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.
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$_2$S$_2$O$_3$).

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**

<table>
<thead>
<tr>
<th>Range</th>
<th>1.0–5,000 μg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Throughput</td>
<td>30 samples/hour</td>
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<tr>
<td>Precision</td>
<td></td>
</tr>
<tr>
<td>10 μg/L</td>
<td>&lt;3% RSD</td>
</tr>
<tr>
<td>100 μg/L</td>
<td>&lt;1% RSD</td>
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<tr>
<td>1,000 μg/L</td>
<td>~1.5% RSD</td>
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</tbody>
</table>

**Chemicals**

- Brij®-35 (21% Solution) (Part #A21-0110-33)
- Cupric Sulfate Pentahydrate, CuSO$_4$•5H$_2$O
- Deionized Water (ASTM Type I or II)
- Hydrochloric Acid, concentrated, HCl
- Imidazole, C$_3$H$_4$N$_2$
- Magnesium Sulfate Heptahydrate, MgSO$_4$•7H$_2$O
- N-(1-naphthy)ethylenediamine Dihydrochloride, C$_{12}$H$_{14}$N$_2$•2HCl
- Potassium Nitrate, KNO$_3$
- Potassium Nitrite, KNO$_2$
- Sodium Bicarbonate, NaHCO$_3$
- Sodium Chloride, NaCl
- Sodium Hydroxide, NaOH
- Sulfanilamide, C$_6$H$_8$N$_2$O$_2$S

**Note**

This method complies with USEPA Method 353.4.

**Selected References**


**Figures**

![Figure 1. Nitrate Calibration (1.0–5,000 µg/L)](image-url)
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)
Method Abstract

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

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

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

Figure 13. Nitrate plus Nitrite Nitrogen ERA QC (100 ppb)
Figure 14. Nitrate Calibration Results (1.0–5,000 μg/L)
Figure 15. Nitrite Calibration Results (1.0–5,000 μg/L)
**Method Abstract**

Table 1. Nitrate plus Nitrite in Seawater Validation Results Table

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Calibrant 1.0 μg/L</th>
<th>Calibrant 10 μg/L</th>
<th>Calibrant 100 μg/L</th>
<th>Calibrant 1,000 μg/L</th>
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<td><strong>% Accuracy</strong></td>
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