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# VALIDATION OF THE USE OF ION CHROMATOGRAPHY FOR ANALYSIS OF FLUORIDE IN WATER

**Hazil Anwar [1], Vivi Sisca[2], Elfizari P Siregar[1], Elizarni[1], Fejri Subriadi[1] and Sri Elfina[1,✉]**

[1]ATI Padang Polytechnic Jl. Bungo Pasang Tabing, Padang Sumatera Barat, Indonesia
[2]Research Centre for Chemical, Research Organisation for nanotechnology and materials, National Research and Innovation Agency (BRIN), Puspiptek, Serpong-Tangerang Selatan, Indonesia
✉Corresponding Author: srielfina73@gmail.com

**ABSTRACT**

This research aims to validate the use of ion chromatography to determine fluoride anions. Determining the fluoride anion content in water is crucial to ensure its quality. Ion chromatography has proven to be efficient in this analysis, adhering to the APHA 4110B-2017 standard. Before being routinely used, the method must be verified to ensure adequate linearity, precision, accuracy, and detection limits. Testing shows a high correlation value (0.9986), with a detection limit (LoD) of 0.0034 mg/L and a quantification limit (LoQ) of 0.1132 mg/L. Precision (%RSD) is 1.02%, while accuracy (recovery) reaches 105%. Linearity analysis indicates F-calculated < F-table (1.42 < 3.18), validating the method for determining fluoride levels in water through ion chromatography.

**Keywords:** Method Validation; Ion Chromatography; Water Quality; Fluoride.

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## INTRODUCTION

Water is a vital element for life across the globe, providing essential benefits for humans, animals, and plants.[1] The significance of water in life makes it a crucial environmental component for sustenance.[2] Water also serves as a relatively high source of fluoride intake, emphasizing the importance of monitoring fluoride levels to prevent potential overexposure. Fluoride, as a chemical compound in water, has significant health implications. While it has beneficial effects in preventing tooth decay at specific concentrations, excessive exposure can lead to adverse effects.[3] Adverse effects range from mild dental fluorosis (discoloration and spots on teeth) to skeletal fluorosis, depending on the level and duration of exposure. Conversely, fluoride deficiency can result in tooth decay. Excessive exposure, in extreme cases, can cause severe poisoning with serious health impacts, including bone deformities and even death.[4] Government regulations, such as Minister of Health Regulation No. 32 of 2017, reinforce the importance of monitoring fluoride levels in water, which sets a limit of 1.5 mg/L for water used in sanitation. This standard aligns with the World Health Organization's guidelines.[5] Various methods, including spectrophotometry and ion chromatography, can determine fluoride anion levels. Ion chromatography, as an efficient method, requires no additional substances in the sample preparation process and has been recognized as a standard method, as exemplified by the American Public Health Association (APHA) 4110B-2017 method.[6,7] However, method verification is necessary before routine use to ensure its suitability and reliability. In light of this framework, this study aims to verify the method for analyzing fluoride anions in clean water using ion chromatography at the PT Medialab Indonesia laboratory. The verification aims to ensure that the method is suitable and reliable for everyday fluoride anion analysis.

## EXPERIMENTAL

### Materials and Equipment

The materials used include a standard fluoride ion solution of 1000 mg/L, ultrapure water, sodium carbonate, sodium bicarbonate, and clean water samples.

The equipment includes CIC-D120 ion chromatography, vacuum, ultrasonicator, micropipette, volumetric flask, watch glass, analytical balance, spatula, spray bottle, syringe, filter membrane, vial, and eluent bottle.


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**Preparation of Solutions**

1. Prepare a standard Fluoride solution at 10 mg/L by adding 1 ml of standard fluoride ion solution 1000 mg/L to a 100 mL volumetric flask and diluting with ultrapure water.
2. Preparation of Fluoride working solutions (0.2; 0.5; 1; 1.5) mg/L by taking specific volumes of standard solution and diluting with ultrapure water.
3. Preparation of the mobile phase/eluent by dissolving sodium carbonate and sodium bicarbonate, followed by vacuum filtration.
4. Preparation of test samples by extracting clean water using a syringe, filtering with a filter membrane, and transferring it into vials.

**Instrument Optimization Stage**

Gradual increase in flow rate in ion chromatography, ensuring pump pressure remains stable, and column and detector temperatures are optimal.

**Testing Phase**

A linearity test is used to measure the concentration of working solutions using ion chromatography and statistically process measurement results to check correlation, slope, and intercept.

Linearity Level Test by measuring the lowest and highest concentrations of fluoride ion working solutions ten times each and calculating the F-value.

Precision Test (Repeatability) by measuring precision test solutions and processing results to determine the relative standard deviation (%RSD).

Accuracy Test by measuring accuracy test solutions and calculating the % recovery.

LoD Test by measuring LoD test solutions and checking for a positive response during measurement.

MDL and LoQ by measuring MDL and LoQ test solutions and processing statistical results to obtain percent relative standard deviation (%RSD), percent recovery (%Recovery), signal-to-noise, method detection limit, and quantification limit.

LoQ Confirmation Test by measuring LoQ confirmation test solutions and calculating percent standard deviation (%RSD) and % recovery.

## RESULTS AND DISCUSSION

Based on the method verification tests that have been conducted, the results include parameters of linearity, Linearity Level, Limit of Detection (LoD), Method Detection Limit (MDL), Limit of Quantification (LoQ), Precision (Repeatability), and accuracy (% Recovery). The verification results can be seen in Table-1.

Table-1: Method Verification Results

| No | Parameter | Results | Acceptance Conditions | Source | Conclusion |
|---|---|---|---|---|---|
| 1 | Linearity (r) | r = 0.9986 | r ≥ 0.990 | 7 | MS* |
| 2 | *Levels of Linearity* | 1.42 < 3.18 | F count < F table | 8 | MS* |
| 3 | Precision (Repeatability) | 1.02 ≤ 10.97 | %RSD ≤ 0.5 CV Horwitz | 8 | MS* |
| 4 | Accuracy (Recovery) | 105 | 75-125 | 7 | MS* |
| 5 | *Limit of Detection (LoD)* | 0.0034 mg/L | Positive Response | 9 | MS* |
| 6 | *Limit Detection Method*(MDL) and Limit of Quantification | Theoretical MDL= 0.0356 mg/L | Reads positive | 7<br>8 | MS* |
| | | S/N = 9.89 | 2.5-10 | | |
| | | %Recovery = 102% | %Recovery= (75-125)% | 9 | |
| | | 0.0356 < 0.1221 < 0.3558 | MDL < Spike < 10MDL | | |
| | | 0.1132 < 1.5 | LoQ ≤ BML | | |
| | | 10.1 ≤ 14.59 | %RSD≤0.67 CV Horwitz | | |

| | | | | | |
|---|---|---|---|---|---|
| 7 | Confirmation *Limit of Quantification (LoQ)* | 10.9 ≤ 14.8 | %RSD≤0.67 CV Horwitz | 8 | MS* |

MS*: Meets the requirements

The results of linearity determination are expressed in correlation and determination coefficients. The calibration curve for the fluoride ion standard solutions can be seen in Fig.-1. The measurements in Fig.-1 show a correlation coefficient (r) value of 0.9986, exceeding the acceptance criterion of ≥0.990 according to APHA 4110B 2017. The relationship between the concentration of the anion and the linear area indicates a positive correlation. The linearity results also note an intercept value (a) of 326813[10], showing the influence of the sample matrix in the solution. Although ideally, the intercept is smaller than the slope value (b) [11], Hadi and Asiah consider the ideal intercept value zer.[12] The slope value (b) of 24514428 reflects the high sensitivity of a testing method, and the intercept and slope values for fluoride anion analysis meet the acceptance criteria, providing linear results.[9] The Linearity Test measured the standard fluoride solution ten times at 0.2 mg/L and 1.5 mg/L concentrations. The results from two standard deviations were compared using the F-ratio, and the measurements of the linearity curve limits can be found in Table-2.

Table-2: Level of Linearity

| Sample | Area | |
|---|---|---|
| | Lowest Standard | Highest Standards |
| 1 | 5012534.00 | 38374947.00 |
| 2 | 4927771.00 | 38439840.00 |
| 3 | 5026344.00 | 38463306.00 |
| 4 | 5016075.00 | 38389834.00 |
| 5 | 5008460.00 | 38368160.00 |
| 6 | 5033601.00 | 38406451.00 |
| 7 | 5059400.00 | 38463306.00 |
| 8 | 5015214.00 | 38399107.00 |
| 9 | 5058731.00 | 38448906.00 |
| 10 | 5018957.00 | 38336256.00 |
| SD | 36453,446 | 43503.23 |
| $SD^2$ | 1328853756.5 | 1892531420 |
| F count | 1.42 | |
| F table (df = n-1, □ 95%) | 3.18 | |

Based on Table-2, the calculated F-value is 1.42, while the critical F-value at a 95% confidence level with degrees of freedom n-1 is 3.18. Since the calculated F-value < critical F-value, H0 is accepted, indicating that the precision level of the lowest working solution area is not significantly different from the accuracy level of the highest working solution area. This result confirms that ion chromatography's concentration range of 0.2 mg/L to 1.5 mg/L exhibits linear regression in the fluoride testing method.[13] If the sample has a high concentration, diluting it to fall within the established working range is recommended. Precision describes the analysis error resulting from random errors, representing uncertainty in measurements due to disturbances and measurement conditions.[14] Precision can be measured as the Relative Standard Deviation (RSD) value, and the results of determining the standard deviation of the anion can be found in Table-3.

Based on the results of ten repeated measurements of the precision test samples in Table-3, it is observed that %RSD ≤ 0.5 CV Horwitz, precisely 1.02 ≤ 10.97. These values meet the precision acceptance criteria established by PT Medialab Indonesia. The obtained RSD values in this precision test indicate that the precision test results fall into the category of high accuracy. Accuracy is measured as the percentage recovery (%recovery) obtained by comparing the difference in analyte concentration in the sample before and after the addition of the standard, considering the standard added to the sample.[11] The results of the accuracy test can be found in Table-4.

In the accuracy data processing, the calculation of recovery values indicates that out of ten repetitions, the %recovery values fall within the range of (103-108%) with an average of 105%. These results meet the acceptance criteria set by the Testing Laboratory of PT Medialab Indonesia, which is within the range of (75-125%).[7] According to EURACHEM[15], the detection limit can be determined by measuring a blank

sample, a sample matrix without the analyte content. Testing the limit of detection involves calculating the theoretical LoD, and the academic LoD concentrations can be found in Table-5.

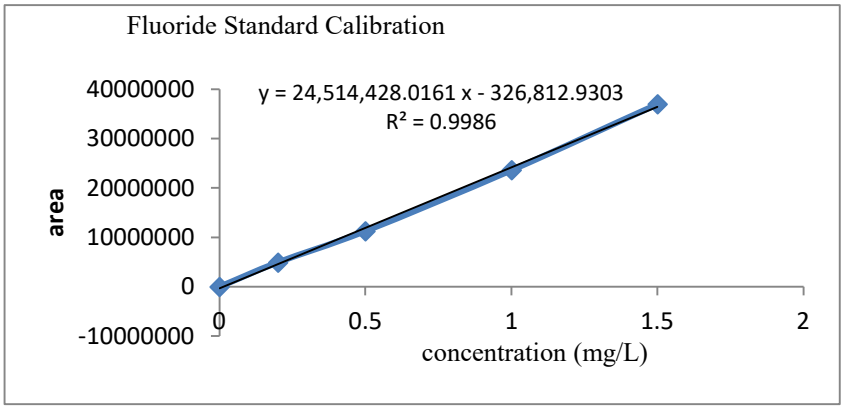


Fig.-1: Relationship between Concentration and Area Size

Table-3: Precision (Repeatability)

| Repetition | C Actual (mg/L) |
|---|---|
| 1 | 0.1215 |
| 2 | 0.1215 |
| 3 | 0.1221 |
| 4 | 0.1222 |
| 5 | 0.1222 |
| 6 | 0.1230 |
| 7 | 0.1235 |
| 8 | 0.1241 |
| 9 | 0.1247 |
| 10 | 0.1247 |
| Average | 0.1230 |
| Standard Deviation (SD) | 0.0013 |
| %RSD | 1.02 |
| CV Horwitz | 21.93 |
| 0.5 CV Horwitz | 10.97 |

Table-4: Accuracy

| Test | Cx | Cs | Vx | vs | C Targets | Area | C Actual | Recovery |
|---|---|---|---|---|---|---|---|---|
| | (mg/L) | (mg/L) | (mL) | (mL) | (mg/L) | | (mg/L) | %Rec |
| 1 | 0.1023 | 0.50 | 9.50 | 0.50 | 0.1222 | 3873288 | 0.1252 | 103 |
| 2 | | | | | 0.1222 | 3735397 | 0.1254 | 103 |
| 3 | | | | | 0.1222 | 3241259 | 0.1265 | 104 |
| 4 | | | | | 0.1222 | 3301012 | 0.1275 | 104 |
| 5 | | | | | 0.1222 | 3561802 | 0.1288 | 105 |
| 6 | | | | | 0.1222 | 3903331 | 0.1291 | 106 |
| 7 | | | | | 0.1222 | 3836870 | 0.1293 | 106 |
| 8 | | | | | 0.1222 | 3717528 | 0.1304 | 107 |
| 9 | | | | | 0.1222 | 3804683 | 0.1307 | 107 |
| 10 | | | | | 0.1222 | 3855842 | 0.1322 | 108 |
| Average | | | | | | | 0.1285 | 105 |
| Acceptance Conditions | | | | | | | (75 - 125)% | |

Table-5: LoD

| Test | Area | Concentration (mg/L) |
|---|---|---|
| 1 | 137810 | 0.0055 |
| 2 | 143138 | 0.0057 |
| 3 | 223082 | 0.0089 |
| 4 | 116492 | 0.0047 |
| 5 | 121822 | 0.0049 |
| 6 | 4567 | 0.0002 |
| 7 | 239309 | 0.0096 |
| 8 | 191293 | 0.0076 |
| 9 | 1679 | 0.0001 |
| 10 | 8375 | 0.0003 |
| Average | | 0.0048 |
| SD | | 0.0035 |
| SD' | | 0.0011 |
| LoD | | 0.0034 |

The instrument can read analyte concentrations greater than or equal to the limit of detection (LOD) value. However, if the analyte concentration is smaller than the LOD, the signal cannot be relied upon as an analyte signal[16] The LOD value is 3 times the standard deviation (SD'). Testing against this performance parameter meets the acceptance criteria set by PT Medialab Indonesia, which requires producing a positive response.[9] The Quantification Limit Detection method can be established by measuring prepared solutions using the spiking technique. In this test, the theoretical concentrations of MDL and LoQ can be found in Table-6.

Table-6: MDL and LoQ

| Test | Area | Concentration (mg/L) | %Rec |
|---|---|---|---|
| 1 | 4030065.00 | 0.1068 | 87 |
| 2 | 3802423.00 | 0.1166 | 95 |
| 3 | 3886836.00 | 0.1214 | 99 |
| 4 | 3739588.00 | 0.1111 | 91 |
| 5 | 3750990.00 | 0.1241 | 102 |
| 6 | 3825264.00 | 0.1166 | 95 |
| 7 | 2742104.00 | 0.1307 | 107 |
| 8 | 3148000.00 | 0.1347 | 110 |
| 9 | 3255736.00 | 0.1407 | 115 |
| 10 | 2938525.00 | 0.1445 | 118 |
| Average | | 0.1247 | 102 |
| SD | | 0.0126 | |
| %RSD | | 10.1 | |
| %Recovery | | 102 | |
| S/N | | 9.9 | |
| 0.67 CV Horwitz | | 14.59 | |
| MDLtheoretical | | 0.0356 | |
| 10MDL | | 0.3558 | |
| LoQtheoretical | | 0.1132 | |

The criteria and results of the performance parameter testing of the analysis method. The method's detection limit is acceptable if it meets several criteria, such as %RSD < 0.67 Horwitz, %Recovery 75-125%, S/N 2.5-10, MDL < Spike < 10MDL, and LoQ ≤ BML.[12] The average relative standard deviation (%RSD) values meet the acceptance limit of 10.10%, less than the Horwitz coefficient of 0.67.[10,9] Accuracy, measured in percentage recovery, also meets the acceptance criteria, with an average percentage recovery in the 87-118% range.[7] The signal-to-noise (S/N) ratio of 9.9 meets the acceptance criteria of 2.5-10.[17] Determining the spike level in MDL ensures that the results meet the acceptance criteria, where the spike

level should be 1/10 of the MDL. The spike level used meets the acceptance criteria, 0.0356 < 0.1221 < 0.3558 (MDL < Spike level < 10 MDL). Furthermore, the quantification limit test results show that the theoretical LoQ is 0.1132 mg/L, reflecting the low concentration of the analyte that can be read by the instrument with acceptable precision and accuracy.[12] The LoQ value meets the acceptance criteria of PT Medialab Indonesia, and the overall performance parameter test results meet the established standards. Based on the quantification limit test, the theoretical LoQ obtained is 0.1132 mg/L, according to the literature.[18] To ensure the success of the instrument readings, this value needs to be confirmed by measuring samples added to the standard fluoride anion solution with a concentration of 2.0 mg/L using Ion Chromatography instrumentation. The results of the confirmation test for the quantification limit can be found in Table-7.

Table-7: LoQ Confirmation

| Test | Area | Concentration (mg/L) | %Rec |
|---|---|---|---|
| 1 | 3077933.00 | 0.1229 | 111.48 |
| 2 | 3249632.00 | 0.1297 | 117.70 |
| 3 | 3366102.00 | 0.1344 | 121.92 |
| 4 | 3219585.00 | 0.1285 | 116.62 |
| 5 | 2938525.00 | 0.1173 | 106.43 |
| 6 | 2675240.00 | 0.1068 | 96.90 |
| 7 | 2414341.00 | 0.0964 | 87.45 |
| 8 | 2552167.00 | 0.1019 | 92.44 |
| 9 | 2762740.00 | 0.1103 | 100.07 |
| 10 | 3061067.00 | 0.1222 | 110.87 |
| Average | | 0.1170 | 106 |
| Standard Deviation (SD) | | 0.0127 | |
| Method Accuracy | | 106 | |
| %RSD | | 10.9 | |
| CV Horwitz | | 22.1 | |
| 0.67 CV Horwitz | | 14.8 | |

In this test, precision is measured by the percentage of relative standard deviation (RSD) at 10.9%, the Horwitz coefficient of variation at 14.8%, and an average accuracy of 106.19%. The quantification limit values obtained meet the acceptance criteria set by PT Medialab Indonesia, which include producing a positive response NATA 2018, RSD (%) ≤ 0.67 CV Horwitz (%) NATA 2012, and recovery (%) values within the range of 75-125%.[7]

## CONCLUSION

In the linearity test, the correlation was 0.9986. The Limit of Detection (LoD) and Method Detection Limit (MDL) gave a positive response, namely, Limit of Detection (LoD) 0.0034 mg/L and Method Detection Limit (MDL) 0.0356 mg/L. The Limit of Quantification (LoQ) obtained was 0.1132 mg/L. The %RSD obtained in the Precision (Repeatability) test was 1.02%. The accuracy (Recovery) obtained was 105%, and in the Level of Linearity test, the Fcount was obtained, which was smaller than the Ftable, namely 1.42 < 3.18. Based on the results of the analysis, the method for determining fluoride ion levels using ion chromatography can be used for analysis of testing fluoride ion levels.

## CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

## AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID IDs, given below:

Hazil Anwar http://orchid.org/ 0009-0000-4416-817X
Vivi Sisca http://orchid.org/ 0000-0001-9325-5990
Elfizari P. Siregar http://orchid.org/ 0009-0002-3612-6951

Elizarni http://orchid.org/0000-0002-2022-5691
Fejri Subriadi http://orchid.org/0009-0005-1485-3252
Sri Elfina http://orchid.org/0009-0003-0788-3010



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