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A solid phase extraction method for analysis of the iron-humic acid complex in natural water

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Abstract: Extracting the iron-humic acid (Fe-HA) complex from natural waters is difficult, since there is a lack of standards and many impurities are co-extracted with the target analytes. In this study, a laboratory synthesized Fe-HA complex was used as a standard to develop a simple solid phase extraction (SPE) method for analysis of Fe-HA complex in natural water. The Fe-HA complex in the SPE extract was separated from the matrix and analyzed with size exclusion chromatography (SEC) hyphenated with ultraviolet spectrophotometry. The Fe-HA complex was quantified as molar concentration of HA bound Fe, which was determined by analyzing the SEC eluent with inductively coupled plasma mass spectrometry. The results suggested that optimized extraction could be achieved with an ENVI-18 cartridge as the sorbent, methanol as the eluent, and a flow rate of sample loading of 10±2 mL·min⁻¹, and without adjusting the sample pH. Under the proposed SPE condition, the linear range of the Fe-HA complex was 0.052 to 0.301 µmol·L⁻¹. The detection limit (S/N=3.0) was found to be 0.0012 µmol·L⁻¹. The recovery of spiked Fe-HA complex from a natural river water sample was 76.8% and acceptable repeatability with a relative standard deviation of 5.2% was achieved. Moreover, the developed SPE method was successfully applied to analyze the Fe-HA complex in the Jiulongjiang River. It was found that the concentrations of the complex were in the range 0.023～0.085 µmol·L⁻¹, showing a gradual decrease from upstream to downstream.

Keywords: Iron, humic acid, complex, solid phase extraction, size exclusion chromatography, inductively coupled plasma mass spectrometry
1. Introduction

As an essential micronutrient, iron (Fe) plays a crucial role in controlling marine primary production, nitrogen fixation and even the global carbon cycle [1-4]. Previous studies find that more than 99% of dissolved Fe is bound to dissolved organic matter (DOM) in natural water [5-8]. It is widely accepted that humic substances (HS), as the most abundant DOM in the aquatic environment [9-11], have become the major candidates for Fe complexation due to their multifunctional groups [12-14]. Therefore, the study of the Fe-HS complex has attracted widespread attention.

Nowadays, the most popular method for studying the Fe-HS complex in natural water is competitive ligand exchange-adsorptive cathodic stripping voltammetry (CLE-ACSV) [12, 15]. Unfortunately, this electrochemical method can reflect only the conditional stability constant and concentration of the Fe-HS complex. Other essential properties of Fe-HS complexes, such as chemical structure, cannot be analyzed with CLE-ACSV. Common structural analytical techniques, including nuclear magnetic resonance and mass spectrometry, often require milligram per liter to gram per liter concentration [6]. However, the concentration of the Fe-HS complex in most waters is extremely low, and obtaining sufficient quantities of the complex is the bottleneck in the structural study of the complex. Therefore, it is necessary to develop a simple and reliable enrichment method for extracting the Fe-HS complex from natural water. In recent years, the solid phase extraction (SPE) technique, with its advantage of low blanks for both trace metals and organic carbon and its high reproducibility, is regarded as a prior enrichment method [16-19].

Some studies report that the Fe complex can be extracted using SPE [17, 18], but the studies are very preliminary. Extraction of the Fe-HS complex from natural water has not been systematically studied probably because of the limitations discussed below. (1) Without the availability of a Fe-HS complex standard, the operational parameters of SPE, such as the type of SPE sorbent and elution solvent, are difficult to optimize. Neither can the concentration of the complex in natural water be quantified owing to the lack of a standard or surrogate. (2) Because many types of
hydrophobic organic matter can be co-extracted and eluted with the Fe-HS complex, the complex in the SPE extracts needs to be separated from the complicated matrix before further analysis.

Humic acid (HA), as a representative of HS, is widely used as a ligand in the study of metal complexation [20, 21]. With abundant functional groups, HA can easily bind with Fe and form the stable Fe-HA complex [22-24]. The Fe-HA complex can be synthesized in the laboratory and used as a potential standard to develop the SPE method. After forming the Fe-HA complex, its molecular weight is larger than either Fe or HA [25, 26]. Based on this, size exclusion chromatography hyphenated with ultraviolet spectrophotometry (SEC-UV), in which analytes are separated based on molecule size [27], might be applied for separation. Inductively coupled plasma mass spectrometry (ICP-MS) is a typical elemental analyzer and is widely used in the analysis of various heavy metals, and could be a suitable detector to quantify the HA bound Fe by analyzing the Fe in the Fe-HA complex.

The objective of this study was to develop a simple and reliable SPE method for extracting the Fe-HA complex from natural water. A laboratory synthesized Fe-HA complex was used as a lab standard and the operation parameters of SPE were optimized. In the study, identification of the Fe-HA complex was carried out using SEC-UV hyphenated with ICP-MS (SEC-UV-ICP-MS), where the UV spectrophotometer was used to record the HA peak while the ICP-MS was used to determine the Fe species in sequence. The Fe-HA complex was quantified as the molar concentration of HA bound Fe by analyzing the acidified SEC eluent of the Fe-HA complex with off-line ICP-MS. The optimized SPE method was applied to the analysis of natural water samples.

2. Experimental

2.1 Materials

Sodium hydroxide (NaOH, ACS grade), phosphoric acid (H₃PO₄, 85%, trace analysis grade), potassium hydrogen phthalate (C₈H₅KO₄, HPLC grade) and ferric chloride hexahydrate (FeCl₃·6H₂O, ACS grade) were obtained from Sigma-Aldrich
Co. (St. Louis, MO, USA) and used as received. Nitric acid (HNO₃, 65%, G. R. grade) and methanol (HPLC grade) were purchased from Merck Co. (Darmstadt, Germany). Hydrochloric acid (HCl, 39%, G. R. grade) was supplied by Kunshan Jincheng Chemical Reagent Co. (Kunshan, China). Several of the commercial SPE cartridges available for use are listed in Table 1.

(Table 1)

Ultra pure water (resistivity 18.2 MΩ·cm) from a Millipore Purification Water System (Millipore Co., MA, USA) was used throughout the experiments. The Fe working solution at the desired concentration was prepared by dissolving FeCl₃·6H₂O in acidified ultra pure water (HCl, pH 1.7) daily.

All containers, except those especially mentioned, were made of low density polyethylene and cleaned by soaking in 4mol·L⁻¹ HCl solution for one week, 2 mol·L⁻¹ HCl solution for another week, and finally storing in 0.01mol·L⁻¹ HCl solution. They were thoroughly washed with ultra pure water before use. Amber glass bottles used for storing DOC samples were combusted at 450°C for 6 h. 0.45 µm hydrophilic polyethersulfone (PES) membrane (Pall Co., Michigan, USA) was soaked in 2% (v/v) HNO₃ for a week and washed with ultra pure water before use.

2.2 Instrumentation

All samples were filtered with 0.22 µm PES membrane before SEC-UV analysis. SEC-UV measurement was carried out with a liquid chromatography system (Shimadzu, Japan) equipped with an LC-20ADXR pump, an SPD-M20A diode array detector, a CBM-20A communication bus module and an SIL-20AXR autosampler. A guard column SWXL (6.0×40 mm) and a TSK G3000SWXL SEC column (5 µm, 7.8×300 mm) were chosen for the separation. SEC chromatograms were recorded at a wavelength of 254 nm. Ultra pure water was employed as the mobile phase with a flow rate of 0.5 mL·min⁻¹. The injection volume was 50 µL.

A7700x ICP-MS (Agilent, USA) was used in the study. The instrumental parameters are summarized in Table 2. For SEC-UV-ICP-MS measurement, the outlet
of the SEC-UV was directly connected to the nebulizer inlet of the ICP-MS via PEEK tubing (0.25mm i.d.).

(Table 2)

The dissolved organic carbon (DOC) content of natural water samples was measured as non purgeable organic carbon with a TOC vCHP total organic carbon analyzer (Shimadzu, Japan) and a high-sensitivity platinum catalyst. Samples were acidified to pH 2 with H₃PO₄ before DOC analysis. A five point calibration curve was prepared with C₈H₅KO₄ as standard.

2.3 Purification of HA

Humic acid supplied by Sigma-Aldrich (St. Louis, MO, USA) was purified based on an optimized procedure, in which HA was dissolved in 0.01 mol·L⁻¹ NaOH solution and centrifuged at 5000 rpm for 30 min. The residue was discarded to remove the associated inorganic solids and insoluble humin. Then the solution was acidified to pH 1 with 3 mol·L⁻¹ HCl solution. The supernatant was removed after centrifugation (10000 rpm, 40 min) to eliminate metals, and the residue was purified HA. This procedure was repeated several times and the resulting low-ash, low-metal HA was dissolved in 0.01mol·L⁻¹ HCl solution as the HA stock solution, which had 32.6 mmol·L⁻¹ of DOC. It was stored in the dark at 4°C until use.

2.4 Preparation of Fe-HA complex solution

For synthesis of the Fe-HA complex, the HA stock solution was diluted with ultra pure water and spiked with Fe working solution to produce a testing solution with 5 mmol·L⁻¹ DOC and 15.91 μmol·L⁻¹ Fe. Then the solution pH was adjusted to 6 with HCl and NaOH solutions and placed in the dark at 25°C for 10 h. The Fe-HA complex concentration in the solution was 3.02 ± 0.07 μmol·L⁻¹ and the complex was found to be stable for 20 h. The Fe-HA complex solution was used as the lab standard solution for the optimization of SPE parameters.
2.5 SPE procedure

Extraction of the Fe-HA complex was performed as follows: the SPE cartridge was preconditioned by passing 20 mL methanol and then 30 mL ultra pure water through it. If the sample volume was 10 mL or less, the sample was passed through the cartridge simply by gravity. When the sample volume was 1 L, the sample was loaded at a flow rate of 10±2 mL·min⁻¹ using a vacuum pump. The loaded cartridge was blown to dryness with a compressed air flow. Finally, a certain volume of elution solvent was used to elute the Fe-HA complex from the cartridge.

2.6 Quantification of Fe-HA complex

The Fe-HA complex was quantified as the molar concentration of HA bound Fe in this study. To do this, the Fe-HA complex in the water sample or SPE extract was separated with SEC-UV, and the SEC eluent from 6~10.5 min was collected, acidified with HNO₃ (2% (v/v) HNO₃ in the final solution) and analyzed with ICP-MS.

2.7 Collection of water samples

Surface river water samples were collected from eight sites (N1~N8), from upstream to downstream along the Jiulongjiang River of Fujian Province, China, on 3 November 2013. Three duplicate water samples were collected in each sampling site. Water samples were stored in the dark at 4°C immediately after collection. All water samples were filtered through 0.45 µm PES membrane to remove suspended particles within 24 h. Twenty mL of filtered water sample was frozen immediately at -20 °C in the dark until DOC analysis. Ten mL of the filtered water sample was acidified with HNO₃ (2% (v/v) HNO₃ in final solution) and stored at 4 °C for analysis of the total dissolved Fe using ICP-MS. One L of the filtered water was stored in the dark at 4°C until extraction within the next 48 h. The salinity, temperature and pH of the water samples were monitored in situ using a WTW340i portable multi-parameter instrument (Xylem, Germany). Information regarding the sampling sites is listed in Table 3.
The SPE extract of the water sample was collected and divided into two parts and treated as follows: (1) A 2 mL extract was subjected to separation with SEC-UV and quantification of the Fe-HA complex with ICP-MS; and (2) the remaining extract was gently evaporated under high purity nitrogen gas flow to 1 mL for analyzing the Fe species with SEC-UV-ICP-MS.

3. Results and Discussion

3.1 Detection of Fe-HA complex

The Fe-HA complex solution was analyzed with the SEC-UV-ICP-MS for identification of the Fe-HA complex. With the UV detector, organic compounds absorbing light at 254 nm could be recorded (Fig. 1a). Two chromatographic peaks, peak I with a larger molecular weight at a retention time of 6–10.5 min and peak II with a smaller molecular weight at a retention time of 24–28 min, are shown in Fig. 1a. From the chromatogram recorded with ICP-MS, as shown in Fig. 1b, Fe was mainly concentrated in peak I and almost no Fe was present in peak II. By comparing Fig. 1a and Fig. 1b, it could be concluded that only peak I consisted of both Fe and organic compounds, indicating the existence of the Fe-HA complex. A control group, which was prepared in the same way as described in section 2.4 except for adding HA working solution, was analyzed with the SEC-UV-ICP-MS. Without HA in the control group, only unbound Fe could exist in the solution. Therefore, the broad peak III recorded with ICP-MS and shown in Fig. 1c corresponded to the unbound Fe. This result further confirmed that peak I was due to the bound Fe-HA compound because of its totally different retention time from the unbound Fe in Peak III.

(Fig. 1)

3.2 Optimization of SPE for extraction of Fe-HA complex
For optimization of the SPE parameters, laboratory prepared Fe-HA complex solution was used as the standard and extracted under different operation conditions. The extraction efficiency was evaluated using the recovery of the Fe-HA complex, which was calculated as the ratio of the amount of Fe-HA complex in the SPE extract to that in the laboratory prepared Fe-HA complex solution. The effect of extraction parameters, including type of SPE sorbent, elution solvent, sample pH values and flow rate of sample loading, on the extraction performance was investigated in detail. Unfortunately, ionic strength, as an important impact factor of extraction, could not be studied because the Fe-HA complex was greatly disassociated in the presence of NaCl or other ionic reagents.

### 3.2.1 Selection of SPE cartridge

Since the composition of the Fe-HA complex was very complicated, a variety of commercial SPE cartridges with different extraction ability were compared to select a suitable one. For each SPE cartridge, 10 mL of laboratory prepared Fe-HA complex solution was extracted, and the results are shown in Fig. 2. ENVI-18, as the typical C18 cartridge, presented SPE cartridges greatest retention ability for the Fe-HA complex and the recovery was about 85.2%. The ENVI-carb, LC-SCX, DSC-MCAX and Oasis HLB cartridges extracted a lower amount of Fe-HA complex. Not surprisingly, almost no Fe-HA complex was extracted on Isolute ENV+ and typical ion-exchange SPE cartridges, Bond Elut PSA and Bond Elut SAX, which were designed for the extraction of polar compounds. The result confirmed that the Fe-HA complex was mainly composed of non-polar groups, which could be easily extracted on a C18 cartridge. In this study, the ENVI-18 cartridge was selected for extraction.

(Fig. 2)

### 3.2.2 Elution

Studies show that the most effective elution solvents for eluting Fe complex from C18 sorbent are acidic solutions, such as HNO₃ and acidic methanol [28-30].
However, disassociation of the Fe complex may inevitably happen when using these acidic solvents, thus influencing further speciation analysis. To avoid such disassociation, the mixture of methanol and water, which is used for eluting the Cu-DOM complex from the C18 cartridge since the 1980’s [31, 32], has also been adopted to elute the Fe-DOM complex [18] and thus was chosen in our study.

Ten mL laboratory prepared Fe-HA complex solution was extracted with the ENVI-18 cartridge. A mixture of methanol and water with different percentages of methanol (0~100%) was tested as the eluting solvent for Fe-HA complex elution, and the solvent volume was also investigated. The elution efficiency was evaluated with recoveries of the Fe-HA complex as listed in Table 4. The experimental results suggested that recovery of the Fe-HA complex increased as the methanol proportion in the mixture increased, and 100% methanol gave the best recovery. When 6 mL of 100% methanol was applied as the elution solvent, the recovery of the Fe-HA complex reached 84.5%, indicating that the targets were well eluted. As a result, we chose 6 mL of 100% methanol to elute the Fe-HA complex.

(Table 4)

3.2.3 Sample pH

Appropriate sample pH may enhance extraction efficiency, and so the pH of water sample should be checked before extraction. In this study, the laboratory prepared Fe-HA complex solution pH was adjusted to 4~9 before extraction. It is well known that the Fe-HA complex dissociates at low pH and Fe hydrolyzes at high pH, so that the concentration of the Fe-HA complex varies with pH adjustment [33-35]. As shown in Fig. 3a, in the different pH adjusted solutions, the concentration of the Fe-HA complex varied and ranged from 0.88 ~ 3.02 \( \mu \text{mol} \cdot \text{L}^{-1} \). The concentration was higher at pH 5~7, which was the range of most natural waters in southern China.

When 10 mL of the pH adjusted solution was extracted, the average recovery could reach 85%. It can be seen from Fig. 3b that the extraction recovery was slightly
decreased at higher pH, which may be explained by the following possibilities. HA contains abundant functional groups and, except for binding with Fe, some functional groups such as hydroxyl and carboxyl may remain on the Fe-HA complex. Under higher pH, these acidic groups on the Fe-HA complex would be deprotonated, which would increase the polarity of some of the Fe-HA complex and lead to a slightly decreased recovery.

Since no significant difference in recovery was observed among different pH solutions, and the pH of most natural waters in southern China is below 8, it could be concluded that pH adjustment was unnecessary when extracting the Fe-HA complex from natural water samples.

(Fig. 3)

3.2.4 Flow rate of sample loading

The flow rate of sample loading not only determines the time of analysis, but also has some effect on the extraction efficiency. In this study, 1 L of ultra pure water was spiked with Fe working solution and HA stock solution as described in section 2.4. The concentration of Fe-HA complex in the spiked sample was 0.086 µmol·L⁻¹. Then, the spiked sample was extracted with flow rates varying from 5±2 to 20±2 mL·min⁻¹. The results in Fig. 4 indicate that low flow rate (≤10±2 mL·min⁻¹) was beneficial to the extraction of the Fe-HA complex, with an average recovery of 82.2%. When the flow rate was higher than 15±2 mL·min⁻¹, the recovery showed a small decrease compared with that of low flow rates. Overall, the flow rate had no significant effect on the extraction efficiency, and 10±2 mL·min⁻¹ was used as an appropriate flow rate for sample loading.

(Fig. 4)

3.2.5 SPE performance

With the optimized extraction method, i.e., using an ENVI-18 cartridge, 6 mL of
100% methanol as eluent, a flow rate of $10 \pm 2 \text{ mL} \cdot \text{min}^{-1}$ and without adjusting the sample pH, 1 L ultra pure water spiked with different concentration of Fe-HA complex was extracted. The spiked samples were prepared by adding an appropriate amount of Fe working solution and HA stock solution to ultra pure water as described in section 2.4. Fig. 5 showed the relationship between the concentration of the Fe-HA complex detected with the proposed method and that of the original spiked sample. The linear dynamic range ($R^2>0.96$) of 0.052 to 0.301 $\mu$mol·L$^{-1}$ indicated a good recovery for Fe-HA complex, which could be presented as the curve slope as 0.855, i.e. 85.5%. The detection limits, estimated as three times the signal to noise ratio, was 0.0012 $\mu$mol·L$^{-1}$. The limit of quantification, estimated as ten times the signal to noise ratio, was 0.0039 $\mu$mol·L$^{-1}$.

(Fig. 5)

One L of filtered water sample collected from the Jiulongjiang River was spiked with Fe working solution and HA stock solution as described in section 2.4. The concentration of the Fe-HA complex in the spiked river water sample was 0.130 $\mu$mol·L$^{-1}$. Extraction of the spiked river water sample was carried out to validate the feasibility of the proposed SPE method. The result showed that the recovery was 76.8% and the relative standard deviation for reproducibility was 5.2% (number of replicates=3), which demonstrated that the proposed SPE method was applicable for natural water sample analysis.

3.3 Method application

Jiulongjiang River water samples were collected and treated using the proposed SPE method. The Fe species in the SPE extracts were determined with SEC-UV-ICP-MS. The analytical result of the N3 sample, as the representative of all samples, is shown in Fig. 6. With the UV detector, a peak with a retention time of 6 to 10.5 min, corresponding to the Fe-HA complex, was observed (Fig. 6a). A very high and broad peak with a retention time of 20 to 40 min indicated a large amount of
co-extracted organic matter in the extract, which had relatively smaller molecular weights based on SEC principles. Fig. 6b shows a chromatogram recorded with ICP-MS, where the Fe-HA complex contained the majority of the Fe, indicating that the Fe-HA complex was the main organic Fe compound extracted using the proposed SPE method. Only a small part of the Fe was eluted in the retention time of 25 to 33 min. The characteristics of this latter part of the compounds remained unknown and needs to be further studied.

(Fig. 6)

The amount of Fe-HA complex in the sample was also detected by analyzing the acidified SEC eluent with ICP-MS. As shown in section 3.2.5, the recovery of the Fe-HA complex from the spiked natural river sample was 76.8%, which was used to correct the quantity of the Fe-HA complex. The result showed that the concentrations of Fe-HA complex in all sampling sites were in the range 0.023~0.085 µmol·L⁻¹. The Fe-HA complex distribution tendency revealed that Fe-HA concentration was higher upstream and lower downstream, as shown in Fig. 7a. Site N1 was located in Longyan City, while N2, N3 and N4 were at traditional livestock farming areas in the Longyan district. These sample sites received a great amount of organic matter from not properly treated waste water. On the other hand, N5, N6, N7 and N8 were far away from the city and were close to the sea with less human impact. Therefore, a decreasing trend of DOC from upstream to downstream could be observed, as shown in Fig. 7b. The total dissolved Fe also had the same distribution trend as DOC (Fig. 7c). Upstream, high concentrations of DOC and Fe led to more of the Fe-HA complex being formed. Therefore, a decreasing trend in the Fe-HA complex from upstream to downstream was observed.

(Fig. 7)
4. Conclusions

In this study, an SPE method for extracting the Fe-HA complex from natural water was developed. The optimized extraction was achieved with an ENVI-18 cartridge, 6 mL of 100% methanol as eluent, $10 \pm 2 \text{ mL} \cdot \text{min}^{-1}$ as sample loading flow rate, and without pH adjustment. The method detection limit was found to be 0.0012 µmol·L$^{-1}$. The recovery of the Fe-HA complex from natural river samples was 76.8%. The proposed SPE method was successfully applied in the analysis of the Fe-HA complex in river water samples. The results showed that the concentrations of Fe-HA complex in the Jiulongjiang River were in the range 0.023~0.085 µmol·L$^{-1}$. The distribution of the Fe-HA complex in the river showed a trend of gradually decreasing from upstream to downstream, which was the same as the distribution pattern of total dissolved Fe and DOC.

Acknowledgments

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References


**Table and Figure Captions**

[1]**Table 1** Information on commercially available SPE cartridges.

[2]**Table 2** Instrumental parameters of ICP-MS.

[3]**Table 3** Information on sampling sites.

[4]**Table 4** Recovery of distribution Fe-HA complex with different proportions of elution solvent and volume (number of replicates=3).

[5]**Fig. 1** SEC-UV-ICP-MS chromatogram of the Fe-HA complex solution and the control group. (a) Fe-HA complex solution analyzed with UV detector; (b) Fe-HA complex solution analyzed with ICP-MS; (c) control group analyzed with ICP-MS.

[6]**Fig. 2** Recovery of Fe-HA complex using different SPE cartridges (number of
Conditions: 6 mL of 100% methanol as eluent, flow rate of sample loading of 10 ± 2 mL·min⁻¹, and without sample pH adjustment.

[7] **Fig. 3** Concentration of Fe-HA complex in pH adjusted solutions (a) and recovery of Fe-HA complex at different pH levels (b) (number of replicates=3).

Conditions: with the ENVI-18 cartridge, 6 mL methanol as eluent, and flow rate of sample loading of 10 ± 2 mL·min⁻¹.

[8] **Fig. 4** Recovery of Fe-HA complex using different flow rates (number of replicates=3).

Conditions: with the ENVI-18 cartridge, 6 mL of 100% methanol as eluent, and without sample pH adjustment.

[9] **Fig. 5** The linearity between the concentration of the Fe-HA complex detected with the proposed method and that of the original spiked sample (number of replicates = 3).

[10] **Fig. 6** Typical SEC-UV-ICP-MS chromatograms of SPE extract. (a) analyzed with UV detector; (b) analyzed with ICP-MS.

[11] **Fig. 7** Distribution of the Fe-HA complex (a), DOC (b) and total dissolved Fe (c) at different sampling sites (number of samples at a site=3).
### Table 1

<table>
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### Table 2

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### Table 3

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With the ENVI-18 cartridge, 10±2 mL·min\(^{-1}\) as sample loading flow rate and without sample pH adjustment.
Fig. 1

(a) Signal intensity

(b) Counts

(c) Counts

Time (min)
Fig. 2

Fig. 3
Fig. 4

 Recovery of Fe-HA complex (%)

 Flow rate (mL·min⁻¹)

 Fig. 5

 Fe-HA complex detected with proposed method (μmol·L⁻¹)

 Fe-HA complex in original spiked sample (μmol·L⁻¹)

 y=0.855x-0.007

 R²=0.967
Fig. 6

(a) Signal intensity
(b) Counts

Time (min)

Fig. 7

(a) Concentration of Fe-HA complex (μmol L⁻¹)
(b) Concentration of DOC (μmol L⁻¹)
(c) Concentration of total dissolved Fe (μmol L⁻¹)

Sampling site