Detection of Iron Ligands in Seawater and Marine Cyanobacteria Cultures by High-Performance Liquid Chromatography–Inductively Coupled Plasma-Mass Spectrometry

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Abstract: Organic ligands dominate the speciation of iron in the ocean. Little is known, however, about the chemical composition and distribution of these compounds. Here, we describe a method to detect low concentrations of organic Fe ligands using reverse-phase high-performance liquid chromatography (HPLC) tandem multicollector inductively coupled plasma mass spectrometry. This technique can be used to screen seawater and marine cultures for target compounds that can be isolated and structurally characterized. Sensitive detection (<1 picomole Fe) is achieved using an iron-free HPLC system to reduce background Fe levels, minimizing 40Ar16O+ interferences on 56Fe with a hexapole collision cell, and introducing oxygen into the sample carrier gas to prevent the formation of reduced carbon deposits that decrease sensitivity. This method was tested with a chromatographic separation of five trace metal complexes that represent the polarity range likely found in seawater. Good separation was achieved with a 20 min water/methanol gradient, although sensitivity decreased by a factor of 2 at high organic solvent concentrations. Finally, Fe ligand complexes were detected from the organic extract of surface South Pacific seawater and from culture media of the siderophore producing cyanobacteria Synechococcus sp. PCC 7002.

Iron (Fe) is an essential micronutrient for marine microorganisms, yet the concentrations of dissolved Fe in surface waters are low (0.02 nM to <1 nM). As a result, primary productivity and carbon export are limited by Fe in regions where the inputs from dust deposition and upwelling are scarce. The solubility and bioavailability of iron and other trace metals such as cobalt, nickel, copper, zinc, and cadmium in the ocean are regulated by organic ligands. Electrochemical measurements of bulk Fe(III) ligand concentration and binding strength suggest that this ligand pool ranges from 1 to 2 nM and complexes over 99% of dissolved iron (Fe) in the ocean. However, the molecular structures, sources, and fates of these ligands are currently unknown.

The biogeochemical importance of organic ligands has sparked interest in developing sensitive, trace metal clean analytical methods that can be coupled with chromatography for the characterization of specific ligands. High-performance liquid chromatography (HPLC) tandem electrospray ionization mass spectrometry (HPLC–ESI-MS) has been used to characterize specific ligands from bulk organic samples. With this method, recent studies have detected putative Fe ligands in seawater by identifying compounds with distinctive metal isotope patterns. MS/MS analysis of these compounds suggest that some are similar to hydroxamate siderophores, which are produced by microbes to facilitate Fe uptake under low Fe conditions.

In this study, we present a complementary method for directly detecting the Fe associated with specific organic compounds by HPLC–tandem inductively coupled plasma-mass spectrometry (ICP-MS). HPLC–ICP-MS offers universal detection of metal complexes in an organic extract sample with minimal sample manipulation and can simultaneously detect multiple metals. With this technique, seawater samples can be rapidly screened to determine the number and relative polarities of low concentration metal ligand complexes. By splitting the flow eluting from the HPLC column, Fe-ligand complexes can be detected in real time and recovered by fraction collection, allowing structural characterization by ESI-MS and other methods. Although HPLC–ICP-MS has previously been shown to provide rapid and sensitive detection of metal–organic complexes from environmental samples (such as Ag, Cd, Cu, Mo, Ni, Pb, Ti, U, W, Zn, and Zr), monitoring Fe by HPLC–ICP-MS is complicated by high procedural blanks, isobaric argon oxide mass interferences in ICP-MS, and the very low concentrations of Fe in marine samples.

The method described here employs several strategies for reducing 56Fe backgrounds and interferences to achieve sensitive, quantitative detection of Fe ligands. Low Fe blanks...
are achieved with the use of a bioinert titanium/peek HPLC system and distilled organic solvents. Second, a hexapole collision cell in the ICP-MS minimizes $^{40}$Ar$^{2+}$O$^{+}$ interferences on $^{56}$Fe.$^{26,27}$ Oxygen gas was introduced into the sample carrier gas to mitigate the formation of reduced carbon species that leave carbon deposits and degrade instrument performance.$^{28,29}$

We used this method to investigate Fe ligand production in seawater and in culture media of the siderophore-producing cyanobacteria Synechococcus PCC sp. 7002. These applications demonstrate the utility of HPLC–ICP-MS for rapidly screening seawater samples for the presence of low concentration Fe ligands.

## METHODS

**Materials, Standards, and Reagents.** Ultrahigh purity water (18.2 MΩ cm), Optima-grade methanol (MeOH), and ammonium hydroxide (NH$_4$OH) (Fisher scientific) were used in this study. For the analysis of standard compound sensitivity and low concentration (<1 μM) naturally occurring ligands in seawater, the methanol was purified by sub-boiling-point distillation in a polytetrafluoroethylene (PTFE) still, which reduced Fe concentrations from 180 to 2 nM (as measured by ICP-MS).$^{27}$ Fe(III)-ethylenediaminetetraacetic acid (Fe-EDTA) was obtained from Alfa Aesar as the monosodium salt. Ferric chloride (FeCl$_3$·6H$_2$O) was purchased from Fisher Scientific. Ferrioxamine E, desferrioxamine B (as deferoxamine mesylate salt), iron-free ferrichrome, Fe-heme (as hemin, from porcine), cyano-cobalamin (vitamin B$_{12}$), nutrient salts and vitamins for culture media were obtained from Sigma Aldrich.

Stock solutions (250 μM) of the standard compounds were made within 24 h of analysis and stored at 4°C. Solutions of Fe-EDTA, ferrioxamine E, and cobalamin were prepared in water. Ferrioxamine E and ferrichrome stock solutions were prepared as free ligands in water to which 1 mol equiv of freshly made 1 mM FeCl$_3$ solution was added. The Fe-heme solution was made by dissolving hemin in 1% NH$_4$OH under ultrasonication for 10 min and then diluting to 0.1% NH$_4$OH with H$_2$O.

PTFE or polycarbonate plastic vials were used for preparing standard solutions, culturing, seawater collection, and ligand extraction. Plasticware was soaked overnight in 0.1% detergent (Citranox), rinsed 5× with H$_2$O, and then soaked in 1 N HCl (J.T. Baker) for at least 2 days followed by a final 5× rinse with H$_2$O. Polyethersulfone and polycarbonate membrane filters (0.2 μm) were soaked in 1 N HCl overnight, rinsed once with pH 2 water, and 5 times with H$_2$O. All samples for HPLC–ICP-MS analysis were placed in 2 mL amber glass autosampler vials or 250 μL vial inserts (Agilent).

**Standard Separation and Isocratic Standard Injections.** Stock solutions of 250 μM Fe-EDTA, ferrioxamine E, ferrichrome, Fe-heme in 0.1% optima grade NH$_4$OH, and cobalamin were combined and diluted to prepare six concentrations of a standard mixture: 5 μM, 2.5 μM, 1.25 μM, and 0.05 μM. These solutions were sequentially analyzed by HPLC–ICP-MS. Quantification of the ICP-MS signal was based on peak area.

**Isocratic Standard Injections.** Solutions of 250 μM Fe-EDTA, ferrioxamine B, ferrioxamine E, ferrichrome, Fe-heme, and cobalamin were diluted to 5 μM in H$_2$O, 50% MeOH in H$_2$O, or 100% MeOH. 0.1% NH$_4$OH was added to the 5 μM Fe-heme solution to prevent precipitation. In order to monitor the effect of solvent on sensitivity, replicates of each solution were injected directly into the multi-collector ICP-MS (MC-ICP-MS) without a column or flow splitter while pumping solvent with the same composition as the sample at a flow rate of 0.05 mL/min. Injections were repeated 2–4 times to evaluate reproducibility. After switching the solvent composition, it took 40 min for the ICP-MS signal to stabilize under the new conditions. Quantification of the ICP-MS signal was based on peak area and normalized to the peak area of Fe-EDTA in 100% H$_2$O.

**Seawater and Culture Samples.** To test the sensitivity of the HPLC–ICP-MS method, organic compounds were extracted and analyzed from seawater and a marine cyanobacteria culture. Seawater was collected with an air driven PTFE deck pump from 100 m in the South Pacific Subtropical Gyre ($26°14.9′$S, $103°57.6′$W; BiG-RAPA cruise, Nov 18–Dec 14, 2010), filtered (0.2 μm, polyethersulfone), and stored in the dark at room temperature in an acid-washed barrel. Duplicate twenty liter samples were transferred into acid-cleaned carboys, and one sample was spiked with 10 pM of ferrichrome and ferrioxamine E to test the recovery and detection of siderophores with this method. The remaining seawater (100 L) was extracted and analyzed for the presence of naturally occurring ligands.

*Synechococcus* sp. PCC 7002 (75 mL) was grown under continuous light at 23°C in Sargasso seawater-based medium (3/4 Sargasso seawater diluted with 1/4 H$_2$O containing 1.5 × 10$^{-6}$ M EDTA, 8 × 10$^{-6}$ M phosphoric acid, 5 × 10$^{-8}$ M ferric citrate, 10$^{-7}$ M MnSO$_4$, 10$^{-8}$ M ZnCl$_2$, 10$^{-8}$ M NaMoO$_4$, 10$^{-10}$ M CoCl$_2$, 10$^{-10}$ M NiCl$_2$, 10$^{-10}$ M NaSeO$_3$, and 1.5 μg of cobalamin per liter)$^{30}$ plus 10$^{-5}$ M HNO$_3$. After seven days, three 1 L acid cleaned polycarbonate flasks containing 600 mL of medium without iron citrate were prepared. Two flasks were inoculated with equal amounts of the Synechococcus culture. The third flask was incubated alongside without inoculation as a media blank. After seven days, the culture media was centrifuged and filtered through a 0.2 μm acid cleaned polycarbonate filter to remove the cells. The absence of heterotrophic contamination was confirmed using a marine purity broth (8 g MgSO$_4$·7H$_2$O, 1.5 g CaCl$_2$, 2H$_2$O, 20 g NaCl, and 17 g AC Difco broth per liter of H$_2$O).

**Solid Phase Organic Extraction.** The high salt concentration in seawater and the marine culture media can interfere with ICP-MS analysis when salt crystals accumulate at the cone interface and reduce the signal throughput (sensitivity). To prevent this as well as concentrate the sample, ligands were extracted from the filtered samples onto a polystyrene-divinylbenzene solid phase extraction column (Isolute, ENV+, Biotage). This resin was selected based on its ability to bind a range of strong Fe ligands.$^3$

The columns were first rinsed with 5 mL of MeOH and 5 mL of H$_2$O. Then, the organic compounds from seawater samples were extracted on a 1 g column at a flow rate of 8 mL/ min, while compounds from Synechococcus media and media blank were extracted on a 0.5 g column at a flow rate of 6 mL/ min. The columns were then rinsed with 25 mL H$_2$O to remove salts and eluted with 5 mL MeOH. To determine the procedural blank associated with the extraction process, a 1 g resin column was rinsed with 5 mL MeOH, 30 mL of H$_2$O, and then eluted with 5 mL of MeOH. The SPE eluent from all samples was dried under high purity N$_2$. The residue was dissolved in 0.5 mL H$_2$O and filtered through a 0.2 μm polyvinylidene fluoride filter for analysis by HPLC–ICP-MS. All analyses were performed at least twice.
To quantify the recoveries of ferrioxamine E and ferrichrome from seawater, a three-point calibration curve was created by analyzing three standard solutions containing 1 μM, 0.5 μM, and 0.25 μM of ferrichrome and ferrioxamine E dissolved in H2O. Signal quantification was based on integrated peak areas from the HPLC–ICP-MS chromatogram. Coeluting organic carbon can potentially enhance the sensitivity of the ICP-MS toward Fe. To account for this matrix effect, we dissolved the solid phase extract from 20 L of South Pacific seawater in a solution of 0.5 μM ferrichrome and ferrioxamine E and compared the ICP-MS response to the original (no dissolved extract) 0.5 μM standard mixture.

High-Performance Liquid Chromatography. HPLC separation was performed with a bioinert HPLC pump and autosampler (Agilent 1260 series) along with titanium or PEEK (polyether ether ketone) tubing to reduce background Fe levels. Samples (20 μL volumes) were injected and separated on a PEEK C18 reverse-phase column (250 × 2.1 mm with 5 μm particle size for the *Synechococcus* sp. 7002 culture, 150 × 2.1 mm with 3 μm particle size for all other analyses, Hamilton) at a flow rate of 0.2 mL/min. Iron-binding ligands were separated using a 20 min linear gradient from 100% H2O to 100% MeOH, followed by a 10 min isocratic elution with MeOH. We tested the effect of 0.1%, 0.01%, and 0.001% formic acid (optima grade, Fisher Scientific) on the chromatographic separation of the standard compounds. The addition of formic acid increased the Fe background and did not significantly change the retention times of the standards, so we chose to exclude it from our mobile phases. A postcolumn PEEK flow splitter and a micrometering valve (Upchurch Scientific) diverted 25% of the eluent to the MC-ICP-MS and 25% to a UV–vis diode array detector and fraction collection (Figure 1).

![Figure 1. Schematic of high-performance liquid chromatography–inductively coupled plasma mass spectrometry (HPLC–ICP-MS) instrumentation and workflow. Compounds are separated by HPLC with a gradient from H2O to MeOH. Twenty five percent of the eluent from the HPLC column is nebulized, desolvated, and analyzed by ICP-MS. Oxygen is added to the sample carrier gas before entering the ICP-MS to mitigate incomplete combustion of organic carbon in the plasma.](image)

For the *Synechococcus* sp. PCC 7002 culture, the major Fe-containing fractions were collected from five injections, dried under N2, dissolved in water, and analyzed by HPLC–ESI-MS (Agilent 6130 series), using the same column and solvent gradient described above.

**Interface Between HPLC and ICP-MS.** The use of organic solvents such as methanol can affect plasma stability, temperature, and shape and also promote the formation of reduced carbon species that interfere with ICP-MS sensitivity when they coat the cone interface. Three measures were taken to reduce the organic solvent interference: (1) lowering the flow rate entering the plasma to 50 μL/min, (2) desolvation of the sample aerosol to remove most of the organic solvent, and (3) addition of oxygen into the plasma to promote complete combustion of organic solvents to carbon dioxide. Desolvation was induced using an APEX Q inlet system (Elemental Scientific, Inc.) with a 50 μL/min quartz Micromist nebulizer. A heated macroporous PTFE membrane desolvator (Spiro TMD, Elemental Scientific) was coupled to the APEX outlet to ensure that most of the solvent was removed before it entered the plasma. Organic solvent combustion and Fe detection were optimized at a 50 mL/min high purity O2 flow introduced post-Spiro TMD, using a mass flow controller (Cole-Parmer).

**ICP-MS Instrumentation.** For trace metal analysis 55Mn, 56Fe, and 59Co were determined simultaneously on Faraday cups on a magnetic sector MC-ICP-MS (GV/Micromass IsoProbe), as described previously. The instrument was equipped with X-sampler and X-skimmer cones (Spectron Inc.). Platinum cones are preferred over nickel cones because they require less frequent cleaning when using O2 in the plasma. A hexapole collision cell with Ar and H2 collision gases was used to minimize 40Ar16O+ interferences on 56Fe and to thermalize the ion beam. Operating conditions are listed in Table 1.

**Table 1. Operating Conditions for HPLC–ICP-MS Analysis**

<table>
<thead>
<tr>
<th>HPLC settings</th>
<th>ICP-MS settings</th>
</tr>
</thead>
<tbody>
<tr>
<td>mobile phase A</td>
<td>H2O</td>
</tr>
<tr>
<td>mobile phase B</td>
<td>MeOH</td>
</tr>
<tr>
<td>flow rate</td>
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<td>APEX condensation temperature</td>
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<td>oxygen gas flow</td>
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<tr>
<td>reflected power</td>
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<td>hexapole Ar gas flow</td>
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<tr>
<td>hexapole H2 gas flow</td>
<td>2.5 mL min⁻¹</td>
</tr>
<tr>
<td>expansion pressure</td>
<td>3 × 10⁻³ mbar</td>
</tr>
<tr>
<td>hexapole pressure</td>
<td>4 × 10⁻³ mbar</td>
</tr>
<tr>
<td>analyzer pressure</td>
<td>2 × 10⁻³ mbar</td>
</tr>
<tr>
<td>nebulizer gas flow</td>
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<tr>
<td>cool gas flow</td>
<td>14 L min⁻¹</td>
</tr>
<tr>
<td>auxiliary gas flow</td>
<td>10 L min⁻¹</td>
</tr>
<tr>
<td>integration time</td>
<td>2 s</td>
</tr>
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</table>

The ICP-MS was tuned daily with a 100 ppb Mn, Fe, Co, and Ni solution dissolved in 0.2 N Vycor distilled HNO3 that was taken up by free aspiration with PTFE sipper tubing. Instrumental sensitivity was typically 2.0 V for 56Fe and 3.55 V for 59Co.

**RESULTS AND DISCUSSION**

Separation of Standard Compounds. To assess the chromatographic resolution, the limit of detection, and the linearity of the ICP-MS detector response, a mixture of 5
standard compounds was analyzed by HPLC–ICP-MS at four concentrations (100 pmol, 50 pmol, 25 pmol, and 1 pmol per 20 μL injection). The standard mixture was composed of four Fe complexes (Fe-EDTA, ferrichrome, ferrioxamine E, and Fe-heme) and one cobalt complex (cobalamin) (Figure 2). These compounds represent major classes of Fe ligands that may be present in seawater.

These compounds were separated with a gradient from water to methanol over 20 min. This gradient resulted in good peak resolution with little tailing. Four distinct peaks appear in the 56Fe trace of the chromatogram (Figure 2) with retention times of 2.1, 13.6, 15.2, and 26.5 min. These retention times correspond to Fe-EDTA, ferrichrome, ferrioxamine E, and Fe-heme, respectively. A single peak appears in the 59Co trace at 14.6 min, corresponding to cobalamin.

Minimum detectable signals of the HPLC–ICP-MS measurements were calculated as three standard deviations above the mean background signal from the H2O blank. For 56Fe, the minimum detectable signal was 3.5 × 10^4 cps. This is 4–32 times lower than the peak heights from 1 pmol Fe-EDTA, ferrichrome, ferrioxamine E, and Fe-heme. In the case of 59Co, the limit was found to be 2.1 × 10^4 counts per second (cps), which is 31 times lower than the peak height from 1 pmol cobalamin. On the basis of these results, the detection limit of this method is in the range of 30–250 femtomoles for Fe and Co complexes.

The ICP-MS signal for each standard compound varies linearly with standard concentration over the range of 1–100 pmol per 20 μL with R^2 values > 0.99 (Table 2). The ICP-MS signal appears to decrease at later retention times, resulting in a lower sensitivity for Fe-Heme than for the other compounds.

### Table 2. Retention Time, Linear Sensitivity, and Correlation Coefficient of Standard Compound Concentration vs ICP-MS Signal

<table>
<thead>
<tr>
<th>compound</th>
<th>retention time (min)</th>
<th>sensitivity (10^10 cps min M^-1)</th>
<th>R^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe-EDTA</td>
<td>2.1</td>
<td>3.1 ± 0.2</td>
<td>0.990</td>
</tr>
<tr>
<td>ferrichrome</td>
<td>13.6</td>
<td>3.3 ± 0.06</td>
<td>0.999</td>
</tr>
<tr>
<td>ferrioxamine E</td>
<td>15.2</td>
<td>3.4 ± 0.1</td>
<td>0.998</td>
</tr>
<tr>
<td>Fe-heme</td>
<td>26.5</td>
<td>2.5 ± 0.2</td>
<td>0.987</td>
</tr>
<tr>
<td>cobalamin</td>
<td>14.6</td>
<td>4.0 ± 0.09</td>
<td>0.999</td>
</tr>
</tbody>
</table>

This suggests that solvent composition influences the ICP-MS sensitivity, as discussed below.

### Effects on ICP-MS Sensitivity

To study the effect of solvent and organic ligand composition on the 56Fe and 59Co signal intensity during analysis, a series of standard compounds were injected directly into the ICP-MS with different mixtures of H2O/MeOH. The identity of the organic ligand appears to have little effect on the ICP-MS signal intensity. For each solvent condition, the mean ICP-MS signal of each Fe compound deviates from the highest signal by 5 to 26%. These variations are small compared to the difference observed between the signal from the same compound in methanol versus water. In general, the ICP-MS signal decreased by a factor of 2, as the proportion of MeOH increased from 0 to 100% (Figure 3). Since Fe-heme elutes in 100% MeOH, this solvent effect on sensitivity may explain why the response factor for Fe-heme is always smaller than other Fe complexes.

There are several mechanisms that can explain this organic solvent effect. The nebulization and desolvation efficiency may vary for ligands in different solvents. Organics can also be a potential source of interferences or C^+ ion modifiers that can affect signal intensity. Finally, organic solvents can influence the atomization and ionization of the analytes by affecting the central channel of the plasma. In culture and seawater extract samples, there is an additional organic matrix effect that enhances sensitivity, discussed below.

### Fe Ligand Detection from Seawater

Previous studies have reported siderophore concentrations of 3–20 μM in seawater from the North Atlantic. To evaluate the detection of Fe ligands present in this concentration range using HPLC–ICP-MS, 20 L of South Pacific surface seawater was spiked with 10 μM of two hydroxamate siderophores. Organic compounds were then concentrated by solid phase extraction and brought up to 0.5 mL QH2O. The chromatogram of the extract containing the ferrichrome and ferrioxamine E spike contained two large peaks superimposed on the broad seawater background (Figure 4a). We found that the organic matrix of the seawater extract enhances the sensitivity of the ICP-MS. By measuring the ICP-MS signal of 5 μM ferrichrome and ferrioxamine E standards dissolved in H2O versus the same concentration dissolved in the seawater control extract, we calculated enhancement factors of 1.6 for ferrichrome and 1.5 for ferrioxamine E relative to the signal achieved in H2O. This may occur through charge-transfer reactions between the Fe and C^+ species formed in the plasma from organic compounds that coelute with the siderophores. Recoveries for...
ferrichrome and ferrioxamine E were 56% and 74%, respectively, which is consistent with previously reported hydroxamate siderophore recoveries after solid phase extraction.13 Other peaks also appear in the seawater sample between 10 and 20 min, suggesting the presence of a suite of naturally occurring Fe ligands at low concentrations. Analysis of the organic extract from South Pacific surface seawater with a longer gradient (0–50% methanol over 30 min) reveals the presence of four major and several minor naturally occurring ligands that elute between 16 and 27 min (Figure 4b). On the basis of the calibration curves of the standard compounds, these peaks represent ligands that are present in seawater from 0.3 to 0.7 pM concentrations.

**Synechococcus sp. PCC 7002.** To further demonstrate the utility of HPLC–ICP-MS for detecting multiple strong iron ligands that are naturally produced in seawater, we analyzed culture media from *Synechococcus* sp. PCC 7002. Under low Fe conditions, this coastal cyanobacteria strain produces a suite of amphiphilic hydroxamate-type siderophores (synechobactins A–C) that have been previously isolated and structurally characterized.35–37 These synechobactins can be extracted from spent culture media and detected by HPLC–ICP-MS.

The chromatogram for the *Synechococcus* sp. PCC 7002 media extract shows three large peaks with retention times of 18.7, 23.3, and 26.7 min (Figure 5, peaks f, h, and i, respectively). The fractions containing these peaks were collected and analyzed by HPLC–ESI-MS. They yielded spectra with parent ions at masses that correspond to the Fe form of synechobactins A (26.7 min; C_{26}H_{46}N_{4}O_{9}Fe^+ = 614.3 m/z), B (23.3 min; C_{24}H_{42}N_{4}O_{9}Fe^+ = 586.2), and C (18.7 min; C_{23}H_{40}N_{4}O_{9}Fe^+ = 558.2), respectively.37 In addition to the three major peaks, there is a small peak at 3.2 min (Figure 5, peak a) that also appears in the blank media extract, and which may correspond to uncomplexed Fe, Fe-EDTA, or Fe-citrate used in the culture medium to buffer free ion concentrations or from the ENV+ resin. There are also additional peaks with retention times at 9.3, 10.6, 11.6, 14.8, and 21.0 min that indicate the presence of uncharacterized Fe ligands (Figure 5, peaks b–e and g, respectively).

The presence of uncharacterized Fe ligands in the HPLC–ICP-MS chromatograms of seawater and the *Synechococcus* sp. PCC 7002 extract demonstrates the strength of this method for screening marine samples for Fe ligands. As in the original studies that characterized synechobactins A–C, siderophores are commonly detected in cell culture media using spectrophotometric tests, such as the chrome azurol S assay which has a detection limit of 2 nmol of free ligand.38,39 Since HPLC–ICP-MS has a lower detection limit and measures the complexed rather than free form of the ligand, this method can detect the presence of strong metal ligands that cannot be detected by spectrophotometric assays.

### CONCLUSIONS

The determination of Fe ligands in seawater requires a targeted approach to better-understand ligand diversity and variability. As a first step to identify target complexes, HPLC–ICP-MS is ideal for rapidly screening seawater and marine cultures for the presence of distinct Fe ligands. While precise quantification based on this method is still hindered by sensitivity changes due to mobile-phase composition and organic matrix effects, it is capable of estimating ligand concentrations within a factor of 2. The major components can then be isolated and structurally characterized with complementary techniques such as ESI-MS analyses.

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