Online Supplementary Material

A comparison of simple analytical methods for determination of fluoride in microlitre-volume plasma samples

FV Zohoori[1], A Maguire [2] , EA Martinez-Mier [3], M Buzalaf [4], R Sanderson [5], GJ Eckert [6],

[1] School of Health and Social Care, Teesside University, UK

[2] Centre for Oral Health Research (COHR), School of Dental Sciences, Newcastle University, UK

[3] Operative Dentistry and Dental Public Health, Indiana University School of Dentistry, US

[4] Bauru School of Dentistry, University of São Paulo, Brazil

[5] Evidence & Policy Research Group, School of Natural & Environmental Science, Newcastle University, UK

[6] Biostatistician Supervisor in Biostatistics, School of Medicine, Indiana University, US

Short title: Determination of fluoride in microlitre-volume plasma

Key words: Fluoride, plasma, method, microlitre-volume

Corresponding author:
Professor FatemehVida Zohoori;
Health and Social Care Institute
Teesside University
Middlesbrough, TS1 3BA
UK

The current supplement presents the three analytical protocols which were used in this manuscript: 1) gold standard HMDS-diffusion method [Martínez-Mier et al., 2011]; 2) Micro-diffusion method [Martinez-Mier et al., 2010]; and 3) Known-addition technique [Ekstrand, 1977; Thermo Electron Corporation Instruction Manual, 2003].

References:

Ekstrand J: A micromethod for the determination of fluoride in blood plasma and saliva. Calcif Tissue Res 1977;23:225-228.

Martinez-Mier E, Buckley C, Chandrappa P, Soto-Rojas AE: Development of a Standard Fluoride Analytical Method for Dental Plaque. J Dent Res 2010;89:931.

Martínez-Mier EA, Cury J.A., Heilman J.R. , Katz B.P., Levy S.M., Li Y., Maguire A., Margineda J., O'Mullane D., Phantumvanit P., Soto-Rojas A.E., Stookey G.K., Villa A., Wefel J.S., Whelton H. , Whitford G.M., Zero D.T., Zhang W., Zohouri V: Development of gold standard ion-selective electrode-based methods for fluoride analysis Caries Res 2011;45:3-12.

Thermo Electron Corporation. Fluoride electrode instruction manual. Thermo Orion, 235238-001 rev. B; 2003.

HMDS-diffusion Method

Step 1) Setting up Diffusion

i) Make a small hole in the lid of each polyethylene petri dish using a soldering iron.

ii) Place 1.0 g of each F standard, sample or blank into the bottom of individual petri dishes. Add 2.0 mL of $DDiH_2O$.

iii) Pipette **50** μL of the 0.05N NaOH (i.e. trapping solution) on the inside surface of the top portion (lid) of the petri-dish in **3-5** equal drops. Position the lid on the dish. Seal the petri dish with Vaseline and/or parafilm, ensuring a **tight seal is formed**.

iv) Pipette **1.0** mL of 3N HMDS-saturated H_2SO_4 through the hole and **immediately** seal the hole with Vaseline and/or seal with parafilm. Gently rotate each dish to assure mixing (this should be repeated periodically during the diffusion period). Samples may be placed on a rotary shaker to be gently swirled throughout the diffusion process). Allow the standards and samples to diffuse for **16-24** hours at room temperature.

Step 2) Preparation of NaOH Trap for Analysis

Gently remove the lid from the first petri dish. Add **25** μL of 0.2N Acetic Acid. Collect the buffered solution in the lid of the petri dish, and adjust the final volume to 75 μL by the addition of $DDiH_2O$.

Step 3) Fluoride measurement using Fluoride Ion-Selective Electrode (ISE) and meter

i) Place the electrode directly on the solution in the petri dish lid, making sure the solution being analysed 'wets' the entire tip of the electrode.

ii) Verify the meter is in the **mV mode.**

iii) Allow the electrode to stabilize as defined by manufacturer.

iv) Record the millivolt reading for the standard or sample or blank on a data sheet.

v) Construct a calibration line to convert millivolt readings into concentration values

Note: Checking the drift in the electrode

It is recommended to run a low concentration standard sample (e.g. 0.1 ppmF) alongside each series of analyses to check for a possible drift in the electrode. If the drift in the electrode is more than 3% for the lowest standard, then a new calibration line should be derived using a fresh set of fluoride standards. Therefore;

- Read a low concentration F standard (0.1 ppmF) at the beginning of every run and record the millivolt reading.
- Repeat the reading for the same F standard (0.1 ppmF) after every 21st aliquot analysed (i.e. 7th sample, in triplicate).
- If the change in mV readings was more than +/- 3%, construct a new calibration line using another set of fluoride standards.

Micro-diffusion Method

Step 1) Setting up Diffusion

i) Make a small hole in the lid of each polyethylene petri dish using a soldering iron.

ii) Place **200** µL of each F standard, sample or blank into the bottom of individual petri dishes. Add **400** µL of $DDiH_2O$.

iii) Pipette **10** μL of the 0.05N NaOH (i.e. trapping solution) on the inside surface of the top portion (lid) of the petri-dish in 2 equal drops. Position the lid on the dish. Seal the petri dish with Vaseline and/or parafilm, ensuring a **tight seal is formed**.

iv) Pipette **200** µL of 3N HMDS-saturated H_2SO_4 through the hole and **immediately** seal the hole with Vaseline and/or seal with parafilm. Gently rotate each dish to assure mixing (this should be repeated periodically during the diffusion period). Samples may be placed on a rotary shaker to be gently swirled throughout the diffusion process). Allow the standards and samples to diffuse for **16-24** hours at room temperature.

Step 2) Preparation of NaOH Trap for Analysis

Gently remove the lid from the first petri dish. Add **5** μL of 0.2N Acetic Acid. Collect the buffered solution in the lid of the petri dish, and adjust the final volume to 20 μL by the addition of $DDiH_2O$.

Step 3) Fluoride measurement using Fluoride Ion-Selective Electrode (ISE) and meter

i) Place the electrode directly on the solution in the petri dish lid, making sure the solution being analysed 'wets' the entire tip of the electrode.

ii) Verify the meter is in the **mV mode.**

iii) Allow the electrode to stabilize as defined by manufacturer.

iv) Record the millivolt reading for the standard or sample or blank on a data sheet.

v) Construct a calibration line to convert millivolt readings into concentration values

Note: Checking the drift in the electrode

It is recommended to run a low concentration standard sample (e.g. 0.1 ppmF) alongside each series of analyses to check for a possible drift in the electrode. If the drift in the electrode is more than 3% for the lowest standard, then a new calibration line should be derived using a fresh set of fluoride standards. Therefore;

- Read a low concentration F standard (0.1 ppmF) at the beginning of every run and record the millivolt reading.
- Repeat the reading for the same F standard (0.1 ppmF) after every 21st aliquot analysed (i.e. 7th sample, in triplicate).
- If the change in mV readings was more than +/- 3%, construct a new calibration line using another set of fluoride standards.

Known-addition Technique

Step 1) Calculation of slope (m) using two F standards

i) Prepare two F standards: 0.1 and 1 ppm F standards

ii) Mix each standard with TISAB III (10:1 v/v)

iii) Measure the mv readings of the standards, directly

iv) Record the mV readings of the two standards in the excel data sheet

v) Subtract the first reading from the second reading to find the electrode slope (m)

Step 2) Preparation of standards for known-addition

Notes:

- Sample concentration should roughly be known (within a factor of three)
- Concentration (µg/mL) of fluoride standards **(Cst)** for each individual sample should be 100 times more concentrated than the sample's concentration.

i) Prepare fluoride standard solutions for each individual sample according to the estimated concentration of each particular sample: e.g. **Cst** should be 2 µg/mL, if the fluoride concentration of the sample is projected to be around 0.02 µg/mL

Step 3) Sample preparation and analysis

For each individual sample, prepare two aliquots:

I. Aliquot (I) with no added fluoride standard: Pipette 200 µL **(Vu)** of a sample into a small container and add 20 µL of TISAB III. Measure and record mV **(E1)** of the sample using a fluoride combination electrode and pH/mV meter.

II. Aliquot (II) with added fluoride standard: Pipette 200 µL **(Vu)** of the same sample into a small container, add 20 µL **(Vs)** of appropriate fluoride standard **(Cst)** and **22 µL** TISAB III. Measure and record mV **(E2)** of the sample using a fluoride combination electrode and pH/mV meter.

Step 4) Calculation of sample concentration

Use the following formulas to calculate the F concentration of samples:

$$Cu = Cs \times [Vs/(Vu + Vs)] / [(10^{(E2-E1)/m}) - (Vu/(Vu + Vs))]$$

Abbreviations:

Cu = F concentration of the unknown sample (ppm)

Cs = F concentration of the F standard (ppm)

Vs = volume of standard (µl)

Vu = volume of sample (µl)

E1 = electrode potential (mV) of the sample (aliquot I)

E2 = electrode potential (mV) of the sample after the addition of F standard (aliquot II)

m = the electrode slope.