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DETERMINATION OF FLUORIDE IN SOFT TISSUES

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SUMMARY: Two analytical procedures, dry combustion and acid extraction, were compared for the preparation of soft animal tissues and other biological samples for determination of fluoride with an ion selective electrode. Both methods were suitable for determining F in the range of 0.5 to 10.0 µg/g with less than 6% error. The combustion method is less laborious but is more accurate. The extraction method is faster and less expensive but is slightly less accurate.

Keywords: Combustion method; Extraction method; Fluoride determination; Fluoride in soft tissue.

INTRODUCTION

Fluoride accumulation in bones and teeth as well as its impact on their mechanical properties is well documented by many authors.[1-4] More controversial, sometimes even contradictory, are data concerning fluoride disposition in soft tissues. Exposure to fluoride compounds results in fluoride absorption and transportation via the blood to tissues and organs causing in them structural changes and disturbance in their function.[5-9] Therefore the assessment of fluoride content and its distribution in tissues of humans and animals may be of practical significance.

Because fluoride is usually present only in trace amounts in soft tissues, its determination in such material often encounters difficulties. Moreover, determination of F in tissues usually requires its transformation into ionic form,[10-14] and these aspects are addressed in this study for final determination of F potentiometrically with an ion specific electrode.

Here we compare results of two methods for soft tissue sample preparation: dry combustion and acid extraction. We have modified our procedures for both methods many times, and we now believe they are suitable for toxicological F analyses.

EXPERIMENTAL PART

Materials: Samples of porcine liver fortified with sodium fluoride and livers of rats exposed for 30 days to sodium fluoride in drinking water containing 0.3, 25, and 100 mg/L were used in this study. These materials were stored at –20º C and thawed at the time of analysis. The accuracy of measurement of fluoride in biological material was tested with Certified Reference Material: Serum Control (Clin Chek).

Solutions and Reagents: Sodium fluoride, standard solution – 1.9 mg F^-/mL (Orion). Diluted sodium fluoride solutions – 1 µg F^-/mL, 10 µg F^-/mL, and 40 µg F^-/mL. Sodium versenate (EDTA), analytical grade – 5 % solution.

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Perchloric acid, analytical grade – 2 M solution. Sodium citrate, analytical grade – 1 M solution. TISAB buffer (pH = 5.2). Magnesium acetate, analytical grade – 5 % solution.

METHODS

Digestion:[12] Specimens of liver were cut into pieces, and weighed aliquots (0.5 or 1.0 g) were transferred into platinum crucibles. The appropriate NaF solution was then added, and the sample was treated with 1 mL of 5 % magnesium acetate solution (to avoid swelling during combustion), dried at 105º C for 12 hr, and pyrolyzed in an oven at 500º C for 24 hr. Cooled samples were transferred into polyethylene measuring vessels by rinsing the crucibles successively with 1 mL of perchloric acid solution, 1.5 mL of 1 M solution of sodium citrate, and 1.5 mL of TISAB buffer (pH = 5.2). Samples of the reference material were treated similarly.

Extraction:[13] Specimens of liver were cut into pieces, mixed, and homogenized. Weighed aliquots (0.5 or 1.0 g) of the homogenate were transferred into polyethylene containers and spiked with an appropriate volume of NaF standard solution before addition of 6.25 mL of 5 % solution of disodium versenate (EDTA) and 6.25 mL of TISAB buffer (pH = 5.2) The containers were then closed and heated with shaking at 95º C in a water bath for 15 min. Cooled samples were filtered on a suction funnel and quantitatively transferred into polyethylene measuring vessels and adjusted to 25 mL with redistilled water. Samples of the reference material were treated similarly.

Final determination: In both methods the final determination of fluoride was performed potentiometrically using a combined fluoride ion selective electrode. Fluoride concentrations were read from calibration curves prepared with standard solutions. Depending on the expected fluoride concentrations in the analyzed samples, calibration curves were prepared in two concentration ranges 0.5 – 2.5 μg F^-/0.5 g tissue and 1.0 – 10.0 μg F^-/1.0 g tissue.[6,14,15]

RESULTS AND DISCUSION

Dry combustion and acid extraction were chosen among available methods for preparation of biological material for the final determination of fluoride with an ion specific electrode. For improvement, some minor modifications in the original form of both procedures were introduced. In this study, good results were obtained with both methods. However, there are some differences in analytical parameters: the amount of labor needed and the amount of time required. Recoveries of fluoride from biological material (spiked with NaF standard solution) and from samples originating from exposed animals and from reference material are slightly higher with the combustion method.

Probably the differences result from the more complicated extraction procedure during sample preparation. In addition to losses that may occur during filtration, another cause of lower results from the extraction method is the fact that not all the fluoride is in the ionic form necessary for potentiometric determination. An advantage of the combustion method is its lower coefficient variation as seen in Tables 1–3. On the other hand, it has the disadvantage of requiring more time, especially during drying and combustion of the tissue sample, as well as the requirement for expensive platinum crucibles.

Table 1. Fluoride content of fortified porcine liver samples

Method	Number of samples per determination	Added μg F^-/g	Determined μg F^-/g ± SD	Coefficient of variation %	Recovery %
Combustion	n = 6	0.5	0.47 ± 0.31	4.8	94
		2.5	2.48 ± 0.12	5.2	99
		5.0	4.8 ± 0.14	5.2	96
		8.0	7.6 ± 0.11	5.0	95
		10.0	9.8 ± 0.21	4.9	98
Extraction	n = 6	0.5	0.44 ± 0.03	5.1	88
		2.5	2.29 ± 0.14	5.7	92
		5.0	4.4 ± 0.17	5.8	88
		8.0	7.1 ± 0.21	5.8	89
		10.0	8.9 ± 0.22	5.6	89

Original liver samples contained 1.4 ± 0.12 μg F^-/g. These values are not included in this table.

Table 2. Fluoride content in reference serum containing 0.503 μg F^-/mL

Method	Number of Samples	Determined μg F^-/mL ± SD	Coefficient of variation %	Recovery %
Combustion	n = 4	0.523 ± 0.036	6.1	103.9
Extraction	n = 4	0.513 ± 0.041	6.8	101.9

Table 3. Fluoride content (μg F^-/g tissue) in liver of rats exposed to F^- in drinking water for 30 days

Method	Animals receiving F^- in drinking water			Coefficient of variation in %
	0.3 mg F^-/L X ± SD. n=22	25 mg F^-/L x ± SD. n=24	100 mg F^-/L x ± SD. n=24	
Combustion	0.69 ± 0.082	2.96 ± 0.13	4.86 ± 0.14	5.2
Extraction	0.54 ± 0.116	2.58 ± 0.18	4.34 ± 0.25	5.8

CONCLUSION

Both the combustion and extraction methods are suitable for determination of trace amounts of fluoride in soft tissues.

The combustion method is less laborious and has better analytical parameters. The extraction method is less time consuming and does not require expensive platinum crucibles.

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