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Determination of fluoride in food by the use of alkali fusion and fluoride ion-selective electrode

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Abstract

A simple, rapid, and reliable method was developed for determination of fluoride in biological samples. Fluoride was determined by alkali fusion and fluoride ion-selective electrode. The influence of concentration and volume of sodium hydroxide as ashing aid, sample weight, lifetime of the electrode and storage time of the sample solutions on analytical results were studied. Fluoride contents of various marine biological samples and certified reference material were determined.

Keywords: Fluoride; Alkali fusion; Fluoride ion-selective electrode

1. Introduction

Increasing awareness of food as a possible fluoride source has accentuated the need for more appropriate analytical procedures for measuring fluoride in food items. The fluoride selective electrode was designed to measure fluoride ion activity in aqueous solutions (Frant & Ross, 1966), and has proved to be a convenient and efficient tool for analysing fluoride in water samples. However, getting fluoride in food into the necessary water soluble ionic form has proved to be difficult.

Various methods for isolating ionic, complex and covalent bound fluoride have been suggested. The most common methods for decomposition of biological materials used are open ashing, fusion, oxygen combustion and acid digestion (Haldinmann & Zimmerli, 1993). The fluoride concentration is most often determined using an ion selective electrode or by gas chromatography. Several publications using dry ashing with alkali as ashing aid have shown that this method is well suited for analysing fluoride in plant and food materials (McQuaker & Gurney, 1977; Singer & Ophaug, 1986; Stevens, McLaughlin & Alston, 1995). Consequently, alkali fusion was chosen as the ashing procedure in this study.

The procedure described in this paper is a modification of a method proposed by Baker (1972). The main purpose of his method was to omit the time consuming Willard–Winter distillation step (Willard & Winter, 1933). This was done by combining NaOH fusion with standard addition. McQuaker and Gurney (1977) and Zarcinas (1979) both published modifications of Baker's method by including samples of soil and vegetation.

The aim of the present study was to develop an analytical method for determination of fluoride by alkali fusion and fluoride ion-selective electrode to be used by the Norwegian Food Control Authorities for analysing food and feed.

2. Materials and methods

2.1. Apparatus

Biological samples were dry ashed in a Carbolite CWF 12/13 muffle furnace using 70 ml nickel crucibles. An Orion fluoride ion-selective electrode (model 9609) was used for fluoride analysis together with an Orion Benchtop pH-meter/ISE meter (model 920 A) and a magnetic stirrer. In addition, a Mettler balance (AG 204 Delta Range), a hot plate (220 V, 200 W), a Shott-Geräter titrator (TitroLine 96), and 50-ml plastic tubes

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(Sarstedt), were used for sample preparation prior to analysis.

2.2. Samples

Three Certified Reference Materials (CRM) in addition to two in-house reference materials were used in this study. Fluoride in Vegetation (Timothy grass, 2695), from the National Institute of Standards and Technology (NIST; Gaithersburg, MD, USA), was provided at two concentrations (referred to as Timothy high and Timothy low). Oyster tissue, 1566a, also purchased from NIST was available for use only in the early phase of the study, Prawns (GBW 08572) were purchased from State Bureau of Technical Supervision People's Republic of China. The two in-house reference materials used were cod flour (0271 Cod Powder) supplied by TORO A/S (Rieber & Søn A/S, Bergen, Norway) and a tea sample (African Pride) supplied by Tanzania Tea Blender Ltd. (Dar es Salaam, Tanzania). Fluoride content of various samples; fish feed (0.6 and 1.0 mm), saithe (*Pollachius virens*), shrimps (*Crangon septemspinosa*), krill (*Euphausia superba*) and tea (Addis tea, Tea Developing & Marketing Enterprise, Addis Abeba, Ethiopia) were also analysed.

2.3. Reagents

All solutions were prepared with analytical-reagent grade chemicals and distilled water. The following reagents were used: 8 mol/l sodium hydroxide solution, prepared by adding 320 g NaOH pellets (Merck) to 1 l of distilled water, and concentrated hydrochloric acid (37%; sp. gr. 1.19), Merck. A 100 mg F^-/l fluoride stock standard solution stabilised in NaF, TISAB III, and buffer solutions for pH calibration, purchased from Orion Research Inc (Boston, USA) were also used.

2.4. General analytical procedure

Samples of approximately 0.25- or 0.50-g samples, depending on the expected fluoride content, were weighed to the nearest 0.0001 g directly into nickel crucibles. The samples were covered with 2.5 or 5.0 ml of 8 mol/l sodium hydroxide solution, depending on the amount of sample weighed into the crucibles. The sample and sodium hydroxide solution were carefully mixed.

The crucibles were put on a hot plate for evaporation to dryness before they were covered and put into the muffle furnace for combustion. The temperature program for the muffle furnace was set at 200°C for approximately 16 h after which the temperature was increased to 525°C and kept there for 3 h. The crucibles were cooled, 10–15 ml distilled water was added, and the crucibles were put on a hot plate in order to aid the dissolution of the fusion cake. After approximately 2 h, the sample solutions were transferred to 50-ml capped plastic tubes.

The sample solutions were neutralised using concentrated and then diluted hydrochloric acid. Concentrated hydrochloric acid was added drop-wise until the pH decreased from 12.0–13.0 to 8.0–8.5. A titrator then adjusted the pH to 7.2–7.5 by adding dilute hydrochloric acid. The titrator was calibrated using pH-buffers 7.0 and 10.0. The sample solution was transferred to a 50-ml volumetric flask (plastic) and diluted to volume with distilled water. The solutions were stored in 50-ml air-tight plastic tubes until analysed.

Aliquots of 5 ml were taken out for analysis after the ash had settled and the solutions were clear. Care was taken to avoid the settled ash. Before analysing, 0.5 ml TISAB III was added to obtain a pH of 5.2–5.4, which is the optimal pH-range for fluoride determination. Reagent blanks were always prepared together with the samples and were brought through the whole procedure. Reagent blanks were used for blank determination as well as for preparing the standard solutions with fluoride concentrations of 0.100, 1.00 and 10.0 mg F^-/l. The standard solutions and sample solutions were analysed using a fluoride ion-selective electrode. The electrode was soaked in distilled water and blotted dry before each measurement.

3. Results and discussion

3.1. Working procedures

3.1.1. Sample weight

The amount of sample needed for determining the fluoride concentration in food depends on both homogeneity of the sample and the concentration of fluoride present. The sample taken for analysis should be representative of the whole sample, however, homogeneity is seldom a problem when working with CRM. When using a routine method in a control laboratory, CRM may be substituted by an in-house standard material of vegetable or animal origin in order to ensure the accuracy of the analytical method. The lower analytical quality of authentic in-house standard samples compared to verified CRM should, however, be kept in mind.

Most food items of vegetable origin have a relatively low fluoride content (Rao, 1984; Sherlock & Corr, 1984). It is, therefore, advantageous to use sample weights of 1–2 g to ensure the quality of the analysis. Experiments to determine the optimal sample weight were carried out (Tables 1 and 2). The results show little difference in concentration due to sample weight. Samples of 1 and 2 g were, however, difficult to handle. This was mainly due to difficulties in transferring the sample solution from nickel crucibles to plastic tubes as the ash adhered to the walls of the crucibles. This resulted in a

excessive sample volume because additional water was required for complete transfer of the ash. There was also a relationship between sample size and the risk of spill-over when neutralising; the risk being higher with a larger sample size. Due to these difficulties, results were not obtained for sample weights of 2 g (Table 2).

Stevens et al. (1995) found no difference in fluoride concentration in tea samples when sample weight was increased from 50 to 400 mg and using acid digestion. They found, however, that the fluoride concentration decreased significantly with increasing sample weight when using Timothy (high concentration) samples.

3.1.2. NaOH as ashing aid and sample weight

Alkali was added to the samples prior to fusion to avoid loss of fluoride during the ashing process. Several publications have used NaOH with a molarity of 16 mol/l (Baker, 1972; Singer & Ophaug, 1986; Stevens et al., 1995). The influence of molarity and NaOH volume on the final fluoride concentration was tested in a series of experiments. The molarity of NaOH was varied from 4 to 16 mol/l (Table 3). The lowest fluoride concentrations were found using 4 mol/l NaOH, no difference in fluoride concentration could be detected between the samples treated with 8, 12 or 16 mol/l NaOH, respectively. As Oyster tissue is not a certified material, and only carries an indicative value, no preference for molarity can be given based on the results. From a purely economical point of view, a concentration of 4 mol/l NaOH should be used. This would also require less acid for the neutralisation process. However, a concentration of 8 mol/l NaOH, caused the fusion cake to dissolve more easily in water, and is therefore, easier to work with. Thus, 8 mol/l NaOH as ashing aid is recommended.

The sample weight used in the experiment shown in Table 3 was approximately 0.2 g and 2 ml of NaOH was added to each sample. In the next experiment, the sample weight varied from 0.2–2.0 g (Table 2). Based on the results from Table 3 and the fact that 2 ml 8 M NaOH was sufficient to avoid loss of fluoride during the ashing process, we calculated the weight ratio of NaOH to sample to be 3:1. According to Bock (1979) the weight ratio of hydroxide to sample usually is around 5–6:1, and occasionally even higher. The results given in Table 2 show little difference in the final fluoride concentration. The following volume and concentration of NaOH are recommended for different sample sizes: a volume of 2.5 ml of 8 mol/l NaOH for 0.25 g, and 5 ml of 8 mol/l NaOH for 0.5 g.

3.1.3. Ashing time

For practical reasons, a long fusion time was chosen at 200°C (Table 4). The preparation of samples for fusion takes approximately 1 working day. It is, therefore, convenient to put the samples in the oven overnight. A temperature of 300°C was tried out for the

Table 1
Effect of sample weight on the concentration of fluoride in Oyster tissue (SRM-1566a) ($n=2$) using 2 ml 16 mol/l NaOH as ashing aid

Weight (g)	F^{-}[a] (mg/kg)
0.0200	287
0.0400	274
0.0800	275
0.1600	286
0.2400	289
0.3000	240

[a] Indicative value, 240 mg F/kg.

Table 2
Effect of sample weight, volume and molarity of NaOH on the concentration of fluoride in cod flour ($n=2$)

Sample weight (g)	NaOH (ml)	NaOH (mol/l)	F^{-} (mg/kg)
0.2000	1.9	8	38
0.4000	3.8	8	45
0.6000	5.6	8	45
0.8000	3.8	16	48
1.000	4.7	16	43
1.200	5.6	16	45
2.000	9.3	16	[a]

[a] Both samples were lost due to excessive sample weight and spill-over during neutralisation.

Table 3
Effect of NaOH concentration as an ashing aid on fluoride concentration in Oyster tissue (SRM-1566a)[a]

N	NaOH (mol/l)	pH	F^{-} (mg/kg)	S.D.
2	4	11.4	245[b]	
4	4	11.4	226	8
10	4	11.5	192	28
3	8	12.5	283	11
5	8	12.4	273	21
3	12	13.0	278	20
4	12	13.0	271	1
10	12	12.8	321	21
5	16	13.3	290	12
6	16	13.2	289	17
14	16	13.0	284	15

[a] Measurements of pH was made before neutralisation with acid and addition of TISAB. Sample weights of 0.2 g were used. Indicative value, 240 mg F/kg.

[b] The fusion cake was difficult to dissolve 4 mol/l NaOH. Because of this, one sample was rejected before neutralisation.

Table 4
Temperature programme for the muffle furnace

Step	Temperature (°C)	Time (h)
1	20	0
2	200	16
3	525	3

second step of the ashing procedure (Table 4; results not shown), but losses were seen because of problems with spill-over and ash coating the crucibles.

The influence of ashing time at 525°C on fluoride concentration in cod tissue was examined (Table 5). Little difference in fluoride concentration was found when ashing at this temperature for 1, 2 or 3 h, respectively. It was, however, much easier to work with samples ashed for 3 h because the ash was finer in structure. Based on these practical reasons, it was chosen to ash at 525°C for 3 h.

Keerthisinghe, McLaughlin and Randall (1991) found that the recovery decreased with fusion time. Highest sample concentrations were, however, found after 2 h fusion. The samples used by Keerthisinghe et al. (1991; subterranean clover) were fused in a muffle furnace at 600°C for 1, 2, 3 and 4 h and Na_2CO_3/NaB_4O_7 was used to aid ashing.

3.1.4. Neutralisation process

The neutralising process was found to be a critical step in the preparation of sample solutions. Since TISAB was not strong enough to adjust the pH in the sample down to the optimal pH range for fluoride analysis of 5.0–5.5, acid was added. To simplify neutralisation, a tritrator was used. Shott-Geräter (1996) recommends solutions with a molarity of 1 mol/l. In the present study, this molarity proved to be too strong to prevent the pH from fluctuating and dropping below 7.0. Fluoride may then be lost as gaseous hydrogen fluoride. The optimal molarity was found to be 7.76×10^{-4} mol/l, but solely using this acid dilution resulted in an excessive final sample volume. A coarse adjustment of the pH, using concentrated acid, was therefore chosen, and the titrator was subsequently used to finally adjust the pH to the correct level.

The ash should be mixed into the solution by using a magnetic stirrer before starting the neutralisation process. If the solution is neutralised before the ash is mixed, the pH has a tendency to fluctuate and drop below 7.0 It is also important to add the concentrated hydrochloric acid drop-wise to prevent spill-over.

The neutralisation process is the most time consuming step in fluoride analysis. Ideally, the titrator would complete the entire process of neutralisation without any need for continuous attention.

Table 5
Fluoride concentration (mg/kg) in cod tissue after ashing in a muffle furnace at 525°C for 1, 2 and 3 h[a]

	Ashing time (h)			
	1	2	3	3
Number (*n*)	5	6	6	5
F^-	40	45	43	44
S.D.	4	4	6	2

[a] The first two steps of the ashing procedure were done as described in Table 4.

3.1.5. Effect of storage time

The stability of fluoride concentrations in the sample solutions over time was examined using the proposed method (Fig. 1). Aliquots of 5 ml from each sample solution (volume of 50 ml after neutralising and dilution) were added 0.5 ml TISAB and analysed once a week for 8 weeks. The results show that the fluoride concentrations varied, but there were no trends of either increasing or decreasing concentrations over this time period. The precision given as standard deviation varied between 2.2 and 5.5 mg F^-/kg, giving a RSD (%) of 3.8 and 7.7, respectively.

Samples prepared for analysis at week one in Table 6 (sample added TISAB), were also analysed after one week storage. The result was 64.1 ± 4.4 mg F^-/kg ($n=7$).

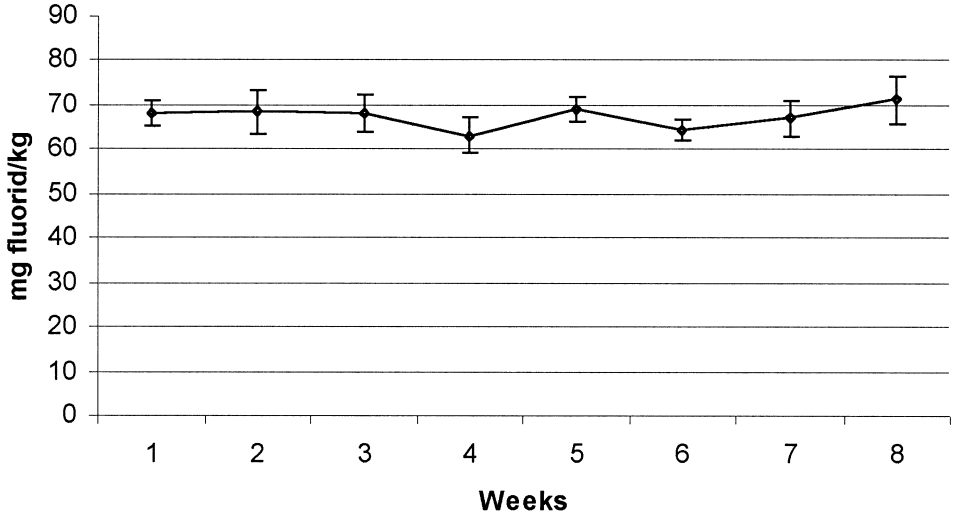

Fig. 1. Effect of storage time on fluoride concentration in samples of Timothy (SRM-2695); $n=7$. TISAB III added gave a pH of 5.2 ± 0.1.

To test sample solution homogeneity, seven replicate aliquots of 5 ml were taken from the same sample solution (cod tissue), TISAB was added, and samples were analysed for fluoride. This gave a fluoride concentration of 39.3 ± 0.5 mg F^-/kg. The fluoride concentration in this sample seemed to be homogenous even after the ash had settled. The results indicate a satisfactory stability of the sample solutions over time.

3.1.6. Standard curve

The standard curve of fluoride reflects the ratio between the content of fluoride in a solution and the resulting measurement response. In preparing a standard curve, the following concentrations were used: 0.0001, 0.001, 0.01, 0.1, 1.0 and 10.0 mg F^-/l. Thus, the fluoride concentration chosen covers both sides of the working range (Fig. 2). The calibration curves (Fig. 2) show a linear relationship between fluoride concentration in the standards and mV-reading between 0.1 and 10 mg/l (from −1 to 1 using the log scale). This is in agreement with the Orion Instruction Manual (Orion, 1991). The regression coefficient for this range of the curve was 0.999 for the 1997 curve and 0.997 for the 1998 curve (for three measuring points). The regression coefficient (R) should be greater than 0.999 (NMKL, 1996). The tests depicted in Fig. 2 were performed with the same electrode 15 months apart. The results show that the electrode's regression coefficient decreased with time and use. The response after 15 months was no longer linear down to the concentration of 0.1 mg F^-/l. According to producer, the electrode should last at least for a period of 12 months. The decrease in the electrode's slope and increased drifting of readings are gradual processes. Care should therefore be taken if older electrodes are used.

The concentration of the blank value that was calculated by the instrument is not given. As the fluoride standards are diluted by reagent blanks in the present method, it was found unnecessary to correct for a blank. The calibration was therefore based on two-point calibration without a blank correction.

3.1.7. Trueness (recovery)

To demonstrate the applicability of the present analytical procedure, a series of experiments was performed to determine the recovery of added fluoride. The recovery study gives information about possible interference with fluoride from other analytes in the sample. The amount of fluoride added should correspond to the level normally present in the sample material.

Table 6 shows the effect of sample weight on the recovery of added fluoride to different samples. Recovery

Table 6
Effect of sample weight on the recovery of spiked fluoride to Timothy (SRM-2695), tea and cod flour (each series comprises five sets of replicates; $n = 5$)

Sample	No. series	Sample weight (g)	F^- added (μg)	F^- found (μg)	Recovery (%)
Timothy low	3	0.2500	0	16.1	
			16	31.7	98
Timothy low	2	0.5000	0	32.8	
			32	66.0	104
Timothy high	3	0.2500	0	73.5	
			69	130	82
Timothy high	3	0.5000	0	126	
			138	238	81
Tea	1	0.2000	0	27.6	
			28	57.0	105
Cod	1	0.6000	0	24.5	
			30	47.3	95

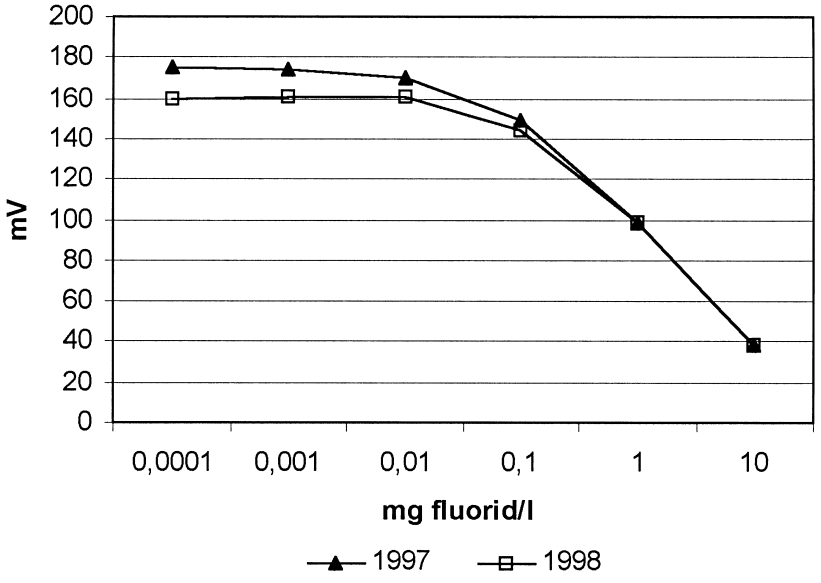

Fig. 2. Typical calibration curves obtained using a fluoride ion-selective electrode over a period of 15 months.

was within the range of 80–110%, which is acceptable (NMKL, 1996). As previously mentioned, Stevens et al. (1995) found significantly decreased fluoride concentrations with increasing sample weight of Timothy (high concentration) samples. In contrast, there were no apparent differences in the recovery between the two sample weights used in the present study.

The tea and cod samples were also analysed using distilled water instead of blank solution as dilutant when preparing the fluoride standards. The recovery of fluoride added to tea samples and cod samples decreased to 72±5% for the tea samples and 76±7% for the cod samples, respectively. This is not surprising as the standard solutions should have a composition as close as possible to the sample solutions.

A weakness of using Timothy for validating purposes is that the fluoride concentration in this material is high, compared to the concentration expected in most food items. However, the use of CRM available for fluoride is of great value when validating analytical methods, and there are few certified fluoride reference materials available. Several methodology studies on fluoride determination in biological material, have also used recovery tests as proof of trueness. It is important to bear in mind that recovery tests offer limited control of systematic error. A recovery test is not sufficient to tell whether or not total fluoride has been determined, but rather that fluoride added, usually as sodium fluoride, has not been lost through the analytical process.

3.1.8. Precision (repeatability)

The repeatability of a method is evaluated by analysing replicate samples, in the same laboratory, using the same equipment and within a short time period (NMKL, 1996). Since precision depends on the concentration of the analyte, materials with a concentration ranging from 5.3 to 280 mg F^-/kg were analysed in the present study (Table 7). Table 7 shows precision given as RSD (%) ranged from 2.2 for high fluoride sample and 6.2 for low fluoride sample.

Table 7
Fluoride concentrations (mean±S.D.) in Certified Reference Materials (mg/kg dry sample)

Sample type	*n*	Mean (mg/kg)	S.D. (mg/kg)	RSD (%)	Certified value[a] (mg/kg)
Prawns (GBW-8572)	4	4.8	0.3	6.2	5.31±0.39
Timothy low (SRM-2695)	10	66	5	7.0	64.0±8.4
Timothy high (SRM-2695)	10	260	10	5.5	277±27
Oyster tissue (SRM-1566a)	10	280	10	2.2	240[b]

[a] Mean and 95% confidence interval.
[b] Informative value only.

3.1.9. Limit of detection and limit of determination

The mV values of standards varying from 0.0001 to 10.0 mg F^-/l were determined and used for constructing a standard curve. The standard curve was not linear below the concentration of 0.1 mg F^-/l (Fig. 2). The fluoride concentration of the reagent blank was therefore determined using the slope between 0.1 and 1.0 mg F^-/l. This is the part of the standard curve used for determining the fluoride concentration in the food samples analysed in this study. Two different fluoride ion-selective electrodes (A and B) were tested using the same blank solutions, 15 months apart (Table 8). New blank solutions were prepared and analysed with both electrodes the second time. Electrode A had been used frequently between the measurements, while electrode B had been stored according to instructions given by the producer (Orion, 1991).

The limit of detection (LOD) is defined as the amount of fluoride corresponding to the lowest measurement signal which, with a certain statistical confidence, may be interpreted as indicating that the analyte is present in the solution, but not necessarily allowing exact quantification (NMKL, 1996). LOD was calculated to be three times the standard deviation of the average blank ($n=15$). Electrode B showed a higher LOD (0.04 μg/ml) than electrode A (0.02 μg/ml), and the limit increased during storage. For electrode B, the LOD was in both cases above the limit of 0.02 mg F^-/l given by the producer (Orion, 1991).

The limit of quantification (LOQ) is defined as the lowest amount of fluoride in a sample that can be quantitatively determined with a certain confidence (NMKL, 1996). LOQ was calculated as 10 times the standard deviation of the average reagent blank.

For electrode A, the LOQ was lower after 15 months. Despite correct storage, LOQ of electrode B also decreased over time. Electrode B had too high a LOQ to be used for analysing most food samples, even when it was new.

The quality of electrodes varies, and correct storage may not be sufficient to maintain electrode effectiveness. These results support the importance of testing the electrodes in use on a regular basis. It is advisable to make a control card based on a control material.

Table 8
Limit of detection (LOD) and limit of quantification (LOQ) for the described method using two electrodes A and B

Electrode	*n*	LOD (mg/l) May 1997	LOD (mg/l) August 1998	LOQ (mg/kg) May 1997	LOQ (mg/kg) August 1998
A	15	0.022	0.015	0.072	0.051
B	15	0.036	0.061	0.117	0.203

Table 9
Fluoride concentration (mg F^-/kg dry wt.) in various samples using the described method

Sample	Latin name	*n*	F^- found (mg/kg)
Fish feed (1.0 mm)		2	32
Fish feed (0.6 mm)		2	79
Saithe	*Pollachius virens*	2	60
Shrimp (meal)	*Crangon septemspinosa*	3	84
Shrimp (meal)	*C. septemspinosa*	3	147
Shrimp (meal)	*C. septemspinosa*	2	318
Krill	*Euphausia superba*	4	1137
Addis tea		2	536

3.1.10. Fluoride concentration in foods

Table 9 gives the fluoride concentration in different food samples. Most of the material is seafood and therefore high in fluoride.

4. Conclusion

Development of methods carried out in different laboratories, should be performed according to defined analytical guidelines. There is an obvious need for documentation of the reliability of analytical procedures used for the determination of fluoride in food. To enable results from different studies to be compared, analyses that give the total fluoride concentration are preferred. This is because different acids and mixtures of acids may dissolve the fluoride in organic matter to varying degrees. CRM based on cereals or vegetables, with a broad range of variation of fluoride concentrations, are required.

The alkali fusion method tested in this study is well suited for analysing fluoride in food. The neutralisation process is, however, time consuming and procedures to simplify this step should be encouraged.

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