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## Estimation of Total Fluoride in Foodstuffs

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A procedure is described for estimation of total fluoride in foodstuffs for levels down to 0.3 mg/kg. The sample is mixed with a prepared calcium hydroxide, as an ashing additive to immobilise volatile fluoride, and ashed in a stainless-steel lined muffle. Fluoride is separated by steam-distillation from a sulphuric acid medium and collected, when necessary, on an anion-exchange resin. After elution, the fluoride is measured potentiometrically using a fluoride ion-sensitive electrode. Standard deviations of the results for levels normally found in foodstuffs have been calculated and derived confidence intervals are given.

### Introduction

Few methods have been described for the estimation of total fluoride in foodstuffs. The procedure used historically has been decomposition of organic material by ashing with an ashing additive to immobilise volatile fluoride and to break down complex fluoride minerals, isolation of the fluoride by a Willard-Winter type distillation and measurement of fluoride in the distillate. A typical method is described in *Official Methods of the Association of Official Analytical Chemists*<sup>1</sup>. In this laboratory, difficulty was encountered in selecting an ashing additive which had a suitably low fluoride content and which was convenient to use both in limiting contamination during ashing and in measurement of the low fluoride levels in the distillate. A prerequisite for estimating the low levels present in many foodstuffs was to obtain low method blanks.

A prepared calcium hydroxide was used as an ashing additive whose fluoride level was estimated from the average of a series of method blanks obtained using the same prepared batch. Ashing was made in nickel dishes in a muffle furnace fully lined with stainless-steel sheet. Fluoride isolated by steam distillation at 145–150°C was collected, concentrated when necessary, on a column of anion-exchange resin<sup>2</sup>. Measurement of fluoride in the final solution was made potentiometrically in a manner which minimised effects of drift from the measurement system.

### Experimental

#### APPARATUS

*Nickel dishes*, of capacity approximately 70 ml.

*Muffle furnace*, which has a complete stainless steel lining.

*Semi-automatic temperature controlled still*: consisting of 150 ml distillation flask fitted with three ground glass jointed necks and connected to a condenser

by a ground glass jointed distillation head through which is constructed a steam inlet tube which extends nearly to the bottom of the flask. The other neck contains a thermometer and a sheath to hold a temperature-sensitive element connected through suitable ground glass joints. All glass joints are sealed with PTFE-sleeves (no fluoride was leached from these sleeves) and retained by external springs. The steam inlet is connected through two demountable 2-way glass taps to a steam generator. Steam is produced from water made just alkaline using a few drops of phenolphthalein solution as indicator. The still is heated by an electric radiant heater (Electro-thermal BA6101) through a proportional current controller (RFL Industries Inc.).

*Ion-exchange column.* Prepared using  $1.00 \text{ g} \pm 0.01 \text{ g}$  of resin in a similar manner to that previously described by Evans and Sergeant<sup>2</sup>.

Measure fluoride concentration in final solutions potentiometrically using a fluoride ion-sensitive electrode (Orion model 94-09) and the Ag/AgCl reference half of a normal pH glass electrode together with a millivolt meter for use with ion-sensitive electrodes having a resolution of at least 0.5 mV.

#### REAGENTS

All reagents should be of analytical grade.

*Calcium hydroxide ashing additive:* Prepare in batches of approximately 50 g in a similar manner to that described in *Official Methods of the AOAC*<sup>1</sup> except that the initial reagent is calcium carbonate precipitated by ammonia (BDH Ltd.) and selected for low fluoride level. Filter the precipitated calcium hydroxide, wash at least five times with water to remove sodium hydroxide, then dry in an oven at 120°C overnight and grind to a homogeneous fine powder.

*Anion-exchange resin Zerolit FF(IP)* (BDH Ltd.)

*Total Ionic Strength Buffer (TISB):* Dissolve 1 g of 1,2-diaminocyclohexane-N,N,N',N'-tetracetic acid (CDTA) in approximately 15 ml of water with addition of sufficient sodium hydroxide. Add this to a solution containing 82 g of sodium acetate and 58 g of sodium chloride in approximately 700 ml of water, adjust the pH of this combined solution to pH 5.8 with acetic acid and dilute to 1000 ml.

*Working standard fluoride solutions:* Prepare at concentrations of 10, 4, 2, 1, 0.4, 0.2, 0.1, 0.04, 0.02 and 0.01  $\mu\text{g}$  ml for calibration and measurement and store in pre-conditioned polythene bottles.

#### Procedure

##### IGNITION

Weigh  $10.00 \text{ g} \pm 0.01 \text{ g}$ , or other suitable quantity of homogenised foodstuff, followed by  $0.50 \text{ g} \pm 0.01 \text{ g}$  of prepared calcium hydroxide into the nickel dish. Add water to make a volume of approximately 30 ml and stir well to produce a saturated solution of calcium hydroxide distributed throughout the sample.

Evaporate the slurries to dryness and if much sugary matter is present char in an oven at about 150°C. Place in the lined muffle and raise the temperature at a rate of 50°C/30 min to 600°C and ash at this temperature overnight. Include at least one dish containing 0.50 g  $\pm$  0.01 g of the calcium hydroxide alone in each ignition set and treat as a method blank.

#### DISTILLATION AND CONCENTRATION

Add 200 mg of silver sulphate and some glass beads to the distillation flask, followed by the ash residue from the nickel dish. Wash the dish twice, with 5 ml portions of water, transferring into the flask. Add 40 ml of sulphuric acid solution (1 + 1) and assemble into the steam distillation train.

Admit steam from the steam generator when the contents of the distillation flask are at a temperature of 150°C, and allow the distillate to pass through the ion-exchange column, maintaining the distillation temperature within the range 145–150°C. Collect 200 ml of distillate which has passed through the ion-exchange column. (If the fluoride content of the sample is believed to be greater than 2 mg/kg, collect the distillate directly in a 200 ml volumetric flask to which has been added phenolphthalein indicator solution and 1M sodium hydroxide solution to maintain the collected distillate just alkaline). Recover the fluoride on the resin by eluting with 0.2M sodium acetate solution, collecting the eluate in a 25 ml volumetric flask.

#### CALIBRATION

Stabilise the fluoride ion electrode response at constant temperature (the electrode having been kept dry when not in use) by immersing in a solution containing 25.0 ml of a 1.0  $\mu\text{g/ml}$  fluoride solution and 25.0 ml of buffer solution for approximately 1 h. Dispense 25.0 ml of buffer solution into a 100 ml polythene beaker and add 25.0 ml of a working standard fluoride solution. Stir for 1 min, then measure the steady potential after 10 min, or longer for the more dilute standard solutions, using the fluoride ion-sensitive electrode. Prepare a standard calibration graph of log (concentration) against potential, which should be linear from 10  $\mu\text{g/ml}$  to 0.1  $\mu\text{g/ml}$  fluoride, and retain for the following measurements.

#### MEASUREMENT

Stabilise the fluoride electrode as above and in a similar manner measure the steady potential of a higher working standard fluoride solution, followed by a lower standard solution for the linear part of the calibration graph. Repeat these measurements in reverse order at the end of the measurement sequence. Measure the potential of an intermediate standard during the measurement sequence. A working calibration graph for the measurement sequence may be drawn from these measurements parallel to the standard calibration graph. Similarly, measure the 25.0 ml column eluates from sample and method blank distillates (or 25.0 ml of the distillates obtained directly from samples containing high fluoride levels) between measurements for the working calibration graph,

to lie if possible in the linear part of the graph. The corresponding fluoride concentrations ( $\mu\text{g/ml}$ ) may be read from the working calibration graph. The average for each calcium hydroxide batch is subtracted from the fluoride concentrations found for corresponding samples.

### Results and Discussion

In the presence of acid, labile fluoride will be evolved as hydrofluoric acid from any matrix and this is the basis of techniques proposed for separation of fluoride in foods by diffusion such as that described by Dabeka *et al.*<sup>3</sup> Such techniques use small samples and there is the possibility of incomplete separation of the fluoride. Fluoride has been separated from rocks and minerals techniques use small samples and there is the possibility of only a fractional separation of the fluoride. Fluoride has been separated from rocks and minerals by pyrohydrolysis using steam and an acidic oxide catalyst<sup>4</sup>. This approach was found to be unsatisfactory for foodstuffs because of the need for small samples which would contain a high proportion of organic matter. It was decided that a procedure for total fluoride must involve destruction of organic matter by ashing prior to a separation stage and would require fluoride to be stabilised, alkaline ashing additives having been used for this purpose in the past.

In preliminary work for this procedure difficulty was found in selecting an ashing additive which had a suitably low fluoride level and gave a tolerable variation in measurement for the method blank. Attempts to prepare a low fluoride level calcium oxide according to a previously described method<sup>5</sup> were unsuccessful, perhaps because the reagents containing low fluoride levels are no longer available. Additionally, a sufficient quantity for a series of estimations was required so that the variation in measurement of the method blank could be found. Alkali hydroxides gave glassy residues after ignition which were difficult to remove from the nickel dish, and some were found to have variable fluoride contents. A calcium hydroxide prepared as described was used. The average fluoride level of method blanks obtained using seven batches of this reagent was  $2.59 \mu\text{g}$  of F, with a range for the mean of eight to twelve determinations of  $2.52$  to  $2.67 \mu\text{g}$  of F. The standard deviation within the eight to twelve determinations was typically  $0.50 \mu\text{g}$  of F.

Initially measurement was attempted using a lanthanum-alizarin-fluorine-blue colorimetric procedure. This has a small linear calibration range (0 to  $20 \mu\text{g}$  of F in 25 ml of measurement solution) and is not sufficiently sensitive for the low fluoride levels found in many foodstuffs and was replaced by potentiometric measurements. The total ionic strength buffer solution was prepared using sodium acetate and acetic acid to avoid the use of sodium hydroxide possibly containing fluoride which would have restricted the range of linearity.

Recoveries of  $50 \mu\text{g}$  of fluoride by distillation at three different temperature ranges and collecting the distillate directly are shown in Table I. Recoveries of  $10 \mu\text{g}$  of fluoride similarly and collecting the fluoride retained on 1 g of resin column are also shown. These indicate little difference between the

TABLE I  
DISTILLATION RECOVERIES OF FLUORIDE AT DIFFERENT DISTILLATION  
TEMPERATURE RANGES

| Temperature range, °C                                     | 1st 100 ml distillate, µg of F | 2nd 100 ml distillate, µg of F | 3rd 100 ml distillate, µg of F | Recovery from 200 ml distillate, per cent. |
|-----------------------------------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------------------|
| 50 µg of F: distillate collected directly                 |                                |                                |                                |                                            |
| 135-140                                                   | 46.0                           | 3.3                            | <1                             | 98.6                                       |
| 140-145                                                   | 45.0                           | 2.2                            | <1                             | 94.4                                       |
| 145-150                                                   | 45.0                           | 2.1                            | <1                             | 94.2                                       |
| 10 µg of F: distillate passed through 1 g of resin column |                                |                                |                                |                                            |
| 135-140                                                   | 7.43                           | 1.30                           | 0.55                           | 87.3                                       |
| 140-145                                                   | 7.83                           | 0.75                           | 0.12                           | 85.8                                       |
| 145-150                                                   | 8.00                           | 0.85                           | 0.73                           | 88.5                                       |

temperature ranges but the 145-150°C range was preferred as the fluoride may distil more quickly and was used in this work. The distillation recoveries from the ion-exchange column are lower and are consistent with recoveries from foodstuffs of added fluoride (Table II). Recovery of fluoride added

TABLE II  
MEAN RECOVERIES OF ADDED FLUORIDE FROM FOODSTUFFS  
HOMOGENATES

| Food group | Mean recovery per cent. | Food group       | Mean recovery per cent. |
|------------|-------------------------|------------------|-------------------------|
| Cereal     | 92                      | Root vegetables  | 87                      |
| Meat       | 76                      | Other vegetables | 79                      |
| Fish       | 93                      | Beverages        | 86                      |
| Fats       | 79                      | Milk             | 83                      |
| Preserves  | 82                      |                  |                         |

Mean recovery for fish and beverages homogenate groups where distillate collected directly = 90 per cent.

Mean recovery for other homogenate groups where distillates passed through resin = 83 per cent.

directly to an anion exchange column was 99 per cent. for a range of 5-50 µg of fluoride.

Interferences likely in the distillation stage are from gelatinous silica, aluminium salts and boron compounds which tend to hold back fluoride evolution, but these are very low in foodstuffs. Volatile acids which would interfere with fluoride retention in the resin are also low except for chloride, which is a constituent of many foodstuffs. Chloride is immobilised during distillation by addition of silver sulphate. Table II shows the recovery of 10 µg of fluoride in the presence of 200 mg of silver sulphate with increasing amounts of chloride ion. Up to 80 mg of chloride ion in a 10-g sample (0.8 per cent.) could be tolerated in this work.

The accuracy of the procedure was assessed from a series of recovery determinations made on nine dietary foodstuff homogenates (Table III). Amounts of 5 and 10 µg of fluoride were added to 10 g each of the food groups with the exception of fish and beverage groups where 10 and 25 µg of fluoride were added; recovery at each level was made in duplicate. The calculated statistical assessments shown in Table IV indicate for the three levels of addition, the

TABLE III  
CHLORIDE INTERFERENCE IN THE DISTILLATION FLUORIDE

| Added chloride (as Cl)<br>mg | Recovery per cent.                                           |                                                                 |
|------------------------------|--------------------------------------------------------------|-----------------------------------------------------------------|
|                              | without $\text{Ag}_2\text{SO}_4$<br>in distillation<br>flask | with 0.2 g $\text{Ag}_2\text{SO}_4$<br>in distillation<br>flask |
| 0                            | 100.7                                                        | 91.7                                                            |
| 20                           | 89.6                                                         | 94.1                                                            |
| 40                           | 86.2                                                         | 85.8                                                            |
| 60                           | 0                                                            | 90.6                                                            |
| 80                           | 0                                                            | 85.9                                                            |
| 100                          | 0                                                            | 42.0                                                            |
| 120                          | 0                                                            | 25.5                                                            |

10  $\mu\text{g}$  of F: distillation temperature 145–150°C: 1 g resin column.

TABLE IV  
STATISTICAL ANALYSIS OF RECOVERY RESULTS (DISTILLATION OF  
ADDED FLUORIDE)

| Fluoride<br>added/<br>$\mu\text{g}$ | Mean<br>recovery<br>per cent. | Range<br>per cent. | No. of<br>results | Repeatability<br>per cent. $\mu\text{g}$ |      | Reproducibility<br>per cent. $\mu\text{g}$ |      | 95 per cent.<br>confidence<br>intervals |       |
|-------------------------------------|-------------------------------|--------------------|-------------------|------------------------------------------|------|--------------------------------------------|------|-----------------------------------------|-------|
|                                     |                               |                    |                   |                                          |      |                                            |      | mg/kg                                   | mg/kg |
| 5                                   | 84                            | 56–114             | 12                | 15.6                                     | 0.78 | 15.7                                       | 0.79 | 0.19                                    | 0.17  |
| 10                                  | 83                            | 70–107             | 18                | 7.2                                      | 0.72 | 11.4                                       | 1.14 | 0.16                                    | 0.24  |
| 25                                  | 86                            | 78–96              | 6                 | 6.2                                      | 1.54 | 8.8                                        | 2.21 | 0.49                                    | 0.57  |

Each array represents recovery from 10 g of homogenate and confidence intervals are similarly based on a 10 g sample mass derived from the repeatability (first value) and reproducibility (second value).

TABLE V  
REPLICATE ANALYSES FOR TOTAL FLUORIDE IN SINGLE FOODSTUFFS AND  
STANDARD MATERIALS

| Foodstuff<br>material  | Sample<br>mass/g | Mean<br>content<br>mg/kg | Range of<br>content<br>mg/kg | Amount<br>$\mu\text{g}$ | Repeatability<br>per cent. $\mu\text{g}$ |      | Reproduci-<br>bility<br>per cent. $\mu\text{g}$ |      | 95 per cent.<br>confidence<br>intervals† |       |
|------------------------|------------------|--------------------------|------------------------------|-------------------------|------------------------------------------|------|-------------------------------------------------|------|------------------------------------------|-------|
|                        |                  |                          |                              |                         |                                          |      |                                                 |      | mg/kg                                    | mg/kg |
| Liver                  | 10               | 0.095                    | 0.05–0.17                    | 0.95                    | 9.1                                      | 0.09 | 69                                              | 0.65 | 0.03                                     | 0.17  |
| Instant dried<br>milk  | 10               | 0.152                    | 0.00–0.46                    | 1.52                    | —                                        | 1.57 | —                                               | 1.67 | 0.50                                     | 0.43  |
| NBS orchard<br>leaves* | 1                | 4.9                      | 3.8–5.8                      | 4.9                     | 17                                       | 0.83 | 17                                              | 0.83 | 0.21                                     | 0.21  |
| White flour            | 10               | 0.49                     | 0.13–0.71                    | 4.9                     | 41                                       | 2.0  | 41                                              | 2.0  | 0.51                                     | 0.51  |
| Bowen's kale*          | 2                | 4.6                      | 3.3–5.3                      | 9.2                     | 3.2                                      | 0.3  | 20                                              | 1.8  | 0.09                                     | 0.49  |
| Canned<br>herring      | 4                | 7.9                      | 6.7–8.9                      | 31.6                    | 8.1                                      | 2.6  | 14                                              | 4.4  | 0.80                                     | 1.1   |
| Tea‡                   | 1                | 124                      | 119–127                      | 124                     | 2.6                                      | 3.2  | 2.6                                             | 3.2  | 0.83                                     | 0.83  |

Each array is the mean of 6 results.

\* NBS orchard leaves and Bowen's kale corrected for moisture content.

† Confidence intervals are based on a 10 g sample mass derived from the repeatability (first value) and reproducibility (second value).

‡ Distillate collected directly.

standard deviation due to analytical uncertainty (the repeatability by one analyst on one matrix), that due to all experimental variables (the reproducibility by any analyst on any matrix) and the 95 per cent. confidence intervals, calculated from the standard deviations.<sup>6</sup>

Assessment of the accuracy of results in application to foodstuffs was completed by applying the procedure to suitable standard reference materials (Bowen's kale and NBS orchard leaves) and retail foods used as laboratory standards for various levels, and the results are shown in Table V. These are uncorrected for the method recovery of 83 per cent. for low levels recovered from the resin column and 90 per cent. for higher levels recovered from direct distillation. Applying the method efficiency correction gives means of 5.5 and 5.9 mg/kg for Bowen's kale and NBS orchard leaves which compare with consensus or certified levels of 5.0 and 4.0 mg/kg.

Ninety-nine per cent. confidence intervals may be calculated for the procedure in application to foodstuffs. In general, for this exercise and for the recovery of added fluoride, these are considered to be 0.4 mg/kg up to 1 mg/kg and 40 per cent. above 1 mg/kg. Similarly, from the values of the reproducibility for both exercises the limit of detection can be derived, namely for recovery at 5  $\mu$ g of F and at 10  $\mu$ g of F the limit of detection is 0.25 and 0.33 mg/kg and for application to foodstuffs (liver and NBS orchard leaves) 0.26 and 0.33 mg/kg.

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