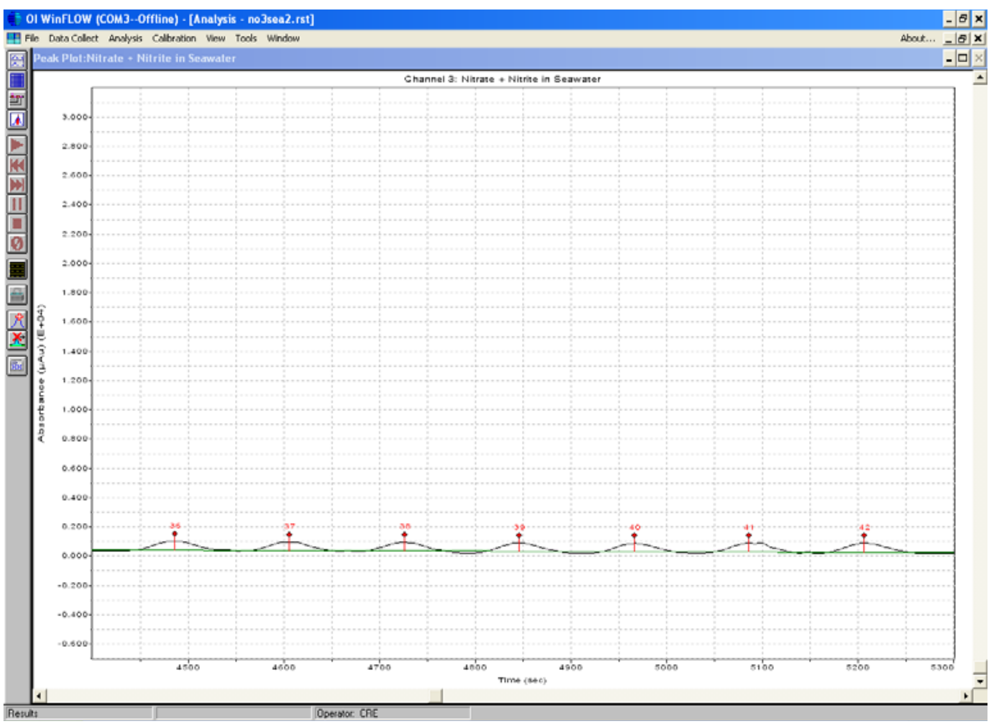

Figure 5. Nitrate Nitrogen Method Detection Limit (at 1.0 ppb)

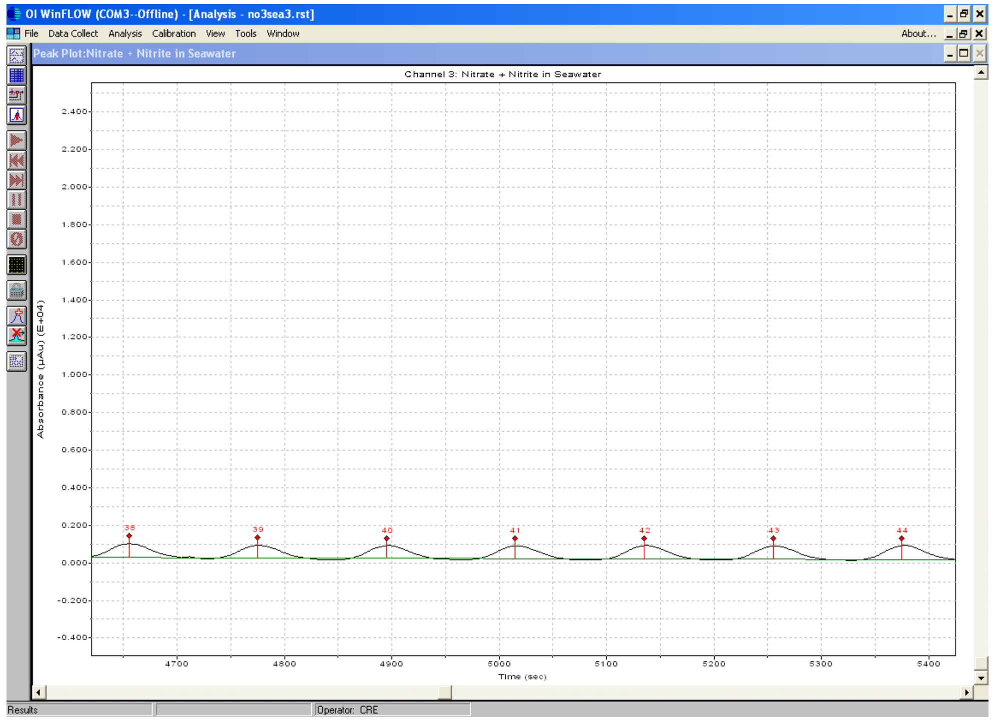

Figure 6. Nitrite Nitrogen Method Detection Limit (at 1.0 ppb)

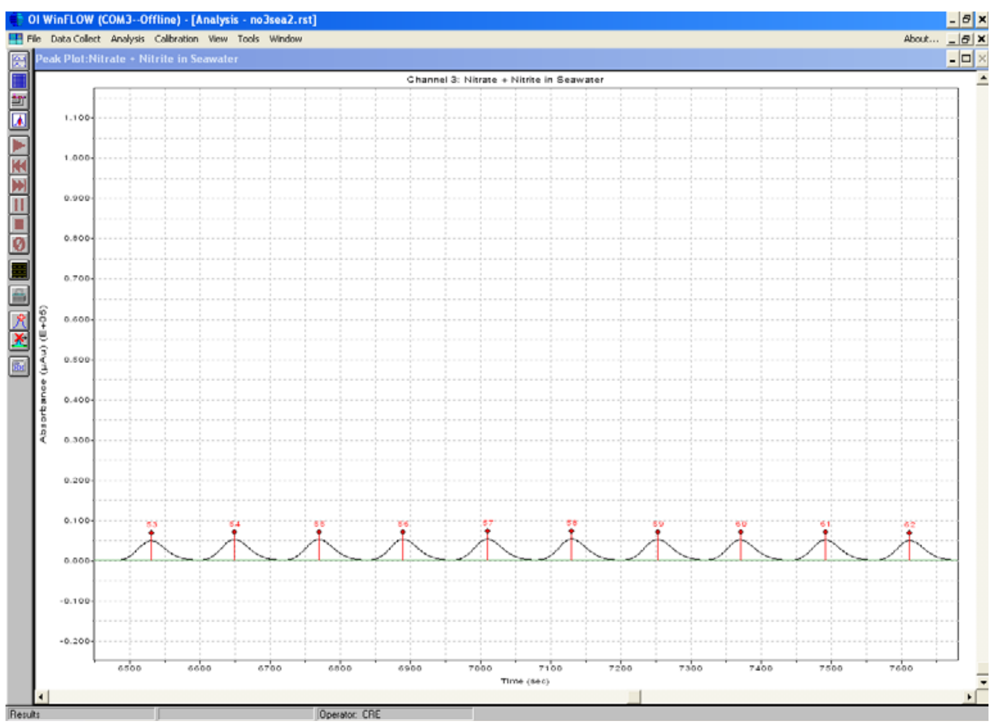

Figure 7. Nitrate Precision at 10 ppb (<3% RSD)

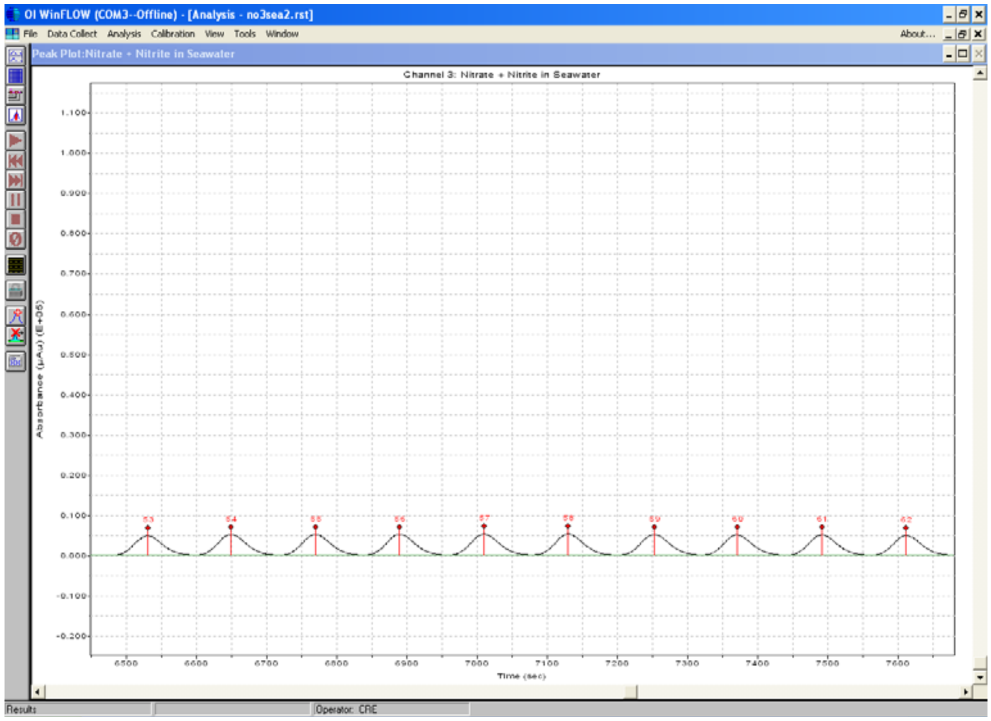

Figure 8. Nitrate Precision at 100 ppb (<1% RSD)

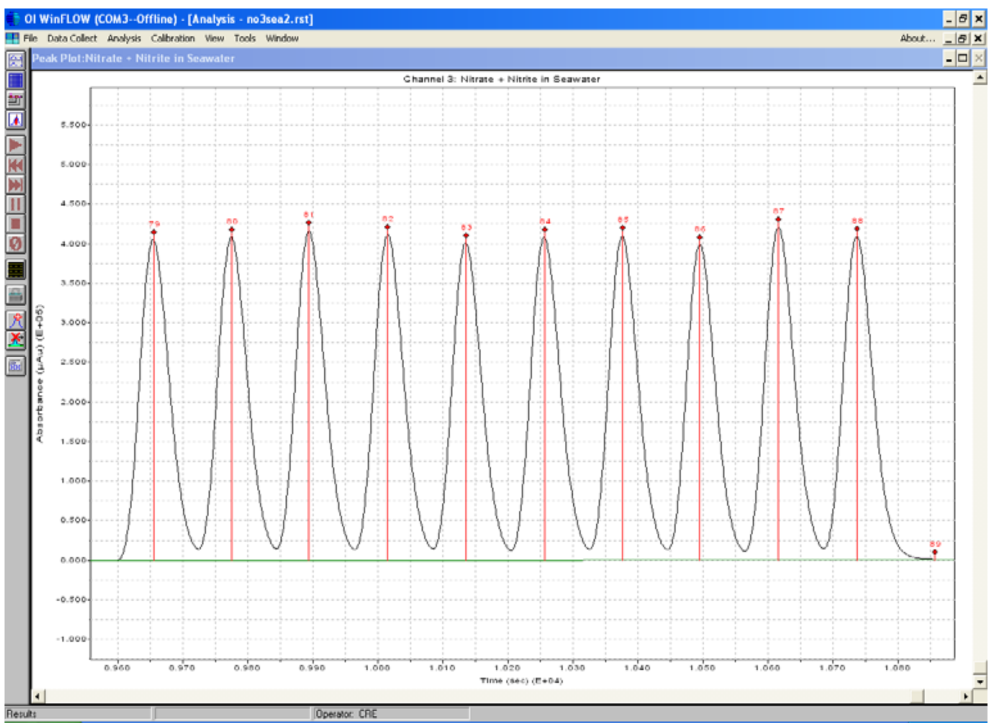

Figure 9. Nitrate Precision at 1,000 ppb (~1.5% RSD)

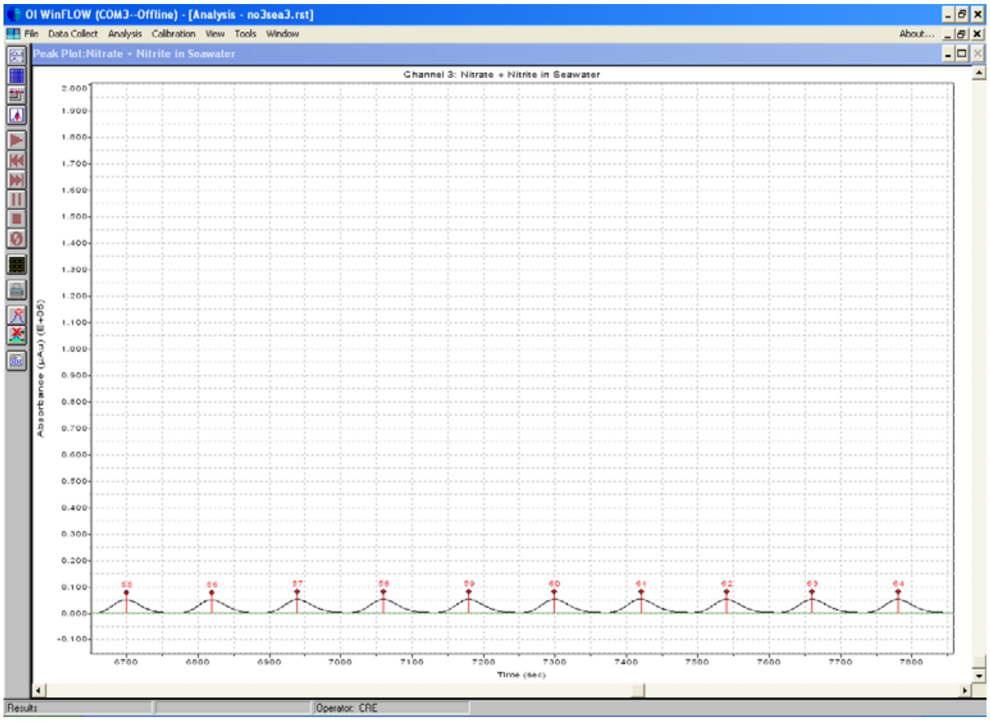

Figure 10. Nitrite Precision at 10 ppb (<3% RSD)

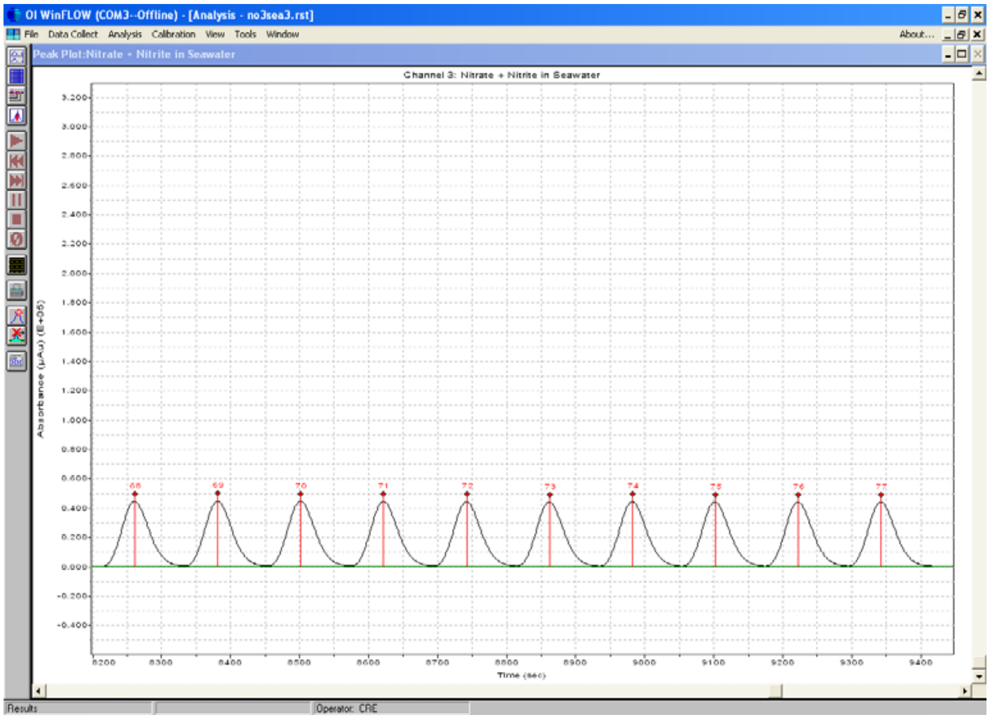

Figure 11. Nitrite Precision at 100 ppb (<1% RSD)

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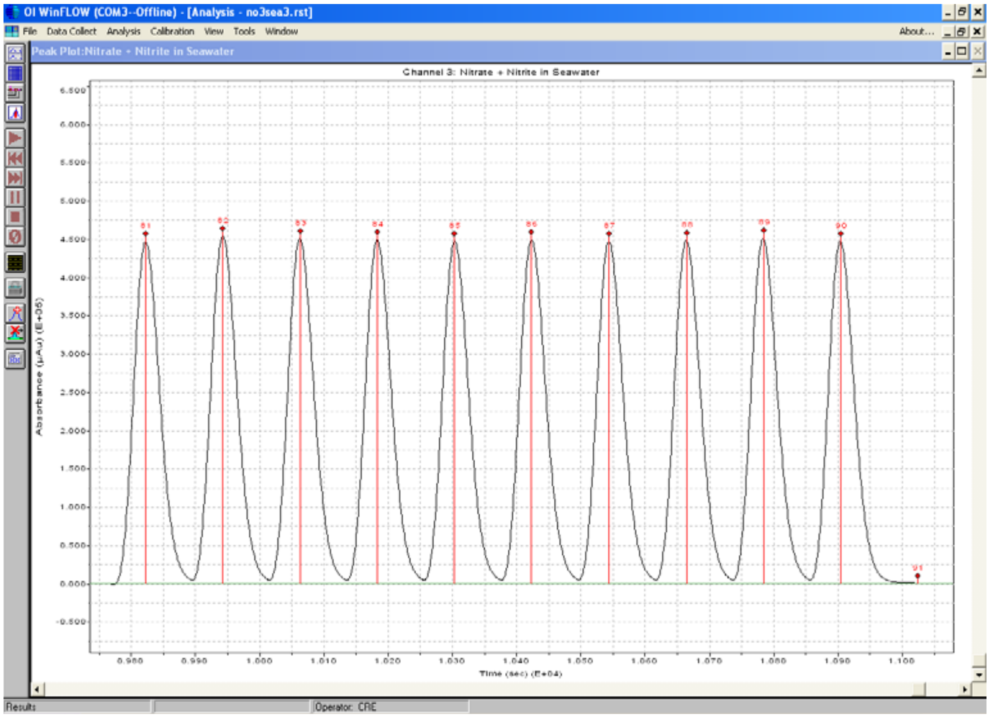

Figure 12. Nitrite Precision at 1,000 ppb (~1.5% RSD)

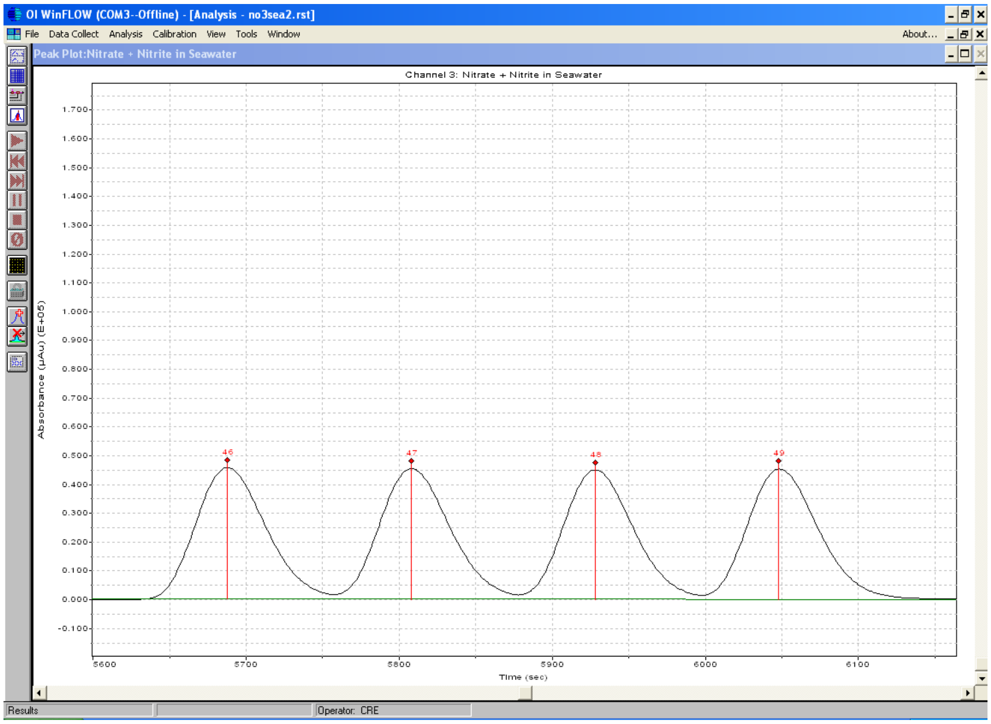

Figure 13. Nitrate plus Nitrite Nitrogen ERA QC (100 ppb)

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File Data Collect Analysis Calibration View Tools Window

Nitrate + Nitrite in Seawater:Calibration 1: Peak 6-9

1,1 | *

*	Name	Conc	Height
*	Cal 0.00 ppb	0.000000	-67.213966
*	Cal 0.00 ppb	0.000000	-85.742355
*	Cal 0.00 ppb	0.000000	-120.93712
*	Cal 1.00 ppb	1.000000	602.407898
*	Cal 1.00 ppb	1.000000	580.881409
*	Cal 1.00 ppb	1.000000	561.878967
*	Cal 5.00 ppb	5.000000	2271.86816
*	Cal 5.00 ppb	5.000000	2252.54418
*	Cal 5.00 ppb	5.000000	2268.96167
*	Cal 10.0 ppb	10.000000	4702.73974
*	Cal 10.0 ppb	10.000000	4969.27441
*	Cal 10.0 ppb	10.000000	4897.42138
*	Cal 50.0 ppb	50.000000	21616.6621
*	Cal 50.0 ppb	50.000000	20624.0078
*	Cal 50.0 ppb	50.000000	20599.0625
*	Cal 100 ppb	100.000000	47301.9101
*	Cal 100 ppb	100.000000	47388.7773
*	Cal 100 ppb	100.000000	48095.0039
*	Cal 500 ppb	500.000000	212678.812
*	Cal 500 ppb	500.000000	212065.218
*	Cal 500 ppb	500.000000	209925.859
*	Cal 1000 ppb	1000.000000	411542.468
*	Cal 1000 ppb	1000.000000	416152.093
*	Cal 1000 ppb	1000.000000	412898.125
*	Cal 5000 ppb	5000.000000	1902221.75
*	Cal 5000 ppb	5000.000000	1869295.50
*	Cal 5000 ppb	5000.000000	1872536.00
	Calib Coef:		
	y=bx+a		
	a: (intercept)	1.4118e+02	
	b:	4.2013e+02	
	Corr Coef:	0.999726	
	Carryover:	0.276%	
	No Drift Peaks		

Figure 14. Nitrate Calibration Results (1.0–5,000 μg/L

OI WinFLOW (COM3--Offline) - [Analysis - no3sea3.rst]

File Data Collect Analysis Calibration View Tools Window

Nitrate + Nitrite in Seawater:Calibration 1: Peak 6-9

1,1 | *

*	Name	Conc	Height
*	Cal 0.00 ppb	0.000000	-48.611000
*	Cal 0.00 ppb	0.000000	-41.275944
*	Cal 0.00 ppb	0.000000	-59.779350
*	Cal 1.00 ppb	1.000000	668.145386
*	Cal 1.00 ppb	1.000000	690.251587
*	Cal 1.00 ppb	1.000000	697.822510
*	Cal 5.00 ppb	5.000000	2542.09228
*	Cal 5.00 ppb	5.000000	2546.89892
*	Cal 5.00 ppb	5.000000	2554.11865
*	Cal 10.0 ppb	10.000000	5000.74755
*	Cal 10.0 ppb	10.000000	5032.20410
*	Cal 10.0 ppb	10.000000	4975.51025
*	Cal 50.0 ppb	50.000000	23209.9843
*	Cal 50.0 ppb	50.000000	23271.1718
*	Cal 50.0 ppb	50.000000	23206.8964
*	Cal 100 ppb	100.000000	44482.9804
*	Cal 100 ppb	100.000000	44363.5195
*	Cal 100 ppb	100.000000	44521.9687
*	Cal 500 ppb	500.000000	230683.203
*	Cal 500 ppb	500.000000	230580.093
*	Cal 500 ppb	500.000000	231808.484
*	Cal 1000 ppb	1000.000000	455960.000
*	Cal 1000 ppb	1000.000000	456004.000
*	Cal 1000 ppb	1000.000000	452984.062
*	Cal 5000 ppb	5000.000000	2052974.62
*	Cal 5000 ppb	5000.000000	2052710.25
*	Cal 5000 ppb	5000.000000	2046710.12
	Calib Coef:		
	y=bx+a		
	a: (intercept)	2.4951e+02	
	b:	4.4214e+02	
	Corr Coef:	0.999717	
	Carryover:	0.188%	
	No Drift Peaks		

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Figure 15. Nitrite Calibration Results (1.0–5,000 μg/L)

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Table 1. Nitrate plus Nitrite in Seawater Validation Results Table

Parameter	Calibrant 1.0 µg/L	Calibrant 10 µg/L	Calibrant 100 µg/L	Calibrant 1,000 µg/L	ERA QC Standard 4.57 mg/L
Rep 1	1.1694	11.3812	114.3497	964.7306	108.4315
Rep 2	1.0794	12.0808	112.2962	968.9868	107.2808
Rep 3	1.0483	12.1946	111.6742	988.1341	106.2350
Rep 4	1.0167	12.1867	113.6816	975.8376	107.1274
Rep 5	0.9712	12.2650	113.4459	951.1946	—
Rep 6	1.0754	12.2844	113.6391	967.9583	—
Rep 7	1.1181	11.9765	113.1731	972.2074	—
Rep 8	—	11.8504	113.1038	945.9189	—
Rep 9	—	11.7968	112.9442	998.1563	—
Rep 10	—	11.4141	113.3402	969.9337	—
Average	1.0683376	11.943057	113.16482	970.30583	107.26866
Standard Deviation	0.0650156	0.3313849	0.7479468	15.370037	0.9019555
% RSD	6.085675	2.7747074	0.6609358	1.5840404	0.8408379
MDL	0.2041488	—	—	—	—
% Accuracy	—	—	—	—	107.82

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