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Determination of Fluoride in Antarctic Krill (*Euphausia superba*) Using Ion Chromatography and its Pretreatments Selection

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Abstract

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A rapid, sensitive and reliable method to quantify fluoride in Antarctic krill has been established. Four different pretreatment methods were used for the extraction of fluoride: double-deionised water extraction, sulphuric acid distillation, hydrochloric acid extraction, and pH adjustment with buffer after hydrochloric acid extraction. Four methods of comparative analysis revealed that sulphuric acid distillation was suitable preparation for ion chromatography determination of fluoride in Antarctic krill (fluoride content 288.7 ± 10.2 mg/kg). The method was partially validated in linearity, accuracy, and precision. The linear range was from 0.1 to 10.0 mg/l with the regression coefficient of 0.99998. The accuracy expressed as the recoveries of standard addition ranged from 95.3% to 101.3%, the relative standard deviation (n = 8) was 1.8–1.9%. With this method, the 3σ limit of detection was 0.06 mg/l of fluoride in Antarctic krill. Our results indicate that the method (limit of quantification 0.2 mg/l) could be well applied for the determination of fluoride in Antarctic krill.

Keywords: fluoride in krill; fluoride quantification; extraction methods; preparations

Fluoride is widely present in nature, and it is an essential trace element both for humans and animals with two thresholds. To a certain extent, fluoride plays an important role in the normal construction and growth of organisms. In contrast, too high or too low fluoride content could result in fluorosis or caries (De Sousa *et al.* 2002; Rocha-Amador *et al.* 2007; Lam & Chu 2012).

Antarctic krill (*Euphausia superba*) has been the most abundant animal species on the planet and the largest animal protein source in the world (Wang *et al.* 2011; Parker & Tyedmers 2012). The protein derived from Antarctic krill is a nutritionally excellent protein source, containing all of the essential amino acids (Chen *et al.* 2009). Despite its huge biomass and valuable protein source, Antarctic krill has not been a large-scale protein source in human food for its high fluoride content (Suzuki & Shibata 1990; Sands *et al* 1998; Jung *et al.* 2013). For human food, FDA recommended an upper limit of 100 mg/kg as sodium fluoride (FDA 2012). Our preliminary study using the ion selective electrode (ISE) method indicated that the concentration of fluoride is 267–330 mg/kg in the whole body of Antarctic krill, 405–440 mg/kg in head, 550–700 mg/kg in carapace, and 53–80 mg/kg in muscle. The concentrations are given on the wet basis. Fluoride is present in both free and bound forms in Antarctic krill including fluorapatite, calcium fluoride, and organic fluoride. Different forms of fluorine have different absorption utilisation. Water-soluble fluoride and fluorion are easy to be absorbed through transmembrane transport into the

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blood circulation. Organic fluoride such as fluoroacetic is not easily absorbed. There is no unified view on existing forms of fluoride in the Antarctic krill at home and abroad. The fluoride content can be determined by ISE (Frant & Ross 1966; Čápka *et al.* 2004; Xie *et al.* 2012), spectrophotometry (Khalifa & Hafez 1998), gas chromatography (GC) (Chiba *et al.* 1982; Haldimann & Zimmerli 1993), ion chromatography (IC) (Jeyakumar *et al.* 2008; Koch *et al.* 2010; Lefler & Ivey 2011), and high performance liquid chromatography (HPLC) (Lipka *et al.* 2000; Musijowski *et al.* 2010). IC has been chosen in this paper, as it is a rapid, selective, sensitive and preferred method for the determination of a variety of anions at trace and ultra-trace levels (Pereira 1992; Lopez-Ruiz 2000).

Although studies have reported IC being used to detect fluoride in water (Miyake *et al.* 2007), forage, toothpaste (Potter *et al.* 1986) and vegetation, this is the first report on the application of IC to determination of fluoride in Antarctic krill. The goal of the present study was to develop and validate an analytical method for determination of fluoride in Antarctic krill based on four different sample preparations by IC.

MATERIAL AND METHODS

Reagents and chemicals. Except the standard substance sodium fluoride was a guaranteed reagent (GR), all of the chemical reagents were analytical reagents (AR). Double-deionised water (DDI H_2O, specific conductance < 1 µS/m) was used for the reagent preparation. Sodium fluoride was obtained from Sigma-Aldrich (St. Louis, USA). Sodium acetate, total ion strength adjustment buffer (pH 5.8, TISAB, 0.75 mol/l sodium citrate equivalent mix with 3 mol/l sodium acetate buffer pH 7.0), sodium hydroxide, sulphuric acid, hydrochloric acid, potassium hydroxide (30 mmol/l), and magnesium nitrate were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Ion chromatography (IC). All experiments utilised a DIONEX ICS-3000 IC system (Dionex, Sunnyvale, USA), equipped with a continuously regenerated trap column (CR-TC), an ASRS-Ultra II 4 mm self-regenerating suppressor (SRS) and a conductivity detector (CD). The separation was performed on an anion exchange column IonPac AS14 (4 × 250 mm, particle size 7 µm), analytical column equipped with an IonPac AG14 (4 × 50 mm, particle size 7 µm) and guard column, at a constant column temperature of 30°C. Hydroxyl (OH^-) was chosen as its elution strength is weaker than that of CO_3^{2-}/HCO_3^-, and it exhibits good selectivity for the weak anions (e.g. F^-, Cl^-). In this paper, potassium hydroxide solution was applied as an eluent at 30 mmol/l with a flow of 1.00 ml/minutes. The sample was introduced through a 25 µl loop fitted with a 0.2 µm membrane filter injector. The suppression mode of SRS was recycle, SRS current 75 mA and test sample time 10 minutes. The Dionex Chromeleon Xpress 6.8 chromatography work-station was used for instrument control, data collection and data processing.

Antarctic krill samples. Frozen Antarctic krill was provided by the Dalian Ocean Fishery Group of Corporations (Liaoning, China). They were stored at –20°C until use.

Antarctic krill preparation. Dry ashing with alkali was adopted for isolating ionic, complex, and covalent fluoride in Antarctic krill. Briefly, 5.00 g Antarctic krill was ground and dissolved in 5.0 ml magnesium nitrate (100 g/l) and 0.5 ml sodium hydroxide (100 g/l). The mixture was dried off at 105°C for 4 h in a drying cabinet firstly, then carbonised and ashed at 550°C for 4 h in a muffle furnace.

There were four different pretreatments to the ash, namely method A–D. Method A: 20 ml DDI H_2O was added to the ash for 2 h to extract fluoride, then all ash and water were transferred to a 50-ml volumetric flask. 20 ml DDI H_2O was used to wash a crucible more than once and incorporated into the volumetric flask. Finally, water was added to 50.0 ml. Method B: 10 ml DDI H_2O was added to the ash and then a few drops of sulphuric acid (2 : 1) were slowly added until no bubbles were produced. The liquid was transferred to a 125-ml distillation bottle, then 20 ml DDI H_2O was used to wash a crucible more than once and incorporated into the distillation bottle. Then 60 ml sulphuric acid (2 : 1) was added to the distillation bottle. Distillation until the temperature of 190°C was started. The distillate was absorbed by 5 ml DDI H_2O and 10 drops of sodium hydroxide (100 g/l) solution with one drop of phenolphthalein indicator using a 50-ml beaker. 10 ml HCl (1 : 11) was used to neutralize sodium hydroxide until the red just disappeared. Then the distillate was diluted with water to 50.0 ml. Method C: the ash was extracted with hydrochloric acid (1 : 11) for an hour at room temperature and diluted with H_2O to 50 ml. Method D: the residues were added TISAB (pH 5.8) to adjust pH to 5~6 after HCl (1 : 11) extraction. Prior to IC analyses, each of the sample supernatants was diluted 100 times and filtered through 0.45-μm membrane filter. All stock solutions were stored in high-density polyethylene bottles at 4°C until use.

Standard solution preparation. Fluoride stock solution (1.0 g/l) was made from sodium fluoride (dried for 4 h at 105°C and cooled in a desiccator) and prepared by dissolving 0.2210 g sodium fluoride in DDI water and diluting to 100 ml. The fluoride stock solution was stored in a polyethylene container at 4°C. Standard fluorine solutions were prepared by appropriate dilution of the stock solution to get concentrations of 0.1, 0.5, 1.0, 5.0, and 10.0 mg/l. Standard fluorine solutions should be prepared before analysis that very day and stored in high-density polyethylene bottles at 4°C until use.

Quantification and quality analyses. Identification of F^- was done by comparing the retention time against known standard sodium fluoride solutions. The F^- content was quantified against an external five-point calibration curve.

Data analysis. Data was analysed with the Dionex Chromeleon Xpress 6.8 chromatography work-station. Values were expressed as the mean ± standard deviation. Differences between variables were tested for significance by one-way ANOVA using SPSS 17 (SPSS Inc., Chicago, USA). A difference was considered to be statistically significant when $P < 0.05$.

RESULTS AND DISCUSSION

Determination of fluoride in Antarctic krill by IC. he aim of this study was to establish a valid qualitative and quantitative method for fluoride in Antarctic krill. Many studies have focused on determination of fluoride in drinking water being present in an ionic form (F^-), which could be determined by the fluoride ion selective electrode (ISE) method. While fluoride is present in both free and bound forms in Antarctic krill. In order to analyse fluoride concentration via more stable F^-, pretreatment to dissolve fluoride into the necessary water soluble ionic form is required, which seems to be the critical step of the entire analytical procedure.

Table 1. Retention time and fluoride content in Antarctic krill by ion chromatography[a] (wet basis)

Sample preparations	*n*	Retention time (min)	F^- Concentration (mg/kg)	RSD (%)
A	6	3.027^a	87.6 ± 8.7^a	9.9
B	8	3.023^a	288.7 ± 10.2^b	3.5
C	4	3.033^a	301.7 ± 6.7^c	2.2
D	4	3.047^a	362.7 ± 18.2^d	5.0

[a]results in a column with different letters are significantly different ($P < 0.05$); A – extracted with DDI H_2O; B – distilled with sulphuric acid; C – extracted with hydrochloric acid; D – extracted with HCl for an hour and then TISAB added to adjust pH to 5–6

In the process of dry ashing, nitrate has been brought in the analyte. To avoid introducing other impurity ions, the ash was extracted with DDI H_2O (method A). In the process of distillation, sulphate has been brought in the sample (method B). Fluoride extraction with HCl, citric acid (Cheng *et al.* 2012), perchloric acid, NaOH, NH_4Cl, KCl or carbonate (Ponikvar *et al.* 2007) has been widely studied. In method C, HCl was chosen as an extraction agent for its high efficiency. Method D, the residues were added hydrochloric acid for an hour firstly and then

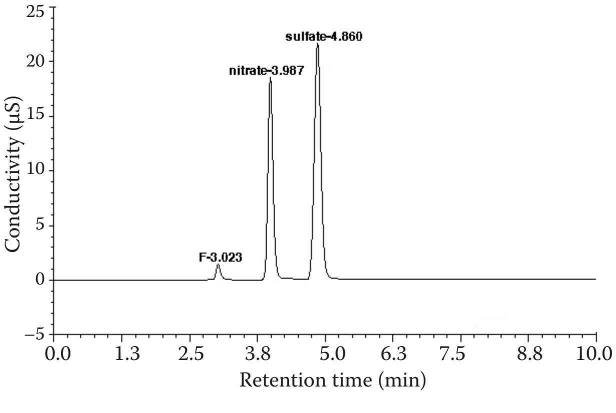

Figure 1. Ion chromatogram of distillation contained fluoride, nitrate, and sulphate. Retention time was the abscissa, while conductivity was the ordinate

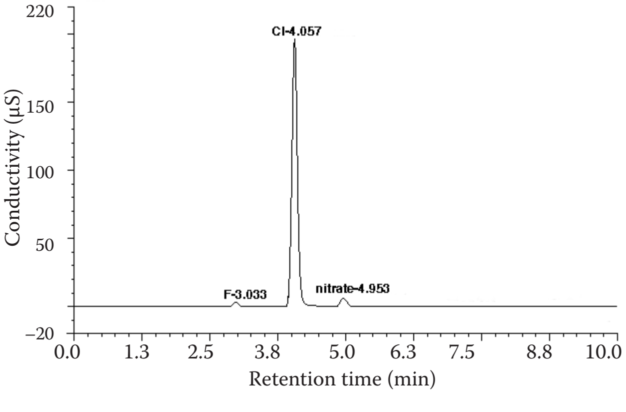

Figure 2. Ion chromatogram of HCl extraction contained fluoride, chloride, and nitrate

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they were added TISAB to adjust pH to 5–6, intending to eliminate the influence of acidity and interference ion (like aluminium, ferric ion or silicate root ion). Results of these experiments are presented in Table 1.

As shown in Table 1, the retention time varied non-significantly. H_2O extraction gave a fluoride concentration of 87.6 ± 8.7 mg/kg, the lowest of four sample preparations. Because this method mainly extracted water-soluble fluoride, extraction of organic fluoride was incomplete. The fluoride concentration of method B was 288.7 ± 10.2 mg/kg (n = 8). In spite of the introduction of nitrate and sulphate in the process of ashing and distillation, neither of them coeluted with the F^- peak (Figure 1). The retention time is 3.023 min for F^-, 3.987 min for nitrate, and 4.860 min for sulphate, so interferences caused by nitrate and sulphate could be excluded. HCl was used as an extracting agent with fluoride concentration 301.7 ± 6.6 mg/kg. HCl extraction gave a higher fluoride concentration than distillation, but it introduced Cl^- into the analyte. As shown in Figure 2, the conductivity of Cl^- was as high as 200 µS, which resulted in the column overloaded with Cl^-. OnGuard II Ag cartridges were used to remove the unwanted chloride ions. OnGuard II Ag cartridges could also reduce F^- concentration because silver fluoride is insoluble, so HCl was not applicable as an F^- extraction agent.

Method D was designed to investigate the influence of pH on the extraction efficiency of F^-. The highest fluoride concentration (362.9 ± 18.2 mg/kg) was obtained after adding TISAB. The retention time (3.047 min) of fluoride anion was longer than in the other three preparations when acetate was present. It was found that the retention time of acetate ion was close to that of fluoride anion, which makes the analysis of fluoride concentration difficult (Figure 3A–C). Therefore, this method was not recommended for determination of fluoride. Moreover, the conductivity of the acetate ion was much lower than that of the fluoride ion from Figures 3A and 3C. Because the acetate anion coeluted with the F^- peak, we tried phosphate buffer (pH 5.8) to adjust pH to 5–6. Phosphate buffer resulted in a floccose sediment in the analyte because part of F^- combined with calcium and phosphate to form fluorapatite [$Ca_5(PO_4)_3F$] and decreased the concentration of F^- (205.0 mg/kg). It can be concluded that adding dietary calcium supplementation or phosphate could reduce the bioavailability of fluoride (Hansen *et al.* 2011). This inference provides a way to solve the problem of Antarctic krill as aquatic feed being limited by a high fluoride level.

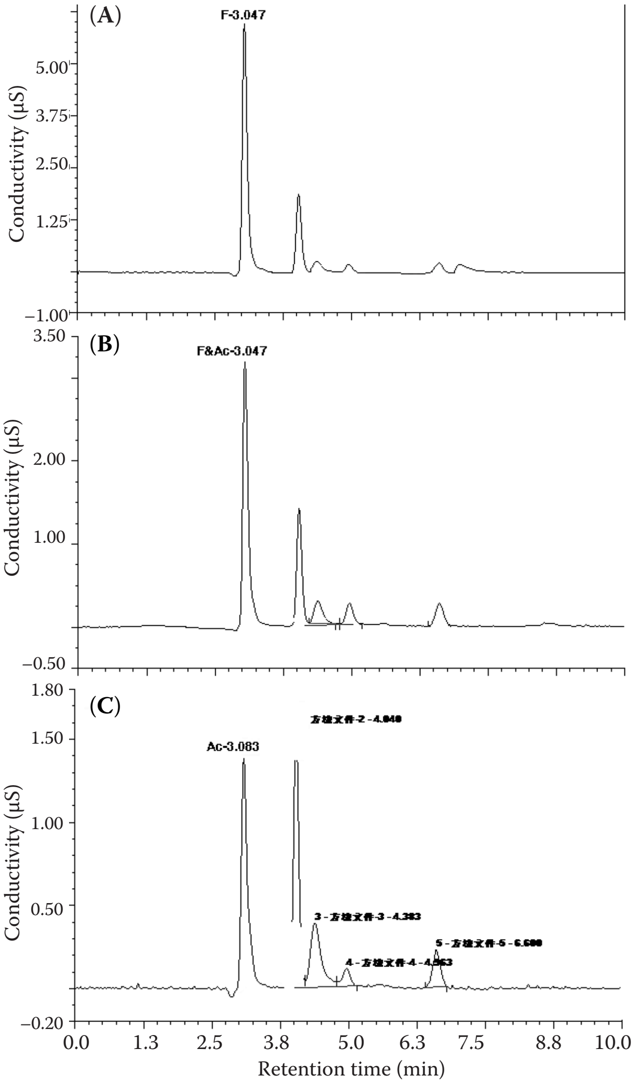

Figure 3. Fragments of chromatograms of the retention time of acetate ion and fluoride ion: (**A**) ion chromatograms of 1.0 mg/l fluoride; (**B**) ion chromatograms of 0.5 mg/l acetate ion and 0.5 mg/l fluoride; (**C**) on chromatograms of 1.0 mg/l acetate ion

Table 1 also shows the precision given as relative standard deviations (RSDs) for four sample preparations ranged from 2.2% to 9.9%. In conclusion, sulphuric acid distillation was the optimum sample preparation for determination of fluoride in Antarctic krill with RSD 3.5%.

Table 2. Precision for fluoride standard solutions (n = 8)

$C_{(F)}$ (mg/l)	Mean (mg/l)	SD (mg/l)	RSD (%)
0.5	0.5060	0.0099	1.9
1.0	1.0094	0.0180	1.8
5.0	5.0361	0.0887	1.8
10.0	9.9753	0.1791	1.8

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Table 3. Recoveries of fluoride from Antarctic krill (n = 4)

Samples	$C_{(F^-)}$ (mg/l)	$C_{(F^-)}$ added (mg/l)	$C_{(F^-)}$ found (mg/l)	Recovery (%)
Water extraction	0.2766 ± 0.0132	0.025	0.3005 ± 0.0133	95.3 ± 2.8
	0.2452 ± 0.0133	0.250	0.4984 ± 0.0123	101.3 ± 3.2
Sulphuric acid distillation	0.0601 ± 0.0022	0.025	0.0848 ± 0.0023	98.6 ± 2.8
	0.0768 ± 0.0038	0.250	0.3172 ± 0.0031	96.3 ± 1.0

Method validation. Parameters like linearity, linear range, precision, accuracy, limit of quantification, and limit of detection analysis were required in order to evaluate this method. Limit of detection was 0.06 mg/l. Limit of quantification was 0.2 mg/l based on the limit of detection. The linear range of this method was 0.1–10.0 mg/l, and the linear correlation coefficient was 0.99998. Precision was calculated as RSD of eight measurements of standard solutions (Table 2). The RSDs were less than 2.0% for all the fluoride concentration levels investigated. The recoveries of standard addition are given in Table 3. Recoveries were acceptable with a range of 95.3–101.3%.

The DIONEX ICS-3000 system was applied to determination of fluoride in Antarctic krill. Potassium hydroxide (30 mmol/l with a flow of 1.00 ml/min) was chosen as an eluent for its good selectivity for the weak fluoride anion. Additionally, standard addition experiments were performed in order to confirm the identity of fluorine and to calculate its recovery. Four kinds of sample preparations were studied based on dry ashing including water extraction, sulphuric acid distillation, hydrochloric acid extraction, and pH adjustment with buffer. Water extraction was inappropriate because of the incomplete extraction of organic fluoride. HCl extraction was not applicable to high amounts of Cl^- which would do harm to the IC separation column. Adjustment of pH with buffer was rejected for interferences caused by acetate. In spite of the introduction of nitrate and sulphate in the process of dry ashing and distillation, neither of them coeluted with the F^--peak, so sulphuric acid distillation would be suitable for determination of fluoride in Antarctic krill.

The conclusion that can be drawn from all these aspects is that the presented method with sulphuric acid distillation meets the requirement for simple and rapid determination of the fluoride concentration in Antarctic krill by IC.

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