A SENSITIVE DETERMINATION OF IODINE SPECIES, INCLUDING ORGANO-IODINE, FOR FRESHWATER AND SEAWATER SAMPLES USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY AND SPECTROPHOTOMETRIC DETECTION.

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Abstract

In order to more effectively use iodine isotope ratios, $^{129}I/^{127}I$, as hydrological and geochemical tracers in aquatic systems, a new HPLC method was developed for the determination of iodine speciation. The dissolved iodine species that dominate natural water systems are iodide, iodate, and organic iodine. Using this new method, iodide was determined directly by combining anion exchange chromatography and spectrophotometry. Iodate and the total of organic iodine species are determined as iodide, with minimal sample preparation, compared to existing methods. The method has been successfully applied to determine iodide, iodate as the difference of total inorganic iodide and iodide after reduction of the sample, and organic iodine as the difference of total iodide (after organic decomposition and reduction) and total inorganic iodide.

Analytical accuracy was tested (1) against certified reference material SRM 1549, powdered milk (NIST), (2) through the method of standard additions, and (3) by comparison to values of environmental waters measured independently by ICP-MS. The method has been successfully applied to measure the concentrations of iodide species in rain, surface and groundwater, estuarine and seawater samples. The detection limit was ~1 nM (0.2 ppb), with less than 3% relative standard deviation for samples determined by standard additions to an iodide solution of 20 nM in 0.1 M NaCl.

This technique is one of the few methods sensitive enough to accurately quantify stable iodine species at nanomolar concentrations in aquatic systems across a range of matrices, and to quantitatively measure organic iodine.

Keywords: iodide, iodate, organic iodine, HPLC, seawater, fresh water, estuarine water

1. Introduction

Recent studies have employed ^{129}I and the isotopic ratio of $^{129}I/^{127}I$ as a tracer of water mass mixing in oceanography and hydrology [1- 6]. Another study has applied inorganic speciation to $^{129}I/^{127}I$ ratios for tracing the transport and mixing of marine sources for ^{129}I [5]. The possible use of the iodine isotopic ratio as a tracer in other aquatic systems [7, 8] as well as the extension of its current use to provide a new geochronometer for organic matter [8, 9] will require a thorough characterization of iodine speciation. There are only a few methods sensitive enough to accurately quantify iodine species (including organo-iodine) at nanomolar concentrations and these do not apply to the whole range of matrices found in aquatic systems, nor do they quantify organic iodine.

Many methods to measure iodine species have been proposed in the literature, but most are applicable only to one particular matrix, e.g., specifically to fresh water, blood, urine, or seawater. A review shows that these literature methods are not applicable to a range of matrices. Analysis of iodine and trace elements in seawater by inductively coupled plasma-mass spectrometry (ICP-MS) is often impractical due to the loss of sensitivity from the build-up of salts [10]. However, recently developed methods using IC-ICP-MS for iodine speciation in fresh waters have successfully been employed [11, 12]. Ion chromatography (IC) and high performance liquid chromatography (HPLC) methods for iodide analysis at trace levels in seawater face the difficulty of separation of high matrix concentrations of chloride and bromide from the much

smaller iodide peak [13, 14]. Some IC methods use the addition of NaCl to the mobile phase to remove interferences by the variability in the composition of inorganic ions in the matrix, but require a post-column reaction [15] or large volume samples of 2 to 6 ml with a pre-concentrator column [13,14]. IC or HPLC methods have been developed to improve the detection limit of iodide in mineral water samples, but again involve post-column reactions for iodide and for iodate [16]. None of these methods are flexible enough to measure iodine species, including organo-iodine, in both fresh water and seawater samples. Results from reverse phase columns are generally not as robust as anion exchange; however, a method using a zwitterionic surfactant coating as the stationary phase was shown to measure iodide at ppb levels, albeit with a long retention time [17]. IC methods using anion exchange and coulometric detection are subject to interferences from natural organic matter [18] that reduce the exchange capacity of the column or foul the electrodes. Voltammetry methods, while suitable for open ocean waters with relatively high concentrations of iodate and total inorganic iodide (reduced fraction used to measure iodide by difference) [19, 20] are subject to similar organic fouling of the electrodes [21] in estuarine and fresh waters, and are very time-consuming. In addition, voltammetry of fresh water samples requires even more argon purge time than seawater in removal of oxygen for iodide analysis [22, 23]. Voltammetry for fresh water samples also requires the addition of electrolyte and trace level metal clean techniques in order to avoid a decrease in signal to noise ratio to a level too low to adequately detect trace iodine species concentrations [24]. Neutron activation methods are more

more expensive, not readily accessible to all labs, and have potential interference problems from bromide [25].

The purpose of this paper is therefore to develop a practical, sensitive method for the quantification of iodide, iodate, and organic iodine species for fresh water and seawater samples.

2.Experimental

2.1 Reagents and standards

Eluents, standard solutions, and reagent solutions were all made up with doubly distilled de-ionized waters of 18.3 MΩ resistance produced from two sequential Barnstead NANOpure systems.

All chemicals used were salts of sodium and were analytical grade or equivalent. The iodide and iodate salts were purchased from Fluka and the metabisulfite, chloride, phosphates, perchlorate, hypochlorite, and hydroxide were purchased from Fisher and Spectrum Corporation.

Reagent solutions were made with water degassed by ultrasonication, particularly the iodide solutions. All iodide and iodate solutions were stored in amber polyethylene bottles that were capped, sealed with parafilm, and refrigerated until use. A primary iodide stock solution of 10 mM was freshly made every three weeks, while the secondary and working solutions of 200 μM and 2 μM were made each day. Large volume standards for iodide were made

up daily when many samples were run. It was determined that peak heights for the same concentration of iodide were dependent on chloride concentration (Figure 1). Since the peak heights were equivalent for the range of 0.1 to 0.3 M NaCl (salinity range of approximately 8 to 20), sample, blank, and standard solutions were made up in a 0.1 M NaCl solution to optimize sensitivity and to approach standardization of samples to the same salinity. Iodide oncentrations in samples from differing environments can be quantified through standard additions of a solution of iodide in 0.1 M NaCl, while iodide in samples with a similar matrix, e.g., open ocean seawater, can be calibrated to the same standards curve.

2.2 Instrumentation

Iodine species were separated, identified, and quantified by means of a Waters HPLC. Millenium[32] software was utilized to operate the system components and to acquire and integrate the chromatograms. Components used for the analysis of iodine species were a 600S gradient controller, a 626 non-metallic pump, a 200 µL sample loop, a 717-plus auto-sampler, and a dual wavelength 2487 UV spectrophotometer. The UV wavelength used was 226 nm.

The column used was a strong anion exchanger, 250 x 4 mm Dionex AS11, 13 µm particle size, preceded by a 50 x 4 mm Dionex AG11 guard

column. The guard and analytical columns were kept at ambient temperature, about 22°C. After each 46-sample tray, the column was cleaned with 0.5 h rinses each of water, 20 mM NaOH, water, 20 mM HNO_3, water, 20% methanol, water, 10% acetonitrile, with a final water rinse to extend the column life and maintain resolution when analyzing waters containing natural organic matter.

The injection volume used for iodide concentrations of 0 to 350 nM was 140 µL. Sample dilutions were, at times, required to fit into this concentration range, or lower injection volumes were used at higher iodide concentrations in order to avoid overloading the column. Table 1 shows ranges of total iodine values for Texas soils [7, 8] and rains [7, 8], United States rivers [8, 9, 26, 27, 28, 29] and sediments [27], and seawater [8, 19, 20, 23, 28, 29, 30, 31, and this work] taken from the literature.

The mobile phase gradient consisted of a unique selection and sapplication of elements from other published methods. The mobile phase had 4 components used in a gradient elution that included column regeneration and equilibration phases. Mobile phase A was 18.3 MΩ water, B was 200 mM NaCl [13, 15], C was 10 mM NaOH (in a solution that was degassed and exposed to the atmosphere as little as possible to avoid carbonate absorption from the air) [32], and D was 75 mM sodium perchlorate and 12.5 mM sodium phosphate [13, 14]. A stock solution for D was prepared by adding 47.5 g of $NaClO_4$, 5.5 g Na_2HPO_4 and 2.1 g NaH_2PO_4, into 2 L, adjusting the pH to 6.1, and finally diluting the solution by 50% with the addition of 18.3 MΩ water. These solutions were run as the gradient profile shown in Table 2.

Unlike other applications, the mobile phase is primarily deionized water with dilute reagent solutions, which were more cost effective and easier to fine-tune when necessary. If the large volume blank and standard solutions of iodide are not used, or if the sample matrices are very different from those analyzed here, the iodide peak resolution can be adjusted. Change in the amount of mobile phase B, sodium chloride, adjusted for changes in sample ionic strength, modified the retention time of the iodide peak. Figure 2 is an example of optimizing the percent gradient and concentration of mobile phase B. In Figure 2, chromatogram “a” represents a change from the gradient in Table 2 to a gradient of 14% B at 0 minutes and 20% B at 10 minutes; chromatogram “b” is a change to 10% B at 0 minutes and 18% B at 10 minutes; and chromatogram “c” is a decrease to 8% B at 0 minutes and 15% B at 10 minutes. Since the % composition of all the components in the mobile phase solution must equal 100%, the difference in solution was made up in the deionized water, or % A. The decrease in the mobile phase B (NaCl) concentration produces chromatograms showing progressively slower elution with increasing resolution of the iodide peak.

Regulation of the mobile phase C, sodium hydroxide, changed the peak shape of other solution components and enhanced separation from other matrix components. An increased percentage of mobile phase D, sodium perchlorate and phosphate buffer, can sharpen the iodide peak shape.

2.3 Method

The analytical scheme is described in Figure 3. Iodide was measured directly diluted in standard and blank solutions of 0.1 M NaCl solution to optimize sensitivity and to approach standardization of samples to the same salinity.

Iodate was analyzed directly as a separate peak in some waters, but most often was difficult to resolve due to its early elution and the significant interference by other anions. The procedure used to determine iodate concentrations in this study was, therefore, first to quantify iodide, next to reduce iodate to iodide by $NaHSO_3$, and finally to calculate iodate as the difference between iodide and the total inorganic iodine (TII) concentration. Iodate was reduced to iodide by $NaHSO_3$ addition to a final concentration of 0.07 mM $NaHSO_3$. After vortexing for a few minutes, the solution was heated at 95^{o}C for 0.5 h in Teflon® bombs that were set into the wells of an Environmental Express Hot Block. After the sample was cooled to room temperature, it was acidified to a final solution concentration of 0.24 mM HCl for less than 5 min. (both amounts of reductant and acid were for an estimated concentration of 500 nM iodate). Then, the treated sample was neutralized with NaOH and diluted to an estimated concentration of 150 nM, and analyzed by HPLC. Analysis should take place as soon as possible after the addition of the SO_3^{2-}, in order to minimize the potential for breaking C-I bonds [20], which could cause an overestimation in the inorganic IO_3^- concentration and an underestimation in the organic iodine fraction. There was no significant difference ($\leq \pm 3\%$) between our duplicate runs from the same

container of prepared sample, but the potential for slow decomposition of more labile DOI may be there even at neutral pH [20]. This potential error, which may be sample-dependent, would cause an overestimation of inorganic IO_3^- and an underestimation of DOI. Such an effect, however, was not seen in our data as our DOI values were high relative to other published values, and in agreement with ICP-MS measurements (see Results and Discussion section).

Organic iodine was determined by the difference of total iodine (TI) and total inorganic iodine (TII) after dehydrohalogenation. For the determination of total iodine, 2 mL of 5M NaOH and 2 mL of Pharmco 200 proof ethanol were added to 7 mL of sample in Teflon® bombs. The bombs were ultrasonicated for three hours at 65°C and allowed to react overnight. An additional 1 mL of 5 M NaOH and 1 mL of Pharmco 200 proof ethanol were added and the solution was again ultrasonicated for three hours at 65°C. The bombs were placed in the wells of an Environmental Express hot block and heated for 0.5 h at 60°C, after the addition of 0.7 mL 1 M $NaHSO_3$ for the reduction of iodate species to iodide. Next, the samples were placed in a freezer for 0.5 h to cool. The process of alkaline hydrolysis, ultrasonication and heating, and finally cooling, was intended to disaggregate colloidal micellular material and break ester bridges in macromolecular humic and fatty acids [33, 34].

After the addition of 3 mL 6 M HCl, the bombs were weighed, heated while uncapped for 1 h at 80°C to drive off ethanol (boiling point, 78°C), and reweighed. The samples were prepared for HPLC analysis by loading the sample onto a pre-conditioned Waters solid-phase extraction (SPE) tC18 cartridge. The

tC18 cartridge was prepared by wetting with 9 mL each of deionized water, methanol, and deionized water. The first 2 mL of sample were discarded, and the remaining effluent retained for analysis. Refractory organic matter and remaining ethanol were retained on the tC18 cartridge, which was then disposed of in an appropriate manner.

Iodide standard solutions and calibrations were run before and after every 15 to 20 samples. Two blanks were run before each sample to insure that no memory effect was present. All samples were run in duplicate, except for fresh water samples of low iodide concentration, which were run in triplicate.

2.4 Certified reference material and standard additions

The organic decomposition technique, and the accuracy of the HPLC analysis was first tested with certified reference organic material, SRM 1549, powdered milk (NIST). The milk solution was prepared by using 500 mg for a stock solution, as recommended by NIST, then diluted to 200 nM of iodide. A 15-mL aliquot of the 200-nM iodide milk solution was adjusted to pH 12 with 120 µL 5 M NaOH, after which 200 µL 4-6% NaClO was added. The solution was ultrasonicated for 20 minutes, and placed into a screw-top Teflon® bomb that was heated to 95°C in an Environmental Express Hot Block for 6 h. After cooling, the milk solution was then transferred to a clear-50 mL Teflon® bottle with an addition of 500 µL 50% Spectrum Ultrapurex H_2O_2. The milk solution in the Teflon® bottle

was then irradiated with two 15-W 254 nm UV tubes and a 15-W 185 nm UV tube. The Teflon® bottles were exposed to 9 h of UV irradiation that was applied in three repetitions of 3 h of irradiation at 85°C, followed by 2 h of cooling.

Then 1 mL of the sample solution, 50 µL of 1 M HSO_3, and 50 µL of 6 M HCl were added to 9 mL of iodide standard additions solutions in 0.1 M NaCl. The respective concentrations of iodide in the standard addition solutions were a) 0 (blank solution), b) 17.8, c) 71.3, and d) 142.6 nM. The blank solution with the sample was run twice, so the respective chromatogram peak height for each of these standard additions was a) 175 and 169, b) 322, c) 799, and d) 1467 µAU. The standard additions curve for the milk had a correlation coefficient of 0.9997 with a slope of 9 and a y-intercept of 166. Procedures for performing standard additions calculations are described in [35]. Examples on how to carry out these calculations and how to calculate the standard error using an Excel spreadsheet are given in [36].

The calculations for recovery determination were as follows: 15 mL of 200 nM milk solution were diluted with 120 µL 5 M NaOH, 200 µL 4-6% NaClO, and 500 µL 50% Spectrum Ultrapurex H_2O_2, to a new iodide concentration of 189.6 nM in 15.82 mL. The x-intercept of the standard additions curve is the value of the negative of the intercept over the slope, which represents a diluted concentration of 18.32 nM. Since 1 mL of the 15.82 mL was added to 9 mL of standard solution with the added 50 µL of 1M HSO_3 and 50 µL of 6M HCl, the final dilution factor was 10.1 mL to 1 mL. When corrected for dilution, the calculated recovery is 18.32 nM multiplied by 10.1/1 or 185 nM. The 185 nM

iodide recovered from a 189.6 nM iodide concentration in the milk solution represented a 98% recovery with a standard error of $\pm$ 0.84 nM.

The chromatograms for iodide in the milk solution are shown in Figure 4. The initial iodide concentration or sample in blank standard addition solution is designated as “a”. The respective iodide standard solutions, where “b” was 17.8 nM, “c” 71.3 nM, and “d” 142.6 nM, were overlain to indicate the ease of iodide peak identification using this method.

While this organic UV decomposition technique in the presence of H_2O_2 yielded an excellent recovery for iodide in milk, the recovery was not quantitative for aquatic samples. The dehydrohalogenation technique using the NaOH/ethanol digestion, described in section 2.3, gave, however, excellent results for all aquatic systems, and therefore became the method of choice for organic iodine determinations.

2.5 Comparison with measurements by ICP-MS

The recovery of total iodine, used for the determination of dissolved organic iodine (DOI), was compared with measurements by ICP-MS. Given the high temperature of the ICP-MS plasma, all organic sample material was completely and quantitatively digested. Other published methods currently used to determine TI, i.e., by UV irradiation [20, 37, 38], chlorination (TI determination by oxidizing I^- to IO_3^- using NaClO [39]), TI by reduction of IO_3^- to I^- using

ascorbic acid, or by catalytic reduction of IO_3^- to I^- using As^{3+} - Ce^{4+} under acidic conditions, were primarily for the total inorganic species and do not quantitatively convert the organic iodine species to a measurable inorganic form [20; 40].

While our UV irradiation technique gave good results for DOC, we did not find it to be quantitative for organo-iodine. However, our UV source was two15-W 254 nm tubes and a 15-W 185 nm tube versus the 700-W mercury lamp used by Wong and Cheng [20] or the 1000-W mercury lamp used by Truesdale et al [38]. Although our UV irradiation apparatus was different, we maintain that after a longer irradiation time, our method resulted in DOC values equivalent to those of Wong and Cheng (~1 ppm), while recoveries of $^{125}I^-$ from radio-iodinated humic material remained relatively low at 60 to 85%. In a similar experiment, Spokes and Liss [37] removed ~ 85% of the absorbance from organic matter (Aldrich humic acid) in seawater after 3 h of irradiance from a mercury arc lamp. Furthermore, Wong and Cheng [40] determined that, while artificial UV irradiation or natural sunlight effectively release inorganic iodide from some subfractions of DOI, as much as 60 nM remained in other samples having an expected (initial) DOI value of 120 nM. Truesdale et al [38] reported that some anoxic waters turned yellow immediately after UV irradiation, and then yielded lower apparent iodine concentrations than untreated samples. This suggests that while some inorganic iodine species were liberated from organic iodine, other organic iodine species were either too recalcitrant for decomposition by this method, or more likely, were newly formed through UV-induced radical reactions.

The lack of quantitative evaluation of organic iodine speciation in fresh waters suggests that many reported TI values are underestimated. Therefore, an optimized dehydrohalogenation technique using the NaOH/ethanol digestion, described in section 2.3, was developed and applied for quantitative organic iodine determinations and was tested by comparison to measurements by ICP-MS.

The comparison is shown for end-member waters, deep sea (1787 m) and surface sea (1 m) waters from the Gulf of Mexico (Table 3), and fresh surface water from the Trinity River (Table 4). The total iodine average value (TIAV) for the surface seawater sample for ICP-MS was 494.5 nM $\pm$ 2% relative standard deviation (RSD) and 4.8% precision (n=2), whereas the TI value for the HPLC was 526.2 nM $\pm$ 0.5% RSD (n=1), which is +6% of the ICP-MS value. The TIAV for the deep seawater sample was 537.0 nM $\pm$ 2% RSD, with a 4.8% precision (n=2) by ICP-MS versus 562.6 nM $\pm$ 0.5% RSD (n=2) by HPLC, with a 1.1% precision, which is within 5% of the ICP-MS value. The TIAV for the Trinity River fresh water sample was 280.3 nM $\pm$ 0.4% RSD (n=1) by ICP-MS, compared to 294.7 nM $\pm$ 0.4% RSD with 2.6% precision (n=2) by HPLC, which is within 5% of the ICP-MS value.

Comparison of HPLC iodide concentrations to those measured by ICP-MS for the same aquatic samples was always within 6% recovery of the ICP-MS values. Therefore, the recovery of TI including the organic iodine species by the dehydrohalogenation technique (described in section 2.3) was shown to be

quantitative and superior to the UV irradiation and chlorination method described in section 2.4.

2.6 Linear dynamic range, detection limits, and relative standard deviation

The linear dynamic range for iodide analysis, 6 μAU or 0.44 nM (lower limit equal to twice the detector background) to beyond 2700 μAU or 300 nM, was determined as the linear response to concentration for iodide in 0.1 M NaCl solution. However, with other anions in the sample matrix, the best iodide peak separation was within the iodide concentration range of 0 to150 nM. Sensitivity, as the slope of the linear range for the response to concentration curve was 9 to 12 μAU/nM, depending on sample matrix. The background within the first 10 minutes of elution (iodide retention is between 4.8 to 6 minutes depending on sample matrix) was very stable at 3 μAU or less. Blanks, 0.1 M NaCl solution with no added iodide, were usually equal to background for fresh waters. For salt waters, however, the background may run as high as 12 μAU, which is equivalent to 0.87 nM or 0.14 ppb. Detection limits (e.g., 3 times relative standard deviation for five samples) for iodide and iodate (as TII) were less than 0.3 nM (0.05 ppb) for fresh water, and lower than 3 nM (0.5 ppb) for seawater samples. The relative standard deviation (RSD) for the inorganic iodide species in all waters was about 4% for a concentration of 3 nM (0.5 ppb) iodide in a 0.1 M NaCl blank solution, and 3% for an iodide concentration of 20 nM (3.3 ppb).

Total iodine values had a detection limit of 3 nM or 0.5 ppb and RSD of 7% for the initial concentration of 3 nM in a 0.1 M NaCl blank solution, and 5% for an iodide concentration of 20 nM (3.3 ppb). On average over all aquatic samples, the detection limit was ~1 nM (0.2 ppb), with less than 3% RSD, when determined using standard additions to an iodide solution of 20 nM in 0.1 M NaCl.

Precision was lower than 10% for all species, but usually was $\leq$ 5% for a clean column.

3. Results and Discussion

3.1 Sample preparation and storage

All containers, polyethelene, fluorcarbonated-polyethylene, or Teflon®, and Teflon® tubing for sample collection were pre-cleaned by ultrasonication at 40°C in a 2% Micro-90® cleaning solution for 1.5 h, triple-rinsed with 18 MΩ water, soaked in 2% Micro-90® solution for 1 week, triple-rinsed with 18 MΩ water, then cleaned with 6 M HCl using the same procedures, and finally, dried in a clean bench. If the cleaned bottles were not used immediately, they were stored in cleaned, sealed plastic bags with a 0.1% HNO_3 solution in the bottle. Prior to collection, the bottles were again triple-rinsed with 18 MΩ water, then triple-rinsed with the sample water down-stream or down-current from the collection

site. Since this study was done in tandem with collection for ^{129}I species, large volume samples were obtained (from 5 to 20 L). The filters used for large volume collection were in-line, hydrophilic polysulfone capsule filters in a polypropylene housing, from Supor or Gellman. The filters were also cleaned by soaking in and pumping through several liters each of Micro-90® solution, 18 MΩ water, 2 M HCl solution, 18 MΩ water, then conditioned with sample water prior to retaining waters for collection.

Samples were either filtered immediately after collection or transported on ice and filtered within a few hours of collection using a 0.45 μm or a 0.1 μm filter. Iodide was run after filtration. The samples were then stored refrigerated or in a dark cool room, after the addition of the $NaHSO_3$ reductant until the remaining species were measured. Some samples were frozen for later analysis. All samples chosen for freezing were filtered with the 0.1 μm filter, stored in containers larger than 250 ml [22], and showed no significant change in iodide or total iodide after three years storage, which is in agreement with other literature [22].

3.2 Sample data

Sample data for iodide, iodate, total inorganic iodine, organic iodine, and total iodine are presented in Tables 3, 4, and 5, respectively, for seawaters, estuarine, and fresh water samples. Chromatograms for the three types of

sample waters are shown as an overlay of standard additions in Figures 5 through 7 for the different iodine species, I^-, TII, and TI. These figures are described with the sample types they represent.

The seawater samples measured were from a vertical profile in a nutrient-poor warm core ring (WCR) in the Gulf of Mexico, Table 3. Since other studies have effectively used total inorganic iodine (TII) as an operational measurement for total iodine (TI), we expected results that show TI values that are slightly higher than 500 nM, with specific iodine (iodine normalized to salinity) values higher than 13 nM. The TI values measured ranged from ~ 522 to 671 nM, with a median value of 575 nM. This was 5.5% higher than the average Arabian Sea value of 545 nM [30] and 12.3% higher than the highest value reported for the North Atlantic [20]. The specific iodine values for the WCR samples were 16.1 nM for the samples from 0 to 121 m depth, and 15.7 nM for the samples deeper than 121 m. Our TI values for both surface and deep water samples have been verified independently by ICP-MS, a method that completely combusts organic iodine species. In support of our contention that other studies have reported TI values equivalent to our TII values, we compare their specific iodine values (of TI), normalized to salinity, to our specific TII values. Our TII concentrations, listed in Table 3, ranged from 480 to 547 nM with a median value of 497 nM, which correspond well to TII literature values, listed in Table 1. The specific iodine values for TII normalized to salinity were 13.4 nM for a depth range of 0 to 121 m and 14.5 nM below 121 m. These specific iodine values are in excellent

agreement with 12.0 to 13.4 for 0-100 m, 13.0 to 14.4 for 100-500 m, and 14.4 to 14.8 for 500-800 m water samples [30, and references therein].

To our knowledge, this is the first presentation of a vertical profile of all three species, iodate, iodide, and dissolved organic iodine in the ocean. Note that while Tian and Nicolas [23] pioneered the measurement of vertical profile for organic iodine in seawater using NaClO oxidation, their method does not, as we show here, recover the more resistant organic iodine compounds, e.g., L-thyroxine [19, 20].

Figure 5 is an example of TII in the Gulf of Mexico WCR sample collected at 1787 m. This sample was run for 12 min., using an isocratic mobile phase as described for the 0.1 min. portion of the gradient mobile phase in Table 2. Without the regeneration and equilibration stages for the gradient mobile phase in Table 2, blanks show a "memory" effect from sample to sample. Therefore, the sample TII concentration has been corrected in a ramp fashion for the blanks "a" which has a peak height value of 70 μAU and "b", peak height value of 107 μAU, Figure 5. The chromatograms for the sample in Figure 5 are the diluted sample (DF = 7.25 in a 0.1M NaCl solution) "c", and the I^- standard additions to the diluted sample of 35.6 nM for "d" and 142.6 nM for "e". The uncorrected peak heights in μAU are 1297 (n = 2) for "c", 1874 for "d", and 3633 for "e". Since blank "b" was run before the sample and "a" after the sample, the blank-corrections for "c" through "e" are 107, 88.5, and 70 μAU, respectively, yielding blank corrected peak height values of 1190, 1785.5, and 3563 μAU. The linear regression line for the plot of peak height versus concentration is y = 16.6x +

1191 (R^2 = 1) and the calculated concentration for “c” is then the intercept divided by the slope or 71.6 nM. The TII for this deep sea water sample is the DF multiplied by the “c” concentration or 519.3 nM, which is < 1% deviation from the average TII value of 519.7 nM listed in Table 3.

The results from our estuarine water samples, presented in Table 4, appear to be very similar to values for other coastal waters [20, 40]. Iodide concentrations are slightly higher or similar to iodate values. Organic iodine concentrations ranged from about 7% to 64% of the total iodine, with an average value of 37% and a median of 42%.

Interestingly, TI in the estuarine samples, excluding the Trinity River sample, is inversely related to salinity (TI = -13.7 Salinity + 111, R^2 = 0.8). Since the Trinity River value was different, this implies that the Bay and riverine TI did not have the same source during the autumn season. Also, the specific iodine value is similar to values for the open ocean. Iodate is proportional to salinity, but with a low specific iodate value, IO_3^- = 5.5 Salinity – 4, R^2 = 0.7, which suggests microbial reduction of iodate to iodide during the oxidation of organic matter.

Figure 6 is the measurement of TI in the Galveston Bay sample with a salinity of 18, Table 4. Chromatogram “a” is the blank. The sample has been diluted 12.2 times and is shown in the “b” chromatogram with no I^- addition. The “c” chromatogram is the sample with an addition of 20 nM of I^- in a 0.1M NaCl solution, and the additions to the “d” and “e” injections are 40 nM and 160 nM, respectively. Peak heights for “a” through “e” are 3 (n=2), 158 (n=2), 312, 813,

and 1583 μAU, respectively. Again, for the graph of peak height as a function of concentration, the equation for the linear regression is y = 8.7x + 239.6 (R^2 = 0.94). This yields an initial sample TI concentration of 27.6 nM, which results in an actual sample TI of 337.9 nM after multiplying by the dilution factor, DF, 12.2. The TI value of 337.9 nM is + 1.9% of the average value for this sample, 331.5 nM, shown in Table 4. No correction was made for the blank value.

The fresh surface and ground water results given in Table 5 are generally within the range of the literature values presented in Table 1 [7, 8, 9, 26, 29].

Figure 7 shows an overlay of chromatograms that depicts the standard additions method for determining I^- concentration in a surface water sample from the Trinity River near its mouth at the Galveston Bay, Table 5. Standard additions were made to a 0.1M NaCl solution, wherein the DF is 10. Chromatogram “a” is the blank and “b” is the sample with no I^- addition. The standard additions of I^- are 3.6 nM (0.6 ppb) for “c”, 17.8 nM for “d”, 35.6 nM for “e”, and 142.6 nM for “f”. The chromatogram peak heights for “b” through “f” are 183 (average of 2 runs), 191, 351, 507, and 1551 μAU, respectively. The linear regression for the plot of the peak height versus the concentration is y = 9.7x + 170.5 (R^2 = 0.9996). From this plot, the I^- concentration in the diluted sample is 17.6 nM (or the intercept divided by the slope). Multiplying this value by the DF, the original sample I^- concentration is 176 nM. The peak height value for the blank is 3 μAU, so no blank correction is needed.

The TI value of 49 nM for the rainwater sample from Galveston, Texas, was higher than the previously published value of 12 nM from the same location.

Seasonal variations depending on local weather conditions can contribute to greater concentrations of iodine due to the close proximity of the sampling location to the ocean, the source region for atmospheric iodine. The surface water value from Gorman Springs of 119 nM and the ground water value from CO_2 Alley, Gorman Cave, of 74 nM were within the range for surface waters of the USA [8, 26]. Major variations in TI concentrations are mostly due to evapotranspiration effects [9], and also due to release from source rocks, variable Eh/pH conditions, sorption to soil minerals, complexation with organic matter, or imbalance of natural salts due to poor irrigation practices in arid climates [26, 28]. The Colorado Bend State Park area, from which these waters are from, is in an arid region. Therefore, TI values higher than the median are expected due to evapotranspiration effects [9]. The Gorman Springs sample collection site has algae, aquatic plants, and swimming organisms. It is reasonable then that the water in the springs shows more DOI than the CO_2 Alley cave water that has been filtered through alluvial sands and karstic gravels during its percolation from the surface to the cave. With the exception of the rain sample, all fresh water samples also showed relatively high iodide to iodate concentration ratios, likely due to biological activities and biogeochemical processes in soils.

Conclusions

We describe here a new and sensitive method for the detection of iodine species at nanomolar concentrations in different aquatic systems, and to quantitatively measure organic iodine. The method has a useful linear range from 1 to 150 nM that is applicable to fresh, estuarine, and seawater samples. The detection limit for iodide, taken as 3 times standard deviation, was found to be less than 1 nM (0.2 ppb), with a relative standard deviation of 3% for all waters, using standard additions to an iodide solution of 20 nM in 0.1 M NaCl.

Recovery for total inorganic and organic iodine species was validated through the use of the certified reference material SRM 1549 powdered milk, the methods of standard additions and reference standards, and by comparison to results from total iodine determinations by ICP-MS.

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Fig. 1. Peak height for the same concentration of iodide, as a function of NaCl concentration.

Fig. 2. Chromatograms depicting a change in % B NaCl mobile phase component. A slower elution and better I^- peak resolution evolves as the % B is decreased from chromatogram “a” to chromatogram “c”.

Fig. 3. Analytical sample processing scheme for the determination of iodine species.

Fig. 4. HPLC chromatograms showing standard additions to a 200 nM TI solution of certified reference material, powdered milk, NIST SRM 1549. The letters “a” through “d” represent the overlain chromatograms of progressively increasing concentrations. The effective concentrations of the standard additions were a) no addition (sample concentration plus blank iodide solution), b) 17.8 nM, c) 71.3 nM, and d) 142.6 nM. Calculations are presented in the text.

Fig. 5. Sea water sample from 1787 m depth from a WCR in the Gulf of Mexico that displays TII analysis using an isocratic mobile phase. The chromatogram curves “a” and “b” represent blanks run before and after the sample which are much higher than blanks using the recommended gradient, Table 2. Chromatogram “c” is the diluted sample (DF = 7.25 in a 0.1M NaCl solution) “c”,

and the I^- standard additions to the diluted sample of 35.6 nM for “d” and 142.6 nM for “e”.

Fig. 6. This chromatogram series depicts the measurement of TI in the Galveston Bay sample with a salinity of 18, Table 4. Chromatogram “a” is the blank. The sample has been diluted 12.2 times and is shown in the “b” chromatogram with no I^- addition. The “c” chromatogram is the sample with an addition of 20 nM of I^- in a 0.1M NaCl solution, and the additions to the “d” and “e” injections are 40 nM and 160 nM, respectively.

Fig. 7. Surface water sample from the Trinity River near its mouth to Galveston Bay, Table 5. This overlay of chromatograms depicts the standard additions method for determining I^- concentration. Standard additions were made to a 0.1M NaCl solution, wherein the dilution factor was 10. Chromatogram “a” is the blank and “b” is the sample with no I^- addition. The standard additions of I^- are 3.6 nM (0.6 ppb) for “c”, 17.8 nM for “d”, 35.6 nM for “e”, and 142.6 nM for “f”.

Table 1
Range of total iodine values in geological matrices.

Sample type	Range of Iodine (nM) Values	Median of Iodine (nM) Values	References
Texas soils	840 – 5600	1911	[7, 8]
Mississippi River Delta sediments	10000 –270000	85000	[27]
Texas rains	0.6 – 12	2	[7, 8,28]
Mississippi River	2.2 – 16.9	5.7	[9, 26-28]
United States Rivers	0.5 – 212	10.7	[8, 9, 26-29]
United States South Coast Rivers	5.5 – 212	16.6	[26]
Surface seawater, Gulf of Mexico	–	500	[8, 29]
Seawater, North Atlantic	354 – 512	440	[20]
Seawater, Arabian Sea	545 – 945[a]	545	[30]
Seawater, specific iodine (total iodine[b] normalized to salinity) (nM/$^{o}/_{oo}$)	12.7 – 14.9	~13	[19, 24, 30, 31, this work]

[a]Values (> 545 nM) in excess of specific iodine in this study are attributed to lateral transport of iodide diffusing out from margin sediments [30]. [b]Total iodine refers to total inorganic iodine in most previous studies; see discussion in text.

Table 2
Mobile phase gradient elution profile.

Time (min)	Flow rate (mL/min)	Mobile phase solution % A	% B	% C	% D	Comment
0.1	1	76.5	8	10	5.5	Analysis
10	1	68	15	10	7	
11	1	58	15	20	7	Regeneration
13	1	76.5	8	10	5.5	
25	1	76.5	8	10	5.5	Equilibration

Mobile phase solution A is distilled water, B is 0.2 M NaCl,
C is 10 mM NaOH, and D is 75 mM $NaClO_4$
in 12.5 mM phosphate buffer of pH 6.1.

Table 3
Iodine speciation in the vertical profile of a warm core ring, Gulf of Mexico, 26° 0.04' N, 95° 20' W.
Collection was July 9, 2000, aboard the R/V Gyre.

Depth (m)	Salinity	DOC [a] (μM-C)	Chl a [b] (μg L^{-1})	[TI][c] (nM)	[TII][d] (nM)	[I^-] (nM)	[IO_3^-] (nM)	[DOI][e] (nM)	% [DOI]
1	36.7	90	0.033	526.2 [f]	491.0	227.8	263.2	35.2	7.2
10	36.7	64	0.046	670.8	496.5	230.0	266.7	174.1	35.1
30	36.7	73	0.024	597.5	481.3	228.4	252.9	116.2	24.1
50	36.5	72	0.056	557.9	480.3	207.6	272.6	77.6	16.2
80	36.5	72	0.119	640.3	489.1	204.8	284.3	151.2	30.9
121	36.5	67	0.192	553.6	495.5	182.4	313.1	58.1	11.7
150	36.4	59	0.089	521.6	496.7	169.1	327.6	24.9	5.0
200	36.4	67	0.014	550.6	508.3	24.3	484.0	42.3	8.3
500	36.8	53	n.d.	588.1	519.3	18.2	501.1	68.8	13.2
1001	34.9	53	n.d.	587.2	523.5	6.7	516.8	63.7	12.2
1501	35.0	47	n.d.	595.1	547.1	28.6	518.5	48	8.8
1787	35.0	43	n.d.	562.6 [f]	519.7	3.4	516.3	42.9	8.3

[a] Data provided by [41]; [b] Data provided by J.L. Pinckney and S.E. Lumsden, n.d. represents concentrations not detectible; [c] [TI]: concentration of total iodine; [d] [TII]: concentration of total inorganic iodine; [e] [DOI]: concentration of dissolved organic iodine; [f] [TI]: independently analyzed by ICP-MS and HPLC.

Table 4
Iodine speciation in estuarine surface waters from Galveston Bay.

Location	Distance from mouth of Bay (km)	Date	Salinity	Chl a [a] (μg/L)	[TI][b] (nM)	[TII][c] (nM)	[I^-] (nM)	[IO_3^-] (nM)	[DOI][d] (nM)	% [DOI]
29.7°N, 94.7°W	45 (near Trinity River mouth)	Sep. 1999	16.5	< 5	300.5	280.6	213.5	67.1	19.9	6.6
29.6°N, 94.8°W	40	Nov. 1999	9	< 5	241.0	127.0	103.6	23.4	114.0	47.3
29.5°N, 94.9°W	22 (mid-Bay)	Sep. 1999	21	10 – 17	438.0 [e]	219.0	112.0	107.0	219.0	50.0
29.5°N, 94.9°W	22 (mid-Bay)	Oct. 1999	12	7	295.4	106.7	54.1	52.6	188.7	63.9
29.5°N, 94.9°W	22 (mid-Bay)	Nov. 1999	18	< 5	331.5	271.4	135.1	136.3	60.1	18.1

[a] Reference [42] and E. Ornolfsdottir, J. Pinkney, S. Lumsden, unpublished data; [b] [TI]: concentration of total iodine; [c] [TII]: concentration of total inorganic iodine; [d] [DOI]: concentration of dissolved organic iodine; [e] [TI]: concentration independently analyzed by ICP-MS and HPLC.

Table 5
Iodine speciation in surface and ground waters from central and southeastern Texas. Gorman Springs and CO_2 Alley (Gorman Cave) are in the Colorado Bend State Park. The Trinity River is the major inflow into Galveston Bay.

Station	Location	Date	DOC (μM)	[TI][b] (nM)	[TII][c] (nM)	[I^-] (nM)	[IO_3^-] (nM)	[DOI][d] (nM)	% [DOI]
Galveston rain	29.3°N, 94.8°W	Aug. 2001		48.9	24.3	4.9	19.4	24.6	48.0
Gorman springs	30.9°N, 99.8°W	Jan. 2000		118.6	67.3	62.8	4.5	51.3	40.0
CO_2 Alley	30.9°N, 99.8°W	Jan. 2000		74.0	64.4	40.4	24.0	9.6	13.0
Trinity River	29.8°N, 94.7°W	Sep. 2000	457[a]	294.7 [e]	187.3	176.1	11.2	107.4	36.4

[a] DOC value from K. W. Warnken, unpublished data. [b] [TI]: concentration of total iodine; [c] [TII]: concentration of total inorganic iodine; [d] [DOI]: concentration of dissolved organic iodine; [e] [TI]: independently analyzed by ICP-MS and HPLC.

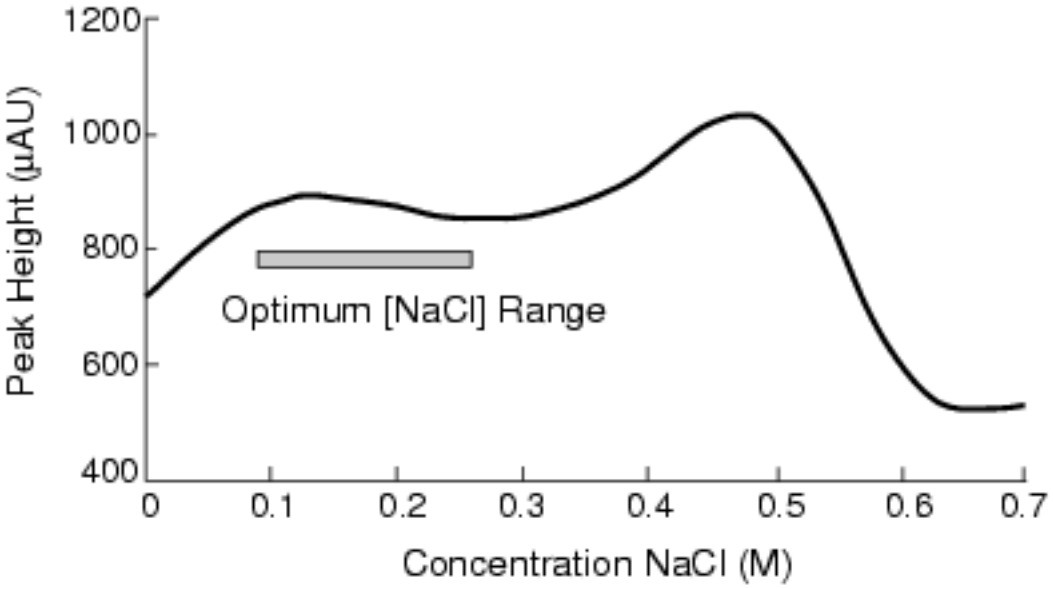
1200
1000
800
600
400
Peak Height (µAU)
Optimum [NaCl] Range
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
Concentration NaCl (M)

Fig. 1

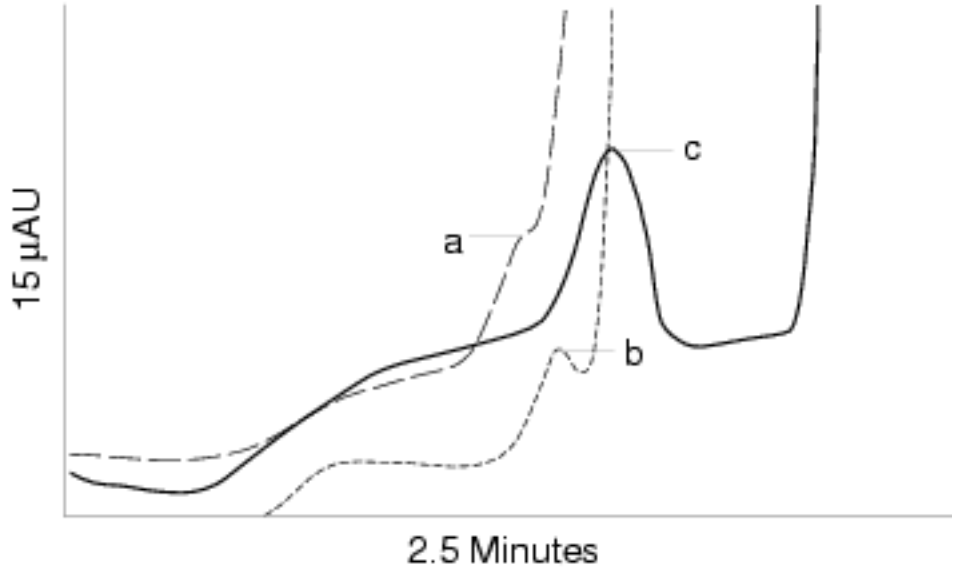

Fig. 2

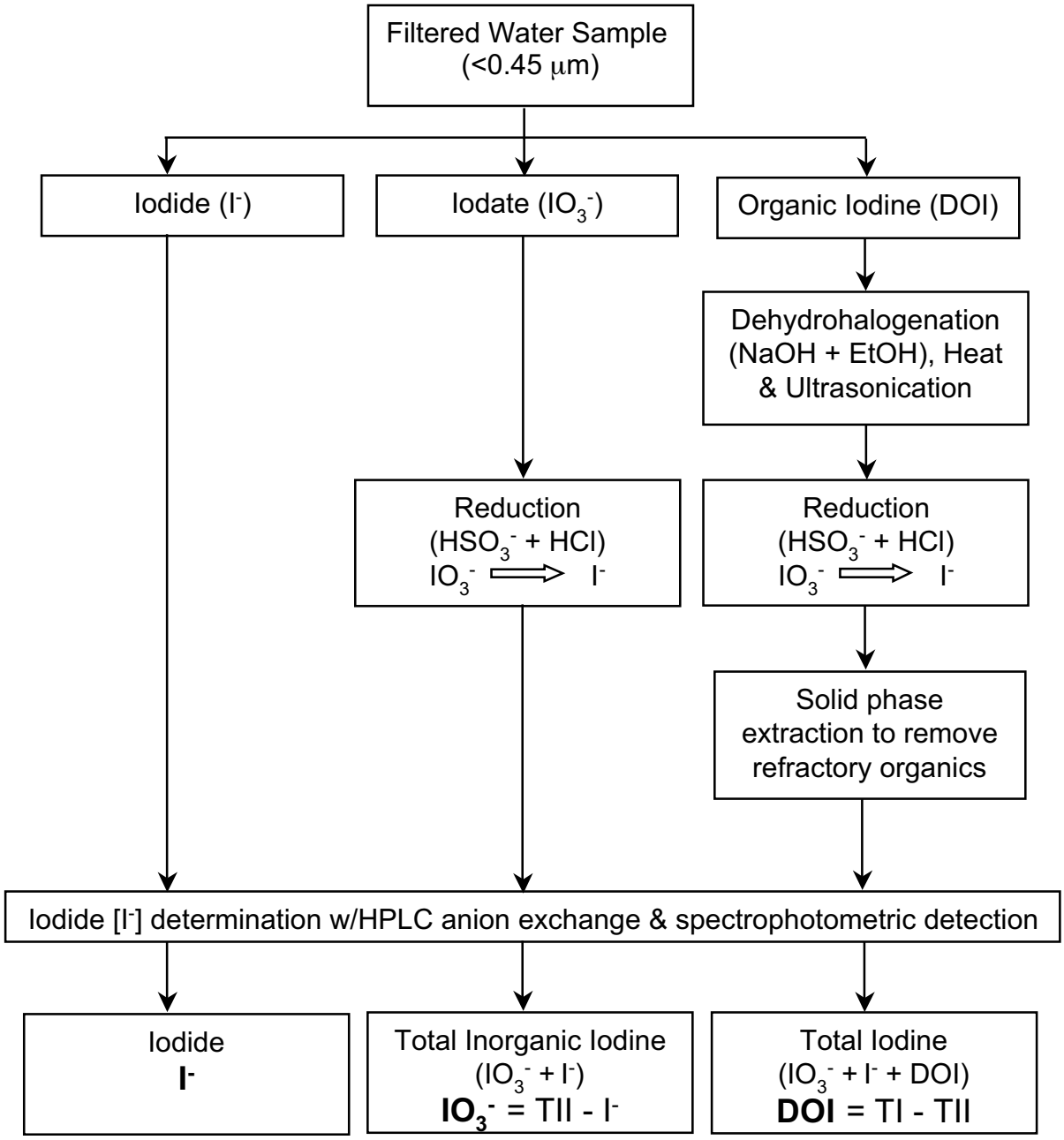
Filtered Water Sample
(<0.45 μm)
Iodide (I^-)
Iodate (IO_3^-)
Organic Iodine (DOI)
Dehydrohalogenation
(NaOH + EtOH), Heat
& Ultrasonication
Reduction
(HSO_3^- + HCl)
IO_3^- ⇒ I^-
Reduction
(HSO_3^- + HCl)
IO_3^- ⇒ I^-
Solid phase
extraction to remove
refractory organics
Iodide [I^-] determination w/HPLC anion exchange & spectrophotometric detection
Iodide
I^-
Total Inorganic Iodine
(IO_3^- + I^-)
IO_3^- = TII - I^-
Total Iodine
(IO_3^- + I^- + DOI)
DOI = TI - TII

Fig. 3

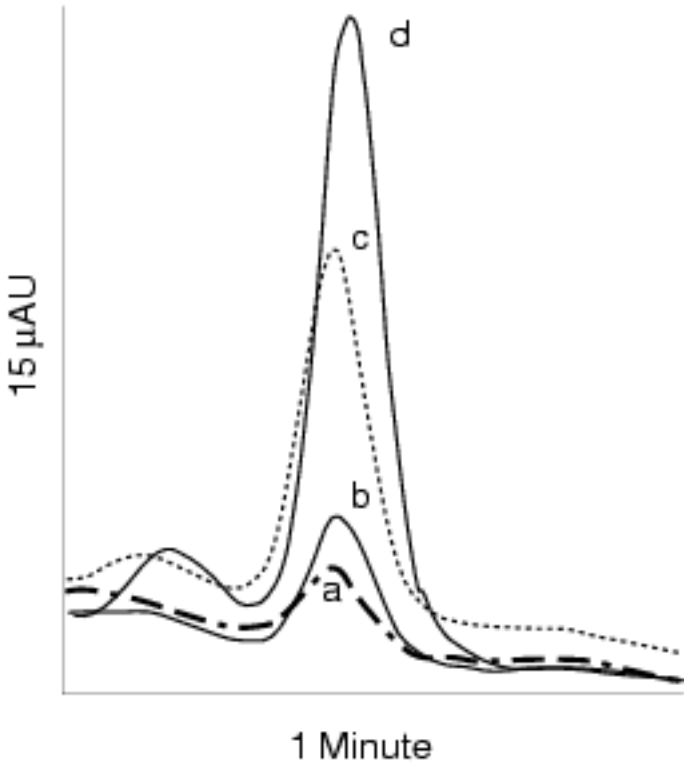

Fig. 4

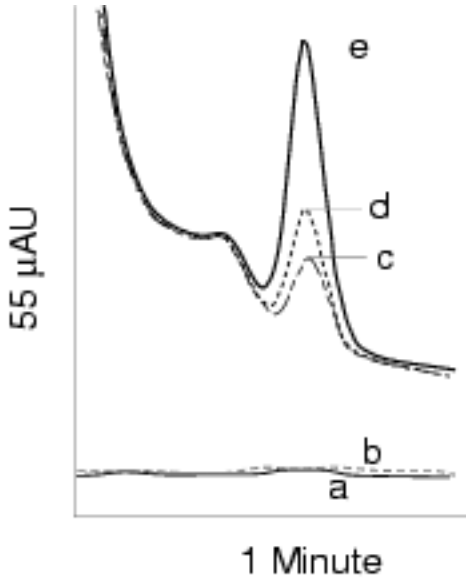

Fig. 5

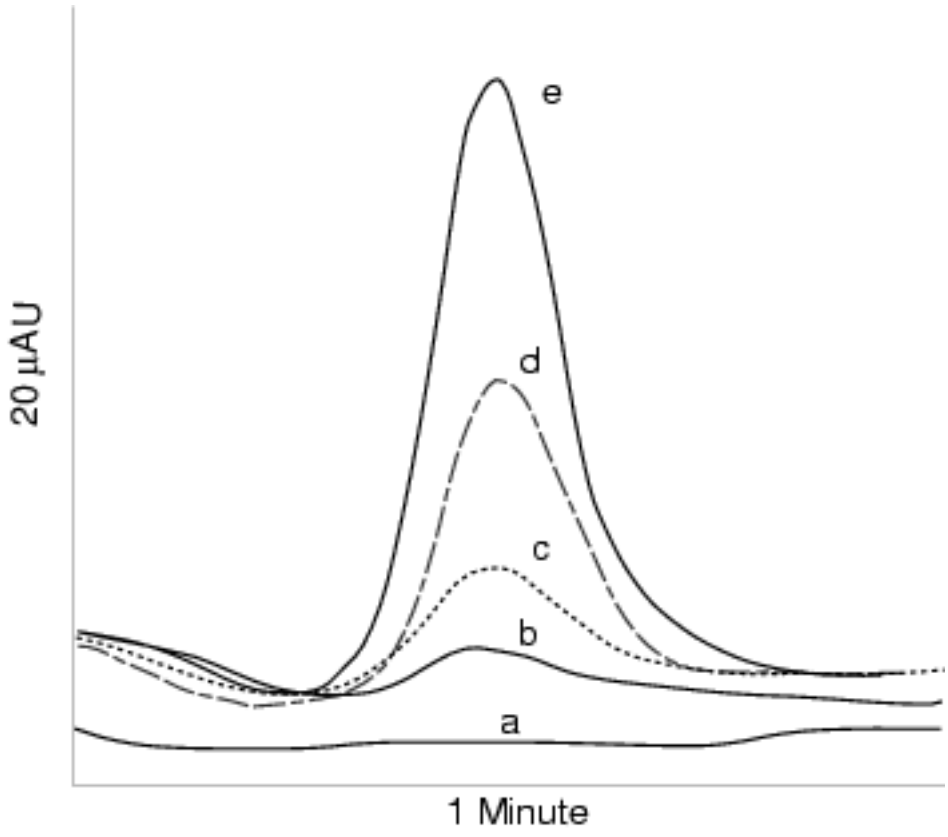

Fig. 6

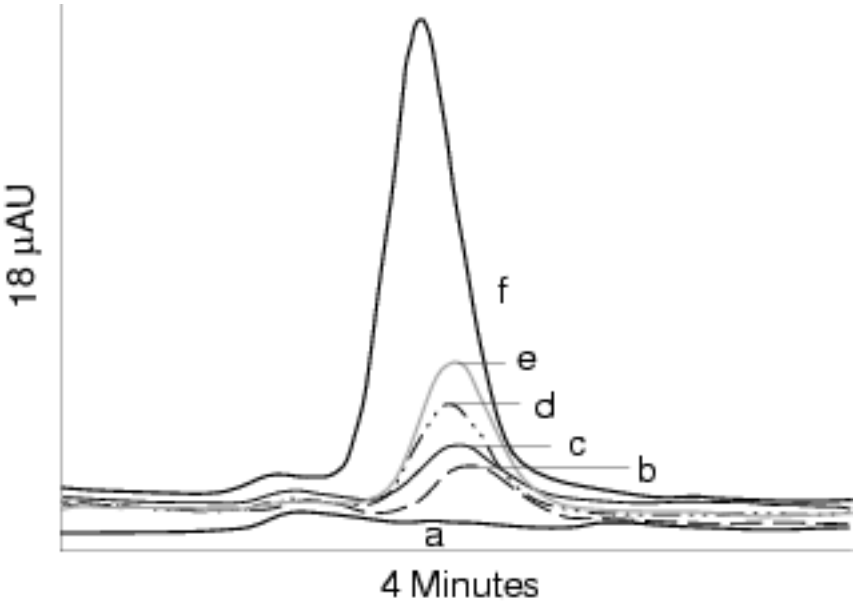

Fig. 7