# Ion Chromatography Detection of Fluoride in Calcium Carbonate

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## Abstract

**Fluoride in aquatic systems is increasing due to anthropogenic pollution, but little is known about how this fluoride affects organisms that live in and around aquatic habitats. Fluoride can bioaccumulate in structures comprised of calcium carbonate, such as shells and skeletons of both freshwater and saltwater species as diverse as snails, corals, and coccolithophorid algae. In this article, ion chromatography (IC) techniques are developed to detect and quantify fluoride in a matrix of calcium carbonate. Solid samples are dissolved in hydrochloric acid, pretreated to remove the majority of the chloride ions, and then analyzed using IC. With these methods, the 3σ limit of detection is 0.2 mg of fluoride/kg of calcium carbonate.**

## Introduction

Fluoride is present throughout the environment, yet the effect of fluoride on animals and organisms is poorly understood (1). The concentration of fluoride in surface, ground and seawater can vary significantly, depending on the local geology as well as anthropogenic sources of fluoride. In this paper, techniques used to determine fluoride concentrations in a calcium carbonate matrix, which can be used for studying fluoride incorporation into the shells and skeletons of both freshwater and saltwater species as diverse as snails, corals, and coccolithophorid algae, are described.

Fluoride in natural waters is due to the weathering of minerals such as fluorite ($CaF_2$) and fluorapatite [$Ca_5(PO_4)_3F$], thus the concentration of fluoride will depend on the geology and the sources of the watershed (2,3). In general, the concentration of fluoride in unpolluted surface water has been found to be between 0.01–0.3 mg/L (1), and in the United States, well water has concentrations that range from 0.02–1.5 mg/L (4). In contrast, seawater has a much higher concentration of fluoride: 1.2–1.5 mg/L (1). In regions where there is a high level of geothermal or volcanic activity, water can have even higher concentrations of fluoride (5–7). Hot springs and geysers in Yellowstone National Park contain 25–50 mg/L of fluoride (7), and a study of lakes in Kenya found concentrations of up to 57 mg/L, coinciding with regions with volcanic rock (5).

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Increased fluoride concentrations can also be due to anthropogenic sources. Aluminum smelters release fluoride as a byproduct, leading to an increase in fluoride in nearby aquatic systems (8–13). Phosphate fertilizers contain fluoride (14), and effluent from fertilizer plants (15) and runoff from agricultural areas (16) can contain elevated levels of fluoride. Since 1945, fluoride has been added to public drinking water systems to help prevent dental caries (cavities). The Center for Disease Control recommends a concentration range from 0.7–1.2 mg/L, with a maximum allowable limit of 4 mg/L (17). By 2006, 69.2% of the United States population using community systems received water that was fluoridated to the optimal level (18). Even though tap water is fluoridated to prevent tooth decay, the majority of tap water is not ingested or used for cooking; over 80% of indoor water usage is for other uses, such as clothes washing, bathing, flushing toilets, and leaks (19). Water used for these purposes will end up at sewage and wastewater treatment centers, where the water is treated to remove physical, chemical, and biological contaminants before the water is released into a local body of water. Additional fluoride is added to these waste streams from fluoride in human waste, originating from fluoride in food (20). Masuda et al. (20) found that during secondary treatment of wastes, biological organism digestion will remove, on average, 57% of the fluoride. The rest is not removed in further treatment steps, and will be introduced into the environment. Camargo et al. (21) measured the fluoride concentration before and after a wastewater treatment plant in Colorado, and found that at 1.6 km downstream from the plant, the fluoride concentration in the river (0.84 mg/L) had almost tripled from the concentration measured upstream from the plant (0.31 mg/L). In addition to indoor water usage, in the United States, 7 billion gallons of water per day are used for home landscape irrigation (22), which will eventually make its way into the local groundwater.

Due to the anthropogenic sources, fluoride concentrations in natural water habitats is increasing, and it is important to understand how these rising levels will affect the animals and other organisms that live in and around these waters. The World Health Organization reviewed studies on the effect of fluoride on plants, animals, and other organisms (1). The majority of these studies have focused on the effects to mammals, and especially humans. Fewer studies have examined the effects of fluoride on aquatic species (23). In fish, fluoride tends to accumulate primarily in the bone tissue (7,24–26), and cartilage (25). In marine crustaceans such as crab, shrimp, and krill, the fluoride accu-

mulates in the exoskeleton (11,27,28). Zhang et al. proposed that fluoride combines with calcium and phosphorus to give fluorapatite [$Ca_5(PO_4)_3F$], which acts as a hardener in the exoskeleton (29,30). A study of Antarctic krill (*Euphausia superba*), found the mouthparts contained the highest amounts of fluoride, which supports Zhang's hypothesis that fluoride acts as a hardener (27). There has been limited research on marine mollusks, mainly studies on growth rate and mortality as a function of fluoride concentration in mussels (8,11,15) and oysters (31). Wright and Davison found that in the blue mussel (*Mytilus edulis*), fluoride concentration increased in the gills, mantle, shell, gut, foot, and adductor muscle (11). Terrestrial mollusks, such as slugs and snails, living in an area of high fluoride content have shown increased levels of fluoride (10). In garden snails (*Helix aspersa maxima*), fluoride accumulated in the foot, hepatopancreas, and shell, but the distribution depended on the concentration of the fluoride dose and the time of exposure. For short exposure times (40 days) at high (133–1330 mg/kg of food) exposures, the majority of the fluoride bioaccumulated in the shell, where smaller fluoride doses (1.5–150 mg/kg of food) over 109 days resulted in the highest concentrations of fluoride being found in the foot (32).

### Fluoride analysis techniques

To further understand how fluoride is incorporated into the shells and skeletons of marine organisms, it is necessary to be able to quantify fluoride in a matrix of calcium carbonate. To analyze samples using ion chromatography (IC), the calcium carbonate must be dissolved in a strong acid, such as hydrochloric acid. The carbonate is converted to carbonic acid, and then to carbon dioxide gas. As a result, the carbonate ion concentration is only slightly higher than the amount normally found in aqueous solutions, due to dissolved carbon dioxide from the atmosphere. The resulting solution will contain calcium ions, carbonate, chloride (from the acid), and any other ions that were incorporated into the calcium carbonate structure.

Fluoride Ion Selective Electrodes (33) have been used to measure a wide variety of matrices (5,8–13,15,24–28,31,32); however, they are not ideal for studying fluoride in a calcium carbonate matrix. After dissolution of the shell with a strong acid, the solution would have a pH ~1. Determination of fluoride at this pH using an electrode will be inaccurate, because most of the fluoride is in the form of HF, $HF_2^-$, and $[HF]_n$ and will not be detected by the electrode, which only measures fluoride, $F^-$ (34). This problem can be overcome by adding a total ionic strength adjustment buffer, which serves to buffer the solution at an optimal pH, releasing fluoride from complexes with cations such as iron(III) and aluminum, and it ensures that all measurements are done at the same ionic strength (35–37). Unfortunately, this technique does not allow for the identification of other ions present in the matrix. One crucial question is whether fluoride is being incorporated into the shells of mollusks as fluorapatite [$Ca_5(PO_4)_3F$], which could act as a hardener, similar to what was seen in the exoskeleton of invertebrates (27,29,30), or as $CaF_2$.

IC offers several advantages for measuring fluoride in a calcium carbonate matrix. After dissolution of the shell in strong acid, the pH of the sample is ~1, and most fluoride would be is in the form of HF, $HF_2^-$, and $[HF]_n$ (34). For IC systems employing potassium hydroxide eluent, low pH samples can be analyzed without the addition of a total ionic strength buffer, because once the sample has been introduced into the basic eluent, all fluoride species will be converted into $F^-$. Therefore, regardless of what form the fluoride was in the original samples, all the fluoride will be detected using IC. IC has been used to detect fluoride in environmental samples, and is both sensitive and selective for detecting fluoride (6,36). The main advantage of IC is the ability to detect other ions, such as phosphate, simultaneously with fluoride, which can be used to determine if fluoride is being incorporated into the calcium carbonate structure as $CaF_2$, or as $Ca_5(PO_4)_3F$.

This paper will discuss IC techniques for analyzing fluoride in a calcium carbonate matrix. The challenges of detecting fluoride, expected to be present at low concentrations (mg/kg of shell) will be addressed, as well as the need to pretreat the samples to remove chloride ions, present due to dissolving the calcium carbonate in hydrochloric acid. IC is a sensitive technique that allows the detection of fluoride in a matrix of calcium carbonate, and offers the advantage that other ions can also be detected.

## Methods

All solution were prepared using 18.2 MΩ cm Nanopure (Barnstead) water. A 1.5 M hydrochloric acid solution was prepared from concentrated hydrochloric acid (Certified ACS Plus, Fisher Scientific). Fluoride stock solutions (1 mM) were made from 0.1 M sodium fluoride (Spex CertiPrep), and used within 2 days of preparation. Samples were prepared in 25-mL volumetric flasks, using 0.25 g of powdered calcium carbonate (Fisher Scientific), and spiked with the fluoride stock solution. Using a glass pipette, 5.00 mL of 1.5 M hydrochloric acid was added, and after dissolution of the calcium carbonate, the solution was diluted to 25.00 mL, yielding final fluoride concentrations between 0–50 µM.

All experiments utilized a Dionex IC-2000 IC system, equipped with a CR-ATC trap column, an ASRS 300 suppressor, and a DS6 heated conductivity cell. The system generated potassium hydroxide electrolytically, with concentrations ranging from 0–66 mM. The eluent was at a constant flow rate of 1.5 mL/min$^{-1}$. The gradient was as follows: 0.45 mM from 0 to 5 min, followed by a ramp from 0.45 mM to 35 mM between 5 to 10 min. After the ions of interest had eluted, the concentration was immediately increased to 66 mM for a column washout, followed by another 5 min at 0.45 mM to re-equilibrate the column. Conductivity data was collected during the first 10 min of the run. Anion separation was accomplished with an AS17 4 mm analytical column coupled with an AG17 guard column.

Samples were transferred using disposable glass pipettes, which were rinsed with Nanopure water at least 6–7 times, or using micropipettes (Eppendorf). Sample filtration to remove particulates was done using 0.2-µm Whatman (GD/X, PES membrane) syringe filters. Prior to use, the filters were rinsed with 60 mL of Nanopure water, and then the first 5 mL of sample was discarded, to insure that the sample was not diluted with water that remained in the syringe filter.

Pretreatment to remove chloride was done with OnGuard II Ag cartridges (Dionex). The cartridges were first hydrated with at least 10 mL of Nanopure water, delivered using a peristaltic pump at a flow rate that did not exceed 2 mL/min. Unless otherwise noted, the first 3 mL of sample was discarded, following the manufacture's instructions (38). The sample was collected in 1.5-mL disposable glass autosampler vials (Dionex). An AS50 autosampler equipped with a 12-µL loop (unless otherwise noted) was used to introduce the samples into the ion chromatograph.

## Results and Discussion

Figure 1 shows the ion chromatogram of a standard solution run with the method previously described. During the first 5 min of the separation, the potassium hydroxide eluent concentration was held constant at 0.45 min, so there is a very flat baseline during the time that the fluoride elutes. Fluoride [Peak 1, retention time (RT) 2.7 min] was well-separated from chloride (Peak 2, 6.9 min), the other major component expected after dissolution of the calcium carbonate in hydrochloric acid. Bromide (Peak 3, 7.9 min) and carbonate (Peak 4, 8.5 min) were also well-separated. Sulfate (Peak 5, 9.1 min) appears as a peak superimposed on the tail of the carbonate peak, but can easily be used to quantify the amount of this ion. If better resolution is needed, a plateau in the gradient could be added, but this would come at the expense of a longer analysis time. In addition to the 10 min required to separate the ions, there is also a 5 min column washout at a potassium hydroxide concentration of 66 mM (the maximum concentration that can be electrolytically generated at a flow rate of 1.5 min), followed by a 5 min equilibration at a potassium hydroxide concentration at 0.45 min.

For samples containing dissolved calcium carbonate, 25 mL of solution was prepared, to produce a sufficient quantity of sample so that, if necessary, at least two pretreated samples could be prepared with sufficient amounts remaining to analyze the sample without implementing an additional pretreatment procedure. To ensure that all of the calcium carbonate reacts, the amount of hydrochloric acid added is 1.5× the minimum amount needed for dissolution. The upper trace in Figure 2 is a typical chromatogram of a sample of approximately 0.25 g of $CaCO_3$, spiked with 9.5 mg of fluoride, after dissolution in hydrochloric acid. Chloride, which normally has a RT of 7.0 min, appears in this sample as a very broad peak that elutes between 3–8 min. The peak at a RT of 1 min is not fluoride; it is actually chloride that was not retained by the column. Because the column is completely overloaded, fluoride cannot be detected and quantified. There are two ways that column overload could be minimized: decrease the injection volume, or prepare samples with a smaller amount of calcium carbonate, and therefore less hydrochloric acid will be needed for dissolution of the sample. Neither of these options is viable, as both will lead to a smaller amount of fluoride being analyzed, compromising the ability to detect fluoride in a matrix of calcium carbonate. Instead, a third option, removing the chloride ions, was employed.

OnGuard II Ag cartridges were used to remove the unwanted chloride ions. These cartridges contain a resin embedded with silver, and chloride is removed by the formation of silver chloride, which is insoluble. Figure 2 shows the ion chromatogram of a sample before and after pretreatment. Only a small amount of chloride (RT = 7.0 min) remains after pretreatment, and the peak corresponding to fluoride (RT = 2.9 min) can now be seen. Because carbonate is more strongly bound to the column than chloride, the carbonate peak is not affected by the presence of the chloride, which moves though the column much faster. The carbonate peak (RT = 8.5 min) can clearly be seen in chromatograms obtained before and after pretreatment, and is unchanged by pretreatment to remove chloride.

The silver cartridges have a limited capacity for chloride removal, and this limitation must be balanced by the need to maximize the fluoride signal. Higher concentrations of calcium carbonate yield higher fluoride signals; however, dissolution requires more hydrochloric acid, which causes the silver car-

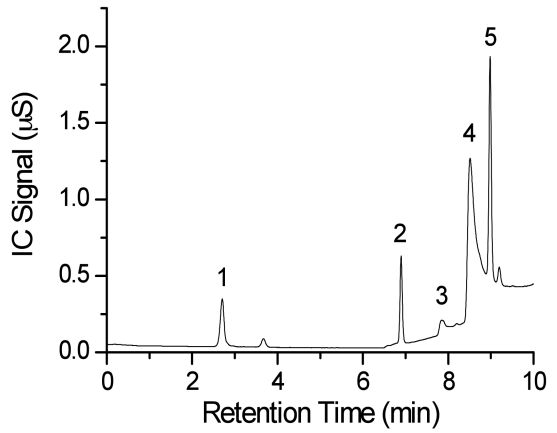


**Figure 1.** Ion chromatogram of standard containing NaF, NaCl, NaBr, $NaHCO_3$, and $Na_2SO_4$ each at concentrations of 40 µM. The peak assignments are: 1, fluoride; 2, chloride; 3, bromide; 4, carbonate; and 5, sulfate.

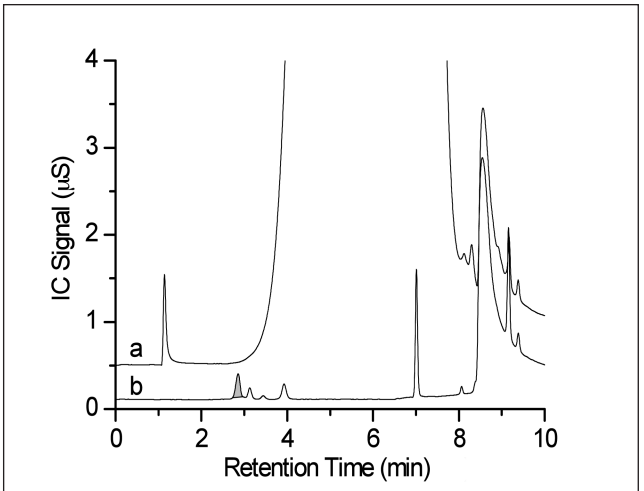


**Figure 2.** Chromatograms (10.0 µL injection) of a sample prepared from 0.25 g of $CaCO_3$, spiked with 9.5 µg of fluoride, and dissolved in hydrochloric acid. (a) Untreated sample and (b) Pretreated to remove chloride. After pretreatment, fluoride (shaded peak) can now be seen. The chromatograms have been offset for clarity.

tridge to reach capacity faster. Once the cartridge has exceeded its capacity to remove chloride, pretreatment will no longer remove chloride, and the sample will once again contain an excess of chloride ions. When using the OnGuard Ag cartridges, the first 3 mL of the sample must be discarded to ensure that the analyte (in this case, fluoride) is not diluted by the water used to hydrate the cartridge (38). Therefore, to ensure that there is 1–2 mL of pretreated sample for IC analysis, the column capacity must not be exceeded until after 5 mL of sample has been pretreated.

To ensure that the choice of 0.25 g of sample was the maximum sample size that could be used to produce a sufficient amount of chloride-free sample for IC analysis, a chloride breakthrough experiment was performed. In this experiment, 0.25 g of $CaCO_3$ spiked with 23.75 mg of fluoride, was dissolved in 5.00 mL of 1.50 M HCl, and then diluted to a final volume of 25 mL. The OnGuard Ag cartridge was hydrated with Nanopure water according to package instructions (38), and then a disposable syringe was used to push the sample through the cartridge at a flow rate that did not exceed 2 mL/min. Fractions (0.5 mL each) were collected directly in glass autosampler vials, and analyzed using IC. Figure 3 shows the cartridge capacity was exceeded and chloride breakthrough occurred after 5 mL of the sample had been collected (note the logarithmic scale). Because the first 3 mL of the sample were discarded, 2 mL of the sample can then be collected before chloride breakthrough occurs. Typically, only 1–1.5 mL of the sample is collected for analysis. This breakthrough experiment shows that 0.25 g of $CaCO_3$ truly is the optimal amount of sample for dissolution with hydrochloric acid. If the sample size was any larger, chloride breakthrough would occur before a sufficient amount of sample could be collected for analysis. If less calcium carbonate was used, then the amount of fluoride in the pretreated sample would be less, and therefore the ability to detect this ion in small concentrations would be compromised.

Figure 4 shows the ion chromatograms of fluoride samples prepared in Nanopure water, with fluoride concentrations ranging from 0–50 µM of fluoride. The fluoride peak (RT = 2.75 min) gets larger, as the fluoride concentration increases. The other main feature of the chromatograms is a peak due to carbonate (RT = 8.6 min), which is present in all samples exposed to air, due to dissolved carbon dioxide. Calcium carbonate samples were spiked with the same amounts of fluoride as the samples prepared in Nanopure water (Figure 4). The calcium carbonate was then dissolved in hydrochloric acid, and then diluted to a final volume of 25 mL. The samples were then pretreated as previously described, and analyzed using IC. The results are shown in Figure 5. For samples of fluoride prepared by the dissolution of calcium carbonate in hydrochloric acid, the carbonate peak is only ~2× the height of the carbonate peak for fluoride samples prepared in Nanopure water (Figure 4), where carbonate is present due to dissolved atmospheric carbon dioxide.

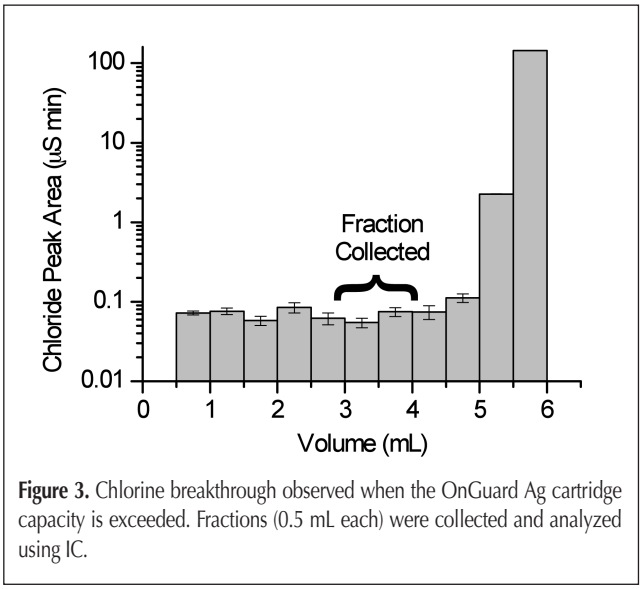


**Figure 3.** Chlorine breakthrough observed when the OnGuard Ag cartridge capacity is exceeded. Fractions (0.5 mL each) were collected and analyzed using IC.

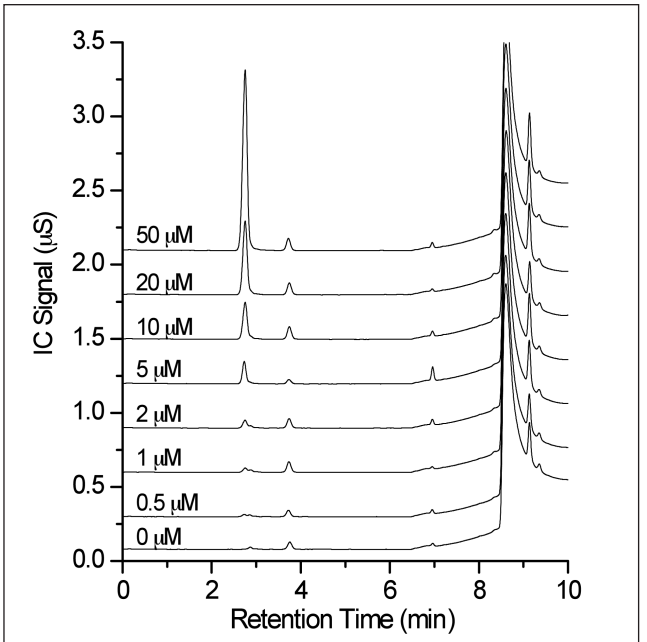


**Figure 4.** Ion chromatograms of fluoride (0–50 µM) solvated in Nanopure water.

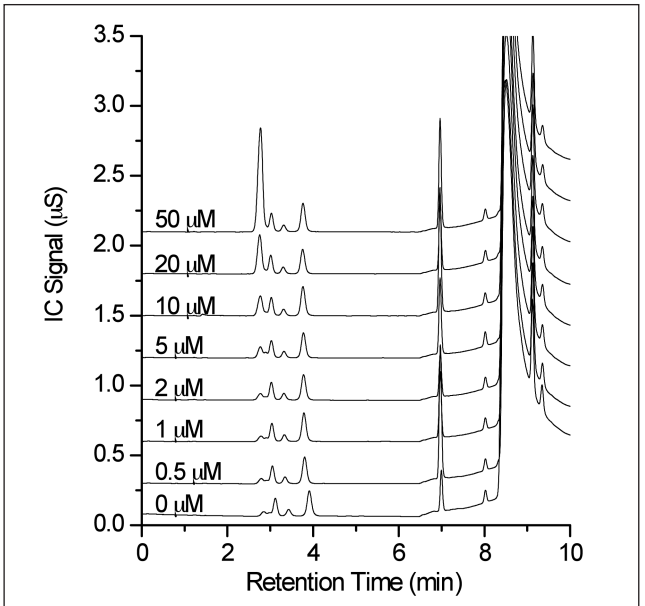


**Figure 5.** Ion chromatograms of fluoride (0–50 µM) in a matrix of calcium carbonate, dissolved in hydrochloric acid, and pretreated to remove chloride.

The chromatograms in Figure 5 show that the peak corresponding to fluoride (RT = 2.85 min) gets larger as the concentration of fluoride increases. There are three other peaks (RT = 3.0, 3.3, 3.8) that do not appear in the chromatograms of fluoride in Nanopure water. These small peaks are not present in samples containing only fluoride and hydrochloric acid and are not due to pretreatment with silver cartridges. These impurities were introduced when the 0.2-µm filters were employed (data not shown). Similar impurities also appeared when glass pipettes were used without rinsing with Nanopure water prior to use. These small peaks are attributed to organic acids, such as lactate, acetate, glycolate, and propionate, which are known to have very similar RTs to fluoride (39). Trace amounts of these acids were believed to remain after the manufacturing of the glass pipettes and the 0.2-µm filters. The contaminants on the glass pipettes were eliminated by rinsing them with Nanopure water before use. Unfortunately, when the 0.2-µm filters are used with acidic solutions, these peaks can only be minimized by rinsing with 60 mL of Nanopure water, and cannot be eliminated entirely. For higher concentrations of fluoride, the fluoride peak (RT = 2.85) was not well-resolved from the small peak with a RT of 3.0 min. Better resolution could be achieved by lowering the hydroxide concentration of the mobile phase from its current concentration of 0.45 mM, which would shift both peaks to higher RTs, but would increase the overall runtime of the analysis. The use of a different column, such as the Dionex AS19 or AS20 could potentially improve the separation of these low retention species (40,41).

The chromatograms in Figures 4 and 5 show that when the peak areas of fluoride are compared to the same concentrations prepared in Nanopure water, the peak areas are smaller in the calcium carbonate samples. Table I shows the calibration data for fluoride in both Nanopure water and in pretreated calcium carbonate samples. From the slopes of the linear calibrations, it can be seen that the fluoride peak has decreased by ~42%. It is necessary to determine whether this decrease is due to fluoride not being detected, or to fluoride that is being removed from the sample. Studies using the fluoride selective electrode have shown that in very acidic solutions, most of the fluoride is in the forms of HF, $HF_2^-$, and $[HF]_n$ (34), thus it cannot be detected because the fluoride selective electrode is only able to detect fluoride when it is $F^-$. However, this is not a concern for IC, because as soon as the sample is introduced into the eluent stream, the sample will equilibrate with the eluent, which is at a pH of 10.65. Therefore, regardless of what form the fluoride exists in the sample, all fluoride will be in the form of $F^-$ when being separated and detected using IC.

Another concern is that fluoride could be removed by precipitation as $CaF_2$ ($K_{sp} = 1.46 \times 10^{-10}$) (42). The concentration of calcium from the dissolution of $CaCO_3$ is high (0.1 M); therefore, in neutral solutions, $CaF_2$ would only precipitate at fluoride concentrations above 38 µM. However, given that the samples in this study samples are very acidic, $CaF_2$ becomes more soluble due to the formation of HF and other fluoride species. Once the solution is injected into the chromatography column, the solution becomes very basic, but because the calcium ions are not retained by the column, there is no possibility that $CaF_2$ will form.

Therefore, the fluoride peak area is smaller due to fluoride being removed during pretreatment of the sample. Silver fluoride is soluble, and when solutions of sodium fluoride in Nanopure water were pretreated using OnGuard Ag cartridges, no fluoride was removed (data not shown). However, when chloride was present in the solution, and precipitated as AgCl, soluble ions were removed by incorporation into the precipitating AgCl (38). The amount of fluoride removed by pretreatment depends on the amount of chloride present. To quantify the amount of fluoride present in samples of unknown concentration, both samples and standards must be prepared using the same amounts of hydrochloric acid. Standard addition, spiking a portion of an unknown sample with a known amount of fluoride

**Table I. Peak Area Calibration Parameters for Fluoride***

| | | Fluoride in Calcium Carbonate† | | |
|---|---|---|---|---|
| | In Nanopure | All Conc. | Low Conc. | High Conc. |
| Range (µM) | 0.0–50.0 | 0.0–50.0 | 0.0–5.0 | 5.0–50.0 |
| Linear regression coefficient, $R^2$ | 0.999 | 0.990 | 0.996 | 0.996 |
| Number of data points | 8 | 8 | 5 | 4 |
| Number of replicates each concentrationv | 3 | 3 | 3 | 3 |
| Slope (× $10^3$) (µS × min/µM) | 2.92 ± 0.04 | 1.86 ± 0.08 | 0.72 ± 0.03 | 1.99 ± 0.09 |
| Intercept (× $10^3$) (µS × min) | –1.3 ± 0.8 | –0.9 ± 1.5 | 1.97 ± 0.06 | –5.5 ± 2.4 |

* 12 µL injection loop.
† Pretreated to remove Cl.

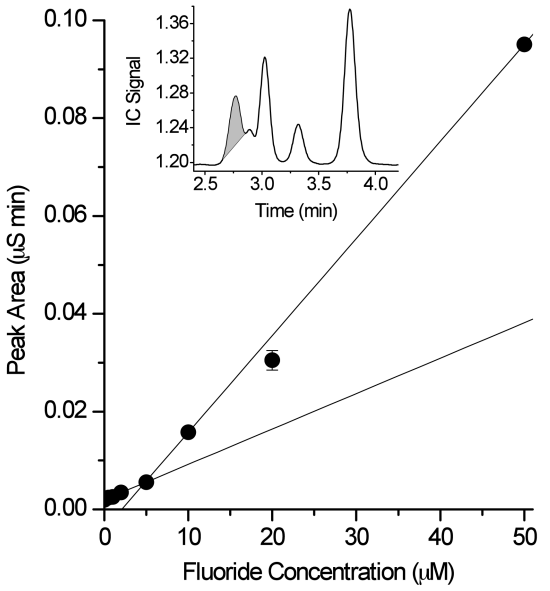


**Figure 6.** Linear calibrations for the fluoride peak area for fluoride (0–50 µM) in a matrix of calcium carbonate, dissolved in hydrochloric acid, and pretreated to remove chloride. The inset shows the region of the ion chromatogram around the RT of fluoride (shaded peak) for a sample with a fluoride concentration of 5 µM. Above this concentration, the fluoride peak engulfs the small peak appearing to the right of fluoride.

prior to dissolution and pretreatment, can also be used to quantify the amount of fluoride in the original sample, as well as confirm the identification of the fluoride peak.

In the ion chromatograms of fluoride in a matrix of calcium carbonate (Figure 5), the fluoride is not baseline resolved from the small peak at a RT of 3.0 min. The peak area of the fluoride peak was determined by baseline integration, where the endpoint of the fluoride ion was set as the valley between the two peaks. Figure 6 shows that when the peak area is plotted as a function of fluoride concentration, there are two regions of linearity, one at low concentrations (1–5 µM) and one at higher concentrations (5–50 µM). The inset of Figure 6 shows a blowup of the fluoride region of the ion chromatogram for the solution that contained 5 µM of fluoride. The inset shows that there is a very small peak halfway between fluoride (shaded peak, RT = 2.85 min) and the peak with the RT of 3.0 min. For fluoride concentrations above 5 µM, this small peak was engulfed by the fluoride peak, which changes not only the area under the peak, but how the baseline is determined in that region as well. Thus, the areas of the peaks for concentrations above 5 µM are artificially high. The change in baseline will also affect how the height of the peak is calculated; calibration curves (not shown) employing height instead of area display similar features as the calibration done with peak area. For samples of unknown fluoride concentration, the appropriate (low concentration or high concentration) calibration curve should be utilized. Conversely, samples with higher fluoride concentrations can be diluted prior to analysis.

To determine the limits of detection and quantification, only the calibration data for low concentrations (0–5 µM) was used. The limit of detection is defined as the concentration that yields a peak area that is three times the signal-to-noise (3σ) of the background signal level, and the limit of quantification is equal to 10σ. In a matrix of calcium carbonate that has been dissolved in hydrochloric acid, the limit of detection is a fluoride concentration of 0.08 µM, and the limit of quantification is 0.3 µM. These concentrations correspond to a limit of detection of 0.2 mg of fluoride/kg of calcium carbonate, and a limit of quantification of 0.5 mg $F^-$/kg $CaCO_3$. These limits compare well with the fluoride selective electrode, which can be used to detect fluoride down to 0.1 µM (33). However, unlike the fluoride selective electrode, IC offers the ability to detect other ions in solution. Despite the analytical challenges of having high ionic concentrations and very acidic solutions after dissolution, IC allows for the quantification of the amount of fluoride in a calcium carbonate matrix.

## Conclusions

It is important to understand the role of fluoride in the environment, especially the fate of fluoride in organisms that live in and around waters that are experiencing an increase of fluoride due to anthropogenic sources. To quantify fluoride in the shells and skeletons of marine organisms, it is necessary to detect fluoride in a matrix of calcium carbonate. This matrix presents several analytical challenges, because when the samples are dissolved in hydrochloric acid, the resulting solution has a calcium ion concentration of 0.1 M, a chloride concentration of 0.3 M, and a pH ~1. To detect fluoride, it was necessary to remove the chloride prior to IC analysis. Even though some fluoride is removed by the pretreatment process, fluoride can still be detected and quantified. IC offers limits of detection and quantification that are very close to those of the fluoride selective electrode, but with the superior advantage of being able to simultaneously detect and quantify other ions of interest. IC is a powerful tool for understanding the fate of fluoride in the environment.

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