The Significance Of Method Validation In The Determination Of The Best Analytical Techniques For The Analysis Of Fluoride In Various Water Matrices.

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Abstract: There are various analytical methods that can be used for the determination of fluoride in water samples. The best method for a particular use can be best ascertained using method validation based on the needs of the customer and the affordability to conduct the said tests. The following factors are critical in determination of which method to select viz. quality, cost, turn around time and competency of personnel conducting the tests, etc. The best method was selected using method validation concept for the determination fluoride concentration in potable water, borehole, leachate, catchments and industrial effluent samples. The method covers the range from 0.1 – 2.0 mg/L F^-, this is the concentration range for most of different water samples analyzed.

Introduction

Fluoride determination in water samples can be done using various analytical techniques. These techniques range from fairly simple to complex methods requiring a some knowledge of fundamentals of chemistry. The ion selective electrode techniques is widely the most used, cost effective and reliable method. Over the years this techniques has been modified to suite various condition of application. However, more advance techniques over the last three two decades have been developed, which prove to be more reliable, competitive, inexpensive, shorter turnaround times, better accuracy and precision, etc. The need to utilize method validation has also increased over the years as a requirement of ISO/IEC 17025:2005 and as basis to establish point of reference to know what the capabilities of the method is. Many handbook of chemistry addresses method validation at length, to determine which method is best for a particular situation.

Fluoride method was validated using two analytical techniques viz, ion selective electrode (ISE) method and the flow injection analyzer (FIA). was The following analytical techniques were validated and

2. PRINCIPLE

Ion selective electrode is used as an indicator electrode in potentiometric titrations. The ion activity is interpolated by means of a calibration curve. The calibration curve is established with standard solutions. The determined analytical measurement is normally the concentration of a substance (and not its activity). Measurements are therefore executed at fixed ionic strength. The ionic strength is fixed with a TISAB (Total Ionic Strength Adjustment Buffer).

Fluoride is an essential trace element of a normal diet and is required from birth until permanent teeth have been formed. Fluoride is therefore essential for the development of resistance to dental caries. The Department of Health has legislated regulations indicating that water service providers are obliged to fluoridate water to a concentration level of up to 0,7 mg F/l.

The present fluoride concentration in potable water put into the reticulation system varies from **0,18 to 0,2 mg F/l**. To meet the 0,7 mg F/l required by the new legislation it would require Water Boards to increase the concentration level of fluorides in its potable water supply by an average of 0,5 - 0,6 mg F/l.

3. INTERFERENCES

The fluoride interferences may occur due to $(OH^-) < 10^{-1}$.

4. PRESERVATION AND STORAGE

4.1 No sample preservation is necessary before the analysis is done.

5. SAFETY PRECAUTIONS

4.1 Wear gloves and when handling the reagents.

6. SAMPLE PREPARATION

6.1 No sample preparation is necessary before the analysis is done.

7. APPARATUS

7.1 Mettler Delta 350 ion meter

7.2 Volumetric flasks, 1000ml, 200ml, 100ml

7.3 Pipettes, graduated, 1ml

7.4 Pipettes, bulb, 5ml, 10ml, 20ml, 25ml

7.5 Measuring cylinders, 100ml

8. REAGENTS

Water used in the preparation of reagents and standard solutions is deionised water prepared by reverse osmosis, with conductivity< 1 µS/cm.

9.1 TISAB (Total Ionic Strength Adjustment Buffer). TISAB is made using the following reagents:

9.1 Sodium Chloride, NaCl

9.2 Complexion IV, (*1, 2 di-amino cyclohexane N, N, N', N" tetraaceticacid*).

9.3 Sodium Hydroxide, NaOH.

9.4 Glacial acetic acid, C_2H_5OOH.

Preparation of TISAB

9.5 Dissolve 116g of NaCl in approximately 1000ml distilled water in a 2L volumetric flask.

9.6 Add 10g of Complexion IV, (*1, 2 di-amino cyclohexane N, N, N', N" tetraaceticacid*) into the solution.

9.7 Mix well and dissolve by drop wise addition of 8mol/L NaOH*.

9.8 Add 114mL of glacial acetic acid.

9.9 Adjust the pH to between 5.0 and 5.5 by adding more 8mol/L NaOH.

9.10 Make the solution up to volume with distilled water and mix well.

9.11 The shelf life of the buffer solution is 6 months.

Preparation of 8mol/L NaOH*

9.12 issolve 320g of NaOH pellets into approximately 500mL distilled water in a1000mL glass beaker.

9.13 This is an exothermic process, cautiously stir mix and add more distilled water to about 850mL.

9.14 Cool the beaker in a water bath of cold water and after cooling transfer into a 1L volumetric flask and make up to volume with distilled water.

10. Sodium Fluoride, NaF.

10.1 Stock solution of 1000mg F/L:

Dissolve 2.21 g NaF in a 1000mL volumetric flask and make up to volume with distilled water. This is the stock solution of 1000 mg F/L. The shelf life of the stock solutions is 12 months and is are kept in the refrigerator.

10.2 Intermediate stock of 10 mg F/L:

Pipette 10.0mL of 1000 mg F/L stock solution into 1000mL volumetric flask and make up to volume with distilled water. This is the working stock solution of 10mg F/L. The shelf life of the intermediate stock solutions is 6 months and is are kept in the refrigerator.

10.3 Calibration Standards of the following concentrations are used for calibration: The shelf life for the standards is 1 month.

Table 1. Calibration standards

Concentration (mg/L)	Volume to pipette from working stock.	Volumetric flask to be used for standards.
0.1	10 mL	1000 mL
0.2	20 mL	1000 mL
0.5	50 mL	1000 mL
1.0	100 mL	1000 mL
2.0	200 mL	1000 mL

11. METHOD

11.1 Switch on the instrument.

11.2 The default screen comes on with concentration display.

11.3 Start by checking your calibration points. Press “Prog”.

11.4 A screen with three options “calibration”, “display” and “system” appears.

11.5 Choose “calibration” by pressing “enter” key, press “enter” again to end up on “Cal. Points”.

11.6 A table of 5 calibration points appears on the top right hand corner. The original values are 1, 10, 100, 1 000 and 10 000. You need to change them by pressing “enter” key then use the keyboard to enter new values (use the arrows to move the curser to where you need to enter values). Currently we use 0.1, 0.2, 0.5, 1.0, and 2.0 mg/L.

11.7 After typing the value press “enter” key to accept, until all the values have been entered.

11.8 Go back to the measurement mode by pressing the up arrow three times and the default screen will appear.

12. CALIBRATION

12.1 Calibration must be performed before analysis is done.

12.2 Press “cal” key and a calibration table appear on the screen.

12.3 Place your first standard with a stirrer. Note to only start stirring once the electrodes are immersed in the solution to avoid air bubbles building up on the electrode membrane causing unstable readings.

12.4 Wait for approximately three minutes for the ion meter to stabilize then press, “read” key. A millivolt reading will appear on the screen, record this value on the logbook to plot the calibration curve.

12.5 Rinse the probe with distilled water and blot gently with soft paper towel. Put in your second standard and repeat the procedure until all the calibration standards are used.

12.6 When calibrating the last standard the “read” key must be pressed twice to end up on the default screen with the concentration of the last standard displayed.

12.7 When calibrating the last standard the “read” key must be pressed twice to end up on the default screen with the concentration of the last standard displayed.

12.8 Calibration is now complete, plot the calibration curve using MS-Excel and the slope must be within the lower limit (**-62.12**) and the upper limit (**-56.20**). The ideal slope of the calibration curve from the Nernst equation is –59.16.

12.9If the calibration slope does not fall within the above limits, re-calibrate the ion meter and follow the procedure outlined from 10.1 above.

12.10 Once re-calibration has been performed and the calibration slope still does not fall within the limits, re-prepare calibration standards, and follow step 12.1 and 12.2 on maintenance of instrument. Re-calibrate the ion meter and follow the procedure outlined from 10.1 above.

12.11 The ion meter is now ready to read samples, starting with quality control sample.

12.12 Remember to use 1 ml of TISAB for every 1 ml of sample, including QC (usually 20ml/20ml).

12.13 Let the sample stabilize then record the reading, report the results to the nearest 0.01mg/L.

14. QUALITY ASSURANCE

14.1 The Quality Assurance (QA) standard solution of 0.5 mg F/l is prepared from 10.0 mg/l F intermediate QA stock solution that is also prepared from 1000.0 mg F/L QA stock solution. The preparation of QA stock solution is done using an alternative supplier and these are kept in the refrigerator.

14.2 Insert a QA standard solution at the start of the analysis and then after each batch, a batch being 10 samples. In the event of there being less than 10 samples in a batch, a QA solution will be run after the last sample.

14.3The analyst who performed the validation of the method shall be deemed competent to perform the analysis. Continuous competence of analysts will be determined by using Proficiency Testing samples from any organizer such as SABS.

15. MAINTENANCE

Rinse the probe with distilled water and blot gently with soft paper towel before every reading is taken.

Wash the fluoride electrode with liquid dish washing soap using your hand when necessary and rinse thoroughly with distilled water.

Please refer to Instrument Manual page 40 for other detailed maintenance.

RESULTS

1. Method Limit of Detection (MLD) and Limit of Quantification (LQC)

A blank solution of de-ionized water was used to determine the MLD and LQC. The summary is as tabulated below:

Table 2: Method parameters

Min	**0.0014**
Max	**0.0135**
Mean	**0.0066**
stdev	**0.0032**
RSD (%)	**48.01**
MLD (mg/l F)	**0.02**
MLQ (mg/l F)	**0.04**

The lowest quantifiable concentration for fluoride will be reported as

< 0.04 mg F/L.

2. Calibration Range

The ion meter was calibrated using the following standards with varying concentration; 0.1, 0.2, 0.5, 1.0, and 2.0 mg F/ L. The calibration range covers all the types of samples analysed by the ion meter. The calibration curve produced has a negative slope since z = -1 for the fluoride ion, F^-. The calibration is the function of the potential difference in mV versus the concentration. See attached graph:

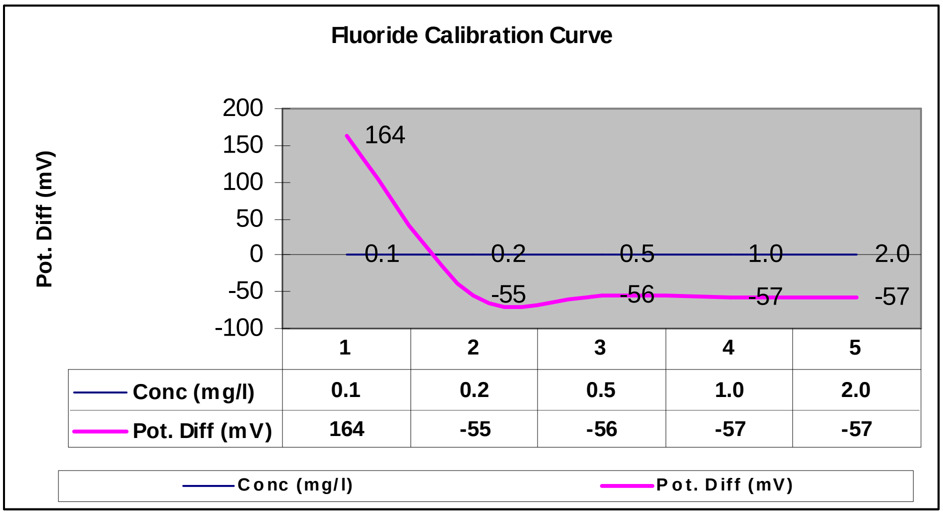

Figure 1: Calibration curve

3. Accuracy

Accuracy was determined using the lowest, middle and the highest range of standard solutions over a period of three consecutive days. The summary is as tabulated below:

Table 3: A. Lowest standard (0.10 mg F/ L)

	11 April 2006	12 April 2006	13 April 2006
Mean	0.0990	0.0993	0.0993
stdev	0.012	0.009	0.009
% RSD	12.35	9.56	9.48
Min	0.0850	0.0881	0.0859
Max	0.1190	0.1182	0.1112

Table 4: B. Middle standard (0.50 mg F/ L)

	11 April 2006	12 April 2006	13 April 2006
Mean	**0.504**	**0.501**	**0.498**
stdev	**0.015**	**0.009**	**0.009**
% RSD	**2.89**	**1.74**	**1.83**
Min	**0.480**	**0.489**	**0.482**
Max	**0.520**	**0.519**	**0.512**

Table 5: C Highest standard (2.00 mg F/ L)

	11 April 2006	12 April 2006	13 April 2006
Mean	**2.00**	**1.99**	**2.01**
stdev	**0.012**	**0.030**	**0.029**
% RSD	**0.58**	**1.52**	**1.46**
Min	**1.98**	**1.96**	**1.95**
Max	**2.02**	**2.05**	**2.04**

From the above tables the % RSD for the middle and highest standard is well below the method uncertainty of 8.34%. Lowest standard has the high % RSD since the results reported for 0.10 mg F/ L are close to the detection limit. The accuracy is acceptable in all three cases.

% Recovery was determined by spiking the unknown samples with known concentration (0.50 mg F/ L) of the analyte. The % recovery of **99.8 %** was achieved. See the attached table:

Table 6: Method Recovery

	Unspike tap water sample (mg F/L)	Spiked Tap Water with 0.5 mg/l (mg/l F)
Mean	**0.181**	**0.681**
Stdev	**0.01**	**0.01**
% RSD	**3.43**	**0.92**
	% RECOVERY	**99.8**

4. Precision

Precision was determined using the real reticulation water sample analyzed over a period of three consecutive days. The summary is as tabulated below:

Table 7: Precision of the method summary

	11 April 2006	12 April 2006	13 April 2006
Mean	**0.181**	**0.181**	**0.183**
stdev	**0.006**	**0.003**	**0.003**
% RSD	**3.43**	**1.74**	**1.88**
Min	**0.168**	**0.175**	**0.178**
Max	**0.190**	**0.186**	**0.189**

From the above tables the % RSD for the sample is less than 3.5% which is far less than the method uncertainty of 8.34%. The precision is acceptable.

5. Uncertainty of measurement

The determination of the uncertainty of measurement was done by making 25 replicate measurements of the quality assurance sample with the known concentration of 0.50 mg F /L

The % relative standard deviation for the measurements was found to be 4.17%. The uncertainty was ±6.62%. The following formula was used to calculate the uncertainty of measurement.

U = $\left(\frac{2s}{x}\right)X100$, where U – is the uncertainty.

s – is the standard deviation.

X – is the mean.

6. Conclusion

All the results obtained during the validation are attached hereunder as proof of analysis done and presented in MS Excel spreadsheet. From the above calculations of the tolerances, the method is deemed fit for intended use.

REFERENCE

1. Mettler Delta 350 Instruction Manual.
2. Standard Method for the Examination of water and wastewater by L.S. Clesceri, A.E.Greenberg and R.R. Trusell, 20th Edition.